was diluted by the careful addition of 1 L of methylene chloride so as to lower the temperature to about 55 °C. The warm solution was quenched into 4 L of a stirred ice–water slurry, and the resulting mixture was extracted with three 1-L portions of methylene chloride. The combined extracts were dried with magnesium sulfate and concentrated under reduced pressure. The resulting orange oil was triturated with 8 L of petroleum ether and chilled in ice water. The resulting crude yellow solid was collected by suction filtration and dried under vacuum. Purification by column chromatography (Merck Silica Gel 60, 40–63 μm, 5–10% acetone/methylene chloride) afforded 265 g (79% yield) of **8b** as a buff white solid, mp 110–112 °C: 90% ee;[9] $[\alpha]^{25}_D$ = –61.7° (c = 1.98, CH_2Cl_2); IR (KBr, cm^{-1}) 3400–3100, 3100–3000, 3000–2800, 1670, 1586, 1537, 1410, 1313, 858, 660; NMR 8.00 (2 H, d, 8.7 Hz), 7.65 (2 H, d, 8.7 Hz), 5.22 (1 H, m) 2.23 (3 H, s), 1.74 (3 H, d, 6.7 Hz). Anal. Calcd: C, 58.54; H, 5.36; N, 6.21; Cl, 15.71. Found: C, 58.35; H, 5.33; N, 6.11; Cl, 15.36.

Preparation of 8c. A 100-mL glass round-bottom flask equipped with a reflux condenser, immersion thermometer, and a mechanical stirrer was charged with 4.0 g (17.7 mmol) of **8b**, 100 mL of methanol, and 100 mL of 6 N sulfuric acid. The solution was heated at reflux for 2 h and then cooled to room temperature. The reaction solution was treated with excess aqueous saturated sodium carbonate to make it basic and then extracted with three 100-mL portions of diethyl ether. The combined organic extracts were washed with 50 mL of saturated aqueous sodium carbonate, dried over sodium sulfate, and concentrated under vacuum to yield 2.9 g (89% yield) of yellow solid **8c**: mp 101–104 °C; HPLC[12] 84% ee; $[\alpha]^{25}_D$ = –85.27° (c = 2.295, CH_2Cl_2); IR (KBr, cm^{-1}) 3411, 3329, 3212, 3100–3000, 3000–2800, 1664, 1643, 1589, 1564, 1348, 1261, 843, 615, 584; NMR 7.88 (2 H, d, 8.7 Hz), 6.66 (2 H, d, 8.7 Hz), 5.20 (1 H, q, 6.7 Hz), 4.21 (2 H, bs), 1.71 (3 H, d, 6.7 Hz). Anal. Calcd: C, 58.87; H, 5.49; N, 7.63; Cl, 19.31. Found: C, 59.03; H, 5.53; N, 7.58; Cl, 19.23.

Preparation of 9h. A 3-L flask equipped with a dropping addition funnel, inert gas inlet/outlet, and a mechanical stirrer was charged with 55.8 g (1.4 mol) of 60% sodium hydride dispersion in oil. The oil was removed under inert atmosphere by washing with three 150-mL portions of hexanes, and the residue was dried in vacuo. The hydride was suspended in 1.5 L of dimethylformamide and the suspension was chilled with an ice–water bath. With good stirring 305 g (1.5 mol) of neat benzyl methyl malonate was added via dropping addition funnel over 1 h so as to control the hydrogen evolution. The mixture was stirred at room temperature for 12 h, and a solution of 64 g (0.35 mol) of **8c** in 800 mL of dimethylformamide was added in one portion. The resulting brown suspension was stirred at room temperature for 25 h and quenched into 2 L of pH 7 phosphate buffer. About 20 mL of 10% aqueous HCl was added to raise the pH to 5.5. The mixture was extracted with three 500-mL portions of methylene chloride. The combined extracts were dried over magnesium sulfate and concentrated in vacuo to afford 300 g of a crude oil, which was subjected to column chromatography (Merck Silica Gel 60, 40–63 μm, 3:1 hexane/acetone) to afford 103.7 g (84% yield) of **9h** as a crude yellow oil, which was used directly in the next step.

Preparation of 10b. A solution of **9h** (1.0 g, 2.8 mmol) in 29 mL of ethyl acetate was charged to a flask containing 40 mg of 10% Pd on carbon in 11 mL of ethyl acetate. The mixture was stirred under 1 atm of hydrogen at room temperature, and the reaction was complete after 2.5 h. The catalyst was removed by filtration through SuperCel, and the filtrate was concentrated under reduced pressure to afford 0.8 g of **9i** as a crude oil. This crude oil (0.7 g) was dissolved in 6 mL of diglyme and heated to reflux for 15 min. The solution was cooled and the solvent was removed in vacuo to afford a crude oil. The oil was purified by flash chromatography (hexane/acetone 3:1) to afford 300 mg (55% yield from **9h**) of **10h** as a white solid: mp 96–99 °C; HPLC[12] 86% ee; IR (KBr, cm^{-1}) 3436, 3346, 3244, 3100–3000, 3000–2800, 1728, 1654, 1643, 1590, 1345, 1175, 841; NMR 7.85 (2 H, d, 8.6 Hz), 6.66 (2 H, d, 8.6 Hz), 4.11 (2 H, bs) 3.86 (1 H, m), 3.64 (3 H, s), 291 (1 H, dd, 8.0 Hz and 16.6 Hz), 2.42 (1 H, dd, 6.2 Hz and 16.6 Hz), 1.21 (3 H, d, 7 Hz). Anal. Calcd: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.09; H, 6.90; N, 6.34.

Preparation of (*R*)-(–) SK&F 93505. A solution of **10b** (11.2 g, 50.6 mmol) dissolved in 90 mL of methanol was treated with with 860 mL of a solution of 1:9 v/v hydrazine/water, which was adjusted to pH 6.5 with glacial acetic acid. The reaction solution was heated at reflux for 1 h and cooled to room temperature. The solution was treated with 150 mL of saturated aqueous sodium carbonate and was extracted with 3 × 300 mL portions of ethyl acetate. The combined organic phases were washed with 100 mL of saturated sodium carbonate, dried over magnesium sulfate, and concentrated in vacuo to afford 9.34 g (91% yield) of (*R*)-(–) SK&F 93505 as a crude white solid: HPLC[12] 84% ee.

This crude solid was twice recrystallized by dissolving in ethyl acetate, boiling off solvent until the cloud point, and cooling. (*R*)-(–) SK&F 93505 was obtained in 50% overall recovery: mp 204–206 °C; HPLC[12] 98% ee; $[\alpha]^{25}_D$ = –469.4° (c = 1.14, MeOH); IR (KBr, cm^{-1}) 3367, 3333, 3219, 3100–3000, 3000–2800, 1664, 1609, 1593, 1520, 1349, 1307, 1185, 844; NMR 8.36 (bs, 1 H), 7.57 (d, 8.7 Hz, 2 H), 6.69 (d, 8.7 Hz, 2 H), 3.90 (bs, 2 H), 3.30 (m, 1 H), 2.68 (dd, 1 H, 6.7 Hz and 16.5 Hz), 2.43 (d, 1 H, 16.5 Hz), 1.23 (d, 3 H, 7.4 Hz). Anal. Calcd: C, 65.01; H, 6.45; N, 20.67. Found: C, 64.89; H, 6.49; N, 20.62.

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Amplification of Enantioselectivity in Biocatalyzed Kinetic Resolution of Racemic Alcohols

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Introduction

Recently, much effort in the field of asymmetric biocatalysis has been directed toward identifying lipases with useful enantioselectivity against a broad array of organic compounds.[1] For many unnatural substrates, the catalysis is often effected with low to moderate selectivity, yielding products with enantiomeric excess ranging from 0.5 to 0.8. To improve the enantiomeric discrimination, several strategies have been proposed which entail physical and/or chemical manipulations of enzyme preparations. Presumably, these treatments led to selectivity enhancement by diminishing competing reactions caused by contaminating enzymes[2] or by altering enzyme molecules to assume new conformation.[3] More recently, Sih and his co-workers have proposed a useful concept of "sequential biocatalytic resolution"[4] in the preparation of axially disymmetric diols such as binaphthol and *threo*-2,4-pentanediol with high optical purity. As these molecules possess two functional sites for enzymatic action, the synergistic coupling of two consecutive enantiospecific steps allows stereoselectivity enhancement.

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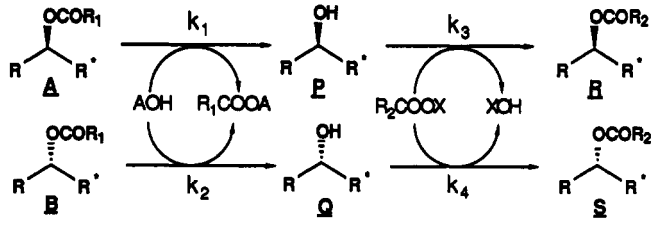
Scheme I



(k_1, k_2, k_3, and k_4 are apparent second-order rate constants)

In principle, this concept may be applied to any coupled enantioselective systems mediated by enzymatic or chemical catalysts. In the course of our exploration of lipase utility, we examined the application of this method to the general cases of lipase-mediated resolutions of racemic alcohols.

Coupled Enantioselective Action in the Resolution of Racemic Alcohols in Organic Media. In view of the mechanistic symmetry of biocatalyzed acyl-transfer reactions in organic media,[5] the antipodal discrimination may take place either preceding or succeeding the step of acyl–enzyme formation, depending on the type of acyl donor/acceptor.[6] Accordingly, the resolution of racemic alcohols can be effected by enantioselective deacylation of the corresponding esters (eq 1) or by its esterification counterpart (eq 2). In many cases, either approach fails

$$(\pm)\text{-}R_1COOR^* + AOH \rightarrow R^*OH + R_1COOA \quad (1)$$

$$R_2COOX + (\pm)\text{-}R^*OH \rightarrow XOH + R_2COOR^* \quad (2)$$

(R*OH, optically active alcohol; AOH, achiral acyl acceptor; R_2COOX, achiral acyl donor)

to achieve satisfactory selectivity, and the products are often subjected to recycling to improve the optical yields. Nonetheless, it is conceivable that in eq 1, provided the reaction is carried out in organic media and an achiral acyl donor is added to the system midway through the reaction, the alcohol intermediate which has been optically enriched in part will undergo ester synthesis with some degree of selectivity. The two consecutive kinetic resolutions can be described by Scheme I. As the biocatalyst displays the same sense of stereochemical preference in both reactions,[7] the desired product can thus be obtained with higher optical yields, albeit at the expense of decreased chemical yields. The basis of this approach parallels that of product recycling in hydrolytic reactions;[8] however, without tedious chromatographic separation of the alcohol intermediates and accompanied chemical transformations. Generally, it is preferable to introduce the achiral acyl acceptor and donor into the system in a stepwise manner to prevent substrate competition which often results in low chemical yields. Also, to facilitate the acylation step, addition of excess amounts of the acyl donor (R_2COOX) is desirable. In the presence of saturating levels of the achiral donor, the majority of the free enzyme will exist in the acylated form, and the concurrent deacylation becomes negligible. In this study, the biocatalytic resolution of racemic 2-phenyl-1-propanol (1) by porcine pancreatic lipase (PPL) was used to test the validity of our approach.

Results and Discussion

(±)-1 was subjected to resolution mediated by PPL in hexane using two different modes of reactions: deacylation with butanol and acylation with vinyl acetate. The results obtained are summarized in Scheme II. In accordance with the data reported by Klibanov and his co-workers,[9] PPL exhibits modest enantioselectivity toward 1 with preference for the *S* enantiomer. The *E* values[10] observed were 5.6 ± 0.2 and 9.8 ± 0.1 for the deacylation of the butyryl ester (2) and the acetylation of 1, respectively. The enantiomeric excess of the resulting products was relatively low, ranging from 0.5 to 0.65 at about 50% conversion. Nevertheless, through the aforementioned coupled procedure, optical purity enhancement in the product fraction became feasible.

The racemic butyrate (2) was first exposed to the crude PPL powder in hexane containing 1.5 equiv of butanol as the acyl acceptor. At approximately 50% conversion, 3 equiv of vinyl acetate was added to the reaction mixture to allow the biocatalytic acylation of *S*-1 (ee_o = 0.55) to proceed. At various intervals, the chemical yield and enantiomeric excess of *S*-3 were determined. As dipicted in Figure 1, the experimental data coincided well with the theoretical prediction[11] based on the known *E* value. Also in line with our assumption, the enantiomeric purity of the butyrate *R*-2 remained virtually unchanged (ee = 0.5), indicating the absence of the deacylation reaction in the presence of excess amounts of vinyl acetate. As noted in Figure 1, the ee value of *S*-3 declined sharply as the acylation approached completion. To obtain *S*-3 with an ee value of 0.9, the reaction was terminated at about 60% of the complete acylation of *S*-1. The combined recovery of *S*-3 (ee = 0.9) in this coupled process was 30% based on the racemic starting material. In comparison, ee values obtained at 30% conversion for biocatalytic systems without this coupled action and with *E* values of 5.6 and 9.8 are 0.62 and 0.75, respectively. On the other hand, to prepare *R*-2 with high optical purity (ee ≥ 0.98), one can simply extend the acetylation reaction (*E* = 9.8) beyond 70%, of which the principle is well understood.[8,12]

This general procedure now allows the preparation of optically active alcohols in moderate yields even when highly enantioselective biocatalysts are not available. To achieve an efficient process, it is essential that the rate of deacylation and that of subsequent acylation be comparable. This may impose a potential restriction for sterically hindered alcohols since the enzymatic deacylation of the corresponding esters is often sluggish. However, one can utilize activated esters[9] or the formate ester[13] of these substrates to circumvent this problem. Generally, enol esters such as vinyl acetate[14] or isopropenyl acetate[15] are

(5) The mechanism of biocatalyzed acyl-transfer reaction can be described as

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(10) The enantiomeric ratio (*E*) is calculated from

$$E = \ln [1 - c(1 + ee(P))]/\ln [1 - c(1 - ee(P))],$$

where ee(P) is the ee value of product. See ref 8.
(11) The theoretical plot (curve a) was generated from the equation

$$\{1 - c[(1 + ee')/(1 + ee_o)]\} = \{1 - c[(1 - ee')/(1 - ee_o)]\}^E$$

where $c = 1 - [(P + Q)/(P_o + Q_o)]$, extent of conversion; $ee_o = [(P_o - Q_o)/(P_o + Q_o)]$, the ee of the alcohol species at the onset of the second reaction; $ee' = [(R - S)/(R + S)]$, the ee of the ester product; $E = k_3/k_4$. A detailed account of this quantitative expression will appear in a full paper.
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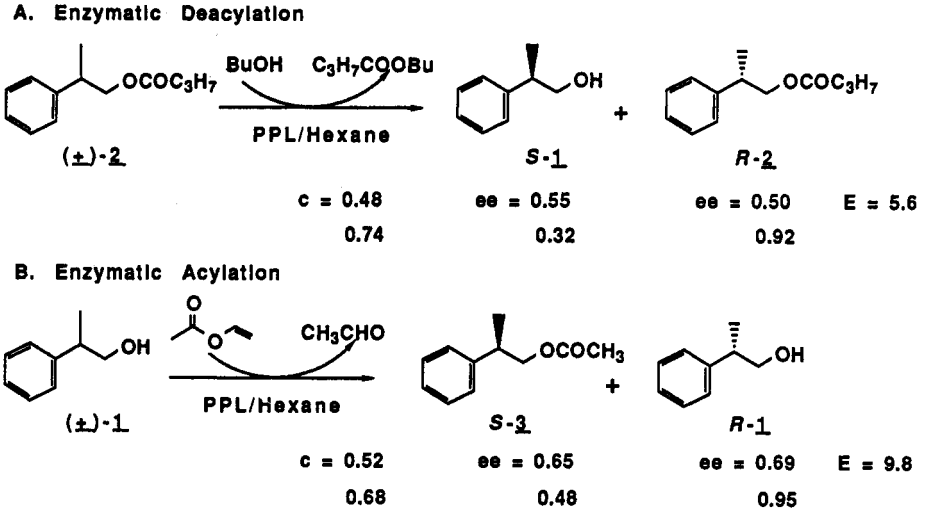
Scheme II



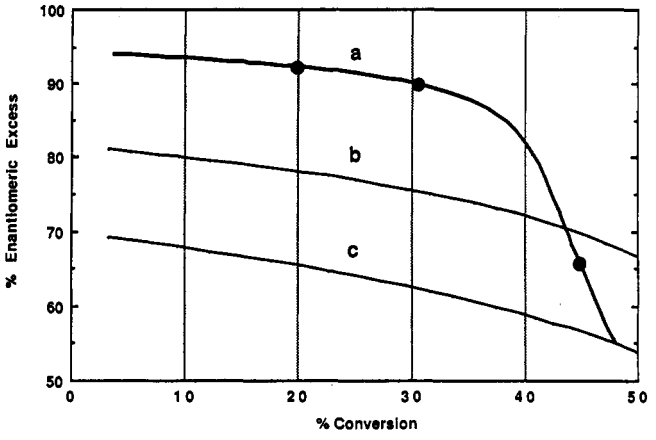


Figure 1. Plot of the percent enantiomeric excess of product as a function of the percent recovery. Curve a represents the coupled system with $ee_o = 0.55$ and $E = 9.8$, and was generated from the equation in ref 11. The extent of recovery is based on the amount of racemic substrate. (●) experimentally determined values. Curves b and c are theoretical predictions for biocatalytic systems with E values of 9.8 and 5.6, respectively. The calculation was according to the equation

$$\ln [1 - c(1 + ee(P))]/\ln [1 - c(1 - ee(P))] = E,$$ in ref 8.

preferably employed as acyl donors due to their ready availability and high turnover rate. Nevertheless, the types of achiral acyl donor/acceptor can be varied in order to optimize the optical and/or chemical yields. In principle, it is possible to introduce the achiral acceptor and donor to the system simultaneously if the following two requirements are satisfied: (1) the catalytic efficiency toward the racemic substrate should be comparable to that toward the acyl donor, and (2) the acyl enzyme derived form the achiral donor should have a preference for the antipodal carbinol over the achiral acyl acceptor. If these requirements are not met then low chemical yields may result from substrate competition. Currently, the application of the technique to the preparation of various optically active alcohols is under investigation in this laboratory.

Experimental Section

Enantioselective Deacylation of (±)-2 with 1-Butanol in Hexane by PPL. Crude PPL powder (500 mg) (Sigma, Type II) was added to a mixture of (±)-2 (1 g, 4.9 mmol) and 1-butanol (538 mg, 7.3 mmol) in hexane (10 mL) which was saturated with doubly distilled water. The resulting suspension was incubated at 30 °C with stirring. At various intervals, aliquots of sample were taken, and the reaction was terminated by removing the protein powder through centrifugation. The organic solvent was taken to dryness under reduced pressure, and the residue was chromatographed over a silica gel column (hexane–ethyl acetate, 30:1 to 10:1) to afford *R*-2 and *S*-1. The remaining butyryl ester was subjected to alkaline hydrolysis (1 N KOH/CH_3OH; room temperature; 3 h) to yield *R*-1 for optical purity determination. The enantiomeric excess of each fraction was determined according to the procedure described below, from which the conversion could be determined according to the equation,[7] c = ee(S)/[ee(S) + ee(P)], where ee(S) and ee(P) denote the values of enantiomeric excess of substrate and product fractions. At 22, 54, and 79 h, the conversion was found to be 32%, 48%, and 61%, respectively; ee(P)'s were 0.6, 0.55, and 0.45, respectively; ee(S)'s were 0.28, 0.5, and 0.7, respectively. The E value was calculated to be 5.6 ± 0.2.

Enantioselective Acylation of (±)-1 with Vinyl Acetate by PPL. To a solution of (±)-1 (1 g, 7.4 mmol) and vinyl acetate (1.58 g, 18.5 mmol) in water-saturated hexane (10 mL) was added 500 mg of crude PPL powder. The reaction mixture was incubated with stirring at 30 °C. The workup procedure was similar to that described above to yield *S*-3 and *R*-1. At 12, 16, and 24 h, the extent of conversion was 52%, 54%, and 59%, respectively; ee(P)'s were 0.65, 0.64, and 0.59, respectively; ee(S)'s were 0.69, 0.75, and 0.87, respectively. The E value was calculated to be 9.8 ± 0.1.

Coupled Procedure for Kinetic Resolution of (±)-1 by PPL. (±)-2 (3 g, 14.6 mmol) and 1-butanol (1.62 g, 21.8 mmol) in water-saturated hexane (30 mL) was incubated with crude PPL powder (1.5 g). After stirring the suspension at 30 °C for 56 h, the ee values of *S*-1 and *R*-2 were 0.55 and 0.5, respectively. Vinyl acetate (3.76 g, 43.8 mmol) was then added in one portion to the reaction mixture. After the addition was complete, aliquots of the solution were withdrawn at various time intervals and centrifuged to remove the protein powder. The organic solvent was evaporated under reduced pressure. The residue was subjected to silica gel chromatography to yield *S*-3, *S*-1, and *R*-2. The chemical yield and the optical purity of each fraction were determined. At 33, 63, and 120 h after the addition of vinyl acetate, the yields of *S*-3 were 20%, 31%, and 45%, respectively, based on the total weight of starting material, and the corresponding ee values were 0.92, 0.90, and 0.66, respectively.

Enantiomeric Purity Determination. The antipodal alcohol 1 was treated with (*S*)-(−)-2-methoxy-2-(trifluoromethyl)-phenylacetyl (MTPA) chloride to form the corresponding (−)-MTPA ester. The MTPA derivatives were analyzed by HPLC using three silica gel columns (10 μm) in tandem (3 × 4.6 mm × 25 cm) with hexane–ethyl ether (100:1) as the mobile phase at a flow rate of 1 mL/min. The retention times for the (−)-MTPA esters of the alcohol 1 were as follows: *R* isomer, 64 min; *S* isomer: 66 min, 20 s.

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