CHIRALITY 16:267–271 (2004)

Utilizing Hydrolases of Opposite Enantiopreference for the Preparation of Both Enantiomers of (1*R*,7a*R*)-(–)- and (1*S*,7a*S*)-(+)-3,6,7,7a-Tetrahydro-1-hydroxy-7a-methyl-1*H*-inden-5(2*H*)-one

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ABSTRACT Four racemic esters of (1*R**,7a*R**)-3,6,7,7a-tetrahydro-1-hydroxy-7a-methyl-1*H*-inden-5(2*H*)-one were prepared and subjected to hydrolysis with two types of hydrolases, including alcalase and three lipases. Alcalase and lipase showed opposite enantiopreference on these esters. Based on this result, we developed a gram-scale procedure using butanoate as the substrate, which was treated consecutively with alcalase and lipase from *Candida rugosa* (CRL), to give both enantiomers of the title compound in high yields and high enantiomeric excess. *Chirality 16:267–271, 2004.*

KEY WORDS: kinetic resolution; chemoenzymatic; enantiopreference; alcalase; lipase; bicyclic hydroxyenone

Hydrolase-catalyzed kinetic resolution is one of the major approaches for the preparation of enantiopure alcohols. Proteases and lipases are among the most used enzymes in this application due to their low cost, stability, and availability.[1,2] Besides, structural knowledge regarding the active sites helps greatly in understanding how the enzymes work and offers useful guidelines on substrate selection and product prediction.[3–6] Alcalase is of special interest, as it is an inexpensive additive widely used in the food industry and detergent formulations.[7] It is therefore well suited in large-scale applications.

Enantiopure bicyclic hydroxyenones **1, 2** and their enantiomers are important building blocks for the synthesis of a variety of natural products, including terpenoids and steroids.[8–14] Currently, they are mainly derived from the corresponding enantiopure Hajos-Parrish ketone and Wieland-Miescher ketone through a regio- and diastereoselective reduction with $NaBH_4$. We previously reported a convenient chemoenzymatic method for the preparation of enantiomerically enriched hydroxyenone (4a*S*,5*S*)-(+)-**2** and found that the acyl moiety played an important role in the alcalase-catalyzed hydrolysis of the racemic esters.[15] Here we further exploit this chemoenzymatic approach and develop a practical method for the large-scale preparation of both enantiomers of compound **1** in high enantiomeric excess.

EXPERIMENTAL

General Methods

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded at 400 and 100 MHz in $CDCl_3$, respectively. Analytical TLC (silica gel, 60F-54, Merck, Darmstadt, Germany) and spots were visualized under UV light and/or phosphomolybdic acid-ethanol. Flash column chromatography was performed with silica gel 60 (70–230 mesh, Merck). HPLC was performed on a Chiralcel OJ column (4.6 × 250 mm, *n*-hexane/*i*-PrOH = 92/8, 1 mL/min) monitored at 235 nm. Racemic hydroxyenone **1** was prepared according to literature procedures.[24,25]

Acetic acid (1*S,7a*S**)-(±)-7a-methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1H-inden-1-yl ester (3).**[24] To an ice-cooled solution of the racemic hydroxyenone **1** (760 mg, 4.22 mmol) in 6 mL of pyridine was slowly added 0.5 mL of Ac_2O (5.29 mmol). The reaction was kept at room temperature (RT) and stirred overnight. After the reaction was complete (~12 h), a few drops of H_2O were added to quench the reaction. The mixture was stirred for another 30 min and pyridine was removed under reduced pressure. The residual oil was dissolved in EtOAc and washed consecutively with 5% citric acid (×3), 5% $NaHCO_3$ (×3), H_2O (×2) and brine. After drying over anhydrous Na_2SO_4 and filtration, the desired ester **3** (890 mg, 93%) was purified by silica gel column chromatography eluted with hexane/EtOAc (8/2). R_f = 0.27 (hexane/EtOAc = 6/4). ^1H NMR (400 MHz, $CDCl_3$): δ 5.76 (s, 1 H), 4.76 (dd, *J* = 9.9, 7.8 Hz,

Contract grant sponsor: National Science Council; Contract grant number: NSC 92-2113-M-002-011 (to L.C.L.).
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Received for publication 31 October 2003; Accepted 9 January 2004

1 H), 2.71 (dddd, J = 19.5, 11.7, 2.6, 2.5 Hz, 1 H), 2.49-2.37 (m, 2 H), 2.32 (m, 1 H), 2.23 (m , 1 H), 2.04 (s, 3 H), 2.01 (ddd, J = 13.2, 5.4, 2.1 Hz, 1 H), 1.89-1.77 (m, 2 H), 1.14 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.5, 172.8, 170.5, 123.5, 80.8, 44.5, 34.1, 33.1, 26.5, 26.2, 20.9, 16.5. IR (neat): 1740, 1670, 1242, 1207, 1044 cm^{-1}. HRMS calcd for $C_{12}H_{17}O_3$ $(M+1)^+$ 209.1178, found 209.1176.

General Procedure for the Preparation of Racemic Esters 4–6

The procedure is similar to that for acetate **3,** except suitable acyl chlorides (butanoyl, hexanoyl, and octanoyl) instead of anhydrides were used as the acylating agents. The desired esters **4–6** were purified by silica gel column chromatography eluted with hexane/EtOAc (8/2 → 6/4).

Butanoic acid (1*S,7a*S**)-(±)-7a-methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1H-inden-1-yl ester (4).** Yield 86%, R_f = 0.42 (hexane/EtOAc = 6/4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.78 (s, 1 H), 4.80 (dd, J = 9.9, 7.9 Hz, 1 H), 2.71 (m, 1 H), 2.52-2.20 (m, 6 H), 2.01 (m, 1 H), 1.96-1.80 (m, 2 H), 1.65 (q, J = 7.4 Hz, 2 H), 1.16 (s, 3 H), 0.94 (t, J = 7.4 Hz, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.6, 173.2, 173.0, 123.5, 80.6, 44.6, 36.2, 34.2, 33.2, 26.6, 26.3, 18.5, 16.6, 13.6. IR (neat): 2923, 2855, 1735, 1675, 1647, 1243, 1178 cm^{-1}. HRMS calcd for $C_{14}H_{21}O_3$ $(M+1)^+$ 237.1491, found 237.1488.

Hexanoic acid (1*S,7a*S**)-(±)-7a-methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1H-inden-1-yl ester (5).** Yield 88%. R_f = 0.47 (hexane/EtOAc = 6/4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.76 (s, 1 H), 4.77 (dd, J = 9.9, 7.9 Hz, 1 H), 2.69 (m, 1 H), 2.52-2.18 (m, 6 H), 1.98 (m, 1 H), 1.90-1.73 (m, 2 H), 1.64-1.52 (m, 2 H), 1.36-1.18 (m, 4 H), 1.14 (s, 3 H), 0.88-0.79 (m, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.7, 173.4, 173.1, 123.5, 80.6, 44.6, 34.3, 34.2, 33.1, 31.2, 26.6, 26.3, 24.7, 22.3, 16.5, 13.9. IR (neat): 2963, 2930, 2860, 1740, 1674, 1653, 1458, 1223, 1170 cm^{-1}. HRMS calcd for $C_{16}H_{25}O_3$ $(M+1)^+$ 265.1840, found 265.1801.

Octanoic acid (1*S,7a*S**)-(±)-7a-methyl-5-oxo-2,3,5,6,7,7a-hexahydro-1H-inden-1-yl ester (6).** Yield 85%. R_f = 0.55 (hexane/EtOAc = 6/4). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.74 (s, 1 H), 4.76 (dd, J = 10.0, 8.0 Hz, 1 H), 2.69 (m, 1 H), 2.49-2.14 (m, 6 H), 1.96 (m, 1 H), 1.88-1.72 (m, 2 H), 1.64-1.50 (m, 2 H), 1.32-1.16 (m, 8 H), 1.12 (s, 3 H), 0.89-0.74 (m, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 198.7, 173.4, 173.1, 123.5, 80.6, 44.6, 34.3, 34.2, 33.1, 31.6, 29.0, 28.8, 26.6, 26.3, 25.0, 22.5, 16.8, 14.0. IR (neat): 2936, 2864, 1740, 1668, 1642, 1451, 1234, 1221, 1169, 1024 cm^{-1}. HRMS calcd for $C_{18}H_{29}O_3$ $(M+1)^+$ 293.2117, found 293.2114.

Enzyme-catalyzed hydrolysis of racemic esters 3–6 (analytical scale). For alcalase-catalyzed reactions, two stock solutions were first prepared. Solution A contained 0.125 M of individual substrate in DMF and solution B contained 25% alcalase 2.4L[7] (v/v) in 0.3 M phosphate buffer (pH 7.0). For each reaction, 80 μL of solution A and 280 μL of phosphate buffer (0.3 M, pH 7.0) were first mixed. It was then added to 40 μL of solution B (total volume = 400 μL) and placed in a shaker at 37°C. The reactions were terminated after 12 h by addition of EtOAc (20 mL) and H_2O (10 mL). The organic layer was collected, washed with H_2O (10 mL ×2), brine (10 mL). It was dried over anhydrous Na_2SO_4, filtered, and concentrated to dryness. The residue was dissolved in *n*-hexance/*i*-PrOH for HPLC analysis. For lipase-catalyzed reactions, 80 μL of solution A and 320 μL of phosphate buffer (0.3 M, pH 7.0) were first mixed. Individual lipase (3 mg) was then added to start the reaction. After 12 h, lipase was filtered off and the rest of the workup procedure was the same as described above.

Gram-scale preparation of both enantiomers of 3,6,7,7a-Tetrahydro-1-hydroxy-7a-methyl-1*H*-inden-5(2*H*)-one (1). To a solution of (±)-butanoate-**4** (1.22 g, 5.16 mmol) in 51 mL of DMF, was added 153 mL of phosphate buffer (0.3 M, pH 7.0) and 1 mL of alcalase. The mixture was stirred at RT for 12 h. Another portion of alcalase (1 mL) was then added. The reaction was terminated when the conversion (aliquots were taken and analyzed with HPLC) was close to 50%. The reaction mixture was extracted with *n*-hexane (200 mL ×6). The remaining ester **4** goes to the hexane layer and the hydroxyenone product **1** stays in the aqueous layer. The aqueous layer was extracted with EtOAc (×6) and the desired hydroxyenone product was purified by silica gel column chromatography eluted with hexane/EtOAc (6/4) to give (1*S*,7a*S*)-(+)-**1** (399 mg, 93% yield). Mp: 63–64°C. R_f = 0.28 (hexane/EtOAc = 4/6). Its ee was found to be 97.8% by HPLC analysis, $[\alpha]_D^{22}$ = +97.1 (c 1.0, benzene). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.75 (s, 1 H), 3.81 (dd, J = 10.0, 7.6 Hz, 1 H), 2.66 (m, 1 H), 2.52-2.28 (m, 3 H), 2.17-2.01 (m, 2 H), 1.89-1.70 (m, 3 H), 1.11 (s, 3 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 199.4, 175.3, 123.4, 80.6, 45.2, 34.1, 33.3, 29.1, 26.5, 15.1. IR (neat): 3422, 1648, 1444, 1365, 1234, 1083 cm^{-1}. HRMS calcd for $C_{10}H_{15}O_2$ $(M+1)^+$ 167.1072, found 167.1077. The remaining ester **4** recovered from the hexane layer was concentrated to dryness. Its ee was found to be 98.8%, and it was further subjected to lipase-catalyzed hydrolysis by dissolving in 13 mL of DMF and 36 mL of phosphate buffer (0.3 M, pH 7.0). CRL (400 mg) was added and the reaction mixture was stirred at RT for 46 h. The lipase was then filtered off. The hydroxyenone product was extracted and purified as described above to give (1*R*,7a*R*)-(–)-**1** (334 mg, 83% yield). Mp: 68–69°C. Its ee was found to be greater than 99.9% by HPLC analysis, $[\alpha]_D^{22}$ = -98.5 (c 1.0, benzene). Its spectroscopic data were identical to those reported in the literature.[26,27]

RESULTS AND DISCUSSION

Although many factors, including temperature, pH, additives, and immobilization on supports, have been

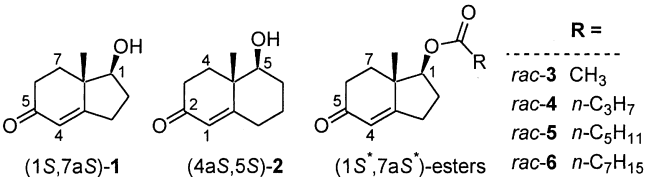


Fig. 1. Structures of bicyclic hydroxyenones **1, 2,** and racemic esters 3–6.

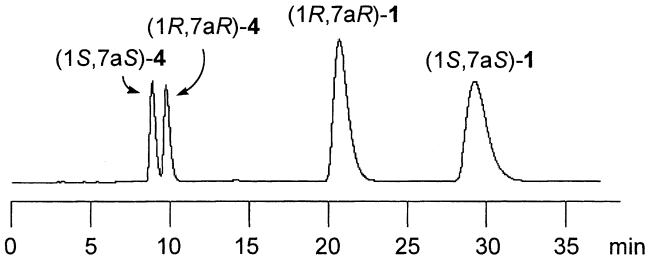


Fig. 2. A representative HPLC chromatogram showing the separation of enantiomeric pairs **1** and **4.** Conditions: Chiralcel OJ (4.6 × 250 mm), *n*-hexane/*i*-PrOH (92/8), 1 mL/min, 235 nm.

studied to improve enzymatic performance, a more effective approach might be changing the acyl moieties of the substrates.[16,17] For preliminary screening purposes, we prepared racemic esters **3–6,** differing in the length of their acyl groups (Fig. 1). They were obtained by acylation of the racemic hydroxyenone **1** with suitable acylating agents in high yields. Two different types of hydrolases, including alcalase and three lipases, were surveyed in this study. Alcalase, prepared from submerged fermentation of a selected strain of *Bacillus licheniformis*, contains a serine protease, subtilisin Carlsberg, as its major enzyme component. Alcalase also possesses esterase activity with a high turnover rate and shows good stability in various organic solvents.[18–20] Lipases from the following sources, *Candida rugosa* (CRL), porcine pancreas (PPL), and wheat germ (WGL), were used. Prior to the screening study, a convenient analytical method was established employing HPLC with a Chiralcel OJ column for the separation of the enantiomeric pairs of remaining ester and hydroxyenone product. As a typical example, the chromatogram for the separation of a mixture of racemic butanoate **4** and hydroxyenone **1** is shown in Figure 2. The ee_s and ee_p for each hydrolytic reaction could be simultaneously determined in a single analysis after a simple extraction procedure, where ee_s and ee_p represent the enantiomeric excess of ester starting material and alcohol product, respectively. Therefore, the progress of each individual

TABLE 1. Results of enzyme-catalyzed hydrolysis of the racemic esters 3–6[a]

		% Conversion[b] (*E* value)[c]			
Ester	R	Alcalase	CRL	PPL	WGL
3	CH_3	13 (12)	NR	NR	29 (5)
4	n-C_3H_7	54 (61)	61 (10)	23 (< 2)	NR
5	n-C_5H_{11}	47 (60)	55 (8)	35 (< 2)	NR
6	n-C_7H_{15}	16 (> 600)	95 (< 2)	40 (8)	NR

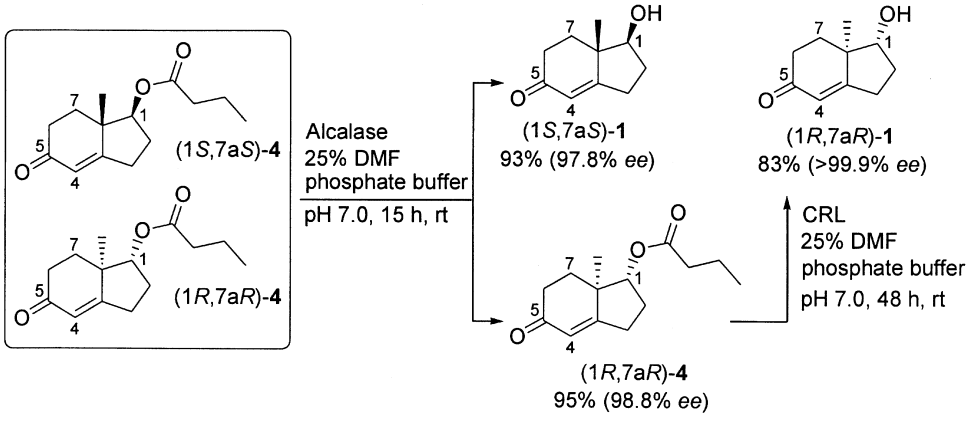
[a]Individual substrate (25 mM) was incubated with the corresponding enzyme in 20% DMF/0.3 M phosphate buffer (pH 7.0) at 37°C for 12 h. The reactions were subjected to HPLC analysis for determination of conversion and *E* value as described in the text.
[b]NR indicates no reaction.
[c]Alcalase favors the formation of (1*S*, 7a*S*)-product, whereas all three lipases showed (1*R*, 7a*R*)-enantiopreference.

hydrolytic reaction, including conversion (c) and enantioselectivity (*E* value), could easily be monitored and calculated by the following equations:[21,22]

$$c = ee_s/(ee_s + ee_p)$$

$$E(\text{enantioselectivity}) = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$$

The preliminary screening reactions were performed by incubating individual racemic ester substrate (25 mM) with the corresponding enzyme in 20% DMF/0.3 M phosphate buffer (pH 7.0) at 37°C. Reactions were stopped and extracted with EtOAc for HPLC analysis after 12 h. The results are shown in Table 1. Alcalase accepted all four esters as its substrate. The reaction rates were in the order of butanoate **4** > hexanoate **5** > octanoate **6** > acetate **3,** and the enantioselectivity increased with the length of the acyl moiety. This result is slightly different from that for compound **2,** in which case only the butanoate was hydrolyzed.[15] On the other hand, all three lipases showed only low enantioselectivity (*E* < 10), and we observed a different trend in their substrate preference. CRL and PPL preferred longer acyl chains and the rate of hydrolysis increased with the length of the acyl moiety, whereas WGL



Scheme 1. A gram-scale preparation of both enantiomers of hydroxyenone **1** using (±)-butanoate **4** as the substrate, which was subjected to consecutive hydrolysis with alcalase and CRL.

only worked on the short chain acetate **4.** The low enantioselectivity of lipases on this series of substrates was consistent with that for compound **2** series. In that case, replacement of DMF with other organic solvents such as DMSO and acetone did not result in improvement in the enantioselectivity.

It is interesting to note that alcalase and lipase displayed opposite enantiopreference. Alcalase favors the formation of (1*S*,7a*S*)-product, while all three lipases favor the (1*R*,7a*R*)-product. This result is in agreement with Kazlauskas' rule for resolution of secondary alcohols.[4,5] Nevertheless, it is important to point out that much higher enantioselectivity of alcalase over lipases observed in this system is unique and contrary to previous reports.[5,23] Recently, a transition-state model was introduced to rationalize the enantioselectivity of subtilisin and lipases toward secondary alcohols.[23] This model, which used a short acetyl group as the acyl moiety, proposes that the conformational requirements of the transition state is critical. During the enzymatic reaction, the large substituent will point toward the external surface of the active site pocket to minimize steric repulsion. Lower enantioselectivity of subtilisin in those systems was probably due to the lack of the protein wall corresponding to the "triangular wall" of lipases. Since alcalase displayed the same enantiopreference on acetate **3** and butanoate **4,** a dramatic improvement in the enantioselectivity when the acyl group was changed from acetyl (E = 12) to butanoyl (E = 61) suggests that the longer butanoyl group should provide an important interaction point with the protein in the active site pocket. This specific interaction site deserves further study and could serve as a useful reference point for modifying the catalytic model of the enzyme.

Based on the above screening results, we chose (±)-butanoate **4** as the substrate and developed a practical method combining the uses of alcalase and CRL for the gram-scale preparation of both enantiomers of hydroxyenone **1** (Scheme 1). Since alcalase displays better enantioselectivity (E > 60) on this substrate and retains good reaction rates and enantioselectivity in the presence of 5–45% DMF, it was adopted as the primary enzyme in the kinetic resolution and CRL was used as an auxiliary enzyme. For a gram-scale resolution, (±)-butanoate **4** (25 mM) was first subjected to alcalase-catalyzed hydrolysis in 25% DMF/0.3 M phosphate buffer (pH 7.0) at RT. Alcalase was added in two portions for better performance. The reaction was stopped at around 50% conversion (checked with HPLC) and the mixture was extracted with *n*-hexane. The remaining ester **4** goes to the hexane layer, while (1*S*,7a*S*)-**1** stays in the aqueous phase. This simple extraction/partition procedure is especially convenient and suitable for a large-scale operation to separate the hydroxyenone product from the remaining ester. After the separation, (1*S*,7a*S*)-**1** was extracted from the aqueous phase with EtOAc and was obtained in high enantiomeric excess (97.8% ee) and yield (93%) after silica gel column chromatography. The recovered ester from the hexane layer was found to be highly enriched (1*R*,7a*R*)-**4** (98.8% ee). It was further subjected to second hydrolysis with CRL in 25% DMF/0.3 M phosphate buffer (pH 7.0) at RT to give (1*R*,7a*R*)-**1** in 83% yield. The optical purity of the product was further raised to >99.9% ee.

CONCLUSION

We have developed a simple and convenient chemoenzymatic method combining the uses of inexpensive alcalase with lipase for the preparation of both enantiomers of 3,6,7,7a-tetrahydro-1-hydroxy-7a-methyl-1*H*-inden-5(2*H*)-one (**1**). By taking advantage of the opposite enantiopreference of these two hydrolases on butanoate **4,** both enantiomers of the hydroxyenone **1** were obtained in high chemical yields and in high optical purities. The acyl moiety of butanoate **4** not only plays an important role in the rate and enantioselectivity of the enzyme-catalyzed resolution, it also offers the advantage of simplifying the purification procedure. An *n*-hexane/H_2O partition easily separates the hydroxyenone product from the remaining ester. The procedure described in this study was intended to give both enantiomers in high chemical yields and in high optical purities. Although CRL showed lower E value than that of alcalase, when used as the secondary hydrolase in this study it is enough to raise the optical purity of the (1*R*,7a*R*)-**1** product to >99.9% ee. More importantly, by simply changing the acyl moiety of the substrates to a butanoyl group, we would be able to expand the use of alcalase in kinetic resolutions and offer an opportunity to reinvestigate some old systems where a short acetyl group was used and poor resolutions were reported in the literature.

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