Pig Liver Esterase Catalyzed Hydrolyses of Racemic α-Substituted α-Hydroxy Esters

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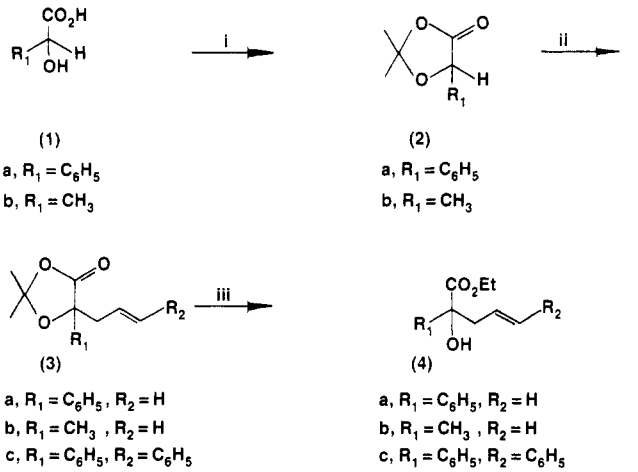
Pig liver esterase catalyzed hydrolyses of some α-substituted α-hydroxy esters give product acids and recovered esters in 9–94% enantiomeric excess. The observed enantiomeric selectivity could be rationalized using a recently proposed active site model, which proved to be of predictive value.

Enantiomerically pure α-alkylated α-hydroxy carboxylic acids are valuable synthetic intermediates in asymmetric synthesis.[1] Such acids in optically active form have thus far been synthesized by more or less elaborate stereoselective syntheses.[2] To the best of our knowledge no attempts to use enzymatic methods to prepare such sterically hindered α-hydroxy acids have been reported in the literature, although the synthetic opportunities provided by enzymes as chiral catalysts for the preparation of enantiomerically pure compounds are now widely exploited.[3] Especially hydrolytic enzymes such as lipases and esterases have proved to be very attractive because they operate without the need for coenzymes. The broad substrate specificity and high stereoselectivity of one of these enzymes, pig liver esterase (PLE) is well documented.[4] Many examples of the use of PLE in the stereoselective hydrolysis of meso diesters have been reported.[5] Much less is known, however, about the PLE-catalyzed resolution of racemic monoesters.[6]

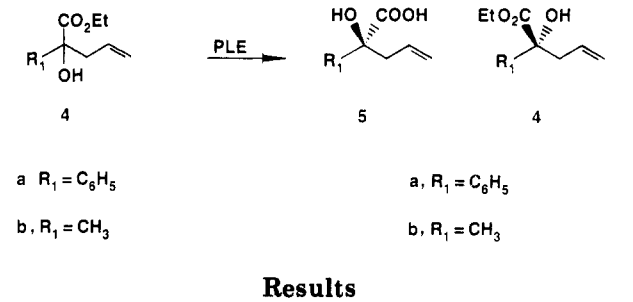
In this paper we describe the results of PLE-catalyzed hydrolyses of racemic 2-substituted 2-hydroxy-4-pentenoic esters (**4**). These α-allyl-substituted esters are attractive synthetic intermediates owing, among other aspects, to the possibilities for further elaboration of the double bond. The data found provide additional information about the three-dimensional structure of PLE, as recently proposed in an active-site model by Jones.[7]

Scheme I[a]



[a] Reagents: (i) $(CH_3)_2C(OCH_3)_2$, benzene; (ii) LDA, $R_2CH{=}CHCH_2Br$; (iii) EtOH, HCl.

Scheme II



Results

The α-substituted α-hydroxy esters were synthesized in good overall yields from the corresponding unbranched α-hydroxy acids, using conventional methods. Thus, protection of the α-hydroxy acids **1a,b** as their acetonides **2a,b**, followed by alkylation with allylic bromides, gave dioxolanones **3a–c**. Acid-catalyzed deprotection of **3a–c** in EtOH afforded the desired α-hydroxy esters **4a–c** (Scheme I).

These esters were subjected to PLE-catalyzed hydrolysis. Two commercially available pig liver esterases were used (PLE-EC 3.1.1.1 [Sigma] and PLE-A [Amano]). The enzymatic hydrolyses of **4a–c** were carried out in 0.05 M phosphate buffer at pH 8. The pH was maintained at this level by addition of 2 N aqueous NaOH from an autoburette. When the conversions reached 20–50% the reactions were terminated and both the optically active unchanged esters and optically active acid products were isolated in

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Table I. Hydrolysis of α-Hydroxy Esters 4a–c by PLE

entry	substrate	PLE[a]	% conv (c)[b]	recovered ester	chem yield, %	% ee (S)	product	chem yield, %	% ee (P)	E[c]
1	(±)-**4a**	S	0.23	(*R*)-**4a**	64	25	(*S*)-**5a**	19	86	17
2	(±)-**4a**	A	0.34	(*R*)-**4a**	52	49	(*S*)-**5a**	29	94	52
3	(±)-**4a**	A	0.51	(*R*)-**4a**	41	86	(*S*)-**5a**	44	83	30
4	(±)-**4b**	S	0.29	(*S*)-**4b**	43	17	(*R*)-**5b**	27	42	3
5	(±)-**4b**	A	0.29	(*S*)-**4b**	55	9	(*R*)-**5b**	26	22	2
6	(±)-**4c**	S	no hydrolysis							
7	(±)-**4c**	A	no hydrolysis							

[a] Pig liver esterase (S: Sigma; A: Amano). [b] Calculated from the ee values of acid and remaining ester. [c] This enantiomeric ratio is calculated from the equation $E = \ln [1 - c(1 + \text{ee(P)})]/\ln[1 - c(1 - \text{ee(P)}]$ and provides a measure of the enzyme's ability to discriminate between the two enantiomers of the substrate.[8]

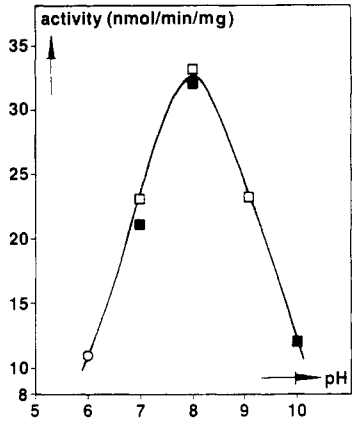

Figure 1. Effect of pH on activity of PLE-A on substrate **4a** at 28 °C. The following buffer solutions (0.05 M) were used: (○) citrate; (□) phosphate; (■) titrisol.

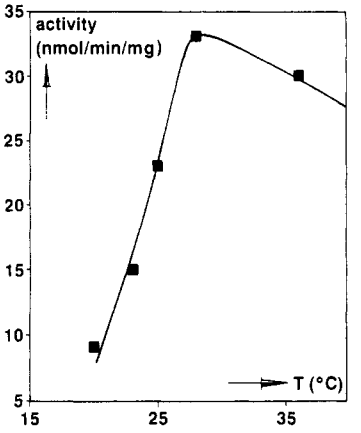

Figure 2. Effect of temperature on activity of PLE-A on substrate **4a** at pH 8.

good yields (Scheme II). The results are summarized in Table I.

The data in the table show that α-hydroxy esters **4a–b** are substrates for PLE. When racemic **4a** was treated in aqueous buffer with PLE-S, the product (*S*)-**5a** could be isolated in good enantiomeric excess (entry 1), with the moderate enantiomeric ratio E[8] of 17. However, it should be noted that the activity dropped considerably after about 5% conversion, making this approach less attractive. Much better results in terms of activity and stereoselectivity were obtained when PLE-A (recently made available) instead of PLE-S was used. During the enzymatic hydrolysis of compound **4a** the enzymatic activity remained quite constant. The *S* ester is hydrolyzed preferentially with a high E value of 52 (entry 2). By optimization of pH and temperature, applied during enzymatic hydrolysis of **4a** by PLE-A, the initial activity could be increased. Optimal conditions (see Figures 1 and 2) proved to be a pH of 8, independent of the type of buffer used, and a temperature of 28 °C.

When the hydrolysis of **4a** was allowed to proceed to 50% conversion, both unreacted ester and acid were isolated in high yield and high ee (entry 3). Enantiomerically pure (*S*)-**5a** could be obtained after one recrystallization from $CHCl_3$. Hydrolysis of the recovered optically active ester **4a** in KOH/MeOH, followed by recrystallization from $CHCl_3$, yielded enantiomerically pure (*R*)-**5a**. The decrease in E value with time may be due to a time dependent decrease in enantioselectivity of the enzyme. It is tempting to attribute the observed change in enantioselectivity to the known fact that PLE consists of a mixture of isoenzymes. However, this can seemingly be ruled out based on findings reported by Jones.[9] Based on representative monocyclic and acyclic diester substrates the enantioselectivity of the isoenzyme components of commercially available PLE proved to be comparable, which suggests that PLE behaves as a single protein.

The ee's of the acids **5a,b** and esters **4a,b** were determined by ^1H NMR analysis, involving derivatization with (*S*)-2-chloropropionyl chloride.[10] The stereochemical configurations of the compounds in Table I were assigned by correlation of optical rotations with known compounds.[2a,11] The absolute configuration of (+)-**5a** was further confirmed by hydrogenation to the known acid (+)-α-*n*-propylmandelic acid (**6**). Two values for the rotation of enantiomerically pure (+)-(*S*)-**6** have been reported in the literature ($[\alpha]_D$ +28.9° (c = 2, EtOH)[11] and $[\alpha]_D$ +21.6° (c = 2.5, EtOH)[2b,12]). We believe that the higher one is correct (see the Experimental Section).

Discussion

We were interested to see if an explanation could be given for the observed enantioselectivity, using an active site model. In this respect, several models have been developed for PLE, e.g. by Tamm,[13] Ohno,[14] Lam,[15] Norin,[16] and Zemlicka.[5b] The most recent model constructed with

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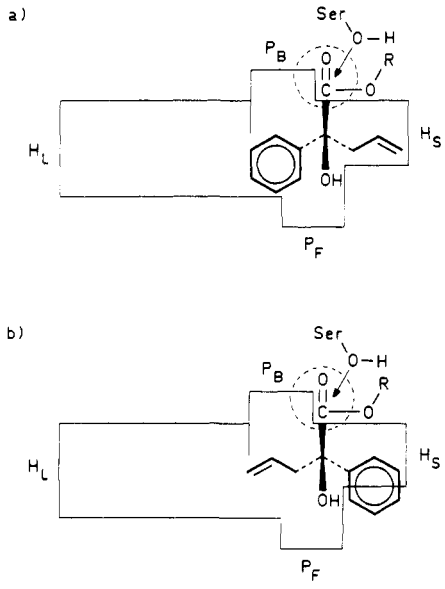

Figure 3. The top-perspective view of the active site model of PLE is shown. When the ester group to be hydrolyzed is placed in the serine sphere, only the orientation of **4a** shown in (a), with the allyl group nicely located in the H_S pocket, represents the sterically allowed fit, resulting in *S* center ester hydrolysis. Binding leading to hydrolysis of the *R* ester would require the phenyl group to be positioned in H_S, which is precluded since this pocket is too small to accept a group of this size (b). The high stereoselectivity of PLE-catalyzed *S* center ester hydrolysis, which would be predicted from these pictures, is observed experimentally.

cubic space descriptors, based on analysis of the reactions of more than a hundred esters with a broad structural range, was proposed by Jones et al.[7] In essence this model of the catalytic site is composed of five binding regions, i.e. (i) a nucleophilic serine site; (ii and iii) two hydrophobic zones, which in general interact with both aliphatic and aromatic portions of a substrate represented by $H_{L(arge)}$ and $H_{S(mall)}$; and (iv and v) two hydrophilic zones located at the front (P_F) and the back (P_B) of the active site as depicted in Figure 3.

The observed stereoselective hydrolysis of compound **4a** by PLE-A and -S may be explained as follows (see Figure 3): In order to be hydrolyzed, the carboxylic ester group should be located at the serine site of the enzyme. As the aromatic phenyl group is structurally too demanding for H_S, which has a volume of about 5.5 Å^3, it will be positioned in H_L. The remaining allyl group will fit comfortably in H_S, which is unable to accept groups larger than four carbon atoms. The polar hydroxyl group will be directed into P_F. In this favored enzyme–substrate complex where the hydrophobic interactions are maximized, the enzyme-catalyzed hydrolysis of compound **4a** should lead to highly enantioselective formation of (*S*)-**5a** and (*R*)-**4a**, as observed experimentally. The predictive value of the PLE active site model as developed by Jones was further illustrated by the correct predictions concerning the enzymatic hydrolysis of esters **4b** and **4c** (entries 4–7). For compound **4b** it may be anticipated that due to the Circe effect criteria on binding rules[17] both the hydrophobic methyl and allyl group may fit in and thus compete for the smaller H_S pocket, which will result in a low enantioselectivity. This was verified by experiment (entries 4 and 5, $E \approx 2$). However, although the enantioselectivity is low, still there is a slight preference for location of the allyl group in H_S, which gives the better hydrophobic binding resulting in preferential *R* ester hydrolysis. Further, when compound **4c**, which has hydrophobic phenyl and phenylallyl groups, was treated with PLE-A and PLE-S in aqueous buffer for prolonged periods of time, no hydrolysis occurred. The two hydrophobic groups, which are both too bulky to be positioned in H_S, appear to inhibit the enzymatic hydrolysis.

In conclusion we observe that sterically hindered α-hydroxy esters may be hydrolyzed successfully using PLE. The stereochemical outcome can be rationalized using an active-stie model proposed by Jones. Both enantiomers of α-allylmandelic acid could be obtained in enantiomerically pure form using this enzymatic hydrolysis. Further investigations of the selectivity of PLE for other α-substituted α-hydroxy esters are in progress.

Experimental Section

Pig liver esterase (PLE EC 3.1.1.1, suspension in 3.2 M $(NH_4)_2SO_4$, activity 100 units/mg protein) was a product of Sigma Chemical Co. and PLE-A (4370 units/g) was obtained from Amano Pharmaceutical Co., Ltd.

2,2-Dimethyl-5-phenyl-1,3-dioxolan-4-one (2a). A mixture of mandelic acid (**1a**) (15.2 g, 0.10 mol), 2,2-dimethoxypropane (12.5 g, 0.12 mol), and benzene (100 mL) was refluxed for 2 h with azeotropic removal of methanol. The resulting solution was concentrated under reduced pressure, and the residue was purified by Kugelrohr distillation (150 °C (20 mmHg)) to give 18.8 g (98% yield) of **2a** as a colorless oil, which solidified on standing: mp 42.5–43.5 °C; ^{1}H NMR ($CDCl_3$) δ 2.7 (2 s, 6 H), 6.3 (s, 3 H), 8.4 (s, 5 H); ^{13}C NMR ($CDCl_3$) δ 25.78 (q), 26.90 (q), 75.86 (d), 110.66 (s), 126.19 (d), 128.43 (d), 128.66 (d), 134.20 (d), 171.20 (s). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.68; H, 6.36.

2,2,5-Trimethyl-1,3-dioxolan-4-one (2b). This compound was prepared from lactic acid (**1b**, 14.7 g, 0.16 mol) and 2,2-dimethoxypropane (22.1 g, 0.21 mol), following the procedure described above. Kugelrohr distillation at 50 °C (50 mmHg) of the crude product afforded 17.7 g (88% yield) of **2b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.48 (d, 3 H), 1.54 (s, 3 H), 1.61 (s, 3 H), 4.48 (q, 1 H); ^{13}C NMR ($CDCl_3$) δ 16.91 (q), 25.08 (q), 26.95 (q), 69.94 (d), 109.83 (s), 173.34 (s). Anal. Calcd for $C_6H_{10}O_3$: C, 55.37; H, 7.74. Found: C, 55.01; H, 7.72.

General Procedure for the Alkylation of the Enolate from the Dioxolanones 2a and 2b with Electrophiles. A 0.10-mol run is described. A solution of **2a,b** (0.10 mol) in THF (30 mL) was added to a solution of LDA (0.11 mmol) in THF-hexane (1:1, 135 mL) at −78 °C. After being stirred for 30 min, the mixture was cooled to −78 °C, and the electrophile (0.14 mol) was added. The reaction mixture was allowed to warm to room temperature (in about 3 h), poured into a half-saturated ammonium chloride solution (150 mL), and diluted with ether. After the organic layer was separated, the aqueous layer was extracted with ether (2 × 100 mL); the ether extracts were combined and dried over $MgSO_4$. Removal of the solvent in vacuum gave the alkylated dioxolanone, which was purified by either distillation or crystallization. Specific details for each compound are given below.

2,2-Dimethyl-5-allyl-5-phenyl-1,3-dioxolan-4-one (3a). From allyl bromide (25.4 g, 0.21 mol) and 28.8 g (0.15 mol) of **2a** was obtained 34.2 g (98% yield) of **3a** after Kugelrohr distillation at 110 °C (0.1 mmHg) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3 H), 2.7 (d, 2 H), 4.9–6.1 (m, 3 H), 7.2–7.8 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 27.60 (q), 27.70 (q), 45.82 (t), 83.12 (s), 110.05 (s), 120.09 (t), 124.61 (d), 127.85 (d), 128.20 (d), 131.17 (d), 139.55 (s), 172.27 (s). Anal. Calcd for $C_{14}H_{16}O_3$: C, 72.39; H, 6.94. Found: C, 72.23; H, 7.04.

2,2,5-Trimethyl-5-allyl-1,3-dioxolan-4-one (3b). Allyl bromide (14.0 g, 0.12 mol) and 12 g (0.09 mol) of **2b** yielded 14.2 g (91% yield) of **3b** after Kugelrohr distilation (60 °C (15 mmHg)) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 3 H), 1.52 (s, 6 H), 2.4 (d, 2 H), 4.8–6.0 (m, 3 H); ^{13}C NMR ($CDCl_3$) δ 24.52 (q), 27.94 (q), 28.60 (q), 42.98 (t), 79.89 (s), 109.42 (s), 119.34 (s). Anal. Calcd for $C_9H_{14}O_3$: C, 55.37; H, 7.74. Found: C, 55.01; H, 7.72.

2,2-Dimethyl-5-phenyl-5-(3′-phenylallyl)-1,3-dioxolan-4-one (3c). Cinnamyl bromide (10.5 g, 53 mmol) and 7.7 g of **2a** (40 mmol) gave after recrystallization from absolute ethanol 9.0 g

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(73% yield) of **3c**: mp 92–93 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.35 (s), 1.57 (s), 2.72, 2.86 (2 dd, 2 H), 6.02–6.14 (m, 1 H), 6.42 (d, 1 H), 7.12–7.64 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 27.64 (q), 45.00 (t), 83.35 (s), 110.21 (s), 122.46 (d), 124.58 (d), 126.02 (d), 127.30 (d), 127.88 (d), 128.23 (d), 128.31 (d), 135.07 (d), 136.71 (s), 139.46 (s), 172.32 (s). Anal. Calcd for $C_{20}H_{20}O_3$: C, 77.90; H, 6.54. Found: C, 77.67; H, 6.62.

Ethyl 2-Hydroxy-2-phenyl-4-pentenoate (4a). HCl gas was bubbled through a solution of **3a** (9.3 g, 0.04 mmol) in absolute ethanol (100 mL) for 2 min. After being refluxed for 2.5 h, the solution was cooled and poured into saturated aqueous sodium bicarbonate (100 mL). Ethanol was removed under reduced pressure, and the remaining aqueous layer was extracted with ether (3 × 100 mL). The combined organic layer was dried over $MgSO_4$ and concentrated in vacuum. Kugelrohr distillation at 79 °C (0.01 mmHg) afforded **4a** (8.4 g, 95% yield) as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.15 (t), 2.74, 2.95 (2 dd, 2 H), 3.64 (s, 1 H), 3.11–4.31 (m, 2 H), 5.09–5.17 (m, 2 H), 5.71–5.85 (m, 1 H), 7.24–7.61 (m, 5 H); ^{13}C NMR ($CDCl_3$) δ 13.98 (q), 44.07 (t), 62.31 (t), 77.80 (s), 119.06 (t), 125.37 (d), 127.59 (d), 128.06 (d), 132.29 (d), 141.31 (s), 174.40 (s).

Ethyl 2-Hydroxy-2-methyl-4-pentenoate (4b). The procedure described above was followed, using a solution of **3b** (11.0 g, 0.065 mol) in absolute ethanol (100 mL), to afford, after Kugelrohr distillation at 75 °C (35 mmHg), 8.7 g (85% yield) of **4b** as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.21 (t, 3 H), 1.25 (s, 3 H), 2.36, 2.44 (2 dd, 2 H), 3.22 (s, 1 H), 4.14–4.21 (m, 2 H), 5.04–5.08 (m, 2 H), 5.66–5.80 (m, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.03 (q), 25.29 (q), 44.46 (t), 61.55 (t), 74.01 (s), 118.68 (t), 132.21 (d), 176.19 (s).

Ethyl 2-Hydroxy-2,5-diphenyl-4-pentenoate (4c). This compound was prepared analogously to **4a**, using a solution of **3a** (8.0 g, 0.026 mol) in absolute ethanol (100 mL). Kugelrohr distillation (150 °C (0.03 mmHg)) of the crude product gave 6.2 g (81% yield) of **4c** as a colorless oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, 3 H), 2.88, 3.11 (2 dd, 2 H), 3.83 (s, 1 H), 4.13–4.30 (m, 2 H), 6.15–6.25 (m, 2 H), 6.50 (d, 1 H), 7.18–7.65 (m, 10 H); ^{13}C NMR ($CDCl_3$) δ 14.07 (q), 43.43 (t), 62.38 (t), 78.13 (s), 123.70 (d), 125.35 (d), 126.08 (d), 127.18 (d), 127.67 (d), 128.12 (d), 128.31 (d), 134.08 (d), 137.01 (s), 141.34 (s), 174.36 (s).

PLE-Catalyzed Hydrolyses of α-Hydroxy Esters 4a-c. The following procedure is representative. PLE was added to a rapidly stirred suspension of α-hydroxy ester in 0.05 M KH_2PO_4 buffer of pH 8 at 28 °C. The pH was maintained at 8 by pH stat-controlled addition of 2 N aqueous NaOH. The reaction was allowed to proceed until the desired extent of hydrolysis, as determined by the volume of base added, had been achieved. The pH of the mixture was then adjusted to 2 by addition of 6 N HCl. EtOAc (20 mL) was added, and the mixture was filtered over Celite. After separation of the organic layer, the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic layers were partly concentrated in vacuum (to a volume of approximately 20 mL) and washed with 5% aqueous $NaHCO_3$ solution (3 × 15 mL). Evaporation of the dried (Na_2SO_4) organic solution yielded the unreacted α-hydroxy ester. The aqueous layer was acidified to pH 2 with 6 N HCl and was then extracted with ether (3 × 20 mL). The organic phase was dried over Na_2SO_4, and removal of the solvent in vacuo gave the α-hydroxy acid. Specific details are given below.

(*S*)-2-Hydroxy-2-phenyl-4-pentenoic Acid (5a). (a) From α-hydroxy ester **4a** (4.0 g, 18 mmol) in buffer (16 mL) with PLE (EC 3.1.1.1, 1.0 mL, 2860 units) was isolated, after a conversion of 0.21, ester (−)-(*R*)-**4a** (2.57 g, 64% yield, 25% ee), $[\alpha]_{436}$ −1.7° (*c* = 1, EtOH), and α-hydroxy acid (+)-(*S*)-**5a** (0.67 g, 19% yield, 86% ee), $[\alpha]_{578}$ +16.7° (*c* = 1, EtOH). (b) From **4a** (7.3 g, 33 mmol) in buffer (30 mL) with PLE (Amano, 470 mg) was isolated, after 34% conversion, ester (−)-(*R*)-**4a** (3.81 g, 52% yield, 49% ee), $[\alpha]_{436}$ −3.4° (*c* = 1, EtOH), and α-hydroxy acid (+)-(*S*)-**5a** (1.87 g, 29% yield, 94% ee), $[\alpha]_{578}$ +18.2° (*c* = 1, EtOH). (c) **4a** (5.5 g, 25 mmol) in buffer (23 mL) with PLE (Amano, 290 mg) yielded, after 51% conversion, ester (−)-(*R*) **4a** (2.25 g, 41% yield, 86% ee), $[\alpha]_{436}$ −5.9° (*c* = 1, EtOH), and α-hydroxy acid (+)-(*S*)-**5a** (2.10 g, 44% yield, 83% ee), $[\alpha]_{578}$ +16.1° (*c* = 1 EtOH). Crystallization from $CHCl_3$ afforded enantiomerically pure α-hydroxy acid (+)-(*S*)-**5a**: mp 130.2–130.7 °C; $[\alpha]_{578}$ +19.4° (*c* = 1, EtOH); $[\alpha]_D$ +27.6° (*c* = 1, $CHCl_3$) (lit.[11] mp 130 °C; $[\alpha]^{22}_D$ +29.0° (*c* = 1, $CHCl_3$)); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.77, 3.00 (2 dd, 2 H), 5.18 (m, 2 H), 5.70–5.84 (m, 1 H), 7.24–7.38 (m, 3 H), 7.57–7.63 (m, 2 H); ^{13}C NMR ($CDCl_3$) δ 43.85 (t), 77.70 (s), 120.06 (t), 125.31 (d), 127.97 (d), 128.21 (d), 131.45 (d), 139.99 (s), 179.09 (s). Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.74; H, 6.20.

(*R*)-2-Hydroxy-2-phenyl-4-pentenoic Acid (5a). Optically active ester (−)-(*R*)-**4a** (2.2 g, 10 mmol, $[\alpha]_{436}$ −5.9° (*c* = 1, EtOH)) was added at room temperature to a solution of KOH (1.7 g, 30 mmol) in CH_3OH (20 mL). After stirring overnight the solvent was removed in vacuo. The residue was dissolved in water (20 mL) and acidified with 6 N HCl. A white solid was formed, which dissolved after addition of ether (20 mL). After separation of the organic phase, the aqueous layer was extracted with ether (2 × 20 mL). Drying (Na_2SO_4) and removal of the solvent in vacuo gave (−)-(*R*)-**5a** (1.9 g, 99% yield, 86% ee), $[\alpha]_{578}$ −16.7° (*c* = 1, EtOH). Crystallization from $CHCl_3$ afforded enantiomerically pure α-hydroxy acid (−)-(*R*)-**5a**, mp 130.0–130.5 °C, $[\alpha]_{578}$ −19.3° (*c* = 1, EtOH).

(*R*)-2-Hydroxy-2-methyl-4-pentenoic Acid (5b). (a) **4b** (2.68 g, 17.0 mmol) in buffer (13 mL) with PLE (EC 3.1.1.1, 1.0 mL, 2860 units) gave after 29% conversion, (+)-(*S*)-**4b** (1.15 g, 43% yield, 17% ee), $[\alpha]_{578}$ +3.3° (*c* = 1, EtOH), and α-hydroxy acid (−)-(*R*)-**5b** (0.88 g, 27% yield, 42% ee) as a colorless oil: $[\alpha]_D$ −5.2° (*c* = 1, EtOH). (b) From ester **4b** (3.16 g, 20.0 mmol) in buffer (15 mL) with PLE (Amano, 166 mg) were isolated, after 29% conversion, (+)-(*S*)-**4b** (1.74 g, 55% yield, 9% ee), $[\alpha]_{578}$ +1.7° (*c* = 1, EtOH), and α-hydroxy acid (−)-(*R*)-**5b** (0.68 g, 26% yield, 22% ee) as a slightly colored oil, which was purified by Kugelrohr distillation (70 °C (0.1 mmHg)): $[\alpha]_D$ −2.5° (*c* = 1, EtOH) [lit.[11] $[\alpha]^{22}_D$ +11.3° (*c* = 1, EtOH) (*S*)]; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.50 (s, 3 H), 2.44–2.58 (2 dd, 2 H), 5.19 (m, 2 H), 5.75–5.90 (m, 1 H), 6.6–8.0 (br, 2 H); ^{13}C NMR ($CDCl_3$) δ 25.10 (q), 44.21 (t), 74.39 (s), 119.65 (t), 131.54 (d), 180.71 (s). Anal. Calcd for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.22; H, 7.82.

Attempted Resolution of 4c. To a suspension of ester **4c** (3.2 g, 13.7 mmol) in buffer (13 mL) was added PLE (Amano, 162 mg). No activity was measured after stirring for 20 h. The same lack of activity was observed when PLE (EC 3.1.1.1, 1.0 mL) was used.

(*S*)-2-Hydroxy-2-phenylpentanoic Acid (6). A mixture of (*S*)-2-hydroxy-2-phenyl-4-pentenoic acid (**5a**, 0.48 g, 2.5 mmol), 20 mg of Pd/C (5%), and EtOAc (20 mL) was shaken in a Parr apparatus, at 3 atm of H_2 pressure, for 20 h. After filtration, to remove the catalyst, the solvent was removed in vacuum, to give (*S*)-**6** as a white solid (0.46 g, 95% yield, >98% ee): mp 100–101 °C; $[\alpha]_D$ +29.3° (*c* = 1, EtOH). (lit.[11] mp 101–102 °C; $[\alpha]^{22}_D$ +28.9° (*c* = 2, EtOH); lit.[12] mp 97–99 °C; $[\alpha]^{25}_D$ +21.6° (*c* = 2.5, EtOH)); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.83 (t, 3 H), 1.18–1.50 (m, 2 H), 1.90–2.20 (m, 2 H), 7.23 (m, 3 H), 7.53 (s, 2 H), 6.2–8.8 (br, 2 H); ^{13}C NMR ($CDCl_3$) δ 13.97 (q), 16.88 (t), 41.62 (t), 78.33 (s), 125.32 (d), 127.80 (d), 128.18 (d), 140.82 (s), 180.34 (s). Anal. Calcd for $C_{11}H_{14}O_3$: C, 68.02; H, 7.27. Found: C, 67.88; H, 7.21.

Enantiomeric Excess Determination. The ee's of the optically active esters **4a,b** and acids **5a,b** were determined by derivatization with (*S*)-2-chloropropionyl chloride followed by 300-MHz ^{1}H NMR analysis.[10] The racemic esters and acids (obtained after basic hydrolysis of the 1,3-dioxolanones **3a,b**) were used as reference standards.

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Registry No. **1a**, 90-64-2; **1b**, 50-21-5; **2a**, 111819-62-6; **2b**, 74262-60-5; **3a**, 129286-24-4; **3b**, 129286-25-5; **3c**, 129286-26-6; **4a**, 129286-27-7; (−)-(*R*)-**4a**, 129388-29-0; **4b**, 129286-28-8; (+)-(*S*)-**4b**, 129388-30-3; **4c**, 129286-29-9; (+)-(*S*)-**5a**, 81037-04-9; (*R*)-**5a**, 62696-41-7; (−)-(*R*)-**5b**, 129286-30-2; (*S*)-**6**, 73698-05-2; PLE, 9016-18-6; $H_2C{=}CHCH_2Br$, 106-95-6; $PhCH{=}CHCH_2Br$, 4392-24-9.