Tetrahedron: Asymmetry Vol. 2, No. 7, pp. 705-720, 1991
Printed in Great Britain

0957-4166/91 $3.00+.00
Pergamon Press plc

PLE CATALYZED HYDROLYSES OF α-SUBSTITUTED α-HYDROXY ESTERS: THE INFLUENCE OF THE SUBSTITUENTS.

Henk Moorlag and Richard M. Kellogg*

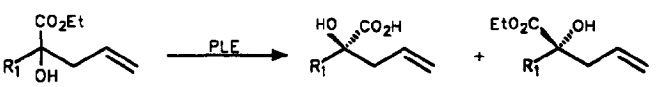
Department of Organic Chemistry, University of Groningen
Nijenborgh 16, 9747 AG Groningen, The Netherlands

(*Received* 16 *April* 1991)

Abstract: The enzymatic hydrolyses of a variety of α-substituted mandelic and lactic esters using pig liver esterase (PLE) have been investigated. High to moderate enantioselectivity was found for various α-substituted mandelic esters, whereas PLE showed low to no enantioselectivity for α-substituted lactic esters. We observed that the enantioselectivity of PLE depends strongly on the length and nature of the substituent at the α-position. Some consequences for an active site model of PLE are discussed.

INTRODUCTION.

During the past two decades enzymes have been recognized more and more as synthetically useful tools in asymmetric synthesis.[1] Of the hydrolytic enzymes pig liver esterase (PLE) has received particular interest mainly due to its broad substrate specificity and accompanying high enantioselectivity often found.[2] PLE has been used mostly for the hydrolysis of meso and prochiral diesters,[3] but some resolutions of more challenging racemic monoesters have been accomplished also.[4] We recently reported, for example, on the PLE catalyzed hydrolysis of α-substituted α-hydroxy esters.[5] It was found that PLE showed a high degree of enantioselectivity (E^6 = 30) for the ester of α-allyl-mandelic acid (**1a**) but that the hydrolysis of the corresponding lactic ester (**1b**), although it proceeded readily, was much less selective (E = 3) (Scheme 1).



1 2 1

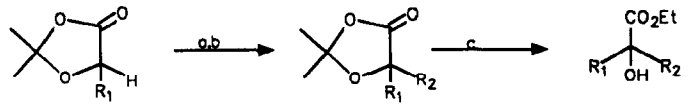
Scheme 1 a: $R_1 = C_6H_5$ a: $R_1 = C_6H_5$
b: $R_1 = CH_3$ b: $R_1 = CH_3$

In order to gain more information about the factors that determine both the reactivity and enantioselectivity of PLE , we investigated the enzymatic hydrolyses of various α-substituted mandelic and lactic esters wherein the size and nature of the substituent adjacent to the carbonyl group were varied. The

influence of the polar hydroxyl function of the α-hydroxy esters was examined by derivatization to the corresponding less polar silylated compounds. The results provide information on the catalytically active site of PLE. The results will be discussed on the basis of a recently proposed active site model.[7]

RESULTS.

The ester substrates were prepared straightforwardly from the acetonides of racemic mandelic and lactic acid as outlined in Scheme 2.



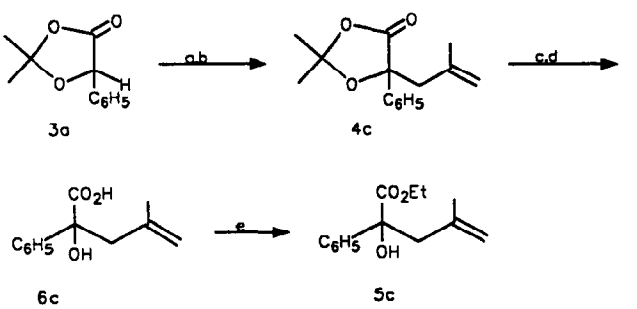
3 4 5

a: R_1 = C_6H_5
b: R_1 = CH_3

a: R_1 = C_6H_5, R_2 = $CH_2CH{=}CH(CH_3)$
b: R_1 = C_6H_5, R_2 = $CH_2C_6H_5$
e: R_1 = CH_3, R_2 = $CH_2CH{=}CH(CH_3)$
f: R_1 = CH_3, R_2 = $CH_2CH{=}CH(C_6H_5)$
h: R_1 = C_6H_5, R_2 = $CH_2CH{=}CH(C_6H_5)$

Scheme 2 Reagents: a: LDA; b: R_2Br; c: EtOH, HCl.

A different approach had to be followed for the synthesis of **5c** because alkylation of **3a** with methallylchloride failed. However, we found that **3a** could be alkylated with methallyl acetate in the presence of a catalytic amount of a Pd^0 catalyst (Scheme 3).

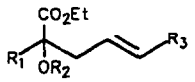


3a 4c 6c 5c

Scheme 3 Reagents: a: LDA; b: $Pd(dba)_2$, dppe, $CH_2{=}C(CH_3)CH_2OAc$; c: KOH, MeOH; d: HCl; e: Cs_2CO_3, EtI.

This approach has been extended to other allylic substrates and provides a catalytic entry to α-substituted α-hydroxy acids, as will be described elsewhere.[8] The allylated dixolanone **4c** was subsequently hydrolyzed under basic conditions to give α-hydroxy acid **6c**, which was esterified. Attempts to esterify **6c** under acidic conditions (EtOH/HCl) led to γ-lactone formation. The synthesis of the O-

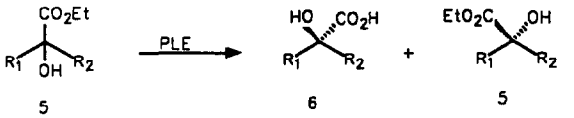
protected derivatives **5g-m** (Figure 1) was accomplished following literature procedures (see Experimental Section).



5g: $R_1 = CH_3$, R_2 = TMS, $R_3 = C_6H_5$
5i: $R_1 = CH_3$, R_2 = TMS, R_3 = H
5j: $R_1 = CH_3$, R_2 = TBDMS, R_3 = H
5k: $R_1 = CH_3$, R_2 = TMS, $R_3 = CH_3$
5l: $R_1 = CH_3$, $R_2 = CH_2C_6H_5$, R_3 = H
5m: $R_1 = C_6H_5$, R_2 = TMS, R_3 = H

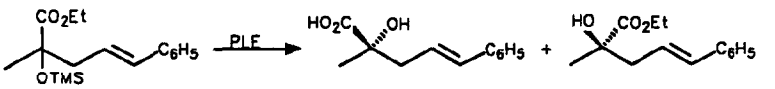
Figure 1

The PLE catalyzed hydrolyses were performed in 0.05 M aqueous phosphate buffer at pH 8 or 7 (the silylated compounds) at 28 °C. The hydrolysis was stopped after 40-50% conversion and the acidic product and unhydrolyzed ester were isolated in good yields (Scheme 4).



5 6 5

a: $R_1 = C_6H_5$, $R_2 = CH_2CH{=}CH(CH_3)$
b: $R_1 = C_6H_5$, $R_2 = CH_2C_6H_5$
c: $R_1 = C_6H_5$, $R_2 = CH_2C(CH_3){=}CH_2$
d: $R_1 = C_6H_5$, $R_2 = CH_3$
e: $R_1 = CH_3$, $R_2 = CH_2CH{=}CH(CH_3)$
f: $R_1 = CH_3$, $R_2 = CH_2CH{=}CH(C_6H_5)$
h: $R_1 = C_6H_5$, $R_2 = CH_2CH{=}CH(C_6H_5)$



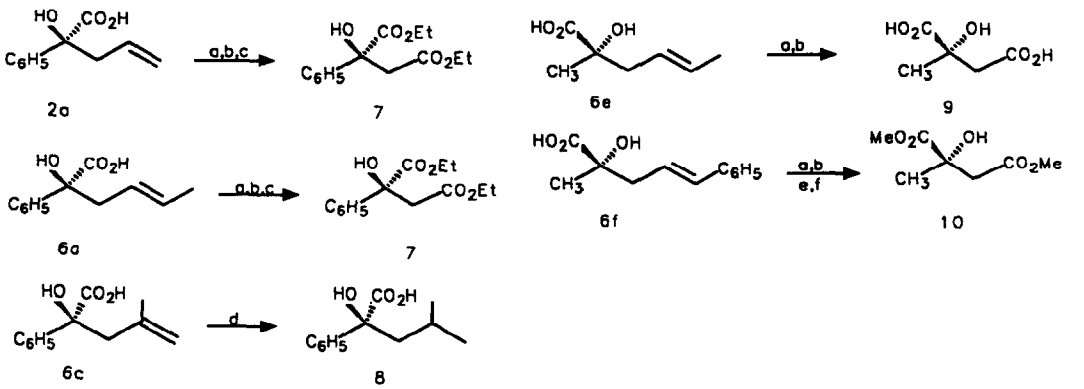
5g 6f 5f

Scheme 4

The results of the hydrolyses are summarized in Table I.

The absolute configurations of the hydrolyzed esters **6a-f** were determined by chemical correlation (Scheme 5). Thus, ozonolysis of **2a**, the absolute configuration and maximum rotation of which are known,[5] resulted, after oxidative work-up and esterification in (S)-2-hydroxy-2-phenyl-succinic acid diethylester (**7**). Oxidative degradation and esterification of **6a** yielded the same compound. Hydrogenation of **6c** afforded (S)-2-hydroxy-4-methyl-2-phenyl-valeric acid (**8**), the absolute configuration of which has been established.[9] The configurations of optically active lactic acid derivatives **6e** and **6f** were established by oxidation to citramalic acid (**9**) and dimethyl citramalate (**10**), respectively. The absolute configuration of **6d** is known from the literature,[10] whereas those of **6b** and **6h** remain undetermined. The ee 's of nonracemic products were determined by conversion into the corresponding (S)-2-chloropropanoyl derivatives, followed by ^1H NMR analysis.[11] This

method has proved to be very suitable for these compounds.



Scheme 5. Reagents: a: O_3; b: H_2O_2, HCO_2H; c: EtOH, HCl; d: Pd/C, H_2; e: MeOH, HCl; f: chrom. (SiO_2).

The data in Table I show that with exception of silylated hydroxy ester **5m**, the substituted mandelic esters all react with PLE, and are hydrolyzed with greater enantiospecificity than the substituted lactic esters. For the substituted mandelic esters the length and size of the fourth substituent on the stereogenic carbon atom has a dramatic influence on the stereospecificity. In this series PLE exhibits an optimum in specificity for an allylic substituent with a C_3 chain length (entry 3). In this case, both unhydrolyzed ester and acid were isolated in high enantiomeric excess. (S)-**6c** could be obtained in enantiomerically pure form after one recrystallization. The enantiospecificity for **6a**, which has a C_4 length chain substituent, is significantly lower, although still acceptable for most synthetic purposes. A dramatic decrease in E value was observed going from a methallyl substituent to a much smaller methyl (entry 4) or a much larger benzyl (entry 2) substituent. In all cases in which the absolute configuration of the products could be established, the enzyme hydrolyzed the (S)-enantiomer preferentially (except **5g**). The rate of hydrolysis was comparable for **5a,c** and **d** (about 30 nmol/min/mg PLE), but was much slower for substrate **5b**. This is probably due to steric factors. Interestingly, when the hydroxyl group of **1a** is converted to a silyloxy group (**5m**) PLE does not accept the compound as a substrate (entry 10) again probably because of steric factors. Even in the presence of a cosolvent (up to 30% DMSO), which may overcome solubility problems of the substrate, the ester is still not hydrolyzed. On the contrary, the corresponding free hydroxy ester was hydrolyzed with high enantioselectivity.[5] It was found that ester **5h**, which we had reported not to be hydrolyzed by PLE,[5] does react, although very slowly and with low enantiospecificity, when DMSO was added as a colsolvent in the hydrolytic reaction.

Table I. **Hydrolysis of α-Hydroxy Esters 5a-l by PLE.**

entry	substrate (racemic)	conv.(c)[a]	recovered ester	%chem yield	%ee(5)	product	%chem yield	%ee(6)	E[b]	rel. rate[c]
1	**5a**	0.46	(R)-**5a**	53	64	(S)-**6a**	38	75	13.4	1.3
2	**5b**	0.49	(-)-**5b**	41	40	(+)-**6b**	43	38	3.3	0.3
3	**5c**	0.52	(R)-**5c**	44	86	(S)-**6c**	39	80	25.0	1.0
4	**5d**	0.42	(R)-**5d**	47	40	(S)-**6d**	41	51	4.5	0.9
5	**5e**	0.50	(R)-**5e**	32	2	(S)-**6e**	49	2	1.1	25.4
6	**5f**	0.47	(R)-**5f**	46	3	(S)-**6f**	46	3	1.1	2.4
7	**5g**	0.42	(S)-**5f**	57	3	(R)-**6f**	41	3	1.1	0.3
8	**5h**	0.46	(-)-**5h**	53	10	(+)-**6h**	30	12	1.4	0.2[d]
9	**5i-l**	non-enantioselective hydrolysis								
10	**5m**	no hydrolysis								

[a] Calculated from the ee values of acid and remaining ester: c = ee(5)/ee(5)+ee(6).
[b] Enantiomeric ratio, calculated from the equation E = ln[1-c(1+ee(6))]/ln[1-c(1-ee(6))].[6]
[c] All relative to **5c** = 1.0.
[d] Hydrolysis performed in the presence of 33% DMSO.

PLE exhibited very low or no enantiospecificity in the hydrolysis of the alkylated lactic esters. The lactic esters were hydrolyzed more rapidly than the mandelic esters. In contrast to the mandelic esters silylation of the lactic esters led to compounds that still were hydrolyzed by PLE, albeit sometimes slowly (**5g** and **5j**). However, protection of the hydroxyl group resulted in general in complete loss of stereospecificity. This may be attributed to the loss of a possible binding mode of the substrate via a polar interaction. Recently it has been shown that α-benzyloxy ester **5l** (as methyl ester) can be hydrolyzed enantioselectively using Lipase OF.[12] However, with PLE we isolated only racemic products.

DISCUSSION.

The results presented here help delineate the fine balance between reactivity and enantioselectivity of PLE. In this respect, more information about the active site of PLE would greatly facilitate the design of substrates for PLE that could be hydrolyzed with high stereoselectivity. Recently Jones and coworkers proposed an active site model for PLE that reflects the topography of the enzyme pocket.[7] This model, which can rationalize many reported PLE catalyzed hydrolyses, consists, in addition to the serine containing ester hydrolysis region, of four binding sites. Two of these sites can accommodate hydrophobic portions of the substrate and two can accept more polar groups. One of the hydrophobic sites is relatively small and can accommodate groups up to C_4 in size. A group which fits well in this pocket will experience the best hydrophobic interaction and will contribute strongly in binding one of the enantiomers of the substrate. However, there has to be another effective interaction between a portion of the substrate molecule and the active site in order to control the stereochemical outcome of the hydrolysis. This can be a polar interaction as shown to occur in the stereoselective hydrolysis of diester substrates.[7] For our substrates, however, a polar interaction seems to be less dominant. This can be concluded from the large difference in stereospecificity of PLE for **1a** and **5a** compared with **1b** and **5e**. A phenyl group seems to interact far better than a methyl group, which may be indicative for a π-interaction from the aromatic group with the active site. Recently it has been suggested that the presence of unsaturation near the chiral center may contribute strongly in the factors that determine the enantioselectivity of PLE in the hydrolysis.[13] Such an interaction indeed may be responsible for the better recognition of the substituted mandelic esters compared to the substituted lactic esters.

Finally, since the enantiomeric ratio for substrates **5a-d** is greatly influenced by the size of the substituent, one must conclude that the interactions that determine the enantioselectivity of PLE are extremely subtle for compounds with a great degree of conformational flexibility. We clearly observe a significant interaction of an allyl or methallyl group with the active site. This is in accordance with findings reported by Jones et al.,[7] who suggest the presence of a small hydrophobic pocket in the active site, which will maximize its hydrophobic binding potential if an entering moiety completely fills

the pocket. A C_4 fragment (entry 1) seems to be marginally larger than the size of the small pocket and gives rise to another binding mode of the substrate, leading to the hydrolysis of the other enantiomer. Still there is a prevalent binding of the crotyl moiety in the small hydrophobic pocket resulting in a reasonable enantiomeric excess of the product. From these experiments it is also clear that a substrate with a substituent that will not maximize the hydrophobic binding potential, because it is too large to fit in the pocket (benzyl) or too small to interact well (methyl), will give low enantioselectivity in PLE catalyzed hydrolysis. Although Jones' active site model seems to work well in rationalizing the outcome of PLE catalyzed hydrolysis of mainly diester substrates, it is less adequate to interpret fully the stereoselectivity observed in the hydrolysis of racemic monoesters. Furthermore the enzyme can accept substrates that will not bind effectively in the active site but still can be located near the active hydrolytic region. This makes the difference between hydrophobic and polar pockets less clearly defined.

In summary it is clear that PLE may be successfully used for the resolution of racemic α-substituted α-hydroxy esters. Not surprisingly there is a question of optimal fit for optimal enantioselectivity of hydrolysis. The lactic acid family seems to be characterized by an improper set of substituents about the stereogenic carbon; the compounds are accepted by PLE but the interplay of substituents with the enzyme is too weak, too aselective, to allow good enantiorecognition. On the other hand, the mandelic acid family has members that are hydrolyzed with good to excellent enantioselectivities. However, likely because of much tighter and more selective interplay of substituents with the enzyme, the risk of total loss of reactivity becomes higher.

Such a conclusion seems inherently logical - a "fit" can be too small, about right, or too large. The present work provides some definition of what "too small", "about right", and "too large" may be for PLE.

EXPERIMENTAL SECTION.

General remarks. Dioxolanones **3a,b** and esters **1a,b** and **5h** were synthesized as described previously.[5] All enzymatic reactions were performed with a Radiometer PHM 82 pH stat, equipped with a TTT 80 titrator and a ABU 80 autoburette. PLE-A was obtained from Amano Pharmaceutical Co.

General procedure for the alkylation of the enolate from the dioxolanones 3a or 3b, with electrophiles. A 0.10 mol run is described. A solution of **3a** or **3b** (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 (ca. 0.14 mol) was added. The reaction mixture was allowed to warm to room temperature (in about 3h) and 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 x 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 crystallisation. Specific details for each compound are given below.

2,2-Dimethyl-5-phenyl-5-(2-butenyl)-1,3-dioxolan-4-one (4a). From crotyl bromide (1.2 g, 9.0 mmol, mixture of cis and trans isomers) and 1.16 g (6.0 mmol) of **3a**, there was obtained 1.2 g (82%) of **4a** after Kugelrohr distillation at 85 °C (0.01 mm Hg). The product consisted of a 4:1 mixture of E,Z isomers. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.41 (s, 3H), 1.58 (m (minor isomer), 3H), 1.65 (s, 3H), 1.67 (m (major isomer), 3H), 2.51-2.81 (m, 2H), 5.33-5.73 (m, 2H), 7.48-7.60 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 17.82 (q), 27.41 (q, isomer), 27.53 (q), 27.71 (q), 39.03 (t, isomer), 44.68 (t), 83.47 (s), 109.94 (s), 123.70 (d, isomer), 124.52 (d), 124.62 (d), 127.67 (d), 128.10 (d, isomer), 128.86 (d), 130.90 (d), 139.63 (s). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 73.19; H, 7.37.

2,2-Dimethyl-5-phenyl-5-benzyl-1,3-dioxolan-4-one (4b). Benzyl bromide (1.54 g, 9.0 mmol) and 1.16 g (6.0 mmol) of **3a** afforded after vacuum distillation 1.45 g (85%) of **4b** as a colorless oil; bp 147-150 °C (0.1 mm Hg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.10 (s, 3H), 1.35 (s, 3H), 3.06 (d, 1H, J = 15 Hz), 3.40 (d, 1H, J = 15 Hz), 7.20-7.73 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 26.86 (q), 27.77 (q), 47.61 (t), 84.10 (s), 110.19 (s), 124.61 (d), 126.95 (d), 127.85 (d), 128.20 (d), 130.82 (d), 134.82 (s), 139.90 (s), 172.25 (s). Anal. Calcd for $C_{18}H_{18}O_3$: C, 76.57, H, 6.43. Found: C, 76.32, H, 6.40.

2,2-Dimethyl-5-methyl-5-(2-butenyl)-1,3-dioxolan-4-one (4e). Crotyl bromide (9.9 g, 73.3 mmol) and 8.0 g (61.5 mmol) of **3b** yielded 10.0 g (88%) of **4e** after Kugelrohr distillation (80 °C, 2.0 mm Hg) as a colorless oil. The product consisted of a mixture of E,Z isomers. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.41 (s, 3H), 1.51 (s, 3H), 1.54 (s, 3H), 1.65 (d, 3H, J = 6.6 Hz), 2.32-2.43 (m, 2H), 5.37-5.51 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 17.85 (q), 24.58 (q), 27.79 (q, isomer), 27.90 (q), 28.67 (q), 36.00 (t, isomer), 41.74 (t), 80.30 (s), 109.34 (s), 122.98 (d, isomer), 123.93 (d), 128.58 (d, isomer), 130.59 (d) 174.88 (s). Anal. Calcd for $C_{10}H_{16}O_3$: C, 65.19; H, 8.73. Found: C, 65.46; II, 8.85.

2,2-Dimethyl-5-methyl-5-(3'-phenylallyl)-1,3-dioxolan-4-one (4f). From cinnamyl bromide (12.4 g, 62.9 mmol) and 6.3 g (48.8 mmol) of **3b** was obtained 9.5 g (80 %) of **4f** after Kugelrohr distillation at 64 °C (0.01 mm Hg) as a slightly yellow oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.51 (s, 3H), 1.53 (s, 3H), 1.60 (s, 3H), 2.54-2.72 (m, 2H), 6.13-6.23 (m, 1H), 6.50 (d, 1H, J = 15 Hz), 7.20-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 24.79 (q), 28.01 (q), 28.65 (q), 42.14 (t), 80.24 (s), 109.60 (s), 122.69 (d), 126.04 (d), 127.35 (d), 128.36 (d), 134.76 (d), 136.66 (s), 174.70 (s). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 72.90; H, 7.41.

2,2-Dimethyl-5-(2'-methylallyl)-5-phenyl-1,3-dioxolan-4-one (4c). A solution of **3a** (1.16 g, 6.0 mmol) in THF (3 mL) was added to a solution of LDA (6.6 mmol) in THF/hexane (3:2, 5 mL) at -78 °C. The mixture was allowed to warm to room temperature and a solution prepared in a second flask by mixing $Pd(dba)_2$ (34.4 mg, 60 μmol), dppe (24.0 mg, 60 μmol) and methallyl acetate (0.75 g, 6.6 mmol) in THF (2 mL) was added. A precipitate was formed during the addition. The mixture was

allowed to stir overnight and was then poured into a half saturated aqueous ammonium chloride solution (10 mL) followed by extraction with ether (3 x 15 mL). The organic layer was dried (Na_2SO_4) and the solvent was removed in vacuum. Kugelrohr distillation (84 °C, 0.02 mm Hg) of the residue afforded **4c** (1.20 g, 82%) as a colorless oil. ^1H NMR (C_6D_6, 300 MHz) δ 1.16 (s, 3H), 1.45 (s, 3H), 1.83 (s, 3H), 2.67 (d, 1H, J = 14.0 Hz), 2.93 (d, 1H, J = 14.0 Hz), 5.00 (s, 1H), 5.04 (s, 1H), 7.16-7.30 (m, 3H), 7.85-7.89 (m, 2H). ^{13}C NMR (C_6D_6) δ 22.63 (q), 23.50 (q), 25.64 (q), 47.17 (t), 82.07 (s), 107.66 (s), 114.57 (t), 123.27 (d), 126.06 (d), 126.53 (d), 138.10 (s), 138.73 (s), 170.41 (s). Anal. Calcd for $C_{15}H_{18}O_3$: C, 73.15; H, 7.37. Found: C, 73.34; H, 7.44.

General Procedure for the Deprotection of the Dioxolanones and Esterification. HCl gas was bubbled through a solution of the dioxolanone in absolute ethanol for 5 min. After being refluxed overnight, the solution was cooled and poured into saturated aqueous sodium bicarbonate. Ethanol was removed under reduced pressure and the remaining aqueous layer was extracted with ether. The combined organic layers were dried over Na_2SO_4 and concentrated in vacuum. Kugelrohr distillation of the residue afforded the desired ethyl ester.

Ethyl 2-Hydroxy-2-phenyl-4-hexenoate (5a). **4a** (0.77 g, 3.1 mmol) in absolute ethanol (5 mL), afforded, after Kugelrohr distillation (100 °C, 0.05 mm Hg) 0.52 (74%) of **5a** as a colorless oil. The product consisted of a 4:1 mixture of E,Z isomers. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, 3H), 1.66 (d, 3H, J = 6 Hz), 2.60-3.08 (m, 2H), 3.72 (s, 1H), 4.12-4.34 (m, 2H), 5.36-5.72 (m, 2H), 7.24-7.68 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 13.92 (q), 14.01 (q), 17.98 (q), 37.17 (t), 43.00 (t), 62.17 (t), 77.95 (s), 123.61 (d), 124.46 (d), 125.41 (d), 127.52 (d), 127.82 (d), 128.00 (d), 130.05 (d), 141.46 (s), 174.52 (s).

Ethyl 2-Hydroxy-2-phenyl-phenylpropanoate (5b). Using a solution of **4b** (2.4 g, 8.5 mmol) in absolute ethanol (10 mL) (in this particular case it was necessary to reflux the solution for 48 h) , there was obtained after Kugelrohr distillation at 145 °C (0.1 mm Hg) 1.83 g (80%) of **5b** as a colorless viscous oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.36 (t, 3H), 3.22 (d, 1H, J = 15 Hz), 3.60 (d, 1H, J = 15 Hz), 3.66 (s, 1H), 4.18 (q, 2H), 7.24 (s, 5H), 7.32-7.72 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 13.92 (q), 45.78 (t), 62.29 (t), 78.50 (s), 125.51 (d), 126.72 (d), 127.62 (d), 127.82 (d), 128.05 (d), 130.37 (d), 135.61 (s), 141.55 (s), 174.04 (s).

Ethyl 2-Hydroxy-2-methyl-4-hexenoate (5e). Starting from **4e** (6.8 g, 37.0 mmol) in absolute ethanol (100 mL), 5.5 g (85%) of **5e** was obtained as a colorless oil, after Kugelrohr distillation (55 °C, 1.5 mm Hg). The product consisted of a mixture of E,Z isomers. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, 3H), 1.36 (s, 3H), 1.63 (d, 2H, J = 6.6 Hz), 2.23-2.43 (m, 2H), 3.11 (s, 1H), 4.14-4.23 (m, 2H), 5.27-5.48 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.13 (q), 17.89 (q), 25.21 (q), 25.40 (q), 37.75 (t), 43.36 (t), 61.50 (t), 74.30 (s), 123.80 (d), 124.51 (d), 127.57 (d), 129.66 (d), 176.39 (s).

Ethyl 2-Hydroxy-2-methyl-5-phenyl-4-pentenoate (5f). **4f** (8.7 g, 35.4 mmol) in absolute ethanol yielded after distillation (bp 97-101 °C, 0.05 mm Hg), 6.7 g (81%) of **5f** as a colorless oil. ^1H NMR ($CDCl_3$, 300 MHz) δ 1.28 (t, 3H), 1.46 (s, 3H), 2.51-2.69 (m, 2H), 3.3 (s, 1H), 4.18-4.28 (m, 2H). 6.13-

6.24 (m, 1H), 6.45 (d, 1H, J = 15.4 Hz), 7.20-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.16 (q), 25.45 (q), 43.79 (t), 61.68 (t), 74.44 (s), 123.76 (d), 126.02 (d), 127.15 (d), 128.31 (d), 133.77 (d), 136.98 (s), 176.26 (s).

2-Hydroxy-2-phenyl-4-methyl-4-pentenoic-acid (6c). To a solution of dioxolanone **4c** (0.9 g, 3.7 mmol) in MeOH (8 mL) was added a solution of KOH (1.0 g, 17.9 mmol) in H_2O (5 mL). After stirring overnight MeOH was removed in vacuum and H_2O (5 mL) was added. The resulting solution was acidified with conc. aqueous HCl. The white solid which precipitated was taken up in ether (5 mL). After separation of the organic layer the aqueous layer was extracted with ether (3 x 5 mL). Drying (Na_2SO_4) of the combined organic layers and evaporation of the solvent afforded **6c** (0.7 g, 92%) as a white crystalline compound. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.70 (s, 3H), 2.74 (d, 1H, J = 14 Hz), 3.10 (1H, J = 14 Hz), 4.85 (s, 1H), 4.95 (s, 1H), 7.18-7.67 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 23.75 (q), 47.31 (t), 99.38 (s), 116.25 (t), 125.30 (d), 127.96 (d), 128.22 (d), 140.61 (s), 178.38 (s).

Ethyl 2-Hydroxy-2-phenyl-4-methyl-4-pentenoate (5c). To a stirred suspension of 2-hydroxy-2-phenyl-4-methyl-4-pentenoic acid (**6c**, 0.61 g, 3.0 mmol) and Cs_2CO_3 (1.95 g, 6.0 mmol) in DMF (15 mL) was added dropwise a solution of ethyl iodide (2.34 g, 15 mmol) in DMF (25 mL). The mixture was stirred for 24 h. Water was added (20 mL) and the resulting solution was extracted with ethyl acetate (3 x 30 ml). The combined organic layers were washed with a saturated NH_4Cl solution (3 x 40 mL) and brine (40 mL). After drying (Na_2SO_4), the solvent was removed in vacuum. An oil pump (1 mm Hg) was used to remove the last trace of DMF. Kugelrohr distillation (0.05 mm Hg, 90 °C) of the residue afforded **5c** (0.65 g, 93%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.21 (t, 3H), 1.67 (s, 3H), 2.62 (d, 1H, J = 13 Hz), 2.98 (d, 1H, J = 13 Hz), 3.70 (s, 1H), 4.10-4.20 (m, 2H), 4.72 (s, 1H), 4.82 (s, 1H), 7.16-7.59 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 13.88 (q), 23.96 (q), 47.21 (t), 62.20 (t), 78.04 (s), 114.97 (t), 125.41 (d), 127.48 (d), 127.97 (d), 141.07 (s), 142.07 (s), 174.64 (s).

Ethyl 2-Hydroxy-2-phenylpropionate (5d). 2-Hydroxy-2-phenylpropionic acid hemihydrate (atrolactic acid hemihydrate, 3.5 g, 20.0 mmol) was dried under reduced pressure at 55 °C (1-2 mm Hg). The acid was dissolved in absolute ethanol (50 mL) and HCl gas was bubbled through for 5 min. After refluxing overnight and workup as described above 3.2 g (83%) of **5d** was isolated as a colorless oil, after Kugelrohr distillation (80 °C, 0.05 mm Hg). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, 3H), 2.79 (s, 3H), 1.84 (s, 1H), 4.16-4.32 (m, 2H), 7.24-7.60 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 13.88 (q), 26.58 (q), 62.23 (t), 75.51 (s), 124.98 (d), 127.55 (d), 128.09 (d), 142.71 (s), 175.43 (s).

Ethyl 2-Benzyloxy-2-methyl-4-pentenoate (5l). This compound was prepared analogously to a literature procedure.[12] To a suspension of NaH (0.78 g, 55% in mineral oil, 18 mmol) in THF (5 mL) was added to a solution of **1b** (2.37 g, 15 mmol) in THF (5 mL) at 0 °C. The mixture was stirred at room temperature for 1.5 h and a solution of benzyl bromide (3.5 g, 21 mmol) in THF (5 mL) and tetra-n-butylammonium iodide (0.55 g, 2.0 mmol) were added at 0 °C. Stirring was continued at room temperature for 2 h, and then the mixture was heated under reflux for 2 h. After cooling, the mixture

was quenched by addition of a saturated ammonium chloride solution and extracted with ether. The combined extracts were washed with a 10% $Na_2S_2O_3$ solution and brine, dried (Na_2SO_4) and concentrated in vacuum. Distillation of the residue afforded **5l** (2.3 g, 62%) as a colorless oil: bp 108-110 °C (0.1 mm Hg); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.24 (t, 3H), 1.43 (s, 3H), 2.47-2.61 (m, 2H), 4.16 (q, 2H), 4.43 (s, 2H), 5.02-5.14 (m, 2H), 5.73-5.87 (m, 1H), 7.18-7.35 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 14.19 (q), 21.27 (q), 42.87 (t), 60.80 (t), 66.66 (t), 72.01 (s),118.34 (t), 127.27 (d), 127.39 (d), 128.09 (d), 132.50 (d), 138.48 (s), 173.64 (s).

General procedure for the silylation of α-hydroxy esters 1a,b and 5e,f. A procedure analogous to that described for silylation with silyl perchlorates[14] was followed. A 10 mmol run is described. A solution of the α-hydroxy ester (10 mmol) and trimethylsilyl triflate (TMSOTf) or t-butyl dimethylsilyl triflate (TBDMSOTf) (15 mmol) in acetonitrile (4 ml) was cooled to 0 °C. Pyridine (20 mmol) was added dropwise and the solution was stirred overnight. The reaction mixture was then poured into pentane (15 mL). The pentane layer was extracted with a saturated $NaHCO_3$ solution (3 x 15 mL). The organic layer was dried (K_2CO_3) and concentrated in vacuum. The residue was purified by Kugelrohr distillation.

Ethyl 2-Trimethylsilyloxy-2-methyl-5-phenyl-4-pentenoate (5g). Starting from **5f** (1.1 g, 4.7 mmol), TMSOTf (1.6 g, 7.1 mmol) and pyridine (0.75 g, 9.4 mmol), 1.1 g (77%) of **5g** was isolated as a colorless oil after Kugelrohr distillation (100 °C, 0.01 mm Hg). ^{1}HNMR ($CDCl_3$, 300 MHz) δ 0.15 (s, 9H), 1.25 (t, 3H), 1.44 (s, 3H), 2.48-2.64 (m, 2H), 4.08-4.17 (m, 2H), 6.13-6.23 (m, 1H), 6.37-6.43 (m, 1H), 7.17-7.35 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 2.06 (q), 14.19 (q), 26.06 (q), 45.20 (t), 60.83 (t), 77.55 (s), 124.96 (d), 125.97 (d), 126.96 (d), 128.14 (d), 128.34 (d), 132.96 (d), 137.40 (s), 174.92 (s).

Ethyl 2-Trimethylsilyloxy-2-methyl-4-pentenoate (5i). α-Hydroxy ester **1b** (1.6 g, 10 mmol), TMSOTf (3.3 g, 15 mmol) and pyridine (1.6 g, 10 mmol) afforded after Kugelrohr distillation (90 °C, 15 mm Hg), 1.6 g (69%) of **5i** as a colorless oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.11 (s, 9H), 1.25 (t, 3H), 1.39 (s, 3H), 2.39-2.42 (m, 2H), 4.15 (q, 2H), 5.00-5.06 (m, 2H), 5.70-5.82 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 1.97 (q), 14.13 (q), 25.88 (q), 46.02 (t), 60.72 (t), 77.34 (s), 117.84 (t), 133.13 (d), 174.86 (s).

Ethyl 2-(t-Butyl)dimethylsilyloxy-2-methyl-4-pentenoate (5j). Starting from **1b** (1.6 g, 10 mmol), TBDMSOTf (4.0 g, 15 mmol) and pyridine (1.6 g, 20 mmol), 2.5 g (92%) of **5j** was obtained as a colorless oil by Kugelrohr distillation (60 °C, 0.1 mm Hg) after removal of some not further identified reaction products from the crude reaction mixture by distillation at 100 °C (12 mm Hg). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.06 (s, 3H), 0.10 (s, 3H), 0.87 (s, 9H), 1.25 (t, 3H), 1.39 (s, 3H), 2.33-2.49 (m, 2H), 4.13 (q, 2H), 5.00-5.05 (m, 1H). ^{13}C NMR ($CDCl_3$) δ 14.15 (q), 18.85 (s), 25.59 (q), 25.71 (q), 25.93 (q), 46.45 (t), 60.63 (t), 77.06 (s), 117.74 (t), 133.23 (d), 174.82 (s).

Ethyl 2-Trimethylsilyloxy-2-methyl-4-hexenoate (5k). α-Hydroxy ester **5e** (0.5 g, 2.9 mmol), TMSOTf (0.96 g, 4.3 mmol) and pyridine (0.45 g, 5.8 mmol) afforded after Kugelrohr distillation 0.35

g (48%) of **5k** as a colorless oil, consisting of a mixture of E,Z isomers in a 4:1 ratio. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.12 (s, 9H), 1.25 (t, 3H), 1.38 (major isomer), 1.40 (minor isomer) (s, 3H), 1.58 (minor isomer), 1.63 (major isomer) (d, 3H, J = 7.5 Hz), 2.25-2.36 (major isomer), 2.36-2.47 (minor isomer) (m, 2H), 4.13 (q, 2H), 5.32-5.60 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 2.00 (q), 14.15 (q), 17.87 (q), 17.93 (q), 25.82 (q), 38.90 (t), 44.82 (t), 60.61 (t), 77.31 (s), 77.52 (s), 124.52 (d), 125.44 (d), 126.51 (d), 128.52 (d), 175.02 (s).

Ethyl 2-Trimethylsilyloxy-2-phenyl-4-pentenoate (5m). From α-hydroxy ester **1a** (1.1 g, 5.0 mmol), TMSOTf (1.7 g, 7.5 mmol) and pyridine (0.8 g, 10 mmol), 1.2 g (82%) of **5m** was obtained after Kugelrohr distillation at 100 °C (0.01 mm Hg) as a colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.16 (s, 9H), 1.26 (t, 3H), 2.81-2.98 (m, 2H), 4.10-4.28 (m, 2H), 5.00-5.10 (m, 2H), 5.66-5.78 (m, 2H), 7.25-7.52 (m, 5H). ^{13} C NMR ($CDCl_3$) δ 1.89 (q), 13.91 (q), 45.26 (t), 61.22 (t), 81.40 (s), 118.08 (t), 125.41 (d), 127.24 (d), 127.79 (d), 132.95 (d), 142.16 (s), 173.2 (s).

PLE Catalyzed Hydrolyses of α-Hydroxy Esters 5a-f,h,l. 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 2N 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 6N HCl. Ethyl acetate (20 mL) was added and the mixture was filtered over Celite. After separation of the organic layer, the aqueous phase was extracted with ethylacetate (3 x 20 mL). The organic phase was partly concentrated in vacuum (to a volume of approximately 20 mL) and washed with 5% aqueous $NaHCO_3$ solution (3 x 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 6N HCl and was then extracted with ether (3 x 20 mL). The organic phase was dried over Na_2SO_4 and removal of the solvent in vacuum gave the α-hydroxy acid. Specific details are given below.

(S)-2-Hydroxy-2-phenyl-4-hexenoic acid (6a). Ester **5a** (0.85 g, 3.6 mmol) in buffer (45 mL) with PLE-A (50 mg) yielded after 46% conversion, (R)-**5a** (0.45 g, 53% yield, 64% ee), $[\alpha]_{578}$ -3.4 (*c* 1, EtOH) and α-hydroxy acid (S)-**6a** (0.28 g, 38% yield, 75% ee) as a slightly colored oil, $[\alpha]_{578}$ +14.8 (*c* 1, $CHCl_3$). The acid was isolated as a 4:1 mixture of E,Z isomers. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.63 (d, J = 6.0 Hz), 2.63-3.08 (m, 2H), 5.32-5.71 (m, 2H), 7.24-7.61 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 18.04 (q), 37.20 (t, isomer), 43.08 (t), 77.80 (s), 123.76 (d), 125.36 (d), 127.97 (d), 128.08 (d), 128.26 (d), 129.25 (d), 131.65 (d), 140.15 (s), 178.23 (s). Exact mass: m/e calculated for $C_{12}H_{14}O_3$: 206.094. Found: 206.094.

(+)-2-Hydroxy-2,3-diphenylpropanoic acid (6b). Ester **5b** (0.44 g, 1.6 mmol) in buffer (30 mL) with PLE-A (50 mg) afforded after 49% conversion, (-)-**5b** (0.18 g, 41% yield, 40% ee), $[\alpha]_{578}$ -16.4 (*c* 1, $CHCl_3$) and α-hydroxy acid (+)-**6b** (0.17 g, 43% yield, 38% ee) as a slightly colored solid, $[\alpha]_{578}$ +15.5 (*c* 1, $CHCl_3$). Recrystallization of the acid from $CHCl_3$ resulted in enantiomeric enrichment of

the enantiomer which remained in solution. It was not possible to obtain enantiomerically pure material in this way. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.20 (d, 1H, J = 15 Hz), 3.64 (d, 1H, J = 15 Hz), 7.20 (m, 5H), 7.35-7.42 (m, 3H), 7.66-7.70 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 45.70 (t), 77.32 (s), 125.52 (d), 127.13 (d), 128.10 (d), 128.21 (d), 128.29 (d), 130.36 (d), 134.82 (s), 140.39 (s), 178.32 (s). Exact mass: m/e calculated for $C_{15}H_{14}O_3$: 242.093. Found: 242.094.

(S)-2-Hydroxy-2-phenyl-4-methyl-4-pentenoic acid (6c). From ester **5c** (0.50 g, 2.1 mmol) in buffer (35 mL) with PLE-A (70 mg) was obtained after 52% conversion, ester (R)-**5c** (0.22 g, 44% yield, 86% ee), $[\alpha]_{578}$ -17.4 (*c* 1, $CHCl_3$), $[\alpha]_{578}$ +1.5 (*c* 1, EtOH), $[\alpha]_{365}$ -12.8 (*c* 1, EtOH) and α-hydroxy acid (S)-**6c** (0.17 g, 39% yield, 80% ee), $[\alpha]_{578}$ +21.5 (*c* 1, $CHCl_3$). Hydrolysis of the recovered ester (0.15 g, 0.64 mmol) in MeOH/KOH afforded α-hydroxy acid (R)-**6c** (0.11g, 83% yield, 86% ee) as a white solid, $[\alpha]_{578}$ -23.2 (*c* 1, $CHCl_3$). Enantiomerically pure material was obtained after one recrystallization from $CHCl_3$/hexane (1:1), mp 129.4-129.6 °C, $[\alpha]_{578}$ -27.0 (*c* 1, $CHCl_3$). Spectroscopic data were identical with those described for racemic **6c**. Anal. Calcd for $C_{12}H_{14}O_3$: C, 69.88; H, 6.84. Found: C, 69.75; H, 6.75. Exact mass: m/e calculated for $C_{12}H_{14}O_3$: 206.094. Found: 206.094.

(S)-Atrolactic acid (6d). From ester **5d** (1.0 g, 5.2 mmol) in buffer (40 mL) with PLE-A (90 mg) was isolated after a conversion of 42% (R)-**5d** (0.48g, 47% yield, 40% ee); $[\alpha]_D$ -9.4 (neat). [lit[15] $[\alpha]_D$ -23.7 (neat) (R)] and (S)-Atrolactic acid (**6d**, 0.35 g, 41% yield, 51% ee); $[\alpha]_D$ +19.1 (*c* 1, EtOH). [lit[10] $[\alpha]_D$ +37.7 (EtOH) (S)]; ^{1}H NMR (C_6D_6, 300 MHz) δ 1.71 (s, 3H), 6.34-6.94 (br, 2H), 7.04-7.62 (m, 5H). ^{13}C NMR (C_6D_6) δ 26.96 (q), 76.10 (s), 125.54 (d), 128.11 (d), 128.59 (d), 142.76 (s), 179.84 (s). Anal. Calcd for $C_9H_{10}O_3$: C: 65.05; H, 6.07. Found: C: 64.93; H: 6.03.

(S)-2-Hydroxy-2-methyl-4-hexenoic acid (6e). **5e** (2.6 g, 15.1 mmol) in buffer (15 mL) with PLE-A (260 mg) gave after 49% conversion, (R)-**5e** (0.83 g, 32% yield, 2% ee), $[\alpha]_{578}$ +2.4 (*c* = 1, EtOH), and α-hydroxy acid (S)-**6e** (1.10 g, 49% yield, 2% ee) as a slightly colored oil, which was purified by Kugelrohr distillation (77 °C, 1.0 mm Hg): $[\alpha]_{578}$ -2.9 (*c* = 1, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.44 (s, 3H), 1.68 (d, 3H, J = 6.7 Hz), 2.29-2.54 (m, 2H), 5.35-5.65 (m, 2H), 6.2-8.2 (br, 2H); ^{13}C NMR ($CDCl_3$) δ 17.89 (q), 24.94 (q), 43.10 (t), 74.40 (s), 123.76 (d), 130.76 (d), 180.82 (s). The product consisted of a mixture E,Z isomers. Exact mass: m/e calculated for $C_7H_{12}O_3$: 144.078. Found: 144.079.

(S)-2-Hydroxy-2-methyl-5-phenyl-4-pentenoic acid (6f). Ester **5f** (2.50 g, 10.7 mmol) in buffer (14 mL) with PLE-A (450 mg) gave after 47% conversion, ester (R)-**5f** (1.15 g, 46% yield, 3% ee), $[\alpha]_{578}$ +2.4 (*c* = 1, EtOH), and acid (S)-**6f** (1.0 g, 46% yield, 3% ee), $[\alpha]_{578}$ -6.3 (*c* = 1, $CHCl_3$) as a slightly colored solid. The product consisted of a 6:1 mixture of regioisomers. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.66 (s, 3H0, 2.64-3.10 (m, 2H), 5.34-5.72 (m, 2H), 7.18-7.43 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 18.03 (q), 43.12 (t), 77.83 (s), 123.79 (d), 125.38 (d), 127.97 (d), 128.28 (d), 131.73 (d), 140.21 (s), 177.84 (s). Exact mass: m/e calculated for $C_{12}H_{14}O_3$: 206.094. Found: 206.094.

(+)-2-Hydroxy-2,5-diphenyl-4-pentenoic acid (6h). A solution of ester **5h** (0.59 g, 1.99 mmol) in

DMSO (10 mL) was added to a buffer solution (pH 8, 20 mL). The pH of the mixture was adjusted to 8 by addition of 1.0 N HCl and PLE-A (135 mg) was added. After a conversion of 46% there was islolated, (-)-**5h** (0.31 g, 53% yield, 10% ee), $[\alpha]_{578}$ -10.7 (*c* 1, $CHCl_3$) and α-hydroxy acid (+)-**6h** (0.16 g, 30% yield, 12% ee), $[\alpha]_{578}$ +6.3 (*c* 0.8, $CHCl_3$). ^1H NMR (CD_3OD, 300 MHz) δ 2.91-3.23 (m, 2H), 5.03 (s, 2H), 6.24-6.34 (m, 1H), 6.54 (d, 1H, J = 16.1 Hz), 7.17-7.41 (m, 8H), 7.70-7.73 (m, 2H). ^{13}C NMR (CD_3OD) δ 44.50 (t), 79.57 (s), 125.44 (d), 126.69 (d), 127.06 (d), 128.10 (d), 128.55 (d), 129.07 (d), 129.38 (d), 134.84 (d), 138.81 (s), 143.60 (s), 177.11 (s). Exact mass: m/e calculated for $C_{17}H_{16}O_3$: 268.109. Found: 268.110.

2-Benzyloxy-2-methyl-4-pentenoic acid (6l). 2-Benzyloxy ester **5l** (1.0 g, 4.0 mmol) in buffer (40 mL) with PLE-A (65 mg) gave after 50% conversion **6l** (0.40 g, 45% yield, 0% ee) and ester **5l** (0.45 g, 45% yield, 0% ee). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.42 (s, 3H), 2.54-2.69 (m, 2H), 4.43 (s, 2H), 5.03-5.09 (m, 2H), 5.63-5.78 (m, 1H), 7.15-7.24 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 21.39 (q), 41.63 (t), 66.26 (t), 72.25 (s), 119.18 (s), 127.61 (d), 127.76 (d), 128.34 (d), 131.64 (d).**PLE Catalyzed Hydrolyses of the Silylated α-Hydroxy Esters 5g-k, 5m.** The general procedure described above was followed, with this difference that a 0.05 M KH_2PO_4 buffer of pH 7 was employed. During the isolation procedure the silylated acid was desilylated; the unreacted ester was isolated as a mixture of silylated and desilylated material. Details are given below.

Silylated-2-Methyl-4-pentenoic-α-hydroxy esters 5i-k. These compounds were all substrates for PLE-A. When the conversion reached 50%, the reaction was stopped and the acidic products were isolated in 39-46% chemical yield. The unreacted esters were recovered in 30-36% chemical yield. Only racemic compounds were isolated.

Silylated-2-Phenyl-4-pentenoic-α-hydroxy ester 5m. No activity of PLE-A was observed for this compound.

(R)-2-Hydroxy-2-methyl-5-phenyl-4-pentenoic acid (6f). Sililoxy ester **5g** (0.75 g, 2.5 mmol) in buffer (40 mL) with PLE-A (100 mg) gave after 42% conversion, α-hydroxy acid (R)-**6f** (0.21 g, 41% yield, 3% ee) as a slightly colored solid; $[\alpha]_{578}$ +6.3 (*c* 1, EtOH) and ester **5f** (partly desilylated). The ester was stirred in a dry ether/HCl solution for 15 min. and after concentration in vacuum (S)-**5f** (0.33 g, 57% yield, 3% ee) was isolated as a colorless oil; $[\alpha]_{578}$ -2.2 (*c* 1, EtOH).

(S)-Diethyl-2-Hydroxy-2-phenyl-butanedioate (7). A solution of enantiomerically pure (S)-2-hydroxy-2-phenyl-4-pentenoic acid (**2a**, 0.45 g, 2.34 mmol) in methanol (17 mL) and CH_2Cl_2 (8 mL) was cooled to -70 °C. Ozone was bubbled through the solution until a pale blue colour developed (about 30 min.). The system was flushed with oxygen and the solvents were removed in vacuum using an oil pump, while maintaining the solution at -10 °C. The resulting oil was treated with formic acid (10 mL, 90%) followed by H_2O_2 (5 mL). The resulting solution was cautiously warmed until a vigorous reaction began (65 °C). After the reaction had subsided, the solution was cooled to 30 °C and again formic acid (10 mL, 90%) and H_2O_2 (5 mL) was added. The solution was heated at 100-

110 °C for 45 min. After cooling (peroxide test: negative) and removal of the solvents in vacuum (an oil pump was used to evaporate the formic acid that was formed), a slightly yellow solid remained. The crude acid was dissolved in absolute EtOH (5 mL) and HCl gas was bubbled through the solution for 2 min. After being refluxed overnight, the reaction mixture was poured into a saturated aqueous $NaHCO_3$ solution (10 mL). EtOH was removed under reduced pressure and the remaining layer was extracted with ether. Drying (Na_2SO_4) and evaporation of the ether layers in vacuum, yielded the ethyl ester as a slightly yellow solid, which was purified by Kugelrohr distillation (125 °C, 0.03 mm Hg) to yield the product as a colorless oil which crystallized on standing. (S)-**7** (0.45 g, 72% yield, >98% ee) was obtained white crystalline material, mp. 65.9-66.4 °C; $[\alpha]_{578}$ -39.7 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.26 (2 x t, 6H), 2.89 (d, 1H, J = 16.5 Hz), 3.45 (d, 1H, J = 16.5 Hz), 4.14-4.28 (m, 4H), 4.38 (s, 1H), 7.32-7.61 (m, 5H). ^{13}C NMR ($CDCl_3$) δ 13.86 (q), 13.98 (q), 44.27 (t), 60.84 (t), 62.31 (t), 77.34 (s), 124.93 (d), 127.94 (d), 128.26 (d), 140.19 (s), 170.82 (s). 173.53 (s). Anal. Calcd for $C_{14}H_{18}O_5$: C, 63.14; H, 6.81. Found: C, 63.02; H, 6.72.

Ozonolysis of (+)-2-Hydroxy-2-phenyl-4-hexenoic acid (6a). A solution of optically active acid **6a** (0.24 g, 1.17 mmol, $[\alpha]_{578}$ +14.8 (*c* 1, $CHCl_3$) in MeOH (10 mL) and CH_2Cl_2 (4 mL) was ozonized as described above. The crude acid was esterified in EtOH/HCl to yield after Kugelrohr distillation (125 °C, 0.03 mm Hg) (S)-**7** (0.18 g, 58% yield, 75% ee) as a colorless oil, which crystallized on standing, $[\alpha]_{578}$ -29.8 (*c* 1, $CHCl_3$).

(S)-2-Hydroxy-2-phenyl-4-methyl-2-phenyl-valeric acid (8). A mixture of **6c** (0.12 g, 0.58 mmol, $[\alpha]_{578}$ +20.6 (*c* 1, $CHCl_3$), 20 mg of Pd/C (5%) and EtOAc (20 mL) was shaken in a Parr apparatus, at 3 atm H_2 pressure, for 20 h. After filtration, to remove the catalyst, the solvent was removed in vacuum, to give (S)-**8** as a white solid (0.10 g, 83% yield, 75% ee) as a white solid: $[\alpha]_D$ +15.0 (*c* = 1, EtOH). (lit[9] $[\alpha]_D$ +20.0 (*c* = 2, EtOH). ^1H NMR ($CDCl_3$, 300 MHz) δ 0.89 (d, 3H, J = 6.59 Hz), 0.95 (d, 3H, J = 6.59 Hz), 1.84 (s, 1H), 2.00-2.20 (m, 2H), 7.29-7.37 (m, 3H), 7.60-7.3 (m, 2H). ^{13}C NMR ($CDCl_3$) δ 23.10 (q), 24.23 (q), 24.47 (d), 47.58 (t), 78.19 (s), 125.32 (d), 127.76 (d), 128.19 (d), 141.55 (s), 180.90 (s). Exact mass: m/e calculated for $C_{12}H_{16}O_3$: 208.110. Found: 208.110.

Ozonolysis of (-)-2-Hydroxy-2-methyl-4-hexenoic acid (6e). Ozonolysis of a solution of **6e** (0.46 g, 3.2 mmol, $[\alpha]_{578}$ -2.9 (*c* 1, EtOH) in MeOH (18 mL) and CH_2Cl_2 (9 mL) as described for **7** gave after workup and evaporation of the formic acid and acetic acid formed, citramalic acid (**9**, 0.33 g, 69% yield, 2% ee (S)) as a slightly yellow syrup, $[\alpha]_D$ +0.51 (*c* 3, H_2O); lit[16] $[\alpha]_D$ +23.1 (*c* 3, H_2O). ^1H NMR (D_2O, 300 MHz) δ 1.50 (s, 3H, 2.74 (d, 1H, J = 17 Hz), 3.08 (d, 1H, J = 17 Hz). ^{13}C NMR (D_2O) δ 26.85 (q), 44.95 (t), 73.55 (s), 174.96 (s), 179.52 (s). Exact mass: m/e calculated for $C_5H_8O_3$ (-CH_3): 133.014. Found: 133.014.

Ozonolysis of (-)-2-Hydroxy-2-methyl-5-phenyl-4-pentenoic acid (6f). Optically active α-hydroxy-acid **6f** (0.31 g, 1.34 mmol, $[\alpha]_{578}$ -5.5 (*c* 1, $CHCl_3$) in MeOH (7 mL) and CH_2Cl_2 (3 mL) was ozonized following the procedure described above. The crude product, isolated after the ozonolysis,

contained also benzoic acid and formic acid. Formic acid was removed from this mixture by evaporation using an oil pump. In order to separate the product from benzoic acid, the entire mixture was esterified in MeOH/HCl analogously to the esterification described above. A slightly yellow oil was isolated, which gave after chromatography over silica gel (ethyl acetate/hexane 1:1) dimethyl citramalate (**10**, 0.13 g, 55% yield, 3% ee, (S)) as a colorless oil, $[\alpha]_{578}$ + 0.36 (*c* 1, CH_3OH); lit[17] $[\alpha]_{578}$ + 10.7 (*c* 1, CH_3OH) (S). ^1H NMR ($CDCl_3$, 300 MHz) δ 1.41 (s, 3H), 2.65 (d, 1H, J = 16.5 Hz), 2.94 (d, 1H, J = 16.5 Hz), 3.44 (s, 1H), 3.65 (s, 3H), 3.77 (s, 3H). ^{13}C NMR ($CDCl_3$) δ 26.12 (q), 43.83 (t), 51.73 (q), 52.77 (q), 72.36 (s), 171.25 (s), 175.77 (s). Exact mass: m/e calculated for $C_7H_{12}O_5$ ($-OCH_3$): 145.050. Found: 145.050.

Enantiomeric excess determination. The ee 's of the optically active esters **5** and acids **6** were determined by derivatization with (S)-2-chloropropanoyl chloride followed by 300 MHz ^1H NMR analysis.[11] The racemic esters and acids were used as reference standards.

Acknowledgement. We are grateful to DSM for support of this research.

References.

1. (a) Jones, J.B. *J. Org. Chem.* **1986**, *42*, 3351. (b) Biocatalysis in Organic Media; Laane, C.; Tramper, J.; Lilly, M.D., Eds.; Elsevier: Amsterdam, 1987. (c) Wong, C.H.; Whitesides, G.M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 617.
2. a) Zhu, L.-M.; Tedford, M.C. *Tetrahedron* **1990**, *46*, 6587. b) Davies, H.G.; Green, R.H.; Kelly, D.R.; Roberts, S.M. In: Best Synthetic Methods. *Biotransformations in Preparative Organic Chemistry*; Academic Press: London, 1989; Chapter 2.
3. For example: (a) Gais, H.-J.; Bülow, G.; Zatorski, A.; Jentsch, M.; Maidonis, P.; Hemmerle, H. *J. Org. Chem.* **1989**, *54*, 5115. (b) Sabbioni, G.; Jones, J.B. *J. Org. Chem.* **1987**, *52*, 4565. (c) Carda, M.; Van der Eycken, J.; Vandewalle, M. *Tetrahedron: Asymmetry* **1990**, *1*, 17.
4. (a) Mohr, P.; Roesslein, L.; Tamm, C. *Tetrahedron Lett* **1989**, *30*, 2513. (b) Wilson, W.K.; Shawn, B.B.; Barber, Y.J.; Scallen, T.J.; Morrow, C.J. *J. Org. Chem.* **1983**, *48*, 3960. (c) Ramaswamy, S.; Hui, R.A.H.F.; Jones, J.B. *J. Chem. Soc. Chem. Commun.* **1986**, 1545. (d) Whitesell, J.K.; Lawrence, R.M. *Chimia* **1986**, *40*, 318. (e) Klunder, A.J.H.; van Gastel, F.J.C.; Zwanenburg, B. *Tetrahedron Lett.* **1988**, *29*, 2697.
5. Moorlag, H,; Kellogg, R.M.; Kloosterman, M.; Kaptein, B.; Kamphuis, J.; Schoemaker, H.E. *J. Org. Chem.* **1990**, *55*, 5878.
6. Chen, C.-S; Fujimoto, Y.; Girdaukas, G.; Sih, C.J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
7. Toone, E.J.; Werth, M.J.; Jones, J.B. *J. Am. Chem. Soc.* **1990**, *112*, 4946.
8. Moorlag, H.; Kellogg, R.M. manuscript in preparation.
9. Meyers, A.I.; Slade, J. *J. Org. Chem.* **1980**, *45*, 2785, 2912.
10. McKenzie, A.; Ritchie, A. *Berichte* **1937**, *70*, 23.
11. Moorlag, H,; Kruizinga, W.H.; Kellogg, R.M. *Recl. Trav. Chim. Pays-Bas* **1990**, *109*, 479.
12. Sugai, T.; Kakeya, H.; Ohta, H. *J. Org. Chem.* **1990**, *55*, 4643.
13. Nakada, M.; Kobayashi, S.; Ohno, M.; Iwasaki, S.; Okuda, S. *Tetrahedron Lett.* **1988**, *29*, 3951.
14. Barton, J.; Tully, C.R. *J. Org. Chem.* **1978**, *43*, 3649.
15. Bonner, W.A; Zderic, J.A.; Casaletto, G.A. *J. Am. Chem. Soc.* **1952**, *74*, 5086.
16. Staring, E.G.J.; Moorlag, H.; Wynberg, H. *Recl. Trav. Chim. Pays-Bas* **1986**, *105*, 374.
17. Staring, E.G.J. *Ph.D. Thesis*, **1985**, Groningen, The Netherlands.