Chiral α-substituted α-hydroxy acids

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CHAPTER IV

PIG LIVER ESTERASE CATALYZED
HYDROLYSES OF α-SUBSTITUTED α-HYDROXY ESTERS

4.1   INTRODUCTION

In Chapter II we described a catalytic entry to α-alkylated α-hydroxy acids using palladium coordinated π-allyl complexes. However, our principal goal of achieving complete stereoselectivity in the alkylation reaction, was not established. We then decided to focus on other catalytic approaches that would enable us to obtain α-alkylated α-hydroxy acids in enantiomerically pure form. As we have in principle a route to these acids in racemic form, via catalytic allylation (Chapter II) as well as via stoichiometric alkylation (Section 4.4.2), a resolution method could be employed to get to both enantiomers of α-alkylated α-hydroxy acids in enantiomerically pure form.

Biocatalysts are in this regard very interesting. Enzymes are capable of rate enhancements (up to 10^{10}) for the reactions they promote. Enzymes can effect reactions under mild conditions. They can exert unique control in regio- and stereo-chemistry thereby enabling stereoselective transformations to be achieved that for many cases cannot currently be matched by nonenzymatic catalysis.[1] Many biocatalysts are now readily accessible either as isolated enzymes or as whole cells. The use of enzymes is even more attractive thanks to the contributions of Klibanov[2] who demonstrated that enzymes, which were long thought to act only in aqueous solutions, can even exert their stereoselective catalysis in organic solvents. Enzymes are therefore now widely accepted as practical tools in stereoselective synthesis and the synthetic opportunities provided by them to act as chiral catalysts in the synthesis of enantiomerically pure compounds are now being exploited rapidly.[3]

The specificity and catalytic properties of enzymes are a result of their structure. The active site of the enzyme binds the substrate and binds even better the transition state of the reaction to be catalyzed (complementarily) which results in

lowering of the Gibbs energy of activation ($\Delta G^{\neq}$), thereby catalyzing the reaction (Figure 4.1).[4]
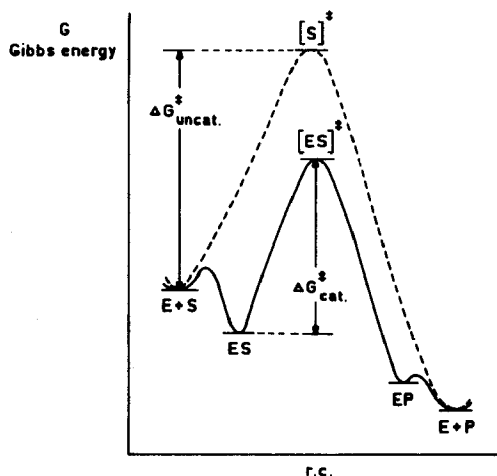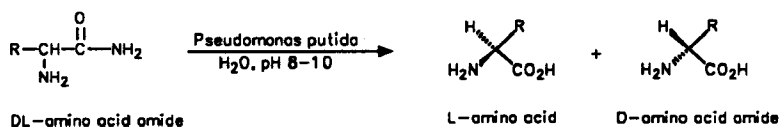

Figure 4.1

Enzyme reactions are classified, according to the transformations to be catalyzed, into six main groups: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. The most useful to organic chemists are those enzymes which can accept a broad structural range of substrates while retaining the ability to operate stereoselectively in every case (this property is often meant by the somewhat confusing term of 'broad substrate specificity'). Enzymes that meet this criterium more or less are the oxidoreductases (which catalyze oxidation-reduction reactions), hydrolases (which catalyze hydrolysis reactions) and lyases (which catalyze addition reactions).

Enzymes can act as such or can require coenzymes in order to be catalytically active. Coenzymes are cosubstrates (for example NAD(H) and ATP) that undergo a chemical transformation during the reaction and need to be recycled to the active form in order for the catalysis to continue.[5] Although coenzyme dependent enzymes are more tedious to handle, coenzyme recycling is nowadays in some cases efficient enough to permit use of these enzymes as economically feasible catalysts in stereoselective transformations on large scale.[6]

An example of a commercialized procedure for the production of enantiomeri-

cally pure α-amino acids using a generally applicable enzymatic resolution process is depicted in Scheme 4.1. A hydrolytic enzyme, an aminopeptidase from *Pseudomonas putida*, hydrolyses stereoselectively racemic amino acid amides to give L-amino acids and D-amino acid amides, which can either be racemized or converted to the D-amino acids.[7]

$$R-\underset{\underset{NH_2}{|}}{CH}-\overset{\overset{O}{\|}}{C}-NH_2 \quad \xrightarrow[\text{H}_2\text{O, pH 8-10}]{\text{Pseudomonas putida}} \quad \underset{H_2N}{\overset{H}{\diagup}}\!\!\!\overset{R}{\diagdown}_{CO_2H} \quad + \quad \underset{H_2N}{\overset{H}{\diagup}}\!\!\!\overset{R}{\diagdown}_{CO_2H}$$

DL—amino acid amide   L—amino acid   D—amino acid amide
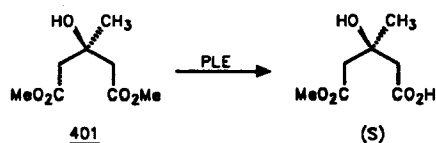
Scheme 4.1

The group of enzymes that have been most successfully applied in stereoselective synthesis are the hydrolases. Hydrolytic enzymes such as lipases and esterases are especially attractive because they do not require coenzymes, they are fairly stable, available at low cost and they convert a wide range of substrates stereoselectively. One of the most studied enzymes used in stereoselective hydrolysis of esters, pig liver esterase (PLE), will be treated more extensively in the following Sections where attention is focussed on the application of PLE in the synthesis of enantiomerically pure α-substituted α-hydroxy acids.

4.2 PIG LIVER ESTERASE IN STEREOSELECTIVE SYNTHESIS

Esterases have been used for two basic transformations: firstly, to cleave a racemic ester to give an optically active recovered ester and an optically active acid, and secondly to remove an acyl group from a racemic acylated alcohol to give an optically active alcohol.

As early as 1904 Dakin reported that pig liver esterase (PLE) catalyzed hydrolysis of racemic ethyl mandelate proceeded with some enantioselectivity.[8] However, it was not until the 1960s that the synthetic opportunities provided by enzymes became to be recognized, since it became then clear that not only natural but also many unnatural substrates prepared by chemical synthesis are accepted by enzymes.[9] Mainly during the past two decades, after the first documented report by Sih and co-

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workers on the stereoselective hydrolysis of dimethyl ß-hydroxy-ß-methylglutarate (**401**, Scheme 4.2),[10] the potential of PLE in stereoselective synthesis has been exploited extensively.[11]

Scheme 4.2

PLE has been used mostly for the hydrolysis of meso and prochiral diesters.[12] Hydrolysis of meso compounds has the intrinsic advantage that in principle a 100% yield of the pure enantiomer can be obtained, whereas the maximum yield of a desired enantiomer in the resolution of racemic mono esters will be 50%. This concept in enzymatic transformations, known as the 'meso trick' is unequalled in conventional organic chemistry. The ability of enzymes to discriminate between centres of opposite configuration in meso compounds is a unique and powerful tool. Some selected examples of applications of the 'meso trick' are shown in Scheme 4.3. PLE exhibits high stereoselectivity in the hydrolysis of diacetate **402** to give the prostaglandin precursor 4-hydroxy-2-cyclopentenyl acetate (**403**).[13] Tricyclic compound **404** undergoes enantioselective hydrolysis with PLE to produce nucleoside synthons **405**[14] The synthesis of (+)-biotin (**406**), a growth-promoting factor at cellular level, demonstrates another useful application of PLE.[15]

In contrast to the well studied catalyzed hydrolysis of (cyclic) diesters substrates much less research has been carried out on the PLE catalyzed hydrolysis of racemic mono esters, although some resolutions have successfully been accomplished.[16] Before describing the results of the PLE catalyzed hydrolysis of the racemic mono esters in which we are interested, some attention will be given to a quantitative treatment of kinetic resolution.
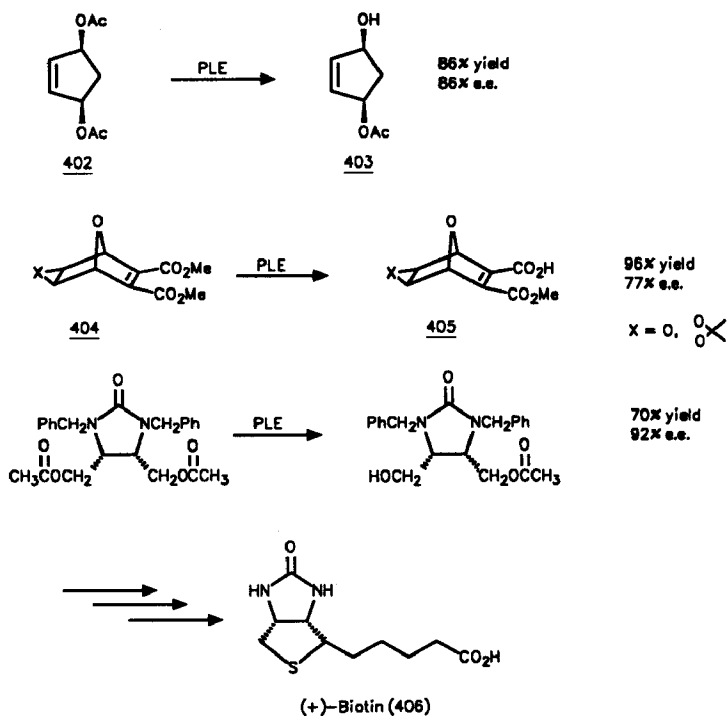
Scheme 4.3

4.3 ENZYMATIC KINETIC RESOLUTION

Most hydrolytic enzymes operate by binding the enantiomers (R and S) of a racemic substrate to form two diastereomeric complexes (ER and ES) that can be converted to enzyme product complexes (EP and EQ) which dissociate to afford the free enzyme and the released products (P and Q) (Equation 1).

$$\text{enz} + \text{R} \rightleftharpoons \text{ER} \rightarrow \text{EP} \rightarrow \text{enz} + \text{P} \qquad (\text{Eq. 1})$$
$$\text{enz} + \text{P} \rightleftharpoons \text{ES} \rightarrow \text{EQ} \rightarrow \text{enz} + \text{Q}$$
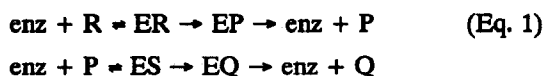
Consider that R and S are the fast and slow reacting enantiomer that compete for the enzyme and that the reaction is irreversible and that there is no product inhibition. It

can be shown[17] that the discrimination between the two competing enantiomers is given by Equation 2, where E is the enantiomeric ratio and V_R, K_R and V_S, K_S are the maximal velocities and Michaelis constants[1] of the reacting enantiomers. R_0 and S_0 are the initial concentrations of R and S.

$$E = \frac{\ln(R / R_0)}{\ln(S / S_0)} = \frac{V_R / K_R}{V_S / K_S}$$ (Eq. 2)

According to the transition state theory,[18] the relation between enantiomeric ratio and the Gibbs energy difference of the diastereomeric transition states is dictated by Equation 3. Hence, an E value of 100 corresponds to a $\Delta\Delta G^{\neq}$ value of approximately 3 kcal/mol.

$$\Delta\Delta G^{\neq} = -RT\ln E$$ (Eq. 3)

In order to compare the enantiomeric specificity of two different enzymatic hydrolyses, the E value, which is a constant independent of time and substrate concentration (under ideal conditions), can be related to the extent of conversion of the hydrolytic reaction (c) and the enantiomeric excess of the product fraction (ee(P)) or recovered substrate fraction (e.e.(S)) (Equation 4).

$$E = \frac{\ln[1 - c(1 + ee(P))]}{\ln[1 - c(1 - ee(P))]} = \frac{\ln[(1 - c)(1 - ee(S))]}{\ln[(1 - c)(1 + ee(S))]}$$ (Eq. 4)

The Gibbs energy difference ($\Delta\Delta G^{\neq}$) required to obtain the product with 99.9% e.e is relatively small, approximately 4.4 kcal/mol. By using these equations it is possible to predict the value of conversion at which the enzymatic hydrolysis should be terminated in order to obtain the desired enantiomer of either product or remaining substrate with maximun e.e. Figure 4.2 (A), which shows a theoretical plot of e.e.(P) as a function of c for various E values, reveals that the e.e. of the product decreases abruptly for values of c beyond 0.5. Therefore, the reaction should be stopped prior to a conversion of 50% irrespective of the value of E. If on the contrary, the remaining

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substrate fraction is of interest, the conversion may proceed to higher values of c as e.e.(S) increases with extent of conversion, to obtain optically highly enriched material (Fig. 4.2 (B)).[17]
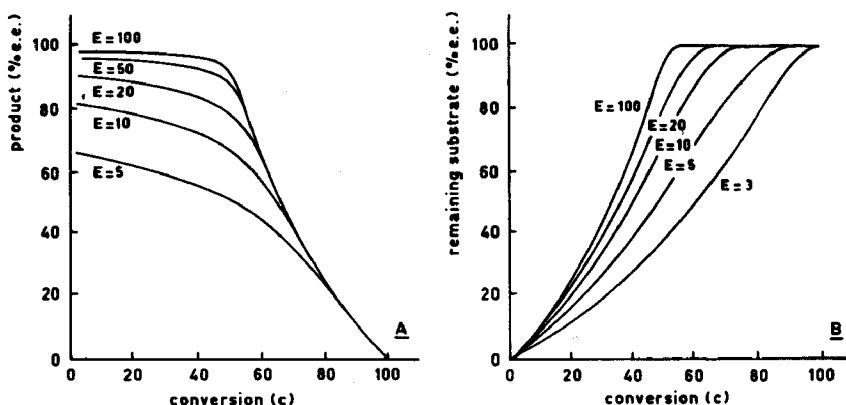
Figure 4.2

Although the extent of conversion can be determined experimentally via GC, HPLC or via titration, it may be more convenient to calculate c, using the relationship expressed in Equation 5.

$$c = \frac{ee(S)}{ee(S)+ee(P)} \qquad \text{(Eq. 5)}$$

In order for an enzymatic resolution to be of practical use, E values greater than 10 are mostly required. However, if necessary, strategies to enhance the optical purity of material provided by enzymes with low enantioselectivity, may bring relief.[19] For example reesterification of optical enriched product, followed by a second enzymatic ester hydrolysis can be carried out, or organic solvents may be employed.[20]

4.4.1   *Enzymes in the synthesis of hydroxy acids*

Although some successful enzymatic resolutions in the synthesis of β-hydroxy acids[16f,21] and α-β-dihydroxy acids[22] have been reported, a general enzymatic route to α-hydroxy acids is not known. Results on the enzymatic hydrolysis of α-hydroxy esters have been disappointing. An exception is the hydrolysis of **407** with a lipase from *Pseudomonas fluorescens*, which yielded after 55% conversion the nonhydrolyzed ester with 99% e.e. (Scheme 4.4).[23]
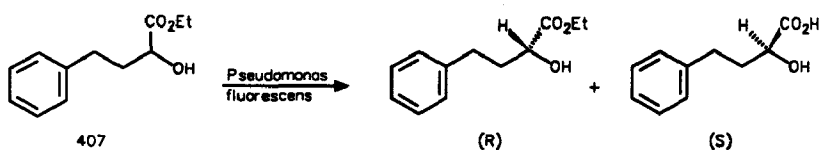
Scheme 4.4

No reports on enzymatic hydrolysis of tertiary hydroxy esters have appeared. This may be the result of the general perception that tertiary esters are difficult to hydrolyze enzymatically. However, recently an effective enantioselective hydrolysis of ketone cyanohydrin acetates **408** with *Pichia miso* was found to afford optically active cyano acetates, which can be hydrolyzed to α-substituted α-hydroxy acids (Scheme 4.5).[24]
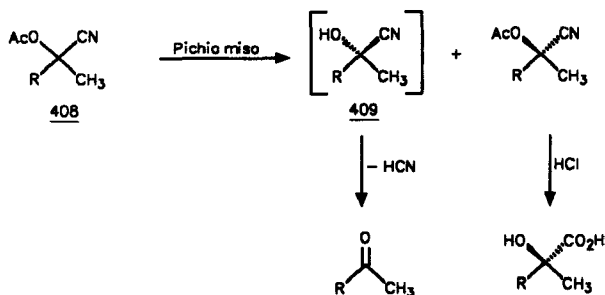
Scheme 4.5

The primary hydrolysis product cyanohydrin **409** is not stable under the reaction conditions and decomposes to a ketone and hydrogen cyanide. Therefore, only one enantiomer of the α-hydroxy acids is accessible with this strategy.

We turned to a more direct route, namely hydrolysis of substituted α-hydroxy esters, as a way to obtain in high e.e. both enantiomers of these important building blocks.

4.4.2 *Synthesis of the substrates: α-substituted α-hydroxy esters*

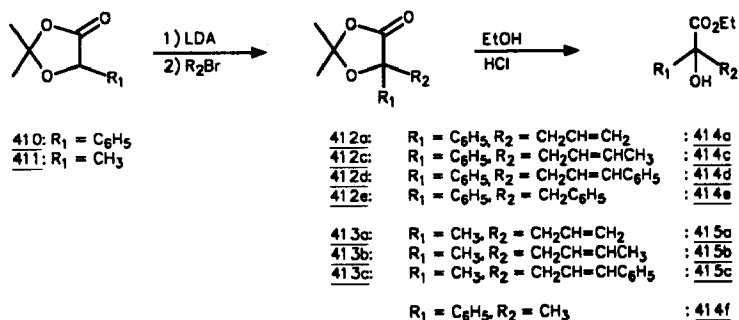The α-substituted α-hydroxy esters were synthesized in good overall yields from the corresponding unbranched α-hydroxy acids, using conventional methods. Thus, protection of mandelic acid and lactic acid as their acetonides **410** and **411**, followed by alkylation with allylic bromides (or, in one case, benzylbromide), gave dioxolanones **412** and **413**. Acid catalyzed deprotection of these dioxolanones in EtOH afforded the desired α-hydroxy esters **414a,c-e** and **415a-c** in good overall yields (71-96%, Scheme 4.6).

410: $R_1 = C_6H_5$
411: $R_1 = CH_3$

|	
412a: $R_1 = C_6H_5, R_2 = CH_2CH=CH_2$	: **414a**
412c: $R_1 = C_6H_5, R_2 = CH_2CH=CHCH_3$	: **414c**
412d: $R_1 = C_6H_5, R_2 = CH_2CH=CHC_6H_5$	: **414d**
412e: $R_1 = C_6H_5, R_2 = CH_2C_6H_5$	: **414e**
413a: $R_1 = CH_3, R_2 = CH_2CH=CH_2$	: **415a**
413b: $R_1 = CH_3, R_2 = CH_2CH=CHCH_3$	: **415b**
413c: $R_1 = CH_3, R_2 = CH_2CH=CHC_6H_5$	: **415c**
$R_1 = C_6H_5, R_2 = CH_3$	: **414f**

Scheme 4.6

The ethyl ester of atrolactic acid (**414f**) was prepared from commercially available atrolactic acid.

A different approach had to be followed for the synthesis of α-methallyl mandelate (**414b**) because alkylation of **410** with methallylchloride failed. Therefore

412b was synthesized using the synthetic methodology described in Chapter II. The acetonide **410** was alkylated with methallyl acetate in the presence of a catalytic amount of a Pd^0 catalyst to afford **412b** in 82% chemical yield. The allylated dioxolanone **412b** was subsequently hydrolyzed under basic conditions to give α-hydroxy acid **416b** (82% yield), which was esterified under basic conditions to afford α-methallyl mandelic acid ethyl ester (**414b**, 93% yield, Scheme 4.7). Attempts to esterify **412b** under acidic conditions (EtOH/HCl) led to formation of γ-lactone **417**, probably due to initial protonation of the double bond to afford a tertiary carbonium ion which then cyclises to form a lactone (Scheme 4.7).
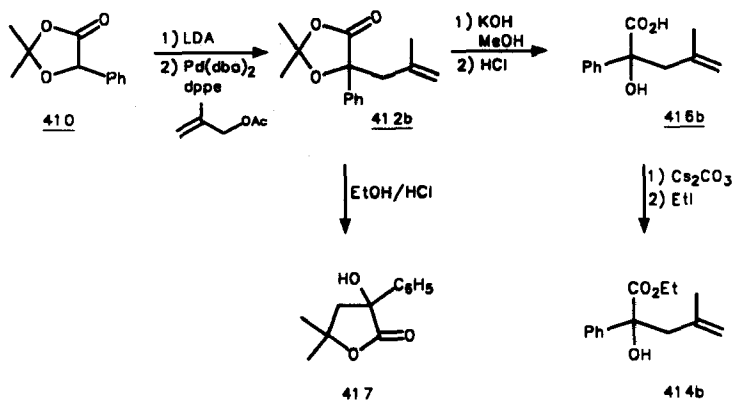
Scheme 4.7

In order to investigate the role of the hydroxyl group of the esters in enzymatic hydrolysis, some O-protected derivatives **414g, 415d-h** were prepared (Fig. 4.3). It has been reported that protection of a polar hydroxy group with a bulky, hydrophobic silyl function can improve the stereoselectivity in PLE catalyzed hydrolysis of substituted dimethyl malonates.[25]

414g: R$_1$ = C$_6$H$_5$, R$_2$ = TMS, R$_3$ = H
415d: R$_1$ = CH$_3$, R$_2$ = TMS, R$_3$ = H
415e: R$_1$ = CH$_3$, R$_2$ = TBDMS, R$_3$ = H
415f: R$_1$ = CH$_3$, R$_2$ = TMS, R$_3$ = CH$_3$
415g: R$_1$ = CH$_3$, R$_2$ = TMS, R$_3$ = C$_6$H$_5$
415h: R$_1$ = CH$_3$, R$_2$ = CH$_2$C$_6$H$_5$, R$_3$ = H
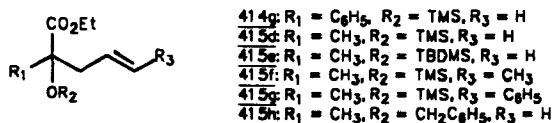
Figure 4.3

The synthesis of the silylated compounds was accomplished analogously to a literature procedure (see Experimental Section). However, it was necessary to use trimethylsilyl-(TMS) or *t*-butyldimethylsilyl (TBDMS) triflates as reagents, because probably for steric reasons the tertiary hydroxyl functionality of the esters did not react with the chlorides of the silylating agents. These esters were subjected to PLE catalyzed hydrolysis (Table 4.1. and 4.2). The next Sections describe the results obtained in the hydrolysis.

4.4.3 *Enzymatic hydrolysis of the substituted α-hydroxy esters*

Initially over 25 available hydrolytic enzyme-preparations were screened for activity with α-allyl mandelate (**414a**). Only two commercially available pig liver esterases, (PLE-EC 3.1.1.1: PLE-S [Sigma] and PLE-A [Amano]), were found to show activity for this substrate. We then performed on larger (preparative) scale the enzymatic hydrolysis of the synthesized α-substituted esters to examine the activity as well as the enantioselectivity of these esterases for the synthesized ester substrates. The enzymatic hydrolyses of the esters **414** were carried out in 0.05 M aqueous phosphate buffer at pH 8. The pH was maintained at this level by addition of 2N aqueous NaOH from an autoburette. When the conversion reached 20-50% the reaction was stopped and both the opticall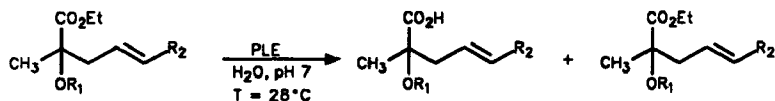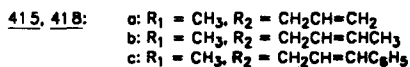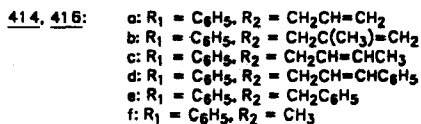y active unchanged esters and optically active acid products were isolated in good yields (Scheme 4.8). If the enzyme is highly enantioselective, the activity will steadily decrease as the conversion approaches 50% and finally stop at this level of conversion.

The hydrolysis of the silylated hydroxy esters was performed at pH of 7. It was established that the silylated esters underwent no desilylation during the enzymatic hydrolysis. During the isolation procedure, however, the acidic product was desilylated as a result of the strongly acidic conditions employed.

414, 416:

a: $R_1 = C_6H_5$, $R_2 = CH_2CH=CH_2$
b: $R_1 = C_6H_5$, $R_2 = CH_2C(CH_3)=CH_2$
c: $R_1 = C_6H_5$, $R_2 = CH_2CH=CHCH_3$
d: $R_1 = C_6H_5$, $R_2 = CH_2CH=CHC_6H_5$
e: $R_1 = C_6H_5$, $R_2 = CH_2C_6H_5$
f: $R_1 = C_6H_5$, $R_2 = CH_3$

415, 418:

a: $R_1 = CH_3$, $R_2 = CH_2CH=CH_2$
b: $R_1 = CH_3$, $R_2 = CH_2CH=CHCH_3$
c: $R_1 = CH_3$, $R_2 = CH_2CH=CHC_6H_5$

415:

d: $R_1 = TMS$, $R_2 = H$
e: $R_1 = TBDMS$, $R_2 = H$
f: $R_1 = TMS$, $R_2 = CH_3$
h: $R_1 = CH_2C_6H_5$, $R_2 = H$
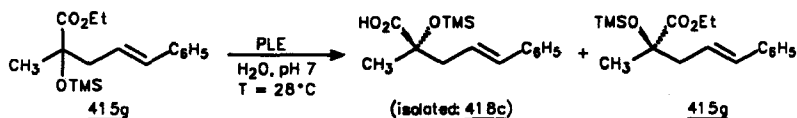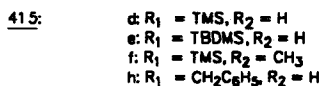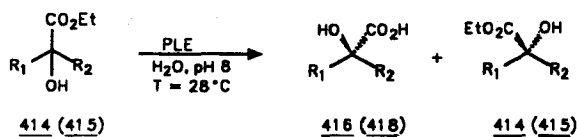
Scheme 4.8

4.4.4 Results of the PLE catalyzed hydrolyses of substituted mandelates 414a-g

The results of the hydrolysis of the substituted mandelates are summarized in Table 4.1. The data in the Table show that all α-hydroxy esters (except silylated **414g**) are substrates for PLE, indicating that PLE can accept and hydrolyze ester moieties attached to a quaternary carbon atom. PLE exhibited a high enantioselectivity for α-allyl mandelate (**414a**) and α-methallyl mandelate (**414b**). When racemic **414a** was treated in aqueous buffer with PLE-S, the product (S)-**416a** could be isolated in good enantiomeric excess (entry 1), with a moderate enantiomeric ratio E of 17. However,

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it should be noted that the activity (rate of conversion) of the enzyme dropped considerably after about 5% conversion, making this approach less attractive. It was found that the reason for this decrease in activity is not due to product inhibition, as the same observation was made when the substrate was treated with PLE-S in the presence of some added acidic product. 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 **414a** 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 **414a** by PLE-A, the initial activity could be increased. Optimal conditions (see Figures 4.4 and 4.5) proved to be a pH of 8, independent of the type of buffer used, and a temperature of 28 °C.
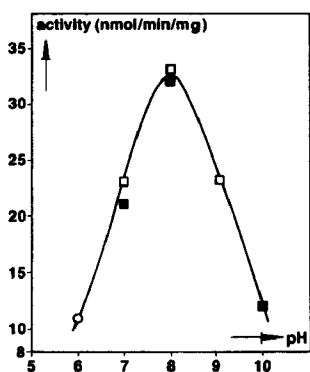
Figure 4.4   Figure 4.5

Figure 4.4:   *Effect of pH on activity of PLE-A on substrate 414a at 28 °C. The following buffer solutions (0.05 M) were used: citrate (○); phosphate (□); titrisol (■).*
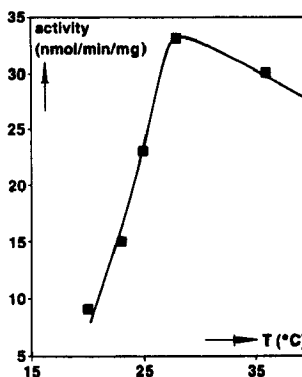
Figure 4.5:   *Effect of temperature on activity of PLE-A on substrate 414a at pH 8.*

When the hydrolysis of **414a** was allowed to proceed to 50% conversion both unreacted ester and acid were isolated in high yield and high e.e. (entry 3, Scheme 4.9). Enantiomerically pure (S)-**416a** could be obtained after one recrystallization from $CHCl_3$. Hydrolysis of the recovered optically active ester **414a** in KOH/MeOH, followed by recrystallization from $CHCl_3$ yielded enantiomerically pure (R)-**416a**.

Table 4.1   *Hydrolysis of α-Substituted Mandelates 414 by PLE*

entry[a]	substrate (racemic)	conv.(c)[b]	recovered ester	%chem. yield	%e.e.	product	%chem. yield	%e.e.	E[c]	rel. rate[d]
1 (S)	414a	0.23	(R)-414a	64	25	(S)-416a	19	86	17	0.3
2 (A)	414a	0.34	(R)-414a	52	49	(S)-416a	29	94	52	0.8
3 (A)	414a	0.51	(R)-414a	41	86	(S)-416a	44	83	30	1.0
4 (S)	414b	0.50	(R)-414b	43	77	(S)-416b	43	76	17	1.0
5 (A)	414b	0.52	(R)-414b	44	86	(S)-416b	39	80	25	0.8
6 (A)	414c	0.46	(R)-414c	53	64	(S)-416c	38	75	13	1.1
7 (A)	414d	0.46	(-)-414d	53	10	(+)-416d	30	12	10	0.2[e]
8 (S)	414e	0.45	(-)-414e	40	50	(+)-416e	42	62	7	0.2
9 (A)	414e	0.49	(-)-414e	41	40	(+)-416e	43	38	3	0.2
10 (A)	414f	0.42	(R)-414f	47	40	(S)-416f	41	51	5	0.8
11 (A)	414g	no hydrolysis								

a)   (S): PLE-S; (A): PLE-A
b)   Conversion: calculated from the e.e. values of acid and remaining ester: $c = e.e.(S)/e.e.(S)+e.e.(P)$.
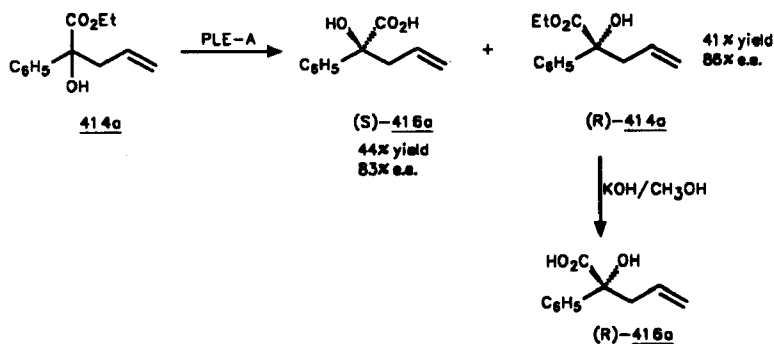c)   Enantiomeric ratio, calculated from the equation $E = \ln[1-c(1+e.e.(P))]/\ln[1-c(1-e.e.(P))]$.
d)   For PLE-A relative to **414a** = 1.0 (entry 3) and for PLE-S relative to **414b** = 1.0 (entry 4).
e)   Hydrolysis performed in the presence of 33% DMSO.

Scheme 4.9

Hydrolysis on a 25 mmol scale conveniently gives access to both enantiomers of α-allyl mandelic acid (**416a**) in quantities of about 1.5 gram. Scaling up these enzymatic resolutions to about 100 mmol should be possible without appreciable difficulties.

The decrease in E value with time (entries 2 and 3) 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 fact that PLE is known to consist of a mixture of isozymes. However, this can probably be ruled out according to findings reported by Jones.[26] Based on representative monocyclic and acyclic diester substrates the enantioselectivity of the isozyme components of commercially available PLE proved to be comparable, which suggests that PLE behaves as a single protein (see, however, Chapter V).

The hydrolysis of **414b** proceeded also with high enantioselectivity (entry 5, Scheme 4.10). In this case, both unhydrolyzed ester and acid were isolated in high enantiomeric excess. (S)-α-methallyl mandelic acid (**416b**) could be obtained in enantiomerically pure form after one recrystallization. The enantioselectivity for α-crotyl mandelate (**414c**, entry 6, Scheme 4.10), is significantly lower, although still acceptable for most synthetic purposes (E = 13). (S)-α-crotyl mandelic acid (**416c**) could be obtained after 46% of conversion with 75% e.e.

CO₂Et R₁ ... PLE-A → HO HO₂C R₁ ... + EtO₂C OH R₁

C₆H₅ ... OH

414b: R₁ = CH₃, R₂ = H
414c: R₁ = H, R₂ = CH₃

(S)-416b
44% yield, 86% e.e.
(S)-416c
38% yield, 75% e.e.

(R)-414b
39% yield, 80% e.e.
(R)-414c
53% yield, 64% e.e.
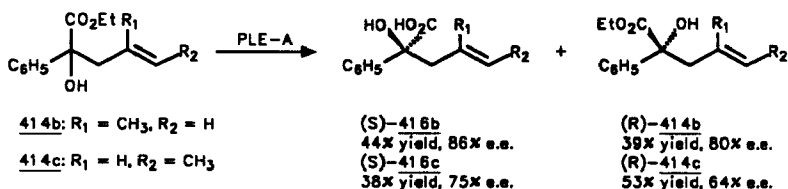
Scheme 4.10

A low E value was observed for hydrolysis of α-benzyl mandelate (**414e**, entries 8 and 9) and α-methyl mandelate (atrolactate **414f**, entry 10). Although the enantioselectivity of PLE-S for **414e** (entry 8) was higher than the selectivity of PLE-A, problems were encountered when PLE-S was used as the activity of this enzyme decreased rapidly during hydrolysis to almost zero (see Experimental Section). In all cases in which the absolute configuration of the products could be established, the (S)-enantiomer was formed preferentially. The rate of hydrolysis was comparable for **414a,b** and **c** (about 30 nmol/min/mg PLE-A), but was much lower for substrate **414e**. This is probably due to steric factors. Interestingly, when the hydroxyl group of **414a** is converted to a silyloxy group (**414g**) PLE does not accept the compound as a substrate (entry 11), 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. Finally, it was found that ester **414d**, which we had initially reported not to be hydrolyzed by PLE[27], does react, although very slowly and with low enantioselectivity, when DMSO was added as a cosolvent in the hydrolytic reaction (entry 7).

Preliminary results indicate that PLE may be used as well in the hydrolysis of another very interesting class of compounds: α-substituted α-amino acid esters. It was found that PLE-A catalyzed hydrolysis of α-allyl phenylglycine ethyl ester (which is the amine analogue of **414a**) proceeds with very high enantioselectivity (E = 87).[28] As not much is known about enzymatic amino ester hydrolysis as a means of obtaining optically active α-amino acids, this strategy deserves more attention in the future.

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The enzymatic hydrolyses of the substituted lactates were carried out in an aqueous buffer solution as described in Section 4.4.3

PLE exhibited very low or no enantioselectivity in the hydrolysis of the allylated lactic esters (Table 4.2). The best result was obtained in the hydrolysis of α-allyl lactate (**415a**) for which PLE showed a slight enantioselectivity for hydrolysis of the (R)-ester (E = 3, entry 1). 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 (**415g**, entry 6 and **415e**). However, protection of the hydroxyl group generally resulted in complete loss of stereospecificity. This may be attributed to the loss of a binding mode of the substrate via a polar interaction. Very recently it has been shown that α-benzyloxy ester **415h** (as methyl ester) can be hydrolyzed enantioselectively using Lipase OF.[29] However, with PLE we isolated only racemic products.

4.4.6   Determination of enantiomeric excesses and absolute configurations

The e.e.'s of the optically active acids **416**, **418** and esters **414**, **415** were determined by ^{1}H NMR analysis, involving derivatization with (S)-2-chloropropanoyl chloride, as described in Chapter III (Section 3.4).

The stereochemical configurations of **416a** and **418a** were assigned by correlation of optical rotations with known compounds.[30,31] The absolute configuration of (+)-**416a** was further confirmed by hydrogenation to the known acid (+)-α-n propyl mandelic acid (**419**, Scheme 4.11). Two values for the rotation of enantiomerically pure (+)-(S)-**419** have been reported in the literature ($[\alpha]_D$ +28.9° (c = 2, EtOH)[30] and $[\alpha]_D$ +21.6° (c = 2.5, EtOH).[32] We found that the higher one is correct (see Experimental Section).

Ozonolysis of (S)-α-allylmandelic acid (**416a**) followed by oxidative work-up and esterification gave a new enantiomerically pure α-hydroxy compound: (S)-2-hydroxy-2-phenylsuccinic acid diethylester (α-phenylmalic acid diethylester, (**420**), Scheme 4.11).

Table 4.2   *Hydrolysis of α-Substituted Lactates 415 by PLE*

entry[a]	substrate (racemic)	conv.(c)[b]	recovered ester	%chem. yield	%e.e.	product	%chem. yield	%e.e.	E[c]	rel. rate[d]
1 (S)	**415a**	0.29	(S)-**415a**	43	17	(R)-**418a**	27	42	3	156
2 (A)	**415a**	0.29	(S)-**415a**	55	9	(R)-**418a**	26	22	2	14.7
3 (S)	**415b**	0.50	(S)-**415b**	32	4	(R)-**418b**	49	4	1	585
4 (A)	**415b**	0.50	(S)-**415b**	32	2	(R)-**418b**	49	2	1	21.5
5 (A)	**415c**	0.47	(S)-**415c**	46	3	(R)-**418c**	46	3	1	2.0
6 (A)	**415g**	0.42	(S)-**415g**	57	3	(R)-**418c**	41	3	1	0.1
7 (A)	**415h**	0.50	**415h**	45	0	**418h**	45	0	-	7.0
8 (A)	**415d-f**	non-enantioselective hydrolysis								

a)   (S): PLE-S; (A): PLE-A.
b)   Conversion: calculated from the e.e. values of acid and remaining ester: $c = e.e.(S)/e.e.(S)+e.e.(P)$.
c)   Enantiomeric ratio, calculated from the equation $E = \ln[1-c(1+e.e.(P))]/\ln[1-c(1-e.e.(P))]$.
d)   Relative to **414a** = 1.0 (PLE A, Table 4.1: entry 3) and relative to **414b** = 1.0 (PLE S, Table 4.1: entry 4).
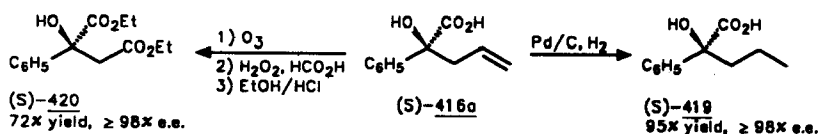
Scheme 4.11

The absolute configurations of the other hydrolyzed esters were determined by chemical correlation (Scheme 4.12). Thus, oxidative degradation and esterification of **416c** yielded compound **420** which is described above. Hydrogenation of **416b** afforded (S)-2-hydroxy-4-methyl-2-phenylpentanoic acid (**421**), the absolute configuration of which has been established.[32] The configurations of optically active lactic acid derivatives **418b** and **418c** were established by oxidation to citramalic acid (**422**)[33] and dimethyl citramalate (**423**),[34] respectively. The absolute configuration of **414f** is known from the literature,[35] whereas those of **416d** and **416e** remain undetermined.
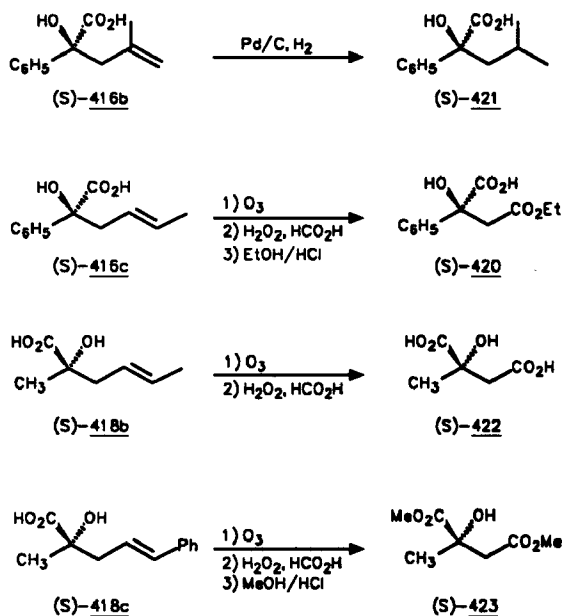
Scheme 4.12

Some preliminary experiments were performed on the epoxidation of α-allyl mandelic acid (Scheme 4.13). It was hoped that due to the stereogenic center in **414a** epoxidation might proceed with high diastereoselectivity. However, studies on epoxidation of racemic **414a** revealed that the presence of the stereogenic center did not influence the diastereomeric ratio of the products formed. Epoxidation with m-chloroperbenzoic acid (mCPBA), at room temperature as well as at -10 °C, yielded epoxide **424** in a 50:50 ratio of diastereomers.
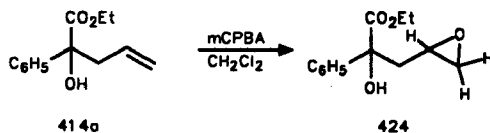
Scheme 4.13

It may be worthwile to perform the epoxidation of enantiomerically pure **416a** under 'racemic' Sharpless conditions[36] which may result in complexation of the substrate with the Ti metal to form a complex that gives diastereoselectivity in the subsequent epoxidation.

4.5 CONCLUDING REMARKS

We have shown that PLE can be succesfully used for resolution of sterically hindered racemic α-substituted α-hydroxy esters. Especially in the substituted mandelate series, PLE can hydrolyse a number of them with very high selectivity to afford enantiomerically pure α-substituted α-hydroxy acids (**416a** and **416b**), whereas other compounds can be obtained in optically enriched form. Both enantiomers are accessible using this type of kinetic resolution. Although PLE seemed to be quite aselective as far as chemical hydrolysis of α-hydroxy esters concerns, more discrimination is observed when the enantioselectivity of the hydrolysis is at issue. This is clearly demonstrated in the PLE catalyzed hydrolysis of the substituted lactates, where hydrolysis proceeded almost without stereoselectivity. This phenomenon in hydrolysis

deserves more attention and in Chapter V we will try to rationalize the observed stereochemical outcome in PLE catalyzed hydrolysis of α-substituted α-hydroxy esters.

4.6   *EXPERIMENTAL SECTION*

General remarks.

Dioxolanones **410** and **411** were synthesized as described in Chapter II, Section 2.9 (compounds **206** and **207**). All enzymatic reactions were performed with a Radiometer PHM 82 pH stat, equipped with a TTT 80 titrator and a ABU 80 autoburette. PLE-S (PLE-EC 3.1.1.1, suspension in 3.2 M $(NH_4)_2SO_2$, activity 100 units/mg protein) was a product of Sigma Chemical Company and PLE-A (4370 units/g) was obtained from Amano Pharmaceutical Co., Ltd.

For other general remarks see Section 2.9.

General procedure for the alkylation of the enolate from the dioxolanones 410 or 411 with electrophiles

A 0.10 mol run is described. A solution of **410** or **411** (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) and poured into a half-saturated ammonium chloride solution (150 mL) and diluted with ether (50 mL). 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 under 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-allyl-5-phenyl-1,3-dioxolan-4-one (412a)

From allyl bromide (25.4 g, 0.21 mol) and 28.8 g (0.15 mol) of **410**, 34.2 g (98% yield) of **412a** was obtained after Kugelrohr distillation at 110 °C (0.1 mm Hg) as a colorless oil; for spectroscopic data see Section 2.9 (compound **209**).

2,2-Dimethyl-5-(2-methylallyl)-5-phenyl-1,3-dioxolan-4-one (412b)

Alkylation of **410** with methallyl chloride failed. This compound was synthesizid as described in Section 2.9 (compound **215**).

2,2-Dimethyl-5-phenyl-5-(2-butenyl)-1,3-dioxolan-4-one (412c)

From crotyl bromide (1.2 g, 9.0 mmol, mixture of cis and trans isomers) and 1.16 g (6.0 mmol) of **410**, there was obtained 1.2 g (82%) of **412c** 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-(3-phenyl-allyl)-1,3-dioxolan-4-one (412d)

Cinnamyl bromide (10.5 g, 53 mmol) and 7.7 g **410** (40 mmol) gave after recrystallization from absolute ethanol 9.0 g (73% yield) of **412d**, mp 92-93 °C; for spectroscopic data see Section 2.9 (compound **214**).

2,2-Dimethyl-5-phenyl-5-benzyl-1,3-dioxolan-4-one (412e)

Benzyl bromide (1.54 g, 9.0 mmol) and 1.16 g (6.0 mmol) of **410** afforded after vacuum distillation 1.45 g (85%) of **412e** 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.

2,2,5-Trimethyl-5-allyl-1,3-dioxolan-4-one (413a)

Allyl bromide (14.0 g, 0.12 mol) and 12 g (0.09 mol) of **411** yielded 14.2 g (91% yield) **413a** after Kugelrohr distillation (60 °C, 15 mm Hg) as a colorless oil:

spectroscopic data were identical with those described in Section 2.9 (compound **222**).

2,2-Dimethyl-5-methyl-5-(2-butenyl)-1,3-dioxolan-4-one (413b)

Crotyl bromide (9.9 g, 73.3 mmol) and 8.0 g (61.5 mmol) of **411** yielded 10.0 g (88%) of **413b** 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; H, 8.85.

2,2-Dimethyl-5-methyl-5-(3'-phenylallyl)-1,3-dioxolan-4-one (413c)

From cinnamyl bromide (12.4 g, 62.9 mmol) and 6.3 g (48.8 mmol) of **411** was obtained 9.5 g (80 %) of **413c** after Kugelrohr distillation (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.

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$_2$SO$_4$ and concentrated under vacuum. Kugelrohr distillation of the residue afforded the desired ethyl ester.

Ethyl 2-hydroxy-2-phenyl-4-pentenoate (414a)

From **412a** (9.3 g, 0.04 mol) in absolute ethanol (100 mL) was isolated after Kugelrohr distillation at 79 °C (0.01 mm Hg) **414a** (8.4 g, 95% yield) as a colorless oil:

¹H NMR (CDCl₃, 300 MHz) δ 1.15 (t), 2.74, 2.95 (2 x dd, 2H), 3.64 (s, 1H), 3.11-4.31 (m, 2H), 5.09-5.17 (m, 2H), 5.71-5.85 (m, 1H), 7.24-7.61 (m, 5H); ¹³C NMR (CDCl₃) 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).

2-Hydroxy-2-phenyl-4-methyl-4-pentenoic-acid (416b)

To a solution of dioxolanone **412b** (0.9 g, 3.7 mmol) in MeOH (8 mL) was added a solution of KOH (1.0 g, 17.9 mmol) in H₂O (5 mL). After stirring overnight MeOH was removed under vacuum and H₂O (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 (Na₂SO₄) of the combined organic layers and evaporation of the solvent afforded **416b** (0.7 g, 92%) as a white crystalline compound. ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃) 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 (414b)

To a stirred suspension of 2-hydroxy-2-phenyl-4-methyl-4-pentenoic acid (**416b**, 0.61 g, 3.0 mmol) and Cs₂CO₃ (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 NH₄Cl solution (3 x 40 mL) and brine (40 mL). After drying (Na₂SO₄), the solvent was removed under vacuum. An oil pump (1 mm Hg) was used to remove the last trace of DMF. Kugelrohr distillation (90 °C, 0.05 mm Hg) of the residue afforded **414b** (0.65 g, 93%) as a colorless oil. ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃) 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-phenyl-4-hexenoate (414c)

A solution of **412c** (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 **414c** as a colorless oil. The product consisted of a 4:1 mixture of E,Z isomers. ^{1}H 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-2,5-diphenyl-4-pentenoate (414d)

Using a solution of **412d** (8.0 g, 0.026 mol) in absolute ethanol (100 mL) yielded after Kugelrohr distillation (150 °C, 0.03 mm Hg) 6.2 g (81% yield) of **414d** as a colorless oil: ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.25 (t, 3H), 2.88, 3.11 (2 x dd, 2H), 3.83 (s, 1H), 4.13-4.30 (m, 2H), 6.15-6.25 (m, 2H), 6.50 (d, 1H), 7.18-7.65 (m, 10H); ^{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).

Ethyl 2-hydroxy-2-phenyl-phenylpropanoate (414e)

Using a solution of **412e** (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 **414e** as a colorless viscous oil. ^{1}H 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-phenylpropionate (414f)

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 **414f** 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-hydroxy-2-methyl-4-pentenoate (415a)

From **413a** (11.0 g, 0.065 mol) in absolute ethanol (100 mL) was isolated after Kugelrohr distillation at 75 °C (35 mm Hg) 8.7 g (85% yield) of **415a** as a colorless oil: ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.21 (t, 3H), 1.25 (s, 3H), 2.36, 2.44 (2 x dd, 2H), 3.22 (s, 1H), 4.14-4.21 (m, 2H), 5.04-5.08 (m, 2H), 5.66-5.80 (m, 1H); ^{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-methyl-4-hexenoate (415b)

Starting from **413b** (6.8 g, 37.0 mmol) in absolute ethanol (100 mL), 5.5 g (85%) of **415b** 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. ^{1}H 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 (415c)

A solution of **413c** (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 **415c** as a colorless oil. ^{1}H 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).

Ethyl 2-benzyloxy-2-methyl-4-pentenoate (415h)

This compound was prepared analogously to a literature procedure.[29] To a

suspension of NaH (0.78 g, 55% in mineral oil, 18 mmol) in THF (5 mL) was added a solution of **415a** (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 brine, dried (Na$_2$SO$_4$) and concentrated under vacuum. Distillation (108-110 °C, 0.1 mm Hg) of the residue afforded **415h** (2.3 g, 62%) as a colorless oil; ^{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 414 and 415

A procedure analogous to that described for silylation with silyl perchlorates was followed.[37] A 10 mmol run is described. A solution of the α-hydroxy ester (10 mmol) and trimethylsilyl triflate (TMSOTf) or t-butyldimethylsilyl triflate (TBDMS-OTf) (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$_2$CO$_3$) and concentrated under vacuum. The residue was purified by Kugelrohr distillation.

Ethyl 2-trimethylsilyloxy-2-phenyl-4-pentenoate (414g)

From α-hydroxy ester **414a** (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 **414g** 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).

Ethyl 2-trimethylsilyloxy-2-methyl-4-pentenoate (415d)

α-Hydroxy ester **415a** (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 **415d** 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 (415e)

Starting from **415a** (1.6 g, 10 mmol), TBDMSOTf (4.0 g, 15 mmol) and pyridine (1.6 g, 20 mmol), 2.5 g (92%) of **415e** 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 (415f)

α-Hydroxy ester **415b** (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 **415f** 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-methyl-5-phenyl-4-pentenoate (415g)

Starting from **415e** (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 **415g** 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).

PLE catalyzed hydrolyses of α-hydroxy esters 414a-f and 415a-c,h

The following procedure is representative. PLE was added to a rapidly stirred suspension of α-hydroxy ester in 0.05 M KH$_2$PO$_4$/K$_2$HPO$_4$ 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 ethyl acetate (3 x 20 mL). The organic phase was partly concentrated under 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$_2$SO$_4$) 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$_2$SO$_4$ and removal of the solvent under vacuum gave the α-hydroxy acid. Specific details are given below.

(S)-2-Hydroxy-2-phenyl-4-pentenoic acid (416a)

(a) From α-hydroxy ester 414a (4.0 g, 18 mmol) in buffer (16 mL) with PLE-S (1.0 mL, 2860 units) was isolated after a conversion of 0.21, ester (R)-414a (2.57 g, 64% yield, 25% e.e.); $[\alpha]_{436}$ -1.7° (c = 1, EtOH) and α-hydroxy acid (S)-416a (0.67 g, 19% yield, 86% e.e.); $[\alpha]_{578}$ +16.7° (c = 1, EtOH).

(b) From 414a (7.3 g, 33 mmol) in buffer (30 mL) with PLE-A (470 mg) was isolated after 34% conversion, ester (R)-414a (3.81 g, 52% yield, 49% e.e.); $[\alpha]_{436}$ -3.4° (c = 1, EtOH) and α-hydroxy acid (S)-416a (1.87 g, 29% yield, 94% e.e.); $[\alpha]_{578}$ +18.2° (c = 1, EtOH).

(c) 414a (5.5 g, 25 mmol) in buffer (23 mL) with PLE-A (290 mg) yielded after

51% conversion, ester (R)-**414a** (2.25 g, 41% yield, 86% e.e.); $[\alpha]_{436}$ -5.9° (c = 1, EtOH) and α-hydroxy acid (S)-**416a** (2.10 g, 44% yield, 83% e.e.); $[\alpha]_{578}$ +16.1° (c = 1, EtOH). Crystallization from CHCl$_3$ afforded enantiomerically pure α-hydroxy acid (S)-**416a**, mp 128.6-128.8 °C. $[\alpha]_{578}$ +19.4° (c = 1, EtOH); $[\alpha]_D$ +27.6° (c = 1, CHCl$_3$). (lit[30] 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 x dd, 2H), 5.18 (m, 2H), 5.70-5.84 (m, 1H), 7.24-7.38 (m, 3H), 7.57-7.63 (m, 2H); ^{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 (416a)

Optically active ester (R)-**414a** (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$_3$OH (20 mL). After stirring overnight the solvent was removed under vacuum. The residu was dissolved in water (20 mL) and acidified with 6N 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 x 20 mL). Drying (Na$_2$SO$_4$) and removal of the solvent under vacuum gave (R)-**416a** (1.9 g, 99% yield, 86% e.e.); $[\alpha]_{578}$ -16.7° (c = 1, EtOH). Crystallization from CHCl$_3$ afforded enantiomerically pure α-hydroxy acid (R)-**416a**, mp 130.0-130.5°C; $[\alpha]_{578}$ -19.3° (c = 1, EtOH).

(S)-2-Hydroxy-2-phenyl-4-methyl-4-pentenoic acid (416b)

(a) Ester **414b** (0.69 g, 2.95 mmol) in buffer (40 mL) with PLE-S (0.4 mL, 1012 units) afforded after 50% conversion, ester (R)-**414b** (0.30 g, 43% yield, 77% e.e.), $[\alpha]_{578}$ -16.7° (c = 1, CHCl$_3$) and α-hydroxy acid (S)-**416b** (0.26 g, 43% yield, 76% e.e.), $[\alpha]_{578}$ +20.6° (c = 1, CHCl$_3$). Hydrolysis of the recovered ester (0.28 g, 1.20 mmol) in MeOH/KOH gave (R)-**416b** (0.23 g, 93% yield, 77% e.e.), $[\alpha]_{578}$ -20.7° (c = 1, CHCl$_3$) as a white solid.

(b) From ester **414b** (0.50 g, 2.1 mmol) in buffer (35 mL) with PLE-A (70 mg) was obtained after 52% conversion, ester (R)-**414b** (0.22 g, 44% yield, 86% e.e.), $[\alpha]_{578}$ -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)-**416b** (0.17 g, 39% yield, 80% e.e.), $[\alpha]_{578}$ +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)-**416** (0.11g, 83% yield, 86% e.e.) 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 **416b**. 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)-2-Hydroxy-2-phenyl-4-hexenoic acid (416c)

Ester **414c** (0.85 g, 3.6 mmol) in buffer (45 mL) with PLE-A (50 mg) yielded after 46% conversion, (R)-**414c** (0.45 g, 53% yield, 64% e.e.), $[\alpha]_{578}$ -3.4° (c = 1, EtOH) and α-hydroxy acid (S)-**416c** (0.28 g, 38% yield, 75% e.e.) 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-2,5-diphenyl-4-pentenoic acid (416d)

(a) To a suspension of ester **414d** (3.2 g, 13.7 mmol) in buffer (13 ml) was added PLE-A (162 mg). No activity was measured after stirring for 20 h. The same lack of activity was observed when PLE-S (1.0 mL) was used.

(b) A solution of ester **414d** (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, (-)-**414d** (0.31 g, 53% yield, 10% e.e.), $[\alpha]_{578}$ -10.7° (c = 1, CHCl$_3$) and α-hydroxy acid (+)-**416d** (0.16 g, 30% yield, 12% e.e.), $[\alpha]_{578}$ +6.3° (c 0.8, CHCl$_3$). ^{1}H NMR (CD$_3$OD, 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$_3$OD) 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 calcula-

ted for $C_{17}H_{16}O_3$: 268.109. Found: 268.110.

(+)-2-Hydroxy-2,3-diphenylpropanoic acid (416e)

(a) Ester **414e** (0.44 g, 1.6 mmol) in buffer (30 mL) with PLE-A (50 mg) afforded after 49% conversion, (-)-**414e** (0.18 g, 41% yield, 40% e.e.), $[\alpha]_{578}$ -16.4° (*c* = 1, CHCl₃) and α-hydroxy acid (+)-**416e** (0.17 g, 43% yield, 38% e.e.) as a slightly colored solid, $[\alpha]_{578}$ +15.5° (*c* = 1, CHCl₃).

(b) To ester **414e** (1.50 g, 5.6 mmol) in buffer (45 mL) was added PLE-S (0.5 mL, 1265 units). After about 20% conversion the activity of the enzym decreased rapidly and additonal PLE-S (0.3 mL, 760 units) was added to the reaction mixture. The hydrolysis started to continue until a conversion of about 35% was reached (after 15 h) after which the activity of the enzym dropped to almost zero. A conversion of 45% was reached after 90 h. After the usual workup, there was isolated (-) **414e** (0.60 g, 40% yield, 50% e.e.), $[\alpha]_{578}$ -21.6° (*c* = 1, CHCl₃) and α-hydroxy acid (+)-**416e** (0,56 g, 41% yield, 62% e.e.), $[\alpha]_{578}$ +23.8° (*c* = 1, CHCl₃) as a white solid. Recrystallization of the acid from CHCl₃ resulted in enantiomeric enrichment of the enantiomer which remained in solution. It was not possible to obtain enantiomerically pure material in this way. ¹H NMR (CDCl₃, 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). ¹³C NMR (CDCl₃) 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)-Atrolactic acid (416f)

From ester **414f** (1.0 g, 5.2 mmol) in buffer (40 mL) with PLE-A (90 mg) was isolated after a conversion of 42% (R)-**414f** (0.48g, 47% yield, 40% e.e.); $[\alpha]_D$ -9.4° (neat). [lit[38] $[\alpha]_D$ -23.7° (neat) (R)] and (S)-Atrolactic acid (**416f**, 0.35 g, 41% yield, 51% e.e.); $[\alpha]_D$ +19.1° (*c* = 1, EtOH). [lit[35] $[\alpha]_D$ +37.7 (EtOH) (S)]; ¹H NMR (C₆D₆, 300 MHz) δ 1.71 (s, 3H), 6.34-6.94 (br, 2H), 7.04-7.62 (m, 5H). ¹³C NMR (C₆D₆) 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 $C_9H_{10}O_3$. 1/2 H₂O: C: 61.88; H, 6.35. Found: C: 61.99; H: 6.40.

(R)-2-Hydroxy-2-methyl-4-pentenoic acid (418a)

(a) 415a (2.68 g, 17.0 mmol) in buffer (13 mL) with PLE-S (1.0 ml, 2860 units) gave after 29% conversion, (S)-415a (1.15 g, 43% yield, 17% e.e.); $[\alpha]_{578}$ +3.3° (c = 1, EtOH) and α-hydroxy acid (R)-418a (0.59 g, 27% yield, 42% e.e.) as a colorless oil; $[\alpha]_D$ -5.2° (c = 1, EtOH).

(b) From ester 415a (3.16 g, 20.0 mmol) in buffer (15 mL) with PLE-A (166 mg) was isolated after 29% conversion, (S)-415a (1.74 g, 55% yield, 9% e.e.); $[\alpha]_{578}$ +1.7° (c = 1, EtOH) and α-hydroxy acid (R)-418a (0.68 g, 26% yield, 22% e.e.) as a slightly colored oil, which was purified by Kugelrohr distillation (70 °C, 0.1 mm Hg): $[\alpha]_D$ -2.5° (c = 1, EtOH). [lit[30] $[\alpha]^{22}_D$ +11.3° (c = 1, EtOH) (S)]; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.50 (s, 3H), 2.44-2.58 (2 x dd, 2H), 5.19 (m, 2H), 5.75-5.90 (m, 1H), 6.6-8.0 (br, 2H); ^{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$_6$H$_{10}$O$_3$: C, 55.37; H, 7.75. Found: C, 55.22; H, 7.82.

(S)-2-Hydroxy-2-methyl-4-hexenoic acid (418b)

(a) Ester 415b (1.83 g, 10.6 mmol) in buffer (12 mL) with PLE-S (0.4 mL, 1012 units) afforded after 49% conversion, (R)-415b (0.58 g, 32% yield, 4% e.e.), $[\alpha]_{578}$ +5.0° (c = 1, EtOH), and (S)-418b (0.75 g, 49% yield, 4% e.e.) as a colorless oil: $[\alpha]_{578}$ -5.9° (c = 1, EtOH).

(b) Ester 415b (2.6 g, 15.1 mmol) in buffer (15 mL) with PLE-A (260 mg) gave after 49% conversion, (R)-415b (0.83 g, 32% yield, 2% e.e.), $[\alpha]_{578}$ +2.4° (c = 1, EtOH), and α-hydroxy acid (S)-418b (1.10 g, 49% yield, 2% e.e.) 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$_7$H$_{12}$O$_3$: 144.078. Found: 144.079.

(S)-2-Hydroxy-2-methyl-5-phenyl-4-pentenoic acid (418c)

Ester 415c (2.50 g, 10.7 mmol) in buffer (14 mL) with PLE-A (450 mg) gave after 47% conversion, ester (R)-415c (1.15 g, 46% yield, 3% e.e.), $[\alpha]_{578}$ +2.4° (c = 1,

EtOH), and acid (S)-618c (1.0 g, 46% yield, 3% e.e.), $[\alpha]_{578}$ -6.3° (c = 1, CHCl$_3$) as a slightly colored solid. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.66 (s, 3H), 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-Benzyloxy-2-methyl-4-pentenoic acid (418h)

2-Benzyloxy ester 415h (1.0 g, 4.0 mmol) in buffer (40 mL) with PLE-A (65 mg) gave after 50% conversion 418h (0.40 g, 45% yield, 0% e.e.) and ester 415h (0.45 g, 45% yield, 0% e.e.). ^{1}H 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 (t), 127.61 (d), 127.76 (d), 128.34 (d), 131.64 (d).

PLE catalyzed hydrolyses of the silylated α-hydroxy esters 414g, 415d-g

The general procedure described above was followed, with this difference that a 0.05 M KH$_2$PO$_4$/K$_2$HPO$_4$ 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-hydroxy-2-phenyl-4-pentenoic ester 414g

No activity of PLE-A was observed for this compound.

Silylated-2-hydroxy-2-methyl-4-pentenoic esters 415d-f

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.

(R)-2-Hydroxy-2-methyl-5-phenyl-4-pentenoic acid (418c)

Sililoxy ester 415g (0.75 g, 2.5 mmol) in buffer (40 mL) with PLE-A (100 mg) gave after 42% conversion, α-hydroxy acid (R)-418c (0.21 g, 41% yield, 3% e.e.) as a

slightly colored solid; $[\alpha]_{578}$ +6.3° (c = 1, EtOH) and ester **415g** (partly desilylated). The ester was stirred in a dry ether/HCl solution for 15 min. and after concentration under vacuum (S)-**415c** (0.33 g, 57% yield, 3% e.e.) was isolated as a colorless oil; $[\alpha]_{578}$ -2.2° (c = 1, EtOH).

(S)-2-Hydroxy-2-phenyl-pentanoic acid (419)

A mixture of (S)-2-hydroxy-2-phenyl-pentenoic acid (**416a**, 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 H_2 pressure, for 20 h. After filtration, to remove the catalyst, the solvent was removed under vacuum, to give (S)-**419** as a white solid (0.46 g, 95% yield, >98% e.e.): mp 100-101 °C; $[\alpha]_D$ +29.3° (c = 1, EtOH). (lit[30] mp 101-102 °C; $[\alpha]^{22}_D$ +28.9° (c = 2, EtOH), [32]mp 97-99 °C; $[\alpha]^{25}_D$ +21.6° (c = 2.5, EtOH)); ^{1}H NMR (CDCl$_3$, 300 MHz) δ 0.83 (t, 3H), 1.18-1.50 (m, 2H), 1.90-2.20 (m, 2H), 7.23 (m, 3H), 7.53 (s, 2H), 6.2-8.8 (br, 2H). ^{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.

(S)-Diethyl 2-hydroxy-2-phenylbutanedioate (420)

A solution of enantiomerically pure (S)-2-hydroxy-2-phenyl-4-pentenoic acid (**416a**, 0.45 g, 2.34 mmol) in methanol (17 mL) and CH_2Cl_2 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 under 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 under 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$_2$SO$_4$) and evaporation of the ether layers under 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)-420 (0.45 g, 72% yield, >98% e.e.) was obtained white crystalline material, mp 65.9-66.4 °C; [α]$_{578}$ -39.7° (c = 1, CHCl$_3$). ^{1}H 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.

(S)-2-Hydroxy-4-methyl-2-phenylpentanoic acid (421)

A mixture of 416b (0.12 g, 0.58 mmol, [α]$_{578}$ +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 under vacuum, to give (S)-421 as a white solid (0.10 g, 83% yield, 75% e.e.) as a white solid: [α]$_D$ +15.0° (c = 1, EtOH). (lit[32] [α]$_D$ +20.0° (c = 2, EtOH). ^{1}H 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-phenyl-4-hexenoic acid (416c)

A solution of optically active acid 416c (0.24 g, 1.17 mmol, [α]$_{578}$ +14.8° (c = 1, CHCl$_3$) in MeOH (10 mL) and CH$_2$Cl$_2$ (4 mL) was ozonolyzed as described for 420. The crude acid was esterified in EtOH/HCl to yield after Kugelrohr distillation (125 °C, 0.03 mm Hg) (S)-420 (0.18 g, 58% yield, 75% e.e.) as a colorless oil, which crystallized on standing, [α]$_{578}$ -29.8° (c = 1, CHCl$_3$).

Ozonolysis of (-)-2-hydroxy-2-methyl-4-hexenoic acid (418b)

Ozonolysis of a solution of 418b (0.46 g, 3.2 mmol, [α]$_{578}$ -2.9° (c = 1, EtOH) in MeOH (18 mL) and CH$_2$Cl$_2$ (9 mL) as described for 420 gave after workup and

evaporation of the formic acid and acetic acid formed, citramalic acid (**422**, 0.33 g, 69% yield, 2% e.e. (S)) as a slightly yellow syrup, $[\alpha]_D$ +0.51° (c = 3, H_2O); lit[33] $[\alpha]_D$ +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 (418c)

Optically active α-hydroxy-acid **418c** (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 (**423**, 0.13 g, 55% yield, 3% e.e., (S)) as a colorless oil, $[\alpha]_{578}$ + 0.36° (c = 1, CH_3OH); lit[34] $[\alpha]_{578}$ + 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

Ethyl 2-hydroxy-2-phenyl-4,5-epoxypentanoate (424)

To a solution of ester **414a** (0.55 g, 2.5 mmol) in CH_2Cl_2 (3 mL) was added at room temperature a mixture of 0.70 g of an mCPBA solution (70-75 % in water) and CH_2Cl_2 (3 mL). After stirring for 3 h a precipitate was formed. This was removed by filtration after stirring overnight. In order to destroy the excess of peroxides, dimethyl-sulfide was added to the filtrate and the solution was subsequently washed with a 5% aqueous $NaHCO_3$ solution and water and the organic layer was separated. Drying of the organic layer over Na_2SO_4, followed by evaporation of the solvent afforded **424** (0.54 g, 92%) as a slightly yellow oil. Based on the NMR spectra the ratio of diastereomers formed is 50:50. The same ratio was found when the reaction was performed at

-10 °C. ^{1}H NMR (CDCl$_3$, 300 MHz) δ 1.17 (t, 3H), 2.21-2.73 (m, 4H), 3.00-3.10 (m, 1H), 3.86, 4.02 (2 x s, 1H), 4.18-4.32 (m, 2H), 7.28-7.60 (m, 5H). ^{13}C NMR (CDCl$_3$) δ 13.84, 13.90 (q), 42.10, 42.20 (t), 46.77, 46.89 (t), 48.21, 48.57 (d), 62.46, 62.55 (t), 77.29, 77.39 (s), 125.03, 125.12 (d), 127.71, 127.81 (d), 128.17 (d), 141.05, 141.30 (s), 174.17, 174.38 (d).

Enantiomeric excess determination

The e.e. 's of the optically active esters and acids were determined by derivatization with (S)-2-chloropropanoyl chloride followed by 300 MHz ^{1}H NMR analysis (see Chapter III, Section 3.4). The racemic esters and acids (obtained after basic hydrolysis of the substituted dioxolanones) were used as reference standards.

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