Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 1401–1412

TETRAHEDRON: *ASYMMETRY*

Lipase-catalyzed resolution of esters of 4-chloro-3-hydroxybutanoic acid: effects of the alkoxy group and solvent on the enantiomeric ratio

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Received 11 March 1999; accepted 1 April 1999

Abstract

Various lipases have been investigated for their potential use as catalysts for the resolution of esters of 4-chloro-3-hydroxybutanoic acid via transesterification in organic solvents. *Rhizomucor miehei* lipase was found to be the most efficient lipase, with the enantiomeric ratio (*E*) being dependent upon of the nature of the alkoxy group of the ester and the resolution medium. Higher *E*-values were obtained when transesterification was performed in benzene or carbon tetrachloride than was the case in hexane. In mixtures of benzene and hexane the trend in *E*-values followed a linear relationship.

1. Introduction

The (*R*)-enantiomers of alkyl 4-chloro-3-hydroxybutanoates are valuable synthons for the production of pharmaceuticals, such as L-carnitine[1,2] and (*R*)-4-amino-3-hydroxybutanoic acid (GABOB). Methods for producing enantiomerically enriched alkyl 4-chloro-3-hydroxybutanoates include reduction of the corresponding 3-oxobutanoates using either microorganisms[3–7] or chiral diphosphine ruthenium catalysts.[8–10] However, lipase-catalyzed resolutions have also been reported.[7,11–13] In contrast to asymmetric synthesis, resolution may give access to both enantiomers in the same process, and may give a high enantiomeric excess (*ee*) of the two products even if the *E*-value is not excellent. The drawback, however, is that the maximum yield is only 50%.

One of the benefits of using low-water reaction systems is the shift of equilibrium, such that hydrolysis is avoided. Since the substrates in the present work are bifunctional, it was essential to exclude hydrolysis and/or alcoholysis of the starting ester and direct acylation of the C-3 hydroxy group. For lipase-catalyzed kinetic resolutions in organic media, the choice of solvent often affects the enantiomeric ratio.[14,15] In this

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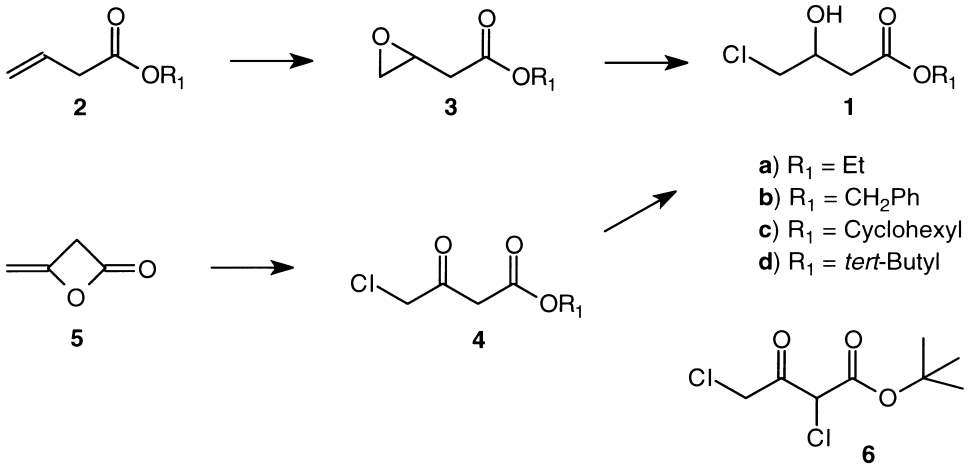
0957-4166/99/$ - see front matter
PII: S0957-4166(99)00126-3

study, the effect of the solvent on the *E*-value is investigated using *Rhizomucor miehei* lipase (RML), one of the more popular biocatalysts for performing kinetic resolutions.[16] RML has been shown to be active at water activities as low as 0.0001[17] and it is relatively stable toward acetaldehyde.[18] The solvent effect on *E*-values in the resolution of 1-(2-furyl) ethanol[19] and 1-phenylethanol,[16] using vinyl acetate as an acyl donor, has been studied previously. Dichloromethane was found to be the best solvent for ring opening of 4-substituted 2-phenyloxazolin-5-one with 1-butanol as the acyl acceptor.[20]

2. Results and discussion

Racemic substrates **1a**, **1b**, **1c** and **1d** were synthesized by various routes (Scheme 1). Esterification of 3-butenoic acid (**2**, R_1=H) with benzyl alcohol or cyclohexanol gave **2b** and **2c**, respectively. Subsequent epoxidation gave **3b** and **3c** which, on further hydrochlorination, gave **1b** and **1c**. Since ethyl 4-chloro-3-oxobutanoate **4a** is commercially available, **1a** was easily obtained by sodium borohydride reduction. The *tert*-butyl ester **1d** was not obtainable by these methods, however, treatment of diketene **5** with chlorine gas in CCl_4 followed by addition of *tert*-butyl alcohol/pyridine gave **4d**.[21] The reaction gave several side-products, the most abundant being *tert*-butyl 2,4-dichloro-3-oxobutanoate **6** as revealed by GC–MS and NMR.

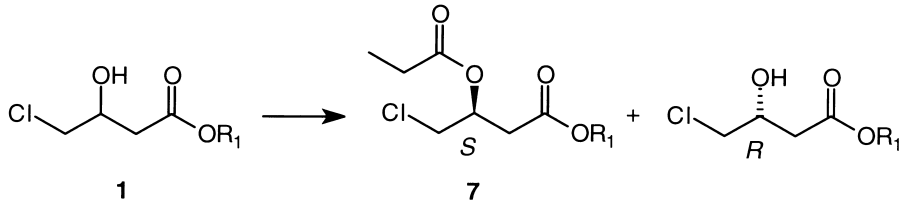


Scheme 1.

The yield of **4d** was increased by adding chlorine to diketene, in contrast to the reverse order of addition. The reduction of **4d** to yield **1d** using $NaBH_4$ gave 10–16% of *tert*-butyl 3,4-epoxybutanoate **3d** and minor amounts of *tert*-butyl 3-hydroxybutanoate (co-elution, GLC).

Since enzymatic reactions usually proceed with higher enantiomeric ratios when the site of reaction is close to the stereocenter, resolutions of 4-chloro-3-hydroxybutanoates **1** were chosen to be performed through transesterification of the secondary hydroxy group (Scheme 2).

In an enzyme-catalyzed transesterification reaction, an acyl donor is needed in order to acylate (esterify) the enzyme. Since the present substrates are bifunctional, hydroxy esters, the ester group of the substrate is also a possible acyl donor. This may lead to formation of side-products and thus lower the yield. This problem was addressed firstly by letting ethyl 3-hydroxybutanoate react with itself using lipase B from *Candida antarctica* (CALB) as a catalyst in the absence of an acyl donor. After 24 h, 25% of the substrate had reacted to form dimers indicating that the substrate may indeed function as an acyl donor. Therefore, it was judged necessary to use an acyl donor which is a much better acylating agent



Scheme 2.

than the substrate to be resolved. Vinyl alkanoates are known to be rapid acylating agents, and reactions with these acyl donors did not lead to any detectable dimerization. When 2-chloro-, 2,2,2-trichloro or 2,2,2-trifluoro butanoates were used as acyl donors, small amounts of dimers were formed. Dimerization of **1a**, **1b** and **1c** was investigated in the absence of an acyl donor in a benzene solution using RML as the catalyst. Substrates **1a** and **1b** dimerized quite readily, converting by 13 and 16%, respectively, after 48 h, while **1c** only dimerized to 3% during the same time. The reaction was particularly simple to monitor for **1b** since it liberated benzyl alcohol. The dimerization reaction was found to reduce the *ee* of the resolution. A slight preference for the (*R*)-enantiomer was observed in the dimerization process as opposed to that observed using a regular acyl donor. In the resolution of **1b** using an acyl donor, no benzyl alcohol was detected in either of the solvents. Since **1b** dimerizes most readily, it is likely that no dimerization took place for **1a**–**1d** under the same conditions. Thus, the different *E*-values in the different solvents cannot be attributed to different rates of dimerization in the different solvents.

Initially, several lipases were investigated for the resolution of ethyl 4-chloro-3-hydroxybutanoate **1a** and phenylmethyl 4-chloro-3-hydroxybutanoate **1b** in hexane using vinyl acetate as the acyl donor (Table 1).

Table 1
Resolution of substrates **1a** and **1b** using different lipases in hexane; acyl donor vinyl acetate

Lipase	*E*, **1a**	*E*, **1b**	Faster reacting enantiomer
CALB	5	4	S
RML	8	20	S
Aspergillus niger lipase	4	25	S
Lipase PS Amano	4	12	S
Candida rugosa lipase	nd	4	R
Chromobacterium viscosum	nd	1	-
Lipase GC, Amano	nd	2	R

nd = not determined

All the lipases tested gave low *E*-values for the resolution of **1a**. RML gave the best result with an *E*-value of 8. For the resolution of **1b** the *E*-values generally increased compared to the resolution of **1a**, for which RML and *Aspergillus* lipase gave the highest *E*-values. CALB showed almost the same *E*-value for both substrates.

Based on these results it was decided to focus on RML as the catalyst. Resolutions of **1a** were performed in different solvents using vinyl acetate as acyl donor. The results are summarized in Table 2.

The reaction medium affected the enantiomeric ratio as well as the rate of reaction. The more hydrophobic solvents gave a higher rate of reaction. An increase in the *E*-value was observed on going from hexane to *tert*-BuOMe, toluene, benzene and CCl_4. In THF, dioxane and $CHCl_3$, the reaction rates were low, probably due to inactivation of the enzyme.[22] *E*-values for the resolution of **1a** in these solvents

Table 2
Resolution of **1a** in different solvents using vinyl acetate as the acyl donor and RML as the catalyst

Solvent	*E*	Solvent	*E*
t.-BuOMe	17	Dioxane	5
Benzene	18	THF	5
Toluene	18	Hexane	8
CCl_4	18	$CHCl_3$	12

were not higher than for reactions in more hydrophobic solvents, and they were, therefore, excluded from this study.

When different acylating agents were tested, it became evident that using vinyl propanoate, instead of vinyl acetate as the acyl donor, increased the rate of reaction considerably. Hence, vinyl propanoate was chosen as the acyl donor for further studies.

Resolutions of **1a**, **1b**, **1c** and **1d** by transesterification using vinyl propanoate as the acyl donor in various solvents were performed using RML as the catalyst (Table 3). Preparative resolutions were performed in order to determine the absolute configuration of the enantiomers. The (*R*)-enantiomers of **1b**, **1c** and **1d**, were isolated in high enantiomeric purity, *ee* >96–99%.

Table 3
E-values obtained during resolution of hydroxyesters **1a**, **1b**, **1c** and **1d** using *Rhizomucor miehei* lipase, RML, and vinyl propanoate or lipase B from *Candida antarctica*, CALB, and vinyl acetate in different solvents. Since ee_p-values were not easily determined, the *E*-values of **1d** were determined from ee_s-values and the degree of conversion using internal standards. Hence these *E*-values are not as accurate as the others

	Rhizomucor miehei lipase vinyl propanoate					CALB vinyl acetate
	Hexane	Benzene	Toluene	CCl_4	*t*.-BuOMe	Hexane
1a	5	15	16	20	20	5
1b	14	28	28	33	17	4
1c	14	74	34	77	20	1.3
1d	>100	>100	>100	>100	>100	33

2.1. Effects of solvent on the enantiomeric ratio

From Table 3 it is evident that there is an effect of solvent on the *E*-value. Reactions in hexane gave, for **1a**–**1c**, lower enantiomeric ratios than reactions in other solvents. In order to investigate the nature of this effect, transesterifications were performed in mixtures of hexane in benzene (0, 50, 80, 95 and 100%), using ethyl 4-chloro-3-hydroxybutanoate **1a** as the substrate. Fig. 1 shows ee_s and ee_p versus the degree of conversion for the resolution of **1a**. Each set of curves represents one *E*-value obtained for each of the three solvent systems: benzene (*E*=15), hexane:benzene, 1:1 (*E*=10), and hexane (*E*=5). The *E*-value decreased linearly with the increased amount of hexane (Fig. 2). The same effect was found for the resolution of phenylmethyl 4-chloro-3-hydroxybutanoate **1b**. The observed effect of solvent on the enantiomeric ratio is obviously not a general trend for this lipase. Hexane was one of the better solvents

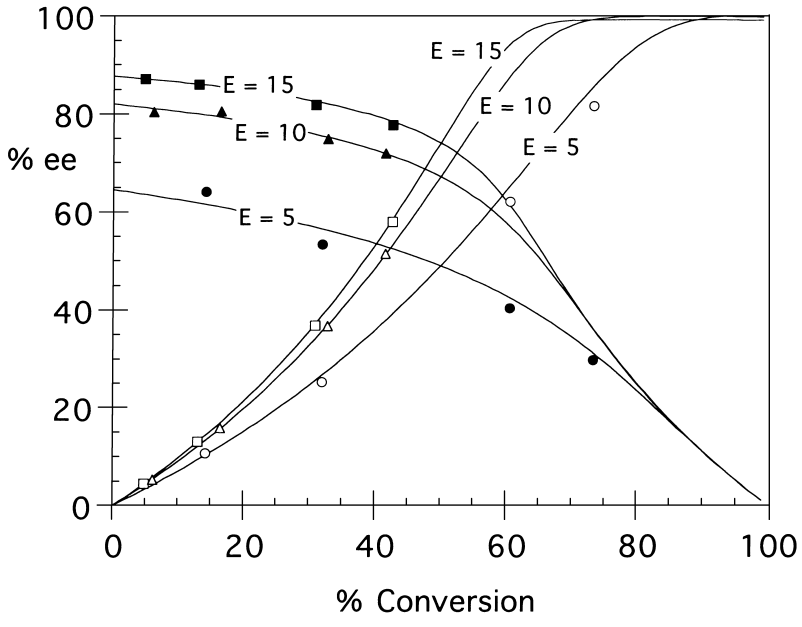


Figure 1. Effect of solvent on the enantiomeric ratio in resolution of **1a**. Plot of ee_s and ee_p versus degree of conversion. Squares=benzene; triangles=benzene:hexane 1:1; circles=hexane. Filled symbols=product fraction; open symbols=substrate fraction

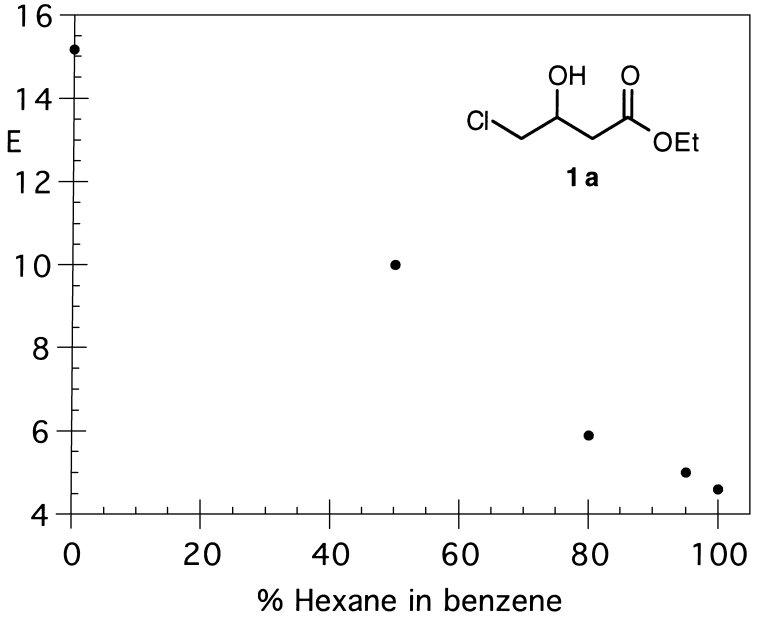


Figure 2. Enantiomeric ratio (*E*) obtained in various mixtures of hexane in benzene (0, 50, 80, 95 and 100%, respectively) in resolution of **1a**

tested in terms of *E*-values for the resolution of 1-(2-furyl)ethanol, while benzene, toluene and CCl_4 gave lower *E*-values.[19]

Various explanations have been offered for the effect of solvent on the *E*-value, such as change of enzyme conformation[23] or the fact that solvent molecules have different affinities for sites inside the

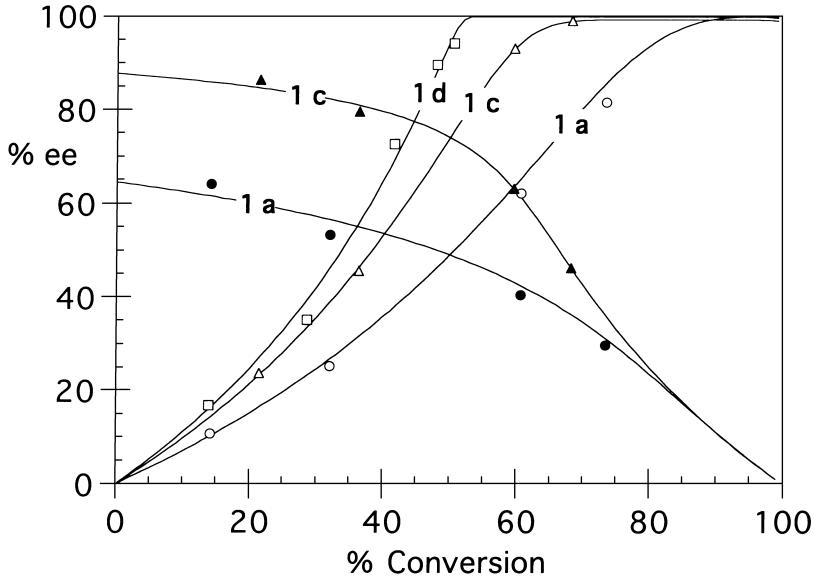


Figure 3. Resolution of **1a**=circles, **1c**=triangles and **1d**=squares, using RML, vinyl propanoate and hexane. Filled symbols are product fraction while empty symbols are substrate fraction

active site.[24] Since there is a linear change of the *E*-value with increasing amounts of hexane in benzene, we think that this indicates a more general solvation effect of the substrate.

The rate of reaction depended strongly on the nature of the solvent. Reactions in hexane gave high rates compared to reactions performed in other solvents. Moreover, for resolution in solvents other than hexane, the rate of reaction after ca. 40% conversion was extremely slow when performed on a small scale. This problem was less serious when the resolution was performed on a preparative scale, in a reaction vessel with a large head space.

2.2. Effect of the alkoxy group

The reason for varying the alkyl group of the ester was to improve the enantiomeric ratio by introduction of a more bulky group, thereby increasing enantiomeric discrimination. Moreover, the competing dimerization would be slowed down and, in the case of *tert*-butyl ester, be negligible.

The effect of the alkoxy group on the *E*-value can be read from Table 3. As can be seen, the effect is solvent-dependent, but *tert*-butyl gave the highest *E*-value independent of the solvent used. For resolution of **1a**–**1c**, reactions in benzene and CCl_4 gave an increase in *E*-value going from **1a** → **1b** → **1c**, while in *tert*-BuOMe the *E*-value seemed independent of the three alkoxy groups. For resolutions in hexane and toluene an increase in *E*-value was observed going from **1a** to **1b**, while no clear difference in *E*-value was observed for the resolutions of **1b** and **1c** in these solvents. The plot of *ee* versus conversion is shown in Fig. 3 for the resolution of **1a**, **1c** and **1d** in hexane. The resolutions of **1a** and **1d** in hexane were further analyzed by plotting the remaining percentage of the individual substrate enantiomers versus time (see Fig. 4). The (*R*)-enantiomers appear to react equally fast, and hence the increase in *E*-values, when going from **1a** to **1d**, is caused by a slower rate of reaction of the (*S*)-enantiomer of **1d**.

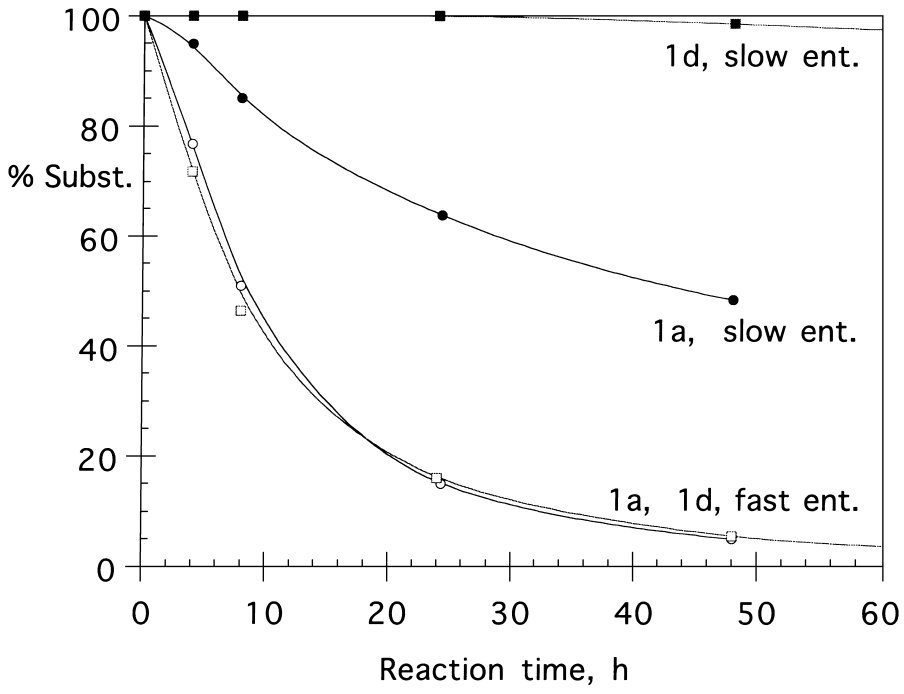


Figure 4. Percent remaining of each enantiomer plotted against reaction time in resolution of **1a**=circles and **1d**=squares. Filled symbols are slow reacting enantiomers and empty symbols are fast reacting enantiomers

3. Experimental

3.1. General

3-Butenoic acid, ethyl (*R*)-4-chloro-3-hydroxybutanoate, ethyl 4-chloro-3-oxobutanoate, *m*-chloroperoxybenzoic acid, DCC, 4-pyrrolidinopyridine, diketene (stab. w. cupric sulfate) and cyclohexanol were purchased from Fluka; vinyl propanoate and benzylalcohol were from Aldrich. The pet. ether had a boiling range of 60–80°C. Column chromatography was performed using silica gel 60 from Fluka. Immobilized *Rhizomucor miehei* lipase (Lipozyme IM, Novo-Nordisk) had specific activity 60 B.i.u./g.

3.2. Analyses

Optical rotation was determined using an AA-10 automatic polarimeter from Optical Activity Ltd, and concentrations are given in g/100 mL. Chiral analyses were performed using CP-Chiracil-Dex CB columns from Chrompack, and a G-TA column from Astec. **1a**–**1c**: for detailed description see the literature.[25] For **1d**: derivatized to its TMSi-derivative and analyzed on CP-Chiracil-Dex CB, DF: 0.25 mm, 8 psi, split: 85 mL/min, temp. prog.: 110–124, 1°C/min, t_1: 10.70, t_2: 11.04, R_s: 2.3. NMR spectroscopy was performed in $CDCl_3$ solutions, using Bruker DPX 300 and 400 instruments, operating at 300 and 400 MHz for ^1H and 75 and 100 MHz for ^{13}C, respectively. Chemical shifts are in ppm rel. to TMS and coupling constants in hertz. Enantiomeric ratios, E and equilibrium constants, K_{eq} were calculated using the computer program *E and K calculator version 2.03*.[26,27]

3.3. Transesterifications, small scale

Substrate (1.31×10^{-4} mol) was dissolved in solvent (3 mL) and vinyl propanoate (5 equiv.) was added. The reaction was started by adding immobilized *Rhizomucor miehei* lipase (20 mg) to the reaction mixture. The reactions were performed in a shaker incubator at 30°C. Chiral GLC analysis gave ee_s- and ee_p-values from which conversion, c, was calculated, $c=ee_s/(ee_s+ee_p)$. For substrate **1a**, the conversion was also measured using *n*-hexadecane as an internal standard, giving the same values as obtained using the above-mentioned method. For substrate **1d**, the conversion was measured using tetradecane as the internal standard. In control experiments without enzyme, no acylation was observed using vinyl propanoate as the acyl donor.

3.4. Dimerization

The degree of dimerization was detected by monitoring the decrease of the starting substrate, using tetradecane as internal standard.

3.5. Ethyl 4-chloro-3-hydroxybutanoate *1a*

Ethyl 4-chloro-3-oxobutanoate **4a** (4.73 g, 26.1 mmol) was dissolved in EtOH (50 mL) and cooled to 0°C and $NaBH_4$ (0.5 equiv.) was added. The reaction mixture was stirred for 1.5 h at room temperature and HCl (0.1 M) was added. When the evolution of H_2 had ceased, EtOH was removed in vacuo. The concentrated reaction mixture was extracted with Et_2O (4×75 mL), the organic fraction washed twice with saline water (saturated), dried over $MgSO_4$ and concentrated in vacuo. To remove yellow impurities the substrate was purified by column chromatography using CH_2Cl_2:acetone, 20:1, giving 3.41 g (78%). ^{1}H NMR, ethoxy group: 1.28 (t, 3H) and 4.18 (q, 2H) J=7.1, acyl part ABMXY-syst.: 2.60 (1H, A), 2.65 (1H, B), 3.59 (1H, X), 3.61 (1H, Y), 4.26 (m, 1H, M), J_{AB} 16.5, J_{AM} 7.4, J_{BM} 4.9, J_{XY} 11.2, J_{XM} 5.5, J_{YM} 5.2, 3.34 (b, 1H); ^{13}C NMR, 14.5, 38.9, 48.5, 61.4, 68.3 and 172.2.

3.6. Phenylmethyl 3-butenoate *2b*

3-Butenoic acid (**2**, R_1=H) (5 g, 58 mmol) and benzylalcohol (1 equiv.) were dissolved in CH_2Cl_2 (260 mL). The reaction mixture was cooled to 0°C and DCC (1 equiv.) and 4-pyrrolidinopyridine (0.78 g, 5.3 mmol) were added. The reaction was stirred at 0°C for 1 h, and further at room temperature for 20 h. The reaction mixture was filtered to remove solid *N*,*N*-dicyclohexyl urea and concentrated in vacuo. The residue was dissolved in hexane and more solid material precipitated. The reaction mixture was further worked up by extraction with water (3×150 mL), 5% AcOH (2×100 mL) and water (2×100 mL). The product was purified by column chromatography using pet. ether:acetone, 8:2, yielding 6.34 g (62%) of phenylmethyl 3-butenoate **2b**. ^{1}H NMR, acyl part: 3.14 (td, 2H, J=6.9 and 1.4), 5.15 (m, 1H), 5.18 (m, 1H), 5.95 (m, 1H), benzyl part: 5.12 (s, 2H), 7.29–7.39 (m, 5H); ^{13}C NMR, 39.5, 66.9, 119.1, 128.6, 128.7, 129.0, 130.5, 136.3 and 171.8.

3.7. Phenylmethyl 3,4-epoxybutanoate *3b*

Phenylmethyl 3-butenoate **2b** (5.06 g, 28.7 mmol) was dissolved in $CHCl_3$ (200 mL) and *m*-chloro peroxybenzoic acid (70% purity, 9.3 g) was added. The reaction was left stirring at room temperature for 7 days. The reaction mixture was extracted with phosphate buffer (pH 7.5, 2×100 mL) and

Na_2CO_3/$NaHCO_3$ solution (pH 10, 4×100 mL). The product was further purified by column chromatography using pet. ether:acetone, 2:1, yielding **3b**, 3.94 g (71%). ^{1}H NMR, acyl part ABMPX-syst.: 2.59 (dd, 1H, P), 2.63 (1H, A), 2.66 (1H, B), 2.87 (dd, 1H, X), 3.33 (m, 1H, M), J_{AB} 16.5, J_{AM} 6.1, J_{BM} 5.9, J_{PX} 4.8, J_{PM} 2.6, J_{XM} 4.4, benzyl part: 5.19 (s, 2H) and 7.35–7.40 (m, 5H); ^{13}C NMR, 38.4, 47.0, 48.3, 67.0, 128.7, 128.8, 129.0, 136.1 and 170.6.

3.8. *Phenylmethyl 4-chloro-3-hydroxybutanoate* ***1b***

LiCl (2.14 g, 50.4 mmol) and $CuCl_2$ (3.4 g, 25.2 mmol) were added to dry THF (50 mL). The mixture was stirred for 20 min at room temperature and **3b** (1.90 g, 9.9 mmol) in THF (10 mL) was added. The reaction mixture was stirred at room temperature under N_2 for 18 h. The reaction was quenched with phosphate buffer (pH 7.0, 50 mL) and THF was removed in vacuo. The residue was diluted with water (25 mL) and extracted with Et_2O (5×50 mL). The organic phase was washed with water (2×50 mL), dried over $MgSO_4$, and concentrated in vacuo. The product was purified by column chromatography using pet. ether:$CHCl_3$:acetone, 3:7:1, yielding 1.95 g (86%) of **1b**. ^{1}H NMR, acyl part ABMXY-syst.: 2.70 (1H, A), 2.73 (1H, B), 3.62 (1H, X), 3.64 (1H, Y), 4.30 (m, 1H, M), J_{AB} 16.6, J_{AM} 7.1, J_{BM} 5.1, J_{XY} 11.2, J_{XM} 5.5, J_{YM} 5.1, 3.05 (b, 1H), benzyl part: 5.19 (s, 2H), 7.30–7.43 (m, 5H); ^{13}C NMR, 38.9, 48.5, 67.2, 68.3, 128.7, 128.9, 129.1, 135.7 and 172.0.

3.9. *Cyclohexyl 3-butenoate* ***2c***

The cyclohexyl ester was synthesized as described for **2b** (Section 3.6) using cyclohexanol to yield 3.14 g (32%) of **2c**. ^{1}H NMR cyclohexyl part: 1.20–1.85 (m, 10H), 4.75 (m, 1H), acyl part: 3.04 (td, 2H, J=7.0 and 1.3), 5.10 (m, 1H), 5.14 (m, 1H), 5.90 (m, 1H); ^{13}C NMR, 24.1, 25.7, 31.9, 39.9, 73.2, 118.5, 131.0 and 171.3.

3.10. *Cyclohexyl 3,4-epoxybutanoate* ***3c***

The epoxide **3c** was synthesized from **2c** as described in Section 3.7. ^{1}H NMR, cyclohexyl part: 1.23–1.88 (m, 10H), 4.83 (m, 1H), acyl part ABMPX-syst.: 2.53 (1H, A), 2.57 (1H, B), 2.56 (1H, P), 2.84 (1H, X), 3.29 (m, 1H), J_{AB} 16.2, J_{AM} 5.5, J_{BM} 6.1, J_{PX} 4.4, J_{XM} 4.4; ^{13}C NMR, 24.1, 25.7, 32.0, 38.8, 47.1, 48.5, 73.6 and 170.2.

3.11. *Cyclohexyl 4-chloro-3-hydroxybutanoate* ***1c***

The hydroxy ester **1c** was synthesized from **3c** as described in Section 3.8. The column chromatography step for **3c** could be omitted, leading to 3.05 g, 74% overall yield from **2c**. ^{1}H NMR, cyclohexyl part: 1.24–1.89 (m, 10H), 4.84 (m, 1H), acyl part ABMXY-syst.: 2.63 (1H, A), 2.66 (1H, B), 3.62 (1H, X), 3.64 (1H, Y), 4.27 (m, 1H, M), J_{AB} 16.4, J_{AM}7.4, J_{BM} 4.9, J_{XY} 11.2, J_{XM} 5.5, J_{YM} 5.2, 3.2 (b, 1H); ^{13}C NMR, 24.0, 25.6, 31.9, 39.2, 48.6, 68.4, 73.9 and 171.6.

3.12. tert-*Butyl 4-chloro-3-oxobutanoate* ***4d***

To a solution of diketene (1.65 g, 19.7 mmol) in CCl_4 (6 mL) cooled to −20 to −25°C was added a cooled solution of Cl_2 (1 equiv.) in CCl_4 (9 mL). The reaction was stirred at this temperature for 30 min. *tert*-Butyl alcohol (1 equiv.) and pyridine (1 equiv.) in CCl_4 (9 mL) were subsequently added at −5°C.

The reaction was stirred for 1 h. After removal of solid precipitate and evaporation of solvent the reaction mixture was diluted in EtOAc and washed twice with water. After drying over $MgSO_4$, the product was purified by column chromatography using CH_2Cl_2 yielding 1.49 g (39%) of **4d**. ^{1}H NMR, keto tautomer: 1.50 (s, 9H), 3.59 (s, 2H), 4.23 (s, 2H), enol tautomer: 1.52 (s), 4.00 (s), 5.25 (s) and 12.21 (OH); ^{13}C NMR, keto tautomer: 28.3, 47.9, 48.5, 83.1, 166.0 and 196.3.

3.13. tert-*Butyl 3,4-dichloro-3-oxobutanoate* ***6***

tert-Butyl 3,4-dichloro-3-oxobutanoate **6** was isolated under the conditions used to purify **4d**. ^{1}H NMR, keto tautomer: 1.53 (s, 9H), CH_2Cl AB-syst.: 4.45 (1H), 4.49 (1H), J_{AB}=16.2, 5.01 (1H), enol tautomer: 1.57, 4.32, 12.35; ^{13}C NMR: 28.0, 46.2, 60.1, 85.7, 163.4 and 192.0.

3.14. tert-*Butyl 4-chloro-3-hydroxybutanoate* ***1d***

The hydroxy ester **1d** was synthesized from **4d** as described in Section 3.5. Reaction time was 1 h. After column chromatography using CH_2Cl_2:pet. ether:acetone, 7:3:1, *tert*-butyl 4-chloro-3-hydroxybutanoate **1d** was isolated yielding 1.00 g (72%). ^{1}H NMR, *tert*-butyl group: 1.50 (s, 9H), acyl part ABMXY-syst.: 2.56 (1H, A), 2.59 (1H, B), 3.60 (1H, X), 3.62 (1H, Y), 4.23 (m, 1H, M), J_{AB} 16.6, J_{AM} 7.0, J_{BM} 5.0, J_{XY} 11.0, J_{XM} 5.0, J_{YM} 5.5, 3.27 (b, 1H); ^{13}C NMR, 28.5, 39.9, 48.5, 68.5, 82.1 and 171.6.

3.15. tert-*Butyl 3,4-epoxybutanoate* ***3d***

The epoxide **3d** was isolated under the conditions used for purification of **1d**. ^{1}H NMR, *tert*-butyl group: 1.48 (s, 9H), acyl part ABMPX-syst.: 2.46 (1H, A), 2.52 (1H, B), 2.55 (1H, P), 2.84 (1H, X), 3.26 (m, 1H), J_{AB} 16.2, J_{AM} 5.4, J_{BM} 6.1, J_{PX} 4.9, J_{PM} 2.7; ^{13}C NMR, 28.4, 39.6, 47.1, 48.6, 81.6 and 170.1

3.16. *Preparative resolutions*

Substrate and vinyl propanoate (5 equiv.) were dissolved in the organic solvent. The reaction was started by adding immobilized RML (300 mg) in the case of **1a**–**1c** and 100 mg for **1d**. The reaction was shaken at room temperature until the desired conversion was reached. The enzyme was filtered off, and the residue concentrated in vacuo. The alcohol and propanoate were separated by column chromatography using CH_2Cl_2:acetone, 20:1.

3.17. *Resolution of ethyl 4-chloro-3-hydroxybutanoate* ***1a***

Resolution of **1a** (2.17 g, 13.2 mmol) was performed using benzene (200 mL) as the solvent. The reaction was stopped after 5 days when a conversion of 30% was reached, yielding (*S*)-**7a**, 0.76 g (24%), *ee* 86%, $[\alpha]_D^{25}$=−13.7 (*c* 1.02, $CHCl_3$). ^{1}H NMR, propanoate: 1.15 (t, 3H), 2.36 (q, 2H), *J*=7.5, for ethoxy: 1.26 (t, 3H), 4.16 (q, 2H), *J*=7.1, acyl part ABMXY-syst.: 2.73 (1H, A), 2.77 (1H, B), 3.71 (1H, X), 3.75 (1H, Y), 5.41 (m, 1H, M), J_{AB} 16.3, J_{AM} 6.9, J_{BM} 6.1, J_{XY} 11.8, J_{MX} 4.7, J_{MY} 4.8; ^{13}C NMR, 9.3, 14.2, 27.8, 36.7, 45.4, 61.2, 69.3, 169.9 and 173.6.

3.18. Resolution of phenylmethyl 4-chloro-3-hydroxybutanoate **1b**

Resolution of **1b** (1.03 g, 4.5 mmol) was performed in benzene:hexane, 7:1 (80 mL), as the solvent. After 5 days the reaction was stopped. Phenylmethyl (*R*)-(+)-4-chloro-3-hydroxybutanoate (*R*)-**1b** was isolated yielding 0.30 g (29%), *ee* 96%, $[\alpha]_D^{25}$=+17.3 (*c* 5.02, $CHCl_3$), $[\alpha]_D^{25}$=+17.8 (*c* 1.5, $CHCl_3$). Phenylmethyl (*S*)-(−)-4-chloro-3-propanoyloxybutanoate (*S*)-**7b** was isolated in a yield of 0.55 g (43%), *ee* 77%, $[\alpha]_D^{25}$=−8.2 (*c* 1.5, $CHCl_3$). ^{1}H NMR, propanoate ABX_3-syst.: 1.12 (t, 3H, X), 2.28 (1H, A), 2.33 (1H, B), J_{AB} 16.6, J_{AX} 7.5, J_{BX} 7.5, acyl part ABMXY-syst.: 2.82 (1H, A), 2.84 (1H, B), 3.72 (1H, X), 3.75 (1H, Y), 5.43 (m, 1H, M), J_{AB} 16.3, J_{AM} 6.9, J_{BM} 6.1, J_{XY} 11.7, J_{XM} 4.6, J_{YM} 4.9, benzyl group: 5.16 (s, 2H) and 7.32–7.41 (m, 5H); ^{13}C NMR, 9.4, 27.8, 36.8, 45.5, 67.1, 69.4, 128.7, 128.8, 129.0, 136.0, 169.9 and 173.7.

3.19. Resolution of cyclohexyl 4-chloro-3-hydroxybutanoate **1c**

Resolution of **1c** (1.09 g, 4.9 mmol) was performed in CCl_4 (150 mL) as the solvent. The reaction was stopped after 4 days. Cyclohexyl (*R*)-(+)-4-chloro-3-hydroxybutanoate, (*R*)-**1c**, was isolated in 0.48 g (44%) yield, *ee* 96%, $[\alpha]_D^{25}$=+17.6 (*c* 1.02, $CHCl_3$). Cyclohexyl (*S*)-(−)-4-chloro-3-propanoyloxybutanoate, (*S*)-**7c**, was isolated in a yield of 0.61 g (45%), *ee* 89%, $[\alpha]_D^{25}$=−9.7 (*c* 1.03, $CHCl_3$). ^{1}H NMR, propanoate ABX_3-syst.: 1.16 (t, 3H, *J*=7.6) and 2.36 (m, 2H), cyclohexyl group: 1.20–1.87 (m, 10H) and 4.79 (m, 1H), acyl part ABMXY-syst.: 2.74 (1H, A), 2.77 (1H, B), 5.43 (m, 1H, M), 3.72 (1H, X), 3.76 (1H, Y), J_{AB} 16.1, J_{AM} 6.9, J_{BM} 6.2, J_{XY} 11.8, J_{XM} 4.6, J_{YM} 4.8; ^{13}C NMR, 9.3, 24.0, 25.6, 27.8, 31.8, 37.1, 45.4, 69.4, 73.6, 169.3 and 173.6.

3.20. Resolution of tert-butyl 4-chloro-3-hydroxybutanoate **1d**

Resolution of **1d** (488 mg, 2.51 mmol) was performed using hexane (60 mL) as the solvent. The reaction was stopped after five days. *tert*-Butyl (*R*)-(+)4-chloro-3-hydroxybutanoate, (*R*)-**1d** was isolated in a yield of 205 mg (42%), *ee* 99%, $[\alpha]_D^{25}$=+22.0 (*c* 1.00, $CHCl_3$). *tert*-Butyl (*S*)-(−)-4-chloro-3-propanoyloxybutanoate, (*S*)-**7d**, was isolated in a yield of 301 mg (48%), *ee* >97%, $[\alpha]_D^{25}$=−8.5 (*c* 1.00, $CHCl_3$). ^{1}H NMR, propanoate ABX_3-syst.: 1.17 (t, 3H, *J*=7.6), 2.37 (m, 2H), *tert*-butyl group: 1.46 (s, 9H), acyl part ABMXY-syst.: 2.67 (1H, A), 2.70 (1H, B), 5.40 (m, 1H, M), 3.70 (1H, X), 3.74 (1H, Y), J_{AB} 16.1, J_{AM} 7.2, J_{BM} 6.0, J_{XY} 11.7, J_{XM} 4.7, J_{YM} 4.7; ^{13}C NMR, 9.4, 27.8, 28.3, 38.0, 45.5, 69.5, 169.1 and 173.6.

3.21. Absolute configurations

The faster reacting enantiomer of ethyl 4-chloro-3-hydroxybutanoate was identified by co-injection of commercially available ethyl (*R*)-4-chloro-3-hydroxybutanoate (*R*)-**1a** with the racemic sample. The (*R*)-enantiomer of phenylmethyl 4-chloro-3-hydroxybutanoate (*R*)-**1b** has been described previously.[1] The reported specific rotation of +8.7 (*c* 5.26, $CHCl_3$) indicates, however, that the enantiomeric excess of this product was only moderate. The absolute configuration of the enantiomers of cyclohexyl 4-chloro-3-hydroxybutanoate has not been described. (*R*)- and (*S*)-**1c** were verified by synthesizing **1c** by alcoholysis of racemic ethyl 4-chloro-3-hydroxybutanoate **1a** and ethyl (*R*)-4-chloro-3-hydroxybutanoate (*R*)-**1a** on a small scale. Cyclohexanol was used as an acyl acceptor (5 equiv.) in hexane, and lipase B from *Candida antarctica* was used as the catalyst. In the resolution of ethyl 4-chloro-3-hydroxybutanoate **1a** by alcoholysis the (*S*)-enantiomer was transformed to products faster than the (*R*)-enantiomer (Chiral

GLC), E=6. It is therefore concluded that the most abundant enantiomer seen in the chiral analysis of **1c** must be (*S*)-**1c**. Cyclohexyl (*R*)-4-chloro-3-hydroxybutanoate (*R*)-**1c** formed in the alcoholysis of ethyl (*R*)-4-chloro-3-hydroxybutanoate (*R*)-**1a** coinjected with the racemic sample, and eluted with the enantiomer which reacts slower in these transesterifications. The specific rotation of (*R*)-*tert*-butyl-4-chloro-3-hydroxybutanoate (*R*)-**1d** was compared to the one previously reported, +18.09 (*c* 5.1, $CHCl_3$).[2]

Acknowledgements

We gratefully acknowledge a fellowship to BHH from the Norwegian Research Council (NFR). We also want to thank Novo-Nordisk A/S for a gift of *Rhizomucor miehei* lipase and lipase B from *Candida antarctica*, and Julie Jackson for linguistic help and comments.

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