Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 1712–1720

Lipase catalyzed acylation of primary alcohols with remotely located stereogenic centres: the resolution of (±)-4,4-dimethyl-3-phenyl-1-pentanol

Sunil Sabbani, Erik Hedenström* and Jimmy Andersson

Chemistry, Department of Natural Sciences, Mid Sweden University, SE-851 70 Sundsvall, Sweden

Received 19 June 2007; accepted 5 July 2007

Abstract—Enantioselective acylation of some (±)-3-alkyl-3-phenyl-1-propanols was performed with enzymes as catalysts. Moderate enantiomeric ratios (*E*), ranging up to $E = 11.6$, were obtained. In the resolution, some of the lipases selectively acylated the (+)-enantiomer while others acylated the (−)-enantiomer of the γ-substituted primary alcohols **1**–**4**. Thus, it is possible to obtain both enantiomers of the alcohols as remaining substrate with high enantiomeric purity. The resolution of (±)-4,4-dimethyl-3-phenyl-1-pentanol **4** was extensively studied and screening experiments were conducted to select suitable lipase(s), reaction medium, acyl donor and appropriate temperature combinations to increase the enantiomeric ratio. Chirazyme® L-6/chloroform/vinyl propionate/38 °C and Chirazyme® L-7/di-iso-propyl ether/vinyl propionate/0 °C were chosen to obtain both enantiomers, (*R*)-(+)-**4** and (*S*)-(−)-**4**, respectively, via sequential resolutions in excellent enantiomeric excess (>98%) and in 25% and 22% yield, respectively.
© 2007 Elsevier Ltd. All rights reserved.

1. Introduction

Enantiopure primary alcohols are valuable intermediates in asymmetric synthesis of pharmaceuticals,[1,2] pheremones[3,4] and perfumes.[3,5] Enantiomerically pure 3-alkyl-3-phenyl propanols are used as intermediates in the asymmetric synthesis of antibacterial agents such as curcuphenol, curcuphenone and curcuhydroquinone,[6] in the synthesis of bisabolene sesquiterpenes such as turmerone, a natural cytotoxic agent from *Curcuma longa*[1] and in the synthesis of the synthetic fragrant florhydral.[7] 3-Alkyl-3-phenyl propanols can be oxidized into the corresponding acids or aldehydes, which are used in the synthesis of some diazoketone derivatives, which in turn have been used in Buchner cyclization reactions.[8] We have previously obtained, via lipase catalysis, pure enantiomers of β-alkyl substituted primary alcohols with good results. However, the enantioselectivity of lipase catalyzed resolutions of primary alcohols are known to be considerably lower compared to when secondary alcohols are used as substrates.[9,10] However, some successful examples of lipase catalyzed resolutions of chiral racemic alcohols with an alkyl group at the β-position are known from our group[11] and from others.[12,13] Very few examples are known from the literature when primary alcohols, with even more remotely located stereogenic centres, are resolved by enzymes in acylation reactions.[7,14–18] *Pseudomonas cepacia* lipase (PCL) catalyzed resolution of (±)-3-phenyl-1-butanol **1** has been reported[15] with a very low *E*-value of 2 but the enantioselective acylation of substrates **2**, **3** and **4** via lipase catalysis has not previously been reported. Herein, we report a study on the preparation of such alkyl γ-substituted primary alcohols in high enantiomeric purity via enzymatic resolution.

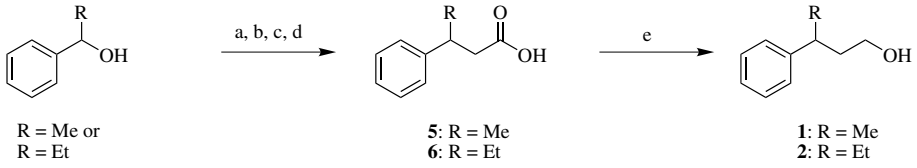
2. Results and discussion

Racemic alcohols **1** and **2** were prepared via $LiAlH_4$ reduction of the corresponding acids **5** and **6**, which were obtained as described[8] from a malonic ester reaction sequence starting from 1-phenyl-ethanol or 1-phenyl-propanol, respectively (see Scheme 1).

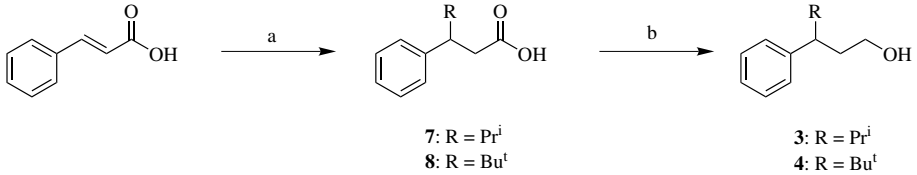
The γ-substituted primary alcohols **3** and **4** were obtained from cinnamic acid via conjugate addition[19] of an *iso*-propyl or *tert*-butyl Grignard reagent followed by $LiAlH_4$ reduction of the acids obtained **7** and **8**, respectively (see Scheme 2).

* Corresponding author. E-mail: erik.hedenstrom@miun.se

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.07.002



Scheme 1. Synthesis of 3-pheny-l-butanol **1** and 3-phenyl-1-pentanol **2**. Reagents and conditions: (a) PBr_3, pyridine, Et_2O; (b) $CH_2(CO_2Et)_2$, $Na_{(S)}$/EtOH; (c) KOH/EtOH, reflux, then HCl; (d) reflux 180–190 °C; (e) (i) $LiAlH_4/Et_2O$; (ii) H_2O/H_3O^+.



Scheme 2. Synthesis of 4-methyl-3-phenyl-1-pentanol **3** and 4,4-dimethyl-3-phenyl-1-pentanol **4**. Reagents: (a) (i) RMgCl, THF or Et_2O; (ii) H_3O^+/ice; (b) (i) $LiAlH_4/Et_2O$; (ii) H_2O/H_3O^+.

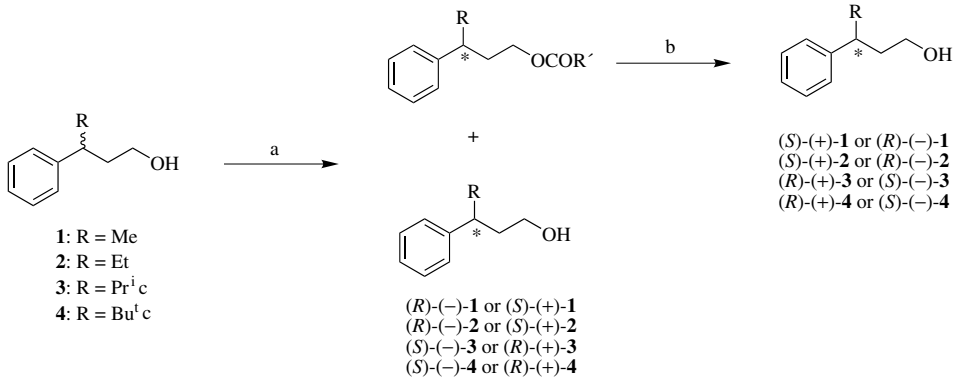
The procedure (see Scheme 3) for the enantioselective acylation of the γ-substituted primary alcohols **1**, **2**, **3** or **4** was started by the addition of an enzyme (stored over dry silica gel at 4 °C)[20] to a stirred solution containing the primary alcohol, a vinyl ester and an organic solvent all pre-equilibrated at a water activity (a_w)[21] of 0.32 or with 4 Å molecular sieves added to obtain dry conditions. All reactions were carried out under an argon atmosphere at a specific temperature. In most cases, the reaction was interrupted below 40% conversion[22] when the enzyme was removed by filtration. The remaining substrate and the product ester were separated via liquid chromatography (LC) and after $LiAlH_4$ reduction to the corresponding alcohols, the enantiomeric excesses (ee_s and ee_p, respectively) were determined on a β-Dex 120 chiral GC-column.

The absolute configurations of the enantiomers of alcohols **1**, **2**, **3** and **4** were confirmed by comparing measured specific rotation values to the ones previously reported (see Table 1).

Substrate **4** was extensively studied; first we investigated the enantioselectivity of different enzymes (see Section 3.1 for a list of enzymes tested) for this substrate in the acylation reaction. In total 15 lipases and 1 esterase from different sources were screened in the resolution of **4** at an initial water activity[21,27] of 0.32 with vinyl propionate as the acyl donor and di-*iso*-propyl ether or *n*-hexane as the reaction medium at room temperature. Most of the enzymes showed low or very low enantioselectivity while the calculated enantiomeric ratio[22] ranged from 1.1 to 9.3.

However, interestingly when using some enzymes, a preference for the (*R*)-(+)-**4** enantiomer was obtained (Lip-PS, E-2, L-5, Lip-FAP 15, Lip-M, L-8, L-3, L-10, Lip-AK and L-7 with $E = 1.1$–9.3) while others showed a preference for the (*S*)-(−)-**4** enantiomer (Lip-AS, Nov-435, L-9, Lip-AL, Lip-TL and L-6 with $E = 1.2$–6.1). Thus, it is possible to use two different enzymes to obtain both enantiomers as the remaining substrate in good enantiomeric purity and in a tolerable yield (see Table 2 for some of the results). Chirazyme® L-7 (entry 15, $E = 9.3$) and Chirazyme® L-6 (entry 16, $E = 6.1$) with the highest enantioselectivity and opposite enantiomer selectivity were chosen in further studies together with Chirazyme® L-10 (entry 14, $E = 3.4$) aiming to increase the *E*-value by altering the reaction conditions (see Table 2).

Different vinyl esters (vinyl acetate, vinyl propionate, vinyl butyrate, vinyl 2,2-dimethyl-propionate and vinyl



Scheme 3. Enantioselective lipase resolution of γ-substituted primary alcohols of the type Ph–CH(R)–CH_2CH_2OH. Reagents: (a) lipase, vinyl ester, organic solvent, initial $a_w = 0.32$ or ∼0; (b) (i) $LiAlH_4/Et_2O$; (ii) H_2O/H_3O^+.

Table 1. GC-retention time, sign of specific rotation and configuration for the enantiomers of alcohol **1**, **2**, **3** and **4**

Substrate	Temperature programme	Carrier gas: He pressure (split)	t_1[a] t_2[a]	Specific rotation $[\alpha]_D^{20}$	Configuration
1	80 °C (3 min), 170 °C (0.3 °C/min)	20 psi (6)	124.08	−10.8 (*c* 0.4, $CDCl_3$) lit.[23] $[\alpha]_D^{25} = -25.4$ (neat)	(*R*)
			125.41	+11.5 (*c* 0.4, $CDCl_3$) lit.[24] $[\alpha]_D^{20} = +25.5$ (*c* 1.52, $CHCl_3$)	(*S*)
2	80 °C (3 min), 170 °C (0.5 °C/min)	20 psi (6)	102.17	−2.4 (*c* 0.8, $CDCl_3$) lit.[25] $[\alpha]_D^{20} = -7.9$ (*c* 4.79, CCl_4)	(*R*)
			103.75	+2.5 (*c* 0.8, $CDCl_3$) lit.[23] $[\alpha]_D^{25} = +14.2$ (neat)	(*S*)
3	80 °C (3 min), 170 °C (0.5 °C/min)	20 psi (6)	155.81	−5.5 (*c* 4.0, $CDCl_3$) lit.[25] $[\alpha]_D^{20} = -10.3$ (neat)	(*S*)
			156.96	+7.3 (*c* 4.0, $CDCl_3$)	(*R*)
4	100 °C (3 min), 170 °C (1 °C/min)	20 psi (6)	56.69	+11.3 (*c* 4.0, benzene) lit.[26] $[\alpha]_D^{25} = +7.31$ (benzene)	(*R*)
			57.12	−11.0 (*c* 4.0, benzene)	(*S*)

[a] t_1 and t_2 are the retention times in minutes for the two enantiomers of compounds **1**–**4**, respectively on GC-column β-dex 120.

Table 2. The enantiopreference and enantioselectivity of some lipases when different γ-substituted alcohols **1**–**4** were stirred at different temperatures in di-*iso*-propyl ether or chloroform with vinyl propionate, at an initial water activity of 0.32 and under argon atmosphere

Entry	Substrate	Lipase	Solvent	Reaction rate (%/h)	Conversion *c*[a] (%)	ee_s[b] (%)	ee_p[b] (%)	*E*[a]	Enantiopreference of the lipase
1	**1**	L-6	Chloroform	1.86	26.0	7.0	20.0	1.6	(*S*)-(+)
2	**1**	L-7	Di-*iso*-propyl ether	1.35	32.5	5.6	11.0	1.3	(*R*)-(−)
3	**1**	L-10	Di-*iso*-propyl ether	22.1	44.2	35.0	44.0	3.6	(*S*)-(+)
4	**2**	L-6	Chloroform	1.25	30.0	12.0	27.0	1.9	(*S*)-(+)
5	**2**	L-7	Di-*iso*-propyl ether	1.68	38.7	19.0	30.0	2.2	(*R*)-(−)
6	**2**	L-10	Di-*iso*-propyl ether	7.75	46.5	40.9	47.0	4.1	(*S*)-(+)
7	**3**	L-6	Chloroform	2.04	49.0	18.4	19.0	1.7	(*R*)-(+)
8	**3**	L-7	Di-*iso*-propyl ether	2.50	37.5	15.0	25.0	1.9	(*R*)-(+)
9	**3**	L-10	Di-*iso*-propyl ether	19.3	33.7	39.2	77.0	11.3	(*R*)-(+)
10	**3**	Lipas-AS	Di-*iso*-propyl ether	0.24	13.0	1.8	12.0	1.3	(*R*)-(+)
11	**3**	Lipas-AL	Di-*iso*-propyl ether	5.70	17.0	4.7	22.7	1.6	(*R*)-(+)
12	**3**	N-435	Di-*iso*-propyl ether	69.0	69.0	2.0	0.90	1.0	(*S*)-(−)
13	**3**	L-9	Di-*iso*-propyl ether	8.73	26.2	10.7	30.2	2.1	(*S*)-(−)
14	**4**	L-10	Di-*iso*-propyl ether	1.75	42.1	31.6	43.4	3.4	(*R*)-(+)
15	**4**	L-7	Di-*iso*-propyl ether	1.29	29.8	31.5	74.2	9.3	(*R*)-(+)
16	**4**	L-6	Di-*iso*-propyl ether	0.81	38.3	38.0	61.3	6.1	(*S*)-(−)
17	**4**	L-6	Chloroform	0.41	29.3	29.9	72.0	8.2	(*S*)-(−)
18	**4**	L-6	Chloroform 38 °C	1.20	35.0	39.0	72.0	9.2	(*S*)-(−)
19	**4**	L-7	Di-*iso*-propyl ether −25 °C	0.08	12.4	11.7	82.2	11.6	(*R*)-(+)
20	**4**	L-7	Di-*iso*-propyl ether 0 °C	0.39	19.0	19.0	81.0	11.4	(*R*)-(+)
21[c]	**4**	L-7	Di-*iso*-propyl ether 0 °C	6.70	32.0	30.0	64.0	6.1	(*R*)-(+)
22[d]	**4**	L-7	Di-*iso*-propyl ether 0 °C	1.38	33.0	34.0	68.0	7.3	(*R*)-(+)

[a] Calculated according to the equation $c = ee_s/(ee_s + ee_p)$ and $E = \ln[1 - c(1 + ee_p)]/\ln[1 - c(1 - ee_p)]$.[22]
[b] Measured by GC on a β-Dex 120 chiral column.
[c] Dry conditions when 266 mg of molecular sieves (4 Å)/ml of substrate solution was added.
[d] Dry conditions when 10.7 mg of molecular sieves (4 Å)/ml of substrate solution was added.

4-*tert*-butyl-benzoate) were tested as acyl donors[28–30] in the lipase catalyzed acylation of **4**. Vinyl propionate gave somewhat higher *E*-values together with these lipases and was used as an acyl donor in further screening experiments for a suitable reaction media. The two most sterically demanding acyl donors tested (vinyl 2,2-dimethyl-propionate and vinyl 4-*tert*-butyl-benzoate) did not react when used along with Chirazyme® L-7 while vinyl 4-*tert*-butyl-benzoate did not react when used with Chirazyme® L-6.

The enantioselectivity of Chirazymes® L-6, L-7 and L-10 were more or less sensitive to changes of reaction media;[31,32] the most influenced was the enantioselectivity of Chirazyme® L-7. The lipases were tested in organic solvents such as di-*iso*-propyl ether, *tert*-butyldimethyl ether, dichloromethane, THF, *n*-hexane and chloroform; some of the results are shown in Table 2. Two combinations of a solvent and a lipase with the highest and reversed enantioselectivity together with a useful reaction rate

(Chirazyme® L-6/chloroform, entry 17 $E = 8.2$, and Chirazyme® L-7/di-*iso*-propyl ether, entry 15 $E = 9.3$) were then used to study the lipase catalyzed acylation of **4** at five different temperatures[33] to investigate the effect[34] on the *E*-value.

Chirazyme® L-6 in $CHCl_3$ gave a higher *E*-value (entry 18, $E = 9.2$) at the higher temperature (38 °C) while Chirazyme® L-7 in di-*iso*-propyl ether gave somewhat higher enantioselectivity when lowering the temperature to −25 °C (entry 19, $E = 11.6$) (see Table 2 and Fig. 1 for the results). The water activity is probably changed, at least when the temperature is lowered far below 0 °C, as the water might freeze, which probably results in a lower water activity than expected. Thus, we added molecular sieves (4 Å)[35,36] to the reaction at 0 °C to obtain $a_w \sim 0$ and interestingly, we found that the enantiomeric ratio was lowered from 11.4 (entry 20) to 7.3 (entry 22). It was also obvious that *E*-value changed with the quantity of molecular sieves added as addition of more molecular sieves gave an even lower *E*-value ($E = 6.1$, entry 21). The rate of the enzymatic reaction in the presence of molecular sieves is higher than the reactions without molecular sieves (see Table 2, entry 20, 21 and 22). However, no product was observed when running the reaction with molecular sieves and without lipase; this might be explained by the fact that the molecular sieves in conjunction with the lipase are able to non-selectively catalyze the reaction.

Structurally different γ-substituted alcohols **1**–**4** were tested as substrates in order to investigate the substrate structure relationship with the lipase active site (see Table 2). When alcohols **1**, **2** or **3** are used as substrates, the enantiopreference of Chirazyme® L-6 is the (+)-enantiomer, but when alcohol **4** is used, the enantiopreference switches to the (−)-enantiomer. When using Chirazyme® L-7 the enantiopreference for alcohols **1** and **2** was the (−)-enantiomer but we see a switch in selectivity for alcohols **3** and **4** as now the (+)-enantiomer reacted faster. A switch is also noticed in the elution order of the enantiomers from the chiral GC-column (see Table 1). Thus, when alcohol **1**, **2** or **3** are analyzed, the (−)-enantiomer is the first eluting one but when analyzing alcohol **4** the (+)-enantiomer is first eluting. Obviously when a very space demanding and more electron donating alkyl group, such as *tert*-butyl, is introduced in the molecule, the chiral recognition of the lipases and the chiral column changes. When using Chirazyme® L-6, the enantiomeric ratio increases ($E = 1.6$ for alcohol **1**, entry 1 to $E = 8.2$ for alcohol **4**, entry 17) when a *tert*-butyl group is introduced as the γ-substituent. The same observation is made when using Chirazyme® L-7 ($E = 1.3$ for alcohol **1**, entry 2 to $E = 9.3$ for alcohol **4**, entry 15) and in both cases the change from *iso*-propyl to *tert*-butyl seems to be most important for the enantiopreference of the lipase. Disappointingly, both Chirazyme® L-6 (entry 7, $E = 1.7$) and L-7 (entry 8, $E = 1.9$) show enantiopreference for the (+)-enantiomer of alcohol **3** with low selectivity. Consequently, we tested some other lipases to find one with the reversed selectivity and we found that with both Novozym-435 (entry 12, $E = 1.0$) and Chirazyme® L-9 (entry 13, $E = 2.1$) the (−)-enantiomer was the faster reacting enantiomer of this alcohol. For substrate **1**, the best results ($E = 3.6$) were obtained with lipase Chirazyme® L-10 (entry 3) which is somewhat higher than obtained by others in a similar reaction.[15] This lipase seems to show an enantioselectivity of the same magnitude for three of the alcohols tested **1**, **2** (entry 6, $E = 4.1$) and **4**, (entry 14, $E = 3.4$) independent of the alkyl group at the stereogenic centre. Interestingly, when Chirazyme® L-10 was used with substrate **3**, an unexpectedly high *E*-value value of 11.3 (entry 9) was obtained with the (+)-enantiomer reacting faster. An increase in the enantiomeric ratios is envisaged for each of the substrate alcohols **1**–**3** if a thorough investigation of the reaction conditions for each of them is undertaken as for alcohol **4**.

We tried to extend the empirical rule proposed by Kazlauskas et al. and others[37] to our substrates **1**–**4** with γ-alkyl substituents and thus predict the faster reacting enantiomer in the lipase catalyzed acylation. Our discussion is based on the assumption that the *tert*-butyl group is a large group with a slightly larger volume (calculated in chemprop from Cambridgesoft) in substrate **4** and the phenyl group is the large group in substrates **1**, **2** and **3**. Based on the above

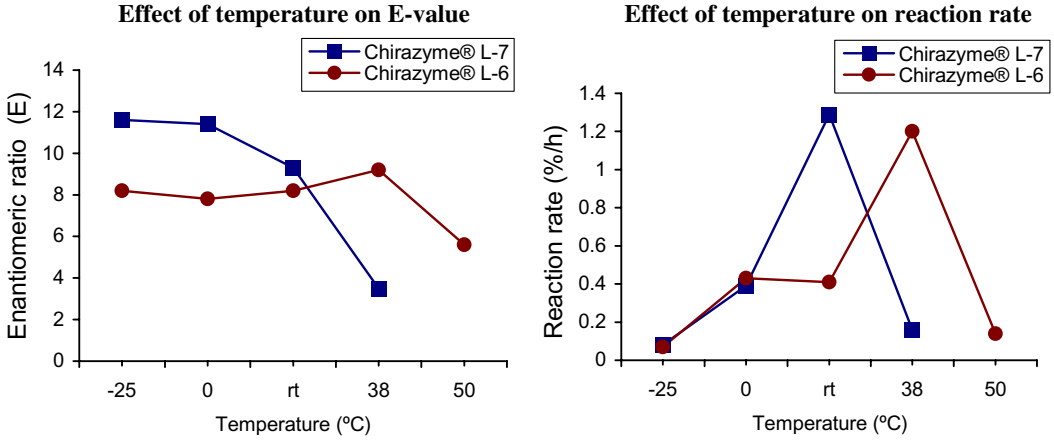
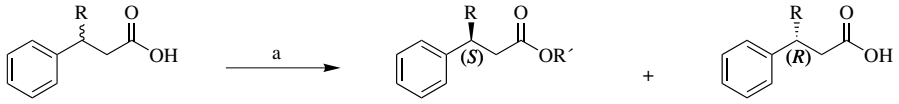


Figure 1. The effect of temperature on the *E*-value and reaction rate when using Chirazyme® L-6/chloroform/vinyl propionate/and Chirazyme® L-7/di-*iso*-propyl ether/vinyl propionate at different temperatures in the acylation of substrate **4**.

assumptions, we can conclude that if the (+)-enantiomer of **1** is the faster reacting enantiomer for a particular lipase, then (+)-**2**, (+)-**3** and (−)-**4** should be the faster reacting enantiomers with the same lipase if the empirical rule is true for our substrates. Chirazyme® L-6, L-7 and L-10 are lipases, which we tested on substrates **1**, **2**, **3** and **4**. Our results show that the empirical rule is true only for the lipase from *Pseudomonas species* (Chirazyme® L-6).

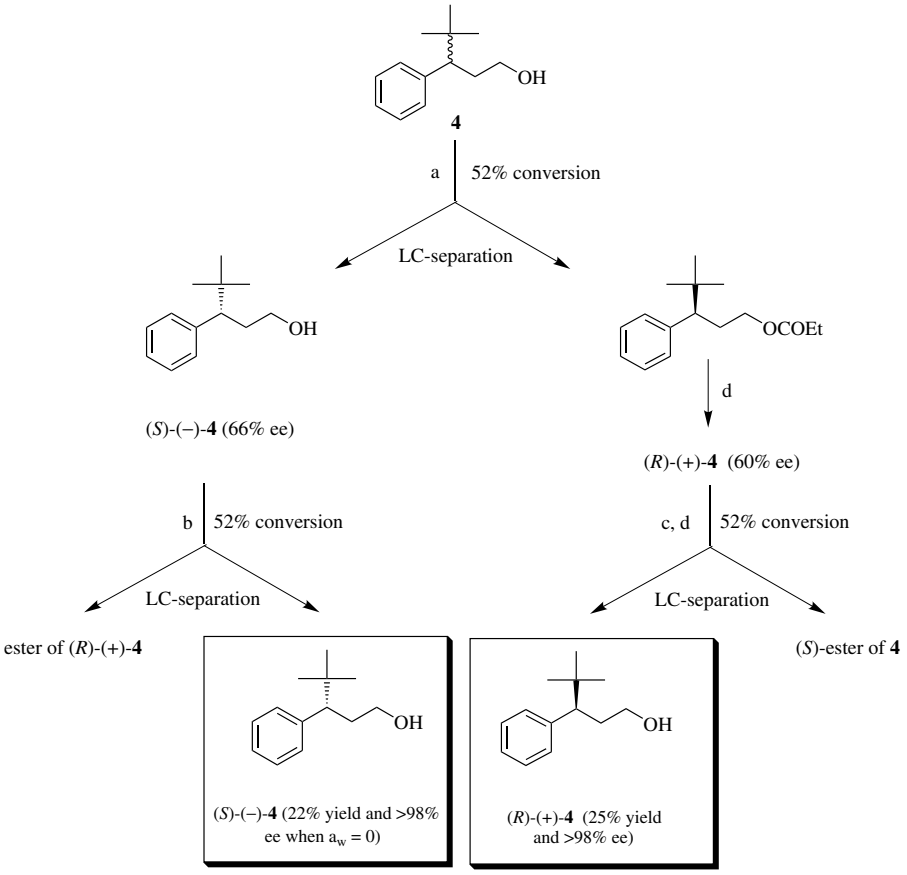
We have previously successfully resolved several alkanoic acids, with remotely located methyl-branching, using *Candida rugosa* lipase as the enantioselective catalyst in esterification reactions.[38,39] Thus, we attempted to esterify enantioselectively the four racemic substrate acids **5**, **6**, **7** and **8** as described for other methyl-branched acids (Scheme 4).[38,39] Substrate acids **5** and **6** were esterified with a modest to slow rate and with a low preference for the (*S*)-enantiomer resulting in very low *E*-values ($E < 2$). Although hydrolysis of the methyl ester of **5** with high *E*-values has been reported[40] no successful resolution of substrate acid **5** was possible either with immobilized CRL, Amano PS or CALB (Chirazyme® L-2). No ester was observed under our conditions for acids **7** and **8**, even when running the reaction for several days.

Finally, in a sequential resolution of (±)-4,4-dimethyl-3-phenyl-1-pentanol **4** (see Scheme 5), two lipases (Chirazyme® L-6 and Chirazyme® L-7) with opposite



5: R = Me
6: R = Et
7[1]: R = Pr^{i} : no reaction
8[1]: R = Bu^{t}: no reaction

Scheme 4. Enantioselective enzymatic resolution of γ-substituted alkanoic acids of the type Ph–CH(R)–CH_2COOH. Reagents: (a) lipase, 1-decanol, isooctane, initial $a_w = 0.32$.



Scheme 5. Lipase catalyzed resolution strategy to obtain >98% ee of both enantiomers of the gamma-substituted primary alcohol (±)-4,4-dimethyl-3-phenyl-1-pentanol **4**. Reagents and conditions: (a) L-7, vinyl propionate, di-*iso*-propyl ether, initial $a_w = 0.32$, 0 °C; (b) L-7, vinyl propionate, di-*iso*-propyl ether, initial $a_w = 0.32$ or $a_w = 0$, 0 °C; (c) L-6, vinyl propionate, chloroform, initial $a_w = 0.32$, 38 °C; (d) (i) $LiAlH_4/Et_2O$; (ii) H_2O/H_3O^+.

enantioselectivity were chosen to obtain both the enantiomers (*S*)-(−)-**4** and (*R*)-(+)-**4** enantiomerically pure. Thus, Chirazyme® L-6/chloroform/vinyl propionate/38 °C/a_w = 0.32 and Chirazyme® L-7/di-*iso*-propyl ether/vinyl propionate/0 °C/a_w = 0.32 combinations gave the best *E*-values at reasonable reaction rates and were used with an aim to obtain both (*S*)-(−)-**4** and (*R*)-(+)-**4** with ee >98%. Consequently, racemic substrate **4** was subjected to lipase catalyzed acylation at an initial water activity of 0.32 in the presence of vinyl propionate, di-*iso*-propyl ether and Chirazyme® L-7 at 0 °C. The reaction was stopped at 52% conversion (see Scheme 5). The product ester of (*R*)-(+)-**4** (60% ee) was reduced, after LC separation of the remaining (*S*)-(−)-**4**, with $LiAlH_4$ and the resulting alcohol (*R*)-(+)-**4** (60% ee) was subjected to Chirazyme® L-6, vinyl propionate and chloroform at 38 °C. Thus, we obtained (*R*)-(+)-**4** with >98% ee and in a yield of 25% from the racemate. The enantiomerically enriched substrate from above (*S*)-(−)-**4** (66% ee) was subjected further to lipase catalysis with Chirazyme® L-7, vinyl propionate, di-*iso*-propyl ether at 0 °C, which gave (*S*)-(−)-**4** with >90% ee. It was not possible to obtain (*S*)-(−)-**4** with an ee greater than 90%, probably due to hydrolysis of the product ester of (*R*)-(+)-**4** back to substrate (*R*)-(+)-**4** and thereby lowering the ee of remaining substrate (*S*)-(−)-**4**.[41] This problem is more pronounced in polar solvents than in non-polar as the water content at the same water activity is higher.[27] To obtain (*S*)-(−)-**4** in high enantiomerically pure form, the reaction was repeated in the presence of 4 Å molecular sieves to avoid the presumed hydrolysis of the produced ester of (*R*)-(+)-**4** (see Scheme 5). With this method, we successfully obtained (*S*)-(−)-**4** with an ee >98% although with a lower *E*-value in presence of molecular sieves vide supra (see Table 2, entries 21, 22 and 20).

3. Experimental

3.1. General

The glassware was dried overnight at 150 °C and the reactions were performed under an argon atmosphere. Diethyl ether used in the chemical syntheses was obtained in a dry form by distillation using $LiAlH_4$ as a drying agent. Thin layer chromatography (TLC) was carried out on precoated silica gel plates (Merck 60 F_{254}). Preparative liquid chromatography (LC) was performed on silica gel (Fluka Silica Gel 60, 0.040–0.063 mm, 230–400 mesh ASTM) employing a gradient of pentane/diethyl ether as eluant (0–100%). Mass spectra were recorded on a Saturn 2000 instrument, operating in the EI mode, coupled to a Varian 3800 GC instrument. NMR spectra were recorded on a Bruker DMX 250 (250 MHz ^{1}H and 62.9 MHz ^{13}C) or a Bruker Digital 500 (500 MHz ^{1}H and 125.8 MHz ^{13}C) spectrophotometer using $CDCl_3$ as solvent and TMS as internal reference. Optical rotations were determined using a Perkin–Elmer 241 and Perkin–Elmer 341 polarimeters using a 1 dm or a 0.1 dm cell. Gas chromatography on a Varian 3400 C_X was used to monitor the progress of enzymatic reactions (Alltech column EC1, 30 m × 0.32 mm; N_2, 5.0 bar). Enantiomeric excesses of compounds **1**–**4** were determined using GC Varian 3400 (Supelco column β-dex 120, 30 m × 0.25 mm × 0.25 μm; He, 20 Psi). Chirazyme® E-2 (*Hogliver esterase*), Chirazyme® L-3 (*Candida rugosa* Lipase), Chirazyme® L-5 (*Candida antarctica* lipase A), Chirazyme® L-6 (Lipase from *Pseudomonas species*), Chirazyme® L-7 (*Porcine pancreatic* lipase), Chirazyme® L-8 (*Thermomyces lanuginosa* lipase), Chirazyme® L-9 (*Mucor miehei* lipase) and Chirazyme® L-10 (Lipase from *Alcaligenes species*) were bought from Boehringer Mannheim GmbH, Germany but these Chiralzymes are no longer commercially availably from the Roche company. Lipase AS (*Aspergillus niger*), Lipase M 10 (*Mucor javanicus*), Lipase FAP 15 (*Rhizopus oryzae*), Lipase AK 20 (*Pseudomonas fluorescens*) and Lipase PS (*Burkholderia cepacia*) were obtained from Amano Enzyme Inc., Nagoya, Japan. Novozym 435 (*Candida antarctica* lipase B) was bought from Novo Nordisk (now Novozymes), Denmark. Lipase AL (*Achromobacter* sp.) and Lipase TL (*Pseudomonas stutzeri*) were obtained from Meito Sangyo co., Ltd, Tokyo, Japan.

3.2. (±)-3-Phenylbutanoic acid 5 and (±)-3-phenylpentanoic acid 6

The two title acids were prepared according to the malonic ester procedure[8] described for (±)-3-phenylpentanoic acid **6**.

3.2.1. (±)-3-Phenylbutanoic acid 5. Acid **5** (7.4 g, 88% yield) was isolated as a white solid: mp 36–38 °C (lit.[42] 35–38 °C); MS (EI): *m*/*z*: 164 (18) (M^+), 147 (3), 131 (2), 119 (5), 118 (29), 105 (100), 104 (11), 91 (10); IR (KBr) 2800–3100, 1707, 1494, 1431, 1320, 1280, 1217, 932, 758, 700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.31 (3H, d, J = 7.0), 2.54–2.58 (1H, d of d, J = 6.8 and J = 15.5), 2.64–2.68 (1H, d of d, J = 8.3 and J = 15.5), 3.20–3.30 (1H, apparent sextet J = 7), 7.18–7.22 (5H, m), 11.4–10.8 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 21.9, 36.18, 42.67, 126.57, 126.76, 128.63, 145.48, 179.06. IR data were similar to published data.[42]

3.2.2. (±)-3-Phenylpentanoic acid 6. Acid **6** (1.6 g, 74% yield) was isolated as a white solid: mp 48–50 °C (lit.[8] 47–49 °C); MS (EI): *m*/*z*: 178 (20) (M^+), 161 (17), 149 (9), 133 (4), 132 (16), 119 (48), 118 (84), 107 (100), 91 (87); IR (KBr) 2500–3100, 1696, 1454, 1405, 1279, 1178, 950, 757, 696; ^{1}H NMR and ^{13}C NMR spectra were similar to published data.[8]

3.3. (±)-4-Methyl-3-phenylpentanoic acid 7 and (±)-4,4-dimethyl-3-phenylpentanoic acid 8

The title acids were prepared from cinnamic acid as described for acid **7**.[19]

3.3.1. (±)-4-Methyl-3-phenylpentanoic acid 7. Acid **7** (2.3 g, 75% yield) was isolated as a white solid: mp 44–46 °C (lit.[8] 46–48 °C); MS (EI): *m*/*z*: 192 (10) (M^+), 177 (6), 149 (11), 133 (12), 132 (57), 117 (10), 115 (10), 107 (100), 104 (69), 91 (41); IR (KBr) 3050–2500, 1696, 1495, 1417, 1386, 1295, 950, 780, 748, 703; ^{1}H NMR ($CDCl_3$) δ 0.74–0.75 (3H, d, J = 6.7), 0.92-0.93 (3H, d, J = 6.7), 1.80–1.89 (1H, m), 2.56–2.61 (1H, q, J = 15.6), 2.76–2.80

(1H, q, $J = 15.6$), 2.84–2.88 (1H, m), 7.10–7.30 (5H, m), 9.5–10.5 (1H, br s); ^{13}C NMR ($CDCl_3$) δ 20.16, 20.54, 33.08, 38.11, 48.39, 126.41, 128.11, 128.19, 142.53, 179.04. ^{13}C NMR spectra were similar to published data.[8]

3.3.2. (±)-4,4-Dimethyl-3-phenylpentanoic acid 8. Acid **8** (3.0 g, 55% yield) was isolated as a white solid: mp 104–105 °C (lit.[43] 114–116 °C); MS (EI): *m/z*: 206 (2) (M^+), 189 (11), 173 (5), 150 (96), 149 (11), 132 (4), 131 (13), 104 (100), 91 (33), 90 (2); IR (KBr) 3050–2500, 1725, 1635, 1477, 1453, 1269, 1166, 1085, 893, 744, 707; ^{1}H NMR ($CDCl_3$) δ 0.86 (9H, s), 2.68–2.81 (2H, m), 2.91–2.94 (1H, q, $J = 4.4$), 7.12–7.25 (5H, m, ArH) ^{13}C NMR spectra were similar to published data.[8]

3.4. (±)-3-Phenyl-1-butanol 1, (±)-3-phenyl-1-pentanol 2, (±)-4-methyl-3-phenyl-1-pentanol 3 and (±)-4,4-dimethyl-3-phenyl-1-pentanol 4

The title alcohols were all obtained via $LiAlH_4$ reduction of the corresponding acid using a published method described for other types of acids.[44]

3.4.1. (±)-3-Phenyl-1-butanol 1. Alcohol **1** (6.5 g, 96% yield) was obtained as a colourless oil: bp 85 °C/2.0 mbar (lit.[45] 117 °C/8 mmHg); MS (EI): *m/z*: 150 (2) (M^+), 133 (6), 132 (36), 118 (8), 117 (72), 105 (100), 104 (12), 91 (40); IR (neat) 3333, 3027, 2959, 2929, 1063, 1494, 1452, 1047, 762, 700; ^{1}H NMR and ^{13}C NMR spectra were similar to published data for (3*R*)-(−)-3-phenyl-1-butanol.[25]

3.4.2. (±)-3-Phenyl-1-pentanol 2. Alcohol **2** (1.4 g, 93% yield) was obtained as a colourless oil: bp 130 °C/5 mbar (lit.[45] 108 °C/1 mmHg); MS (EI): *m/z*: 164 (5) (M^+), 147 (37), 146 (93), 131 (6), 119 (22), 117 (100), 105 (87), 104 (15), 91 (28); IR, ^{1}H NMR and ^{13}C NMR data were similar to published data for the pure enantiomers.[25,46]

3.4.3. (±)-4-Methyl-3-phenyl-1-pentanol 3. Alcohol **3** (2.2 g, 96% yield) was obtained as a colourless oil: bp 170 °C/4 mbar (lit.[25] 125 °C/0.2 Torr); MS (EI): *m/z* 178 (2) (M^+), 161 (28), 160 (76), 145 (6), 133 (12), 118 ((13), 117 (57), 105 (100), 104 (9), 91 (4); IR (neat) 3358, 2956, 1734, 1493, 1453, 1385, 1113, 1045, 743, 701; ^{1}H NMR ($CDCl_3$) δ 0.73 (3H, d, $J = 6.7$), 0.97 (3H, d, $J = 6.7$), 1.2–1.3 [1H, br s, disappears on shaking with D_2O), 1.77–1.86 (2H, m), 2.04–2.11 (1H, m), 2.38–2.42 (1H, m), 3.36–3.50 (2H, m), 7.12–7.29 (5H, m); ^{13}C NMR data was similar to published data for (3*S*)-(−)-4-methyl-3-phenyl-1-pentanol.[47]

3.4.4. (±)-4,4-Dimethyl-3-phenyl-1-pentanol 4. Alcohol **4**[48] (2.7 g, 100% yield) was obtained as a white solid: mp 48–50 °C; MS (EI): *m/z* 192 (2) (M^+), 159 (7), 136 (11), 135 (5), 118 (100), 117 (73), 105 (63), 104 (15), 91 (43); IR (KBr) 3319, 2951, 1478, 1451, 1231, 1051, 1022, 788, 722, 701; ^{1}H NMR ($CDCl_3$) δ 0.88 (9H, s), 1.55 (1H, s), 1.89–2.08 (2H, m), 2.44–2.47 (1H, d of d, $J = 12.2$ and $J = 3.0$), 3.29–3.45 (2H, m), 7.14–7.27 (5H, m); ^{13}C NMR ($CDCl_3$) δ 28.19, 32.47, 33.67, 52.87, 62.09, 126.16, 127.75, 129.44, 142.33.

3.5. General procedure for obtaining pre-equilibrated water activity

One vial containing a solution of 0.26 mmol of the substrate, a γ-substituted alcohol **1**, **2**, **3** or **4** in organic solvent (1.0 ml), one vial containing the vinyl ester and one vial containing the organic solvent were equilibrated in a desiccator containing a saturated aqueous solution of $MgCl_2 \cdot 6H_2O$ ($a_w = 0.32$)[41] for 16 h. The enzymes were stored over dry silica gel at 4 °C and used directly in the enzyme catalysis without any further treatment.

3.6. General procedure for enzyme catalyzed acylation of the γ-substituted alcohols 1, 2, 3 or 4

After the pre-equilibration step as above vinyl ester (1.0 mmol) was added to the vial containing the substrate solution and the volume was made up to 1.0 ml by adding some of the pre-equilibrated organic solvent. Alternatively, when dry conditions were desired and molecular sieves (4 Å) were used, the above equilibration step was excluded. The acylation reaction was started by the addition of 3–5 mg of enzyme. The reaction was carried out under an argon atmosphere. After stirring for the appropriate time at a specific temperature, the reaction was stopped by removing the enzyme by filtration. The solvent from the filtrate was evaporated under reduced pressure and the residue was chromatographed on silica gel (using a gradient of Et_2O in *n*-pentane as eluant) to separate the unreacted substrate from the product ester. The product ester obtained was reduced[44] back to alcohol with $LiAlH_4$ and then the enantiomeric excesses (ee_s and ee_p) were determined using GC Varian 3400 (column β-dex 120, 30 m × 0.25 mm × 0.25 μm; N_2, 5 Psi; He, 20 Psi). For retention times of substrates **1**, **2**, **3** and **4** see Table 1.

3.6.1. (*R*)-(−)- and (*S*)-(+)-3-phenyl-1-butanol (*R*)-(−)-1 and (*S*)-(+)-1. (±)-3-Phenyl-1-butanol **1** was acylated following the general procedure above using Chirazyme® L-10 in di-*iso*-propyl ether. The reaction was stopped after 4 h at 49% conversion to give the remaining substrate (*R*)-(−)-3-phenyl-1-butanol in a yield of 51% and in 43% ee, $[\alpha]_D^{20} = -10.75$ (*c* 0.4, $CDCl_3$) {lit.[23] $[\alpha]_D^{25} = -25.4$ (neat)} and (*S*)-(+)-3-phenyl-1-butanol (obtained after reduction from the produced ester) in a yield of 49% and in 45% ee, $[\alpha]_D^{20} = +11.5$ (*c* 0.4, $CDCl_3$) {lit.[24] $[\alpha]_D^{20} = +25.5$ (*c* 1.52, $CHCl_3$)}. Analytical data were identical with the data for the racemic compound **1** above.

3.6.2. (*R*)-(−)- and (*S*)-(+)-3-Phenyl-1-pentanol (*R*)-(−)-2 and (*S*)-(+)-2. (±)-3-Phenyl-1-pentanol **2** was acylated following the general procedure above using Chirazyme® L-10 in di-*iso*-propyl ether. The reaction was stopped after 5 h at 46.5% conversion to give (*R*)-(−)-3-phenyl-1-pentanol as remaining substrate in a yield of 54% and in 41% ee, $[\alpha]_D^{20} = -2.4$ (*c* 0.8, $CDCl_3$) {lit.[25] $[\alpha]_D^{20} = -7.9$ (*c* 4.79, CCl_4)} and (*S*)-(+)-3-phenyl-1-pentanol (obtained after reduction of the produced ester) in a yield of 47% and in 47% ee, $[\alpha]_D^{25} = +2.5$ (*c* 0.8, $CDCl_3$) {lit.[23] $[\alpha]_D^{25} = +14.2$ (neat)}. Analytical data were identical with the data for the racemic compound **2** above.

3.6.3. (*R*)-(+)- and (*S*)-(−)-4-methyl-3-phenyl-1-pentanol (*R*)-(+)-3 and (*S*)-(−)-3. (±)-4-Methyl-3-phenyl-1-pentanol **3** was acylated following the general procedure as described above using Chirazyme® L-10 in di-*iso*-propyl ether. The reaction was stopped after 1.75 h at 33.7% conversion to give (*S*)-(−)-4-methyl-3-phenyl-1-pentanol as the remaining substrate in a yield of 66.3% and in 39.2% ee, $[\alpha]_D^{20} = -5.5$ (*c* 4.0, $CDCl_3$) {lit.[25] $[\alpha]_D^{20} = -10.30$ (neat)} and (*R*)-(+)-4-methyl-3-phenyl-1-pentanol (obtained after reduction of the ester produced) in a yield of 33.7% and in 77% ee, $[\alpha]_D^{25} = +7.25$ (*c* 4.0, $CDCl_3$). Analytical data were identical with the data for the racemic compound **3** above.

3.6.4. (*R*)-(+)-4,4-Dimethyl-3-phenyl-1-pentanol (*R*)-(+)-4. (±)-4,4-Dimethyl-3-phenyl-1-pentanol **4** was first acylated following the general procedure above using Chirazyme® L-7 in di-*iso*-propyl ether. The reaction was kept under continuous stirring for 12 days and the reaction then stopped at 52% conversion, by filtering off the enzyme. The filtrate was evaporated and the residue was chromatographed (using a gradient of Et_2O in pentane as eluant) to separate the unreacted substrate (*S*)-(−)-**4** from the product ester of (*R*)-(+)-**4**. The product ester of (*R*)-(+)-**4** (60% ee) was reduced with $LiAlH_4$ to obtain the enantiomerically enriched alcohol (*R*)-(+)-**4** (60% ee) and this alcohol was subjected to lipase catalyzed acylation as described above but with Chirazyme® L-6 in chloroform. The reaction was stopped after 50 h at a conversion of 52%. After general work up as above (*R*)-(+)-**4**, 4-dimethyl-3-phenylpentan-1-ol (*R*)-(+)-**4** was obtained in an enantiomeric excess of >98% and in 25% total yield calculated from the racemate. $[\alpha]_D^{20} = +11.3$ (*c* 4.0, benzene) {lit.[26] $[\alpha]_D^{25} = +7.3$ (benzene)}.

3.6.5. (*S*)-(−)-4,4-Dimethyl-3-phenyl-1-pentanol (*S*)-(−)-4. The unreacted substrate (*S*)-(−)-**4** (66% ee) from above was once more subjected to Chirazyme® L-7 as described above but for 80 h and with 4 Å molecular sieves. The reaction was stopped at 52% conversion and after general work up as described above (*S*)-(−)-**4**, 4-dimethyl-3-phenyl-1-pentanol (*S*)-(−)-**4** was obtained in an enantiomeric excess of >98% and in 22% total yield calculated from the racemate. $[\alpha]_D^{20} = -11.0$ (*c* 4.0, benzene).

3.7. General procedure for enzyme catalyzed esterification of the γ-substituted acids 5, 6, 7 or 8

To γ-substituted acids **5**, **6**, **7** or **8** (67 mg, 0.44 mmol) and 1-decanol (69 mg, 0.44 mmol) in isooctane (2.5 ml) the salt pair Na_2SO_4 (80 mg, 0.5 mmol) and $Na_2SO_4{\cdot}10H_2O$ (85 mg, 0.26 mmol) was added to maintain a water activity of 0.76.[49] The esterification was started by addition of lipase either 170 mg of Amano® lipases (AK and PS) or 80 mg of CALB (Chirazyme® L-2) or CRL (immobilized on Accurel 200–300 μm).[50] After stirring for an appropriate time in room temperature, the reactions were stopped below 50% conversion by filtering off and washing the lipase several times with Et_2O. The product ester and remaining acid were separated via liquid chromatography using an increasing gradient of distilled EtOAc in distilled cyclohexane as eluant. After $LiAlH_4$ reduction in dry Et_2O the enantiomeric excess of both product and substrate alcohols were determined on a β-dex 120 chiral GC-column.

Acknowledgements

We thank Amano Enzyme Inc., Nagoya, Japan for the kind gift of the lipases AS, Lipase M 10, Lipase FAP 15, Lipase AK 20 and Lipase PS and we also thank Meito Sangyo co., Ltd, Tokyo, Japan for the kind gift of the lipases Lipase AL and Lipase TL. Financial support from Vinnova is gratefully acknowledged. We also thank Professor Anita Maguire and Dr. Francis Harrington for the synthesis of the first batch of (±)-4,4-dimethyl-3-phenylpentanoic acid.

References

1. Fuganti, C.; Serra, S.; Dulio, A. *J. Chem. Soc., Perkin Trans. 1* **1999**, 279–282.
2. Fuganti, C.; Serra, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 97–101.
3. Zada, A.; Harel, M. *Tetrahedron: Asymmetry* **2004**, *15*, 2339–2343.
4. Hedenström, E.; Edlund, H.; Lund, S.; Abersten, M.; Persson, D. *J. Chem. Soc., Perkin Trans. 1* **2002**, 1810–1817, and references cited therein.
5. Brenna, E.; Fuganti, C.; Serra, S. *Tetrahedron: Asymmetry* **2003**, *14*, 1–42.
6. Fuganti, C.; Serra, S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3758–3764.
7. Abate, A.; Brenna, E.; Negri, C. D.; Fuganti, C.; Serra, S. *Tetrahedron: Asymmetry* **2002**, *13*, 899–904.
8. Maguire, A. R.; Buckley, N. R.; O'Leary, P.; Ferguson, G. *J. Chem. Soc., Perkin Trans. 1* **1998**, 4077–4091.
9. Ghanem, A.; Aboul-Enein, H. Y. *Tetrahedron: Asymmetry* **2004**, *15*, 3331–3351.
10. For an extensive review on the use of lipases in organic chemistry see: Kazlauskas, R. J.; Bornscheuer, U. T. In *Biotransformations with Lipases*; Rehm, H. J., Reed, G., Eds.; Biotechnology: Biotransformations I; VCH, 1998; Vol. 8a, pp 38–191.
11. Nordin, O.; Nguyen, B.-V.; Vorde, C.; Hedenström, E.; Högberg, H.-E. *J. Chem. Soc., Perkin Trans. 1* **2000**, 367–376.
12. Baczko, K.; Larpent, C. *J. Chem. Soc., Perkin Trans. 1* **2000**, 521–526.
13. Mezetti, A.; Keith, C.; Kazlauskas, J. R. *Tetrahedron: Asymmetry* **2003**, *14*, 3917–3924.
14. Sankaranarayanan, S.; Sharma, A.; Chattopadhyay, S. *Tetrahedron: Asymmetry* **2002**, *13*, 1373–1378.
15. Kawasaki, M.; Goto, M.; Kawabata, S.; Kometani, T. *Tetrahedron: Asymmetry* **2001**, *12*, 585–596.
16. Im, D. S.; Cheong, C. S.; Lee, S. H.; Park, H.; Youn, B. H. *Tetrahedron: Asymmetry* **1999**, *10*, 3759–3767.
17. Gamalevich, G. D.; Serebryakov, E. P. *Russ. Chem. Bull.* **1997**, *46*, 171–183.
18. Cambou, B.; Klibanov, A. M. *J. Am. Chem. Soc.* **1984**, *106*, 2687–2692.
19. Iserloh, U.; Curran, D. P.; Kanemasa, S. *Tetrahedron: Asymmetry* **1999**, *10*, 2417–2428.
20. Högberg, H.-E.; Edlund, H.; Berglund, P.; Hedenström, E. *Tetrahedron: Asymmetry* **1993**, *4*, 2123–2126.
21. Valivetty, R. H.; Halling, P. J.; Macrae, A. R. *Indian J. Chem.* **1992**, *31B*, 914–916.

22. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
23. Hashimoto, S.-I.; Yamada, S.-I.; Koga, K. *Chem. Pharm. Bull.* **1979**, *27*, 771–782.
24. Shindo, M.; Koga, K.; Tomioka, K. *J. Org. Chem.* **1998**, *63*, 9351–9357.
25. Ahlbrecht, H.; Enders, D.; Santowski, L.; Zimmermann, G. *Chem. Ber.* **1989**, *122*, 1995–2004.
26. Menicagli, R.; Lardicci, L. *Chem. Ind.* **1974**, 576–577.
27. Jacobsen, E. E.; Anthonsen, T. *Can. J. Chem.* **2002**, *80*, 577–581.
28. Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *Tetrahedron: Asymmetry* **1996**, *7*, 625–628.
29. Ottosson, J.; Hult, K. *J. Mol. Cat. B: Enzym.* **2001**, *11*, 1025–1028.
30. Hirose, K.; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. *Tetrahedron: Asymmetry* **2000**, *11*, 1199–1210.
31. Wescott, C. R.; Klibanov, A. M. *Biochim. Biophys. Acta* **1994**, *1206*, 1–9.
32. Tanaka, K.; Yasuda, M. *Tetrahedron: Asymmetry* **1998**, *9*, 3275–3282.
33. Sakai, T.; Kishimoto, T.; Tanaka, Y.; Ema, T.; Utaka, M. *Tetrahedron Lett.* **1998**, *39*, 7881–7884.
34. Phillips, R. S. *Enzyme Microb. Technol.* **1992**, *14*, 417–419.
35. Giacometti, J.; Giacometti, F.; Milin, C.; Vasic-Racki, D. *J. Mol. Catal. B: Enzym.* **2001**, *11*, 921–928.
36. Kuwabara, K.; Watanabe, Y.; Adachi, S.; Nakanishi, K.; Matsuno, R. *Biochem. Eng. J.* **2003**, *16*, 17–22.
37. Kazlauskas, R. J. *Curr. Opin. Chem. Biol* **2000**, *4*, 81–88, and references cited therein.
38. Nguyen, B.-V.; Hedenström, E. *Tetrahedron: Asymmetry* **1999**, *10*, 1821–1826.
39. Lundh, M.; Smitt, O.; Hedenström, E. *Tetrahedron: Asymmetry* **1996**, *7*, 3277–3284.
40. Varadharaj, G.; Hazell, K.; Reeve, C. D. *Tetrahedron: Asymmetry* **1998**, *9*, 1191–1195.
41. Lundh, M.; Nordin, O.; Hedenström, E.; Högberg, H.-E. *Tetrahedron: Asymmetry* **1995**, *6*, 2237–2244.
42. Sigma–Aldrich website: http://www.sigmaaldrich.com/catalog/search/ProductDetail/ALDRICH/116807.
43. Koelsch, C. F. *J. Am. Chem. Soc.* **1943**, *65*, 1640–1643.
44. Hedenström, E.; Edlund, H.; Lund, S. *J. Mol. Catal. B: Enzym.* **2003**, *23*, 53–59.
45. Levene, P. A.; Marker, R. E. *J. Biol. Chem.* **1931**, *93*, 749–774.
46. Pridgen, L. N.; Mokhallalati, M. K.; Wu, M.-J. *J. Org. Chem.* **1992**, *57*, 1237–1241.
47. Azzena, C. U.; Pilo, L. *Synthesis* **1999**, *4*, 664–668.
48. Ibarra, C. A.; Gonzalez, F.; Perez-Ossorio, R. *Anal. Quim.* **1976**, *72*, 661–669.
49. Valivety, R. H.; Halling, P. J.; Peilow, A. D.; Macrae, A. R. *Biochim. Biophys. Acta* **1992**, *1122*, 143–146.
50. Sabbani, S.; Hedenström, E.; Nordin, O. *J. Mol. Catal. B: Enzym.* **2006**, *42*, 1–9.