Pergamon

SCIENCE DIRECT®

Tetrahedron: *Asymmetry* 14 (2003) 3917–3924

TETRAHEDRON: *ASYMMETRY*

Highly enantioselective kinetic resolution of primary alcohols of the type Ph-X-CH(CH_3)-CH_2OH by *Pseudomonas cepacia* lipase: effect of acyl chain length and solvent

Alessandra Mezzetti, Curtis Keith[†] and Romas J. Kazlauskas*[,‡]

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montréal, Québec, Canada H3A 2K6

Received 1 September 2003; accepted 18 September 2003

Abstract—Although lipase from *Pseudomonas cepacia* (PCL) shows high enantioselectivity towards many secondary alcohols, it usually exhibits only low to moderate enantioselectivity towards primary alcohols. To increase this enantioselectivity, we optimised the reaction conditions for the PCL-catalysed hydrolysis of esters of three chiral primary alcohols: 2-methyl-3-phenyl-1-propanol **1**, 2-phenoxy-1-propanol **2** and solketal **3**. The enantioselectivity towards **1**-acetate increased from $E=16$ to 38 upon changing the solvent from ethyl ether/phosphate buffer to 30% *n*-propanol in phosphate buffer and increased again to $E \geq 190$ upon changing the substrate from **1**-acetate to **1**-heptanoate. The same changes increased the enantioselectivity towards alcohol **2** from $E=17$ to 70, but did not significantly increase the enantioselectivity towards alcohol **3**. The best solvent was similar to the solvent used to crystallise the open form of PCL and likely stabilises the open form of PCL. This stabilisation may increase the enantioselectivity by removing kinetic contributions from a non-enantioselective lid-opening step. We determined the kinetic contribution of the lid-opening step by measuring the interfacial activation of PCL. The activation energy for the PCL-catalysed hydrolysis of ethyl acetate was at least 2.6 kcal/mol lower in the presence of a water–organic solvent interface.

1. Introduction

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) usually successfully resolve chiral secondary alcohols, but resolution of chiral primary alcohols is more difficult due to low enantioselectivities. Fewer lipases show any enantioselectivity toward primary alcohols and the enantioselectivities are usually low to moderate.[1] Such reactions often require the optimisation of the reaction conditions to increase the enantioselectivity.

Changing the acyl group often increases the enantioselectivity of lipases towards primary alcohols. Different acyl groups lead to different structures for the transition state, which determines the enantioselectivity. For example, Miyazawa et al.[2] improved the enantioselectivity of lipase AL towards 2-phenoxy-1-propanol **2** from 1.1 to 19 by replacing vinyl trifluoroacetate with vinyl butanoate as acylating agent. However, for PCL, vinyl acetate gave higher E ($E=42$) than the butanoate ($E=35$) or the trifluoroacetate ($E=1.5$).[3] Hirose et al.[4] improved the resolution of 2-phenyl-1-propanol by using chiral acyl donors. With lipase QL in hexane racemic vinyl-3-phenylbutanoate gave $E=32$, vinyl (*R*)-3-phenylbutanoate yielded low enantioselectivity ($E=4$) and slow reaction (41 h for 27% conversion), while the (*S*) enantiomer provided higher E ($E=30$) and faster reaction (1 h for 58% conversion). These results suggest that the alcohol and acyl chain moieties interact during enantiorecognition by lipases.

Changing the solvent also increases the enantioselectivity, especially for transesterification reactions. Indeed, transesterification in organic solvents has been more successful for resolving chiral primary alcohols than the hydrolysis of the corresponding esters.[3–5] Miyazawa et al.[5] increased the enantioselectivity of the lipase AK-catalysed transesterification of **2** with vinyl butanoate from 4.5 in benzene to 35 in diisopropyl ether. Hirose et al.[4] improved the enantioselectivity of the lipase

* Corresponding author. Tel.: +1-612-624-5904; fax: +1-612-625-5780; e-mail: rjk@umn.edu

† Present address: CombinatoRx Inc., 650 Albany Street, Boston, MA 02118, USA.

‡ Present address: Department of Biochemistry, Molecular Biology & Biophysics and The Biotechnology Institute, University of Minnesota, 1479 Gortner Avenue, St. Paul, MN 55108, USA.

0957-4166/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2003.09.049

SL-catalysed transesterification of 2-phenyl-1-propanol with racemic vinyl-3-phenylbutanoate from $E=40$ in hexane to $E=98$ in diisopropyl ether. Despite the numerous attempts to relate the change in enzyme enantioselectivity to a solvent property such as log P, trial and error is still the best method to choose the best solvent.[6–9]

Herein, we study the PCL-catalysed hydrolysis of three chiral primary alcohols: 2-methyl-3-phenyl-1-propanol **1**, 2-phenoxy-1-propanol **2** and solketal **3**. Pure enantiomers of **1** have been used for the preparation of fungicidal compounds,[10] for the synthesis of adenosine receptor agonists and antagonists[11] and for the synthesis of the side chain of zaragozic acid A.[12] Pure enantiomers of **2**, structurally related to **1**, have proven useful for the synthesis of juvenoids, analogues of the insect juvenile hormone[13] and pure enantiomers of **3** have been used as building blocks in the synthesis of bioactive compounds such as the β-adrenergic blocker timolol.[14]

(*R*)-**1** has been prepared in ee >98% by baker's yeast-catalysed reduction of (2-dimethoxymethylallyl)benzene.[15] High enantioselectivity ($E=172$) was also obtained in the *Pseudomoas fluorescens* lipase-catalysed transesterification of **1** with vinyl acetate in methylene chloride.[16] Furthermore, while the PCL-catalysed hydrolysis of **1**-acetate proceeded with only moderate enantioselectivity ($E=16$),[17] the esterification of **1** with vinyl acetate in chloroform was more successful ($E=116$[18] and $E=120$[19]). Similarly, while the PCL-catalysed hydrolysis of **2**-acetate proceeds with modest enantioselectivity ($E=17$),[20] PCL catalyses the transesterification of **2** with vinyl acetate in diisopropyl ether with higher enantioselectivity ($E=42$).[5] The resolution of alcohol **3** has been the subject of extensive research in the past 15 years. The best results have been achieved in the enantioselective oxidation ($E>100$) of solketal to solketalic acid by two alcohol dehydrogenases[21] and in the lipase AK-catalysed acylation of **3** with vinyl butanoate at −40°C ($E=55$).[22] The PCL-catalysed hydrolysis of **3**-benzoate, however, gave low enantioselectivities, ranging from $E=3.7$ to 9.[23]

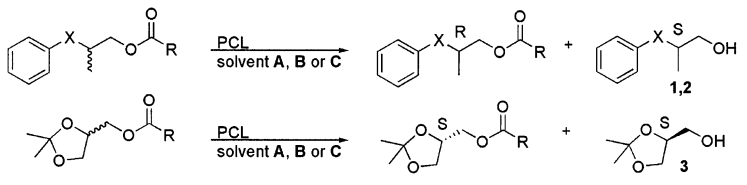
To increase the enantioselectivity of PCL towards esters of chiral primary alcohols **1**, **2** and **3**, we optimised both the acyl chain length of the substrate and the reaction conditions. We also verified the rate enhancement of PCL-catalysed hydrolysis in condition of interfacial activation using several achiral esters.

2. Results

We investigated how the acyl chain length influences the enantioselectivity of PCL towards alcohols **1**, **2**, and **3**. We tested the acetate, butanoate and heptanoate of alcohol **1** and the acetate and heptanoate of alcohols **2** and **3** in potassium phosphate buffer, pH 7.0, containing 30 vol% of *n*-propanol (solvent **A**) (Scheme 1 and Table 1, entries 1, 6, 7, 9, 10, 12 and 13). A similar solvent previously promoted the crystallisation of the open form of PCL.[24] Here, however, the concentration of PCL in solvent **A** is kept below 10 mg/mL to avoid its crystallisation while still stabilising the open form. The concentration of the substrates in all reactions was above their solubility limit so that some organic phase was always present to promote interfacial activation of PCL.

In the case of alcohol **1**, PCL showed the highest enantioselectivity with the heptanoate ($E\geq190\pm30$, Table 1, entry 1) and the lowest with the acetate ($E=38\pm6$, Table 1, entry 7). For the butanoate, PCL exhibited an intermediate enantioselectivity ($E=130\pm30$). In the case of alcohol **2** however, the *E* values were within experimental error ($E=70\pm20$ and $E=52\pm6$, Table 1, entries 9 and 10). We did not detect any reaction for (±)-**3**-acetate, while the enantioselectivity of PCL towards (±)-**3**-heptanoate ($E=5.2\pm0.1$) was significantly lower than for substrates **1** and **2** and similar to what previously reported for the hydrolysis of solketal benzoate by PCL ($E=3.7$ to 9).[23] For all the alcohols the reactions were ca. two-fold faster with long acyl chain esters.

The enantioselectivity for the PCL-catalysed hydrolysis of **1**-heptanoate also varied with the solvent. In biphasic 30 vol% *t*-butyl methyl ether (*t*-BME)/potassium phosphate buffer, pH 7.0 (solvent **B**) the enantioselectivity dropped to 120±20 and in only potassium phosphate buffer, pH 7.0 (solvent **C**), the enantioselectivity further dropped to 61±4 (Table 1, entries 4 and 5).



1 X= CH_2, **2** X= O
R = C_6H_{13}, C_3H_7, CH_3
A 30 vol% *n*-propanol in potassium phosphate 0.4 M, pH 7.0; **B** 30 vol% *t*-butyl methyl ether/potassium phosphate 0.4 M, pH 7.0; **C** potassium phosphate 0.4 M pH 7.0

Scheme 1.

Table 1. PCL-catalysed hydrolysis of esters of **1**, **2** and **3** in different solvents

Entry	Substrate	Enzyme source	Solvent[a]	Conv. (%)	Ee_p (%)	Ee_s (%)	E[b]
1	(±)-**1**-Heptanoate	GPCL	**A**	39.2±0.1	≥98.0±0.2	63.0±0.2	≥190±30 (*S*)
2	(±)-**1**-Heptanoate	LPL 200S	**A**	36.4±0.1	≥98.0±0.2	56±5	≥180±30 (*S*)
3	(±)-**1**-Heptanoate	Lipase PS	**A**	35.1±0.1	≥98.0±0.2	52.5±0.7	≥170±30 (*S*)
4	(±)-**1**-Heptanoate	GPCL	**B**	15.5±0.5	98.0±0.2	19.0±0.2	120±20 (*S*)
5	(±)-**1**-Heptanoate	GPCL	**C**	32.0±0.1	95.0±0.2	45.0±0.2	61±4 (*S*)
6	(±)-**1**-Butanoate	GPCL	**A**	17±2	98.0±0.2	21±3	130±30 (*S*)
7	(±)-**1**-Acetate	GPCL	**A**	28.0±0.5	93±1	35.0±0.3	38±6 (*S*)
8	(±)-**1**-Acetate[17]	LPL 200S or PS30	n.r.[c]	44	79	62	16 (*S*)
9	(±)-**2**-Heptanoate	GPCL	**A**	51.0±0.1	90±1	94±1	70±20 (*S*)
10	(±)-**2**-Acetate	GPCL	**A**	18±3	95.5±0.4	21±4	52±6 (*S*)
11	(±)-**2**-Acetate[20]	LPL 200S	**B**	n.a.	n.a.	n.a.	17 (*S*)
12	(±)-**3**-Heptanoate	GPCL	**A**	27±4	62±2	23±4	5.2±0.1 (*S*)
13	(±)-**3**-Acetate	GPCL	**A**	n.r.[d]	–	–	–

[a] **A** = 30 vol% *n*-propanol in potassium phosphate 0.4 M, pH 7.0; **B** = 30 vol% *t*-butyl methyl ether in potassium phosphate 0.4 M, pH 7.0; **C** = potassium phosphate 0.4 M, pH 7.0

[b] Determined from ee_p% and ee_s% according to Chen et al.;[38] enantiomeric purities of the starting materials and products were measured by gas chromatography directly or after conversion to the **1**-acetate or **2**-trifluoroacetate; alcohol enantiopreference in parentheses.

[c] 'a rapidly stirred suspension of substrate dissolved in a small amount of ether and phosphate buffer (10 mM, pH 7) containing lipase from *P. cepacia*…'[17]

[d] No enzymatic hydrolysis was observed after 48 h.

All three commercial samples of PCL tested catalysed the hydrolysis of **1**-heptanoate, favouring the (*S*)-enantiomer, with high enantioselectivity, $E \geq 180\pm30$ (Table 1, average of entries 1–3). PCL from Genzyme Diagnostics (GPCL), Amano LPL200S and Amano lipase PS, all gave E values that were within experimental error of each other, $E \geq 190\pm30$, $E \geq 180\pm30$ and $E \geq 170\pm30$, respectively.

To verify the interfacial activation of PCL, i.e. the rate enhancement upon changing from a soluble to an insoluble substrate form, we measured its reaction rate of ester hydrolysis in potassium phosphate buffer with increasing concentration of the substrate for ethyl acetate, isopropenyl acetate, *n*-pentyl acetate and ethyl butanoate, as well as for methyl acetate (Fig. 1A). While methyl acetate, a soluble ester, displayed a steady increase in reaction rate with increasing concentration, the other four substrates showed a sharp rate enhancement at a particular substrate concentration. This concentration matched the solubility limit of the substrate as seen by an increase in cloudiness of the solution. The rate increase ranged from about 10-fold for ethyl butanoate to about 40-fold for *n*-pentyl acetate. Isopropenyl acetate showed almost a 15-fold increase upon reaching the solubility limit and ethyl acetate exhibited a 25-fold increase.

These experiments demonstrate the classical interfacial activation phenomenon that is characteristic of lipases,[25,26] but only estimate its magnitude. One reason for an underestimate is that the experiments contained an interface even at concentrations where the ester was still soluble. The ester may absorb to the polyethylene surface of the reaction vessel or to the surface of bubbles formed by stirring. Since a surface is present while we measure the soluble rate, this rate is likely higher than the true soluble rate. A second reason why the experiments may not be accurate is that the substrate concentration also changes abruptly at the solubility limit. The concentration changes from the soluble concentration in the aqueous phase to neat substrate concentration in the insoluble phase. It is not clear how this concentration change might influence the measured interfacial activation.

To eliminate the concentration effect, we compared the rates of hydrolysis of ethyl acetate by PCL at identical substrate concentrations with and without an interface present. The ethyl acetate was either dissolved in water or in a second phase of heptane. The rates with interface were seventy-five times higher than those without interface (Fig. 1B). The experiment discussed in the previous paragraphs showed that the rate of hydrolysis of ethyl acetate increased only 25-folds at the solubility limit of ethyl acetate. The difference in the two experiments is due to the different organic phase—pure ethyl acetate or ethyl acetate dissolved in heptane. The heptane interface lowers the activation energy by 2.6 kcal/mol (RTln75). This value is a lower limit since the without-interface experiments still contained the unavoidable air–water interface and the water–reaction vessel interface. Solubility limits prevented the measurement of the kinetic parameters k_{cat} and K_m. Other esters were not soluble enough in water to measure the without-interface initial rate under pseudo-first order conditions.

3. Discussion

The largest change in enantioselectivity, observed during the resolution of esters of alcohol **1** (from 38 to ≥190, corresponding to a free energy change of ~3.1 kcal/mol in the transition state), occurred upon extending the ester acetyl group to a heptanoyl group. Weissfloch and Kazlauskas[17] reported $E = 16$ for the resolution of **1**-acetate in an ether/water mixture. The

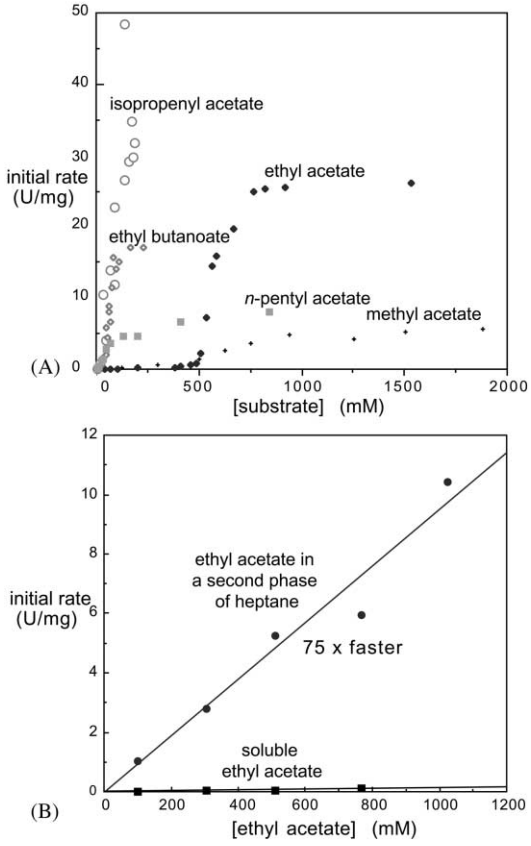


Figure 1. Initial rate versus substrate concentration for PCL-catalysed hydrolysis of several esters. (A) The observed rates of hydrolysis increase 10 to 40-fold at a particular concentration (~50 mM for ethyl butanoate, isopropenyl acetate and pentyl acetate; ~600 mM for ethyl acetate). These concentrations match the solubility limit of the ester as seen by the increased cloudiness of the solution. The maximum rate for isopropenyl acetate was ~300 U/mg, but this is beyond the *y*-axis limit of this chart. (B) Initial rate of hydrolysis of ethyl acetate, either dissolved or present in a separate heptane phase, as a function of ethyl acetate concentration. At the same substrate concentration, the initial rate was 75-fold faster in heptane. This increase is due to interfacial activation. All experiments used Amano LPL-200S, a purified lipase preparation.

fact that PCL exhibited, in all the solvents used, higher enantioselectivity for **1**-heptanoate ($E=120$ in the very similar solvent **B**, $E \geq 190$ in solvent **A** and $E=61$ in solvent **C**) shows not only the important role of the solvent, but also of the acyl chain length in determining PCL enantioselectivity.

The long heptanoyl chain may reduce the flexibility of the alcohol moiety in the transition state. X-Ray crystal structures show that the acyl chain binds to the large hydrophobic pocket (hydrophobic groove HA) of PCL.[27] This pocket comprises PCL hydrophobic residues Pro113, Phe119, Leu164, Leu167, Val266 and Val267. With a short acyl chain like an acetyl, the substituents from the alcohol moiety can also bind in this pocket.[28] The longer heptanoyl chain prevents the alcohol moiety from binding in this pocket and thus restricts the flexibility of the alcohol moiety.

The solvent effect on enantioselectivity may involve the phenomenon of interfacial activation. PCL enantioselectivity towards **1**-hepanoate was lowest in aqueous buffer where the only organic phase present was the undissolved substrate ($E=61$, solvent **C**). Upon adding *t*-butyl methyl ether as organic phase the enantioselectivity increased to $E=120$ (solvent **B**). Finally, upon changing to a solvent mixture that favoured crystallisation of the open form of PCL, its enantioselectivity increased even further ($E \geq 190$, solvent **A**).

Overbeeke et al.[29] suggested a mechanism by which interfacial activation could contribute to enantioselectivity even though it precedes the transition state for the chemical reaction. They found a similar increase in enantioselectivity (from $E=8$ to 16) for the porcine pancreatic lipase-catalysed hydrolysis of glycidyl butanoate when passing from water to a biphasic water/substrate system, i.e. in conditions of interfacial activation.[30] They showed that when the interfacial activation is slow (rate similar to that for the chemical reaction), it will also contribute to the rate of the overall reaction. Since interfacial activation is presumably non-enantioselective, this contribution lowers the overall enantioselectivity. Reaction conditions that speed up interfacial activation may thus also increase the enzyme enantioselectivity.[29] Increasing the amount of organic phase (solvent **C** to **B**) or using a solvent mixture that favours the open form of the enzyme (solvent **A**) might increase the lipase enantioselectivity by speeding up its interfacial activation. The higher reactivity of primary alcohols as compared to secondary alcohols[31] may also make interfacial activation more important to the enantioselectivity of lipases towards primary alcohols than towards secondary alcohols.

We also measured the minimum contribution of the interfacial activation to the reaction rate of PCL-catalysed hydrolysis of ethyl acetate. At the same substrate concentrations, ethyl acetate in a separate organic phase reacts at least 75-times faster (≥ 2.6 kcal/mol contribution to the stabilisation of the transition state) than ethyl acetate dissolved in the aqueous phase.

The value of ≥ 2.6 kcal/mol for the interfacial activation of PCL is consistent with its proposed molecular basis. In the closed inactive form,[§] the active site is blocked and a key catalytic residue-Leu17, which stabilises the oxyanion of the tetrahedral intermediate, is

[§] Several groups have solved the X-ray crystal structure of the open form of PCL,[24,32] but not of the closed form. However, we assume that this structure would be similar to that of *Pseudomonas glumae* lipase (PGL), which shares 83% identity with PCL and whose closed form X-ray crystal structure has been solved.[33]

incorrectly oriented. In the open form of PCL,[24,32] the active site is accessible and Leu17 is correctly oriented. A value of ≥ 2.6 kcal/mol is reasonable for a hydrogen bond contribution to catalysis, but it is difficult to predict how much the unblocking of the active site would contribute to catalysis.

Another explanation for the increased enantioselectivity upon changing the solvent is decreased enzyme flexibility. The increase in enzyme enantioselectivity in organic solvents has been suggested to be due to decreased enzyme flexibility.[34,35] The lower dielectric constant of organic solvents as compared to an aqueous environment increases the strength of the intramolecular electrostatic interactions and thus decreases the flexibility of the enzyme. The crystallization solvent used in our experiments may also decrease the flexibility of PCL since it promotes crystallisation of the lipase open form.

This is the first time that esters of chiral primary alcohols **1** and **2** have been resolved with high E via PCL-catalysed hydrolysis. By the accurate choice of solvent and acyl chain length, we increased the enantioselectivity of PCL from 16[17] to ≥ 190 for the hydrolysis of (±)-**1**-heptanoate and from 17[20] to 70 for the hydrolysis of (±)-**2**-heptanoate. Unfortunately this strategy did not improve the enantioselectivity of PCL toward alcohol **3**. The resolution of esters of chiral primary alcohols remains more difficult than the resolution of secondary alcohols, probably due to their greater flexibility. These results show that the enantioselectivity of PCL is highly substrate dependent and each primary alcohol must be evaluated individually to determine the best set of conditions for its resolution.

4. Experimental

All chemicals were purchased from Sigma-Aldrich Co. (Oakville, ON) unless otherwise specified. Lipases from *Pseudomonas cepacia* were purchased from Genzyme Diagnostics (Cambridge, MA) (2190 U/mg powder) and Amano Enzyme USA Co., Ltd. (Lombard, IL) (LPL200S 2260 U/mg powder or Lipase PS ≥ 30 U/mg powder). (±)-2-Phenoxy-1-propanol was purchased from TCI America (Portland, OR). Rates of hydrolysis of achiral substrates were measured by a Radiometer Copenhagen pH-stat (Copenhagen, Denmark). The chiral column used for the GC analyses was a Chirasil-DEX CB, 25 m×0.25 mm×0.25 μm (Life Sciences, Peterborough, ON). The chiral column used for the HPLC analysis was a Chiracel OD, 25 cm×4.6 mm (Daicel Chemical Industries, Ltd., Exton, PA). NMR spectra were collected at 270 Mz for ^{1}H and 68 MHz for ^{13}C.

4.1. Interfacial activation

Rates of ester hydrolysis were measured by pH-stat in potassium phosphate buffer (10 mM, pH 7.0), with an endpoint set at pH 7.15. PCL (Amano LPL200S) in buffer (10 mL, 0.5 mg lipase/mL) in a polypropylene cup was stirred vigorously with the mechanical stirrer until a constant pH was attained (5 min). Upon addition of substrate, the recorder was started and the initial rate measured. The activity of the enzyme was calculated from these slopes and a [substrate]-rate profile plotted for each ester assayed. In some cases with soluble substrates, a magnetic stirrer replaced the mechanical stirrer.

4.2. (±)-2-Methyl-3-phenyl-1-propanol, 1

Lithium aluminium hydride (4.30 g, 110 mmol) was placed in a flame-dried reaction flask under argon atmosphere. The flask was cooled to 0°C and anhydrous tetrahydrofuran (100 mL) was added. 2-Methyl-3-phenyl propenal (3.70 g, 25 mmol) dissolved in anhydrous tetrahydrofuran (25 mL) was added dropwise. The reaction was stirred overnight under argon atmosphere, quenched with a saturated solution of ammonium chloride, filtered, extracted with ethyl acetate, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. The product was purified by flash chromatography on a silica gel column (eluent: 7:3 hexane:ethyl acetate). Yield 89%. $R_f = 0.55$ (silica gel, 7:3 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.15–7.30 (m, 5H, Ph), 3.42–3.55 (m, 2H, CH_2), 2.75 (dd, 1H, CH_2, $J_1 = 6.2$ Hz, $J_2 = 13.4$ Hz), 2.41 (dd, 1H, CH_2, $J_1 = 8.2$ Hz, $J_2 = 13.4$ Hz), 1.87–2.00 (m, 1H, CH), 0.91 (d, 3H, CH_3, $J = 6.7$ Hz); ^{13}C NMR ($CDCl_3$) δ 16.5, 37.9, 39.8, 67.5, 125.9, 128.3, 129.3, 140.9; MS (EI) m/z (rel. int.) 150 (26, M^+), 132 (16, $M-H_2O$), 117 (39, $M^+-H_2O-CH_3$), 91 (100, $M^+-CH(CH_3)CH_2OH$), 77 (8, Ph).

4.3. (±)-2-Methyl-3-phenyl-1-propyl heptanoate, 1-heptanoate

A solution of (±)-2-methyl-3-phenyl-1-propanol (0.50 g, 3.3 mmol) and pyridine (0.42 mL, 5 mmol) in dry ether (50 mL) was stirred at 4°C. Heptanoyl chloride (0.97 mL, 6.7 mmol) was added dropwise to the solution at 4°C. After 2 h the reaction mixture was washed with a saturated solution of $NaHCO_3$, water and brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. The product was purified by flash chromatography on a silica gel column (eluent: 8:2 hexane:ethyl acetate) and isolated as a yellow oil. Yield 90%. $R_f = 0.78$ (silica gel, 8:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.07–7.30 (m, 5H, Ph), 3.87–4.00 (m, 2H, CH_2), 2.40–2.76 (m, 2H, CH_2), 2.31 (t, 2H, CH_2, $J = 7.4$ Hz), 2.04–2.17 (m, 1H, CH), 1.57–1.68 (m, 2H, CH_2), 1.23–1.35 (m, 6H, $3CH_2$), 0.86–0.93 (m, 6H, $2CH_3$); ^{13}C NMR ($CDCl_3$) δ 14.1, 16.7, 22.6, 25.1, 28.9, 31.6, 34.4, 34.7, 39.9, 68.5, 126.1, 128.3, 129.2, 140.1, 174.0; MS (CI/NH_3) m/z (rel. int.) 263 (4, M+H$^+$), 132 (100, $M^+-C_6H_{13}COOH$), 91 (44, tropylium), 77 (2, Ph).

4.4. (±)-2-Methyl-3-phenyl-1-propyl butanoate, 1-butanoate

A solution of (±)-2-methyl-3-phenyl-1-propanol (0.50 g, 3.3 mmol), butyric anhydride (0.55 g, 3.5 mmol), triethylamine (0.48 mL, 6.7 mmol) and 4-(dimethylamino)pyridine (10 mg, 0.08 mmol) in methylene

chloride (25 mL) was stirred at room temperature for 3 h. The reaction mixture was washed with a saturated solution of $NaHCO_3$, water and brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. The product was purified by flash chromatography on a silica gel column (eluent: 8:2 hexane:ethyl acetate) and isolated as a yellow oil. Yield 89%. R_f= 0.72 (silica gel, 8:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.06–7.30 (m, 5H, Ph), 3.87–4.00 (m, 2H, CH_2), 2.40–2.76 (m, 2H, CH_2), 2.29 (t, 2H, CH_2, J=7.4 Hz), 2.04–2.17 (m, 1H, CH), 1.59–1.73 (m, 2H, CH_2), 0.90–0.98 (m, 6H, $2CH_3$); ^{13}C NMR ($CDCl_3$) δ 13.8, 16.8, 18.6, 34.7, 36.3, 39.9, 68.5, 126.1, 128.4, 129.2, 140.1, 173.8; MS (CI/NH_3) m/z (rel. int.) 221 (9, M+H$^+$), 132 (>81, M$^+$–C_3H_7COOH), 117 (>100, M$^+$-), 91 (>84, $PhCH_2$), 77 (3, Ph), 71 (27, C_4H_7O).

4.5. (±)-2-Methyl-3-phenyl-1-propyl acetate, 1-acetate

A solution of (±)-2-methyl-3-phenyl-1-propanol (2.50 g, 16.7 mmol), acetic anhydride (1.85 mL, 16.7 mmol), triethylamine (2.37 mL, 33.4 mmol) and 4-(dimethylamino) pyridine (10 mg, 0.08 mmol) in methylene chloride (30 mL) was stirred at room temperature for 3 h. The reaction mixture was washed with a saturated solution of $NaHCO_3$, water and brine, dried over anhydrous $MgSO_4$ and the solvent was evaporated under vacuum. The product was purified by flash chromatography on a silica gel column (eluent: 7:3 hexane:ethyl acetate) and isolated as a yellow oil. Yield 94%. R_f= 0.76 (silica gel, 7:3 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.06–7.30 (m, 5H, Ph), 3.85–3.98 (m, 2H, CH_2), 2.40–2.76 (m, 2H, CH_2), 2.02–2.11 (m, 1H, CH), 2.01 (s, 3H, CH_3), 0.91 (d, 3H, CH_3, J=6.7 Hz); ^{13}C NMR ($CDCl_3$) δ 14.3, 16.7, 34.6, 40.1, 68.7, 126.1, 128.4, 129.2, 141.6, 171.1; MS (CI/NH_3) m/z (rel. int.) 193 (15, M+H$^+$), 133 (48, M$^+$–CH_3COOH), 117 (100, M$^+$–H_2O–CH_3), 91 (84, tropylium), 77 (4, Ph).

4.6. (±)-2-Phenoxy-1-propyl heptanoate, 2-heptanoate

The reaction was performed as for **1**-heptanoate. Yield 89% (yellow oil). R_f=0.77 (silica gel, 8:2 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 6.90–7.30 (m, 5H, Ph), 4.54–4.65 (m, 1H, CH), 4.12–4.29 (m, 2H, CH_2), 2.29 (t, 2H, CH_2, J=7.4 Hz), 1.53–1.68 (m, 2H, CH_2), 1.22–1.36 (m, 9H, $3CH_2$+CH_3), 0.86 (t, 3H, CH_3, J= 5.2 Hz); ^{13}C NMR ($CDCl_3$) δ 14.1, 16.9, 22.5, 24.9, 28.8, 31.5, 34.3, 66.8, 71.8, 116.2, 121.2, 129.6, 157.8, 173.8; MS (EI) m/z (rel. int.) 264 (4, M$^+$), 171 (100, M$^+$–PhO), 113 (27, $C_6H_{13}CO$), 94 (20.6, PhOH), 77 (9, Ph).

4.7. (±)-2-Phenoxy-1-propyl acetate, 2-acetate

The reaction was performed as for **1**-acetate. Yield 91% (yellow oil). R_f=0.59 (silica gel, 7:3 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 7.23–7.30 (m, 2H, Ph), 6.88–6.95 (m, 3H, Ph), 4.55–4.66 (m, 1H, CH), 4.25 (dd, 1H, CH_2, J_1=6.4 Hz, J_2=11.6 Hz), 4.15 (dd, 1H, CH_2, J_1=4.3 Hz, J_2=11.6 Hz), 2.06 (s, 3H, CH_3), 1.32 (d, 3H, CH_3, J=6.2 Hz); ^{13}C NMR ($CDCl_3$) δ 16.7, 20.9, 67.0, 71.8, 116.2, 121.3, 129.6, 157.8, 171.0; MS (EI) m/z (rel. int.) 194 (8, M$^+$), 101 (89, M$^+$–PhO), 94 (83, PhOH), 77 (19, Ph), 43 (>100, CH_3CO).

4.8. (±)-2,2-Dimethyl-[1,3]dioxolan-4-methyl heptanoate, 3-heptanoate

The reaction was performed as for **1**-heptanoate. Yield 92% (yellow oil). R_f=0.71 (silica gel, 7:3 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 4.23–4.31 (m, 1H, CH), 3.99–4.15 (m, 2H, CH_2), 3.67–3.71 (m, 2H, CH_2), 2.30 (t, 2H, CH_2, J=7.7 Hz), 1.52–1.63 (m, 2H, CH_2), 1.39 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.21–1.30 (m, 6H, $3CH_2$), 0.84 (t, 3H, CH_3, J=6.9 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.5, 24.9, 25.4, 26.7, 28.8, 31.5, 34.2, 64.5, 66.4, 73.7, 109.8, 173.6; MS (CI/NH_3) m/z (rel. int.) 245 (12, M+1), 229 (61, M$^+$–CH_3), 113 (33, M$^+$–(2,2-dimethyl-[1,3]dioxolan-4-methanol).

4.9. (±)-2,2-Dimethyl-[1,3]dioxolan-4-methyl acetate, 3-acetate

The reaction was performed as for **1**-acetate. Yield 93% (yellow oil). R_f=0.71 (silica gel, 7:3 hexane:ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 4.22–4.31 (m, 1H, CH), 3.99–4.15 (m, 2H, CH_2), 3.65–3.70 (m, 2H, CH_2), 2.10 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.29 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 20.8, 25.4, 26.7, 64.9, 66.3, 73.6, 109.9, 170.8; MS (CI/NH_3) m/z (rel. int.) 175 (27, M+1), 159 (83, M–CH_3).

4.10. PCL-catalysed kinetic resolution of esters of alcohol 1

Substrates (±)-**1**-heptanoate, (±)-**1**-butanoate and (±)-**1**-acetate (0.08 mmols) were suspended or dissolved in the appropriate solvent (3.28 mL) (Table 1). PCL solutions (0.05 mL) in 0.4 M potassium phosphate buffer at pH 7.0 (GPCL and LPL200S 0.0084 mg/mL, PS 0.2050 mg/mL) were added to the reaction vials. The mixtures were stirred at room temperature and monitored by thin layer chromatography (TLC) (hexane:ethyl acetate 8:2) until about 50% conversion (visual estimate). The reactions were quenched with 2% HCl until pH 2, extracted with ethyl ether and the solvent evaporated under vacuum. Starting material ester and product alcohol were separated by preparative TLC (hexane:ethyl acetate 8:2). The recovered esters **1**-heptanoate and **1**-butanoate were converted to the corresponding alcohol **1** by NaOH promoted hydrolysis in aqueous ethanol (15 w/v% NaOH in 50 vol% aqueous ethanol) at room temperature. The alcohols were converted to **1**-acetate by pyridine-catalysed esterification using acetic anhydride. The enantiomeric excesses (ee) of the non-reacted starting materials (**1**-heptanoate, **1**-butanoate and **1**-acetate) and of the product (**1**) of the reactions were derived from the ee of **1**-acetate measured by gas chromatograpy: 120°C isothermal for 35 min and raised to 180°C at a rate of 10°C/min, 8.5 psi. Retention times were 31.2 min for (*S*)-3-methyl-2-phenyl-1-propyl acetate ((*S*)-**1**-acetate) and 31.9 min for (*R*)-3-methyl-2-phenyl-1-propyl acetate ((*R*)-**1**-acetate); α=1.02. (*S*)-**1** $[\alpha]_D^{20}$=−11.3 (*c* 0.124, C_6H_6), lit.[36] $[\alpha]_D^{20}$=−11.1 (*c* 4.6, C_6H_6).

4.11. PCL-catalysed kinetic resolution of esters of alcohol 2

Kinetic resolutions were carried out as for the esters of alcohol **1**. Starting ester and product alcohol were separated by preparative TLC (hexane:ethyl acetate 8:2). The recovered esters **2**-heptanoate and **2**-acetate were converted to the corresponding alcohol **2** by NaOH promoted hydrolysis in aqueous ethanol (15% NaOH in 50 vol% aqueous ethanol) at room temperature. The alcohols thus obtained were derivatised to the corresponding trifluoroacetates by pyridine-catalysed esterification using trifluoroacetic anhydride. The enantiomeric excesses (ee) of the non-reacted starting materials **2**-heptanoate and **2**-acetate and of the product (**2**) of the reactions were derived from the ee of the trifluoroacetates measured by gas chromatograpy: 120°C isothermal for 10 min and raised to 180°C at a rate of 10°C/min, 10 psi. Retention times were 10.3 min for (*S*)-2-phenoxy-1-propyl trifluoroacetate and 10.5 min for (*R*)-2-phenoxy-1-propyl trifluoroacetate; $\alpha = 1.03$. The absolute configuration of the resolved alcohol was determined by oxidising the alcohol product of a kinetic resolution to the corresponding carboxylic acid by means of the Jones' reagent and analysing it by HPLC using hexane:isopropanol:formic acid (90:10:1) as mobile phase. Retention times were 10 min for (*S*)-2-phenoxypropionic acid and 16.1 min for (*R*)-2-phenoxypropionic acid; $\alpha = 1.61$.[37]

4.12. PCL-catalysed kinetic resolution of esters of alcohol 3

The reactions were performed as for the esters of alcohol **1** and were submitted to gas chromatographic analysis to determine the enantiomeric excesses of non-reacted starting materials and products using 100°C for 10 min, then raised to 180°C at a rate of 5°C/min, 8 psi. Retention times were 15.2 min for (*S*)-**3**, 15.0 min for (*R*)-**3** ($\alpha = 1.02$), 28.3 min for (*S*)-**3**-heptanoate and 28.5 min for (*R*)-**3**-heptanoate ($\alpha = 1.01$). The retention time of the enantiomers was confirmed by comparison with commercially available samples of pure enantiomers of (*R*)-**3** and (*S*)-**3**.

Acknowledgements

A.M. thanks Les Fonds Québécois de la Recherche sur la Nature et les Technologies (FCAR) for two postgraduate fellowships. We thank the National Sciences and Engineering Research Council of Canada (NSERC) for financial support and Dr. Enikö Forro for her work on the PCL-catalysed transesterifications of alcohols **1** and **2** (unpublished results).

References

1. Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis*; Wiley-VCH: Weinheim, Germany, 1999; pp. 65–106.
2. Miyazawa, T.; Yukawa, T.; Koshiba, T.; Ueji, S; Yanagihara, R.; Yamada, T. *Biotechnol. Lett*. **2001**, *23*, 1547–1550.
3. Miyazawa, T.; Yukawa, T.; Ueji, S.; Yanagihare, R.; Yamada, T. *Biotechnol. Lett*. **1998**, *20*, 235–238.
4. Hirose, K; Naka, H.; Yano, M.; Ohashi, S.; Naemura, K.; Tobe, Y. *Tetrahedron: Asymmetry* **2000**, *11*, 1199–1210.
5. Miyazawa, T.; Yukawa, T.; Koshiba, T.; Sakamoto, H.; Ueji, S.; Yanagihara, R.; Yamada, T. *Tetrahedron: Asymmetry* **2001**, *12*, 1595–1602.
6. Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc*. **1991**, *113*, 3166–3171.
7. Ducret, A.; Trani, M.; Lortie, R. *Enzyme Microb. Technol*. **1998**, *22*, 212–216.
8. Carrea, G.; Ottolina, G.; Riva, S. *Trends Biotechnol*. **1995**, *13*, 63–70 (erratum 122).
9. Wescott, C. R.; Klibanov, A. M. *Biochim. Biophys. Acta* **1994**, *1206*, 1–9.
10. Martin, C.; Himmele, W.; Siegel, H. US Pat. 4,410,734, 1983.
11. Delinck, D. L.; Margolin, A. L. *Tetrahedron Lett*. **1990**, *31*, 6797–6798.
12. Stoermer, D.; Caron, S.; Heathcock, C. H. *J. Org. Chem*. **1996**, *61*, 9115–9125.
13. Wimmer, Z.; Saman, D.; Francke, W. *Helv. Chim. Acta* **1994**, *77*, 502–508.
14. Weinstock, L. M.; Mulvey, D. M.; Tull, R. *J. Org. Chem*. **1976**, *41*, 3121–3124.
15. Ferraboschi, P; Reza-Elahi, S; Verza, E; Santaniello, E. *Tetrahedron: Asymmetry* **1999**, *10*, 2639–2642.
16. Ferraboschi, P; Casati, S.; De Grandi, S.; Grisenti, P.; Santaniello, E. *Biocatalysis* **1994**, *10*, 279–288.
17. Weissfloch, A. N. E.; Kazlauskas, R. J. *J. Org. Chem*. **1995**, *60*, 6959–6969.
18. Nordin, O.; Nguyen, B.-V.; Vörde, C.; Hedenstrom, E.; Högberg, H.-E. *J. Chem. Soc., Perkin Trans. 1* **2000**, 367–376.
19. Högberg, H.-E.; Berglund, P.; Edlund, H.; Fggerhag, J.; Hedenstrijm, E; Lundh, M; Nordin, O.; Servi, S; Vörde, C. *Catal. Today* **1994**, *22*, 591–606.
20. Tuomi, W. V.; Kazlauskas, R. J. *J. Org. Chem*. **1999**, *64*, 2638–2647.
21. Geerlof, A.; van Tol, B. A.; Jongejan, J. A.; Duine, J. A. *Biosci. Biotechnol. Biochem*. **1994**, *58*, 1028–1036.
22. Sakai, T.; Kishimoto, T.; Tanaka, Y.; Ema, T.; Utaka, M. *Tetrahedron Lett*. **1998**, *39*, 7881–7884.
23. Bosetti, A.; Bianchi, D.; Cesti, P.; Golini, P. *Biocatalysis* **1994**, *9*, 71–77.
24. Schrag, J. D.; Li, Y.; Cygler, M.; Lang, D.; Burgdorf, T.; Hecht, H.-J.; Schmid, R.; Schomburg, D.; Rydel, T. J.; Oliver, J. D.; Strickland, L. C.; Dunaway, C. M.; Larson, S. B.; Day, J.; McPherson, A. *Structure* **1997**, *5*, 187–202.
25. Brzozowski, A. M.; Derewenda, U.; Derewenda, Z. S.; Dodson, G. G.; Lawson, D. M.; Turkenburg, J. P.; Bjorkling, F.; Huge-Jensen, B.; Patkar, S. A.; Thim, L. *Nature* **1991**, *351*, 491–494.
26. Derewenda, U.; Brzozowski, A. M.; Lawson, D. M.; Derewenda, Z. S. *Biochemistry* **1992**, *31*, 1532–1541.
27. Lang, D. A.; Mannesse, M. L. M.; de Haas, G. H.; Verheij, H. M.; Dijkstra, B. W. *Eur. J. Biochem*. **1998**, *254*, 333–340.

28. Luić, M.; Tomić, S.; Leščić, I.; Ljubović, E.; Šepac, D.; Šunjić, V.; Vitale, L.; Saenger, W.; Kojić-Prodić, B. *Eur. J. Biochem.* **2001**, *268*, 3964–3973.
29. Overbeeke, P. L. A.; Govardhan, C.; Khalaf, N.; Jongejan, J. A.; Heijnen, J. J. *J. Mol. Cat. B: Enzym.* **2000**, *10*, 385–393.
30. Van Tol, J. B. A.; Jongejan, J. A.; Duine, J. A. *Biocatal. Biotransform.* **1995**, *12*, 99–118.
31. Nishizawa, K.; Ohgami, Y.; Matsuo, N.; Kisida, H.; Hirohara, H. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1293–1298.
32. Kim, K. K.; Song, H. K.; Shin, D. H.; Hwang, K. Y.; Suh, S. W. *Structure* **1997**, *5*, 173–185.
33. Lang, D.; Hofmann, B; Haalck, L; Hecht, H. J.; Spener, F.; Schmid, R. D.; Schomburg, D. *J. Mol. Biol.* **1996**, *259*, 704–717.
34. Affleck, R.; Haynes, C. A.; Clark, D. S. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5167–5170.
35. Klibanov, A. *Trends Biotechnol.* **1997**, *15*, 97–101.
36. Evans, D. A.; Ennis, M. D.; Mathre, D. J. *J. Am. Chem. Soc.* **1982**, *104*, 1737–1739.
37. Wu, S.-H.; Lay, S.-Y.; Lin, S.-L.; Chu, F.-Y.; Wang, K.-T. *Chirality* **1991**, *3*, 67–70.
38. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.