Pergamon

SCIENCE DIRECT®

Tetrahedron: *Asymmetry* 14 (2003) 317–323

TETRAHEDRON: *ASYMMETRY*

Lipase-catalyzed enantioselective transesterification toward esters of 2-bromo-tolylacetic acids

David Guieysse,[a] Christophe Salagnad,[b] Pierre Monsan[a] and Magali Remaud-Simeon[a,*]

[a]*Centre de Bioingénierie Gilbert Durand, Département de Génie Biochimique et Alimentaire, UMR CNRS 5504, UMR INRA 792, INSA, 135 Avenue de Rangueil, F-31077 Toulouse cedex 4, France*

[b]*Aventis Pharma, Process Development Biotechnology, 9, quai Jules Guesde, F-94400 Vitry sur Seine, France*

Received 29 October 2002; revised 13 November 2002; accepted 21 November 2002

Abstract—Lipases from *Candida antarctica*, *Pseudomonas cepacia* and *Rhizomucor miehei* were tested in the resolution of seven racemic substrates belonging to the (*RS*)-2-bromo tolyl acetate ester category, but differing either in the position of the methyl substituent on the acyl part of the aromatic ring, or in the structure of the alkyl group. Lipase-catalyzed kinetic resolution via transesterification reaction between the ester and octanol in octane revealed that, of the three enzymes tested, *P. cepacia* lipase is the most efficient for resolution of the various racemates, with *R*-enantiopreference. In addition, the position of the methyl substituent was found to play a key role in governing the enantioselectivity of the reaction. Using *P. cepacia* lipase and 2-bromo-*m*/*p*-tolyl- or 2-bromophenylacetic acid esters *E*-values of >50 were measured, whereas with the *ortho* derivatives, *E*-values dramatically decreased to <6.

1. Introduction

Lipase stereoselectivity is widely used in organic synthesis for the preparation of chiral building blocks or the production of valuable enantiomers such as pheromones and antibiotics.[1,2] In numerous cases, these enzymes have been revealed as excellent asymmetric catalysts.[3–5] However, only a few examples of lipase-catalyzed resolution of 2-halogeno aromatic carboxylic esters[6–10] have been reported. CLEC (cross-linked enzyme crystals) lipases from *Candida rugosa* and *Pseudomonas cepacia* were shown described to catalyze the kinetic resolution of 2-bromo esters[8] and 2-chloro esters[6] via hydrolysis reaction but afforded moderate levels of selectivity. These types of compounds are important intermediates found in the synthetic pathways of a number of drugs such as prostaglandin, prostacyclin, semi-synthetic penicillin, thiazolium salts.[11,12] In particular, methyl and ethyl ester derivatives of 2-bromo-*o*-tolylacetic acid are used as precursors for the synthesis of analgesics,[13] and non-peptide angiotensin II-receptor antagonists.[14,15]

Recently, we screened lipases from different organisms (*P. cepacia*, *Rhizomucor miehei*, *Candida antarctica*, *C. rugosa* and *Humicola lanuginosa*) for their ability to catalyze the kinetic resolution of 2-bromo-*o*-tolyl ethyl acetate through transesterification reaction.[7] Among the enzymes tested, lipases from *P. cepacia* and from *R. miehei* immobilized on polypropylene were the most stereoselective catalysts. Operating conditions for *R. miehei* lipase-catalyzed transesterification between 2-bromo-*o*-tolyl ethyl acetate and octanol in octane were established to resolve the racemic mixture. However, the moderate enantioselectivity value (11.2) limited the product yield. Moreover, varying the solvent, the reaction temperature, or the water activity used in the reaction had no marked effect on the enantioselectivity. As would be expected, lipase enantiopreference appeared to be mainly governed by the preferential structural recognition of one of the two enantiomers. However, the molecular basis that controls lipase enantioselectivity toward 2-halogeno aromatic carboxylic acids has never been extensively explored in the literature, and to date, only one study provides an empirical rule for predicting crude *C. rugosa* lipase enantioselectivity toward chiral carboxylic acids.[9]

In the work reported herein, the goal was to improve the lipase-catalyzed kinetic resolution of 2-bromo-tolyl acetate esters and to acquire new insights into the phenomenon of enantiorecognition. To this end, 21

* Corresponding author. Tel.: +33 561559446; fax: +33 561559400; e-mail: remaud@insa-tlse.fr

0957-4166/03/$ - see front matter
PII: S0957-4166(02)00784-X

enzyme/substrate combinations were tested in transesterification reaction using the lipases from *P. cepacia*, *R. miehei* or *C. antarctica* and a set of seven 2-bromo-aromatic acetic acid ester derivatives, modified either on the acyl or on the alkyl part. Enzyme activities, *E*-values and enantiomeric excess were determined and are discussed for each combination.

2. Results and discussion

Enantioselectivity toward 2-bromo-tolyl acetate esters may be influenced both by the enzyme active site structure and by the substrate structure. For this reason, transesterification reactions of esters modified either on their acyl part or on their alkyl part were carried out with the lipases from *R. miehei* (RML), *C. antarctica* (CAL-B) and *P. cepacia* (PCL), these enzymes being considered as representative of three different classes on the basis of the distinctive topology of their active sites.

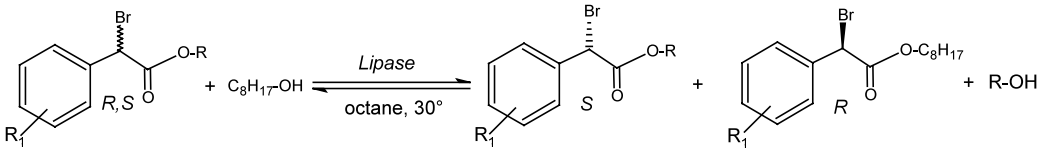
2.1. Effect of changes in the acyl part of the substrate

To evaluate the influence of the acyl part of the substrate on lipase enantioselectivity, (±)-2-bromophenyl ethyl acetate **1** was synthesized from 2-bromophenylacetic acid. (±)-2-Bromo-*o*/*m*/*p*-tolyl ethyl acetate **2**–**4** were synthesized from *o*/*m*/*p*-tolualdehyde, via their corresponding 2-bromo-tolylacetic acid. This was then followed by time course transesterification of these racemic substrates catalyzed by the three selected enzymes, the reaction being carried out in *n*-octane using 1-octanol as the alcohol, as shown in Scheme 1. In addition, each of the racemic 2-bromo carboxylic acids were chemically resolved to obtain a reference and to determine their absolute configuration (see Section 4).

For all the substrates, RML and CAL-B preferentially catalyzed the transformation of the (*S*)-enantiomer of racemates **1**–**4**, whereas PCL always displayed the opposite enantiopreference, favouring transformation of the (*R*)-enantiomer. The set of results in Table 1 reveals that the position of the methyl substitution influences both the lipase activities and enantioselectivities to various degrees depending on the enzymes concerned.

In the case of CAL-B, very low conversion rates are seen for *ortho*-substituted substrate **2**, indicating poor recognition. Transesterification rates significantly increased for non-, *meta*- and *para*-substituted substrates (**1**, **3** and **4**, respectively) as shown by conversion rate values obtained after 120 h of reaction, but still remained moderate. For all substrates, very low *E*-values (<2) were observed, showing that CAL-B is not enantioselective. In addition, the optimization of reaction parameters such as solvent, alcohol, water activity and temperature did not increase the enantioselectivity of the reaction (data not shown).

For RML, lower conversion rates were again obtained in the case of the *ortho*-substituted substrate since only



1: R_1= H, R= C_2H_5

2: R_1= *ortho*-CH_3, R =C_2H_5 **5**: R_1= *ortho*-CH_3, R =CH_2-C_6H_5

3: R_1= *meta*-CH_3, R= C_2H_5 **6**: R_1= *meta*-CH_3, R= CH_2-C_6H_5

4: R_1= *para*-CH_3, R= C_2H_5 **7**: R_1= *para*-CH_3, R= CH_2-C_6H_5

Scheme 1.

Table 1. Enantioselectivity of free CAL-B, RML and PCL in transesterification between the substrates **1**–**4** and 1-octanol in *n*-octane

Substrate	Free *C. antarctica* lipase			Free *R. miehei* lipase			Free *P. cepacia* lipase		
	$E^b-(S)^c$	% Conv.[a]	% e.e.$_s$	$E^b-(S)^c$	% Conv.	% e.e.$_s$	E^b–$(R)^c$	% Conv.[a]	% e.e.$_s$
1	1.3 (±0.2)	32	2	1.3 (±0.5)	51 (72 h)	3	57 (±7)	49	77
2	n.d.	7	2	2.8 (±0.1)	22 (72 h)	9	4.3 (±0.5)	15	10
3	1 (±0.1)	28	1	1.9 (±0.1)	55 (72 h)	8	59 (±2)	46	75
4	1.6 (±0.3)	17	4	3.4 (±0.1)	58 (48 h)	33	50 (±3)	41	67

[a] After 120 h.

[b] *E*=ratio of initial rates, n.d: not determined.

[c] Fast reacting enantiomer. The absolute configuration was determined by circular dichroism (exciton method)[16,17]

22% of racemic **2** was transesterified after 72 h. *meta*-, *para*- and Non-methyl-substituted substrates (**3**, **4**, **1**) were converted much more rapidly, confirming that *ortho* substitution disfavors the reaction. This effect can be attributed to steric hindrance effects that slow the reaction. However, irrespective of the substrate considered, *E*-values always remain moderate, ranging from 1.3 to 3.4. As in the case of CAL-B, optimization of reaction parameters did not allow the increase in lipase enantioselectivity.

Compared to CAL-B and RML, PCL also converted the *para*-, *meta*- and unsubstituted substrates almost three times faster than the *ortho*-substituted one. Interestingly, PCL enantioselectivity was also strongly influenced by the position of the methyl substitution on the aromatic ring. Like the other enzymes tested, PCL displays a low enantioselectivity toward the *ortho*-substituted ester with an *E*-value of 4.3, but a remarkable tenfold increase in lipase enantioselectivity was found toward *para*-, *meta*-, and non-substituted substrates, the maximum *E*-value of 59 being attained in the transesterification of the *meta*-substituted substrate. To better appreciate the effect of the size of the halide substituent on PCL enantioselectivity toward the *ortho*-substituted substrate, it could be interesting to study the resolution of 2-chloro esters, these compounds being resolved by the CLEC form of PCL[6] with 65% $e.e._s$ at 50% conversion. In addition, during the transesterification reaction of substrate **1**, the enantiomeric excesses of the product and substrate reached 79 and 77%, respectively (at 49% conversion). During kinetic resolution of 2-bromophenylacetic acid methyl ester via hydrolysis reaction,[8] enantiomeric excesses of product and substrate of 65 and 51%, respectively, were obtained at 32% conversion. Although the latter substrate possesses a slightly different structure (a methyl instead of an ethyl group), our results suggest that PCL is more enantioselective when catalyzing transesterification.

2.2. Effect of changes in the alkyl part of the substrate

Racemic benzyl esters **5**, **6** and **7** were then synthesized from 2-bromo-*o*/*m*/*p*-tolylacetic acids in order to determine the influence of the alkyl part of the substrate on the selectivity of the lipase. Changing the ethyl for a benzyl group enabled the size of the alkyl part to be significantly increased. In addition, a π–π interaction (electronic interaction) with active site amino acid residues may also occur and induce a change in the enantioselectivity. The results obtained with this set of substrates and the three selected enzymes are reported in Table 2.

Very low conversion rates and *E*-values were measured with CAL-B, indicating that this enzyme is clearly not adapted to accommodate these substrates. A small binding pocket limited by Trp-104 is known to fix the small alkyl part of the substrate. The size of the benzyl group probably hinders the binding in this pocket.

Conversely, transesterification with RML was more efficient for most of the benzyl esters. Excepting for the 2-bromo-*o*-tolyl benzyl ester, all the benzyl esters were converted faster than the ethyl ester, as indicated by the higher conversion rate (up to 57%) reached after 48 h. Regarding *E*-values, no general trend was observed and the enantioselectivity remained moderate (between 1.5 and 5.1).

In the case of the reaction catalyzed by PCL, the presence of a benzyl group always favored the conversion of the esters. This effect was particularly accentuated for the 2-bromo-*o*-tolyl benzyl ester, for which the conversion rate was twice as high as that observed for the ethyl ester **2** but the *E*-value remained very low (<6). Notably, for the two other benzyl esters **6** and **7**, the increase in reaction rate appears to be correlated to a decrease in *E*-values, *E*-values of 36 and 30 being measured for the *meta*- and *para* substituted benzyl derivatives, respectively.

3. Conclusion

In conclusion, we observed that simple methyl substitution on the aromatic ring in the acyl part of the substrate has a significant influence on lipase activity and enantioselectivity for the resolution of esters of 2-bromo-tolyl carboxylic acids **2**–**7**. Not one of the three lipases was efficient in resolving the *ortho*-substituted substrates **2** and **5**. *ortho*-Substitution seems to induce strong steric hindrance, reducing the discrimination between the two enantiomers and decreasing the reaction rate for the two species. However, PCL was the most enantioselective enzyme for the resolution of the non-, *meta*- and *para*-substituted substrates.

Table 2. Enantioselectivity of free CAL-B, RML and PCL in transesterification between the substrates **5**–**7** and 1-octanol in *n*-octane

Substrate	Free *C. antarctica* lipase			Free *R. miehei* lipase			Free *P. cepacia* lipase		
	$E^b-(S)^c$	% Conv.[a]	% $e.e._s$	E^b–$(S)^c$	% Conv.	% $e.e._s$	$E^b-(R)^c$	% Conv.	% $e.e._s$
5	n.d.	<2	1	1.5 (±0.1)	15 (72 h)	1	6 (±0.2)	33 (120 h)	35
6	1 (±0.2)	19	1	5.1 (±0.3)	57 (48 h)	52	36 (±5)	51 (48 h)	86
7	n.d.	<5	2	1.9 (±0.5)	57 (48 h)	19	30 (±4)	50 (96 h)	78

[a] After 120 h.
[b] E = ratio of initial rates, n.d: not determined.
[c] Fast reacting enantiomer. The absolute configuration was determined by circular dichroism (exciton method).

To attempt to understand, improve and predict *P. cepacia* lipase enantioselectivity toward (*R*)-2-bromo-tolyl/phenylacetic acids, a complementary tool, using molecular modeling of the first tetrahedral intermediate of the reaction in these different cases, may lead to an explanation for the phenomena observed during the enzymatic resolution. In addition, this approach could be helpful with a view to recommending what mutation site could improve lipase enantioselectivity.

4. Experimental

4.1. Biological reagents

All lipases were purchased from Roche Diagnostics (Germany). Chirazyme L-9, lyo., 360 U/mg (free lipase from *R. miehei*); Chirazyme L-1, lyo., 352 U/mg (free lipase from *P. cepacia*); Chirazyme L-2, lyo., 175 U/mg (free lipase from *C. antarctica*).

4.2. Chemical reagents

All reagents were of commercial quality and were purchased from Sigma/Aldrich. *n*-Octane was dried over molecular sieve (3 Å) before use.

4.3. General procedure for the preparation of 2-bromo-tolylacetic acid and their derivative esters

4.3.1. (±)-2-Bromo-*o*-, *m*- and *p*-tolylacetic acids. (±)-2-Bromophenylacetic acid is commercially available (Aldrich).

The bromoacetic acid was synthesized according to a method previously described which was slightly modified.[18–20] A saturated solution of sodium metabisulphite was prepared by stirring finely powdered sodium metabisulphite (207.5 g) with water (280 mL) for 0.5 h and then filtering to remove the excess salt. In a 1 L beaker, sodium cyanide (20.4 g, 0.41 mol) in water (82.5 mL) was mixed with *o*- or *m*- or *p*-tolualdehyde (50 g, 0.41 mol). The sodium metabisulphite solution was added from a dropping funnel, slowly at first and then more rapidly (addition time: 10–15 min). During the initial stage of the addition, crushed ice (125 g) was added to the reaction mixture in several portions. The two-phase liquid mixture was transferred to a separating funnel. The organic layer was removed and placed in a large evaporating dish and concentrated hydrochloric acid (62.5 mL) was added immediately with stirring. Hydrolysis was allowed to proceed at rt for 12 h. The solution was then evaporated to dryness on a steam bath and stirred from time to time to break up the deposit of ammonium chloride and tolylacetic acid. The residue was washed with cold toluene (2–3× 100 mL). Inorganic salts were separated from tolylacetic acid by toluene extraction using a Soxhlet apparatus. The 2-hydroxy-tolylacetic acid crystallized at rt and was collected on a Buchner funnel and dried in air to afford the 2-hydroxy-*o*- or *m*- or *p*-tolylacetic acid as a white solid.

The 2-hydroxy-*o*- or *m*- or *p*-tolylacetic acid (10 g) and sulphuric acid (1.63 mL) were added successively to a stirred solution of 48% aqueous hydrobromic acid (12.5 g) and concentrated sulfuric acid (2 mL). The solution was stirred under reflux for 3 h, cooled and added to water (50 mL). The organic phase was isolated by extraction with ether and evaporated, giving an oil, which was purified by silica gel column chromatography (*n*-hexane:ethyl acetate, 98:2) to afford the 2-bromo-*o*- or *m*- or *p*-tolylacetic acid as a white solid.

4.3.2. 2-Bromo-tolyl or phenyl acetate esters 1–7

4.3.2.1. Ethyl ester. The bromo acid (43.6 mmol), ethanol (100 mL) and *p*-toluenesulphonic acid (0.2 g) were stirred under reflux (4–5 h). The reaction was followed by TLC using *n*-hexane:ethyl acetate (9:1) as eluent. Ethanol was evaporated at 35°C under water-pump vacuum. The residual oil was dissolved in dichloromethane (25 mL) and washed with a saturated sodium bicarbonate solution (3×10 mL) and finally with distilled water (10 mL). The dichloromethane solution was dried over magnesium sulphate, filtered and then evaporated to dryness at 35°C under water pump vacuum.

4.3.2.2. Benzyl and octyl esters. Benzyl or octyl alcohol (50 mmol), bromo acid (60 mmol), *p*-toluenesulphonic acid (0.2 g) and toluene (80 mL) were stirred and heated under reflux. The water formed in the reaction was continuously removed azeotropically using a Dean–Stark apparatus. The toluene solution was cooled to rt and washed with saturated sodium bicarbonate solution (3×25 mL) and finally with distilled water (25 mL). The toluene solution was dried over magnesium sulfate, filtered and evaporated to dryness at 50°C under water pump vacuum.

4.4. Spectroscopic data

Infrared spectra were recorded on a Perkin Elmer, 1310 infrared spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC-200.1 (^{1}H 200.1 MHz and ^{13}C 50.3 MHz) spectrometer.

4.4.1. (±)-2-Bromo phenyl ethyl acetate, 1. Yield: 8.83 g (79%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 1.24–1.31 (t, $J=7.1$ Hz, 3H, -C$\underline{H}_3$), δ 4.17–4.29 (qd, $J=2.8$ and 7.1 Hz, 2H, -OC$\underline{H}_2$CH$_3$), δ 5.34 (s, 1H, -C$\underline{H}$Br), δ 7.33–7.57 (m, 5H, Ar$\underline{H}$). **^{13}C NMR** ($CDCl_3$), δ 13.98 (-CH$_2$C$\underline{H}_3$), 46.91 (-CHBr), 62.57 (-OC$\underline{H}_2$CH$_3$), 128.60 (×2), 128.71 (×2), 128.86, 129.30, 168.35 ($\underline{C}$OO). Elemental anal. calcd for $C_{10}H_{11}O_2Br$: C, 49.41; H, 4.56. Found: C, 49.65; H, 4.32%.

4.4.2. (±)-2-Bromo phenyl octyl acetate, 8. Yield: 10.52 g (70%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1460 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 0.85–0.91 (t, $J=6.7$ Hz, 3H, -CH$_2$C$\underline{H}_3$), δ 1.26 (s, 10H, C$\underline{H}_2$), δ 1.64 (m, 2H, C$\underline{H}_2$), δ 4.14–4.21 (td, $J=6.6$ -OC$\underline{H}_2$CH$_2$-), δ 5.35 (s, 1H, -C$\underline{H}$Br), δ 7.33–7.58 (5H, Ar$\underline{H}$). **^{13}C NMR** ($CDCl_3$), δ 14.18 (-CH$_2$C$\underline{H}_3$), 22.70, 25.75, 28.41, 29.14, 29.18, 31.79, 47.02 (-$\underline{C}$HBr), 66.66

(-OCH$_2$-), 128.72 (×2), 128.84 (×2), 129.28, 135.97, 168.40 (COO). Elemental anal. calcd for $C_{16}H_{23}O_2Br$: C, 58.72; H, 7.08. Found: C, 58.91; H, 6.90%.

4.4.3. (±)-2-Bromo-*o*-tolylacetic acid, 9. Yield: 5.39 g (39%). **IR** (KBr, neat) 3300–2800 (ν_{O-H}), 1710 ($\nu_{C=O}$), 1600 ($\nu_{C=C}$), 1310–1240 (ν_{C-O}) cm^{-1}. **^{1}H NMR** (*d*, DMSO), δ 2.35 (s, 3H, ArCH$_3$), δ 5.95 (s, 1H, -CHBr), δ 7.20–7.51 (m, 4H, ArH), δ 13.24 (s, 1H, COOH); **^{13}C NMR** (*d*, DMSO), δ 18.74 (ArCH$_3$), 47.39 (-CHBr), 126.420, 128.59, 128.81, 130.66, 135.39, 138.16, 169.06 (COOH). Elemental anal. calcd for $C_9H_9O_2Br$: C, 47.19; H, 3.96. Found: C, 47.69; H, 3.58%.

4.4.4. (±)-2-Bromo-*o*-tolyl ethyl acetate, 2. Yield: 8.70 g (78%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 1.24–1.31 (t, $J=7$ Hz, 3H, -OCH$_2$CH$_3$), δ 2.41 (s, 3H, ArCH$_3$), δ 4.19–4.31 (qd, $J=3.2$–7 Hz, 2H, -OCH$_2$CH$_3$), δ 5.63 (s, 1H, -CHBr), δ 7.19–7.26 (m, 3H, ArH), δ 7.59–7.64 (m, 1H, ArH); **^{13}C NMR** ($CDCl_3$), δ 14.05 (-CH$_2$CH$_3$), 19.36 (ArCH$_3$), 44.83 (-CHBr), 62.68 (-OCH$_2$CH$_3$), 126.90, 128.79, 129.25, 130.87, 134.48, 136.13, 168.30 (COO). Elemental anal. calcd for $C_{11}H_{13}O_2Br$: C, 51.38; H, 5.10. Found: C, 51.40; H, 4.98%.

4.4.5. (±)-2-Bromo-*o*-tolyl benzyl acetate, 5. Yield: 14.16 g (74%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 2.4 (s, 3H, ArCH$_3$), δ 5.17–5.32 (2H, d, $J=12.2$ Hz, -OCH$_2$ph), δ 5.71 (s, 1H, -CHBr), δ 7.18–7.27 (m, 3H, ArH), δ 7.34–7.36 (m, 5H, ArH), δ 7.60–7.64 (m, 1H, ArH); **^{13}C NMR** ($CDCl_3$), δ 19.38 (ArCH$_3$), 44.71 (-CHBr), 68.21 (-OCH$_2$ph), 126.92, 128.34 (×2), 128.60 (×2), 128.68, 128.95, 129.33, 130.91, 134.30, 135.07, 136.18, 168.16 (COO). Elemental anal. calcd for $C_{16}H_{15}O_2Br$: C, 60.21; H, 4.74. Found: C, 60.58; H, 4.62%.

4.4.6. (±)-2-Bromo-*o*-tolyl octyl acetate, 10. Yield: 13.87 g (68%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1460 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 0.85–0.91 (t, $J=6.8$ Hz, 3H, -CH$_2$CH$_3$), δ 1.25 (s, 10H, CH$_2$), δ 1.63 (m, 2H, CH$_2$), δ 2.41 (s, 3H, ArCH$_3$), δ 4.15–4.22 (t, $J=6.8$ Hz, -OCH$_2$CH$_2$-), δ 5.63 (s, 1H, -CHBr), δ 7.18–7.25 (m, 3H, ArH), δ 7.60 (m, 1H, ArH); **^{13}C NMR** ($CDCl_3$), δ 14.16 (-CH$_2$CH$_3$), 19.37 (ArCH$_3$), 22.69, 25.74, 28.42, 29.13, 29.17, 31.78, 44.85 (-CHBr), 66.75 (-OCH$_2$-), 126.87, 128.88, 129.21, 130.82, 134.51, 136.05, 168.36 (COO). Elemental anal. calcd for $C_{17}H_{25}O_2Br$: C, 59.83; H, 7.38. Found: C, 60.20; H, 7.36%.

4.4.7. (±)-2-Bromo-*m*-tolylacetic acid, 11. Yield: 5.79 g (42%). **IR** (KBr, neat) 3300–2800 (ν_{O-H}), 1710 ($\nu_{C=O}$), 1600 ($\nu_{C=C}$), 1310–1240 (ν_{C-O}) cm^{-1}. **^{1}H NMR** (CD_3COCD_3), δ 2.33 (s, 3H, ArCH$_3$), δ 5.59 (s, 1H, -CHBr), δ 7.15–7.44 (m, 4H, ArH), δ 13.19 (s, 1H, COOH). **^{13}C NMR** (CD_3COCD_3), δ 21.31 (ArCH$_3$), 48.21 (-CHBr), 126.70, 129.47, 130.13, 130.62, 137.65, 139.19 169.55 (COOH). Elemental anal. calcd for $C_9H_9O_2Br$: C, 47.19; H, 3.96. Found: C, 47.60; H, 4.38%.

4.4.8. (±)-2-Bromo-*m*-tolyl ethyl acetate, 3. Yield: 8.40 g (75%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 1.24–1.31 (t, $J=7$ Hz, 3H, -OCH$_2$CH$_3$), δ 2.36 (s, 3H, ArCH$_3$), δ 4.17–4.26 (qd, $J=3.2$ and 7 Hz, 2H, -OCH$_2$CH$_3$), δ 5.32 (s, 1H, -CHBr), δ 7.17–7.36 (m, 4H, ArH). **^{13}C NMR** ($CDCl_3$), δ 13.99 (-CH$_2$CH$_3$), 21.40 (ArCH$_3$), 47.10 (-CHBr), 62.55 (-OCH$_2$CH$_3$), 125.76, 128.76, 129.30, 130.13, 135.80, 136.67, 168.42 (COO). Elemental anal. calcd for $C_{11}H_{13}O_2Br$: C, 51.38; H, 5.10. Found: C, 51.46; H, 4.89%.

4.4.9. (±)-2-Bromo-*m*-tolyl benzyl acetate, 6. Yield: 10.01 g (72%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 2.35 (s, 3H, ArCH$_3$), δ 5.21–5.24 (d, $J=4.8$ Hz, 2H, -OCH$_2$ph), δ 5.39 (s, 1H, -CHBr), δ 7.17–7.36 (m, 9H, ArH). **^{13}C NMR** ($CDCl_3$), δ 21.41 (ArCH$_3$), 46.95 (-CHBr), 68.09 (-OCH$_2$ph), 125.85, 128.29 (×2), 128.58 (×2), 128.66, 128.80, 129.34, 130.21, 135.08, 135.61, 138.72, 168.23 (COO). Elemental anal. calcd for $C_{16}H_{15}O_2Br$: C, 60.21; H, 4.74. Found: C, 60.59; H, 4.72%.

4.4.10. (±)-2-Bromo-*m*-tolyl octyl acetate, 12. Yield: 10.25 g (69%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1460 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 0.85–0.91 (t, $J=6.5$ Hz, 3H, -CH$_2$CH$_3$), δ 1.26 (s, 10H, CH$_2$), δ 1.64 (m, 2H, CH$_2$), δ 2.36 (s, 3H, ArCH$_3$), δ 4.13–4.21 (td, $J=1$ and 6.5 Hz, -OCH$_2$CH$_2$-), δ 5.32 (s, 1H, -CHBr), δ 7.12–7.36 (m, 4H, ArH). **^{13}C NMR** ($CDCl_3$), δ 14.16 (-CH$_2$CH$_3$), 21.40 (ArCH$_3$), 22.69, 25.77, 28.41, 29.17, 29.17, 31.79, 47.20 (-CHBr), 66.63 (-OCH$_2$-), 125.76, 128.73, 129.29, 130.11, 135.84, 136.64, 166.46 (COO). Elemental anal. calcd for $C_{17}H_{25}O_2Br$: C, 59.83; H, 7.38. Found: C, 60.20; H, 7.21%.

4.4.11. (±)-2-Bromo-*p*-tolylacetic acid, 13. Yield: 5.10 g (37%). **IR** (KBr, neat) 3300–2800 (ν_{O-H}), 1710 ($\nu_{C=O}$), 1600 ($\nu_{C=C}$), 1310–1240 (ν_{C-O}) cm^{-1}. **^{1}H NMR** (CD_3COCD_3), δ 2.31 (s, 3H, ArCH$_3$), δ 5.61 (s, 1H, -CHBr), δ 7.18–7.54 (2d, 2+2H, ArH). **^{13}C NMR** (CD_3COCD_3), δ 21.21 (ArCH$_3$), 48.04 (-CHBr), 129.56 (×2), 130.16 (×2), 134.75, 139.93, 169.62 (COOH). Elemental anal. calcd for $C_9H_9O_2Br$: C, 47.19; H, 3.96. Found: C, 47.51; H, 3.81%.

4.4.12. (±)-2-Bromo-*p*-tolyl ethyl acetate, 4. Yield: 8.47 g (78%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 1.23–1.31 (t, $J=7$ Hz, 3H, -OCH$_2$CH$_3$), δ 2.34 (s, 3H, ArCH$_3$), δ 4.17–4.29 (qd, $J=3$ and 7 Hz, 2H, -OCH$_2$CH$_3$), δ 5.33 (s, 1H, -CHBr), δ 7.15–7.46 (2d, 2+2H, ArH). **^{13}C NMR** ($CDCl_3$), δ 14.00 (-CH$_2$CH$_3$), 21.30 (ArCH$_3$), 46.97 (-CHBr), 62.50 (-OCH$_2$CH$_3$), 128.61 (×2), 129.57 (×2), 132.97, 139.41 168.44 (COO). Elemental anal. calcd for $C_{11}H_{13}O_2Br$: C, 51.38; H, 5.10. Found: C, 51.35; H, 5.00%.

4.4.13. (±)-2-Bromo-*p*-tolyl benzyl acetate, 7. Yield: 9.73 g (70%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 2.37 (s, 3H, $ArCH_3$), δ 5.22–5.24 (2H, d, $J=4.1$ Hz, $-OCH_2ph$), δ 5.41 (s, 1H, -CHBr), δ 7.16–7.20 (d, 2H, ArH), δ 7.34–7.37 (m, 5H, ArH), δ 7.44–7.48 (d, 2H, ArH). **^{13}C NMR** ($CDCl_3$), δ 21.35 ($ArCH_3$), 46.80 (-CHBr), 68.07 ($-OCH_2ph$), 128.29 (×2), 128.58 (×2), 128.71 (×3), 129.63 (×2), 132.79, 135.11, 139.52, 168.31 (COO). Elemental anal. calcd for $C_{16}H_{15}O_2Br$: C, 60.21; H, 4.74. Found: C, 60.56; H, 4.77%.

4.4.14. (±)-2-Bromo-*p*-tolyl octyl acetate, 14. Yield: 9.51 g (64%). **IR** (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1460 ($\nu_{C=C}$), 1280–1140 (ν_{C-O}) cm^{-1}. **^{1}H NMR** ($CDCl_3$), δ 0.85–0.92 (t, $J=6.8$ Hz, 3H, $-CH_2CH_3$), δ 1.26 (s, 10H, CH_2), δ 1.60 (m, 2H, CH_2), δ 2.34 (s, 3H, $ArCH_3$), δ 4.13–4.20 (td, $J=6.8$ and 0.9 Hz, $-OCH_2CH_2-$), δ 5.33 (s, 1H, -CHBr), δ 7.14–7.46 (2d, 2+2H, ArH). **^{13}C NMR** ($CDCl_3$), δ 14.16 ($-CH_2CH_3$), 21.30 ($ArCH_3$), 22.70, 25.76, 28.41, 29.18 (×2), 31.79, 47.06 (-CHBr), 66.59 ($-OCH_2-$), 128.61 (×2), 129.54 (×2), 133.01, 139.38, 168.51 (COO). Elemental anal. calcd for $C_{17}H_{25}O_2Br$: C, 59.83; H, 7.38. Found: C, 59.98; H, 7.32%.

4.5. General procedure for enzymatic transesterification

A typical transesterification was carried out in *n*-octane (5 ml) containing the ester (0.25 mmol) (50 mM), 1-octanol (0.75 mmol) (150 mM) and lipase (5 mg/ml). The temperature was maintained at 30°C. The mixture was shaken at 250 rpm for the time indicated in Tables 1 and 2. The progress of the reaction was followed by sampling the reaction at regular intervals.

4.6. HPLC analysis

The Chiral HPLC device was equipped with a chiral column: Chiralpack AD, AS or OJ (25 cm×4.6 mm) (Daicel Chemical Industries Ltd, Japan) connected to a UV detector (at 254 nm). The conditions were: *n*-hexane/isopropanol, 99.8/0.2, v/v for **1**–**6**, **8**, **10** and **12** with column AD; *n*-hexane/isopropanol, 99.8/0.2, v/v for **7** with column AS; *n*-hexane/isopropanol/TFA, 96/4/0.1, v/v/v for **9**, **11** and **13** with column AS; *n*-hexane/isopropanol, 98/2, v/v for **14** with column OJ. Each sample of transesterification reaction was diluted 10–12 times in the mobile phase, filtered before injection (20 μl) and analyzed at a flow rate of 1.0 ml/min at rt. The retention times (t_R/min) were as follows: **1**: 7.98 (*S*), 8.59 (*R*); **2**: 7.01 (*S*), 7.44 (*R*); **3**: 7.14 (*S*), 7.50 (*R*); **4**: 8.68 (*S*), 9.42 (*R*); **5**: 8.30 (*S*), 10.01 (*R*); **6**: 8.22 (*S*), 8.70 (*R*); **7**: 8.51 (*S*), 9.32 (*R*); **8**: 7.30 (*S*), 7.82 (*R*); **9**: 10.65 (*R*), 12.92 (*S*); **10**: 6.38 (*S*), 6.81 (*R*); **11**: 10.86 (*R*), 11.85 (*S*); **12**: 6.55 (*S*), 6.65 (*R*); **13**: 11.50 (*R*), 12.72 (*S*); **14**: 9.51 (*S*), 10.0 (*R*).

4.7. Resolution of 2-bromo tolyl- or phenylacetic acid

(1*R*,2*S*)-(−)-Ephedrine was used as resolving agent. (−)-Ephedrine (21.8 mmol) was dissolved in ethanol at (95% v/v, 10.6 g) at 20°C under magnetic stirring. After complete dissolution of (−)-ephedrine, 2-bromo tolyl- or phenylacetic acid (21.8 mmol) were added and the solution was mixed for 15 min at 20°C. In the absence of crystallization, the mixture was placed at 4°C. The solution was filtered through a glass funnel of porosities 2 and the salt [(−)-ephedrine·(*R*)-bromo tolylacetic acid] was dried efficiently. The salt was washed (4–6 times) with a small amount of iced ethanol and filtered as previously described. The salt was subsequently dissolved in 30 ml of water at pH 0–1 (hydrochloric acid) for 1 h in an ice bath to liberate the pure carboxylic acid. The liberated oil ((*R*)-bromo tolyl- or phenylacetic acid) was extracted three times with 20 ml of diethyl ether. The organic layer was washed twice in 10 ml of water and then was left to stand overnight at 5°C after adding anhydrous sodium sulfate.[21] The organic layer was filtered and evaporated to dryness under reduced pressure to give (*R*)-bromo tolyl- or phenylacetic acid. The specific rotation was obtained and the absolute configuration was determined by the circular dichroism exciton method.

(*R*)-Bromophenylacetic acid: Yield 0.32 g; e.e. = 97%; $[\alpha]_D^{20}$ −92 (*c* 0.5, diethyl ether) (lit.[21] $[\alpha]_D^{20}$ −105 (diethyl ether)). Elemental anal. calcd for $C_8H_7BrO_2$: C, 44.68; H, 3.28. Found: C, 44.93; H, 3.32%.

(*R*)-Bromo-*o*-tolylacetic acid: Yield 0.35 g; e.e. = 96%; $[\alpha]_D^{20}$ −3.6 (*c* 0.1, diethyl ether). Elemental anal. calcd for $C_9H_9BrO_2$: C, 47.19; H, 3.96. Found: C, 47.41; H, 4.12%.

(*R*)-Bromo-*m*-tolylacetic acid: Yield 0.38 g; e.e. = 95%; $[\alpha]_D^{20}$ −101 (*c* 0.6, diethyl ether). Elemental anal. calcd for $C_9H_9BrO_2$: C, 47.19; H, 3.96. Found: C, 47.58; H, 3.60%.

(*R*)-Bromo-*p*-tolylacetic acid: Yield 0.29 g; e.e. = 95%; $[\alpha]_D^{20}$ −76 (*c* 0.5, diethyl ether). Elemental anal. calcd for $C_9H_9BrO_2$: C, 47.19; H, 3.96. Found: C, 47.07; H, 3.78%.

4.8. Determination of enantiomeric excess (e.e.), conversion rate and enantioselectivity (*E*)

From HPLC results, enantiomeric excess (e.e.) was calculated as defined below: $e.e._s = \{[R]-[S]\}_s/\{[R]+[S]\}_s$ (s = substrate) and the conversion rate: $C = 1-[(R+S)_t/(R+S)_{t=0}]\times 100$.

The enantioselectivity value was the ratio of the initial rate of (*R*)-enantiomer production (vi*R*) versus the initial rate of (*S*)-enantiomer production (vi*S*): $E = (viR/viS)$. The initial rates were determined, before 10% of substrate conversion, by linear regression over at least five points.

Acknowledgements

The authors would like to thank Dr. Lorenzo Di Bari and Dr. Gennaro Pescitelli of the Dipartimento di

Chimica e Chimica Industriale (Pisa, Italy) for the determination of absolute configurations by the circular dichroism exciton method.

References

1. Ueji, S.; Fujino, R.; Okubo, N.; Miyazawa, T.; Kurita, S.; Kitadani, M.; Muromatsu, A. *Biotechnol. Lett*. **1992**, *14*, 163–168.
2. Tsai, S.-W.; Lin, S.-F.; Chang, C.-S. *J. Chem. Technol. Biotechnol*. **1999**, *74*, 751–758.
3. Bornscheuer, U. T.; Bessler, C.; Srinivas, R.; Krishna, S. H. *Trends Biotechnol*. **2002**, *20*, 433–437.
4. Reetz, M. T. *Curr. Opin. Chem. Biol*. **2002**, *6*, 145–150.
5. Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolts, M.; Witholt, B. *Nature* **2001**, *409*, 258–268.
6. Haughton, L.; Williams, J. M. J. *Synthesis* **2001**, *6*, 943–946.
7. Guieysse, D.; Salagnad, C.; Monsan, P.; Remaud-Simeon, M. *Tetrahedron: Asymmetry* **2001**, *12*, 2473–2480.
8. Jones, M. M.; Williams, M. J. *Chem. Commun*. **1998**, 2519–2520.
9. Ahmed, S. N.; Kazlauskas, R. J.; Morinville, A. H.; Grochulski, P.; Schrag, J. D.; Cygler, M. *Biocatalysis* **1994**, *9*, 209–225.
10. Kirchner, G.; Scollar, M. P.; Klibanov, A. M. *J. Am. Chem. Soc*. **1985**, *107*, 7072–7076.
11. Kalaritis, P.; Regenye, R. W.; Partridge, J. J.; Coffen, D. L. *J. Org. Chem*. **1990**, *55*, 812–815.
12. Bouisset, M.; Radisson, J. *Patent*, 1991, *E.P. 420706 B1*.
13. Epstein, J. W.; Brabander, H. J.; Fanshawe, W. J.; Hofmann, C. M.; McKenzie, T. C.; Safir, S. R.; Osterberg, A. C.; Cosulich, D. B.; Lovell, F. M. *J. Med. Chem*. **1981**, *24*, 481–490.
14. Dhanoa, D. S.; Bagley, S. W.; Chang, R. S. L.; Lotti, V. J.; Chen, T.-B.; Kivlighn, S. D.; Zingaro, G. J.; Siegl, P. K. S.; Chakravarty, P. K.; Patchett, A. A.; Greenlee, W. J. *J. Med. Chem*. **1993**, *36*, 3738–3742.
15. Dhanoa, D. S.; Bagley, S. W.; Chang, R. S. L.; Lotti, V. J.; Chen, T.-B.; Kivlighn, S. D.; Zingaro, G. J.; Siegl, P. K. S.; Patchett, A. A.; Greenlee, W. J. *J. Med. Chem*. **1993**, *36*, 4239–4249.
16. Adam, W.; Lukacs, Z.; Viebach, K.; Humpf, H. U.; Saha-Möller, C. R.; Schreier, P. *J. Org. Chem*. **2000**, *65*, 186–190.
17. Hör, K.; Gimple, O.; Schreier, P.; Humpf, H. U. *J. Org. Chem*. **1998**, 322–325.
18. Bordwell, F. G.; Knipe, A. C. *J. Org. Chem*. **1970**, *35*, 2956–2959.
19. Malcolm Bruce, J.; Sutcliffe, F. K. *J. Chem. Soc*. **1957**, 4789–4793.
20. Furniss, B. S.; Hannaford, A. J.; Smith, P. W. J.; Tatchell, A. R. *VOGEL'S, Textbook of Practical Organic Chemistry*; 5th ed.; Longman, 1989; pp. 729–730.
21. Shiraiwa, T.; Baba, Y.; Miyazaki, H.; Sakata, S.; Kawamura, S.; Uehara, M.; Kurokawa, H. *Bull. Chem. Soc. Jpn*. **1993**, *66*, 1430–1437.