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Resolution of 2-bromo-*o*-tolyl-carboxylic acid by transesterification using lipases from *Rhizomucor miehei* and *Pseudomonas cepacia*

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Abstract—Several lipases were screened for their ability to catalyze the enantioselective transesterification of 2-bromo-*o*-tolyl acetic acid. Amongst the preparations tested, the lipases from *Rhizomucor miehei* and *Pseudomonas cepacia* were selected. The best enantioselectivity was obtained with *Rhizomucor miehei* lipase immobilized on polypropylene ($E=11.3$), which was more stereoselective than the free form. Hydrophobic solvents with log P higher than 2.5 were the most suitable giving the highest E-values. In addition, factors such as the water activity and the reaction temperature had little effect on the resolution of the racemic mixture. The selectivity of the enzymes with respect to the substrate was also only weakly affected by the structure of the leaving alcohol except in the case of the *iso*-propyl group, which causes high steric hindrance. Operating conditions under reduced pressure were defined to resolve the racemic mixture with immobilized *Rhizomucor miehei* lipase.

1. Introduction

Today many organic chemists employ lipases as catalysts for the synthesis of enantiomercally pure compounds, because of their remarkable stability in organic media and their high stereoselectivity.[1,2] Enantiopure carboxylic acids are important building blocks for the synthesis of many pharmaceuticals, pesticides, and natural compounds such as pheromones.[3,4] Lipases have been successfully employed for the resolution of various racemic alcohols via hydrolysis, esterification or transesterification but only a limited amount of information exists on the chiral resolution of 2-substituted acids,[5–10] especially 2-halogeno-carboxylic acids.[11–15] Most studies concern the resolution of 2-arylpropionic acids, an important group of non-steroid anti-inflammatory drugs,[16–20] or 2-hydroxy carboxylic acids that are also important building blocks.[21] The lipases of *Candida antarctica B*,[5] *Candida rugosa*[22] and *Rhizomucor miehei*[16] have been successfully employed for the resolution of 2-arylpropionic acids and *Pseudomonas cepacia* lipase was used for the resolution of 2-hydroxy acids.[21] These studies report that many factors can modify the stereoselectivity of lipases for a given reaction. However, no rules have been established to enable the prediction of the enantioselectivity of a particular enzyme towards a specific substrate.

The 2-bromo-*o*-tolyl-acetic acid, methyl and ethyl esters derivatives, are used as precursor for the synthesis of drugs including benzylpenicillins,[23] analgesics[24] and non-peptide angiotensin II-receptor antagonists.[25,26] Here, the stereoselectivity of five commercial lipases for the resolution of 2-bromo-*o*-tolyl-acetic acid was investigated. 2-Bromo-*o*-tolyl-acetic acid was resolved by enzymatic transesterification of its esters (*RS*)-**1** to give the corresponding octyl esters (Scheme 1) and the influence of the solvent, the water activity and the temperature on the enzyme selectivity were examined. Operating conditions optimizing the production of enantiomerically pure (*R*)-**1b** are proposed.

2. Results and discussion

2.1. Lipase selection

The biocatalytic activity and enantioselectivity of commercial lipases from *P. cepacia*, *R. miehei*, *C. antarctica*, *C. rugosa* and *H. lanuginosa* were tested in transesterifi-

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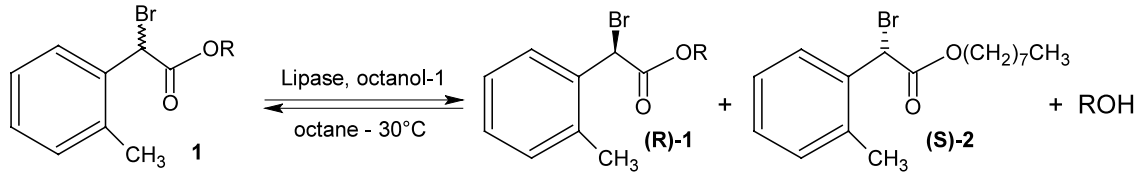

1a: R= CH_3 **1b**: R= C_2H_5 **1c**: R= C_3H_7 **1d**: R= $CH(CH_3)_2$ **1e**: R= $CH_2(C_6H_5)$

Scheme 1.

cation reactions between 2-bromo-*o*-tolyl-ethyl acetate and *n*-octanol in octane at 30°C (Scheme 1).

As shown in Table 1, among the five lipases tested, only the lipases from *R. miehei* (RML) and *P. cepacia* (PCL) were selected for the transesterification of the racemic acid. The reactions catalyzed by both enzymes proceed with *E*-values of 2.8–11.3. But, the enzymes display an opposite enantiopreference. Indeed, the (*S*)-enantiomer is preferred by RML whereas the (*R*)-enantiomer is preferred by PCL. The opposing preferences observed for these lipases is probably related to the structure of their active sites. Although the amino acids involved in catalysis are similar for both enzymes, the topology of their active sites is very different.[29–31] The binding pocket of RML at the surface of the protein is obstructed by the 'so-called' lid, whereas the binding pocket of PCL forms an elliptical funnel which is more accessible than the binding pocket of RML.

The enantioselectivity of immobilized forms of PCL and RML was also investigated as shown in Table 1. The *E*-values increased from 2.8 to 11.3 when RML was immobilized on a hydrophobic support such as polypropylene. In contrast, PCL immobilized on polypropylene was much less active than the free enzyme, with less than 2% of the substrate being consumed in 120 h. This illustrates how immobilization influences (and in some cases improves) the selectivity and activity of a catalyst. These effects can be attributed to conformational changes, which would be highly dependent on the catalyst and on the immobilization methods involved. Edlund et al.[32] observed similar effects with *C. rugosa* lipase, which was found to be more enantioselective after immobilization on polypropylene for the esterification of (±)-2-methyldecanoic acid with long chain alcohols. When immobilized CRL was used, *E*-values were higher ($E=28^a$ or $E=120$–150^b) than *E*-values of reactions using crude lipase ($E=14^a$ or $E=23$–24^b) with 1-hexadecanol[a] or 1-octadecanol[b], respectively. More recently Fernandez-Lorente et al.[33,34] showed that immobilization of *Pseudomonas fluorescens* on different supports is a valuable method for modulation of the activity and selectivity. Indeed, the enantioselectivity of the free enzyme and of some immobilized derivatives was increased by more than one order of magnitude (from $E=7$ to 80) by using lipase immobilized on hydrophobically-modified matrices such as octyl agarose or decaoctyl Sepabeads.

Table 1. Lipase-catalyzed transesterification between α-bromo-*o*-tolyl-ethyl acetate **1b** and *n*-octanol in octane in the presence of different free or immobilized lipases

Lipase	Support	Conversion (%)	e.e.$_p$[c] (%)	E[d]	f.e.[e]
R. miehei	No	22[a]	41	2.8 (±0.1)	*S*
R. miehei	Polypropylene PP 100	25[a]	74	11.3 (±0.7)	*S*
R. miehei	Duolite A568	34[a]	65	9.5 (±0.2)	*S*
P. cepacia	No	15[b]	57	4.3 (±0.5)	*R*
P. cepacia	Polypropylene PP 100	<2[b]	n.d.	n.d.	n.d.
C. antarctica B	No	7[b]	23	n.d.	*S*
C. antarctica B	Polypropylene PP 100	<2[b]	n.d.	n.d.	n.d.
C. rugosa	No	5[b]	19	n.d.	*S*
H. lanuginosa	No	<2[b]	n.d.	n.d.	n.d.

[a] After 72 h.
[b] After 120 h.
[c] Enantiomeric excess of product determined by chiral HPLC.
[d] *E* (ratio of initial rates of product appearance (vi*R*/vi*S*)).
[e] Fast reacting enantiomer. The absolute configuration was determined by circular dichroism (Exciton method); as explained in Refs. 27 and 28 n.d.: not determined.

2.2. Influence of the structure of the leaving alcohol

To study the influence of the structure and size of the leaving alcohol of the ester substrate on the enantioselectivity of the studied lipases (RML and PCL), the transesterification reaction was carried out using various esters and *n*-octanol as substrate. Substituents (Scheme 1) of increasing size were tested: methyl, ethyl, propyl, *iso*-propyl and benzyl esters. The results of the transesterification with *n*-octanol in octane are summarized in Table 2.

- The first observation is that the *iso*-propyl ester reacts very slowly with both lipases. This is probably due to the steric hindrance induced by this substituent.
- Except for the *iso*-propyl ester, increasing the size of the -OR substituent did not drastically modify the activity or the enantioselectivity of free RML. The immobilized enzyme was more sensitive to the variation of structure of the acyl moiety. A moderate enantioselectivity of 11.3±0.7 was measured with an ethyl substituent but there was no correlation between the size of the leaving alcohol and the enantioselectivity measured.
- Low *E*-values ranging from 3.7 to 6 were obtained with the free PCL, benzyl ester being better resolved than all the other esters.

The best substrate for RML, **1b** and the best substrate for PCL, **1e**, were used for the remainder of the study.

2.3. Effect of the organic solvent

In order to investigate solvent effects, transesterification reactions were carried out in a wide variety of solvents with log *P* values of 1.3 to 4.5. For each reaction, the *E* value was measured. Table 3 shows the results obtained with immobilized RML and free PCL for the transesterification of substrates **1b** and **1e** with *n*-octanol.

Table 2. Enantioselectivity of *R. miehei* and *P. cepacia* lipase in transesterification reaction in *n*-octane with different leaving alcohols (-OR) (Scheme 1)

Acyl donor	*R. miehei* lipase				*P. cepacia* lipase	
	Free		Immobilized on polypropylene		Free	
	E^a	Conversion[b] (%)	E^a	Conversion[b] (%)	E^a	Conversion[b] (%)
1a	2.8 (±0.1)	18	6.2 (±0.1)	23	5.2 (±0.3)	17
1b	2.8 (±0.1)	32	11.3 (±0.7)	34	4.3 (±0.5)	15
1c	2.2 (±0.1)	26	6.3 (±0.5)	30	3.7 (±0.2)	21
1d	n.d.	<4	n.d.	<2	n.d.	<4
1e	1.5 (±0.1)	26	1.1 (±0.1)	25	6.0 (±0.2)	33

[a] $E = (viR/viS)$.
[b] After 216 h n.d.: not determined.

Table 3. Effect of organic solvent on enantioselectivity of RML- and PCL-catalyzed transesterification of **1b** and **1e**, respectively with *n*-octanol

Solvents	Log *P*	*R. miehei* lipase immobilized on polypropylene				Free *P. cepacia* lipase			
		E^a	Conversion (%)	e.e.$_s$ (%)[e]	e.e.$_p$ (%)[e]	E^a	Conversion (%)	e.e.$_s$ (%)[e]	e.e.$_p$ (%)[e]
Octane	4.5	11.3	37[b]	45	73	6.0	39[b]	43	63
Isooctane	4.5	9.5	43[b]	48	63	8.0	43[b]	49	65
Cyclooctane	4.5	8.6	44[b]	49	62	8.2	49[b]	62	61
Heptane	4.0	10.3	36[b]	41	73	5.7	45[b]	47	56
Hexane	3.5	10.6	43[b]	52	69	5.7	45[b]	45	54
Cyclohexane	3.2	8.9	49[b]	57	59	7.4	41[b]	50	67
Toluene	2.5	12.6	30[b]	34	79	8.0	14[b]	10	73
Di*iso*-propyl ether	1.9	3.4	46[c]	33	39	7.1	51[d]	62	60
MTBE	1.9	5.6	55[c]	58	47	8.7	54[b]	68	57
MIBK	1.3	2.2	26[b]	2	6	2.0	19[b]	8	10
2M2B	1.3	n.d.	<5[b]	n.d.	n.d.	6.4	16[b]	16	79

[a] Ratio of initial rates.
[b] After 216 h.
[c] After 72 h.
[d] After 120 h. n.d.: not determined.
[e] Enantiomeric excess of substrate (e.e.$_s$) and product (e.e.$_p$) determined by chiral HPLC. MTBE = methyl *tert*-butyl ether. MIBK = methyl isobutyl ketone. 2M2B = 2-methyl-2-butanol

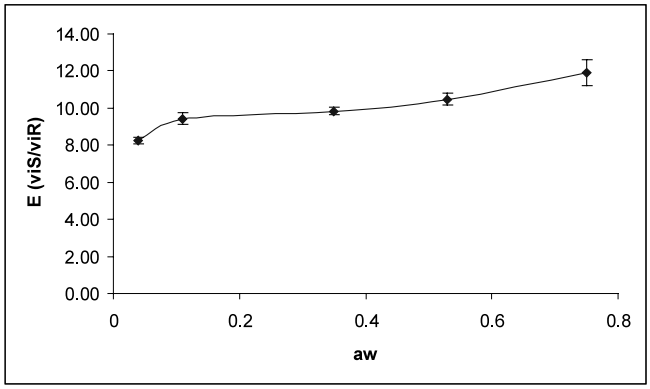

Figure 1. Enantioselectivity of RML immobilized on polypropylene as a function of water activity in transesterification between **1b** and *n*-octanol in octane.

- Whatever the solvent used, lipases from RML and PCL always showed the same preference for an enantiomer, (the (*S*)-enantiomer for RML and the (*R*)-enantiomer for PCL).
- Enzyme activities varied with the solvent used. However, no straightforward correlation can be established between the activity measured and the hydrophobicity of the solvent. From the results shown in Table 3, the solvents can be classified into three categories. The first category includes hydrophilic solvents with log P<1.9. With these solvents, both conversion and enantioselectivity were low. 2-Methylbutan-2-ol seems to denature RML because the conversion was less than five percent after reaction of 216 h duration. The second category of solvents, ethers, have intermediate log P values ranging from 1.3 to 2.5. In their presence the lipases were more active, but the enantioselectivity was low. These solvents may change the enzyme/substrate interaction, by modifying for example the organization of water molecules in the active site, thus increasing or decreasing the preference for one of the enantiomers. The last category of solvents includes more hydrophobic solvents with log P higher than 2.5. The overall reaction rate decreased in their presence as indicated by the values obtained after 216 h. They are undoubtedly the most suitable solvents for the resolution of **1b** as they represent a good compromise between reaction rates and E-values.

2.4. Effect of water activity

It is well established that the hydration level of the reaction medium influences the properties of enzymes, especially their catalytic activity, but also in some circumstances the enantiomeric ratio.[35,36] The influence of water activity on the resolution of **1b** by RML immobilized on polypropylene was investigated. The initial rate of production of both the (*R*)- and (*S*)-enantiomers decreased with increasing activity, but no significant effect was observed on the resulting enantioselectivity as shown in Fig. 1.

2.5. Effect of temperature

Temperature is another factor, which, in certain cases affects the enantioselectivity of enzymatic reactions. Consequently, the effect of temperature on the RML- and PCL-catalyzed transesterification of **1b** and **1e**, respectively with *n*-octanol in octane was investigated. As shown in Fig. 2, the increase in temperature reduced the enantioselectivity of RML, whereas it had no effect on the enantioselectivity of the reactions using PCL.

2.6. Operating conditions for the resolution of 1b

The best conditions for enantioselective transesterification of **1b** were obtained with immobilized RML in the presence of *n*-octanol at 30°C. In these conditions, (*R*)-**1b** cannot be separated from the (*S*)-**1b** because the reaction equilibrium, is reached when the enantiomeric excess of substrate is only about 45% and it is not possible to convert more (*S*)-enantiomer at that point. One way to improve the resolution involves shifting the reaction equilibrium in order to ensure complete conversion of the (*S*)-enantiomer. The reaction equilibrium was thus shifted by carrying out the reaction under reduced pressure. In this way, the ethanol produced was continuously removed and the equilibrium shifted

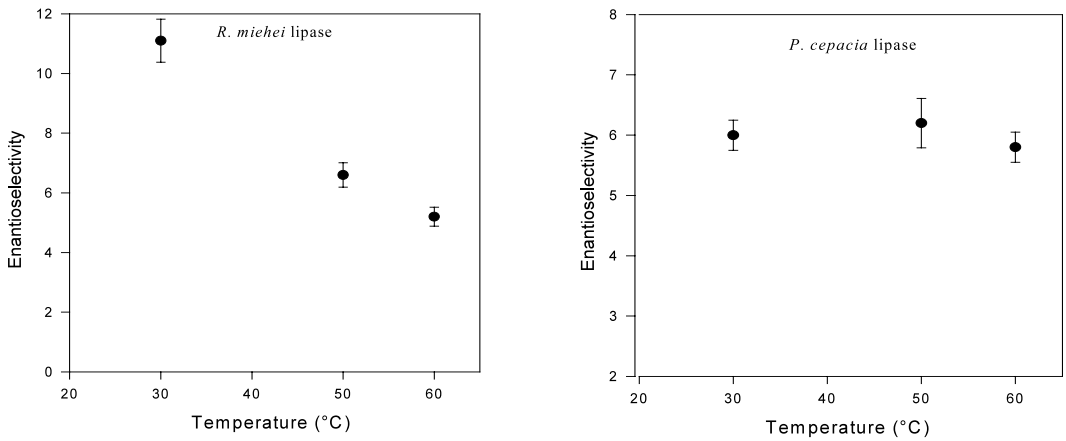

Figure 2. Effect of temperature on the enantioselectivity of RML (immobilized on polypropylene) and PCL (free lipase) lipase-catalyzed transesterification of **1b** and **1e**, respectively with *n*-octanol in octane.

Table 4. Resolution of racemic **1b** under reduced pressure catalyzed by RML immobilized on polypropylene

Conditions	E[a]	e.e.$_s$%[c]	e.e.$_p$%[c]	% Conversion
30°C, 1 atm[b]	11.3	45	73	37 (216 h)
30°C, 260 mBar[b]	9.5	66	63	51 (139 h)

[a] Ratio of initial rates.
[b] [ester] = 50 mM, [octanol] = 150 mM, 10 mg/mL of immobilized RML in octane.
[c] Enantiomeric excess of substrate (e.e.$_s$) and product (e.e.$_p$) determined by chiral HPLC.

towards the product side. The results are summarized in Table 4.

After incubation for 360 h (>75% conversion) under 260 mBar pressure at 30°C, unreacted substrate (*R*)-**1b** was obtained with an e.e.$_s$ of >97 and 20% yield after separation by column chromatography on silica gel.

3. Conclusions

In conclusion the stereoselectivity of RML and PCL towards α-bromo-*o*-tolyl acetic esters is poorly influenced by the solvent used, the water activity and the reaction temperature.

The best enantioselectivity $E = 11.3$ was obtained with an immobilized form of RML, whereas the value of $E = 2.8$ was obtained with free RML for **1b** as substrate showing that immobilization can improve the selectivity of the catalyst.

Even with moderate enantioselectivity, controlling the operating conditions enables the shift of reaction equilibrium towards the products to prepare enantiopure (*R*)-**1b** in 20% yield with e.e.$_s$ of >97. To improve the yield the effect of enzyme immobilization and substrate structure on the enantioselectivity and activity of the enzyme will be further investigated.

4. Experimental

4.1. Biological reagents

All lipases were purchased from Roche Diagnostics (Germany). Chirazyme L-9, lyo. (free lipase from *R. miehei*), Chirazyme L-9, c-f, C2, lyo. (lipase from *R. miehei* immobilized by adsorption on porous polypropylene), Chirazyme L-9, c-f, dry, (lipase from *R. miehei* immobilized by adsorption on a macroporous anion exchange resin). Chirazyme L-1, lyo. (free lipase from *P. cepacia*), Chirazyme L-1, c-f, C2, lyo. (lipase from *P. cepacia* immobilized by adsorption on porous polypropylene). Chirazyme L-2, lyo. (free lipase from *C. antarctica*), Chirazyme L-2, c-f, C2, lyo. (lipase from *C. antarctica* immobilized by adsorption onto an acrylic resin), Chirazyme L-2, c-f, C3, lyo. (lipase from *C. antarctica* immobilized by adsorption onto porous polypropylene). Chirazyme L-8, lyo (free lipase from *H. lanuginosa*). Chirazyme L-3, lyo. (free lipase from *C. rugosa*).

4.2. Chemical reagents

All reagents were of commercial quality and were purchased from Sigma/Aldrich. All solvents were dried over molecular sieve (3 Å) before use.

4.3. General procedure for the preparation of 2-bromo-*o*-tolyl acetic acid alkyl ester *o*-tolylacetic acid

The bromoacetic acid was synthesized according to a method previously described which was slightly modified as follows.[37–39]

(N.B. The preparation must be carried out in an efficient fume cupboard).

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*-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 *o*-tolyl-acetic acid. The residue was washed with cold toluene (2–3×100 mL). Inorganic salts were separated from *o*-tolylacetic acid by toluene extraction using a Soxhlet apparatus. The acid crystallized at rt and was collected on a Buchner funnel and dried in air to afford the product as a white solid (25.90 g, 38% yield).

4.3.1. α-Bromo-*o*-tolylacetic acid 1. Compound (±)-**1** (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 product as a white solid with a (5.39 g, 39%).

4.3.2. 2-Bromo-*o*-tolyl-acetate esters 1a–1e

4.3.2.1. Methyl, ethyl, propyl and *iso*-propyl esters. The bromo acid (43.6 mmol), alcohol (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. The alcohol was evaporated at 35°C under water-pump vacuum. The residual oil was dissolved in dichloromethane (25 mL) and washed with 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. Mass spectra were recorded on a Micro Mass, auto-spect EBEQ (EI+, 70 ev).

4.4.1. (±)-2-Bromo-*o*-tolyl-acetic acid. Yield: 5.39 g, (39%): IR (KBr, neat) 3300 to 2800 (ν_{O-H}), 1710 ($\nu_{C=O}$), 1600 ($\nu_{C=C}$), 1310 to 1240 (ν_{C-O}) cm^{-1}. ^{1}H NMR (*d*, DMSO): δ 2.35 (s, 3H, ArC$\underline{H}_3$): δ 5.95 (s, 1H, -C$\underline{H}$Br): δ 7.20–7.51 (m, 4H, Ar$\underline{H}$): δ 13.24 (s, 1H, COO$\underline{H}$); ^{13}C NMR (*d*, DMSO): δ 18.74 (Ar$\underline{C}$H$_3$), 47.39 (-$\underline{C}$HBr), 126.420, 128.59, 128.81, 130.66, 135.39, 138.16, 169.06 ($\underline{C}$OOH). Mass: calcd M$^{+\bullet}$=227.97859, found M$^{+\bullet}$=227.97803. Mp 106–108°C.

4.4.2. (±)-2-Bromo-*o*-tolyl methyl acetate. Yield: 8.12 g, (77%). IR (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280 to1140 (ν_{C-O}) cm^{-1}. ^{1}H NMR (CDCL$_3$): δ 2.41 (s, 3H, ArC$\underline{H}_3$): δ 3.79 (s, 3H, -OC$\underline{H}_3$): δ 5.65 (s, 1H, -C$\underline{H}$Br): δ 7.19–7.26 (m, 3H, Ar$\underline{H}$): δ 7.58–7.62 (m, 1H, Ar$\underline{H}$); ^{13}C NMR (CDCL$_3$): δ 19.34 (Ar$\underline{C}$H$_3$), 44.52 (-$\underline{C}$HBr), 53.53 (-O$\underline{C}$H3), 126.95, 128.85, 129.34, 130.91, 134.37, 136.11, 168.80 ($\underline{C}$OO). Mass: calcd M$^{+\bullet}$=241.99424, found M$^{+\bullet}$=241.99324.

4.4.3. (±)-2-Bromo-*o*-tolyl ethyl acetate. Yield: 8.70 g, (78%). IR (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280 to 1140 (ν_{C-O}) cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 1.24–1.31 (t, *J*=*7Hz*, 3H, -OC$\underline{H}_3$): δ 2.41 (s, 3H, ArC$\underline{H}_3$): δ 4.19–4.31 (qd, *J*=*3.2–7 Hz*, 2H, -OC$\underline{H}_2$CH$_3$): δ 5.63 (s, 1H, -C$\underline{H}$Br): δ 7.19–7.26 (m, 3H, Ar$\underline{H}$): δ 7.59–7.64 (m, 1H, Ar$\underline{H}$); ^{13}C NMR (CDCl$_3$): δ 14.05 (-CH$_2\underline{C}$H$_3$), 19.36 (Ar$\underline{C}$H$_3$), 44.83 (-CHBr), 62.68 (-O$\underline{C}$H$_2$CH$_3$), 126.90, 128.79, 129.25, 130.87, 134.48, 136.13, 168.30 ($\underline{C}$OO). Mass: calcd M$^{+\bullet}$=256.00989, found M$^{+\bullet}$=256.00961.

4.4.4. (±)-2-Bromo-*o*-tolyl propyl acetate. Yield: 9.06 g, (77%). IR (neat), 1750 and 1725 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280 to 1140 (ν_{C-O}) cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 0.86–0.94 (t, *J*=7.2 Hz, 3H, -CH$_2$C$\underline{H}_3$): δ 1.60–1.74 (m, *J*=7.2 Hz, 2H, -OCH$_2$C$\underline{H}_2$CH$_3$): δ 2.41 (s, 3H, ArC$\underline{H}_3$): δ 4.11–4.18 (td, *J*=1.2–7.2 Hz, 2H, -OC$\underline{H}_2$): δ 5.64 (s, 1H, -C$\underline{H}$Br): δ 7.15–7.26 (m, 3H, Ar$\underline{H}$): δ 7.60–7.64 (m, 1H, Ar$\underline{H}$); ^{13}C NMR (CDCl$_3$): δ 10.25 (-CH$_2\underline{C}$H$_3$), 19.32 (Ar$\underline{C}$H$_3$), 21.82 (-$\underline{C}$H2-), 44.78 (-$\underline{C}$HBr), 68.12 (-O$\underline{C}$H2-), 126.83, 128.85, 129.16, 130.76, 134.47, 136.03, 166.34 ($\underline{C}$OO). Mass: calcd M$^{+\bullet}$=270.02554, found M$^{+\bullet}$=270.02493.

4.4.5. (±)-2-Bromo-*o*-tolyl *iso*-propyl acetate. Yield: 2.35 g, (20%). IR (neat) 1745 and 1720 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280 to 1100 (ν_{C-O}) cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 1.19–1.23 (d, *J*=6.3 Hz, 3H, -CH(C$\underline{H}_3$): δ 1.27–1.30 (d, *J*=6.3 Hz, 3H, -CH(C$\underline{H}_3$): δ 2.41 (s, 1H, ArC$\underline{H}_3$): δ 5.04–5.12 (m, 1H, CH): δ 5.59 (s, 1H, -C$\underline{H}$Br),. δ 7.16–7.26 (m, 3H, Ar$\underline{H}$): δ 7.59–7.63 (m, 1H, Ar$\underline{H}$); ^{13}C NMR (CDCl$_3$): δ 19.32 (ArCH3), 21.46 (-OCH($\underline{C}$H$_3$)$_2$), 21.56 (-OCH($\underline{C}$H$_3$)$_2$), 45.14 (-$\underline{C}$HBr), 70.44, (-O$\underline{C}$H(CH$_3$)$_2$ 126.60, 128.66, 129.12, 130.79, 134.53, 136.09, 167.74 ($\underline{C}$OO). Mass: calcd M$^{+\bullet}$=270.02554, found M$^{+\bullet}$=270.02615.

4.4.6. (±)-2-Bromo-*o*-tolyl benzyl acetate. Yield: 14.16 g, (74%). IR (neat) 1750 and 1730 ($\nu_{C=O}$), 1600 and 1475 ($\nu_{C=C}$), 1280 to 1140 (ν_{C-O}) cm^{-1}. ^{1}H NMR (CDCl$_3$): δ 2.4 (s, 3H, ArC$\underline{H}_3$): δ 5.17–5.32 (2H, d, *J*=12.2 Hz, -OC$\underline{H}_2$ph): δ 5.71 (s, 1H, -C$\underline{H}$Br): δ 7.18–7.27 (m, 3H, Ar$\underline{H}$): δ 7.34–7.36 (m, 5H, Ar$\underline{H}$): δ 7.60–7.64 (m, 1H, Ar$\underline{H}$); ^{13}C NMR (CDCl$_3$): δ 19.38 (Ar$\underline{C}$H3), 44.71 (-$\underline{C}$HBr), 68.21 (-O$\underline{C}$H$_2$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 ($\underline{C}$OO). Mass: calcd M$^{+\bullet}$=318.02554, found M$^{+\bullet}$=318.02649.

4.4.7. (±)-2-Bromo-*o*-tolyl octyl acetate. 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$_2$C$\underline{H}_3$): δ 1.25 (s, 10H, C$\underline{H}_2$): δ 1.63 (m, 2H, C$\underline{H}_2$): δ 2.41 (s, 3H, ArC$\underline{H}_3$): δ 4.15–4.22 (t, *J*=6.8 Hz, -OC$\underline{H}_2$CH$_2$-): δ 5.63 (s, 1H, -C$\underline{H}$Br): δ 7.18–7.25 (m, 3H, Ar$\underline{H}$): δ 7.60 (m, 1H, Ar$\underline{H}$); ^{13}C NMR (CDCl$_3$): δ 14.16 (-CH$_2\underline{C}$H$_3$), 19.37 (ArCH3), 22.69, 25.74, 28.42, 29.13, 29.17, 31.78, 44.85 (-$\underline{C}$HBr), 66.75 (-O$\underline{C}$H2-), 126.87, 128.88, 129.21, 130.82, 134.51, 136.05, 168.36 ($\underline{C}$OO). Mass: calcd M$^{+\bullet}$=340.10379, found M$^{+\bullet}$=340.10298.

4.5. Pre-equilibration of water activity (a_w)

Enzyme preparations and organic solvent were equilibrated with saturated salt solutions for at least four days at 4°C in separate containers. The salts used were LiBr (water activity, a_w=0.06), LiCl (a_w=0.11), $MgCl_2 \cdot 6H_2O$ (a_w=0.32), $Mg(NO_3)_2 \cdot 6H_2O$ (a_w=0.53), NaCl (a_w=0.75), KNO_3 (a_w=0.92), $K_2Cr_2O_7$ (a_w=0.98). With molecular sieve (3 Å) a_w=0.04.

4.6. General procedure for the enzymatic transesterification

A typical transesterification was carried out in solvent (5 mL) containing the ester (0.25 mmol) (50 mM), octanol (150 mM) (0.75 mmol) and lipase (5 mg/mL for free lipase and 10 mg/mL for immobilized lipase). The temperature was maintained at 30°C unless otherwise stated in the text. The mixture was shaken at 250 rpm for the time indicated in Tables 1–4. The progress of the reaction was followed by taking samples at regular intervals.

4.7. Enzymatic transesterification under reduced pressure

(±)-2-Bromo-*o*-tolyl ethyl acetate **1b** (128 mg, 0.5 mmol) and octanol (195.3 mg, 1.5 mmol) were dissolved in *n*-octane (10 mL). Then, *R. miehei* lipase immobilized on polypropylene (10 mg/mL) was added. The reaction was carried out in a rotative apparatus using the conditions specified in Table 4. The reaction progress was followed by taking samples at regular intervals.

The reaction carried out at 260 mBar pressure was terminated after 360 h (>75% conversion) by removing the immobilized enzyme by filtration. The enzyme was washed twice with *n*-octane. Both the filtrate and the washing solutions were evaporated. The pale yellow oil obtained was then applied to a column of silica gel (60 M, 230–400 mesh), the products **2** and **1b** were separated by chromatography using *n*-hexane–ethyl acetate (98:2, v/v) as eluent. (*R*)-**1b** was obtained as a colorless oil (26.1 mg, 20%); $[\alpha]_D^{20} = -3.4$ (*c* 0.015, *n*-hexane); e.e.$_s$ >97% by HPLC. The product was obtained as a colorless oil (88.6 mg of octyl ester, 52%).

4.8. HPLC analysis

The Chiral HPLC device was equipped with a chiral column: Chiralpack AD or AS (25 cm×4.6 mm) (Daicel Chemical Industries Ltd, Japan) connected to a UV detector (at 254 nm). Each sample was diluted 16 times in the mobile phase (hexane/isopropanol 99.8/0.2, v/v), filtered before injection (20 μL) and analyzed at a flow rate 1.0 mL/min at rt.

4.9. 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: $C = 1-[(R-S)_t/(R-S)_{t=0}]*100$.

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*): $E = (viR/viS)$.[40] The initial rates were determined by linear regression over at least five points before 10% of substrate conversion.

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