Received: 21 June 2017 | Revised: 29 August 2017 | Accepted: 1 October 2017

DOI: 10.1002/chir.22779

REGULAR ARTICLE

WILEY

Convenient enzymatic resolution of (*R*,*S*)-2-methylbutyric acid catalyzed by immobilized lipases

Mateus Mittersteiner[1] | Bruna Luiza Linshalm[1] | Ana Paula Furlan Vieira[1] | Patrícia Bulegon Brondani[2] | Dilamara Riva Scharf[1] | Paulo Cesar de Jesus[1]

[1] Departamento de Química, Universidade Regional de Blumenau, Blumenau, SC, Brazil

[2] Departmento de Ciências Exatas e Educação, Universidade Federal de Santa Catarina, Blumenau, SC, Brazil

Correspondence
P. C. de Jesus, Departamento de Química, Universidade Regional de Blumenau, Antonio da Veiga Street, 140, Blumenau, SC 89019-917, Brazil.
Email: pcj@furb.br

Abstract

The application of several immobilized lipases has been explored in the enantioselective esterification of (*R*,*S*)-2-methylbutyric acid, an insect pheromone precursor. With the use of *Candida antarctica* B, using hexane as solvent, (*R*)-pentyl 2-methylbutyrate was prepared in 2 h with c 40%, ee_p 90%, and $E = 35$, while *Thermomyces lanuginosus* leads to c 18%, ee_p 91%, and $E = 26$. The (*S*)-enantiomer was obtained by the use of *Candida rugosa* or *Rhizopus oryzae* (2-h reaction, c 34% and 35%, ee_p 75 and 49%, and $E = 10$ and 4, respectively). Under optimal conditions, the effect of the solvent, the molar ratio, and the nucleophile were evaluated.

KEYWORDS

enantioselective esterification, enzymatic acylation, immobilized enzymes, pheromones, racemic acids

1 | INTRODUCTION

Enzymes are very efficient catalysts; the rates of enzyme-mediated processes are generally faster (by a factor of approximately 10^{12}) than chemically catalyzed processes. In addition, enzyme-catalyzed reactions are environmentally friendly, occurring under mild conditions. Lipases are considered stable and robust enzymes, even though they can be sensitive to reaction conditions (such as pressure, temperature, and pH). Given their high specificity, commercial availability, and versatility to catalyze a wide range of different reactions (such as esterification, transesterification, and interesterification), they became the most explored class of enzymes in organic synthesis.[1]

Enantiopure alcohols,[2-4] amines,[5,6] and carboxylic acids[7-9] are key compounds for the synthesis of many fine-chemistry derivatives, such as pharmaceuticals, agrochemicals, and pheromones.[9] In particular, steriochemically pure carboxylic acids are extremely useful building blocks, being easily converted into many of the other functional groups.

As an example, consider the (*S*)-2-methylbutyric acid is found in the sexual pheromones of the pink hibiscus mealybug, *Maconellicoccus hirsutus*. It is also a precursor of (*S*)-2-methyl-*N*-((*S*)-2-methylbutyl)butanamide, the sexual pheromone of *Migdolus fryanus*,[10,11] a beetle considered a pest in sugarcane crops. The (*R*)-enantiomer has been successfully applied by Zhang et al[12] in the synthesis of liquid crystals, leading to good luminescent and liquid crystalline properties. Additionally, the esterification of carboxylic acids in general leads to ester derivatives that are as well interesting building blocks for the synthesis of pheromones and flavored compounds used in the food industry.

In spite of the fact that several racemic carboxylic acids have been resolved by lipases,[13-22] a literature survey shows only few methodologies for the enantioselective esterification of 2-methylbutyric acid. This may be due to the fact that methyl and ethyl moieties have a small size difference making it difficult for the efficient recognition by the lipase.[23] In an attempt to resolve the 2-methylbutyric acid, Holmberg et al[24] prepared (*S*)-heptyl

2-methylbutyrate using *Candida cylindracea* in the free form as a catalyst. They observed 77% of conversion with enantioselectivity (*E*) of 4.6 and the remaining (*R*)-acid with 85% of enantiomeric excess (*ee*). Lipase originating from *Chromobacterium viscosum* immobilized in a microemulsion-based organogel was also applied in the preparation of (*S*)-ethyl 2-methylbutyrate. The ester was obtained with an *ee* of 31.7% in a reaction time of 21 days, while the nonreactive (*R*)-acid was obtained with 22.2% of *ee*.[25] Better enantioselectivity values were obtained when the enzymatic hydrolysis was performed on the (*R*,*S*)-ethyl 2-methylbutyrate, obtaining the (*R*)-2-methylbutyric acid with 95% of enantiomeric excess at 4°C.[23] Despite the high *ee* values, this protocol requires a previous chemical preparation of the racemic ester for posterior enzymatic hydrolysis.

In this context, encouraged by the few publications regarding methodologies for the enantioselective esterification and resolution of this racemic acid, we present herein an efficient and direct methodology for this purpose.

2 | MATERIALS AND METHODS

2.1 | General

(*R*,*S*)-2-Methylbutyric acid (98%) was purchased from Acros Organic. The solvents 1,4-dioxan (99%), cyclohexane (99%), heptane (99%), and ethyl acetate (99.5%) were obtained from Vetec. *n*-Hexane (98.5%), acetonitrile (99%), and chloroform (99.5%) were obtained from Dinâmica. All reagents and solvents were obtained from commercial suppliers and used without further purification. The alcohols 1-butanol (99.4%), 1-pentanol (98%), 1-hexanol (98%), and 1-octanol (98%) were obtained from Vetec. Silica gel (0.063-0.200 mm; 70-230 mesh) was obtained from Macharey-Nagel. The crude sugarcane bagasse was obtained from a local commercial source. Commercially available lipases from *Candida antarctica* B (CaLB) immobilized in resin (10 U mg^{-1}), Lipozyme 435 (10 U mg^{-1}), and Lipozyme CaLB-L (L. CaLB-L; 5 KLU g^{-1}) were kindly donated by Novozymes and lipases originating from *Rhizopus oryzae* (ROL; 150 U g^{-1}), *Mucor javanicus* (MJL; 10 U g^{-1}), and *Candida rugosa* (CRL; 30 U g^{-1}) were donated by Amano Pharmaceuticals Co. Lipase from *Thermomyces lanuginosus* (TLL; 50 U g^{-1}) was obtained from Sigma-Aldrich.

2.2 | Analytical methods

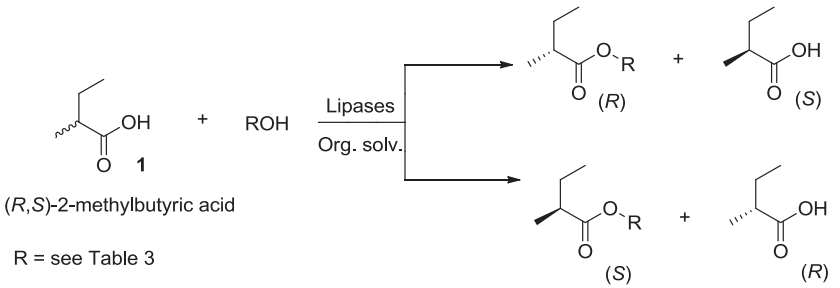
NMR was used to calculate the conversion and characterize the compounds. Spectra were recorded on a Bruker Ultrashield spectrometer operating at a frequency of 300 MHz (^{1}H NMR) and 75 MHz (^{13}C NMR). Data are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, sex = sextet, dq = double quartet, and m = multiplet), and coupling constants (*J*) in Hertz and integrated intensity. Gas chromatography (GC) analysis for the determination of the enantiomeric excesses of the product (ee_p) and substrate (ee_s) was carried out using a GC Agilent 7890B and a Shimadzu 14B (FID detector). A β-DEX 120 column (30 m × 0.25 mm × 0.25 μm) (Supelco) was used, and the method applied was column temperature program of 70°C (3 min) to 200 °C (7°C min^{-1}) and 200°C (15 min). The injector and detector temperatures were set at 250°C and 300°C, respectively. The flow rate for the carrier gas (helium) was 2 mL min^{-1}. The retention times under these conditions were 9.34 min for (*S*)-2-methylbutyric acid, 9.41 min for (*R*)-2-methylbutyric acid, 9.58 min for (*R*)-pentyl 2-methylbutyrate, and 9.65 min for (*S*)-pentyl 2-methylbutyrate. The parameters (ee_p and ee_s) were calculated according the integrated area of the enantiomers. The absolute configuration was determined by comparison with data previously reported for these enzymes.[9,26,27]

2.3 | Immobilization of the lipases

Prior to the immobilization, a pre-treatment was applied to the raw bagasse in order to standardize the solid support. Firstly, the peel was removed and discarded, and the remaining parts were placed in a flask containing water for 24 h, changing the water periodically. The fibers were then cut into pieces of approximately 0.8 cm and boiled in water for 2 h to remove the saccharose from the residue. The bagasse was then washed with running water and left to dry at room temperature. The dry bagasse was placed in an Erlenmeyer flask (1 g) along with phosphate buffer (100 mL) (Na_2HPO_4/KH_2PO_4 0.07 mol L^{-1}; pH 7.2) and the lipase, that is, CRL, MJL, or ROL (200 mg). The mixture was shaken (150 rpm) at 35°C (optimal temperature of most lipases herein used) for 2 h. Lipozyme CaLB-L and lipase from *Thermomyces lanuginosus* were immobilized onto SiO_2 according to Mittersteiner et al.[28]

2.4 | General procedure for enzymatic resolution

The immobilized lipase was placed in a 125-mL Erlenmeyer flask containing an organic solvent (25 mL), (*R*,*S*)-2-methylbutyric acid (10 mmol), and an aliphatic alcohol (10 mmol). The reaction mixture was then placed in an orbital shaker (Technal TE-420) at 150 rpm and 37°C for pre-determined times. The formation of the products



SCHEME 1 Enantioselective esterification of (*R*,*S*)-1 with aliphatic alcohols catalyzed by immobilized lipases

was monitored by thin layer chromatography using *n*-hexane and ethyl acetate (15:1 *v*/v) as eluent. The reactions were interrupted, the enzyme was filtered out, and the reaction medium was analyzed by ^{1}H NMR and GC-FID. Control reactions were carried out in the absence of the lipases, and no product was detected under the same experimental conditions. After column chromatography (the same eluent as cited above), (*R*)-pentyl 2-methylbutyrate (yield: 30%) and (*S*)-2-methylbutyric acid (yield: 60%) were isolated and characterized by ^{1}H and ^{13}C NMR.

(*R*)-pentyl 2-methylbutyrate: $[\alpha]_D^{25} = +51$ (c 0.1, $CHCl_3$) *ee* 90%; IR (KBr) cm^{-1}: 2962, 1734, 1463; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 4.06 (t, 2H, $J = 6.75$ Hz); 2.35 (sex, 1H, $J = 6.9$ Hz); 1.65 (m, 3H); 1.46 (m, 1H); 1.32 (m, 4H); 1.13 (d, 3H, $J = 6.96$ Hz); 0.90 (t, 6H, $J = 6.99$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 176.8; 64.2; 41.1; 28.3; 28.0; 26.7; 22.2; 16.6; 13.9; 11.5.

(*S*)-2-methylbutyric acid: $[\alpha]_D^{25} = -10$ (c 0.1, $CHCl_3$) *ee* 60%; IR (KBr) cm^{-1}: 3032, 2970, 2879, 1707, 1465, 1226; ^{1}H NMR (300 MHz, $CDCl_3$): δ (ppm) 11.0 (s, 1H); 2.42 (sex, 1H, $J = 6.81$ Hz); 1.72 (dq, 1H, $J = 13.62$ Hz); 1.50 (dq, 1H, $J = 13.71$ Hz); 1.18 (d, 3H, $J = 6.99$ Hz); 0.95 (t, 3H, $J = 7.44$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) 183.6; 40.9; 26.5; 16.3; 11.4.

TABLE 1 Effect of time course on the esterification reaction between (*R*,*S*)-2-methylbutyric acid and 1-pentanol catalyzed by CaLB

		(*R*)-pentyl 2-methylbutyrate			
Entry	**Time, h**	**c, %**	**ee_p, %**	**ee_s, %**	***E***
1	0.25	19	39	9	2
2	0.50	36	43	24	3
3	1.0	38	74	45	10
4	2.0	40	90	60	35
5	3.0	60	46	69	5
6	4.0	79	22	81	3
7	5.0	89	11	89	3
8	6.0	94	6	91	2

Conditions: hexane, 10 mmol of the racemic acid, 10 mmol of the alcohol, 100 mg of CaLB, 37°C, 150 rpm.

[a]Calculated by using the formula: $E = \{\ln [ee_p(1 - ee_s)]/(ee_p + ee_s)\}/\{\ln [ee_p(1 + ee_s)]/(ee_p + ee_s)\}$.[24]

3 | RESULTS AND DISCUSSION

The immobilized lipases from *Rhizopus oryzae*, *Mucor javanicus*, *Candida rugosa*, *Thermomyces lanuginosus*, and *Candida antarctica* B were applied to the resolution of (*R*,*S*)-2-methylbutyric acid. A schematic representation of the biocatalyzed resolution reaction is presented below (Scheme 1).

3.1 | Effect of time course in the resolution reaction

Initially, in order to obtain the best time conditions regarding enantiomeric excesses, CaLB was chosen as the catalyst due to the high enantiomeric excesses it usually provides. The alcohol 1-pentanol was chosen to evaluate the parameters of this first step, according to Jesus et al[16] where (*R*,*S*)-2-methylpentanoic acid, a structurally similar acid, obtained conversion of 28% and *ee* of 75% for the corresponding ester. Table 1 presents the data obtained for the different reaction times.

The data in Table 1 suggest that a kinetic competition is occurring between the enantiomers at the active site of the enzyme. The ee_p increases for up to 2 h of reaction, in which case it was possible to obtain a good conversion (40%) along with high ee_p (90%), which decays after this period of time. The longer the reaction time, the higher the ee_s value was obtained (Figure 1).

The results obtained are in agreement with values reported in the literature[24] in which (*S*)-heptyl 2-

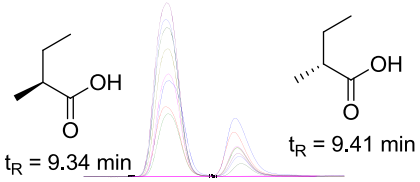


FIGURE 1 Expanded region of the chromatogram of the kinetic resolution of (*R*,*S*)-2-methylbutyric acid with 1-pentanol catalyzed by CaLB

methylbutyrate was prepared with 77% of conversion and the remaining (*R*)-acid was obtained with an enantiomeric excess of 85%.

3.2 | Screening of lipases

Based on the results obtained with CaLB, regarding the optimal time for the formation of the ester, several lipases immobilized on different supports were tested for the resolution of the racemic acid (Table 2).

As shown in Table 2, all lipases were able to catalyze the esterification reaction. The best enantiomeric excesses were observed when using CaLB (entry 4, Table 1) and TLL (entry 4, Table 2). When CRL and CaLB-L were the catalysts, moderated selectivity was observed. ROL and Lipozyme 435 showed a lower selectivity under the same conditions, while MJL provided the racemic ester. ROL and CRL catalyzed the formation of the (*S*)-esters, while TLL, CaLB, CaLB-L, and Lipozyme 435 formed the (*R*)-esters. In order to confirm the selectivity, (*R*)-pentyl 2-methylbutyrate catalyzed by CaLB was isolated by column chromatography (eluent: hexane and AcOEt 90:10) and analyzed by polarimetry, leading to $[\alpha]_D^{25} = +51$ (0.1, $CHCl_3$). The non-reactive (*S*)-acid of the corresponding reaction obtained $[\alpha]_D^{25} = -10$ (0.1, $CHCl_3$).

The reuse of lipases immobilized on SiO_2 has been previously described for our group.[28] Here, we evaluated as well the reuse of ROL immobilized on sugarcane bagasse. We observed the results for four consecutive reactions in the preparation of (*S*)-pentyl 2-methylbutyrate (Figure 2).

As can be seen in Figure 2, the immobilized lipase could be reused for two cycles without the diminishment of the conversion rate for the ester formation. After the second cycle, it was observed a decay of the conversion, which could indicate that the lipase is suffering inactivation by the organic solvent, or, due to a desorption process. Nevertheless, it is important to note that the enantioselectivity was maintained through all the four cycles of reaction.

TABLE 2 Effect of different lipases on the preparation of pentyl 2-methylbutyrate

Entry	Enzyme	Enantiopreference	c, %	ee_p, %	ee_s, %	*E*
1	ROL[a]	(*S*)	35	49	26	4
2	MJL[a]	-	32	-	-	-
3	CRL[a]	(*S*)	34	75	39	10
4	TLL[b]	(*R*)	18	91	20	26
5	CaLB-L[b]	(*R*)	09	81	8	10
6	L. 435	(*R*)	45	56	46	6

Conditions: immobilized lipases, hexane, 10 mmol of (*R*,*S*)-2-methylbutyric acid, 10 mmol of the alcohol, 2 h, 37°C, 150 rpm.

[a]Immobilized on sugarcane bagasse.

[b]Immobilized on SiO_2.

[c]The lipase did not show any selectivity.

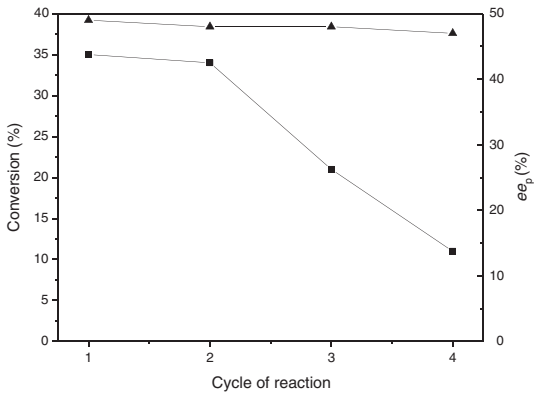


FIGURE 2 Reuse of ROL immobilized on sugarcane bagasse through four cycles of reaction in the preparation of (*S*)-pentyl 2-methylbutyrate: (■) conversion and (▲) ee_p

3.3 | Effect of the solvent and molar ratio

The polarity of the solvent, given by log P, considerably affects the activity and enantioselectivity of enzymes.[29-31] For this reason, different solvents with different log P values were evaluated under the optimal conditions defined in Table 1. The results can be observed in Figure 3.

Several biocatalytic studies have shown that reactions using solvents with log P > 3.0 are usually more efficient, since these non-polar solvents are able to trap water around the enzyme, creating a micro-aqueous layer, maintaining the conformation of the lipase, therefore preserving its catalytic activity. Polar solvents, on the other hand, tend

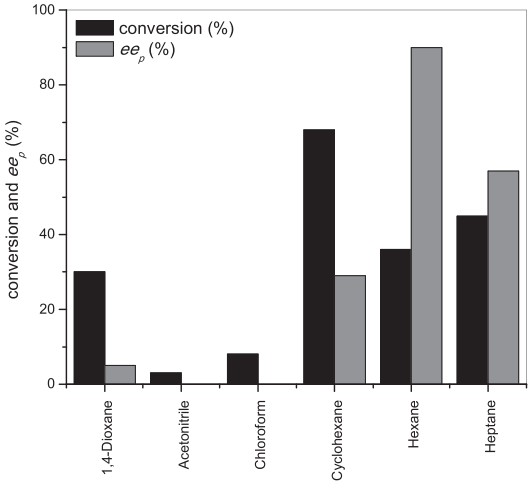


FIGURE 3 Influence of organic solvents on the esterification reaction for the formation of (*R*)-pentyl 2-methylbutyrate catalyzed by CaLB. Conditions: 100 mg of immobilized lipase, solvent, 10 mmol of (*R*,*S*)-1, 10 mmol of the alcohol, 2 h, 37°C, 150 rpm

to alter the amount of water that surrounds the enzyme, promoting a destabilization of the biocatalyst.[29,31,32] The observed results were in agreement with this information, and the enzyme activity and selectivity were highly influenced by the choice of the organic solvent.

Having established the best conditions regarding time (2 h), type of lipase (CaLB), and solvent (hexane), the molar ratio (alcohol/acid) was studied in the proportions 0.5 (excess of acid) and 2.0 (excess of alcohol).

The excess of acid (ratio 0.5) provided great conversion but poor selectivity (c 82%, ee_p 13%, ee_s 60%, $E = 2$). A possible explanation for this is the fact that the media is saturated with the racemic substrate (acyl donor) and the enzyme is no longer able to selectively differentiate between the enantiomers before the formation of the acyl-enzyme complex. This being, when the nucleophile attacks the site, there is low selectivity for the product. On the other hand, when using excess of the alcohol (ratio 2.0), moderate conversion and selectivity were obtained (c 40%, ee_p 52%, ee_s 35%, $E = 4$). We believe that in the excess of the alcohol, the nucleophilic attack is happening too fast for full selectivity control, showing that the ratio 1, as demonstrated in Table 1, is ideal for this substrate.

3.4 | Effect of the nucleophile

It is well reported in the literature that the chain size of the alcohol can affect the reaction yield, conversion, and enantioselectivity in acylation reactions catalyzed by lipases.[24] This being, a homologous series of *n*-alcohols was reacted with (*R*,*S*)-**1** to investigate the selectivity of the lipase towards different alkyl chain sizes. The obtained results can be observed in Table 3.

The conversion for the corresponding ester decreased as the chain of the alcohol was increased. This behavior was already demonstrated by Jesus et al[16] in the esterification of (*R*,*S*)-2-methylpentanoic acid with 1-pentanol, which had the best conversion for ester. Regarding enantiomeric excesses, it would be expected that in a homologous series of *n*-alcohols, the enantiomeric excess would be maintained. However, in the preparation of the different alkyl 2-methylbutyrates, the lipase showed good selectivity only when 1-pentanol was employed as the reactant, and moderate to low selectivity was presented as the alcohol moiety was increased. This suggests that the lipase is not able to accommodate the substrates without suffering loss of selectivity, thus a conformational change in the active site might be happening.

TABLE 3 Effect of the nucleophile moiety in the esterification of (*R*,*S*)-2-methylbutyric acid

Entry	Alcohol	c, %	ee_p, %	ee_s, %	*E*
1	1-butanol	54	11	13	1
2	1-pentanol	40	90	60	35
3	1-hexanol	24	44	14	3
4	1-octanol	18	50	11	3

Conditions: CaLB (100 mg), hexane (25 mL), (*R*,*S*)-2-methylbutyric acid (10 mmol), the alcohol (10 mmol), 2 h, 37°C, 150 rpm.

4 | CONCLUSION

We have demonstrated a convenient enantioselective esterification of (*R*,*S*)-2-methylbutyric acid with several immobilized lipases. A critical part of the study was the observation of a kinetic competition occurring in the formation of the enantiopure esters. In this manner, the time of reaction was proved to be a key variable. The best results were observed when TLL and CaLB were applied as catalysts, for 2 h of reaction time. (*R*)-pentyl 2-methylbutyrate was obtained by TLL with c 18% and ee_p of 91% and by CaLB with c 40% and ee_p of 90%. CRL and ROL catalyzed the (*S*)-ester with conversions of 34% to 35% and ee_p of 75% and 49%, respectively.

We expect that this enzymatic protocol will prove to be useful in the synthesis of enantiopure esters and acid derivatives, including carboxylic acids where most lipases showed low selectivity. Further kinetic studies involving other racemic compounds with small difference of the substituents should be performed.

ACKNOWLEDGMENTS

The authors are grateful to INCT Catalysis Brazil and the Chemistry Department at the Universidade Regional de Blumenau (FURB). We are also thankful to Novozymes Latin America for donating the lipases and Prof. Maria da Graça Nascimento (UFSC-Florianópolis—Brazil) for the polarimetric analysis.

ORCID

Mateus Mittersteiner http://orcid.org/0000-0002-8501-844X
Paulo Cesar de Jesus http://orcid.org/0000-0002-1314-3444

REFERENCES

1. Sheldon RA, Van Pelt S. Enzyme immobilisation in biocatalysis: why, what and how. *Chem Soc Rev*. 2013;42:6223-6235.
2. Bandeira PT, Alnoch RC, Oliveira ARM, et al. Enzymatic kinetic resolution of aliphatic sec-alcohols by LipG9, a metagenomic lipase. *J Mol Catal B: Enzym*. 2016;125:58-63.
3. Suan C, Sarmidi MR. Immobilised lipase-catalysed resolution of (*R*,*S*)-1-phenylethanol in recirculated packed bed reactor. *J Mol Catal B: Enzym*. 2004;28:111-119.

4. Silva JC, Nascimento MG. The influence of organic medium and supports in the resolution of (*R*,*S*)-menthol catalyzed by lipases. *J Braz Chem Soc*. 2016;27:2226-2233.
5. Boros Z, Falus P, Márkus M, et al. How the mode of *Candida antarctica* lipase B immobilization affects the continuous-flow kinetic resolution of racemic amines at various temperatures. *J Mol Catal B: Enzym*. 2013;85–86:119-125.
6. Youshko MI, Van Rantwijk F, Sheldon RA. Enantioselective acylation of chiral amines catalysed by aminoacylase I. *Tetrahedron Asymmetry*. 2001;12:3267-3271.
7. Fish PV, Mackenny M, Bish G, et al. Enantioselective synthesis of (*R*)- and (*S*)-N-Boc-morpholine-2-carboxylic acids by enzyme-catalyzed kinetic resolution: application to the synthesis of reboxetine analogs. *Tetrahedron Lett*. 2009;50:389-391.
8. Chen X, Wu Q, Zhu D. Enzymatic synthesis of chiral 2-hydroxy carboxylic acids. *Process Biochem*. 2015;50:759-770.
9. Tsai SW. Enantiopreference of *Candida antarctica* lipase B toward carboxylic acids: substrate models and enantioselectivity thereof. *J Mol Catal B: Enzym*. 2016;127:98-116.
10. Zhang A, Amalin D, Shirali S, et al. Sex pheromone of the pink hibiscus mealybug, *Maconellicoccus hirsutus*, contains an unusual cyclobutanoid monoterpene. *Proc Natl Acad Sci U S A*. 2004;101:9601-9606.
11. Leal WS, Bento JMS, Vilela EF, Della Lucia TMC. Female sex pheromone of the longhorn beetle *Migdolus fryanus* Westwood: N-(2′*S*)-methylbutanoyl 2-methylbutylamine. *Experientia*. 1994;50:853-856.
12. Bo C, Li Y, Ke W, Quan H. Synthesis, liquid crystalline and luminescent properties of trimeric phenylenevinylene with end-groups of (*R*)-(+)-2-methylbutyric acid ester. *Chem Chin Lett*. 2009;20:1423-1426.
13. Gandomkar S, Habibi Z, Mohammadi M, Yousefi M, Salimi S. Enantioselective resolution of racemic ibuprofen esters using different lipases immobilized on epoxy-functionalized silica. *Biocatal Agric Biotechnol*. 2015;4:550-554.
14. Tsai SW, Wei HJ. Enantioselective esterification of racemic naproxen by lipases in organic solvent. *Enzyme Microb Technol*. 1994;16:328-333.
15. García R, García T, Martínez M, Aracil J. Kinetic modelling of the synthesis of 2-hydroxy-5-hexenyl 2-chlorobutyrate ester by an immobilised lipase. *Biochem Eng J*. 2000;5:185-190.
16. Jesus PC, Silva PLF, João JJ, Nascimento MG. Enantioselective esterification of 2-methylpentanoic acid catalised via immobilized lipases in chrysotile and microemulsion-based gels. *Synth Commun*. 1998;28:2893-2901.
17. Engel K. Lipase-catalyzed enantioselective esterification of 2-methylalkanoic acids. *Tetrahedron Asymmetry*. 1991;2:165-168.
18. García R, Renedo A, Martínez M, Aracil J. Enzymatic synthesis of n-octyl (+)-2-methylbutanoate ester from racemic (+/-)-2-methylbutanoic acid by immobilized lipase: optimization by statistical analysis. *Enzyme Microb Technol*. 2002;30:110-115.
19. Nguyen BV, Hedenström E. Candida rugosa lipase as an enantioselective catalyst in the esterification of methyl branched carboxylic acids: resolution of rac-3,7-dimethyl-6-octenoic acid (citronellic acid). *Tetrahedron Asymmetry*. 1999;10:1821-1826.
20. Ahmad AL, Low EM, Shukor SRA. Immobilization of phenylalanine dehydrogenase onto Eupergit CM for the synthesis of (*S*)-2-amino-4-phenylbutyric acid. *J Mol Catal B: Enzym*. 2013;88:26-31.
21. Ujang Z, Husain WH, Seng MC, Rashid AHA. The kinetic resolution of 2-(4-chlorophenoxy)propionic acid using *Candida rugosa* lipase. *Process Biochem*. 2003;38:1483-1488.
22. Ghanem A, Aboul-Enein MN, El-Azzouny A, El-Behairy MF. Lipase-mediated enantioselective kinetic resolution of racemic acidic drugs in non-standard organic solvents: direct chiral liquid chromatography monitoring and accurate determination of the enantiomeric excesses. *J Chromatogr A*. 2010;1217:1063-1074.
23. Xiaolu LW, Wang D, Xu Y, Geng Y, Cong CN. Kinetic resolution of 2-methylbutyric acid and its ester by esterification and hydrolysis with lipases. *Chinese J Catal*. 2009;30:951-957.
24. Holmberg E, Holmquist M, Hedenström E, et al. Reaction conditions for the resolution of 2-methylalkanoic acids in esterification and hydrolysis with lipase from *Candida cylindracea*. *Appl Microbiol Biotechnol*. 1991;35:572-578.
25. Uemasu I, Hinze WL. Enantioselective esterification of 2-methylbutyric acid catalyzed via lipase immobilized in microemulsion-based organogels. *Chirality*. 1994;6:649-653.
26. Velasco-Lozano S, López-Gallego F, Rocha-Martin J, Guisán JM, Favela-Torres E. Improving enantioselectivity of lipase from *Candida rugosa* by carrier-bound and carrier-free immobilization. *J Mol Catal B: Enzym*. 2016;130:32-39.
27. Hoffmann I, Silva VD, Nascimento MG. Enantioselective resolution of (*R*,*S*)-1-phenylethanol catalyzed by lipases immobilized in starch films. *J Braz Chem Soc*. 2011;22:1559-1567.
28. Mittersteiner M, Machado TM, Jesus PC, Brondani PB, Scharf DR, Junior RW. Easy and simple SiO_2 immobilization of Lipozyme CaLB-L: its use as a catalyst in acylation reactions and comparison with other lipases. *J Braz Chem Soc*. 2017;28:1185-1192.
29. Nascimento MG, Silva JMR, Silva JC, Alves MM. The use of organic solvents/ionic liquids mixtures in reactions catalyzed by lipase from *Burkholderia cepacia* immobilized in different supports. *J Mol Catal B: Enzym*. 2015;112:1-8.
30. Silva JMR, Bitencourt TB, Moreira MA, Nascimento MG. Enzymatic epoxidation of β-caryophyllene using free or immobilized lipases or mycelia from the Amazon region. *J Mol Catal B: Enzym*. 2013;95:48-54.
31. Herbst D, Peper S, Niemeyer B. Enzyme catalysis in organic solvents: influence of water content, solvent composition and temperature on *Candida rugosa* lipase catalyzed transesterification. *J Biotechnol*. 2012;162:398-403.
32. Laane C, Boeren S, Vos K, Veeger C. Rules for optimization of biocatalysis in organic solvents. *Biotechnol Bioeng*. 1987;30:81-87.

How to cite this article: Mittersteiner M, Linshalm BL, Vieira APF, Brondani PB, Scharf DR, de Jesus PC. Convenient enzymatic resolution of (*R*,*S*)-2-methylbutyric acid catalyzed by immobilized lipases. *Chirality*. 2017;1–6. https://doi.org/10.1002/chir.22779