

LIPASE CATALYZED PARALLEL KINETIC RESOLUTION OF IBUPROFEN

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ABSTRACT. A series of commercially available lipases in various solvents using several alcohols as nucleophiles were studied for the stereoselective esterification of ibuprofen and alcoholysis of ibuprofen esters respectively. Novel methods were developed for the stereoselective synthesis of both enantiomers of the target compound. The (*S*)-selective alcoholysis and (*R*)-selective hydrolysis in a parallel kinetic resolution procedure in presence of lipase from *Mucor miehei* as catalyst were performed.

Keywords: *lipase, Ibuprofen, enzymatic kinetic resolution, stereoselectivity*

INTRODUCTION

Ibuprofen is one of the most important members of Nonsteroidal anti-inflammatory drugs (NSAIDs) that belongs to the family of propanoic acid. Their anti-inflammatory action resides primarily in the (*S*)-enantiomer. The undesired (*R*)-profens might bring some health problems, e.g. accumulation in fatty tissues, with unknown effects [1].

The preparation of (*S*)-Ibuprofen on industrial scale has been studied in the last three decades, different strategies based on asymmetric synthesis [2] and (enzymatic) kinetic and dynamic kinetic resolution [3] being employed. The resolution methods are preferred while the racemate synthesis on a large scale has been optimized and efficient technologies were developed.

Biocatalysis offers a green alternative for the resolution of racemic profens using usual kinetic resolution processes. Large series of lipases and esterases have been shown to be highly enantioselective towards several

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profens. Not only hydrolytic approaches performed in aqueous media, but also alcoholysis and aminolysis reactions in non-conventional media were reported in the last two decades [4].

Usually, esterifications are limited by the lower activity of the biocatalysts, by the need to shift the equilibrium to the product-side (e.g. by removing the water formed in the process) and as well by long reaction times (days) and only moderate enantioselectivities and yields. Lipase-catalyzed esterification [5] or alcoholysis [6] could overcome the disadvantages of hydrolytic procedures due to the limited solubility of ibuprofen-esters in aqueous systems.

The medium engineering is a generally accepted method for fine tuning of the enzymatic activity. The used solvent can modify the enzymatic activity in two ways, namely by enhancing the substrate solubility and by causing conformational changes of the protein [7]. The water content of the reaction mixture is one of the most influent factors on the activity of lipases in organic media. Moreover, the solubility of the residual water of enzymes in the used organic solvents can significantly influence the enzyme activity, selectivity and product distribution in a certain biocatalytic procedure. Nevertheless, down-stream procedures involving the isolation and purification of the reaction products could be significantly improved when enzymatic kinetic resolutions are performed in organic solvents.

In concordance with the mechanism of action of lipases, firstly the serine from the catalytic triad will react with an esteric substrate generating an acyl-enzyme intermediate by releasing the alcoholic residue from the substrate. In the second stage the formed acyl-enzyme can easily react with a nucleophile present in the reaction mixture, generating the reaction product while the acyl-free enzyme is released. The enzyme activity and stereoselectivity could be significantly improved when esters derived from fatty (medium or long chain) alcohols are used as starting materials, by miming the structure of the natural substrates of lipases. In addition, esters derived from profens and fatty alcohols are highly soluble in less polar solvents, which were found to be the most proper reaction media for lipases when they are acting as stereoselective catalysts in non-aqueous systems. Compared with short-chain alcohols which are stripping the essential water from the enzyme and are acting as dead-end inhibitors [8], fatty alcohols are well tolerated by enzymes and also they can be considered as green chemicals.

While in the last decades several lipase-catalyzed biocatalytic reactions (including synthesis and/or hydrolysis) for synthetic natural like fatty acid esters were developed, [9] only a few enzyme mediated (trans)esterification using long chain alcohols as nucleophile are known.

Lipase catalyzed synthesis of non-toxic, non-skin-irritant, odorless and tasteless non-ionic surfactants like biodegradable *n*-Alkyl (C8-C14) esters of glucuronic have been already reported [10]. Further, the optimal conditions

for the enzymatic esterification of lactic and glycolic acids with fatty alcohols (C8–C16) in the presence of a lipase from *Candida antarctica* were also studied [11]. In this way a rapid, nearly complete conversion into the desired ester with high volumetric productivity was achieved. Alternatively, the enzymatic transesterification of ethyl lactate with fatty alcohols increased the yield for the desired (C8–C16) type alkyl lactate (e.g. 87% in 24 h for dodecyl lactate).

Generally, lipases are versatile stereoselective catalysts since are able to mediate the kinetic resolution of several chiral alcohols, carboxylic acids, esters, amides and lactones by hydrolysis, alcoholysis, acidolysis or transesterification [12].

Encouraged by all these results, we turn our attention to the development of an enzymatic procedure for the synthesis of both enantiomers of Ibuprofen. For this scope commercially available lipases were tested as potential catalysts for the efficient parallel kinetic resolution of various ibuprofen based esters. The method was further improved by substrate and medium engineering tools.

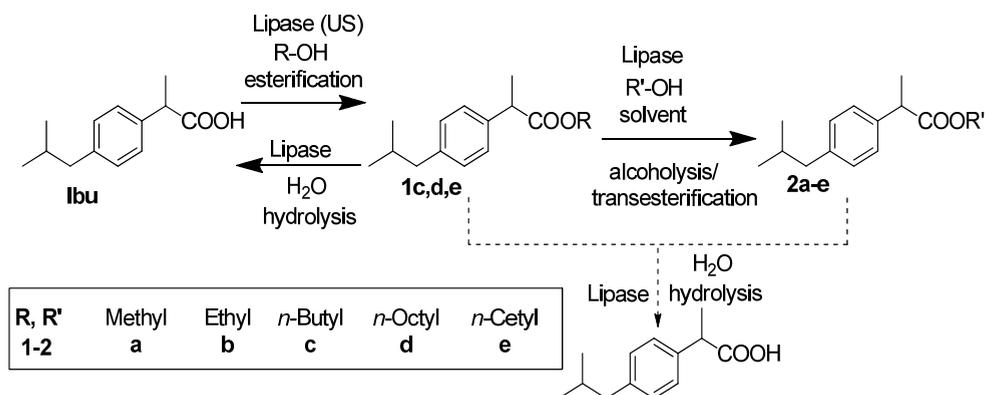
RESULTS AND DISCUSSION

Due to the residual water content of the used solvent and/or enzyme, the biocatalytic alcoholysis can be often concurred by secondary hydrolytic reactions, both in a stereoselective manner. Moreover, varying the reaction conditions, enzymes could display opposite stereoselectivities for the same substrate-product pair. Consequently both, *R* or *S* enantiomers of the reaction counterparts could be produced. Last, but not least, the enzyme activity is strongly influenced by the length of the alkyl chain attached to substrate or nucleophile. Increasing enzyme activity with the length of the acyl residue was reported [13] when sixteen different amides were hydrolyzed in presence of lipase B from *C. antarctica*.

Taking into account these facts the potential biocatalytic paths, when Ibuprofen or its (C1-C16) *n*-alkyl-ester are used as substrates in kinetic resolutions, are depicted in Scheme 1.

In order to explore how length of the alkyl chain of nucleophilic alcohols and of the ibuprofen esters influence the sense and the grade of stereoselectivity of lipases but also the product distribution all possible enzymatic routes: esterification, alcoholysis/transesterification and hydrolysis were subjected for investigations.

Moreover, the enzymatic alcoholysis *versus* hydrolysis were both studied with the aim to produce optimally both, optically pure enantiomers of ibuprofen and ibuprofen esters, respectively.



Scheme 1. Possible reaction routes for the biocatalytic transformation of Ibuprofen and its various esters

1. Lipase-catalyzed esterification of *rac*-ibuprofen

Since lipases from *Aspergillus oryzae* and *Rhizopus oryzae* were successfully used for the esterification of racemic phenylacetic and 2-phenyl-1-propanoic acid [14], first the lipase-catalyzed esterification of racemic ibuprofen (*rac*-Ibu) was tested under various conditions. Commercial lipases, such as lipases A and B from *Candida antarctica* CaL-A and CaL-B), lipase from *Candida rugosa* (CrL), lipase from *Pseudomonas cepacia* (PcL), lipase from *Pseudomonas fluorescens* (LAK), *Pancreatic porcine* lipase (PpL), lipase from *Mucor miehei* (MmL) were used as potential biocatalysts for the esterification of ibuprofen in several neat alcohols at 55°C with and without sonication. Using different alcohols as nucleophile (*n*-butanol, *iso*-butanol, *n*-pentanol, *n*-octanol, *n*-cetanol) it was found that the enzymatic activity continuously decreased with the increasing of the alkyl chain length of the alcohols. Best results were obtained with immobilized CaL-B on Eupergite (Novozym 435) in *n*-butanol. Only the *R*-selective CaL-B displayed satisfactory results for esterification, leading *R*-ibuprofen butyl ester (ee=85%) with a conversion of 10% after 1 h. By sonication the reaction rate increased slowly (*c* >13% after 1 h), but the enzyme displayed lower selectivity (ee=73% for *R*-ibuprofen butyl ester). Next this reaction was performed at room temperature in several recommended solvents like MTBE, *n*-octane, acetonitrile, toluene and THF, using 5 *equiv.* of butanol. Best results were obtained in MTBE when the reaction undergoes without a significant improvement of the enzyme activity, but the enzyme selectivity slightly increased of (ee=89% for *R*-ibuprofen butyl ester).

2. Lipase-catalyzed hydrolysis of racemic ibuprofen esters

Next the hydrolysis of three bulkier racemic ibuprofen esters (*rac-1c-e*) were performed in the presence of the above mentioned lipases in the same organic solvents. For this scope water, saturated octane, MTBE, toluene or acetonitrile, THF containing 5 *equiv.* of water were used. It was found that at lower conversions ($c < 5\%$) the weakly active CaL-B and MmL displayed both high but surprisingly opposite stereoselectivities ($E > 200$) as depicted in Scheme 2. When higher conversions were reached ($c > 10\%$), the enantiopurities of the formed ibuprofen decreased dramatically as shown in Table 1.

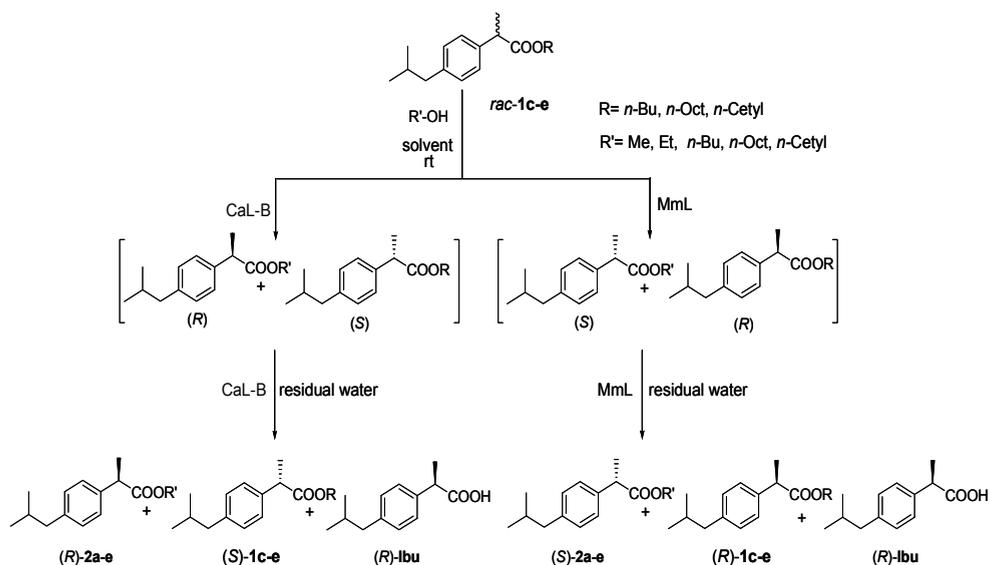
Table 1. Hydrolysis of racemic ibuprofen esters (*rac-1c-e*, 10 mg) with two lipases (10 mg) in several organic solvents saturated with water or containing 5 *equiv.* of water (after 12 h).

Substrate/solvent		CaL-B				MmL			
		Products		c	E	Products		c	E
		ee (S)-1	ee (S)-Ibu			ee (R)-1	ee (S)-Ibu		
<i>rac-1c</i>	MTBE	6	99	5.7	>200	67	53	55.8	6
	Octane	1	99	1.0	>200	6	99	5.7	>200
	CH ₃ CN	5	73	6.4	7	1	59	1.7	4
	Toluene	15	75	16.7	8	4	78	4.9	8
	THF	2	99	2.0	>200	1	99	1.0	>200
<i>rac-1d</i>	MTBE	7	46	13.2	3	60	40	60.0	4
	Octane	1	99	1.0	>200	3	99	2.9	>200
	CH ₃ CN	4	34	10.5	2	27	39	40.9	3
	Toluene	1	99	1.0	>200	7	71	9.0	6
	THF	1	78	1.3	8	1	99	1.0	>200
<i>rac-1e</i>	MTBE	1	88	1.1	16	51	8	86.4	2
	Octane	1	99	1.0	>200	6	99	5.7	>200
	CH ₃ CN	5	67	6.9	5	58	14	80.6	2
	Toluene	1	99	1.0	>200	10	99	9.2	>200
	THF	4	99	3.9	>200	1	81	1.2	10

The use of a triple-folded mutant of CaL-A with a 30-fold enhanced activity towards profens, which displayed high enantioselectivities for (*R*)-selective hydrolysis of ibuprofen esters, was earlier reported [15]. Beside the enhanced selectivity, the activity of the mutant remains unsatisfactory as found also for the native lipases. This information and our herein presented results determined us to investigate also the alcoholysis of the racemic bulkier ibuprofen esters with various aliphatic alcohols (Scheme 2).

3. Enantioselective alcoholysis of racemic ibuprofen esters

Next an analytical scale extensive screening for the alcoholysis of *rac*-Ibuprofen octyl ester (*rac*-**1d**) with *n*-butanol in the same five solvents (MTBE, *n*-octane, acetonitrile, THF, toluene) in presence of several lipases was investigated. Most of the lipases (CaL-A, PpL, LAK, CrL, lipase from *Burkholderia cepacia* LPS, and Alcalase) were inactive. Good results were displayed by the *R*-enantioselective CaL-B and by the (*S*)-selective MmL (Scheme 2). Similarly to MmL, lipases from lyophilized mycelia of *Aspergillus oryzae* displayed *S*-selectivity when *rac*-Flurbiprofen based esters [16] were subjected for alcoholysis.



Scheme 2. The opposite enantioselectivity of CaL-B and MmL in the alcoholysis of racemic esters of Ibuprofen

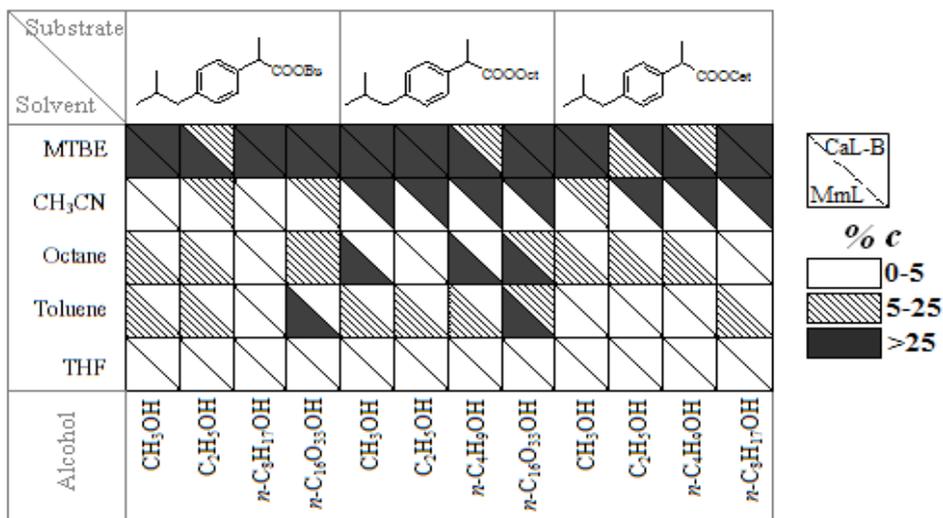


Figure 1. Conversions for the CaL-B and MmL mediated alcoholysis of *rac*-**1c,d,e** in various solvents at room temperature, after 17h

Based on these results further other nucleophiles (methanol, ethanol, *n*-octanol and *n*-cetyl alcohol) were tested for the alcoholysis of all three bulky racemic esters (*rac*-**1c-e**) in presence of these two lipases with opposite enantioselectivity in the same solvents. The obtained most relevant results are presented in Figure 1 (conversion) and Figure 2 (enantiomeric excesses).

Due to the presence of the residual water from the enzymes, a secondary enzymatic hydrolysis of the produced kinetic resolution products occurred in all cases. For these reaction both lipases (CaL-B and MmL) were *R*-selective biocatalyst.

In almost all cases the optical purity of the produced (*R*)-ibuprofen by hydrolytic side reactions was high, while the enantiomeric excesses of the formed (*S*-**2** or (*R*)-**2** esters and of the remained (*R*-**1** or (*S*-**1** esters were moderate to good.

Highest conversions for the MmL catalysed alcoholysis for all three *rac*-**1d-e** esters were obtained in MTBE, excepting the ethanolysis of the cetyl ester *rac*-**1e** which undergoes similarly and slowly both in MTBE and *n*-octane. Generally, the same solvent was appropriate also for the CaL-B mediated transesterifications, excepting the butanolysis of octyl- and cetyl ester *rac*-**1d-e**, when acetonitrile proved to be most convenient reaction media. The CaL-B mediated ethanolysis of butyl ester *rac*-**1c** proceeded slowly in both MTBE and acetonitrile. While *n*-octane was the adequate solvent for the MmL catalysed alcoholysis of octyl ester *rac*-**1d**, in THF all reactions underwent with insignificant conversions.

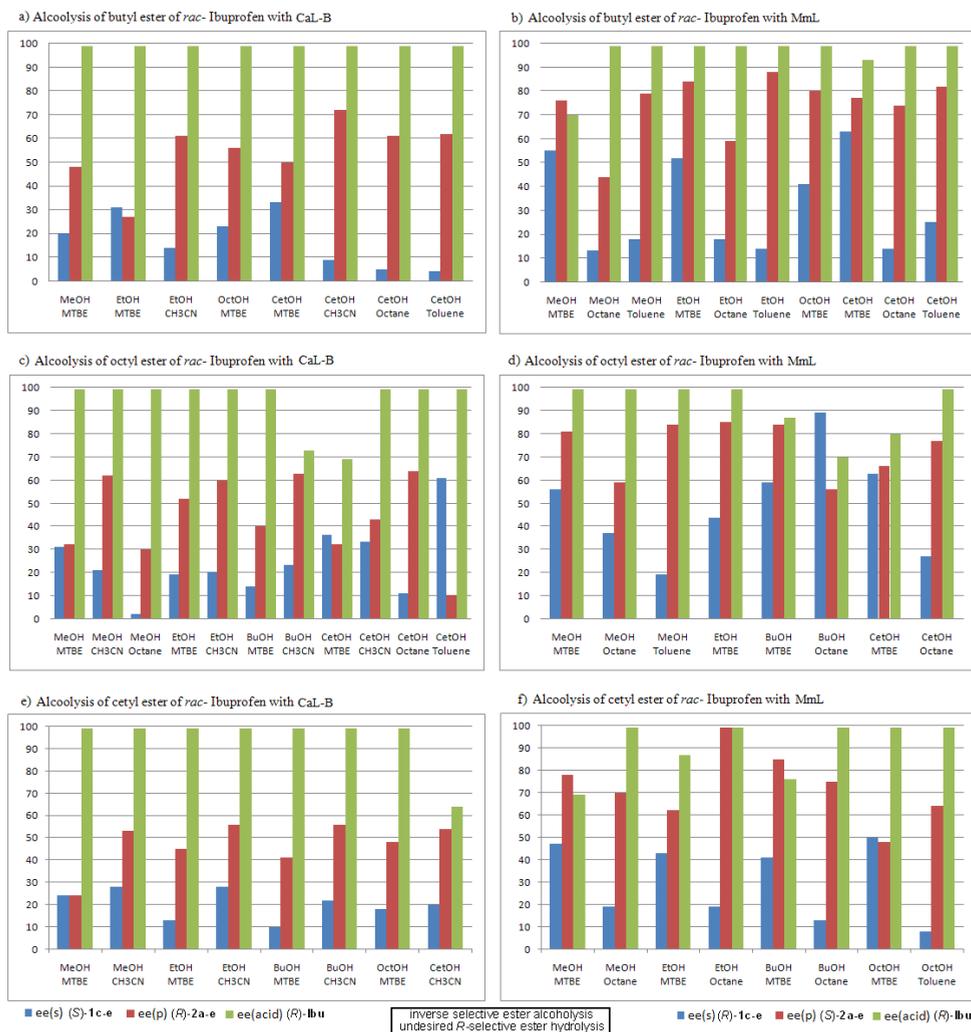
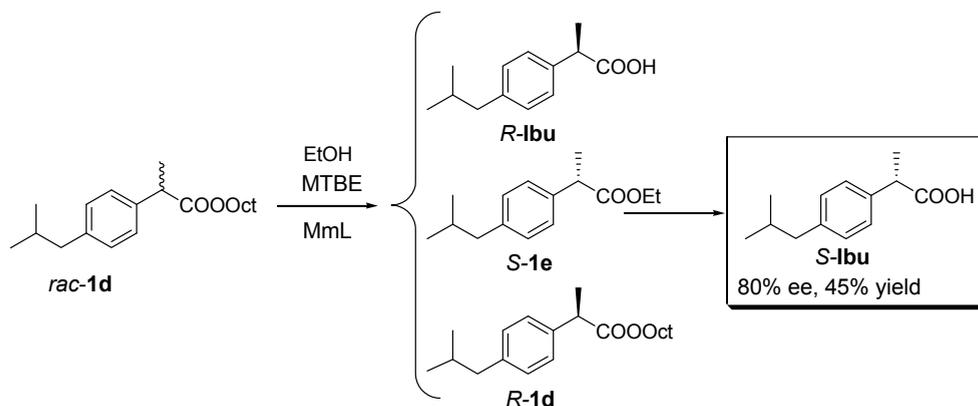


Figure 2. Enantiomeric excesses for the alcoholysis of the *rac*-1c,d,e (5 μ L) with five different alcohols (5 equiv.) in presence of CaL-B and MmL (25 mg/mL) in various solvents (500 μ l) at room temperature, after 17h

Considering all analytical scale results as schematically depicted in Figure 1 and 2 the optimal biotransformation is catalyzed by MmL in MTBE when the octyl ester of ibuprofen (*rac*-1d) was the substrate and ethanol was the nucleophile. In this reaction (*S*)-2b (ee 86%) and (*R*)-1d (ee 44%) were formed. However, by a parallel enzymatic hydrolytic resolution, the (*R*)-Ibuprofen was also formed as by product in a highly enantiomerically enriched form (ee 99%).



Scheme 3. Preparative scale synthesis of (*S*)-Ibuprofen

Using the optimal conditions (enzymatic ethanolysis of racemic octyl ester (*rac*-1d) catalyzed by MmL in MTBE, followed by hydrolysis of the obtained *S*-ethyl ester (*S*-2b), the preparative scale procedure was performed as depicted in Scheme 3.

CONCLUSIONS

All possible enzymatic routes involving the ibuprofen esters with long chain alcohols *rac*-1 as product or substrate were studied. A selective and facile strategy to prepare both enantiomers of ibuprofen by lipase mediated kinetic resolution of racemic *n*-octyl ester was developed. Two enzymes with opposite enantioselectivity were tested and the influence of the chain length of alcoholic moiety on the obtained conversion and optical purity of products were systematically investigated.

EXPERIMENTAL SECTION

1. Analytical methods

The ^1H and ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz and 75 MHz, respectively, at 25 °C using tetramethylsilane (TMS) as an internal standard and CDCl_3 as solvent. Thin Layer Chromatography (TLC) was carried out using Merck Kieselgel 60F²⁵⁴ sheets. Spots were visualized

by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60Å (63-200 μm).

All reagents were purchased from Merck or Sigma-Aldrich and used as received. Solvents and alcohols for enzymatic reactions were stored over molecular sieves unless otherwise stated. Commercially lipases were purchased from Sigma or Novozym.

The enantiomeric separations were performed by HPLC using an Agilent 1200 instrument with DAD detector using a Chiralpak IB column, with *n*-hexane-2-propanol (99:1, v/v) as eluent for all esteric compounds and *n*-hexane-2-propanol with 1% acetic acid content (99:1, v/v) for Ibuprofen, at 1 mL/min flow rate.

Table 2. Chromatographic enantiomers separation on IB HPLC column

t_R (min)	Ibu	1,2a	1,2b	1,2c	1,2d	1,2e
<i>R</i>	11.99	11.6	8.33	9.45	9.15	8.3
<i>S</i>	14.45	13.35	10.51	11.27	11.19	11.27

Determination of *E* was based on the Chen equation [17]:

$$E = \ln[(1-c)(1-ee_s)] / \ln[(1-c)(1+ee_s)]$$

Racemic esters were obtained by direct esterification with the appropriate alcohol in dioxane as solvent, in the presence of DCC at room temperature. The structures of the obtained purified esters were confirmed by analytical and spectral data which are in agreement with those reported in the literature [18].

2. Lipase mediated kinetic resolutions

2.1. Selective enzymatic esterification

Racemic ibuprofen (200 mg) was solved in the tested solvent (0.4 mL) and the alcohol (5 equiv.) and the lipases (100 mg) were added. The reactions were carried out at 55°C in a US bath. Samples (50 μL) were taken and diluted with *n*-hexane (1 mL), than filtered and analyzed by HPLC.

2.1.2. Lipase-catalyzed hydrolysis of ibuprofen esters

Racemic esters (*rac*-**1c-e**, 10 mg) were solved in the tested solvent (1 mL) saturated with water and the lipases (10 mg) were added. The reactions was carried out at 25°C under shaking at 1250 rpm. Samples were taken and diluted with *n*-hexane, than filtered and analyzed by HPLC.

2.1.3. Lipase-catalyzed alcoholysis of ibuprofen esters

Racemic esters (*rac*-**1c-e**, 10 mg) were solved in the solvent (1 mL) and then the alcohol used as nucleophile (5 equiv.), 3-4 pieces of molecular sieves and t lipase (10 mg) were added. The reaction was carried out at 25°C under shaking at 250 rpm. Samples were taken and diluted with *n*-hexane, then filtered and analyzed by HPLC.

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