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Application of ionic liquids in enzymic resolution by hydrolysis of cycloalkyl acetates

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Abstract—A comparative study was performed in the enzymic resolution of the isomers of 2-(4-methoxybenzyl)cyclohexyl acetates **1** and **2**. The investigation consisted in application of three commercially available lipases (Novozyme 435, Lipozyme IM and non-immobilized powdered lipase from *Candida antarctica*), two ionic liquids (1-butyl-4-methylpyridinium chloride and 1,3-dimethylimidazolinium methyl sulfate), three modifications of the reaction conditions and two respective isomers of the racemic substrate (**1** and **2**), and resulted in our finding the appropriate conditions to get both of the products, stereoisomers of 2-(4-methoxybenzyl)cyclohexanol (**3**; 1*S*,2*S*) or (**5**; 1*S*,2*R*), and (in some cases) also the stereoisomers of the deracemized substrate (**4**; 1*R*,2*R*) or (**6**; 1*R*,2*S*) with high or acceptable enantiomeric purity.

1. Introduction

Biocatalytic processes, employing either enzymes or whole cells for the biotransformation of many natural or synthetic substrates, have been useful tools for the preparation of important enantiopure synthons for decades.[1] The majority of organic compounds used as substrates in those processes are insoluble in aqueous media, and, therefore, useful procedures were elaborated for application of enzyme technology in organic media, miscible or immiscible with water.[2] Organic solvents, which are not miscible with water, often allow application of higher reaction temperatures for enzymic processes than the enzyme would allow in aqueous media.[1,2] Most of the organic solvents which may be used as media for enzymic processes are not friendly for the environment. It seems that ionic liquids represent certain environmentally friendly alternatives to organic solvents. It was found that ionic liquids can be used either as co-solvents in aqueous systems or in biphasic systems or even as pure solvents.[3]

The objective of our study was to compare the lipase-mediated process for enzymic resolution of racemic substrates under several modified conditions:

(i) Performing the enzymic reaction in the presence of the selected ionic liquids, 1-butyl-4-methylpyridinium chloride ([BMPy]Cl) or 1,3-dimethylimidazolinium methyl sulfate ([DMIM]$MeSO_4$), as co-solvents in an aqueous system.
(ii) Performing the experiments under the conditions given in (i), with the additional presence of acetonitrile, an organic solvent miscible with water.
(iii) Performing the experiments without the presence of both ionic liquids and acetonitrile (the reference experiments).

The separated *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexyl acetate (**1** and **2**) were employed as substrates for the above-described modifications of the lipase-mediated processes. The *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexanol are used as intermediates in the synthesis of several series of biologically active compounds displaying effect mostly on morphology or oviposition of insects.[4] These biologically active compounds are considered to represent environmentally friendly insect pest

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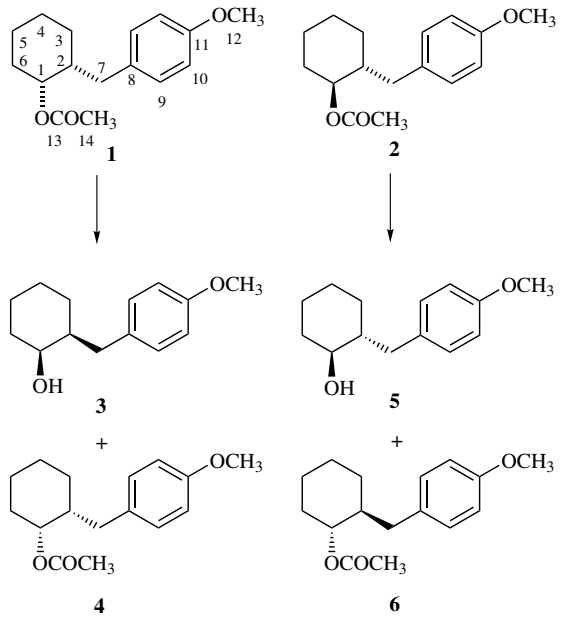


Scheme 1. Lipase-mediated resolution by hydrolytic reactions. Numbers of carbon atoms in the compound **1** show carbon atom numbering used in the assignment of the ^{1}H and ^{13}C NMR signals.

management agents.[4] In this investigation, attention was also focused on the preparation of enantiopure compounds. Enzymic processes designed under different conditions are often used for the preparation of convenient enantiopure intermediates or final products with biological activity. For that reason, isomers of 2-(4-methoxybenzyl)cyclohexanol are often used as convenient model substrates in designing novel procedures and/or their modifications in our projects.[5]

In this investigation, a comparative study was performed consisting of the application of three commercially available lipases [Novozyme 435 (lipase from *Candida antarctica*, immobilized on acrylic resin), Lipozyme IM (lipase from *Mucor miehei*, immobilized on a macroporous ion-exchange resin), and powdered, non-immobilized lipase from *C. antarctica*], two ionic liquids ([BMPy]Cl and [DMIM]-$MeSO_4$), three modifications of the reaction conditions (cf. above) and two respective isomers of the racemic substrates **1** and **2** (Scheme 1).

2. Results and discussion

The starting isomers of substrates **1** and **2** were prepared from the respective racemic *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexanol, using acetic anhydride and pyridine, and subsequent chromatographic purification in quantitative yields.

The key results of this investigation are summarized in Table 1. The discussion of the results is presented according to the lipases applied (entries mentioned in the paragraphs below can be found in Table 1):

(a) Novozyme 435: This enzyme showed remarkable selectivity in transformation of the *cis*-isomer of substrate **1**. Under the presence of [BMPy]Cl (entry 1), product **3** [(1*S*,2*S*); ee >99%] and the remaining substrate **4** [(1*R*,2*R*); ee >99%] were received in high yields and with excellent enantiomeric purity. In turn, when [DMIM]$MeSO_4$ was used as a medium (entry 2) in the same reaction, no progress was observed, and racemic starting substrate **1** remained untouched in the reaction mixture. In the presence of acetonitrile as an auxiliary solvent, the hydrolysis of **1** proceeded quantitatively, giving almost racemic product **3** (entries 4 and 5). A reference experiment (entry 3), that is, the experiment performed with the absence of both, the ionic liquid and the auxiliary solvent, resulted in receiving product **3** [(1*S*,2*S*); ee >99%] with high enantioselectivity but lower chemical yield, and due to that fact, the remaining substrate **4** [(1*R*,2*R*); ee >62.5%] had a lower enantiopurity. The same lipase mediated resolution of the *trans*-isomer of substrate **2** with excellent enantioselectivity [**5**; (1*S*,2*R*); ee >99%] in the presence of both ionic liquids (entries 6 and 7), and also in the reference experiment (entry 8). A quantitative ester fission of **2** was observed. When the reaction was performed in the presence of either ionic liquid tested in the presence of acetonitrile as an auxiliary solvent, the product was almost racemic (entries 9 and 10).

(b) Lipozyme IM: The effect of this enzyme on the deracemization reaction tested was remarkably different from the effect of Novozyme 435. The reference experiment (entry 13) was the only modification of the resolution reaction through which product **3** [(1*S*,2*S*); ee >99%] was received with high enantioselectivity and high chemical yield. This enzyme was blocked by the presence of [BMPy]Cl (entry 11). The presence of the alternative ionic liquid, [DMIM]$MeSO_4$ (entry 12), resulted in poor chemical yield but high enantiomeric purity of product **3** [(1*S*,2*S*); ee >99%]. The presence of acetonitrile together with either of the ionic liquids resulted in no reaction progress (entries 14 and 15). A similar scheme was observed with substrate **2**. No reaction was observed in the presence of [BMPy]Cl (entry 16), and in the modification of the reaction conditions using acetonitrile as an auxiliary solvent (entries 19 and 20). However, using [DMIM]-$MeSO_4$ (entry 17) yielded more than double the quantity of product **5** [(1*S*,2*R*); ee >99%] than the reference experiment (entry 18).

(c) Non-immobilized lipase from *C. antarctica*: Using substrate **1**, this enzyme was blocked by [DMIM]-$MeSO_4$ (entry 22). No reaction was also observed in the presence of acetonitrile (entries 24 and 25). The presence of [BMPy]Cl (entry 21) resulted in substantial ester **1** fission, however, with a poor enantioselectivity [**3**, (1*S*,2*S*); ee = 19%]. A higher chemical yield of **3** [(1*S*,2*S*); ee >99%] and excellent enantiomeric purity were found only in the reference experiment (entry 23). Using substrate **2**, this enzyme was also blocked by the presence of [DMIM]$MeSO_4$ (entry 27), and by the simultaneous presence of either of the ionic liquids tested and acetonitrile (entries 29 and 30). Almost quantitative ester **2** splitting, with poor enantioselec-

Table 1. Results of the enzymic process

Entry	Substrate	Enzyme[a] (specific activity; quantity of the enzyme used for the reaction)	Ionic liquid/auxiliary organic solvent	Products (yield[b] [%]; ee[c]); absolute configuration
1	**1**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[BMPy]Cl/none	**3** (48; >99); (1*S*,2*S*); **4** (44; 92); (1*R*,2*R*)
2	**1**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/none	**3** (—; —); (1*S*,2*S*); **4** (96; —); (1*R*,2*R*)
3	**1**	Novozyme 435 (7 U mg^{-1}; 20 mg)	None/none	**3** (30; >99); (1*S*,2*S*); **4** (65; 62.5); (1*R*,2*R*)
4	**1**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[BMPy]Cl/acetonitrile	**3** (94; 0); (1*S*,2*S*); **4** (—; —); (1*R*,2*R*)
5	**1**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/acetonitrile	**3** (95; 0); (1*S*,2*S*); **4** (—; —); (1*R*,2*R*)
6	**2**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[BMPy]Cl/none	**5** (45; >99); (1*S*,2*R*); **6** (48; 97.5); (1*R*,2*S*)
7	**2**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/none	**5** (43; >99); (1*S*,2*R*); **6** (49; 97); (1*R*,2*S*)
8	**2**	Novozyme 435 (7 U mg^{-1}; 20 mg)	None/none	**5** (49.5; >99); (1*S*,2*R*); **6** (49; >99); (1*R*,2*S*)
9	**2**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[BMPy]Cl/acetonitrile	**5** (93; 0); (1*S*,2*R*); **6** (—; —); (1*R*,2*S*)
10	**2**	Novozyme 435 (7 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/acetonitrile	**5** (92; 0); (1*S*,2*R*); **6** (—; —); (1*R*,2*S*)
11	**1**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[BMPy]Cl/none	**3** (—; —); (1*S*,2*S*); **4** (95; —); (1*R*,2*R*)
12	**1**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/none	**3** (17; >99); (1*S*,2*S*); **4** (72; 60); (1*R*,2*R*)
13	**1**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	None/none	**3** (45; >99); (1*S*,2*S*); **4** (49; 90.5); (1*R*,2*R*)
14	**1**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[BMPy]Cl/acetonitrile	**3** (—; —); (1*S*,2*S*); **4** (95; —); (1*R*,2*R*)
15	**1**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/acetonitrile	**3** (—; —); (1*S*,2*S*); **4** (94; —); (1*R*,2*R*)
16	**2**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[BMPy]Cl/none	**5** (—; —); (1*S*,2*R*); **6** (96; —); (1*R*,2*S*)
17	**2**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/none	**5** (25; >99); (1*S*,2*R*); **6** (70; 55); (1*R*,2*S*)
18	**2**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	None/none	**5** (11; >99); (1*S*,2*R*); **6** (74.5; 31.5); (1*R*,2*S*)
19	**2**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[BMPy]Cl/acetonitrile	**5** (—; —); (1*S*,2*R*); **6** (96; —); (1*R*,2*S*)
20	**2**	Lipozyme IM (0.03 U mg^{-1}; 20 mg)	[DMIM]$MeSO_4$/acetonitrile	**5** (—; —); (1*S*,2*R*); **6** (97; —); (1*R*,2*S*)
21	**1**	CAL (3.1 U mg^{-1}; 4 mg)	[BMPy]Cl/none	**3** (85; 19); (1*S*,2*S*); **4** (12; 20); (1*R*,2*R*)
22	**1**	CAL (3.1 U mg^{-1}; 4 mg)	[DMIM]$MeSO_4$/none	**3** (—; —); (1*S*,2*S*); **4** (94; —); (1*R*,2*R*)
23	**1**	CAL (3.1 U mg^{-1}; 4 mg)	None/none	**3** (40; >99); (1*S*,2*S*); **4** (55; 75); (1*R*,2*R*)
24	**1**	CAL (3.1 U mg^{-1}; 4 mg)	[BMPy]Cl/acetonitrile	**3** (—; —); (1*S*,2*S*); **4** (96; —); (1*R*,2*R*)
25	**1**	CAL (3.1 U mg^{-1}; 4 mg)	[DMIM]$MeSO_4$/acetonitrile	**3** (—; —); (1*S*,2*S*); **4** (93; —); (1*R*,2*R*)
26	**2**	CAL (3.1 U mg^{-1}; 4 mg)	[BMPy]Cl/none	**5** (90; 6); (1*S*,2*R*); **6** (8; —); (1*R*,2*S*)
27	**2**	CAL (3.1 U mg^{-1}; 4 mg)	[DMIM]$MeSO_4$/none	**5** (—; —); (1*S*,2*R*); **6** (95; —); (1*R*,2*S*)
28	**2**	CAL (3.1 U mg^{-1}; 4 mg)	None/none	**5** (25; 89); (1*S*,2*R*); **6** (69; 58.5); (1*R*,2*S*)
29	**2**	CAL (3.1 U mg^{-1}; 4 mg)	[BMPy]Cl/acetonitrile	**5** (—; —); (1*S*,2*R*); **6** (95; —); (1*R*,2*S*)
30	**2**	CAL (3.1 U mg^{-1}; 4 mg)	[DMIM]$MeSO_4$/acetonitrile	**5** (—; —); (1*S*,2*R*); **6** (94; —); (1*R*,2*S*)

[a] CAL = non-immobilized lipase from *C. antarctica*.
[b] Hyphen in the column of yields means that the appropriate compound was not found during HPLC analysis.
[c] Hyphen in the column of enantiomeric excess (ee) means that this value could not be determined, that is, the appropriate product was either racemic or its yield was too low to determine its ee value.

tivity, to the product **5** [(1*S*,2*R*); ee = 6%] was observed in the presence of [BMPy]Cl (entry 26). Even the reference experiment resulted in moderate enantiomeric purity of product **5** [(1*S*,2*R*); ee = 89%].

3. Conclusion

Novozyme 435 was able to mediate enzymic resolution by hydrolysis of the racemic substrates **1** and **2** under the presence of [BMPy]Cl (Table 1, entries 1 and 6) affording either products **3** [(1*S*,2*S*); ee >99%] and **4** [(1*R*,2*R*); ee = 92%] or **5** [(1*S*,2*R*); ee >99%] and **6** (1*R*,2*S*; ee = 97.5%). In addition, this reaction catalyzed by Novozyme 435 was successful under the presence of [DMIM]$MeSO_4$ only for substrate **2** (Table 1, entry 7), affording products **5** [(1*S*,2*R*); ee >99%] and **6** [(1*R*,2*S*); ee = 97%]. The chemical yields and the enantiomeric purity of products **3**–**6** resulting from these reactions (Table 1, entries 1, 6 and 7) overcame the results obtained from the appropriate reference experiments (Table 1, entries 3 and 8).

Evaluating the rest of the experiments, only Lipozyme IM in the presence of [DMIM]$MeSO_4$ was able to produce **5** (Table 1, entry 17) in higher chemical yield than in the comparable reference experiment (Table 1, entry 18). Summarizing the results from the rest of the experiments presented in Table 1, the reference experiments (entries 13, 23 and 28) gave better results than any modification of this process performed under the presence of the tested ionic liquids. The presence of acetonitrile in the reaction system contributed to the quantitative course of the enzymic process (entries 4, 5, 9 and 10) mediated by Novozyme 435 in the presence of either ionic liquid. In turn, the presence of this organic co-solvent blocked the rest of the reactions (entries 14, 15, 19, 20, 24, 25, 29 and 30), in which either Lipozyme IM or non-immobilized lipase from *C. antarctica* was used as a biocatalyst.

In summary, it is possible to evaluate this complex study as successful, because we found modifications of this enzymic process by employing selected ionic liquids, through which we obtained the requested products **3**–**6** enantiomerically pure (ee >99%) and in high chemical yields.

4. Experimental

4.1. General

The ^{1}H NMR and the ^{13}C NMR spectra were recorded on a Bruker AVANCE 500 spectrometer (in FT mode) at 500.1 and 125.8 MHz, respectively, in $CDCl_3$ using either tetramethylsilane (δ 0.0 for ^{1}H NMR) or a solvent signal ($CDCl_3$—δ 77.00 for ^{13}C NMR) as internal reference at a temperature 303 K. The ^{19}F NMR spectra were recorded on a Varian UNITY 500 spectrometer at 470.3 MHz in deuteriochloroform using hexafluorobenzene as external reference (δ −162.9). 2D NMR experiments were measured using the following characteristic parameters: ^{1}H,^{1}H-PFG-COSY—spectral width 9 ppm in both f_1, f_2 dimensions, delay 1 s, data matrix for processing 2048 × 2048 data points; ^{1}H,^{13}C-PFG-HSQC—spectral width 9 ppm in f_2 and 180 ppm in f_1, delay 1 s, data matrix for processing 2048 × 2048 data points. IR spectra were recorded in a solution (CCl_4) on a Bruker IFS 88 instrument. Mass spectra (FAB) were recorded on a VG analytical 70–250 SE mass spectrometer, ZAB–EQ (BEQQ configuration) at 70 eV. Preparative column chromatography was performed on a silica gel type 60 (particle size 0.04–0.063 mm; Fluka, Switzerland). TLC was performed on aluminium sheets precoated with silica gel 60 (Merck, Germany). Analytical HPLC was carried out on a TSP (Thermoseparation Products, USA) instrument equipped with a ConstaMetric 4100 Bio pump and a SpectroMonitor 5000 UV DAD. The analyses of the products were performed on a chiral Nucleodex β-OH column (150 × 4 mm; Macherey-Nagel, Germany) using a methanol/water mixture (9:1, v/v) as mobile phase at 0.3 mL min^{-1}. The eluate was monitored at 220, 254 and 275 nm, and the UV spectra were run from 200 to 300 nm. An Autopol IV polarimeter (Rudolph Research Analytical, USA) was used for the measurement of optical rotation. A Unimax 1010 incubator (Heidolph, Germany) equipped with controlled heating was used to accommodate a magnetic stirrer under its cover to keep the requested reaction temperature for proceeding of the enzymic reactions.

4.2. Enzymes

Three enzymes were used in this study. Lipozyme IM (Novo Nordisk, Denmark) is the lipase from *Rhizomucor miehei* immobilized by adsorption on a macroporous ion exchange resin, showing specific activity 0.03 U mg^{-1}; 1 U corresponds to the amount of enzyme liberating 1 μmol of oleic acid per minute from triolein used as a substrate at pH = 8.0 and at 40 °C. Novozyme 435 (Novo Nordisk, Denmark) is the lipase from *C. antarctica* immobilized on a polyacrylate resin, showing specific activity 7 U mg^{-1}; 1 U corresponds to the amount of enzyme able to synthesize 1 μmol of propyl laurate per minute at 60 °C. Non-immobilized lipase from *C. antarctica* (Fluka) was used as the third enzyme tested, showing specific activity 3.1 U mg^{-1}; 1 U corresponds to the amount of enzyme liberating 1 μmol of oleic acid per minute from triolein used as a substrate at pH = 8.0 and at 40 °C.

4.3. 2-(4-Methoxybenzyl)cyclohexyl acetates 1 and 2

In a typical experiment, either of the respective isomers of 2-(4-methoxybenzyl)cyclohexanol (4.3 g; 19.52 mmol) was dissolved in dry pyridine (20 mL), and acetic anhydride (10 mL) was added. The reaction mixture was allowed to stand overnight and then poured onto a mixture of ice and hydrochloric acid (1:1; 30 mL). The organic layer was extracted with toluene, dried over sodium sulfate and the solvent was evaporated, leaving the crude residue. Products **1** or **2** were received by a small column chromatography in 95% yields [4.86 g (**1**) and 4.90 g (**2**), respectively]. Their analytical data are summarized below.

Compound **1**: ^{1}H NMR ($CDCl_3$): 1.19–1.27 (m, 1H, C-4), 1.35–1.44 (m, 1H, C-6), 1.40–1.53 (m, 2H, C-3), 1.45–1.54 (m, 2H, C-5), 1.65–1.75 (m, 1H, C-4), 1.70 (m, 1H, C-2), 1.88–1.94 (m, 1H, C-6), 2.10 (s, 3H, C-14) 2.40 (dd, J = 8.0, 13.7, 1H, C-7), 2.56 (dd, J = 6.9, 13.7, 1H, C-7), 3.78 (s, 3H, C-12), 4.90 (dt, J = 2.0, 2.0, 4.6, 1H, C-1), 6.81 (m, 2H, C-10), 7.01 (m, 2H, C-9); ^{13}C NMR ($CDCl_3$): 20.89 (t, C-5), 21.30 (q, C-14), 24.96 (t, C-4), 26.95 (t, C-3), 29.89 (t, C-6), 37.64 (t, C-7), 42.42 (d, C-2), 55.21 (q, C-12), 72.21 (d, C-1), 113.73 (d, C-10), 129.87 (d, C-9), 132.49 (s, C-8), 157.84 (s, C-11), 170.66 (s, C-13); IR (CCl_4): 3064 (w), 3032 (w), 2936 (s), 1737 (s), 1613 (m), 1513 (s), 1246 (s), 1041 (s), 948 (w); FAB MS: (m/z) 262 ([M]$^{+}$, 17), 203 (24), 121 (100), 91 (5). For $C_{16}H_{22}O_3$ (262.34) calcd: C, 73.25; H, 8.46. Found: C, 73.29; H, 8.44.

Compound **2**: ^{1}H NMR ($CDCl_3$): 0.97 (ddt, J = 3.5, 12.3, 12.3, 14.0, 1H, C-3), 1.06–1.15 (m, 1H, C-5) 1.24–1.35 (m, 2H, C-4 and C-6), 1.57–1.63 (m, 1H, C-4), 1.66–1.74 (m, 3H, C-2, C-3 and C-5), 1.99–2.02 (m, 1H, C-6), 2.02 (s, 3H, C-14), 2.22 (dd, J = 9.0, 13.7, 1H, C-7), 2.83 (dd, J = 4.0, 13.7, 1H, C-7), 3.78 (s, 3H, C-12), 4.56 (dt, J = 4.7, 10.0, 10.0, 1H, C-1), 6.82 (m, 2H, C-10), 7.03 (m, 2H, C-9); ^{13}C NMR ($CDCl_3$): 21.30 (q, C-14), 24.53 (t, C-5), 25.10 (t, C-4), 30.01 (t, C-3), 31.86 (t, C-6), 37.95 (t, C-7), 43.83 (d, C-2), 55.23 (q, C-12), 76.89 (d, C-1), 113.61 (d, C-10), 130.04 (d, C-9), 132.29 (s, C-8), 157.79 (s, C-11), 170.82 (s, C-13); IR (CCl_4): 3064 (w), 3033 (w), 2936 (s), 1735 (s), 1613 (m), 1513 (s), 1244 (s), 1028 (s), 876 (w), 854 (w); FAB MS: (m/z) 262 ([M]$^{+}$, 13), 203 (15) 202 (16), 121 (100), 91 (6). For $C_{16}H_{22}O_3$ (262.34) calcd: C, 73.25; H, 8.46. Found: C, 73.22; H, 8.49.

4.4. Enzymic resolution of 2-(4-methoxybenzyl)cyclohexyl acetates 1 and 2

In a typical reference experiment (Table 1, entries 3, 8, 13, 18, 23 and 28), the racemic substrate (**1** or **2**; 20 mg; 0.09 mmol) was mixed with phosphate buffer (1.7 mL; pH = 6.5), and the enzyme was added (for its quantity see Table 1). Either an ionic liquid (382 mg; 0.3 mL) was

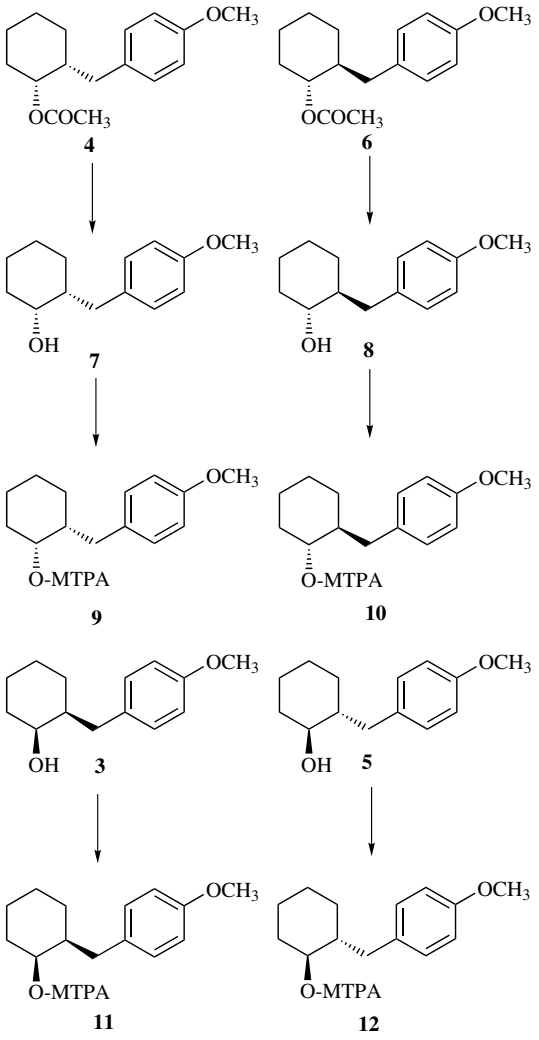
added, where needed (Table 1, entries 1, 2, 4–7, 9–12, 14–17, 19–22, 24–27, 29 and 30), or even acetonitrile (0.7 mL), the auxiliary solvent, was added (Table 1, entries 4, 5, 9, 10, 14, 15, 19, 20, 24, 25, 29 and 30). Complete miscibility of either aqueous phase with ionic liquid, or aqueous phase, ionic liquid and acetonitrile was observed. The reaction mixture was stirred for 72 h at 40 °C. Work-up of the reaction mixture consisted of filtering the enzyme off the reaction mixture. The rest of the mixture was extracted with ether, dried over sodium sulfate, then the solvent was evaporated and the residue was either separated by column chromatography (Table 1, entries 1, 3, 6–8, 13, 17, 23 and 28) to give pure products (**3**–**6**; Scheme 1) and/or analyzed by chiral HPLC. The chemical yields of products **3**–**6**, as well as their enantiomeric purity and absolute configuration are given in Table 1. The analytical data of **3** and **5** are summarized below.

Compound **3**: ^{1}H NMR ($CDCl_3$): 1.18–1.25 (m, 1H, C-4), 1.34–1.44 (m, 2H, C-3), 1.42–1.48 (m, 2H, C-5 and C-6), 1.54–1.62 (m, 1H, C-5), 1.64–1.70 (m, 2H, C-2 and C-4), 1.73–1.79 (m, 1H, C-6), 2.48 (dd, $J = 7.6$, 13.6, 1H, C-7), 2.66 (dd, $J = 7.6$, 13.6, 1H, C-7), 3.78 (s, $w = 11$, 1H, C-1), 3.79 (s, 3H, C-12), 6.82 (m, 2H, C-10), 7.10 (m, 2H, C-9); ^{13}C NMR ($CDCl_3$): 20.34 (t, C-5), 25.31 (t, C-4), 26.37 (t, C-3), 33.27 (t, C-6), 37.75 (t, C-7), 43.67 (d, C-2), 55.23 (q, C-12), 68.51 (d, C-1), 113.66 (d, C-10), 129.95 (d, C-9), 133.01 (s, C-8), 157.75 (s, C-11); IR (CCl_4): 3631 (w), 3501 (w), 3064 (w), 3032 (w), 2934 (s), 2835 (m), 1176 (s), 1041 (s), 974 (m); FAB MS: (m/z) 220 ([M]$^+$). For $C_{14}H_{20}O_2$ (220.30) calcd: C, 76.32; H, 9.15. Found: C, 76.29; H, 9.12. $[\alpha]_D^{20} = +34.4$ (c 0.116, $CHCl_3$).

Compound **5**: ^{1}H NMR ($CDCl_3$): 0.90 (ddt, $J = 3.5$, 11.5, 11.5, 16.5, 1H, C-3), 1.09 (dtt, $J = 3.5$, 3.5, 11.7, 11.7, 15.0, 1H, H-5), 1.19–1.29 (m, 2H, C-4 and C-6) 1.46 (m, 1H, C-2), 1.56–1.60 (m, 1H, H-5), 1.64 (ddt, $J = 2.0$, 3.5, 3.5, 16.5, 1H, C-3), 1.68–1.73 (m, 1H, C-4), 1.95–2.00 (m, 1H, C-6), 2.33 (dd, $J = 9.0$, 13.5, 1H, C-7), 3.07 (dd, $J = 4.1$, 13.5, 1H, C-7), 3.28 (dt, $J = 4.3$, 9.9, 9.9, 1H, C-1), 3.79 (s, 3H, C-12), 6.82 (m, 2H, C-10), 7.10 (m, 2H, C-9); ^{13}C NMR ($CDCl_3$): 24.88 (t, C-4), 25.43 (t, C-5), 30.00 (t, C-3), 35.77 (t, C-6), 38.07 (d, C-7), 47.08 (t, C-2), 55.22 (q, C-12), 74.51 (d, C-1), 113.61 (d, C-10), 130.25 (d, C-9), 132.67 (s, C-8), 157.76 (s, C-11); IR (CCl_4): 3624 (w), 3604 (w), 3064 (w), 3033 (w), 2835 (m), 1177 (m), 1042 (s), 1025 (m); FAB MS: (m/z) 220 ([M]$^+$). For $C_{14}H_{20}O_2$ (220.30) calcd: C, 76.32; H, 9.15. Found: C, 76.35; H, 9.11. $[\alpha]_D^{20} = -21.3$ (c 0.083, $CHCl_3$).

4.5. Chemical hydrolysis of 2-(4-methoxybenzyl)cyclohexyl acetate (4 and 6) to 2-(4-methoxybenzyl)cyclohexanols 7 and 8

For analytical purpose, samples of the deracemized substrates **4** and **6** were chemically hydrolyzed to the corresponding stereoisomers of 2-(4-methoxybenzyl)cyclohexanol. A solution of the respective compounds **4** or **6** (10 mg; 0.018 mmol) in a mixture of methanol (1 mL) and water (0.25 mL) was heated at reflux in the presence



Scheme 2. Reactions performed in the synthesis of the (*R*)-MTPA ester of chiral alcohols for analytical purposes.

Table 2. Selected ^{1}H and ^{19}F NMR parameters (signals and coupling constants) of **9**–**12**

Ester of the (*R*)-MTPA	Parameter					Product (absolute configuration)
	^{1}H NMR				^{19}F NMR	
	δ (H-7a)	δ (H-7b)	J (2,7a)	J (2,7b)	δ (CF_3)	
9	2.34	2.51	8.2	6.7	−67.31	**7** (1*R*,2*R*)
10	2.18	2.89	9.9	3.2	−67.55	**8** (1*R*,2*S*)
11	2.25	2.45	8.1	6.8	−67.13	**3** (1*S*,2*S*)
12	2.08	2.70	9.8	3.1	−67.44	**5** (1*S*,2*R*)

of potassium carbonate (15 mg) for 2 h. Methanol and water were removed under reduced pressure, the residue was applied onto a silica gel column and purified, affording the pure products **7** and **8** in the yields $\geqslant 95\%$.

4.6. Synthesis of 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid esters of 2-(4-methoxybenzyl)cyclohexanols 9–12

A general procedure used for the synthesis of the (*R*)-MTPA (3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid; Mosher's acid) esters on a milligram scale starting from the (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride (MTPCl, Mosher's chloride) was carried out as described previously.[5,6] Esters **9**–**12** (Scheme 2) were obtained in quantitative yields and their ^{1}H and ^{19}F NMR data used for the assignment of the absolute configuration of the parent major enantiomers **3**, **5**, **7** and **8** of 2-(4-methoxybenzyl)cyclohexanol are given in Table 2 and discussed in Section 2.

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