Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 15 (2004) 3911–3917

Enzymic resolution of 2-substituted cyclohexanols through lipase-mediated esterification

Zdeněk Wimmer,[a,*] Vasso Skouridou,[b] Marie Zarevúcka,[a] David Šaman[a] and Fragiskos N. Kolisis[b]

[a]*Institute of Organic Chemistry and Biochemistry AS CR, Flemingovo náměstí 2, CZ-16610 Prague 6, Czech Republic*
[b]*Biotechnology Laboratory, School of Chemical Engineering, National Technical University of Athens, 9 Iroon Polytechniou Street, Zografou Campus, GR-15700 Athens, Greece*

Received 24 September 2004; accepted 4 November 2004

Abstract—Several lipases were used for the kinetic resolution of the racemic *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexanol, by lipase-mediated esterification of the substrates to the corresponding acetate isomers. Conversion of the products and the remaining deracemized substrates into diastereoisomeric esters of 3,3,3-trifluoromethyl-2-methoxy-2-phenylpropanoic acid, their analysis by chiral HPLC and assignment of their absolute configurations through their ^{1}H and ^{19}F NMR spectra, were the basis of evaluation of the studied enzymic process. Lipase from *Rhizomucor miehei* (RML) was found to be the most efficient enzyme regarding enantiomeric excess (ee) and yield of the desired products, while resolution by lipase from *Rhizopus arrhizus* (RAL) resulted in satisfactory ee and lower yields.

1. Introduction

Biocatalysis in non-aqueous media has received considerable attention over the past decade,[1,2] especially for the synthesis of enantiopure compounds of biological interest by lipases (triacylglycerol hydrolases, EC 3.1.1.3).[3,4] In particular, the enzymic resolution by lipases of chiral 2-substituted cycloalkanols, precursors of the insect juvenile hormone bioanalogues, has been studied extensively.[5–14] Among the lipases tested to date, lipase B from *Candida antarctica* was the only one able to resolve both separated racemic isomers of 2-(4-methoxybenzyl)cyclohexanol[15] with almost quantitative enantiomeric excess (ee >99%) of the products, that is, 2-(4-methoxybenzyl)cyclohexyl acetates. This enzyme is known to be a highly enantioselective catalyst for the resolution of secondary alcohols, and a study[15] has proven its ability in connection with the studied substrates.

In fact, two of the stereoisomers [(1*S*,2*S*)- and (1*S*,2*R*)-2-(4-methoxybenzyl)cyclohexanol] of the possible are easily accessible through asymmetric reduction of 2-(4-methoxybenzyl)cyclohexanone mediated by different microorganisms, mostly yeasts.[16–22] The opposite stereoisomeric pair of the alcohols, however, is accessible through laborious processes, and, generally, in lower chemical yields and enantiomeric excesses, even if successful resolutions have already been performed as well.[6,8–11,13,14]

Therefore, the objective of the present study was to find lipases and reaction conditions, through which the required stereoisomers, (1*R*,2*R*)- and (1*R*,2*S*)-2-(4-methoxybenzyl)cyclohexanol, would be accessible with high enantiomeric excesses and chemical yields, preferably among remaining substrates in the enzymic transesterification. Starting from the separated racemic *cis*- and *trans*-isomers of 2-(4-methoxybenzyl)cyclohexanol, suitable lipases are expected to catalyze the esterification of the (1*R*,2*R*)- and (1*R*,2*S*)-2-(4-methoxybenzyl)cyclohexanol isomers to the corresponding acetates, while (1*S*,2*S*)- and (1*S*,2*R*)-2-(4-methoxybenzyl)cyclohexanol isomers will remain intact in the reaction mixture. The basic goal of this study was to answer the question, if the enzymic resolution can be managed in a way to afford both the products and the remaining substrates, with as high as possible enantiomeric excess and chemical yields.

* Corresponding author. Tel.: +42 0220 183 281; fax: +42 0224 310 090; e-mail: wimmer@uochb.cas.cz

0957-4166/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2004.11.009

2. Results and discussion

Several lipases were used for the resolution of racemic *cis*- and *trans*-2-(4-methoxybenzyl)cyclohexanol. Lipases from *Candida cylindracea* (CCL), *Penicillium roquefortii* (PRL) and *Rhizopus niveus* (RNL) were unable to catalyze the desired transesterification. The rest of the lipases tested were able to resolve the racemates; Table 1 summarizes the results of the enzymic reactions, which resulted in obtaining certain quantities of the products and deracemized substrates. These reactions were subjected to a more detailed study, during which the reaction course was monitored using chiral HPLC, with the products analyzed after final work-up of the reaction mixtures in order to assign the absolute configuration of both the major enantiomers of the products and the deracemized substrates.

Determination of the enantiomeric purity of the major enantiomers **3a** and **4a** of the products **3ab** and **4ab** (Scheme 1) was accomplished in two ways. The first method consisted of a synthesis of convenient diastereoisomeric derivatives of the alcohols **5a** and **5b**, **6a** and **6b**, **7a** and **7b** and **9a** and **9b** (Scheme 2). The second method consisted of the application of a chiral stationary phase of a cyclodextrin type to separate enantiomers of the chiral alcohols (cf. Schemes 1 and 2) using chiral HPLC analysis. A combination of both methods is convenient to have as it gives two independent analytical methods for assigning the absolute configuration of the major enantiomers of chiral alcohols.

Convenient diastereoisomeric derivatives are the diastereoisomeric esters of the chiral alcohols with the respective pure enantiomers of (*R*)-(+)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (MTPA, Mosher acid).[23–26] The diastereoisomeric mixtures of the MTPA esters **8a** and **8b** and **10a** and **10b**–**12a** and **12b** (Scheme 2) can be separated by analytical HPLC and the content of each enantiomer of the chiral alcohol present in the samples calculated, based on the respective peak areas of the diastereoisomeric MTPA esters in the HPLC chromatograms. The absolute configurations of the major enantiomers of the MTPA esters **8a** and **10a**–**12a** can be assigned by measuring their ^{1}H and ^{19}F NMR spectra.[27] Enantiomeric purity and the absolute configuration of the major enantiomers of all products and deracemized substrates (Schemes 1 and 2) were determined by applying this approach. Assignment of the absolute configuration at the C(2) stereogenic centre was based on the differences of the chemical shifts of the signals of both hydrogen atoms of the CH_2–Ar (benzyl) group, which are not equivalent due to their chiral environment (Fig. 1). This corresponds with the literature data,[23] and it has been proven several times during our studies[12–18] that an upfield shift of the signals of benzyl hydrogen atoms in the ^{1}H NMR spectra of the diastereoisomeric esters derived from (*R*)-MTPA, with the esters derived from (*S*)-MTPA consistent only with the (*S*)-absolute configuration at the C(1) carbon centre of 2-(4-methoxybenzyl)cyclohexanol, as in **5a**, **6a**, **7b** and **9b**.[12–18,23] The evaluation of the ^{19}F NMR data of the diastereoisomeric esters resulted in the same conclusion (Fig. 1). A downfield shift of the signal of the CF_3 group in the (*R*)-MTPA esters in comparison with the same shift observed in the (*S*)-MTPA esters is in agreement with the expected displacement of the CF_3 group from the eclipsed arrangement with the carbonyl group as a consequence of steric interactions of the bulkier groups, that is, from the phenyl group of the MTPA

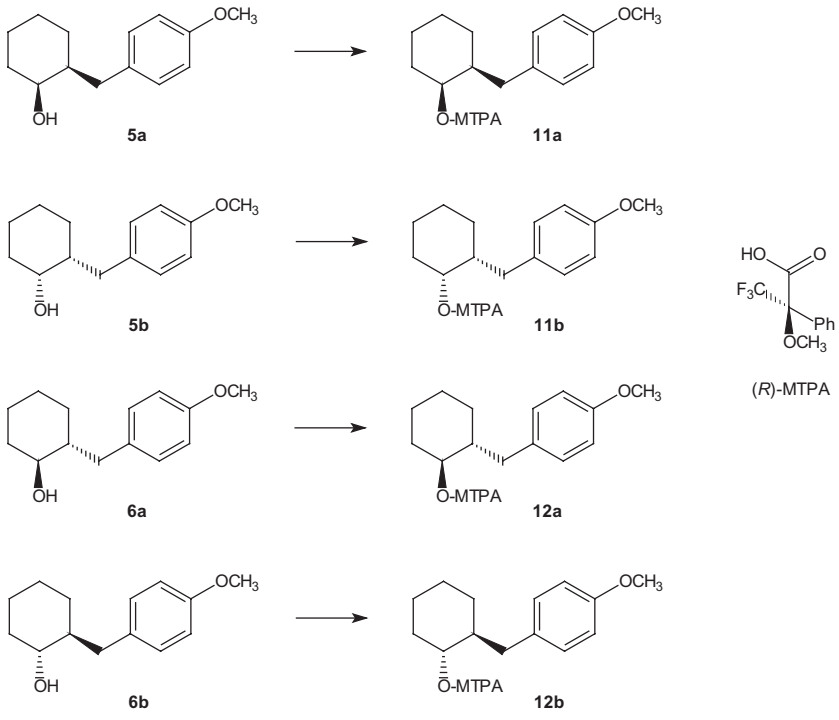
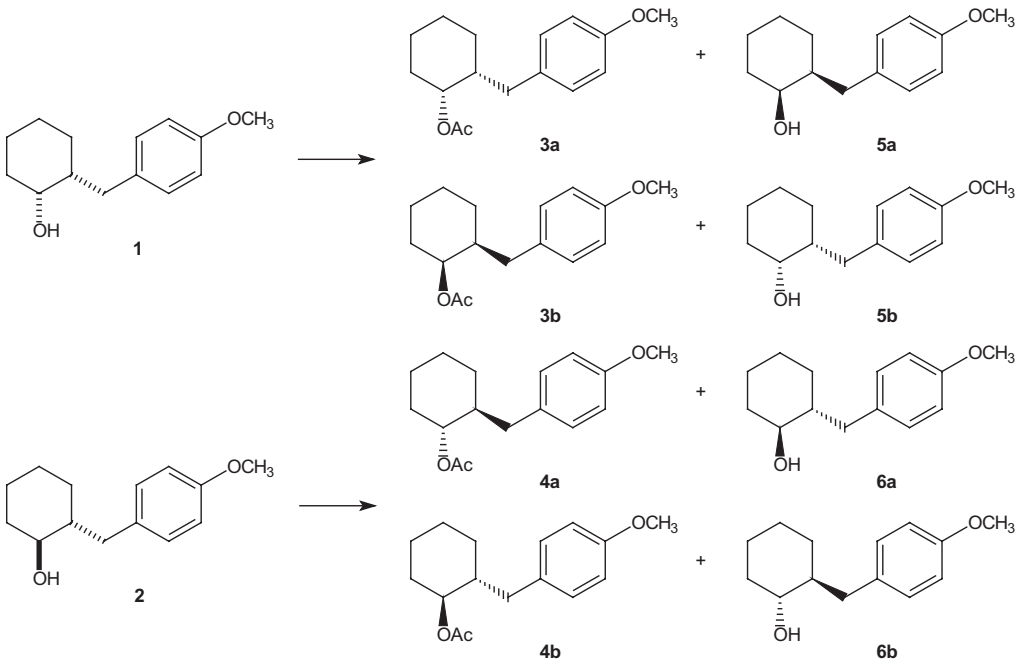


Table 1. Results of the enzyme-mediated resolutions applied to the respective racemic isomers **1** and **2** of 2-(4-methoxybenzyl)cyclohexanol

Entry	Substrate	Enzyme (appl. activity; weight)	Solvent	Products (yield [%]; enantiomeric excess [%]); absolute configuration	
1	**1**	RML (600 mU; 20 mg)	Hexane	**3a** (36.5; 92.2); (1*R*,2*R*)	**5a** (48.0; 51.2); (1*S*,2*S*)
2	**2**	RML (600 mU; 20 mg)	Hexane	**4a** (38.3; 85.3); (1*R*,2*S*)	**6a** (50.1; 62.4); (1*S*,2*R*)
3	**1**	RML (600 mU; 20 mg)	Toluene	**3a** (5.8; >99.0); (1*R*,2*R*)	**5a** (49.7; 5.52); (1*S*,2*S*)
4	**2**	RML (600 mU; 20 mg)	Toluene	**4a** (8.0; 95.1); (1*R*,2*S*)	**6a** (50.7; 10.2); (1*S*,2*R*)
5	**1**	RAL (40 mU; 20 mg)	Hexane	**3a** (18.3; 94.6); (1*R*,2*R*)	**5a** (50.6; 23.9); (1*S*,2*S*)
6	**2**	RAL (40 mU; 20 mg)	Hexane	**4a** (17.7; 73.5); (1*R*,2*S*)	**6a** (54.0; 31.2); (1*S*,2*R*)
7	**1**	MJL (40 mU; 8 mg)	Hexane	**3a** (4.6; 95.0); (1*R*,2*R*)	**5a** (50.9; 6.7); (1*S*,2*S*)
8	**2**	MJL (40 mU; 8 mg)	Hexane	**4a** (6.7; 93.6); (1*R*,2*S*)	**6a** (51.0; 9.3); (1*S*,2*R*)
9	**1**	WGL (4 U; 40 mg)	Hexane	No reaction	
10	**2**	WGL (4 U; 40 mg)	Hexane	**4a** (1.8; n.d.[a]); —	**6a** (50.9; 3.6); (1*S*,2*R*)
11	**1**	PPL (561.2 U; 40 mg)	Hexane	**3a** (4.6; 93.0); (1*R*,2*R*)	**5a** (51.9; 8.8); (1*S*,2*S*)
12	**2**	PPL (561.2 U; 40 mg)	Hexane	**4a** (8.1; 90.4); (1*R*,2*S*)	**6a** (53.2; 14.5); (1*S*,2*R*)
13	**1**	CLL (40 mU; 40 mg)	Hexane	No reaction	
14	**2**	CLL (40 mU; 40 mg)	Hexane	No reaction	
15	**1**	CLL (75 mU; 75 mg)	No solvent[a]	**3a** (0.2; n.d.[b]); —	**5a** (50.3; 0.8); (1*S*,2*S*)
16	**2**	CLL (75 mU; 75 mg)	No solvent[a]	**4a** (1.8; n.d.[b]); —	**6a** (50.8; 3.5); (1*S*,2*R*)

[a] Vinyl acetate was used both, as the solvent and the acyl donor.
[b] n.d. = not determined.

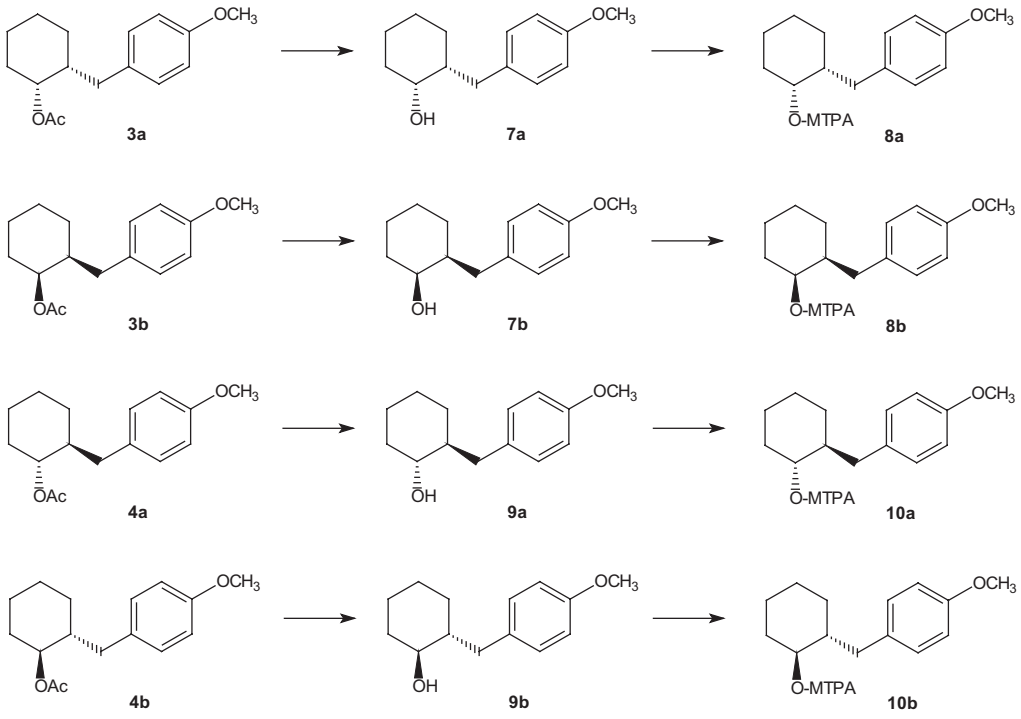


Scheme 1.

part of the esters, and from the benzyl substituent at C(2) of the alcoholic part of the MTPA esters. Selected data are given in Table 2.

The latter method, based on the chromatographic behaviour of the enantiomeric mixtures of the alcohols, in which the major enantiomers correspond to the structures **5a**, **6a**, **7a** and **9a** using a chiral HPLC Nucleodex β-OH column, filled with a chiral β-cyclodextrin-based stationary phase, was applied to confirm the above-described structure assignment. The results obtained from the chiral HPLC analysis were in good agreement with those obtained by analyzing the diastereoisomeric MTPA esters of the studied enantiomers of the alcohols.

Detailed analysis of the samples derived from all the reaction systems, as described above, showed that the most successful enzyme in mediating the desired resolution was lipase from *Rhizomucor miehei* (RML) and two reaction systems were used: (a) in the first system, hexane was used as solvent and vinyl acetate as the acyl donor (Table 1, entries 1 and 2). RML gave the best



Scheme 2.

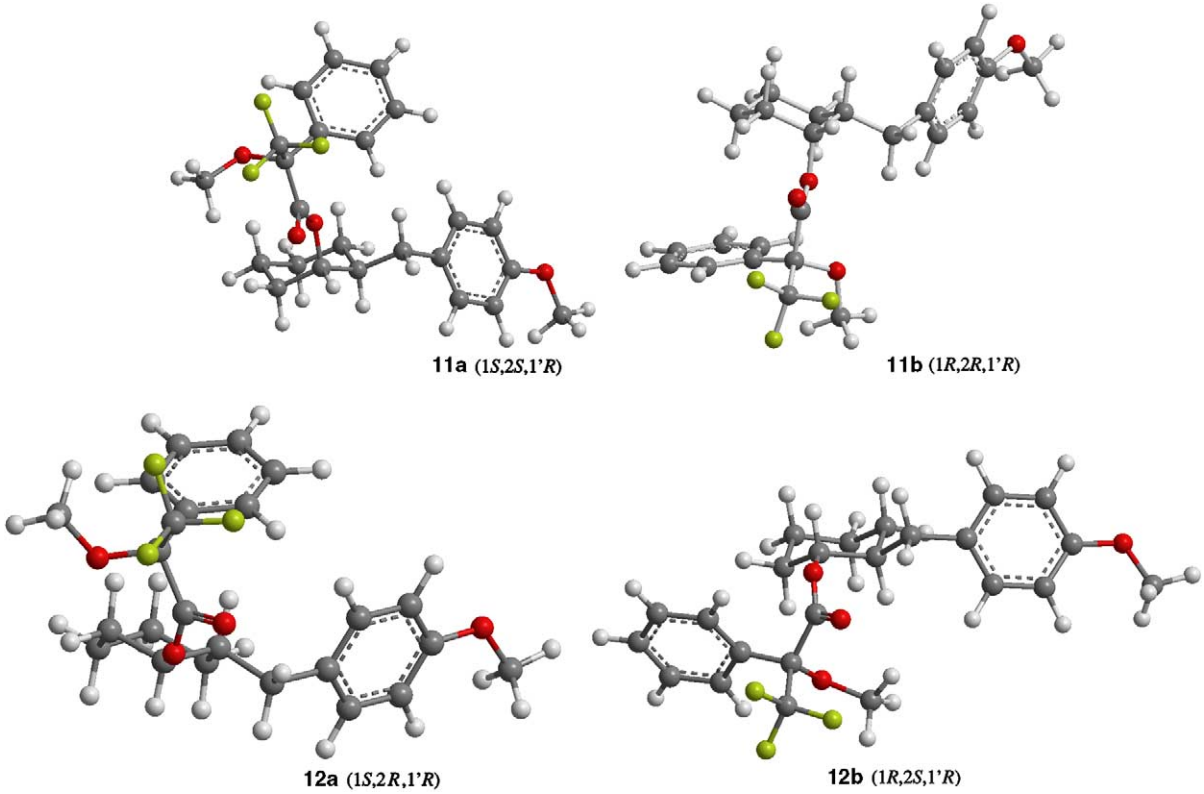


Figure 1. Models of the MTPA esters **11a**–**12b**. More shielded H–CH′Ar, H′–CHAr hydrogen atoms and C–F_3 atoms **11a** and **12a**; less shielded H–CH′Ar, H′–CHAr hydrogen atoms and C–F_3 atoms **11b** and **12b**.

results using both substrates. The maximum conversion was achieved after 140 h and the reactions were stopped after 213 h (for substrate **1**) or 288 h (for substrate **2**). The absolute configuration of the products was assigned using NMR analysis of the diastereoisomeric MTPA esters. Products **3a** and **3b** and **4a** and **4b**, were subjected

Table 2. The ^{1}H and ^{19}F NMR based assignment of the absolute configuration of the (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (MTPA) esters

Compound	δ [H–CH′Ar][a]	δ [H′–CHAr][a]	δ (CF_3)[b]	AC[c]
5a	2.25	2.45	−67.13	1*S*,2*S*
7a	2.33	2.52	−67.31	1*R*,2*R*
6a	2.07	2.70	−67.44	1*S*,2*R*
9a	2.17	2.89	−67.55	1*R*,2*S*

[a] ^{1}H NMR data based on the chemical shifts of the signals of the hydrogen atoms in the benzyl (CH_2Ar) group of the alcohol part of the MTPA esters.

[b] ^{19}F NMR data based on the chemical shifts of the signals of the fluorine atoms in the CF_3 functionality of the acid part of the MTPA esters.

[c] AC = absolute configuration of the alcohols **5a**, **6a**, **7a** and **9a**, the major enantiomers of 2-(4-methoxybenzyl)cyclohexanol.

to alkaline removal of the acyl functionality, and to subsequent esterification of the resulting chiral alcohols **7a** and **7b** and **9a** and **9b** with (*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl chloride affording the (*R*)-MTPA esters **8a** and **8b** and **10a** and **10b**–**12a** and **12b** (Scheme 2). These (*R*)-MTPA esters were analyzed through their ^{1}H and ^{19}F NMR spectra as described above. The absolute configurations of the products were assigned, and are given in Table 1. Chiral HPLC analysis of the MTPA esters resulted in the calculation of the enantiomeric purity of the parent products and deracemized substrates, which are summarized in Table 1; (b) in the second reaction system, toluene was used as the solvent and vinyl acetate as the acyl donor (Table 1, entries 3 and 4). The results indicate that toluene is relatively inconvenient solvent for the enzyme, since only 6–8% of the products were obtained. The data on the absolute configurations and the ee values are shown in Table 1.

The next successful enzyme was lipase from *Rhizopus arrhizus* (RAL). Using hexane as solvent and vinyl acetate as the acyl donor, this enzyme was able to mediate the requested chiral resolution of substrates **1** and **2** (Table 1, entries 5 and 6). Maximum conversion was achieved after 140 h and the products obtained being **3a** and **3b** (18.3%) and **4a** and **4b** (17.7%). The conversion of the enzymic reaction mediated by lipase from *Mucor javanicus* (MJL) was lower than 10%, using hexane as solvent, and vinyl acetate as acyl donor (Table 1, entries 7 and 8). The yields of the products isolated from this reaction were 4.6% (**3a** and **3b**) and 6.7% (**4a** and **4b**). The data on the absolute configurations and the ee values are shown in Table 1. Low yields of both products were achieved in the processes of resolution mediated by lipase from porcine pancreas (PPL): 4.6% (**3a** and **3b**) and 8.1% (**4a** and **4b**), respectively (Table 1, entries 11 and 12). In this enzymic process, hexane was used as solvent and vinyl acetate as the acyl donor. Lipase from wheat germ (WGL) did not seem able to accommodate substrate **1**, since there was no evidence of reaction using TLC analysis (Table 1, entry 9). Moreover, only traces of the products **4a** and **4b** were found when substrate **2** was used in the hexane/vinyl acetate system (Table 1, entry 10). When lipase from *Candida lipolytica* (CLL) was used, with hexane as solvent and vinyl acetate as acyl donor, no products were detected (Table 1, entries 13 and 14). Therefore, the reaction system was altered and vinyl acetate used to act as both solvent and acyl donor (Table 1, entries 15 and 16), and the amount of the enzyme increased to about double quantity. In that case, small quantities of both products were obtained as indicated in Table 1. Finally, when lipases from *C. cylindracea* (CCL), *Rhizopus niveus* (RNL) and *P. roquefortii* (PRL) were employed as reaction mediators, no reaction or low yields of the products were observed. RNL was used in two reaction systems (hexane/vinyl acetate and vinyl acetate/vinyl acetate system). No detailed analysis of the reaction products was performed.

3. Conclusion

Two of the enzymes tested, lipases from *R. miehei* (RML) and *R. arrhizus* (RAL), gave the only positive results in mediating of the requested reaction. Both enzymes accommodated the substrates in the hexane or toluene (solvents)/vinyl acetate (acyl donor) system. RML proved the most successful enzyme and the achieved enantiomeric purity values (ee 92% for **3a**, and 85% for **4a**) at 36% or 38% yields, respectively, are encouraging results for obtaining the enantiomers with the requested absolute configuration; (1*R*,2*R*) for **3a** and (1*R*,2*S*) for **4a**. In fact, when changing hexane as the solvent for toluene, the enantiomeric excesses of the products **3a** and **4a** increased to even more successful values, exceeding ee >95% for both, **3a** and **4a**, however the conversion of the enzymic process decreased to 6–8% yield of **3a** and **4a**. Lipase RAL also showed its ability to mediate this enzymic resolution with quite good results regarding the enantiomeric purity of products **3a** and **4a**, however, their chemical yields were about a half of those achieved with RML in hexane. All other enzymes tested in this study showed results of lower importance due to low or no conversions found.

The (1*S*,2*S*)- and (1*S*,2*R*)-enantiomers of 2-(4-methoxybenzyl)cyclohexanol are accessible with acceptable enantiomeric excess (ee >95%) through enzymic reduction of 2-(4-methoxybenzyl)cyclohexanone with yeasts.[16–22] There have been several lipases tested so far (those from *Rhizopus oryzae*, *C. cylindracea*, porcine pancreas or *Geotrichum candidum*), which enable us to prepare the additional existing stereoisomers of 2-(4-methoxybenzyl)cyclohexanol, that is, (1*R*,2*R*)- and (1*R*,2*S*)-enantiomers with acceptable enantiomeric excess (ee >95%), but lower conversion (~30%).[6,8–11,13,14] Herein we have extended the number of lipases, which can be employed in the processes of resolution of 2-substituted cyclohexanols. Due to the changes in the strains of the yeasts available for enzymic reductions, and due to the modifications in commercial availability of isolated lipases, accompanied by the existence of lipases prepared in laboratories (not available commercially), we are required to periodically perform the most recent studies of enzymic reductions and enzymic resolutions to investigate the potential of novel microorganisms and lipases for substrates, which had been studied in the past.

4. Experimental

4.1. General

Small vials (up to 5 mL inner volume) were used for performing the enzymic reactions. The vials were equipped with screw stopcocks and septa covered by a Teflon layer. The ^{1}H NMR and the ^{19}F NMR spectra were recorded on a Varian UNITY 500 spectrometer (in a FT mode) at 499.8 and 470.3 MHz, respectively, in deuteriochloroform using either tetramethylsilane (δ 0.0) as the internal reference or hexafluorobenzene as external reference (δ −162.9). 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. Analysis of the chiral products was performed on a chiral Nucleodex β-OH column (150 × 4 mm; Macherey-Nagel, Germany) using methanol/water (4:1, v/v) as the mobile phase at 0.3 mL min^{-1}. The eluate was monitored at 220, 254 and 275 nm while the UV spectra were run from 200 to 300 nm.

4.2. Lipases

Nine lipases were used for the kinetic resolution of the racemic *cis*- and *trans*-2-substituted cyclohexanols: lipase from *C. cylindracea* (CCL; 1.6 U mg^{-1}), *C. lipolytica* (CLL; 0.001 U mg^{-1}), *M. javanicus* (MJL; 0.005 U mg^{-1}), *P. roquefortii* (PRL; 0.002 U mg^{-1}), *Rhizomucor miehei* (Lipozyme IM; RML; 0.03 U mg^{-1}), *R. arrhizus* (RAL; 0.002 U mg^{-1}), *Rhizopus niveus* (RNL; 0.0025 U mg^{-1}), lipase from porcine pancreas (type II; PPL; 14.03 U mg^{-1}) and from wheat germ (WGL; 0.1 U mg^{-1}). Lipase RML (adsorbed on a macroporous resin) was kindly offered by Novo Nordisk (Baegsvaerd, Denmark) and lipase PPL obtained from Sigma (Steinheim, Germany). All other lipases were used in their free forms and were generous gifts from Fluka Chemie GmbH (Buchs, Switzerland).

4.3. Enzymic transesterification

Substrates **1** or **2** (20 mg; 0.092 mmol) and vinyl acetate (20 μL; 0.216 mmol) were dissolved in the appropriate organic solvent (2 mL), and the lipase preparation added to the reaction mixture as described in Table 1. The reactions were performed at 40 °C under continuous magnetic stirring and monitored for 9 days (216 h). Maximum conversions were reached after 140 h (cf. Table 1).

4.4. Chemical hydrolysis of 2-(4-methoxybenzyl)cyclohexyl acetate

A solution of the respective compounds **3a** and **3b** or **4a** and **4b** (10 mg; 0.018 mmol) in a mixture of methanol (1 mL) and water (0.25 mL) was heated to reflux in the presence of potassium carbonate (15 mg) for 2 h. Methanol and water were removed under reduced pressure and the residue applied onto a silica gel column and purified, to afford the pure products **7a** and **7b** and **9a** and **9b** in the yields ⩾90%.

4.5. Synthesis of 3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid esters of 2-(4-methoxybenzyl)cyclohexanol

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 previously described.[23–26] The esters **8a** and **8b** and **10a** and **10b**–**12a** and **12b** (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 **5a** and **5b**, **6a** and **6b**, **7a** and **7b** and **9a** and **9b** of 2-(4-methoxybenzyl)cyclohexanol, given in Table 2 and discussed in Section 2.

Acknowledgements

This work was supported by the Czech-Greek project KONTAKT. Its Czech part (project ME 692) was funded by the Czech Ministry of Education and its Greek part (project EPAN 4.3.6.1) funded by the Greek General Secretariat of Research and Technology.

References

1. Klibanov, A. M. *Nature* **2001**, *409*, 241–246.
2. Kontogianni, A.; Skouridou, V.; Sereti, V.; Stamatis, H.; Kolisis, F. N. *Eur. J. Lipid Sci. Technol.* **2001**, *103*, 655–660.
3. Patel, R. N. *Enzyme Microb. Technol.* **2002**, *31*, 804–826.
4. Skouridou, V.; Chrysina, E. D.; Stamatis, H.; Oikonomakos, N. G.; Kolisis, F. N. *J. Mol. Catal. B: Enzym.* **2004**, *29*, 9–12.
5. Wimmer, Z. *Tetrahedron* **1992**, *48*, 8431–8436.
6. Zarevúcka, M.; Rejzek, M.; Wimmer, Z.; Streinz, L.; Demnerová, K. *Biotechnol. Lett.* **1993**, *15*, 1139–1144.
7. Zarevúcka, M.; Rejzek, M.; Pavlík, M.; Wimmer, Z.; Zima, J.; Legoy, M.-D. *Biotechnol. Lett.* **1994**, *16*, 807–812.
8. Zarevúcka, M.; Žalská, Z.; Rejzek, M.; Streinz, L.; Wimmer, Z.; Macková, M.; Demnerová, K. *Enzyme Microb. Technol.* **1995**, *17*, 866–869.
9. Zarevúcka, M.; Rejzek, M.; Wimmer, Z.; Zima, J.; Zhao, Q.; Legoy, M.-D. *Biotechnol. Lett.* **1995**, *17*, 1317–1320.
10. Zarevúcka, M.; Rejzek, M.; Šaman, D.; Wimmer, Z.; Vaněk, T.; Zhao, Q.; Legoy, M.-D. *Enantiomer* **1996**, *1*, 227–231.
11. Wimmer, Z.; Zarevúcka, M.; Rejzek, M.; Šaman, D.; Zhao, Q.; Legoy, M.-D. *Helv. Chim. Acta* **1997**, *80*, 818–831.
12. Zarevúcka, M.; Rejzek, M.; Hoskovec, M.; Svatoš, A.; Wimmer, Z.; Koutek, B.; Legoy, M.-D. *Biotechnol. Lett.* **1997**, *19*, 745–750.
13. Rejzek, M.; Wimmer, Z.; Zarevúcka, M.; Kuldová, J.; Šaman, D.; Vacek, M. *Tetrahedron: Asymmetry* **1998**, *9*, 3657–3665.

14. Brunet, C.; Zarevúcka, M.; Wimmer, Z.; Legoy, M.-D. *Biotechnol. Lett.* **1999**, *21*, 605–610.
15. Brunet, C.; Zarevúcka, M.; Wimmer, Z.; Legoy, M.-D. *Enzyme Microb. Technol.* **2002**, *31*, 609–614.
16. Wimmer, Z.; Buděšinský, M.; Macek, T.; Svatoš, A.; Šaman, D.; Vašičková, S.; Romaňuk, M. *Collect. Czech. Chem. Commun.* **1987**, *52*, 2326–2337.
17. Wimmer, Z.; Vaněk, T.; Macek, T.; Šaman, D.; Svatoš, A. *Enzyme Microb. Technol.* **1992**, *14*, 197–202.
18. Wimmer, Z.; Macek, T.; Svatoš, A.; Šaman, D. *J. Biotechnol.* **1992**, *26*, 173–181.
19. Zarevúcka, M.; Rejzek, M.; Šaman, D.; Streinz, L.; Wimmer, Z. *Biocatal. Biotrans.* **1996**, *13*, 233–243.
20. Novák, J.; Zarevúcka, M.; Wimmer, Z.; Tykva, R. *Biotechnol. Lett.* **2001**, *23*, 1517–1522.
21. Zarevúcka, M.; Sochůrková, P.; Wimmer, Z.; Šaman, D. *Biotechnol. Lett.* **2003**, *25*, 987–992.
22. Zarevúcka, M.; Wimmer, Z.; Šaman, D.; Demnerová, K.; Macková, M. *Tetrahedron: Asymmetry* **2004**, *15*, 1325–1330.
23. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549.
24. Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1970**, *35*, 4002–4003.
25. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.
26. Sullivan, G. R.; Dale, J. A.; Mosher, H. S. *J. Org. Chem.* **1973**, *38*, 2143–2147.
27. Rinaldi, P. L. *Prog. Nucl. Magn. Reson. Spectrosc.* **1982**, *15*, 291–351.