Journal of Molecular Catalysis B: Enzymatic 57 (2009) 145–148

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

The role of different anions in ionic liquids on *Pseudomonas cepacia* lipase catalyzed transesterification and hydrolysis

P. Vidya [a], Anju Chadha [a,b,*]

[a] *Laboratory of Bioorganic Chemistry, India*
[b] *Department of Biotechnology, National Center for Catalysis Research, Indian Institute of Technology Madras, Chennai 600036, India*

ARTICLE INFO

Article history:
Received 14 April 2008
Received in revised form 7 August 2008
Accepted 11 August 2008
Available online 22 August 2008

Keywords:
Ionic liquids
Transesterification
Pseudomonas cepacia lipase
Ethyl 3-phenylpropanoate

ABSTRACT

Lipase *Pseudomonas cepacia* (*PS*) catalyzed transesterification of ethyl 3-phenylpropanoate with eleven alcohols was investigated in three ionic liquids [ILs], [Bmim]BF_4, [Bmim]PF_6, and [Bmim]Tf_2N, consisting of an identical cation and different anions. The yields were higher in hydrophobic ILs [Bmim]Tf_2N (55–96%) and [Bmim]PF_6 (22–95%), than in hydrophilic [Bmim]BF_4 (0–19%). The incubation of lipase *PS* in hydrophobic ILs for a period of 20–300 days at room temperature resulted in an increased yield of 62–98% in [Bmim]Tf_2N and 45–98% in [Bmim]PF_6, respectively. The lipase *PS*-hydrophobic IL mixture was recycled five times without any decrease in the yield of the products. In another set of experiments, the hydrolytic activity of the enzyme was determined after incubation in each of the three ILs and in hexane for 20 days at room temperature. It was found to be 1.8- and 1.6-fold higher in [Bmim]Tf_2N and [Bmim]PF_6, respectively, remained unchanged in [Bmim]BF_4 and was 1.6 times lower in hexane as compared to the non-incubated enzyme.

1. Introduction

Enzymatic reactions carried out in ionic liquids [ILs] reportedly have high activity, good enantioselectivity, high reaction rates, high thermal and operational stability [1–4]. It is also known that the effect of various ILs on enzymatic reactions varies widely and unpredictably [5–10]. The ILs commonly used for biocatalysis contain an imidazolium based cation with anions such as BF_4, PF_6 and Tf_2N [11]. Most properties of ILs such as hydrophobicity, miscibility with organic solvents, ability to dissolve organic and inorganic solutes mainly depend on the anion [12–14].

Earlier work from our laboratory reported that lipase *Pseudomonas cepacia* (*PS*) catalyzed transesterification of ethyl 3-phenylpropanoate, when carried out with a variety of alcohols, in different organic solvents resulted in transesterification only with butanol in 92% yield [15,16]. 3-Phenylpropanoic acid esters are important organic molecules and find application in the flavor and fragrance industry [17]. Since ILs were used as suitable media for carrying out lipase catalyzed reactions [7,13,18–20], and as part of our continuing interest in developing 'green' methods for the preparation of important organic molecules, we carried out the biocatalyzed transesterification of ethyl 3-phenylpropanoate in ILs with eleven alcohols (Scheme 1). In order to evaluate the influence of anions on the catalytic performance of lipase *PS*, three ILs, [Bmim]BF_4, [Bmim]PF_6 and [Bmim]Tf_2N, with an identical cation (Bmim) and different anions [BF_4, PF_6 and Tf_2N] were selected as reaction media for this transesterification reaction. The long-term stability of the enzyme in hydrophobic ILs up to a period of 10 months was also examined. This is the first report demonstrating that lipase *PS* incubated in hydrophobic ILs for long periods (20–300 days) resulted in an increase in activity. The recycling of lipase *PS*-hydrophobic IL mixture was also investigated.

2. Experimental procedures

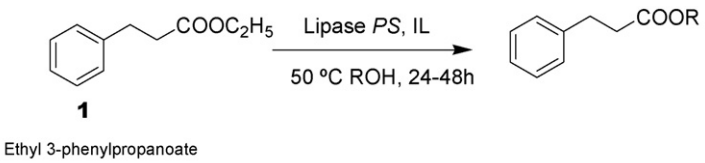
2.1. Materials

ILs [Bmim]BF_4, [Bmim]PF_6 and [Bmim]Tf_2N were prepared and purified following reported procedures [7,8]. Lipase *PS* was bought from Amano Pharmaceuticals Co., Nagoya, Japan. All other chemicals were purchased from commercial sources and were of

Abbreviations: IL, ionic liquids; lipase *PS*, *Pseudomonas cepacia* lipase; [Bmim]BF_4, 1-butyl-3-methylimidazolium tetrafluoroborate; [Bmim]PF_6, 1-butyl-3-methylimidazolium hexafluorophosphate; [Bmim]Tf_2N, 1-butyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; S.D., standard deviation; DMF, *N*,*N*-dimethylformamide; CAL-B, *Candida Antarctica* lipase B; [Emim]Tf_2N, 1-ethyl-3-methylimidazolium bis(trifluoromethylsulfonyl)imide; [Bm_3N]Tf_2N, butyltrimethylammonium bis(trifluoromethylsulfonyl)imide.

* Corresponding author at: Laboratory of Bioorganic Chemistry, Department of Biotechnology, Indian Institute of Technology Madras, Chennai 600036, India. Tel.: +91 44 2257 4106; fax: +91 44 2257 4102.
E-mail address: anjuc@iitm.ac.in (A. Chadha).

1381-1177/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.molcatb.2008.08.007



Scheme 1. Lipase *PS* catalyzed transesterification of ethyl 3-phenylpropanoate in ILs.

analytical grade. The transesterified products were identified by a Nucon Gas Chromatograph (5700 series) using Flame Ionization Detector (FID). An SE-30 column operating isothermally at 160 °C was used to identify the enzymatically formed ester. The injector and detector were kept at 280 °C. Column chromatography was done on silica gel (100–200 mesh). All UV measurements were carried out on a Jasco V 530 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ on a JEOL GSX-400 MHz spectrometer. Chemical shifts are expressed in ppm values using TMS as an internal standard. IR spectra were recorded on a Shimadzu IR 470 Instrument. Mass spectra were recorded on a Q TOF micromass spectrometer. TLC was done by using Kieselgel 60 F_{254} aluminium sheets (Merck 1.05554). The water content in the ILs was determined with an 831 Metrohm Karl-Fischer coulometer.

2.2. Preparation of standard esters

3-Phenylpropanoic acid esters were prepared by the reported procedure [15]. All the 3-phenylpropanoic acid esters, except decyl-3-phenylpropanoate (Table 1 entry 10) are reported earlier. These were characterized by ^{1}H and ^{13}C NMR and compared with literature data [21]. Decyl-3-phenylpropanoate was obtained as a colorless liquid in 61% yield after purification on a silica gel column using hexane as solvent. ^{1}H NMR ($CDCl_3$, 400 MHz) δ ppm: 7.33–7.22 (m, 5H, Ph), 4.10–4.07 (t, 2H, CH_2, J = 6.8 Hz), 3.00–2.96 (t, 2H, CH_2, J = 7.8 Hz), 2.67–2.63 (t, 2H, CH_2, J = 7.3 Hz), 1.29–1.61 (m, 16H, CH_2), 0.93–0.89 (t, 3H, CH_3, J = 7.3 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ ppm: 172.9, 140.5, 128.4, 128.2, 126.1, 64.5, 35.8, 31.8, 30.9, 29.4, 29.2, 29.2, 28.5, 25.8, 22.6, 14.0; IR (neat, cm^{-1}): 2925, 1736, 1455, 1162; HRMS (EI): calculated for $C_{19}H_{30}O_2Na$ $(M+Na)^+$, 313.2151; found 313.2144.

The retention times [in minutes] for the esters on GC were as follows: methyl 3-phenylpropanoate: 1.59, ethyl 3-phenylpropanoate: 2.35, propyl 3-phenylpropanoate: 3.28, butyl 3-phenylpropanoate: 4.45, pentyl 3-phenylpropanoate: 7.25, hexyl 3-phenylpropanoate: 13.26, heptyl 3-phenylpropanoate: 20.24, octyl 3-phenylpropanoate: 32.14, decyl 3-phenylpropanoate: 44.05, 3-methyl-1-butyl 3-phenylpropanoate: 7.05, benzylpropanoate: 7.00, 1-methyl ethyl 3-phenylpropanoate: 4.07.

Table 1
Lipase *PS* catalyzed transesterification of **1** in ILs

	ROH	log *P*	Yield % of ester formed in		
			[Bmim]BF_4 48 h	[Bmim]PF_6 48 h	[Bmim]Tf_2N 24 h
1	CH_3OH	−0.76	0	22	60
2	$CH_3(CH_2)_2OH$	0.344	19	55	66
3	$(CH_3)_2CHOH$	0.691	11	42	55
4	$CH_3(CH_2)_3OH$	0.875	19	95	96
5	$CH_3(CH_2)_4OH$	1.407	14	65	83
6	$(CH_3)_2CHCH_2CH_2OH$	1.223	19	70	88
7	$CH_3(CH_2)_5OH$	1.938	17	58	73
8	$CH_3(CH_2)_6OH$	2.469	10	52	64
9	$CH_3(CH_2)_7OH$	3.001	13	33	78
10	$CH_3(CH_2)_9OH$	4.063	10	30	54
11	$C_6H_5CH_2OH$	1.035	10	62	80

The reaction mixture was kept in shaker at 50 °C at 250 rpm for 24–48 h.

2.3. Typical procedure for lipase PS catalyzed transesterification of ethyl 3-phenylpropanoate in ILs

To screw capped test tubes of 3 mL capacity ethyl 3-phenylpropanoate (50 mg, 0.28 mmol), alcohol (0.56 mmol), 1 mL of IL and 50 mg of lipase *PS* were added. This mixture was kept in an orbital shaker at 50 °C at 250 rpm for 48 h (Scheme 1). At intervals of 8 h, aliquots of 50 μL were taken in 1 mL of hexane–ethyl acetate (90:10). This biphasic mixture was vortexed for 2 min to extract all the reactants and product to the organic layer and analyzed by GC to monitor the time course of the reaction (extraction efficiency was >99%). After the completion of the reaction, the reaction mixture was extracted into hexane–ethyl acetate (90:10; 2 mL × 3), concentrated, purified by column chromatography and characterized as given in Section 2.2.

2.4. Lipase PS-IL recyclability for transesterification

On completion of the transesterification reaction and separation of the products from the reaction medium, [Bmim]PF_6 and [Bmim]Tf_2N containing the lipase were recharged with a fresh set of substrates and the transesterification was carried out as given in Section 2.3.

2.5. Determination of hydrolytic activity of lipase PS

Enzyme activity was determined by the of 4-nitrophenyl palmitate assay [22], with modifications. The reaction was started by adding 100 μL of a uniform suspension of lipase *PS* in IL or hexane (0.5 mg in 500 μL) to 100 μL 0.8 mM solution of 4-nitrophenyl palmitate (6.04 mg dissolved in 500 μL of DMF, made up to 20 mL with water). The final volume was made up to 1 mL with IL or hexane. This mixture was incubated at 37 °C for 10 min. The reaction was arrested by addition of 2 mL of 0.3 M sodium carbonate solution. A blank correction for each sample was done. Enzyme activity was determined by measuring the increase in absorbance at 412 nm produced by the release of 4-nitrophenol by the enzymatic hydrolysis of 4-nitrophenyl palmitate. All experiments were carried out in duplicate providing a S.D. of less than 0.5%. One unit of hydrolytic activity is expressed as μmol of the product (4-nitrophenol) formed per minute by 1 mg of lipase *PS*.

2.6. Stability of lipase PS in ILs

2.6.1. Transesterification with lipase PS incubated in IL

To screw capped test tubes of 3 mL capacity, 50 mg of lipase *PS* and 1 mL of [Bmim]PF_6 or [Bmim]Tf_2N were added. The resulting

mixture was vortexed for 2 min and kept at room temperature. At selected intervals of time, i.e. every 5 days up to 1 month and then every 20 days up to 10 months, a fresh set of substrates (**1** and alcohol) were added to each lipase-IL mixture and the transesterification reaction was carried out as given in Section 2.3.

2.6.2. Hydrolytic activity of lipase PS incubated in IL

To 1.5 mL screw-capped vials, 0.5 mg of lipase *PS*, 500 μL of [Bmim]BF_4, [Bmim]PF_6, [Bmim]Tf_2N or hexane were added. This mixture was vortexed for 1 min and kept at room temperature for 20 days. A fresh mixture of lipase *PS* with IL or hexane was also prepared as described above. The hydrolytic activity of the enzyme before and after incubation was determined as in Section 2.5.

3. Results and discussion

*3.1. Influence of the anions on lipase PS catalyzed transesterification of **1***

Earlier work from our laboratory reported that lipase *PS* catalyzed transesterification of ethyl 3-phenylpropanoate with alcohols, viz. butanol, 1-phenyl ethanol, benzyl alcohol and octanol in a variety of organic solvents, resulted in transesterification only with butanol in 92% yield [15,16]. However, replacement of organic solvents by the IL, [Bmim]PF_6 resulted in the formation of transesterified products with all the alcohols. Since the results clearly proved that [Bmim]PF_6 is an excellent medium for this transesterification, two more ILs, [Bmim]BF_4 and [Bmim]Tf_2N were used to examine the role of the anion in the IL. Eleven different alcohols were used. The results are summarized in Table 1. [Bmim]Tf_2N and [Bmim]PF_6 proved to be the best choice for this reaction [yields 22–96%], whereas in [Bmim]BF_4 the yields were found to be very low (0–19%). These three ILs influence the conformation of the enzyme and consequently its activity [23,24] due to the differences in their properties, viz. polarity, hydrophobicity, hydrogen bonding, basicity and viscosity, It is reported that hydrophilic polar organic solvents impede the rate of enzyme catalyzed reactions by stripping the tightly bound water from the enzyme molecule causing the deactivation of the enzyme [25,26]. Even though [Bmim]BF_4 is the most polar of the three ILs studied, all of them are more polar than methanol or acetonitrile [27,28]. Hence, high polarity alone is insufficient to explain the low yields in [Bmim]BF_4.

[Bmim]BF_4 is hydrophilic whereas [Bmim]PF_6 and [Bmim]Tf_2N are hydrophobic. The hydrophobicity increases in the order [Bmim]Tf_2N > [Bmim]PF_6 [8]. Since this transesterification reaction was carried out under anhydrous conditions (only IL was used as the medium, without any addition of water as a co-solvent), it is possible that [Bmim]BF_4 removes the tightly bound water from the enzyme molecule thus reducing its catalytic activity under anhydrous conditions leading to poor yield. In addition, [Bmim]BF_4, with its ability to form strong hydrogen bonds [29] may react readily with both—the internal hydrogen bonds of the enzyme and the tightly bound water molecules of the enzyme, resulting in conformational changes that decrease the activity of the enzyme.

Of the two hydrophobic ILs, better yields were obtained in [Bmim]Tf_2N, possibly because [Bmim]Tf_2N (27 cP, at 25 °C) is less viscous than [Bmim]PF_6 (397 cP, 25 °C). The high viscosity of [Bmim]PF_6 may limit the mass transfer of the substrates and products to and from the active site of the enzyme and may lead to a decrease in conversion yield [8]. Moreover, the reaction in [Bmim]Tf_2N was found to be faster, i.e. completed within 24 h, while with the other two ILs, it took 48 h for completion of the reaction.

Table 2
Transesterification of **1** after incubation of lipase *PS* in IL for 20 days

Entry	ROH	Yield % of ester formed in	
		[Bmim]PF_6 48 h	[Bmim]Tf_2N 24 h
1	CH_3OH	45 (2.04)	78 (1.30)
2	$CH_3(CH_2)_2OH$	65 (1.18)	70 (1.06)
3	$(CH_3)_2CHOH$	62 (1.47)	65 (1.18)
4	$CH_3(CH_2)_3OH$	96 (1.01)	98 (1.02)
5	$CH_3(CH_2)_4OH$	96 (1.47)	98 (1.18)
6	$(CH_3)_2CHCH_2CH_2OH$	98 (1.40)	98 (1.11)
7	$CH_3(CH_2)_5OH$	98 (1.68)	98 (1.34)
8	$CH_3(CH_2)_6OH$	98 (1.88)	98 (1.53)
9	$CH_3(CH_2)_7OH$	61 (1.84)	98 (1.25)
10	$CH_3(CH_2)_9OH$	60 (2.00)	62 (1.14)
11	$C_6H_5CH_2OH$	90 (1.45)	92 (1.15)

Values in brackets indicate the fold increase compared to the non-incubated lipase *PS* (Table 1). The reaction mixture was kept in shaker at 50 °C at 250 rpm for 24–48 h.

The stability of the lipase *PS* in IL for this transesterification reaction was studied [Section 2.6.1]. [Bmim]PF_6 and [Bmim]Tf_2N were considered for this study since the yields were very low in [Bmim]BF_4. It was observed that the enzyme was stable in these ILs for a period of at least 10 months. Interestingly, the hydrophobic ILs, [Bmim]PF_6 and [Bmim]Tf_2N are hygroscopic and get saturated at low water concentration even after drying at 70 °C for 4 h at room temperature [8,29]. It is suggested that even at mole fraction of water less than 0.25, for [Emim]Tf_2N (hydrophobic) nearly half the water is associated with other water molecules, and exists as water clusters [30]. The water content in the ILs for this transesterification reaction, as determined by Karl-Fischer coulometer was found to be in the range 0.72–0.77% for [Bmim]Tf_2N and 1.09–1.13% for [Bmim]PF_6. Hence lipase *PS* in these water-immiscible ILs at saturation of water ('wet IL') can be considered as existing as an aqueous solution, in which there are free water clusters which preserve the critical water molecules in the microenvironment of the enzyme, thus preventing its deactivation due to the loss of the essential water shell [31]. Dupont describes that wet ILs are nano-structured materials which allow neutral molecules to reside in less polar regions and ionic or polar species to undergo faster diffusion in the more polar or wet regions [32]. In this context, enzymes in water immiscible ILs should also be considered as included into hydrophilic gaps of the network, where the observed stabilization of enzymes could be attributed to the maintenance of this strong network around the protein displaying exceptional synthetic activity and operational stability [31]. The storage stability of cytochrome *c* in aqueous buffer is modest, but in a 20 wt% solution of choline dihydrogen phosphate IL in water, the activity was maintained for at least 6 months at room temperature [33]. In another set of experiments, the thermal stability of CAL-B at 50 °C over 4 days was found to be 3–4 times higher in [Emim]Tf_2N and [Bm_3N]Tf_2N than in water or hexane [34].

In the present study it was observed that lipase-*PS* incubated in the ILs for 20 days to 10 months resulted in increased yields of 45–98% (1–2-fold) in [Bmim]PF_6 and 62–98% (1–1.5-fold) in [Bmim]Tf_2N (Table 2). Recyclability of the lipase *PS*-hydrophobic IL mixture [Section 2.4], showed that both the lipase *PS*-IL mixtures can be reused five times without any decrease in the yields (data not shown). Thus [Bmim]PF_6 and [Bmim]Tf_2N function as excellent stabilizing agents for lipase *PS* at room temperature resulting in a remarkable increase in the yields for transesterification when stored for long time. The results indicate that apart from preserving the essential water shell, the IL provides a suitable microenvironment for the enzyme leading to a more compact conformation, capable of exhibiting high activity and stability.

Table 3
Hydrolytic activity of lipase *PS* in IL/hexane as determined by 4-nitrophenyl palmitate assay

Medium	Hydrolytic activity (μmol/min/mg)		Fold change in activity
	Before incubation	After incubation	
Hexane	26.0	16.2	1.6[a]
[Bmim]BF_4	16.6	16.7	1.0
[Bmim]PF_6	28.3	45.2	1.6[b]
[Bmim]Tf_2N	35.3	63.5	1.8[b]

[a] Fold decrease.
[b] Fold increase.

3.2. Influence of IL on hydrolytic activity of lipase PS due to the pretreatment of Lipase

Since the lipase *PS*-IL mixture, incubated for 20 days resulted in an increase in the yields of the transesterification reaction, we also investigated the hydrolytic activity of lipase *PS* due to incubation in IL or hexane for 20 days, as mentioned in Section 2.6.2. The hydrolytic activity of lipase *PS* for the hydrolysis of 4-nitrophenyl palmitate was high in the hydrophobic ILs than in hydrophilic [Bmim]BF_4 (Table 3). On incubation for 20 days, activity increased to 1.8- and 1.6-fold in [Bmim]Tf_2N and [Bmim]PF_6, respectively, while in [Bmim]BF_4 the activity remained unchanged and in hexane, a 1.6-fold decrease in activity was observed. A possible reason could be that the IL might be interacting with charged groups of the enzyme, either in the active site or at its periphery, causing changes in the enzyme's structure [35]. Lipase *PS* exhibited the same hydrolytic activity in [Bmim]BF_4 even after 20 days, which might be due to the ability of this IL to conserve these electrostatic interaction with the enzyme for long periods, whereas [Bmim]Tf_2N and [Bmim]PF_6 might be maximizing the interactions required for stabilization of the enzyme and the maintenance of the water shell around it, thus resulting in increased activity and stability. In another study, *Mucor janvanicus* lipase pretreated with ILs such as [Bmim]BF_4, [Bmim]PF_6, [Emim]Tf_2N and [Emim]BF_4 for 20 min was found to be higher than that of untreated lipase for a hydrolysis reaction in an aqueous medium [36].

3.3. Effect of alcohols on the lipase PS catalyzed transesterification of **1**

The effect of alcohols on conversion yields in hydrophobic ILs, [Bmim]Tf_2N and [Bmim]PF_6 present some interesting observations. With straight chain alcohols, the yield increases as the number of carbon atoms increases, reaching a maximum with butanol (Table 1 entries 1, 2, 4). Further increase in the number of carbon atoms of the alcohols resulted in decreased yield of the transesterified product giving lowest yields with decanol (Table 1 entries 5, 7–10). Transesterification with 3-methyl-1-butanol and benzyl alcohol yielded 62–88% of the products (Table 1 entries 6, 11) in both the ionic liquids. It is interesting to note that the among the eleven alcohols, transesterification with butanol, pentanol, 3-methyl-1-butanol and benzyl alcohols whose log *P* lies in the range 0.875–1.407, which can be considered as moderately hydrophilic [37] resulted in high yields (62–96%) in both the ILs.

4. Conclusion

Three ILs having identical cation and different anions were tested as reaction media for the lipase *PS* catalyzed transesterification of 3-phenylpropanoate with eleven alcohols. The hydrophobic ILs, [Bmim]PF_6 and [Emim]Tf_2N were found to be very efficient media for this transesterification than hydrophilic [Bmim]BF_4 indicating that the nature of the anion has an impact on the catalytic performance of lipase *PS*. The lipase *PS*-hydrophobic IL mixture incubated for 20–300 days resulted in an increased yield for transesterification. In another set of experiments, the hydrolytic activity of lipase *PS* for 4-nitrophenyl palmitate hydrolysis in these three ILs was found to be high with hydrophobic ILs and resulted in an enhancement in activity when the lipase *PS*-hydrophobic IL mixture was incubated for 20 days. The results reported here clearly demonstrate that hydrophobic IL can be used as excellent medium and also as a reservoir for lipase *PS*, in which the enzyme can be stored with improved activity and stability.

Acknowledgement

One of the authors [P. Vidya], is grateful to the University Grants Commission, Government of India for research fellowship.

References

[1] Y. Yuan, S. Bai, Y. Sun, Food Chem. 97 (2006) 324–330.
[2] W.Y. Lou, M.H. Zong, Y.Y. Liu, J.F. Wang, J. Biotechnol. 125 (2006) 64–74.
[3] M.H. Katsoura, A.C. Polydera, L. Tsironis, A.D. Tselepis, H. Stamatis, J. Biotechnol. 123 (2006) 491–503.
[4] R.A. Sheldon, R.M. Lau, M.J. Sorgedrager, F.V. Rantwijk, K.R. Seddon, Green Chem. 4 (2002) 147–151.
[5] J.A. Laszlo, D.L. Compton, Biotechnol. Bioeng. 75 (2001) 181–186.
[6] S.J. Nara, J.R. Harjani, M.M. Salunkhe, A.T. Mane, P.P. Wadgaonkar, Tetrahedron Lett. 44 (2003) 1371–1373.
[7] S. Park, R.J. Kazlauskas, J. Org. Chem. 66 (2001) 8395–8401.
[8] J.G. Huddleston, A.E. Visser, W.M. Reichert, H.D. Willauer, G.A. Broker, R.D. Rogers, Green Chem. 3 (2001) 156–164.
[9] M. Eckstein, P. Wasserscheid, U. Kragl, Biotechnol. Lett. 24 (2002) 763–767.
[10] F.V. Rantwijk, R.M. Lau, R.A. Sheldon, Trends Biotechnol. 21 (2003) 131–138.
[11] P. Lozano, T.D. Diego, J.P. Guegan, M. Vaultier, J.L. Iborra, Biotechnol. Bioeng. 75 (2001) 563–569.
[12] Z. Yang, W. Pan, Enzyme Microb. Technol. 37 (2005) 19–28.
[13] F.V. Rantwijk, F. Secundo, R.A. Sheldon, Green Chem. 8 (2006) 282–286.
[14] C. Pilissao, M.G. Nascimento, Tetrahedron: Asymmetry 17 (2006) 428–433.
[15] K. Priya, A. Chadha, Enzyme Microb. Technol. 32 (2003) 485–490.
[16] K. Priya, T. Venugopal, A. Chadha, Indian J. Biochem Biophys. 39 (2002) 259–263.
[17] http://www.fragrance and products network.com.
[18] T. Itoh, E. Akasaki, K. Kudo, S. Shirakami, Chem. Lett. 30 (2001) 262–263.
[19] R.M. Lau, F.V. Rantwijk, K.R. Seddon, R.A. Sheldon, Org. Lett. 2 (2000) 4189–4191.
[20] K.W. Kim, B. Song, M.Y. Choi, M.J. Kim, Org. Lett. 3 (2001) 1507–1509.
[21] C.J. Pouchert, The Aldrich Library of NMR Spectra, vols. second ed., Aldrich Chemical Co., Inc., Milwaukee, 1983.
[22] U.K. Winkler, M. Stuckmann, J. Bacteriol. 138 (1979) 663–670.
[23] Y. Zhou, Curr. Nanosci. 1 (2005) 35–42.
[24] C. Chiappe, E. Leandria, S. Lucchesia, D. Pieraccinia, B.D. Hammock, C. Morisseau, J. Mol. Catal. B: Enzym. 27 (2004) 243–248.
[25] A. Zaks, A.M. Klibanov, J. Biol. Chem. 263 (1988) 3194–3201.
[26] S.J. Nara, J.R. Harjani, M.M. Salunkhe, Tetrahedron Lett. 43 (2002) 2979–2982.
[27] A.J. Carmichael, K.R. Seddon, J. Phys. Org. Chem. 13 (2000) 591–595.
[28] M.J. Earley, B.S. Engal, K.R. Seddon, Aust. J. Chem. 57 (2004) 149–150.
[29] M.G. Freire, P.J. Carvalho, A.M. Fernandes, I.M. Marrucho, A.J. Queimada, J.A.P. Coutinho, J. Colloid Interface Sci. 314 (2007) 621–630.
[30] M.S. Kelkar, E.J. Maginn, J. Phys. Chem. B 111 (2007) 4867–4876.
[31] P. Lozano, T.D. Diego, S. Gmouh, M. Vaultier, J.L. Iborra, Biocatal. Biotransform. 23 (2005) 169–176.
[32] J. Dupont, J. Braz. Chem. Soc. 15 (2004) 341–350.
[33] K. Fujita, M. Forsyth, D.R. MacFarlane, R.W. Reid, G.D. Elliott, Biotechnol. Bioeng. 94 (2006) 1209–1213.
[34] T.D. Diego, p. Lozano, S. Gmouh, M. Vaultier, J.L. Iborra, Biomacromolecules 6 (2005) 1457–1464.
[35] W.Y. Lou, M.H. Zong, H. Wu, Biocatal. Biotransform. 22 (2005) 171–176.
[36] D.T. Dang, S.H. Ha, S.M. Lee, W.J. Chang, Y.M. Koo, J. Mol. Catal. B: Enzym. 45 (2007) 118–121.
[37] C. Laane, S. Boeren, K. Vos, C. Veeger, Biotechnol. Bioeng. 30 (1987) 81–87.