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Biocatalysis in ionic liquids: the stereoconvergent hydrolysis of *trans*-β-methylstyrene oxide catalyzed by soluble epoxide hydrolase

Cinzia Chiappe [a,*], Elsa Leandri [a], Serena Lucchesi [a], Daniela Pieraccini [a], Bruce D. Hammock [b,1], Christophe Morisseau [b]

[a] *Dipartimento di Chimica Bioorganica e Biofarmacia, Università di Pisa, via Bonanno 33, 56126 Pisa, Italy*
[b] *Department of Entomology & Cancer Research Center, University of California, Davis, CA 95616, USA*

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Abstract

Soluble epoxide hydrolase (sEH) was shown to catalyze hydrolysis of epoxides using the ionic liquids (ILs) [bmim][PF_6], [bmim][$N(Tf)_2$], and [bmim][BF_4] (where bmim = 1-butyl-3-methylimidazolium, PF_6 = hexafluorophosphate, $N(Tf)_2$ = bis(trifluoromethylsulfonyl)imide, and BF_4 = tetrafluoroborate) as reaction medium. Reaction rates were generally comparable with those observed in buffer solution, and when the cress enzyme was used the hydrolysis of *trans*-β-methylstyrene oxide gave, through a stereoconvergent process, the corresponding optically active (1*S*,2*R*)-*erythro*-1-phenylpropane-1,2-diol.

Keywords: Biocatalysis; Ionic liquids; Stereoconvergent hydrolysis

1. Introduction

Non-aqueous biocatalysis provides a useful component of methodology in organic synthesis [1]. Although water is one of the most commonly proposed green alternatives to organic solvents, the use of enzymes in non-aqueous media eliminates several obstacles that limit their application in water. Consequently, once it was established that enzymes could work in organic solvents, with little or no-water, research in this area surged. However, biocatalysts in non-aqueous media often suffer from reduced activity, selectivity or stability, and the use of organic solvents has raised questions of environmental concern, in particular when they are employed on preparative scale [2].

Recently ionic liquids (ILs), organic salts liquid at or near room temperature, have emerged as alternative green media for biotransformations, using both whole cell systems and isolated enzymes. The use of ionic liquids has showed many advantages, such as better enzyme stability, substrate and/or product selectivity, and suppression of side reactions [3]. As solvents for chemical reactions, ILs exhibit excellent physico-chemical properties. In particular, they have the ability to dissolve polar and non-polar organic, inorganic, and polymeric materials, high thermal stability and a lack of significant vapor pressure [4]. Furthermore, all the physico-chemical properties of ILs can be modified by altering the cation or anion, and in principle the optimal IL may be designed for each specific reaction system. These features are important also for enzymatic reactions, where they are associated with the ability to enhance enzyme stability and selectivity, and reduce side reactions.

Although the majority of enzymes reported to be active in ILs are lipases [5], other biocatalysts have been investigated in ILs with improved results compared to water. For example galactosylation of lactose with β-galactosidase in the presence of *N*-acetylglucosamine, has been recently studied for the synthesis of *N*-acetyllactosamine [6]. Because the secondary hydrolysis of the reaction product was greatly reduced, the reaction occurs with a higher yield in the IL compared to the aqueous natural medium. Enantioselective reductions of prochiral ketones using Baker's yeast have been also recently described [7].

As a part of our research program, we became interested in ionic liquids as alternative solvents for biotransformations using epoxide hydrolases (EHs) as catalyst.

* Corresponding authors. Tel.: +39-050-44074; fax: +39-050-43321.
E-mail addresses: cinziac@farm.unipi.it (C. Chiappe), bdhammock@ucdavis.edu (B.D. Hammock).
[1] Co-corresponding author.

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A current topic in synthetic organic chemistry is the preparation of optically pure epoxides as well as their corresponding vicinal diols [8]. These compounds are highly versatile chiral synthons, often used in the synthesis of biologically active molecules. One of the emerging approaches to obtain such building blocks is the enantioselective hydrolysis of racemic epoxides catalyzed by EHs [9]. These enzymes have long been known from mammalian systems, and stereochemical studies have shown that the reaction catalyzed by EHs generally proceeds with a high product and/or substrate enantioselectivity [10]. However, the interest of the synthetic chemist towards the application of these biocatalysts has increased only recently, due to two new factors that arose only in the past decade: (i) the discovery that these enzymes are ubiquitous in nature, since they have been detected in plants, fungi, microorganisms and they are, therefore, easily accessible [11]; (ii) the discovery that different EHs may be cloned, modified, and expressed in cells or microorganisms [12]. The existence of various EHs, and their availability in preparative scale, has surely contributed to increase the interest towards these biocatalysts. However, in contrast to other enzymatic systems, practically no data have been reported on the use of these enzymes in organic solvents. To the best of our knowledge, only one study has been recently reported on the use of whole cells of an isolated *Aspergillus niger* sp. in an organic solvent for the enantioselective hydrolysis of racemic epichlorohydrin [13].

Having extensively investigated EH catalyzed hydrolysis in water we were interested in the study of the activity of such systems in ionic liquids. Thus, here we report our preliminary results on the hydrolysis of *trans*-β-methylstyrene oxide (**1**) by soluble EHs in ionic liquids. With this substrate, the reaction catalyzed by cress soluble EH is stereoconvergent giving the corresponding (1*S*,2*R*)-*erythro*-1-phenylpropane-1,2-diol (**2**) as the main product.

2. Experimental

The ^{1}H and ^{13}C NMR spectra were registered in $CDCl_3$ with a Bruker AC 200 instrument using TMS as the internal reference. The enantiomeric excesses (ee) of the unreacted epoxide and of the resulting diol (after transformation into the corresponding bis(trifluoroacetyl) derivatives) were determined by GLC analysis using a Carlo Erba HRGC 5300 instrument equipped with a 20 m Chiraldex G-TA (ASTEC) column, evaporator and detector 220 °C, helium flow 50 kPa at 98 °C. The conversions were determined by GLC analysis, under the same conditions used for the determination of the ee's, by adding phenylmethyl ketone as an internal standard.

2.1. Chemicals

trans-β-Methylstyrene oxide (1*R*,2*R*)- and (1*S*,2*S*)-β-methylstyrene oxide were purchased from Aldrich. Racemic *trans*-β-methylstyrene oxide was prepared by oxidation of *trans*-β-methylstyrene with *m*-chloroperbenzoic acid, as previously reported [14]. The corresponding diols were synthesized from the epoxide via acid catalyzed hydrolysis, as previously reported [14]. [bmim][BF_4] was purchased from Solvent Innovation (GmbH). [bmim][PF_6] and [bmim][$N(Tf)_2$] were prepared following the reported procedures [15]: close attention was paid to the elimination of bases and Cl^- ions which may be present in the solvents as impurities. The purity of imidazolium salts was always checked by UV measuring the absorption spectra between 240 and 400 nm. Purified $[bmim]^+$ salts (containing $Cl^- < 0.1$ ppm) have practically no absorption band in the 250–300 nm region [16]. After drying (2 h at 80 °C under vacuum) the water amount in ILs was determined by Karl–Fisher technique using an apparatus composed of a stand titrator and a coulometer. The water content of dried ILs ranged from 150 to 350 ppm.

2.2. Enzyme preparation and purification

The recombinant soluble epoxide hydrolase (sEH) enzymes of mouse and cress were prepared and purified as previously described [17]. Recombinant cDNA of each enzyme was cloned into the baculovirus expression system. Insect cells from *Trichoplusia ni* were transfected with prepared baculovirus in order to express the desired enzyme and subsequently were purified from cell lysate via affinity chromatography.

2.3. Enantioselectivity assays

Epoxide **1** (13–26 mg) and epoxide hydrolase (0.08–3.1 mg) were mixed with ionic liquid (2 ml) or buffer solution (Tris–HCl, pH = 7.4) and the resulting mixture was stirred at 37 °C. After a prefixed time (2–4 h) the reaction mixture was first extracted with hexane (five times with 4 ml portions). The combined hexane phases were analyzed by GC on a chiral column, after addition of phenylmethylketone as an internal standard, to determine the concentration of unreacted substrate. The ionic liquid was then extracted with ethyl ether (five times with 4 ml portions) and the combined ethereal phases, containing the formed diol, were analyzed by GC on the same chiral column, under the same conditions, after transformation of the diol into the corresponding bis-trifluoroacetyl derivative.

Blank experiments carried out without enzyme or using a deactivated preparation showed that the spontaneous hydrolysis did not contribute to diol formation under the incubation conditions.

Finally, after extraction of the unreacted epoxide and the product diol the ionic liquids were always filtered, dried under vacuum at 80 °C for 2 h and reused.

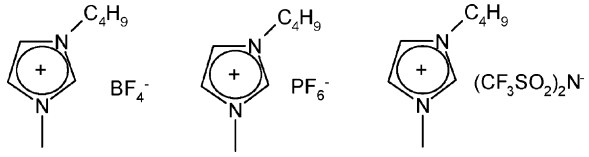
The water content of the dried ILs, determined by Karl–Fisher titration ranged from 150 to 350 ppm. When purified enzyme was used the amount of water was around 1% while in the case of crude extracts it was around 10%.

2.4. Product isolation

Epoxide **1** (1.0 g, 7.5 mmol) and crude cress sEH (120 mg of proteins) were added to 10 ml of [bmim][PF_6] and the mixture was incubated at 37 °C for 8 h. The reaction, stopped by filtration of the enzyme, was extracted first with hexane (6 × 5 ml) to isolate the residue epoxide (350 mg, 2.6 mmol), then with ethyl ether (6 × 5 ml) to obtain the formed diol (1*S*,2*R*)-**2** (410 mg, 2.7 mmol). The enantiomeric excesses of the isolated products were determined by GC, as reported above.

3. Result and discussion

Three ionic liquids [18], [bmim][PF_6], [bmim][$N(Tf)_2$], and [bmim][BF_4] (where bmim = 1-butyl-3-methylimidazolium, PF_6 = hexafluorophosphate, $N(Tf)_2$ = bis(trifluoromethylsulfonyl)imide and BF_4 = tetrafluoroborate) have been tested as media for sEH catalyzed hydrolysis and compared with the generally used Tris–HCl buffer solution (pH = 7.4).



We decided to start our investigation using these salts since they are the most common ILs applied both in organic reactions and catalyzed processes. There is extensive information on these three ILs. Therefore, they were selected for our initial studies in the hope that we could correlate our results with the physico-chemical properties of the new reaction media. Finally, not all ILs are always suitable for biocatalysis, but many enzymes are active in ILs containing BF_4, PF_6, and $N(Tf)_2$ anions [3a]. Interestingly, while these three ILs have the same cation, they display quite different properties. In particular, [bmim][BF_4] is highly hydrophilic in nature and the other two are hydrophobic. Furthermore, they are characterized by a different polarity and viscosity [19]. These properties may be of primary importance in enzyme-catalyzed reactions, since they are capable to affect the conformation of the enzymes and consequently their reactivity.

The evaluation of the enzyme activity in these media was carried out at 37 °C, using a typical good substrate for sEH, *trans*-β-methylstyrene oxide, and two soluble EHs were chosen as the enzyme. Baculovirus infected cell lysates of recombinant cress or mouse enzymes, or an affinity purified recombinant cress sEH, were used as biocatalysts. The enzyme activity and the enantioselectivity of these reactions were evaluated by GC, using a chiral column.

In typical analytic experiments, the enzymatic reactions were performed with a solution containing substrate (0.1–0.2 mmol), enzyme (0.08–3.1 mg) in solvent (2 ml). Since the crude enzyme extracts were suspended in 0.1 M sodium phosphate buffer (pH 7.4) approximately 10% water (0.2 ml) was added to the final reaction volume of 2 ml. When the purified enzyme was used the amount of water added was around 1%. At prefixed times (2–4 h), the enzyme was removed by filtration and the solution was first extracted with hexane to remove the unreacted epoxide, then with ethyl ether to isolate the resulting diol. After addition of a proper internal standard the organic phases were analyzed by GC using a chiral column, which allowed us to measure the enantiomeric excesses of the remaining epoxide and of the diol, and to determine their absolute configurations by comparison of the retention times of the two enantiomers of epoxide and diol with those of samples of known configuration. The results are given in Table 1.

Only *erythro*-1-phenylpropane-1,2-diol (**2**) was formed as the hydrolysis product of epoxide **1**, in agreement with the expected anti-stereoselective opening of the oxirane ring. The absence of the corresponding *threo*-diol suggested that only the sEH catalyzed hydrolysis was responsible for diol formation, both in buffer solution and in ILs. Blank experiments, carried out in the three ionic liquids without enzyme

Table 1
Hydrolysis of *trans*-β-methylstyrene oxide (**1**) catalyzed by crude cress or mouse soluble EH (csEH, msEH) or purified csEH in ionic liquids

Solvent	[**1**] mmol	Enzyme			Time (h)	Conversion (%)	Residue (1*S*,2*S*)-**1** ee (%)	Formed (1*S*,2*R*)-**2** ee (%)
		Source	Prepared type	mg				
[bmim][PF_6]	0.1	Cress	Crude	3.1	4	32	40	90
[bmim][$N(Tf)_2$]	0.1	Cress	Crude	3.1	4	38	34	60
[bmim][BF_4]	0.1	Cress	Crude	3.1	4	25	4	60
Tris–HCl	0.1	Cress	Crude	3.1	4	38	40	72
[bmim][PF_6]	0.2	Cress	Purified	0.08	2	39	10	78
[bmim][$N(Tf)_2$]	0.2	Cress	Purified	0.08	2	40	4	74
Tris–HCl	0.2	Cress	Purified	0.08	2	37	10	70
[bmim][PF_6]	0.1	Mouse	Crude	2.8	4	17	0	20
[bmim][$N(Tf)_2$]	0.1	Mouse	Crude	2.8	4	14	0	6
[bmim][BF_4]	0.1	Mouse	Crude	2.8	4	22	0	6
Tris–HCl	0.1	Mouse	Crude	2.8	4	50	0	0

or in the presence of deactivated enzyme, further confirm that no spontaneous hydrolysis occurred in these media. It is worth noting that preliminary experiments, carried out on α-methylstyrene oxide, show that in these media the EH catalyzed hydrolysis of highly reactive epoxides can be conducted without competition of the non-enzymatic process, which generally occurs with a different regiochemistry and without any stereoselectivity. To facilitate comparisons, the reactions in buffer solutions and in ILs were carried out at the same substrate concentration. However, the solubility of epoxides in ILs is much higher than in aqueous solutions. For example epoxide **1** is completely soluble in [BMIM][$N(Tf)_2$] at ≅4 M in the preparative experiment described below.

It is worth noting that the data reported in Table 1 show for the first time that sEH works also in ILs. When crude preparations were used, the hydrolysis was only slightly slower in the ionic liquids than in Tris–HCl buffer, however, purified cress sEH showed practically the same activity in ionic liquids and in buffer solution. Attempts to carry out the same process in some common organic solvents were unsuccessful. No activity was found when the incubations of **1** with crude cress were carried out both in *tert*-butanol or methyl-*tert*-butylether.

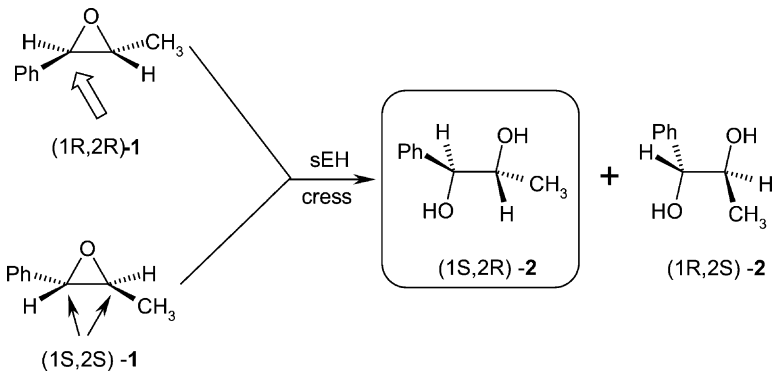
Related to the reactions in ILs the data reported in Table 1 show the anion nature had only a moderate effect on the reaction rate: the conversions were generally slightly lower in [bmim][BF_4], the more hydrophilic IL. The ionic liquids [bmim][PF_6] and [bmim][$N(Tf)_2$], despite being polar, are hydrophobic. This feature is opposite to what was observed for most of the common organic solvents. Therefore, in these ILs, at constant water content, the increase in hydrophobicity may determine an increase in water activity around the protein, favoring the enzyme action by enhancement of free water molecules, which can act as nucleophilic reagent [20].

The reactivity data related to the reactions in [bmim][PF_6] and [bmim][$N(Tf)_2$] indicate that viscosity, which is significantly different for these two ILs [15], has only a minor effect on reactivity or the outcome of the reaction is balanced by other factors. The increase in viscosity on going from [bmim][$N(Tf)_2$] to [bmim][PF_6] might be a mass-transfer limiting parameter that, in principle, could reduce the enzymatic activity. Similar conversion rates were obtained both in [bmim][$N(Tf)_2$] and [bmim][PF_6]. Ionic liquids are, however, solvents characterized by a salt-like structure even in the liquid state. It has been, therefore, proposed that viscosity, which represents the whole diffusion of molecules and ions, does not adequately represent the diffusion of reactants in ILs. These salts, which cannot form ordered crystals, contain probably voids that can accommodate small solute molecules. The different nature of the anion (cation being equal) affecting the cation–anion interaction, may determine the cavity size distribution, and therefore, the solute diffusion [21].

It is also interesting to note that, at least for this epoxide, both the substrate and product enantioselectivity depend primarily on the enzyme source, being very low with the mouse enzyme and relatively high with the cress soluble EH. When this latter enzyme was used as catalyst diol formation was a stereoselective process arising from the stereoconvergent oxirane ring opening of the two enantiomers (Scheme 1). Experiments carried out using pure (1*R*,2*R*)-**1** and (1*S*,2*S*)-**1** as substrates and cress sEH as catalyst have shown that, both in buffer solution or in ILs, the formal nucleophilic attack of water occurs selectively at C1 in the case of (1*R*,2*R*) enantiomer (<98%), while the same enzyme is practically non-selective for the same carbon of (1*S*,2*S*)-**1**. This substrate give the two enantiomeric diols, (1*R*,2*S*)-**2** and (1*S*,2*R*)-**2**, in a 1:1 ratio. Similar results were also found for the cress sEH catalyzed hydrolysis of 3-phenylglycidol [10b].

Product enantioselectivity, characterizing the reaction in buffer solution, is maintained or slightly enhanced in all three ionic liquids. The highest enantiomeric ratios have been found in [bmim][PF_6], in particular when crude cress preparations have been used. The inclusion of the enzyme in the ionic liquid matrix may drive the protein toward a more active conformation for expressing its synthetic activity.

The possibility of applying this procedure in the preparative synthesis of chiral compounds was explored. The reaction of **1** with crude cress sEH in [bmim][PF_6] was repeated in a larger scale. Epoxide **1** (1.0 g, 7.5 mmol) and crude cress sEH (120 mg of proteins) were added to 10 ml



Scheme 1. Regioselectivity for the cress sEH catalyzed hydrolysis of epoxides (1*R*,2*R*)-**1** and (1*S*,2*S*)-**1**.

of [bmim][PF_6] ([***1***] = 0.75 M) and the mixture was incubated at 37 °C for 8 h. The extraction of products with hexane and ethyl ether, as reported above for the reactions on analytical scale, allowed isolation of the residual epoxide (1*S*,2*S*)-**1** (350 mg, 2.6 mmol; ee 40%) and the resulting diol (1*S*,2*R*)-**2** (410 mg, 2.7 mmol, ee 90%). This latter result (not yet optimized) strongly supports our hypothesis that EHs can be used in ionic liquids to prepare chiral synthons.

There are limited data on toxicity of ILs [22]. Anyway, the lack of measurable vapor pressure means that ionic liquids do not release harmful vapors and the ecological advantage arising from their application in organic reactions and catalyzed processes increases if they can be recycled. For example with the exception of [bmim][BF_4], we demonstrate that ionic liquids can be reused at least four times without loss in enzyme activity or selectivity. In the case of [bmim][BF_4], the use of the recycled ionic liquid must be avoided since it dramatically increases the non-desirable racemic hydrolysis process.

Finally, since the use of organic solvents for product isolation reduces the "greenness" of ILs, investigations are in progress to develop suitable methods for product isolation, as well as the possibility to use other ILs bearing "greener" anions.

4. Conclusions

In summary, we have shown for the first time that epoxide hydrolases catalyze the hydrolysis of epoxides in unnatural ionic liquids. Reaction rates and stereoselectivity are comparable with those observed in buffer solution. It is noteworthy that in these solvents spontaneous oxirane hydrolysis is also depressed. For example the highly reactive epoxide, α-methylstyrene oxide yields exclusively the diols arising from the enzymatic reaction.

We envisage that ionic liquids could, therefore, have benefits for performing biotransformation of epoxides which cannot be hydrolyzed in aqueous buffer due to problems with solubility or stability in water. The use of ionic liquids may open up a new prospect for the application of epoxide hydrolases in organic synthesis.

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References

[1] A.M.P. Koshinen, A.M. Klibanov, Enzymatic Reactions in Organic Media, Blackie Academic & Professional, Glasgow, 1996; K. Faber, Biotransformtion in Organic Chemistry, 3rd ed., Springer, Berlin, Germany, 1997.
[2] A.M. Klibanov, Trends Biotechnol. 15 (1997) 97.
[3] (a) U. Kragl, M. Eckstein, N. Kraftzik, Chem. Biotechnol. 13 (2002) 565;
(b) F. van Rantwijk, R.M. Lau, R.A. Sheldon, Trends Biotechnol. 21 (2003) 131;
(c) S. Park, R.J. Kazlauskas, Curr. Opin. Biotechnol. 14 (2003) 1.
[4] J.D. Holbrey, K.R. Seddon, Clean Prod. Process. 1 (1999) 223;
M.J. Earle, K.R. Seddon, Pure Appl. Chem. 72 (2000) 1391;
T. Welton, Chem. Rev. 99 (1999) 2071;
P. Wasserscheid, W. Keim, Angew. Chem. Int. Ed. 39 (2000) 3773;
R.A. Sheldon, Chem. Commun. (2001) 2399;
H. Olivier-Bourbigou, L. Magna, J. Mol. Catal. A 182 (2002) 419;
J. Dupont, R.F. de Souza, P.A.Z. Suarez, Chem. Rev. 102 (2002) 3667.
[5] R.M. Lau, F. van Rantwijk, K.R. Seddon, R.A. Sheldon, Org. Lett. 2 (2000) 4189;
S. Park, R.J. Kazlauskas, J. Org. Chem. 66 (2001) 8395;
S.H. Schöfer, N. Kaftzik, P. Wassersheid, U. Kragl, Chem. Commun. (2001) 425;
K.W. Kim, B. Song, M.J. Kim, Org. Lett. 3 (2001) 1507;
S.J. Nara, J.R. Harjani, M.M. Salunkhe, Tetrahedron Lett. 43 (2002) 2979;
P. Lozano, T. de Diego, D. Carrié, M. Vaultier, J.L. Iborra, J. Mol. Catal. B 21 (2003) 9.
[6] N. Kafzik, P. Wassersheid, U. Kragl, Org. Process. Res. Dev. 6 (2002) 553.
[7] J. Howarth, P. James, J. Dai, Tetrahedron Lett. 42 (2001) 7517.
[8] P.S. Savle, M.J. Lamoereaux, J.F. Berry, R.D. Gandour, Tetrahedron Asym. 9 (1998) 1843.
[9] A. Steinraber, K. Faber, Curr. Opin. Biotechnol. 12 (2001) 552;
A. Archelas, R. Furstoss, Curr. Opin. Chem. Biol. 5 (2001) 112;
I.V.J. Archer, Tetrahedron 53 (1997) 15617.
[10] (a) C. Chiappe, G. Cordoni, G. Lo Moro, C.D. Palese, Tetrahedron Asym. 9 (1998) 341;
(b) K.C. Williamson, C.M. Morisseau, J.E. Maxwell, B.D. Hammock, Tetrahedron Asym. 11 (2000) 4451.
[11] W. Kroutil, M. Mischitz, K. Faber, J. Chem. Soc., Perkin 1 (1997) 3629;
P. Moussou, A. Archelas, R. Baratti, R. Furstoss, J. Org. Chem. 63 (1998) 3532;
C.A.G.M. Weijers, A.L. Botes, M.S. van Dick, M. de Bont, Tetrahedron Asym. 9 (1998) 467;
C. Morisseau, J.K. Beetham, F. Pinot, S. Debernard, J.W. Mewman, B.D. Hammock, Arch. Biochem. Biophys. 387 (2000) 321.
[12] A. Stapleton, J.K. Beetham, F. Pinot, J.E. Garbarino, D.R. Rockhold, M. Friedman, B.D. Hammock, W.R. Belknap, Plant J. 6 (1994) 251;
L. Zhao, E. Mathur, D. Weiner, T. Richardson, A. Milaln, M. Burk, B. Han, PCT Int. Appl. (2003) 400.
[13] W.J. Choi, E.Y. Lee, S.J. Yoon, S.-T. Yang, C.Y. Choi, J. Biosci. Bioeng. 88 (1999) 339.
[14] G. Bellucci, C. Chiappe, A. Cordoni, Tetrahedron Asym. 7 (1996) 197.
[15] R.T. Fuller, H.C. Carlin, D. de Long, J. Haworth, J. Chem. Soc., Chem. Commun. (1994) 299;
L. Cammarata, S.G. Kazarian, P.A. Salter, T. Welton, Phys. Chem. Chem. Phys. (2001) 5192;
C. Chiappe, D. Pieraccini, P. Saullo, J. Org. Chem. 68 (2003) 6710.
[16] I. Billard, G. Moutiers, A. El Azzi, C. Gaillard, C. Mariet, J. Lützenkinchen, Inorg. Chem. 42 (2003) 1726.
[17] D.F. Grant, D.H. Stroms, B.D. Hammock, J. Biol. Chem. 3268 (1993) 17628;
C. Morisseau, J.K. Beetham, F. Pinot, S. Debernard, J.W. Newman, B.D. Hammock, Arch. Biochem. Biophys. 378 (2000) 321.

[18] In this work we used both commercial (Solvent Innovation GmbH) and synthesized ILs but always particular attention was paid to the purity.
[19] J.G. Huddleston, A.E. Visser, W.M. Reichert, H.D. Willauer, G.A. Broker, R.D. Rogers, R.D. Green, Green Chem. 3 (2001) 156; S.V. Dzyuba, R.A. Bartsch, Chem. Phys. Chem. 3 (2002) 161.
[20] P. Halling, J. Enzyme Microb. Technol. 16 (1994) 178.
[21] For a discussion on the effect of the IL structure on the rate constants for diffusion in these solvents see: A.J. McLean, M.J. Muldoon, C.M. Gordon, I.R. Dunkin, Chem. Commun. (2002) 1880.
[22] B. Jastorff, R. Störmann, J. Ranke, K. Mölter, F. Stock, B. Oberheitmann, W. Hoffmann, J. Hoffmann, M. Nüchter, B. Ondruschka, J. Filser, Green Chem. 5 (2003) 136.