

## Enzyme-catalyzed reactions in ionic liquids

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**Abstract**—Ionic liquids have been suggested as potential “green solvents” due to their unique properties such as non-volatility, nonflammability, and a wide temperature range for liquid phase. This review describes recent advances of biocatalyst reactions in ionic liquids. Enzyme-catalyzed reactions in ionic liquids—transesterification, synthesis, conversion, ammoniolysis, hydrolysis, epoxidation, resolution, and oxidation are presented. The use of ionic liquids for protein folding/refolding and the toxicity of ionic liquids are also discussed.

**Key words:** Ionic Liquids, Enzymatic Reactions, Protein Folding/Refolding, Toxicity of Ionic Liquids

### INTRODUCTION

The development of safe and environmentally benign processes plays an important role for the clean manufacturing process and environmental remediation. Even though the first IL, [EtNH<sub>3</sub>][NO<sub>3</sub>] was reported in 1974 [Park and Kazlauskas, 2003], room temperature ionic liquids (RTILs) are recently emerging as desirable substituents for volatile, toxic, and flammable organic solvents, which are major causes of environmental pollution.

Ionic liquids (ILs) are usually composed of organic cations and inorganic or organic anions (see Fig 1).

Some of the typical properties of ionic liquids are as follows.

1. ILs are low melting point (<100 °C) salts that represent a new class of non-aqueous but polar solvent.
2. ILs are ‘green solvents’ because ILs exhibit non-flammable, non-volatile under ambient conditions.
3. ILs that remain as liquids within a broad temperature (<300 °C) have high thermal stability.
4. ILs are ‘designer solvents’, as their chemical and physical properties such as melting point, viscosity, density, hydrophobicity and polarity can be modified by altering the nature of their cations and anions. By manipulating the solvent properties, one is able to design an ionic liquid for specific reaction conditions, such as to increase the substrate solubility, to modify the enzyme selectivity, to tailor the reaction rate, or to increase liquid-liquid extraction efficiency.
5. ILs are able to dissolve a wide range of organic, inorganic, organometallic compounds and polymers.
6. As ILs serve as a good medium to solubilize gases such as H<sub>2</sub>, CO<sub>2</sub> and O<sub>2</sub>, many reactions and separations are now being performed using ionic liquids and supercritical CO<sub>2</sub>.
7. ILs can be potentially reused and recycled.
8. The immiscibility of ionic liquids with either water or organic solvents can be used to form a two-phase system.
9. Normally, an ionic liquid with longer alkyl chains on the cation and a larger anion size presents a higher viscosity and also a lower

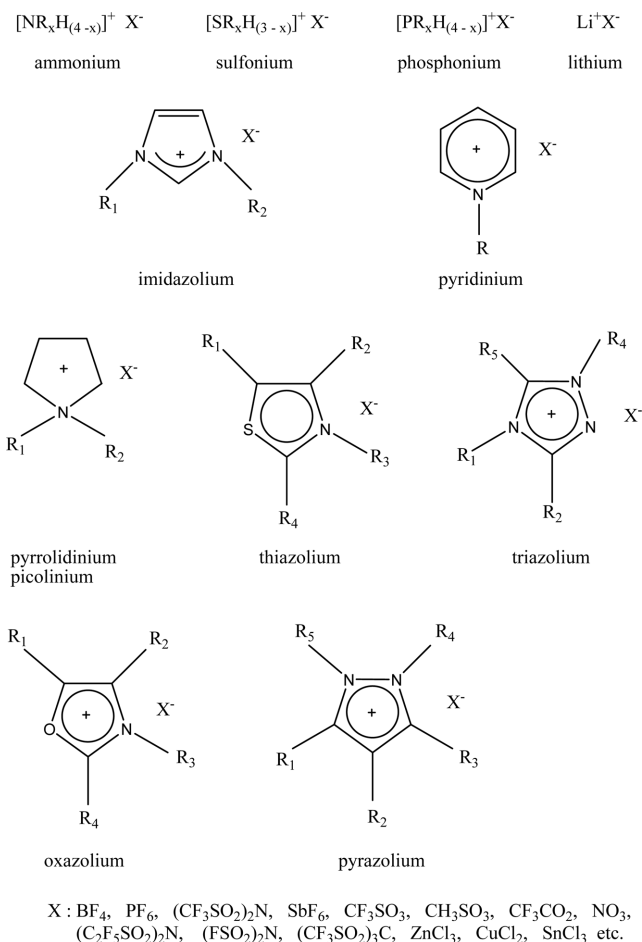


Fig. 1. Some examples of cations and anions in ionic liquids.

polarity.

ILs may play a role as ideal solvents for biocatalytic reactions, separations, enzyme immobilization and protein folding/refolding in biotechnology. Generally, there are three types of operation for ILs in biocatalytic processes: they can be used as a pure solvent, as

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a co-solvent in aqueous systems and in a biphasic system [Kragl et al., 2002; Marsh et al., 2002].

However, they cannot necessarily be considered as green solvents because of their low biodegradability and high toxicological properties, although ILs can be applied as solvents and catalysts [de Diego et al., 2005].

Recent studies show that the enzyme activity may be influenced by the solvent properties of ILs [Yang and Pan, 2005]. Water-immiscible ILs have been shown to be excellent nonaqueous media for enzyme-catalyzed reactions because ionic liquids possessing a longer hydrophobic alkyl chain hold less tendency to strip off the essential water from the enzyme, which results in a high enzyme

**Table 1. Examples of biocatalytic reactions in ionic liquids**

Biocatalyst	Ionic liquids	Reaction
Screening of nine lipases and two esterases	Ten different ILs	Kinetic resolution of 1-phenylethanol by transesterification
Supported CALB, free CALB	[bmim][PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ]	Transesterification of ethyl butanoate with butan-1-ol
Immobilized CALB, native PCL	[emim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Transesterification of alcohol with vinyl acetate
$\alpha$ -chymotrypsin	[omim][PF <sub>6</sub> ] [bmim][PF <sub>6</sub> ]	Transesterification of <i>N</i> -acetyl-L-phenylalanine ethyl ester into propyl ester
Free PCL, immobilized PCL	[bmim][PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ]	Transesterification of 2-hydroxymethyl-1,4-benzodioxane using vinyl acetate
CALB	[bmim][PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ] [bmim][CF <sub>3</sub> SO <sub>3</sub> ] [bmim][SbF <sub>6</sub> ] [bmim][TFA] in the absence of added water	Kinetic resolution of 5-phenyl-1-penten-3-ol by transesterification
Novozyme 435	[bdmim][BF <sub>4</sub> ]/[PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ]/[PF <sub>6</sub> ]	Transesterification of 5-phenyl-1-penten-3-ol using vinyl acetate
Immobilized esterases from <i>Bacillus subtilis</i> , <i>Bacillus stearothermophilus</i>	[bmim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N] [bmim][PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ]	Transesterification of 1-phenylethanol catalyzed by esterases
Free CALB and $\alpha$ -chymotrypsin	[emim][BF <sub>4</sub> ]/[PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ]/[PF <sub>6</sub> ] [emim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N] [bmim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N] [mtoa][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N]	Enzymatic ester synthesis by transesterification
Commercial lipases	[emim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N] [bmim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N] [bmim][PF <sub>6</sub> ] [troma][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N]	Production of glycidyl esters by transesterification in ILs-scCO <sub>2</sub>
Lipases	various ILs	Physical properties of ILs about the activity and stability of lipase for transesterification
CALB	Ten different ILs	Esterification of glucose
CRL	[bmim][PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ] [onim][PF <sub>6</sub> ]	Esterification of 2-substituted-propanoic acids with 1-butanol
CRL	[bmim][PF <sub>6</sub> ] [mnim][PF <sub>6</sub> ] [bmim][BF <sub>4</sub> ] [hmim][BF <sub>4</sub> ]	Pervaporation for removal of water produced during enzymatic esterification
CRL	[bmim][PF <sub>6</sub> ] [moemim][PF <sub>6</sub> ]	Acylation of methyl 6- <i>O</i> -trityl- $\alpha$ -D-glucopyranoside and methyl 6- <i>O</i> -trityl- $\alpha$ -D-galactopyranoside
Immobilized CALB	ILs	Acylation of amines
Supported PCL	[bmim][PF <sub>6</sub> ]	Acylation of racemic alcohols using succinic anhydride

Table 1. Continued

Biocatalyst	Ionic Liquids	Reaction
Immobilized CALB	[bmim][PF <sub>6</sub> ] [emim][BF <sub>4</sub> ]	Acylation of amines with carboxylic acids
Lipase AK, PFL	[bmim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Acetylation of racemic <i>P</i> -chiral hydroxymethanephosphinates and hydroxymethylphosphine oxides
PEG-CALB, free CALB	[bmim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Lipase-catalyzed glucose fatty acid ester synthesis
Thermolysin, which dissolves in ILs	[bmim][PF <sub>6</sub> ] containing 5% (v/v) water	Synthesis of <i>Z</i> -aspartame
Free CALB	[emim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ] [emim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N] [bmim][(CF <sub>3</sub> SO <sub>2</sub> ) <sub>2</sub> N]	Synthesis of butyl butyrate
Free CALB	[bmim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Polycondensation of dicarboxylic acid esters with 1,4-butanediol
PCL, supported on celite	[bmim][PF <sub>6</sub> ]	Polyester synthesis of diethyl octane-1,8-dicarboxylate and 1,4-butanediol
Hydroxynitrile lyases from <i>Prunus amygdalus</i> and <i>Hevea brasiliensis</i>	[emim][BF <sub>4</sub> ]/buffer [pmim][BF <sub>4</sub> ]/buffer [bmim][BF <sub>4</sub> ]/buffer biphasic IL/water (1 : 1)	Synthesis of cyanohydrins
$\beta$ -galactosidase from <i>Bacillus cirulans</i>	[mmim][MeSO <sub>4</sub> ]	Synthesis of <i>N</i> -acetylactosamine
Whole cells of <i>Rhodococcus</i> R312	[bmim][PF <sub>6</sub> ]	Conversion of 1,3-dicyanobenzene to cyanobenzamide and 3-cyanobenzoic acid
CALB	[bmim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Alcoholysis, ammoniolysis, hydrolysis
PEG-lipase	ILs	Alcoholysis of 2-phenyl-1-propanol and vinyl acetate
Lipase	ILs	Hydrolysis of the racemic naproxen methyl ester
PCL, supported on celite	[bmim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Hydrolysis and alcoholysis of 3,4,6-tri- <i>O</i> -acetyl-D-glucal
CRL	[bmim][BF <sub>4</sub> ]/buffer [hmim][BF <sub>4</sub> ]/buffer [bmim][PF <sub>6</sub> ]/buffer	Hydrolysis of butyl 2-(4-chlorophenoxy)propionate
Epoxide hydrolase from cress and mouse	[bmim][PF <sub>6</sub> ] [bmim][Tf <sub>2</sub> N] [bmim][BF <sub>4</sub> ]	Hydrolysis of <i>trans</i> - $\beta$ -methylstyrene oxide
CALB	[bmim][BF <sub>4</sub> ] [bmim][PF <sub>6</sub> ]	Epoxidation of cyclohexene
Commercial protease	[EtPy][TFA]/ water (15 : 85)	Resolution of <i>N</i> -acetyl amino acid esters
Alcalase from <i>Bacillus licheniformis</i>	[emim][BF <sub>4</sub> ] [EtPy][BF <sub>4</sub> ]	Resolution of homophenylalanine ester
Glucose oxidase, peroxidase	[bmim][PF <sub>6</sub> ]	Sulfoxidation of thioanisole and methyl 2-naphthyl sulphide
Whole cells of immobilized baker' yeast	[bmim][PF <sub>6</sub> ]/ water (10 : 1)	Reduction of ketones
<i>Pseudomonas</i> sp. lipase, mandelate racemase from <i>Pseudomonas putida</i>	Ten different ILs	Deracemization of ( $\pm$ )-mandelic acid

activity. Enzymes are usually active in ionic liquids containing anions of  $\text{BF}_4$ ,  $\text{PF}_6$  and  $\text{Tf}_2\text{N}$ , but inactive in those containing anions such as  $\text{NO}_3$ ,  $\text{CH}_3\text{CO}_2$ ,  $\text{CF}_3\text{CO}_2$  and  $\text{CF}_3\text{SO}_3$ . It is unclear that the hydrophobicity and polarity of ILs have an influence on the enzyme activity. An ionic liquid with a higher viscosity may correspond to a lower reaction rate due to the mass-transfer limitation. Traces of silver ion or acidic impurities in ILs are the causes of slow or no reaction.

Currently, ionic liquids have been applied to various fields. In petrochemistry, ILs are also used in crude petroleum separation and separation of pollutants such as sulfur and phosphorus. In the pharmaceutical industry, they are used in medicine synthesis. In precision chemistry, they are used as solvents of various material synthesis, separation without factory waste water and catalysts with low price and low toxicity, while chemical catalysts have high price and toxicity. In France, they are used in the manufacture of plasticizer which softens brittle plastics, and the purification of industrial waste water by removing cadmium and mercury from water. At the University of Southern Alabama, ILs were reused by reversing the binding process after ILs bind with impurities such as carbon dioxide and hydrogen sulfide from natural gas that are the factors of lowering its fuel value and contributing to acid rain. Oxidation of organic compounds is one of the most fundamental reactions in chemistry. At the University of California, Los Angeles, Dr. Abu-Omar has created a system that uses an ionic liquid and hydrogen peroxide to create epoxides without generating waste. In electrochemistry, Korea succeeded in the production of ethylene carbonate, which is the main component in mobile phones, PDA's (personal digital assistant) and

a notebook batteries. ILs function as catalysts for the formation of ethylene carbonate using carbon dioxide and ethylene oxide. Through this process, global warming can be reduced.

In this article, an attempt has been made to present a detailed review of the versatility of ionic liquids as environmentally friendly green solvents for various biochemical transformations.

## ENZYME REACTION IN IONIC LIQUIDS

Unlike conventional organic solvents, the use of enzymes and substrates in ionic liquids has shown the advantages of increased activity, stability or selectivity of the enzyme and an extensive solubility of substrates and enzymes. Furthermore, enzyme immobilization has been developed to prevent deactivation and enhance enzyme activity. Biocatalytic reactions in ionic liquids include transesterification, synthesis, conversion, alcoholysis, ammoniolysis, hydrolysis, epoxidation, resolution, oxidation, reduction, deracemization and so on. The above-mentioned reactions, which are listed in Table 1 and Fig. 2, will be explained.

Kinetic resolution of 1-phenylethanol by transesterification with vinyl acetate [Schfer et al., 2001; Eckstein et al., 2002]; The best results were obtained for CALB in  $[\text{bmim}][\text{CF}_3\text{SO}_3]$  and  $[\text{bmim}][(\text{CF}_3\text{SO}_2)_2\text{N}]$ . The enzyme (CALB) was reused three times with less than 10% loss of activity per cycle while the enantioselectivity was not influenced. It is worth noting that virtually no reaction occurred in  $[\text{bmim}][\text{BF}_4]/[\text{PF}_6]$ . For the esterases, no activity was observed (scheme 1).

Transesterification of ethyl butanoate with butan-1-ol [Lau et al.,

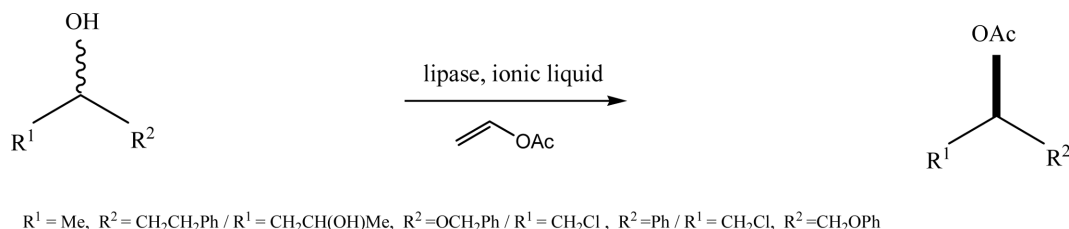
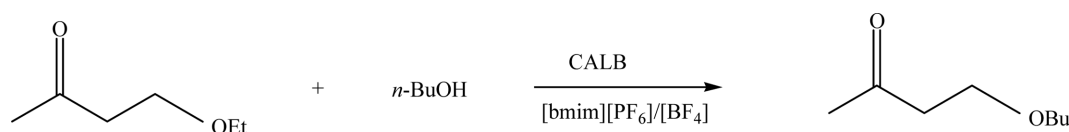
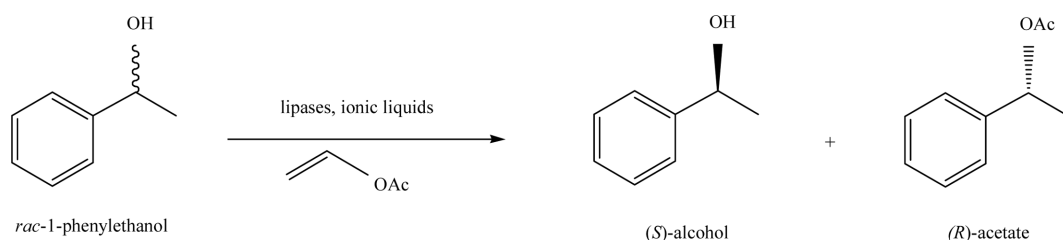


Fig. 2. Biocatalytic reactions in ionic liquids.

2000]; This reaction resulted in 81% yield after 4 h using a supported CALB and 78-79% yield after 24 h using free CALB in [bmim][PF<sub>6</sub>] and [bmim][BF<sub>4</sub>], respectively (scheme 2).

Transesterification of alcohol in the presence of vinyl acetate [Kim et al., 2001]; Lipases were up to 25 times more enantioselective in ionic liquids than in conventional organic solvents such as THF or toluene (scheme 3).

Transesterification of *N*-acetyl-L-phenylalanine ethyl ester into propyl ester [Laszlo et al., 2001; Lozano et al., 2001; Eckstein et al., 2002]; With an appropriate level of water added to the ionic liquid, the reaction rates are of the same order of magnitude for both ionic liquids and organic solvents such as acetonitrile or hexane. Despite the fact that enzyme activity in the ILs reached only 10 to 50% of the value in 1-propanol, the increased stability led to higher final product. In ILs, the enzyme is active at lower water activities compared with organic solvents (scheme 4).

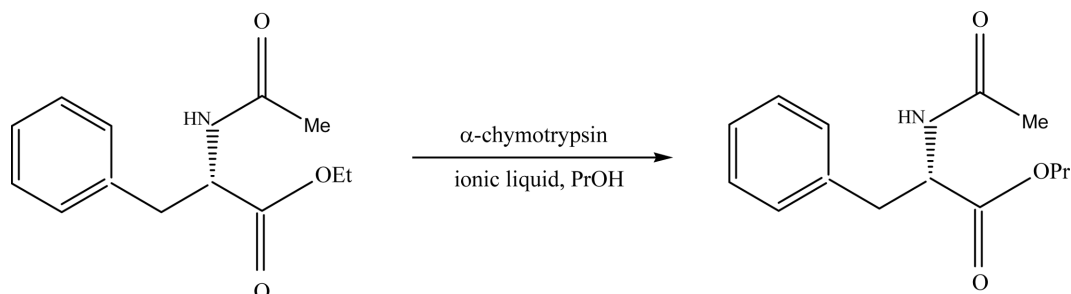
Transesterification of 2-hydroxymethyl-1,4-benzodioxane using vinyl acetate [Nara et al., 2002]; Transesterification of 2-hydroxymethyl-1,4-benzodioxane was catalyzed by free- or immobilized PCL in [bmim][PF<sub>6</sub>], [bmim][BF<sub>4</sub>], and organic solvents, respectively. As the amount of [bmim][PF<sub>6</sub>] in dichloromethane, which is miscible with [bmim][PF<sub>6</sub>], was progressively increased from 0 to 90% (v/v) in the reaction mixture, a slight increase in initial rate was observed. The supported PCL gave better results than the unsupported counterpart PCL in terms of initial rates of transesterification in all the reaction media. [Bmim][PF<sub>6</sub>] served as a relatively better medium for both immobilized PCL and free PCL-catalyzed esterification.

Lipases in ILs can be recycled for several runs without a substantial diminution in lipase activity (scheme 5).

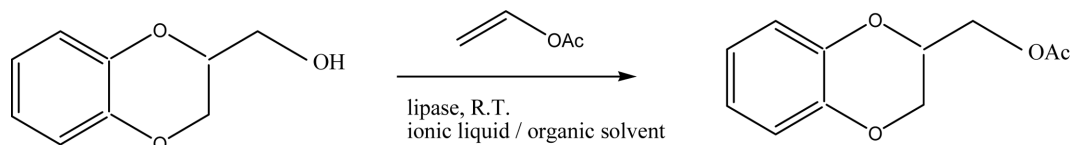
Kinetic resolution of 5-phenyl-1-penten-3-ol by transesterification [Itoh et al., 2001, 2002]; A high reaction rate and high enantioselectivity was reached in [bmim][PF<sub>6</sub>] and [bmim][BF<sub>4</sub>]. But the reaction rate dropped considerably. It was caused by the inhibitory action of an acetaldehyde oligomer, which was accumulated in the solvent system. This problem was solved while performing acylation with methyl esters catalyzed by CALB under reduced pressure at 40 °C. The acylated compounds were obtained in an optically pure form and the lipase was recycled for three consecutive runs without loss in the reactivity, enantioselectivity and reaction rate, but this system could not be applied for volatile substrates (scheme 6).

Transesterification of 5-phenyl-1-penten-3-ol using vinyl acetate as the acyl donor [Itoh et al., 2003]; [Bdmim][BF<sub>4</sub>] was the best solvent in this reaction. The accumulation of the acetaldehyde oligomer was not observed and a volatile substrate could also be used under normal pressure. The enzyme was repeatedly used 10 times, retaining perfect enantioselectivity and high activity. No reaction took place when [bdmim][PF<sub>6</sub>] was used as the solvent.

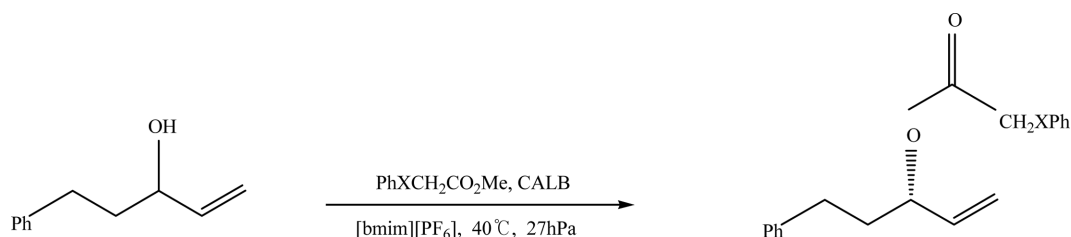
Transesterification of 1-phenylethanol catalyzed by esterases from *Bacillus subtilis* and *Bacillus stearothermophilus* [Persson and Bornscheuer, 2003]; [Bmim][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N], [bmim][PF<sub>6</sub>], and [bmim][BF<sub>4</sub>] were used as reaction media for this transesterification. By immobilizing the esterases onto celite, it was only possible to obtain activity in the ILs. Highest specific activity was obtained in *n*-hexane for both enzymes, while the specific activity was similar in MTBE,



Scheme 4.



Scheme 5.



Scheme 6.

Fig. 2. Continued.

vinyl acetate and ILs. The enantioselectivities ( $E \sim 7.0$  and  $3.0$  for *B. subtilis* and *B. stearothermophilus* esterase, respectively) were however independent of the solvent. Kinetic resolution was also performed with CALB and *Pseudomonas* sp. lipase. For these two enzymes, the enantioselectivity was also not affected by the solvent and high enantioselectivity ( $E > 100$ ) was obtained in all solvents investigated. The stability of the esterase from *B. stearothermophilus* at  $40^\circ\text{C}$  was considerably increased in [bmim][BF<sub>4</sub>] and [bmim][PF<sub>6</sub>] compared to in *n*-hexane and MTBE. A half-life of  $>240$  h was obtained in [bmim][PF<sub>6</sub>], which was  $>30$ - and  $>3$ -fold higher as compared to in *n*-hexane and MTBE, respectively.

Enzymatic ester synthesis by transesterification [Lozano et al., 2003]; Six different ionic liquids were used as reaction media for ester synthesis catalyzed by both free CALB and  $\alpha$ -chymotrypsin at 2% (v/v) water content and  $50^\circ\text{C}$ . All of the assayed ionic liquids proved adequate media for enzyme-catalyzed transesterification, and in the case of lipase, the synthetic activity was clearly enhanced compared to that obtained in organic solvents of similar polarity. In general, all the ionic liquids increased the thermal stability of both enzymes. For example, [emim][BF<sub>4</sub>] enhanced 5 times in the synthetic activity and 4 times half-life time, respectively, of lipase compared to 1-butanol.

Production of glycidyl esters by transesterification in ILs-supercritical carbon dioxide (sc CO<sub>2</sub>) [Lozano et al., 2004]; Free and im-

mobilized commercial CALA, CALB, and MML were assayed as catalysts in the synthesis of glycidyl esters from *rac*-glycidol in non-aqueous conditions. Four different ionic liquids such as [emim][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N], [bmim][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N], [bmim][PF<sub>6</sub>], and [troma][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N] with supercritical carbon dioxide ( $40$ – $50^\circ\text{C}$  and  $100$ – $150$  bar) were used as reaction media. All lipases were able to catalyze glycidyl ester synthesis. Enzyme activities were greatly enhanced (up to 95 times) by the use of ILs compared to a classical organic solvent (toluene) and the increase in the alkyl chain length of the acyl donor ester (alkyl vinyl ester). The activity and enantioselectivity exhibited by each lipase were practically independent of the ILs. *R*-glycidyl esters were preferentially obtained by both CALA and MML, while *S*-glycidyl ester synthesis was favored by CALB, which showed the highest degree of activity in all cases. Continuous processes for glycidyl butyrate synthesis in sc CO<sub>2</sub>, catalyzed by each lipase suspended in [emim][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N], also showed excellent results. Although sc CO<sub>2</sub>/IL biphasic systems slightly reduced the activities of lipases, the enantioselectivity remained unchanged (scheme 7).

Physical properties of ILs about the activity and stability of lipase for the transesterification of methylmethacrylate and 2-ethyl-1-hexanol and the polytransesterification of divinyl adipate and 1,4-butanediol [Karr et al., 2003]; Lipase activity and stability were investigated in dialkylimidazolium and pyrrolidinium-based ionic liquids with a variety of anions including hexafluorophosphate, acetate,

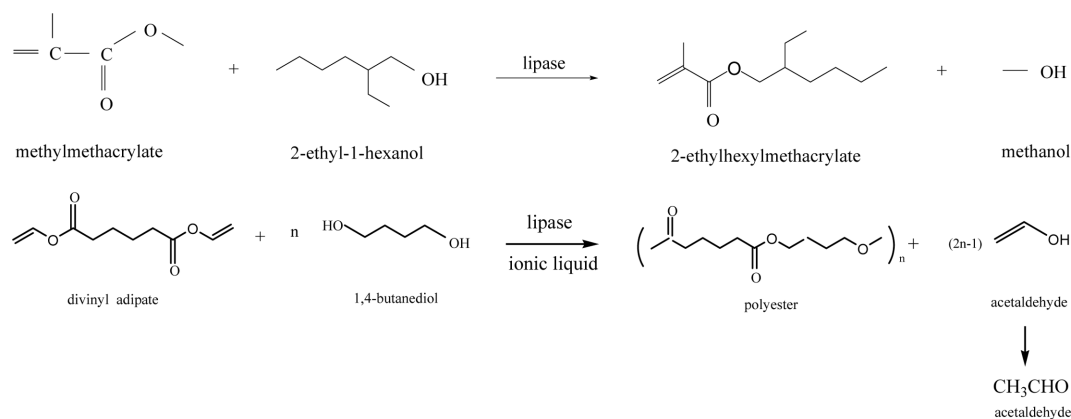
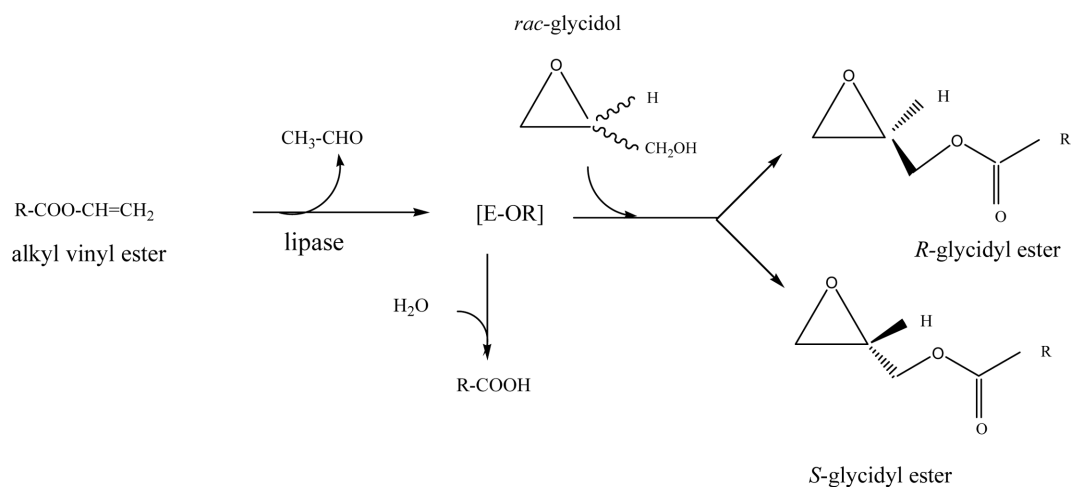


Fig. 2. Continued.

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nitrate, methanesulfonate, trifluoroacetate, and trifluoromethylsulfonate. The initial rates in these ionic liquids and several organic solvents were examined for the lipase-catalyzed transesterification of methyl methacrylate and the polytransesterification of divinyl adipate and 1,4-butanediol. Free lipase (CRL) catalyzed the transesterification of methyl methacrylate in [bmim][PF<sub>6</sub>] at a rate 1.5 times greater than in hexane. However, no detectable activity was observed in all the “hydrophilic” ionic liquids studied. Methods of enzyme stabilization including adsorption, PEG-modification, and immobilization in polyurethane foam were ineffective in improving enzymatic activity in the hydrophilic ionic liquids. Solvatochromic studies and partition coefficient measurements suggest that ionic liquids are more polar and hydrophilic than organic solvents such as hexane, acetonitrile, and tetrahydrofuran. Stability studies indicate that lipases exhibit greater stability in ionic liquids than in organic solvents including hexane (scheme 8).

Esterification of glucose [Park and Kazlauskas, 2001]; Esterification of glucose in [emim][BF<sub>4</sub>] was selective and no formation of the diester was observed. The reaction was much faster in [moemim][BF<sub>4</sub>] in which, at 55 °C, glucose was 100-fold more soluble than in acetone or THF (scheme 9).

Enantioselective esterification of 2-substituted-propanoic acids with 1-butanol [Ulbert et al., 2004]; The roles of solvent hydrophobicity (log P), water content and the effect of substituents were evaluated. First, water amount necessary in the reaction media for op-

timal hydration of CRL was determined in each solvent by adjusting different water concentrations (0.08-0.77 mol/dm<sup>3</sup> water concentrations according to solvent log P) in the reaction mixtures prior to starting the reactions. Optimal water concentration in the reaction media was determined, where the enzyme shows maximal activity and enantioselectivity. Enantioselectivity could be improved when chlorine substituent was replaced by slightly bigger size bromine. Contrary to reactions in common organic solvents, CRL in [bmim][PF<sub>6</sub>] and [onim][PF<sub>6</sub>] could be recycled five times without appreciable activity or enantioselectivity losses (scheme 10).

Pervaporation for removal of water produced during enzymatic esterification [Bélafi-Bakó et al., 2002]; The experimental results on CRL esterification at different initial water content with and without water removal by pervaporation in [bmim][PF<sub>6</sub>], [nmim][PF<sub>6</sub>], [bmim][BF<sub>4</sub>], and [hmim][BF<sub>4</sub>] are summarized. The best result was obtained at 0.5% constant water content with the pervaporation which removed water produced continuously in [bmim][PF<sub>6</sub>]. The values of enantioselectivity were found slightly smaller than that in *n*-pentane.

Acylation of methyl 6-*O*-trityl- $\alpha$ -D-glucopyranoside and methyl 6-*O*-trityl- $\alpha$ -D-galactopyranoside [Kim et al., 2003]; The reaction in ionic liquid was fast and gave a higher yield than that in THF and chloroform. The regioselectivity was significantly increased in ionic liquids. The 2-*O*-acylated products were exclusively obtained (scheme 11).

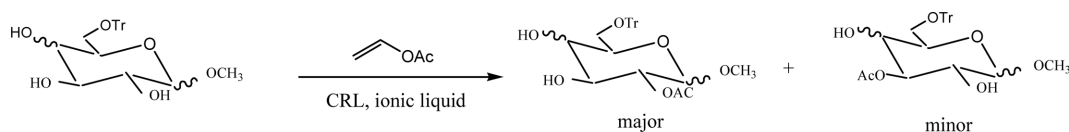
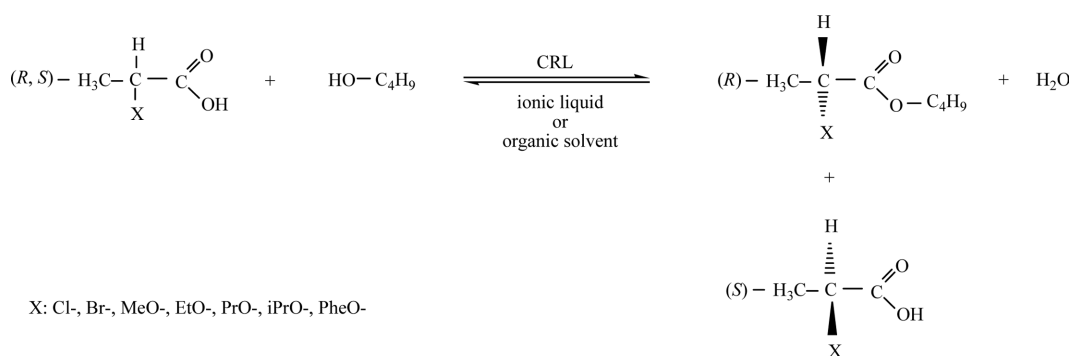
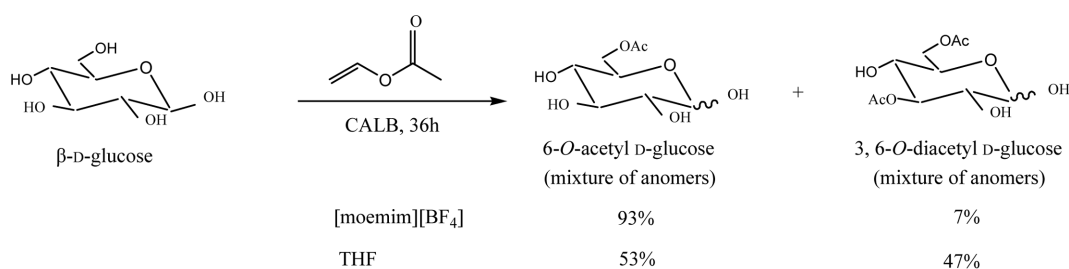


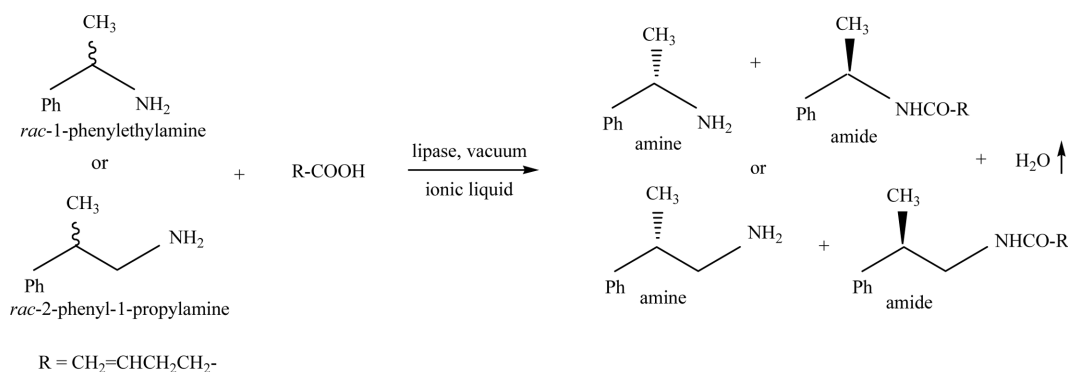
Fig. 2. Continued.

Enantioselective acylation of amines [Irimescu and Kato, 2004]; The use of ionic liquids as reaction media for lipase-catalyzed enantioselective acylation of 1-phenylethylamine and 2-phenyl-1-propylamine with 4-pentenoic acid was investigated. The best performing ionic liquids for each of these amines as well as its solvent properties were very different. Preparative scale kinetic resolution of 1-phenylethylamine was performed efficiently in [bdmim][CF<sub>3</sub>SO<sub>3</sub>] (scheme 12).

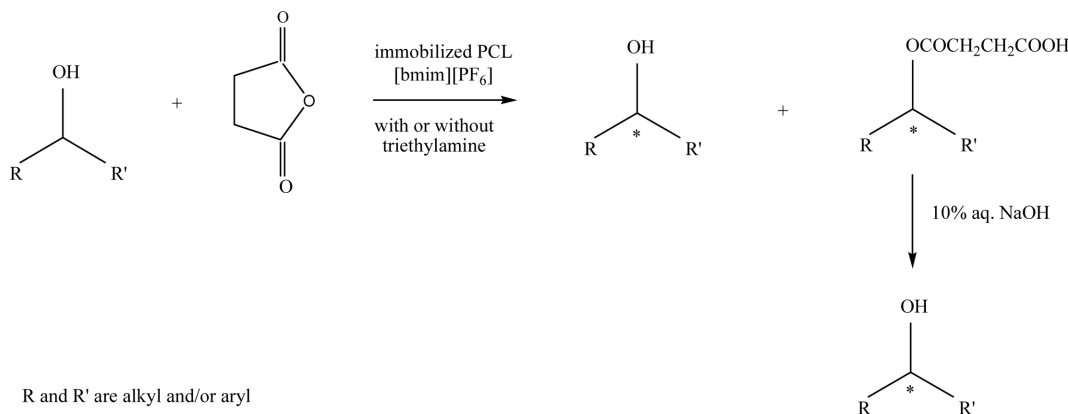
Acylation of racemic alcohols using succinic anhydride [Rasalkar et al., 2004]; Racemic secondary alcohols were resolved via enantioselective acylation using succinic anhydride as acyl donor

catalyzed by PCL supported on celite in [bmim][PF<sub>6</sub>]. Organic base, namely triethylamine as an additive in ionic liquid has been found to enhance the rate of the reaction (scheme 13).

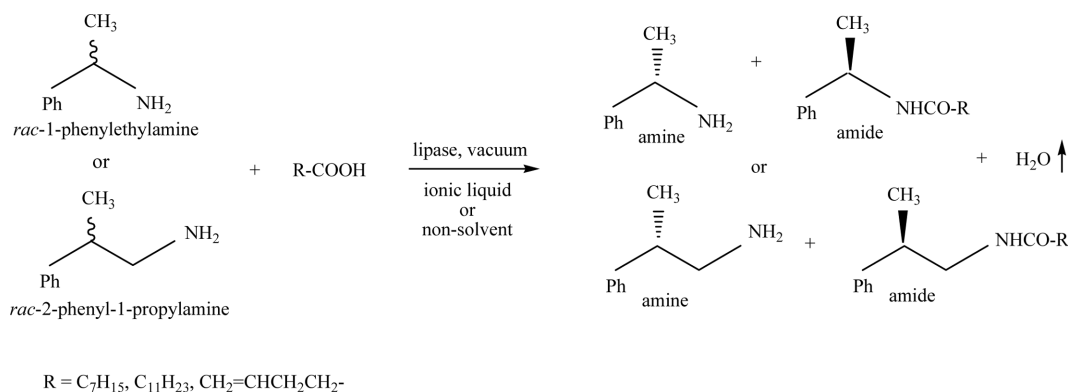
Acylation of amines with carboxylic acids [Irimescu and Kato, 2004]; The immobilized CALB-catalyzed enantioselective acylation of 1-phenylethylamine or 2-phenyl-1-propylamine was performed by reacting the amines with carboxylic acids such as octanoic acid, dodecanoic acid, and 4-pentenoic acid in a non-solvent or in [bmim][PF<sub>6</sub>] or [emim][BF<sub>4</sub>] as reaction media. The reaction equilibrium was shifted toward amide synthesis by the removal of by-product water under reduced pressure (scheme 14).



Scheme 12.



Scheme 13.



Scheme 14.



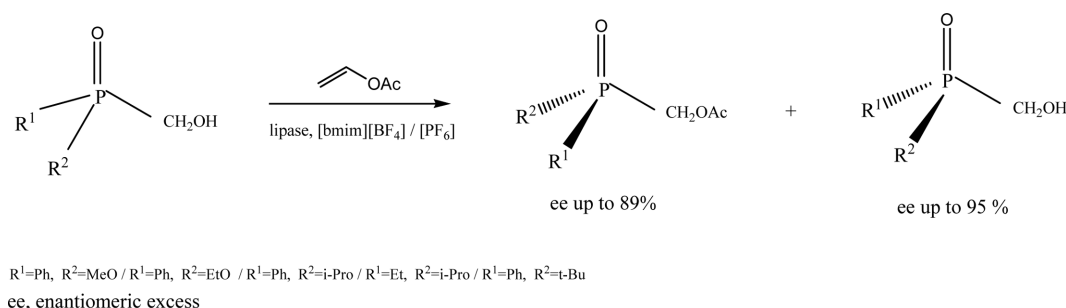
Acetylation of racemic *P*-chiral hydroxymethanephosphinates and hydroxymethylphosphine oxides [Kielbasinski et al., 2002]; The acetates were produced in up to 89% enantiomeric excess and the recovered alcohols in up to 95% enantiomeric excess using Amano lipase AK (*Pseudomonas fluorescens*) in [bmim][PF<sub>6</sub>]. Lipase AK (Amano) and lipase from *Pseudomonas fluorescens* (Fluka) were proven to be six times more enantioselective in [bmim][PF<sub>6</sub>] than in common organic solvents. On the contrary, the analogous reactions performed in [bmim][BF<sub>4</sub>] were practically non-stereoselective (scheme 15).

Lipase-catalyzed glucose fatty acid ester synthesis in ILs [Ganske and Bornscheuer, 2005]; Glucose fatty acid ester synthesis by using glucose, lauric- and myristic acid vinyl ester with PEG-modified CALB was performed in pure [bmim][BF<sub>4</sub>] (30% conversion) and in pure [bmim][PF<sub>6</sub>] (35% conversion). However, no sugar ester synthesis occurred when free fatty acids were used as substrate. Con-

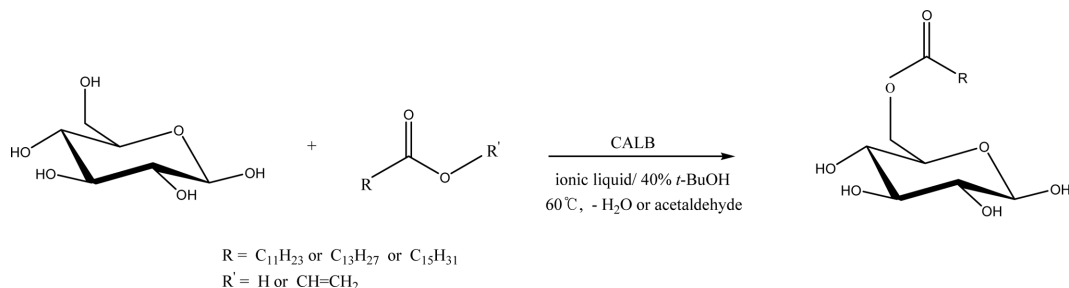
version of up to 90% and isolated yields of up to 89% in a solvent system composed of ionic liquid, [bmim][BF<sub>4</sub>] containing 40% *t*-butanol were achieved by using glucose, fatty acid vinyl esters, such as lauric- and myristic acid vinyl ester as acyl donors and commercial CALB. Moreover, free fatty acids also reacted (scheme 16).

Synthesis of *Z*-aspartame by the reaction of carbobenzoxy-L-aspartate and L-phenylalanine methyl ester hydrochloride [Erbel-dinger et al., 2000]; *Z*-aspartame is a precursor of aspartame, the artificial sweetener. The yield was 95% which is similar to that reported for enzymatic aspartame synthesis in organic solvents such as ethyl acetate with low water content (scheme 17).

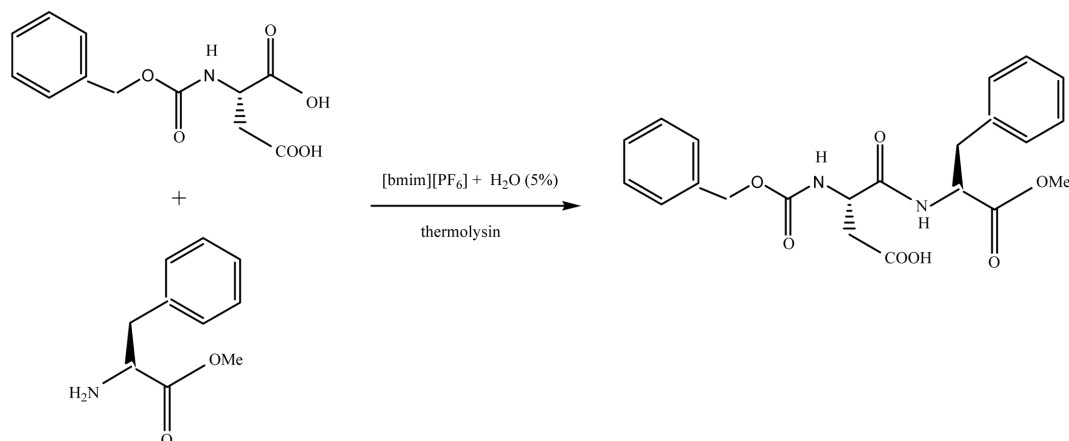
Synthesis of butyl butyrate [Lozano et al., 2001]; Free CALB at 2% (v/v) water content and 50 °C showed an enhanced activity in four different ionic liquids compared to inorganic solvents such as hexane and 1-butanol. The enhanced activity was related to the increase in polarity of ILs. The reuse of free lipase in [bmim][PF<sub>6</sub>]



Scheme 15.



Scheme 16.



Scheme 17.

Fig. 2. Continued.

through the continuous operation cycles showed a half-life time of 2,300 times greater than that observed when the enzyme was incubated in the absence of substrate (3.2 h), and a selectivity higher than 90%.

Polycondensation of dicarboxylic acid esters with 1,4-butanediol [Uyama et al., 2002]; This reaction generated oligomer ( $M_n=350$  Da) at ambient conditions. The oligomers of increased molecular weight ( $M_n=1,500$  Da) were formed at reduced pressure and increased polymerization times.

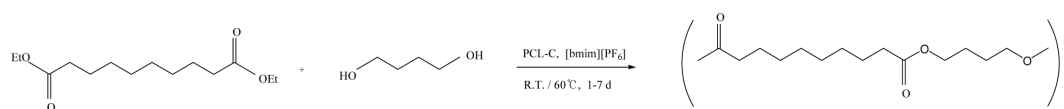
Polyester synthesis of diethyl octane-1,8-dicarboxylate and 1,4-butanediol [Nara et al., 2003]; The polymer formed in the [bmim][PF<sub>6</sub>] exhibited insolubility after it exceeded a certain molecular weight. The molecular weight of the polymer improved from 2150 at room temperature for 7 days to 5,400 at 60°C for 7 days as determined by GPC analysis (scheme 18).

Synthesis of cyanohydrins by hydrogen cyanide addition to aldehydes or ketones [Gaisberger et al., 2004]; Benzaldehyde, decanal, undecanal, and dodecanal were reacted with hydrogen cyanide in a

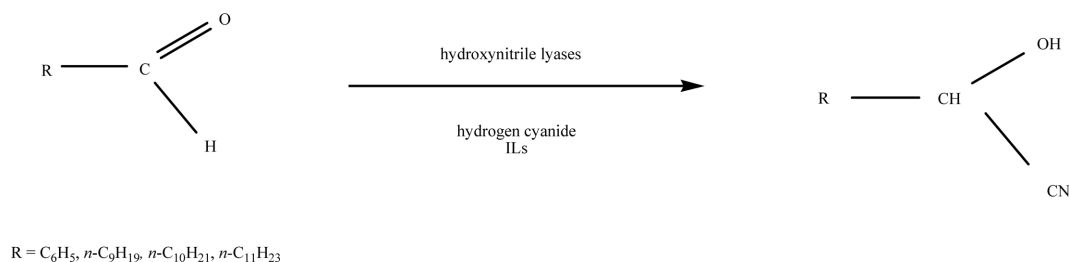
two-phase solvent system formed by aqueous buffer and ionic liquids such as [emim][BF<sub>4</sub>], [pmim][BF<sub>4</sub>], and [bmim][BF<sub>4</sub>] (1 : 1) in the presence of the hydroxynitrile lyases from *Prunus amygdalus* and *Hevea brasiliensis*. When compared to organic solvents as the nonaqueous phase, the reaction rate was significantly increased and the enantioselectivity remained good (scheme 19).

Synthesis of *N*-acetylglucosamine from lactose and *N*-acetylglucosamine [Kaftzik et al., 2002; Kragl et al., 2001]; The activity in pure [mmim][MeSO<sub>4</sub>] was low. In an aqueous system, the enzyme catalyzed the secondary hydrolysis of the product and its yield was less than 30%. The addition of 25% (v/v) of [mmim][MeSO<sub>4</sub>] as a water-miscible co-solvent suppressed the secondary hydrolysis of the formed product, resulting in doubling the yield to almost 60% (scheme 20).

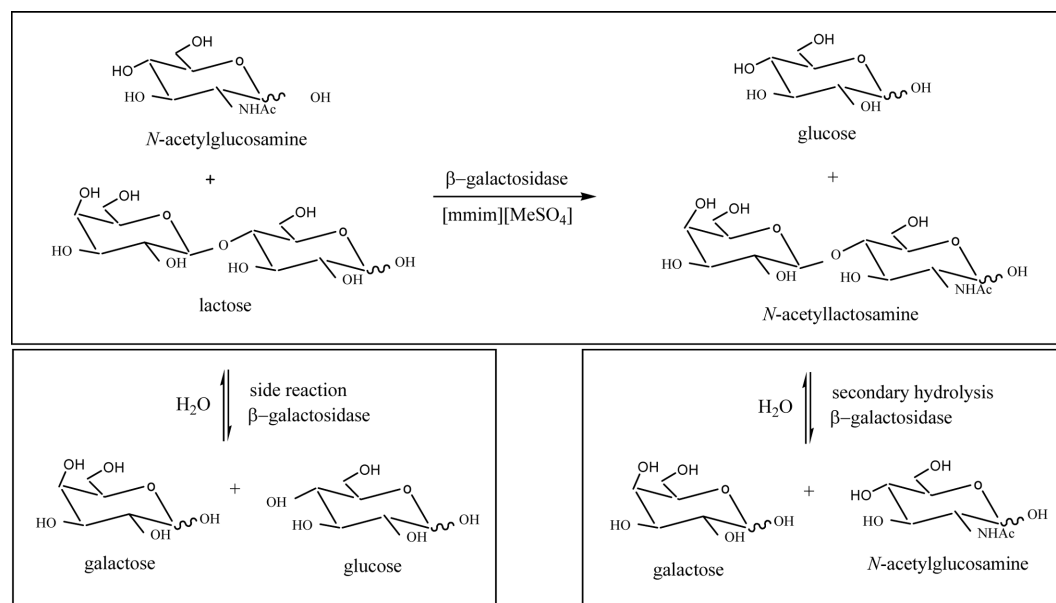
Conversion of 1,3-dicyanobenzene to 3-cyanobenzamide and 3-cyanobenzoic acid [Cull et al., 2000]; Conversion of 1,3-dicyanobenzene to 3-cyanobenzamide and 3-cyanobenzoic acid in both water-[bmim][PF<sub>6</sub>] and water-toluene systems showed similar profiles. The



Scheme 18.



Scheme 19



Scheme 20.

initial rate of 3-cyanobenzamide production in the water-[bmim][PF<sub>6</sub>] system was somewhat lower due to the reduced mass transfer rate of 1,3-dicyanobenzene in the more viscous [bmim][PF<sub>6</sub>] phase. The ionic liquid acts as a reservoir for the substrates. Nitrile hydratase from *Rhodococcus* R312 remains in the aqueous phase, where the reaction takes place.

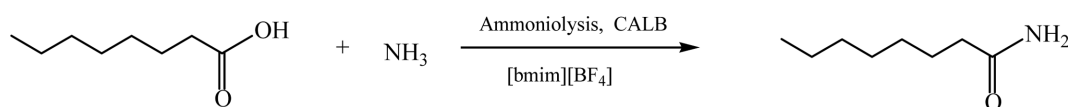
Alcoholysis, ammoniolysis and hydrolysis reactions [Lau et al., 2000]; Almost all cases for these reactions were comparable with, or better than, those observed in conventional organic reaction media (scheme 21).

Enantioselective alcoholysis between 2-phenyl-1-propanol and vinyl acetate [Maruyama et al., 2004]; Although native lipase powder exhibited very low activity in the ILs, a PEG-lipase complex was higher in the ILs than in common organic solvents such as *n*-

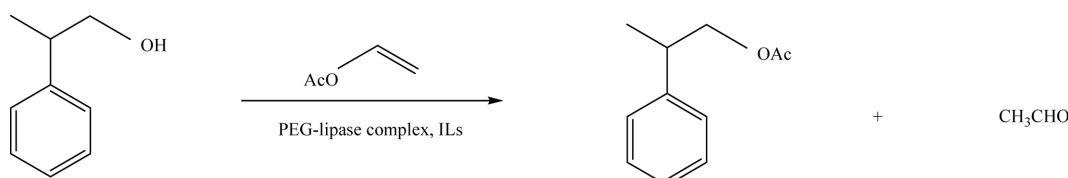
hexane, isooctane, and dimethylsulfoxide. A kinetic study of lipase-catalyzed alcoholysis in an [bmim][PF<sub>6</sub>] revealed that the Michaelis constant for 2-phenyl-1-propanol in the IL was half of that in *n*-hexane. Enantioselective alcoholysis of 1-phenylethanol in the ILs using the PEG-lipase complex gave rise to high enantioselectivity comparable to that in *n*-hexane (scheme 22).

Stereoselective hydrolysis of the racemic naproxen methyl ester [Zhao et al., 2004]; The enantioselectivity of the lipase was higher in an aqueous-ionic liquid biphasic system than that in an aqueous-organic biphasic system. The best activity and enantioselectivity were observed when the ratio of the aqueous phase to the ionic liquid phase was 1 : 1 (scheme 23).

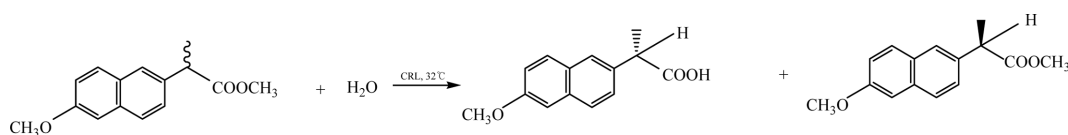
Regioselective hydrolysis and alcoholysis of 3,4,6-tri-*O*-acetyl- $\beta$ -D-glucal [Nara et al., 2004]; A marked regioselectivity toward the



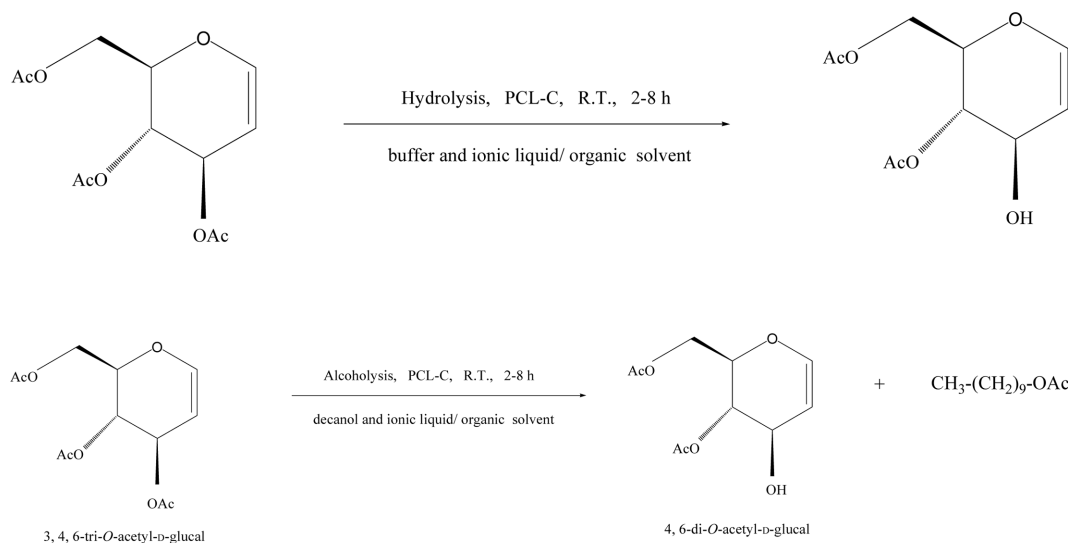
Scheme 21.



Scheme 22.



Scheme 23.



Scheme 24.

Fig. 2. Continued.



optimum organic solvent for this process.

Resolution of *N*-acetyl amino acid esters [Zhao and Malhotra, 2002]; 15% (v/v) [EtPy]<sup>+</sup>[CF<sub>3</sub>COO<sup>-</sup>] or acetonitrile in water was used with alcalase to resolve *N*-acetyl amino acid esters at 25 °C and for 24 h. The yield (29-39%) and enantiomeric excess (86-97%) obtained in the IL were comparable to the yield (15-35%) and enantiomeric excess (63-95%) obtained in acetonitrile. The results showed that with low concentration of this IL, the enzymatic resolution could be increased considerably depending upon the substrate used.

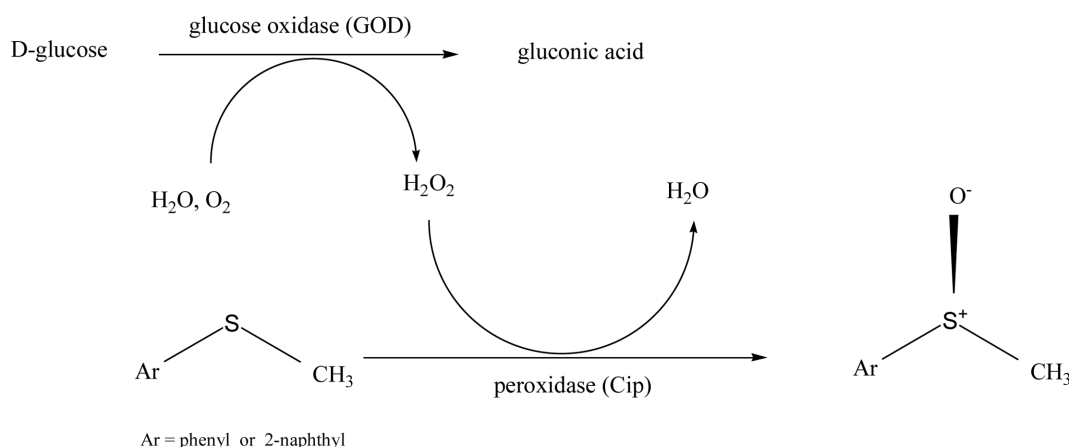
Resolution of homophenylalanine ester [Zhao et al., 2003]; [Emim][BF<sub>4</sub>] and [EtPy][BF<sub>4</sub>] were investigated as novel media for the enzymatic resolution of amino acid ester to obtain enantiomeric amino acid homophenylalanine. The use of mixed solvents such as water/ionic liquid led to lower enantioselectivity than water alone. It was assumed that high concentration of ionic liquid in water denatures the enzyme and thus decreases the enantioselectivity (scheme 27).

Sulfoxidation of thioanisole and methyl-2-naphthyl sulfide [Okrasa et al., 2003]; The rates of oxidation for both sulfides (thioanisole

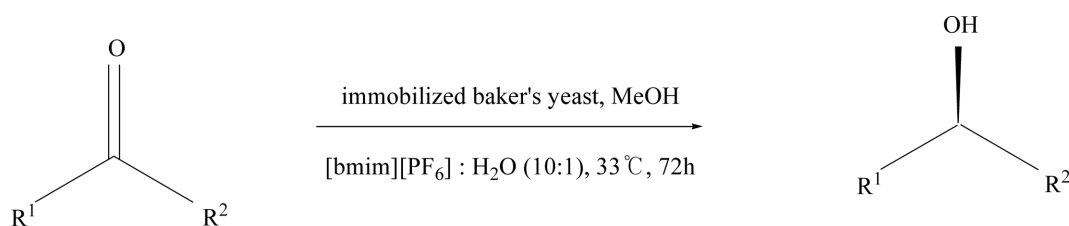
and methyl-2-naphthyl sulfide) were comparable. The glucose oxidase and peroxidase in [bmim][PF<sub>6</sub>] were re-used. Further isolation of the sulfoxides from the IL was easier than that from water. The oxidation of methyl-2-naphthyl sulfide showed a conversion of up to 36% and enantiomeric excess of up to 91% by glucose oxidase (GOD) from *Aspergillus niger* and peroxidase from *Coprinus cinereus* (Cip) with adding 10% water, for 32 hr, and at room temperature. The oxidation of thioanisoles in the IL by GOD/Cip was comparable to those in water. All substrates (glucose and sulfides) of enzymes and their products (gluconic acid and sulfoxides) are perfectly soluble in [bmim][PF<sub>6</sub>] (scheme 28).

Reduction of ketones [Howardh et al., 2004]; The results showed that the product yield varied over the range of substrates. Some gave poor yields while others gave good yields. In general, the enantioselectivity obtained in [bmim][PF<sub>6</sub>] was comparable to those obtained in other media (scheme 29).

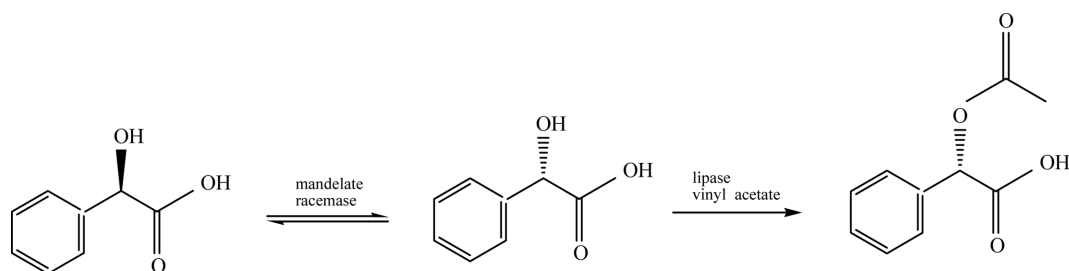
Deracemization of (±)-mandelic acid using a lipase-mandelate racemase two-enzyme system in ionic liquids [Kaftzik et al., 2004];



Scheme 28.



Scheme 29.



Scheme 30.

Fig. 2. Continued.

This deracemization was used to investigate the scopes and limitations of ILs for a dynamic resolution approach. *Pseudomonas* sp. lipase catalyzed *O*-acylation of ( $\pm$ )-mandelic acid and mandelate racemase from *Pseudomonas putida* (ATCC 12633) catalyzed racemization of the remaining unreacted (*R*)-mandelic acid. Mandelate racemase was observed to be active in [mmim][MeSO<sub>4</sub>] or [bmim][OctSO<sub>4</sub>] at water activity  $a_w > 0.74$ . Mandelate racemase activity could also be obtained in a biphasic system consisting of water and ionic liquids such as [mmim][MeSO<sub>4</sub>], [emim][CH<sub>3</sub>-C<sub>6</sub>H<sub>4</sub>-SO<sub>3</sub>], [bmim][BF<sub>4</sub>], [bmim][OctSO<sub>4</sub>], and [pmim][BF<sub>4</sub>] (scheme 30).

Comparison of peroxidase activities of hemin, cytochrome *c*, and microperoxidase-11 [Laszlo and Compton et al., 2002]. The ability of Fe(III) protoporphyrin(IX) chloride (hemim), microperoxidase-11 (MP-11), and cytochrome *c* (cyt-*c*) to oxidize 2-methoxyphenol (guaiacol) was examined in [bmim][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N], [bmim][PF<sub>6</sub>], and [omim][PF<sub>6</sub>]. All three biocatalysts displayed peroxidase activity when activated by an electron acceptor, *tert*-butyl hydroperoxide for hemin and hydrogen peroxide for MP-11 and cyt-*c*. Hemin required the addition of a coordinating base, pyridine or *N*-methylimidazole (NMI), to produce an active complex. Cyt-*c* did not require exogenous ligands for activity in ILs, although their addition increased peroxidase activity. MP-11 could not be solubilized without an exogenous ligand. Pyridine provided higher activities than NMI for these three catalysts. The activities of hemin and MP-11 peroxidase in ILs were markedly higher compared to those in organic solvents with similar polarity, as characterized by probe solvatochromic behavior, while cyt-*c* activity was comparable between both types of solvents. There was no consistent preference by the catalysts for a particular IL.

Solubilization of cellulose [Swatloski et al., 2002]. Solutions containing up to 25 wt% cellulose can be formed as viscous pastes in [bmim]Cl. Chloride-containing ILs appear to solubilize cellulose through hydrogen bonding from the hydroxyl functions to the anions of the solvent. Cellulose could be precipitated from the ionic liquid solution by the addition of water, or other precipitating solutions including ethanol and acetone.

Understanding structure-stability relationships of CALB in ionic liquids [de Diego et al., 2005]. The stabilization of CALB in the [btma][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N] and [emim][(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N] was associated with both the maintenance of the 50% of initial  $\alpha$ -helix content and the enhancement of  $\beta$ -strands. Fluorescence studies clearly showed how an enzyme unfolding was occurring with time in both water and hexane. Both ILs might be attributed to a compact and active enzyme conformation, resulting in an enhancement of the stability.

### PROTEIN FOLDING/REFOLDING USING IONIC LIQUIDS

Genetic engineering has produced proteins via prokaryotic expression systems. Although the expression in *Escherichia coli* and other hosts is a convenient method for the production of large amounts of protein, the absence of the proper refolding mechanism in the host may lead to nonnative conformations and the formation of inclusion bodies [Armstrong et al., 1999]. Due to the revolutionary advances in genetic expression processes, more general renaturation strategies are required. Generally, the renaturation of inactive protein begins with the isolation of the inclusion bodies followed by

dissolution of the proteins promoted with a chemical denaturant such as urea and guanidine hydrochloride. Dialysis or dilution of the denaturant then initiates refolding of the protein. Unfortunately, during this dilution process the protein may reform as inactive aggregates that further complicate isolation and purification. Therefore, it is essential to optimize the conditions that minimize the formation of inactive aggregates during refolding. The dissolution of aggregates or prevention of protein aggregation can be promoted by small molecule additives.

The denaturation of hen egg white lysozyme (HEWL) during its dissolution in [EtNH<sub>3</sub>][NO<sub>3</sub>] was observed by using fluorescence spectroscopy. The structural change that takes place when an enzyme dissolves in the IL is often reversible. Therefore, when a solution of the lysozyme in [EtNH<sub>3</sub>][NO<sub>3</sub>] was diluted with water, the denatured enzyme was found to regain 75% of its activity (5% [EtNH<sub>3</sub>][NO<sub>3</sub>]; 95% water) and resulted in over 90% recovery of active protein. An important aspect of this process is that renaturation of HEWL occurs at concentrations of 1.6 mg/mL, whereas other renaturation processes occur at significantly lower protein concentrations. The use of [EtNH<sub>3</sub>][NO<sub>3</sub>] as a refolding additive is advantageous because the renaturation in the IL is a one-step process [Summers and Flowers, 2000].

### TOXICITY OF IONIC LIQUIDS

From the viewpoint of environmental science, ILs are of interest because of their non-volatile character and thermal stability which make them potentially 'green solvents'. The general questions of 'greenness' and of sustainability of the use of ionic liquids as alternative reaction media are discussed in a companion paper presented at the 2002 DECHEMA conference, 'Green Solvents for Catalysis' [Jastorff et al., 2003]. Therefore, the overall aim of our studies is focused on screening their general toxicity by quick and inexpensive methods, and to obtain information on the influence of structural variations on their toxicity.

Specifically, biotransformation in a two-phase system is the promising application of RTILs in the field of biotechnology. Imidazolium-based ionic liquids can replace the conventional organic solvents in the extractive fermentation of lactate by investigating their extraction behaviors and solvent toxicity. It was reported that the extraction behaviors of lactic acid with imidazolium-based ionic liquids containing tri-*n*-butylphosphate extractant were similar to those of conventional organic solvents. Lactic acid producing bacterium, *Lactobacillus rhamnosus* (NBRC 3863) consumed glucose and produced lactate in the presence of imidazolium-based ionic liquids [Matsumoto et al., 2004]. The goal of such processes is to enhance the yield of bioconversion by continuously removing the water insoluble compounds from the aqueous reaction solution. Generally, microorganisms grow in aqueous media. Luminescent bacteria, IPC-81 (leukemia cells), and C<sub>6</sub> (glioma cells) rat cell lines are tested in methyl- and some ethylimidazolium ionic liquids. Effective concentrations in these test systems are generally some orders of magnitude lower than the toxicity of conventional solvents such as acetone, acetonitrile, methanol, and methyl *t*-butyl ether. No general influence of the anionic compound in the ionic liquids on toxicity could be found, although they seem to modulate toxicity in some cases. [Lee et al., 2005] The clear influence of the alkyl chain

length on toxicity was quantified by linear regression analysis [Ranke et al., 2004; Swatloski et al., 2004]. There are only a few results on the toxicity of RTILs, while many researches have been performed on that of organic solvents. The appropriate selection of the ionic liquid for bioprocesses requires general knowledge of the toxicity of RTILs, including whole cell fermentation and enzymatic reaction system [Matsumoto et al., 2004; Ranke et al., 2004; Lee et al., 2005].

## CONCLUSIONS

Aforementioned research results demonstrated the potential of ILs as solvents for biotransformations. There are many simple and complex ionic liquids, the concept of tailor-made solvent, which can be synthesized, but selecting the best system for a particular process is a real problem and challenge. The ionic liquids take part in dissolving reactants and stabilizing biocatalyst. A researcher can obtain a large quantity of products with highly stereo-, regio-, enantioselectivities in ionic liquids and observe better enzyme activity and stability or suppression of side reactions. The disadvantage, however, is the need to separate solvent and catalyst from the product. Product separation has been carried out by pervaporation, nanofiltration, and extraction with supercritical CO<sub>2</sub> [Blanchard and Brennecke, 2001]. Pervaporation has been reported for less volatile compounds, such as phenylethanol [Schäfer et al., 2001]. Nanofiltration has been used for the isolation of non-volatile compounds such as carbohydrate or charged compounds [J Kröckel, unpublished results]. The efficient re-use and purification of ionic liquids and the reduction in the cost of the ionic liquids are further important issues to be considered of their industrial applications in the future.

## NOMENCLATURE

### Abbreviations

[bmim] : 1-butyl-3-methylimidazolium  
 [PF<sub>6</sub>] : hexafluorophosphate  
 [(CF<sub>3</sub>SO<sub>2</sub>)<sub>2</sub>N]=[Tf<sub>2</sub>N] : bis(trifluoromethylsulfonyl) amide  
 CALA : *Candida antarctica* lipase type A  
 CALB : *Candida antarctica* lipase type B  
 [BF<sub>4</sub>] : tetrafluoroborate  
 [emim] : 1-ethyl-3-methylimidazolium  
 PCL : *Pseudomonas cepacia* lipase  
 THF : Tetra Hydro Fouran  
 [omim] : 1-octyl-3-methylimidazolium  
 ILs : ionic liquids  
 CRL : *Candida rugosa* lipase  
 [CF<sub>3</sub>SO<sub>3</sub>]=[TfO] : trifluoromethanesulfonate  
 [SbF<sub>6</sub>] : hexafluoroantimonate  
 [TFA] : trifluoroacetate  
 PFL : *Pseudomonas fluorescens* lipase  
 PEG : Poly(Ethylene Glycol)  
 GPC : Gel Permeation Chromatography  
 [mmim] : 1-methyl-3-methylimidazolium  
 [CH<sub>3</sub>SO<sub>4</sub>]=[MeSO<sub>4</sub>]=[MS] : methylsulfate  
 [EtPy] : *N*-ethyl pyridinium  
 [bdmim] : 1-butyl-2,3-dimethylimidazolium  
 [moemim] : 1-methoxyethyl-3-methyl imidazolium

[onim] : 1-octyl-3-nonyl-imidazolium  
 [pmim] : 1-phenylpropyl-3-methylimidazolium  
 [hmim] : 1-hexyl-3-methylimidazolium  
 [OctSO<sub>4</sub>] : octylsulfate  
 [troma] : trioctylmethylammonium  
 [mtoa] : methyltrioctylammonium  
 MTBE : *tert*-butyl-methylether  
 MML : *Mucor miehei* lipase  
 [EtNH<sub>3</sub>] : ethylammonium  
 [NO<sub>3</sub>] : nitrate  
 [btma] : butyltrimethylammonium  
 [mnim] : 1-methyl-3-nonylimidazolium

## REFERENCES

- Armstrong, N., Lencastre, A. D. and Gouaux, E., "A new protein folding screen: application to the ligand binding domains of a glutamate and kainate receptor and to lysozyme and carbonic anhydrase," *Protein Sci.*, **8**, 1475 (1999).
- Baudequin, C., Baudoux, J., Levillain, J., Cahard, D., Gaumont, A. C. and Plaquevent, J. C., "Ionic liquids and chirality: opportunities and challenges," *Tetrahedron: Asymmetry*, **14**, 3081 (2003).
- Blanchard, L. A. and Brennecke, J. F., "High-pressure phase behavior of ionic liquid/CO<sub>2</sub> system," *J. Phys. Chem. B*, **105**(12), 2437 (2001).
- Bélafi-Bakó, K., Dörmö, N., Ulbert, O. and Gubicza, L., "Application of pervaporation for removal of water produced during enzymatic esterification in ionic liquids," *Desalination*, **149**, 267 (2002).
- Chiappe, C., Leandri, E., Lucchesi, S. and Pieraccini, D., "Biocatalysis in ionic liquids: the stereoconvergent hydrolysis of *trans*- $\beta$ -methylstyrene oxide catalyzed by soluble epoxide hydrolase," *J. Mol. Catal. B: Enzym.*, **27**, 243 (2004).
- Cull, S. G., Holbrey, J. D., Vargas-Mora, V., Seddon, K. R. and Lye, G. J., "Room-temperature ionic liquids as replacements for organic solvents in multiphase bioprocess operations," *Biotechnol. Bioeng.*, **69**(2), 227 (2000).
- de Diego, T., Lozano, P., Vaultier, M. and Iborra, J. L., "Understanding structure-stability relationships of *Candida antarctica* lipase B in ionic liquids," *Biomacromolecules*, **6**, 1457 (2005).
- Eckstein, M., Sesing, M., Kragl, U. and Adlercreutz, P., "At low water activity  $\alpha$ -chymotrypsin is more active in an ionic liquid than in non-ionic organic solvents," *Biotechnol. Lett.*, **24**, 867 (2002).
- Eckstein, M., Wasserscheid, P. and Kragl, U., "Enhanced enantioselectivity of lipase from *Pseudomonas* sp. at high temperatures and fixed water activity in the ionic liquid 1-butyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl] amide," *Biotechnol. Lett.*, **24**, 763 (2002).
- Erbeldinger, M., Mesiano, A. J. and Russel, A. J., "Enzymatic catalysis of formation of *Z*-aspartame in ionic liquid- an alternative to enzymatic catalysis in organic solvents," *Biotechnol. Prog.*, **16**(6), 1129 (2000).
- Gaisberger, R. P., Fechter, M. H. and Griengl, H., "The first hydroxynitrile lyase catalyze cyanohydrin formation in ionic liquids," *Tetrahedron: Asymmetry*, **15**, 2959 (2004).
- Ganske, F. and Bornscheuer, U. T., "Lipase-catalyzed glucose fatty acid ester synthesis in ionic liquids," *Org. Lett.*, **0**(0), A-B (2005).
- Gordon, C. M., "New developments in catalysis using ionic liquids," *Appl. Catal.*, **222**, 101 (2001).
- Howardh, J., James, P. and Dai, J., "Immobilized Baker's yeast reduc-

- tion of ketones in an ionic liquid, [BMIM][PF<sub>6</sub>] and water mix," *Tetrahedron Lett.*, **42**, 7517 (2001).
- Irimescu, R. and Kato, K., "Investigation of ionic liquids as reaction media for enzymatic enantioselective acylation of amines," *J. Mol. Catal. B: Enzym.*, **30**, 189 (2004).
- Irimescu, R. and Kato, K., "Lipase-catalyzed enantioselective reaction of amines with carboxylic acids under reduced pressure in non-solvent system and in ionic liquids," *Tetrahedron Lett.*, **45**, 523 (2004).
- Itoh, T., Akasaki, E., Kudo, K. and Nishimura, Y., "Efficient lipase-catalyzed enantioselective acylation under reduced pressure conditions in an ionic liquid solvent system," *Chem. Lett.*, **31**(2), 154 (2002).
- Itoh, T., Akasaki, E., Kudo, K. and Shirakami, S., "Lipase-catalyzed enantioselective acylation in the ionic liquid solvent system: reaction of enzyme anchored to the solvent," *Chem. Lett.*, **30**(3), 262 (2001).
- Itoh, T., Nishimura, Y., Kashiwagi, M., Onaka, M., In Rogers, R. D. and Seddon, K. R., *Ionic Liquids as Green Solvents: Progress and Prospects*, ACS Symposium Series, American Chemical Society, Oxford University Press, Oxford, Chapter 21 (2003).
- Itoh, T., Nishimura, Y., Ouchi, N. and Hayase, S., "1-Butyl-2,3 dimethylimidazolium tetrafluoroborate: the most desirable ionic liquid solvent for recycling use of enzyme in lipase-catalyzed transesterification using vinyl acetate as acyl donor," *J. Mol. Catal. B: Enzym.*, **26**, 41 (2003).
- Jain, N., Kumar, A., Chauhan, S. and Chauhan, S. M. S., "Chemical and biochemical transformations in ionic liquids," *Tetrahedron*, **61**(5), 1015 (2004).
- Jastorff, B., Störmann, R., Ranke, J., Mölter, K., Stock, F., Oberheitmann, B., Hoffmann, W., Hoffmann, J., Nüchter, M., Ondruschka, B. and Filser, J., "How hazardous are ionic liquids? Structure-activity relationships and biological testing as important elements for sustainability evaluation," *Green Chem.*, **5**, 136 (2003).
- Kaar, J. L., Jesionowski, A. M., Berberich, J. A., Moilto, R. and Russell, A. L., "Impact of ionic liquid physical properties on lipase activity and stability," *J. Am. Chem. Soc.*, **125**(14), 4125 (2003).
- Kaftzik, N., Kroutil, W., Faber, K. and Kragl, U., "Mandelate racemase activity in ionic liquids: scopes and limitations," *J. Mol. Catal. A: Chem.*, **214**, 107 (2004).
- Kaftzik, N., Wasserscheid, P. and Kragl, U., "Use of ionic liquids to increase the yield and enzyme stability in the  $\beta$ -galactosidase catalyzed synthesis of *N*-acetylglucosamine," *Organ. Proc. Res. Dev.*, **6**(4), 553 (2002).
- Kielbasinski, P., Albrycht, M., Luczak, J. and Mikolajczyk, M., "Enzymatic reactions in ionic liquids: lipase-catalyzed kinetic resolution of racemic, *P*-chiral hydroxymethanephosphinates and hydroxymethylphosphine oxides," *Tetrahedron: Asymmetry*, **13**, 735 (2002).
- Kim, K. W., Song, B., Choi, M. Y. and Kim, M. J., "Biocatalysis in ionic liquids: markedly enhanced enantioselectivity of lipase," *Org. Lett.*, **3**(10), 1507 (2001).
- Kim, M. J., Choi, M. Y., Lee, J. K. and Ahn, Y. J., "Enzymatic selective acylation of glycosides in ionic liquids: significantly enhanced reactivity and regioselectivity," *J. Mol. Catal. B: Enzym.*, **26**, 115 (2003).
- Kragl, U., Eckstein, M. and Kaftzik, N., "Enzyme catalysis in ionic liquids," *Curr. Opin. Biotechnol.*, **13**, 565 (2002).
- Kragl, U., Kaftzik, N., Schöfer, S. H., Eckstein, M., Wasserscheid, P. and Hilgers, C., "Enzyme catalysis in the presence of ionic liquids," *Chem. Today*, **19**, 22 (2001).
- Laszlo, J. A. and Compton, D. L., " $\alpha$ -Chymotrypsin catalysis in imidazolium-based ionic liquids," *Biotechnol. Bioeng.*, **75**(2), 181 (2001).
- Laszlo, J. A. and Compton, D. L., "Comparison of peroxidase activities of hemin, cytochrome *C* and microperoxidase-11 in molecular solvents and imidazolium-based ionic liquids," *J. Mol. Catal. B: Enzym.*, **18**, 109 (2002).
- Lau, R. M., Rantwijk, F. V., Seddon, K. R. and Sheldon, R. A., "Lipase-catalyzed reactions in ionic liquids," *Org. Lett.*, **2**(26), 4189 (2000).
- Lee, S.-M., Chang, W.-J., Choi, A.-R. and Koo, Y.-M., "Influence of ionic liquids on the growth of *Escherichia coli*," *Korean J. Chem. Eng.*, **22**, 687 (2005).
- Lozano, P., de Diego, T., Carrié, D., Vaultier, M. and Iborra, J. L., "Enzymatic ester synthesis in ionic liquids," *J. Mol. Catal. B: Enzym.*, **21**, 9 (2003).
- Lozano, P., de Diego, T., Carrié, D., Vaultier, M. and Iborra, J. L., "Over-stabilization of *Candida antarctica* lipase B by ionic liquids in ester synthesis," *Biotechnol. Lett.*, **23**, 1529 (2001).
- Lozano, P., de Diego, T., Carrié, D., Vaultier, M. and Iborra, J. L., "Synthesis of glycidyl esters catalyzed by lipases in ionic liquids and supercritical carbon dioxide," *J. Mol. Catal. A: Chem.*, **214**, 113 (2004).
- Lozano, P., de Diego, T., Guegan, J. P., Vaultier, M. and Iborra, J. L., "Stabilization of  $\alpha$ -chymotrypsin by ionic liquids in transesterification reactions," *Biotechnol. Bioeng.*, **75**(5), 563 (2001).
- Marsh, K. N., Boxall, J. A. and Lichtenhaler, R., "Room temperature ionic liquids and their mixtures- a review," *Fluid Phase Equilib.*, **219**, 93 (2004).
- Marsh, K. N., Deev, A., Wu, A. C.-T., Tran, E. and Klamt, A., "Room temperature ionic liquids as replacements for conventional solvents - a review," *Korean J. Chem. Eng.*, **19**, 357 (2002).
- Maruyama, T., Yamamura, H., Kotani, T., Kamiya, N. and Goto, M., "Poly(ethylene glycol)-lipase complexes that are highly active and enantioselective in ionic liquids," *Org. Biomol. Chem.*, **2**(8), 1239 (2004).
- Matsumoto, M., Mochiduki, K., Fukunishi, K. and Kondo, K., "Extraction of organic acids using imidazolium-based ionic liquids and their toxicity to *Lactobacillus rhamnosus*," *Sep. Purif. Technol.*, **40**, 97 (2004).
- Mohile, S. S., Potdar, M. K., Harjani, J. R., Nara, S. J. and Salunkhe, M. M., "Ionic liquids: efficient additives for *Candida rugosa* lipase-catalyzed enantioselective hydrolysis of butyl 2-(4-chlorophenoxy) propionate," *J. Mol. Catal. B: Enzym.*, **30**, 185 (2004).
- Nara, S. J., Harjani, J. R. and Salunkhe, M. M., "Lipase-catalyzed transesterification in ionic liquids and organic solvents: a comprehensive study," *Tetrahedron Lett.*, **43**, 2979 (2002).
- Nara, S. J., Harjani, J. R., Salunkhe, M. M., Mane, A. T. and Wadgaonkar, P. P., "Lipase-catalyzed polyester synthesis in 1-butyl-3-methylimidazolium hexafluorophosphate ionic liquid," *Tetrahedron Lett.*, **44**, 1371 (2003).
- Nara, S. J., Mohile, S. S., Harjani, J. R., Jitendra R., Naik, P. U. and Salunkhe, M. M., "Influence of ionic liquids on the rates and regioselectivity of lipase-mediated biotransformations on 3,4,6-tri-*O*-acetyl-D-glucal," *J. Mol. Catal. B: Enzym.*, **28**, 39 (2004).
- Okrasa, K., Guibe-Jampel, E. and Therisod, M., "Ionic liquids as a new reaction medium for oxidase-peroxidase-catalyzed sulfoxidation," *Tetrahedron Asymmetry*, **14**, 2487 (2003).
- Olivier-Bourbigou, H. and Magna, L., "Ionic liquids: perspectives for organic and catalytic reactions," *J. Mol. Catal. A: Chem.*, **182-183**,



- 419 (2002).
- Panke, S. and Wubbolts, M. G., "Enzyme technology and bioprocess engineering," *Curr. Opin. Biotechnol.*, **13**, 111 (2002).
- Park, S. S. and Kazlauskas, R. J., "Biocatalysis in ionic liquids-advantages beyond green technology," *Curr. Opin. Biotechnol.*, **14**, 432 (2003).
- Park, S. S. and Kazlauskas, R. J., "Improved preparation and use of room temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations," *J. Org. Chem.*, **66**(25), 8395 (2001).
- Persson, M. and Bornscheuer, U. T., "Increased stability of an esterase from *Bacillus Stearothermophilus* in ionic liquids as compared to organic solvents," *J. Mol. Catal. B: Enzym.*, **22**, 21 (2003).
- Poole, C. F., "Chromatographic and spectroscopic methods for the determination of solvent properties of room temperature ionic liquids," *J. of Chromatogr. A*, **1037**, 49 (2004).
- Ranke, J., Molter, K., Stock, F., Bottin-Weber, U., Poczobutt, J., Hoffmann, J., Ondruschka, B., Filser, J. and Jastorff, B., "Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays," *Ecotoxicol. Environ. Saf.*, **58**, 396 (2004).
- Rantwijk, F. V., Lau, R. M. and Sheldon, R. A., "Biocatalytic transformations in ionic liquids," *Trends Biotechnol.*, **21**(3), 131 (2003).
- Rasalkar, M. S., Potdar, M. K. and Salunkhe, M. M., "*Pseudomonas cepacia* lipase-catalyzed resolution of racemic alcohols in ionic liquid using succinic anhydride: role of triethylamine in enhancement of catalytic activity," *J. Mol. Catal. B: Enzym.*, **27**, 267 (2004).
- Schfer, S. H., Kaftzik, N., Wasserscheid, P. and Kragl, U., "Enzyme catalysis in ionic liquids: lipase-catalyzed kinetic resolution of 1-phenylethanol with improved enantioselectivity," *Chem. Commun.*, 425 (2001).
- Schöfer, T., Rodrigues, C. M., Afonso, C. A. M. and Crespo, J. G., "Selective recovery of solutes from ionic liquids by pervaporation-a novel approach for purification and green processing," *Chem. Commun.*, **1**, 1622 (2001).
- Summers, C. A. and Flowers, R. A., "Protein renaturation by the liquid organic salt ethylammonium nitrate," *Protein Sci.*, **9**, 2001 (2000).
- Swatloski, R. P., Holbrey, J. D. and Rogers, R. D., "Using *Caenorhabditis elegans* to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids," *Chem. Commun.*, **6**, 668 (2004).
- Swatloski, R. P., Spear, S. K., Holbrey, J. D. and Rogers, R. D., "Dissolution of cellulose with ionic liquids," *J. Am. Chem. Soc.*, **124**, 4974 (2002).
- Ulbert, O., Fráter, T., Bélafi-Bakó, K. and Gubicza, L., "Enhanced enantioselectivity of *Candida rugosa* lipase in ionic liquids as compared to organic solvents," *J. Mol. Catal. B: Enzym.*, **31**, 39 (2004).
- Uyama, H., Takamoto, T. and Kobayashi, S., "Enzymatic synthesis of polyesters in ionic liquids," *Polym. J.*, **34**, 94 (2002).
- Welton, T., "Ionic liquids in catalysis," *Chem. Rev.*, **248**, 2459 (2004).
- Wilkes, J. S., "Properties of ionic liquid solvents for catalysis," *J. Mol. Catal. A: Chem.*, **214**, 11 (2004).
- Yang, Z. and Pan, W., "Ionic liquids: green solvents for nonaqueous biocatalysis," *Enzyme and Microbial Technology*, **37**, 19 (2005).
- Zhao, D., Wu, M., Kou, Y. and Min, E., "Ionic liquids: applications in catalysis," *Catal. Today*, **74**, 157 (2002).
- Zhao, H. and Malhotra, S. V., "Enzymatic resolution of amino acid esters using ionic liquid *N*-ethylpyridinium trifluoroacetate," *Biotechnol. Lett.*, **24**, 1257 (2002).
- Zhao, H., Luo, R. G. and Malhotra, S. V., "Kinetic study on the enzymatic resolution of homophenylalanine ester using ionic liquids," *Biotechnol. Progress*, **19**(3), 1016 (2003).
- Zhao, Y. J., Xin, J. Y., Li, Z., Xia, C. G. and Li, S. B., "The lipase-catalyzed stereoselective hydrolysis of the racemic naproxen methyl ester in an aqueous organic biphasic system and an aqueous ionic liquid biphasic system," *Fenxi Cuihua*, **18**(1), (2004).