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Effect of ionic liquid [BMIM][PF$_6$] on asymmetric reduction of ethyl 2-oxo-4-phenylbutyrate by *Saccharomyces cerevisiae*

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Abstract The effect of ionic liquid 1-butyl-3-methyl-imidazolium hexafluorophosphate ([BMIM][PF$_6$]) on the asymmetric reduction of ethyl 2-oxo-4-phenylbutyrate (EOPB) to synthesize optical active ethyl 2-hydroxy-4-phenylbutyrate (EHPB) catalyzed by *Saccharomyces cerevisiae* was investigated. (*R*)-EHPB [70.4%, e.e.(*R*)] is obtained using ethyl ether or benzene as the solvent. The main product is (*S*)-EHPB [27.7%, e.e.(*S*)] in [BMIM][PF$_6$]. However, in ionic liquid-water (10:1, v/v) biphasic system, the enantioselectivity of the reduction is shifted towards (*R*)-side, and e.e.(*R*) is increased from 6.6 to 82.5% with the addition of ethanol (1%, v/v). The effect of the use of [BMIM][PF$_6$] as an additive in relatively small amounts on the reduction was also studied. We find that there is a decline in the enantioselectivity of the reduction in benzene. In addition, a decrease in the conversion of EOPB and the yield of EHPB with increasing [BMIM][PF$_6$] concentrations occurs in either organic solvent–water biphasic systems or benzene.

Keywords Baker's yeast · Asymmetric reduction · Ionic liquid · Ethyl 2-oxo-4-phenylbutyrate · 1-Butyl-3-methylimidazolium hexafluorophosphate

Introduction

Ionic liquids had been explored as potential substitutes for traditional reaction medium in biochemical reactions to dissolve a wide range of different substances due to the high polarity [1]. Ionic liquids can be used to provide a nonaqueous, polar alternative for two- or multi-phase systems and enhance the activity, selectivity and stability of enzymes because they are immiscible with either water or organic solvents [2–5]. In some cases, the regioselectivity and enantioselectivity of enzymes are enhanced in ionic liquids compared with the same reaction in conventional organic solvents [6–9]. Some lipases were employed for the kinetic resolution of chiral alcohols in the ionic liquids, and an increase in enantioselectivity was observed [10, 11]. Howarth et al [12] combined the advantages of whole cell bioreagents with the advantages of ionic liquids for the first time. They attempted to reduce seven available ketones in [BMIM][PF$_6$] and water with an immobilized baker's yeast. Because the trends in enantioselectivity and yield of alcohol product are irregularly varied with substrates, it is difficult to evaluate [BMIM][PF$_6$] as a reaction medium instead of conventional organic solvents for the biocatalysis.

Recent researches indicate that ionic liquid is a promising new class of solvents and they can support an efficient whole-cell biocatalytic process [13]. (*R*)-EHPB is a versatile key intermediate for the synthesis of a variety of angiotensin converting enzymes (ACE) inhibitors [14]. In this experiment, baker's yeast asymmetric reduction of EOPB to (*R*)-EHPB was selected as a model reaction. The effect of hydrophobic [BMIM][PF$_6$] as a medium or an additive on a whole-cell biocatalytic process was investigated. As far as we are aware, it is the first example of a whole-cell asymmetric reduction of aromatic ketone ester in an ionic liquid, [BMIM][PF$_6$], and it clearly expands the potential and possibilities for ionic liquids as alternative solvents to support a broad range of whole-cell biotransformations.

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Materials and methods

Enzymes and chemicals

Saccharomyces cerevisiae (Taohua Brand Dry Yeast) from Meishan Mauri Yeast Co. (China). EOPB from Weixing Chemicals Co. (China). (*R*)-EHPB from Aldrich chemical Co. Tetradecane from Sigma (USA). [BMIM][PF$_6$] was prepared following the reported procedures [15]. Ionic liquid was dried in vacuum at 80 °C for 24 h before use. All other chemicals are of analytical grade. All organic solvents were used after drying according to the conventional methods.

Asymmetric reduction by baker's yeast

A typical reduction of EOPB was performed in a 100 mL reactor fitted with a magnetic stirrer and a reflux condenser at 30 °C, 300 rpm for 24 h with 40 mL ionic liquid. 2.0 g dry baker's yeast was suspended in 40 mL [BMIM][PF$_6$] containing 0.050 g EOPB and 4 mL water. As [BMIM][PF$_6$] is immiscible in water, therefore a two-phase system ([BMIM][PF$_6$]-water, 10:1, v/v) formed. The yeast was removed by filtration after the reaction. The desired product was extracted with ethyl ether (10 mL × 3). The organic portion was dried over Na$_2$SO$_4$ for 3 h, followed by concentration in vacuum to give a yellow viscous residue. [BMIM][PF$_6$] was dried in vacuum oven at 80 °C and recycled after use in the reactions.

A reduction of EOPB in organic solvent–water biphasic systems was performed in a 60 mL reactor fitted with a magnetic stirrer and a reflux condenser at 30 °C, 300 rpm. 2 g dry baker's yeast was suspended in 10 mL water. 20 mL organic solvent containing 0.083 g EOPB was added. The organic portion was separated centrifugally from the yeast and dried over Na$_2$SO$_4$ for 3 h, followed by concentration under vacuum to give a yellow viscous residue.

Analytical methods

The conversion of EOPB, the yield of EHPB and enantiomeric excess (e.e.) of (*R*)-EHPB were determined by GC equipped with a chiral GC column (25 m × 0.32 mm × 0.25 μm, CP-Chirasil-DEX CB). The GC conditions are as follows: split ratio 1:100, N$_2$ carrier gas at 30 mL min^{-1}, the column temperature, the injection temperature and the detector temperature were 160, 250 and 250 °C, respectively. The e.e.(*R*) was calculated as follows:

$$\text{e.e.}(R) = \frac{[R] - [S]}{[R] + [S]} \times 100\%$$

where [*R*] and [*S*] is the concentration of (*R*)-EHPB and (*S*)-EHPB, respectively.

Results and discussion

### Asymmetric reduction of EOPB in organic solvents

In our previous research, we found that (*R*)-EHPB was preferentially decomposed to form a byproduct—3-phenylpropanol (PPN), under the catalysis of hydrolytic enzymes within baker's yeast in the aqueous medium, while (*S*)-EHPB remained almost unaffected. As a result, The produced (*S*)-enantiomer was obtained as the major product when the reduction was carried out in the aqueous medium (80%, e.e.(*S*)) [16]. This decomposition pathway has been elucidated by us (Scheme 1) [17]. The enantioselectivity of the bioreduction could be changed by using organic solvents [16–21]. In addition, the amount of water in the reaction system is the key determinant of the enzyme properties (e.g. activity, stability, and specificity) in nonaqueous solvents. Asymmetric reduction of EOPB with baker's yeast was studied in organic solvents and ionic liquids, respectively. Figure 1a shows the enantioselectivity of the reduction in organic solvents shifts to (*R*)-side with the increase of the initial water content of the reaction mixture (≤3%, v/v). The enantioselective decomposition of (*R*)-enantiomer occurred frequently in the aqueous medium is restrained in water-immiscible organic solvents, which makes (*R*)-enantiomer accumulated [16, 20].

Asymmetric reduction of EOPB in ionic liquid

The enzyme activity, stability and enantioselectivity are strongly affected by ionic liquids especially when there is no or little water presence in ionic liquids media [22–24]. The enantioselectivity of the reduction in [BMIM][PF$_6$] shifts to (*S*)-side (Fig. 1a) and the yield of (*S*)-product is gradually improved with the increase of the initial water content, whereas enantioselectivity of the reduction in [BMIM][PF$_6$]-water (10:1, v/v) biphasic system is reversed and shifts towards (*R*)-side [6.6%, e.e.(*R*)]. Furthermore, the conversion of EOPB and yield of EHPB increases to 77.5 and 40.1% when the initial water content in [BMIM][PF$_6$] is up to 3% (v/v) (Fig. 1b, c). The conversion of EOPB (87.4%) and the yield of EHPB (42.3%) is higher in [BMIM][PF$_6$]-water (10:1, v/v) biphasic system than that in [BMIM][PF$_6$] monophase system.

Biocatalyst could not efficiently interact with the substrate because of the relatively high viscosity of ionic liquid. To overcome this problem, a small amount of organic solvents (methanol, ethanol, isopropanol, etc.) is added into the reaction. In addition, short-chain alcohols were used as co-substrates for cell maintenance and coenzyme regeneration. The yield and enantiomeric excess

Scheme 1 Schematic diagram of forming EHPB and byproducts in the microbial reduction of EOPB by baker's yeast
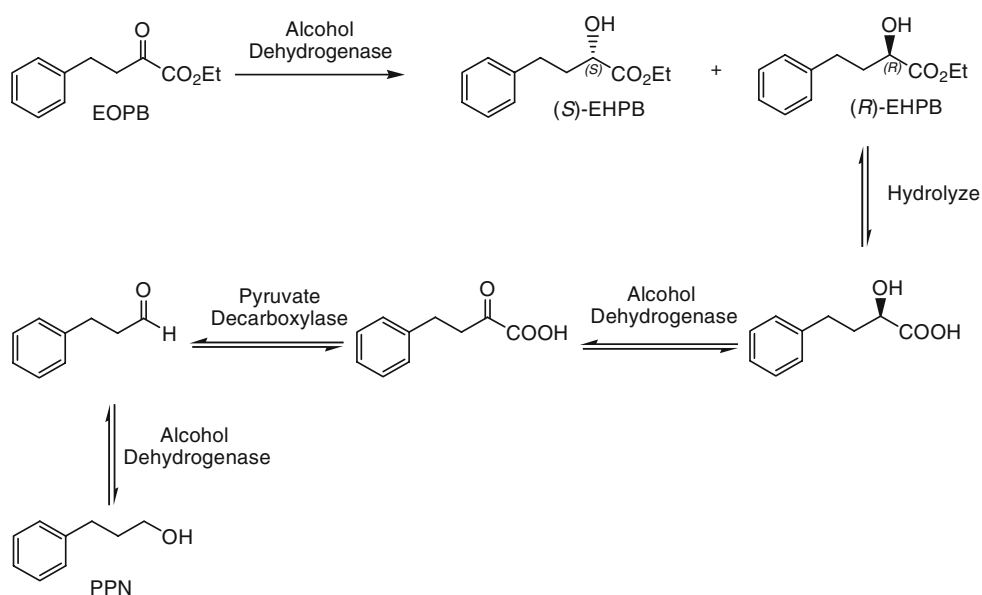
of product could be improved by adding them in small amounts to the reaction mixture [25]. Some short-chain alcohols for this biotransformation have been tested in benzene–water (2:1, v/v) biphasic system, and ethanol (3%, v/v) is more suitable for the reduction than others (Fig. 2). In [BMIM][PF$_6$]-water (10:1, v/v) biphasic system, addition of methanol (1.5%, v/v) resulted in a decline in both the conversion of EOPB and yield of EHPB in spite of the relatively high enantioselectivity of the reduction (75.9%, e.e.(R)). However, ethanol could give better results than other alcohols used. As the initial concentration of ethanol is 1% (v/v), the conversion of EOPB, yield of EHPB and e.e.(R) are increased to 89.6, 45.8 and 82.5%, respectively (Fig. 3). Ethanol is oxidized to form aldehyde catalyzed by yeast alcohol dehydrogenase. Meanwhile, NAD(P)+ is reduced to NAD(P)H which is supplied to oxidoreductases from yeast cell as cofactor.

To further verify the effect of [BMIM][PF$_6$], we tested its ability to affect the enantioselectivity of yeast reduction of EOPB in solvent–water (1:10, v/v) biphasic systems. The results are summarized in Table 1. The (R)-product [68.2%, e.e.(R)] is obtained in benzene–water (1:10, v/v) biphasic system. However, the enantioselectivity of the reduction is reversed and shifted to (S)-side by using toluene as the solvent phase in place of benzene. Although both reductions in [BMIM][PF$_6$]-water biphasic system and in benzene–water biphasic system afford the same isomer [(R)-products], the enantioselectivity of the reduction decreases from 68.2 to 7.0% by changing the nonaqueous phase from benzene to [BMIM][PF$_6$] in biphasic systems. Benzene might affect conformations of yeast alcohol dehydrogenases both (R)- and (S)-specific to alter the catalytic activity and

enantioselectivity of the enzymes in yeast cells [17]. The yield of EHPB was only 7.2% in [BMIM][PF$_6$]-water (1:10, v/v) biphasic system, which might be attributed to the side reaction, hydrolytic reaction, catalyzed by enzymes in yeast cells. This asymmetric decomposition is accelerated in the presence of a relatively large amount of water.

Influence of ionic liquid as an additive on asymmetric reduction of EOPB

One of the most special properties for ionic liquids is their high polarity with an extremely low log P, for example, [BMIM][PF$_6$] (log $P = -2.9$). An obvious trend towards higher reaction rates in more polar ionic liquids was observed, which is to the opposite of the trend shown when the reaction was conducted in traditional organic solvents [26]. Therefore, the general characteristics of reaction (the selectivity or reaction rate) might be modified by the addition of a small amount of ionic liquids. To gain information on how the [BMIM][PF$_6$] affects the reduction as a reaction additive (≤0.1%, v/v), the conversion of EOPB, yield of EHPB and the enantioselectivity was studied in the presence of various amounts of [BMIM][PF$_6$], respectively. The results are shown in Fig. 4. Asymmetric reduction of EOPB by baker's yeast is carried out in organic solvent–water biphasic systems with relatively high e.e.(R). The effect of [BMIM][PF$_6$] as an additive on the enantioselectivity of the reduction is not obvious in the experimental range. The use of 0.04% (v/v) [BMIM][PF$_6$] resulted in a slight increase (maximally 3%) in e.e.(R). However, The conversion of EOPB and yield of EHPB
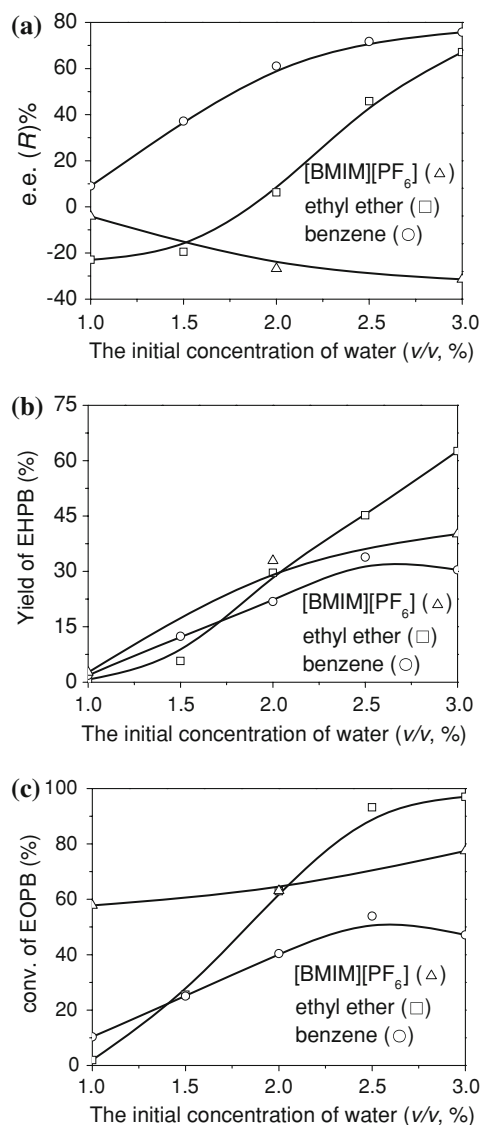
Fig. 1 Effect of the initial concentration of water on the reaction. In ethyl ether or benzene, 2.5 g baker's yeast, 50 mL organic solvent, 0.052 g EOPB, 30 °C, 300 rpm, 24 h. In ionic liquids, 2.0 g baker's yeast, 40 mL [BMIM][PF$_6$], 0.050 g EOPB, 30 °C, 300 rpm, 24 h

Fig. 2 Effect of alcohols on the reaction in benzene–water (2:1, v/v) biphasic system. 8 g baker's yeast, 0.165 g EOPB, 1.28 g α-PC, 20 mL phosphate buffer solution (0.2 M, pH 8.0), 40 mL benzene, 30 °C, 300 rpm, 15 h. The concentration of alcohols is 3% (v/v)

Fig. 3 Effect of alcohols on the reaction in [BMIM][PF$_6$]-water (10:1, v/v) biphasic system 3.0 g baker's yeast, 0.165 g EOPB, 3.0 mL water, 30 mL [BMIM][PF$_6$], 30 °C, 300 rpm, 24 h

decline with increasing in the initial concentration of [BMIM][PF$_6$], and a similar tendency in both the conversion of EOPB and yield of EHPB is observed in benzene (Fig. 6). With the presence of [BMIM][PF$_6$] (0.1%, v/v), the conversion of EOPB and yield of EHPB decrease by 5.7 and 3.0% in benzene–water biphasic system, and more notable decrease can be observed in toluene-water biphasic system (13 and 6.6%).

Increasing ionic strength of buffer solution could decrease the enantioselectivity of the reaction and the catalytic activity of yeast in benzene–water biphasic system (Fig. 5). Ionic liquid partitions in the organic/water mixture as an ion pair could occur, therefore the
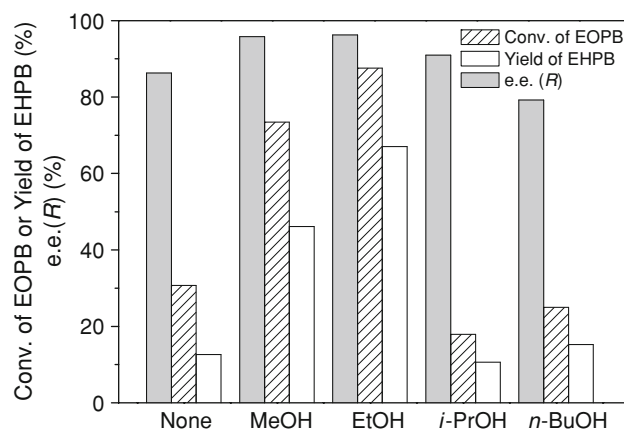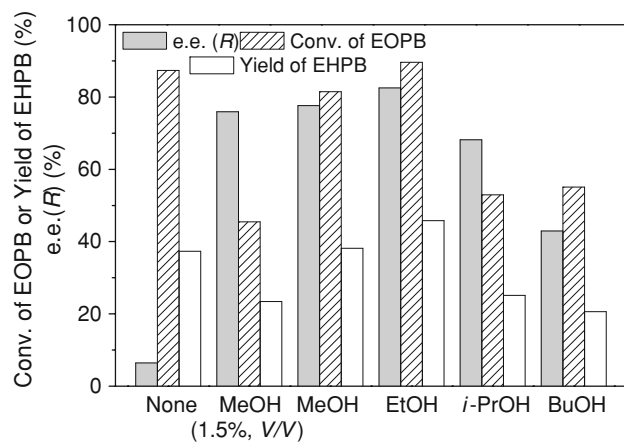
reduction might be affected by ion pairing in both phases. The ionic strength of reaction system could be increased with the addition of ionic liquids, which might lead to the decrease of the conversion of EOPB and yield of EHPB. Toluene could alter the yeast cell membrane permeability to severely damage the cell and brings further handicaps to the biotransformation [27]. The yeast cells immersed in toluene–water biphasic system was more sensitive than those in benzene–water biphasic system by changing the reaction conditions. Therefore, a more remarkable decrease of the conversion of EOPB and yield of EHPB in toluene–water biphasic system could be observed.

Experiments with different initial concentration of [BMIM][PF$_6$] from 0.01 to 0.08% (v/v) were arranged to

Table 1 Reduction of EOPB by baker's yeast in solvent–water (1:10, v/v) biphasic system(s)

Biphasic system	e.e. (%)	Yield of EHPB (%)	Conv. of EOPB (%)	Configuration
Benzene/water	68.2	23.2	60.0	R
Toluene/water	0.2	23.9	74.8	S
[BMIM][PF$_6$]/water	7.0	7.2	63.6	R

2.0 g baker's yeast, 0.124 g EOPB, 10 mL water, 20 mL solvent, 30 °C, 300 rpm, 5 h
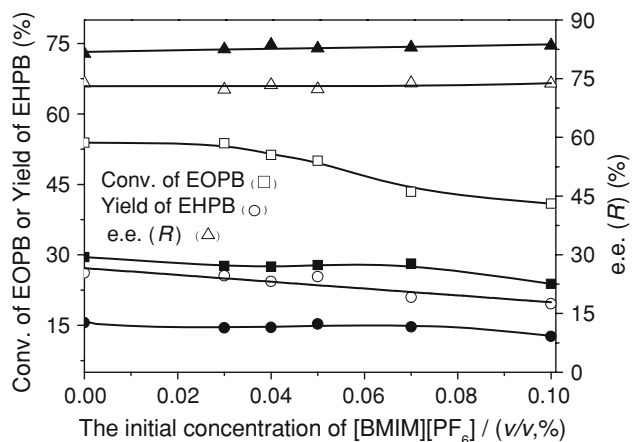
Fig. 4 Effect of [BMIM][PF$_6$] as an additive on the reaction in organic solvent–water (2:1, v/v) biphasic systems. 2.0 g baker's yeast, 0.124 g EOPB, 10 mL water, 20 mL benzene or toluene, 30 °C, 300 rpm, 5 h. Solid point-benzene, hollow point-toluene
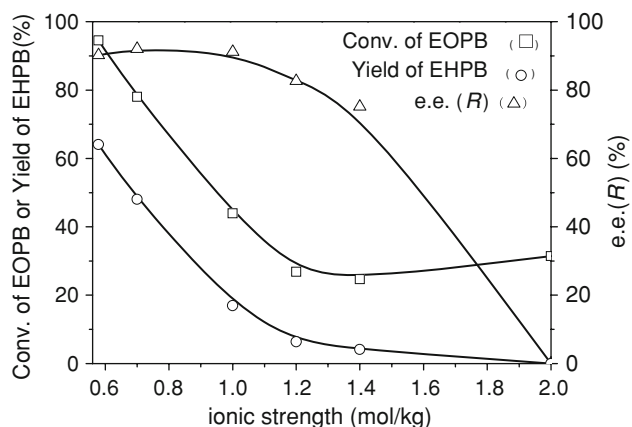
Fig. 5 Effect of ionic strength on the reaction in benzene–water (2:1, v/v) biphasic system. 8 g baker's yeast, 0.165 g EOPB, 20 mL Tris–HCl buffer solution (0.2 M, pH 8.0), 40 mL benzene, 30 °C, 300 rpm, 15 h. NaCl was used to adjust ionic strength

study the effect of [BMIM][PF$_6$] on the reduction in benzene (Fig. 6). The e.e.(R) decreased by more than 28% when the initial concentration of [BMIM][PF$_6$] is up to 0.08% (v/v).
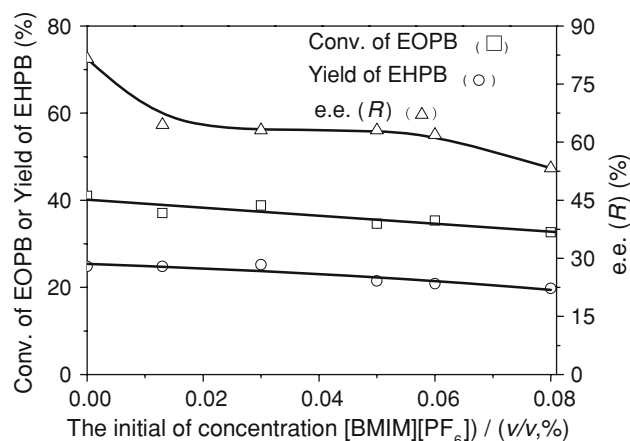
Fig. 6 Effect of [BMIM][PF$_6$] as an additive on the reaction in benzene. 1.2 g baker's yeast, 0.026 g EOPB, 25 mL benzene, 30 °C, 300 rpm, 24 h

Conclusion

The enantioselectivity of the reduction is reversed in [BMIM][PF$_6$]-water (10:1, v/v) biphasic system and shifts to (R)-side from (S)-side in [BMIM][PF$_6$]. Furthermore, catalytic activity of the yeast and the enantioselectivity of the reduction could be improved by using ethanol in [BMIM][PF$_6$]-water (10:1, v/v) biphasic system. These studies elucidated the use of an ionic liquid–water biphasic system for asymmetric reduction of EOPB that resulted in an improvement of reduction despite the relatively high viscosity of ionic liquids. A significant adverse influence on asymmetric reduction of EOPB caused by using a small amount of [BMIM][PF$_6$] as a reaction additive was observed.

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