Lipase-Catalyzed Enantioselective Acylation in the Ionic Liquid Solvent System: Reaction of Enzyme Anchored to the Solvent

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The lipase-catalyzed enantioselective acylation of allylic alcohols in an ionic liquid solvent was demonstrated; the reaction was significantly dependent on the counter anion of the imidazolium salt and good results were obtained when the reaction was carried out in [bmim]PF_6 or [bmim]BF_4 as solvent. We also first demonstrated that it was possible to repeatedly use the enzyme in the ionic liquid solvent system.

Proper choice of the reaction media is very important when developing practical processes in chemistry. Ionic liquids are new class of solvents which have attracted growing interest over the past few years due to their unique physical and chemical properties.[1] It is well known that lipase tolerates non-natural reaction conditions and the lipase-catalyzed transesterification in an organic solvent system has been well recognized as a very useful means of synthesizing optically active compounds.[2] Therefore it should be expected that the lipase-catalyzed reaction should occur in the ionic liquid solvent. The investigations of such possible uses the ionic liquids as the reaction media of the lipase-catalyzed reaction have just begun; Lye and his coworkers reported that the biotransformation is possible in an ionic liquid solvent system for the first time in July 2000[3] and two examples of enzymatic reaction in ionic liquids have been reported late in the same year.[4,5] However, no example of an asymmetric enzymatic reaction in an ionic liquid solvent has been reported.[6] Herein, we report the first example that realizes the recycle use of lipase in an ionic liquid solvent system for the asymmetric transesterification of an allylic alcohol.[7]

We chose the imidazolium salts as the solvent in our enzymatic reaction among various types of ionic liquids based on two criteria. The first is that imidazolium salts are stable under atmospheric conditions and especially tolerant to water.[1] The second is that we are systematically able to investigate the suitable combination of the imidazolium cation and counter anion of the salt for the enzymatic reaction.[1]

First, we investigated the lipase-catalyzed reaction using butylmethylimidazolium pentafluorophosphate ([bmim]PF_6)[8] as the solvent because this salt is insoluble in both water and ether; this means that an easy extraction process of the products is expected. In addition, we also anticipated that it might be possible to anchor the lipase in the ionic liquids during the work up process and this may allow us to repeatedly use the enzyme.

Typically, the reaction was carried out as follows: To a mixture of lipase (25 mg) in the ionic liquid (1.5 mL) were added racemic 5-phenyl-1-penten-3-ol ((±)-**1**)[9] (50 mg, 0.30 mmol) as a model substrate and vinyl acetate (39 mg, 0.45 mmol, 1.5 equiv) as the acyl donor. The resulting mixture was stirred at room temperature (ca. 25 °C) and the reaction course was monitored by GC analysis. The reaction was stopped by the addition of 3 mL of ether when the molar ratio of acetate **2** and alcohol **1** became equal. The reaction mixture was filtered through a glass-sintered filter with a celite pad to remove the enzyme and product, and unreacted alcohol was isolated from the filtrate. It is noteworthy that the ionic solvent was recovered without any loss in the amount after the work-up process and it was possible to reuse it after washing with water and dried under vacuum for several hours at 50 °C. The optical purities of the acetate (*S*)-**2** produced and the remaining alcohol (*R*)-**1** were determined by capillary GC analysis using a chiral column (Chiraldex G-TA).[9b] Acylation of the alcohol was accomplished by three types of enzymes, such as *Candida antarctica* lipase (CAL, Novozym 435), Lipase from *Alcaligenes* sp. (QL), and *Pseudomonas cepacia* lipase (PS). The desired acetate **2** had an extremely high enantioselectivity (Entries 1–3), though reaction rate was slightly inferior compared to those in the usual organic solvent reaction system (Entry 10). On the other hand, no reaction took place when *Candida rugosa* lipase (CRL) or Procine liver lipase (PPL) was used as the catalyst in the [bmim]PF_6 solvent system (Entries 4 and 5).

We next investigated the proper combination of the counter anion with [bmim] cation in the reaction of the CAL-catalyzed acylation (Entries 6–9). It was found that the acylation rate was strongly dependent on the anionic part of the solvent, while the CAL-catalyzed acylation proceeded with high enantioselectivi-

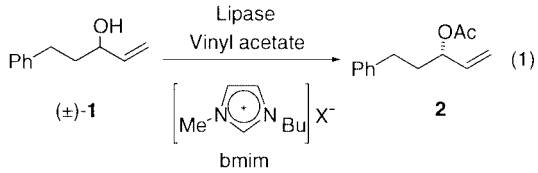

Table 1. Lipase-catalyzed transesterification in an ionic liquid solvent system

Entry	Lipase[a]	Solvent	Time /h	%ee of **2** (Yield/%)[b]	Conv. /c	Rate[c]	E value[d]
1	CAL	[bmim]PF_6	5	>99 (45)	0.47	9.4	>580
2	QL	[bmim]PF_6	25	94 (49)	0.41	1.6	65
3	PS	[bmim]PF_6	168	>99 (17)	0.19	0.11	>250
4	CRL	[bmim]PF_6	168	0	0	0	--------
5	PPL	[bmim]PF_6	168	0	0	0	--------
6	CAL	[bmim]TFA	48	91 (19)	0.12	0.25	227
7	CAL	[bmim]BF_4	3.5	>99 (44)	0.48	14	>640
8	CAL	[bmim]OTf	24	>99 (34)	0.43	1.8	>450
9	CAL	[bmim]SbF_6	48	>99 (31)	0.37	0.77	>360
10	CAL	*i*-Pr_2O	3	>99 (47)	0.50	17	>1000

[a] CAL (Novozym435): *Candida antarctica*; QL: *Alcaligenes* sp.; PS: *Pseudomonas cepacia* (Amano); CRL: *Candida rugosa* (Meito : Lipase OF); PPL: Porcine liver lipase (Sigma Type II). [b] Isolated yield. [c] Rate: %conv./reaction time (h). [d] See ref 10.

ty in all solvents tested. The best result was recorded when [bmim]BF_4[11a] was employed as the solvent (Entry 7) and the reaction rate was nearly equal to that of the reference reaction in *i*-Pr_2O (Entry 10). On the contrary, a significant drop in the reaction rate was obtained when the reaction was carried out in [bmim]TFA[11b] (Entry 6), [bmim]OTf[11c] (Entry 8) or [bmim]SbF_6[11d] (Entry 9). From these obtained results, it was concluded that [bmim]PF_6 and [bmim]BF_4 are suitable solvents for the present lipase-catalyzed reaction. Although the acylation rate in the reaction of [bmim]PF_6 was a slightly inferior to that in [bmim]BF_4, we chose [bmim]PF_6 as the best solvent for the present enzymatic reaction system. Because a very easy work-up process was realized in the reaction of [bmim]PF_6 due to the insolubility of this salt in both water and ether. [bmim]BF_4 was quite soluble in water and, therefore, it was difficult to remove the by-product such as acetic acid by simple work-up processes.

Scheme 1. Lipase-catalyzed reaction system anchored to the solvent.

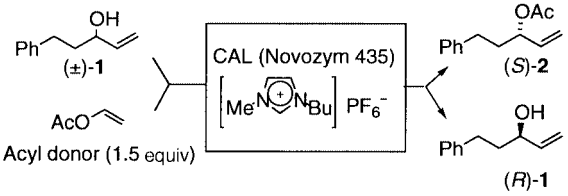

Table 2. Recycle use of lipase in the ionic liquid solvent system

Entry	Recycle no.	Time /h	%ee of **2** (Yield/%)[a]	%ee of **1** /%Yield[a]	Conv. /c	Rate[b]	E value[c]
1	0	3	>99 (47)	86 (44)	0.47	16	>580
2	1	3	>99 (30)	60 (63)	0.37	12	>400
3	2	6	>99 (38)	86 (54)	0.47	7.8	>560
4	3	22	>99 (34)	74 (64)	0.43	2.0	>440
5	4	91	>99 (28)	68 (58)	0.41	0.45	>400

[a] Isolated yield. [b] Rate: %conv./reaction time (h). [c] See ref 10.

Since it was anticipated that lipase might be anchored by the ionic liquid solvent and remained in it after the extraction work-up of the products, we next attempted to repeatedly use the lipase in the [bmim]PF_6 solvent system (Scheme 1). The results are shown in Table 2. A mixture of the substrate, lipase, and vinyl acetate in the [bmim]PF_6 solvent was stirred for 3 h, and then ether was added to the reaction mixture to form the biphasic state. The desired products and unreacted alcohol were quantitatively extracted from the ether (upper layer). To the remained ionic liquid phase, which was placed under reduced pressure for 15 min to remove the ether, a mixture of the substrate and vinyl acetate was again added. This mixture was stirred at rt. As expected, the acylation reaction smoothly took place and the product was obtained without any loss in enantioselectivity (Entry 2). It was thus confirmed that the enzyme was in fact anchored in the ionic liquid solvent after the work-up process. Repeating the same process, we successfully showed that recycling of the enzyme was indeed possible in our ionic liquid solvent system, though the reaction rate gradually dropped by repeating the reaction process (Entries 3–5). This is the first demonstration that the recycle use of enzyme was in fact possible in the ionic liquid solvent system.

In conclusion, we demonstrated the lipase-catalyzed enantioselective transesterification of an allylic alcohol in the ionic liquid solvent system and demonstrate that it is possible to repeatedly use the enzyme in the ionic liquid solvent system. Further investigation of the scope and limitations of this reaction, especially optimization of the reaction conditions for the lipase recycling system in the ionic solvent system, will make it even more beneficial.

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