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Increased Enantioselectivity and Remarkable Acceleration of Lipase-Catalyzed Transesterification by Using an Imidazolium PEG–Alkyl Sulfate Ionic Liquid

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Abstract: Several types of imidazolium salt ionic liquids were prepared derived from poly(oxyethylene)alkyl sulfate and used as an additive or coating material for lipase-catalyzed transesterification in an organic solvent. A remarkably increased enantioselectivity was obtained when the salt was added at 3–10 mol % versus substrate in the *Burkholderia cepacia* lipase (lipase PS-C)-catalyzed transesterification of 1-phenylethanol by using vinyl acetate in diisopropyl ether or a hexane solvent system. In particular, a remarkable acceleration was accomplished by the ionic liquid coating with lipase PS in an iPr$_2$O solvent system while maintaining excellent enantioselectivity; it reached approximately 500- to 1000-fold acceleration for some substrates with excellent enantioselectivity. A similar acceleration was also observed for **IL1**-coated *Candida rugosa* lipase. MALDI-TOF mass spectrometry experiments of the ionic-liquid-coated lipase PS suggest that ionic liquid binds with lipase protein.

Keywords: acceleration · enantioselectivity · ionic liquids · lipids · transesterification

Introduction

The value of an enzymatic reaction in organic synthesis is greatly increased by its environmentally friendly aspect.[1] Lipase PS from *Burkholderia cepacia*[2] is one of the most widely used enzymes applicable for various substrates;[1] however, the activity in nonaqueous media is reduced and it has been reported that the enantioselectivity is significantly dependent on the solvent system.[3] As an example, excellent enantioselectivity was recorded when (±)-1-phenylethanol (**1a**) was acetylated by lipase PS-C in toluene,[4] while significant reduction in enantioselectivity was obtained when the reaction was carried out in diisopropyl ether (iPr$_2$O) as solvent. Therefore, development of a strategy to improve lipase reaction performance in an organic solvent system is desirable.[1,3]

Several methods have been reported for activation of lipases in a nonaqueous medium: lipid-coating-mediated activation by Okahata,[5] entrapment of lipases in hydrophobic sol–gel materials by Reetz,[6] molecular bioimprinting by Braco,[7] and salt-mediated activation by Dordick.[8] Among these, the salt-mediated activation of lipases by the Dordick group is quite interesting;[8] they indicated that enzymes could be activated by a large amount of salts with kosmotropic anions during the lyophilization process.[8] Recently, Kim and Lee reported another very interesting salt-mediated activation of a lipase: Lipase PS was mixed with the ionic liquid and resulted in "ionic-liquid-coated lipase PS", which showed a more increased enantioselectivity than that of commercial lipase PS-C in toluene, though no significant modification of the reaction rate was obtained.[9]

Room-temperature ionic liquids are a new class of solvents and have attracted growing interest recently because

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of their unique physical and chemical properties; they are nonvolatile, nonflammable, have low toxicity, and good solubility for many organic and inorganic materials.[10] We, in the Bioscience and Technology group, established that lipase-catalyzed transesterification could be conducted in an ionic-liquid solvent system;[11,12] the enantioselectivity was dependent on the anionic part of the imidazolium salts.[10–13] It has been reported that some surfactants were able to stabilize enzymes in an ionic liquid solvent system; the groups of Russell[14] and Goto[15] independently reported that PEG treatment significantly stabilized the enzyme reactivity in an ionic-liquid solvent system, and made it possible to modify the enantioselectivity. Lipase reactivity in an organic solvent system can be modified by several reported compounds: crown ethers,[16,17] cyclodextrins,[18] surfactants,[7,19] and a polar solvent such as dimethylsulfoxide (DMSO),[20] or even water.[21]

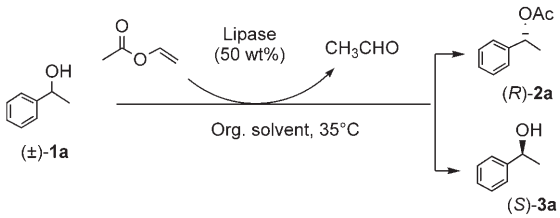
Based on these results, we investigated the possibility of regulating the enantioselectivity and reactivity of lipase PS by using iPr$_2$O as the solvent. We expected that optimization of an appropriate combination of the anionic part and the imidazolium cation of the ionic liquids might make it possible to design a useful regulator of the lipase-catalyzed reaction. We prepared several types of imidazolium poly(oxyethylene) alkyl sulfate ionic liquids and used them as an additive or coating material for lipase PS; a remarkably increased enantioselectivity was obtained when the ionic liquid was added at 3 mol% versus the substrate in the lipase PS-C-catalyzed transesterification of (±)-**1a** by using vinyl acetate as acyl donor in an iPr$_2$O solvent system. Further, we discovered that an extraordinary acceleration was accomplished by our ionic-liquid coating with lipase PS in an iPr$_2$O solvent system, while maintaining excellent enantioselectivity, and even better enantioselectivity was obtained for some substrates.[12] Herein, we wish to report the details of our method of ionic-liquid-promoted activation of the lipase.

Results and Discussion

Additive effect on the lipase-catalyzed transesterification: Several methods are reported to improve enzyme enantioselectivity in a nonaqueous solvent system, the simplest being the addition of a suitable compound that affects the enzyme activity. We previously reported that increased enantioselectivity occurred when the reaction was carried out in the presence of a trace amount of thiocrown ether.[16] Ueji et al. reported that enantioselectivity of *Candida rugosa* lipase (CRL) was increased by the addition of DMSO as cosolvent in toluene.[20] Modified enantioselectivity was also reported if the reaction was carried out in an ionic-liquid solvent instead of a usual organic solvent.[9–13,22] We therefore investigated the lipase-catalyzed acylation of (±)-1-phenylethanol (**1a**) in the presence of various additives, in particular, ionic-liquid additives by using iPr$_2$O as solvent (Table 1).

The enantioselectivity of lipase PS-C for (±)-**1a** is significantly dependent on the reaction medium and the E value[22] is just 16 for iPr$_2$O (entry 1), while it is over 200 for toluene (entry 2). We found that a remarkably increased enantioselectivity was obtained when the reaction was carried out in the presence of 10 mol% (versus substrate) of

Table 1. Results of the additive effect on the lipase PS-catalyzed enantioselective acylation of (±)-1-phenylethanol (**1a**)



	Solvent	Enzyme additive[a]	t[h]	Yield[b] [%] of (*R*)-**2a** (ee_p, [%])[c]	Yield[b] [%] of (*S*)-**3a** (ee_s, [%])[c]	Conversion [%][d]	E value[d]
1	iPr$_2$O	PS-C-none	28	21 (83)	61 (38)	32	16
2	toluene	PS-C-none	28	31 (99)	40 (76)	43	>200
3	iPr$_2$O	PS-C+[14]-ane-S4	28	34 (>99)	58 (66)	40	>200
4	iPr$_2$O	PS-C+DMSO	28	33 (>99)	50 (71)	41	>200
5	iPr$_2$O	PS-C+[bmim][BF$_4$]	24	15 (43)	64 (20)	31	3
6	iPr$_2$O	PS-C+[bdmim]BF$_4$]	24	16 (98)	55 (24)	20	125
7	iPr$_2$O	PS-C+[bdmim][PF$_6$]	24	19 (95)	67 (35)	27	55
8	iPr$_2$O	PS-C+Brij56[e]	26	15 (92)	66 (33)	26	35
9	iPr$_2$O	PS-C+**IL1**	28	33 (>99)	50 (>99)	50	>200
10	iPr$_2$O	PS-C+**IL2**	29	30 (70)	57 (35)	32	10
11	iPr$_2$O	CF-PS[f]-none	26	9 (87)	72 (15)	15	17
12	iPr$_2$O	CF-PS[f]+**IL1**	24	24 (>99)	61 (41)	29	>200

[a] 10 mol% versus (±)-**1a**. [b] Isolated yield. [c] Determined by HPLC (Chiralcel OB, hexane/*i*PrOH=8:1). [d] Calculated from ee_p and ee_s. $E=\ln[(1-c)(1+ee_p)]/\ln[(1-c)(1-ee_p)]$, in which c is conversion, which was calculated by the following formula: $c=ee_p/(ee_p+ee_s)$. See ref. [23]. [e] Brij56=poly[oxyethylene(10)] cetyl ether ($C_{16}H_{33}(OCH_2CH_2)_nOH$, $n\sim10$). [f] The reaction was carried out in the presence of 0.5 wt% (versus substrate) of CF-PS.

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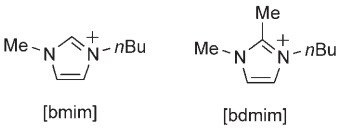
thiocrown ether, 1,4,8,11-tetrathiatetracyclodecane ([14]ane-S4),[16] or DMSO (entries 3 and 4).[20] It was reported that modified stereoselectivity sometimes occurred when the lipase-catalyzed reaction was conducted in an ionic-liquid solvent system,[9–13,22] and it was established that the lipase activity was dependent on the combination of the cationic and anionic parts of the imidazolium salts, in particular, the anionic part of the ionic liquids.[14,24] It is also known that some surfactants, such as poly(oxyethylene)alkyl ether, affect lipase reactivity,[14,15] so we next tested the lipase PS-C-catalyzed acetylation of (±)-**1a** in the presence of a surfactant, poly[ethylenoxy(10)] cetyl ether (Brij56)[25] or ionic liquids. Interestingly, increased enantioselectivity was recorded for 1-butyl-2,3-dimethylimidazolium tetrafluoroborate ([bdmim][BF_4]) (entry 6) or 1-butyl-2,3-dimethylimidazolium hexafluorophosphate [bdmim][PF_6] (entry 7), while a significant decrease was obtained by addition of 1-butyl-2-methylimidazolium tetrafluoroborate [bmim][BF_4] (entry 5).



Further, a slight increase of enantioselectivity was recognized when Brij56 was added to the reaction mixture (entry 8). These results prompted us to prepare a hybrid of Brij56 with an imidazolium salt, and to evaluate its effect on the lipase activity.

Two types of imidazolium salts, [bdmim][cetyl-PEG10-sulfate] (**IL1**) and [bmim][cetyl-PEG10-sulfate] (**IL2**) were synthesized:[12] A mixture of Brij56 with ammonium amidosulfate was stirred for 17 h at 110 °C and dried under reduced pressure at 66.7 Pa and at 60 °C for 3 h to give ammonium Brij56 sulfate as white powder. This was treated with an equivalent amount of imidazolium chloride in acetone with vigorous stirring at room temperature for 6 h to form ammonium chloride as a white precipitate, which was removed by filtration through a glass-sintered filter with a Celite pad. The resulting filtrate was evaporated and the residue was again dissolved in acetone and filtered through an alumina (neutral type I, activated) short column. The filtrate was evaporated and dried under reduced pressure to form **IL1** and **IL2** with 70–74 % overall yield, respectively. The salts showed melting points at 35–37 °C due to their amphiphilic nature and they dissolved easily in many types of organic solvents and in water.

There was a clear contrast in the modification effect between these two ionic liquids on the lipase PS-C-catalyzed reaction (entries 9 and 10); a remarkably increased enantioselectivity was accomplished when the reaction was carried out in the presence of approximately 10 mol % (versus substrate) of **IL1**, which had a methyl group at the 2-position on the imidazolium ring, while no marked change of enantioselectivity was recorded when **IL2**, which has a proton at the 2-position on the imidazolium ring, was used as additive (entry 10). We discovered that ionic liquid **IL1** worked as an excellent additive to increase the enantioselectivity, and a high *E* value was recorded even when **IL1** was used as additive in the Celite-free PS (CF-PS)-catalyzed reaction (entry 12).[26] This additional effect was found to be independent of the presence of Celite as supporting material of the lipase (entries 11 and 12).

Activation of the lipase by the ionic-liquid coating: Dordick, Clark, and co-workers reported that lyophilization was essential to activate the lipase by inorganic salt-mediated activation.[8] Recently, Goto et al. reported that a marked acceleration was obtained for PEG-supported lipase PS, which was prepared by lyophilization of an enzymatic buffer solution in the presence of PEG, in an organic solvent system.[15] However, Kim and Lee prepared their "ionic-liquid-coated lipase" by just mixing the lipase with a melted ionic liquid.[9] Further, Braco established that lyophilization was essential to achieve activation of the enzyme by a bioimprinting protocol.[7] From these results, we hypothesized that the coating method might be the key point for activation of lipases by an ionic liquid; lyophilization of an enzyme in the presence of the ionic liquid might be essential to activate the lipase. We thus prepared "**IL1**-coated PS" by the following method: Commercial lipase PS-C (1.0 g, involving approximately 1 wt % of the enzyme protein) was added to potassium phosphate buffer (10 mL, 0.1 M, pH 7.2); this mixture was stirred for 5 min at room temperature, then centrifuged at 3500 rpm for 5 min. The resulting supernatant was mixed with **IL1** and the solution was lyophilized to give **IL1**-PS (250 mg).[27] Celite-free lipase PS (CF-PS) was also prepared by lyophilization of the supernatant of the mixture of commercial lipase PS-C in 0.1 M phosphate buffer (pH 7.2) after centrifugation.[27] The transesterification of (±)-**1a** by using these ionic-liquid-coated enzymes was extremely successful and the results are summarized in Table 2.

A remarkable acceleration was accomplished by using **IL1**-PS whilst maintaining excellent enantioselectivity. Reaction conversion reached 44 % after just 1.3 h when **IL1**-PS, which was prepared by using about 50 equivalents of **IL1** versus lipase protein, was used as catalyst in *i*Pr_2O (entry 1), while it took 28 h at only 32 % conversion when commercial PS-C was used as catalyst, as mentioned earlier (Table 1, entry 1). The results of the activation of lipase PS depended on the amount of **IL1** in the coating process; the best result was recorded when **IL1**-PS was prepared by using approximately 100 equivalents of **IL1** versus lipase protein (entry 2). The use of a large amount of **IL1** (about 300 or 500 equivalents versus lipase protein) caused a slight decrease of both enantioselectivity and reaction rate (entries 3 and 4). Enantioselectivity significantly depends on the cationic part of the ionic liquids: no marked increased enantioselectivity was recorded for **IL2**-PS (entry 5) and only a slight increase was obtained for Salt**1**-PS (entry 7). A remarkable acceleration was again obtained for Brij56-PS, while this enzyme showed only slightly increased enantioselectivity (entry 8). These results seem to suggest that the

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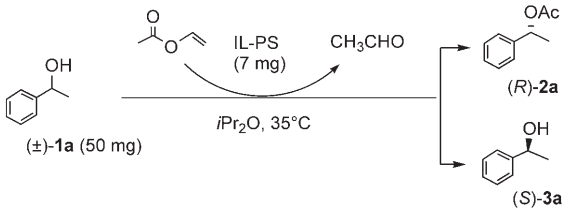
Table 2. Results of additive effect on the lipase PS-catalyzed enantioselective acylation of (±)-1-phenylethanol (**1a**).



	CM[a]	Molar ratio enzyme:CM	t [h]	Yield[b] [%] of (*R*)-**2a** (ee_p, [%])[c]	Yield[b] [%] of (*S*)-**3a** (ee_s, [%])[c]	Conversion [%][d]	E value[d]
1	**IL1**	1: 50	1.3	30 (>99)	46 (79)	44	>200 (470)
2	**IL1**	1:100	1.0	42 (>99)	42 (98)	50	>200 (1060)
3	**IL1**	1:300	2.5	25 (>99)	66 (36)	27	>200 (285)
4	**IL1**	1:500	3.5	27 (>99)	52 (58)	37	>200 (360)
5	**IL2**	1:100	2.0	36 (67)	43 (88)	57	14
6	Salt**1**[e]	1:100	2.0	33 (94)	47 (79)	45	75
7	Brij56	1:100	1.0	27 (84)	77 (58)	48	27
8	**IL1**[f]	1:300	24.0	30 (>99)	46 (32)	24	>200 (270)
9	**IL1**[g]	1:100	48.0	21 (99)	60 (47)	32	>200 (315)
10	**IL1**[g]	1:100+Gly[h]	24.0	27 (98)	56 (78)	43	>200 (217)

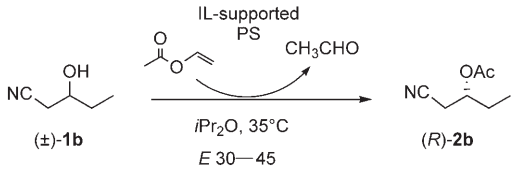
[a] CM = coating material. [b] Isolated yield. [c] Determined by HPLC (Chiralcel OB, hexane/*i*PrOH = 8:1). [d] Calculated by ee_p and ee_s. $E = \ln[(1-c)(1+ee_p)]/\ln[(1-c)(1-ee_p)]$, in which c is conversion, which was calculated by the following formula: $c = ee_p/(ee_p+ee_s)$. [e] Salt1: $[NH_4][C_{16}H_{33}(OCH_2CH_2)_{10}OSO_3]$. [f] CF-PS was mixed with **IL1** melted by warming and used immediately for the reaction. [g] **IL1** coating lipase was prepared by using a pure lipase protein. [h] Lyophilization was carried out in the presence of 100 equiv of **IL1** and glycine (versus lipase protein).

present activation might be caused by the result of an interaction of a poly(oxyethylene)alkyl group and a cationic part of the ionic liquid. It was confirmed that lyophilization of an enzyme in the presence of the ionic liquid was essential to activate the lipase because no significant acceleration was recorded when CF-PS was mixed with **IL1** that had been melted by warming, and the resulting enzyme was used immediately for acylation (entry 8). However, increased enantioselectivity was obtained in this case too (entry 8). These results seem to suggest that there might be a different origin for the increased enantioselectivity and acceleration.

Table 2 shows the results of ionic-liquid-coated lipase PS-catalyzed enantioselective acylation of (±)-1-phenylethanol (**1a**). During these experiments, we found that the amount of **IL1**-PS was about 200–300 mg per 1.00 g of PS-C after lyophilization even though it was dried very carefully. Since it was reported that commercial PS-C was prepared by supporting 1.0 wt% of pure enzyme protein with Celite, the amount of **IL1**-PS was evidently too large based on the information of PS-C provided from Amano Enzyme Ltd: We used only 29 mg of **IL1** per 1.00 g of PS-C. The question was resolved by Amano Enzyme Ltd; commercial PS-C contains 20 wt% of glycine that was used as essential stabilizer during the preparation of PS-C by the lyophilization process. So, we prepared **IL1**-coated PS by using pure enzyme protein that was provided by Amano Enzyme Ltd and tested its activity; no acceleration was obtained though excellent enantioselectivity was still recorded (entry 9). We next prepared **IL1**-pure PS by lyophilization in the presence of 100 equivalents of glycine. An increased reaction rate was indeed obtained (entry 10), but it was not significant compared to that of **IL1**-PS (entry 2). This was assumed to be because lipase activity was rapidly lost during the lyophilization process without glycine.

Since it was anticipated that the alkyl sulfate anion might have an impact on the lipase reactivity, we next attempted to evaluate the poly(oxyethylene)alkyl sulfate anion by using (±)-**1b**[16c,d] as a model substrate; the reaction of (±)-**1b** proceeds very rapidly, making it possible to determine the reliability of the kinetic parameters, although only a small modification of the enantioselectivity was found for the **1L1**-PS-catalyzed acetylation of (±)-**1b**. Ten types of ionic-liquid-coated lipase PS and Brij56-PS were prepared and used as catalysts in the transesterification of (±)-**1b** (Table 3). We discovered that **IL1** was the best ionic liquid to activate lipase PS (entry 8). The reaction rate was drastically accelerated and reached 98-fold acceleration over commercial lipase PS-C when **IL1**-supported lipase PS was used as catalyst (entry 8). The second choice was **IL1c**-PS with which 90-fold accelera-

Table 3. Results of ionic-liquid-coated lipase PS-catalyzed enantioselective acylation of (±)-3-hydroxypentanenitrile (**1b**).



	Coating material[a]	Rate [M h^{-1} mg^{-1}]	Specific activity
1	PS-C	0.10	1
2	[bmim][BF_4]	0.85	9
3	[bdmim][BF_4]	3.9	39
4	[bdmim][*n*-PentOSO_3]	6.4	64
5	Brij56	7.9	79
6	**IL1a** (from Brij35)	4.1	41
7	**IL1b** (from Brij52)	6.7	70
8	**IL1** (from Brij56)	9.8	98
9	**IL1c** (from Brij58)	9.0	90
10	**IL1d** (from Brij92)	8.1	81
11	**IL1e** (from Brij97)	6.4	64
12	**IL1f** (from Brij700)	6.8	68

[a] **IL1** = [bdmim][$C_{16}H_{33}(OCH_2CH_2)_{10}OSO_3$]; **IL1a** = [bdmim][$C_{12}H_{25}(OCH_2CH_2)_{23}OSO_3$]; **IL1b** = [bdmim][$C_{16}H_{33}(OCH_2CH_2)_2OSO_3$]; **IL1c** = [bdmim][$C_{16}H_{33}(OCH_2CH_2)_{20}OSO_3$]; **IL1d** = [bdmim][$C_{18}H_{35}(OCH_2CH_2)_2OSO_3$]; **IL1e** = [bdmim][$C_{18}H_{35}(OCH_2CH_2)_{10}OSO_3$]; **IL1f** = [bdmim][$C_{18}H_{35}(OCH_2CH_2)_{100}OSO_3$].

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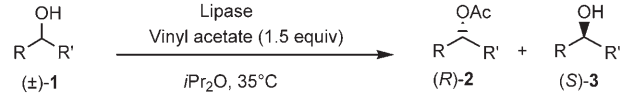
tion was recorded (entry 9). It is interesting that [bdmim][BF_4] gave better results than [bmim][BF_4] (entries 2 and 3). It was thus confirmed that the cationic group of the ionic liquid played an important role in the increased enantioselectivity of lipase PS.

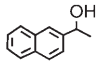
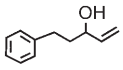
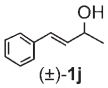
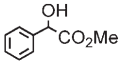
We further discovered a very interesting activation property of **IL1**-PS: the acceleration of the rate was significantly dependent on the substrates (Table 4). A truly remarkable acceleration was accomplished for **1a**, **1b**, **1d**,[29] **1e**,[30] **1h**,[33] **1k**,[36] and **1i**;[35] 1100-fold acceleration was obtained for alcohol, (±)-**1d**, 500-fold for (±)-**1e**, 98-fold for (±)-**1b**, 26-fold for (±)-**1l**,[11f] and 12–18-fold accelerations were recorded for (±)-**1i**, **1a**, and **1h** while excellent enantioselectivity was maintained. On the other hand, only a slight acceleration was recorded for **1g**[32] and **1j**,[35] and none was observed for **1c**[28] or **1f**.[31] The effect of **IL1** on the enantioselectivity was also dependent on the substrate: a remarkably increased enantioselectivity was obtained for **1a**, **1j**, and **1l**, while only a small modification occurred for **1b**.

Since it is anticipated that the supporting effect would be general for lipases, we next investigated the **IL1**-supporting effect of *Candida rugosa* lipase (CRL). Ueji and co-workers recently reported that lyophilization of *Candida rugosa* lipase in the presence of some ionic compounds resulted in dramatic improvement of its enantioselectivity for the esterification of 2-arylpropionic acid derivatives in an organic solvent system.[20c] Therefore, we chose the CRL-catalyzed transesterification of 2-(4-ethylphenoxy)propionic acid ((±)-**4**)[20] with *n*-butanol (*n*BuOH) as a model reaction[20] and the results are shown in Table 5.

Both enantioselectivity and reaction rate were improved when the reaction was carried out by using **IL1**-supported CRL as catalyst (entries 3–5). The effect was significantly dependent on the amount of the supporting **IL1**: high enantioselectivity and marked acceleration were obtained for **IL1**-CRL-**b** (entry 4) and **IL1**-CRL-**c** (entry 5), while only a small activation was recorded for **IL1-a**. Reaction conversion was 50% after 24 h reaction when **IL1**-CRL-**b** was used as catalyst in iPr_2O (entry 4), but it took 60 h at 26% conversion when commercial CRL was used as catalyst (entry 1). Interestingly, better enantioselectivity was obtained when the reaction was carried out in the presence of 10 mol% (versus substrate) **IL1**, while the reaction rate decreased under the reaction conditions of entry 2. Further, it was found that the **IL1** support significantly stabilized CRL in the organic solvent system. No drop in reactivity was observed when **IL1**-CRL-**c** was placed in dry hexane for a week at room temperature, while the reactivity was completely lost if commercial CRL was placed in hexane for the same period. Since similar stabilization was observed for PS-C, we expect that the stabilization effect of **IL1** might be general for various lipases.

Table 4. Results of the activation effect by the **IL1** coating on the lipase PS-catalyzed enantioselective acylation of (±)-**1**.



Substrate	lipase PS[a]	**IL1**-PS[a]	Specific activity[c,d]
 (±)-**1a**	rate: 65 *E* 16	rate: 1000 *E* >200	15
 (±)-**1b**	rate: 100 *E* 39	rate: 9800 *E* 40	98
 (±)-**1c**	rate: 13 *E* >200	rate:14 *E* >200	1
 (±)-**1d**	rate: 1.0×10^{-2} *E* 199	rate: 11 *E* >200	1100
 (±)-**1e**	rate: 1.2×10^{-2} *E* >200	rate: 6 *E* >200	500
 (±)-**1f**	rate[b]: 52 *E* >200	rate[b]: 85 *E* >200	2
 (±)-**1g**	rate[b]: 32 *E* >200	rate[b]: 163 *E* >200	5
 (±)-**1h**	rate[b]: 7.3 *E* >200	rate[b]: 133 *E* >200	18
 (±)-**1i**	rate: 2.6 *E* >200	rate: 31 *E* >200	12
 (±)-**1j**	rate[b]: 12 *E* 138	rate[b]: 300 *E* >200	26
 (±)-**1k**	rate: 3.2 *E* 12	rate: 19 *E* 123	6
 (±)-**1l**	rate: 1.1 *E* >200	rate: 12 *E* >200	11

[a] Rate: $\mu mol\,h^{-1}\,mg(enzyme)^{-1}$. Rate was determined by GC analysis. [b] Rate was determined from the % conversion data, because the reaction proceeded slowly. [c] Specific activity of **IL1**-PS versus PS-C. [d] Specific activity was calculated by the rate of **IL1**-PS divided by that of PS-C.

Recycling use of IL1-PS: We recently reported the preparation of fluorine-substituted hydrophobic ionic liquid, 1-butyl-3-methylimidazolium 2,2,3,3,4,4,5,5-octafluoropentyl sulfate ([bmim][C5F8]), and demonstrated that efficient lipase-catalyzed transesterification occurred in [bmim][C5F8] as solvent.[34] We attempted to demonstrate the recycling use of the enzyme in the [bmim][C5F8] solvent system by using (±)-**1j** as substrate (Table 6). As shown in Table 6, both the reaction rate and enantioselectivity gradually decreased with

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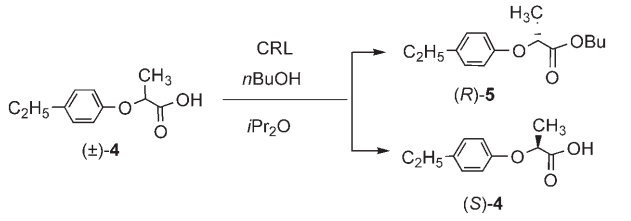
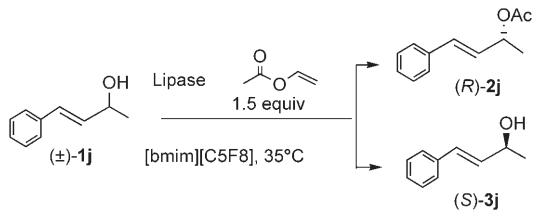
Table 5. Results of esterification of 2-(4-ethylphenoxy)propionic acid ((±)-**4**) catalyzed by the ionic-liquid-supported CRL (*Candida rugosa* lipase).



	Lipase[a]	Additive	*t* [h]	Conversion [%][b]	*ee* [%] (*R*)-**5**[c]	*E* value[b]
1	CRL	none	60	26	62	5
2	CRL	**IL1**[d]	96	14	98	96
3	**IL1**-CRL-**a**	none	32	27	92	32
4	**IL1**-CRL-**b**	none	24	50	>99	>200
5	**IL1**-CRL-**c**	none	24	36	>99	>200

[a] Lipase MY (Meito); **IL1**-CRL-**a**: **IL1**/MY = 1.0 g:50 mg, **IL1**-CRL-**b**: **IL1**/MY = 1.0 g:500 mg, **IL1**-CRL-**c**: **IL1**/MY = 1.0 g:1.0 g. [b] Calculated by % *ee* of (*R*)-**5** (ee_p) and % *ee* of (*S*)-**4** (ee_s). $E = \ln[(1-c)(1+ee_p)]/\ln[(1-c)(1-ee_p)]$, here *c* means conversion which was calculated by the following formula: $c = ee_p/(ee_p+ee_s)$. See ref. [23]. [c] Determined by HPLC (Chiralcel OB, hexane/*i*PrOH = 200:1). [d] 10 mol % of **IL-1** was added.

Table 6. Results of recyclable use of PS-C and **IL1**-PS.



	Run	Lipase	*t* [h]	*ee* [%] of (*R*)-**2j**[a] (yield [%])[b]	*ee* [%]of (*S*)-**3j**[a] (yield [%])[b]	Conversion [%][c]	*E* value[c]
1	1st	PS-C	24.0	98 (13)	25 (87)	20	148
2	2nd	PS-C	24.0	96 (22)	16 (77)	14	62
3	3rd	PS-C	24.0	93 (10)	10 (67)	10	31
4	4th	PS-C	24.0	93 (6)	9 (75)	9	31
5	5th	PS-C	24.0	88 (5)	5 (60)	5	16
6	1st	**IL1**-PS	2.5	98 (32)	51 (62)	34	165
7	2nd	**IL1**-PS	4.5	96 (22)	29 (66)	23	65
8	3rd	**IL1**-PS	6.0	96 (30)	39 (61)	29	72
9	4th	**IL1**-PS	8.0	97 (20)	29 (77)	23	87
10	5th	**IL1**-PS	13.0	97 (18)	23 (78)	19	82

[a] Isolated yield. [b] The enantiomeric excess was determined by HPLC analysis by using OJ-H (∅ 4.6 × 250 mm, hexane/*i*PrOH = 9:1, 35 °C). [c] See ref. [23].

repetition of the reaction process in the system when commercial PS-C was used as catalyst (entries 1–5).[34] To our delight, the desired product was obtained with excellent enantioselectivity by using **IL1**-PS as catalyst, and five repetitions of this process showed no significant drop in the reaction rate (entries 6–10). We thus succeeded in demonstrating the recyclable use of lipase in [bmim][C5F8] by using **IL1**-PS as catalyst.

Investigation of the origin of this IL1-mediated activation: To investigate the remarkable acceleration accomplished by an ionic-liquid coating of the enzyme we next measured the kinetic parameters of *Burkholderia cepacia* (PS-C)-catalyzed transesterification of **1b**. Enantiomerically pure alcohols (*R*)-**1b**[16c,d] and (*S*)-**1b**[16c,d] were subjected to the lipase-catalyzed transesterification by using vinyl acetate as acyl donor in the *i*Pr$_2$O solvent system, and the progress of the reaction was monitored by capillary gas chromatography to obtain the initial rate (v_0). The reaction was typically done as follows: dry *i*Pr$_2$O (2.0 mL) was added to alcohol (*R*)-**1b** or (*S*)-**1b** together with the lipase (25 mg for PS-C or 7.0 mg for **IL1**-PS). The resulting mixture was stirred at 35 °C and sampled at one-minute intervals when **IL1**-PS was used as catalyst. However, since the PS-C-catalyzed reaction proceeded slowly, we sampled at 30-minute intervals. The concentration of the acetate [S_0] was systematically changed, and the plot of v_0 against [S_0] afforded a typical saturation curve.

Table 7 shows the kinetic parameters for the PCL-catalyzed transesterification of 3-hydroxybutanenitrile (**1b**) by using vinyl acetate as an acyl donor. The ionic-liquid coating significantly accelerated the reaction by using two enantiomers of similar magnitude (approximately tenfold). However, the difference in ratio of V_{max} values of (*R*)-**1b**/(*S*)-**1b** of **IL1**-PS was slightly inferior to that of PS-C (Table 4). It is well recognized that the K_m of the lipase-catalyzed reaction shows a generally large value, indicating that the binding of lipases to the substrates is weak.[38] A large K_m value was, in fact, obtained by the present reaction. A slightly reduced K_m was found in **IL1**-PS for

Table 7. Kinetic parameters[a] for the PCL-catalyzed transesterification of 3-hydroxybutanenitrile (**1b**) by using vinyl acetate as an acyl donor.

	Substrate	Lipase	V_{max} [M min^{-1} mg(lipase)$^{-1}$]	K_{cat} [min^{-1}]	K_m [M]	K_{cat}/K_m [min^{-1} M^{-1}]
1	(*R*)-**1b**	PS-C	3.1×10^{-1}	2.0×10^{4}	1.6	1.3×10^{4}
2	(*R*)-**1b**	**IL1**-PS	3.0	2.0×10^{5}	2.5	8.0×10^{4}
3	(*S*)-**1b**	PS-C	7.0×10^{-3}	4.6×10^{2}	6.0×10^{-1}	7.5×10^{2}
4	(*S*)-**1b**	**IL1**-PS	9.1×10^{-2}	6.0×10^{3}	2.9×10^{-1}	2.1×10^{4}

[a] Because of the heterogenous reaction, the nonlinear least-squares method was applied to the Michaelis–Menten-type of equations: $v_0 = V_{max}(E)_{mg}[S_0]/(K_m+[S_0])$, in which V_{max} is normalized by the weight of lipase protein $(E)_{mg}$.

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the (*S*)-**1b** (entry 4) compared to that of PS-C (entry 3). As a result, catalytic efficiency (V_{max}/K_m) between **IL1**-PS and PS-C was modified: a slightly higher value was obtained for **IL1**-PS than for PS-C. This corresponded to the increased enantioselectivity of **IL1**-PS. As mentioned earlier, the cationic part of the coating materials significantly modified the enantioselectivity of the enzyme (Table 2). We anticipated that the cationic part of the ionic liquid might bind with the lipase protein; this may cause conformational change of the enzyme and contribute to increasing the difference of K_m between enantiomers. To confirm the binding of the ionic liquid with the lipase protein, MALDI-TOF mass experiments were carried out (Figure 1).

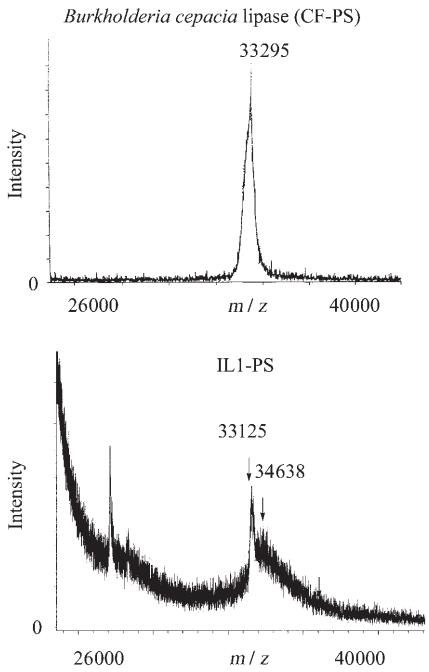

Figure 1. Results of MALDI-TOF MS experiments on CF-PS and **IL1**-PS.

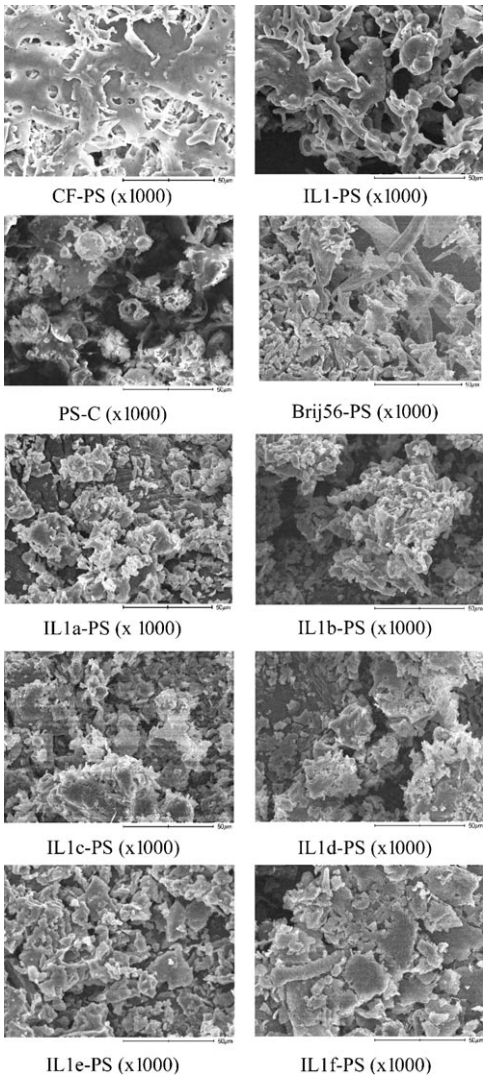

Figure 2. Scanning electron micrographs (SEM) of CF-PS, PS-C, Brij56-PS, and seven types of ionic-liquid-coated PS, **IL1**–**IL f** at 1000-fold magnitude.

The molecular peak of the lipase PS protein was found at 33295 Da, which coincided with the reported molecular weight of *Burkholderia cepacia* lipase.[26] As clearly shown in Figure 1, lipase PS protein made a complex with **IL1** by its appearance, such as, that at 34638 Da, though determination of the number of ionic-liquid molecules binding with the enzyme was unsuccessful because **IL1** has polymeric parts of different molecular weight.[40]

Since **IL1**-PS is not soluble in iPr_2O, it is assumed that the interface property of lipase protein with the substrate molecules might be important for the efficiency of the present transesterification. Hence we looked at the surface properties of CF-PS, Brij56-PS, and ionic-liquid-coated lipase PS (Figure 2). Significant differences were found in the surfaces of CF-PS and **IL1**-PS. Lipase powders of ionic-liquid-coated PS and Brij56-PS have a highly porous nature, while CF-PS has a flat surface. **IL1**-PS appears to have a wider surface area than that of native lipase PS (CF-PS) or commercial PS-C, while all ionic-liquid-coated enzymes and Brij56-PS appear to have similar-looking surface areas. Since significant acceleration was obtained for these enzymes, the surface-area expansion may take place during the lyophilization process in the presence of ionic liquids or surfactant Brij56; this may contribute to the production of highly porous lipase powder. It is not surprising that the substrate alcohol can access the enzyme more easily than the less-porous CF-PS, and cause the rate acceleration of **IL1**-PS in the transesterification. However, since enantioselectivity was dependent on the nature of the coating material, particularly the cationic part of the ionic liquid, it is impossible to explain the present lipase activation only by the surface-area increase of the enzyme protein powder.

We currently assume that at least three factors are involved in the origin of the modification of the present

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lipase-catalyzed reaction. One is the modified flexibility of the enzyme by the ionic liquid. Ueji suggested that flexibility change of the lipase protein reflects on the stereoselectivity of the enzyme,[20] and an organic medium causes modification of the flexibility of the lipase protein.[39] Since MALDI-TOF mass experiments suggest that **IL1** binds with the enzyme protein, it seems possible that a polymer part of the **IL1** binding with the lipase protein may have a certain impact on the flexibility of the lipase protein. The other factor is the influence of the presence of water molecules that might occur in **IL1**. The water content of the reaction mixture (Table 2, entry 2), estimated by the Karl–Fischer method, was 680 ppm. This level of water content does not seem to be an important factor in increased enantioselectivity.[21b] From MALDI-TOF mass experiments on **IL1**, we estimated that this salt had at least 23 water molecules,[40] though it was impossible to determine the exact number. A small amount of water reportedly significantly influenced the flexibility of enzymes and accelerated the transesterification of lipase in the organic solvent system.[21] The third possible origin of the present lipase activation is that a cationic part of the ionic liquid binds with lipase protein and causes preferential modification of the enzyme conformation; this may explain why the present activation of the enzyme depends on the substrate.

Conclusion

We discovered that the novel ionic liquid [bdmim][cetyl-PEG10-sulfate](**IL1**) worked as an excellent activator of lipase PS-catalyzed acetylation of various types of secondary alcohols by using vinyl acetate as acyl donor in iPr$_2$O, while maintaining excellent enantioselectivity. More than 1000-fold rate acceleration was accomplished for some substrates. Lyophilization was essential to activate the lipase effectively by the ionic liquid. Our activation system made it possible to realize both increased enantioselectivity and rate acceleration. In contrast, the previously established activation protocol, such as PEG coating of a lipase could accelerate the reaction, but caused no significant increased enantioselectivity; also, using toluene as solvent sometimes provided increased enantioselectivity for some substrates, but other times caused a significant reduction in the reaction rate. Since similar activation was observed for CRL, we expect that the activation effect of **IL1** might be general for various lipases. We believe that this work represents not only a significant advance in the manner of preparation of optically active compounds by using an enzymatic reaction, but also provides a new aspect in the application of an ionic liquid for such a reaction. Since the present activation was significantly dependent on the substrate, we are also hoping that further optimization of the cationic part of the ionic liquids may make it possible to apply the present protocol of lipase activation for various broader types of substrates.

Experimental Section

Reagents and solvents were purchased from common commercial sources and were used as received or purified by distillation over appropriate drying agents. ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM MH-500 or JNM MH-400 MHz spectrometer. Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in $CDCl_3$ as an internal reference. IR spectra were obtained on SHIMADZU FTIR 8000 spectrometers. Optical rotation was measured with a JASCO DIP-370 digital polarimeter. The rate was determined by gas chromatography analysis (Quadrex-bonded fused-silica methyl silicone, ∅ 0.25 mm × 25 m, N_2). The optical purity was determined by HPLC analysis by using OD, OD-H, OB, AD, or OJ-H, and capillary gas chromatography (Chiraldex G-TA, ∅ 0.25 mm × 20 m, 100 °C, He). MALDI-TOF MS spectra were obtained by using a BRUKER AutoFLEX-T2 apparatus. SEM images were recorded by using a JEOL JSM-6390 LV instrument.

1-Butyl-2,3-dimethylimidazolium chloride: A solution of 1,2-dimethylimidazole (55.4 g, 0.58 mol) and 1-chlorobutane (53.3 g, 0.58 mol) was stirred for 24 h under reflux conditions. After being cooled to RT, excess 1-chlorobutane was removed under reduced pressure to give 1-butyl-2,3-dimethylimidazolium chloride ([bdmim][Cl]) as a half-melted white solid and it was used in the next reaction without further purification.

Ammonium poly[oxyethylene(*n*)] alkyl sulfate: A mixture of Brij56 (poly[oxyethylene(10)] cetyl ether) (13.7 g, 20.0 mmol) and sulfamic acid (1.94 g, 20.0 mmol) was stirred for 17 h at 110 °C under argon and dried under reduced pressure at 66.7 Pa, at 60 °C, for 3 h, to give ammonium Brij56 sulfate as a white solid.

1-Butyl-2,3-dimethylimidazolium poly(oxyethylene) alkyl sulfate (IL1): After the preparation of ammonium poly[oxyethylene(10)] cetyl sulfate and 1-butyl-2,3-dimethylimidazolium chloride, these two crude products were added to acetone (20 mL) and the mixture was stirred for 24 h at RT. The ammonium chloride that precipitated was removed by filtration through a sintered glass filter with a Celite pad. The filtrate was concentrated under vacuum for a little while, and then activated carbon was added, and the mixture was stirred for 10 min. The activated carbon was filtered through a sintered glass filter with a Celite pad and the filtrate was filtered through an Al_2O_3 (neutral type I, activated) short column. The filtrate was evaporated and dried under reduced pressure at 5 Torr, for 24 h, at 60 °C, to give 1-butyl-2,3-dimethylimidazolium poly[oxyethylene(10)] cetyl sulfate (13.6 g, 0.015 mol) as a yellowish solid at RT in 74 % yield.

1-Butyl-2,3-dimethylimidazolium poly[oxyethylene(10)] cetyl sulfate (IL1) (from Brij56): M.p. 35–37 °C; ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.80$ (t, $J = 7.4$ Hz, 3H), 0.88 (t, $J = 7.3$ Hz, 3H), 1.10–1.30 (m, 32H), 1.29–1.31 (m, 2H), 1.47–1.50 (m, 4H), 1.70–1.73 (m, 2H), 2.61 (s, 3H), 3.36 (t, $J =$ 5.1 Hz, 4H), 3.49–3.62 (m, 34H), 3.81 (s, 3H), 4.00 (t, $J = 5.1$ Hz, 2H), 4.07 (t, $J = 7.8$ Hz, 2H), 7.24 (s, 1H), 7.38 ppm (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 9.48$, 13.20, 13.77, 19.23, 22.30, 25.73, 28.98, 29.11, 29.23, 29.31, 31.35, 31.54, 35.06, 48.01, 61.20, 65.78, 69.68, 69.05, 70.19, 71.12, 72.26, 120.17, 122.65, 143.57 ppm; IR (neat): $\tilde{\nu} = 2916$, 2851, 1468, 1350, 1252, 1115, 951, 845 cm^{-1}; MALDI-TOF MS (matrix: SA): m/z calcd for $C_{45}H_{90}N_2O_{14}S$ (average MW): 915.3; found: 1344.

Preparation of IL1-supported lipase PS (IL1-PS) by lyophilization: Commercial lipase PS-C (1.0 g; Amano) was added to a potassium phosphate buffer (pH 7.2) solution (0.1 M, 10 mL). The mixture was centrifuged at 3500 rpm for 5 min. **IL1** (29.0 mg, approximately 3.1×10^{-2} mmol) was dissolved into the resulting supernatant, that contains approximately 3.1×10^{-4} mmol of lipase PS protein, and the mixture was lyophilized to give **IL1**-PS (344 mg) as a white powder. By using the same procedure, thirteen types of supported lipase PS compounds were prepared (for details, see Supporting Information).

Lipase PS-C-catalyzed acylation of (±)-1-phenylethanol (1a) with additive in iPr$_2$O: Lipase PS-C (25 mg) was added to a solution of (±)-**1a** (50 mg, 0.41 mmol), vinyl acetate (52.9 mg, 0.62 mmol), and an additive (0.04 mmol) in iPr$_2$O (2.0 mL) and the mixture was stirred at 35 °C. The reaction course was monitored by capillary GC analysis and silica gel

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TLC. Compounds (*R*)-**2a** and (*S*)-**3a** were obtained by preparative silica gel thin-layer chromatography (TLC). The enantioselectivity was determined by HPLC analysis on a chiral column (Chiralcel OB, hexane/2-propanol = 8:1, 200:1).

(*R*)-**2a**: $R_f = 0.55$ (hexane/ethyl acetate 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 1.47$ (d, $J = 6.9$ Hz, 3H), 2.00 (s, 3H), 5.81 (q, $J = 6.9$ Hz, 1H), 7.19–7.29 ppm (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 21.25$, 22.12, 72.22, 126.00, 127.77, 128.40, 141.59, 170.21 ppm; IR (neat): $\tilde{\nu} = 2980$, 1730, 1495, 1370, 1240, 1030, 940, 760 cm^{-1}.

(*S*)-**3a**: $R_f = 0.25$ (hexane/ethyl acetate = 4:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.43 (d, $J = 6.4$ Hz, 3H), 1.75 (s, 1H; OH), 4.83 (q, $J = 6.4$ Hz, 1H), 7.28–7.30 ppm (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 25.01$, 70.13, 125.30, 127.26, 128.33, 145.76 ppm; IR (neat): $\tilde{\nu} = 3330$, 3030, 2970, 2890, 1490, 1450, 1010, 900 cm^{-1}.

IL1-PS-catalyzed acylation of 1-phenylethanol (1a) in *i*Pr$_2$O: **IL1**-PS (7.0 mg) was added to a solution of (±)-**1a** (50 mg, 0.41 mmol) and vinyl acetate (52.9 mg, 0.62 mmol) in *i*Pr$_2$O (2.0 mL) and the mixture was stirred at 35 °C. The reaction course was monitored by capillary GC analysis and silica gel TLC. Compounds (*R*)-**2a** and (*S*)-**3a** were obtained by preparative silica gel thin-layer chromatography (TLC). The enantioselectivity was determined by HPLC analysis by using a chiral column (Chiralcel OB, hexane/2-propanol = 8:1, 200:1).

1-Butyl-3-methylimidazolium 2,2,3,3,4,4,5,5-octafluoropentyl sulfate ([bmim][C5F8]):[35] A mixture of 2,2,3,3,4,4,5,5-octafluoropentanol (33.0 g, 0.14 mol) and sulfamic acid (13.6 g, 0.14 mol) was stirred at 130 °C for 24 h under argon atmosphere and was cooled to RT to give ammonium 2,2,3,3,4,4,5,5-octafluoropentyl sulfate as a white precipitate. The ammonium salt was washed with hexane three times and evaporated to dryness. [bmim][Cl] (23.6 g, 0.135 mol) was added to a solution of the salt in acetone (135 mL) and the resulting solution was stirred 24 h at RT to form ammonium chloride (NH_4Cl) as a white precipitate. Precipitated NH_4Cl was removed by filtration through a sintered glass filter with a Celite pad and the filtrate was concentrated under vacuum to give [bmim][C5F8] as viscous oil. This was washed with a mixed solvent of hexane and ethyl acetate (4:1) and water, then diluted with acetone, and treated with activated charcoal. The activated charcoal was removed by filtration through a sintered glass filter with a Celite pad and the filtrate was filtered through an Al_2O_3 (neutral type I, activated) short column, then was finally lyophilized to give [bmim][C5F8] (40.2 g, 0.089 mol) as yellowish oil in 66% yield: ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 0.95$ (t, $J = 7.3$ Hz, 3H), 1.32–1.40 (m, 2H), 1.83–1.89 (m, 2H), 3.98 (s, 3H), 4.21 (t, $J = 7.4$ Hz, 2H), 4.45 (t, $J = 14.7$ Hz, 2H), 5.99–6.23 (m, 1H), 7.32 (s, 1H), 7.39 (s, 1H), 9.18 ppm (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 12.99$, 19.16, 31.78, 36.02, 49.65, 62.95 (t, $J_{C,F} = 24.9$ Hz), 107.53 (2t, $J_{C,F} = 30.5$ Hz, $J_{C,F} = 253.3$ Hz; 2-CF_2), 109.93 (2t, $J_{C,F} = 25.8$ Hz, $J_{C,F} = 261.3$ Hz; 3-CF_2), 110.63 (2t, $J_{C,F} = 30.5$ Hz, $J_{C,F} = 263.2$ Hz; 4-CF_2), 114.85 (2t, $J_{C,F} = 30.5$ Hz, $J_{C,F} = 253.3$ Hz; CF_2H), 122.07, 123.62, 136.56 ppm; ^{19}F NMR (470 MHz, $CDCl_3$): $\delta = 24.31$ (t, $J = 51.8$ Hz, 2F), 31.61 (s, 2F), 36.25 (s, 2F), 41.57 ppm (t, $J = 11.5$ Hz, 2F); IR (neat): $\tilde{\nu} = 2880$, 1510, 1460, 1270, 1240, 1170, 1130, 1040 cm^{-1}; elemental analysis calcd (%) for $C_{13}H_{18}F_8N_2O_3S$: C 35.95, H 4.18, N 6.45; found: C 35.88, H 4.19, N 6.46.

Lipase PS-C-catalyzed acylation of 4-phenyl-3-butene-2-ol (1j) in [bmim][C5F8]—recyclable use of enzyme: Lipase PS-C (27.2 mg) was added to a mixture of 4-phenyl-3-butene-2-ol (±)-**1j** (59.0 mg, 0.35 mmol) and vinyl acetate (50.2 mg, 0.58 mmol) in [bmim][C5F8] (1.0 mL) and the mixture was stirred at 35 °C for 24 h. The reaction course was monitored by capillary GC analysis and silica gel TLC. Diethyl ether (1.5 mL) was added to the reaction mixture of to form biphasic layers and the product acetate (*R*)-**2j** and alcohol (*S*)-**3j** were isolated from the ether layer. It was essential to repeat the extraction with ether from the reaction mixture ten times. Since the lipase remained in the ionic liquid layer, it was possible to use the lipase repeatedly; the ionic layer was placed under reduced pressure at RT for 5 h to remove the ether and (±)-**1j** (59.0 mg, 0.35 mmol) and vinyl acetate (50.2 mg, 0.58 mmol) was added to the resulting ionic layer and the mixture was stirred at 35 °C.

Acknowledgements

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[40] See Supporting Information for MALDI-TOF MS spectra of **IL1**.

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