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Enhanced activity and modified substrate-favoritism of *Burkholderia cepacia* lipase by the treatment with a pyridinium alkyl-PEG sulfate ionic liquid

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ABSTRACT

Three types of pyridinium salts, i.e., 1-ethylpyridin-1-ium cetyl-PEG$_{10}$ sulfate (PY$_{ET}$), 1-butylpyridin-1-ium cetyl-PEG$_{10}$ sulfate (PY$_{BU}$), and 1-(3-methoxypropyl)pyridin-1-ium cetyl-PEG$_{10}$ sulfate (PY$_{MP}$), have been prepared and evaluated for their activation property of *Burkholderia cepacia* lipase by comparison to the control IL-coated enzymes, 1-butyl-2,3-dimethylimidazolium cetyl-PEG$_{10}$ sulfate-coated lipase PS (IL1-PS). Among the tested pyridinium salt-coated lipases, the PY$_{ET}$-coated lipase PS (PY$_{ET}$-PS) exhibited the best results; the transesterification of 1-(pyridin-2-yl)ethanol, 1-(pyridin-3-yl)ethanol, 1-(pyridin-4-yl)ethanol, or 4-phenylbut-3-en-2-ol proceeded faster than those of the IL1-PS-catalyzed reaction while maintaining an excellent enantioselectivity ($E > 200$). This improved efficiency was found to be dependent on the increased K_{cat} value.

1. Introduction

Lipases (triglycerol acylhydrolases EC 3.1.1.3) are the most widely used enzymes as a synthetic tool because they can catalyze a number of reactions including hydrolysis, transesterification, alcoholysis, acidolysis, esterification, and aminolysis [1]. Lipases are generally tolerable to a wide number of substrates, however, very slow reactions or poor enantioselective reactions are sometimes obtained [1]. The sciences of ionic liquids (IL) have significantly progressed in various fields during the past two decades [2,3]. The most fascinating property of the ILs is their structural diversity, which allows their utilization in various stages of chemistry in both chemical [4–11] and enzymatic reactions [12–17]. We have thus demonstrated that ionic liquid-engineering in lipase-catalyzed reactions allows a possible solution to the problems in the use of lipase-catalyzed reactions for organic synthesis, increasing the reaction rate of transesterification and modifying the enantioselectivity [13].

In 2002, Lee and Kim reported the example of a modified enantioselectivity of the lipase-catalyzed transesterification by the ionic liquid coating process, though no acceleration had been accomplished by their method [18]. We found that the key solution to realize the IL-mediated improved performance of lipase-catalyzed reactions was in both the appropriate design of the ILs and the preparation method of "the ionic liquid-coated enzyme" in 2004 [19]; we synthesized 1-butyl-2,3-dimethylimidazolium 3,6,9,12,15,18,21,24,27,30-decaoxacetyltriacontyl sulfate (cetyl-PEG$_{10}$ sulfate)(IL1) and prepared the IL1-coated-*Burkholderia cepacia* lipase (IL1-PS) through lyophilization (IL1-PS) [19,20]. The resulting enzyme worked as an excellent catalyst for the enantioselective transesterification of various types of secondary alcohols in both conventional organic solvents or IL solvent systems and a significant acceleration was recorded for many substrates while maintaining the original enantioselectivity [20–25]. The chirality of the imidazolium cation influenced the activation property of the ILs; 1-((*R*)- pyrroridin-2-yl)methyl-2,3-dimethylimidazolium cetyl-PEG$_{10}$ sulfate (D-ProMe) (Fig. 1) more strongly activated the lipase compared to that of the (*S*)-isomer [25]. We next discovered the cooperative activation of IL1 with amino acids [26]; the coating on the lipase PS using IL1 with L-proline or L-tyrosine effectively activated the lipase while maintaining an excellent enantioselectivity (E value [27] >200). We next found that the lipase PS coated with tributyl(2-methoxyethyl)phosphonium cetyl-PEG$_{10}$ sulfate (PL1-PS) showed a different substrate specificity to that of the IL1-PS

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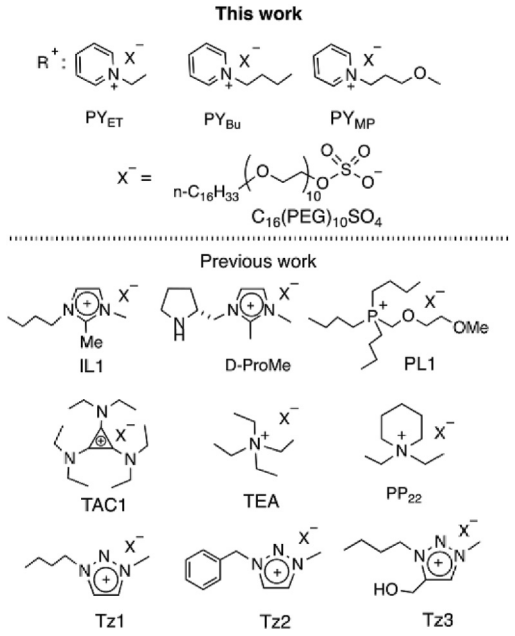


Fig. 1. Ionic liquids as activating agents of the lipase-catalyzed transesterification of secondary alcohols.

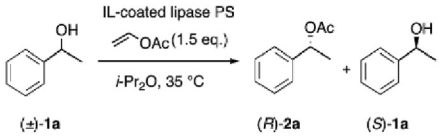
(Fig. 1) [28]. Kim and co-workers reported the activation of a lipase by immobilization of the enzyme with ionic surfactant compounds that have an alkyl PEG moiety through lyophilization [29–31]. Rahman and co-workers also reported that amino acid ILs worked as good activating agents of *Candida rugose* lipase [32].

We further investigated the design of ILs that affect the reactivities of lipases focusing on the cationic part of cetyl-PEG_{10} sulfate; the substrate specificity of lipase PS was significantly modified when the enzyme was coated with the quaternary ammonium type cetyl-PEG_{10} sulfates (TAC1, TEA, or PP_{22}) (Fig. 1) [33]. In particular, tris(diethylamino)cyclopropenium cetyl-PEG_{10} sulfate-coated lipase PS (TAC1-PS) displayed a different substrate specificity compared to the previously reported IL1-, D-ProMe-, or PL1-PS. We next prepared 1-butyl-3-methyl-1,2,3-triazolium cetyl-PEG_{10} sulfate (Tz1)-coated lipase PS (Tz1-PS) and revealed that Tz1-PS exhibited almost the same activity as that of the freshly-prepared one even after storage for 2 years in $[N_{221MEM}][Tf_2N]$ (See Fig. 1) [34].

Lee and co-worker recently reported that the preparation of a glucose-headed surfactant (GHS)-coated lipoprotein lipase from *Burholderia* species, which has three aromatic rings and alkyl PEG moieties, displayed a significant reactivity in organic solvents [35,36]. Although it was postulated that the authors used the aromatic group as a template of the PEG moiety [35,36], we anticipated that the phenyl group may contribute to the increased binding affinity on a certain part of the amino acid residue of the enzyme by a π-π or CH-π interaction. We thus paid attention to the pyridinium salt IL [37]. We postulated that pyridinium cetyl-PEG_{10} sulfate ILs might act as activating agents of enzymes because the pyridinium cation exhibits an aromatic functionality. Hence, we prepared three types of pyridinium ILs, i.e. 1-ethylpyridin-1-ium (PY_{ET}), 1-butylpyridin-1-ium (PY_{BU}), and 1-(3-methoxy)propylpyridin-1-ium (PY_{MP}) (Fig. 1, upper) and investigated their activating property against lipase PS. As expected, the resulting IL-coated enzymes displayed superior results versus those of the previously reported IL-coated lipase PSs for the enantioselective transesterification of several secondary alcohols.

2. Results and discussion

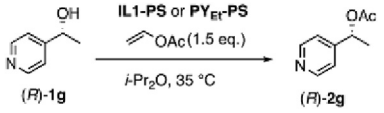
We prepared the cetyl-PEG_{10} sulfate salts with three types of pyridinium cations, i.e., 1-ethylpyridin-1-ium (PY_{ET}), 1-butylpyridin-1-ium (PY_{BU}), and 1-(3-methoxypropyl)pyridine-1-ium (PY_{PM}), then used them as supporting materials of *Burkholderia cepacia* lipase (the lipase PS). Their catalytic ability was evaluated by comparison of the results obtained by the commercial lipase PS (native PS-C), IL1-PS [20], TAC1-PS [33], and PP_{22}-PS [33] using 1-phenylethanol (**1a**) as a model substrate in diisopropyl ether (*i*-Pr_2O) as the solvent in the presence of 1.5 equivalents of vinyl acetate as the acyl donor at 35 °C (Eq. (1) and Table 1).



(1)

As noted in Table 1, the three novel IL-coated lipase PSs (Entries 5–7) worked well as catalysts for all the substrates tested and 44- to 57-fold accelerations were obtained compared to the native lipase PS (entry 1), though the reaction catalyzed by IL1-PS proceeded with the fastest reaction rate for this substrate (entry 2). Compared to the results obtained from the quaternary ammonium type salts-coated PS, TAC1-PS (entry 3) or 1,1-diethylpiperidinium cetyl-PEG_{10} sulfate-coated PS (PP_{22}-PS) (entry 4), the pyridinium salt-coated lipase PSs afforded better results (entries 5–7). In particular, PY_{ET}-PS gave the best results among the three tested pyridinium cetyl-PEG_{10} sulfate-coated enzymes (entry 5). Therefore, we choose PY_{ET}-PS for further study and investigated the kinetic resolution of various secondary alcohols.

Table 2 shows the PY_{ET}-PS-catalyzed kinetic resolution for 12 types of secondary alcohols. We also demonstrated the native PS-C and IL1-PS-catalyzed reactions as control experiments (Table 2). PY_{ET}-PS gave excellent results for all the substrates compared to those of the native PS-C, though IL1-PS gave results superior to those of PY_{ET}-PS for (±)-**1a**, (±)-**1b**, and (±)-**1c** (entries 1–3, 6, 9). To our delight, PY_{ET}-PS provided the best results for the three pyridinyl alcohols, (±)-**1g**, (±)-**1h**, and (±)-**1i** (entries 7 to 9) and (±)-4-phenylbut-3-en-2-ol (**1j**) (entry 10). For the reaction of (±)-**1k**, a 47-fold faster reaction was accomplished when using PY_{ET}-PS compared to the native PS-C, though a slight reduction of the enantioselectivity was observed (entry 11). In the reaction of (±)-5-phenylpent-1-en-2-ol (**1l**), PY_{ET}-PS exhibited results superior to those of the native PS and IL1-PS (entry 12).



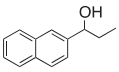
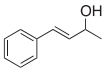
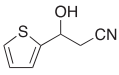
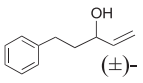
(2)

Table 1
Enantioselective transesterification of 1-phenylethanol (**1a**) using various types of IL-coated lipase PSs in the presence of 1.5 eq. of vinyl acetate as an acyl donor.

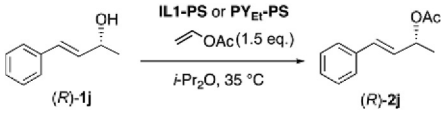
Entry	Enzyme	Rate[a]	conv. (%)[b]	(*R*)-**2a** (%ee)	(*S*)-**1a** (% ee)	E value
1	native-PS-C	14	43[c]	>99	74	>200
2	IL1-PS	884	43	>99	77	>200
3	TAC1-PS	605	30	>99	44	>200
4	PP_{22}-PS	593	27	>99	37	>200
5	PY_{ET}-PS	799	42	>99	70	>200
6	PY_{BU}-PS	613	30	>99	44	>200
7	PY_{MP}-PS	708	35	>99	53	>200

[a] Rate: mM h^{-1} mg $enzyme^{-1}$.
[b] Reaction time: 30 min.
[c] Reaction time: 24 h.

Table 2
Results of enantioselective transesterification of secondary alcohols using lipase PS, IL1-PS, and PY_{ET}-PS in the presence of 1.5 eq. of vinyl acetate as an acyl donor.

Entry	Substrate	Enzyme		
		native PS-C	IL1-PS	PY_{ET}-PS
		Rate[a] (E value)[b]	Rate[a] (E value)[b]	Rate[a] (E value)[b]
1	 (±)-**1a**	16 (>200)[c]	**890** (>200)	799 (>200)
2	 (±)-**1b**	60 (>200)[c,d]	**1950** (>200)[c,d]	**1903** (>200)
3	 (±)-**1c**	13 (>200)[c,d]	**569** (>200)[c,d]	455 (>200)
4	 (±)-**1d**	0.01 (>200)[c,d]	22 (>200)[c,d]	**33** (>200)
5	 (±)-**1e**	0.01 (>200)[c,d]	**14** (>200)[c,d]	**15** (>200)
6	 (±)-**1f**	2.5 (>200)[c]	**68** (147)[c]	57 (131)
7	 (±)-**1g**	7.2 (>200)	500 (112)	**1274** (>200)
8	 (±)-**1h**	8.0 (>200)[c,d]	341 (132)[c,d]	**430** (>200)
9	 (±)-**1i**	6.0 (>200)[c,d]	**383** (>200)[c,d]	**312** (>200)
10	 (±)-**1j**	6.8 (>200)	285 (>200)	**778** (>200)
11	 (±)-**1k**	6.6 (189)[c,d]	295 (40)[c,d]	**313** (121)
12	 (±)-**1l**	3.0 (>200)	33 (>200)	**100** (>200)

[a] Rate: mM h^{-1} mg $enzyme^{-1}$. Results using bold font showed the best ones among the tested enzymes.
[b] Reaction time: 30 min.
[c] Reaction time: 24 h.
[d] Reported results in ref. 33.



(3)

In order to obtain further insight into the PY_{ET}-PS mediated activation of the native PS-C, we next conducted a kinetic experiment using (*R*)-1-(pyridn-3-yl)ethanol (**1g**) (Eq. (2)) and (*R*)-4-phenylbut-3-en-2-ol (**1j**) (Eq. (3)). As we previously reported [33,34], the activation profile depended on both the cationic part of the coating materials and the substrate (Table 3). Significantly increased V_{max} and K_{cat} values were recorded for the PY_{ET}-PS-catalyzed reactions for both substrates compared to those of IL1-PS. In particular, the K_{cat} value of PY_{ET}-PS for substrate (*R*)-**1g** was 30-fold higher than that of IL1-PS. However, increased K_m values were also obtained for the PY_{ET}-PS-catalyzed reactions by both substrates. In the case of the reaction using (*R*)-**1g**, K_m value of PY_{ET}-PS was 35-fold larger than that of IL1-PS. Therefore, as results, no significant difference was found for the resulting K_{cat}/K_m values between IL1-PS and PY_{ET}-PS for this substrate. It has been reported that lipases from different organisms have a closed conformation, and catalytic triads are buried beneath a helical lid segment [38–40]. Studies with lipases covalently complexed compounds and organic solvents indicated a large rigid movement of the lid part that plays an important role in the catalytic activity of the lipases [41,42]. The substrate is then introduced into the catalytic site when the lid part has an open conformation [41]. It was suggested that both the K_m values and K_{cat} values might be increased in the open form [35,36,42]. The present kinetic experiments thus indicate that the cationic part of the coated ILs strongly influence the enzyme specificity towards the substrates by changing its structure for the open form by binding with a certain part of the enzyme protein surface.

The changing of the open form of the lid part of the enzyme seemed to be the origin of the activation of the pyridinium alkyl PEG sulfate coating, thus we anticipated that the reaction might be dependent on the acyl donor. Therefore, we next investigated the preference of acyl donor compounds against PY_{ET}-PS by comparison with those of the native PS-C and IL1-PS using four types of acyl donors, i.e., vinyl acetate, vinyl butyrate, vinyl octanoate, and 2,2,2-trifluoroethyl octanoate (Table 4). For the enantioselectivity of these reactions, all the reactions proceeded with E values over 200. However, as expected, the reaction rate depended on the employed acyl donor. Both IL1-PS and PY_{ET}-PS gave better results when using vinyl octanoate in the reaction of (±)-**1a** (entry 3). On the other hand, PY_{ET}-PS had a higher reaction rate using vinyl acetate (entry 5) or vinyl butanoate in the reaction of (±)-**1g** (entry 6). For the reaction of (±)-**1j**, the reactions mediated IL1-PS were faster than those of PY_{ET}-PS for vinyl butanoate or vinyl octanoate, PY_{ET}-PS proceeded faster when using vinyl acetate (entry 9). It was thus found that optimization of the acyl donor was also important for the IL-coated lipase catalyzed transesterification.

Table 3
Kinetic parameters of the PY_{ET}-PS -catalyzed transesterification using vinyl acetate as an acyl donor for the two types of (*R*)-alcohols.

Substrate	Enzyme	V_{max}[a]	K_m[a]	K_{cat}[a]	K_{cat}/K_m[a]
(*R*)-**1g**	IL1-PS	4.4×10^{-3}	0.074	2.0×10^{3}	2.6×10^{4}
	PY_{Et}-PS	1.9×10^{-1}	2.6	6.2×10^{4}	2.4×10^{4}
(*R*)-**1j**	IL1-PS	2.2×10^{-2}	0.25	7.8×10^{3}	3.2×10^{4}
	PY_{ET}-PS	4.1×10^{-1}	0.58	1.3×10^{4}	2.3×10^{4}

[a] V_{max}: M min^{-1}, K_m: M, K_{cat}: min^{-1}, K_{cat}/K_m: min^{-1} M^{-1}.

Table 4
Acyl donor preference on the reaction rate for the lipase-catalyzed transesterification of three types of alcohols.

Entry	Substrate	Acyl donor	native PS-C	IL1-PS	PY_{ET}-PS
			Reaction rate[a]	Reaction rate[a]	Reaction rate[a]
1	(±)-**1a**	Vinyl acetate	19	884	799
2		Vinyl butanoate	19	917	497
3		Vinyl octanoate	26	1183	911
4		2,2,2-trifluoroethyl octanoate	7.1	690	618
5	(±)-**1g**	Vinyl acetate	11	821	1274
6		Vinyl butanoate	7.2	578	778
7		Vinyl octanoate	46	1019	969
8		2,2,2-trifluoroethyl octanoate	7.1	616	517
9	(±)-**1j**	Vinyl acetate	7.2	372	778
10		Vinyl butanoate	12	487	395
11		Vinyl octanoate	24	752	739
12		2,2,2-trifluoroethyl octanoate	6.3	504	482

[a] Rate: mM h^{-1} mg $enzyme^{-1}$.

3. Conclusions

We have synthesized three types of pyridinium cetyl-PEG10 sulfate ILs and evaluated their property for activation of *Burkholderia cepacia* lipase by the coating process through lyophilization. Among the tested IL-coated enzymes, the PY_{ET}-coated lipase PS (PY_{ET}-PS) was especially suitable for the transesterification of 1-(pyridin-4-yl)ethanol (**1g**), 1-(pyridin-3-yl)ethanol (**1h**), 1-(pyridin-2-yl)ethanol (**1i**), and 4-phenylbut-3-en-2-ol (**1j**). To the best of our knowledge, the fastest transesterification was accomplished while maintaining a perfect enantioselectivity (E value > 200) and this was postulated to be due to the increasing K_{cat} value. It should be noted that the lipase reactivity could be enhanced only by coating with these ionic liquids, and the cationic part of the IL plays an important role in the substrate preference of the enzyme.

4. Experimental

4.1. General procedures

The reagents and solvents were purchased from common commercial sources and used as received or purified by distillation over the appropriate drying agents. *Burkholderia cepacia* lipase (native PS-C) was purchased from Amano Enzyme Ltd. The reactions requiring anhydrous conditions were carried out under argon with dry, freshly distilled solvents, and magnetic stirring. The reactions, except for the preparation of the ionic liquids, were monitored by thin layer chromatography using a silica gel plate and GC. Thin layer chromatography was performed with the indicated solvents and Wako gel B-5F. The ^1H NMR spectra and ^{13}C NMR spectra were recorded by a JEOL JNM MH-500 (500 MHz for ^1H and 125 MHz for ^{13}C). Chemical shifts are expressed in ppm downfield from tetramethylsilane (TMS) in $CDCl_3$ as the internal reference. The IR spectra were obtained using a SHIMADZU FT-IR 8000 spectrometers. High resolution mass spectra were recorded by a Thermo Fisher Scientific EXACTIVE mass spectrometer. The rate was determined by a gas chromatography analysis (Quadrex bonded fused silica methyl silicone, ϕ 0.25 mm × 25 m, N_2). The optical purity was determined by HPLC analysis using Daicel OD, OD-H, OB, AD, or OJ-H.

4.2. Preparation of PY_{ET}

A mixture of pyridine (0.80 g, 10 mmol) and bromoethane (1.30 g, 12 mmol) was stirred ar rt for 43 h, then the mixture was washed with ether twice and treated with active charcoal for 12 h by stirring. After removing active charcoal by filtration and washed with ether, the filtrate was dried under reduced pressure at rt to give 1-ethylpyridin-1-ium bromide (0.60 g, 3.2 mmol) in 32% yield. Mp 120 °C, ^1H NMR (500 MHz, ppm, $CDCl_3$ J = Hz) δ 1.75 (3H, t, J = 7.5), 5.10 (2H, q, J = 7.5), 8.15 (2H, t, J = 7), 8.53 (1H, s), 9.63 (2H, d, J = 6.5); ^{13}C NMR (125 MHz, ppm, $CDCl_3$ J = Hz) δ 17.37, 57.12, 57.39, 128.48, 128.64, 144.74, 144.92, 145.08, 145.60.

A mixture of 1-ethylpyridin-1-ium bromide (150 mg, 0.80 mmol) and ammonium cetyl-PEG_{10} sulfate [20] (624 mg, 0.80 mmol) in dichloromethane (CH_2Cl_2) (8.0 ml) was stirred at rt for 24 h to form the precipitate of ammonium bromide (NH_4Br). The precipitate was removed by filtration and the extract was diluted with CH_2Cl_2 and cooled to −25 °C to form the precipitate of NH_4Br which was removed by filtration. This process was repeated several times until no more precipitate was formed. The resulting filtrate was dried by lyophilization to give 666 mg (0.77 mmol) of **PY_{ET}** in 96% yield: mp 35.2 °C (DSC); ^1H NMR (500 MHz, ppm, $CDCl_3$ J = Hz) δ 0.88 (3H, t, J = 7.5), 1.25–1.58 (28H, m) 1.73 (1H, t, J = 7.5), 2.77 (2H, s), 3.44 (2H, t, 7), 3.58–3.74 (40H, m), 3.59–3.73 (46H+α, m), 5,03 (2H, q, J = 7.5), 8.14 (2H, t, J = 7.5), 8.50 (1H, t, 8), 9.47 (2H, d, J = 5.5); ^{13}C NMR (125 MHz, ppm, $CDCl_3$) δ 14.10, 17.18, 22.64, 26.00, 29.30, 29.44, 29.56, 29.63, 31.86, 61.42, 69.92, 70.41, 71.49, 72.56, 128.44, 144.91; IR (neat, cm^{-1}) 2917, 2887, 2849, 1725, 1633, 1467, 1346, 1278, 1246, 1112, 965, 846, 781, 713, 686, 630, 580, 530; ESI-MS m/z: 108.0802 (M^+); calcd for $C_7H_{10}N^+$ (M^+) 108.0814.

PY_{BU} and PY_{MP} were prepared by a similar method.

PY_{BU}: mp 33.5 °C (DSC); ^1H NMR (500 MHz, ppm, $CDCl_3$ J = Hz) δ 0.88 (3H, t, J = 7.5), 0.96 (3H, t, J = 7.5), 1.25–1.41 (28H, m), 1.55 (2H, d, J = 7.0) 1.98 (2H, m), 3.42 (2H, t, J = 7.0), 3.55–3.72 (40H, m) 4.16(1H, t, J = 4.5), 4.89 (2H, t, J = 7.0), 8.12 (2H, t, J = 7.0), 8.48 (1H, t, J = 7.5), 9.33 (2H, d, J = 5.5); ^{13}C NMR (125 MHz, ppm, $CDCl_3$) δ 13.49, 14.08, 19.34, 22.65, 26.04, 29.31, 29.46, 29.58, 29.65, 31.38, 33.69, 61.56, 61.89, 66.28, 69.98, 70.19, 70.36, 70.49, 71.51, 72.50, 128.38, 144.92, 145.26; IR (neat, cm^{-1}) 2917, 2887, 2852, 2470, 1636, 1464, 1343, 1281, 1237, 1109,1033, 962, 846, 775, 719, 689, 719, 689, 630, 580, 541; ESI-MS m/z: 136.1113 (M^+); calcd for $C_9H_{14}N^+$ (M^+) 136.1127.

PY_{MP}: mp 35.4 °C (DSC); ^1H NMR (500 MHz, ppm, $CDCl_3$ J = Hz) δ 0.88 (3H, t, J = 7.5), 1.25–1.28 (26H, m), 1.57 (2H, q, J = 6.5), 2.34 (2H, m), 3.24 (3H, s), 3.45 (4H, m), 3.57–3.74 (38H+α, m), 4.16 (1H, t, J = 5), 5.01 (2H, t, J = 6.5), 8.10 (2H, t, J = 7.5), 8.50 (1H, t, J = 7.5), 9.36 (2H, d, J = 6.0); ^{13}C NMR (125 MHz, ppm, $CDCl_3$) δ 14.06, 22.63, 26.02, 29.29, 29.44, 29.56, 29.63, 31.14, 31.85, 58.62, 59.79, 61.59, 66.27, 68.55, 69.98, 70.18, 70.22, 70.49, 71.49, 72.48, 128.13, 145.00, 145.54; IR (neat, cm^{-1}) 2914, 2887, 2852, 1636, 1464, 1346, 1281, 1106, 1027, 959, 843, 772, 716, 630, 580, 530, 476; ESI-MS m/z: 152.1060 (M^+); calcd for $C_9H_{14}NO^+$ (M^+) 152.1076.

4.3. Preparation of PY_{ET}-PS, PY_{BU}-PS, and PY_{MP}-PS

Commercial lipase PS (native PS-C) (Amano Enzyme Ltd.: 1.002 g, enzyme protein 10 mg; 3.1×10^{-4} mmol) was dissolved in 10 ml of 0.1 M phosphate buffer (pH 7.2) and the mixture was centrifuged twice at 3500 rpm for 5 min, then the resulting supernatant was mixed with PY_{ET} (23.3 mg, 3.1×10^{-2} mmol). The mixture was stirred for 15 min at rt, then lyophilized to afford PY_{ET} -PS (283 mg). The native PS-C included 1.0% lipase protein (3.1×10^{-4} mmol), 20 wt % glycine, and the rest Celite. The 10 ml of the 0.1 M potassium phosphate buffer included 124.9 mg of K_2HPO_4 and 38.5 mg of KH_2PO_4, thus the theoretical amount of PY_{ET} -PS after lyophilization was calculated to be 396.7 mg. However, the amount of the obtained powder was 283 mg. We suppose that the glycine and K_2HPO_4 or KH_2PO_4 form complexes, some of which were removed by Celite absorption during the process. Because the enzyme protein and PY_{ET} might not be removed during the process, we estimated the enzyme content in this PY_{ET}-PS powder was 3.5% (w/w) and the remaining was PY_{ET} and very fine inorganic materials derived from the Celite or potassium phosphate buffer. PY_{BU}-PS and PY_{PM}-PS were prepared by a similar method and they contained enzyme proteins of 3.7%(w/w) and 3.9%(w/w), respectively. Since the IL-coated enzymes are very hygroscopic, these were stored in a desiccator. We also prepared IL1-PS [20] and TAC1-PS [33] according to our previous reported method and used as the control experiments.

4.4. Enzymatic reaction

4.4.1. Kinetic resolution of (±)- 1-phenylethanol (**1a**)

4.4.1.1. Results of PY_{ET}-PS-catalyzed reaction. The reaction was typically carried out as follows: To a mixture of 5.0 mg of PY_{ET}-PS (0.19 mg of the enzyme protein) in 2.0 mL of solvent (*i*-Pr_2O or IL) was added (±)-**1a** (50 mg, 0.41 mmol) and 53 mg of vinyl acetate (1.5 equiv.) and the resulting mixture was stirred at 35 °C. To evaluate the initial reaction rate, the reaction was conducted in the presence of 0.5 mmol of hexadecane as the internal reference. An aliquot of the reaction mixture was sampled after a 30 min reaction and extracted with a mixed solvent of diethyl ether and hexane (1:4) and the rate was determined by capillary GC analysis. The reaction course was monitored by silica gel thin layer chromatography (TLC) and the product (*R*)-**2a** and unreacted alcohol (*S*)-**1a** were extracted with a mixed solvent of diethyl ether and hexane (1:4) when the spots became the same size, then purified by silica gel TLC. The enantiomeric excess of the product acetate and unreacted alcohol were determined by HPLC (Chiralcel OB-H, n-hexane: 2-propanol = 9:1 or 20:1). The reaction rate was determined by GC analysis after a 30 min reaction in the presence of an internal reference. The enantioselectivity of the reaction was shown as the E value which was calculated by the %ee of (*R*)-**2** (eeP_p) and %ee of (*S*)-**1** (eeS_s). $E = \ln[(1-c(1+eeP_p))/\ln[(1-c(1-eeP_p))$; where, c means conv. which was calculated by the following formula according to the reference: $c = eeP_p/(eeP_p+eeS_s)$ [27].

(*R*)-**2a**: 21.8 mg, 0.13 mmol, Y = 32%, >99% *ee*.
(*S*)-**1a**: 23.3 mg, 0.19 mmol, Y = 46%, 70% *ee*.
Conv. 41%, *E* value > 200, Rate: 799 mM h^{-1} mg $enzyme^{-1}$
Results of PY_{BU}-PS catalyzed reaction.
(*R*)-**2a**: 17.3 mg, 0.11 mmol, Y = 27.0%, >99% *ee*.
(*S*)-**1a**: 31.8 mg, 0.26 mmol, Y = 63%, 44% *ee*.
Conv. 30%, *E* value > 200, Rate: 613 mM h^{-1} mg $enzyme^{-1}$
Results of PY_{MP}-PS catalyzed reaction.
(*R*)-**2a**: 17.1 mg, 0.10 mmol, Y = 24%, >99% *ee*.
(*S*)-**1a**: 24.8 mg, 0.20 mmol, Y = 49%, 53% *ee*.
Conv. 35%, *E* value > 200, Rate: 708 mM h^{-1} mg $enzyme^{-1}$

4.4.2. Kinetic resolution of (±)- 1-indanole (**1b**) [20] using PY_{ET}-PS

A mixture of (±)-**1b** (54.8 mg, 0.41 mmol), vinyl acetate (52.0 mg, 0.61 mmol), and PY_{ET}-PS (5.5 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 15 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate. After removal of the enzyme by filtration through a glass sintered filter with a Celite pad, silica gel TLC (hexane/ethyl acetate = 4:1) gave (*R*)-**2b** [20] and (*S*)-**1b**.

(*R*)-**2b**: 32.8 mg, 0.19 mmol, Y = 46%, >99% *ee*.
(*S*)-**1b**: 21.3 mg, 0.16 mmol, Y = 39%, >99% *ee*.
Conv. 50%, *E* value > 200, Rate: 1909 mM h^{-1} mg $enzyme^{-1}$

4.4.3. Kinetic resolution of (±)-1-(naphthalen-2-yl)ethan-1-ol (**1c**) [20] using PY_{ET}-PS

A mixture of (±)-**1c** (70.6 mg, 0.41 mmol), vinyl acetate (53.2 mg, 0.61 mmol), and PY_{ET}-PS (7.4 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 20 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate. After removal of the enzyme by filtration through a glass sintered filter with a Celite pad, silica gel TLC (hexane/ethyl acetate = 7:1) gave (*R*)-**2c** and (*S*)-**1c.**

(*R*)-2c: 12.8 mg, 0.060 mmol, Y = 15%, >99% *ee*.
(*S*)-1c: 45.8 mg, 0.27 mmol, Y = 65%, 27% *ee*.
Conv. 21%, *E* value > 200, Rate: 445 mM h^{-1} mg $enzyme^{-1}$

4.4.4. Kinetic resolution of (±)-1-(naphthalen-2-yl)propan-1-ol (**1d**) [20] using PY_{ET}-PS

A mixture of (±)-**1d** (76.1 mg, 0.41 mmol), vinyl acetate (52.7 mg, 0.61 mmol), and PY_{ET}-PS (7.5 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 40 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (hexane/ethyl acetate = 6:1) gave (*R*)-**2d** and (*S*)-**1d**.

(*R*)-2d: 6.7 mg, 0.030 mmol, Y = 7%, >99% *ee*.
(*S*)-1d: 62.1 mg, 0.33 mmol, Y = 81%, 6.4% *ee*.
Conv. 6%, *E* value > 200, Rate: 33 mM h^{-1} mg $enzyme^{-1}$

4.4.5. Kinetic resolution of (±)-1-(naphthalen-1-yl)ethan-1-ol (**1e**) [20] using PY_{ET}-PS

A mixture of (±)-**1e** (70.8 mg, 0.41 mmol), vinyl acetate (52.8 mg, 0.61 mmol), and PY_{ET}-PS (7.1 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 140 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (hexane/ethyl acetate = 4:1) gave (*R*)-**2e** and (*S*)-**1e**.

(*R*)-**2e**: 10.1 mg, 0.050 mmol, Y = 12%, >99% *ee*.
(*S*)-**1e**: 49.4 mg, 0.23 mmol, Y = 56% *ee*,
Conv. 12%, *E* value > 200, Rate: 15 mM h^{-1} mg $enzyme^{-1}$

4.4.6. Kinetic resolution of (±)- 2-chloro-1-phenylethan-1-ol (**1f**) [20] using PY_{ET}-PS

A mixture of (±)-**1f** (64.1 mg, 0.41 mmol), vinyl acetate (54.0 mg, 0.61 mmol), and PY_{ET}-PS (6.5 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 240 min. The reaction was quenched by addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (hexane/ethyl acetate = 6:1) gave (*S*)-**2f** and (*R*)-**1f.** Although acetate 2f was assigned to as the (*S*)-form and alcohol as the (*R*)-isomer following to the Cahn-Ingold-Prelog priority *rule*, the stereoselectivity of the enzyme was the same as the others.

(*S*)-**2f**: 18.7 mg, 0.090 mmol, Y = 22%, 97% *ee*.
(*R*)-**1f**: 38.0 mg, 0.24 mmol, Y = 59%, 34.7% *ee*.
Conv. 26%, *E* value > 200, Rate: 57 mM h^{-1} mg $enzyme^{-1}$

4.4.7. Kinetic resolution of (±)-1-(pyridine-4-yl)ethan-1-ol (**1g**) [20] using PY_{ET}-PS

A mixture of (±)-**1g** (50.3 mg, 0.41 mmol), vinyl acetate

(53.8 mg, 0.61 mmol), and PY_{ET}-PS (5.1 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 20 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (ethyl acetate) gave (*R*)-**2g** and (*S*)-**1g**.

(*R*)-**2g**: 23.9 mg, 0.050 mmol, Y = 34%, >99% *ee*.

(*S*)-**1g**: 28.9 mg, 0.23 mmol, Y = 56%, 80% *ee*.

Conv. 45%, *E* value > 200, Rate: 1274 mM h^{-1} mg $enzyme^{-1}$

4.4.8. Kinetic resolution of (±)-1-(pyridin-3-yl)ethan-1-ol (**1h**) [20] using PY_{Et}-PS

A mixture of (±)-**1h** (50.2 mg, 0.41 mmol), vinyl acetate (52.0 mg, 0.61 mmol), and PY_{ET}-PS (5.4 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 15 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (ethyl acetate only) gave (*R*)-**2h** and (*S*)-**1h**.

(*R*)-**2h**: 9.3 mg, 0.060 mmol, Y = 15%, >99% *ee*.

(*S*)-**1h**: 22.9 mg, 0.19 mmol, Y = 46%, 15% *ee*.

Conv. 23%, *E* value > 200, Rate: 430 mM h^{-1} mg $enzyme^{-1}$

4.4.9. Kinetic resolution of (±)-1-(pyridin-2-yl)ethan-1-ol (**1i**) [20] using PY_{ET}-PS

A mixture of (±)-**1i** (50.3 mg, 0.40 mmol), vinyl acetate (53.4 mg, 0.61 mmol), and PY_{ET}-PS (5.2 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 15 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (ethyl acetate only) gave (*R*)-**2i** and (*S*)-**1i**.

(*R*)-**2i**: 7.4 mg, 0.050 mmol, 12%, >99 %ee.

(*S*)-**1i**: 24.7 mg, 0.20 mmol, 49%, 20 %ee.

Conv. 17%, *E* value > 200, Rate: 319 mM h^{-1} mg $enzyme^{-1}$

4.4.10. Kinetic resolution of (±)- (*E*)-4-phenylbut-3-en-2-ol (**1j**) [20] using PY_{Et}-PS

A mixture of (±)-**1j** (60.6 mg, 0.41 mmol), vinyl acetate (53.5 mg, 0.61 mmol), and PY_{ET}-PS (6.3 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 20 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (ethyl acetate only) gave (*R*)-**2j** and (*S*)-**1j**.

(*R*)-**2j**: 23.4 mg, 0.12 mmol, 29%, >99 %ee.

(*S*)-**1j**: 38.5 mg, 0.26 mmol, 63%, 44%ee.

Conv. 31%, *E* value > 200, Rate: 778 mM h^{-1} mg $enzyme^{-1}$

4.4.11. Kinetic resolution of (±)-1-3-hydroxy-3-(thiophen-2-yl) propanenitrile (**1k**) using PY_{ET}-PS

A mixture of (±)-**1k** [33] (62.5 mg, 0.41 mmol), vinyl acetate (53.4 mg, 0.61 mmol) and PY_{ET}-PS (6.3 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 60 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (hexane/ethyl acetate = 1:1) gave (*R*)-**2k** and (*S*)-**1k**.

(*R*)-**2k**: 27.3 mg, 0.14 mmol, Y = 34%, 97% *ee*.

(*S*)-**1k**: 39.8 mg, 0.26 mmol, Y = 63%, 60% *ee*.

Conv. 38%, *E* value 121, Rate: 313 mM h^{-1} mg $enzyme^{-1}$

4.4.12. Kinetic resolution of (±)-5-phenylpent-1-en-3-ol (**1l**) [20] using PY_{ET}-PS

A mixture of (±)-**1l** (66.6 mg, 0.41 mmol), vinyl acetate (53.5 mg, 0.61 mmol) and PY_{ET}-PS (6.5 mg) in *i*-Pr_2O (2.0 ml) was stirred at 35 °C for 60 min. The reaction was quenched by the addition of 1.0 ml of ethyl acetate and the enzyme was removed by filtration through a glass sintered filter with a Celite pad, then silica gel TLC (hexane/ethyl acetate = 1:1) gave (*R*)-**2l** and (*S*)-**1l**.

(*R*)-2l: 7.9 mg, 0.04 mmol, Y = 10%, >99% *ee*.

(*S*)-1l: 48.0 mg, 0.30 mmol, Y = 73%, 15% *ee*.

Conv. 13%, *E* value > 200, Rate: 100 mM h^{-1} mg $enzyme^{-1}$

For the HPLC profiles of compounds **1a**~**1l**, and **2a**~**2l**, see SI of our reference 33.

4.5. Determination of kinetic parameter in Table 3

We used IL1-PS (enzyme contents = 3.7% (w/w)) and freshly prepared PY_{ET}-PS (enzyme contents = 4.0% (w/w)) for the reaction. The reaction was carried out using 5.0 mg of IL1-PS or PY_{ET}-PS in 2.0 ml of *i*-Pr_2O. The reaction rate was determined by GC-analysis in the presence of an internal standard. For details, see the Supplementary material.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2018.12.028.

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