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Enzymatic production of (*S*)-3-cyano-5-methylhexanoic acid ethyl ester with high substrate loading by immobilized *Pseudomonas cepacia* lipase

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ABSTRACT

(*S*)-3-Cyano-5-methylhexanoic acid ethyl ester is a valuable synthetic intermediate for pregabalin. Immobilized lipase PS from *Pseudomonas cepacia* was screened and shown to be the best biocatalyst for the enantioselective hydrolysis of 3-cyano-5-methylhexanoic acid ethyl ester, a racemic mixture involving a β-stereocenter. The optimum temperature and pH for the biocatalytic process were 35 °C and 6.0, respectively. Lipase PS IM exhibited a strong tolerance toward high substrate concentrations of up to 2.0 M (366 g/l). In the scaled-up biotransformation, (*S*)-3-cyano-5-methylhexanoic acid ethyl ester was produced in 0.89 M (162.9 g/l), 99.2% *ee*, and 44.5% yield. These results indicated that lipase PS IM catalyzed the preparation of (*S*)-3-cyano-5-methylhexanoic acid ethyl ester and could be used as an efficient route for the large-scale production of pregabalin.

1. Introduction

(*S*)-3-(Aminomethyl)-5-methylhexanoic acid (pregabalin, Lyrica® API) is a lipophilic 4-aminobutyric acid (GABA) analogue and has been developed for the treatment of several central nervous system disorders including neuropathic pain, epilepsy, anxiety, and social phobia.[1] Due to its high and broad therapeutic activity, Lyrica attained sales reaching $1.2 billion in 2006 and $3.6 billion in 2011.[2] Since the pharmacological activity of pregabalin resides in the (*S*)-enantiomer,[3] considerable efforts have been devoted to the synthesis of enantiomerically pure (*S*)-pregabalin.

The first-generation manufacturing process started with a Knoevenagel condensation of isovaleraldehyde and diethyl malonate, followed by cyanation, hydrolysis, hydrogenation, and decarboxylation to yield racemic pregabalin, which underwent resolution with (*S*)-(+)-mandelic acid in a three-step crystallization process.[4] As a result, the overall yield of the process is only 20% and the undesired (*R*)-pregabalin cannot be efficiently recycled. Other chemical routes mostly comprise of the use of chiral auxiliaries or chiral catalysts, such as (*S*)-phenylethyl amine,[5] Evans' oxazolidinones,[6] aluminum salen catalysts,[7] rhodium Me-DuPHOS catalyst,[8] D-mannitol acetonide,[9] and so on. Although these routes afforded high enantiomeric purity, they have practical limitations for large-scale application because of multiple steps, extensive use of solvent, expensive reagents, and harsh conditions.

Biocatalysis is becoming one of the greenest technologies for the synthesis of pharmaceutical intermediates due to its high enantioselectivity, mild reaction conditions, and environmental friendliness.[10] Due to these advantages, several enzymatic approaches have been reported for the preparation of chiral intermediates of pregabalin. For example, Xie et al. reported the regio- and stereospecific hydrolysis of racemic isobutylsuccinonitrile by nitrilase from *Arabidopsis thaliana*, resulting in (*S*)-3-cyano-5-methylhexanoic acid in 45% yield and 97% *ee*.[11] Hedvati et al. described the kinetic resolution of 3-acetamido-5-methylhexanoic acid ethyl ester (CMH) with commercial lipases, leading to (*R*)-CMH and (*S*)-CMH in 98% and 79% *ee*, respectively.[12] Sterimbaum et al. attempted the enantioselective esterification of 3-isobutylglutaric acid by *Candida antarctic* lipase B (CALB), which afforded (*S*)-isobutyglutaric methyl ester in 96% yield and 95.5% *ee*.[13] In another synthetic route, CALB was involved in the enantioselective hydrolysis of 2-(nitromethyl)alkanoates. Reduction of the nitro group of the resulting enantiomerically pure γ-nitro acids gave directly GABA analogues including (*S*)-pregabalin.[14] Thijs et al. reported the kinetic resolution of methyl 4-isobutyl-2-oxo-pyrrolidine-3-carboxylate by pig liver esterase for the preparation of (*S*)-pregabalin (97.3% *ee*).[15] However, these approaches have drawbacks such as low substrate concentration tolerance and catalytic efficiency, thus restricting their industrial application.

The most successful scalable chemoenzymatic strategy for (*S*)-pregabalin involved a lipolase-catalyzed resolution of *rac*-2-carboxyethyl-3-cyano-5-methylhexanoic acid ethyl ester (CNDE, 3.0 M) to produce the desired (*S*)-mono acid enantiomer in high resolution yields (45%) and enantioselectivity (98% *ee*).[16]

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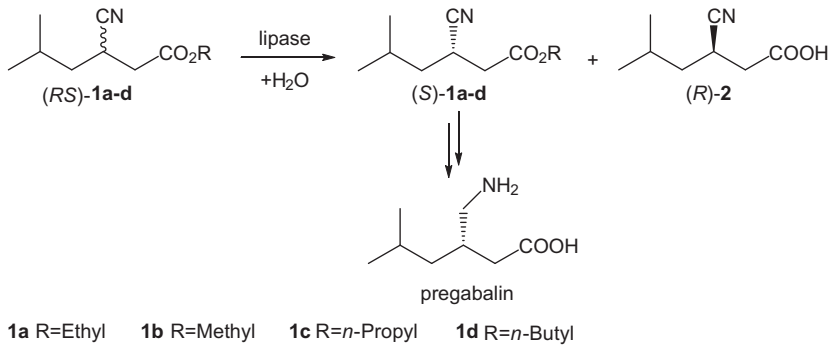

Scheme 1. Lipase-mediated resolution of *rac*-3-cyano-5-methyl-hexanoic acid ester, a valuable synthetic intermediate for pregabalin.

The second-generation route improved the process efficiency compared to the first-generation process by setting the stereocenter early in the synthesis, thus enabling the reuse of (*R*)-CNDE, and reducing organic solvent usage. However, a closer look at the chemoenzymatic process made us realize that the atom economy of the strategy was still not satisfactory. Since the route involved enzymatic hydrolysis and decarboxylation steps, 255 g of CNDE theoretically produced only 91.5 g of the desired (*S*)-3-cyano-5-methylhexanoic acid ethyl ester.

(*S*)-3-Cyano-5-methylhexanoic acid ethyl ester **1a** is a valuable portion of (*S*)-pregabalin. An efficient route for preparing (*S*)-**1a** is the enantioselective hydrolysis of the racemic mixture mediated by lipases or esterases (Scheme 1), which exhibits higher atom economy than the second-generation chemoenzymatic route. However, since the stereogenic center of **1a** is located on the β-carbon atom of the ester group, there has been no report on enzymes that are capable of the enantioselective kinetic resolution of (*R*,*S*)-**1a** until now.

Herein our aim was to develop a novel biocatalytic process for (*R*,*S*)-3-cyano-5-methylhexanoic acid ethyl ester. A suitable biocatalyst for the enantioselective hydrolysis of the substrate with a β-stereogenic center was explored. The operating parameters including reaction temperature, pH, and substrate concentration on commercial lipase PS IM were examined. The tolerance of lipase PS IM against high substrate concentration was also investigated.

2. Results and discussion

2.1. Enzyme screening

Eight commercially available lipases were screened for their capacity for the kinetic resolution of **1a** and four enzymes showed activity toward the substrate (Table 1).

Of these enzymes, lipase PS IM was the best in terms both of enantioselectivity ($E = 44$) and activity. Novozym 435 showed the highest activity toward (*R*,*S*)-**1a**, but no stereoselectivity discrimination was observed during the hydrolytic process. Lipase AK and lipase TL IM exhibited only low to moderate enantioselectivity. Therefore, lipase PS IM was selected for further study based on its high enantioselectivity and activity for the hydrolysis of (*R*,*S*)-**1a**. The screening results also suggested that lipase-mediated enantioselective hydrolysis of the substrate bearing the β-stereogenic center away from the carbonyl carbon atom is more difficult than its counterpart with an α-stereocenter.[17]

Racemic 3-cyano-5-methylhexanoic acid esters **1b–d** were synthesized with different alkyl chains in order to study the effect of the alcohol moiety on the enantioselectivity and activity of lipase PS IM. As can be seen from Table 2, lipase PS IM exhibited the highest activity toward *n*-propyl ester **1c**. Meanwhile, no remarkable change in enantioselectivity was observed when the carbon chains of the alcohol moiety became longer except for that of ethyl ester **1a** ($E = 40$).

2.2. Effect of temperature

In general, the reaction temperature influences the enantioselectivity and stability of biocatalysts;[18,19] the thermal stability of biocatalysts is always taken as one of the most important criteria for industrial applications.[20] Therefore, the effect of temperature on the enantioselective hydrolysis of (*R*,*S*)-**1a** was studied. The conversion notably increased from 9.4% to 41.5% with an increase of temperature from 20 °C to 65 °C (Fig. 1). The enantioselectivity did not significantly change from 20 °C to 35 °C ($E = 45$), however, it rapidly decreased with a further increase of the temperature up to 65 °C ($E = 24$). Xia et al. also reported that the enantioselectivity of lipase PS IM decreased when temperature was above 40 °C in the kinetic resolution of 2-chloro-1-phenylethanol.[21] Based on the above results, 35 °C was chosen as the optimal temperature in the following work.

2.3. Effect of pH

It is well known that the enantioselectivity and activity of enzymes can be remarkably influenced by pH.[22] In order to

Table 1
Performance of eight commercial available lipases for the hydrolytic resolution of (*RS*)-**1a**

Entry	Enzyme	*T* (h)	ee_S (%)	ee_P (%)	*c* (%)	*E*	Stereoselectivity
1	Lipase PS IM	8	69	91	43	44	(*R*)
2	Lipase AK	72	31	85	26	17	(*R*)
3	Lipase TL IM	12	50	41	45	5	(*S*)
4	Novozym 435	10	—	—	100	—	(*R*)/(*S*)
5	Lipase A	24	—	—	—	—	—
6	Lipase G50	24	—	—	—	—	—
7	Rhizopus niveus lipase	24	—	—	—	—	—
8	Lipase AY	24	—	—	—	—	—

Reaction conditions: 5 ml of sodium phosphate buffer (100 mM, pH 7.0), 80 mg of lipase, 91.5 mg of (*R*,*S*)-**1a** (100 mM), and 30 °C.

Table 2
Enzymatic hydrolysis of 3-cyano-5-methylhexanoic acid esters **1a–d**

Substrate	T (h)	ee_S (%)	ee_P (%)	c (%)	E
1a	4	32	93	25	40
1b	4	43	86	33	20
1c	4	63	87	42	28
1d	4	48	86	36	21

Reaction conditions: 5 ml of sodium phosphate buffer (100 mM, pH 7.0), 80 mg of lipase, 0.5 mmol of esters **1a–d**, and 30 °C.

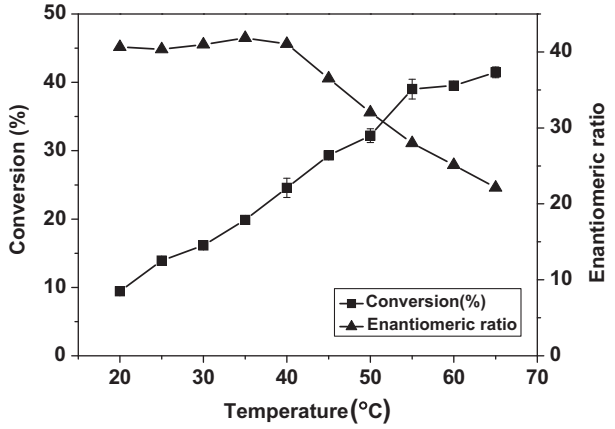

Figure 1. Effect of temperature on the conversion and enantioselectivity of lipase PS IM catalyzed hydrolysis. Symbols: conversion (■); enantiomeric ratio (▲). Reaction conditions: 5 ml of sodium phosphate buffer (100 mM, pH 7.0), 80 mg of lipase PS IM, 91.5 mg of (*R,S*)-**1a** (100 mM), and 3 h.

determine the optimum pH for the lipase PS IM-mediated resolution of (*R,S*)-**1a**, the hydrolytic reaction was performed under different pH values at 35 °C. As shown in Figure 2, the lipase PS IM activity was low at pH< 5.0 or pH> 8.5; the optimum pH of lipase PS IM for (*R,S*)-**1a** was 6.0. With respect to the enantioselectivity, the ee_P was above 92.0% and remained stable over the whole pH range. Hence, pH 6.0 was chosen as the favorable pH for the enzymatic resolution of (*R,S*)-**1a**.

2.4. Optimization of the substrate loading

A high substrate concentration would be beneficial for the practical application of a biocatalytic process because it could improve the space–time yield and reduce the cost of product isolation to a large extent. The effect of substrate concentration on the initial reaction rate was investigated at a fixed amount of enzyme (16.0 g/l). As shown in Figure 3, the initial reaction rate was increased from 3.59 mM/h to 6.55 mM/h when the substrate concentration was increased from 0.01 M to 0.1 M. No inhibitory effect of the substrate on the enzyme activity was observed, even when the substrate concentration was increased to 0.5 M. However, the enantiomeric purity of the product decreased from 93.7% to 82.3% when the substrate concentration exceeded 0.4 M (73.2 g/l). A similar dependence of the enzyme enantioselectivity on the substrate concentration was also found in other lipases and nitrilase-catalyzed reactions,[20,23,24] which probably resulted from the strengthened hydrophobic interactions between the excess substrate and enzyme, leading to a conformational change in the active site of the biocatalyst.

In order to enhance the volumetric productivity of the biocatalytic process, the substrate concentration was further increased at a fixed ratio of substrate to enzyme (S/E). The pH was kept at approximately 6.0 by the automatic addition of NaOH (1.0 M). As

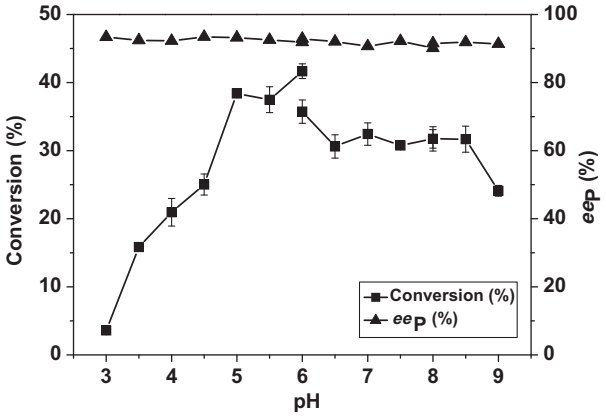

Figure 2. Effect of pH on (*RS*)-**1a** hydrolysis catalyzed by lipase PS IM. Symbols: conversion (■); ee_P (▲). Reaction conditions: 5 ml of different buffer (citrate buffer solution, 3.0–6.0; sodium phosphate buffer, 6.0–8.0; Tris-HCl buffer, 8.0–9.0.), 80 mg of lipase PS IM, 91.5 mg of (*RS*)-**1a** (100 mM), 35 °C, and 3 h.

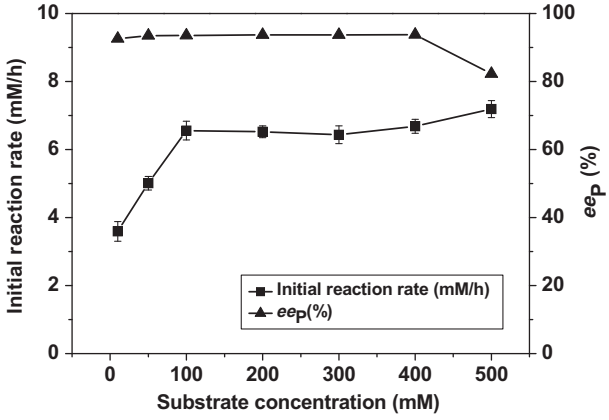

Figure 3. Effect of substrate concentration on the initial reaction rate and enantioselectivity of lipase PS IM catalyzed hydrolysis. Symbols: initial reaction rate (■) and ee_P (▲). Reaction conditions: 5 ml of sodium phosphate buffer (100 mM, pH 6.0), 80 mg of lipase PS IM, 35 °C, and 0.5 h.

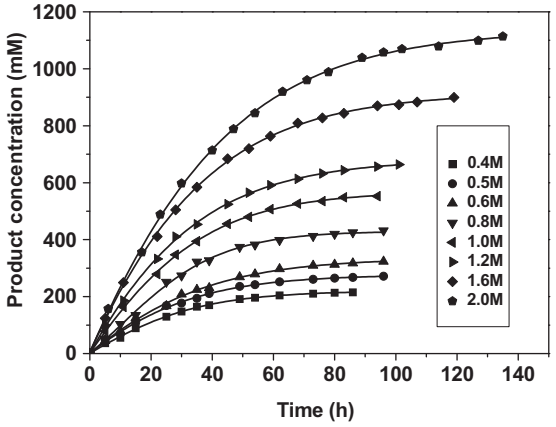

Figure 4. Time courses of (*RS*)-**1a** hydrolysis catalyzed by lipase PS IM with the same substrate (S) to enzyme (E) ratio (S/E ratio). Reaction conditions: 10 ml of sodium phosphate buffer (100 mM, pH 6.0), 35 °C, and pH 6.0.

shown in Figure 4, the product formation was significantly increased from 0.22 M to 1.01 M when the substrate concentration was raised from 0.4 M to 2.0 M (366 g/l) after 86 h, thus indicating

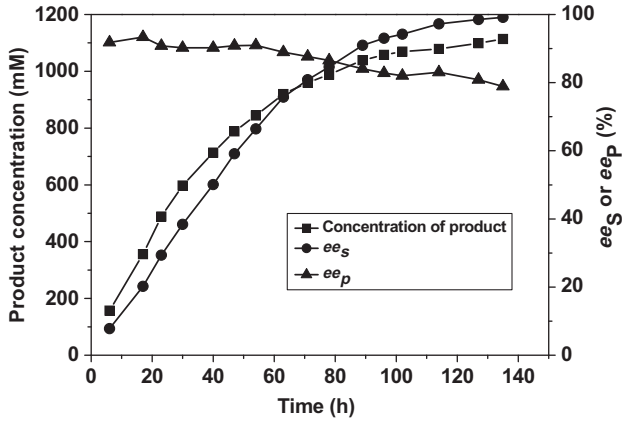

Figure 5. Hydrolytic resolution progress of (*RS*)-**1a** by lipase PS IM. Reaction conditions: 1000 ml of sodium phosphate buffer (100 mM, pH 6.0), 2.0 mol (386 g) of (*RS*)-**1a**, 80 g of lipase PS IM, and 35 °C.

that lipase PS IM was tolerant against high concentrations of the substrate and product. More importantly, lipase PS IM exhibited high enantioselectivity even at a substrate concentration of up to 2.0 M in a fixed *S*/*E* mode, giving (*S*)-**1a** in 0.90 M and 99.0% *ee*.

In order to further evaluate the feasibility of the optimized bioprocess for the production of (*S*)-**1a** in practical use, a 1–l scale biotransformation was performed in a 2–l stirred-tank reactor. Figure 5 plots the kinetic resolution progress of (*R,S*)-**1a** under the above optimal conditions. A conversion of 49.4% with an ee_P of 86.5% and ee_S of 84.6% was achieved after 78 h, after which the hydrolytic reaction rate became slow. In order to obtain (*S*)-**1a** with high enantiomeric purity, the reaction time was prolonged to 135 h and (*S*)-**1a** was finally afforded in 0.89 M (162.9 g/l), 99.2% *ee*, and 44.5% yield. Therefore, the feasibility of the bioprocess for the practical production of enantiomerically pure (*S*)-**1a** was confirmed.

3. Conclusion

Herein a novel biocatalytic process for the production of (*S*)-3-cyano-5-methylhexanoic acid ethyl ester has been developed. Immobilized lipase PS from *Pseudomonas cepacia* was explored as the best enzyme for the hydrolytic resolution of the racemic mixture with a β-stereocenter. The substrate loading was increased as high as 2.0 M (366 g/l) and (*S*)-3-cyano-5-methylhexanoic acid ethyl ester was produced in 99% *ee* and 44.5% yield. These results imply that lipase PS IM is a potentially ideal and promising biocatalyst for the practical production of (*S*)-3-cyano-5-methylhexanoic acid ethyl ester. Future work will be focused on the reuse of the immobilized enzymes in order to reduce production costs.

4. Experimental

4.1. General

Lipase PS IM (*Pseudomonas cepacia* lipase, immobilized on diatomaceous earth) was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan); lipase AK (*Pseudomonas fluorescens* lipase), lipase A (*Aspergillus niger* lipase), lipase G50 (*Penicillium camemberti* lipase), *Rhizopus niveus* lipase, and lipase AY (*Candida rugosa* lipase) were obtained from Sigma-Aldrich (Shanghai) Trading Co., Ltd. (Shanghai, China); lipase CALB (*Candida antarctica* lipase fraction B immobilized on macroporous polyacrylate resin) and lipase TL IM (silica-granulated *Thermomyces lanuginosus* lipase) were supplied by Novozymes A/S (Bagsvaerd, Denmark). Compound (*R,S*)-**1a** was kindly provided by Zhejiang Apeloa Medical Technology Co. Ltd. (Jinhua, China). The other chemicals used herein were of analytical grade from local suppliers.

4.2. Methods

4.2.1. Screening for lipases capable of the enantioselective hydrolysis of (*R,S*)-1a

Suspensions of different commercially available lipases (80 mg) in 5 ml solution of (*R,S*)-**1a** (91.5 mg, 0.5 mmol) in sodium phosphate buffer (100 mM, pH 7.0) were shaken at 180 rpm in sealed glass vials at 30 °C. Samples (0.5 ml each) were withdrawn and the reaction was quenched by the addition of 100 μl of HCl (1 M). After centrifugation, the supernatant was extracted with 800 μl of ethyl acetate. The ethyl acetate layer was dried over anhydrous Na_2SO_4. The enantiomeric excess of the substrate (ee_S), and product (ee_P) were determined by chiral GC analysis. All experiments were conducted in triplicate if not specified.

4.2.2. General procedure for the chemical synthesis of racemic esters

For the synthesis of compound **1b**, thionyl chloride (1.1 equiv) was added dropwise to methanol (40 ml) cooled in an ice bath. After stirring at room temperature for 0.5 h, racemic 3-cyano-5-methylhexanoic acid (4.65 g, 0.03 mol) was added and the mixture was heated to 60 °C for 4 h. The solvent was removed under vacuum to obtain the product as a yellow oil. For the synthesis of compounds **1c** and **1d**, racemic 3-cyano-5-methylhexanoic acid (4.65 g, 0.03 mol), thionyl chloride (1.2 equiv), and dichloromethane (40 ml) were combined. After stirring at 60 °C for 2 h, additional solvent was removed by vacuum distillation. *n*-Propanol or *n*-butanol (30 ml) was then added and the batch temperature was held at 60 °C for 2 h. The solvent was removed in vacuo to give the product as a yellow oil.

4.2.2.1. 3-Cyano-5-methylhexanoic acid methyl ester 1b. This compound was obtained as a yellow liquid; yield: 4.66 g (92%); ^{1}H NMR (CD_3Cl): δ 3.74 (s, 3H), 3.07–3.02 (m, 1H), 2.72–2.68 (m, 1H), 2.57–2.53 (m, 1H), 1.88–1.85 (m, 1H), 1.67–1.61 (m, 1H), 1.36–1.31 (m, 1H), 0.98–0.95 (m, 6H, $-CH_3$); MS (EI): m/z = 169 (M^+), 154, 138, 126, 113, 96, 85, 74, 54.

4.2.2.2. 3-Cyano-5-methylhexanoic acid *n*-propyl ester 1c. This compound was obtained as a yellow liquid; yield: 5.20 g (88%); ^{1}H NMR (CD_3Cl): δ 4.12–4.09 (m, 2H), 3.08–3.04 (m, 1H), 2.73–2.70 (m, 1H), 2.57–2.52 (m, 1H), 1.88–1.85 (m, 1H), 1.70–1.64 (m, 3H), 1.37–1.31 (m, 1H), 0.98–0.96 (m, 9H, $-CH_3$); MS (EI): m/z = 197 (M^+), 156, 138, 120, 96, 83, 61.

4.2.2.3. 3-Cyano-5-methyl hexanoic acid *n*-butyl ester 1d. This compound was obtained as a yellow liquid; yield: 5.25 g (83%); ^{1}H NMR (CD_3Cl): δ 4.16–4.13 (m, 2H), 3.08–3.03 (m, 1H), 2.73–2.68 (m, 1H), 2.57–2.52 (m, 1H), 1.89–1.85 (m, 1H), 1.66–1.61 (m, 3H), 1.42–1.34 (m, 3H), 0.99–0.93 (m, 9H, $-CH_3$); m/z = 211 (M^+), 182, 168, 156, 138, 120, 99, 83, 69, 56.

4.2.3. General procedure for the lipase PS IM catalyzed resolution of esters 1a–d

The reaction medium consisted of sodium phosphate buffer (100 mM, pH 7.0), lipase PS IM (80 mg), and the corresponding ester **1a–d** (0.5 mmol). The biotransformations were carried out at 30 °C on a rotary shaker. The enantiomeric excess of the substrate and product was determined by chiral GC analysis.

4.2.4. Enantioselective hydrolysis of (*R,S*)-1a by lipase PS IM

Compound (*R,S*)-**1a** (91.5 mg, 0.5 mmol) and lipase PS IM (80 mg) were homogenized in 5 ml of buffer, and the hydrolysis

reaction was carried out in an orbital shaker at 180 rpm in a temperature range of 20–65 °C and a pH range of 3.0–9.0 (100 mM citrate buffer solution, 3.0–6.0; 100 mM sodium phosphate buffer, 6.0–8.0; and 100 mM Tris-HCl buffer, 8.0–9.0). The enantiomeric excess of the product and conversion of the substrate were determined by chiral GC analysis.

The substrate tolerance of lipase PS IM was studied with substrate concentrations ranging from 0.4 M to 2.0 M at pH 6.0 and 35 °C. The ratio of the substrate to the enzyme ratio (*S*/*E*) was fixed in the reactions.

4.2.5. Chiral GC analysis

Enantiomeric compositions of the residual ester and the corresponding acid were determined by GC-14C gas chromatography (Shimadzu, Japan) equipped with FID detector and chiral capillary column BGB-174 (BGB Analytik, Switzerland, 30 m × 0.25 mm, 0.25 μm film thickness) using helium as the carrier gas. The injector and detector temperatures were set at 220 °C. For compounds **1a**–**c**, the initial column temperature of 130 °C was held for 17 min, then raised to 170 °C at a rate of 10 °C/min and finally held at 170 °C for 15 min. Retention times for (*R*)-**1a**, (*S*)-**1a**, (*R*)-**1b**, (*S*)-**1b**, (*R*)-**1c**, (*S*)-**1c**, (*S*)-**2**, and (*R*)-**2** were 14.62 min, 15.01 min, 11.54 min, 11.85 min, 19.72 min, 19.84 min, 32.22 min, and 33.10 min, respectively. For compound **1d**, the initial temperature 110 °C was held for 30 min, then raised to 170 °C at a rate of 5 °C/min and finally held at 170 °C for 14 min. Retention times: (*R*)-**1d**, 38.35 min; (*S*)-**1d**, 38.45 min; (*S*)-**2**, 47.28 min; and (*R*)-**2**, 48.34 min.

The conversion (*c*) and enantiomeric ratio (*E*) were calculated based on ee_S and ee_P using the method developed by Rakels.[25]

4.2.6. (*S*)-3-Cyano-5-methylhexanoic acid ethyl ester (*S*)-1a

The enzymes were removed from the reaction mixture by centrifugation, and the substrate and products were extracted with ethyl acetate. Compound (*S*)-**1a** was separated by silica gel chromatography using petroleum ether/ethyl acetate (20:1) as the mobile phase. The eluent was evaporated under reduced pressure to afford a light yellow oil; $[\alpha]_D^{20} = -12.2$ (*c* 1.1, CH_3OH); ^1H NMR (CD_3OD): δ 4.22–4.18 (m, 2H), 3.09–3.03 (m, 1H), 2.71–2.67 (m, 1H), 2.56–2.51 (m, 1H), 1.90–1.84 (m, 1H), 1.67–1.61 (m, 1H), 1.37–1.31 (m, 1H), 1.30–1.28 (m, 3H, $-CH_3$), 0.98–0.96 (m, 6H, $-CH_3$); MS (EI): *m*/*z* = 183 (M^+), 168, 156, 138, 127, 120, 112, 96, 88, 82, 70, 60, 54.

4.2.7. Potassium (*S*)-3-cyano-5-methylhexanoate

A glass reactor was charged with a solution of (*S*)-**1a** (5.0 g) in ethanol (50 ml). A solution of potassium hydroxide (1.53 g) in ethanol (6 ml) was added, with the addition rate being controlled in order to maintain the temperature below 25 °C. The mixture was stirred at 20–25 °C for 5 h. The solvent was removed in vacuo to give the product as a white crystalline solid (4.80 g). $[\alpha]_D^{20} = -22.5$ (*c* 1.1, CH_3OH) {$[\alpha]_D^{20} = -20.6$ (*c* 1.1, CH_3OH)[26]}; ^1H NMR (CD_3OD): δ 3.12–3.08 (m, 1H), 2.51–2.46 (m, 1H), 2.42–2.37 (m, 1H), 1.85–1.81 (m, 1H), 1.62–1.56 (m, 1H), 1.43–1.37 (m, 1H), 1.0–0.98 (m, 6H, $-CH_3$); ^{13}C NMR (CD_3OD): δ 177.70(O=C), 124.22 (CN), 42.27, 41.94, 28.46, 27.63, 23.51,21.87; IR (KBr) ν_{max} (cm^{-1}): 3355, 2958, 2913, 2870, 2241, 1579, 1422, 1402, 1314, 1301, 638. MS (EI): *m*/*z* = 155 (M^+), 137, 122, 112, 96, 85, 71, 67, 60, 54.

Acknowledgments

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