Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 1493–1502

TETRAHEDRON: *ASYMMETRY*

Enantioselective synthesis of (2*S*)-5-{4-[2-(4-fluorophenoxy)ethyl]piperazin-1-yl}-2-isopropyl-2-phenyl-pentanenitrile dihydrochloride (E2050) using enzyme-catalyzed kinetic resolution

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Received 10 May 2002; accepted 27 June 2002

Abstract—Immobilized lipase (*Pseudomonas* sp.)-catalyzed transesterification of (*RS*)-4-cyano-4-isopropyl-4-phenyl-1-butanol **4** in vinyl acetate gave (*S*)-4-cyano-4-isopropyl-4-phenyl-1-butyl acetate **3a** with high enantiomeric excess values and conversions (95–97% ee, 30–47%, $E=90$–93), leaving the enantiomerically pure (*R*)-alcohol **4** (>99% ee) unreacted. As **3a** can be efficiently converted to E2050, use of the immobilized lipase should provide an economical route for large-scale synthesis of E2050.

1. Introduction

The neuron-selective Ca^{2+} channel blocker E2050 ((2*S*)-5-{4-[2-(4-fluorophenoxy)ethyl]piperazin-1-yl}-2-isopropyl-2-phenylpentanenitrile dihydrochloride) was discovered in our research program directed towards novel neuroprotective agents.[1] It possesses the same moiety (with a quaternary stereogenic carbon) as the well-known L-type calcium antagonist, emopamil. Several reports[2] have described the synthesis of the chiral moiety **4** and its congeners, but these approaches are limited to small-scale preparation because of the need for many reaction steps or time-consuming chromatography. Although the kinetic resolution of racemic 2-phenyl-1-propanol using enzymatic transesterification was previously reported by Kawasaki et al.,[3a] the enzyme-catalyzed kinetic resolution of racemic 4-cyano-4-isopropyl-4-phenyl-1-butanol, which has a longer alkyl chain, has not been reported. Herein, we report a practical enantioselective synthesis of chiral 4-cyano-4-isopropyl-4-phenyl-1-butanol as a basis for practical asymmetric synthesis of E2050. Recently, Im et al. reported an improved enzyme-catalyzed resolution of (*RS*)-4-cyano-4-(3,4-dimethoxyphenyl)-4-isopropyl-1-butanol using enzymatic transesterification.[3b] However, in their report, a second enzymatic resolution is necessary to obtain the enantiomerically pure (*S*)-enantiomer (E value[4] = 49). Our method affords pure (*S*)- and (*R*)-enantiomers by single-step enzymatic kinetic resolution without using additives (E value = 93).

2. Results and discussion

Our strategy to prepare enantiomerically pure E2050 is outlined in Fig. 1. Substrate types I and II were chosen as key intermediates and subjected to enzymatic resolution.

(*RS*)-4-Cyano-4-isopropyl-4-phenyl-1-butanoates (substrate type I) and (*RS*)-4-cyano-4-isopropyl-4-phenyl-1-butanol derivatives (substrate type II) were prepared as shown in Scheme 1.

First, we examined the kinetic resolution of the carboxylates **1a**,**b**,**c** (substrate type I) using enzymatic hydrolysis. Since the resultant carboxylic acid was obtained as a crystalline solid, its enantiomeric purity might be easily improved by recrystallization. However, preliminary studies gave disappointing results with many kinds of lipase and esterase, such as Lipase AK, Lipase PSA-30, porcine pancreatic lipase (PPL), *Rhizopus delemar* lipase (RDL), immobilized lipase (*Pseu-*

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PII: S0957-4166(02)00364-6

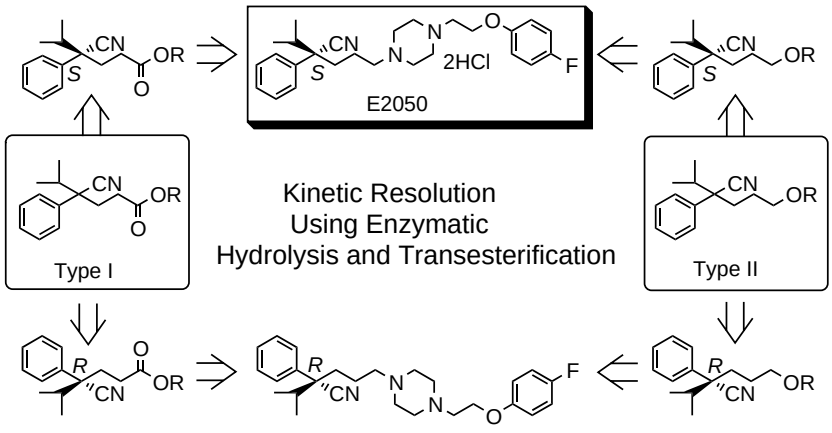
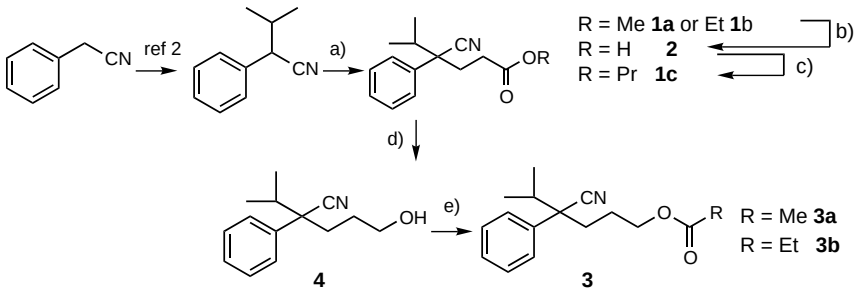


Figure 1. Enantioselective synthetic route to E2050.



Scheme 1. Synthesis of substrates. *Reagents and conditions*: (a) methyl acrylate (for **1a**) or ethyl acrylate (for **1b**), KOBu^t, THF; (b) 5N NaOH, aq. THF; (c) 1-propanol, 2-chloro-3,5-dinitropyridine, pyridine; (d) $LiAlH_4$; (e) Ac_2O, pyridine (for acetate (**3a**)) or propionyl chloride, Et_3N, THF (for propionate (**3b**)).

domonas sp.), and pig liver esterase (PLE).[†] Therefore, enzyme-catalyzed kinetic resolution of (*RS*)-4-cyano-4-isopropyl-4-phenyl-1-butanol derivatives (substrate type II) was investigated.

2.1. Kinetic resolution of the acylates 3a,b using enzymatic hydrolysis in phosphate buffer or organic solvent

Enzymatic hydrolysis of **3a**,**b** was performed in phosphate buffer (pH 6.89) at room temperature using several lipases (Lipase AK, immobilized lipase, PPL, RDL, Lipase PSA-30) and PLE. The results are summarized in Table 1. PLE did not significantly hydrolyze the acetate **3a** and propionate **3b**. Lipase PSA-30 predominantly hydrolyzed (*R*)-**3a**,**b**, while other lipases favored (*S*)-**3a**,**b**. Immobilized lipase gave the best result ((*S*)-**4**, 85% ee, 21% yield; (*R*)-**3a**, 30% ee, 78%).

[†] When lipases other than RDL were used, the reaction did not proceed within 180 h. With PLE, the reaction proceeded within 24 h in all cases. The (*S*)-carboxylates **1** were predominantly hydrolyzed to the (*S*)-carboxylic acid **2**, but these enzymatic hydrolyses showed low enantioselectivity. Although PLE-catalysed hydrolysis of the methyl ester **1a** gave the (*S*)-carboxylic acid **2** in high conversion yield (51%), the enantiomeric excess of the obtained (*S*)-**2** was only 24% ee.

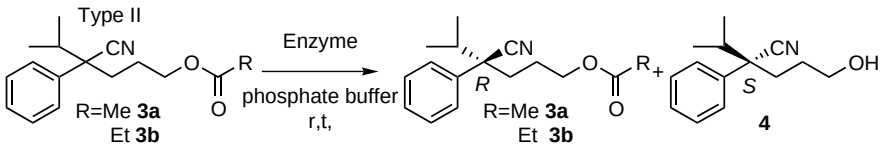
Next, we tried the enzymatic hydrolysis of the acylated alcohols **3a**,**b** in *tert*-butyl methyl ether (*t*BuOMe) or diisopropyl ether saturated with water in the presence of immobilized lipase (Table 2).

In these cases, the enzymatic hydrolyses of **3a**,**b** afforded (*S*)-**4** with improved enantiomeric purity compared with the reaction in phosphate buffer. The absolute configurations of the hydrolyzed products were determined by comparison with authentic samples derived from the reported[2a] chiral aldehyde **6** by HPLC using a chiral column (Scheme 2).

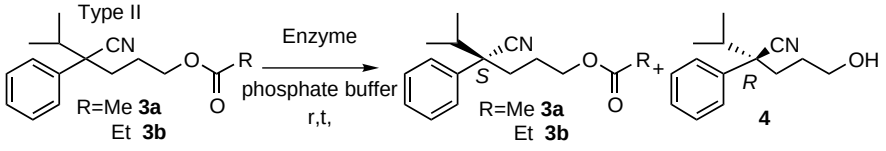
2.2. Kinetic resolution of the alcohol 4 using enzymatic transesterification

The substrate **4** was subjected to enzymatic transesterification in vinyl acetate or vinyl propionate at room temperature using several lipases (lipase AK, immobilized lipase, PPL, RDL, lipase PSA-30) and PLE. The ratio of substrate (alcohol **4**), enzyme, and vinyl acetate was ca. 100 mg:10 mg:10 ml. The results are summarized in Table 3. Immobilized lipase gave superior enantiomeric excesses and yields. The transesterification of **4** in vinyl acetate using immobilized lipase gave the corresponding (*S*)-**3a** (43% ee, 70%) and (*R*)-**4** (99.3% ee, 30%), while the same process in vinyl propionate gave (*S*)-**3b** (99% ee, 3%) and (*R*)-**4** (16% ee, 83%).

Table 1. Enzymatic hydrolysis in phosphate buffer



Entry	R	Enzyme[a]	Reaction Time (h)	Yield of (*R*)-**3**		Yield of (*S*)-**4**		*E*
				c.y.%	% ee[b]	c.y.%	% ee[b]	
1	Me	Lipase AK	18	48	70	52	72	13
2	Me	Immobilized lipase	168	78	30	21	85	17
3	Me	PPL	24	67	8.1	23	13	1
4	Me	RDL	9	70	11	21	39	3
5	Et	Lipase AK	26	48	57	40	64	8
6	Et	Immobilized lipase	143	70	24.4	23	90	24
7	Et	PPL	8	65	4.7	11	0.2	1
8	Et	RDL	9	64	12	20	31	2

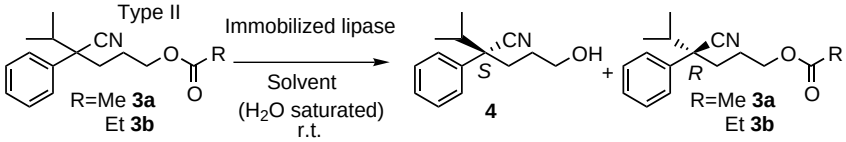


Entry	R	Enzyme[a]	Reaction Time (h)	Yield of (*S*)-**3**		Yield of (*R*)-**4**		*E*
				c.y.%	% ee[b]	c.y.%	% ee[b]	
9	Me	Lipase PSA-30	18	50	23	49	21	2
10	Et	Lipase PSA-30	119	54	13	46	15	2

a) Lipase AK (*Pseudomonas fluorescens*), Immobilized lipase (*Pseudomonas* sp.), PPL; porcine pancreatic lipase, RDL; *Rhizopus delemar* lipase, Lipase PSA-30 (*Burkholderia cepacia*)

b) Enantiomeric excess (% ee) was determined by HPLC (CHIRALCEL OJ, hexane:IPA:EtOH= 85:10:5)

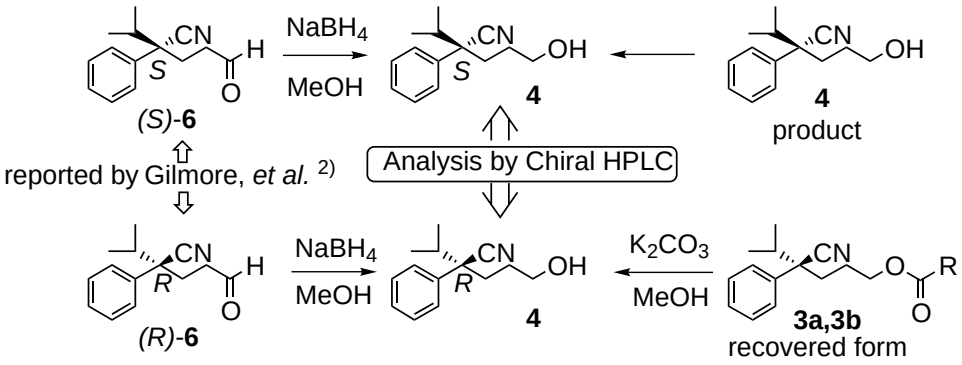
Table 2. Enzymatic hydrolysis in organic solvent



Entry	R	Solvent	Reaction time (h)	Yield of (*S*)-**4**		Yield of (*R*)-**3**		*E*
				c.y. (%)	% ee[a]	c.y. (%)	% ee[a]	
1	Me	*t*-BuOMe	24	22	95	66	35	55
2	Et	*t*-BuOMe	144	20	94	74	–	–
3	Me	Diisopropyl ether	163	20	93	75	–	–
4	Et	Diisopropyl ether	>163	–	–	–	–	–

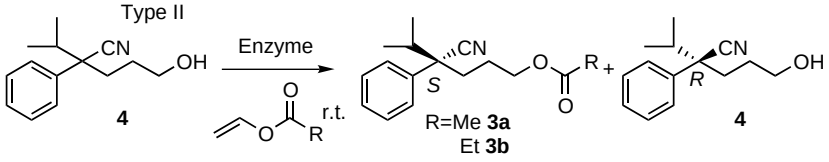
[a] Enantiomeric excess (% ee) was determined by HPLC (CHIRALCEL OJ, hexane:IPA:EtOH = 85:10:5).

To optimize the reaction conditions, we varied the ratio of substrate, enzyme and vinyl acetate (entries 7–9). Decreasing the amount of enzyme or vinyl acetate resulted in high enantiomeric purity of (*S*)-**3a**, probably because of the prolonged reaction time (94–96% ee and 17–44%). The reaction conditions detailed in entry 9 of Table 3 were selected as the optimum.



Scheme 2. Determination of absolute configuration.

Table 3. Enzymatic transesterification of (±)-**4**



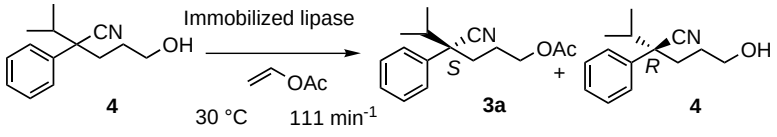
Entry	R	Enzyme	Reaction time (h)	Yield of (*S*)-**3**		Yield of (*R*)-**4**		*E*
				c.y. (%)	% ee	c.y. (%)	% ee	
1	Me	Lipase Ak	45	52	64	48	57	8
2	**Me**	**Immobilized lipase**	**0.3**	**70**	**43**	**30**	**99.3**	12
3	Me	PPL	168	29	26	70	9.2	2
4	Me	RDL	>168	–	–	–	–	–
5	Et	PPL	24	48	9.8	52	7.1	1
6	Et	**Immobilized lipase**	**15**	**3**	**99**	**83**	**16**	230
7	**Me**	**Immobilized lipase**	**27**	**17**	**95**	**83**	**19**	47
8	**Me**	**Immobilized lipase**	**6**	**18**	**96**	**79**	**26**	63
9	**Me**	**Immobilized lipase**	**1.5**	**44**	**94**	**54**	**71**	69

Entries 1–6: Substrate (20 mg), lipase (2 mg added all at once), vinyl acetate or vinyl propionate (excess).
Entry 7: Substrate (200 mg), lipase (4×0.2 mg added portionwise), vinyl acetate (excess).
Entry 8: Substrate (20 mg), lipase (2 mg added all at once), vinyl acetate (1.2 equiv.), EtOAc (excess).
Entry 9: Substrate (200 mg), lipase (2 mg added all at once), vinyl acetate (excess).

2.3. Enzymatic transesterification on a large scale

Based on the above results, the ratio of substrate **4**, immobilized lipase and vinyl acetate was fixed at 1 g:2 mg:1 ml. A preparative-scale transesterification was performed in a Taitec Personal-10 Incubator® (111 min^{-1}, at 30°C). The reaction was monitored by ^1H NMR to avoid undesired over-reaction. The best result was obtained when the enzymatic transesterification was terminated at the point of 60–70% conversion. Isolation of the product was carried out by silica gel chromatography. These results suggest that this process should be feasible for larger scale synthesis on kilogram scale (Table 4).

Table 4. Enzymatic transesterification on a large scale



Entry	Substrate (g)	Reaction time (h)	Yield of (*S*)-**3a**		Yield of (*R*)-**4**		*E*
			c.y. (%)	% ee	c.y. (%)	% ee	
1	18	**3.7**	**30**	**97**	67	33	91
2	35	**7.2**	**32**	**94.7**	68	39	54

2.4. Large-scale double-step resolution of (±)-4 by transesterification using immobilized lipase

In order to improve the enantiomeric purity of (*S*)-**3a** obtained through enzymatic esterification, subsequent enzymatic resolution was investigated. Ideally, direct enzymatic hydrolysis by switching the solvent from vinyl acetate to buffer solution would give the desired (*S*)-**4**, which can be used in the next process to synthesize E2050. However, the enzymatic hydrolysis required a long reaction time on large scale. Thus, re-transesterification was investigated. Termination of the reaction after 80–90% conversion of the substrate gave (*S*)-**3a** in >99% ee (11.5 g) and 29% yield from (±)-**4** (35 g) (theoretical maximum 50%), as shown in Scheme 3.

2.5. Reuse of immobilized lipase

The feasibility of reusing the immobilized lipase was investigated. As shown in Table 5, transesterification using fresh immobilized lipase afforded (*S*)-**3a** in 95% ee and 47% yield in 23 h ($E=93$).

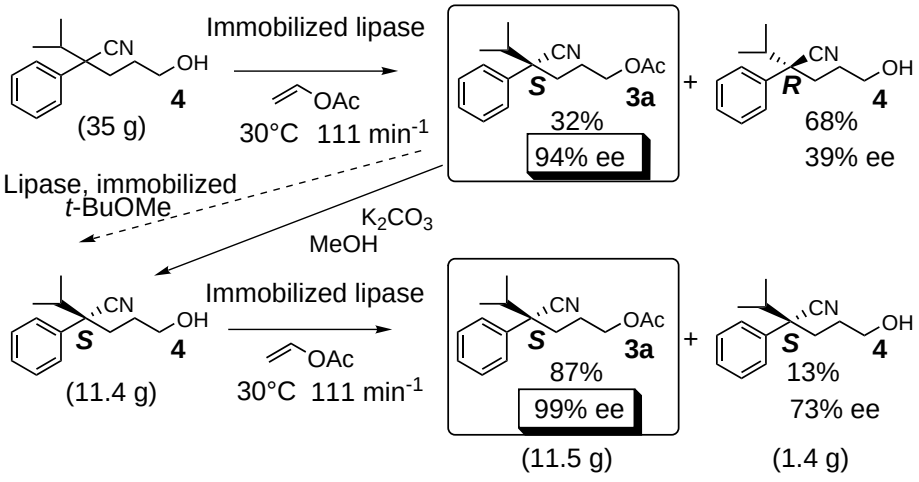
On the other hand, reuse of immobilized lipase gave almost the same result ((*S*)-**3a** >94% ee and 38%), but the reaction rate decreased ten-fold. This may have been due to acetaldehyde produced during the transesterification in vinyl acetate. Utilization of an alternative acetylating agent or reaction in a column loaded with lipase is under investigation.

2.6. Asymmetric synthesis of E2050 from (*S*)-3a

The enantiomerically pure (*S*)-**3a** (99% ee) was then used for the synthesis of E2050. Hydrolysis of (*S*)-**3a** afforded the corresponding alcohol (*S*)-**4** in 95% isolated yield. (*S*)-**4** was converted to the corresponding mesylate, followed by coupling with *p*-fluorophenoxyethylpiperazine to afford the free form of E2050 in 84% yield (two steps from (*S*)-**4**). The HCl salt of E2050 was prepared by a standard procedure and had 99.9% ee after one recrystallization (Scheme 4).

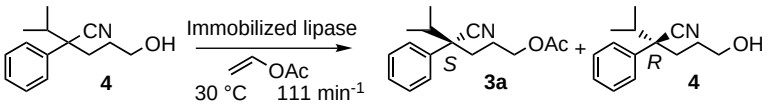
3. Summary

An asymmetric synthesis of the neuron-selective calcium channel blocker E2050 was established by using immobilized lipase-catalyzed transesterification to prepare the key intermediate. This method is expected to be suitable for kilogram-scale synthesis.



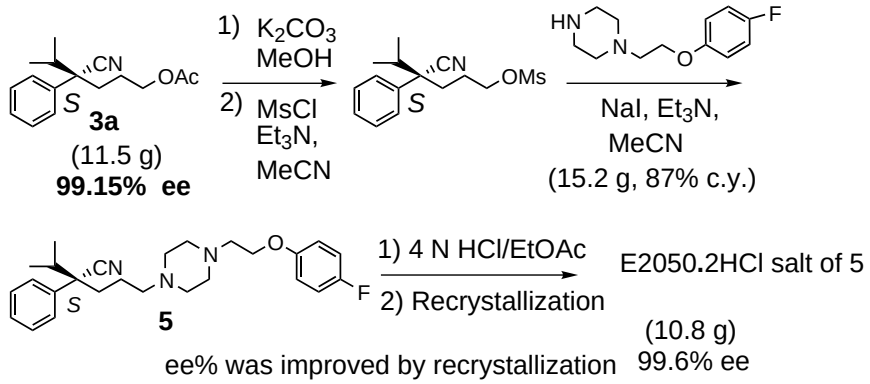
Scheme 3. Double-step resolution using transesterification.

Table 5. Recovery and re-use of immobilized lipase



Entry	Immobilized lipase	Substrate (g)	Reaction time (h)	Yield of (*S*)-**3a**		Yield of (*R*)-**4**		*E*
				c.y. (%)	% ee	c.y. (%)	% ee	
1	Fresh	1	**23**	**47**	**95**	53	78	93
2	Recovered	0.6	**257**	**38**	>**94**	62	>**57**	58

Entry 1: substrate:lipase:vinyl acetate = 1 g:5 mg:1 ml.
Entry 2: substrate:lipase:vinyl acetate = 0.6 g:3 mg:1 ml.



Scheme 4. Enantioselective synthesis of E2050.

4. Experimental

^{1}H NMR spectra were measured at 400 MHz on a Varian UNITY400 spectrometer. Chemical shifts (ppm) were reported relative to internal $CDCl_3$ (residual $CHCl_3$, ^{1}H, 7.26 ppm). EI(+)HRMS spectra were recorded on a Thermoquest Finnigan Mat SSQ7000. High-performance liquid chromatography for the determination of enantiomeric purity was performed on a Shimadzu SIL-10AD VP with a Shimadzu SPD-10A VP UV–vis detector set at 200 nm and Chiralcel OJ 4.6×250 mm (eluent, hexane:ethanol:isopropanol (850:100:50), flow rate; 0.7 ml/min). Specific rotations were measured on a JASCO DIP-1000 digital polarimeter. Vinyl acetate (monomer) and vinyl propionate were purchased from Tokyo Kasei Corp. (Japan), Immobilized lipase (EC 3.1.1.3) was purchased from Wako Pure Chemical industries Ltd. (Japan), PPL (type II) was purchased from Sigma Corp., RDL (EC 3.1.1.3) was purchased from Seikagaku Kogyo Corp. (Japan). Pig liver esterase (EC 3.1.1.1) was purchased from Sigma Corp., and Lipase AK (Amano 20) and lipase PSA-30 (Amano 20) were given courtesy of Amano Pharmaceutical Corp. (Japan). All materials were used as received.

4.1. (±)-Methyl 4-cyano-5-methyl-4-phenylhexanoate, 1a

Acrylic acid methyl ester (16.7 g, 96.9 mmol), a solution of potassium *tert*-butoxide in 2-methyl-2-propanol (1.0 M, 19.4 ml, 19.4 mmol) and 18-crown-6 (512 mg, 1.94 mmol) were added to a stirred solution of 3-methyl-2-phenylbutyronitrile (15.4 g, 96.9 mmol) in THF (150 ml). The reaction mixture was heated under reflux overnight, then cooled to room temperature, and ice-water was added. This mixture was extracted with EtOAc. The combined extracts were dried over Na_2SO_4. Removal of the solvent in vacuo gave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 50% EtOAc in hexane afforded (±)-**1a** as a colorless oil (21.7 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.79 (d, 6.8 Hz, 3H), 1.23 (d, $J=6.8$ Hz, 3H), 1.96 (ddd, $J=4.8$ Hz, 12.0 Hz, 16.4 Hz, 1H), 2.10–2.24 (m, 2H), 2.37–2.61 (m, 2H), 3.60 (s, 3H), 7.29–7.41 (m, 5H); EI(+)HRMS calcd for $C_{15}H_{19}O_2N$ (M^+) 245.1416, found 245.1444.

4.2. (±)-Ethyl 4-cyano-5-methyl-4-phenylhexanoate, 1b

1b was prepared from acrylic acid ethyl ester in a similar manner to that described above (75%). ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.79 (d, $J=6.8$ Hz, 3H), 1.20 (t, $J=7.1$ Hz, 3H), 1.23 (d, $J=6.6$ Hz, 3H), 1.89–2.00 (m, 1H), 2.10–2.26 (m, 2H), 2.34–2.56 (m, 2H), 3.98–4.13 (m, 2H), 7.29–7.42 (m, 5H); EI(+)-HRMS calcd for $C_{16}H_{21}O_2N$ (M^+) 259.1572, found 259.1601.

4.3. (±)-4-Cyano-5-methyl-4-phenylhexanoic acid, 2

(±)-**2** was prepared from (±)-**1a** or **1b** by non-enzymatic hydrolysis (76 or 78% yield, respectively). ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.79 (d, $J=6.8$ Hz, 3H), 1.23 (d, $J=6.6$ Hz, 3H), 1.94–2.06 (m, 1H), 2.08–2.23 (m, 2H), 2.40–2.55 (m, 2H), 7.29–7.42 (m, 5H); EI(+)-HRMS calcd for $C_{14}H_{17}O_2N$ (M^+) 231.1259, found 231.1266.

4.4. (±)-Propyl 4-cyano-5-methyl-4-phenylhexanoate, 1c

2-Chloro-3,5-dinitropyridine (278.4 mg, 0.14 mmol) was added to a stirred solution of (±)-4-cyano-5-methyl-4-phenylhexanoic acid **2** (316 mg, 0.14 mmol) and 1-propanol (0.2 ml, 2.68 mmol) in pyridine (1.4 ml) under an N_2 atmosphere at room temperature. The mixture was stirred at room temperature overnight, then the reaction was quenched with saturated $NaHCO_3$ and the whole mixture was extracted with EtOAc. The combined extracts were dried over $MgSO_4$. Removal of the solvent in vacuo gave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 20% EtOAc in hexane afforded (±)-**1c** (283 mg, 76%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.79 (d, $J=6.6$ Hz, 3H), 0.89 (t, $J=7.4$ Hz, 3H), 1.23 (d, $J=6.6$ Hz, 3H), 1.52–1.63 (m, 2H), 1.95 (ddd, $J=7.2$ Hz, 12.0 Hz, 16.4 Hz, 1H), 2.01–2.24 (m, 2H), 2.35–2.53 (m, 2H), 3.89–4.02 (m, 2H), 7.28–7.41 (m, 5H); EI(+)HRMS calcd for $C_{17}H_{23}O_2N$ (M^+) 273.1729, found 273.1740.

4.5. (±)-4-Cyano-5-methyl-4-phenylhexanol, 4

A solution of (±)-ethyl 4-cyano-5-methyl-4-phenylhexanoate **1a** (9.4 g, 36.4 mmol) in THF (60 ml) was added dropwise to a solution of $LiAlH_4$ (1.03 g, 27.3 mmol) in THF (60 ml) at 0°C, and the mixture was stirred at the same temperature for 1 h. After stirring at room temperature overnight, the mixture was cooled to 0°C, then the reaction was quenched with saturated $NaHCO_3$ (10 ml) and 1N NaOH (12 ml). After removal of the precipitate, the organic layer was dried over $MgSO_4$. Removal of the solvent in vacuo gave an oily residue, which was purified by column chromatography on silica gel (50% EtOAc in hexane) to afford (±)-**4** as a colorless oil (5.01 g, 64%). ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.79 (d, $J=6.6$ Hz, 3H), 1.22 (d, $J=6.6$ Hz, 3H), 1.17–1.28 (m, 1H), 1.62–1.66 (m, 1H), 1.98 (td, $J=4.4$ Hz, 12.5 Hz, 1H), 2.14 (qu, $J=6.8$ Hz, 1H), 2.25 (ddd, $J=4.4$ Hz, 12.0 Hz, 13.6 Hz, 1H), 3.59 (m, 2H), 7.26–7.40 (m, 5H); EI(+)HRMS calcd for $C_{14}H_{19}ON$ (M^+) 217.1467, found 217.1470.

4.6. (±)-4-Cyano-5-methyl-4-phenylhexyl acetate, 3a

A mixture of (±)-4-cyano-5-methyl-4-phenylhexanol **4** (1.32 g, 6.12 mmol), acetic anhydride (1 ml, 9.05 mmol) and pyridine (2 ml) was stirred at room temperature overnight. After having been cooled to 0°C, the reaction mixture was neutralized with saturated $NaHCO_3$, then extracted with EtOAc. The combined extracts were dried over $MgSO_4$. Removal of the solvent in vacuo gave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% EtOAc in hexane afforded (±)-**3a** as a colorless oil (1.3 g, 80%). ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.79 (d, $J=6.8$ Hz, 3H), 1.21 (d, $J=6.8$ Hz, 3H), 1.28 (m, 1H), 1.70 (m, 1H), 1.90 (ddd, $J=4.4$ Hz, 12.4 Hz, 13.6 Hz, 1H), 2.02 (s, 3H), 2.13 (qu, $J=6.8$ Hz, 1H), 2.21 (ddd, $J=4.4$ Hz, 12.0 Hz, 13.6 Hz, 1H), 3.99 (t, $J=6.2$ Hz, 2H), 7.28–7.41 (m, 5H); EI(+)-HRMS calcd for $C_{16}H_{21}O_2N$ (M^+) 259.1572, found 259.1575.

4.7. (±)-4-Cyano-5-methyl-4-phenylhexyl propionate, 3b

Propionyl chloride (773 mg, 8.35 mmol) was added to a stirred solution of (±)-4-cyano-5-methyl-4-phenylhexanol **4** (1.21 g, 5.57 mmol) and Et_3N (1.6 ml, 11.1 mmol) in THF (10 ml). The reaction mixture was stirred at room temperature overnight, then the reaction was quenched with brine, and extracted with EtOAc. The combined extracts were dried over $MgSO_4$. Removal of the solvent in vacuo gave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 10% EtOAc in hexane afforded (±)-**3b** as a colorless oil (1.3 g, 87%). ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.78 (d, $J=4.8$ Hz, 3H), 1.12 (d, $J=7.6$ Hz, 3H), 1.20 (d, $J=4.8$ Hz, 3H), 1.24–1.34 (m, 1H), 1.65–1.76 (m, 1H), 1.91 (td, $J=4.4$ Hz, 12.8 Hz, 1H), 2.13 (qu, $J=6.8$ Hz, 1H), 2.20 (td, $J=4.4$ Hz, 13.0 Hz, 1H), 2.30 (q, $J=7.6$ Hz, 2H), 3.99 (t, $J=6.0$ Hz, 2H), 7.29–7.41 (m, 5H); EI(+)HRMS calcd for $C_{17}H_{23}O_2N$ (M^+) 273.1729, found 273.1737.

4.8. General method for enzymatic hydrolysis of the (±)-acyl alcohol 3a,b (substrate type II) in phosphate buffer

A suspension of the (±)-acetete **3a** (or (±)-propionate **3b**) (100 mg) and enzyme (10 mg) in acetone (0.1 ml) (in the case of long reaction times, acetonitrile (10 ml) was added instead of acetone (0.1 ml)) and phosphate buffer (10 ml, pH 6.8) was stirred at 23°C, and the reaction was monitored by TLC. After the period of time noted in Table 1, hydrolysis was terminated by extracting the mixture with $CHCl_3$. The combined extracts were dried over $MgSO_4$ and then concentrated in vacuo to leave an oily residue, which was purified by column chromatography on silica gel (hexane:ethyl acetate = 4:1) to give the hydrolyzed alcohol **4** and recovered acetate **3a** (or propionate **3b**) as colorless oils.

4.8.1. (*S*)-(−)-4-Cyano-5-methyl-4-phenylhexanol, 4. 52% yield, 72% ee, by Lipase AK hydrolysis of 4-cyano-5-methyl-4-phenylhexyl acetate in phosphate buffer, $[\alpha]_D^{27}$ −5.3 (*c* 0.8, $CHCl_3$).

4.8.2. (*R*)-(+)-4-Cyano-5-methyl-4-phenylhexanol, 4. 49% yield, 21% ee, by Lipase PSA-30 hydrolysis of 4-cyano-5-methyl-4-phenylhexyl acetate in phosphate buffer, $[\alpha]_D^{27}$ +3.9 (*c* 0.9, $CHCl_3$).

4.8.3. (*R*)-(+)-4-Cyano-5-methyl-4-phenylhexyl acetate, 3a. 48% yield (recovery), 70% ee, by Lipase AK hydrolysis of 4-cyano-5-methyl-4-phenylhexyl acetate in phosphate buffer, $[\alpha]_D^{27}$ +19.1 (*c* 0.9, $CHCl_3$).

4.8.4. (*S*)-(−)-4-Cyano-5-methyl-4-phenylhexyl acetate, 3a. 50% yield (recovery), 23% ee, by Lipase PSA-30 hydrolysis of 4-cyano-5-methyl-4-phenylhexyl acetate in phosphate buffer, $[\alpha]_D^{27}$ −3.8 (*c* 0.8, $CHCl_3$).

4.8.5. (*R*)-(+)-4-Cyano-5-methyl-4-phenylhexyl propionate, 3b. 48% yield (recovery), 57% ee, by Lipase AK hydrolysis of 4-cyano-5-methyl-4-phenylhexyl propionate in phosphate buffer, $[\alpha]_D^{28}$ +12.0 (*c* 0.88, $CHCl_3$).

4.8.6. (*S*)-(−)-4-Cyano-5-methyl-4-phenylhexyl propionate, 3b. 54% yield (recovery), 13% ee, by Lipase PSA-30 hydrolysis of 4-cyano-5-methyl-4-phenylhexyl propionate in phosphate buffer, $[\alpha]_D^{28}$ −2.2 (*c* 0.88, $CHCl_3$).

4.9. General method of enzymatic hydrolysis of the (±)-acyl alcohol 3a,b (substrate type II) in organic solvent

A suspension of (±)-acetete **3a** (or (±)-propionate **3b**) (100 mg) and immobilized lipase (10 mg) in water-saturated *tert*-butyl methyl ether (10 ml) or water-saturated diisopropyl ether (10 ml) was stirred at 23°C, and the reaction was monitored by TLC. After the time period noted in Table 2, immobilized lipase was removed by filtration, and the filtrate was concentrated in vacuo to leave an oily residue, which was purified by column chromatography on silica gel (hexane:ethyl acetate = 4:1) to give the hydrolyzed alcohol **4** and recovered acetate **3a** (or propionate **3b**) as colorless oils.

4.10. Determination of enantiomeric excess of hydrolyzed products 4 and 3a or 3b

The enantiomeric excess (% ee) of the hydrolyzed alcohol **4** was determined by HPLC (CHIRALCEL OJ, eluent, hexane:IPA:EtOH = 850:100:50, flow rate; 0.7 ml/min, retention time (min) 9.5 for (*R*)-alcohol **4** and 7.5 for (*S*)-alcohol **4**).

The enantiomeric excess (% ee) of the recovered acetate **3a** or propionate **3b** was determined after conversion into the corresponding alcohol **4**, which was prepared by the method described below.

4.11. Conversion of recovered acetate 3a (or propionate 3b) into the corresponding alcohol 4

A mixture of the recovered (*R*)- or (*S*)-acetate **3a** (or propionate **3b**) (14 mg) and K_2CO_3 (3 mg) in methanol (10 ml) was stirred at room temperature for 3 h. It was neutralized with 5N aqueous HCl, then concentrated in vacuo to give an oily residue, to which water was added, and the mixture was extracted with EtOAc. The combined extracts were dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by short column chromatography on silica gel (50% EtOAc in hexane) to give (*R*)- or (*S*)-alcohol **4** as a colorless oil for HPLC analysis.

4.12. Determination of absolute configuration of the hydrolyzed products 3a or 3b and 4

The absolute configuration of the hydrolyzed products was determined by comparison of the retention time on chiral HPLC with the value for the (*R*)-alcohol **4** (or (*S*)-alcohol **4**) derived from the reported (*R*)-aldehyde **6** (or (*S*)-aldehyde **6**). The conversion of the reported (*R*)-aldehyde **6** (or (*S*)-aldehyde **6**)[2a] into the corresponding (*R*)-alcohol **4** (or (*S*)-alcohol **4**) is described below.

4.13. (*S*)-4-Cyano-5-methyl-4-phenylhexnol, 4

$NaBH_4$ (20 mg, 0.53 mmol) was added to a stirred solution of (*S*)-4-cyano-5-methyl-4-phenylhexanal **6** (18 mg, 0.08 mmol) prepared by the reported method.[2a] After stirring at room temperature for 1 h, the reaction mixture was concentrated in vacuo to give an oily residue, to which water was added, and the mixture was extracted with EtOAc. The combined extracts were dried over Na_2SO_4. After removal of the solvent in vacuo, the residue was purified by short column chromatography on silica gel (50% EtOAc in hexane) to give (*S*)-(−)-4-cyano-5-methyl-4-phenylhexanoic acid **4** as a colorless oil for HPLC analysis.

4.14. (*R*)-4-Cyano-5-methyl-4-phenylhexanol, 4

(*R*)-(+)-4-Cyano-5-methyl-4-phenylhexanol **4** was prepared from (*R*)-4-cyano-5-methyl-4-phenylhexanal **6** (18 mg), which was prepared by the reported method in the same manner as described above for the HPLC analysis sample.

4.15. General method of enzymatic transesterification of the (±)-alcohol 4 (substrate type II)

A suspension of the (±)-alcohol **4** (100 mg, 0.46 mmol) and enzyme (10 mg) in vinyl acetate (or vinyl propionate) (10 ml) was stirred at 23°C. The reaction was monitored by TLC. After the time period noted in Table 3, the enzyme was removed by filtration, and the filtrate was concentrated in vacuo to leave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 25% EtOAc in hexane afforded the acetate **3a** (or propionate **3b**) and the recovered alcohol **4** as colorless oils.

4.15.1. (*S*)-(−)-4-Cyano-5-methyl-4-phenylhexyl acetate, 3a. 18% yield, 96% ee, by immobilized lipase (*Pseudomonas* sp.)-catalysed transesterification of 4-cyano-5-methyl-4-phenylhexanol, $[\alpha]_D^{29}$ −25 (*c* 0.558, $CHCl_3$).

4.15.2. (*S*)-(−)-4-Cyano-5-methyl-4-phenylhexyl propionate, 3b. 48% yield, 9.8% ee, by PPL-catalysed transesterification of 4-cyano-5-methyl-4-phenylhexanol, $[\alpha]_D^{30}$ −2.7 (*c* 1.2, $CHCl_3$).

4.15.3. (*R*)-(+)-4-Cyano-5-methyl-4-phenylhexanol, 4. 30% yield (recovery), 99.3% ee, by immobilized lipase (*Pseudomonas* sp.) transesterification of 4-cyano-5-methyl-4-phenylhexanol in vinyl acetate, $[\alpha]_D^{29}$ +19.4 (*c* 0.42, $CHCl_3$).

The enantiomeric excess of the resolved products **3a** or **3b** and **4** was determined in a similar manner to that described for the determination of the enantiomeric excess of hydrolyzed products **4** and **3a** or **3b**.

4.16. Large-scale enzymatic transesterification of alcohol (±)-4 using immobilized lipase

A suspension of (±)-4-cyano-5-methyl-4-phenylhexanol **4** (18 g) and immobilized lipase (35 mg) in vinyl acetate (20 ml) was stirred at 30°C in Taitec Personal-10 Incubator® (111 min^{-1}). The reaction was monitored by ^1H NMR at intervals of 1 h. Immobilized lipase was removed by filtration at the point of 60% conversion, and the filtrate was concentrated in vacuo to leave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 25% EtOAc in hexane afforded the (*S*)-acetate **3a** of 97% ee (6.4 g, 30%) and recovered (*R*)-alcohol **4** of 33% ee (12 g, 67%) as colorless oils.

4.17. Re-resolution of (*S*)-acetate 3a of 94.7% ee by means of immobilized lipase-catalyzed transesterification

The (*S*)-acetate **3a** of 94.7% ee (13.4 g), prepared from the (±)-alcohol **4** (35 g) in a similar manner to that described above, was converted into the corresponding (*S*)-alcohol **4** in the same manner as described for the conversion of the recovered acetate **3a** (or propionate **3b**) into the corresponding alcohol **4**. The obtained (*S*)-alcohol **4** (11.4 g) was submitted to the same enzymatic procedure as described above. After 80–90% con-

version of the substrate, immobilized lipase was removed by filtration, and the filtrate was concentrated in vacuo to leave an oily residue, which was purified by column chromatography on silica gel. The fraction eluted with 25% EtOAc in hexane afforded the (*S*)-acetate **3a** of 99.15% ee (11.5 g, 87%) and the recovered (*S*)-alcohol **4** of 73% ee (1.4 g, 13%) as colorless oils.

4.18. (2*S*)-5-{4-[2-(4-Fluorophenoxy)ethyl]piperazin-1-yl}-2-isopropyl-2-phenylpentanenitrile dihydrochloride (E2050)

To a solution of the (*S*)-acetate **3a** of 99.15% ee (11.51 g, 44.5 mmol) in methanol (500 ml) was added K_2CO_3 (11 g, 79.6 mmol). The mixture was stirred at room temperature overnight, then neutralized with 5N aqueous HCl, and concentrated in vacuo to give an oily residue, to which water was added. The mixture was extracted with EtOAc. The combined extracts were dried over Na_2SO_4. Removal of the solvent in vacuo gave the (*S*)-alcohol **4** as a colorless oily residue (9.16 g), which was dissolved in acetonitrile (70 ml). To this solution, Et_3N (12.8 ml, 91.8 mmol) was added. The mixture was cooled to 0°C, and a solution of methanesulfonyl chloride (3.66 ml, 46.3 mmol) in acetonitrile (70 ml) was added dropwise. The reaction mixture was stirred at 0°C for 1 h, then at room temperature overnight, and concentrated in vacuo to give an oily residue, which was dissolved in ether (300 ml). This solution was washed with brine. The organic layer was dried over Na_2SO_4. Removal of the solvent in vacuo gave 12.13 g of the mesylate as a dark reddish oil, which was dissolved in acetonitrile (113 ml). To this solution, sodium iodide (6.97 g, 46.5 mmol), Et_3N (6.4 ml, 45.9 mmol) and *p*-fluorophenoxyethylpiperazine (10.41 g, 46.4 mmol) were added. The mixture was stirred at 70°C for 3 h. The reaction mixture was cooled to room temperature and concentrated in vacuo to give an oily residue, which was dissolved in EtOAc (400 ml). This solution was washed with water (400 ml), and the organic layer was dried over Na_2SO_4. Removal of the solvent in vacuo left an oily residue, which was purified by column chromatography on Cromatorex NH silica gel. The fraction eluted with 17% EtOAc in hexane afforded the free form of E2050 as a colorless oil (15.22 g, 80.2% yield from compound **4**). 99.6% ee, $[\alpha]_D^{20}$ −3.9 (*c* 1.46, $CHCl_3$), ^{1}H NMR (400 MHz, $CDCl_3$); δ (ppm) 0.77 (d, $J=6.8$ Hz, 3H), 1.05–1.17 (m, 1H), 1.20 (d, $J=6.8$ Hz, 3H), 1.50–1.60 (m, 1H), 1.88 (dt, $J=4.4$ Hz, 12.4 Hz, 1H), 2.06–2.19 (m, 2H), 2.24–2.30 (m, 2H), 2.30–2.43 (m, 4H), 2.46–2.62 (m, 4H), 2.77 (t, $J=5.8$ Hz, 2H), 4.04 (t, $J=5.8$ Hz, 2H), 6.80–6.85 (m, 2H), 6.91–6.99 (m, 2H), 7.25–7.32 (m, 1H), 7.32–7.40 (m, 4H).

4N HCl/EtOAc (18 ml) was added dropwise to a solution of the free form of E2050 (15.2 g) in EtOAc (150 ml), and the mixture was stirred at room temperature for 30 s. After removal of the solvent, the residual white precipitate was dissolved in 1-propanol (88.5 ml) under reflux. The solution was stirred while being gradually cooled to room temperature overnight. The precipitated colorless crystals were collected by filtration and dried in an oven at 60°C for 9 days to give E2050 as colorless crystals (10.77 g, 61%), >99.9% ee, $[\alpha]_D^{29}$ −5.2 (*c* 0.73, EtOH), ^{1}H NMR (400 MHz, DMSO-d_6); δ (ppm) 0.68 (d, $J=6.6$ Hz, 3H), 1.11 (d, $J=6.6$ Hz, 3H), 1.22–1.34 (m, 1H), 1.58–1.72 (m, 1H), 2.06–2.30 (m, 3H), 3.00–3.25 (m, 2H), 3.30–3.80 (m, 10H), 4.36 (brs, 2H), 6.98–7.07 (m, 2H), 7.11–7.20 (m, 2H), 7.32–7.40 (m, 1H), 7.40–7.50 (m, 4H), ESI-Mass; 424 (MH^+), mp 183–187°C. HPLC impurities: 0.68%, residual solvent: 0.09%, water content: 0.30%, chloride ion content: 14.56% (% anhydrous/desolvated theoretical value = 14.28%)).

A second crop of crystals of E2050 was obtained; 1.19 g (6.7%), 99.05% ee. Filtrate; 2.58 g (14.5%), 99.47% ee.

Acknowledgements

We wish to thank members of the Analytical Chemistry Section of Eisai Tsukuba Research Laboratories for interpretation of NMR and mass spectra and for obtaining elemental analysis data.

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