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Enzyme-catalyzed kinetic resolution of piperidine hydroxy esters

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Abstract—The highly enantioselective (E >200) kinetic resolution of (±)-ethyl *cis*-(±)-**4** and *trans*-1-(*tert*-butoxycarbonyl)-4-hydroxypiperidine-3-carboxylate (±)-**5** was achieved by *Pseudomonas fluorescens* lipase-catalyzed asymmetric acylation with vinyl acetate in diisopropyl ether at room temperature. *Candida antarctica* lipase A-catalyzed asymmetric acylation of (±)-ethyl *cis*-1-benzyl-3-hydroxypiperidine-4-carboxylate (±)-**11** was performed with vinyl propanoate in diisopropyl ether at 3 °C, with good enantioselectivity (E = 75).

1. Introduction

Among the alkaloids and their analogues, esters and hydroxy esters containing a piperidine ring can modify neurotransmission, resulting in wide-ranging effects on the central nervous system. Piperidine-based compounds, such as nipecotic acid and its hydroxy- or amino-substituted derivatives, have therefore been synthetized[1] and investigated for their GABA-agonist activity,[2] their binding capability to the cocaine receptor on the dopamine transporter[3] and their potential cholinergic[4] and anticonvulsant[5] activities. The binding sites involved in these effects exhibit stereoselectivity, as (*R*)-(−)-nipecotic acid has proved to be a more effective GABA-uptake inhibitor[2d] and the affinity of (*R*)-cocaine is higher for the cocaine receptor.[6] Hydroxylated and polyhydroxylated piperidines have been found to be effective glucosidase inhibitors[7] and have been used in the synthesis of oligosaccharide mimetics.[8] The *trans*-4-hydroxy ester **5** is of importance as a potential intermediate in the synthesis of the first reported selective ORL1-receptor antagonist.[9] Orthogonally protected *trans*-4-aminopiperidine-3-carboxylic acid has been utilized as a conformationally restricted β-amino acid building block in foldamer research.[10] These facts highlight the significance of the syntheses of chiral piperidines as synthetic building blocks and members of the chiral pool.

The enantioselective synthesis of variably substituted piperidine compounds has been widely investigated.[11] Baker's yeast has been utilized with moderate to good diastereoselectivities for the reduction of ethyl 1-(*tert*-butoxycarbonyl)-4-oxopiperidine-3-carboxylate **3** under fermenting and nonfermenting conditions, when the (3*R*,4*S*)-enantiomer was obtained.[8a,12,13] This absolute configuration is identical with that of the corresponding stereogenic carbons of natural (−)-cocaine. The absolute configuration of the resulting hydroxy ester has been proved,[12a] but the specific rotation data on the products with different enantiomeric excesses remain unclear. Enzymatic kinetic resolution has been successfully used in the cases of the *N*-methyl and *N*-isopropyl analogues of **4** and **5**,[14] as earlier for the resolution of racemic cocaine,[15] through the pig liver esterase-catalyzed (*R*)-selective hydrolysis of the phenylacetyl group. Baker's yeast also catalyzes the reduction of ethyl 1-benzyl-3-oxopiperidine-4-carboxylate (the base of **6**) in an (*R*)-selective manner.[16]

In order to prepare the enantiomers of piperidine-based 4-hydroxy and 3-hydroxy esters, we set out to investigate the *O*-acylation reactions of *N*-Boc-protected 4-hydroxy esters **4** and **5**, and their 3-hydroxy ester regioisomers **9** and **10**, in organic media by lipase catalysis.

2. Results and discussion

2.1. Synthesis of model racemic hydroxy esters

N-Benzyl-substituted oxo esters **1** and **6** were synthesized by Dieckmann condensation[17] from ethyl

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3-(*N*-benzyl-*N*-carbethoxyethyl)aminopropanoate[18] and ethyl 4-(*N*-benzyl-*N*-carbethoxymethyl)aminobutanoate,[17] respectively, which were debenzylated to give hydrochlorides **2** and **7**. Boc-protected oxo esters **3** and **8** were subjected to reduction with $NaBH_4$ to give hydroxy esters **4**, **5** and **9**, **10**, which were isolated by column chromatography. Compounds **9** and **10** were formed in a ratio of 7:3 in the reduction step; **9** could be isolated with de = 96%, and **10** with de = 90%, but in a very low (2%) yield. The diastereomeric mixture of *N*-benzyl-substituted hydroxy esters **11** and **12** was prepared directly from the liberated base of **6** under the same reduction conditions. Pure **11** was isolated by column chromatography, whereas pure **12** could not be isolated; it was obtained only in a 1:1 diastereomeric mixture (Scheme 1).

2.2. Lipase-catalyzed acylation of (±)-4 and (±)-5

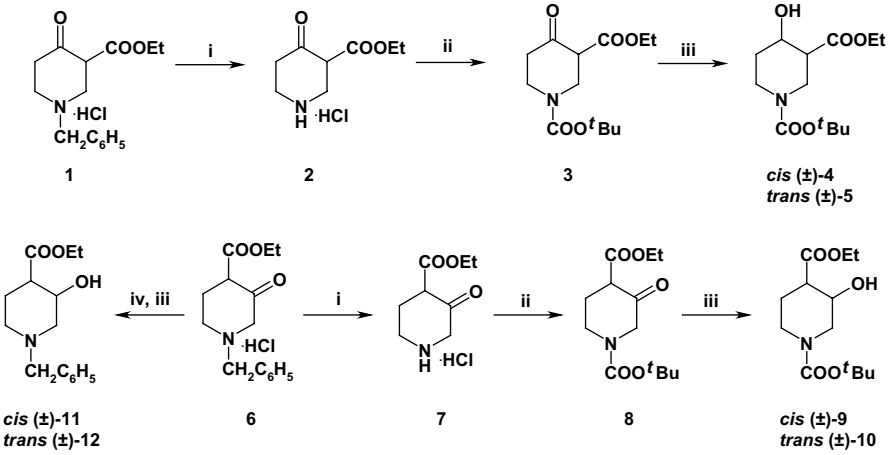
After a lipase screening for the acylation of (±)-**4** at room temperature, the effects of solvent, acyl donor, temperature and enzyme concentration were studied (Table 1).

With vinyl acetate (VA) as acyl donor in diisopropyl ether (*i*-Pr_2O) at room temperature, *Candida antarctica* lipase A (CAL-A) catalyzed the acylation with moderate enantioselectivity (entry 1). Lipolase (immobilized *C. antarctica* lipase B preparation), lipase PS (from *P. cepacia*), CAL-B (*C. antarctica* lipase B, Novozym 435) and lipase AK (from *P. fluorescens*) demonstrated excellent enantioselectivities, but lower catalytic activity, under the same conditions (entries 2–5). The best combination of enantioselectivity and reaction rate was observed for immobilized lipase AK catalysis. In an effort to increase the reaction rate, different solvents were tested: low reaction rates and low enantioselectivities were observed in CH_2Cl_2, THF and acetone (entries 6, 7 and 9), whereas the reaction rate was considerably higher in *i*-Pr_2O and in hexane (entries 5 and 8), with excellent enantioselectivities, *i*-Pr_2O giving the best results. On change of the acyl donor to isopropenyl acetate or vinyl propanoate (VP), the reaction became slower (after 144 h, $c = 48\%$, or after 116 h, $c = 50\%$), but the enantioselectivity was not affected ($E > 200$). Increase of the temperature was accompanied by a slight decrease in enantioselectivity (entries 10–12). As the lipase concentration was increased from 20 mg/mL (entry 10) to 30 mg/mL (after 10 h, $c = 37\%$, $E > 200$), the reaction rate increased considerably, but a further increase to 40 mg/mL (after 10 h, $c = 41\%$, $E = 86$) resulted in a drop in enantioselectivity.

In the small-scale acylation of (±)-**5** under the conditions optimized for (±)-**4** (Table 1, entry 10), a higher reaction rate (about five times) was observed, with excellent enantioselectivity (after 2 h, $c = 41\%$, $E > 200$). This observation is in accordance with the literature data concerning the *O*-acylation of the *cis*- and *trans*-cyclohexane-based analogues by lipase PS[19] and the hydrolysis of the *O*-acetates by *P. fluorescens* lipase.[20]

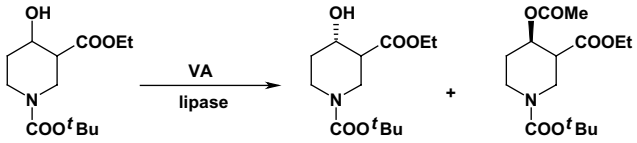
2.3. Lipase-catalyzed acylation of (±)-11

Since the *O*-acylated derivatives of the 3-hydroxy regioisomer **9** could not be separated by chiral gas chromatography on either a β-cyclodextrin, an L-valine or a γ-cyclodextrin column (Chrompack CP-Chirasil-DEX-CB, Chirasil-L-Val and GAMMA DEX 120), we decided to continue with the *N*-benzyl analogue **11**, the enantiomers of which could be easily analyzed through baseline separation of the acylated derivatives. In an enzyme screening, with 0.2 M VA as acyl donor in *i*-Pr_2O at 0.1 M substrate concentration, with 70 mg/mL enzyme at 45 °C, lipase AK, lipase PS and Chirazyme L-2 were found not to catalyze the *O*-acylation of (±)-**11**; only CAL-A displayed considerable catalytic activity, but low enantioselectivity (Table 2, entry 1). To increase the enantioselectivity, the effects of solvent, acyl donor, temperature and enzyme concentration were tested (Table 2). The enantioselectivity increased with decreasing temperature, but the reaction rate decreased (entries 1–3). As the reaction rate did not decrease at



Scheme 1. Reagents and conditions: (i) Pd/C, EtOH, H_2 atm; (ii) Boc_2O, Et_3N, CH_2Cl_2; (iii) $NaBH_4$, abs EtOH; (iv) Et_3N, CH_2Cl_2.

Table 1. Enzymatic *O*-acylation (20 mg/mL enzyme) of (±)-**4** and (±)-**5** (0.1 M) with vinyl acetate (VA) (0.2 M)

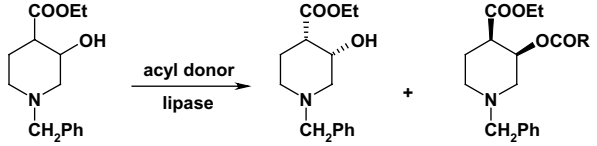


(±)-**4** *cis* (±)-**5***trans* **4a** **5a** **4b** **5b**

Entry	Enzyme	T (°C)	Solvent	Time (h)	Conv.[a] (%)	Ee_s (%)	Ee_p (%)	E[b]
1	CAL-A[c]	rt	*i*-Pr_2O	3.6	51	89	85	37
2	Lipase PS[c]	rt	*i*-Pr_2O	72	33	97	48	122
3	CAL-B	rt	*i*-Pr_2O	72	39	61	97	131
4	Lipolase	rt	*i*-Pr_2O	70	43	72	98	>200
5	Lipase AK[c]	rt	*i*-Pr_2O	72	49	95	98	>200
6	Lipase AK[c]	rt	CH_2Cl_2	180	2	2	93	27
7	Lipase AK[c]	rt	THF	180	13	11	76	8
8	Lipase AK[c]	rt	Hexane	180	50	99	98	>200
9	Lipase AK[c]	rt	Acetone	180	8	2	19	1
10	Lipase AK[c]	45	*i*-Pr_2O	11	27	37	>99	>200
11	Lipase AK[c]	55	*i*-Pr_2O	43	49	96	99	>200
12	Lipase AK[c]	60	*i*-Pr_2O	28	41[d]	67	96	100

[a] Conv. = $ee_s/(ee_s + ee_p)$.
[b] $E = \ln[(1 - ee_s)/(1 - ee_s/ee_p)]/\ln[(1 - ee_s)/(1 + ee_s/ee_p)]$.[21]
[c] Contains 20% (w/w) of lipase adsorbed on Celite in the presence of sucrose.[22]
[d] The reaction stopped at this conversion.

Table 2. CAL-A-catalyzed *O*-acylation of (±)-**11** (0.1 M) with different acyl donors (0.2 M) in organic solvents



(±)-**11** *cis* **11a** **11b**

R = Me, Et, *n*-Pr

Entry	CAL-A (mg/mL)	T (°C)	Solvent	Acyl donor	Time (h)	Conv.[a] (%)	Ee_s (%)	Ee_p (%)	E[b]
1	70	rt	*i*-Pr_2O	VA	5	50	60	60	7
2	70	rt	*i*-Pr_2O	VA	10	38	50	83	18
3	70	3	*i*-Pr_2O	VA	145	40	57	86	23
4	40	rt	*i*-Pr_2O	VA	9	43	60	79	15
5	40	rt	Toluene	VA	166	40	47	69	9
6	40	rt	MeCN[c]	VA	166	29	21	52	4
7	40	rt	*i*-Pr_2O	VP	4	48	71	77	16
8	40	3	*i*-Pr_2O	VP	4	27	36	96	75
9	40	rt	*i*-Pr_2O	VB	3	48	63	69	10
10	40	rt	*i*-Pr_2O	Isopropenyl acetate	50	36	45	79	13
11	40	45	$PrCO_2Et$	$PrCO_2Et$	128	21	10	38	2

[a] Conv. = $ee_s/(ee_s + ee_p)$.
[b] $E = \ln[(1 - ee_s)/(1 - ee_s/ee_p)]/\ln[(1 - ee_s)/(1 + ee_s/ee_p)]$.[21]
[c] Opposite enantioselectivity.

40 mg/mL enzyme concentration (entry 4 vs entry 2), from economic considerations 40 mg/mL enzyme was used in the further reactions. On change of the solvent to toluene or MeCN, the enantioselectivity and the reaction rate decreased (entries 5 and 6 vs entry 4). The enzyme exhibited the opposite enantiopreference in MeCN, but low enantioselectivity and a low reaction rate (entry 6). With increase of the acid side-chain length in the acyl donor from VA to VP, the reaction rate increased without a loss in enantioselectivity at room temperature (entries 4 and 7), but when vinyl butanoate (VB) was used, the enantioselectivity did decrease (entry 9). The application of isopropenyl acetate, possessing a bulkier side-chain in the alcohol part of the ester, decreased both the enantioselectivity and the reaction rate (entry 10 vs entry 4).

Table 3. Gram-scale resolution of (±)-**4**, (±)-**5** and (±)-**11**

Substrate	Time (h)	Conv.[c] (%)	4a–11a				4b–11b			
			Abs. config.	Ee (%)	$[\alpha]_D^{25}$	Yield[d] (%)	Abs. config.	Ee (%)	$[\alpha]_D^{25}$	Yield[d] (%)
(±)-**4**[a]	41.5	50	3*R*,4*S*	>99	+57.9[e]	30	3*S*,4*R*	99	−41.0[e]	47
(±)-**5**[a]	14	50	3*S*,4*S*	99	−24.4[f]	41	3*R*,4*R*	>99	+6.5[g]	39
(±)-**11**[b] R=CH_2CH_3	Resolution in two steps		3*S*,4*S*	92	+37.7[h]	28	3*R*,4*R*	91	−24.0[i]	33

[a] 0.1 M substrate, 0.2 M VA in *i*-Pr_2O, 30 mg/mL lipase AK at 45 °C.
[b] 0.1 M substrate, 0.2 M VP in *i*-Pr_2O, 40 mg/mL CAL-A at 3 °C.
[c] Conv. = $ee_s/(ee_s + ee_p)$.
[d] Total yield starting from racemic substrate.
[e] *c* 1, CH_2Cl_2.
[f] *c* 0.91, CH_2Cl_2.
[g] *c* 1.01, CH_2Cl_2.
[h] *c* 0.77, $CHCl_3$.
[i] *c* 1, $CHCl_3$.

For the isolation of enantiomeric compounds, resolution could be achieved in two consecutive steps, under the same conditions (entry 8).

As a result of the above small-scale experiments, the preparative-scale resolution of (±)-**4** and (±)-**5** was performed through *O*-acetylation by lipase AK with VA in *i*-Pr_2O at 45 °C (Table 1, conditions of entry 10), while (±)-**11** was resolved through *O*-acylation by CAL-A with VP in *i*-Pr_2O at 3 °C (Table 2, entry 8), and the enantiomers were isolated (Table 3).

2.4. Determination of absolute configuration

When the specific rotation data on **4a** are compared with literature values,[8a,12,13] we can conclude from the sign of rotation that lipase AK exhibited (*R*) selectivity in the *O*-acylation. Our results reveal $[\alpha]_D^{25} = +57.9$ (*c* 1, CH_2Cl_2) for the hydroxy ester **4a** (ee >99%), which is in accordance with the literature values of $[\alpha]_D^{25} = +15$ (*c* 0.75, CH_2Cl_2, ee = 24%)[8a] and $[\alpha]_D^{25} = +23$ (*c* 0.75, CH_2Cl_2, ee = 41%) for the (3*R*,4*S*) enantiomer.[13] For determination of the absolute configuration, **11b** was transformed to its methyl ester and *N*-Boc-protected analogue, which is known in the literature. Comparison of the specific rotation $[\alpha]_D^{25} = -27$ (*c* 0.12, $CHCl_3$) with that given in the literature, $[\alpha]_D^{25} = -32.7$ (*c* 1, $CHCl_3$, ee not given)[12b] proved the absolute configuration (3*R*,4*R*) for the product.

3. Conclusions

The resolution of highly valuable *N*-protected piperidine-based hydroxy esters has been achieved by (*R*)-selective lipase catalysis. Enantiomerically pure (ee ⩾ 99%) (3*R*,4*S*)-ethyl *cis*-**4a** and (3*S*,4*S*)-ethyl *trans*-1-(*tert*-butoxycarbonyl)-4-hydroxypiperidine-3-carboxylate **5a** and (3*S*,4*R*)-ethyl *cis*-**4b** and (3*R*,4*R*)-ethyl *trans*-1-(*tert*-butoxycarbonyl)-4-acetyloxypiperidine-3-carboxylate **5b** were prepared by lipase AK-catalyzed (*R*)-selective *O*-acetylation with VA in *i*-Pr_2O at 45 °C. (3*S*,4*S*)-Ethyl *cis*-1-benzyl-3-hydroxypiperidine-4-carboxylate **11a** (ee = 92%) and (3*R*,4*R*)-ethyl *cis*-1-benzyl-3-propanoyloxypiperidine-4-carboxylate **11b** (ee = 91%) were prepared by CAL-A-catalyzed (*R*)-selective *O*-acylation with VP in *i*-Pr_2O at 3 °C. The isolated enantiomeric compounds were characterized, and the previously described literature data for **4a** were clarified. Enantiomerically pure **5a**, **5b** and **11b** had not been reported in the literature previously. All the enantiomerically pure compounds isolated are of great pharmaceutical interest for further investigations.

4. Experimental

In a typical small-scale experiment, the substrate (0.1 M) was dissolved in an organic solvent, and a lipase or a lipase preparation, 20% (w/w) immobilized on Celite in the presence of sucrose,[22] and 2 equiv of an acyl donor were added. The progress of the reactions and the ee values were followed by taking samples at intervals and analyzing them by gas chromatography on a Chrompack CP-Chirasil-DEX-CB (25 m). For good baseline separation, the unreacted hydroxy group in the sample was derivatized with acetic or propanoic anhydride in the presence of pyridine containing 1% 4-*N*,*N*-dimethylaminopyridine before injections.

Melting points were determined with a Kofler apparatus at a heating rate of 4 °C/min. ^{1}H NMR spectra were recorded in $CDCl_3$ at ambient temperature on a Bruker DRX400 spectrometer. Chemical shifts are given in δ (ppm) relative to TMS as internal standard; multiplicities were recorded as s (singlet), br s (broad singlet), d (doublet), dd (double doublet), ddd (double double doublet), t (triplet), dt (double triplet), ddt (double double triplet), q (quartet) or m (multiplet). MS spectra were recorded on a Finnigan MAT 95 S instrument. Elemental analyses were performed with a Perkin–Elmer CHNS-2400 Ser II Elemental Analyzer. Optical rotations were measured with a Perkin–Elmer 341 polarimeter.

4.1. Ethyl 4-oxopiperidine-3-carboxylate hydrochloride, 2

Ethyl 1-benzyl-4-oxopiperidine-3-carboxylate hydrochloride[18] **1** (2.5 g, 8.40 mmol) was hydrogenated in the

presence of 10% Pd/C catalyst at atmospheric pressure in abs EtOH (250 mL) for 8 h until the hydrogen uptake was over. The catalyst was then filtered off and the solvent was evaporated off. The residue was recrystallized from EtOH/Et_2O to give light-brown crystals (1.64 g, 94%), mp 159–162 °C, lit. mp[23] 164–166 °C. ^{1}H NMR δ 1.31 (3H, t, J = 7.1 Hz, CH_2CH_3), 2.77 (2H, t, J = 5.7, 6-CH_2), 3.37 (2H, d, J = 5.2 Hz, 5-CH_2), 3.87 (2H, s, 2-CH_2), 4.23 (2H, q, J = 7.1 Hz, OCH_2), 10.12 (2H, s, $NH_2^+Cl^-$), 12.16 (1H, br s, OH). Anal. Calcd for $C_8H_{14}ClNO_3$: C, 46.27; H, 6.80; N, 6.75. Found: C, 46.64; H, 6.99; N, 6.27%.

4.2. Ethyl 1-(*tert*-butoxycarbonyl)-4-oxopiperidine-3-carboxylate, 3

Ethyl 4-oxopiperidine-3-carboxylate hydrochloride **2** (6.05 g, 29.23 mmol) was dissolved in CH_2Cl_2, and Et_3N (11.86 g, 117.21 mmol) was added. The mixture was stirred for 2 h and di-*tert*-butyl dicarbonate (7.04 g, 32.22 mmol) was then added to the mixture, which was next stirred overnight. The solvent was evaporated off and the product was purified by silica gel column chromatography, using EtOAc/hexane = 1:3 as eluent, to give colourless crystals (7.06 g, 89%), mp 64–65 °C (crystallized from hexane), lit. mp[12b] 62 °C. ^{1}H NMR δ 1.31 (3H, t, J = 6.6 Hz, CH_2CH_3), 1.48 (9H, s, *t*Bu), 2.32–2.40 (2H, m, 5-CH_2), 3.56 (2H, t, J = 5.8 Hz, 6-CH_2), 4.06 (2H, s, 2-CH_2), 4.23 (2H, q, J = 6.8 Hz, OCH_2), 12.05 (1H, br s, OH). Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.55; H, 7.80; N, 5.16. Found: C, 57.06; H, 8.00; N, 4.72%.

4.3. (±)-Ethyl *cis*- and *trans*-1-(*tert*-butoxycarbonyl)-4-hydroxypiperidine-3-carboxylate, (±)-4 and (±)-5

Ethyl 1-(*tert*-butoxycarbonyl)-4-oxopiperidine-3-carboxylate (3 g, 11.06 mmol) was dissolved in abs EtOH (ca. 100 mL), and $NaBH_4$ (0.25 g, 6.61 mmol) was added in small portions during 30 min at room temperature.[2g] The mixture was stirred overnight, and the solvent was then evaporated off. The residue was dissolved in water and extracted with EtOAc (3 × 25 mL). The combined organic layer was dried on Na_2SO_4 and concentrated. NMR indicated that the crude product was a mixture of 87% *cis* and 13% *trans* isomers. The diastereomers were separated by column chromatography, using EtOAc/hexane = 1:3 as eluent.

(±)-**4**. First-eluting isomer, de = 98%: colourless crystals, mp 62–63 °C (crystallized from hexane), lit. mp[12b] 58–60 °C. ^{1}H NMR (DMSO-d_6, 400 MHz, 333 K) δ 1.19 (3H, t, J = 7.3 Hz, CH_2CH_3), 1.39 (9H, s, *t*Bu), 1.49–1.59 (1H, m, 5-H_{ax}), 1.67 (1H, ddd, J = 3.8, 13.6 Hz, 5-H_{eq}), 2.46 (1H, dd, J = 3.3, 4.3 Hz, 3-H_{ax}), 3.06–3.26 (2H, m, 6-H_{ax}, 2-H_{ax}), 3.58 (1H, ddt, J = 1.3, 4.8, 13.1 Hz, 6-H_{eq}), 3.77 (1H, dd, J = 4.0, 13.4 Hz, 2-H_{eq}), 4.02–4.12 (2H, m, CH_2CH_3), 4.19 (1H, dd, J = 3.5, 6.0 Hz, 4-H), 4.70 (1H, br s, OH). ^{13}C NMR δ 13.7, 27.8, 31.5, 38.0, 39.6, 45.4, 59.4, 64.1, 78.3, 154.4, 170.8. MS (*m*/*z*, EI) (rel. abund.) 273 (2, [M^+]), 216 (32), 200 (13), 172 (23), 154 (37), 126 (50), 100 (24), 82 (79), 57 (100), 41 (21). Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.22; H, 8.98; N, 5.26%.

(±)-**5**. de = 90%: light-yellow oil, ^{1}H NMR (DMSO-d_6, 400 MHz, 333 K) δ 1.19 (3H, t, J = 7.1 Hz, CH_2CH_3), 1.22–1.32 (1H, m, 5-H), 1.39 (9H, s, *t*Bu), 1.81 (1H, ddd, J = 4.3, 7.6, 12.6 Hz, 5-H), 2.23 (1H, ddd, J = 4.0, 9.1, 9.8 Hz, 3-H), 2.72–3.13 (2H, m, 2-H, 6-H), 3.73–3.80 (2H, m, 2-H, 6-H), 3.89 (1H, dd, J = 2.3, 13.4 Hz, 4-H), 4.08 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.79 (1H, d, J = 5.5, OH). ^{13}C NMR δ 13.7, 27.7, 32.5, 41.3, 43.5, 49.7, 59.6, 67.59, 78.6, 153.5, 171.5. MS (*m*/*z*, EI) (rel. abund.) 273 (4, [M^+]), 216 (67), 200 (32), 172 (66), 154 (37), 126 (72), 100 (65), 82 (82), 57 (100), 41 (51). Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.25; H, 8.64; N, 5.20%.

4.4. Preparative-scale resolution of (±)-4

Five hundred milligrams (1.83 mmol) of (±)-**4** was dissolved in 18 mL of *i*-Pr_2O, and 540 mg (30 mg/mL) of 20% lipase AK preparation and 340 μL (3.67 mmol) of VA were added. The reaction mixture was shaken at 45 °C for 41.5 h. The reaction was stopped at 50% conversion by filtering off the enzyme. After evaporation of the solvent, the crude mixture was purified by column chromatography, using EtOAc/hexane = 1:3 as eluent.

(3*R*,4*S*)-**4a** (150 mg, 30%), a slowly crystallizing oil, ee >99%, $[\alpha]_D^{25}$ = +57.9 (*c* 1, CH_2Cl_2). The ^{1}H NMR, ^{13}C NMR and MS data were identical with those for (±)-**4**. HRMS (EI): 273.15839 (calcd 273.15762). Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H; 8.48; N, 5.12. Found: C, 56.77; H, 7.93; N, 4.73%.

(3*S*,4*R*)-**4b** (160 mg, 47%), a colourless oil, ee = 99%, $[\alpha]_D^{25}$ = −41.0 (*c* 1, CH_2Cl_2). ^{1}H NMR δ 1.23 (3H, t, J = 7.0 Hz, CH_2CH_3), 1.46 (9H, s, *t*Bu), 1.64–1.75 (1H, m, 5-H_{ax}), 1.95 (1H, ddd, J = 3.0, 7.3, 12.1 Hz, 5-H_{eq}), 2.04 (3H, s, $OCOCH_3$), 2.66 (1H, ddd, J = 3.3, 4.3, 11.1 Hz, 3-H_{ax}), 3.05 (1H, t, J = 12.3, 6-H_{ax}), 3.31 (1H, t, J = 12.6, 2-H_{ax}), 3.86 (1H, d, J = 10.3 Hz, 6-H_{eq}), 4.05–4.21 (3H, m, 2-H_{eq}, CH_2CH_3), 5.46 (1H, dd, J = 3.0, 6.3 Hz, 4-H). ^{13}C NMR δ 14.8, 21.6, 29.1, 29.8, 39.6, 41.4, 45.0, 61.4, 68.8, 80.7, 155.6, 171.1, 170.6. MS (*m*/*z*, EI) (rel. abund.) 315 (3, [M^+]), 258 (68), 242 (15), 214 (65), 199 (70), 182 (26), 170 (25), 154 (88), 126 (87), 110 (29), 82 (92), 57 (100), 43 (43). HRMS (EI): 315.17090 (calcd 315.16818). Anal. Calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.63; H, 8.23; N, 4.74%.

4.5. Preparative-scale resolution of (±)-5

Compound (±)-**5** [168 mg (0.61 mmol)] was dissolved in 6 mL of *i*-Pr_2O, and 180 mg (30 mg/mL) of 20% lipase AK preparation and 114 μL (1.23 mmol) of VA were added. The reaction mixture was shaken at 45 °C for 14 h. The reaction was stopped at 50% conversion by filtering off the enzyme. After evaporation of the solvent, the crude mixture was purified by column chromatography, using EtOAc/hexane = 1:3 as eluent.

(3*S*,4*S*)-**5a** (68 mg, 41%), a light-yellow oil, ee = 99%, $[\alpha]_D^{25} = -24.4$ (*c* 0.91, CH_2Cl_2). The ^1H NMR, ^{13}C NMR and MS data were identical with those for (±)-**5**. HRMS (EI): 273.15965 (calcd 273.15762). Anal. Calcd for $C_{13}H_{23}NO_5$: C: 57.13, H: 8.48, N: 5.12. Found: C: 56.64, H: 8.06, N: 4.96%.

(3*R*,4*R*)-**5b** (67 mg, 39%), a light-yellow oil, ee >99%, $[\alpha]_D^{25} = +6.5$ (*c* 1.01, CH_2Cl_2). ^1H NMR δ 1.25 (3H, t, $J = 7.1$ Hz, CH_2CH_3), 1.46 (9H, s, *t*Bu), 1.50–1.70 (1H, m, 5-H_{ax}), 2.03 (3H, s, OCH_3), 2.04–2.10 (1H, m, 5-H_{eq}), 2.60 (1H, ddd, $J = 4.3$, 9.8 Hz, 3-H_{ax}), 3.01 (1H, ddd, $J = 3.3$, 11.1, 13.9 Hz, 6-H_{ax}), 3.14 (1H, br s, 2-H_{ax}), 3.91 (1H, dt, $J = 4.0$, 13.9 Hz, 6-H_{eq}), 4.12 (3H, q, $J = 7.1$ Hz, 2-H, CH_2CH_3), 5.14 (1H, ddd, $J = 4.3$, 9.6 Hz, 4-H). ^{13}C NMR δ 14.8, 21.7, 29.0, 30.3, 42.3, 44.7, 47.6, 61.6, 71.7, 80.9, 155.0, 170.6, 171.5. MS (*m*/*z*, EI) (rel. abund.) 315 (3, [M^+]), 256 (16), 242 (15), 214 (42), 198 (77), 182 (42), 170 (26), 154 (88), 126 (87), 110 (32), 82 (93), 57 (100), 43 (46). HRMS (EI): 315.16915 calcd 315.16818). Anal. Calcd for $C_{15}H_{25}NO_6$: C, 57.13; H, 7.99; N, 4.44. Found: C, 57.08; H, 7.88; N, 4.22%.

4.6. (±)-Ethyl *cis*-1-benzyl-3-hydroxypiperidine-4-carboxylate, (±)-11

1-Benzyl-3-oxopiperidine-4-carboxylate (1.5 g, 5.74 mmol) (liberated from **6**[17] with Et_3N and purified by silica gel column chromatography, using EtOAc/hexane = 1:3 as eluent) was dissolved in abs EtOH (ca. 100 mL), and $NaBH_4$ (0.25 g, 6.61 mmol) was added in small portions during 30 min at room temperature.[2g] The mixture was stirred overnight, and the solvent was then evaporated off. The residue was dissolved in water and extracted with EtOAc (3 × 20 mL). The combined organic layer was dried on Na_2SO_4 and concentrated. NMR indicated that the crude product was a mixture of 74% *cis* and 26% *trans* isomers. The *cis* isomer was isolated after silica gel column chromatography, using EtOAc/hexane = 1:3 as eluent; the *trans* isomer could be obtained only in a 1:1 diastereomeric mixture.

(±)-**11**. First-eluting isomer, de = 98%: a light-brown oil, ^1H NMR (DMSO-d_6, 400 MHz) δ 1.16 (3H, t, $J = 7.09$ Hz, CH_2CH_3), 1.48–1.60 (1H, m, 5-H), 1.92 (2H, t, $J = 8.46$ Hz, 5-H, 6-H), 2.10 (1H, d, $J = 11.37$ Hz, 2-H), 2.35–2.45 (1H, m, 4-H), 2.68–2.80 (2H, m, 2-H, 6-H), 3.45 (2H, d, $J = 1.51$ Hz, CH_2Ph), 3.95–4.10 (3H, m, CH_2CH_3, 3-H), 4.32 (1H, d, $J = 6.92$ Hz, OH), 7.18–7.35 (5H, m, aromatic). ^{13}C NMR (DMSO-d_6, 400 MHz) δ 14.6, 22.6, 45.6, 51.9, 59.2, 60.0, 62.3, 66.4, 127.2, 128.5, 129.1, 138.7, 173.0. MS (*m*/*z*, EI) (rel. abund.) 263 (10, [M^+]), 245 (6), 234 (4), 218 (12), 186 (8), 172 (50), 146 (8), 126 (8), 118 (4), 98 (6), 91 (100). HRMS (EI): 263.15238 (calcd 263.152141). Anal. Calcd for $C_{15}H_{21}NO_3$: C, 68.42; H, 8.04; N, 5.32. Found: C, 68.35; H, 7.99; N, 5.36%.

4.7. Preparative-scale resolution of (±)-11

Compound (±)-**11** [165 mg (0.63 mmol)] was dissolved in 6 mL of *i*-Pr_2O, and 240 mg (40 mg/mL) of 20% CAL-A preparation and 137 μL (1.26 mmol) of VA were added. The reaction mixture was stirred in a cold room for 5 h. The reaction was stopped at 36% conversion by filtering off the enzyme. After evaporation of the solvent, the crude mixture was purified by column chromatography, using EtOAc as eluent, to afford (3*S*,4*S*)-**11a** (100.4 mg, 61%), ee = 51%, and (3*R*,4*R*)-**11b** (66.9 mg, 33%), ee = 91%, $[\alpha]_D^{25} = -24.0$ (*c* 1, $CHCl_3$).

^1H NMR data for **11b**: δ 1.13 (3H, t, $J = 7.6$ Hz, $OCOCH_2CH_3$), 1.21 (3H, t, $J = 7.0$ Hz, CH_2CH_3), 1.81–1.88 (1H, m, 4-H), 2.06–2.23 (3H, m, 1-H, 4-H, 5-H), 2.28–2.36 (2H, m, $OCOCH_2CH_3$), 2.47–2.54 (1H, m, 3-H), 2.94–3.00 (1H, m, 5-H), 3.07 (1H, dd, $J = 3.0$, 12.6 Hz, 1-H), 3.50 (1H, d, $J = 13.4$ Hz, 6-H), 3.60 (1H, d, $J = 13.4$ Hz, 6-H), 4.04–4.19 (2H, m, CH_2CH_3), 5.32 (1H, dd, $J = 3.0$, 5.3 Hz, 2-H), 7.22–7.32 (5H, m, aromatic). ^{13}C NMR δ 9.9, 14.8, 23.9, 28.4, 44.5, 52.4, 55.9, 61.2, 62.9, 127.9, 128.9, 129.6, 138.2, 172.5, 174.4. MS (*m*/*z*, EI) (rel. abund.) 319 (6, [M^+]), 290 (2), 274 (30), 256 (8), 245 (80), 228 (8), 218 (28), 190 (4), 172 (100), 149 (12), 118 (16), 97 (10), 91 (96). HRMS (EI): 319.17810 (calcd 319.17835).

Enantiomerically enriched **11a** [90 mg (0.34 mmol)] was dissolved in 3 mL of *i*-Pr_2O, and 120 mg (40 mg/mL) of 20% CAL-A preparation and 75 μL (0.68 mmol) of VA were added. The reaction mixture was stirred at 3 °C for 7.5 h. The reaction was stopped at 23% conversion by filtering off the enzyme. After evaporation of the solvent, the crude mixture was purified by column chromatography, using EtOAc as eluent, to afford (3*S*,4*S*)-**11a** (41.6 mg, 46%), ee = 92%, $[\alpha]_D^{25} = +37.7$ (*c* 0.77, $CHCl_3$), and (3*R*,4*R*)-**11b** (24.5 mg, 23%), ee = 62%.

The ^1H NMR, ^{13}C NMR and MS data for **11a** were identical with those for (±)-**11**. HRMS (EI): 263.15261 (calcd 263.15214).

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References

1. (a) Krogsgaard-Larsen, P.; Thyssen, K.; Schaumburg, K. *Acta Chem. Scand.* **1978**, *32*, 327–334; (b) Jacobsen, P.; Schaumburg, K.; Krogsgaard-Larsen, P. *Acta Chem. Scand.* **1980**, *34*, 319–326.
2. (a) Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. *Nature* **1975**, *258*, 627–628; (b) Krogsgaard-Larsen, P.; Johnston, G. A. R.; Curtis, D. R.; Game, C. J. A.; McCulloch, R. M. *J. Neurochem.* **1975**, *25*, 803–809; (c) Johnston, G. A. R.; Stephanson, A. L.; Twitchin, B. *J. Neurochem.* **1976**, *26*, 83–87; (d) Johnston, G. A. R.; Krogsgaard-Larsen, P.; Stephanson, A. L.; Twitchin, B. *J. Neurochem.* **1976**, *26*, 1029–1032; (e) Krogsgaard-Larsen, P.; Roldskov-Christiansen, T. *Eur. J. Med. Chem.* **1979**, *14*, 157–164; (f) Krogsgaard-Larsen, P. *J. Med. Chem.* **1981**, *24*, 1377–1383; (g) Jacobsen, P.; Labouta, I. M.; Schaumburg, K.; Falch, E.; Krogsgaard-Larsen, P. *J. Med. Chem.* **1982**, *25*, 1157–1162; (h) Ali, F. E.; Bondinell,

W. E.; Dandridge, P. A.; Frazee, J. S.; Garvey, E.; Girard, G. R.; Kaiser, C.; Ku, T. W.; Lafferty, J. J.; Moonsammy, G. I.; Oh, H.-J.; Rush, J. A.; Setler, P. E.; Stringer, O. D.; Venslavsky, J. W.; Volpe, B. W.; Yunger, L. M.; Zirkle, C. L. *J. Med. Chem.* **1985**, *28*, 653–660; (i) Pavia, M. R.; Lobbestael, S. J.; Nugiel, D.; Mayhugh, D. R.; Gregor, V. E.; Taylor, C. P.; Schwarz, R. D.; Brahce, L.; Vartanian, M. G. *J. Med. Chem.* **1992**, *35*, 4238–4248; (j) Krogsgaard-Larsen, P.; Frolund, B.; Frydenvang, K. *Curr. Pharm. Des.* **2000**, *6*, 1193–1209.

3. (a) Singh, S.; Basmadjian, G. P.; Avor, K. S.; Pouw, B.; Seale, T. W. *J. Med. Chem.* **1997**, *40*, 2474–2481; (b) Kozikowski, A. P.; Araldi, G. L.; Boja, J.; Meil, W. M.; Johnson, K. M.; Flippen-Anderson, J. L.; George, C.; Saiah, E. *J. Med. Chem.* **1998**, *41*, 1962–1969; (c) Hoepping, A.; Babich, J.; Zubieta, J. A.; Johnson, K. M.; Machill, S.; Kozikowski, A. P. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3211–3216; (d) Petukhov, P. A.; Zhang, M.; Johnson, K. J.; Tella, S. R.; Kozikowski, A. P. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2079–2083; (e) Feng, X.; Fandrick, K.; Johnson, R.; Janowsky, A.; Cashman, J. R. *Bioorg. Med. Chem.* **2003**, *11*, 775–780.
4. Takemura, S.; Miki, Y.; Komada, F.; Takahashi, K.; Suzuki, A. *Chem. Pharm. Bull.* **1979**, *27*, 1893–1900.
5. (a) Löscher, W.; Frey, H.-H. *Drug Res.* **1984**, *34*, 1484–1488; (b) Crider, A. M.; Tita, T. T.; Wood, J. D.; Hinko, C. N. *J. Pharm. Sci.* **1982**, *71*, 1214–1219.
6. (a) Carroll, F. I.; Lewin, A. H.; Abraham, P.; Parham, K.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1991**, *34*, 883–886; (b) Carroll, F. I.; Lewin, A. H.; Boja, J. W.; Kuhar, M. J. *J. Med. Chem.* **1992**, *35*, 969–981.
7. (a) Liang, X.; Lohse, A.; Bols, M. *J. Org. Chem.* **2000**, *65*, 7432–7437; (b) Jensen, H. H.; Lohse, A.; Petersen, B. O.; Duus, J. O.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2000**, 667–670; (c) Lillelund, V. H.; Jensen, H. H.; Liang, X.; Bols, M. *Chem. Rev.* **2002**, *102*, 515–553; (d) Dhavale, D. D.; Matin, M. M.; Sharma, T.; Shabharwal, S. G. *Bioorg. Med. Chem.* **2003**, *11*, 3295–3305.
8. (a) Byrgesen, E.; Nielsen, J.; Willert, M.; Bols, M. *Tetrahedron Lett.* **1997**, *38*, 5697–5700; (b) Carstensen, E. V.; Phytera, S. A.; Bols, M.; Nielsen J.; Willert M. Patent WO 9,903,832, 1999, *Chem. Abstr.* **1999**, *130*, 153577; (c) Liang, X.; Petersen, B. O.; Duus, J. O.; Bols, M. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2764–2773.
9. (a) Kawamoto, H.; Ozaki, S.; Itoh, Y.; Miyaji, M.; Arai, S.; Nakashima, H.; Kato, T.; Ohta, H.; Iwasawa, Y. *J. Med. Chem.* **1999**, *42*, 5061–5063; (b) Kawamoto, H.; Nakashima, H.; Kato, T.; Arai, S.; Kamata, K.; Iwasawa, Y. *Tetrahedron* **2001**, *57*, 981–986.
10. Schinnerl, M.; Murray, J. K.; Langenhan, J. M.; Gellman, S. H. *Eur. J. Org. Chem.* **2003**, 721–726.
11. (a) Bailey, P. D.; Millwood, P. A.; Smith, P. D. *Chem. Commun.* **1998**, 633–640; (b) Laschat, S.; Dickner, T. *Synthesis* **2000**, 1781–1813.
12. (a) Knight, D. W.; Lewis, N.; Share, A. C.; Haigh, D. *Tetrahedron: Asymmetry* **1993**, *4*, 625–628; (b) Knight, D. W.; Lewis, N.; Share, A. C.; Haigh, D. *J. Chem. Soc., Perkin Trans. 1* **1998**, 3673–3683.
13. Willert, M.; Bols, M. *Acta Chem. Scand.* **1998**, *52*, 461–468.
14. Roberti, M.; Rondanin, R.; Ferroni, R.; Baruchello, R.; Invidiata, F. P.; Andrisano, V.; Bertucci, C.; Bertolasi, V.; Grimaudo, S.; Tolomeo, M.; Simoni, D. *Tetrahedron: Asymmetry* **2000**, *11*, 4397–4405.
15. Kozikowski, A. P.; Simoni, D.; Baraldi, P. G.; Lampronti, I.; Manfredini, S. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 441–444.
16. Seebach, D.; Roggo, S.; Maetzke, T.; Braunschweiger, H.; Cercus, J.; Krieger, M. *Helv. Chim. Acta* **1987**, *70*, 1605–1615.
17. Iselin, B. M.; Hoffman, K. *Helv. Chim. Acta* **1954**, *37*, 178–184.
18. Morosawa, S. *Bull. Chem. Soc. Jpn.* **1958**, *31*, 418–422.
19. Kanerva, L. T.; Sundholm, O. *Acta Chem. Scand.* **1993**, *47*, 823–825.
20. Xie, Z.-F.; Nakamura, I.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 966–967.
21. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
22. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
23. Moreno-Manas, M.; Pérez, M.; Pleixats, R. *Tetrahedron* **1994**, *50*, 515–528.