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Lipase-catalyzed kinetic and dynamic kinetic resolution of 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid

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Abstract—A dynamic kinetic resolution method for the preparation of enantiopure 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (*R*)-**2** was developed involving the CAL-B-catalyzed enantioselective hydrolysis of the corresponding ethyl ester (±)-**1** in toluene/acetonitrile (4:1) containing 1 equiv of added water and 0.25 equiv of dipropylamine. This method allowed the preparation of (*R*)-**2** (ee = 96%) with 80% isolated yield. The kinetic resolution of (±)-**1** in diisopropyl ether at 3 °C afforded both enantiomers with ee ⩾92%.

1. Introduction

The isoquinoline skeleton is of great chemical and biological importance. Drugs of either natural or synthetic origin that contain this skeleton can be applied to a wide range of therapies. Noscapine, praziquantel and quinapril are the best-known therapeutic agents based on the 1,2,3,4-tetrahydroisoquinoline moiety.[1] Noscapine, a well-known antitussive agent, was recently discovered to have microtubule-targeting anticancer properties.[2] Some derivatives of the 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid (1-TIC) occur naturally, for example, (−)-salsolinol-1-carboxylic acid, the 6,7-dihydroxy-1-methyl-substituted derivative, has been identified from the human brain.[3] 1-TIC can be used in the synthesis of promising matrix metalloproteinase (MMP) inhibitors[4] and in gonadotropine-releasing hormone (GnRH) receptor antagonists.[5] (*S*)-1,2,3,4-Tetrahydroisoquinoline-3-carboxylic acid (3-TIC) is part of the constitution of quinapril.[1] Both 1-TIC and 3-TIC are conformationally constrained amino acids. The use of these types of building blocks has gained ground in the design of peptide ligands, with improved selectivity towards a certain type of receptor: the side-chain topology of these amino acids is rather restricted relative to the peptide backbone, allowing less conformational variability.[6] 1-TIC can be obtained in an enantiopure form by the diastereomer salt formation of its N-protected derivatives[7] and the diastereoselective synthesis of Reissert compounds.[8]

Enzyme chemistry has led to the development of numerous simple, sustainable and widely applicable methods for the resolution of amino acids. Lipases may act enantioselectively on both the amino and carboxylic functionalities, involving many possible resolution pathways.[9] Our aim was to elaborate a method for the enzyme-catalyzed resolution of (±)-**1**, relying on methods applied successfully for the two structural analogues of 1-TIC ethyl ester (Fig. 1), as 1-TIC is a benzologue of pipecolic acid and a conformationally constrained, cyclic analog of phenylglycine. Thus, methyl pipecolate was previously resolved by the CAL-A (*Candida antarctica* lipase A)-catalyzed acylation of its secondary amino group with vinyl esters, and the system was later transformed via dynamic kinetic resolution via Schiff base formation with in situ formed acetaldehyde.[10] Studies of the dynamic kinetic resolution by the CAL-B (*Candida antarctica* lipase B)-catalyzed ammonolysis of phenylglycine methyl ester were also previously reported using

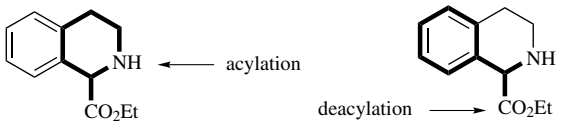

Figure 1. Possible resolution strategies for the kinetic resolution of the ethyl ester of 1-TIC, (±)-**1**.

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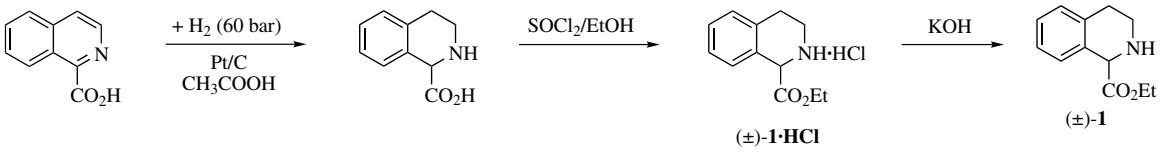


Scheme 1. Synthesis of (±)-**1.**

pyridoxal for the imine formation.[11] For our resolution studies, we prepared substrate (±)-**1** from isoquinoline-1-carboxylic acid by the platinum-catalyzed reduction under a H_2 atmosphere,[12] followed by esterification with EtOH/$SOCl_2$ and liberation of the free amino ester with KOH (Scheme 1).

2. Results and discussion

According to the above-mentioned method for the resolution of methyl pipecolate,[10] we started our investigations with the enzymatic N-acylation of (±)-**1**, using CAL-A (adsorbed on Celite in the presence of sucrose)[13] as a catalyst. The reaction proved to be extremely slow, even at 47 °C. Among the four acyl donors tested (trifluoroethyl acetate, trifluoroethyl chloroacetate, trifluoroethyl butanoate and isopropenyl acetate), isopropenyl acetate afforded the fastest N-acylation (5% conversion in 72 h).

Considering (±)-**1** as a phenylglycine ester analogue,[11] the CAL-B-catalyzed aminolyses of (±)-**1** with butyl- and benzylamine in diisopropyl ether (DIPE) were studied at 25 °C. The substrate reacted smoothly, but to our surprise, the product was not the expected amide **3** (Scheme 2), but instead was hydrolysis product (*R*)-**2,** which formed enantioselectively. The water for the hydrolysis originated from the CAL-B preparation. It is well known that CAL-B in the Novozym 435 preparation contains considerable amounts of water adsorbed on the immobilization material, exposing carboxylic acid esters to hydrolysis.[14,15] Accordingly, other lipases (see Section 4) adsorbed on Celite[13] were screened for the reactions of (±)-**1** in DIPE in the presence of amines. The substrate reacted through slow hydrolysis (conversion $\leqslant$ 5% after 24 h) without any sign of simultaneous aminolysis.

2.1. Kinetic resolution of (±)-1

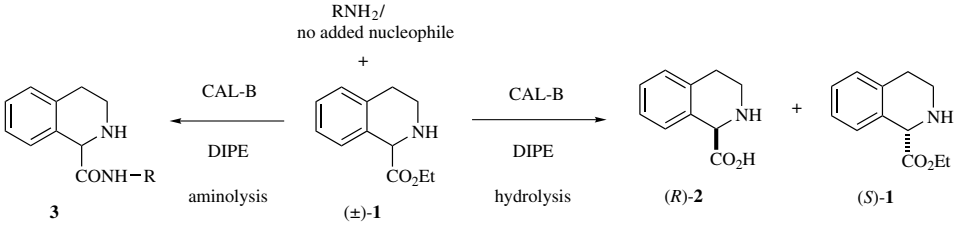
Since the enantioselectivity for the hydrolysis of (±)-**1** by CAL-B in DIPE in the presence of amines was relatively good, we optimized the kinetic resolution through hydrolysis (Scheme 2). In these reactions, an important observation was the decreasing trend in the values of enantiomer ratios (*E*) with time. For instance in the presence of benzylamine, *E*-values decreased from 35 at 52% conversion (reaction time 16 h) to 23 at 55% conversion (reaction time 40 h). This indicated the occurrence of a side-reaction, which we confirmed to be the racemization of the less reactive **1** in the presence of basic compounds. Thus, the basic reagents should be avoided in the optimization of the kinetic resolution. In fact, a drop in $ee^{(S)\text{-}\mathbf{1}}$ with time was evident, even in the sample where CAL-B was removed, indicating that ester **1** itself is basic enough to cause slow racemization with time. Due to the racemization tendency enantioselectivity is not considered in the terms of *E* values in this work.

For the hydrolysis of (±)-**1** in DIPE in the presence of water (0.5 equiv) at 25 °C, CAL-B proved to be the most enantioselective ($ee^{(R)\text{-}\mathbf{2}} = 92\%$, $ee^{(S)\text{-}\mathbf{1}} = 72\%$ at 52% conversion) from the enzymes screened. There was no significant difference in either the reactivity or selectivity between commercially available CAL-B preparations. Solvents were screened for the hydrolysis of (±)-**1** in the presence of water (0.5 equiv) and CAL-B (Table 1). Hydrolysis in *t*-butyl methyl ether (TBME) or in DIPE afforded the highest reactivities in terms of the time needed to reach 50% conversions (Table 1, entries 3 and 5). The best selectivity was observed for the reaction in DIPE (entries 4 and 5). Dichloromethane (DCM) and acetonitrile promoted the racemization of the substrate most strongly as seen from the very low $ee^{(S)\text{-}\mathbf{1}}$ values (entries 7 and 8).

Table 1. Solvent effects on the enzymatic hydrolysis of (±)-**1**[a]

Entry	Solvent	Time (h)	Conv.[b] (%)	$ee^{(S)\text{-}\mathbf{1}}$ (%)	$ee^{(R)\text{-}\mathbf{2}}$[c] (%)
1	THF	38	29	18	87
2	Dioxane	40	31	26	82
3	TBME	4	50	64	88
4	DIPE	1.5	29	38	98
5	DIPE	6	52	72	92
6	Toluene	7	30	28	87
7	DCM	38	29	3	79
8	Acetonitrile	22	30	7	86

[a] (±)-**1** (0.05 M) in a solvent in the presence of water (0.5 equiv) and CAL-B (50 mg mL^{-1}), at 25 °C.
[b] Determined by using octadecane as an internal standard.
[c] According to GC, after derivatization with diazomethane.



Scheme 2. Kinetic resolution of (±)-**1**; R = Bu or Bz.

The addition of 1–5 equiv of water in DIPE only slightly affected the reactivity for the CAL-B-catalyzed hydrolysis of (±)-**1**, leading to 51–54% conversions at 6 h, the effect on enantioselectivity being within the experimental errors as seen from the $ee^{(R)\text{-}2}$ values at the given conversions (Table 2). On the other hand, the racemization rate decreased markedly with an increase in the amount of water as shown when the solution of (*S*)-**1** (0.05 M) in DIPE was studied in the presence of various amounts of water. For further studies, we chose to add 1 equiv of water in DIPE.

Table 2. Effect of added water on the enzymatic hydrolysis of (±)-**1** in DIPE[a]

Entry	Water amount (equiv)	Conv.[b] (%)	$ee^{(S)\text{-}1}$ (%)	$ee^{(R)\text{-}2}$[c] (%)
1	0	51	63	92
2	0.5	52	72	92
3	1	53	76	91
4	2	53	81	90
5	5	54	84	87

[a] (±)-**1** (0.05 M), CAL-B (50 mg mL^{-1}), at 25 °C, reaction time 6 h.
[b] Determined by using octadecane as an internal standard.
[c] According to GC, after derivatization with diazomethane.

The temperature effect on the hydrolysis of (±)-**1** in DIPE in the presence of 1 equiv of water was also studied. Lowering the reaction temperature to 3 °C considerably improved the enantioselectivity (Table 3, entry 3 compared to entries 1 and 2). The racemization of the less reactive starting material **1** was also hindered in the resolution mixture (around 1% during the given reaction time at 3 °C). As a drawback, the enzymatic reactivity notably slowed down when the temperature was decreased (50% conversion was reached only after 4 days).

Table 3. Preparative-scale kinetic resolution of (±)-**1** in DIPE in the presence of water (1 equiv)[a]

Entry	*T* (°C)	Time (h)	Conv.[b] (%)	Y[(*S*)-**1**][c] (%)	Y[(*R*)-**2**][c] (%)	$ee^{(S)\text{-}1}$ (%)	$ee^{(R)\text{-}2}$[d] (%)
1	25	6	53	—	—	76	91
2	25	48	62	36	57	88	88
3	3	92	50	42	46	92	94

[a] (±)-**1** (0.05 M), CAL-B (50 mg mL^{-1}).
[b] Determined by using octadecane as an internal standard.
[c] Isolated chemical yield.
[d] According to GC, after derivatization with diazomethane.

Finally, the gram-scale resolution of (±)-**1** was performed at 25 °C and 3 °C (Table 3, entries 2 and 3). The advantage of using a lower temperature was also evident from the gram-scale resolutions. At 25 °C, $ee^{(S)\text{-}1} = 88\%$ could be reached only at 62% conversion. It is important to note that the (*S*)-**1** amino ester underwent spontaneous racemization as a neat oil ($ee^{(S)\text{-}1} = 52\%$ after 15 h at 3 °C). During the purification of (*S*)-**1** by column chromatography on silica, rapid racemization was observed. The racemization was also accelerated by strong mineral acids (with HCl/EtOH, (±)-**1**·HCl crystallized out from (*S*)-**1**). Finally, we succeeded in isolating the picrate of (*S*)-**1** with ee = 92% at 42% chemical yield when calculated from the initially racemic starting material; (*R*)-**2** was isolated with ee = 94% at a yield of 46%.

2.2. Dynamic kinetic resolution of (±)-1

The above observations that ester (*S*)-**1** spontaneously and in the presence of added amines racemized with time led us to develop a dynamic kinetic resolution process for the preparation of (*R*)-**2**. In order to achieve this, the racemization had to be accelerated. It appeared most obvious to test additives with a basic character, for example, secondary and tertiary aliphatic amines (Table 4). There was also another problem to overcome: in DIPE and in toluene, the hydrolysis reaction tended to stop at 63–65% conversion. At this point there are two competing nucleophiles, water and methanol. Evidently the reactions stop at an equilibrium where the two nucleophiles compete for the same acyl-enzyme intermediate (TIC-1 esterified with the serine hydroxyl of the active site of CAL-B), leading to the acid and ethylester, respectively.

The test-reactions with amine additives were carried out without added water, in accordance with the observation stated above that added water decreased the racemization tendency of (*S*)-**1** without affecting the enzymatic reactivity and the formation of acid (*R*)-**2**. The use of secondary amines (Table 4, entries 3 and 4) furnished a twofold advantage: the racemization rate was increased while at the same time the equilibrium position (ca 65%) was changed more to hydrolysis. In aprotic solvents, bases are less solvated and therefore more reactive. For a further increase of the racemization rate, aprotic polar solvents were tested. Moreover, the polarity enhances charge separation, which is crucial for the carbanion formation during the base-catalyzed racemization.[16] In accordance with this, we observed a relatively fast racemization in the acetonitrile during solvent screenings (Table 1, entry 8). Since the reaction in acetonitrile started to cease already at around 30% conversion, toluene as a non-water-miscible, aprotic solvent was added in acetonitrile.

Table 4. Effect of amine additives (1 equiv) on the enzymatic hydrolysis of (±)-**1** in DIPE[a]

Entry	Additive	Conv.[b] (%)	$ee^{(S)\text{-}1}$ (%)	$ee^{(R)\text{-}2}$[c] (%)
1	No additive	63	68	88
2	DIPEA[d]	65	83	88
3	Dipropylamine	89	71	91
4	Piperidine	91	62	84

[a] (±)-**1** (0.05 M), CAL-B (50 mg mL^{-1}), 25 °C, reaction time 5 days.
[b] Determined by using octadecane as an internal standard.
[c] According to GC, after derivatization with diazomethane.
[d] *N*,*N*-Diisopropylethylamine.

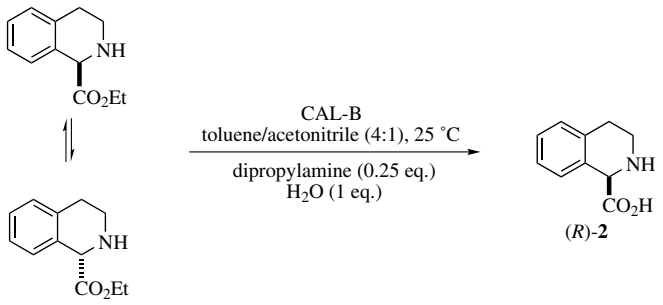
Toluene/acetonitrile mixtures containing acetonitrile (10–50%-v/v) and water (1 equiv) were tested in the presence of dipropylamine (0.25 equiv) and CAL-B (20 mg mL^{-1}). Compared to the case of the kinetic resolution studies above, the enzyme content was lowered in order to maneu-

ver the racemization of (*S*)-**1** and the formation of (*R*)-**2** to proceed at similar rates. Increasing the acetonitrile contents (10–50%-v/v) caused decreased conversions from 56% to 41% (Table 5). As expected on the basis of the results in Table 1, racemization of (*S*)-**1** became slower with the more toluene that was present. Accordingly, the values of $ee^{(S)\text{-}1}$ in Table 5 decreased from 31% to 8% when the amount of toluene was increased. The toluene/acetonitrile ratio did not affect the enantiopurity of (*R*)-**2**. We chose toluene/acetonitrile (4:1) as the optimum mixture for the preparative scale dynamic kinetic resolution (Scheme 3).

Table 5. Effect of acetonitrile in toluene on the dynamic kinetic resolution of (±)-**1**[a]

Entry	Toluene/acetonitrile	Conversion (%)	$ee^{(S)\text{-}1}$ (%)	$ee^{(R)\text{-}2}$ [b] (%)
1	9:1	56	31	94
2	4:1	53	23	95
3	7:3	49	16	95
4	3:2	47	11	95
5	1:1	41	8	95

[a] (±)-**1** (0.05 M) in a solvent in the presence of water (1 equiv) and CAL-B (20 mg mL^{-1}), at 25 °C, reaction time 2 days.
[b] According to GC, after derivatization with diazomethane.



Scheme 3. Dynamic kinetic resolution of (±)-**1**.

The gram-scale dynamic kinetic resolution was started at 25 °C with 1 equiv of water, 0.25 equiv of dipropylamine and 20 mg mL^{-1} CAL-B in toluene/acetonitrile (4:1). In order to transform the substrate ester completely into (*R*)-**2** within a reasonable time scale, another portion of the enzyme was added when the reaction had already been carried out for 2 days. By doing this, we obtained (*R*)-**2** (ee = 96%) with an isolated yield of 80% after a total reaction time of 6 days.

2.3. Absolute configuration

The absolute configurations of the enantiomers obtained were determined by comparing the observed $[\alpha]_D^{25}$ values −63.1 (*c* 1, 1 mol dm^{-3} HCl) and +18.8 (*c* 2, 1 mol dm^{-3} NaOH) for (*R*)-**2** (ee = 96%) with the literature data {$[\alpha]_D^{25}$ − 68.05 (*c* 1, 1 mol dm^{-3} HCl)[17] and $[\alpha]_D^{25}$ + 22.0 (*c* 2, 1 mol dm^{-3} NaOH)[7,17]}. For the picrate of (*S*)-**1**, we obtained $[\alpha]_D^{25} = -28.2$ (*c* 0.5, $CHCl_3$) and $[\alpha]_D^{25} = -30.2$ (*c* 0.5, toluene).

3. Conclusions

In conclusion, we have developed a CAL-B-catalyzed kinetic resolution procedure for the kinetic resolution of (±)-**1** by hydrolysis in DIPE, which yielded (*R*)-**2** (ee = 94%) in a high isolated yield (46%). (*S*)-**1**, as the picrate (ee = 92%), was isolated at 42% isolated yield. The transformation of kinetic into dynamic kinetic resolution was accomplished via base-catalyzed racemization of amino ester (*S*)-**1**. In this method, (±)-**1** provided (*R*)-**2** (ee = 96%) at 80% isolated yield. In the dynamic kinetic resolution method, the slow racemization rate compared to the rate of enzymatic hydrolysis was solved by starting the hydrolysis in the presence of low enzyme content and by adding more enzyme during the course of the reaction.

4. Experimental

4.1. Materials and methods

CAL-B (lipase B from *Candida antarctica*), produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous acrylic resin, was purchased from Sigma–Aldrich, Novo Nordisk (Novozym 435) and from Roche Diagnostics (Chirazyme L-2). All CAL-B preparations stand for the same enzyme preparations from Novozyme Corp. Herein we mainly used CAL-B from Sigma. Lipase PS (*Burkholderia cepacia* lipase) and lipase AK (*Pseudomonas fluorescence* lipase) were products of Amano Pharmaceuticals and CAL-A (lipase A from *Candida antarctica*) that of Novo Nordisk. The latter enzymes were all adsorbed on Celite in the presence of sucrose as previously described.[13] The immobilized preparations contained 20% (w/w) of lipase.

Isoquinoline-1-carboxylic acid and benzylamine were purchased from Aldrich, and octadecane was from Fluka. Solvents were of the highest analytical grade. Diisopropyl ether (DIPE) was from Fluka, *t*-butyl methyl ether (TBME) was from Sigma–Aldrich.

In a typical small-scale experiment, an achiral reagent (0.1 M) and water (0.5–5.0 equiv when added) were added to the suspension of (±)-**1** (0.05 M) in an organic solvent (1.5 mL) and lipase preparation (10–75 mg mL^{-1}). In the case of the dynamic kinetic resolution, one of the amines (0.25 equiv) was added. In each experiment, octadecane was used as an internal standard. The progress of the reaction and $ee^{(S)\text{-}1}$ for the unreacted substrate enantiomer were determined by gas chromatography on a CP Chirasil-DEX CB-coated capillary column at a constant temperature of 155 °C and a pressure of 140 kPa. The $ee^{(R)\text{-}2}$ values were determined in the same way after derivatization with diazomethane.

Optical rotations were measured with a Perkin–Elmer 341 polarimeter. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus.

4.2. Preparation of the starting material (±)-1

4.2.1. 1,2,3,4-Tetrahydroisoquinoline-1-carboxylic acid (±)-2. Isoquinoline-1-carboxylic acid (5.65 g, 32.63 mmol) was dissolved in glacial acetic acid (70 mL) and Pt/C (0.5 g) was added to the solution. The mixture was stirred under a H_2 atmosphere (60 bar) at 25 °C for 24 h. The catalyst was then filtered off and the solvent evaporated. From the residue, after washing with diethyl ether and drying, (±)-**2** (5.70 g, 99%; mp 268–270 °C, lit.[12] mp 269–271 °C) was obtained.

4.2.2. Ethyl 1,2,3,4-tetrahydroisoquinoline-1-carboxylate (±)-1. The esterification of (±)-**2** was carried out in absolute ethanol. To ethanol cooled to below −10 °C, $SOCl_2$ (3.31 mL, 45.39 mmol) was added dropwise under stirring, while the temperature was maintained below −10 °C. After the $SOCl_2$ had been consumed, (±)-**2** (5.70 mg, 32.17 mmol) was added in one portion and the mixture allowed to warm up to room temperature. After stirring at room temperature for 3 h, the mixture was refluxed for 1 h. On evaporation of the solvent and recrystallization from ethanol/diethyl ether, (±)-**1**·HCl (4.81 g, 61%; mp 134–137 °C, lit.[18] mp 132–134 °C) was obtained as a white crystalline powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.30–1.34 (t, 3H, $J = 7.13$; –CH_2–CH_3), 3.02–3.34 (m, 2H, H-4), 3.65–3.79 (m, 2H, H-3), 4.27–4.33 (m, 2H, –CH_2–CH_3), 5.39 (s, 1H, H-1), 7.15–7.52 (m, 4H, Ph). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 14.63, 25.86, 40.10, 56.08, 63.82, 126.25, 127.77, 128.69, 129.47, 129.92, 132.87, 168.07.

Compound (±)-**1** was prepared by the release of the amino ester from (±)-**1**·HCl (2.00 g, 8.27 mmol) with aqueous KOH (1.40 g, 24.95 mmol). The base was extracted with diethyl ether (3 × 20 mL). After drying, the solvent was evaporated off and (±)-**1** (1.51 g, 54% overall yield) was obtained as a pale-orange dense oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.28–1.31 (t, 3H, $J = 7.12$; –CH_2–CH_3), 2.0–2.2 (br s, 1H, NH), 2.78–2.84 (m, 2H, H-4), 3.03–3.33 (m, 2H, H-3), 4.19–4.24 (m, 2H, –CH_2–CH_3), 4.71 (s, 1H, H-1), 7.11–7.35 (m, 4H, Ph).

4.3. Gram-scale kinetic resolution of (±)-1

CAL-B (2.44 g, 50 mg mL^{-1}) was added to DIPE (48.5 mL), followed by the addition of (±)-**1** (500 mg, 2.44 mmol) and water (44 μL, 2.44 mmol). The mixture was shaken at 3 °C. At 50% conversion ($ee^{(S)\text{-}1} = 92\%$, $ee^{(R)\text{-}2} = 94\%$), the lipase was filtered off and washed first with DIPE and then with water. A saturated solution of picric acid (558 mg, 2.44 mmol) in diethyl ether was added dropwise to the organic phase and the solution was left to concentrate at room temperature. The picrate of (*S*)-**1** crystallized out {441 mg, 42% yield, mp 142–143 °C, ee = 92%, $[\alpha]_D^{25} = -28.2$ (*c* 0.5, $CHCl_3$) and $[\alpha]_D^{25} = -30.2$ (*c* 0.5, toluene)} as orange needles. After evaporation of the aqueous phase and recrystallization from water/acetone, (*R*)-**2** [196 mg, 46% yield, mp 238–239 °C, lit.[7] mp 273 °C (recrystallized from ethanol–water), ee = 94%] was isolated. The picrate of (*S*)-**1**: ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.20–1.24 (t, 3H, $J = 7.12$; –CH_2–CH_3), 2.92–2.99 (m, 2H, H-4), 3.71–3.76 (m, 2H, H-5), 4.07–4.17 (m, 2H, H-3), 5.37 (s, 1H, H-1), 7.03–7.43 (m, 4H, Ph), 8.83 (s, 2H, aromatic protons of the picrate anion). ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm): 14.42, 25.40, 40.52, 86.93, 64.32, 125.68, 127.37, 128.13, 129.34, 129.59, 129.87, 130.51, 131.50, 141.96, 162.48, 167.96. Compound (*R*)-**2**: ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.07–3.11 (m, 2H, H-4), 3.45–3.63 (m, 2H, H-3), 4.97 (s, 1H, H-1), 7.29–7.54 (m, 4H, Ph). ^{13}C NMR (400 MHz, D_2O) δ (ppm): 25.00, 40.31, 59.04, 127.39, 128.32, 128.59, 128.76, 129.14, 132.20, 172.34.

4.4. Gram-scale dynamic kinetic resolution of (±)-1

(±)-**1** (500 mg, 2.44 mmol), dipropylamine (83.5 μL, 0.61 mmol), water (44 μL, 2.44 mmol) and CAL-B (970 mg, 20 mg mL^{-1}) were added to a mixture of toluene/acetonitrile (4:1) (48.5 mL). The mixture was shaken at 25 °C for 2 days and then another portion of CAL-B (970 mg) was added. The $ee^{(S)\text{-}1}$ remained below 20% throughout the reaction. After 6 days the reaction was worked up by filtering off the enzyme, which was washed with DIPE and then with water (at 40 °C). After evaporation of the aqueous phase and recrystallization from water/acetone, (*R*)-**2** {346 mg, 80% yield, mp 239–240 °C, lit.[7] mp 273 °C (recrystallized from ethanol–water), ee = 96%, $[\alpha]_D^{25} = -63.1$ (*c* 1, 1 mol dm^{-3} HCl) and $[\alpha]_D^{25} = +18.8$ (*c* 2, 1 mol dm^{-3} NaOH)} was isolated. Compound (*R*)-**2**: ^{1}H NMR (400 MHz, D_2O) δ (ppm): 3.06–3.10 (m, 2H, H-4), 3.43–3.63 (m, 2H, H-3), 4.95 (s, 1H, H-1), 7.28–7.54 (m, 4H, Ph). ^{13}C NMR (400 MHz, D_2O) δ (ppm): 25.00, 40.31, 59.04, 127.39, 128.32, 128.59, 128.76, 129.14, 132.20, 172.34.

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References

1. Kleemann, A.; Engel, J.; Kutscher, B.; Reichert, D. *Pharmaceutical Substances: Syntheses, Patents, Applications*; Thieme: Stuttgart, 2001; pp 1689–1770.
2. Joshi, H. C.; Zhou, J. *Drug News Perspect.* **2000**, *13*, 543–546.
3. (a) Naoi, M.; Maruyama, W.; Dostert, P.; Kohda, K.; Kaiya, T. *Neurosci. Lett.* **1996**, *212*, 183–186; (b) Tóth, B. E.; Bodnár, I.; Homicskó, K. G.; Fülöp, F.; Fekete, M. I. K.; Nagy, G. M. *Neurotoxicol. Teratol.* **2002**, *24*, 655–666.
4. Hofmeister, A.; Schudok, M.; Matter, H.; Breitschopf, K.; Ugolini, A. PCT Int. Appl. WO 2006002763, 2006.
5. Zhu, Y.-F.; Regan, C.; Rueter, J. K.; Guo, Z.; Chen, Y.; Huang, C. Q. PCT Int. Appl. WO 2005007164, 2005.
6. (a) Gibson, S. E.; Guillo, N.; Tozer, M. J. *Tetrahedron* **1999**, *55*, 585–615; (b) Strominger, J. L.; Fridkis-Hareli, M. PCT Int. Appl. WO 2002059143, 2002.
7. Bajusz, S.; Mohai, L.; Fehér, A.; Lavich, J.; Széll, G.; Véghelyi, B. PCT Int. Appl. WO 199312091, 1993.

8. Sieck, O.; Schaller, S.; Grimme, S.; Liebscher, J. *Synlett* **2003**, 337–340.
9. Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854.
10. (a) Liljeblad, A.; Lindborg, J.; Kanerva, A.; Katajisto, J.; Kanerva, L. T. *Tetrahedron Lett.* **2002**, *43*, 2471–2474; (b) Liljeblad, A.; Kiviniemi, A.; Kanerva, L. T. *Tetrahedron* **2004**, *60*, 671–677.
11. Wegman, M. A.; Hacking, M. A. P. J.; Rops, J.; Pereira, P.; van Rantwijk, F.; Sheldon, R. A. *Tetrahedron: Asymmetry* **1999**, *10*, 1739–1750.
12. Ho, B.; Crider, A. M.; Stables, J. P. *Eur. J. Med. Chem.* **2001**, *36*, 265–286.
13. Kanerva, L. T.; Sundholm, O. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2407–2410.
14. Gyarmati, Z. C.; Liljeblad, A.; Argay, G.; Kálmán, A.; Bernáth, G.; Kanerva, L. T. *Adv. Synth. Catal.* **2004**, *346*, 566–572.
15. Veum, L.; Kanerva, L. T.; Halling, P. J.; Maschmeyer, T.; Hanefeld, U. *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.
16. Ebbers, E. J.; Ariaans, G. J. A.; Bruggink, A.; Zwanenburg, B. *Tetrahedron: Asymmetry* **1999**, *10*, 3701–3718.
17. Fauszt, I.; Fehér, A.; Horváth, G.; Juhász, A.; Széll, E.; Bajusz, S. In *Peptides 1994, Proceedings of the 23rd European Peptide Symposium, Braga, Portugal, 1994*; Escom Science: Leiden, 1995; pp 678–679.
18. Klutchko, S.; Blankley, C. J.; Fleming, R. W.; Hinkley, J. M.; Werner, A. E.; Nordin, I.; Holmes, A.; Hoefle, M. L.; Cohen, D. M.; Essenburg, A. D.; Kaplan, H. R. *J. Med. Chem.* **1986**, *29*, 1953–1961.