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Efficient lipase-catalysed route for the kinetic resolution of salsolidine and its ß-carboline analogue

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ABSTRACT

Racemic 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline **1** and 1-methyl-1,2,3,4-tetrahydro-ß-carboline **3** were resolved through lipase-catalysed asymmetric acylation on the secondary amino group. High enantioselectivities (*E* >200) were observed when the acylation of racemic **1** was performed with phenyl allyl carbonate in the presence of *Candida rugosa* lipase in toluene at 40 °C or with *Candida antarctica* lipase B in *tert*-butyl methyl ether at 50 °C. Excellent enantioselectivity (*E* >200) characterised the CAL-B-catalysed acylation of racemic **3** with phenyl allyl carbonate in the presence of triethylamine in *tert*-butyl methyl ether at 50 °C. The product (*R*)-carbamates (*ee* >97%) were hydrolysed into the corresponding (*R*)-enantiomers of the free amines **1** and **3** (*ee* = 99%) with the use of $Pd_2(dba)_3{\cdot}CHCl_3$ catalyst.

1. Introduction

Interest in the research of compounds containing the 1-substituted 1,2,3,4-tetrahydroisoquinoline ring system has recently come into view, since compounds bearing this skeleton exert major biological activities. Several derivatives possess a common nucleus of synthetic structures, while other compounds are extracted from natural sources. All of them are associated with important pharmacological effects. The naturally occurring (*S*)-norcoclaurine [(1*S*)-1-(4-hydroxybenzyl)-1,2,3,4-tetrahydro-6,7-isochinolindiol] is an intermediate in the synthesis of morphine, papaverine or the antibacterial berberine.[1] Racemic norcoclaurine has α- and ß-adrenoreceptor activity.[2] Solifenacin [(1*S*,3′*R*)-3′quinuclidinyl-1-phenyl-1,2,3,4-tetrahydro-2-isoquinolinecarboxylate] containing a 1-phenyl-substituted tetrahydroisoquinoline core is an example of synthetic compounds. It shows urinary antispasmodic effect.[3] Both enantiomers of 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline **1** (salsolidine) are naturally occurring compounds.[4] The (*R*)-enantiomer was isolated from *Genista pungens*,[4] while the (*S*)-enantiomer was found in *Salsola richteri*.[4] Many pharmacological properties have been described to salsolidine; *e.g.*, it inhibits the uptake of 5-hydroxytryptamine by human blood platelets.[4] The (*R*)-enantiomer has a monoamine oxidase A (MAO A) inhibitoring effect.[4] On the other hand, as a structural feature of a trimethoprim analogue, it acts as a dihydrofolate reductase inhibitor.[4] The pharmaceutically valuable 1-substituted 1,2,3,4-tetrahydro-ß-carboline skeleton is also a common building block of several alkaloids, including the naturally occurring reserpine, which shows antitumor activity, as well as antihypertensive and neuroprotective effects.[5] Synthetic 1-substituted *N*-acylated tetrahydro-ß-carbolines have inhibitory activity against the Breast Cancer Resistance Protein (ABCG2).[6] The (*S*)-enantiomer of salsolidine analogue 1-methyl-1,2,3,4-tetrahydro-ß-carboline **3** (eleagnine) was isolated from *Eleagnus angustifolia* and *Petalostyles labicheoides*.[7] The racemic form can bind to the $GABA_A$ receptors in the benzodiazepine binding site, but it has an inverse agonist effect.[8]

It is not surprising, therefore, that a large number of synthetic routes have been developed for the preparation of **1** and **3**, in particular, in enantiomeric forms.[7,9–13] As an example, enantiomeric **1** was synthesized by Ding and et al. through enantioselective acylation of the racemic mixture in a batch process.[14]

In this work, our aim was to devise a new enzymatic strategy for the preparation of enantiomeric **1** and **3**, through lipase-catalysed kinetic resolution (Scheme 1). In addition to the batch reactions, we planned to examine the possibilities for their enzymatic reactions in a continuous-flow system. The use of this novel method has clear advantages, such as short reaction times, rapid heating and pressure screening.[15] In the literature, there are various examples using this innovative technique for resolution. For example, the asymmetric acylation of 1-phenylethylamine[16,17] and an imidazole derivative[18] as well as the esterification of flurbiprofen[19] have been reported.

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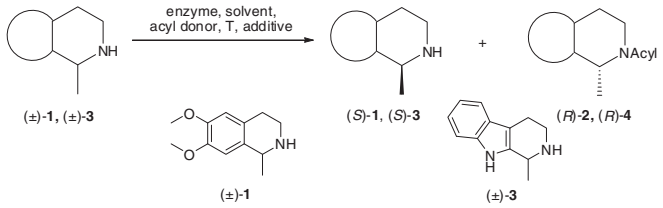


Scheme 1. Kinetic resolution of (±)-**1** and (±)-**3** through lipase-catalysed *N*-acylation on the secondary amino group.

2. Results and discussion

2.1. Synthesis of (±)-1 and (±)-3

1-Methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (±)-**1** was prepared from 3,4-dimethoxyphenylethylamine and acetic anhydride through Bischler–Napieralski cyclization followed by reduction, while racemic 1-methyl-1,2,3,4-tetrahydro-ß-carboline (±)-**3** was synthesized utilizing the Pictet–Spengler reaction via a microwave-assisted procedure according to known literature methods.[9,11]

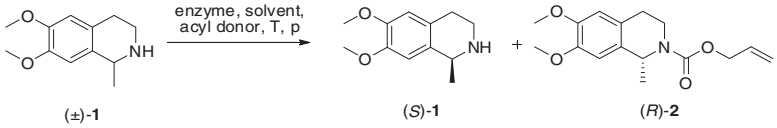
2.2. Enzymatic resolution of (±)-1 and (±)-3

Ding et al. reported the asymmetric *N*-acylation of racemic **1** ($E > 200$, conv. = 50% in 72 h, yield > 46%) by using CAL-A (*Candida antarctica* lipase A), 3-methoxyphenyl allyl carbonate in toluene at 40 °C.[14] The dynamic kinetic resolution of (±)-**1** was also described by Page *et al.* using a combination of catalysts AY (*Candida rugosa* lipase) and pentamethylcyclopentadienyliridium(III) iodide dimer in the presence of 3-methoxyphenyl propyl carbonate at 40 °C (conv. = 90% in 23 h, *ee* = 96%, yield = 82%).[20]

We started the resolution of (±)-**1** by an enzyme screening. First, the reaction was performed in batch with phenyl allyl carbonate in toluene at 40 °C (Scheme 2, Table 1, entries 1–4). Low enantioselectivity was observed with CAL-A ($E = 9$, entry 1) and with PS-IM (*Burkholderia cepacia* lipase) ($E = 2$, entry 2). In contrast, an improved $E = 46$ was found with CAL-B (*Candida antarctica* lipase B) 49% conversion in 4 days (entry 4). Lipase AY was the best catalyst with a conversion of 50% in 72 h and an excellent E (>200) (entry 3). Since the tested CAL-B was purchased as an immobilized enzyme (from Sigma) and also in view of its potential use in continuous-flow system, CAL-B was chosen for further optimization.

Next, the CAL-B-catalysed reaction was performed at 50 and then 60 °C (Table 1, entries 5 and 6). As the temperature was increased, the reaction rate increased considerably (entries 4–6). However, the best combination of reaction rate and enantioselectivity was found at 50 °C (entry 5). When toluene was replaced with *t*-BuOMe, a much faster reaction with excellent E (>200) was observed (entry 7).

Having the optimized conditions in the batch process (CAL-B, phenyl allyl carbonate, *t*-BuOMe, 50 °C, 1 bar), we decided to perform a reaction under these conditions in a continuous-flow reactor (an *H*-Cube in 'no H_2 mode'). A 70-mm-long heat- and pressure-resistant stainless-steel CatCart was filled with CAL-B. Unfortunately, only 20% conversion was reached after a cycle, although an excellent E (>200) was observed (Table 2, entry 1).



Scheme 2. Kinetic resolution of (±)-**1** through enantioselective *N*-acylation.

Table 1
N-Acylation of (±)-**1** in batch[a]

Entry	Enzyme	T (°C)	Solvent	Reaction time (h)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	CAL-A	40	Toluene	96	42	50	68	9
2	PS-IM	40	Toluene	96	3	13	25	2
3	AY	40	Toluene	72	50	99	98	>200
4	CAL-B	40	Toluene	96	49	85	89	46
5	CAL-B	50	Toluene	48	50	96	95	154
6	CAL-B	60	Toluene	48	51	96	92	94
7	CAL-B	50	*t*-BuOMe	2.5	50	99	97	>200

[a] 0.025 M (±)-**1**, phenyl allyl carbonate.
[b] According to HPLC after a derivatisation with Ac_2O.
[c] According to HPLC.

Table 2
Effect of pressure and temperature on the acylation of (±)-**1** with phenyl allyl carbonate in a continuous-flow system[a]

Entry	p (bar)	T (°C)	Conv. (%)	ee_s[b] (%)	ee_p[c] (%)	E
1	1	50	20	24	99	>200
2	30	50	3	3	99	>200
3	60	50	9	10	99	>200
4	1	60	21	26	99	>200
5	1	70	25	33	99	>200
6	1	80	7	7	99	>200

[a] 0.025 M (±)-**1**, 244 mg CAL-B (70 mm CatCart); *t*-BuOMe, 0.1 mL min^{-1}.
[b] According to HPLC after a derivatisation with Ac_2O.
[c] According to HPLC.

In order to improve the reaction rate, the pressure was increased from 1 to 30, and then 60 bar. The *E* remained excellent (>200), but the reaction rate decreased significantly (compare entries 2 and 3 to entry 1). In a further attempt to increase the rate, we examined the effect of temperature on the reaction. As the temperature was increased from 50 °C to 60 °C and then 70 °C, the rate increased slightly (entries 1, 4 and 5), while at 80 °C the activity of the enzyme decreased drastically (entry 6) after a single cycle. Thus, 70 °C was selected as the optimal temperature for further experiments.

Because of the relatively modest conversion of 25% obtained after a cycle (Table 2, entry 5), the enzymatic mixture was pumped through the reactor five times consecutively, using the same CatCart filled with CAL-B. As the number of the cycles increased, the conversion increased progressively (Fig. 1) reaching 41% with an excellent final *E* (>200) after the 5th cycle. However, it is noteworthy, that the activity of the enzyme decreased significantly after the 2nd cycle.

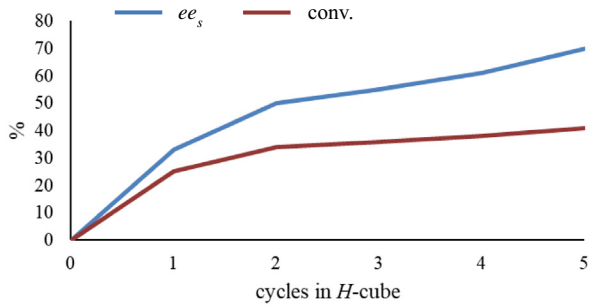
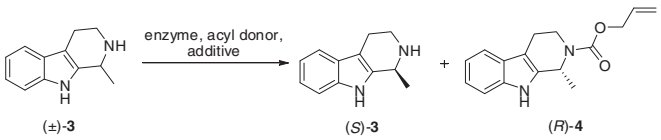


Figure 1. The influence of the number of cycles on reaction rate in the case of (±)-**1**, 1st cycle: ee_s = 33%, conv. = 25%, 2nd cycle: ee_s = 50%, conv. = 34%, 3rd cycle: ee_s = 55%, conv. = 36%, 4th cycle: ee_s = 61%, conv. = 38%, 5th ee_s = 70%, conv. = 41%, ee_p = 99% in each cycle.

Finally, the kinetic resolution of (±)-**1** was also tested with lipase AY and phenyl allyl carbonate in *H*-Cube (toluene, 40 °C, 1 bar). Both the reaction rate and the enantioselectivity were rather low after a cycle (ee_s = 3%, ee_p = 53%, conv. = 5%, *E* = 3), since the enzyme was compressed due to its powdery structure.

On the basis of the preliminary experiments, the preparative-scale resolution of (±)-**1** was performed with both lipase AY (conv. = 50%, *E* > 200, *ee* = 99%) and CAL-B (conv. = 50%, *E* > 200, *ee* > 97%) enzymes under the optimized conditions in batch (Table 3).

In view of the above results, *N*-acylation of racemic **3** (Scheme 3) was performed with phenyl allyl carbonate in the presence of CAL-B in *t*-BuOMe at 50 °C. Excellent *E* (>200) was observed but the reaction stopped after a long run (conv. = 47% after a week, ee_s = 89%, ee_p = 99%). To solve this problem, 3-methoxyphenyl allyl carbonate[21] was tested in the reaction. Excellent *E* (> 200), but rather low reaction rate (conv. = 4% after a week) was observed. Therefore, phenyl allyl carbonate was used in further reactions.



Scheme 3. Kinetic resolution of (±)-**3** through lipase-catalysed enantioselective *N*-acylation.

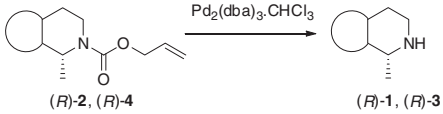
When a catalytic amount of Et_3N was added to the reaction mixture,[22] a relatively fast reaction (conv. = 50% after 36 h) was observed without the earlier-mentioned deactivation stop before 50% (ee_s = 97%, ee_p = 98% at a conversion of 50%).

Lipase AY was also tested for the acylation of racemic **3** with phenyl allyl carbonate in toluene at 40 °C. Unfortunately, the enzyme proved to be inactive and only racemic **3** was detected in the reaction media even after a week.

In view of these preliminary results, the CAL-B-catalysed preparative-scale resolution of racemic **3** was performed (phenyl allyl carbonate, *t*-BuOMe, Et_3N, 50 °C) and 50% conversion was observed in 48 h (*E* > 200, *ee* > 97%) (Table 3).

2.3. Hydrolysis of (*R*)-2 and (*R*)-4

The removal of the *N*-allyloxycarbonyl (Alloc) moiety from carbamates (*R*)-**2** and (*R*)-**4** was also investigated (Scheme 4).



Scheme 4. Hydrolysis of (*R*)-**2** and (*R*)-**4**.

First, the hydrolysis was carried out with triethanolamine in 50% aqueous NaOH solution at 120 °C,[14,23] but the removal of the protecting groups was not observed even after a one-week treat-

Table 3
Preparative-scale resolution of (±)-**1** and (±)-**3**

Substrate	Reaction time (h)	Enzyme	Conv. (%)	*E*	Enantiomer	*ee* (%)	Yield (%)	$[\alpha]_D^{25}$
(±)-**1**[a]	24	AY	50	>200	(*S*)-**1**	99[d]	40	−59[g]
					(*R*)-**2**	99[e]	39	−104[g]
(±)-**1**[b]	49	CAL-B	50	>200	(*S*)-**1**	99[d]	41	−58.9[h]
					(*R*)-**2**	97[e]	38	−102[g]
(±)-**3**[c]	48	CAL-B	50	>200	(*S*)-**3**	98[f]	40	−63[g]
					(*R*)-**4**	97[e]	41	−99[g]

[a] 0.48 M (±)-**1**, 30 mg mL^{-1} AY, 30 mL toluene, 4 equiv. of phenyl allyl carbonate, 40 °C.
[b] 0.48 M (±)-**1**, 30 mg mL^{-1} CAL-B, 30 mL *t*-BuOMe, 4 equiv. phenyl allyl carbonate, 50 °C.
[c] 0.54 M (±)-**3**, 30 mg mL^{-1} CAL-B, 15 mL toluene, 4 equiv. phenyl allyl carbonate, 5 μl Et_3N, 50 °C.
[d] According to HPLC after derivatisation with Ac_2O.
[e] According to HPLC.
[f] According to HPLC after derivatisation with $(CH_3CH_2CH_2CO)_2O$.
[g] *c* 0.3, EtOH.
[h] *c* 0.55, EtOH.

ment. Then iodine, a non-transition metal catalyst, was tested for deprotection,[24] in dry acetonitrile, in the presence of water at room temperature. After 3 days, a relatively small amount of (*R*)-**3** (yield = 24%, *ee* = 92%) and no (*R*)-**1** were detected. Next, a Pd catalyst [tris(dibenzylideneacetone)dipalladium-chloroform adduct, $Pd_2(dba)_3{\cdot}CHCl_3$] was used for the removal of the Alloc moiety,[25] in the presence of formic acid (HCOOH) and triphenylphosphine (PPh_3) in tetrahydrofuran (THF) at 40 °C, under Ar. In both cases (*R*)-**1** and (*R*)-**3** were formed without any loss in enantiopurity (*ee* = 99%) and in good yields [85% in 2 h for (*R*)-**3** and 60% in 16 h for (*R*)-**1**].

2.4. Absolute configurations

The stereochemistry of the enantiomers was determined by comparing the specific rotation values of the prepared free amines **1** and **3** with literature data for (*R*)-salsolidine and the (*R*)-enantiomer of its ß-carboline analogue (Experimental). Both enzymes were found to display (*R*)-selectivity during acylation reactions.

3. Conclusions

Efficient new enzymatic methods have been developed for the synthesis of the enantiomers of salsolidine and its ß-carboline analogue. Excellent selectivity *E* (>200) was observed upon performing the acylation of (±)-**1** in the presence of CAL-B with phenyl allyl carbonate in *t*-BuOMe in both batch mode and a continuous-flow system. The same high *E* (>200) characterised the resolution of (±)-**1**, when the acylation was carried out in the presence of lipase AY with phenyl allyl carbonate in toluene at 40 °C. CAL-B catalysed the enantioselective acylation of (±)-**3** with excellent *E* (>200), when the reaction was performed with phenyl allyl carbonate, in the presence of Et_3N in *t*-BuOMe at 50 °C. To the best of our knowledge, (±)-**3** was resolved for the first time by using enzymes. Carbamate (*R*)-**2** and (*R*)-**4** were hydrolysed into the corresponding amines with $Pd_2(dba)_3{\cdot}CHCl_3$ as catalyst in THF, in the presence of PPh_3 and HCOOH. The products were formed with excellent enantiopurity [*ee* = 99% for both (*R*)-**1** and (*R*)-**3**] and in good yields [60% of (*R*)-**1** and 85% of (*R*)-**3**].

4. Experimental

4.1. Materials and methods

CAL-B (lipase B from *Candida antarctica*) immobilized on acrylic resin was purchased from Sigma, and lipase PS-IM (*Burkholderia Cepacia*) immobilized on diatomaceous earth was from Amano Enzyme Europe Ltd. Lipase AY (*Candida rugosa*) was from Fluka and CAL-A (lipase A from *Candida antarctica*) from Novo Nordisk.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter. ^{1}H NMR spectra were recorded on a Burker Avance DRX 400 spectrometer. Melting points were determined on a Kofler apparatus. Microwave (MW) reactions were performed in a CEM Discover MW reactor (Matthews, NC, USA). The elemental analysis was measured by means of a Perkin-Elmer CHNS-2400 Ser II Elemental Analyzer. The *H*-Cube reactor used in 'no H_2 mode' and equipped with a stainless steel enzyme-charged cartridge (70 mm length, 4 mm inside diameter) was from ThalesNano Inc.

The *ee* values of the enantiomers were determined by HPLC [Chiralpak IA column (4.6 mm × 250 mm)]. Eluent: *n*-hexane/*i*Pa (80:20), flow rate: 0.5 mL min^{-1}, 260 nm; retention times (min) for Ac_2O-derivatised form of (*S*)-**1**: 21.10, for (*R*)-**1**: 26.96 and eluent: *n*-hexane/*i*Pa (96:4), flow rate: 0.5 mL min^{-1}, 220 nm; retention times (min) for (*R*)-**2**: 67.53, (*S*)-**2**: 73.73. In the case of (±)-**3**, the eluent ratio was *n*-hexane/*i*Pa (90:10), 240 nm; retention times (min) for the $(CH_3CH_2CH_2CO)_2O$-derivatised form of (*R*)-**3**: 23.71, (*S*)-**3**: 26.89 and for (*R*)-**4**: 28.43 and (*S*)-**4**: 32.43.

4.2. Syntheses of (±)-1 and (±)-3

Racemic **1** was synthesized from 3,4-dimethoxyphenylethylamine (18.12 g, 99.98 mmol) in three steps according to a known literature method.[10] Pure (±)-**1** was obtained as a white solid (7.40 g, 36% yield, mp: 48 °C, lit.:[9] 48–49 °C).

^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.41–1.51 (d, *J* = 6.8 Hz, 3H, CH-C*H*$_3$); 1.60–1.75 (br s, 1H, N*H*); 2.62–2.72, 2.77–2.87 (2 m, 2 × 1H, NH-CH$_2$-C*H*$_2$); 2.96–3.08, 3.23–3.33 (2 m, 2 × 1H, NH-C*H*$_2$-CH$_2$); 3.81–3.93 (d, *J* = 1.8 Hz, 6H, 2 × CH-O-C*H*$_3$); 4.04–4.12 (q, *J* = 6.4 Hz, 1H, NH-C*H*-CH$_3$) 6.60 (s, 1H, Ar); 6.66 (s, 1H, Ar). Anal. Calcd for $C_{12}H_{17}NO_2$: C, 69.54, H 8.27, N 6.76. Found: C, 69.52, H 8.20, N 6.81.

Racemic **3** was synthesized from tryptamine (600 mg, 3.75 mmol) in a microwave reactor.[13] (±)-**3** was isolated in good yield (560 mg, 80% yield, mp: 177–179 °C, lit.:[26] 177–178 °C) as a pale yellow solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 1.41–1.53 (d, *J* = 3.3 Hz, 3H, CH-C*H*$_3$); 1.54–1.67 (br s, 1H, CH-N*H*); 2.67–2.83 (m, 2H, NH-CH$_2$-C*H*$_2$); 2.99–3.11, 3.31–3.43 (2 m, 2 × 1H, NH-C*H*$_2$-CH$_2$), 4.14–4.24 (m, 1H, NH-C*H*-CH$_3$), 7.06–7.18 (m, 2H, Ar); 7.28–7.35 (d, *J* = 8 Hz, 1H, Ar) 7.45–7.51 (d, *J* = 8 Hz, 1H, Ar); 7.68–7.85 (br s, 1H, Ar-N*H*). Anal. Calcd for $C_{12}H_{14}N_2$: C, 77.38, H 7.58N 15.04. Found: C, 77.33, H 7.62, N 15. 09.

4.3. Small-scale enzymatic reactions

Preliminary small-scale experiments were carried out in both batch and continuous-flow systems. In batch, racemic (±)-**1** or (±)-**3** (0.025 mmol) was dissolved in an organic solvent (1 mL). 4 equiv. acyl donor, 30 mg enzyme and 1 μL Et_3N used as an additive in the case of (±)-**3** were added and the reaction mixtures were shaken in an incubator shaker at 40–60 °C. During continuous-flow investigations, (±)-**1** (0.025 mmol) was dissolved in *t*-BuOMe (1 mL) and phenyl allyl carbonate (4 equiv.) was added. The solution was pumped through the compressed (1–60 bar) and heated (50–80 °C) CAL-B-filled cartridge (244 mg) in *H*-Cube in 'no H_2 mode' with 0.1 mL min^{-1} flow rate. In the case of the AY-catalysed reaction (250 mg), the solution was pumped through the CatCart cartridge (1 bar, 40 °C) with 1.8 mL min^{-1} flow rate.

4.4. Preparative-scale resolution of (±)-1

Racemic **1** (100 mg, 0.48 mmol) was dissolved in toluene (30 mL). After adding lipase AY (900 mg, 30 mg mL^{-1}) and phenyl allyl carbonate (0.31 mL, 1.91 mmol, 4 equiv.), the reaction mixture was shaken in an incubator shaker at 40 °C for 24 h. The reaction was stopped at 50% conversion by filtering off the enzyme and washing it with toluene (2 × 30 mL) followed evaporation of the solvent. The products [(*S*)-**1**, (*R*)-**2**] were separated by column chromatography on silica, with the elution of CH_2Cl_2/MeOH (1:1), affording carbamate (*R*)-**2** {55 mg, 39%, $[\alpha]_D^{25}$ = −104 (*c* 0.3, EtOH) colourless oil, *ee* = 99%}. The free amine (*S*)-**1** was crystallized from hexane/EtOAc (2:1) {40 mg, 40%, $[\alpha]_D^{25}$ = −59 (*c* 0.3, EtOH) lit.:[14] $[\alpha]_D^{25}$ = −58.5 (*c* 0.5, EtOH) white crystalline product, mp: 47 °C, lit.:[14] 47–48 °C, *ee* = 99%}.

(±)-**1** (100 mg, 0.48 mmol) was dissolved in *t*-BuOMe (30 mL). After adding CAL-B (900 mg, 30 mg mL^{-1}) and phenyl allyl carbonate (0.31 mL, 1.91 mmol, 4 equiv.), the reaction was carried out in an incubator shaker at 50 °C in 49 h. Separation of the enantiomers was performed as above, affording carbamate (*R*)-**2** {53 mg, 38%, $[\alpha]_D^{25}$ = −102 (*c* 0.3, EtOH), colourless oil, *ee* = 97%} and free amine

(*S*)-**1** crystallized in hexane/EtOAc (2:1) {41 mg, 41%, $[\alpha]_D^{25}$ = −58.9 (*c* 0.55, EtOH), white crystalline product, mp: 46–47 °C, *ee* = 99%}.

^{1}H NMR (400 MHz, $CDCl_3$) for (*R*)-**2**: δ (ppm): 1.42–1.49 (d, *J* = 6.0 Hz, 3H, CH-C*H*$_3$); 2.58–2.72, 2.79–2.96 (2m, 2 × 1H, NH-CH$_2$-C*H*$_2$); 3.12–3.37, 3.49–3.69 (2 m, 2 × 1H, NH-C*H*$_2$-CH$_2$); 3.80–3.89 (d, *J* = 1.1 Hz, 6H, 2 × CH-O-C*H*$_3$), 4.04–4.31 (m, 1H, NH-C*H*-CH$_3$), 4.54–4.73 (d, *J* = 5.2 Hz, 2H, C*H*$_2$-CH=CH$_2$); 5.17–5.26 (dd, *J* = 1.3 Hz, 10.4 Hz, 1H, CH=C*H*$_2$); 5.28–5.37 (dd, *J* = 1.5 Hz, 17.5 Hz, 1H, CH=C*H*$_2$); 5.91–6.02 (m, 1H, C*H*=CH$_2$); 6.59 (br s, 2H, Ar). Anal. Calcd for $C_{16}H_{21}NO_4$: C, 65.96, H 7.27, N 4.81. Found: C, 65.92, H 7.21, N 4.86.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**1** were similar to those for (±)-**1**.

4.5. Preparative-scale resolution of (±)-3

(±)-**3** (100 mg, 0.54 mmol) dissolved in *t*-BuOMe (15 mL) was mixed with CAL-B (450 mg, 30 mg mL^{-1}), phenyl allyl carbonate (0.35 mL, 2.15 mmol, 4 equiv.) and Et_3N (5 μL). The reaction mixture was shaken for 48 h in an incubator shaker at 50 °C. When a conversion of 50% was observed, the enzyme was filtered off and washed with *t*-BuOMe (2 × 15 mL). Carbamate (*R*)-**4** was separated from free amine (*S*)-**3** by column cromatography with CH_2Cl_2/MeOH (1:1). Carbamate (*R*)-**4** was obtained as a colourless oil {60 mg, 41%, $[\alpha]_D^{25}$ = −99 (c = 0.3, EtOH), *ee* = 97%}, and secondary amine (*S*)-**3** was crystallized in hexane {40 mg, 40%, $[\alpha]_D^{25}$ = −63 (*c* 0.3, EtOH), lit.:[7] $[\alpha]_D^{25}$ = −65.8 (*c* 2.0, EtOH), lit.:[27] $[\alpha]_D^{25}$ = −56.8 (*c* 2.0, EtOH), pale yellow solid, mp: 177–178 °C, lit.:[27] 179–181 °C, *ee* = 98%}.

^{1}H NMR (400 MHz, $CDCl_3$) for (*R*)-**4**: δ (ppm): 1.46–1.53 (d, *J* = 6.8 Hz, 3H, CH-C*H*$_3$); 2.67–2.89 (m, 2 × 1H, NH-CH$_2$-C*H*$_2$); 3.11–3.28, 4.29–4.56 (m, 2 × 1H, NH-C*H*$_2$-CH$_2$); 4.6–4.72 (m, 2H, C*H*$_2$-CH=CH$_2$); 5.17–5.26 (dd, *J* = 1.1 Hz, 10.5 Hz, 1H, CH$_3$-C*H*-NH); 5.27–5.43 (dd, *J* = 0.8 Hz, 17.2 Hz, 1H, CH=C*H*$_2$); 5.91–6.04 (m, 1H, C*H*=CH$_2$); 7.05–7.20 (m, 2H, Ar), 7.28–7.34, 7.41–7.5 (d, J = 8.0 Hz, 2H, Ar); 7.66–7.95 (br s, 1H, Ar-N*H*). Anal. Calcd for $C_{16}H_{18}N_2O_2$: C, 71.09, H 6.71, N 10.36. Found: C, 71.13, H 6.75, N 10.29.

The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*S*)-**3** were similar to those for (±)-**3**.

4.6. Hydrolysis of (*R*)-2 and (*R*)-4

(*R*)-**4** (60 mg, 0.22 mmol) was dissolved in dry acetonitrile (0.72 ml) and water (12 μL, 0.66 mmol, 3 equiv.) and then iodine (167.6 mg, 0.66 mmol, 3 equiv.) were added to the reaction mixture. After stirring during 72 h at room temperature, it was cooled down to 4 °C and a 20% solution of Na_2SO_3 was added, followed by extraction with CH_2Cl_2 (3 × 5 mL). Product (*R*)-**3** was purified by column chromatography with CH_2Cl_2/MeOH (1:1) and it was crystallized in hexane giving a pale yellow solid with a low yield {10 mg, 24%, $[\alpha]_D^{25}$ = +50, (*c* 0.3, EtOH), lit.:[27] $[\alpha]_D^{25}$ = +55.6 (*c* 2.0, EtOH), mp: 177–178 °C, lit.:[27] 179–181 °C, *ee* = 92%}.

Carbamate (*R*)-**2** (55 mg, 0.19 mmol) was dissolved in THF (2 mL). PPh_3 (10 mg, 0.04 mmol), HCOOH (28 μL, 0.74 mmol) and $Pd_2(dba)_3{\cdot}CHCl_3$ (14.5 mg, 0.014 mmol) were added and the solution was stirred overnight at 40 °C. After evaporation the catalyst was removed on a celite column with MeOH. Product (*R*)-**1** was purified by column chromatography with CH_2Cl_2/MeOH/Et_3N (89:10:1). The amine was dissolved in 5 mL H_2O and saturated $NaHCO_3$ solution was added until pH > 9. The aqueous media was extracted with $CHCl_3$ (2 × 7 mL). After evaporation of the organic media, (*R*)-**1** was crystallized from hexane/EtOAc (2:1) as a white solid {23.4 mg, 60%, $[\alpha]_D^{25}$ = +60, (c 0.3, EtOH), lit.:[14] $[\alpha]_D^{25}$ = +59.2 (*c* 1.0, EtOH), mp: 46 °C, lit.:[14] 47–48 °C, *ee* = 99%}. The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*R*)-**1** were similar to those for (±)-**1**.

In the case of (*R*)-**4** (60 mg, 0.22 mmol), the reaction was completed in 2 h, providing (*R*)-**3** as a pale yellow solid (crystallized from hexane) {34.9 mg, 85%, $[\alpha]_D^{25}$ = +62, (*c* 0.3, EtOH), lit.:[27] $[\alpha]_D^{25}$ = +55.6 (*c* 2.0, EtOH), mp: 178–180 °C, lit.:[27] 179–181 °C, *ee* = 99%}. The ^{1}H NMR (400 MHz, $CDCl_3$) spectroscopic data for (*R*)-**3** were similar to those for (±)-**3**.

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