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An Efficient Enzymatic Synthesis of Benzocispentacin and Its New Six- and Seven-Membered Homologues

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Abstract: A very efficient enzymatic method was developed for the synthesis of new enantiomeric benzocispentacin and its six- and seven-membered homologues through the Lipolase (lipase B from *Candida antarctica*) catalyzed enantioselective ($E>200$) ring opening of 3,4-benzo-6-azabicyclo-[3.2.0]heptan-7-one, 4,5-benzo-7-azabicyclo[4.2.0]octan-8-one, and 5,6-benzo-8-azabicyclo[5.2.0]nonan-9-one with H_2O in iPr_2O at 60 °C. The (1*R*,2*R*)-β-amino acids ($ee \geq 96\,\%$, yields $\geq 40\,\%$) and (1*S*,6*S*)-, (1*S*,7*S*)-, and (1*S*,8*S*)-β-lactams ($ee > 99\,\%$, yields $\geq 44\,\%$) produced could be easily separated. The ring opening of racemic and enantiomeric β-lactams with 18 % HCl afforded the corresponding β-amino acid hydrochlorides.

Keywords: amino acids · cleavage reactions · enantioselectivity · enzymes · lactams

Introduction

Cyclic β-lactams and β-amino acids have been intensively investigated owing to their potential biological activity (e.g., monobactams and cispentacin)[1] and their utility in synthetic chemistry.[2] For example, they can serve as building blocks for the synthesis of modified peptides with increased activity and stability,[3–5] and with well-defined three-dimensional structures (e.g., β-peptides with possible antibiotic activity) similar to those of natural peptides.[6] Alicyclic β-amino acids can also be used in heterocyclic[7] and combinatorial[8] chemistry.

Several direct and indirect enzymatic methods have been described for the preparation of enantiopure β-amino acids or their derivatives. The indirect enzymatic method consisting of the lipase-catalyzed asymmetric acylation of the primary hydroxy group of *N*-hydroxymethylated β-lactams, or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening to give the β-amino ester or acid, respectively, are not too efficient and relatively long procedures, but they ensure the simultaneous preparation of both β-lactam enantiomers.[9] The direct β-lactam ring opening, through lipase-catalyzed enantioselective alcoholysis, furnishes the β-amino acids in low yields.[10] We recently discovered a simple and efficient direct enzymatic hydrolysis method for the enantioselective ($E>200$) ring cleavage of β-lactams in an organic medium (e.g., the synthesis of cispentacin).[11] The great advantages of this method are that the lactam ring need not necessarily be activated, and the β-amino acid and β-lactam products are obtained in good chemical yields and can easily be separated. Shintani and Fu[12] described a copper-catalyzed intramolecular Kinugasa reaction (in the presence of planar chiral phosphaferrocene-oxazoline as the ligand) and prepared tricyclic β-lactams, our target β-lactam fused to a six-membered ring being one of them ($ee = 88\,\%$).

Because of the earlier extensive investigations of alicyclic β-lactams and β-amino acids, the synthesis of new benzocispentacin homologues in both racemic and enantiopure forms is very attractive. Our present aim was the synthesis of the title bicyclic β-amino acids through the lipase-catalyzed ring opening of the corresponding β-lactams.

Results and Discussion

Syntheses of (±)-4, (±)-5, and (±)-6: The β-lactams (±)-**4** to (±)-**6** were prepared by 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to the corresponding cycloalkenes (**1**–**3**; Scheme 1), by using slightly modified literature procedures.[13–15] In order to synthesize the seven-membered β-lactam (±)-**6**, benzosuberene (**3**), the substrate for CSI addition, was first prepared from 1-benzosuberone by means of $NaBH_4$ reduction and subsequent H_2O elimina-

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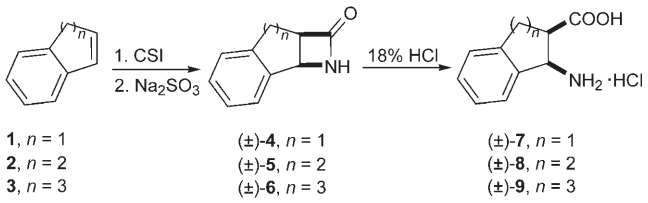


1, *n* = 1 (±)-**4**, *n* = 1 (±)-**7**, *n* = 1
2, *n* = 2 (±)-**5**, *n* = 2 (±)-**8**, *n* = 2
3, *n* = 3 (±)-**6**, *n* = 3 (±)-**9**, *n* = 3

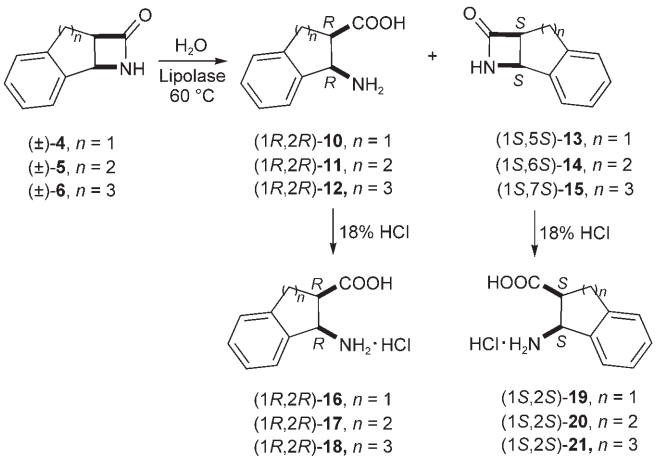
Scheme 1. Syntheses of compounds (±)-**4** to (±)-**9**.

tion.[16] All the reactions took place regio- and stereoselectively: not even traces of other regio- or stereoisomers were detected. As the racemic six- and seven-membered β-amino acids are new compounds, the transformations of (±)-**5** and (±)-**6** by ring opening with 18% HCl were also performed, resulting in β-amino acid hydrochlorides (±)-**8** and (±)-**9**, respectively (Scheme 1).

Lipase-catalyzed enantioselective ring opening of (±)-4, (±)-5, and (±)-6: The earlier results[11] on the lipase-catalyzed enantioselective hydrolysis of β-lactams suggested the possibility of the enantioselective ring opening of (±)-**4** to (±)-**6** with H_2O in an organic solvent (Scheme 2).

We started with the ring cleavage of (±)-**5** with one equivalent of H_2O, and Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a geneticallly modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) in iPr_2O at 60 °C (Table 1, entry 8), but also tested the reactions with Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*; Table 1, entry 3) and CAL-A (lipase A from *Candida antarctica*; Table 1, entries 10 and 11). High enantioselectivities ($E > 200$) were observed in the cases of Chirazyme L-2 and Lipolase, whereas CAL-A (neither the free enzyme nor the immobilized preparation) did not exhibit any catalytic activity (no product was detected after 15 h).

The Chirazyme L-2 catalyzed reactions were performed not only at 60 °C, but also at 50 °C (Table 1, entry 1) and 70 °C (Table 1, entry 6). With increasing temperature, the reaction rate increased without a drop in enantioselectivity ($E > 200$).



(±)-**4**, *n* = 1 (1*R*,2*R*)-**10**, *n* = 1 (1*S*,5*S*)-**13**, *n* = 1
(±)-**5**, *n* = 2 (1*R*,2*R*)-**11**, *n* = 2 (1*S*,6*S*)-**14**, *n* = 2
(±)-**6**, *n* = 3 (1*R*,2*R*)-**12**, *n* = 3 (1*S*,7*S*)-**15**, *n* = 3

(1*R*,2*R*)-**16**, *n* = 1 (1*S*,2*S*)-**19**, *n* = 1
(1*R*,2*R*)-**17**, *n* = 2 (1*S*,2*S*)-**20**, *n* = 2
(1*R*,2*R*)-**18**, *n* = 3 (1*S*,2*S*)-**21**, *n* = 3

Scheme 2. Lipase-catalyzed enantioselective ring opening of compounds (±)-**4** to (±)-**6**.

Table 1. Conversion and enantioselectivity of ring opening of (±)-**5**.[a]

Entry	Enzyme (30 mg mL^{-1})	T [°C]	H_2O [equiv]	Conv. [%]	ee_s[b] [%]	ee_p[c] [%]	E
1	Chirazyme L-2	50	1	9	10	>99	>200
2	Chirazyme L-2	60	–	24	32	>99	>200
3	Chirazyme L-2	60	1	24	32	>99	>200
4	Chirazyme L-2	60	2	19	23	>99	>200
5	Chirazyme L-2	60	4	11	12	>99	>200
6	Chirazyme L-2	70	1	27	37	>99	>200
7	Lipolase	60	–	24	31	>99	>200
8	Lipolase	60	1	23	29	>99	>200
9	Lipolase	60	2	17	20	>99	>200
10	CAL-A	60	1	no reaction			
11	CAL-A[d]	60	1	no reaction			

[a] 0.05 M substrate in iPr_2O after 15 h. [b] According to GC. s = substrate. [c] According to GC after double derivatization. p = product. [d] Contains 20% (w/w) of lipase adsorbed on Celite in the presence of sucrose.

A set of experiments was performed to determine the effect of H_2O present in the reaction medium on the enzymatic activity (Table 1, entries 4, 5 and 9). The catalytic activities of the tested Chirazyme L-2 and Lipolase were progressively lowered on increasing the amount of H_2O, although the enantioselectivity was apparently not affected. Furthermore, the hydrolysis of **5** in the presence of Chirazyme L-2 or Lipolase was complete even without the addition of any H_2O (Table 1, entries 2 and 7), because the H_2O present in the enzyme preparation or in the solvent used was sufficient for the β-lactam ring opening.

Even though the enantioselectivity in the Lipolase-catalyzed ring opening of **5** was excellent ($E > 200$), the reactivity for the hydrolysis clearly increased as the quantity of enzyme was increased (Table 2). When merely 10 mg mL^{-1} of enzyme was used, the reaction reached 50% conversion over a relatively long period of time (236 h; entry 1). In spite of the fact that the optimal enzyme quantity (resulting in the shortest reaction time needed to reach 50% conver-

Abstract in Hungarian: *Hatékony enzimes módszert dolgoztunk ki benzociszpentacin és homológjai enantiomertiszta formában történő előállítására. A 3,4-benzo-6-azabiciklo-[3.2.0]heptán-7-on, 4,5-benzo-7-azabiciklo[4.2.0]oktán-8-on és 5,6-benzo-8-azabiciklo[5.2.0]nonán-9-on enantioszelektív gyűrűnyitását (*E *> 200) vízzel Lipolase (*Candida antarctica B *lipáz-katalízissel, diizopropil-éterben, 60 °C-on végeztük. A nagy enantiomerfelesleggel és jó termeléssel kapott (1*R*,2*R*)-β-aminosav (*ee *≥ 96%, termelés ≥ 40%) és (1*S*,6*S*)-, (1*S*,7*S*)-valamint (1*S*,8*S*)-β-laktám (*ee *> 99%, termelés ≥ 44%) enantiomerek elválasztása egyszerűen, szerves-vizes extrakcióval történt. Mind a racém, mind az enantiomertiszta β-laktámokból előállítottuk a megfelelő aminosav hidrokloridokat.*

Table 2. Effect of quantity of Lipolase on ring opening of (±)-**5**.[a]

Entry	Lipolase [g mL^{-1}]	Conv. [%]	ee_s[b] [%]	ee_p[c] [%]	E
1	10	9	7	>99	>200
		(50 after 236 h)	(98)	(>99)	(>200)
2	20	12	13	>99	>200
3	30	15	17	>99	>200
4	40	22	28	>99	>200
5	50	24	31	>99	>200
6	75	29	40	>99	>200
7	100	31	45	>99	>200

[a] 0.05 M substrate in iPr$_2$O, with 1 equiv H_2O, at 60 °C, after 15 h. [b] According to GC. [c] According to GC after double derivatization.

sion) proved to be 100 mg mL^{-1} (entry 7), for reasons of economy, 50 mg mL^{-1} Lipolase was chosen for use in the preparative-scale resolutions of (±)-**4** to (±)-**6**.

Several solvents were tested in order to study the solvent effect in the Lipolase-catalyzed hydrolysis of (±)-**5** at 60 °C (Table 3). Lipolase was practically inactive in acetone

Table 3. Effect of solvent on ring opening of (±)-**5**.[a]

Entry	Solvent	Conv. [%]	ee_s[b] [%]	ee_p[c] [%]	E
1	acetone	no reaction			
2	tetrahydrofuran	no reaction			
3	diethyl ether	33	49	>99	>200
4	*tert*-amyl alcohol	no reaction			
5	diisopropyl ether	44	75	>99	>200
6	toluene	31	44	>99	>200
7	*n*-hexane	no reaction			

[a] 0.05 M substrate in the solvent tested, 30 mg mL^{-1} Lipolase, and 1 equiv H_2O, at 60 °C, after 22 h. [b] According to GC. [c] According to GC after double derivatization.

(entry 1), tetrahydrofuran (entry 2), and *n*-hexane (entry 7). The reaction proceeded more slowly in Et$_2$O (entry 3) and toluene (entry 6) than in iPr$_2$O (conversion 44% after 22 h).

On the basis of the results of the above experiments, the gram-scale resolutions of (±)-**4** to (±)-**6** were performed in iPr$_2$O with Lipolase (50 mg mL^{-1}) as the catalyst and H_2O (1 equiv) as the nucleophile, at 60 °C. The results are presented in Table 4 and in the Experimental Section.

Table 4. Lipolase-catalyzed ring opening of (±)-**4** to (±)-**6**.[a]

				β-Amino acid (**10**–**12**)				β-Lactam (**13**–**15**)			
	t [h]	Conv. [%]	E	Yield [%]	Isomer	ee[b] [%]	$[\alpha]_D^{25}$	Yield [%]	Isomer	ee[c] [%]	$[\alpha]_D^{25}$
(±)-**4**	6	50	>200	40	1*R*,2*R*	96	−6[d]	44	1*S*,5*S*	99	+224[e]
(±)-**5**	54	50	>200	45	1*R*,2*R*	99	+25.1[f]	45	1*S*,6*S*	99	+313[g]
(±)-**6**	51	50	>200	44	1*R*,2*R*	97	+7[h]	45	1*S*,7*S*	99	−137[i]

[a] 50 mg mL^{-1} enzyme in iPr$_2$O and 1 equiv H_2O, at 60 °C. [b] Determined by GC after double derivatization with 1) diazomethane or 2) acetic anhydride, in the presence of 4-dimethylaminopyridine and pyridine (see the Experimental Section). [c] According to GC (see the Experimental Section). [d] c = 0.11 in H_2O. [e] c = 0.33 in $CHCl_3$. [f] c = 0.30 in H_2O. [g] c = 0.35 in $CHCl_3$. [h] c = 0.09 in H_2O. [i] c = 0.28 in $CHCl_3$.

Transformations of the enantiomers: The transformations involving the ring opening of β-lactam enantiomers **13**–**15** with 18% HCl resulted in the enantiomers of the β-amino acid hydrochlorides **19**–**21** ($ee \geq 99$%; Scheme 2). Treatment of enantiomeric β-amino acids **10**–**12** with 18% HCl resulted in amino acid hydrochlorides **16**–**18**, respectively ($ee \geq$ 98%). As the β-homooligomers constructed from the building block *trans*-2-aminocycloalkanecarboxylic acid display stable helical structures,[6] the preparation of *trans*-β-amino acid hydrochlorides (racemic **26** and enantiopure **23** and **27**) was also investigated (Scheme 3). The transformation of **11** with $SOCl_2$ afforded amino ester **22** (ee > 99%), whereas that of **14** by ring opening with 22% HCl/EtOH resulted in the enantiomer of the β-amino ester **25** (ee = 99%). NaOEt isomerization of racemic **24** and enantiopure **22** and **25**, followed by acidic hydrolysis, resulted in the corresponding *trans*-amino acid hydrochlorides **23**, **26**, and **27** (ee = 99%, containing ≈30% *cis* isomer; Scheme 3). The physical data of the enantiomers prepared are reported in Table 5.

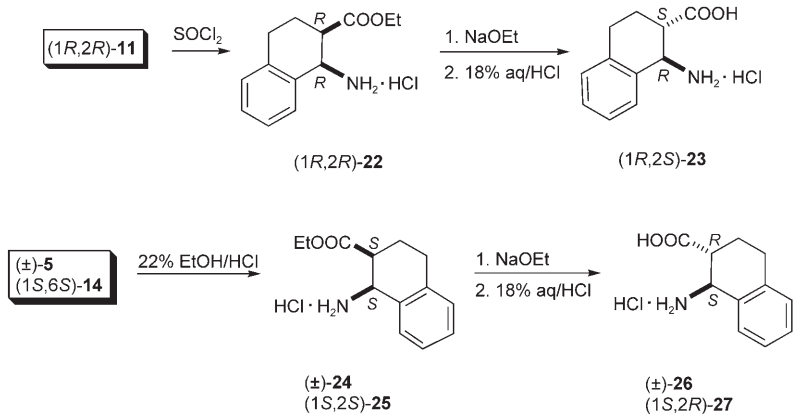
The absolute configuration in the case of **16** was proved by comparing the $[\alpha]$ value with the literature data[13] (Table 5), whereas for its six- and seven-membered homologues the analyzed chromatograms indicated the same enantioselectivity preference for Lipolase.

Conclusion

In conclusion, an efficient direct enzymatic method was developed for the synthesis of benzocispentacin and its new, optically pure six- and seven-membered homologues by enantioselective ring cleavage of the corresponding β-lactams in an organic medium. The Lipolase-catalyzed highly enantioselective reactions (E > 200) with H_2O (1 equiv) as the nucleophile in iPr$_2$O at 60 °C produced β-amino acid and β-lactam enantiomers ($ee \geq 96$%) in good chemical yields (40–45%). The products were also easily separated. Transformations by the ring opening of β-lactams with 18% HCl and 22% EtOH/HCl resulted in the corresponding β-amino acid and ester hydrochlorides, respectively ($ee \geq 99$%). Isomerization of the esters, followed by hydrolysis, resulted in the corresponding *trans*-amino acid hydrochlorides. The present method of formation of new enantiopure β-lactams and β-amino acids (benzocispentacin and its homologues) proved to be a very simple, inexpensive route, and could be easily scaled up. The synthesized enantiopure β-amino acids are promising building blocks for the synthesis of peptides and peptidomimetics, as potential pharmacons.

Experimental Section

Materials and methods: Lipolase (lipase B from *Candida antarctica*),



Scheme 3. Epimerization of esters.

Table 5. Specific rotations of enantiomers prepared.

Amino acid hydrochloride	*ee* [%]	$[\alpha]_D^{25}$
(1*R*,2*R*)-**16**	98	−5.8 ($c=0.4$ in H_2O)
		−5.7 ($c=0.5$ in MeOH)[a]
(1*S*,2*S*)-**19**	>99	+5.7 ($c=0.4$ in H_2O)
(1*R*,2*R*)-**17**	>99	+27.8 (c=0.35 in H_2O)
(1*S*,2*S*)-**20**	99	−27.5 ($c=0.37$ in H_2O)
(1*R*,2*R*)-**18**	98	+8.1 ($c=0.4$ in H_2O)
(1*S*,2*S*)-**21**	99	−8 ($c=0.12$ in H_2O)
(1*R*,2*R*)-**22**	>99	+40 ($c=0.15$ in EtOH)
(1*S*,2*S*)-**25**	>99	−39 ($c=0.17$ in EtOH)

[a] $[\alpha]_D^{25}=-5.3$ ($c=0.5$ in MeOH).[13]

produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, was from Sigma-Aldrich. CAL-A (lipase A from *Candida antarctica*) and Novozym 435 as an immobilized lipase (lipase B from *Candida antarctica*) on a macroporous acrylic resin were from Novo Nordisk. Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Before use, CAL-A (5 g) was dissolved in Tris-HCl buffer (0.02 M; pH 7.8) in the presence of sucrose (3 g), followed by adsorption on Celite (17 g; Sigma). The lipase preparation thus obtained contained 20% (w/w) lipase. 1-Benzosuberone, CSI, and the cycloalkenes (indene and 1,2-dihydronaphthalene) were from Aldrich. The solvents were of the highest analytical grade.

In a typical small-scale experiment, racemic β-lactam (0.05 M solution) in an organic solvent (2 mL) was added to the lipase tested (10, 20, 30, 40, 50, 75, or 100 mg mL^{-1}). H_2O (0, 1, 2, or 4 equiv) was added. The mixture was shaken at 50, 60, or 70 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by means of gas chromatography (GC). The *ee* values for the unreacted β-lactam enantiomers were determined by gas chromatography on a Chromopack Chiralsil-Dex CB column (25 m) [120 °C for 7 min→190 °C, temperature rise 20 °C min^{-1}; 140 kPa; retention times (min): **13**, 13.74 (antipode: 13.38); **14**, 16.57 (antipode: 15.82); **15**, 20.14 (antipode: 19.84)], while the *ee* values for the β-amino acids produced were determined by using a gas chromatograph equipped with a chiral column after double derivatization with 1) diazomethane (Caution! The derivatization with diazomethane should be performed under a well-working hood); 2) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine [CP-Chirasil-Dex CB column, 120 °C for 7 min→190 °C, temperature rise 20 °C min^{-1}; 140 kPa; retention times (min): **10**, 13.73 (antipode: 13.38); **11**, 15.71 (antipode:15.52); **12**, 17.86 (antipode: 17.09)].

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

Synthesis of racemic 3,4-benzo-6-azabicyclo[3.2.0]heptan-7-one, 4,5-benzo-7-azabicyclo[4.2.0]octan-8-one, and 5,6-benzo-8-azabicyclo[5.2.0]-nonan-9-one ((±)-4, (±)-5, and (±)-6)

*Synthesis of (±)-**4***: By using the literature procedure[13] and indene (11.62 g, 0.10 mol), (±)-**4** was obtained (11.44 g, 72%; recrystallized from AcOEt and MeOH, m.p. 190–191 °C).

Data for (±)-**4**: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=3.07$ (dd, $J=17.5$, 10.5 Hz, 1H; CH_2), 3.35 (d, $J=17.3$ Hz, 1H; CH_2), 4.03 (d, $J=10.5$ Hz, 1H; *CH*CO), 5.03 (d, $J=4.2$ Hz, 1H; *CH*N), 6.23 (br s, 1H; N*H*), 7.21–7.34 ppm (m, 4H; C_6H_4); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta=$ 31.1, 54.9, 59.2, 125.8, 127.0, 127.8, 129.8, 141.2, 144.5, 172.0 ppm; elemental analysis calcd (%) for $C_{10}H_9NO$: C 75.45, H 5.70, N 8.80; found: C 75.39, H 5.60, N 8.85.

*Synthesis of (±)-**5***: A solution of CSI (15.57 g, 0.11 mol) in dry Et_2O (40 mL) was added dropwise to 1,2-dihydronaphthalene (13.02 g, 0.1 mol) dissolved in dry Et_2O (160 mL) at 0 °C. The solution was stirred for 0.5 h at 0 °C, and then for 4 h at room temperature. The resulting liquid was added dropwise to a vigorously stirred solution of Na_2SO_3 (1.87 g) and K_2CO_3 (43.28 g) in H_2O (200 mL). The organic layer was separated and the phase was extracted with AcOEt. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting yellowish oil was crystallized from *n*-hexane to give (±)-**5** (16.15 g, 78%; recrystallized from *i*Pr_2O, m.p. 96–99 °C).

Data for (±)-**5**: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=1.59$–2.86 (m, 4H; $2\times CH_2$), 3.71–3.73 (m, 1H; *CH*CO), 4.68 (d, $J=4.9$ Hz, 1H; *CH*N), 5.96 (br s, 1H; N*H*), 7.19–7.3 ppm (m, 4H; C_6H_4); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta=23.6$, 27.5, 50.8, 52.2, 127.3, 129.2, 129.7, 130.2, 134.7, 140.0, 170.4 ppm; elemental analysis calcd (%) for $C_{11}H_{11}NO$: C 76.28, H 6.40, N 8.09; found: C 76.32, H 6.44, N 8.11.

*Synthesis of (±)-**6***: A solution of CSI (2.79 mL, 32.1 mmol) in dry Et_2O (20 mL) was added dropwise to benzosuberene (1.56 g, 10.71 mmol), prepared from 1-benzosuberone by means of $NaBH_4$ reduction and subsequent H_2O elimination,[16] dissolved in dry Et_2O (20 mL) at room temperature. The solution was stirred for 2 days at room temperature, and the mixture was then quenched with H_2O. The organic layer was separated and poured into a solution of 20% Na_2SO_3 (24 mL). The pH was adjusted to 8 by the addition of 10% KOH. The organic layer was separated and the phase was extracted with Et_2O. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated. The resulting white solid was recrystallized from *n*-hexane and Et_2O to give (±)-**6** (0.52 g, 26%; m.p. 158–161 °C).

Data for (±)-**6**: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): $\delta=1.31$–2.75 (m, 6H; $3\times CH_2$) 3.41 (d, $J=13.4$ Hz, 1H; *CH*CO), 4.95 (d, $J=5.4$ Hz, 1H; *CH*N), 6.79 (br s, 1H; N*H*), 7.04–7.29 ppm (4H; m, C_6H_4); ^{13}C NMR (100.62 MHz, D_2O): $\delta=21.7$, 23.4, 31.0, 53.3, 54.2, 126.4, 127.5, 128.6, 129.4, 136.7, 137.1, 171.5 ppm; elemental analysis calcd (%) for $C_{12}H_{13}NO$: C 76.98, H 7.00, N 7.48; found: C 76.91, H 6.88, N 7.36.

Gram-scale resolution of racemic (±)-4, (±)-5, and (±)-6

*Gram-scale resolution of (±)-**4***: Racemic **4** (1.00 g, 6.28 mmol) was dissolved in *i*Pr_2O (40 mL). Lipolase (2 g, 50 mg mL^{-1}) and H_2O (113 μL, 6.28 mmol) were added and the mixture was shaken in an incubator shaker at 60 °C for 6 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residue (1*S*,5*S*)-**13** was crystallized out [441 mg, 44%; recrystallized from *i*Pr_2O $[\alpha]_D^{25}=+224$ ($c=0.33$ in $CHCl_3$); m.p. 232–233 °C; *ee* = 99%]. The filtered enzyme was washed with distilled H_2O (3×15 mL), and the H_2O was evaporated off, yielding the crystalline β-amino acid (1*R*,2*R*)-**10**

[446 g, 40%; $[\alpha]_D^{25}=-6$ ($c=0.11$ in H_2O); m.p. 263–264°C with sublimation (recrystallized from H_2O and Me_2CO), $ee=96\%$]. When **10** (200 mg) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*R*)-**16** was obtained [181 mg, 75%; $[\alpha]_D^{25}=-5.8$ ($c=0.4$ in H_2O); $[\alpha]_D^{25}=-5.7$ ($c=0.5$ in MeOH); ref. [13]: $[\alpha]_D^{25}=-5.3$ ($c=0.5$ in MeOH); m.p. 210–217°C with sublimation (recrystallized from EtOH and Et_2O), $ee=98\%$] was formed.

Data for **13**: The ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS) data are similar to those for (±)-**4**; elemental analysis calcd (%) for $C_{10}H_9NO$: C 75.45, H 5.70, N 8.80; found: C 75.42, H 5.59, N 8.77.

Data for **10**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta=3.25$ (d, $J=9.6$ Hz, 1H; CH_2), 3.3 (d, $J=8.6$ Hz, 1H; CH_2), 3.52 (d, $J=7.2$ Hz, 1H; C*H*CO), 4.85 (d, $J=6.8$ Hz, 1H; C*H*N), 7.35–7.53 ppm (m, 4H; C_6H_4); elemental analysis calcd (%) for $C_{10}H_{11}NO_2$: C 67.78, H 6.26, N 7.90; found: C 67.47, H 6.25, N 7.98.

Data for **16**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta=3.38$ (d, $J=8.9$ Hz, 2H; CH_2), 3.78 (dd, $J=16$, 8.6 Hz, 1H; C*H*CO), 5.01 (d, $J=6.8$ Hz, 1H; C*H*N), 7.38–7.47 ppm (m, 4H; C_6H_4); elemental analysis calcd (%) for $C_{10}H_{11}NO_2$·HCl: C 56.21, H 5.66, N 5.56; found: C 55.88, H 5.40, N 5.58.

*Gram-scale resolution of (±)-**5***: By using the procedure described above, the reaction of racemic **5** (1.00 g, 5.77 mmol) and H_2O (103 μL, 5.77 mmol) in *i*Pr_2O (40 mL) in the presence of Lipolase (2 g, 50 mg mL^{-1}) at 60°C afforded unreacted (1*S*,6*S*)-**14** [452 mg, 45%; $[\alpha]_D^{25}=+313$ ($c=0.35$ in $CHCl_3$); m.p. 142–143°C (recrystallized from *i*Pr_2O); $ee=99\%$] and β-amino acid (1*R*,2*R*)-**11** [490 mg, 45%; $[\alpha]_D^{25}=+25.1$ ($c=0.30$ in H_2O); m.p. 269–271°C with sublimation (recrystallized from H_2O and Me_2CO); $ee=99\%$] in 54 h. When **11** (200 mg) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*R*)-**17** [198 mg, 82%; $[\alpha]_D^{25}=+27.8$ ($c=0.35$ in H_2O); m.p. 237–240°C with sublimation (recrystallized from EtOH and Et_2O), $ee=99\%$] was formed.

Data for **14**: The ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS) data are similar to those for (±)-**5**; elemental analysis calcd (%) for $C_{11}H_{11}NO$: C 76.28, H 6.40, N 8.09; found: C 76.39, H 6.55, N 7.94.

Data for **11**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta=1.98$ (dd, $J=9.9$, 4.8 Hz, 1H; CH_2), 2.22 (dd, $J=11.1$, 1.9 Hz, 1H; CH_2), 2.84–3.01 (m, 3H; CH_2 and C*H*CO), 4.67 (br s, 1H; C*H*N), 7.29–7.39 ppm (m, 4H; C_6H_4); ^{13}C NMR (100.62 MHz, D_2O): $\delta=21.7$, 28.1, 43.4, 50.3, 127.0, 129.6, 129.8, 130.1, 131.3, 137.6, 181.4 ppm; elemental analysis calcd (%) for $C_{11}H_{13}NO_2$: C 69.09, H 6.85, N 7.32; found: C 69.11, H 6.85, N 7.33.

Data for **17**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta=2.06$–2.31 (m, 2H; CH_2), 2.91–2.99 (m, 2H; CH_2), 3.13–3.18 (m, 1H; C*H*CO), 4.83 (d, $J=3.4$ Hz, 1H; C*H*N), 7.24–7.37 ppm (m, 4H; C_6H_4); ^{13}C NMR (100.62 MHz, D_2O): $\delta=20.7$, 27.5, 41.7, 50.0, 127.2, 129.2, 130.0, 130.2, 130.5, 137.1, 177.0; elemental analysis calcd (%) for $C_{11}H_{13}NO_2$·HCl: C 58.03, H 6.20, N 6.15; found: C 58.17, H 6.31, N 6.10.

*Gram-scale resolution of (±)-**6***: By using the procedure described above, the reaction of racemic **6** (500 mg, 2.67 mmol) and H_2O (48 μL, 2.67 mmol) in *i*Pr_2O (20 mL) in the presence of Lipolase (1 g, 50 mg mL^{-1}) at 60°C afforded unreacted (1*S*,7*S*)-**15** (219 mg, 45%; $[\alpha]_D^{25}=-137$ ($c=0.28$ in $CHCl_3$); m.p. 208°C (recrystallized from *i*Pr_2O); $ee=99\%$] and β-amino acid (1*R*,2*R*)-**12** [256 mg, 43%; $[\alpha]_D^{25}=+7$ ($c=0.12$ in H_2O); m.p. 241–243°C (recrystallized from H_2O and Me_2CO); $ee=97\%$] in 51 h. When **12** (100 mg) was treated with 22% HCl/EtOH (3 mL), (1*R*,2*R*)-**18** [93 mg, 79%; $[\alpha]_D^{25}=+8.1$ ($c=0.4$ in H_2O); m.p. 262–267°C (recrystallized from EtOH and Et_2O), $ee=98\%$] was formed.

Data for **15**: The ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS) data are similar to those for (±)-**6**; elemental analysis calcd (%) for $C_{12}H_{13}NO$: C 76.98, H 7.00, N 7.48; found: C 76.88, H 7.36, N 7.41.

Data for **12**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta=1.73$–2.18 (m, 4H; CH_2) 2.86–2.93 (m, 3H; CH_2 and C*H*CO), 4.83 (br s, 1H; C*H*N), 7.26–7.34 ppm (m, 4H; C_6H_4); elemental analysis calcd (%) for $C_{12}H_{15}NO_2$: C 70.22, H 7.37, N 6.82; found: C 70.24, H 7.18, N 6.55.

Data for **18**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta=1.67$–2.89 (m, 6H; 3×CH_2), 3.23 (br s, 1H; C*H*CO), 5.0 (br s, 1H; C*H*N), 7.28–7.34 ppm (m, 4H; C_6H_4); elemental analysis calcd (%) for $C_{12}H_{15}NO_2$·HCl: C 59.63, H 6.67, N 5.79; found: C 59.58, H 6.53, N 5.41.

Ring opening of (±)-4, (±)-5, (±)-6, (1*S*,5*S*)-13, (1*S*,6*S*)-14, and (1*S*,7*S*)-15 with HCl: Racemic (±)-**4** (500 mg, 3.14 mmol), (±)-**5** (500 mg, 2.88 mmol), or (±)-**6** (500 mg, 2.67 mmol) was dissolved in 18% HCl (15 mL) and placed under reflux for 3 h. The solvent was evaporated off, and the product was recrystallized from EtOH and Et_2O, which afforded white crystals of (±)-**7** (597 mg, 85%, m.p. 219–222°C), (±)-**8** (571 mg, 87%, m.p. 237–240°C), or (±)-**9** (520 mg, 85%, m.p. 158–160°C), respectively.

By following the procedure described above, the ring-opening reactions of (1*S*,5*S*)-**13** (200 mg, 1.25 mmol), (1*S*,6*S*)-**14** (200 mg, 1.15 mmol), and (1*S*,7*S*)-**15** (100 mg, 0.53 mmol) afforded white crystals of (1*S*,2*S*)-**19** [213 mg, 79%, $[\alpha]_D^{25}=+5.7$ ($c=0.4$ in H_2O); m.p. 204–211°C with sublimation; $ee=99\%$], (1*S*,2*S*)-**20** [229 mg, 87%; $[\alpha]_D^{25}=-27.5$ ($c=0.37$ in H_2O); m.p. 260–262°C; *ee* 99%], and (1*S*,2*S*)-**21** [102 mg, 79%; $[\alpha]_D^{25}=-8$ ($c=0.12$ in H_2O); m.p. 252–259°C; $ee=99\%$], respectively.

The ^{1}H NMR (400 MHz, D_2O, 25°C, TMS) data for (1*S*,2*S*)-**19** and (±)-**7**, (1*S*,2*S*)-**20** and (±)-**8**, and (1*S*,2*S*)-**21** and (±)-**9** are similar to those for (1*R*,2*R*)-**16**, (1*R*,2*R*)-**17**, and (1*R*,2*R*)-**18**.

Elemental analysis calcd (%) for (1*S*,2*S*)-**19** ($C_{10}H_{11}NO_2$·HCl): C 56.21, H 5.66, N 5.56; found: C 56.11, H 5.64, N 6.69.

Elemental analysis calcd (%) for (±)-**7** ($C_{10}H_{11}NO_2$·HCl): C 56.21, H 5.66, N 5.56; found: C 55.96, H 5.60, N 6.44.

Elemental analysis calcd (%) for (1*S*,2*S*)-**20** ($C_{11}H_{13}NO_2$·HCl): C 58.03, H 6.20, N 6.15; found: C 57.88, H 6.39, N 6.12.

Elemental analysis calcd (%) for (±)-**8** ($C_{11}H_{13}NO_2$·HCl): C 58.03, H 6.20, N 6.15; found: C 57.96, H 5.98, N 6.10.

Elemental analysis calcd (%) for (1*S*,2*S*)-**21** ($C_{12}H_{15}NO_2$·HCl): C 59.63, H 6.67, N 5.79; found: C 59.51, H 6.86, N 5.64.

Elemental analysis calcd (%) for (±)-**9** ($C_{12}H_{15}NO_2$·HCl): C 59.63, H 6.67, N 5.79; found: C 59.53, H 6.64, N 5.77.

Preparation of esters (±)-24 and (1*S*,2*S*)-25: (±)-**5** or (1*S*,6*S*)-**14** (200 mg, 1.15 mmol) was dissolved in 22% EtOH/HCl (10 mL) and placed under reflux for 9 h. The solvent was evaporated off, and the product was recrystallized from EtOH and Et_2O, which afforded white crystals of (±)-**24** [219 mg, 77%, m.p. 161–164°C with sublimation] or (1*S*,2*S*)-**25** [230 mg, 80%, $[\alpha]_D^{25}=-39$ ($c=0.17$ in EtOH); m.p. 188–191°C with sublimation], respectively.

Data for (±)-**24**: ^{1}H NMR (400 MHz, $CDCl_3$, 25°C, TMS): $\delta=1.25$ (t, $J=7.1$ Hz, 3H; CH_3), 2.08–3.01 (m, 4H; 2×CH_2), 3.22–3.25 (m, 1H; C*H*CO), 4.21 (q, $J=7.1$ Hz, 2H; OCH_2), 4.55 (br s, 1H; C*H*N), 7.11–7.26 (m, 3H; C_6H_4), 7.71 ppm (d, $J=7.2$ Hz, 1H; C_6H_4); elemental analysis calcd (%) for $C_{13}H_{17}NO_2$·HCl: C 61.05, H 7.09, N 5.48; found: C 61.00, H 6.93, N 5.49.

Data for (1*S*,2*S*)-**25**: The ^{1}H NMR (400 MHz, D_2O, 25°C, TMS) data are similar to those for (±)-**24**; elemental analysis calcd (%) for (1*S*,2*S*)-**25** ($C_{13}H_{17}NO_2$·HCl): C 61.05, H 7.09, N 5.48; found: C 60.88, H 7.02, N 5.56.

Preparation of ester (1*R*,2*R*)-22: $SOCl_2$ (0.21 mL, 2.86 mmol) was added dropwise to dry EtOH (15 mL) at −15°C. Amino acid **11** (250 mg, 1.3 mmol) was added in one portion to the mixture. After stirring for 30 min at 0°C, and then for 3 h at room temperature, the mixture was placed under reflux for a further 30 min and then evaporated. Ester **22** was recrystallized from EtOH and Et_2O [203 mg, 60%, $[\alpha]_D^{25}=+40$ ($c=0.2$ in EtOH); m.p. 189–192°C with sublimation].

Data for (1*R*,2*R*)-**22**: The ^{1}H NMR (400 MHz, D_2O, 25°C, TMS) data are similar to those for (±)-**24**; elemental analysis calcd (%) for (1*R*,2*R*)-**22** ($C_{13}H_{17}NO_2$·HCl): C 61.05, H 7.09, N 5.48; found: C 60.91, H 7.22, N 5.38.

Isomerization of (±)-24, (1*R*,2*R*)-22, and (1*S*,2*S*)-25: Na (0.1 g, 4.34 mmol) was dissolved in dry EtOH (5 mL). Base (±)-**24** (500 mg, 2.28 mmol) was added to this solution and the mixture was refluxed for 9 h. After evaporation of the solvent, 18% HCl (5 mL) was added, and then the mixture was refluxed for 10 h. After standing overnight, the solution was filtered, and evaporated to dryness. MeOH (5 mL) was added, and the solution was filtered again. The solvent was evaporated off, and

the residue was recrystallized from EtOH and Et_2O, which afforded white crystals of (±)-**26** (404 mg, 78%; containing ≈30% *cis* isomer).

Data for (±)-**26**: ^{1}H NMR (400 MHz, D_2O, 25°C, TMS): $\delta = 2.11$–3.03 (m, 4H; $2\times CH_2$), 3.17–3.22 (m, 1H; *CH*CO), 4.9 (d, $J = 5.5$ Hz, 1H; C*H*N), 7.26–7.44 ppm (m, 4H; C_6H_4); elemental analysis calcd (%) for (±)-**26** ($C_{11}H_{13}NO_2$·HCl): C 58.03, H 6.20, N 6.15; found: C 58.14, H 6.11, N 6.13.

By following the procedure described above, base (1*R*,2*R*)-**22** (150 mg, 0.68 mmol) and base (1*S*,2*S*)-**25** (150 mg, 0.68 mmol) afforded white crystals of (1*R*,2*S*)-**23** (116 mg, 75%) and (1*S*,2*R*)-**27** (139 mg, 89%) (both containing ≈30% *cis* isomer), respectively.

The ^{1}H NMR (400 MHz, D_2O, 25°C, TMS) data for (1*R*,2*S*)-**23** and (1*S*,2*R*)-**27** are similar to those for (±)-**26**.

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