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Enzymatic Method for the Synthesis of Blockbuster Drug Intermediates – Synthesis of Five-Membered Cyclic γ-Amino Acid and γ-Lactam Enantiomers

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A very efficient enzymatic method was developed for the synthesis of cyclic γ-lactam and γ-amino acid enantiomers, intermediates for drugs with a prominent turnover (e.g., abacavir and carbovir), through the CAL-B-catalysed enantioselective ($E > 200$) hydrolysis of the corresponding *N*-Boc protected and unprotected racemic γ-lactams with H_2O in iPr_2O.

Introduction

In the series of cyclic *cis* γ-amino acids, 4-aminocyclopent-2-ene-1-carboxylic acid, 3-aminocyclopentane-1-carboxylic acid and the enantiomers thereof are known compounds; owing to their GABA analogue structures, they possess GABA receptor agonist, partial agonist and antagonist activities,[1] and they can additionally be used in the synthesis of peptides with definite nonnatural secondary structures and for the design and synthesis of self-assembling peptide nanotubes.[2] (In this specification, the following numbering and nomenclature are applied for the compounds: *cis*-4-aminocyclopent-2-ene-1-carboxylic acid and its (1*S*,4*R*)-(–) and (1*R*,4*S*)-(+) enantiomers, *cis*-3-aminocyclopentane-1-carboxylic acid, 2-azabicyclo[2.2.1]hept-5-en-3-one and 2-azabicyclo[2.2.1]heptan-3-one.)

One of the enantiomers of cyclic *cis* γ-amino acids, (1*S*,4*R*)-4-aminocyclopent-2-ene-1-carbocyclic acid, is a key intermediate used in one of the syntheses of purine and pyrimidine carbonucleosides with significant antiviral activity (e.g., abacavir[3] and carbovir[4]),[5] through known methods, for example by reduction[6] and a subsequent coupling reaction.[7] Abacavir is a selective and potent reverse-transcriptase inhibitor for the treatment of human immunodeficiency virus and hepatitis B virus infections in adults and children.

The antipode γ-amino acid enantiomer is also used as a starting material for substances exerting valuable biological activity.[8] Further, the saturated analogue 3-aminocyclopentane-1-carboxylic acid is a starting material applied in the search for biologically active compounds.[9]

Because formation of the appropriate absolute configuration of the asymmetric centres is essential for both the synthesis of active ingredients of drugs and the search for biologically active compounds, several methods have been developed for the resolution of cyclic γ-amino acids; for example, the conventional method of diastereomeric salt formation is used for resolution. The method leads to pure enantiomers through a multistep process starting from racemic 2-azabicyclo[2.2.1]hept-5-en-3-one.[10]

Besides the conventional resolution methods, enzymatic resolution processes have also been described. For example, Csuk et al.[11] present the preparation of the appropriate (1*S*,4*R*)-4-(acylamino)cyclopent-2-ene-1-carboxylic acid (49.7%*ee*) and methyl (1*R*,4*S*)-4-(acylamino)cyclopent-2-ene-1-carboxylate (>99%*ee*) through pig liver esterase catalysed enantioselective ester cleavage of the racemic methyl *cis*-4-acylaminocyclopent-2-ene-1-carboxylate. By these methods, γ-amino acid enantiomers can be obtained after nonenzymatic hydrolysis of the starting racemic 2-azabicyclo[2.2.1]hept-5-en-3-one in several steps (acylations, esterification and deacylation) and resolution is carried out by selective cleavage of the ester groups linked to the cyclopentene ring with esterase or lipases.

Evans et al.[12] described a process for the resolution of racemic 2-azabicyclo[2.2.1]hept-5-en-3-one by using the lactamase activity of ENZA-1 (*Rhodococcus equi* NCIMB 41213) and ENZA-20 (*Psedomonas solanacearum* NCIMB 40249) strains. The (+) and (–) enantiomers of 2-azabicyclo[2.2.1]hept-5-en-3-one and the (–) and (+) enantiomers of *cis*-4-aminocyclopent-2-ene-1-carboxylic acid were obtained through opening of the lactam ring. Taylor et al.[13] reported that the use of the more selective and stable ENZA-25 and ENZA-22 strains with lactamase activity was more suitable for the resolution of 2-azabicyclo[2.2.1]-hept-5-en-3-one.

In this paper, we report the first direct enzymatic method that is equally applicable for the synthesis of cyclic γ-lactam and γ-amino acid enantiomers through the CAL-B (lipase B from *Candida antarctica*) catalysed enantioselective ($E >$

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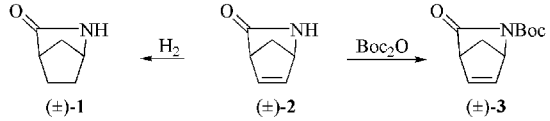
200) hydrolysis of the corresponding *N*-Boc protected and unprotected racemic γ-lactams in an organic solvent (Scheme 2).

Results and Discussion

Syntheses of (±)-1 and (±)-3

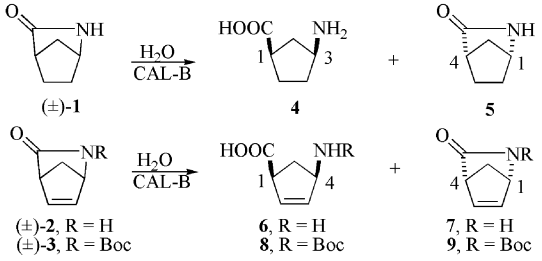
γ-Lactam (±)-**1** was prepared from commercially available 2-azabicyclo[2.2.1]hept-5-en-3-one (Vince lactam[14]) [(±)-**2**] by catalytic transfer hydrogenation in the presence of the hydrogen donor cyclohexene,[15] whereas the reaction of (±)-**2** with di-*tert*-butyl dicarbonate[16] resulted in desired *N*-Boc-protected γ-lactam (±)-**3** (Scheme 1).



Scheme 1. Syntheses of (±)-**1** and (±)-**3**.

Lipase-Catalysed Enantioselective Ring Cleavage of (±)-1–(±)-3

On the basis of the results of the enantioselective hydrolyses of carbocyclic β-lactams[17] and β-amino esters[18] by using lipase B from *Candida antarctica* in an organic solvent, we started preliminary experiments in iPr$_2$O with enzyme screening including lipase PS (*Pseudomonas cepacia*), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*), Chirazyme L-2, Novozym 435 and Lipolase (all three are forms of immobilised lipase B from *Candida antarctica*), Chirazyme L-5 (lipase A from *Candida antarctica*) and PPL (porcine pancreatic lipase) (Scheme 2). The reactions were performed for the model compound (±)-**2** with H_2O (1 equiv.) at 45 or 60 °C. Chirazyme L-5 at 60 °C (Table 1, entry 13) and lipases PS, AK and PPL at 45 °C (Table 1, entries 14–16) catalysed the hydrolysis of (±)-**2** selectively, but the reaction rates were relatively low. Chirazyme L-2 (Table 1, entry 11), Novozym 435 (Table 1, entry 12) and Lipolase (Table 1, entry 1) were all promising catalysts at 60 °C, as they directed the hydrolysis selectively and faster, although with slight differences in reaction rates. Lipolase was chosen as the enzyme for further studies and for the gram-scale resolution.



Scheme 2. Lipase-catalysed enantioselective ring cleavage of (±)-**1**–(±)-**3**.

The catalytic activity of the tested Lipolase was affected by the amount of added H_2O to the reaction mixture (Table 1, entries 2–6). The shortest reaction time (the time needed to reach 50% conversion) was obtained for 0.5 equiv. of added H_2O (Table 1, entry 2). As the amount of added H_2O was increased, the reaction became progressively slower, though E remained high (when the hydrolysis was performed in H_2O, the reaction reached 45% conversion after 19 h; $E > 200$). Furthermore, when the reaction was performed without the addition of H_2O (Table 1, entry 3), the H_2O in the reaction medium (<0.1%) or present in the enzyme preparation (<5%) was still sufficient for the hydrolysis to proceed. On the principle that "the best solvent is no solvent",[19] the hydrolysis of (±)-**2** was also performed in a solvent-free system: a mixture of thoroughly powdered racemic **2** (55 mg, 0.5 mmol), Lipolase (150 mg)

Table 1. Conversion and enantioselectivity of hydrolysis of (±)-**2**.[a]

Entry	Reaction time	Enzyme (30 mg mL^{-1})	Temp. [°C]	H_2O [equiv.]	Conv. [%]	ee_s[b] [%]	ee_p[c] [%]	E
1	140 min	Lipolase	60	1	42	72	>99	>200
2	140 min	Lipolase	60	0,5	50	>99	>99	>200
	(50 min)				(26)	(34)	(>99)	(>200)
3	140 min	Lipolase	60	–	50	>99	>99	>200
4	140 min	Lipolase	60	2	36	55	>99	>200
5	140 min	Lipolase	60	5	24	32	>99	>200
6	140 min	Lipolase	60	10	11	12	>99	>200
7	17 h	Lipolase	30	0.5	43	74	>99	>200
8	50 min	Lipolase	45	0.5	11	12	>99	>200
9	50 min	Lipolase	70	0.5	29	40	>99	>200
10	50 min	Lipolase	80	0.5	48	86	>99	>200
11	140 min	Chirazyme L-2	60	1	41	68	>99	>200
12	140 min	Novozym 435	60	1	40	65	>99	>200
13	64 h	Chirazyme L-5[d]	60	1	15	17	>99	>200
14	64 h	PPL	45	1	20	25	>99	>200
15	64 h	Lipase PS[d]	45	1	10	11	>99	>200
16	64 h	Lipase AK[d]	45	1	14	16	>99	>200

[a] 0.05 M substrate in iPr$_2$O. [b] According to GC (Experimental Section). [c] According to GC after double derivatisation (Experimental Section). [d] Contains 20 wt.-% of lipase adsorbed on Celite in the presence of sucrose.

and added H_2O (0.25 mmol) was vigorously shaken at 60 °C. The reaction led to 47% conversion in 33 h [ee_s = 88%, ee_p > 99%; E > 200 (ee_s and ee_p stand for the enantiomeric excess of the substrates and the products, respectively)].

Next, we analysed the effects of temperature on the enantioselectivity and reaction rate. The Lipolase-catalysed hydrolysis of (±)-**2** with 0.5 equiv. of H_2O in iPr_2O at 30 °C proved to be a relatively slow reaction but the enantioselectivity was high (Table 1, entry 7). When the reaction was performed at 45 °C (Table 1, entry 8), the reaction rate increased; upon further increase of the temperature (to 70 or 80 °C), considerably faster reactions were observed (Table 1, entries 9 and 10) without a drop in the enantioselectivities.

Several solvents were tested to study the solvent effect in the Lipolase-catalysed hydrolysis of (±)-**2** at 60 °C. The enzyme was practically inactive in Me_2CO (Table 2, entry 1). The reaction proceeded faster in *t*BuOMe and *n*-hexane (Table 2, entries 5 and 7), more slowly in 1,4-dioxane, Et_2O, toluene and CH_2Cl_2 (Table 2, entries 2, 4, 6 and 8) and much more slowly in THF (Table 2, entry 3) than that in iPr_2O (Table 1, entry 2), which all afforded high enantioselectivities (E > 200).

Table 2. Conversion and enantioselectivity of hydrolysis of (±)-**2**.[a]

Entry	Reaction time	Solvent	Conv. [%]	ee_s[b] [%]	ee_p[c] [%]	E
1	36 h	Me_2CO		no reaction		
2	36 h	1,4-dioxane	22	28	>99	>200
3	36 h	THF	7	7	>99	>200
4	50 min	Et_2O	18	21	>99	>200
5	50 min	*t*BuOMe	35	54	>99	>200
6	36 h	toluene	33	49	>99	>200
7	50 min	*n*-hexane	34	51	>99	>200
8	36 h	CH_2Cl_2	20	24	>99	>200

[a] 0.05 M substrate in the solvent tested, 30 mg mL^{-1} Lipolase and 0.5 equiv. of H_2O, at 60 °C. [b] According to GC (Experimental Section). [c] According to GC after double derivatisation (Experimental Section).

The reaction rate for the hydrolysis of (±)-**2** increased with increasing the amount of enzyme (Table 3, entries 1–3 and 7–9).

Table 3. Effect of quantity of Lipolase on hydrolysis of (±)-**2**.[a]

Entry	Lipolase [mg mL^{-1}]	Conv. [%]	ee_s[b] [%]	ee_p[c] [%]	E
1	10	15 (50 after 20 h)	18 (98)	>99 (>99)	>200 (>200)
2	20	23	29	>99	>200
3	30	32	46	>99	>200
4	30[d]	28	39	>99	>200
5	30[e]	25	33	>99	>200
6	30[f]	23	30	>99	>200
7	40	33	49	>99	>200
8	50	36	56	>99	>200
9	75	39	64	>99	>200

[a] 0.05 M substrate in iPr_2O, with 0.5 equiv. of H_2O, at 60 °C, after 50 min. [b] According to GC (Experimental Section). [c] According to GC after double derivatisation (Experimental Section). [d] Already used one time. [e] Already used two times. [f] Already used three times.

Finally, we analysed the reusability of the enzyme: the hydrolysis of (±)-**2** was tested with Lipolase that had already been used in 1, 2 or 3 cycles (Table 3, entries 4–6). The catalytic activities of the tested Lipolase were progressively slightly lowered, though the enantioselectivity was apparently not affected.

The hydrolysis of (±)-**1** under the optimised conditions revealed excellent enantioselectivity (E > 200), whereas a drop in enantioselectivity (E = 51) occurred for the hydrolysis of (±)-**3** under these conditions. In order to increase E, the reactions were performed at lower temperatures, and we found E > 200 when the ring cleavage of (±)-**3** was performed at 30 °C.

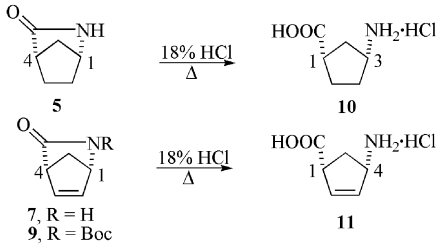
On the basis of the preliminary results, the gram-scale resolutions of (±)-**1**–(±)-**3** were performed with H_2O (0.5 equiv.) in the presence of Lipolase in iPr_2O at 30 °C for (±)-**3** and at 65 °C for (±)-**1** and (±)-**2**. The products were characterised by a very good enantiomeric excess at close to 50% conversion. The results are reported in Table 4 and the Experimental Section.

The transformations involving the hydrolysis of γ-lactams **5**, **7** and **9** with 18% aqueous HCl resulted in the enantiomers of γ-amino acid hydrochlorides **10** and **11** (Scheme 3). Adding 22% HCl/EtOH to amino acids **4**, **6** and **8**, followed by solvent evaporation resulted in enantiopure hydrochloride salts **4**·HCl and **6**·HCl. Physical data on the enantiomers prepared are reported in the Experimental Section.

Table 4. Lipolase-catalysed preparative-scale hydrolyses of (±)-**1**–(±)-**3**.[a]

				γ-Amino acid·(**4**, **6** and **8**)				γ-Lactam (**5**, **7** and **9**)			
	Reaction time [h]	Conv. [%]	E	Yield [%]	Isomer	ee[b] [%]	$[\alpha]_D^{25}$ (H_2O)	Yield [%]	Isomer	ee[c] [%]	$[\alpha]_D^{25}$ ($CHCl_3$)
(±)-**1**	91	50	>200	42	1*R*,3*S*	98	–10.6[d]	47	1*R*,4*S*	>99	+158[e]
(±)-**2**	4	50	>200	48	1*S*,4*R*	>99	–243[f]	46	1*S*,4*R*	>99	+549[g]
(±)-**3**	18	50	>200	44	1*S*,4*R*	96	–40.8[h]	44	1*S*,4*R*	97	+187[i]

[a] 30 mg mL^{-1} enzyme in iPr_2O, 0.5 equiv. of H_2O, 60 °C for (±)-**1** and (±)-**2**, and 30 °C for (±)-**3**. [b] Determined by GC after double derivatisation (Experimental Section). [c] According to GC (Experimental Section). [d] c = 0.35. [e] c = 0.45. [f] c = 0.34. [g] c = 0.26. [h] c = 0.25. [i] c = 0.28.



Scheme 3. Hydrolyses of (+)-**5**, (+)-**7** and (+)-**9**.

The absolute configurations were proved by comparing the [*α*] values with the literature data (Experimental Section), except in the case of **5**, when unsaturated γ-lactam enantiomer (1*S*,4*R*)-**7** was reduced catalytically in the presence of cyclohexene as a hydrogen donor to (1*R*,4*S*)-**5**. It should be mentioned that in all these reactions the same stereochemical demands are fulfilled; only the sequence of priority of the substituents on the substrates differs.

Conclusions

The present enzymatic procedure is equally applicable for the ring cleavage of *N*-Boc protected and unprotected γ-lactams, resulting in γ-amino acid and γ-lactam enantiomers (≥96%*ee*), blockbuster intermediates. The CAL-B-catalysed highly enantioselective reactions ($E > 200$) with added H_2O (0.5 equiv.) in iPr_2O at 30 or 60 °C afforded the products in good chemical yields (≥42%). Further advantages of this method are the facts that the amino group need not necessarily be protected and the products can be easily separated. The amino acid enantiomers produced [**4** and **6**, ≥98%*ee*, where (1*S*,4*R*)-4-aminocyclopent-2-ene-1-carboxylic acid (**6**) is used in the synthesis of Carbovir] precipitates from organic solvents, whereas they are soluble in H_2O, so that they can be easily washed off the surface of the enzyme: they can be extracted in H_2O. Unreacted γ-lactam enantiomers **5** and **7** (>99%*ee*) can be isolated from organic solvent and submitted to acidic hydrolysis to form the corresponding γ-amino acid enantiomers (**10** and **11**; ≥97%*ee*). The separation of the *N*-Boc protected γ-lactam and γ-amino acid (**8** and **9**; ≥96%*ee*) necessitates column chromatography. An advantage of this process is that instead of the lactamases, which are not readily available, it uses lipases, which are commercially available, stable, have high enantioselectivity and can be used on an industrial scale.[20]

Experimental Section

Materials and Methods: Lipolase (lipase B from *Candida antarctica*), produced by the submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin (Catalogue no. L4777), and porcine pancreatic lipase (PPL) were purchased from Sigma–Aldrich. CAL-A (lipase A from *Candida antarctica*) and Novozym 435 as an immobilised lipase (lipase B from *Candida antarctica*) on a macroporous acrylic resin were purchased from Novo Nordisk. Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Lipase PS (*Pseudomonas cepacia*) and lipase AK (*Pseudomonas fluorescens*) were purchased from Amano Pharmaceuticals, whereas lipase AY (*Candida rugosa*) was obtained from Fluka. Racemic 2-azabicyclo[2.2.1]heptan-3-one [(±)-**2**] was purchased from Acros and used after recrystallisation from iPr_2O. The solvents were of the highest analytical grade.

In a typical small-scale experiment, racemic γ-lactam (0.05 M solution) in an organic solvent (1 mL) was added to the lipase tested (10, 20, 30, 40, 50 or 75 mg mL^{-1}). H_2O (0, 0.5, 1, 2, 5 or 10 equiv.) was added. The mixture was shaken at 30, 45, 60, 70 or 80 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analysing them by gas chromatography. The *ee* values for the unreacted γ-lactam enantiomers were determined by gas chromatography on a Chromopack Chiralsil-Dex CB column (25 m) (120 °C for 25 min → 160 °C) {temperature rise 10 °C min^{-1}; 140 kPa; retention times [min], **5**: 29.22 (antipode: 28.82); **7**: 26.74 (antipode: 26.12); **9**: 27.79 (antipode: 28.04)}, whereas the *ee* values for the γ-amino acids produced were determined by using a gas chromatograph equipped with a chiral column after double derivatisation with (i) CH_2N_2[21] (*Caution! Derivatisation with diazomethane should be performed under a well-ventilated hood!*); (ii) Ac_2O in the presence of 4-dimethylaminopyridine and pyridine (Chromopack Chirasil-Dex CB column, 120 °C for 25 min → 160 °C) {temperature rise 10 °C min^{-1}; 140 kPa; retention times [min], **4**: 32.78 (antipode: 33.19); **6**: 31.02 (antipode: 31.65); **8**: 36.66 (antipode: 36.41)}.

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

2-Azabicyclo[2.2.1]heptan-3-one [(±)-1]: Palladium-on-carbon (1.2 g) was added to 2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**2**] (2 g, 18.34 mmol) dissolved in a mixture of MeOH (60 mL) and cyclohexene (16 mL). The mixture was heated at reflux for 3 h, and the catalyst was filtered off. After evaporation, white crystalline 2-azabicyclo[2.2.1]heptan-3-one [(±)-**1**] was recrystallised from iPr_2O [1.8 g, 89%; m.p. 69–74 °C]. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 1.38–1.94 (m, 6 H, 3 × CH_2), 2.74 (s, 1 H, C*H*CO), 3.89 (m, 1 H, C*H*NH), 5.88 (br. s, 1 H, N*H*) ppm. C_6H_9NO (111.14): calcd. C 64.84, H 8.16, N 12.60; found C 64.51, H 8.16, N 12.46.

***N*-(*tert*-Butoxycarbonyl)-2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-3]:** To a solution of 2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**2**] (1 g, 9.17 mmol) in dioxane (10 mL) was added H_2O (10 mL) and a 1 M solution of di-*tert*-butyl dicarbonate (1.74 g, 10.08 mmol) dissolved in 1,4-dioxane (10 mL) dropwise at 0 °C. The solution was stirred for 4 h at room temperature, after which the dioxane was evaporated off and the aqueous solution was acidified with a 10% solution of H_2SO_4 (pH = 2.5). The solution was extracted with EtOAc (3 × 15 mL), and the organic phase was dried (Na_2SO_4), filtered and the solvents evaporated to dryness. The resulting white crystalline *N*-(*tert*-butoxycarbonyl)-2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-**3**] was recrystallised from iPr_2O [1.4 g, 73%; m.p. 60–65 °C]. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 1.37–1.42 (overlapping of the s of 9 H, 3 × CH_3 and m of 2 H, CH_2), 2.04–2.06 (d, J = 8.6 Hz, 1 H, C*H*CO), 2.26–2.28 (d, J = 8.6 Hz, 1 H, C*H*NH), 6.72–6.97 (m, 2 H, C*HCH*) ppm. $C_{11}H_{15}NO_3$ (209.24): calcd. C 63.14, H 7.23, N 6.69; found C 63.32, H 7.18, N 6.77.

Gram-Scale Resolution of 2-Azabicyclo[2.2.1]heptan-3-one [(±)-1]: To a solution of racemic **1** (1.00 g, 9.00 mmol) dissolved in iPr_2O (60 mL) was added Lipolase (2 g, 30 mg mL^{-1}) and H_2O (81 μL, 4.5 mmol), and the mixture was shaken in an incubator shaker at

60 °C for 91 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated, and residue (1*R*,4*S*)-**5** crystallised out (470 mg, 47%); the solid was recrystallised from *i*Pr_2O {$[\alpha]_D^{25}$ = +158 (*c* = 0.45, $CHCl_3$); m.p. 78–81 °C; >99%*ee*}. The filtered off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off to yield crystalline γ-amino acid (1*R*,3*S*)-**4** (488 mg, 42%). $[\alpha]_D^{25}$ = –10.6 (*c* = 0.35, H_2O) {ref.[22] $[\alpha]_D^{21}$ for (1*S*,3*R*) amino acid = +7.0 (*c* = 2, H_2O); m.p. >260 °C with decomposition (recrystallised from H_2O and Me_2CO), 98%*ee*}.

When 22% HCl/EtOH (4 mL) was added to **4** (200 mg) and the solvent was evaporated off (1*R*,3*S*)-**4**·HCl (225 mg, 88%) was obtained. $[\alpha]_D^{25}$ = –10.8 (*c* = 0.6, H_2O). M.p. 177–180 °C (EtOH/Et_2O). 99%*ee*.

5: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): Data were similar to those for (±)-**1**. C_6H_9NO (111.14): calcd. C 64.84, H 8.16, N 12.60; found C 64.66, H 8.19, N 12.51.

4: ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): δ = 1.79–2.26 (m, 6 H, 3 × C*H*$_2$), 2.79–2.84 (m, 1 H, C*H*COOH), 4.85 (m, 1 H, C*H*NH_2) ppm. $C_6H_{11}NO_2$ (129.16): calcd. C 55.80, H 8.58, N 10.84; found C 55.61, H 8.60, N 10.99.

4·HCl: ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): δ = 1.77–2.40 (m, 6 H, 3 × C*H*$_2$), 2.97–3.01 (m, 1 H, C*H*COOH), 3.72–3.75 (m, 1 H, C*H*NH_2) ppm. $C_6H_{11}NO_2$·HCl (165.62): calcd. C 43.51, H 7.30, N 8.46; found C 43.59, H 7.40, N 8.65.

Gram-Scale Resolution of 2-Azabicyclo[2.2.1]hept-5-en-3-one [(±)-2]: The procedure described above was used. The reaction of racemic **2** (1.00 g, 9.16 mmol) and H_2O (82 μL, 4.58 mmol) in *i*Pr_2O (45 mL) in the presence of Lipolase (1.5 g, 30 mg mL^{-1}) at 60 °C afforded unreacted (1*S*,4*R*)-**7** {461 mg, 46%; $[\alpha]_D^{25}$ = +549 (*c* = 0.26, $CHCl_3$), ref.[23] $[\alpha]_D^{25}$ for (1*R*,4*S*) lactam = –513 (*c* = 0.52, $CHCl_3$); m.p. 97–100 °C (*i*Pr_2O) ref.[23] m.p. 89–90 °C; >99%*ee*} and γ-amino acid (1*S*,4*R*)-**6** {559 mg, 48%; $[\alpha]_D^{25}$ = –243 (*c* = 0.34, H_2O); m.p. >260 °C (decomp.; H_2O/Me_2CO); >99%*ee*} in 4 h.

When 22% HCl/EtOH (5 mL) was added to **6** (200 mg) and the solvent was evaporated off (1*S*,4*R*)-**6**·HCl (223 mg, 87%) was obtained. $[\alpha]_D^{25}$ = –110.9 (*c* = 0.55, H_2O); m.p. 205–208 °C (EtOH/Et_2O); >99%*ee*.

7: ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, TMS): δ = 2.23–2.42 (m, 2 H, C*H*$_2$), 3.23 (s, 1 H, C*H*CO), 4.34 (m, 1 H, C*H*NH), 5.54 (br. s, 1 H, N*H*), 6.67–6.80 (m, 2 H, C*HCH*) ppm. C_6H_7NO (109.13): calcd. C 66.04, H 6.47, N 12.84; found C 66.30, H 6.51, N 12.75.

6: ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): δ = 2.00–2.54 (m, 2 H, C*H*$_2$), 3.50–3.52 (m, 1 H, C*H*COOH), 4.33–4.35 (m, 1 H, C*H*NH_2), 5.94–6.28 (m, 2 H, C*HCH*) ppm. $C_6H_9NO_2$ (127.14): calcd. C 56.68, H 7.13, N 11.02; found C 56.89, H 7.10, N 10.92.

6·HCl: ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): δ = 2.05–2.71 (m, 2 H, C*H*$_2$), 3.73 (m, 1 H, C*H*COOH), 4.40 (m, 1 H, C*H*NH_2), 5.97–6.24 (m, 2 H, C*HCH*) ppm. $C_6H_9NO_2$·HCl (163.60): calcd. C 44.05, H 6.16, N 8.56; found C 44.28, H 5.90, N 8.56.

Gram-Scale Resolution of *N*-(*tert*-Butoxycarbonyl)-2-azabicyclo[2.2.1]hept-5-en-3-one [(±)-3]: The procedure described above was used. The reaction of racemic **3** (500 mg, 2.39 mmol) and H_2O (21 μL, 1.18 mmol) in *i*Pr_2O (30 mL) in the presence of Lipolase (0.9 g, 30 mg mL^{-1}) at 30 °C afforded unreacted (1*S*,4*R*)-**9** {223 mg, 44%; $[\alpha]_D^{25}$ = +187 (*c* = 0.28, $CHCl_3$), ref.[24] $[\alpha]_D^{25}$ for (1*R*,4*S*) lactam = –189 (*c* = 0.89, CH_2Cl_2); m.p. 77–80 °C (*i*Pr_2O); 96%*ee*} and γ-amino acid (1*S*,4*R*)-**8** {238 mg, 44%; $[\alpha]_D^{25}$ = –40.8 (*c* = 0.25, H_2O), ref.[24] $[\alpha]_D^{25}$ for (1*S*,4*R*) amino acid = –40.3 (*c* = 2.1, CH_2Cl_2); m.p. 130–132 °C (H_2O/Me_2CO); 96%*ee*} in 18 h. Column chromatographic separation of the above products was performed on silica [Silica gel 60 (0.063–0.200 mm), purchased from Merck KGaA], with elution with ethyl acetate/*n*-hexane (1:3).

When 22% HCl/EtOH (4 mL) was added to **8** (100 mg) and the solvent was evaporated off (1*S*,4*R*)-**6**·HCl (57 mg, 79%) was formed. $[\alpha]_D^{25}$ = –101.6 (*c* = 0.4, H_2O); m.p. 201–204 °C (EtOH/Et_2O); 95%*ee*.

9: ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): Data were similar to those for (±)-**3**. $C_{11}H_{15}NO_3$ (209.24): calcd. C 63.14, H 7.23, N 6.69; found C 62.90, H 7.28, N 6.67.

8: ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 1.15–1.19 (m, 2 H, C*H*$_2$), 1.37 (s, 9 H, 3 × C*H*$_3$), 1.67–1.72 (m, 1 H, C*H*COOH), 2.37–2.42 (m, 1 H, C*H*NH), 4. 48 (br. s, 1 H, COO*H*), 5.70–5.82 (m, 2 H, C*HCH*), 6.85 (br. s, 1 H, N*H*) ppm. $C_{11}H_{17}NO_4$ (227.26): calcd. C 58.14, H 7.54, N 6.16; found C 57.91, H 7.60, N 6.06.

6·HCl: ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): Data for **6**·HCl obtained from **8** were similar to those for **6**·HCl obtained from **6**. $C_6H_9NO_2$·HCl (163.60): calcd. C 44.05, H 6.16, N 8.56; found C 44.04, H 6.00, N 8.48.

Hydrolyses of (+)-5, (+)-7 and (+)-9: Enantiomeric **5** (200 mg, 1.79 mmol), **7** (200 mg, 1.83 mmol) or **9** (100 mg, 0.47 mmol) was dissolved in 18% HCl (10 mL) and heated at reflux for 2 h. The solvent was evaporated off, and the product was recrystallised from EtOH and Et_2O, which afforded white crystals of **10** {241 mg, 81%; m.p. 175–177 °C; $[\alpha]_D^{25}$ = +10.7 (*c* = 0.5, H_2O); 97%*ee*} or **11** {from **7**: 268 mg, 90%; m.p. 208–209 °C; $[\alpha]_D^{25}$ = +111.1 (*c* = 0.35, H_2O); >99%*ee*; from **9**: 64 mg, 82%; m.p. 202–205 °C; $[\alpha]_D^{25}$ = +100.7 (*c* = 0.3, H_2O); 95%*ee*}.

(1*S*,3*R*)-10: ^{1}H NMR (400 MHz, D_2O, 25 °C, TMS): Data were similar to those for (1*R*,3*S*)-**4**·HCl. $C_6H_{11}NO_2$·HCl (165.62): calcd. C 43.51, H 7.30, N 8.46; found C 43.50, H 7.17, N 8.21.

(1*R*,4*S*)-11 (obtained from 7 and 9): Data were similar to those for (1*S*,4*R*)-**6**·HCl. $C_6H_9NO_2$·HCl (163.60): calcd. C 44.05, H 6.16, N 8.56; found (from **7**) C 44.08, H 6.16, N 8.45; found (from **9**) C 43.86, H 6.01, N 8.52.

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