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Advanced procedure for the enzymatic ring opening of unsaturated alicyclic β-lactams

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Abstract—Enantiopure β-amino acids **1a**–**4a** and β-lactams **1b**–**4b** were prepared simultaneously through the lipolase-catalysed enantioselective ring opening of unsaturated racemic β-lactams (±)-**1**-(±)-**4**. High enantioselectivities (E >200) were observed when the reactions were performed with 1 equiv of water in iPr$_2$O at 70 °C. The resolved (1*R*,2*S*)-amino acids (yield ⩾45%) and (1*S*,5*R*)-, (1*S*,6*R*)- and (1*S*,8*R*)-lactams (yield ⩾47%) could be easily separated. The ring opening of lactam enantiomers **1b**–**4b** with 18% HCl afforded the corresponding β-amino acid hydrochlorides **1c**·HCl–**4c**·HCl (ee >95%).
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1. Introduction

Interest in cyclic β-amino acids and β-lactams has greatly increased over the past few years and has become a hot topic in synthetic and medicinal chemistry.[1–4] Some cyclic β-amino acids themselves exhibit biological activity (e.g., cispentacin, with antibacterial activity;[5,6] or PLD-118, with antifungal activity, which is currently under clinical investigation).[7] Such compounds can be used as precursors of β-lactams;[8,9] as building blocks for the synthesis of modified peptides with increased activity and stability[10,11] or in drug research.[12–14] Cyclic β-lactams are also important as intermediates in the synthesis of β-amino acids,[15] short peptide segments,[16] taxoid antitumour agents,[17] alkaloids[18] and heterocycles of biological and medicinal importance.[19]

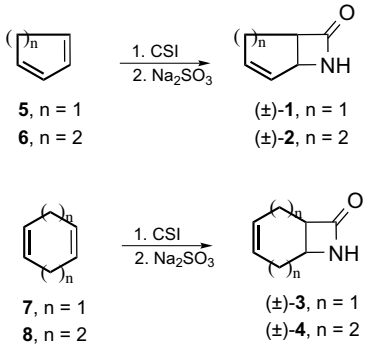
A number of enzymatic syntheses of β-amino acids or their derivatives and β-lactams in enantiomerically pure form have been elaborated over the past few years.[20–28] We have recently developed a direct enzymatic method for the preparation of enantiopure valuable β-amino acids (e.g., cispentacin) through the lipase-catalysed enantioselective hydrolysis of β-lactams in an organic solvent.[29,30]

Herein, we report an easy and efficient lipase-catalysed enantioselective ring opening of racemic 6-azabicyclo[3.2.0]hept-3-en-7-one, (±)-**1**, 7-azabicyclo[4.2.0]oct-4-en-8-one (±)-**2**, 7-azabicyclo[4.2.0]oct-3-en-8-one, (±)-**3** and 9-azabicyclo[6.2.0]dec-4-en-10-one, (±)-**4**, in an organic solvent.

2. Results and discussion

2.1. Syntheses of (±)-1–(±)-4

The racemic β-lactams **1**–**4** were prepared by the cycloaddition of chlorosulfonyl isocyanate (O=C=N–SO_2Cl; CSI) to the corresponding cycloalkadiene **5**–**8** (Scheme 1).[1,31–34] The 1,2-dipolar cycloaddition of CSI to 1,2-cyclopentadiene **5** and 1,3-cyclohexadiene **6** takes place regioselectively, in accordance with the Markovnikov orientation,[1,31,32] resulting in β-lactams (±)-**1** and (±)-**2**.



Scheme 1.

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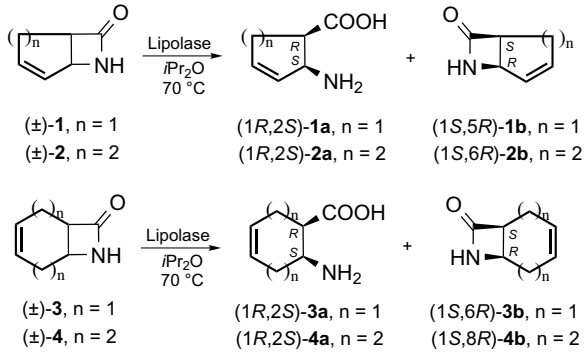
2.2. Lipase-catalysed enantioselective ring opening of (±)-1–(±)-4

We previously resolved model compounds (±)-**2** and (±)-**3** through an indirect enzymatic method: lipase PS (lipase from *Pseudomonas cepacia*)-catalysed enantioselective acylation of the *N*-hydroxymethylated derivatives in acetone at room temperature (E >200, $\text{yield}_{\text{alcohol}} \geqslant 32\%$, $\text{yield}_{\text{ester}} \geqslant 34\%$),[21] and through a direct enzymatic method: Novozym 435 (lipase B from *Candida antarctica*, immobilised on a macroporous acrylic resin)-catalysed enantioselective ring opening alcoholysis in diisopropyl ether at 60 °C (E >200, $\text{yield}_{\beta\text{-lactam}} \geqslant 39\%$, $\text{yield}_{\beta\text{-amino acid}} \leqslant 11\%$).[25] We also recently reported a simple and efficient direct method for the enantioselective ring opening of unactivated β-lactams in an organic medium: lipolase (lipase B from *C. antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin) proved to be applicable for the enantioselective ring opening of 6-azabicyclo[3.2.0]heptan-7-one and the homologues,[29,30] when the reactions were performed with H_2O (1 equiv) in diisopropyl ether at 60 and 70 °C, respectively (E >200, $\text{yield}_{\beta\text{-lactam}} \geqslant 36\%$, $\text{yield}_{\beta\text{-amino acid}} \geqslant 43\%$). These results[29,30] on the lipase-catalysed enantioselective hydrolysis of β-lactams suggested the possibility of the enantioselective ring opening of (±)-**1**–(±)-**4** with H_2O (1 equiv) in diisopropyl ether at high temperature (Scheme 2).

We started the ring-opening experiments on (±)-**2** with lipolase at 60 °C, but also tested the reactions at 25, 40 and 70 °C. In all cases, the enantioselectivities were high (E >200) while the reaction rates gave the following temperature dependence: after 16 h, the conversion was 10% at 25 °C, 30% at 40 °C and 49% at 60 °C, while it was 50% at 70 °C after 5 h. In spite of the high temperature (70 °C), the enzyme did not lose any significant activity. Also it could be reused successfully for a second ring-opening reaction of (±)-**2** (conversion 48% after 5 h, at 70 °C).



Scheme 2.

Even though the E value in the lipolase-catalysed ring opening of (±)-**2** with H_2O (0 or 1 equiv) at 70 °C is excellent (E >200), in order to enhance the reaction rate (conversion 43–46% after 4 h), several nucleophiles (1 equiv of 2-octanol, Et_3N and *N*,*N*-diisopropylethylamine) were also tested for the ring opening of (±)-**2**. Since no significant changes in enantioselectivity (E >200) or reaction rate (conversion 45–46% after 4 h) were observed, we concluded that the water present in the reaction medium (<0.1%) or in the enzyme preparation (<5%) was responsible for the lactam ring opening.

On the basis of the preliminary results, the gram-scale resolutions of (±)-**1**–(±)-**4** were performed with 1 equiv of water in the presence of lipolase in diisopropyl ether at 70 °C. The products were characterised by excellent enantiomeric excesses at 50–51% conversion. The results are reported in Table 1.

Evans et al.[27] investigated the enzymatic ring-opening reactions of (±)-6-azabicyclo[3.2.0]hept-3-en-7-one (±)-**1** and found that ENZA-1 (*Rhodococcus equi NCIB 40213*), catalysed the enantioselective ring opening of this compound in water. After two consecutive incubations of the lactam with *Rhodococcus equi*, the (1*R*,5*S*)-

Table 1. Lipolase-catalysed ring opening of (±)-**1**–(±)-**4**

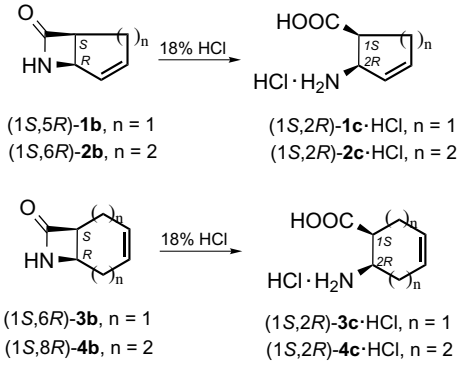
	Time (h)	Conv. (%)	E	β-Lactam **1b**–**4b**				β-Amino acid **1a**–**4a**			
				Yield[a] (%)	Isomer	Ee[b] (%)	$[\alpha]_D^{25}$	Yield[a] (%)	Isomer	Ee[c] (%)	$[\alpha]_D^{25}$
(±)-**1**	5	51	>200	48	1*S*,5*R*	99	−34.8[d]	45	1*R*,2*S*	96	+96.7[e]
(±)-**2**	5	50	>200	48	1*S*,6*R*	99	+161.1[d,f]	46	1*R*,2*S*	98	+121.1[g,h]
(±)-**3**	4.5	50	>200	48	1*S*,6*R*	99	−29.1[d,i]	45	1*R*,2*S*	99	−38.8[g,j]
(±)-**4**	7	51	>200	47	1*S*,8*R*	99	−24.9[k]	46	1*R*,2*S*	95	+23.9[g]

[a] Yield 100% at 50% conversion.
[b] According to GC.
[c] Determined by GC [after double derivatization (i) diazomethane; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine].
[d] *c* 0.45, $CHCl_3$.
[e] *c* 0.3, H_2O.
[f] $[\alpha]_D^{25} = +164$ (*c* 0.13, $CHCl_3$) for (1*S*,6*R*)-**2b**.[21]
[g] *c* 0.5, H_2O.
[h] $[\alpha]_D^{25} = +120$ (*c* 0.25, H_2O) for (1*R*,2*S*)-**2a**.[21]
[i] $[\alpha]_D^{25} = -26.3$ (*c* 0.5, $CHCl_3$) for (1*S*,6*R*)-**3b**.[21]
[j] $[\alpha]_D^{25} = -36.2$ (*c* 0.5, H_2O) for (1*R*,2*S*)-**3a**.[21]
[k] *c* 0.3, $CHCl_3$; $[\alpha]_D^{25} = -19$ (*c* 0.3, MeOH) for (1*S*,8*R*)-**4b**. $[\alpha]_D^{25} = -20.2$ (*c* 0.35, MeOH) for (1*S*,8*R*)-**4b**.[23]

lactam enantiomer was isolated with high ee. Lloyd et al.[28] reported the resolution of 7-azabicyclo[4.2.0]oct-4-en-8-one (±)-**2** using a lactamase (NCIMB 41042) in phosphate buffer at pH 7. Although they specified the formation of β-amino acid in the ring-opening reaction, they isolated and characterised only the (1*R*,6*S*)-β-lactam. Our method not only provides the β-lactams **1b**–**4b** with high ees (⩾99%) in high yields (⩾46%), but also the β-amino acids **1a**–**4a** with high ees (⩾95%) in high yields (⩾45%). It is important to stress that the lipolase-catalysed ring opening of (±)-**1** and (±)-**2** in organic solvent favours the opposite enantiomer than that in the case of NCIMB 41042 or NCIMB 40213 lactamase under aqueous conditions.

2.3. Transformations of the enantiomers

The transformations involving the ring opening of β-lactams **1b**–**4b** with 18% aqueous HCl resulted in the enantiomers of the β-amino acid hydrochlorides **1c**·HCl–**4c**·HCl (Scheme 3). Treatment of amino acids **1a**–**4a** with 18% aqueous HCl resulted in enantiopure hydrochlorides **1a**·HCl–**4a**·HCl. The physical data on the enantiomers prepared are reported in Table 2.



Scheme 3.

The absolute configurations were proven by comparing the $[\alpha]_D$ values with the literature data[21,23,27] (Table 1). The absolute configuration for **1a**–**4a** was (1*R*,2*S*), for **1b** it was (1*S*,5*R*), for **2b** and **3b** it was (1*S*,6*R*), while that for **4b** was (1*S*,8*R*).

3. Conclusions

An efficient and simple direct enzymatic method was developed for the enantioselective ring opening of racemic 6-azabicyclo[3.2.0]hept-3-en-7-one (±)-**1**, 7-azabicyclo[4.2.0]oct-4-en-8-one (±)-**2**, 7-azabicyclo[4.2.0]-oct-3-en-8-one (±)-**3** and 9-azabicyclo[6.2.0]dec-4-en-10-one (±)-**4** with 1 equiv of H_2O in iPr_2O at 70 °C (E >200). The enantioselective ring-opening reactions of unsaturated **1**–**4** reached 50% conversion in a much shorter time (4.5–7 h) than that for their saturated analogues (141–249 h).[29] The lipolase-catalysed ring opening of (±)-**1** and (±)-**2** in organic solvent favours the opposite enantiomer than that in the case of lactamase present in the whole cells of *Rhodococcus globerulus* (NCIMB 41042) or *R. equi* in phosphate buffer at pH 7. The β-lactam (yield = 48%) and β-amino acid (yield ⩾45%) products can be easily separated. Ring opening of the β-lactams **1b**–**4b** with 18% aqueous HCl resulted in the enantiomers of the β-amino acid hydrochlorides **1c**·HCl–**4c**·HCl (ee >95%). All the produced unsaturated enantiomers can be used for further valuable transformations (i.e., they can be used to prepare information-rich chiral scaffolds for elaboration into single enantiomer compounds libraries as the alkene functionality is amenable to a range of transformations).[35]

Table 2. Physical data on enantiomers prepared

Amino acid hydrochloride	Ee (%)	$[\alpha]_D^{25}$
1*R*,2*S*-**1a**·HCl	99	+81.6 (*c* 0.3, H_2O)
1*S*,2*R*-**1c**·HCl	98	−80.2 (*c* 0.3, H_2O)
1*R*,2*S*-**2a**·HCl	99	+121.7 (*c* 0.4, H_2O)
1*S*,2*R*-**2c**·HCl	99	−121.4 (*c* 0.4, H_2O)
1*R*,2*S*-**3a**·HCl	99	−26 (*c* 0.25, H_2O)
1*S*,2*R*-**3c**·HCl	99	+25.8 (*c* 0.4, H_2O)
1*R*,2*S*-**4a**·HCl	95	+14.2 (*c* 0.35, H_2O)
1*S*,2*R*-**4c**·HCl	98	−15.9 (*c* 0.3, H_2O)

4. Experimental

4.1. Materials and methods

Lipolase (lipase B from *C. antarctica*), produced by submerged fermentation of a genetically modified *A. oryzae* microorganism and adsorbed on a macroporous resin, was from Sigma-Aldrich (Catalog no L4777). Chlorosulfonyl isocyanate, and 1,3- and 1,4-cyclohexadiene were purchased from Aldrich. The solvents were of the highest analytical grade.

In a typical small-scale experiment, racemic 2-azetidinone (0.05 M solution) in diisopropyl ether (2 mL) was added to lipolase (50 mg/mL). Water (1 equiv) was added and the mixture was shaken at 70 °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 β-lactam enantiomers were determined by gas chromatography on a Chromopak Chiralsil-Dex CB column (25 m) [140 °C for 7 min → 190 °C (rate of temperature rise 20 °C/min; 100 kPa), retention times (min): **1b**: 6.17 (antipode: 5.64); **2b**: 9.49 (antipode: 9.26); **3b**: 10.04 (antipode: 9.68); **4b**: 11.95 (antipode: 11.85)], while the ee values for the ring-opened amino acids produced (during the preliminary experiments) were calculated by using *n*-hexadecane as an internal standard. The ee values for the isolated β-amino acid enantiomers were determined by using a gas chromatograph equipped with a chiral column after double derivatization with (i) diazomethane; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (Chirasil-L-Val

column, 100 °C for 10 min → 160 °C, rate of temperature rise 10 °C/min; 30 kPa), retention times (min): **1a**: 18.2 (antipode: 18.38); (Chirasil-L-Val column, 120 °C for 4 min → 190 °C, rate of temperature rise 20 °C/min; 140 kPa), retention times (min): **2a**: 6.2 (antipode: 6.3); **3a**: 5.84 (antipode: 5.94); (CP-Chirasil-Dex CB column, 120 °C for 7 min → 160 °C, rate of temperature rise 10 °C/min; 30 kPa), retention times (min): **4a**: 36.92 (antipode: 37.45).

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 on a Kofler apparatus.

4.2. Gram-scale resolution of 7-azabicyclo[4.2.0]oct-3-en-8-one, (±)-1

Crystalline racemic **1** (1 g, 9.16 mmol) was dissolved in diisopropyl ether (40 mL). Lipolase (2 g, 50 mg/mL) and water (0.16 mL, 9.16 mmol) were added and the mixture shaken in an incubator shaker at 70 °C for 5 h. The reaction was stopped by filtering off the enzyme at 50% conversion (ee-**1b** = 99%). The solvent was evaporated off and the residue (1*S*,5*R*)-**1b** crystallized out {0.4 g, 48%; recrystallized from diisopropyl ether, $[\alpha]_D^{25} = -34.8$ (*c* 0.45, $CHCl_3$); mp 76–77 °C, lit.[27b] mp 76–77 °C; ee 99%}. The filtered-off enzyme was washed with distilled water (3 × 15 mL) and the water evaporated off, yielding the crystalline β-amino acid (1*R*,2*S*)-**1a** {0.52 g, 45%; $[\alpha]_D^{25} = +96.7$ (*c* 0.3, H_2O); mp >240 °C with sublimation (recrystallized from methanol); ee = 96%}. When **1a** (0.1 g) was treated with 18% aqueous HCl (3 mL), (1*R*,2*S*)-**1a**·HCl was obtained {0.11 g, 85%; $[\alpha]_D^{25} = +81.6$ (*c* 0.3, H_2O); mp 178–183 °C, ee = 99%}.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **1a**: 2.54–2.56 (1H, m, H-5_A) 2.67–2.74 (1H, m, H-5_B) 3.27 (1H, q, J = 8.4 Hz, H-1) 4.28–4.29 (1H, m, H-2) 5.80–5.81 (1H, m, H-3) 6.23–6.24 (1H, m, H-4). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 35.3, 46.0, 56.5, 126.7, 139.6, 179.8. Analysis: calculated for $C_6H_9NO_2$: C, 56.68; H, 7.13; N, 11.02; found: C, 56.51; H, 6.88; N, 10.91.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **1a**·HCl: 2.76–2.80 (2H, m, H-5_A and H-5_B) 3.51–3.58 (1H, m, H-1) 4.46–4.48 (1H, m, H-2) 5.83–5.85 (1H, m, H-3) 6.28–6.29 (1H, m, H-4). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 34.57, 43.91, 56.25, 126.21, 139.05, 175.9. Analysis: calculated for $C_6H_9NO_2$·HCl: C, 44.05; H, 6.16; N, 8.56; found: C, 43.79; H, 6.26; N, 8.55.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **1b**: 2.42–2.49 (1H, m, H-2_A) 2.69–2.76 (1H, m, H-2_B) 3.82–3.84 (1H, m, H-1) 4.50–4.51 (1H, m, H-5) 5.93–5.95 (1H, m, H-4) 6.01–6.03 (1H, m, H-3) 6.48 (1H, br s, NH). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm) 31.5, 54.0, 59.9, 131.3, 137.7, 173.0. Analysis: calculated for C_6H_7NO: C, 66.04; H, 6.47; N, 12.84; found: C, 66.12; H, 6.42; N, 12.98.

4.3. Gram-scale resolution of 7-azabicyclo[4.2.0]oct-4-en-8-one, (±)-2

Following the procedure described above, the reaction of racemic **2** (2 g, 16.26 mmol) and water (0.29 mL, 16.26 mmol) in diisopropyl ether (80 mL) in the presence of lipolase (4 g, 50 mg/mL) at 70 °C afforded the unreacted (1*S*,6*R*)-**2b** {0.96 g, 48%; $[\alpha]_D^{25} = +161.1$ (*c* 0.45, $CHCl_3$); mp 113–114 °C (recrystallized from diisopropyl ether), lit.[21] mp 106–108 °C; ee = 99%} and β-amino acid (1*R*,2*S*)-**2a** {1.02 g, 46%; $[\alpha]_D^{25} = +121.1$ (*c* 0.5, H_2O); mp 233–236 °C (recrystallized from water–acetone) lit.[25] mp 236–238 °C; ee = 98%} in 5 h. When **2a** (0.2 g) was treated with 18% aqueous HCl (3 mL), (1*R*,2*S*)-**2a**·HCl was obtained {0.24 g, 96%; $[\alpha]_D^{25} = +121.7$ (*c* 0.4, H_2O); mp 190–212 °C (slow melting), ee = 99%}.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **2a**: 1.84–2.18 (4H, m, 2 × CH_2) 2.72–2.77 (1H, m, H-1) 3.98–3.99 (1H, m, H-2) 5.73–6.14 (2H, m, C*HCH*). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 22.1, 24.4, 42.4, 47.1, 122.0, 135.2, 181.2. Analysis: calculated for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92; found: C, 59.47; H, 7.88; N, 9.82.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **2a**·HCl: 1.90–2.20 (4H, m, 2 × CH_2) 3.04–3.08 (1H, m, H-1) 4.09 (1H, m, H-2) 5.73–6.16 (2H, m, C*HCH*). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 20.9, 23.9, 40.8, 46.6, 121.3, 135.4, 179.5. Analysis: calculated for $C_7H_{11}NO_2$·HCl: C, 47.33; H, 6.81; N, 7.89; found: C, 47.47; H, 6.79; N, 7.88.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **2b**: 1.63–2.12 (4H, m, 2 × CH_2) 3.51 (1H, m, H-1) 4.02–4.04 (1H, m, H-6) 5.93–6.14 (2H, m, C*HCH*) 5.95 (1H, br s, NH). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm) 22.4, 22.5, 45.0, 50.5, 126.6, 135.2, 172.7. Analysis: calculated for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37; found: C, 68.13; H, 7.32; N, 11.48.

4.4. Gram-scale resolution of 7-azabicyclo[4.2.0]oct-3-en-8-one, (±)-3

Following the procedure described above, the reaction of racemic **3** (2 g, 16.26 mmol) and water (0.29 mL, 16.26 mmol) in diisopropyl ether (80 mL) in the presence of lipolase (4 g, 50 mg/mL) at 70 °C afforded the unreacted (1*S*,6*R*)-**3b** (0.96 g, 48%; $[\alpha]_D^{25} = -29.1$ (*c* 0.45, $CHCl_3$); mp 152–154 °C (recrystallized from diisopropyl ether), lit.[21] mp 152–153 °C; ee = 99%) and β-amino acid (1*R*,2*S*)-**3a** {1 g, 45%; $[\alpha]_D^{25} = -38.8$ (*c* 0.5, H_2O); mp 232–234 °C (recrystallized from water–acetone) lit.[25] mp 233–235 °C; ee = 99%} in 4.5 h. When **3a** (0.2 g) was treated with 18% aqueous HCl (3 mL), (1*R*,2*S*)-**3a**·HCl was obtained {0.22 g, 88%; $[\alpha]_D^{25} = -26$ (*c* 0.25, H_2O); mp 191–220 (slow melting), ee = 99%}.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **3a**: 2.23–2.51 (4H, m, 2 × CH_2) 2.74–2.78 (1H, m, H-1) 3.77–3.80 (1H, m, H-2) 5.64–5.84 (2H, m, C*HCH*). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 25.2, 27.8, 41.3, 47.5, 122.6,

126.7, 181.3. Analysis: calculated for $C_7H_{11}NO_2$: C, 59.56; H, 7.85; N, 9.92; found: C, 59.52; H, 8.07; N, 9.92.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **3a**·HCl: 2.27–2.55 (4H, m, 2×CH_2) 3.07–3.11 (1H, m, H-1) 3.90–3.91 (1H, m, H-2) 5.66–5.83 (2H, m, C*HCH*). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 24.5, 27.9, 39.8, 46.9, 122.8, 125.9, 176.8. Analysis: calculated for $C_7H_{11}NO_2$·HCl: C, 47.33; H, 6.81; N, 7.89; found: C, 47.59; H, 6.82; N, 7.93.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **3b**: 1.59–2.11 (4H, m, 2×CH_2) 3.49–3.51 (1H, m, H-1) 4.02–4.04 (1H, m, H-6) 5.94–6.17 (2H, m, C*HCH*) 6.01 (1H, br s, NH). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm) 22.0, 27.8, 47.7, 48.8, 124.8, 126.8, 171.2. Analysis: calculated for C_7H_9NO: C, 68.27; H, 7.37; N, 11.37; found: C, 68.22; H, 7.32; N, 11.40.

4.5. Gram-scale resolution of 9-azabicyclo[6.2.0]dec-4-en-10-one, (±)-4

Following the procedure described above, the reaction of racemic **4** (1 g, 6.61 mmol) and water (0.12 mL, 6.61 mmol) in diisopropyl ether (40 mL) in the presence of lipolase (2 g, 50 mg/mL) at 70 °C afforded the unreacted (1*S*,8*R*)-**4b** (0.47 g, 47%; $[\alpha]_D^{25} = -24.9$ (*c* 0.4, $CHCl_3$); $[\alpha]_D^{25} = -19.9$ (*c* 0.3, MeOH); mp 136–140 °C (recrystallized from diisopropyl ether), lit.[23] mp 117–119 °C; ee = 99%) and β-amino acid (1*R*,2*S*)-**4a** {0.51 g, 46%; $[\alpha]_D^{25} = +23.9$ (*c* 0.3, H_2O); mp 218–220 °C (recrystallized from water–acetone); ee = 95%} in 7 h. When **4a** (0.1 g) was treated with 18% aqueous HCl (3 mL), (1*R*,2*S*)-**4a**·HCl was obtained {0.1 g, 82%; $[\alpha]_D^{25} = +14.2$ (*c* 0.35, H_2O); mp 194–205 °C, ee = 95%}.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **4a**: 1.81–2.58 (8H, m, 4×CH_2) 2.79 (1H, m, H-1) 3.70–3.73 (1H, m, H-2) 5.72–5.73 (2H, m, C*HCH*). ^{13}C NMR (100.62 MHz, D_2O) (ppm) 22.9, 24.7, 27.2, 29.8, 45.9, 52.5, 129.6, 129.9, 181.8. Analysis: calculated for $C_9H_{15}NO_2$: C, 63.88; H, 8.93; N, 8.28; found: C, 63.99; H, 8.81; N, 8.08.

^{1}H NMR (400 MHz, D_2O) δ (ppm) for **4a**·HCl: 1.65–2.35 (8H, m, 4×CH_2) 2.94 (1H, m, H-1) 3.67 (1H, m, H-2) 5.55–5.62 (2H, m, C*HCCH*). ^{13}C NMR (100.62 MHz, D_2O) δ (ppm) 23.0, 23.7, 26.9, 30.2, 44.6, 51.8, 129.6, 130.9, 177.0. Analysis: calculated for $C_9H_{15}NO_2$·HCl: C, 52.56; H, 7.84; N, 6.81; found: C, 52.32; H, 7.99; N, 6.79.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) for **4b**: 1.83–2.02 and 2.30–2.37 (8H, m, 4×CH_2) 3.21–3.24 (1H, m, H-1) 3.74–3.78 (1H, m, H-8) 5.58–5.62 (2H, m, C*HCH*) 6.16 (1H, br s, NH). ^{13}C NMR (100.62 MHz, $CDCl_3$) δ (ppm) 23.3, 24.4, 24.9, 31.3, 54.0, 54.6, 130.9, 131.6, 172.2. Analysis: calculated for $C_9H_{13}NO$: C, 71.49; H, 8.67; N, 9.26; found: C, 71.62; H, 8.67; N, 9.23.

4.6. Ring opening of β-lactam enantiomers 1b–4b with aqueous HCl

(1*S*,5*R*)-**1b** (0.1 g, 0.92 mmol), (1*S*,6*R*)-**2b** (0.1 g, 1.63 mmol), (1*S*,6*R*)-**3b** (0.1 g, 1.63 mmol) or (1*S*,8*R*)-**4b** (0.1 g, 0.67 mmol) was dissolved in 18% HCl (7 mL) and the solution refluxed for 3 h. The solvent was then evaporated off and the product recrystallized from ethanol–diethyl ether, which afforded white crystals of (1*S*,2*R*)-**1c**·HCl {0.13 g, 87%, $[\alpha]_D^{25} = +81.6$ (*c* 0.3, H_2O); (168–171 °C); ee 99%}, (1*S*,2*R*)-**2c**·HCl {0.13 g, 90%; $[\alpha]_D^{25} = -121.4$ (*c* 0.4, H_2O); slow melting (192–209 °C); ee 99%}, (1*S*,2*R*)-**3c**·HCl {0.11 g, 76%, $[\alpha]_D^{25} = +25.8$ (*c* 0.4, H_2O); slow melting (180–224 °C); ee 99%}, and (1*S*,2*R*)-**4c**·HCl {0.1 g, 73%; $[\alpha]_D^{25} = -15.9$ (*c* 0.3, H_2O); (a slowly crystallizing oil); ee 98%}. The ^{1}H NMR (400 MHz, D_2O) δ (ppm) data for (1*S*,2*R*)-**1c**·HCl, (1*S*,2*R*)-**2c**·HCl, (1*S*,2*R*)-**3c**·HCl and (1*S*,2*R*)-**4c**·HCl are similar to those for (1*R*,2*S*)-**1a**·HCl, (1*R*,2*S*)-**2a**·HCl, (1*R*,2*S*)-**3a**·HCl and (1*R*,2*S*)-**4a**·HCl. Anal. found for (1*S*,2*R*)-**1c**·HCl: C, 44.11; H, 6.11; N, 8.27. Anal. found for (1*S*,2*R*)-**2c**·HCl: C, 47.22; H, 6.84; N, 7.87. Anal. found for (1*S*,2*R*)-**3c**·HCl: C, 47.49; H, 6.77; N, 7.65. Anal. found for (1*S*,2*R*)-**4c**·HCl: C, 52.51; H, 7.96; N, 6.67.

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