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A New Route to Enantiopure β-Aryl-Substituted β-Amino Acids and 4-Aryl-Substituted β-Lactams through Lipase-Catalyzed Enantioselective Ring Cleavage of β-Lactams

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Abstract: A simple and efficient direct enzymatic method was developed for the synthesis of 4-aryl-substituted β-lactams and the corresponding β-amino acid enantiomers through the CAL-B (lipase B from *Candida antarctica*)-catalyzed enantioselective ($E>200$) ring cleavage of the corresponding racemic β-lactams with 1 equiv. of H_2O in *i*-Pr_2O at 60 °C. The product (*R*)-β-amino acids (ee ≥ 98%, yields ≥ 42%) and unreacted (*S*)-β-lactams (ee ≥ 95%, yields ≥ 41%) could be easily separated. The ring opening of enantiomeric β-lactams with 18% HCl afforded the corresponding enantiopure β-amino acid hydrochlorides (ee ≥ 99%).

Keywords: β-aryl-substituted β-amino acids; 4-aryl-substituted β-lactams; enantioselectivity; enzyme catalysis; lipase; ring cleavage

Introduction

β-Aryl-substituted β-amino acids and the corresponding β-lactams have been intensively investigated, due to their unique biological activity[1] and their utility in synthetic chemistry[2] and drug research.[3] In the free form, they have neurological activity and are known to be receptor antagonists and enzyme inhibitors.[4] They are also components of naturally occurring cyclic peptide astins with antitumor properties.[3] A large number of syntheses for racemic and enantiopure acyclic β-amino acids and β-lactams have already been reported.[5] As an example, 3-amino-3-(4-cyanophenyl)propanoic acid and its heteroaryl-substituted analogues have been successfully prepared in enantiomerically pure form through *Candida antarctica* lipase A-catalyzed *N*-acylation of the corresponding racemic esters.[6] Enantiomers of 4-phenyl- and 4-(*p*-tolyl)-2-azetidinones and β-aryl-substituted β-amino acids or their derivatives have been synthesized *via* lipase PS-catalyzed *R*-selective butyrylation ($E \geq 57$)[7] of the primary hydroxy group of *N*-hydroxymethylated β-lactams, or hydrolysis ($E \geq 89$) of the corresponding ester derivatives, followed by ring opening to the β-amino ester or acid, respectively.[8] This indirect enzymatic method ensures the simultaneous preparation of both β-lactam enantiomers. We have also reported a direct enzymatic method through Novozym 435 (lipase B from *Candida antarctica*)-catalyzed enantioselective alcoholysis *via* the ring opening of β-lactams ($E>200$), leading to enantiopure β-lactams in high yields (39–46%), with high ee (≥ 96%), and β-amino acids with high ee (≥ 96%) but in low yields (7–11%).[9] We recently developed a very simple and efficient new enzymatic hydrolysis method for the enantioselective ($E>200$) ring opening of alicyclic β-lactams (the synthesis of cispentacin, for instance).[10] A great advantage of this method is that the lactam ring does not necessarily need to be activated and the product β-amino acid and β-lactam are obtained in good chemical yields (≥ 36%). These results on the lipase-catalyzed enantioselective hydrolysis of alicyclic β-lactams suggested the possibility of the enantioselective ring cleavage of racemic 4-aryl-substituted β-lactams. Accordingly, in this paper we report the lipase-catalyzed enantioselective ring opening of racemic 4-aryl-substituted β-lactams, in an organic solvent. The aryl substituents were selected with regard to the synthetic applicability of the products, with different electronic characters and in different positions.

Results and Discussion

Syntheses of (±)-1–(±)-7

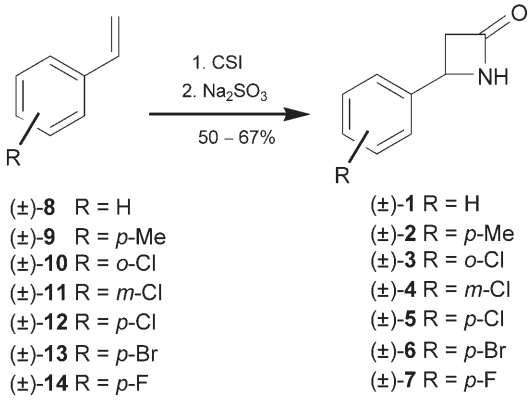
The racemic β-lactams (±)-**1** and (±)-**2** were prepared according to the literature procedure, starting from styrene or 4-methylstyrene by chlorosulfonyl isocyanate (CSI) addition[4] (Scheme 1). β-Lactams (±)-**3**–(±)-**7** were prepared in a similar, but slightly modified way, as described in the Experimental Section.

Lipase-Catalyzed Enantioselective Ring Cleavage of (±)-1–(±)-7

The earlier results[6] on the lipase-catalyzed enantioselective hydrolysis of alicyclic β-lactams suggested the possibility of the enantioselective ring opening of (±)-**1**–(±)-**7** with H_2O in an organic solvent (Scheme 2).

We explored the ring cleavage reactions on (±)-**1** with 1 equiv. of H_2O as nucleophile and Lipolase (lipase B from *Candida antarctica*) as catalyst, in *i*-Pr_2O, at 60 °C (Table 1, entry 7), but also tested the reactions with Chirazyme L-2 (entry 2) and Novozym 435 (entry 8) (both lipase B from *Candida antarctica*). High enantioselectivities ($E > 200$) were observed in all cases, with no significant differences in reactivity. Chirazyme L-5 (lipase A from *Candida antarctica*), lipase AY (*Candida rugosa*), lipase AK (*Pseudomonas fluorescens*) and lipase PS (*Pseudomonas cepacia*) did not exhibit any reactivity (no products detected after 24 h) at 45 °C. When the ring opening reaction of (±)-**1** catalyzed by Chirazyme L-2 was performed at 3 °C, a much slower reaction was observed (the reaction needed 336 h to reach 46% conversion), but with the same high enantioselectivity ($E > 200$).

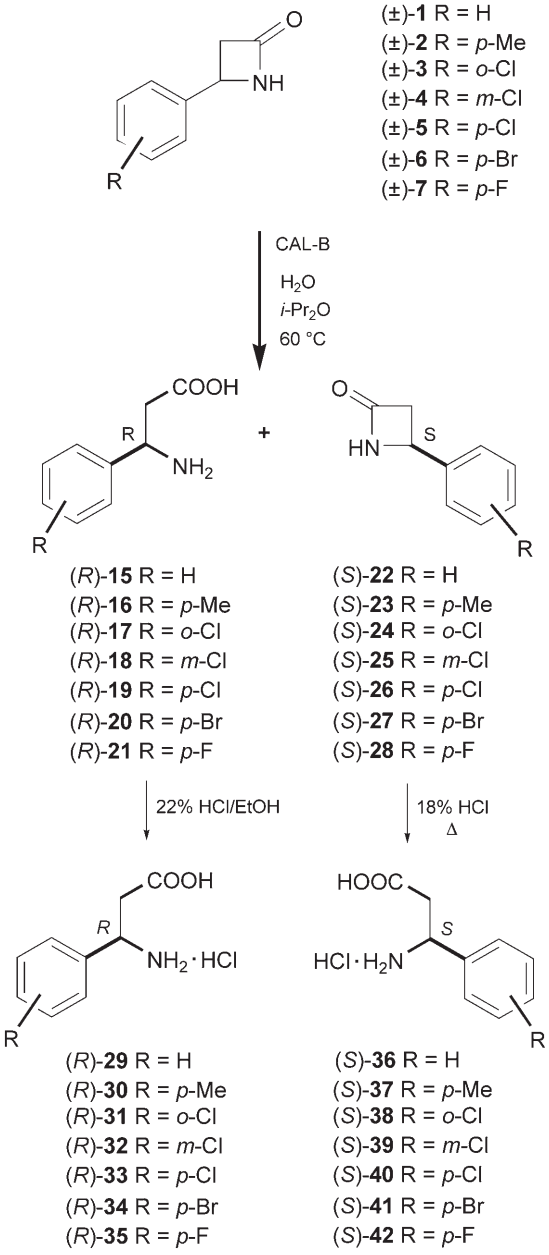
Although the CAL-B-catalyzed ring opening of (±)-**1** with H_2O (1 equiv.) at 60 °C proceeded with excellent enantioselectivity ($E > 200$), in a relatively short time



(±)-**8** R = H
(±)-**9** R = *p*-Me
(±)-**10** R = *o*-Cl
(±)-**11** R = *m*-Cl
(±)-**12** R = *p*-Cl
(±)-**13** R = *p*-Br
(±)-**14** R = *p*-F

(±)-**1** R = H
(±)-**2** R = *p*-Me
(±)-**3** R = *o*-Cl
(±)-**4** R = *m*-Cl
(±)-**5** R = *p*-Cl
(±)-**6** R = *p*-Br
(±)-**7** R = *p*-F

Scheme 1. Syntheses of (±)-**1**–(±)-**7**.



Scheme 2. Lipase-catalyzed enantioselective ring opening of (±)-**1**–(±)-**7**.

(conversion 24–26% after 1 h), several additives (2-OctOH, Et_3N and *i*-Pr_2EtN) were also tested in an attempt to enhance the reaction rate (Table 1, entries 3–5). Since no significant changes in enantioselectivity ($E > 200$) or reaction rate (conversion 25–27% after 1 h) were observed, and as the hydrolysis was complete even without the addition of H_2O (Table 1, entries 1 and 6), we concluded that the H_2O in the reaction medium (< 0.1%) or present in the enzyme preparation (< 5%) was responsible for the lactam ring opening.

We next analyzed the effect of the solvent on the enantiodiscrimination and the reaction rate. The CAL-B-catalyzed ring opening of (±)-**1** with H_2O (1 equiv.) at

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

Entry	Enzyme [50 mg mL^{-1}]	Additive [equivs.]	Conv. [%]	ee$_s$[b] [%]	ee$_p$[c] [%]	E
1	Chirazyme L-2	–	26	35	>99	>200
2	Chirazyme L-2	1 equiv. H_2O	28	38	>99	>200
3	Chirazyme L-2	1 equiv. Et_3N	26	35	>99	>200
4	Chirazyme L-2	1 equiv. *i*-Pr_2EtN	27	37	>99	>200
5	Chirazyme L-2	1 equiv. 2-OctOH	25	33	>99	>200
6	Lipolase	–	24	31	>99	>200
7	Lipolase	1 equiv. H_2O	24	31	>99	>200
8	Novozym 435	1 equiv. H_2O	25	33	>99	>200

[a] 0.05 M substrate in *i*-Pr_2O, at 60 °C, after 1 h.
[b] According to GC.
[c] According to GC after double derivatization.

60 °C was very slow when *i*-Pr_2O was replaced by acetone, acetonitrile, 1,4-dioxan, *tert*-amyl alcohol, tetrahydrofuran or chloroform (1–2% conversion after 24 h; data not shown), but the conversion was acceptable in toluene (22% conversion after 2 h), with excellent enantioselectivity ($E>200$).

The reactivity for the hydrolysis of (±)-**1** clearly increased as the quantity of enzyme was increased (Table 2). In the presence of 10 mg mL^{-1} enzyme, the reaction was relatively slow (49% conversion after 41 h; entry 1). In spite of the fact that the optimal enzyme quantity (resulting in the shortest reaction time needed to reach 50% conversion) proved to be 75 mg mL^{-1} (entry 6), for reasons of economy 30 mg mL^{-1} (entry 3) Lipolase was chosen for the preparative-scale resolutions of (±)-**1**–(±)-**7**.

On the basis of the preliminary results, the gram-scale resolutions of (±)-**1**–(±)-**7** were performed with 1 equiv. of H_2O in the presence of Lipolase (30 mg mL^{-1}) in *i*-Pr_2O at 60 °C. The products were characterized by an excellent enantiomeric excess at 49–50% conversion. The results are reported in Table 3 and in the Experimental Section.

Transformations of the Enantiomers

The transformations involving the ring opening of β-lactams **23**–**28** with 18% HCl resulted in the enantiomers of the β-amino acid hydrochlorides **36**–**42** (Scheme 2). Treatment of amino acids **15**–**21** with 22% EtOH/HCl resulted in enantiopure hydrochlorides **29**–**35**. The physical data on the enantiomers prepared are reported in the Experimental Section.

The absolute configurations in the cases of **21**, **22**, **23**, **33** and **35** were assigned by comparing the [α] values with the literature data[9,11] (see Table 3 and Experimental Section), while for **17**, **18** and **20** the analyzed chromatograms indicated the same enantiopreference for Lipolase.

Conclusions

In conclusion, an efficient direct enzymatic method was developed for the synthesis of optically pure β-aryl-substituted β-amino acids and β-lactams *via* the enantioselective ring cleavage of the corresponding β-lactams in an organic medium. The Lipolase-catalyzed highly enantioselective reactions ($E>200$) when H_2O (1 equiv.) was used as a nucleophile in *i*-Pr_2O at 60 °C led to β-amino acid and β-lactam enantiomers (ee ≥

Table 2. Effect of the quantity of Lipolase on the ring opening of (±)-**1**.[a]

Entry	Lipolase [mg mL^{-1}]	Conv. [%]	ee$_s$[b] [%]	ee$_p$[c] [%]	E
1	10	8 (49 after 41 h)	9	>99	>200
2	20	14	16	>99	>200
3	30	17	20	>99	>200
4	40	22	28	>99	>200
5	50	24	31	>99	>200
6	75	32	47	>99	>200

[a] 0.05 M substrate in *i*-Pr_2O, with 1 equiv. H_2O, at 60 °C, after 1 h.
[b] According to GC.
[c] According to GC after double derivatization.

Table 3. Lipolase-catalyzed ring opening of (±)-**1**–(±)-**7**.[a]

	Time [h]	Conv. [%]	*E*	β-Amino acid (**15**–**21**)				β-Lactam (**22**–**28**)			
				Yield [%]	Isomer	ee[b] [%]	$[\alpha]_D^{25}$	Yield [%]	Isomer	ee[c] [%]	$[\alpha]_D^{25}$
(±)-**1**	24	50	>200	47	*R*	>99	+7[d]	46	*S*	>99	−137[e]
(±)-**2**	30	49	>200	42	*R*	99	+8[f]	45	*S*	95	−113[g]
(±)-**3**	14	50	>200	47	*R*	99	+30.3[h]	48	*S*	99	−271[i]
(±)-**4**	11	50	>200	46	*R*	99	+5[j]	46	*S*	99	−118[k]
(±)-**5**	15	50	>200	48	*R*	99	+16.3[l]	46	*S*	99	−110[m]
(±)-**6**	14	49	>200	47	*R*	98	+4[n]	41	*S*	96	−73[o]
(±)-**7**	13	50	>200	43	*R*	99	+4.9[p]	49	*S*	99	−117[q]

[a] 3 mg mL^{-1} enzyme in *i*-Pr_2O, 1 equiv. H_2O, 60 °C.
[b] Determined by GC [after double derivatization (i) diazomethane; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine (Experimental Section).
[c] According to GC (Experimental Section).
[d] $c=0.17$; H_2O.
[e] $c=0.28$; EtOH, lit.[9] $[\alpha]_D^{25}$: −139 ($c=0.19$; EtOH).
[f] $c=0.20$; H_2O.
[g] $c=0.26$; EtOH, lit.[9] $[\alpha]_D^{25}$: −121.9 ($c=0.5$; EtOH).
[h] $c=0.43$; H_2O.
[i] $c=0.47$; EtOH.
[j] $c=0.51$; H_2O.
[k] $c=0.46$; EtOH.
[l] $c=0.33$; H_2O.
[m] $c=0.49$; EtOH.
[n] $c=0.10$; H_2O.
[o] $c=0.16$; EtOH.
[p] $c=0.45$; H_2O, lit.[11] $[\alpha]_D^{25}$: +3.9 ($c=0.4$; H_2O).
[q] $c=0.45$; EtOH.

95%) in good chemical yields (41–49%). The products could be easily separated. Transformations through the ring opening of β-lactams with 18% HCl resulted in the corresponding β-amino acid hydrochlorides (ee ≥ 99%). It is important to note that no significant correlation was found between the steric and electronic nature of the substituent on the aryl ring and the activation of the ring cleavage. The present method proved to be a very simple, inexpensive route, and could be easily scaled up. The synthetized enantiopure β-amino acids and β-lactams are promising building blocks for the synthesis of peptides, peptidomimetics and potential pharmacons.

Experimental Section

Materials and Methods

Lipolase (lipase B from *Candida antarctica*), produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin, was from Sigma-Aldrich. Novozym 435, as an immobilized lipase (lipase B from *Candida antarctica*) on a macroporous acrylic resin, was from Novo Nordisk. Chirazyme L-2 (a carrier-fixed lipase B from *Candida antarctica*) was purchased from Roche Diagnostics Corporation. Chlorosulfonyl isocyanate, styrene and substituted styrenes were from Aldrich. The solvents were of the highest analytical grade.

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

Synthesis of Racemic β-Lactams, (±)-1 and (±)-2

The racemic β-lactams (±)-**1** and (±)-**2** were prepared according to the literature,[7] starting from styrene **8** (2 g, 19.20 mmol) or 4-methylstyrene **9** (2 g, 16.92 mmol).

(±)-4-Phenyl-2-azetidinone [(±)-1]: yield: 2.22 g (79%); mp 104–105 °C (lit.[8] mp 108–109 °C).

(±)-4-(*p*-Tolyl)-2-azetidinone [(±)-2]: yield: 1.73 g (63%); mp 87–88 °C (lit.[8] mp 85–86 °C).

Synthesis of Racemic β-Lactams (±)-3–(±)-7

β-Lactams (±)-**3**–(±)-**7** were prepared with a slightly modified synthetic procedure: a solution of substituted styrene **10** (2 g, 14.43 mmol) or **11** (2 g, 14.43 mmol) or **12** (2 g, 14.43 mmol) or **13** (2 g, 10.92 mmol) or **14** (2 g, 12.10 mmol) in absolute toluene (10 mL) was added dropwise to a stirred solution of CSI (1 equiv.) in absolute toluene (10 mL) at 0 °C. The reaction mixture was stirred at room temperature for 8 h and then left to stand overnight. The solution was added dropwise to a vigorously stirred solution of Na_2SO_3 (0.8 g) and

Na_2CO_3 (3.6 g) in H_2O (30 mL). The organic layer was separated and the aqueous phase was extracted with toluene. The combined organic layers were dried (Na_2SO_4) and, after filtration, concentrated. The resulting crude β-lactams were recrystallized from *i*-Pr_2O.

(±)-4-(2-Chlorophenyl)-2-azetidinone [(±)-3]: yield: 1.30 g (50%); mp 123–125 °C (lit.[12] mp 121–122 °C).

(±)-4-(3-Chlorophenyl)-2-azetidinone [(±)-4]: yield: 1.75 g (67%); mp 93–95 °C.

(±)-4-(4-Chlorophenyl)-2-azetidinone [(±)-5]: yield: 1.52 g (58%); mp 97–99 °C (lit.[13] mp 98–99 °C).

(±)-4-(4-Bromophenyl)-2-azetidinone [(±)-6]: yield: 1.55 g (50%); mp 104–108 °C (lit.[14] mp 105 °C).

(±)-4-(4-Fluorophenyl)-2-azetidinone [(±)-7]: yield: 1.49 g (55%); mp 58–62 °C.

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 or 75 mg mL^{-1}). H_2O or additive (0 or 1 equiv.) was added. The mixture was shaken at 60 °C. The progress of the reaction was followed by taking samples from the reaction mixture at intervals and analyzing them by gas chromatography. The ee values for the unreacted β-lactam enantiomers were determined by gas chromatography on a Chromopack Chiralsil-Dex CB column (25 m) [160 °C for 4 min → 190 °C (temperature rise 20 °C min^{-1}), 140 kPa; retention times (min); **22**: 18.28 (antipode: 16.85); **23**: 20.48 (antipode: 19.55); 190 °C isothermal, 140 kPa; **24**: 41.77 (antipode: 40.10); **25**: 50.75 (antipode: 47.81); **26**: 53.77 (antipode: 52.01); **27**: 50.76 (antipode: 47.88); **28**: 23.15 (antipode: 12.28)], while the ee values for the β-amino acids produced were determined by using a gas chromatograph equipped with a Chirasil-L-Val column (20 m) after double derivatization with (i) diazomethane [**Caution!** the derivatization with diazomethane should be performed under a well-working hood]; (ii) acetic anhydride in the presence of 4-dimethylaminopyridine and pyridine [120 °C for 2 min → 180 °C (temperature rise 20 °C min^{-1}), 140 kPa; retention times (min); **15**: 11.74 (antipode: 11.95); **16**: 16.27 (antipode: 16.67); 150 °C for 30 min → 180 °C (temperature rise 20 °Cmin^{-1}), 100 kPa: **17**: 35.91 (antipode: 36.27); 120 °C for 10 min → 190 °C (temperature rise 10 °C min^{-1}), 140 kPa; **18**: 27.34 (antipode: 27.75); **21**: 19.61 (antipode: 19.78); 150 °C for 15 min → 180 °C (temperature rise 5 °C min^{-1}), 120 kPa: **19**: 36.62 (antipode: 37.47); 180 °C isothermal, 140 kPa; **20**: 28.68 (antipode: 29.76)].

Gram-Scale Resolution of (±)-1

Racemic β-lactam **1** (0.5 g, 3.39 mmol) was dissolved in *i*-Pr_2O (70 mL). Lipolase (2.1 g, 30 mg mL^{-1}) and H_2O (61 μL, 3.39 mmol) were added, and the mixture was shaken in an incubator shaker at 60 °C for 24 h. The reaction was stopped by filtering off the enzyme at 50% conversion. The solvent was evaporated off and the residue (*S*)-**22** crystallized; yield: 230 mg (46%); recrystallized from *i*-Pr_2O {$[\alpha]_D^{25}$: −137 (*c* 0.28; EtOH) lit.[9] $[\alpha]_D^{25}$: −139 (*c* 0.19; EtOH); mp 116–118 °C, lit.[9] mp 114 °C; ee > 99%}.

The filtered-off enzyme was washed with distilled H_2O (3 × 15 mL), and the H_2O was evaporated off, affording the crystalline β-amino acid (*R*)-**15** [yield: 263 mg (47%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +7 (*c* 0.27; H_2O); mp 242–246 °C; ee > 99%].

When **15** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**29** was obtained [yield: 98 mg (81%); $[\alpha]_D^{25}$: −3 (*c* 0.30; H_2O); mp 195–198 °C, ee > 99%].

Gram-Scale Resolution of (±)-2

Via the procedure described above, the reaction of racemic **2** (0.5 g, 3.10 mmol) and H_2O (56 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**23** {yield: 225 mg (45%); $[\alpha]_D^{25}$: −113 (*c* 0.26; EtOH), lit.[9] $[\alpha]_D^{25}$: −121.9 (*c* 0.5; EtOH); mp 60–61 °C (recrystallized from *i*-Pr_2O), lit.[9] mp 56 °C; ee = 95%} and β-amino acid (*R*)-**16** {yield: 233 mg, 42%; recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +8 (*c* 0.20; H_2O), (lit.[9] $[\alpha]_D^{25}$: given by mistake with the opposite sign); mp 244–248 °C, lit.[9] mp 241–243 °C; ee = 99%} in 30 h.

When **16** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**30** {yield: 97 mg (81%); $[\alpha]_D^{25}$: −4.5 (*c* 0.36; H_2O); mp 197–202 °C (recrystallized from EtOH and Et_2O), ee > 99%} was formed.

Gram-Scale Resolution of (±)-3

Via the procedure described above, the reaction of racemic **3** (0.5 g, 2.75 mmol) and H_2O (50 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**24** {yield: 240 mg (48%); $[\alpha]_D^{25}$: = −271 (*c* 0.47; EtOH); mp 108–112 °C (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**17** {yield: 258 mg (47%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +30.3 (*c* 0.43; H_2O); mp 235–239 °C (with sublimation), ee = 99%} in 14 h.

When **17** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**31** {yield: 104 mg (88%); $[\alpha]_D^{25}$: +8.4 (*c* 0.47; H_2O); mp 175–178 °C (recrystallized from EtOH and Et_2O), ee > 99%} was formed.

Gram-Scale Resolution of (±)-4

Via the procedure described above, the reaction of racemic **4** (0.5 g, 2.75 mmol) and H_2O (50 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**25** {yield: 230 mg (46%); $[\alpha]_D^{25}$: −118 (*c* 0.47; EtOH); mp 95–98 °C, (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**18** {252 mg (46%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +5 (*c* 0.43; H_2O); mp 230–233 °C, ee 99%} in 11 h.

When **18** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**32** {yield: 106 mg (90%); $[\alpha]_D^{25}$: −3.2 (*c* 0.43, H_2O); mp 200–204 °C (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Gram-Scale Resolution of (±)-5

Via the procedure described above, the reaction of racemic **5** (0.5 g, 2.75 mmol) and H_2O (50 μL, 3.10 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at

60 °C afforded the unreacted (*S*)-**26** {yield: 232 mg (46%); $[\alpha]_D^{25}$: −110 (*c* 0.49; EtOH); mp 123–127 °C, (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**19** {yield: 263 mg (48%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +16.3 (*c* 0.33; H_2O); mp 241–244 °C with sublimation, lit.[10] mp 223–225 °C; ee = 99%} in 15 h.

When **19** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**33** {yield: 106 mg, 90%; $[\alpha]_D^{25}$: −5.2 (*c* 0.46, H_2O), lit.[10] $[\alpha]_D^{25}$: −3.33 (1.6 N HCl); mp 184–188 °C (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Gram-Scale Resolution of (±)-6

Via the procedure described above, the reaction of racemic **6** (0.5 g, 2.21 mmol) and H_2O (40 μL, 2.21 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**27** {220 mg (41%); $[\alpha]_D^{25}$: −73 (*c* 0.16; EtOH); mp 151–154 °C, (recrystallized from *i*-Pr_2O); ee = 96%} and β-amino acid (*R*)-**20** {yield: 234 mg (47%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +4 (*c* 0.45; H_2O); mp 258–260 °C, ee = 98%} in 14 h.

When **20** (50 mg) was treated with 18% HCl (3 mL), (*R*)-**34** {yield: 52 mg (90%); $[\alpha]_D^{25}$: −4.1 (*c* 0.45, H_2O); mp 191–193 °C with sublimation (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Gram-Scale Resolution of (±)-7

Via the procedure described above, the reaction of racemic **7** (0.5 g, 3.03 mmol) and H_2O (55 μL, 3.03 mmol) in *i*-Pr_2O (70 mL) in the presence of Lipolase (2.1 g, 30 mg mL^{-1}) at 60 °C afforded the unreacted (*S*)-**28** {yield: 219 mg (44%); $[\alpha]_D^{25}$: = −117 (*c* 0.45; EtOH); mp 97–101 °C (recrystallized from *i*-Pr_2O); ee = 99%} and β-amino acid (*R*)-**21** {yield: 268 mg (48%); recrystallized from H_2O and Me_2CO; $[\alpha]_D^{25}$: +4.9 (*c* 0.45; H_2O), lit.[10] $[\alpha]_D^{25}$: +3.9 (*c* 0.4; H_2O); mp 245–247 °C, ee = 99%} in 13 h.

When **21** (100 mg) was treated with 18% HCl (3 mL), (*R*)-**35** {110 mg (91%); $[\alpha]_D^{25}$: −1.2 (*c* 0.37, H_2O), lit.[10] $[\alpha]_D^{25}$: −1.9 (1.9 N HCl); mp 166–169 °C (recrystallized from EtOH and Et_2O), ee = 99%} was formed.

Ring Opening of (*S*)-22–(*S*)-28 with 18% HCl

The β-lactam enantiomer (*S*)-**22** (50 mg, 0.34 mmol) or (*S*)-**23** (50 mg, 0.31 mmol) or (*S*)-**24** (100 mg, 0.55 mmol) or (*S*)-**25** (100 mg, 0.55 mmol) or (*S*)-**26** (100 mg, 0.55 mmol) or (*S*)-**27** (50 mg, 0.22 mmol) or (*S*)-**28** (100 mg, 0.60 mmol) was dissolved in 18% HCl (12 mL) and the solution was refluxed for 2 h. The solvent was then evaporated off, and the product was recrystallized from EtOH and Et_2O, which afforded white crystals of the β-amino acid hydrochlorides.

(*S*)-**36**: {yield: 49 mg (72%); $[\alpha]_D^{25}$: +3.0 (*c* 0.28; H_2O); mp 197–201 °C}.

(*S*)-**37**: {yield: 52 mg (78%); $[\alpha]_D^{25}$: +4.0 (*c* 0.28; H_2O); mp 196–201 °C}.

(*S*)-**38**: {yield: 104 mg (80%); $[\alpha]_D^{25}$: −8.6 (*c* 0.46; H_2O); mp 176–179 °C}.

(*S*)-**39**: {yield: 111 mg (86%); $[\alpha]_D^{25}$: +3.3 (*c* 0.35; H_2O); mp 201–204 °C}.

(*S*)-**40**: {yield: 109 mg (84%); $[\alpha]_D^{25}$: +5.3 (*c* 0.46; H_2O); mp 185–188 °C}.

(*S*)-**41**: {yield: 57 mg (91%); $[\alpha]_D^{25}$: +3.8 (*c* 0.45; H_2O); mp 191–193 °C with sublimation}.

(*S*)-**42**: {yield: 108 mg (81%); $[\alpha]_D^{25}$: +1.5 (*c* 0.47; H_2O); mp 164–168 °C}.

Supporting Information

The spectroscopic and analytical data for racemates **1**–**7** and enantiomers **15**–**42** are presented in the Supporting Information.

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