Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 2841–2844

Candida antarctica lipase B-catalyzed ring opening of 4-arylalkyl-substituted β-lactams

Gábor Tasnádi,[a] Enikő Forró[a,*] and Ferenc Fülöp[a,b]

[a]*Institute of Pharmaceutical Chemistry, Academy of Sciences, University of Szeged, H-6720 Szeged Eötvös utca 6, Hungary*
[b]*Research Group of Stereochemistry of the Hungarian, Academy of Sciences, University of Szeged, H-6720 Szeged Eötvös utca 6, Hungary*

Received 30 October 2007; accepted 13 November 2007

Abstract—The Lipolase-catalyzed ring opening of racemic 4-benzyl- **3** and 4-phenylethyl-2-azetidinone **4** was performed with 0.5 equiv of H_2O in diisopropyl ether at 45 °C. The resulting (*S*)-β-amino acid **5** or **6** (ee ⩾ 87%) and (*R*)-β-lactam **7** or **8** (ee >99%) enantiomers could easily be separated. The ring opening of enantiomeric β-lactams with 18% aqueous HCl afforded the corresponding enantiopure β-amino acid hydrochlorides **9** and **10** (ee >99%).

1. Introduction

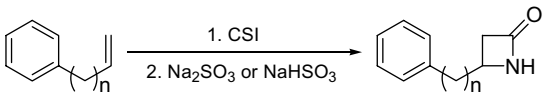
In recent years, β-amino acids have aroused considerable interest as potentially biologically active compounds.[1] (*S*)-Homo-β-phenylalanine increases the μ-type opioid receptor affinity[2] and is a valuable building block for two tripeptidomimics exhibiting angiotensin-converting enzyme inhibitor activity[3] and an optically active poly(β-peptide).[4] It has been tested as a catalyst in intra- and intermolecular aldol reactions.[5] (*R*)-Homo-β-phenylalanine derivatized with a thiazolidine is a potent inhibitor of dipeptidyl peptidase IV, which allows a novel therapeutic approach to the treatment of diabetes type 2.[6] A photochemical application is also known.[7] β-Phenylethyl-β-alanine has been applied as a model compound in analytical studies[8] and its antiseizure activity has also been studied.[9] Valuable antiplatelet[10] and antiviral agents[11] have been synthesized from the title β-lactams.

Since their widespread investigation, over the last few years a number of new enzymatic and asymmetric syntheses of β-amino acids and β-lactams have been published most of which have been reviewed.[12] An important indirect enzymatic method for the preparation of β-amino acid enantiomers is the lipase-catalyzed asymmetric acylation of the primary hydroxy group of the N-hydroxymethylated β-lactams, or the lipase-catalyzed hydrolysis of the corresponding ester derivatives, followed by ring opening to give the desired β-amino acids.[13] We recently discovered a simple and efficient direct enzymatic method for the enantioselective (E >200) ring cleavage of β-lactams.[14] Later, the enantioselective (E >200) ring opening of several 4-aryl-substituted β-lactams was reported.[15]

Herein, we report the lipase-catalyzed ring cleavage of 4-benzyl- and 4-phenylethyl-2-azetidinones (±)-**3** and (±)-**4**.

2. Results and discussion

The starting racemic β-lactams **3** and **4** were prepared by the addition of chlorosulfonyl isocyanate to allylbenzene **1** or 4-phenyl-1-butene **2**, according to a literature method[10,13d] (Scheme 1).



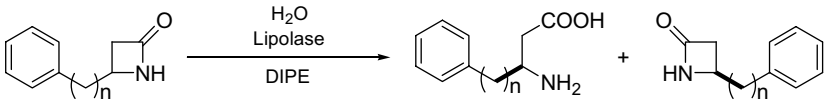
1, n = 1 (±)-**3**, n = 1
2, n = 2 (±)-**4**, n = 2

Scheme 1.

The earlier results on the lipase-catalyzed enantioselective hydrolysis of 4-aryl-substituted β-lactams[15] suggested the possibility of the enantioselective ring opening of (±)-**3** and (±)-**4**. Relatively low enantioselectivity (E = 12) was

* Corresponding author. Tel.: +36 62 544964; fax: +36 62 545705; e-mail: forro@pharm.u-szeged.hu

0957-4166/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2007.11.016



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

Scheme 2.

observed when the ring in (±)-**4** was opened with H_2O in diisopropyl ether (DIPE), with Lipolase (30 mg/mL) as a catalyst at 60 °C (Scheme 2).

To increase the enantioselectivity, several further enzymes were tested. In addition to Lipolase (lipase B from *Candida antarctica*, produced by submerged fermentation of a genetically modified *Aspergillus oryzae* microorganism and adsorbed on a macroporous resin), Chyrazyme L-2 and Novozym 435 (both lipase B from *C. antarctica*) also proved to be promising catalysts, directing the hydrolysis of (±)-**4** with similar enantioselectivities ($E \sim 12$). Lipase A from *C. antarctica*, lipase AY from *Candida rugosa* and Lecitase did not show any reactivity at 45 °C (no conversion after 24 h), while PPL (porcine pancreas lipase) and lipase AK from *Pseudomonas fluorescens* catalyzed the reaction at 45 °C, although the reaction rates and the enantioselectivities were low (after 24 h, conv. ~ 3%, $E \sim 2$). Therefore Lipolase was chosen as the enzyme for further studies.

Next, we tested the ring-cleavage reactions of (±)-**4** at different temperatures: 60, 50, 45, 40 and 30 °C. Decreasing the reaction temperature also caused the reaction rate to decrease as well, but without an increase in enantioselectivity (after 7 h, conv. = 41% at 60 °C; 24% at 50 °C; 20% at 45 °C; and 15% at 40 °C; after 5 days, conv. = 61% at 30 °C). Thus, 45 °C was chosen as the optimal temperature.

Several solvents were also tested. No reaction was observed after 24 h when the Lipolase (50 mg/mL)-catalyzed ring cleavage of (±)-**4** was performed in chloroform, tetrahydrofuran or acetone. The reaction proceeded more slowly in toluene (conv. = 19% after 24 h) and much more slowly in 1,4-dioxane (conv. = 4% after 24 h) than in DIPE (conv. = 58% after 24 h), *tert*-butyl methyl ether (conv. = 60% after 24 h), diethyl ether (conv. = 49% after 24 h) or *n*-hexane (conv. = 69% after 24 h). So we chose to continue our studies in DIPE.

Certain additives can have a beneficial influence by increasing the enantioselectivity and/or the reaction rate.[16] As an attempt, 1 equiv of triethylamine, 2-octanol and *N*,*N*-diisopropylethylamine were added to the reaction mixture. However, no significant changes in the reaction rate or enantioselectivity were observed (conv. ~ 57% after 24 h, $E \sim 10$).

Since the enantioselective ring cleavage of some *N*-Boc-protected cyclic β-lactams has been described,[17] we synthesized *N*-Boc-protected-(±)-**4**. Unfortunately, the Lipolase (50 mg/mL)-catalyzed ring opening of *N*-Boc-(±)-**4** at 45 °C did not give a better result (conv. = 94% after 24 h, $E = 3$).

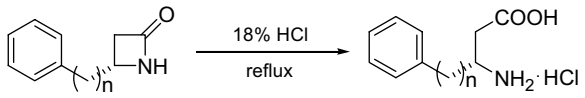
On the basis of the preliminary results, we decided to perform the gram-scale resolutions of (±)-**3** and (±)-**4** in DIPE with Lipolase as catalyst and H_2O (0.5 equiv) as the nucleophile at 45 °C. We planned to stop the reactions at about 25% conversion [ee(β-amino acids) ~60%], and then perform the reactions until about 85% conversion [ee(β-lactams) ~90%], following recrystallization of the crude β-amino acid and β-lactam enantiomers. The results are shown in Table 1 and in Section 4.

Table 1. Lipolase-catalyzed ring opening of (±)-**3** and (±)-**4**

	Time (h)	Conv. at workup (%)	Enantiomer	Yield (%)	Isomer	ee[a] (%)	$[\alpha]_D^{25}$
(±)-**3**	13	24	β-Amino acid **5**	27	(*S*)	89[c]	+7[b,e]
	88	81	β-Lactam **7**	36	(*R*)	>99[d]	+38.8[b,f]
(±)-**4**	11	29	β-Amino acid **6**	31	(*S*)	87[c]	+24[b,g]
	22	89	β-Lactam **8**	30	(*R*)	>99[d]	+19[b,h]

[a] After recrystallization.
[b] Specific rotations were measured with a Perkin–Elmer 341 polarimeter.
[c] According to HPLC [APEX Octadecyl 5 μ column (0.04 cm × 25 cm); precolumn derivatization with (*S*)-NIFE according to the literature;[18] the mobile phases were H_2O (A) and MeCN (B), both of which contained 0.1% TFA; the gradient slopes were 95% A + 5% B at 0 min, increased to 25% A + 75% B within 60 min.; flow rate: 0.8 mL/min; room temperature; detection at 205 nm; retention times (min): **5**, 38.69 (antipode: 39.67); **6**, 40.00 (antipode: 40.90)].
[d] According to GC [Chrompack Chirasil-L-Val column (25 m × 0.25 mm); 90 °C for 20 min (**3**) and 10 min (**4**) → 140 °C; temperature rise 5 °C/min; 140 kPa; retention times (min): **7**, 35.77 (antipode: 36.66); **8**, 38.52 (antipode: 39.27)].
[e] *c* 0.2; H_2O lit.[19] $[\alpha]_D^{25} = -8.5$ (*c* 0.2, H_2O) for (*R*)-**5**, ee = 95%.
[f] *c* 0.65; $CHCl_3$ lit.[13d] $[\alpha]_D^{25} = +30.1$ (*c* 0.65, $CHCl_3$), ee = 98%.
[g] *c* 0.28 lit.[20] $[\alpha]_D^{25} = -28.4$ (*c* 0.56, H_2O) for (*R*)-**6**, ee >99%; H_2O.
[h] *c* 0.21; $CHCl_3$.

The transformations involving the ring opening of β-lactams **7** and **8** with 18% aqueous HCl afforded β-amino acid hydrochlorides **9** and **10** (Scheme 3), while treatment of amino acids **5** and **6** with 18% aqueous HCl resulted in the corresponding β-amino acid hydrochlorides **5**·HCl and **6**·HCl.



7, n = 1
8, n = 2

9, n = 1
10, n = 2

Scheme 3.

The absolute configurations were proven by comparing the specific rotations with the literature data[13d,19,20] (Table 1). Thus, the absolute configuration for **5** and **6** is (*S*), and for **7** and **8** it is (*R*).

3. Conclusion

In conclusion, 4-benzyl- and 4-phenylethyl-2-azetidinones (±)-**3** and (±)-**4** were resolved via opening of the β-lactam ring in an organic medium. The Lipolase-catalyzed reactions when H_2O (0.5 equiv) was used as nucleophile in DIPE at 45 °C led to (*S*)-β-amino acids **5** and **6** (ee ⩾ 87%) and (*R*)-β-lactams **7** and **8** (ee >99%). The products could be separated with ease. Transformations of β-lactams **7** and **8** by ring opening with 18% aqueous HCl gave the corresponding enantiomers of the β-amino acid hydrochlorides **9** and **10** (ee >99%).

4. Experimental

4.1. Small-scale resolutions

In a small-scale experiment, (±)-**4** (0.05 M solution) in DIPE (1 mL) was added to Lipolase (30 or 50 mg/mL). H_2O (0.5 equiv) was added. The mixture was shaken at 30, 40, 45, 50 or 60 °C. The progress of the reaction was followed by taking samples from the mixture at intervals and analyzing them by gas chromatography and HPLC (Table 1).

4.2. Gram-scale resolution of racemic 4-benzyl-2-azetidinone (±)-3

Racemic **3** (1.2 g, 7.44 mmol) was dissolved in DIPE (40 mL). Lipolase (1.2 g, 30 mg/mL) and H_2O (67 μL, 3.72 mmol) were added. The mixture was stirred at 45 °C for 13 h. The reaction was stopped by filtering off the enzyme at 24% conversion. The solvent was evaporated off, affording the unreacted β-lactam **7** (0.85 g, 5.27 mmol, ee = 18%). The filtered enzyme was washed with distilled H_2O (3 × 20 mL), and the H_2O was evaporated off, yielding the crystalline (*S*)-β-amino acid **5** {0.36 g, 27%; $[\alpha]_D^{25} = +7$ (*c* 0.2; H_2O); mp = 207–210 °C (recrystallized from H_2O/acetone); ee = 89%; lit.[19] $[\alpha]_D^{25} = -8.5$ (*c* 0.2, H_2O) for (*R*)-**5**; mp = 222–225 °C; ee = 95%}.

To obtain β-lactam **7** with high ee, the above unreacted lactam (0.85 g) was dissolved in DIPE (30 mL). Lipolase (0.8 g, 27 mg/mL) and H_2O (47 μL, 2.64 mmol) were added. The mixture was stirred at 45 °C for 88 h. The reaction was stopped by filtering off the enzyme at 81% conversion. The solvent was evaporated off, affording (*R*)-β-lactam **7** {0.43 g, 36%; $[\alpha]_D^{25} = +38.8$ (*c* 0.65; $CHCl_3$); mp = 67–69 °C (recrystallized from DIPE); ee >99%; lit.[13d] $[\alpha]_D^{25} = +30.1$ (*c* 0.65, $CHCl_3$); ee = 98%}. When **5** (36 mg) was treated with 18% HCl (5 mL), **5**·HCl was obtained {38 mg, 88%; $[\alpha]_D^{25} = +6$ (*c* 0.21; H_2O); mp = 172–175 °C; ee = 89%; lit.[13d] mp = 176–178 °C}.

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **5**: 2.40–2.46 (dd, *J* = 16.8, 8.2 Hz, 1H, C*H*$_2$CO$_2$H), 2.52–2.57 (dd, *J* = 16.8, 4.9 Hz, 1H, C*H*$_2$CO$_2$H), 2.91–3.04 (m, 2H, C*H*$_2$Ph), 3.73–3.76 (m, 1H, CH), 7.30–7.43 (m, 5H, Ph).

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **5**·HCl: 2.66–2.82 (m, 2H, CH_2CO), 3.03–3.05 (m, 2H, CH_2Ph), 3.90–3.93 (m, 1H, CH), 7.32–7.45 (m, 5H, Ph).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) for **7**: 2.68–2.72 (d, *J* = 14.8 Hz, 1H), 2.82–2.87 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.95–3.00 (dd, *J* = 13.7, 5.7 Hz, 1H), 3.05–3.10 (m, 1H), 3.83–3.86 (m, 1H), 5.83 (br s, 1H), 7.17–7.35 (m, 5H).

4.3. Gram-scale resolution of racemic 4-phenylethyl-2-azetidinone (±)-4

With the procedure described above, the ring cleavage of racemic **4** (0.8 g, 4.57 mmol) in DIPE (30 mL) in the presence of Lipolase (0.8 g, 27 mg/mL) and H_2O (41 μL, 2.29 mmol) afforded (*S*)-β-amino acid **6** [0.27 g, 31%; $[\alpha]_D^{25} = +24$ (*c* 0.28; H_2O); mp = 215–219 °C (recrystallized from H_2O/acetone); ee = 87%; lit.[20] $[\alpha]_D^{25} = -28.4$ (*c* 0.56, H_2O) for (*R*)-**6**; mp = 215–217 °C; ee >99%] in 11 h and (*R*)-β-lactam **8** [0.24 g, 30%; $[\alpha]_D^{25} = +19$ (*c* 0.21; $CHCl_3$); mp = 46–48 °C (recrystallized from DIPE); ee >99%] in 22 h. When **6** (30 mg) was treated with 18% HCl (5 mL), **6**·HCl was obtained [33 mg, 92%; $[\alpha]_D^{25} = +12$ (*c* 0.21; H_2O); mp = 150–152 °C; ee = 87%].

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **6**: 1.96–2.02 (m, 2H), 2.45–2.52 (dd, *J* = 16.8, 8.4 Hz, 1H), 2.61–2.67 (dd, *J* = 16.8, 4.6 Hz, 1H), 2.72–2.79 (m, 2H), 3.48–3.53 (m, 1H), 7.29–7.42 (m, 5H).

^{1}H NMR (D_2O, 400 MHz) δ (ppm) for **6**·HCl: 2.00–2.05 (m, 2H), 2.69–2.86 (m, 4H), 3.62 (m, 1H), 7.28–7.41 (m, 5H).

^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm) for **8**: 1.94–2.00 (dd, *J* = 14.5, 7.5 Hz, 2H, CH_2Ph), 2.55–2.59 (d, *J* = 14.8 Hz, 1H, C*H*H), 2.64–2.73 (m, 2H, CH_2), 3.02–3.08 (m, 1H, CH*H*), 3.61–3.65 (m, 1H, CH), 5.67 (br s, 1H, NH), 7.16–7.32 (m, 5H, Ph).

4.4. Ring opening of β-lactam enantiomers 7 and 8

Compounds **7** (50 mg) or **8** (25 mg) were refluxed in 18% HCl (10 mL) for 3 h. The solvent was evaporated off to afford **9** [60 mg, 90%; $[\alpha]_D^{25} = -8$ (*c* 0.11; H_2O); mp = 182–185 °C; ee >99%] or **10** {26 mg, 79%; $[\alpha]_D^{25} = -15$ (*c* 0.21; H_2O); mp = 146–148 °C; ee >99%}. The ^1H NMR (H_2O, 400 MHz) δ (ppm) data for **9** and **10** are similar to those for **5**·HCl and **6**·HCl.

Acknowledgements

The authors acknowledge the receipt of OTKA Grants T 046440 and T 049407, GVOP-3.1.1.-2004-05-0255/3.0 and a Bolyai Fellowship for E.F.

References

1. Juaristi, E. *Enantioselective synthesis of β-amino acids*; Wiley-VHC: New York, 2005.
2. Longobardo, L.; Melck, D.; Siciliano, R.; Santini, A.; Di Marzo, V.; Cammarota, G. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1185–1188.
3. Pavar, M. C.; Hanif, K.; Azam, A.; Lata, S.; Qadar Pasha, M. A.; Pasha, S. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2117–2121.
4. Cheng, J.; Deming, T. J. *Macromolecules* **2001**, *34*, 5169–5174.
5. Limbach, M. *Tetrahedron Lett.* **2006**, *47*, 3843–3847.
6. Xu, J.; Ok, H. O.; Gonzalez, E. J.; Colwell, L. F., Jr.; Habulihaz, B.; He, H.; Leiting, B.; Lyons, K. A.; Marsilio, F.; Patel, R. A.; Wu, J. K.; Thornberry, N. A.; Weber, A. E.; Parmee, E. R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4759–4762.
7. Rzeska, A.; Malicka, J.; Stachowiak, K.; Szymanska, A.; Lankiewicz, L.; Wiczk, W. *J. Photochem. Photobiol. A: Chem.* **2001**, *140*, 21–26.
8. (a) Hyun, M. H.; Han, S. C.; Whangbo, S. H. *J. Chromatogr., A* **2003**, *992*, 47–56; (b) Árki, A.; Tourwé, D.; Solymár, M.; Fülöp, F.; Armstrong, D. W.; Péter, A. *Chromatographia* **2004**, *60*, S43–S54.
9. Tan, C. Y. K.; Wainman, D.; Weaver, D. F. *Bioorg. Med. Chem.* **2003**, *11*, 113–121.
10. Zablocki, J. A.; Tjoeng, F. S.; Bovy, P. R.; Miyano, M.; Garland, R. B.; Williams, K.; Schretzman, L.; Zupec, M. E.; Rico, J. G.; Lindmark, R. J.; Toth, M. V.; McMackins, D. E.; Adams, S. P.; Panzer-Knodle, S. G.; Nicholson, N. S.; Taite, B. B.; Salyers, A. K.; King, L. W.; Campion, J. G.; Feigen, L. P. *Bioorg. Med. Chem.* **1995**, *5*, 539–551.
11. Yoakim, C.; Ogilvie, W. W.; Cameron, D. R.; Chabot, C.; Guse, I.; Haché, B.; Naud, J.; O'Meara, J. A.; Plante, R.; Déziel, R. *J. Med. Chem.* **1998**, *41*, 2882–2891.
12. (a) Liu, M.; Sibi, M. P. *Tetrahedron* **2002**, *58*, 7991–8035; (b) Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854; (c) Fülöp, F. *Chem. Rev.* **2001**, *101*, 2181–2204; (d) Fülöp, F.; Martinek, T. A.; Tóth, G. K. *Chem. Soc. Rev.* **2006**, *35*, 323–334.
13. (a) Forró, E.; Árva, J.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 643–649; (b) Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2001**, *12*, 2351–2358; (c) Kámán, J.; Forró, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593–1600; (d) Li, X.-G.; Kanerva, L. T. *Adv. Synth. Catal.* **2006**, *348*, 197–205.
14. Forró, E.; Fülöp, F. *Org. Lett.* **2003**, *5*, 1209–1212.
15. Forró, E.; Paál, T.; Tasnádi, G.; Fülöp, F. *Adv. Synth. Catal.* **2006**, *348*, 917–923.
16. Theil, F. *Tetrahedron* **2000**, *56*, 2905–2919.
17. Li, H.; Argade, A.; Singh, R.; Thota, S.; Carroll, D.; Tso, K.; Taylor, V.; McLaughlin, J.; Markovstov, V. PCT Int. Appl. WO 2005/118544 A2, 2005.
18. Péter, A.; Árki, A.; Vékes, E.; Tourwé, D.; Lázár, L.; Fülöp, F.; Armstrong, D. W. *J. Chromatogr., A* **2004**, *1031*, 171–178.
19. Seki, M.; Matsumoto, K. *Tetrahedron Lett.* **1996**, *37*, 3165–3168.
20. Jefford, C. W.; McNulty, J.; Lu, Z.-H.; Wang, J. B. *Helv. Chim. Acta* **1996**, *79*, 1203–1216.