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Enantiopure *N*-Benzyloxycarbonyl-β²-amino Acid Allyl Esters from Racemic β-Lactams by Dynamic Kinetic Resolution using *Candida antarctica* Lipase B

Eleonora Gianolio,[+a] Resmi Mohan,[+a] and Albrecht Berkessel[a,*]

[a] University of Cologne, Department of Chemistry, Organic Chemistry, Greinstraße 4, 50939 Cologne, Germany
Fax: (+49)-221-470-5102; e-mail: berkessel@uni-koeln.de

[+] These authors contributed equally to the work.

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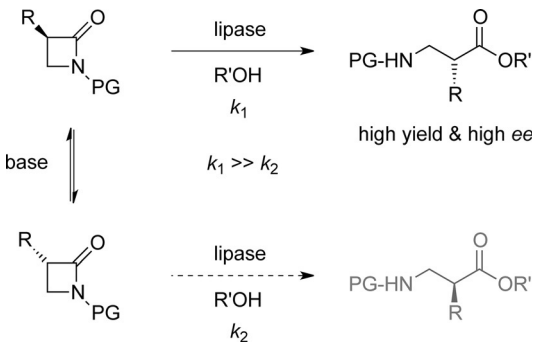
Abstract: The dynamic kinetic resolution of α-substituted racemic β-lactams by alcoholytic ring-opening, catalyzed by immobilized lipase B from *Candida antarctica* is described. With this process, a variety of racemic α-substituted *N*-Cbz-azetidinones (Cbz = benzyloxycarbonyl) was transformed to the corresponding *N*-Cbz-protected β²-amino acid allyl esters with high enantioselectivity (up to 99%) and high yields (up to quantitative) at room temperature.

Keywords: β-amino acids; *Candida antarctica* lipase B (CALB); dynamic kinetic resolution; β-lactams

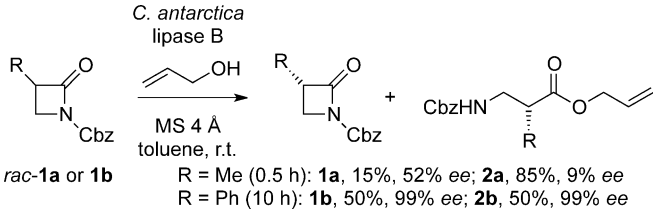
β-Amino acids, although less abundant than their α-analogues, are known to be valuable building blocks for the preparation of pharmaceuticals,[1] peptides[2] and natural products.[3] Established methods[4] for the preparation of β²-amino acids in enanatiopure form are, for example, catalytic asymmetric hydrogenation,[5] conjugate additions of carbon and nitrogen nucleophiles to α,β-unsaturated systems, the Mannich reaction[6] and the kinetic resolution (KR) of suitable racemic precursors.[7] Compared to purely chemical methods, only few biocatalytic options for the production of enantiopure β²-amino acids are described in the literature. The main routes are based on the KR of β-amino acid derivatives by lipases, lactamases, acylases, nitrilases, and nitrile hydrolases.[8] The major disadvantage of kinetic resolution in general, however, is the maximum yield of 50% of the desired enantiopure material. Dynamic kinetic resolution (DKR) overcomes this limitation by combining an enantiodiscriminative transformation with an *in situ* racemization process, affording (in the ideal case) enantiomerically pure product in quantitative yield.

As part of our interest in the development of catalytic asymmetric methods for the preparation of α-amino acids[9] and β-amino acids,[10] we recently focused on the use of suitably substituted β-lactams (2-azetidinones) as substrates for the preparation of enantiopure β²-amino acid derivatives by dynamic kinetic resolution (DKR). 2-Azetidinones are β-amino acid derivatives readily available in racemic form, for which, to the best of our knowledge, no enzymatic DKR has been reported as yet. Herein we present a method for the enantioselective synthesis of *N*-Cbz-protected β²-amino acid allyl esters by DKR of aryl-substituted β-lactams in the presence of *Candida antarctica* lipase B (CALB). In this approach, a catalytic amount of base and allyl alcohol as nucleophile are employed (Scheme 1), affording the corresponding *N*-Cbz β²-amino acid allyl esters in high enantiopurity (up to 99% *ee*) and high yields (up to quantitative).

Inspired by the work of Sharma et al.,[11] we found an efficient access to compounds *rac*-**1b–f** by dehydrative cyclization of the corresponding *N*-Cbz-3-amino-



Scheme 1. Alcoholytic dynamic kinetic resolution (DKR) of α-substituted racemic β-lactams.



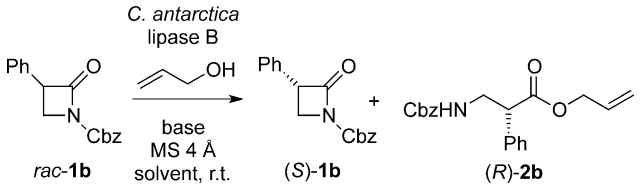
Scheme 2. CALB-catalyzed kinetic resolution of the β-lactams *rac*-**1a/b**.

2-arylpropanoic acids with phosphoryl chloride (see the Supporting Information). For the synthesis of *rac*-**1a** and *rac*-**1g**, the synthesis of the N-unprotected β-lactam followed by N-Cbz protection, as described by Murayama et al., proved superior.[12]

In an initial screening, the racemic α-substituted β-lactams synthesized were subjected to lipase-catalyzed alcoholytic ring opening in order to identify potential biocatalysts as well as suitable substrate structures for the subsequent development of the dynamic kinetic procedure (Scheme 2). For the α-methyl-substituted and *N*-Cbz-protected lactam *rac*-**1a**, from a set of 15 lipases, only five serine hydrolases proved to be active in the alcoholysis (see the Supporting Information). Among those, lipase B from *Candida antarctica* showed very high activity – 85% conversion after 30 min – but rather low selectivity for substrate *rac*-**1a** ($E = 1.8$). In contrast, the same enzyme provided excellent enantioselectivity acting on the α-phenyl-substituted derivative *rac*-**1b**, giving rise to high enantiopurity both for the product ester (*R*)-**2b** as well as for the remaining lactam (*S*)-**1b**. As it turned out, neither *N*-PMP (*p*-methoxyphenyl) nor *N*-unprotected β-lactams underwent this enzymatic alcoholysis efficiently.

Aiming for a dynamic kinetic resolution protocol, various organic bases were evaluated as racemization catalysts in the previously optimized enzymatic ring opening of *rac*-**1b**. The use of 0.5 equivalents of pyridine or Hünig's base (Table 1, entries 1 and 2), did not effect DKR, leading in both cases to simple KR. In the presence of the stronger base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), DKR was achieved, but the ester **2b** was obtained with 29% *ee* only (entry 3). 1.5-Diazabicyclo[4.3.0]non-5-ene (DBN) was finally chosen as racemization catalyst for the substrate *rac*-**1b**. When enantioenriched **1b** was exposed to 0.6 equiv. of this base, racemization was complete within *ca.* 10 min, both in the presence and absence of CALB. In the DKR experiments, the product enantiomeric excess could be further increased to 98%, by employing lower concentrations of the base, albeit at the expense of somewhat longer reaction times (entries 4–6). Variation of the solvent did not lead to further improvement, but identified both toluene and MTBE as the reaction media of choice (entries 6–9). Moreover, the effect of various alcohol nucleophiles

Table 1. Influence of base and solvent on the enzymatic resolution the β-lactam *rac*-**1b**.



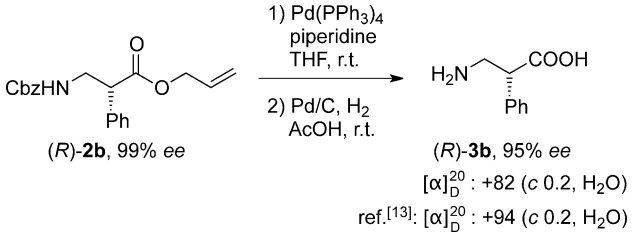
Entry	Base (equiv.)	Solvent	Time [h]	Conv.[a] [%]	*ee*[a] [%]
1	pyridine (0.5)	toluene	21	50	99
2	$(i\text{-Pr})_2$NEt (0.5)	toluene	21	50	99
3	DBU (0.5)	toluene	4	quant.	29
4	DBN (0.5)	toluene	4	82	41
5	DBN (0.2)	toluene	17	quant.	89
6	DBN (0.1)	toluene	20	quant.	98
7	DBN (0.15)	THF	24	50	70
8	DBN (0.15)	MTBE	24	quant.	95
9	DBN (0.15)	MeCN	24	33	79

[a] Conversion of *rac*-**1b** and *ee* of (*R*)-**2b** determined by HPLC.

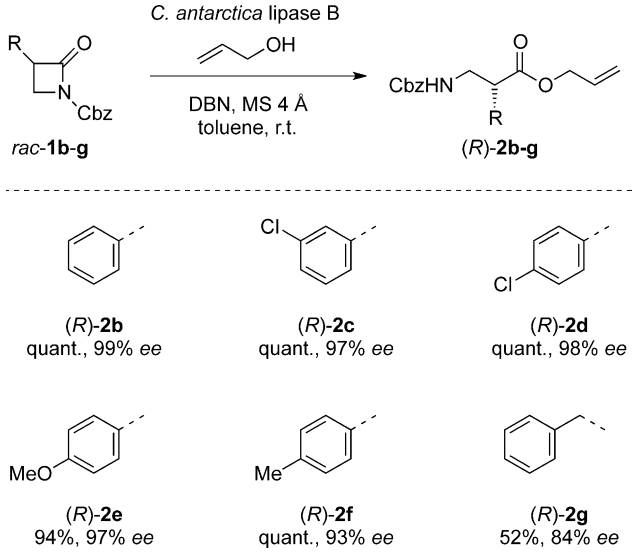
on the DKR protocol was surveyed. We were delighted to see that excellent enantiomeric excesses were obtained with other alcohols (such as ethanol, isobutyl alcohol and isopropyl alcohol, see the Supporting Information) as well.

For some batches of CALB, we observed that the CALB/DBN system effected clean KR, with no dynamic behavior, however. We suspected that the inhibition of racemization might be due to adventitious acidic impurities in some batches of the commercial acrylate resin-bound lipase. To our delight, pre-treatment of the resin-bound enzyme with NEt_3 restored the catalytic activity, and the alcoholytic lactam opening proceeded with the usual excellent yield and enantiopurity.

For the determination of the absolute configuration of the product *N*-Cbz-β²-amino acid allyl ester **2b**, the latter was subjected to global deprotection (Scheme 3). Note that this two-step protocol resulted only in a minor decrease of enantiopurity. Comparison of the specific rotation of the free amino acid **3b**



Scheme 3. Determination of the absolute configuration of the *N*-Cbz-β²-amino acid allyl ester **2b**.



Scheme 4. Scope and limitations for the DKR of racemic α-substituted β-lactams; yields/*ee*s of isolated products are given.

with literature data confirmed the (*R*)-selectivity of the lipase.[13] We assume that the stereoselectivity of CALB is analogous for the other substrates tested, i.e., the β^2-amino acid ester products **2a**–**g** (see below) are assumed to be of the (*R*)-configuration uniformly.

The scope of the chemo-enzymatic dynamic kinetic resolution was studied using the β-lactams *rac*-**1c–g**. In all cases, the *N*-Cbz β-amino acid allyl esters **2c–g** were obtained with very high optical purity and in high yields (Scheme 4). However, this study also revealed that an aromatic substituent is required for efficient dynamic kinetic resolution to occur. In the case of the α-benzyl-substituted β-lactam *rac*-**1g**, only kinetic resolution was observed under our optimized conditions. Most likely, this effect is due to the lower acidity of the lactam's α-proton in the presence of an aliphatic/benzylic substituent, as opposed to aromatic ones.

In conclusion, we report the dynamic kinetic resolution of racemic β-lactams by alcoholytic ring-opening, catalyzed by the commercially available *Candida antarctica* lipase B. The resulting enantiopure *N*-Cbz protected β^2-amino acid allyl esters are valuable building blocks for peptide synthesis, or can easily be deprotected to the parent β^2-amino acid.

Experimental Section

General Methods

All reactions were carried out under an argon atmosphere and with dry solvents, unless otherwise noted. Solvents were dried by standard procedures. All commercially available chemicals (purchased from Sigma Aldrich, Acros or Carbolution Chemicals) were used without further purification. CALB was applied in immobilized form as received from Sigma Aldrich. HPLC analysis was performed on Merck Hitachi HPLC (Chiralpak OJ, AD, AD-H) and PDA (photodiode array) detector. See the Supporting Information for the synthesis of the α-substituted racemic β-lactams *rac*-**1a–g**.

Dynamic Kinetic Resolution

In a typical experiment, the racemic α-substituted β-lactam *rac*-**1b** (281 mg, 1.00 mmol) was dissolved in dry toluene (10 mL), DBN (7.4 μL, 60 μmol), allyl alcohol (102 μL, 1.50 mmol) and immobilized *Candida antarctica* lipase B (CALB, 1.00 g) was added, together with ground molecular sieves 4 Å (300 mg). The reaction mixture was flushed with argon and stirred at room temperature. After 24 h, the mixture was filtered and the solvent was removed from the filtrate under vacuum. The crude product was purified by column chromatography on silica gel (cyclohexane:ethyl acetate 7:3), affording the *N*-Cbz β^2-amino acid allyl ester **2b** as a colorless solid; yield: 339 mg (quant.); 99% *ee*; mp 34 °C. ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 7.27–7.18$ (m, 10H), 5.81–5.68 (m, 1H), 5.13–5.01 (m, 5H), 4.52–4.49 (m, 2H), 3.88–3.83 (m, 1H), 3.62–3.49 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 172.4$ (t), 156.2 (s), 136.4 (s), 136.0 (d), 131.6 (d), 128.9 (d), 128.5 (d), 127.9 (d), 118.3 (t), 66.7 (t), 65.5 (t), 51.5 (d), 43.7 (s); elemental analysis: calcd.: C 70.78, H 6.24, N 4.13; found: C 70.71, H 6.13, N 4.11.

Supporting Information

The Supporting Information contains all experimental details for the synthesis and characterization of the racemic β-lactams (*rac*-**1a–g**) and *N*-Cbz β-amino acid allyl esters (**2a–g**). Furthermore, the procedures for the KR and DKR, methods used for HPLC analysis and retention times of substrates and products are given.

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References

[1] E. Juaristi, V. A. Soloshonok, in: *Enantioselective Synthesis of β-Amino Acids*, (Eds.: E. Juaristi, V. A. Soloshonok), Wiley-Interscience, Hoboken, **2004**, pp 19–90.
[2] a) D. Seebach, J. L. Matthews, *Chem. Commun.* **1997**, 2015–2022; b) J. Kapitán, F. Zhu, L. Hecht, J. Gardiner, D. Seebach, L. D. Barron, *Angew. Chem.* **2008**, *120*, 6492–6494; *Angew. Chem. Int. Ed.* **2008**, *47*, 6392–6394.
[3] a) J. Mittendorf, F. Kunisch, M. Matzke, H. C. Militzer, A. Schmidt, W. Schönfeld, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 433–436; b) F. von Nussbaum, P. Spiteller, in: *β-Amino Acids in Nature* in *Highlights in Bioorganic*

Chemistry: Methods and Applications, (Eds.: C. Schmuck, H. Wennemers), Wiley-VCH, Weinheim, **2004**, pp 63–89.

[4] For a review on β-amino acids, see: B. Weiner, W. Szymanski, D. B. Janssen, A. J. Minnaard, B. L. Feringa, *Chem. Soc. Rev.* **2010**, *39*, 1656–1691.

[5] a) D. Ager, in: *Science of Synthesis, Stereoselective Synthesis 1*, (Ed.: J. G. de Vries), Thieme, Stuttgart, **2011**, pp 185–256; b) H. Shimizu, I. Nagasaki, K. Matsumura, N. Sayo, T. Saito, *Acc. Chem. Res.* **2007**, *40*, 1385–1393; c) W. Tang, X. Zhang, *Chem. Rev.* **2003**, *103*, 3029–3069.

[6] a) N. Z. Burns, in: *Science of Synthesis, Stereoselective Synthesis 2*, (Ed.: G. A. Molander), Thieme, Stuttgart, **2011**, pp 785–834; b) A. E. Taggi, A. M. Hafez, T. Lectka, *Acc. Chem. Res.* **2003**, *36*, 10–19; c) A. Córdova, *Acc. Chem. Res.* **2004**, *37*, 102; d) Q. Wang, M. Leutzsch, M. van Gammeren, B. List, *J. Am. Chem. Soc.* **2015**, *135*, 15334–15337; e) J. W. Yang, C. Chandler, M. Stadtler, D. Kampen, B. List, *Nature* **2008**, *452*, 453–455; f) J. W. Yang, M. Stadler, B. List, *Angew. Chem.* **2007**, *119*, 615–617; *Angew. Chem. Int. Ed.* **2007**, *46*, 609–611.

[7] a) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173–180; b) S. Krauthäuser, L. A. Christianson, D. R. Powell, S. H. Gellman, *J. Am. Chem. Soc.* **1997**, *119*, 11719–11720.

[8] a) R. Sundell, L. T. Kanerva, *Eur. J. Org. Chem.* **2015**, 1500–1506; b) E. Forró, F. Fülöp, *Curr. Med. Chem.* **2012**, *19*, 6178–6187; c) B. A. Wu, W. Szymanski, S. de Wildeman, G. J. Poelarends, B. L. Feringa, D. B. Janssen, *Adv. Synth. Catal.* **2010**, *352*, 1409–1412; d) V. Gotor-Fernández, E. Busto, V. Gotor, *Adv. Synth. Catal.* **2006**, *348*, 797–812; e) A. Liljeblad, L. T. Kanerva, *Tetrahedron* **2006**, *62*, 5831–5854; f) M. Solymár, A. Liljeblad, L. Lazar, F. Fülöp, L. T. Kanerva, *Tetrahedron: Asymmetry* **2002**, *13*, 1923–1928; g) E. Forró, F. Fülöp, *Mini-Rev. Org. Chem.* **2004**, *1*, 93–102; h) R. Brieva, J. Z. Crich, C. J. Sih, *J. Org. Chem.* **1993**, *58*, 1068–1075.

[9] a) A. Berkessel, F. Cleemann, S. Mukherjee, T. N. Müller, J. Lex, *Angew. Chem.* **2005**, *117*, 817–821; *Angew. Chem. Int. Ed.* **2005**, *44*, 807–811; *Angew. Chem.* **2005**, *117*, 817–821; b) A. Berkessel, F. Cleemann, S. Mukherjee, T. N. Müller, J. Lex, *Chem. Commun.* **2005**, 1898–1900; c) A. Berkessel, S. Mukherjee, T. N. Müller, F. Cleemann, K. Roland, M. Brandenburg, J.-M. Neudörfl, J. Lex, *Org. Biomol. Chem.* **2006**, *4*, 4319–4330.

[10] a) A. Berkessel, F. Cleemann, S. Mukherjee, *Angew. Chem.* **2005**, *117*, 7632–7635; *Angew. Chem. Int. Ed.* **2005**, *44*, 7466–7469; b) A. Berkessel, I. Jurkiewicz, R. Mohan, *ChemCatChem* **2011**, *3*, 319–330; c) P. Renzi, C. Kronig, A. Carlone, S. Eröksüz, A. Berkessel, M. Bella, *Chem. Eur. J.* **2014**, *20*, 11768–11775.

[11] S. D. Sharma, R. D. Anand, B. Kaur, *Synth. Commun.* **2004**, *34*, 1855–1862.

[12] T. Murayama, T. Kobayashi, T. Miura, *Tetrahedron Lett.* **1995**, *36*, 3703–3706.

[13] E. Arvanitis, H. Ernst, A. A. Ludwig, A. J. Robinson, P. B. Wyatt, *J. Chem. Soc. Perkin Trans. 1* **1998**, 521–528.

