

PII: S0957-4166(96)00214-5

# Biocatalysis for the Preparation of Optically Active &-Lactam Precursors of Amino Acids

Péter Csomós,<sup>a,b</sup> Liisa T. Kanerva\*,<sup>a</sup> Gábor Bernáth<sup>b</sup> and Ferenc Fülöp<sup>b</sup>

<sup>a</sup>Departments of Chemistry and Biomedicine, University of Turku, FIN-20014 Turku, Finland <sup>b</sup>Institute of Pharmaceutical Chemistry, Albert Szent-Györgyi Medical University, Szeged, Hungary

Abstract: Enantioselective acylation of N-hydroxymethylated  $\beta$ -lactams in the presence of *Pseudomonas* sp. lipase afforded optically active precursors for the preparation of (1R, 2S)- and (1S, 2R)-2-aminocyclopentane- and (1R, 2S, 3R, 4S)- and (1S, 2R, 3S, 4R)-3-aminobicyclo[2.2.1]heptanecarboxylic acids. Due to the high enantio-selectivity (E = 90 and 62) and in order to minimize the enzymatic hydrolysis of the acylated products back to the starting alcohol, the reactions were performed in acetone. Copyright © 1996 Elsevier Science Ltd

Alicyclic  $\beta$ -aminocarboxylic acids are useful intermediates for the synthesis of a number of heterocyclic compounds with potential pharmacological activity.<sup>1,2</sup> Among such amino acids, cispentacin (FR 109615), (-)-(1*R*,2*S*)-2-aminocyclopentanecarboxylic acid, is a simple natural product with effective antifungal properties.<sup>3-6</sup> For *L*-aspartyl-2-aminocyclopentanecarboxylic acid methyl esters, the absolute configuration of the 2-aminocyclopentanecarboxylic acid strongly affects the taste of the dipeptide.<sup>7</sup> Moreover,  $\beta$ -aminocarboxylic acids can be introduced into peptides in order to increase their biological activities.<sup>8-11</sup>

Enantiopurity is a general demand in biologically and pharmaceutically active compounds. In our previous work, ten structurally different mono- and bicyclic  $\beta$ -aminocarboxylic acid esters were resolved through fast lipase-catalysed *N*-acylation at the stereogenic *R*-centre.<sup>12</sup> The products (one enantiomer as an unreacted aminoacid ester and the other as the corresponding amide) were readily transformed to the corresponding free amino acids. In the present work, we exploit a chemoenzymatic route to the enantiomers of *cis*-2-aminocyclopentane and *exo*-3-aminobicyclo[2.2.1]heptanecarboxylic acids as model compounds. 6-Azabicyclo[3.2.0]heptan-7-one **1a** and its labile *N*-hydroxymethyl derivative **2a** are versatile synthons for the preparation of cispentacin **7a** and the (1*S*,2*R*) enantiomer **5a** (Scheme 1). Similarly, starting with racemic *exo*-3-azatricyclo[4.2.1.0<sup>2.5</sup>]nonan-4-one **1b** leads to the enantiomers **5** and **7b** (Scheme 2). Racemic  $\beta$ -lactams **1a** and **b** are readily available, and their *N*-hydroxymethylation with paraformaldehyde affords racemic **2a** and **b**.<sup>13-15</sup> In this work, the enzymatic resolution of racemates **1** and **2** was the main goal, in order to obtain optically active intermediates leading to the final products.

## **Results and Discussion**

Lipases are generally ineffective for the cleavage of an amide bond.<sup>12</sup> To our knowledge, the only exception to this is the lipase-catalysed enantioselective ring opening of *N*-benzoyl-substituted azetidin-2-one by methanol.<sup>16</sup> Encouraged by this, we first studied the enzymatic ring opening of  $\beta$ -lactams **1a** and **b** directly to 2-aminocarboxylic acids **5a** and **b** or **7a** and **b** (Schemes 1 and 2). However, our efforts with various nucleophiles and lipases in organic solvents did not result in success. In accordance with the previous results on the unsaturated analogue of **1a**, commercial lactamases did not work either on the present substrates.<sup>17</sup> It seems that lactamases in a special whole-cell preparation (*Rhodococcus equi*, NCIB 40213) currently constitute the only system capable of enantioselective ring opening.<sup>17,18</sup>

#### Scheme 1



i. Paraformaldehyde, cat. K<sub>2</sub>CO<sub>3</sub>, cat. H<sub>2</sub>O, sonication in tetrahydrofurane. ii. Lipase AK, vinyl butyrate in acetone. iii. NH<sub>4</sub>OH/MeOH. iv.18% HCl

As the second possibility to  $\beta$ -aminocarboxylic acids, we relied upon the lipase-catalysed resolution of *N*-hydroxymethyl- $\beta$ -lactams **2a** and **b**. In this case, the resolution is based on the asymmetric acylation of the primary hydroxy group (Schemes 1 and 2; step ii). Owing to the remote position of the stereogenic centres from the reaction site, enantioselectivity for such compounds is often low. The reverse enzymatic reaction further tends to reduce enantiomeric purities at higher conversions, except when enol esters or acid anhydrides can be used as acyl donors.<sup>19,20</sup> In spite of these predictions, some *N*-hydroxymethylated monocyclic azetidinones and pyrrolidinones, 2-azabicyclo[2.2.1]hept-5-en-3-one and hydantoin derivatives have been successfully resolved by

lipase catalysis.<sup>15,20-24</sup> In accordance with these results, the screening of enzymes revealed the possibility of the use of *Pseudomonas* lipases AK and PS for the asymmetric acylation of **2a** and **b**. Thus, for the lipase AK- and PS-catalysed butyrylations of **2a**, values of E = 45 and 28 (enantioselectivity ratio values<sup>25</sup>), respectively, were observed in the presence of 2,2,2-trifluoroethyl butyrate in diethyl ether at room temperature. On the basis of our previous experience on the resolution of primary alcohols, lipase AK was chosen for further studies.<sup>19</sup>

## Scheme 2



 i. Paraformaldehyde, cat. K<sub>2</sub>CO<sub>3</sub>, cat. H<sub>2</sub>O, sonication in tetrahydrofurane. ii. Lipase AK, vinyl butyrate in acetone. iii. NH<sub>4</sub>OH/MeOH. iv.18% HCl

Lipase AK-catalysed butyrylation with vinyl butyrate in place of 2,2,2-trifluoroethyl butyrate gave E = 49. This value is very close to the above value of 45, indicating that the enzymatic reaction of 2,2,2-trifluoroethanol with **6a** back to **2a** should not disturb the enantiopurities obtainable. Unexpectedly, however, at conversions close to 50% or higher, the *E* values tended to drop dramatically for both vinyl and 2,2,2-trifluoroethyl butyrates as acyl donors. We have earlier demonstrated that in transesterifications the water adsorbed on the enzyme preparation can serve as a nucleophile, leading to ester hydrolysis.<sup>26</sup> It has now emerged that in diethyl ether racemic butyrate **6a** (25 mM) reacts to alcohol **2a** (28% conversion in 3 h; E = 3) in the presence of a lipase AK preparation without any added nucleophile except the water adsorbed on the seemingly dry catalyst. As a consequence of this reaction taking place in parallel with the lipase-catalysed butyrylation of

2a, there should be a decrease in the enantiopurities of the resolved products. In accordance with this, for the lipase AK-catalysed butyrylation of 2a (E = 45) at 55% conversion, for example the observed ee values of 0.72 for the product 6a and 0.88 for the unreacted 2a are lower than the theoretical values<sup>25</sup> of 0.81 and 0.99, respectively.

**Table 1.** Butyrylation of **2a** (25 mM) in the presence of lipase AK preparation<sup>a</sup> (25 mg ml<sup>-1</sup>) and 2,2,2-trifluoroethyl butyrate (0.05 M) in organic solvents at room temperature.

Solvent	log P <sup>b</sup>	Time/h	Conversion/%	E	
Acetonitrile	-0.33	2	37	62	
Acetone	-0.23	2.5	38	97	
Tetrahydrofuran	0.49	0.75	38	83	
Diethyl ether	0.85	0.75	42	45	
		1.5	43	65°	
2-Methyl-2-butanol	1.3	0.75	36	79	
tert-Butyl methyl ether	1.36	0.5	45	21	
3-Methyl-3-pentanol	1.8	1	37	48	
Diisopropyl ether	1.9	0.25	41	60	
Toluene	2.5	1.5	37	34	

<sup>a</sup>Contains 10% (w/w) of the lipase adsorbed on Celite in the presence of sucrose; refs. 12 and 28.  $^{\circ}P$  is the partition coefficient of the solvent between water and 1-octanol. <sup>c</sup>Reaction at 0 °C.

Manipulation of the enzymatic reactivity and enantioselectivity by an appropriate choice of solvent is a general method in optimizing enzyme-catalysed reactions. Clearly, the characteristics of the acyl donor, the acceptor and the solvent affect the enantioselectivity of an individual lipase.<sup>12,19,20</sup> As concerns the present work, this means the need to find a solvent where the enzymatic hydrolysis of **6a** (or **6b**) is minimized and where the enzymatic acylation of **2a** (or **2b**) proceeds with high enantioselectivity. Accordingly, the lipase AK-catalysed butyrylation of **2a** with 2,2,2-trifluoroethyl butyrate was conducted in different solvents (Table 1). The reactions proceeded smoothly in various hydrophobic and hydrophilic solvents, including both water-miscible and water-immiscible media. Interestingly, high enantioselectivities were observed in acetonitrile, acetone and tetrahydrofuran, the behaviour in acetone best following the above requirements. Accordingly, in this case less

than 5% of racemic butyrate **6a** (25 mM) was hydrolysed by the water adsorbed on the lipase AK preparation (25 mg ml<sup>-1</sup>) in 3 h.

**Table 2.** Butyrylation of **2a** and **b** (0.1 M) in the presence of lipase AK preparation<sup>a</sup> (50 mg ml<sup>-1</sup>) and vinyl butyrate (0.2 M) in acetone at  $0^{\circ}$ C.

Compound	Time/h	Conversion/%	ee <sub>p</sub> /%	ee <sub>s</sub> /%	E	_
2a	2.7	52	90 ( <b>6a</b> )	98 ( <b>3a</b> )	90	-
2b	3.5	53	86 ( <b>6b</b> )	98 ( <b>3b</b> )	62	

<sup>a</sup>Contains 10% (w/w) of the lipase adsorbed on Celite in the presence of sucrose; refs. 12 and 28

The enantioselectivity of lipase AK is clearly enhanced for the butyrylation of 2a when the reaction is conducted at lower temperatures (Table 1; row 5). In accordance with the previous results on the lipase AKcatalysed acylation of solketal,<sup>19</sup> butyric acid derivatives were deemed to be the most appropriate acyl donors also in the present work. Although 2,2,2-trifluoroethyl and vinyl butyrates seem to differ as acyl donors only as concerns the reactivity (Tables 1 and 2), there is a minor risk of reversible acyl transfer in the former case. Thus, for the acylation of solketal with 2,2,2-trifluoroethyl esters the reaction reached equilibrium at 96-98% conversion.<sup>19</sup> On the above basis, the gram-scale resolution of **2a** and **b** was performed with vinyl butyrate in acetone in the presence of lipase AK at 0 °C. The results are shown in Table 2 and in the Experimental section. The present resolutions afforded (15.5R)-6-hydroxymethyl-6-azabicyclo[3.2.0]heptan-7-one 3a and (1S,2R,5S,6R)-3-hydroxymethyl-3-azatricyclo[4.2.1.0<sup>2.5</sup>]nonan-4-one **3b** as the less reactive enantiomers (Schemes 1 and 2). Thus, the enzymatic acylation of  $\beta$ -aminoacid esters in the previous work<sup>12</sup> produced the enantiomer which is now obtained as the less reactive N-hydroxymethyl enantiomer. The absolute configuration was confirmed by treating 3a with NH<sub>4</sub>OH/MeOH, resulting in the specific rotation  $[\alpha]_D^{20} = +37.4$  (c = 1.0,  $CHCl_3$ ) for the product 4a. This value is opposite in sign to the value of -38 (c = 1.1,  $CHCl_3$ ) reported for the (1R,5S) enantiomer.<sup>27</sup> The absolute configuration of **3a** was also supported by the value  $\left[\alpha\right]_{D}^{20} = +4.4$  (c = 1.0,  $H_2O$ ) obtained after acid hydrolysis to 5a, compared to the value of -4.5 ( $c = 1.0, H_2O$ ) previously determined for cispentacin hydrochloride 7a.<sup>12</sup> Similarly, 3b was converted to the corresponding azetidinone 4b and amino acid hydrochloride **5b** with  $\left[\alpha\right]_{D}^{20} = -4.1$  (c = 2.0, MeOH). This value is again opposite in sign to the value of  $\left[\alpha\right]_{D}^{20} = -4.1$ +3.9 (c = 2, MeOH) obtained for (1S,2R,3S,4R)-7b in the previous work.<sup>12</sup> It is also worth mentioning that the same enantiopreference was observed for lipase PS and AK catalyses.

## Experimental

Lipases AK (from *Pseudomonas sp.*) and PS (from *Pseudomonas cepacia*) were products of Amano Pharmaceuticals Co. The enzymes were adsorbed on Celite in the presence of sucrose as previously described.<sup>12,28</sup> All the solvents were of the highest analytical grade and were dried over molecular sieves (3 Å) before use. For gram-scale resolution, acetone was dried over  $K_2CO_3$  for one day and distilled just before use. Vinyl butyrate was purchased from Tokyo Kasei Kogyo Co. The ester was redistilled before use. 2,2,2-Trifluoroethyl butyrate was prepared from 2,2,2-trifluoroethanol and butyric anhydride.

In a typical small-scale experiment, a solution (2 ml, 25 mM) of a racemate in an organic solvent was added to the lipase preparation (25 mg ml<sup>-1</sup>) and an acyl donor (50 mM in the reaction mixture) was pipetted in. The progress of the reaction was followed by taking samples from the reaction mixture at intervals. The unreacted *N*-(hydroxymethyl)azetidinone in the sample was derivatized with di*-tert*-butyl dicarbonate in the presence of 4-dimethylaminopyridine and pyridine before the gas chromatographic analysis on a Chirasil-*L*-Val column (25 m). For **4a** and **b**, the column was Chrompack CP-Cyclodextrin- $\beta$ -2,3,6-M-9. Optical rotations were determined with a JASCO Model DIP-360 digital polarimeter. [ $\alpha$ ]<sub>D</sub> values are given in units of 10<sup>-1</sup> deg cm<sup>2</sup> g<sup>-1</sup>.

Preparation of Racemic **2a** and **b**. 6-Azabicyclo[3.2.0]heptan-7-one **1a** (10 mmol, 1.11 g) was dissolved in tetrahydrofuran (25 ml). Paraformaldehyde (12 mmol, 0.36 g),  $K_2CO_3$  (1 mmol, 0.14 g) and  $H_2O$  (1 ml) were added. The solution was sonicated for 4 h. The solvent was evaporated off and the residue was dissolved in diethyl ether (50 ml). The solution was dried over Na<sub>2</sub>SO<sub>4</sub>. Evaporation off of the solvent and recrystallization from diisopropyl ether afforded colourless crystals of **2a** (1.06 g, 7.5 mmol; m.p. 47-48 °C). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 1.33-2.10 (6H, m, 3xCH<sub>2</sub>), 3.47 (1H, q. H-1, J=3.7, 4.5), 4.19 (1H, t, H-5, J=4.2), 4.52 (1H, d, CH<sub>2</sub>OH, J=10.5), 4.68 (1H, d, CH<sub>2</sub>OH, J=9.3).

Similarly, *exo*-3-azatricyclo[4.2.1.0<sup>2,5</sup>]nonan-4-one **1b** (10 mmol, 1.37 g) afforded white crystals of **2b** (1.56 g, 9.3 mmol; m.p. 62-65 °C). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>)  $\delta$  (ppm): 1.00-1.70 (6H, m, 3xCH<sub>2</sub>), 2.44 (1H, d, H-6, J=2.4), 2.53 (1H, d, H-1, J=3.7), 2.94 (1H, d, H-5, J=3.7), 3.56 (1H, d, H.2, J=3.7), 4.5-4.64 (2H, q, CH<sub>2</sub>OH, J=10.4, 11.4).

*Enzymatic Resolution of* **2a** and **b**. Racemic **2a** (4 mmol, 0.56 g) was dissolved in dry acetone (40 ml) and the temperature was decreased to 0 °C. The enzyme preparation (2 g) and vinyl butyrate (8 mmol, 0.91 g) were added and the mixture was stirred at 0 °C. After 160 minutes, triethylamine (10 drops) was added in order to enhance the stability of unreacted acid-labile **2a** and the enzyme was filtered off at 52% conversion. The acetone was evaporated off. The residue was chromatographed on silica, with elution with dichloromethane for separation of the product **6a** (0.38 g, 1.8 mmol;  $[\alpha]_D^{20}$ -33.1 (*c* = 1, CHCl<sub>3</sub>); ee 92%) as a pale-yellow oil. Elution

with dichloromethane:ethyl acetate (1:1) finally afforded the unreacted **3a** (0.23 g, 1.6 mmol;  $[\alpha]_D^{20}$ -32.4 (c = 1, CHCl<sub>3</sub>); ee 98%), also as a pale-yellow oil.

Similarly, racemic **2b** (4 mmol, 0.67 g) afforded the product **6b** (0.46 g, 2.0 mmol;  $[\alpha]_D^{20}$  -30.5 (c = 1, CHCl<sub>3</sub>); ee 90%) and unreacted **3b** (0.26 g, 1.5 mmol;  $[\alpha]_D^{20}$  +13.0 (c = 1, CHCl<sub>3</sub>); ee 98%), as colourless oils.

Acknowledgements. One of us (P.C.) is grateful for grants from the Turku University Foundation and the Centre for International Mobility (CIMO). Support from the Technology Development Centre (TEKES) in Finland is also gratefully acknowledged.

#### References

- 1. Bernáth, G.; Fülöp, F.; Stájer, G. Janssen Chimica Acta 1991, 9, 12.
- 2. Bernáth, G. Acta Chim. Hung. 1992, 129, 107.
- 3. Oki, T.; Hirano, M.; Tomatsu, K.; Numata, K.; Kamei, H. J. Antibiotics 1989, 42, 1756.
- 4. Konishi, M.; Nishio, M.; Saitoh, K.; Miyaki, T.; Oki, T.; Kawaguchi, H. J. Antibiotics 1989, 42, 1749.
- Iwamoto, T.; Tsujii, E.; Ezaki, M.; Fujie, A.; Hashimoto, S.; Okuhara, M.; Kohsaka, M.; Imanaka, H.; Kawabata, K.; Inamoto, Y.; Sakane, K. J. Antibiotics 1990, 43, 1.
- 6. Kawabata, K.; Inamoto, Y.; Sakane, K.; Iwamoto, T.; Hashimoto, S. J. Antibiotics 1990, 43, 513.
- 7. Yamazaki, T.; Zhu, Y.-F.; Probstl, A.; Chadha, R.K.; Goodman, M. J. Org. Chem. 1991, 56, 6644.
- 8. Mierke, D.F.; Nössner, G.; Schiller, P.W.; Goodman, M. Int. J. Pept. Protein Res. 1991, 35, 35.
- 9. Yamazaki, T.; Goodman, M. Chirality 1991, 3, 268.
- 10. Yamazaki, T.; Probstl, A.; Schiller, P.W.; Goodman, M. Int. J. Pept. Protein Res. 1991, 37, 364.
- Huang, Z.; Probstl, A.; Spencer, J.R.; Yamazaki, T.; Goodman, M. Int. J. Pept. Protein Res. 1993, 42, 352.
- Kanerva, L.T.; Csomós, P.; Sundholm, O.; Bernáth, G.; Fülöp, F. Tetrahedron: Asymmetry 1996, 7, submitted.
- 13. Nativ, E.; Rona, P. Isr. J. Chem. 1972, 10, 55.
- 14. Moriconi, E.J.; Crawford, W.C. J. Org. Chem. 1968, 33, 370.
- 15. Jouglet, B.; Rousseau, G. Tetrahedron Lett. 1993, 34, 2307.
- 16. Brieva, R.; Crich, J.Z.; Sih, C.J. J. Org. Chem. 1993, 58, 1068.
- 17. Evans, C.; McCague, R.; Roberts, S.M.; Sutherland, A.G.; Wisdom, R. J. Chem. Soc., Perkin 1 1991, 2276.
- Taylor, S.J.C.; McCague, R.; Wisdom, R.; Lee, C.; Dickson, K.; Ruecroft, G.; O'Brien, F.; Littlechild, J.; Bevan, J.; Roberts, S.M.; Evans, C.T. *Tetrahedron: Asymmetry* 1993, 4, 1117.
- 19. Vänttinen, E.; Kanerva, L.T. J. Chem. Soc., Perkin Trans. 1 1994, 3459.

- Kanerva, L.T. In *Enzymatic Reactions in Organic Media*, Koskinen, A.M.P.; Klibanov, A.M., Eds. Chapman & Hall, London, **1996**, Ch. 7, p 170.
- 21. Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. Chem. Pharm. Bull. 1992, 40, 2227.
- 22. Oumoch, S.; Rousseau, G. Bioorg. Med. Chem. Lett. 1994, 4, 2841.
- 23. Nakano, H.; Okuyama, Y.; Iwasa, K.; Hongo, H. Tetrahedron: Asymmetry 1994, 5, 1155.
- 24. Mizuguchi, E.; Achiwa, K.; Wakamatsu, H.; Terao, Y. Tetrahedron: Asymmetry, 1994, 5, 1407.
- 25.  $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$  or  $E = \ln[1-c(1+ee_p)]/\ln[1-c(1-ee_p)]$ ; Chen, C.-S.; Sih, C.J. Angew. Chem. Int. Ed. Engl. 1989, 28, 695.
- 26. Kanerva, L.T.; Sundholm, O. J. Chem. Soc., Perkin 1 1993, 1385.
- 27. Evans, C.T.; Roberts, S.M.; Sutherland, A.G. PCT Int. Appl. WO. 92/18477.
- 28. Kanerva, L.T.; Sundholm, O. J. Chem. Soc., Perkin Trans. 1 1993, 2407.

(Received in UK 28 March 1996)