Pergamon

Tetrahedron: Asymmetry, Vol. 8, No. 19, pp. 3231–3235, 1997
© 1997 Elsevier Science Ltd. All rights reserved.
Printed in Great Britain
0957-4166/97 $17.00 + 0.00

PII: S0957-4166(97)00405-9

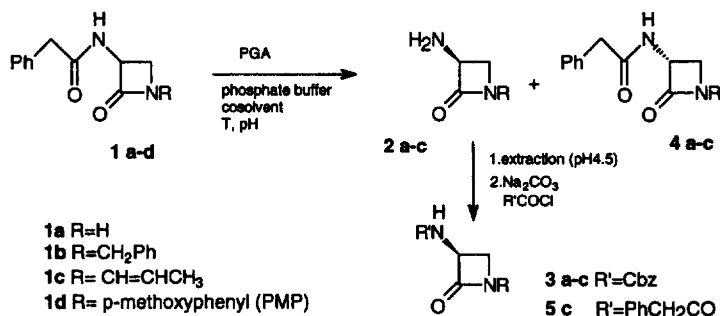
Penicillin G acylase mediated synthesis of the enantiopure (*S*)-3-amino-azetidin-2-one

Gianfranco Cainelli,[a] Daria Giacomini,[a,*] Paola Galletti [a] and Marco DaCol [b]

[a] Dipartimento di Chimica 'G.Ciamician', Università degli Studi di Bologna, Via Selmi 2, 40126 Bologna, Italy
[b] Biochimica OPOS SpA, Via delle Industrie 84, 20041 Agrate Brianza, Italy

Abstract: A detailed study of the influence of temperature, pH and enzyme amount on the enantiomeric excess in the enzymatic resolution of *N*-1 protected 3-amino-azetidin-2-ones with supported PGA is presented. Both enantiomers could be obtained in good enantiomeric excess. © 1997 Elsevier Science Ltd

The (*S*)-3-amino-azetidin-2-one unit is the core of the molecular structure of most important β-lactam antibiotics such as penicillins, cephalosporins and monobactams and could be used as a common intermediate for their synthesis.[1] As part of an ongoing project on the total synthesis of new β-lactam antibiotics, we became interested in the enantioselective synthesis of the (*S*)-3-amino-azetidin-2-one. We thought that an interesting alternative to its classical preparation through biosynthetic penicillin and cephalosporin degradation,[2] could be a facile racemic process using inexpensive starting materials followed by kinetic resolution by Penicillin G Acylase.[3] PGA is an enzyme produced by *E. coli* capable of transferring phenylacetyl groups from esters, amides or acids to acceptor molecules such as alcohols, amines or water; it is used on an industrial scale for the production of 6-APA and 7-ADCA, in peptide protective group chemistry and for the resolution of alcohols.[4] A recent important application of PGA in the field of β-lactams is represented by the kinetic enantioselective acylation of an intermediate in the synthesis of the new carbacephalosporin antibiotic Loracarbef, an analogue of the well known Cefaclor.[5] In our synthetic project, PGA should accomplish the kinetic enantioselective hydrolysis of the phenylacetamide bond in a racemic 3-amino-azetidin-2-one.[6] In this way the (*S*) enantiomer could be obtained as an amino group while the (*R*) enantiomer remains as such and the two species can be separated through a simple acid–base extraction. The (*S*) enantiomer is then recovered as a free amine or a suitable derivative ready for subsequent transformations (Scheme 1).



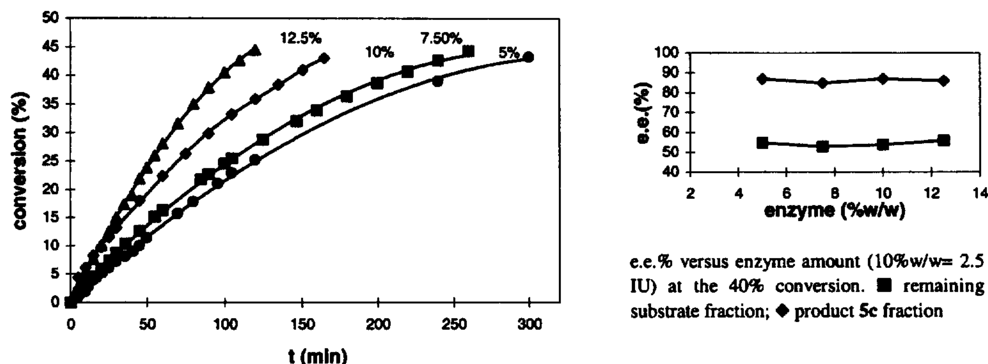
Scheme 1.

Screening for PGA activity was performed with 3-*N*-(phenylacetyloxy)-amino-azetidin-2-ones differently substituted on the *N*-1 position (Table 1).

* Corresponding author. Fax: +39 (51) 259456; Email: giacomin@ciam.unibo.it

Table 1. Enzymatic hydrolysis of β-lactams **1a–d** catalysed by PGA

compound	R	pH	conversion (%)	product	$[\alpha]_D$ (c, solvent)
1a	H	7.4	40	**3a**	-14.3 (1.47, CH_3OH)
1b	CH_2Ph	7.3	45	**3b**	-37.8 (1.03, CH_3OH)
1c	$CH{=}CHCH_3$	7.5	45	**3c**	-20 (1.5, CH_3OH)
1d	PMP	7.3	-	-	-



e.e.% versus enzyme amount (10%w/w= 2.5 IU) at the 40% conversion. ■ remaining substrate fraction; ◆ product **5c** fraction

Figure 1. Effect of variation of enzyme loading (% w/w) on conversion and e.e. in the hydrolysis of (±)-**1c**.

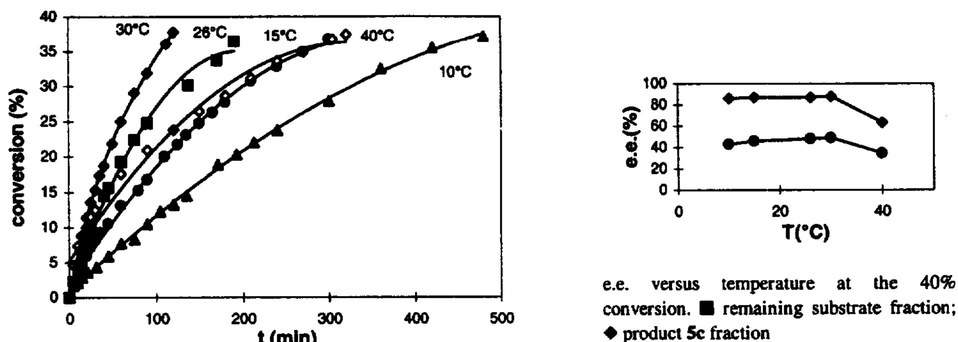
The experiments were carried out by incubating substrates (±) **1a–d** (1 mmol) in phosphate buffer and CH_3CN as a cosolvent (10%) at room temperature with PGA immobilised on Eupergit C beads (25 IU, 100 mg). As the pH of the reaction decreases during the hydrolysis, the rate of reaction and conversion were monitored by the consumption of 0.1 N NaOH added to maintain the initial value.[7] The crude reaction mixture was extracted at pH 4.5 with ethyl acetate, and the (*S*)-3-amino-azetidin-2-one separated in the aqueous phase and directly derivatized as (*S*)-3-carbobenzyloxy-3-amino-β-lactams. In that way the absolute configuration of **1a** was determined by comparison of the specific rotation.[8] Results in Table 1 reveal that PGA has a good tolerance with respect to the *N*-1 protective group because all tested azetidinones are accepted by this enzyme with the exception of **1d**, probably due to the insolubility of this substrate in the aqueous–organic reaction phase.

Time course studies using PGA and compound **1c** in acetone as a cosolvent show that the hydrolysis rate is strongly dependent on the enzyme loading (Figure 1).[9] As expected, the reaction took longer to reach 40% conversion at the lower enzyme amount, therefore the e.e. at 40% conversion did not undergo substantial variation.

The temperature effects on conversion and enantiomeric excess are reported in Figure 2. The range explored has been lower-limited to 10°C due to solubility problems. The enzymatic hydrolysis rate increases until 30°C and the e.e. values have no variation in the same T range. At 40°C there is a fall in the enantiomeric excess and in the rate[10] so that this temperature represents the upper working limit for the hydrolysis.

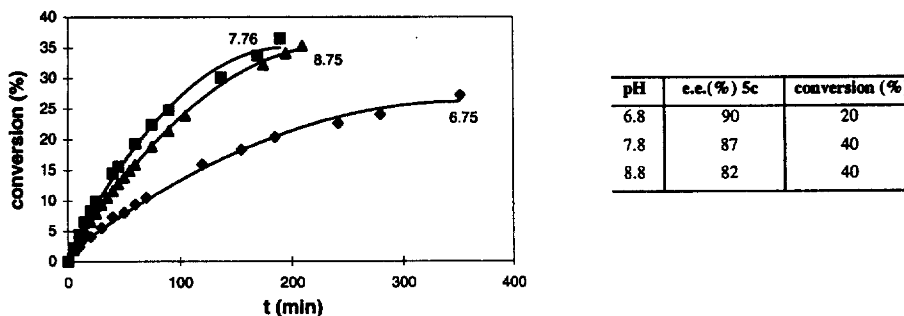
The pH value in the range 7.76–8.75 does not affect the conversion and the enantiomeric excess, while at pH 6.75 the reaction rate was lowered so that a conversion of more than 25% could not be reached (Figure 3).

A detailed study of the resolution of (±) **1c** was performed by monitoring the course of the e.e. in relation to the proceeding conversion at pH=7.8 and T=25°C with 10% w/w of enzyme. During the initial phase of the reaction, enantiomerically pure (*S*)-**5c** was found to be the product of the hydrolysis



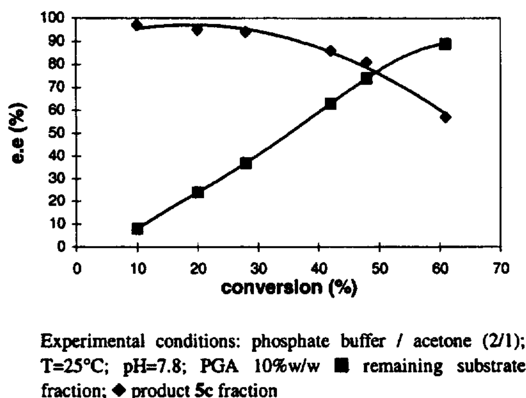
e.e. versus temperature at the 40% conversion. ■ remaining substrate fraction; ◆ product **5c** fraction

Figure 2. Effect of variation of reaction temperature on conversion and e.e. in the hydrolysis of (±)-**1c**.



pH	e.e.(%) 5c	conversion (%)
6.8	90	20
7.8	87	40
8.8	82	40

Figure 3. Effect of variation of pH on conversion and e.e. in the hydrolysis of (±)-**1c**.



Experimental conditions: phosphate buffer / acetone (2/1); T=25°C; pH=7.8; PGA 10%w/w ■ remaining substrate fraction; ◆ product **5c** fraction

Figure 4. Dependance of e.e. of product and substrate on the percentage conversion.

and a good kinetic resolution (e.e.>94%) was achieved until a conversion of 30%. Figure 4 shows that at a conversion of 40% or more, the decrease in e.e. seems to be significant. At 60% conversion, the remaining substrate fraction resulted in an 89% e.e., so that both enantiomers can be obtained with a good enantiomeric excess depending on the rate of conversion.

At various intervals, the values of the enantiomeric excess of the remaining substrate fraction ($e.e._s$) and of the product ($e.e._p$) were determined. The parameter describing the efficiency of the resolution was introduced as the dimensionless enantiomeric ratio E which remains constant throughout the reaction.[11,12] From the experimental values of $e.e._s$ and $e.e._p$ at 42% of conversion the enantiomeric

ratio has been calculated as E=25. This is generally considered a good value for a reliable industrial application.

In conclusion, we obtained the enantiopure (*S*)-3-amino-azetidin-2-one via a biocatalytic process by means of Penicillin G Acylase. The enzyme tolerates different *N*-1 substituents. A more detailed study on pH, temperature and conversion was performed on *N*-1-propenyl derivative obtaining a good enantiomeric ratio on both enantiomers depending on the conversion achieved.

Experimental

General

Penicillin G Amidase (PGA) was kindly furnished by Recordati (Italy). Starting materials were prepared according to the procedure reported by Cainelli *et al.*[6] During hydrolysis, the temperature was set and maintained at a constant value using a thermostatically controlled oil bath while the pH was kept constant with an automatic titrator (Metrohm 718 STAT Titrino). Quantitative analysis and conversions were determined by HPLC on a HP1900 instrument, inverse phase, column Aquapore RP300, 7 micron, H_2O/CH_3CN as mobile phase, λ=254nm. The enantiomeric excess was performed in HPLC: HP1900, direct phase, chiral column S,S-DACH.DNB Lichosorb si.100, 5 micron, 25 cm×4 mm, kindly furnished by Prof. Gasparrini, University of Rome (Italy), mobile phase: hexane/CH_2Cl_2/iPrOH 54/44/2, λ=254nm.

The proton magnetic resonance (^1H NMR) and carbon magnetic resonance (^{13}C NMR) were recorded on a Gemini 300-VARIAN Spectrometer. IR spectra were obtained on a Nicolet 205 FT IR. Optical rotations were measured on a Perkin–Elmer Polarimeter 343. Melting points are uncorrected.

Enzymatic hydrolysis of 1-N-(propen-1-yl)-3-N-(phenylacetyloxy)-amino-azetidin-2-one (±) 1c

Compound **1c** (100 mg, 0.41 mmol) was dissolved in acetone (2 mL) and diluted with 4 mL of 0.1 M phosphate buffer for pH 7.8 and for pH 6.8 or a mixture HCl/Na_3BO_3 for pH 8.8. The enzyme (40 IU, 10% w/w or the chosen amount) was added and the mixture stirred at 1000 rpm. pH was maintained at its initial value with the automatic addition of 0.1 N NaOH. At the desired conversion, the supported enzyme was filtered off, acetone was eliminated *in vacuo*, the pH of the aqueous phase was adjusted to 4.5 with diluted HCl and extracted with EtOAc (3×10 mL). In the organic phase (*R*)-**4c** was collected while the aqueous phase containing (*S*)-**2c** was treated with Na_2CO_3 (till pH 8) and phenylacetylchloride (1.5 eq) in acetone (10 mL) to obtain, after removing acetone *in vacuo* and extracting with AcOEt (3×10 mL), compound (*S*)-**5c**.

5c: $[\alpha]_D$=−30 (c=0.9, $CHCl_3$). e.e. 94%. m.p. 102°C. IR ($CHCl_3$, cm^{-1}): 3300, 1752, 1655. ^1H NMR (300 MHz, $CDCl_3$): 1.68 (dd, 3H, J_1=6.6 Hz, J_2=1.6 Hz, CH_3CH=CH); 3.25 (dd, 1H, J_1=2.6 Hz, J_{AB}=6.1 Hz, $CHCH_A\mathbf{H_B}$;); 3.56 (s, 2H, $PhCH_2CO$); 3.65 (dd, 1H, J_1=6.5 Hz, J_{AB}=6.5 Hz, $CHC\mathbf{H_A}H_B$); 4.9 (m, 1H, **CH**); 5.06 (m, 1H, CH_3CH=CH); 6.42 (dd, 1H, J_1=1.6 Hz, J_2=14.22, CH_3CH=**CH**); 7.08 (d, 1H, J=7.41 Hz, NH); 7.2–7.4 (m, 5H, arom). ^{13}C NMR (300 MHz, $CDCl_3$): 171.4; 162.8; 134.3: 128.2; 126.7; 121.8; 108.5; 55.2; 47.4; 43.0; 14.5.

Compounds (*S*)-**3a–c** were obtained with the same procedure reported above for **1c**, using CH_3CN (10%) as a cosolvent and 25 IU (100 mg) of enzyme for 1 mmol of substrate at pH 7.3–7.5 (see Table 1), at room temperature. Final derivatization of products (*S*)-**2a–c** was accomplished with benzylchloroformate (1.5 eq) instead of phenylacetylchloride.

(*S*)-**3a**: $[\alpha]_D$=−14.3 (c=1.47, CH_3OH). o.p. 80%. m.p. 48°C. IR (nujol, cm^{-1}): 3300, 1730, 1690. ^1H NMR (300 MHz, $CDCl_3$): 3.39 (dd, 1H, $CH\mathbf{H_A}H_B$, J_{AB}=5.0 Hz, J_1=2.0 Hz); 3.65 (dd, 1H, $CHCH_A\mathbf{H_B}$, J_{AB}=5.0 Hz, J_1=5.0 Hz); 4.88 (m, 1H, $\mathbf{CH}CH_AH_B$) 5.14 (s, 2H, $Ph\mathbf{CH_2}O$); 5.34 (bs, 1H, **NH**); 5.72 (bs, 1H, **NH**); 7.37 (m, 5H, **Ph**). ^{13}C NMR (300 MHz, CD_3OD): 171.7; 158.2; 138.3; 129.8; 129.4; 129.2; 68.1; 59.8; 45.1.

(*S*)-**3b**: $[\alpha]_D$=−37.8C (c=1.03, CH_3OH). m.p. 103°C. I.R. ($CHCl_3$, cm^{-1}): 3300, 1752, 1722. ^1H NMR (200 MHz, $CDCl_3$): 3.18 (dd, 1H, $CHC\mathbf{H_A}H_B$, J_{AB}=5.2 Hz, J_1=2 Hz); 3.47 (dd, 1H, $CHCH_A\mathbf{H_B}$, J_{AB}=5.2 Hz, J_1=5.4 Hz); 4.42 (s, 2H, $N\mathbf{CH_2}Ph$) 4.83 (m, 1H, $\mathbf{CH}CH_AH_B$) 5.11 (s, 2H, $Ph\mathbf{CH_2}O$);

5.47 (d, 1H, **NH**, J=5.8 Hz). ^{13}C NMR (200 MHz, $CDCl_3$): 166.3; 155.5; 135.9; 135.0; 128.8; 128.5; 128.2; 128.1; 127.8; 67.2; 57.6; 48.3; 46.0.

(*S*)-**3c**: $[\alpha]_D$=−20 (c=1.5, CH_3OH). m.p. 85°C. I.R. ($CHCl_3$, cm^{-1}): 3300, 1750, 1691. ^{1}H NMR (200 MHz, $CDCl_3$): 1.68 (d, 3H, J=6.6 Hz, $\mathbf{CH_3}$CH=CH); 3.38 (dd, 1H, J_1=2.4 Hz, J_{AB}=5.8 Hz, CHCH$_A$**H$_B$**); 3.7 (dd, 1H, J_1=5.5 Hz; J_{AB}=5.8 Hz, CHC**H$_A$**H$_B$); 4.84 (m, 1H, **CH**); 5.12 (m, 3H, PhC**H$_2$**OCO, CH_3**CH**=CH); 6.5 (d, 1H, J=14.2, CH_3CH=**CH**); 6.5 (d, 1H, J=6.8 Hz, NH); 7.36 (m, 5H, arom). ^{13}C NMR (200 MHz, $CDCl_3$): 162.5; 155.5; 135.9; 128.4; 128.1; 127.9; 122.0; 108.5; 67.2; 56.7; 47.6; 14.5.

Acknowledgements

This work was financially supported by Murst, CNR and University of Bologna (fund for selected topics). The authors are grateful to Miss Micaela Fabbri for HPLC analysis.

References

1. van der Steen, F. H.; van Koten, G. *Tetrahedron* **1991**, *47*, 7503.
2. Stoodley, R. J. in *Recent Advances in the Chemistry of β Lactam Antibiotics* Elks, J., Ed., The Chemical Society, London, **1977**, 190.
3. Duggleby, H. J.; Tolley, S. P.; Hill, C. P.; Dodson, E. J.; Dodson, G.; Moody, P. C. E. *Nature* **1995**, *373*, 264.
4. Baldaro, E.; Fuganti, C.; Servi, S.; Tagliani, A.; Terreni, M. in *Microbial Reagents in Organic Synthesis* Kluwer Academic Publishers, **1992**, 175.
5. Briggs, B. S.; Wright, I. G.; Zmijewski, M. J. *New J. Chem.* **1994**, *18*, 425.
6. Cainelli; G.; DaCol; M.; Galletti; P.; Giacomini, D. *Synlett* **1997**, 923.
7. The correction factor between NaOH titration and real conversion has been established through a calibration plot and a quantitative HPLC analysis.
8. Cimarusti, C. M.; Applegate, H. E; Chang, H. W.; Floyd, D. M.; Koster, W. H.; Slusarchyk, W. A.; Young, M. G. *J. Org. Chem.* **1982**, *47*, 182.
9. Conversion % and e.e.% were determined through chiral HPLC on phenylacetamide derivatives (*R*) **4c** and (*S*) **5c**.
10. Work is in progress in order to understand these results.
11. Chen, C. S.; Fujimoto, Y.; Girdakaus, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
12. The enantiomeric ratio E was calculated with the equation reported in Stecher, H.; Faber, K. *Synthesis* **1997**, 1.

(Received in UK 18 July 1997; accepted 3 September 1997)