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Porcine pancreatic lipase mediated regio- and stereoselective hydrolysis: chemoenzymatic synthesis of (2*S*,3*S*)-2-amino-3,4-dihydroxybutyric acid†

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Abstract—Porcine pancreatic lipase was used in the chemoenzymatic hydrolysis of 2-azido-3-hydroxy-4-methylcarbonyloxybutyl acetate. The reaction occurred with high regio- and stereoselectivity to give enantiomerically pure (2*S*,3*R*)-3-azido-2,4-dihydroxy butyl acetate **5** (e.e. >99%) which was easily converted to (2*S*,3*S*)-2-amino-3,4-dihydroxybutyric acid **1**, an important synthetic intermediate in the synthesis of β-lactam antibiotics and phytosiderophores. © 2001 Elsevier Science Ltd. All rights reserved.

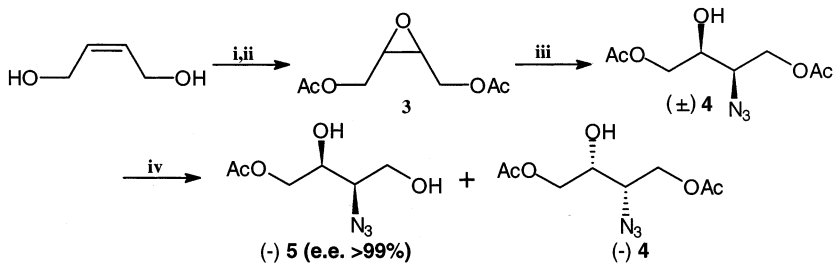
1. Introduction

β-Hydroxy-α-amino acids are constituents of several compounds, such as peptides, polyoxins and enzyme inhibitors, covering a wide range of biological activity from antibiotic to immunosuppressive properties,[1] hence, their stereoselective synthesis is of great interest. In particular, (2*S*,3*S*)-2-amino-3,4-dihydroxybutyric acid **1** has been used as a synthetic intermediate in the synthesis of β-lactam antibiotics and phytosiderophores.[2]

2. Results and discussion

In continuation of our studies[3] on the chemoenzymatic syntheses of biologically active compounds, we present an efficient preparation of the title compound. The stereoselective porcine pancreatic lipase (PPL) mediated hydrolysis of 2-azido-3-hydroxy-4-methylcarbonyloxy butyl acetate was the key step in this synthesis (Scheme 1).

Commercially available *cis*-1,4-butenediol in pyridine was treated with Ac_2O to obtain the diacetate **2** in 90% yield. Epoxidation of the olefin functionality of **2** was achieved with dimethyldioxirane (prepared in situ by the reaction of Oxone and acetone) at 25°C and pH ~7.2 to yield 3-methylcarbonyloxymethyl-2-oxiranyl-methyl acetate **3**, which was then opened cleanly with NaN_3 and NH_4Cl in 80% aqueous ethanol at 80°C to obtain the *syn*-2-azido-3-hydroxy compound **4** in high 86% overall yield from **2**. The racemic azido alcohol (50



Scheme 1. (i) Ac_2O/pyridine; (ii) Oxone, acetone, 27°C, pH 7.2; (iii) NaN_3, NH_4Cl, aq. EtOH; (iv) porcine pancreatic lipase, pH 7.2.

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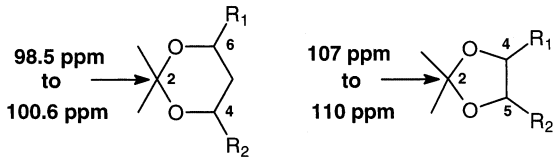


Figure 1.

mmol) was subjected to enzymatic hydrolysis with porcine pancreatic lipase (EC 3.1.1.3, 500 mg, 170 units/mg, Sigma, Type II) at 30°C in 0.01 M Tris–HCl buffer containing 0.1 M aqueous NaCl (50 mL, pH 7.2). The pH of the reaction was maintained by the constant addition of 0.2N aqueous sodium hydroxide and the reaction was followed by monitoring the amount of NaOH consumed. The reaction was stopped after 1 hour when hydrolysis was 35% complete. Extraction with ethyl acetate and purification of the crude product by silica column chromatography with hexane/ethyl acetate (4:1) afforded the 1,3-diol **5** in 30% yield, whilst the unreacted azido alcohol **4** was returned from this process in 62% yield.[4]

2.1. Regioselectivity of enzymatic hydrolysis

For the determination of regioselectivity in the enzymatic hydrolysis, diol **5** was subjected to periodate oxidation. The absence of any cleavage product indicated that **5** was a 1,3-diol. For further confirmation of the structure, diol **5** was converted to its acetonide with 2,2-dimethoxypropane in the presence of catalytic CSA in 92% yield.

Based on the work reported earlier by Rychnovsky et al. for six-membered ring acetonides[5a] and Dana et al. for five-membered ring acetonides (Fig. 1),[5b] using the chemical shifts of the acetonide carbons in ^{13}C NMR spectroscopy allows the differentiation between 1,2- and 1,3-diols.

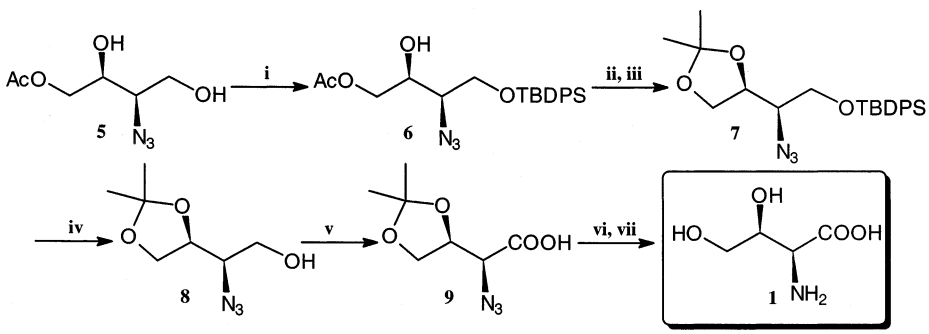
A clear signal at $\delta = 99.46$ and the absence of a peak in the region of 107–110 ppm in the ^{13}C NMR spectrum[6] of acetonide confirmed that the enzymatic hydrolysis reaction led to the formation of the 1,3-diol.

2.2. Stereoselectivity of enzymatic hydrolysis

The absolute stereochemistry at the centre generated in enzyme mediated hydrolysis was determined as (*S*) by preparing the title compound (2*S*,3*S*)-2-amino-3,4-dihydroxybutyric acid **1** from **5**. To determine the e.e., both **4** and **5** were completely acetylated with Ac_2O. The corresponding triacetyl derivative gave excellent resolution on a Chiralcel OJ column (Daicel, Japan, 5×250 mm) with retention times as follows: 2-azido-3,4-di(methylcarbonyloxy)-(2*S*,3*R*)-butyl acetate 30.1 min; 2-azido-3,4-di(methylcarbonyloxy)-(2*R*,3*S*)-butyl acetate 34.5 min with propan-2-ol (10%) in hexane mobile phase and a flow rate of 0.4 mL min^{-1}. Detection of the product was by eluate analysis at 215 nm. Product **5** was observed to be enantiomerically pure with an e.e. of >99%, while recovered starting diacetate **4** was found to have an e.e. of 55%.

2.3. Synthesis of (2*S*,3*S*)-2-amino-3,4-dihydroxybutyric acid

The synthesis of (2*S*,3*S*)-2-amino-3,4-dihydroxybutyric acid **1** was successfully carried out in the following manner (Scheme 2). Protection of the primary alcohol functionality of the 1,3-diol **5** with TBDPSCl in the presence of imidazole occurred with high chemoselectivity and afforded (2*S*,3*R*)-3-azido-2-hydroxy-4-(*tert*-butyldiphenyl)silyloxybutyl acetate **6** in 89% yield. Deacetylation of **6** was accomplished with K_2CO_3 in 50% aq. methanol to furnish the 1,2-diol in 95% yield, which was then cleanly protected as its acetonide **7**. Removal of the silyl ether protecting group by treatment with TBAF in THF then gave alcohol **8**. Oxidation[7] of **8** with $RuCl_3{\cdot}3H_2O$ and $NaIO_4$ at 0°C cleanly provided the requisite acid **9** in 85% yield and the acetonide of **9** was cleaved using an acidic resin (Amberlyst 15, Fluka) at 50°C in methanol to afford a dihydroxyazide which was subjected to catalytic hydrogenation with palladium on carbon catalyst to give the target dihydroxy amino acid **1** in 70% yield with >99% e.e. Additionally, **1** had identical spectral and physical data to those reported in the literature.[2a,8]



Scheme 2. (i) TBDPSCl/imidazole; (ii) K_2CO_3/CH_3OH; (iii) 2,2-dimethoxypropane, CSA; (iv) TBAF/THF; (v) $RuCl_3{\cdot}3H_2O$, $NaIO_4$, CCl_4, CH_3CN, H_2O; (vi) Amberlyst 15, CH_3OH, 50°C; (vii) Pd/C, H_2, CH_3OH.

3. Conclusion

We have presented a simple and convenient approach to the title compound and also demonstrated the preparation of the highly functionalised intermediate, **5**, with high enantiomeric purity using a simple PPL catalysed hydrolytic reaction where high regiospecificity coupled with high stereoselectivity is observed.[9] Earlier reports on the resolution of azido alcohols[10] have been mainly confined to the use of lipases from *Candida cylindracea* and *Pseudomonas* sp. and although excellent enantio- and diastereoselectivities have been observed in the enzymatic reactions, high regioselectivities such as those observed in the present case have not previously been observed because both primary and secondary hydroxyl groups are usually found to react under such conditions.[10g,h] The methodology presented here is amenable to further extension. Additionally, intermediate **5** can be used in the synthesis of several biologically active compounds.

Acknowledgements

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4. **4**: IR (neat) 3600–3200, 2120, 1735 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.1 (s, 6H, $(COCH_3)_2$), 2.3 (br s, 1H, OH), 3.7 (m, 1H, CHN_3), 3.8 (br s, 1H, CHOH), 4.15 (m, 2H, OCH_2CHOH), 4.3 (m, 2H, OCH_2CHN_3); $[\alpha]_D^{25}=-10.0$ (*c* 1.0, $CHCl_3$). **5**: IR (neat) 3600–3250, 2110, 1740 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.1 (s, 3H, $COCH_3$), 2.9 (br s, 1H, OH), 3.15 (br s, 1H, OH), 3.5 (m, 1H, CHN_3), 3.9 (m, 2H, OCH_2CHOH); 4.0 (m, 1H, CHOH), 4.2 (d, 2H, $J=6.5$ Hz, OCH_2CHN_3); mass: 189 M^+; $[\alpha]_D^{25}=-24.7$ (*c* 0.8, $CHCl_3$). **6**: IR (neat): 3450 (br), 2115, 1735 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.1 (s, 9H, $C(CH_3)_3$), 2.05 (s, 3H, $COCH_3$), 2.3 (d, 1H, OH), 3.5 (m, 1H, CHN_3), 3.9 (m, 3H, OCH_2CHO, OCH_2CHO and OCH_2CHN_3), 4.1 (m, 2H, OCH_2CHO and OCH_2CHN_3), 7.4 (m, 6H, Ph), 7.65 (m, 6H, Ph); mass: 427 (M^+); $[\alpha]_D^{25}=-16.4$ (*c* 1, $CHCl_3$). **8**: IR (neat): 3550–3400 (br), 2120, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.38 (s, 3H, CH_3), 1.48 (s, 3H, CH_3), 1.8 (br, 1H, OH), 3.4 (m, 1H, CHN_3), 3.72 (m, 2H, CH_2OH), 3.85 (m, 1H, OCH_2), 4.05 (m, 1H, OCH_2), 4.25 (m, 1H, OCH); mass: 187 (M^+); $[\alpha]_D^{25}=-12.6$ (*c* 1.0, $CHCl_3$). **9**: IR (neat): 2115, 1690 cm^{-1}; ^1H NMR ($CDCl_3$): δ 1.35 (s, 3H, CH_3), 1.5 (s, 1H, CH_3), 3.75 (d, 1H, $J=5$ Hz, CHN_3), 3.98 (m, 1H, OCH_2CHO), 4.15 (m, 1H, OCH_2CHO), 4.55 (m, 1H, $(OCH_2)CHO$); mass: 201 (M^+); $[\alpha]_D^{25}=-8.5$ (*c* 1.0, MeOH).
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6. ^{13}C NMR ($CDCl_3$): 18.35, 20.61, 28.38, 53.53, 63.77, 64.06, 69.60, 99.46, 170.42.
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8. **1**: mp 215°C; (lit[2a] 214°C); $[\alpha]_D^{25}=-13.4$ (*c* 2, water), lit[2a] −13.5 (*c* 2, water). ^1H NMR (D_2O): δ 3.49–3.61 (m, 3H, $CHNH_2$, CH_2OH), 3.98 (m, 1H, $(HOCH_2)CHOH$).
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