COMMUNICATION

Efficient chemoenzymatic synthesis of (2*S*,3*S*)-3-hydroxyleucine mediated by immobilised penicillin G acylase †

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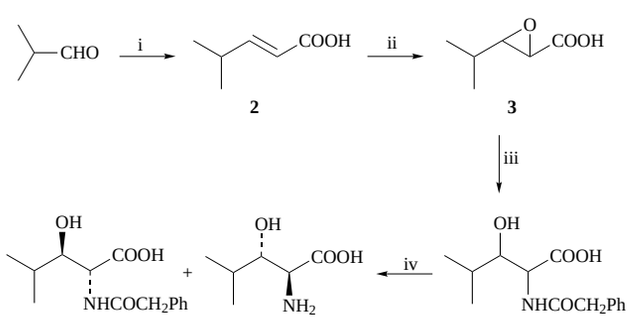
Immobilised penicillin G acylase (EC 3.5.1.11) has been used in the key step to obtain optically pure (2*S*,3*S*)-(+)-hydroxyleucine (ee >99%).

(2*S*,3*S*)-(+)-3-Hydroxyleucine **1** is an important constituent of a range of naturally occurring cyclodepsipeptides that include telomycin,[1] azinothricin,[2] citropeptin,[3] variapeptin,[4] A83586C,[5] L-156,602[6] and verucopeptin.[7] Even though several elegant asymmetric syntheses have been described in the literature,[8] no enzyme-catalysed resolution step has been reported for the synthesis of optically pure **1**. Here we describe a highly convenient and efficient chemoenzymatic synthesis of (2*S*,3*S*)-(+)-3-hydroxyleucine where immobilised penicillin G acylase (EC 3.5.1.11) has been used in the key step for resolution of the racemic hydroxy amino acid to obtain optically pure **1** (ee >99%). Although applications of penicillin acylase in the production of 6-aminopenicillanic acid[9] and kinetic resolution of racemic alcohols,[10] amines[11] and β-amino acids[12] are well-known, ours is the first example where penicillin acylase has been used in the resolution of a β-hydroxy-α-amino acid.

A simple Doebner condensation between isobutyraldehyde and malonic acid in pyridine solution[13] affords the *trans*-α,β-unsaturated acid **2** as a single isomer in 80% yield. Epoxidation of **2** with dimethyldioxirane (prepared *in situ* by the reaction of Oxone and acetone[14]) gave highly stable *cis*-epoxy acid **3** in almost quantitative yield. In agreement with the results of Caldwell and Bondy, opening of epoxy acid **3** with aqueous ammonia occurred regioselectively[15] in a *trans* fashion to give racemic hydroxyleucine (95%). This was converted to its *N*-phenylacetyl derivative **4** (95%) and subjected to enantioselective hydrolysis with immobilised penicillin G acylase in aqueous solution at pH 7.8 to obtain optically pure (2*S*,3*S*)-(+)-3-hydroxyleucine **1** (ee >99%, 40% hydrolysis) (Scheme 1).



Scheme 1 *Reagents and conditions*: i, $CH_2(COOH)_2$, pyridine, 0 °C, 2 h, 80 °C, 8 h, 80%; ii, Oxone, acetone, $NaHCO_3$, 24 °C, 2 h, 98%; iii, aq. NH_3, reflux, 6 h, $PhCH_2COCl$, OH^-, 0 °C, 2 h, 95%; iv, immobilized penicillin G acylase, pH 7.8–8.0, 1 h, 40% conversion, 71% of theoretical yield

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The optical purity of **1** was determined by converting the crude lyophilised product into its *N*-phenylacetyl derivative. (Chiralcel OJ, Daicel, Japan; 5 × 250 mm, λ 254 nm; 15% propan-2-ol in hexane containing 0.1% TFA; flow rate 0.7 ml min^{-1}; retention times (2*R*,3*R*): 7.3 and (2*S*,3*S*): 10.2 min). We have found that this method is very reliable and especially useful in cases of amino acids and amino alcohols whose enantiomers do not separate well on a Crownpack CR (+) column; or where the detection wavelength is less than 230 nm and the presence of impurities in the crude sample interfere in the analysis. Full details about this methodology will be published in due course.

Experimental

(2*S*,3*S*)-3-Hydroxyleucine 1

N-Phenylacetyl-3-hydroxyleucine **4** (530 mg, 2 mmol)‡ was dissolved in water (10 cm^3), the pH adjusted to 7.8 with 5 M aqueous ammonia and immobilised penicillin G acylase (100 mg, 20 units) added. The reaction mixture was shaken in a conical flask at 200 rpm (35 °C) and the reaction was followed by HPLC analysis (Chrompack C8, 5 × 250 mm, λ 254 nm, 40% acetonitrile–water containing 0.2% phosphoric acid, flow rate 1 ml min^{-1}; retention times **4**: 4.5 min, phenylacetic acid: 5.2 min). The reaction was stopped at 40% hydrolysis (1 h) and the enzyme was filtered off and washed with water. The combined aqueous layer was acidified with conc. HCl and then extracted with dichloromethane to remove unreacted amide and phenylacetic acid. The aqueous solution was lyophilised to get a white powder which after recrystallisation from water–methanol[8a] gave white crystalline (2*S*,3*S*)-3-hydroxyleucine **1**: (105 mg, 71% of theoretical), mp 221–223 °C (lit.,[7] 219–221 °C) ee >99%; $[\alpha]_D^{20}$ +26.0 (*c* 2.0 in water) (lit.,[1] $[\alpha]_D$ +22 (*c* 2.0 in water); $\delta_H(D_2O$, 200 MHz) 3.90 (1H, d, *J* 2.5,§ C*H*NH), 3.50 (1H, dd, *J* 9.2, 3.1, C*H*OH), 1.93 [1H, m, C*H*$(CH_3)_2$], 1.00 (3H, d, *J* 6.2, CH_3), 0.97 (3H, d, *J* 6.2, CH_3). Compound **4** (300 mg, 94%); mp 189–191 °C, ee 45%; $[\alpha]_D^{20}$ +1.1 (*c* 1.0 in CH_3OH).

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‡ Selected ^{1}H NMR spectral data for **4** and the intermediates leading to **4**: **2** $\delta_H(CDCl_3$, 200 MHz) 10 (1H, br, COOH), 6.95 (1H, dd, *J* 15.5, 6.5, C*H*COOH), 5.70 [1H, d, *J* 15.5, C*H*$CH(CH_3)_2$], 2.51 [1H, m, C*H*$(CH_3)_2$], 1.01 [6H, d, *J* 6.45, $(CH_3)_2$CH]; **3** $\delta_H(CDCl_3$, 200 MHz) 8.65 (1H, br s, COOH), 3.28 (1H, d, *J* 2.4, C*H*COOH), 2.95 [1H, dd, *J* 6.0, 2.4, C*H*$CH(CH_3)_2$], 1.65 [1H, m, C*H*$(CH_3)_2$], 1.06 (3H, d, *J* 6.7, CH_3), 1.01 (3H, d, *J* 6.7, CH_3); **4** $\delta_H[CDCl_3 + (CD_3)_2SO]$ 7.56 (1H, br d, NH), 7.28 (5H, m, Ph), 4.55 (1H, dd, *J* 6.2, 3.1, C*H*COOH), 3.60 (2H, 2s, CH_2Ph), 3.39 (1H, dd, *J* 6.2, 2.9, C*H*OH), 1.89 [1H, m, C*H*$(CH_3)_2$], 1.05 (3H, d, *J* 6.2, CH_3), 1.00 (3H, d, *J* 6.2, CH_3).

§ *J* Values are given in Hz.

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