Enzymatic Synthesis of Bicyclic γ-Lactams using Clavaminic Acid Synthase

Jack E. Baldwin,^a Robert M. Adlington,^a Justin S. Bryans,^a Matthew D. Lloyd,^a Timothy J. Sewell,^a Christopher J. Schofield,^a K. H. Baggaley^b and R. Cassels^b

^a The Dyson Perrins Laboratory and the Oxford Centre for Molecular Sciences, South Parks Road, Oxford OX1 3QY, UK ^b SmithKline Beecham Pharmaceuticals, Brockham Park, Betchworth, Surrey RH3 7AJ, UK

Incubation of a γ -lactam analogue of proclavaminic acid with clavaminic acid synthase, gave two novel bicyclic γ -lactams.

Clavulanic acid 1 is a commercially important β -lactamase inhibitor.¹ Studies on the biosynthesis of 1 by the SmithKline Beecham group resulted in the isolation of clavaminic acid 2 which was shown to be a precursor of clavulanic acid 1. Furthermore a monocyclic β -lactam, proclavaminic acid 3 was also isolated, which in turn was shown to be a precursor of clavaminic acid 2.^{2,3} The enzyme responsible for the catalytic oxidative cyclisation and desaturation of proclavaminic acid 1 to give clavaminic acid 2 has been purified from *Streptomyces clavuligerus* and has been partially characterised.⁴ The enzyme, clavaminic acid synthase (CAS) was shown to require dioxygen and α -ketoglutarate as cosubstrates and to be dependent on iron(11) for activity. Hence, it belongs to the family of 2-oxo acid dependent dioxygenases, some of which also catalyse key steps in the biosynthesis of other β -lactam microbial metabolites. The gene encoding CAS has been identified, cloned and CAS has been over expressed in *Escherichia coli*.⁵ Recent *in vitro* experiments using CAS have determined that cyclisation precedes desaturation and that the saturated clavam 4 is an intermediate in the conversion of 3 into 2 (Scheme 1).⁶

Extensive substrate analogue studies on oxygenases involved in the biosynthesis of the penicillins and cephalosporins have established that they have a lax specificity with regard to unnatural substrates and have resulted in mechanistic proposals.⁷ We speculated that a similar approach





Scheme 2 Z = PhCH₂OCO; Bn = PhCH₂ Reagents i, NaH, dimethylformamide (DMF), BrCH₂CO₂Bn (77%); ii, (Me₃Si)₂NLi, tetrahydrofuran (THF), -78 °C; iii, OHC(CH₂)₂NHZ 7 then H₃O⁺ (30% for 8a); iv, H₂, 10% Pd/C, EtOH-H₂O (1:1) (65%, in each case)

may prove fruitful both in the study of CAS and its *in vitro* use as a synthetic reagent. Herein, we report the enzymatic synthesis of bicyclic γ -lactams exploiting CAS, from a monocyclic γ -lactam substrate $\pm 5a$.

The requisite substrate was synthesised in racemic form using an extension of methodology developed for the synthesis of proclavaminic acid 3.³ Thus, the pyrrolidinone 6 was selectively deprotonated on the exocyclic methylene group and the resultant enolate quenched with the aldehyde 7 to give a mixture of *threo* 8a and *erythro* 8b alcohols 8a:8b, 3:1, in moderate yield, which were separated by HPLC. Deprotection gave the desired *threo* 5a and *erythro* 5b compounds (Scheme 2).

Analysis by ¹H NMR (500 MHz) of incubations of **5a** with CAS[†] and the appropriate cofactors⁴ indicated the presence of



Scheme 3 Reagents: i, 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, CH_2Cl_2 , (81%); ii, $NaIO_4$, OsO_4 (cat.), THF, H_2O (3:1); iii, CH_2Cl_2 , CF_3CO_2H (cat.) (30% from 11 plus *ca*. 25% recovered starting material); iv, H_2 , 10% Pd/C, THF, H_2O (1:1) (85%)

two new bicyclic materials. These were purified by reversedphase HPLC and assigned as the saturated bicyclic y-lactam 9 and the γ -lactam analogue of clavaminic acid 10.[‡] For 9: HPLC Waters Bondapak amine column, 0.015% HCO₂H in 95% H₂O-5% MeOH; δ_H (500 MHz, D₂O) 1.74-1.93 (2 H, m, 9-H and 6-H), 2.02-2.09 (1 H, m, 9-H), 2.24-2.32 (1 H, m, 6-H), 2.34–2.40 (1 H, m, 7-H), 2.57–2.75 (1 H, m, 7-H), 3.05 (2 H, ca. t, J 7 Hz, 2 × 10-H), 3.92 (1 H, d, J, 6.5 Hz, 2-H), 4.10-4.15 (1 H, m, 3-H) and 5.25 (1 H, dd, J 6.5 and 3 Hz, 5-H); A 2D COSY (correlation spectroscopy) experiment was consistent with the connectivity as indicated; m/z (electrospray) 215 (MH⁺). For 10: (i) HPLC as for 9, then (ii) HPLC octadecylsilane reversed-phase column, 25 mmol dm-3 NH_4HCO_3 in H_2O ; δ_H (500 MHz, D_2O) 2.06–2.11 (1 H, m, 6-H), 2.42–2.73 (2 H, m, 6-H and 7-H), 2.7 (1 H, dd, J 16 and 8 Hz, 7-H), 3.46-3.58 (2 H, m, 2 × 10-H), 4.75 (1 H, dt, J 7 and 1 Hz, 9-H), 4.86 (1 H, ca. s, 2-H) and 5.66 (1 H, dd, J 6.5 and 3 Hz, 5-H); A 2D COSY experiment was consistent with the connectivity as indicated; m/z (electrospray) 213 (MH⁺). The relative stereochemical assignment of 9 was initially made on the basis of NOE (nuclear Overhauser effect) experiments [selected data only: irradiation at 5.25 (5-H) enhanced signals at 4.10-4.15 (3-H, 8%) and 2.24-2.32 (6-H, 9%); irradiation at 4.10-4.15 (3-H) enhanced signals at 5.25 (5-H, 8%), 3.05 (2 × 10-H, 4%), 2.02-2.09 (9-H, 4%) and 1.74-1.93 (9-H and 6-H 3%); irradiation at 3.92 (2-H) enhanced signals at 3.05 (2 × 10-H, 4%), 2.02–2.09 (9-H, 4%) and 1.74–1.93 (6-H and 9-H, 6%)]. The erythro material \pm 5b was found not to give any bicyclic lactams upon incubation with CAS. Neither 9 not 10 showed significant biological activity either as antibacterial agents or as β -lactamase inhibitors.

The structural assignments and relative stereochemistry of the new products were further substantiated by synthesis of ± 9 . The strategy employed used methodology previously developed for the synthesis of y-lactam analogues of the oxa-penams.8 Thus, the amide 11, synthesised from diprotected racemic threo-β-hydroxyornithine9 and pent-4-enoic acid, was oxidised and cyclised to give a single isolated bicyclic lactam 13, via the epimeric alcohols 12. Lactam 13 was deprotected to give the desired γ -lactam ± 9 (Scheme 3), which was shown by doping experiments to be indistinguishable by ¹H NMR (500 MHz) from the biosynthetic sample. Furthermore, incubation of the racemic synthetic 9 with CAS also gave 10, confirming 9 to be an intermediate between 5a and 10.

This synthesis of bicyclic γ -lactams utilising CAS is the first report of the transformation of a novel substrate by CAS and indicates that, like isopenicillin N synthase, CAS may have a relatively relaxed specificity towards unnatural substrates.

[†] The CAS used in this study was obtained from both recombinant and wild-type sources. Full details will be published elsewhere. In a typical incubation protocol, 2 mg of \pm **5a** were incubated with 0.2 IU partially purified 'recombinant' CAS (specific activity: 2.5 IU mg⁻¹) according to previously published protocols,⁶ to give after HPLC purification **9** (*ca.* 15%) and **10** (*ca.* 15%). Longer incubation times increased the ratio of **10** to **9**.

[‡] The absolute stereochemistry of products **9** and **10** was preliminarily assigned by analogy to the conversion of the natural substrate **3** to **2**.

Future studies will attempt to further exploit this propensity in investigations concerning the mechanism of CAS. We thank the SERC for a CASE award to M. D. L.

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