Enzymatic Synthesis of Bicyclic γ-Lactams using Clavaminic Acid Synthase

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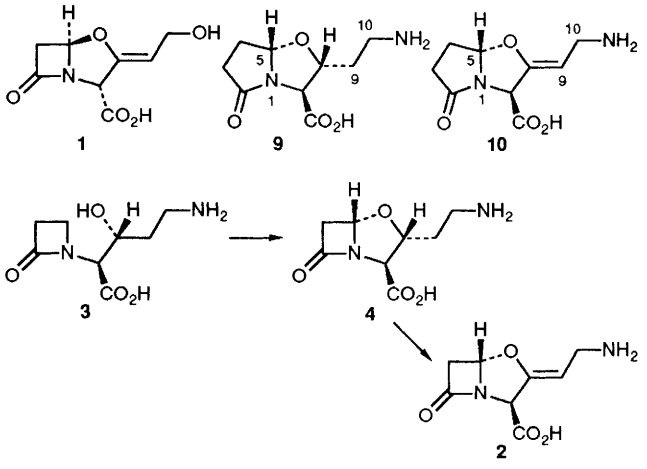
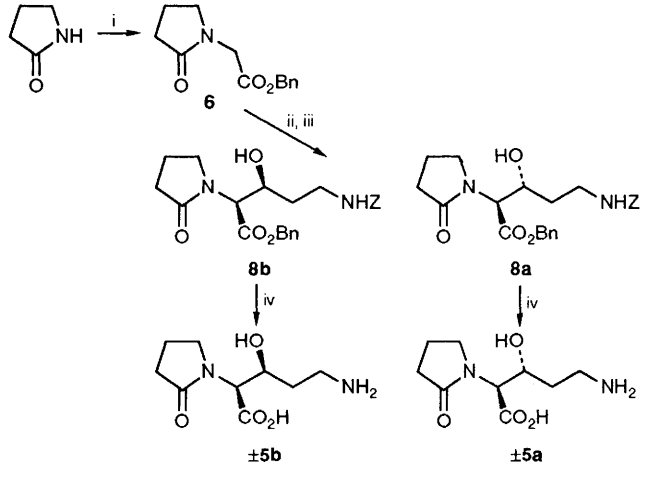
Incubation of a γ-lactam analogue of proclavaminic acid with clavaminic acid synthase, gave two novel bicyclic γ-lactams.

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(II) 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 1



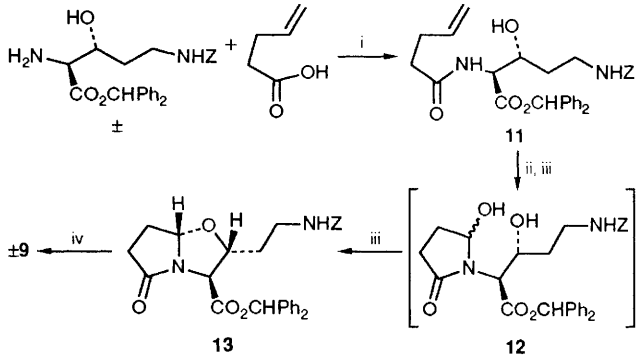
Scheme 2 Z = $PhCH_2OCO$; Bn = $PhCH_2$ *Reagents* i, NaH, dimethylformamide (DMF), $BrCH_2CO_2Bn$ (77%); ii, $(Me_3Si)_2NLi$, tetrahydrofuran (THF), −78 °C; iii, $OHC(CH_2)_2NHZ$ **7** then H_3O^+ (30% for **8a**); iv, H_2, 10% Pd/C, EtOH–H_2O (1 : 1) (65%, in each case)

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 ±**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 cofactors[4] indicated the presence of

† 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 ±**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**.



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 reversed-phase HPLC and assigned as the saturated bicyclic γ-lactam **9** and the γ-lactam analogue of clavaminic acid **10**.‡ For **9**: HPLC Waters Bondapak amine column, 0.015% HCO_2H in 95% H_2O–5% MeOH; δ_H (500 MHz, D_2O) 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 ±**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 γ-lactam analogues of the oxa-penams.[8] Thus, the amide **11**, synthesised from diprotected racemic *threo*-β-hydroxyornithine[9] 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 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.

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