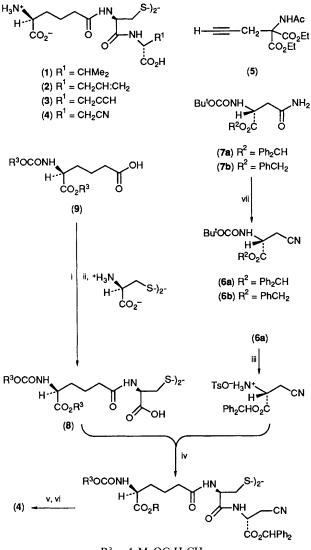
## Formation of Novel Unsaturated Side Chain Penicillins with Isopenicillin N Synthase

Jack E. Baldwin,\* Mark Bradley, Shaun D. Abbott, and R. M. Adlington The Dyson Perrins Laboratory and the Centre for Molecular Sciences, South Parks Road, Oxford OX1 3QY, UK

Incubation of  $\delta_{-L-\alpha}$ -aminoadipoyl-L-cysteinyl-D-propargylglycine (3) and  $\delta_{-L-\alpha}$ -aminoadipoyl-L-cysteinyl-D-cyanoalanine (4) with isopenicillin N synthase resulted in the formation of three novel penicillin antibiotics, possessing unsaturated side chains (10), (11), and (12).

Many analogues of the natural substrate  $\delta$ -L- $\alpha$ -aminoadipoyl-L-cysteinyl-D-valine (1) have on incubation with isopenicillin N synthase (IPNS) given new  $\beta$ -lactam products.<sup>1</sup> Of particular interest is the analogue  $\delta$ -L- $\alpha$ -aminoadipoyl-L-cysteinyl-D-allylglycine (2), which gave six  $\beta$ -lactam products.<sup>2</sup> three of which were oxygenated species, oxygen being derived from the co-substrate  $O_2$ .<sup>3</sup> This surprising result illuminated the unexpected mono-oxygenase pathway for IPNS with unsaturated substrates and raised the fundamental question, *i.e.*, what active site species arising from the iron-dioxygen



 $R^3 = 4 - MeOC_6H_4CH_2 -$ 

**Scheme 1.** Reagents and conditions: i,  $ClCO_2Bu^i/Et_3N$ /tetrahydrofuran; iii, TsOH; iv,  $EEDQ/Et_3N$ ; v, trifluoroacetic acid/anisole, 0 °C, 1 h; vi, reverse phase HPLC; vii, TsCl/pyridine. Ts =  $OSO_2C_6H_4Me$ .

redox cycle could, on the one hand, abstract hydrogen from the valine  $\beta$ -position in (1), yet when presented with an alkenic functionality, as in (2), presumably in a similar spatial location, would proceed along this mono-oxygenase pathway? The proposal of an iron-oxene (Fe<sup>IV</sup>=O) has been invoked to explain this duality of mechanism.<sup>4</sup> To explore the behaviour of triple bonds in this system the two tripeptides  $\delta$ -L- $\alpha$ aminoadipoyl-L-cysteinyl-D-propargylglycine (3) and  $\delta$ -L- $\alpha$ aminoadipoyl-L-cysteinyl-D-cyanoalanine (4) were synthesised and incubated with IPNS.

The D-propargylglycine functionality was synthesised via the diethylacetamidomalonate adduct (5), produced by alkylation of diethylacetamidomalonate with propargyl bromide using NaH, and resolved as the N-acyl compound using hog kidney acylase.<sup>5</sup> The amino acid was protected and converted to the tripeptide (3) by standard techniques.<sup>6</sup>

D-3-Cyanoalanine was produced in the N-t-butyloxycarbonyl, benzhydryl ester or N-t-butyloxycarbonyl, benzyl ester

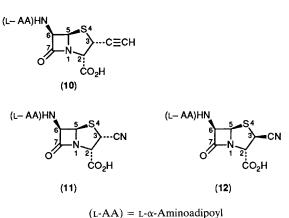


Table 1.D-Valine substitute	Penicillins formed $(\beta : \alpha \text{ ratio})$		Ref.
D-Norvaline	Ethyl	(>10:1) (7:1) (4:1) (1:2) (<1:15) (1:1)	8ª
D-α-Aminobutyrate	Methyl		9
D-Allyglycine (2)	Vinyl		2
D-Allenylalanine	Allenyl		10
D-Propargylglycine	Ethynyl		This work
D-Cyanoalanine	Cyano		This work

<sup>a</sup> The penicillin stereochemistry was only assigned after recent work involving a deuteriated version of the substrate, results of which will appear separately.

protected form (**6a**, **b**) (Scheme 1) by the method of Christie *et al.*<sup>7</sup> by dehydration of the similarly protected D-asparagines (**7a**, **b**) using toluene-*p*-sulphonyl chloride and pyridine. However, the nitrile functionality proved incompatible with standard deprotection techniques [refluxing with CF<sub>3</sub>-CO<sub>2</sub>H/anisole for an acid labile protecting scheme; and selective removal with Na/NH<sub>3</sub>(l) for a benzyl protecting scheme], and so an alternative strategy was sought. Thus (**6a**) was deprotected using toluene-*p*-sulphonic acid in Et<sub>2</sub>O/ EtOH and coupled using ethyl 1,2-dihydro-2-ethoxy-1-quinoline carboxylate (EEDQ) with bis [*N*-(4-methoxybenzyloxycarbonyl)- $\alpha$ -(4-methoxybenzyl)- $\delta$ -(L- $\alpha$ -aminoadipoyl)]-L-

cystine (8), produced by isobutylchloroformate mediated coupling of N-(4-methoxybenzyloxycarbonyl)- $\alpha$ -(4-methoxybenzyl)-L- $\alpha$ -aminoadipic acid (9) and L-cystine. The protected tripeptide disulphide thus formed was smoothly deprotected at 0 °C with CF<sub>3</sub>CO<sub>2</sub>H/anisole, with no hydration of the nitrile to the amide observable by 500 MHz <sup>1</sup>H NMR and mass spectrometry.

Incubation of  $\delta$ -L- $\alpha$ -aminoadipoyl-L-cysteinyl-D-propargylglycine (3) under standard conditions† produced, in

<sup>&</sup>lt;sup>†</sup> The tripeptide (typically 1–2 mg) in NH<sub>4</sub>HCO<sub>3</sub> buffer (3 ml, 25 mM) was pretreated with dithiothreitol (DTT) (100  $\mu$ l, 100 mM solution) for 10 min at 27 °C, treated with the necessary cofactors (sequentially: 100  $\mu$ l, 50 mM L-ascorbate solution; 50  $\mu$ l catalase at one tenth dilution of standard Sigma C-100 supply; and 100  $\mu$ l, 5 mM Fe<sup>2+</sup> solution) and IPNS solution (typically 10–12 IU in 2 ml 25 mM NH<sub>4</sub>HCO<sub>3</sub> buffer), divided into two portions, and incubated in air at 27 °C, 250 rpm shake rate for 40 min, with additional DTT (50  $\mu$ l) and Fe<sup>2+</sup> (50  $\mu$ l) being added to each portion after 20 min. Acetone precipitation of the enzyme (70% acetone), centrifugation (12 000 rpm, 10 min), and evaporation of the supernatant *in vacuo*, gave the crude incubation mixture.

virtually quantitative yield, a mixture of three new  $\beta$ -lactam containing metabolites (ratio 15:1:<0.5). The major product possessed antibiotic activity similar to isopenicillin N against the organisms Staphylococcus aureus and Escherichia coli, activity which was destroyed by the addition of  $\beta$ -lactamase I. This product was purified by reverse phase HPLC and characterised by 500 MHz 1H NMR and mass spectrometry, as an acetylenic penicillin. By NOE experiments and by the magnitude of the coupling constant between H-2 and H-3 of 7 Hz, the relative stereochemistry of the penicillin acetylene group was assigned as  $\alpha$  (10). Thus irradiation of the resonance associated with the H-2 proton gave an NOE to H-3 (10%) but no NOE to either H-6 or H-5. Irradiation of the resonance associated with H-3 gave an NOE to H-2 (8%) only, whilst irradiation of the resonances assigned to H-6 or H-5 gave no NOE to either H-2 or H-3.‡

Incubation of  $\delta$ -L- $\alpha$ -aminoadipoyl-L-cysteinyl-D-cyanoalanine (4) under standard conditions gave only 10% conversion, leading to the formation of two  $\beta$ -lactam products (ratio 1 : 1), both of which displayed antibiotic activity against *S. aureus* and *E. coli*, which was destroyed by the addition of  $\beta$ -lactamase I. These products were purified by reverse phase HPLC and assigned by HPLC retention properties and 500 MHz <sup>1</sup>H NMR as the  $\alpha$  and  $\beta$  penicillins (11) and (12)‡ (Table 1).

Provided that in each series both the  $\alpha$ - and  $\beta$ -penam products are equistable to the conditions of incubation and work-up, then it is apparent that alkyl substituents (ethyl and methyl) preferentially form  $\beta$ -penams, whereas unsaturated entities (vinyl, allenyl, and ethynyl) assume increasingly  $\alpha$ -oriented products. In contrast the highly polar cyano group shows no geometric preferences. Since our previous studies have provided evidence for a radical intermediate in the carbon–sulphur bond forming step, which in the formation of monosubstituted penams rotates faster than ring closure, \$\$ then the results of Table 1 suggest a preferential binding of unsaturated substituents to the  $\alpha$ -site and of saturated substituents to the  $\beta$ -site. This difference may arise from the juxtaposition of aromatic vs. aliphatic amino acid side chains in the  $\alpha$ - and  $\beta$ -sites respectively. Not surprisingly the polar cyano group does not respond to such association, and its orientation probably results from electrostatic influences.

We thank the SERC and Eli Lilly and Co., Indianapolis, USA, for financial support.

Received, 26th March 1990; Com. 0/01317J

## References

- 1 J. E. Baldwin and E. P. Abraham, Nat. Prod. Rep., 1988, 129.
- 2 J. E. Baldwin, R. M. Adlington, M. Bradley, A. R. Pitt, and N. J. Turner, J. Chem. Soc., Chem. Commun., 1989, 978, and references cited therein.
- 3 J. E. Baldwin, R. M. Adlington, S. L. Flitsch, H.-H. Ting, and N. J. Turner, J. Chem. Soc., Chem. Commun., 1986, 1305.
- 4 J. E. Baldwin, in 'Selectivity a Goal for Synthetic Efficiency,' proceedings of the Fourteenth Workshop Conference Hoechst, Schloss Reisenburg, 18th—22nd September 1983, ed. W. Bartmann and B. M. Trost, Verlag Chemie, 1984, 391; for a summary of more recent evidence see ref. 1 above.
- 5 O. Leukart, M. Caviezel, A. Eberle, E. Escher, A. Tun-Kyi, and R. Schwyzer, *Helv. Chim. Acta*, 1976, **59**, 2181.
- 6 J. E. Baldwin, S. R. Herchen, B. L. Johnson, M. Jung, J. J. Usher, and T. Wan, J. Chem. Soc., Perkin Trans. 1, 1981, 2253.
- 7 B. D. Christie and H. Rapoport, J. Org. Chem., 1985, 50, 1239.
- 8 J. E. Baldwin, R. M. Adlington, N. J. Turner, B. P. Domayne-
- Hayman, H.-H. Ting, A. E. Derome, and J. A. Murphy, J. Chem. Soc., Chem. Commun., 1984, 1167.
- 9 J. E. Baldwin, E. P. Abraham, R. M. Adlington, J. A. Murphy, and (in part) N. B. Green, H-H. Ting, and J. J. Usher, *J. Chem. Soc.*, *Chem Commun.*, 1983, 1319; ratio of β-lactam products reassessed in later unpublished work by J. E. Baldwin and S. Flitsch; see S. Flitsch, D.Phil. (Oxon), 1985.
- 10 J. E. Baldwin, R. M. Adlington, A. Basak, and H-H. Ting, J. Chem. Soc., Chem. Commun., 1986, 1280.
- 11 J. E. Baldwin, R. M. Adlington, B. P. Domayne-Hayman, G. Knight, and H-H. Ting, J. Chem. Soc., Chem. Commun., 1987, 1661.

§ A radical intermediate is thought most likely in the light of the result of incubating the two isomers  $\delta$ -L- $\alpha$ -aminoadipoyl-L-cysteinyl-D-(3R) and (3S)-(2-amino-3-deuteriobutyrate) with IPNS which both give the same  $\alpha$ -deuterio- $\beta$ -methylpenam,<sup>9</sup> and the results with the D-cyclopropylalanine containing tripeptide.<sup>11</sup>

<sup>‡</sup> Spectroscopic data for (10): δ<sub>H</sub> (500 MHz, D<sub>2</sub>O) 1.66–1.96 (4H, 2 × m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 2.38–2.41 (2H, m, CH<sub>2</sub>CO), 2.91 (1H, d, J 2.5 Hz, HCC), 3.73–3.75 (1H, m, CHCH<sub>2</sub>), 4.90 (1H, dd, J7, 2.5 Hz, H-3), 5.53 and 5.66 (2H, ABq, J4 Hz, H-5 and H-6); use of CD<sub>3</sub>CN : D<sub>2</sub>O (1:1) shifted the HOD peak, revealing δ<sub>H</sub> 4.83 (1H, d, J7 Hz, H-2); m/z (+ve argon FAB) 356 (MH<sup>+</sup>). For (11) (more mobile isomer): δ<sub>H</sub> (500 MHz, D<sub>2</sub>O) 1.66–1.96 (4H, 2 × m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 2.38–2.42 (2H, m, CH<sub>2</sub>CO), 3.72–3.75 (1H, m, CHCH<sub>2</sub>), 5.39 and 5.51 (2H, ABq, J 4 Hz, H-5 and H-6); H-2 and H-3 resonances obscured by residual solvent peak. For (12) (less mobile isomer): δ<sub>H</sub> (500 MHz, D<sub>2</sub>O) 1.66–1.96 (4H, 2 × m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>CO), 2.38–2.42 (2H, m, CH<sub>2</sub>CO), 3.72–3.75 (1H, m,