

Formation of Novel Unsaturated Side Chain Penicillins with Isopenicillin N Synthase

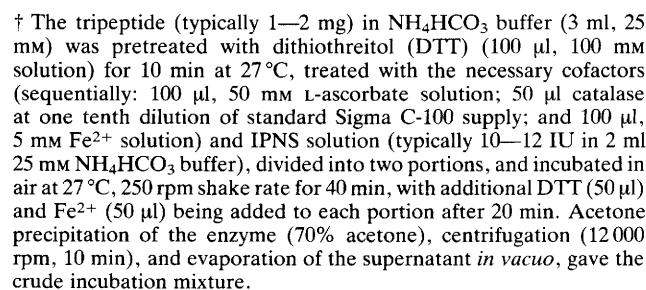
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Incubation of δ -L- α -aminoadipoyl-L-cysteinyl-D-propargylglycine (**3**) and δ -L- α -aminoadipoyl-L-cysteinyl-D-cyanoalanine (**4**) with isopenicillin N synthase resulted in the formation of three novel penicillin antibiotics, possessing unsaturated side chains (**10**), (**11**), and (**12**).

Many analogues of the natural substrate δ -L- α -aminoadipoyl-L-cysteinyl-D-valine (**1**) have on incubation with isopenicillin N synthase (IPNS) given new β -lactam products.¹ Of particular interest is the analogue δ -L- α -aminoadipoyl-L-cysteinyl-D-allylglycine (**2**), which gave six β -lactam products,²

three of which were oxygenated species, oxygen being derived from the co-substrate O₂.³ 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



virtually quantitative yield, a mixture of three new β -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 β -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 α (**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 δ -L- α -aminoadipoyl-L-cysteinyl-D-cyanoalanine (**4**) under standard conditions gave only 10% conversion, leading to the formation of two β -lactam products (ratio 1 : 1), both of which displayed antibiotic activity against *S. aureus* and *E. coli*, which was destroyed by the addition of β -lactamase I. These products were purified by reverse phase HPLC and assigned by HPLC retention properties and 500 MHz ^1H NMR as the α and β penicillins (**11**) and (**12**)[‡] (Table 1).

Provided that in each series both the α - and β -penam products are equistable to the conditions of incubation and work-up, then it is apparent that alkyl substituents (ethyl and methyl) preferentially form β -penams, whereas unsaturated entities (vinyl, allenyl, and ethynyl) assume increasingly α -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 α -site and of saturated substituents to the β -site. This difference may arise from the juxtaposition of aromatic vs. aliphatic amino acid side chains in the α - and β -sites respectively. Not surprisingly the polar cyano group does not respond to such association, and its orientation probably results from electrostatic influences.

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[‡] Spectroscopic data for (**10**): δ_{H} (500 MHz, D_2O) 1.66–1.96 (4H, $2 \times \text{m}$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}$), 2.38–2.41 (2H, m, CH_2CO), 2.91 (1H, d, J 2.5 Hz, HCC), 3.73–3.75 (1H, m, CHCH_2), 4.90 (1H, dd, J 7, 2.5 Hz, H-3), 5.53 and 5.66 (2H, ABq, J 4 Hz, H-5 and H-6); use of $\text{CD}_3\text{CN}:\text{D}_2\text{O}$ (1 : 1) shifted the HOD peak, revealing δ_{H} 4.83 (1H, d, J 7 Hz, H-2); m/z (+ve argon FAB) 356 (MH^+). For (**11**) (more mobile isomer): δ_{H} (500 MHz, D_2O) 1.66–1.96 (4H, $2 \times \text{m}$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}$), 2.38–2.42 (2H, m, CH_2CO), 3.72–3.75 (1H, m, CHCH_2), 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_{H} (500 MHz, D_2O) 1.66–1.96 (4H, $2 \times \text{m}$, $\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}$), 2.38–2.42 (2H, m, CH_2CO), 3.72–3.75 (1H, m, CHCH_2), 5.34 and 5.38 (2H, ABq, J 4 Hz, H-5 and H-6); H-2 and H-3 resonances obscured by residual solvent peak.

[§] A radical intermediate is thought most likely in the light of the result of incubating the two isomers δ -L- α -aminoadipoyl-L-cysteinyl-D-(3*R*) and (3*S*)-(2-amino-3-deuteriobutyrate) with IPNS which both give the same α -deuterio- β -methylpenam,⁹ and the results with the D-cyclopropylalanine containing tripeptide.¹¹