Formation of a Novel Bicyclic γ-Lactam with Isopenicillin N Synthase

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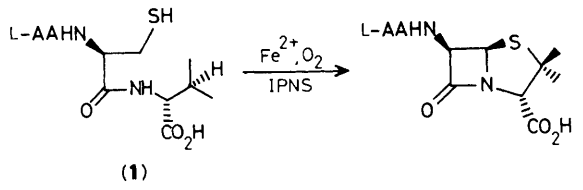
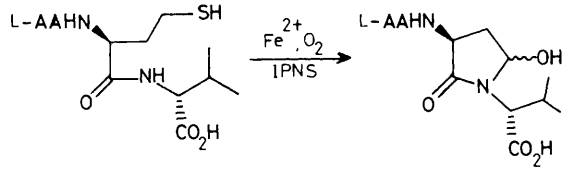
Incubation of isopenicillin N synthase (IPNS) with [(5*S*)-5-amino-5-carboxypentanoyl]-L-homocysteinyl-L-cysteine (**14**) resulted in the formation of a novel bicyclic γ-lactam (**17**) containing an intramolecular disulphide linkage.

We have previously demonstrated that incubation of isopenicillin N synthase (IPNS) with analogues of the natural substrate for IPNS, [(5*S*)-5-amino-5-carboxypentanoyl]-L-cysteinyl-L-valine, [L,L,D-A·C·V (**1**)], can provide a wide range of bicyclic β-lactam structures.[1] Recently we reported that incubation of L,L,D-A·homocysteinyl·V [L,L,D-A·HC·V (**2**)] with IPNS resulted in a change in the basic product type, from the bicyclic β-lactams normally observed (*e.g.* Scheme 1) to the monocyclic 5-hydroxy-γ-lactams (**3**)[2] (Scheme 2). We also demonstrated that the oxygen of the hydroxy group in



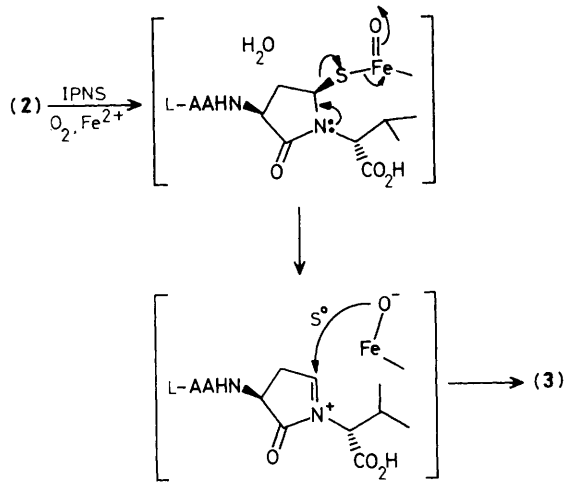
(1)

Scheme 1

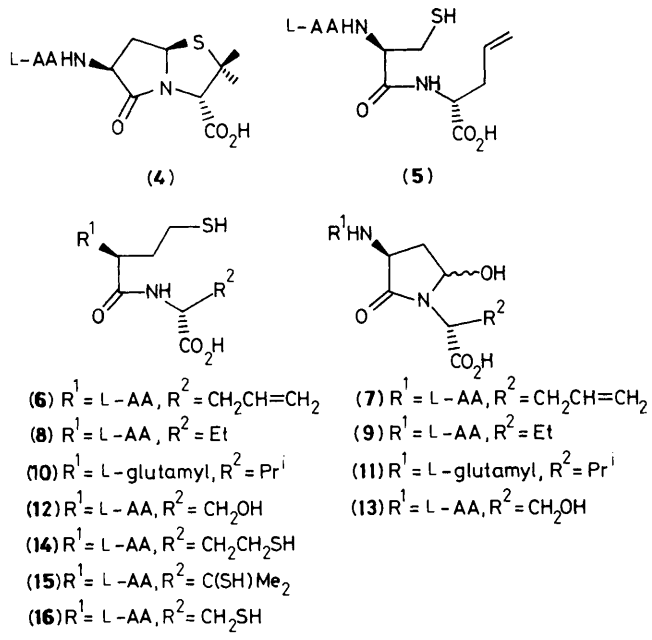


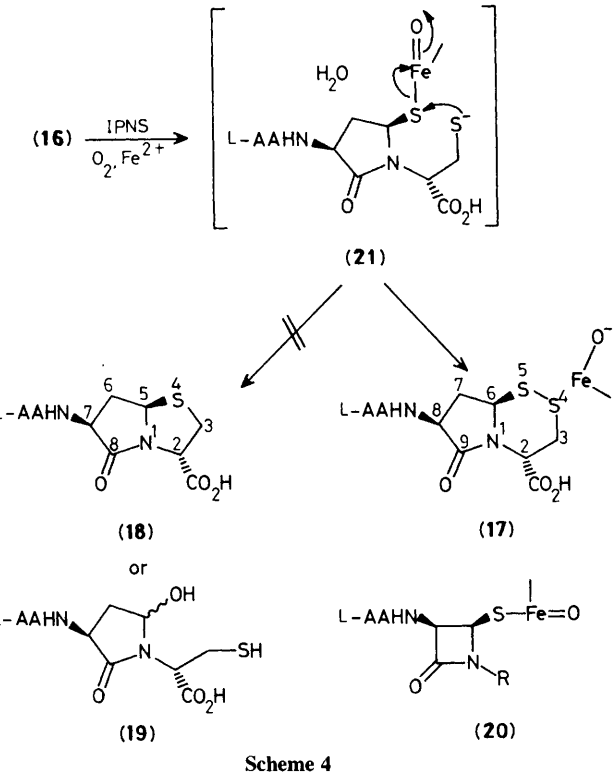
(2) (3)

Scheme 2. L-AA = L-α-aminoadipoyl, [(5*S*)-5-amino-5-carboxypentanoyl].



Scheme 3





Scheme 4

these products was derived, at least in part, from dioxygen. We were unable to detect any bicyclic lactams [*e.g.* (**4**)] in the incubation products. Here we report the results of our initial attempts to modify the mode of cyclisation of homocysteinyl tripeptides by IPNS to produce bicyclic γ-lactams.

We have previously argued[2] that these new monocyclic species arise through the more rapid collapse of the intermediate thioferryl entity than occurs in the β-lactam forming process (Scheme 3). We have thus designed a number of homocysteine-containing peptides in the hope of accelerating the second ring formation to provide bicyclic products.

L,L,D-A·C·allylglycine (**5**) has been shown to give six different products with IPNS;[3] thus we synthesised and incubated L,L,D-A·HC·allylglycine (**6**). However, only the epimeric hydroxy lactams (**7**) analogous to those previously obtained were observed. Incubation of L,L,D-A·HC·aminobutyrate (**8**) similarly gave conversion to (**9**), whilst L,L,D-glutamyl·HC·valine (**10**) [which contains the same number of methylenes as L,L,D-A·C·V (**1**)] gave only a low yield of the corresponding monocyclic lactams (**11**).

We then reasoned that incorporation of a nucleophile at the valinyl position of a homocysteinyl tripeptide might result in the intramolecular trapping of an enzyme-bound intermediate. Incubation of L,L,D-A·HC·serine (**12**) resulted only in a low yield of monocyclic lactams (**13**), whilst L,L,D-A·HC·HC (**14**) and L,L,D-A·HC·penicillamine (**15**) did not give any detectable [by ^{1}H n.m.r. (500 MHz)] products. However, incubation of L,L,D-A·HC·C (**16**) with IPNS gave as the single isolated product after h.p.l.c. [C18 octadecylsilane reverse phase, 25 mM ammonium hydrogencarbonate] the novel

bicyclic γ-lactam disulphide (**17**): retention time 24 min; δ_H (500 MHz, D_2O)† 1.50—1.55 (2H, m, $CH_2CH_2CH_2CO$), 1.65—1.80 (3H, m, CH_2CH_2CO and 7-H), 2.24 (2H, *ca.* t, *J* 7 Hz, CH_2CO), 2.82 (1H, ddd, *J* 15, 9.5, 7.5 Hz, 7-H), 3.11 (1H, dd, *J* 14, 3 Hz, 3-H), 3.29 (1H, dd, *J* 14, 3 Hz, 3-H), 3.60 (1H, *ca.* t, *J* 6.5 Hz, $CHCH_2CH_2$), 4.39 (1H, dd, *J* 9.5, 9 Hz, 8-H), 4.83 (1H, dd, *J ca.* 3, 3 Hz, 2-H), 5.15 (1H, dd, *J ca.* 7.5, 7.5 Hz, 6-H); COSY[4] and nuclear Overhauser enhancement (n.O.e.) experiments were used to establish the connectivity and relative stereochemistry as indicated; *m/z* (fast atom bombardment) 378 (MH^+).

There was no evidence for the 5,5-bicyclic γ-lactam (**18**), which was synthesised for comparison,‡ or the hydroxy lactam (**19**).

We have proposed the monocyclic β-lactam–iron–oxene complex (**20**) as an enzyme bound intermediate in penicillin biosynthesis. As noted above, we previously reasoned that the lack of bicyclic products from homocysteinyl substrates reflected the more rapid collapse of the analogous γ-lactam intermediate resulting in the release of a monocyclic lactam with loss of atomic sulphur (Scheme 3). This new result indicates that the homocysteinyl residue is directly coupled to an electron withdrawing moiety and thus the formation of (**17**) may result from the intramolecular cleavage by thiolate of the Fe–S bond of the enzyme substrate complex (**21**) (Scheme 4).

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† Referenced to sodium (2,2,3,3-$^2H_4$)-3-trimethylsilylpropanoate.

‡ Synthesised by methodology previously reported by us:[2,5] h.p.l.c. [C18 octadecylsilane reverse phase, 25 mM ammonium hydrogencarbonate], retention time 8.5 min; δ_H (500 MHz, D_2O)† 1.4—1.8 (4H, m, $CH_2CH_2CH_2CO$), 2.05 (1H, ddd, *J* 13, 10.5, 7.5 Hz, 6-H), 2.28 (2H, *ca.* t, *J* 7 Hz, CH_2CO), 2.91 (1H, ddd, *J* 13, 8.5, 7 Hz, 6-H), 3.30 (1H, dd, *J* 11.5, 5 Hz, 3-H), 3.41 (1H, dd, *J* 11.5, 7 Hz, 3-H), 3.71 (1H, *ca.* t, *J* 6 Hz, $CHCH_2CH_2$), 4.70 (1H, dd, *J* 10.5, 8.5 Hz, 7-H), 4.75 (1H, dd, *J* 7, 5 Hz, 2-H), 5.05 (1H, dd, *J* 7.5, 7 Hz, 5-H); COSY[4] and n.O.e. experiments were used to establish the connectivity and relative stereochemistry as indicated; *m/z* (fast atom bombardment) 346 (MH^+).

References

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