Penicillin Biosynthesis: Structure–Reactivity Profile of Allenic Substrates for Isopenicillin N Synthetase

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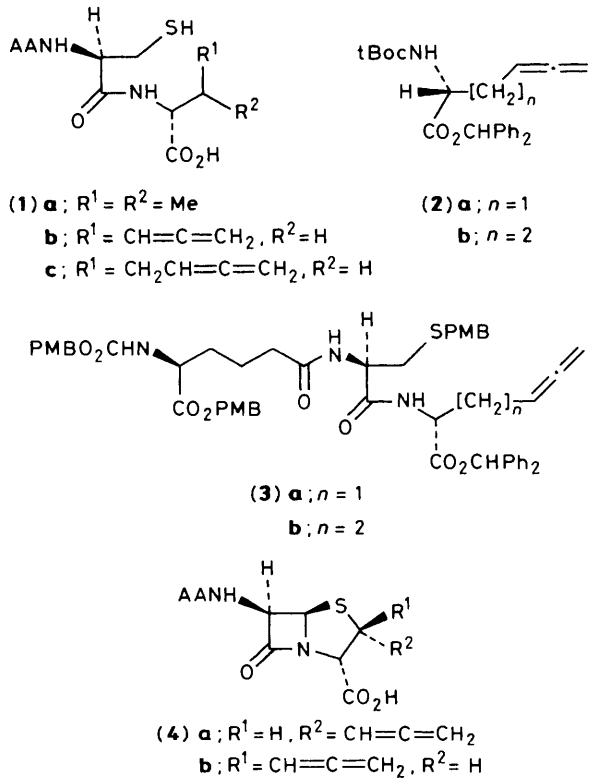
Two allene-containing tripeptides (**1b**) and (**1c**) have been prepared and evaluated as substrates for isopenicillin N synthetase; the formation of penam, cepham, and dienyl products is consistent with the operation of desaturative ring closures.

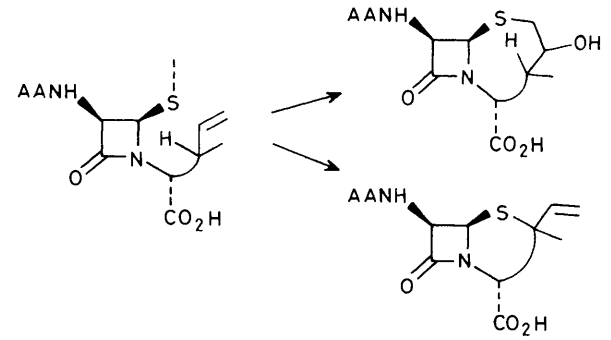
Recent results from this laboratory have revealed that the non-haem iron enzyme isopenicillin N synthetase (IPNS) can tolerate quite large structural variations within the valine moiety of the natural substrate (**1a**) whilst still providing bicyclic β-lactam products.[1] When saturated valine analogues were employed as substrates, following the initial β-lactam formation,[2] both penam and cepham ring closures occurred by an overall desaturative (−4H) path.[3] On the other hand unsaturated valine analogues gave products both from desaturative *and* hydroxylative (−2H + 1O) paths,[1,4] Scheme 1. The hydroxy group was shown to originate from dioxygen.[5] In order to explore the effect of other types of unsaturation on these enzymatic ring closures we have now prepared peptides containing allenyl moieties. Thus peptides (**1b**) and (**1c**) were obtained from the (2*R*)-amino acids (**2a**) and (**2b**) which in turn were synthesised by allene transfer[6] to protected 3-iodo-(2*S*)-aminopropanoic acid and 4-bromo-(2*R*)-aminobutanoic acid[7] respectively. The so-derived amino acids were coupled[8] to give protected peptides (**3a**) and (**3b**), which were deprotected (CF_3CO_2H, anisole, reflux, 30 min) to give (**1b**) and (**1c**).

Incubation of (**1b**) with homogeneous IPNS[9] from *Cephalosporium acremonium* CO 728 gave, after protein precipitation, a crude product for which ^{1}H n.m.r. analysis (500 MHz) indicated 3 β-lactam containing products (**4a**), (**4b**), and (**5**) (ratio 20:10:7).† Purification by h.p.l.c. (reverse phase octadecylsilane, 50 mM aqueous NH_4HCO_3 as eluant) gave in addition to the expected[4] 2α- and 2β-allenyl penams, (**4a**), (**4b**) respectively, the 2-*exo*-allenyl cepham (**5**), $\nu_{max.}$ (CaF_2 cells, D_2O) 1950 w (allene), 1746 m cm^{-1} (β-lactam C=O); *m/z* (positive argon fast atom bombardment) 370(MH^+); δ_H (D_2O, 500 MHz)‡ 1.65—1.83, 1.84—2.01(4H, 2 × m, CH_2CH_2-CH_2CO), 2.45(2H, t, *J* 7 Hz, CH_2CO), 2.65—2.70(1H, m, 3β-H), 2.91(1H, dd, *J* 14, 3 Hz, 3α-H), 3.77(1H, t, *J* 7 Hz, $CH[CH_2]_3$–), 4.96—5.01(2H, AB part of ABX, δ_A 4.96, δ_B 5.01, J_{AB} 12, J_{AX} 3.5, J_{BX} 2.5 Hz, C=CH_2), 5.38, 5.40(2H, ABq, *J* 4 Hz, 6,7-H), (4-H obscured by HOD.) Simultaneous irradiation of both allene protons, δ_H 4.96—5.01 collapsed the multiplet at δ_H 2.65—2.70 (3β-H) to a double doublet whilst a Jeener n.m.r. experiment[10] established the connectivity CH_2=C=C–CH_2–CH–. (**5**) showed no antibacterial activity against *Staphylococcus aureus* N.C.T.C. 6571 at a concentration of 100 μg ml^{-1}.§

Incubation of (**1c**) with IPNS in analogous fashion gave a crude product devoid of antibacterial activity. Purification by h.p.l.c. (reverse phase octadecylsilane, 10 mM aqueous NH_4HCO_3 as eluant) gave in addition to the expected[1] 2α- and 2β-allenyl cephams (**6a**), (**6b**), the diene (**7**) (ratio 3:1:7).† The structure as (**7**) follows from n.m.r. and mass spectral data, δ_H (D_2O, 500 MHz)‡ 1.66—1.81, 1.82—1.99(4H, 2 × m, $CH_2CH_2CH_2CO$), 2.43(2H, t, *J* 7 Hz, CH_2CO), 3.06—3.31(2H, br.m, 5-H), 3.72(1H, dd, *J* 5.5, 7 Hz, $CH[CH_2]_3$), 4.45—4.51(1H, m, 6-H), 5.35, 5.42(2H, ABq, *J* 4.5 Hz, 8,9-H), 5.47, 5.56(2 × 1H, 2 × s, C=CH_2), 5.72(1H, dt, *J* 12, 9 Hz, 4-H),



AA = L-α-aminoadipoyl
tBOC = t-butyloxycarbonyl
PMB = *p*-methoxybenzyl



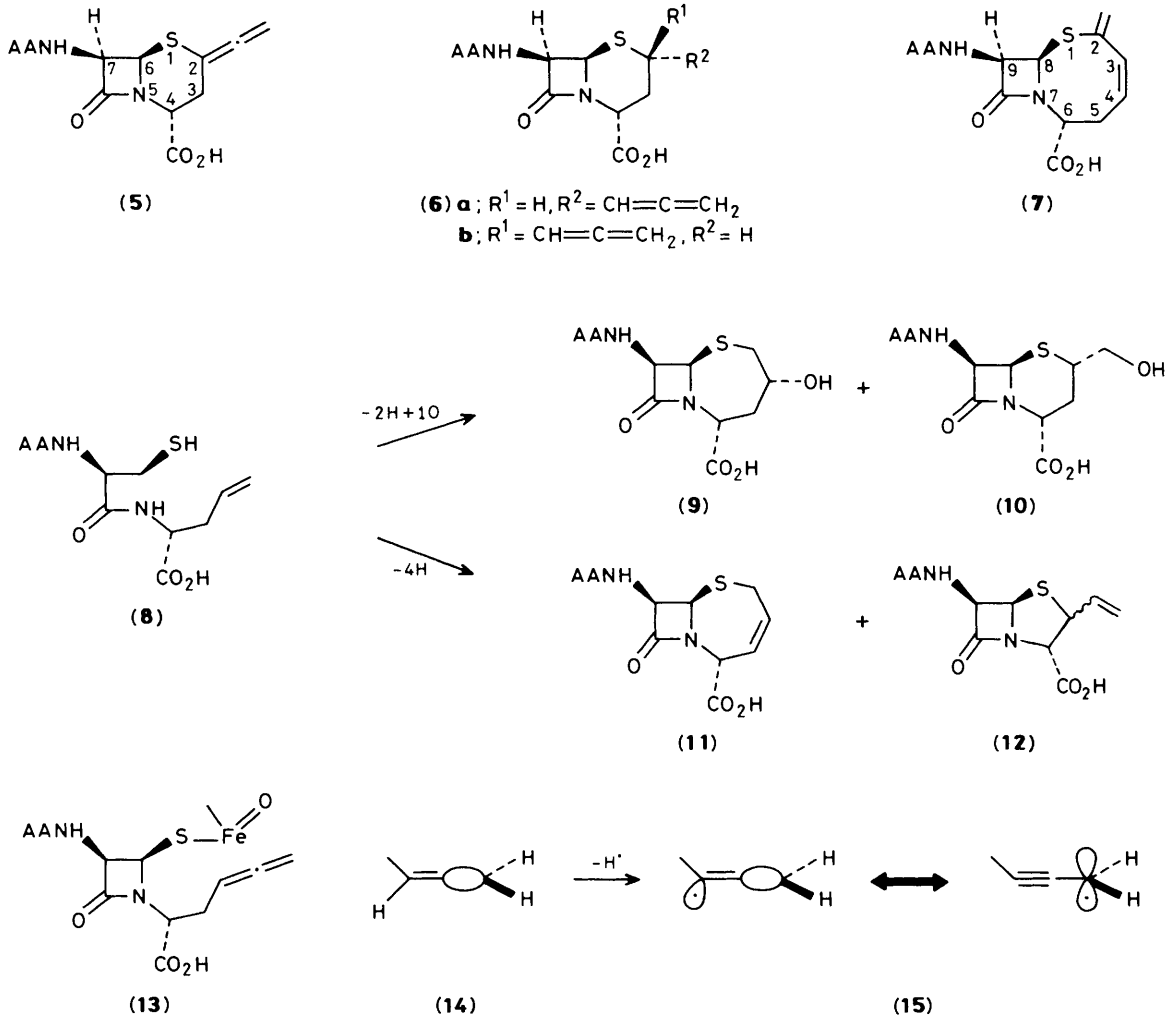
AA = L-α-aminoadipoyl

Scheme 1

† In a comparison study, incubation of (**1a**), (**1b**), and (**1c**), (1 mg each) with IPNS (5 International Units) under standard conditions, gave *ca.* 100%, 90%, and 20% total conversion into β-lactam products respectively.

‡ Chemical shifts were referenced to internal sodium (2,2,3,3-$^2H_4$)-3-trimethylsilylpropionate (TSP) = 0.00 p.p.m.

§ 100 μl of this solution was used for bioassay analysis by the 'holed plate' assay method.



AA = L-α-aminoadipoyl

6.50(1H, d, *J* 12 Hz, 3-H); *m/z* (positive argon fast atom bombardment) 384(MH^+). The connectivities CH_2=C–CH=CH–CH_2–CH– were consistent with a Jeener n.m.r. experiment[10] and with proton decoupling experiments where irradiation of 5-H(δ_H 3.06—3.31) collapsed 6-H(δ_H 4.49) from a multiplet to a singlet and 4-H(δ_H 5.72) from a double-triplet to a doublet (*J* 12 Hz). (**7**) showed no antibacterial activity against *S. aureus* N.C.T.C. 6571 at a concentration of 50 μg ml^{-1}.§

The enzymatic transformation of allene (**1b**) differs significantly from that of the corresponding alkene (**8**), which gave both products of hydroxylation, (**9**), (**10**) and desaturation, (**11**), (**12**), whereas (**1b**) with the same relative location of a double bond gave only desaturation products *i.e.* (**4a**), (**4b**), and (**5**). This lack of hydroxylation products from the allene-containing substrates suggests that the hydroxylating entity, possibly an iron-oxo species as (**13**),[5] behaves as a source of electrophilic oxygen. The lower electron density of the allenic structure relative to an isolated double bond would then disfavour this mode of hydroxylative cyclisation. Interestingly, the product (**5**) must arise from loss of the allenic hydrogen which although initially surprising can be accommodated when the allylic nature of the C–H bond is recognised, *i.e.* (**14**) to (**15**).

In summary these results show that the allene functionality can participate in desaturative ring closures on the IPNS enzyme, giving rise to a range of unusual bicyclic β-lactams.

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