Penicillin Biosynthesis: Active Substrates derived by Methoxy Substitution in the Valinyl Residue of the Natural Substrate

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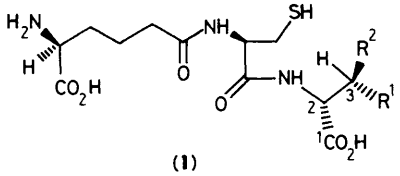
The structure–reactivity profile of tripeptides modified by methoxy substitution in the valinyl moiety of L-(α-aminoadipoyl)-L-cysteinyl-D-valine with the enzyme isopenicillin N synthase has been examined; substrate bulk and absolute configuration at the oxygen-substituted carbon were found to play crucial roles in determining substrate reactivity.

Recently we described that a new antibacterial penicillin containing a 2α-methoxy group (**2a**) was obtained by enzymatic synthesis from the tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-(*O*-methyl-allothreonine) (**1a**) with isopenicillin N synthase (IPNS) from *Cephalosporium acremonium* CO 728.[1] The absolute configuration at C-3 of so-modified peptides was found to be an important factor since the *O*-methyl threonyl tripeptide (**1b**) was not a β-lactam producing substrate with IPNS, *i.e.*, (**1b**)—⤫→ (**2b**). In order to evaluate the generality of methoxy substitution for methyl in the valine moiety of the natural substrate (**1c**), we have synthesised and tested a series of methoxy-modified tripeptides.

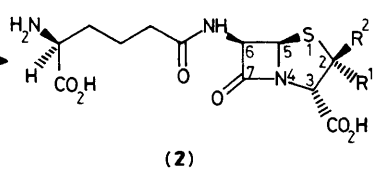
Initially the *O*-methylserinyl tripeptide (**1d**) was synthesised following standard peptide coupling and deprotection procedures[2] (Scheme 1). Incubation of (**1d**) with purified IPNS under the usual conditions[3] gave after protein precipitation two new β-lactam products. Purification by h.p.l.c. [reverse phase octadecylsilane column, i, 25 mM NH_4HCO_3 as eluant; ii, 0.05% HCO_2H in H_2O as eluant] gave firstly the 2β-methoxypenam (**2c**), δ_H (500 MHz, D_2O)† 1.65—1.95 (4H, 2 × m, $CH_2CH_2CH_2CO$), 2.38—2.45 (2H, m, CH_2CO),

† ^1H N.m.r. spectra were referenced to sodium 3-trimethylsilyltetradeuteriopropionate = 0.00 p.p.m.



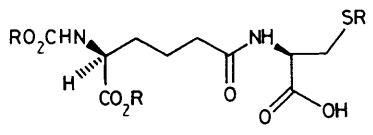
(1)

a; R^1 = OMe, R^2 = Me
b; R^1 = Me, R^2 = OMe
c; R^1 = R^2 = Me
d; R^1, R^2 = H, OMe
e; R^1 = OMe, R^2 = CH=CH_2
f; R^1 = CH=CH_2, R^2 = OMe
g; R^1 = Me, R^2 = Et
h; R^1 = Et, R^2 = Me
j; R^1 = Me, R^2 = H



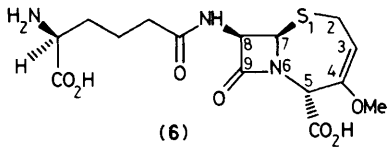
(2)

a; R^1 = OMe, R^2 = Me
b; R^1 = Me, R^2 = OMe
c; R^1 = H, R^2 = OMe
d; R^1 = OMe, R^2 = H
e; R^1, R^2 = OMe, CH=CH_2
g; R^1 = Me, R^2 = Et
h; R^1 = Et, R^2 = Me
j; R^1, R^2 = H, Me



(3)

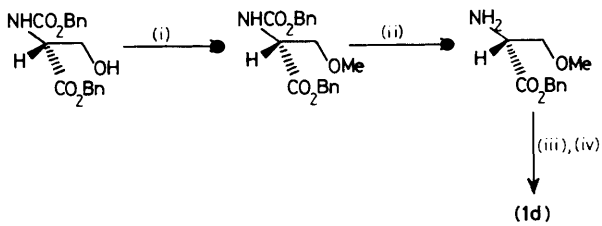
a; R = CH_2Ph
b; R = p-$MeOC_6H_4CH_2$



(6)

3.32 (3H, s, OMe), 3.70—3.75 [1H, m, C*H*(CH_2)$_3$], 4.98 (1H, s, 3-H), 5.38, 5.48 (2H, ABq, *J* 4 Hz, 5,6-H), 5.63 (1H, s, 2-H); *m/z* (positive argon fast atom bombardment) 362 (*M*H$^+$), which showed no detectable antibacterial activity towards *Staphylococcus aureus* N.C.T.C. 6571 or *Micrococcus luteus* DS 292 at a concentration of 50 μg ml^{-1},‡ and secondly [h.p.l.c. system i, only] the 2α-methoxypenam (**2d**) δ_H (500 MHz, D_2O)† 1.63—1.93 (4H, 2 × m, C*H*$_2$C*H*$_2$C*H*$_2$CO), 2.35—2.40 (2H, m, CH_2CO), 3.36 (3H, s, OMe), 3.68—3.73 [1H, m, C*H*(CH_2)$_3$], 5.47, 5.59 (2H, ABq, *J* 4 Hz, 5,6-H), 5.84 (1H, d, *J* 6 Hz, 2-H), (3-H obscured), *m/z* (positive argon fast atom bombardment) 362 (*M*H$^+$), which showed antibacterial activity towards *S. aureus* N.C.T.C. 6571 and *M. luteus* DS 292 at a concentration of 50 μg ml^{-1}.‡ The stereochemistries of (**2c**) and (**2d**) as the 2β- and 2α-methoxy penams respectively follow from the observed couplings *J*(H-2, H-3) of *ca.* 0 and 6 Hz respectively, and by comparison with literature coupling constant values.[4]

Secondly we studied the effect of methoxy substitution at C-3 of unsaturated tripeptides, *via* synthesis of the tripeptides (**1e**) and (**1f**) (Scheme 2). Incubation of (**1e**) with purified IPNS[3] gave, after protein precipitation, two new β-lactam products. Purification by h.p.l.c. (reverse phase octadecylsilane column, 10 mM NH_4HCO_3 as eluant) gave firstly the 2-methoxy-2-vinyl penam (**2e**), δ_H (500 MHz, D_2O)† 1.63—1.72, 1.82—1.92 (4H, 2 × m, C*H*$_2$C*H*$_2$C*H*$_2$CO), 2.37—2.43 (2H, m, CH_2CO), 5.24, 5.67 (2H, ABq, *J* 4 Hz, 5,6-H), 5.42 (1H, d, *J* 11 Hz, CH=C*H*$_2$), 5.52 (1H, d, *J* 17 Hz, CH=C*H*$_2$), 5.88 (1H, dd, *J* 17, 11 Hz, C*H*=CH_2), [3-H, *Me*OC*H*(CH_2)$_3$ obscured], as a very minor product which gave antibacterial activity towards *Staphylococcus aureus* N.C.T.C. 6571 at a concentration of 25 μg ml^{-1},‡ and secondly the 4-methoxyhomoceph-3-em (**6**),§ ν_{max} (CaF_2 cells, D_2O) 1740s (C=O); δ_H (500 MHz, D_2O)† 1.62—1.92 (4H, 2 × m, C*H*$_2$C*H*$_2$C*H*$_2$CO), 2.34—2.42 (2H, m, CH_2CO), 3.31 (2H, *ca.* d, *J* 7.5 Hz, 2-H), 3.52 (3H, s, OMe), 3.65—3.72 [1H, m, C*H*(CH_2)$_3$], 4.93 (1H, s, 5-H), 5.13 (1H, *ca.* t, *J* 7.5 Hz, 3-H), 5.35, 5.58 (2H, ABq, *J* 4 Hz, 7,8-H), as a major product which showed no detectable antibacterial activity towards *S. aureus* N.C.T.C. 6571 at a concentration of 100 μg ml^{-1},‡ *m/z* (positive argon fast atom bombardment) 388 (*M*H$^+$). The structure as (**6**) was consistent with selective ^{1}H n.m.r. spectroscopy decoupling experiments since irradiation of 2-H (δ_H 3.31) caused collapse of 3-H (δ_H 5.13) from a triplet to a singlet, and irradiation of 3-H caused collapse of 2-H from a doublet to a singlet. Incubation of the diastereoisomeric tripeptide (**1f**) with IPNS gave no detectable conversion to β-lactam products.



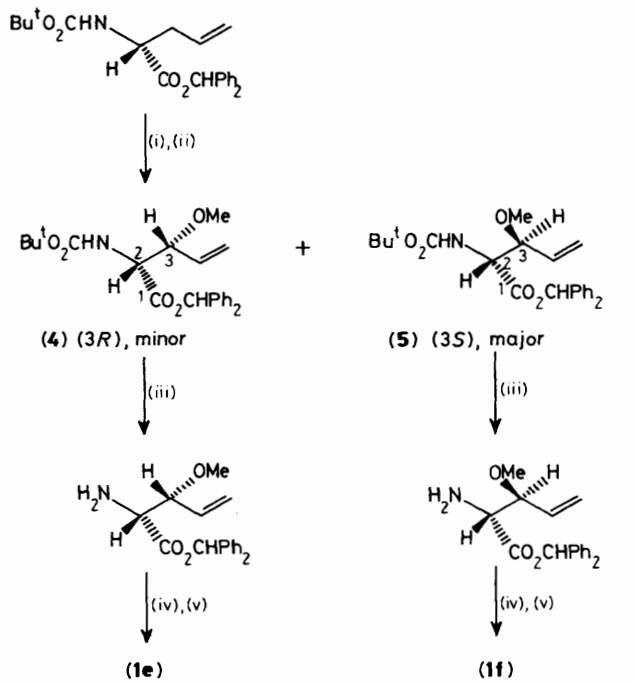
Bn = CH_2Ph

Scheme 1. *Reagents:* i, BF_3·OEt_2, CH_2N_2; ii, HBr/HOAc, then NEt_3; iii, coupling to (**3a**), ref. 2; iv, Na/NH_3, ref. 2.

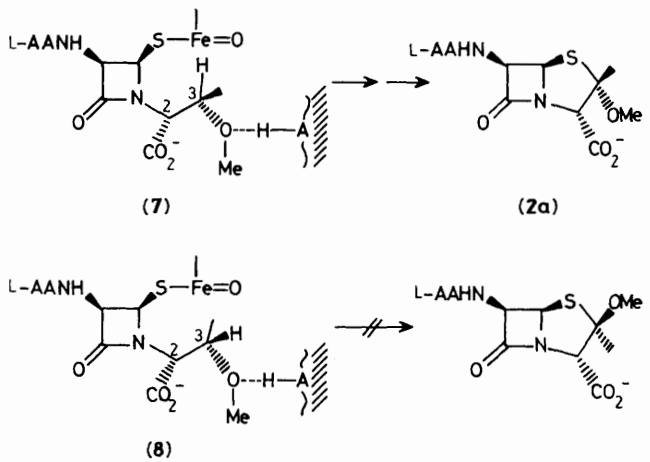
In summary these results and others[1,7] demonstrate that if methoxy substitution at C-3 within the valinyl equivalent of the tripeptide provides the *allo* configuration, then such tripeptides can act as substrates for IPNS, whereas the corresponding diastereoisomeric *threo* configured tripeptides do not. In sharp contrast the tripeptides containing isoleucine, (**1g**) and *allo*-isoleucine, (**1h**), *both* cyclise to penicillins, (**2g**) and (**2h**) respectively, with retention of configuration in the

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

§ For (**6**) an α-configuration of the carboxy group is assumed, not proven.



Scheme 2. *Reagents:* i, SeO_2, Bu^tO_2H, $C_2H_4Cl_2$, ref. 5; ii, TlOEt, MeI, dimethylformamide; iii, toluene-4-sulphonic acid, then $NaHCO_3$; iv, coupling to (**3b**), ref. 6; v, CF_3CO_2H, anisole, ref. 6.



Scheme 3. L-AA = L-α-aminoadipoyl.

carbon–sulphur bond-forming step.[8] Since an ethyl group must be approximately isosteric with a methoxy group the difference between the two series must derive from some property of the methoxy function. One possibility is that a hydrogen bond between the methoxy group and an active site group restricts rotation around C(2)–C(3) in the intermediate,[7,9] as in Scheme 3. Thus, the species (**7**) derived from the *allo*-threonine peptide can be cyclised, but that derived from the threonine, (**8**), is restrained in a conformation in which the β-hydrogen atom cannot be attacked by the active species. The formation of two epimeric methoxy penams, (**2c**) and (**2d**), from the *O*-methyl serine peptide (**1d**) may be compared with the α-aminobutyrate peptide (**1j**) which gave both epimeric monomethylpenams (**2j**), presumably through a rapidly rotating free radical,[4,10] a process which is hindered by the presence of two substituents at the radical centre.

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