Studies on Side Chain Interactions during the Isopenicillin N Synthase Catalysed Biosynthesis of Penicillins

J. Chem. Research (S), 1997, 200–201
J. Chem. Research (M), 1997, 1401–1411

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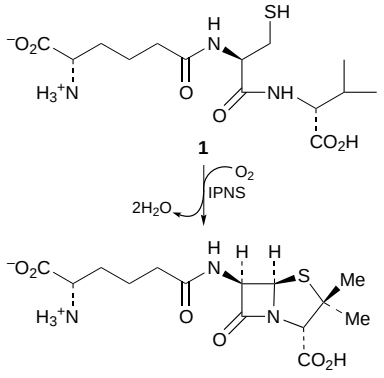
The role of the L-δ-(α-aminoadipoyl)–L-cysteinyl amide bond in isopenicillin N synthase catalysis is probed by the synthesis of two analogues of its tripeptide substrate L-δ-α-aminoadipoyl-L-cysteinyl-D-valine.

Isopenicillin N synthase (IPNS) catalyses the reaction of dioxygen and the tripeptide L-δ-α-aminoadipoyl-L-cysteinyl-D-valine (ACV, **1**) to give isopenicillin N (IPN, **2**) and two water molecules (Scheme A).[1,2] The L-δ-α-aminoadipoyl side chain of IPNS may be replaced with a variety of analogues,[1,2] but for efficient conversion to a penicillin a linear 6-carbon chain (or equivalent) is required.[4]



Scheme A

The puckered nature of the penicillin products and presumably intermediates during IPNS catalysis may place the L-δ-α-aminoadipoyl–cysteinyl amide link proximate to the reactive iron centre and it was considered possible that this amide plays a catalytic role. The analogue **6** in which the NH of the amide link is 1,3-transposed into the side chain of ACV may thus act as a mechanistic probe for the involvement of the L-δ-α-aminoadipoyl-L-cysteinyl amide link in catalysis.

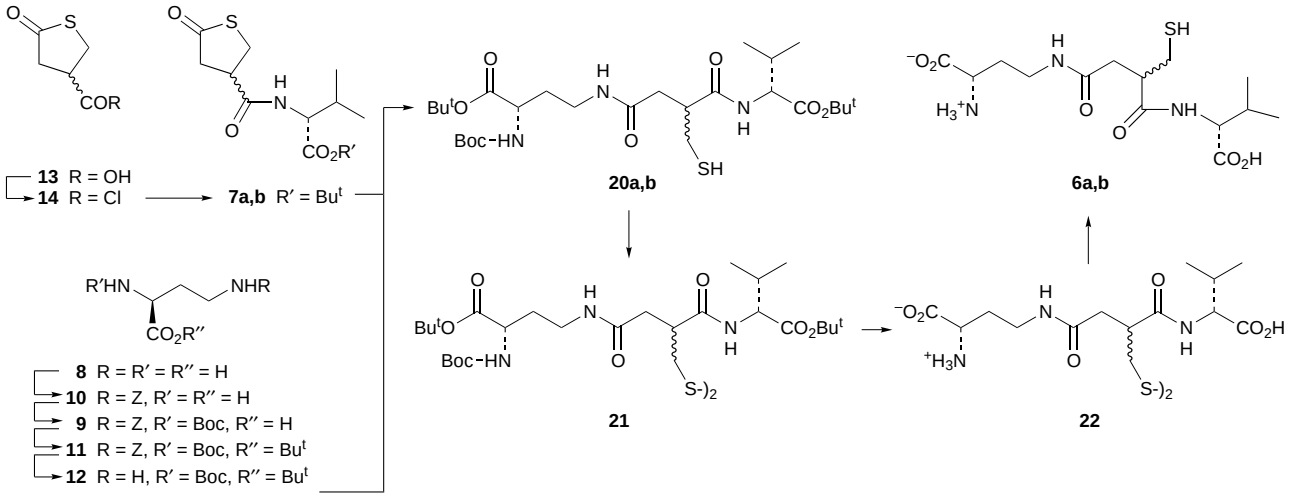
Diaminobutyric acid (**8**) was sequentially *N*-protected with benzyloxycarbonyl (Z) and *tert*-butyloxycarbonyl (Boc) groups, on its δ- and α-amino groups respectively to give **9** *via* **10**.[8] *tert*-Butyl ester **11** formation using *tert*-butyl alcohol and dimethylformamide neopentyl acetal,[9,10] followed by hydrogenolysis of the δ-amino protecting group gave **12**. Racemic thioparaconic acid (**13**), synthesised[7] from itaconic acid, was activated as its acid chloride **14** and reacted with D-valine *tert*-butyl ester to give the lactones **7a**,**b** (70%).

Diprotected L-diaminobutyric acid (**12**) was treated (EtOH, Cairos tube, 110 °C, overnight)[13] with the lactones **7a**,**b** to give the epimeric thiols **20a**,**b** in low yield (16%). Thiols **20a**,**b** were oxidised [$PhI(OAc)_2$] to a mixture of disulfides **21** (36%) in order to prevent intramolecular attack of the thiol on the side chain amide link resulting in reformation of thiolactones **7a**, **b** and **12** (Scheme B). Acid mediated deprotection gave the desired epimeric peptides as a mixture of disulfides **22** (98%), which were subsequently reduced using dithiothreitol to give crude epimeric thiols **6a**,**b** which were purified by HPLC. Neither **6a** nor **6b** were found to be substrates or inhibitors of IPNS.

The influence of the substrate side chain linkage in IPNS catalysis was also investigated by removal of the carbonyl of the side chain amide link, *i.e.* by the synthesis and incubation of **24**. Since the amino group of the L-δ-α-aminoadipoyl side chain is not required for IPNS turnover,[4] the synthetic target was simplified to **25**.

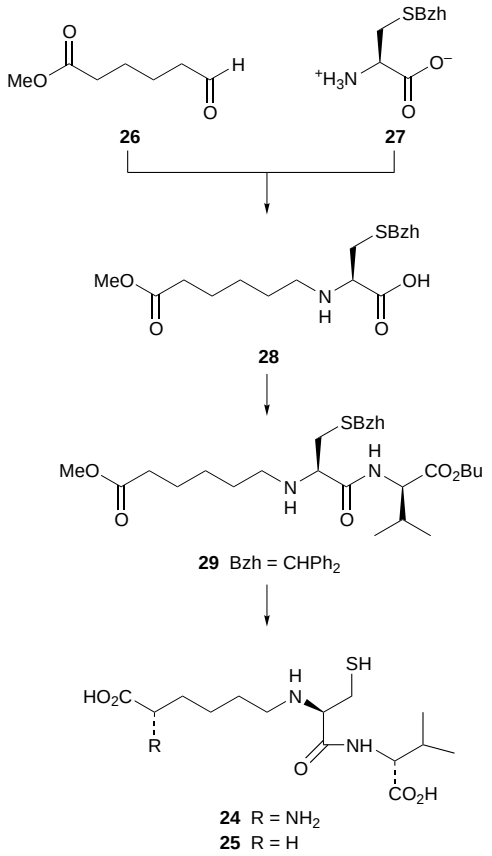
Thus, aldehyde **26**[14] and *S*-benzhydrylcysteine (**27**) were reacted under reductive amination conditions ($NaBH_3CN$, NaOH, MeOH) to give acid **28** (40%), which was coupled with D-valine *tert*-butyl ester to give **29** (62%) (Scheme C). Deprotection *via* basic cleavage of the methyl ester, followed by acid mediated removal of the benzhydryl and ester groups gave tripeptide **25** (97% prior to HPLC).

Incubation of **25** with IPNS led again only to recovered starting material with no evidence for the formation of products (by ^1H NMR, bioassay, or HPLC analyses). Preincubation experiments of **25** with IPNS did not lead to any increased inactivation relative to controls with ACV **1**. However, preliminary kinetic studies indicated that amine **25**



Scheme B

*To receive any correspondence.



Scheme C

reversibly inhibited IPNS with an IC_{50} of between 35 and 45 μM.

Techniques used: IR, ^1H and ^{13}C NMR, MS, TLC, polarimetry, elemental analysis

References: 14

Schemes: 1

Received, 29th January 1997; Accepted, 11th March 1997
Paper E/7/00662D

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