

Absolute Stereochemistry of Proclavaminic Acid, the Monocyclic Biosynthetic Precursor of Clavulanic Acid

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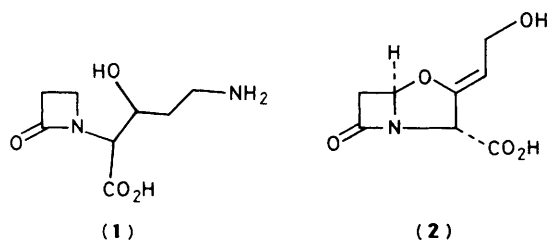
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Proclavaminic acid (**1**) has been synthesized by a route which indicated the absolute stereochemistry of the two chiral centres to be (2*S*,3*R*).

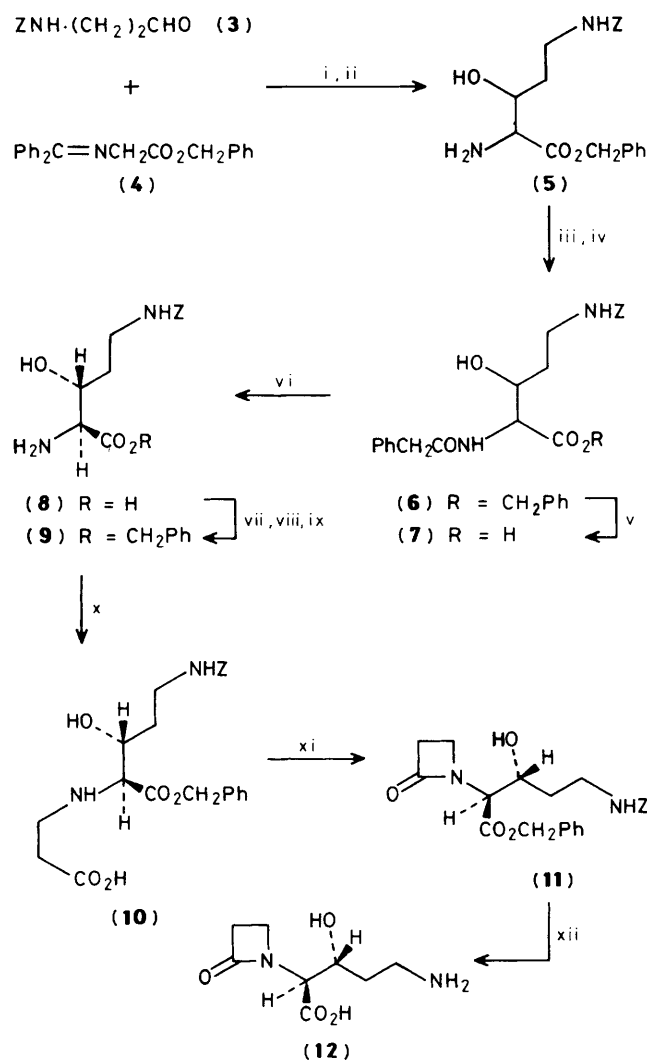
In previous communications^{1,2} we have described the isolation of proclavaminic acid (**1**) and its involvement in the biosynthesis of clavulanic acid (**2**) in *Streptomyces clavuligerus* ATCC 27064. A synthesis of the bioactive enantiomer of proclavaminic acid which did not enable the absolute stereochemistry to be deduced was also reported.³ In order to shed further light on the absolute stereochemistry of proclavaminic acid we report here a further synthesis *via* a resolved β -hydroxyornithine and elaborating the β -lactam ring without altering the chiral centres.

Aldol condensation of 3-(benzyloxycarbonylamino)propanaldehyde⁴ (**3**) with the masked glycine ester⁵ (**4**) followed by acid hydrolysis⁶ of the intermediate imine yielded the *N*⁵-protected β -hydroxyornithine (**5**)† in 95% yield (diastereoisomer ratio *ca.* 1:1, *erythro:threo*) (Scheme 1). Separation of the diastereoisomers was accomplished *via* the differential solubilities of the hydrochloride salts of (**5**) or by column chromatography of the phenylacetyl derivatives (**6**). The less polar diastereoisomer of (**6**) was assigned the *threo* configuration by conversion to the deprotected amino acid and showing this material to be identical to *threo*- β -hydroxyornithine synthesized by an independent route.⁷ Mild base hydrolysis of *threo*-(**6**) followed by enzymic deacylation of the resulting acid (**7**) with immobilised *Escherichia coli* acylase (E.C. 3.5.1.11) yielded enantiomerically pure (chiral h.p.l.c.) *threo*-*N*⁵-benzyloxycarbonyl- β -hydroxyornithine (**8**) (31%). The known hydrolytic specificity⁸ of the *E. coli* acylase coupled with the *threo* relative stereochemistry indicated that the absolute stereochemistry of (**8**) was (2*S*,3*R*).

The benzyl ester of (**8**), prepared by the method of Maclaran,⁹ was treated with an excess of acrylic acid in acetonitrile to yield the β -amino acid (**10**) [58% from (**9**)]. Ring closure using the Ohno procedure¹⁰ gave enantiomerically pure β -lactam (**11**) (51%). Model reactions on enantiomerically pure benzyl threoninates and benzyl *N*⁵-benzyloxycarbonyl-(*S*)-ornithinate demonstrated that this method of elaborating the β -lactam ring did not affect the stereochemistry of the chiral centres. Deprotection of (**11**) afforded proclavaminic acid (**12**) (85%). In the presence of partially purified clavaminic acid synthase,¹ Fe²⁺, O₂, and 2-ketoglutarate this material was converted to clavaminic acid (**13**).

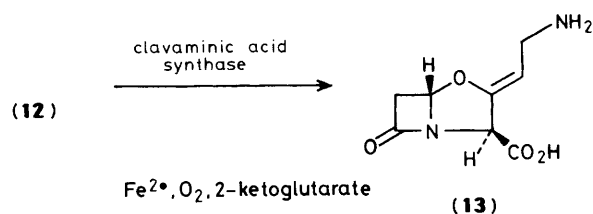


The synthesis of the racemic *erythro* analogue of proclavaminic acid from *erythro*-(**5**) was carried out using the route described above. The product was identical to the inactive diastereoisomer described previously³ and similarly was not converted to clavaminic acid (**13**) by the clavaminic acid synthase system.



Scheme 1. Reagents and conditions: Z = PhCH₂OCO-; i, (Me₃Si)₂NLi, tetrahydrofuran (THF), -70°C; ii, 2 M HCl-ether, NaHCO₃; iii, PhCH₂CO₂H, Et-N=C=N(CH₂)₃NMe₂, HCl, THF; iv, separate diastereoisomers; v, 1 equiv. NaOH, THF-H₂O (2:1); vi, immobilised *E. coli* acylase, pH 7.5, 37°C; vii, 1 equiv. KOH, MeCOCH₂CO₂Me, MeOH; viii, PhCH₂Br, dimethylformamide (DMF); ix, *p*-MeC₆H₄SO₃H, dioxane-ethyl acetate (3:1), NaHCO₃; x, 10 equiv. CH₂=CHCO₂H, MeCN; xi, di-2-pyridyl disulphide, Ph₃P, MeCN; xii, H₂, Pd-C (10%), EtOH.

† Satisfactory analytical and/or spectroscopic data were obtained for all new compounds.



On the basis of the above evidence we conclude that proclavaminic acid possesses the (2*S*,3*R*) absolute stereochemistry. Therefore the ring closure of the monocyclic proclavaminic acid (12) to the bicyclic clavaminic acid (13) which has the (3*S*,5*S*) stereochemistry by clavaminic acid synthase proceeds with retention of stereochemistry at the carbon bearing the carboxy function.

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