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

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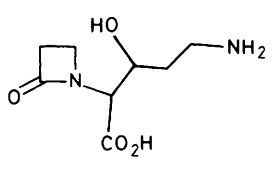
Proclavaminic acid (**1**) has been synthesized by a route which indicated the absolute stereochemistry of the two chiral centres to be (2*S*,3*R*).

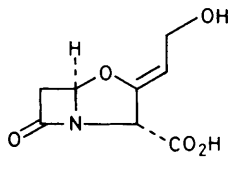
In previous communications[1,2] we have described the isolation of proclavaminic acid (**1**) and its involvement in the biosynthesis of clavulanic acid (**2**) in *Streptomyces clavuligerus* ATCC 27064. A synthesis of the bioactive enantiomer of proclavaminic acid which did not enable the absolute stereochemistry 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)propionaldehyde[4] (**3**) with the masked glycine ester[5] (**4**) followed by acid hydrolysis[6] 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 specificity[8] 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 procedure[10] 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] Fe^{2+}, O_2, and 2-ketoglutarate this material was converted to clavaminic acid (**13**).



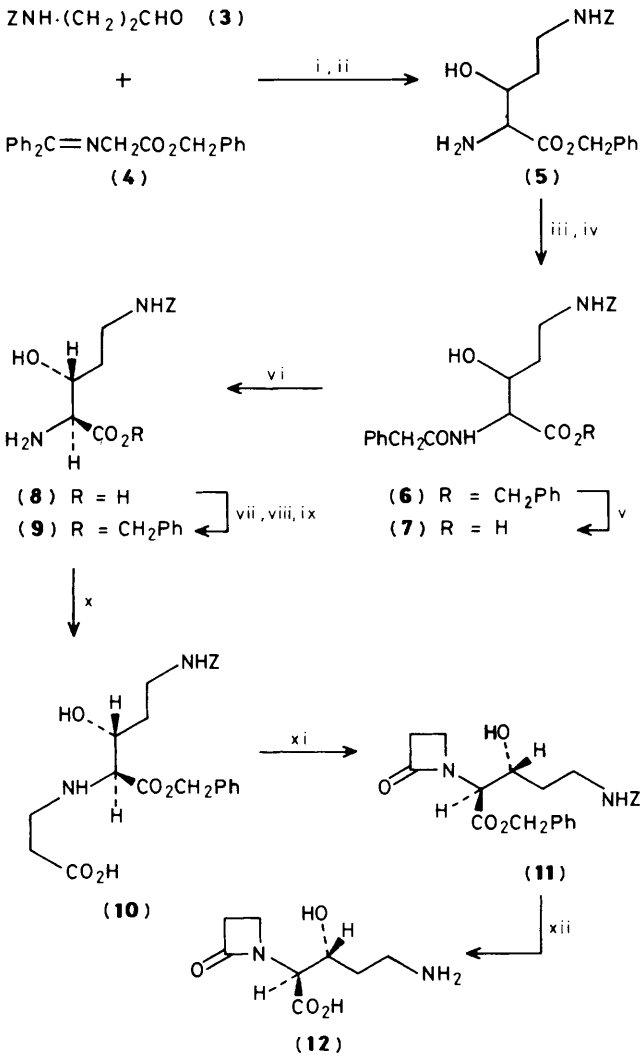
(**1**)



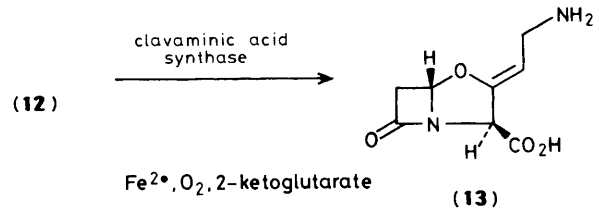
(**2**)

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

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 previously[3] and similarly was not converted to clavaminic acid (**13**) by the clavaminic acid synthase system.



Scheme 1. *Reagents and conditions*: Z = $PhCH_2OCO$–; i, $(Me_3Si)_2NLi$, tetrahydrofuran (THF), −70 °C; ii, 2 M HCl–ether, $NaHCO_3$; iii, $PhCH_2CO_2H$, Et–N=C=N$(CH_2)_3NMe_2$, HCl, THF; iv, separate diastereoisomers; v, 1 equiv. NaOH, THF–H_2O (2:1); vi, immobilised *E. coli* acylase, pH 7.5, 37 °C; vii, 1 equiv. KOH, $MeCOCH_2CO_2Me$, MeOH; viii, $PhCH_2Br$, dimethylformamide (DMF); ix, *p*-$MeC_6H_4SO_3H$, dioxane–ethyl acetate (3:1), $NaHCO_3$; x, 10 equiv. CH_2=$CHCO_2H$, MeCN; xi, di-2-pyridyl disulphide, Ph_3P, MeCN; xii, H_2, Pd–C (10%), EtOH.



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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