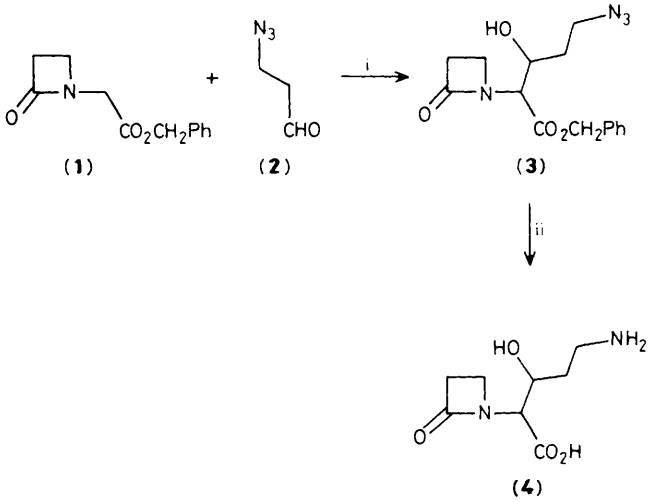
Synthesis of the Novel Monocyclic β-Lactam Proclavaminic Acid

Keith H. Baggaley, John T. Sime, Neville H. Nicholson, Stephen W. Elson,* Janet Gillett, Susan Holland, and Stefan R. Woroniecki

Beecham Pharmaceuticals Research Division, Brockham Park, Betchworth, Surrey RH3 7AJ, U.K.

Proclavaminic acid has been chemically synthesised and one pure enantiomer separated which was shown to possess identical physicochemical and biochemical properties to the natural material isolated from *Streptomyces clavuligerus.*

In the previous Communication[1] we described the isolation of proclavaminic acid, a novel monocyclic β-lactam, which is an intracellular product of the clavulanic acid producer *Streptomyces clavuligerus* ATCC 27064. Proclavaminic acid was tentatively assigned the structure (**4**). In order to confirm this assignment we have synthesised (**4**) *via* the route shown in Scheme 1.† The azetidinone (**1**) was synthesised by known methods[2] and condensed with the aldehyde (**2**).[3] The two diastereoisomers of the aldol product (**3**) were separated by silica gel column chromatography, and each was converted to (**4**) by catalytic hydrogenation. The ^1H n.m.r. spectra of the two diastereoisomers of (**4**) were different, especially with regard to the shift for the proton attached to the α-carbon of the amino acid moiety, as would be expected. For one diastereoisomer (**4a**), the doublet of the α-proton was centred at δ 4.08‡ ($^1J_{HH}$ 5.5 Hz), whereas for the other diastereo-



Scheme 1. *Reagents and conditions:* i, $[Me_3Si]_2Li$, tetrahydrofuran, −70 °C; ii, 10% Pd on charcoal, H_2.

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

‡ Spectra of (**4**) were run in D_2O and referenced to external HOD. Spectra of (**3**) were run in $CDCl_3$ and referenced to internal Me_3Si.

isomer (**4b**), the corresponding value was δ 4.18. The ^{1}H n.m.r. spectrum of natural proclavaminic acid was identical to that of (**4a**). When (**4a**) and (**4b**) were individually incubated with a partially purified preparation of clavaminic acid synthetase,[1] with the appropriate cofactors,[1] it was found that 50% of (**4a**) was cyclised to clavaminic acid, whereas there was no conversion of (**4b**). It was concluded that one enantiomer of (**4a**) corresponded to the natural metabolite.

The two enantiomers of (**4a**) were prepared by treating the appropriate diastereoisomer (**3a**), with the esterase subtilisin Carlsberg (EC 3.4.21.14). The unhydrolysed ester was examined by ^{1}H n.m.r. in the presence of the enantioselective solvating reagent *S*-(+)-2,2,2-trifluoro-1-(9-anthryl)ethanol and was thereby shown to be enantiomerically pure. The hydrolysed and unhydrolysed products of the enzymatic resolution were converted to the two enantiomers of (**4a**) then tested in the clavaminic acid synthetase cyclising system. The enantiomer derived from the hydrolysable enantiomer of (**3a**) was not converted into clavaminic acid, whereas that derived from the non-hydrolysable enantiomer was converted into clavaminic acid with high efficiency (>80%).

Physiochemical data (^{1}H and ^{13}C n.m.r., i.r. spectra, and h.p.l.c. retention times) for the synthetic, biologically active enantiomer and for the natural proclavaminic acid were found to be identical. Thus the original assignment of the structure of proclavaminic acid as (**4**) has been shown to be correct. At this stage the absolute stereochemistry of the biologically active enantiomer is not known.

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