Tetrahedron Letters,Vol.24,No.29,pp 2941-2944,1983 0040-4039/83 $3.00 + .00
Printed in Great Britain

SYNTHESIS OF 1-(1-D-CARBOXY-2-METHYLPROPYL)-3-L-(5-L-AMINOADIPAMIDO)-4-L-MERCAPTOAZETIDIN-2-ONE (SECO-ISOPENICILLIN N), A POTENTIAL INTERMEDIATE IN PENICILLIN BIOSYNTHESIS.

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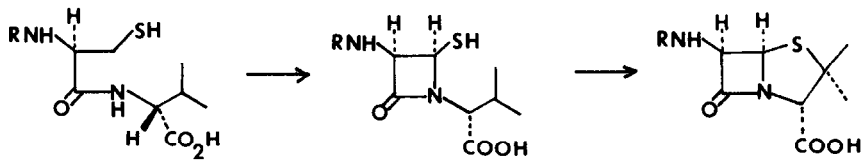
Abstract. The title compound has efficiently been synthesized by the skeletal rearrangement of the protected isopenicillin N sulfoxide and the subsequent hydrolysis of the thiazoline ring.

Although the LLD-ACV tripeptide (1) has been successfully converted into penicillin N via isopenicillin N by a preparation of lysed Cephalosporium acremonium protoplasts, [1] no clear-cut evidence has so far been obtained for an intermediate between the tripeptide and isopenicillin N(3) or for any particular cyclization mechanism leading to the β-lactam thiazolidine ring systems. Two different biosynthetic pathways are possible in theory for the penam ring formation from 1. In the first pathway, a monocyclic β-lactam (2) is formed first, while the seven-membered ring is presumed to be the key intermediate in the second pathway (Scheme 1).[2-4]

In an experiment involving the cell-free system of Penicillium chrysogenum protoplasts, the ACV-tripeptide (1) was reportedly converted to a compound, to which a monocyclic β-lactam-containing structure (2) was tentatively proposed.[5] A recent report on the synthesis of 2[6] prompted us to communicate our continuing efforts on the synthetic and biosynthetic work in this area.[7]



1 2 3

R = δ-(L-α-Aminoadipyl)

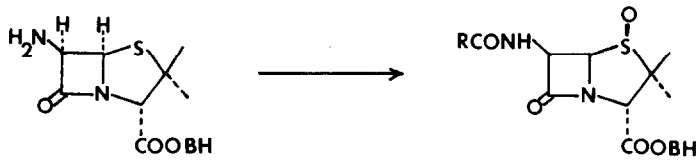
Scheme 1

In the synthetic plan of 2 (L and D,L) in their dimeric forms, we opted for the acid-labile protecting groups for both the amino and the carboxylate groups, which could be removed at the very last stage of the synthetic executions (Scheme 2). Thus, the readily available 6-aminopenicillanic acid (6-APA) in its 6-salicylideneamino protected form[8], was esterified with di-

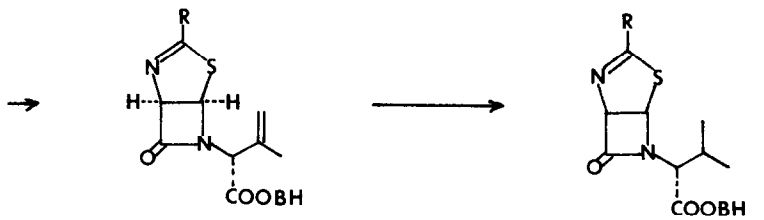
phenyldiazomethane to the benzhydryl ester (4) of 6-APA: IR (film) 3360, 1775, 1740 cm^{-1}; ^{1}H-NMR($CDCl_3$) δ 1.23(s, 3H), 1.53 (s, 3H), 1.81 (br, 2H, NH_2), 4.51 (s, 1H, C_3-H), 4.53 (d, J=4.2 Hz, 1H, C_5-H), 5.51 (d, J=4.2 Hz, 1H, C_6-H), 6.87 (s, 1H, $CHPh_2$) and 7.03-7.4 (m, 10H, aromatic). The ester was then coupled with α-benzhydryl ester of N-Boc-(L)-α- aminoadipic acid[9] by means of the methyl chloroformate activation to give the protected iso-penicillin N in 60% yield as foamy solid after a silica-gel column. The corresponding sulfoxide (5a) was prepared in quantitative yield by treating the protected iso-penicillin N with m-chloroperbenzoic acid in CH_2Cl_2.

When the sulfoxide (5a) was heated with $P(OMe)_3$ in refluxing benzene for 36 h,[10] the rearranged product, thiazolinoazetidinone (6a) was obtained in 57% yield after purification on silica-gel: mp 101-103°; $[\alpha]_D$ - 79.05° (c=1.0, EtOH); IR(KBr) 3380, 1770, 1740 and 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$)δ1.41 (s, 9H), 1.58-1.9 (br. m. 4H), 1.79 (s, 3H), 2.45 (br, 2H), 4.15 (br, 1H), 4.76 (br s, 1H), 4.95 (br s, 1H, C_3-H), 5.03 (br d, J=1,4 Hz, 2H), 5.83 (d, J=4.5 Hz, 1H, β-lactam), 5.85 (d, J=4.5 Hz, 1H, β-lactam), 6.87 (s, 1H), 6.89 (s, 1H) and 7.25-7.36 (m, 20H). Hydrogenation of 6a in EtOH/PhH (1/5) over the Wilkinson catalyst at 20 psi of H_2 gave 7a in 63% yield after purification on silica-gel: mp 119°; $[\alpha]_D$ -19.4° (c=0.67, EtOH); IR (KBr) 3380, 1770, 1735 and 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$) 0.85 and 0.95 (each d, J=6.5 Hz, 3H), 1.43 (s, 9H) 1.5-2.5 (m, 7H), 4.3 (d, J=9 Hz, 1H, C_3-H), 4.43 (br, 1H), 5.06 (br d, J=8 Hz, 1H), 5.6 (d, J=4.2 Hz, 1H, β-lactam), 5.77 (d, J=4.2 Hz, 1H, β-lactam), 6.88 and 6.90 (each s, 1H), and 7.28-7.39 (m, 20H); MS (FAB Ionization) m/e 776 ($M+H^+$). The hydrolytic ring-opening of the thiazoline part of 7a to the β-lactam mercaptan (8a) could be accomplished under well-controlled conditions (1N-HCl in MeOH). But the product (8a) was found to be too unstable for the subsequent handling. The mercaptan (8a) was, therefore, immediately oxidized to the corresponding disulfide (9a) by treatment with I_2 and $NaHCO_3$ in aqueous THF.[11] It was found possible to perform the hydrolysis and the oxidative dimerization in a single step procedure involving I_2 in DMSO and 1N. HCl.[12] The disulfide (9a) showed the following properties: foamy solid; $[\alpha]_D$ -49.6° (c=1.35, EtOH); IR (KBr) 3340, 1770, 1730 and 1690 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 0.86 and 1.01 (each d, J=6.8 Hz, 3H), 1.41 (br s, 9H), 1.5-2.5 (m, 7H), 4.16 (d, J=9 Hz, 1H, C_3-H), 4.37 (br, 1H) 5.0-5.23 (m, 3H), 6.34 (br, 1H), 6.86 (s, 1H), 6.91 (s, 1H) and 7.23-7.35 (m, 20H); MS (FAB ionization) m/e 1585 ($M+H^+$).

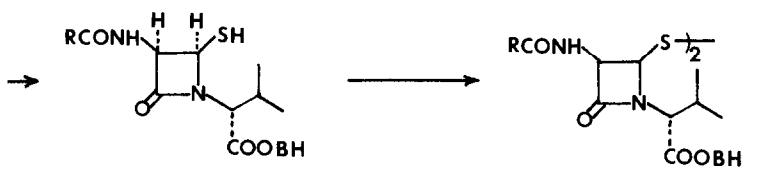
The protecting groups were removed by subjecting disulfide 9a to trifluoroacetic acid containing an excess amount of anisole at 0° for 30 min. The dimer of seco-isopenicillin N (10a) appeared to be reasonably stable as solid over several weeks at 0°C and displayed the following properties: mp 158-162°C (decomp.); homogeneous on HPLC (C-18 reverse phase, CH_3CN-0.1M phosphate buffer (pH7); IR (KBr) 3350, 1760 and 1660 cm^{-1}; ^{1}H-NMR (D_2O containing DCl) δ 1.02 and 1.07 (each d, J=6.4 Hz, 3H), 1.85 (br, 2H), 2.08 (br, 2H), 2.53 (br, 3H), 4.06 (d, J=9.5 Hz, 1H), 4.21 (br t, 1H), 5.2 (d, J=4.5 Hz, 1H) and 5.33 (d, J=4.5 Hz, 1H); MS (FAB ionization) m/e 721 ($M+H^+$). The key features of ^{1}H-NMR of 10a are consistent with the expectations based on ^{1}H-NMR of isopenicillin N.[13] The diastereomeric mixture 10b was analogously synthesized from the benzhydryl ester of 6-APA and the α-benzhydryl ester of N-Boc-(D,L)-α-aminoadipic acid.[14] The reductive cleavage of 10a to 2 could be accomplished either by treatment with dithiothreitol (DTT) in a pH7 buffer or by treatment with Zn in DCl/D_2O. Compound 2 has been found to be extremely unstable (lifetime being less than a few minutes) at pH7.0, but appeared to be considerably more



4 5a. R = (L)-tBocNHCH(COOBH)$(CH_2)_3$-
b. (DL)



6 7



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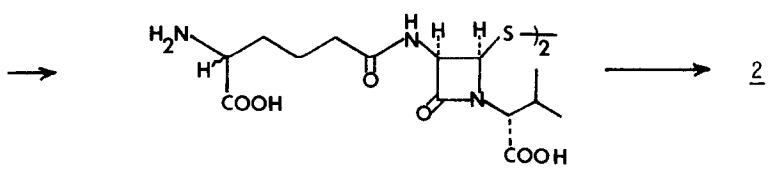

10a. (L)
b. (DL)

SCHEME 2

stable in DCl/D_2O solution: ^{1}H-NMR δ 1.0328 and 1.0517 (each d, J = 7.1 Hz, 3H), 1.6-2.6 (7H), 4.0784 (d, J = 9.5 Hz, 1H), 4.2598 (t, J = 6.4 Hz, 1H), 5.6338 (d, J = 3.8 Hz, 1H) and 5.9740 (d, J = 3.8 Hz, 1H). Treating compound 2 with I_2 yielded the well-characterized disulfide (10a).

In the preliminary study, incubation of 10a or 2 with the cell-free preparation of C. acremonium did not yield a detectable quantity of isopenicillin N.[15] However, the feeding experiments are continuing with P. chrysogenum[5] and Streptomyces clavuligerus cell-free systems[16] at various acidities.

Acknowledgement. We thank Dr. K. B. Tomer, Midwest Center for Mass Spectrometry at University of Nebraska for the Fast Atom Bombardment (FAB) mass spectra. This research has been supported by NIH (A1-14481) and Robert A. Welch Foundation (A-752).

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(14) The spectral properties (IR and NMR) of the diastereomeric mixture (5b-10b) are very similar to those of 5a-10a. A significant ^{1}H-NMR resolution of the diastereomeric pairs was observed only in 7b and the HPLC resolution only in 9b and 10b.

(15) Results obtained by Robert L. Baxter and A. Ian Scott at University of Edinburgh.

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(Received in USA 10 January 1983)