CONCERNING THE INTERMEDIACY OF MONOCYCLIC β-LACTAMS IN PENICILLIN BIOSYNTHESIS: SYNTHESIS AND ATTEMPTED BIO-CONVERSION OF SECO-ISOPENICILLIN N†

A. Ian Scott,* Ramaswamy Shankaranarayan, and Sung-Kee Chung[1]

Center for Biological NMR, Department of Chemistry, Texas A&M University, College Station, Texas 77843, USA

Abstract - The title compound (**3a**) was synthesized as its dimer by the skeletal rearrangement of protected isopenicillin N-sulfoxide, hydrogenation, hydrolysis of the thiazoline ring, oxidative dimerization and deprotection. The reductive conversion of the dimer to monomer (**3a**) was clearly demonstrated. Seco-isopenicillin N (**3a**) gave no bioactive products on incubation with cell-free systems from either *Cephalosporium acremonium* or *Streptomyces clavuligerus*.

INTRODUCTION

The primary β-lactam antibiotics such as isopenicillin N and cephalosporin C are known to be derived from L-α-aminoadipic acid, L-cysteine and L-valine. Although the isolation of δ-(L-α-aminoadipoyl)-(L)-cysteinyl-(D)-valine (ACV) from *Penicillium chrysogenum* and *Cephalosporium acremonium* strongly suggested the possible involvement of the LLD-ACV (Arnstein) tripeptide as a key biosynthetic intermediate of the β-lactam antibiotics, the hypothesis was difficult to test until recently because the intact microorganisms do not take up the tripeptide.[2] It has been demonstrated that cell-free preparations from various microorganisms are capable of converting the tripeptide to isopenicillin N[3] and it has been shown that penicillin N is converted to cephalosporin C by a cell-free system.[4] The cyclizing enzymes (isoPen synthetase - IPNS) have been purified[5] and IPNS gene has been sequenced and cloned from several organisms including *C. acremonium* and *S. clavuligerus*.[6]

Despite these developments, the mechanistic processes involved in the *in vivo* conversion of the tripeptide (**1**) to the bicyclic penam and cephem structures still remain largely unknown. Thus no intermediate has been established between **1** and isopenicillin N (**2**), the first bicyclic structure in the proven biosynthetic pathway of β-lactams. Nevertheless, a large number of labeling experiments have set fairly rigid requirements for any proposed oxidative cyclization mechanism of **1**. For example, Bycroft,[7a] Young[7b] and Aberhart[7c] have shown by use of L-cysteine chirally labeled at

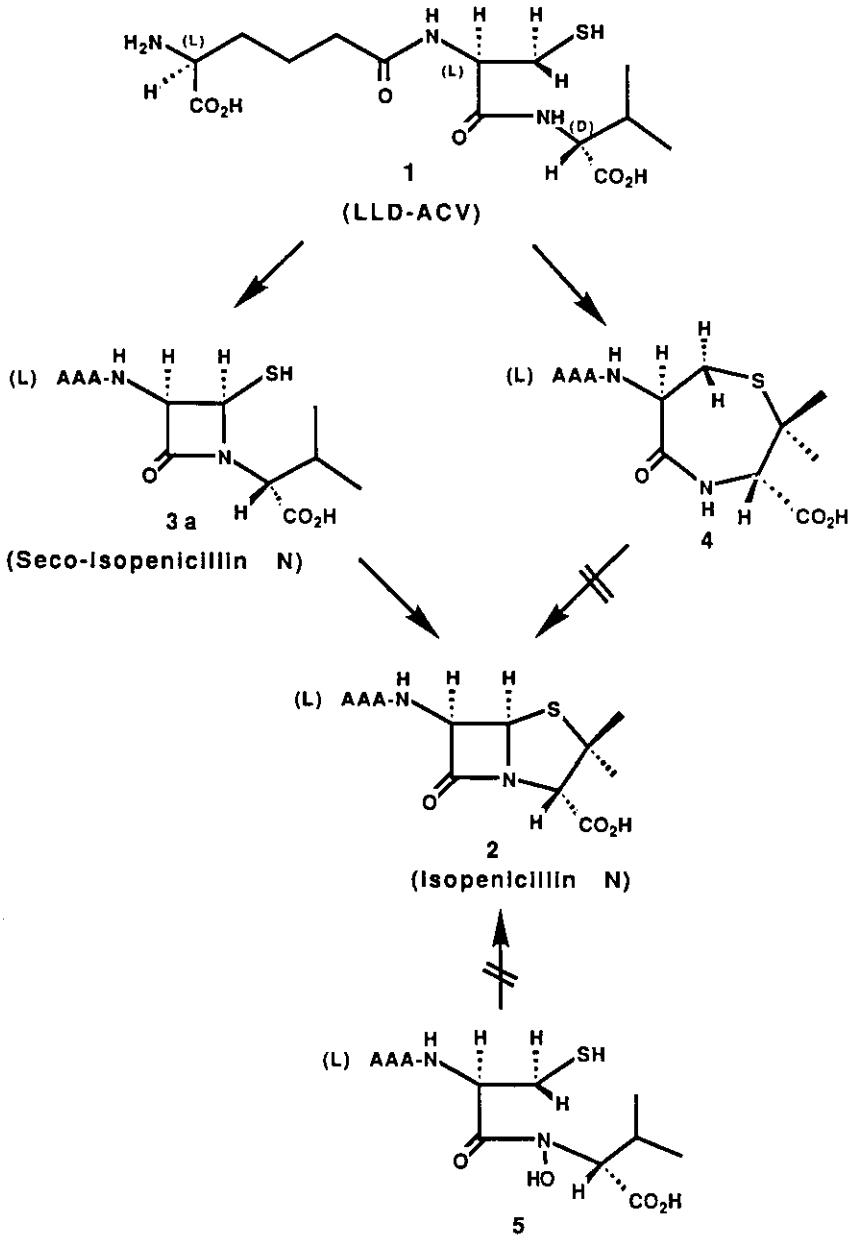
† Dedicated to the memory of Professor Tetsuji Kametani.

C-3 that this carbon becomes C-5 of the penam structure with overall retention of configuration. Sih, Abraham and their colleagues have found that in the bioconversion of (2S,3R)- and (2S,3S)-[4,4,4- $^2H_3$]-valine into penicillin N, the deuterium atoms are located exclusively in the β-methyl and α-methyl group of penicillin N, respectively, thus indicating that formation of the thiazolidine ring should occur with retention of configuration at C-3 of valine.[8] The incorporation of the LLD-ACV tripeptide into isopenicillin N was previously shown to proceed without loss of the α-hydrogen of the D-valine moiety, and this precluded the possible involvement of the dehydrovaline analog of **1** as a biosynthetic intermediate.[3a] For the bioconversion of **1** to **2**, two oxidative pathways have been suggested to rationalize the stoichiometry of the conversion:[9]

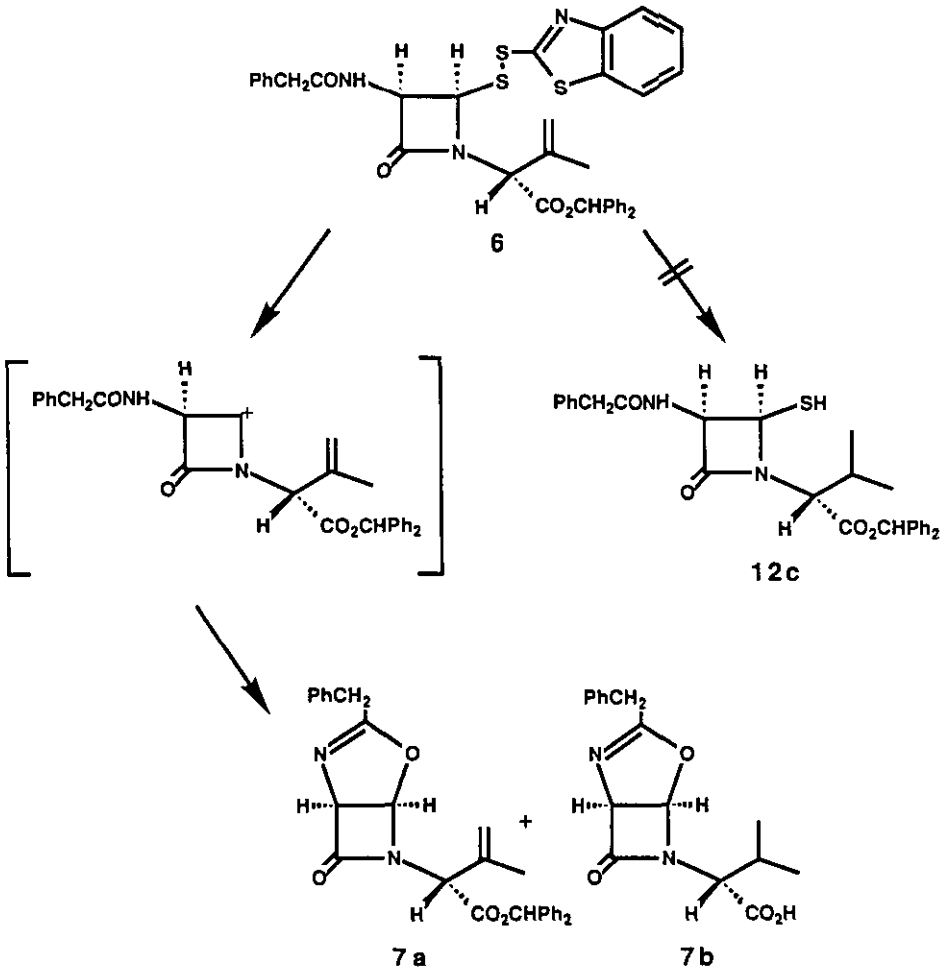
$$ACV + O_2 \longrightarrow \text{IsoPen N} + 2H_2O$$

In the first pathway, a monocyclic β-lactam intermediate (**3a**) is first formed, followed by thiazolidine ring formation. A second pathway involving a 7-membered ring intermediate was tested by Baldwin, Abraham *et al.* who showed that the synthetic thiazepinone - containing peptide (**4**) could not be converted to isopenicillin N in a cell-free system from *C. acremonium*.[9b] Furthermore, the synthetic hydroxamic acid (**5**) which could have served as an intermediate for either pathway (based on biomimetic chemistry[10a]) was not incorporated.[10b] However, Meesschaert *et al.*[11] reported that in an experiment employing a cell-free preparation of *P. chrysogenum* protoplasts, **1** was converted to "compound Y", to which the monocyclic β-lactam structure (**3a**) was assigned (Scheme 1). We describe herein a synthesis of seco-isopenicillin N (**3a**)[12,13] and the results of its incubation with cell-free systems from *C. acremonium* and *S. cavuligerus*.

In the initial synthetic plan, we examined the possibility of direct access to the β-mercaptoazetidinone structure by hydrogenating the Fujisawa disulfide **6** over Wilkinson catalyst. However, when **6**, prepared from the corresponding penicillin sulfoxide according to the literature procedure,[14] was hydrogenated in benzene-ethanol over $Rh(PPh_3)_3Cl$, the desired product (**12c**) was not obtained. Instead, the product mixture was found to be primarily composed of **7a** and **7b**. The fact that the rearranged oxazolino-azetidinone (**7b**) rather than the desired β-mercaptoazetidinone was formed during the hydrogenation was revealed by the ^{1}H-nmr resonances of the β-lactam hydrogens of (**7b**) at δ 5.10 (d, J=3.4 Hz, 1H) and 6.04 ppm (d, J=3.4 Hz, 1H). Additionally, product **7b** was found to be identical in all respects to an authentic sample prepared by hydrogenating **7a**, which in turn was independently prepared by literature procedure.[15] Oxazolino-azetidinones have previously been prepared from the corresponding sulfoxides or disulfides by treatment with tervalent phosphorus compounds.[15,16] It was found that treatment of disulfide **6** with $Rh(PPh_3)_3Cl$ in ethanol-benzene yielded the rearranged product (**7a**) and that PPh_3 alone was not as effective as the Wilkinson catalyst in promoting this rearrangement. It seems reasonable to suggest that the Rh catalyst rather than dissociated PPh_3 behaves as an electrophile in the interaction with the sulfur atom of **6**, thus generating a carbonium ion center at the C-4 position of the azetidinone ring. The carbonium ion is then captured by the internal nucleophile, an amide functionality, in oxazoline ring formation (Scheme 2).

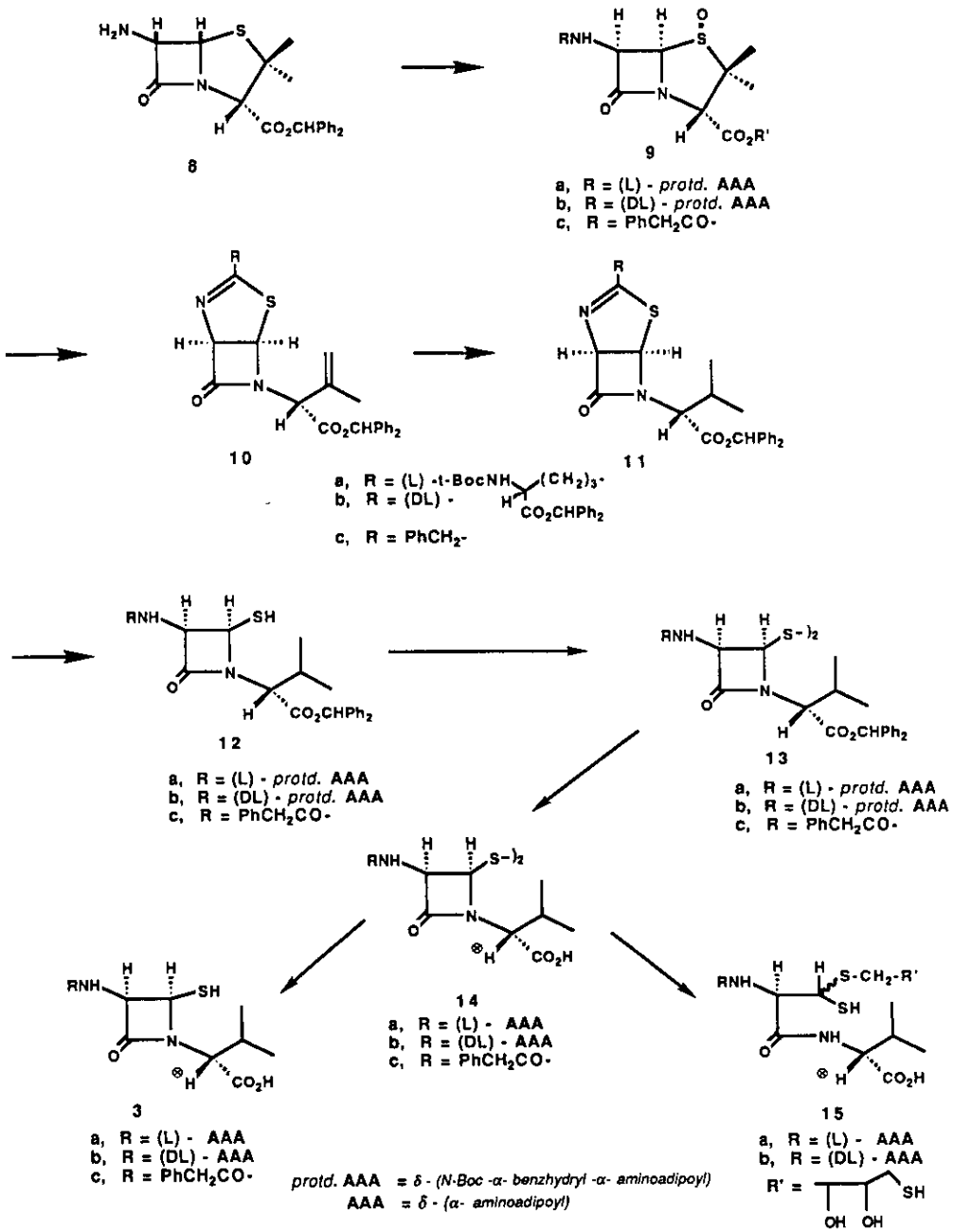

1
(LLD-ACV)
3 a
(Seco-Isopenicillin N)
4
2
(Isopenicillin N)
5
AAA = α-aminoadipoyl

Scheme 1



Scheme 2

The above rearrangement problem was circumvented via the synthetic route outlined in Scheme 3. The readily available 6-aminopenicillanic acid (6-APA) was converted to the 6-salicylideneamino protected form[17] and esterified with CPh_2N_2 to the benzhydryl ester of 6-APA. In separate experiments, ester **8** was coupled with the α-benzhydryl esters of N-Boc(L)- and N-Boc-(DL)- α-aminoadipic acid[18] by means of chloroformate activation, to provide the fully protected isopenicillin N [(a) series] and the diastereomeric mixture of Isopenicillin N and Penicillin N [(b)series] respectively. Oxidation with mCPBA gave the corresponding β-sulfoxides (**9a,b**) in good yields. Sulfoxide **9a** was heated with $P(OMe)_3$ in refluxing benzene for 36 h[19] to effect the rearrangement to the thiazolino-azetidinone (**10a**). Hydrogenation of **10a** in ethanol/benzene over Wilkinson catalyst proceeded without complication to give **11a** in 82% yield. The hydrolytic ring-opening of the thiazoline moiety of **11a** to **12a** could be accomplished under well-controlled



Scheme 3

conditions (1N HCl in MeOH). The product (**12a**) was found to be somewhat unstable and was therefore immediately oxidized to its disulfide (**13a**) with I_2 and $NaHCO_3$ in aqueous THF.[20] It was subsequently found possible to perform the hydrolytic ring-opening and the oxidative dimerization in a single step procedure involving I_2 in 1N HCl and DMSO.[21] The protected disulfide (**13a**) was fully characterized by spectroscopic methods, including the FAB mass spectrum which showed m/z 1585 (MH^+).

All protecting groups were removed in a single step by dissolving the dimer (**13a**) in CF_3CO_2H containing excess anisole at 0° C for 30 min. The resultant dimer of seco-isopenicillin N(**14a**) was homogeneous on reverse phase hplc and paper electrophoresis, and appeared to be reasonably stable as a solid at 0°C. The spectral data are in agreement with the structure **14a**. The key features of the ^{1}H-nmr of **14a** are consistent with expectations based on the spectroscopic data for isopenicillin N,[22] and the FAB mass spectrum showed m/z 721 (MH^+). The diastereomeric mixture **14b** was analogously synthesized from **9b**. A significant ^{1}H-nmr resolution of the diastereomeric pairs was observed only in **11b**, and the hplc resolution was seen only in **13b** and **14b**.

The reductive conversion of the dimer (**14a**) to seco-isopenicillin N (**3a**) turned out to be problematic because of the instability of **3a**. After considerable experimentation, it was found that the reduction could be most conveniently carried out with Zn dust in 20% HCl(DCl was used for ^{1}H-nmr experiments), and that the stability of **3a** was considerably enhanced at acidic pH. Although compound **3a** was too unstable for isolation, its formation could be readily monitored either by ^{1}H-nmr or hplc. It was very important to maintain the proper ratio of Zn to DCl volume (the ideal being 1-3 mg of Zn for ~0.8 ml of soln) and also essential to carry out the reduction at low temperatures (0-5° C) to achieve reproducible results. We found that with slow addition of Zn powder at ice-bath temperature over 10 min, we could conveniently monitor the progressive reduction of **14a** to **3a** by ^{1}H-nmr. Under these conditions no other products could be detected. Reduction of **14a** is accompanied by very significant changes in its ^{1}H-nmr spectrum, the most characteristic feature being the downfield shift of the two β-lactam proton resonances at δ 5.09 (d, J=4.6Hz, 1H) and 5.31ppm (d, J=4.6 Hz, 1H) in **14a** to 6.17 (d, J=4.5 Hz, 1H) and 6.39 (d, J=4.5 Hz, 1H) in **3a**. In addition, the C1-H of the 1-D-Carboxy-2-methylpropyl sidechain at N1 ($H^{\circledR}$ in **14a**) resonating at δ 4.06 ppm (d, J=9.5 Hz,1H) moved downfield to 4.31 (d, J=7.4 Hz, 1H) upon reduction to **3a**. Another notable feature of the reduction was the perturbation of proton resonances of methylenes in the α-amino adipamido sidechain: β-, γ- and δ-CH_2 signals at δ 1.89 (br, 2H), 2.02 (br, 2H) and 2.52 (br t, 2H) in **14a** moved downfield to 2.11 (br, 2H), 2.5 (br, 2H) and 3.18(br t, 2H) in **3a**. Similar changes, though of lesser magnitude, in the proton resonances were observed in model studies of reduction of ACV and analogous peptide disulfides (**16-20**); (Table 1). The preparation, chemistry and results of incubation studies of the disulfides **17 - 20** with cell-free systems from *S. clavuligerus* will be described elsewhere.[12b]

The reduction mixtures of the thiol (**3a**) and zinc salts were stable for several hours. In the case of **3a**, β-lactam proton signals could be detected by ^{1}H-nmr after 7 days at pH < 4 and 5° C. The disulfide **14a** could be safely regenerated from the reduction mixture containing **3a** by stepwise addition of iodine (to excess) to give a product identical (by ^{1}H-nmr) with **14a** (Figure 1). In studies with ACV (**1**), the regenerated disulfide (**16**) was isolated and transformed *in vivo* to **2** with cell-free extracts from *Streptomyces clavuligerus*.[3b] However, if a large excess of Zn was initially added in one portion or if the temperature was allowed to rise above 10° C, reduction of **14a** gave a product with ^{1}H-nmr features (Figure 1f) quite different from those of **3a**: two β-lactam signals at δ 5.63 and 5.97 ppm (each d, J=3.8 Hz, 1H); other signals at δ 4.06 (d, J=7.2 Hz, 1H), 1.60 (br, 2H), 2.10 (br, 2H) and 2.50 ppm

Table 1. Effect of Zn/DCl reduction on proton resonances (δ ppm) in peptide disulfides (**16-20**)

peptide	Disulfide		Monosulfide	
	5-H	3'-CH	5-H	3'-CH
16	4.28 (d, J=6Hz, 1H)	2.58 (br t, 2H)	4.33 (d, J=6Hz, 1H)	2.61 (br t, 2H)
17[12b]	4.42 (br t, 1H)	2.54 (br t, 2H)	4.43 (br t,1H)	2.58 (br t, 2H)
18[12b]	4.64 (s, 1H)	2.36 (br t, 2H)	4.32 (s, 1H)	2.40 (br t, 2H)
19[12b]	compound decomposed in DCl soln.			
20[12b]	4.28 (d, J=6Hz, 1H)		4.40 (d, J=6Hz, 1H)	
14a	4.06 (d, J=9.5Hz, 1H)	2.53 (br t, 2H)	4.31 (d, J=7.4Hz, 1H)	3.18 (br t, 2H)

(br, 2H). No attempt was made to isolate this product whose proton spectrum was unaffected by excess iodine. The relatively clean reduction - oxidation cycle between **14a** (hplc retention time 10.5 min) and **3a** (retention time 7.5 min) could be followed by reverse phase hplc analysis. Although the lifetime of **3a** in acidic solution appears to be significantly longer than a previous estimate[12a] (based on ^{1}H-nmr and hplc data on reoxidation), it completely decomposed to uncharacterized products as the pH was raised above 6. In fact, it has been suggested[13b] that the reduction of the disulfide **14a** with Zn/DCl-D_2O leads to uncharacterized products which may not correspond to the thiol (**3a**). However, the conditions described elsewhere[13b] in attempts to repeat the reduction are not the same as those employed in our laboratory.[12a] Thus, the reoxidation of (**3a**) back to (**14a**) is clearly demonstrated by the nmr time course shown in Figure 1 and also by hplc analysis, thus resolving any previously suggested discrepancy.[13b]

After initial unsuccessful attempts, we found that dithiothreitol (DTT) provided a practical method of converting **14a** to **3a** *in situ*, suitable for incubation studies with IPNS enzyme. Although DTT has been reported to reduce **14a** to non β-lactam product **15a**[13b] irreversibly, we could demonstrate the reduction of **14a** to **3a** and reoxidation back to **14a** under controlled conditions. Addition of 2.5 equivalents of DTT (cf. 6 equivalents employed in the reduction of ACV dimer for incorporation studies) to **14a** in 0.05M Tris buffer (pH 7.5 - 7.8, 22° C) immediately led to the appearance of pairs of doublets at δ 0.94, 1.00, 5.09 and 5.11 ppm characteristic of **3a**. These new signals appear to grow at the expense of those from **14a** (Figure 2a - d). However, upon addition of iodine (excess should be avoided!), the broad signals at δ 5.10 and 5.12 ppm collapse to sharp doublets as do the methyl doublets at δ 0.94 and 1.00 ppm (Figure 2d). No further changes could be observed even after 45 min in the reoxidised mixture (Figure 2e). The reduction and reoxidation of the monolactam disulfide was rigorously demonstrated when the DL- analog, **14b**

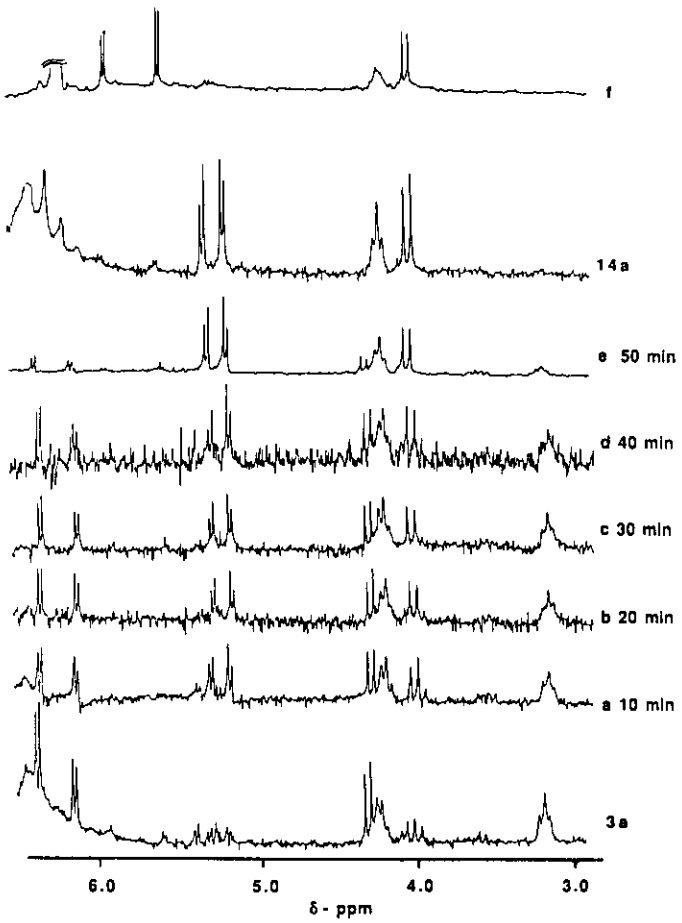


Figure 1. ^{1}H-Nmr time course of the reduction of **14a** (2 mg in 0.4 ml of 20% DCl in D_2O) with Zn (~3 mg, 0° C,15 min) to **3a** followed by oxidation of **3a** back to **14a** with iodine (**a** → **e**). 200.057 MHz; data were accumulated using a modified DANTE pulse sequence[32] for selective solvent excitation (180° selective pulse on H_2O, 2 sec. τ period and 90° pulse); size = 16 K, NS = 64, LB = 0.0 Hz and then Fourier transformed. Each spectrum (**a** → **e**) was recorded at 10 min intervals (→ 50 min) after addition of iodine to **3a** (bottom trace). The spectrum of pure **14a** is shown in the second trace from top. The spectrum of the product from irreversible reduction of **14a** is shown in the top trace (f).

was used (Figure 3a -c). Reversibility of the DTT reduction of β-lactam disulfides is further borne out by the reduction- reoxidation of the unsaturated monolactam disulfide (**23** → **24**) present as an impurity (nmr signals shown as * in Figure 3.). Survival of the β-lactam during DTT reduction is further confirmed by the FTir analysis of the freeze-dried reduction mixtures. No significant loss of the β-lactam ring in numerous trials was observed although the isolation of **3a** or **3b** was not attempted. Thus, **3a** can be generated *in situ* with sufficient life time under conditions suitable for incubation with IPNS. Although these results are in apparent contrast with the findings of the Oxford

group[13] the method of preparation of **14a** and conditions of its attempted reduction are quite different from those described above. However, it is clear that **3a** (and **3b**) completely decompose to non β-lactam products (not characterized) on standing for extended periods (>15 min at pH >7, 22° C) or in presence of excess DTT. It is thus unlikely that **3a** could survive the extensive isolation procedures described by Meesschaert *et al.*[11]

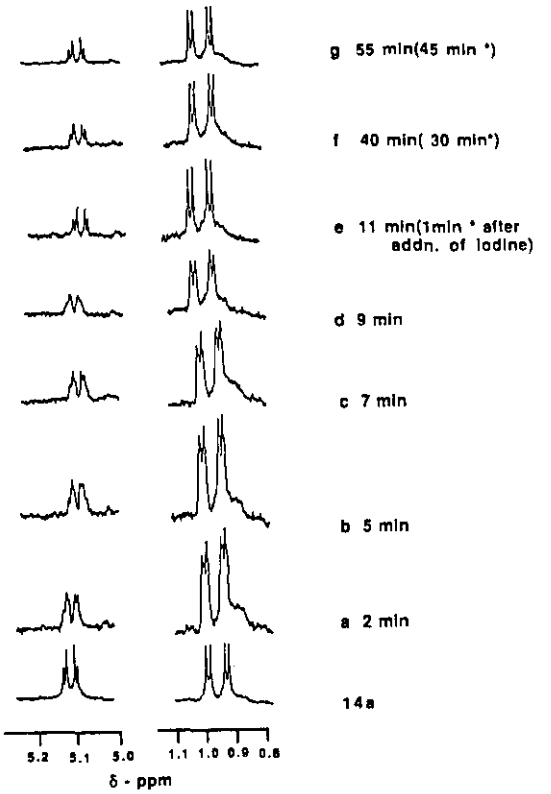


Figure 2. ^{1}H-Nmr time course of the reduction of **14a** (1 mg in 0.4 ml of 0.05 M Tris buffer, pH 7.8) with DTT (0.5 mg)at 22° C (a → d) and oxidation of the reduction mixture with iodine (2 mg, e → g). 500.138 MHz; data were accumulated with a 45° pulse, size = 16 K, NS = 50, LB = 0.0 Hz and then Fourier transformed. Times in parentheses refer to times of incubation with iodine.

Incubation of **3a** (generated *in situ* from **14a** with DTT) with the *C. acremonium* system reported earlier[23] or with *S.clavuligerus* cell-free system [3b] did not produce any isopenicillin N nor did it interfere with the conversion of ACV to isopenicillin N when co-incubated (see Table 2).

The involvement of **3a** in penicillin biosynthesis has been suggested based on competitive isotope effects.[24] In our hands all efforts to detect **3a** either free or enzyme-bound with cell-free systems from *S.clavuligerus* using high-field *nmr spectroscopy have been unsuccessful so far. It is quite likely that the monocyclic* β-lactam species **3a** does not exist free but rather is present as an enzyme-bound iron-oxo intermediate (**25**)[26] or equivalent species with sufficient long half life to lead to iso penicillin N and also to unusual penams and cephams with modified ACV substrates.[27]

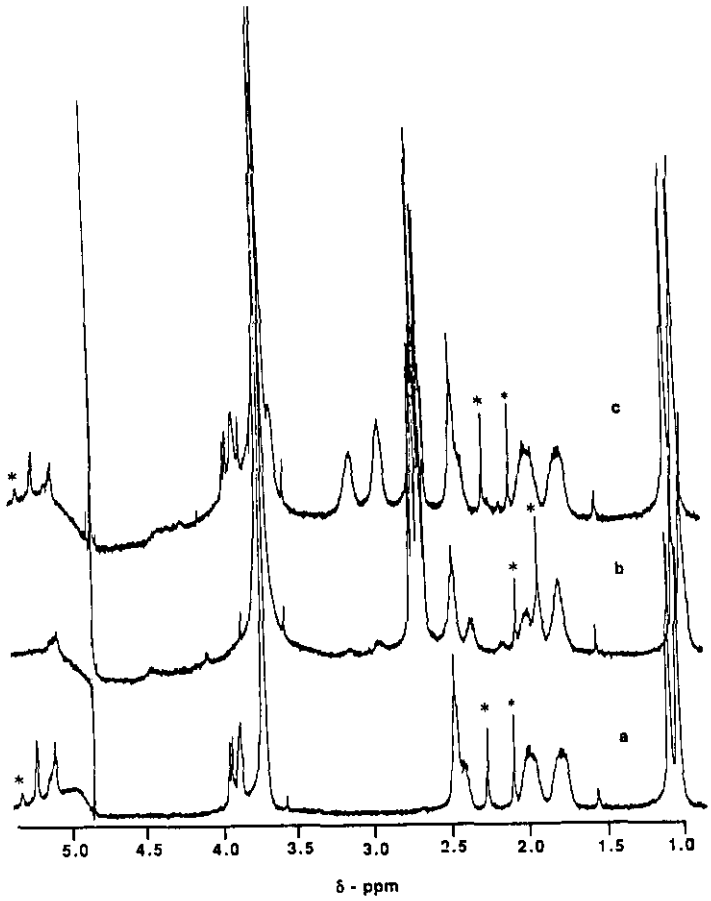


Figure 3. ^{1}H-Nmr time course of reduction and reoxidation of **14b**. (a) **14b** in Tris buffer($^{2}H_2O$, pH 7.5). (b) **14b** (10 mg in 0.5 ml buffer) reduced with DTT (5mg in 0.5 ml of buffer, 8 min, 22° C), freeze-dried and resuspended in $^{2}H_2O$. (c) sample (b) oxidised with iodine; 500.138 MHz ; data accumulated with selective solvent (HOD) suppression; size = 16K, NS = 512, LB = 0.0 Hz. Signals from **23** and **24** are marked '*'.

Recent reports[25] on the conversion of phenylacetyl-L-cysteinyl-D-valine (**21**) and phenoxyacetyl -L-cysteinyl-D- valine (**22**) peptides to bioactive compounds(presumably Penicillin G and Penicillin V) by cell-free extracts from *C.acremonium* and *S.clavuligerus* prompted us to test the fate of the monolactam **3c** under similar conditions. 6-phenylacetamidopenicillin β- sulfoxide (**9c**)[28] was converted to **14c** through a series of reactions analogous to the

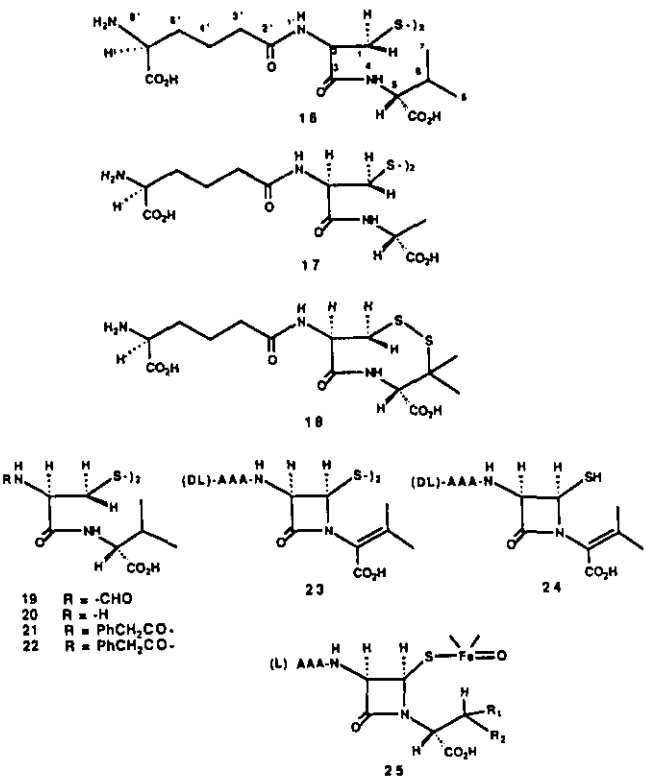


Chart 1

preparation of **14a** described above (Scheme 3). Monomer **3c** prepared *in situ* by reduction of **14c** with DTT in Tris buffer (pH 7.5) has a much higher stability at pH 7.5 compared with **3a** as evidenced by ^{1}H-nmr.[29] No bioactive products were detected however, when **14c** (preincubated with DTT) was incubated with partially purified IPNS enzyme from *S. clavuligerus*. Also **14c** did not interfere with the Isopenicillin N biosynthesis when co-incubated with ACV disulfide (**16**) in presence of IPNS (Table 2.).

Table 2. Bioassay of incubation mixtures.

substrate	Zone of inhibition (mm)	
	Micrococcus luteus	*Comamonas terrigena*
16	29	30
14a	12	0
14c	12	0
14a + 16	27	28
14c + 16	28	29
none	11	0

EXPERIMENTAL SECTION

Melting points were determined on a Buchi 510 capillary melting point apparatus and are uncorrected. ^{1}H Nmr and ^{13}C-nmr were recorded on Varian EM-390 and XL-200, Bruker WM300 and AM-500 and Jeol PFT-100 spectrometers. Chemical shifts are reported in δ ppm values with Me_4Si or 3-(Trimethylsilyl)propionic- 2,2,3,3-d_4 acid, sodium salt as reference. Ir spectra were measured on Perkin-Elmer 297 and Pye-Unicam 2-300 spectrophotometers. FTir analyses were done on a DIGILAB FT-60 spectrometer equipped with 3200 data station. EI mass spectra were recorded on a Hewlett-Packard Model 5982-A instrument, and FAB mass spectral analyses were done at the Midwest Center for Mass Spectrometry, University of Nebraska. Optical rotations were measured on a Perkin-Elmer 241 polarimeter at ambient temperature. Electrophoresis was carried out in formic acid/acetic acid/H_2O (1:4:45 by vol; pH 2.0) on Whatman No. 1 paper at 100 V/cm over 45 min. Hplc analyses were performed on a Perkin-Elmer 2B instrument using Waters Associates μ-Bondapak-C18 reverse phase column (0.39 X 30 cm) at flow rate of 1 ml/min and λ_{max} 220 nm.

Cell-free extracts from *Streptomyces clavuligerus* ATCC 27064 were prepared from 40-h old cells according to Jensen *et al.*[3b] and partially purified by streptomycin sulfate or protamine sulfate and ammonium sulfate precipitation, desalting on Sephadex G-25 followed by concentration in an amicon ultrafiltration cell using PM-10 and PM-30 membranes.

Bioassays were run using agar-diffusion procedures against *Micrococcus luteus* ATCC 9341 and *Comamonas terrigena* ATCC 8461. Direct *in vitro* ^{1}H-nmr studies on incubation mixtures were carried out in a Bruker AM-500 NMR spectrometer in a 5-mm nmr tube under non-spinning conditions with oxygen purging at 2 bubbles/min.

Hydrogenation of Fujisawa Disulfide (**6**). Fujisawa disulfide (**6**) was prepared from the corresponding penicillin sulfoxide essentially according to the literature procedure[14] and showed the following properties: mp 142-146° C; ir (KBr) 3290, 1775, 1740, 1665 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 1.93 (s, 3H), 3.66 (s, 2H), 4.89 and 5.04 (each s, 1H), 5.13 (br s, 1H), 5.23 (dd, J=8.1, 4.8 Hz, 1H), 5.51 (d, J=4.8 Hz, 1H), 6.56 (d, J=8.1 Hz, 1H), 6.88 (s, 1H), 7.2-7.83 (m, 19H). A solution of **6** (0.8 g, 1.26 mmol) in benzene/ethanol (5:1 by vol. 30 ml) was hydrogenated over $Rh(PPh_3)_3Cl$ (1.73 g, 1.87 mmol) at 20 psi of H_2 and room temperature for 18 h. Removal of solvents and a column chromatography on silica gel (hexane/EtOAc) yielded *ca.* 250 mg of the crude product mixture which consisted mainly of **7a** and **7b** in *ca.* 2:1 ratio. Analytically pure samples of **7a** and **7b** were obtained by preparative hplc. Compound **7b** recrystallized from MeOH showed the following properties: mp 120-121° C; ir($CHCl_3$) 1780, 1740, 1645 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.64 (d, J=6.9 Hz, 3H), 0.67 (d, J=6.9 Hz, 3H), 2.18 (m, 1H), 3.61 and 3.64 (each d, J=14 Hz, 1H), 4.29 (d, J=6.7 Hz, 1H), 5.10 (d, J=3.4 Hz, 1H), 6.04 (d, J=3.4 Hz, 1H), 6.87 (s, 1H), 7.20-7.32 (m, 15H); ms(FAB) m/z 469 (MH^+).

Benzhydryl 6-Aminopenicillanate (8). To a solution of 6-APA (4 g, 18.5 mmol) in 50 ml water (adjusted to pH 7.5 with 0.1N NaOH) at 0° C was added salicylaldehyde (18 g, 148 mmol), and the mixture was stirred for 45 min at ambient temperature. The yellow solution was cooled to *ca.* 5° C, acidified with 3N H_2SO_4 to pH 2.5 and extracted with EtOAc. The yellow oily residue, obtained from the EtOAc extract after washing, drying(Na_2SO_4) and concentration, was triturated with petroleum ether (100 ml) and kept in a refrigerator. The separated solid product was washed with petroleum ether and recrystallized from $CHCl_3$ and pet. ether, 4.0 g (70%) of yellow solid: mp 155-160° C(dec); ir ($CHCl_3$) 3200-2850, 1780, 1720, 1620 cm^{-1}; ^{1}H-nmr ($CDCl_3$ + DMSO-d_6) 1.60 (s, 3H), 1.67 (s, 3H), 4.35 (s, 1H), 5.33 (d, J=4.5 Hz, 1H), 5.63 (br d, 1H), 6.70-7.50 (m, 4H), 8.53 (s, 1H), 11.07 (br, 2H). The crude *Schiff's base* (4.0 g, 12.5 *mmol*) *in dry acetone* (30 ml) was treated with diphenyldiazomethane [30] (2.8 g, 14.4 mmol) at room temperature. After the purple color was discharged (in *ca.* 30 min), the solution was extractively worked up with EtOAc. The extract was treated with p-TsOH (3 g) in wet acetone(2 ml) to furnish the p-toluenesulfonate salt of 8. The free base (8, 2.72 g, 58%) was obtained by washing the salt in EtOAc with 5% $NaHCO_3$: ir (film) 3360, 1775, 1740 cm^{-1} ; ^{1}H-nmr ($CDCl_3$) 1.23 (s, 3H), 1.53 (s, 3H), 1.81 (br, 2H), 4.51 (s, 1H), 4.53 (d, J=4.2 Hz, 1H), 5.51 (d, J=4.2 Hz, 1H), 6.87 (s, 1H), 7.03-7.40 (m, 10H); ^{13}C-nmr ($CDCl_3$) 26.697, 30,030, 62.90, 64.066, 69.972, 70.132, 78.340, 126.90, 127.15, 127.54, 128.07, 128.30, 128.51, 128.98, 139.09, 139.15, 167.08, 177.57.

Benzhydryl N- δ-[N-Boc-(L)- α-amino- α-benzhydryladipoyl]-6-aminopenicillanate β-sulfoxide (9a). To a solution of α-benzhydryl ester of N-Boc-(L)- δ-aminoadipic acid (4.8 g, 11.24 mmol) and Et_3N (1.135 g, 11.24 mmol) in dry acetone (100 ml) at -15° C (ice-salt bath) was added methyl chloroformate (1.068 g, 11.24 mmol). To this mixture, after stirring for 5 min, was added 8 (4.5 g, 11.78 mmol) in 15 ml of dry acetone. The mixture was stirred for 6 h at the ice-bath temperature and worked up with EtOAc. A column chromatographic separation on silica gel *(hexane/EtOAc) gave the coupled product* as a colorless foam, 6.4 g (72%): $[\alpha]_D$ +127.2° C (c, 2.09 EtOH); ir (KBr) 3350,1780, 1740, 1710, 1680 cm^{-1} ; ^{1}H-nmr ($CDCl_3$) 1.26 and 1.62 (each s, 3H), 1.42 (s, 9H), 1.50-2.30 (m, 6H), 4.39-4.45 (m, 1H), 4.52 (s, 1H), 5.12 (br d, J=8 Hz, 1H), 5.54 (d, J=4.2 Hz, 1H), 5.67 (dd, J=8.8, 4.2 Hz, 1H), 6.16 (br d, J=8.8 Hz, 1H), 6.89 and 6.95 (each s, 1H), 7.26-7.36 (m, 20H); hplc RT 6.8 min (MeCN/H_2O: 80/20). The coupled product (4.0 g, 5.06 mmol) in $CHCl_3$ (100 ml) at -15° C was treated with mCPBA (1.12 g, 5.56 mmol) for 40 min, and extractively worked up in EtOAc to give 9a (4.28 g) as white foam: $[\alpha]_D$ + 76.57° C (c, 1.1 EtOH); ir (KBr) 1800, 1745, 1710 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.90 and 1.69 (each s, 3H), 1.42 (s,9H), 1.30-2.30 (m, 6H), 4.43 (br, 1H), 4.75 (s, 1H), 4.97 (d, J=4.7 Hz, 1H), 5.11 (br d, J=8 Hz, 1H), 1H, 6.05 (dd, J=10.4, 4.7 Hz, 1H), 6.89 (br s, 1H), 6.97 (br d, J=10.4 Hz, 1H), 6.99 (br s, 1H), 7.28-7.39 (m, 20H); hplc RT 6.0 min (MeCN/H_2O: 80/20). 9b:[31] $[\alpha]_D$ + 110.34° C (c, 0.73 EtOH); ir ($CHCl_3$) 3450, 1795, 1750, 1715,1680 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.88 and 1.67 (each s, 3H), 1.38-1.70 (m, 4H), 1.42 (s, 9H), 2.20 (br m, 2H), 4.40 (br m, 1H), 4.75 (s, 1H), 4.95 (br d, 1H), 5.28 (br, 1H), 6.08 (br m, 1H), 6.88 (br s, 1H), 6.99 (s, 1H), 7.28-7.35 (m, 20H); ^{13}C-nmr ($CDCl_3$) 17.908, 19.176, 20.670, 28.087, 31.434, 35.044, 53.202, 56.088, 65.965, 75.168, 76.468, 77.625, 78.607, 79.622,

126.246, 139.49, 155.138, 166.734, 171.30, 171.57, 173.368; ms (EI) m/z 514, 428, 262, 168, 167, 165, 152, 121, 105, 98.

Rearrangement of **9a**. A solution of **9a** (4.0 g, 4.95 mmol) and $P(OMe)_3$ (3 ml) in 100 ml of benzene was refluxed for 36 h. An extractive workup followed by silica gel chromatography(hexane/EtOAc) gave **10a** (1.58 g, 43%) as pale yellow solid: mp 101-103° C; $[\alpha]_D$ -79.05° (c, 1.0 EtOH); ir (KBr) 3380, 1770, 1740, 1710 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 1.41 (s, 9H), 1.58-1.90 (m, 4H), 1.79 (s, 3H), 2.45 (br, 2H), 4.15 (br, 1H), 4.76 (br s, 1H), 4.95 (br s, 1H), 5.03 (br d, J=1.4 Hz, 2H), 5.83 (d, J=4.5 Hz, 1H), 5.85 (d, J=4.5 Hz, 1H), 6.87 (s, 1H), 6.89 (s, 1H), 7.25-7.36 (m, 20H); hplc RT 8.6 min (MeCN/H_2O: 80/20). **10b**: [31] mp 54-56° C; $[\alpha]_D$ - 75° (c, 0.84 EtOH); ir (KBr) 3400, 1780, 1745, 1720, 1620 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 1.42 (s, 9H), 1.60-1.75 (m, 4H), 1.80 (s, 3H), 2.45 (m, 2H), 4.40 (m, 1H), 4.77 (br s, 1H), 4.97 (br s, 1H), 5.00-5.18 (m, 2H), 5.86 (br s, 2H), 6.88 (s, 1H), 6.90 (s, 1H), 7.24-7.33 (m, 20H).

Hydrogenation of **10a**. A solution of **10a** (1.58 g, 2.1 mmol) and Wilkinson catalyst (2.4 g, 2.5 mmol) in benzene/ethanol (5/1, 120 ml) was hydrogenated in a Parr hydrogenator at 20 psi H_2 and room temperature for 5.5 h. Removal of the solvents and a silica gel column chromatography(hexane/EtOAc) gave **11a** (1.3 g, 82%) as white solid: mp 119° C; $[\alpha]_D$ - 19.4° (c, 0.67 EtOH); ir (KBr) 3380, 1770, 1735, 1710 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.85 and 0.95 (each d, J=6.5 Hz, 3H), 1.43 (s, 9H), 1.50-2.50 (m, 7H), 4.30 (d, J=9 Hz, 1H), 4.43 (br, 1H), 5.06 (br d, J =8 Hz, 1H), 5.60 (d, J=4.2 Hz, 1H), 5.77 (d, J=4.2 Hz, 1H), 6.88 and 6.90 (each s, 1H), 7.28-7.39 (m, 20H); ms (FAB) m/z 776 (MH^+); hplc RT 10 min (MeCN/H_2O: 80/20). **11b**: [31] Yellow foam; $[\alpha]_D$ - 23.07° (c, 0.52 EtOH); ir (KBr) 3400, 1775, 1735, 1705, 1610 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.84 and 0.94 (each d, J=6.7 Hz, 3H), 1.42 (br s, 9H), 1.50-2.00 (m, 4H), 2.20-2.50 (m, 3H), 4.22 (d, J=9 Hz, 1H), 4.40 (m, 1H), 5.10 (br d, J=8 Hz, 1H), 5.60 (m, 1H), 5.76 (m, 1H), 6.88 (s, 1H), 6.89 (s, 1H), 7.26-7.37 (m, 20H).

Direct Conversion of **11a** to **13a**. A solution of **11a** (0.5 g, 0.65 mmol) in DMSO (50 ml) containing iodine(0.1 g, 0.8 mmol) was treated with 1N HCl(~5 ml) at room temperature for 30 min. An extractive workup in EtOAc followed by a column chromatography on silica gel(hexane/EtOAc) gave **13a** (0.2 g, 40%) as a white foam: $[\alpha]_D$ - 49.6° (c, 1.35, EtOH); ir (KBr) 3340, 1770, 1730, 1690 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.86 and 1.01 (each d, 3H, J=6.8 Hz), 1.41 (br s, 9H), 1.50-2.50 (m, 7H), 4.16 (d, J=9 Hz, 1H), 4.37 (br, 1H), 5.00-5.23 (m, 3H), 6.34 (br, 1H), 6.86 (br s, 1H), 6.91 (s, 1H), 7.23-7.35 (m, 20H); ms (FAB) m/z 1585 (MH^+); hplc RT 17.4 min (MeCN/H_2O: 85/15). **13b**:[31] $[\alpha]_D$ - 45.22° (c, 1.15, EtOH); ir (KBr) 3350, 1770, 1740, 1710 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.83 and 0.98 (each d, J=6.7 Hz, 3H), 1.39 (br s, 9H), 1.40-2.50 (m, 7H), 4.13 (d, J=9.2 Hz, 1H), 4.35 (m, 1H), 5.00 (br d, J=3.2 Hz, 1H), 5.05-5.30 (br m, 2H), 6.30-6.55 (m, 1H), 6.83 (s, 1H), 6.88 (s, 1H), 7.25-7.30 (m, 20H); hplc RT 15.7 and 17.4 min (MeCN/H_2O: 85/15).

Deprotection of **13a**. The protected dimer (**13a**, 55 mg) dissolved in dry anisole (0.2 ml) was cooled in an ice-bath and treated with CF_3CO_2H (2 ml) for 30 min. Trifluoroacetic acid was removed under a stream of N_2 and the residue was thoroughly washed with dry ether to give **14a** (22 mg) as white powder: mp 158-162° C (dec); ir (KBr) 3350, 1760, 1660 cm^{-1}; ^{1}H-nmr (20% DCl/D_2O) 1.00 and 1.04 (each d, J=6.6 Hz, 3H), 1.89 (br, 2H), 2.02 (br, 2H), 2.52 (br m, 3H), 4.06 (d, J=9.5 Hz, 1H), 4.20 (br t, 1H), 5.09 (d, J=4.6 Hz, 1H), 5.31 (d, J=4.6 Hz, 1H); (0.05 M Tris, pH 7.8) 0.95 and 1.01 (each d, J=6.6 Hz, 3H), 1.77 (br m, 2H), 1.90(br m, 1H), 1.98 (br m, 1H), 2.34 (br m, 1H), 2.46 (br m, 2H), 5.10 (d, J=4.6 Hz, 1H), 5.13 (d, J=4.6 Hz, 1H) (other signals hidden under buffer); ms (FAB) m/z 721 (MH^+); hplc RT 4.9 min [MeCN/0.05 M potassium phosphate buffer, pH 7.0: 5/95]. **14b**:[31] mp 179-182° C (dec); ir (KBr) 3300, 1770, 1660 cm^{-1}; ^{1}H-nmr (20% DCl/D_2O) 0.99 and 1.04 (each d, J=6.8 Hz, 3H), 1.65-2.65 (m, 7H), 4.08 (br t, 1H), 4.23 (br s, 1H), 5.28 (br s, 1H), 5.33 (br s, 1H); (0.05 M Tris buffer, ^2H_2O; pH 7.5) 0.98 and 1.04 (each d, J=6Hz, 3H), 1.75 and 1.95 (each m, 2H), 2.40 (m, 1H), 2.45 (m, 1H), 3.89 (m, 1H), 3.94 (d, J= 9.9 Hz, 1H), 5.12 (m, 1H) and 5.24 (br s, 1H); hplc retention time 4.1 (D) and 4.9 (L) min [MeCN/0.05 M potassium phosphate buffer, pH 7.0: 5/95].

Reductive Cleavage of **14a** to **3a** (with Zn/DCl-D_2O (20%)). Disulfide **14a** (2 mg) dissolved in 20% DCl/D_2O (0.4 ml) in a 5 mm nmr tube and cooled in an ice-bath was treated with Zn dust (~3 mg) over 5 min. At the end of 10 min all the Zn had dissolved to give a clear soln. Reduction to monomer was complete in 15 min as monitored by ^{1}H-nmr: 1.03 and 1.04 (each d, J=6.8 Hz, 3H), 2.11 (br, 2H), 2.50 (br, 2H), 3.18 (br t, 2H), 4.31 (d, J=7.4 Hz, 1H), 4.20 (br t, 1H), 6.17 (d, J=4.5 Hz, 1H), 6.39 (d, J=4.5 Hz, 1H).

Reoxidation of **3a** to **14a**. Batches of iodine (1 mg) were added to the above solution at 10 min intervals and ^{1}H-nmr recorded. About 70% reoxidation had been achieved in about an hour (see Figure 1). Reoxidation **3a** to **14a** was also monitored by reverse phase hplc: C_{18} reverse phase column, 0.39 x 30 cm; MeOH/0.05M KH_2PO_4 (pH 4.6): 5/95, 1ml/min at λ_{max} 220nm; retention times **14a**: 10.5 min; **3a**: 7.5 min.

Reductive Cleavage of **14a** to **3a** (with DTT in 0.05M Tris buffer (^2H_2O; pH 7.5)). Disulfide **14a** (1 mg) in Tris buffer (0.4 ml) in a 5 mm nmr tube at 22° C was treated with DTT (0.5 mg, 3 μmol). The course of reduction was followed by ^{1}H-nmr every 2 min. After 10 min, the reduction mixture [^{1}H-nmr (Figure 2d) 0.94, 0.95, 1.00 and 1.01 (each d, J=6.2 Hz), 5.09 and 5.11 (each d, J=3.8 Hz), 5.10 and 5.13 (each d, J=4.6 Hz)] was treated with iodine (2 mg). The proton spectrum of the yellow solution was recorded immediately, and after 30 and 45 min (Figure 2e → 2g). ^{1}H-nmr (Figure 2g): 0.96 and 1.01 (each d, J=6.2 Hz, 3H), 5.11 and 5.13 (each d, J=4.6 Hz, 1H). This preparation was used for incubation experiments with IPNS.

Reduction of **14b** to **3b** (with DTT in 0.05M Tris buffer (^2H_2O, pH 7.5)). To disulfide **14b** (10 mg) in Tris buffer (0.5 ml) was added a solution of DTT (5 mg) in Tris (0.5 ml) and the mixture was allowed to stand with occasional

shaking at room temp. for 8 min. The mixture was immediately freeze-dried. FTir of the solid was recorded as KBr discs. For reoxidation, the solid was resuspended in ^2H_2O [^{1}H-nmr (Figure 3b): 0.85 and 1.00 (each d, J=6 Hz, 3H), 1.75 (m, 2H), 1.90 and 1.95 (each m, 1H), 2.34 (m, 1H), 2.45 (m, 1H) and 5.15 (br m)] and just enough iodine was added to ensure complete oxidation [^{1}H-nmr (Figure 3c) identical with that of **14b** (Figure 3a)].

Benzhydryl-6-phenylacetamido penicillin β-sulfoxide (**9d**): Sulfoxide (**9c**)[28] (16.4 g, 46.0 mmol) was esterified with diphenyldiazomethane (10.26 g, 1.15 eq.) in acetone. Ester was purified by chromatography on silica gel(hexane/EtOAc): 22.9 g(96%), mp 118-120° C; ir (KBr) 3400, 1790, 1750, 1660, 1495, 1290, 1210 and 1150 cm^1; ^{1}H-nmr ($CDCl_3$) 0.87 and 1.65 (each s, 3H), 3.54 and 3.60 (each d, J=16.5Hz, 1H), 4.71 (s,1H), 4.94 (d, J=4.5Hz, 1H), 6.04 (dd, J=4.5Hz, 9Hz, 1H), 6.97 (s, 1H), 7.06 (d, J=9Hz, 1H) and 7.20-7.40 (m, 15H).

Rearrangement of **9d**: Ester **9d** (14.3 g) was refluxed in benzene (100 ml) containing P $(OMe)_3$ (30 ml) for 40 h. The product on purification by chromatography on silica gel(hexane/EtOAc) gave **10c** (10.7g, 80%) as a pale yellow gum: ir (film) 1770,1740 cm^{-1}; ^{1}H-nmr($CDCl_3$) 1.67 (s, 3H), 3.80 and 3.87 (d, J=14.7Hz, 1H), 4.7 (s, 1H), 4.95 (br d, 2H), 5.87 and 5.93 (d, J=4.5Hz, 1H), 6.87 (s, 1H) and 7.22-7.35 (m, 15H).

Hydrogenation of **10c**: Thiazolidino-azetidinone **10c** (2.84 g, 5.9 mmol) was hydrogenated over $(Ph_3P)_3RhCl$ (6.4 g, 1.15 eq.) in benzene/ethanol (5/1, 120 ml) at 20 psi H_2 and at room temp. for 11 h. Solvent removal and chromatographic fractionation on silica-gel(hexane/EtOAc) gave **11c** (2.6 g, 91%) as an off-white solid which was recrystallised from $CHCl_3$-hexane: mp 81-83° C; ir (KBr) 1760, 1730 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.87 and 0.93 (each d, J=7.6Hz, 3H), 2.24 (m, 1H), 3.78 and 3.86 (each d, J=14.9Hz, 1H), 4.20(d, J=9.0Hz, 1H), 5.80 and 5.83 (each d, J=4.5Hz, 1H), 6.87 (s, 1H) and 7.20-7.40 (m, 15H).

Oxidative dimerisation of **11c**: Dihydro derivative **11c** (0.2 g) in DMSO (5 ml) containing iodine (0.1 g) was stirred with 1N HCl (2 ml) at room temp. for 3 h. After extractive workup in EtOAc followed by silica gel chromatography (hexane/EtOAc) **13c** was obtained as a white foam (0.17 g); ir (KBr) 3320, 1770, 1730, 1670 cm^{-1}; ^{1}H-nmr ($CDCl_3$) 0.83 and 1.06 (each d, J=6.5Hz, 3H), 2.47(m, 1H), 3.36 and 3.54(each s, J=16Hz, 1H), 4.05(br d, 1H), 4.11(d, J=9Hz, 1H), 4.61(d, J=4.5Hz, 1H), 6.07(d, J=9Hz, 1H), 6.94(s, 1H) and 7.20-7.40(m, 15H).

Deprotection of **13c**: The protected disulfide **13c** (0.1 g) was dissolved in dry anisole (0.5 ml), cooled in ice and treated with dry TFA (1 ml) for 2 h under N_2. TFA was removed under a stream of N_2 and residue thoroughly washed with ether to give a white solid (70 mg); ir (KBr) 1760, 1660 cm^{-1}; ^{1}H-nmr (Acetone-d_6 + ^2H_2O) 1.02 and 1.05(each d, J=7Hz, 3H), 2.37(m, 1H), 3.60 and 3.67(each d, J=13.9Hz, 1H), 3.94(d, J=9.3Hz, 1H), 4.49 and 4.89(each d, J=3.9Hz, 1H) and 7.20-7.40(m, 15H).

Incubations with IPNS from *S. clavuligerus*:[3b]

14a: To **14a** (1.0 mg) in Tris buffer (0.2 ml)preincubated with DTT (0.5 mg) at room temp. for 10 min was added freshly prepared solutions of sodium ascorbate (60 mmol, 25 µl), $FeSO_4$ (7mmol, 10 µl) followed by Tris buffer (0.1ml) and partially purified IPNS enzyme (50 µl, 2 mg protein/ml). The mixture was incubated at 22° C for 90 min. with occasional mixing. The reaction was terminated by addition of methanol (0.4 ml). 20 µl of this solution was used for bioassay.

14c: Disulfide **14c** (1.0 mg) in Tris buffer (0.2 ml) preincubated with DTT (0.5 mg) and containing other cofactors as above was incubated with partially purified IPNS enzyme (50 µl) for 90 min. at 22° C. Methanol (0.4 ml)-deactivated mixture (20µl) was used for bioassay.

Co-incubations with ACV: Disulfide **14a** or **14c** (1 mg) and ACV **16** (250 µg) were preincubated with DTT (0.7 mg) for 10 min at room temp. (22° C). Solutions of sodium ascorbate (60mmol, 25 µl) and $FeSO_4$ (7 mmol, 10 µl) were added followed by Tris buffer (0.2 ml) and partially purified IPNS enzyme (50 µl). After incubating the mixture at 22° C for 90 min the reaction was terminated by addition of methanol (0.4 ml). 20 µl of this mixture was used for bioassay.

Bioassay of incubation mixtures: The methanol-deactivated incubation mixture was applied to a sterile 5mm paper disk on nutrient agar containing the indicator organism. After incubation of the plates for 16 h at 27° C, the zone of inhibition was measured (Table 2).

ACKNOWLEDGMENTS

We thank Dr. K. B. Tomer, Midwest Center for Mass Spectrometry at the University of Nebraska for the FAB mass spectra. This research has been supported by the National Institutes of Health (AI-14481 to AIS and SKC) and by the Robert A. Welch Foundation (A-752 to SKC and A- 943 to AIS).

REFERENCES AND NOTES

1. Present address: Dept. of Chemistry, Pohang Institute of Science and Technology, Pohang, 680, S. Korea
2. J. O'Sullivan and E. P. Abraham, "Antibiotics-Biosynthesis", Vol. 4 J. W. Corcoran, Ed., Springer-Verlag: Berlin, 1981; p. 101. D. J. Aberhart, Tetrahedron, **1977**, *33*, 1545.
3. (a) P. A. Fawcett, J. J. Usher, J. A. Huddleston, R. C. Bleaney, J. J. Nisbet, and E. P. Abraham, Biochem. J. **1976**, *157*, 651.
   (b) S. E. Jensen, D. W. S. Westlake, and S. Wolfe, J. Antibiot., **1982**, *35*, 483.

4. (a) M. Kohsaka and A. L. Demain, Biochem. Biophys. Res. Comm., **1976**, *70*, 465.
(b) A. Scheidegger, M. T. Kuenzi, and J. Nuesch, J. Antibiot., **1984**, *37*, 522.
(c) S. E. Jensen, D. W. S. Westlake, R.J. Bowers, and S. Wolfe, J. Antibiot., **1985**, *35*, 1351.

5 From *C. acremonium*: C.-H. Pang, B. Chakravarti, R. M. Adlington, H.-H. Ting, R. L. White, G. S. Jayatilake, J. E. Baldwin, and E. P. Abraham, Biochem. J., **1984**, *222*, 789. I. J. Hollander, Y.-Q Shen, J. Heim, A. L. Demain, and S. Wolfe, Science, **1984**, *224*, 610. From *P. chrysogenum*, F. R. Ramos, M. J. Lopez-nieto, and J. F. Marlin, Antimicrob. Agents Chemother., **1985**, 380. From *S. clavuligerus*: S. E. Jensen, B. K. Leskin, L. C. Vining, Y. Aharonowitz, D. W. S. Westlake, and S. Wolfe, Can. J. Microbiol., **1986**, *32*, 953.

6. (a) J. R. Miller and T. D. Ingolia, Mol. Microbiol., **1989**, *3*, 689.
(b) T. D. Ingolia and S. W. Queener, Med. Res. Reports, **1989**, *9*, 245.
and references cited therein.

7. (a) B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, J. Chem. Soc., Chem. Commun., **1975**, 123.
(b) D. J. Morecombe and D. W.Young, J. Chem. Soc., Chem. Commun., **1975**, 198. (c) D. J. Aberhart, J. Y. R. Chu, and L. J. Lin, J. Chem. Soc., Perkin I, **1975**, 2517.

8. H. Kluender, F. C. Huang, A. Fritzberg, H. Schnoes, C. J. Sih, P. Fawcett, and E. P. Abraham, J. Am. Chem. Soc., **1974**, *96*, 4054. However, recent studies have shown that retention or inversion of configuration during second ring closure is rather dictated by the nature of substitution at C-3 in valine.[27b,c]

9. (a) R. L.White, E. M. John, J. E. Baldwin, and E. P. Abraham, Biochem. J., **1982**, *203*, 791.
(b) G. Bahadur, J. E. Baldwin, T. Wan, M. Jung, E. P. Abraham, J. A. Huddleston, and R. L. White, J. Chem. Soc., Chem. Commun., **1981**, 1146.

10 (a) A. I. Scott, S. E. Yoo, S. K. Chung, and J. A. Lacadie, Tetrahedron lett., **1975**, 4103.
(b) R. L. Baxter, G. A. Thomson, and A. I. Scott, J. Chem. Soc., Chem. Commun., **1984**, 32.

11. B. Meesschaert, P. Adriaens, and H: Eyssen, J. Antibiot., **1980**, *33*, 722.

12. (a) A preliminary report of this work has been published: S. K. Chung, R. Shankaranarayan, and A. I. Scott, Tetrahedron Lett., **1983**, 2941.
(b) R. Shankaranarayan and A. I. Scott, to be published.

13. Another synthesis of this compound has also been reported: (a) E. P. Abraham, R. M. Adlington, J. E. Baldwin, M. J. Crimmin, L. D. Field, G. S. Jayatilake, and R. L. White, J. Chem. Soc., Chem. Commun., **1982**, 1180. (b) J. E. Baldwin, E. P. Abraham, R. M. Adlington, M. J. Crimmin, L. D. Field, G. S. Jayatilake, R. L. White, and J. J. Usher, Tetrahedron , **1984**, *40*, 1907.

14. T. Kamiya, T. Teraji, Y. Saito, M. Hashimoto, O. Nakaguchi, and T. Oku, Tetrahedron Lett., **1973**,3001.

15. S. Yamamoto, S. Kamata, N. Haga, Y. Hamashima, and W. Nagata, Tetrahedron Lett., **1981**, *22*, 3089.

16. Y. Maki, N. Mitsumori, M. Sako, and M. Suzuki, J. Chem. Soc., Perkin Trans. I, **1981**, 2087.

17. V. R. Reiner and P. Zeller, Helv. Chem. Acta , **1968**, *51*, 1805.

18. S. Wolfe and M. G. Jokinen, Can. J. Chem., **1979**, *57*, 1388.

19. R. D. G. Cooper and F. L.Jose, J. Am. Chem. Soc., **1970**, *92*, 2575.

20. J. E. Baldwin and M. A. Christie, J. Chem. Soc., Chem. Commun., **1978**, 239.

21. A. G. Long, "Recent Advances in Chemistry of β-Lactam Antibiotics"; J. Elks, Ed., The Chemical Society: London, 1977; p. 226.

22. H. R. Felix, H. H. Peter, and H. J. Treichler, J. Antibiot.,**1981**, *34*, 567

23. R. L. Baxter and A. I. Scott, unpublished results.

24. J. E. Baldwin, R. M. Adlington, S. E. Moroney, L. D. Field, and H.-H. Ting, J. Chem. Soc., Chem. Commun.,**1984**, 984. J. E. Baldwin, E. P. Abraham, C. G. Lovel, and H.-H. Ting, J. Chem. Soc., Chem. Commun., **1984**, 902.

25. J. E. Baldwin, E. P. Abraham, G. L. Burge, and H.-H. Ting, J. Chem. Soc., Chem. Commun., **1985**, 1808. S. E. Jensen, W. S. Westlake, R. J. Bowers, L. Lyubechansky, and S. Wolfe, J. Antibiot., **1986**, *39*, 822. J. M. Luengo, M. J. Lopez-Nieto, and F. Salto, J. Antibiot., **1986**, *39*, 1144.

26. J. A. Robinson, Chem. Soc. Rev., **1988**, *17*, 383.

27 e.g., see

a) J. E. Baldwin, R. M. Adlington, M. Bradley, N. J Turner, and A. R. Pitt, J. Chem. Soc., Chem. Commun., **1989**, 978

b) J. E. Baldwin, R. M. Adlington, C. J. Schofield, and H-H. Ting, J. Chem. Soc., Chem. Commun., **1989**, 758.

c) J. E. Baldwin , Nat. Pdts. Reports,**1988**, 129.

28. J. M. Essery, K. Dadabo, W. J. Gottstein, A. Hallstrand, and L. C. Cheney, J. Org. Chem., **1965**, *30*, 4388.

29. A. I. Scott and R. Shankaranarayan, unpublished results.

30. L. I. Smith and K. L. Howard, "Organic Synthesis, Collective Volume III"; John Wiley & Sons: New York, 1955; p. 351.

31. Only the physical data for the (DL)-series **9b-14b** is given, since the method of preparations of each is identical with that of corresponding L-series (**9a-14a**).

32. R. Benn and H. Gunther, Angew. Chem., Int. Ed. Engl., **1983**, *22*, 350.

Received, 4th September, 1989