

The xanthine oxidase signal exhibits proton superhyperfine splitting,¹ and ESR signals of nonoxo sulfur complexes, such as Mo(S₂CNEt₂)(HNSC₆H₄)₂, exhibit both proton and nitrogen superhyperfine splitting.¹¹ These nonoxo complexes have distorted trigonal prismatic geometry¹² and a short intramolecular Mo-N distance, indicating considerably multiple-bond character. It has been suggested the consequently short Mo-H distance may be an important factor in the large ¹H splittings observed.¹² The MoOCIL₂ complex, however, gave no evidence for such splitting over a wide temperature range, from ambient to frozen in both DMF and CHCl₃. This lack of superhyperfine splitting may have its origin in differences in geometry, or in the absence in MoOCIL₂ of extensive delocalization of the Mo(V) electron into an aromatic system in comparison with Mo(S₂CNEt₂)(HNSC₆H₄)₂.

Cyclic voltammetry and controlled potential coulometry at a platinum cathode indicate that both complexes are readily reduced in a one electron step to Mo(IV) species (Table II). In the voltage range used (+0.50 to -2.50 V vs. SCE), however, the complexes could not be oxidized to the Mo(VI) state, indicating the Mo(VI)-Mo(V)-Mo(IV) complexes may not represent a simple redox series.¹⁴ This is most likely due to the necessity of adding a second oxo group to the Mo(VI) dioxo complexes, requiring an oxo donor not present in the electrochemical system.¹⁴ A detailed electrochemical study of these and other Mo(V) complexes will be published elsewhere.¹⁴

While the complexes reported here model reasonably well some of the ESR parameters of xanthine oxidase, the differences in $\langle g \rangle$ and, particularly, $\langle A \rangle$, as well as a lack of proton superhyperfine splitting, suggest the Mo(V) site of the enzyme is, for whatever reasons, somewhat different. Work with a variety of other complexes, which may provide additional evidence concerning this important question, is underway and will be reported later.

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Descysteaminythienamycin

Sir:

Thienamycin (**1**), isolated from *Streptomyces cattleya*,¹ is a rather unusual bicyclic β -lactam antibiotic. In contrast to the well-known penam and cephem antibiotics, it contains no sulfur atom in the ring system and the two β -lactam ring protons are trans to one another.² More interestingly, thienamycin displays potent, broad spectrum antibacterial activities against both Gram-positive and Gram-negative microorganisms (including *Pseudomonas spp.*) and is resistant to bacterial β -lactamases.³

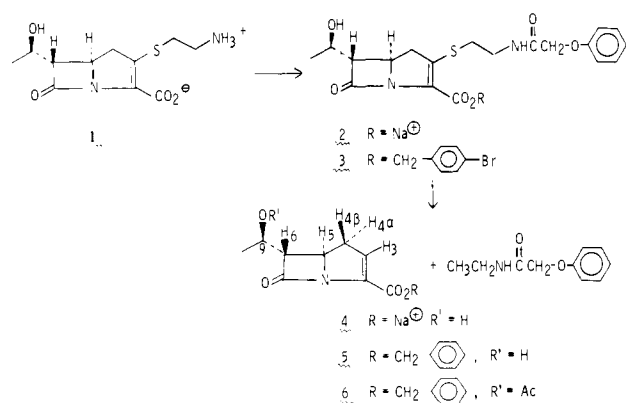
One of our objectives in chemically modifying thienamycin was to substitute the aminoethylthio (cysteamine) side chain with a hydrogen atom and determine the antibacterial activities of the resultant product, i.e., of descysteaminythienamycin (**4**). However, despite intensive efforts to carry out a reductive cleavage reaction directly on thienamycin under a variety of conditions known to cleave carbon-sulfur bonds, all reactions destroyed the β -lactam.⁴ We now report the successful preparation of this simple but novel bicyclic β -lactam ring system and its potent antibacterial activity against most microorganisms.

The previously unsuccessful attempts to cleave directly the thienamycin side chain led us to believe that nucleophilicity of the cleavage products, ethylamine or cysteamine, was responsible for the destruction of the β -lactam ring. Therefore, instead of operating directly upon thienamycin, the amino group of thienamycin was first protected with a phenoxyacetyl group before hydrogenolysis. This protecting group serves two purposes: (1) the phenoxyacetyl group diminishes the nucleophilicity of the nitrogen, and (2) the aromatic ring of this group facilitates the final purification step in which an XAD-2 resin is used.⁵

N-Acylation of thienamycin was accomplished under Schotten-Baumann conditions in the presence of 2.5 equiv of phenoxyacetyl chloride (0 °C for 20 min in a 1:1 0.1 M aqueous sodium phosphate buffer-dioxane solvent system maintained at pH 8.5-9.0 with 2.5 N sodium hydroxide), followed by chromatography on an XAD-2 column eluted first with water and then with 10% tetrahydrofuran (THF) in water, to

Table I. Inhibitory Zone Diameters (Millimeters) vs. Penicillin-Sensitive and Resistant Bacterial Strains

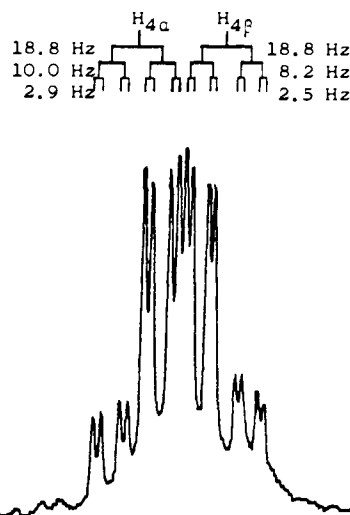
compd	disc content, μg (nmol)	<i>S. aureus</i>		<i>E. coli</i>		<i>Enterobacter cloacae</i>		<i>Pseudomonas aeruginosa</i>	
		MB2985	MB2314	MB2482	MB2964	MB2647	MB2646	MB3835	MB3350
carbenicillin	50 (118)	34	18	24	0	22	16	21	0
thienamycin	25 (92)	41	40	28	29	26	26	26	24
4	25.6 (117)	40	38	31	33	31	30	23	20



give *N*-phenoxyacetylthienamycin sodium salt (**2**, 70%); UV (H₂O) 302 nm (ϵ 7800); NMR (D₂O, 60 MHz), 1.25 (d, 3, J = 6 Hz, CH₃), 2.65–3.80 (m, 7), 4.10 (m, 2, H₅ and H₉), 4.53 (s, 2, CH₂O), 7.12 ppm (m, 5, aromatic protons). Treatment of **2** with *p*-bromobenzyl bromide in hexamethylphosphoramide (HMPA) for 30 min at room temperature followed by TLC purification (silica gel GF, ethyl acetate eluant, R_f 0.20) gave the expected *p*-bromobenzyl ester **3** (80%); mass spectrum 574 (M⁺), 530 (M – 44), and 488 (M – 86); IR (CHCl₃) 1776 cm⁻¹ (β -lactam); UV (EtOH) 320 nm (ϵ 8000); NMR (CDCl₃, 60 MHz) 1.28 (d, 3, CH₃), 2.76–3.72 (m, 7), 4.15 (m, 2, H₅ and H₉), 4.46 (s, 2, CH₂O), 5.12 (q, 2, *p*-bromobenzyl CH₂), 6.70–7.40 ppm (m, 9, aromatic protons). The reductive cleavage of **2** was conducted in aqueous solution (pH 7.0) at room temperature under 1 atm of hydrogen in the presence of five times its weight of palladium oxide. The progress of the reaction was monitored by reverse-phase high-pressure liquid chromatography⁶ and electrophoresis,⁷ until 90% of the sodium *N*-phenoxyacetylthienamycin was consumed (2.5 h). After filtration, the aqueous solution (pH 9.5) was adjusted to pH 7.0 with 2.5 N aqueous phosphoric acid and chromatographed on an XAD-2 column, eluted with water, to give the desired product, sodium descysteaminythienamycin (**4**, 40% yield). Subsequent elution of the column with 20% THF–H₂O gave the expected cleavage product, *N*-ethylphenoxycetamide (50% yield); mass spectrum 179 (M⁺); NMR (CDCl₃, 60 MHz), 1.20 (t, 3, CH₃), 3.40 (quintet, 2, CH₂), 4.50 (s, 2, CH₂O), 6.10 (NH), 7.10 (m, 5, aromatic protons).

The structural assignment of sodium descysteaminythienamycin is based on the spectral data and chemical derivatization: UV (H₂O) 265 nm (ϵ 6500); IR (Nujol mull) 1750 cm⁻¹ (β -lactam); NMR (D₂O, 300 MHz) 1.27 (d, 3, J = 6.0 Hz, CH₃), 2.83 (octet, 1, $J_{4\beta-3} = 2.5$, $J_{4\beta-5} = 8.2$, $J_{4\beta-\alpha} = 18.8$ Hz, H_{4 β}), 2.95 (octet, 1, $J_{4\alpha-3} = 2.9$, $J_{4\alpha-5} = 10.0$, and $J_{4\alpha-\beta} = 18.8$ Hz, H_{4 α}), 3.42 (q, 1, $J_{6-5} = 3.1$, $J_{6-9} = 6.2$ Hz, H₆), 4.26 (m, H₅ and H₉), 6.29 ppm (q, 1, $J_{3-4\alpha} = 2.9$, $J_{3-4\beta} = 2.5$ Hz, H₃). The 300-MHz NMR of **4**, in which the AB pattern of H_{4 α} and H_{4 β} is completely resolved, makes the structure assignment unequivocal (Figure 1).⁸

Attempts to obtain a field desorption mass spectrum of **4** were unsuccessful. However, esterification of **4** with benzyl bromide in HMPA yielded descysteaminythienamycin benzyl ester (**5**, 75%) which showed a molecular ion in the EI mass spectrum at m/e 287, and the expected fragments at m/e 243

**Figure 1.** 300-MHz NMR spectrum of H_{4 α} and H_{4 β} of sodium descysteaminythienamycin

(M – 44) and 202 (M – 85); IR (CHCl₃) 1781 (β -lactam), 1726 cm⁻¹ (ester); UV (EtOH) 276 nm (ϵ 7800); NMR (CDCl₃, 100 MHz), 1.33 (d, 3, J = 6.1 Hz, CH₃), 2.84 (m, 2, H₄), 3.18 (q, 1, $J_{6-5} = 2.9$, $J_{6-9} = 6.8$ Hz, H₆), 4.20 (m, 2, H₅ and H₉), 5.18 (d, 1, J = 12 Hz, benzyl CH₂), 5.32 (d, 1, J = 12.0 Hz, benzyl CH₂), 6.46 (t, 1, $J_{3-4} = 3.0$ Hz), 7.34 ppm (m, 5, aromatic protons). Treatment of **5** with acetic anhydride–pyridine at room temperature for 3 h provided *O*-acetyl-descysteaminythienamycin benzyl ester (**6**) in 68% yield: IR (CHCl₃) 1780 (β -lactam), 1740 cm⁻¹ (ester); NMR (CDCl₃, 60 MHz), 1.38 (d, 3, CH₃), 2.02 (s, 3, OAc), 2.82 (m, 2, H₄), 3.30 (q, 1, H₆), 4.20 (d of t, 1, H₅), 5.19 (m, 1, H₉), 5.28 (s, 2, benzyl CH₂), 6.49 (q, 1, H₃), 7.40 ppm (m, 5, aromatic protons).

Sodium descysteaminythienamycin possesses *in vitro* activity comparable with that of the parent thienamycin against both Gram-positive species and the *Enterobacteriaceae* Gram-negative microorganisms. Although the average activity of **4** against thienamycin-sensitive *pseudomonas* strains is one third that of thienamycin itself, descysteaminythienamycin is nevertheless still superior to carbenicillin and active against Gram-negative strains resistant to carbenicillin (Table I). Descysteaminythienamycin represents the simplest bicyclic β -lactam antibiotic to date which still possesses such extraordinary potency.

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- (6) The reverse-phase HPLC analysis of the reaction mixture was performed using a Waters Associates high pressure liquid chromatograph equipped with a 2.5-mm i.d. X 61 cm Bondapak C₁₈/Corosil column. Using 10% THF-H₂O as solvent at 1.0-mL/min flow rate, sodium *N*-phenoxyacetylthienamycin and sodium descysteaminythienamycin showed retention times of 9 and 3 min, respectively.
- (7) High voltage electrophoresis was also used to analyze the reaction mixture. In a typical electrophoretic separation (0.05 M, pH 7.0, sodium phosphate buffer at 2 kv for 20 min on Whatman chromatography paper, followed by bioautographic visualization), sodium *N*-phenoxyacetylthienamycin and sodium descysteaminythienamycin showed mobilities of 5 and 8 cm, respectively, toward the anode.
- (8) The H_α protons of **4** appeared as two doublets in the 60-MHz NMR spectrum, and as two triplets in the 100-MHz spectrum. Since the outer peaks of the AB pattern could not be observed in the 60- and 100-MHz spectra, the assignment of the chemical shifts of the H_{4α} and H_{4β} protons remain uncertain: K. M. Silverstein and G. C. Bassler, "Spectrometric Identification of Organic Compounds", Wiley, New York, 1967, p 130.

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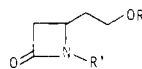
Total Synthesis of Thienamycin Analogues. 1. Synthesis of the Thienamycin Nucleus and *dl*-Descysteaminythienamycin

Sir:

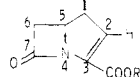
Thienamycin (**16**), a novel β -lactam antibiotic isolated from *Streptomyces cattleya*, is an unusually potent antibiotic.¹ It has three structural features not found in the classical β -lactam antibiotics, the penicillins and cephalosporins:² (1) an α -hydroxyethyl side chain instead of a β -amido side chain at C-6, (2) an unusual cysteamine side chain at position 2, and (3) a highly strained nucleus consisting of an unsaturated five-membered ring fused to a β -lactam in which a methylene replaces the sulfur at position 1, found in conventional β -lactam antibiotics.

Introduction of the hydroxyethyl side chain at the 6(7) position of a penicillin (cephalosporin)³ or of a cysteamine side chain at position 3 of a cephalosporin⁴ did not increase the activity of these nuclei, leading us to believe that the nucleus of thienamycin, 1-carba-2-penam-3-carboxylic acid, may have high antibiotic activity and may be a major contributor to the unusual antibiotic activity of thienamycin.

We describe below the synthesis of sodium 1-carba-2-penam-3-carboxylate (**7**) and its (*R*)-6 α -hydroxyethyl ana-



- R = Ac, R' = H
- R = Ac, R' = CHOHC(O)NB
- R = Ac, R' = CHClCO₂NB
- R = Ac, R' = CPh₁₃CO₂NB
- R = H, R' = CPh₁₃CO₂NB

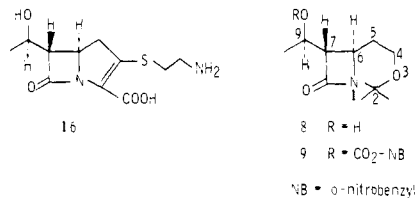


- R = NB
- R = Na

logue (**15**) and report on the antibiotic activity of **7**.⁵ Condensation of 4-(2-acetoxyethyl)-azetidin-2-one (**1**)⁶ with 2 equiv of *o*-nitrobenzyl glyoxalate, (C₆H₆, Δ , Dean-Stark, CaH₂, 3 h) gave the hydroxy acetate **2** (70%) after silica gel chromatography (50% EtOAc-C₆H₆): IR⁷ 3300 (OH), 1760 (β -lactam), 1740 (ester), 1530 (NO₂); NMR⁷ 7.5-8.4 (m, *o*-C₆H₄NO₂), 5.66 (s, CH₂Ar), 5.5 (d, -CHOH), 4.2 (2t, CH₂OAc), 3.95 (m, C-4 H), 3.15 (q, *J* = 5, *J* = 15 Hz, C-3 α H), 2.65 (q, *J* = 3, *J* = 15 Hz, C-3 β H), 2.05 (s, CH₃C), 1.95 (m, CH₂CH₂O). Hydroxy acetate **2** gave the unstable chloro compound **3** (SOCl₂, pyridine, THF, -20 to 25 °C, 25 min) which without purification was converted to the ylide-acetate **4** (Ph₃P, DMF, 1 hr, 25 °C) in 82% yield after silica gel chromatography (EtOAc): IR 1740 (β -lactam, ester), 1620 (benzyl ester), 1525 (NO₂); NMR 7.2-8.2 (m, aromatic H), 5.1-5.8 (m, CH₂Ar), 4.12 (q, CH₂OAc), 1.96 (s, CH₃C=O).

The negative charge on the ylide carbon decreases the bond energy of both the β -lactam and ester carbonyl to which it is adjacent; this is reflected in the shift of both carbonyl absorptions to longer wavelengths (1740 and 1620 cm⁻¹ in **4** vs. 1760 and 1740 cm⁻¹ in **2**). Hydrolysis of **4** with 1.2 equiv of NaOMe in MeOH (25 °C, 1 h), followed by chromatography on silica gel (5% MeOH, EtOAc), gave alcohol **5** (86%; IR (3600 (OH), 1740 (β -lactam), 1630 (benzyl ester), 1530 (NO₂)) with no evidence of ester exchange or hydrolysis of the azetidinone, both functions being protected against nucleophilic attack by the presence of the negative charge on the adjoining ylide carbon. Oxidation of the ylide-alcohol **5** (Me₂SO, Ac₂O, 25 °C, 3.5 h) gave the corresponding aldehyde which spontaneously cyclized to the carbapenam **6** (30%, after preparative TLC on silica gel): IR 1772 (β -lactam), 1722 (ester), 1660 (C=C), 1530 (NO₂); NMR 7.3-8.5 (m, -O-C₆H₄NO₂), 6.61 (t, C-2 H), 5.73 (q, AB, CH₂Ar), 4.35 (m, *J* = 3, *J* = 5, *J* = 8 Hz, C-3 H), 3.58 (q, *J* = 5, *J* = 14 Hz, C-6 α H), 2.99 (q, *J* = 3, *J* = 14 Hz, C-6 β H), 2.96 (t, C-1 H); UV $\lambda_{\max}^{\text{EtOH}}$ 265 nm ϵ 9200; mass spectrum *m/e* 288. Photolysis of **6** (degassed dioxane-water, 1 equiv of NaHCO₃, 350 nm, Rayonet reactor, 25 °C, cold finger cooling, 55 min) gave the desired carbapenam sodium salt **7**: 19%;⁸ UV $\lambda_{\max}^{\text{H}_2\text{O}}$ 262 nm (extinguishable by addition of NH₂OH).

The antibiotic activity of **7**, shown in Table I, is compared with those of thienamycin and ampicillin. The nucleus of thienamycin is itself a powerful antibiotic and contributes a major factor toward the remarkable activity of **16**; however



it is susceptible to β -lactamases as shown by its low activity against penicillinase-producing strains.

The above synthesis can be modified to prepare analogues of thienamycin; this is demonstrated below by the synthesis of descysteaminythienamycin (**15**) which can also be obtained

Table 1. Inhibitory Zone Diameters (Millimeters) vs. Penicillin-Sensitive and Resistant Bacterial Strains

compd	disc content, μg (nmol)	<i>S. aureus</i>		<i>E. coli</i>		Enterobacter cloacae	
		MB2985	MB2314	MB2482	MB2964	MB2647	MB2646
ampicillin	10 (28)	33.5	13	20.3	0	18	0
thienamycin	6.3 (23)	38.5	38.5	25	25.5	22.5	22
7 ^a	8.2 (54)	20.5	0	20	0	21	16.5

^a 54 nmol of racemic **7** corresponds to 27 nmol of active **7**.