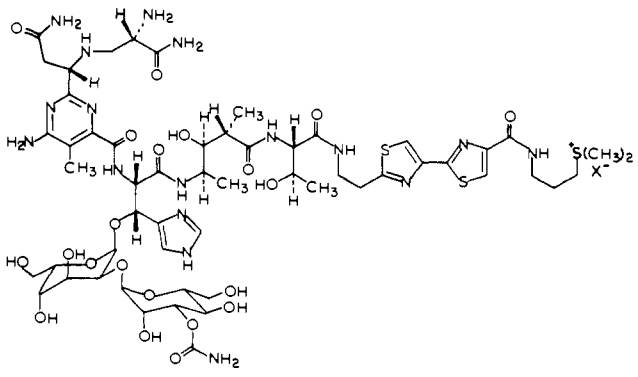
Bleomycin: Synthesis and Structural Verification of the Tripeptide S and Tetrapeptide S Moieties

Sir:

The antitumor antibiotic bleomycin has attracted considerable interest because of its activity against certain human malignancies and the prospect that new bleomycin analogues may find even greater utility in the clinic.[1,2] A structure was first proposed for bleomycin in 1972[3] and has recently been revised to that shown (**1**, bleomycin A_2).[4] Additionally, the



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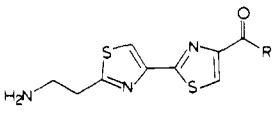
structure proposed by Dabrowiak et al.[5] for the Cu(II) complex of bleomycin has been revised following X-ray crystallographic analysis of a biosynthetic intermediate,[6] and the structure of the "active" Fe(II)-bleomycin complex, proposed by extension of that of the revised Cu(II)-bleomycin complex,[6] has recently been questioned.[7] Given the lack of success in obtaining a sample of bleomycin in a form suitable for X-ray crystallography, it appears inevitable that unambiguous structural verification must await a total synthesis of bleomycin. As part of such an effort, we have prepared quantities of tripeptide S (**3d**) and tetrapeptide S (**7c**), accessible previously only by partial hydrolysis of bleomycin. The identity of the synthetic and natural compounds serves to verify the structure proposed for this portion of the bleomycin molecule.[8]

We have previously described the preparation of 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylic acid (**2**, R = OH)



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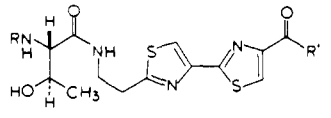
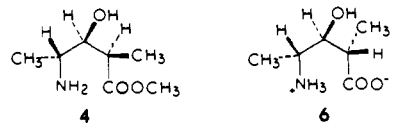


3a R=H, R′=OCH_3
3b R=o-NO_2-C_6H_4S, R′=$NH(CH_2)_3Br$
3c R=H, R′=$NH(CH_2)_3Br$
3d R=H, R′=$NH(CH_2)_3\overset{+}{S}(CH_3)_2$

from β-alanine and cysteine.[9] As the bithiazole moiety of bleomycin represents the C terminus of bleomycinic acid, the common structural unit from which all of the naturally occurring bleomycins and second generation bleomycin analogues[2] are accessible, the initial strategy for the elaboration of bleomycin focused on the coupling of ester **2** (R = OCH_3)[10] with activated derivatives of *o*-nitrophenylsulfenylthreonine. Although high yields of coupled products were obtained, and these were convertible into peptide **3a** ($CHCl_3$, 2 equiv of aqueous 10 N HCl), this approach was abandoned when the product was found to undergo remarkably facile hydrolysis to afford the (zwitterionic) carboxylic acid. The obvious limitations on the variety of appropriate protecting groups[11] and the recognition that all naturally occurring bleomycins are *n*-C_3 or *n*-C_4 amide derivatives of bleomycinic acid prompted us to consider the use of bithiazole derivatives containing "activated" C-terminal substituents, each convertible into those of several naturally occurring bleomycins.

Bithiazole **2** [R = $NH(CH_2)_3Br$], a potential "precursor"[12] of bleomycins A_1, A_2, demethyl A_2, A_2′-b, A_5, and A_6,[13] was prepared in 75% overall yield from 2′-(2-aminoethyl)-2,4′-bithiazole-4-carboxylic acid by successive N-trifluoroacetylation [CH_3OH, $(CF_3CO)_2O$, Hünigs base, 1.5 h] and condensation with 3-bromopropylamine.[14] Deblocking (20 equiv of K_2CO_3, aqueous THF, 15 h) gave the desired bithiazole **2** [R = $NH(CH_2)_3Br$],[15] which was treated directly with 2,4-dinitrophenyl *N*-(*o*-nitrophenylsulfenyl)threoninate;[16] extractive workup afforded **3b** as yellow needles in 83% yield, mp 104–107 °C. A chloroform solution of **3b** was stirred with 2 equiv of 10 N hydrochloric acid (25 °C, 2 h), depositing key intermediate **3c** HCl as colorless crystals (92%, mp 208.5–211 °C after recrystallization from methanol-ether). Tripeptide derivative **3c** was converted readily [aqueous $(CH_3)_2S$, $Pb(NO_3)_2$, 24 h] into **3d**, identical with a sample of tripeptide S obtained by degradation of bleomycin.[17]

The synthesis of tetrapeptide S involved the condensation of tripeptide analogue **3c** with (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvaleric acid. The latter has been prepared by Yoshioka et al.[18] as part of a mixture of four isomeric amino acids. While the desired isomer is a minor component of the mixture, the corresponding 2*R*,3*S*,4*R* isomer is a major component and may be obtained in optically pure form as the corresponding methyl ester (**4** HCl).[19] Treatment of **4** with 0.3 equiv of $NaOCH_3$ in methanol at 25 °C effected cyclization to the lactam of the same absolute configuration within 4 h; after 72 h at reflux, epimerization at C-2 gave a 1:3 mixture (quantitative yield) of **5a** and the thermodynamically more



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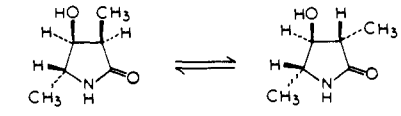

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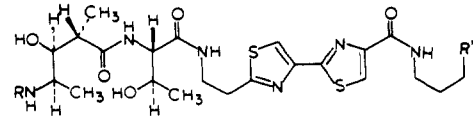
stable species **5b** (having the desired 2*S*,3*S*,4*R* configuration). Hydrolysis of the mixture (4 N HCl, 100 °C, 4 h) and fractional crystallization afforded the required amino acid (**6**).[20]

The expectation that bithiazole derivative **3c** might undergo polymerization, in analogy to **2**,[15] prompted us to convert **6** into a derivative with strongly electrophilic character at C-1 to facilitate the desired intermolecular reaction. Several N(O)-blocked activated esters of **6** were found to be insufficiently reactive, to undergo facile epimerization at C-2 or to afford tripeptide analogues that could not be deblocked. However, the *tert*-butyloxycarbonyl derivative of **6** (di-*tert*-butyl carbonate, dioxane, 25 °C, 12 h) could be obtained as colorless cubes in 91% yield, mp 142–143 °C, and converted cleanly into the corresponding 2,4-dinitrophenyl ester. The ester was isolated by extractive workup and utilized directly for condensation with **3c** in CH_2Cl_2; tetrapeptide analogue **7a** was ob-



7a R=$(CH_3)_3CO\overset{O}{\overset{\|}{C}}$, R′=Br
7b R=H, R′=Br
7c R=H, R′=$\overset{+}{S}(CH_3)_2$

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tained in 86% crude yield.[21] After crystallization from chloroform-pentane, treatment of a chloroform solution of **7a** with 2 equiv of 10 N HCl gave **7b** HCl, isolable by filtration in 92% yield after precipitation from methanol-ether. In analogy to the conversion **3c** → **3d**, treatment of tetrapeptide analogue **7b** with aqueous dimethyl sulfide containing $Pb(NO_3)_2$ gave **7c** in ~40% yield. The identity of the synthetic and authentic materials verifies the structural assignment for this component of bleomycin.[8,22]

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(7) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616.
(8) The ^1H and ^{13}C NMR spectra of bleomycin A_2 contain resonances identical with those of isolated tripeptide S and tetrapeptide S. Since these species were also isolated by different procedures (treatment with acid and *N*-bromosuccinimide, respectively), it seems unlikely that any rearrangement accompanied their isolation.
(9) McGowan, D. A.; Jordis, U.; Minster, D. K.; Hecht, S. M. *J. Am. Chem. Soc.* **1977**, *99*, 8078.
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(11) Bleomycin is both acid and base sensitive and contains a bithiazole moiety that poisons hydrogenation catalysts. Additionally, the presence of highly polar or nucleophilic substituents on each of the naturally occurring bleomycins precludes the use of the analogue of **3a** with "preformed" C-terminal substituents.
(12) E.g., dissolution of the *N*-acetyl derivative of **2** [R = $NH(CH_2)_3Br$] in neat 1,4-diaminopropane effected conversion into the intended product [**2**, R = $NH(CH_2)_3NH(CH_2)_3NH_2$] in quantitative yield within 5 min, as judged by TLC and NMR analyses.
(13) Umezawa, H. *Lloydia* **1977**, *40*, 67.
(14) Conversion of the crystalline *N*-trifluoroacetyl derivative into the corresponding acid chloride [$SOCl_2$, $(C_2H_5)_3N$, CH_2Cl_2, 25 °C, 18 h], followed by treatment with 3-bromopropylamine hydrochloride [$(C_2H_5)_3N$, $(CH_3)_2N(C_6H_4N)$, CH_2Cl_2, 25 °C, 2 h], afforded the fully blocked bithiazole derivative as colorless needles in 99% yield, mp 151–152 °C after crystallization from ethanol-water. This compound could be stored at room temperature for extended periods of time without decomposition. The structures of all new synthetic intermediates were verified by appropriate spectral measurements and by elemental analysis.
(15) The deblocking was effected in 95% (spectrophotometric) yield; as anticipated, the deblocked compound could not be isolated as the free base owing to its ready polymerization. However, solutions of the free base having concentrations of <0.4 M were sufficiently stable for routine manipulations.
(16) Prepared from *N*-(*o*-nitrophenylsulfenyl)threonine (Zervas, L.; Borovas, D.; Gazis, E. *J. Am. Chem. Soc.* **1963**, *85*, 3660) by treatment (CH_3CN, 0 °C) with equal amounts of *N,N'*-dicyclohexylcarbodiimide and 2,4-dinitrophenol. Crystallization (ethyl acetate–pentane) afforded the ester in 92% yield, mp 125.5–128 °C.
(17) Authentic tripeptide S was obtained by treatment of bleomycin A_2 with 12 N HCl at 25 °C for 7 days (Umezawa, H. *Pure Appl. Chem.* **1971**, *28*, 665), followed by chromatography on Sephadex C-25. The synthetic and authentic samples had the same mobilities on Sephadex C-25 and on paper and thin layer chromatograms using five different solvent systems. Their ultraviolet and ^1H and ^{13}C NMR spectra were also identical.
(18) Yoshioka, T.; Hara, T.; Takita, T.; Umezawa, H. *J. Antibiot. (Tokyo)* **1974**, *27*, 356.
(19) Under optimal conditions, the isolated mixture of 4-amino-3-hydroxy-2-methylvaleric acids was 86% 2*R*,3*S*,4*R*. After conversion into methyl ester **4** in quantitative yield, virtually all of the major isomer was obtained by crystallization from methanol–ether. See Hecht, S. M.; Burlett, D. J.; Mushika, Y.; Kuroda, Y.; Levin, M. D., ref 6a, p 48 ff.
(20) Workup of the hydrolyzed mixture of lactams (Dowex 50, H^+ form, elution with water and then with 2.9% aqueous NH_4OH) afforded a mixture of 2*R*,3*S*,4*R* and 2*S*,3*S*,4*R* amino acids in 77% yield, from which **6** could be obtained in nearly theoretical yield.
(21) Workup involved extraction of the reaction mixture with cold aqueous Na_2CO_3, concentration of the dried organic phase to a small volume, and precipitation of the product with excess pentane.
(22) Tetrapeptide S was obtained by treatment of bleomycin A_2 with 6 equiv of *N*-bromosuccinimide (Muraoka, Y.; Takita, T.; Maeda, K.; Umezawa, H. *J. Antibiot. (Tokyo)* **1972**, *25*, 185). The isolated peptide was purified by chromatography on Sephadex C-25 and shown to have the same chromatographic properties as the synthetic material on this support and also when analyzed by TLC and paper chromatography in several solvent systems, as well as the same properties by ultraviolet and ^1H and ^{13}C NMR spectroscopy.
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Ammoximation: Direct Synthesis of Oximes from Ammonia, Oxygen, and Ketones

Sir:

Ketoximes have been prepared by a reaction according to the equation

$$NH_3 + {}^1/_2O_2 + \text{ketone} \rightarrow \text{ketoxime} + H_2O \qquad (1)$$

The further oxidation of a mixture of NH_3 and an olefin (propylene) or an aldehyde (acrolein) to form a nitrile (acrylonitrile) is termed ammoxidation. The present process referring to the synthesis of ketoximes as described by equation 1 is termed ammoximation. It is well known that cyclohexanone oxime can be rearranged to caprolactam in high yields in the vapor phase over aluminosilicate catalysts.[1] Therefore, a direct synthesis of caprolactam could be envisioned for converting cyclohexanone, NH_3, and air directly into caprolactam by placing a reactor for the vapor-phase rearrangement of the oxime directly after the ammoximation reactor.

Oximes are normally prepared by reaction of ketones with hydroxylamine. The source of NH_2OH is the oxidation of NH_3 to NO (or NO_2) followed by reduction with H_2 or SO_2.[2] The formation of NH_2OH from NH_3 and O_2 has been reported.[3,4] The reactants were passed over a Pt catalyst at low pressures and high temperatures (>800 °C). Small amounts of NH_2OH and N_2O were found as the major products collected in a (liquid air) cold trap. While NH_2OH is thermally unstable,[5-7] it was felt that scavenging the NH_2OH or its precursors with ketones to form the more stable oximes would be a much more favorable process. However, we have not yet been able to identify NH_2OH as an intermediate in reaction 1. It is also known that oximes can be prepared from ketones, NH_3, and H_2O_2 or organic peroxides.[8] However, the present synthesis is thought not to involve peroxides since addition to the feed of a variety of peroxides or of radical inhibitors[9] did not influence the ammoximation reaction.

Vapor-phase reactions[10] were conducted in a borosilicate glass tube of ~14-mm o.d. containing a glass frit or plug of glass wool to hold the catalyst in place. The reactor was inside an electrically heated tube furnace, with concurrent, down-flow

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