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Registry No. *p*BrBz-Aib-(L-Pro-Aib)$_3$-OMe, 141613-74-3; *p*BrBz-Aib-(L-Pro-Aib)$_4$-OMe, 141613-75-4.

Supplementary Material Available: Tables of final atomic coordinates, bond lengths, and torsion angles for *p*BrBz-Aib-(L-Pro-Aib)$_3$-OMe and *p*BrBz-Aib-(L-Pro-Aib)$_4$-OMe (15 pages). Ordering information is given on any current masthead page.

On the Role of Individual Bleomycin Thiazoles in Oxygen Activation and DNA Cleavage

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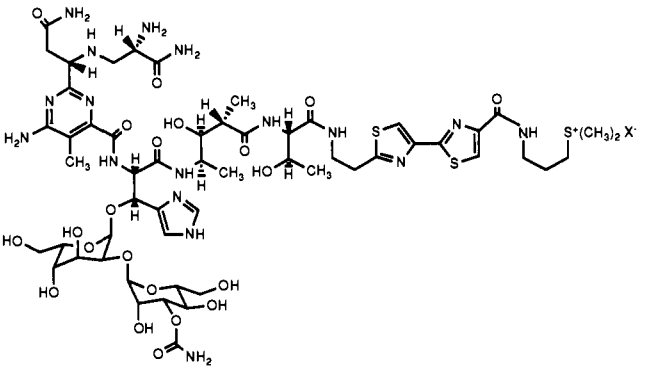
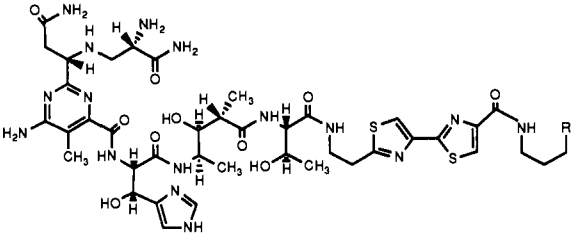
Abstract: Two structurally novel bleomycin (BLM) analogs were prepared by total synthesis to permit the evaluation of the role of individual thiazole moieties in the processes of bleomycin-mediated oxygen activation and DNA degradation. Each of the compounds was structurally related to deglycobleomycin demethyl A_2 but contained an *S*-methyl-L-cysteine moiety in lieu of one of the two thiazoles normally present in bleomycin. In common with bleomycin and deglycobleomycin, both monothiazole BLMs were found to be excellent catalysts for the oxygenation of low molecular weight substrates such as naphthalene and styrene and also mediated the demethylation of *N,N*-dimethylaniline. However, both of the monothiazole BLMs were much less effective than bleomycin or deglycobleomycin in promoting DNA degradation. Analysis of the effects of the monothiazole BLMs on 5′- and 3′-^{32}P end labeled DNA duplexes indicated that cleavage occurred without discernible sequence selectivity. These results demonstrate that the bithiazole moiety in BLM is not required for O_2 activation or for the oxygenation and oxidation of low molecular substrates in what are presumably biomolecular processes. However, the bithiazole clearly does contribute to the efficiency of bleomycin-mediated DNA degradation and to the sequence selectivity of DNA strand scission by bleomycin.

The bleomycins are a family of structurally related, glycopeptide-derived antibiotics with significant antitumor activity.[1] Blenoxane, the clinically used mixture of bleomycins, contains bleomycin A_2 as its major constituent. The therapeutic effect of BLM is believed to result from its ability to mediate DNA degradation,[2,3] although it has been shown recently that BLM is also capable of mediating RNA degradation in a highly selective fashion.[4] The ability of BLM to degrade DNA is dependent on the participation of a redox-active metal ion cofactor such as Fe, Cu or Mn and a source of oxygen.[2,3,5] The ferrous ion–bleomycin complex, Fe(II)·BLM, combines with O_2 to produce a reactive and unstable oxygenated metallobleomycin species termed "activated bleomycin".[3,6] The available evidence suggests that activated Fe·BLM contains a high valent, metal–oxo species that is formed by 2e$^-$ reduction of Fe·BLM-bound oxygen to a peroxide, followed by heterolysis of the peroxide O–O bond.[6,7] Support for representation of activated Fe·BLM as a perferryl species derives from the similarities in the chemistry noted for activated

Chart I



bleomycin A_2



deglycobleomycin A_2 R = $S^+(CH_3)_2$

deglycobleomycin demethyl A_2 R = SCH_3

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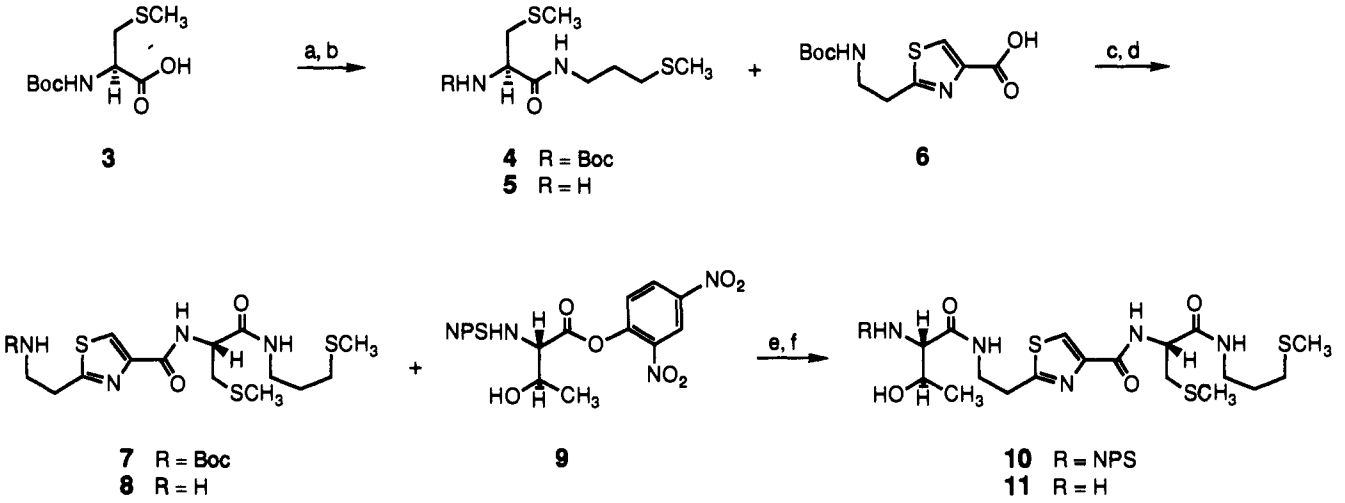
Scheme I[a]



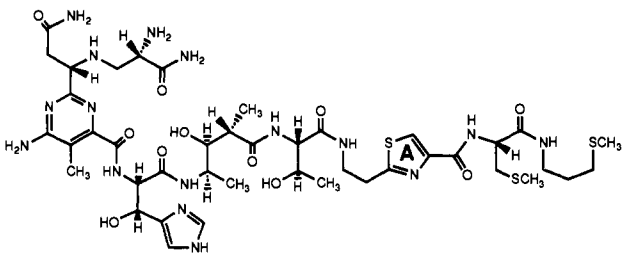
[a] a, $H_2N(CH_2)_3SCH_3$, DCC, HOBt, 25 °C; b and d, 4.5 M HCl, dioxane, 25 °C; c, DCC, HOBt, 25 °C; e, 4-(dimethylamino)pyridine, CH_2Cl_2, 25 °C; f, concentrated HCl.

bleomycin[8] and cytochrome P-450 systems.[9] In a reaction analogous to the "peroxide shunt" pathway in cytochrome P-450 activation, it has also been shown that activated Fe·BLM can be formed by admixture of Fe(III)·BLM and H_2O_2.[6d]

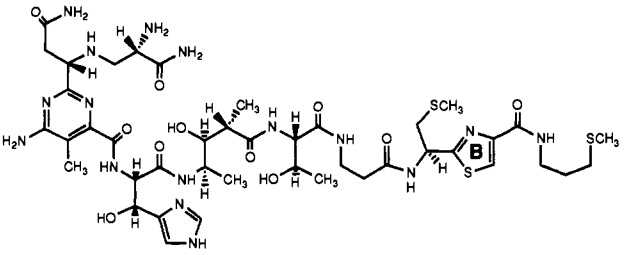
Bleomycin-mediated DNA degradation occurs predominantly at 5′-GC-3′ and 5′-GT-3′ sequences[10] and involves oxidative degradation of deoxyribose.[3] Two sets of products are formed, apparently from a common C-4′ deoxyribose radical.[11] One set of products involves strand scission; the other results in formation of a lesion that undergoes strand scission upon treatment with alkali. Both lesions have been characterized structurally.[12,13]

Sequence selective DNA degradation by bleomycin involves initial binding of the drug; the lifetimes of the DNA complexes formed by Fe(III)·BLM and Cu(II)·BLM have been measured.[14] Both the bithiazole moiety and metal binding domain contribute to the DNA affinity of bleomycin.[15-17] Because recent findings

Chart II



bleomycin monothiazole A (1)



bleomycin monothiazole B (2)

suggest that the observed sequence specificity of DNA binding by bleomycin may actually be due to the metal binding domain,[16a,b,d] the nature of bithiazole–DNA interaction is unclear. It has been shown that the bithiazole moiety is necessary for BLM-mediated DNA helix elongation[17] and unwinding,[16c,17] consistent with the intercalation of the bithiazole moiety. The observed quenching of bithiazole fluorescence[18] and broadening of the two ^1H NMR resonances corresponding to the nonex-

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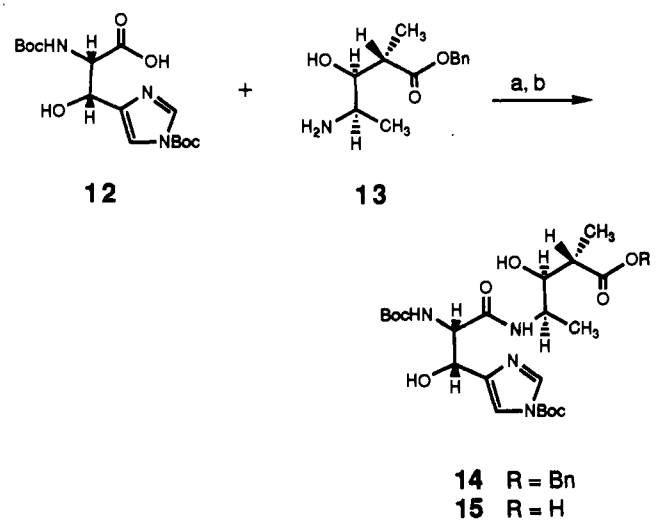
Scheme II[a]



[a] a, DCC, HOBt; b, H_2, 10% Pd/C.

changeable bithiazole H's upon DNA binding[19] are also consistent with this thesis. However, certain DNA minor groove binders such as distamycin can alter the specificity of DNA cleavage by BLM, while intercalators such as ethidium bromide have no effect.[20] Recently, Sugiura et al. have proposed a model for BLM binding to DNA which involves H-bonding between the thiazole nitrogens and the guanosine 2-amino group.[21]

While the bithiazole moiety of BLM is clearly essential for DNA binding, little work has been done to define the minimum structural requirements at the C-terminus of BLM for efficient DNA binding. It is not known, for example, whether both thiazoles are essential. In this context it may be noted that phleomycin, which contains a thiazolinylthiazole moiety, exhibits essentially the same sequence selectivity of DNA cleavage as bleomycin.[22] Further, Morii et al.[23] have shown that two BLMs altered in the bithiazole moiety by phototransformation also exhibited similar sequence selectivity of DNA cleavage.

To delineate the role of each thiazole ring in BLM, we have synthesized two bleomycin analogs (**1** and **2**), each of which contains a single thiazole. The ability of these synthetic BLM analogs to support the oxygenation of low molecular weight substrates as well as the degradation of DNA has been evaluated. Presently, we demonstrate that neither thiazole ring was required for efficient oxygen activation or for the oxidative transformation of low molecular weight substrates such as naphthalene, styrene, or *N*,*N*-dimethylaniline. On the other hand, neither of the monothiazole analogs of bleomycin effected DNA degradation efficiently nor in a sequence-selective fashion.

Results

Synthesis of Bleomycin Analogs. The preparation of bleomycin monothiazoles A and B (**1** and **2**) was carried out in analogy with the synthesis of bleomycin demethyl A_2 and deglycobleomycin demethyl A_2.[24] The synthesis of bleomycin monothiazole A (**1**) is outlined in Schemes I–III. Thus Boc-protected *S*-methylcysteine[25] (**3**) was condensed with 3-(methylthio)propylamine in 90% yield via the agency of DCC-HOBt, and the resulting amide (**4**) was deprotected in good yield using 4.5 M hydrogen chloride in dioxane. *S*-Methyl-(*S*)-cysteine [3-(methylthio)propyl]amide (**5**) was then condensed with 2-[*N*-((*tert*-butoxycarbonyl)-amino)ethyl]thiazole-4-carboxylic acid (**6**), the latter of which was readily accessible from the known[26] benzoylated thiazole-4-carboxylic acid. (Butoxycarbonyl) thiazolyl *S*-methylcysteine amide derivative **7** was obtained in 85% yield as an amorphous solid. Subsequent deblocking of **7** (4.5 M hydrogen chloride in dioxane) provided the respective amine **8** in 82% yield. The introduction of the requisite threonine moiety was accomplished using *N*-(*o*-nitrophenylsulfenyl)threonine 2,4-dinitrophenyl ester (**9**);[27] fully protected tripeptide derivative **10** was obtained as the expected yellow solid in 82% yield. Following removal of the NPS protecting group (concentrated HCl, 0 °C, 95% yield), the respective amine (**11**) was condensed with known[24a] dipeptide **15** (DCC, HOBt; DMF; 40 h), providing fully protected derivative **16** as a colorless glass in 37% yield.

The synthesis of bleomycin monothiazole A (**1**) was completed (Scheme III) by initial removal of the Boc protecting groups from **16** (CF_3COOH, $(CH_3)_2S$; 0 °C; 3 h), which was accomplished in 90% yield. Condensation of peptide **17** with pyrimidoblamic acid (**18**)[28] in the presence of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent)[29] afforded crude Boc BLM monothiazole A (**19**). This intermediate was treated with CF_3COOH–$(CH_3)_2S$ at 0 °C to effect its conversion to **1**. Purification of the final product was achieved by chromatography on Amberlite XAD-2 and then by fractionation of a Cu chelate on CM Sephadex C-25. Separation of the fractionated product from Cu(II) and salt was then accomplished on an Amberlite XAD-2 column, affording bleomycin monothiazole A (**1**) as a colorless glass in 25% overall yield from **17**. The structure of **1** was verified by ^1H NMR spectroscopy and mass spectral analysis.

The route employed for the preparation of BLM monothiazole B is outlined in Schemes IV and V. *N*-Boc-*S*-methylcysteine methyl ester (**20**)[30] was reduced to the respective aldehyde (**21**) with diisobutylaluminum hydride, and the crude product was condensed directly with (*S*)-cysteine methyl ester in benzene for 2 days. Thiazolidine **22** was obtained as a colorless solid in 71% yield following purification by silica gel chromatography. Dehydrogenation of the thiazolidine was accomplished by the use of chemical manganese dioxide, in analogy with the work of Hamada et al.[31] Vigorous stirring of thiazolidine **22** with this reagent in a benzene solution containing pyridine (55–58 °C, 6 h) effected conversion to the respective thiazole in 54% yield. Following removal of the Boc protecting group with CF_3COOH-$(CH_3)_2S$, aminothiazole **24** was condensed with Boc-β-alanine (DCC, HOBt) in dry acetonitrile, affording dipeptide analog **25**. This thiazole derivative was obtained as colorless microcrystals in 70% yield and was characterized fully, including an assessment of optical integrity at the asymmetric center under conditions required for completion of the synthesis of BLM derivative **2**. The latter was accomplished in part by analysis of the product resulting from saponification of **25** in aqueous CH_3OH containing 2 M NaOH. Thiazole carboxylic acid **26**, obtained as a colorless solid in essentially quantitative yield, could be reconverted to methyl ester

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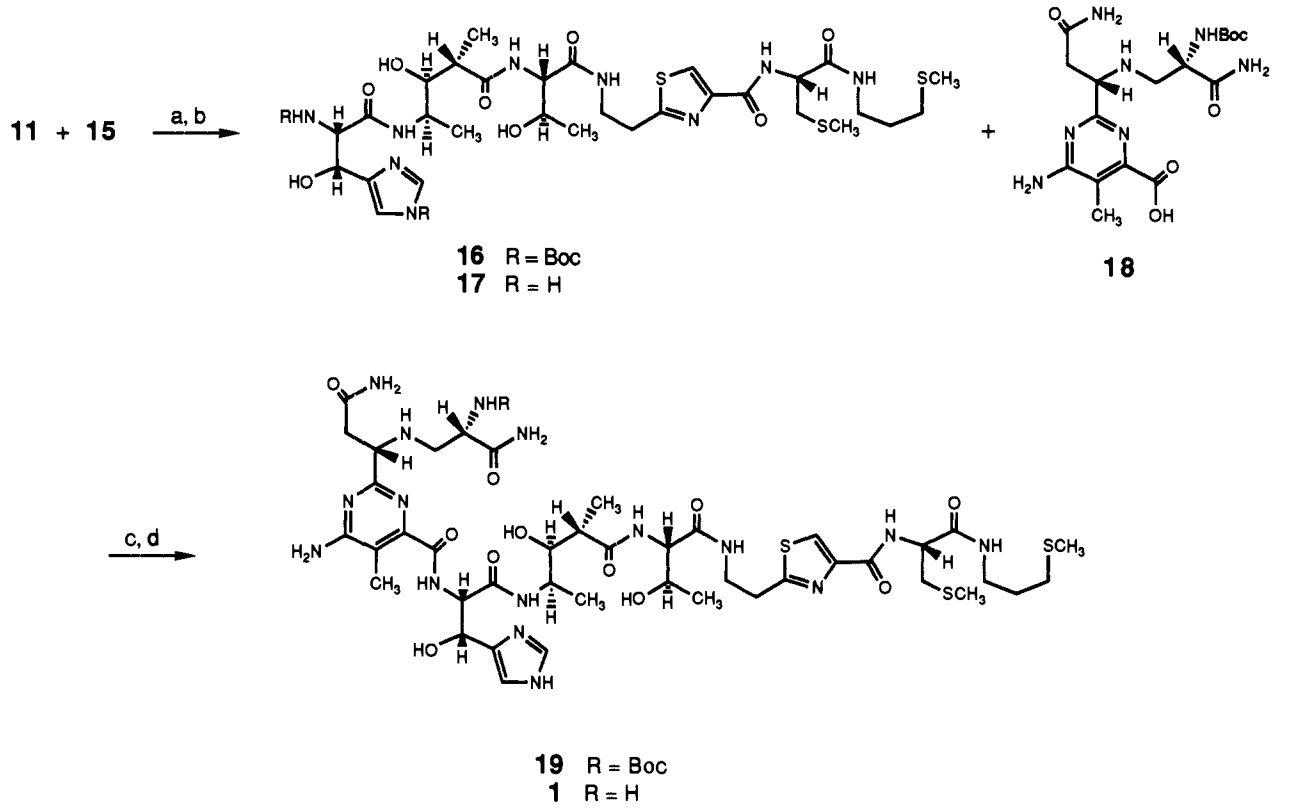
Scheme III[a]



[a] a, DCC, HOBt, 25 °C; b and d, CF_3COOH, $(CH_3)_2S$, 0 °C; c, BOP reagent, *i*-Pr_2NEt, 25 °C.

25 via the agency of diazomethane; this methyl ester was identical in all respects, including optical rotation, with the sample of **25** employed initially for saponification. Compound **26** was also condensed with [3-(methylthio)propyl]amine, providing the dipeptide analog (**27**) required for the synthesis of BLM monothiazole B. The use of threonine derivative **9** for the elaboration of the requisite tripeptide analog was unsatisfactory, owing to the formation of byproducts. Therefore, after dipeptide **27** was deblocked (4.5 M hydrogen chloride in dioxane), **28** was condensed with threonine derivative **29** in DMF via the agency of DCC-HOBt. Protected tripeptide analog **30** was obtained as colorless microcrystals in 82% overall yield from **27**. Deprotection of **30** was accomplished with Zn-HOAc, providing tripeptide analog **31** as a light yellow foam in 95% yield.

The remainder of the synthesis of BLM monothiazole was parallel to that of BLM monothiazole A (cf. Schemes III and V). Thus tripeptide **31** was condensed with dipeptide **15**, providing di-Boc peptide **32** as a colorless solid in 55% yield. Following deblocking of **32** (CF_3COOH, $(CH_3)_2S$; 88% yield), peptide derivative **33** was condensed with pyrimidoblamic acid (**18**) in the presence of the BOP reagent. Crude Boc BLM monothiazole B (**34**) was deblocked at 0 °C with CF_3COOH-$(CH_3)_2S$ and purified chromatographically as described above for isomeric analog **1**. BLM monothiazole B (**2**) was obtained as a colorless glass in 30% overall yield from **33**. The assigned structure was fully supported by the ^1H NMR and mass spectra.

Oxygen Transfer to Low Molecular Weight Substrates. In addition to mediating the oxidative destruction of DNA[2,3] and RNA,[4] BLM also serves as a reasonably efficient catalyst for the oxygenation of certain low molecular weight substrates. In the presence of oxidants such as iodosobenzene and H_2O_2, Fe(III)·BLM is capable of mediating the oxidation and oxygenation of substrates such as *cis*-stilbene and styrene.[8,32] Activated BLM has also been shown to mediate the hydroxylation of aromatic substrates such as naphthalene and anisole.[8c] Therefore, as part of the characterization of BLM monothiazoles A and B (**1** and

Table I. Hydroxylation of Naphthalene by Fe(III)·BLMs[a]

catalyst	1-naphthol, mM	2-naphthol, mM	total product, mM
Fe(III)·BLM	1.14	1.21	2.35
Fe(III)·BLM monothiazole A	1.74	1.64	3.38
Fe(III)·BLM monothiazole B	0.71	0.59	1.30
Fe(III)	0	0	0

[a] Carried out in 4:1 CH_3OH–H_2O containing 500 μM Fe(III)·bleomycin or BLM monothiazole analog, 50 mM naphthalene, and 30 mM H_2O_2 at 25 °C for 1.0–1.5 h.

Table II. Styrene Oxygenation by Fe(III)·BLMs[a]

catalyst	styrene oxide, mM	phenylacetaldehyde, mM	total product, mM
Fe(III)·BLM	1.68	1.18	2.86
Fe(III)·BLM monothiazole A	2.44	1.21	3.65
Fe(III)·BLM monothiazole B	1.34	0.75	2.09
Fe(III)	0	0	0

[a] Carried out in 4:1 CH_3OH–H_2O containing 500 μM Fe(III)·bleomycin or BLM monothiazole analog, 50 mM styrene, and 30 mM H_2O_2 at 0 °C for 1.5 h.

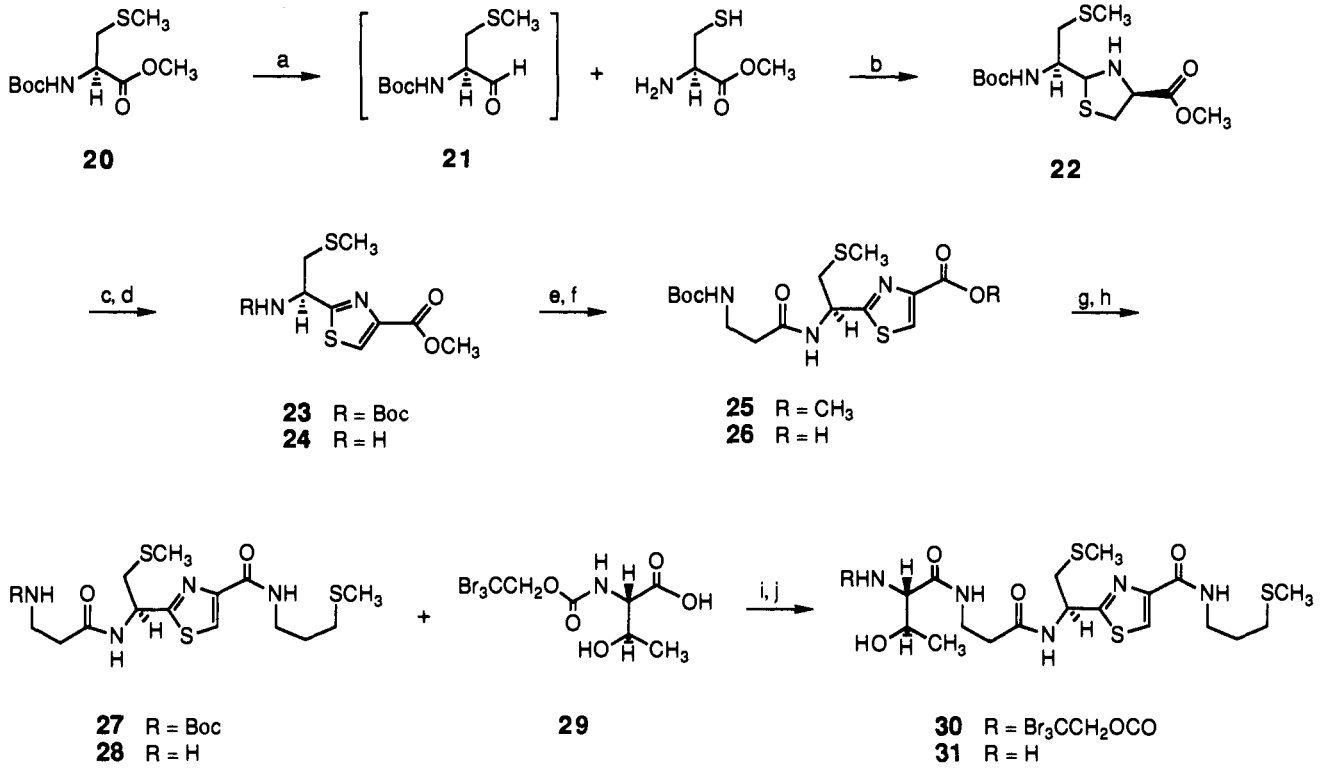
Table III. N-Demethylation of *N,N*-Dimethylaniline by Fe(III)·BLMs[a]

catalyst	*N*-methylaniline, mM
Fe(III)·BLM	2.38
Fe(III)·BLM monothiazole A	2.02
Fe(III)·BLM monothiazole B	1.55
Fe(III)	0

[a] Carried out in 4:1 CH_3OH–H_2O containing 500 μM Fe(III)·bleomycin or BLM monothiazole analog, 50 mM *N,N*-dimethylaniline, and 30 mM H_2O_2 at 25 °C for 1.5 h.

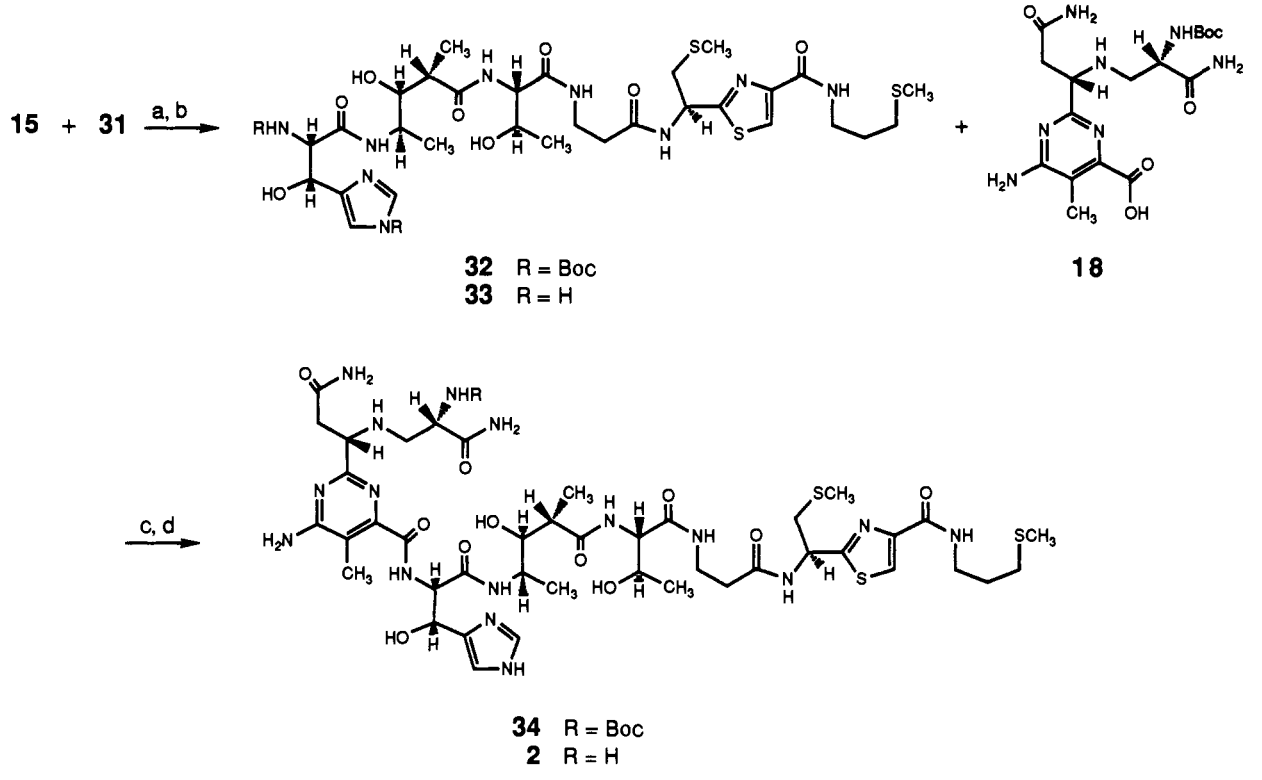
(32) (a) Heimbrook, D. C.; Mulholland, R. L., Jr.; Hecht, S. M. *J. Am. Chem. Soc.* **1986**, *108*, 7839. (b) Ehrenfeld, G. M.; Shipley, J. B.; Heimbrook, D. C.; Sugiyama, H.; Long, E. C.; van Boom, J. H.; van der Marel, G. A.; Oppenheimer, N. J.; Hecht, S. M. *Biochemistry* **1987**, *26*, 931. (c) Heimbrook, D. C.; Carr, S. A.; Mentzer, M. A.; Long, E. C.; Hecht, S. M. *Inorg. Chem.* **1987**, *26*, 3835.

Scheme IV[a]



[a] a, DIBAL-H, −78 °C; b, C_6H_6, 25 °C, 2 days; c, chemical MnO_2, pyridine, 55 °C; d, CF_3COOH, $(CH_3)_2S$, 0 °C; e, $BocNH(CH_2)_2COOH$, DCC, HOBt, 25 °C; f, CH_3OH, 2 M aqueous NaOH; g, $H_2N(CH_2)_3SCH_3$, DCC, HOBt, 25 °C; h, 4.5 M HCl, dioxane, 0 °C; i, DCC, HOBt, 25 °C; j, Zn/HOAc, 0 °C.

Scheme V[a]



[a] a, DCC, HOBt, 25 °C; b and d, $CF_3COOH/(CH_3)_2S$, 0 °C; c, BOP reagent, *i*-Pr_2NEt, 25 °C.

2), we assessed the ability of these species to effect the oxidative transformation of low molecular weight substrates following activation in the presence of Fe(III) and H_2O_2 (Tables I–III).

As shown in Table I, in the presence of H_2O_2 and naphthalene, 500 μM Fe(III)·BLM catalyzed the formation of 1.14 mM 1-naphthol and 1.21 mM 2-naphthol, i.e., about 4.7 hydroxylated naphthalene molecules for each Fe(III)·BLM employed. The products were identified by direct comparison of each with an authentic sample. Repetition of the experiment using Fe(III)·BLM monothiazole A afforded the same hydroxylated naphthols

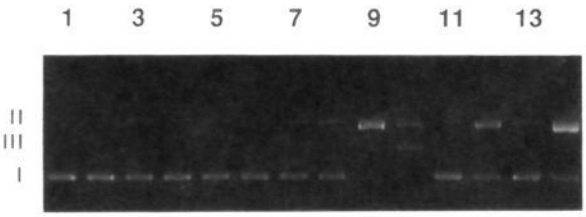


Figure 1. Relaxation of pBR322 form I DNA by BLMs in the presence of Fe(III) + H_2O_2. Lane 1, DNA alone; lane 2, 10 μM Fe(III); lane 3, 10 μM Fe(III)·deglygo BLM demethyl A_2; lane 4, 10 μM Fe(III)·BLM monothiazole A; lane 5, 10 μM Fe(III)·monothiazole B; lane 6, 250 μM H_2O_2; lane 7, 5 μM Fe(III) + 250 μM H_2O_2; lane 8, 10 μM, Fe(III) + 250 μM H_2O_2; lane 9, 5 μM Fe(III)·deglyco BLM demethyl A_2 + 250 μM H_2O_2; lane 10, 10 μM Fe(III)·deglyco BLM demethyl A_2 + 250 μM H_2O_2; lane 11, 5 μM Fe(III)·BLM monothiazole A + 250 μM H_2O_2; lane 12, 10 μM Fe(III)·BLM monothiazole A + 250 μM H_2O_2; lane 13, 5 μM Fe(III)·BLM monothiazole B + 250 μM H_2O_2; lane 14, 10 μM Fe(III)·BLM monothiazole B + 250 μM H_2O_2.

in a total yield of 3.38 mM; this was slightly greater than the total yield obtained with Fe(III)·BLM itself. Fe(III)·BLM monothiazole B was also found to effect naphthalene hydroxylation in the presence of H_2O_2, producing 1.30 mM hydroxylated naphthols.

The oxygenation of styrene by bleomycin produces not only the epoxide but also phenylacetaldehyde. As summarized in Table II, 500 μM Fe(III)·BLM + H_2O_2 effected the formation of 1.68 mM styrene oxide and 1.18 mM phenylacetaldehyde or about six oxygenated product molecules for each Fe(III)·BLM utilized. Once again, the same products were observed when Fe(III)·BLM monothiazole A + H_2O_2 was tested, and the yields were slightly greater than those obtained with BLM itself. Fe(III)·BLM monothiazole B also effected styrene oxygenation in the presence of H_2O_2, producing 1.34 mM styrene oxide and 0.75 mM phenylacetaldehyde.

Also studied was the ability of the BLM monothiazoles to effect the oxidative demethylation of *N*,*N*-dimethylaniline. As shown in Table III, 500 μM Fe(III)·BLM monothiazoles A and B produced *N*-methylaniline in 2.02 and 1.55 mM yields, respectively. This was slightly less than the amount of *N*-methylaniline produced by Fe(III)·BLM + H_2O_2 (2.38 mM) but indicated that both BLM monothiazoles were reasonably efficient catalysts for this oxidative transformation.

DNA Degradation by BLM Monothiazoles. Having established that BLM monothiazoles A and B were both effective in supporting the oxygenation of low molecular weight substrates, each was assayed for its ability to effect the oxidative degradation of DNA. This is illustrated in Figure 1, which employed **1** and **2** as well as deglyco BLM demethyl A_2, for the relaxation (i.e., nicking) of a supercoiled plasmid DNA. When employed at 10 μM concentration in the presence of 250 μM H_2O_2, deglyco BLM demethyl A_2 effected the conversion of form I (supercoiled) DNA to form II (relaxed circular) and form III (linear duplex DNA) (lane 10). Although no form III DNA resulted when 5 μM Fe(III)·deglyco BLM demethyl A_2 was used, quantitative conversion of form I → form II DNA was still observed (lane 9). DNA cleavage was dependent on the presence of Fe(III), H_2O_2, and the BLM congener, since the omission of any of these resulted in the absence of DNA relaxation (e.g., lanes 1–3 and 6–8). In comparison, when assayed at 5 μM concentrations under the same conditions used for deglyco BLM demethyl A_2, neither BLM monothiazole A (**1**) nor BLM monothiazole B (**2**) effected DNA relaxation (lanes 11 and 13), although significant DNA relaxation was obtained at 10 μM concentrations of Fe(III)·**1** and Fe(III)·**2** (lanes 12 and 14). Essentially the same results were obtained for these three BLM congeners when activation was effected in the presence of Fe^{2+} + O_2 + dithiothreitol (DTT) (not shown).

Fe·bleomycin-mediated DNA cleavage has been shown to occur in a sequence-selective fashion, producing damage primarily at $^{5'}$-GT-$^{3'}$ and $^{5'}$-GC-$^{3'}$ sequences.[10] In order to assess the effect of the structural alteration within the bithiazole moiety of BLM on the sequence selectivity of DNA cleavage, a 40-nucleotide DNA oligomer was synthesized, 5′-^{32}P end labeled, and employed as a substrate for the structurally modified BLMs. As shown in Figure 2, admixture of 10 μM Fe(III)·deglyco BLM A_2 + 100 μM H_2O_2 produced DNA damage in a sequence-selective fashion; as expected,[10] the most efficient cleavage occurred at $^{5'}$-GT-$^{3'}$ and $^{5'}$-GC-$^{3'}$ sequences. The same cleavage pattern was obtained when this DNA substrate was treated with Fe(III)·deglyco BLM demethyl A_2 + H_2O_2 or Fe(III)·BLM A_2 + H_2O_2, although the efficiencies of cleavage varied somewhat (data not shown).

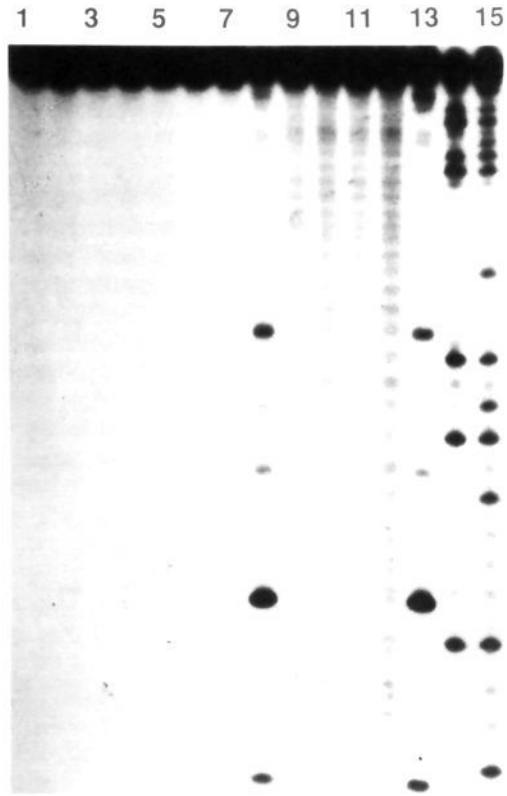


Figure 2. Cleavage of a 5′-^{32}P end-labeled 40 base-pair DNA duplex by BLMs in the presence of Fe(III) + H_2O_2. Lane 1, DNA alone; lane 2, 10 μM Fe(III)·deglyco BLM A_2; lane 3, 100 μM Fe(III)·BLM monothiazole A; lane 4, 100 μM Fe(III)·BLM monothiazole B; lane 5, 1.0 mM H_2O_2; lane 6, 100 μM Fe(III); lane 7, 100 μM Fe(III) + 1.0 mM H_2O_2; lane 8, 10 μM Fe(III)·deglyco BLM A_2 + 100 μM H_2O_2; lane 9, 50 μM Fe(III)·BLM monothiazole A + 1.0 mM H_2O_2; lane 10, 100 μM Fe(III)·BLM monothiazole A + 1.0 mM H_2O_2; lane 11, 50 μM Fe(III)·BLM monothiazole B + 1.0 mM H_2O_2; lane 12, 100 μM Fe(III)·BLM monothiazole B + 1.0 mM H_2O_2; lane 13, 50 μM Fe(II)·deglyco BLM A_2 + O_2; lane 14, Maxam–Gilbert G-lane; lane 15, G + A lane.

Cleavage of the same duplex DNA was studied following aerobic activation of the same BLMs in the presence of Fe^{2+}. As shown in Figure 2 (lane 13), 50 μM Fe(II)·deglyco BLM A_2 + O_2 produced the same pattern of cleavage observed when the same BLM congener was activated in the presence of Fe(III) and H_2O_2 (cf. lanes 8 and 13). The lesser efficiency of DNA cleavage obtained upon aerobic activation in this experiment reflected the absence of a reducing agent; when aerobic activation of Fe(II)·deglyco BLM A_2 or Fe(II)·deglyco BLM demethyl A_2 was carried out in the presence of 100 μM DTT, more efficient cleavage occurred at the same sites apparent in Figure 2. The ability of reducing agents to potentiate DNA cleavage by aerobically activated Fe(II)·BLM is well documented[2,3] and undoubtedly reflects the need for a reducing equivalent to effect Fe(II)·BLM activation under aerobic conditions.

In contrast to the foregoing results, cleavage of the DNA duplex by BLM monothiazoles A and B required higher (50–100 μM) concentrations of the Fe(III) complexes + 1.0 mM H_2O_2 to obtain comparable amounts of DNA degradation. Further, unlike the sequence-selective cleavage observed for BLM and deglyco BLMs, BLM monothiazoles A and B produced cleavage of DNA at essentially every position, with no clear sequence selectivity. In

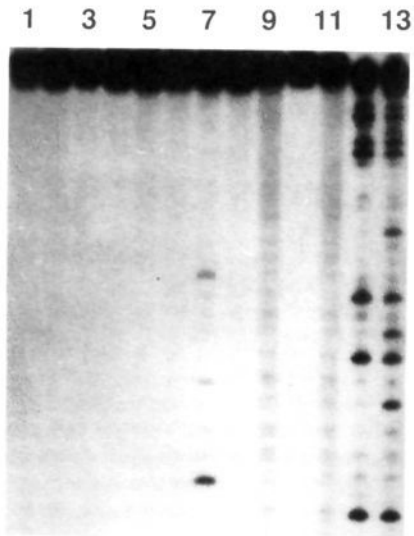


Figure 3. Cleavage of a 5′-^{32}P end labeled 40 base-pair DNA duplex by BLMs in the presence of Fe(II) + O_2 + dithiothreitol (DTT). Lane 1, DNA alone; lane 2, 10 μM deglyco BLM A_2; lane 3, 100 μM BLM monothiazole A; lane 4, 100 μM monothiazole B; lane 5, 100 μM Fe^{2+}; lane 6, 100 μM Fe^{2+} + 1 mM DTT; lane 7, 10 μM Fe(II)·deglyco BLM A_2 + 100 μM DTT; lane 8, 100 μM Fe(II)·BLM monothiazole A; lane 9, 100 μM Fe(II)·BLM monothiazole A + 1 mM DTT; land 10, 100 μM Fe(II)·BLM monothiazole B; lane 11, 100 μM Fe(II)·BLM monothiazole B + 1 mM DTT; lane 12, G-lane; lane 13, G + A-lane.

addition, cleavage at each site produced two sets of products, the faster moving of which had the same mobility as the lesions produced by Fe·deglyco BLM A_2 and presumably had 3′-phosphoroglycolate termini.[3,12] The slower moving bands comigrated with the products of the Maxam–Gilbert sequencing reactions, which are known to be 3′-phosphates.[3,12c,13] The same results were obtained using aerobically activated Fe(II)·BLM monothiazoles A and B. Cleavage was apparent only when these congeners were present at 100 μM concentrations and only when dithiothreitol was also present (1.0 mM concentration) (Figure 3).

In order to assess the nature of DNA damage mediated by BLM monothiazoles A and B at the 5′-termini of the lesions produced, we also employed a 3′-^{32}P end-labeled 140-nt DNA duplex as a substrate for cleavage (Figure 4). Consistent with earlier findings, aerobically or anaerobically activated Fe·deglyco BLM A_2 produced lesions in a sequence selective fashion giving bands that comigrated with the bands in Maxam–Gilbert sequencing lanes; the presence of 5′-phosphate termini were, therefore, inferred. In analogy with the results obtained in Figures 2 and 3, the cleavage produced by Fe(III)·BLM monothiazoles A and B occurred at every position on the DNA duplex and required much higher concentrations to produce the amount of damage obtained with deglyco BLM A_2. Once again, the bands produced by BLM monothiazoles A and B comigrated with the Maxam–Gilbert bands, suggesting that they contained 5′-phosphate termini.

The observation that BLM monothiazoles A and B cleaved DNA without any clear sequence selectivity, and only when employed at high concentrations, suggested that DNA cleavage by these species might take place in a bimolecular fashion due to poor DNA binding by the BLM monothiazoles, rather than by a sequential process involving initial selective DNA binding, followed by cleavage. To test this hypothesis, we employed as a DNA substrate a single-stranded DNA oligonucleotide identical in sequence with the radiolabeled strand of the DNA duplex employed in Figure 2. Consistent with earlier findings that single-strand DNA is not a good substrate for cleavage by BLM, none of the BLM congeners cleaved the 40-nt substrate efficiency. As shown in Figure 5 for 100 μM Fe(III)·deglyco BLM A_2 + 1.0 mM H_2O_2, the use of high concentrations of BLM congener capable of effecting sequence selective cleavage of double-stranded DNA afforded random cleavage of the single-stranded substrate. Cleavage of the single-strand substrate was also effected in a random fashion by the same high concentrations of Fe(III)·BLM monothiazoles sufficient to effect cleavage of double-strand substrate. For all three Fe(III)·BLMs shown in Figure 5, cleavage at each site produced two products (vide supra).

Discussion

Bleomycin-mediated DNA strand scission may be viewed as a two-step process.[3,33] Following initial noncovalent binding of the DNA substrate by an activated metallobleomycin, DNA is degraded by oxidative transformations that involve the C-4′ H of deoxyribose.[3,11–13] That DNA binding by bleomycin requires the bithiazole moiety is suggested by physicochemical studies that have demonstrated interaction of the bithiazole with DNA.[15b–d] Further, an analog of BLM lacking the bithiazole could not degrade DNA, in spite of the fact that it formed an Fe(II) complex which reductively activated O_2 and effected the oxygenation of *cis*-stilbene.[15a]

While it seems clear that the bithiazole moiety of BLM is required for DNA binding, recent studies have suggested somewhat greater complexity in the interaction of bleomycin with DNA. For example, it has been shown that both the N- and C-termini of metallobleomycins participate in DNA unwinding, suggesting strongly that both must be capable of DNA binding.[16c] For a series of deglyco BLM analogs in which the N- and C-terminal binding domains were separated by spacers of varying length, Carter et al. demonstrated that the N-terminal (i.e., metal binding) domain was the principal determinant of sequence selectivity of DNA cleavage.[16d] Nonetheless, Otsuka et al. have recently described a BLM analog containing distamycin moiety in lieu of the bithiazole; this analog effected cleavage of DNA in AT-rich regions, indicating that the sequence selectivity of DNA cleavage was controlled by the distamycin moiety, a known AT binder.[15e]

In order to obtain additional information concerning the nature of BLM–DNA interaction and to define the roles of individual thiazole rings in DNA binding, we have prepared two bleomycin analogs, each of which contains one of the two thiazoles present in bleomycin. In each case, the "missing" thiazole has been replaced by a (methylated) *S*-cysteinyl peptide, the putative biosynthetic precursor of the thiazole.[26,34] To facilitate the syntheses, the compounds designed were analogs of deglyco BLM, i.e., lacking the carbohydrate moiety. Deglyco BLM exhibits DNA binding and cleavage characteristics very similar to those of BLM itself, including production of the same DNA degradation products.[32b,35] The syntheses of BLM monothiazoles A and B (**1** and **2**, respectively) are outlined in Schemes I–V and paralleled closely the strategy employed for the synthesis of deglyco BLM demethyl A_2.[24a]

The initial studies with these synthetic analogs focused on their ability to form Fe chelates and activate oxygen, which would indicate whether the bithiazole moiety has any essential role in metal chelation and oxygen activation by BLM. As outlined in Table I, in the presence of Fe(III) and H_2O_2, BLM monothiazoles A and B were both able to catalyze the hydroxylation of naphthalene, affording 1-naphthol and 2-naphthol in roughly equal amounts. Formation of the same products in similar proportion was also catalyzed by BLM itself under the same experimental conditions; the yield of products formed by BLM was intermediate between those formed by BLM monothiazole A and BLM monothiazole B. Similarly, the same three BLMs effected the conversion of styrene to styrene oxide and phenylacetaldehyde (Table II). Once again, BLM monothiazole A was the most

(33) (a) Van Atta, R. B.; Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 2722. (b) Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 3997.

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efficient catalyst of the three, and BLM monothiazole B was the least efficient. Also investigated was the ability of the BLMs to catalyze the oxidative demethylation of *N,N*-dimethylaniline. As shown in Table III, all three compounds effected the conversion of *N,N*-dimethylaniline to *N*-methylaniline; the yields ranged from 2.38 mM for Fe·BLM to 1.55 mM for BLM monothiazole B.

These results demonstrate clearly that the bithiazole moiety of BLM is not required for the formation of an activated BLM analog. The efficient transfer of oxygen from these activated BLMs to low molecular weight substrates is not altogether surprising since the reactions are presumably all bimolecular in nature, i.e., no prebinding of substrate by the BLMs is required. It may be noted that the specific reactions investigated were chosen in part because each is known to be of a type catalyzed efficiently by cytochrome P-450 and porphyrin model systems functionally related to cytochrome P-450.[9] That the bleomycins carry out chemical transformations analogous to those of cytochrome P-450[9] constitutes one important line of evidence that the reactive species responsible for mediating these transformations must be similar. In conjunction with more direct physicochemical evidence,[6,7,33a] this suggests that activated metallobleomycins, like activated cytochrome P-450,[9] must be high valent metal–oxo species. Given the results outlined in Tables I–III, this analogy can be extended to include BLM monothiazole A and BLM monothiazole B.

Having shown that BLM monothiazoles A and B could form activated Fe complexes capable of oxygenating suitable low molecular weight substrates in a fashion similar to Fe·BLM itself, the ability of these BLM analogs to effect DNA degradation was studied. As shown in Figure 1, 10 μM Fe(III)·BLM monothiazoles A and B + H_2O_2 effected relaxation of a supercoiled plasmid DNA with comparable efficiencies, but each was significantly less efficient than Fe(III)·deglyco BLM demethyl A_2. The same observation was made when these three deglyco BLMs were activated for plasmid DNA relaxation in the presence of Fe(II) and O_2 (data not shown). Further, it has been shown previously that the deglyco BLMs are somewhat less efficient in effecting DNA degradation than the respective bleomycins.[32b,35] Therefore, in contrast to their ability to transfer oxygen to low molecular weight substrates with efficiencies comparable to that of bleomycin (Tables I–III), the BLM monothiazoles were much less efficient than BLM in nicking DNA. Clearly, the difference might logically be thought to be due to differences in efficiency of DNA binding between those analogs having an intact bithiazole or only single bithiazole moieties. The lesser efficiencies of the BLM monothiazoles as reagents for DNA cleavage was also apparent in experiments that employed linear DNA duplexes as substrates (Figures 2–4).

Also determined from the experiments that employed a 5′-^{32}P-end labeled 40-base pair DNA duplex as substrate was the sequence selectivity of DNA cleavage by the BLM monothiazoles as well as the chemical nature of the DNA lesions. Previous studies by Henner et al.[36] and Hertzberg and Dervan[37] showed that the cleavage of 5′-^{32}P-end labeled DNA by hydroxyl radicals generated either by ionizing radiation in water or Fe(II)·EDTA + O_2 + DTT produced two types of DNA lesions in roughly equal amounts. The formation of these lesions was detected by high resolution, denaturing polyacrylamide gel electrophoresis which revealed the presence of two closely spaced bands at each cleavage site. The slower moving of these two bands was found to have the same mobility as bands formed in Maxam–Gilbert sequencing reactions, i.e., having 3′-phosphate termini.[38] The faster moving band was found to contain a 3′-phosphoroglycolate, identified by chemical and enzymatic degradation of the lesion and characterization of the resulting glycolic acid,[37] and also by direct comparison at the nucleotide level with a synthetic nucleoside 3′-phosphoroglycolate.[36]

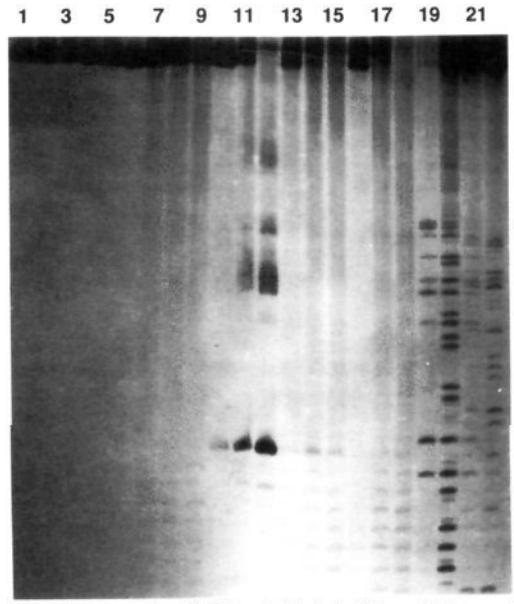

Figure 4. Cleavage of a 3′-^{32}P end labeled 140-nt DNA restriction fragment by BLMs. Lane 1, DNA; lane 2, 10 μM Fe(III)·deglyco BLM A_2; lane 3, 100 μM Fe(III)·BLM monothiazole A; lane 4, 100 μM Fe(III)·BLM monothiazole B; lane 5, 100 μM Fe^{3+}; lane 6, 1 mM H_2O_2; lane 7, 25 μM Fe^{3+} + 1 mM H_2O_2; lane 8, 50 μM Fe^{3+} + 1 mM H_2O_2; lane 9, 100 μM Fe^{3+} + 1 mM H_2O_2; lane 10, 100 μM Fe(II)·deglyco BLM A_2; lane 11, 5 μM Fe(III)·deglyco BLM A_2 + 100 μM H_2O_2; lane 12, 10 μM Fe(III)·deglyco BLM A_2 + 100 μM H_2O_2; lane 13, 25 μM Fe(III)·BLM monothiazole A + 1 mM H_2O_2; lane 14, 50 μM Fe(III)·BLM monothiazole A + 1 mM H_2O_2; lane 15, 100 μM Fe(III)·BLM monothiazole A + 1 mM H_2O_2; lane 16, 25 μM Fe(III)·BLM monothiazole B + 1 mM H_2O_2; lane 17, 50 μM Fe(III)·BLM monothiazole B + 1 mM H_2O_2; lane 18, 100 μM Fe(III)·BLM monothiazole B + 1 mM H_2O_2; lane 19, G-lane; lane 20, G + A-lane; lane 21, C-lane; lane 22, C + T-lane.

Analysis of the cleavage products resulting from BLM treatment of the 5′-^{32}P-end labeled DNA duplex is shown in Figures 2 and 3. In the presence of 100 μM H_2O_2, 10 μM Fe(III)·deglyco BLM A_2 produced cleavage of the DNA substrate at a small number of sites; the same was also true when we used 10 μM Fe(II)·deglyco BLM A_2 in the presence of 100 μM dithiothreitol. Cleavage was most efficient at a $^{5'}$-GC-$^{3'}$ site, consistent with the known sequence specificity of BLM. These bands migrated ahead of the corresponding Maxam–Gilbert sequencing bands, consistent with the production of 3′-phosphoroglycolate termini as the predominant products of BLM-mediated DNA strand scission.[36,37] In sharp contrast, cleavage of the same substrate by the BLM monothiazoles was completely random as regards position of cleavage and resulted in the formation of comparable amounts of products having 3′-phosphate and 3′-phosphoroglycolate termini. The pattern of cleavage was thus quite similar to that produced by hydroxyl radicals (vide supra). All of the BLMs tested gave 5′-phosphates at the end of the 3′-fragments produced (Figure 4).

Although the ability of the activated BLM monothiazoles to transfer oxygen to low molecular weight substrates is suggestive of the intermediacy of a high valent metal–oxo species,[7-9,33] there have been occasional observations consistent with the presence of diffusible oxygen radicals as minor constituents of activated bleomycin preparations.[39] Further, since the chemistry of DNA degradation mediated by the BLM monothiazoles is observed only at high concentrations of these compounds, it seemed plausible that even minor reactive species might be present at concentrations

(35) (a) Oppenheimer, N. J.; Chang, C.; Chang, L.-H.; Ehrenfeld, G.; Rodriguez, L. O.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 1606. (b) Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869.

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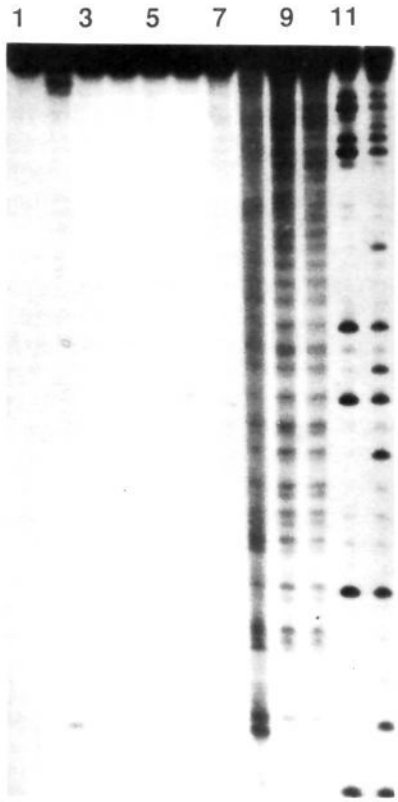

Figure 5. Cleavage of a 5′-^{32}P end-labeled 40-nucleotide single-stranded DNA by BLMs in the presence of Fe(III) + H_2O_2. Lane 1, DNA alone; lane 2, 100 μM Fe(III)·deglyco BLM A_2; lane 3, 100 μM Fe(III)·BLM monothiazole A; lane 4, 100 μM Fe(III)·BLM monothiazole B; lane 5, 100 μM Fe(III); lane 6, 1.0 mM H_2O_2; lane 7, 100 μM Fe(III) + 1.0 mM H_2O_2; lane 8, 100 μM Fe(III)·deglyco BLM A_2 + 1.0 mM H_2O_2; lane 9, 100 μM Fe(III)·BLM monothiazole A + 1.0 mM H_2O_2; lane 10, 100 μM Fe(II)·BLM monothiazole B + 1.0 mM H_2O_2; lane 11, G-lane; lane 12, G + A-lane.

sufficient to produce significant DNA damage. To assess the possible participation of •OH in DNA damage mediated by the BLM monothiazoles, we studied DNA damage in the presence of 5% DMSO, which is known to scavenge •OH at this concentration.[40] The presence of DMSO failed to diminish DNA damage to a significant extent;[39a] further, the use of a sensitive colorimetric assay[41] for methylsulfinic acid, the product formed by reaction of DMSO and •OH, failed to detect any of this product. Thus, in spite of the similarity in DNA cleavage patterns produced by the BLM monothiazoles and by •OH, the accumulated evidence suggests that the BLM monothiazoles do not function via the intermediacy of •OH.

It seems more likely that the lack of sequence specificity in DNA cleavage mediated by BLM monothiazoles A and B simply results from poor DNA binding. Consistent with this interpretation was the observation that both of these congeners gave random cleavage of a single-stranded DNA at the same high concentration (Figure 5), suggesting lack of well ordered binding to the oligomer prior to cleavage. In fact, deglyco BLM A_2 also cleaved the single-stranded substrate in a random fashion, and only at high concentration, consistent with the earlier finding that single-stranded DNA is a poor substrate for BLM.[22]

It may be noted that the formation of oligonucleotide 3′-phosphates and 3′-phosphoroglycolates in comparable amounts by •OH has been suggested to result from reaction both at C-1′ and C-4′ of deoxyribose. While the same two products are formed by Fe·BLM as a consequence of attack at C-4′,[3,11-13] oligonucleotide 3′-phosphoroglycolates predominate under ambient conditions,[3,11-13] and 3′-phosphates form efficiently from alkali labile lesions only if subsequent chemical workup of the reaction mixtures is optimized to effect conversion to oligonucleotide 3′-phosphates.[13e] It is conceivable that the putative oligonucleotide 3′-phosphates observed in the reaction employing BLM monothiazoles A and B actually do result from attack at the C-1′ position of deoxyribose, as a consequence of lack of DNA binding that would otherwise orient the BLM monothiazoles in a fashion conducive to specific abstraction of the deoxyribose C-4′ H. If this were true, it would suggest that appropriate alteration of the structure of BLM molecule, or its oligonucleotide substrate, could result in oxidative attack at C-1′ of deoxyribose by BLM.

Experimental Section

General Methods. Elemental analyses were carried out by Chemalytics, Inc. or by Atlantic Microlab, Inc. Melting points were taken on a Thomas Hoover apparatus and are not corrected. Optical rotations were determined on a Perkin-Elmer Model 141 polarimeter. ^{1}H NMR spectra were recorded on a Varian EM-390, Nicolet-NT-360, or a GE-300 MHz spectrometer. Chemical shifts are referenced to $CHCl_3$ at 7.26 ppm, CH_3OH at 3.30 ppm, or HOD at 4.80 ppm. The following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet, td, triplet of doublets; qn, quintet; br, broad, dd; doublets of doublets. Chemical ionization mass spectra (CIMS) were recorded on a Perkin-Elmer Hitachi RMU-6 or Varian MAT-44 mass spectrometer using isobutane. High resolution peak matching was carried out on Finnigan MAT 8230 and Kratos IH mass spectrometers. Liquid secondary ion mass spectra (LSIMS) were recorded on a Quadropole-FT mass spectrometer. Gas chromatography was performed on a Varian Model 3400 gas chromatograph with a J & W Scientific DB-1701, 15 m capillary column with helium as the carrier gas, and flame ionization detection. Plasmid pBR322 supercoiled DNA was obtained from Bethesda Research Laboratories, and [γ-^{32}P]ATP was obtained from ICN Radiochemicals. Deglycobleomycin A_2 was obtained as described.[24a,42]

***N*-(*tert*-Butoxycarbonyl)-*S*-methyl-(*S*)-cysteine [3-(Methylthio)propyl]amide (4).** To a suspension of 14.1 g (0.06 mol) of *N*-(*tert*-butoxycarbonyl)-*S*-methyl-(*S*)-cysteine (**3**)[25] and 8.1 g (0.06 mol) of 1-hydroxybenzotriazole in 600 mL of dry CH_3CN at 0 °C was added 12.4 g (0.06 mol) of *N*,*N*′-dicyclohexylcarbodiimide and 6.94 g (66 mmol) of [3-(methylthio)propyl]amine. The reaction mixture was stirred at room temperature for 3 days, and the resulting precipitate was filtered. The filtrate was diluted with 1 L of ethyl acetate and then washed successively with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and water. The organic layer was dried ($MgSO_4$) and concentrated to afford an oil. Trituration with hexane provided *N*-(*tert*-butoxycarbonyl)-*S*-methyl-(*S*)-cysteine [3-(methylthio)propyl]amide (**4**) as a solid suitable for further synthetic transformation: yield 17.4 g (90%). Crystallization from ether–hexane provided **4** as colorless needles: mp 54–55 °C; $[\alpha]^{25}_D$ −26.1° (*c* 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9), 1.85 (qn, 2, *J* = 6 Hz), 2.02 (s, 3), 2.10 (s, 3), 2.51 (t, 2, *J* = 6 Hz), 2.79–2.86 (m, 2), 3.37 (q, 2, *J* = 6 Hz), 4.17 (q, 1, *J* = 6 Hz), 5.31 (d, 1, *J* = 6 Hz, ex D_2O) and 6.55 (m, 1, ex D_2O); mass spectrum (chemical ionization), *m*/*z* 323 (M + 1)$^+$ and 267. Anal. Calcd for $C_{13}H_{26}N_2O_3S_2$: C, 48.42; H, 8.12; N, 8.68. Found: C, 48.52; H, 8.31; N, 8.66.

***S*-Methyl-(*S*)-cysteine [3-(Methylthio)propyl]amide (5).** To a solution of 6.45 g (0.02 mol) of cysteineamide **4** in 24 mL of CH_2Cl_2 at 0 °C was added 24 mL of a 4.5 M solution of hydrogen chloride in dioxane. The combined solution was stirred at 0 °C for 5 min and then at 25 °C for 1 h. The reaction mixture was concentrated under diminished pressure, and the residue was washed with ether (3 × 50 mL) and then dissolved in 80 mL of 1:1 CH_3OH–H_2O. The solution was neutralized (Amberlite IRA-45 resin) and then concentrated to dryness under diminished pressure, and the residue was triturated with ether (2 × 200 mL). The combined ether extract was concentrated to afford an oily residue, which was purified by chromatography on a silica gel column (30 × 4.5 cm). Elution with 2% CH_3OH in CH_2Cl_2 gave *S*-methylcysteine [3-(methylthio)propyl]amide (**5**) as a yellow syrup: yield 3.56 g (80%); $[\alpha]^{25}_D$ +5.3° (*c* 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.81 (m, 4), 2.15 (s, 6), 2.41–2.76 (m, 4), 3.01 (dd, 1, *J* = 14, 4 Hz), 3.30 (q, 2, *J* = 6 Hz) and 7.59 (br s, 1, ex D_2O); mass spectrum (chemical ionization), *m*/*z* 222 (M^+) and 205; mass spectrum (electron impact), *m*/*z* 222.085 ($C_8H_{18}N_2OS_2$ requires 222.085).

2-[(*N*-(*tert*-Butoxycarbonyl)amino)ethyl]thiazole-4-carboxylic Acid (6). A solution containing 3.92 g (13 mmol) of ethyl 2-[(*N*-benzoylamino)ethyl]thiazole-4-carboxylate[26] in 200 mL of 6 M HCl was heated

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(41) Steiner, M. G.; Babbs, C. F. *Arch. Biochem. Biophys.* **1990**, *278*, 478.
(42) Muraoka, Y.; Suzuki, M.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Takita, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 353.

at reflux with stirring for 24 h. The cooled reaction mixture was filtered to remove benzoic acid, and the filtrate was concentrated under diminished pressure. Crystallization of the residue from EtOH–ether provided 2-(aminoethyl)thiazole-4-carboxylic acid hydrochloride as colorless needles: yield 2.67 g (99%); mp 221–222 °C (dec); ^{1}H NMR (D_2O) δ 3.45 (m, 4) and 8.33 (s, 1); mass spectrum (chemical ionization) m/z 173 $(M + 1)^+$ and 156. Anal. Calcd for $C_6H_9ClN_2O_2S$: C, 34.54; H, 4.34; N, 13.43. Found: C, 34.63; H, 4.41; N, 13.26.

To a solution of 2.65 g (12.7 mmol) of 2-(aminoethyl)thiazole-4-carboxylic acid hydrochloride in 17 mL of water was added a mixture of 17 mL of DMF and 17 mL of Et_3N. The combined solution was cooled in an ice bath and treated with 4.44 g (21 mmol) of di-*tert*-butyldicarbonate.[43] The resulting solution was stirred at room temperature for 24 h, diluted with 170 mL of water, and extracted with hexane (2 × 170 mL). The aqueous layer was acidified to pH 4 with 2 M HCl and extracted repeatedly with ethyl acetate (12 × 200 mL). The combined organic extract was dried ($MgSO_4$) and concentrated under diminished pressure to provide a colorless oil. Crystallization of the product from 10% MeOH in H_2O gave 2-[(*N*-(*tert*-butoxycarbonyl)amino)ethyl]thiazole-4-carboxylic acid (**6**) as colorless needles: yield 2.51 g (72%); mp 82–83 °C; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9), 3.20 (t, 2, J = 6 Hz), 3.55 (q, 2, J = 6 Hz), 4.90 (br s, 1, ex D_2O), and 8.11 (s, 1); mass spectrum (chemical ionization), m/z 273 $(M + 1)^+$, 217 and 127; mass spectrum (electron impact), m/z 272.083 ($C_{11}H_{16}N_2O_4S$ requires 272.083).

***N*-*t*-Boc Thiazolyl *S*-Methylcysteine Amide 7.** A solution containing 1.66 g (6.1 mmol) of thiazolecarboxylic acid **6** and 0.83 g (6.1 mmol) of 1-hydroxybenzotriazole in 55 mL of dry CH_3CN was cooled in an ice bath and treated under Ar with 1.26 g (6.1 mmol) of *N*,*N*′-dicyclohexylcarbodiimide and a solution containing 1.36 g (6.1 mmol) of *S*-methylcysteine [3-(methylthio)propyl]amide (**5**) in 20 mL of dry CH_3CN. The combined solution was stirred at room temperature for 2 days. The precipitate that had formed was filtered, and the filtrate was diluted with 250 mL of ethyl acetate and washed successively with 5% aqueous HCl and saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford an oily residue. The residue was dissolved in 200 mL of ether and filtered to remove insoluble material, and the filtrate was concentrated under diminished pressure. The product was purified by chromatography on a silica gel column (45 × 4.5 cm). Elution with 2% CH_3OH in CH_2Cl_2 gave compound **7** as a colorless solid: yield 2.47 g (85%); $[\alpha]^{25}_D$ −13.1° (c 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9), 1.82 (qn, 2, J = 7 Hz), 2.06 (s, 3), 2.20 (s, 3), 2.54 (t, 2, J = 7 Hz), 3.00 (m, 2), 3.20 (t, 2, J = 7 Hz), 3.38–3.70 (m, 4), 4.66 (q, 1, J = 7 Hz), 4.85 (m, 1, ex D_2O), 6.75 (br s, 1, ex D_2O), 7.99 (s, 1) and 8.03 (d, 1, J = 7 Hz, ex D_2O); mass spectrum (chemical ionization), m/e 476 (M^+) and 402; mass spectrum (electron impact), m/z 476.156 ($C_{19}H_{32}N_4O_4S_3$ requires 476.158).

Thiazolyl *S*-Methylcysteine Amide 8. *N*-*t*-Boc thiazolyl-*S*-methylcysteine amide **7** (238 mg, 0.50 mmol) was dissolved in a cooled (0 °C) solution of anhydrous dioxane containing 4.5 M hydrogen chloride. The resulting solution was stirred at 0 °C for 3 min and then at 25 °C for 20 min. The solution was concentrated under diminished pressure, and the residue was triturated with three 5-mL portions of ether. The residue was dissolved in 5 mL of 50% aqueous CH_3OH and neutralized with Amberlite IRA-45 resin. Concentration of the solution provided an oil, which was purified by chromatography on silica gel (20 × 2.5 cm); elution was with 8% CH_3OH in CH_2Cl_2. Thiazolyl *S*-methylcysteine amide **8** was isolated as an amorphous solid: yield 155 mg (82%); $[\alpha]^{25}_D$ −12.3° (c 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.79 (qn, 2), 2.02 (s, 3), 2.15 (s, 3), 2.50 (t, 2, J = 7 Hz), 2.95–3.45 (m, 10), 4.63 (q, 1, J = 7 Hz), 6.81 (br t, 1, ex D_2O), 7.95 (s, 1), and 8.20 (d, 1, J = 7 Hz, ex D_2O); mass spectrum (chemical ionization), m/z 377 $(M + 1)^+$ and 172; mass spectrum (electron impact), m/z 376.108 ($C_{14}H_{24}N_4O_2S_3$ requires 376.106).

[((*N*-NPS-threonyl)amino)ethyl]thiazolyl *S*-Methylcysteine Amide 10. Method A. To a solution containing 106 mg (0.28 mmol) of thiazole derivative **8** in 15 mL of dry CH_2Cl_2 was added 122 mg (0.28 mmol) of 2,4-dinitrophenyl *N*-(*o*-nitrophenylsulfenyl)threonine (**9**)[27] and 10 mg of 4-(dimethylamino)pyridine. The resulting solution was stirred at 25 °C for 24 h under argon. The reaction mixture was treated with an additional 70 mg (0.16 mmol) of NPS threonine derivative **9**, and stirring was continued for an additional 3 h. The reaction mixture was diluted with 50 mL of CH_2Cl_2, and the solution was washed successively with aqueous $NaHCO_3$ and brine. The dried ($MgSO_4$) organic phase was concentrated under diminished pressure, and the residue was purified by repeated chromatography on silica gel (20 × 2.5 cm); elution with 2% CH_3OH in CH_2Cl_2 provided threonylthiazole derivative **10** as a yellow solid: yield 146 mg (82%); $[\alpha]^{25}_D$ −42° (c 1.0, CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.26 (d, 3, J = 7 Hz), 1.83 (qn, 2, J = 7 Hz), 2.07 (s, 3), 2.18 (s, 3), 2.53 (t, 2, J = 7 Hz), 2.85 (d, 2, J = 7 Hz), 3.22 (m, 2), 3.40 (m, 2), 3.60 (t, 1, J = 3.5 Hz), 3.75 (m, 1), 3.87 (m, 1), 4.33 (m, 1), 4.36 (d, 1, J = 3 Hz, ex D_2O), 4.70 (q, 1, J = 7.5 Hz), 5.31 (d, 1, J = 3.5 Hz, ex D_2O), 6.72 (br t, 1, ex D_2O), 7.23 (t, 1), 7.62 (t, 1), 7.85 (d, 1), 8.01 (s, 1), 8.13 (d, 1, J = 9 Hz, ex D_2O), and 8.27 (d, 2, J = 8 Hz); mass spectrum (chemical ionization), m/z 631 $(M + 1)^+$, 478, 225 and 156. Anal. Calcd for $C_{24}H_{34}N_6O_6S_4 \cdot 0.5H_2O$: C, 45.05; H, 5.51; N, 13.13. Found: C, 45.24; H, 5.48; N, 13.10.

Method B. To a cooled solution of 238 mg (0.50 mmol) of *N*-*t*-Boc thiazolyl *S*-methylcysteine amide **7** in 1 mL of anhydrous CH_2Cl_2 was added 0.7 mL of cold dioxane containing 4.5 M hydrogen chloride. The combined solution was stirred at 0 °C for 3 min and then at 25 °C for 1.5 h. The reaction mixture was cooled, which led to the separation of a solid precipitate, the latter of which was isolated by decantation of the solvent. The residue was dissolved in 10 mL of 50% aqueous CH_3OH, and the resulting solution was neutralized (Amberlite IRA-45 resin). The methanolic solution was concentrated to provide an oily residue, which was applied to a small silica gel column (5 × 2.5 cm). Elution with 15% CH_3OH in CH_2Cl_2 provided thiazolyl *S*-methylcysteine amide **8** as a yellow solid: yield 0.177 g. The crude, deblocked thiazole was dissolved in 25 mL of dry CH_2Cl_2 and treated with 190 mg (0.47 mmol) of 2,4-dinitrophenyl *N*-(*o*-nitrophenylsulfenyl)threonine (**9**)[27] and 16.7 mg of 4-(dimethylamino)pyridine. The resulting solution was stirred vigorously at 25 °C under argon for 24 h. The reaction mixture was treated with an additional 117 mg (0.27 mmol) of NPS threonine derivative **9**, and stirring was continued at 25 °C for an additional 3 h. The reaction mixture was diluted with CH_2Cl_2 and worked up in the same fashion as indicated under method A. Threonylthiazole derivative **10** was obtained as a yellow solid: yield 231 mg (73%). This material was identical with that obtained by method A, as judged by optical rotation, behavior on silica gel TLC, and ^{1}H NMR spectroscopy.

[(Threonylamino)ethyl]thiazolyl *S*-Methylcysteine Amide 11. To a cold (0–5 °C) solution of NPS threonyl thiazole derivative **10** (126 mg, 0.20 mmol) in 3 mL of dry chloroform was added 41 μL (0.5 mmol) of cold, concentrated HCl under argon. The reaction mixture was stirred at 25 °C for 4 h, then concentrated to 2 mL, and cooled to −78 °C for 30 min to effect precipitation of the product. The solvent was removed by decantation and the residue was washed with two 3-mL portions of cold ether. The residue was purified by chromatography on a column of silica gel (12 × 1 cm). Elution with 10% CH_3OH in CH_2Cl_2 provided [(threonylamino)ethyl]thiazolyl *S*-methylcysteine amide **11** as a colorless foam: yield 93 mg (95%); $[\alpha]^{25}_D$ −22° (c 0.35, CH_3OH); ^{1}H NMR (D_2O) δ 1.07 (d, 3, J = 7 Hz), 1.80 (qn, 2, J = 7 Hz), 2.03 (s, 3), 2.13 (s, 3), 2.52 (t, 2, J = 7 Hz), 2.95 (dd, 1, J = 14, 7 Hz), 3.06 (dd, 1, J = 14, 7 Hz), 3.25–3.40 (m, 4), 3.53 (d, 1, J = 6 Hz), 3.62 (qn, 1, J = 6 Hz), 3.77 (qn, 1, J = 6 Hz), 3.95 (qn, 1, J = 6 Hz), 4.66 (dd, 1, J = 8, 6 Hz) and 8.15 (s, 1); mass spectrum (chemical ionization), m/z 478 $(M + 1)^+$; mass spectrum (electron impact), m/z 477.150 ($C_{18}H_{31}N_5O_4S_3$ requires 477.153).

N^{α},N^{im}-Bis(*tert*-butoxycarbonyl)-(*S*)-*erythro*-β-hydroxyhistidine (12). To a cold (0–5 °C), stirred suspension of (*S*)-*erythro*-β-hydroxyhistidine dihydrochloride[44] (244 mg, 1.0 mmol) in 0.45 mL of water and 2.4 mL of DMF was added successively 873 mg (4.0 mmol) of di-*tert*-butyldicarbonate and 545 mg (2.5 mmol) of triethylamine. The resulting solution was stirred at 0–5 °C for 1 h and then at 25 °C for 3.5 h. The reaction mixture was cooled, diluted with 30 mL of cold water, and then extracted with two 80-mL portions of ether. The combined ether layer was back-extracted with two 5-mL portions of cold water, and all of the aqueous extracts were combined. The cold aqueous layer was adjusted to pH 3 with cold 1 M citric acid (~10 mL). The acidified solution was extracted with three 100-mL portions of cold CH_2Cl_2. The combined organic extract was washed with cold water (three 3-mL portions), dried over $MgSO_4$, and concentrated under diminished pressure at room temperature. The residue was triturated with three 3-mL portions of hexane and then treated with 3 mL of benzene, which was evaporated from the residue in vacuo. N^{α},N^{im}-bis(*tert*-butoxycarbonyl)-(*S*)-*erythro*-β-hydroxyhistidine (**12**) was obtained as a colorless solid: yield 334 mg (90%); $[\alpha]^{25}_D$ +82.6° (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9), 1.60 (s, 9), 4.65 (br s, 1), 5.33 (br s, 1), 5.74 (d, 1), 7.45 (s, 1) and 8.16 (s, 1); chemical ionization, m/z 372 $(M + 1)^+$ and 328; mass spectrum (electron impact), m/z 309.167 $(M - CO_2, H_2O)^+$ ($C_{15}H_{23}N_3O_4$ requires 309.167).

Benzyl (2*S*,3*S*,4*R*)-4-Amino-3-hydroxy-2-methylvalerate (13). A stirred solution containing 147 mg (1.0 mmol) of (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvaleric acid[27] in 9.5 mL of benzyl alcohol was cooled to 0 °C and saturated with dry hydrogen chloride for 1 h. The solution

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(44) Hecht, S. M.; Rupprecht, K. M.; Jacobs, P. M. *J. Am. Chem. Soc.* **1979**, *101*, 3982.

was allowed to warm to room temperature and was stirred at 25 °C for 15 h. The reaction mixture was diluted with 25 mL of water and washed with two 70-mL portions of ether. The combined ether layer was extracted with two 5-mL portions of water, and all of the aqueous extracts were combined and concentrated to dryness. The residue was purified by chromatography on a silica gel column (12 × 1 cm). Elution with 5% CH_3OH in CH_2Cl_2 gave benzyl (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvalerate hydrochloride as a semisolid: yield 218 mg (80%); ^1H NMR (D_2O) δ 1.16 (d, 3, J = 7 Hz), 1.25 (d, 3, J = 7 Hz), 2.66 (dd, 1, J = 9.5, 7 Hz), 3.25 (td, 1, J = 7, 2.5 Hz), 3.85 (dd, 1, J = 9.5, 2.5 Hz), 5.16, 5.17 (2 s, 2), and 7.43 (br s, 5); mass spectrum (chemical ionization), m/z 238 $(M + 1)^+$; mass spectrum (electron impact), m/z 237.136 ($C_{13}H_{19}NO_3$ requires 237.135).

Benzyl (2*S*,3*S*,4*R*)-4-[(N^{α},N^{im}-Bis(*tert*-butoxycarbonyl)-(*S*)-*erythro*-β-hydroxyhistidyl)amino]-3-hydroxy-2-methylvalerate (14). A solution containing 88 mg (0.23 mmol) of Bis(*t*-Boc)-β-hydroxyhistidine (**12**) and 59 mg (0.44 mmol) of 1-hydroxybenzotriazole in 2 mL of dry CH_2Cl_2 was cooled to 0 °C and treated with 90 mg (0.44 mmol) of *N*,*N*'-dicyclohexycarbodiimide. The reaction mixture was then treated successively with a solution containing 50 mg (0.18 mmol) of benzyl valerate **13** in 2 mL of dry CH_2Cl_2 and with 95 μL (0.55 mmol) of *N*,*N*-(diisopropylethyl)amine. The combined solution was stirred at 0 °C for 1 h and then at 25 °C for 24 h. The solvent was concentrated under diminished pressure, and the residue was treated with 4 mL of ethyl acetate. The insoluble material was removed by filtration, and the filtrate was concentrated under diminished pressure. The residue was purified by flash chromatography[45] on a silica gel column (15 × 3 cm). Elution with 25% acetone in CH_2Cl_2 provided dipeptide analog **14**, which was isolated as a colorless solid: yield 35 mg (32%); $[\alpha]^{25}_D$ +23.2° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.03 (d, 3, J = 7 Hz), 1.21 (d, 3, J = 7 Hz), 1.39 (s, 9), 1.58 (s, 9), 2.50 (m, 1), 3.57 (br s, 1), 3.77 (m, 1), 3.93 (m, 1), 4.44 (br s, 1), 4.49 (dd, 1), 4.96 (br s, 1), 5.11 (s, 2), 5.88 (d, 1), 6.70 (m, 1), 7.34 (br s, 5), 7.37 (s, 1) and 8.03 (s, 1); mass spectrum (LSIMS), m/z 613 $(M + Na)^+$, 591 $(M + H)^+$ and 491.

(2*S*,3*S*,4*R*)-4-[(N^{α},N^{im}-Bis(*tert*-butoxycarbonyl)-(*S*)-*erythro*-β-hydroxyhistidinyl)amino]-3-hydroxy-2-methylvaleric Acid (15). A solution of Bis(*t*-Boc) dipeptide benzyl ester **14** (35 mg, 59 μmol) and 25 mg of 10% palladium-on-carbon in 2.5 mL of ethanol was stirred under 1 atm of H_2 at 25 °C for 4 h. The catalyst was removed by filtration, and the filtrate was concentrated under diminished pressure. Following evaporation of two 3-mL portions of benzene from the residue, Bis(*t*-Boc) dipeptide **15** was isolated as a colorless solid: yield 29 mg (98%); $[\alpha]^{25}_D$ +21.0° (*c* 1, CH_3OH); ^1H NMR (CD_3OD) δ 1.03 (d, 3, J = 7 Hz), 1.10 (d, 3, J = 7 Hz), 1.41 (s, 9), 1.60 (s, 9), 2.27 (m, 1), 3.74 (dd, 1, J = 7, 4 Hz), 3.90 (m, 1), 4.36 (d, 1, J = 7 Hz), 4.89 (d, 1, J = 7 Hz), 7.37 (s, 1) and 8.11 (s, 1); mass spectrum (LSIMS), m/z 523 $(M + Na)^+$, 501 $(M + H)^+$ and 401; mass spectrum (FABMS), m/z 501.258 ($C_{22}H_{37}N_4O_9$ requires 501.256).

This material was dried (P_2O_5, 12 h) and then used directly for condensation with threonine derivative **11**.

Condensation of [(Threonylamino)ethyl]thiazolyl *S*-Methylcysteine Amide 11 with Bis(*t*-Boc) β-Hydroxyhistidinyl Valeric Acid 15. To a cold (0–5 °C), stirred solution containing 38 mg (76 μmol) of bis(*t*-Boc) dipeptide **15**, 38 mg (280 μmol) of 1-hydroxybenzotriazole, and 91 mg (440 μmol) of *N*,*N*'-dicyclohexylcarbodiimide in 2 mL of dry DMF under argon was added dropwise a cold solution of 47 mg (91 μmol) of threonine derivative **11** in 2 mL of dry DMF. The combined solution was treated with 88 μL (64 mg, 630 μmol) of triethylamine, and the reaction mixture was stirred under argon at 0 °C for 3 h and then at 25 °C for 37 h. The solution was concentrated under diminished pressure at 25 °C, and the residue was dissolved in 100 mL of CH_2Cl_2 and washed with 2 mL of water. The organic phase was dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by flash chromatography[45] on a silica gel column (12 × 2.5 cm, elution was with 10:1 CH_2Cl_2–EtOH) and by preparative silica gel TLC (development was with 5:1 ethyl acetate–CH_3OH). Bis(*t*-Boc) peptide **16** was isolated as a colorless glass: yield 27 mg (37%); silica gel TLC R_f 0.52 (5:1 ethyl acetate–CH_3OH); $[\alpha]^{25}_D$ +1.5° (*c* 1.0, CH_3OH); ^1H NMR (CD_3OD) δ 1.01 (d, 3, J = 7 Hz), 1.11 (d, 3, J = 7 Hz), 1.14 (d, 3, J = 7 Hz), 1.36 (s, 9), 1.60 (s, 9), 1.79 (qn, 2, J = 7 Hz), 2.03 (s, 3), 2.14 (s, 3), 2.50 (t, 2, J = 7), 2.55 (m, 1), 2.91 (dd, 1, J = 13, 7 Hz), 2.99 (dd, 1, J = 13, 7 Hz), 3.61–3.67 (m, 3), 3.85 (qn, 1, J = 6 Hz), 4.14 (m, 1), 4.27 (d, 1, J = 5 Hz), 4.35 (d, 1, J = 6 Hz), 7.38 (s, 1), 8.10 (s, 1) and 8.13 (s, 1); mass spectrum (LSIMS), m/z 983 $(M + Na)^+$, 961 $(M + H)^+$, 860 and 760.

Peptide 17. To a cold (0–5 °C), stirred solution of Bis(*t*-Boc) peptide **16** (25 mg, 26 μmol) in 220 μL of dimethyl sulfide under argon was added 0.45 mL of trifluoroacetic acid. The reaction mixture was stirred under argon at 0–5 °C for 3 h. The solution was concentrated under diminished pressure, and the residue was treated with 1 mL of water and again concentrated under diminished pressure. The residue was applied to an Amberlite XAD-2 column (19 × 2 cm), which was washed successively with water (200 mL) and methanol (100 mL). The methanol eluate was concentrated to afford a syrup from which several 2-mL portions of benzene were evaporated. The residue was dried over P_2O_5, affording peptide **17** as a colorless solid: yield 23 mg (90%); silica gel TLC R_f 0.42 (4:1:1 1-butanol–HOAc–H_2O); $[\alpha]^{25}_D$ −7.2° (*c* 0.9, CH_3OH); ^1H NMR (D_2O) δ 0.97 (d, 3, J = 7 Hz), 1.07 (d, 3, J = 7 Hz), 1.15 (d, 3, J = 7 Hz), 1.80 (qn, 2, J = 7 Hz), 2.04 (s, 3), 2.13 (s, 3), 2.47–2.55 (m, 3), 2.97 (dd, 1, J = 13, 7 Hz), 3.05 (dd, 1, J = 13, 7 Hz), 3.24–3.38 (m, 4), 3.60–3.70 (m, 3), 3.77 (m, 1), 4.04 (qn, 1, J = 4 Hz), 4.20 (d, 1, J = 4 Hz), 4.26 (d, 1, J = 5 Hz), 4.65 (dd, 1, J = 6, 4 Hz), 5.17 (d, 1, J = 5 Hz), 7.23 (s, 1), 7.89 (s, 1) and 8.10 (s, 1); mass spectrum (LSIMS), m/z 782 $(M + Na)^+$, 760 $(M + H)^+$ and 607; mass spectrum (FABMS), m/z 760.293 ($C_{30}H_{50}N_9O_8S_3$ requires 760.294).

Condensation of Peptide 17 with Pyrimidoblamic Acid (18). To a cold (0–5 °C), stirred solution containing 8 mg (19 μmol) of pyrimidoblamic acid, 21 mg (21 μmol) of peptide **17** and 16 mg (37 μmol) of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP reagent[29]) in 1 mL of dry DMF under argon was added 65 μL (48 mg, 371 μmol) of *N*,*N*-diisopropylethylamine. The reaction mixture was stirred under argon at 0–5 °C for 10 min and then at 25 °C for 12 h. The solution was concentrated at 25 °C under diminished pressure, and the residue was dissolved in 5 mL of water and extracted with two 5-mL portions of ethyl acetate. The aqueous phase was then extracted with three 10-mL portions of *n*-butanol, and the combined *n*-butanol layer was washed with 3 mL of water. The *n*-butanol extract was concentrated under diminished pressure to provide a syrup. This residue was applied to an Amberlite XAD-2 column (20 × 2 cm), which was washed successively with 80 mL of water and 100 mL of methanol. The methanol fractions containing the desired product were combined and concentrated, providing crude Boc bleomycin monothiazole A (**19**) as a syrup: yield 22 mg; silica gel TLC R_f 0.24 (4:1:1 *n*-butanol–acetic acid–water).

This compound was used in the next step without further purification.

Bleomycin Monothiazole A (1). A cold (0–5 °C) solution containing 22 mg of crude Boc bleomycin monothiazole A (**19**) in 0.5 mL of trifluoroacetic acid and 0.3 mL of dimethyl sulfide was stirred under argon for 6 h. The reaction mixture was diluted with 5 mL of water and extracted twice with 5-mL portions of ethyl acetate. The combined ethyl acetate extract was back-extracted with 4 mL of water, and all of the aqueous extracts were combined and concentrated to 2 mL under diminished pressure. The concentrated aqueous solution was applied to an Amberlite XAD-2 column (20 × 2 cm) which was washed with water until the eluate was neutral (~120 mL) and then with 100 mL of methanol. The methanol fractions containing the desired product were combined and concentrated under diminished pressure, affording 19 mg of a solid product. The solid and 15 mg of $CuSO_4 \cdot 5H_2O$ were dissolved in 1 mL of 0.05 M Na citrate buffer, pH 4.5, and the resulting solution was applied to a column of CM Sephadex C-25 (36 × 1.5 cm). The column was washed with 0.05 M citrate buffer, pH 4.5, containing a linear gradient of NaCl (0 → 2 M; total volume 2 L) at a flow rate of 70 mL/h. The fractions containing the desired product, which were blue in color, were treated with 600 mg of EDTA·2Na·$2H_2O$, and the resulting solution was maintained at 25 °C for 1 h. The solution was then applied to an Amberlite XAD-2 column (19 × 2 cm), which was washed successively with 50 mL of 5% aqueous NaCl solution, 300 mL of water, and 100 mL of methanol. The methanol fractions containing the desired product (as judged by UV spectroscopy) were combined and concentrated under diminished pressure at 25 °C. Bleomycin monothiazole A (**1**) was obtained as a colorless glass: yield 5 mg (25% from **17**); silica gel TLC R_f 0.40 (1:1:1 1-butanol–HOAc–H_2O); ^1H NMR (D_2O) δ 1.07 (d, 6, J = 7 Hz), 1.13 (d, 3, J = 7 Hz), 1.77 (qn, 2), 1.88 (s, 3), 2.02 (s, 3), 2.11 (s, 3), 2.47–2.57 (m, 3), 2.65 (m, 1), 2.91–3.07 (m, 3), 3.22–3.37 (m, 5), 3.61–3.73 (m, 4), 3.85 (q, 1), 3.95 (dd, 1), 4.05 (m, 2), 4.21 (d, 1), 4.65 (m, 1), 4.87 (d, 1, J = 5 Hz), 5.09 (d, 1, J = 5 Hz), 7.21 (s, 1), 7.88 (s, 1), and 8.10 (s, 1); mass spectrum (LSIMS), m/z 1089 $(M + Na)^+$ and 1067 $(M + H)^+$.

***N*-(*tert*-Butoxycarbonyl)-*S*-methyl-(*S*)-cysteine Methyl Ester (20).** A solution containing 19.2 g (0.08 mol) of *N*-(*tert*-butoxycarbonyl)-*S*-methyl-(*S*)-cysteine (**3**)[25] in 120 mL of ether was cooled to −10 °C. To this solution was added ~10 mL of a solution of diazomethane in ether (prepared from 26 g (0.20 mol) of *N*-methyl-*N*-nitrosourea and 50% aqueous KOH). The combined solution was maintained at 25 °C for 5 h, and the reaction mixture was then diluted with 300 mL of ethyl acetate. The combined organic solution was washed with water, dried ($MgSO_4$), and concentrated under diminished pressure. The residue was dissolved in ethyl acetate and washed through a small silica gel column (13 × 5 cm). The ethyl acetate eluate was concentrated to afford *N*-(*tert*-butoxycarbonyl)-*S*-methyl-(*S*)-cysteine methyl ester (**20**)[30] as a colorless syrup: yield 20.1 g (98%); $[\alpha]^{25}_D$ −27.6° (*c* 1.0, CH_3OH); ^1H

NMR ($CDCl_3$) δ 1.44 (s, 9), 2.11 (s, 3), 2.93 (m, 2), 3.77 (s, 3), 4.55 (m, 1) and 5.35 (m, 1).

Methyl (1′*S*,4*S*)-2-[1′-((*tert*-Butoxycarbonyl)amino)-2′-(methylthio)ethyl]thiazolidine-4-carboxylate (22). A stirred solution containing 4.33 g (17.3 mmol) of Boc cysteine derivative **20** in 85 mL of dry THF was cooled to −78 °C and treated dropwise with 38 mmol of diisobutylaluminum hydride (38 mL of a 1 M solution) in hexane over a period of 1.5 h under argon. The combined solution was stirred for an additional 30 min at −78 °C, and 20 mL of water was added to the reaction mixture, which was maintained at 0 °C for 2 h. The precipitate was filtered and washed with 300 mL of ethyl acetate. The filtrate and washings were combined, washed with two 50-mL portions of water, and dried ($MgSO_4$). Concentration of the organic phase under diminished pressure afforded crude aldehyde **21** as a colorless syrup: yield 3.72 g. This material was used directly for condensation with (*S*)-cysteine methyl ester.

A solution of 3.72 g of crude aldehyde **21** and 1.70 g (12.6 mmol) of (*S*)-cysteine methyl ester in 25 mL of benzene was stirred at 25 °C for 2 days. The reaction mixture was diluted with 300 mL of ethyl acetate, washed with two 50-mL portions of water, and dried ($MgSO_4$). Concentration under diminished pressure afforded a syrupy residue, which was purified by flash column chromatography[45] on silica gel (30 × 3.5 cm). Elution with 27% ethyl acetate in hexane provided 0.84 g (19% recovery) of cysteine derivative **20**. Further elution with 30% ethyl acetate in hexane afforded methyl (1′*S*,4*S*)-2-[1′-((*tert*-butoxycarbonyl)amino)-2′-(methylthio)ethyl]thiazolidine-4-carboxylate (**22**) as a colorless solid: yield 3.36 g (57%; 71% based on consumed starting material); ^1H NMR ($CDCl_3$) δ 1.42, 1.45 (2 s, 9), 2.12, 2.15 (2 s, 3), 2.63–2.75 (m, 3), 3.20–3.30 (m, 2), 3.75, 3.78 (2 s, 3), 4.32 (m, 1), 4.87 (d, 1, J = 6 Hz) and 5.00 (m, 2); mass spectrum (chemical ionization) m/z 337 $(M + 1)^+$, 263 and 237; mass spectrum (electron impact), m/z 336.118 ($C_{13}H_{24}N_2O_4S_2$ requires 336.118).

Methyl (*S*)-2-[1-((*tert*-Butoxycarbonyl)amino)-2-(methylthio)ethyl]thiazole-4-carboxylate (23). To a solution containing 3.36 g (10 mmol) of thiazolidine **22** and 1.03 g (13 mmol) of pyridine in 95 mL of benzene was added 43.5 g (0.5 mol) of chemical manganese dioxide (Chemetals, Inc., Baltimore, MD). The reaction mixture was stirred vigorously with a mechanical stirrer at 55–58 °C for 6 h. The cooled reaction mixture was filtered, and the filtrate was concentrated under diminished pressure to afford a syrupy residue, which was purified by flash column chromatography on silica gel (20 × 3 cm). Elution with 27% ethyl acetate in hexane provided thiazole derivative **23** as a light yellow syrup: yield 1.8 g (54%); $[\alpha]^{25}_D$ −38.0° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.44 (s, 9), 2.01 (s, 3), 3.12 (d, 2, J = 6 Hz), 3.95 (s, 3), 5.21 (q, 1, J = 7 Hz), 5.61 (m, 1) and 8.12 (s, 1); mass spectrum (chemical ionization), m/z 333 $(M + 1)^+$, 277 and 220; mass spectrum (electron impact), m/z 332.087 ($C_{13}H_{20}N_2O_4S_2$ requires 332.086).

Methyl (*S*)-2-[1-Amino-2-(methylthio)ethyl]thiazole-4-carboxylate (24). A cold (0–5 °C) solution of 0.94 g (2.8 mmol) of thiazole **23** in 20 mL of dimethyl sulfide was treated with 33 mL of cold trifluoroacetic acid. The reaction mixture was stirred in an ice bath for 7 h, and the solution was then concentrated under diminished pressure. The syrupy residue was coevaporated with three 5-mL portions of benzene. The residue was dissolved in 100 mL of 50% aqueous methanol, stirred with 15 g of Amberlite IRA-45 resin for 30 min to effect neutralization, then filtered, and concentrated under diminished pressure at room temperature. The residue was purified by chromatography on a column of silica gel (20 × 3 cm). Elution with ethyl acetate and then with 5% CH_3OH in ethyl acetate, provided the trifluoroacetate salt of methyl (*S*)-2-[1-amino-2-(methylthio)ethyl]thiazole-4-carboxylate (**24**), which was isolated as a yellow syrup after concentration of the eluate: yield 0.61 g (93%); $[\alpha]^{25}_D$ −25° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.57 (br s, 2), 2.10 (s, 3), 2.74 (dd, 1, J = 13, 9 Hz), 3.17 (dd, 1, J = 13, 3 Hz), 3.95 (s, 3), 4.50 (dd, 1, J = 9, 3 Hz), and 8.12 (s, 1); mass spectrum (chemical ionization), m/z 233 $(M + 1)^+$, 216 and 187; mass spectrum (electron impact), m/z 232.034 ($C_8H_{12}N_2O_2S_2$ requires 232.034).

Methyl (*S*)-2-[1-(((*N*-(*tert*-Butoxycarbonyl)amino)propionyl)amino)-2-(methylthio)ethyl]thiazole-4-carboxylate (25). To a cold (0–5 °C) solution of 1.96 g (10 mmol) of *N*-(*tert*-butoxycarbonyl)-β-aminoalanine and 0.56 g (4 mmol) of 1-hydroxybenzotriazole in 20 mL of dry acetonitrile under argon was added 0.85 g (4 mmol) of *N*,*N*′-dicyclohexylcarbodiimide. The reaction mixture was stirred at 0 °C for 30 min, and then treated with a solution containing 1.15 g (3 mmol) of aminoalkylthiazole derivative **24** in 50 mL of dry acetonitrile. The combined solution was stirred at 0 °C for 30 min, and then at 25 °C for 40 h. The solvent was concentrated under diminished pressure and 200 mL of ethyl acetate was added to the residue. Insoluble material was removed by filtration, and the filtrate was washed successively with 10% aqueous citric acid, saturated aqueous $NaHCO_3$ and water. The dried ($MgSO_4$) organic phase was concentrated under diminished pressure and the residue was purified by chromatography on a silica gel column (25 × 2.5 cm). Elution with 2% CH_3OH in CH_2Cl_2 provided thiazole derivative **25**, obtained as colorless microcrystals from CH_2Cl_2: yield 0.95 g (70%); mp 84–85 °C; $[\alpha]^{25}_D$ −48° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9), 2.05 (s, 3), 2.52 (t, 2, J = 6 Hz), 3.04–3.17 (m, 2), 3.44 (q, 2), 3.95 (s, 3), 5.15 (s, 1), 5.50 (q, 1, J = 6 Hz), 6.67 (d, 1, J = 6 Hz) and 8.13 (s, 1); mass spectrum (chemical ionization), m/z 404 $(M + 1)^+$. Anal. Calcd for $C_{16}H_{25}N_3O_5S_2$: C, 47.63; H, 6.24; N, 10.41. Found: C, 47.74; H, 6.20; N, 10.38.

(*S*)-2-[1-(((*N*-(*tert*-Butoxycarbonyl)amino)propionyl)amino)-2-(methylthio)ethyl]thiazole-4-carboxylic Acid (26). To a cold (0–5 °C) solution of 1.21 g (3.0 mmol) of thiazole methyl ester **25** in 10 mL of CH_3OH was added 3 mL (6 mmol) of aqueous 2 M NaOH. The reaction mixture was stirred at 25 °C for 1 h and then neutralized with Amberlite IRA-120 resin (H^+-form). The solution was concentrated under diminished pressure, and the residue was dissolved in 20% CH_3OH in CH_2Cl_2 and filtered through a small silica gel column. The eluate was concentrated, affording thiazole carboxylic acid derivative **26** as a colorless solid: yield 1.17 g (99%); $[\alpha]^{25}_D$ −46° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.41 (s, 9), 2.06 (s, 3), 2.54 (m, 2), 3.07 (m, 2), 3.46 (m, 2), 5.20 (br s, 1), 5.50 (m, 1), 7.17 (br s, 1) and 8.19 (s, 1); mass spectrum (chemical ionization), m/z 390 $(M + 1)^+$, 225 and 133; mass spectrum (electron impact), m/z 389.108 ($C_{15}H_{23}N_3O_5S_2$ requires 389.108).

Treatment of Thiazole Carboxylic Acid Derivative 26 with Diazomethane. A solution containing 22 mg (0.056 mmol) of thiazole carboxylic acid **26** in 1 mL of THF was treated with a solution containing approximately 5.6 mmol of diazomethane in 2 mL of 1:1 THF–ether. The combined solution was maintained at 25 °C for 3 h and then concentrated under diminished pressure. The residue was applied to a preparative silica gel TLC plate, which was developed with 5% CH_3OH in $CHCl_3$. Thiazole methyl ester **25** was recovered from the TLC plate and provided colorless microcrystals from CH_2Cl_2: yield 15 mg (68%); mp 84–85 °C; $[\alpha]^{25}_D$ −47.4° (*c* 0.5, CH_3OH). This compound had the same chromatographic properties on TLC, optical rotation, and ^1H NMR spectrum as the sample of **25** prepared by condensation of **24** and Boc-β-aminoalanine (vide supra).

2-[(((*N*-Boc-amino)propionyl)amino)alkyl]thiazole-4-carboxamide 27. To a cold (0–5 °C) solution containing 1.17 g (3.0 mmol) of Boc thiazole carboxylic acid **26** and 0.49 g (3.6 mmol) of 1-hydroxybenzotriazole in 35 mL of acetonitrile under argon was added 0.74 g (3.6 mmol) of *N*,*N*′-dicyclohexylcarbodiimide, followed by 0.38 g (3.6 mmol) of [3-(methylthio)propyl]amine. The reaction mixture was stirred at 0 °C for 30 min and then at 25 °C for 45 h. The cooled reaction mixture was filtered, and the filtrate was diluted with 100 mL of ethyl acetate. The organic extract was washed with three 30-mL portions of 10% aqueous citric acid, saturated aqueous $NaHCO_3$, and brine. The dried ($MgSO_4$) organic extract was concentrated under diminished pressure. The residue was purified by flash chromatography on a silica gel column (20 × 2.5 cm). Elution of the column with 4% CH_3OH in CH_2Cl_2 provided pure thiazole carboxamide **27**, which was obtained as colorless microcrystals from CH_2Cl_2–hexane: yield 1.15 g (80%); mp 102–103 °C; $[\alpha]^{25}_D$ −35° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9), 1.94 (qn, 2, J = 6 Hz), 2.05 (s, 3), 2.13 (s, 3), 2.54 (t, 2, J = 6 Hz), 2.59 (t, 2, J = 6 Hz), 3.02–3.15 (m, 2), 3.47 (dd, 2, J = 10, 6 Hz), 3.56 (dd, 2, J = 10, 6 Hz), 5.09 (m, 1), 5.50 (dd, 1, J = 10, 6 Hz), 6.71 (m, 1), 7.46 (m, 1), and 8.04 (s, 1); mass spectrum (chemical ionization), m/z 477 $(M + 1)^+$ and 377. Anal. Calcd for $C_{19}H_{32}N_4O_4S_3$: C, 47.90; H, 6.77; N, 11.76. Found: C, 47.91; H, 6.69; N, 11.83.

2-[((Aminopropionyl)amino)alkyl]thiazole-4-carboxamide 28. To a cold (0–5 °C), stirred solution of 82 mg (0.17 mmol) of thiazole carboxamide **27** in 0.6 mL of dry CH_2Cl_2 was added 0.6 mL of cold 4.5 M hydrogen chloride in dioxane. The combined solution was stirred at 0 °C for 5 h and then concentrated under diminished pressure. The residue was dissolved in 4 mL of 1:1 CH_3OH–H_2O, neutralized with Amberlite IRA-45 resin, and concentrated under diminished pressure. Three 3-mL portions of benzene were successively added to the residue and evaporated in vacuo, providing the hydrochloride salt of 2-(aminoalkyl)thiazole-4-carboxamide **28** as a colorless solid: yield 68 mg (95%); $[\alpha]^{25}_D$ −41.7° (*c* 1.0, CH_3OH); ^1H NMR (D_2O) δ 1.89 (qn, 2, J = 7 Hz), 2.06 (s, 3), 2.08 (s, 3), 2.58 (t, 2, J = 7 Hz), 2.78 (m, 2), 3.04 (dd, 1, J = 10, 6 Hz), 3.19–3.34 (m, 3), 3.47 (t, 2, J = 7 Hz), 5.43 (dd, 1, J = 10, 6 Hz) and 8.13 (s, 1); mass spectrum (chemical ionization), m/z 377 $(M + 1)^+$ and 89; mass spectrum (electron impact), m/z 376.104 ($C_{14}H_{24}N_4O_2S_3$ requires 376.106).

***N*-[(2,2,2-Tribromoethoxy)carbonyl]-(*S*)-threonine (29).** To a cold (0–5 °C) solution containing 2.26 g (19 mmol) of (*S*)-threonine and 1.53 g (14.4 mmol) of Na_2CO_3 in 30 mL of water was added a solution of 5.09 g (14 mmol) of 2,2,2-tribromoethyl azidoformate[46] in 65 mL of dioxane.

(45) Still, W. C.; Khan, J.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

The combined solution was stirred at 0 °C for 3 h and then at 25 °C for 6 h. The reaction mixture was diluted with 20 mL of water and extracted with three 50-mL portions of ether. The aqueous phase was acidified to pH 1 with concentrated HCl and extracted with three 100-mL portions of CH_2Cl_2. The combined CH_2Cl_2 extract was washed with brine and water and then dried ($MgSO_4$). Concentration of the organic phase under diminished pressure gave a solid that crystallized from ether–hexane to provide *N*-[(2,2,2-tribromoethoxy)carbonyl]-(*S*)-threonine (**29**) as colorless needles: yield 3.27 g (53%); mp 165–166 °C; $[\alpha]^{25}_D$ −4.7° (*c* 1.0, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.32 (d, 3, *J* = 7 Hz), 4.42 (dd, 1, *J* = 10, 2 Hz), 4.51 (qn, 1, *J* = 7, 2 Hz), 4.89 (d, 1, *J* = 14 Hz), 5.03 (d, 1, *J* = 14 Hz) and 5.88 (d, 1, *J* = 10 Hz); mass spectrum (chemical ionization), *m/z* 432, 430, 428, and 426 $(M + 1)^+$; mass spectrum (electron impact), *m/z* 424.810 ($C_7H_{10}Br_3NO_5$ requires 424.810).

***N*-[(2,2,2-Tribromoethoxy)carbonyl]threonyl Thiazole Derivative 30.** To a cold (0–5 °C) solution containing 92 mg (0.21 mmol) of *N*-[(2,2,2-tribromoethoxy)carbonyl]threonine (**29**) and 73 mg (0.54 mmol) of 1-hydroxybenzotriazole in 1 mL of dry DMF under argon was added 143 mg (0.54 mmol) of *N*,*N*′-dicyclohexylcarbodiimide. The resulting solution was stirred at 0 °C for 20 min and then treated dropwise with a solution containing 68 mg (0.16 mmol) of (aminoalkyl)thiazole derivative **28** in 1.5 mL of dry DMF. *N*-Methylmorpholine (19 μL, 0.18 mmol) was added as a neat liquid, and the reaction mixture was stirred at 0 °C for 2 h and then at 25 °C for 45 h. The reaction mixture was concentrated under diminished pressure, and the residue was treated with 4 mL of ethyl acetate. The insoluble material was filtered, and the filtrate was washed successively with 10% citric acid, saturated aqueous $NaHCO_3$, and water. The dried ($MgSO_4$) organic phase was concentrated, and the residue was purified by flash chromatography on a silica gel (15 × 2.5 cm). Elution with 5% CH_3OH in CH_2Cl_2 afforded the desired thiazole derivative **30**, which was obtained as colorless microcrystals from hexane–CH_2Cl_2: yield 112 mg (86%); mp 71–72 °C; $[\alpha]^{25}_D$ −30.4° (*c* 0.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.21 (d, 3, *J* = 7 Hz), 1.93 (qn, 2, *J* = 7 Hz), 2.07 (s, 3), 2.12 (s, 3), 2.55 (m, 2), 2.60 (t, 2, *J* = 7 Hz), 3.08 (d, 2, *J* = 7 Hz), 3.51–3.63 (m, 4), 4.12 (dd, 1, *J* = 7, 1 Hz), 4.39 (m, 1), 4.87–4.97 (m, 2), 5.47 (q, 1, *J* = 7 Hz), 6.12 (d, 1, *J* = 7 Hz), 6.97 (m, 1), 7.09 (m, 1), 7.50 (m, 1) and 8.03 (s, 1); mass spectrum (chemical ionization), *m/z* 790, 788, 786 and 784 $(M + 1)^+$; mass spectrum (electron impact), *m/z* 782.905 ($C_{21}H_{32}Br_3N_5O_6S_3$ requires 782.906).

2-[(((Threonylamino)propionyl)amino)alkyl]thiazole-4-carboxamide 31. To a cold (0–5 °C), stirred solution containing 65 mg (0.08 mmol) of N-protected threonyl thiazole derivative **30** and 520 mg of HOAc in 5 mL of 9:1 CH_3OH–H_2O was added 52 mg (0.8 mmol) of Zn. The reaction mixture was stirred at 0 °C for 3 h and was then concentrated under diminished pressure. The residue was purified by chromatography on a silica gel column (20 × 2 cm). Washing with 3% CH_3OH in CH_2Cl_2 effected elution of unreacted **30** (8 mg). Washing of the column with 20% CH_3OH in CH_2Cl_2 afforded crude **31** as a solid. This solid was dissolved in 5 mL of water, treated with 200 mg of EDTA, and stirred at 25 °C for 30 min. The aqueous solution was then applied to an Amberlite XAD-2 column (15 × 2.5 cm), which was washed successively with 50 mL of saturated brine, 50 mL of H_2O, and 50 mL of CH_3OH. The appropriate CH_3OH fractions were combined and concentrated under diminished pressure. The solid residue was dissolved in 3 mL of CH_3OH, and the solution was acidified ("pH 1", as measured by pH paper) by addition of a solution containing 1 M hydrogen chloride in ether. The solution was concentrated under diminished pressure, and the residue was triturated with 3 mL of CH_2Cl_2. The solvent was decanted, and the residue was dried in vacuo, affording the hydrochloride salt of **31** as a light yellow foam: yield 35 mg (95%); $[\alpha]^{25}_D$ −40.4° (*c* 0.5, CH_3OH); ^1H NMR (CD_3OD) δ 1.22 (d, 3, *J* = 7 Hz), 1.88 (qn, 2, *J* = 7 Hz), 2.07 (s, 3), 2.09 (s, 3), 2.54 (t, 2, *J* = 7 Hz), 2.55 (t, 2, *J* = 7 Hz), 2.97 (dd, 1, *J* = 15, 10 Hz), 3.23 (dd, 1, *J* = 15, 5 Hz), 3.44–3.52 (m, 3), 3.55–3.62 (m, 2), 3.97 (m, 1), 5.40 (dd, 1, *J* = 10, 5 Hz) and 8.10 (s, 1); mass spectrum (chemical ionization), *m/z* 478 $(M + 1)^+$ and 460; mass spectrum (electron impact), *m/z* 477.152 ($C_{18}H_{31}N_5O_4S_3$ requires 477.154).

Condensation of 2-[(((Threonylamino)propionyl)amino)alkyl]thiazole-4-carboxamide 31 with Bis(*t*-Boc) β-Hydroxyhistidinyl Valeric Acid 15. To a cold (0–5 °C), stirred solution containing 37 mg (74 μmol) of Bis(*t*-Boc) dipeptide **15**, 37 mg (274 μmol) of 1-hydroxybenzotriazole, and 88 mg (426 μmol) of *N*,*N*′-dicyclohexylcarbodiimide in 1.5 mL of dry DMF under argon was added dropwise a cold solution containing 45 mg (88 μmol) of threonylthiazole derivative **31** in 2.5 mL of dry DMF. The combined solution was treated with 85 μL (62 mg, 610 μmol) of triethylamine, and the reaction mixture was stirred under argon at 0 °C for 3 h and then at 25 °C for 48 h. The reaction mixture was concentrated under diminished pressure at 25 °C, and the residue was treated with 5 mL of ethyl acetate. The ethyl acetate solution was cooled and filtered through Celite, and the filtrate was washed with 5 mL of H_2O. The dried ($MgSO_4$) organic phase was concentrated under diminished pressure, and the residue was purified by chromatography on a silica gel column (15 × 3 cm; elution was with 5% CH_3OH in ethyl acetate) and then by preparative TLC (development was with 6:1 ethyl acetate–CH_3OH). Bis(*t*-Boc) peptide **32** was isolated as a colorless solid: yield 39 mg (55%); silica gel TLC R_f 0.53 (6:1 ethyl acetate–CH_3OH); $[\alpha]^{25}_D$ −3.6° (*c* 0.5, CH_3OH); ^1H NMR (CD_3OD) δ 1.09 (d, 3, *J* = 7 Hz), 1.10 (d, 3, *J* = 7 Hz), 1.14 (d, 3, *J* = 7 Hz), 1.37 (s, 9), 1.59 (s, 9), 1.87 (qn, 2, *J* = 7 Hz), 2.07 (s, 3), 2.10 (s, 3), 2.50 (m, 1), 2.53 (t, 4, *J* = 7 Hz), 2.97 (dd, 1, *J* = 15, 9 Hz), 3.23 (dd, 1, *J* = 15, 6 Hz), 3.46 (t, 2, *J* = 7 Hz), 3.50 (t, 2, *J* = 7 Hz), 3.65 (m, 1), 3.87 (qn, 1), 4.12 (m, 1), 4.27 (d, 1, *J* = 3 Hz), 4.35 (d, 1, *J* = 7 Hz), 5.41 (dd, 1, *J* = 9, 6 Hz), 7.37 (s, 1), 8.08 (s, 1), and 8.12 (s, 1); mass spectrum (LSIMS), *m/z* 983 (M + Na)$^+$, 961 $(M + H)^+$, 860, and 760.

Peptide 33. Bis(*t*-Boc) peptide **32** (25 mg, 26 μmol) was converted to peptide **33** in analogy with the conversion of **16** → **17**. Peptide **33** was obtained as a colorless solid: yield 22 mg (88%); $[\alpha]^{25}_D$ −11.6° (*c* 0.5, CH_3OH); ^1H NMR (D_2O) δ 1.01 (d, 3, *J* = 7 Hz), 1.11 (d, 3, *J* = 7 Hz), 1.18 (d, 3, *J* = 7 Hz), 1.90 (qn, 2, *J* = 7 Hz), 2.10 (s, 6), 2.54 (m, 1), 2.59 (t, 4, *J* = 7 Hz), 3.05 (dd, 1, *J* = 15, 9 Hz), 3.23 (dd, 1, *J* = 15, 6 Hz), 3.45–3.57 (m, 4), 3.68 (dd, 1, *J* = 8, 4 Hz), 3.80 (m, 1), 4.06 (m, 1), 4.23 (d, 1, *J* = 5 Hz), 4.26 (d, 1, *J* = 6 Hz), 5.20 (d, 1, *J* = 6 Hz), 5.42 (dd, 1, *J* = 9, 6 Hz), 7.27 (s, 1), 8.00 (s, 1), and 8.14 (s, 1); mass spectrum (LSIMS), *m/z* 782 $(M + Na)^+$, 760 $(M + H)^+$, and 607.

Condensation of Peptide 33 with Pyrimidoblamic Acid (18). Compound **34** was prepared by condensation of 8 mg (19 μmol) of pyrimidoblamic acid and 22 mg (22 μmol) of peptide **33**, in analogy with the synthesis of **19**. Boc bleomycin monothiazole B (**34**) was obtained as a colorless syrup: yield 32 mg; silica gel TLC R_f 0.71 (2:1:1 1-butanol–HOAc–H_2O). This compound was used in the next step without further purification.

Bleomycin Monothiazole B (2). Crude Boc bleomycin monothiazole B (**34**) (32 mg) was deblocked with trifluoroacetic acid and dimethyl sulfide, in analogy with synthesis of bleomycin monothiazole A (**1**). Bleomycin monothiazole B (**2**) was obtained as a colorless glass: yield 6 mg (30% from **33**); silica gel TLC R_f 0.11 (2:1:1 *n*-butanol–HOAc–H_2O); $[\alpha]^{25}_D$ −12.8° (*c* 0.25, H_2O); ^1H NMR (D_2O) δ 1.12 (d, 6, *J* = 7 Hz), 1.15 (d, 3, *J* = 7 Hz), 1.90 (qn, 2, *J* = 7 Hz), 1.93 (s, 3), 2.05 (s, 3), 2.07 (s, 3), 2.57 (m, 5), 2.67 (m, 2), 2.93–3.03 (m, 3), 3.16 (dd, 1, *J* = 15, 6 Hz), 3.44–3.52 (m, 4), 3.72 (m, 1), 3.87 (dd, 1, *J* = 13, 5 Hz), 3.96 (m, 1), 4.04 (t, 1, *J* = 4 Hz), 4.10 (m, 1), 4.24 (d, 1, *J* = 6 Hz), 4.87 (d, 1, *J* = 8 Hz), 5.11 (d, 1, *J* = 8 Hz), 5.37 (dd, 1, *J* = 9, 6 Hz), 7.19 (s, 1), 7.81 (s, 1) and 8.12 (s, 1); mass spectrum (LSIMS), *m/z* 1089 $(M + Na)^+$ and 1067 $(M + H)^+$.

General Procedure for the Hydroxylation of Naphthalene. The reaction mixture included 5 μL of a 5 mM aqueous solution of Fe(III)·BLM, 5 μL of a 21 mM methanolic solution of 1-decanol (internal standard) and 10 μL of a 0.25 M methanolic solution of naphthalene in 25 μL of CH_3OH. The reaction was initiated by the addition of 5 μL of 0.3 M H_2O_2 and maintained at 25 °C for 1–1.5 h. The reaction mixture was diluted with 400 μL of H_2O and extracted with 100 μL of $CHCl_3$. The $CHCl_3$ extract was analyzed by gas chromatography. The following temperature program was employed at a He gas flow rate of 5.0 mL/min: 100 °C for 2 min; 100 → 200 °C at 40 °C/min; and then 200 °C for 15 min. Under these conditions, the observed retention times were as follows: naphthalene, 3.4 min; 1-decanol, 5.4 min; 1-naphthol, 12.8 min; and 2-naphthol, 12.9 min.

General Procedure for the Oxygenation of Styrene. The reaction mixture included 5 μL of a 5 mM aqueous solution of Fe(III)·BLM, 5 μL of a 28 mM methanolic solution of ethyl benzoate (internal standard), and 10 μL of a 0.25 M methanolic solution of styrene in 25 μL of CH_3OH. This solution was cooled in an ice bath, and the reaction was initiated by the addition of 5 μL of 0.3 M H_2O_2. The reaction mixture was maintained at 0 °C for 1.5 h, then diluted with 400 μL of H_2O, and extracted with 100 μL of $CHCl_3$. The $CHCl_3$ extract was analyzed by gas chromatography using the following temperature program at a He gas flow rate of 5.0 mL/min: 40 °C for 2 min; 40 → 70 °C at 10 °C/min; 70 °C for 10 min; 70 → 85 °C at 10 °C/min; and then 85 °C for 10 min. Under these conditions, the observed retention times were as follows: styrene, 3.8 min; styrene oxide, 10.1 min; phenylacetaldehyde, 10.5 min; and ethyl benzoate, 16.5 min.

General Procedure for the Demethylation of *N*,*N*-Dimethylaniline. The reaction mixture included 5 μL of a 5 mM aqueous solution of Fe(III)·BLM, 5 μL of a 28 mM methanolic solution of ethyl benzoate (internal standard), and 10 μL of a 0.25 M methanolic solution of *N*,*N*-dimethylaniline in 25 μL of CH_3OH. The reaction was initiated by the addition of 5 μL of 0.3 M H_2O_2 and maintained at 25 °C for 1.5 h. The reaction mixture was diluted with 400 μL of water and extracted with 100 μL of $CHCl_3$. The $CHCl_3$ extract was analyzed by gas chroma-

tography using the same conditions described above for the analysis of styrene oxygenation products. Under these conditions, the observed retention times were as follows: *N,N*-dimethylaniline, 9.3 min; *N*-methylaniline, 10.3 min; and ethyl benzoate, 14.7 min.

Preparation of a 5′-^{32}P-End Labeled 40-Nucleotide DNA Oligonucleotide. A 40-nucleotide (nt) DNA oligomer having the sequence 5′-CCC TCC CGA CTG CCT ATG ATG TTT ATC CTT TGG ATG GTC G-3′ was prepared on a Biosearch Model 8700 DNA synthesizer using phosphoramidite chemistry.[47] Also prepared was the complementary 40-nt sequence. 5′-End labeling of the DNA sequence shown above was carried out using 10 μg of the DNA in 50 μL of 50 mM Tris-HCl, pH 7.6, containing 10 mM $MgCl_2$, 5 mM dithiothreitol (DTT), 100 μM spermidine, 2.5 μM [γ-^{32}P] ATP (7000 Ci/mmol), and 500 units of T4 polynucleotide kinase (Bethesda Research Laboratories; 1 unit is defined as the amount of enzyme that will incorporate 1 nmol of labeled ATP into acid-precipitable material in 30 min). The reaction mixture was incubated at 37 °C for 1 h and then treated with 2.5 volumes of cold ethanol to effect precipitation of the DNA. The DNA was maintained at −80 °C for 12 h, recovered by centrifugation, and purified by gel electrophoresis on a 20% denaturing polyacrylamide gel.[32b] The recovered, 5′-^{32}P labeled DNA was used directly as a single-stranded substrate or else converted to the respective DNA duplex by annealing to its complementary 40-nt DNA oligomer (via heating in the presence of the complementary strand and 100 mM NaCl at 80 °C for 3 min, followed by slow cooling to 25 °C).

Cleavage of ^{32}P-End Labeled DNA by BLM Analogs. Reaction mixtures contained 2.5 pmol (~50 000 cpm) of 5′-^{32}P- or 3′-^{32}P-end labeled DNA and 200 μM sonicated calf thymus DNA in 10 μL of 10–20 mM Na cacodylate, pH 5.8 or 7.4. The reactions were initiated by the addition of the appropriate concentration of BLM and either Fe(III) + H_2O_2 or Fe(II) + dithiothreitol (DTT). The reaction mixtures were maintained at 25 °C for 1 h, quenched by the addition of 5 μL of loading buffer (10 M urea, 1.5 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), and applied to 20% polyacrylamide gels containing 8 M urea. Electrophoresis was carried out in 100 mM Tris-borate buffer, pH 8.4, containing 100 mM EDTA at 25 mA for 4 h. Autoradiography (Kodak XAR-2 film) was carried out at −80 °C for 24–48 h.

Preparation of a 3′-^{32}P-End Labeled 140-Nucleotide DNA Restriction Fragment. Plasmid pBR322 (12.5 μg) was digested simultaneously with 100 units each of *Hind* III and *Nci* I (Bethesda Research Laboratories; 1 unit is defined as that amount required to digest 1 μg of the substrate DNA in 1 h under the reaction conditions) in 50 μL of 0.5 M Tris-HCl, pH 8.0, containing 0.3 M NaCl, 0.1 M $MgCl_2$ and 1.0 mM dithiothreitol at 37 °C for 1 h. To this solution was added 125 μCi of [α-^{32}P]dATP and 100 units of AMV reverse transcriptase (Bethesda Research Laboratories; 1 unit incorporates 1 nmol of deoxynucleotide into acid-precipitable material in 10 min at 37 °C). The reaction mixture was incubated at 37 °C for 2 h. The 3′-^{32}P end-labeled 140-nt DNA fragment was purified on a nondenaturing 8% polyacrylamide gel and isolated by a crush and soak technique.

Relaxation of Supercoiled DNA by Bleomycin Analogs. Reactions were carried out in 16 μL (total volume) of 5 mM Na cacodylate buffer, pH 5.8, containing 200 ng of supercoiled pBR322 plasmid DNA and the appropriate concentration of Fe(III)·BLM analog and H_2O_2. Reaction mixtures were incubated at 25 °C for 1 h and then treated with 10 μL of loading buffer (40 mM Tris-OAc buffer, pH 7.8, containing 5 mM EDTA, 40% glycerol, 0.4% sodium dodecyl sulfate and 0.3% bromophenol blue) and applied to a 1.2% agarose gel containing 1 μg/mL of ethidium bromide. Horizontal gel electrophoresis was carried out in 40 mM Tris-OAc, pH 7.8, containing 5 mM EDTA at 130 V for 3–4 h (UV visualization).

Conclusions

Analogs of deglyco BLM, each of which lack one of the thiazole rings present in the parent molecule, were found to form Fe complexes that activated oxygen as well as BLM and deglyco BLM. Although both BLM monothiazole analogs effected the oxygenation and oxidation of low molecular weight substrates as well as BLM in what are believed to be bimolecular processes, neither could mediate sequence selective cleavage of DNA. We conclude that the bithiazole moiety is not required for Fe binding or oxygen activation, but that both rings must be present to mediate the sequence selective DNA cleavage characteristic of BLM group antibiotics.

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Correlating Backbone Amide and Side Chain Resonances in Larger Proteins by Multiple Relayed Triple Resonance NMR

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Abstract: A new three-dimensional triple resonance NMR experiment is described that correlates the amide ^{1}H and ^{15}N resonances of one residue simultaneously with both the $^{13}C_\alpha$ and $^{13}C_\beta$ resonances of its preceding residue. Sensitivity of the new experiment is comparable with that of the HN(CO)CA experiment (Bax, A.; Ikura, M. *J. Biomol. NMR* **1991**, *1*, 99–105), but the additional correlation to the C_β resonance of the preceding residue provides invaluable assignment information, previously inaccessible. The technique is demonstrated for interferon-γ, a homodimeric protein of 31.4 kDa, enriched uniformly with ^{13}C and ^{15}N.

The most difficult part in using the recently developed 3D triple resonance NMR for obtaining complete backbone assignments in larger proteins[1] involves the degeneracy in the H_α–C_α region of the ^{1}H–^{13}C shift correlation spectrum. Particularly for α-helical proteins with a narrow dispersion of H_α chemical shifts, this type of degeneracy frequently can make it difficult to establish in an unambiguous manner which backbone amide is correlated with which side chain. Even for the relatively well resolved spectrum of calmodulin, 40 such cases were reported.[1] Here we report a new 3D triple resonance technique that provides connectivity

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