Total Synthesis of Bleomycin Group Antibiotics. Total Syntheses of Bleomycin Demethyl A_2, Bleomycin A_2, and Decarbamoyl Bleomycin Demethyl A_2

Kiyoaki Katano, Haoyun An, Yoshiaki Aoyagi, Mark Overhand, Steven J. Sucheck, William C. Stevens, Jr., Cynthia D. Hess, Xiang Zhou, and Sidney M. Hecht*

Contribution from the Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22901

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Abstract: The total syntheses of bleomycin A_2 (**1**) by two routes are described. The final step in the synthesis of bleomycin A_2 involves methylation of bleomycin demethyl A_2 (**2**). This bleomycin derivative is of interest mechanistically, and can also provide access to other bleomycins via its known chemical conversion to bleomycinic acid. Accordingly, the synthetic strategy presented represents a particularly versatile approach for the elaboration of a wide variety of BLM congeners. Bleomycin was constructed from five key intermediates, the syntheses of which are described. 1,6-Di-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-β-L-gulopyranose (**3**) was converted quantitatively to its disaccharide chloride (**4**), the latter of which was condensed with N^{α},N^{im}-bis(*t*-Boc)-(*S*)-*erythro*-β-hydroxyhistidine (**7**) to provide α-*O*-glycosidated product **16**. The subsequent couplings with benzyl valerate **8**, threonylbithiazole **9**, and N^{α}-*t*-Boc-pyrimidoblamic acid (**10**) afforded access to bleomycin demethyl A_2 (**2**) and decarbamoyl bleomycin demethyl A_2 (**26**). Although it was obtained as a byproduct of the synthesis of BLM, the synthesis of decarbamoyl bleomycin demethyl A_2 nonetheless constitutes the first report of synthetic access to this BLM congener that is of particular importance from a mechanistic perspective. This BLM can be used to resolve the issue of the participation of the carbamoyl group as a metal ligand in metallobleomycins. Also reported is a new route to bleomycin demethyl A_2 that employed an unprotected carbamoyl group throughout the synthesis. In conjunction with the use of the uncharged methylthiopropylamide C-substituent, the latter strategy permitted improved functional group manipulation and thereby afforded intermediates that could be purified with greater facility. Because the synthesis of bleomycin is limited by the efficiency of elaboration of the carbohydrate moiety, a study was carried out to define improved methods for the preparation of this constituent of BLM. Three new routes were explored, and a route involving the intermediacy of disaccharide activated as a glycosyl bromide was found to be particularly efficient and convenient. Also of importance was the finding that activation of carbohydrate intermediates as their glycosyl trichloroacetimidates permitted the requisite couplings to be carried out conveniently and in good yields. Synthetic BLM demethyl A_2 was shown to have the same potency as a naturally derived sample in a plasmid DNA relaxation assay. The sequence selectivity of DNA cleavage by synthetic and authentic Fe(II)·BLMs was also shown to be the same. Also established for the first time for any synthetic bleomycin was the actual chemistry of DNA degradation, which is the same as that for naturally derived bleomycins.

The bleomycins (BLMs) are a family of structurally related glycopeptide-derived antibiotics with significant antitumor activity.[1] They were originally isolated from fermentation broths of *Streptomyces verticillus* by Umezawa and co-workers.[2] Blenoxane, the clinically used mixture of bleomycins, contains mainly bleomycin A_2 and bleomycin B_2, in addition to smaller amounts of other bleomycins.[3] The bleomycins were initially thought to be β-lactam-type antibiotics.[4] However, this structure was later revised,[1,5] and the revised structure was confirmed by total synthesis in 1982 by the Umezawa[6] and Hecht[7] laboratories. Subsequently, Umezawa and co-workers reported improvements in their synthesis.[8] More recently, Boger and co-workers have also reported a total synthesis of BLM A_2.[9]

The therapeutic effects of bleomycins are believed to derive from their ability to mediate the degradation of DNA[10−12] and possibly also RNA.[12,13] Polynucleotide degradation mediated by BLM is metal ion and oxygen dependent. Of particular interest has been the sequence selectivity of DNA cleavage,

* To whom correspondence should be addressed at the Department of Chemistry.

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which involves predominantly 5′-GC-3′ and 5′-GT-3′ sequences.[10,11] BLM has also been shown to recognize nucleic acid tertiary structure, as exemplified by the cleavage of a duplex−triplex junction in DNA,[14] bulges in DNA[15] and RNA,[13c] and the putative junctions between single- and double-stranded regions in RNA.[13]

As a consequence of their clinical utility, as well as their interesting structures and mechanism of action, the BLMs have been the focus of considerable attention. In addition to the bleomycin analogues and fragments accessible by degradation or modification of the natural product,[16−18] ongoing synthetic efforts have facilitated the preparation of synthetic[19−23] and semisynthetic[24] BLMs as potential therapeutic agents and as tools for dissecting the mechanism(s) of bleomycin action. Studies of the mechanism of action, in particular, have benefited from access to BLM subunits and analogues.[19−25]

Presently, we report the full details of two total syntheses of bleomycin A_2, using bleomycin demethyl A_2 as a key intermediate. One of these syntheses employed carbohydrate intermediates containing a protected mannose carbamoyl group; the other synthesis was achieved without protection of the carbamoyl group. Also obtained as a byproduct from one synthetic route was decarbamoyl bleomycin demethyl A_2, a species of special interest for mechanistic studies of BLM metal ion binding and activation. In addition, we report three new routes for the elaboration of the carbohydrate moiety of bleomycin, two of which gave particularly favorable results from the perspectives of efficiency and convenience for the elaboration of the carbohydrate moiety.

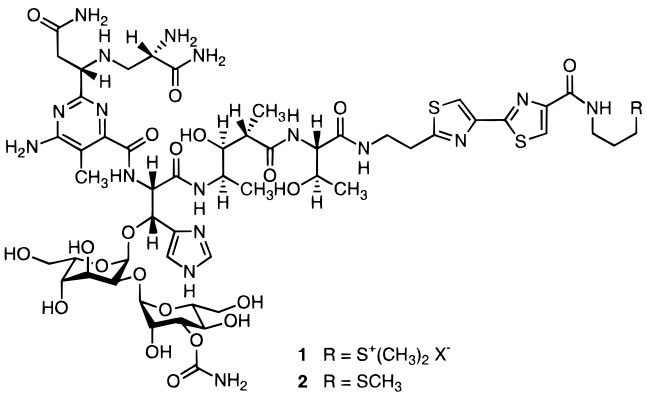

Figure 1. Structures of bleomycin A_2 (**1**) and bleomycin demethyl A_2 (**2**).

Results and Discussion

From the perspective of total synthesis, bleomycin A_2 (**1**) and bleomycin demethyl A_2 (**2**) (Figure 1) may be envisioned to consist of five key structural elements, the coupling of which could provide synthetic access to the natural products. These constituents, shown as intermediates protected in a fashion suitable for the elaboration of bleomycin (Figure 2), include the disaccharide moiety (**3**−**6**), (*S*)-*erythro*-*β*-hydroxyhistidine (**7**) (cf. Scheme 1), (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvaleric acid (**8**), the threonylbithiazole moiety (**9**) (cf. Scheme 2), and pyrimidoblamic acid (**10**). Synthetic studies in three laboratories have demonstrated amply that the success of strategies for the synthesis of bleomycin depends critically on the choice of protecting groups and the order in which the coupling of key intermediates is effected. The modified approach described by Umezawa and co-workers[8] includes the *O*-glycosidation of *erythro*-N^{α}-*t*-Boc-N^{im}-tos-(*S*)-*β*-hydroxyhistidine methyl ester with the glycosyl bromide analogous to disaccharide derivative **6**, subsequent coupling to tetrapeptide

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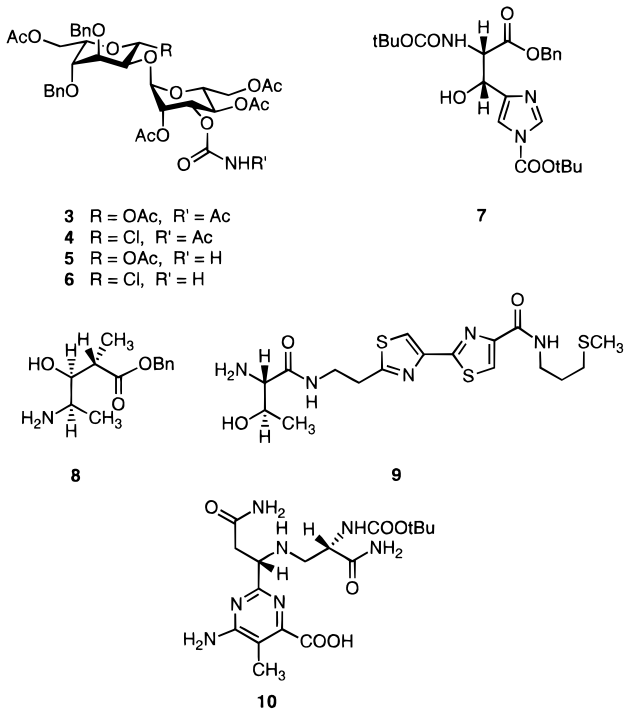


Figure 2. Key intermediates employed for the synthesis of bleomycin.

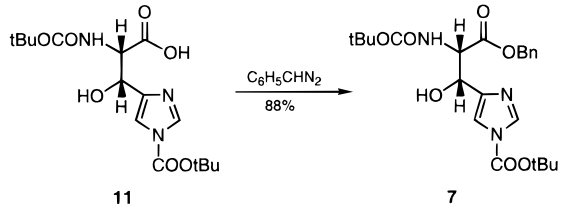
S and N^{α}-*t*-Boc-pyrimidoblamic acid, followed by deprotection to give bleomycin A_2. Boger and co-workers,[9] in their initial efforts, found that the *O*-glycosidation of N^{α}-*t*-Boc-N^{im}-trityl-(*S*)-*β*-hydroxyhistidine methyl ester with the *β*-glycosyl diphenyl phosphate provided a mixture of at least three components with the N^{α}-Boc deprotection product. However, this reaction was conducted in good yield by using an N^{α}-CBz protecting group on (*S*)-*β*-hydroxyhistidine instead of the N^{α}-Boc group to avoid the competitive N^{α}-Boc deprotection. Subsequent coupling with tetrapeptide S and N^{α}-*t*-Boc-pyrimidoblamic acid similarly afforded bleomycin A_2.[9] Our strategy included successful *O*-glycosylation of N^{α},N^{im}-di-*t*-Boc-(*S*)-*erythro*-*β*-hydroxyhistidine benzyl ester (**7**) with disaccharide chlorides **4** and **6** (Schemes 3 and 7) and subsequent, sequential coupling with benzyl valerate **8**, tripeptide S derivative **9**, and N^{α}-*t*-Boc-pyrimidoblamic acid (**10**) (Schemes 4−6, 8, and 9). This synthetic strategy has also proven useful for the synthesis of four other BLM congeners.

A unique feature of the synthetic scheme employed here involves the intermediacy of bleomycin demethyl A_2. In addition to providing uncharged synthetic intermediates, amenable to purification with greater facility, bleomycin demethyl A_2 is of great interest intrinsically for its DNA binding and cleaving properties[26] and has also been used as a convenient starting material to obtain BLMs modified within the C-substituent via chemical transformation to bleomycinic acid.[3,27,28]

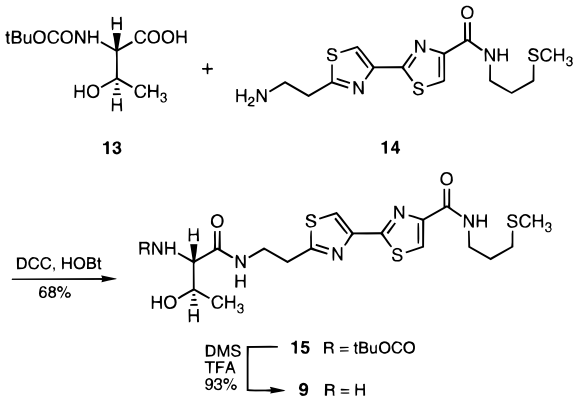
Synthesis of Key Intermediates. The requisite disaccharides, 1,6-di-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(*N*-acetylcarbamoyl)-α-D-mannopyranosyl]-*β*-L-gulopyranose (**3**) and 1,6-di-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-*β*-L-gulopyranose (**5**), were synthesized according to our previously reported procedures.[29] N^{α},N^{im}-bis(*tert*-butoxycarbonyl)-(*S*)-*erythro*-*β*-hydroxyhistidine (**11**) (Scheme 1) was prepared from (*S*)-*erythro*-*β*-hydroxyhistidine.[30] Benzylation of **11** with phenyldiazomethane, prepared in situ from benzaldehyde tosylhydrazone, provided the corresponding benzyl ester **7** in 88% yield.

(2*S*,3*S*,4*R*)-4-Amino-3-hydroxy-2-methylvaleric acid (**12**) ($[\alpha]^{25}_D = +10.5°$ (*c* 1.0, H_2O), lit.[31] $[\alpha]^{23}_D = +10.7°$ (*c* 7.25, H_2O), lit.[32] $[\alpha]^{25}_D = +12.1°$ (*c* 1.03, H_2O)) was synthesized according to the reported procedures.[31,33] Benzylation of this acid with benzyl alcohol in the presence of anhydrous hydrogen chloride afforded the corresponding benzyl ester (**8**) in 89% yield.[21c] Threonylbithiazole derivative **9** was synthesized using reported procedures[19a,21a,34,35] (Scheme 2). Specifically, 2′-(2-aminoethyl)-2,4′-bithiazole-4-[(3-methylthio)propyl]carboxamide (**14**) was prepared by the reported method from the corresponding carboxylic acid.[19a,34,35] The coupling of **14** with commercially available (*S*)-N^{α}-*t*-Boc-threonine (**13**) via the agency of DCC−HOBt afforded the coupled product N^{α}-*t*-Boc-threonylbithiazole **15**[35] in 68% yield. Deprotection of **15** with trifluoroacetic acid in the presence of dimethyl sulfide gave threonylbithiazole derivative **9** in 93% yield. N^{α}-*t*-Boc-pyrimidoblamic acid (**10**) was prepared by our reported procedure;[36]

Scheme 1



Scheme 2



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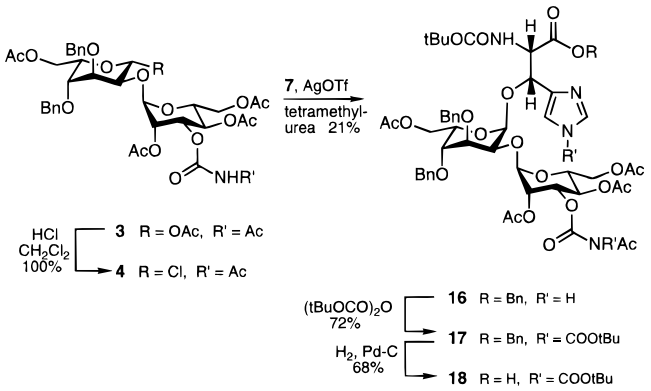
Scheme 3

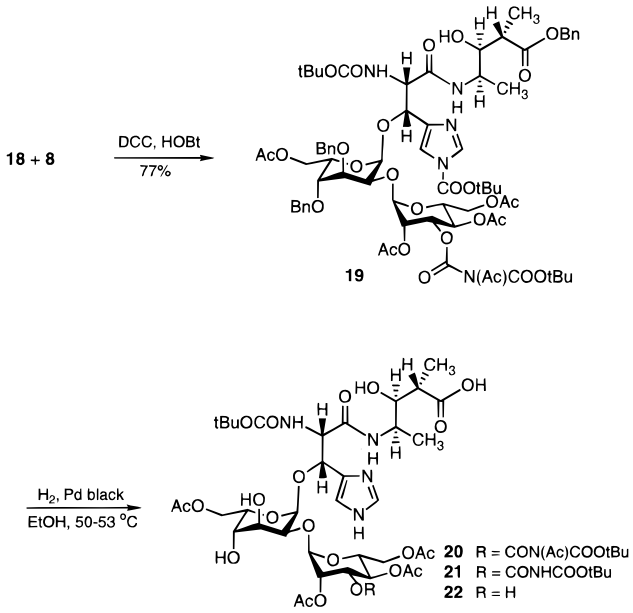


this species is also accessible using other synthetic strategies.[37] The key intermediates were all characterized thoroughly, including NMR spectroscopic and mass spectral analyses.

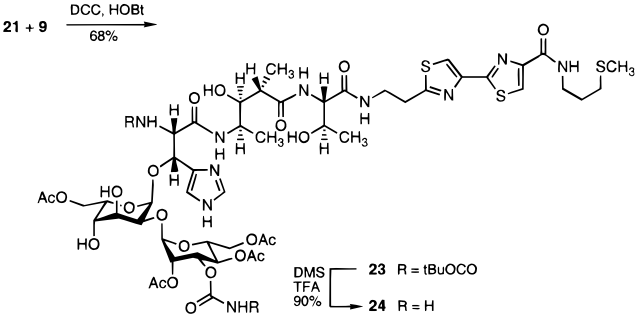
Elaboration of BLM from a Disaccharide Having an *N*-Protected Carbamoyl Group. Disaccharide **3** was converted to the corresponding gulopyranosyl chloride (**4**) by using hydrogen chloride gas in dichloromethane (Scheme 3). Gulopyranosyl chloride **4** was isolated as a colorless foam in quantitative yield after workup, and was used directly in the next step. The condensation of **4** with benzyl N^{α},N^{im}-di-*t*-butoxycarbonyl-(*S*)-*erythro*-β-hydroxyhistidine (**7**) (CF_3SO_3Ag, $(CH_3)_2NCON(CH_3)_2$) in anhydrous 1,2-dichloroethane at 45 °C for 24 h afforded the coupling product **16** in 21% yield. Treatment of **16** with di-*tert*-butyl dicarbonate in dry pyridine at 25 °C for 2 h provided tri-*t*-Boc product **17** in which the N^{α},N^{im} and carbamoyl nitrogens were all protected by Boc groups. Compound **17** underwent hydrogenolysis selectively over 5% palladium-on-carbon in ethyl acetate to afford the corresponding free carboxylic acid **18** in 68% yield without the loss of the Boc protecting groups.

The tri-Boc derivative **18** was condensed with benzyl (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvalerate (**8**) using DCC−HOBt in the presence of *N*,*N*-diisopropylethylamine at 25 °C for 3 h (Scheme 4). Dipeptide disaccharide **19** was obtained as a colorless foam in 77% yield ($[\alpha]^{24}_D$ −12.2° (c 2.3, EtOH)). Benzyl ester **19** was deesterified by hydrogenolysis over commercially available palladium black at 50−53 °C for 20 h. The N^{im}-Boc protecting group was also solvolyzed under these conditions, affording compound **20**. An attempt to purify this compound by simple extractive procedures under neutral conditions resulted in conversion to compound **21**, in which the acetyl group of the *O*-carbamoyl substituent had been lost, as judged by NMR spectroscopy. Also formed to some extent was dipeptide disaccharide derivative **22**, in which the *O*-carbamoyl group itself underwent solvolysis. The mixture of *N*-Boc carbamoyl protected **21** and **22** (the latter of which is a precursor of decarbamoyl BLM) was used for the next coupling step without further attempts at purification. As shown in Scheme 5, condensation of **21** and threonylbithiazole **9** was carried out at 25 °C for 20 h in dry DMF in the presence of DCC−HOBt. Pentapeptide disaccharide **23** was obtained as a colorless glass in 68% yield and characterized by ^{1}H NMR spectroscopy. The Boc protecting group was removed by treatment with trifluoroacetic acid in the presence of dimethyl sulfide, affording **24** in 90% yield as a glass.

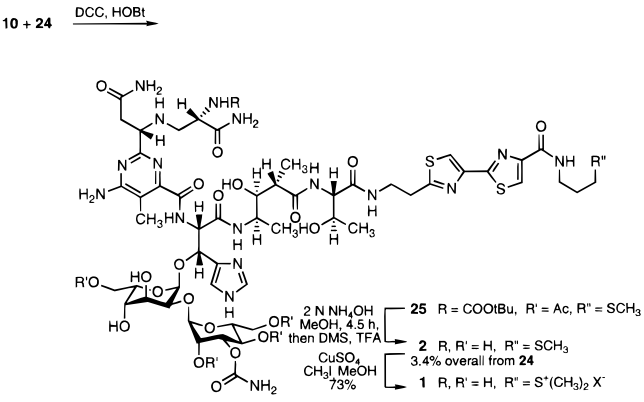
Scheme 4



Scheme 5



Scheme 6



Final coupling of pentapeptide disaccharide **24** with N^{α}-*t*-Boc-pyrimidoblamic acid (**10**)[36] was conducted at 25 °C for 2 days using DCC−HOBt in anhydrous DMF (Scheme 6). The resulting product (**25**) was deprotected with a 1:1 mixture of 2 N aqueous NH_4OH−methanol at 25 °C for 4.5 h (to remove the acetyl groups), followed by treatment with trifluoroacetic acid−dimethyl sulfide at 0 °C for 75 min (to effect removal of the *N*-*t*-Boc protecting group).

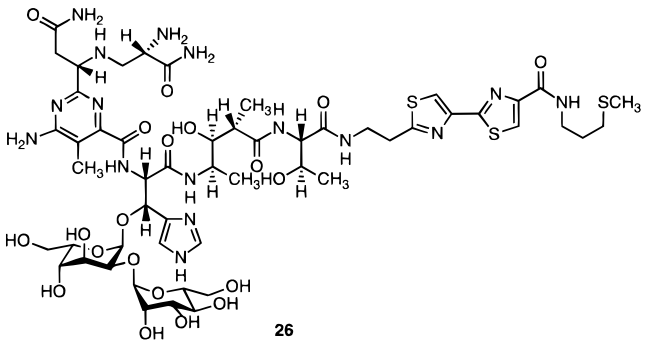
Crude bleomycin demethyl A_2 (**2**), putatively containing decarbamoyl bleomycin demethyl A_2 as a consequence of adventitious solvolysis of the carbamoyl group (vide supra), was initially purified by chromatography on an Amberlite XAD-2 column, followed by chromatographic fractionation of the Cu-

(36) Aoyagi, Y.; Chorghade, M. S.; Padmapriya, A. A.; Suguna, H.; Hecht, S. M. *J. Org. Chem.* **1990**, *55*, 6291.

(37) (a) Umezawa, Y.; Morishima, H.; Saito, S.; Takita, T.; Umezawa, H.; Kobayashi, S.; Otsuka, M.; Narita, M.; Ohno, M. *J. Am. Chem. Soc.* **1980**, *102*, 6630. (b) Boger, D. L.; Honda, T.; Dang, Q. *J. Am. Chem. Soc.* **1994**, *116*, 5619.

(II) complex on a CM Sephadex C-25 column. The first peak to elute was treated with EDTA disodium salt to remove Cu(II), and then purified on an Amberlite XAD-2 column, affording pure bleomycin demethyl A_2 (**2**) as a colorless powder. The purified product **2** was found to have chromatographic properties identical with those of authentic bleomycin demethyl A_2 on CM-Sephadex C-25 and silica gel TLC in several solvent systems. This compound also had the same retention time as authentic BLM demethyl A_2 on C_{18} reversed phase HPLC. The structure of synthetic BLM demethyl A_2 was also confirmed by high-resolution FAB mass spectrometry and by its ^{1}H NMR spectrum, which was identical with that of an authentic sample.

The second peak to elute from the CM Sephadex C-25 was also treated with EDTA to remove Cu(II) and then purified on an XAD-2 column to afford decarbamoyl bleomycin demethyl A_2 (**26**). Decarbamoyl BLM demethyl A_2 was obtained as a



26

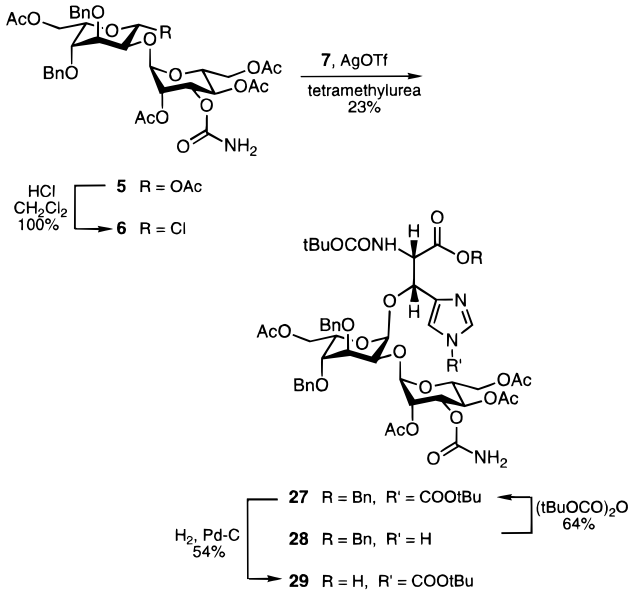
colorless glass. Although this BLM derivative has not been reported previously, it was shown to be identical with an authentic sample[38] on silica gel TLC using several solvent systems.

Synthetic BLM demethyl A_2 (**2**) was converted to BLM A_2 (**1**) by methylation of its Cu(II) chelate, as described.[7,39] The sample of **1** so obtained was identical in all respects with naturally derived bleomycin A_2.

Synthesis of Decarbamoyl BLM Demethyl A_2. Although obtained here as a byproduct during our initial synthesis of BLM demethyl A_2, the synthesis of decarbamoyl BLM demethyl A_2 represents a particularly important achievement. The carbamoyl group has been postulated to function as a metal ligand for at least three different metal ions that bind to BLM. This includes the Fe(II)·CO complex of BLM,[40] for which two groups have assigned the role of ligand to this group. It has also been suggested that Zn(II)·BLM[41] and Co(II)·BLM[42] complexes employ the carbamoyl moiety as metal ligands, although alternative ligands have been suggested as well.[43]

Decarbamoyl BLM represents a particularly valuable species for determining the possible participation of the carbamoyl group as a metal ligand since it can be compared functionally with the corresponding BLM derivative itself. In fact, Sugiyama et al.[18c] demonstrated that in the absence of the carbamoyl group there was a dramatic change in the strand selectivity of DNA oligonucleotide degradation by Fe(II)·BLM. More recently, it has been shown that the absence of the carbamoyl group produced fundamental changes in the spectral properties of Fe(II)·BLM, again suggesting an important role for the carbamoyl moiety in metal binding by BLM.[44]

Scheme 7



Despite the synthesis of numerous analogues of BLM, to date there has been no report on the synthesis of decarbamoyl BLM. This species has been accessible in low yield exclusively by treatment of BLM under basic aqueous conditions over a period of several months.[45] The sample of decarbamoyl BLM demethyl A_2 prepared in this study was shown to be identical in structure and behavior with a sample derived from BLM demethyl A_2 by base treatment, and also with an authentic synthetic sample prepared recently by an unambiguous total synthesis.[38]

Synthesis of Bleomycin without Protection of the Carbamoyl Group. Because the *N*-protected *O*-carbamoyl group employed in our original synthesis of bleomycin proved to be unstable hydrolytically during routine manipulations, we explored another route for the synthesis of bleomycin. This route, which is outlined in Schemes 7−9, incorporated a few important modifications, including (i) the use of an unprotected carbamoyl group throughout the entire synthesis, (ii) successful retention of the N^{im}-*t*-Boc protecting group until its intentional removal prior to the last peptide coupling, and (iii) the use of freshly prepared palladium black which permitted the clean removal of all benzyl groups in a single step. As a consequence of these changes, the intermediates obtained could be purified readily and exhibited excellent spectral characteristics.

Initially, disaccharide **5**, having an unprotected *O*-carbamoyl group, was converted to the respective gulopyranosyl chloride **6** in quantitative yield by the use of gaseous hydrogen chloride in CH_2Cl_2 (Scheme 7). Derived chloride **6** was condensed with benzyl N^{α},N^{im}-di-*t*-Boc-(*S*)-*erythro*-β-hydroxyhistidine (**7**) at 48

(38) An, H.-Y.; Katano, K.; Padmapriya, A. A.; Hess, C. D.; Hecht, S. M. Manuscript in preparation.

(39) Roy, S. N.; Orr, G. A.; Brewer, C. F.; Horwitz, S. B. *Cancer Res.* **1981**, *41*, 4471.

(40) (a) Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 5616. (b) Akkerman, M. A. J.; Neijman, E. W. J. F.; Wijmenga, S. S.; Hilbers, C. W.; Bermel, W. *J. Am. Chem. Soc.* **1990**, *112*, 7462.

(41) (a) Akkerman, M. A. J.; Haasnoot, C. A. G.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *173*, 21l. (b) Manderville, R. A.; Ellena, J. F., Hecht, S. M. *J. Am. Chem. Soc.* **1994**, *116*, 10851. (c) Manderville, R. A.; Ellena, J. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 7891.

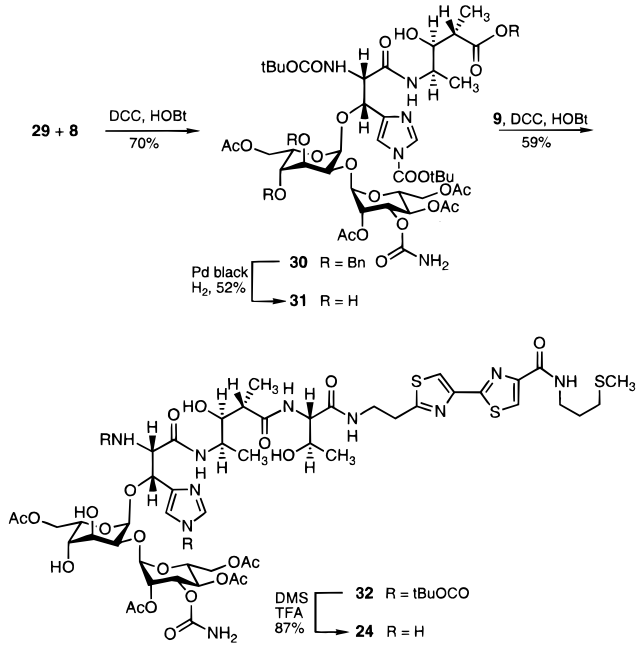
(42) Cortes, J. C.; Sugiyama, H.; Ikudome, K.; Saito, I.; Wang, A. H.-J. *Eur. J. Biochem.* **1997**, *224*, 818.

(43) See, e.g. (a) Stubbe, J.; Kozarich, J. W.; Wu, W.; Vanderwall, D. E. *Acc. Chem. Res.* **1996**, *29*, 322 and references therein. (b) Calafat, A. M.; Won, H.; Marzilli, L. G. *J. Am. Chem. Soc.* **1997**, *119*, 3656.

(44) Loeb, K. E.; Zaleski, J. E.; Hess, C. D.; Hecht, S. M.; Solomon, E. I. *J. Am. Chem. Soc.* **1998**, *120*, 1249.

(45) Naganawa, H.; Muraoka, Y.; Takita, T.; Umezawa, H. *J. Antibiot.* **1977**, *30*, 388.

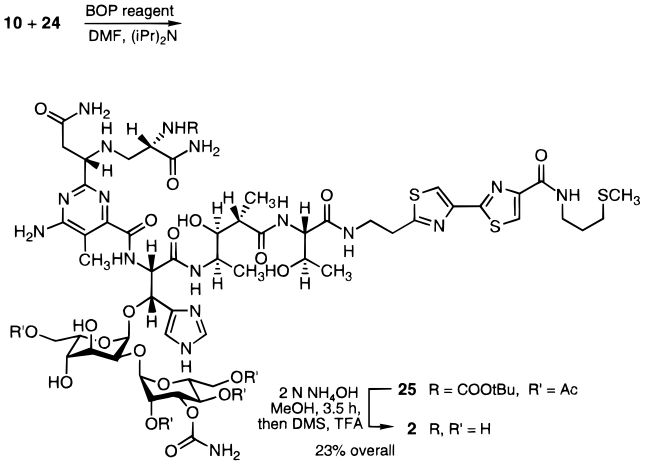
Scheme 8



°C for 22 h by using silver trifluoromethanesulfonate as the catalyst. Chromatographic separation of the reaction mixture on a silica gel flash column gave the desired di-Boc disaccharide **27** in 12% yield, as well as mono-Boc disaccharide **28** (11% yield). The conversion of **28** to the corresponding di-Boc derivative **27** was effected by treatment of **28** with di-*tert*-butyl dicarbonate at 25 °C for 1 h; this limited Boc substitution exclusively to the N^{im} nitrogen, instead of both N^{im} and carbamoyl nitrogen atoms (cf. **16** → **17**, Scheme 3). Compound **27** obtained in this fashion had exactly the same spectroscopic properties as compound **27** isolated directly from the coupling of **6** and **7**. Compounds **27** and **28** were characterized by their ^{1}H NMR and high-resolution FAB mass spectra. Compound **27** was then debenzylated selectively by hydrogenation over 10% palladium-on-carbon at 25 °C for 20 h. The pure carboxylic acid **29** was obtained as a colorless foam characterized by its ^{1}H and ^{13}C NMR spectra as well as a molecular ion in the high-resolution FAB mass spectrum.

The condensation of carboxylic acid **29** with the hydrochloride of benzyl (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvalerate (**8**) was effected by the use of DCC−HOBt in the presence of *N*,*N*-diisopropylethylamine at 25 °C for 5 h (Scheme 8). The corresponding benzyl ester **30** was obtained as a colorless foam in 70% yield and was characterized by its ^{1}H and ^{13}C NMR spectra as well as its high-resolution FAB mass spectrum. To debenzylate **30** effectively, the palladium black catalyst was freshly made according to the reported procedure[46] with some improvement. In particular, freshly prepared palladium black had to be washed thoroughly with water and then anhydrous methanol to preclude the solvolysis of the N^{im}-Boc protecting group.[47] Benzyl ester **30** was hydrogenated over the freshly prepared palladium black in absolute ethanol under 1 atm of hydrogen at 25 °C for 40 h. The completely debenzylated product (**31**) was obtained as a colorless foam in 52% yield. ^{1}H NMR spectroscopy confirmed that the two benzyl groups on gulose had been removed, and that no solvolysis of either Boc group had occurred. The structure of **31** was also

Scheme 9



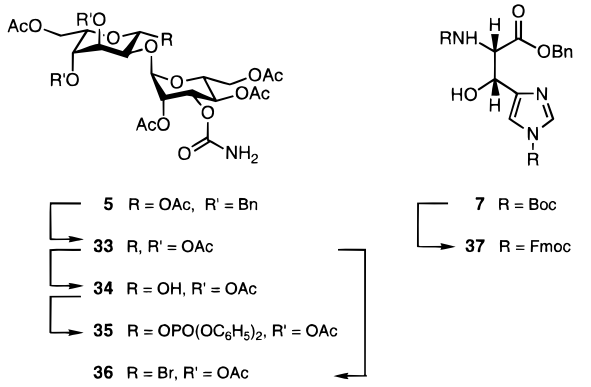
confirmed by ^{13}C NMR spectroscopy, and by low- and high-resolution FAB mass spectrometry. Dipeptide disaccharide **31** was coupled with threonylbithiazole **9** at 25 °C for 24 h using DCC−HOBt in the presence of *N*,*N*-diisopropylethylamine in anhydrous DMF. Di-Boc pentapeptide **32** was obtained as a colorless foam in 59% yield and was characterized fully. The Boc protecting groups on **32** were removed using trifluoroacetic acid at 0 °C for 3 h to provide pentapeptide disaccharide **24** as the free amine in 87% yield after chromatographic purification on an Amberlite XAD-2 column. This intermediate was also characterized by ^{1}H NMR spectroscopy and high-resolution FAB mass spectrometry. The final coupling of **24** with N^{α}-*t*-Boc-pyrimidoblamic acid (**10**)[36] was effected by the use of benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate[48] (Scheme 9). The reaction was conducted in anhydrous DMF in the presence of *N*,*N*-diisopropylethylamine at 25 °C for 28 h. The condensation product **25** so obtained was the same as compound **25** obtained by the use of *N*-protected carbamoyl intermediates (cf. Schemes 6 and 9). However, the material obtained by the latter route was a single compound. After extractive workup, crude **25** was deacetylated directly with a 1:1 mixture of 2 N aqueous NH_4OH−methanol at 25 °C for 3.5 h. The Boc-protected intermediate was treated further with trifluoroacetic acid in dimethyl sulfide at 0 °C for 1.5 h to remove the Boc protecting group. Crude bleomycin demethyl A_2 (**2**) was converted to its Cu(II) complex, which was purified by chromatography on a CM Sephadex C-25 column. The purified Cu(II) complex of bleomycin demethyl A_2 (**2**) was decomplexed with Na EDTA and purified by flash chromatography on a reversed-phase (C_{18}) column. Compound **2**, $[\alpha]^{25}_D = 17.1°$ (*c* 0.14, H_2O), was obtained as a colorless foam in 23% overall yield from **24**. The purified product had chromatographic properties identical with those of an authentic, naturally derived sample, as well as with those of BLM demethyl A_2 (**2**) obtained by the protected carbamoyl route. The structure of bleomycin demethyl A_2 was further confirmed by its ^{1}H NMR spectrum, which was identical with that of an authentic sample, and by its high-resolution FAB mass spectrum. Intermediates **27**−**32** and **25** were all obtained in a good state of purity after chromatographic separation, as judged by HPLC analysis. These compounds were characterized by their ^{1}H NMR and high-resolution FAB mass spectral analyses. Compounds **29** and **30** were also characterized by their ^{13}C NMR spectra. The

(46) Felix, A. M.; Jimenez, M. H.; Meienhofer, J.; Revesz, L.; Büchi, G. *Org. Synth.* **1979**, *59*, 159.

(47) The catalyst should be saturated with solvent at all times; the dry catalyst is pyrophoric.

(48) (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219. (b) Castro, B.; Dormoy, J. R.; Dourtoglou, B.; Evin, G.; Selve, C.; Ziegler, J. C. *Synthesis* **1976**, 751. (c) Castro, B.; Evin, G.; Selve, C.; Seyer, R. *Synthesis* **1977**, 413.

Scheme 10



absence of racemization during the peptide couplings was verified both by detailed NMR spectral analysis and by hydrolysis of key intermediates following peptide coupling and analysis of the recovered fragments for loss of optical integrity.

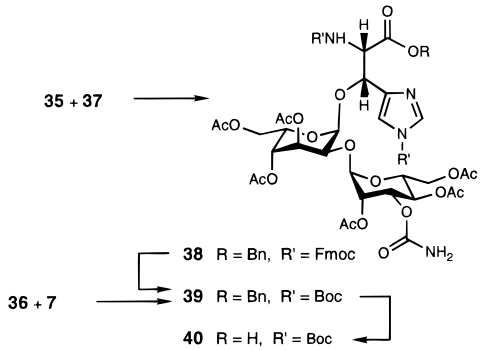
In addition to the syntheses of BLM demethyl A_2, BLM A_2, and decarbamoyl BLM demethyl A_2 reported here, the methods outlined here have been applied successfully for the synthesis of a number of novel BLM analogues.[21,38,49]

Optimization of the Synthesis of the Carbohydrate Moiety of Bleomycin. While the route employed for the synthesis of bleomycin without protection of the carbamoyl group is entirely workable, facets of the overall scheme that may be considered less than optimal include the low yield associated with the introduction of β-hydroxyhistidine (i.e., **6** + **7** → **27**, Scheme 7) and the partial loss of the imidazole protecting group in the same transformation, as well as the occasional difficulty in effecting the debenzylation of intermediate **30** (Scheme 8). Further, although the glycosylated intermediates isolated were single isomers having the anomeric configurations indicated in each case, it was not possible to analyze the anomeric composition of the products in most cases. Accordingly, we have investigated alternative synthetic approaches.

As outlined in Scheme 10, the difficulty in removing the benzyl protecting groups from advanced synthetic intermediates can be obviated by replacing the benzyl groups prior to introduction of the β-hydroxyhistidine moiety. Because the additional acetyl groups introduced into the disaccharide deactivate the anomeric center, the anomeric center must be converted to an intermediate more reactive than the species accessible by treatment of a glycosyl chloride with AgOTf. Accordingly, dibenzyl disaccharide **5** was converted to peracetylated disaccharide **33** in essentially quantitative yield by debenzylation over 20% palladium-on-carbon followed by acetylation.[9] Removal of the anomeric acetate (**33** → **34**) followed by conversion to the diphenyl phosphate derivative (**34** → **35**) also followed literature precedent[9] and proceeded in 80% overall yield. As an alternative, the anomeric center in **33** was also converted directly to the bromide (**33** → **36**) in 93% yield via the agency of 30% HBr in HOAc.

To circumvent the difficulty encountered with loss of the N^{im} protecting group during introduction of the β-OH histidine moiety, the latter was protected as the di-Fmoc derivative **37**. The Fmoc group is generally easily removed with amine bases, but is stable under acidic conditions.[50] Compound **37** was readily accessible by deprotection of **7** (CF_3COOH) and reprotection with *N*-(9-fluorenylmethoxycarbonyl)succinimide[51] (67% overall yield) (Scheme 10).

Scheme 11



Coupling of glycosyl disaccharide **35** with di-Fmoc β-hydroxyhistidine derivative **37** in 2:1 ether−CH_2Cl_2 in the presence of TMSOTf gave the desired product **38** after 20 min at 0 °C. Compound **38** was obtained as an 8:1 mixture of α and β anomers from which the desired α anomer was isolated in 58% yield by preparative silica gel TLC (Scheme 11). Compound **38** was deblocked with piperidine at 25 °C and then converted to the di-Boc derivative by treatment with di-*tert*-butyl dicarbonate.

Compound **39** was isolated as a foam in 70% yield and converted promptly to the corresponding carboxylic acid **40** by hydrogenolysis over 10% palladium-on-carbon (99% yield).

Compound **40** was also accessible in a more direct fashion via the coupling of glycosyl bromide **36** with di-Boc β-hydroxyhistidine derivative **7** in the presence of tetramethylurea (28% conversion; 54% yield based on consumed **7**). Because this transformation was carried out at a lower temperature over a shorter period of time (40 °C for 14 h) than the analogous coupling of **6** and **7** (48 °C for 22 h) (cf. Schemes 7 and 11), loss of the N^{im} protecting group was not an important side reaction. While the overall yields of **40** accessible from **35** and **36** are comparable, the route involving glycosyl bromide **36** is shorter and more convenient operationally and has been used successfully to prepare quantities of intermediates for the elaboration of bleomycin congeners.

Also explored with quite favorable results was application of the carbohydrate trichloroacetimidate intermediates pioneered by Schmidt[52] for the elaboration of the carbohydrate moiety of bleomycin (Scheme 12). Accordingly, key saccharide intermediates 1,2,4,6-tetra-*O*-acetyl-3-*O*-(*p*-nitrophenoxycarbonyl)-α-D-mannopyranose (**41**)[29c] and 3,4-di-*O*-benzyl-1,6-*O*-diacetyl-β-L-gulopyranose (**44**)[29b] were prepared as described previously. Treatment of saccharide **41** with anhydrous ammonia afforded the 3-*O*-carbamoylated product after 1 h; deacetylation of the anomeric position occurred after extended reaction to afford 2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranose (**42**) cleanly in 74% yield. The Schmidt trichloroacetimidate **43** was prepared essentially quantitatively from saccharide **42** using trichloroacetonitrile in the presence of anhydrous potassium carbonate; the product was predominantly the β-imidate.[52] Treatment of glycosyl acceptor **44**[29b] with imidate **43** in the presence of 5 mole percent of $BF_3{\cdot}OEt_2$ provided the 1,2-*trans*-linked disaccharide **5** in 86% yield as a >10:1 mixture of α

(49) (a) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193. (b) Kane, S. A.; Sasaki, H.; Hecht, S. M. *J. Am. Chem. Soc.* **1995**, *117*, 9107. (c) Zuber, G.; Quada, Jr., J. C.; Hecht, S. M. *J. Am. Chem. Soc.* **1998**, *120*, 9368.

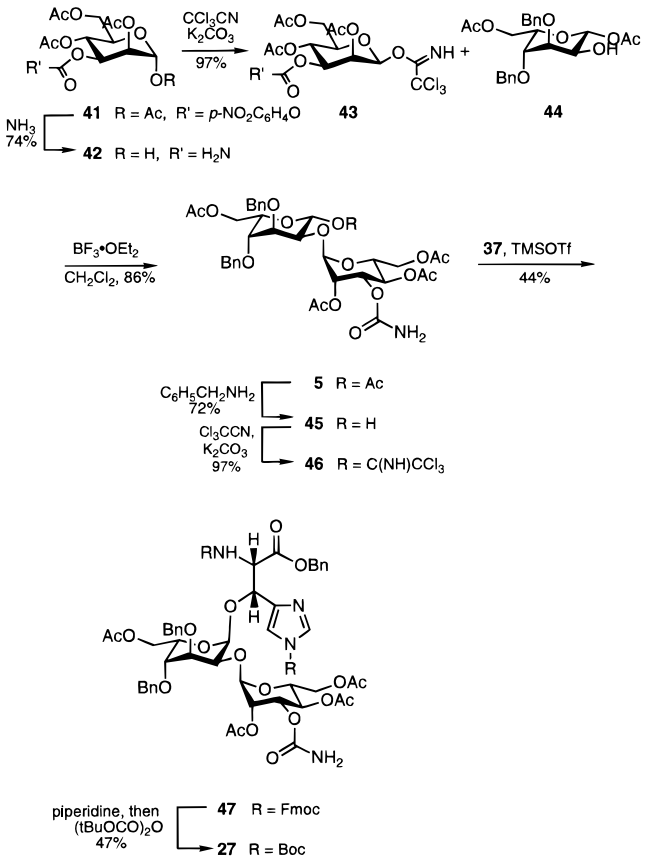
(50) Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1980**, 45, 72.

(51) Lapatsanis, L.; Milias, G.; Froussios, K.; Kolovos, M. *Synthesis* **1983**, 671.

(52) Schmidt, R. R. *Angew Chem., Int. Ed. Engl.* **1986**, *25*, 212.

(53) Sakai, T. T.; Riordan, J. M.; Glickson, J. D. *Biochim. Biophys. Acta* **1983**, *758*, 176.

Scheme 12



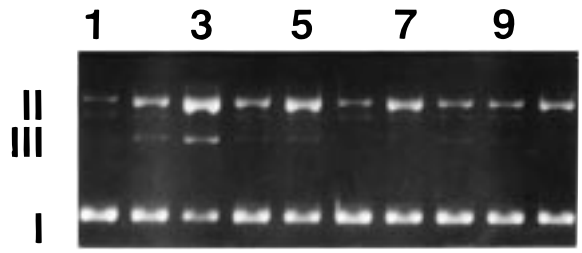


Figure 3. Relaxation of pBR322 Form I DNA by BLM congeners: lane 1, DNA alone; lane 2, 1 μM Fe(II)·BLM A_2; lane 3, 5 μM Fe(II)·BLM A_2; lane 4, 1 μM Fe(II)·BLM demethyl A_2; lane 5, 5 μM Fe(II)·BLM demethyl A_2; lane 6, 1 μM synthetic Fe(II)·BLM demethyl A_2; lane 7, 5 μM synthetic Fe(II)·BLM demethyl A_2; lane 8, 5 μM BLM A_2; lane 9, 5 μM BLM demethyl A_2; lane 10, 5 μM Fe^{2+}.

and β anomers, respectively. Disaccharide **5** was then deacylated at the anomeric position ($C_6H_5CH_2NH_2$, 25 °C, 72%) and activated as trichloroacetimidate **46** in 97% yield.[52]

To prepare glycosylated N^{α},N^{im}-bis(*t*-Boc)-(*S*)-*erythro*-β-hydroxyhistidine benzyl ester (**27**), a key intermediate in the synthesis of BLM A_2 (cf. Scheme 7), imidate **46** was used to glycosylate N^{α},N^{im}-bis(Fmoc)-(*S*)-*erythro*-β-hydroxyhistidine benzyl ester (**37**). The α glycoside was formed using TMSOTf under thermodynamic conditions.[52] Glycosylated N^{α},N^{im}-bis-(Fmoc)-(*S*)-*erythro*-β-hydroxyhistidine benzyl ester (**47**), obtained in 44% yield as a colorless glass, was deprotected with piperidine and refunctionalized using di-*tert*-butyl dicarbonate to provide key intermediate **27**.

Biochemical Characterization of Synthetic BLM Demethyl A_2. Synthetic BLM demethyl A_2 was also characterized for its ability to mediate the cleavage of DNA. As shown in Figure 3, synthetic and naturally derived samples of bleomycin demethyl A_2 effected the relaxation of supercoiled plasmid DNA to essentially the same extent when incubated in the presence of equimolar Fe^{2+}. As reported previously,[26a,b] both samples of **2** were less potent than Fe(II)·BLM A_2 in mediating DNA cleavage. Also studied was the sequence selectivity of DNA cleavage by synthetic bleomycin demethyl A_2. As shown in Figure 1 (Supporting Information), incubation of a 158-base pair 3′-^{32}P-end-labeled DNA duplex with Fe(II)·BLM demethyl A_2 afforded the same pattern of cleavage as that obtained with Fe(II)·BLM A_2 itself. The essentially identical patterns of cleavage, as well as the lesser potency of BLM demethyl A_2, were both as anticipated on the basis of literature precedent.[26a,b,53] Also apparent from the better resolved bands on the polyacrylamide gel was the appearance of products having both 3′-phosphate and 3′-phosphoroglycolate termini.[10,11] This finding confirms that synthetic BLM demethyl A_2 also mediates the same chemical transformations of DNA noted for naturally derived bleomycins. Although the syntheses of naturally occurring BLMs have been reported previously, this represents the first confirmation of the nature of the actual chemistry of DNA degradation mediated by a synthetic BLM.

Experimental Section

General Methods. NMR chemical shifts are referenced to $CHCl_3$ at 7.24 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; br, broad; q, quartet; br s, broad singlet; br d, broad doublet; dd, doublet of doublets; dq, doublet of quartets. Optical rotation data are reported as $[\alpha]_D^{temp}$ (concentration in g/100 mL of solvent). Chemical ionization (CI) mass spectra were recorded using isobutane. Some of the high-resolution data were obtained with the assistance of the Michigan State University Mass Spectrometry Facility (supported by NIH/NCRR Grant RR0480) or the Washington University Mass Spectrometry Resource, supported by the NIH/NCRR (Grant RR0954). All organic chemicals, as well as palladium black, were purchased from Aldrich Chemical Co., as were all deuterated NMR solvents. Pyridine was heated at reflux for 3 h over KOH and then distilled prior to use.

Ferrous ammonium sulfate [$Fe^{II}(NH_4)_2(SO_4)_2$] was purchased from EM Science. Agarose was obtained from Bethesda Resarch Laboratories. Acrylamide, *N*,*N*′-methylenebisacrylamide, ethidium bromide, piperidine, cacodylic acid, Trizma base, bromophenol blue, and herring sperm DNA (carrier for Maxam−Gilbert sequencing reactions) were purchased from Sigma Chemicals. The disodium salt of (ethylenedinitrilo)tetraacetic acid (EDTA) was purchased from J. T. Baker, and xylene cyanol was from Bio-rad Laboratories. Endonuclease *Hin*dIII was purchased from Promega; *Eco*RV was from New England BioLabs. Alkaline phosphatase, Sephadex G-50 spin columns, and plasmid pBR322 were obtained from Boehringer Mannheim Biochemicals. T4 Polynucleotide kinase was purchased from United States Biochemicals. [γ-^{32}P]ATP (7000 Ci/mmol) was from ICN Radiochemicals.

Coupling of Pentapeptide Disaccharide 24 with *N*-*t*-Boc-pyrimidoblamic Acid (10). Deprotection and Purification of Bleomycin Demethyl A_2 (2) and Decarbamoyl Bleomycin Demethyl A_2 (26). To a solution containing 29 mg (23 μmol) of *N*-*t*-Boc-pyrimidoblamic acid (**10**)[36,37] in 0.3 mL of anhydrous DMF were added successively 5 mg (39 μmol) of *N*,*N*-diisopropylethylamine, 3.5 mg (26 μmol) of 1-hydroxybenzotriazole, and 6 mg (26 μmol) of *N*,*N*′-dicyclohexylcarbodiimide (DCC). The reaction mixture was stirred at 25 °C for 2 days. The solvent was concentrated under diminished pressure, and the residue was partitioned between water and ethyl acetate. The aqueous phase was extracted with ethyl acetate, and the combined organic phase was back-extracted with water. The combined aqueous phase was concentrated to dryness under diminished pressure to afford the crude coupled product (**25**).

Crude **25** was treated with 2 mL of 2 N ammonium hydroxide and 2 mL of methanol at 25 °C for 4.5 h to remove the acetyl groups. After concentration of the solution, the residue was treated with 1 mL of 5:3 trifluoroacetic acid−dimethyl sulfide at 0 °C for 75 min to remove the Boc protecting group. The solvent was concentrated, and

the residue was dissolved in 5 mL of water. The aqueous solution was neutralized (to pH 7.3) with aqueous sodium bicarbonate solution and applied to an Amberlite XAD-2 column (5 × 1.4 cm). The column was washed with 30 mL of water and then with 100 mL of methanol. The methanol eluate was concentrated under diminished pressure to afford crude bleomycin demethyl A_2.

The crude product and 4 mg of $CuSO_4$ were dissolved in 1 mL of 0.05 M ammonium formate. The dark blue solution was applied to a CM-Sephadex C-25 column (75 × 1 cm, buffered with 0.05 M ammonium formate). After washing with 35 mL of 0.05 M ammonium formate, the column was washed with a linear gradient of 0.05−0.5 M ammonium formate. The fractions were monitored by UV absorbance at 292 nm. The first product peak was collected, treated with 350 mg of EDTA disodium salt for 30 min, and then applied to an Amberlite XAD-2 column (5 × 1.4 cm). After washing successively with 20 mL of water, 30 mL of 50% sodium chloride, and 30 mL of water, the product was eluted with 50 mL of 4:1 methanol−0.002 N hydrochloric acid. The solvent was concentrated, and the residue was dissolved in a small amount of water. The frozen aqueous solution was lyophilized to give bleomycin demethyl A_2 (**2**) as a colorless powder: yield 1.1 mg (3.4% from **24**); silica gel TLC R_f 0.63 (1:2 10% NH_4OAc−EtOH); R_f 0.47 (1:3 10% NH_4OAc−EtOH); ^1H NMR (D_2O) δ 1.06 (d, 3 H, J = 6 Hz), 1.10 (d, 3 H, J = 7 Hz), 1.13 (d, 3 H, J = 7 Hz), 1.87−1.94 (m, 2 H), 1.97 (s, 3 H), 2.07 (s, 3 H), 2.53−2.60 (m, 4 H), 2.67 (s, 1 H), 2.72−2.86 (m, 2 H), 3.15−3.30 (m, 4 H), 3.44−3.52 (m, 2 H), 3.54−3.63 (m 3 H), 3.65−3.83 (m, 6 H), 3.87−3.92 (m, 2 H), 3.97−4.06 (m, 4 H), 4.11−4.15 (m, 1 H), 4.17−4.24 (m, 2 H), 4.99 (s, 1 H), 5.06 (d, 1 H, J = 6.5 Hz), 5.22 (d, 1 H, J = 3.5 Hz), 5.45 (d, 1 H, J = 6.5 Hz), 7.57 (s, 1 H), 8.00 (s, 1 H), 8.14 (s, 1 H), and 8.76 (s, 1 H); mass spectrum (FAB), m/z 1400 $(M + H)^+$; mass spectrum (FAB), m/z 1400.497 $(M + H)^+$ ($C_{54}H_{82}N_{17}O_{21}S_3$ requires 1400.503).

A second peak from the Sephadex column was collected and treated with 400 mg of EDTA disodium salt. This solution was also purified by Amberlite XAD-2 column chromatography as described above to give decarbamoyl bleomycin demethyl A_2 (**26**) as a colorless powder: yield 2.7 mg (8.6%); silica gel TLC R_f 0.33 (1:2 10% NH_4OAc−EtOH); R_f 0.26 (1:3 10% NH_4OAc−EtOH); ^1H NMR (D_2O) δ 0.95 (d, 3 H, J = 7 Hz), 1.03 (d, 3 H, J = 7 Hz), 1.07 (d, 3 H, J = 7 Hz), 1.92 (s, 3 H), 2.08 (m, 2 H), 2.67 (q, 1 H, J = 7 Hz), 2.70 (s, 3 H), 2.67−2.92 (m, 4 H), 3.15 (m, 3 H), 3.19 (s, 3 H), 3.25 (m, 1 H), 3.35−3.58 (m, 6 H), 3.60 (t, 2 H, J = 6 Hz), 3.75 (m, 2 H), 3.75 (s, 1 H), 3.85 (m, 2 H), 3.80−4.00 (m, 4 H), 4.10 (d, 1 H, J = 6 Hz), 4.18 (m, 2 H), 4.97 (s, 1 H), 5.09 (d, 1 H, J = 9 Hz), 5.18 (d, 1 H, J = 2 Hz), 5.29 (d, 1 H, J = 9 Hz), 7.60 (s, 1 H), 8.05 (s, 1 H), 8.18 (s, 1 H), and 8.77 (s, 1 H). This material was identical in all respects with an authentic sample of decarbamoyl bleomycin demethyl A_2.[38]

Cu(II)·BLM Demethyl A_2. A solution of 1.0 mg (7.1 μmol) of BLM demethyl A_2 in 120 μL of 50 mM citrate buffer, pH 4.7, was treated with 1.2 mg (7.3 μmol, 1.1 equiv) of $CuSO_4$. The solution was maintained at 0−4 °C for 30 min, and the resulting blue solution was purified by reversed-phase HPLC on an Alltima C_{18} column (250 × 4.6 mm) using a linear gradient of 0.1 M NH_4OAc, pH 5.5, containing increasing amounts of CH_3CN (0 → 25 min, linear gradient from 0 to 88% CH_3CN in aqueous NH_4OAc) at a flow rate of 1.0 mL/min. The eluate was monitored at 290 nm; the product eluted at 13.3 min. Concentration of the eluate under diminished pressure provided Cu(II)·BLM demethyl A_2 as a blue powder: yield 0.75 mg (73%).

Bleomycin A_2 (1). A solution of 0.75 mg (5.1 μmol) of Cu·BLM demethyl A_2 in 200 μL of dry MeOH under Ar was treated with 30 μL (510 μmol) of CH_3I. The reaction mixture was stirred in the dark at room temperature. The reaction was monitored by reversed-phase HPLC on an Alltima C_{18} column (250 × 4.6 mm) using a linear gradient of 0.1 M NH_4OAc, pH 5.5, containing increasing amounts of CH_3CN (0 → 25 min, linear gradient from 0 to 88% CH_3CN in NH_4OAc) at a flow rate of 1.0 mL/min. The eluate was monitored at 290 nm. After 6 days, the reaction was complete. After the reaction mixture was concentrated under diminished pressure, crude Cu(II)·BLM A_2 was isolated by preparative reversed-phase HPLC using the same mobile phase described above. The product eluted at 11.1 min. Concentration of the eluate afforded Cu(II)·BLM A_2 as a blue powder: yield 0.55 mg (76%).

The synthesized Cu(II)·BLM A_2 was dissolved in 15% aqueous EDTA solution and stirred at 25 °C for 12 h. The mixture was applied to a C_{18} open column (7 × 1 cm) and washed with 250 mL of deionized water. Then BLM A_2 was eluted from the column with 4:1 MeOH−2 mM HCl. After concentration of the eluate, BLM A_2 was purified further by reversed-phase HPLC on an Alltima C_{18} column (250 × 4.6 mm) using a linear gradient of 0.1 M NH_4OAc, pH 4.5, containing increasing amounts of CH_3CN (0 → 30 min, linear gradient from 5 to 12% CH_3CN in NH_4OAc) at a flow rate of 1.5 mL/min. The eluate was monitored by 290 nm; the product eluted at 28.8 min. Concentration of the eluate under diminished pressure to a small volume, followed by lyophilization, afforded BLM A_2 as a colorless solid: yield 0.38 mg (61% from Cu(II)·BLM A_2); ^1H NMR (D_2O) δ 1.00−1.10 (m, 9H), 1.92 (s, 3H), 2.10 (m, 1H), 2.40 (m, 1H), 2.50−2.70 (m, 4H), 2.83 (s, 6H), 2.91 (m, 1H), 3.18 (m, 1H), 3.25−4.04 (m, 23H), 4.12 (d, 1H, J = 5 Hz) 4.65 (m, 1H), 4.85 (br s, 1H), 5.00 (d, 1H, J = 2 Hz), 5.20 (m, 2H), 7.20 (s, 1H), 7.78 (s, 1H), 7.95 (s, 1H), and 8.15 (s, 1H); mass spectrum (FAB), m/z 1414.5 (M^+); mass spectrum (FAB), m/z 1414.523 (M^+) ($C_{55}H_{84}N_{17}O_4S_3$ requires 1414.519) and 1415.530 $(M + H)^+$ ($C_{55}H_{85}N_{17}O_4S_3$ requires 1414.527).

Coupling of Disaccharide Chloride 6 with Benzyl N^{α},N^{im}-Bis-(*tert*-butoxycarbonyl)-(*S*)-*erythro*-β-hydroxyhistidine (7). β-Hydroxyhistidine benzyl ester derivative **7** (1.0 g, 2.16 mmol) and 855 mg (3.32 mmol) of silver trifluoromethanesulfonate were dried overnight under vacuum and dissolved in 3 mL of anhydrous 1,2-dichloroethane and 0.5 mL (0.48 g, 4.1 mmol) of 1,1,3,3-tetramethylurea. A solution of 2.27 g (3.02 mmol) of disaccharide chloride **6** in 10 mL of anhydrous 1,2-dichloroethane was added dropwise to this solution at 0 °C. The reaction mixture was stirred at 48 °C for 22 h. To the cooled reaction mixture was added 100 mL of brine. The well-shaken mixture was diluted with 400 mL of ethyl acetate, filtered through a bed of Celite, and washed with ethyl acetate. The organic phase was washed with saturated sodium bicarbonate and water. The dried ($MgSO_4$) solution was filtered, and the filtrate was concentrated. The residue was purified by flash chromatography on a silica gel column (30 × 2 cm). Elution with 1:2 toluene−ethyl acetate afforded the di-Boc-protected disaccharide **27** as a colorless foam: yield 308 mg (12%); silica gel TLC R_f 0.33 (10:1 chloroform−methanol); $[\alpha]^{25}_D$ −19.0° (c 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.39 (s, 9 H), 1.60 (s, 9 H), 1.84 (s, 3 H), 2.00 (s, 3 H), 2.02 (s, 3 H), 2.10 (s, 3 H), 3.67 (m, 1 H), 3.86 (m, 1 H), 3.99 (m, 1 H), 4.04 (m, 1 H), 4.10 (m, 1 H), 4.30 (m, 2 H), 4.41 (m, 1 H), 4.53 (m, 1 H), 4.61 (m, 1 H), 4.66 (m, 2 H), 4.81 (m, 1 H), 4.90 (m, 1 H), 4.99 (m, 1 H), 5.07 (m, 1 H), 5.16 (m, 1 H), 5.21 (m, 1 H), 5.25 (m, 1 H) 5.39 (m, 1 H), 6.42 (br d, 1 H), 7.12−7.40 (m, 16 H), and 7.92 (s, 1 H); mass spectrum (FAB), m/z 1177 $(M + H)^+$; mass spectrum (FAB), m/z 1177.467 $(M)^+$ ($C_{58}H_{73}N_4O_{22}$ requires 1177.471).

The flash silica gel column was further eluted with 20:1 chloroform−methanol, affording mono-Boc-protected disaccharide **28** as a pale yellow foam: yield 252 mg (11%); silica gel TLC R_f 0.20 (10:1 $CHCl_3$−CH_3OH); ^1H NMR ($CDCl_3$) δ 1.37 (s, 9 H), 1.86 (s, 3 H), 2.00 (s, 3 H), 2.04 (s, 3 H), 2.10 (s, 3 H), 3.67 (m, 1 H), 3.86 (m, 1 H), 3.99 (m, 1 H), 4.04 (m, 1 H), 4.10 (m, 1 H), 4.30 (m, 2 H), 4.41 (m, 1 H), 4.53 (m, 1 H), 4.61 (m, 1 H), 4.66 (m, 2 H), 4.81 (m, 1 H), 4.90 (m, 1 H), 4.99 (m, 1 H), 5.07 (m, 1 H), 5.16 (m, 1 H), 5.21 (m, 1 H), 5.25 (m, 1 H), 5.39 (m, 1 H), 6.71 (s, 1 H), 7.10−7.40 (m, 16 H), and 7.92 (s, 1 H); mass spectrum (FAB), m/z 1077.418 $(M + H)^+$ ($C_{53}H_{65}N_4O_{20}$ requires 1077.419).

Reprotection of Mono-Boc-Protected Disaccharide 28. Compound **28** (252 mg, 0.234 mmol) in 2 mL of dry pyridine was treated with 140 mg (0.64 mmol) of di-*tert*-butyl dicarbonate at 25 °C for 1 h. The solution was concentrated under diminished pressure with co-evaporation of portions of toluene; the foamy residue was purified by flash chromatography on a silica gel column (20 × 2 cm). Elution with 1:2 toluene−ethyl acetate gave compound **27** as a white foam: yield 177 mg (64%). The di-Boc product obtained in this fashion was identical to the compound described above, as judged by silica gel TLC and ^1H NMR spectroscopy.

Selective Debenzylation of Disaccharide Benzyl Ester 27. A mixture of 480 mg (0.408 mmol) of disaccharide benzyl ester **27** and 0.11 g of 10% palladium-on-carbon in 10 mL of absolute ethanol was stirred at 25 °C under 1 atm of hydrogen for 20 h. The catalyst was filtered through Celite and washed with ethanol. The filtrate was concentrated under diminished pressure, and the residue was purified by flash chromatography on a silica gel column (20 × 2 cm). Elution with 1:2 toluene−ethyl acetate, and then 15:1 and finally 10:1 chloroform−methanol, afforded disaccharide derivative **29** as a colorless foam: yield 241 mg (54%); silica gel TLC R_f 0.41 (5:1 chloroform−methanol); $[\alpha]_D^{25}$ +0.33° (*c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 9 H), 1.59 (s, 9 H), 1.87 (s, 3 H), 2.03 (s, 3 H), 2.07 (s, 3 H), 2.13 (s, 3 H), 3.40 (br s, 1 H), 3.65 (s, 1 H), 3.77−4.18 (m, 6 H), 4.20−4.60 (m, 5 H), 4.65−4.84 (m, 2 H), 5.15−5.37 (m, 4 H), 5.47−5.57 (m, 1 H), 5.85 (br s, 1 H), 7.14 (br s, 2 H), 7.22−7.40 (m, 10 H), 7.42 (s, 1 H), and 8.04 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 21.08, 21.33, 28.24, 28.73, 62.68, 62.84, 62.97, 65.33, 66.12, 69.67, 69.72, 70.37, 70.78, 73.16, 73.26, 74.14, 80.11, 86.37, 98.70, 115.33, 128.54, 128.91, 129.05, 129.42, 137.12, 137.67, 137.85, 138.11, 142.32, 147.24, 155.99, 170.48, 170.56, 170.68, 171.03, 171.24, 171.68, and 172.40; mass spectrum (FAB), *m*/*z* 1125 $(M + Na)^+$; mass spectrum (FAB), *m*/*z* 1125.380 $(M + Na)^+$ ($C_{51}H_{66}N_4O_{22}Na$ requires 1125.380).

Coupling of Disaccharide 29 with Benzyl (2*S*,3*S*,4*R*)-4-Amino-3-hydroxy-2-methylvalerate (8). A mixture of 72 mg (66 μmol) of disaccharide **29** and 27 mg (98 μmol) of benzyl valerate hydrochloride **8** was coevaporated with portions of toluene, dried under vacuum overnight, and then dissolved in 2 mL of anhydrous CH_2Cl_2. To the stirred solution were added successively 27 μL (20 mg, 150 μmol) of *N*,*N*-diisopropylethylamine, 15 mg (110 μmol) of 1-hydroxybenzotriazole, and 25 mg (120 μmol) of *N*,*N*′-dicyclohexylcarbodiimide. The reaction mixture was stirred at 25 °C under Ar for 5 h and then diluted with 80 mL of ethyl acetate. The insoluble material was removed by filtration through Celite and washed with 20 mL of ethyl acetate. The filtrate was washed successively with 30 mL of 0.5 M aqueous citric acid solution, 30 mL of saturated sodium bicarbonate, 30 mL of water, and 30 mL of brine. The combined organic phase was dried (Na_2SO_4) and concentrated. The residue was purified by flash chromatography on a silica gel column (15 × 2 cm). Elution with 80:1 and then 40:1 chloroform−methanol afforded the desired product **30** as a colorless foam: yield 60 mg (70%); silica gel TLC R_f 0.41 (10:1 chloroform−methanol); $[\alpha]^{25}_D$ −30° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 3 H, J = 6 Hz), 1.19 (d, 2 H, J = 6.5 Hz), 1.41 (s, 9 H), 1.58 (s, 9 H), 1.94 (s, 3 H), 1.98 (s, 3 H), 2.07 (s, 3 H), 2.16 (s, 3 H), 2.40−2.51 (m, 1 H), 3.50−3.65 (m, 1 H), 3.78−3.96 (m, 3 H), 4.00−4.15 (m, 4 H), 4.20−4.50 (m, 6 H), 4.54−4.70 (m, 4 H), 4.76−4.90 (m, 2 H), 5.05−5.18 (m, 4 H), 5.24−5.44 (m, 3 H), 6.45 (m, 1 H), 7.17 (br s, 2 H), 7.22−7.40 (m, 15 H), 7.44 (s, 1 H), and 7.99 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 12.66, 16.08, 21.35, 21.17, 28.22, 28.69, 42.48, 48.16, 62.84, 66.59, 66.71, 67.75, 69.59, 70.22, 70.42, 70.57, 70.76, 72.54, 73.31, 73.44, 73.54, 74.09, 74.61, 75.30, 75.63, 80.46, 86.66, 97.66, 116.78, 128.57, 128.94, 136.40, 136.52, 137.53, 137.82, 137.95, 138.27, 138.68, 140.43, 146.97, 155.82, 168.10, 168.77, 170.39, 170.49, 170.68, 171.11, and 175.66; mass spectrum (FAB), *m*/*z* 1328 $(M + Na)^+$; mass spectrum (FAB), *m*/*z* 1328.526 $(M + Na)^+$ ($C_{64}H_{83}N_5O_{24}Na$ requires 1328.532).

Debenzylation of Disaccharide Dipeptide Benzyl Ester 30. To a suspension of 80 mg (0.45 mmol) of $PdCl_2$ in 40 μL of 2 N HCl were added 4 mL of boiling water, 19.2 μL (23 mg, 0.51 mmol) of formic acid, and 1.25 mL of 10% potassium hydroxide solution. The pH of the mixture was adjusted to ∼7−8 with formic acid, and the mixture was boiled for 7−9 min to form the palladium black as big chunks. The liquid phase was removed, and the catalyst was washed successively with 30 mL of water and 50 mL of methanol. The disaccharide dipeptide benzyl ester **30** (29 mg, 22 μmol) was dissolved in 3 mL of absolute ethanol and added to the flask containing the freshly prepared palladium black catalyst. The reaction mixture was stirred at 25 °C under 1 atm of hydrogen for 40 h. The catalyst was filtered through Celite and washed with methanol. The filtrate was concentrated under diminished pressure, and the residue was purified by flash chromatography on a silica gel column (12 × 2 cm). Elution with 5:1 and then 1:1 chloroform−methanol afforded the debenzylated product **31** as a colorless foam: yield 12 mg (52%); silica gel TLC R_f 0.35 (3:1 chloroform−methanol); $[\alpha]_D^{25}$ +11.8° (*c* 0.5, methanol); ^{1}H NMR (CD_3OD) δ 1.11 (d, 3 H, J = 6.5 Hz), 1.27 (d, 3 H, J = 5 Hz), 1.40 (s, 9 H), 1.60 (s, 9 H), 1.99 (s, 3 H), 2.04 (s, 6 H), 2.11 (s, 3 H), 2.40−2.50 (m, 1 H), 3.20−3.32 (m, 2 H), 3.60−3.70 (m, 1 H), 3.70−3.80 (m, 1 H), 3.90−4.18 (m, 8 H), 4.50−4.65 (m, 1 H), 5.00−5.15 (m, 4 H), 5.20−5.35 (m, 3 H), 5.38−5.50 (m, 2 H), 7.58 (s, 1 H), and 8.15 (s, 1 H); mass spectrum (FAB), *m*/*z* 1036 $(M + H)^+$; mass spectrum (FAB), *m*/*z* 1036.407 $(M + H)^+$ ($C_{43}H_{66}N_5O_{24}$ requires 1036.409).

Coupling of Compound 31 with Threonylbithiazole 9. A mixture of 20 mg (19 μmol) of disaccharide dipeptide acid **31**, 20 mg (45 μmol) of threonylbithiazole **9**, and 6 mg (44 μmol) of 1-hydroxybenzotriazole was coevaporated with portions of toluene, dried overnight under vacuum in the presence of P_2O_5, and then dissolved in 1 mL of anhydrous DMF. To this reaction mixture were added 10 mg (48 μmol) of *N*,*N*′-dicyclohexylcarbodiimide and 10 μL (7.3 mg, 5.6 μmol) of *N*,*N*-diisopropylethylamine. The reaction mixture was stirred at 25 °C under Ar for 24 h. The solvent was concentrated, and the residue was coevaporated with portions of toluene under diminished pressure. The residue was purified by flash chromatography on a silica gel column (17 × 2 cm). Elution with 20:1 and then 15:1 chloroform−methanol afforded Boc-pentapeptide disaccharide **32** as a colorless foam: yield 16 mg (59%); silica gel TLC R_f 0.45 (5:1 chloroform−methanol); $[\alpha]^{25}_D$ +18.8° (*c* 1.0, methanol); ^{1}H NMR ($CDCl_3$) δ1.18 (d, 9 H, J = 6.5 Hz), 1.42 (s, 9 H), 1.59 (s, 9 H), 1.90 (m, 2 H), 2.00 (s, 3 H), 2.06 (s, 6 H), 2.10 (s, 3 H), 2.13 (s, 3 H), 2.60 (t, 2 H, J = 6.5 Hz), 3.20−3.44 (m, 4H), 3.44−3.53 (m, 2 H), 3.54−3.63 (m, 2 H), 3.65−3.83 (m, 6 H), 3.87−3.92 (m, 2 H), 3.97−4.06 (m, 4 H), 4.11−4.15 (m, 1 H), 4.17−4.24 (m, 2 H), 4.99 (m, 1 H), 5.06 (m, 1 H), 5.22 (m, 1 H), 5.45 (m, 1 H), 7.18−7.30 (m, 3 H), 7.49−7.58 (m, 2 H), 7.67−7.75 (m, 1 H), 7.75 (s, 1 H), 8.02 (s, 1 H), and 8.65 (s, 2 H); mass spectrum (FAB), *m*/*z* 1462 $(M + H)^+$; mass spectrum (FAB), *m*/*z* 1461.509 $(M + H)^+$ ($C_{60}H_{89}N_{10}O_{26}S_3$ requires 1461.510).

Partial Deprotection of Pentapeptide Disaccharide 32. Boc-pentapeptide disaccharide **32** (16 mg, 10.9 μmol) was treated with 0.4 mL of dimethyl sulfide and 0.8 mL of trifluoroacetic acid at 0 °C under Ar for 3 h. The reaction mixture was concentrated under diminished pressure, and the residue was coevaporated with portions of toluene. The crude product was dissolved in 1.0 mL of water and loaded onto an Amberlite XAD-2 column (20 × 1 cm). The column was washed with 80 mL of water, and the product was eluted from the column by washing with 100 mL of methanol. The combined methanol eluate was concentrated under diminished pressure, and the residue was coevaporated with portions of toluene to afford the pentapeptide disaccharide **24** as a pale yellow foam: yield 12 mg (87%); ^{1}H NMR (CD_3OD) δ 1.07−1.37 (m, 9 H), 1.97 (m, 3 H), 2.17 (s, 9 H), 2.24 (s, 3 H), 2.60 (m, 2 H), 3.20−3.45 (m, 4 H), 3.44−3.52 (m, 2 H), 3.54−3.63 (m, 2 H), 3.65−3.83 (m, 6 H), 3.87−3.92 (m, 2 H), 3.97−4.06 (m, 4 H), 4.11−4.15 (m, 1 H), 4.17−4.24 (m, 2 H), 4.99 (m, 1 H), 5.06 (m, 1 H), 5.22 (m, 1 H), 5.45 (m, 1 H), 7.67 (s, 1 H), 8.05 (s, 1 H), 8.15 (s, 1 H), and 8.94 (s, 1 H); mass spectrum (FAB), *m*/*z* 1261 $(M + H)^+$; mass spectrum (FAB), *m*/*z* 1261.407 $(M + H)^+$ ($C_{50}H_{73}N_{10}O_{22}S_3$ requires 1261.406).

This product was employed in the next transformation without further purification.

Coupling of Pentapeptide Disaccharide 24 with *N*-*t*-Boc-pyrimidoblamic Acid (10). Deprotection and Purification of Bleomycin Demethyl A_2 (2). A mixture of 12 mg (9.5 μmol) of pentapeptide disaccharide **24** and 8 mg (18 μmol) of *N*-*t*-Boc-pyrimidoblamic acid (**10**)[36] was coevaporated with portions of toluene and then dried overnight under vacuum in the presence of P_2O_5. To this mixture was added 14 mg (32 μmol) of benzotriazol-1-yloxytris(dimethylamino)-phosphonium hexafluorophosphate. The reaction mixture was dissolved in 1 mL of anydrous DMF and cooled to 0 °C. *N*,*N*-Diisopropylethylamine (33 μL, 24.5 mg, 0.18 mmol) was added, and the reaction mixture was stirred at 25 °C under Ar for 28 h. The solution was concentrated under diminished pressure. The residue was coevaporated with portions of toluene and then treated successively with 30 mL of water and 30 mL of ethyl acetate. The aqueous phase was extracted with ethyl acetate. The combined organic phase was washed with three

10-mL portions of water. The combined aqueous phase was concentrated and coevaporated with toluene to give crude product **25**.

Crude **25** was dissolved in 2 mL of methanol and treated with 2 mL of 2 N NH_4OH at 25 °C for 3.5 h to remove the acetyl groups. The solvent was concentrated and coevaporated with portions of toluene to give a colorless glass. This glass was treated with 1 mL of dimethyl sulfide and 2 mL of trifluoroacetic acid at 0 °C for 1.5 h. The solvent was concentrated, and the residue was treated with 20 mL of ethyl acetate and 20 mL of water. The organic phase was washed with three 8-mL portions of water. The combined aqueous phase was concentrated to afford crude bleomycin demethyl A_2 (**2**).

Crude BLM **2** and 8 mg of $CuSO_4 \cdot 5H_2O$ were dissolved in 1 mL of 50 mM sodium citrate buffer, pH 4.5, and applied to a CM Sephadex C-25 column (24 × 1.5 cm). The column was washed with a linear gradient of NaCl (0 → 2 N) in 50 mM sodium citrate buffer, pH 4.5. The column was monitored at 292 nm, and the appropriate fractions were pooled and concentrated. This Cu(II)·BLM complex and 0.2 g of Na_2EDTA salt in 7 mL of water were stirred at 25 °C for 1 h. The solution was purified by chromatography on a C_{18} reversed-phase column (9.5 × 1 cm). The column was first washed with 70 mL of water and then with 60 mL of 4:1 MeOH−0.002 N HCl. The methanol−0.002 N HCl eluate was pooled and concentrated. The residue was dissolved in 4 mL of water, frozen, and lyophilized to give bleomycin demethyl A_2 (**2**) as a colorless powder: yield 3 mg (23% from **24**); silica gel TLC R_f 0.47 (1:3 10% NH_4OAc−EtOH); λ_{max} 292 nm (ϵ 14 000); $[\alpha]^{25}_D$ −17.1° (*c* 0.14, H_2O); ^{1}H NMR (D_2O) δ 1.06 (d, 3 H, J = 6 Hz), 1.10 (d, 3 H, J = 7 Hz), 1.13 (d, 3 H, J = 7 Hz), 1.87−1.94 (m, 2 H), 1.97 (s, 3 H), 2.07 (s, 3 H), 2.53−2.60 (m, 4 H), 2.67 (s, 1 H), 2.72−2.86 (m, 2 H), 3.15−3.30 (m, 4 H), 3.44−3.52 (m, 2 H), 3.54−3.63 (m, 3 H), 3.65−3.83 (m, 6 H), 3.87−3.92 (m, 2 H), 3.97−4.06 (m, 4 H), 4.11−4.15 (m, 1 H), 4.17−4.24 (m, 2 H), 4.99 (s, 1 H), 5.06 (d, 1 H, J = 6.5 Hz), 5.22 (d, 1 H, J = 3.5 Hz), 5.45 (d, 1 H, J = 6.5 Hz), 7.57 (s, 1 H), 8.00 (s, 1 H), 8.14 (s, 1 H), and 8.76 (s, 1 H); mass spectrum (FAB), *m*/*z* 1400 $(M + H)^+$; mass spectrum (FAB), *m*/*z* 1400.500 $(M + H)^+$ ($C_{54}H_{82}N_{17}O_{21}S_3$ requires 1400.503).

Benzyl *erythro*-N^{α},N^{im}-Bis(9-fluorenylmethoxycarbonyl)-β-[3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-*S*-histidine (38). Compound **35** (11.5 mg, 13 μmol) was coevaporated with three 1-mL portions of toluene. To the residue was added 300 μL of CH_2Cl_2 containing 11.7 mg (16 μmol) of di-Fmoc β-hydroxyhistidine **37**, which had been coevaporated with three 1-mL portions of toluene. The stirred reaction mixture was diluted with 600 μL of ether, cooled (0 °C), and treated with 10.3 μL (12.2 mg, 53 μmol) of trimethysilyl trifluoromethanesulfonate. Stirring was continued for 20 min at 0 °C, and then the reaction mixture was poured into 8 mL of a 2:1 ethyl acetate−aqueous $NaHCO_3$ mixture with vigorous stirring. The organic phase was washed twice with 5 mL of brine, dried ($MgSO_4$), and concentrated under diminished pressure. The residue was purified using preparative silica gel TLC (0.5-mm plate thickness). Elution with 8:2 ethyl acetate−hexanes gave compound **38** as a colorless foam: yield 10.2 mg (58%); silica gel TLC R_f 0.38 (4:1 ethyl acetate−hexanes); ^{1}H NMR ($CDCl_3$) δ 1.77, 1.87, 2.02, 2.07, 2.08, and 2.10 (each s, 3H), 3.9−4.4 (m, 10H), 4.5 (br s, 2H), 4.70−4.75 (m, 3H), 4.77 (d, 1H, J = 12 Hz), 4.97 (d, 1H, J = 12 Hz), 5.0−5.35 (m, 8H), 5.4 (t, 1H, J = 2 Hz), 5.68 (m, 1H), 6.33 (d, 1H, J = 8.5 Hz), 7.2−7.5 (m, 14H), 7.5−7.7 (m, 4H), 7.77 (dd, 4H, J = 13, 7.5 Hz), and 7.95 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.91, 21.00, 21.11, 47.02, 47.54, 57.05, 60.80, 62.01, 62.58, 62.91, 65.28, 65.82, 66.63, 67.64, 67.85, 68.86, 69.65, 69.99, 70.27, 71.20, 73.35, 96.35, 96.94, 116.10, 120.35, 125.15, 125.64, 125.70, 127.44, 127.58, 127.82, 128.08, 128.58, 128.72, 128.79, 135.71, 137.35, 141.35, 141.52, 141.70, 141.79, 143.05, 144.26, 144.57, 148.54, 155.61, 156.63, 169.54, 169.78, 169.89, 170.44, 170.49, 170.79, 170.91, and 171.03; mass spectrum (FAB), *m*/*z* 1325.426 $(M + H)^+$ ($C_{68}H_{69}N_4O_{24}$ requires 1325.430).

6-*O*-Acetyl-3,4-di-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-β-L-gulopyranosyl Trichloroacetimidate (46). A sample of 120 mg (0.164 mmol) of 6-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α/β-gulopyranoside (**45**) was dissolved in 5 mL of CH_2Cl_2. To this solution were added 236 mg (1.64 mmol) of trichloroacetonitrile and 122 mg (0.884 mmol) of anhydrous potassium carbonate. The solution was stirred vigorously for 6 h and then filtered. The filtered solid was washed with 10 mL of CH_2Cl_2. The organic filtrate was partitioned against 5 mL of water. The water layer was back-extracted with 5 mL of CH_2Cl_2. The combined organic phase was then concentrated under diminished pressure to afford trichloroacetimidate **46** as a colorless foam: yield 140 mg (97%); silica gel TLC R_f 0.42 (1:1 hexanes−acetone); $[\alpha]^{20}_D$ 26.1° (*c* 0.20, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.05 (s, 3H), 2.05 (s, 3H), 2.20 (s, 3H), 3.55 (m, 1H), 4.01 (m, 1H), 4.08−4.13 (m, 3H), 4.16−4.22 (m, 3H), 4.31 (m, 1H), 4.34 (d, 1H, J = 12.5 Hz), 4.37 (d, 1 H, J = 11.5 Hz), 4.50 (d, 1H, J = 12.5 Hz), 4.57 (br s, 2H), 4.60 (m, 1H), 4.68 (d, 1H, J = 11 Hz), 4.87 (m, 1H), 5.21−5.31 (m, 2 H) 6.10 (d, 1H, J = 8 Hz), 7.20−7.38 (m, 10H), and 8.67 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 20.52, 20.61, 20.72, 20.88, 61.81, 62.56, 65.98, 68.44, 69.49, 70.18, 72.12, 72.83, 73.83, 73.34, 73.72, 93.10, 94.45, 95.13, 128.14, 128.23, 128.53, 137.32, 137.09, 155.11, 160.74, 169.79, 169.95, 170.45, and 170.53; mass spectrum (FAB), *m*/*z* 899.157 $(M + Na)^+$ ($C_{37}H_{43}N_2O_{16}Cl_3Na$ requires 899.157).

Benzyl *erythro*-N^{α},N^{im}-Bis(9-fluorenylmethoxycarbonyl)-β-[6-*O*-acetyl-3,4-di-*O*-benzyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-*S*-histidine (47). A sample of 59.2 mg (67.1 μmol) of disaccharide imidate **46** and 15 mg (21.3 μmol) of β-hydroxyhistidine derivative **37** were combined and dried under vacuum. Dichloromethane (0.5 mL) was added to the mixture, and the solution was treated with 13.3 μL (16.9 mg, 73.8 μmol) of TMSOTf. The reaction mixture was stirred at 25 °C for 1.5 h and then quenched with saturated $NaHCO_3$ solution. The reaction mixture was partitioned against 5 mL of CH_2Cl_2, and the aqueous layer was extracted with three 10-mL portions of ethyl acetate. The combined organic fraction was dried ($MgSO_4$). The solvent was concentrated under diminished pressure, and the product was purified by preparative silica gel TLC; development was with 3:1 ethyl acetate−hexanes. The product was eluted from the silica gel with ethyl acetate to afford the Fmoc-protected histidinyldisaccharide **47** as a colorless glass: yield 12.5 mg (44%); silica gel TLC R_f 0.38 (3:1 ethyl acetate−hexanes); $[\alpha]^{23}_D$ −6.4° (*c* 0.14, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.82 (s, 3H), 1.87 (s, 3H), 1.99 (s, 3H), 2.13 (s, 3H), 3.60 (m, 1H), 3.90−4.12, 4.09 (m, 6H), 4.22 (m, 3H), 4.28−4.42 (m, 5H), 4.50−4.66 (m, 4H), 4.75−4.87 (m, 1H), 5.11 (m, 3H), 5.19−5.32 (m, 6H), 5.39 (m, 1H), 6.63 (d, 1H, J = 8.5 Hz), 7.20−7.81 (m, 32H), and 7.93 (s, 1H); mass spectrum (FAB), *m*/*z* 1443.487 $(M + Na)^+$ ($C_{78}H_{76}N_4O_{22}Na$ requires 1443.483).

Relaxation of Supercoiled DNA by BLM. DNA relaxation was measured using 200 ng of supercoiled pBR322 plasmid DNA in 5 μL of 10 mM sodium cacodylate buffer, pH 7.2. The reactions contained equimolar BLM and $Fe^{II}(NH_4)_2(SO_4)_2$ at the concentration indicated in the figure legend. The reactions were initiated by the addition of $Fe^{II}(NH_4)_2(SO_4)_2$ from a freshly prepared solution, and incubated at 23 °C for 10 min. The reactions were quenched by the addition of 10 μL of a 1:1 mixture of 100 mM EDTA, pH 7.4, and 40% sucrose loading solution containing 0.1% (w/v) bromophenol blue. A portion of each reaction (7 μL) was analyzed by horizontal electrophoresis on a 1% agarose gel containing 1 μg/mL ethidium bromide (30 v, 2 h; then 40 v, 12 h). The bands were visualized by UV transillumination.

Preparation of a 5′-^{32}P-End-Labeled DNA Restriction Fragment. Plasmid pBR322 (50 μg) was incubated with 100 units of restriction endonuclease *Hin*dIII in 10 mM Tris HCl, pH 8.0, containing 5 mM $MgCl_2$, 100 mM NaCl, and 1 mM β-mercaptoethanol. The digestion reaction was carried out at 37 °C for 1 h, and then the DNA was dephosphorylated with 5 units of alkaline phosphatase (37 °C). The DNA was recovered by ethanol precipitation following phenol extraction. The linearized DNA (0.5 A_{260} unit; 10 pmol) was 5′-^{32}P-end-labeled by incubation with 320 μCi of [γ-^{32}P]ATP and 6 units of T4 polynucleotide kinase at 37 °C for 30 min. The DNA was purified on a Sephadex G-50 column and then digested with 300 units of restriction enzyme *Eco*RV in 350 μL (total volume) of 10 mM Tris−HCl, pH 8.0, containing 5 mM $MgCl_2$, 100 mM NaCl, and 1 mM β-mercaptoethanol at 37 °C for 1 h. The 158-base pair 5′-^{32}P-end-labeled DNA duplex was isolated from an 8% nondenaturing polyacrylamide gel.

Cleavage of ^{32}P-End Labeled Duplex DNA by BLM. Reactions contained ~30 000 cpm of 158-base pair 5′-^{32}P-end-labeled DNA duplex in 5 μL of 10 mM sodium cacodylate, pH 7.4. The reactions containing BLM at the concentrations indicated in the figure legend were initiated by the addition of equimolar Fe^{2+} from a freshly prepared solution of $Fe^{II}(NH_4)_2(SO_4)_2$. The reactions were incubated at 23 °C for 20 min, then quenched by the addition of 5 μL of loading buffer (7 M urea, 1 mM EDTA, 0.1% bromophenol blue, and 0.1% xylene cyanol), and applied to a 14% polyacrylamide gel containing 8 M urea. Electrophoresis was carried out at 10 W for 1 h and then at 50 W for 2 h. The gel was analyzed using a phosphorimager; autoradiography (Kodak XAR-2 film) was carried out at −80 °C.

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Supporting Information Available: Polyacrylamide gel showing the DNA cleavage activities of synthetic bleomycin congeners and synthetic methods for compounds **4**−**7**, **9**, **15**−**24**, **27**, **36**, **37**, **39**, **40**, **42**, **43**, and **45** (17 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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