Total Synthesis of Bleomycin A_2 and Related Agents. 3. Synthesis and Comparative Evaluation of Deglycobleomycin A_2, Epideglycobleomycin A_2, Deglycobleomycin A_1, and Desacetamido-, Descarboxamido-, Desmethyl-, and Desimidazolyldeglycobleomycin A_2

Dale L. Boger,* Takeshi Honda, Royce F. Menezes, and Steven L. Colletti

Contribution from the Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, California 92037

Received October 5, 1993[⊗]

Abstract: Full details of the completion of the total synthesis of deglycobleomycin A_2 (**2**) and deglycobleomycin A_1 (**3**), the algycones of two naturally occurring bleomycins, are provided. Extensions of the studies to the preparation of epideglycobleomycin A_2 (**4**), desacetamidodeglycobleomycin A_2 (**5**), descarboxamidodeglycobleomycin A_2 (**6**), desmethyldeglycobleomycin A_2 (**7**), and desimidazolyldeglycobleomycin A_2 (**8**) are described. The agents **4–8**, which are not accessible through structural modification of the natural products themselves, constitute key substructure analogs incorporating deep-seated and mechanistically relevant structural modifications in the metal chelation subunit. Extensions of the studies to the preparation of GABA,Gly-deglycobleomycin A_2 (**43**) and GABA,Gly-desacetamidodeglycobleomycin A_2 (**45**) are detailed in efforts that confirm a subtle and significant role for the C2 acetamido side chain detected in the initial comparisons of **2** with **4** and **5** and additionally provide an initial assessment of the role of the tetrapeptide S backbone substituents (**43** versus **2**, **45** versus **5**). The comparative examination of the DNA cleavage properties of the Fe(II) or Fe(III) complexes of **1–8** and **43**, **45** has been conducted, and four properties have been assessed including the relative DNA cleavage efficiency and ratio of double- to single-strand DNA breaks with supercoiled ΦX174 DNA as well as the relative DNA cleavage efficiency and the DNA cleavage selectivity with 5′-end-labeled w794/w836 duplex DNA. The results of the relative DNA cleavage studies and the assessment of double- to single-strand DNA cleavage events demonstrate important, productive roles for the terminal sulfonium salt, and the pyrimidoblamic acid C2 acetamido side chain, a more subtle but perceptible role for the C2 side chain β-amino-L-alanine carboxamide, no role for the pyrimidine C5 methyl group, and an expected essential role for the histidine imidazole. Similarly, the tetrapeptide S backbone substituents absent in **43** and **45** were determined to substantially but not dramatically diminish DNA cleavage efficiency without altering DNA binding affinity or the characteristic DNA cleavage selectivity. In contrast to the relative impact that the structural changes within the bleomycin A_2 metal binding domain had on the relative DNA cleavage efficiencies and with the important exception of **8**, they had no perceptible impact on the observed selectivity of DNA cleavage, 5′-GC, 5′-GT > 5′-GA. Similarly, deglycobleomycin A_1, lacking the C-terminal sulfonium salt, or **43** and **45**, lacking all the tetrapeptide S backbone substituents, exhibited the identical and characteristic 5′-GC, 5′-GT > 5′-GA DNA cleavage selectivity of **1** and **2**, indicating that they affect DNA cleavage efficiency but not cleavage selectivity. Only **8**, lacking the essential imidazole, was found to exhibit an altered and nonselective DNA cleavage pattern presumably derived from oxidative cleavage of duplex DNA via generation of a diffusable oxidant.

The bleomycins are a family of glycopeptides possessing clinically useful antitumor activity thought to be mediated by their metal-dependent oxidative cleavage of duplex DNA[1-10] (Figure 1). In the accompanying articles, we have detailed syntheses of the bleomycin A_2 C-terminus including tri-, tetra- and pentapeptide S and related agents[11] as well as its amino terminus including pyrimidoblamic acid and a series of structurally related agents.[12] Herein, we detail the incorporation of the individual subunits into the total synthesis of deglycobleomycin A_2 (**2**)[13-17] and A_1 (**3**), the aglycones of two naturally occurring bleomycins.[9] Inherent in the formulation of the approach was the control of the relative and absolute stereochemistry of the nine acyclic stereogenic centers found in **2** and **3** in a sufficiently

⊗ Abstract published in *Advance ACS Abstracts*, May 15, 1994.

(1) Ohno, M.; Otsuka, M. In *Recent Progress in the Chemical Synthesis of Antibiotics*; Lukacs, G., Ohno, M., Eds.; Springer-Verlag: New York, 1990; p 387.
(2) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.
(3) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383.
(4) Sugiura, Y.; Takita, T.; Umezawa, H. *Met. Ions Biol. Syst.* **1985**, *19*, 81.
(5) Twentyman, P. R. *Pharmacol. Ther.* **1984**, *23*, 417.
(6) Povirk, L. F. In *Molecular Aspects of Anti-Cancer Drug Action*; Neidle, S., Waring, M. J., Eds.; MacMillian: London, 1983.
(7) Hecht, S. M. In *Bleomycin: Chemical, Biochemical and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979.
(8) Umezawa, H. In *Bleomycin: Current Status and New Developments*; Carter, S. K., Crooke, S. T., Umezawa, H., Eds.; Academic Press: New York, 1978.
(9) Umezawa, H. *Pure Appl. Chem.* **1971**, *28*, 665.
(10) Dedon, P. C.; Goldberg, I. H. *Chem. Res. Toxicol.* **1992**, *5*, 311.
(11) Boger, D. L.; Colletti, S. L.; Honda, T.; Menezes, R. F. *J. Am. Chem. Soc.*, companion paper in this issue. Boger, D. L.; Menezes, R. F. *J. Org. Chem.* **1992**, *57*, 4331.
(12) Boger, D. L.; Honda, T.; Dang, Q. *J. Am. Chem. Soc.*, companion paper in this issue.
(13) Structure determination: Takita, T.; Muraoka, Y.; Nakatani, T.; Fujii, A.; Umezawa, Y.; Naganawa, H.; Umezawa, H. *J. Antibiot.* **1978**, *31*, 801.
(14) Boger, D. L.; Menezes, R. F.; Honda, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 273.
(15) Takita, T.; Umezawa, Y.; Saito, S.; Morishima, H.; Umezawa, H.; Muraoka, Y.; Suzuki, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1981**, *22*, 671. Saito, S.; Umezawa, Y.; Morishima, H.; Takita, T.; Umezawa, H.; Narita, M.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron Lett.* **1982**, *23*, 529. Saito, S.; Umezawa, Y.; Yoshioka, T.; Takita, T.; Umezawa, H.; Muraoka, Y. *J. Antibiot.* **1983**, *36*, 92.
(16) Aoyagi, Y.; Suguna, H.; Murugesan, N.; Ehrenfeld, G. M.; Chang, L.-H.; Ohgi, T.; Shekhani, M. S.; Kirkup, M. P.; Hecht, S. M. *J. Am. Chem. Soc.* **1982**, *104*, 5237.

0002-7863/94/1516-5631$04.50/0 © 1994 American Chemical Society

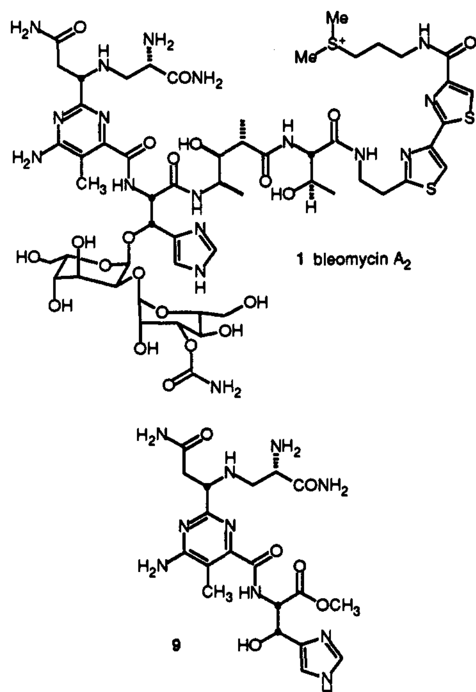


Figure 1.

concise manner so as to reduce the logistical challenges of the synthesis to a manageable problem readily adaptable to the concurrent preparation of structural analogs possessing deep-seated changes in the parent structure. In realization of this objective and in efforts that represent the initiation of a detailed examination of the functional role of the individual bleomycin subunits and substituents, we also describe herein the extension of studies to the synthesis and comparative examination of epideglycobleomycin A_2 (**4**), desacetamidodeglycobleomycin A_2 (**5**),[18] descarboxamidodeglycobleomycin A_2 (**6**), desmethyldeglycobleomycin A_2 (**7**), and desimidazolyldeglycobleomycin A_2 (**8**), representing key substructure analogs incorporating deep-seated structural modifications in the metal chelation subunit **9**. Further extensions of the studies to the preparation of GABA,-Gly-deglycobleomycin A_2 (**43**) and GABA,Gly-desacetamidodeglycobleomycin A_2 (**45**)[19] are detailed in efforts that confirm a subtle and significant role for the C2 acetamido side chain first detected in the comparisons of **2** with **4** and **5**. These latter agents have also served to provide an initial probe of the role and relative importance of the tetrapeptide S backbone substituents. Despite the interest in bleomycin and the importance of understanding the structural origin of its properties, only a limited but classic set of chemical derivatives[20] and chemical degradation products[21–23] of the metal binding domain have been characterized. The study of these agents revealed the importance of the C2 pyrimidoblamic acid side chain primary amine for metal chelation ($NH_2 > NHMe \gg NMe_2$),[20] the importance of the electron-donating properties of the pyrimidine C4 amine for efficient oxygen activation,[1] the relative importance of the C2 acetamido side chain stereochemistry (natural > epi),[21] and the competitive inactivation of the agent through β-amino-L-alanine carboxamide hydrolysis ($CONH_2 \gg CO_2H$) with conversion to a metal complex possessing a carboxylate ligand that proved incapable of activating O_2.[22,23] Consequently, the agents **4–8**, **43**, and **45** represent key substructures not accessible through degradation or derivatization of the natural product itself which have not yet been accessible for evaluation but are especially relevant to understanding the metal chelation, oxygen activation, and DNA cleavage properties of the natural product. Their preparation and evaluation could be anticipated to complement past studies of synthetic agents incorporating simplified metal binding domains[1,24,25] and the studies of agents incorporating C-terminus tri- and tetrapeptide S structural modifications.[1,25–28]

Pertinent to the studies detailed herein, prior studies have established that the disaccharide subunit of bleomycin A_2 does not contribute to the DNA binding affinity or the characteristic 5′-GC, 5′-GT DNA cleavage selectivity although it enhances DNA cleavage efficiency (2–5×) and biological potency.[1,17,21b] Consequently, we have elected to examine a full range of analogs of deglycobleomycin A_2 rather than the less accessible bleomycin A_2 analogs themselves with the intent of addressing the unresolved questions of duplex DNA molecular recognition and functional reactivity. In addition to the unresolved questions of duplex DNA recognition and the origin of the bleomycin DNA cleavage selectivity, we anticipate that the modest potency (μM IC_{50}) and efficacy, the limiting pulmonary toxicity,[29] the problematic ineffective cellular penetration,[30] the rapid specific (bleomycin

(17) Oppenheimer, N. J.; Chang, C.; Chang, L.-H.; Ehrenfeld, G.; Rodriguez, L. O.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 1606. Sugiura, Y.; Suzuki, T.; Otsuka, M.; Kobayashi, S.; Ohno, M.; Takita, T.; Umezawa, H. *J. Biol. Chem.* **1983**, *258*, 1328. Sugiura, Y.; Kuwahara, J.; Suzuki, T. *FEBS Lett.* **1985**, *182*, 39. Kenani, A.; Lamblin, G.; Henichart, J.-P. *Carbohydr. Res.* **1988**, *177*, 81. Boger, D. L.; Menezes, R. F.; Yang, W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 959. Freyder, C. P.; Zhou, W.; Doetsch, P. W.; Marzilli, L. G. *Prep. Biochem.* **1991**, *21*, 257.

(18) Desacetamidodeglycobleomycin A_2: Boger, D. L.; Menezes, R. F.; Dang, Q. *J. Org. Chem.* **1992**, *57*, 4333.

(19) Boger, D. L.; Menezes, R. F.; Dang, Q.; Yang, W. *Bioorg. Med. Chem. Lett.* **1992**, *2*, 261.

(20) N^{α}-Acetylbleomycin A_2: Oppenheimer, N. J.; Rodriguez, L. O.; Hecht, S. M. *Biochemistry* **1980**, *19*, 4096. N^{α}-Methylbleomycin A_2 and N^{α},N^{α}-dimethylbleomycin A_2: Fukuoka, T.; Muraoka, Y.; Fujii, A.; Naganawa, H.; Takita, T.; Umezawa, H. *J. Antibiot.* **1980**, *33*, 114.

(21) (a) Epibleomycin A_2: Muraoka, Y.; Kobayashi, H.; Fujii, A.; Kunishima, M.; Fujii, T.; Nakayama, Y.; Takita, T.; Umezawa, H. *J. Antibiot.* **1976**, *29*, 853. (b) Shipley, J. B.; Hecht, S. M. *Chem. Res. Toxicol.* **1988**, *1*, 25.

(22) Deamidobleomycin A_2: Umezawa, H.; Hori, S.; Sawa, T.; Yoshioka, T.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 419.

(23) Deamidobleomycin A_2 and depyruvamidobleomycin A_2: Sugiura, Y. *J. Am. Chem. Soc.* **1980**, *102*, 5208.

(24) PYML analogs: Otsuka, M.; Yoshida, M.; Kobayashi, S.; Ohno, M.; Sugiura, Y.; Takita, T.; Umezawa, H. *J. Am. Chem. Soc.* **1981**, *103*, 6986. Sugano, Y.; Kittaka, A.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3635. Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3631. Otsuka, M.; Kittaka, A.; Ohno, M.; Suzuki, T.; Kuwahara, J.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3639. Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2811, 2821. Suga, A.; Sugiyama, T.; Sugano, Y.; Kittaka, A.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Maeda, K. *Synlett* **1989**, 70. Otsuka, M.; Masuda, T.; Haupt, A.; Ohno, M.; Shiraki, T.; Sugiura, Y.; Maeda, K. *J. Am. Chem. Soc.* **1990**, *112*, 838. Owa, T.; Haupt, A.; Otsuka, M.; Kobayashi, S.; Tomioka, N.; Itai, A.; Ohno, M.; Shiraki, T.; Uesugi, M.; Sugiura, Y.; Maeda, K. *Tetrahedron* **1992**, *48*, 1193. Umezawa, H.; Takita, T.; Sugiura, Y.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron* **1984**, *40*, 501. Ohno, M.; Otsuka, M.; Kittaka, A.; Sugano, Y.; Sugiura, Y.; Suzuki, T.; Kuwahara, J.; Umezawa, K.; Umezawa, H. *Int. J. Exp. Clin. Chemother.* **1988**, *1*, 12.

(25) Kenani, A.; Lohez, M.; Houssin, R.; Helbecque, N.; Bernier, J. L.; Lemay, P.; Henichart, J. P. *Anti-Cancer Drug Des.* **1987**, *2*, 47. Kenani, A.; Bailly, C.; Helbecque, N.; Houssin, R.; Bernier, J.-L.; Henichart, J.-P. *Eur. J. Med. Chem.* **1989**, *24*, 371.

(26) For additional chemical derivative, degradative, and semisynthetic analogs: Isobleomycin A_2: Nakayama, Y.; Kunishima, M.; Omoto, S.; Takita, T.; Umezawa, H. *J. Antibiot.* **1973**, *26*, 400. Decarbamoylbleomycin A_2: Sugiyama, H.; Ehrenfeld, G. M.; Shipley, J. B.; Kilkuskie, R. E.; Chang, L.-H.; Hecht, S. M. *J. Nat. Prod.* **1985**, *48*, 869. Peplomycin and liblomycin (semisynthetic bleomycins): Umezawa, H.; Takita, T.; Saito, S.; Muraoka, Y.; Takahashi, K.; Ikemoto, H.; Minamide, S.; Nishikawa, K.; Fukuoka, T.; Nakatani, T.; Fujii, A.; Matsuda, A. In *Bleomycin Chemotherapy*; Sikic, B. I., Rozenweig, M., Carter, S. K., Eds.; Academic Press: Orlando, FL, 1985; p 289. Takita, T.; Maeda, K. *J. Heterocycl. Chem.* **1980**, *17*, 1799. Vloon, W. J.; Kruk, C.; Pandit, U. K.; Hofs, H. P.; McVie, J. G. *J. Med. Chem.* **1987**, *30*, 20.

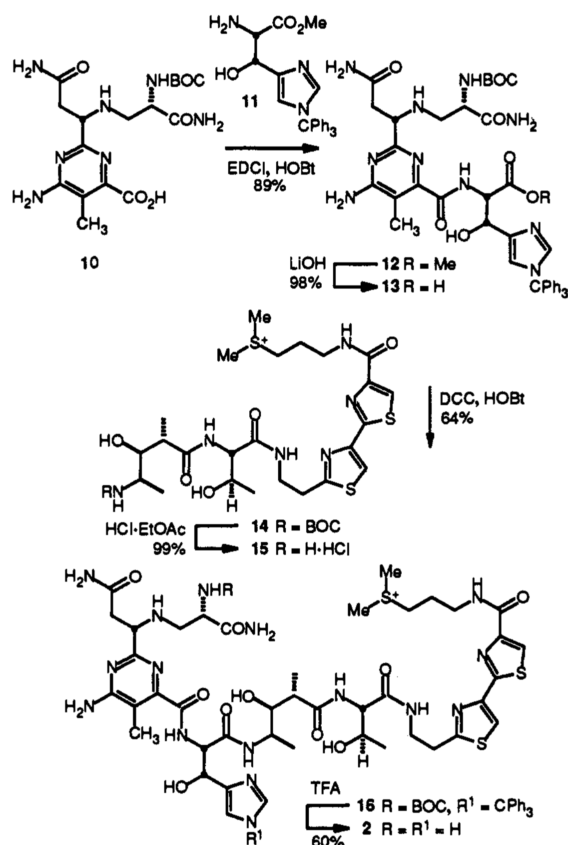
(27) Carter, B. J.; Murty, V. S.; Reddy, K. S.; Wang, S.-N.; Hecht, S. M. *J. Biol. Chem.* **1990**, *265*, 4193. Carter, B. J.; Reddy, K. S.; Hecht, S. M. *Tetrahedron* **1991**, *47*, 2463.

(28) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278.

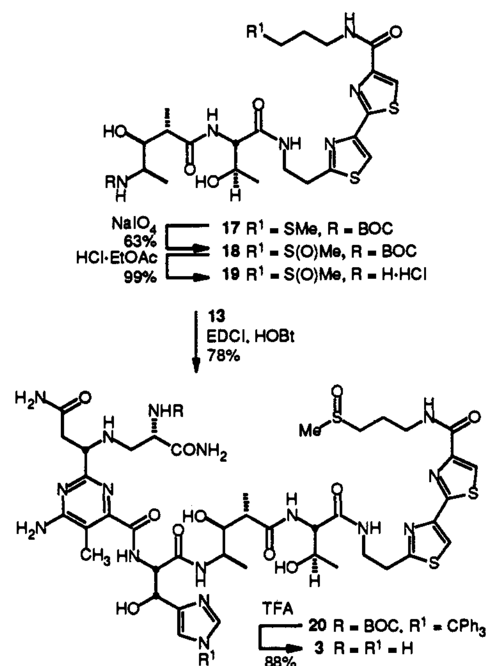
(29) Raisfeld, I. H. *Toxicol. Appl. Pharmacol.* **1980**, *56*, 326.

(30) Poddevin, B.; Orlowski, S.; Belehradek, J., Jr.; Mir, L. M. *Biochem. Pharmacol.* **1991**, *42*, S67.

Scheme 1



Scheme 2



hydrolase)[31] and nonspecific inactivation, and the inherent chemical instability of the naturally occurring bleomycins may be addressed through the preparation and evaluation of agents bearing such deep-seated structural changes.

Deglycobleomycin A_2. Direct coupling of N^{α}-BOC-pyrimidoblamic acid (**10**)[12] with *erythro*-N^{π}-(triphenylmethyl)-β-hydroxy-L-histidine methyl ester (**11**)[11] provided **12** (1.05 equiv of EDCI, 1.0 equiv of HOBt, THF–DMF 2:1, 25 °C, 72 h, 89%) and was found to be conveniently conducted without protection of the primary or secondary amine of **10** (Scheme 1). Acid-catalyzed deprotection of **12** (TFA, 25 °C, 45 min, 93%) provided **9**, $[\alpha]^{25}_{D}$ +13 (*c* 0.01, CH_3OH), constituting the amine terminus metal chelation subunit of bleomycin A_2. Hydrolysis of the methyl ester of **12** (1.5 equiv of LiOH, THF–CH_3OH–H_2O 3:1:1, 0 °C, 1.5 h, 98%) followed by *direct* coupling of **13** with synthetic tetrapeptide S (**15**)[11] provided **16**, $[\alpha]^{25}_{D}$ −21 (*c* 0.03, CH_3OH), and was found to be conveniently conducted without additional protection of the adorning functionality (3 equiv of DCC, 1.0 equiv of HOBt, 2.5 equiv of $NaHCO_3$, DMF, 25 °C, 72 h, 64%). Final deprotection of **16** (TFA, 25 °C, 45 min, 60%) afforded deglycobleomycin A_2 (**2**),[17] $[\alpha]^{25}_{D}$ −15 (*c* 0.025, 0.1 N HCl) (lit[15] $[\alpha]^{25}_{D}$ −15 (*c* 0.5, 0.1 N HCl)), identical in all compared respects with a sample of authentic material (^1H NMR, IR, MS/HRMS, $[\alpha]_D$, TLC, HPLC). Notably, the final coupling and deprotection reactions were conducted employing the intact sulfonium salt rather than postponing its introduction to a penultimate stage.[15,16] This provided a more convergent synthesis of the agent, further simplified the purification of the coupling product **16**, and did not introduce additional competitive side reactions in route to **2**.

Deglycobleomycin A_1. The importance of the bleomycin C-terminus cation and its electrostatic contribution to DNA binding affinity has been well recognized from studies of the relative DNA binding affinities of such agents as well as from studies of the relative DNA cleavage efficiencies of demethyldeglycobleomycin A_2[27] and bleomycinic acid,[21b] both lacking the terminal sulfonium salt. With the accessibility to tetrapeptide S and its precursors, we elected to prepare deglycobleomycin A_1, the aglycone of a naturally occurring bleomycin, and to examine its properties in order to assess the relative impact of the substitution of a sulfoxide for the sulfonium salt. Despite the relative importance of this simple comparison especially in light of the reported altered DNA cleavage selectivity of bleomycinic acid,[21b] neither synthetic or degradatively derived deglycobleomycin A_1 had been described previously. Deliberate oxidation[32] of the tetrapeptide S methyl sulfide precursor **17**[11] (1 equiv of $NaIO_4$, H_2O, 0 °C, 4 h) proceeded cleanly to provide the sulfoxide **18** (63%) and the corresponding sulfone (Scheme 2). Acid-catalyzed deprotection of **18** (3 N HCl–EtOAc, 25 °C, 75 min, 93%) and immediate coupling of **19**, $[\alpha]^{25}_{D}$ +41 (*c* 0.01, CH_3OH), with **13** (3 equiv of EDCI, 1 equiv of HOBt, 2.6 equiv of $NaHCO_3$, DMF, 25 °C, 65 h, 78%) provided **20**. Final acid-catalyzed deprotection of **20** (TFA, 25 °C, 45 min, 88%) provided deglycobleomycin A_1 (**3**), $[\alpha]^{25}_{D}$ +32 (*c* 0.015, CH_3OH).

Epideglycobleomycin A_2. The C2 acetamido side chain of bleomycin A_2 has been shown not to be intimately involved in the key metal chelation and subsequent oxygen activation event[33–36] required of DNA cleavage and yet appears to be important to the properties of the natural product, since epibleomycin A_2[21] exhibits diminished biological activity and a reportedly altered DNA cleavage selectivity.[21b] Our own preliminary studies revealed a significant albeit subtle role for the C2 acetamido side chain in

(31) Umezawa, H.; Hori, S.; Sawa, T.; Yoshioka, T.; Takeuchi, T. *J. Antibiot.* **1974**, *27*, 419. Nishimura, C.; Suzuki, H.; Tanaka, N.; Yamaguchi, H. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 788. Sebti, S. M.; DeLeon, J. C.; Lazo, J. S. *Biochemistry* **1987**, *26*, 4213. Nishimura, C.; Tanaka, N.; Suzuki, H.; Tanaka, N. *Biochemistry* **1987**, *26*, 1574. Enenkel, C.; Wolf, D. H. *J. Biol. Chem.* **1993**, *268*, 7036. Sebti, S. M.; Mignano, J. E.; Jani, J. P.; Srimatkandada, S.; Lazo, J. S. *Biochemistry* **1989**, *28*, 6544.

(32) Prolonged storage of **17** led to inadvertent air oxidation to **18** even with storage under N_2.

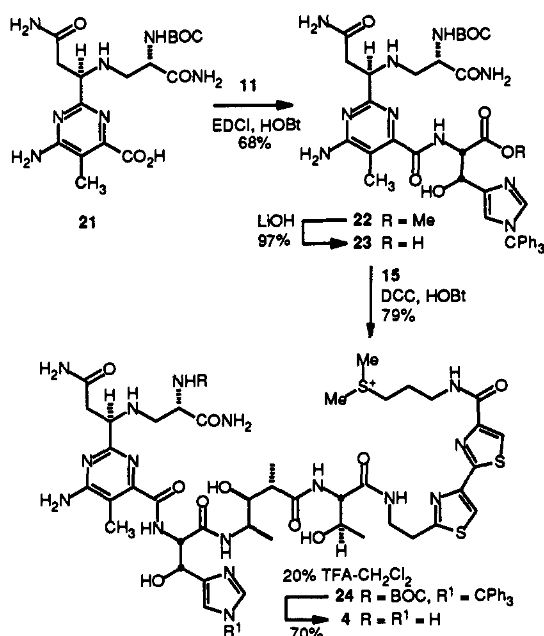
(33) Iitaka, Y.; Nakamura, H.; Nakatani, T.; Muraoka, Y.; Fujii, A.; Takita, T.; Umezawa, H. *J. Antibiot.* **1978**. *31*, 1070.

(34) Dabrowiak, J. C.; Tsukayama, M. *J. Am. Chem. Soc.* **1981**, *103*, 7543.

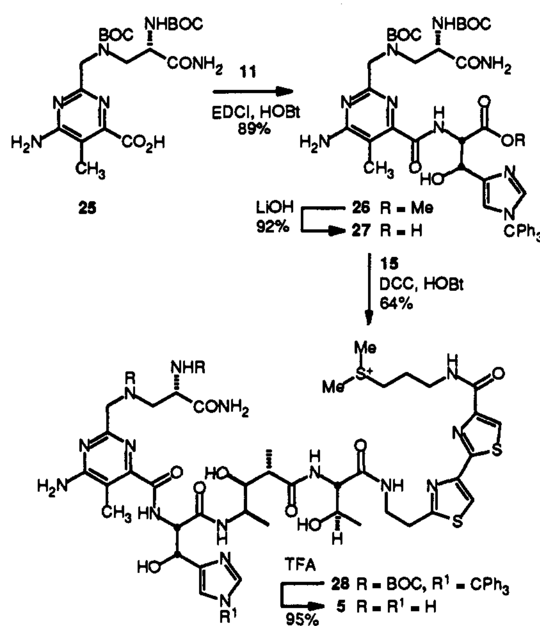
(35) Akkerman, M. A. J.; Neijman, E. W. J. F.; Wijmenga, S. S.; Hilbers, C. W.; Bermel, W. *J. Am. Chem. Soc.* **1990**, *112*, 7462.

(36) Akkerman, M. A. J.; Haasnoot, C. A. G.; Hilbers, C. H. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 434. Akkerman, M. A. J.; Haasnoot, C. A. G.; Hilbers, C. W. *Eur. J. Biochem.* **1988**, *173*, 211. Akkerman, M. A. J.; Haasnoot, C. A. G.; Pandit, U. K.; Hilbers, C. W. *Magn. Reson. Chem.* **1988**, *26*, 793.

Scheme 3



Scheme 4



the DNA cleavage efficiency of related agents.[18,37] Consequently, in efforts to address the role of the C2 acetamido side chain directly and in studies which unambiguously distinguish the structures of natural **2** from the epimer **4**, epideglycobleomycin A_2 (**4**) was prepared for direct comparison with **2** and **5**. Coupling of **21**,[12,14] epimeric with natural pyrimidoblamic acid at the C2 acetamido side chain center, with the protected *erythro*-β-hydroxy-L-histidine **11**[11] provided **22** (1.05 equiv of EDCI, 1 equiv of HOBt, THF-DMF 1:1, 25 °C, 72 h, 68%) (Scheme 3). Hydrolysis of the methyl ester (1.5 equiv of LiOH, THF-CH_3OH-H_2O 3:1:1, -10 °C, 1 h, 97%) followed by coupling of **23** with tetrapeptide S (**15**)[11] afforded **24** in excellent yield (3 equiv of DCC, 1 equiv of HOBt, 3.4 equiv of $NaHCO_3$, DMF, 25 °C, 72 h, 79%). Final deprotection of **24** (TFA, CH_2Cl_2, 0 °C, 4–5 h, 70%) provided epideglycobleomycin A_2 (**4**), $[\alpha]^{23}_D$ +24 (*c* 0.025, 0.1 N HCl). Both **24** [R_f, $[\alpha]^{23}_D$, ^1H NMR (CD_3OD)] and **4** [R_f, $[\alpha]^{23}_D$, ^1H NMR (D_2O)] were readily distinguishable from **16** [R_f, $[\alpha]^{25}_D$, ^1H NMR (CD_3OD)] and **2** [R_f, $[\alpha]^{25}_D$, ^1H NMR (D_2O)], respectively. Subsequent to the efforts to prepare **2** and **3** but in conjunction with efforts to assemble **4**, the final acid-catalyzed deprotection reaction conducted at 25 °C with 3 N HCl-EtOAc was observed to provide an occasional contaminant or predominant H_2O elimination product. Although the structure of this material was not established, it was found that its generation could be minimized or eliminated by conducting the deprotection at 0 versus 25 °C with 20% TFA-CH_2Cl_2 (2–5 h).

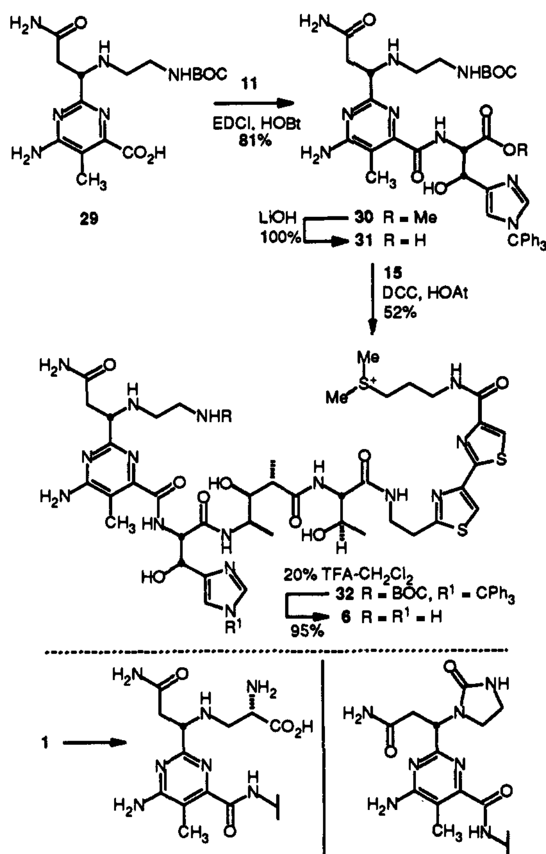
Desacetamidodeglycobleomycin A_2. The direct comparison of desacetamidodeglycobleomycin A_2 (**5**)[18] with **2** and **4** was required to provide a complete assessment of the role the C2 acetamido side chain may play in the DNA cleavage efficiency of bleomycin A_2. In addition, β-elimination of the β-amino-L-alanine carboxamide side chain activated by the C2 acetamido side chain has been suggested to contribute to the inherent chemical instability of bleomycin A_2. Thus, the removal of the C2 acetamido side chain could be anticipated to eliminate this chemical degradation pathway and potentially enhance the chemical stability of the agent. Unlike **4** and epibleomycin A_2, which may be derived from epimerization of bleomycin A_2 and chemical degradation, **5** constitutes an important substructure of the natural product which had not yet been evaluated and that may be accessible only through chemical synthesis.[18]

(37) Boger, D. L.; Honda, T.; Menezes, R. F.; Colletti, S. L.; Dang, Q.; Yang, W. *J. Am. Chem. Soc.* **1994**, *116*, 82. Boger, D. L.; Yang, W. *BioMed. Chem. Lett.* **1992**, *2*, 1649.

Direct coupling of **25**,[12,18] lacking the pyrimidoblamic acid C2 acetamido side chain, with *erythro*-N^{π}-(triphenylmethyl)-β-hydroxy-L-histidine methyl ester (**11**)[11] provided **26** (1.05 equiv of EDCI, 1.0 equiv of HOBt, THF-DMF 2:1, 25 °C, 72 h, 89%) (Scheme 4). Methyl ester hydrolysis (2 equiv of LiOH, THF-CH_3OH-H_2O 3:1:1, 25 °C, 3 h, 92%) followed by *direct* coupling of **27** with tetrapeptide S (**15**)[11] provided **28**, $[\alpha]^{25}_D$ +51 (*c* 0.035, CH_3OH), and was found to be conveniently conducted without protection of the potentially reactive functionality (3.0 equiv of DCC, 1.0 equiv of HOBt, 2.5 equiv of $NaHCO_3$, DMF, 25 °C, 72 h, 64%). Final exhaustive deprotection of **28** with TFA (25 °C, 1.5 h, 95%) proved more effective than deprotection with 3 N HCl-EtOAc and afforded desacetamidodeglycobleomycin A_2 (**5**), $[\alpha]^{23}_D$ +83 (*c* 0.03, CH_3OH).

Descarboxamidodeglycobleomycin A_2. The terminal α-carboxamido group found within the pyrimidoblamic acid C2 side chain has long been suggested not to be intimately involved in the key metal chelation of bleomycin A_2[33–36] and has no obvious role in the subsequent oxygen activation and duplex DNA cleavage properties of the agent although recent NMR studies[35] have implicated a potential role for carboxamide metal coordination. It does, however, undergo a rapid in vivo hydrolysis catalyzed by bleomycin hydrolase[31] to provide the corresponding *inactive* carboxylic acid in which the carboxylate has been proposed to displace N^{α}-amine metal coordination and serves as one ligand of the inactivated metal complex.[23] In efforts to determine if there is a productive and unappreciated role for the problematic terminal carboxamide substituent, descarboxamidodeglycobleomycin A_2 (**6**) was prepared by chemical synthesis for subsequent evaluation (Scheme 5). Coupling of **29**,[12] lacking the C2 side chain α-carboxamide, with **11**[11] provided **30** in excellent yield (1.05 equiv, EDCI, 1.0 equiv of HOBt, DMF, 25 °C, 48 h, 81%). Although this was not examined in detail, the coupling of **29** with **11** proved less satisfactory when conducted with DCC (1.05 equiv, 2.0 equiv of $NaHCO_3$, DMF, 23 °C, 24 h, 66%) in the absence of HOBt. Methyl ester hydrolysis (1.5 equiv of LiOH, THF-CH_3OH-H_2O 3:1:1, -10 °C, 2 h, 100%) followed by coupling of **31** with tetrapeptide S (**15**)[11] provided **32** (1.0 equiv of DCC, 1.5 equiv of HOAt, 3.0 equiv of $NaHCO_3$, DMF, 23 °C, 96 h, 52%). Final deprotection of **32** (20% TFA-CH_2Cl_2, 0 °C, 4 h, 95%) provided descarboxamidodeglycobleomycin A_2 (**6**), $[\alpha]^{25}_D$ -9 (*c* 0.05, 0.1 N HCl). Consistent with observations in our preceding efforts, the β-amino group of the pyrimidoblamic acid C2 side chain could be taken through this reaction sequence without

Scheme 5



competitive coupling reactions that would require its deliberate protection and, notably, without its competitive or inadvertent closure onto the proximal *tert*-butyl carbamate with five-membered urea generation even in the final deprotection reaction (Scheme 5).

Desmethyldeglycobleomycin A_2. A key steric interaction of the pyrimidoblamic acid C5 methyl group with the ortho C6 carboxamide may favor its proper orientation for metal chelation and consequently enhance the metal coordination, oxygen activation, and subsequent DNA cleavage efficiency of the natural product. However, the inherent electronic destabilization of the syn conformation derived from a well-established lone pair–lone pair repulsion coupled with an additional inherent destabilizing NH_2/H steric interaction of the syn conformation of **7** suggests a strong preference for even its adoption of the anti conformation (ΔE 5.4–7.2 kcal)[38] (Figure 2). Although the difference between the syn and anti conformations of **7** is smaller than that observed with **2** (ΔE = 8.4–10.4 kcal, $\Delta\Delta E$ = 3.0–3.2 kcal),[38] the anti preference is still sufficient to ensure its exclusive conformational population. Consequently, the C5 methyl substituent of **2** was anticipated to be unnecessary for the agent to preferentially adopt the anti amide carbonyl conformation. In efforts to directly assess the role of the pyrimidine C5 methyl substituent, desmethyldeglycobleomycin A_2 (**7**) was prepared by chemical synthesis. The *N*-BOC protected C5 desmethylpyrimidoblamic acid **33**[37] available from our prior studies was coupled with **11**[11] to provide **34** (1.05 equiv of EDCI, 1.0 equiv of HOBt, THF–DMF 2:1, 25 °C, 72 h, 67%) (Scheme 6). Methyl ester hydrolysis (1.5 equiv of LiOH, 0 °C, 1.5 h, 98%) followed by direct coupling (1.2 equiv of DCC, 1 equiv of HOBt, 1.4 equiv of $NaHCO_3$, DMF, 25 °C, 72 h, 55%) of **35** with tetrapeptide S (**15**)[11] provided **36**, $[\alpha]^{25}_D$ −9.5 (*c* 0.055, CH_3OH). Acid-catalyzed deprotection of **36** (20% TFA–CH_2Cl_2, 0 °C, 4 h, 90%) provided desmethyldeglycobleomycin A_2 (**7**), $[\alpha]^{25}_D$ −7 (*c* 0.03, 0.1 N HCl).

(38) The range represents values derived from MM2, AM1, and MNDO calculations.

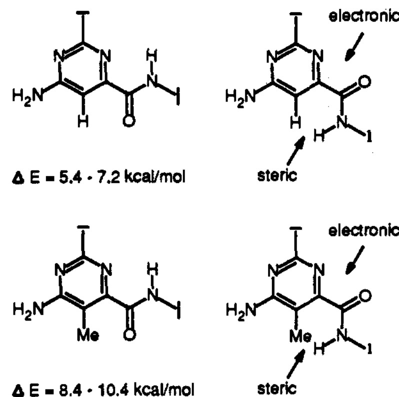
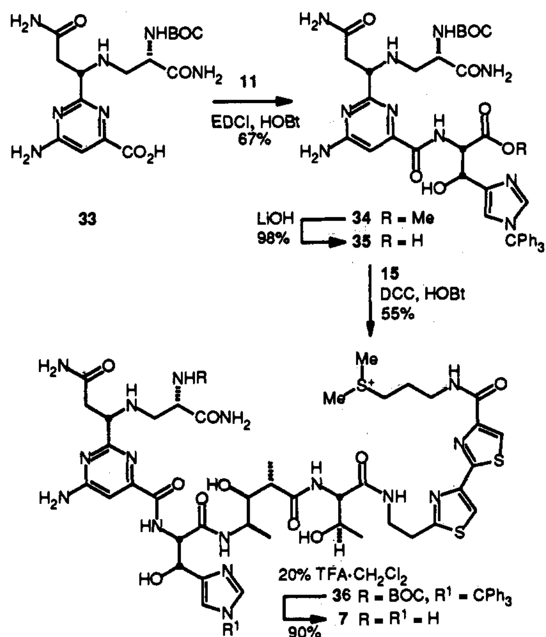


Figure 2.

Scheme 6

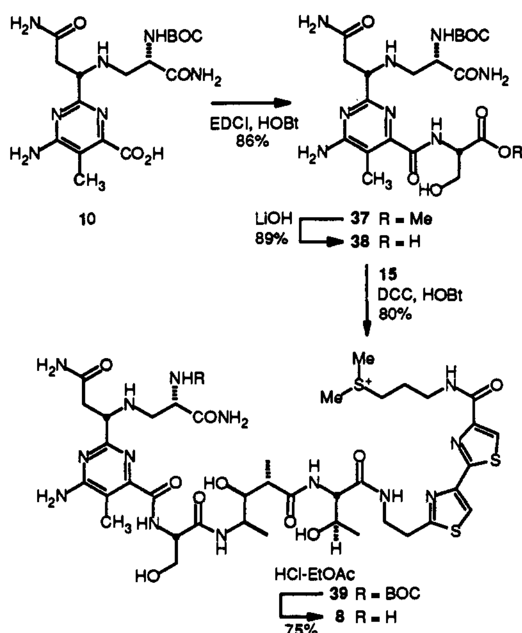


Desimidazolyldeglycobleomycin A_2. The imidazole of the *erythro*-β-hydroxy-L-histidine subunit in conjunction with the adjacent pyrimidoblamic acid subunit functions as a key ligand in the bleomycin A_2 metal complexes and has been assumed to play a pivotal role in the subsequent oxygen activation properties of the natural product metal complexes. In efforts to confirm this pivotal role of the imidazole, desimidazolyldeglycobleomycin A_2 (**8**) was prepared by chemical synthesis for subsequent evaluation (Scheme 7).

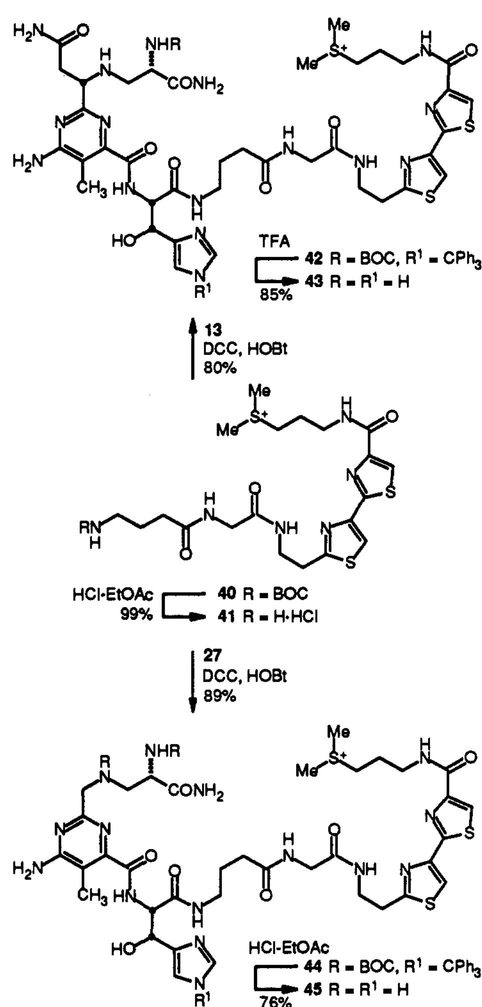
Coupling of **10**[12] with L-serine methyl ester (1.05 equiv of EDCI, 1.0 equiv of HOBt, 3.5 equiv of $NaHCO_3$, DMF, 25 °C, 50 h, 86%) provided **37**. Sequential methyl ester hydrolysis (1.5 equiv of LiOH, 3:1:1 THF–CH_3OH–H_2O, 0 °C, 1.5 h, 89%), coupling of **38** with tetrapeptide S (**15**, 3.0 equiv of DCC, 1.0 equiv of HOBt, 3.2 equiv of $NaHCO_3$, DMF, 25 °C, 60 h, 80%),[11] and acid-catalyzed deprotection (3 N HCl–EtOAc, 25 °C, 1 h, 75%) provided **8**, $[\alpha]^{25}_D$ −21 (*c* 0.008, H_2O), lacking the imidazole substituent of deglycobleomycin A_2.

GABA,Gly-deglycobleomycin A_2 and GABA,Gly-desacetamidodeglycobleomycin A_2. In the preliminary examinations of **2** and **5**,[18] a significant role for the C2 acetamido side chain was detected in the comparisons of their relative efficiency of ΦX174 DNA cleavage. To determine whether the observations were general or intimately linked to the specific comparisions of the

Scheme 7



Scheme 8



fully functionalized deglycobleomycins **2** and **5**, the further simplified agents **43** and **45**[19] were prepared for additional examination. The agents **43** and **45** differ only in the presence or absence of the key C2 acetamido side chain, and both lack all the backbone substituents of the tetrapeptide S chain linking the bithiazole C-terminus and the metal chelation subunit. Both agents maintain the five metal coordination centers within the metal chelation subunit and the full skeletal structure of the natural product in which γ-aminobutyric acid (GABA) and glycine (Gly) have been substituted for the (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylpentanoic acid and L-threonine subunits of bleomycin A_2, respectively. In addition to the comparison of **43** with **45**, which was anticipated to provide an independent assessment of the role of the C2 acetamido side chain, the comparisons of **43** with **2** and **45** with **5** were anticipated to provide an initial assessment of the role of tetrapeptide S linking chain substituents. Notably, the tetrapeptide analog **40** lacking all the backbone substituents of the natural agent was found to possess a DNA binding affinity (K_B) and stoichiometry of binding indistinguishable from those of *N*-BOC-tetrapeptide S.[11] Consequently, the examination of **45** was anticipated to provide an assessment of the important or subtle role of the tetrapeptide S linking chain substituents. In addition, the agent **45** represents the basal substitution of deglycobleomycin A_2, and its assessment provides an important comparative baseline resulting from removal of all the natural product peripheral substituents.

Penultimate coupling of **13** with **41**[11] (3 equiv of DCC, 1 equiv of HOBt, 2.7 equiv of $NaHCO_3$, DMF, 25 °C, 72 h, 80%) provided **42** in excellent yield, and subsequent deprotection (TFA, 25 °C, 45 min, 85%) provided **43** (Scheme 8). Similarly, coupling of **41**[11] with **27** (3 equiv of DCC, 1 equiv of HOBt, 2.5 equiv of $NaHCO_3$, DMF, 25 °C, 51 h, 89%) provided **44** in excellent yield. The use of diphenyl phosphorazidate (DPPA) provided much lower coupling conversions, and the removal of excess reagent and the urea byproduct derived from the use of EDCI proved problematic in alternative preparations of **44**. Exhaustive acid-catalyzed deprotection of **44** provided **45** in excellent yield (3 N HCl–EtOAc, 25 °C, 2 h, 76%).

DNA Cleavage Properties. The first study of the relative efficiency of the Fe(II) complexes of **1–9**, **16**, **43**, and **45** to cleave DNA in the presence of O_2 and an appropriate reducing agent (2-mercaptoethanol) was conducted through examination of the single- and double-strand cleavage of supercoiled ΦX174 RFI DNA (Form I) to produce relaxed (Form II) and linear (Form III) DNA, respectively. Like Fe(II)–bleomycin A_2 (**1**)[2-4,10,39-50] and deglycobleomycin A_2 (**2**),[17] the Fe(II) complexes of **3–8**, **43**, and **45** produced both single- and double-strand cleavage of ΦX174 RFI DNA (Table 1). The lack of DNA cleavage by the agents alone in the absence of Fe(II) in control studies is consistent with expectations that the agents are cleaving DNA by a metal-

(39) Ishida, R.; Takahashi, T. *Biochem. Biophys. Res. Commun.* **1975**, *66*, 1432.

(40) Lloyd, R. S.; Haidle, C. W.; Robberson, D. L. *Biochemistry* **1978**, *17*, 1890.

(41) Sausville, E. A.; Stein, R. W.; Peisach, J.; Horwitz, S. B. *Biochemistry* **1978**, *17*, 2740, 2746. Burger, R. M.; Horwitz, S. B.; Peisach, J.; Wittenberg, J. B. *J. Biol. Chem.* **1979**, *254*, 1229. Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636. Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 8612. Burger, R. M.; Blanchard, J. S.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1985**, *260*, 15406. Burger, R. M.; Projan, S. J.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1986**, *261*, 15955.

(42) D'Andrea, A. D.; Haseltine, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 3608. Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310.

(43) Takeshita, M.; Grollman, A. P.; Ohtsubo, E.; Ohtsubo, H. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 5983. Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608.

(44) Kuramochi, H.; Takahashi, K.; Takita, T.; Umezawa, H. *J. Antibiot.* **1981**, *34*, 576. Umezawa, H.; Takita, T.; Sugiura, Y.; Otsuka, M.; Kobayashi, S.; Ohno, M. *Tetrahedron* **1984**, *40*, 501.

(45) Murugesan, N.; Ehrenfeld, G. M.; Hecht, S. M. *J. Biol. Chem.* **1982**, *257*, 8600. Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *Biochemistry* **1988**, *27*, 58. Sugiyama, H.; Kilkuskie, R. E.; Chang, L.-H.; Ma, L.-T.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1986**, *108*, 3852. Murugesan, N.; Wu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R. E.; Rodriguez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735. Sugiyama, H.; Kilkuskie, R. E.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1985**, *107*, 7765. Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104. Long, E. C.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 5272.

Table 1. Summary of ΦX174 RFI DNA Cleavage Properties: Fe(II)–Complexes, O_2 Activation, Mercaptoethanol Initiation

	relative efficiency of DNA cleavage[a]	ratio of double- to single-stranded DNA cleavage[b]
1 (bleomycin A_2)	2–5	1:6
2 (deglycobleomycin A_2)	1.0	1:12
3 (deglycobleomycin A_1)	0.3	1:28
4 (epideglycobleomycin A_2)	0.25	1:29
5 (desacetamido-deglycobleomycin A_2)	0.25	1:29
6 (descarboxamido-deglycobleomycin A_2)	0.55	1:18
7 (desmethyldeglyco-bleomycin A_2)	1.0	1:18
8 (desimidazolyl-deglycobleomycin A_2)	0.1	1:57
9	<0.04[c]	nd
Fe(II)	0.04	1:98
16	<0.04[c]	nd
43	0.29 (1)[d]	1:33
45	0.08 (0.28)	1:35
(+)-P-3A	0.8–0.5 (1)[d]	1:30
(–)-*epi*-P-3A	0.25–0.18 (0.25)	1:38
(–)-desacetamido-P-3A	0.25–0.18 (0.25)	1:40

[a] Relative efficiency of supercoiled ΦX174 DNA cleavage. [b] Ratio of double- to single-stranded cleavage of supercoiled ΦX174 DNA cleavage calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$. [c] DNA cleavage indistinguishable from background Fe(II). [d] The values in parentheses refer to relative efficiency within the **43–45** series or P-3A series, respectively.

dependent oxidative process in a manner analogous to that of **1** and **2**. Although both single- and double-strand DNA lesions[10,47–49] result from the radical-mediated oxidative cleavage of DNA by bleomycin A_2, the latter have often been considered to be the more significant biological event.[10] Consequently, the relative extent of double- to single-stranded DNA cleavage was established in a study of kinetics of cleavage to produce linear and circular DNA for the Fe(II) complexes of **1–9**, **43**, and **45** with the supercoiled ΦX174 DNA.[47] Typical results are summarized in Table 1. A statistical treatment of the kinetics of the generation of circular and linear DNA was used to assess the ratio of double- to single-stranded DNA cleavage events. The reactions show initial fast kinetics in the first 1–10 min depending on the substrate, and the subsequent decreasing rate of DNA cleavage may reflect conversion to a less active or inactive agent or metal complex reactivation kinetics. We assumed a Poisson distribution for the formation of single- and double-stranded breaks to calculate the average number of double- and single-strand cuts per DNA molecule using the Freifelder–Trumbo equation.[51] The data for the first few minutes (2–10 min, agent dependent) could be fitted to a linear equation, and the ratios of double- to single-strand cuts observed with the Fe(II) complexes of **1–9**, **16**, **43**, and **45** are summarized in Table 1. A theoretical

(46) Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Absalon, M. J.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1993**, *32*, 2601. McGall, G. H.; Rabow, L. E.; Ashley, G. W.; Wu, S. H.; Kozarich, J. W. *J. Am. Chem. Soc.* **1992**, *114*, 4958. Rabow, L. E.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3196. Rabow, L. E.; McGall, G. H.; Stubbe, J.; Kozarich, J. W. *J. Am. Chem. Soc.* **1990**, *112*, 3203. Kozarich, J. W.; Worth, L., Jr.; Frank, B. L.; Christner, D. F.; Vanderwall, D. E.; Stubbe, J. *Science* **1989**, *245*, 1996. Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562, 7569; *J. Biol. Chem.* **1983**, *258*, 4694.
(47) Povirk, L. F.; Wubker, W.; Kohnlein, W.; Hutchinson, F. *Nucl. Acids Res.* **1977**, *4*, 3573. Povirk, L. F.; Han, Y.-H.; Steighner, R. J. *Biochemistry* **1989**, *28*, 5808. Steighner, R. J.; Povirk, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8350.
(48) Huang, C.-H.; Mirabelli, C. K.; Jan, Y.; Crooke, S. T. *Biochemistry* **1981**, *20*, 233. Mirabelli, C. K.; Huang, C.-H.; Crooke, S. T. *Cancer Res.* **1980**, *40*, 4173. Mirabelli, C. K.; Ting, A.; Huang, C.-H.; Mong, S.; Crooke, S. T. *Cancer Res.* **1982**, *42*, 2779.
(49) Keller, T. J.; Oppenheimer, N. J. *J. Biol. Chem.* **1987**, *262*, 15144.
(50) Guan, L. L.; Kuwahara, J.; Sugiura, Y. *Biochemistry* **1993**, *32*, 6141.
(51) Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681.

Table 2. Relative w794 DNA Cleavage Efficiency[a]

conditions	bleomycin A_2	deglycobleomycin A_2
Fe(II), Tris-HCl (25 mM, pH 7), O_2, mercaptoethanol, 37 °C, 30 min	0.05 (10 μM)	0.02 (100 μM)
Fe(III), Tris-HCl (10 mM, pH 7), H_2O_2 (100 μM), 37 °C, 30 min	0.5 (1 μM)	0.2 (10 μM)
Fe(III), 10 mM phosphate (pH 7), 10 mM KCl, H_2O_2 (100 μM), 37 °C, 10 min	1.0 (0.5 μM)	1.0 (2 μM)

[a] Relative extent of 5′-end-labeled w794 DNA cleavage observed and the value in parentheses represents the concentration of agent required to provide 50% cleavage.

ratio of approximately 1:100 is required in order for the linear DNA to be the result of the random accumulation of single-strand breaks within the 5386 base pair size of ΦX174 DNA assuming that sequential cleavage on the complementary strands within 15 base pairs is required to permit formation of linear DNA from the hybridized duplex DNA. Experimentally it was determined that Fe(II) alone produced a ratio of 1:98 double-strand–single-strand breaks under our conditions of assay, consistent with the theoretical ratio.

The selectivity of DNA cleavage was examined within duplex w794 DNA and its complement w836 DNA[52,53] by monitoring strand cleavage of singly ^{32}P 5′-end-labeled double-stranded DNA after exposure to the Fe(III) complexes of the agents following activation with H_2O_2[54,55] in 10 mM phosphate buffer (pH 7.0). Thus, incubation of the labeled duplex DNA with the agents in the presence of equimolar $FeCl_3$ and H_2O_2 (100 μM) led to DNA cleavage. Removal of the agent by EtOH precipitation of the DNA, resuspension of the treated DNA in aqueous buffer, and high resolution polyacrylamide gel electrophoresis (PAGE) of the resultant DNA under denaturing conditions adjacent to Sanger sequencing standards permitted the identification of the sites of DNA cleavage. Initially, the DNA cleavage reactions were conducted in TE buffer (pH 8) or Tris-HCl buffer (pH 6–8, 10–50 mM) in the presence of equimolar Fe(II) and O_2 initiated by treatment with mercaptoethanol or dithiothreitol (DTT). Unlike the use of end-labeled restriction fragments, the protocol employed utilizes a 10-fold excess of unlabeled DNA as carrier DNA.[53] Under these conditions, bleomycin A_2 (10 μM) and deglycobleomycin A_2 (100 μM) proved to be sufficiently effective at producing DNA cleavage to provide a distinguishable cleavage pattern within the labeled w794/w836 but less effective analogs were not. In contrast, the use of the Fe(III) complexes activated by treatment with H_2O_2 (100 μM) provided a much more rapid and efficient cleavage of duplex DNA, and distinguishable cleavage patterns for each of the agents examined were observed when experiments were conducted under the optimized conditions. The DNA cleavage intensities proved to be sensitive to the reaction conditions of time, temperature, and pH, and they proved to be especially dependent upon the choice of buffer. Reactions conducted in 10 mM Tris-HCl (pH 7 or 8, 37 °C, 10–30 min) proved better than those conducted in TE buffer (pH 8, 25 or 37 °C, 10–30 min) but not nearly as effective as those conducted in 10 mM phosphate buffer (pH 7.0, 10 mM KCl, 37 °C, 15 min). Table 2 summarizes representative comparisons of the relative w794 cleavage efficiencies under different reaction conditions.

(52) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. *J. Mol. Biol.* **1989**, *209*, 255.
(53) Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.
(54) Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 3997.
(55) Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 4532.

Table 3. Summary of DNA Cleavage Sites of Fe(III)–Bleomycins within w794 and w836 DNA

cleavage sites	no. of cleavage sites	total no. of dinucleotide sites	%	cleavage sites	no. of cleavage sites	total no. of dinucleotide sites	%
5'-GC	29	29	100	5'-TT	1	13	8
5'-GT	5	5	100	5'-TA	1	15	7
5'-GA	11	14	79	5'-TC	0	19	0
5'-GG	0	28	0	5'-TG	0	10	0
5'-AT	7	18	39	5'-CT	1	20	5
5'-AC	2	7	28	5'-CC	0	38	0
5'-AA	3	24	13	5'-CA	0	18	0
5'-AG	0	22	0	5'-CG	0	17	0

A statistical treatment of the observed and available dinucleotide DNA cleavage sites detected with the agents is summarized in Table 3, and the observed sites of DNA cleavage within w794/w836 are illustrated in Figure 3. The w794 and w836 PAGE from which this data were taken are shown in Figures 4 and 5 and illustrate clearly that no distinguishing differences in the sequence selectivity of DNA cleavage were observed with the agents **1–7**, **43**, and **45**.

Quantitation of the consumption of labeled DNA representing an accurate measure of the extent of DNA cleavage provided an additional assessment of the relative efficiency of DNA cleavage under a second set of conditions (Fe(III) complex, H_2O_2 activation versus Fe(II) complex, O_2, 2-mercaptoethanol initiation). The results of the quantitative assessment for both w794 and w836 DNA are summarized in Table 4 and take into account the different concentrations of complex employed in the DNA cleavage reaction. The same relative order and the same relative quantitative trends in the DNA cleavage efficiency were observed with the w794/w836 DNA protocol that were observed with ΦX174 DNA although the absolute magnitudes of the differences were expectedly different. This, no doubt, reflects the two different procedures employed for agent activation and initiation of DNA cleavage and the conditions of the assay (temperature, time, buffer).

Discussion. As detailed in the early seminal studies, bleomycin A_2 (K_B 1.0 × 10^5 M^{-1}, 3.9 base pair binding site size) and deglycobleomycin A_2 (K_B 1.1 × 10^5 M^{-1}, 3.8 base pair binding site size) exhibit the same DNA binding affinity, the same DNA cleavage selectivity, and similar DNA cleavage efficiencies (for example, see Tables 1 and 2). Thus, the role of the disaccharide is subtle and appears to not substantially affect the DNA cleavage properties of the agents. As a consequence, the relative comparisons among the deglyco agents including deglycobleomycin A_2 detailed herein are expected to be representative of the same relative comparisons that would be observed with the bleomycin A_2 analogs themselves. The examination of the agents **4–8**, **43**, and **45**, in which a single key substituent or functionality within the metal chelation subunit of **2** has been systematically *removed* while keeping the remaining portion of the molecule constant, permits an accurate assessment of the role of that substituent. Notably, the *successful* identification of an important substituent role derived from this systematic study of the authentic metal chelation subunit within the deglycobleomycin A_2 structure itself is derived from a *poor* analog lacking the one important substituent.

Analogous to the observations first made by Hecht and his colleagues,[56] Fe(II)–**9** proved indistinguishable from the background DNA cleavage reaction of Fe(II) itself. Similarly, **16**, in which the primary α-amino group of the pyrimidoblamic acid C2 side chain is protected as a BOC derivative and the histidine imidazole is protected as its trityl derivative, failed to cleave duplex DNA above background Fe(II), indicating the key role of one or both of the functional substituents.

(56) Kilkuskie, R. E.; Suguna, H.; Yellin, B.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 260.

The comparison of the ΦX174 DNA cleavage efficiency of the Fe(II) complexes of **4** and **5** with **2** permits the first accurate assessment of the relative importance and functional role of the pyrimidoblamic acid C2 acetamido side chain. Although the side chain has been shown not to be apparently involved in the metal chelation, it has been suggested to contribute to the efficiency of DNA cleavage by constituting one side or component of an oxygen binding pocket thereby sterically shielding or protecting the reactive iron–oxo intermediate or more subtly by enhancing binding affinity or orientation with duplex DNA. Consistent with an important role, Fe(II)–**2** proved to be 4 times more effective than Fe(II)–**4** or Fe(II)–**5** in its ability to cleave supercoiled ΦX174 DNA (Table 1). In addition, the ratio of double- to single-strand DNA cleavage events was reduced from 1:12 for Fe(II)–**2** to 1:29 for both Fe(II)–**4** and Fe(II)–**5**. In fact, Fe(II)–**4** and Fe(II)–**5** proved indistinguishable in the assays, and both displayed diminished DNA cleavage properties relative to those of Fe(II)–**2**. Similar observations were made in the w794/w836 DNA cleavage assays with the Fe(III) complexes (Table 4). Both **4** and **5** proved significantly less effective at producing DNA cleavage, with desacetamidodeglycobleomycin A_2 (**5**) being slightly (2–3 times) more effective than epideglycobleomycin A_2 (**4**). Significant are the observations that both **4** and **5** are 10–30 times less effective at producing the w794/w836 DNA cleavage and that the selectivity of the DNA cleavage reaction is unaffected by the removal or epimerization of the C2 acetamido side chain. These observations are especially significant and suggest an important and productive role for the C2 acetamido side chain which increases DNA cleavage efficiency and significantly increases the ratio of double- to single-strand DNA cleavage events *without* affecting the DNA cleavage selectivity.

In efforts to independently assess the role of the C2 acetamido side chain, comparisons of **43** possessing the C2 acetamido side chain with **45** lacking the C2 acetamido side chain were conducted employing the same protocols. Like the comparisons of **2** versus **5**, the Fe(*II*) complex of **43** proved to be approximately 4 times more effective than **45** at cleaving ΦX174 DNA and slightly more effective at producing double- versus single-strand DNA cleavage events (Table 1). Similarly, the Fe(III) complex of **43** proved to be 2–3 times more effective than **45** at cleaving w794/w836 DNA without a perceptible alteration of the characteristic bleomycin A_2 DNA cleavage selectivity (Table 4). A comparative measurement of the DNA binding constants of **43** (K_B = 2.4 × 10^5 M^{-1}), **45** (K_B = 2.4 × 10^5 M^{-1}), and **2** (K_B = 1.1 × 10^5 M^{-1}) revealed that the distinctions observed were not the consequence of diminished DNA binding affinity. Thus, the comparisons of **43** with **45** confirm a useful and productive role for the C2 acetamido side chain which increases the DNA cleavage efficiency and significantly enhances double- to single-strand cleavage without affecting the DNA cleavage selectivity or DNA binding affinity.

These results proved to be exactly analogous to those made in comparisons of (+)-P-3A, *epi*-(−)-P-3A, and (−)-desacetamido-P-3A, in which the epimerization or removal of the C2 acetamido side chain reduced the DNA cleavage efficiency (4 times) and signficiantly reduced the ratio of double- to single-strand DNA cleavage events[37] (Table 1). Although there are a number of attractive explanations for these observations, the comparison of the (+)-P-3A results with those derived herein has proven especially revealing. (+)-P-3A and the related agents exhibit the same C2 acetamido side chain behavior but have been found to cleave DNA with no discernible sequence selectivity. Although these results need to be interpreted with caution in light of the observations of Mascharak,[57] they do suggest that the role of the C2 acetamido side chain is unlikely to be the result of a specific DNA interaction or orientation effect especially suited for a particular cleavage site or bleomycin bound conformation.

(57) Guajardo, R. J.; Hudson, S. E.; Brown, S. J.; Mascharak, P. K. *J. Am. Chem. Soc.* **1993**, *115*, 7971.

```
w836   5'-AGGCGGCCTCGGCCTCTGCATAAATAAAAAAAATTAGTCAGCCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGCGGGATGGCGGAG-3'
w794   3'-TCCGCCGGAGCCGGAGACGTATTTATTTTTTTTAATCAGTCGGTACCCCGCCTCTTACCCGCCTTGACCCGCCTCAATCCCCGCCCTACCGCCTC-5'
```

Figure 3. Summary of cleavage sites for the bleomycins within w794 and w836 DNA. The missing terminal regions of the DNA not shown, from which some of the data for Table 3 were taken, represent the nonoverlapping regions not present on the complementary clones.

Figure 4. Cleavage of double-stranded DNA (SV40 DNA fragment, 156 base pairs, nucleotide no. 5239–150, clone w794) in 10 mM phosphate buffer, pH 7.0, containing 1.47 mM H_2O_2 by Fe(III)–bleomycins. The DNA cleavage reactions were run for 10 min at 37 °C, and electrophoresis was conducted at 1100 V (5.5 h) on an 8% denaturing PAGE and visualized by autoradiography: lane 1, control DNA; lane 2, 128 μM Fe(III) control; lane 3, 0.5 μM Fe(III)–bleomycin A_2 (**1**); lane 4, 2 μM Fe(III)–deglycobleomycin A_2 (**2**); lane 5, 16 μM Fe(III)–deglycobleomycin A_1 (**3**); lane 6, 32 μM Fe(III)–epideglycobleomycin A_2 (**4**); lane 7, 16 μM Fe(III)–desacetamidodeglycobleomycin A_2 (**5**); lane 8, 8 μM Fe(III)–descarboxamidodeglycobleomycin A_2 (**6**); lane 9, 4 μM Fe(III)–desmethyldeglycobleomycin A_2 (**7**); lane 10, 128 μM Fe(III)–desimidazolyldeglycobleomycin A_2 (**8**); lane 11, 64 μM Fe(III)–GABA,Gly-deglycobleomycin A_2 (**43**); lane 12, 128 μM Fe(III)–GABA,Gly-desacetamidodeglycobleomycin A_2 (**45**).

Figure 5. Cleavage of double-stranded DNA (SV40 DNA fragment, 146 base pairs, nucleotide no. 5189–91, clone w836) in 10 mM phosphate buffer, pH 7.0, containing 1.47 mM H_2O_2 by Fe(III)–bleomycins. The DNA cleavage reactions were run for 10 min at 37 °C, and electrophoresis was conducted at 1100 V (5.5 h) on an 8% denaturing PAGE and visualized by autoradiography. The first 60 5′ base pairs are shown for clarity: lane 1, control DNA; lane 2, 128 μM Fe(III) control; lane 3, 0.5 μM Fe(III)–bleomycin A_2 (**1**); lane 4, 2 μM Fe(III)–deglycobleomycin A_2 (**2**); lane 5, 16 μM Fe(III)–deglycobleomycin A_1 (**3**); lane 6, 32 μM Fe(III)–epideglycobleomycin A_2 (**4**); lane 7, 16 μM Fe(III)–desacetamidodeglycobleomycin A_2 (**5**); lane 8, 8 μM Fe(III)–descarboxamidodeglycobleomycin A_2 (**6**); lane 9, 4 μM Fe(III)–desmethyldeglycobleomycin A_2 (**7**); lane 10, 128 μM Fe(III)–desimidazolyldeglycobleomycin A_2 (**8**); lane 11, 64 μM Fe(III)–GABA,Gly-deglycobleomycin A_2 (**43**); lane 12, 128 μM Fe(III)–GABA,Gly-desacetamidodeglycobleomycin A_2 (**45**).

Rather, the observations are consistent with a productive role for the C2 acetamido side chain in increasing the effective cleavage of DNA by stabilizing the activated metal complex, increasing its effective catalytic turnover, or diminishing nonproductive decomposition perhaps through shielding or protecting the activated metal from solvent or through an unrecognized complementary coordination to the metal.

In addition to serving as an independent confirmation of the important role of the C2 acetamido side chain, the comparative examination of **43** with **2** and **45** with **5** serves to provide two independent assessments of the cumulative role of the tetrapeptide S substituents. The metal complexes of **2** proved to be 3–4 times more effective than **43** at cleaving ΦX174 DNA, 40–50 times more effective at cleaving w794/w836 DNA, and substantially more effective at producing double- versus single-strand DNA cleavage. While the DNA cleavage efficiency differences between **2** and **43** are substantial and significant, they are not nearly of the magnitude one might expectedly attribute to the cumulative effect of *all* the tetrapeptide S substituents. In addition, while the tetrapeptide S substituents affected the DNA cleavage efficiency, no perceptible alteration in the DNA cleavage selectivity was observed (Figures 4 and 5). In an independent confirmation of these effects, **5** proved to be 3–4 times more effective than **45** at cleaving ΦX174 DNA, 10 times more effective at cleaving w794/w836 DNA, and more effective at producing double- versus single-strand DNA cleavage events, and both agents cleaved DNA with the same characteristic 5'-GC, 5'-GT selectivity as **1** and **2**. Thus, the tetrapeptide S substituents affect DNA cleavage efficiency *and* the ratio of double- to single-strand cleavages but have no perturbing effects on DNA cleavage selectivity. Notably, **45** represents the parent backbone structure of bleomycin A_2 lacking nearly all the nonessential substituents and the disaccharide subunit. Bleomycin A_2 (**1**) and deglycobleomycin A_2 (**2**) proved to be 25–60 times and 10–15 times, respectively, more effective than **45** at cleaving ΦX174 DNA and 500–1000 times and 100 times more effective than **45** at cleaving w794/w836, and the comparisons of **45** with **1** and **2** represent a cumulative assessment of the nonessential peripheral substituents. Notably, the DNA cleavage selectivity is unaffected by removal of the substituents, and it is only the DNA cleavage efficiency that is reduced. Two potential explanations may be advanced for these observations. The removal of the backbone substituents may significantly alter the DNA binding affinity, thereby lowering the effective relative DNA cleavage efficiency, or the backbone substituents may alter or restrict the DNA bound conformation or orientation of the agent and enhance DNA cleavage efficiency. Consequently, we measured the apparent DNA binding constants and binding site sizes for **43** and **45** (apparent K_B, calf thymus DNA)[11] for comparison with those of **1**–**2** following the protocol detailed in the accompanying article.[11] Both **43** ($K_B = 2.4 \times 10^5$ M^{-1}, 3.3 base pairs) and **45** (2.4×10^5 M^{-1}, 3.3 base pairs) possessed comparable and slightly *higher*

Table 4. Summary of w794/w836 DNA Cleavage Properties: Fe(III)–Complexes, H_2O_2 Activation

agent	relative efficiency of DNA cleavage		DNA cleavage selectivity[a]
	w794	w836	
1 (bleomycin A_2)	5.8	9.1	5′-GC, 5′-GT > 5′-GA
2 (deglycobleomycin A_2)	1.0	1.0	5′-GC, 5′-GT > 5′-GA
3 (deglycobleomycin A_1)	0.10	0.13	5′-GC, 5′-GT > 5′-GA
4 (epideglycobleomycin A_2)	0.04	0.03	5′-GC, 5′-GT > 5′-GA
5 (desacetamidodeglycobleomycin A_2)	0.09	0.08	5′-GC, 5′-GT > 5′-GA
6 (descarboxamidodeglycobleomycin A_2)	0.20	0.14	5′-GC, 5′-GT > 5′-GA
7 (desmethyldeglycobleomycin A_2)	0.60	0.50	5′-GC, 5′-GT > 5′-GA
8 (desimidazolyldeglycobleomycin A_2)	0.008	0.006	none
43	0.02	0.02	5′-GC, 5′-GT > 5′-GA
45	0.009	0.009	5′-GC, 5′-GT > 5′-GA
Fe(III)	0.006	0.005	none
(+)-P-3A			none
(−)-*epi*-P-3A			none
(−)-desacetamido P-3A			none

[a] See Table 3: 5′-GC, 5′-GT > 5′-GA > 5′-AT > 5′-AC > 5′-AA, 5′-TT, 5′-TA, 5′-CT.

binding constants than bleomycin A_2 (**1**, 1.0×10^5 M^{-1}, 3.9 basepairs) and deglycobleomycin A_2 (1.1×10^5 M^{-1}, 3.8 base pairs) but exhibited smaller and perhaps significantly reduced binding site sizes. The comparable binding constants for **1**, **2** and **43**, **45** are consistent with expectations based on a comparison of the corresponding binding constants for the tetrapeptide subunits,[11] highlight the observation that the tetrapeptide S backbone substituents as well as the disaccharide do not productively contribute to DNA binding affinity, illustrate that the differences in **43** versus **45** are not due to a distinguishing binding affinity derived from the C2 acetamido side chain, and similarly illustrate that the distinctions in **1**, **2** versus **43**, **45** are not derived from differences in DNA binding affinity. The observations are consistent with the tetrapeptide S backbone substituents altering or restricting the DNA bound conformation of **1** and **2**, leading to enhanced DNA cleavage efficiency and an enhanced ratio of double- to single-strand DNA cleavage events.

The comparison of descarboxamidodeglycobleomycin A_2 (**6**), which lacks the pyrimidoblamic acid C2 side chain α-carboxamido group, with **2** proved exceptionally interesting. Rapid and selective bleomycin hydrolase hydrolysis of this carboxamido group has been suggested to account for the rapid metabolic inactivation of **1**.[31] Provided this group does not contribute to the productive properties of **1**, analogs lacking this group might represent more potent, more efficacious, or longer acting antitumor agents. The agent **6** proved to be only 2 times less effective than **2** at cleaving ΦX174 DNA, only slightly less effective at producing double- versus single-strand DNA cleavage in the ΦX174 DNA assay (1:18 vs 1:12; Table 1), and only 5–7 times less effective than **2** in the more sensitive w794/w836 DNA cleavage assay (Table 4). No differences in the DNA cleavage selectivity of **6** and **2** were observed (Figures 4 and 5). The impact that these small and subtle distinctions in the cleavage efficiency which result from removal of the α-carboxamido groups might have on analogs of **1** relative to their increased stability toward bleomycin hydrolase metabolic inactivation should prove especially interesting and is under current investigation.

The comparison of desmethyldeglycobleomycin A_2 (**7**), lacking only the pyrimidine C5 methyl group, with **2** proved exceptionally nice. The two agents proved indistinguishable in the ΦX174 DNA cleavage assays, exhibiting the same DNA cleavage efficiency and nearly the same ratio of double- to single-strand DNA cleavage (Table 1). Only a very subtle difference in the relative efficiency of DNA cleavage was detected in the more sensitive w794/w836 DNA cleavage protocol (2 times), and the distinctions are within the experimental error of the assays (Table 4). As with the preceding agents, **7** and **2** exhibited the same profile of DNA cleavage, indicating that the C5 methyl substituent of **1** and **2** does not contribute productively to DNA cleavage efficiency or selectivity (Figures 4 and 5).

Consistent with its assumed pivotal role in the metal chelation properties of bleomycin A_2, desimidazolyldeglycobleomycin A_2 (**8**) proved to be substantially less efficient in cleaving ΦX174 DNA and only slightly better than background Fe(II). Similarly, it exhibited a poor efficiency for double-stranded DNA cleavage (1:57 double-stranded–single-stranded cleavage), being only slightly better than background Fe(II) (Table 1). Similar and more revealing observations were made in the studies with w794/w836 DNA. DNA cleavage by **8** was observed above background Fe(III) but at a substantially reduced (120–150 times, Table 4) efficiency relative to **2** and was found to occur with *no* discernible sequence selectivity (Figures 4 and 5). Although this was not investigated in detail, this most likely may be attributed to a change in the mechanism for the Fe(III)–**8** cleavage of duplex DNA which, unlike that of **2**, may be mediated through Fenton chemistry with generation of diffusable oxidants including hydroxyl radical. Thus, consistent with expectations, the removal of the imidazole from deglycobleomycin A_2 dramatically reduces the productive DNA cleavage capabilities of the agent. In addition to the confirmation of the pivotal role of the histidine imidazole, the observations also highlight a problematic experimental feature of affinity cleavage agents based on the bleomycin A_2 structure. Because the cleavage selectivity of bleomycin A_2 is relatively low and entails several small dinucleotide sites, bleomycin A_2 affinity cleavage agents employing a reactive diffusable oxidant are unlikely to provide sequence-selective binding information about the agents.[58]

Finally, similar to observations made in the comparisons of deglycobleomycin A_2, (**2**) and demethyl deglycobleomycin A_2 lacking the sulfonium salt, deglycobleomycin A_1 (**3**), bearing the C-terminus sulfoxide, proved to be 3–4 times less effective than **2** at cleaving ΦX174 DNA, exhibited a diminished double- to single-strand DNA cleavage ratio (1:28 versus 1:12), and proved to be 8–10 times less efficient at cleaving w794/w836 DNA but did so with the identical and characteristic 5′-GC, 5′-GT > 5′-GA selectivity. Thus, consistent with expectations but in notable contrast to the reported behavior of bleomycinic acid,[21b] the C-terminus modification found in **3** did not alter the cleavage selectivity but simply reduced DNA cleavage efficiency attributable to a reduced DNA binding affinity.

In Vitro Cytotoxic Activity. Representative agents within the series studied were examined for in vitro cytotoxic activity[59,60] (Table 5). Consistent with past observations, the deglyco agents including **2** proved less potent than **1** itself. As has been previously

(58) Kane, S. A.; Natrajan, A.; Hecht, S. M. *J. Biol. Chem.* **1994**, *269*, 10899.
(59) Terasima, T.; Takabe, Y.; Katsumata, T.; Watanabe, M.; Umezawa, H. *J. Natl. Cancer Inst.* **1972**, *49*, 1093. Terasima, T.; Umezawa, H. *J. Antibiot.* **1970**, *23*, 300. Suzuki, H.; Nagai, K.; Yamaki, H.; Tanaka, N.; Umezawa, H. *J. Antibiot.* **1968**, *21*, 379.
(60) Nicolaou, K. C.; Dai, W.-M.; Tsay, S.-C.; Estevez, V. A.; Wrasidlo, W. *Science* **1992**, *256*, 1172.

Table 5. Cytotoxic Activity

agent	IC_{50}, L1210 (μM)[a]	IC_{50}, 786-O (μM)[a]
1 (bleomycin A_2)	2.5	17–25
2 (deglycobleomycin A_2)	110	nt
6 (descarboxamidodeglycobleomycin A_2)	390	440
43 (GABA, Gly-deglycobleomycin A_2)	960	nt

[a] Inhibiting concentration (IC) of agent required for 50% inhibition of cell growth, 72-h cell culture assay starting with 1×10^4 cells/200 μL, ref 60.

described in the early studies of Umezawa, the relative cytotoxic activities follow trends observed in the relative efficiency of DNA cleavage (*i.e.*, **1** > **2**). However, it is important to note that this increased DNA cleavage efficiency is *not* derived from an enhanced DNA binding affinity and that the lack of the disaccharide does not alter the DNA cleavage selectivity. Consistent with the trends observed in the relative DNA cleavage efficiency, deglycobleomycin A_2 proved to be 3–4 times and 9 times more potent (L1210) than **6** and **43**, respectively.

The 786-O renal cell line contains elevated levels of bleomycin hydrolase. Consequently, if carboxamide hydrolysis effected by bleomycin hydrolase is uniquely responsible for inactivation of **1**, the relative sensitivity of this cell line to **1** would be reduced while the sensitivity to **6** could be expected to remain unaltered. Consistent with expectations, **1** proved to be approximately 7–10 times less potent against 786-O. In contrast, no significant change in the relative potency of **6** toward 786-O versus L1210 was observed, consistent with the lack of opportunity for bleomycin hydrolase inactivation of **6**.

Conclusions. The past observations that agents related to the iron chelation subunit of bleomycin A_2 may effectively cleave DNA above a control Fe(II) background with an altered selectivity[57] or with no discernible sequence selectivity[37] illustrate that a bleomycin related metal chelation subunit alone is not sufficient for reproducing the bleomycin A_2 sequence-selective DNA cleavage.[37] The observations are consistent with studies which suggest that the C-terminus of bleomycin A_2 including the bithiazole and tri- or tetrapeptide subunits contributes to the bleomycin A_2 DNA cleavage selectivity.[28] The major structural deletions to the metal binding domain described herein including removal or epimerization of the C2 acetamido side chain, removal of the β-amino-L-alanine carboxamide, and removal of the pyrimidine C5 methyl substituent had no effect on the selectivity of DNA cleavage. In contrast, removal of the histidine imidazole provided an agent that exhibited substantially reduced and nearly abolished DNA cleavage capabilities including a loss of DNA cleavage selectivity, illustrating the pivotal role this substituent plays within **1** and **2**. In addition, removal of the tetrapeptide S backbone substituents did not perceptibly alter the DNA cleavage selectivity or DNA binding affinity observed with **1** and **2**.

Despite the lack of impact on DNA cleavage selectivity or binding affinity, the structural alterations in the metal chelation subunit had a significant impact on the relative efficiency of DNA cleavage and the observed ratio of double- versus single-strand DNA cleavage events. The comparisons of the DNA cleavage properties of **2** with those of **4** and **5** and the independent comparisons of **43** with **45** illustrate a prominent and important role for the C2 acetamido side chain of **1** and **2**. Like observations made in the comparisons of P-3A, *epi*-P-3A, and desacetamido P-3A,[37] both the DNA cleavage efficiency and the ratio of double- to single-strand DNA cleavage events are reduced significantly by the removal or epimerization of the C2 acetamido side chain. In contrast, removal of the C2 side chain β-amino-L-alanine carboxamide only subtly reduced the efficiency of DNA cleavage and the ratio of double- to single-strand DNA breaks. This small impact coupled with the cytotoxic studies summarized in Table 5 suggests potential improvements in the biological properties of **1** (half-life, efficacy) may be derived from such agents which are incapable of the proposed metabolic inactivation by bleomycin hydrolase. In addition, the studies illustrate that the pyrimidine C5 methyl group does not contribute to the efficiency or selectivity of DNA cleavage and that the histidine imidazole is essential. Finally, removal of the tetrapeptide S backbone substituents substantially reduced the efficiency of DNA cleavage. Since this removal of the tetrapeptide S backbone substituents does not alter the DNA binding affinity of the agents,[11] such observations are consistent with the tetrapeptide S backbone substituents altering or restricting the DNA bound conformation of **1** and **2** in such a way as to lead to enhanced DNA cleavage.

The extension of the technology developed herein to the total synthesis of bleomycin A_2 (**1**) is detailed in the following article,[61] and the extension of the efforts to the further exploration of the C-terminus tetrapeptide S subunit of **1** and **2** is in progress and will be reported in due course.

Experimental Section

***N*$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-*N*$^{\beta}$-[1-amino-3(*S*)-(4-amino-6-(amido-*N*^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl methyl ester)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (12).** A solution of **10**[12] (8.1 mg, 0.019 mmol) in THF–DMF (2:1, 0.34 mL) was treated with **11**[11] (8.9 mg, 0.021 mmol, 1.1 equiv), HOBt (2.6 mg, 0.019 mmol, 1.0 equiv), and EDCI (3.8 mg, 0.020 mmol, 1.05 equiv), and the mixture was stirred under Ar at 25 °C (72 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 1 mm PCTLC, 10% CH_3OH-CH_2Cl_2) afforded **12** (13.9 mg, 15.6 mg theoretical, 89%) as a white film: R_f 0.25 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]^{25}_D$ +18 (*c* 0.08, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.54 (s, 1H), 7.38 (m, 9H), 7.13 (m, 6H), 6.95 (s, 1H), 5.16 (d, J = 6.0 Hz, 1H), 5.02 (d, J = 6.0 Hz, 1H), 4.15 (m, 1H), 4.00 (m, 1H), 3.70 (s, 3H), 2.88 (m, 1H), 2.80 (m, 1H), 2.72 (m, 1H), 2.55 (dd, J = 7.5, 14.0 Hz, 1H), 2.34 (s, 3H), 1.47 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.7, 171.9, 167.9, 167.1, 166.6, 157.0, 152.5, 143.6, 141.3, 139.9, 130.9, 130.8, 129.4, 129.3, 129.2, 120.9, 113.3, 80.2, 79.0, 76.9, 69.2, 61.7, 59.4, 55.6, 52.9, 41.7, 28.7, 11.6; IR (neat) ν_{max} 3394, 3236, 3142, 2966, 1737, 1649, 1443, 1390, 1243, 1208, 1079, 1014, 755 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 967.2819 (M^+ + Cs, $C_{43}H_{50}N_{10}O_8$ requires 967.2867).

***N*$^{\beta}$-(1-Amino-3(*S*)-(4-amino-6-(amido-*erythro*-β-hydroxy-L-histidyl methyl ester)-5-methylpyrimidin-2-yl)propion-3-yl)-(*S*)-β-aminoalanine Amide (9).** A solution of **12** (1.2 mg, 0.0015 mmol) in TFA (2 mL) was stirred at 25 °C (45 min) under Ar. The mixture was concentrated in vacuo. Chromatography (reverse phase C-18, 1 × 1 cm, H_2O eluant) with collection of the UV-active fractions afforded **9** (0.65 mg, 0.70 mg theoretical, 93%) as a thin film: $[\alpha]^{25}_D$ +13 (*c* 0.01, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.92 (s, 1H), 7.55 (s, 1H), 5.34 (d, J = 6.0 Hz, 1H), 5.12 (d, J = 6.0 Hz, 1H), 4.21 (m, 2H), 3.78 (s, 3H), 3.25 (m, 1H), 3.15 (dd, J = 5.0, 10.0 Hz, 1H), 2.92 (m, 1H), 2.80 (m, 1H), 2.31 (s, 3H); IR (neat) ν_{max} 3383, 3219, 2973, 1740, 1737, 1695, 1512, 1497, 1367, 1213, 1062, 853, 703 cm^{-1}; FABHRMS (NBA) *m/e* 493.2277 (M^+ + H, $C_{19}H_{28}N_{10}O_6$ requires 493.2272).

***N*$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-*N*$^{\beta}$-[1-amino-3(*S*)-(4-amino-6-(amido-*N*^{im}-triphenylmethyl-*erythro*-β-hydroxy-L-histidyl)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (13).** A solution of **12** (5.8 mg, 0.007 mmol) in THF–CH_3OH–H_2O (3:1:1, 0.2 mL) was treated with aqueous 1 N LiOH (0.011 mL, 0.011 mmol, 1.5 equiv), and the mixture was stirred at 0 °C (1.5 h). After most of the THF and CH_3OH was evaporated, the aqueous phase was extracted with $CHCl_3$ (0.5 mL). The aqueous phase was acidified with aqueous 1.2 N HCl to pH 7, and the mixture was passed through a short C-18 reverse-phase plug using H_2O for elution. The UV-active fractions were combined, and the solvent was removed in vacuo to afford **13** (5.6 mg, 5.7 mg theoretical, 98%) as a white foam: $[\alpha]^{25}_D$ −13 (*c* 0.07, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.48 (s, 1H), 7.33 (m, 9H), 7.14 (m, 6H), 7.00 (s, 1H), 5.06 (d, J = 6.0 Hz, 1H), 4.75 (d, J = 6.0 Hz, 1H), 4.14 (m, 1H), 3.93 (m, 1H), 3.22 (m, 2H), 3.00 (m, 1H), 2.82 (dd, J = 7.0, 14.0 Hz, 1H), 2.35 (s, 3H), 1.47 (s, 9H); IR (neat) ν_{max} 3400, 3341, 2977, 2929, 1737, 1663, 1644, 1531, 1408, 1364, 1196, 1157, 1054, 956, 852 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 953.2779 (M^+ + Cs, $C_{42}H_{48}N_{10}O_8$ requires 953.2711).

(61) Boger, D. L.; Honda, T. *J. Am. Chem. Soc.*, following paper in this issue.

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)deglycobleomycin A_2 (16). A solution of **13** (3.6 mg, 0.0044 mmol) in DMF (0.1 mL) was treated sequentially with DCC (2.75 mg, 0.013 mmol, 3 equiv), HOBt (0.6 mg, 0.0044 mmol, 1 equiv), $NaHCO_3$ (1.3 mg, 0.015 mmol, 2.5 equiv), and **15**[11] (4.2 mg, 0.0062 mmol, 1.4 equiv) dissolved in DMF (0.1 mL), and the mixture was stirred under Ar at 25 °C (72 h). The reaction mixture was concentrated in vacuo to give an oily solid. The crude mixture was dissolved in CH_3OH (3 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH solution was then evaporated, and the sample was triturated with $CHCl_3$ (3 × 1 mL). Chromatography (reverse-phase C-18, 0.5 × 2.0 cm, 5–70% CH_3OH–H_2O gradient elution) gave **16** (3.9 mg, 6.1 mg theoretical, 64%) as a thin film: R_f 0.5 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ −21 (*c* 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 8.10 (s, 1H), 7.56 (s, 1H), 7.35 (m, 9H), 7.16 (m, 6H), 6.90 (s, 1H), 4.35 (d, *J* = 4.5 Hz, 1H), 4.10 (m, 1H), 4.07 (d, *J* = 7.0 Hz, 1H), 4.03 (d, *J* = 7.0 Hz, 1H), 3.79 (m, 1H), 3.77 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.62 (m, 3H), 3.58 (m, 3H), 3.37 (m, 2H), 3.24 (m, 2H), 2.98 (s, 6H), 2.80 (m, 1H), 2.69 (dd, *J* = 13.0, 6.5 Hz, 1H), 2.56 (m, 2H), 2.42 (dd, *J* = 14.5, 9.0 Hz, 1H), 2.23 (s, 3H), 2.13 (m, 2H), 1.41 (s, 9H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 6.5 Hz, 3H), 1.10 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3448, 2989, 2907, 1625, 1478, 1419, 1261, 1102, 1043, 996, 914, 749 cm^{-1}; FABHRMS (NBA) *m/e* 1389.5737 (M^+, $C_{66}H_{85}N_{16}O_{12}S_3$ requires 1389.5695).

Deglycobleomycin A_2 (2). The solid **16** (1.2 mg, 0.0008 mmol) was treated with TFA (1 mL), and the mixture was stirred under Ar at 25 °C (45 min). The solvent was evaporated in vacuo to give an oily solid. Chromatography (SiO_2, 0.5 × 2 cm, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH eluant, TLC R_f 0.16) followed by chromatography over Amberlite XAD-2 (2 × 1 cm), first desalting the absorbed sample with H_2O and then eluting the agent with CH_3OH, afforded **2** (0.54 mg, 0.9 mg theoretical, 60%) as a white solid identical in all respects with an authentic sample of deglycobleomycin A_2:[17] $[\alpha]^{25}_D$ −15 (*c* 0.025, 0.1 N HCl) (lit[15] $[\alpha]^{24}_D$ −15 (*c* 0.5, 0.1 N HCl)); ^1H NMR (D_2O, 400 MHz) δ 8.10 (br s, 1H), 8.02 (s, 1H), 7.82 (s, 1H), 7.08 (br s, 1H), 4.02 (d, *J* = 4.0 Hz, 1H), 3.89 (m, 1H), 3.85 (m, 1H), 3.67 (m, 1H), 3.50 (m, 3H), 3.42 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.39 (t, *J* = 6.5 Hz, 2H), 3.18 (t, *J* = 7.0 Hz, 2H), 3.06 (t, *J* = 7.0 Hz, 2H), 2.92 (m, 2H), 2.71 (s, 6H), 2.35 (m, 2H), 2.15 (m, 1H), 1.95 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.78 (s, 3H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.95 (d, *J* = 6.0 Hz, 3H), 0.88 (d, *J* = 7.0 Hz, 3H); IR (nujol) ν_{max} 3323, 2819, 2844, 1651, 1547, 1458, 1374, 1307, 1261, 1063, 969 cm^{-1}; FABHRMS (NBA) *m/e* 1047.3981 (M^+, $C_{42}H_{63}N_{16}O_{10}S_3$ requires 1047.4075).

(2″*S*,3″*S*,4″*R*)-3-[2′-(2‴-((*N*-(4″-((*tert*-Butyloxy)carbonyl)amino)-3″-hydroxy-2″-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfoxide (18). A solution of $NaIO_4$ (0.67 mg, 0.0031 mmol, 1 equiv) in H_2O (16 μL) was added to **17**[11] (2 mg, 0.0030 mmol) in EtOH (43 μL) at 0 °C, and the reaction mixture was stirred at 0 °C for 4 h (TLC: SiO_2, 20% CH_3OH–CH_2Cl_2; R_f (**17**) = 0.62, R_f (sulfone) = 0.58, and R_f (**18**) = 0.51). The crude reaction mixture was filtered, the solids were washed with EtOH, and the filtrate was concentrated in vacuo. Purification of the residue (SiO_2, 0.5 × 2.0 cm, 10% CH_3OH–CH_2Cl_2) afforded the sulfone (1 mg, R_f 0.41) and **18** (1.3 mg, 2.1 mg theoretical, 63%, R_f 0.24) as white films. For **18**: R_f 0.24 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ +22 (*c* 0.065, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 8.14 (s, 1H), 4.30 (d, *J* = 4.0 Hz, 1H), 4.12 (m, 1H), 3.66 (m, 4H), 3.58 (m, 4H), 2.93 (m, 1H), 2.85 (m, 1H), 2.65 (s, 3H), 2.58 (dq, *J* = 9.0, 7.0 Hz, 1H), 2.09 (tt, *J* = 6.0, 6.0 Hz, 2H), 1.41 (s, 9H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3312, 2977, 1631, 1454, 1367, 1166, 1088, 825 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 821.1445 (M^+ + Cs, $C_{28}H_{44}N_6O_8S_3$ requires 821.1437).

For the sulfone: R_f 0.4 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ +60 (*c* 0.050, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.19 (s, 1H), 8.14 (s, 1H), 4.30 (d, *J* = 4.0 Hz, 1H), 4.15 (m, 1H), 3.65 (m, 3H), 3.57 (m, 3H), 3.20 (m, 4H), 2.98 (s, 3H), 2.58 (dq, *J* = 9.0, 7.0 Hz, 1H), 2.14 (m, 2H), 1.41 (s, 9H), 1.19 (d, *J* = 7.0 Hz, 3H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3362, 2949, 1629, 1459, 1381, 1088, 824 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 837.1383 (M^+ + Cs, $C_{28}H_{44}N_6O_9S_3$ requires 837.1386).

(2″*S*,3″*S*,4″*R*)-3-[2′-(2‴-((*N*-(4″-Amino-3″-hydroxy-2″-methylpentanoyl)-L-threonyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido]propyl Methyl Sulfoxide Hydrochloride (19). The solid **18** (3.8 mg, 0.0055 mmol) was treated with 3 N HCl–EtOAc (1 mL), and the mixture was stirred under Ar at 25 °C (75 min). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (1 × 1 mL) to give **19** (3.2 mg, 3.45 mg theoretical, 93%) as a hygroscopic solid: $[\alpha]^{25}_D$ +41 (*c* 0.01, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.22 (s, 1H), 8.19 (s, 1H), 4.24 (d, *J* = 4.5 Hz, 1H), 4.11 (m, 1H), 3.75 (m, 3H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.56 (m, 1H), 3.39 (m, 2H), 2.92 (m, 1H), 2.85 (m, 1H), 2.70 (s, 3H), 2.58 (m, 1H), 2.21 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.35 (d, *J* = 6.0 Hz, 3H), 1.34 (d, *J* = 6.0 Hz, 3H), 1.15 (d, *J* = 6.0 Hz, 3H); IR (neat) ν_{max} 3440, 3006, 1643, 1525, 1478, 1433, 1254, 1165, 1046, 1024, 998, 826, 765 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 721.0926 (M^+ + Cs, $C_{23}H_{36}N_6O_6S_3$ requires 721.0913).

Deglycobleomycin A_1 (3). A solution of **13** (3.92 mg, 0.0048 mmol) in DMF (0.5 mL) was treated sequentially with EDCI (2.74 mg, 0.014 mmol, 3 equiv), HOBt (0.65 mg, 0.0048 mmol, 1 equiv), $NaHCO_3$ (1.05 mg, 0.012 mmol, 2.6 equiv), and **19** (3.1 mg, 0.005 mmol, 1.05 equiv) dissolved in DMF (0.1 mL), and the mixture was stirred under Ar at 25 °C (65 h). The reaction mixture was concentrated in vacuo to give an oily solid. The crude residue was dissolved in CH_3OH (2 mL), the insoluble inorganic salts were removed by centrifugation, and the CH_3OH was evaporated in vacuo. Chromatography (reverse-phase C-18, 1–15% CH_3OH–H_2O gradient elution) gave **20** (5.2 mg, 6.65 mg theoretical, 78%) as a thin film: R_f 0.32 (SiO_2, 10:9:1 CH_3OH-10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.15 (s, 1H), 8.11 (s, 1H), 7.58 (s, 1H), 7.33 (m, 9H), 7.13 (m, 6H), 6.95 (s, 1H), 4.32 (d, *J* = 4.5 Hz, 1H), 4.15 (m, 1H), 4.01 (m, 2H), 3.80 (dd, *J* = 6.0, 8.0 Hz, 1H), 3.78 (m, 1H), 3.70 (m, 3H), 3.61 (m, 3H), 3.28 (t, *J* = 7.0 Hz, 2H), 2.85 (m, 2H), 2.76 (m, 2H), 2.67 (s, 3H), 2.55 (m, 2H), 2.42 (m, 1H), 2.34 (s, 3H), 2.12 (m, 2H), 1.45 (s, 9H), 1.28 (d, *J* = 6.0 Hz, 3H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.14 (d, *J* = 6.0 Hz, 3H).

The solid **20** (2.5 mg, 0.0018 mmol) was treated with TFA (1 mL), and the mixture was stirred under Ar at 25 °C (45 min). The solvent was evaporated in vacuo to give an oily solid. Chromatography (reverse-phase C-18, H_2O) gave **3** (1.66 mg, 1.88 mg theoretical, 88%) as a thin film: R_f 0.24 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ +32 (*c* 0.015, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.60 (s, 1H), 8.01 (s, 1H), 7.88 (s, 1H), 6.95 (s, 1H), 4.38 (d, *J* = 6.0 Hz, 1H), 4.22 (m, 1H), 4.15 (d, *J* = 4.5 Hz, 1H), 4.10 (m, 1H), 3.92 (m, 1H), 3.70 (m, 1H), 3.34 (m, 8H), 2.94 (m, 2H), 2.80 (m, 2H), 2.54 (s, 3H), 2.42 (m, 2H), 2.04 (s, 3H), 1.98 (m, 3H), 1.06 (d, *J* = 6.0 Hz, 3H), 1.04 (d, *J* = 6.5 Hz, 3H), 0.95 (d, *J* = 6.0 Hz, 3H); IR (neat) ν_{max} 3355, 2974, 2923, 1653, 1638, 1547, 1525, 1483, 1440, 1410, 1385, 1294, 1162, 1124 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 1181.2857 (M^+ + Cs, $C_{41}H_{60}N_{16}O_{11}S_3$ requires 1181.2845).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*R*)-(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl methyl ester)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (22). Thoroughly dried **21**[12] (11.0 mg, 0.026 mmol) was placed in a reaction vessel followed by the addition of EDCI (5.2 mg, 0.027 mmol, 1.05 equiv) and HOBt (3.5 mg, 0.026 mmol, 1.0 equiv). A solution of **11**[11] (15 mg, 0.035 mmol, 1.35 equiv) in THF–DMF (1:1, 0.5 mL) was added under Ar, and the reaction mixture was stirred at 23 °C (72 h). The solvents were removed in vacuo to provide an oily solid. Chromatography (SiO_2, 1 mm PCTLC, 10% CH_3OH–CH_2Cl_2) afforded **22** (14.3 mg, 21 mg theoretical, 68%) as a white film: R_f 0.13 (SiO_2, 10% CH_3OH-CH_2Cl_2); $[\alpha]^{23}_D$ −32 (*c* 0.06, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.51 (s, 1H), 7.34 (m, 9H), 7.12 (m, 6H), 6.87 (s, 1H), 5.14 (d, *J* = 5.0 Hz, 1H), 4.99 (d, *J* = 5.0 Hz, 1H), 4.12 (m, 1H), 3.91 (m, 1H), 3.63 (s, 3H), 2.85 (m, 1H), 2.73 (m, 1H), 2.57 (m, 1H), 2.50 (m, 1H), 2.29 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.7, 171.8, 168.0, 167.2, 166.7, 157.8, 151.9, 143.5, 141.0, 140.0, 130.9, 130.8, 129.4, 129.3, 129.2, 121.0, 113.1, 80.8, 77.0, 69.0, 61.7, 59.3, 55.6, 52.9, 50.3, 41.6, 28.7, 11.7; IR (neat) ν_{max} 3341, 3219, 3021, 1737, 1667, 1552, 1497, 1445, 1392, 1367, 1251, 1216, 1164, 1065, 859 cm^{-1}; FABHRMS (NBA) *m/e* 835.3888 (M^+ + H, $C_{43}H_{50}N_{10}O_8$ requires 835.3891).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*R*)-(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (23). A solution of **22** (7 mg, 0.008 mmol) in THF–CH_3OH–H_2O (3:1:1, 0.2 mL) was cooled to −10 °C and treated with aqueous 1 N LiOH (13 μL, 0.013 mmol, 1.5 equiv). The reaction mixture was stirred at −10 °C (1 h) with monitoring by TLC (SiO_2, 10% CH_3OH–CH_2Cl_2). After the THF and CH_3OH were evaporated under a N_2 stream, the aqueous phase was extracted with EtOAc (1 × 0.5 mL). The aqueous phase was separated and acidified to pH 4 with the addition of aqueous 1.2 N HCl. The milky aqueous phase was thoroughly extracted with 30% *i*PrOH–$CHCl_3$ (5 × 0.5 mL) until no more UV activity was detected in the H_2O layer. The combined organic phases were concentrated in vacuo to provide **23** (6.7 mg, 6.9 mg theoretical, 97%) as a white film: $[\alpha]^{23}_D$ +28 (*c* 0.085, CH_3OH); ^1H

Table 6. Distinguishing Features of **2** versus **4** and Related Agents

agent	R_f (SiO_2)[a]	$[\alpha]^{25}_D$	^1H NMR[b]
2	0.16	−15 (*c* 0.025, 0.1 N HCl)	2.92 (m, 2H, CH_2CONH_2) 3.89 (m, 1H, $CHCONH_2$)
4	0.18	+24 (*c* 0.025, 0.1 N HCl)	3.00 (m, 2H, CH_2CONH_2) 3.94 (m, 1H, $CHCONH_2$)
6	0.22	−9 (*c* 0.05, 0.1 N HCl)	2.93 (m, 2H, CH_2CONH_2) 2.87 (m, 2H, CH_2NH_2)
16	0.50	−21 (*c* 0.03, CH_3OH)	3.79 (m, 1H, $CHCONH_2$) 3.77 (dd, *J* = 6.0, 6.0 Hz, 1H, $CHNHCH_2$)
24	0.46	+3 (*c* 0.33, CH_3OH)	3.84 (m, 1H, $CHCONH_2$) 3.79 (dd, *J* = 6.5, 6.5 Hz, 1H, $CHNHCH_2$)
32	0.43	−10 (*c* 0.07, CH_3OH)	3.01 (m, 2H, CH_2NHBOC) 3.78 (m, 1H, $CHNHCH_2$)

[a] SiO_2, 10:9:1 CH_3OH–10% aqueous NH_4OAc–10% aqueous NH_4OH. [b] D_2O, 400 MHz for **2**, **4**, and **6**; CD_3OD, 400 MHz for **16**, **24**, and **32**.

NMR (CD_3OD, 400 MHz) δ 7.37 (s, 1H), 7.25 (m, 9H), 7.07 (m, 6H), 6.91 (s, 1H), 5.03 (d, *J* = 4.5 Hz, 1H), 4.70 (d, *J* = 4.5 Hz, 1H), 4.08 (m, 1H), 3.91 (dd, *J* = 6.5, 4.5 Hz, 1H), 2.76 (m, 1H), 2.71 (dd, *J* = 11.5, 6.5 Hz, 1H), 2.57 (m, 1H), 2.46 (dd, *J* = 14.0, 7.5 Hz, 1H), 2.28 (s, 3H), 1.40 (s, 9H); IR (neat) ν_{max} 3329, 1676, 1617, 1508, 1449, 1338, 1101, 743 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 953.2711 (M^+ + Cs, $C_{42}H_{48}N_{10}O_8$ requires 953.2711).

N^α-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)epideglycobleomycin A_2 (24). Thoroughly dried **23** (4.1 mg, 0.005 mmol) was placed in a reaction vessel followed by the addition of DCC (3.1 mg, 0.015 mmol, 3.0 equiv), HOBt (0.67 mg, 0.005 mmol, 1.0 equiv), and $NaHCO_3$ (1.4 mg, 0.017 mmol, 3.4 equiv). A solution of freshly prepared **15**[11] (4.8 mg, 0.007 mmol, 1.4 equiv) in DMF (0.125 mL) was added, and the reaction mixture was stirred under Ar at 23 °C (72 h) before the solvent was removed in vacuo. The crude mixture was dissolved in CH_3OH (0.5 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH was evaporated, and the residue was triturated with neutralized $CHCl_3$ (3 × 0.5 mL) with centrifugation to remove the soluble DCC byproducts. The remaining residue was purified by reverse phase chromatography (C-18, 0.5 × 2.0 cm, 5–70% CH_3OH–H_2O gradient elution) to afford **24** (5.5 mg, 6.9 mg theoretical, 79%) as a white film: R_f 0.46 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{23}_D$ +3 (*c* 0.33, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 8.10 (s, 1H), 7.58 (s, 1H), 7.32 (m, 9H), 7.10 (m, 6H), 6.86 (s, 1H), 4.29 (d, *J* = 4.0 Hz, 1H), 4.11 (dq, *J* = 4.0, 6.5 Hz, 1H), 4.07 (d, *J* = 6.5 Hz, 1H), 4.04 (d, *J* = 6.5 Hz, 1H), 3.84 (m, 1H), 3.79 (dd, *J* = 6.5, 6.5 Hz, 1H), 3.63 (m, 1H), 3.62 (t, *J* = 7.5 Hz, 2H), 3.59 (m, 1H), 3.58 (t, *J* = 7.0 Hz, 2H), 3.36 (t, *J* = 7.5 Hz, 2H), 3.25 (t, *J* = 7.0 Hz, 2H), 2.92 (s, 6H), 2.79 (dd, *J* = 13.0, 6.5 Hz, 1H), 2.69 (dd, *J* = 13.0, 6.5 Hz, 1H), 2.55 (m, 2H), 2.41 (m, 1H), 2.29 (s, 3H), 2.13 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.38 (s, 9H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3322, 2926, 2851, 1628, 1449, 1380, 1088, 824 cm^{-1}; FABHRMS (glycerol) *m/e* 1389.5700 (M^+, $C_{66}H_{85}N_{16}O_{12}S_3$ requires 1389.5695).

Epideglycobleomycin A_2 (4). A solution of solid **24** (0.7 mg, 0.0005 mmol) in CH_2Cl_2 (58 μL) was cooled to 0 °C and treated with TFA (14 μL) under Ar. The yellow homogenous reaction mixture was stirred at 0 °C for 4–5 h and monitored by TLC (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH; **24** R_f 0.46, **4** R_f 0.18). The TFA and CH_2Cl_2 were evaporated under a N_2 stream at 0 °C, and the yellow residue was dried in vacuo. The white residue was diluted with H_2O (200 μL, 2×) and filtered to remove the insoluble trityl byproducts. The aqueous filtrate was lyophilized to a glassy solid and triturated with neutralized $CHCl_3$ (100 μL, 3×) to afford **4** (0.37 mg, 0.53 mg theoretical, 70%) as a white film: R_f 0.18 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{23}_D$ +24 (*c* 0.025, 0.1 N HCl); ^1H NMR (D_2O, 400 MHz) δ 8.50 (s, 1H), 7.99 (s, 1H), 7.85 (s, 1H), 7.59 (s, 1H), 4.08 (d, *J* = 5.5 Hz, 1H), 3.94 (m, 1H), 3.88 (m, 1H), 3.80 (m, 1H), 3.61 (m, 1H), 3.44 (m, 3H), 3.38 (m, 2H), 3.19 (m, 2H), 3.09 (m, 2H), 3.00 (m, 2H), 2.71 (s, 6H), 2.59 (m, 2H), 2.46 (m, 1H), 1.97 (s, 3H), 1.95 (m, 2H), 1.02 (d, *J* = 6.0 Hz, 3H), 1.01 (d, *J* = 6.0 Hz, 3H), 0.92 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3317, 2933, 1728, 1656, 1354, 1056, 1015, 836 cm^{-1}; FABMS (NBA) *m/e* 1029 (M^+ − 18, $C_{42}H_{63}N_{16}O_{10}S_3$). See also Table 6

Alternatively the agent could be purified by chromatography (SiO_2, 0.5 × 1.0 cm, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH) followed by chromatography over Amberlite XAD-2 (H_2O wetted, 6 × 0.5 cm) for removal of buffer salts by first eluting with H_2O to remove the buffer salts followed by sample elution with CH_3OH to afford **4** albeit with some loss of agent.

N^α,N^β-Bis((*tert*-butyloxy)carbonyl)-N^β-[(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl methyl ester)-5-methylpyrimidin-2-yl)methylene]-(*S*)-β-aminoalanine Amide (26). A solution of **25**[12] (24 mg, 0.051 mmol) in THF–DMF (2:1, 0.5 mL) was treated with **11**[11] (26 mg, 0.061 mmol, 1.2 equiv), HOBt (6.9 mg, 0.051 mmol, 1 equiv), and EDCI (10.2 mg, 0.053 mmol, 1.05 equiv), and the reaction mixture was stirred under Ar at 25 °C (72 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 1 × 3 cm, 5–15% CH_3OH–$CHCl_3$ gradient elution) afforded **26** (40 mg, 45 mg theoretical, 89%) as a white solid: R_f 0.52 (SiO_2, 15% CH_3OH–$CHCl_3$); mp 123–125 °C (EtOAc–hexane); $[\alpha]^{23}_D$ −15 (c 0.3, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 7.52 (br s, 1H), 7.35 (m, 9H), 7.16 (m, 6H), 7.05 (br s, 1H), 5.53 (s, 2H), 5.10 (d, *J* = 6.0 Hz, 1H), 5.02 (m, 1H), 4.52 (m, 1H), 4.35 (m, 1H), 3.82 (m, 1H), 3.71 (s, 3H), 2.30 (s, 3H), 1.48 (s, 9H), 1.28 (s, 9H); IR (neat) ν_{max} 3347, 3197, 2950, 1682, 1504, 1366, 1248, 1163, 910, 732 cm^{-1}; FABHRMS (DTT–DTE) *m/e* 878.4229 (M^+ + H, $C_{46}H_{55}N_9O_9$ requires 878.4201).

N-BOC and N^π-trityl deprotection of **26** provided N^β-[(4-amino-6-(amido-*erythro*-β-hydroxy-L-histidyl methyl ester)-5-methylpyrimidin-2-yl)methylene]-(*S*)-β-aminoalanine amide. A solution of **26** (4 mg, 0.0045 mmol) in TFA (2 mL) was stirred at 25 °C (1 h) under Ar. The mixture was concentrated in vacuo. Chromatography (reverse-phase C-18, 1 × 1 cm, H_2O eluant) with collection of the UV-active fractions afforded the methyl ester (1.96 mg, 1.98 mg theoretical, 99%) as a solid: ^1H NMR (CD_3OD, 400 MHz) δ 8.87 (s, 1H), 7.54 (s, 1H), 5.36 (d, *J* = 6.0 Hz, 1H), 5.06 (d, *J* = 6.0 Hz, 1H), 4.42 (m, 1H), 4.29 (s, 2H), 3.81 (s, 3H), 3.68 (dd, *J* = 6.0, 14.0 Hz, 1H), 3.60 (dd, *J* = 7.0, 14.0 Hz, 1H), 2.26 (s, 3H); IR (neat) ν_{max} 3381, 2973, 2960, 1741, 1700, 1512, 1447, 1367, 1213, 1166, 1062, 921, 853 cm^{-1}; FABHRMS (NBA) *m/e* 436.2070 (M^+ + H, $C_{17}H_{25}N_9O_5$ requires 436.2057).

N^α,N^β-Bis((*tert*-butyloxy)carbonyl)-N^β-[(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl)-5-methylpyrimidin-2-yl)methylene]-(*S*)-β-aminoalanine Amide (27). A solution of **26** (7.8 mg, 0.0089 mmol) in THF–CH_3OH–H_2O (3:1:1, 0.2 mL) was treated with 1 N aqueous LiOH (0.018 mL, 0.018 mmol, 2 equiv), and the mixture was stirred at 25 °C (3 h). After most of the THF and CH_3OH was evaporated, the aqueous phase was extracted with $CHCl_3$ (2 × 0.5 mL). The aqueous phase was acidified with 1.2 N aqueous HCl to pH 4–5, and the mixture was extracted with 25% 2-propanol–$CHCl_3$ (5 × 1 mL). The combined organic extracts were dried ($MgSO_4$), and the solvent was removed in vacuo to afford **27** (7.0 mg, 7.6 mg theoretical, 92%), which was carried into the subsequent coupling with **15** without further purification: ^1H NMR (CD_3OD, 400 MHz) δ 7.55 (br s, 1H), 7.35 (m, 9H), 7.16 (m, 6H), 7.05 (br s, 1H), 5.54 (s, 2H), 5.22 (m, 1H), 5.03 (m, 1H), 4.60 (m, 1H), 4.45 (m, 1H), 3.80 (m, 1H), 2.31 (s, 3H), 1.48 (s, 9H), 1.29 (s, 9H); IR (neat) ν_{max} 3410, 2969, 2897, 1698, 1682, 1510, 1445, 1365, 1249, 1160, 732 cm^{-1}.

N^α,N^β-Bis((*tert*-butyloxy)carbonyl)-N^{im}-(triphenylmethyl)desacetamidodeglycobleomycin A_2 (28). A solution of **27** (5.95 mg, 0.0069 mmol) in DMF (0.1 mL) was treated with **15**[11] (4.8 mg, 0.0073 mmol, 1.1 equiv), HOBt (0.93 mg, 0.0069 mmol, 1 equiv), DCC (4.3 mg, 0.021 mmol, 3 equiv), and $NaHCO_3$ (1.5 mg, 0.017 mmol, 2.5 equiv), and the reaction mixture was stirred under Ar at 25 °C (72 h). The reaction mixture was concentrated in vacuo to give an oily solid. The crude mixture was dissolved in CH_3OH (3 mL) and passed through a cotton plug. The CH_3OH was evaporated, and the sample was triturated with $CHCl_3$ (3 × 1 mL). Chromatography (reverse phase C-18, 5–30% CH_3OH–H_2O gradient elution) gave **28** (6.5 mg, 10.1 mg theoretical, 64%) as a thin film: R_f 0.45 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{23}_D$ +51 (*c* 0.035, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.22 (br s, 1H), 8.12 (br s, 1H), 7.45 (br s, 1H), 7.31 (m, 9H), 7.10 (m, 6H), 6.92 (br s, 1H), 4.70 (br s, 2H), 4.44 (m, 2H), 4.22 (m, 4H), 3.78 (m, 3H), 3.64 (m, 4H), 3.42 (t, *J* = 7.0 Hz, 2H), 2.97 (s, 6H), 2.62 (m, 1H), 2.27 (s, 3H), 2.18 (m, 2H), 1.47 (s, 9H), 1.21 (s, 9H), 1.16 (m, 9H); IR (neat) ν_{max} 3423, 2978, 2919, 2849, 1649, 1625, 1543, 1484, 1425, 1367, 1249, 1167, 1049, 1008, 820 cm^{-1}; FABHRMS (NBA) *m/e* 1433.6055 (M^+, $C_{69}H_{90}N_{15}O_{13}S_3$ requires 1433.6083).

Desacetamidodeglycobleomycin A_2 (5). The solid **28** (3.1 mg, 0.0021 mmol) was treated with TFA (1 mL), and the mixture was stirred under Ar at 25 °C (1.5 h). The solvent was evaporated in vacuo, and the oily solid was triturated with $CHCl_3$ (3 × 1 mL). Chromatography (reverse-phase C-18, H_2O) afforded **5** (2.2 mg, 2.3 mg theoretical, 95%) as a thin

film: R_f 0.2 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{23}_D$ +83 (*c* 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.89 (br s, 1H), 8.26 (br s, 1H), 8.15 (br s, 1H), 7.54 (br s, 1H), 5.36 (d, *J* = 6.0 Hz, 1H), 5.15 (m, 1H), 4.40 (m, 4H), 4.00 (m, 4H), 3.80 (m, 4H), 3.65 (t, *J* = 7.0 Hz, 2H), 3.42 (t, *J* = 6.5 Hz, 2H), 2.98 (s, 6H), 2.65 (m, 1H), 2.31 (s, 3H), 2.20 (m, 3H), 1.30 (m, 6H), 1.15 (d, *J* = 6.0 Hz, 3H); IR (neat) ν_{max} 3350, 2955, 1673, 1512, 1365, 1160, 1048, 1008, 820 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 1123.2927 (M^+ + Cs, $C_{40}H_{60}N_{15}O_9S_3$ requires 1123.2915).

N^α-((*tert*-Butyloxy)carbonyl)-N^β-[1-amino-3(*S*)-(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl methyl ester)-5-methylpyrimidin-2-yl)propion-3-yl]-2-amino-1-ethylamine (30). Dry 29[12] (1.8 mg, 0.005 mmol) was placed in a reaction vessel followed by the addition of EDCI (1.0 mg, 0.005 mmol, 1.1 equiv) and HOBt (0.6 mg, 0.005 mmol, 1.0 equiv). A solution of 11 (2.0 mg, 0.005 mmol, 1.0 equiv) in DMF (0.03 mL) was added under Ar, and the reaction mixture was stirred at 23 °C (48 h). The solvents were removed in vacuo to provide an oily solid. Chromatography (SiO_2, 0.5 × 2 cm, 10% CH_3OH–CH_2Cl_2) afforded **30** (3.0 mg, 3.7 mg theoretical, 81%) as a white film: R_f 0.30 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]^{23}_D$ +12 (*c* 0.050, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.48 (s, 1H), 7.34 (m, 9H), 7.11 (m, 6H), 6.89 (s, 1H), 5.09 (d, *J* = 5.5 Hz, 1H), 4.95 (d, *J* = 5.5 Hz, 1H), 4.32 (dd, *J* = 6.5, 6.5 Hz, 1H), 3.65 (s, 3H), 3.08 (dd, *J* = 6.0, 6.0 Hz, 2H), 2.69 (dd, *J* = 6.0, 14.0 Hz, 1H), 2.55 (m, 3H), 2.31 (s, 3H), 1.39 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 171.7, 167.5, 166.8, 152.3, 143.5, 141.2, 140.0, 130.8, 129.3, 125.3, 121.0, 118.6, 114.3, 112.4, 80.7, 77.0, 68.8, 60.6, 59.4, 56.4, 52.9, 28.7, 11.7; IR (neat) ν_{max} 3344, 2950, 1630, 1452, 1383, 1088, 824 cm^{-1}; FABHRMS (NBA) *m/e* 792.3845 (M^+ + H, $C_{42}H_{49}N_9O_7$ requires 792.3833).

N^α-((*tert*-Butyloxy)carbonyl)-N^β-[1-amino-3(*S*)-(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl)-5-methylpyrimidin-2-yl)propion-3-yl]-2-amino-1-ethylamine (31). A solution of **30** (4.6 mg, 0.006 mmol) in THF–CH_3OH–H_2O (3:1:1, 0.15 mL) was cooled to −10 °C and treated with aqueous 1 N LiOH (9 μL, 0.009 mmol, 1.5 equiv). The reaction mixture was stirred at −10 °C (2 h) with monitoring by TLC (SiO_2, 10% CH_3OH–CH_2Cl_2). After the THF and CH_3OH were evaporated under a N_2 stream at −10 °C, H_2O (0.5 mL) and EtOAc (0.5 mL) were added at 23 °C, and the organic phase was removed. The aqueous layer was acidified to pH 4 with the addition of aqueous 1.2 N HCl and concentrated in vacuo. The acid was desalted by reverse-phase chromatography (C-18, 0.5 × 4.0 cm, H_2O and then CH_3OH elution) to afford pure **31** (4.5 mg, 4.5 mg theoretical, 100%) as a white film: $[\alpha]^{25}_D$ +3 (*c* 0.23, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.91 (s, 1H), 7.35 (m, 9H), 7.10 (m, 6H), 7.04 (s, 1H), 5.09 (d, *J* = 5.0 Hz, 1H), 4.95 (d, *J* = 5.0 Hz, 1H), 4.55 (m, 1H), 3.45 (m, 4H), 3.21 (dd, *J* = 12.0, 7.0 Hz, 1H), 3.12 (dd, *J* = 12.0, 3.0 Hz, 1H), 2.36 (s, 3H), 1.41 (s, 9H); IR (neat) 3401, 1639, 1506, 1446, 1407, 1259, 1170, 1125, 659 cm^{-1}; FABHRMS (NBA) *m/e* 778.3665 (M^+ + H, $C_{41}H_{47}N_9O_7$ requires 778.3677).

N^α-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)descarboxamidodeglycobleomycin A$_2$ (32). A solution of **31** (1.0 mg, 0.001 mmol), HOAt (0.30 mg, 0.002 mmol, 1.5 equiv), $NaHCO_3$ (0.40 mg, 0.004 mmol, 3.0 equiv), and **15** (1.2 mg, 0.002 mmol, 1.4 equiv) in DMF (0.033 mL) at 0 °C was treated with DCC (0.30 mg, 0.001 mmol, 1.0 equiv) under Ar, and the reaction mixture stirred at 0 °C (1.5 h) and 23 °C (96 h). The crude reaction mixture was concentrated in vacuo and dissolved in CH_3OH (0.5 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH was evaporated, and the remaining residue was triturated with neutralized $CHCl_3$ (3 × 0.5 mL) with centrifugation to remove the soluble DCC byproducts. The remaining residue was purified by reverse-phase chromatography (C-18, 0.5 × 2.0 cm, 5–70% CH_3OH–H_2O gradient elution) to afford **32** (0.9 mg, 1.7 mg theoretical, 52%) as a white film: R_f 0.43 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{23}_D$ −10 (*c* 0.070, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 8.10 (s, 1H), 7.59 (s, 1H), 7.33 (m, 9H), 7.10 (m, 6H), 6.79 (s, 1H), 4.60 (m, 1H), 4.29 (d, *J* = 4.0 Hz, 1H), 4.12 (dq, *J* = 4.0, 6.5 Hz, 1H), 4.05 (m, 1H), 3.78 (m, 1H), 3.63 (m, 1H), 3.62 (t, *J* = 7.5 Hz, 2H), 3.60 (m, 1H), 3.58 (t, *J* = 7.0 Hz, 2H), 3.38 (m, 2H), 3.24 (t, *J* = 7.0 Hz, 2H), 3.01 (m, 2H), 2.92 (s, 6H), 2.70 (m, 1H), 2.52 (m, 3H), 2.31 (s, 3H), 2.27 (m, 1H), 2.13 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.37 (s, 9H), 1.22 (d, *J* = 6.5 Hz, 3H), 1.20 (d, *J* = 6.5 Hz, 3H), 1.10 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3313, 2933, 2872, 1651, 1549, 1451, 1390, 1251, 1174, 1123 cm^{-1}; FABMS (NBA) *m/e* 1329 (M^+ − 18, $C_{65}H_{84}N_{15}O_{11}S_3$).

Descarboxamidodeglycobleomycin A$_2$ (6). A solution of solid **32** (1.4 mg, 0.001 mmol) in CH_2Cl_2 (80 μL) was cooled at 0 °C and treated with TFA (20 μL) under Ar excluding light. The yellow homogenous reaction mixture was stirred at 0 °C for 4 h and monitored by TLC (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH; **32** R_f 0.43, **6** R_f 0.22). The solvent was evaporated at 0 °C, and the yellow residue was dried in vacuo (1 h). The white residue was dissolved in H_2O (0.5 mL, 2×) and filtered to remove the insoluble trityl byproducts. The aqueous filtrate was lyophilized to a white film to afford **6** (0.95 mg, 1.0 mg theoretical, 95%): R_f 0.22 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{23}_D$ −9 (*c* 0.05, 0.1 N HCl); ^1H NMR (D_2O, 400 MHz) δ 8.56 (s, 1H), 7.98 (s, 1H), 7.87 (s, 1H), 7.56 (s, 1H), 4.04 (d, *J* = 5.0 Hz, 1H), 3.88 (dq, *J* = 4.0, 6.5 Hz, 1H), 3.78 (dd, *J* = 6.5, 6.5 Hz, 1H), 3.59 (dd, *J* = 7.0, 5.0 Hz, 1H), 3.45 (m, 1H), 3.37 (m, 2H), 3.26 (m, 2H), 3.16 (m, 2H), 3.07 (m, 2H), 2.93 (m, 2H), 2.87 (m, 2H), 2.69 (s, 6H), 2.44 (m, 2H), 2.00 (s, 3H), 1.98 (m, 1H), 1.95 (m, 2H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3415, 1667, 1436, 1195, 1133, 795 cm^{-1}; FABHRMS (NBA) *m/e* 1004.4035 (M^+, $C_{41}H_{62}N_{15}O_9S_3$ requires 1004.4017).

N^α-((*tert*-Butyloxy)carbonyl)-N^β-[1-amino-3(*S*)-(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl methyl ester)pyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (34). A solution of 33[37] (7.2 mg, 0.017 mmol) in THF–DMF (2:1, 0.2 mL) was treated with 11[11] (8.2 mg, 0.019 mmol, 1.1 equiv), HOBt (2.4 mg, 0.017 mmol, 1.0 equiv), and EDCI (3.6 mg, 0.018 mmol, 1.05 equiv), and the mixture was stirred under Ar at 25 °C (72 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 1 × 3 cm, 10% CH_3OH–CH_2Cl_2) afforded **34** (9.6 mg, 14.3 mg theoretical, 67%) as a foam: R_f 0.45 (SiO_2, 20% CH_3OH–CH_2Cl_2); $[\alpha]^{25}_D$ +3 (*c* 0.10, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.40 (s, 1H), 7.15 (m, 9H), 7.00 (m, 6H), 6.86 (s, 1H), 6.79 (s, 1H), 5.04 (d, *J* = 5.5 Hz, 1H), 4.88 (d, *J* = 5.5 Hz, 1H), 4.06 (m, 1H), 3.92 (dd, *J* = 5.0, 8.0 Hz, 1H), 3.53 (s, 3H), 2.75 (m, 2H), 2.64 (dd, *J* = 5.0, 15.0 Hz, 1H), 2.46 (dd, *J* = 8.0, 15.0 Hz, 1H), 1.31 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.0, 171.7, 168.5, 167.1, 165.6, 157.0, 155.8, 143.6, 141.1, 140.1, 130.9 (2C), 129.4, 129.3, 120.9, 116.4, 81.1, 77.0, 69.1, 61.6, 59.5, 54.1, 53.0, 49.8, 39.6, 28.7; IR ($CHCl_3$) ν_{max} 3402, 3218, 3052, 2984, 1734, 1699, 1684, 1653, 1457, 1420, 1265, 1183, 1157, 896 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 953.2711 (M^+ + Cs, $C_{42}H_{48}N_{10}O_8$ requires 953.2711).

N^α-((*tert*-Butyloxy)carbonyl)-N^β-[1-amino-3(*S*)-(4-amino-6-(amido-N^{im}-(triphenylmethyl)-*erythro*-β-hydroxy-L-histidyl)pyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (35). A solution of **34** (8.0 mg, 0.010 mmol) in THF–CH_3OH–H_2O (3:1:2, 0.2 mL) was treated with aqueous 1 N LiOH (0.015 mL, 0.015 mmol, 1.5 equiv), and the mixture was stirred at 0 °C (1.5 h). After most of the THF and CH_3OH was evaporated, the aqueous phase was acidified to pH 7 with the addition of aqueous 1.2 N HCl and the aqueous phase was extracted with 20% *i*PrOH–$CHCl_3$ (3 × 0.5 mL). The solvent was removed in vacuo to afford **35** (7.6 mg, 7.8 mg theoretical, 98%) as a white foam: $[\alpha]^{25}_D$ −10.0 (*c* 0.02, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.36 (s, 1H), 7.25 (m, 9H), 7.10 (m, 6H), 6.88 (s, 1H), 6.87 (s, 1H), 4.95 (d, *J* = 5.6 Hz, 1H), 4.67 (d, *J* = 5.6 Hz, 1H), 4.26 (m, 2H), 3.52 (m, 1H), 3.09 (m, 1H), 2.85 (m, 1H), 2.65 (m, 1H), 1.32 (s, 9H); IR (neat) ν_{max} 3442, 3210, 2965, 1682, 1675, 1445, 1260, 1127, 1051 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 939.2560 (M^+ + Cs, $C_{41}H_{46}N_{10}O_8$ requires 939.2554).

N^α-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)desmethyldeglycobleomycin A$_2$ (36). A solution of **35** (3.4 mg, 0.0040 mmol) in DMF (25 μL) was treated sequentially with DCC (1.1 mg, 0.0050 mmol, 1.2 equiv), HOBt (0.62 mg, 0.0040 mmol, 1 equiv), $NaHCO_3$ (0.6 mg, 0.0064 mmol, 1.4 equiv), and 15[11] (4.3 mg, 0.0064 mmol, 1.4 equiv) dissolved in DMF (25 μL), and the mixture was stirred under Ar at 25 °C (72 h). The crude mixture was dissolved in CH_3OH (2 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH solution was evaporated, and the sample was triturated with $CHCl_3$ (3 × 1 mL). Chromatography (reverse-phase C-18, 20–90% CH_3OH–H_2O gradient elution) gave **36** (3.0 mg, 5.4 mg theoretical, 55%) as a thin film: R_f 0.5 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ −9.5 (*c* 0.055, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.09 (s, 1H), 7.99 (s, 1H), 7.53 (s, 1H), 7.15 (m, 9H), 6.95 (m, 7H), 6.71 (s, 1H), 4.78 (m, 2H), 4.23 (d, *J* = 3.6 Hz, 1H), 4.05 (dq, *J* = 4.0, 6.8 Hz, 1H), 3.90–3.85 (m, 2H), 3.75 (dd, *J* = 4.8, 9.2 Hz, 1H), 3.66 (dd, *J* = 5.2, 7.2 Hz, 1H), 3.55 (m, 4H), 3.28 (m, 2H), 3.12 (m, 2H), 2.83 (s, 6H), 2.68 (m, 2H), 2.50 (m, 2H), 2.34 (dd, *J* = 9.2, 14.8 Hz, 1H), 2.04 (m, 2H), 1.31 (s, 9H), 1.13 (d, *J* = 6.8 Hz, 3H), 1.12 (d, *J* = 6.8 Hz, 3H), 1.04 (d, *J* = 6.4 Hz, 3H); IR (neat) ν_{max} 3425, 2932, 1721, 1637, 1542, 1253, 1161 cm^{-1}; FABMS (NBA) *m/e* 1375 (M^+, $C_{65}H_{83}N_{16}O_{12}S_3$).

Desmethyldeglycobleomycin A_2 (7). The solid **36** (0.8 mg, 0.0006 mmol) was treated with 20% TFA–CH_2Cl_2 (200 μL), and the mixture was stirred at 0 °C (4 h) under Ar. The solvent was evaporated in vacuo to give an oily solid. The sample was triturated with $CHCl_3$ (2 × 0.5 mL), and the crude mixture was extracted with H_2O (3 × 1 mL). The H_2O solution was evaporated to give **7** (0.54 mg, 0.60 mg theoretical, 90%) as a thin film: R_f 0.20 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ -6.7 (*c* 0.03, 0.1 N HCl); ^1H NMR (D_2O, 400 MHz) δ 8.16 (s, 1H), 8.03 (s, 1H), 7.62 (br s, 1H), 7.04 (br s, 1H), 6.98 (s, 1H), 4.78 (m, 2H), 4.24 (d, *J* = 4.4 Hz, 1H), 4.12 (m, 3H), 3.95 (dd, *J* = 5.7, 6.8 Hz, 1H), 3.75 (dd, *J* = 4.8, 5.3 Hz, 1H), 3.60 (m, 4H), 3.31 (m, 4H), 3.10 (m, 2H), 2.88 (s, 6H), 2.75 (m, 4H), 2.65 (m, 1H), 2.13 (m, 2H), 1.18 (d, *J* = 6.8 Hz, 6H), 1.10 (d, *J* = 6.8 Hz, 3H); IR (neat) ν_{max} 3415, 2913, 1715, 1632, 1428, 1248, 1580, 972 cm^{-1}; FABMS (NBA) *m/e* 1015 (M^+ - 18, $C_{41}H_{61}N_{16}O_{10}S_3$).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(amido-L-seryl methyl ester)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (37). A solution of **10**[12] (4.5 mg, 0.011 mmol) in DMF (0.2 mL) was treated with L-serine methyl ester (2.3 mg, 0.015 mmol, 1.4 equiv), HOBt (1.43 mg, 0.011 mmol, 1.0 equiv), EDCI (2.13 mg, 0.011 mmol, 1.05 equiv), and $NaHCO_3$ (3.16 mg, 0.038 mmol, 3.5 equiv), and the mixture was stirred under Ar at 25 °C (50 h). The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 1 × 2 cm, 15% CH_3OH–CH_2Cl_2) afforded **37** (4.8 mg, 5.6 mg theoretical, 86%) as a foam: R_f 0.22 (SiO_2, 15% CH_3OH–CH_2Cl_2); $[\alpha]^{25}_D$ -8.0 (*c* 0.013, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 4.70 (dd, *J* = 5.0, 5.5 Hz, 1H), 4.18 (m, 1H), 4.08 (dd, *J* = 7.0, 14.0 Hz, 1H), 3.98 (m, 2H), 3.83 (s, 3H), 2.85 (m, 2H), 2.65 (dd, *J* = 6.0, 14.0 Hz, 1H), 2.58 (dd, *J* = 7.0, 14.0 Hz, 1H), 2.37 (s, 3H), 1.49 (s, 9H); IR (neat) ν_{max} 3357, 2978, 2940, 1684, 1650, 1562, 1444, 1350, 1110 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 659.1550 (M^+ + Cs, $C_{21}H_{34}N_8O_8$ requires 659.1554).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(amido-L-seryl)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine Amide (38). A solution of **37** (3.0 mg, 0.006 mmol) in THF–CH_3OH–H_2O (3:1:1, 0.2 mL) was treated with aqueous 1 N LiOH (0.009 mL, 0.009 mmol, 1.5 equiv), and the mixture was stirred at 0 °C (1.5 h). After evaporation of most of the THF and CH_3OH, the aqueous phase was extracted with $CHCl_3$ (0.5 mL). The aqueous phase was acidified with aqueous 1.2 N HCl to pH 7, and the mixture was passed through a C-18 reverse-phase plug using H_2O for elution. The UV-active fractions were combined, and the solvent was removed in vacuo to afford **38** (2.90 mg, 2.92 mg theoretical, 98%) as a white foam, which was used directly in the next reaction: ^1H NMR (CD_3OD, 400 MHz) δ 4.72 (dd, *J* = 5.0, 5.5 Hz, 1H), 4.15 (m, 4H), 2.78 (m, 4H), 2.38 (s, 3H), 1.52 (s, 9H).

Desimidazolyldeglycobleomycin A_2 (8). A solution of **38** (2.9 mg, 0.0057 mmol) in DMF (0.1 mL) was treated sequentially with DCC (3.53 mg, 0.017 mmol, 3.0 equiv), HOBt (0.77 mg, 0.0057 mmol, 1.0 equiv), $NaHCO_3$ (1.53 mg, 0.018 mmol, 3.2 equiv), and **15**[11] (4.8 mg, 0.0073 mmol, 1.3 equiv) dissolved in DMF (0.1 mL), and the mixture was stirred under Ar at 25 °C (60 h). The reaction mixture was concentrated in vacuo to give an oily solid. The crude mixture was dissolved in CH_3OH (3 mL), and the insoluble inorganic salts were removed by centrifugation. The CH_3OH solution was then evaporated, and the sample was triturated with $CHCl_3$ (3 × 1 mL). Chromatography (reverse-phase C-18, 1–20% CH_3OH–H_2O gradient elution) gave **39** as a thin film: R_f 0.3 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.95 (br s, 1H), 7.72 (s, 1H), 4.38 (m, 1H), 4.11 (m, 1H), 3.94 (m, 2H), 3.75 (m, 3H), 3.50 (m, 6H), 3.18 (m, 1H), 3.10 (m, 2H), 2.77 (s, 6H), 2.45 (m, 5H), 1.95 (m, 6H), 1.26 (br s, 9H), 0.98 (m, 9H).

The solid **39** was treated with 3 N HCl-EtOAc (1 mL), and the mixture was stirred under Ar at 25 °C (1 h). The solvent was evaporated in vacuo to give an oily solid. Chromatography (SiO_2, 0.5 × 2 cm, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH eluant, TLC R_f 0.12) followed by chromatography over Amberlite XAD-2 (2 × 1 cm), first desalting the absorbed sample with H_2O and then eluting the agent with CH_3OH, afforded **8** (3.3 mg, 5.6 mg theoretical, 59% for two steps) as a thin film: $[\alpha]^{25}_D$ -21 (*c* 0.008, H_2O); ^1H NMR (D_2O, 400 MHz) δ 8.09 (s, 1H), 7.91 (s, 1H), 4.41 (dd, *J* = 5.0, 5.5 Hz, 1H), 4.10 (d, *J* = 5.0 Hz, 1H), 4.00 (m, 1H), 3.77 (m, 5H), 3.55 (m, 4H), 3.45 (t, *J* = 6.0 Hz, 2H), 3.26 (t, *J* = 5.0 Hz, 2H), 3.15 (m, 2H), 2.78 (s, 6H), 2.70 (m, 1H), 2.47 (m, 3H), 2.02 (tt, *J* = 6.0, 6.0 Hz, 2H), 1.92 (s, 3H), 1.02 (d, *J* = 6.0 Hz, 3H), 0.98 (d, *J* = 6.0 Hz, 3H), 0.94 (d, *J* = 6.0 Hz, 3H); IR (neat) ν_{max} 3434, 2986, 2905, 1684, 1635, 1475, 1419, 1367, 1251, 1161, 1059 cm^{-1}; FABHRMS (NBA) *m/e* 981.3810 (M^+, $C_{39}H_{61}N_{14}O_{10}S_3$ requires 981.3857).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{im}-(triphenylmethyl)-GABA,Gly-deglycobleomycin A_2 (42). A solution of **13** (5.5 mg, 0.0067 mmol) in DMF (0.5 mL) was treated sequentially with DCC (4.15 mg, 0.02 mmol, 3.0 equiv), HOBt (0.91 mg, 0.0067 mmol, 1.0 equiv), $NaHCO_3$ (1.5 mg, 0.018 mmol, 2.7 equiv), and **41**[11] (4.1 mg, 0.0072 mmol, 1.08 equiv) dissolved in DMF (0.1 mL), and the mixture was stirred under Ar at 25 °C (72 h). The reaction mixture was concentrated in vacuo to give an oily solid. The crude mixture was dissolved in CH_3OH (3 mL), and the inorganic salts were removed by centrifugation. The CH_3OH solution was evaporated, and the sample was triturated with $CHCl_3$ (3 × 1 mL). Chromatography (reverse-phase C-18, 5–50% CH_3OH–H_2O gradient elution) gave **42** (7.0 mg, 8.7 mg theoretical, 80%) as a thin film: R_f 0.4 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{25}_D$ -38 (*c* 0.02, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.22 (s, 1H), 8.05 (s, 1H), 7.62 (s, 1H), 7.32 (m, 9H), 7.10 (m, 6H), 6.91 (s, 1H), 4.70 (m, 1H), 4.05 (m, 1H), 3.87 (s, 2H), 3.82 (m, 1H), 3.68 (t, *J* = 6.0 Hz, 2H), 3.60 (t, *J* = 6.5 Hz, 2H), 3.55 (m, 2H), 3.46 (t, *J* = 6.0 Hz, 2H), 3.30 (m, 2H), 2.98 (s, 6H), 2.84 (m, 1H), 2.62 (t, *J* = 6.0 Hz, 2H), 2.43 (m, 2H), 2.33 (s, 3H), 2.16 (m, 2H), 1.79 (m, 4H), 1.45 (s, 9H); IR (neat) ν_{max} 3434, 2967, 2923, 1645, 1628, 1489, 1456, 1422, 1261, 1122, 1067, 923 cm^{-1}; FABHRMS (NBA) *m/e* 1301.5119 (M^+, $C_{62}H_{77}N_{16}O_{10}S_3$ requires 1301.5171).

GABA,Gly-deglycobleomycin A_2 (43). The solid **42** (2.1 mg, 0.0016 mmol) was treated with TFA (1 mL), and the mixture was stirred under Ar at 25 °C (45 min). The solvent was evaporated in vacuo to give an oily solid. Chromatography (SiO_2, 0.5 × 2 cm, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH eluant, TLC R_f 0.12) followed by chromatography over Amberlite XAD-2 (3 × 1 cm) first desalting the absorbed sample with H_2O and then eluting the agent with CH_3OH, afforded **43** (1.3 mg, 1.55 mg theoretical, 85%) as a thin film: $[\alpha]^{25}_D$ -17 (*c* 0.01, CH_3OH); ^1H NMR (D_2O, 400 MHz) δ 8.07 (s, 1H), 7.94 (s, 1H), 7.92 (s, 1H), 7.19 (s, 1H), 5.15 (d, *J* = 6.0 Hz, 1H), 3.97 (m, 1H), 3.65 (m, 1H), 3.56 (m, 3H), 3.41 (m, 6H), 3.24 (t, *J* = 7.0 Hz, 2H), 3.19 (s, 2H), 3.04 (t, *J* = 7.0 Hz, 2H), 2.77 (s, 6H), 2.60 (m, 2H), 2.32 (s, 3H), 2.12 (t, *J* = 7.0 Hz, 2H), 2.05 (m, 4H); IR (neat) ν_{max} 3441, 3151, 2995, 1627, 1526, 1418, 1212, 1045, 898 cm^{-1}; FABHRMS (NBA) *m/e* 959.3560 (M^+, $C_{38}H_{55}N_{16}O_8S_3$ requires 959.3551).

N^{α},N^{β}-Bis((*tert*-butyloxy)carbonyl)-N^{im}-(triphenylmethyl)-GABA,Gly-desacetamidodeglycobleomycin A_2 (44). A solution of **27** (4.4 mg, 0.0051 mmol) in DMF (0.5 mL) was treated with **41**[11] (3.2 mg, 0.0056 mmol, 1.1 equiv), HOBt (0.69 mg, 0.0051 mmol, 1.0 equiv), DCC (3.16 mg, 0.015 mmol, 3.0 equiv), and $NaHCO_3$ (1.1 mg, 0.013 mmol, 2.5 equiv), and the reaction mixture was stirred under Ar at 25 °C (51 h). The reaction mixture was concentrated in vacuo to give an oily solid. The crude mixture was dissolved in CH_3OH (1 mL), and the inorganic salts were removed by centrifugation. The CH_3OH was evaporated, and the sample was triturated with $CHCl_3$ (2 × 1 mL). Chromatography of the solid (reverse-phase C-18, 5–25% CH_3OH–H_2O gradient elution) gave **44** (6.1 mg, 6.8 mg theoretical, 89%) as a thin film: R_f 0.28 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{24}_D$ -24 (*c* 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.22 (s, 1H), 8.15 (s, 1H), 7.50 (br s, 1H), 7.33 (m, 9H), 7.16 (m, 6H), 7.05 (br s, 1H), 5.40 (d, *J* = 6.5 Hz, 1H), 5.35 (d, *J* = 6.5 Hz, 1H), 4.33 (m, 4H), 3.85 (br s, 2H), 3.55 (m, 5H), 3.45 (t, *J* = 7.0 Hz, 2H), 3.22 (m, 4H), 2.99 (s, 6H), 2.30 (m, 2H), 2.18 (br s, 3H), 1.75 (m, 4H), 1.47 (br s, 9H), 1.27 (br s, 9H); IR (neat) ν_{max} 3373, 2978, 2922, 1636, 1550, 1433, 1367, 1250, 1167, 1062 cm^{-1}; FABMS (NBA) *m/e* 1345 (M^+, $C_{65}H_{82}N_{15}O_{11}S_3$).

GABA,Gly-desacetamidodeglycobleomycin A_2 (45). The solid **44** (3.5 mg, 0.0026 mmol) was treated with 3 N HCl-EtOAc (1 mL), and the mixture was stirred under Ar at 25 °C (2 h). The solvent was evaporated in vacuo to give an oily solid. Purification by trituration with $CHCl_3$ (2 × 2 mL) and recrystallization from CH_3OH–Et_2O gave **45** (1.78 mg, 2.35 mg theoretical, 76%) as a hygroscopic solid: R_f 0.15 (SiO_2, 10:9:1 CH_3OH–10% aqueous $CH_3CO_2NH_4$–10% aqueous NH_4OH); $[\alpha]^{24}_D$ +89 (*c* 0.095, CH_3OH); ^1H NMR (D_2O, 400 MHz) δ 8.75 (s, 1H), 8.21 (s, 1H), 8.08 (s, 1H), 7.42 (s, 1H), 5.50 (d, *J* = 6.0 Hz, 1H), 5.25 (d, *J* = 6.0 Hz, 1H), 4.40 (m, 1H), 4.19 (m, 3H), 3.82 (s, 2H), 3.60 (m, 5H), 3.39 (t, *J* = 6.5 Hz, 2H), 3.25 (m, 2H), 3.18 (m, 2H), 2.92 (s, 6H), 2.30 (m, 2H), 2.16 (t, *J* = 7.0 Hz, 2H), 1.95 (s, 3H), 1.75 (m, 2H); IR (neat) ν_{max} 3383, 2923, 2851, 1678, 1441, 1200, 1138, 800 cm^{-1}; FABHRMS (NBA) *m/e* 902.3381 (M^+, $C_{36}H_{52}N_{15}O_7S_3$ requires 902.3336).

General Procedure for the DNA Cleavage Reactions: Supercoiled ΦX174 DNA Relative Efficiency Study. All reactions were run with freshly prepared agent–Fe(II) complexes. The agent–Fe(II) complexes

were prepared by combining 1 μL of a H_2O solution of agent at the 10 times specified concentration with 1 μL of a freshly prepared equimolar aqueous $Fe(NH_4)_2(SO_4)_2$ solution followed by vortex mixing. Each of the agent–Fe(II) complex solutions was treated with 7 μL of a buffered DNA solution containing 0.25 μg of supercoiled ΦX174 RFI DNA (1.4×10^{-8} M) in 50 mM Tris-HCl buffer solution (pH 8). The DNA cleavage reactions were initiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol. The final concentrations of the agents employed in the study were 0.2–5 μM bleomycin A_2 (**1**), 0.2–5 μM deglycobleomycin A_2 (**2**), 0.2–10 μM **3**, 1–50 μM **4**, 0.5–20 μM **5** and **6**, 0.5–10 μM **7**, 1–50 μM **8**, 1–10 μM **43**, and 1–50 μM **45**. The DNA reaction solution was incubated at 25 °C for 1 h. The reactions were quenched with the addition of 5 μL of loading buffer formed by mixing Keller buffer (0.4 M Tris-HCl, 0.05 M NaOAc, 0.0125 M EDTA, pH 7.9) with glycerol (40%), sodium dodecyl sulfate (0.4%), and bromophenol blue (0.3%). Electrophoresis was conducted on a 1% agarose gel containing 0.1 μg/mL ethidium bromide at 40 V for 2.5 h, and the gel was immediately visualized on a UV transilluminator and photographed using polaroid T667 black and white instant film. Direct fluorescence quantitation of DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator taking into account the relative fluorescence intensities of Forms I–III ΦX174 DNA (Forms II and III fluorescence intensities are 0.7 times that of Form I).

General Procedure for Quantitation of Double-Stranded and Single-Stranded Supercoiled ΦX174 RFI DNA Cleavage. The agent–Fe(II) complexes were formed by mixing 1 μL of a selected concentration of a H_2O solution of agent with 1 μL of a freshly prepared equimolar aqueous $Fe(NH_4)_2(SO_4)_2$ solution. Seven microliters of a buffered DNA solution containing 0.25 μg of supercoiled ΦX174 RFI DNA (1.4×10^{-8} M) in 50 mM Tris-HCl buffer solution (pH 8) was added to each of the agent–Fe(II) complex solutions. The final concentrations of the agents employed in the study were 1 μM bleomycin A_2 (**1**), 2.5 μM deglycobleomycin A_2 (**2**), 10 μM **3**, 20 μM **4**, 10 μM **5**, 8 μM **6**, 4 μM **7**, 20 μM **8**, 10 μM **43**, and 20 μM **45**. The DNA cleavage reactions were intiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol to each of the reaction mixtures. The solutions were thoroughly mixed and incubated at 25 °C for 40, 30, 20, 15, 10, 8, 6, 4, 2, and 1 min, respectively. The reactions were quenched with the addition of 5 μL of loading buffer, and electrophoresis was run on a 1% agarose gel containing 0.1 μg/mL ethidium bromide at 50 V for 2.5 h. Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system taking into account the relative fluorescence intensities of Forms I–III ΦX174 DNA (Forms II and III fluorescence intensities are 0.7 times that of Form I). The ratio of the double- to single-strand cleavage was calculated with use of the Freifelder–Trumbo equation[51] assuming a Poisson distribution.

General Procedure for Cleavage of 5′-End-Labeled w794 DNA: Relative Efficiency and Selectivity. All reactions were run with freshly prepared agent–Fe(III) complexes. The agent–Fe(III) complexes were prepared by combining 1 μL of a H_2O solution of agent at the 10 times specified concentration with 1 μL of a freshly prepared equimolar aqueous $FeCl_3$ solution. Each of the agent–Fe(III) complex solutions was treated with 7 μL of a buffered DNA solution containing the ^{32}P 5′-end-labeled w794 or w836 DNA[53] in 10 mM phosphate buffer, 10 mM KCl solution (pH 7.0, Na_2HPO_4–NaH_2PO_4). The final concentrations of the agents employed in the study were 0.5 μM bleomycin A_2 (**1**), 2 μM deglycobleomycin A_2 (**2**), 16 μM **3**, 32 μM **4**, 16 μM **5**, 8 μM **6**, 4 μM **7**, 128 μM **8**, 64 μM **43**, and 128 μM **45**. The DNA cleavage reactions were initiated by adding 1 μL of 50% aqueous H_2O_2. The DNA reaction solutions were incubated at 37 °C for 10 min. The reactions were quenched with the addition of 2 μL of glycerol followed by EtOH precipitation and isolation of the DNA. The DNA was resuspended in 10 μL of TE buffer, and formamide dye (10 μL) was added to the supernatant. Prior to electrophoresis, the samples were warmed at 100 °C for 5 min, placed in an ice bath, and centrifuged and the supernatant was loaded onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the agent-treated DNA. Gel electrophoresis was conducted using a denaturing 8% sequencing gel (19:1 acrylamide–*N*,*N*-methylenebisacrylamide, 8 M urea). Formamide dye contained xylene cyanol FF (0.03%), bromophenol blue (0.3%), and aqueous Na_2EDTA (8.7%, 250 mM). Electrophoresis running buffer (TBE) contained Tris base (100 mM), boric acid (100 mM), and Na_2EDTA–H_2O (0.2 mM). Gels were prerun for 30 min with formamide dye prior to loading the samples. Autoradiography of dried gel was carried out at −78 °C using Kodak X-Omat AR film and a Picker Spectra intensifying screen. Quantitation of the DNA cleavage reaction was conducted on a Millipore Bio Image 60S RFLP system measuring the remaining uncleaved w794/w836 DNA.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (Grant CA42056, D.L.B.; Grant F32-GM15302, S.L.C.) and a Glaxo fellowship (T.H.). We wish to thank Dr. W. Yang for conducting the DNA cleavage studies detailed herein and Dr. T. Doyle of Bristol-Myers Squibb for samples of blenoxane, from which authentic samples of bleomycin A_2 and deglycobleomycin A_2 were secured.