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Solid-Phase Synthesis of Bleomycin Group Antibiotics. Construction of a 108-Member Deglycobleomycin Library

Christopher J. Leitheiser, Kenneth L. Smith, Michael J. Rishel, Shigeki Hashimoto, Kazuhide Konishi, Craig J. Thomas, Chunhong Li, Michael M. McCormick, and Sidney M. Hecht*

Contribution from the Departments of Chemistry and Biology, University of Virginia, Charlottesville, Virginia 22904

Received November 27, 2002; E-mail: sidhecht@virginia.edu

Abstract: The bleomycins (BLMs) are structurally related glycopeptide antibiotics isolated from *Streptomyces verticillus* that mediate the sequence-selective oxidative damage of DNA and RNA. Deglycobleomycin, which lacks the carbohydrate moiety, cleaves DNA analogously to bleomycin itself, albeit less potently, and has been used successfully for analyzing the functional domains of bleomycin. Although structural modifications to bleomycin and deglycobleomycin have been reported, no bleomycin or deglycobleomycin analogue having enhanced DNA cleavage activity has yet been described. The successful synthesis of a deglycobleomycin on a solid support has permitted the facile solid-phase synthesis of 108 unique deglycobleomycin analogues through parallel solid-phase synthesis. Each of the deglycobleomycin analogues was synthesized efficiently; the purity of each crude product was greater than 60%, as determined by HPLC integration. The solid-phase synthesis of the deglycobleomycin library provided near-milligram to milligram quantities of each deglycobleomycin, thereby permitting characterization by ^{1}H NMR and high-resolution mass spectrometry. Each analogue demonstrated supercoiled plasmid DNA relaxation above background cleavage; the library included two analogues that mediated plasmid relaxation to a greater extent than the parent deglycobleomycin molecule.

Introduction

The bleomycins (BLMs) are a family of glycopeptide-derived antitumor antibiotics that were originally isolated from a culture broth of *Streptomyces verticillus* as copper chelates.[1] A mixture of bleomycins, consisting primarily of bleomycin A_2 (~60%) and bleomycin B_2 (~30%) (Figure 1),[2] are used for the treatment of cancers.[3] The therapeutic effects of bleomycin are believed to derive in part from damage to chromosomal DNA in the presence of a metal ion cofactor.[4] Bleomycin can also degrade some RNAs at micromolar concentrations,[5] in both the presence[5a,c] and absence of Fe^{2+},[5b] and RNA may represent another therapeutic locus.[4e,5a,d]

Deglycobleomycin (Figure 2) cleaves DNA in a sequence-selective manner quite similar to that of bleomycin itself.[6] Despite a lesser cleavage efficiency and a decreased double- to single-strand cleavage ratio for deglycobleomycin, the aglycon is more readily accessible synthetically and has been used successfully to define the essential structural elements necessary to perform sequence-selective oxidative DNA degradation.[7] Over the past two decades, numerous analogues of bleomycin and deglycobleomycin have been synthesized to better understand the mechanism of DNA cleavage by bleomycin.[7] Most of these have differed from the parent (deglyco)bleomycin only within a single amino acid constituent.

Recently, the solid-phase synthesis of deglycobleomycin A_5 has been reported.[8] This has also permitted the solid-phase syntheses of deglycobleomycins having varied C-termini[9] and

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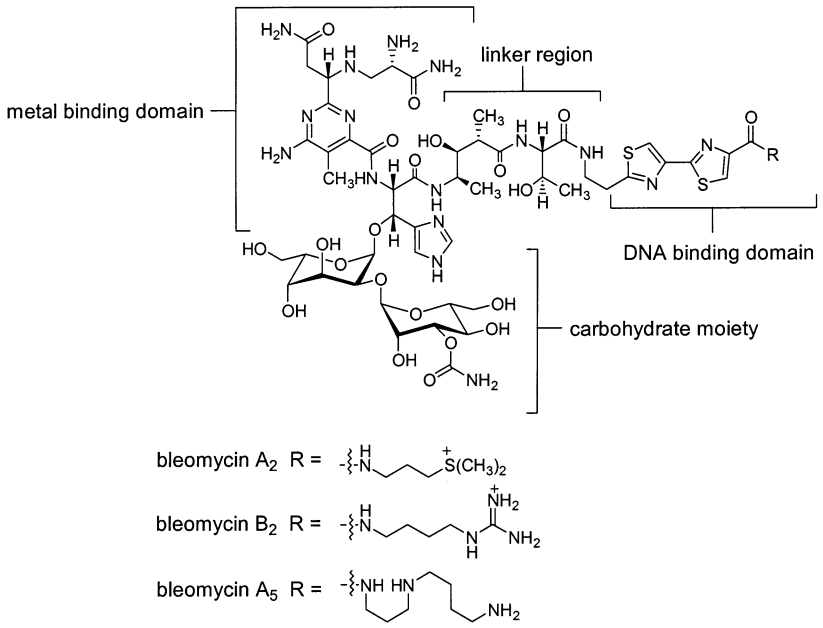

Figure 1. Structures of bleomycin A_2, B_2, and A_5.

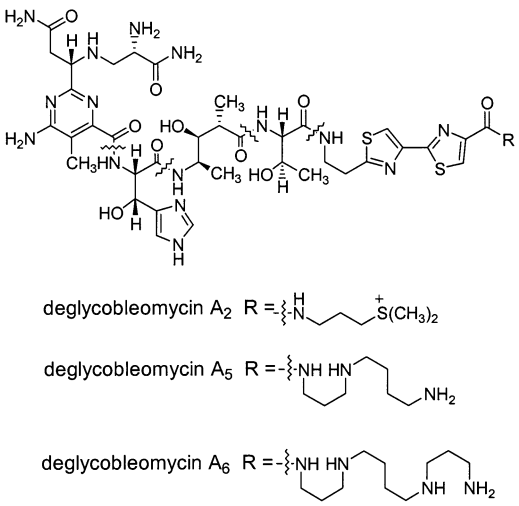

Figure 2. Structures of deglycobleomycin A_2, A_5, and A_6.

the synthesis of bleomycin A_5 itself.[10] As a result of these efforts, the preparation of a combinatorial library of deglycobleomycins seemed plausible and could further reveal the roles of individual bleomycin constituents. Presently, we report the combinatorial synthesis, purification, and structural characterization of 108 deglycobleomycin analogues.

Results

It was hoped that the incorporation of multiple substitutions simultaneously within the bleomycin core structure would afford analogues having improved biochemical properties. These might include enhanced potency of DNA cleavage, enhanced double-strand DNA cleavage, preferential RNA cleavage, or resistance to the action of bleomycin hydrolase,[11] an enzyme responsible for BLM catabolism in a therapeutic setting. In an effort to achieve these changes, the initial deglycobleomycin library included analogues of the bithiazole moiety, the threonine/methylvalerate linker structure, and the β-hydroxyhistidine moiety (Figure 1). The individual building blocks for the modified deglycobleomycins were either commercially available or readily accessible through existing synthetic methods. To prepare 108 analogues, three bithiazole, three threonine, three methylvalerate, and four β-hydroxyhistidine substitutions were made (Figure 3). The constituents present in deglycobleomycin were also included in this library, so that the effects of the modifications could be analyzed systematically. The selection of the amino acid fragments was based on previously reported observations as discussed in the following.

Selection of Fragment Analogues for Library Synthesis. In addition to bithiazole itself, a chlorobithiazole analogue was selected, since previous reports of deglycobleomycins containing halobithiazoles demonstrated DNA cleavage in the presence of light.[12] A trithiazole-containing deglycobleomycin analogue recently synthesized demonstrated diminished 5′-GC-3′ cleavage patterns when incorporated into deglycobleomycin, indicating a preference for 5′-GT-3′ cleavage.[13] In addition, the analogue containing the trithiazole demonstrated enhanced RNA cleavage. It was hoped that incorporating these two analogues into the bleomycin library would provide biochemically interesting new analogues.

The role of the threonine moiety of bleomycin has been studied in two laboratories.[14] The data suggested that simple

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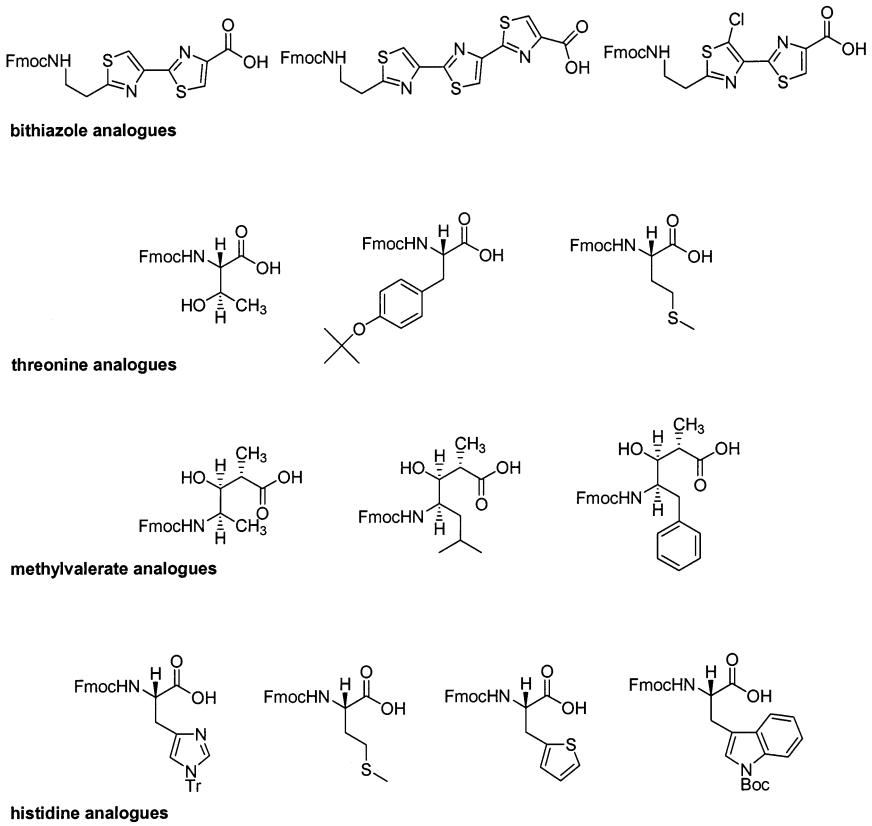
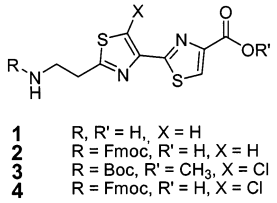


Figure 3. Deglycobleomycin fragment analogues used for combinatorial library synthesis.

modifications of threonine do not affect sequence selectivity but do affect the efficiency of strand scission. It was also concluded that conformational effects imposed by this constituent can play an important role in the efficiency of DNA cleavage. Since previous studies did not address the range of structural parameters that can affect BLM function, additional structural factors were examined in this study in an attempt to define the effects of hydrogen bonding or π-stacking interactions with DNA and RNA. As a result, tyrosine and methionine were selected for use in the library.

A systematic study of the methylvalerate moiety revealed that, in addition to the optimized four-carbon chain length, substituents at the 2- and 4-positions were important for efficient DNA strand scission.[15] The modifications provided important insights into the preferred conformation of the methylvalerate moiety.[15] On this basis, it was felt that increased steric bulk at the C-4 position might provide a better preferential fit to DNA compared to the natural methylvalerate moiety. As a result, the isopropylmethylvalerate and phenylmethylvalerate moieties were utilized for analogue synthesis.

The histidine moiety provides two potential metal coordination sites for metal-dependent DNA strand scission.[16] Replacements for this amino acid should logically contain analogous potential metal coordination sites. To expedite deglycobleomycin library synthesis, these amino acids were also chosen from among those that are commercially available. Accordingly, tryptophan, methionine, thienylalanine, and histidine were used. Each of these amino acids has a nitrogen or sulfur atom in a position roughly equivalent to those of the imidazole nitrogens of β-hydroxyhistidine. While previous studies have defined the importance of the τ-nitrogen for sequence selectivity,[17] it was believed that the altered heterocyclic and nonheterocyclic substitutions might demonstrate alternative RNA binding motifs, a property not explored previously. Using these criteria for selection of the amino acid fragments, the synthesis of the required amino acid fragments was performed.



Synthesis of the Fmoc Amino Acid Fragments. The Fmoc−bithiazole moiety was synthesized by treating 2′-aminoethyl-2,4′-bithiazole-4-carboxylic acid hydrochloride (**1**) with 9-fluorenylmethyl-succinimidyl carbonate in a 1:1 aqueous 5% K_2CO_3-dioxane solution.[18] The desired product **2** was obtained in 72% yield. The synthesis of the Fmoc−chlorobithiazole moiety was accomplished by adding an additional step to the reported procedure[12d] for the preparation of Boc-chlorobithiazole methyl ester. Fully protected methyl ester **3** was saponified (3:1 methanol−NaOH (1 N aq)). The Boc protecting group was then

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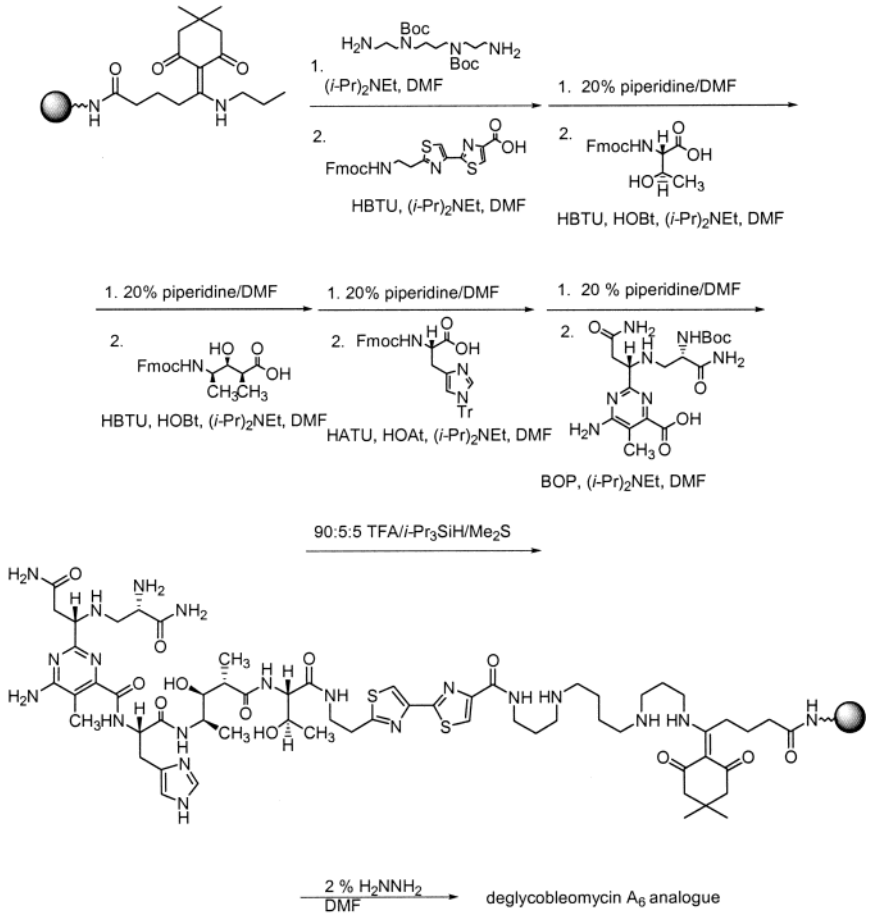
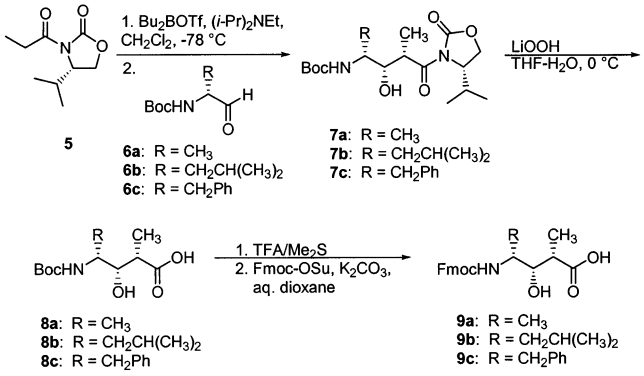
Scheme 2



removed with TFA in the presence of dimethyl sulfide, and the resulting free amino acid was treated with *N*-(9-fluorenylmethoxycarbonyloxy)-succinimide in alkaline aqueous dioxane. After precipitation by addition of TFA, desired Fmoc-protected chlorobithiazole analogue **4** was obtained in 49% yield.

Scheme 1



The synthesis of the Fmoc−methylvalerate moiety was accomplished by adding an additional step to the previously reported route for Boc-methylvalerate synthesis.[19,20] The *Z*-boron enolate of the acylated chiral auxiliary **5**[21] was condensed with Boc-D-alanal[22] (**6a**) (Scheme 1). The stereoselective aldol condensation gave the desired product **7a** in 71% yield. Likewise, **7b** and **7c** were obtained in 40 and 27% yields, respectively. The chiral auxiliary was removed with lithium peroxide in 3:1 THF−H_2O at 0 °C and gave amino acid derivative **8a** in 88% yield (84 and 87% yields for **8b** and **8c**, respectively). The Boc group was then removed with TFA in the presence of dimethyl sulfide and treated with *N*-(9-fluorenylmethoxycarbonyloxy)succinimide in alkaline aqueous dioxane, which yielded the requisite Fmoc-protected amino acid **9a** in 63% yield. Amino acid derivatives **9b** and **9c** were obtained analogously in yields of 63 and 37%, respectively. This method proved to be extremely versatile and readily applicable to the synthesis of additional methylvalerate analogues, including conformationally constrained valerates.[23] It may be noted that further modifications were made in the synthesis of the methylvalerate analogues following Evans' groundbreaking work in the use of chiral enolates to mediate highly stereocontrolled aldol reactions.[21] Most notable was the stereoselective generation of (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvalerate in 4 steps and 46% overall yield from Boc-*R*-alanine anhydride.[24] It was demonstrated that the outcome of the crucial $Zn(BH_4)_2$ mediated reduction of the 3-keto intermediate leading

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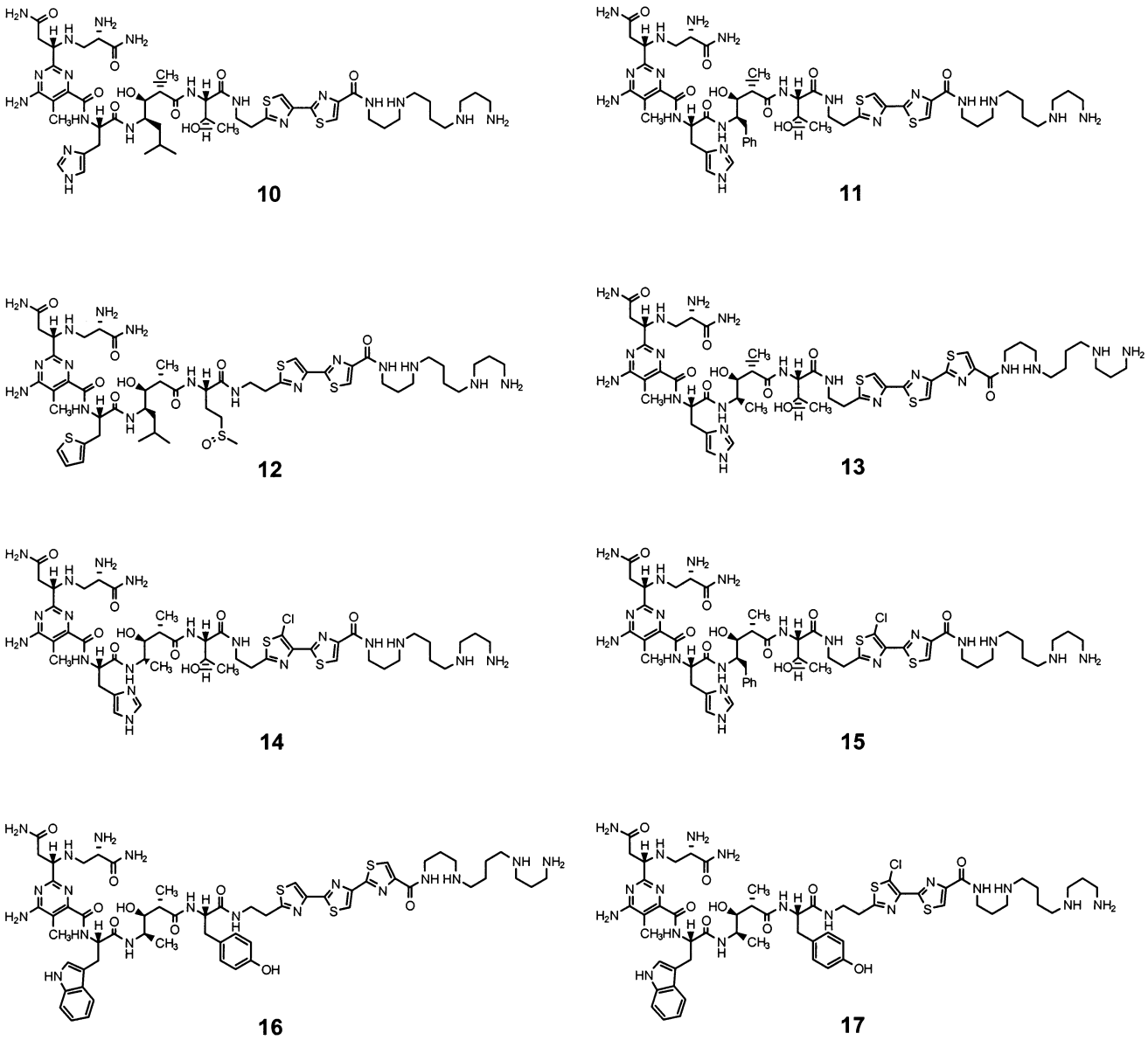

Figure 4. Representative deglycobleomycin analogues **10**−**17** characterized and listed in the Experimental Section in the Supporting Information.

to **7a** was directed almost exclusively by the presence and absolute stereochemistry of the group at C2 and not by the chirality at C4 or that present in the chiral auxiliary. Ohno[19] and Boger[20] further refined this method for the synthesis (2*S*,3*S*,4*R*)-4-amino-3-hydroxy-2-methylvalerate as they demonstrated that the synthesis could be accomplished from Boc-*R*-alanal[22] in three steps and 61% overall yield.

Synthesis of a 108-Member Deglycobleomycin Library. The synthesis of the deglycobleomycin A_6 library was carried out as outlined in Scheme 2 for deglycobleomycin A_6 itself. The linker-bound resin[25] was treated with di-Boc spermine[26] in the presence of Hunig's base in DMF for 24 h.[25b] This provided the spermine-functionalized resin. The bithiazole moiety of deglycobleomycin was immediately added to the solid support via HBTU-mediated coupling in DMF. After washing the resin with DMF and CH_2Cl_2, we assayed a small sample using the Kaiser test[27] and the bromophenol blue test.[28] The coupling was quantified by Fmoc cleavage and gave a loading of 0.17 mmol/g, representing a transamination and coupling efficiency of 40%.[29] Since neither test indicated the presence of any free amine, the resin was treated with a solution of 20% piperidine in DMF, and the threonine moiety was coupled to the resin by the use of HBTU, HOBt, and Hunig's base, providing the tripepide with a coupling efficiency of 0.17 mmol/g (>98%). The methylvalerate moiety was then added by the same sequence of treatments; the coupling efficiency was 0.15 mmol/g (>98%). Following an additional treatment with piperidine, the histidine moiety was coupled to the resin using HATU, HOAt,

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(29) Fmoc cleavage was based on the dibenzylfulvene−piperidine adduct formed upon treatment of the resin with piperidine. The optical densities of 5540 M^{-1} at 290 nm and 7300 M^{-1} at 300 nm were used to calculate the loading from a known weight of dry resin.

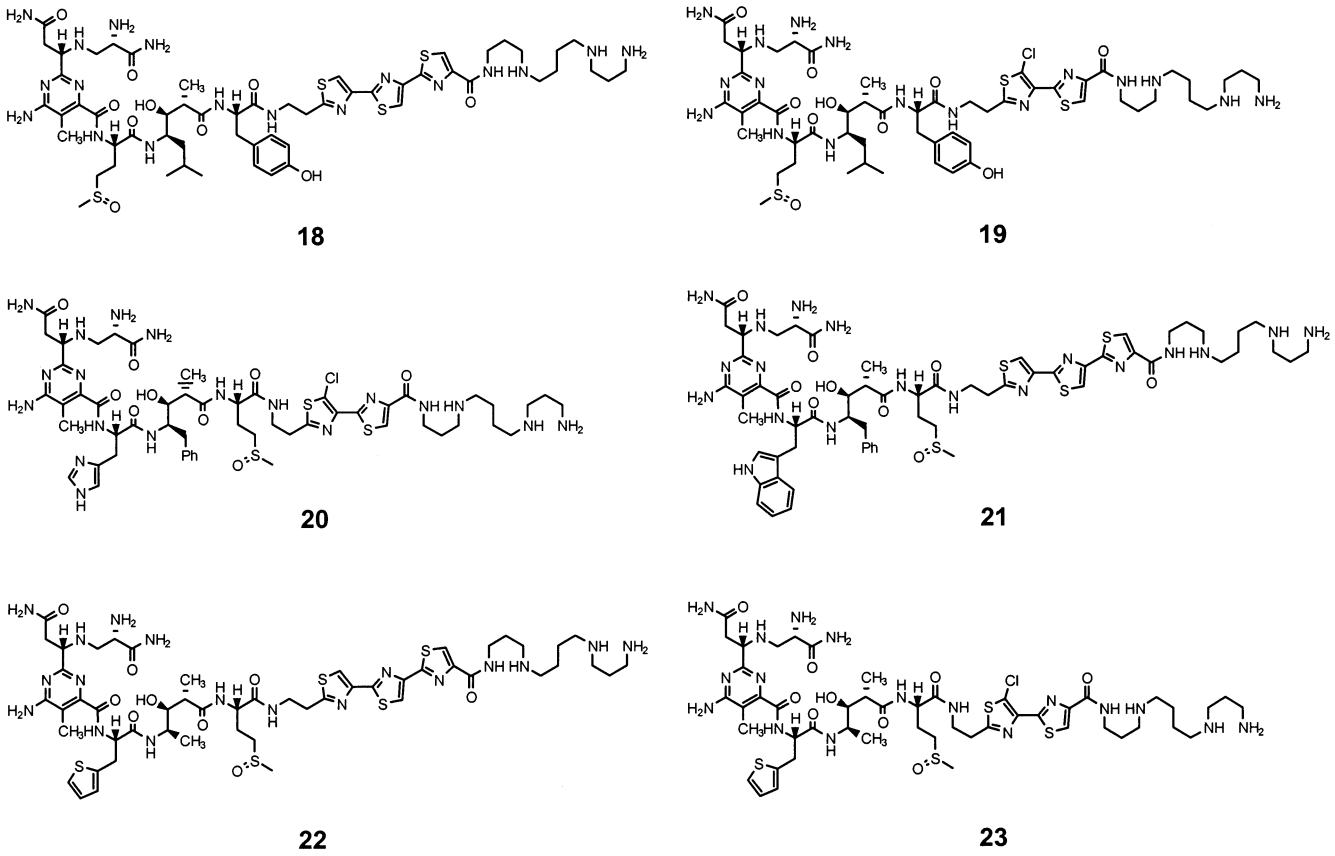

Figure 5. Representative deglycobleomycin analogues **18**−**23** characterized and listed in the Experimental Section in the Supporting Information.

and Hunig's base which yielded resin-bound pentapeptide resin with a final loading of 0.13 mmol/g (>95%).

Initial attempts to couple Boc pyrimidoblamic acid at room temperature demonstrated less than quantitative coupling efficiencies, typically less than 50%. Additional attempts to improve the coupling efficiency with more active coupling reagents or heat were not successful, and in fact, the attempted coupling of Boc pyrimidoblamic acid via the agency of HATU eliminated coupling altogether. However, when the final coupling of Boc pyrimidoblamic acid was performed at 0 °C in the presence of BOP and Hunig's base, nearly quantitative coupling was observed, as determined by reversed phase HPLC. ^{1}H NMR spectroscopy was used to determine the overall yield for deglycobleomycin A_6 by careful integration of the exocyclic C5-methyl substituent on pyrimidoblamic acid compared to a known amount of added *tert*-butyl alcohol. The overall yield of the desired deglycobleomycin A_6 analogue was calculated to be 66%, which corresponded to a stepwise coupling yield of 92% from the attached bithiazole moiety. This demonstrated that the coupling efficiency of pyrimidoblamic had improved greatly compared to that of the previous reported procedure.[8] The fully protected deglycobleomycin A_6 was then treated with a 90:5:5 TFA−Me_2S−triisopropylsilane[30] solution and gave the fully functionalized resin-bound deglycobleomycin A_6. The resin-bound deglycobleomycin was then liberated from the resin with a solution of 2% hydrazine in DMF.

The remaining deglycobleomycins in the library were synthesized in analogy with deglycobleomycin A_6 (Scheme 2). A parallel, that is, spatially separated, library was constructed in order to test the robustness of the synthetic methodology. The spatially separated library was based on 36 bithiazole-containing deglycobleomycins, 36 chlorobithiazole-containing deglycobleomycins, and 36 trithiazole-containing deglycobleomycins. The resin was initially functionalized with the protected spermine and the bithiazole moieties. Each resin was then divided into three separate vessels, and the appropriate threonine analogue was then added. The splitting process was repeated with the addition of the methylvalerate and histidine moieties. This continuous division technique minimized the number of reactions required and also provided flexibility in the amounts of resin employed for specific analogues, thus affording a larger quantity of final products compared to other combinatorial techniques. The final deglycobleomycin analogues were readily deprotected under acidic conditions and cleaved from the resin using 2% hydrazine in DMF.

Each of the deglycobleomycin analogues was purified by reversed phase HPLC. In every case, the major peak was subsequently identified as the desired product. The purity of the crude products, as determined by HPLC peak integration, was >60% and in some cases >70%. All of the purified deglycobleomycin analogues were obtained in near-milligram or milligram quantities after reversed phase HPLC purification. The deglycobleomycin analogues were characterized by ^{1}H NMR spectroscopy, electrospray mass spectrometry, and, in some cases, high-resolution mass spectrometry. Representative deglycobleomycins are shown in Figures 4 and 5, and characterization data for these compounds can be found in the Experimental Section in the Supporting Information. Electro-

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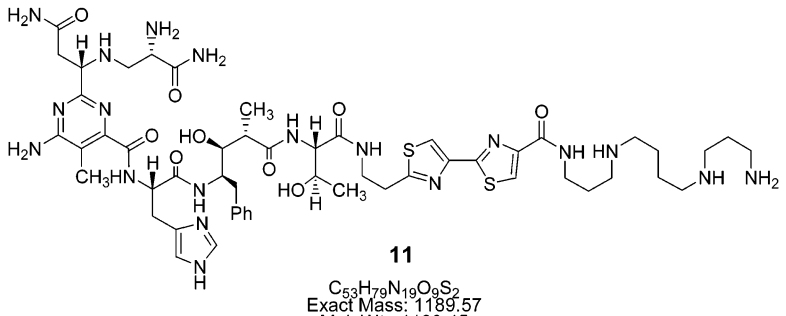

11

$C_{53}H_{79}N_{19}O_9S_2$
Exact Mass: 1189.57
Mol. Wt.: 1190.45

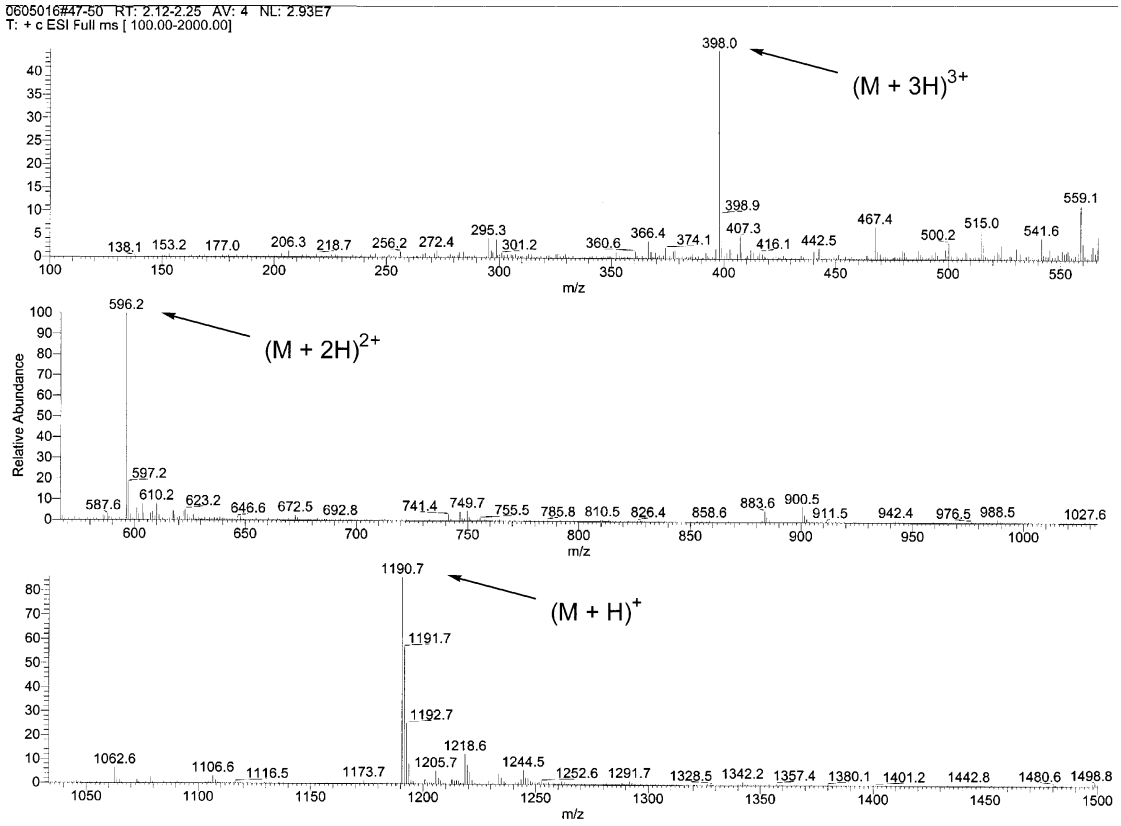

Figure 6. Electrospray mass spectrum of crude deglycobleomycin analogue **11**.

spray mass spectra, HPLC chromatograms, and ^{1}H NMR spectra are shown in Figures 6 and 7 for deglycobleomycin analogue **11** and Figures S1 and S2 (Supporting Information) for deglycobleomycin analogue **23**. Electrospray mass spectrometric analysis of the crude sample was used in order to determine whether the desired deglycobleomycin analogue had been synthesized. Clearly present in Figures 6 and S1 are the desired molecular ions for deglycobleomycin analogues **11** and **23**, respectively. In addition to the presence of the molecular ion, the di- and tripositive ions are also present, which was expected as a result of the multiple positions on deglycobleomycin that could be protonated. The absence of any undesired peptides resulting from incomplete coupling reactions was also verified, suggesting that the coupling reactions were nearly quantitative. Figures 7 and S2 show the reversed phase HPLC chromatograms and ^{1}H NMR spectra for deglycobleomycin analogues **11** and **23**. The major peak isolated from each reversed phase HPLC purification was analyzed by ^{1}H NMR spectroscopy. Clearly present in these ^{1}H NMR spectra are protons having chemical shifts characteristic of the amino acid fragments present within the deglycobleomycin analogue. Thus, we verified that this methodology was robust and could be employed for the synthesis of a large number of deglycobleomycin analogues. The derived deglycobleomycin analogues could be readily identified through classical chemical characterization techniques.

Methionine-Containing Deglycobleomycin Analogues. The incorporation of methionine into the deglycobleomycin library was accompanied by an additional transformation. Methionine has been reported to oxidize to the sulfoxide during the protocol used for acidic cleavage.[31] All deglycobleomycin congeners containing a methionine moiety were isolated as the respective methionine oxide species, as exemplified by deglycobleomycin analogue **23** (Figure S1). Analysis of these products by reversed phase HPLC and electrospray mass spectrometry indicated that

(31) (a) Hofmann, K.; Haas, W.; Smithers, M. J.; Wells, R. D.; Wolman, Y.; Yanaihara, N.; Zanetti, G. *J. Am. Chem. Soc.* **1965**, *87*, 620. (b) Joppich-Kuhn, R.; Corkill, J. A.; Giese, R. W. *Anal. Biochem.* **1982**, *119*, 73. (c) Hammer, R. P.; Alberico, F.; Gera, L.; Barany, G. *Int. J. Pept. Protein Res.* **1990**, *36*, 31.

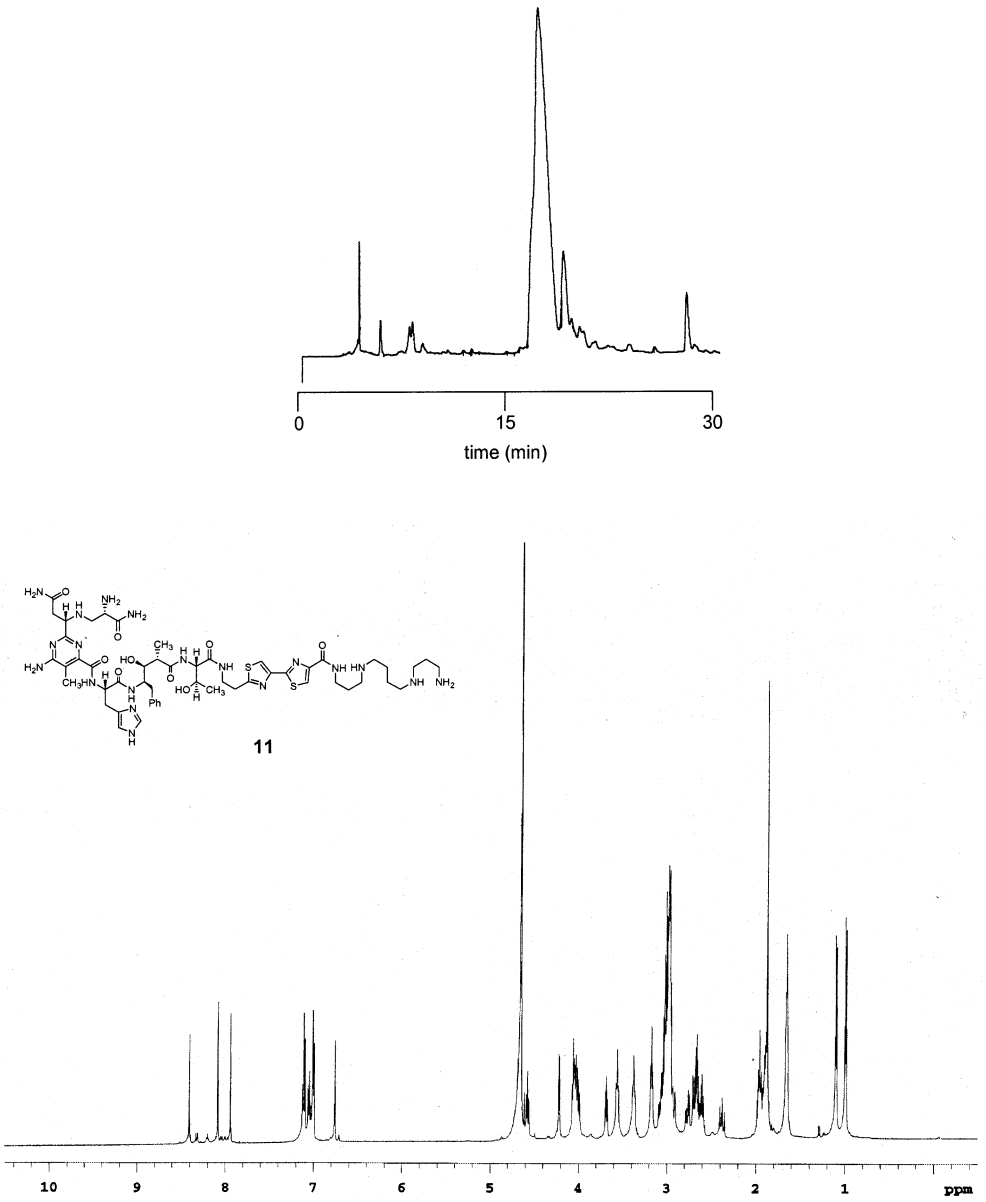

Figure 7. HPLC chromatogram and ^{1}H NMR spectrum of **11**. The major peak at 17.4 min was found to be the desired product: HPLC analysis was carried out on a C_{18} reversed phase HPLC column (250 × 10 mm) using a linear gradient of 14 → 20% CH_3CN in 0.1% aq TFA at a flow rate of 4 mL/min over a period of 30 min.

the oxidized species were, in fact, the major products, analogous to **23** (Figure S2). In most cases, the methionine-containing species was not present at all. Fortunately, methionine-oxide-containing peptides can be reduced in high yields with excess ammonium iodide in the presence of methyl sulfide in neat TFA.[32] This reduction procedure could be used for the facile conversion of methionine oxide to methionine in our deglycobleomycin analogues. For example, the methionine-oxide-containing deglycobleomycin **24** shown in Figure 8 was purified by HPLC and reduced quantitatively to **24a** using the aforementioned protocol, as demonstrated quantitatively by reversed phase HPLC (Figure S3) and high-resolution mass spectrometry.

To prevent reoxidation upon storage, the methionine-containing deglycobleomycin analogues were characterized and assayed initially as the oxidized species. Those species of special interest in any given assay can be reduced to the respective methionine-

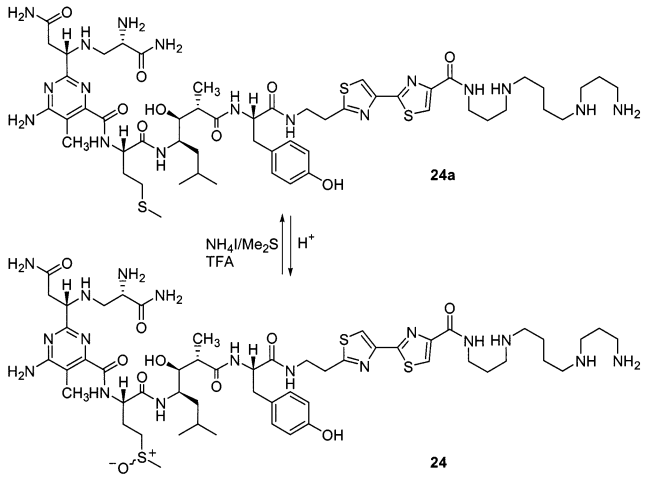

Figure 8. Oxidation and reduction of methionine-containing deglycobleomycins.

(32) Nicolás, E.; Vilaseca, M.; Giralt, E. *Tetrahedron* **1995**, *51*, 5701.

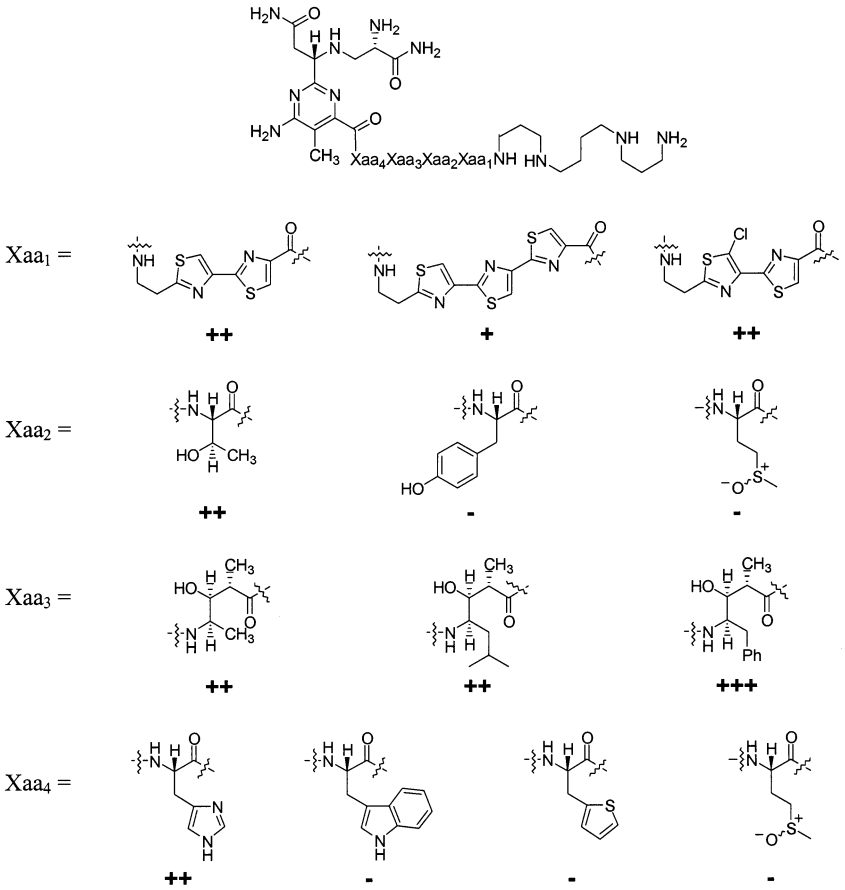

Figure 9. Effect of single and multiple substitutions in deglycobleomycin A_6 on the efficiency of relaxation of a supercoiled DNA plasmid. Combinations of + and ++ constituents yielded deglycoBLMs that produced Form III supercoiled plasmid breaks. Combinations of ++ and +++ constituents yielded deglycoBLMs that produced Form III supercoiled plasmid breaks with good and sometimes enhanced potency. Any analogue containing one or more − constituents yielded deglycoBLMs that did not produce Form III DNA but did produce Form II DNA.

containing analogues. While the presence of methionine oxide in the analogues initially isolated was not planned, the wherewithal to convert these species readily to those containing methionine does provide facile access to an additional 34 deglycobleomycin analogues.

DNA Cleavage Mediated by Members of the Deglycobleomycin A_6 Library. The deglycobleomycin library was utilized in the supercoiled DNA plasmid relaxation assay to identify compounds having altered DNA cleavage activity. In the presence of equimolar Fe^{2+}, all compounds synthesized as part of the library mediated plasmid relaxation to some extent. Two analogues (**11** and **15**) demonstrated supercoiled DNA plasmid relaxation to an extent greater than deglycobleomycin A_6 itself.

Discussion

Synthesis and Characterization of Analogues. In recent years, the properties of a number of DNA binding agents have been explored by the preparation of libraries of compounds.[33] The ability to synthesize (deglyco)bleomycin on a solid support has permitted the preparation of a significant number of analogues for evaluation. From a synthetic perspective, the most important observations relate to coupling efficiencies and the facility of analogue preparation. As judged by the Fmoc cleavage assay, all couplings except those involving the Boc pyrimidoblamic acid moiety were calculated to be greater than 90%. Since the major product isolated by reversed phase HPLC was the desired deglycobleomycin conjugate, it was apparent that coupling of the pyrimidoblamic acid moiety was also quite efficient, when the low temperature coupling protocol was employed. While the basis for the success of the low temperature protocol is uncertain, it may relate to the stability of the activated intermediate. In any case, the procedure was generally applicable for those species studied. This suggests that the solid-phase synthetic methodology is robust and that additional (deglyco)-bleomycins can be synthesized without undue concern over incomplete coupling reactions. The individual deglycobleomycins were obtained in near-milligram and milligram quantities after purification, which is altogether sufficient for chemical and biochemical evaluation. Also notable was the facility with which individual analogues can be prepared. Once the protected amino acid constituents and functionalized resin are obtained, synthesis and purification of a deglycobleomycin analogue can be accomplished routinely in 2−3 days.

All deglycobleomycin analogues were purified readily by reversed phase HPLC and characterized by ^1H NMR spectroscopy and low-resolution (electrospray) or high-resolution (FAB) mass spectrometry. Each compound was identified readily by mass spectrometry, as a consequence of the presence of a

(33) (a) Sagnou, M. J.; Howard, P. W.; Gregson, S. J.; Eno-Amooquaye, E.; Burke, P. J.; Thurston, D. E. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2083. (b) Baraldi, P. G.; Balboni, G.; Pavani, M. G.; Spalluto, G.; Tabrizi, M. A.; De Clercq, E.; Balzarini, J.; Bando, T.; Sugiyama, H.; Romagnoli, R. *J. Med. Chem.* **2001**, *44*, 2536. (c) Kers, I.; Dervan, P. B. *Bioorg. Med. Chem.* **2002**, *10*, 3339.

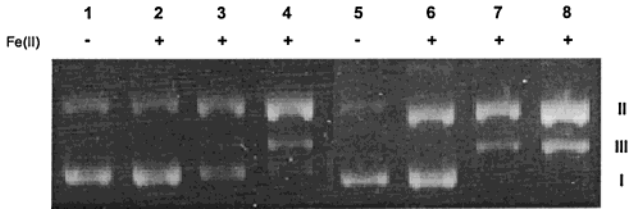

Figure 10. Relaxation of supercoiled pBR322 plasmid DNA by Fe(II)•deglycoBLM **11**. Lane 1, DNA + 10 μM deglycoBLM; lane 2, 1 μM deglycoBLM; lane 3, 5 μM deglycoBLM; lane 4, 10 μM deglycoBLM; lane 5, 10 μM deglycoBLM **11**; lane 6, 1 μM deglycoBLM **11**; lane 7, 5 μM deglycoBLM **11**; lane 8, 10 μM deglycoBLM **11**. Lanes 2−4 and 6−8 also contained 10 μM Fe^{2+}; essentially no cleavage was observed in the presence of Fe^{2+} alone.

molecular ion, in addition to the presence of the di- and tripositive molecular ions. Characterization by ^{1}H NMR afforded definitive evidence for the structures of individual deglycobleomycins synthesized. Chemical shifts corresponding to the individual amino acid fragments were easily identifiable throughout the entire range of analogues synthesized, providing more conclusive evidence for the actual structures of individual analogues.

As noted previously, methionine-containing deglycobleomycins were isolated as the respective methionine S-oxides. A protocol for the reduction of these species to deglycobleomycins containing methionine has been demonstrated utilizing deglycobleomycin **24**. The facile reduction of methionine-oxide-containing deglycobleomycins will clearly permit the introduction of methionine into additional deglycobleomycin libraries. Electrospray mass spectrometry was helpful in characterizing both the oxidized and unoxidized methionine-containing species. Purification of the oxidized and unoxidized methionine compounds was accomplished readily by reversed phase HPLC, and the individual species were easily identifiable by a dramatic shift in retention time. As anticipated, the less polar unoxidized methionine-containing analogues were retained several minutes longer than the respective oxidized methionine-containing species under identical gradient conditions. HPLC analysis of the reduced species also indicated quantitative reduction, providing an exceptionally clean product.

Preliminary Biochemical Evaluation. Each member of the deglycobleomycin library was assayed and found to mediate concentration-dependent plasmid DNA relaxation to some extent. The efficiency of cleavage by individual analogues was strongly dependent upon the specific substitutions made to the parent molecule. To permit a semiquantitative evaluation of the effects of individual amino acid substitutions, the individual amino acid constituents in deglycobleomycin A_6 were each assigned a score of ++. Each of the modified amino acid constituents employed for analogue synthesis was then assigned a score (−, +, ++, or +++) by evaluating the potency of cleavage of the deglycobleomycin resulting from the single substitution of that amino acid constituent in place of the one normally present in deglycobleomycin A_6. The results of the plasmid relaxation assays utilizing all of the analogues are summarized in Figure 9. As shown, analogues prepared from constituents having scores of + or ++ produced deglycobleomycin analogues of reasonable potency that generally mediated double-strand DNA cleavage. Deglycobleomycin analogues prepared from constituents having scores of ++ and +++ exhibited a potency of cleavage of supercoiled plasmid DNA comparable to or greater than that of deglycobleomycin itself. While all of the deglycobloemycin analogues prepared were capable of relaxing supercoiled plasmid DNA to some extent, the presence of an amino acid having a score of − resulted in a substantial diminution of DNA relaxation potency, and the presence of two such constituents afforded weakly active deglycobleomycins that produced minimal amounts of Form II DNA and no Form III DNA. Significantly, when an amino acid having a score of − was present, no combination of other constituents could restore the potency of DNA relaxation.

In terms of the effects of specific substitutions, the replacement of threonine with tyrosine or methionine afforded deglycobleomycin analogues that mediated plasmid relaxation with minimal efficiency; those deglycoBLMs tested for cleavage of a linear DNA duplex gave only sequence neutral cleavage. Thus, while the structure of the threonine moiety is not unique in its ability to afford deglycoBLM analogues capable of sequence-selective DNA cleavage,[14] the range of amino acid structures that can be employed in this position are clearly somewhat limited and require more precise definition. The same conclusion was reached for the β-hydroxyhistidine moiety in which replacement of the imidazole moiety of histidine with an indole, thiophene, or dialkylsulfoxide moiety greatly diminished the supercoiled DNA relaxation potential.

Deglycobleomycin analogues **11** and **15** exhibited potencies of supercoiled plasmid DNA relaxation greater than those of deglycobleomycin A_6 itself. Further, each of these analogues produced Form III DNA with enhanced efficiency. This is illustrated in Figure 10 for deglycoBLM derivative **11**. These represent the first examples of the preparation of (deglyco)-bleomycin analogues having potencies greater than those of the parent molecule from which they were derived. It seems reasonable to assert that the use of solid-phase synthesis of additional bleomycin libraries, employing the principles defined in the present study, may afford bleomycins having improved properties in the several types of assays used to characterize bleomycin function.

Acknowledgment. This paper is dedicated to the memory of Professor Claude Hélène. This work was supported by NIH Research Grants CA76297 and CA77284 awarded by the National Cancer Institute.

Supporting Information Available: Experimental procedures for the synthesis and bioassay of deglycoBLM derivatives. ^{1}H NMR and mass spectral data for members of the combinatorial library. This material is available free of charge via the Internet at http://pubs.acs.org.

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