Synthesis and Evaluation of the Fully Functionalized Bleomycin A_2 Metal Binding Domain Containing the 2-*O*-(3-*O*-Carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl Disaccharide

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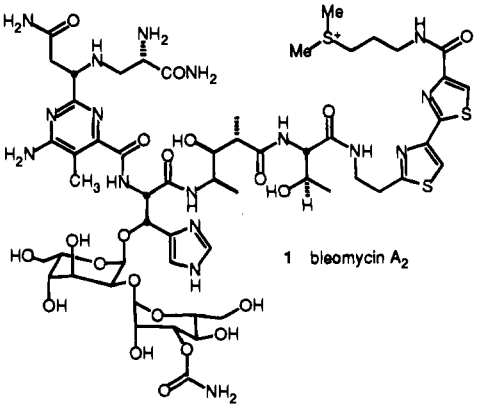
Received January 17, 1995[⊗]

Abstract: The synthesis of the full metal binding domain of bleomycin A_2 complete with linkage to the 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl disaccharide is detailed. Metal complexes (Fe(II), Fe(III)) of this full metal binding domain which includes the putative carbamoyl ligand residing in the disaccharide were found to cleave DNA in the presence of O_2 (Fe(II)) or H_2O_2 (Fe(III)) well above background cleavage and only 8−10× less efficiently than deglycobleomycin A_2 or 30−40× less efficiently than bleomycin A_2 and to do so in a non-sequence-specific manner with significantly reduced ratios of double versus single strand DNA cleavage (1:48 versus 1:6 for bleomycin A_2). Thus, although the metal binding domain of bleomycin A_2 may play a role in determining the selectivity observed in DNA cleavage when incorporated into the full natural product structure, the metal binding domain alone or in the presence of noncovalently linked tri- and tetrapeptide S failed to exhibit the sequence-selective DNA cleavage characteristic of bleomycin A_2.

Bleomycin A_2 (**1**), the major naturally occurring constituent of the clinical antitumor drug blenoxane, is thought to derive its therapeutic effects from the ability to mediate the oxidative cleavage of double-stranded DNA[1−15] or RNA[16−18] by a process that is metal ion and oxygen dependent. Consequently bleomycin A_2,[19] its naturally occurring congeners,[20] its degradation products,[21−27] and its semisynthetic derivatives[28−30] as well as



1 bleomycin A_2

synthetic analogs[31−36] have been the subject of extensive and continued examination in efforts to define the fundamental functional roles of the individual subunits. The pyrimidoblamic acid subunit along with the adjacent *erythro*-β-hydroxy-L-histidine provides the metal chelation coordination sites required

[⊗] Abstract published in *Advance ACS Abstracts*, June 15, 1995.

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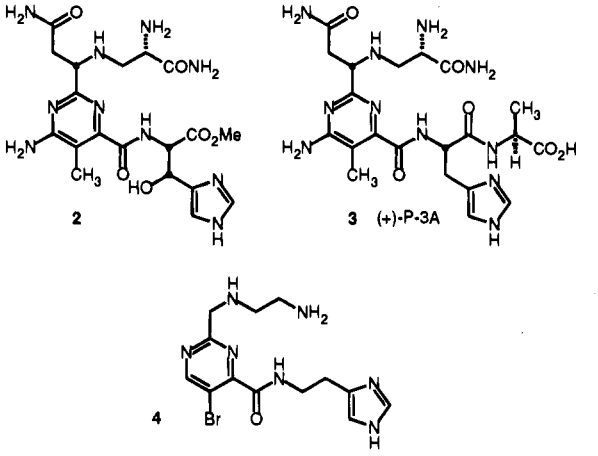
for Fe(II) complexation and molecular oxygen activation responsible for the subsequent DNA cleavage. The small contribution that the metal binding domain makes to the DNA binding affinity has long been recognized,[37] and the potential contribution that this segment may make in polynucleotide recognition remains an active topic of consideration.[18,32,33,36] The C-terminus tri- and tetrapeptide S subunits including the terminal sulfonium cation and the bithiazole provide the majority of the bleomycin A_2 DNA binding affinity[37,38] and may contribute to polynucleotide recognition and the DNA cleavage selectivity. Despite the extensive studies on the bleomycins, the nature of the relevant bleomycin A_2 bithiazole binding with duplex DNA remains unresolved and has been proposed to involve intercalation or minor groove binding.[39] Similarly, the origin of the DNA cleavage selectivity remains unresolved, and both the tri- and tetrapeptide C-terminus[40] and the N-terminus metal binding domain[32,33] have been suggested to be independently responsible for the characteristic bleomycin A_2 5′-GC/5′-GT DNA cleavage selectivity.

Efforts to directly assess the role of the metal binding domain have been limited. Metal complexes of **2**[41] and related agents[32] constituting the N-terminus of bleomycin A_2 fail to cleave DNA in the presence of O_2 (Fe(II)) or H_2O_2 (Fe(III)) above background cleavage. However, (+)-P-3A (**3**) and two structurally related agents were recently found to cleave DNA significantly above background and to do so in a non-sequence-selective fashion.[36] Although not identical to the bleomycin A_2 metal binding domain, their close structural similarity was taken to suggest that this portion of the bleomycin A_2 metal binding domain is insufficient for observation of the characteristic DNA cleavage selectivity. In contrast, the observation that Fe(II)–PMA (**4**) produced a DNA cleavage pattern strikingly similar though not identical to bleomycin A_2 has suggested that the metal binding domain may be responsible for or dominate the characteristic bleomycin A_2 cleavage selectivity.[33]



These preceding studies potentially suffer from the lack of incorporation of the bleomycin A_2 disaccharide into the metal binding domain. The commonly accepted depiction of the bleomycin A_2 metal chelation is derived from the X-ray crystal structure of the Cu(II) complex of P-3A (**3**) in which the primary and secondary amines of the β-aminoalaninamide side chain, pyrimidine-N1, and β-hydroxy-L-histidine imidazole-N3 and its deprotonated amide were coordinated to the metal in a square planar, pyramidal complex.[42] This provided the basis for a proposed structure of bleomycin A_2 metal complexes in which the mannose C3 carbamoyl group occupies a sixth coordination site.[42] NMR studies of a range of bleomycin A_2 metal complexes have indicated that carbamoyl coordination may or may not be observed, and extensive studies have contributed to the consensus that the pyrimidine, imidazole, and secondary amine are bound to the metal.[43–45] The remaining metal ligands and their arrangement within activated bleomycin A_2 remain unresolved. The importance of these issues has been highlighted by the observation that Fe(II)– and Cu(I)–bleomycin A_2 are structurally distinct and that the observed differences in the metal coordination geometries in the two complexes may contribute to the observed differences in DNA cleavage selectivity.[46] Similarly, the metal coordination geometries of Fe(II)–bleomycin A_2 and deglycobleomycin A_2 are believed to substantially differ[47] and may account for the differences in their relative

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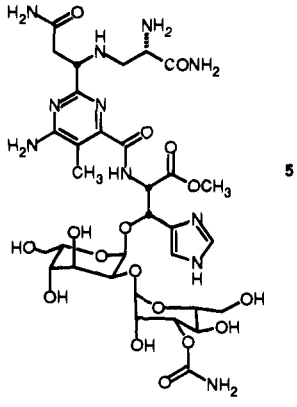
cleavage selectivities.[48] Since deglycobleomycin A_2 and decarbamoylbleomycin A_2 were found to behave similarly in this comparative examination, their distinctions from bleomycin A_2 itself may be attributable to the absence of the carbamoyl group and its potential metal coordination.[48] Although it has been shown that the disaccharide does not contribute to DNA binding affinity,[37,38] it possesses additional functional roles that enhance DNA cleavage efficiency and increase the double to single strand DNA cleavage ratios.[38] In addition to potentially providing the carbamoyl group as a sixth metal binding ligand, it has been suggested to provide a cavity for and stabilize the O_2-bound activated metal complex.[49]

In efforts to resolve such issues and in an effort to directly assess the potential polynucleotide recognition features of the bleomycin A_2 metal binding domain, herein we report the synthesis and evaluation of **5**, the fully functionalized N-

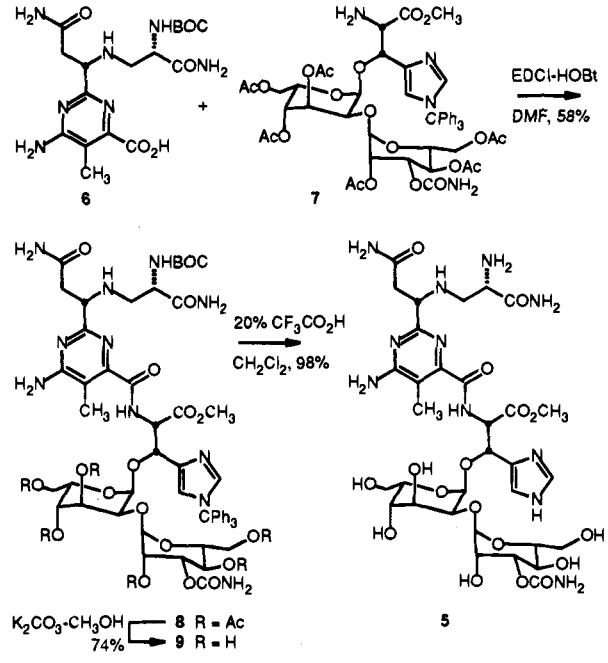


5

terminus metal binding domain of bleomycin A_2 complete with linkage to the 2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl disaccharide. Metal complexes of **5** which includes the putative carbamoyl metal ligand residing in the disaccharide were found to cleave duplex DNA in the presence of O_2 (Fe(II)) or H_2O_2 (Fe(III)) well above background cleavage and to do so in a nonspecific manner with a significantly reduced double to single strand cleavage ratio. These observations which were made with **5** alone or in the presence of noncovalently linked tri- and tetrapeptide S demonstrate that the metal binding domain alone is insufficient for observation of the characteristic bleomycin A_2 DNA cleavage selectivity.

Synthesis of N^{β}-[1-Amino-3(*S*)-[4-amino-6-[[*erythro*-β-[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-*N*-L-histidyl Methyl Ester]carbonyl]-5-methylpyrimidin-2-yl]-1-oxo-3-propyl]-(*S*)-β-aminoalaninamide (5). Coupling of synthetic N^{α}-BOC-pyrimidoblamic acid (**6**)[50] with synthetic **7**[51] (1.1 equiv of EDCI, 1.1 equiv of HOBt, DMF, 25 °C, 48 h, 58%) provided **8**, the linked and protected intact metal binding domain of bleomycin A_2 (Scheme 1). Methanolysis of the six *O*-acetates of **8** effected by treatment with 6.3 equiv of powdered K_2CO_3 in rigorously dried CH_3OH (25 °C, 30 min) cleanly afforded **9** (74%). The use of excess K_2CO_3 or substantially longer reaction times resulted in competitive β-elimination of the disaccharide and the use of nonrigorously dried CH_3OH led

Scheme 1



to competitive methyl ester hydrolysis. Final acid-catalyzed deprotection of **9** (20% CF_3CO_2H–CH_2Cl_2, 0 °C, 3 h, 98%) cleanly provided **5**. The synthesis outlined in Scheme 1 concisely provided **5** in excellent conversions from the precursors **6**[50] and **7**[51] which in turn required nine steps (20–25% overall) and seven steps (16–19% overall from the respective monosaccharides), respectively, to prepare. In turn, the suitably protected and appropriately activated monosaccharides for use in the synthesis of **7** required eight steps (36% overall) and nine steps (13% overall) to assemble from α-D-mannose.[51]

DNA Cleavage Properties of 5. The initial study of the relative efficiency of DNA cleavage was conducted with Fe(II)–**5** and supercoiled ΦX174 DNA in the presence of O_2 and 2-mercaptoethanol. Like Fe(II)–bleomycin A_2 and deglycobleomycin A_2, the Fe(II) complex of **5** produced both single and double strand cleavage to afford relaxed (form II) and linear (form III) DNA, respectively, Table 1. The Fe(II) complex of **5** was found to be 8–10× less effective than deglycobleomycin A_2 and 30–40× less effective than bleomycin A_2 but 2–4× more effective than Fe(II) at cleaving the supercoiled DNA. The lack of DNA cleavage by **5** in the absence of Fe(II) in control studies was consistent with expectations that it was cleaving DNA by a metal dependent process in a manner analogous to that of **1**. The relative extent of double strand to single strand DNA cleavage was established in a study of the kinetics of supercoiled ΦX174 DNA cleavage to produce linear and circular DNA. The results are summarized in Table 1. The reaction exhibited initial fast kinetics in the first 1–10 min, and the subsequent decreasing rate may reflect conversion to a less active or inactive agent or metal complex reactivation. We assumed a Poisson distribution for the formation of single strand and double strand breaks to calculate the average number of double and single strand cuts per DNA molecule using the Freifelder–Trumbo equation.[52] The data for the first few minutes (1–10 min) could be fitted to a linear equation, and the ratios of double strand to single strand cuts observed with the Fe(II) complex of **5** and related agents are summarized in Table 1. The ratio of double to single strand DNA cleavage for **5** was established to be 1:48 which was substantially lower

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Table 1. Summary of DNA Cleavage Properties of **5**

agent	relative efficiency of DNA cleavage[a] ΦX174[a]	w794[b]	w836[b]	ratio of double to single strand DNA cleavage[c]	DNA cleavage selectivity[b]
bleomycin A_2 (**1**)	2−5	5.8	9.1	1:6	5′-GC, 5′-GT > 5′-GA
deglycobleomycin A_2	1.0	1.0	1.0	1:12	5′-GC, 5′-GT > 5′-GA
(+)-P-3A (**3**)[d]	0.3−0.2	nd	nd	1:30	none
(−)-epi-P-3A[d]	0.07−0.06	nd	nd	1:38	none
(−)-desacetamido-P-3A[d]	0.07−0.06	nd	nd	1:40	none
2	≥ 0.04[e]	0.008	0.007	nd	none
5	0.13−0.10	0.03	0.03	1:48	none
Fe(II)	0.04	0.006	0.005	1:98	none

[a] Relative efficiency of supercoiled ΦX174 DNA cleavage, Fe(II)−O_2, 2-mercaptoethanol. [b] Examined within 5′-^{32}P-end-labeled w794 and w836 DNA, Fe(III)−H_2O_2. [c] Ratio of double- to single-stranded cleavage of supercoiled ΦX174 DNA calculated as $F_{III} = n_2\exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$. [d] Taken from ref 36. [e] DNA cleavage indistinguishable from background Fe(II).

than bleomycin A_2 (1:6) or deglycobleomycin A_2 (1:12) and similar to the ratio derived from uncomplexed Fe(II) cleavage (1:98). A theoretical 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:single strand breaks under our conditions of assay consistent with the theoretical ratio.

The selectivity of DNA cleavage along with an additional assessment of the relative efficiency of DNA cleavage was examined within duplex w794 DNA and its complement w836 DNA[53,54] by monitoring strand cleavage of singly ^{32}P 5′-end-labeled double-stranded DNA after exposure to the Fe(III) complex followed by activation with H_2O_2[55] in 10 mM phosphate buffer (pH 7.0).[38] This protocol has proven to be much more sensitive to the distinctions between the relative efficiency of DNA cleavage by related agents. Thus, incubation of the labeled duplex DNA with **5** in the presence of equimolar $FeCl_3$ and excess H_2O_2 led to DNA cleavage. Following a quench of the reaction with the addition of glycerol, 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. An extensive range of conditions for the DNA cleavage reactions were examined including TE (pH 8) or Tris−HCl buffer (pH 6−8, 10−50 mM) in the presence of equimolar Fe(III) and H_2O_2 or Fe(II) and O_2 initiated by treatment with mercaptoethanol or dithiothreitol (DTT).[38] The effect of hydrogen peroxide concentration on the DNA cleavage efficiency and sequence selectivity was also examined. The DNA cleavage efficiency of both Fe(III)−**5** and Fe(III) increased with increasing hydrogen peroxide concentration from 300 μM[33] to 5 mM[55] (3−5×) and again from 5 mM to 1.5 M[41] H_2O_2 (2−3×). No selectivity for cleavage was observed under any of the activation conditions. The same increase in DNA cleavage efficiency was observed with Fe(III)−bleomycin A_2 (**1**) and Fe(III)−deglycobleomycin A_2, and the clarity of the sequence selectivity of the DNA cleavage reactions for both Fe(III)−**1** and Fe(III)−deglycobleomycin A_2 improved at the higher concentrations of H_2O_2 under our conditions. When the DNA cleavage reactions were run in the presence of DMSO[33] as a radical scavenger, the DNA cleavage efficiency of Fe(III)−**5** was reduced 2−3× as was that of Fe(III) itself, and again no evidence of DNA cleavage selectivity by **5** was observed. In additional efforts, the order of addition of the reagents to the DNA cleavage reactions was examined in detail including the initial incubation (30 min) of the DNA mixture with **5** and deglycobleomycin A_2, prior to the addition of H_2O_2 and Fe(III).[33] No distinctions were observed for both **5** or deglycobleomycin A_2 with the latter producing the characteristic DNA cleavage of bleomycin A_2 and the former cleaving DNA in a nonselective manner. In analogy to the studies that led to the observation that preincubation of DNA with the full carbohydrate domain of calicheamicin γ_1^1 followed by incubation with the enediyne aglycon leads to sequence-selective cleavage not observed in the absence of the carbohydrate domain,[56] the DNA cleavage reactions of Fe(III)−**5** were also conducted in the presence of an equimolar amount of added tri- and tetrapeptide S, the C-terminus of bleomycin A_2. Although the differences were quite subtle, the DNA cleavage efficiency of **5** increased slightly when an equivalent amount of tri- or tetrapeptide S was present, but for all practical purposes, no distinctions were observed.

Under all conditions examined, **5** was found to cleave DNA well above background Fe(II) or Fe(III) and to do so in a non-sequence-selective fashion (Figure 1 and Table 1). The Fe(III) complex of **5** was 5−6× more effective than Fe(III) itself, 30−35× less effective than deglycobleomycin A_2, and 200−300× less effective than bleomycin A_2. Comparisons alongside the Fe(II) or Fe(III) complexes of bleomycin A_2 and deglycobleomycin A_2 assured that the protocols employed would permit detection of the characteristic sequence-selective DNA cleavage reaction.

Conclusions. The preparation and evaluation of **5** has permitted the first direct assessment of the independent properties of the metal binding domain of bleomycin A_2. The Fe(II) and Fe(III) complexes of the fully functionalized metal binding domain of bleomycin A_2, **5**, complete with linkage to the disaccharide which includes the putative carbamoyl ligand were found to cleave duplex DNA in the presence of O_2 or H_2O_2 well above background Fe(II) or Fe(III), respectively, and to do so in a non-sequence-selective fashion with a significantly reduced double to single strand DNA cleavage ratio. Thus, although the metal binding domain of bleomycin A_2 may play a role in determining the selectivity observed in DNA cleavage when incorporated into the natural product structure, the metal binding domain alone or in the presence of noncovalently linked tri- or tetrapeptide S is not capable of producing the character-

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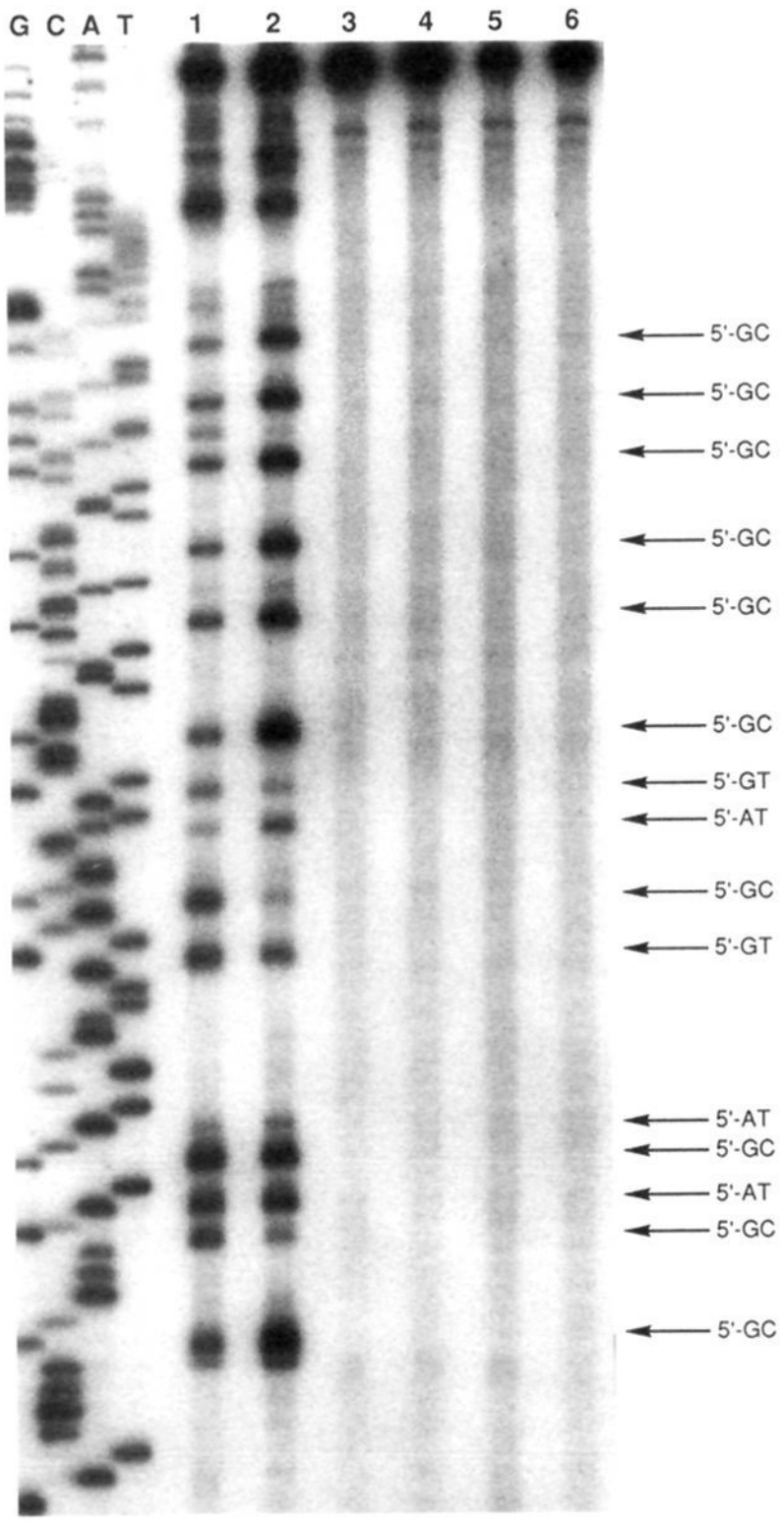

Figure 1. Cleavage of double-stranded DNA (SV40 DNA fragment, 156 base pairs, nucleotide no. 5239-150, clone w794) in 10 mM phosphate/10 mM KCl buffer, pH 7.0, containing excess H_2O_2 by Fe(III)−bleomycin A_2 (**1**), Fe(III)−deglycobleomycin A_2, and Fe(III)−**5**: lane 1, 0.5 μM Fe(III)−bleomycin A_2 (**1**); lane 2, 2.0 μM Fe(III)−deglycobleomycin A_2; lane 3, control DNA; lane 4, 128 μM Fe(III) control; lanes 5 and 6, 64 and 32 μM Fe(III)−**5**. The DNA cleavage reactions were run for 30 min at 37 °C, and electrophoresis was conducted at 1100 V (5.5 h) on an 8% denaturing PAGE and visualized by autoradiography. Quantitation of the DNA cleavage in lanes 1, 2, and 4−6 is provided in Table 3.

istic 5′-GC/5′-GT DNA cleavage selectivity or efficiency of bleomycin A_2.

Experimental Section

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\beta}$-[1-amino-3(*S*)-[4-amino-6-[[*N*^{im}-(triphenylmethyl)-*erythro*-β-[3,4,6-tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-*N*-L-histidyl methyl ester]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalaninamide (8).** A solution of **6**[50] (0.0074 mmol, 3.2 mg) in DMF (100 μL) was treated with **7**[51] (0.0074 mmol, 7.7 mg), HOBt (0.0090 mmol, 1.2 mg, 1.1 equiv), and EDCI (0.0090 mmol, 1.75 mg, 1.1 equiv), and the mixture was stirred under Ar at 25 °C for 48 h. The reaction mixture was concentrated in vacuo to give an oily solid. Chromatography (SiO_2, 0.5 × 5 cm, 10−20% CH_3OH−$CHCl_3$ gradient elution) afforded **8** (6.2 mg, 10.7 mg theoretical, 58%) as a foam: R_f 0.18 (SiO_2, 20% CH_3OH-$CHCl_3$); $[\alpha]_D^{25}$ −13.8 (*c* 0.13, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.47 (1H, d, J = 1.2 Hz), 7.23−7.37 (9H, m), 7.03−7.12 (7H, m), 5.29 (1H, d, J = 3.8 Hz), 5.11−5.26 (7H, m), 4.96 (1H, d, J = 1.6 Hz), 4.03−4.27 (5H, m), 4.00 (1H, t, J = 3.7 Hz), 3.87−3.95 (2H, m), 3.80 (3H, s), 3.57−3.67 (1H, m), 2.52−2.89 (3H, m), 2.41 (1H, dd, J = 8.8, 14.8 Hz), 2.26 (3H, s), 2.10 (3H, s), 2.09 (3H, s), 1.99 (3H, s), 1.99 (3H, s), 1.90 (3H, s), 1.85 (3H, s), 1.43 (9H, s); IR (neat) ν_{max} 3453, 1762, 1492, 1418, 1210 cm^{-1}; FAB HRMS (NBA) *m/e* 1454.5620 (M^+ + H, $C_{68}H_{83}N_{11}O_{25}$ requires 1454.5640).

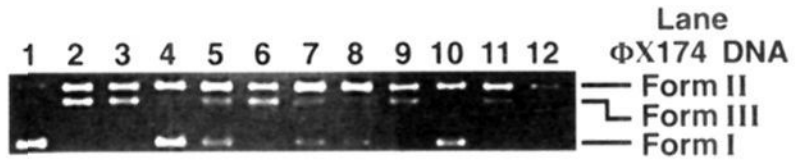

Figure 2. Cleavage of supercoiled ΦX174 DNA by Fe(II)−**5** and related agents: lane 1, control ΦX174 DNA, 95% form I (supercoiled), 5% form II (circular); lane 2, 0.5 μM Fe(II)−**1** (bleomycin A_2); lane 3, 2.0 μM Fe(II)−deglycobleomycin A_2; lanes 4−6, 8.0, 32.0, and 48.0 μM Fe(II)−**2**; lanes 7−9, 4.0, 8.0, and 16.0 μM Fe(II)−**5**; lanes 10−12, 8.0, 32.0, and 48.0 μM Fe(II) control. Solutions contained 0.25 μg of supercoiled ΦX174 DNA (1.4×10^{-8} M) in 50 mM Tris−HCl (pH 8.0) containing 10 mM 2-mercaptoethanol. The DNA cleavage reactions were run for 1 h at 25 °C, and electrophoresis was conducted at 50V for 3 h on a 1% agarose gel containing 0.1 μg/mL ethidium bromide. Form I = supercoiled DNA, form II = relaxed DNA (single strand cleavage), and form III = linear DNA (double strand cleavage). The gel was immediately visualized on a UV transilluminator and photographed using Polaroid T667 black and white instant film and a Millipore BioImage 60S RFLP system in the presence of ethidium bromide. The results are tabulated in Table 2.

Table 2. Cleavage of Supercoiled ΦX174 RFI DNA by **1**, **2**, and **5**[a]

		concn (μM)		% form		
lane	agent	agent	Fe(II)	I	II	III
1			0	95	5	0
2	bleomycin A_2 (**1**)	0.5	0.5	0	61	39
3	deglycobleomycin A_2	2.0	2.0	0	67	33
4	**2**	8.0	8.0	49	51	0
5	**2**	32.0	32.0	10	84	6
6	**2**	48.0	48.0	0	67	33
7	**5**	4.0	4.0	6	88	6
8	**5**	8.0	8.0	5	88	7
9	**5**	16.0	16.0	0	68	32
10	none		8.0	50	50	0
11	none		32.0	7	71	22
12	none		48.0	0	74	26

[a] The quantitation of forms I−III DNA present at each lane of Figure 2 was conducted by direct fluorescence quantitation of the DNA in the presence of ethidium bromide on a Millipore BioImage 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× that of form I).

***N*$^{\alpha}$-(*tert*-Butyloxycarbonyl)-*N*$^{\beta}$-[1-amino-3(*S*)-[4-amino-6-[[*N*^{im}-(triphenylmethyl)-*erythro*-β-[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]-*N*-L-histidyl methyl ester]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalaninamide (9).** A solution of **8** (1.25 mg, 0.86 μmol) in dry CH_3OH (35 μL) was treated with powdered K_2CO_3 (0.75 mg, 5.43 μmol, 6.3 equiv) at 25 °C under N_2. After stirring for 30 min, HOAc (0.7 μL) was added to the reaction mixture, and the solution was concentrated in vacuo. Chromatography (reverse-phase C-18, 0−70% CH_3OH−H_2O gradient elution) afforded **9** (0.77 mg, 1.03 mg theoretical, 74%) as a foam: R_f 0.2 (SiO_2, 8:2:1 BuOH−HOAc−H_2O); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.50 (1H, d, J = 1.3 Hz), 7.28−7.36 (9H, m), 7.07−7.13 (6H, m), 7.05 (1H, d, J = 1.3 Hz), 5.23−5.27 (3H, m), 4.92−5.00 (1H, m, partially obscured by solvent), 4.76−4.81 (1H, m, partially obscured by solvent), 4.06−4.13 (1H, m), 4.05 (1H, dd, J = 2.0, 3.0 Hz), 4.02 (1H, t, J = 3.4 Hz), 3.85−4.00 (5H, m), 3.64−3.83 (4H, m), 3.70 (3H, s), 3.44−3.52 (1H, m), 2.61−2.88 (3H, m), 2.50 (1H, dd, J = 8.4, 14.8 Hz), 2.27 (3H, s), 1.42 (9H, s); FABHRMS (NBA) *m/e* 1202.4955 (M^+ + H, $C_{56}H_{71}N_{11}O_{19}$ requires 1202.5006).

Table 3. Relative Efficiency of 5′-End-Labeled w794 DNA Cleavage by **5** and Related Agents[a]

lane	agent	concn (μM)	IOD	% DNA cleavage	concn × IOD	relative efficiency
1	bleomycin A_2 (**1**)	0.5	5.8	65	2.9	5.2
2	deglycobleomycin A_2	2.0	7.5	54	15.0	1
3	control DNA		16.4			
4	Fe(III)	128	15.1	8	1933	0.008
5	**5**	64	6.1	63	390	0.038
6	**5**	32	7.6	54	243	0.062

[a] Quantitation of the consumption of the 5′-^{32}P-end-labeled w794 DNA based on the autoradiograph of the sequencing gel shown in Figure 1 with quantitation conducted using a Millipore BioImage 60S RFLP system; IOD = integrated optical density.

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[*erythro*-β-[2-*O*-(3-*O*-carbamoyl-α-D-mannopyranosyl)-α-L-gulopyranosyl]carbonyl]-*N*-L-histidyl methyl ester]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-β-aminoalaninamide (5).** Compound **9** (0.68 mg, 0.57 μmol) was treated with 20% CF_3CO_2H–CH_2Cl_2 (120 μL) at 0 °C under N_2. After stirring for 3 h, the reaction mixture was concentrated under a N_2 stream. Chromatography (reverse-phase C-18, 0–10% CH_3OH–H_2O gradient elution) afforded **5** (0.48 mg, 0.49 mg theoretical, 98%): R_f 0.7 (SiO_2 10:9:1 CH_3OH–10% NH_4OAc–10% NH_4OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.72 (1H, d, J = 0.9 Hz), 7.20 (1H, d, J = 0.9 Hz), 5.46 (1H, d, J = 5.5 Hz), 5.32 (1H, d, J = 5.5 Hz), 5.27 (1H, d, J = 3.6 Hz), 4.93–4.98 (1H, m, partially obscured by solvent), 4.69 (1H, dd, J = 3.6, 9.6 Hz), 4.06–4.13 (3H, m), 4.05 (1H, dd, J = 2.0, 3.2 Hz), 3.88–4.00 (4H, m), 3.68–3.86 (4H, m), 3.72 (3H, s), 3.49–3.54 (1H, m), 2.83–3.10 (3H, m), 2.62 (1H, d, J = 8.8, 11.6 Hz), 2.26 (3H, s); FABHRMS (NBA) *m/e* 859.3300 (M^+ + H, $C_{32}H_{49}N_{11}O_{17}$ requires 859.3311).

General Procedure for the Supercoiled ΦX174 DNA Cleavage Reactions: Relative Efficiency Study. All reactions were run with freshly prepared Fe(II) complexes. The Fe(II) complexes were prepared by combining 1 μL of a H_2O solution of the agent at the 10× specified concentration with 1 μL of a freshly prepared equimiolar aqueous $Fe(NH_4)_2(SO_4)_2$ solution followed by vortex mixing and centrifugation. Each of the 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 8, 16, 32, and 48 μM Fe(II) control, 0.1, 0.2, and 0.5 μM bleomycin A_2 (**1**), 0.5, 1.0, 2.0, and 4.0 μM deglycobleomycin A_2, and 2.0, 4.0, 16.0, and 32.0 μM **5**. The DNA reaction solutions were 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 50 V for 3 h, and the gel was immediately visualized on a UV transilluminator and photographed using Polaroid T667 black and white instant film (Figure 2). Direct fluorescence quantitation (Table 2) 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× that of form I).

General Procedure for Quantitation of Double Strand and Single Strand Supercoiled ΦX174 DNA Cleavage. The Fe(II) complexes were formed by mixing 1 μL of an aqueous 320 μM solution of **5** with 1 μL of a freshly prepared 320 μM aqueous $Fe(NH_4)_2(SO_4)_2$ solution. A 7 μL sample 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 Fe(II) complex solutions. The final concentration of **5** employed in the study was 32 μM. The DNA cleavage reactions were initiated 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 60, 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 3 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 flluorescence intensities of forms I–III ΦX174 DNA (forms II and III fluorescence intensities are 0.7× that of form I). The ratio of double to single strand DNA cleavage was calculated with use of the Freifelder–Trumbo equation,[52] assuming a Poisson distribution.

General Procedure for Cleavage of 5′-End-Labeled w794 and w836 DNA: Relative Efficiency and Selectivity. All reactions were run with freshly prepared Fe(III) complexes. The Fe(III) complexes were prepared by combining 1 μL of a H_2O solution of the agent at 10× the specified concentration with 1 μL of a freshly prepared equimolar aqueous $FeCl_3$ solution. Each of the 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[54] in a 10 mM phosphate buffer solution (Na_2HPO_4–NaH_2PO_4, pH 7.0) containing 10 mM KCl. The final concentrations of the agents employed in the study were 32, 64, and 128 μM control Fe(III), 0.5 μM bleomycin A_2 (**1**), 2.0 μM deglycobleomycin A_2, and 32 and 64 μM **5**. 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 30 min. The reactions were quenched with the addition of 2 μL of 50% aqueous glycerol followed by EtOH precipitation and isolation of the DNA. The DNA was resuspended in 6 μL of TE buffer (pH 8.0), and formamide dye (6 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 (3 μL) 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) at 1100 V for 5.5 h. 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 the dried gel was carried out at −78 °C using Kodak X-Omat AR film and a Picker spectrum-intensifying screen. Quantitation of the DNA cleavage reaction was conducted on a Millipore Bio Image 60S RFLP system measuring the remaining uncleaved DNA, and the values recorded in Table 1 are the average of three (**5**, **2**) or six experiments (**1**, deglycobleomycin A_2, and Fe(III)). The quantitated values for Figure 1 are tabulated in Table 3.

Acknowledgment. We gratefully acknowledge the financial support of the National Institutes of Health (D.L.B., Grant CA42056) and the sabbatical leave of S.T. sponsored by Otsuka Pharmaceutical Co., Ltd.

JA950155K