Definition of the Effect and Role of the Bleomycin A_2 Valerate Substituents: Preorganization of a Rigid, Compact Conformation Implicated in Sequence-Selective DNA Cleavage

Dale L. Boger,*,† Timothy M. Ramsey,† Hui Cai,† Silvia T. Hoehn,‡ and JoAnne Stubbe‡

Contribution from the Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, and Departments of Chemistry and Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139

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Abstract: The synthesis and a comparative study of deglycobleomycin A_2 analogues containing key modifications in the valerate subunit are described in efforts that define the role of the linker length and its substituents. In addition to demonstrating that the C3-hydroxy group exhibits only a modest effect (1−2×) that may be attributed to an intermolecular H-bond with DNA and that the length of the linker is important (C4 > C5 > C3 > C2), the studies illustrate that the substantial impact of the C4-methyl group is linked to the presence of the C2-methyl group, while the modest impact of the C2-methyl group itself (ca. 2×) is not coupled to the presence of the C4-methyl group. These effects may be explained by the conformational properties of the agents resulting from the substitutions and beautifully fit the models of DNA-bound Co(III)-OOH bleomycin A_2 and deglycobleomycin A_2. The magnitude of the effects suggests that an important functional role of the linker region is to facilitate adoption of a rigid, compact conformation productive for DNA cleavage. The studies also identify a second potential site that may adopt two nearly equivalent conformations which may constitute a swivel point that permits access to a class of related bound structures adaptable to the conformational characteristics of the multiple cleavage sites or access to both strands of DNA from a common intercalation site important for both primary (single strand) and secondary (double strand) cleavage.

Bleomycin A_2 (**1**, Figure 1) is the major constituent of the clinical antitumor drug Blenoxane and is thought to derive its therapeutic effects from the ability to mediate the cleavage of double-stranded DNA or RNA by a metal ion- and oxygen-dependent process.[1−8] Studies employing derivatives of the natural product, its degradation products, or semisynthetic analogues, as well as its analogues, have contributed to an understanding of the structural features contributing to the sequence-selective cleavage of DNA.[9] In our efforts, this has entailed single changes in the structure of bleomycin A_2 or deglycobleomycin A_2 (**2**), conducted with the intent of defining the role of each subunit, functional group, or substituent.[9] These studies, carried out in conjunction with recent structural studies,[10−13] have begun to unravel the subtle structural features contributing to the properties of the natural product.

Despite these studies, the role of the linker region joining the metal binding domain with the bithiazole DNA binding domain and its substituents is poorly defined. In conjunction with the systematic examination of the L-threonine subunit detailed in the accompanying article,[9] herein we describe a detailed study of the valerate linker region. Early studies with a select set of bleomycin A_2 analogues disclosed by Umezawa, Ohno, and co-workers illustrated the importance of the presence and absolute stereochemistry of the C4-methyl group in the substituted pentanoic acid (valerate) subunit in the DNA

† The Scripps Research Institute.

‡ Massachusetts Institute of Technology.

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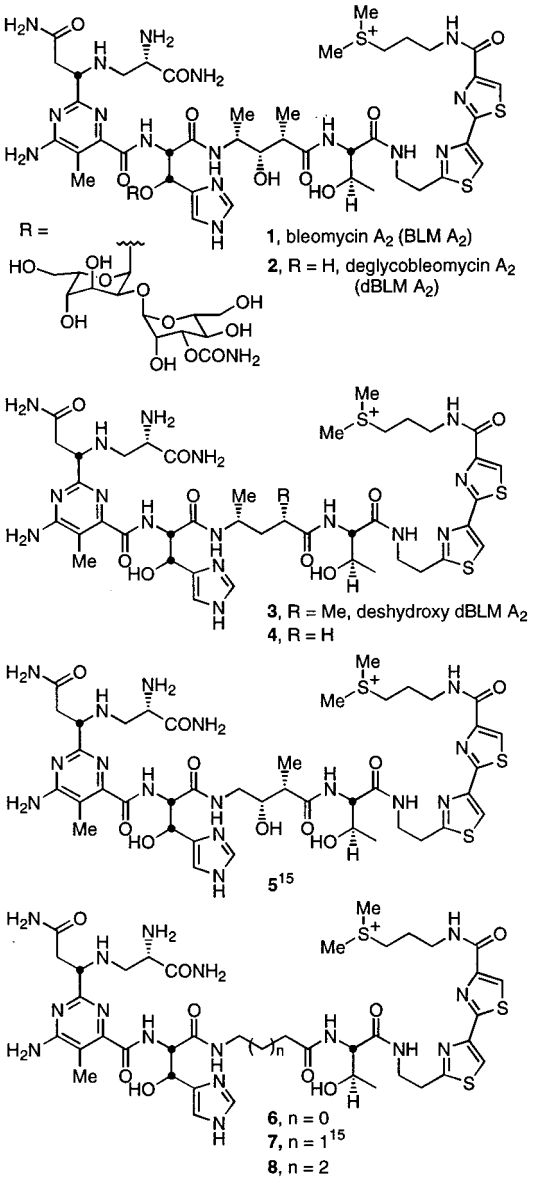

Figure 1.

cleavage efficiency of the resulting analogues.[3] Similarly, Hecht and co-workers have shown that substitution of $(gly)_n$, $n = 0-4$, for the L-threonine subunit caused no significant change in the characteristic 5′-GC, 5′-GT cleavage selectivity of bleomycin A_2 although the cleavage occurred with altered efficiencies.[14] More recently, we disclosed the examination of a number of linker modifications of deglycobleomycin A_2 (**2**) that, while not altering the cleavage selectivity of **2**, revealed an unusually large impact of its length and substituents on the cleavage efficiency.[15] Notably, replacement of the L-threonine subunit with glycine substantially reduced the cleavage efficiency and the double strand/single strand (ds/ss) cleavage ratio, and the studies in the accompanying paper further clarify the role of this subunit's substituents.[9] Additionally, the studies revealed an important role (ca. 7×) for the C4-methyl substituent of the valerate subunit and less prominent roles for the C3-hydroxy group (1−2×) and C2-methyl substituent (1−2×). These effects were cumulative, and removal of all of the valerate and threonine substituents led to a >100-fold reduction in DNA cleavage efficiency. In these studies and prior to the recent structural studies, we suggested this may be due to their effect on the adoption of a compact, bent bound conformation productive for DNA cleavage[15] and consistent with the 3.5 base-pair binding site size of **1** and **2**[17] although disruption of the bleomycin A_2 interaction with duplex DNA or altered activation kinetics could also account for the diminished properties.

Herein, we detail the synthesis and examination of **3**−**8** that, in conjunction with recent structural studies, clearly define the effects of the valerate subunit and the impact of its substituents. Analogue **3** permitted the assessment of the functional importance of the C3-hydroxy group, not directly addressed in our earlier studies. The examination of **3** and **4** relative to our prior analogues **5**[15] and **7**[15] was anticipated to further clarify the role of the C2- and C4-methyl substituents. Similarly, the direct comparison of **6** and **8** with **7**[15] permitted an assessment of the length of the valerate linker. The results of the studies were more revealing than anticipated. In addition to demonstrating that the C3-hydroxy group exhibits only a modest effect (1−2×) and that the length of the linker is important (C4 > C5 > C3), the studies illustrate that the significant impact of the C4-methyl substituent is directly coupled to the presence of the C2-methyl substituent, while the more modest impact of the C2-methyl group itself is not coupled to the presence of the C4-methyl group. These results beautifully fit the expected effects of the substitutions on the conformational properties of the analogues and agree remarkably well with the models of DNA-bound Co(III)OOH bleomycin A_2[10] and deglycobleomycin A_2.[11] This suggests that the linker substituent effects can be ascribed principally to their role in facilitating the adoption of a rigid, compact DNA-bound conformation that leads to productive sequence-selective strand cleavage. Moreover, the magnitude of the effects suggests that the functional role of the linker region extends well beyond that of simply connecting the bithiazole DNA binding domain to the pyrimidoblamic acid metal chelation subunit. Rather, its length and substituents serve to facilitate preorganization of the metal complexes into a compact conformation productive for DNA cleavage. The studies also identify a potential site that may adopt two nearly equivalent turn conformations. This site may constitute a swivel point in the structure that permits access to a class of related bound conformations adaptable to multiple cleavage sites or perhaps access to both strands of duplex DNA from a single intercalation site important for both primary single strand (ss) and secondary double strand (ds) DNA cleavage.

Synthesis. The analogues were prepared employing a recent modification[16] of our original synthesis of **2**.[17−20] The coupling of the pentapeptide S analogues **15a**−**e** with N^{α}-BOC-pyrimidoblamic acid avoids the occasional dehydration reaction of the β-hydroxy-L-histidine subunit that accompanies the coupling of tetrapeptide S with the prelinked pyrimidoblamic acid/*erythro*-β-hydroxy-L-histidine. It is accomplished with the sulfonium salt installed, on substrates incorporating only one protecting group, without deliberate protection of the imidazole, and without competitive imidazole acylation. Thus, coupling of the

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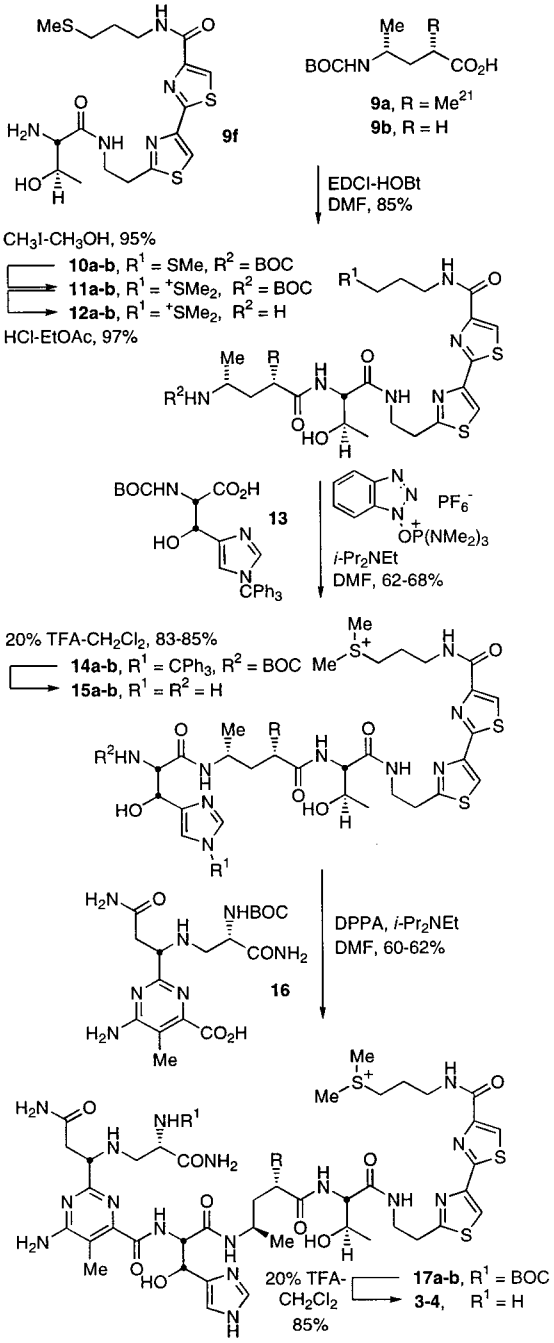
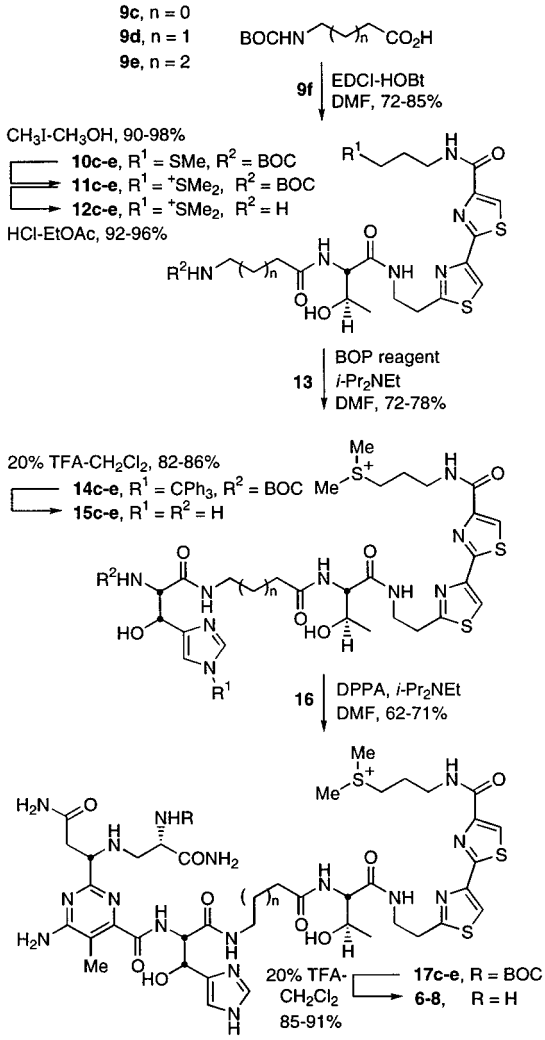
(20) Boger, D. L.; Honda, T. *J. Am. Chem. Soc.* **1994**, *116*, 5647.

Scheme 1



valerate replacement subunits **9a**[21] and **9b** with the tripeptide S precursor **9f**[17] (1.2 equiv of EDCI,[22] 1 equiv of HOBt, DMF, 23 °C, 24 h, 85%) followed by *S*-methylation (50 equiv of CH_3I, CH_3OH, 23 °C, 24−26 h, 95%) and acid-catalyzed *N*-BOC deprotection (3 N HCl-EtOAc, 23 °C, 1.5 h, 97%) provided the tetrapeptide S analogues **12a**−**b** (Scheme 1). Subsequent coupling of **12a**−**b** with *N*-BOC-*erythro*-β-hydroxy-L-histidine[17] (**13**, 1.4 equiv of BOP reagent,[22] 2 equiv of *i*-Pr_2NEt, DMF, 23 °C, 8 h, 62−68%) and acid-catalyzed *N*-BOC deprotection (20% TFA−CH_2Cl_2, 0 °C, 4 h, 83−85%) cleanly afforded the pentapeptide S analogues **15a**−**b**. Diphenylphosphoryl azide (DPPA) promoted coupling of **15a**−**b** with N^{α}-BOC-pyrimidoblamic acid (**16**,[18] 1.4 equiv of DPPA, 2 equiv of *i*-Pr_2NEt, DMF, 0 °C, 10 h, 60−62%) and acid-catalyzed *N*-BOC deprotection of **17a**−**b** (20% TFA−CH_2Cl_2, 0 °C, 2.5 h, 85%) provided **3** and **4**.

Scheme 2



Similarly, the unsubstituted *N*-BOC amino acids **9c**−**9e** were converted to the deglycobleomycin A_2 analogues **6**−**8** incorporating 3−5 carbon linkers in place of the valerate subunit (Scheme 2).

In preliminary efforts, the alternative approach of coupling **18**[19,23] with the tetrapeptide S analogues **12** was explored (Scheme 3). This coupling generally proved less effective regardless of the reagents employed and was often accompanied by competitive elimination of water from the β-hydroxy-L-histidine subunit. This presumably results from the enhanced acidity of the C^{α} proton when the L-histidine amine is N-acylated as an amide (i.e., **18**) rather than a carbamate (i.e., **13**), and the approach outlined in Schemes 1−2 typically proved superior.

DNA Cleavage Properties. The assessment of the DNA cleavage properties of **3**−**8** were conducted using four different assays. The initial examination was conducted with the Fe(II) complexes of **3**−**8** in the presence of O_2 and 2-mercaptoethanol as an appropriate reducing agent. The assessment was made through the determination of the relative concentrations of the Fe(II) complex required to produce comparable ss and ds cleavage of supercoiled ΦX174 DNA (Form I) to generate relaxed (Form II) and linear (Form III) DNA, respectively

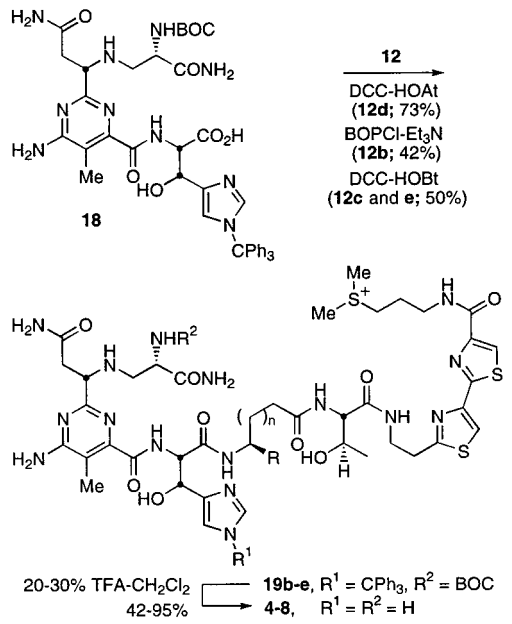
(21) Koskinen, A. M. P.; Pihko, P. M. *Tetrahedron Lett.* **1994**, *35*, 7417.
(22) EDCI = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; BOP reagent = benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate.; DCC = 1,3-dicyclohexylcarbodiimide.
(23) Boger, D. L.; Teramoto, S.; Honda, T.; Zhou, J. *J. Am. Chem. Soc.* **1995**, *117*, 7338.

Table 1. Summary of ΦX174 and w794 DNA Cleavage Properties

agent	relative efficiency of DNA cleavage ΦX174[a]	relative efficiency of DNA cleavage w794[b]	ratio of double to single strand cleavage[c]	DNA cleavage selectivity[b]
1, bleomycin A_2	2−5	5.8	1:6	5′-GC, 5′-GT > 5′-GA
2, deglycobleomycin A_2	1.0	1.0	1:12	5′-GC, 5′-GT > 5′-GA
3, deshydroxy dBLM A_2	0.8	0.40	1:14	5′-GC, 5′-GT > 5′-GA
4, C4-Me dBLM A_2	0.20	0.20	1:25	5′-GC, 5′-GT > 5′-GA
5, C4-desmethyl dBLM A_2	0.40	0.20	1:15	5′-GC, 5′-GT > 5′-GA
6, C3 dBLM A_2	0.08	0.10	1:43	very weak 5′-GC, 5′-GT > 5′-GA
7, C4 dBLM A_2	0.13	0.13	1:31	5′-GC, 5′-GT > 5′-GA
8, C5 dBLM A_2	0.08	0.13	1:38	weak 5′-GC, 5′-GT > 5′-GA
Fe[a,b]	0.04	0.03	1:98	none

[a] Relative efficiency of supercoiled ΦX174 DNA cleavage, Fe(II)−O_2, 2-mercaptoethanol. The results are the average of eight experiments. [b] Examined within [^{32}P]-5′-end-labeled w794, Fe(III)−H_2O_2. The results are the average of 10 experiments. [c] Ratio of double- to single-stranded cleavage of supercoiled ΦX174 DNA calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$.

Scheme 3



(Figure 2). Like Fe(II)−bleomycin A_2 and deglycobleomycin A_2, the Fe(II) complexes of **3**−**8** produced both ss and ds DNA cleavage, and the results are summarized in Table 1. Removal of the C3-hydroxyl group gave **3** which was only slightly less effective (0.8×) than deglycobleomycin A_2 (**2**) at cleaving the supercoiled DNA. The observation that **5** which lacks the C4-methyl group of **2** was found to be substantially less effective than deglycobleomycin A_2 (0.4−0.5×) proved analogous to observations in our prior studies.[15] Removal of C2-methyl and C3-hydroxyl groups with **4** led to a more substantial 5-fold reduction in the DNA cleavage efficiency, while further removal of the remaining C4-methyl substituent of **4** to give **7** resulted in an even greater impact on the cleavage efficiency (0.13×). Shortening and extending the linker length by one carbon gave agents **6** and **8** which were less effective than **7**, and each proved to be only 2−3× more efficient than Fe(II) cleavage itself.

Although both ss and ds DNA lesions result from the oxidative cleavage of DNA by bleomycin A_2, the latter have been considered to be the more significant biological event. For **3**−**8**, the extent of ds to ss DNA cleavage was established in a study of the kinetics of supercoiled ΦX174 DNA cleavage to produce linear and circular DNA, indicating that **2** and its analogues do not promote ds cleavage to the same extent as bleomycin A_2. We assumed a Poisson distribution for the formation of ss and ds breaks to calculate the average number of double and single strand cleavages per DNA molecule using the Freifelder−Trumbo equation.[24] The results are illustrated in Figure 3 for **7** and are summarized for the full set of agents in Table 1. Consistent with the relative efficiencies of DNA cleavage, the ratio of ds to ss cleavage for **3** and **5** were 1:14 and 1:15, being slightly lower but nearly indistinguishable from that of deglycobleomycin A_2 (1:12). The extent of ds cleavage was lower for **4** (1:25), and that of **6**−**8** was considerably lower (1:43, 1:31, and 1:38, respectively).[25]

The sequence selectivity of DNA cleavage was examined with duplex w794 DNA[26] by monitoring strand cleavage of singly [^{32}P]-5′-end-labeled duplex DNA after exposure to the Fe(III) complexes of the agents following activation with H_2O_2[19,23,27] in 1 mM phosphate buffer (pH 7.0).[26] This assay is more

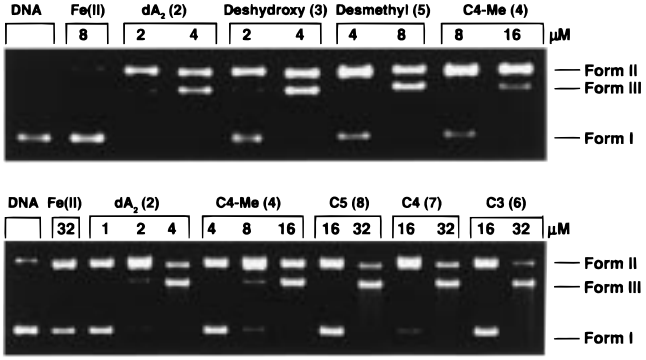


Figure 2. Agarose gel illustrating the cleavage reactions of supercoiled ΦX174 DNA by Fe(II)-agents at 25 °C for 1 h in buffer solutions containing 2-mercaptoethanol. After electrophoresis on a 1% agarose gel, the gel was stained with 0.1 μg/mL ethidium bromide and visualized on a UV transilluminator and quantified on a Millipore BioImage 60S RFLP system. The results are tabulated in Table 1.

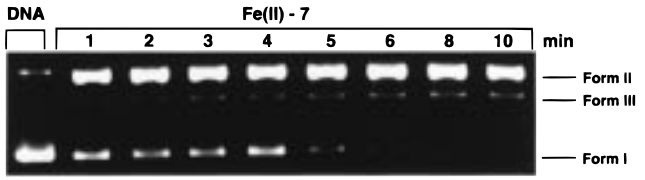


Figure 3. Representative kinetics of supercoiled ΦX174 DNA cleavage by Fe(II)−**7** (16 μM) in buffer solution containing 2-mercaptoethanol. The DNA cleavage reactions were run at 25 °C for various lengths of time, and electrophoresis was conducted on a 1% agarose gel. Direct fluorescence quantitation of the percentage of forms I−III DNA present at each time point was conducted using a Millipore BioImage 60S RFLP system visualized on a UV (312 nm) transilluminator in the presence of 0.1 μg/mL ethidium bromide, taking into account the relative fluorescence intensities of forms I−III ΦX174 DNA (forms II and III have fluorescence intensities that are 0.7 times that of form I).

(24) Freifelder, D.; Trumbo, B. *Biopolymers* **1969**, *7*, 681.

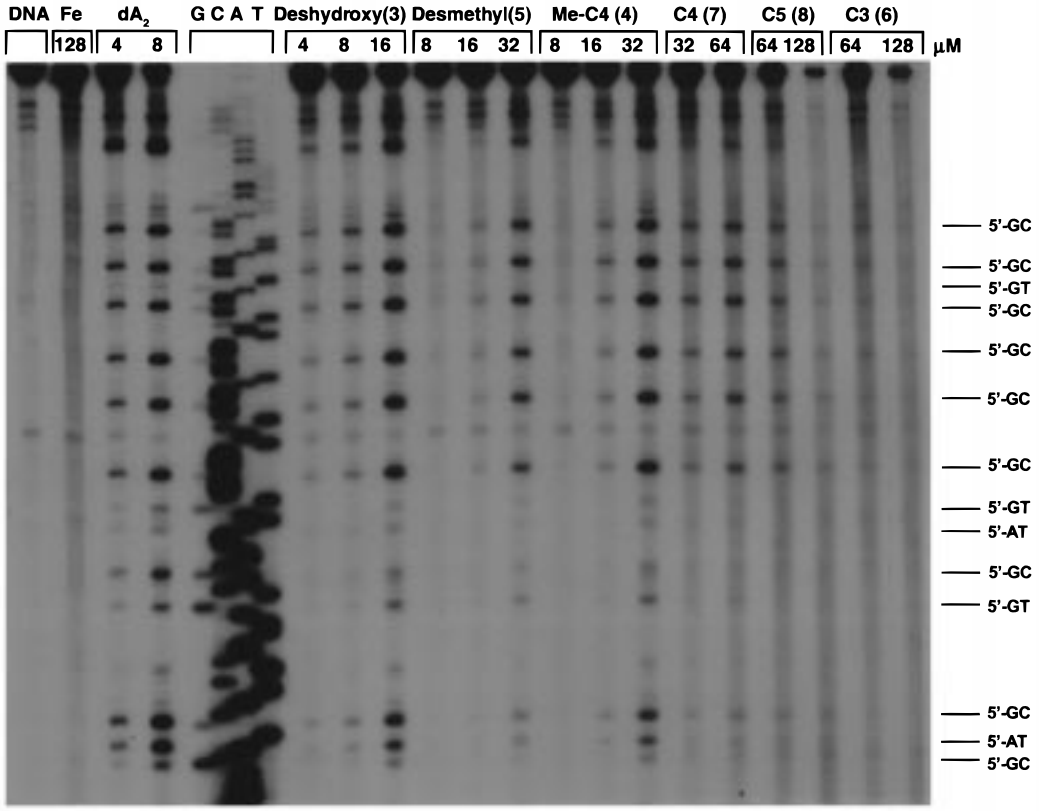


Figure 4. Cleavage of double-stranded DNA by Fe(III)−agent (SV40 DNA fragment, 144 base pairs, nucleotide no. 5238-138, clone w794) in phosphate/KCl buffer containing H_2O_2. The DNA cleavage reactions were run for 90 min at 4 °C, and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.

sensitive to differences in the cleavage efficiency than the supercoiled DNA cleavage assay and dependent upon the reaction conditions and amount of radiolabeled DNA employed.[25b] Thus, while the magnitude of the efficiency differences may vary, the relative trends in efficiency have not been observed to be altered (Table 1). An extensive range of conditions were examined, including variations in reaction temperature, time, and amount of DNA employed. Under all conditions, **3** was found to be nearly half as effective as deglycobleomycin A_2 and to cleave DNA in the sequence-selective fashion characteristic of **1** and **2** (Figure 4). In a manner similar to that seen with ΦX174 DNA, **4** and **5** showed more significant reductions (4−5-fold), and **6**−**8** exhibited even greater reductions (8−10-fold, **7** > **8** > **6**) in the DNA cleavage efficiency. Notably, the side-by-side comparison of **5** illustrated once again that the removal of the C4-methyl group from the valerate linker has a very substantial impact on the cleavage efficiency (0.2×), analogous to our prior observation (0.15×).[15] The distinctions between **4** and **5** observed in the ΦX174 DNA cleavage assay (**5** > **4**, 2×) essentially disappeared in the w794 assay, and the two analogues were nearly indistinguishable (**5** ≥ **4**). The significance of these subtle distinctions is not yet known but cannot be attributed to the samples themselves since both assays were conducted concurrently with the same sample stock solutions. With the exception of **6**, all displayed the characteristic DNA cleavage selectivity of bleomycin A_2. However, the clarity of the sequence-selective cleavage diminished as the efficiency of DNA cleavage decreased. The nonselective cleavage of DNA competes more effectively with selective cleavage with the less effective agents **6**−**8**, and the ability to detect the selective cleavage nearly disappears with **6**. Thus, in the series of C3−C5 linkers (**6**−**8**), not only was **7** (C4) more efficient than **6** and **8** (**7** > **8** > **6**, C4 > C5 > C3) but the DNA cleavage selectivity was more pronounced as well.

The cleavage properties of **3**−**8** were also investigated using an internally labeled hairpin oligonucleotide assay as described previously that is useful for quantitating ds cleavage.[28] The results of this investigation are presented in Table 2. The cleavage efficiency values are somewhat lower than those detected in the other assays,[25b] but qualitatively they suggest the same trends. These results indicate that removal of the valerate substituents at the α, β, and γ positions significantly diminishes the cleavage efficiency. However, the trends in terms of the general importance of these positions are analogous. The trends for the length of the linker are also very similar, but no sequence specificity above background was observed for longer or shorter linker analogues. The ds cleavage efficiency

(25) (a) A theoretical ratio of approximately 1:100 is required 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. (b) The larger increases in the magnitude of differences observed with the hairpin assay and, to a lesser extent, with the w794 DNA versus φX174 DNA can be attributed in part to a corresponding amount of unlabeled carrier DNA which competes for the consumption of the agent and its oxidizing capability. Additionally, the hairpin assay is run under single turnover conditions which may affect the magnitude of the differences. For the discussion of the impact of a substituent, the φX174 DNA cleavage results which entail no added carrier DNA are used for the comparisons.

(26) Ambrose, C.; Rajadhyaksha, A.; Lowman, H.; Bina, M. *J. Mol. Biol.* **1989**, *209*, 255. Boger, D. L.; Munk, S. A.; Zarrinmayeh, H.; Ishizaki, T.; Haught, J.; Bina, M. *Tetrahedron* **1991**, *47*, 2661.

(27) Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 3997. Natrajan, A.; Hecht, S. M.; van der Marel, G. A.; van Boom, J. H. *J. Am. Chem. Soc.* **1990**, *112*, 4532.

(28) Absalon, M. J.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1995**, *34*, 2065.

Table 2. Hairpin DNA Cleavage Efficiency

agent	μM[a]	cleavage efficiency[b]	cleavage specificity
2	5	1.0	5′-GT, GC
3	50	0.1	5′-GT, GC
4	235	<0.02	5′-GT, GC
5	100	<0.05	5′-GT, GC
6	187	<0.03	nd
7	100	<0.05	5′-GT, GC
8	200	<0.02	nd

[a] For assay, see refs 9 and 28. Agent concentration needed to achieve a comparable amount of total DNA cleavage as deglycobleomycin A_2 (**2**, 5 μM). [b] Total DNA cleavage relative to deglycobleomycin A_2, nd = none detected.

was not determined for each of the analogues using this assay since the ss cleavage efficiency was so low. Only analogue **3** showed a significant amount of an oligonucleotide fragment of appropriate size to be associated with ds cleavage (data not shown). However, this analogue shows the fragment only at a high concentration which suggests that this band may also be caused by two separate instances of ss cleavage since the activation conditions for the analogue allow multiple turnovers.

Length of the Valerate Linker and Cumulative Substituent Effect. Whereas removing the valerate substituents showed no impact on the DNA cleavage selectivity, changing the chain length diminished or abolished sequence specificity, depending on the analogue and assay used. All modifications substantially altered the cleavage efficiency and the ratio of ds to ss DNA cleavage. The length of the linker followed a well-defined relationship of C4 > C5 > C3 where the length of the natural linker was established to be optimal. When these results are combined with our prior observation of the further diminished cleavage efficiency of the analogue containing a D-Ala substitution (C2) for the valerate subunit,[15] the trend is extended (C4 > C5 > C3 > C2), indicating that linkers longer than four carbons exhibit a diminished DNA cleavage efficiency and those shorter than four carbons are progressively and more seriously impacted. This would be consistent with the adoption of a well-defined compact conformation productive for DNA cleavage where the agents possessing a chain shorter than four carbons may have progressively more difficulty in adopting effective conformations spanning the linker region. In turn, those longer than four carbons possess greater flexibility and less of an opportunity to adopt the productive bound conformation required for selective cleavage. Moreover, the substantially reduced cleavage efficiency and ds/ss cleavage ratio of even C4 (**7**, ca. 0.1×) established that the valerate substituents contribute substantially to properties of **1**−**2** and, as detailed below, may do so by facilitating the adoption of a compact bound conformation implicated in recent structural studies.

Conformational Effects of the Valerate Substituents. The valerate subunit of both free and DNA-bound Co(III)OOH bleomycin A_2 and deglycobleomycin A_2 adopts a well-defined, rigid conformation (Figure 5). Important characteristics of this conformation are two turns, one at the C2 center and one at the C4 center. At both sites, the methyl substituents adopt the extended conformation position, inducing turns in the linker and producing a compact conformation. Diagnostic of this conformation is the small coupling constant for C2−H/C3−H ($J \cong 1.8 \pm 1.2$ Hz) and the large coupling constant for C3−H/C4−H ($J = 9.5 \pm 1.2$ Hz).[10]

As a consequence of the C2 substitution, the subunit is rigidly held in a compact conformation **A** but could adopt two equivalent conformations consisting of a compact carbonyl/ C2-methyl eclipsed conformation **D** or an extended conformation

Figure 5. DNA-bound conformation of Co(III)OOH BLM A_2 taken from ref 10, highlighting the compact, rigid conformation of the valerate linker region enlisting two turns, one at C2 and one at C4, and a C3−OH H-bond to the phosphate spanning the intercalation site. At the C2 and C4 positions, the valerate methyl substituents adopt the extended conformation, inducing the two turns.

E (Figure 6).[29] These differ only in the ability of the extended conformation to benefit from an intramolecular H-bond from the C3-hydroxyl group to the valerate carbonyl. Despite this potential, both free and bound Co(III)OOH bleomycin A_2 adopt the compact carbonyl/C2-methyl eclipsed conformation **D**, presumably compensated for by the adoption of H-bonds from the valerate NH (1.8 Å, 155°) and the threonine NH (1.9 Å, 152°) to the metal-complexed hydroperoxide.[10] However, it is this site within the valerate linker that could adopt two near equivalently accessible conformations. Since there is more than one energetically favorable conformation, it is possible that this site constitutes a swivel point within the molecule which complements one found in the threonine subunit.[9] This swivel point would allow access to several related bound conformations that could meet the conformational requirements of multiple cleavage sites as well as those of ds cleavage from a common intercalation site. Alternative conformations about the C3 (≥2.65−3.35 kcal, not shown) or C4 centers (Figure 6 top, ≥2.4−2.65 kcal) are much less stable and constitute noncontributing conformations.[29]

An important ramification of this rigid conformation of the valerate linker is the defined placement and orientation of the amide bond linking the valerate and β-hydroxy-L-histidine subunits. This is due to the presence of the C4-methyl

(29) The following *A*-values of 0.9 kcal/mol (OH), 1.8 kcal/mol (CH_3), and 1.6 kcal/mol (NHCOPh for NHCOR) were employed. Values of $^1/_2$ the *A*-value were employed to represent a gauche interaction with a methyl group: OH/CH_3 (0.45 kcal/mol), CH_3/CH_3 (0.9 kcal/mol), and NHCOR/CH_3 (0.8 kcal/mol). The latter was also used to approximate NHCOR/CH_2R and NHCOR/CHR_2 gauche interactions. A value of 0.4 kcal/mol was used to approximate an OH/NHCOR gauche interaction (versus 0.45 kcal/mol for OH/CH_3) and should prove sufficiently accurate for the purposes used herein. It was approximated by using a value $^1/_4$ of the *A*-value of NHCOPh (0.4 versus 1.6 kcal/mol) in analogy to that of CH_3 (0.45 versus 1.8 kcal/mol). The 1,3-diaxial NHCOR/CH_3 was estimated to be >3.5 kcal/mol, slightly smaller than a 1,3-diaxial CH_3/CH_3 interaction of 3.7 kcal/mol. It was approximated by using a value that was 0.1 kcal/mol higher than 2× the *A*-value of the substituents (1.6 + 1.8 kcal/mol) in analogy to that of a CH_3/CH_3 1,3-diaxial interaction (2× 1.8 + 0.1 = 3.7 kcal/mol). 1,2-Eclipsing interactions of H/CO and CH_3/CO were approximated, enlisting the corresponding H/CH_3 and CH_3/CH_3 values of 1.3 and 3.7 kcal/mol, respectively.

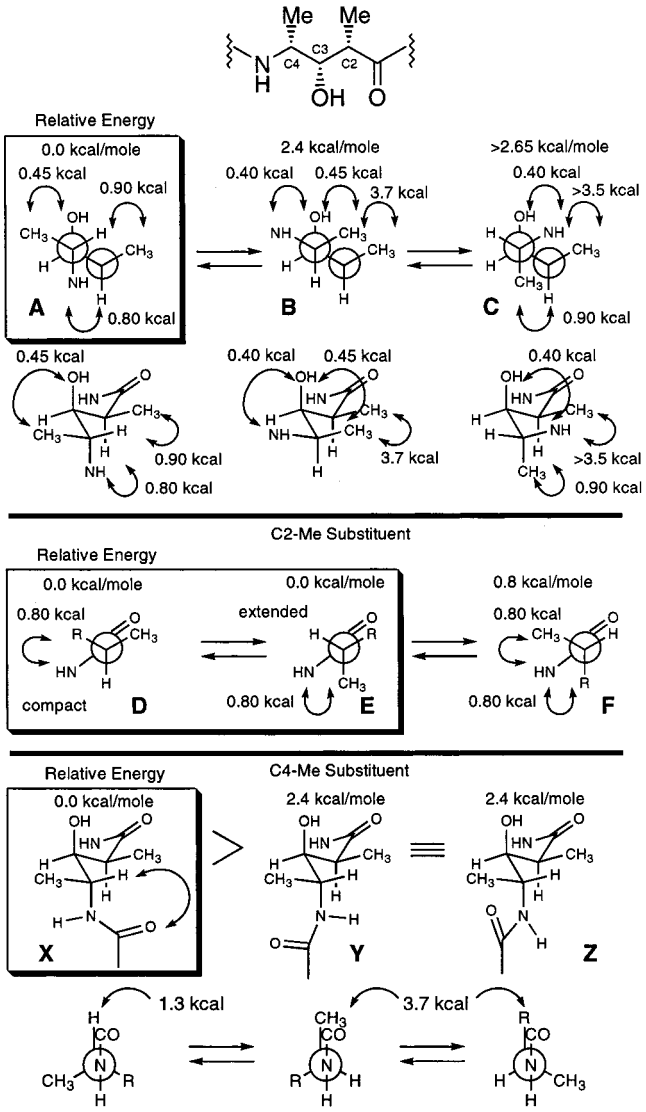


Figure 6. Conformational analysis of the natural valerate subunit.

substituent which, when combined with the presence of the C2-methyl group, must adopt the extended orientation **A** on the valerate chain, inducing a second turn in the valerate linker (Figure 6, $\geq$2.4−2.65 kcal). In the absence of the C4-methyl group, the C4 amide would preferentially adopt the extended **B** versus turn conformation **A**. In the absence of the C2-methyl group, either the C4-methyl group or the C4-NHCO could equivalently adopt the extended orientation. Just as important as this placement of the amide, the preferential adoption of the H-eclipsed carbonyl conformation **X** (ΔE ca. 2.4 kcal, Figure 6 bottom) sets a defined orientation for the β-hydroxy-L-histidine subunit and the entire metal binding domain. In total, this provides the core rigid structure about which the DNA binding domain (C-terminus) and the metal chelation domain (N-terminus) are linked.

Intuitively, the most unexpected effect of the substituents is the modest contribution of the C3-hydroxy group.[10] Its removal to provide **3** afforded an agent that exhibited a modest reduction in the DNA cleavage efficiency (1−0.5×), and the change had almost no impact on the ds/ss cleavage ratio. Consistent with this, the removal of the C3-hydroxy group does not directly impact the conformational properties of the valerate subunit. Its removal has little effect on the conformation about the C3 center (ΔE alternatives = $\geq$2.25−2.45 kcal) or the C4 center (Figure 7, $\geq$2.0−2.7 kcal).

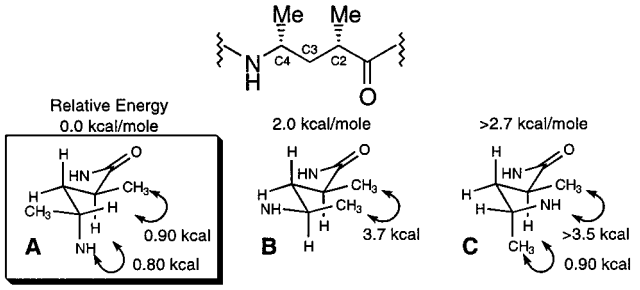


Figure 7. Conformational analysis of **3** and impact of the C3-hydroxy group.

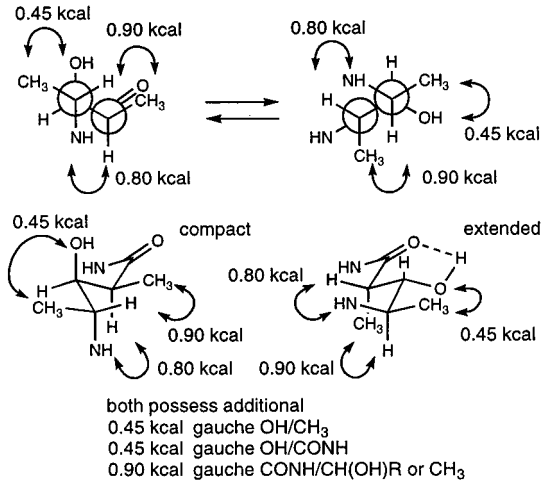


Figure 8.

The presence of the C3-hydroxy group could preferentially stabilize a nonproductive extended versus compact conformation through a H-bond to the valerate carbonyl (Figure 8). Its disruption, observed in free and bound Co(III)OOH bleomcyin A_2, through the use of a compensating threonine NH H-bond to the metal-bound hydroperoxide frees up this hydroxyl group which is enlisted to engage in a H-bond with a DNA backbone phosphate spanning the intercalation site (see Figure 5). This intermolecular H-bond with DNA may contribute to the affinity and alignment of the DNA-bound agent as well as compensate for the lost H-bond resulting from adoption of the compact versus extended conformation. It is this lost intermolecular H-bond with the nonbridging oxygen of the phosphate backbone that may account for the modest decrease in the cleavage efficiency with **3** (0.8×).

As detailed in our earlier study,[15] removal of the C4-methyl group resulted in a larger reduction in the cleavage efficiency of the resulting agent **5**. This impact may be attributed to its conformational effects on the valerate subunit. Its removal allows the agent to adopt two nearly equivalent conformations about the C4 center including both the productive turn conformation **A** (ΔE = 0.4 kcal) and that where the amide adopts the now more stable extended conformation **B** (Figure 9). It additionally lowers the barrier to adopting alternative C3 conformations (ΔE = $\geq$0.45−3.9 kcal, not shown). Even upon adoption of the productive compact conformation with the correct positioning of the β-hydroxy-L-histidine linking amide, it may now adopt either of two orientations (**X** and **Y**) with equivalent energies (Figure 9, bottom). This further increases the number of accessible, potentially nonproductive conformations available to the agent, contributing significantly to the reduced efficiency of DNA cleavage by **5** and constitutes part of the important role attributed to the C4-methyl substituent.

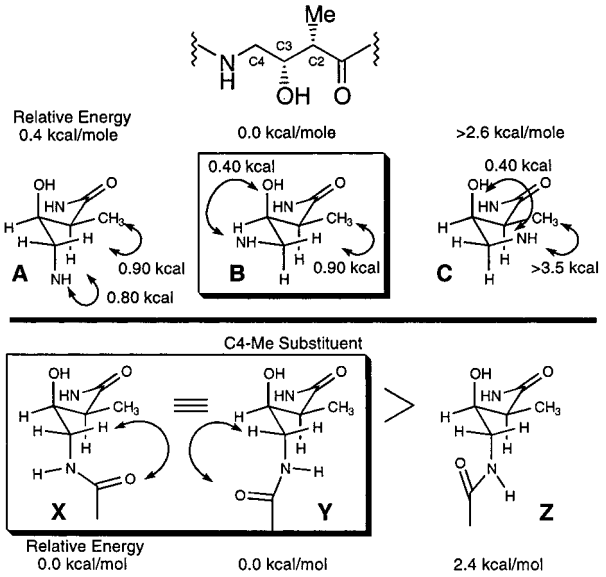


Figure 9. Conformational analysis of **5** and the impact of the C4-methyl substituent.

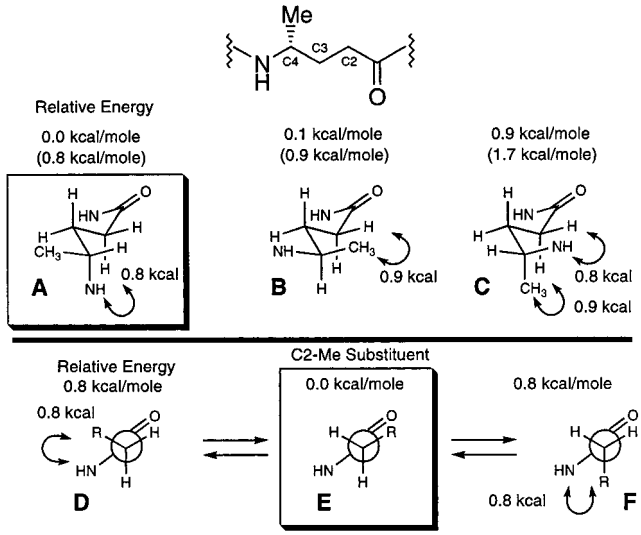


Figure 10. Conformational analysis of **4**.

Despite the substantial impact of removing the C4-methyl group and the small impact of removing the C3-hydroxy group, its presence alone with the agent **4** lacking both the C2-methyl and the C3-hydroxy group resulted in an even larger reduction in the cleavage efficiency. The properties of this agent, which constitutes the further removal of the C2-methyl group from **3**, indicates that a large portion of the impact of the C4-methyl group is intimately linked to the presence of one or both of the additional substituents and suggests that its effect is principally related to the presence of the C2-methyl group (Figure 10). Agent **4** now preferentially adopts the C2 extended R eclipsed conformation (Figure 10, conformation **E**) rather than the productive compact conformation ($\Delta E = 0.8$ kcal), conformation **D**. This agent may also adopt two C4 conformations, constituting either the productive turn (**A**) or the extended amide conformation **B** ($\Delta E = 0.1$ kcal). The gauche/gauche conformation **C** ($\Delta E = 0.9$ kcal) and alternative C3 conformations ($\Delta E = 1.4-2.7$ kcal, not shown) are also accessible. Nonetheless, upon adoption of the productive valerate conformation, the C4-methyl group does ensure the correct orientation of the β-hydroxy-L-histidine linking amide. The net effect of the removal of the C2-methyl group and the C3-hydroxy group is the potential for adopting a greater ensemble of accessible conformations of which those derived from the extended C2 conformation **E** would be preferred over the productive conformation **D**.

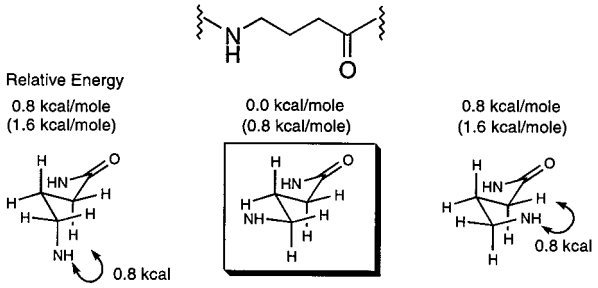


Figure 11. Conformational analysis of **7**.

Table 3

agent	rel ΔE^a (kcal/mol)	rel DNA-cleavage efficiency ΦX174	rel DNA-cleavage efficiency w794[b]
2, dBLM A_2	0.0	1.0	1.0 (1.0)
3, deshydroxy dBLM A_2	0.0[c]	0.8[c]	0.5[c](nd)
5, C4-desmethyl dBLM A_2	0.4[d]	0.4[d]	0.2[d](0.14)
4, C4-Me dBLM A_2	0.8	0.2	0.2 (nd)
7, C4 dBLM A_2	1.6	0.13	0.13 (0.14)

[a] E (low energy conformation) $-$ E (DNA-bound conformation adopted from Co(III)OOH BLM A_2). [b] Data in parentheses taken from ref 15, conducted under alternate assay conditions (0.3× DNA, 37 °C, 30 min). nd = not done. [c] Lacks capability for intermolecular C3−OH/phosphate H-bond, see text. [d] Possesses two equivalent L-histidine H-eclipsed amide orientations versus one for **2**−**4**, further contributing to a reduction in the cleavage efficiency, see text.

The agent **7**, lacking all the valerate substituents which constitutes the further removal of the C4-methyl group from **4**, exhibits an even further diminished DNA cleavage efficiency. Like **4**, **7** preferentially adopts the C2 extended versus compact conformation ($\Delta E = 0.8$ kcal), preferentially adopts the C4 amide extended versus turn conformation ($\Delta E = 0.8$ kcal), and has accessible the alternative turn (gauche) conformation ($\Delta E = 0.8$ kcal). It also possesses two more alternative accessible C3 conformations ($\Delta E = 0.9$ kcal, not shown) and may adopt either of two orientations for the linking β-hydroxy-L-histidine amide ($\Delta E = 0.0$ kcal, not shown), Figure 11. This larger ensemble of accessible conformations and the larger preference for the fully extended versus compact conformation ($\Delta E = 1.6$ kcal) accounts for the larger reduction in DNA cleavage efficiency with **7**.

Although the most appropriate correlation would be calculated Boltzmann probabilities for adoption of the productive bound conformation, the number of potential conformations for agents such as **7** precludes such an accurate assessment. However, a rough correlation derived simply by calculation of the relative energy difference between the low-energy conformation and the DNA-bound conformation adopted from Co(III)OOH bleomycin A_2 nicely accounts for the differences in the relative DNA cleavage efficiencies (Table 3). The exception to this is **3** which lacks the capabilities for formation of the intermolecular H-bond of the C3-hydroxy group with the nonbridging oxygens of the DNA backbone phosphate spanning the intercalation site.

Conclusions

The synthesis and examination of **3**−**8** containing key modifications in the valerate subunit of deglycobleomycin A_2 revealed that each of the substituents contributes in a significant

or substantial manner to the DNA cleavage efficiency but has minimal impact on the primary 5′-GPy cleavage selectivity. The length of the valerate linker appears to be optimal (C4 > C5 > C3 > C2) although the effect was established in the absence of linker substituents which have a substantial effect (ca. 10×). The most modest of the effects is that of the C3-hydroxy group[10] (1.2×). Its presence has little or no impact on the conformational properties of the valerate linker, and its effect may be attributed to its participation in an intermolecular H-bond with the nonbridging oxygens of a phosphate spanning the intercalation site that enhances binding affinity and/or alignment of the DNA-bound agent. The more substantial impact of the C4-methyl group is linked to the presence of the C2-methyl group, while the modest impact of the C2-methyl group itself (ca. 2×) is not coupled to the presence of the C4-methyl group. These effects are consistent with the expected conformational properties of the agents resulting from the substitutions and beautifully fit models of DNA-bound Co(III)OOH bleomycin A_2 and deglycobleomycin A_2. In addition to indicating that it is not an extended conformation, the cumulative magnitude of the effects is substantial and suggests that an important functional role of the valerate substituents is to preorganize bleomycin A_2 into a rigid, compact conformation productive for DNA cleavage. One site, the C2 position possessing a methyl substituent, may constitute a flexible position capable of adopting two equivalently accessible conformations that may accommodate conformational variations among cleavage sites, facilitate conformational reorganization to accommodate ds cleavage from a single intercalation site, or enhance the mechanics of DNA binding/decomplexation required for sampling multiple sites.

Experimental Section[30]

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4(*S*)-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2-(*R*)-hydroxy-1-propyl)amino)carbonyl)-2(*R*)-pentyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine Amide (3).**[30] A solution of **17a**[30] (6 mg, 0.005 mmol, 1 equiv) in CH_2Cl_2 (100 μL) at 0 °C was treated with 20% TFA−CH_2Cl_2 (200 μL), and the mixture was stirred at 0 °C (2.5 h). The solvent was evaporated in vacuo and the product was purified by reverse phase chromatography (C-18, 1.2 × 4 cm, 0−90% CH_3OH-H_2O gradient elution) affording **3** (4.6 mg, 5.5 mg theoretical, 85%) as a white solid: R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{23}_D$ −5 (*c* 0.1, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.27 (s, 1H), 8.22 (s, 1H), 8.13 (s, 1H), 7.31 (s, 1H), 5.16 (d, *J* = 7.5 Hz, 1H), 4.73 (d, *J* = 7.5 Hz, 1H), 4.30 (d, *J* = 4.5 Hz, 1H), 4.05 (m, 4H), 3.69 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.60 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.38 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.28 (dd, *J* = 7.5, 7.5 Hz, 2H), 2.94 (s, 6H), 2.76 (m, 1H), 2.56 (m, 1H), 2.47 (m, 1H), 2.25 (s, 3H), 2.16 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.79 (m, 1H), 1.49 (m, 1H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.25 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3333, 2943, 1676, 1641, 1548, 1430, 1194, 1133, 1112 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 1031.4100 (M^+, $C_{42}H_{63}N_{16}O_9S_3$ requires 1031.4126).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2(*R*)-hydroxy-1-propyl)amino)carbonyl)-2(*R*)-butyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine amide (4):**[30] (85%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{23}_D$ −13.6 (*c* 0.28, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.81 (s, 1H), 8.24 (s, 1H), 8.11 (s, 1H), 7.48 (s, 1H), 5.20 (d, *J* = 8.0 Hz, 1H), 4.71 (d, *J* = 8.0 Hz, 1H), 4.25 (d, *J* = 4.5 Hz, 1H), 4.09 (dq, *J* = 6.5, 4.5 Hz, 2H), 4.05 (m, 2H), 3.96 (dd, *J* = 6.0, 4.0 Hz, 2H), 3.70 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.59 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.37 (dd, *J* = 7.5, 7.5 Hz, 2H), 3.25 (dd, *J* = 7.5, 7.5 Hz, 2H), 2.93 (s, 6H), 2.78 (m, 1H), 2.59 (m, 1H), 2.33 (m, 2H), 2.21 (s, 3H), 2.15 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.82 (m, 1H), 1.73 (m, 1H), 1.17 (d, *J* = 6.5 Hz, 3H), 1.11 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3313, 2923, 1668, 1550, 1506, 1432, 1195, 1130 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 1017.3928 (M^+, $C_{41}H_{61}N_{16}O_9S_3$ requires 1017.3970).

Preparation of 4 from 19b. A solution of **19b** (2.0 mg, 0.0014 mmol) in CH_2Cl_2 (150 μL) at 0 °C was treated with CF_3CO_2H (50 μL) under Ar, and the solution was stirred at 0 °C (4 h). The solvents were evaporated under a stream of N_2, and the residue was dissolved in CH_3OH (200 μL). Aqueous NH_4OH (28%, 15 μL) was added, and the colorless solution was stirred at 23 °C (1 h). The solvent was evaporated in vacuo and the residue was purified by reverse phase chromatography (C-18, 0−70% CH_3OH-H_2O to afford **4** (0.88 mg, 1.4 mg theoretical, 62%) as a white film.

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((2-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2(*R*)-hydroxy-1-propyl)amino)carbonyl)-1-ethyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine amide (6):**[30] (91%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{23}_D$ −33 (*c* 0.16, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.85 (s, 1H), 8.40 (s, 1H), 8.12 (s, 1H), 7.45 (s, 1H), 5.20 (d, *J* = 8.0 Hz, 1H), 4.74 (d, *J* = 8.0 Hz, 1H), 4.26 (m, 2H), 4.21 (d, *J* = 4.5 Hz, 1H), 4.09 (dq, *J* = 6.5, 4.5 Hz, 2H), 3.69 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.60 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.45 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.37 (t, *J* = 7.5 Hz, 2H), 3.26 (t, *J* = 7.5 Hz, 2H), 2.92 (s, 6H), 2.81 (m, 1H), 2.56 (dd, *J* = 6.5, 6.5 Hz, 2H), 2.21 (s, 3H), 2.14 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.10 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3334, 3018, 2936, 1665, 1548, 1507, 1431, 1202, 1132 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 989.3699 (M^+, $C_{39}H_{57}N_{16}O_9S_3$ requires 989.3657).

Preparation of 6 from 19c: (52%).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((3-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2(*R*)-hydroxy-1-propyl)amino)carbonyl)-1-propyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine amide (7):**[30] (87%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{23}_D$ −35 (*c* 0.13, CH_3OH); $[\alpha]^{23}_D$ −8 (*c* 0.05, 0.1 N aqueous HCl); ^1H NMR (CD_3OD, 400 MHz) δ 8.85 (s, 1H), 8.32 (s, 1H), 8.13 (s, 1H), 7.50 (s, 1H), 5.21 (d, *J* = 8.0 Hz, 1H), 4.74 (d, *J* = 8.0 Hz, 1H), 4.26 (d, *J* = 4.5 Hz, 1H), 4.20 (m, 2H), 4.12 (dq, *J* = 6.5, 4.5 Hz, 1H), 3.73 (m, 2H), 3.64 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.59 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.37 (t, *J* = 7.5 Hz, 2H), 3.27 (dd, *J* = 6.0, 6.0 Hz, 2H), 2.93 (s, 3H), 2.70 (m, 1H), 2.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 2.21 (s, 3H), 2.16 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.82 (dd, *J* = 6.5, 6.5 Hz, 2H), 1.12 (d, *J* = 6.5 Hz, 3H); ^1H NMR (D_2O, 400 MHz) δ 8.54 (s, 1H), 8.02 (s, 1H), 7.89 (s, 1H), 7.11 (s, 1H), 5.03 (d, *J* = 8.0 Hz, 1H), 4.41 (m, 1H), 4.28 (m, 1H), 4.17 (m, 1H), 3.97 (d, *J* = 4.5 Hz, 1H), 3.89 (m, 1H), 3.50 (m, 1H), 3.39 (m, 4H), 3.26 (m, 1H), 3.18 (t, *J* = 7.5 Hz, 2H), 3.08 (t, *J* = 6.5 Hz, 2H), 2.98 (t, *J* = 7.0 Hz, 2H), 2.80 (m, 2H), 2.70 (s, 6H), 2.09 (m, 2H), 1.96 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.77 (s, 3H), 1.54 (t, *J* = 7.0 Hz, 2H), 0.88 (d, *J* = 6.5 Hz, 3H); IR (neat) 3325, 2923, 2852, 1669, 1556, 1538, 1515, 1450, 1426, 1201, 1130 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 1003.3810 (M^+, $C_{40}H_{59}N_{16}O_9S_3$ requires 1003.3813).

Preparation of 7 from 19d: (95%).

***N*$^{\beta}$-[1-Amino-3(*S*)-[4-amino-6-[[[1(*S*)-(((4-(((1(*S*)-(((2-(4′-((((3-dimethylsulfonio)-1-propyl)amino)carbonyl)-2′,4-bithiazol-2-yl)-1-ethyl)amino)carbonyl)-2(*R*)-hydroxy-1-propyl)amino)carbonyl)-1-butyl)amino)carbonyl)-2(*R*)-hydroxy-2-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]propion-3-yl]-(*S*)-*β*-aminoalanine amide (8):**[30] (85%) R_f = 0.2 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{23}_D$ −3.5 (*c* 0.25, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.21 (s, 1H), 8.12 (s, 1H), 8.00 (s, 1H), 7.20 (s, 1H), 5.15 (d, *J* = 8.0 Hz, 1H), 4.79 (d, *J* = 8.0 Hz, 1H), 4.25 (d, *J* = 4.5 Hz, 1H), 4.10 (dq, *J* = 6.5, 4.5 Hz, 1H), 4.02 (m, 2H), 3.68 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.63 (dd, *J* = 6.5, 6.5

(30) Full characterization of intermediates **9b**−**e**, **10a**−**e**, **11a**−**e**, **12a**−**e**, **14a**−**e**, **15a**−**e**, **17a**−**e**, and **19a**−**e** is provided in Supporting Information.

Hz, 2H), 3.59 (dd, $J = 6.5, 6.5$ Hz, 2H), 3.37 (t, $J = 7.5$ Hz, 2H), 3.26 (dd, $J = 6.0, 6.0$ Hz, 2H), 3.21 (dd, $J = 6.5, 6.5$ Hz, 2H), 2.93 (s, 6H), 2.73 (m, 1H), 2.53 (m, 1H), 2.26 (m, 2H), 2.23 (s, 3H), 2.14 (tt, $J = 7.0, 7.0$ Hz, 2H), 1.54 (m, 2H), 1.49 (m, 2H), 1.11 (d, $J = 6.5$ Hz, 3H); IR (neat) ν_{max} 3325, 2923, 2864, 1668, 1651, 1556, 1515, 1426, 1201, 1183, 1130 cm^{-1}; FABHRMS (NBA−CsI) *m/z* 1017.3930 (M^+, $C_{41}H_{61}N_{16}O_9S_3$ requires 1017.3970).

Preparation of 8 from 19e: (42%).

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Supporting Information Available: Experimental procedures and characterization of **9b**−**e**, **10a**−**e**, **11a**−**e**, **12a**−**e**, **14a**−**e**, **15a**−**e**, **17a**−**e**, and **19a**−**e** and experimental details for the DNA cleavage studies are provided (14 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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