Assessment of the Role of the Bleomycin A_2 Pyrimidoblamic Acid C4 Amino Group

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Abstract: The preparation and examination of **3** and **4** and their unnatural epimers **6** and **7** by following a new and alternative synthesis of **2** was conducted to assess the role of the pyrimidine C4 amine of bleomycin A_2 (**1**) and deglycobleomycin A_2 (**2**). The agent **3** bearing a pyrimidine C4 dimethylamino substituent exhibited a substantially diminished DNA cleavage efficiency (10−15×) relative to **2** and the loss of the characteristic 5′-GC/5′-GT cleavage selectivity. The agent **4** in which the pyrimidine C4 amino group was removed exhibited an even greater diminished DNA cleavage efficiency (30×) relative to that for **2**. For this agent, the characteristic cleavage selectivity is either slightly or significantly reduced depending on the assay conditions. Even in the instances where it was not substantially altered, the ability to detect it required a temperature of 4 versus 25−37 °C. This information and temperature dependence suggest a reduced binding interaction and are consistent with the participation of the pyrimidine C4 amine in one of two critical hydrogen bonds of a minor groove triplex-like recognition between the metal binding domain of **1** and **2** and guanine at the 5′-GC/5′-GT cleavage sites responsible for the characteristic cleavage selectivity. These observations have further implications on the potential origin of inherent 5′-GPy > 5′-APy cleavage selectivity of bleomycin A_2 itself. This cleavage preference of 5′-GPy > 5′-APy may be analogously attributed to a reduced binding affinity at the 5′-APy sites resulting from one versus two triplex-like hydrogen bonds to adenine.

Bleomycin A_2 (**1**, Figure 1),[1−11] the major 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 or RNA by a process that is metal ion and oxygen dependent.[12−18] Extensive studies employing derivatives of the natural product,[19,20] its degradation products or semisynthetic analogues,[21−30] and closely related or substantially simplified analogues[31−34] have contributed to

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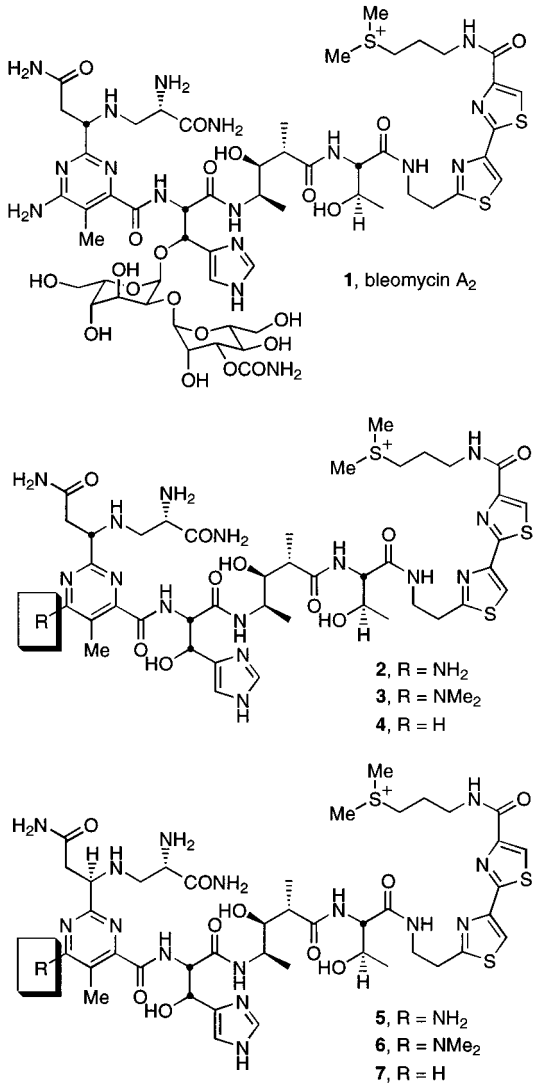

Figure 1.

an emerging model of the structural features responsible for the sequence-selective cleavage of duplex DNA. Recent structural studies have demonstrated that the Co(III) hydroperoxide complexes of bleomycin A_2 (**1**) and deglycobleomycin A_2 (**2**) bind to an oligonucleotide at a cleavage site in essentially identical manners albeit with **2** exhibiting a lower affinity (30×).[35−37] Both **1** and **2** exhibit comparable DNA cleavage selectivities but with **2** exhibiting lower efficiencies (2−6×). This comparable behavior suggests that the disaccharide contributes to the cleavage efficiency of **1** but not its DNA cleavage selectivity and that it may do so by increasing DNA binding affinity or altering the kinetic parameters for activation. Regardless of the role, these and related studies illustrate that deglycobleomycin A_2 analogues may provide important and relevant information on the nature of the interaction of **1** with duplex DNA. In our own efforts,[38−48] this has entailed single point changes in the structure of deglycobleomycin A_2 conducted with the intention of defining the role of each subunit, functional group, or substituent. These studies, carried out in conjunction with structural studies, have begun to unravel many of the subtle structural features contributing to the properties of the natural product which suggest that they are greater than the sum of its parts.

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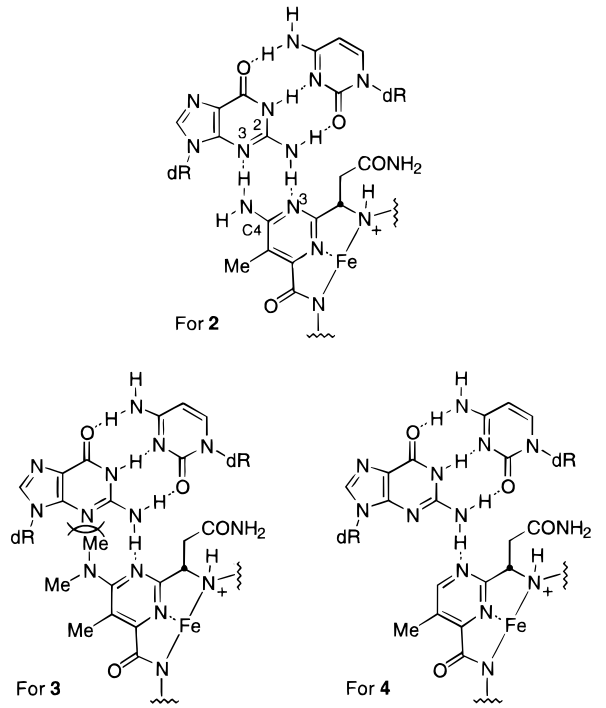

Figure 2.

Herein we report the synthesis and evaluation of **3** and **4** as well as their epimers **6** and **7** in which the pyrimidoblamic acid C4 primary amine of **2** has been replaced with a tertiary dimethylamine or removed altogether. In addition to the impact this may have on the metal chelation and oxygen activation properties of **1** and **2**, their examination allows the functional assessment of the consequences of preventing formation of a key hydrogen bond from this primary amine to guanine N3 implicated in recent structural studies.[35,36] These studies suggested two previously unrecognized and critical hydrogen bonds between the pyrimidine of the metal binding domain of bleomycin A_2 and guanine of the cleavage sites 5′-GC/5′-GT. The pyrimidoblamic acid N3 was found to be hydrogen bonded to the non-base-pairing hydrogen of the guanine C2 amine, and one of the pyrimidoblamic acid C4 amine hydrogens was hydrogen bonded to guanine N3, providing a triplex-like recognition interaction in the minor groove (Figure 2). These observations provide a structural basis for the metal binding domain control of the cleavage sequence selectivity which had been implicated in preceding studies.[32]

This key interaction potentially provides the basis for the sequence-selective cleavage of DNA by **1** and **2** and explains the requirement for the guanine C2 amine for 5′-GC/5′-GT cleavage.[49−51] However, this interaction differs from the earlier Sugiura and Dickerson models that enlist bithiazole minor groove binding and its hydrogen bonding to the cleavage site guanine C2 amine.[50,51] Rather, it defined a previously unrecognized role of the bleomycin A_2 pyrimidine C4 amine which the analogues **3** and **4** and their epimers **6** and **7** may address directly. As detailed herein, both changes dramatically reduce the DNA cleavage efficiency (10−30×). The C4 dimethylamino substitution with **3** and **6** results in the loss of the characteristic DNA cleavage selectivity, while its removal with **4** and **7** maintained most of the characteristic 5′-GC/5′-GT cleavage selectivity but did substantially diminish the ability to detect it (4 versus 25 or 37 °C) for one set of assays whereas very little cleavage specificity could be detected in another set of assays even at 4 °C. These results suggest that the basis for sequence selectivity defined by the structural models obtained with the cobalt bleomycins is indicative of the interaction of the physiologically important iron bleomycins with DNA.

Alternative Synthesis of Deglycobleomycin A_2 (2). In our preceding efforts culminating in the total synthesis of deglycobleomycin A_2 and bleomycin A_2, the final coupling to link the full agent was conducted at the tetrapeptide S and β-hydroxy-L-histidine juncture.[44,45] This convergent assembly proved advantageous for the synthesis of the natural product and was adopted for the preparation of a range of analogues. For the efforts herein, a more direct preparation would entail a final coupling of N^{α}-BOC-pyrimidoblamic acid (BOC = *tert*-butoxycarbonyl) or its analogues to pentapeptide S. In an effort to establish the viability of this approach and the level of required protecting groups, an alternative synthesis of deglycobleomycin A_2 (**2**) was developed (Scheme 1). Coupling of tetrapeptide S (**8**)[42] and N^{α}-BOC,N^{im}-CPh_3-β-hydroxy-L-histidine (**9**)[42] effected by (benzotriazol-1-yloxy)tris(dimethylamino)-phosphonium hexafluorophosphate (BOP reagent, 1.5 equiv, 2 equiv of *i*-Pr_2NEt, DMF, 25 °C, 8 h, 75−88%) cleanly provided **10**. Acid-catalyzed deprotection of **10** (20% TFA−CH_2Cl_2, 0 °C, 2.5 h) followed by liberation of the amine free base provided pentapeptide S (**11**, 88%)[42] resulting from both BOC and trityl removal. Subsequent coupling of **11** with N^{α}-BOC-pyrimidoblamic acid (**12**)[43] proceeded smoothly when effected by diphenylphosphoryl azide (DPPA) treatment (1.5 equiv, 3 equiv of *i*-Pr_2NEt, DMF, 25 °C, 10 h, 72%) providing N^{α}-BOC-deglycobleomycin A_2 (**13**) incorporating only one protecting group with the sulfonium salt installed. This was accomplished without deliberate protection or competitive acylation of the imidazole. In preceding studies with bleomycin A_2,[45] we reported that a related coupling effected by activation with dicyclohexylcarbodiimide (DCC−HOBt, DMF, 25 °C, 36 h) provided predominately imidazole versus primary amine coupling. Whether these observations are unique to bleomycin A_2 and result from the attempted reaction of a more hindered primary amine or whether they are related to the activation conditions (DPPA versus DCC−HOBt) has not been established. However, the same coupling of **11** with **12** conducted with DCC−HOBt (DMF, 25 °C, 10 h) provided **13** in much lower conversions (25−29%). It is even plausible that the acylated imidazole serves as an intermediate but is effectively converted to **13** under the DPPA reaction conditions (3 equiv of *i*-Pr_2-NEt). Acid-catalyzed deprotection of **13** (20% TFA−CH_2Cl_2, 0 °C, 2 h, 86−89%) provided deglycobleomycin A_2 (**2**) identical in all respects with authentic material. The success of this approach provided the basis for our synthesis of **3** and **4**.

Synthesis of C4 (Dimethylamino)pyrimidoblamic Acid and Its Epimer and Their Incorporation into 3 and 6. Displacement of the 4-chloro substituent of **15**[52] by treatment with dimethylamine (2 M in THF, 25 °C, 1.5 h, 97%) cleanly provided **16**, and subsequent acetal hydrolysis afforded the aldehyde **17** and our key intermediate for diastereoselective introduction of the C2 side chain (Scheme 2). Following protocols developed in our synthesis of **1** and **2**,[43,53] condensation of **17** with N^{α}-BOC-β-amino-L-alanineamide (**18**)[41] followed by

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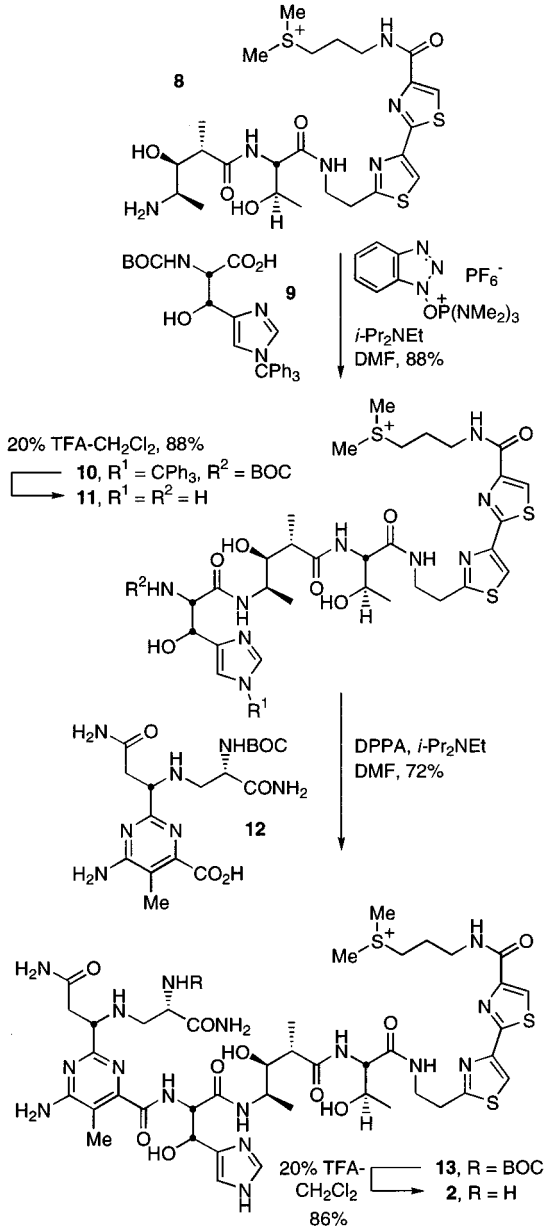
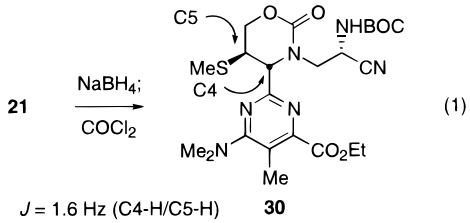
Scheme 1

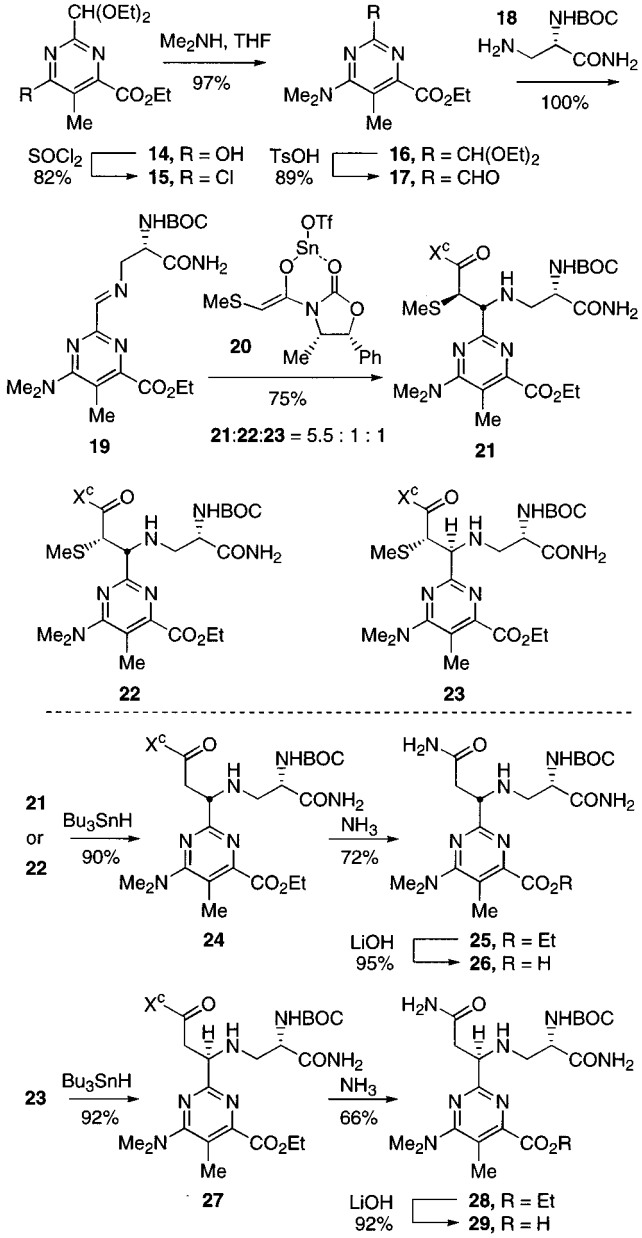


addition of **19** to the stannous (*Z*)-enolate **20**, generated by treatment of the corresponding oxazolidinone with *i*-Pr_2NEt (2.2 equiv) in the presence of $Sn(OTf)_2$ (2.0 equiv), provided a separable mixture of **21**−**23** (75%, 5.5:1:1 ratio). Both **21** and **22** provided **24** upon reductive removal of the thiomethyl group (Bu_3SnH, C_6H_6, 80 °C, 1 h, 90−92%) and thus possess the same configuration at the newly introduced amine center. In contrast, **23** provided the diastereomer **27** upon reductive removal of the thiomethyl group and the unnatural (*R*)-configuration at the newly introduced amine center. Analogous to observations made in our synthesis of pyrimidoblamic acid,[43] ^{1}H NMR analysis (*J*, C4−H/C5−H) of the cyclic carbamate **30** ($J = 1.6$ Hz, eq 1) established that the major product **21** is the anti aldol imine addition product ($J = 1.5$ Hz typically), while **22** constitutes the minor syn addition product ($J = 5.6$ Hz typically). The remaining minor diastereomer **23**, which possessed the unnatural (*R*)-configuration at the newly introduced amine center, was assigned the anti aldol imine addition product stereochemistry by analogy to our prior work.[43] Although we did not unambiguously establish the absolute configuration at the newly introduced amine center, the diastereoselection of 87:13 (**21** + **22**:**23**) corresponds exactly to that observed in our efforts on the synthesis of pyrimidoblamic acid (87:13)[43] where the relative and absolute configurations were unambiguously established for all three diastereomers by independent synthesis. Thus, they would appear to be safely assigned. Nonetheless, to ensure that such an ambiguity might not inadvertently alter the interpretation of the properties, both diastereomers **24** and **27** were carried forward to provide the final agents **3** and **6**. Aminolysis of the acyloxazolidinone (16% NH_3−EtOH, 0 °C, 1 h, 65−70%) followed by ethyl ester hydrolysis of **25** and **28** provided **26** and **29**, respectively.

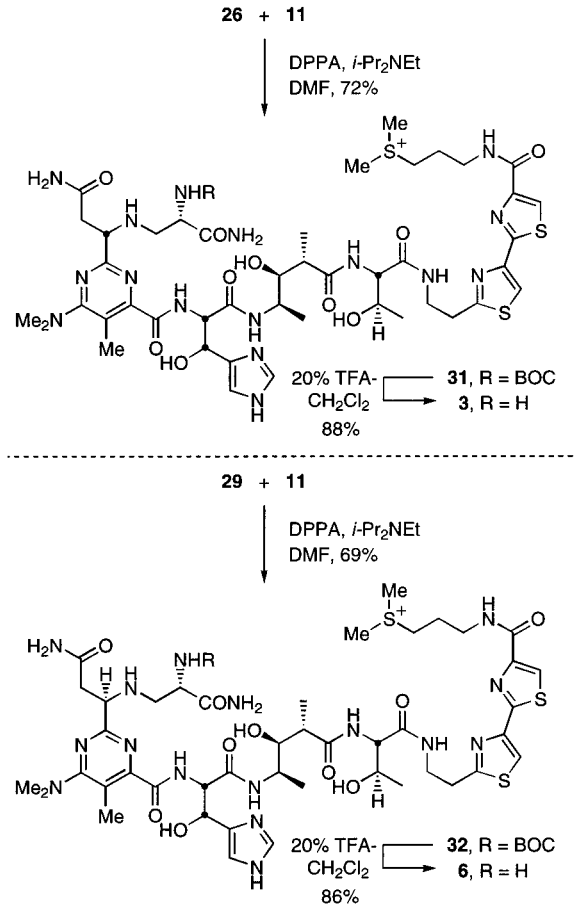


Coupling of both **26** and **29** with pentapeptide S (**11**, 1.5 equiv of DPPA, 3 equiv, *i*-Pr_2NEt, DMF, 0 °C, 12 h, 69−72%) and

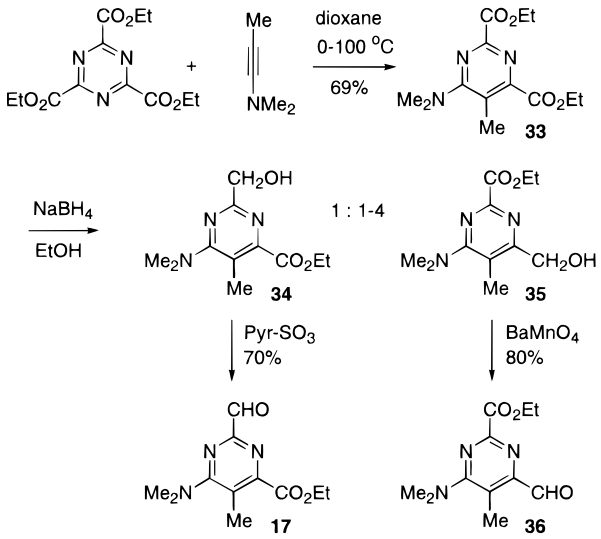
Scheme 2



Scheme 3



Scheme 4

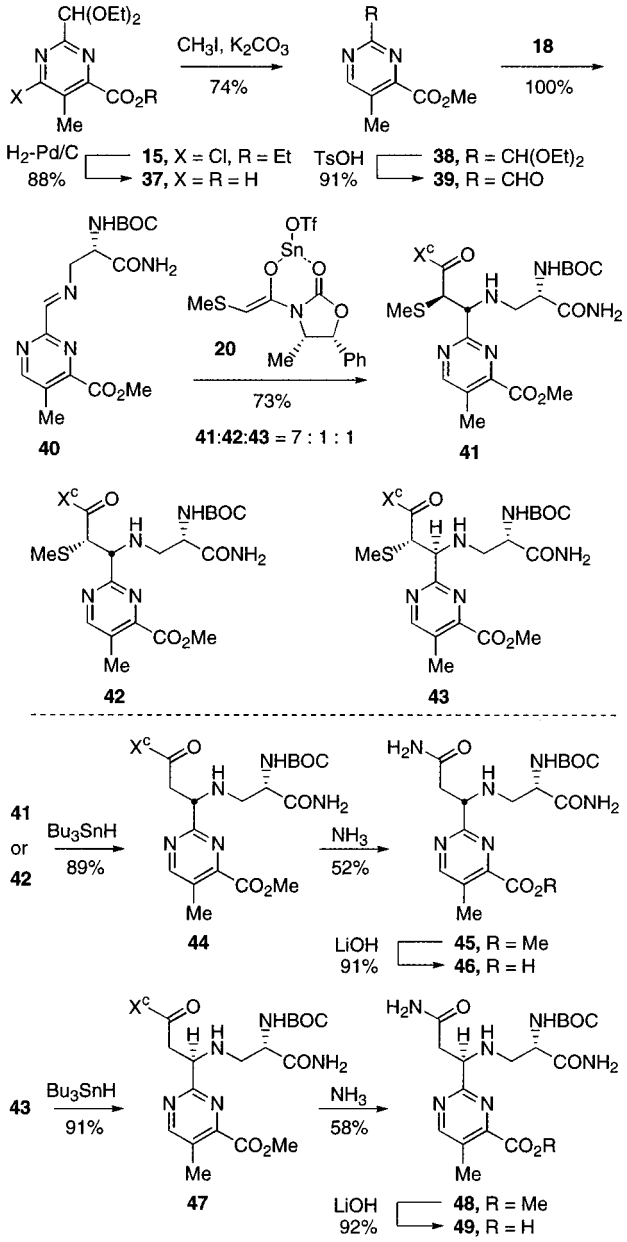


subsequent acid-catalyzed deprotection of the N^{α}-BOC group (20% TFA−CH_2Cl_2, 0 °C, 1.5 h, 86−88%) provided **3** and **6**, respectively (Scheme 3).

An alternative and less satisfactory preparation of **17** was also accomplished from the key intermediate **33** derived from the [4 + 2] cycloaddition of 1-(dimethylamino)propyne with 2,4,6-tris(ethoxycarbonyl)-1,3,5-triazine (Scheme 4).[43,54,55] Attempted selective reduction of **33** (1 equiv of $NaBH_4$, EtOH, 20−25 °C, 5 h) analogous to the successful efforts employed

(54) Boger, D. L.; Dang, Q. *Tetrahedron* **1988**, *44*, 3379.
(55) Ott, E. *Chem. Ber.* **1919**, *52*, 656.

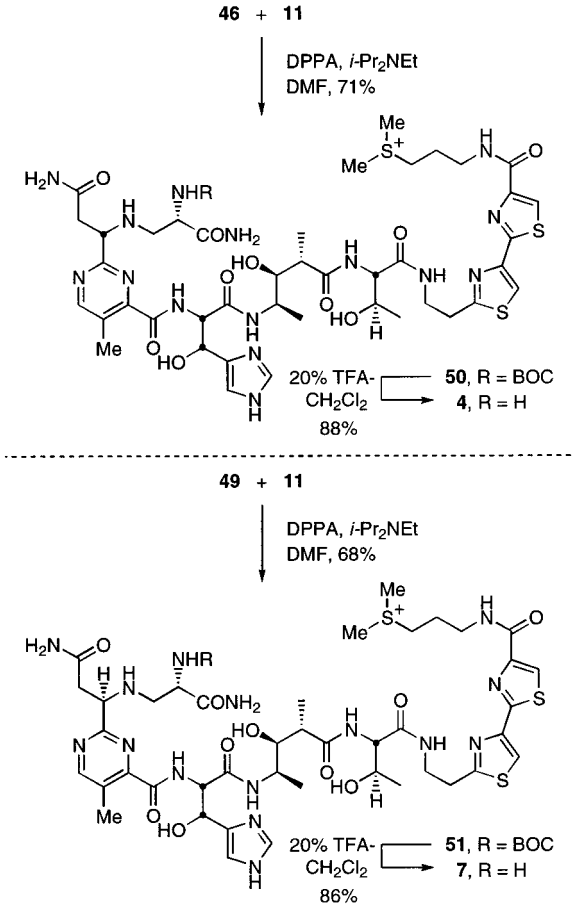
Scheme 5



for **1** and **2**[43] failed to provide clean C2 ethyl ester reduction and typically provided both C2 and C6 reduction (1:1−4, respectively) under a range of reaction conditions. Subsequent oxidations of the corresponding alcohols provided **17** (pyr-SO_3, Et_3N, DMSO−CH_2Cl_2, 0 °C, 1 h, 70%) and **36** ($BaMnO_4$, CH_2Cl_2, 20−25 °C, 12 h, 80%).

Synthesis of C4 Desaminopyrimidoblamic Acid and Its Epimer and Their Incorporation into 4 and 7. Following unsuccessful efforts at reductive deamination of a pyrimidoblamic acid precursor, the synthesis of **46** and its epimer **49** was accomplished as shown in Scheme 5. Reductive dechlorination of **15** (H_2, Pd−C, 2 N aqueous KOH−Et_2O 1:1, 25 °C, 1 h, 88%) under conditions that resulted in ethyl ester hydrolysis cleanly provided **37**. Methyl ester formation (CH_3I, K_2CO_3, DMF, 25 °C, 10 h, 74%) followed by acetal hydrolysis (91%) of **38** provided the key aldehyde **39** (Scheme 5). Condensation of **39** with **18**[41] followed by addition of **40** to the stannous (*Z*)-enolate **20** provided a separable 7:1:1 mixture of **41**−**43** (73%). Both **41** and **42** provided **44** upon reductive removal of the thiomethyl group, indicating that they both

Scheme 6



possess the same natural (*S*)-configuration at the newly introduced amine center while **43** provided the diastereomer **47**. Although we did not unambiguously establish the absolute or relative stereochemistry for the imine aldol addition products, the diastereoselection of 89:11 (**41** + **42**:**43**) and relative amounts of each of the three products parallel those observed in our preceding efforts on pyrimidoblamic acid (87:13)[43] where they were unambiguously established and are analogous to those defined in Scheme 2. However, to ensure that an inadvertent misassignment might not alter the interpretation of the properties, both diastereomers were carried forward to provide **4** and **7**. Following reductive desulfurization (3 equiv of Bu_3SnH, C_6H_6, 80 °C, 1 h, 89−91%), aminolysis of the acyloxazolidinones **44** and **47** (16% NH_3−EtOH, 0 °C, 52−58%) and final methyl ester hydrolysis of **45** and **48** (LiOH, *t*-BuOH−H_2O (2:1), 0 °C, 1 h, 91−92%) afforded **46** and **49**, respectively.

Coupling of both **46** and **49** with pentapeptide S (**11**, 1.5 equiv of DPPA, 3 equiv of *i*-Pr_2NEt, DMF, 0 °C, 12 h, 68−71%) and subsequent acid-catalyzed deprotection of the N^{α}-BOC group (20% TFA−CH_2Cl_2, 0 °C, 1.5 h, 86−88%) cleanly provided **4** and **7**, respectively (Scheme 6).

DNA Cleavage Properties. Four assays were used to examine the DNA cleavage properties of **3** and **4** and their epimers. The initial study of the relative efficiency of DNA cleavage was conducted with the Fe(II) complexes 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) complexes of all four agents produced single- and double-strand cleavage to afford relaxed (form II) and linear (form III) DNA, respectively (Figure 3, Table 1). Both **3** and **4** were found to be substantially less effective (12× and 17×)

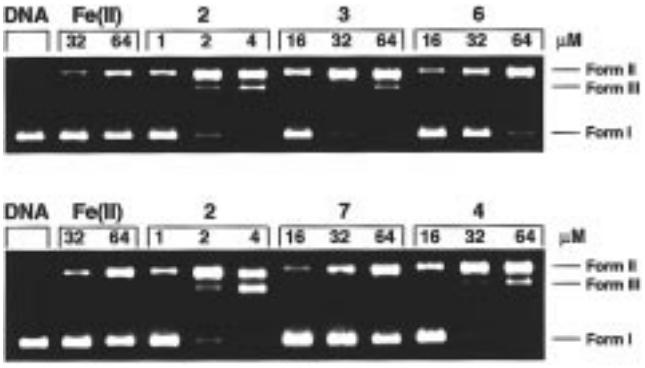


Figure 3. Agarose gel illustrating the cleavage reactions of supercoiled ΦX174 DNA by Fe(II)-**3** and **6** (top) or **4** and **7** (bottom) at 25 °C for 1 h in buffer solutions containing 2-mercaptoethanol. After electrophoresis on a 1% agarose gel, the gel was stained with 0.1 μg/mL ethidium bromide and visualized on a UV transilluminator and quantified on a Millipore BioImage 60S RFLP system. The results are tabulated in Table 1.

at cleaving ΦX174 RFI DNA than deglycobleomycin A_2 and only 1.5−2× more effective than uncomplexed Fe(II) itself. Their epimers **6** and **7** were even less effective and were nearly indistinguishable from Fe(II) itself.

The relative extent of double-strand (ds) to single-strand (ss) DNA cleavage was established in a study of the kinetics of supercoiled ΦX174 DNA cleavage to produce linear and circular DNA. The reactions exhibit initial fast kinetics in the first 1−5 min, and the subsequent decreasing rate may reflect conversion to a less active or inactive agent or the kinetics of metal complex reactivation. We assumed a Poisson distribution for the formation of ss and ds breaks to calculate the average number of ds and ss cuts per DNA molecule using the Freifelder−Trumbo equation.[56] The ratios of ds to ss cleavages observed with the Fe(II) complexes are summarized in Table 1. The ratios for **3** and **4** were established to be 1:53 and 1:45, and those of their epimers **6** and **7** were 1:61 and 1:48, respectively. Consistent with the relative efficiencies of DNA cleavage, this was substantially lower than that of bleomycin A_2 (1:6) or deglycobleomycin A_2 (1:12) and is nearly indistinguishable from the ratio derived from uncomplexed Fe(II) cleavage (1:98). A theoretical ratio of approximately 1:100 is required for the linear DNA to be the result of the random accumulation of ss breaks within the 5386 base-pair size of ΦX174 RFI DNA assuming that sequential cleavage on the complementary strands within 15 base pairs is required to permit formation of linear DNA. Thus, all four agents have effectively lost their ability to promote ds cleavage of DNA. The significance of these observations is not yet completely understood. It is not yet clear whether the second cleavage adheres to a special sequence selectivity. Early studies examining a limited number of ds cleavage sites implied no special selectivity,[57] while more recent studies have clearly defined hot spots for ds cleavage that include 5′-GT cleavage on both strands, i.e. 5′-G*T*AC.[58] It could be argued that the ds:ss cleavage ratio with **3** and **4** and their epimers should not diminish with the decreased cleavage efficiency if the former is accurate while the later would be substantially effected if its basis for selectivity also involved the triplex-like hydrogen bond model. While the results suggest that the latter may prove accurate, typically, the ratio of ds:ss cleavage diminishes as the cleavage efficiency decreases, and the results with **3** and **4** and

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(57) Povirk, L. F.; Han, Y.-H.; Steighner, R. J. *Biochemistry* **1989**, *28*, 5808. Steighner, R. J.; Povirk, L. F. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 8350.

Table 1. Summary of ΦX174 and w794 DNA Cleavage Properties

agent	relative efficiency of DNA cleavage[a] ΦX174[a]	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	0.08	0.09	1:53	disrupted
4	0.06	0.03	1:45	weak 5′-GC, 5′-GT > 5′-GA
5[44]	0.25	0.04	1:29	5′-GC, 5′-GT > 5′-GA
6	0.04	0.10	1:61	disrupted
7	0.03	0.03	1:48	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 six experiments. [b] Examined within 5′ ^{32}P-end-labeled w794, Fe(III)−H_2O_2. The results are the average of four experiments. [c] Ratio of double- to single-stranded cleavage of supercoiled ΦX174 DNA calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$.

their epimers also suggest that they may simply conform to this established trend.[40−48]

Consistent with past observations,[44] the natural epimers **3** and **4** were slightly more effective (2×) than the unnatural epimers **6** and **7**. In past studies where this has been examined (e.g., **5** versus **2**),[44] the unnatural epimers proved to be 2−4× less effective than the natural epimers and indistinguishable from the agents that lack the acetamido side chain altogether. The comparisons of **3** and **4** with **6** and **7** conform nicely to these past trends, further establishing the stereochemical assignments. The prior studies suggested that the acetamido side chain natural configuration may contribute to adoption of a metal chelate or DNA-bound conformation productive for cleavage while the unnatural epimers lack this enhancement but are not adversely affected by the presence of the acetamido side chain. Finally, in both the assays with ΦX174 supercoiled DNA, **3** and **4** and their epimers **6** and **7** are among the worst analogues of deglycobleomycin A_2 examined to date that contain a single site structural change.

Most revealing was the comparison of the DNA cleavage selectivity of **3** and **4** determined using end-labeled duplex DNA[59] and a hairpin oligonucleotide previously used to study ds cleavage.[58] The selectivity of DNA cleavage and an additional assessment of the relative efficiency of DNA cleavage were examined with duplex w794 DNA[59] by monitoring strand cleavage of singly ^{32}P 5′-end-labeled double-stranded DNA by the Fe(III) complexes upon activation with H_2O_2[60] in 10 mM phosphate buffer (pH 7.0). This protocol has proven to be more sensitive to the distinctions in the relative efficiency of DNA cleavage than the ΦX174 supercoiled DNA cleavage assays, but both have always provided the same trends in our hands. Thus, incubation of the labeled duplex DNA with the agents 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. Typical comparisons for agent **3** and its epimer **6** are illustrated in Figure 4.

Under all conditions examined, **3** and **6** were found to cleave DNA only slightly above background Fe(III) (Table 1). The

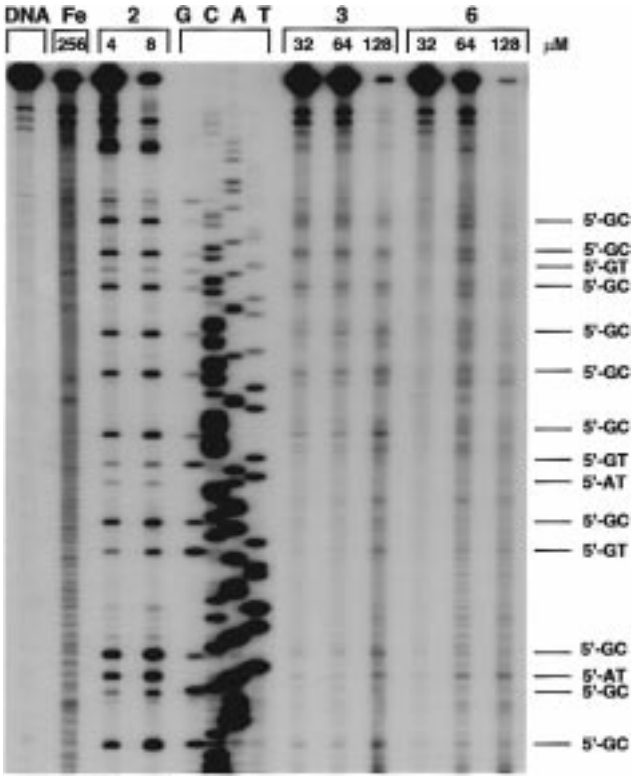

Figure 4. Cleavage of double-stranded DNA by Fe(III)-**3** and **6** (SV40 DNA fragment, 144 base pairs, nucleotide no. 5238−138, clone w794) in phosphate/KCl buffer containing H_2O_2. The DNA cleavage reactions were run for 90 min at 4 °C, and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.

Fe(III) complexes of both agents were 3× more effective than Fe(III) itself but 10× less effective than deglycobleomycin A_2. Under a range of experimental conditions, the DNA cleavage selectivity characteristic of bleomycin A_2 and deglycobleomycin A_2 was essentially lost. Only when the assay was conducted at 4 versus 25 or 37 °C were the remnants of the characteristic cleavage detectable with **3**, and even then, the cleavage pattern is substantially altered (Figure 4). Within w794 DNA small enhancements above nonselective cleavage were observed at sites characteristic of **2** but typically appeared as doubled bands indicating proximal or adjacent cleavage sites. The clear, crisp pattern of **2** is not observed, and this slight cleavage preference barely rises above nonselective cleavage. At 25 or 37 °C, this slight preference disappears. The epimer **6** was even less selective and cleaves DNA with essentially no selectivity. We have interpreted these observations to indicate that the characteristic sequence-selective cleavage of **1** and **2** is disrupted by the C4 dimethylamino substitution of the pyrimidoblamic acid subunit.

(58) Absalon, M. J.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1995**, *34*, 2065. Absalon, M. J.; Wu, W.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1995**, *34*, 2076.

(59) 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.

(60) 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.

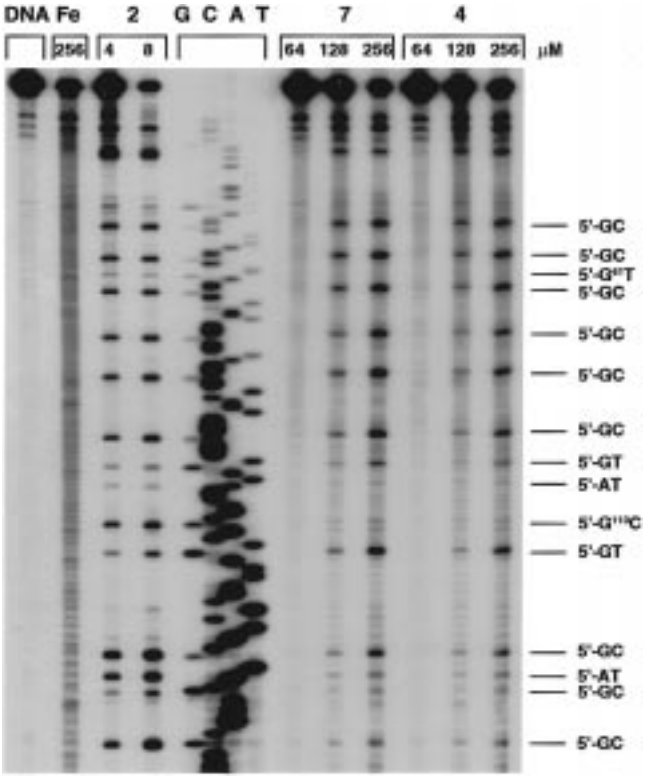

Figure 5. Cleavage of double-stranded DNA by Fe(III)-**4** and **7** (SV40 DNA fragment, 144 base pairs, nucleotide no. 5238−138, clone w794) in phosphate/KCl buffer containing H_2O_2. The DNA cleavage reactions were run for 90 min at 4 °C, and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.

A comparison of the DNA cleavage sequence selectivity for the desamino agent **4** and its epimer **7** proved even more interesting, and a representative examination is illustrated in Figure 5 while the results are summarized in Table 1. Both agents were found to cleave w794 double-stranded DNA substantially less effectively (30×) than deglycobleomycin A_2 or even **3** and **6**. Unlike **3** and **6**, the DNA cleavage sequence selectivity characteristic of **1** and **2** remained when the amino group on the pyrimidine ring was removed. However, the ability to detect the cleavage selectivity was most pronounced at 4 °C and, unlike **1** and **2**, diminished as the assay temperature was raised to 25 or 37 °C. Even at 4 °C, the cleavage selectivity was not nearly as clear or crisp as that of **1** or **2**, and suffers from competitive nonselective cleavage. Although similar, the cleavage selectivity of both **4** and **7** is distinguishable from that of **1** and **2**. For example, the clean and pronounced 5′-GC cleavage of deglycobleomycin A_2 at G^{113} and the weak 5′-GT cleavage at G^{61} are not observed with either **4** or **7**. The significance of this subtle alteration is not yet known. In addition, the relative efficiency of cleavage at the minor 5′-AT sites was diminished and essentially disappeared. Our interpretation of these observations is that the removal of the pyrimidoblamic acid C4 amine removes the key guanine N3 hydrogen bond to the C4 amine but does not preclude formation of the second hydrogen bond from the guanine C2 NH_2 to the pyrimidine N3 of **4**. Thus, the effectiveness of the interaction is diminished by the loss of one hydrogen bond but not completely lost. Both epimers **4** and **7** were nearly indistinguishable in their efficiency, and both were comparable to, or perhaps even slightly less effective than, uncomplexed Fe(III).

The Co(III) hydroperoxide complex of bleomycin A_2 stoichiometrically versus catalytically cleaves DNA only in the presence of light by a mechanism that precludes double-stranded DNA cleavage but does so with largely the same sequence and chemical specificity as the Fe bleomycins.[61] Thus, it is not surprising that at 25 °C the Co(III) hydroperoxide complexes of **3**, **4**, **6**, and **7** exhibited analogous decreases in cleavage efficiency and all four resulted in a loss of the cleavage selectivity relative to **2** (data not shown).[61]

```
5'   CGAATTCTGCGGTACCTTTCCCA A
                              A
3'   GCTTAAGACGCCATGGGAAAGGGA A
                    *
```

Figure 6. Hairpin oligonucleotide containing GTAC hot spot for double-strand cleavage. ^{32}P labeling site is marked by ∗. Good ss cleavage sites are underlined; the ds cleavage site is in bold.

Recently, a method for quantitatively evaluating the ratio of ss versus ds cleavage has been developed using a hairpin (51mer) oligonucleotide in which a hot spot for ds cleavage, a GTAC box, is built into the center of the duplex region and is flanked by good ss cleavage sites at C10 and C43 (Figure 6).[58] Initially, efforts focused on the use of Fe(II) and O_2 to generate the activated bleomycin analogues. Since essentially no cleavage had been observed under these conditions even in the absence of carrier DNA for ineffective analogues, compounds **3** and **4** were activated by an alternative protocol using Fe(III) and H_2O_2. The cleavage efficiency with this construct was estimated to be at least 50 times less than that of bleomycin. Furthermore, even at 4 °C, the cleavage pattern resembled that of hydroxide radical with essentially no specificity for cleavage at GT or GC's in this DNA shown to be excellent sites for ss cleavage (Figure 7). In addition no apparent ds cleavage band was observed.

The lack of significant sequence specificity for **4** seems to differ somewhat from the studies with w794 DNA. A careful examination of the w794 sequence reveals a few GC and GT sites cleaved by **2**, but not by **4** or **7** (see Figure 5). Around these sites nonselective chemistry appears to occur. Even with the selective w794 cleavage conducted at 4 °C, the selectivity is weakened and suffers from competitive nonselective strand breakage. Since there are only two GC and GT cleavage sites in the hairpin oligonucleotide, these results suggest that different sequence contexts may have different affinities for the analogues which could lead to different cleavage efficiencies. Moreover, since the method of activation required to detect cleavage is effecting multiple turnovers, the results suggest that **3** and **4** can shift between selective iron−oxo chemistry and nonselective iron−oxo or hydroxide radical chemistry and that, in the absence of an effectively bound conformation, the latter may dominate.

Importantly, these results provide additional support that the putative H-bonding interaction between the 4-amino group of the pyrimidine plays a key role in the specificity and efficiency of both ss and ds cleavage.

Oxidation Capabilities of 2−4 and 6 and 7. In final efforts to characterize the properties of the agents, the abilities of their

(61) Chang, C.-H.; Meares, C. F. *Biochemistry* **1982**, *21*, 6332. Chang, C.-H.; Meares, C. F. *Biochemistry* **1984**, *23*, 2268. Suzuki, T.; Kuwahara, J.; Goto, M.; Sugiura, Y. *Biochim. Biophys. Acta* **1985**, *824*, 330. Saito, I.; Morii, T.; Sugiyama, H.; Matsura, T.; Meares, C. F.; Hecht, S. M. *J. Am. Chem. Soc.* **1989**, *111*, 2307. Worth, Jr., L.; Frank, B. L.; Christner, D. F.; Absalon, M. J.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1993**, *32*, 2601. The cleavage studies were conducted by formation of the Co complexes (equimolar $CoCl_2$, 2 h, 25 °C, open to air, 0.5−128 μM), addition of w794 end-labeled DNA in pH 7 phosphate buffer, and illumination of the mixture with a 312-nm UV illuminator (20 min, 25 °C). Workup and PAGE of the cleavage reactions was conducted as described for the Fe(III) complex cleavages of DNA. Appropriate controls of $CoCl_2$ alone, agents in the absence of metal, and reactions conducted in the dark or under room light ensured that the DNA cleavage reactions were due to the Co(III)−agent complexes initiated by 312-nm irradiation. Under these conditions 0.5−2 μM deglycobleomycin A_2 selectively cleaved the w794 DNA.

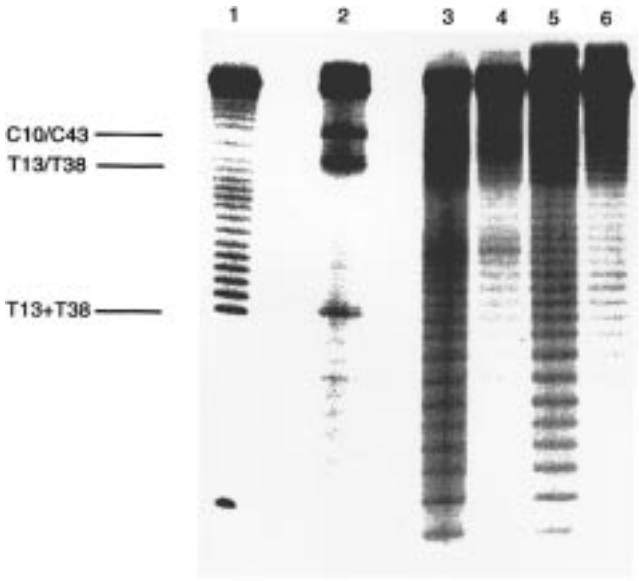

Figure 7. Cleavage of internally radiolabeled hairpin oligonucleotide by Fe(III)-**3** and **4** in HEPES/NaCl buffer containing H_2O_2: Lane 1, G+A Maxam−Gilbert standard (5′-radiolabeled hairpin oligonucleotide); lane 2, 1 μM BLM; lane 3, 400 μM **3**; lane 4, 200 μM **3**; lane 5, 400 μM **4**; lane 6, 200 μM **4**. Lanes 1 and 2 were run on a separate gel under identical conditions and lined up according to an internal standard.

Table 2. Styrene Oxidation[a]

agent	styrene oxide (mM)	phenylacetaldehyde (mM)	ratio	total product (mM)	rel efficiency
Fe(III)−**2**	1.80	1.32	1.36	3.12	1.0
Fe(III)−**3**	1.67	1.21	1.38	2.88	0.92
Fe(III)−**4**	1.11	0.88	1.26	1.99	0.64
Fe(III)−**6**	1.55	1.00	1.55	2.55	0.82
Fe(III)−**7**	1.07	0.79	1.35	1.86	0.59
Fe(III)[b]	0	0		0	0
Fe(III)−**2** (no H_2O_2)	0	0		0	0

[a] 500 μM Fe(III)−agent, 50 mM styrene, 30 mM H_2O_2, 0 °C, 1.5 h, 80% CH_3OH-H_2O. Results reported are the average of two runs. [b] 500 μM Fe(III) under identical conditions with H_2O_2 present.

Fe(III) complexes to mediate the oxidation of styrene were investigated.[62] The oxidation of styrene by deglycobleomycin A_2 produces both styrene epoxide and phenylacetaldehyde. A solution of 500 μM Fe(III)-**2**, 50 mM styrene, and 30 mM H_2O_2 (0 °C, 1.5 h) produced 1.80 mM styrene epoxide and 1.32 mM phenylacetaldehyde constituting slightly over six oxidations for each Fe(III)−**2** utilized (Table 2).

Although the pyrimidine C4 amine of bleomycin A_2 is not directly engaged in the metal chelation, the electronic character of C4 substituents has been shown to affect the oxygen activation properties of the agents.[31,63] Electron-donating substituents have been found to increase the O_2 activation properties of a series of related synthetic metal chelation subunits.[2] Notably, a C4 dimethylamino substituent incorporated into a pyridine analogue (PYML-8) of the pyrimidoblamic acid subunit has been reported to be superior at activating O_2.[2,31] The examination of **3**, **4**, **6**, and **7** revealed that the same products and same product distribution were observed with all four analogues and **2**. Thus, like **2**, **3**, **4**, **6**, and **7** provide competent Fe−oxo intermediates distinguishable from hydroxide radical oxidations. The relative efficiencies of the two dimethylamino analogues (**3** and **6**) were nearly indistinguishable from that of **2** itself, while the efficiencies of the two desamino analogues (**4** and **7**) were somewhat lower. This is consistent with the expectation that a pyrimidine C4 electron-donating substituent would improve the oxygen activation properties of the metal complexes.[2,31] However, both **4** and **7** were still quite effective, providing roughly four oxidations per Fe(III) complex, and this subtle distinction from **2** is insufficient to account for the distinctions in the DNA cleavage efficiencies of the agents. This is especially true of **3** and **6**, which possess the oxidation capabilities of **2** but which failed to produce the characteristic DNA cleavage selectivity.

Discussion. The synthesis and examination of **3** and **4** and their unnatural epimers **6** and **7** by following an alternative approach to the preparation of **2** provide direct evidence for the critical role that the pyrimidoblamic acid C4 amine plays in the polynucleotide recognition of bleomycin A_2. These results support the proposal that CoBLM A_2 is a good mimic of "activated iron BLM" and that the NMR spectrum of the CoBLM species bound site specifically to DNA provides a useful model for the physiologically important FeBLM A_2.[35] Substitution of **2** with a pyrimidine C4 dimethylamino group did not significantly alter the inherent metal chelation, oxygen activation, or oxidation capabilities of the resulting agent **3** but did substantially diminish the DNA cleaving efficiency of the agent (10−15×) and resulted in the loss of the characteristic 5′-GC/5′-GT cleavage selectivity of the parent agent **2**. Similarly, the removal of the C4 pyrimidine amino group of **2** only subtly reduced the oxidation capabilities of the resulting agent **4** (1.6×) but had a much more substantial diminishing effect on the DNA cleavage efficiency of the agent (30×). The characteristic 5′-GC/5′-GT cleavage selectivity of the agent **4** was substantially diminished, and the ability to detect the selectivity above nonselective cleavage was greatly reduced, requiring assay conditions of 4 versus 25−37 °C. Both of these observations are consistent with the involvement of the pyrimidine C4 amine in one of a pair of hydrogen bonds in a triplex-like recognition of the cleavage site guanine (Figure 2). Substitution with the C4 dimethylamino group not only precludes the formation of a pyrimidine C4 amine/guanine N3 hydrogen bond but also sterically prevents formation of the remaining guanine C2 amine/pyrimidine N3 hydrogen bond, reducing the cleavage efficiency and destroying the cleavage selectivity of the agent. In contrast, removal of the C4 amino only precludes formation of the pyrimidine C4 amine/guanine N3 hydrogen bond but does not prevent formation of the remaining guanine C2 amine/pyrimidine N3 hydrogen bond. The resulting interaction involving only one but not both of the key hydrogen bonds is weaker. Consequently, the DNA cleavage efficiency is substantially reduced (30× relative to **2**) and the cleavage selectivity more difficult to detect (4 versus 25−37 °C).

These observations have further implications on the inherent DNA cleavage selectivity of bleomycin A_2 itself. Bleomycin A_2 not only cleaves essentially all 5′-GT and 5′-GC sites in duplex DNA but also cleaves 5′-AT and 5′-AC sites albeit less effectively (25−40% versus 100% of available sites) and typically does so with a weaker relative efficiency of cleavage (Table 3). Although many of the minor or unusual cleavage

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(63) Loeb, K. E.; Zaleski, J. M.; Westre, T. E.; Guajardo, R. J.; Mascharak, P. K.; Hedman, B.; Hodgson, K. O.; Solomon, E. I. *J. Am. Chem. Soc.* **1995**, *117*, 4545.

(64) Full characterization of intermediates are provided in the Supporting Information.

Table 3. Summary of DNA Cleavage Sites for Fe(III)−Bleomycin A_2[a]

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

[a] Within w794 and w836 DNA, ref 44.

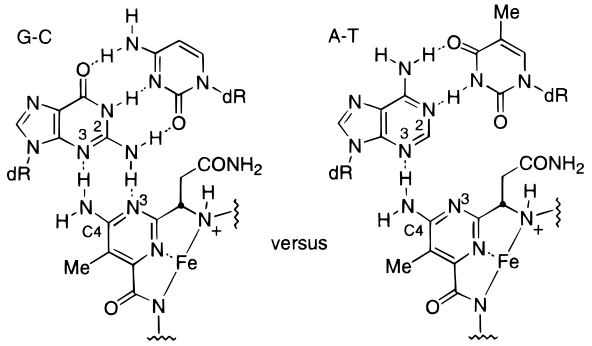

Figure 8.

sites could result from ds cleavage originating from primary sites on the complementary strand, the more general cleavage preference of 5′-GPy versus 5′-APy may be analogously attributed to reduced binding affinity at the 5′-APy sites resulting from one versus two triplex-like hydrogen bonds to adenine (Figure 8). Thus, the behavior of **4** has shed further light on the potential origin of the inherent 5′-GPy > 5′-APy cleavage selectivity of bleomycin A_2. These results complement the studies where Waring and co-workers have shown that the cleavage is greatly diminished but not lost when guanine is replaced with inosine and that when adenine is replaced with 2-aminoadenine the cleavage at pyrimidines 3′ to this site is greatly enhanced.[49] Both of these complementary single site modifications in DNA also support the basis of the hydrogen-bonding specificity proposed.

Experimental Section

(3-(2′-(2-((2(*S*)-(*N*-(4(*R*)-(*N*-(2(*S*)-((*tert*-Butyloxycarbonyl)amino)-3(*R*)-hydroxy-3-(*N*-(triphenylmethyl)imidazol-4-yl)propanoyl)amino)-3(*S*)-hydroxy-2(*S*)-methylpentanoyl)amino)-3(*R*)-hydroxybutanoyl)-amino)ethyl)-2,4′-bithiazole-4-carboxamido)propyl)dimethylsulfonium Chloride (10). A solution of tetrapeptide S[42] (**8**, 26.4 mg, 0.042 mmol), **9**[42] (25 mg, 0.049 mmol, 1.15 equiv), and BOP reagent (27.8 mg, 0.063 mmol, 1.5 equiv) in DMF (450 μL) at 0 °C was treated with *i*-Pr_2NEt (14.6 μL, 0.084 mmol, 2 equiv) under Ar and stirred for 8 h. The DMF was removed in vacuo and the resulting oil triturated with H_2O (2 mL) and filtered through Celite. The resulting solid was dissolved and eluted from the Celite with CH_3OH and concentrated to provide a white solid. Chromatography (C-18, 1.0 × 4.0 cm, 0−90% CH_3OH-H_2O gradient elution) afforded **10** (35.2 mg, 46.9 mg theoretical, 75%; 75−88%) as a white solid: R_f = 0.5 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{25}_D$ +21.5 (*c* 0.06, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 8.12 (s, 1H), 7.42 (br s, 1H), 7.35 (m, 6H), 7.11 (m, 4H), 6.91 (br s, 1H), 4.81 (d, *J* = 6.5 Hz, 1H), 4.36 (d, *J* = 6.5 Hz, 1H), 4.32 (d, *J* = 4.0 Hz, 1H), 3.91 (m, 1H), 3.66 (m, 4H), 3.58 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.36 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.27 (dd, *J* = 6.5, 6.5 Hz, 2H), 2.92 (s, 6H), 2.60 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.13 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.40 (s, 9H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.06 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 168.0, 161.4, 156.9, 154.9, 148.6, 146.7, 140.9, 128.3, 126.7, 122.7, 116.2, 73.6, 66.9, 65.8, 57.1, 41.6, 39.9, 37.4; IR (neat) ν_{max} 3338, 2927, 1651, 1549, 1497, 1446, 1364, 1251, 1148 cm^{-1}; FABHRMS (NBA-CsI) *m*/*z* 1082.4361 (M^+, $C_{54}H_{68}N_9O_9S_3$ requires 1082.4302).

(3-(2′-(2-((2(*S*)-(*N*-(4(*R*)-(*N*-(2(*S*)-Amino-3(*R*)-hydroxy-3-imidazol-4-ylpropanoyl)amino)-3(*S*)-hydroxy-2(*S*)-methylpentanoyl)amino)-3(*R*)-hydroxybutanoyl)amino)ethyl)-2,4′-bithiazole-4-carboxamido)-propyl)dimethylsulfonium Chloride (11, Pentapeptide S). A suspension of **10** (20 mg, 0.018 mmol) in CH_2Cl_2 (200 μL) at 0 °C was treated with 20% TFA−CH_2Cl_2 (200 μL) and the mixture stirred at 0 °C for 2.5 h before the solvent was evaporated in vacuo under a stream of N_2. The residue was dissolved in CH_3OH, and the solution was treated with aqueous NH_4OH (28%, 20 μL) and stirred at 23 °C (1 h). The solvent was removed in vacuo and the mixture purified by reverse-phase chromatography (C-18, 1.0 × 4 cm, 0−60% CH_3OH-H_2O), affording **11** (12.3 mg, 14.0 mg theoretical, 88%) as a white film: R_f = 0.25 (SiO_2, 10:9:1 CH_3OH−10% aqueous $CH_3CO_2NH_4$−10% aqueous NH_4OH); $[\alpha]^{25}_D$ +6.3 (*c* 0.20, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.21 (s, 1H), 8.13 (s, 1H), 7.79 (br s, 1H), 7.15 (br s, 1H), 5.04 (d, *J* = 5.5 Hz, 1H), 4.29 (d, *J* = 4.0 Hz, 1H), 4.14 (d, *J* = 5.5 Hz, 1H), 4.09 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.87 (m, 1H), 3.67 (m, 4H), 3.59 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.37 (dd, *J* = 7.5, 7.5 Hz, 2H), 3.28 (dd, *J* = 6.5, 6.5 Hz, 2H), 2.93 (s, 6H), 2.52 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.14 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.18 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.05 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 177.8, 172.7, 170.8, 166.2, 164.1, 161.2, 152.4, 149.5, 136.7, 132.3, 125.4, 118.6, 117.8, 75.5, 68.5, 66.2, 59.7, 58.4, 44.8, 42.6, 40.1, 38.6, 33.6, 25.6, 25.3, 20.1, 15.6, 14.3; IR (neat) ν_{max} 3266, 2937, 1662, 1549, 1431, 1199, 1127 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 740.2662 (M^+, $C_{30}H_{46}N_9O_7S_3$ requires 740.2682).

Deglycobleomycin A_2 (2). A solution of **12** (3.6 mg, 8 μmol, 1.2 equiv) and **11** (5 mg, 7 μmol) in DMF (100 μL) at 0 °C under Ar was treated with DPPA (2.2 μL, 10 μmol, 1.5 equiv) followed by *i*-Pr_2NEt (3.8 μL, 14 μmol, 3 equiv). The reaction mixture was stirred at 0 °C for 10 h in the dark before the solvent was removed in vacuo. Chromatography (C-18, 1.2 × 4 cm, 0−70% CH_3OH-H_2O gradient elution) afforded **13** (6 mg, 8.1 mg theoretical, 72%) as a white solid.

A solution of **13** (6 mg, 5 μmol) in CH_2Cl_2 (100 μL) at 0 °C was treated with 20% TFA−CH_2Cl_2 (100 μL), and the reaction mixture was stirred at 0 °C for 2 h. The solvent was removed in vacuo. Chromatography (C-18, 1.2 × 4 cm, 0−90% CH_3OH-H_2O gradient elution) afforded **2** (4.6 mg, 5.5 mg theoretical, 86%) as a white solid identical in all respects with authentic material.[21,44]

Ethyl 4-Chloro-2-(diethoxymethyl)-5-methylpyrimidine-6-carboxylate (15). A solution of **14**[52] (2.2 g, 7.64 mmol) in DMF (32 mL) at 23 °C under Ar was treated with freshly distilled $SOCl_2$ (distilled over collidine, 1.1 mL, 15.1 mmol, 2 equiv), and the mixture was stirred (2 h). The mixture was treated with K_2CO_3 (0.5 g) and concentrated to a red oil. Chromatography (SiO_2, 40% EtOAc−hexane) afforded **15** (2.0 g, 2.3 g theoretical, 82%) as a pale yellow oil: R_f = 0.5 (SiO_2, 50% Et_2O−hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.51 (s, 1H), 4.45 (q, *J* = 7.0 Hz, 2H), 3.79 (dq, *J* = 9.5, 7.0 Hz, 2H), 3.66 (dq, *J* = 9.5, 7.0 Hz, 2H), 2.47 (s, 3H), 1.40 (t, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 164.5, 163.8, 163.7, 157.5, 128.0, 101.4, 63.0, 62.6, 15.14, 15.08, 14.0; IR (neat) ν_{max} 2978, 2931, 1732, 1558, 1532, 1381, 1334, 1225, 1115, 1060 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 303.1122 ($M + H^+$, $C_{13}H_{19}N_2ClO_4$ requires 303.1111).

Ethyl 2-(Diethoxymethyl)-4-(dimethylamino)-5-methylpyrimidine-6-carboxylate (16). A solution of **15** (2.0 g, 6.50 mmol) in THF (5 mL) at 23 °C was treated with $HN(CH_3)_2$ (2 M in THF, 6.8 mL, 2.1 equiv), and the mixture was stirred (1.5 h). The reaction mixture was diluted with H_2O (10 mL) and extracted with EtOAc (5 × 10 mL), dried (Na_2SO_4), and concentrated to a pale yellow oil affording **16** (1.98 g, 2.0 g theoretical, 97%) which required no further purification: R_f = 0.45 (SiO_2, 50% Et_2O−hexane); ^1H NMR ($CDCl_3$, 400 MHz) δ 5.48 (s, 1H), 4.39 (q, *J* = 7.0 Hz, 2H), 3.76 (dq, *J* = 9.5, 7.0 Hz, 2H), 3.63 (dq, *J* = 9.5, 7.0 Hz, 2H), 3.13 (s, 6H), 2.28 (s, 3H), 1.38 (t, *J* = 7.0 Hz, 3H), 1.21 (t, *J* = 7.0 Hz, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 166.7, 166.6, 161.7, 156.6, 113.7, 102.4, 62.6, 61.8, 40.6, 16.2, 15.2, 14.1; IR (neat) ν_{max} 2975, 2931, 1735, 1565, 1535, 1400, 1379, 1215,

1116, 1059 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 444.0898 (M + Cs^+, $C_{15}H_{25}N_3O_4$ requires 444.0899).

4-(Dimethylamino)-6-(ethoxycarbonyl)-5-methylpyrimidine-2-carboxaldehyde (17). A solution of **16** (1.97 g, 6.25 mmol) in acetone−H_2O (2:1, 25 mL) was treated with TsOH (108 mg, 0.62 mmol, 0.10 equiv), and the mixture was warmed at 80 °C (8 h). The mixture was neutralized with the addition of 10% aqueous $NaHCO_3$ and extracted with EtOAc (5 × 20 mL), dried (Na_2SO_4), and concentrated to an oil. Chromatography (SiO_2, 30% EtOAc−hexane) afforded **17** (1.42 g, 1.60 g theoretical, 89%): R_f = 0.35 (SiO_2, 50% Et_2O−hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.93 (s, 1H), 4.99 (q, *J* = 7.0 Hz, 2H), 3.16 (s, 6H), 2.38 (s, 3H), 1.41 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.4, 166.5, 165.8, 156.7, 155.7, 117.0, 62.5, 40.6, 16.7, 14.1; IR (neat) ν_{max} 2938, 1728, 1568, 1403, 1380, 1203, 1061 cm^{-1}; FABHRMS (NBA−NaI) *m*/*z* 260.1017 (M + Na^+, $C_{11}H_{15}N_3O_3$ requires 260.1011).

***N*$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-*N*$^{\beta}$-(((4-(dimethylamino)-6-(ethoxycarbonyl)-5-methylpyrimidin-2-yl)methylene)amino)-(*S*)-β-aminoalanineamide (19).** A solution of **17** (35 mg, 0.15 mmol) and **18**[41,43] (33.5 mg, 0.17 mmol, 1.1 equiv) in anhydrous CH_3CN (1.5 mL) was stirred at 23 °C (1 h). The reaction mixture was concentrated in vacuo and pumped dry, affording **19** (63.4 mg, 63.4 mg theoretical, 100%) as a white foam: $[\alpha]^{25}_D$ +38 (*c* 0.85, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 8.18 (s, 1H), 4.40 (q, *J* = 7.0 Hz, 2H), 4.14 (m, 1H), 4.03 (dd, *J* = 5.0, 7.5 Hz, 1H), 3.87 (dd, *J* = 5.0, 12.0 Hz, 1H), 3.15 (s, 6H), 2.28 (s, 3H), 1.37 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.7, 172.9, 169.6, 166.6, 166.3, 163.6, 160.6, 157.3, 156.6, 155.5, 115.2, 70.1, 62.3, 61.5, 54.3, 40.6, 16.5, 14.1; IR (neat) ν_{max} 3333, 2977, 1682, 1564, 1510, 1403, 1366, 1249, 1167, 1062 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 423.2369 (M + H^+, $C_{19}H_{30}N_6O_5$ requires 423.2356).

Diastereoselective Reaction of the Stannous (*Z*)-Enolate of (4*S*,5*R*)-3-((Methylthio)acetyl)-4-methyl-5-phenyl-2-oxazolidinone with 19. A solution of $Sn(OTf)_2$ (245 mg, 0.58 mmol, 4 equiv) dissolved in anhydrous THF (1.0 mL) under Ar was cooled to −78 °C and treated sequentially with (4*S*,5*R*)-3-((methylthio)acetyl)-4-methyl-5-phenyl-2-oxazolidinone (78 mg, 0.29 mmol, 2 equiv) in anhydrous THF (1 mL) and *i*-Pr_2NEt (115 μL, 0.66 mmol, 4.5 equiv). The mixture was stirred for 1 h at −20 °C for complete enolate formation, and the reaction mixture was recooled to −78 °C. A solution of **19** (62 mg, 0.14 mmol, 1 equiv) in anhydrous THF (1.5 mL) was slowly added, and the reaction mixture allowed to warm to 0 °C where it was stirred for 7.5 h. The reaction mixture was poured into a two-layer solution of $CHCl_3$ (15 mL) and saturated aqueous $NaHCO_3$ (7 mL) with vigorous stirring. The organic layer was separated off, dried ($MgSO_4$), and concentrated in vacuo. Chromatography (SiO_2, 3 × 6 cm, 5% CH_3OH−$CHCl_3$) gave 70.2 mg (96.2 mg theoretical, 75%) of a 5.5:1:1 mixture of the diastereomers. Further chromatography (PCTLC, 4 mm SiO_2, 4% CH_3OH−$CHCl_3$) afforded **21** (51 mg), **22** (10 mg), and **23** (9 mg) as white solids.

Ethyl 2(*R*)-(1-((2(*S*)-((*tert*-Butoxycarbonyl)amino)-2-carbamoylethyl)amino)-2-(((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinon-3-yl)carbonyl)-2(*R*)-(methylthio)ethyl)-4-(dimethylamino)-5-methylpyrimidine-6-carboxylate (21): R_f = 0.35 (SiO_2, 15% CH_3OH−$CHCl_3$); $[\alpha]^{25}_D$ −26.3 (*c* 0.315, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42 (m, 5H), 5.82 (d, *J* = 7.5 Hz, 1H), 5.22 (d, *J* = 11 Hz, 1H), 4.85 (m, 1H), 4.41 (q, *J* = 7.0 Hz, 2H), 4.10 (m, 1H), 4.03 (dd, *J* = 5.0, 5.0 Hz, 1H), 3.16 (s, 6H), 2.71 (dd, *J* = 5.0, 5.0 Hz, 2H), 2.31 (s, 3H), 2.05 (s, 3H), 1.39 (t, *J* = 7.0 Hz, 3H), 1.37 (s, 9H), 0.97 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.8, 173.7, 170.4, 166.8, 166.4, 156.1, 155.9, 152.7, 133.2, 129.0, 128.6, 125.7, 113.1, 79.8, 78.7, 64.1, 61.7, 54.9, 53.4, 49.1, 46.5, 40.6, 28.3, 16.3, 14.7, 14.2, 11.9; IR (neat) ν_{max} 3347, 2078, 1777, 1690, 1566, 1365, 1196, 1061 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 820.2118 (M + Cs^+, $C_{32}H_{45}N_7O_8S$ requires 820.2105).

Ethyl 2(*R*)-(1-((2(*S*)-((*tert*-Butoxycarbonyl)amino)-2-carbamoylethyl)amino)-2-(((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinon-3-yl)carbonyl)-2(*S*)-(methylthio)ethyl)-4-(dimethylamino)-5-methylpyrimidine-6-carboxylate (22): R_f = 0.34 (SiO_2, 15% CH_3OH−$CHCl_3$); $[\alpha]^{25}_D$ −9.3 (*c* 0.82, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.37 (m, 5H), 5.72 (d, *J* = 7.5 Hz, 1H), 5.47 (d, *J* = 11 Hz, 1H), 4.35 (m, 1H), 4.40 (q, *J* = 7.0 Hz, 2H), 4.25 (m, 1H), 4.15 (d, *J* = 11.0 Hz, 1H), 3.16 (s, 6H), 2.91 (m, 2H), 2.30 (s, 3H), 2.04 (s, 3H), 1.45 (s, 9H), 1.38 (t, *J* = 7.0 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.9, 171.1, 166.8, 166.4, 165.7, 156.6, 155.8, 133.2, 128.8, 125.6, 112.4, 78.9, 78.8, 64.1, 61.9, 60.5, 54.8, 53.5, 49.9, 41.5, 40.6, 28.3; IR (neat) ν_{max} 2919, 1772, 1681, 1558, 1365, 1193, 1054 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 820.2122 (M + Cs^+, $C_{32}H_{45}N_7O_8S$ requires 820.2105).

Ethyl 2(*S*)-(1-((2(*S*)-((*tert*-Butoxycarbonyl)amino)-2-carbamoylethyl)amino)-2-(((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinon-3-yl)carbonyl)-2(*S*)-(methylthio)ethyl)-4-(dimethylamino)-5-methylpyrimidine-6-carboxylate (23): R_f = 0.33 (SiO_2, 15% CH_3OH−$CHCl_3$); $[\alpha]^{25}_D$ +21.1 (*c* 0.085, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.38 (m, 5H), 5.74 (d, *J* = 8.5 Hz, 1H), 5.12 (d, *J* = 11.0 Hz, 1H), 4.42 (q, *J* = 7.0 Hz, 2H), 4.23 (m, 2H), 4.15 (d, *J* = 11.0 Hz, 1H), 4.09 (m, 1H), 3.15 (s, 6H), 2.89 (m, 2H), 2.31 (s, 3H), 2.04 (s, 3H), 1.42 (s, 9H), 1.39 (t, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.5, 173.5, 171.0, 168.0, 167.6, 166.5, 157.0, 154.5, 135.3, 128.8, 126.5, 125.6, 113.2, 80.3, 64.6, 63.0, 56.0, 55.7, 50.9, 41.0, 30.7, 28.7, 28.3, 16.6, 14.5; IR (neat) ν_{max} 3337, 2929, 1778, 1687, 1563, 1359, 1193, 1059 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 820.2120 (M + Cs^+, $C_{32}H_{45}N_2O_8S$ requires 820.2105).

Ethyl 2(*S*)-(1-((2(*S*)-((*tert*-Butoxycarbonyl)amino)-2-carbamoylethyl)amino)-2-(((4*S*,5*R*)-4-methyl-5-phenyl-2-oxazolidinon-3-yl)carbonyl)ethyl)-4-(dimethylamino)-5-methylpyrimidine-6-carboxylate (24). A solution of **21** (50 mg, 0.72 mm) in C_6H_6 (0.5 mL) was treated with Bu_3SnH (63 μL, 0.23 mmol, 3 equiv) and AIBN (2.5 mg, 0.015 mmol, 2.0 equiv), and the reaction mixture was warmed at 80 °C (1 h) under Ar. The mixture was cooled to 23 °C and the solvent evaporated in vacuo. Chromatography (SiO_2, 2 × 5 cm, 5% CH_3OH−$CHCl_3$) afforded **24** (45 mg, 50 mg theoretical, 90%) as a white solid: R_f = 0.30 (5% CH_3OH−$CHCl_3$); $[\alpha]^{25}_D$ −38 (*c* 0.15, $CHCl_3$); ^{1}H NMR (CD_3OD, 400 MHz) δ 7.41 (m, 5H), 5.74 (d, *J* = 7.0 Hz, 1H), 4.77 (dq, *J* = 6.5, 7.0 Hz, 1H), 4.39 (q, *J* = 7.0 Hz, 2H), 4.16 (dd, *J* = 6.0, 8.0 Hz, 1H), 4.04 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.54 (dd, *J* = 8.0, 16.0 Hz, 1H), 3.25 (dd, *J* = 6.0, 16.0 Hz, 1H), 3.14 (s, 6H), 2.84 (dd, *J* = 6.5, 12.0 Hz, 1H), 2.76 (dd, *J* = 5.5, 12.0 Hz, 1H), 2.28 (s, 3H), 1.41 (s, 9H), 1.37 (t, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 173.6, 170.3, 168.0, 167.6, 166.7, 164.4, 162.3, 157.5, 136.9, 129.5, 129.4, 127.1, 126.6, 113.5, 82.4, 63.0, 61.5, 55.5, 54.8, 53.5, 50.2, 40.9, 28.7, 17.5, 16.4, 14.4; IR (neat) ν_{max} 3333, 2974, 1733, 1687, 1564, 1405, 1379, 1225, 1164 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 774.2217 (M + Cs^+, $C_{31}H_{43}N_7O_8$ requires 774.2227).

Similar treatment of **22** provided **24**, identical in all respects.

***N*$^{\alpha}$-(*tert*-Butoxycarbonyl)-*N*$^{\beta}$-(1-amino-3(*S*)-(4-(dimethylamino)-6-(ethoxycarbonyl)-5-methylpyrimidin-2-yl)propion-3-yl)-(*S*)-β-aminoalanineamide (25).** Solid **24** (24.7 mg, 0.036 mmol) was treated with 15% NH_3-EtOH (4.5 mL), and the solution was stirred for 1.0 h at 0 °C. The solvent was evaporated in vacuo. Chromatography (SiO_2, 2 × 5 cm, 10% CH_3OH−$CHCl_3$) afforded **25** (12.5 mg, 17.3 mg theoretical, 72%) as a white solid: R_f = 0.25 (10% CH_3OH−$CHCl_3$); $[\alpha]^{25}_D$ −28 (*c* 0.28, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.39 (q, *J* = 7.0 Hz, 2H), 4.12 (m, 1H), 4.01 (dd, *J* = 5.0, 8.5 Hz, 1H), 2.82 (dd, *J* = 12.0, 6.0 Hz, 1H), 2.78 (dd, *J* = 12.0, 6.0 Hz, 1H), 2.62 (dd, *J* = 5.5, 15.0 Hz, 1H), 2.54 (dd, *J* = 8.5, 15.0 Hz, 1H), 2.28 (s, 3H), 1.43 (s, 9H), 1.38 (t, *J* = 7.0 Hz, 3H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.5, 176.4, 168.0, 167.7, 167.0, 157.5, 113.5, 102.5, 80.7, 63.1, 61.9, 55.6, 41.9, 41.0, 28.7, 16.4, 14.4; IR (neat) ν_{max} 3323, 2927, 1666, 1531, 1400, 1255, 1164 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 482.2715 (M + H^+, $C_{21}H_{35}N_7O_6$ requires 482.2727).

***N*$^{\alpha}$-(*tert*-Butoxy)carbonyl)-*N*$^{\beta}$-(1-amino-3(*S*)-(6-carboxy-4-(dimethylamino)-5-methylpyrimidin-2-yl)propion-3-yl)-(*S*)-β-aminoalanineamide (26).** A solution of **25** (10.6 mg, 0.002 mmol) in *t*-BuOH−H_2O (2:1, 0.30 mL) at 0 °C was treated with aqueous 1 N LiOH (49 μL, 0.044 mmol, 2 equiv), and the mixture was stirred for 1 h. The solution was acidified to pH 4−5 with the addition of aqueous 1 N HCl, and the solvent was evaporated in vacuo. Chromatography (C-18, 1.2 × 5 cm, 0−40% CH_3OH−H_2O) afforded **26** (9.5 mg, 10 mg theoretical, 95%) as a white solid: R_f = 0.75 (SiO_2, 4:1:1 *i*-PrOH−H_2O−HOAc); $[\alpha]^{25}_D$ −31 (*c* 0.90, CH_3OH); ^{1}H NMR (CD_3OD, 400 MHz) δ 4.18 (m, 2H), 3.16 (s, 6H), 3.14 (m, 1H), 2.94 (dd, *J* = 4.0, 16.0 Hz, 1H), 2.84 (dd, *J* = 8.0, 16.0 Hz, 1H), 2.33 (s, 3H), 1.45 (s,

9H); IR (neat) ν_{max} 3328, 3201, 2975, 1668, 1580, 1448, 1367, 1252, 1160 cm^{-1}; FABHRMS (NBA−NaI) *m*/*z* 476.2247 (M + Na$^+$, $C_{19}H_{31}N_7O_6$ requires 476.2234).

N^{α}-(*tert*-Butoxycarbonyl)-N^{β}-(1-amino-3(*S*)-(4-(dimethylamino)-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)-3(*S*)-hydroxy-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-aminoalanineamide (31). A solution of **26** (7.5 mg, 0.016 mmol, 1.15 equiv) and pentapeptide S (**11**, 11.0 mg, 0.014 mmol, 1.0 equiv) in DMF (200 μL) at 0 °C under Ar was treated with DPPA (4.5 μL, 0.022 mmol, 1.4 equiv) followed by *i*-Pr$_2$NEt (7.5 μL, 0.043 mmol, 2 equiv). The reaction mixture was stirred for 10 h at 0 °C in the dark before the solvent was removed in vacuo. Chromatography (C-18, 1.2 × 4.5 cm, 0−70% CH$_3$OH−H$_2$O gradient elution) afforded **31** (11.5 mg, 16.4 mg theoretical, 72%): R_f = 0.45 (SiO$_2$, 10:9:1 CH$_3$OH−10% aqueous CH$_3$CO$_2$NH$_4$-10% aqueous NH$_4$OH); $[\alpha]^{25}_D$ −5.0 (*c* 0.05, CH$_3$OH); ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.21 (s, 1H), 8.11 (s, 1H), 7.93 (br s, 1H), 7.17 (br s, 1H), 5.17 (d, *J* = 7.0 Hz, 1H), 4.76 (d, *J* = 7.0 Hz, 1H), 4.40 (m, 1H), 4.29 (dd, *J* = 4.0, 5.0 Hz, 1H), 4.11 (m, 1H), 3.97 (dd, *J* = 12.5, 6.5 Hz, 1H), 3.71 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.64 (m, 4H), 3.56 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.35 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.27 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.08 (s, 6H), 2.91 (s, 6H), 2.52 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.31 (s, 3H), 2.12 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.43 (s, 9H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.09 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3291, 1659, 1590, 1487, 1401, 1246, 1206, 1092, 914 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1175.4966 (M$^+$, $C_{49}H_{75}N_{16}O_{12}S_3$ requires 1175.4912).

N^{β}-(1-Amino-3(S)-(4-(dimethylamino)-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)-3(S)-hydroxy-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-aminoalanineamide (3). A solution of **31** (5 mg, 0.005 mmol) in CH$_2$Cl$_2$ (100 μL) at 0 °C was treated with 20% TFA−CH$_2$Cl$_2$ (200 μL) and the mixture stirred at 0 °C (1.5 h). The solvent was evaporated in vacuo. Chromatography (C-18, 1.2 × 2.0 cm, 0−60% CH$_3$OH−H$_2$O gradient elution) afforded **3** (4.7 mg, 5.4 mg theoretical, 88%): R_f = 0.20 (SiO$_2$, 10:9:1 CH$_3$OH−10% aqueous CH$_3$CO$_2$NH$_4$−10% aqueous NH$_4$OH); $[\alpha]^{25}_D$ −18 (*c* 0.078, CH$_3$OH); ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.20 (s, 1H), 8.11 (s, 1H), 7.68 (br s, 1H), 7.10 (br s, 1H), 5.11 (d, *J* = 7.0 Hz, 1H), 4.78 (d, *J* = 7.0 Hz, 1H), 4.11 (dd, *J* = 6.0, 4.0 Hz, 1H), 4.06 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.93 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.83 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.69 (m, 1H), 3.65 (m, 4H), 3.57 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.27 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.07 (s, 6H), 2.92 (s, 6H), 2.76 (dd, *J* = 11.0, 5.0 Hz, 1H), 2.56 (dd, *J* = 15.0, 9.5 Hz, 1H), 2.48 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.30 (s, 3H), 2.16 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.08 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3352, 1647, 1588, 1481, 1255, 1200, 1066, 900 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1075.4366 (M$^+$, $C_{44}H_{67}N_{16}O_{10}S_3$ requires 1075.4388).

N^{β}-(1-Amino-3(*R*)-(4-(dimethylamino)-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)-3(*S*)-hydroxy-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 (6):[64] (86%); R_f = 0.20 (SiO$_2$, 10:9:1 CH$_3$OH-10% aqueous CH$_3$CO$_2$NH$_4$-10% aqueous NH$_4$-OH); $[\alpha]^{25}_D$ −9.0 (*c* 0.090, CH$_3$OH); ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.20 (s, 1H), 8.11 (s, 1H), 7.67 (br s, 1H), 7.10 (br s, 1H), 5.11 (d, *J* = 7.0 Hz, 1H), 4.77 (d, *J* = 7.0 Hz, 1H), 4.10 (m, 1H), 4.07 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.92 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.66 (m, 2H), 3.58 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.27 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.06 (s, 6H), 2.92 (s, 6H), 2.76 (dd, *J* = 11.0, 5.0 Hz, 1H), 2.55 (dd, *J* = 15.0, 9.0 Hz, 1H), 2.48 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.30 (s, 3H), 2.13 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.10 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3280, 1641, 1587, 1550, 1487, 1366, 1241, 1066 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1075.4333 (M$^+$, $C_{44}H_{67}N_{16}O_{10}S_3$ requires 1075.4388).

N^{α}-(*tert*-Butoxy)carbonyl)-N^{β}-(1-amino-3(*S*)-(4-(((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)-3(*S*)-hydroxy-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-aminoalanineamide (50). A solution of **46**[64] (4.0 mg, 0.01 mmol, 1.15 equiv) and pentapeptide S (**11**, 7.0 mg, 0.009 mmol, 1.0 equiv) in DMF (100 μL) at 0 °C under Ar was treated with DPPA (3.2 μL, 0.015 mmol, 1.4 equiv) followed by *i*-Pr$_2$NEt (5.2 μL, 0.03 mmol, 2 equiv). The reaction mixture was stirred for 10 h at 0 °C in the dark before the solvent was removed in vacuo. Chromatography (C-18, 1.2 × 4.5 cm, 0−70% CH$_3$OH−H$_2$O gradient elution) afforded **50** (8.0 mg, 11.3 mg theoretical, 71%): R_f = 0.50 (SiO$_2$, 10:9:1 CH$_3$OH−10% aqueous CH$_3$CO$_2$NH$_4$−10% aqueous NH$_4$OH); $[\alpha]^{25}_D$ +29 (*c* 0.15, CH$_3$OH); ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.70 (s, 1H), 8.19 (s, 1H), 8.11 (s, 1H), 8.04 (br s, 1H), 7.14 (br s, 1H), 4.98 (d, *J* = 7.0 Hz, 1H), 4.59 (d, *J* = 7.0 Hz, 1H), 4.43 (m, 1H), 4.31 (d, *J* = 4.0 Hz, 1H), 4.27 (m, 1H), 4.12 (dd, *J* = 6.5, 4.0 Hz, 1H), 3.92 (dd, *J* = 6.5, 6.5 Hz, 1H), 3.66 (m, 3H), 3.57 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.34 (dd, *J* = 7.5, 7.5 Hz, 2H), 3.27 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.10 (m, 1H), 2.97 (m, 1H), 2.90 (s, 6H), 2.55 (s, 3H), 2.49 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.11 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.42 (s, 9H), 1.16 (d, *J* = 6.5 Hz, 3H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.09 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3313, 2892, 1650, 1587, 1542, 1485, 1241, 1201, 1086 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1132.4471 (M$^+$, $C_{47}H_{70}N_{15}O_{12}S_3$ requires 1132.4491).

N^{β}-(1-Amino-3(*S*)-(4-(((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)-3(*S*)-hydroxy-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-aminoalanineamide (4). A solution of **50** (5 mg, 0.005 mmol) in CH$_2$-Cl$_2$ (100 μL) at 0 °C was treated with 20% TFA−CH$_2$Cl$_2$ (200 μL) and the mixture stirred at 0 °C (1.5 h). The solvent was evaporated in vacuo. Chromatography (C-18, 1.2 × 2.0 cm, 0−60% CH$_3$OH−H$_2$O gradient elution) afforded **4** (4.4 mg, 5.2 mg theoretical, 88%) as a white film: R_f = 0.20 (SiO$_2$, 10:9:1 CH$_3$OH−10% aqueous CH$_3$CO$_2$-NH$_4$−10% aqueous NH$_4$OH); $[\alpha]^{25}_D$ −16 (*c* 0.011, CH$_3$OH); ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.70 (s, 1H), 8.20 (s, 1H), 8.11 (s, 1H), 7.64 (s, 1H), 7.01 (s, 1H), 4.97 (d, *J* = 7.0 Hz, 1H), 4.62 (d, *J* = 7.0 Hz, 1H), 4.31 (d, *J* = 4.0 Hz, 1H), 4.23 (dd, *J* = 6.5, 6.5 Hz, 1H), 4.11 (m, 1H), 4.01 (dd, *J* = 9.0, 4.0 Hz, 2H), 3.93 (dd, *J* = 7.0, 7.0 Hz, 1H), 3.69 (m, 1H), 3.63 (m, 4H), 3.59 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.36 (dd, *J* = 8.0, 7.0 Hz, 2H), 3.27 (m, 2H), 2.92 (s, 6H), 2.68 (dd, *J* = 13.0, 5.0 Hz, 1H), 2.63 (m, 1H), 2.55 (s, 3H), 2.49 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.13 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.17 (d, *J* = 6.5 Hz, 3H), 1.13 (d, *J* = 6.5 Hz, 3H), 1.06 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3304, 1652, 1590, 1549, 1488, 1242, 1206, 1088, 913 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1165.3011 (M$^+$ + Cs, $C_{42}H_{62}N_{15}O_{10}S_3$ requires 1165.3021).

N^{β}-(1-Amino-3(*R*)-(4-(((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)-3(*S*)-hydroxy-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-aminoalanineamide (7):[64] (86%); R_f = 0.20 (SiO$_2$, 10:9:1 CH$_3$OH-10% aqueous CH$_3$CO$_2$NH$_4$−10% aqueous NH$_4$OH); $[\alpha]^{25}_D$ −11 (*c* 0.15, CH$_3$OH); ^{1}H NMR (CD$_3$OD, 400 MHz) δ 8.70 (s, 1H), 8.20 (s, 1H), 8.11 (s, 1H), 7.64 (s, 1H), 7.01 (s, 1H), 4.95 (d, *J* = 7.0 Hz, 1H), 4.61 (d, *J* = 7.0 Hz, 1H), 4.30 (d, *J* = 4.0 Hz, 1H), 4.23 (dd, *J* = 6.5, 6.5 Hz, 1H), 4.11 (m, 1H), 4.00 (m, 2H), 3.92 (dd, *J* = 7.0, 7.0 Hz, 2H), 3.66 (m, 5H), 3.61 (dd, *J* = 6.5, 6.5 Hz, 1H), 3.57 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.36 (dd, *J* = 8.0, 7.5 Hz, 2H), 2.93 (s, 6H), 2.71 (dd, *J* = 11.5, 7.0 Hz, 1H), 2.64 (m, 1H), 2.55 (s, 3H), 2.48 (dq, *J* = 7.0, 7.0 Hz, 1H), 2.15 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.17 (d, *J* = 6.5 Hz, 3H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.06 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3304, 2925, 1647, 1590, 1544, 1488, 1236, 1201, 1093, 913 cm^{-1}; FABHRMS (NBA−CsI) *m*/*z* 1032.3960 (M$^+$, $C_{42}H_{62}N_{15}O_{10}S_3$ requires 1032.3973).

DNA Cleavage and Oxidation Properties. Experimental details for the DNA cleavage studies and the oxidation of styrene may be found in the Supporting Information.

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Supporting Information Available: Full experimental details for the preparation of **2** and **37**−**46**, characterization of **30** and the intermediates **27**−**29**, **32**, **47**−**49**, and **51** leading to the epimeric series **6** and **7**, and experimental details for the DNA cleavage and styrene oxidation studies (8 pages). See any current masthead page for ordering and Internet access instructions.

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