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Synthesis and Evaluation of Potential N^{π} and N^{σ} Metal Chelation Sites within the β-Hydroxy-L-Histidine Subunit of Bleomycin A_2: Functional Characterization of Imidazole N^{π} Metal Complexation

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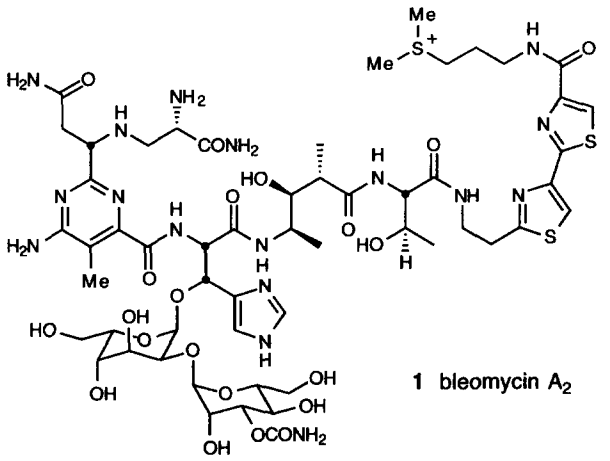
Abstract—The synthesis and evaluation of **4** and **5**, fully functionalized deglycobleomycin A_2 (**2**) analogues incorporating an oxazole and a pyrrole in place of the β-hydroxy-L-histidine imidazole, are detailed. The oxazole agent is only capable of N^{π} metal complexation through a form related to the N^1-H imidazole tautomer of bleomycin A_2 (**1**) while the pyrrole agent may potentially mimic the N^{σ} metal complexation capabilities of the imidazole N^3-H tautomer. Metal complexes (Fe-II, Fe-III) of **4** and **5** were found to cleave duplex DNA in the presence of O_2 (Fe-II) or H_2O_2 (Fe-III). The oxazole agent **4** which is incapable of N^{σ} metal chelation was found to behave analogous to, albeit slightly less effectively than, deglycobleomycin A_2 resulting in the characteristic 5′-GC/5′-GT sequence selective cleavage of duplex DNA directly confirming that imidazole/oxazole N^{π} metal chelation is sufficient for functional reactivity. Importantly, the effective substitution of the oxazole O-1 for the histidine N-1 further illustrates that this group does not require deprotonation upon metal complexation, oxygen activation, or the ensuing oxidation reactions, that the functional bleomycin A_2 tautomer is the imidazole N^1-H tautomer, and that the imidazole N^1-H functionality is not contributing to the polynucleotide recognition through H-bonding to the phosphate backbone or nucleotide bases. In contrast, the pyrrole agent **5** which is incapable of N^{π} metal chelation, but possesses the capabilities of functioning as a N^{σ} metal donor was also found to cleave duplex DNA, but does so in a nonsequence selective fashion with a significantly reduced efficiency and a diminished double to single strand cleavage ratio both only slightly above that of background iron itself. These observations are analogous to those made with **3** which lacks the imidazole altogether and further support the observations that N^{π} coordination, not N^{σ} coordination, of the imidazole is required for the functional activity of bleomycin A_2.

Introduction

Bleomycin A_2 (**1**), the major naturally occurring constituent of the clinical antitumor drug Blenoxane, is thought to derive its therapeutic effects from the ability to mediate the oxidative cleavage of double-stranded DNA[1–15] or RNA[1,16–18] by a process that is metal ion and oxygen dependent. Consequently bleomycin A_2,[19] its naturally occurring congeners,[20] its degradation products,[21–27] semisynthetic derivatives,[28–30] as well as synthetic analogues[31–39] have been the subject of extensive and continued examination in efforts to define the essential functional roles of the individual subunits. The pyrimidoblamic acid subunit along with the adjacent *erythro*-β-hydroxy-L-histidine provide the metal chelation coordination sites required for Fe(II) complexation and molecular oxygen activation responsible for the subsequent DNA cleavage. The small contribution that the metal binding domain makes to the DNA binding affinity has long been recognized[41] and the contribution that this segment may make in polynucleotide recognition remains an active topic of investigation.[18,32,33,36,38,40] The C-terminus tri- and tetrapeptide S subunits including the terminal sulfonium cation and the bithiazole provide the majority of the bleomycin A_2 DNA binding affinity,[41,42] substantially enhance the DNA cleavage efficiency and may contribute to polynucleotide recognition and the resulting DNA cleavage selectivity. Despite the extensive studies on the bleomycins, the nature of the relevant bleomycin A_2 bithiazole binding with duplex DNA is only recently being unraveled and has been proposed to involve intercalation, partial intercalation or minor groove binding.[43,44]

Central to the properties of bleomycin is the metal chelation and subsequent O_2 activation. The commonly accepted depiction of the bleomycin A_2 metal chelation is derived from the X-ray crystal structure of the Cu(II) complex of P-3A, a related natural product, in which the primary and secondary amines of the β-aminoalanine amide side chain, pyrimidine-N1, the β-hydroxy-L-histidine imidazole-N3 and its deprotonated amide were found coordinated to the metal in a square planar, pyramidal complex with the primary amine occupying an axial coordination site.[45] This provided the basis for a proposed structure of bleomycin A_2 metal complexes in which the mannose C3 carbamoyl group occupies a sixth coordination site which is displaced by bound O_2 in the activated complex.[45] NMR[40,46–49] and related spectroscopic



studies[50] of a range of bleomycin A_2 metal complexes have contributed to the consensus that pyrimidine-N1, imidazole-N3 and the secondary amine are bound to the metal. However, their arrangement and the remaining metal ligands remain to be unambiguously established and, more importantly, their relevance to the coordination geometry within the catalytically active metal complex of activated bleomycin A_2 remain unclear.

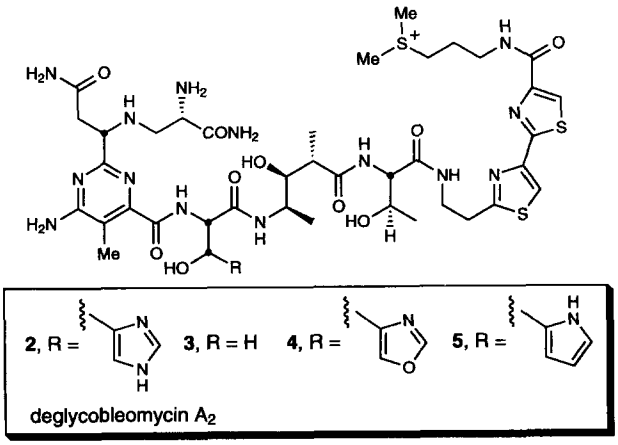
In addition, the nature of the imidazole-N3 complexation may involve either N^{π} or N^{σ} metal coordination. Potentiometric titration of the bleomycin copper complex indicated that between pH 4 and 9 one deprotonated functional group must occupy one of the coordination sites.[45] Since the histidine amide is in a favorable position to form a metal ligand, the deprotonated amide nitrogen was inferred to be one of the coordination sites despite the relative pK_a's of imidazole (14.4) and an amide (17) in the absence of a metal.[45] Moreover, while metal coordination to either the amide or imidazole can be expected to lower their pK_a, the coordination of an imidazole to Fe(II) in a multidentate complex has been reported to lower the pK_a to 8.5–9.0.[46] Though never directly addressed, this was concurrently supported by the X-ray crystal structure of the Cu(II) complex of P-3A and more recently by those of simple model complexes which support both the β-hydroxyhistidine imidazole N^{π} complexation and its deprotonated amide N^{σ} complexation.[33,45] While early NMR studies have addressed this issue and would seem to confirm imidazole N^{π} complexation, these and related spectroscopic studies[46–50] on a range of metal complexes have also suggested that the β-hydroxyhistidine amide nitrogen may not always be involved in bleomycin A_2 complexation and thus cast further doubt on both the nature of the catalytically relevant complex as well as the site of the deprotonated metal ligand. The most recent and impressive studies of Stubbe[40] conducted with the functionally relevant Co—OOH complex of bleomycin A_2 illustrate the accepted pyrimidine-N1 and secondary amine coordination, further establish histidine imidazole N^{π} and deprotonated amide complexation, and favor axial primary amine coordination over the mannose carbamoyl.

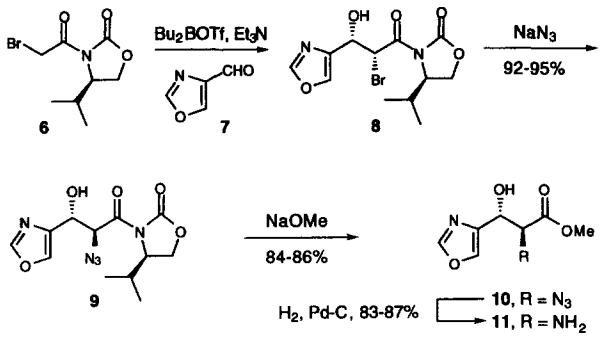
In efforts to help directly resolve such issues and to directly assess the potential functional features of the bleomycin A_2 metal binding domain, herein we report the synthesis and evaluation of **4** and **5**, fully functionalized deglycobleomycin A_2 analogues incorporating an oxazole and a pyrrole in place of the β-hydroxy-L-histidine imidazole. The oxazole agent is only capable of N^{π} metal complexation through a form related to the N^1-H imidazole tautomer of bleomycin A_2 while the later pyrrole agent may potentially mimic the N^{σ} metal complexation capabilities of the imidazole N^3-H tautomer. The results of the studies establish that N^{π} metal chelation is sufficient and required for functional activity, that the functional activity of the imidazole does not require deprotonation and that the functional bleomycin A_2 tautomer is the N^1-H tautomer.

Results

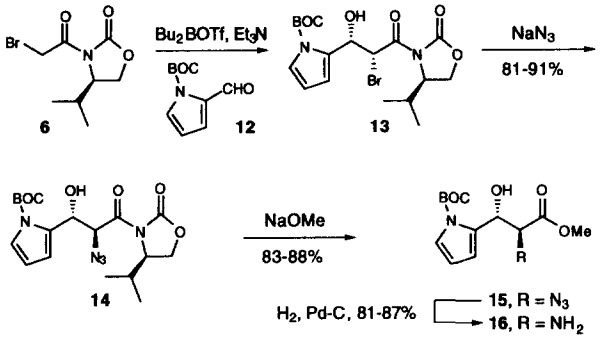
Synthesis of the modified *erythro*-β-hydroxy-L-histidine subunits

Methyl (3*R*,2*S*)-2-amino-3-hydroxy-3-(4′-oxazolyl)propanoate (11) and methyl (3*R*,2*S*)-N^{π}-BOC-2-amino-3-hydroxy-3-(2′-pyrrolyl)propanoate (16). The preparation of **4** and **5** required the synthesis of the appropriately protected *erythro*-β-hydroxy-L-histidine analogues **11** and **16** in which the imidazole was replaced with an oxazole and pyrrole, respectively. Both were prepared according to the approach first implemented for the authentic histidine subunit[42,51] based on the diastereoselective syn aldol addition of the optically active α-bromoacetyl oxazolidinone **6**[52] including modifications that suppress competitive retro aldol reactions, epimerization, and which provide the appropriately protected free amine derivative suitable for direct coupling with the pyrimidoblamic acid subunit without further functionalization. Treatment of oxazole-4-carboxaldehyde (**7**)[53] with the dibutylboronyl *Z*-enolate of **6** (1.1 equiv Bu_2BOTf, 1.4 equiv Et_3N,





Scheme 1.



Scheme 2.

CH_2Cl_2) provided the expected syn aldol product **8** (−78 to 0 °C, 2 h, 57%) as the exclusive product (Scheme 1). Azide displacement of the bromide (5 equiv NaN_3, DMF, 40 °C, 30 min, 92–95%) followed by low temperature methanolysis of the acyl oxazolidinone **9** (0.05 equiv $NaOCH_3$, CH_3OH, −10 °C, 3 min, 84–89%) cleanly provided **10**. Use of LiN_3 in the azide displacement resulted in a competitive retro aldol reaction and the use of stoichiometric $NaOCH_3$, longer reaction periods, or higher reaction temperatures in the conversion of **9** to the methyl ester **10** resulted in competitive epimerization of the C2 center. Azide reduction (cat 10% Pd–C, H_2, 20% EtOH–EtOAc, 25 °C, 1 h, 83–87%) cleanly provided the free amine **11**, $[\alpha]_D^{23}$ −4.9 (*c* 0.25, $CHCl_3$).

Similarly, treatment of N-BOC-pyrrole-2-carboxaldehyde (**12**)[54] with the dibutylboronyl *Z*-enolate of **6** provided the syn aldol product **13** (−78 to 0 °C, 2 h, CH_2Cl_2, 45–56%), Scheme 2. Azide displacement of the bromide (5 equiv NaN_3, DMF, 40 °C, 30 min, 82–91%), methanolysis of the acyl oxazolidinone (0.05 equiv $NaOCH_3$, CH_3OH, 0 °C, 3 min, 83–88%) and reduction of the azide (10% Pd–C, H_2, 20% EtOH–EtOAc, 81–87%) provided the free amine **16**, $[\alpha]_D^{23}$ +21.2 (*c* 0.6, $CHCl_3$). In this instance, the potential that the order of the last two steps could be reversed was also investigated since it offered the advantage that the methanolysis may prove less sensitive to competitive racemization. This proved to be the case and reduction of the azide **14** to the corresponding amine (H_2, Pd–C, 20% EtOH–EtOAc, 23 °C, 1 h, 45%) followed by methanolysis of the acyl oxazolidinone with stoichiometric $NaOCH_3$ or $LiOCH_3$ (0 °C, 5–10 min, 80–84%) cleanly provided **16** under conditions that resulted in racemization of **15**.

Synthesis of 4: the oxazole containing deglycobleomycin A_2

The incorporation of **11** into the oxazole containing deglycobleomycin A_2 analogue **4** proved straightforward. Direct coupling of **11** with N^{α}-BOC-pyrimidoblamic acid (**17**,[55] 1.05 equiv EDCI, 1.0 equiv HOAt, DMF:THF 1:2, 23 °C, 72 h, 54%) provided **18** (Scheme 3). Methyl ester hydrolysis (1.5 equiv LiOH, THF:CH_3OH:H_2O 3:1:1, 0 °C, 1.5 h, 97%) followed by direct coupling of the resulting carboxylic acid **19** with tetrapeptide S hydrochloride (**20**,[42] 1.0 equiv DCC, 1.5 equiv HOAt, 3.0 equiv $NaHCO_3$, DMF, 23 °C, 72 h, 59%) cleanly provided **21**. Notably, this coupling was conducted with no tetrapeptide S protecting groups and the sulfonium salt installed in the substrate and with a single N^{α}-BOC protecting group incorporated into the pyrimidoblamic acid subunit thereby simplifying the final stages of the synthesis of **4**.[37,56] Acid-catalyzed deprotection of **21** (20% TFA–CH_2Cl_2, 0 °C, 4 h, 55–65%) provided **4**, $[\alpha]_D^{23}$ −19 (*c* 0.1, CH_3OH).

Synthesis of 5: the pyrrole containing deglycobleomycin A_2

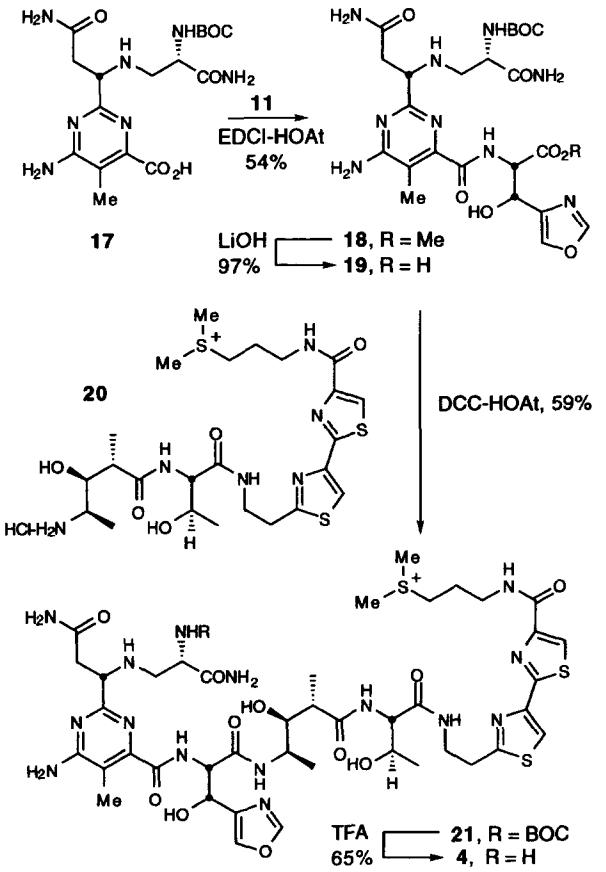
The incorporation of **16** into the pyrrole containing deglycobleomycin A_2 analogue **5** proved more challenging. Direct coupling of **16** with N^{α}-BOC-pyrimidoblamic acid (**17**,[55] 1.05 equiv EDCI, 1.0 equiv HOAt, DMF:THF 1:2, 23 °C, 72 h, 51–56%) proceeded uneventfully to provide **22** in good yield (Scheme 4). However, the subsequent methyl ester hydrolysis of **22** to provided **23** proved problematic and provided competitive removal of the pyrrole N^{π}-BOC protecting group. In initial studies, the best conversions achieved in the attempted selective methyl ester hydrolysis were 20–26% yield of **23** (1.5 equiv LiOH, THF:CH_3OH:H_2O 3:1:1, 0 °C, 45 min) and required its separation from both recovered **22** and **24**. After considerable effort, the selective hydrolysis was accomplished under surprisingly mild reaction conditions (1 equiv $NaHCO_3$, 35% H_2O–*t*-BuOH, 23 °C, 8 h) and provided **23** (61%) in good yield. Following the initial unsuccessful efforts to achieve the selective methyl ester hydrolysis of **22**, we had also elected to remove both the methyl ester and N^{π}-BOC anticipating that the latter could be reinstalled if necessary. Thus, the deliberate hydrolysis of both the methyl ester and N^{π}-BOC was also accomplished by treatment of **22** with excess LiOH (2.5 equiv, THF:CH_3OH:H_2O 3:1:1, 0 °C, 1.5 h, 73%) to provide **24**. Subsequent coupling of **23** or **24** (1.0 equiv DCC, 1.5 equiv HOAt, 3.0 equiv $NaHCO_3$, DMF, 25 °C, 72 h) with tetrapeptide S hydrochloride (**20**)[42] provided **25** (59%) or **26** (57%), respectively, in good yields although the latter coupling without the pyrrole N^{π}-BOC protecting group was

capricious. Acid-catalyzed deprotection of **25** or **26** (20% TFA:CH_2Cl_2, 0 °C, 4 h) both provided **5**, $[\alpha]_D^{23}-10$ (*c* 0.1, CH_3OH) in 60% or 65% yield, respectively.

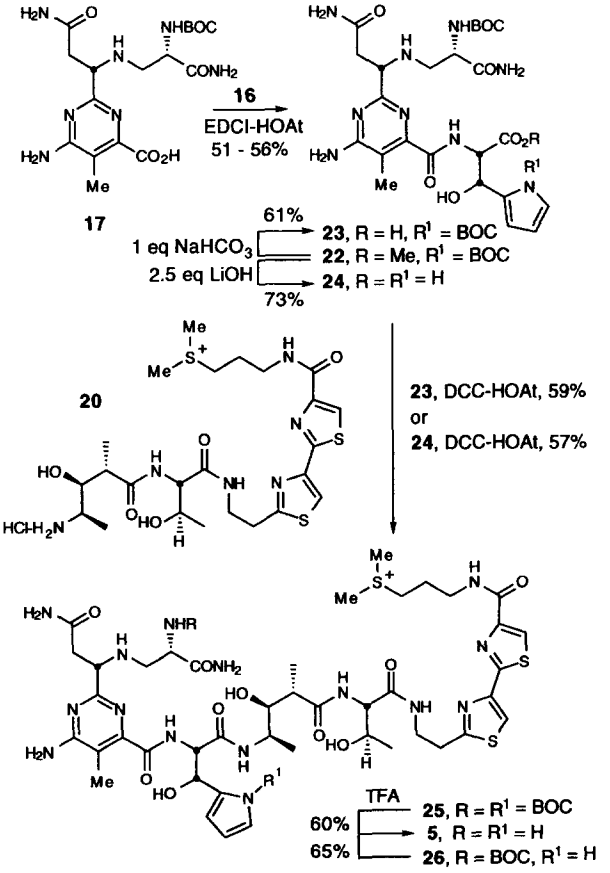
DNA cleavage properties of 4 and 5

Although both single and double strand DNA lesions result from the radical-mediated oxidative cleavage of DNA by bleomycin A_2, the latter have often been considered to be the more significant biological event.[57-60] The relative extent of double strand to single strand DNA cleavage was established in a study of the kinetics of supercoiled ΦX174 DNA cleavage to produce linear and circular DNA. The results are illustrated in Figure 1 for **4**, and the results obtained with the full set of agents are summarized in Table 1. 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 metal complex reactivation kinetics. We assumed a Poisson distribution for the formation of single strand and double strand breaks to calculate the average number of double and single strand cuts per DNA molecule using the Freifelder–Trumbo equation.[61] The data for the first few minutes could be fitted to a linear equation and the ratio of double strand to single strand cuts observed with the Fe(II) complexes of **4–5** and related agents are summarized in Table 1. Provided the reaction is not taken to high percentage DNA cleavage, even the latter data points provide comparable albeit slightly less accurate estimates of the ds:ss cleavage ratio. The ratio of double to single strand DNA cleavage for the oxazole containing agent **4** (1:26) was reduced by only a factor of 2 relative to deglycobleomycin A_2 (1:12) indicating a minor but potentially significant alteration. The ratio of double to single strand DNA cleavage for **5** was established to be 1:44 which was substantially lower than bleomycin A_2 (1:6) or deglycobleomycin A_2 (1:12), essentially identical to that established for **3** (1:57)[37] which lacks the entire imidazole unit and approaches the ratio derived from uncomplexed Fe(II) cleavage (1:98). A theoretical ratio of approximately 1:100 is required in order for the linear DNA to be the result of the random accumulation of single strand breaks within the 5386 base-pair size of ΦX174 DNA assuming that sequential cleavage on the complementary strands within 15 base-pairs is required to permit formation of linear DNA from the hybridized duplex DNA. Experimentally it was determined that Fe(II) alone produced a ratio of 1:98 double:single strand breaks under our conditions of assay consistent with the theoretical ratio.

Most revealing was the comparison of the DNA cleavage selectivity of **2–5**. The selectivity of DNA cleavage along with an assessment of the relative efficiency of DNA cleavage were examined within duplex w794 DNA[62,63] by monitoring strand cleavage of



Scheme 3.



Scheme 4.

singly ^{32}P 5′-end-labeled double-stranded DNA after exposure to the Fe(III)-complex followed by activation with H_2O_2[64] in 10 mM phosphate buffer (pH 7.0).[38,42] This protocol has proven to be more sensitive to the distinctions in the relative efficiency of DNA cleavage by related agents than the ΦX174 supercoiled DNA cleavage assays but both assays have to date provided qualitatively similar results. Thus, incubation of the labeled duplex DNA with **4** or **5** in the presence of equimolar $FeCl_3$ and excess H_2O_2 led to DNA cleavage. Following a quench of the reaction with the addition of glycerol, removal of the agent by EtOH precipitation of the DNA, resuspension of the treated DNA in aqueous buffer and high resolution polyacrylamide gel electrophoresis (PAGE) of the resultant DNA under denaturing conditions adjacent to Sanger sequencing standards permitted the identification of the sites of DNA cleavage. The behavior of Fe(III)-**4** was exactly analogous to deglycobleomycin A_2 and produced the identical 5′-GC/5′-GT cleavage selectivity with no substantive distinctions (Fig. 2). Moreover, even in this sensitive assay where few of the extensive range of agents examined to date approximate the relative efficiency of **2**,[35–39] Fe(III)-**4** was nearly as efficient (0.5×) as deglycobleomycin A_2. In sharp contrast, Fe(III)-**5** was at least 6× less efficient than deglycobleomycin A_2 and was found to cleave the DNA in a nonsequence selective fashion exhibiting no preference for reacting at the characteristic 5′-GC/5′-GT sites (Fig. 2).

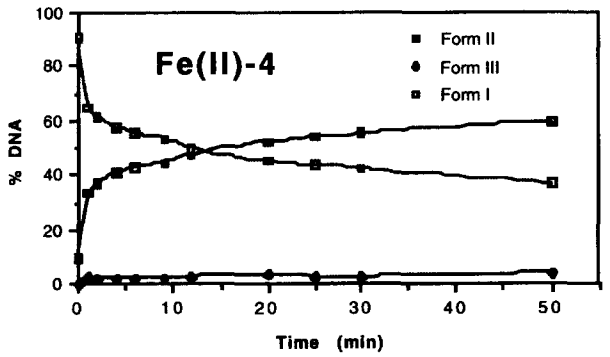


Figure 1. Kinetics of supercoiled ΦX174 DNA cleavage by Fe(II)-**4** (12 μM) in buffer solutions containing 2-mercaptoethanol. The DNA cleavage reactions were run at 25 °C for various lengths of time and electrophoresis was conducted on a 1.0% agarose gel. Direct fluorescence quantitation of the percentage 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^{-1} 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).

Over a wide range of conditions, no changes in the observations were found for both **5** or deglycobleomycin A_2 with the latter producing the characteristic DNA cleavage of bleomycin A_2 and the former cleaving DNA in a nonselective manner. Under all conditions examined, **5** was found to cleave DNA above background Fe(III) and to do so in a nonsequence selective fashion. The Fe(III) complex of **5** was 3× more effective than Fe(III) itself, comparable in effectiveness to **3** lacking the imidazole altogether and 6× less effective than deglycobleomycin A_2 and 10–25× less effective than bleomycin A_2. Comparisons alongside the Fe(II) or Fe(III) complexes of bleomycin A_2 and deglycobleomycin A_2 assured that the protocols employed would permit detection of the characteristic sequence selective DNA cleavage reaction.

Discussion

Metal complexes of **4** and **5** were found to cleave duplex DNA in the presence of O_2 (Fe-II) or H_2O_2 (Fe-III) above background cleavage. The oxazole agent **4** which is incapable of N^{σ} metal chelation was found to behave in a manner analogous to to deglycobleomycin A_2 and, although it proved slightly less effective, it produced the characteristic 5′-GC/5′-GT sequence selective cleavage of duplex DNA establishing that N^{π} metal chelation is sufficient for functional reactivity. The effective substitution of the oxazole O-1 for the histidine imidazole N-1 further illustrated that this group does not require deprotonation upon metal complexation, O_2 activation, or the ensuing oxidation reactions, that the functional bleomycin A_2 tautomer is the N^1-H tautomer (Fig. 3) and that the imidazole N^1-H functionality is not contributing to the polynucleotide recognition through H-bonding to phosphate backbone or nucleotide bases. Like **3**[37] which

Table 1. Summary of DNA cleavage properties of **4–5** and related agents

Agent	Relative efficiency of DNA cleavage[a]	Ratio of double to single strand DNA cleavage[b]	DNA cleavage selectivity[a]
	w794[a]	ΦX174	
1, Bleomycin A_2	5.8	1:6	5′-GC, 5′-GT > 5′-GA
2, Deglycobleomycin A_2	1.0	1:12	5′-GC, 5′-GT > 5′-GA
3[c]	0.08	1:57	None
4	0.5	1:26	5′-GC, 5′-GT > 5′-GA
5	0.15	1:44	None
Fe[a,b]	0.06	1:98	None

[a]Examined within 5′-^{32}P-end-labelled w794 DNA, Fe(III)-H_2O_2.
[b]Ratio of double to single stranded cleavage of supercoiled ΦX174 DNA calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$.
[c]Taken from ref. 37.

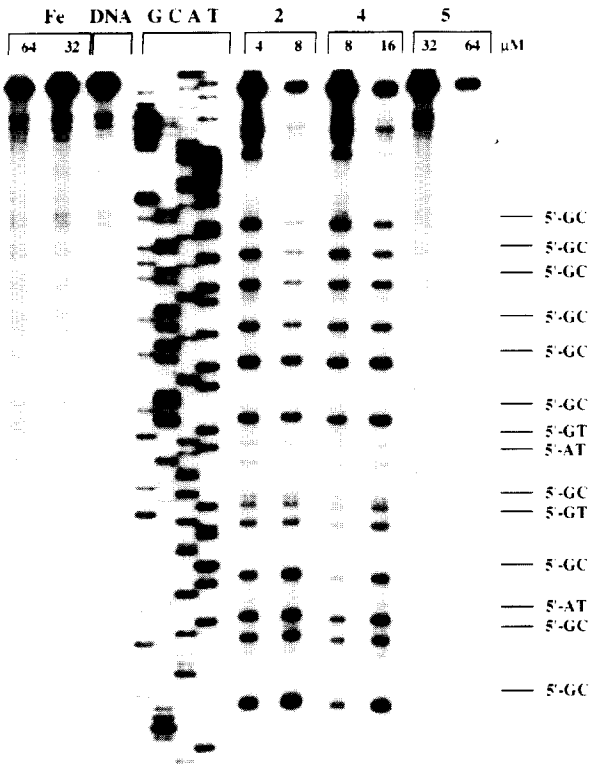


Figure 2. Cleavage of double strand DNA by Fe(III)-agents (SV40 DNA fragment, 144 base-pairs, nucleotide no. 5238–138, clone w794) in phosphate–KCl buffer containing H_2O_2. The DNA cleavage reactions were run for 30 min at 37 °C and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.

lacks the imidazole altogether, the pyrrole agent **5** which is incapable of N^{π} metal chelation but possesses potential N^{σ} coordination capabilities was also found to cleave duplex DNA but does so in a nonsequence selective fashion with a significantly reduced efficiency and a diminished double to single strand cleavage ratio further supporting the conclusion that N^{π} coordination, not N^{σ} coordination, is required for the functional activity of bleomycin A_2. It is likely that the pyrrole agent **5** behaves analogous to the agent **3** lacking the imidazole or a substitution group altogether and that their nonsequence selective cleavage of DNA above that of background iron is derived from a diffusible oxidant (e.g., HO·)[37] generated through oxygen activation by a bound or partially bound metal that is nonfunctional. Regardless of the origin of these latter observations, the ineffectual behavior of **3** and **5** versus **4** establish the requirement for the imidazole and are consistent with the proposals that imidazole N^{π} metal coordination is required for functional activity. These studies, in conjuction with those that demonstrate that the functional activity of **2** also requires the L-histidine secondary amide[65] should prove useful in establishing the catalytically relevant species responsible for the functional behavior of the deglycobleomycin A_2 metal complexes.

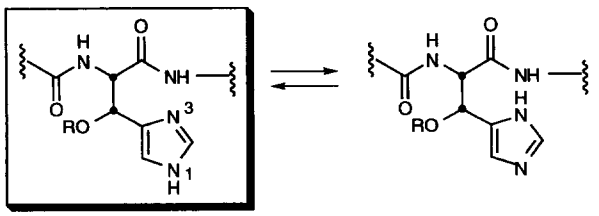


Figure 3.

Experimental

(4*R*,3′*R*,2′*R*)-3-[2′-Bromo-3′-hydroxy-3′-(oxazol-4″-yl) propionyl]-4-isopropyl-2-oxazolidinone (8). A solution of **6** (0.52 mmol, 104 mg) in anhydrous CH_2Cl_2 (0.5 mL) under Ar at −78 °C was treated with Bu_2BOTf (1.1 equiv, 0.57 mmol, 0.099 mL) followed by Et_3N (1.4 equiv, 0.73 mmol, 0.080 mL), and the mixture was stirred at −78 °C (30 min) and 23 °C (2 h). The mixture was recooled to −78 °C and **7**[53] (1.0 equiv, 0.52 mmol, 40 mg) in CH_2Cl_2 (0.5 mL) was added slowly. The mixture was stirred at −78 °C (30 min) and 0 °C (1.5 h). The reaction mixture was diluted with Et_2O:CH_2Cl_2 (10 mL, 2:1) and extracted with 1 N aqueous $KHSO_4$ (1 × 5 mL) and saturated aqueous NaCl (1 × 5 mL) before the organic phase was concentrated in vacuo. The crude oil was dissolved in CH_3OH (2 mL) at 0 °C and 30% aqueous H_2O_2 (0.74 mL) was added slowly. The mixture was stirred at 0 °C (1 h) before being concentrated in vacuo to remove the CH_3OH. Water (3 mL) was added and the residue was extracted with Et_2O:CH_2Cl_2 (3 × 10 mL, 2:1). The combined organic phase was washed with 5% aqueous $NaHCO_3$ (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, 50% EtOAc–hexane) gave **8** (102 mg, 179 mg theoretical, 57%) as a white solid: R_f 0.30 (SiO_2, 50% EtOAc–hexane); mp 169–170 °C; $[\alpha]_D^{23}$ −15.8 (*c* 0.1, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.83 (s, 1H), 7.75 (s, 1H), 6.03 (d, *J* = 4.0 Hz, 1H), 5.18 (d, *J* = 4.0 Hz, 1H), 4.50 (m, 1H), 4.32 (m, 2H), 2.43 (m, 1H), 0.95 (d, *J* = 6.0 Hz, 3H), 0.91 (d, *J* = 6.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 169.5, 152.4, 151.0, 138.9, 136.9, 67.3, 63.5, 58.2, 47.8, 29.7, 27.9, 17.8, 14.8; IR (neat) ν_{max} 3514, 2917, 1792, 1693, 1513, 1478, 1384, 1217, 1111, 1056, 914 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 369.0024 ($M^+ + Na$, $C_{12}H_{15}BrN_2O_5$ requires 369.0062).

(4*R*,3′*R*,2′*S*)-3-[2′-Azido-3′-hydroxy-3′-(oxazol-4″-yl) propionyl]-4-isopropyl-2-oxazolidinone (9). A solution of **8** (0.25 mmol, 85 mg) in anhydrous DMF (8 mL) was treated with NaN_3 (5 equiv, 1.23 mmol, 80 mg) and the mixture was stirred at 40 °C (30 min). The solution was poured into H_2O (10 mL), and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with H_2O (5 × 20 mL) and saturated aqueous NaCl (1 × 20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 8 cm, 40% EtOAc–hexane) afforded **9** (70 mg, 76 mg theoretical, 92%) as a white solid; R_f 0.50 (SiO_2 50% EtOAc–hexane); mp 80–81 °C; $[\alpha]_D^{23}$ −21 (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.88 (s, 1H),

7.76 (s, 1H), 5.52 (d, $J=8.0$ Hz, 1H), 5.04 (d, $J=8.0$ Hz, 1H), 4.50 (m, 1H), 4.38 (m, 2H), 2.41 (m, 1H), 0.93 (d, $J=6.0$ Hz, 3H), 0.90 (d, $J=6.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 169.2, 151.5, 138.8, 136.5, 76.5, 67.7, 63.9, 61.5, 58.9, 28.2, 17.8, 14.5; IR (neat) ν_{max} 3451, 2963, 2114, 1775, 1702, 1388, 1202, 1056, 915 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 332.0983 ($M^+ + Na$, $C_{12}H_{15}N_5O_5$ requires 332.0971).

Methyl (3*R*,2*S*)-2-azido-3-hydroxy-3-(4′-oxazolyl) propanoate (10). A solution of **9** (0.178 mmol, 55 mg) in CH_3OH (0.050 mL) under Ar at −10 °C was treated with $NaOCH_3$ (0.05 equiv, 0.007 mL, 2 M solution in CH_3OH) and the mixture was stirred at −10 °C (3 min). The reaction mixture was quenched by the addition of pH 7 phosphate buffer (0.70 mL) and the mixture was partitioned between saturated aqueous NaCl:NH_4Cl (1:1, 0.70 mL) and CH_2Cl_2 (2 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 2 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, 45–75% EtOAc–hexane gradient elution) afforded **10** (32 mg, 38 mg theoretical, 84%) as an oil: R_f 0.45 (SiO_2, 50% EtOAc–hexane); $[\alpha]_D^{23}$ −33 (*c* 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.86 (s, 1H), 7.68 (s, 1H), 5.09 (dd, $J=12.0$, 6.0 Hz, 1H), 4.38 (d, $J=6.0$ Hz, 1H), 3.81 (s, 3H), 3.13 (d, $J=6.0$ Hz, 1H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 168.7, 151.3, 138.5, 136.3, 67.5, 65.1, 52.8; IR (neat) ν_{max} 3381, 2940, 2160, 1715, 1505, 1418, 1243, 1089 cm^{-1}; FABHRMS (NBA) *m/e* 213.0617 ($M^+ + H$, $C_7H_8N_4O_4$ requires 213.0624).

Methyl (3*R*,2*S*)-2-amino-3-hydroxy-3-(4′-oxazolyl) propanoate (11). A solution of **10** (0.24 mmol, 50 mg) in 20% EtOH–EtOAc (10 mL) at 23 °C was combined with 10% Pd–C (20% by weight, 10 mg), and the mixture was vacuum-purged with H_2 (20 ×). The reaction mixture was stirred under a H_2 atmosphere at 23 °C for 1 h. The mixture was filtered through a pad of Celite, the catalyst washed with EtOAc and the filtrate was concentrated in vacuo. Chromatography (SiO_2, 2 × 10 cm, 10% CH_3OH–CH_2Cl_2) gave **11** (36 mg, 44 mg theoretical, 82%) as an oil: R_f 0.50 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]_D^{23}$ −4.9 (*c* 0.25, $CHCl_3$); ^{1}H NMR (CD_3OD, 250 MHz) δ 8.04 (s, 1H), 7.76 (s, 1H), 4.80 (d, $J=12.0$ Hz, 1H), 3.75 (d, $J=12.0$ Hz, 1H), 3.61 (s, 3H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 173.4, 151.1, 139.8, 136.3, 67.3, 58.2, 52.2; IR (neat) ν_{max} 3381, 2940, 2160, 1715, 1505, 1418, 1243, 1089 cm^{-1}; FABHRMS (NBA) *m/e* 187.0712 ($M^+ + H$, $C_7H_{10}N_2O_4$ requires 187.0719).

(4*R*,3′*R*,2′*R*)-N$^{\pi}$-BOC-3-[2′-Bromo-3′-hydroxy-3′-(pyrrol-2″-yl)propionyl]-4-isopropyl-2-oxazolidinone (13). A solution of **6** (0.60 mmol, 150 mg) in anhydrous CH_2Cl_2 (0.56 mL) under Ar at −78 °C was treated with Bu_2BOTf (1.1 equiv, 0.66 mmol, 0.144 mL) followed by Et_3N (1.4 equiv, 0.84 mmol, 0.117 mL) and the mixture was stirred at −78 °C (30 min) and 23 °C (2 h). The mixture was recooled to −78 °C, and **12**[54] (1.0 equiv, 0.60 mmol, 117 mg) in CH_2Cl_2 (0.56 mL) was added slowly. The mixture was stirred at −78 °C (30 min) and 0 °C (1.5 h). The reaction mixture was diluted with Et_2O:CH_2Cl_2 (10 mL, 2:1) and extracted with 1 N aqueous $KHSO_4$ (1 × 5 mL) and saturated aqueous NaCl (1 × 5 mL) before the organic phase was concentrated in vacuo. The crude oil was dissolved in CH_3OH (2 mL) at 0 °C and 30% aqueous H_2O_2 (0.84 mL) was added slowly. The mixture was stirred at 0 °C (1 h) before being concentrated in vacuo to remove the CH_3OH. Water (3 mL) was added and the residue was extracted with Et_2O:CH_2Cl_2 (3 × 10 mL, 2:1). The combined organic phase was washed with 5% aqueous $NaHCO_3$ (1 × 10 mL) and saturated aqueous NaCl (1 × 10 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 5 cm, 10–20% EtOAc–hexane gradient elution) gave **13** (143 mg, 266 mg theoretical, 54%) as a white solid: R_f 0.33 (SiO_2, 25% EtOAc–hexane); mp 64 °C; $[\alpha]_D^{23}$ +9.2 (*c* 0.13, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.16 (dd, $J=3.0$, 1.5 Hz, 1H), 6.26 (dd, $J=3.0$, 1.5 Hz, 1H), 6.22 (t, $J=7.5$ Hz, 1H), 6.08 (t, $J=3.0$ Hz, 1H), 5.39 (dd, $J=8.5$, 7.5 Hz, 1H), 4.60 (d, $J=7.5$ Hz, 1H), 4.32 (m, 1H), 4.15 (d, $J=6.0$ Hz, 1H), 2.32 (m, 1H), 1.58 (s, 9H), 0.90 (d, $J=6.0$ Hz, 3H), 0.88 (d, $J=6.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.1, 131.7, 122.8, 114.9, 110.5, 85.3, 76.5, 67.7, 63.8, 63.2, 58.2, 47.1, 27.9, 27.8, 17.8, 17.7, 14.7, 14.6; IR (neat) ν_{max} 3458, 2967, 1781, 1731, 1703, 1484, 1372, 1202, 1061 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 467.0799 ($M^+ + Na$, $C_{18}H_{25}BrN_2O_6$ requires 467.0794).

(4*R*,3′*R*,2′*S*)-N$^{\pi}$-BOC-3-[2′-Azido-3′-hydroxy-3′-(pyrrol-2″-yl)propionyl]-4-isopropyl-2-oxazolidinone (14). A solution of **13** (0.47 mmol, 208 mg) in anhydrous DMF (14 mL) was treated with NaN_3 (5 equiv, 2.33 mmol, 152 mg) and the mixture was stirred at 40 °C (30 min). The solution was poured into H_2O (10 mL), and the aqueous phase was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with H_2O (5 × 20 mL) and saturated aqueous NaCl (1 × 20 mL), dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 8 cm, 50% EtOAc–hexane) afforded **14** (158 mg, 190 mg theoretical, 82%) as a white solid; R_f 0.25 (SiO_2, 25% EtOAc–hexane); mp 63–64 °C; $[\alpha]_D^{23}$ −35 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 250 MHz) δ 7.19 (dd, $J=3.0$, 1.5 Hz, 1H), 6.34 (dd, $J=3.0$, 1.5 Hz, 1H), 6.13 (t, $J=7.5$ Hz, 1H), 5.66 (t, $J=8.5$ Hz, 1H), 5.22 (dd, $J=8.5$, 7.5 Hz, 1H), 4.52 (m, 1H), 4.25 (m, 2H), 2.34 (m, 1H), 1.58 (s, 9H), 0.91 (d, $J=6.0$ Hz, 3H), 0.89 (d, $J=6.0$ Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 169.9, 153.4, 150.1, 132.4, 123.1, 114.6, 110.6, 85.3, 68.6, 64.3, 63.4, 59.3, 58.7, 52.5, 28.1, 27.8, 17.8, 14.5; IR (neat) ν_{max} 3474, 2946, 2092, 1779, 1738, 1712, 1389, 1338, 1200, 1123, 1056 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 430.1695 ($M^+ + Na$, $C_{18}H_{25}N_5O_6$ requires 430.1703).

Methyl (3*R*,2*S*)-N$^{\pi}$-BOC-2-azido-3-hydroxy-3-(2′-pyrrolyl)propanoate (15). A solution of **14** (0.74 mmol, 300 mg) in CH_3OH (1.5 mL) under Ar at −10 °C was treated with $NaOCH_3$ (0.05 equiv, 0.019 mL, 2 M solution in CH_3OH) and the mixture was stirred at −10 °C (3 min). The reaction mixture was quenched

by the addition of pH 7 phosphate buffer (3.0 mL), and the mixture was partitioned between saturated aqueous NaCl:NH_4Cl (1:1, 3.0 mL) and CH_2Cl_2 (5 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL). The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. Chromatography (SiO_2, 2 × 10 cm, 15–20% EtOAc–hexane gradient elution) afforded **15** (192 mg, 228 mg theoretical, 83%) as an oil: R_f 0.25 (SiO_2, 20% EtOAc–hexane); $[\alpha]_D^{23}$ −5.5 (*c* 0.36, $CHCl_3$); ^1H NMR ($CDCl_3$, 250 MHz) δ 7.18 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.34 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.14 (t, *J* = 3.0 Hz, 1H), 5.21 (dd, *J* = 8.5, 7.5 Hz, 1H), 4.54 (d, *J* = 7.5 Hz, 1H), 4.22 (d, *J* = 8.5 Hz, 1H), 3.79 (s, 3H), 1.59 (s, 9H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 169.1, 150.2, 132.7, 123.1, 122.6, 115.0, 113.9, 110.6, 85.2, 69.2, 64.9, 52.7, 27.9; IR (neat) ν_{max} 3465, 2980, 2359, 2110, 1741, 1343, 1166, 1129, 1059 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 333.1164 (M^+ + Na, $C_{13}H_{18}N_4O_5$ requires 333.1175).

Methyl (3*R*,2*S*)-N$^{\alpha}$-BOC-2-amino-3-hydroxy-3-(2′-pyrrolyl)propanoate (16). A solution of **28** (0.13 mmol, 39 mg) in 20% EtOH–EtOAc (8 mL) at 23 °C was combined with 10% Pd–C (20% by weight, 8 mg), and the mixture was vacuum-purged with H_2 (20 ×). The reaction mixture was stirred under a H_2 atmosphere at 23 °C for 1.5 h. The mixture was filtered through Celite, the catalyst was washed with EtOAc and the filtrate was concentrated in vacuo. Chromatography (SiO_2, 2 × 10 cm, 50% EtOAc–hexane) gave **16** (29 mg, 36 mg theoretical, 81%) as an oil: R_f 0.12 (SiO_2 50% EtOAc–hexane); $[\alpha]_D^{23}$ +21.2 (*c* 0.6, $CHCl_3$); ^1H NMR (CD_3OD, 400 MHz) δ 7.15 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.25 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.07 (t, *J* = 3.0 Hz, 1H), 5.39 (d, *J* = 6.0 Hz, 1H), 3.78 (d, *J* = 6.0 Hz, 1H), 3.51 (s, 3H), 1.54 (s, 9H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 174.3, 150.1, 133.6, 122.6, 121.4, 113.9, 111.5, 110.5, 84.9, 70.7, 58.2, 52.1, 27.9; IR (neat) ν_{max} 3372, 2979, 1737, 1371, 1332, 1163, 1125, 1053 cm^{-1}; FABHRMS (NBA) *m/e* 285.1455 (M^+ + H, $C_{13}H_{20}N_2O_5$ requires 285.1450).

N$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-N$^{\beta}$-[1-amino-3(*S*)-(4-amino-6-(amido-(3-(4′-oxazolyl)-3(*R*)-hydroxy-2(*S*)-amino) methylpropionyl)-5-methylpyrimidin-2-yl) propion-3-yl]-(*S*)-β-aminoalanine amide (18). A solution of **17**[55] (0.008 mmol, 3.3 mg) in THF:DMF (2:1, 0.008 mL) was treated with **16** (0.008 mmol, 1.4 mg, 1.1 equiv), HOAt (0.008 mmol, 1.0 mg, 1.0 equiv) and EDCI (0.008 mmol, 1.5 mg, 1.05 equiv), and the mixture was stirred under Ar at 23 °C (72 h). The reaction mixture was concentrated in vacuo to give an oil. Chromatography (SiO_2, 0.5 mm PCTLC, 20% CH_3OH–CH_2Cl_2) afforded **18** (2.5 mg, 4.6 mg theoretical, 54%) as a white film: R_f 0.30 (SiO_2, 20% CH_3OH–CH_2Cl_2); $[\alpha]_D^{23}$ −36 (*c* 0.05, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.09 (s, 1H), 7.81 (s, 1H), 5.12 (d, *J* = 5.0 Hz, 1H), 4.92 (d, *J* = 5.0 Hz, 1H), 4.05 (dd, *J* = 8.5, 5.5 Hz, 1H), 3.89 (dd, *J* = 6.5, 5.5 Hz, 1H), 3.61 (s, 3H), 2.79 (dd, *J* = 12.5, 8.5 Hz, 2H), 2.67 (dd, *J* = 12.0, 5.5 Hz, 1H), 2.56 (dd, *J* = 15, 5.5 Hz, 3H), 2.49 (dd, *J* = 15.0, 8.5 Hz, 1H), 2.21 (s, 3H), 1.34 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.1, 167.5, 165.5, 153.7, 152.2, 151.0, 148.9, 136.0, 128.7, 123.5, 120.1, 110.1, 84.6, 76.2, 64.9, 59.2, 57.4, 56.2, 53.2, 50.1, 27.8, 26.1, 23.5, 9.0; IR (neat) ν_{max} 3374, 2923, 2851, 1733, 1666, 1553, 1512, 1441, 1394, 1364, 1256, 1164 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 726.1593 (M^+ + Cs, $C_{24}H_{35}N_9O_9$ requires 726.1612).

N$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-N$^{\beta}$-[1-amino-3(*S*)-(4-amino-6-(amido-(3-(4′-oxazolyl)-3(*R*)-hydroxy-2(*S*)-amino) propanoic acid)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine amide (19). A solution of **18** (0.0016 mmol, 1.0 mg) in THF:CH_3OH:H_2O (3:1:1, 0.042 mL) was cooled to 0 °C and treated with aqueous 1 N LiOH (0.002 mmol, 2.6 μL, 1.5 equiv). The reaction mixture was stirred at 0 °C (1.5 h) with monitoring by TLC (SiO_2, 10% CH_3OH–CH_2Cl_2). After the THF and CH_3OH were evaporated under a stream of N_2 at 0 °C, H_2O (0.5 mL) and EtOAc (0.5 mL) were added at 23 °C and the organic phase was removed. The aqueous layer was acidified to pH 5 with the addition of aqueous 1.2 N HCl and concentrated in vacuo. The acid **19** was purified by reverse-phase chromatography (C-18, 0.5 × 8.0 cm, 0–80% CH_3OH–H_2O gradient elution) to afford **19** (0.94 mg, 0.98 mg theoretical, 97%) as a white film: $[\alpha]_D^{23}$ −22 (*c* 0.05, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.03 (s, 1H), 7.77 (s, 1H), 5.14 (d, *J* = 5.0 Hz, 1H), 4.73 (d, *J* = 5.0 Hz, 1H), 4.05 (dd, *J* = 8.5, 5.5 Hz, 1H), 3.91 (dd, *J* = 8.5, 5.5 Hz, 1H), 2.80 (dd, *J* = 12.0, 8.5 Hz, 1H), 2.68 (dd, *J* = 12.0, 5.5 Hz, 1H), 2.55 (dd, *J* = 15.0, 5.5 Hz, 1H), 2.49 (dd, *J* = 15.0, 8.5 Hz, 1H), 2.29 (s, 3H), 1.36 (s, 9H); IR (neat) ν_{max} 3341, 3223, 2918, 2850, 1663, 1643, 1614, 1550, 1506, 1392, 1162, 1058; FABHRMS (NBA–CsI) *m/e* 712.1427 (M^+ + Cs, $C_{23}H_{33}N_9O_9$ requires 712.1456).

N$^{\alpha}$-((*tert*-Butyloxy)carbonyl)-N$^{\beta}$-[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)-3 (*S*)-hydroxy-2 (*R*)-pentyl) amino) carbonyl)-2-(oxazol-4″-yl)-2(*R*)-hydroxy-1(*S*)-ethyl]amino]carbonyl-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (21). A solution of **19** (0.002 mmol, 1.2 mg), HOAt (0.002 mmol, 0.26 mg, 1.5 equiv), $NaHCO_3$ (0.007 mmol, 0.64 mg, 3.0 equiv) and **20**[42] (0.003 mmol, 1.8 mg, 1.4 equiv) in DMF (0.026 mL) at 0 °C was treated with DCC (0.002 mmol, 0.37 mg, 1.0 equiv) under Ar and the reaction mixture was stirred at 0 °C (1.5 h) and 23 °C (72 h). The crude reaction mixture was concentrated in vacuo, dissolved in CH_3OH and the insoluble inorganic salts were removed by centrifugation. The CH_3OH was evaporated and the remaining residue was treated with neutralized $CHCl_3$ (3 × 0.5 mL) with centrifugation to remove the soluble DCC byproducts. The remaining residue was purified by reverse-phase chromatography (C-18, 0.5 × 2.0 cm, 0–80% CH_3OH–H_2O gradient elution) to afford **23** (1.3 mg, 2.3 mg theoretical, 59%) as a white film: $[\alpha]_D^{23}$ −43 (*c* 0.007, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.19 (s, 1H), 8.09 (s, 1H), 8.03 (s, 1H), 7.98 (s, 1H), 5.11 (d, *J* = 5.5 Hz, 1H), 4.20 (d,

$J=4.5$ Hz, 1H), 4.03 (dq, $J=4.5$, 6.5 Hz, 1H), 3.89 (m, 1H), 3.85 (m, 1H), 3.78 (m, 1H), 3.61 (m, 1H), 3.51 (dd, $J=6.0$, 6.0 Hz, 1H), 3.48 (dd, $J=6.5$, 6.5 Hz, 2H), 3.24 (dd, $J=6.5$, 6.5 Hz, 2H), 2.83 (s, 6H), 2.81 (m, 1H), 2.70 (dd, $J=12.0$, 9.5 Hz, 1H), 2.65 (dd, $J=12.0$, 5.0 Hz, 1H), 2.56 (dd, $J=15.0$, 5.0 Hz, 1H), 2.44 (dd, $J=15.0$, 9.0 Hz, 1H), 2.21 (s, 3H), 2.06 (tt, $J=7.0$, 7.0 Hz, 2H), 1.31 (s, 9H), 1.21 (d, $J=6.5$ Hz, 3H), 1.19 (d, $J=6.5$ Hz, 3H), 1.10 (d, $J=6.5$ Hz, 3H); IR (neat) ν_{max} 3333, 2923, 2851, 1630, 1574, 1548, 1451, 1379, 1312, 1251, 1164, 1082 cm^{-1}; FABMS (NBA) *m/e* 1149 (M^+, weak); FABHRMS (NBA) *m/e* 1130.4386 (M^+-18, $C_{47}H_{70}N_{15}O_{13}S_3$ requires 1130.4333).

N^{β}-[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)-3(*S*)-hydroxy-2(*R*)-pentyl)-amino)carbonyl)-2-(oxazol-2″-yl)-2(*R*)-hydroxy-1(*S*)-ethyl]amino]carbonyl-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (4). A solution of **21** (0.003 mmol, 4.0 mg) in CH_2Cl_2 (280 μL) was cooled to 0 °C and treated with TFA (70 μL) under Ar. The yellow homogenous reaction mixture was stirred at 0 °C (4 h) and monitored by TLC (SiO_2, 10:9:1 CH_3OH:10% aqueous $CH_3CO_2NH_4$:10% aqueous NH_4OH; **21** R_f 0.50; **4** R_f 0.20). The TFA and CH_2Cl_2 were evaporated under a stream of N_2 at 0 °C and the yellow residue dried in vacuo. Chromatography (SiO_2, 0.5 × 2.0 cm, 10:9:1 CH_3OH:10% aqueous $CH_3CO_2NH_4$:10% aqueous NH_4OH gradient elution) followed by chromatography over Amberlite XAD-2 (2 × 1 cm), first desalting the absorbed sample with H_2O and then eluting with 80% CH_3OH–H_2O gave **4** (2.0 mg, 3.6 mg theoretical, 65%) as a white solid: $[\alpha]_D^{23}-19$ (*c* 0.1, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.08 (s, 1H), 8.06 (s, 1H), 8.04 (s, 1H), 8.01 (s, 1H), 5.10 (d, $J=5.5$ Hz, 1H), 4.20 (d, $J=4.5$ Hz, 1H), 4.14 (m, 1H), 4.01 (m, 1H), 3.78 (m, 1H), 3.65 (m, 1H), 3.60 (dd, $J=6.0$, 6.0 Hz, 2H), 3.49 (dd, $J=6.5$, 6.5 Hz, 3H), 3.32 (m, 2H), 3.20 (dd, $J=6.5$, 6.5 Hz, 2H), 2.82 (s, 6H), 2.80 (m, 1H), 2.71 (dd, $J=12.0$, 9.5 Hz, 1H), 2.63 (dd, $J=12.0$, 5.0 Hz, 1H), 2.55 (dd, $J=15.0$, 5.0 Hz, 1H), 2.44 (dd, $J=15.0$, 9.5 Hz, 2H), 2.25 (s, 3H), 2.11 (tt, $J=7.0$, 7.0 Hz, 1H), 1.20 (d, $J=6.5$ Hz, 3H), 1.15 (d, $J=6.5$ Hz, 3H), 1.04 (d, $J=6.5$ Hz, 3H); IR (neat) ν_{max} 3333, 2912, 2851, 1635, 1594, 1553, 1451, 1410, 1384, 1312, 1092 cm^{-1}; FABHRMS (NBA) *m/e* 1048.3915 (M^+, $C_{42}H_{62}N_{15}O_{11}S_3$ requires 1048.3905).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(amido-(3-(*tert*-butyloxy)carbonyl-2′-pyrrolyl)-3(*R*)-hydroxy-2(*S*)-amino methyl propionyl)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine amide (22). A solution of **17**[55] (0.009 mmol, 4.1 mg) in THF:DMF (2:1, 0.096 mL) was treated with **16** (0.010 mmol, 2.8 m g, 1.1 equiv), HOAt (0.009 mmol, 1.2 mg, 1.0 equiv) and EDCI (0.0095 mmol, 2.0 mg, 1.05 equiv), and the mixture was stirred under Ar at 23 °C (72 h). The reaction mixture was concentrated in vacuo to give an oil. Chromatography (SiO_2, 1 mm PCTLC, 10% CH_3OH–CH_2Cl_2) afforded **22** (3.4 mg, 6.6 mg theoretical, 51%) as a white film: R_f 0.27 (SiO_2, 10% CH_3OH–CH_2Cl_2); $[\alpha]_D^{23}+40$ (*c* 0.3, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.15 (dd, $J=3.0$, 1.5 Hz, 1H), 6.28 (dd, $J=3.0$, 1.5 Hz, 1H), 6.05 (t, $J=3.0$ Hz, 1H), 5.48 (d, $J=6.0$ Hz, 1H), 4.92 (d, $J=6.0$ Hz, 1H), 4.19 (d, $J=4.5$ Hz, 1H), 3.91 (dq, $J=6.5$, 6.5 Hz, 1H), 3.49 (s, 3H), 3.09 (m, 2H), 2.78 (m, 2H), 2.56 (dd, $J=5.0$, 15.0 Hz, 1H), 2.47 (dd, $J=15.0$, 9.0 Hz, 1H), 2.18 (s, 3H), 1.54 (s, 9H), 1.38 (s, 9H); ^{13}C NMR ($CDCl_3$, 63 MHz) δ 175.5, 172.3, 167.5, 166.6, 153.3, 153.1, 151.0, 149.9, 135.1, 126.1, 125.8, 124.5, 118.7, 115.9, 114.3, 112.1, 111.4, 85.8, 78.9, 76.2, 68.9, 61.3, 58.0, 53.4, 28.6, 28.4, 28.3, 28.2, 28.1, 11.7; IR (neat) ν_{max} 3360, 2954, 1736, 1672, 1505, 1370, 1336, 1162, 1059 cm^{-1}; FABHRMS (NBA) *m/e* 692.3380 (M^++H, $C_{30}H_{45}N_9O_{10}$ requires 692.3368).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(amido-(3-(((*tert*-butyloxy)carbonyl)-2′-pyrrolyl)-3(*R*)-hydroxy-2(*S*)-amino)propionic acid)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine amide (23). A solution of **22** (0.004 mmol, 2.8 mg) in 35% H_2O–*t*-BuOH (0.10 mL) was treated with aqueous 1 N $NaHCO_3$ (0.004 mmol, 4 μL, 1.0 equiv). The reaction mixture was stirred at 23 °C (8 h) with monitoring by TLC (SiO_2, 10% CH_3OH–CH_2Cl_2). After the *t*-BuOH was evaporated, H_2O (0.5 mL) and EtOAc (0.5 mL) were added at 23 °C and the organic phase was removed. The aqueous layer was acidified to pH 5 with the addition of aqueous 1.0 N HCl. The acid was purified by reverse-phase chromatography (C-18, 0.5 × 4.0 cm, 0–50% CH_3OH–H_2O gradient elution) to afford **22** (1.6 mg, 2.7 mg theoretical, 61%) as a white film: $[\alpha]_D^{23}-20$ (*c* 0.05, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 7.07 (dd, $J=3.0$, 1.5 Hz, 1H), 6.32 (dd, $J=3.0$, 1.5 Hz, 1H), 5.97 (t, $J=3.0$ Hz, 1H), 5.36 (d, $J=7.5$ Hz, 1H), 4.70 (d, $J=7.5$ Hz, 1H), 4.03 (dd, $J=4.0$, 9.5 Hz, 1H), 3.85 (dd, $J=9.0$, 5.0 Hz, 1H), 2.72 (dd, $J=12.0$, 9.5 Hz, 1H), 2.69 (dd, $J=12.0$, 5.0 Hz, 1H), 2.56 (dd, $J=15.0$, 5.0 Hz, 1H), 2.44 (dd, $J=15.0$, 9.0 Hz, 1H), 2.11 (s, 3H), 1.43 (s, 9H), 1.33 (s, 9H); IR (neat) ν_{max} 3361, 3036, 2977, 1658, 1633, 1569, 1540, 1506, 1461, 1407, 1299, 1162 cm^{-1}; FABHRMS (NBA–NaI) *m/e* 700.3055 (M^++Na, $C_{29}H_{43}N_9O_{10}$ requires 700.3031).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[1-amino-3(*S*)-(4-amino-6-(amido-(3-(2′-pyrrolyl)-3(*R*)-hydroxy-2(*S*)-amino)propionic acid)-5-methylpyrimidin-2-yl)propion-3-yl]-(*S*)-β-aminoalanine amide (24). A solution of **22** (0.009 mmol, 6.6 mg) in THF:CH_3OH:H_2O (3:1:1, 0.250 mL) was cooled to 0 °C and treated with aqueous 1 N LiOH (0.021 mmol, 21 μL, 2.5 equiv). The reaction mixture was stirred at 0 °C (1.5 h) with monitoring by TLC (SiO_2, 10% CH_3OH–CH_2Cl_2). After the THF and CH_3OH were evaporated under a stream of N_2 at 0 °C, H_2O (0.5 mL) and EtOAc (0.5 mL) were added at 23 °C and the organic phase was removed. The aqueous layer was acidified to pH 5 with the addition of aqueous 1.2 N HCl and concentrated in vacuo. The acid was purified by reverse-phase chromatography (C-18, 0.5 × 8.0 cm, 0–80% CH_3OH–H_2O) to afford **24**

(4.0 mg, 5.5 mg theoretical, 73%) as a white film: $[\alpha]_D^{23}$ −22 (*c* 0.05, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 6.56 (dd, *J* = 3.0, 1.5 Hz, 1H), 5.96 (dd, *J* = 3.0, 1.5 Hz, 1H), 5.88 (t, *J* = 3.0 Hz, 1H), 5.10 (d, *J* = 7.5 Hz, 1H), 4.63 (d, *J* = 7.5 Hz, 1H), 4.22, (d, *J* = 4.5 Hz, 1H), 4.07 (m, 1H), 3.83 (m, 1H), 2.71 (dd, *J* = 12.0, 9.5 Hz, 1H), 2.68 (dd, *J* = 12.0, 5.0 Hz, 1H), 2.55 (dd, *J* = 15.0, 5.0 Hz, 1H), 2.41 (dd, *J* = 15.0, 9.0 Hz, 1H), 2.10 (s, 3H), 1.33 (s, 9H); IR (neat) ν_{max} 3343, 3210, 2923, 2841, 1661, 1641, 1600, 1559, 1518, 1400, 1312, 1256, 1164 cm^{-1}; ESMS *m/e* 578 ($M^+ + H$, $C_{24}H_{35}N_9O_8$).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[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)-3 (*S*)-hydroxy-2 (*R*)-pentyl) amino) carbonyl)-2 (((1″-*tert*-butyloxy)carbonyl)pyrrol-2″-yl)-2(*R*)-hydroxy-1(*S*)-ethyl] amino] carbonyl-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (25). A solution of **23** (0.002 mmol, 1.2 mg), HOAt (0.002 mmol, 0.26 mg, 1.5 equiv), $NaHCO_3$ (0.005 mmol, 0.44 mg, 3.0 equiv) and **20**[42] (0.003 mmol, 1.7 mg, 1.4 equiv) in DMF (0.022 mL) at 0 °C was treated with DCC (0.002 mmol, 0.37 mg, 1.0 equiv) under Ar and the reaction mixture was stirred at 0 °C (1.5 h) and 23 °C (72 h). The crude reaction mixture was concentrated in vacuo, dissolved in CH_3OH and the insoluble inorganic salts were removed by centrifugation. The CH_3OH was evaporated and the remaining residue was treated with neutralized $CHCl_3$ (3 × 0.5 mL) with centrifugation to remove the soluble DCC byproducts. The remaining residue was purified by reverse-phase chromatography (C-18, 0.5 × 2.0 cm, 0–80% CH_3OH–H_2O gradient elution) to afford **25** (1.3 mg, 2.2 mg theoretical, 59%) as a white film: $[\alpha]_D^{23}$ −19.4 (*c* 0.03, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.10 (s, 1H), 8.00 (s, 1H), 7.30 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.85 (dd, *J* = 3.0, 1.5 Hz, 1H), 6.21 (t, *J* = 3.0 Hz, 1H), 5.00 (d, *J* = 7.5 Hz, 1H), 4.65 (d, *J* = 7.5 Hz, 1H), 4.31 (d, *J* = 4.5 Hz, 1H), 4.12 (dq, *J* = 4.5, 6.5 Hz, 1H), 3.93 (m, 2H), 3.85 (m, 1H), 3.65 (m, 1H), 3.63 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.50 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.26 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.00 (m, 2H), 2.83 (s, 6H), 2.62 (dd, *J* = 9.5, 4.5 Hz, 2H), 2.57 (m, 2H), 2.45 (dq, *J* = 6.5, 6.5 Hz, 1H), 2.25 (s, 3H), 2.06 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.51 (s, 9H), 1.32 (s, 9H), 1.25 (d, *J* = 6.5 Hz, 3H), 1.12 (d, *J* = 6.5 Hz, 3H), 1.02 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3331, 2977, 2918, 2850, 1741, 1653, 1550, 1456, 1368, 1324, 1250, 1162, 1122, 1068 cm^{-1}; FABMS (NBA) *m/e* 1246 (M^+, $C_{53}H_{80}N_{15}O_{14}S_3$).

N^{α}-((*tert*-Butyloxy)carbonyl)-N^{β}-[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)-3 (*S*)-hydroxy-2 (*R*)-pentyl) amino) carbonyl)-2-(pyrrol-2″-yl)-2(*R*)-hydroxy-1(*S*)-ethyl]amino]carbonyl-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (26). A solution of **24** (0.0015 mmol, 1.0 mg), HOAt (0.002 mmol, 0.28 mg, 1.5 equiv) $NaHCO_3$ (0.004 mmol, 0.37 mg, 3.0 equiv), and **20**[42] (0.002 mmol, 1.4 mg, 1.4 equiv) in DMF (0.021 mL) at 0 °C was treated with DCC (0.0016 mmol, 0.33 mg, 1.0 equiv) under Ar and the reaction mixture was stirred at 0 °C (1.5 h) and 23 °C (72 h). The crude reaction mixture was concentrated in vacuo, dissolved in CH_3OH and the insoluble inorganic salts were removed by centrifugation. The CH_3OH was evaporated, and the remaining residue was treated with neutralized $CHCl_3$ (3 × 0.5 mL) with centrifugation to remove the soluble DCC byproducts. The remaining residue was purified by reverse-phase chromatography (C-18, 0.5 × 2.0 cm, 0–80% CH_3OH–H_2O gradient elution) to afford **26** (0.91 mg, 1.6 mg theoretical, 57%) as a white film: ^1H NMR (CD_3OD, 400 MHz) δ 8.10 (s, 1H), 8.02 (s, 1H), 6.51 (dd, *J* = 3.0, 1.5 Hz, 1H), 5.95 (dd, *J* = 3.0, 1.5 Hz, 1H), 5.90 (t, *J* = 3.0 Hz, 1H), 5.02 (d, *J* = 7.5 Hz, 1H), 4.66 (d, *J* = 7.5 Hz, 1H), 4.23 (d, *J* = 4.5 Hz, 1H), 4.14 (m, 1H), 4.03 (m, 1H), 3.90 (m, 2H), 3.87 (m, 1H), 3.67 (dd, *J* = 6.0, 6.0 Hz, 1H), 3.62 (m, 1H), 3.49 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.36 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.25 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.03 (m, 2H), 2.83 (s, 6H), 2.69 (dd, *J* = 9.5, 4.5 Hz, 1H), 2.60 (m, 2H), 2.43 (dq, *J* = 6.5, 6.5 Hz, 1H), 2.22 (s, 3H), 2.04 (tt, *J* = 7.0, 7.0 Hz, 2H), 1.33 (s, 9H), 1.21 (d, *J* = 6.5 Hz, 3H), 1.14 (d, *J* = 6.5 Hz, 3H), 1.09 (d, *J* = 6.5 Hz, 3H); IR (neat) ν_{max} 3333, 2976, 2900, 2860, 1741, 1653, 1560, 1520, 1456, 1360, 1300, 1260, 1120, 1056 cm^{-1}; FABMS (NBA) *m/e* 1146 (M^+, weak); FABHRMS (NBA) *m/e* 1128.4593 ($M^+ - 18$, $C_{48}H_{72}N_{15}O_{12}S_3$ requires 1128.4646).

N^{β}-[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)-3(*S*)-hydroxy-2(*R*)-pentyl) amino) carbonyl)-2-(pyrrol-2″-yl)-2 (*R*)-hydroxy-1 (*S*)-ethyl] amino] carbonyl-5-methylpyrimidin-2-yl] -1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (5). A solution of **25** (0.002 mmol, 3 mg) in CH_2Cl_2 (250 μL) was cooled to 0 °C and treated with TFA (60 μL) under Ar. The yellow homogeneous reaction mixture was stirred at 0 °C (4 h) and monitored by TLC (SiO_2, 10:9:1 CH_3OH:10% aqueous $CH_3CO_2NH_4$:10% aqueous NH_4OH (**24** R_f 0.50, **5** R_f 0.20). The TFA and CH_2Cl_2 were evaporated under a stream of N_2 at 0 °C. Chromatography (SiO_2, 0.5 × 2.0 cm, 10:9:1 CH_3OH: 10% aqueous $CH_3CO_2NH_4$:10% aqueous NH_4OH gradient elution) followed by chromatography over Amberlite XAD-2 (2 × 1 cm), first desalting the absorbed sample with H_2O, then eluting the product with 80% CH_3OH–H_2O gave **5** (1.3 mg, 2.1 mg theoretical, 60%) as a white film: $[\alpha]_D^{23}$ −10 (*c* 0.10, CH_3OH); ^1H NMR (CD_3OD, 400 MHz) δ 8.10 (s, 1H), 8.05 (s, 1H), 6.56 (dd, *J* = 3.0, 1.5 Hz, 1H), 5.96 (dd, *J* = 3.0, 1.5 Hz, 1H), 5.88 (t, *J* = 3.0 Hz, 1H), 5.09 (d, *J* = 7.5 Hz, 1H), 4.65 (d, *J* = 7.5 Hz, 1H), 4.25 (d, *J* = 4.5 Hz, 1H), 4.10 (dq, *J* = 4.5, 6.5 Hz), 3.93 (dd, *J* = 9.5, 4.5 Hz, 1H), 3.91 (dq, *J* = 5.5, 6.5 Hz, 1H), 3.87 (m, 1H), 3.62 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.66 (m, 1H), 3.51 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.35 (dd, *J* = 6.5, 6.5 Hz, 2H), 3.28 (dd, *J* = 6.0, 6.0 Hz, 2H), 3.01 (m, 2H), 2.80 (s, 6H), 2.69 (dd, *J* = 9.5, 4.5 Hz, 2H), 2.57 (m, 2H), 2.46 (dq,

J=6.5, 6.5 Hz, 1H), 2.25 (s, 3H), 2.11 (tt, J=7.0, 7.0 Hz, 2H), 1.20 (d, J=6.5 Hz, 3H), 1.17 (d, J=6.5 Hz, 3H), 1.05 (d, J=6.5 Hz, 1H); IR (neat) ν_{max} 3370, 2925, 1656, 1549, 1370, 1210, 1066 cm^{-1}; FABHRMS (NBA–CsI) *m/e* 1179.3176 (M^++Cs, $C_{43}H_{64}N_{15}O_{16}S_3$ requires 1179.3170).

General procedure for quantitation of double strand and single strand supercoiled ΦX174 DNA cleavage

The Fe(II) complexes were formed by mixing 1 μL of an aqueous 120 and 140 μM solutions of **4** or **5** with 1 μL of a freshly prepared 120 or 140 μM aqueous $Fe(NH_4)_2(SO_4)_2$ solution, respectively. Seven microliters of a buffered DNA solution containing 0.25 μg of supercoiled ΦX174 RFI DNA (1.4×10^{-8} M) in 50 mM Tris–HCl buffer solution (pH 8) were added to each of the Fe(II) complex solutions. The final concentrations of **4** and **5** employed in the study were 12 and 14 μM. The DNA cleavage reactions were initiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol to each of the reaction mixtures. The solutions were thoroughly mixed and incubated at 25 °C for 60, 50, 40, 30, 25, 20, 15, 12, 10, 8, 6, 4, 2 and 1 min, respectively. The reactions were quenched with the addition of 5 μL of loading buffer, and electrophoresis was run on a 1% agarose gel at 50 V for 3 h. Direct fluorescence quantitation of the DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system taking into account the relative fluorescence intensities of Forms I–III ΦX174 DNA (Forms II and III fluorescence intensities are 0.7 times that of Form I). The ratio of double to single strand DNA cleavage was calculated with use of the Freifelder–Trumbo equation[61] assuming a Poisson distribution and the results are summarized in Table 1. For **4**, the ratio was established to be 1:25 at 12 μM and 1:27 at 14 μM. For **5**, the ratio was established to be 1:46 at 12 μM and 1:42 at 14 μM.

General procedure for cleavage of 5′-end-labeled w794: relative efficiency and selectivity

All reactions were run with freshly prepared Fe(III) complexes. The Fe(III) complexes were prepared by combining 1 μL of a H_2O solution of the agent at 10 times the specified concentration with 1 μL of a freshly prepared equimolar aqueous $FeCl_3$ solution. Each of the Fe(III) complex solutions were treated with 7 μL of a buffered DNA solution containing the ^{32}P 5′-end-labeled w794[63] in a 10 mM phosphate buffer solution (Na_2HPO_4–NaH_2PO_4, pH 7.0) containing 10 mM KCl. The final concentrations of the agents employed in the study were 32 and 64 μM control Fe(III), 4.0 and 8.0 μM deglycobleomycin A_2, 8 and 16 μM **4** and 32 and 64 μM **5**. The DNA cleavage reactions were initiated by adding 1 μL of 50% aqueous H_2O_2. The DNA reaction solutions were incubated at 37 °C for 30 min. The reactions were quenched with the addition of 1 μL of 50% aqueous glycerol followed by EtOH precipitation and isolation of the DNA. The DNA was resuspended in 6 μL of TE buffer (pH 8.0) and formamide dye (6 μL) was added to the supernatant. Prior to electrophoresis, the samples were warmed at 100 °C for 5 min, placed in an ice bath, centrifuged and the supernatant (3 μL) was loaded onto the gel. Sanger dideoxynucleotide sequencing reactions were run as standards adjacent to the agent-treated DNA. Gel electrophoresis was conducted using a denaturing 8% sequencing gel (19:1 acrylamide:*N*,*N*-methylenebisacrylamide, 8 M urea) at 1100 V for 5.5 h. Formamide dye contained xylene cyanol FF (0.03%), bromophenol blue (0.3%), and aqueous Na_2EDTA (8.7%, 250 mM). Electrophoresis running buffer (TBE) contained Tris base (100 mM), boric acid (100 mM) and Na_2EDTA–H_2O (0.2 mM). Gels were prerun for 30 min with formamide dye prior to loading the samples. Autoradiography of the dried gel was carried out at −78 °C using Kodak X-Omat AR film and a Picker spectra intensifying screen. Quantitation of the DNA cleavage reaction was conducted on a Millipore Bio Image 60S RFLP system measuring the remaining uncleaved DNA and the values recorded in Table 1 are the average of 3 (**4**, **5**, **2**) or 6 experiments (**1** and Fe(III)).

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