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# Synthesis and Evaluation of Deglycobleomycin $A_2$ Analogues Containing a Tertiary N-Methyl Amide and Simple Ester Replacement for the L-Histidine Secondary Amide: Direct Functional Characterization of the Requirement for Secondary Amide Metal Complexation

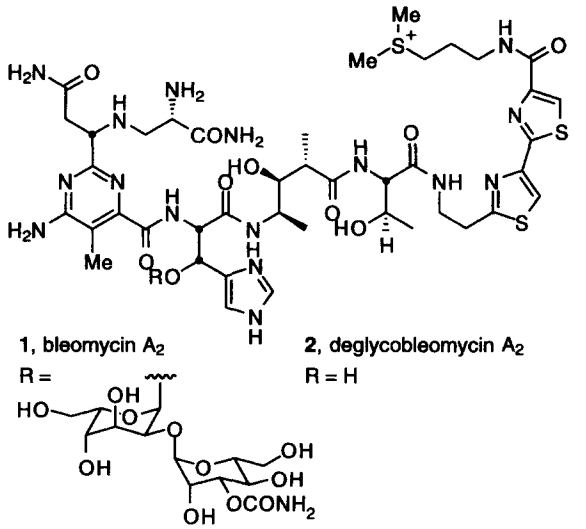
Dale L. Boger,* Shuji Teramoto and Hui Cai

*Department of Chemistry, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037, U.S.A.*

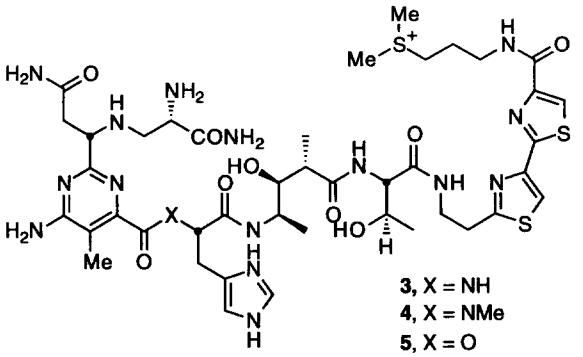
**Abstract**—The synthesis and comparative examination of **3–5**, analogues of deglycobleomycin $A_2$ (**2**) which address the inferred importance of the L-histidine secondary amide directly, are detailed. The agent **3** lacks only the L-histidine β-hydroxy group of deglycobleomycin $A_2$ and the corresponding agents **4** and **5** incorporate a tertiary N-methyl amide and simple ester in place of the L-histidine secondary amide. The DNA cleavage properties of **3** proved essentially indistinguishable from those of deglycobleomycin $A_2$ (**2**) confirming that the distinctions between bleomycin $A_2$ (**1**) and deglycobleomycin (**2**) are due to the removal of the disaccharide and not the introduction of the L-histidine free β-hydroxy group. The agents **4** and **5** containing a tertiary N-methyl amide and ester in place of the L-histidine secondary amide were found to cleave duplex DNA but to do so in a nonsequence selective fashion with a substantially reduced efficiency and a diminished double to single strand cleavage ratio that are only slightly greater than that of free iron itself. These latter observations establish the functional requirement for the L-histidine secondary amide and are consistent with the proposals that the L-histidine deprotonated secondary amide is required for functional metal chelation and activity.

## 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[31–41] 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 iron 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[42] and its contribution to polynucleotide recognition and the structural origin of this effect[43] remain active topics of investigation.[18,32,33,36,38,43] 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[42,44] and has been suggested to contribute to polynucleotide recognition and the DNA cleavage selectivity. Despite the extensive studies on the bleomycins, only recently are the details of the nature of the relevant bleomycin $A_2$ bithiazole binding with duplex DNA being unraveled[43,45] and through the years has been proposed to involve intercalation, partial intercalation or minor groove binding.[42–43,45–46]




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.[47] This provided the basis for a proposed structure of bleomycin $A_2$ metal complexes in which the mannose C3 carbamoyl group occupies a putative sixth coordination site which is displaced by bound $O_2$ in the activated complex.[48] NMR and related spectroscopic studies 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.[43,48–51] However, their arrangement and the remaining metal ligands remain controversial and, importantly, their relevance to the coordination geometry within activated bleomycin $A_2$ remain unclear. The most recent and impressive studies of Stubbe[43] 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 $N^{\pi}$ and deprotonated amide complexation, and favor axial primary amine coordination over the mannose carbamoyl.

In initial studies, potentiometric titration of the bleomycin copper complex indicated that one deprotonated functional group occupies one of the coordination sites between pH 4 and 9.[47] 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 anticipated relative $pK_a$ of imidazole (14.4) and an amide (17) in the absence of a metal.[47] 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 illustrate both the β-hydroxyhistidine imidazole $N^{\pi}$ complexation and its deprotonated amide $N^{\sigma}$ complexation.[33,47] While NMR studies have addressed this issue and would seem to confirm imidazole $N^{\pi}$ complexation,[48] these and related spectroscopic studies conducted on a range of metal complexes have also suggested that the β-hydroxyhistidine amide nitrogen may not always be involved and cast doubt on the nature and site of the deprotonated metal ligand. In recent studies conducted to address this issue directly,[41] we demonstrated that imidazole $N^{\pi}$ metal complexation is sufficient and required for functional reactivity, that imidazole deprotonation is not required for functional reactivity, and that the functional bleomycin $A_2$ imidazole tautomer is the imidazole $N^1$-H tautomer.

Herein we report the synthesis and evaluation of **3–5**, analogues of deglycobleomycin $A_2$ (**2**) which further address the inferred importance of the L-histidine secondary amide directly. The agent **3** lacks only the L-histidine β-hydroxy group of **2** and the corresponding agents **4–5** incorporate a tertiary N-methyl amide or ester in place of the L-histidine secondary amide of **3**. The N-methyl amide **4** is only capable of amide $N^{\pi}$ complexation and is incapable of deprotonation and metal complexation through the accepted $N^{\sigma}$ coordination. Similarly, the ester **5** is incapable of providing the key secondary amide metal coordination site. The results of the studies establish that β-hydroxy-L-histidine amide $N^{\pi}$ metal chelation is not sufficient for functional activity and that **1–3** require the secondary amide for functional activity.

## Results

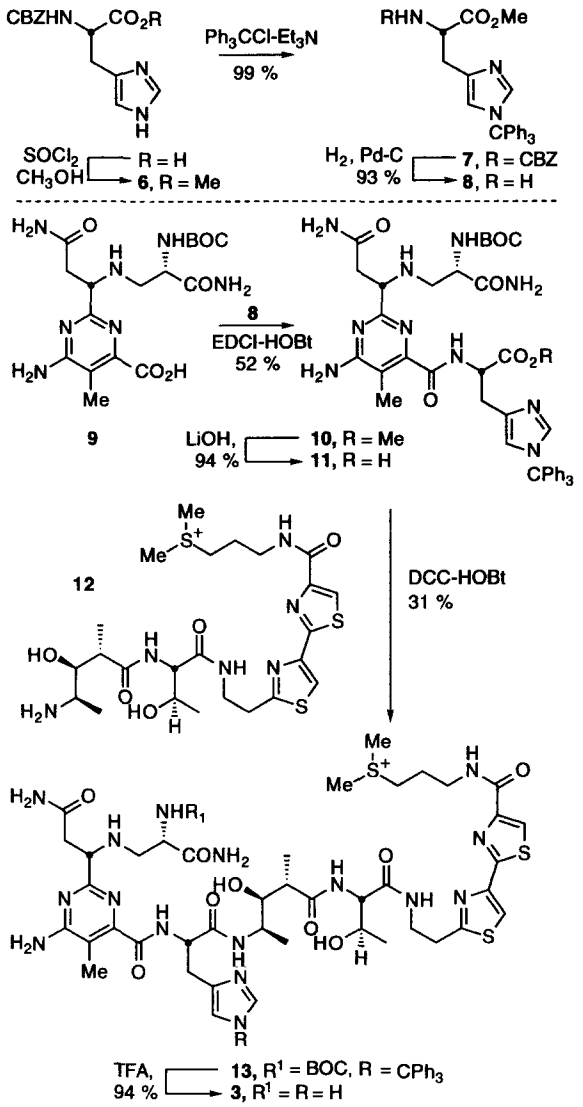
### Synthesis of deshydroxy deglycobleomycin $A_2$ (3)

Agent **3** was required for direct comparison with **4–5** and its examination provided the additional opportunity to directly assess the impact of the free hydroxy group of deglycobleomycin $A_2$. Past assessments have assumed that the diminished properties of **2** relative to those of **1** may be attributed to the removal of the disaccharide and are not derived from the newly introduced hydroxyl group. We felt this was especially important to establish given our recent demonstration of the important role of the disaccharide L-gulose subunit but the lack of significant functional role for the terminal D-mannose subunit.[39]

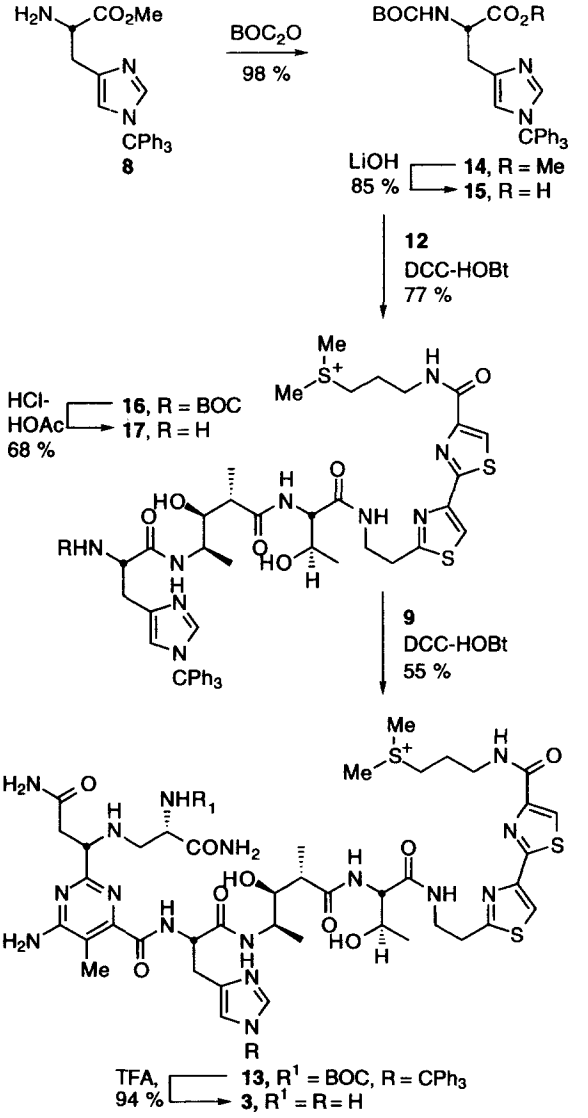
Regioselective $N^{im}$-tritylation at imidazole N-1 of $N^{\alpha}$-Cbz-L-histidine methyl ester (**6**)[52] effected by treatment with $Ph_3CCl$ (1.0 equiv, 1.0 equiv $Et_3N$, $C_6H_6$, 80 °C, 1 h, 99%) followed by clean hydrogenolysis ($H_2$, 10% Pd–C, $CH_3OH$, 25 °C, 2.5 h, 93%) of the Cbz protecting group provided $N^{im}$-trityl-L-histidine methyl ester (**8**) without competitive $N^{im}$-trityl hydrogenolysis (Scheme 1). Direct coupling of **8** with $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**,[53] 1.5 equiv EDCI, 1.1 equiv HOBt, DMF, 25 °C, 24 h, 52%) provided **10**. Methyl ester hydrolysis (LiOH, $H_2O$–$CH_3OH$, 4 °C, 2.5 h, 94%), coupling of resulting carboxylic acid **11** with tetrapeptide S (**12**)[44] and final acid-catalyzed

deprotection of **13** provided **3** (94%), $[\alpha]_D^{25} -10$ (*c* 0.07, $CH_3OH$).

While this approach provided **3**, the coupling of **11** with tetrapeptide S proved unexpectedly problematic. The formation of the coupling product **13** was accompanied by the generation of an additional isomeric product (3:1 ratio) and required careful reverse phase chromatography with some loss of material in order to isolate pure material. Although it was not unambiguously established, this was presumed to be the result of partial racemization of the activated ester of **11** produced for coupling with **12** and a limited survey of alternative amide coupling protocols provided similar (DPPA) or less satisfactory results (BOPCl). Recognizing that racemization of the L-histidine α-center is more problematic with the more acidic $N^{\alpha}$-acyl versus $N^{\alpha}$-carbamate derivatives, we simply reversed the order of tetrapeptide S and $N^{\alpha}$-BOC pyrimidoblamic acid couplings[39,54] which averted the partial racemization (Scheme 2). Coupling of $N^{\alpha}$-BOC,$N^{im}$-trityl-L-histidine (**15**) with tetrapeptide S (**12**)[44] cleanly provided **16** (1.5 equiv DCC, 1.1 equiv HOBt, 3.0 equiv $NaHCO_3$, DMF, 25 °C, 12 h, 77%) with no evidence of racemization. Notably, the fragment **12** contains the installed sulfonium salt and no peripheral protecting groups thereby simplifying the final stages of the preparation of **3**. Selective N-BOC deprotection under conditions introduced by Sieber and Riniker[55] (1 N HCl, 90% HOAc–$H_2O$, 25 °C, 5 min)[54] proceeded cleanly without $N^{im}$-trityl deprotection and provided **17** (68%) along with a small amount of recovered **16** (14%). Subsequent coupling of **17** with $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**,[53] 2.9 equiv DCC, 1.1 equiv HOBt, DMF, 25 °C, 15 h, 55%) provided **13** and acid-catalyzed deprotection afforded **3**, both of which proved identical to the material described above.



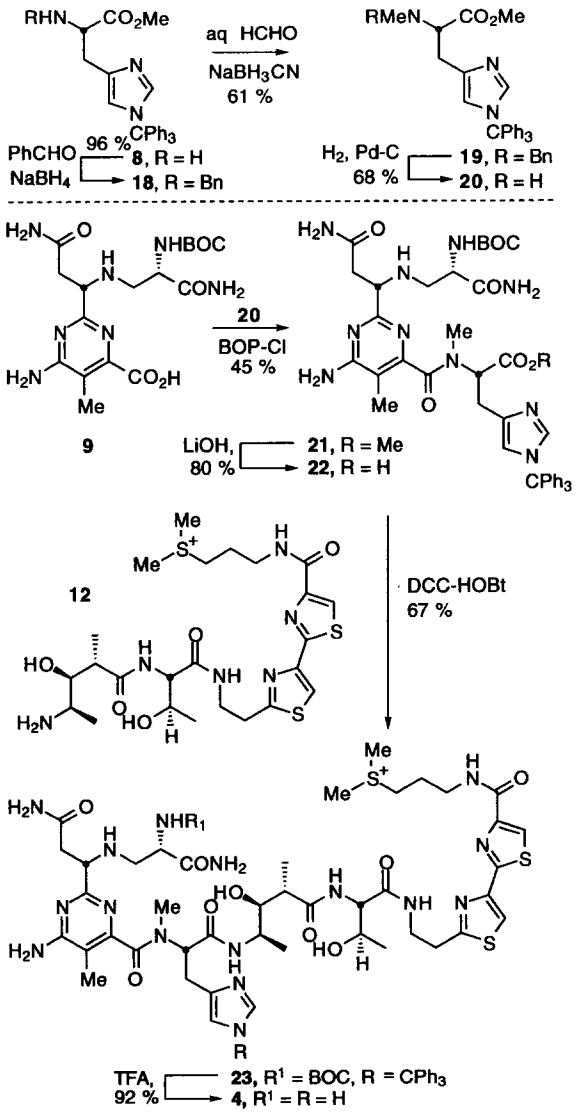

Scheme 1.




Scheme 2.

### Synthesis of N-methyl-L-histidine deglycobleomycin $A_2$ (4)

The appropriately protected N-methyl-L-histidine methyl ester **20** for direct incorporation into **4** was prepared from $N^{im}$-trityl-L-histidine methyl ester (**8**) by reductive benzylation (PhCHO, $CH_3OH$, 25 °C, 1.5 h; $NaBH_4$, 5–10 °C, 30 min, 96%) followed by reductive methylation of **18** (37% aqueous HCHO, $NaBH_3CN$, HOAc–$CH_3CN$, 25 °C, 20 min, 61%) and subsequent hydrogenolysis ($H_2$, 10% Pd–C, 25 °C, 10 h, 38%, 68% based on recovered **19**) of the benzyl amine **19** (Scheme 3). Coupling of **20** with $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**,[53] 1.2 equiv BOPCl, 2.5 equiv $Et_3N$, $CH_2Cl_2$, 25 °C, 6 h, 45%) provided **21** with formation of the key tertiary amide. Other coupling protocols including EDCI–HOBt (17%), DCC–HOBt (0%) and DCC–DMAP (0%) provided less satisfactory results. Methyl ester hydrolysis (LiOH, $H_2O$–$CH_3OH$, 4 °C, 3.5 h, 80%) followed by coupling of **22** with tetrapeptide S (**12**,[44] 3 equiv DCC, 0.5 equiv HOBt, 1.1 equiv $NaHCO_3$, 25 °C, 35 h, 67%) cleanly provided **23** with no evidence of competitive racemization. Final acid-catalyzed deprotection of **23** provided **4** (92%), $[\alpha]_D^{25} - 32$ (*c* 0.09, $CH_3OH$).




Scheme 3.

### Synthesis of ester 5

The synthesis of the ester **5** which was viewed as a second complement to the agent **4** required the selectively protected alcohol **28** incorporating an ester which could be deprotected following coupling with **9** without inadvertent base-catalyzed epimerization of the α center or competitive hydrolysis of the newly formed ester. For this reason, the benzyl ester **28** was selected and prepared from L-histidine as outlined on Scheme 4. Coupling of **28** with preformed $Et_3N$ salt of $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**,[53] 1.1 equiv BOPCl, $CH_2Cl_2$, 4 days, 25 °C) provided the ester **29** which proved unusually sensitive to subsequent hydrolysis. Although **29** was isolated cleanly, exposure to protic solvents during its purification on $SiO_2$ (10% $CH_3OH$–$CH_2Cl_2$) led to partial hydrolysis of the newly formed sensitive ester. Benzyl ester deprotection by catalytic hydrogenolysis ($H_2$, cat 10% Pd–C, $CH_3OH$, 30 min, 25 °C, 76%) provided the carboxylic acid **30**. While **30** was cleanly isolated and characterized, partial hydrolysis of **30** was observed upon purification by C18-reverse phase chromatography (30–70% $CH_3OH$–$H_2O$ gradient). Sequential coupling with tetrapeptide S (**12**, 2 equiv DCC, 0.25 equiv HOBt, 45 h, 25 °C, 89%) and acid-catalyzed deprotection (20% TFA–$CH_2Cl_2$, 2 h, 4 °C, quant) provided **5**, $[\alpha]_D^{25} - 13$ (*c* 0.15, DMF). Both **31** and **5** proved unusually sensitive to exposure to protic solvents including $CH_3OH$ and $H_2O$ and significant amounts of hydrolysis of the key ester were observed. When this occurred during attempted chromatographic purification, the resulting hydrolysis products were found to contaminate the samples of **31** and **5**. Consequently, pure samples (>95% pure) of **31** and **5** were better obtained by trituration purification.

### DNA Cleavage properties of 3–5

Three assays were used to examine the DNA cleavage properties of **3–5**. 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 **3–5** produced single and double strand cleavage to afford relaxed (Form II) and linear (Form III) DNA, respectively (Fig. 1 and Table 1). The Fe(II) complex of **3** lacking only the histidine β-hydroxy group was found to be essentially as effective at cleaving the ΦX174 DNA as deglycobleomycin $A_2$ itself. The Fe(II) complex of the tertiary amide **4** was found to be 12 × less effective than deglycobleomycin $A_2$ but 2 × more effective than Fe(II) itself . Similarly, the

Fe(II) complex of **5** was found to be 8 × less effective than deglycobleomycin $A_2$ but 3 × more effective than Fe(II). The lack of DNA cleavage by **3–5** in the absence of Fe(II) in control studies was consistent with expectations that they were cleaving DNA by a metal dependent process.

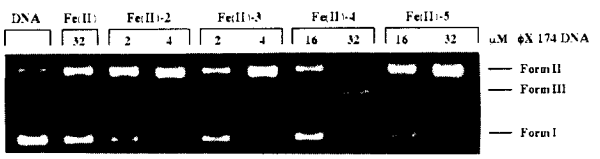
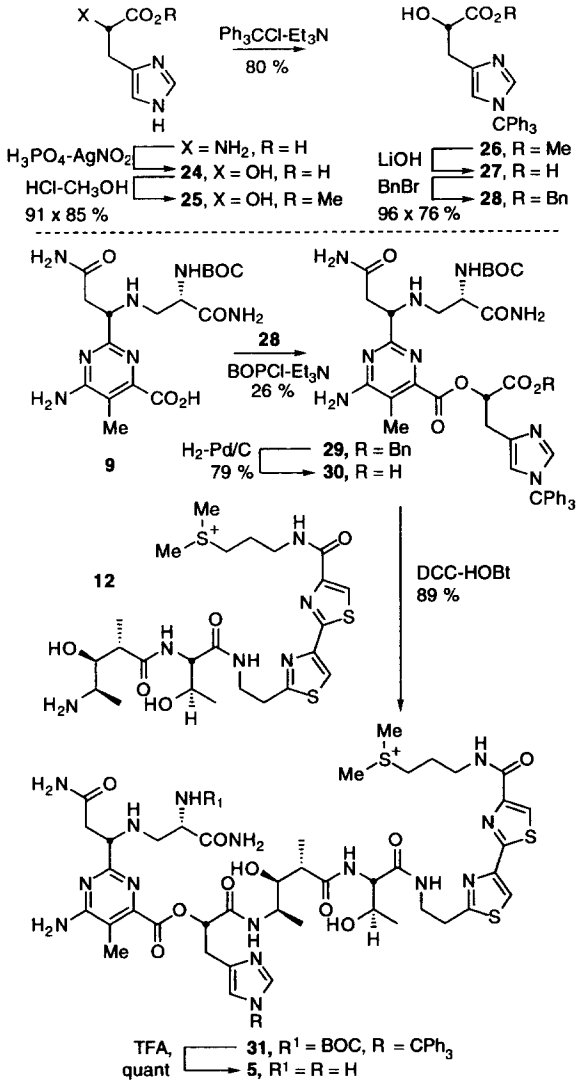



**Figure 1.** Agarose gel illustrating the cleavage reactions of supercoiled ΦX174 DNA by Fe(II)-agents at 25 °C for 1 h in buffer solutions containing 2-mercaptoethanol. After electrophoresis on a 1% agarose gel, the gel was stained with 0.1 μg mL$^{-1}$ ethidium bromide and visualized on a UV transilluminator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




**Scheme 4.**

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 2 for **3** and the full set of results obtained for **1–5** 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 the kinetics of metal complex reactivation. We assumed a Poisson distribution for the formation of single strand and double strand breaks to calculate the average number of double and single strand cuts per DNA molecule using the Freifelder–Trumbo equation.[56] 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 is summarized in Table 1. The ratio of double to single strand DNA cleavage for **3** (1:12) was indistinguishable from that of deglycobleomycin $A_2$ (1:12) indicating that the removal of the L-histidine β-hydroxy group did not cause a significant alteration. The ratio of double to single strand DNA cleavage for **4** and **5** was established to be 1:61 and 1:49, respectively, which was substantially lower than bleomycin $A_2$ (1:6) or deglycobleomycin $A_2$ (1:12) and is similar to the ratio derived from uncomplexed Fe(II) cleavage (1:98). A theoretical ratio of approximately 1:100 is required in order for the linear DNA to be the result of the random

**Table 1.** Summary of DNA cleavage properties of **2–5**

| Agent | Relative efficiency of DNA cleavage[a] | | Ratio of double to single strand DNA cleavage[c] | DNA cleavage selectivity[b] |
|---|---|---|---|---|
| | ΦX174[a] | w794[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**[c] | 0.73 | 0.75 | 1:12 | 5′-GC, 5′-GT > 5′-GA |
| **4** | 0.08 | 0.04 | 1:61 | None |
| **5** | 0.13 | 0.08 | 1:49 | None |
| Fe[a,b] | 0.04 | 0.03 | 1:98 | None |

[a]Relative efficiency of supercoiled ΦX174 DNA cleavage, Fe(II)-$O_2$, 2-mercaptoethanol.
[b]Examined within 5′$^{32}$P-end-labelled w794 DNA, Fe(III)-$H_2O_2$. The results with **2–4** and Fe(III) are the average of six experiments, those of **5** are the average of two experiments.
[c]Ratio of double to single stranded cleavage of supercoiled ΦX174 DNA calculated as $F_{III} = n_2 \exp(-n_2)$, $F_I = \exp[-(n_1 + n_2)]$.

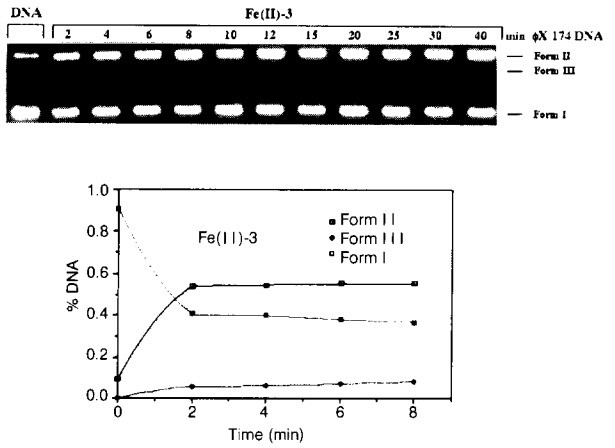



**Figure 2.** Representative kinetics of supercoiled ΦX174 DNA cleavage by Fe(II)-**3** (4 μM) in buffer solution containing 2-mercaptoethanol. The DNA cleavage reactions were run at 25 °C for various lengths of time and electrophoresis was conducted on a 1% agarose gel. Direct fluorescence quantitation of the percentage of forms I–III DNA present at each time point was conducted using a Millipore BioImage 60S RFLP system visualized on a UV (312 nm) transilluminator in the presence of 0.1 μg $mL^{-1}$ 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).

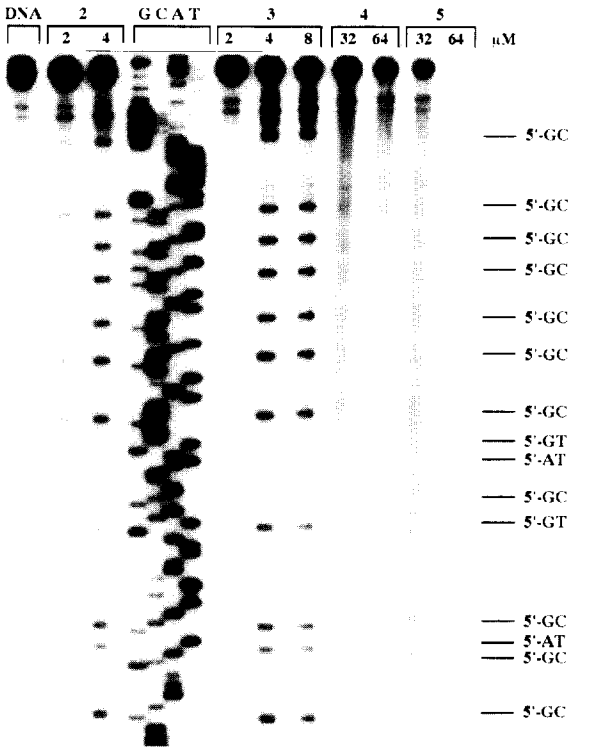



**Figure 3.** Cleavage of double-stranded DNA by Fe(III)-agents (SV40 DNA fragment, 144 base pairs, nucleotide no. 5238–138, clone w794) in phosphate–KCl buffer containing $H_2O_2$. The DNA cleavage reactions were run for 30 min at 37 °C, and electrophoresis was run on an 8% denaturing PAGE and visualized by autoradiography.

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 additional assessment of the relative efficiency of DNA cleavage were examined within duplex w794 DNA[57,58] by monitoring strand cleavage of singly $^{32}P$ 5′-end-labeled double-stranded DNA after exposure to the Fe(III)-complex followed by activation with $H_2O_2$[59] in 10 mM phosphate buffer (pH 7.0). This protocol has proven to be much more sensitive to the distinctions in the relative efficiency of DNA cleavage by related agents than the ΦX174 supercoiled DNA cleavage assays but both have always provided the same trends. Thus, incubation of the labeled duplex DNA with **2–5** 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. A typical comparison is illustrated in Figure 3.

Over a wide range of conditions, no changes in the observations were found for **2–5** with **2** and **3** being nearly indistinguishable and producing the sequence selective DNA cleavage characteristic of bleomycin $A_2$ and with **4** and **5** cleaving DNA in a nonselective manner. Under all conditions examined, **4** and **5** were found to cleave DNA above background Fe(III) and to do so in a nonsequence selective fashion (Fig. 3 and Table 1). The Fe(III) complex of **4** was 1.5× more effective than Fe(III) itself, 25× less effective than deglycobleomycin $A_2$ and Fe(III)-**5** was found to be 2× more effective than **4**. Comparisons alongside the 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

The results of the comparisons of **2–5** were revealing. Metal complexes of **3–5** were found to cleave duplex DNA in the presence of $O_2$ (Fe-II) or $H_2O_2$ (Fe-III) and it is significant that both activation procedures provided comparable results. The agent **3** which lacks only the histidine β-hydroxy group of deglycobleomycin $A_2$ was found to behave in a manner nearly indistinguishable from deglycobleomycin $A_2$. It produced the

characteristic 5′-GC/5′-GT sequence selective cleavage of duplex DNA with a comparable efficiency directly confirming that the distinctions between bleomycin $A_2$ (**1**) and deglycobleomycin $A_2$ (**2**) are due to the removal of the disaccharide and not the introduction of the L-histidine free β-hydroxy group. The tertiary amide **4** and ester **5** which are incapable of histidine amide deprotonation and $N^{\sigma}$ metal coordination were also found to cleave duplex DNA but to do so in a nonsequence selective fashion with a significantly reduced efficiency and a diminished double to single strand cleavage ratio that were only slightly greater than that of free iron itself. These latter observations establish that **2–3** require the L-histidine secondary amide for functional activity and would seem to confirm that the histidine $N^{\sigma}$ metal coordination is central to the functional activity of bleomycin $A_2$ itself. While much of the DNA cleavage by **4** and **5** observed in the presence of iron is not distinguishable from that of background iron itself, it is likely that the nonsequence selective cleavage of DNA by **4** and **5** above that observed for background iron is derived from a diffusible oxidant (e.g., $HO\cdot$)[37,41] generated through oxygen activation by a bound or partially bound but nonfunctional metal complex. Regardless of the origin of these observations, the ineffectual behavior of **4–5** establish the functional requirement for the L-histidine secondary amide and are consistent with the proposals that its deprotonation is required for functional metal chelation and activity.

## Experimental

**$N^{\alpha}$-Benzyloxycarbonyl-$N^{im}$-triphenylmethyl-L-histidine methyl ester (7).** A solution of **6** (85 mg, 0.26 mmol) in $C_6H_6$ (1 mL) was treated with $Ph_3CCl$ (75 mg, 0.26 mmol) and $Et_3N$ (37 μL, 0.26 mmol) at 25 °C. After stirring for 1 h at 80 °C, the reaction mixture was diluted with EtOAc (5 mL). The organic layer was washed with $H_2O$ (2 mL), saturated aqueous NaCl (2 mL), dried ($Na_2SO_4$) and concentrated in vacuo. Chromatography ($SiO_2$, 0.8 × 13 cm, 2% $CH_3OH$–$CH_2Cl_2$) provided **7** (140 mg, 142 mg theoretical, 99%) as a colorless syrup: $[\alpha]_D^{25}$ +15.7 (*c* 1.0, $CHCl_3$), lit[52] $[\alpha]_D^{25}$ +12.8 (*c* 1.0, $CHCl_3$); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.26–7.38 (15H, m), 7.04–7.13 (6H, m), 6.52 (1H, s), 6.37 (1H, d, *J* = 8.2 Hz), 5.10 (1H, d, *J* = 12.4 Hz), 5.09 (1H, d, *J* = 12.4 Hz), 4.60 (1H, ddd, *J* = 4.6, 5.0, 8.2 Hz), 3.61 (3H, s), 3.06 (1H, dd, *J* = 5.0, 14.6 Hz), 2.99 (1H, dd, *J* = 4.6, 14.6 Hz).

**$N^{im}$-Triphenylmethyl-L-histidine methyl ester (8).** A solution of **7** (1.22 g, 2.25 mmol) in $CH_3OH$ (15 mL) containing 10% Pd–C (0.20 g) was stirred under an atmosphere of $H_2$ (1 atm) for 2.5 h at 25 °C. The reaction mixture was filtered through a Celite pad, and washed with 1% $Et_3N$–$CH_3OH$ (10 mL) and $CH_3OH$ (30 mL). Chromatography ($SiO_2$, 2 × 20 cm, 2–5% $CH_3OH$–$CH_2Cl_2$ gradient elution) gave **8** (0.86 g, 0.93 g theoretical, 93%) as a colorless syrup. Crystallization from $CH_2Cl_2$–$Et_2O$ provided **8** as a white powder (0.78 g): $[\alpha]_D^{25}$ +9.1 (*c* 1.0, $CH_3OH$), lit[60] $[\alpha]_{578}^{25}$ +7.0 (*c* 1.0, $CH_3OH$); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.37 (1H, d, *J* = 1.2 Hz), 7.28–7.36 (9H, m), 7.07–7.16 (6H, m), 6.58 (1H, d, *J* = 1.2 Hz), 3.81 (1H, dd, *J* = 4.9, 6.9 Hz), 3.64 (3H, s), 2.97 (1H, dd, *J* = 4.9, 14.3 Hz), 2.87 (1H, dd, *J* = 6.9, 14.3 Hz), 1.70 (2H, s).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[1-amino-1-oxo-3(*S*)-[4-amino-6-(1(*S*)-(methoxycarbonyl)-2-(1-(triphenylmethyl)imidazol-4-yl)-1-ethylaminocarbonyl)-5-methylpyrimidin-2-yl]-3-propyl]-(*S*)-β-aminoalanine amide (10).** DMF (200 μL) was added to a mixture of **8** (5.9 mg, 14.1 μmol), $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**,[53] 5.0 mg, 11.8 μmol), EDCI (3.4 mg, 17.6 μmol) and HOBt (1.7 mg, 12.9 μmol) at 0 °C under $N_2$. After stirring for 24 h at 25 °C, the reaction mixture was concentrated in vacuo. The residue was dissolved with EtOAc (2 mL) and washed with $H_2O$ (2 mL). The $H_2O$ layer was extracted with EtOAc (2 × 2 mL) and the combined organic layer was washed with $H_2O$ (2 mL), saturated aqueous NaCl (2 mL), dried ($Na_2SO_4$) and concentrated in vacuo. Chromatography ($SiO_2$, 0.5 × 4 cm, 10–17% $CH_3OH$–$CH_2Cl_2$ gradient elution) afforded **10** (5.0 mg, 9.7 mg theoretical, 52%) as a white amorphous solid: $R_f$ 0.6 ($SiO_2$, 20% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{24}$ +9.2 (*c* 0.25, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 7.40 (1H, d, *J* = 1.4 Hz), 7.23–7.34 (9H, m), 7.03–7.11 (6H, m), 6.76 (1H, d, *J* = 1.4 Hz), 4.81 (1H, dd, *J* = 5.1, 8.3 Hz), 4.09 (1H, dd, *J* = 4.9, 5.2 Hz), 3.86 (1H, dd, *J* = 4.9, 8.8 Hz), 3.70 (3H, s), 3.18 (1H, dd, *J* = 5.1, 14.8 Hz), 3.13 (1H, dd, *J* = 8.3, 14.8 Hz), 2.80 (1H, dd, *J* = 4.9, 12.1 Hz), 2.68 (1H, dd, *J* = 5.2, 12.1 Hz), 2.53 (1H, dd, *J* = 4.9, 14.9 Hz), 2.38 (1H, dd, *J* = 8.8, 14.9 Hz), 2.28 (3H, s), 1.43 (9H, s); $^{13}C$ NMR ($CD_3OD$, 100 MHz) δ 176.7, 176.5, 173.3, 167.8, 167.0, 166.6, 157.7, 152.7, 143.6, 139.6, 137.4, 130.8, 129.33, 129.29, 121.5, 113.2, 80.8, 76.8, 61.8, 55.5, 54.0, 53.0, 50.1, 41.8, 30.9, 28.7, 11.7; IR ($CH_2Cl_2$) $\nu_{max}$ 3340, 3210, 1678, 1504, 1446, 1267, 1171, 737 $cm^{-1}$; FABHRMS (NBA–CsI) *m/e* 951.2879 ($M^+$ + Cs, $C_{43}H_{50}N_{10}O_7$ requires 951.2918).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[1-amino-1-oxo-3(*S*)-[4-amino-6-(1 (*S*)-carboxyl-2-(1-(triphenylmethyl)imidazol-4-yl)-1-ethylaminocarbonyl)-5-methylpyrimidin-2-yl]-3-propyl]-(*S*)-β-aminoalanine amide (11).** A solution of **10** (4.4 mg, 5.4 μmol) in $CH_3OH$ (0.5 mL) was treated with 0.1 N aqueous LiOH (100 μL) at 4 °C. After stirring for 4 h at 4 °C, the reaction mixture was concentrated under a $N_2$ stream. The mixture was diluted with $H_2O$ (0.5 mL) and extracted with EtOAc (0.5 mL). After acidification with 10% aqueous HCl (pH 4), reverse phase chromatography (C-18, 0.5 × 3 cm, 0–90% $CH_3OH$–$H_2O$ gradient elution) gave **11** (4.1 mg, 4.3 mg theoretical, 94%) as a white amorphous solid: $R_f$ 0.1 ($SiO_2$, 20% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{25}$ −3.0 (*c* 0.2, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 7.44 (1H, s), 7.23–7.34 (9H, m), 7.03–7.12 (6H, m), 6.83 (1H, s), 4.67 (1H, dd, *J* = 4.0, 9.1 Hz), 4.24–4.34 (1H, m), 4.13–4.22 (1H, m), 3.26 (1H, dd, *J* = 4.0, 14.7 Hz), 3.04 (1H, dd, *J* = 9.1, 14.7 Hz), 2.98–3.19 (2H, m), 2.74–2.84 (1H, m), 2.62 (1H, dd, *J* = 9.1, 15.4 Hz), 2.28 (3H, s), 1.42 (9H, s);

$^{13}C$ NMR ($CD_3OD$, 100 MHz) δ 177.2, 175.7, 175.5, 166.9, 166.6, 163.3, 157.7, 152.8, 143.5, 138.8, 138.1, 130.9, 129.3, 129.2, 121.3, 114.1, 81.2, 77.1, 61.4, 55.7, 53.7, 38.8, 32.2, 30.8, 28.7, 11.9; IR (KBr) $\nu_{max}$ 3354, 1673, 1497, 1447, 1391, 1146, 1132, 750 $cm^{-1}$; FABHRMS (NBA) *m/e* 805.3758 ($M^+ + H$, $C_{44}H_{48}N_{10}O_7$ requires 805.3786).

**$N^{\alpha}$-((*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-(1-(triphenylmethyl)imidazole-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (13)**. Method A: DMF (100 μL) was added to a mixture of **11** (3.0 mg, 3.7 μmol), tetrapeptide S (**12**,[44] 3.5 mg, 5.6 μmol), DCC (1.5 mg, 7.5 μmol), and HOBt (0.6 mg, 4.1 μmol) at 4 °C, and the mixture was stirred for 24 h at 25 °C under $N_2$. After concentration in vacuo, the residue was triturated with $CH_2Cl_2$ (3 × 1 mL) to remove DCC and reaction byproducts. Chromatography (C-18, 0.5 × 5 cm, 0–90% $CH_3OH$–$H_2O$ gradient elution) provided **13** (1.6 mg, 5.1 mg theoretical, 31%) as a white amorphous solid: $R_f$ 0.6 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$ +7.5 (*c* 0.08, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 8.18 (1H, s), 8.07 (1H, s), 7.38 (1H, s), 7.22–7.31 (9H, m), 7.02–7.08 (6H, m), 6.80 (1H, s), 4.69 (1H, dd, *J* = 5.5, 8.8 Hz), 4.31 (1H, d, *J* = 4.4 Hz), 4.10 (1H, dq, *J* = 4.4, 6.4 Hz), 4.06–4.15 (1H, m), 3.93 (1H, dq, *J* = 5.2, 6.7 Hz), 3.84 (1H, dd, *J* = 4.9, 8.7 Hz), 3.70 (1H, dd, *J* = 5.2, 6.8 Hz), 3.61–3.67 (2H, m), 3.59 (2H, t, *J* = 6.4 Hz), 3.37 (2H, t, *J* = 7.4 Hz), 3.25 (2H, t, *J* = 6.6 Hz), 3.12 (1H, dd, *J* = 5.5, 14.6 Hz), 3.06 (1H, dd, *J* = 8.8, 14.6 Hz), 2.92 (6H, s), 2.65–2.86 (2H, m), 2.59 (1H, dq, *J* = 6.8, 6.9 Hz), 2.52 (1H, dd, *J* = 4.9, 14.9 Hz), 2.37 (1H, dd, *J* = 8.7, 14.9 Hz), 2.27 (3H, s), 2.14 (2H, tt, *J* = 6.4, 7.4 Hz), 1.41 (9H, s), 1.17 (3H, d, *J* = 6.9 Hz), 1.12 (3H, d, *J* = 6.4 Hz), 1.07 (3H, d, *J* = 6.7 Hz); IR (KBr) $\nu_{max}$ 3422, 1664, 1616, 1157, 1123, 750 $cm^{-1}$; FABMS (NBA) *m/e* 1373 ($M^+$, $C_{66}H_{85}N_{16}O_{11}S_3$).

Method B: DMF (100 μL) was added to a mixture of **17** (3.1 mg, 3.2 μmol), $N^{\alpha}$-BOC-pyrimidoblamic acid **9** (1.5 mg, 3.5 μmol), DCC (1.9 mg, 9.2 μmol) and HOBt (0.5 mg, 3.5 μmol) at 25 °C, and the mixture was stirred for 15 h at 25 °C under $N_2$. The reaction mixture was concentrated in vacuo and triturated with $CH_2Cl_2$ (2 × 0.5 mL). After drying under a $N_2$ stream, reverse phase chromatography (C-18, 0.5 × 2.5 cm, 0–70% $CH_3OH$–$H_2O$ gradient elution) gave **13** (2.4 mg, 4.4 mg theoretical, 55%) as a white amorphous solid.

**$N^{\beta}$-[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-(imidazol-4-yl)-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (3)**. The compound **13** (2.4 mg, 1.76 μmol) was treated with 20% $CF_3CO_2H$–$CH_2Cl_2$ (250 μL) at 4 °C and the mixture was stirred for 2 h at 4 °C. After concentration under a $N_2$ stream, the mixture was treated with a solution of 29% aqueous $NH_4OH$ (4 μL) in $CH_3OH$ (0.5 mL). After stirring for 3 h at 25 °C, the mixture was concentrated under a $N_2$ stream. Chromatography (C-18, 0.5 × 2 cm, 0–20% $CH_3OH$–$H_2O$ gradient elution) afforded **3** (1.7 mg, 1.8 mg theoretical, 94%), as a white amorphous solid: $R_f$ 0.1 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{24}$ −10.0 (*c* 0.07, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 8.20 (1H, s), 8.10 (1H, s), 7.63 (1H, d, *J* = 1.1 Hz), 6.94 (1H, d, *J* = 1.1 Hz), 4.66 (1H, dd, *J* = 6.9, 7.4 Hz), 4.31 (1H, d, *J* = 4.3 Hz), 4.10 (1H, dq, *J* = 4.3, 6.4 Hz), 3.97 (1H, dd, *J* = 4.4, 9.7 Hz), 3.92 (1H, dq, *J* = 5.8, 6.8 Hz), 3.82–3.89 (1H, m), 3.62–3.72 (3H, m), 3.59 (2H, t, *J* = 6.5 Hz), 3.37 (2H, t, *J* = 7.4 Hz), 3.28 (2H, t, *J* = 7.0 Hz), 3.07–3.14 (2H, m), 2.93 (6H, s), 2.86–2.92 (2H, m), 2.70 (1H, dd, *J* = 4.4, 15.4 Hz), 2.58 (1H, dq, *J* = 6.8, 6.9 Hz), 2.53 (1H, dd, *J* = 9.7, 15.4 Hz), 2.26 (3H, s), 2.14 (2H, tt, *J* = 6.5, 7.4 Hz), 1.17 (3H, d, *J* = 6.9 Hz), 1.13 (3H, d, *J* = 6.4 Hz), 1.07 (3H, d, *J* = 6.8 Hz); IR (KBr) $\nu_{max}$ 3328, 1651, 1628, 1560, 1447, 1243, 1088, 760 $cm^{-1}$; ESMS *m/e* 1031 ($M^+$, $C_{42}H_{63}N_{16}O_9S_3$).

**$N^{\alpha}$-(*tert*-Butyloxy)carbonyl-$N^{im}$-triphenylmethyl-L-histidine methyl ester (14)**. Di-*tert*-butyl dicarbonate (0.19 mL, 0.80 mmol) was added to a suspension of **8** (0.11 g, 0.27 mmol) and $NaHCO_3$ (0.14 g, 1.6 mmol) in THF:$H_2O$ (3:1, 6 mL) at 0 °C and the mixture was stirred for 2 h at 25 °C. The reaction mixture was diluted with $H_2O$ (10 mL), and extracted with EtOAc (2 × 10 mL). The organic layer was washed with $H_2O$ (10 mL), saturated aqueous NaCl (10 mL), dried ($Na_2SO_4$) and concentrated in vacuo. Chromatography ($SiO_2$, 1 × 13 cm, 5% $CH_3OH$–$CH_2Cl_2$) provided **14** (0.136 g, 0.138 g theoretical, 98%) as a white amorphous solid: $[\alpha]_D^{25}$ +11.7 (*c* 0.95, $CHCl_3$); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.37 (1H, d, *J* = 1.3 Hz), 7.29–7.36 (9H, m), 7.07–7.14 (6H, m), 6.53 (1H, d, *J* = 1.3 Hz), 6.02 (1H, d, *J* = 8.4 Hz), 4.53 (1H, ddd, *J* = 4.7, 5.0, 8.4 Hz), 3.60 (3H, s), 3.04 (1H, dd, *J* = 5.0, 14.5 Hz), 2.97 (1H, dd, *J* = 4.7, 14.5 Hz), 1.43 (9H, s); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 172.4, 155.6, 142.3, 138.7, 136.5, 129.8, 128.0, 119.5, 79.5, 75.2, 53.7, 52.0, 30.3, 28.3.

**$N^{\alpha}$-(*tert*-Butyloxy)carbonyl-$N^{im}$-triphenylmethyl-L-histidine (15)**. A solution of **14** (47 mg, 92 μmol) in $CH_3OH$ (3 mL) was treated with 0.1 N aqueous LiOH (1.4 mL, 140 μmol) at 4 °C and the mixture was stirred for 2 h at 4 °C. The reaction mixture was neutralized with the addition of 10% aqueous HCl and the $CH_3OH$ was removed under a $N_2$ stream. Filtration provided solid **15** (31 mg). The filtrate was concentrated in vacuo and chromatography of the residue (C-18, 1 × 2 cm, 0–100% $CH_3OH$–$H_2O$ gradient elution) gave additional **15** (8 mg, total 39 mg, 46 mg theoretical, 85%) as a white solid: $[\alpha]_D^{24}$ +14.6 (*c* 0.67, EtOH), lit[61] $[\alpha]_D^{20}$ +5.9 (*c* 1.0, EtOH); $^1H$ NMR

($CD_3OD$, 400 MHz) δ 7.60 (1H, s), 7.33–7.42 (9H, m), 7.10–7.19 (6H, m), 6.83 (1H, s), 4.29 (1H, dd, $J$=4.8, 8.4 Hz), 3.06 (1H, dd, $J$=4.8, 14.4 Hz), 2.88 (1H, dd, $J$=8.4, 14.4 Hz), 1.38 (9H, s).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-1-[[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]-$N^{im}$-triphenylmethyl-L-histidine (16).** DMF (200 μL) was added to a mixture of **15** (5.2 mg, 10.5 μmol), tetrapeptide S (**12**,[44] 8.5 mg, 13.2 μmol), DCC (4.1 mg, 19.9 μmol), HOBt (1.6 mg, 11.5 μmol) and $NaHCO_3$ (3.1 mg, 36.9 mmol) at 25 °C, and the mixture was stirred for 12 h at 25 °C under $N_2$. After concentration in vacuo, $H_2O$ (2 mL) was added to the mixture. The insoluble material was removed by filtration through a Celite pad and washed with $H_2O$ (3 mL). The $H_2O$ layer was subjected to C-18 chromatography (0.5×5 cm, 0–80% $CH_3OH$–$H_2O$ gradient elution) which provided **16** (8.6 mg, 11.2 mg theoretical, 77%) as a white amorphous solid: $R_f$ 0.6 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{24}$+8.4 (*c* 0.25, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 8.19 (1H, s), 8.09 (1H, s), 7.31–7.39 (10H, m), 7.06–7.14 (6H, m), 6.77 (1H, s), 4.32 (1H, d, $J$=4.2 Hz), 4.24 (1H, dd, $J$=6.0, 8.3 Hz), 4.11 (1H, dq, $J$=4.2, 6.4 Hz), 3.88 (1H, dq, $J$=5.6, 6.9 Hz), 3.62–3.69 (3H, m), 3.59 (2H, t, $J$=6.4 Hz), 3.37 (2H, t, $J$=7.5 Hz), 3.26 (2H, t, $J$=6.8 Hz), 2.94 (1H, dd, $J$=6.0, 14.4 Hz), 2.93 (6H, s), 2.77 (1H, dd, $J$=8.3, 14.4 Hz), 2.57 (1H, dq, $J$=6.6, 6.8 Hz), 2.14 (2H, tt, $J$=6.4, 7.5 Hz), 1.38 (9H, s), 1.15 (3H, d, $J$=6.9 Hz), 1.13 (3H, d, $J$=6.4 Hz), 1.03 (3H, d, $J$=6.8 Hz); IR (KBr) $\nu_{max}$ 3405, 1654, 1546, 1252, 1164, 749 $cm^{-1}$; FABHRMS (NBA) *m/e* 1066.4387 ($M^+$+H, $C_{54}H_{68}N_9O_8S_3$ requires 1066.4353).

**1-[[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]-$N^{im}$-triphenylmethyl-L-histidine (17).** 1 N HCl in 90% aqueous HOAc (150 μL) was added to **16** (5.0 mg, 4.7 μmol) at 25 °C and the mixture was stirred for 5 min at 25 °C. After concentration in vacuo, the reaction mixture was dissolved in $CH_3OH$ (0.5 mL), and treated with 29% aqueous $NH_4OH$ (5 μL). After stirring for 1.5 h at 25 °C, the reaction mixture was concentrated under a $N_2$ stream. Chromatography (C-18, 0.5×3 cm, 0–70% $CH_3OH$–$H_2O$ gradient elution) gave **17** (3.1 mg, 4.5 mg theoretical, 68%) as a white amorphous solid and 0.7 mg (14%) of recovered **16**. For **17**: $R_f$ 0.2 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$+5.0 (*c* 0.08, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 8.19 (1H, s), 8.09 (1H, s), 7.42 (1H, s), 7.32–7.39 (9H, m), 7.06–7.14 (6H, m), 6.77 (1H, s), 4.31 (1H, d, $J$=4.2 Hz), 4.10 (1H, dq, $J$=4.2, 6.4 Hz), 3.90 (1H, dq, $J$=5.7, 6.8 Hz), 3.84 (1H, dd, $J$=6.0, 6.8 Hz), 3.62–3.70 (3H, m), 3.59 (2H, t, $J$=6.5 Hz), 3.37 (2H, t, $J$=7.5 Hz), 3.25 (2H, t, $J$=6.8 Hz), 2.98 (1H, dd, $J$=6.0, 14.8 Hz), 2.93 (6H, s), 2.86 (1H, dd, $J$=6.8, 14.8 Hz), 2.54 (1H, dq, $J$=6.9, 7.0 Hz), 2.14 (2H, tt, $J$=6.5, 7.5 Hz), 1.20 (3H, d, $J$=6.9 Hz), 1.13 (3H, d, $J$=6.4 Hz), 1.00 (3H, d, $J$=6.8 Hz); IR (KBr) $\nu_{max}$ 3341, 1654, 1545, 1445, 1250, 1131, 750 $cm^{-1}$; FABHRMS (NBA) *m/e* 966.3790 ($M^+$+H, $C_{49}H_{60}N_9O_6S_3$ requires 966.3829).

**$N^{\alpha}$-Benzyl-$N^{im}$-triphenylmethyl-L-histidine methyl ester (18).** A solution of **8** (0.31 g, 0.75 mmol) in $CH_3OH$ (4 mL) was treated with benzaldehyde (91 μL, 0.90 mmol) at 0 °C and the mixture was stirred for 1.5 h at 25 °C. $NaBH_4$ (29 mg, 0.75 mmol) was added to the resulting mixture at 0 °C. After stirring for 30 min at 5–10 °C, the reaction mixture was treated with 10% aqueous HCl (pH 4) and concentrated in vacuo. The residue was dissolved in $H_2O$ (10 mL), basified with 1 N aqueous NaOH, and extracted with EtOAc (2×10 mL). The organic layer was washed with $H_2O$ (10 mL), saturated aqueous NaCl (10 mL), dried ($Na_2SO_4$) and concentrated in vacuo. Chromatography ($SiO_2$, 2×15 cm, 2% $CH_3OH$–$CH_2Cl_2$) provided **18** (0.36 g, 0.38 g theoretical, 96%) as a colorless syrup: $R_f$ 0.6 ($SiO_2$, 10% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{24}$+2.0 (*c* 0.5, $CH_3OH$); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.36 (1H, d, $J$=1.3 Hz), 7.28–7.34 (9H, m), 7.18–7.28 (5H, m), 7.07–7.14 (6H, m), 6.58 (1H, d, $J$=1.3 Hz), 3.82 (1H, d, $J$=13.0 Hz), 3.66 (1H, d, $J$=13.0 Hz), 3.63 (1H, dd, $J$=6.2, 6.6 Hz), 3.62 (3H, s), 2.94 (1H, dd, $J$=6.2, 14.2 Hz), 2.91 (1H, dd, $J$=6.6, 14.2 Hz), 2.12 (1H, s); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 174.9, 142.4, 139.8, 138.4, 137.1, 129.7, 128.23, 128.15, 127.9, 126.8, 119.3, 75.0, 60.8, 51.9, 51.5, 32.2; IR (neat) $\nu_{max}$ 3060, 1733, 1495, 1445, 1197, 1169, 748 $cm^{-1}$; FABHRMS (NBA) *m/e* 502.2478 ($M^+$+H, $C_{33}H_{31}N_3O_2$ requires 502.2495).

**$N^{\alpha}$-Benzyl-$N^{\alpha}$-methyl-$N^{im}$-triphenylmethyl-L-histidine methyl ester (19).** 37% Aqueous HCHO (92 μL, 1.23 mmol), $NaBH_3CN$ (23 mg, 0.37 mmol) and HOAc (22 μL, 0.37 mmol) were added sequentially to a solution of **18** (126 mg, 0.25 mmol) in $CH_3CN$ (4 mL) at 0 °C. After stirring for 20 min at 25 °C, 0.1 N aqueous NaOH (20 mL) was added and the mixture was extracted with EtOAc (2×20 mL). The organic layer was washed with $H_2O$ (20 mL), saturated aqueous NaCl (20 mL), dried ($Na_2SO_4$) and concentrated in vacuo. Chromatography ($SiO_2$, 0.8×14 cm, 1–2% $CH_3OH$–$CH_2Cl_2$ gradient elution) afforded **21** (80 mg, 129 mg theoretical, 61%) as a colorless syrup: $R_f$ 0.4 ($SiO_2$, 5% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{24}$−22.6 (*c* 0.5, $CH_3OH$); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.39 (1H, d, $J$=1.3 Hz), 7.26–7.34 (9H, m), 7.18–7.24 (5H, m), 7.09–7.16 (6H, m), 6.61 (1H, d, $J$=1.3 Hz), 3.80 (1H, dd, $J$=6.9, 8.4 Hz), 3.74 (1H, d, $J$=13.6 Hz), 3.64 (3H, s), 3.60 (1H, d, $J$=13.6 Hz), 3.09 (1H, dd, $J$=8.4, 14.5 Hz), 2.95 (1H, dd, $J$=6.9, 14.5 Hz), 2.24 (3H, s); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 172.3, 142.3, 139.3, 138.1, 137.6, 129.7, 128.5, 128.1, 127.9, 126.7, 119.2, 75.1, 66.3, 58.6, 50.9, 37.6, 28.8; IR (neat) $\nu_{max}$ 3028, 1733, 1495, 1446, 1158, 747 $cm^{-1}$; FABHRMS (NBA) *m/e* 516.2663 ($M^+$+H, $C_{34}H_{33}N_3O_2$ requires 516.2651).

**$N^{\alpha}$-Methyl-$N^{im}$-triphenylmethyl-L-histidine methyl ester (20).** A solution of **19** (128 mg, 0.25 mmol) in

$CH_3OH$ (2.5 mL) containing 10% Pd–C (50 mg) was stirred under an atmosphere of $H_2$ (1 atm) at 25 °C for 10 h. The reaction mixture was filtered through a Celite pad and washed with 1% $Et_3N$–$CH_3OH$ (10 mL). After concentration in vacuo, chromatography ($SiO_2$, 1 × 10 cm, 2–10% $CH_3OH$–$CH_2Cl_2$ gradient elution) gave **20** (40 mg, 106 mg theoretical, 38%) as a colorless oil and 56 mg (44%) of recovered **19**. Crystallization from $Et_2O$–$iPr_2O$ afforded **20** as a white powder: mp 107–109 °C; $R_f$ 0.5 ($SiO_2$, 10% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{24}$ +16.0 (*c* 0.5, $CH_3OH$); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.36 (1H, d, *J* = 1.4 Hz), 7.28–7.35 (9H, m), 7.08–7.15 (6H, m), 6.56 (1H, d, *J* = 1.4 Hz), 3.62 (3H, s), 3.52 (1H, t, *J* = 6.4 Hz), 2.91 (2H, d, *J* = 6.4 Hz), 2.38 (3H, s), 1.95 (1H, br s); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 174.8, 142.4, 138.6, 137.1, 129.7, 127.9, 119.3, 75.1, 63.3, 51.5, 34.6, 31.9; IR (neat) $\nu_{max}$ 2928, 1730, 1489, 1443, 1323, 1207, 1129, 1027, 751 $cm^{-1}$; FABHRMS (NBA) *m/e* 426.2172 ($M^+$ + H, $C_{27}H_{27}N_3O_2$ requires 426.2182).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[1-amino-1-oxo-3(*S*)-[4-amino-6-[(N-methyl-N-(1(*S*)-(methoxycarbonyl)-2-(1-(triphenylmethyl)imidazol-4-yl)-1-ethyl)amino)carbonyl]-5-methylpyrimidin-2-yl]-3-propyl]-(*S*)-β-aminoalanine amide (21).** A suspension of **20** (9.9 mg, 23.3 μmol), $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**,[53] 6.6 mg, 15.5 μmol) and BOPCl (4.8 mg, 18.3 μmol) in $CH_2Cl_2$ (200 μL) was treated with $Et_3N$ (5.3 μL, 38.0 μmol) and stirred for 6 h at 25 °C. After concentration under a $N_2$ stream, EtOAc (2 mL) was added to the mixture and the solution was washed with $H_2O$ (1 mL), saturated aqueous NaCl (1 mL) and dried ($Na_2SO_4$). Chromatography ($SiO_2$, 0.5 × 3 cm, 5–20% $CH_3OH$–$CH_2Cl_2$ gradient elution) afforded **21** (5.8 mg, 12.9 mg theoretical, 45%) as a white amorphous solid: $R_f$ 0.1 ($SiO_2$, 10% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{25}$ −20.8 (*c* 0.13, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) mixture of rotamers δ 7.31–7.43 (10H, m), 7.10–7.17 (6H, m), 6.90 (0.6H, s), 6.84 (0.4H, s), 5.14 (0.6H, dd, *J* = 5.0, 10.4 Hz), 4.61 (0.4H, dd, *J* = 7.3, 8.4 Hz), 4.02–4.08 (1H, m), 3.80 (1H, dd, *J* = 5.1, 8.7 Hz), 3.75 (3H, s), 3.28–3.36 (0.4H, m, partially obscured by solvent), 3.18 (0.6H, dd, *J* = 10.4, 15.1 Hz), 3.08–3.14 (1H, m), 3.00 (1.2H, s), 2.75 (1.8H, s), 2.60–2.82 (2H, m), 2.35–2.54 (2H, m), 1.82 (1.8H, s), 1.74 (1.2H, s), 1.43 (9H, s); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.28–7.39 (10H, m), 7.06–7.15 (6H, m), 6.75 (0.5H, s), 6.64 (0.5H, s), 5.26 (0.5H, dd, *J* = 4.9, 9.8 Hz), 4.62 (0.5H, dd, *J* = 6.2, 9.4 Hz), 4.01–4.16 (1H, m), 3.83–3.97 (1H, m), 3.73 (1.5H, s), 3.70 (1.5H, s), 3.36 (0.5H, dd, *J* = 4.9, 15.1 Hz), 3.17 (0.5H, dd, *J* = 9.8, 15.1 Hz), 3.17 (0.5H, dd, *J* = 6.2, 14.5 Hz), 3.01 (1.5H, s), 2.99 (0.5H, dd, *J* = 9.4, 14.5 Hz), 2.87–3.04 (1H, m), 2.81 (1.5H, s), 2.39–2.67 (3H, m), 1.89 (1.5H, s), 1.87 (1.5H, s), 1.41 (9H, s); IR (KBr) $\nu_{max}$ 3350, 1653, 1581, 1491, 1445, 1166, 1057, 751 $cm^{-1}$; FABHRMS (NBA–NaI) *m/e* 855.3940 ($M^+$ + Na, $C_{44}H_{52}N_{10}O_7$ requires 855.3918).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[1-amino-1-oxo-3(*S*)-4-[amino-6-[(N-methyl-N-(1(*S*)-carboxy-2-(1-(triphenylmethyl)imidazol-4-yl)-1-ethyl)amino)carbonyl]-5-methylpyrimidin-2-yl]-3-propyl]-(*S*)-β-aminoalanine amide (22).** A solution of **21** (5.8 mg, 7.0 μmol) in $CH_3OH$ (0.7 mL) was treated with 0.1 N aqueous LiOH (140 μL) at 4 °C and stirred for 3.5 h at 4 °C. After concentration under a $N_2$ stream, the reaction mixture was diluted with $H_2O$ (1 mL) and extracted with EtOAc (1 mL). The $H_2O$ layer was acidified with the addition of 10% aqueous HCl (pH 4) and charged to C-18 chromatography (0.5 × 2 cm, 0–70% $CH_3OH$–$H_2O$ gradient elution) which provided **22** (4.6 mg, 5.7 mg theoretical, 80%) as a white amorphous solid: $R_f$ 0.1 ($SiO_2$, 20% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{25}$ +0.4 (*c* 0.2, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) mixture of rotamers δ 7.31–7.46 (10H, m), 7.09–7.17 (6H, m), 6.94 (0.34H, s), 6.85 (0.66H, s), 5.17–5.30 (0.34H, m), 4.32–4.41 (0.66H, m) 4.29 (0.66H, dd, *J* = 4.5, 11.2 Hz), 3.92–4.26 (1.34H, m), 3.33–3.42 (0.34H, m), 3.04 (2H, s), 3.00–3.23 (3H, m), 2.93 (0.66H, d, *J* = 6.4 Hz), 2.80 (1H, s), 2.53–2.85 (2H, m), 1.84 (1H, s), 1.75 (2H, s), 1.44 (9H, s); IR (KBr) $\nu_{max}$ 3390, 1623, 1492, 1399, 1167, 751 $cm^{-1}$; FABHRMS (NBA–CsI) *m/e* 951.2939 ($M^+$ + Cs, $C_{43}H_{50}N_{10}O_7$ requires 951.2918).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[3(*S*)-[4-amino-6-[[[-methyl-N-[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-(triphenylmethyl)imidazol-4-yl)]-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (23).** DMF (150 μL) was added to a mixture of **22** (4.6 mg, 5.6 μmol), tetrapeptide S (**12**,[44] 5.3 mg, 8.4 μmol), DCC (3.1 mg, 15.0 μmol), HOBt (0.4 mg, 2.8 μmol) and $NaHCO_3$ (0.5 mg, 6.0 μmol) at 25 °C, and stirred for 35 h at 25 °C under $N_2$. After concentration in vacuo, the reaction mixture was diluted with $H_2O$ (1 mL) and extracted with EtOAc (0.5 mL). The $H_2O$ layer was charged to C-18 chromatography (0.5 × 4 cm, 0–80% $CH_3OH$–$H_2O$ gradient elution) which provided **23** (5.2 mg, 7.8 mg theoretical, 67%) as a white amorphous solid: $R_f$ 0.6 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$ −15.0 (*c* 0.16, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) mixture of rotamers δ 8.19 (0.34H, s), 8.18 (0.66H, s), 8.10 (0.66H, s), 8.08 (0.34H, s), 7.31–7.41 (10H, m), 7.07–7.14 (6H, m), 6.87 (0.34H, s), 6.83 (0.66H, s), 5.21–5.32 (0.34H, m), 4.71 (0.66H, dd, *J* = 5.1, 10.6 Hz), 4.31 (0.66H, d, *J* = 4.4 Hz), 4.29 (0.34H, d, *J* = 4.3 Hz), 3.85–4.15 (4H, m), 3.61–3.77 (3H, m), 3.59 (2H, t, *J* = 6.4 Hz), 3.37 (2H, t, *J* = 7.4 Hz), 3.22–3.29 (3H, m), 2.97–3.11 (1H, m), 2.94 (2H, s), 2.93 (2H, s), 2.92 (4H, s), 2.82 (1H, s), 2.46–2.73 (5H, m), 2.14 (2H, tt, *J* = 6.4, 7.4 Hz), 1.75 (1H, s), 1.67 (2H, s), 1.42 (9H, s), 1.22 (2H, d, *J* = 6.9 Hz), 1.09–1.20 (7H, m); IR (KBr) $\nu_{max}$ 3331, 1650, 1548, 1250, 1163, 752 $cm^{-1}$; FABMS (NBA) *m/e* 1387 ($M^+$, $C_{67}H_{87}N_{16}O_{11}S_3$).

**$N^{\beta}$-[3(*S*)-[4-Amino-6-[[[N-methyl-N-[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-(imidazol-4-yl)]-1-ethyl]amino]carbonyl]-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (4)**. The compound **23** (3.1 mg, 2.2 μmol) was treated with 20% $CF_3CO_2H$–$CH_2Cl_2$ (0.5 mL) at 4 °C and the mixture was stirred for 2 h at 4 °C. After concentration under a $N_2$ stream, the mixture was treated with a solution of 29% aqueous $NH_4OH$ (5 μL) in $CH_3OH$ (0.5 mL). After stirring for 2 h at 25 °C, the mixture was concentrated under a $N_2$ stream. Chromatography (C-18, 0.5 × 2 cm, 0–70% $CH_3OH$–$H_2O$ gradient elution) afforded **4** (2.1 mg, 2.3 mg theoretical, 92%) as a white amorphous solid: $R_f$ 0.1 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$ −32 (*c* 0.09, $CH_3OH$); $^1H$ NMR ($CD_3OD$, 400 MHz) mixture of rotamers δ 8.21 (1H, s), 8.12 (0.6H, s), 8.11 (0.4H, s), 7.63 (0.4H, d, *J* = 1.0 Hz), 7.56 (0.6H, d, *J* = 1.0 Hz), 6.98 (0.4H, br s), 6.89 (0.6H, br s), 5.37–5.47 (0.4H, m), 4.58 (0.6H, dd, *J* = 4.0, 11.0 Hz), 4.30 (0.6H, d, *J* = 4.6 Hz), 4.29 (0.4H, d, *J* = 4.8 Hz), 4.10 (0.6H, dq, *J* = 4.4, 6.4 Hz), 3.91–4.06 (2.4H, m), 3.72–3.85 (2H, m), 3.63–3.72 (2H, m), 3.59 (2H, t, *J* = 6.4 Hz), 3.36 (2H, t, *J* = 7.2 Hz), 3.22–3.39 (3H, m), 3.05–3.20 (1H, m), 3.00 (1.8H, s), 2.93 (2.4H, s), 2.92 (3.6H, s), 2.84 (1.2H, s), 2.71–3.02 (2.4H, m), 2.48–2.68 (2.6H, m), 2.14 (2H, tt, *J* = 6.4, 7.2 Hz), 1.67 (1.2H, s), 1.48 (1.8H, s), 1.24 (1.8H, d, *J* = 7.0 Hz), 1.11–1.22 (7.2H, m); IR (KBr) $\nu_{max}$ 3328, 1628, 1575, 1446, 1159, 760 $cm^{-1}$; ESMS *m/e* 1045 ($M^+$, $C_{43}H_{65}N_{16}O_9S_3$).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[1-amino-1n-oxo-3(*S*)-[4-amino-6-(1(*S*)-(benzyloxycarbonyl)-2-(1-(triphenylmethyl)imidazol-4-yl)-1-ethoxycarbonyl)-5-methylpyrimidin-2-yl]-3-propyl]-(*S*)-β-aminoalanine amide (29)**. A suspension of $N^{\alpha}$-BOC-pyrimidoblamic acid (**9**, 10 mg, 23.5 μmol) in $CH_3OH$ (0.5 mL) was treated with $Et_3N$ (3.3 μL, 23.5 μmol), concentrated with $N_2$ stream and dried under vacuum. A solution of the resulting salt and **28**[62] (14.5 mg, 28.2 μmol) in $CH_2Cl_2$ (0.5 mL) was treated with BOPCl (6.7 mg, 25.9 μmol) and stirred for 4 days at 25 °C. The reaction mixture was diluted with EtOAc (5 mL) and washed with saturated aqueous $NaHCO_3$ (2 mL). The water layer was extracted with EtOAc (5 mL). The combined organic layer was washed with $H_2O$ (2 mL), saturated aqueous NaCl (2 mL), dried ($Na_2SO_4$) and concentrated in vacuo. Chromatography ($SiO_2$, 0.5 × 5 cm, 10% $CH_3OH$–$CH_2Cl_2$) gave **6** (5.7 mg, 21.1 mg theoretical, 26%) as a colorless syrup: $R_f$ 0.4 ($SiO_2$, 10% $CH_3OH$–$CH_2Cl_2$); $[\alpha]_D^{25}$ −3.9 (*c* 0.33, DMF); $^1H$ NMR ($CDCl_3$, 400 MHz) δ 7.23–7.44 (15H, m), 7.00–7.06 (6H, m), 6.72 (1H, d, *J* = 1.1 Hz), 5.51 (1H, t, *J* = 5.9 Hz), 5.15 (1H, d, *J* = 12.4 Hz), 5.14 (1H, d, *J* = 12.4 Hz), 4.00–4.11 (1H, m), 3.85 (1H, dd, *J* = 4.6, 9.1 Hz), 3.25 (2H, d, *J* = 5.9 Hz), 2.70–2.82 (1H, m), 2.55–2.66 (1H, m), 2.47 (1H, dd, *J* = 4.6, 15.0 Hz), 2.41 (1H, dd, *J* = 9.1, 15.0 Hz), 2.02 (3H, s), 1.43 (9H, s); $^{13}C$ NMR ($CDCl_3$, 100 MHz) δ 174.0, 173.7, 168.9, 166.8, 165.3, 163.9, 155.9, 152.1, 142.2, 138.4, 135.3, 134.9, 129.7, 128.6, 128.5, 128.2, 128.1, 128.0, 119.9, 111.7, 79.8, 75.3, 73.5, 67.3, 60.8, 53.7, 49.5, 41.0, 30.3, 28.4, 11.6; IR (neat) $\nu_{max}$ 3340, 3206, 1740, 1674, 1175, 1080, 733 $cm^{-1}$; FABHRMS (NBA–CsI) *m/e* 1028.3030 ($M^+$ + Cs, $C_{49}H_{53}N_9O_8$ requires 1028.3071).

**$N^{\alpha}$-((*tert*-Butyloxy)carbonyl)-$N^{\beta}$-[1-amino-1-oxo-3(*S*)-[4-amino-6-(1(*S*)-carboxyl-2-(1-(triphenylmethyl)imidazol-4-yl)-1-ethoxycarbonyl)-5-methylpyrimidin-2-yl]-3-propyl]-(*S*)-β-aminoalanine amide (30)**. A solution of **29** (6.8 mg, 7.6 μmol) in $CH_3OH$ (1 mL) was stirred over 10% Pd–C (3.0 mg) under an atmosphere of $H_2$ (1 atm) for 30 min at 25 °C. The reaction mixture was filtered through a Celite pad (washed with 1% $Et_3N$–$CH_3OH$, 5 mL) and concentrated in vacuo. Chromatography (C-18, 0.5 × 4 cm, 30–70% $CH_3OH$–$H_2O$ gradient elution) provided **30** (4.8 mg, 6.1 mg theoretical, 79%) as a white amorphous powder: $R_f$ 0.9 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$ −7.9 (*c* 0.24, DMF); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 7.45 (1H, s), 7.27–7.34 (9H, m), 7.06–7.12 (6H, m), 6.87 (1H, s), 5.39 (1H, dd, *J* = 3.2, 9.1 Hz), 4.22–4.31 (1H, m), 4.05–4.14 (1H, m), 3.26 (1H, dd, *J* = 3.2, 15.1 Hz), 3.15 (1H, dd, *J* = 9.1, 15.1 Hz), 2.91–3.08 (2H, m), 2.70–2.81 (1H, m), 2.62 (1H, dd, *J* = 8.8, 15.6 Hz), 2.09 (3H, s), 1.43 (9H, s); $^{13}C$ NMR (DMF-$d_7$, 100 MHz) δ 174.0, 173.4, 171.5, 168.5, 166.6, 165.0, 162.9, 156.4, 154.2, 143.3, 138.5, 137.2, 130.2, 128.8, 128.7, 120.2, 110.2, 78.8, 75.6, 74.4, 61.8, 55.5, 50.2, 42.1, 31.1, 28.4, 12.1; IR (KBr) $\nu_{max}$ 3421, 1678, 1636, 1399, 1165, 750 $cm^{-1}$; FABHRMS (NBA–CsI) *m/e* 938.2581 ($M^+$ + Cs, $C_{42}H_{47}N_9O_8$ requires 938.2602).

**$N^{\alpha}$-((*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-(1-(triphenylmethyl)imidazol-4-yl)-1-ethoxy]carbonyl]-5-methylpyrimidin-2-yl] 1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (31)**. DMF (200 μL) was added to a mixture of **30** (4.2 mg, 5.2 μmol), tetrapeptide S (**12**, 3.2 mg, 5.2 μmol), DCC (2.3 mg, 11.1 μmol), and HOBt (0.2 mg, 1.5 μmol) at 4 °C and the mixture was stirred for 45 h at 25 °C under $N_2$. After concentration in vacuo, the residue was suspended with $CHCl_3$ (0.5 mL). The suspension was filtered through a Celite pad and washed with $CHCl_3$ (0.5 mL). Collection of the insoluble product by washing the Celite with DMF (3 × 0.4 mL) and concentration in vacuo gave **31** (6.4 mg, 7.2 mg theoretical, 89%, >95% pure) as a pale yellow amorphous solid: $R_f$ 0.6 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$ −2.5 (*c* 0.32, DMF); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 8.19 (1H, s), 8.10 (1H, s), 7.40 (1H, d, *J* = 1.4 Hz), 7.25–7.38 (9H, m), 7.03–7.11 (6H, m), 6.82 (1H, d, *J* = 1.4 Hz), 5.46 (1H, dd, *J* = 4.8, 8.6 Hz), 4.30 (1H, d, *J* = 4.2 Hz), 4.24–4.34 (1H, m), 4.11 (1H, dq, *J* = 4.2, 6.4 Hz), 4.06–4.17 (1H, m), 3.97 (1H, dq, *J* = 5.6, 6.6 Hz), 3.62–3.77 (3H, m), 3.59 (2H,t, *J* = 6.4 Hz), 3.38 (2H, t, *J* = 7.1 Hz), 3.26 (2H, t. *J* = 6.9 Hz), 3.19 (1H, dd, *J* = 4.8, 15.1 Hz), 3.17 (1H, dd, *J* = 8.6, 15.1 Hz), 2.94

(6H, s), 2.50–2.83 (5H, m), 2.15 (2H, tt, $J=6.4$, 7.1 Hz), 2.07 (3H, s), 1.42 (9H, s), 1.19 (3H, d, $J=6.9$ Hz), 1.13 (3H, d, $J=6.4$ Hz), 1.10 (3H, d, $J=6.6$ Hz); IR (KBr) $\nu_{max}$ 3405, 1655, 1547, 1225, 1161, 751 $cm^{-1}$; ESMS *m/e* 1375 ($M^+ + H$, $C_{66}H_{84}N_{15}O_{12}S_3$).

**$N^{\beta}$-[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-(imidazol-4-yl)-1-ethoxy]carbonyl]-5-methylpyrimidin-2-yl]-1-amino-1-oxo-3-propyl]-(*S*)-β-aminoalanine amide (5).** Compound **31** (3.2 mg, 2.3 μmol) was treated with 20% $CF_3CO_2H$–$CH_2Cl_2$ (0.8 mL) at 4 °C and the mixture was stirred for 2 h at 4 °C. The reaction mixture was concentrated with a $N_2$ stream and dried under vacuum. The residue was suspended with $CHCl_3$ (0.5 mL) and filtered through a Celite pad. After washing with $CHCl_3$ (2 × 0.5 mL), collection of the insoluble product by washing the Celite with DMF (3 × 0.4 mL) and concentration in vacuo provided **5** (3.1 mg, quant., >95% pure) as a pale yellow amorphous solid: $R_f$ 0.1 ($SiO_2$, 10:9:1 $CH_3OH$:10% aqueous $NH_4OAc$:10% aqueous $NH_4OH$); $[\alpha]_D^{25}$ −13.3 (*c* 0.15; DMF); $^1H$ NMR ($CD_3OD$, 400 MHz) δ 8.21 (1H, s), 8.12 (1H, s), 7.46 (1H, s), 7.37 (1H, s), 5.51 (1H, t, $J=5.9$ Hz), 4.30 (1H, d, $J=4.2$ Hz), 3.59–4.14 (4H, m), 3.62–3.74 (3H, m), 3.59 (2H, t, $J=6.5$ Hz), 3.33–3.42 (4H, m), 3.25–3.30 (2H, m, partially obscured by solvent), 2.93 (6H, s), 2.87–3.03 (2H, m), 2.71 (1H, dd, $J=4.6$, 15.4 Hz), 2.59 (1H, dd, $J=9.4$, 15.4 Hz), 2.49 (1H, dq, $J=6.5$, 6.8 Hz), 2.17 (3H, s), 2.15 (2H, tt, $J=6.5$, 7.5 Hz), 1.17 (3H, d, $J=7.0$ Hz), 1.14 (3H, d, $J=6.5$ Hz), 1.13 (3H, d, $J=6.2$ Hz); IR (KBr) $\nu_{max}$ 3431, 1679, 1554, 1204, 1133, 802 $cm^{-1}$; ESMS *m/e* 1032 ($M^+$, $C_{42}H_{62}N_{15}O_{10}S_3$).

### General procedure for the supercoiled ΦX174 DNA cleavage reactions: relative efficiency study

All reactions were run with freshly prepared Fe(II) complexes. The Fe(II) complexes were prepared by combining 1 μL of a $H_2O$ solution of the agent at the 10 times specified concentration with 1 μL of a freshly prepared equimolar aqueous $Fe(NH_4)_2(SO_4)_2$ solution followed by vortex mixing and centrifugation. Each of the Fe(II) complex solutions was treated with 7 μL of a buffered DNA solution containing 0.25 μg of supercoiled ΦX174 RFI DNA ($1.4 \times 10^{-8}$ M) in 50 mM Tris–HCl buffer solution (pH 8). The DNA cleavage reactions were initiated by adding 1 μL of aqueous 10 mM 2-mercaptoethanol. The final concentrations of the agents employed in the study were 32 μM Fe(II) control, 2.0 and 4.0 μM deglycobleomycin $A_2$, 2.0 and 4.0 μM **3**, 16.0 and 32.0 μM **4** and 16.0 and 32.0 μM **5**. The DNA reaction solutions were incubated at 25 °C for 1 h. The reactions were quenched with the addition of 5 μL of loading buffer formed by mixing Keller buffer (0.4 M Tris–HCl, 0.05 M NaOAc, 0.0125 M EDTA, pH 7.9) with glycerol (40%), sodium dodecyl sulfate (0.4%) and bromophenol blue (0.3%). Electrophoresis was conducted on a 1% agarose gel at 50 V for 3 h and the gel was stained with 0.1 μg $mL^{-1}$ ethidium bromide and visualized on a UV transilluminator and photographed using polaroid T667 black and white instant film (Fig. 1). Direct fluorescence quantitation of DNA in the presence of ethidium bromide was conducted using a Millipore Bio Image 60S RFLP system visualized on a UV (312 nm) transilluminator taking into account the relative fluorescence intensities of Forms I–III ΦX174 DNA (Form II and III fluorescence intensities are 0.7 times that of Form I).

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

The Fe(II) complexes were formed by mixing 1 μL of aqueous 40 μM **3** or 240 μM **4** or **5** solutions with 1 μL of a freshly prepared 40 or 240 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 ($1.4 \times 10^{-8}$ M) in 50 mM Tris–HCl buffer solution (pH 8) were added to each of the Fe(II) complex solutions. The final concentrations of **3**–**5** employed in the study were 4, 24, and 24 μM, respectively. 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 40, 30, 25, 20, 15, 12, 10, 8, 6, 4, 2, 1, and 0.5 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[56] assuming a Poisson distribution and the results are summarized in Table 1. For **3**, the ratio was established to be 1:12 at 4 μM. For **4**, the ratio was established to be 1:61 at 24 μM. For **5**, the ratio was established to be 1:49 at 24 μM.

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

All reactions were run with freshly prepared Fe(III) complexes. The Fe(III) complexes were prepared by combining 1 μL of a $H_2O$ solution of the agent at 10 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 2 μL of a 10 mM phosphate buffer solution ($Na_2HPO_4$–$NaH_2PO_4$, pH 7.0) containing 10 mM KCl and 5 μL of a buffered DNA solution containing the $^{32}P$ 5′-end-labeled w794 DNA.[58] The final concentrations of the agents employed in the study were 32 and 64 μM control Fe(III), 2.0 and 4.0 μM deglycobleomycin $A_2$, 2.0, 4.0, and 8.0 μM **3**, 32 and 64 μM **4** and **5**. The

DNA cleavage reactions were initiated by adding 1 μL of 50% aqueous $H_2O_2$. The DNA reaction solutions were incubated at 37 °C for 30 min. The reactions were quenched with the addition of 2 μL of 50% aqueous glycerol followed by EtOH precipitation and isolation of the DNA. The DNA was resuspended in 6 μL of TE buffer (pH 8.0), and formamide dye (6 μL) was added to the supernatant. Prior to electrophoresis, the samples were warmed at 100 °C for 5 min, placed in an ice bath, 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 6 (**3**, **4**, Fe(III)) or 2 experiments (**5**).

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