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Solid-Phase Synthesis and Biochemical Evaluation of Conformationally Constrained Analogues of Deglycobleomycin A_5

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Abstract—Deglycobleomycin binds to and degrades the self-complementary oligonucleotide $d(CGCTAGCG)_2$ in a sequence selective fashion. A previous modeling study [J. Am. Chem. Soc. 120, (1998), 7450] had shown that, during binding to double stranded DNA, the conformation of the methylvalerate domain of deglycoBLM approximated that of *S*-proline. In the belief that an analogue of deglycoBLM structurally constrained to mimic the DNA-bound conformation might exhibit facilitated DNA binding and cleavage, an analogue of deglycoBLM was prepared in which the methylvalerate moiety was replaced by *S*-proline. This deglycoBLM analogue, as well as the related analogue containing *R*-proline, was synthesized on a TentaGel resin. Both of the analogues were found to be capable of binding Fe^{2+} and activating O_2 for transfer to styrene. However, both deglycoBLM analogues exhibited diminished abilities to effect the relaxation of supercoiled plasmid DNA, and neither mediated sequence selective DNA cleavage.

Introduction

The bleomycins (BLMs), originally isolated from a fermentation broth of *Streptomyces verticillus* by Umezawa and coworkers,[1] are structurally related, glycopeptide derived antitumor antibiotics used extensively in the clinic for the treatment of several types of cancers.[2] It is believed that the anticancer activity of BLM is due to the oxidative degradation of DNA[3,4] and possibly RNA[5–7] by the drug in the presence of a redox active metal ion such as Fe[8,9] or Cu.[10] Metal independent cleavage of RNA has also been reported and could plausibly contribute to the therapeutic effects.[11]

BLM has four main structural domains (Fig. 1); the function(s) of each have been studied by physical measurements such as NMR, and by the structural modification of individual domains. The metal binding domain is responsible for metal binding, oxygen binding and activation, and sequence selective cleavage of DNA.[3,4] The carbohydrate moiety participates in metal binding[12,13] and possibly also in cell surface recognition.[14] In the absence of the carbohydrate moiety the deglycobleomycins exhibit a potency and sequence selective DNA degradation similar to that of bleomycin.[15,16] The C-terminal domain of BLM is important for DNA binding.[17] The importance of the linker domain, comprised of the dipeptide between the DNA binding and metal binding domains, has been the subject of a number of recent studies.

Based on NMR measurements and molecular dynamics calculations of Co·BLM–DNA complexes[18–21] and the study of deglycoBLM analogues structurally modified within the linker domain,[22–24] it has been suggested that the methylvalerate moiety within the linker domain assumes a specific compact conformation during complexation with DNA. Methyl substitutions at positions 2 and 4 also play important roles in stabilizing the compact conformation essential for DNA binding.[24] A parallel study of the binding of Zn·BLM[25,26] and Zn·deglycoBLM[27,28] also provided evidence for a bent conformation of the DNA oligonucleotide-bound metalloBLMs. In an effort to develop a conformationally rigid deglycoBLM analogue that could mimic the behavior of the parent molecule, and thereby provide evidence in support of the putative bent structure of (deglyco)BLM within the complex with DNA, we modeled the conformation of deglycoBLM analogues containing *R*-proline and *S*-proline in place of the methylvalerate moiety normally present in deglycoBLM (Fig. 2). These modeling studies suggested that the deglycoBLM analogue containing *S*-proline (**1a**) was reasonably similar in conformation to the conformation

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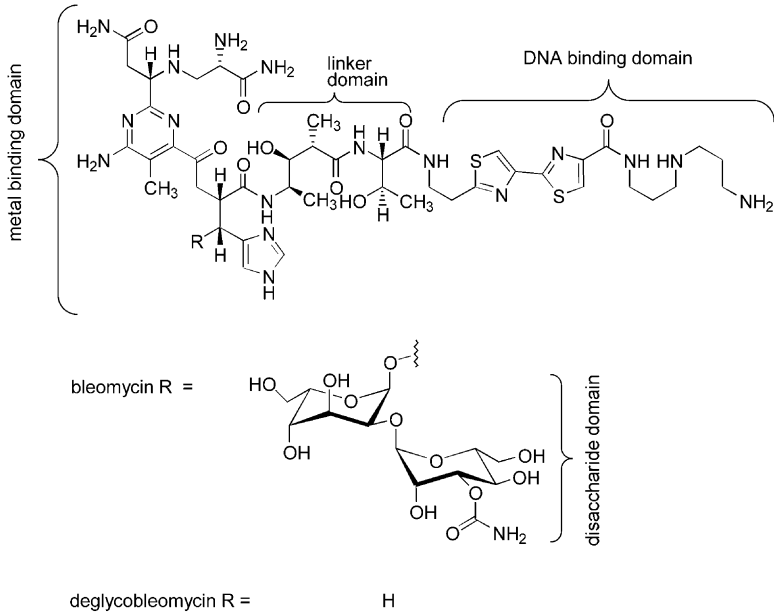


Figure 1. Structures and domains of BLM A_5 and deglycoBLM A_5.

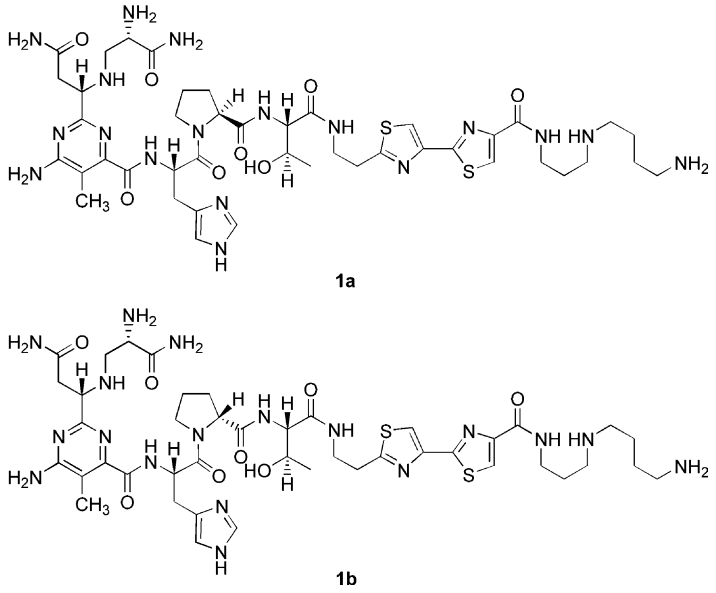


Figure 2. DeglycoBLM analogues containing *S*-proline (**1a**) and *R*-proline (**1b**) in place of the methylvalerate moiety.

calculated for Zn(II)·deglycoBLM A_5 (Fig. 3). Presently we describe the syntheses and biochemical evaluation of the deglycoBLM analogues containing *S*- and *R*-proline in place of the methylvalerate moiety.

Results

Syntheses of deglycoBLM analogues

The deglycoBLM analogues were prepared by solid phase synthesis on a TentaGel resin in analogy with recent efforts from our laboratory.[29–31] Resin **2** (Scheme 1) was prepared as described by Bycroft[32–34] with a loading 0.242 mmol/g. The terminal amine was protected with an *o*-nitrobenzenesulfonyl (NBS) group in the presence of Hunig's base to afford resin **3**. Mitsunobu reaction[35] of resin **3** with *N*-Boc-3-aminopropan-1-ol[36] followed by Boc removal gave resin **4** (Scheme 1). To quantify the loading on resin **4**, a small amount of the resin was treated with an excess of FmocOSu in the presence of Hunig's base. Quantitative Fmoc cleavage[37] indicated that the loading was 0.185 mmol/g, corresponding to a yield of 81% for the three steps involved in resin preparation. Resin **4** was used promptly for the elaboration of deglycoBLMs **1**.

Fmoc-protected bithiazole,[29–31] was coupled to resin **4** in the presence of HOBT, HBTU and Hunig's base (Scheme 2).[38] This coupling proceeded in 88% yield, resulting in a loading of 0.155 mmol/g. Fmoc deprotection (20% piperidine in DMF), followed by condensation with

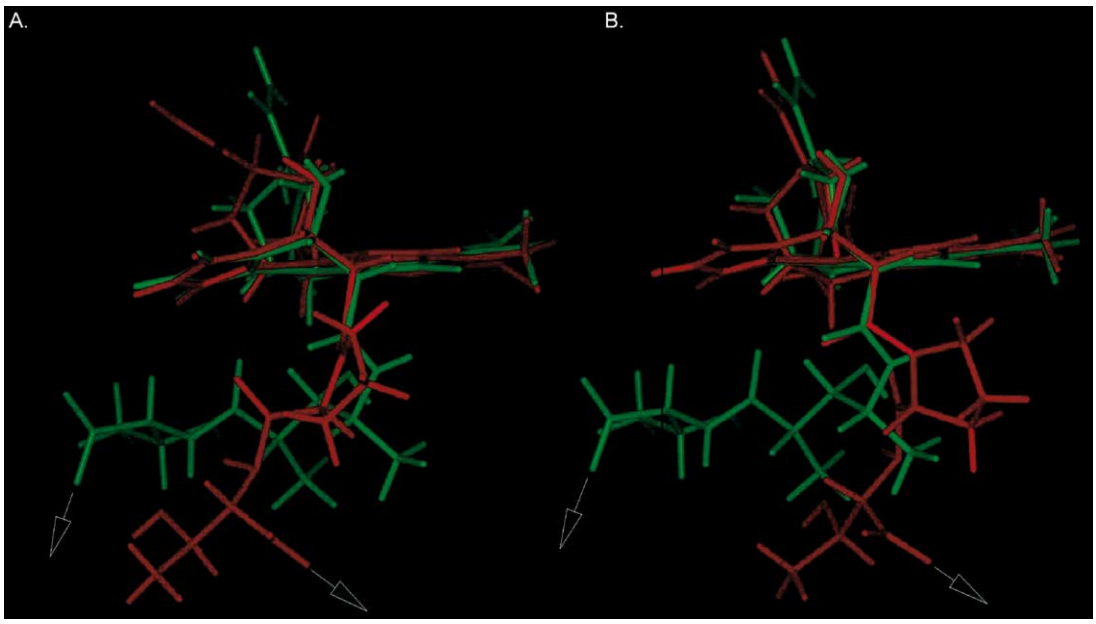
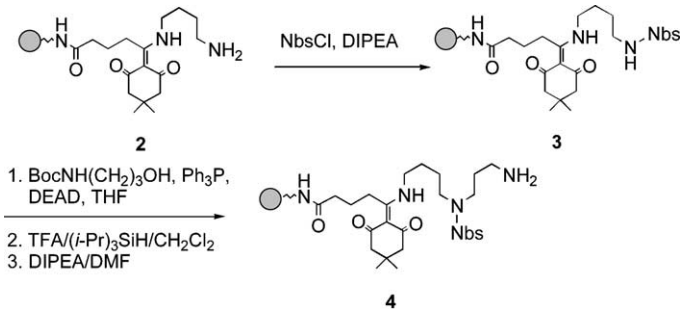


Figure 3. Comparison of Zn(II)·deglycoBLM (green) with (A) Zn(II)·deglycoBLM derivative **1a** (red) and (B) Zn(II)·deglycoBLM derivative **1b** (red). The DNA binding domains (arrows) have been removed for clarity.



Scheme 1. Synthesis of mono-protected spermidine linked to a TentaGel resin.

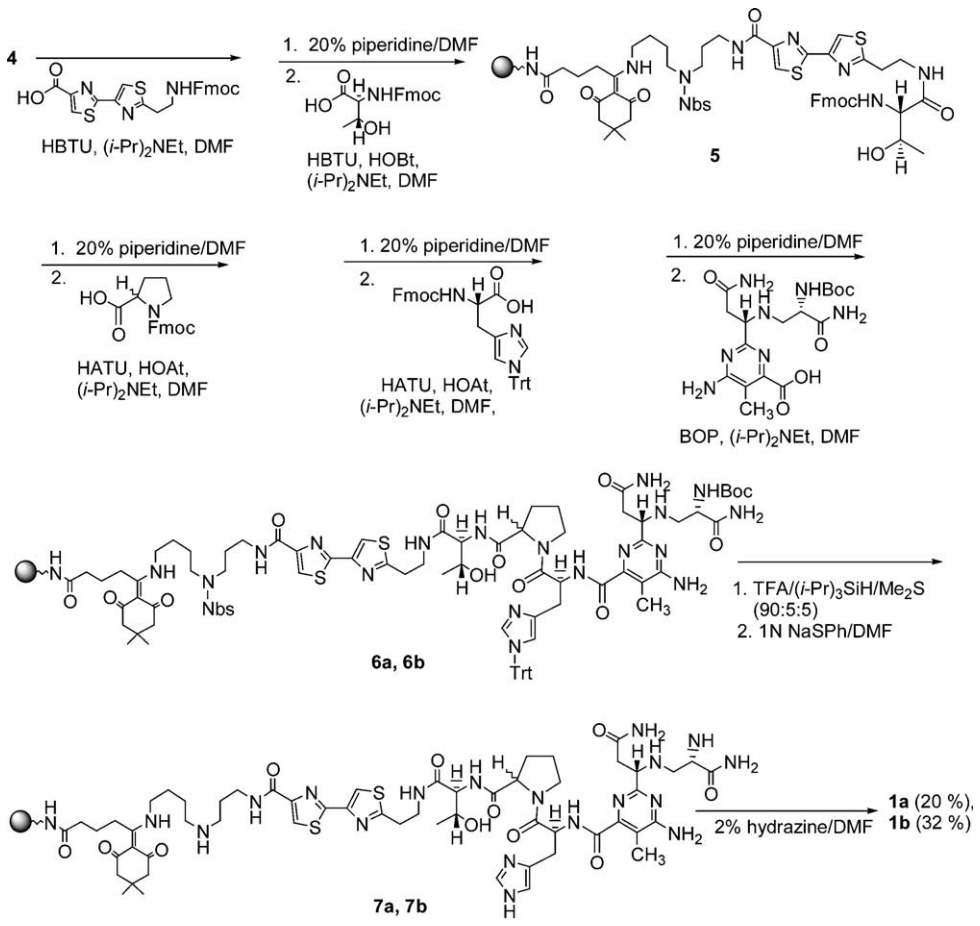
Fmoc-threonine (HOBt, HBTU, and Hunig's base) gave the Fmoc-protected resin **5**. The loading was found to be 0.137 mmol/g for this step, corresponding to a 90% yield.

The tripeptide linked resin **5** was divided into portions to prepare the *R*- and *S*-proline-linked tetrapeptides. Fmoc cleavage was achieved by treating resin **5** with 20% piperidine in DMF. The resin was coupled with Fmoc-*S*-proline to produce the precursor of **1a**, or Fmoc-*R*-proline to produce the precursor of **1b** (HATU, HOAT, and Hunig's base). The loadings for the tetrapeptide-linked resins were 0.132 and 0.136 (97 and 100%), respectively, for the precursors of **1a** and **1b**. After removal of the Fmoc group, both tetrapeptide-linked resins were coupled with Fmoc-tritylhistidine (HATU, HOAT, and Hunig's base). Following Fmoc deprotection, the loadings were found to be 0.129 mmol/g for the precursor of **1a**, and 0.124 mmol/g for the precursor of **1b** (100 and 96%, respectively). The syntheses of the fully protected deglycoBLM A_5 analogues (**6a** and **6b**) were completed by the addition of Boc-pyrimidoblamic acid[40–42] to the resin-bound pentapeptides (excess BOP, and diisopropylethylamine at 0 °C in the absence of light under nitrogen) (Scheme 2).

The acid-labile protecting groups were removed successfully with a 50% solution of 90:5:5 TFA–(*i*-Pr)$_3$SiH–Me_2S in CH_2Cl_2 over a period of 4 h. Removal of the NBS group (1 N NaSPh in DMF) afforded the resin-bound, fully deprotected deglycobleomycin analogues **7a** and **7b**. Cleavage of these deglycobleomycins from the resin was accomplished by three treatments of each resin with 2% hydrazine in DMF. The filtrate was concentrated under an argon atmosphere and the residue was dissolved in 0.5 mL of CF_3COOH. The deglycoBLM analogues precipitated following slow addition of the CF_3COOH solution to ether cooled in dry ice. The isolated solids were washed with 2 mL of cold ether to afford the crude products. DeglycoBLMs analogues **1a** and **1b** were purified by C_{18} reversed-phase HPLC. Purified deglycoBLMs **1a** and **1b** were collected and lyophilized to yield the products as colorless solids. These were characterized by ESI-MS and ^{1}H NMR spectroscopy.

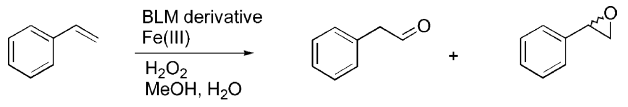
Oxidative transformations mediated by deglycoBLMs 1

In previous studies, it has been reported that Fe(III)·BLM can function as a catalyst to transfer oxygen to olefinic substrates in the presence of peroxide



Scheme 2. Synthesis and cleavage of resin-bound analogues to afford deglycoBLMS **1a** and **1b**.

(Scheme 3).[43,44] Accordingly, deglycoBLM analogues **1a** and **1b** were complexed with Fe^{3+} and used as catalysts to oxidize styrene in the presence of excess hydrogen peroxide. As shown in Table 1, both of these conformationally constrained analogues were capable of oxidizing styrene to styrene oxide and phenylacetaldehyde, albeit with somewhat lower efficiency than that of bleomycin itself.



Scheme 3. Oxidation of styrene by deglycobleomycin A_5 derivatives **1a** and **1b**.

The cleavage of DNA by deglycoBLM analogues **1a** and **1b** was studied initially using supercoiled pSP64 plasmid DNA as a substrate. As shown in Figure 4, the use of increasing concentrations of **1a** or **1b** in the presence of 1.5 μM Fe^{2+} resulted in increasing conversion of Form I (supercoiled) to Form II (nicked circular) DNA. Unlike deglycoBLM A_5 (lanes 13 and 14), neither **1a** nor **1b** produced any detectable Form III (linear duplex) DNA. The conversion of Form I to Form II DNA was comparable for deglycoBLM **1a** than **1b** (cf.

Table 1. Oxygen transfer to styrene mediated by bleomycin and conformationally constrained BLM analogues **1a** and **1b** in the presence of Fe^{3+}[a]

BLM derivative	Oxidized product	Product conc (mM)		
		40 min	80 min	120 min
Bleomycin[b]	Phenylacetaldehyde	2.11±0.61	4.56±2.84	5.31±2.21
	Styrene oxide	0.78±0.04	0.64±0.09	0.51±0.13
DeglycoBLM **1a**[c]	Phenylacetaldehyde	0.56±0.22	0.53±0.04	0.70±0.40
	Styrene oxide	0.68±0.42	0.52±0.04	0.57±0.27
DeglycoBLM **1b**[d]	Phenylacetaldehyde	0.37±0.02	0.38±0.03	0.38±0.04
	Styrene oxide	0.44±0.04	0.40±0.05	0.38±0.05

[a]All concentrations are in mM, and all values are the mean of three experiments.
[b]Final concentration in reaction mixture was 0.79 mM.
[c]Final concentration in reaction mixture was 0.49 mM.
[d]Final concentration in reaction mixture was 0.35 mM.

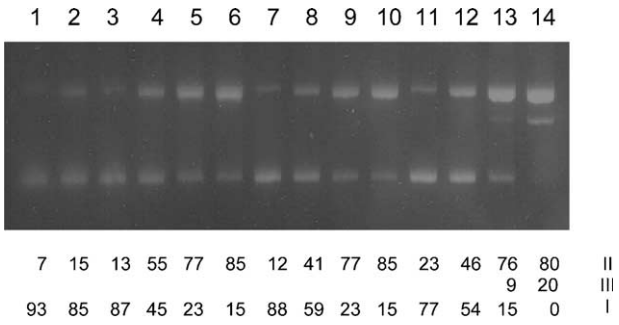


Figure 4. Cleavage of supercoiled pSP64 DNA by deglycoBLM analogues **1a** and **1b**. Lane 1: DNA alone; lane 2: 1.5 μM Fe^{2+}; lane 3: 5 μM **1b**; lane 4: 1 μM **1b** + 1.5 μM Fe^{2+}; lane 5: 3 μM **1b** + 1.5 μM Fe^{2+}; lane 6: 5 μM **1b** + 1.5 μM Fe^{2+}; lane 7: 5 μM **1a**; lane 8: 1 μM **1a** + 1.5 μM Fe^{2+}; lane 9: 3 μM **1a** + 1.5 μM Fe^{2+}; lane 10: 5 μM **1a** + 1.5 μM Fe^{2+}; lane 11: 5 μM deglycoBLM A_5; lane 12: 1 μM dgBLM A_5 + 1.5 μM Fe^{2+}; lane 13: 3 μM deglycoBLM A_5 + 1.5 μM Fe^{2+}; lane 14: 5 μM deglycoBLM A_5 + 1.5 μM Fe^{2+}.

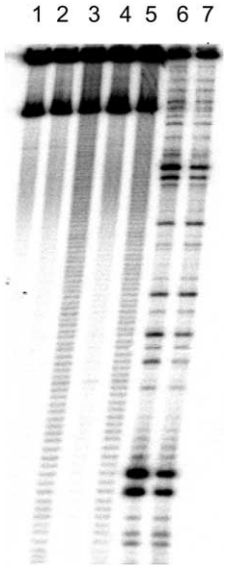


Figure 5. Cleavage of a 5′-^{32}P end labeled DNA duplex by deglycoBLM analogues **1a** and **1b**. Lane 1: DNA alone; lane 2: 1 μM **1b** + 10 μM Fe^{2+}; lane 3: 10 μM **1b** + 10 μM Fe^{2+}; lane 4: 1 μM **1a** + 10 μM Fe^{2+}; lane 5: 10 μM **1a** + 10 μM Fe^{2+}; lane 6: 1 μM deglycoBLM A_5 + 10 μM Fe^{2+}; lane 7: 10 μM deglycoBLM A_5 + 10 μM Fe^{2+}. The band of intermediate mobility in lanes 1–5 was due to adventitious non-denatured DNA.

lanes 6 and 10), but not the result that might have been anticipated based on initial modeling studies (Fig. 3).

Also studied was the cleavage of a 5′-^{32}P end labeled 158-base pair DNA duplex by Fe(II)·deglycoBLMs **1a** and **1b**. As shown in Figure 5, both analogues effected significant DNA cleavage when employed at 10 μM concentration in the presence of equimolar Fe^{2+}, but neither produced sequence selective cleavage of the DNA duplex substrate.

Discussion

Several studies have been reported that analyze the roles of the individual structural domains of BLM in sequence selective DNA cleavage.[3–5] Structurally modified BLM analogues have been used to define the roles of the metal binding domain in DNA recognition. These studies have established that the metal binding domain can determine both sequence[45] and strand selectivity[46] of DNA cleavage. While the metal binding domain of deglycobleomycin alone was not able to cleave DNA in a sequence selective fashion,[47] presumably due to inadequate affinity for DNA, a structural analogue of this domain was shown to mediate DNA cleavage with a selectivity quite similar to that of BLM itself.[48]

While the metal binding domain of BLM is believed to be the primary determinant of sequence selective cleavage by BLM, studies involving photoinduced DNA cleavage by halogenated bithiazoles indicated that these species can bind to DNA in a sequence selective fashion, albeit with a selectivity different than that of BLM.[49,50] When the chlorinated bithiazoles were incorporated within deglycoBLM analogues, these species mediated photoactivated DNA cleavage in the same fashion as the chlorinated bithiazoles. Alternatively, cleavage of the same DNA substrate could be effected in dark reactions by admixture of Fe^{2+} under aerobic conditions. Oxidative cleavage exhibited sequence selectivity of DNA cleavage characteristic of (deglyco)BLM itself.[51]

In the aggregate, these findings suggested that both the metal binding and C-terminal domains of (deglyco)-BLM mediate sequence selective DNA *binding*. It seems likely that DNA *cleavage* is maximally efficient at sites that can accommodate the binding preferences of both of the foregoing structural domains of BLM. In this context, one would expect that the linker region that connects the metal binding and C-terminal domains should be able to facilitate the binding of the attached domains to DNA. In this sense, it is not surprising that both spectroscopic[18–21,25–28] and synthetic studies have suggested a compact folded structure for the linker domain of (deglyco)BLM.

In the present study, two analogues of deglyco(BLM) have been prepared in which proline has replaced the methylvalerate moiety normally present. The analogue containing *S*-proline (**1a**) was predicted to have a conformation reasonably similar to that calculated for Zn(II)·deglycoBLM (Fig. 3). In comparison, analogue **1b** containing *R*-proline was predicted to assume a rather different conformation.

Fe(II)·deglycoBLMs **1a** and **1b** were both found to transfer oxygen to styrene, verifying that both could activate oxygen for DNA cleavage (Table 1). While both analogues mediated concentration dependent relaxation of supercoiled plasmid DNA (Fig. 4), as well as the degradation of linear duplex DNA (Fig. 5), neither deglycoBLM analogue effected DNA cleavage in a sequence selective fashion.

In principle, the lack of sequence selective DNA cleavage may simply reflect the inadequacy of conformationally constrained proline analogues **1a** or **1b** as models of the DNA-bound conformation of (deglyco)-

BLM defined for Zn(II)·deglycoBLM.[27] In this context, it is interesting to note that a parallel study has utilized an analysis of the folded conformation of the methylvalerate moiety of Co(III)·BLM[18-24] to synthesize another series of deglycoBLM analogues containing conformationally constrained analogues designed to mimic elements of that structural motif.[52] Those analogues also failed to mediate sequence selective DNA cleavage.

While the failure of each of the prepared analogues to mediate DNA cleavage in a selective fashion may only reflect the inability of the analogues to assume a precise conformation required for DNA cleavage, it seems possible that the intrinsic lack of flexibility of the conformationally constrained analogues could also contribute to the lack of sequence selective DNA cleavage. If the latter were true, it would suggest that an initially formed metalloBLM complex must undergo a conformational rearrangement before DNA cleavage can occur. While no direct test of this possibility has been made, it has been reported that Co(III)·BLM binds to sites on DNA that it does not cleave[53] and that a conformational rearrangement of Co(III)·BLM bound to DNA may be required for double-stranded DNA cleavage.[54–56]

Experimental

Synthesis of deglycoBLM analogues

Protected resin-bound deglycoBLMs 6a and 6b. To 600 mg (0.27 mmol) of pre-swollen resin **2** was added 239 mg (1.08 mmol) of *o*-nitrobenzenesulfonyl chloride and 281 μL (208 mg, 1.62 mmol) of diisopropylethylamine in 2 mL of dry THF. The reaction mixture was shaken in the absence of light under nitrogen for 5 h to obtain resin **3**. This resin was filtered and washed five times with 2-mL portions of dry THF, followed by five washings with 2-mL portions of DMF, and two with 2-mL portions of CH_2Cl_2. A small amount of resin was subjected to the Kaiser test to verify completion of the coupling reaction. The resin was used in the next step immediately.

To 600 mg (0.27 mmol) of pre-swollen resin was added slowly 439 mg (1.62 mmol) of *N*-Boc-3-aminopropanol, and 364 mg (1.35 mmol) of triphenylphosphine in a 25-mL column fitted with a frit. The resin was shaken gently for 1 min, then 0.33 mL (365 mg, 2.0 mmol) of diethyl azodicarboxylate was added slowly. The resin was shaken for 24 h, then filtered and washed five times with 2-mL portions of DMF, followed by five 2-mL portions of CH_2Cl_2 to afford Nbs- and Boc-protected spermidine resin.

To a suspension of 500 mg (0.121 mmol) of swollen, fully protected spermidine resin was added 1 mL of 0.1 M triisopropylsilane in CH_2Cl_2. Then 1.2 mL (1.78 g, 16 mmol) of trifluoroacetic acid was added and the resin was shaken for 2 h. The resin was filtered and washed twice with 1.5-mL portions of trifluoroacetic acid, followed by five washings with 2-mL portions of DMF, five washings with 2-mL portions of CH_2Cl_2, and two washings with 2-mL portions of DMF. The resin was neutralized by washing five times with 2-mL portions of a 0.1 N solution of diisopropylethylamine in DMF and then five times with 2-mL portions of DMF, five times with 2-mL portions of CH_2Cl_2, and two times with 2-mL portions of DMF to yield resin **4**. A small amount of resin **4** in 0.5 mL of DMF was treated with an excess (10 equiv) of FmocCl and ~30 equiv of Hunig's base; the mixture was shaken for 30 min. The resin was filtered and rinsed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of MeOH, then dried under vacuum. The loading was determined from the Fmoc cleavage assay as 0.185 mmol/g (81% yield).

To 500 mg (0.121 mmol) of resin **4** was added a solution containing 173 mg (0.36 mmol) of Fmoc bithiazole,[29-31] 138 mg (0.36 mmol) of HBTU (*O*-benzotriazolyl-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate), 49 mg (0.36 mmol) of HOBT (*N*-hydroxybenzotriazole), and 126 mL (93 mg, 0.37 mmol) of Hunig's base in 2 mL of DMF. The resulting mixture was shaken for 30 min. The resin was filtered and rinsed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of MeOH, then dried under vacuum over KOH pellets. A small sample was subjected to the Kaiser test, which indicated that the reaction was complete; quantitative Fmoc cleavage indicated a loading of 0.155 mmol/g (88% yield).

A solution containing 2 mL of 20% piperidine in DMF was added to the pre-swollen resin-linked dipeptide. The suspension was shaken for 5 min, filtered and treated again with 2 mL of 20% piperidine in DMF for an additional 5 min. This rinsing/shaking procedure was repeated twice. The resin was washed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. A solution containing 79 mg (0.23 mmol) of Fmoc threonine, 88 mg (0.23 mmol) of HBTU, 31 mg (0.23 mmol) of HOBT, and 81 μL (60 mg, 0.47 mmol) of Hunig's base in 2 mL of DMF was added to the resulting resin, which was shaken gently. After 30 min resin **5** was filtered and rinsed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. Quantitative Fmoc cleavage indicated a loading of 0.137 mmol/g (90% yield).

A solution containing 2 mL of 20% piperidine in DMF was added to 276 mg (0.038 mmol) of pre-swollen resin **5** in a 6-mL column fitted with a frit. The suspension was shaken for 5 min, filtered and again treated with 2 mL of 20% piperidine in DMF for 5 min. This rinsing/shaking procedure was repeated twice. The resin was washed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. A solution containing 38 mg (0.114 mmol) of Fmoc-*S*-proline (for **1a**) or Fmoc-*R*-proline (for **1b**), 43 mg (0.114 mmol) of HATU (*O*-(7-azabenzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate), 16 mg (0.114 mmol) of HOAT (1-hydroxy-7-azabenzo-

triazole), and 40 μL (30 mg, 0.37 mmol) of Hunig's base in 0.5 mL of DMF was added. After 30 min, the resin was filtered and rinsed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of MeOH, and then dried under vacuum. Quantitative Fmoc cleavage indicated a loading of 0.132 mmol/g (97% yield) for *S*-proline, and 0.136 mmol/g (100% yield) for *R*-proline attachment to the resin-bound peptide.

A solution containing 2 mL of 20% piperidine in DMF was added to the pre-swollen resin containing tetrapeptide (0.038 mmol). The suspension was shaken for 5 min, filtered and treated again with 2 mL of 20% piperidine in DMF for an additional 5 min. This rinsing/shaking procedure was repeated twice. The resin was washed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. A solution containing 71 mg (114 mmol) of Fmoc tritylhistidine, 43 mg (0.114 mmol) of HATU, 16 mg (0.114 mmol) of HOAT, and 40 μL (30 mg, 0.37 mmol) of Hunig's base in 0.5 mL of DMF was added. After 30 min, the resin was filtered and rinsed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of MeOH. The resulting resin was dried under diminished pressure over KOH pellets. Quantitative Fmoc removal indicated a loading of 0.129 mmol/g (100% yield) for the *S*-proline derivative, and 0.124 mmol/g (96% yield) for *R*-proline derivative.

A solution containing 2 mL of 20% piperidine in DMF was added to 46 mg (0.006 mmol) of pre-swollen resin containing the pentapeptide in a 6-mL column fitted with a frit. The suspension was shaken for 5 min, filtered and again treated with 2 mL of 20% piperidine in DMF for 5 min. This rinsing/shaking procedure was repeated twice. The resin was washed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. A solution containing 7.5 mg (0.018 mmol) of *N*-Boc pyrimidoblamic acid,[40–42] 24 mg (0.053 mmol) of BOP (benzotriazol-1-yl-oxy-tris–dimethylaminophosphonium hexafluorophosphate), and 18 μL (13 mg, 0.11 mmol) of Hunig's base in 0.3 mL of DMF was added, and protected from light. After 16 h the resin was filtered and rinsed with five 2-mL portions of DMF and five 2-mL portions of CH_2Cl_2 to yield resins **6a** and **6b**.

Deprotection and resin cleavage of deglycoBLM analogues

To the suspension of fully protected resin-linked deglycobleomycins **6a** and **6b** was added 1 mL of 20:1:1 CH_2Cl_2–$(i\text{-}Pr)_3SiH$–Me_2S. Then 1.2 mL (19.2 mmol) of trifluoroacetic acid was added and the resin was shaken for 2 h. The resin was filtered and washed twice with 1.5-mL portions of trifluoroacetic acid, followed by five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. The resin was treated with 2 mL of 1 N sodium thiophenolate in DMF for 20 min to obtain resins **7a** and **7b**. The resins were filtered and washed with five 2-mL portions of DMF, five 2-mL portions of CH_2Cl_2, and two 2-mL portions of DMF. After an additional treatment with 0.1 N sodium thiophenolate solution and washing, deglycoBLMs **1a** and **1b** were cleaved from the resin by three treatments with 0.5 mL of 2% hydrazine in DMF for 3 min. The filtrates were collected in 5-mL round-bottom flasks. The solutions were concentrated under an argon atmosphere. The products were dissolved in 0.5 mL of trifluoroacetic acid and precipitated in cold anhydrous ether (−20 °C) as colorless solids. The precipitates were washed with 2 mL of cold anhydrous ether and dried under diminished pressure. The solids were dissolved in 1 mL of deionized water, frozen and lyophilized to give colorless solids. Chromatographic purification was performed on an Alltech Alltima C_{18} reversed-phase HPLC column (150×4.6 mm) using a gradient of aq 0.1 N trifluoroacetic acid and acetonitrile. A linear gradient was employed (88:12 0.1 N CF_3COOH–CH_3CN→79:21 0.1 N CF_3COOH–CH_3CN over a period of 30 min at a flow rate of 4 mL/min). Fractions containing the desired product were collected, frozen and lyophilized to afford a colorless solid: yield 1.1 mg of **1a**, and 1.8 mg of **1b**.

For **1a**: ^{1}H NMR (D_2O) δ 1.05 (d, 3H, J=6.4 Hz), 1.57–1.77 (m, 5H), 1.80–1.95 (m, 7H), 2.10 (m, 1H), 2.69–2.81 (m, 1H), 2.90–3.20 (m, 13H), 3.45–3.70 (m, 6H), 3.80 (m, 1H), 4.00–4.20 (m, 4H), 4.40 (m, 1H), 5.10 (m, 1H), 7.25 (s, 1H), 8.03 (s, 1H), 8.12 (s, 1H) and 8.56 (s, 1H); mass spectrum (ESI), m/z 1025.5 $(M+H)^+$, 513.4 $(M+H)^{++}$, and 343.0 $(M+H)^{+++}$ (theoretical $(M+H)^+$ 1026.2).

For **1b**: ^{1}H NMR (D_2O) δ 1.00 (d, 3H, J=6.4 Hz), 1.59–1.77 (m, 4H), 1.77–2.00 (m, 8H), 2.20 (m, 1H), 2.59–2.67 (m, 1H), 2.85–3.31 (m, 13H), 3.39–3.64 (m, 6H), 3.80 (m, 1H), 3.95–4.20 (m, 4H), 4.42 (m, 1H), 5.14 (m, 1H), 7.26 (s, 1H), 7.95 (s, 1H), 8.12 (s, 1H) and 8.56 (s, 1H); mass spectrum (ESI), m/z 1025.6 $(M+H)^+$, 513.6 $(M+H)^{++}$, and 342.9 $(M+H)^{+++}$ (theoretical $(M+H)^+$ 1026.2).

General procedure for the oxidation of styrene by Fe(III)·BLM and analogues

To a solution of 0.20 mg (0.19 μmol) of BLM derivative, and 0.09 mg (0.19 μmol) of ferric perchlorate in 220 μL of water was added at 4 °C 100 μL of a methanolic solution containing 277 mM styrene and 6.21 mM ethyl benzoate. To the resulting solution was added a 91 mM H_2O_2 solution until a final concentration of 15 mM H_2O_2 was reached. Aliquots (100-μL) of the reaction mixture were taken at 40-min time intervals, and each was quenched with 300 μL of water, and extracted with three 100-μL portions of $CHCl_3$. The combined organic phase was dried over $MgSO_4$, filtered and analyzed by gas chromatography. Injections were 10 μL in volume (splitless mode) and applied to a 5% phenylsiloxane capillary column (0.25 mm ID×30 m). The following temperature program was employed at a He gas flow rate of 1.0 mL/min: 60 °C for 22 min; 60→85 °C at 10 °C/min; then 85 °C for 15 min. Under these conditions the observed retention times (corrected) were as follows: styrene 4.6 min; phenylacetaldehyde 18.9 min; styrene oxide 21.3 min; ethyl benzoate 31.0 min.

Molecular modeling studies

Modeling studies of the Zn(II)·deglycoBLM and the *R*- and *S*-proline analogues were carried out using Insight II/Discover_3 (Accelrys, San Diego, CA, USA) on a Silicon Graphics Octane using the ESFF forcefield. The screw sense, axial ligand and bond angles around the Zn metal were fixed based on the results of previous modeling studies.[18,27] Molecular dynamics followed by minimization of Zn(II)·deglycoBLM and the respective *R*- and *S*- proline analogues produced significantly different results (Fig. 3). Ten structures were calculated for each analogue. The majority of structures fell within a narrow range for each set of structures. We observed a high degree of structural similarity in the metal binding domain and the methylvalerate and proline peptides, respectively. The threonine and bithiazole regions showed progressively higher variability moving away from the metal binding domain. Similar to the reported structure of HOO·Co(III)·BLM,[18] the bithiazole of Zn(II)·deglycoBLM was folded back underneath the equatorial plane of the metal binding domain and was on the opposite face relative to the axial primary amine ligand. The metal binding domains of the *R*- and *S*-proline analogues were superimposed on the metal binding domain of Zn(II)·deglycoBLM to visualize the similarity of methylvalerate and proline congeners.

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