General Methods

Ultraviolet measurements were taken on a Perkin-Elmer Lambda 20 Lambda Array UV-Vis spectrophotometer. HPLC was performed on a Varian 9012 HPLC using a Perkin-Elmer LC 235 diode array detector. All reactions were carried out under nitrogen. HATU, HOAt, anhydrous grade DMF, anhydrous grade piperidine, $Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$, and anhydrous grade Hunig's base were purchased from Aldrich Chemicals. 8-*N*-(1-*N*-Dde-spermidine)trityl polystyrene resin, HBTU, HOBt, BOP, and 9-fluorenylmethylcarbamoylthreonine were purchased from Calbiochem-NovaBiochem Corp. Methylene chloride was distilled from calcium hydride. $Fe(NH_4)_2(SO_4)_2 \bullet 6H_2O$ was used to prepare aqueous solutions for admixture with bleomycin; these were made immediately prior to use.

Agarose gel electrophoresis was carried out in 40 mM Tris-acetate buffer, pH 7.8, containing 5 mM EDTA. The agarose gel loading solution contained 30% glycerol and 0.05% (w/v) bromophenol blue. Distilled and deionized water from a Milli-Q system was treated with Chelex resin (Sigma Chemicals) and used for all aqueous solutions and manipulations.

Synthesis of DeglycoBLM 2a. To a cold (0 °C) suspension containing 500 mg (0.79 mmol/g) of the resin in 3 mL of CH_2Cl_2 was added 250 μL (182 mg, 1.8 mmol) of Hunig's base followed by 327 mg (1.5 mmol) of di-*t*-butyl dicarbonate. The suspension was allowed to warm to room temperature. After 16 h, the resin was filtered and washed for 30 s with three 10-mL portions of CH_2Cl_2 and three 10-mL portions of methanol. The resulting resin was dried under diminished pressure over KOH pellets. A small portion (25 mg) of the resin was cleaved using 3% TFA in CH_2Cl_2. The TFA solution was concentrated, dissolved in 1 mL of water, frozen and lyophilized to give an off-white solid: mass spectrum (electrospray), *m/z* 410.2 $(M+H)^+$ and 310.5 $(M\text{-}Boc)^+$.

To a suspension containing 100 mg (0.78 mmol/g) of the above resin swollen in 0.2 mL of DMF was added 1 mL of a solution containing 2% hydrazine in DMF. After 3 min, the suspension was filtered and treated again with 1 mL of 2% hydrazine in DMF. After 3 min, the suspension was filtered and treated a third time with 1 mL of 2% hydrazine in DMF. The resulting resin was filtered and washed for 30 s each with five 3-mL portions of DMF, three 3-mL portions of CH_2Cl_2, and again with three 3-mL portions of DMF. Qualitative ninhydrin assay indicated the presence of the primary amine. A solution containing 87 mg (0.18 mmol) of Fmoc-bithiazole, 69 mg (0.18 mmol) of HBTU, 25 mg (0.18 mmol) of HOBt, and 63 μL (47 mg, 0.37 mmol) of Hunig's base in 0.7 mL of DMF was added to the resin. After 1 h, the resin was filtered and rinsed for 30 s with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2 and again with two 5-mL portions of DMF. A small sample (~5 mg) was subjected to the Kaiser test which indicated that the reaction was complete; quantitative Fmoc cleavage indicated a loading of 0.35 mmol/g (65%). A solution containing 2 mL of 20% piperidine in DMF was added. The suspension was shaken for 5 min, filtered and treated again with 2 mL of 20% piperidine in DMF solution for an additional 5 min. This rinsing/shaking procedure was repeated twice. The resin was washed for 30 s with seven 5-mL portions of DMF, five 5-mL portions of CH_2Cl_2 and again with three 5-mL portions of DMF. A solution containing 62 mg (0.18 mmol) of Fmoc-threonine, 69 mg (0.18 mmol) HBTU, 25 mg (0.18 mmol) of HOBt and 63 μL (47 mg, 0.37 mmol) of Hunig's base in 0.7 mL of DMF was added. After 1 h, the resin was filtered and rinsed for 30 s with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2 and again with three 5-mL portions of DMF. Quantitative Fmoc cleavage indicated a loading of 0.34 mmol/g (95%). A solution containing 20% piperidine in DMF was added. The suspension was shaken for 5 min, filtered and treated with 2 mL of 20% piperidine in DMF for an additional 5 min. This

rinsing/shaking procedure was repeated twice. The resin was washed for 30 s with seven 5-mL portions of DMF, five 5-mL portions of CH_2Cl_2 and again with three 5-mL portions of DMF. A solution containing 67 mg (0.18 mmol) of Fmoc-methylvalerate, 69 mg (0.18 mmol) of HBTU, 25 mg (0.18 mmol) of HOBt and 63 μL (47 mg, 0.37 mmol) of Hunig's base in 0.7 mL of DMF was added. After 1 h, the resin was filtered and rinsed for 30 s with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2 and three 5-mL portions of methanol. Quantitative Fmoc removal indicated a loading of 0.30 mmol/g (95%). A suspension containing 20 mg of the above resin was treated with a solution containing 2 mL of 20% piperidine in DMF. The solution was shaken for 5 min, filtered and treated with 2 mL of 20% piperidine in DMF for an additional 5 min. This rinsing/shaking procedure was repeated twice. The resin was washed for 30 s with seven 5-mL portions of DMF, five 5-mL portions of CH_2Cl_2 and again with three 5-mL portions of DMF. A solution containing 31 mg (0.042 mmol) of N^{α}-Fmoc-N^{im}-trityl-β-hydroxyhistidine, 27 mg (0.042 mmol) of HATU, 6 mg (0.042 mmol) of HOAt and 19 μL (15 mg, 0.084 mmol) of Hunig's base in 0.5 mL of DMF was added. After 1 h, the resin was filtered and rinsed successively for 30 s with three 5-mL portions each of DMF, CH_2Cl_2, and ethanol. The resulting resin was dried under diminished pressure over KOH pellets. Quantitative Fmoc cleavage indicated a loading of 0.24 mmol/g (90%). A suspension containing 10 mg of this pentapeptide resin was treated with a solution containing 1 mL of 20% piperidine in DMF. The solution shaken for 5 min, filtered and treated with 1 mL of 20% piperidine in DMF for an additional 5 min. This rinsing/shaking procedure was repeated twice. A qualitative Fmoc cleavage assay indicated that the Fmoc group had been removed. The resin was washed for 30 s with seven 5-mL portions each of DMF, five 5-mL portions of CH_2Cl_2 and again with three 5-mL portions of DMF. A solution containing 5.0 mg (11.6 μmol) of Boc pyrimidoblamic acid,

15.0 mg (34.4 μmol) of BOP, and 4 μL (3 mg, 23.2 μmol) of Hunig's base in 0.3 mL of DMF was added. After 16 h in the absence of light, the resin was filtered and rinsed successively for 30 s with three 5-mL portions each of DMF, CH_2Cl_2, and ethanol. The resin was dried under diminished pressure over KOH pellets. The resin was treated for 3 h with 3 mL of a 90:2.5:2.5:5 TFA- triisopropylsilane-H_2O-Me_2S solution. The resin was filtered and washed with two 2-mL portions of TFA and the eluate was concentrated under a stream of nitrogen. The resulting oil was dissolved in 1 mL of TFA and added dropwise to 10 mL of cold (-20 °C) ether. The precipitate was washed with twice with 2-mL portions of ether and dried under diminished pressure. The solid was dissolved in 1 mL of deionized water, frozen and lyophilized to give an off-white solid. Chromatographic purification was performed on an Alltech Alltima C_{18} (4.6 x 150 mm) reversed phase HPLC column using a gradient of aq 0.1 N NH_4OAc, pH 4.5, and acetonitrile. A linear gradient was employed starting from 94:6 0.1 NH_4OAc-CH_3CN to 90:10 0.1 NH_4OAc over a period of 30 min at a flow rate of 1 mL/min. Fractions containing the desired product were collected, frozen and lyophilized to give a colorless solid, which was purified by C_{18} reversed-phase HPLC: yield 790 μg (35% overall); ^1H NMR (D_2O) δ 1.00 (d, 3H, J = 6.4 Hz), 1.01 (d, 3H, J = 6.5 Hz), 1.08 (d, 3H, J = 7.0 Hz), 1.67 (m, 6H), 1.81 (s, 3H), 1.93 (t, 1H, J = 7 Hz), 2.49 (m, 1H), 2.54 (m, 1H), 2.93 (m, 2H), 3.02 (m, 4H), 3.20 (t, 2H, J = 7.0 Hz), 3.43 (t, 2H, J = 7.0 Hz), 3.45 (t, 2H, J = 7.5 Hz), 3.56 (t, 2H, J = 7.0 Hz), 3.66 (m, 1H), 3.77 (m, 1H), 3.80 (m, 1H), 3.84 (m, 1H), 3.95 (m, 1H), 4.00 (m, 1H), 4.14 (d, 1H, J = 4.5 Hz), 4.77 (m, 1H), 5.17 (d, 1H, J = 8.0 Hz), 7.14 (s, 1H), 7.96 (s, 1H), 8.12 (s, 1H) and 8.66 (s, 1H); mass spectrum (electrospray), m/z 1073.5 $(M + H)^+$.

Relaxation of Plasmid DNA by Fe(II)•DeglycoBLM A_5 Analogues

Reactions were carried out in 15 μL (total volume) of 10 mM sodium cacodylate buffer, pH 7.0, containing 500 ng (0.252 pmol) of pSP64 plasmid DNA and the appropriate concentrations of Fe(II)•deglycoBLM A_5 (**2a**). Reaction mixtures were incubated at 37 °C for 30 min and then treated with 5 μL of loading solution (30% glycerol containing 0.05% (w/v) bromophenol blue) and applied to a 1% agarose gel containing 1 μg/mL of ethidium bromide. Horizontal gel electrophoresis was carried out in 90 mM Tris acetate buffer, pH 8.3, containing 1 mM EDTA at 100 V for 3 h (UV visualization).