Supporting Information for

Solid Phase Synthesis of Bleomycin Group Antibiotics. Construction of a 108-Member Deglycobleomycin Library

Christopher J. Leitheiser, Kenneth L. Smith, Michael J. Rishel, Shigeki Hashimoto, Kazuhide Konishi, Craig J. Thomas, Chunhong Li, Michael M. McCormick, and Sidney M. Hecht*

Experimental Section

General Methods. Ultraviolet measurements were taken on a Perkin-Elmer Lambda 20 Lambda Array UV-Vis spectrophotometer. ^{1}H NMR spectra were recorded on a General Electric QE-300 MHz or a Varian Unity 500 MHz NMR spectrophotometer using residual solvent peaks at 2.50 ppm for DMSO-d_6, 3.31 ppm for methanol-d_4 4.63 ppm for D_2O, and 7.26 ppm for $CDCl_3$ for calibration of chemical shifts. HPLC separations were performed on a Waters 600 series HPLC multi-solvent delivery system using a Kratos 747 UV detector. All reactions were carried out under nitrogen or argon. Anhydrous grade piperidine, HATU, HOAt, and $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ were purchased from Aldrich Chemicals. Anhydrous grade DMF, biochemical grade TFA, hydrazine monohydrate and anhydrous grade Hunig's base were purchased from Acros Organics. HPLC grade water and acetonitrile were purchased from Fisher Chemicals. TentaGel resin, HBTU, HOBt, BOP, and all commercially available Fmoc-amino acids were purchased from NovaBiochem. Methylene chloride was distilled from calcium

hydride. The optical density of 5540 M^{-1} at 290 nm and 7300 M^{-1} at 300 nm was used to calculate the loading from a known weight of dry resin. The optical density of 14,500 M^{-1} at 290 nm for bithiazole and chlorobithiazole-containing deglycobleomycins and 19,100 M^{-1} at 300 nm for trithiazole containing deglycobleomycins was used to calculate the final deglycobleomycin yield. $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ was used to prepare aqueous solutions for admixture with bleomycin; these were made immediately prior to use. High resolution mass spectrometry was performed at the Michigan State University Mass Spectrometry Facility.

[2-(Fluorenylmethoxycarbonyl)amino]ethyl-2,4'-bithiazole-4-carboxylic Acid (2). To a suspension containing 1.45 g (5.00 mmol) of 2-(aminoethyl)-2,4'-bithiazole-4-carboxylic acid (**1**) hydrochloride in 100 mL in 5% aqueous K_2CO_3 was added 1.68 g (5.00 mmol) of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide in 100 mL of dioxane. After stirring for 16 h, the resulting solution was poured into 100 mL of water and acidified with 1 N HCl. The precipitate was filtered, washed with three 15-mL portions of 1 N HCl, and then with water. The resulting solid was dissolved in 30 mL of TFA and added dropwise to ethanol. The resulting solid was filtered and dried under diminished pressure to give an off-white solid: yield 1.72 mg (72 %); mp 171-173 °C; TLC R_f 0.4 (90:10:1 CH_2Cl_2-methanol-acetic acid); ^{1}H NMR (methanol-d_4) δ 3.18 (t, 2H, J = 6.0 Hz), 3.49 (t, 2H, J = 7.0 Hz), 4.14 (t, 1H, J = 6.0 Hz), 4.30 (d, 2H, J = 7.0 Hz), 7.21 (d, 2H, J = 7.0 Hz), 7.30 (dd, 2H, J = 7.0 Hz), 7.55 (dd, 2H, J = 8.0 Hz), 7.71 (dd, 2H, J = 8.0 Hz), 8.13 (s, 1H) and 8.25 (s, 1H); ^{13}C NMR (DMSO) δ 32.8, 46.7, 65.3, 117.9, 120.1, 125.1, 127.1, 127.6, 128.9, 140.8, 143.9, 147.3, 148.2, 156.2, 162.1, 162.3 and 169.2; mass spectrum (FAB), *m/z* 478.0879 $(M + H)^+$ ($C_{24}H_{20}N_3O_4S_2$ requires 478.0895).

5'-Chloro-2'-2-[(fluorenylmethoxycarbonyl)amino]ethyl-2,4'-bithiazole-4-carboxylic Acid (4). To a solution containing 693 mg (1.67 mmol) of 2'-[(2-*tert*-

butoxycarbonyl)amino]ethyl-5'-chloro-2,4'-bithiazolyl-4-carboxylic acid methyl ester (**3**)[12d] in 60 mL of methanol was added 20 mL of 1 N NaOH. After 16 h, the solution was concentrated by 75% under diminished pressure and extracted with three 30-mL portions of ethyl acetate. The resulting aqueous extract was acidified to pH ~ 2 with 1 N HCl and extracted with three 30-mL portions of ethyl acetate. The organic extract was dried over $MgSO_4$, filtered and concentrated under diminished pressure. The resulting solid was treated with 20 mL of dimethyl sulfide followed by 50 mL of TFA. After 16 h, the solution was concentrated under diminished pressure and 50 mL of a 5% aqueous K_2CO_3 solution was added, followed by 563 mg (1.67 mmol) of 9-fluorenylmethyl succinimidyl carbonate in 100 mL of dioxane. After stirring for 16 h, the resulting solution was poured into 100 mL of water and acidified with 1 N HCl. The resulting solid was filtered, washed with three 15-mL portions of 1 N HCl, and then with water. The solid was dissolved in 30 mL of TFA and added dropwise to ethanol. The resulting solid was filtered and dried under diminished pressure to give an off-white solid: yield 430 mg (49 %); mp 126-127 °C; ^{1}H NMR (DMSO-d_6) δ 3.09 (t, 2H, J = 7.0 Hz), 3.45 (t, 2H, J = 7.0 Hz), 4.14 (t, 1H, J = 7.0 Hz), 4.30 (d, 2H, J = 7.0 Hz), 7.23 (dd, 2H, J = 7.0 Hz), 7.31 (dd, 2H, J = 7.0 Hz), 7.54 (d, 2H, J = 7.0 Hz), 7.71 (d, 2H, J = 8.0 Hz) and 7.97 (s, 1H); ^{13}C (DMSO) δ 38.4, 46.7, 65.3, 120.2, 122.7, 124.9, 125.1, 127.1, 129.7, 140.8, 141.5, 143.8, 148.5, 156.2, 160.4, 162.1, 166.9; mass spectrum (FAB), *m/z* 512.0515 $(M + H)^+$ ($C_{24}H_{19}ClN_3O_4S_2$ requires 512.0505).

(1*R*,2*S*,3*S*,4'*S*)-[2-Hydroxy-4-(4'-isopropyl-2'-oxo-oxazolidin-3'-yl)-1,3-dimethyl-4-oxobutyl]carbamic Acid *tert*-Butyl Ester (7a).[19] To a cooled (0 °C) solution containing 926 mg (5.00 mmol) of acylated oxazolidinone **5**[20] in 5 mL of CH_2Cl_2 was added 5.5 mmol of Bu_2BOTf and 1.05 mL (775 mg, 8.00 mmol) of Hunig's base. After 45 min, the solution was cooled to -78 °C and maintained at -78 °C for 15 min. A solution containing 982 mg (5.50

mmol) of *N*-(*tert*-butoxycarbonyl)-*R*-alanal (**6a**)[21] in 5 mL of CH_2Cl_2 was added and the solution was allowed to warm to room temperature overnight. The reaction was quenched by the addition of 10 mL of potassium phosphate buffer, pH 7.0, extracted with three 20-mL portions of ether, and the combined organic extract was washed with three 10-mL portions of saturated aqueous NaCl and concentrated under diminished pressure. The crude oil was dissolved in 15 mL of methanol and cooled to 0 °C, then 5 mL of 30 % H_2O_2 was added slowly. After 4 h, 10 mL of water was added and the reaction mixture was concentrated under diminished pressure. The residue was extracted with three 15-mL portions of ether and the combined organic phase was washed with three 10-mL portions of 5% $NaHCO_3$ and three 10-mL portions of saturated NaCl. The resulting organic phase was dried over $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was applied to a silica gel column (30 x 4 cm), which was washed with 2:3 ethyl acetate-hexanes to afford **7a** as a colorless solid: yield 1.27 g (71 %); silica gel TLC R_f 0.29 (2:3 ethyl acetate-hexanes); $[\alpha]^{20}_D$ +84.7 (*c* 0.23, MeOH), Lit.[19] $[\alpha]^{20}_D$ +84 (*c* 0.26, MeOH); ^{1}H NMR ($CDCl_3$) δ 0.85 (d, 3H, *J* = 7.0 Hz), 0.89 (d, 3H, *J* = 7.0 Hz), 1.20 (d, 3H, *J* = 6.0 Hz), 1.31 (d, 3H, *J* = 6.0 Hz), 1.45 (s, 9H), 2.35 (m, 1H), 2.92 (br s, 1H), 3.75 (br s, 2H), 4.00 (br q, 1H), 4.25 (m, 1H), 4.45 (m, 2H) and 4.52 (br d, 1H).

(2*S*,3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-2-methylpentanoic Acid (8a).[19] To a cooled (0 °C) solution containing 700 mg (1.95 mmol) of **7a** in 40 mL of 3:1 THF-H_2O was added 1.75 mL (6 eq) of 30% H_2O_2 followed by 217 mg (3.90 mmol) of LiOH. After stirring at 0 °C for 3 h, the excess peroxide was quenched at 0 °C by the addition of 10 mL of 1.5 N aq Na_2SO_3, the pH was adjusted to 9-10 with saturated aq $NaHCO_3$, and the oxazolidinone was removed by extraction with three 10-mL portions of CH_2Cl_2. The aqueous phase was then acidified to pH ~2 with 1 N HCl, and extracted with three 20-mL portions of ethyl acetate. The

combined organic phase was dried over $MgSO_4$, filtered, and concentrated under diminished pressure to give **8a**[19] as a colorless sticky solid: yield 354 mg (73%); silica gel TLC R_f 0.8 (ethyl acetate); $[\alpha]^{20}_D$ +8.4 (*c* 1.0, MeOH), Lit.[19] $[\alpha]^{25}_D$ +8.4 (*c* 1.0, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.20 (d, 3H, *J* = 6.0 Hz), 1.26 (d, 3H, *J* = 6.0 Hz), 2.61 (d, 1H, *J* = 6.0 Hz), 3.80 (m, 2H), 4.82 (br d, 1H) and 6.00 (br s, 1H).

(2*S*,3*S*,4*R*)-4-(*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methylpentanoic Acid (9a). To a solution containing 1.42 g (5.84 mmol) of **8a**[19] in 10 mL of dimethyl sulfide was added 30 mL of TFA. After 2 h, the solution was concentrated under a stream of nitrogen and concentrated further under diminished pressure. The resulting oil was dissolved in 15 mL of a 5% aqueous K_2CO_3 solution followed by the addition of 2.13 g (6.30 mmol) of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide in 20 mL of dioxane. After 16 h, the solution was extracted with three 15-mL portions of ether, and three 15-mL portions of ethyl acetate. The combined aqueous extract was acidified to pH ~2 with 1 N HCl. The mixture was extracted with three 15-mL portions of ethyl acetate. The resulting organic extract was dried over $MgSO_4$, filtered, and concentrated under diminished pressure. Chromatography on flash silica gel (35 x 5 cm), elution with 93:5:2 CH_2Cl_2-methanol-acetic acid, gave **9a** as a colorless solid: yield 1.3 g (63%), TLC R_f 0.4 (93:5:2 CH_2Cl_2-methanol-acetic acid); $[\alpha]^{20}_D$ -0.5 (*c* 1.1, methanol); ^{1}H NMR (methanol-d_4) δ 1.08 (d, 3H, *J* = 7.0 Hz), 1.11 (d, 3H, *J* = 7.0 Hz), 2.47-2.43 (m, 1H), 3.63-3.56 (m, 1H), 3.73-3.69 (m, 1H), 4.13 (t, 1H, *J* = 7.0 Hz), 4.37-4.25 (m, 2H), 7.24 (dd, 2H, *J* = 7.0 Hz), 7.34 (dd, 2H, *J* = 7.0 Hz), 7.57 (d, 2H, *J* = 7.0 Hz) and 7.73 (d, 2H, *J* = 7.0 Hz); ^{13}C NMR (methanol-d_4) δ 42.5, 47.2, 47.6, 48.9, 49.2, 66.5, 75.1, 119.9, 125.2, 127.2, 127.8, 141.6, 144.3, 157.1 and 178.0; mass spectrum (FAB), *m/z* 370.1653 $(M + H)^+$ ($C_{21}H_{24}NO_5$ requires 370.1654).

(1*R*,2*S*,3*S*,4'*S*)-[2-Hydroxy-1-isobutyl-4-(4'-isopropyl-2'-oxo-oxazolidin-3'-yl)-3-methyl-4-oxo-butyl]carbamic Acid *tert*-Butyl Ester (7b). To a cooled (0 °C) solution containing 2.49 g (13.5 mmol) of acylated oxazolidinone **5** in 5 mL of CH_2Cl_2 was added 14.9 mmol of Bu_2BOTf and 3.06 mL (2.2 g, 16.2 mmol) of Hunig's base. After 45 min, the solution was cooled to -78 °C and maintained at -78 °C for 15 min. A solution containing 3.2 g (14.9 mmol) of *N*-(*tert*-butoxycarbonyl)-*R*-leucinal (**6b**) in 14 mL of CH_2Cl_2 was added and the solution was allowed to warm to room temperature overnight. The reaction was quenched by the addition of 40 mL of potassium phosphate buffer, pH 7.0, extracted with three 20-mL portions of ether, and the combined organic extract was washed with three 50-mL portions of saturated aqueous NaCl and concentrated under diminished pressure. The crude oil was dissolved in 40 mL of methanol and cooled to 0 °C, then 30 mL of 30 % H_2O_2 was added slowly. After 4 h, 30 mL of water was added and the reaction mixture was concentrated under diminished pressure. The residue was extracted with three 30-mL portions of ether and the combined organic phase was washed with three 30-mL portions of 5% $NaHCO_3$ and three 30-mL portions of saturated NaCl. The resulting organic phase was dried over $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was applied to a silica gel column (30 x 4 cm), which was washed with 1:2 ethyl acetate-hexanes to afford **7b** as a colorless solid. The solid was crystallized from 1:3 ethyl acetate hexanes to provide the product as colorless crystals: yield 2.15 g (40 %); mp 134–135 °C; silica gel TLC R_f 0.36 (1:2 ethyl acetate-hexanes); $[\alpha]^{20}_D$ +92.8 (*c* 1.0, MeOH), ^{1}H NMR ($CDCl_3$) δ 0.87–0.94 (m, 12H), 1.32 (d, 3H, J = 7.0 Hz), 1.44 (s, 9H), 1.70 (m, 2H), 2.32–2.38 (m, 1H), 3.14 (br. s, 1H), 3.76 (m, 2H), 3.98 (m, 1H), 4.19–4.24 (m, 3H), 4.28 (m, 1H), and 4.44 (m, 1H), ^{13}C NMR ($CDCl_3$) δ 12.21, 15.17, 18.41, 22.03, 24.36,

25.27, 28.83, 39.94, 41.31, 51.20, 58.74, 63.78, 75.28, 79.91, 156.20, and 178.11; mass spectrum (chemical ionization) *m/z* 401 $(M + H)^+$.

Anal. Calcd for $C_{20}H_{36}O_6N_2$: C, 59.98; H, 9.06. Found: C, 59.83; H, 8.99.

(2*S*,3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-2,6-dimethylheptanoic Acid (8b). To a cooled (0 °C) solution containing 520 mg (1.30 mmol) of **7b** in 35 mL of 3:1 THF-H_2O was added 1.1 mL (7.8 mmol, 6 eq) of 30% H_2O_2 followed by 62.3 mg (2.6 mmol) of LiOH. After stirring at 0 °C for 3 h, the excess peroxide was quenched at 0 °C by the addition of 10 mL of 1.5 N aq Na_2SO_3, the pH was adjusted to 9-10 with saturated aq $NaHCO_3$, and the oxazolidinone was removed by extraction with three 25-mL portions of CH_2Cl_2. The aqueous phase was then acidified to pH ~2 with 1 N HCl, and extracted with three 20-mL portions of ethyl acetate. The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under diminished pressure to give **8b** as a colorless solid: yield 316 mg (84%); mp 103–105 °C; silica gel TLC R_f 0.3 (49:49:1 ethyl acetate–hexanes–acetic acid); $[\alpha]^{20}_D$ +20 (*c* 0.34, MeOH), ^{1}H NMR ($CDCl_3$) δ 0.87 (m, 6H), 1.12 (d, 3H, *J* = 7.0 Hz), 1.28 (m, 1H), 1.37 (s, 9H), 1.51 (m, 1H), 1.64 (m, 1H) 2.53 (m, 1H), 3.57 (m, 1H), 3.66 (m, 1H) and 6.24 (d, 1H, *J* = 9.2 Hz), ^{13}C NMR ($CDCl_3$) δ 10.06, 20.09, 22.65, 24.11, 26.98, 39.41, 41.63, 50.56, 74.52, 77.99, 156.09, and 177.32; mass spectrum (chemical ionization), *m/z* 290 $(M + H)^+$; mass spectrum (FAB), *m/z* 290.1977 $(M + H)^+$ ($C_{14}H_{28}NO_5$ requires 290.1967).

(2*S*,3*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2,6-dimethylheptanoic Acid (9b). To a solution containing 1.08 g (3.74 mmol) of **8b** in 12 mL of dimethyl sulfide was added 20 mL of TFA. After 2 h, the solvent was evaporated under a stream of nitrogen and then coevaporated with toluene under diminished pressure. The resulting salt was dissolved in 20 mL of a 5% aqueous K_2CO_3 solution followed by the addition of 1.26 g

(3.74 mmol) of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide in 40 mL of dioxane. After 16 h, the solution was washed with three 15-mL portions of ethyl acetate. The combined aqueous extract was then acidified to pH ~2 with 1 N HCl. The mixture was extracted with three 15-mL portions of ethyl acetate. The resulting organic extract was dried over $MgSO_4$, filtered, and concentrated under diminished pressure. Chromatography on flash silica gel (35 x 5 cm), elution with 93:5:2 CH_2Cl_2-methanol-acetic acid, gave **9b** as a colorless solid: yield 1.0 g (63%), TLC R_f 0.15 (65:33:1 hexanes–ethyl acetate–acetic acid); $[\alpha]^{20}_D$ +21 (*c* 0.6, methanol); ^{1}H NMR (methanol-d_4) δ 0.88 (d, 3H, J = 6.0 Hz), 0.91 (d, 3H, J = 6.4 Hz), 1.15 (d, 3H, J = 7.0 Hz), 1.32–1.38 (m, 1H). 1.52–1.62 (m, 2 H), 2.50–2.53 (m, 1H), 3.61–3.64 (m, 1H), 3.75 (dd, 1H, J = 7.9 Hz, J = 4.9 Hz), 4.16 (dd, 1H, J = 6.7 Hz, J = 6.4 Hz), 4.35–4.42 (m, 2H), 7.25–7.29 (m, 2H), 7.33–7.37 (m, 2H), 7.61–7.63 (m, 2H) and 7.74–7.76 (m, 2H); ^{13}C NMR (methanol-d_4) δ 10.33, 19.60, 20.70, 23.31, 24.65, 39.91, 42.13, 51.61, 66.05, 74.99, 119.72, 124.95, 124.97, 126.92, 126.94, 127.56, 157.23 and 177.91; mass spectrum (electrospray), *m/z* 412.0 $(M + H)^+$; mass spectrum (FAB), *m/z* 412.2130 $(M + H)^+$ ($C_{24}H_{29}NO_5$ requires 412.2124).

(1*R*,2*S*,3*S*,4'*S*)-[1-Benzyl-2-hydroxy-4-(4'-isopropyl-2'-oxo-oxazolidin-3'-yl)-3-methyl-4-oxobutyl]carbamic Acid *tert*-Butyl Ester (7c). To a cooled (0 °C) solution containing 5.82 g (31.5 mmol) of acylated oxazolidinone **5**[20] in 27 mL of CH_2Cl_2 was added 34.6 mmol of Bu_2BOTf and 6.55 mL (4.77 g, 37.7 mmol) of Hunig's base. After 45 min, the solution was cooled to -78 °C and maintained at -78 °C for 15 min. A solution containing 8.60 g (34.6 mmol) of *N*-(*tert*-butoxycarbonyl)-*R*-phenylalanal (**6c**)[21] in 15 mL of CH_2Cl_2 was added and the solution was allowed to slowly warm to room temperature overnight. The reaction was quenched by the addition of 50 mL of potassium phosphate buffer, pH 7.0, extracted with three 50-mL portions of ethyl acetate, and the combined organic extract was washed with three 50-mL

portions of saturated aqueous NaCl and concentrated under diminished pressure. The crude oil was dissolved in 105 mL of methanol and cooled to 0 °C, then 32 mL of 30 % H_2O_2 was added slowly. After 4 h, 50 mL of water was added and the reaction mixture was concentrated under diminished pressure. The residue was extracted with three 25-mL portions of ethyl acetate and the combined organic phase was washed with three 10-mL portions of 5% $NaHCO_3$ and three 10-mL portions of saturated NaCl. The resulting organic phase was dried over $MgSO_4$, filtered, and concentrated under diminished pressure. The residue was applied to a silica gel column (30 x 4 cm), which was washed with 1:2 ethyl acetate-hexanes to afford **7c** as a colorless solid. The solid was crystallized from 1:3 ethyl acetate–hexanes to provide **7c** as colorless crystals: yield 3.7 g (27 %); mp 142–143 °C; silica gel TLC R_f 0.24 (1:2 ethyl acetate-hexanes); $[\alpha]^{20}_D$ +94.3 (*c* 1.0, MeOH), ^{1}H NMR ($CDCl_3$) δ 0.84–0.91 (dd, 6H, J = 6.9 Hz, J = 12.3 Hz), 1.32 (d, 3H, J = 6.9 Hz), 1.37 (s, 9H), 2.28–2.35 (m, 1H), 2.90 (m, 1H), 3.02 (m, 1H), 3.40 (m, 1H), 3.76 (m, 1H), 3.92 (m, 2H), 4.18 (m, 2H), 4.38 (m, 2H) and 7.25 (m, 5H), ^{13}C NMR ($CDCl_3$) δ 11.00, 14.30, 17.52, 27.92, 36.22, 38.71, 51.82, 57.75, 62.88, 71.91, 79.15, 125.92, 127.95, 129.50, 137.32, 152.77, 154.97 and 177.52; mass spectrum (chemical ionization) *m/z* 435 $(M + H)^+$.

Anal. Calcd for $C_{23}H_{34}O_6N_2$: C, 63.57; H, 7.89. Found: C, 63.30; H, 8.08

(2*S*,3*S*,4*R*)-4-*tert*-Butoxycarbonylamino-3-hydroxy-2-methyl-5-phenyl-pentanoic Acid (8c). To a cooled (0 °C) solution containing 2.00 g (4.60 mmol) of **7c** in 40 mL of 3:1 THF-H_2O was added 3.9 mL (6 eq) of 30% H_2O_2 followed by 0.48 g (9.2 mmol) of LiOH. After stirring at 0 °C for 3 h, the excess peroxide was quenched at 0 °C by the addition of 10 mL of 1.5 N aq Na_2SO_3, the pH was adjusted to 9-10 with saturated aq $NaHCO_3$, and the oxazolidinone was removed by extraction with three 25-mL portions of CH_2Cl_2. The aqueous phase was then acidified to pH ~2 with 1 N HCl, and extracted with three 25-mL portions of ethyl acetate. The

combined organic phase was dried over $MgSO_4$, filtered, and concentrated under diminished pressure to give **8c** as a colorless powder: yield 1.29 g (87%); mp 139–141 °C, silica gel TLC R_f 0.52 (94:5:1 CH_2Cl_2–MeOH–AcOH); $[\alpha]^{20}_D$ +5.2 (*c* 0.9, MeOH), ^{1}H NMR (methanol-d_4) δ 1.13 (d, 3H, J = 7.22 Hz), 1.21 (s, 9H), 2.48 (m, 1H), 2.60 (m, 1H), 3.12 (dd, 1H, J = 3.1 Hz, J = 13.5 Hz), 3.66 (m, 1H), 3.84 (m, 1H), 4.83 (br s, 1H) and 7.15 (m, 5H); ^{13}C NMR (methanol-d_4) δ 9.38, 26.85, 36.48, 41.57, 54.09, 73.69, 77.97, 125.14, 127.26, 128.81, 138.53, 155.79, and 177.27.

Anal. Calcd for $C_{17}H_{25}O_5N$: C, 63.14; H, 7.79. Found: C, 62.99; H, 7.80.

(2*S*,3*S*,4*R*)-4-(9*H*-Fluoren-9-ylmethoxycarbonylamino)-3-hydroxy-2-methyl-5-phenylpentanoic Acid (9c). To a solution containing 1.29 g (3.99 mmol) of **8c** in 50 mL of dimethyl sulfide was added 20 mL of TFA. After 2 h, the solution was evaporated under a stream of nitrogen and then coevaporated with toluene under diminished pressure. The resulting oil was dissolved in 20 mL of a 5% aqueous K_2CO_3 solution followed by the addition of 1.34 g (3.99 mmol) of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide in 40 mL of dioxane. After 16 h, the solution was extracted with three 15-mL portions of ether, and three 10-mL portions of ethyl acetate. The combined aqueous extract was acidified to pH ~2 with 1 N HCl. The mixture was extracted with three 15-mL portions of ethyl acetate. The resulting organic extract was dried over $MgSO_4$, filtered, and concentrated under diminished pressure. Chromatography on flash silica gel (35 x 5 cm), elution with 95:3:2 CH_2Cl_2-methanol-acetic acid, gave **9c** as a colorless solid: yield 657 mg (37%), TLC R_f 0.23 (95:3:2 CH_2Cl_2-methanol-acetic acid); $[\alpha]^{20}_D$ +45.2 (*c* 0.77, methanol); ^{1}H NMR (methanol-d_4) δ 1.20 (d, 3H, J = 7.0 Hz), 2.57–2.64 (m, 2H), 3.20–3.23 (dd, 1H, J = 2.5 Hz, J = 13.5 Hz), 3.79–3.83 (m, 1H), 3.88-3.99 (m, 2H), 4.04–4.08 (m, 1H), 4.16-4.19 (m, 1H), 7.04–7.32 (m, 9H), 7.47 (d, 2H, J = 5.0 Hz), and 7.68 (d, 2H, J = 7.5

Hz); ^{13}C NMR (methanol-d_4) δ 9.90, 36.96, 42.12, 47.26, 55.24, 66.32, 74.22, 119.73, 124.99, 125.13, 125.99, 126.92, 126.96, 127.54, 127.57, 128.07, 129.46, 139.04, 141.33, 141.37, 143.89, 144.27, 156.98 and 177.94; mass spectrum (chemical ionization), *m/z* 446.0 $(M + H)^+$; mass spectrum (FAB), *m/z* 446.1965 $(M + H)^+$ ($C_{27}H_{27}NO_5$ requires 446.1967).

General Procedure for the Attachment of Bithiazole (Analogues) to the Solid Support. To a suspension containing 2.0 g (0.45 mmol/g) of NovaSyn TentaGel amino functionalized resin was added a solution containing 1.77 g (4.4 mmol) of Boc-protected spermine[26] and 919 μL (682 mg, 5.28 mmol) of Hunig's base in 8 mL of DMF. After 24 h, the resin was filtered, and washed for 30 s each with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2, three 5-mL portions of methanol, and then three 5-mL portions of DMF. A solution containing 1.26 mg (2.64 mmol) of Fmoc-bithiazole, 1.00 mg (2.64 mmol) of HBTU, and 919 μL (682 mg, 5.28 mmol) of Hunig's base was added. After 30 min, the resin was filtered and washed for 30 s each with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2, and three 5-mL portions of methanol. The resulting resin was dried under diminished pressure over KOH pellets. Quantitative Fmoc cleavage analysis indicated a loading of 0.17 mmol/g (57 % over 3 steps).

General Procedure for the Attachment of Threonine (Analogues) to the Resin-Bound Dipeptide. To a suspension containing 1.0 g of bithiazole-functionalized resin was added sequentially for 5 min each, three 4-mL solutions containing 20 % piperidine in DMF. The resulting resin was washed for 30 s each with seven 10-mL portions of DMF, five 10-mL portions of CH_2Cl_2, and then three 10-mL portions of DMF. A solution containing 174 mg (0.51 mmol) of Fmoc-threonine, 193 mg (0.51 mmol) of HBTU, 77 mg (0.51 mmol) of HOBt, and 178 μL (132 mg, 1.02 mmol) of Hunig's base in 4 mL of DMF was added. After 30 min the resin

was filtered and washed with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2, and again with three 5-mL portions of methanol. The resulting resin was dried under diminished pressure over KOH pellets. Quantitative Fmoc cleavage analysis indicated a loading of 0.17 mmol/g (>95%).

General Procedure for the Attachment of Methylvalerate (Analogues) to the Resin-Bound Tripeptide. To a suspension containing 300 mg of the derivatized resin was added three 1.0-mL solutions containing 20% piperidine in DMF. The resulting resin was washed for 30 s each with seven 5-mL portions of DMF, five 5-mL portions of CH_2Cl_2, and then three 5-mL portions of DMF. A solution containing 56 mg (0.153 mmol) of Fmoc-methylvalerate, 58 mg (0.153 mmol) of HBTU, 23 mg (0.153 mmol) of HOBt, and 53 μL (39 mg, 0.306 mmol) of Hunig's base in 1 mL of DMF was added. After 30 min, the resin was filtered and washed for 30 s each with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2, and then three 5-mL portions of methanol. The resulting resin was dried over KOH pellets under diminished pressure. Quantitative Fmoc cleavage analysis indicated a loading of 0.15 mmol/g (94%).

General Procedure for the Attachment of Histidine (Analogues) to the Resin-Bound Tetrapeptide. To a suspension containing 60 mg of the derivatized resin was added successively three 0.5-mL solutions containing 20% piperidine in DMF for 5 min each. The resulting resin was washed for 30 s each with seven 5-mL portions of DMF, five 5-mL portions of CH_2Cl_2, and then three 5-mL portions of DMF. A solution containing 17 mg (0.027 mmol) of Fmoc-trityl-histidine, 10 mg (0.027 mmol) of HATU, 4 mg (0.027 mmol) of HOAt, and 9 μL (7 mg, 0.054 mmol) of Hunig's base in 0.5 mL of DMF was added. After 30 min, the resin was filtered for 30 s each with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2, and

three 5-mL portions of methanol. The resulting resin was dried under diminished pressure over KOH pellets. Quantitative Fmoc cleavage analysis indicated a loading of 0.13 mmol/g (95%).

General Procedure for the Synthesis of Deglycobleomycin A_6 (Analogues). To a suspension containing 40 mg of the pentapeptide resin was added three 0.5-mL solutions containing 20 % piperidine in DMF for 10 min each. The resulting resin was washed for 30 s each with seven 5-mL portions of DMF, five 5-mL portions of CH_2Cl_2, and three 5-mL portions of DMF. The resin was then added to a 10 mL round bottom flask containing 1 mL of DMF and cooled to 0 °C for 10 min. A mixture containing 5 mg (11.6 μmol) of Boc-pyrimidoblamic acid and 15 mg (34.8 μmol) of BOP was added to the resin with an additional 1 mL of DMF. The reaction mixture was cooled for an additional 10 min followed by the addition of 12 μL (9 mg, 70 μmol) of Hunig's base. After 16 h, the resin was filtered and washed for 30 s each with three 5-mL portions of DMF, three 5-mL portions of CH_2Cl_2, and then three 5-mL portions of methanol. The resulting resin was dried under diminished pressure over KOH pellets.

General Procedure for the Cleavage of DeglycoBLM A_6 (Analogues) from the Resin. To a suspension containing 24-30 mg of resin-bound fully protected deglycobleomycin A_6 (analogue) was added a solution containing 200 μL of triisopropylsilane and 200 μL of methyl sulfide. After 5 min, 4 mL of trifluoroacetic acid was added to the suspension. After 4 h, the resin was filtered and washed with five 3-mL portions of CH_2Cl_2 and three 3-mL portions of DMF. The resulting resin was treated with a 0.5 mL of 2% hydrazine in DMF solution. The resin was filtered and then treated with three 0.5-mL portions of 2% hydrazine in DMF solution for an additional 10 min. The eluate was collected and concentrated under diminished pressure. The resulting oil was dissolved in 0.1% aq TFA, frozen and lyophilized.

Deglycobleomycin A_6. The crude product was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 x 10 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (88:12 0.1% aq TFA-CH_3CN → 83:17 0.1% aq TFA-CH_3CN) over a period of 30 min at a flow rate of 4 mL/min. Fractions containing the desired product eluted at 12.0 min and were collected, frozen, and lyophilized to give deglycobleomycin A_6 as a colorless solid: yield 3.1 mg (66%); ^{1}H NMR (D_2O) δ 0.94 (d, 3H, J = 7.0 Hz), 0.99 (d, 3H, J = 7.0 Hz), 1.07 (d, 3H, J = 7.0 Hz), 1.69 (m, 4H), 1.93 (s, 3H), 1.98 (m, 4H), 2.53 (t, 1H, J = 7.5 Hz), 2.63 (m, 2H), 3.03 (m, 12H), 3.21 (m, 4H), 3.44 (t, 2H, J = 7.0 Hz), 3.58 (t, 1H, J = 5.5 Hz), 3.64 (t, 1H, J = 5.0 Hz), 3.77 (t, 1H, J = 6.5 Hz), 3.97 (m, 2H, J = 6.0 Hz), 4.04 (t, 1H, J = 5.5 Hz), 4.12 (d, 1H, J = 5.5 Hz), 7.27 (s, 1H), 7.98 (s, 1H), 8.13 (s, 1H) and 8.59 (s, 1H); mass spectrum (FAB), *m/z* 1114.5559 $(M + H)^+$ ($C_{47}H_{76}O_9N_{19}S_2$ requires 1114.5515).

Deglycobleomycin Analogue 10. The crude product was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 x 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (87:13 0.1% aq TFA-CH_3CN → 80:20 0.1% aq TFA-CH_3CN) over a period of 30 min at a flow rate of 4 mL/min. Fractions containing the desired product eluted at 15.4 min and were collected, frozen, and lyophilized to give **10** as a colorless solid: yield 1.5 mg (39%); ^{1}H NMR (D_2O) δ 0.56 (s, 3H), 0.68 (s, 3H), 0.93 (d, 3H, J = 6.0 Hz), 1.04 (d, 3H, J = 7.0 Hz), 1.16-1.19 (m, 2H), 1.64-1.68 (m, 4H), 1.89-1.99 (m, 7H), 2.49 (m, 1H), 2.60-2.68 (m, 2H), 2.94-3.05 (m, 12H), 3.11-3.23 (m, 4H), 3.42 (dd, 1H, J = 7.0, 6.0 Hz), 3.48 (m, 1H), 3.56 (dd, 1H, J = 6.5, 5.5 Hz), 3.71-3.73 (m, 1H), 3.91 (m, 1H), 3.99 (dd, 1H, J = 6.0 Hz), 4.04 (t, 1H, J = 5.5 Hz), 4.15 (d, 1H, J = 5.5 Hz), 7.27 (s, 1H),

7.99 (s, 1H), 8.12 (s, 1H) and 8.60 (s, 1H); mass spectrum (FAB), *m/z* 1155.5900 $(M + H)^+$ ($C_{50}H_{81}O_9N_{19}S_2$ requires 1155.5984).

Deglycobleomycin Analogue 11. The crude product was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 x 10 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (86:14 0.1% aq TFA-CH_3CN → 80:20 0.1% aq TFA-CH_3CN) over a period of 30 min at a flow rate of 4 mL/min. Fractions containing the desired product eluted at 17.4 min and were collected, frozen, and lyophilized to give **11** as a colorless solid: yield 2.5 mg (55%); ^1H NMR (D_2O) δ 0.99 (d, 3H, J = 6.5 Hz), 1.10 (d, 3H, J = 7.0 Hz), 1.62-1.68 (m, 4H), 1.86-1.97 (m, 7H), 2.37 (t, 1H, J = 10.0 Hz), 2.58-2.70 (m, 3H), 2.75 (t, 1H, J = 6.0 Hz), 2.91-3.06 (m, 14H), 3.17 (t, 2H, J = 6.5 Hz), 3.36-3.38 (m, 2H), 3.56 (t, 2H, J = 6.0 Hz), 3.68 (d, 1H, J = 6.5 Hz), 3.99-4.07 (m, 4H), 4.21 (d, 1H, J = 5.0 Hz), 4.57 (t, 1H, J = 7.5 Hz), 6.75 (s, 1H), 7.0 (d, 2H, J = 7.0 Hz), 7.05 (dd, 1H, J = 7.5, 7.0 Hz), 7.10 (dd, 2H, J = 7.5, 7.0 Hz), 8.08 (s, 1H) and 8.40 (s, 1H); mass spectrum (FAB), *m/z* 1190.5786 $(M + H)^+$ ($C_{53}H_{80}O_9N_{19}S_2$ requires 1190.5828).

Deglycobleomycin Analogue 12. The crude product was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 x 10 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (83:17 0.1% aq TFA-CH_3CN → 68:32 0.1% aq TFA-CH_3CN) over a period of 30 min at a flow rate of 4 mL/min. Fractions containing the desired product eluted at 17.9 min and were collected, frozen, and lyophilized to give **12** as a colorless solid: yield 3.0 mg (68%); ^1H NMR (D_2O) δ 0.47 (dd, 3H, J = 6.5, 3.2 Hz), 0.64 (d, 3H, J = 6.5 Hz), 0.76-0.88 (m, 1H), 1.01 (dd, 3H, J = 6.5, 2.4 Hz), 1.04 (t, 1H, J = 10.0 Hz), 1.17 (t, 1H, J = 10.0 Hz), 1.64-1.67 (m, 4H), 1.84-1.98 (m, 7H), 2.40 (p, 1H, J = 7.0 Hz), 2.45 (d, 3H, J = 8.0 Hz), 2.56-2.75 (m, 4H), 2.95-3.06 (m, 12H), 3.10-3.38 (m, 5H), 3.40

(d, 2H, J = 7.0 Hz), 3.45-3.49 (m, 1H), 3.51-3.54 (m, 1H), 3.58-3.64 (m, 3H), 4.06-4.09 (m, 2H), 4.33-4.37 (m, 1H), 6.18 (d, 1H, J = 3.0 Hz), 6.85 (dd, 1H, J = 5.0, 5.0 Hz), 7.18 (d, 1H, J = 6.0 Hz), 8.00 (s, 1H) and 8.11 (s, 1H); mass spectrum (FAB), *m/z* 1218.5526 $(M + H)^+$ ($C_{52}H_{84}O_9N_{17}S_4$ requires 1218.5521).

Deglycobleomycin Analogue 13. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (87:13 0.1% aq TFA-CH_3CN → 77:23 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 17.8 min and were collected, frozen, and lyophilized to give **13** as a colorless solid: yield 1.4 mg (36%); ^{1}H NMR (D_2O) δ 0.88 (d, 3H, J = 6.5 Hz), 0.96 (d, 3H, J = 6.5 Hz), 1.36 (d, 3H, J = 7.0 Hz), 1.64 (m, 4H), 1.87 (s, 3H), 1.88-1.96 (m, 4H), 2.46-2.59 (m, 3H), 2.95-3.02 (m, 12H), 3.09 (d, 1H, J = 8.0 Hz), 3.11 (d, 1H, J = 7.5 Hz), 3.17 (q, 2H, J = 6.0 Hz), 3.40 (t, 2H, J = 6.5 Hz), 3.54 (t, 2H, J = 6.5 Hz), 3.59 (dd, 1H, J = 5.0, 4.8 Hz), 3.70 (m, 1H), 3.90 (dd, 1H, J = 7.0, 6.8 Hz), 3.95 (m, 1H), 3.99 (dd, 1H, J = 6.0, 6.0 Hz), 4.11 (d, 1H, J = 5.0 Hz), 7.19 (s, 1H), 7.98 (s, 1H), 8.08 (s, 1H), 8.12 (s, 1H) and 8.15 (s, 1H); mass spectrum (FAB), *m/z* 1197.5352 $(M + H)^+$ ($C_{50}H_{77}O_9N_{20}S_3$ requires 1197.5345).

Deglycobleomycin Analogue 14. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (90:10 0.1% aq TFA-CH_3CN → 80:20 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 21.8 min and were collected, frozen, and lyophilized to give **14** as a colorless solid: yield 1.7 mg (45%); ^{1}H NMR (D_2O) δ 0.92 (d, 3H, J = 6.5 Hz), 0.99 (d, 3H, J = 6.5 Hz), 1.05 (d, 3H, J = 7.0 Hz), 1.64-1.70 (m, 4H), 1.92 (s, 3H), 1.93-1.99 (m,

4H), 2.50 (m, 1H), 2.60-2.70 (m, 2H), 2.96-3.06 (m, 12H), 3.10-3.18 (m, 4H), 3.43 (t, 2H, J = 6.5 Hz), 3.54 (t, 2H, J = 6.0 Hz), 3.62 (dd, 1H, J = 5.5 Hz), 3.73, (p, 1H, J = 6.0 Hz), 3.96-4.00 (m, 2H), 4.06 (dd, 1H, J = 6.5, 5.5 Hz), 4.11 (d, 1H, J = 5.5 Hz), 7.24 (s, 1H), 8.19 (s, 1H) and 8.45 (s, 1H); mass spectrum (FAB), *m/z* 1148.5118 $(M + H)^+$ ($C_{47}H_{75}O_9ClN_{19}S_2$ requires 1148.5125).

Deglycobleomycin Analogue 15. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (83:17 0.1% aq TFA-CH_3CN → 73:27 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 15.9 min and were collected, frozen, and lyophilized to give **15** as a colorless solid: yield 1.1 mg (27 %); ^{1}H NMR (D_2O) δ 1.00 (d, 3H, J = 6.5 Hz), 1.08 (d, 3H, J = 7.0 Hz), 1.60-1.68 (m, 4H), 1.84 (s, 3H), 1.86-1.97 (m, 4H), 2.36 (br t, 1H, J = 14.0 Hz), 2.54-2.68 (m, 3H), 2.72-2.76 (m, 1H), 2.86-3.02 (m, 14H), 3.09 (t, 2H, J = 5.5 Hz), 3.38 (t, 2H, J = 6.5 Hz), 3.48-3.58 (m, 2H), 3.69 (t, 1H, J = 6.5, 6.0 Hz), 3.90 (dd, 1H, J = 8.0, 6.0 Hz), 3.96-4.50 (m, 3H), 4.21 (d, 1H, J = 4.5 Hz), 4.55 (t, 1H, J = 6.0 Hz), 6.73 (s, 1H), 6.98 (d, 2H, J = 7.0 Hz), 7.00 (dd, 1H, J = 6.5 Hz), 7.08 (dd, 2H, J = 7.0 Hz), 8.15 (s, 1H) and 8.38 (s, 1H); mass spectrum (FAB), *m/z* 1224.5413 $(M + H)^+$ ($C_{53}H_{79}O_9S_2N_{19}Cl$ requires 1224.5438).

Deglycobleomycin Analogue 16. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (79:21 0.1% aq TFA-CH_3CN → 69:31 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 17.7 min and were collected, frozen, and lyophilized to give **16** as a colorless solid: yield 2.2 mg (44%); ^{1}H NMR (D_2O) δ 0.62 (d, 3H, J = 6.5 Hz),

0.82 (d, 3H, J = 7.0 Hz), 1.62-1.68 (m, 4H), 1.76 (s, 3H), 1.89-1.99 (m, 4H), 2.24 (p, 1H, J = 7.0 Hz), 2.50-2.68 (m, 3H), 2.81-2.85 (m, 1H), 2.92-3.04 (m, 14H), 3.09-3.12 (m, 2H), 3.38-3.41 (m, 3H), 3.48-3.52 (m, 3H), 3.94-3.98 (m, 2H), 4.40 (t, 1H, J = 7.5 Hz), 4.53 (t, 1H, J = 7.5 Hz), 6.57 (d, 2H, J = 8.0 Hz), 6.87-6.91 (m, 3H), 6.95-7.03 (m, 2H), 7.25 (d, 1H, J = 7.5 Hz), 7.38 (d, 1H, J = 7.5 Hz), 7.89 (s, 1H), 7.94 (s, 1H) and 7.99 (s, 1H); mass spectrum (FAB), *m/z* 1308.5725 $(M + H)^+$ ($C_{60}H_{82}O_9S_3N_{19}$ requires 1308.5705).

Deglycobleomycin Analogue 17. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (81:19 0.1% aq TFA-CH_3CN → 71:29 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 20.7 min and were collected, frozen, and lyophilized to give **17** as a colorless solid: yield 3.5 mg (85%); ^1H NMR (D_2O) δ 0.60 (d, 3H, J = 6.5 Hz), 0.84 (d, 3H, J = 6.5 Hz), 1.60-1.68 (m, 4H), 1.83-1.88 (m, 5H), 1.94-1.97 (m, 2H), 2.26 (m, 1H), 2.60-2.72 (m, 3H), 2.79-2.83 (m, 1H), 2.94-3.15 (m, 16H), 3.26-3.42 (m, 4H), 3.48-3.56 (m, 2H), 4.02-4.08 (m, 2H), 4.36 (t, 1H, J = 7.5 Hz), 4.56 (t, 1H, J = 5.5 Hz), 6.54 (d, 2H, J = 6.5 Hz), 6.83 (d, 2H, J = 6.5 Hz), 6.96 (dd, 1H, J = 6.0, 5.0 Hz), 7.08 (dd, 1H, J = 8.0, 7.0 Hz), 7.11 (s, 1H), 7.34 (d, 1H, J = 6.0 Hz), 7.46 (d, 1H, J = 5.5 Hz) and 8.11 (s, 1H); mass spectrum (FAB), *m/z* 1259.5452 $(M + H)^+$ ($C_{57}H_{80}O_9S_2N_{18}Cl$ requires 1259.5486).

Deglycobleomycin Analogue 18. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (83:17 0.1% aq TFA-CH_3CN → 73:27 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 24.9 min and were collected, frozen, and lyophilized to

give **18** as a colorless solid: yield 2.7 mg (58%); ^{1}H NMR (D_2O) δ 0.59 (d, 3H, J = 6.5 Hz), 0.72 (d, 3H, J = 6.5 Hz), 0.85 (d, 3H, J = 6.5 Hz), 1.21 (t, 1H, J = 9.0 Hz), 1.26-1.31 (m, 2H), 1.60-1.67 (m, 4H), 1.88-1.97 (m, 4H), 1.97 (s, 3H), 2.06-2.15 (m, 2H), 2.33 (p, 1H, J = 7.0 Hz), 2.55-2.60 (m, 4H), 2.66-2.74 (m, 4H), 2.82-2.89 (m, 1H), 2.89-3.06 (m, 12H), 3.04-3.13 (m, 3H), 3.34-3.49 (m, 4H), 3.51-3.58 (m, 1H), 3.69-3.72 (m, 1H), 4.07-4.09 (m, 1H), 4.38-4.44 (m, 2H), 6.47 (d, 2H, J = 7.5 Hz), 6.80 (d, 2H, J = 7.5 Hz), 7.99 (s, 1H), 8.04 (s, 1H) and 8.10 (s, 1H); mass spectrum (FAB), *m/z* 1311.5735 $(M + H)^+$ ($C_{57}H_{87}O_{10}S_4N_{18}$ requires 1311.5735).

Deglycobleomycin Analogue 19. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (83:17 0.1% aq TFA-CH_3CN → 73:27 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 19.8 min and were collected, frozen, and lyophilized to give **19** as a colorless solid: yield 1.7 mg (38%); ^{1}H NMR (D_2O) δ 0.63 (d, 3H, J = 6.5 Hz), 0.76 (d, 3H, J = 6.5 Hz), 0.88 (d, 3H, J = 7.0 Hz), 1.15 (t, 1H, J = 11.0 Hz), 1.29-1.35 (m, 2H), 1.63-1.66 (m, 4H), 1.85-1.97 (m, 4H), 1.98 (s, 3H), 2.12-2.17 (m, 2H), 2.35 (p, 1H, J = 6.5 Hz), 2.55-2.58 (m, 1H), 2.65-2.70 (m, 2H), 2.72-2.83 (m, 4H), 2.86-2.94 (m, 2H), 2.95-3.05 (m, 12H), 3.07-3.13 (m, 1H), 3.30-3.40 (m, 3H), 3.49 (dd, 1H, J = 7.0 Hz), 3.58-3.62 (m, 1H), 3.71-3.76 (m, 1H), 4.08 (d, 2H, J = 6.5 Hz), 4.40 (t, 1H, J = 7.0, 6.5 Hz), 4.43-4.46 (m, 1H), 6.49 (d, 2H, J = 8.0 Hz), 6.90 (d, 2H, J = 8.0 Hz) and 8.17 (s, 1H); mass spectrum (FAB), *m/z* 1262.5563 $(M + H)^+$ ($C_{54}H_{85}O_{10}S_3N_{17}Cl$ requires 1262.5516).

Deglycobleomycin Analogue 20. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (86:14 0.1% aq TFA-CH_3CN → 76:24

0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 22.5 min and were collected, frozen, and lyophilized to give **20** as a colorless solid: yield 3.0 mg (60%); ^{1}H NMR (D_2O) δ 1.06 (d, 3H, J = 7.0 Hz), 1.60-1.68 (m, 4H), 1.86 (s, 3H), 1.87-2.02 (m, 4H), 2.40 (d, 1H, J = 12 Hz), 2.45 (d, 3H, J = 9.5 Hz), 2.51 (t, 1H, J = 6.5 Hz), 2.60-2.76 (m, 7H), 2.78-3.05 (m, 14H), 3.09-3.12 (m, 2H), 3.38 (t, 2H, J = 6.5 Hz), 3.46-3.52 (m, 1H), 3.52-3.62 (m, 1H), 3.67 (dd, 1H, J = 6.0, 5.5 Hz), 4.00 (m, 1H), 4.06 (q, 2H, J = 6.0 Hz), 4.37 (m, 1H), 4.56 (t, 1H, J = 7.5 Hz), 6.73 (s, 1H), 7.00 (d, 2H, J = 7.5 Hz), 7.03 (dd, 1H, J = 7.0, 7.0 Hz), 7.10 (dd, 2H, J = 7.5, 7.5 Hz), 8.16 (s, 1H) and 8.38 (s, 1H); mass spectrum (FAB), *m/z* 1270.5297 $(M + H)^+$ ($C_{54}H_{81}O_9S_3N_{19}Cl$ requires 1270.5315).

Deglycobleomycin Analogue 21. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (78:22 0.1% aq TFA-CH_3CN → 68:32 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 20.6 min and were collected, frozen, and lyophilized to give **21** as a colorless solid: yield 2.9 mg (55%); ^{1}H NMR (D_2O) δ 1.04 (d, 3H, J = 6.5 Hz), 1.62-1.67 (m, 4H), 1.73 (d, 3H, J = 3.0 Hz), 1.86-1.96 (m, 4H), 2.04-2.11 (m, 2H), 2.37-2.53 (m, 7H), 2.59-2.63 (m, 1H), 2.70-3.01 (m, 18H), 3.16-3.24 (m, 2H), 3.36 (t, 2H, J = 7.0 Hz), 3.53 (m, 1H), 3.61 (m, 2H), 3.90 (m, 2H), 4.46 (m, 1H), 4.53 (m, 1H), 6.40 (s, 1H), 6.80 (dd, 1H, J = 7.0, 7.0 Hz), 6.93 (dd, 1H, J = 7.0, 7.0 Hz), 6.98 (d, 3H, J = 7.5 Hz), 7.06 (t, 2H, J = 7.0 Hz), 7.16 (d, 1H, J = 8.0 Hz), 7.19 (d, 1H, J = 8.0 Hz), 7.75 (s, 1H), 7.85 (s, 1H) and 7.88 (s, 1H); mass spectrum (FAB), *m/z* 1368.5800 $(M + H)^+$ ($C_{62}H_{86}O_9S_4N_{19}$ requires 1368.5739).

Deglycobleomycin Analogue 22. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) column using aq 0.1 % TFA and

CH_3CN mobile phases. A linear gradient was employed (82:18 0.1% aq TFA-CH_3CN → 72:28 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 16.4 min and were collected, frozen, and lyophilized to give **22** as a colorless solid: yield 2.9 mg (55%); ^{1}H NMR (D_2O) δ 0.83 (d, 3H, J = 7.0 Hz), 1.00 (d, 3H, J = 7.0 Hz), 1.62-1.80 (m, 4H), 1.88-1.99 (m, 7H), 2.38 (p, 1H, J = 7.0 Hz), 2.47 (d, 3H, J = 5.5 Hz), 2.63-2.74 (m, 4H), 2.95-3.08 (m, 13H), 3.12-3.21 (m, 5H), 3.42 (t, 2H, J = 6.5 Hz), 3.52-3.64 (m, 4H), 4.08 (q, 2H, J = 6.0 Hz), 4.32 (dt, 1H, J = 8.5, 4.4 Hz), 6.77 (d, 1H, J = 3.5 Hz), 6.82 (d, 1H, J = 5.0 Hz), 6.83 (d, 1H, J = 5.0 Hz), 7.15 (d, 1H, J = 5.0 Hz), 8.00 (s, 1H), 8.07 (s, 1H) and 8.11 (s, 1H); mass spectrum (FAB), *m/z* 1259.4875 $(M + H)^+$ ($C_{52}H_{79}O_9S_5N_{18}$ requires 1259.4881).

Deglycobleomycin Analogue 23. The crude product was purified on a Supelco HS C_{18} reversed phase semi-preparative (250 x 21.2 mm, 5 μm) column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (85:15 0.1% aq TFA-CH_3CN → 75:25 0.1% aq TFA-CH_3CN) over a period of 40 min at a flow rate of 16 mL/min. Fractions containing the desired product eluted at 23.8 min and were collected, frozen, and lyophilized to give **23** as a colorless solid: yield 2.8 mg (65%); ^{1}H NMR (D_2O) δ 0.85 (d, 3H, J = 6.5 Hz), 1.00 (d, 3H, J = 7.0 Hz), 1.66-1.63 (m, 4H), 2.00-1.91 (m, 7H), 2.39 (m, 1H), 2.66 (d, 3H, J = 8.0 Hz), 2.77-2.60 (m, 4H), 3.21-2.95 (m, 18H), 3.41 (dd, 2H, J = 7.0, 7.0 Hz), 3.57-3.52 (m, 3H), 3.64 (m, 1H), 4.11-4.08 (m, 2H), 4.30 (dt, 1H, J = 8.8, 4.5 Hz), 6.81 (d, 1H, J = 3.0 Hz), 6.86 (d, 1H, J = 5.0 Hz), 6.87 (d, 1H, J = 5.0 Hz), 7.17 (d, 1H, J = 5.0 Hz) and 8.18 (s, 1H); mass spectrum (FAB), *m/z* 1210.4702 $(M + H)^+$ ($C_{49}H_{77}O_9S_4N_{17}Cl$ requires 1210.4662).

Deglycobleomycin Analogue 24. The crude product was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 x 10 mm, 5 μm) HPLC column using aq 0.1 % TFA

and CH_3CN mobile phases. A linear gradient was employed (84:16 0.1% aq TFA-CH_3CN → 74:26 0.1% aq TFA-CH_3CN) over a period of 30 min at a flow rate of 4 mL/min. Fractions containing the desired product eluted at 14.8 min and were collected, frozen, and lyophilized to give **24** as a colorless solid: yield 2.6 mg (58%); ^1H NMR (D_2O) δ 0.61 (d, 3H, J = 6.5 Hz), 0.75 (d, 3H, J = 6.5 Hz), 0.87 (d, 3H, J = 6.5 Hz), 1.13 (t, 1H, J = 12.0 Hz), 1.29-1.34 (t, 2H, J = 11.0 Hz), 1.60-1.66 (m, 4H), 1.85-1.96 (m, 4H), 1.99 (s, 3H), 2.08-2.18 (m, 2H), 2.34 (m, 1H), 2.57 (s, 3H), 2.58-2.61 (m, 1H), 2.69-2.82 (m, 3H), 2.84-2.91 (m, 1H), 2.93-3.01 (m, 12H), 3.05-3.11 (m, 3H), 3.28-3.21 (m, 1H), 3.30-3.36 (m, 1H), 3.73 (t, 2H, J = 6.5 Hz), 3.48 (dd, 1H, J = 7.5, 7.0 Hz), 3.55-3.58 (m, 1H), 3.71-3.73 (m, 1H), 4.11-4.37 (m, 2H), 4.38 (t, 1H, J = 7.5 Hz), 4.43-4.46 (m, 1H), 6.49 (d, 2H, J = 8.5 Hz), 6.80 (d, 2H, J = 8.5 Hz), 7.94 (s, 1H) and 8.06 (s, 1H); mass spectrum (FAB), *m/z* 1228.5934 $(M + H)^+$ ($C_{54}H_{86}O_{10}N_{17}S_3$ requires 1228.5906).

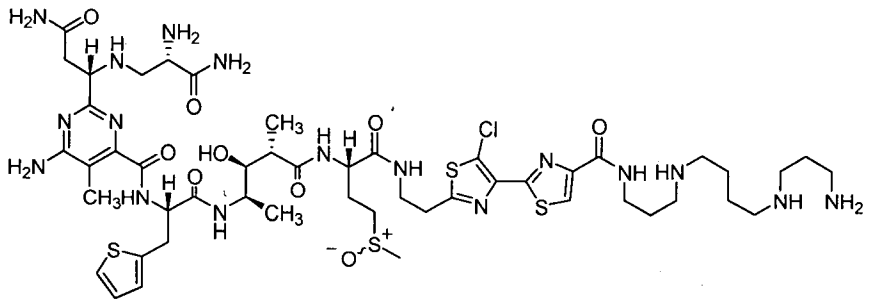
Reduction of Methionine Oxide-Containing Deglycobleomycin Analogue 24. To a cooled (0 °C) solution containing 400 μg of deglycobleomycin analogue **24** in 300 μL of TFA was added 200 μL of dimethyl sulfide followed by a catalytic amount (< 0.5 mg) of ammonium iodide in the absence of light. After 4 h, the mixture was concentrated under a stream of nitrogen, dissolved in 1 mL of water, frozen, and lyophilized. The crude material was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 x 10 mm, 5 μm) HPLC column using aq 0.1 % TFA and CH_3CN mobile phases. A linear gradient was employed (84:16 0.1% aq TFA-CH_3CN → 74:26 0.1% aq TFA-CH_3CN) over a period of 30 min at a flow rate of 4 mL/min. The product eluted at 26.3 min, and was frozen and lyophilized to give **24a** as a colorless solid: mass spectrum (FAB), *m/z* 1212.5890 $(M + H)^+$ ($C_{54}H_{86}O_9S_3N_{17}$ requires 1212.5957).

Plasmid DNA Cleavage by Fe(II)•Deglycobleomycin A_6 Analogues. A typical reaction mixture contained a deglycoBLM derivative and 300 ng of pBR322 or pSP64 DNA in 25 μL (total volume) of 50 mM sodium cacodylate, pH 7.0. The cleavage reaction was initiated by the addition of equimolar $Fe(NH_4)_2(SO_4)_4$ from a freshly prepared solution as the last component; the reaction mixture was incubated at 0 °C for 30 min. Final concentrations of Fe•deglycobleomycin analogues were varied from 1 μM to 10 μM. The reaction was then quenched by the addition of 5 μL loading solution (30% glycerol, 0.25% bromophenol blue, and 25 mM EDTA) and the quenched reaction mixture was applied to a 1.0 % agarose gel containing 0.5 μg/mL of ethidium bromide. Horizontal gel electrophoresis was carried out in 40 mM Tris–HCl, pH 8.0, containing 20 mM sodium acetate and 1 mM EDTA at 136 V for 2.2 h. The gel was destained with H_2O for 30 min and photographed under UV illumination.

Figure S1. Electrospray mass spectrum of crude deglycobleomycin analogue **23**.

Figure S2. HPLC chromatogram and ^{1}H NMR spectrum of **23**. The major peak at 23.8 min was found to be the desired product. HPLC analysis was carried out on a C_{18} reversed phase column (250 x 21.2 mm) using a linear gradient of 15 → 25% 0.1% aqueous TFA in CH_3CN at a flow rate of 16 mL/min over a period of 40 min.

Figure S3. HPLC chromatograms of **24** and **24a**. Analogue **24** (top), present in the crude reaction mixture, eluted at 14.8 min, while purified **24a** (bottom) eluted at 26.3 min. HPLC analysis was carried out on a C_{18} reversed phase column (250 x 10 mm) using a linear gradient of 16 → 26% aqueous TFA in CH_3CN at a flow rate of 4 mL/min over a period of 30 min.



23

$C_{49}H_{76}ClN_{17}O_9S_4$

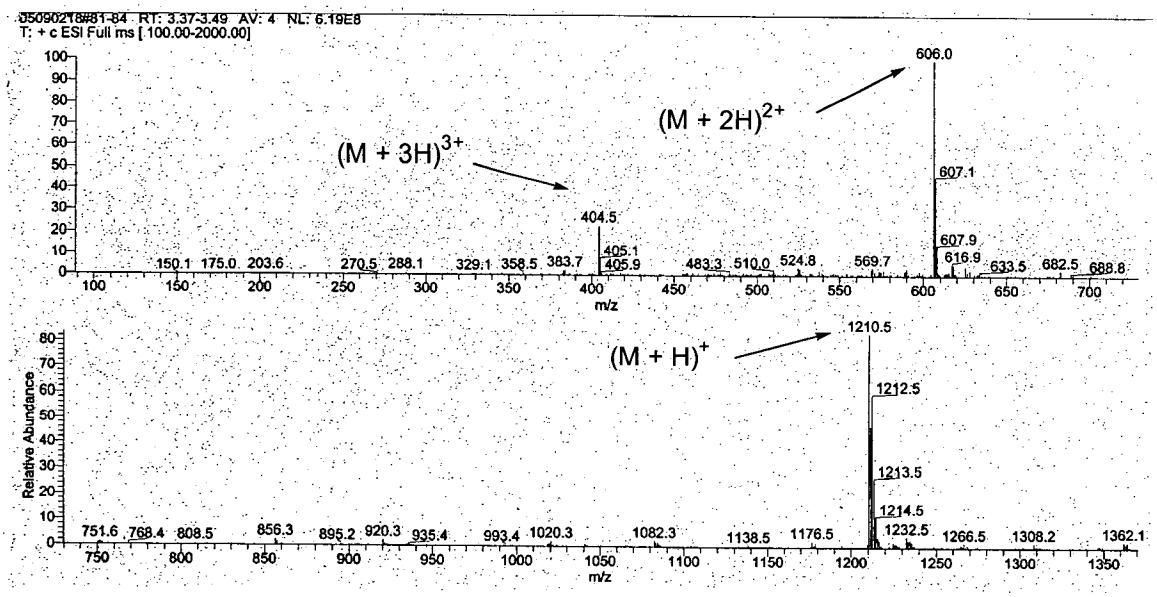
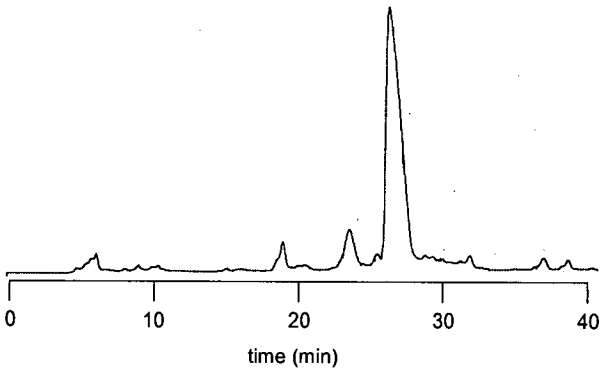


Figure S1.



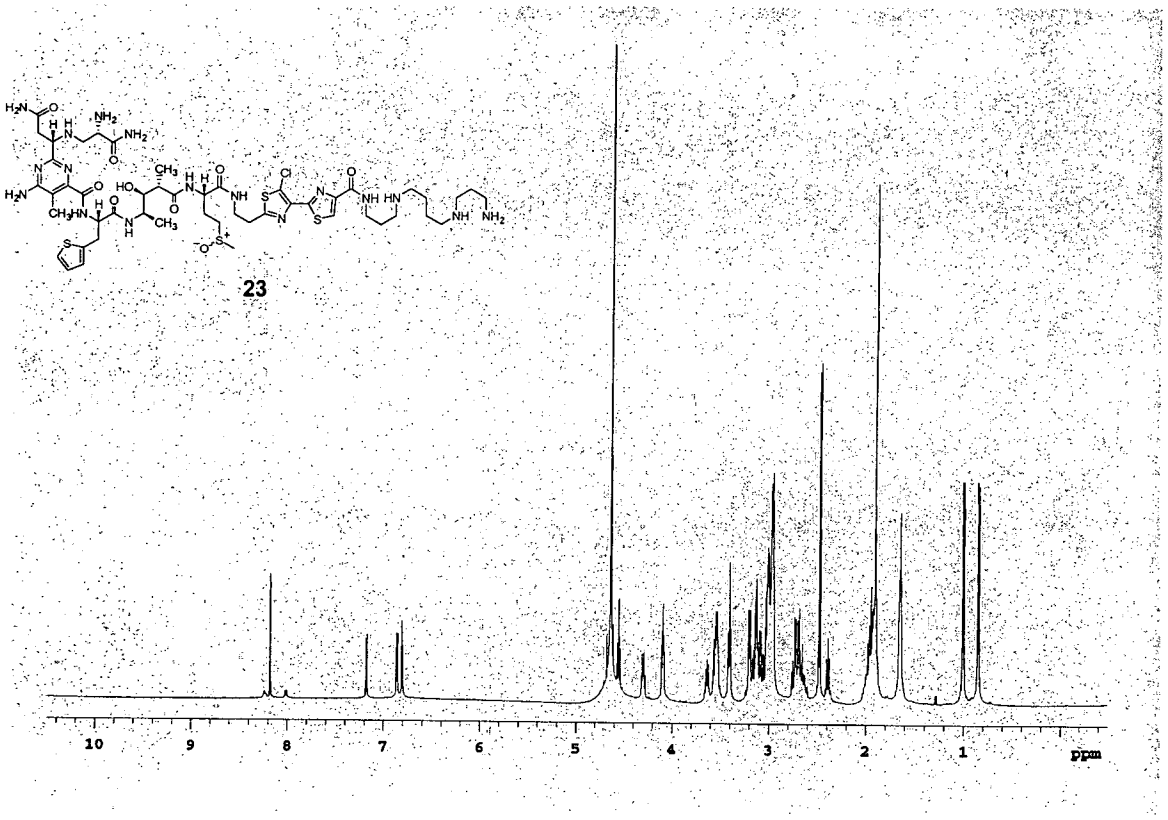


Figure S2.

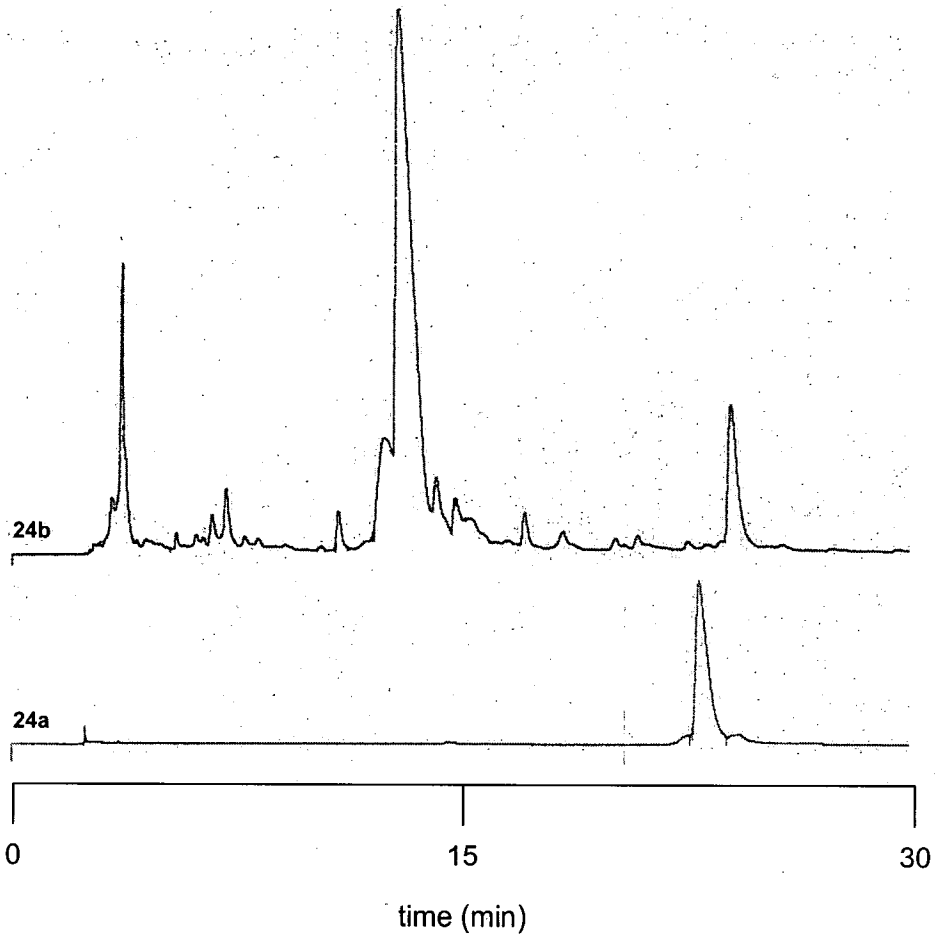
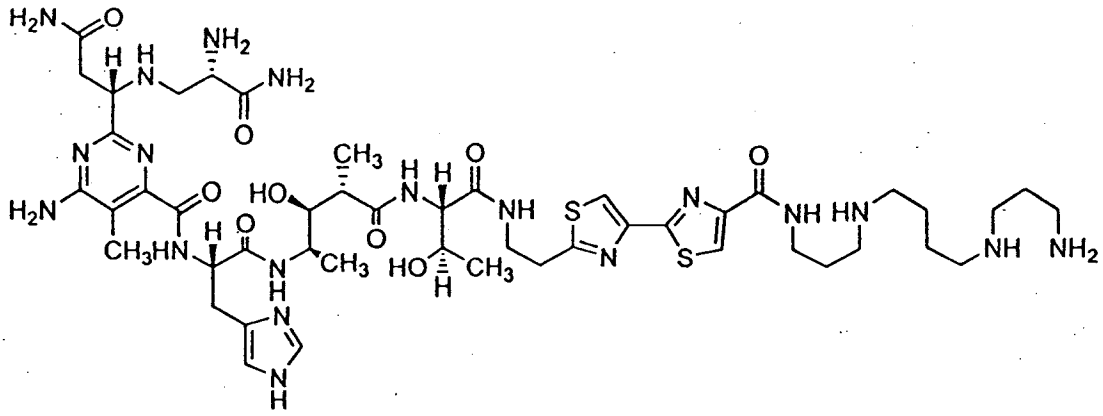


Figure S3.

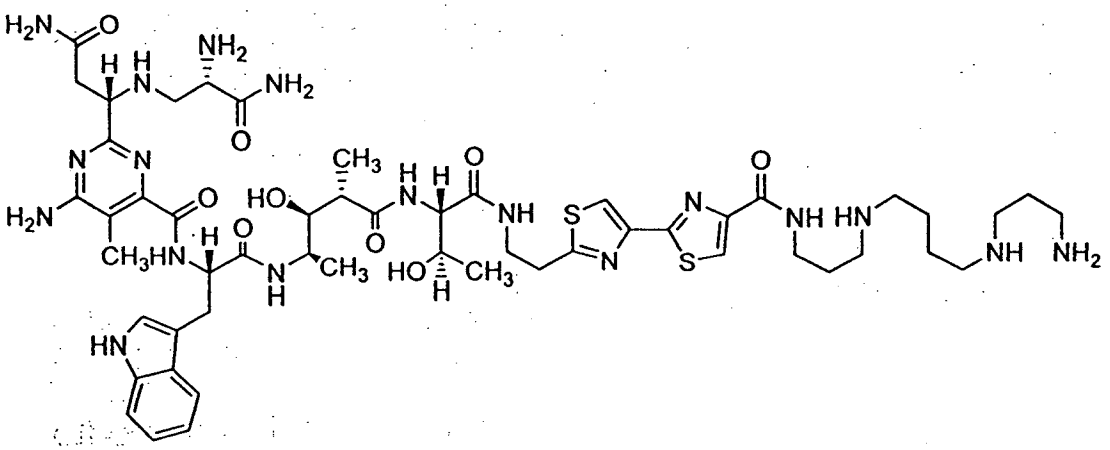
CJL-1 **deglycobleomycin A_6**
Cjl-ii-245



$C_{47}H_{75}N_{19}O_9S_2$
Exact Mass: 1113.5437
Mol. Wt.: 1114.3531

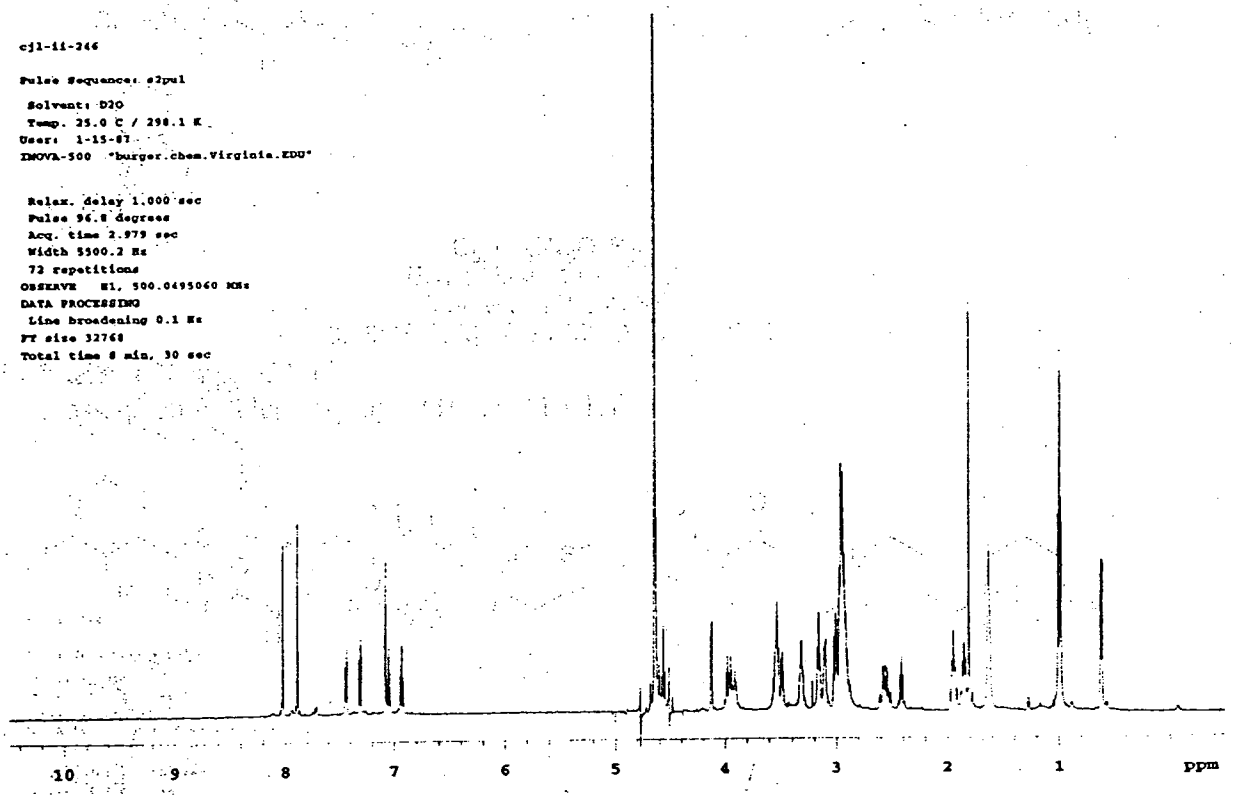
Yield: 1.69 mg (39%)
Mass spectrum (electrospray) 1114.6 $(M + H)^+$

CJL-2
Cjl-ii-246

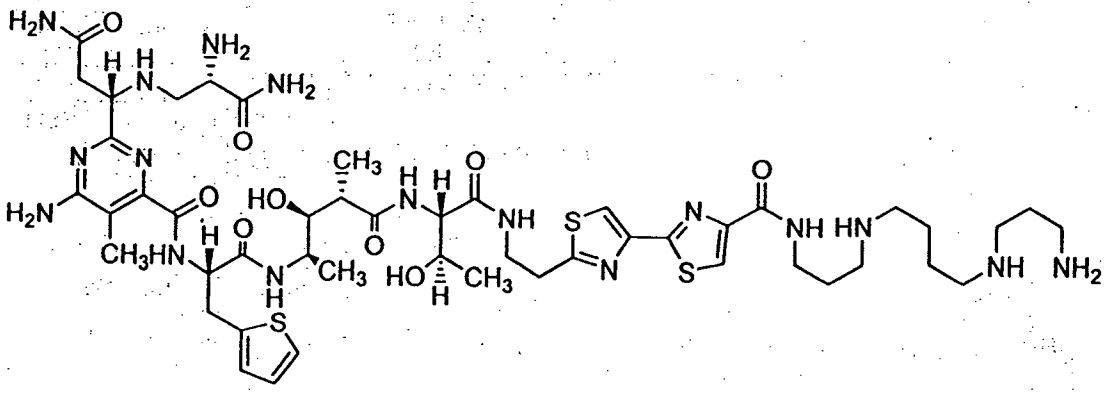


$C_{52}H_{78}N_{18}O_9S_2$
Exact Mass: 1162.5641
Mol. Wt.: 1163.4236
C, 53.68; H, 6.76; N, 21.67; O, 12.38; S, 5.51

Yield 4.30 mg (95%)
mass spectum (electrospray) 1163.7 $(M + H)^+$

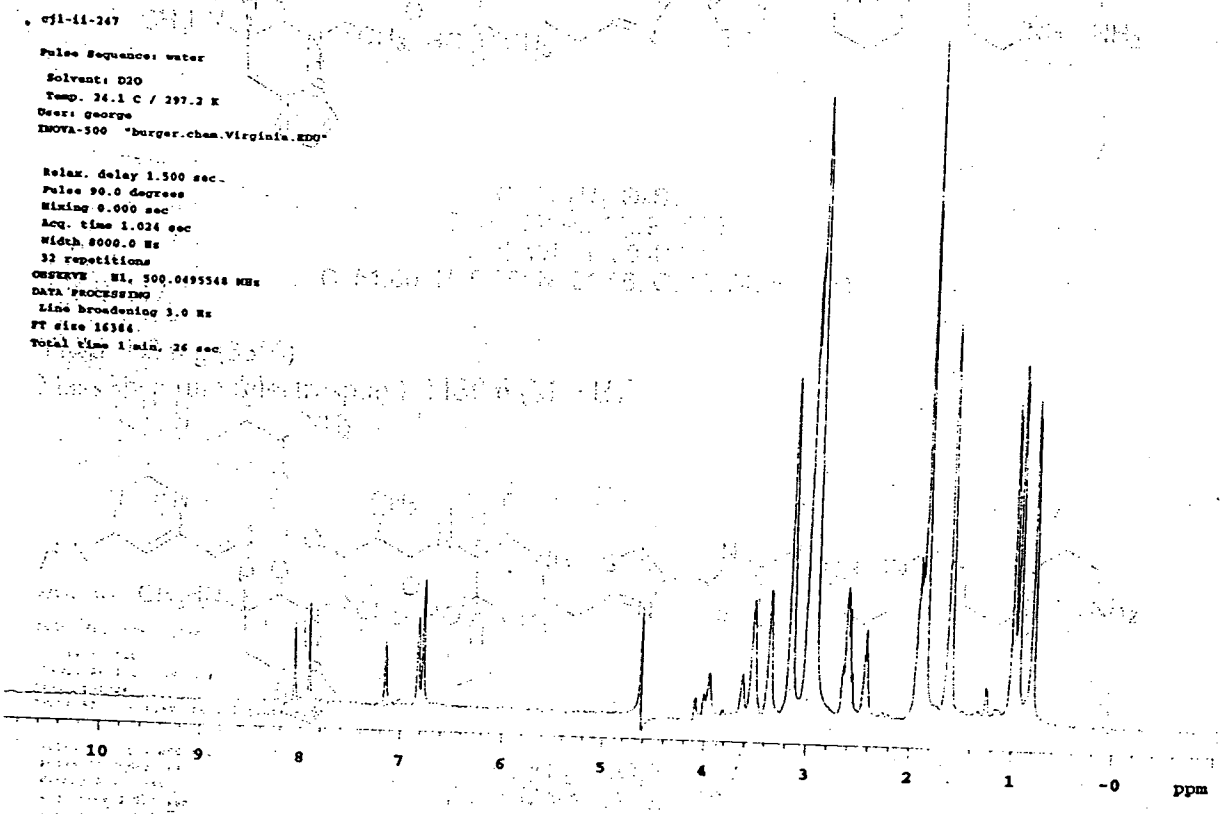


CJL-3
Cjl-ii-247

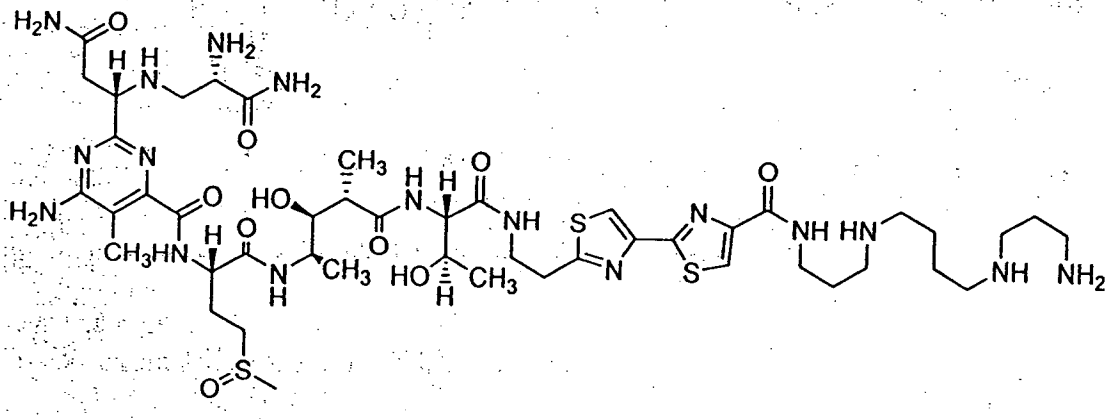


$C_{48}H_{75}N_{17}O_9S_3$
Exact Mass: 1129.5096
Mol. Wt.: 1130.4163
C, 51.00; H, 6.69; N, 21.06; O, 12.74; S, 8.51

Yield 1.2 mg (33%)
Mass spectrum (electrospray) 1130.6 $(M + H)^+$

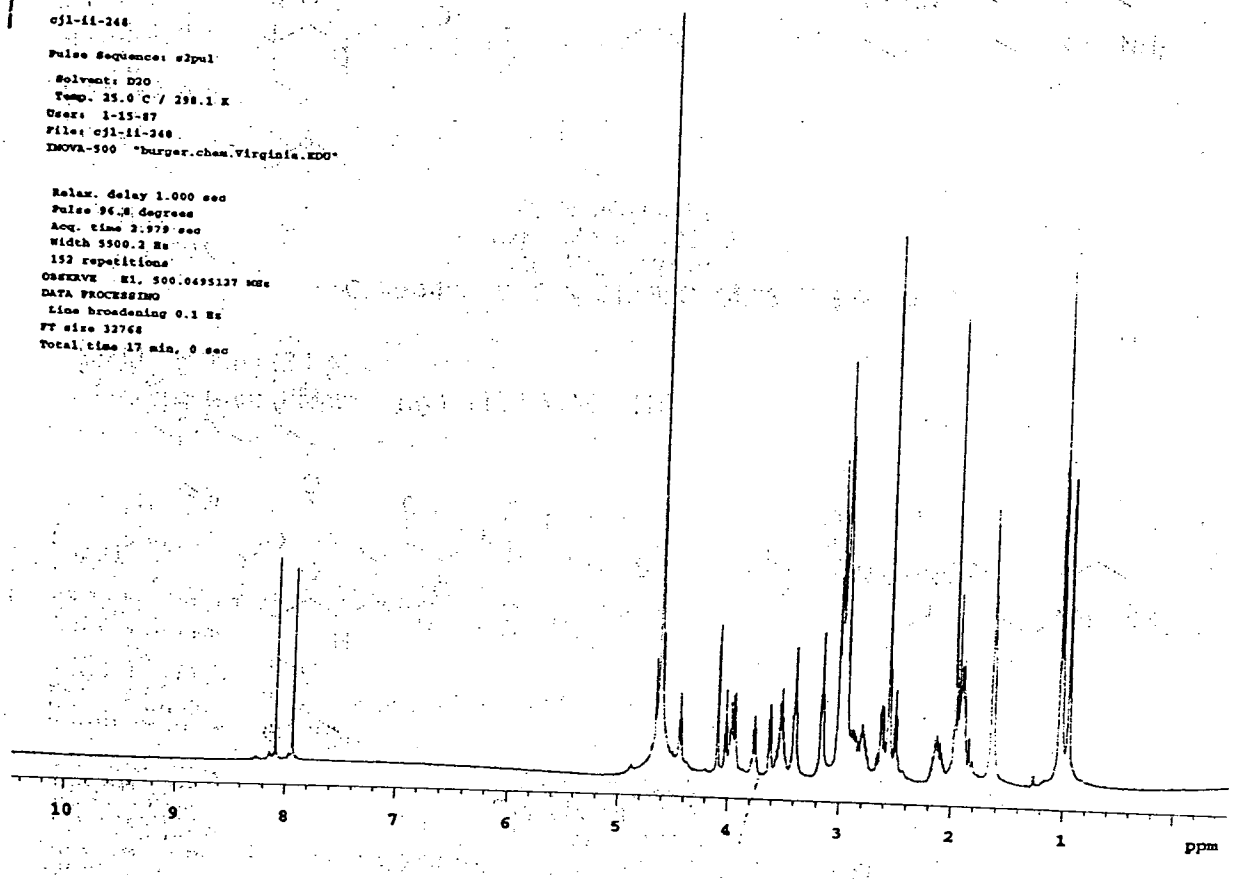


CJL-4
Cjl-ii-248

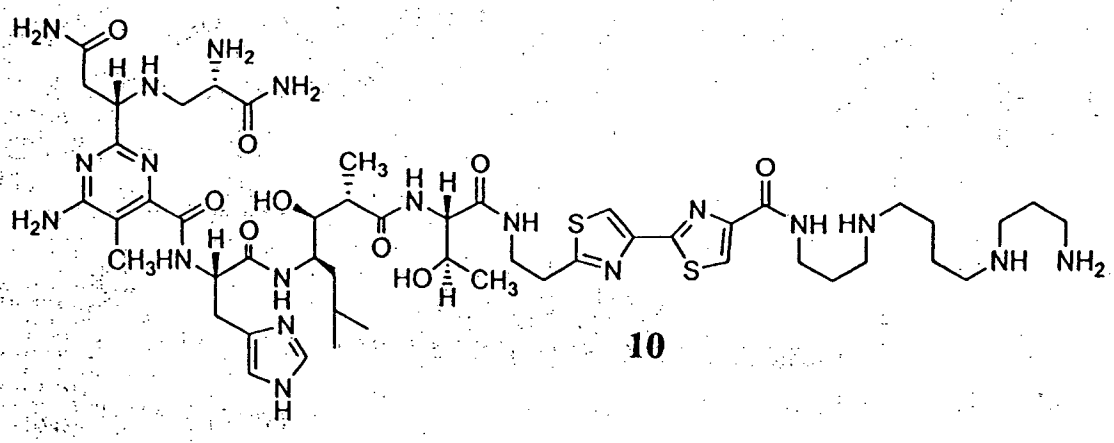


$C_{46}H_{77}N_{17}O_{10}S_3$
Exact Mass: 1123.52
Mol. Wt.: 1124.41
C, 49.14; H, 6.90; N, 21.18; O, 14.23; S, 8.56

Yield 1.50 mg (34%)
Mass spectrum (electrospray) 1124.6 $(M + H)^+$



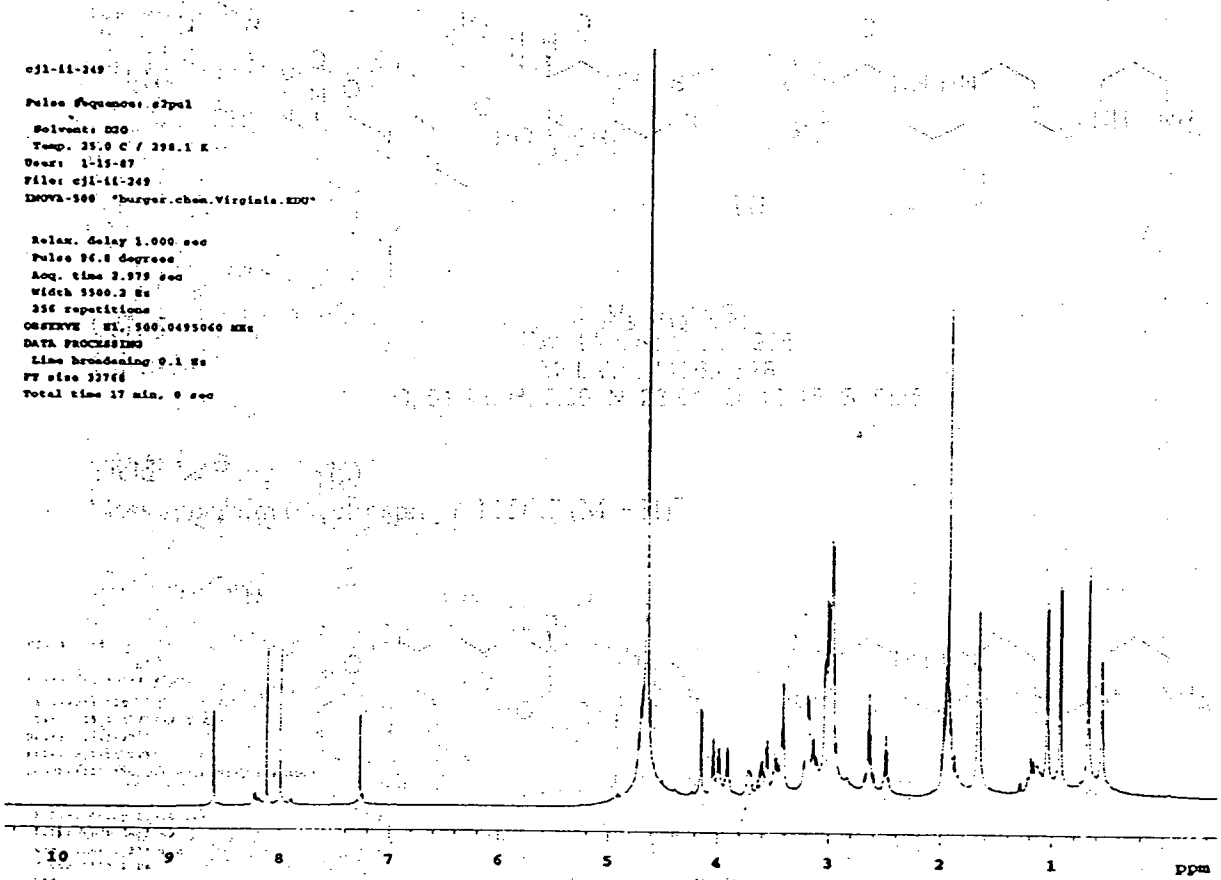
CJL-5
Cjl-ii-249



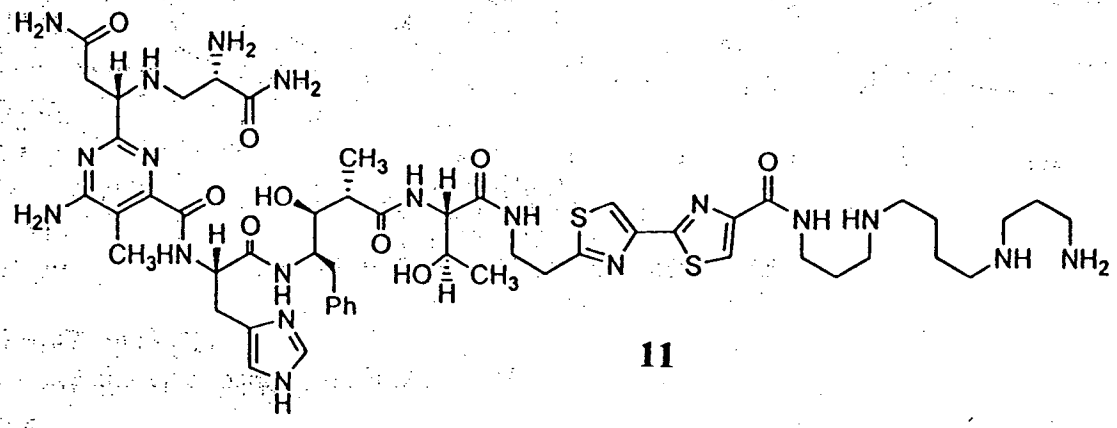
10

$C_{50}H_{81}N_{19}O_9S_2$
Exact Mass: 1155.5906
Mol. Wt.: 1156.4328
C, 51.93; H, 7.06; N, 23.01; O, 12.45; S, 5.55

Yield 1.47 mg (33%)
Mass spectrum (electrospray) 1156.7 $(M + H)^+$



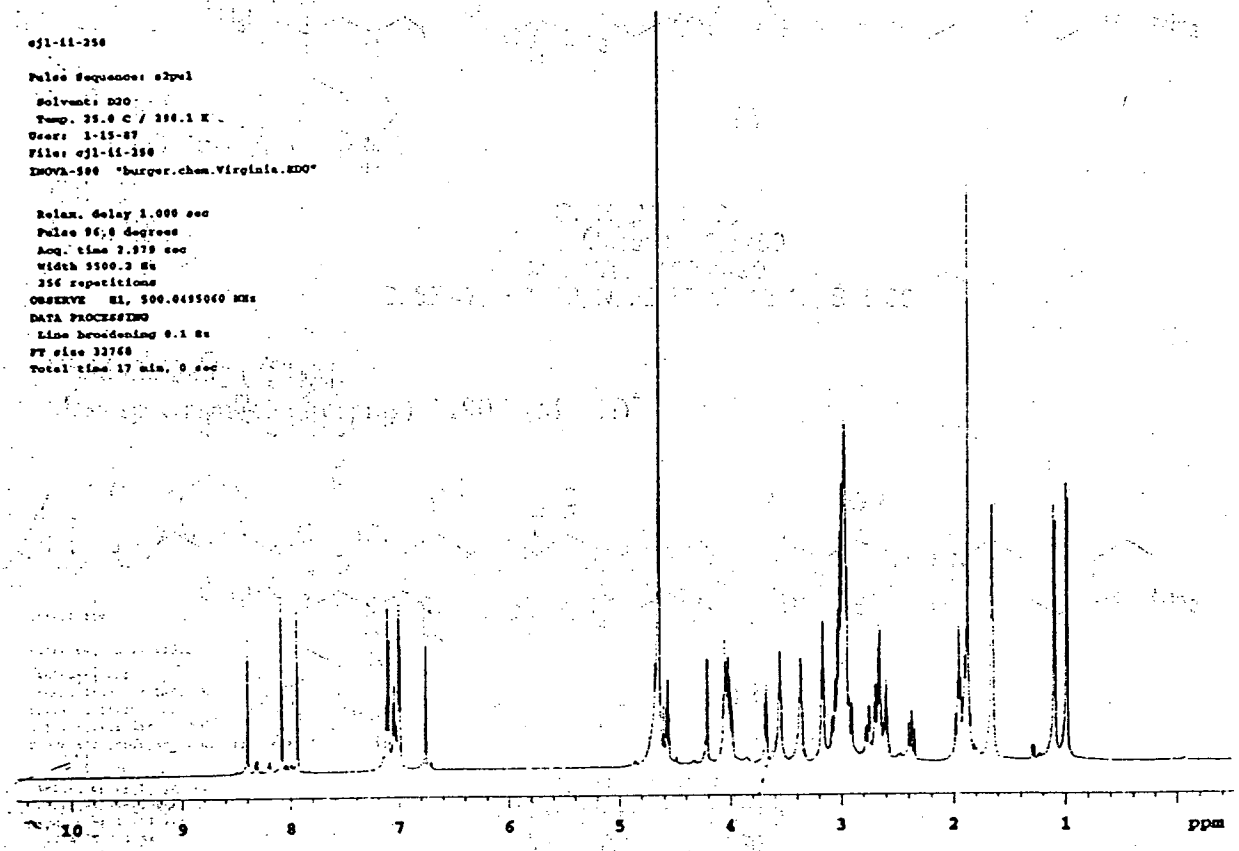
CJL-6*
Cjl-ii-250



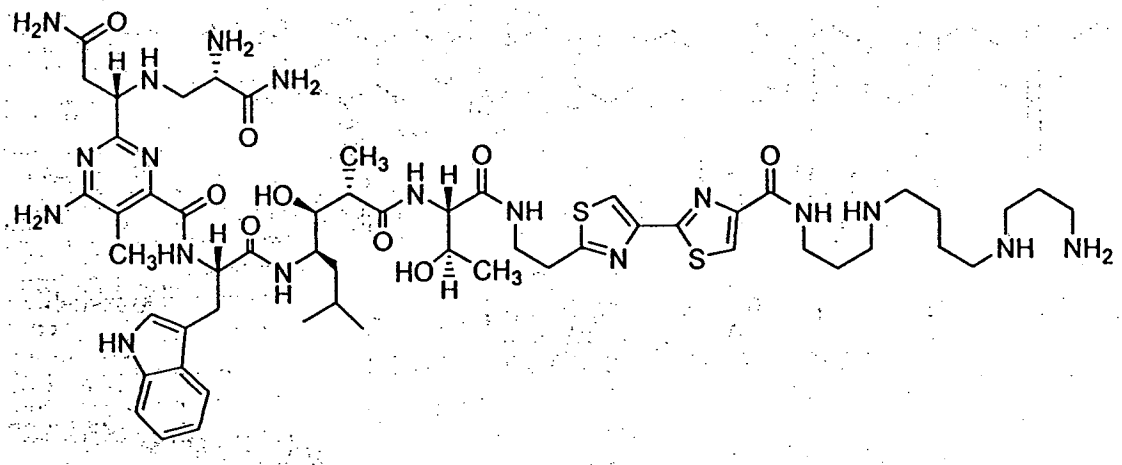
11

$C_{53}H_{79}N_{19}O_9S_2$
Exact Mass: 1189.5750
Mol. Wt.: 1190.4490
C, 53.47; H, 6.69; N, 22.36; O, 12.10; S, 5.39

Yield 2.53 mg (55%)
Mass spectrum (electrospray) 1190.7 $(M + H)^+$

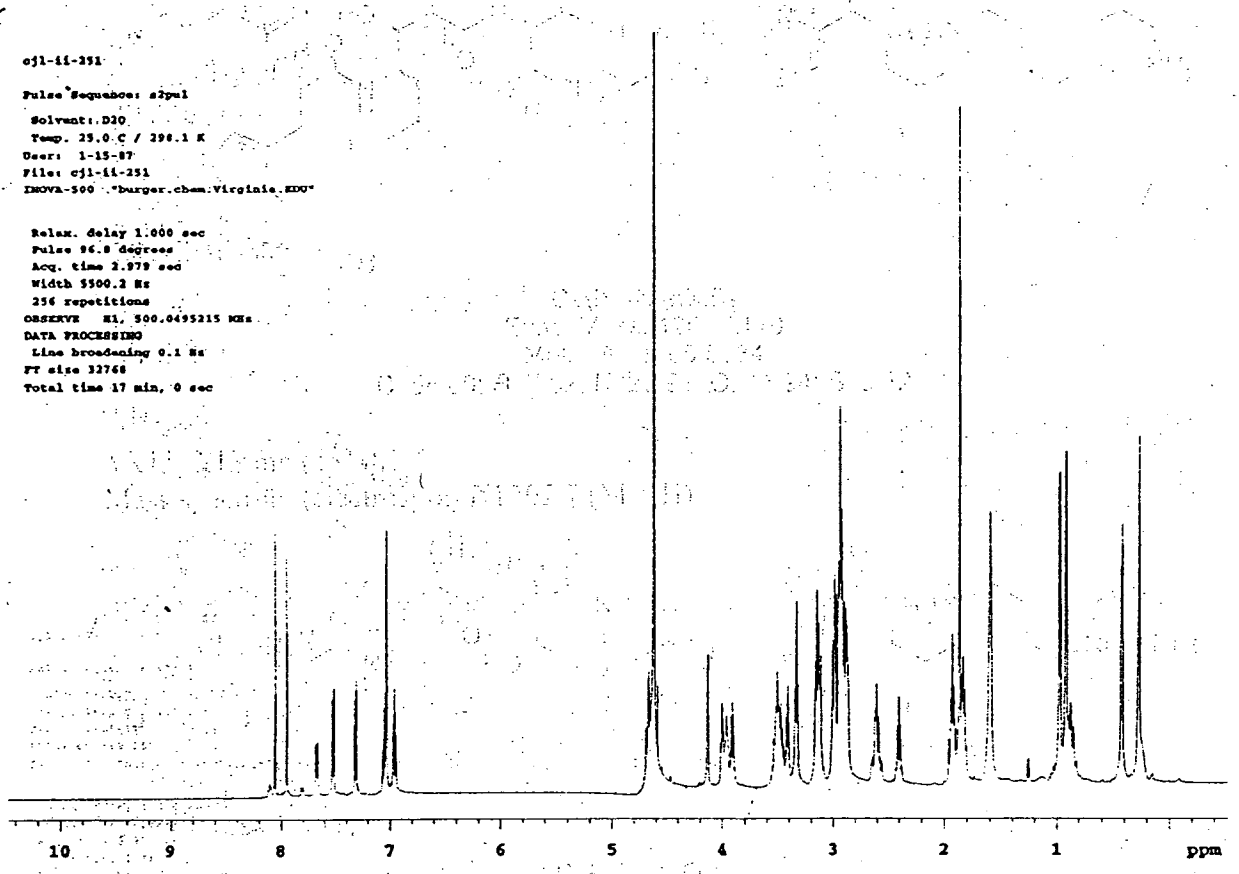


CJL-7
Cjl-ii-251

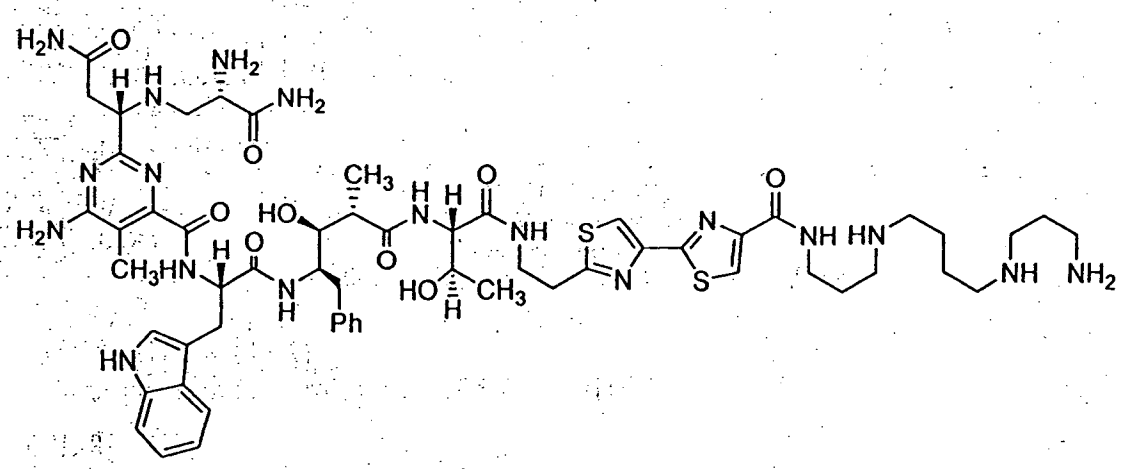


$C_{55}H_{84}N_{18}O_9S_2$
Exact Mass: 1204.6110
Mol. Wt.: 1205.5034
C, 54.80; H, 7.02; N, 20.91; O, 11.94; S, 5.32

Yield 2.13 mg (45%)
Mass spectrum (electrospray) 1205.7 $(M + H)^+$

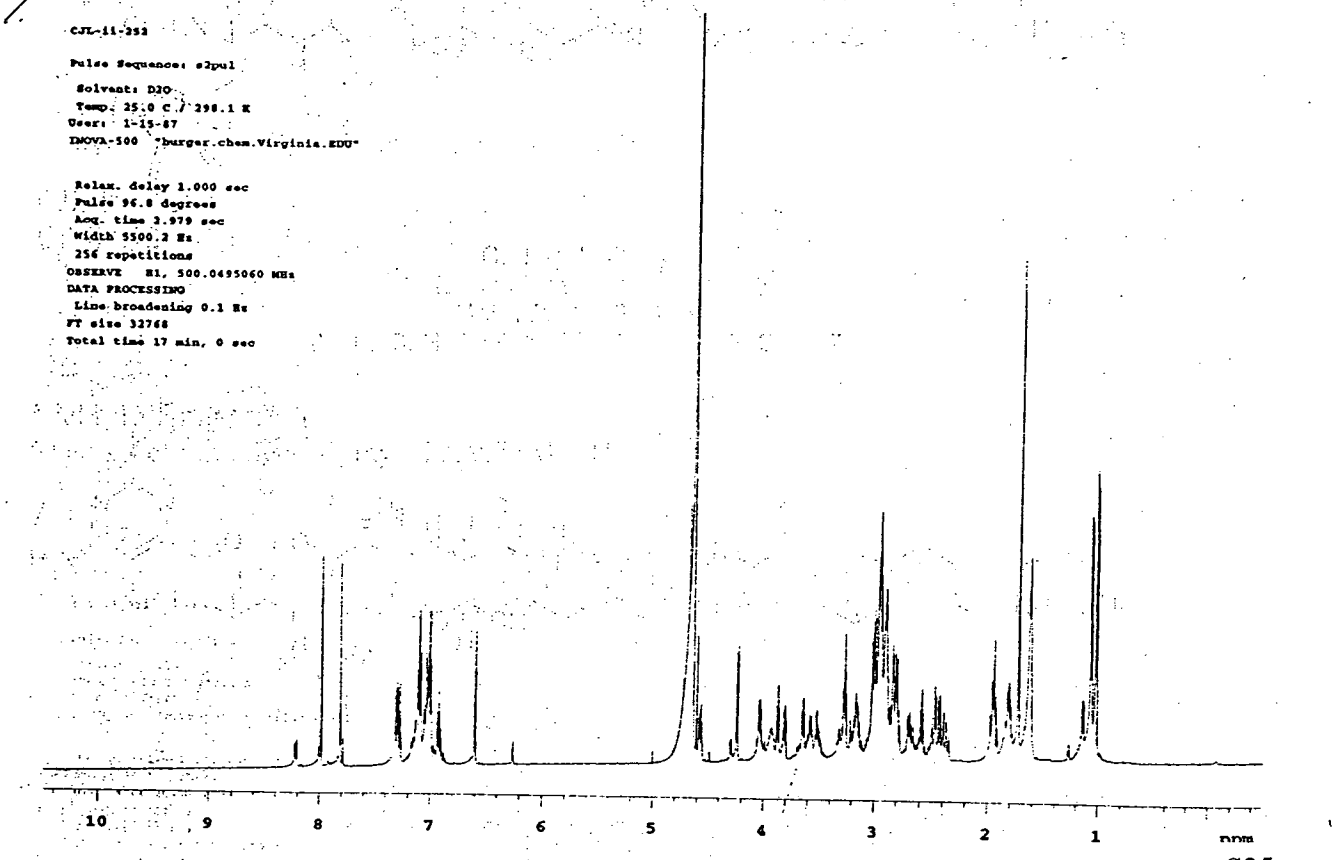


CJL-8
Cjl-ii-252

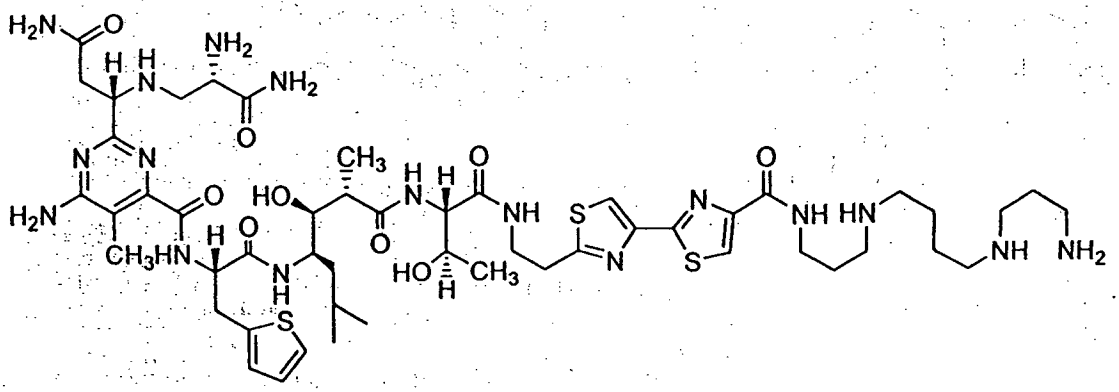


$C_{58}H_{82}N_{18}O_9S_2$
Exact Mass: 1238.5954
Mol. Wt.: 1239.5196
C, 56.20; H, 6.67; N, 20.34; O, 11.62; S, 5.17

Yield 1.05 mg (22%)
Mass spectrum (electrospray) 1239.7 $(M + H)^+$

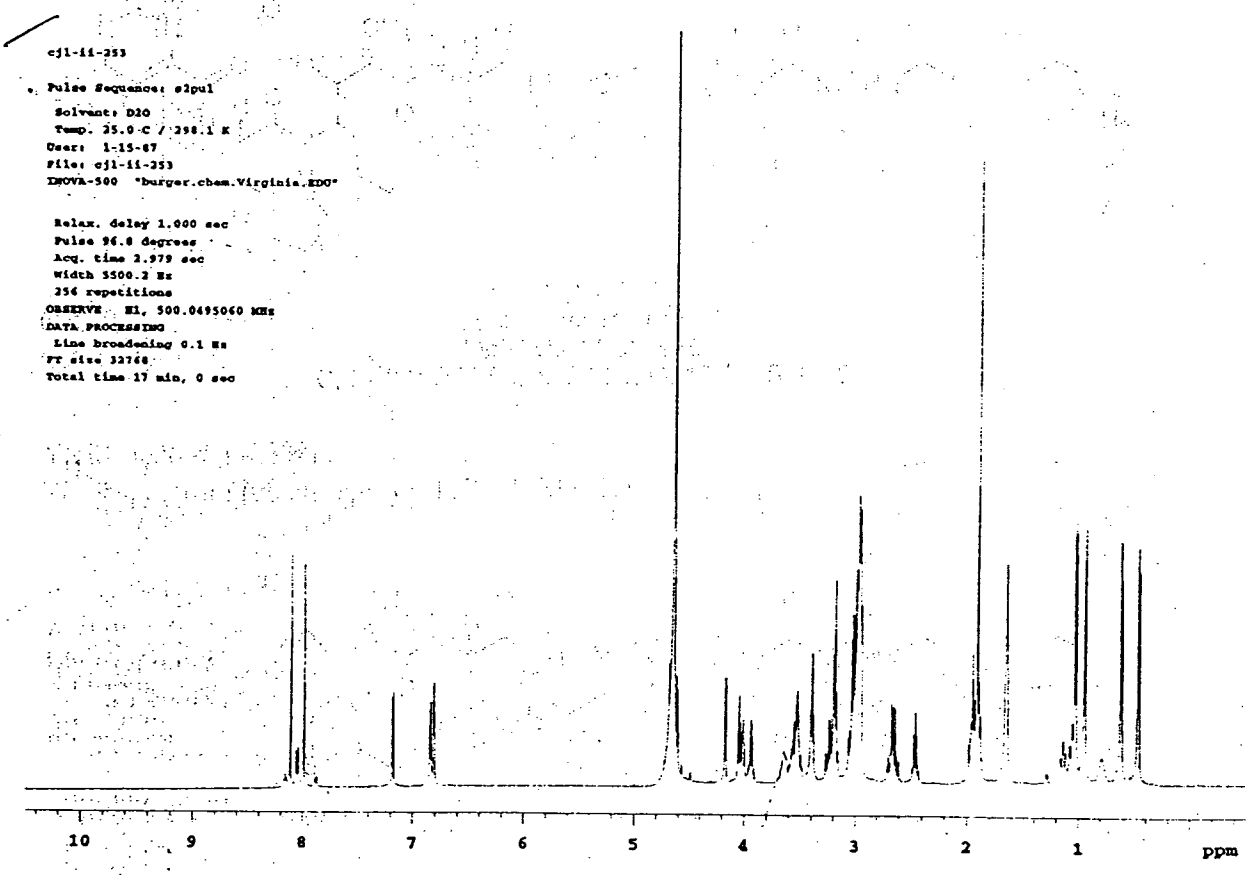


CJL-9
Cjl-ii-253

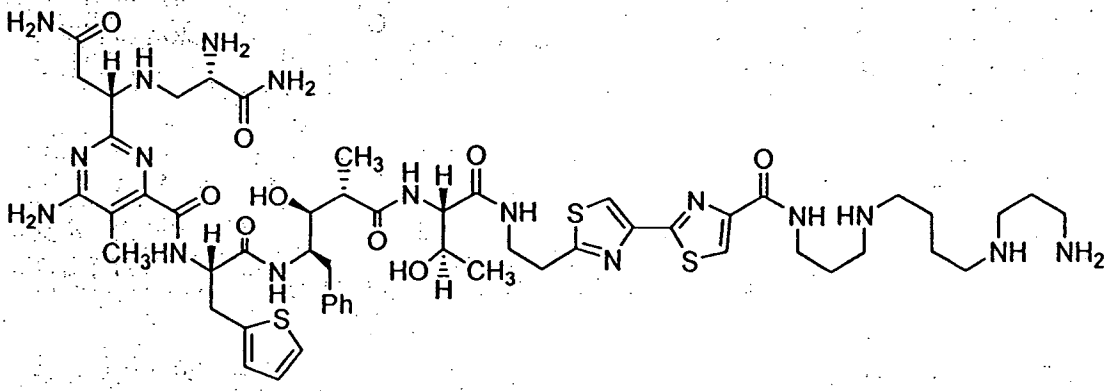


$C_{51}H_{81}N_{17}O_9S_3$
Exact Mass: 1171.5565
Mol. Wt.: 1172.4960
C, 52.24; H, 6.96; N, 20.31; O, 12.28; S, 8.20

Yield 1.06 mg (23%)
Mass spectrum (electrospray) 1172.7 $(M + H)^+$

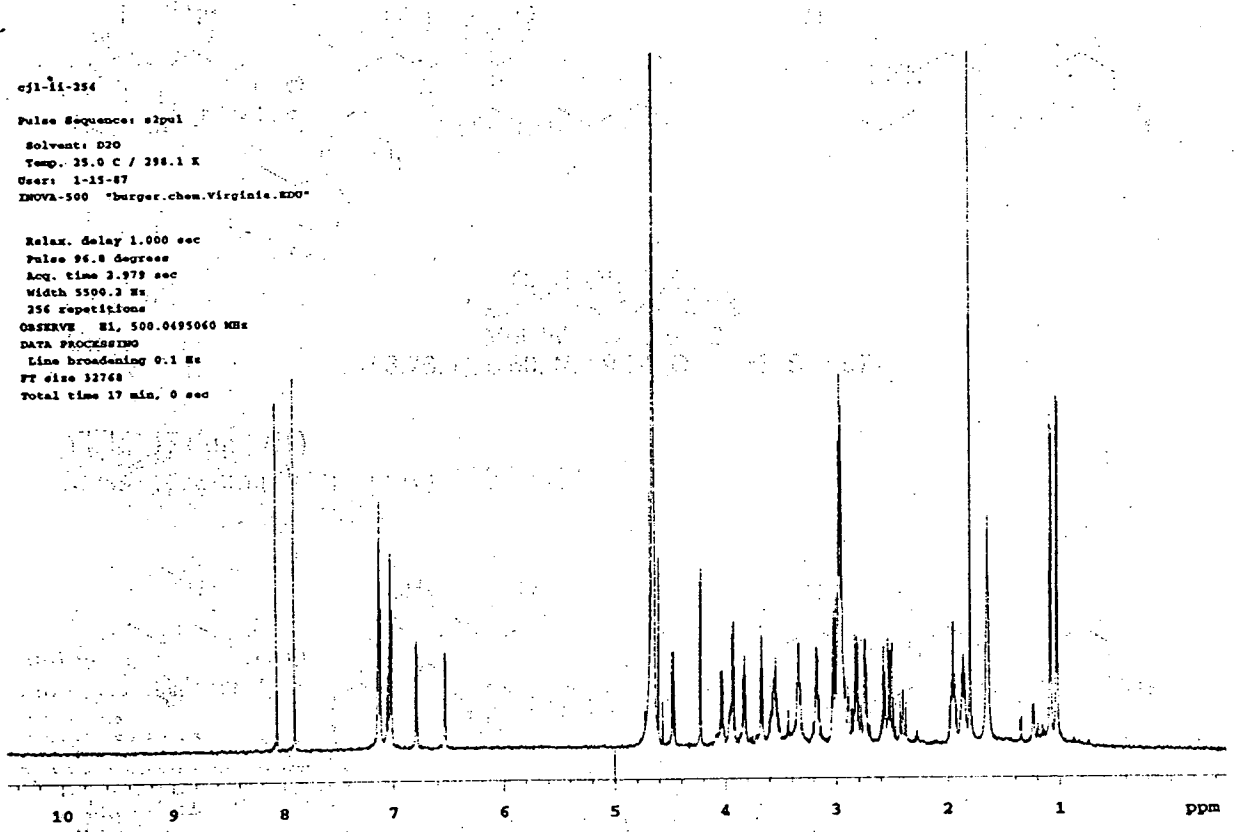


CJL-10
Cjl-ii-254

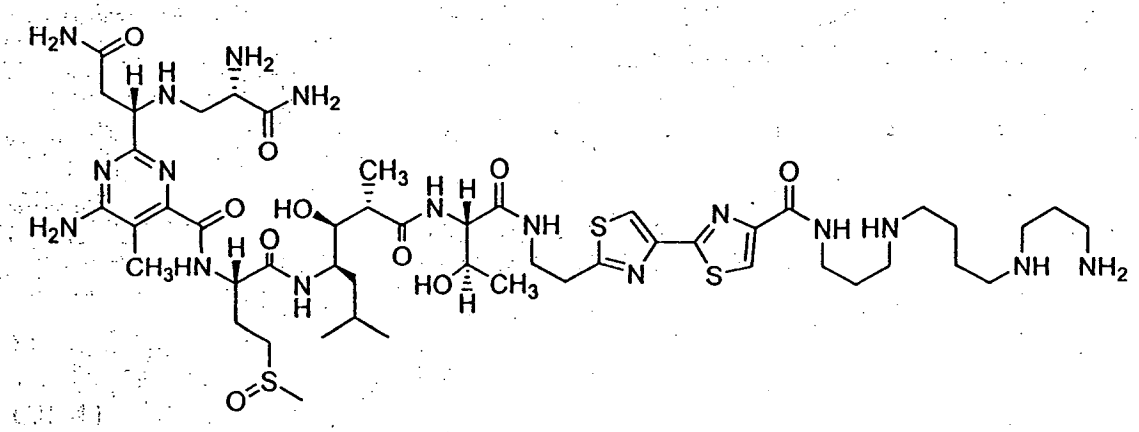


$C_{54}H_{79}N_{17}O_9S_3$
Exact Mass: 1205.5409
Mol. Wt.: 1206.5122
C, 53.76; H, 6.60; N, 19.74; O, 11.93; S, 7.97

Yield 434 μg (9%)
Mass spectrum (electrospray) 1206.7 $(M + H)^+$

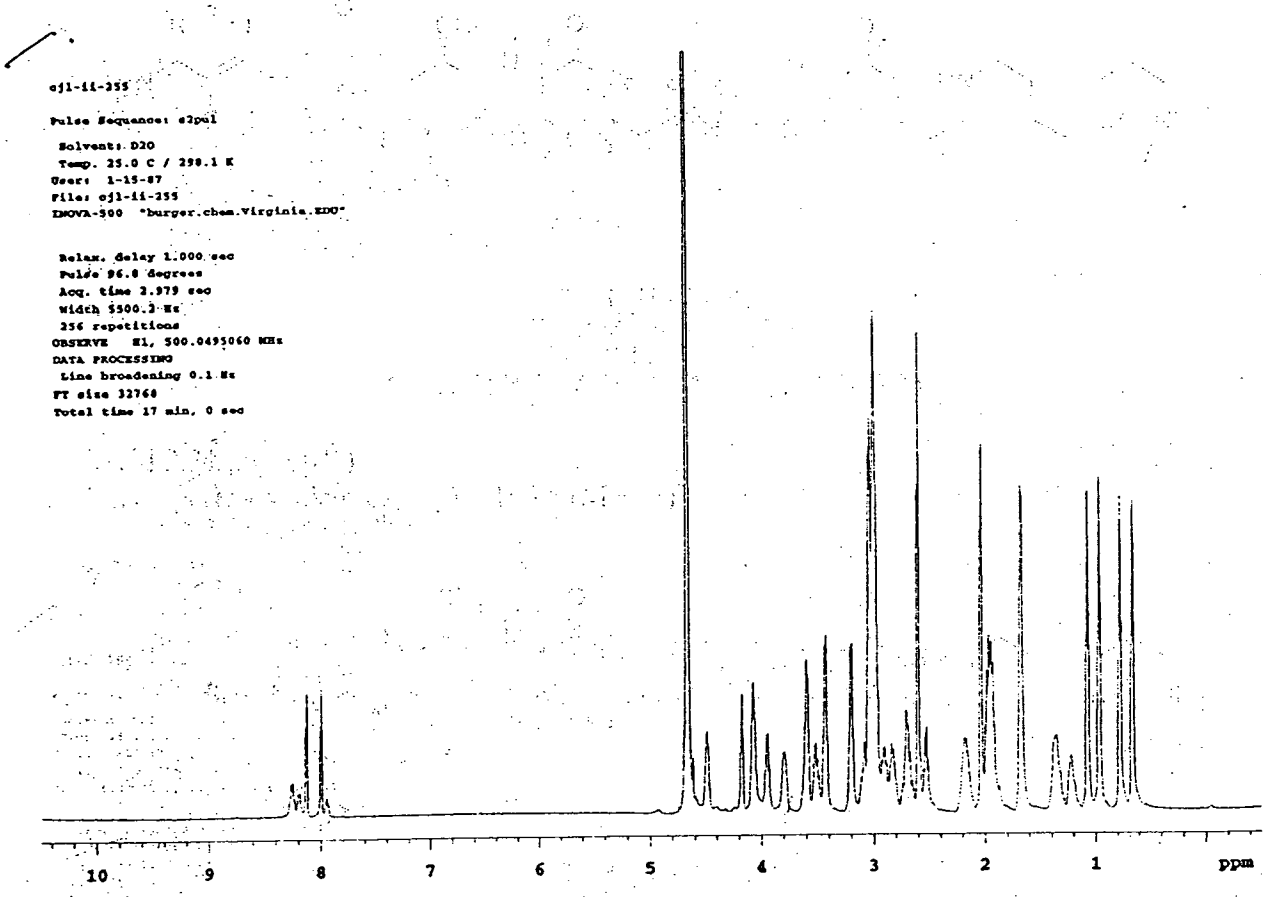


CJL-11
Cjl-ii-255

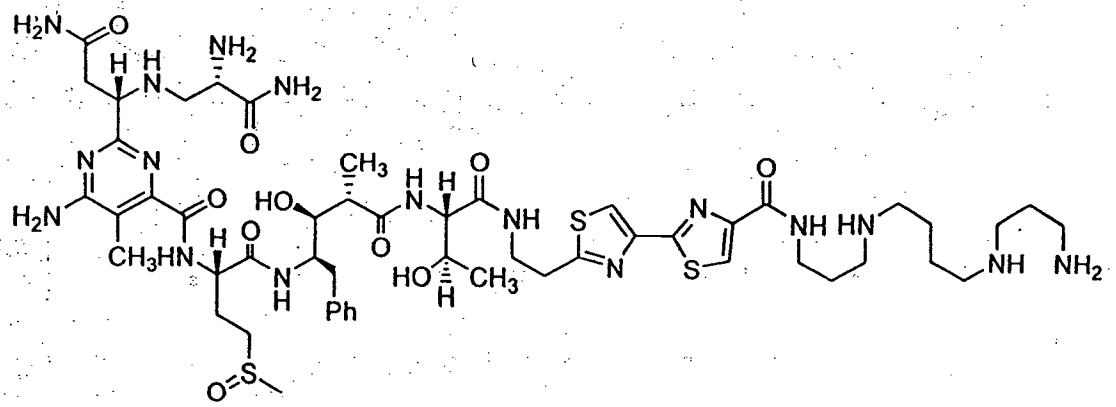


$C_{49}H_{83}N_{17}O_{10}S_3$
Exact Mass: 1165.57
Mol. Wt.: 1166.49
C, 50.45; H, 7.17; N, 20.41; O, 13.72; S, 8.25

Yield 2.31 mg (60%)
Mass spectrum (electrospray) 1166.5 $(M + H)^+$



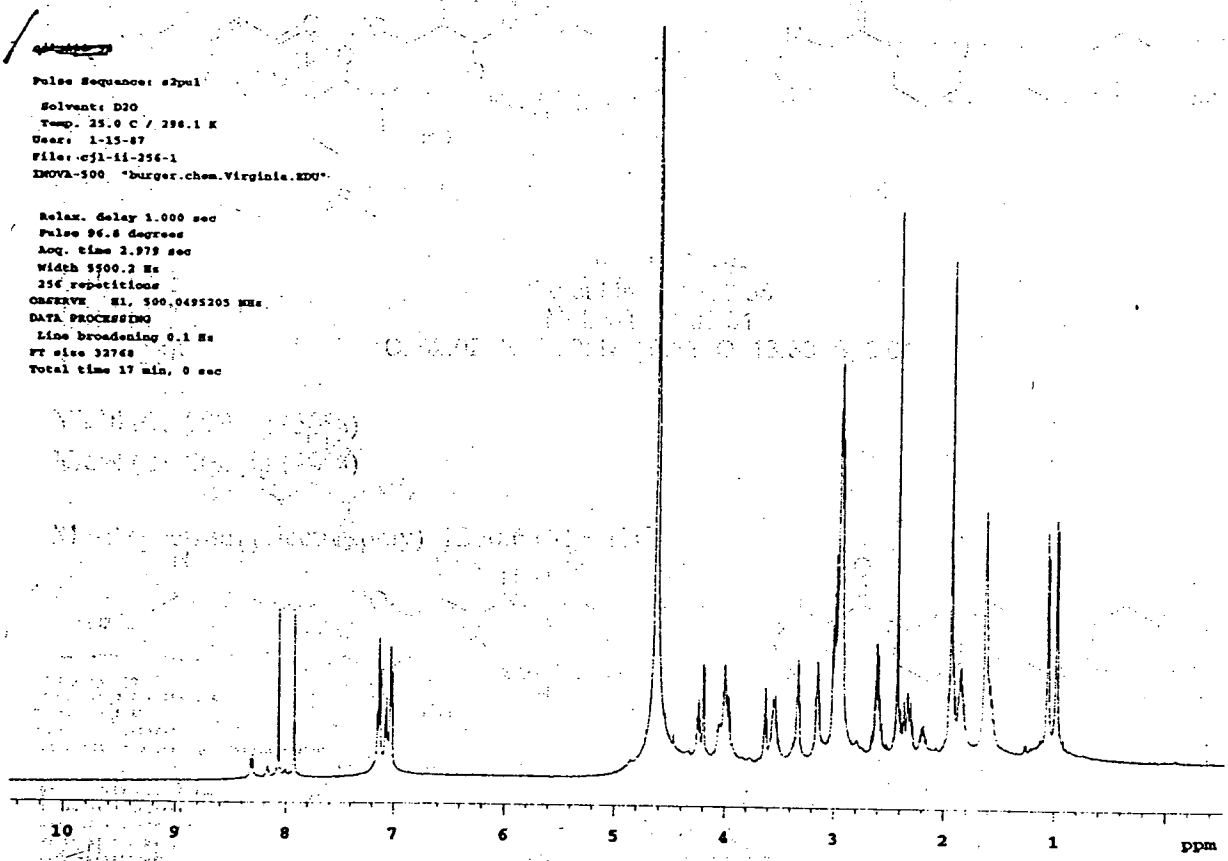
CJL-12-1
CJL-12-2
Cjl-ii-256



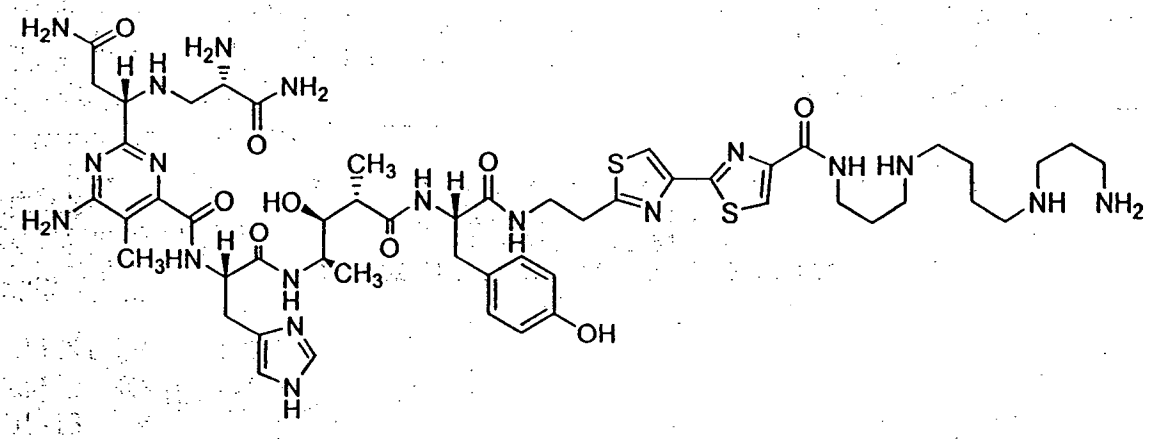
$C_{52}H_{81}N_{17}O_{10}S_3$
Exact Mass: 1199.55
Mol. Wt.: 1200.51
C, 52.02; H, 6.80; N, 19.83; O, 13.33; S, 8.01

Yield (1) 990 µg (50%)
Yield (2) 802 µg (40%)

Mass spectrum (electrospray) 1200.6 $(M + H)^+$



CJL-13
CJL-ii-286



$C_{52}H_{77}N_{19}O_9S_2$
Exact Mass: 1175.56
Mol. Wt.: 1176.42
C, 53.09; H, 6.60; N, 22.62; O, 12.24; S, 5.45

Yield 3.76 mg (82%)
Mass spectrum (electrospray) 1176.7 $(M + H)^+$