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Synthesis, Biological Evaluation and DNA Binding Properties of Novel Bleomycin Analogues

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Abstract—A series of bleomycin analogues was prepared with a facile synthetic method. All the compounds were shown to display significant antitumor activity against HeLa and BGC-823 cell lines in vitro. The binding properties with CT-DNA and cleavage efficiency to pBR322 DNA were investigated, the results indicate that there is a positive relationship between DNA cleavage efficiency and the binding affinity to DNA, and the antitumor activity of the bleomycin analogues is enhanced as the hydrophobicity of the C-terminus substituent side chain increased.

The bleomycins (BLMs) are a family of structurally related glycopeptide-derived antitumor antibiotics originally isolated from *Streptomyces verticillus* by Umezawa and co-workers.[1] A number of BLMs, such as BLM A_2, BLM A_5 and Pepleomycin are now used routinely as antitumor agents for the treatment of several types of neoplasms, notably squamous cell carcinomas and malignant lymphomas.[2,3] These antitumor agents are believed to mediate their therapeutic effects by binding to and oxidatively cleaving DNA and possibly RNA, in the presence of a metal ion cofactor.[4–6] The structure of BLMs (Fig. 1) is commonly divided into four functional domains: the N-terminus domain, the C-terminus domain, the linker domain and the carbohydrate domain.[7] Naturally occurring BLMs differ only in the nature of the C-terminus substituent and are expected to be positively charged at physiological pH.[3,8]

As a consequence of their clinical utility, as well as their mechanism of action and the interesting structures, BLMs have been the focus of considerable attention.[9–12] It was believed that the C-terminus domain of BLMs is related to their renal and lung toxicity and antitumor activity.[13,14] Our prior work showed that the terminal amine moieties of BLMs contribute their binding affinity with DNA.[15] Hitherto, a number of interesting analogues of BLMs altered at the C-terminus have been prepared.[3,16] Some of the reported BLMs obtained by means of: (a) fermentation method in media containing a special amine, (b) semi-synthetic method starting from bleomycinic acid, have diminished pulmonary toxicity relative to blenoxane.[17] BLM A_5 (Pingyangmycin) is a naturally occurring BLMs antibiotics as a major component separated from fermentation solution of *S. Verticillus var, Pingyangensis n. sp*, in which the C-terminus is spermidine. However, like other BLMs, BLM A_5 has a number of drawbacks, notably pulmonary fibrosis side effect. [18,19] Up to now, the C-terminal amine modification of BLM A_5 by carboxylic acid has not been reported. In order to develop new BLMs with more effective and less side effects, we synthesized a series of novel derivatives of BLM A_5 with a facile synthetic method. In addition, the antitumor activity, DNA binding properties and cleavage efficiency to pBR322 DNA in the presence of Fe(II) were also studied.

Chemistry

The method used to prepare the BLM A_5 derivatives **5a–f** is shown in Scheme 1. In order to protect the primary amino groups in N-terminus domain of the BLM A_5, the Cu(II)·BLM A_5 **3** was prepared in 95% yield by coordination reaction of compound **2** with $CuSO_4$ in

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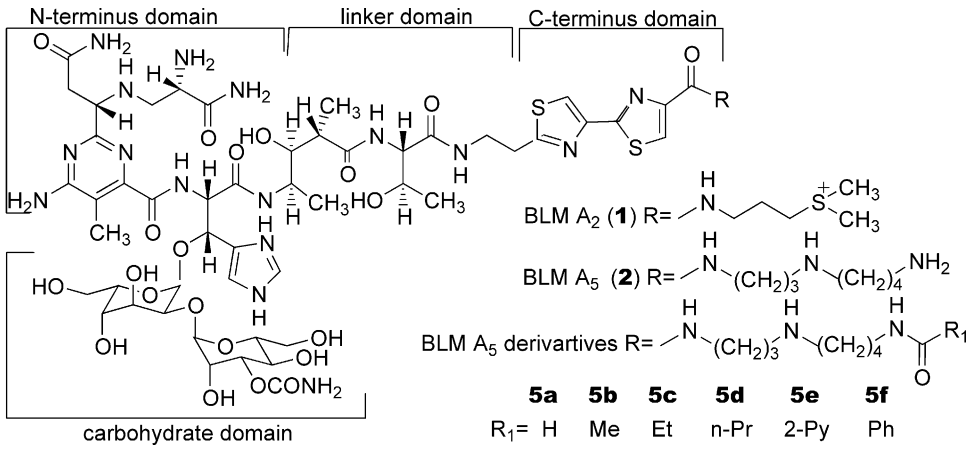
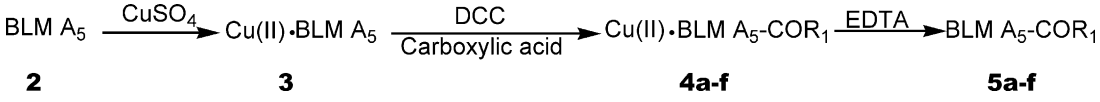


Figure 1. Structure of bleomycin A_2 (**1**), A_5 (**2**) and bleomycin A_5 derivatives.



Scheme 1.

aqueous solution as a blue powder. Compound **3** was an ideal intermediate for further synthetic manipulation, because there were only two free primary amino groups unemployed as metal ligands, one being the terminal amine in C-terminus domain, and the other the amino group in pyrimidine. [20] The coupling of **3** was coupled with large excess corresponding aliphatic and aromatic acid at −5 °C for 12 h in the presence of *N*,*N*′-dicyclohexyl carbodiimide (DCC) in MeOH, providing Cu(II)·BLM A_5-COR_1 **4a–f** as major product in 70–85% yield. Copper complexes **4a–f** were treated with 15% EDTA solution to remove the copper and then desalted on a HP-20 column to afford BLM A_5-COR_1 **5a–f** as colorless powder in 85–90% yield.[21]

Compared with compound **2**, the protons of the terminal methylene within the C-terminal spermidine substituent of compounds **5a–f** shifted downfield (from 2.849 to 3.117–3.406 ppm) in ^{1}H NMR, while its carbons shifted upfield (from 40.852 to 37.846–39.736 ppm) in ^{13}C NMR, indicating that the acylation occurred at the primary amine within the C-terminal spermidine substituent. In addition, the structures of compounds **5a–f** were confirmed by FAB-MS and elemental analysis. [22]

Biological Activity

The synthesized derivatives **5a–f** of BLM A_5 together with BLM A_5 **2** and BLM A_2 **1** were tested in vitro for their antitumor activity against HeLa and BGC-823 cell lines by using the tetrazolium salt (MTT) assay.[23] The 50% inhibition concentrations (IC_{50}) of the compounds are reported in Table 1. Compared with the positive control drugs **1** and **2**, compounds **5a–f** showed significant antitumor activity in the range of 2.62–31.0 μM (IC_{50}). Little difference was observed in the effects of BLM A_5 **2** and its **5a–f** derivatives against HeLa cell line, but noticeable difference in their effects against BGC-823 cell line. Furthermore, compounds **5a–f** exhibited some change in potency in comparison to BLM A_5 **2** in BGC-823 cell line; the antitumor activity was enhanced as the hydrophobicity of the C-terminus substituent side chain increased. Compound **5d** was the most potent agent among the tested compounds. A possible reason for this interesting observation is that the hydrophobic property of the C-terminus substituent of BLM derivatives can affect the binding properties to nuclear DNA, and permeability to cell membrane, thus resulting in various antitumor activities.

Table 1. In vitro antitumor activities of compounds **5a–f**, **1** and **2** against the HeLa and BGC-823 cell lines

Compd	Cell lines IC_{50} (μM)	
	HeLa	BGC-823
5a	12.2	31.0
5b	15.3	22.1
5c	14.4	6.46
5d	13.8	2.62
5e	14.8	10.8
5f	11.7	7.46
2	15.1	8.19
1	16.2	10.1

DNA Cleavage

It is generally believed that the antitumor activities of BLMs are the consequence of a direct damage to nuclear DNA. To further elucidate this issue, the abilities of compounds **5a–f** together with BLM A_5 **2** to cleave duplex DNA were tested through examination of single-strand and double-strand cleavage of supercoiled pBR322 DNA (Form I) to produce relaxed (Form II) DNA in the presence of Fe(II).[11,24] The results of the densitometric analysis of the gel picture (Fig. 2) are shown in Figure 3. Comparison of the

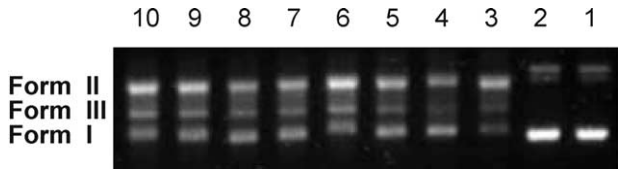


Figure 2. Agarose gel illustrating the cleavage reaction of supercoiled pBR322 by Fe (II)-compound. Lane 1, DNA alone; lane 2, 4.0 μM Fe (II); lane 3, 2.0 μM **1**, 4.0 μM Fe (II); lane 4, 2.0 μM **2**, 4.0 μM Fe (II); lane 5, 2.0 μM **5a**, 4.0 μM Fe (II); lane 6, 2.0 μM **5b**, 4.0 μM Fe (II); lane 7, 2.0 μM **5c**, 4.0 μM Fe (II); lane 8, 2.0 μM **5d**, 4.0 μM Fe (II); lane 9, 2.0 μM **5e**, 4.0 μM Fe (II); lane 10, 2.0 μM **5f**, 4.0 μM Fe (II).

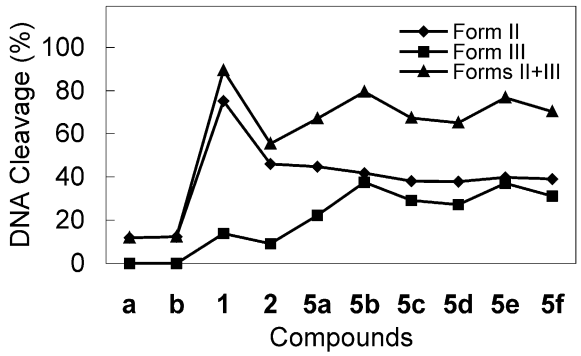


Figure 3. Cleavage efficiency of supercoiled pBR322 plasmid DNA to Form II and Form III DNA in the presence of Fe (II)-BLMs. The cleavage efficiency was calculated by the following equation: Form II = (Form II)$_s$/[(Form I)$_{s+c}$(Form II)$_{s+2}$ ×(Form III)$_s$]×100-(Form II)$_c$/[(Form I)$_{c+c}$(Form II)$_c$]×100; Form III = 2×(Form III)$_s$/[(Form I)$_{s+c}$(Form II)$_{s+2}$×(Form III)$_s$]×100; Forms II + III = Form II + Form III. The subscripts 's' and 'c' refer to the sample and controls, respectively.

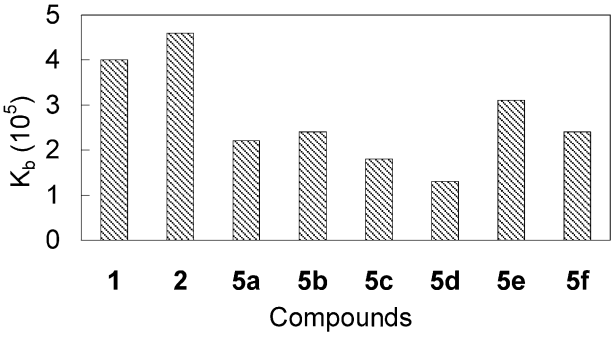


Figure 4. The apparent binding constant K_b for compounds **5a–f**, **1** and **2** with CT-DNA.

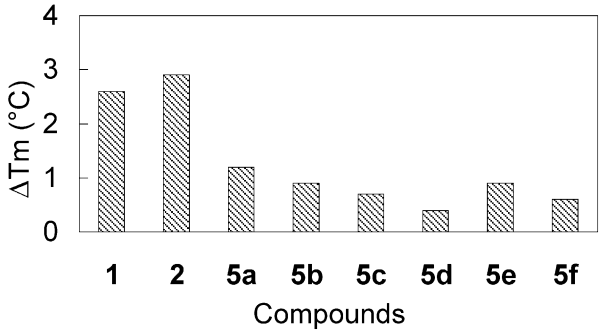


Figure 5. The alteration of the melting temperature ΔT_m (°C) for CT-DNA bind to compounds **5a–f**, **1** and **2**.

extent of cleavage produced by compounds **5a–f** and BLM A_5 **2** indicated that compounds **5a–f** cleft DNA with 1.1–1.2-fold greater efficiency than BLM A_5 **2**, and **5b** was the strongest. It was interesting to note that as the substituted chain from one carbon in **5b** to three carbons in **5d**, there was a decrease in the DNA cleavage efficiency and with the pyridyl and benzyl substituted chain, the cleavage efficiency was decreased too as in the case of **5e** to **5f**. The fact that the trend of the cleavage efficiency in **5b** to **5f** is opposite to that of the antitumor activities indicates that the DNA cleavage is necessary, but not a sufficient condition for antitumor activity. The permeability to cell membrane or the uptake by cells of BLMs may play a key role in its biological activity.

DNA Binding Studies

As a DNA cleavage antitumor agent, BLMs can bind to nuclear DNA by C-terminus domain. In order to compare the effects on DNA binding affinity resulting from the C-terminus alteration, the DNA binding properties which the Co(II) complex of BLM A_5 derivatives **5a–f** together with **2** bound to calf thymus DNA (CT-DNA), including apparent binding constant (K_b) and thermal denaturatation alteration (ΔTm) were determined with the methods reported.[25,26] The alteration of the melting temperature (ΔTm) of CT-DNA and the apparent binding constant (K_b) are shown in Figures 4 and 5. It is observed that compounds **5a–f** and **2** bind to CT-DNA in a similar binding mode and with a slightly different binding affinity. The binding strength of compounds **5a–f** was less strong than BLM A_5 **2**, and at the same time, with an increase in the hydrophobicity of the substituted chain from **5b** to **5d** and **5e** to **5f,** the DNA binding affinity was decreased dramatically. This result can be explained that the hydrophobic group connected to the C-terminus decreased the positivity of the positive charged C-terminus domain, and then decreased the electrostatic binding affinity to DNA, and the long flexible side chain decreased the intercalation stability of the bithiazol domain. The trend is in agreement with that of the DNA cleavage efficiency affected by the substituted chain. The reason why DNA cleavage efficiency of compound **2** was weaker and its DNA binding strength was stronger than that of compounds **5a–f**, may be that compound **2** was positively charged at the C-terminal amine under the tested condition (pH 7.0), and the positive charge decreased its cleavage efficiency.

Conclusion

In conclusion, the procedure described herein provided an easy and efficient method with which to prepare BLM analogues from BLM A_5. The synthesized compounds **5a–f** exhibited significant antitumor activity and the hydrophobic and flexible properties of the C-terminal side chain displayed an important effect on its biological activity and DNA binding affinity. From studying the effects of C-terminus alteration on biological activities, pBR322 cleavage efficiency and binding

properties to CT-DNA of BLMs, we can conclude that there is a positive relationship between DNA cleavage efficiency and the binding affinity to DNA. Not only the DNA binding and DNA cleavage, but other factors, such as permeability to cell membrane or uptake by cell are important for their antitumor activity.

Acknowledgements

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21. General procedure: **Cu (II)·BLM A_5** (**3**) An aqueous solution containing 90 mg (63 μmol) of **2** was treated with 112 mg (70 μmol) $CuSO_4$ and the combined solution was maintained at 0–4 °C for 30 min, the resulting solution was purified on a C-18 column and the product was then lyophilized to obtain **3** as a blue powder (95 mg, 95%). **Cu(II)·BLM A_5-COR_1** (**4a–f**) DCC (290 μmol) was added to 15 mL methanol solution containing carboxylic acid (290 μmol), and the mixture was stirred at 0 °C for 30 min. A solution of **3** (58 μmol) in 5mL methanol was then added to the reaction mixture, and the reaction mixture was stirred at −5 °C for 12 h. After removed the precipitated DCU by filtration, the filtrate was concentrated under diminished pressure, and the crude product was purified by column chromatography (silica gel G) using MeOH/10% NH_4AC/10% NH_3 (100:10:1) as eluent. The product mixture was evaporated to remove the solvent at diminished pressure, and lyophilized to give **4a–f** resulting in 70–85% yield as a blue powder. **BLM A_5-COR_1** (**5a–f**) Demetallation of **4a–f** was accomplished by stirring with 15% EDTA (10 mL) at 30 °C for 1 h. The reaction mixture was passed through an ion exchange resin (HP-20) column, washed with water successively, and then eluted with acidic methanol MeOH/2 mM HCl (4:1). The eluate was evaporated to remove the solvent and lyophilized to afford **5a–f** in 90–95% yield as a white powder.
22. **5a**: mp 163–165 °C; ^{1}H NMR (500 MHz, D_2O) δ ^{13}C NMR (500 MHz, D_2O) δ 1.083 (d, 3H), 1.096 (d, 3H), 1.112 (d, 3H), 1.634 (m, 4H), 1.904 (s, 3H), 2.461 (m, 1H), 2.619 (m, 2H), 2.688 (m, 1H), 2.983 (m, 2H), 3.090 (m, 2H), 3.119 (m, 2H), 3.224 (C-terminal methylene, m, 3H), 3.412 (br, 2H), 3.560 (m, 2H), 3.605 (m, 2H), 3.707 (m, 1H), 3.785 (m, 2H), 3.837 (br, 2H), 3.864 (br, 1H), 3.931 (m, 1H), 3.987 (m, 2H), 4.047 (m, 3H), 4.092 (br, 1H), 4.210 (br, 1H), 4.653 (br, 1H), 4.998 (s, 1H), 5.070 (d, 1H), 5.279 (m, 2H), 7.290 (s, 1H), 7.864 (d, 1H), 7.978 (s, 1H), 8.049 (d, 1H), 8.156 (s, 1H); ^{13}C NMR (500 MHz, D_2O) δ 11.492, 12.681, 15.221, 19.541, 23.612, 24.117, 26.852, 32.617, 37.256, 37.846 (C-terminal methylene), 40.340, 40.791, 43.331, 46.229, 47.581, 47.985, 48.203, 53.074, 57.448, 59.810, 60.238, 61.551, 61.745, 65.342, 67.665, 67.786, 68.403, 68.955, 69.724, 70.804, 73.508, 74.199, 74.976, 98.036, 98.735, 112.961, 118.299, 119.674, 125.485, 135.197, 137.489, 147.613, 149.632, 152.671, 158.568, 163.646, 165.280, 165.941, 166.205, 168.326, 169.569, 171.247, 171.698, 172.623, 176.888, 178.154, 180.796; FAB-MS *m/z* 1469 ($M^+ + 1$). Anal. calcd for $C_{58}H_{89}N_{19}O_{22}S_2 \cdot 8H_2O$: C, 43.20; N, 16.50; H, 6.56. Found: C, 43.19; N, 16.33; H, 6.51%. **5b**: mp 168–170 °C; ^{1}H NMR (500 MHz, D_2O) δ 0.950 (d, 3H), 0.963 (d, 3H), 1.409 (m, 2H), 1.541 (m, 2H), 1.804 (s, 3H), 1.858 (s, 3H), 1.890 (m, 2H), 2.307 (m, 1H), 2.498 (m, 2H), 2.594 (m, 1H), 2.916 (m, 2H), 2.947 (m, 2H), 3.020 (m, 3H), 3.035 (m, 2H), 3.058 (m, 1H), 3.394 (m, 2H), 3.350 (C-terminal methylene, m, 2H), 3.442 (m, 4H), 3.565 (br, 1H), 3.695 (m, 1H), 3.703 (br, 1H), 3.731 (m, 2H), 3.774 (m, 2H), 3.866 (m, 4H), 3.894 (br, 1H), 3.945 (br, 2H), 4.078 (d, 1H), 4.552 (br, 1H), 4.853 (s, 1H), 4.890 (d, 1H), 5.103 (s, 1H), 5.116 (d, 1H), 7.125 (s, 1H), 7.647 (s, 1H), 7.981 (s, 1H), 7.789 (s, 1H); ^{13}C NMR (500 MHz, D_2O) δ 11.518, 12.580, 15.419, 19.603, 22.558, 23.737, 26.078, 26.177, 26.509, 32.652, 37.051, 37.383 (C-terminal methylene), 39.309, 39.741, 41.069, 43.277, 45.767, 47.959, 48.141, 50.532, 54.234, 57.787, 59.813, 60.726, 60.892, 61.572, 65.407, 67.715, 68.545, 68.993, 69.724, 71.002, 73.991, 74.190, 74.970, 98.196, 98.844, 112.806, 119.679, 125.705, 135.536, 137.592, 147.554, 149.396, 152.850, 158.627, 163.226, 164.006, 165.201, 166.264, 168.389, 169.833, 171.195, 172.639, 174.764, 176.823, 177.968, 178.201; FAB-MS *m/z* 1484 ($M^+ + 1$). Anal. calcd for $C_{59}H_{91}N_{19}O_{22}S_2 \cdot 7H_2O$: C, 44.03; N, 16.54; H, 6.64. Found: C, 43.95; N, 16.32; H, 6.58%. **5c**: mp 173–175 °C; ^{1}H NMR (500 MHz, D_2O) δ 1.054 (d, 3H), 1.076 (d, 3H), 1.129 (s, 3H), 1.141 (s, 3H), 1.561 (m, 2H), 1.690 (m, 2H), 1.992 (s, 3H), 2.027 (m, 2H), 2.203 (m, 2H), 2.553 (m, 1H), 2.655 (m, 2H), 3.003 (m, 2H), 3.063 (m, 2H), 3.122 (m, 2H), 3.180 (C-terminal methylene, m, 2H), 3.225 (br, 1H), 3.512 (m, 2H), 3.604 (m, 2H), 3.667 (br, 2H), 3.732 (m, 1H), 3.785 (m, 2H), 3.849 (d, 1H), 3.900 (m, 2H), 3.988 (m, 2H), 4.031 (br, 1H), 4.056 (br, 1H), 4.076 (d, 1H), 4.103 (d, 1H), 4.136 (br, 1H), 4.217 (d, 1H), 4.723 (br, 1H), 5.015 (s, 1H), 5.073 (d, 1H), 5.266 (d, 1H), 5.435 (d, 1H), 7.506 (s, 1H), 7.985 (s, 1H), 8.161 (s, 1H), 8.503 (s, 1H); ^{13}C NMR (500 MHz, D_2O) δ 10.317, 11.576, 13.099, 14.932, 19.532,

23.603, 26.198, 26.400, 29.850, 32.616, 36.998, 39.096 (C-terminal methylene), 39.749, 40.712, 43.463, 45.700, 47.534, 47.891, 48.342, 52.879, 57.028, 59.779, 60.369, 61.193, 61.550, 65.264, 67.704, 67.828, 68.465, 68.947, 69.740, 70.392, 72.677, 74.945, 98.223, 98.488, 112.597, 118.999, 119.637, 125.697, 131.726, 136.217, 147.561, 149.410, 153.093, 158.671, 163.302, 164.079, 165.089, 165.757, 168.244, 168.788, 171.243, 171.460, 172.579, 176.682, 178.080, 178.702; FAB-MS *m/z* 1497 ($M^+ + 1$). Anal. calcd for $C_{60}H_{93}N_{19}O_{22}S_2 \cdot 7H_2O$: C, 44.41; N, 16.40; H, 6.65. Found: C, 44.32; N, 16.31; H, 6.61%. **5d**: mp 180–182 °C; ^{1}H NMR (500 MHz, D_2O) δ 0.839 (d, 3H), 1.072 (d, 3H), 1.130 (s, 3H), 1.135 (s, 3H), 1.517 (m, 2H), 1.556 (m, 2H), 1.684 (m, 2H), 1.983 (s, 3H), 2.021 (m, 2H), 2.155 (m, 2H), 2.565 (m, 1H), 2.662 (m, 2H), 3.022 (br, 2H), 3.056 (m, 2H), 3.122 (m, 2H), 3.177 (C-terminal methylene, m, 2H), 3.190 (br, 1H), 3.492 (br, 2H), 3.563-3.587 (br, 3H), 3.742 (m, 1H), 3.785 (br, 2H), 3.843 (d, 1H), 3.896 (s, 1H), 3.918 (s, 1H), 3.998 (br, 2H), 4.023 (br, 1H), 4.053 (br, 2H), 4.131 (d, 1H), 4.214 (d, 1H), 4.719 (br, 1H), 5.010 (s, 1H), 5.068 (d, 1H), 5.259 (s, 1H), 5.458 (d, 1H), 7.539 (s, 1H), 7.966 (d, 1H), 8.136 (d, 1H), 8.615 (s, 1H); ^{13}C NMR (500 MHz, D_2O) δ 11.623, 13.156, 13.408, 14.942, 19.550, 19.702, 23.654, 26.225, 26.416, 32.633, 37.013, 38.386 (C-terminal methylene), 39.111, 39.751, 40.591, 43.482, 45.694, 47.541, 47.892, 48.349, 52.820, 56.963, 59.762, 60.380, 61.250, 61.563, 65.263, 67.727, 68.582, 68.948, 69.757, 70.321, 72.503, 74.212, 74.967, 98.152, 98.579, 112.594, 119.124, 119.651, 125.678, 131.102, 135.985, 147.535, 149.381, 153.127, 158.689, 163.243, 163.998, 165.059, 165.532, 168.194, 198.652, 171.208, 171.391, 172.566, 176.579, 177.731, 178.074; FAB-MS *m/z* 1511 ($M^+ + 1$). Anal. calcd for $C_{61}H_{95}N_{19}O_{22}S_2 \cdot 4H_2O$: C, 46.29; N, 16.81; H, 6.56. Found: C, 46.35; N, 16.72; H, 6.49%. **5e**: mp 192–194 °C; ^{1}H NMR (500 MHz, D_2O) δ 1.082 (d, 3H), 1.131 (d, 3H), 1.126 (s, 3H), 1.690 (m, 2H), 1.764 (m, 2H), 1.987 (s, 3H), 2.037 (m, 2H), 2.547 (m, 1H), 2.648 (m, 2H), 2.993 (m, 2H), 3.100 (m, 2H), 3.155 (m, 2H), 3.218 (m, 2H), 3.406 (C-terminal methylene, m, 2H), 3.521 (m, 2H), 3.588 (m, 2H), 3.663 (br, 2H), 3.729 (m, 1H), 3.788 (br, 2H), 3.843 (br, 1H), 3.908 (m, 3H), 3.984 (m, 2H), 4.029 (br, 1H), 4.056 (br, 2H), 4.086 (br, 1H), 4.131 (br, 1H), 4.217 (d, 1H), 4.712 (br, 1H), 5.012 (s, 1H), 5.072 (d, 1H), 5.262 (d, 1H), 5.418 (d, 1H), 7.485 (s, 1H), 7.517 (s, 1H), 7.966 (d, 1H), 8.129 (d, 1H), 8.152 (br, 1H), 8.446 (s, 1H), 8.617 (br, 1H), 8.798 (br, 1H); ^{13}C NMR (500 MHz, D_2O) δ 11.577, 13.073, 14.972, 19.534, 23.624, 26.179, 26.370, 32.618, 36.974, 39.736 (C-terminal methylene), 39.820, 40.758, 43.459, 45.588, 47.541, 47.785, 48.326, 52.927, 57.069, 59.770, 60.358, 61.181, 61.555, 65.278, 67.712, 67.872, 68.414, 68.956, 69.749, 70.436, 72.755, 74.212, 74.960, 98.274, 98.457, 112.609, 118.949, 119.635, 125.022, 125.685, 130.881, 132.040, 136.313, 137.045, 147.565, 149.374, 151.739, 153.074, 158.666, 163.289, 164.090, 165.112, 165.845, 168.263, 168.858, 171.231, 171.498, 172.581, 176.747, 178.089; FAB-MS *m/z* 1546 ($M^+ + 1$). Anal. calcd for $C_{63}H_{92}N_{20}O_{22}S_2 \cdot 6H_2O$: C, 45.76; N, 16.94; H, 6.34. Found: C, 45.91; N, 17.00; H, 6.27%. **5f**: mp 180–182 °C; ^{1}H NMR (500 MHz, D_2O) δ 1.079-1.125 (m, 9H), 1.657 (m, 2H), 1.744 (m, 2H), 1.995 (s, 3H), 2.013 (m, 2H), 2.467 (m, 1H), 2.642 (m, 2H), 2.975 (m, 2H), 3.102 (m, 4H), 3.175 (br, 1H), 3.349 (C-terminal methylene, m, 2H), 3.417 (m, 1H), 3.487 (m, 2H), 3.540 (br, 4H), 3.706 (m, 1H), 3.779 (br, 1H), 3.807 (m, 4H), 3.817 (m, 2H), 3.893 (m, 1H), 3.976 (m, 1H), 4.023 (br, 1H), 4.040 (br, 2H), 4.078 (br, 1H), 4.215 (dr, 1H), 4.651 (br, 1H), 4.992 (s, 1H), 5.060 (d, 1H), 5.264 (d, 1H), 5.293 (d, 1H), 7.277 (s, 1H), 7.397 (br, 2H), 7.478 (s, 1H), 7.631 (br, 2H), 7.900 (br, 2H), 8.071 (d, 1H), 8.407 (s, 1H); ^{13}C NMR (500 MHz, D_2O) δ11.514, 12.710, 15.321, 19.563, 23.619, 26.276, 26.369, 32.585, 36.967, 39.718 (C-terminal methylene), 40.774, 43.292, 45.560, 47.549, 47.798, 48.171, 53.050, 57.448, 59.763, 60.230, 60.960, 61.550, 65.342, 67.688, 67.813, 68.372, 68.947, 69.724, 70.796, 73.407, 74.199, 74.961, 98.052, 98.721, 112.924, 118.347, 119.606, 125.604, 127.562, 129.380, 132.721, 134.057, 134.974, 137.398, 147.483, 149.301, 152.658, 158.563, 163.131, 163.955, 165.245, 165.913, 168.290, 169.534, 171.072, 171.258, 171.631, 172.579, 176.853, 178.142; FAB-MS *m/z* 1545 ($M^+ + 1$). Anal. calcd for $C_{64}H_{93}N_{19}O_{22}S_2 \cdot 6H_2O$: C, 46.51; N, 16.10; H, 6.40. Found: C, 46.49; N, 16.03; H, 6.46%. **2**: mp 168–170 °C; ^{1}H NMR (500 MHz, D_2O) δ 0.956 (d, 3H), 0.985 (d, 3H), 1.003 (s, 3H), 1.640 (m, 4H), 1.877 (s, 3H), 1.906 (m, 2H), 2.363 (m, 1H), 2.530 (m, 2H), 2.849 (C-terminal methylene, m, 2H), 2.904 (m, 2H), 2.974 (m, 2H), 3.010 (m, 2H), 3.099 (m, 1H), 3.403 (m, 2H), 3.496 (m, 2H), 3.587 (m, 2H), 3.649 (br, 1H), 3.782 (m, br, 4H), 3.858 (m, 1H), 3.910 (m, 2H), 3.936 (br, 2H), 3.946 (br, 1H), 3.958 (br,1H), 3.974 (br, 1H), 4.080 (d, 1H), 4.543 (m, 1H), 4.872 (s, 1H), 4.937 (d, 1H), 5.139 (d, 1H), 5.191 (d, 1H), 7.214 (s, 1H), 7.876 (d, 1H), 8.060 (d, 1H), 8.301 (s, 1H); ^{13}C NMR (500 MHz, D_2O) δ 11.560, 12.850, 15.196, 19.579, 23.526, 24.644, 26.509, 32.663, 37.029, 39.531, 39.780, 40.852 (C-terminal methylene), 43.400, 45.918, 47.581, 47.720, 48.295, 53.066, 57.401, 59.872, 60.338, 61.053, 61.597, 65.373, 67.704, 67.999, 68.295, 68.994, 69.771, 70.750, 73.345, 74.246, 75.023, 98.192, 98.643, 112.893, 118.533, 119.730, 125.775, 134.368, 137.212, 147.623, 149.488, 152.844, 158.640, 163.427, 164.204, 165.276, 165.975, 168.384, 169.440, 171.352, 171.631, 172.657, 176.899, 178.189; FAB-MS *m/z* 1441 ($M^+ + 1$).

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24. μL reaction mixture contained 400 ng of plasmid DNA in TEA buffer (pH 7.0), 2 μM conresponding compounds, 4 μM $(NH_3)_2Fe(SO_4)_2$ was incubation at 25 °C. After 30 min, the reaction mixtures were analyzed on a 0.8% agarose gel and visualized by ethidium bromide stain.
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