Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Development of Novel Bone Targeting Peptide-Drug Conjugate of 13-aminomethyl-15-thiomatrine for Osteoporosis Therapy

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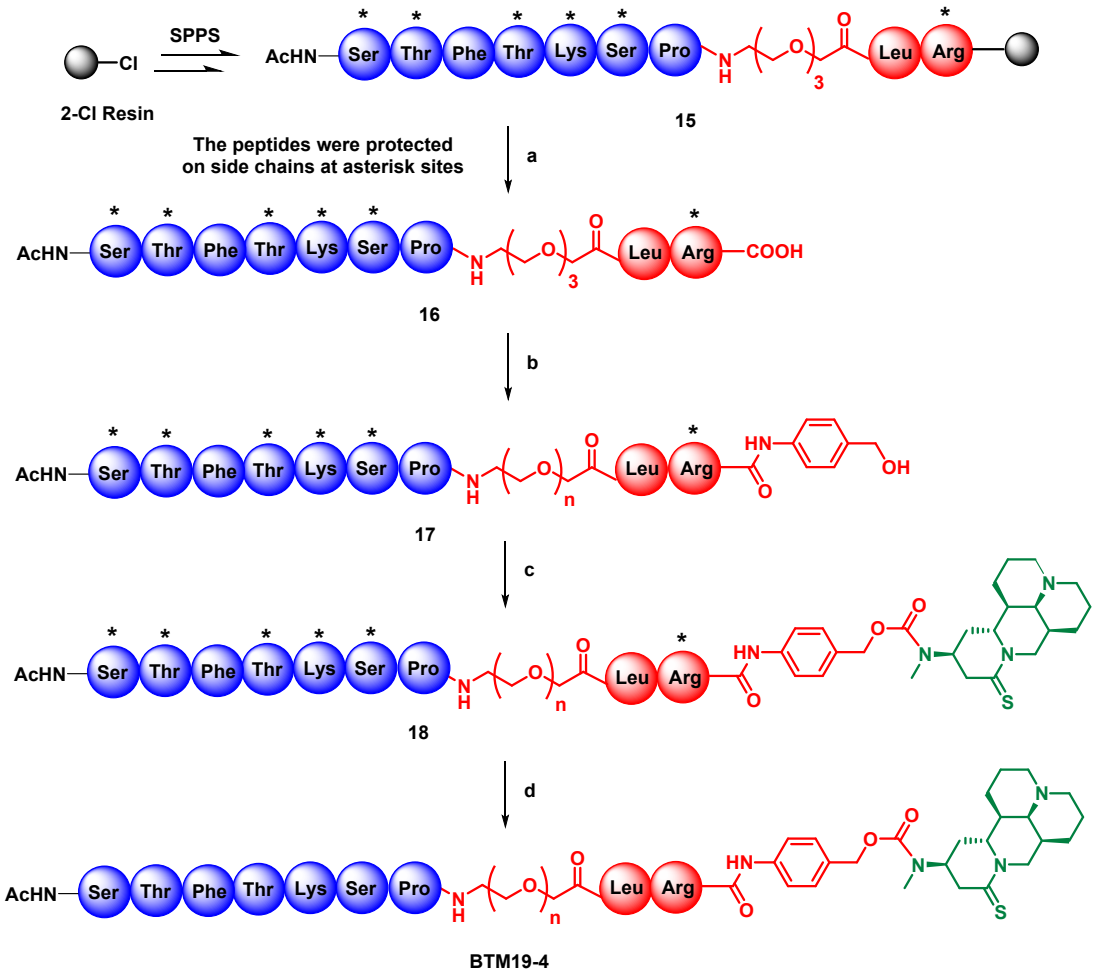

Scheme S1. The synthesis route of **BTM19-4**. Reagents and conditions: a) TFE/DCM (1:3, *v/v*), rt, 4 h, 87%; b) 4-aminophenyl methanol, HOBt, DIC, DMF, rt, 2 h, 74%; c) i) Triphosgene, activated carbon, THF, rt, 12 h; ii) **M19**, Et_3N, DMF, rt, 12 h, 76% in two steps; d) TFA/water/EDT/TIPs (95:2:2:1, *v/v/v/v*), rt, 2 h, 59%.

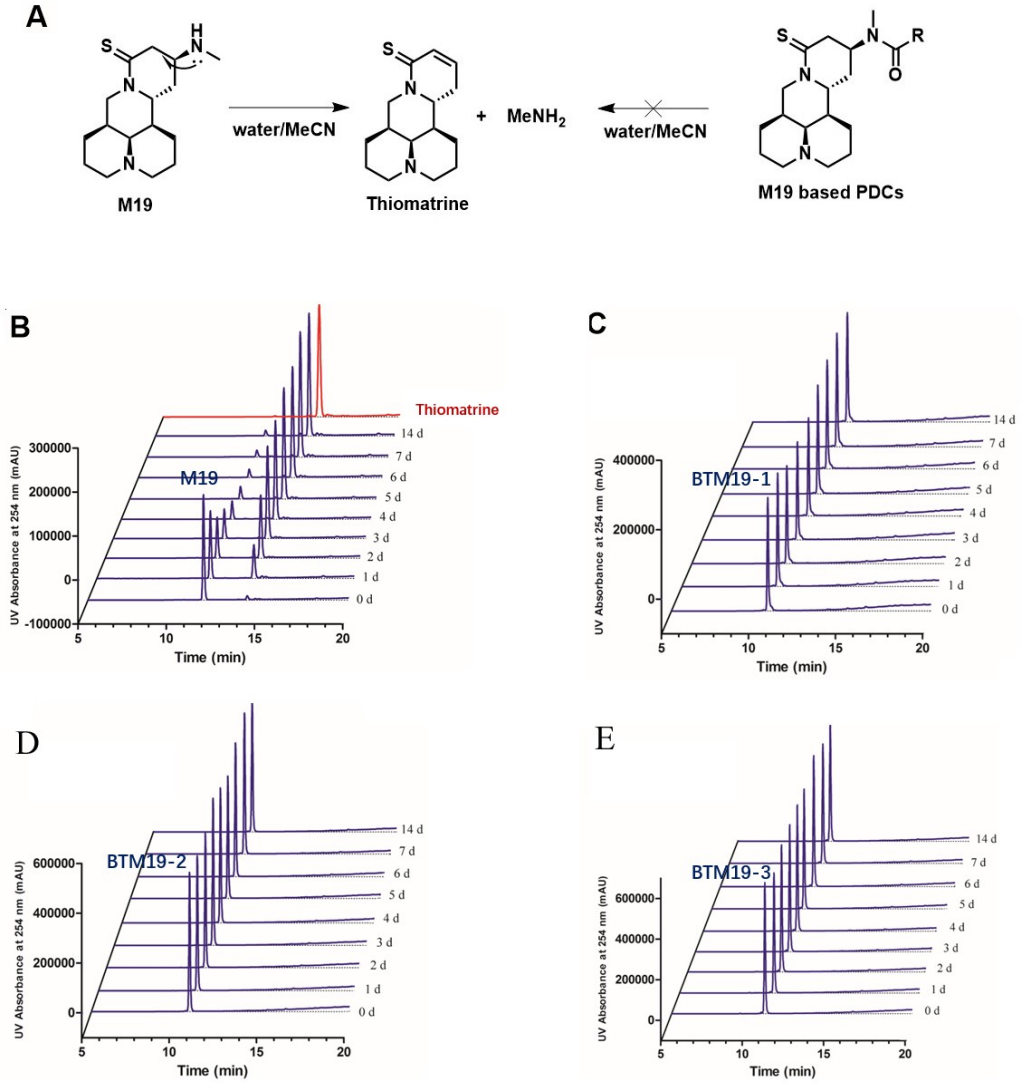

Figure S1. (A) Illustration of the chemical stability study of M19 vs. PDCs in water/MeCN solution at room temperature; Chromatograms of chemical stability study of **M19 (B)**, **BTM19-1** (**C**), **BTM19-2** (**D**), **BTM19-3** (**E**).

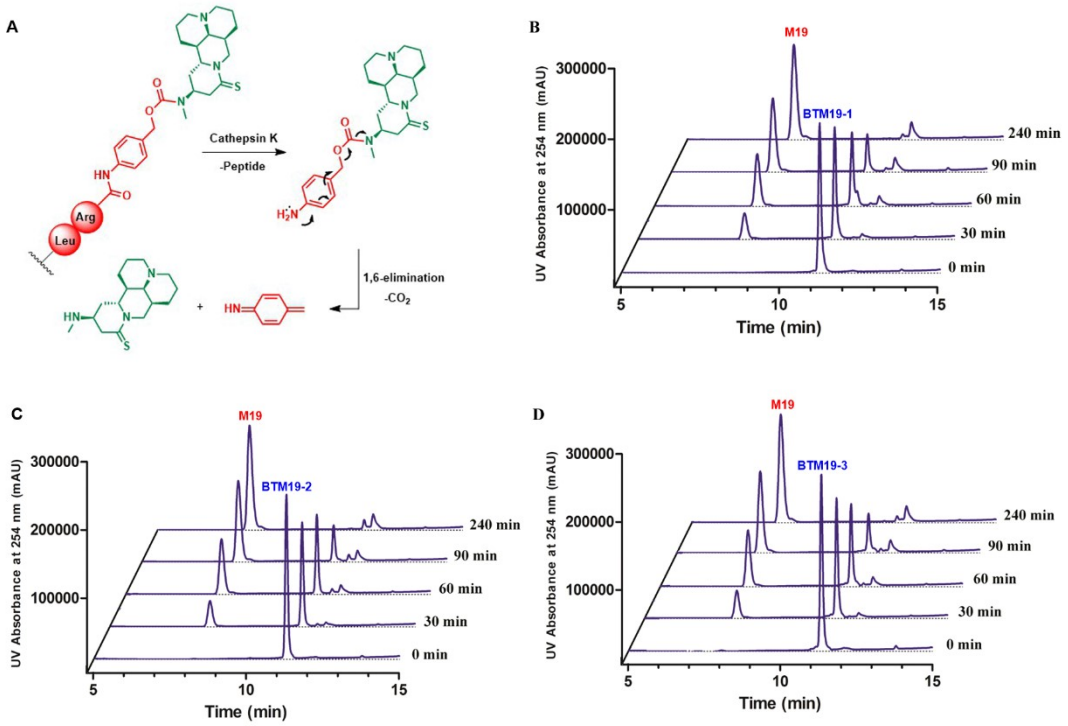

Figure S2. (A) Mechanism of the drug release from self-immolative spacer PABC and Cathepsin K substrate; Chromatograms of drug release study of **BTM19-1** (B), **BTM19-2** (**C**) and **BTM19-3** (**D**) at pH 5.5 (37 °C) with cathepsin K.

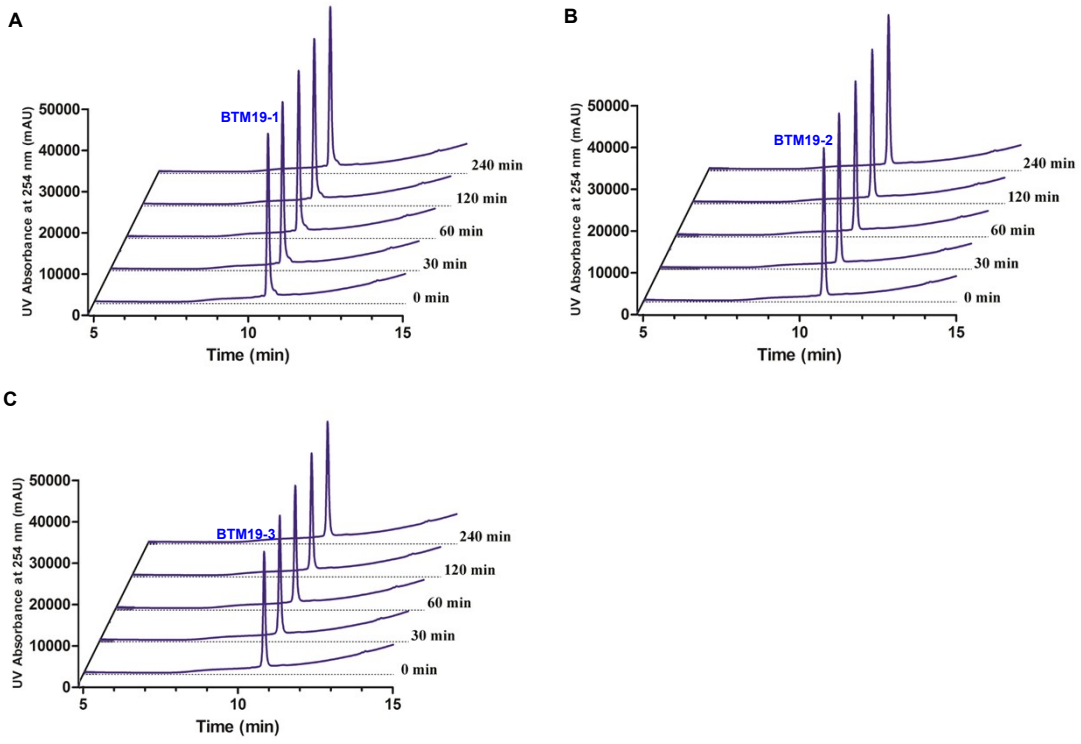

Figure S3. Chromatograms of proteolytic stability study of **BTM19-1** (**A**), **BTM19-2** (**B**), **BTM19-3** (**C**) under a-chymotrypsin treatment.

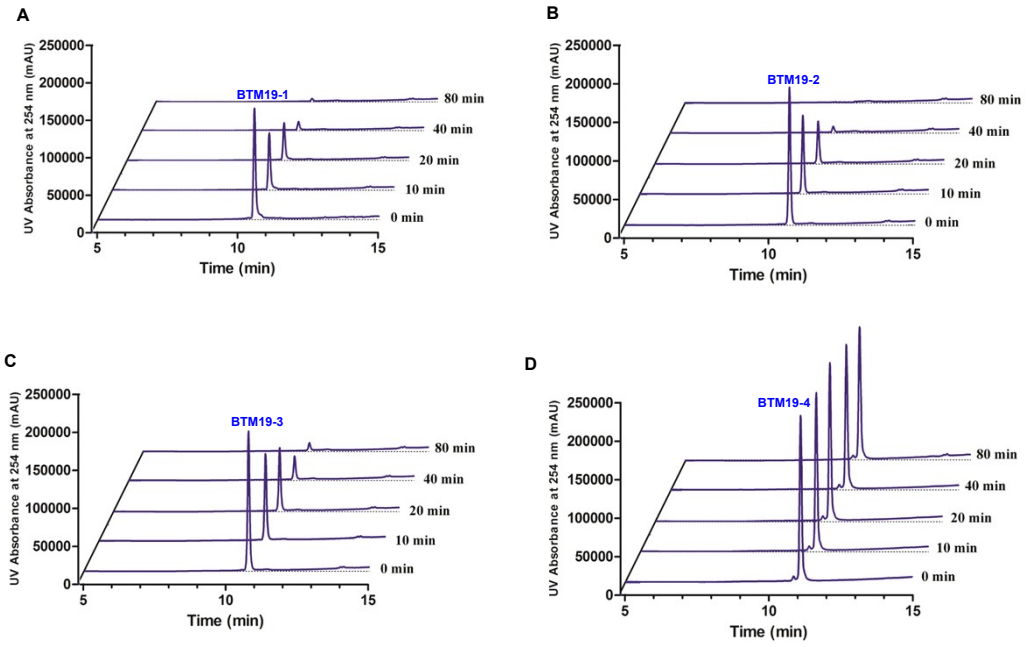

Figure S4. Chromatograms of binding study of **BTM19-1** (**A**), **BTM19-2** (**B**), **BTM19-3** (**C**) and **BTM19-4** (**D**) to hydroxyapatite at pH=5.5 and 37 °C.

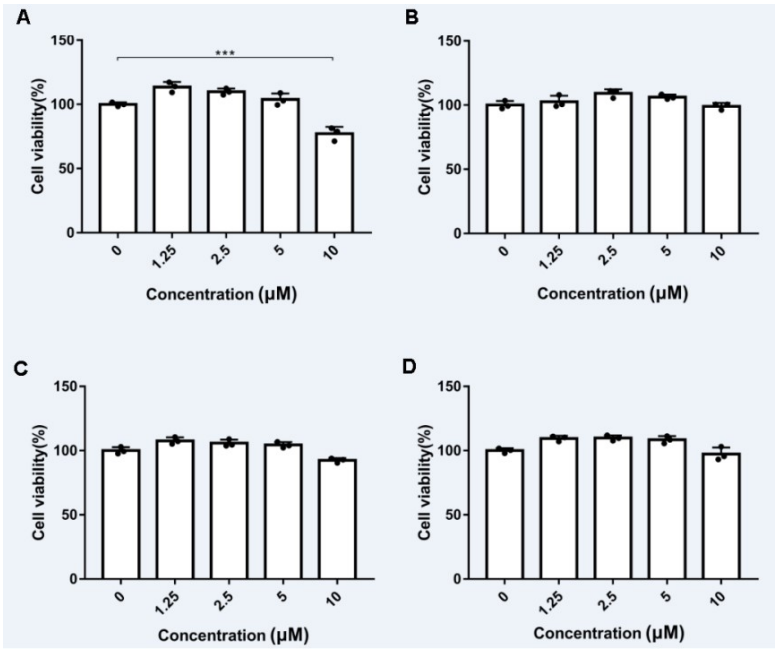

Figure S5. Quantification data of cytotoxic study after **M19** (**A**), **BTM19-1** (**B**), **BTM19-2** (**C**) and **BTM19-3** (**D**) treatment on RAW264.7 cell measured by CCK-8 assay. Data points are displayed as the mean value SEM of duplicate independent experiments. (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$).

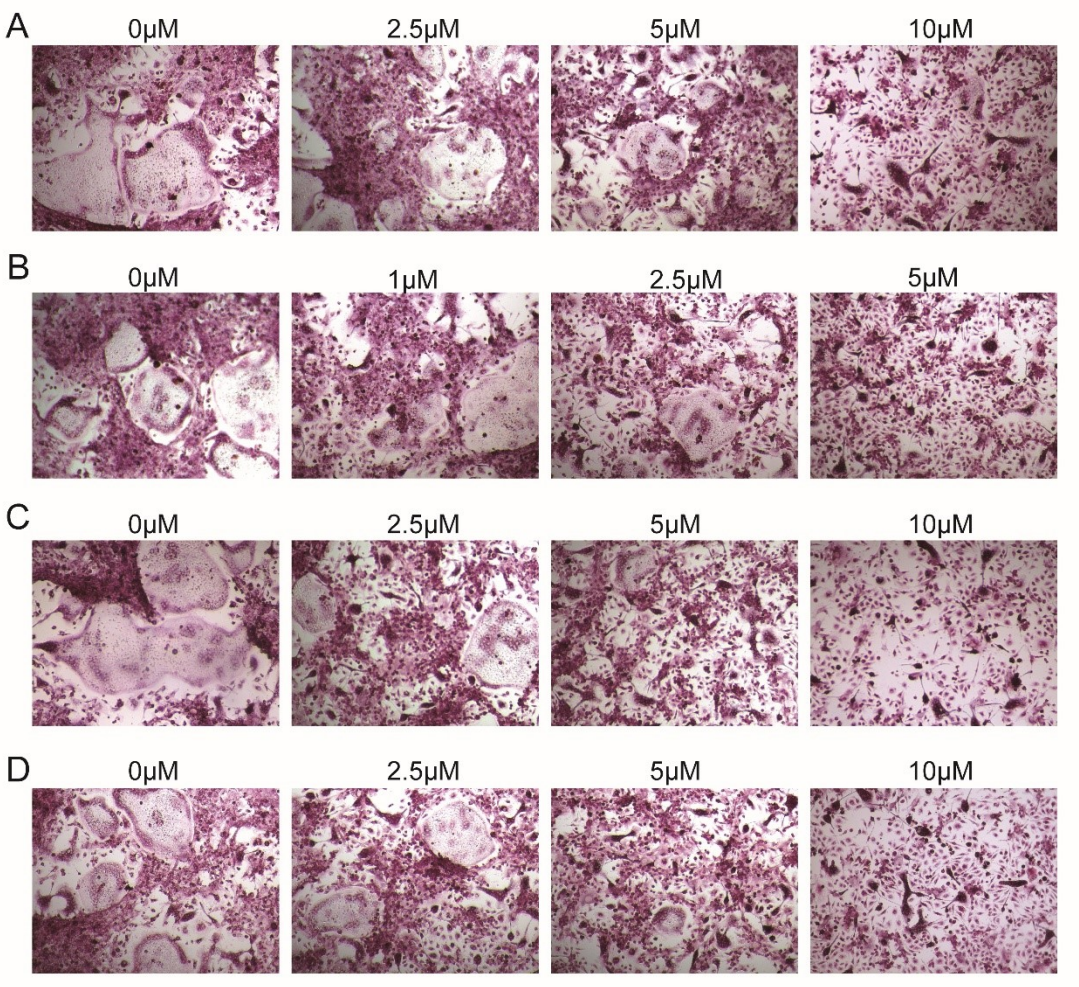

Figure S6. Formation of TRAP-positive cells from RAW264.7 cells with RANKL, M-CSF and treated with **M19** (**A**), **BTM19-1** (**B**), **BTM19-2** (**C**) and **BTM19-3** (**D**).

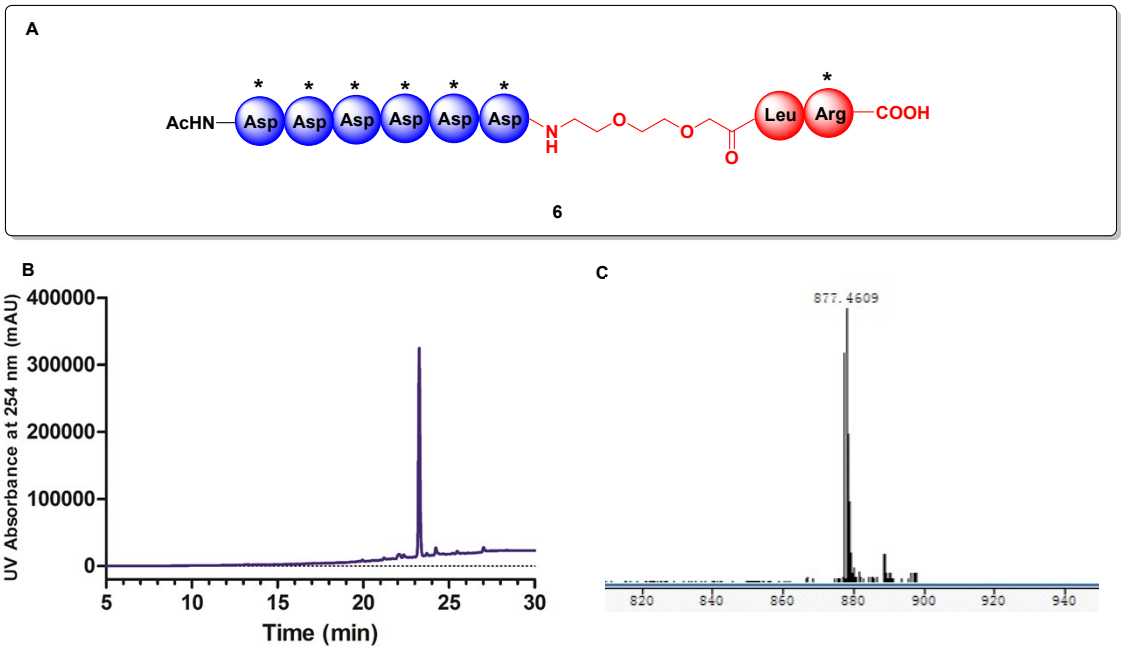

Figure S7. 6 as white powder, 1.43 g, 82% yield. **A**) Structure of **BTM19-1-2**; **B**) HPLC trace of purified **BTM19-1-2**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-1-2** (calcd. for $C_{81}H_{132}N_{12}O_{28}S$ 1752.8995; found $[M+2H]^{2+}$ 877.4609).

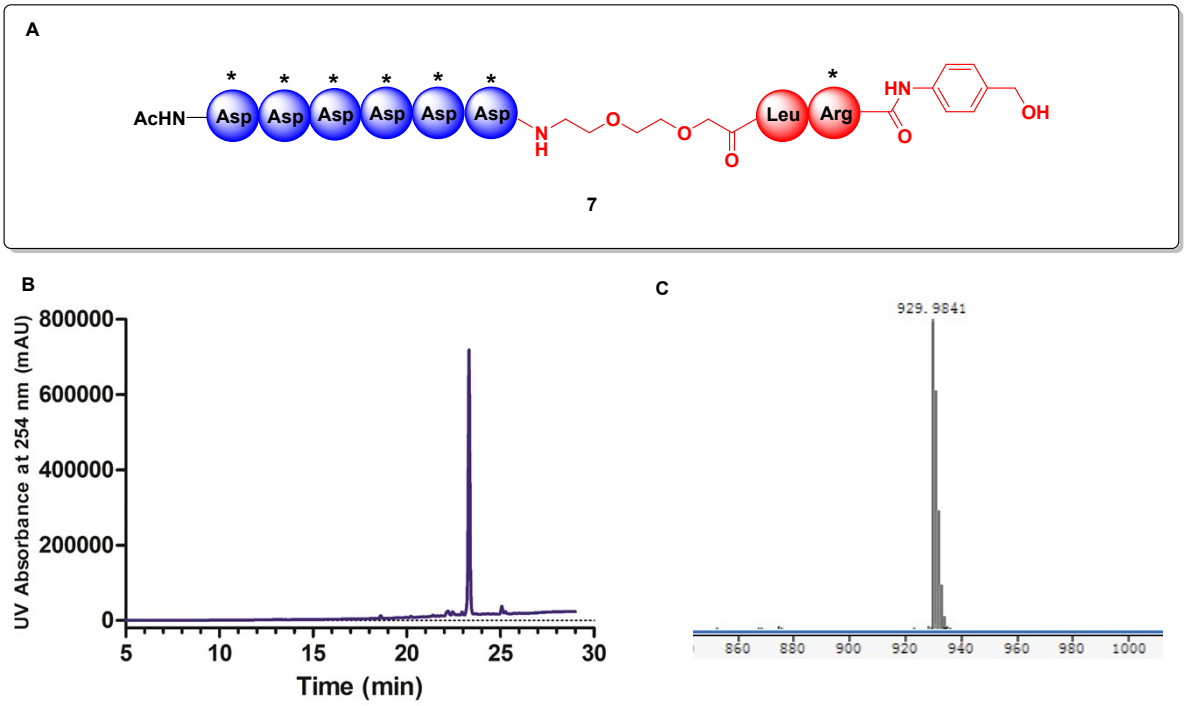

Figure S8. 7 as white powder, 1.12 g, 79% yield. **A**) Structure of **BTM19-1-3**; **B**) HPLC trace of purified **BTM19-1-3**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-1-3** (calcd. for $C_{88}H_{139}N_{13}O_{28}S$ 1857.9573; found $[M+2H]^{2+}$ 929.9841).

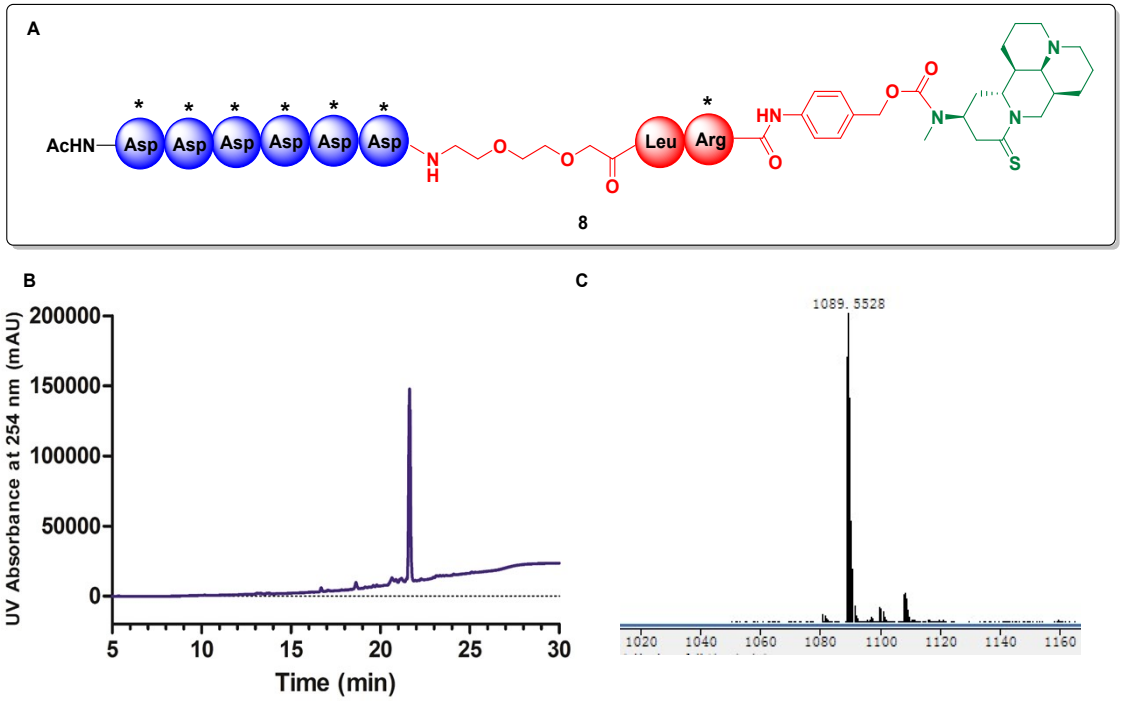

Figure S9. 8 as white powder, 1.1 g, 78% yield. **A**) Structure of **BTM19-1-4**; **B**) HPLC trace of purified **BTM19-1-4**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-1-4** (calcd. for $C_{105}H_{164}N_{16}O_{29}S_2$ 2177.1292; found $[M+2H]^{2+}$ 1089.5528).

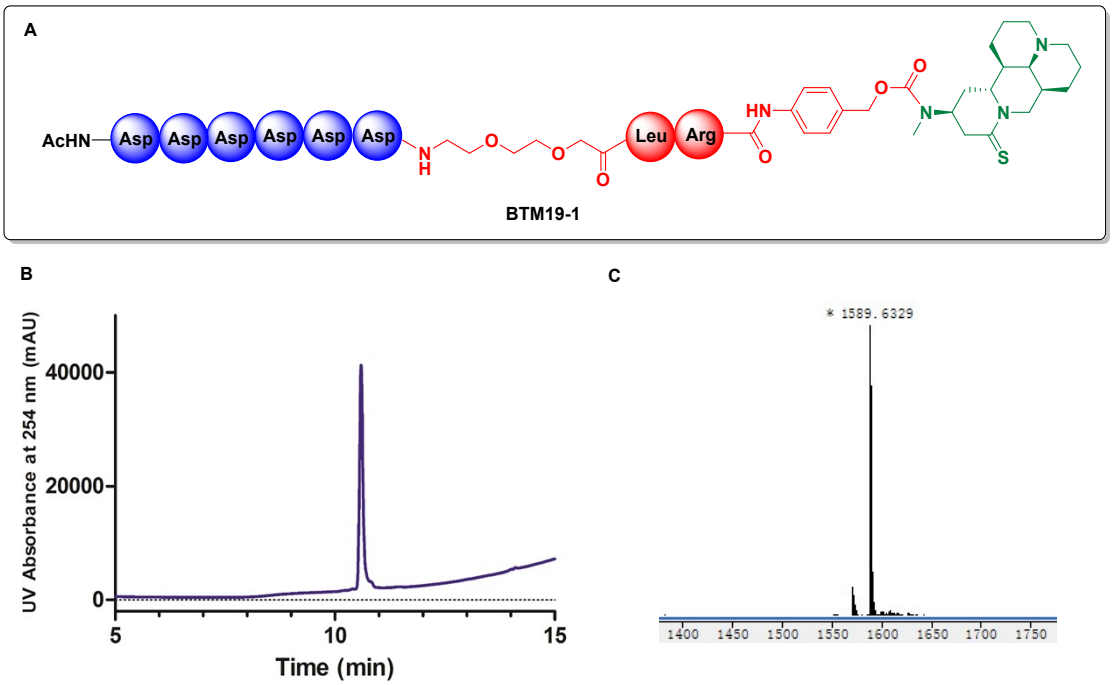

Figure S10. BTM19-1 as lyophilized white powder, 492 mg, 62% yield. **A**) Structure of **BTM19-1**; **B**) HPLC trace of purified **BTM19-1**. Gradient: 90-0% of buffer B in 15 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-**

1 (calcd. for $C_{68}H_{100}N_{16}O_{26}S$ 1588.6715; found $[M+H]^+$ 1589.6329).

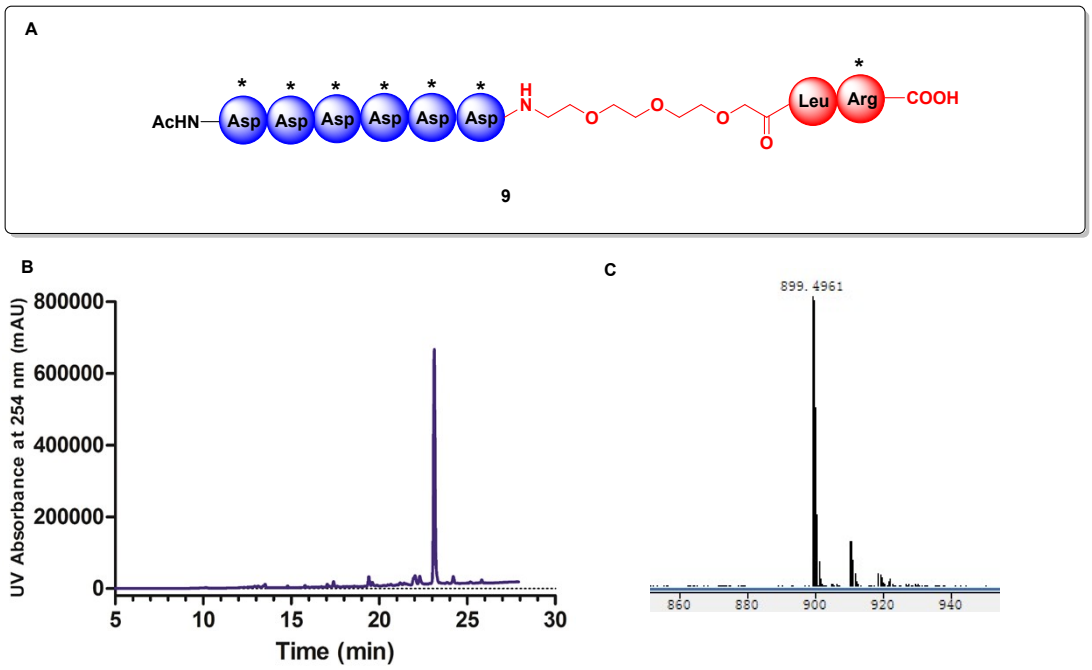

Figure S11. 9 as white powder, 1.5 g, 83% yield. **A**) Structure of **BTM19-2-2**; **B**) HPLC trace of purified **BTM19-2-2**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-2-2** (calcd. for $C_{83}H_{136}N_{12}O_{29}S$ 1796.9257; found $[M+2H]^{2+}$ 899.4961).

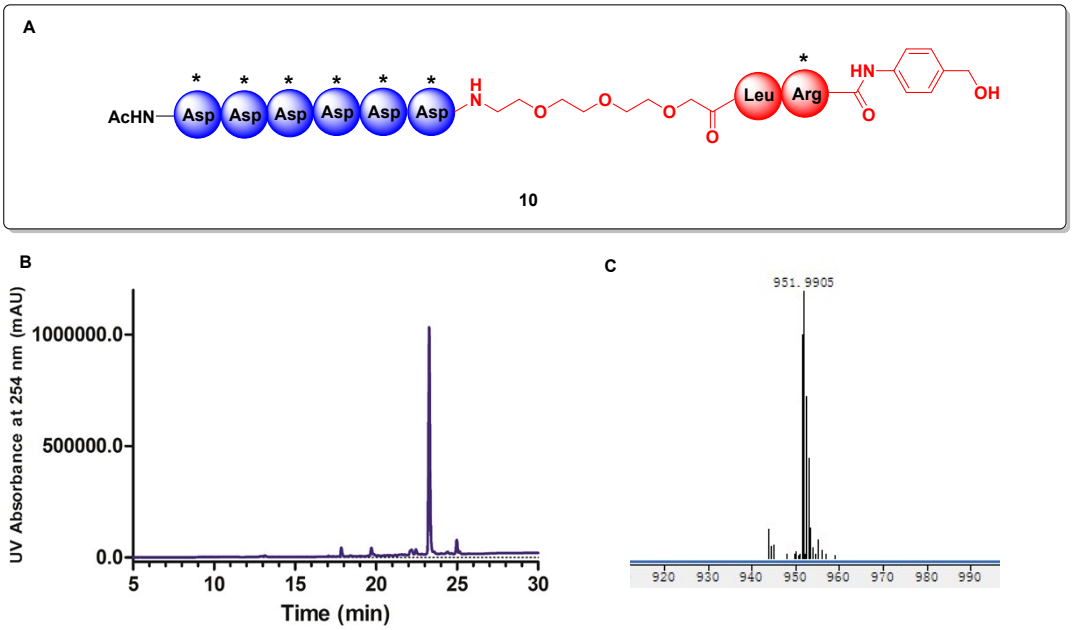

Figure S12. 10 as white powder, 1.18 g, 75% yield. **A**) Structure of **BTM19-2-3**; **B**) HPLC trace of purified **BTM19-2-3**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-2-3** (calcd. for $C_{90}H_{143}N_{13}O_{29}S$ 1901.9835; found $[M+2H]^{2+}$ 951.9905).

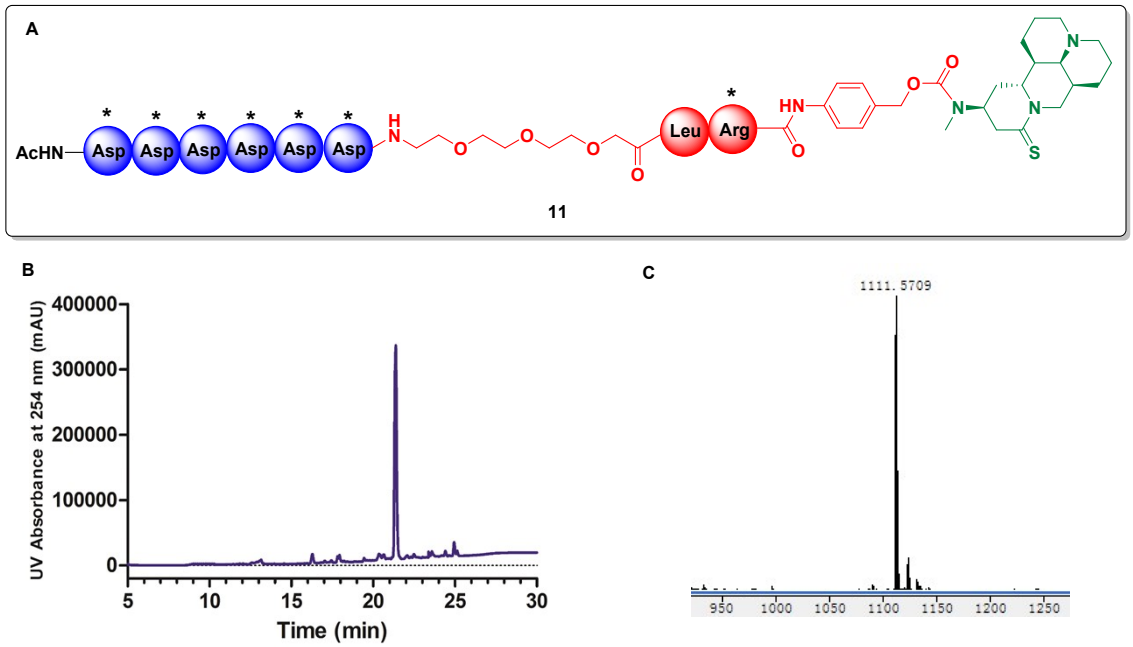

Figure S13. 11 as white powder, 1.07 g, 85% yield. **A**) Structure of **BTM19-2-4**; **B**) HPLC trace of purified **BTM19-2-4**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-2-4** (calcd. for $C_{107}H_{168}N_{16}O_{30}S_2$ 2221.1554; found $[M+2H]^{2+}$ 1111.5709).

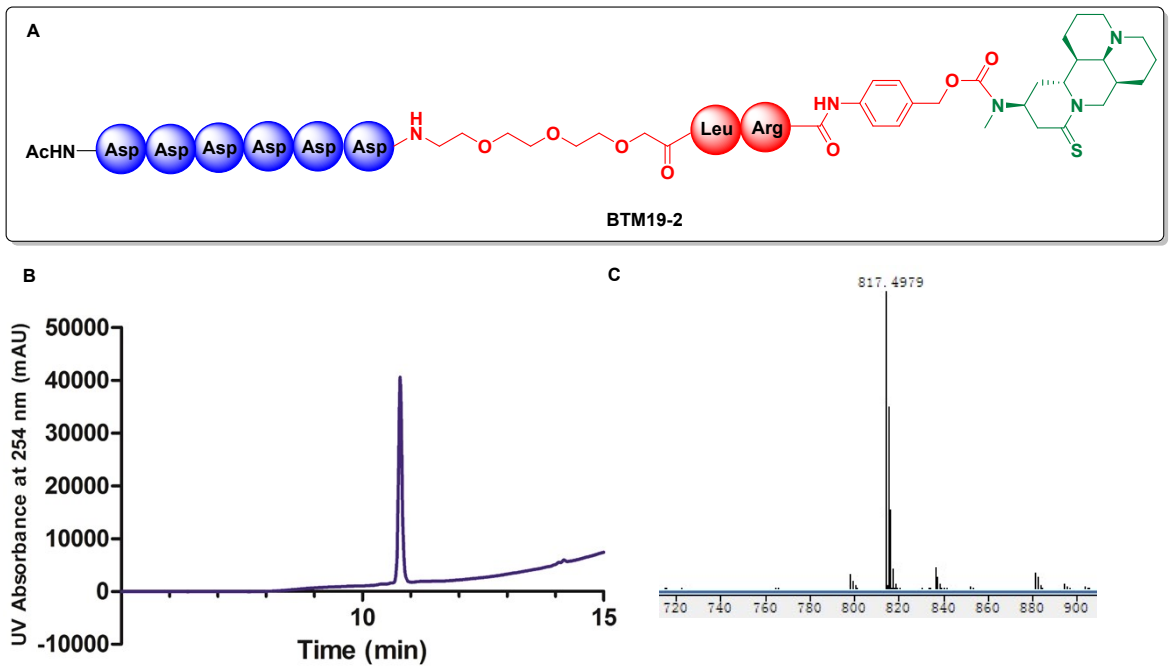

Figure S14. BTM19-2 as lyophilized white powder, 493 mg, 63% yield. **A**) Structure of **BTM19-2**; B) HPLC trace of purified **BTM19-2**. Gradient: 90-0% of buffer B in 15 min with C18 column (5 μm, 2.5 mm×250 mm). C) HR-MS spectrum of **BTM19-2** (calcd. for $C_{70}H_{104}N_{16}O_{27}S$ 1632.9678; found $[M+2H]^{2+}$ 817.4979).

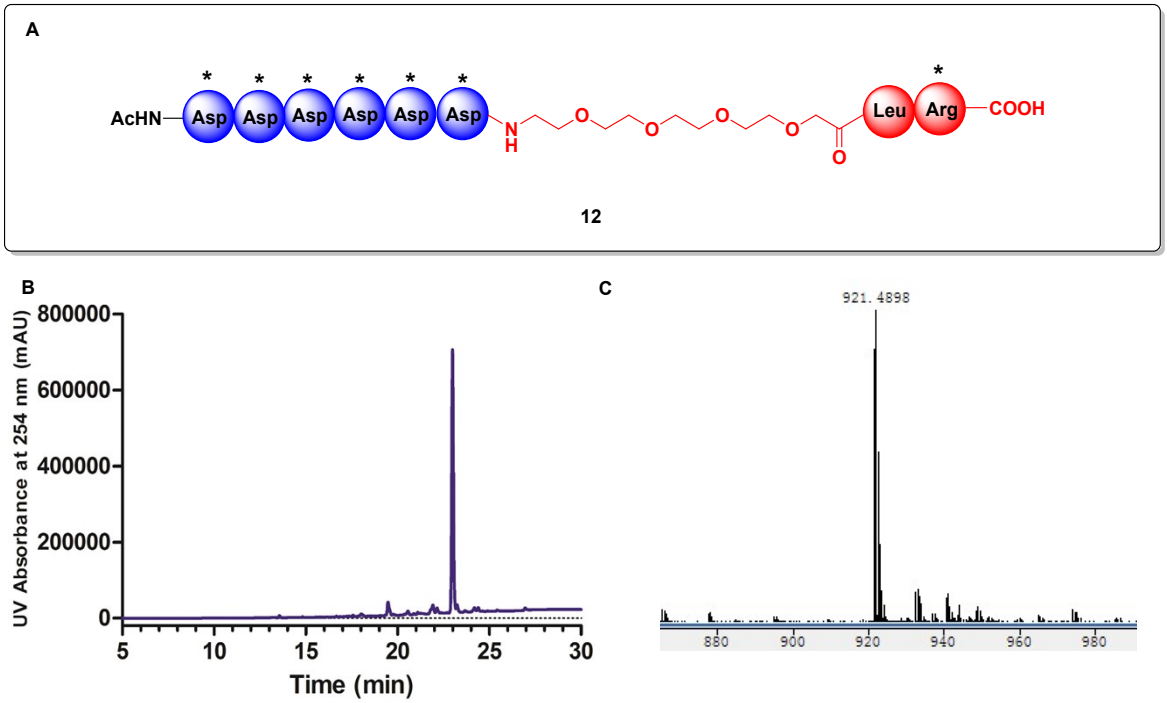

Figure S15. 12 as white powder, 1.47 g, 80% yield. **A**) Structure of **BTM19-3-2**; **B**) HPLC trace of purified BTM19-3-2. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-3-2** (calcd. for $C_{85}H_{140}N_{12}O_{30}S$ 1840.9519; found $[M+2H]^{2+}$ 921.4898).

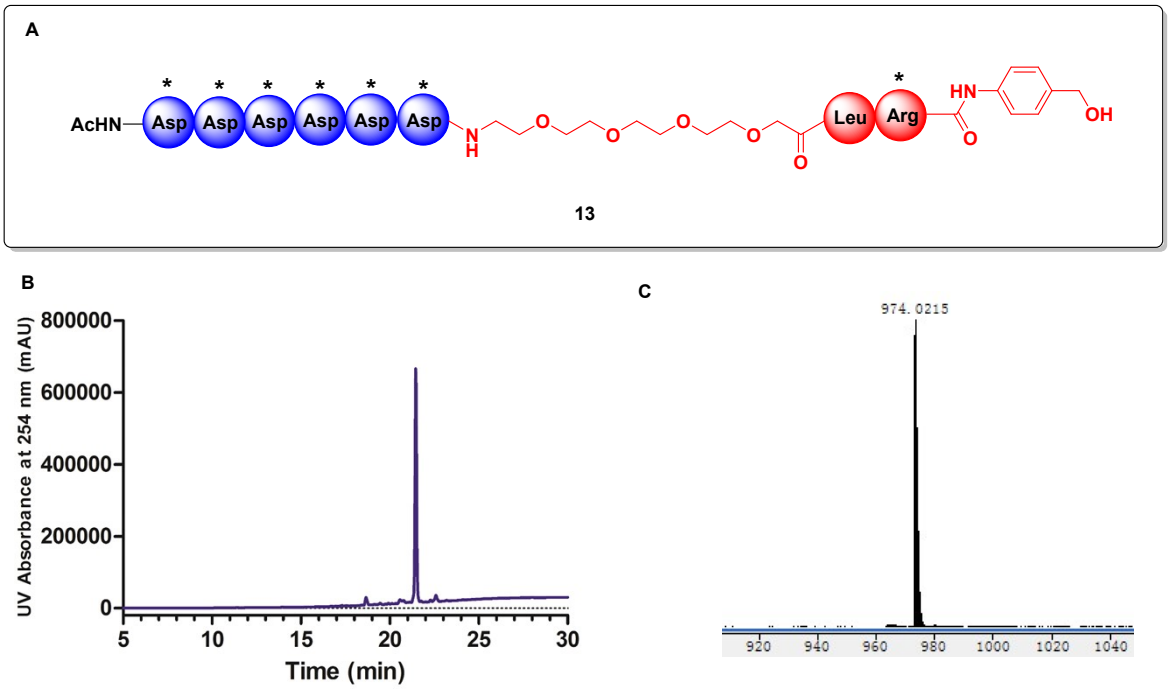

Figure S16. 13 as white powder, 1.15 g, 74% yield. **A**) Structure of **BTM19-3-3**; B) HPLC trace of purified **BTM19-3-3**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). C) HR-MS spectrum of **BTM19-3-3** (calcd. for $C_{92}H_{147}N_{13}O_{30}S$ 1946.0098; found $[M+2H]^{2+}$ 974.0215).

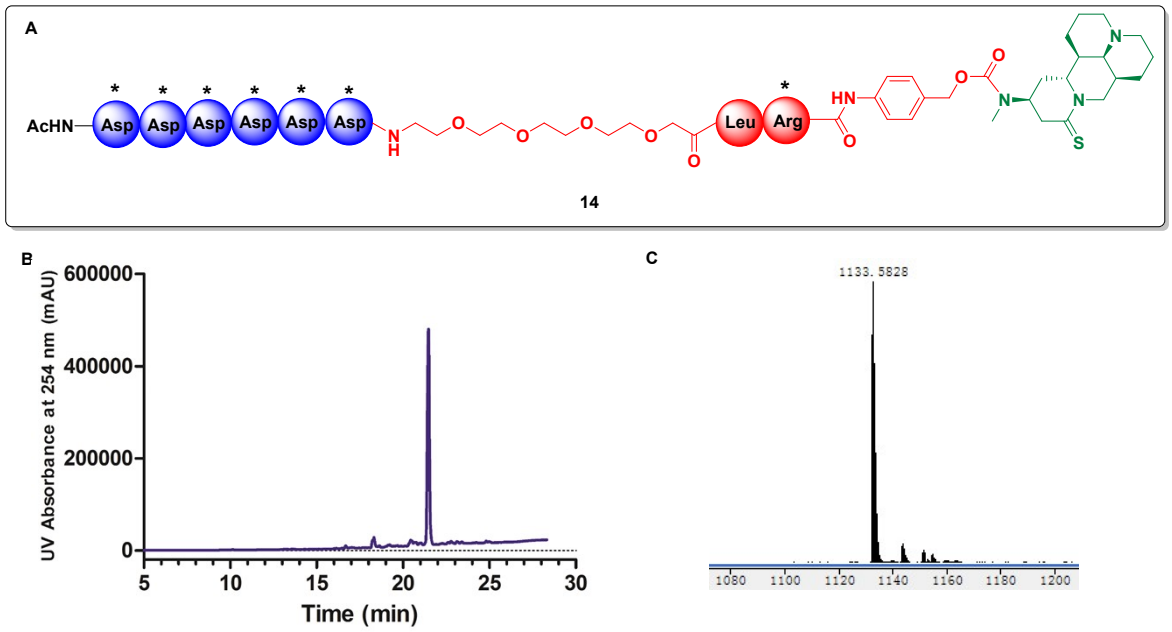

Figure S17. 14 as white powder 1.09 g, 86% yield. **A**) Structure of **BTM19-3-4**; B) HPLC trace of purified **BTM19-3-4**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-3-4** (calcd. for $C_{109}H_{172}N_{16}O_{31}S_2$ 2265.1816; found $[M+2H]^{2+}$ 1133.5828).

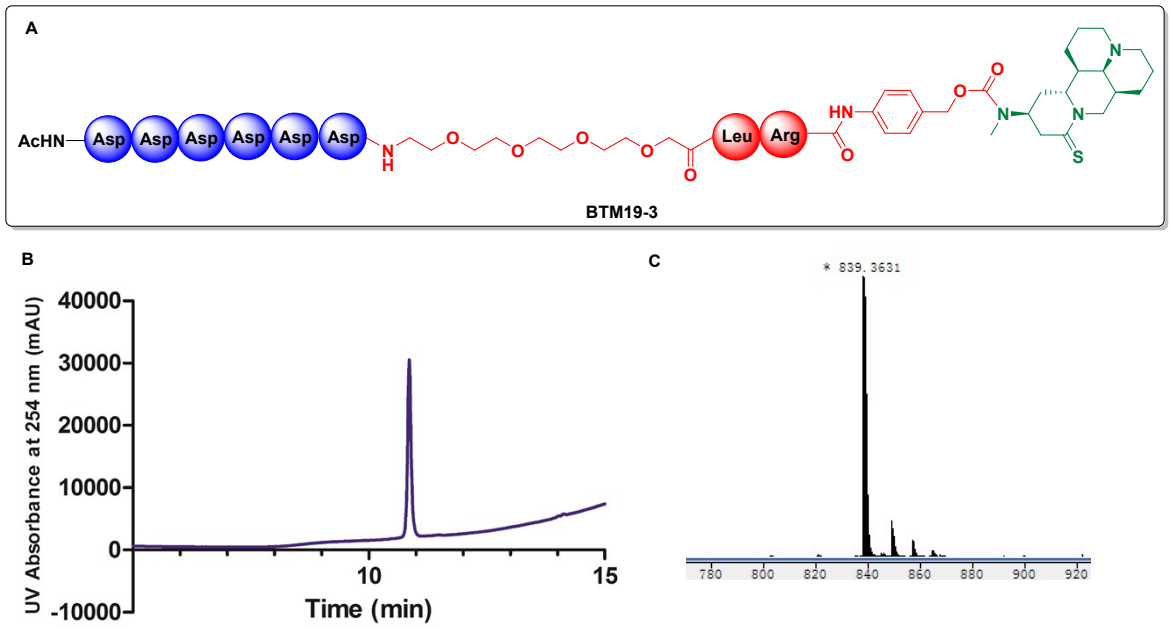

Figure S18. BTM19-3 as lyophilized white powder, 443 mg, 60% yield. **A**) Structure of **BTM19-3**; **B**) HPLC trace of purified **BTM19-3**. Gradient: 90-0% of buffer B in 15 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-3** (calcd. for $C_{72}H_{108}N_{16}O_{28}S$ 1676.7240; found $[M+2H]^{2+}$ 839.3631).

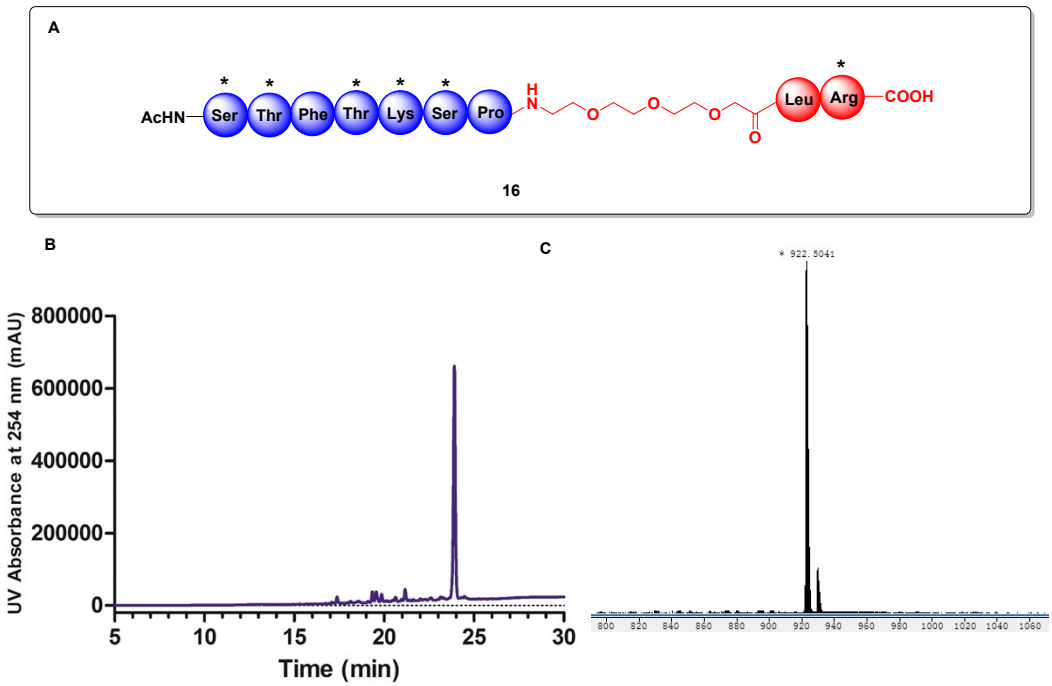

Figure S19. 16 as white powder, 1.6 g, 87% yield. **A**) Structure of **BTM19-4-2**; **B**) HPLC trace of purified BTM19-4-2. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-4-2** (calcd. for $C_{90}H_{150}N_{14}O_{24}S$ 1843.0668; found $[M+2H]^{2+}$ 922.5041).

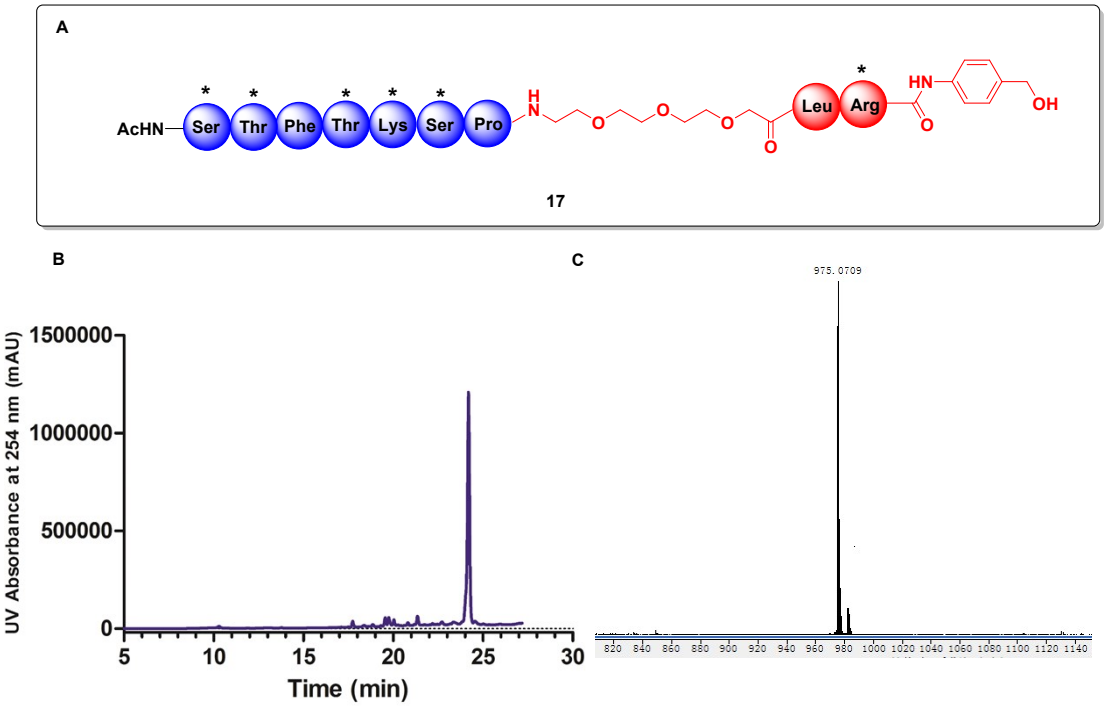

Figure S20. 17 as white powder 1.25 g, 74% yield. **A**) Structure of **BTM19-4-3**; **B**) HPLC trace of purified **BTM19-4-3**. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-4-3** (calcd. for $C_{97}H_{157}N_{15}O_{24}S$ 1948.1247; found $[M+2H]^{2+}$ 975.0709).

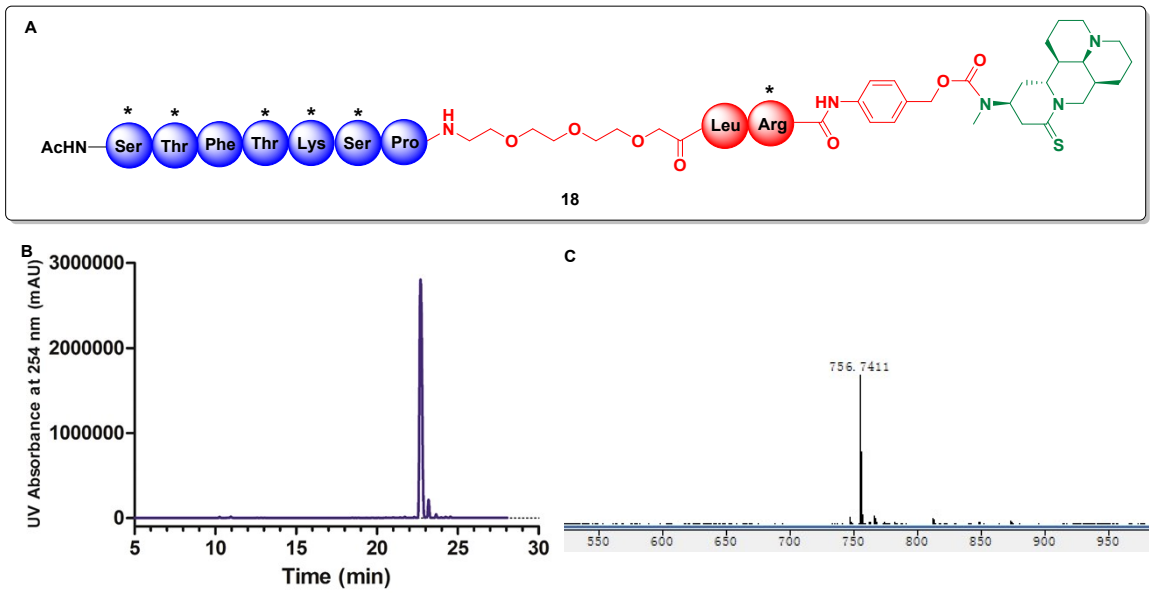

Figure S21. 18 as white powder, 1.05 g, 76% yield. **A**) Structure of **BTM19-4-4**; **B**) HPLC trace of purified BTM19-4-4. Gradient: 90-0% of buffer B in 20 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-4-4** (calcd. for $C_{114}H_{182}N_{18}O_{25}S_2$ 2267.2965; found $[M+3H]^{3+}$ 756.7441).

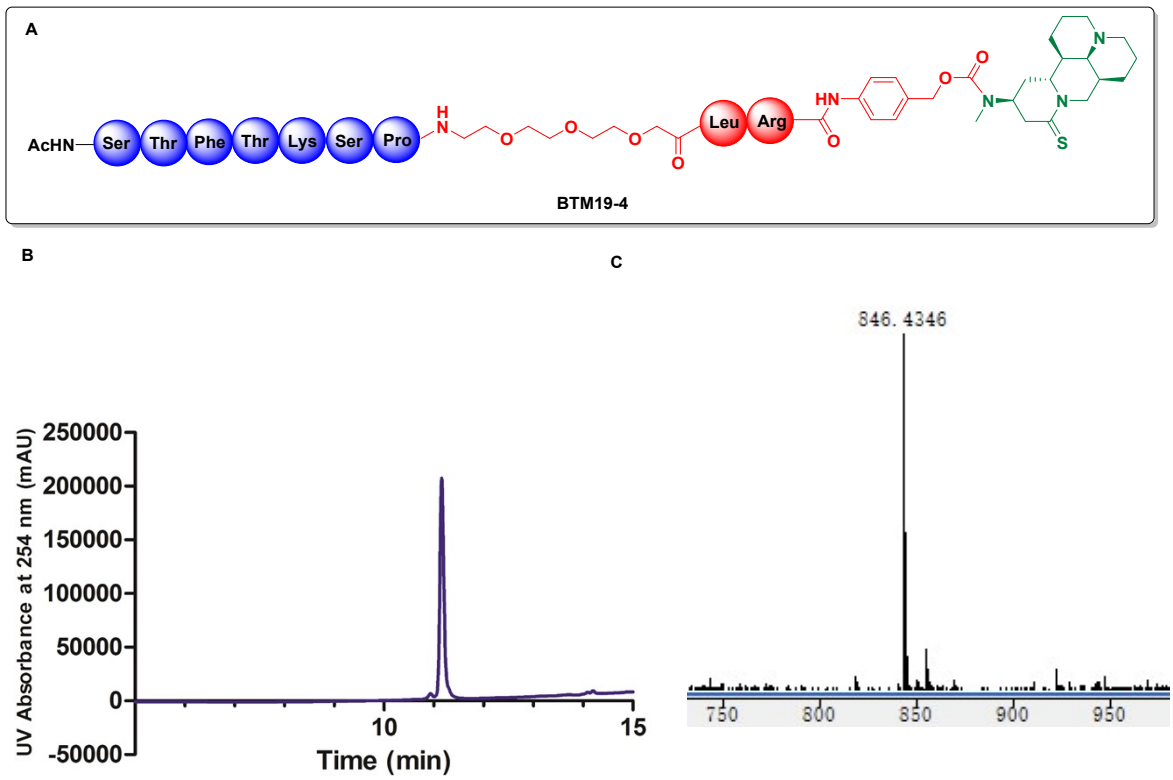

Figure S22. BTM19-4 as lyophilized white powder, 458 mg, 59% yield. **A**) Structure of BTM19-4; **B**) HPLC trace of purified **BTM19-4**. Gradient: 90-0% of buffer B in 15 min with C18 column (5 μm, 2.5 mm×250 mm). **C**) HR-MS spectrum of **BTM19-4** (calcd. for $C_{80}H_{126}N_{18}O_{20}S$ 1690.9116; found $[M+2H]^{2+}$ 846.4346).

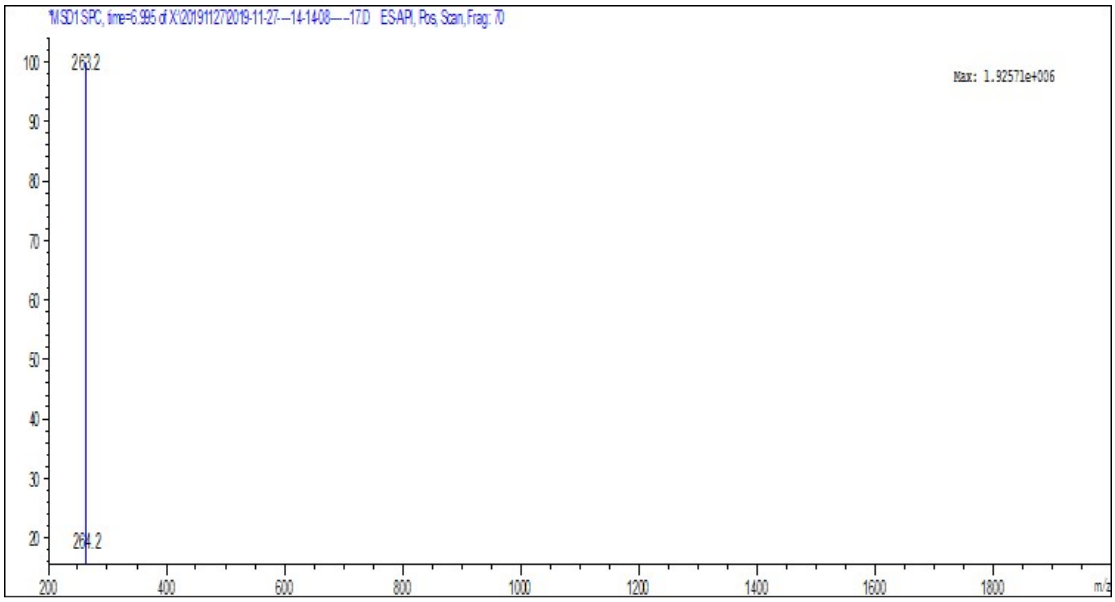

Figure S23. ESI-MS spectrum of **2** (calculated for $C_{15}H_{22}N_2S$ 262.15; found $[M+H]^+$ 263.2).

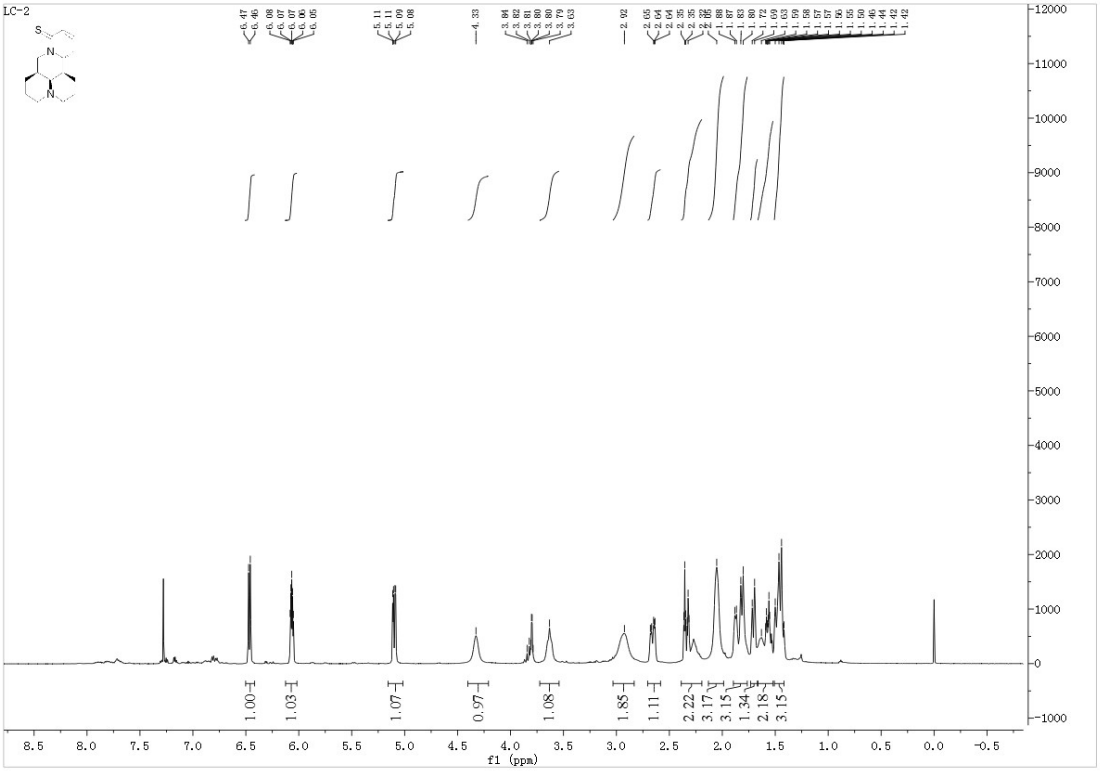

Figure S24. ^{1}H-NMR data of **2**.

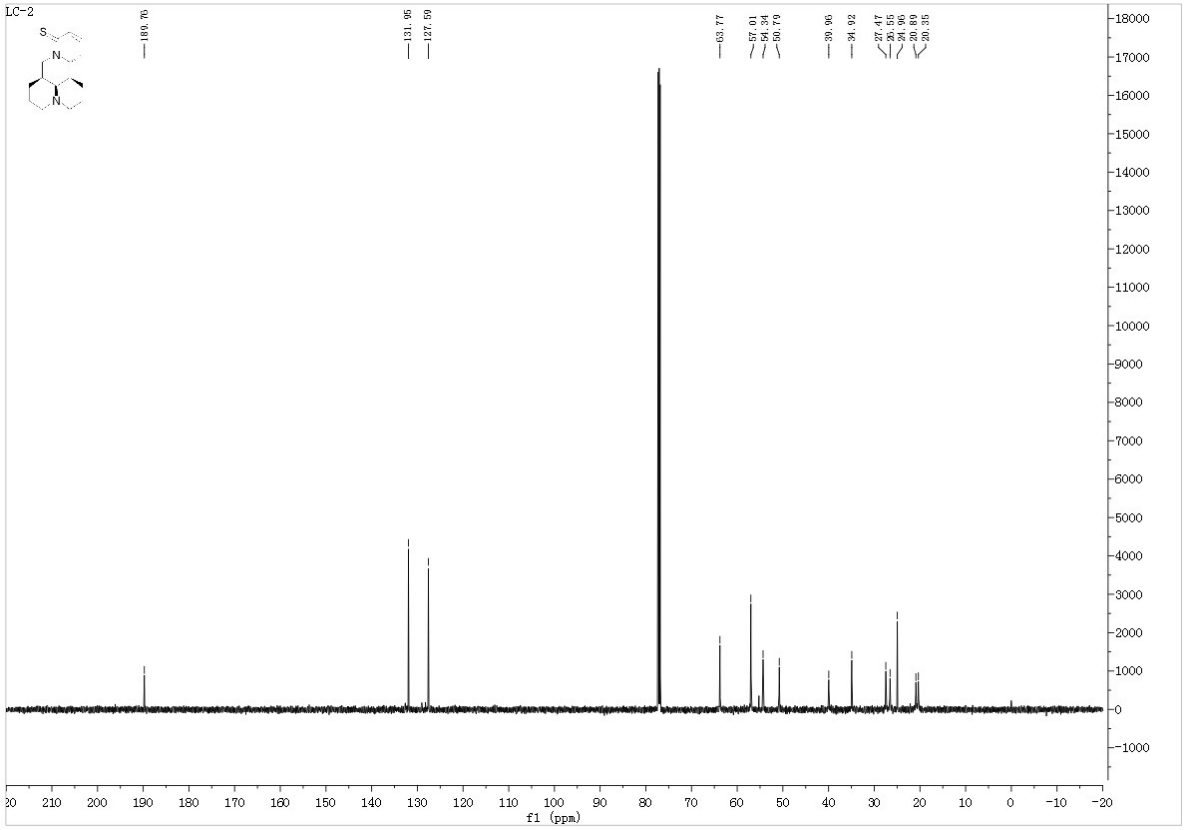

Figure S25. ^{13}C-NMR data of **2**.

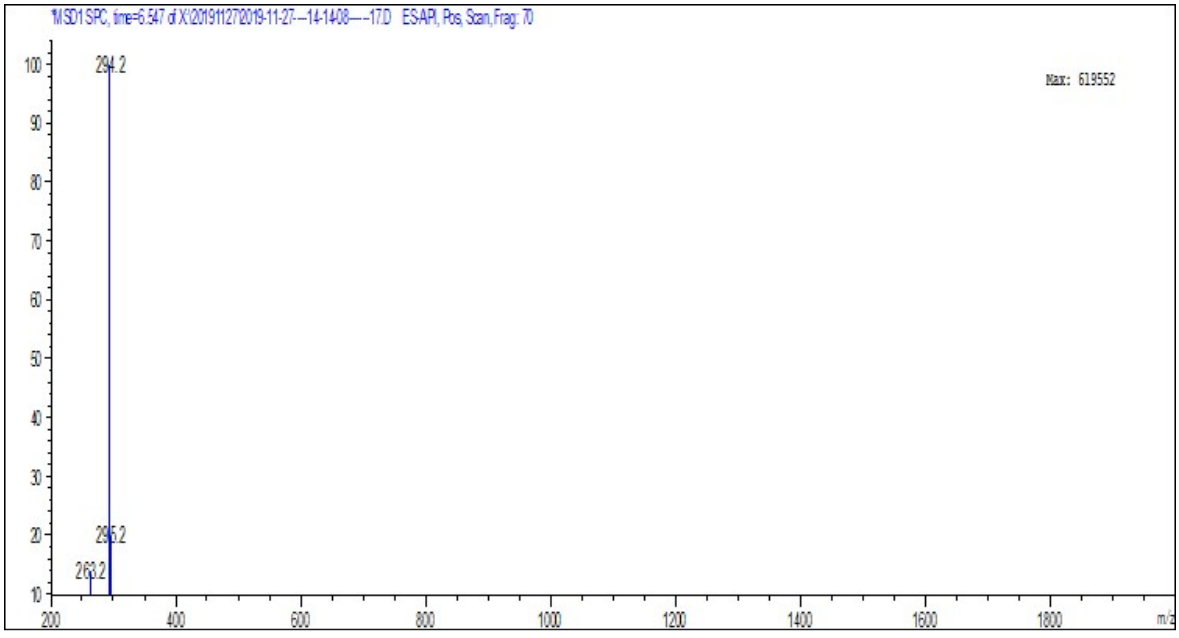

Figure S26. ESI-MS spectrum of **3** (calculated for $C_{16}H_{27}N_3S$ 293.19; found $[M+H]^+$ 294.2).

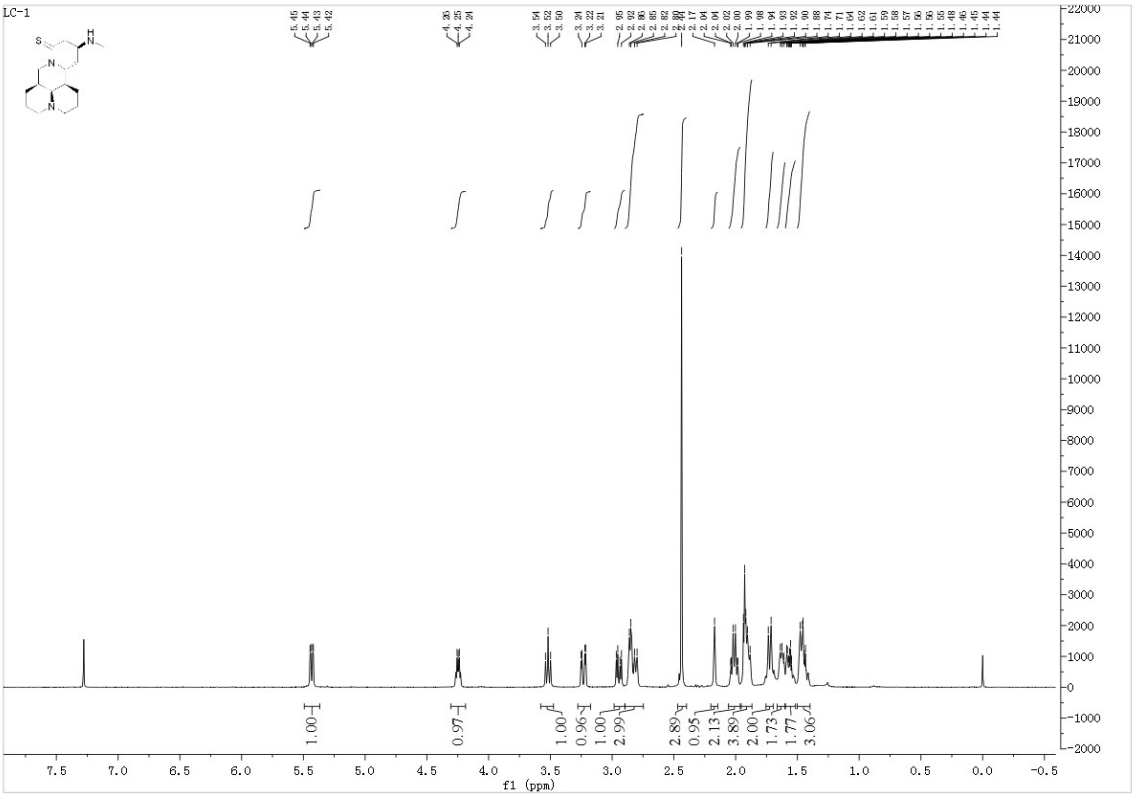

Figure S27. ^{1}H-NMR data of **2**.

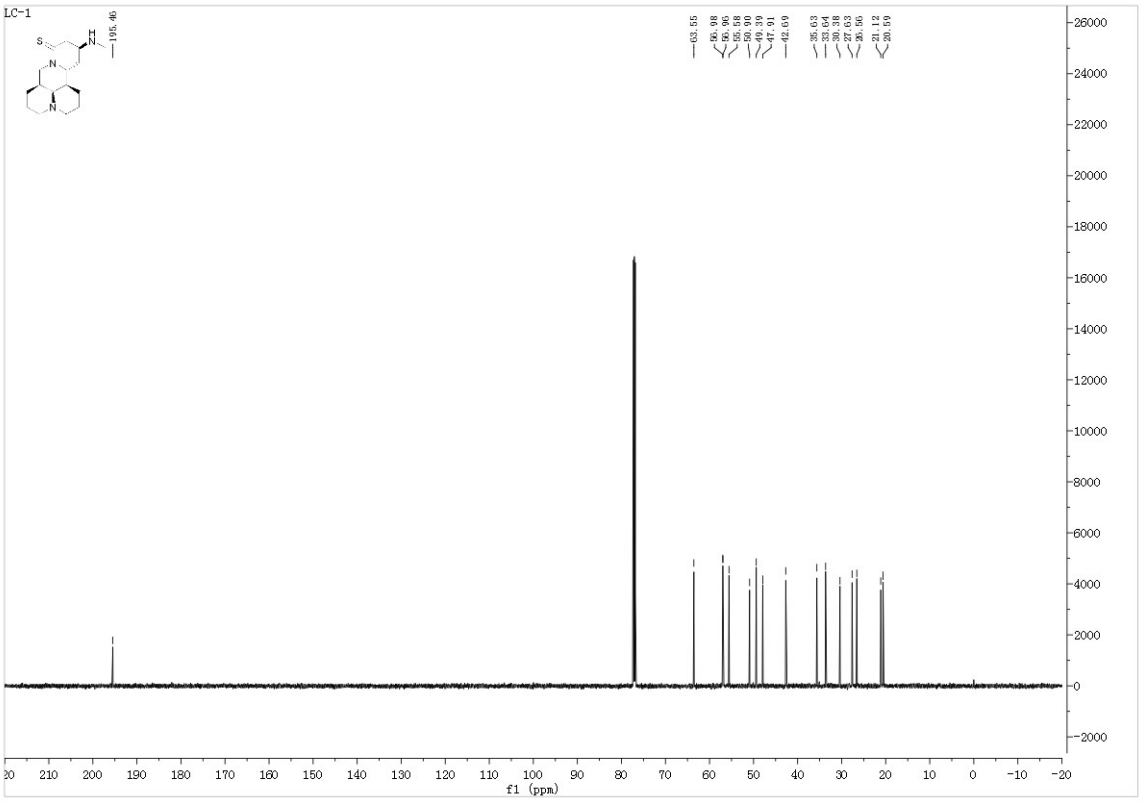

Figure S28. ^{13}C-NMR data of **2**.