Supporting Information

New Insight into the Mode of Action of Vancomycin dimers in Bacterial Cell Wall Synthesis

Osamu Yoshida,[b] Jun Nakamura,[a] Hidenori Yamashiro,[b] Kenji Miura[b]
Sayaka Hayashi,[a] Kosei Umetsu,[a] Shu Xu,[a] Hideki Maki,*[b] Hirokazu Arimoto*[a]

[a]*Graduate School of Life Sciences, Tohoku University, 2-1-1 Katahira, Sendai 980-8577, Japan. Fax: +81-(0)22-217-6204; Tel: +81-(0)22-217-6201; E-mail: arimoto@biochem.tohoku.ac.jp*
[b]*Discovery Research Laboratories, Shionogi & Co., Ltd., Osaka 561-0825, Japan. E-mail: hideki.maki@shionogi.co.jp*

- Table of Contents -

1. General Methods……S2

2. Preparation of Building Blocks

(1) Preparation of Compound **1**……S3

(2) Preparation of Compound **2**……S4

(3) Preparation of Compound **5**……S6

3. Spectra of Compounds

(1) ^{1}H NMR of Compound **1**……S7

(2) MS of Compound **1**……S8

(3) ^{1}NMR of Compound **2**……S9

(4) ^{1}NMR of Compound **5**……S10

(5) ^{1}NMR of Compound **SI-2**…… S11

(6) ^{1}NMR of Compound **SI-3**…… S12

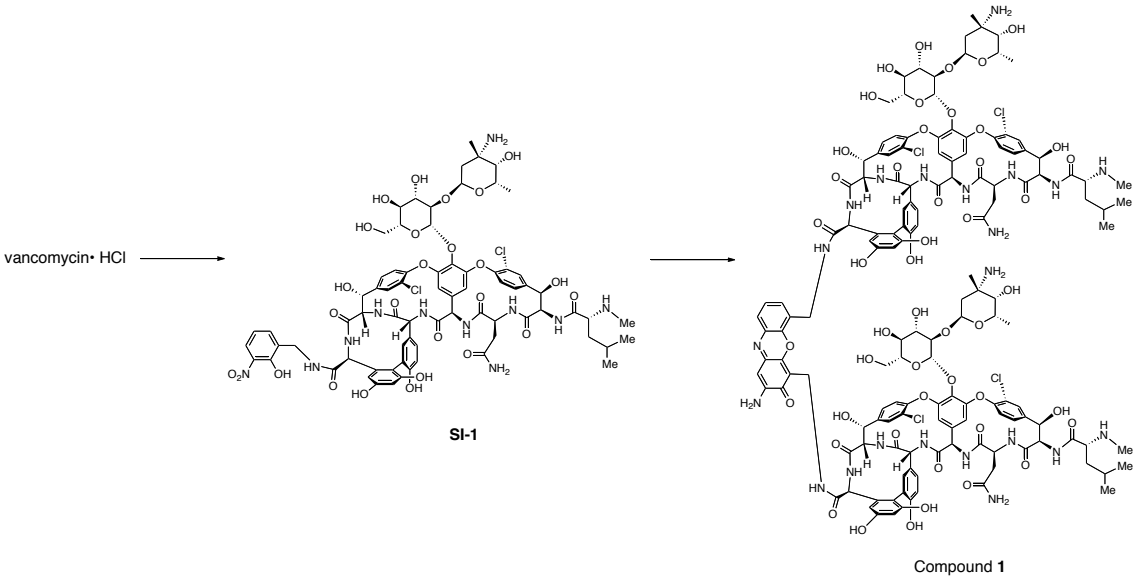
4. Reference…… S13

1. General Methods.

Reagents and solvents were used as provided from commercial suppliers, and all reactions were carried out at room temperature unless otherwise noted. LC-ESI-TOF MS were obtained on a Bruker Daltonics microTOF-TSfocus. LC part was performed on Agilent 1100 Series, by using Imtakt UNISON UK $_3C_8$ column (2 x 150 mm) under the following conditions: flow rate of 0.2 mL/min, 65% to 95% acetonitrile (0.1% formic acid)/water (0.1% formic acid), duration of 5 min, temperature of 40°C, and UV 214, 254, 280, and 190-400 nm. Monoisotopic mass was used for calculation of exact mass (C = 12.0000, H = 1.0078, O = 15.9949, N = 14.0031, Cl = 35.9689, P = 30.9738, Na = 22.9898). Nuclear magnetic resonance (NMR) spectra were recorded at room temperature on a JEOL ECA600 at 600 MHz and Varian Unity INOVA 500 at 500 MHz. Chemical shifts (δ) are reported in ppm in ^{1}H spectra relative to the solvent peak of CD_3OD (3.31 ppm), and DMSO-d_6 (2.5 ppm). Coupling constants are given in Hz. Thin-layer chromatography (TLC) was performed using Merck silica gel 60 F254 precoated plates (0.25 mm). Flash chromatography was carried out using 60–230 mesh silica gel (Kanto Chemical silica gel 60N). Analytical reversed-phase HPLC (RP-HPLC) was performed on equipments as follows, RP-colomn: nacalai tesque Cosmosil 5C_{18}-AR-II (4.6 x 150 mm); conditions: flow rate of 1.0 mL/min, 15% to 100% acetonitrile (0.1% TFA)/water (0.1% TFA), duration of 10 min, temperature of 30°C, and UV 280 nm. Preparative RP-column chromatography was performed with Merck Lobar LiChroprep RP-18 Groβe C (37 x 440 mm, 50 μm particle size) or Yamazen ULTRA PACK ODS-S-50B (26 x 300 mm, 50 μm particle size), methanol/water (0.1% TFA) or acetonitrile/water (0.1 % TFA). Preparative HPLC was performed with nacalai tesque Cosmosil $_5C_{18}$ AR-II-waters (10 x 250 mm), acetonitrile/water (0.1% TFA).

2. Preparation of Building Blocks.

(1) Preparation of Compound **1**



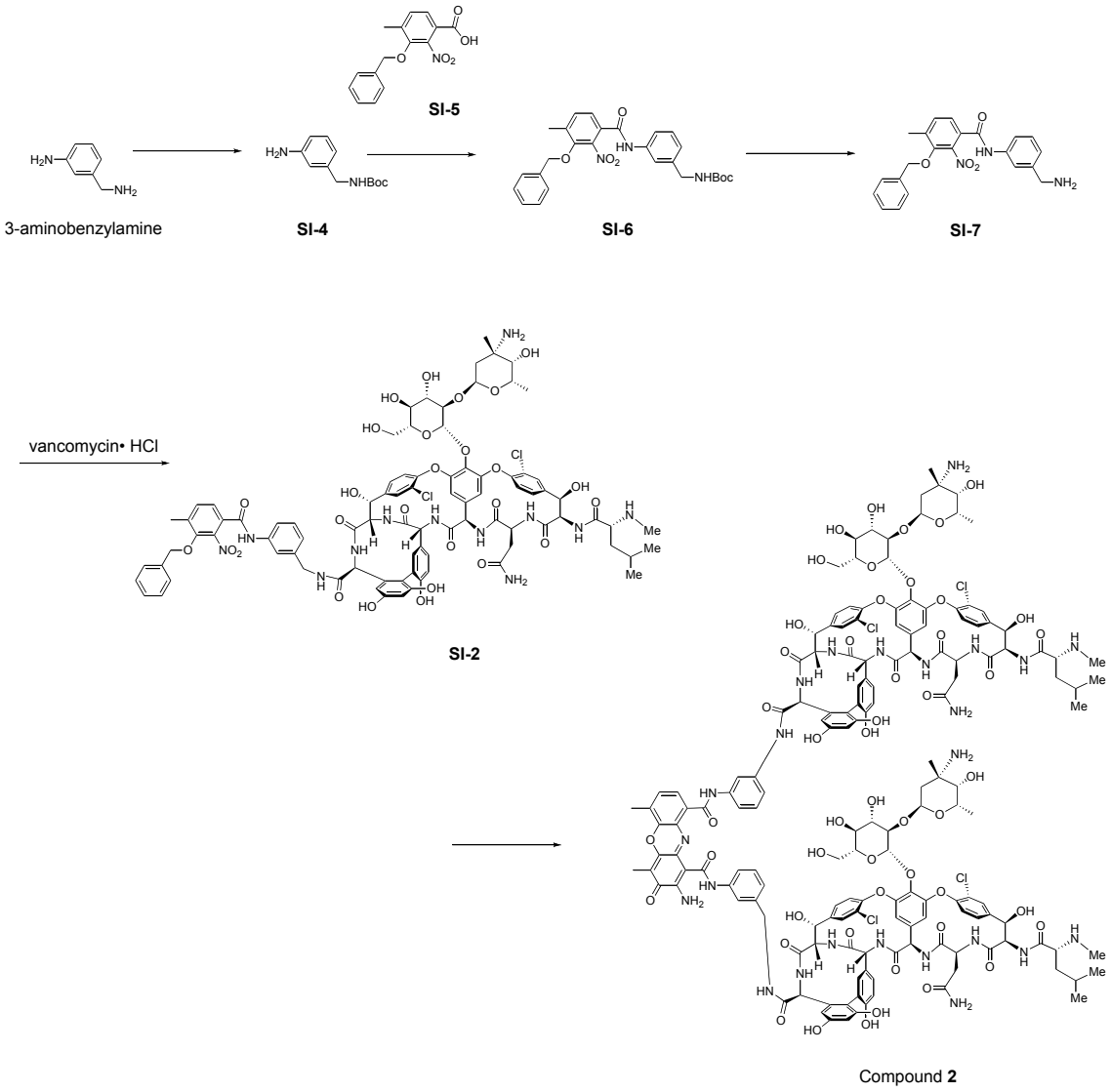
Scheme S1

Compound **SI-1**. A solution of vancomycin•HCl salt (0.19 g, 0.13 mmol, Wako), HOBt (23 mg, 0.17 mmol), PyBOP (81 mg, 0.26 mmol) and *N*-methylmorpholine (0.011 mL, 2.5 mmol) in DMF/DMSO (1:1, 8 mL) was stirred at room temperature for 90 min. 2-Hydroxy-3-nitrobenzylamine (17 mg, 0.11 mmol) was added, and then the reaction mixture was stirred at room temperature for an additional 30 min. The resulting mixture was poured into 7% brine (50 mL) at 0 °C, and the precipitate of crude **SI-1** was collected by filtration (Millipore, pore size 1.0 mm, JAWP04700). The residue was added to ethyl acetate (50 mL), and stirred at room temperature for 10 min. Then, the precipitate of products was collected by filtration (Millipore, pore size 1.0 μm, JAWP04700). The crude solid of **SI-1** was dissolved in acetonitrile/water (2:5, 14 mL), then purified by reverse-phase column chromatography (Lobar LiChroprep RP-18 Große B, Merck, 30% methanol/water (0.1% TFA), to yield compound **SI-1** (38 mg, 18%): 89% purity, R_t = 5.9 min.

Compound **1:** Compound **SI-1** (320 mg, 0.2 mmol) in methanol (11 mL) was reduced for 3 h with hydrogen in the presence of 10% Pd-C catalyst (340 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off by use of celite (rinsed with 5 mL of methanol), and *p*-quinone (39 mg, 1.8 eq., dissolved in 1 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 20 h under darkness, and then poured into ethyl acetate (110 mL). Filtration (Millipore: pore size 1.0 μm, JAWP04700) yielded crude compound **1**. The crude solid of **1** was dissolved in water and acetonitrile, then purified by reverse-phase column

chromatography (Lobar LiChroprep RP-18 Große B, Merck: acetonitrile:water (0.1% TFA)=3:17) to yield 145 mg (46%) of compound **1** (TFA salt). Further purification of this sample (9 mg, 0.0028 mmol) by HPLC (Cosmosil $_5C_{18}$-AR-II waters, Φ 10 mm x 250 mm, 17% acetonitrile (0.1% TFA)/water (0.1% TFA), flow rate 4 mL/min) gave an orange solid (5.6 mg, 0.0018 mmol): 96% purity; R_t= 2.4 min; ^{1}H NMR (600 MHz, CD_3OD, 22˚C, solvent): *∂* 8.95 (br., 2H), 8.7-8.68 (br., 2H), 8.57 (br., 1H), 8.33 (br., 1H), 7.7 (s, 2H), 7.65-7.61 (m, 12H), 7.5 (t, *J*= 7.2 Hz, 2H), 7.42 (t, *J*= 7.9 Hz, 2H), 7.29 (m, 2H), 7.25 (d, *J*= 8.3 Hz, 3H), 7.05 (s, 2H), 7.02 (s, 2H), 6.99 (br., 1H), 6.84 (d, *J*= 8.3 Hz, 1H), 6.78 (d, *J*= 8.6 Hz, 1H), 6.49 (s, 2H), 6.35 (s, 3H), 6.34 (s, 2H), 6.22 (s, 1H), 5.78 (br., 6H), 5.46 (t, *J*= 7.9 Hz, 4H), 5.42 (br., 4H), 5.34-5.31 (m, 12H), 4.69-4.58 (m, 1H), 4.52 (q, *J*= 14 Hz, 2H), 4.32 (br., 2H), 4.23 (s, 1H), 4.16 (s, 1H), 4.08 (q, *J*= 12, 6.8 Hz, 2H), 3.62 (s, 2H), 3.86 (s, 2H), 3.83 (m, 2H), 3.75-3.73(m, 2H), 3.62 (t, *J*= 7.5 Hz, 2H), 3.52 (t, *J*= 9.6 Hz, 2H), 2.91 (br., 1H), 2.88 (br., 1H), 2.27 (br., 2H), 2.07-2.04 (m, 2H), 1.93 (s, 1H), 1.91 (s, 1H), 1.86 (m, 1H), 1.76 (br., 1H), 1.69 (m, 1H), 1.5 (d, *J*= 5.4 Hz, 12H), 1.21 (t, *J*= 5.5 Hz, 12H), 1.01 (br., 12H), 0.97 (br., 12H); HRMS (LC-ESI-TOF) calcd. for $C_{146}H_{160}Cl_4N_{22}O_{48}$: 1565.4833 $[M+2H]^{2+}$; found 1565.4826 $[M+2H]^{2+}$.

(2) Preparation of Compound **2**



Scheme S2

Compound **SI-4**. Boc_2O (0.55 g, 2.5 mmol) in 1,4-dioxane (7 mL) was added to a solution of 3-aminobenzylamine (0.61 g, 5 mmol, Aldrich) in dry 1,4-dioxane (7 mL). The reaction mixture was stirred at room temperature for 4.5 h. Evaporation of the solvent gave crude compound **SI-4** as pale yellow viscous liquid (0.62 g): R_f = 0.41 (SiO_2 TLC, ethyl acetate/hexane = 1:1). This crude compound was used for the next step without further purification.

Compound **SI-6**. Four drops of DMF were added to a mixture of carboxylic acid **SI-5** (1.4 g, 4.8 mmol) and oxalyl chloride (0.57 mL, 6.2 mmol) in dichloromethane (18 mL) at room temperature. The mixture was stirred at room temperature for 55 min, and then dichloromethane and excess oxalyl chloride were distilled off. The viscous, yellow

residue was dissolved in THF and the solution was quickly transferred to a THF solution (18 mL) of compound **SI-4** (1.3 g, 5.9 mmol) at 0 °C (ice-water bath). The solution was stirred at 0 °C to room temperature over 2 h and then stirred for additional 2.5 days. The reaction solution was poured into the aqueous $NaHCO_3$. The resulting mixture was partitioned with ethyl acetate (100 mL). The organic layer was washed with water, brine, and finally dried over $MgSO_4$. Filtration and evaporation afforded crude compound **SI-6** as a pale orange solid. The product was purified by column chromatography (SiO_2, ethyl acetate/hexane = 3:7), to yield compound **SI-6** (1.9 g, 82%): 93% purity, R_t = 9.4 min; HRMS (LC-ESI-TOF) calcd. for $C_{27}H_{29}N_3NaO_6$: 514.1948 $[M+Na]^+$, found 514.1945 $[M+Na]^+$.

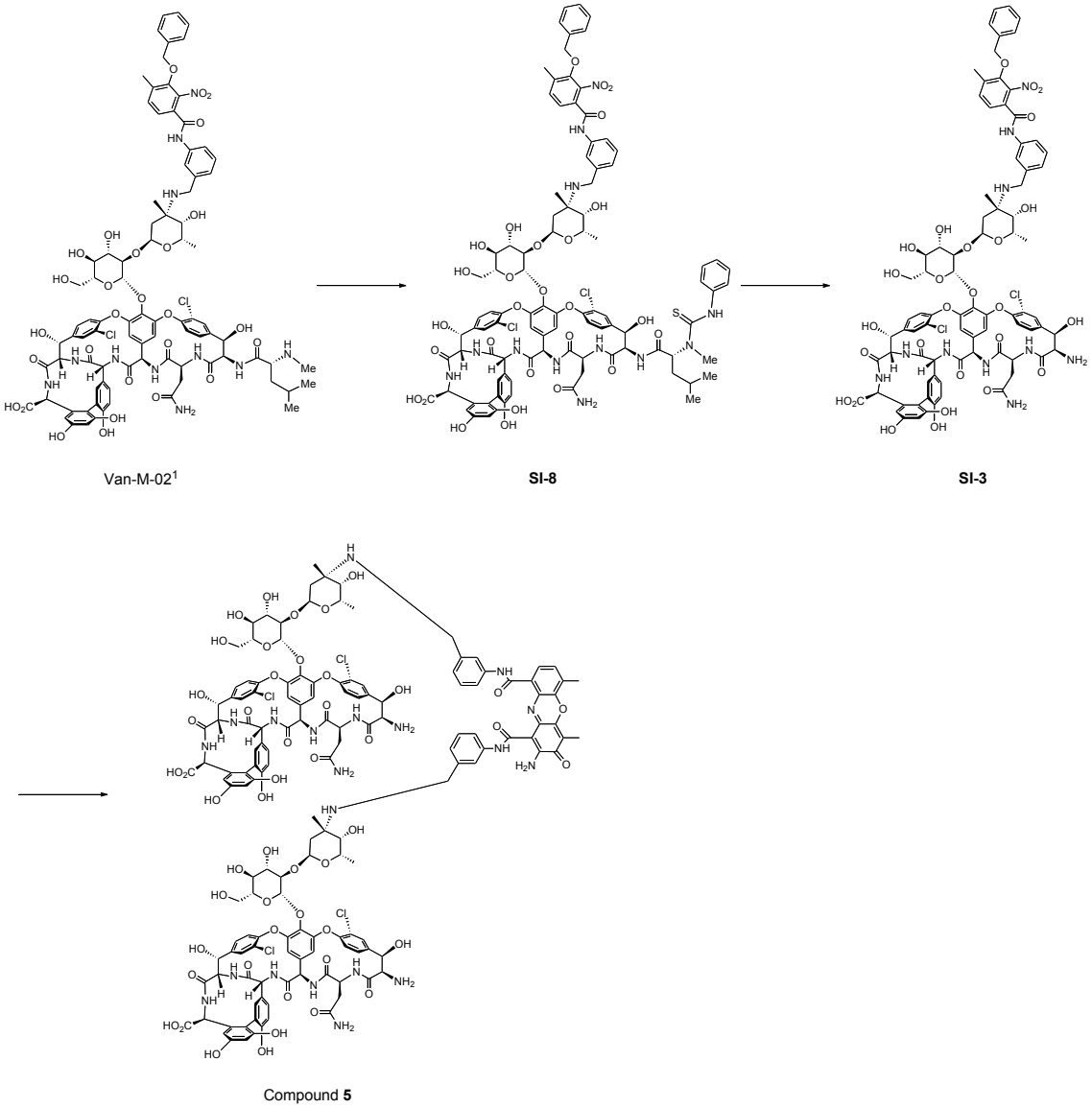
Compound **SI-7**. TFA (17 mL) was added to a solution of **SI-6** (0.5 g, 1 mmol) in THF (10 mL) at 0 °C (ice-water bath). The reaction mixture was stirred at room temperature for 4.5 h at 0 °C under Ar. Evaporation to remove the solvent and excess TFA *in vacuo* gave crude product, then it was purified by recrystallization from methanol and toluene, yielded compound **SI-7** as a pale yellow solid (0.39 g, 97%): 96% purity, R_t = 6.1 min; HRMS (LC-ESI-TOF) calcd. for $C_{22}H_{22}N_3O_4$: 392.1604 $[M+H]^+$, found 392.1592 $[M+H]^+$.

Compound **SI-2**. A solution of vancomycin•HCl salt (0.57 g, 0.38 mmol, Wako), HOBt (0.11 g, 0.83 mmol), PyBOP (0.4 g, 0.77 mmol) and *N*-methylmorpholine (0.58 mL, 4.7 mmol) in DMF/DMSO (4:1, 40 mL) was stirred at room temperature for 35 min. Compound **SI-7** (0.15 g, 0.38 mmol) in DMF/DMSO (4:1, 2.5 mL) was added dropwise, and then the reaction mixture was stirred at room temperature for an additional 16 h. The resulting mixture was poured into 7% brine (300 mL) at 0 °C, and a pale orange precipitate of **SI-2** was collected by filtration (Millipore, pore size 1.0 μm, JAWP04700). The residue was added to ethyl acetate (100 mL), and stirred at room temperature for 10 min. Then, the pale orange precipitate of **SI-2** was collected by filtration (Millipore, pore size 1.0 μm, JAWP04700). The crude solid of **SI-2** (0.74 g) was dissolved in acetonitrile/water (2:5, 70 mL). Purification by reverse-phase column chromatography (Lobar LiChroprep RP-18 Große B, Merck, 30% acetonitrile/aq. HCl (0.005 M) yielded compound **SI-2** (HCl salt) as a pale yellow solid (0.32 g, 46%). Further purification of this sample (25 mg, 0.014 mmol) by HPLC (Cosmosil $_5C_{18}$-AR-II waters, Φ 10 mm x 250 mm, 35% acetonitrile (0.1% TFA)/water (0.1% TFA), flow rate 3 mL/min) gave an orange solid (15 mg, 0.0082 mmol): >99% purity, R_t = 5.5 min; ^{1}H NMR (600 MHz, DMSO-d_6 + D_2O, 22˚C, solvent): ∂ 8.69 (s, 1H), 8.5 (s, 1H), 7.81 (s, 1H), 7.62-7.58 (m, 4H), 7.53 (d, *J*= 8.4 Hz, 1H), 7.05 (d, *J*= 7.8 Hz, 1H), 7.43 (d, *J*= 9 Hz, 1H), 7.04-7.39 (m, 4H), 7.36 (m, 1H), 7.3 (d, *J*= 8.4 Hz, 1H), 7.28 (t, *J*= 8.1 Hz, 1H), 7.18 (s, 1H), 7.17 (d, *J*= 8.4 Hz, 1H), 7.56 (d, *J*= 8.4 Hz, 1H), 6.75 (d, *J*= 9.6 Hz, 1H), 6.7 (d, *J*= 8.4 Hz, 1H), 6.35 (d, *J*= 2.4 Hz, 1H), 6.26 (d, *J*= 2.4 Hz, 1H), 5.71 (s, 1H), 5.55 (s, 1H), 5.24 (d, *J*= 6 Hz, 1H), 5.21 (s, 1H), 5.2 (s, 1H), 5.16 (s, 1H), 5.15 (s, 1H), 4.89 (s, 1H), 4.65 (d,

J= 6.6 Hz, 1H), 4.43 (d, *J*= 5.4 Hz, 2H), 4.34 (s, 2H), 4.21 (s, 1H), 3.92 (s, 1H), 3.53 (q, *J*= 9.2 Hz, 2H), 3.42 (m, 1H), 3.24 (d, *J*= 4.8 Hz, 2H), 3.15 (s, 1H), 2.6 (br., 3H), 2.41 (s, 3H), 2.14 (d, *J*= 7.8 Hz, 1H), 1.88, (d, *J*= 9.6 Hz, 1H), 1.72, (d, 12.6 Hz. 1H), 1.65 (m, 1H), 1.61 (m, 1H), 1.55 (m, 1H), 1.26 (s, 3H), 1.03 (d, *J*= 6 Hz, 3H), 0.88 (d, *J*= 6.6 Hz, 3H), 0.83 (d, *J*= 6 Hz, 3H); HRMS (LC-ESI-TOF) calcd. for $C_{88}H_{95}Cl_2N_{12}O_{27}$: 1821.5806 $[M+H]^+$, found 1821.5731 $[M+H]^+$.

Compound **2:** Compound **SI-2** (100 mg, 0.044 mmol) in methanol (4 mL) and dry DMF (1 mL) was reduced for 1.5 h with hydrogen in the presence of 10% Pd-C catalyst (90 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off by use of celite (rinsed with 6 mL of methanol), and *p*-quinone (9.0 mg, 1.8 eq., dissolved in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 18.5 h under darkness, and then poured into ethyl acetate (100 mL). Filtration (Millipore: pore size 1.0 µm, JAWP04700) yielded 100 mg of crude compound **2** as an orange solid. Purification of this crude sample (33 mg) by HPLC (Cosmosil $_5C_{18}$-AR-II, Φ 10 mm x 250 mm, 32% acetonitrile (0.1% TFA)/water (0.1% TFA), flow rate 4 mL/min) gave an orange solid (1.9 mg, 8%): >99% purity, R_t= 4.2 min; ^{1}H NMR (600 MHz, DMSO-*d*6 + D_2O, 22 ˚C, solvent): ∂ 10.5 (s, 1H), 9.02 (S, 1H), 8.7 (s, 1H), 8.52 (s, 1H), 8.48 (s, 1H), 7.82 (br., 2H), 7,7 (br., 1H), 7.61 (d, *J*= 7.5 Hz, 1H), 7.54-7.52 (m, 6H), 7.44 (d, *J*= 9.3 Hz, 1H), 7.38 (d, *J*= 7.9 Hz, 1H), 7.32 (d, *J*= 8.3 Hz, 2H), 7.24-7.18 (m, 4H), 7.03 (d, *J*= 7.5 Hz, 1H), 6.91 (d, *J*= 6.8 Hz, 1H), 6.85 (t, *J*= 6.4 Hz, 1H), 6.77 (d, *J*= 8.5 Hz, 2H), 6.71 (d, *J*= 8.5 Hz, 2H), 6.37 (t, *J*= 2.4 Hz, 2H), 6.34 (d, *J*= 1.7 Hz, 2H), 5.75 (br., 1H), 5.6 (br., 1H), 5.27-5.23 (m, 6H), 5.17 (br., 4H), 4.91 (br., 1H), 4.67 (br., 2H), 4.46-4.44 (m, 3H), 4.33-4.21 (m, 5H), 3.96 (br., 2H), 3.68 (d, *J*= 10.9 Hz, 2H), 3.55-3.53 (m, 3H), 3,26 (br., 4H), 2.62 (br, 6H), 3.16 (s, 2H), 2.19 (s, 1H), 3.23 (s, 3H), 2.13 (br., 1H), 1.89 (br., 1H), 1.74-1.61 (m, 4H), 1.56 (m, 1H), 1.29 (d, *J*= 2.7 Hz, 6H), 1.06 (d, *J*= 3.7 Hz, 6H), 0.91 (d, *J*= 6.1 Hz, 6H), (d, *J*= 6.1 Hz, 6H); MS (LC-ESI-TOF) calcd. for $C_{162}H_{174}Cl_4N_{24}O_{50}$: 1698.5361 $[M+2H]^{2+}$; found 1698.5178 $[M+2H]^{2+}$.

(3) Preparation of Compound **5**



Scheme S3

Compound **SI-8**. Phenyl isothiocyanate (0.078 ml, 0.66 mmol) was added to a solution of Van-M-02[1] (0.40 g, 0.22 mmol) in pyridine/water (1:1, 15 mL) at 0 °C (ice-water bath). The solution was stirred at 0 °C for 3 h. After Van-M-02[1] had been consumed, the reaction mixture was diluted with methanol and toluene, evaporated to remove pyridine, and poured into ethyl acetate (500 mL). The resulting precipitate was collected by filtration (Millipore, pore size 1.0 μm, JAWP04700), to yield crude compound **SI-8** (0.34 g). This crude compound **SI-8** was used for the next step without further purification.

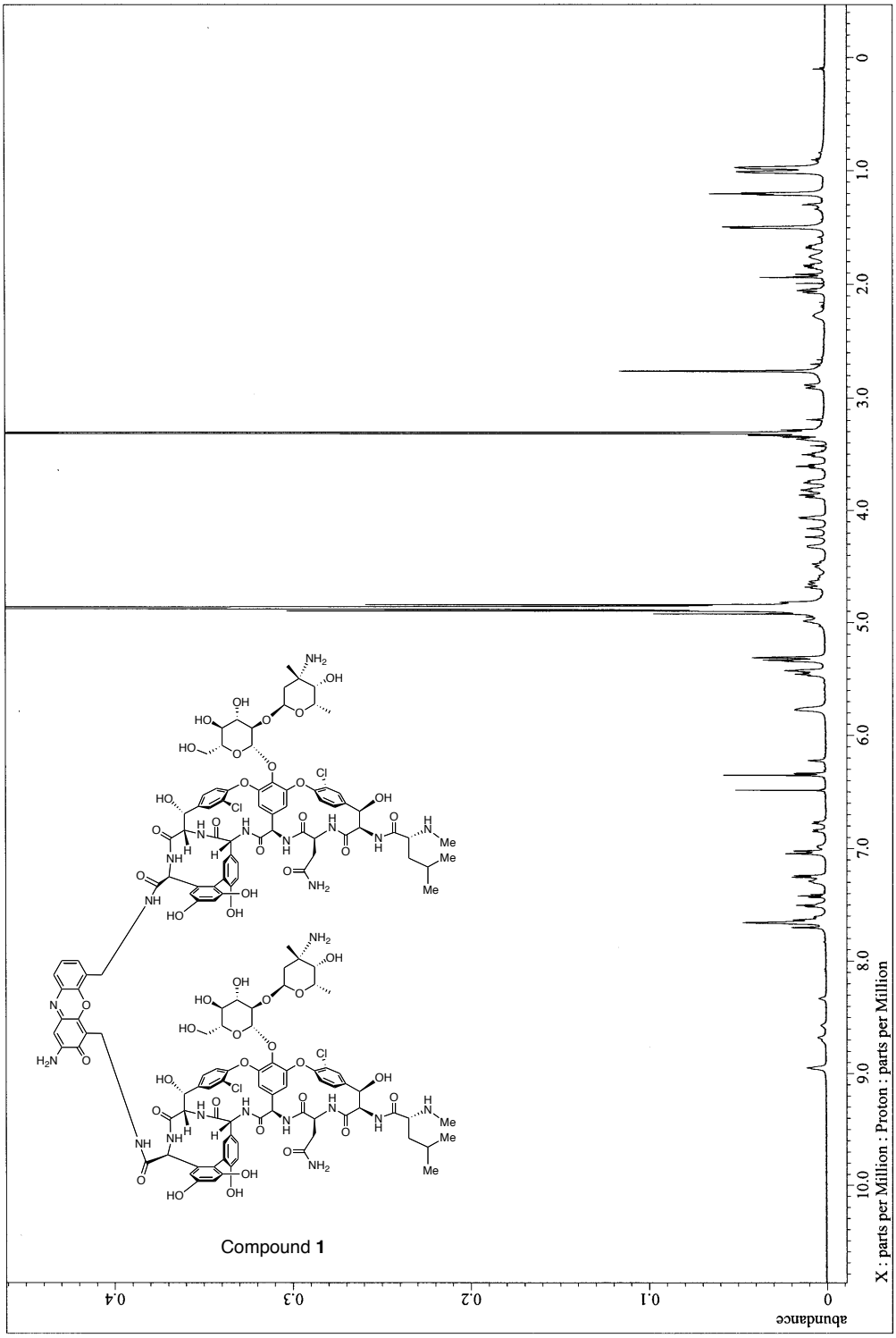
Compound **SI-3**. TFA/methanol (1:1, 11 mL) was added to crude compound **SI-8** (0.34 g) at 0 °C (ice-water bath). The reaction solution was stirred at 0 °C to room

temperature over 2.5 h. TFA (2 mL) was added to the reaction and the reaction solution was stirred for additional 3 h at room temperature. A portion of methanol was added and the reaction mixture was evaporated to remove TFA. The residue was poured into ethyl acetate (300 mL). The resulting whitish precipitate was collected by filtration (Millipore, pore size 1.0 µm, JAWP04700), washed with ethyl acetate, to give a pale brown solid (0.67 g). This crude compound **SI-3** was purified by reverse-phase column chromatography (Yamazen ODS-SM-50B, 26 x 300 mm, 50% methanol/water (0.1% TFA)), to yield compound **SI-3** as a pale red solid (0.19 g, 53%): 99% purity, R_t = 5.0 min; ^{1}H NMR (600 MHz, DMSO-*d*6 + D_2O, 22 ˚C, solvent): ∂ 8.66 (s, 1H), 7.94 (br., 1H), 7.81 (s, 1H), 7.65 (d, *J*= 7.8 Hz, 1H), 7.59 (d, *J*= 6 Hz, 1H), 7.57 (d, *J*= 9 Hz, 1H), 7.52 (d, *J*= 7.8 Hz, 1H), 7.46 (d, *J*= 8.4 Hz, 1H), 7.43-7.37 (m, 8H), 7.31 (d, *J*= 7.8 Hz, 1H), 7.22 (d, *J*= 7.2 Hz, 2H), 7.17 (s, 1H), 7.12 (d, *J*= 7.8 Hz, 1H), 6.77 (d, *J*= 9.6 Hz, 1H), 6.72 (d, *J*= 8.4 Hz, 1H), 6.39 (s, 1H), 6.22 (s, 1H), 5.69 (s, 1H), 5.47 (s, 1H), 5.37 (d, *J*= 6 Hz, 1H), 5.27 (s, 1H), 5.2 (s, 1H), 5.09 (br., 2H), 4.64 (d, *J*= 6 Hz, 1H), 4.43 (br., 1H), 4.39 (s, 1H), 3.96 (br., 2H), 3.48-3.43 (m, 5H), 3.26-3.21 (m, 3H), 2.43 (s, 3H), 2.15 (m, 1H), 2.09 (m, 1H), 1.82 (d, *J*= 12.6 Hz, 1H), 1.47 (s, 3H), 1.42 (m, 1H), 1.1 (d, *J*= 4.8 Hz, 3H), 1.06 (m, 1H); HRMS (LC-ESI-TOF) calcd. for $C_{81}H_{81}Cl_2N_{10}O_{27}$: 1695.4644 $[M+H]^+$, found 1695.4662 $[M+H]^+$.

Compound **5:** Compound **SI-3** (20 mg, 0.012 mmol) in methanol (0.2 mL) and DMF (0.8 mL) was reduced for 1.5 h with hydrogen in the presence of 10% Pd-C catalyst (21 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off by use of membrane filter (rinsed with 20 mL of methanol), and *p*-quinone (29 mg, 3 eq., dissolved in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 4 h under darkness, and then purified by gel permeation chromatography (GE Healthcare, Sephadex LH-20, 15 x 250mm, elution solvent: methanol). Further purification of this crude sample (10 mg) by HPLC (nacalai, Cosmosil $_5C_{18}$ AR-II-waters, 10 x 250 mm, 25% acetonitrile /water (0.1% TFA), flow rate 4 mL/min) gave an orange solid (2.3 mg, 12%): >99% purity, R_t= 4.0 min; ^{1}H NMR (600 MHz, DMSO-*d*6 + D_2O, 22 ˚C, solvent): ∂ 12.3 (s, 1H), 10.8 (s, 1H), 8.92 (s, 1H), 8.84 (s, 1H), 8.6 (s, 1H), 8.01 (s, 1H), 7.86 (s, 1H), 7.85 (s, 1H), 7.64 (s, 1H), 7.61 (s, 1H), 7.56 (s, 1H), 7.55 (s, 1H), 7.5 (d, *J*= 8.3 Hz, 1H), 7.45 (t, *J*= 7.9 Hz, 1H), 7.32 (m, 2H), 7.21 (t, *J*= 8.2 Hz, 1H), 6.84 (t, *J*= 7.2 Hz, 1H), 6.78 (d, *J*= 8.2 Hz, 1H), 6.71 (d, *J*= 7.6 Hz, 1H), 6.4 (d, *J*= 1.9 Hz, 1H), 6.24 (t, *J*= 2.4 Hz, 1H), 4.66 (d, *J*= 5.9 Hz, 1H), 4.52 (s, 1H), 4.46 (s, 1H), 4.41 (t, *J*= 4 Hz, 1H), 4.19 (s, 1H), 4.05 (d, *J*= 9.6 Hz, 1H), 3.45 (m, 2H), 3.26 (m, 2H), 2.87 (d, *J*= 12 Hz, 1H), 2.54 (s, 1H), 2.53 (s, 1H), 2.19 (s, 1H), 2,07 (br., 2H), 1.81 (t, *J*= 12 Hz, 1H), 1.47 (d, *J*= 5.5 Hz, 3H), 1.11 (d, *J*= 5.5 Hz, 3H); MS (LC-ESI-TOF) calcd. for $C_{148}H_{146}Cl_4N_{20}O_{50}$: 1572.4204 $[M+2H]^{2+}$; found 1572.4343 $[M+2H]^{2+}$.

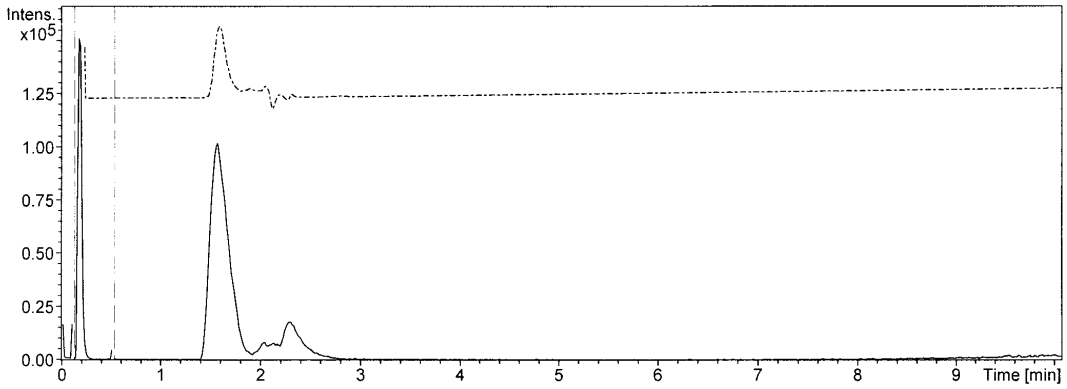
3. Spectra of Compounds.

(1) ^{1}H NMR of Compound **1** (^{1}H NMR, 600 MHz, methanol-d_4)

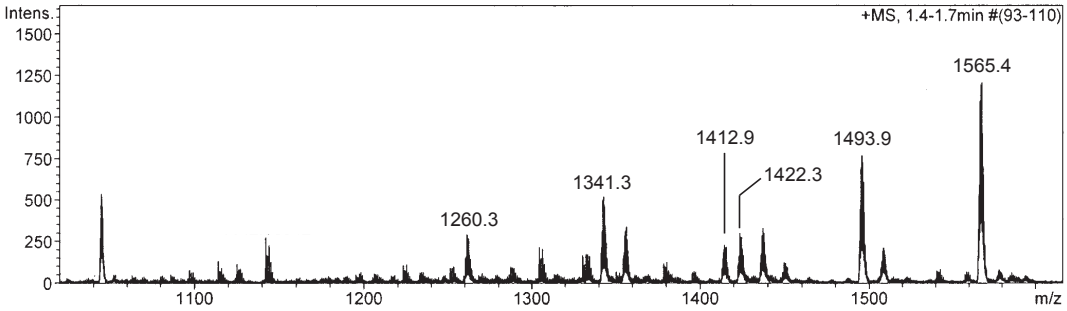


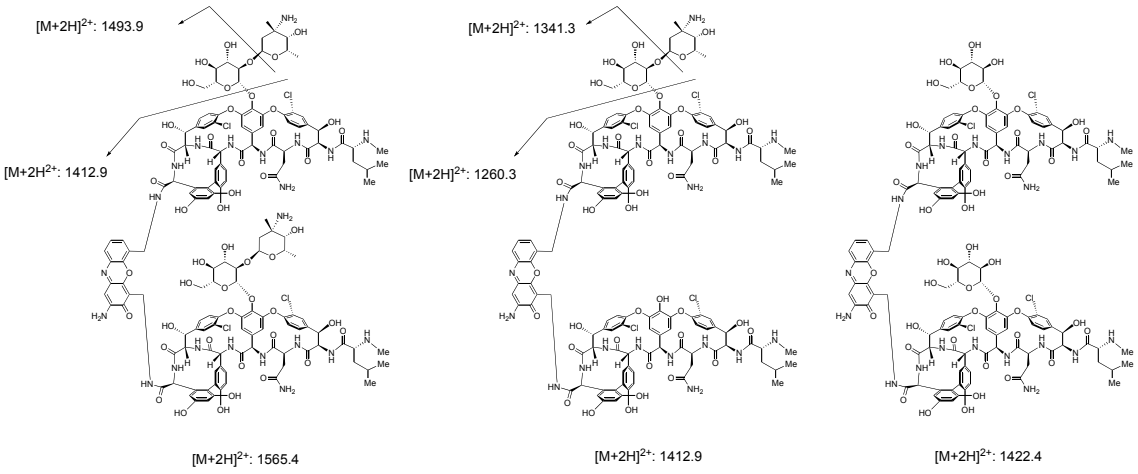
(2) MS of Compound **1** (LC-ESI (pos)-TOF).

- LC chart



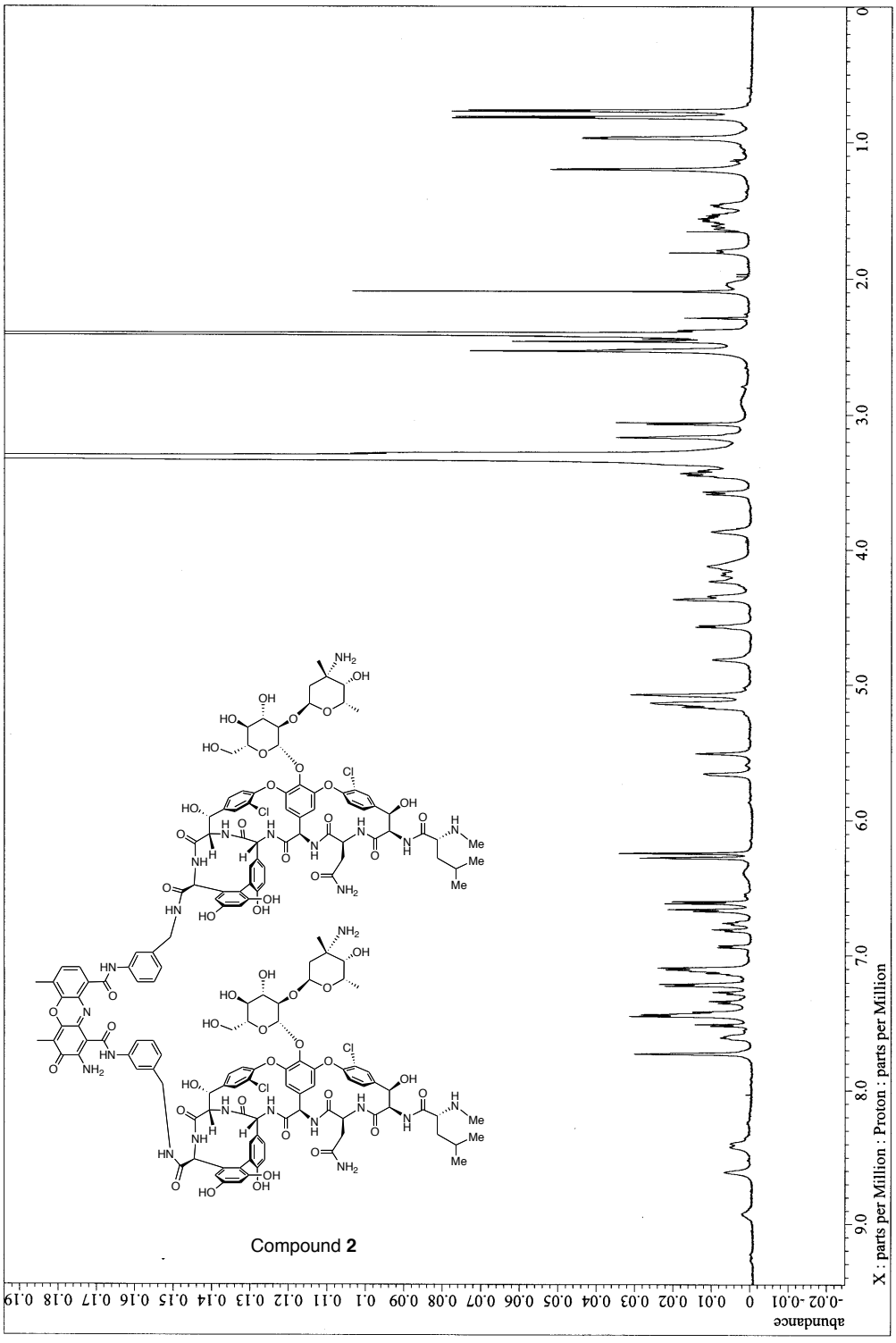
- MS chart (1.4-1.7 min)





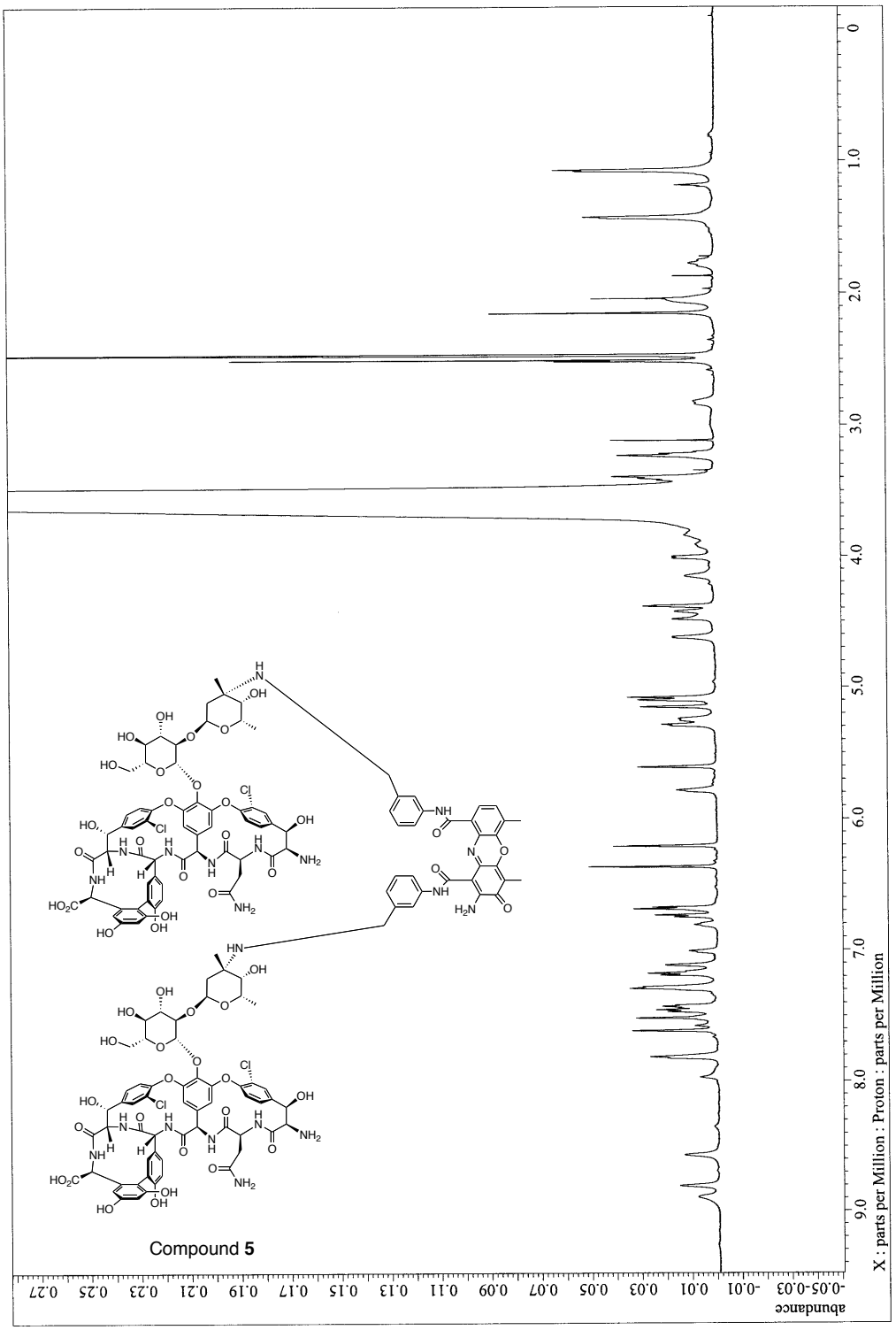
Compound **1**

(3) ^{1}H NMR of Compound **2** (^{1}H NMR, 600 MHz, DMSO-d_6 + one drop of D_2O)

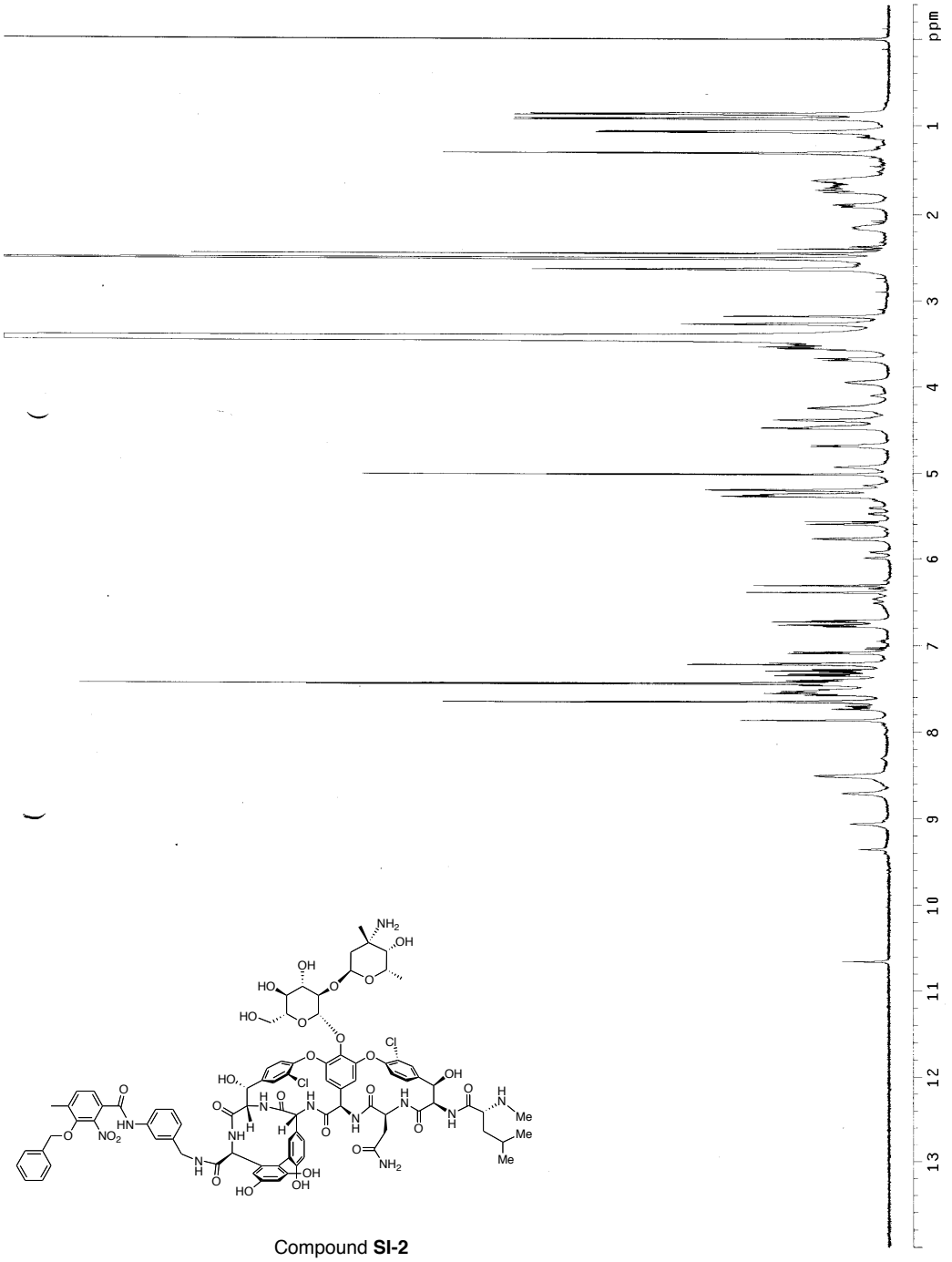


Compound **2**

(4) ^{1}H NMR of Compound **5** (^{1}H NMR, 600 MHz, DMSO-d_6 + one drop of D_2O)

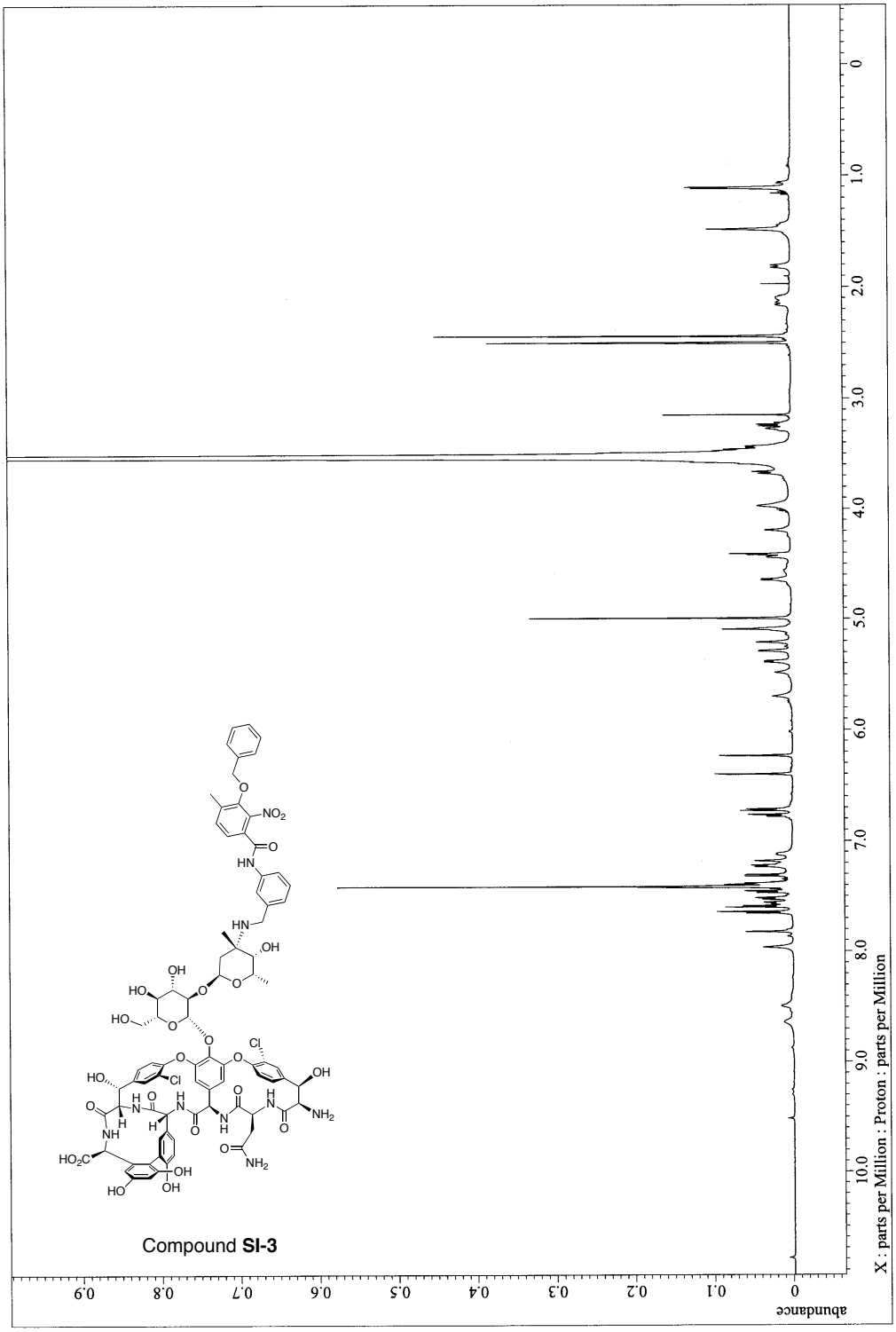


(5) ^{1}H NMR of Compound **SI-2** (^{1}H NMR, 500 MHz, DMSO-d_6 + one drop of D_2O)



Compound **SI-2**

(6) ^{1}H NMR of Compound **SI-3** (^{1}H NMR, 600 MHz, DMSO-d_6 + one drop of D_2O)



Compound **SI-3**

4. Reference

1 K. Miura, H. Yamashiro, K. Uotani, S. Kojima, T. Yutsudo, J. Lu, O. Yoshida, Y. Yamano, H. Maki and H. Arimoto, *Antimicrob. Agents Chemother.,* 2010, **54**, 960-962.