Supplementary Information

Synthesis of rigidly-linked vancomycin dimers and their in vivo efficacy against resistant bacteria

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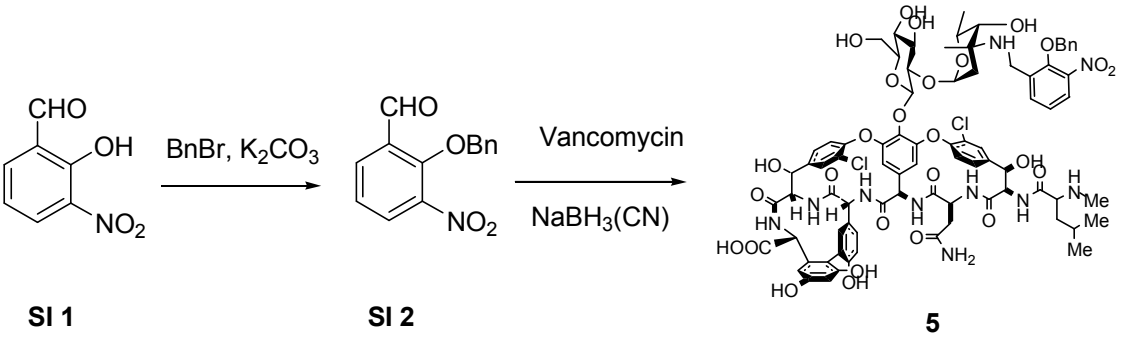
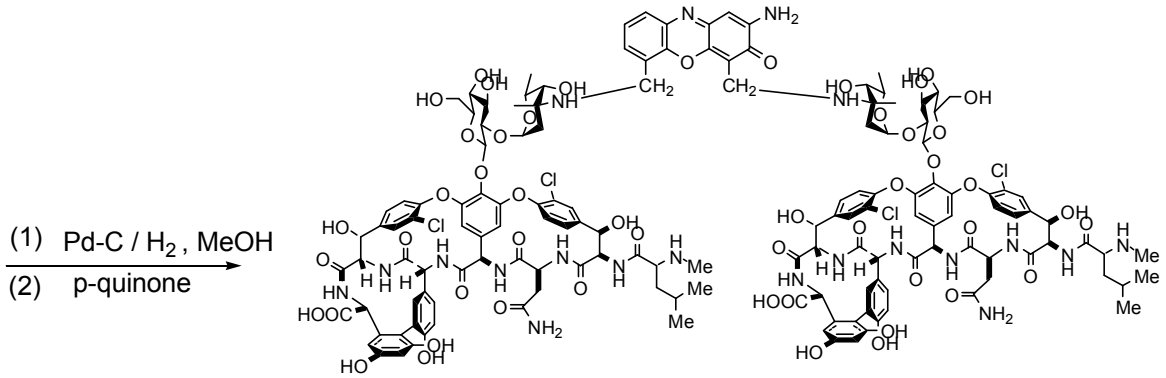
Experimental Section

General procedures

Mass spectrometry: Mass spectra were measured by Voyager (MALDI-TOF, PE Biosystems), API 3000 (ESI triple quadrupole, PE Biosystems), and Q-Tof Ultima (API Waters). Fragment analyses were done by API3000. The MALDI-TOF data of compounds were expressed with average mass (C = 12.0107, H = 1.0079, O = 15.9994, N = 14.0067, Cl = 35.4527, P = 30.9738, Na = 22.9898). The API-TOF and ESI data were expressed with monoisotopic mass.

Synthesis of dimer 1





To a solution of 2-hydroxy-3-nitrobenzaldehyde **SI 1** (0.334 g, 2 mmol, Aldrich) in dry DMF (3 mL) was added benzyl bromide (0.24 mL, 2 mmol) and finely powdered K_2CO_3 (276 mg, 2 mmol). This mixture was stirred at 65℃ under Ar

for 24 h. The reaction was poured into water. The mixture was extracted with chloroform (3×300 mL). The combined chloroform layers were washed with dil. HCl and brine, and dried over $MgSO_4$. Concentration of the chloroform solution *in vacuo* gave a yellow solid. Purification of the residue by a preparative TLC (50% EtOAc/hexane) yielded compound **SI 2** (0.31 g, 61 %). ^{1}H NMR ($CDCl_3$):δ 10.18 (s, 1H), 8.14 (d, 1H, J = 8.0 Hz), 8.08 (d, 1H, J = 7.6 Hz), 7.36-7.40 (m, 6H), 5.19 (s, 2H).

A solution of vancomycin-HCl salt (495 mg, 0.333 mmol), **SI 2** (85.7 mg, 0.333 mmol, 1 eq.), and *i*-Pr_2NEt (0.114 mL, 2 eq.) in DMF/MeOH (1:1, 20 mL) was heated at 70℃ for 3h and then allowed to cool to room temperature. $NaBH_3CN$ (84.0 mg, 1.33 mmol, 4 eq.) was added, and then the reaction mixture was stirred at 70℃ for an additional 24 h, and allowed to cool to ambient temperature. The resulting mixture was poured into Et_2O (300 mL), and the precipitate was collected by a centrifugation. Purification of the precipitate by HPLC (Develosil ODS-HG-5, Φ 20 mm×250 mm, CH_3CN:H_2O:TFA = 1:2:0.1%, flow rate 2 mL/min, UV: 215 nm, t_R = 34.21 min) yielded compound **5** (335 mg, 59%) as a pale green solid: MS (MALDI-TOF, CHCA) calcd for $C_{80}H_{86}Cl_2N_{10}NaO_{27}$ $[M+H]^+$ *m/z* 1713.49, found 1714.07; MS (ESI) $[M+H]^+$, 1599, 1448, 1305, 1143, 846, 565, 547, 403, 385

Compound **5** (61.1 mg, 0.036 mmol) in methanol (3 mL) was reduced for 3h with hydrogen in the presence of 10% Pd-C catalyst (55 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off, and *p*-quinone (7.0 mg, 0.065 mmol, in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 24 h under dark, and then poured into Et_2O (100 mL). A pink precipitate was collected by a centrifugation. Purification of the precipitate by reverse-phase HPLC (Develosil ODS-HG-5, Φ 20 mm×250 mm, CH_3CN:H_2O:TFA = 1:2:0.001, flow rate 2 mL/min, UV: 215 nm, t_R = 21.82 min) yielded 40.7 mg (68%) of compound **1**(TFA salt) as a pink solid: Purity 100 % (HPLC, Develosil ODS-HG-5, CH_3CN:H_2O:TFA = 1:2:0.001, UV: 215 nm), MS (MALDI-TOF, CHCA) calcd for $C_{146}H_{158}Cl_4N_{20}NaO_{50}$ $[M+Na]^+$ *m/z* 3157.72, found 3157.38; MS (ESI) 1827, 1684, 1568 $[M+2H]^{2+}$, 1448, 1305, 1143, 1046 $[M + 3H]^{3+}$, 784 $[M + 4H]^{4+}$

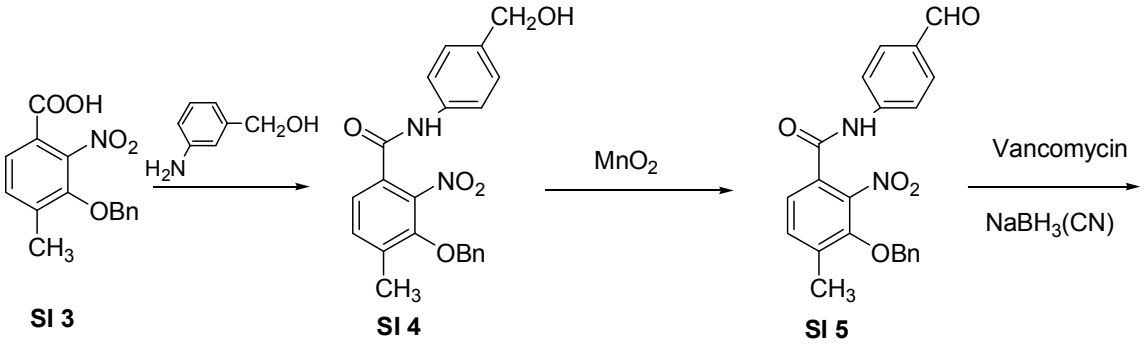
Compound **5** (120 mg, 0.054 mmol) in methanol (6 mL) was reduced for 2h with hydrogen in the presence of 10% Pd-C catalyst (110 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off, and *p*-quinone (14 mg, 0.11 mmol, in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 18 h under dark, and then poured into Et_2O (80 mL). A black solid was collected by a filtration (MILLIPORE, pore size 1.0 μm, JAWP04700). The solid was dissolved in aq. $NaHCO_3$ solution (50 mL, pH 8), which was added 1 M aq. HCl to adjust pH 3. Purification by reverse-phase column chromatography (Lobar LiChroprep RP-18 Groβe B, Merck, CH_3CN:0.005 M aq. HCl =2:8) yielded 34 mg (41%) of compound **1** (HCl salt) as a red solid: Purity 93.4 % (HPLC, Cosmosil ODS-AR-II, Φ 4.6 mm×150 mm, CH_3CN:H_2O:TFA = 15:85:0.001 to 0:100:0.001 for over 10 min, flow rate 1 mL/min, UV: 280 nm), MS(API) calcd for $C_{146}H_{160}Cl_4N_{20}O_{50}$ $[M+2H]^{2+}$ *m/z* 1567.40, found 1567.99.

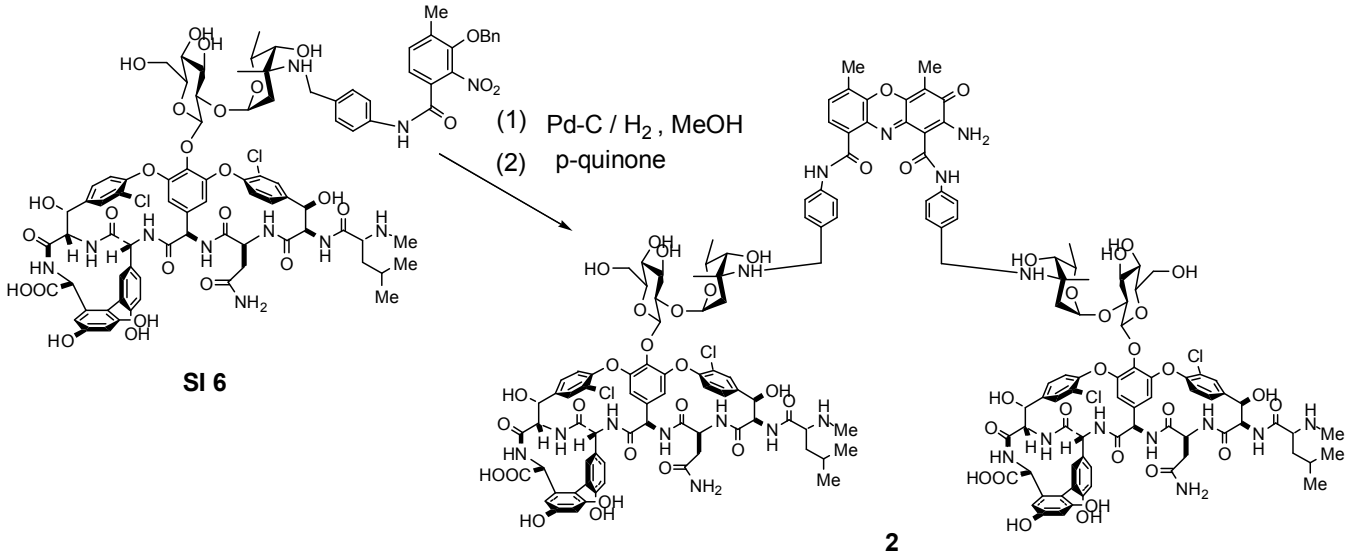
Synthesis of dimer 2

A mixture of acid **SI 3** (0.574 g, 2.0 mmol) and oxalyl chloride (0.226 mL, 2.6 mmol, 1.3 equiv) in dichloromethane (20 mL), was added 2 drops of DMF at room temperature. The mixture was heated to reflux for 40 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 (10 mL) of 4-aminobenzyl alcohol (0.246 g, 2.0 mmol, Wako Chemical), and triethylamine (0.348 mL, 2.5 mmol) at ice cold. The solution was stirred at 0 ℃ for 30 min. The reaction was worked up by pouring the solution into aqueous $NaHCO_3$. The resulting mixture was extracted with ethyl acetate (3×30 mL). The ethyl acetate extracts were combined, and washed with water and brine, and finally dried over $MgSO_4$. Removal of solvents in vacuo afforded **SI 4** as a pale yellow solid. The product was recrystallized from EtOAc/hexane to give **SI 4** (0.588 g, colorless crystal, 75%). ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H,), 7.55 (s, 1H), 7.34 (d,

2H, J = 7.6 Hz), 7.36-7.45 (m, 7 H), 5.02 (s, 2H), 4.67 (s, 2H), 2.40 (s, 3H).

A mixture of **SI 4** (0.588 g), MnO_2 (3.00 g) in dichloromethane (20 mL) was stirred at room temperature for 3 h.. Evaporation of solvent in vacuo gave crude product **SI 5** (0.460 g, 77%), which was used for next step without purification. ^{1}H NMR($CDCl_3$): δ 9.95 (s, 1H,), 7.89 (d, 2H, J = 8.3 Hz), 7.76 (d, 2H, J = 8.3 Hz), 7.39-7.45 (m, 7 H), 5.03 (s, 2H), 2.43 (s, 3H).



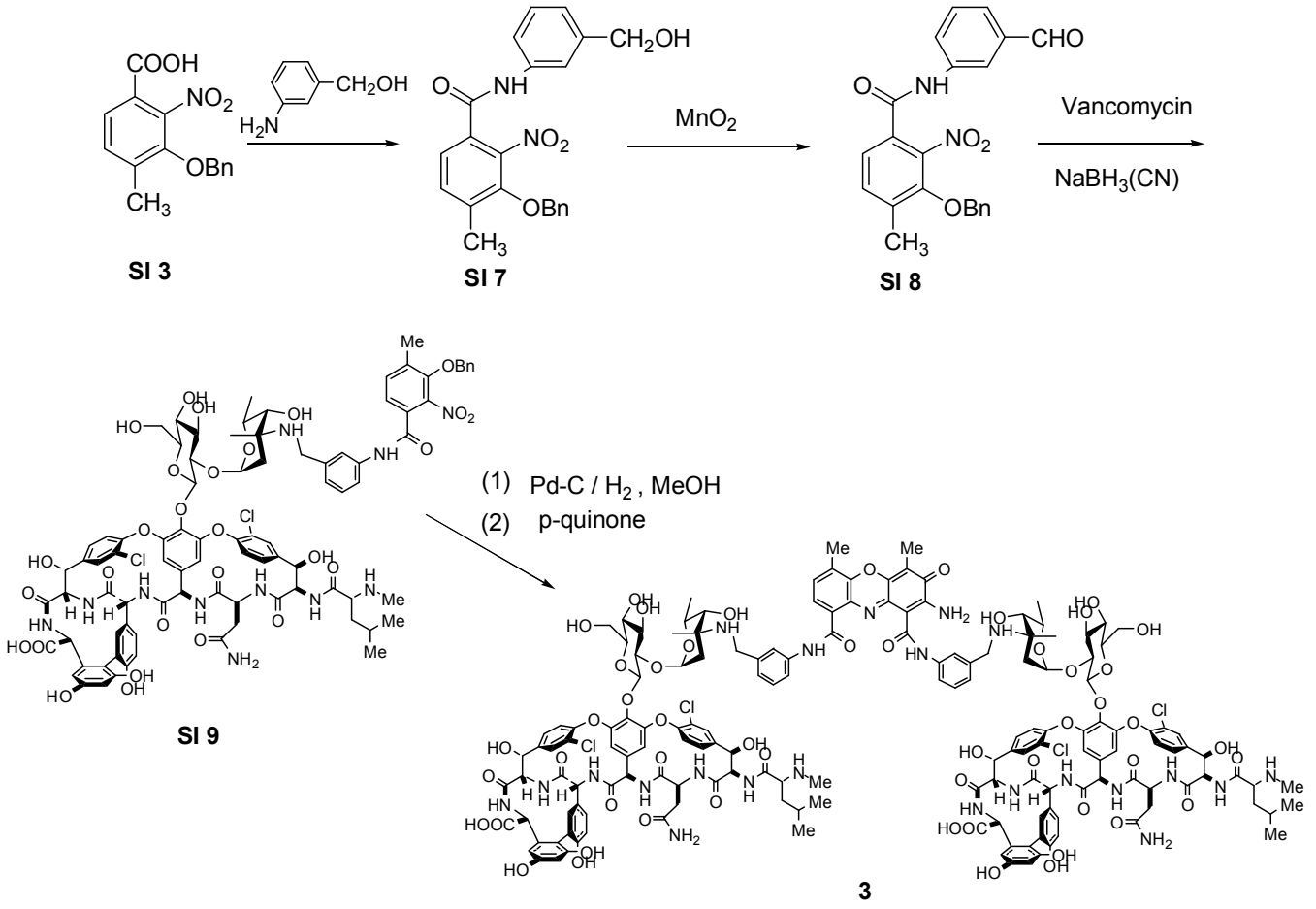


A solution of vancomycin-HCl salt (0.495 mg, 0.333 mmol), **SI 5** (0.130 g), and *i*-Pr_2NEt (0.114 mL, 2 eq.) in DMF/MeOH (1:1, 20 mL) was heated at 70℃ for 2h and then allowed to cool to room temperature. $NaBH_3CN$ (83.6 mg, 1.33 mmol, 4 eq.) was added, and then the reaction mixture was stirred at 70℃ for an additional 24 h, and allowed to cool to ambient temperature. The resulting mixture was poured into Et_2O (400 mL), and a white precipitate of **SI 6** was collected by a centrifugation. Purification of precipitate by reverse-phase column chromatography (Cosmosil ODS-OPN, CH_3CN:H_2O:TFA = 1:1:0.01) yielded **SI 6** (0.376 g, 62 %) as a white solid: R_f = 0.45 (ODS TLC, CH_3CN:H_2O:TFA = 1:1:0.01), MS (MALDI-TOF, CHCA) calcd for $C_{88}H_{93}Cl_2N_{11}NaO_{28}$ $[M+Na]^+$ 1846.63, found 1846.15. This crude compound was used for next step without further purification.

Compound **SI 6** (65.5 mg, 0.036 mmol) in methanol (3 mL) was reduced for 3h with hydrogen in the presence of 10% Pd-C catalyst (55 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off, and *p*-quinone (7.0 mg, 0.065 mmol, in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 24 h under dark, and then poured into Et_2O (50 mL). A pink precipitate was collected by a centrifugation. Purification of the precipitate by reverse-phase HPLC (Develosil ODS-HG-5, Φ 20 mm×250 mm,

CH_3CN:H_2O:TFA = 1:2:0.001, flow rate 2 mL/min, UV: 215 nm, t_R = 43.44 min) yielded 25.8 mg (40%) of compound **2** (TFA salt) as a pink solid: Purity 95.7 % (HPLC, Develosil ODS-HG-5, CH_3CN:H_2O:TFA = 1:2:0.001, UV: 215 nm), MS (MALDI-TOF, CHCA) calced for $C_{162}H_{172}Cl_4N_{22}NaO_{52}$ $[M+Na]^+$ 3424.02, found 3424.77, MS (ESI) 1701 $[M+2H]^{2+}$, 1448, 1305, 1143, 1134 $[M+3H]^{3+}$, 851 $[M+4H]^{4+}$

Synthesis of dimer 3



A mixture of acid **SI 3** (0.574 g, 2.0 mmol) and oxalyl chloride (0.226 mL, 2.6 mmol, 1.3 equiv) in dichloromethane (20 mL), was added 2 drops of DMF at room temperature. The mixture was heated to reflux for 40 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 (10 mL) of 3-aminobenzyl alcohol (0.246 g, 2.0 mmol, Wako Chemical), and triethylamine (0.348 mL, 2.5 mmol) at ice cold. The solution was stirred at 0 ℃ for 30 min. The reaction was worked up by pouring the solution into aqueous $NaHCO_3$. The resulting mixture was extracted with ethyl acetate (3×30 mL). The ethyl acetate extracts were combined, and washed with water and brine, and finally dried over $MgSO_4$. Removal of solvents in vacuo afforded **SI 7** as a pale yellow solid. The product was further purified by column chromatography (EtOAc), yielding **SI 7** (0.678 g, 86%). R_f = 0.62 (SiO_2 TLC, EtOAc), ^{1}H NMR ($CDCl_3$): δ 7.78 (s, 1H,), 7.63 (s, 1H), 7.36-7.45 (m, 9H), 7.19 (d, 1 H, J = 7.6 Hz), 5.03 (s, 2H), 4.72 (s, 2H), 2.42 (s, 3H).

A mixture of **SI 7** (0.678 g), MnO_2 (3.07 g) in dichloromethane (20 mL) was stirred at room temperature for 2 h.. Evaporation of solvent in vacuo gave crude product (0.554 g), which was purified by a column chromatography (EtOAc) yielding compound **SI 8** (0.491 g, 73%). ^{1}H NMR($CDCl_3$): δ 10.01 (s, 1H,), 7.92 (d, 1H, J = 7.6 Hz), 7.85 (s, 1H), 7.71 (d, 1H, J = 8.0 Hz), 7.54 (t, 1H, J = 8.0 Hz), 7.37-7.45 (m, 7 H), 5.03 (s, 2H), 2.42 (s, 3H).

A solution of vancomycin-HCl salt (495 mg, 0.333 mmol), **SI 8** (85.7 mg, 0.333 mmol, 1 eq.), and *i*-Pr_2NEt (0.114 mL, 2 eq.) in DMF/MeOH (1:1, 20 mL) was heated at 70℃ for 2h and then allowed to cool to room temperature. $NaBH_3CN$ (84.0 mg, 1.33 mmol, 4 eq.) was added, and then the reaction mixture was stirred at 70℃ for an additional 24 h, and allowed to cool to ambient temperature. The resulting mixture was poured into Et_2O (400 mL), and a white precipitate of **SI 9** was collected by a centrifugation (0.565 g, 93%). This crude compound was used for next step without further purification: R_f = 0.51 (ODS TLC, CH_3CN:H_2O:TFA = 1:1:0.01), MS (MALDI-TOF, CHCA) calcd for $C_{88}H_{93}Cl_2N_{11}NaO_{28}$ $[M+Na]^+$ 1846.63, found 1846.37.

Compound **SI 9** (51.8 mg, 0.028 mmol) in methanol (3 mL) was reduced for 3h with hydrogen in the presence of 10% Pd-C catalyst (50 mg) to give the corresponding aminophenol intermediate. This was used for next step without purification. MS (MALDI-TOF, CHCA) calcd for $C_{88}H_{93}Cl_2N_{11}NaO_{28}$ $[M+Na]^+$ 1846.63, found 1846.37; MS (ESI) 1822 $[M+H]^+$, 1431, 1305, 1143, 913 $[M+2H]^{2+}$, 680, 518.

To a methanolic solution (3 mL) of the aminophenol, *p*-quinone (5.7 mg, 0.053 mmol, in 0.5 mL of methanol) was added. The mixture was stirred at room temperature for 22 h under dark, and then poured into Et_2O (50 mL). A pink precipitate was collected by a centrifugation. Purification of the precipitate by reverse-phase HPLC (Develosil ODS-HG-5, Φ 20 mm×250 mm, CH_3CN:H_2O:TFA = 1:2:0.1%, flow rate 2 mL/min, UV: 215 nm, t_R = 35.33 min) yielded **3** (TFA salt, 25.9 mg, 50%) as a pink solid: Purity 98.7 % (HPLC, Develosil ODS-HG-5, CH_3CN:H_2O:TFA = 1:2:0.001, UV: 215 nm), MS (MALDI-TOF, CHCA) calcd for $C_{162}H_{172}Cl_4N_{22}NaO_{52}$ $[M+Na]^+$ 3424.02, found 3424.16, MS (ESI) 2093, 1967, 1701 $[M+2H]^{2+}$, 1448, 1305, 1143, 1134 $[M+3H]^{3+}$, 851 $[M+4H]^{4+}$.

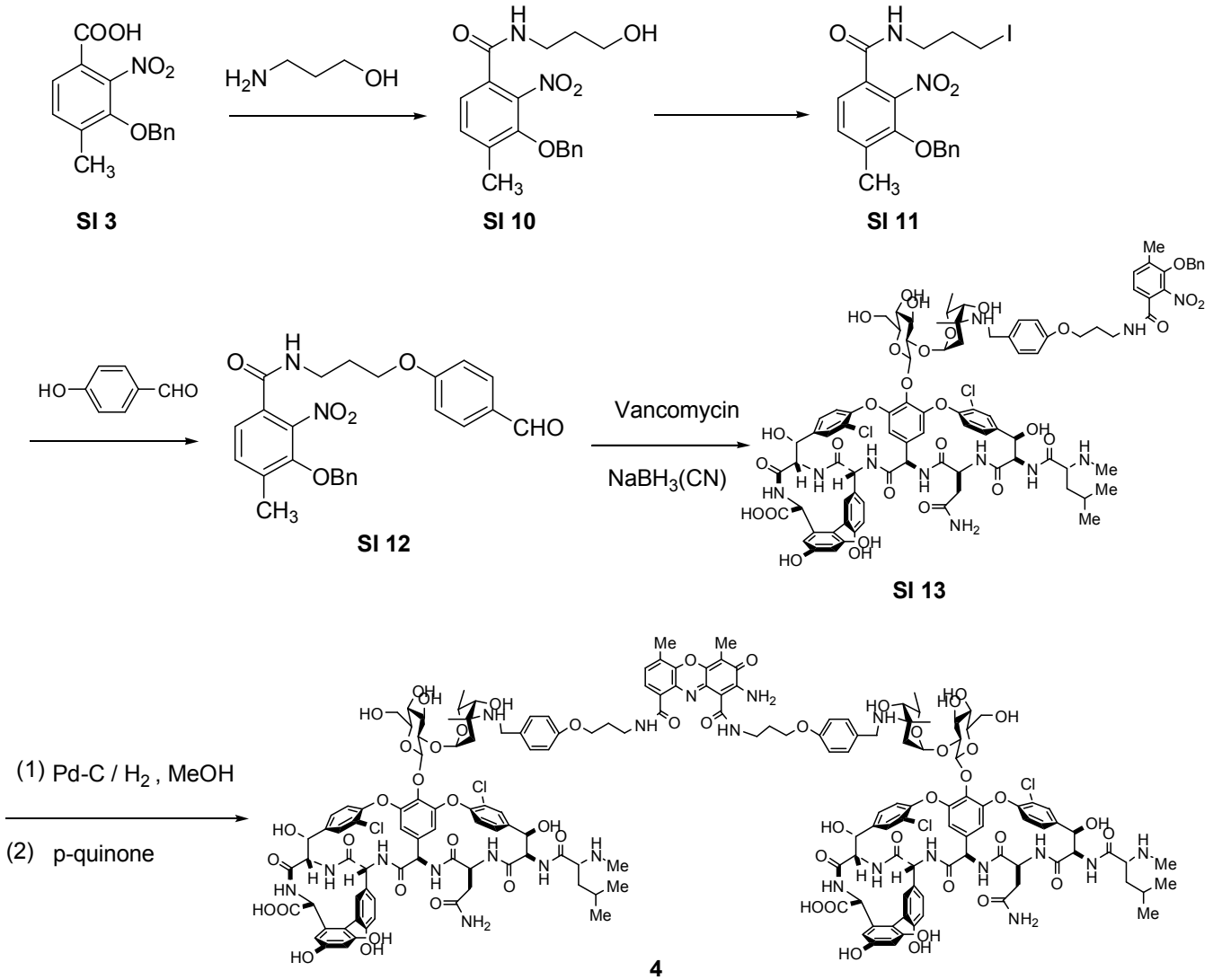
Compound **SI 9** (100 mg, 0.055 mmol) in methanol (3 mL) was reduced for 2 h with hydrogen in the presence of 10% Pd-C catalyst (92 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off, and *p*-quinone (7.5 mg, 0.058 mmol, in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 12 h under dark, and then poured into Et_2O (80 mL). A blown solid was collected by a filtration (MILLIPORE, pore size 1.0 μm, JAWP04700). The solid was dissolved in aq. $NaHCO_3$ solution (20 mL, pH 8), which was added 1 M aq. HCl to adjust pH 4. Purification by reverse-phase column chromatography (Lobar LiChroprep RP-18 Groβe B, Merck, CH_3CN:0.005 M aq. HCl =3:17) yielded 49 mg (53%) of compound **3** (HCl salt) as an orange solid: Purity 88.5 % (HPLC, Cosmosil ODS-AR-II, Φ 4.6 mm×150 mm, CH_3CN:H_2O:TFA = 15:85:0.001 to 0:100:0.001 for over 10 min, flow rate 1 mL/min, UV: 280 nm, MS(API) calcd for $C_{162}H_{172}Cl_4N_{22}O_{52}$ $[M+2H]^{2+}$ *m/z* 1700.5, found 1701.5, MS (ESI) 2093, 1967, 1701 $[M+2H]^{2+}$, 1431, 1305, 1143, 1134 $[M+3H]^{3+}$, 851 $[M+4H]^{4+}$.

Synthesis of dimer 4

A mixture of acid **SI 3** (2.87 g, 10.0 mmol) and oxalyl chloride (1.13 mL, 1.3 equiv) in dichloromethane (80 mL), was added 2 drops of DMF at room temperature. The mixture was heated to reflux for 40 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 (80 mL) of 3-amino-1-propanol (0.92 mL, 12 mmol, 1.2 equiv), and triethylamine (3.48 mL, 25 mmol, 2.5 equiv) at ice cold. The solution was stirred at 0 ℃ for 30 min. The reaction was worked up by pouring the solution into aq. $NaHCO_3$ solution. The resulting mixture was extracted with ethyl acetate (3×40 mL). The ethyl acetate extracts were combined, and washed with water and brine, and finally dried over $MgSO_4$. Removal of solvents in vacuo afforded **SI 10** as a pale yellow solid. This crude material was subjected to next step without further purification. R_f = 0.65 (SiO_2 TLC, EtOAc:EtOH = 4:1), ^1H NMR δ 7.28-7.42 (m, 7H,), 4.98 (s, 2 H), 3.73 (t, 2H, J = 5.6 Hz), 3.55 (q, 2 H, J = 5.6 Hz), 2.36 (s, 3H), 1.77 (m, 2H).

To a stirred solution of thionyl chloride (2.04 mL, 28 mmol) in DMF (10 mL) at 0℃ under argon were added KI (24.5 g, 0.15 mol in 70 mL of DMF) and **SI 10** (10 mmol in 30 mL of DMF) through syringe. The reaction mixture was stirred at 50℃ for 3 h before quenching with water. The mixture was extracted with ether (3×100 mL), and the combined ether extracts were washed successively with aqueous sodium thiosulfate solution and water. The organic solution was dried over anhydrous $MgSO_4$. Evaporation of solvent in vacuo afforded **SI 11** as a pale yellow solid. The product was recrystallized from EtOAc/hexane to give 2.41 g (53.1% in two steps) of pale yellow crystals. ^{1}H NMR δ 7.36-7.43 (m, 6H,), 7.28 (d, 1H, J = 8.4 Hz), 4.99 (s, 2 H), 3.51 (q, 2H, J = 6.4 Hz), 3.24 (t, 2 H, J = 6.4 Hz), 2.38 (s, 3H), 2.12 (m, 2H).

To a solution of 4-hydroxybenzaldehyde (0.336 g, 2.75 mmol) in acetone (10 mL) were added anhydrous potassium carbonate (0.345 g, 2.5 mmol) and compound **SI 11** (1.135 g, 2.5 mmol). The reaction was heated at reflux for 20 h. The mixture was then cooled to room temperature, and poured into water (200 mL). Filtration afforded the product as a white solid, which was purified by a column chromatography (SiO_2, 5% MeOH/$CHCl_3$) yielding **SI 12** (0.504 g, 45%). R_f = 0.50 (SiO_2 TLC, $CHCl_3$:MeOH = 95:5), ^{1}H NMR($CDCl_3$): δ 9.89 (s, 1H,), 7.84 (d, 2H, J = 8.4 Hz), 7.27-7.41 (m, 7H), 7.27 (s, 1H), 7.01 (d, 2H, J = 8.4 Hz), 4.99 (s, 2 H), 4.17 (t, 2H, J = 6.0 Hz), 3.64 (t, 2 H, J = 6.0 Hz), 2.38 (s, 3H), 2.15 (m, 3H).



A solution of vancomycin-HCl salt (495 mg, 0.333 mmol), **SI 12** (149 mg, 0.333 mmol, 1 eq.), and *i*-Pr_2NEt (0.114 mL,

2 eq.) in DMF/MeOH (1:1, 20 mL) was heated at 70℃ for 2h and then allowed to cool to room temperature. $NaBH_3CN$ (83.6 mg, 1.33 mmol, 4 eq.) was added, and then the reaction mixture was stirred at 70℃ for an additional 24 h, and allowed to cool to ambient temperature. The resulting mixture was poured into Et_2O (400 mL), and a white precipitate was collected by a centrifugation. Purification of the precipitate by reverse-phase column chromatography (Cosmosil ODS-OPN, CH_3CN:H_2O:TFA = 2:1:0.01) yielding **SI 13** (0.396 g, 63%) as a white solid: R_f = 0.34 (ODS TLC, CH_3CN:H_2O:TFA = 1:1:0.01), MS (MALDI-TOF, CHCA) calcd for $C_{91}H_{99}Cl_2N_{11}NaO_{29}$ $[M+Na]^+$ 1904.71, found 1905.04; , MS (ESI) 1880 $[M+H]^+$, 1448, 1305, 1143, 942 $[M+2H]^{2+}$, 738, 433, 327.

Compound **SI 13** (54 mg, 0.029 mmol) in methanol (3 mL) was reduced for 3h with hydrogen in the presence of 10% Pd-C catalyst (50 mg) to give the corresponding aminophenol intermediate. The Pd catalyst was filtered off, and *p*-quinone (5.7 mg, 0.053mmol, in 0.5 mL of methanol) was added to the filtrate. The mixture was stirred at room temperature for 24 h under dark, and then poured into Et_2O (50 mL). A pink precipitate was collected by a centrifugation. Purification of the precipitate by reverse-phase HPLC (Develosil ODS-HG-5, Φ 20 mm×250 mm, CH_3CN:H_2O:TFA = 1:2:0.001, flow rate 2 mL/min, UV: 215 nm, t_R = 34.81 min) yielded 31.3 mg (62%) of dimer **4** (TFA salt) as a pink solid: Purity 98.5 % (HPLC, Develosil ODS-HG-5, CH_3CN:H_2O:TFA = 1:2:0.001, UV: 215 nm), (MALDI-TOF, CHCA) calcd for $C_{168}H_{184}Cl_4N_{22}NaO_{54}$ $[M+Na]^+$ 3540.18, found 3541.15, MS (ESI) 2209, 2066, 1759 $[M+2H]^{2+}$, 1448, 1305, 1173, 1143, 880.