Supporting Information (SI) Appendix

Peripheral Modifications of [Ψ[CH_2NH]Tpg^4]Vancomycin with Added Synergistic Mechanisms of Action Provide Durable and Potent Antibiotics

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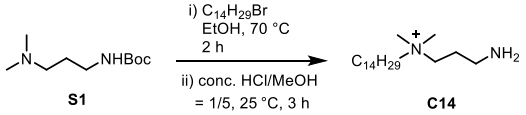
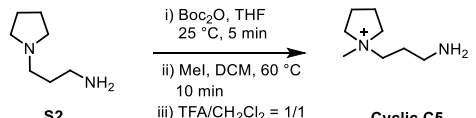
For compounds **9**-**18**: Rapidly exchanging amide NH signals and OH signals are absent in ^{1}H NMR spectra taken in CD_3OD or D_2O and often broadened in ^{1}H NMR spectra taken in DMSO-d_6. This leads to a lower proton count in the ^{1}H NMR spectra listings and in the presented spectra.



S1 **C14**

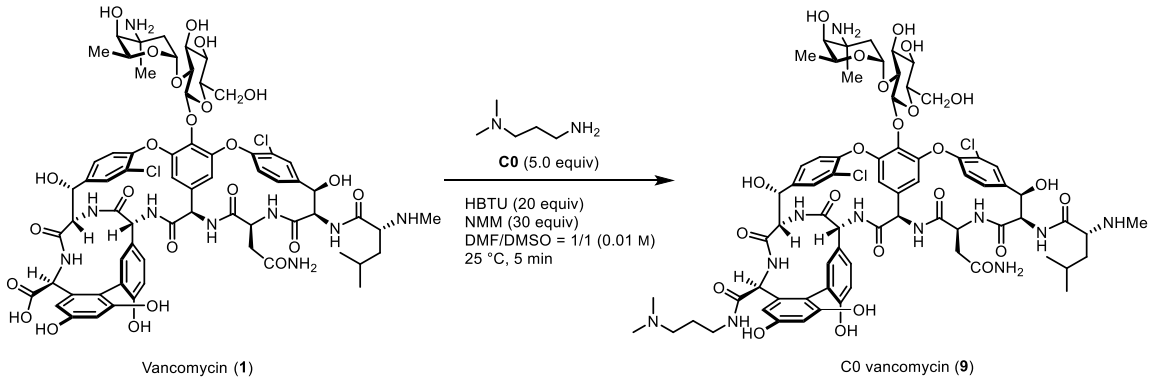
Compound C14: A solution of **S1** (300 mg, 1.5 mmol) in anhydrous EtOH (4 mL) was treated with $C_{14}H_{29}Br$ (0.74 g, 3.0 mmol) at 25 °C and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was cooled to 25 °C and the solvent was removed under a stream of N_2. The residue was purified by flash chromatography (SiO_2, 5–15% MeOH/CH_2Cl_2) to afford the corresponding ammonium salt as a yellow oil. This oil was treated with conc. HCl/MeOH = 1/5 (2 mL) at 25 °C and the mixture was stirred at 25 °C for 3 h. The solvent and HCl were removed under a stream of N_2 to afford **C14** (271 mg, 61%, 2 steps) as a white solid identical in all respects with authentic material (^{1}H NMR, D_2O).[51]



S2 **Cyclic C5**

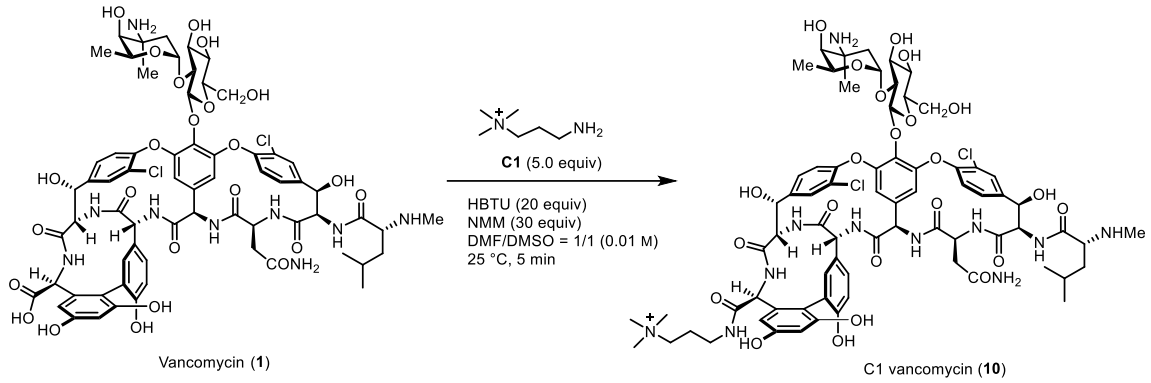
Compound Cyclic C5: A solution of **S2** (300 mg, 2.4 mmol) in anhydrous THF (3 mL) was treated with Boc_2O (510 mg, 2.3 mmol) at 25 °C and the reaction mixture was stirred at 25

°C for 5 min. The solvent was removed under a stream of N_2 to afford the crude Boc protected amine as a colorless oil. This oil was dissolved in anhydrous CH_2Cl_2 (2 mL) and treated with MeI (3.3 g, 23.4 mmol) at 25 °C and the reaction mixture was stirred at 60 °C for 10 min. The reaction mixture was cooled to 25 °C and the solvent and MeI were removed under a stream of N_2. The residue was purified by flash chromatography (SiO_2, 5–20% MeOH/CH_2Cl_2) to afford the corresponding ammonium salt as a yellow oil. This oil was treated with TFA/CH_2Cl_2 (1/1, 2 mL) at 25 °C and the reaction mixture was stirred at 25 °C for 1 h. The solvent and TFA were removed under a stream of N_2 to afford **Cyclic C5** (154 mg, 46%, 3 steps) as a yellow oil: ^{1}H NMR (D_2O, 600 MHz, 298 K) δ 3.50–3.39 (m, 4H), 3.38–3.34 (m, 2H), 2.97 (s, 3H), 2.94 (t, 2H, J = 7.2 Hz), 2.15–2.05 (m, 6H); ESI-TOF HRMS *m/z* 143.1547 (M + H^+, $C_8H_{19}N_2$ requires 143.1548).

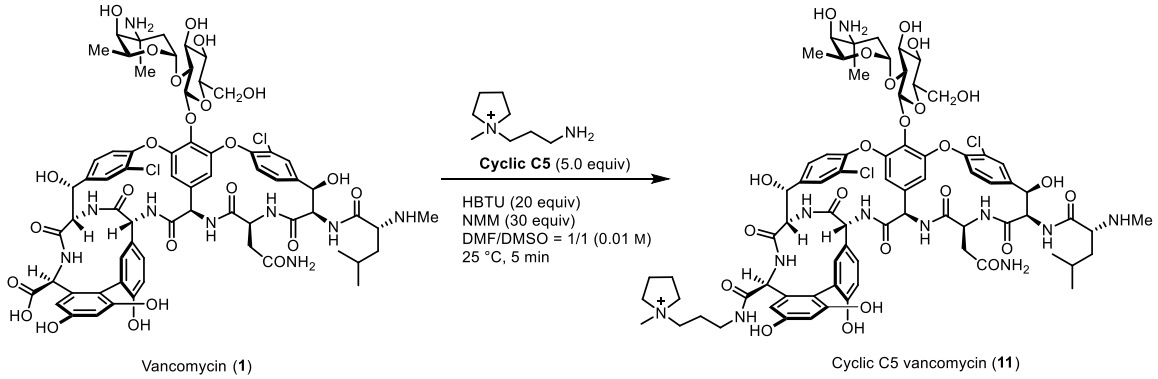


Vancomycin (**1**) C0 vancomycin (**9**)

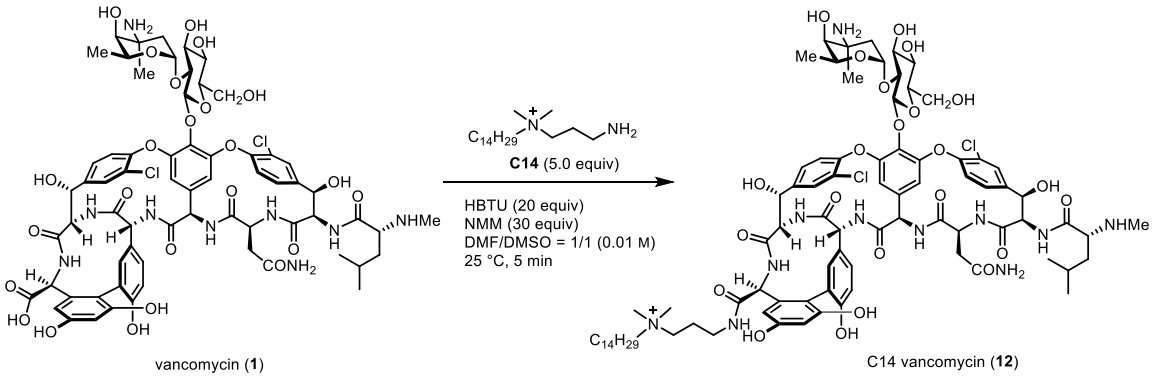
Compound 9: A solution of **1** (2.0 mg, 1.4 μmol) in DMF/DMSO (1/1, 60 μL) was treated with **C0** (1 M in DMF/DMSO = 1/1, 7.0 μL, 7.0 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 41.2 μL, 41.2 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 27.6 μL, 27.6 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 3 mL/min, t_R = 20.8 min) to afford **9** (1.4 mg, 64%) as a white amorphous solid identical in all respects with authentic material (^{1}H NMR, D_2O).[51]



Compound 10: A solution of **1** (1.5 mg, 1.0 μmol) in DMF/DMSO (1/1, 100 μL) was treated with **10** (1 M in DMF/DMSO = 1/1, 5.2 μL, 5.2 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 31.2 μL, 31.2 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 20.8 μL, 20.8 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 3 mL/min, t_R = 20.6 min) to afford **10** (1.1 mg, 68%) as a white film: ^{1}H NMR (DMSO-d_6, 600 MHz, 298 K) δ9.43 (br s, 1H), 9.02 (s, 1H), 8.69 (s, 1H), 8.56 (s, 1H), 8.20 (s, 1H), 7.82 (s, 1H), 7.76 (s, 1H), 7.73 (s, 1H), 7.58 (d, 2H, *J* = 8.4 Hz), 7.47 (d, 2H, *J* = 9.0 Hz), 7.37 (d, 1H, *J* = 8.4 Hz), 7.31 (s, 1H), 7.19 (d, 1H, *J* = 8.4 Hz), 7.02 (br s, 1H), 6.88 (d, 1H, *J* = 10.8 Hz), 6.78 (d, 1H, *J* = 8.4 Hz), 6.71 (d, 1H, *J* = 8.4 Hz), 6.39 (s, 1H), 6.22 (s, 1H), 5.76 (s, 1H), 5.57 (s, 1H), 5.36 (s, 1H), 5.26 (d, 1H, *J* = 7.8 Hz), 5.23 (s, 1H), 5.20 (s, 1H), 4.96 (s, 1H), 4.69 (d, 1H, *J* = 7.8 Hz), 4.48 (s, 1H), 4.28 (s, 1H), 4.25 (d, 1H, *J* = 5.4 Hz), 3.96 (s, 2H), 3.69 (s, 1H), 3.67 (s, 1H), 3.60–3.40 (m, 5H), 3.27 (s, 1H), 3.25–3.10 (m, 4H), 3.09–3.05 (m, 2H), 3.00 (s, 9H), 2.69 (s, 6H), 2.66 (s, 1H), 2.25–2.10 (m, 1H), 1.91 (d, 1H, *J* = 11.4 Hz), 1.85 (s, 2H), 1.74 (d, 1H, *J* = 13.2 Hz), 1.69–1.64 (m, 1H), 1.59–1.51 (m, 2H), 1.30 (s, 3H), 1.07 (d, 3H, *J* = 6.0 Hz), 0.90 (d, 3H, *J* = 6.0 Hz), 0.85 (d, 3H, *J* = 6.0 Hz); ESI-TOF HRMS *m/z* 774.2867 ($[M + 2H]^{+2}$, $C_{72}H_{90}Cl_2N_{11}O_{23}$ requires 774.2861).

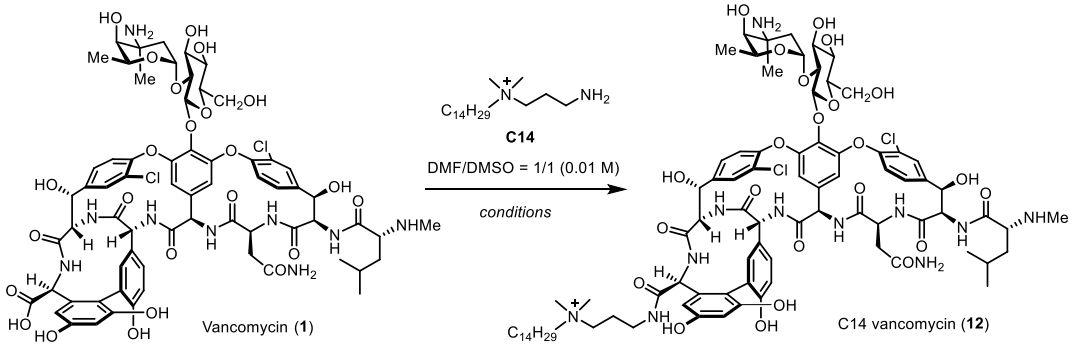


Compound 11: A solution of **1** (1.8 mg, 1.1 μmol) in DMF/DMSO (1/1, 50 μL) was treated with **Cyclic C5** (1 M in DMF/DMSO = 1/1, 5.5 μL, 5.5 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 32.7 μL, 32.7 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 21.8 μL, 21.8 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 3 mL/min, t_R = 20.4 min) to afford **11** (1.2 mg, 61%) as a white film: ^{1}H NMR (DMSO-d_6, 600 MHz, 298 K) δ 9.14 (br s, 2H), 8.99 (s, 1H), 8.80–8.65 (m, 1H), 8.56 (s, 1H), 8.18 (s, 1H), 7.85–7.81 (m, 1H), 7.67 (s, 3H), 7.60–7.45 (m, 4H), 7.35 (dd, 1H, *J* = 6.6, 3.0 Hz), 7.30 (s, 1H), 7.25 (d, 1H, *J* = 8.4 Hz), 7.20 (d, 1H, *J* = 7.8 Hz), 7.08 (br s, 1H), 6.85 (d, 1H, *J* = 11.4 Hz), 6.78 (d, 1H, *J* = 8.4 Hz), 6.70 (d, 1H, *J* = 8.4 Hz), 6.38 (d, 1H, *J* = 2.4 Hz), 6.22 (d, 1H, *J* = 2.4 Hz), 5.77 (d, 1H, *J* = 7.8 Hz), 5.58 (d, 1H, *J* = 13.2 Hz), 5.35–5.15 (m, 6H), 4.93 (br s, 1H), 4.69 (t, 1H, *J* = 5.4 Hz), 4.49 (d, 2H, *J* = 5.4 Hz), 4.27 (d, 2H, *J* = 5.4 Hz), 4.00–3.90 (m, 1H), 3.35–3.25 (m, 3H), 3.24–3.15 (m, 3H), 3.11–3.02 (m, 1H), 2.93 (s, 3H), 2.67–2.60 (m, 4H), 2.54 (s, 1H), 2.20–2.00 (m, 5H), 1.95–1.83 (m, 3H), 1.80–1.50 (m, 4H), 1.29 (d, 3H, *J* = 13.8 Hz), 1.07 (d, 3H, *J* = 6.0 Hz), 0.91 (d, 3H, *J* = 6.6 Hz), 0.86 (d, 3H, *J* = 6.6 Hz). Note: additional peaks in the spectrum are buried under a broad singlet due to H_2O. ESI-TOF HRMS *m/z* 787.2922 ($[M + 2H]^{+2}$, $C_{74}H_{92}Cl_2N_{11}O_{23}$ requires 787.2948).



Compound 12: A solution of **1** (4.0 mg, 2.8 μmol) in DMF/DMSO (1/1, 150 μL) was treated with **C14** (1 M in DMF/DMSO = 1/1, 13.8 μL, 13.8 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 82.8 μL, 82.8 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 55.2 μL, 55.2 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 21.2 min) to afford **12** (2.8 mg, 58%) as a white amorphous solid identical in all respects with authentic material (^{1}H NMR, DMSO-d_6).[51]

Note: This reaction was run on scales of 0.5–10 mg (51–63%) during the optimization of conditions (Table S1). Compounds **13** and **18** were synthesized on 0.7 mg and 0.25 mg scales with respect to their starting material.

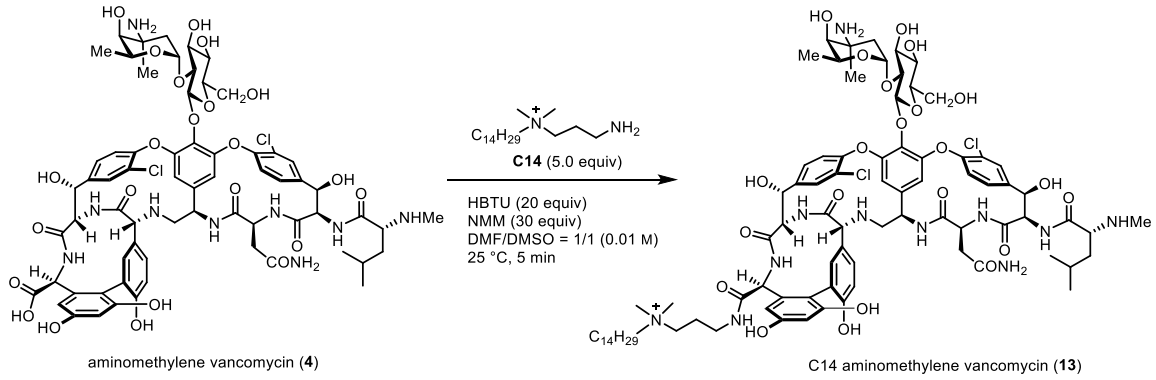


entry	reaction scale	**C14**	HBTU	base	temp.	time	isolated yield
1[a]	10-2 mg	2 equiv	2 equiv	DIEA (5 equiv)	0 °C	12 h	52-58%
2	500 μg	2 equiv	2 equiv	DIEA (5 equiv)	0 °C	12 h	45%
3	500 μg	5 equiv	10 equiv	DIEA (15 equiv)	0 °C	2 h	51%
4	500 μg	5 equiv	20 equiv	DIEA (30 equiv)	0 °C	2 h	58%
5	500 μg	5 equiv	20 equiv	DIEA (30 equiv)	25 °C	5 min	55%
6	500 μg	5 equiv	20 equiv	NMM (30 equiv)	25 °C	5 min	63%

[a] Adapted procedure from: Haldar, J. *et al*. *J. Med. Chem*. **2014**, *57*, 4558.

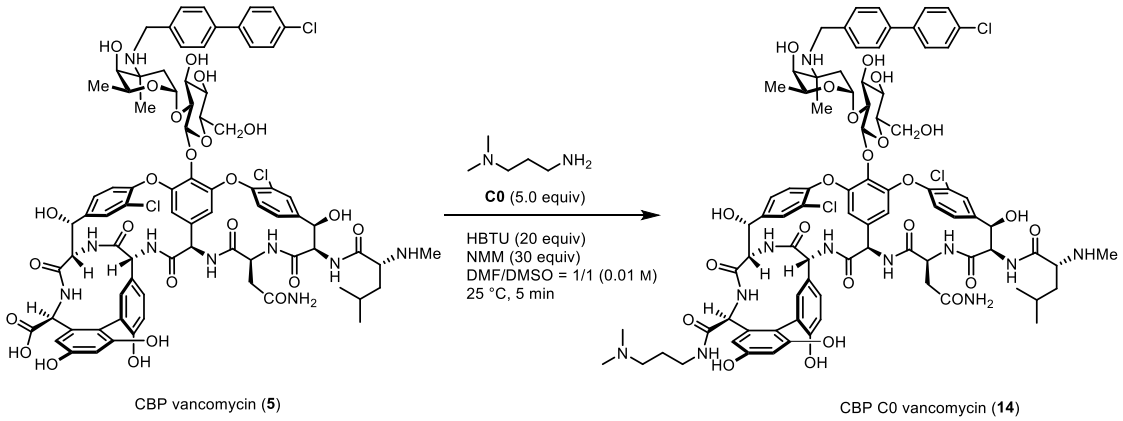
Table S1. Optimization of reaction conditions for the coupling of vancomycin (**1**) and **C14**.

Experimental for the total synthesis of **4**[43,48] has been previously disclosed.

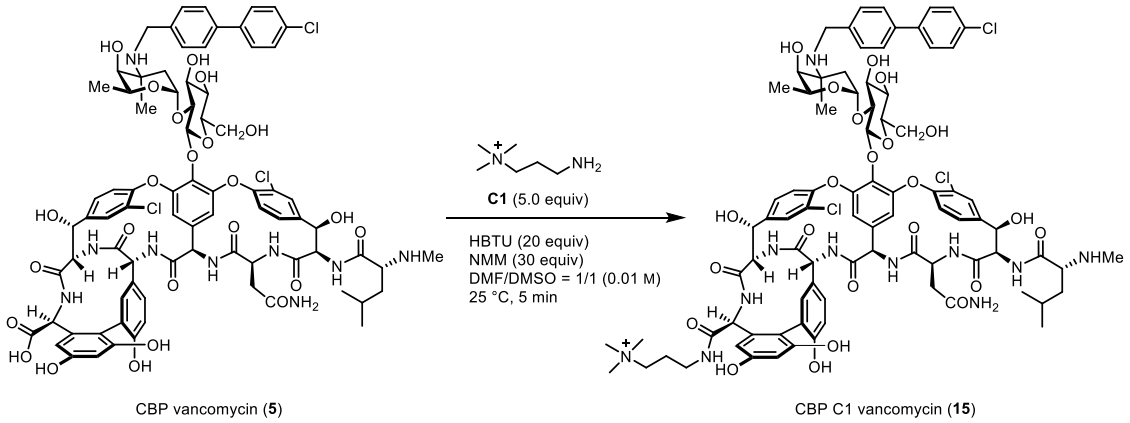


Compound 13: A solution of **4** (0.69 mg, 0.48 μmol) in DMF/DMSO (1/1, 30 μL) was treated with **C14** (1 M in DMF/DMSO = 1/1, 2.4 μL, 2.4 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 14.4 μL, 14.4 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 9.6 μL, 9.6 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.2 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 18.4 min) to afford **13** (0.53 mg, 64%, typically 61–67%) as a white film: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 8.85–8.80 (m, 1H), 8.50–8.45 (m, 1H), 8.35–8.30 (m, 1H), 7.98 (s, 1H), 7.82 (d, 1H, J = 8.7 Hz), 7.74 (dd, 1H, J = 8.4, 1.8 Hz), 7.59 (d, 1H, J = 2.4 Hz), 7.41 (d, 1H, J = 9.0 Hz), 7.31 (d, 1H, J = 2.4 Hz), 7.25–7.17 (m, 2H), 7.15 (dd, 1H, J = 8.4, 2.4 Hz), 7.11–7.06 (m, 1H), 6.93 (d, 1H, J = 8.4 Hz), 6.46 (d, 1H, J = 2.4 Hz), 6.33 (d, 1H, J = 2.4 Hz), 5.54 (d, 1H, J = 3.0 Hz), 5.44 (d, 1H, J = 7.8 Hz), 5.41 (d, 2H, J = 2.4 Hz), 5.37 (d, 1H, J = 5.4 Hz), 4.57–4.54 (m, 1H), 4.43–4.31 (m, 2H), 4.29–4.19 (m, 1H), 4.18–3.98 (m, 1H), 3.88–3.70 (m, 3H), 3.69–3.59 (m, 2H), 3.58–3.49 (m, 2H), 3.40–3.32 (m, 4H), 3.29–3.24 (m, 5H), 3.05 (d, 5H, J = 3.6 Hz), 3.00 (s, 3H), 2.86 (s, 3H), 2.79 (s, 3H), 2.66 (br s, 9H), 2.64–2.56 (m, 1H), 2.33–2.25 (m, 1H), 2.10–1.92 (m, 5H), 1.83–1.70 (m, 3H), 1.67–1.57 (m, 2H), 1.54 (s, 3H), 1.45–1.25 (m, 23H), 1.20 (d, 3H, J = 6.6 Hz), 0.95 (d, 3H, J = 7.2 Hz), 0.93–0.87 (m, 6H); ESI-TOF HRMS *m/z* 857.8965 ($[M + 2H]^{+2}$, $C_{85}H_{117}Cl_2N_{11}O_{22}$ requires 857.8948).

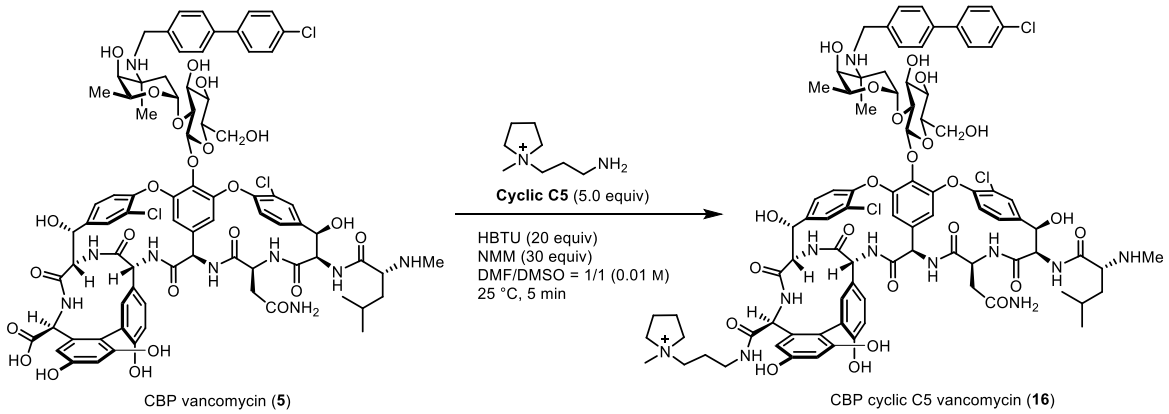
Experimental for the synthesis of CBP vancomycin **5**[48] has been previously disclosed.



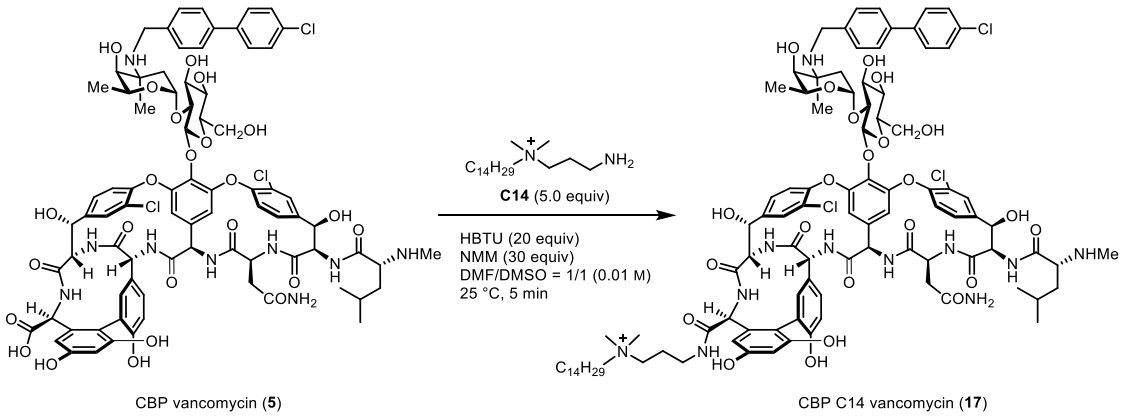
Compound 14: A solution of **5** (2.0 mg, 1.2 μmol) in DMF/DMSO (1/1, 100 μL) was treated with **C0** (1 M in DMF/DMSO = 1/1, 6.1 μL, 6.1 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 36.4 μL, 36.4 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 24.3 μL, 24.3 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 13.1 min) to afford **14** (1.2 mg, 55%) as a white film: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ9.20–8.99 (m, 1H), 8.73 (s, 1H), 8.48–8.38 (m, 1H), 8.01–7.87 (m, 1H) 7.77–7.41 (m, 13H), 7.32–7.30 (br s, 1H), 7.27 (d, 1H, J = 9.0 Hz), 7.21–7.16 (m, 1H), 7.10 (br s, 1H), 7.04–6.90 (m, 1H), 6.86–6.79 (br s, 1H), 6.53–6.29 (m, 3H), 5.80 (s, 1H), 5.58–5.29 (m, 5H), 4.60–4.53 (m, 1H), 4.43–3.98 (m, 5H), 3.93–3.36 (m, 9H), 3.21–3.10 (m, 2H), 3.02–2.98 (m, 1H), 2.93–2.87 (m, 6H), 2.86 (br s, 1H), 2.82 (br s, 1H), 2.78 (s, 3H), 2.74 (br s, 1H), 2.66 (s, 2H), 2.21–2.13 (m, 1H), 2.10–1.93 (m, 3H), 1.90–1.80 (m, 1H), 1.78–1.69 (m, 2H), 1.66 (s, 3H), 1.40–1.22 (m, 6H), 1.00 (d, 3H, J = 7.2 Hz), 0.96 (d, 3H, J = 7.2 Hz); ESI-TOF HRMS *m/z* 866.7940 ($[M + 2H]^{+2}$, $C_{74}H_{96}Cl_3N_{11}O_{23}$ requires 866.7952).



Compound 15: A solution of **5** (1.4 mg, 0.85 μmol) in DMF/DMSO (1/1, 100 μL) was treated with **C1** (1 M in DMF/DMSO = 1/1, 4.3 μL, 4.3 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 25.5 μL, 25.5 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 17.0 μL, 17.0 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 13.7 min) to afford **15** (0.91 mg, 61%) as a white film: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 9.15–8.95 (m, 1H), 8.80–8.70 (m, 1H), 8.45–8.35 (m, 1H), 7.78–7.40 m, 13H), 7.38–7.06 (m, 3H), 7.05–6.95 (m, 1H), 6.82 (d, 1H, J = 8.4 Hz), 6.46 (d, 1H, J = 2.4 Hz), 6.38 (s, 2H), 5.85–5.75 (m, 1H), 5.57–5.30 (m, 5H), 4.62–4.51 (m, 1H), 4.40–4.23 (m, 2H), 4.23–4.03 (m, 4H), 3.93–3.80 (m, 1H), 3.78–3.70 (m, 1H), 3.68–3.40 (m, 5H), 3.22–3.02 (m, 12H), 2.99 (s, 1H), 2.86 (s, 1H), 2.83–2.73 (m, 4H), 2.66 (s, 3H), 2.23–2.14 (m, 1H), 2.10–2.00 (m, 3H), 1.87–1.60 (m, 6H), 1.33–1.25 (m, 4H), 1.05–0.93 (m, 6H); ESI-TOF HRMS *m/z* 873.8042 ($[M + 2H]^{+2}$, $C_{85}H_{98}Cl_3N_{11}O_{23}$ requires 873.8027).

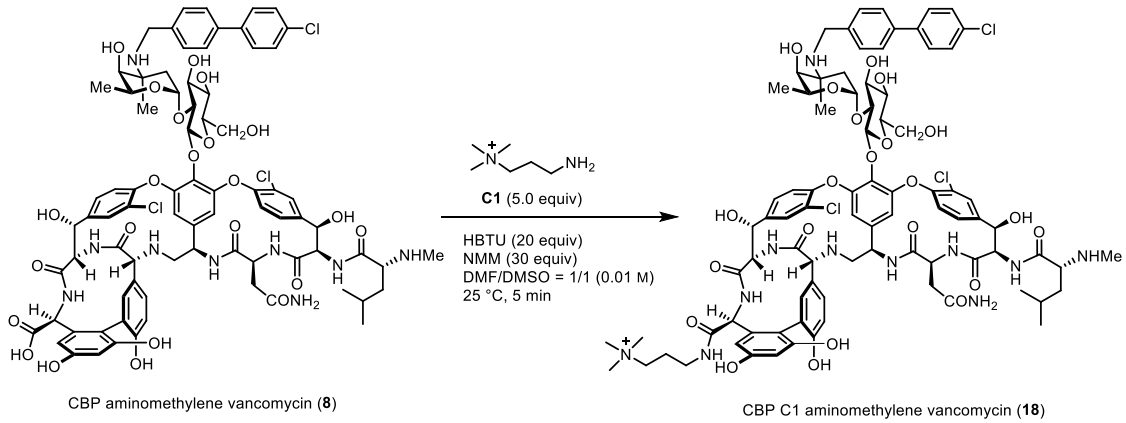


Compound 16: A solution of **5** (1.8 mg, 1.1 μmol) in DMF/DMSO (1/1, 100 μL) was treated with **Cyclic C5** (1 M in DMF/DMSO = 1/1, 5.5 μL, 5.5 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 21.8 μL, 21.8 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 32.7 μL, 32.7 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 12.7 min) to afford **16** (1.2 mg, 61%) as a white film: ^{1}H NMR (CD_3OD, 600 MHz, 298 K, rotamers (4:1)) δ 9.04 (s, 0.25H), 8.74 (s, 1H), 8.35 (s, 0.25H), 8.00 (s, 1H), 7.76–7.56 (m, 18H), 7.50–7.48 (m, 4H), 7.35 (d, 0.25H, J = 9.6 Hz), 7.30 (d, 1H, J = 9.0 Hz), 7.13–7.09 (m, 1.25H), 7.11 (s, 1H), 6.83 (d, 1H, J = 9.0 Hz), 6.48–6.46 (m, 1.25H), 6.40–6.39 (m, 1.25H), 5.84 (br s, 1H), 5.54 (d, 1H, J = 7.8 Hz), 5.49 (d, 1H, J = 4.8 Hz), 5.42 (s, 1H), 5.37–5.34 (m, 2.5H), 4.59 (dd, 0.25H, J = 6.0, 6.0 Hz), 4.37–4.31 (m, 1H), 4.27–4.17 (m, 5H), 4.15–4.04 (m, 4.25H), 3.93–3.86 (m, 1.75H), 3.82–3.75 (m, 1.25H), 3.72 (s, 1H), 3.71–3.64 (m, 3H), 3.59–3.47 (m, 9H), 3.45–3.42 (m, 2.5H), 3.40–3.35 (m, 2H), 3.25–3.21 (m, 1H), 3.18 (d, 1H, J = 2.4 Hz), 3.16–3.12 (m, 1H), 3.09–3.06 (m, 5.5H), 3.04–3.02 (m, 4.25H), 2.97–2.93 (m, 3.5H), 2.89 (s, 3H), 2.82–2.77 (m, 5H), 2.68 (s, 6H), 2.30–2.18 (m, 8.5H), 2.12–2.04 (m, 4.25H), 1.91–1.85 (m, 1.5H), 1.83–1.76 (m, 2H), 1.74–1.65 (m, 3H), 1.42–1.27 (m, 3H), 1.09–0.95 (m, 6H); ESI-TOF HRMS *m/z* 886.8121 ($[M + 2H]^{+2}$, $C_{87}H_{101}Cl_3N_{11}O_{23}$ requires 886.8103).



Compound 17: A solution of **5** (2.1 mg, 1.3 μmol) in DMF/DMSO (1/1, 100 μL) was treated with **C14** (1 M in DMF/DMSO = 1/1, 6.4 μL, 6.4 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 38.3 μL, 38.3 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 25.5 μL, 25.5 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.2 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 18.4 min) to afford **17** (1.9 mg, 76%) as a white film: ^{1}H NMR (CD_3OD, 600 MHz, 298 K, rotamers (2:1)) δ 9.16 (s, 0.5H), 9.09 (s, 0.5H), 8.74 (s, 1H), 8.40 (s, 1H), 7.98 (s, 0.5H), 7.89 (s, 0.5H), 7.80–7.53 (m, 11H), 7.51 (d, 1H, J = 7.8 Hz), 7.48–7.42 (m, 3H), 7.38–7.25 (m, 3H), 7.20 (d, 0.5H, J = 8.4 Hz), 7.17–7.12 (m, 2H), 7.04 (d, 0.5H, J = 2.4 Hz), 6.91 (s, 0.5H), 6.83 (d, 1H, J = 8.4 Hz), 6.51 (s, 0.5H), 6.47 (s, 1H), 6.41 (s, 0.5H), 6.37 (s, 1H), 6.29 (d, 0.5H, J = 7.8 Hz), 5.81 (br s, 1H), 5.65 (s, 0.5H), 5.58–5.51 (m, 1H), 5.48–5.38 (m, 2H), 5.35 (s, 1H), 5.22 (d, 0.5H, J = 2.4 Hz), 5.01 (s, 0.5H), 4.70 (s, 1H), 4.62 (s, 0.5H), 4.60–4.52 (m, 2H), 4.36 (s, 0.5H), 4.28 (s, 1H), 4.19–4.06 (m, 5H), 3.98 (d, 0.5H, J = 10.8 Hz), 3.92–3.80 (m, 1.5H), 3.78–3.71 (m, 2H), 3.63–3.59 (m, 2H), 3.57–3.51 (m, 3H), 3.47 (s, 1H), 3.43–3.38 (m, 1.5H), 3.12–3.06 (m, 11H), 3.02–3.00 (m, 8H), 2.92 (d, 1H, J = 2.4 Hz), 2.89 (s, 1H), 2.87 (s, 0.5H), 2.83–2.79 (m, 4H), 2.66 (s, 6H), 2.22–2.18 (m, 2H), 2.16–2.10 (m, 0.5H), 2.07–1.99 (m, 4H), 1.95–1.83 (m, 2H), 1.81–1.62 (m, 12H), 1.44–1.19 (m, 41H), 1.09–0.96 (m, 3.5H), 0.93–0.88 (m, 7H), 0.81 (d, 1H, J = 4.2 Hz), 0.71 (d, 1H, J = 3.6 Hz); ESI-TOF HRMS *m/z* 1928.7987 (M^+, $C_{98}H_{125}Cl_3N_{11}O_{23}$ requires 1928.8010).

Experimental for the total synthesis of **8**[48] has been previously disclosed.



Compound 18: A solution of **8** (0.24 mg, 0.15 μmol) in DMF/DMSO (1/1, 20 μL) was treated with **C1** (0.1 M in DMF/DMSO = 1/1, 7.4 μL, 0.74 μmol), *N*-methylmorpholine (Acros, distilled, 1 M in DMF/DMSO = 1/1, 4.5 μL, 4.5 μmol), and HBTU (Chem-Impex International, Inc., 1 M in DMF/DMSO = 1/1, 3.0 μL, 3.0 μmol) at 25 °C. The reaction mixture was stirred at 25 °C for 5 min and quenched with the addition of 50% MeOH in H_2O (0.5 mL) at 25 °C. The mixture was purified by semi-preparative reverse-phase HPLC (Nacalai Tesque, Inc., ARII-C18, 5 μm, 10 × 150 mm, 20–80% MeCN/H_2O–0.07% TFA gradient over 30 min, 3 mL/min, t_R = 12.9 min) to afford **18** (0.14 mg, 53%) as a white film: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 9.20–9.05 (m, 1H), 9.01 (s, 1H), 8.64–8.56 (m, 1H), 7.98 (s, 1H), 7.80–7.55 (m, 10H), 7.47 (d, 2H, J = 10.2 Hz), 7.37–7.31 (m, 1H), 7.29 (d, 1H, J = 9.0 Hz), 7.25 (s, 1H), 7.09 (d, 1H, J = 9.6 Hz), 6.86 (d, 1H, J = 9.0 Hz), 6.84 (s, 1H), 6.73 (d, 1H, J = 2.4 Hz), 6.47 (s, 1H), 6.39–6.35 (m, 1H), 5.85–5.75 (m, 2H), 5.55–5.40 (m, 3H), 5.35–5.25 (m, 1H), 4.26 (br s, 2H), 4.20–4.05 (m, 4H), 3.95–3.80 (m, 2H), 3.79–3.70 (m, 2H), 3.68–3.55 (m, 3H), 3.54–3.40 (m, 7H), 3.19 (s, 1H), 3.11 (s, 1H), 3.05 (s, 3H), 3.03–2.95 (m, 7H), 3.00 (s, 3H), 2.92 (s, 1H), 2.87 (s, 2H), 2.76 (s, 3H), 2.66 (s, 4H), 2.30–2.15 (m, 6H), 2.14–2.01 (m, 4H), 1.87–1.73 (m, 2H), 1.68 (s, 3H), 1.26 (d, 3H, J = 7.2 Hz), 1.03 (s, 3H, J = 7.2 Hz), 0.99 (s, 3H, J = 7.2 Hz); ESI-TOF HRMS *m/z* 866.8129 ([M + 2H]$^{+2}$, $C_{85}H_{102}Cl_3N_{11}O_{22}$ requires 866.8130).

***In vitro* antimicrobial assays**[S1]

One day before experiments were run, fresh cultures of vancomycin-sensitive *Staphlococcus aureus* (VSSA strain ATCC 25923), methicillin and oxacillin-resistant *Staphlococcus aureus subsp. aureus* (MRSA strain ATCC 43300), vancomycin-resistant *Enterococcus faecalis* (VanA VRE, BM4166), *Enterococcus faecium* (VanA VRE, ATCC BAA-2317), vancomycin-resistant *Enterococcus faecalis* (VanB VRE, strain ATCC 51299), *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC BAA-1710), *Pseudomonas aeruginosa* (ATCC 15442), *Klebsiella pneumoniae* (ATCC 700603) were inoculated and grown in an orbital shaker at 37 °C in 100% Mueller-Hinton broth (VSSA, MRSA and VanB VRE), 100% brain-heart infusion broth (VanA VRE, *A. baumannii* and *K. pneumoniae*) or 100% Luria broth (*E. coli* and *P. aeruginosa*). After 24 h, the bacterial stock solutions were serial diluted with the culture medium (10% Mueller-Hinton broth for VSSA, MRSA and VanB VRE or 10% brain-heart infusion broth for VanA VRE *A. baumannii* and *K. pneumoniae* or 10% Luria Broth for *E. coli* and *P. aeruginosa*) to achieve a turbidity equivalent to a 1:100 dilution of a 0.5 M McFarland solution. This diluted bacterial stock solution was then inoculated in a 96-well V-shaped glass coated microtiter plate, supplemented with serial diluted aliquots of the antibiotic solution in DMSO (4 μL), to achieve a total assay volume of 0.1 mL. The plate was then incubated at 37 °C for 18 h, after which minimal inhibitory concentrations (MICs) were determined by monitoring the cell growth (observed as a pellet) in the wells. The lowest concentration of antibiotic (in μg/mL) capable of eliminating cell growth in the wells is the reported MIC value. The reported MIC values for the vancomycin analogues were determined against vancomycin as a standard in the first well.

S1. Clinical and Laboratory Standards Institute (2009) *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically,* Approved Standard, 7th ed, CLSI document M07-A8, Clinical and Laboratory Standards Institute: Wayne, PA.

For VanA *E. faecalis* (VanA VRE, BM 4166): resistant to erythromycin, gentamicin, chloramphenicol, and ciprofloxacin as well as vancomycin and teicoplanin; sensitive to

daptomycin.

For VanA *E. faecium* (VanA VRE, ATCC BAA-2317): resistant to ampicillin, benzylpenicillin, ciprofloxacin, erythromycin, levofloxacin, nitrofurantoin, and tetracycline as well as vancomycin and teicoplanin, insensitive to linezolid; sensitive to tigecycline and dalfopristine.

Cell wall permeability assay[S2,S3]

One day before experiments were run, cultures of vancomycin-resistant *Enterococcus faecalis* (VanA VRE, BM4166) and *Enterococcus faecium* (VanA VRE, ATCC BAA-2317), were inoculated and grown in an orbital shaker at 37 °C in 100% brain-heart infusion broth for 12 h. The above bacterial solution was subjected to a subculture to obtain fresh mid log phase bacterial cells (total volume of bacterial suspension = 7 mL, incubation time = 6 h, OD_{600} = 0.6). After the cultured bacteria was harvested (3000 rpm, 4 °C, 20 min), the white bacterial precipitate was washed and resuspended in 5 mM glucose and 5 mM HEPES buffer (1:1, 500 μL, pH = 7.2). This bacterial suspension (130 μL) was charged in a 96-well black plate with a clear bottom (Corning 3650). The propidium iodide dye (10 μL, 150 μM DMSO solution) was added to the above suspension and the fluorescence was monitored at 25 °C for 5 min at 30 second intervals using a microplate reader (Molecular Devices®, Max Gemini EX) at an excitation wavelength of 535 nm and an emission wavelength of 617 nm. The test compound (150 μM, 10 μL) was added to the cell suspension and the fluorescence was monitored at 25 ºC for an additional 15 min.

The impact of the structural modifications on cell wall permeability against both VanA VRE examined herein was also examined (Figure S1). Vancomycin (**1**), C14-vancomycin (**12**), CBP C1-vancomycin (**15**), and CBP C1-aminomethylene vancomycin (**18**) displayed similar induced permeabilities against both vancomycin-resistant *Enterococcus faecalis* (VanA VRE, BM4166) and *Enterococcus faecium* (VanA VRE, ATCC BAA-2317).

S2. Sahal, D *et al*. (2010) *J Med Chem* 53:6079.

S3. Barbeau, B *et al*. (1999) *J. Microbiol Methods* 37:77.

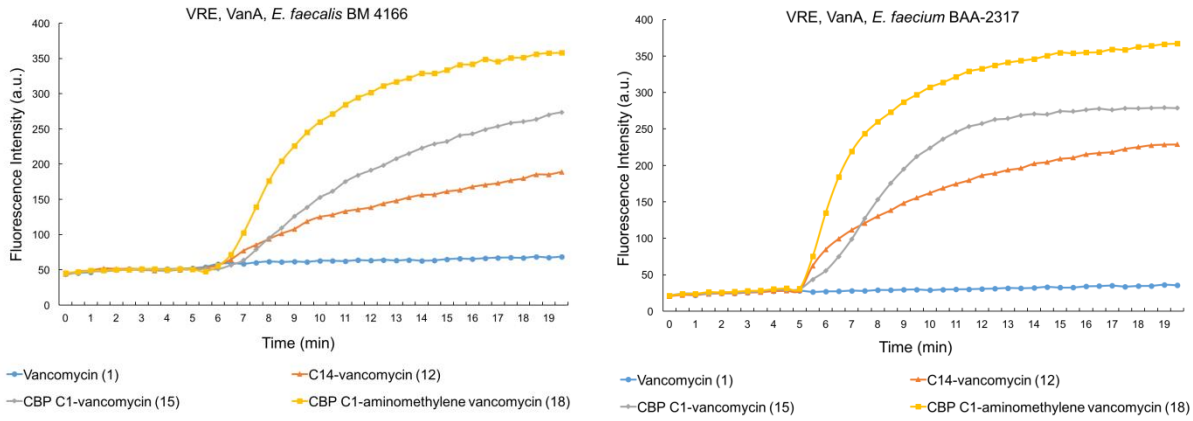


Figure S1. Examination of cell wall permeability against VanA *E. faecalis* BM4166 and *E. faecium* ATCC BAA-2317.

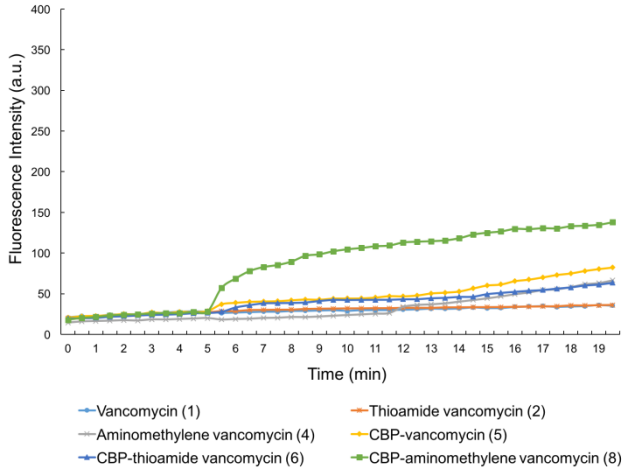


Figure S2. Examination of cell wall permeability induced by compounds **1**–**8** (10 μM added at 5 min) in VanA VRE (*E. faecium* ATCC BAA-2317).

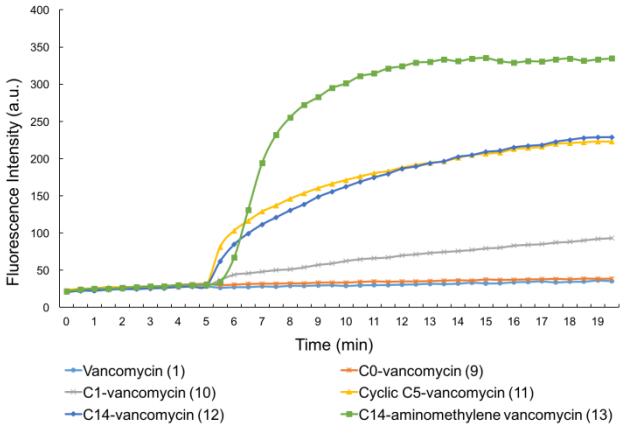


Figure S3. Examination of cell wall permeability induced by compounds **9**, **11**–**13** (10 μM added at 5 min), **10** (100 μM added at 5 min) in VanA VRE (*E. faecium* ATCC BAA-2317).

Cell wall depolarization assay [51,S4,S5]

One day before experiments were run, cultures of vancomycin-resistant *Enterococcus faecium* (VanA VRE, ATCC BAA-2317), were inoculated and grown in an orbital shaker at 37 °C in 100% Brain-Heart Infusion for 12 h. The above bacterial solution was subjected to subculture to obtain fresh mid log phase bacterial cells (total volume of bacterial suspension = 7 mL, incubation time = 6 h, OD_{600} = 0.6). After cultured bacteria media was harvested (3000 rpm, 4 °C, 20 min), this bacterial precipitate was washed and resuspended in a mixture of 5 mM glucose, 5 mM HEPES, and 5 mM KCl buffer (1:1:1, pH = 7.2). This bacterial suspension (130 μL) was charged in a 96-well black plate (Corning 3650). The dye ($DiSC_35$: 3,3'-Dipropylthiadicarbocyanine iodide, 150 μM DMSO solution, 2.5 μL) was added to the above suspension, preloading the dye in the bacterial cell membrane. The fluorescence was monitored for 10 min at 1 min interval using a microplate reader (Molecular devices, Max Gemini EX) at an excitation wave length of 622 nm and an emission wave length of 670 nm. The test compound (150 μM, 10 μL) was added to the cell suspension and the fluorescence was monitored for a further 30 min.

S4. Haldar, J *et al*. (2013) *Chem Commun* 49:9389.
S5. Wimley, WC *et al*. (2010) *J Am Chem Soc* 53:6079.

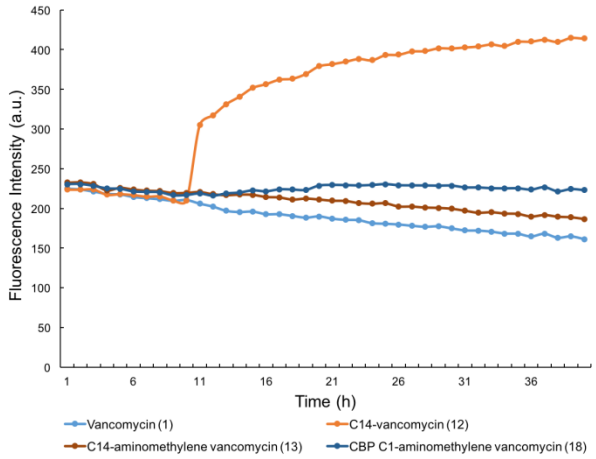


Figure S4. Cell wall depolarization against VanA *E. faecium* ATCC BAA-2317.

Resistance development study[S6]

The MICs of the vancomycin analogues against vancomycin-resistant *Enterococcus faecalis* (VanA VRE, BM4166) and *Enterococcus faecium* (VanA VRE, ATCC BAA-2317) were determined (see page S13 for protocol). The bacterial suspension (40 μL) in the 96-well plate at sub-MIC concentration (MIC/2) was inoculated with 100% brain-heart infusion broth and the bacteria were grown in an orbital shaker at 37 °C for 6 h until the value of OD_{600} became 0.6. A new MIC assay was performed with the same protocol (see S13). This process was repeated for 50 passages, and the fold increase in MIC was determined at each passage.

S6. Savage, PB *et al*. (2012) *J Antimicrob Chemother* 67:2665.

Cell wall biosynthesis inhibition assay[30]

Cultures of vancomycin-resistant *Enterococcus faecalis* (VanA VRE, BM4166) and *Enterococcus faecium* (VanA VRE, ATCC BAA-2317) were inoculated and grown in an orbital shaker at 37 °C in 100% brain-heart infusion broth for 12 h. The above bacterial solution was subjected to a subculture to obtain fresh mid log phase bacterial cells (total volume of bacterial suspension = 5 mL, incubation time = 6 h, OD_{600} = 0.6). Tetracycline (5 mg/mL, 130 μL) was added to the above bacterial suspension to ensure complete inhibition

of protein synthesis and incubated at 37 °C for 30 min. Vancomycin analogues were added and the mixture was incubated at 37 °C for a further 30 min. After the bacteria was harvested (3000 rpm, 4 °C, 20 min), this bacterial precipitate was washed and resuspended in 5 mM glucose and 5 mM HEPES buffer (500 µL, 1:1, pH = 7.2). This bacterial suspension was heated at 100 °C for 15 min and centrifuged (13000 rpm, 25 °C, 10 min). The entire volume of supernatant was directly purified by semi-preparative reverse-phase HPLC without further manipulation (Agilent Technologies, Zorbax SB-C18, 5 µm, 9.4 × 150 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 3 mL/min, t_R = 11.9 min) to afford UDP Mur *N*-Ac depsipentapeptide (**19**) as a white film identical in all respects with authentic material (^{1}H NMR, D_2O).[S7]

The amount of UDP Mur *N*-Ac pentapeptide (**19**) was quantified by use of calibration curves (Figure S5 for *E. faecalis* BM4166, Figure S6 for *E. faecium* ATCC BAA-2317) based on the area under the curve (AUC).

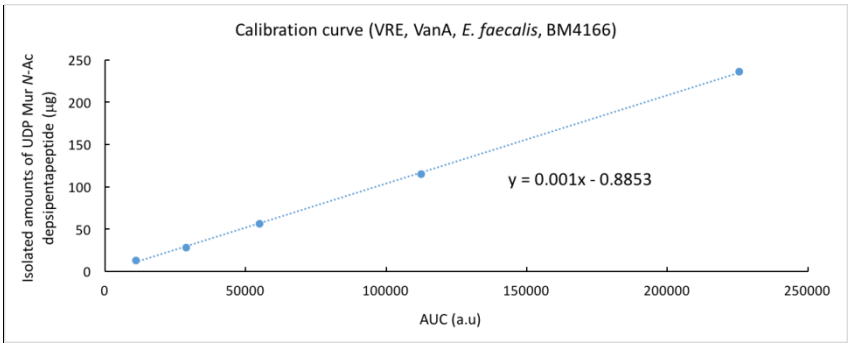


Figure S5. Calibration curve (VRE, VanA *E. faecalis* BM4166).

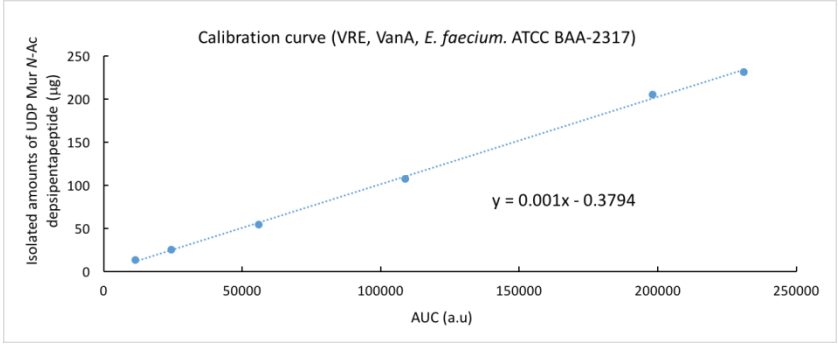


Figure S6. Calibration curve (VRE, VanA *E. faecium* ATCC BAA-2317).

S7. Liu, H, Sadamoto, R, Sears, PS, Wong, CH (2001) *J Am Chem Soc* 123:9916.

Hemolysis assay[S8]

The blood cells in pig whole blood (2 mL, Pel-Free Biologicals, non-sterile, sodium citrate) were harvested (3000 rpm, 4 °C, 20 min), and the red blood precipitate was washed and resuspended in phosphate buffer saline (pH 7.4). This diluted red blood cell stock solution (384 μL) was incubated with the antibiotic solution in DMSO (16 μL) in a 1 mL microtube to achieve the final concentration of the test compounds. The mixture was then incubated at 37 °C for 1 h. The solution was diluted with phosphate buffer saline (pH 7.4, 200 μL) at 25 °C and centrifuged (3000 rpm, 4 °C, 20 min). The supernatant (200 μL) was transferred to a microtiter plate. A positive control (0.2 % vol% Triton X-100, 100% total hemolysis) and the negative control (no antibiotic, 0% hemolysis) were prepared. A_{350} was measured using a microplate reader (Molecular Devices®, Max Gemini EX). The % hemolysis was determined by calculating the following equation shown below (eq. 1).

$$\text{Hemolysis (\%)} = \frac{(A_{test} - A_{zero})}{(A_{total} - A_{zero})} \times 100 \quad \text{(eq. 1)}$$

A_{test} : Absorbance with test compound
A_{total} : Absorbance of 100% hemolysis
A_{zero} : Absorbance of 0% hemolysis

S8. Patch, JA, Barron, AE (2003) *J Am Chem Soc* 125:12092.

The key compounds in the series were examined for in vitro toxicity that might result from the combined mechanisms of action, especially the introduction of structural modifications (quaternary ammonium salt) that might impact host as well as bacterial cell wall integrity. The compounds were examined for red blood cell hemolytic activity, resulting from membrane lysis. Although the differences in mammalian and bacterial cell wall composition are extensive, including the more highly anionic composition of the bacterial cell wall responsible for a preferential and differential cation binding, lysis of mammalian cell membranes (red blood cells) are potential off-target consequences of cationic compounds that impact bacterial cell membrane integrity. The standard red blood cell hemolysis assay was conducted and measures the extent of red blood cell lysis after 1 h exposure to candidate compounds (pH 7.4, PBS, 37 °C, 1 h). No compound in the series, including **18**, exhibit any

hemolytic activity even at concentrations >1000-fold above their MICs (Figure S7A). Because this set of observations did not distinguish between any of the derivatives (no hemolytic activity with any derivative), we extended the time of the assay out to 24 h (Figure S7B). However, red blood cells deteriorate under the conditions of the assay as time progresses and such extended time assays are not recommended or utilized by any in the field. So the results should not be taken as reflective of potential toxicity. However, it is notable that **18** was the best compound in the series even with an extended exposure, displaying little hemolytic activity and behaving no different than the control linezolid which does not act on the bacterial cell membrane. It was also substantially better than vancomycin itself which was no different than the control tigecycline that also does not act on the bacterial cell wall membrane, and it was much better and readily distinguishable from control daptomycin that acts by permeabilizing (not lysing) the bacterial cell membrane. The only compound in the series examined that performed worse than the control daptomycin was the C-terminus C14 quaternary ammonium salt of vancomycin (**12**). Importantly this combined set of studies indicate compound **18**, as well as **15**, have less of an impact on mammalian red blood cell membranes than even vancomycin itself. Finally, the extraordinary potency of the key analog **18** would also be expected to minimize any nonselective toxicity because the amounts required for observation of antimicrobial activity are so low.

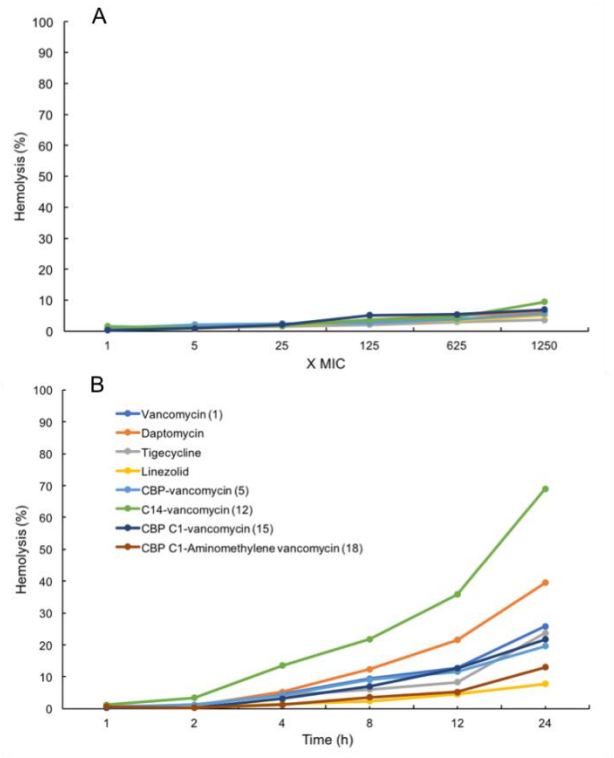
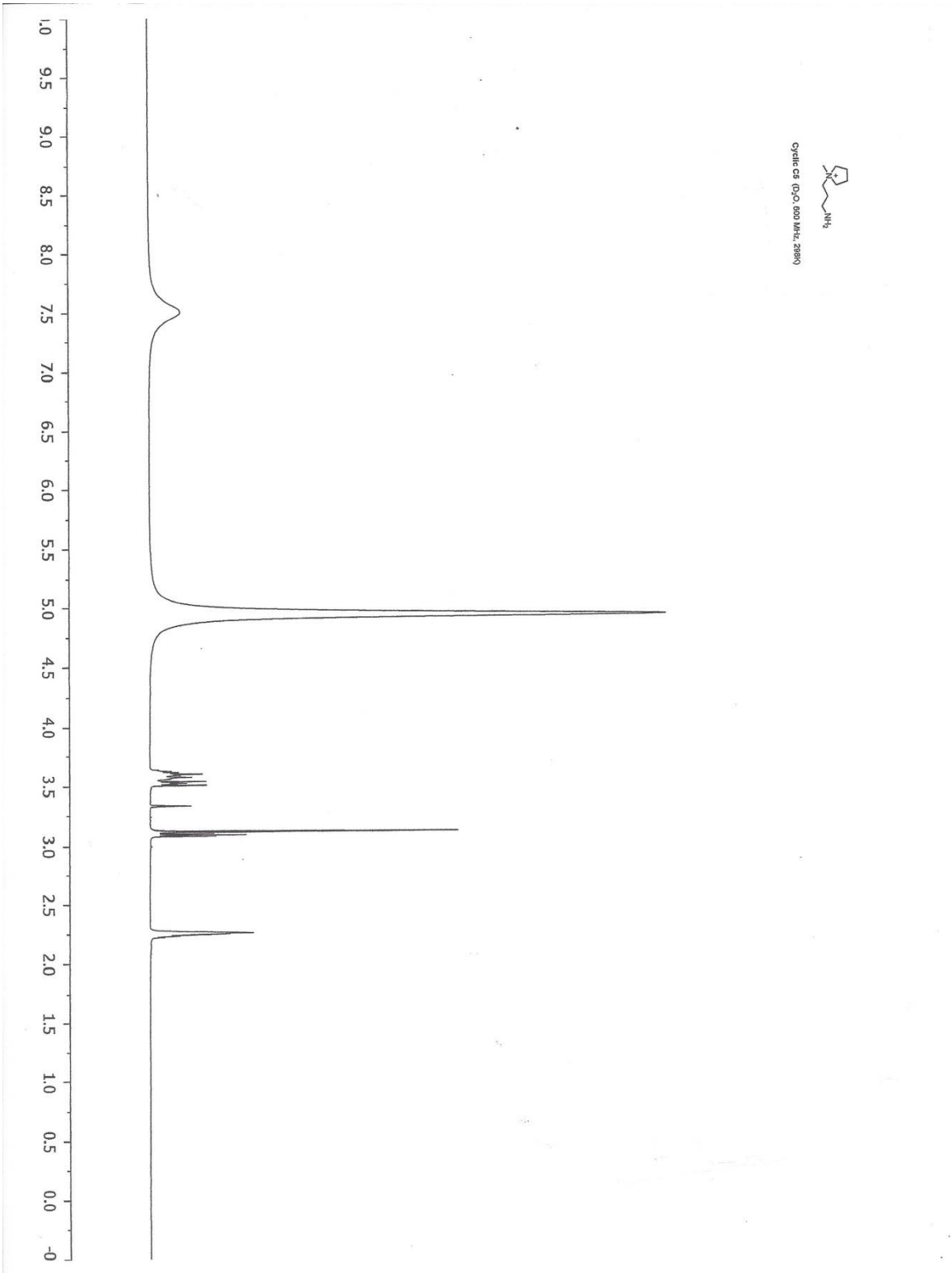
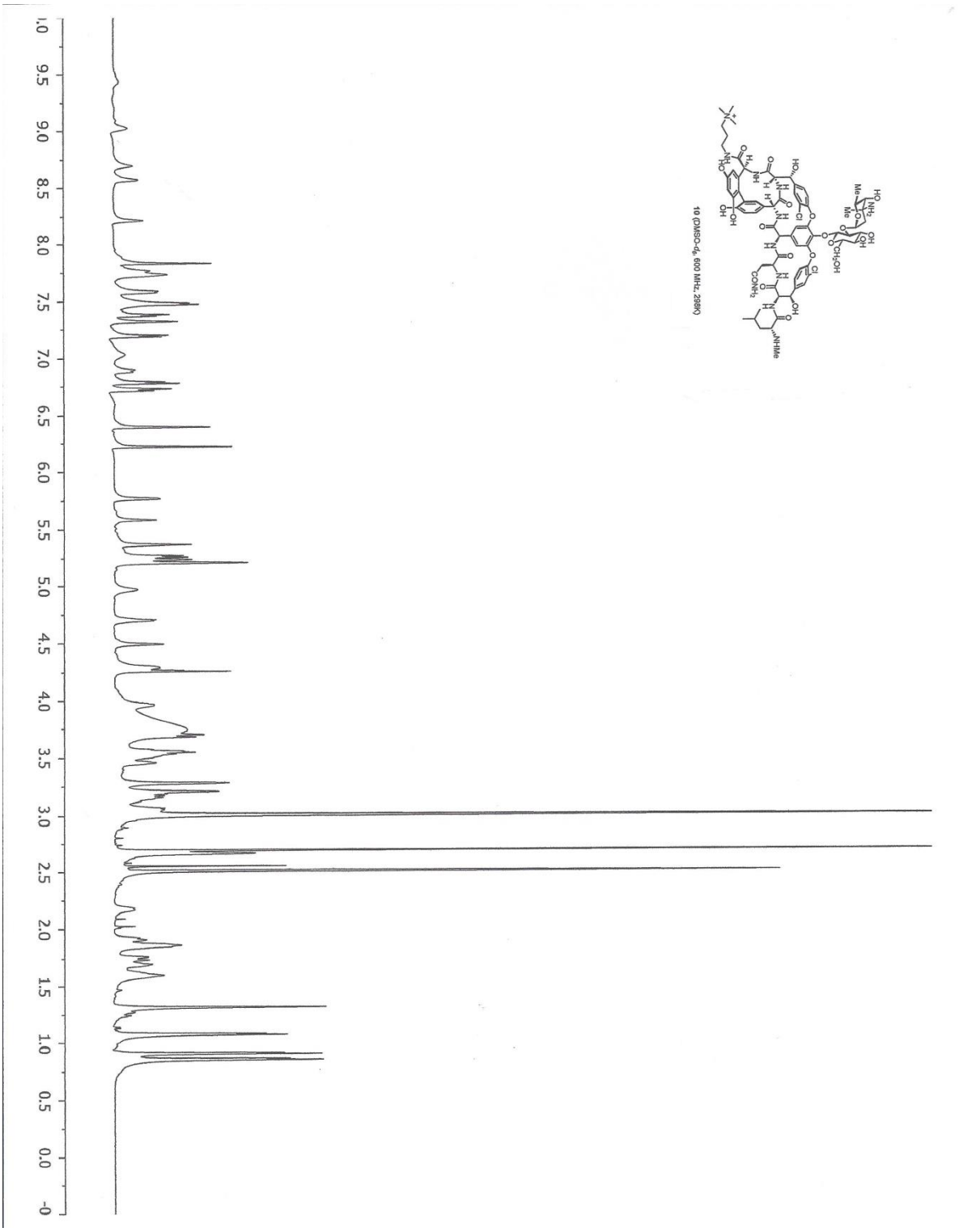


Figure S7. Hemolytic assay of red blood cells. (A) % Hemolysis observed versus concentration expressed as fold concentration over measured MIC alonside vancomycin, daptomycin, linezolid and tigecycline controls. (B) % Hemolysis versus time at MIC concentration alongside vancomycin, daptomycin, linezolid and tigecycline controls.


cyclic C5 (D2O, 600 MHz, 298K)
NH2
10
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0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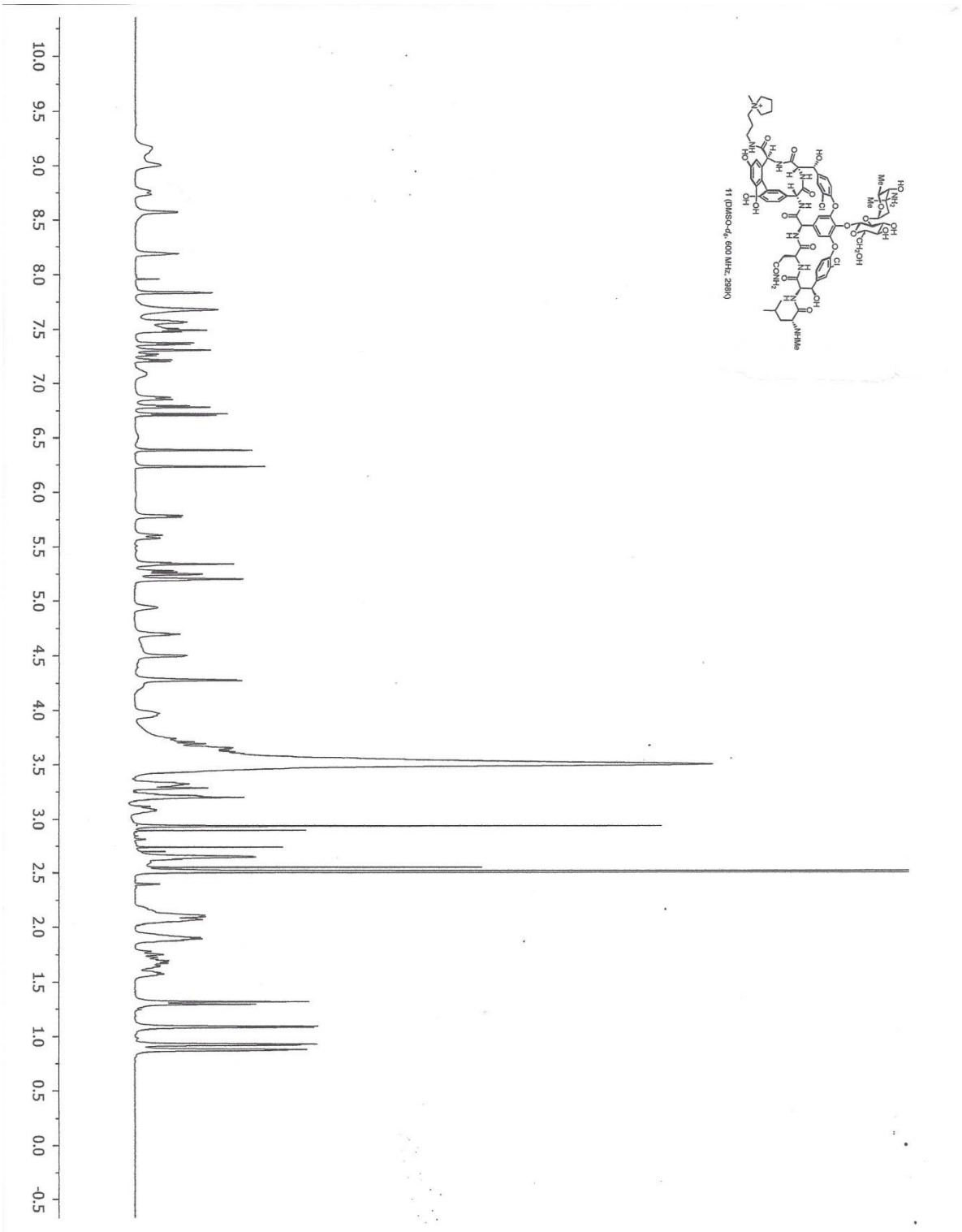
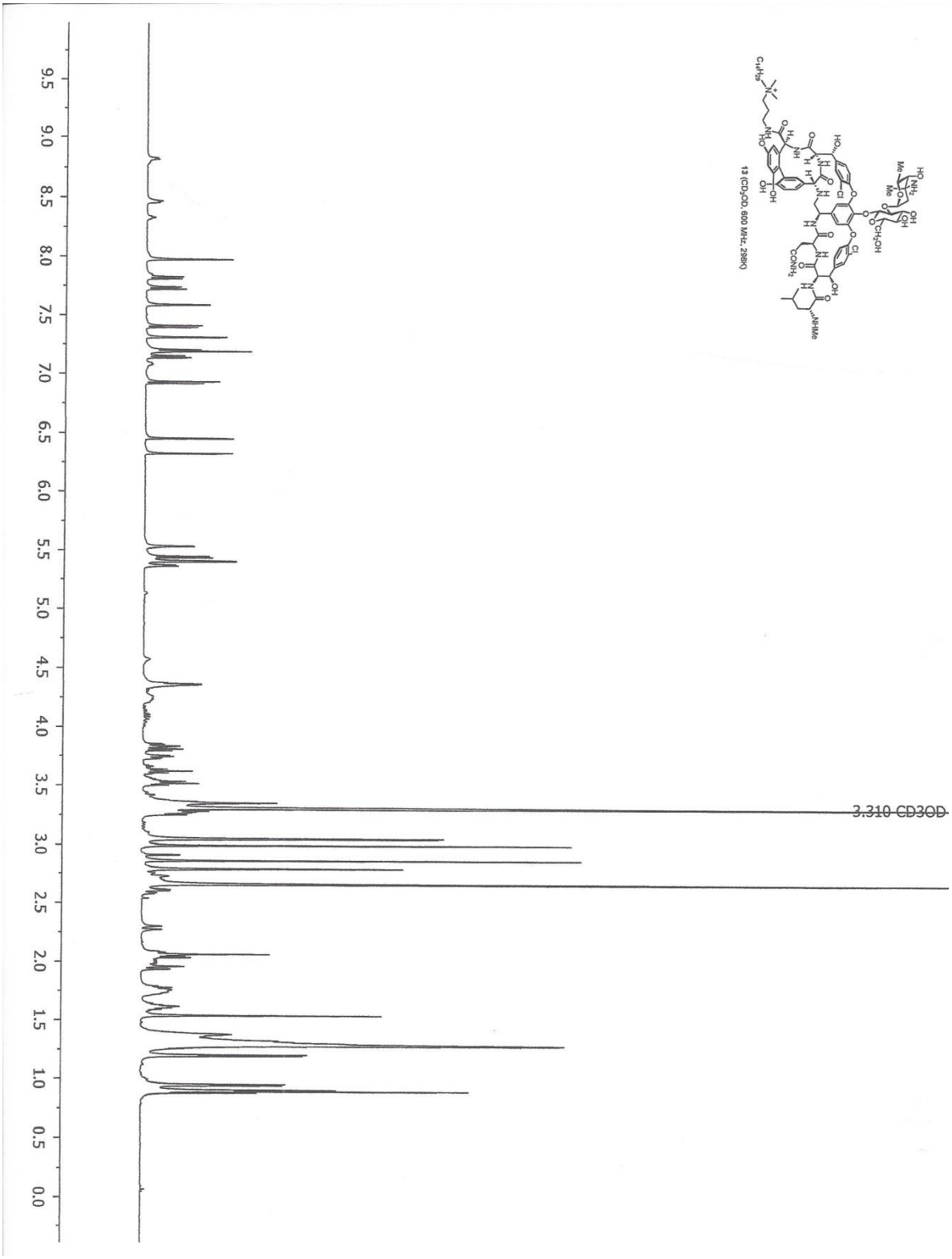
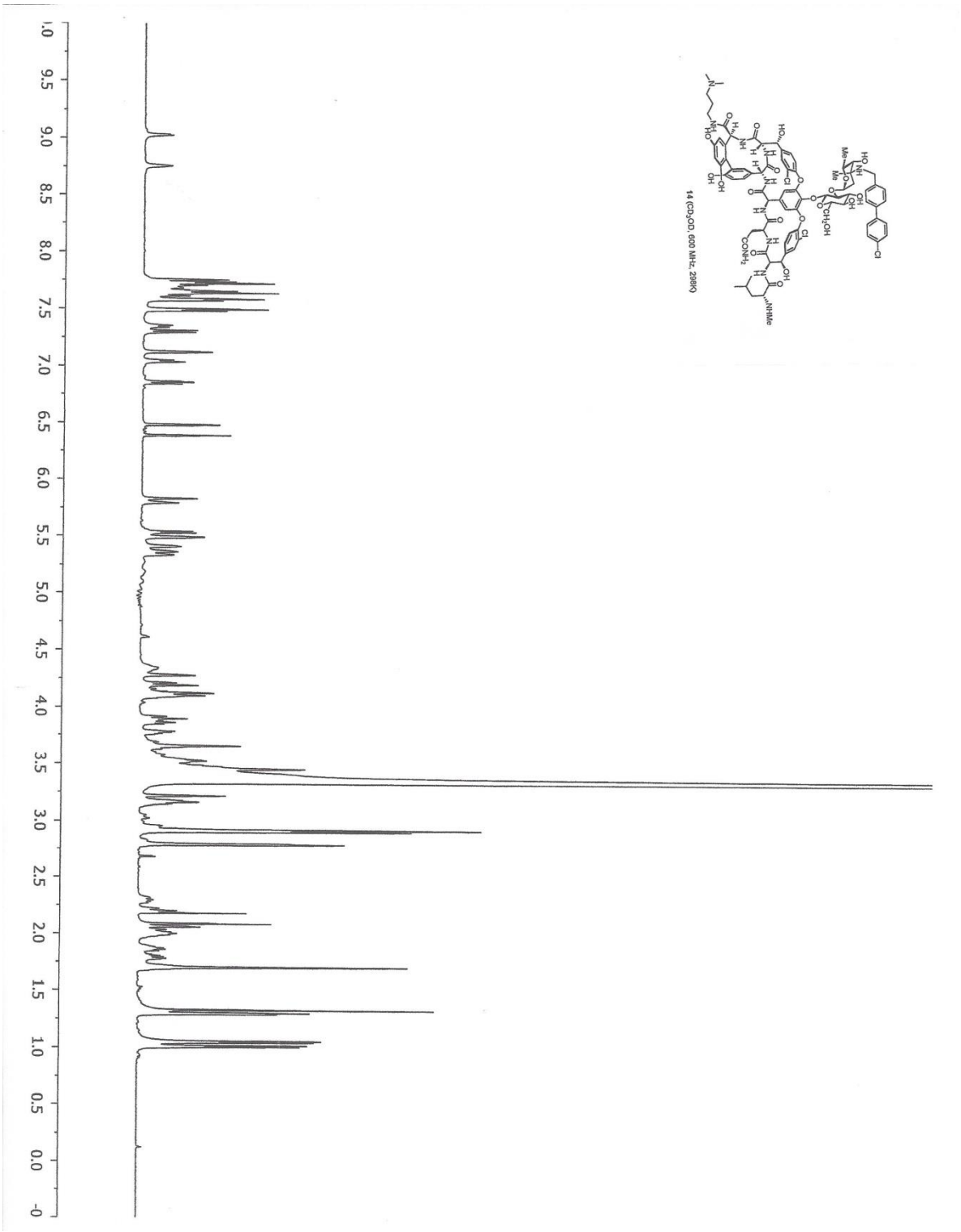
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0


11 (DMSO-d6, 600 MHz, 298K)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5


3.310 CD3OD
13 (CD3OD, 600 MHz, 298K)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


14 (CD3OD, 600 MHz, 298K)
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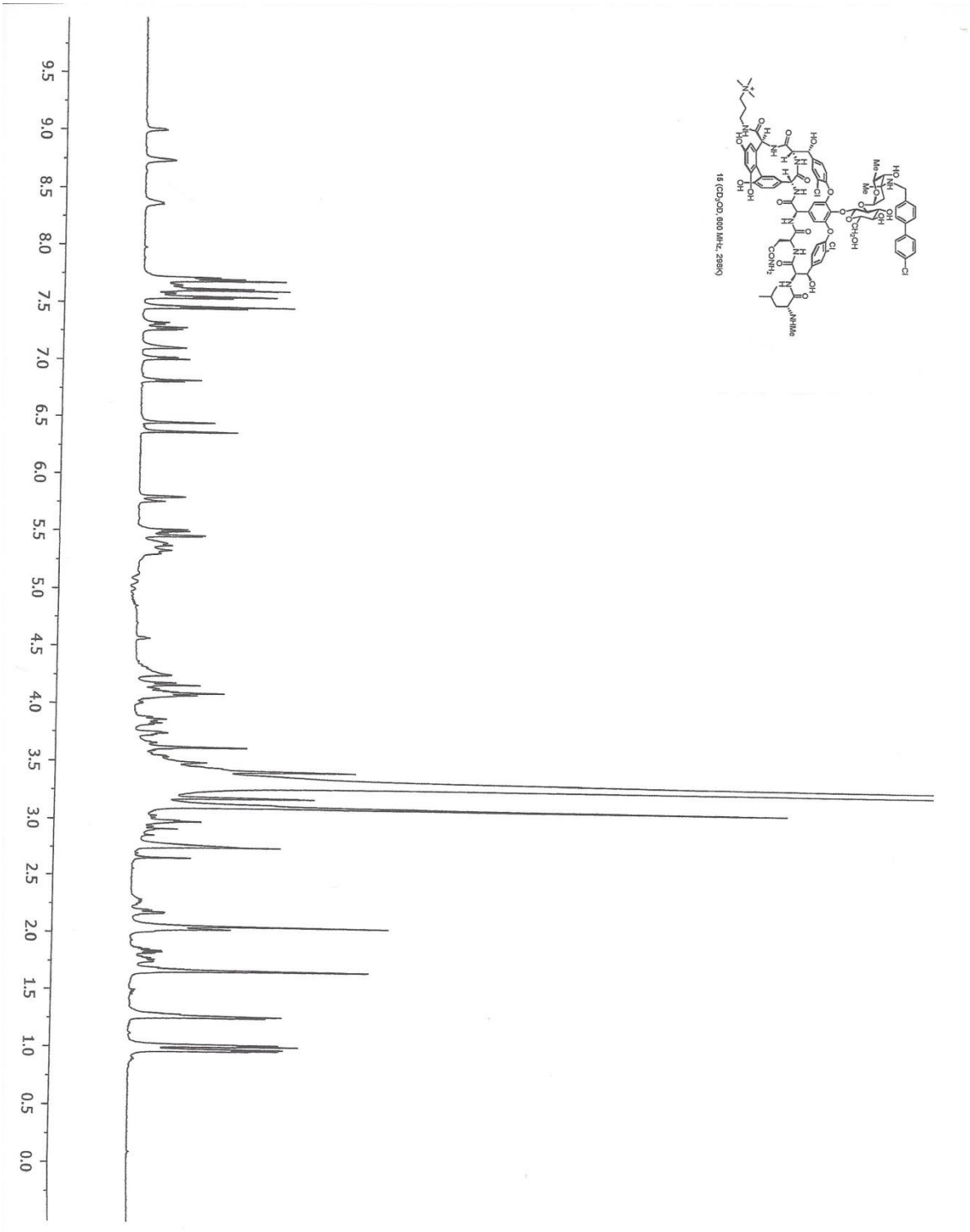
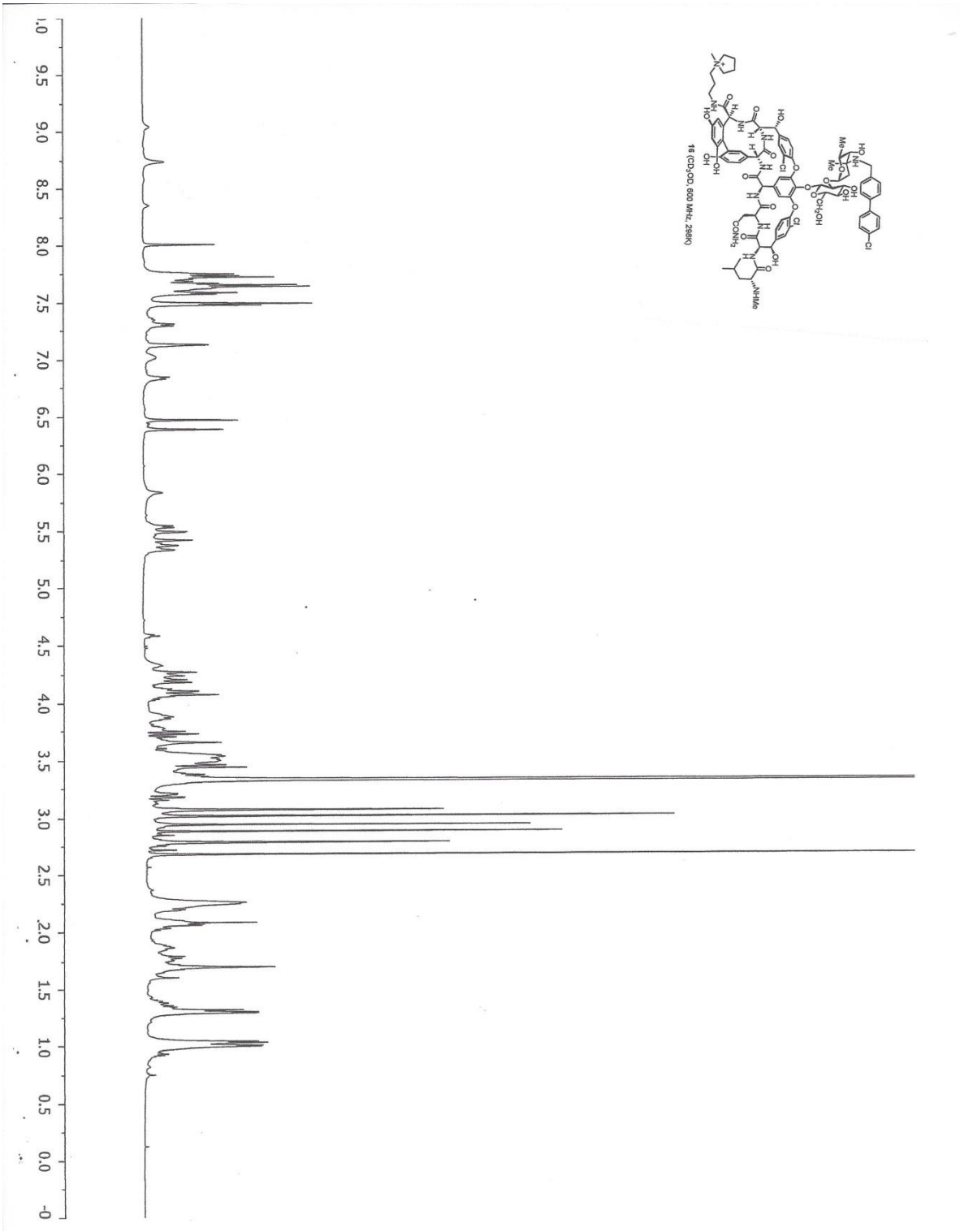
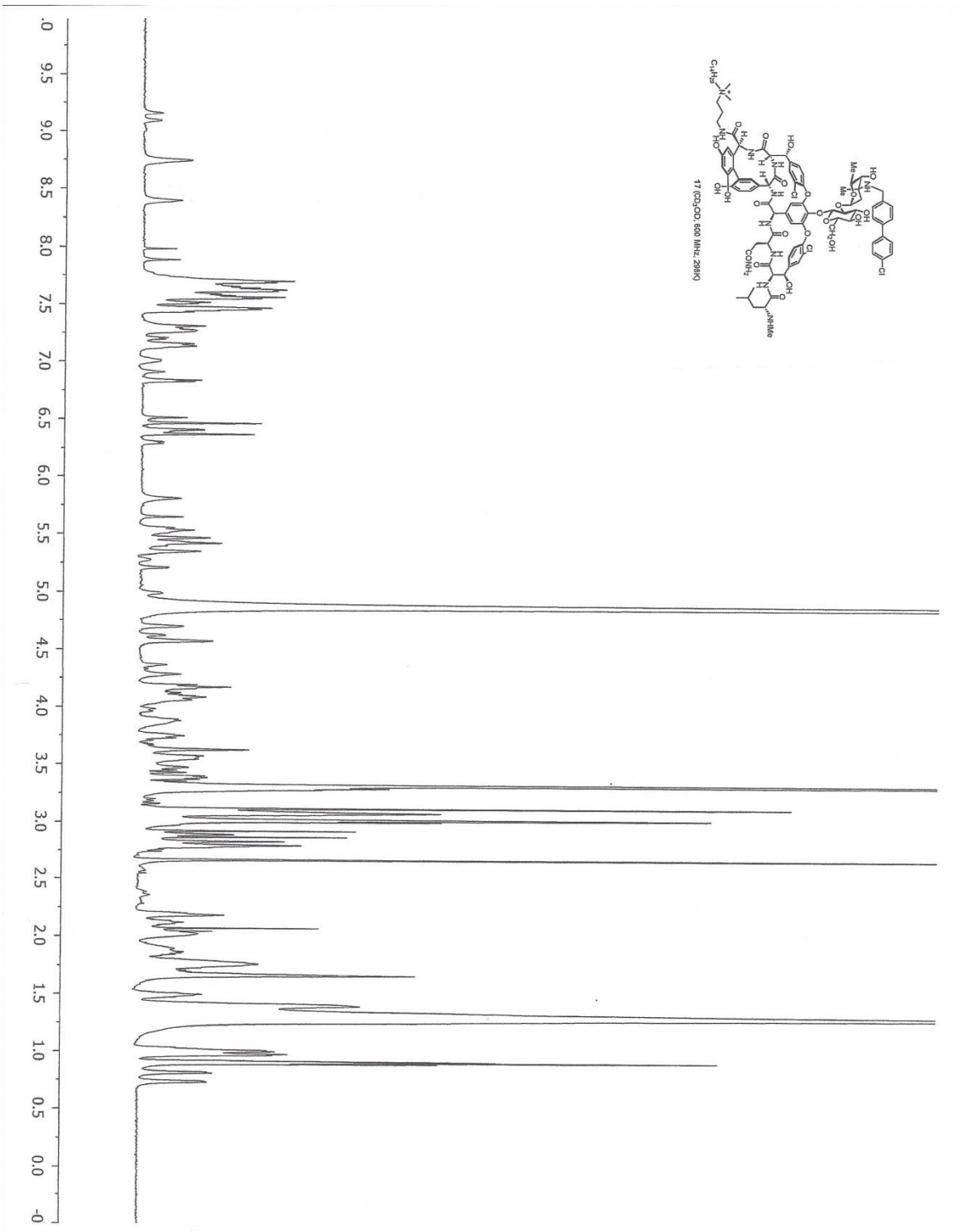
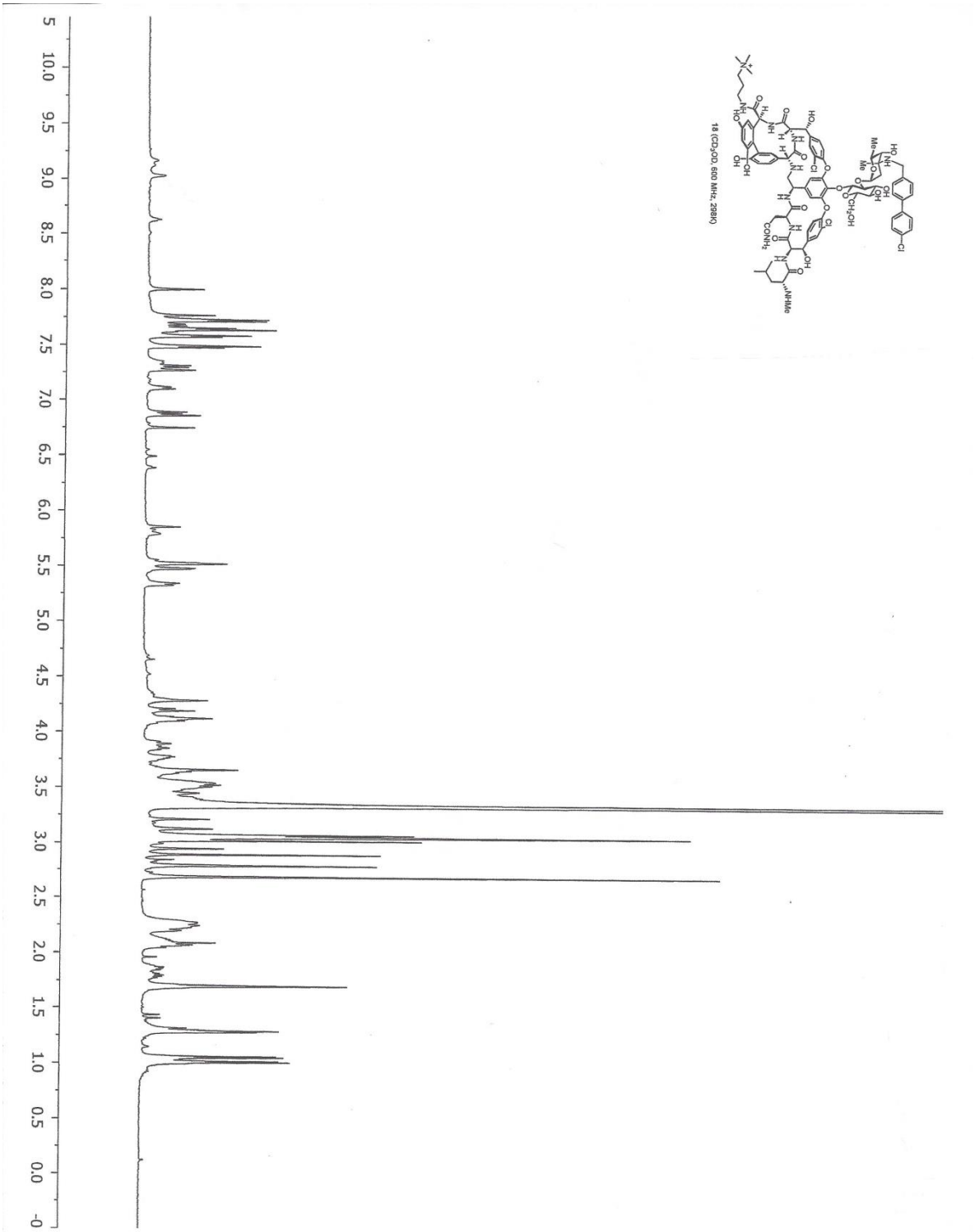
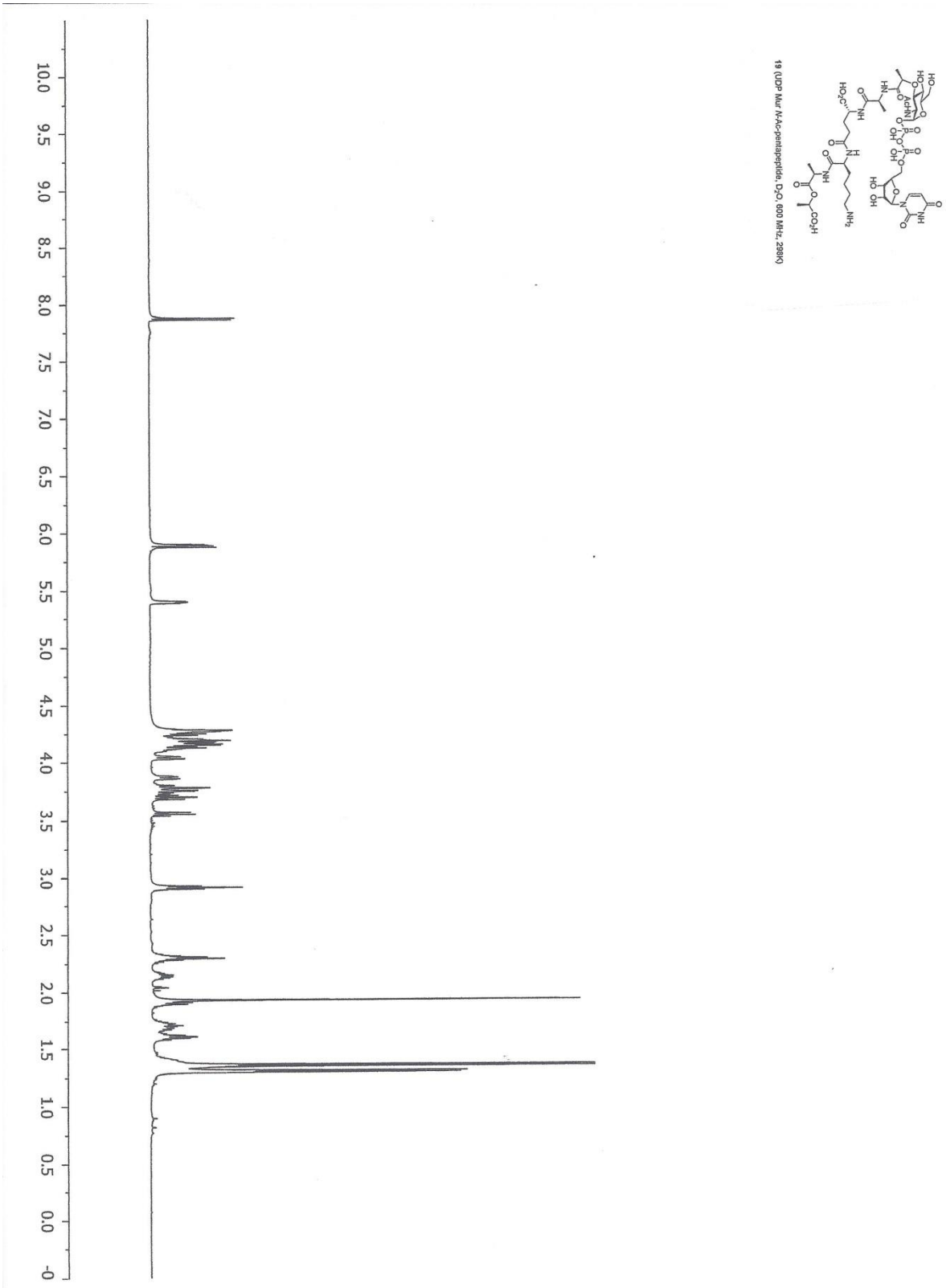
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0


16 (CD3OD, 600 MHz, 298K)
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0


10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0
16 (CD3OD, 600 MHz, 298K)


17 (CD3OD, 600 MHz, 298K)
.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0


18 (CD3OD, 600 MHz, 298K)
5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0


19 (UDP Mur N-Ac-pentapeptide, D2O, 600 MHz, 288K)
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0