Supporting Information

Total Synthesis of [Ψ[C(=NH)NH]Tpg^4] Vancomycin and its (4-Chlorobiphenyl)methyl Derivative: Impact of Peripheral Modifications on Vancomycin Analogs Redesigned for Dual D-Ala-D-Ala and D-Ala-D-Lac Binding

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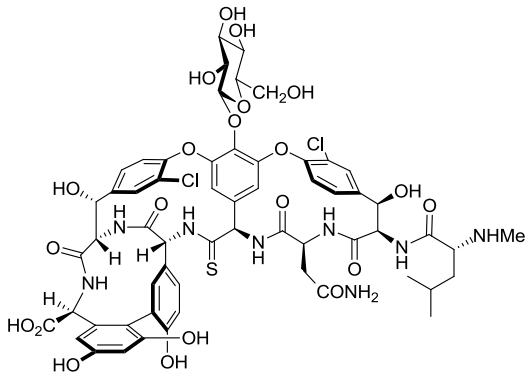
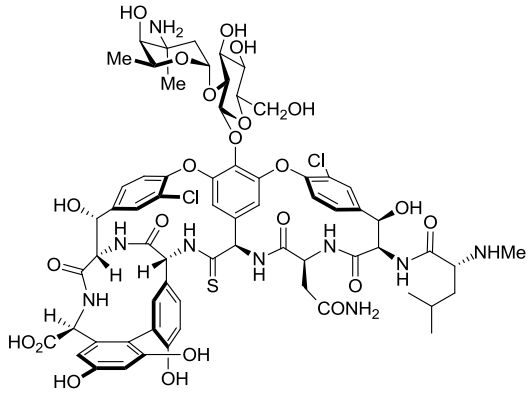


Compound 13: In a total volume of 1.0 mL, 4.0 mM UDP-glucose (2.3 mg, Sigma-Aldrich, 4.0 μmol) and 0.5 mM **8** (0.58 mg, 0.50 μmol) were incubated with 75 mM Tricine-NaOH (pH 9.0), 2 mM tris-(2-carboxyethyl)phosphine, 1 mM $MgCl_2$, glycerol (10% v/v) and 10 μM GtfE for 42 h at 37 °C. The reaction mixture was quenched by the addition of MeOH (9.0 mL) at 0 °C and the residue was passed through a 0.45 μm polyethersulfone membrane filter and concentrated by evaporation to a final volume of *ca*. 1.5 mL. After the addition of H_2O (0.5 mL), the mixture was purified by semi-preparative reverse-phase HPLC (Vydac 218TP1022–C18, 10 μm, 22 × 250 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 10 mL/min, t_R = 23.2 min) to afford **13** (0.48 mg, 75% yield) as a white amorphous solid: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 8.83 (d, J = 6.0 Hz, 1H), 8.42 (br s, 1H), 7.74–7.72 (m, 2H), 7.69–7.65 (m, 2H), 7.64–7.60 (m, 2H), 7.30 (d, J = 8.4 Hz, 1H), 7.24 (br s, 1H), 6.78–6.73 (m, 1H), 6.45 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 1.6 Hz, 1H), 6.26 (s, 1H), 5.95 (s, 1H), 5.41–5.36 (m, 3H), 5.32–5.28 (m, 1H), 4.41 (d, J = 9.0 Hz, 1H), 4.30 (s, 1H), 4.23 (dd, J = 4.8, 4.8 Hz, 1H), 4.07–4.04 (m,

1H), 3.92 (d, J = 11.4 Hz, 1H), 3.82–3.81 (br m, 1H), 3.68–3.64 (m, 1H), 3.57–3.49 (m, 2H), 3.44–3.42 (m, 2H), 3.21–3.20 (m, 1H), 2.79 (s, 3H), 2.78 (s, 1H), 2.67 (s, 1H), 1.90–1.84 (m, 2H), 1.74–1.62 (m, 1H), 1.46–1.43 (m, 1H), 1.40–1.30 (m, 3H), 0.97 (d, J = 6.0 Hz, 3H), 0.95 (d, J = 6.0 Hz, 3H); ESI-TOF HRMS *m/z* 1321.3245 (M + H^+, $C_{59}H_{62}Cl_2N_8O_{21}S$ requires 1321.3206).



Compound 2: In a total volume of 1.3 mL, 3.0 mM UDP-vancosamine[24] (2.2 mg, 3.8 μmol) and 0.5 mM **13** (0.84 mg, 0.64 μmol) were incubated with 75 mM Tricine-NaOH (pH 9.0), 2 mM tris-(2-carboxyethyl)phosphine, 0.2 mg/mL bovine serum albumin, 1 mM $MgCl_2$, glycerol (10% v/v), and 5 μM GtfD for 1 h at 37 °C. The reaction mixture was quenched by the addition of MeOH (8 mL) at 0 °C and was passed through a 0.45 μm polyethersulfone membrane filter and concentrated by evaporation to a final volume of *ca*. 2 mL. After the addition of H_2O (1.0 mL), the mixture was purified by semi-preparative reverse-phase HPLC (Vydac 218TP1022–C18, 10 μm, 22 × 250 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 10 mL/min, t_R = 21.3 min) to afford **2** (0.81 mg, 87% yield) as a white amorphous solid: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 8.80 (d, J = 6.0 Hz, 1H), 8.49 (s, 1H), 7.73–7.72 (m, 1H), 7.68–7.63 (m, 5H), 7.32–7.27 (m, 1H), 7.22 (d, J = 1.6 Hz, 1H), 6.78 (d, J = 8.4 Hz, 1H), 6.46 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 1.6 Hz, 1H), 6.17 (s, 1H), 5.85 (s, 1H), 5.49–5.41 (m, 3H), 5.34–5.32 (m, 5H), 4.45–4.38 (br m, 1H), 4.29 (s, 1H), 4.25–4.21 (m, 1H), 4.08–4.06 (m, 2H), 3.99 (s, 1H), 3.92–3.88 (m, 1H), 3.86–3.83 (m, 2H), 3.80–3.78 (m, 1H), 3.70–3.61 (m, 2H), 3.59–3.51 (m, 2H), 3.44–3.42 (m, 1H), 3.22–3.20 (m, 1H), 3.01–2.98 (m, 1H), 2.77 (s, 3H), 2.36–2.28 (m, 2H), 2.08–2.04 (m, 2H), 1.95–1.93 (m, 1H), 1.90–1.85 (m, 1H), 1.71–1.64 (m, 1H), 1.51

(s, 3H), 1.45–1.35 (m, 3H), 1.20 (d, J = 6.6 Hz, 3H), 1.19–1.12 (m, 1H), 1.00 (d, J = 6.0 Hz, 3H), 0.98 (d, J = 6.6 Hz, 3H); ESI-TOF HRMS *m/z* 1464.4131 (M + H^+, $C_{66}H_{75}Cl_2N_9O_{23}S$ requires 1464.4152).

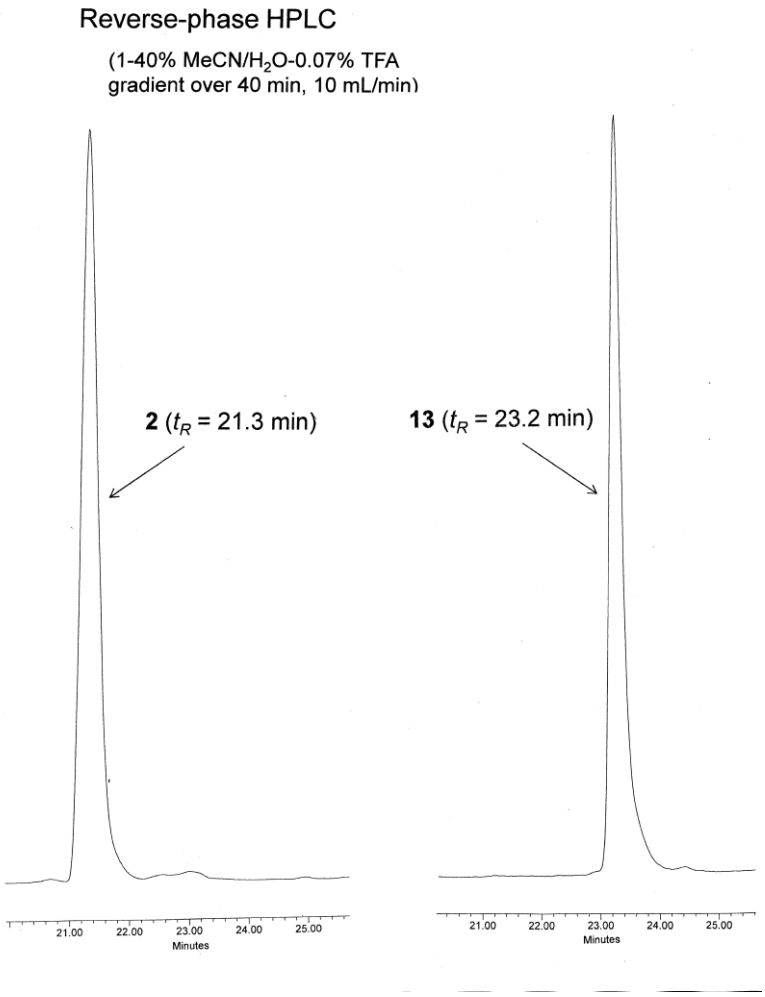
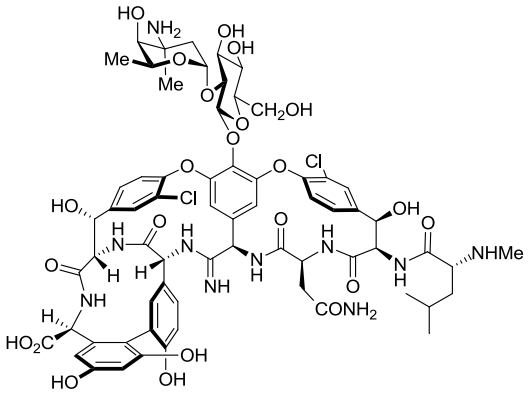
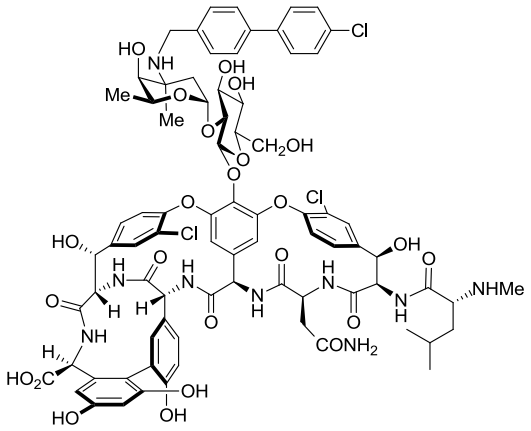


Figure S1. HPLC trace of the crude reaction mixture in the conversion of **8** to **13** and **13** to **2**. For **8** to **13** (Vydac 218TP1022–C18, 10 μm, 22 × 250 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 10 mL/min, t_R = 23.2 min) indicating that the isolated yield (75%) underestimates the extent of the conversion (86–92% by HPLC). For **13** to **2** (Vydac 218TP1022–C18, 10 μm, 22 × 250 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 10 mL/min, t_R = 21.3 min) indicating that the isolated yield (87%) underestimates the extent of the conversion (>95% by HPLC).



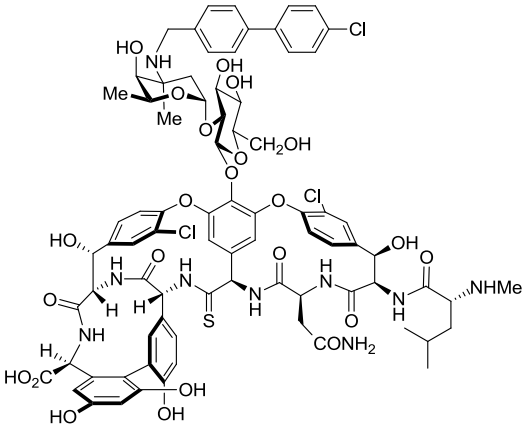
Compound 3: A mixture of **2** (0.27 mg, 0.18 μmol) and AgOAc (0.3 mg, 1.8 μmol) was treated with saturated NH_3−CH_3OH (0.2 mL) at 0 °C. The reaction mixture was stirred for 7 h at 25 °C. The reaction mixture was quenched by the addition of 50% CH_3OH in H_2O (0.2 mL) and the residue was purified by semi-preparative reverse-phase HPLC (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 = 16.0 min) to afford **3** (50 μg, 32% yield brsm, unoptimized) as a white amorphous solid: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 7.82–7.68 (m, 3H), 7.43 (s, 1H), 7.20–7.11 (m, 2H), 7.04 (s, 1H), 6.89 (s, 1H), 6.49–6.45 (m, 2H), 5.59–5.51 (m, 3H), 5.42–5.38 (m, 2H), 4.31–4.16 (m, 3H), 3.82–3.76 (m, 2H), 3.67–3.47 (m, 3H), 3.19 (s, 1H), 2.95–2.82 (m, 5H), 2.45–2.34 (m, 1H), 2.11–1.99 (m, 3H), 1.90–1.75 (br m, 2H), 1.65 (s, 3H), 1.24–1.19 (m, 5H), 0.88 (d, J = 6.0 Hz, 3H), 0.83 (d, J = 6.0 Hz, 3H); ESI-TOF HRMS *m/z* 724.2307 (M + 2H$^+$, $C_{66}H_{76}Cl_2N_{10}O_{23}$ requires 724.2304).



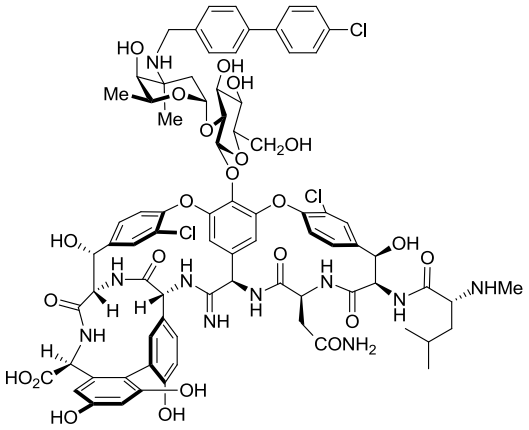
Compound 4: A solution of **1** (vancomycin, 0.45 mg, 0.31 μmol) in anhydrous DMF (30 μL) was treated with 4-(4-chlorophenyl)benzaldehyde (0.1 M in DMF, 4.7 μL, 0.47 μmol) and *i*-Pr_2NEt (distilled, 0.1 M in DMF, 15.6 μL, 1.56 μmol) at 25 °C. The reaction mixture was stirred for 2 h at 70 °C. After the reaction was complete, the mixture was treated with $NaCNBH_3$ (1 M in THF, 31.2 μL, 31.2 μmol) and stirred for 5 h at 70 °C. The reaction mixture was quenched by the addition of 50% CH_3OH in H_2O (0.2 mL) at 25 °C and the residue was purified by semi-preparative reverse-phase HPLC (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 = 34.3 min) to afford **4** (0.31 mg, 61% yield) as a white amorphous solid: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 8.98 (s, 1H), 8.71 (s, 1H), 7.76–7.70 (m, 5H), 7.62 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 7.20 (d, J = 9.0 Hz, 1H), 7.08 (s, 1H), 6.71 (br s, 1H), 6.52 (d, J = 2.4 Hz, 1H), 6.41 (d, J = 2.4 Hz, 1H), 5.63 (s, 1H), 5.52 (s, 1H), 5.40–5.37 (m, 2H), 5.28 (d, J = 2.4 Hz, 1H), 4.77 (s, 1H), 4.73 (d, J = 6.0 Hz, 1H), 4.27 (s, 1H), 4.19–4.15 (m, 3H), 4.08–3.95 (m, 2H), 3.90–3.80 (m, 2H), 3.68–3.62 (m, 3H), 3.43 (s, 1H), 2.92 (d, J = 12.6 Hz, 1H), 2.78 (s, 1H), 2.19 (d, J = 12.0 Hz, 1H), 2.05 (d, J = 13.2 Hz, 1H), 1.90–1.87 (m, 1H), 1.88 (s, 3H), 1.68–1.65 (m, 1H), 1.25 (d, J = 6.6 Hz, 3H), 0.95–0.92 (m, 6H); ESI-TOF HRMS *m/z* 824.7421 (M + $2H^+$, $C_{79}H_{84}Cl_3N_9O_{24}$ requires 824.7420).

This reaction was run on scales of 0.2–1.2 mg (55–61%) as part of the optimization of conditions for use with **5** on the amounts available.

Larger scale procedure: A solution of **1** (vancomycin, 90.0 mg, 62.1 μmol) in anhydrous DMF (8.0 mL) was treated with 4-(4-chlorophenyl)benzaldehyde (19.8 mg, 74.5 μmol) and *i*-Pr_2NEt (51.0 μL, 0.32 mmol) at 25 °C. The reaction mixture was stirred for 2 h at 70 °C. After the reaction was complete, the mixture was treated with $NaCNBH_3$ (1 M in THF, 0.32 mL, 0.32 mmol) and stirred for 5 h at 70 °C. The reaction mixture was quenched by the addition of 50% CH_3OH in H_2O (1.0 mL) at 25 °C and the residue was purified by semi-preparative reverse-phase HPLC (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 = 34.3 min) to afford **4** (75.3 mg, 74% yield) as a white amorphous solid.



Compound 5: Following the procedure detailed for **4** and using **2** (0.42 mg, 0.29 μmol), semi-preparative reverse-phase HPLC (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 = 34.9 min) afforded **5** (0.27 mg, 57% yield) as a white amorphous solid: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 7.72–7.66 (m, 6H), 7.62 (d, J = 8.4 Hz, 2H), 7.55 (d, J = 8.4 Hz, 2H), 7.34 (s, 1H), 7.27 (d, J = 8.4 Hz, 2H), 7.17 (s, 1H), 6.75 (d, J = 9.0 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 6.39 (d, J = 2.4 Hz, 1H), 5.52–5.46 (m, 2H), 5.37–5.31 (m, 3H), 4.59 (s, 1H), 4.24 (s, 1H), 4.16 (d, J = 12.6 Hz, 1H), 4.07 (d, J = 12.6 Hz, 1H), 3.92 (d, J = 6.0 Hz, 1H), 3.85–3.75 (m, 2H), 3.63 (dd, J = 9.0, 9.0 Hz, 1H), 3.60–3.56 (m, 2H), 3.44–3.39 (m, 2H), 3.19 (s, 1H), 2.72 (s, 3H), 2.40–2.25 (m, 1H), 2.20–2.15 (m, 1H), 2.10–1.97 (m, 2H), 1.83–1.73 (m, 2H), 1.69–1.59 (m, 5H), 1.25 (d, J = 6.6 Hz, 3H), 1.00–0.94 (m, 6H); ESI-TOF HRMS *m/z* 832.7286 (M + $2H^+$, $C_{79}H_{84}Cl_3N_9O_{23}S$ requires 832.7306).

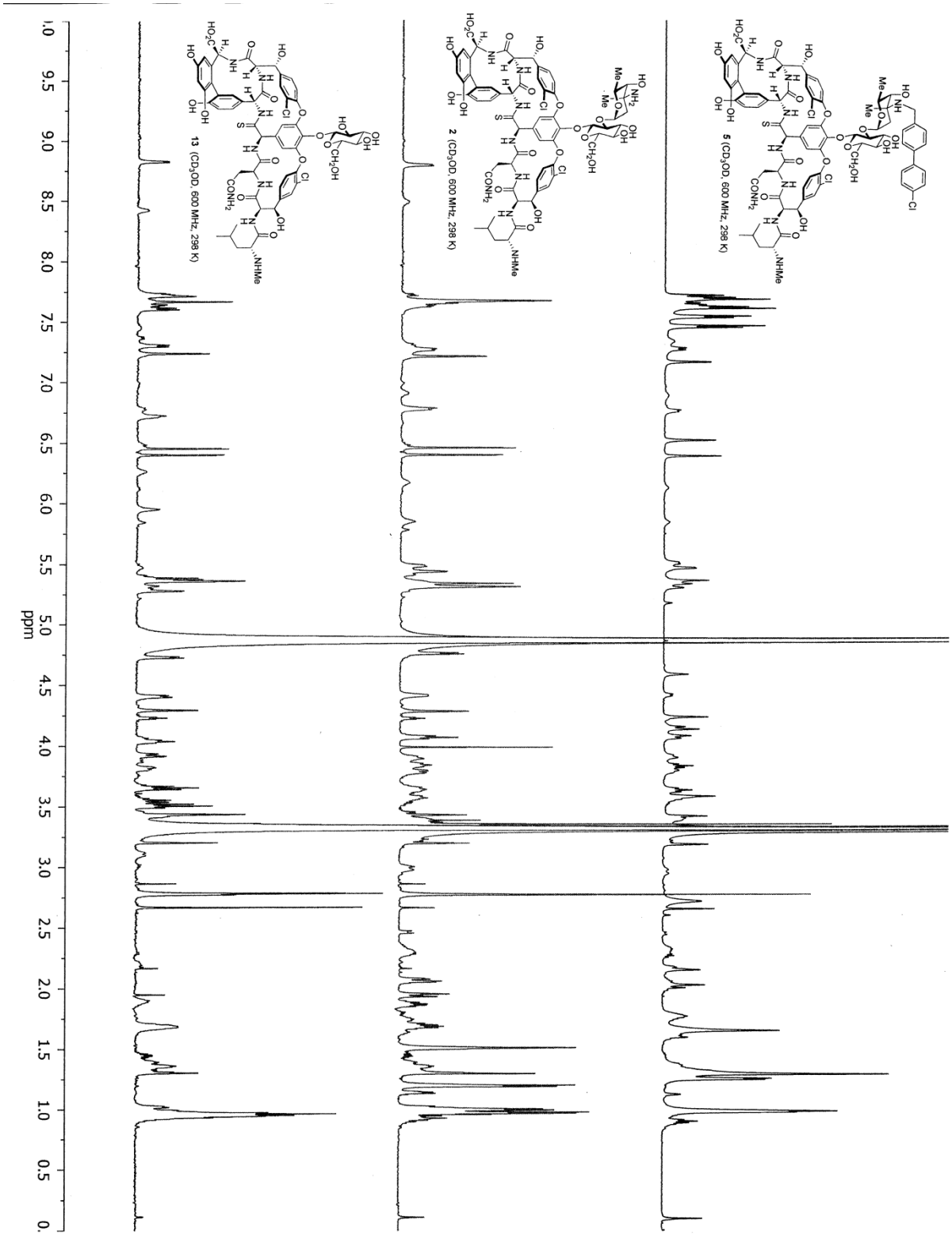


Compound 6: Following the procedure detailed for **3** and using **5** (0.31 mg, 0.19 μmol), semi-preparative reverse-phase HPLC (Zorbax SB–C18, 5 μm, 9.4 × 150 mm, 1–40% MeCN/H_2O–0.07% TFA gradient over 40 min, 3 mL/min, t_R = 33.6 min) afforded **6** (67 μg, 45% yield brsm, unoptimized) as a white amorphous solid: ^{1}H NMR (CD_3OD, 600 MHz, 298 K) δ 7.82–7.72 (m, 3H), 7.64 (d, J = 8.4 Hz, 2H), 7.56 (d, J = 8.4 Hz, 2H), 7.49–7.33 (m, 4H), 7.07 (s, 1H), 6.91 (d, J = 9.0 Hz, 1H), 6.51–6.46 (m, 2H), 5.61–5.37 (m, 5H), 4.33 (br s, 1H), 4.21–4.13 (m, 2H), 4.09–4.03 (m, 2H), 3.88–3.75 (m, 2H), 3.73–3.58 (m, 5H), 3.51–3.49 (m, 1H), 3.37 (s, 1H), 3.21 (s, 1H), 2.88 (s, 3H), 2.81–2.76 (m, 2H), 2.49–2.45 (m, 1H), 2.42–2.28 (m, 2H), 2.21–2.06 (m, 3H), 1.85–1.77 (m, 2H), 1.65 (s, 3H), 1.40–1.30 (m, 4H), 0.92 (d, J = 6.6 Hz, 3H), 0.88 (d, J = 6.6 Hz, 3H); ESI-TOF HRMS *m/z* 824.2539 ($M + 2H^+$, $C_{79}H_{88}Cl_3N_{10}O_{23}$ requires 824.2578).

***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) and vancomycin-resistant *Enterococcus faecalis* (VanB VRE, strain ATCC 51299), were inoculated and grown in an orbital shaker at 37 °C in 100% Mueller-Hinton broth (VSSA, MRSA and VanB VRE) or 100% Brain-Heart Infusion broth (VanA VRE). 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) to achieve the turbidity equivalent of 1:100 dilution of 0.5 M Macfarland solution. This diluted bacterial stock solution was then inoculated into a well of a V-shaped 96-well glass coated microtiter plate, supplemented with serial diluted aliquots of the antibiotic solution 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 the cell growth in the wells is reported as the MIC. The reported MIC values for the new antibiotics were determined against vancomycin as a standard in the first well, which have well-established MIC values.

S1. Clinical and Laboratory Standards Institute. *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, 2009.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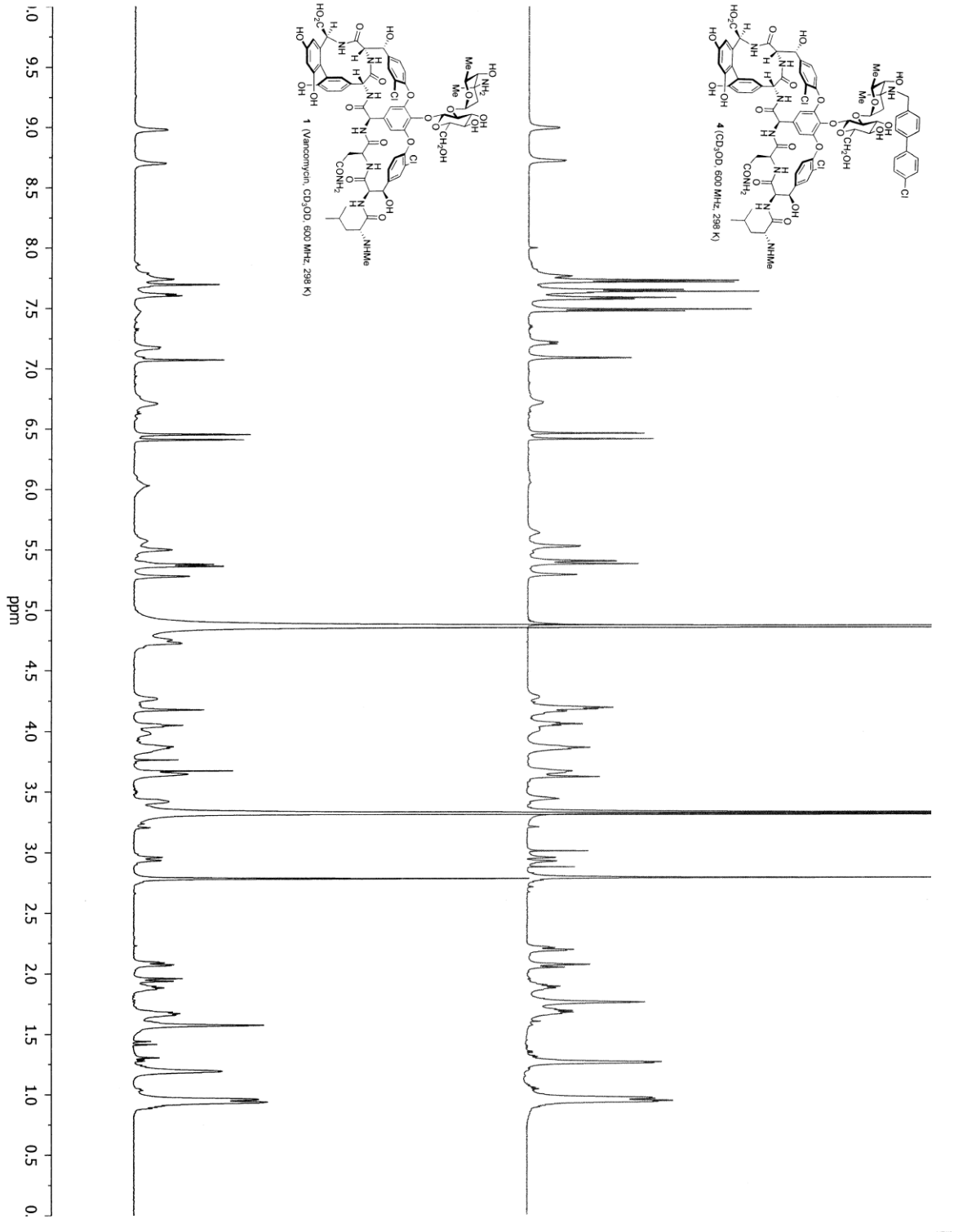
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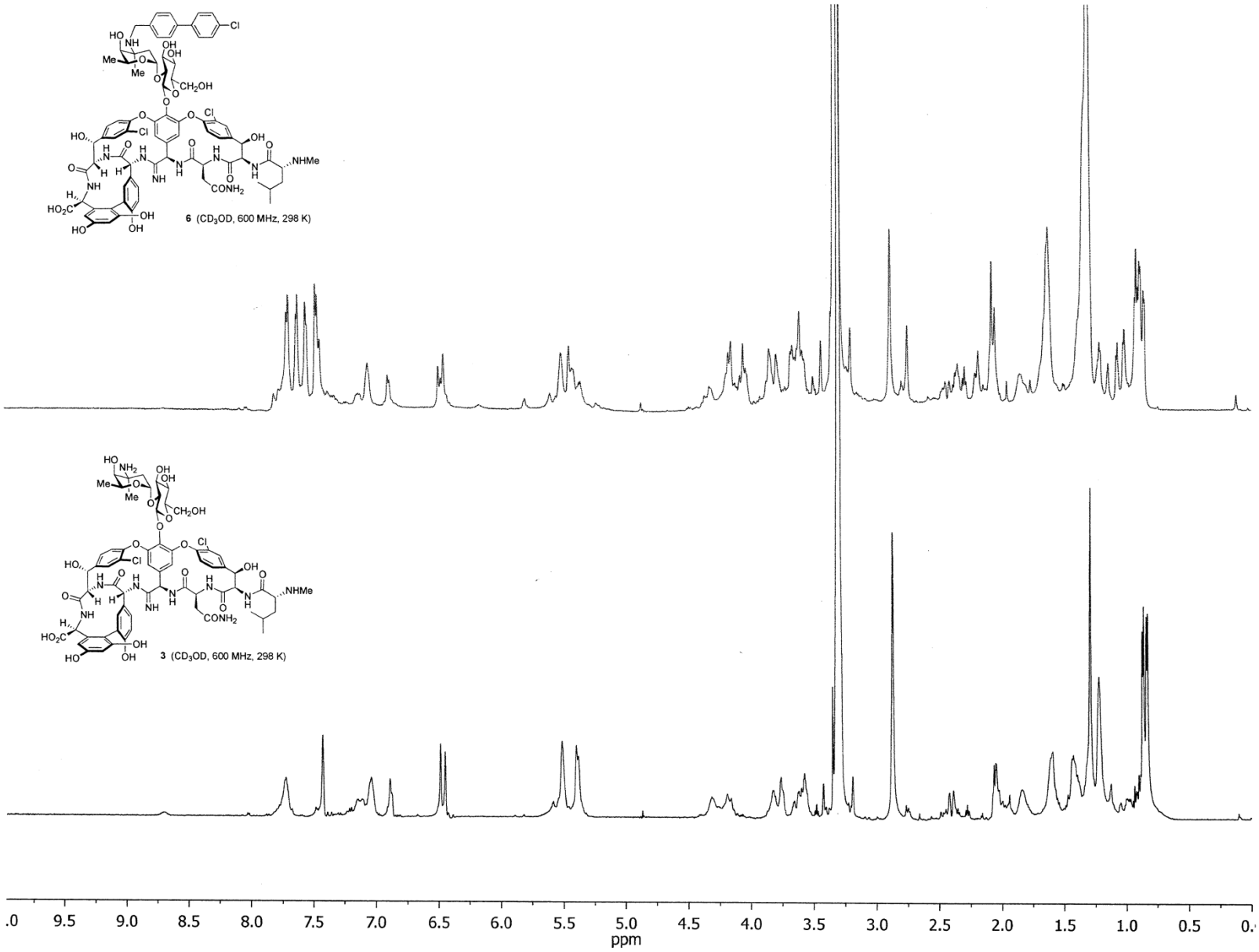
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