Catalytic Site-Selective Thiocarbonylations and Deoxygenations of Vancomycin Reveal Hydroxyl-Dependent Conformational Effects

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Supporting Information

I. General Procedures

Proton NMR spectra were recorded on a 400 or 500 MHz spectrometer. Proton chemical shifts were reported in ppm (δ) with the residual protium in the NMR solvent as a reference ($CHCl_3$, δ 7.26 relative to tetramethylsilane, TMS; DMSO, δ 2.50 relative to TMS). The listed spectral data are reported as follows: chemical shift (multiplicity [singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m)], coupling constants [Hz], integration; *assignment if determined*). Carbon NMR spectra were recorded on a 100 or 126 MHz spectrometer with complete proton decoupling. Carbon chemical shifts are reported in ppm (δ) relative to the solvent signal ($CDCl_3$, δ 77.0; DMSO-d_6, δ 39.5). NMR data were collected at 25 °C except when indicated. Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 Å F254 pre-coated plates (0.25 mm thickness). TLC visualization was accomplished by irradiation with a UV lamp. Liquid chromatography-mass spectrometry (LC/MS) was performed on a Waters Acquity instrument equipped with dual atmospheric pressure chemical ionization (API)/electrospray ionization (ESI), a SQ mass spectrometer, and a photodiode array detector. High-resolution liquid chromatography-mass spectrometry (HR-LC/MS) was performed on a Waters XEVO instrument equipped with ESI, a QToF mass spectrometer, and a photodiode array detector. Analytical high-performace liquid chromatography (HPLC) was performed on an Agilent 1100 Series instrument equipped with a diode array detector. Preparative HPLC was performed on a Gilson instrument equipped with a diode array detector and liquid handler. Medium-performance liquid chromatography (MPLC) was performed on a Biotage SP4 instrument equipped with a diode array detector and liquid handler.

LC/MS Method

Method 1: Column = Waters Acquity UPLC® BEH C_{18} 1.7 μm, 2.1 × 100 mm; Temperature = 25 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 0.8 mL/min
Start at 20% B, ramped to 100% B over 3 min, held at 100% B for 1 min.

HR-LC/MS Method

Method 2: Column = Waters Acquity UPLC® BEH C_8 1.7 μm, 2.1 × 100 mm; Temperature = 25 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 0.3 mL/min
Held at 5% B for 1 min, ramped to 95% B over 5 min and held for 1.5 min.

Analytical HPLC Methods

Method 3: Column = Phenomenex Luna 5 μm C_{18} (2), 250 × 10 mm; Temperature = 25 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 4.0 mL/min
Held at 5% B for 2 min, ramped to 67% B over 28 min, held at 67% B over 10 min, ramped to 95% B over 10 min, held at 95% B for 3 min, ramped to 5% B over 3 min and held for 5 min.

Method 4: Column = Waters SymmetryPrep C_8 7 μm, 300 × 7.6 mm; Temperature = 25 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 3.5 mL/min
Held at 0% B for 3 min, ramped to 15% B over 25 min, ramped to 95% B over 3 min, held at 95% B for 7 min, ramped to 0% B over 2 min and held for 7 min.

Preparative HPLC Methods

Method 5: Column = Waters SymmetryPrep C_8 7 μm, 300 × 19 mm; Temperature = 21-23 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 21 mL/min
Held at 0% B for 1.5 min, ramped to 13% B over 25 min, held at 13% B for 1.5 min, ramped to 85% B over 1 min, held at 85% B for 5 min, ramped to 0% B over 0.5 min and held for 7 min.

Method 6: Column = Waters SymmetryPrep C_8 7 μm, 300 × 19 mm; Temperature = 21-23 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 21 mL/min
Held at 0% B for 2 min, ramped to 15% B over 25 min, held at 15% B for 1.5 min, ramped to 85% B over 1 min, held at 85% B for 5 min, ramped to 0% B over 1 min and held for 7 min.

Method 7: Column = Waters SymmetryPrep C_8 7 μm, 300 × 19 mm; Temperature = 21-23 °C
Solvent A = H_2O (0.1% formic acid); Solvent B = MeCN (0.1% formic acid); Flow Rate = 21 mL/min
Held at 0% B for 1.5 min, ramped to 10% B over 25 min, held at 10% B for 1.5 min, ramped to 85% B over 1 min, held at 85% B for 5 min, ramped to 0% B over 0.5 min and held for 7 min.

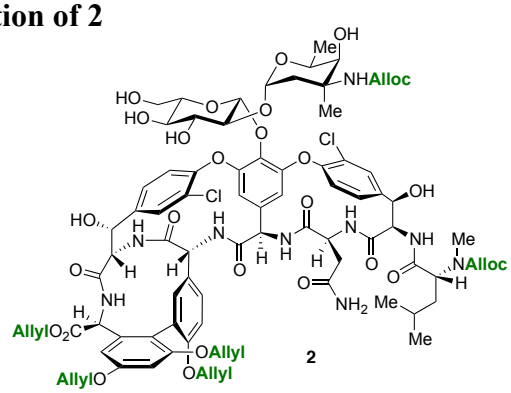
Initial studies were carried out using vancomycin hydrochloride that was provided as a gift from Eli Lilly and Company (Indianapolis, IN). Additionally, vancomycin hydrochloride was purchased from ChemImpex International, Inc (Wood Dale, IL). Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), and toluene were purified by a Seca Solvent Purification System from GlassContour (Nashua, NH). 1,4-Dioxane was refluxed over sodium borohydride for 3 h and distilled under N_2-atmosphere. The distilled dioxane was stored under positive pressure of N_2, away from light, and over 4 Å molecular sieves and was used within 1 month of distillation. All other chemicals were commercially available and used as received.

Minimum inhibitory concentrations (MICs) were determined by Micromyx, LLC (Kalamazoo, MI) in accordance with CLSI guidelines.[1]

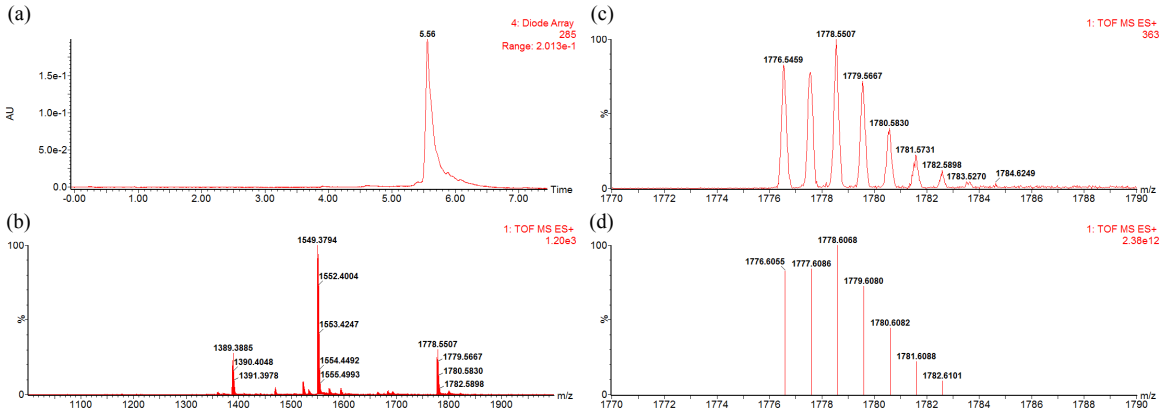
Chemical Abbreviations

PCTF = *O*-phenyl chlorothionoformate
PEMP = 1,2,2,6,6-pentamethylpiperidine
AIBN = 2,2′-azobis(2-methylpropionitrile) (“azobisisobutyronitrile”)
HBTU = *O*-(benzotriazol-1-yl)-*N,N,N′,N′*-tetramethyluronium hexafluorophosphate
Cle = 1-aminocyclopentane-1-carboxylic acid (“cycloleucine”)
Pmh = π-methylhistidine
DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene
EDC•HCl = *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride
HOBt•H_2O = 1-hydroxybenzotriazole hydrate

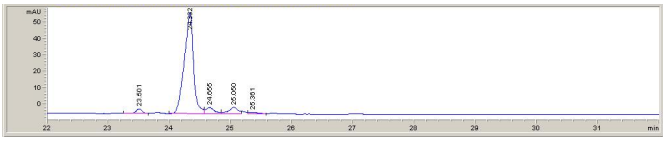
II. Purification and Characterization of 2



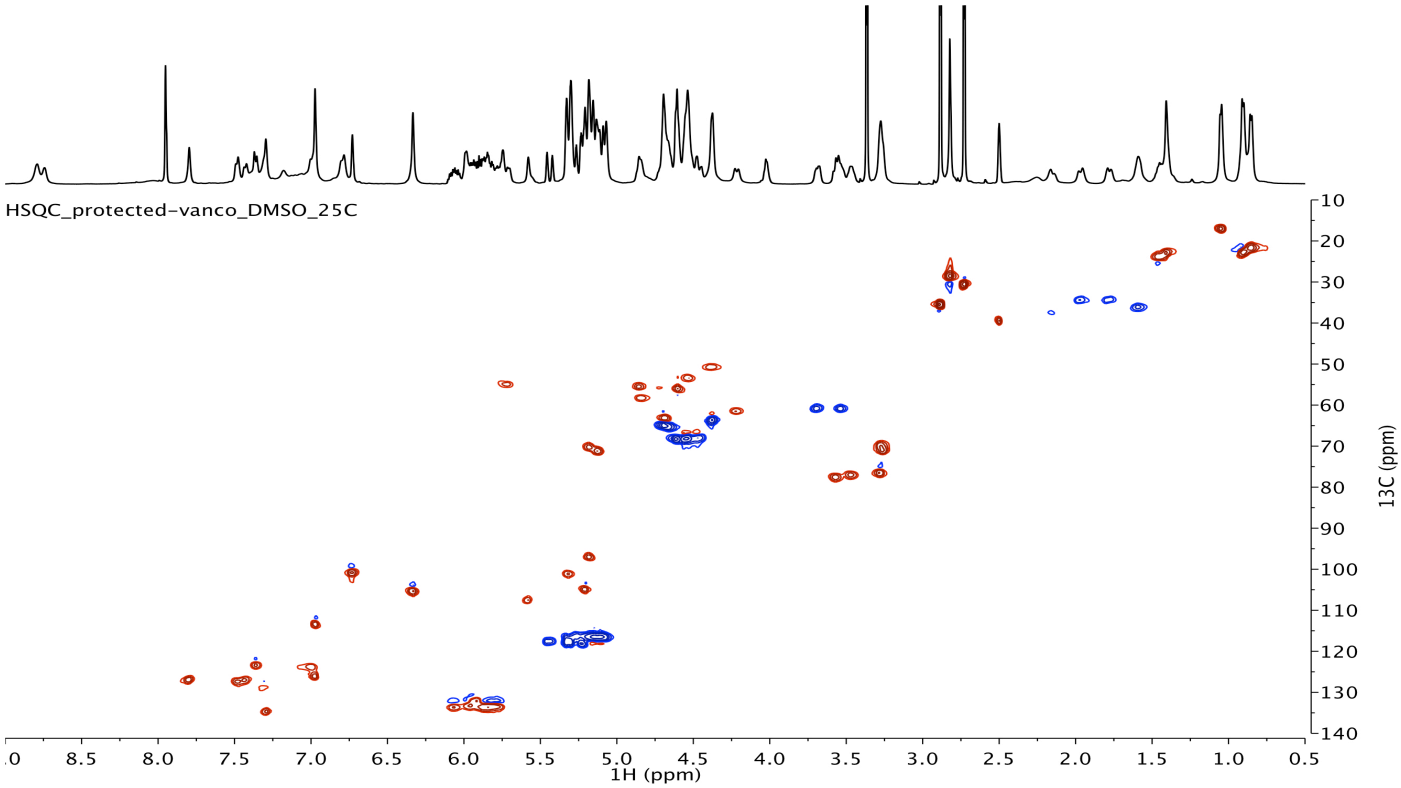
Minimally protected vancomycin **2** was synthesized by both of two reported procedures.[2] This was partially purified by MPLC (SNAP-C18 120-g column with a 12-g samplet; solvent A = H_2O, solvent B = MeOH, flow = 45 mL/min, λ = 210 nm; loaded 750 mg of crude **2** in 3:1 MeOH/H_2O; held at 75% B for 1 column volume (CV), ramped to 80% B over 4 CV, held at 80% B for 6 CV, ramped to 90% B over 1 CV and held for 2 CV; **2** eluted at 8-10 CV).



HR-LC/MS Data from Purified 2: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 5.56 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{86}H_{99}Cl_2N_9O_{28} + H]^+$.

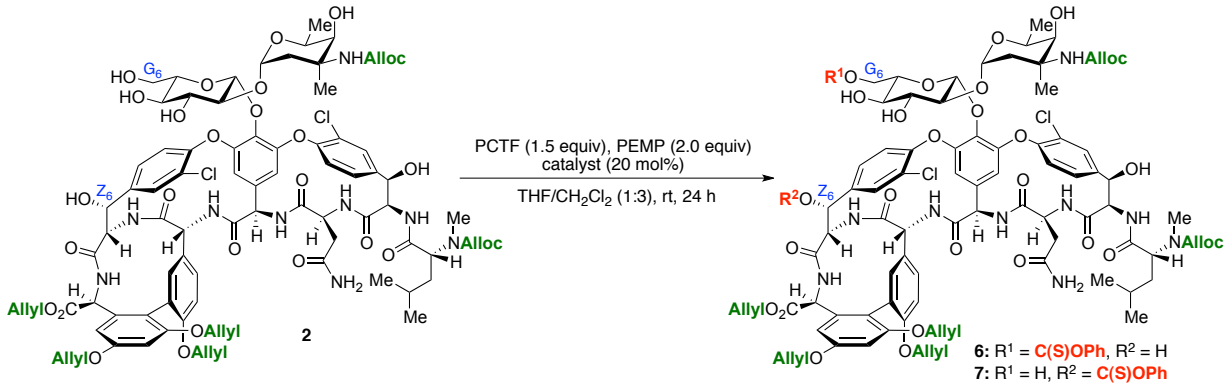


HPLC Trace of Purified 2: Analytical HPLC Method 3, λ = 285 nm, t_R (**2**) = 24.2 min.



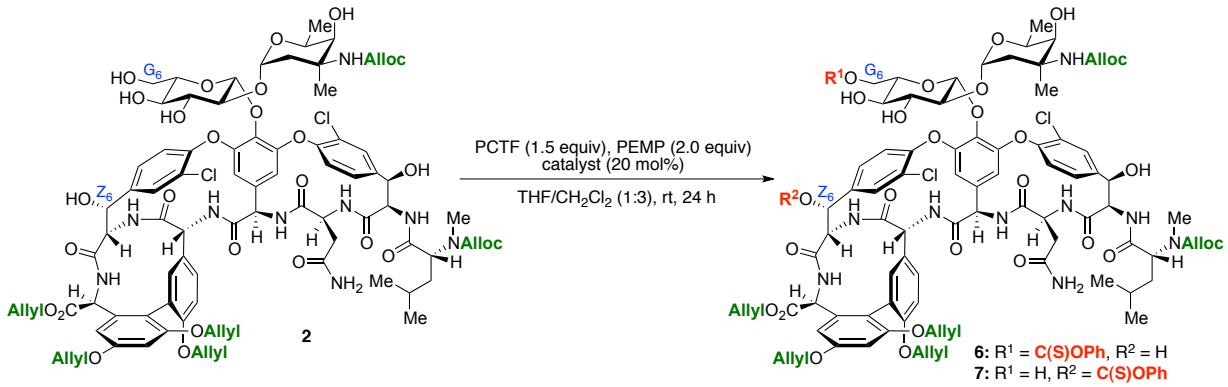
Full HSQC Spectrum of 2: Expanded from manuscript Figure 4 DMSO-d_6, 25 °C, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Blue phase = methylenes. Red phase = methines and methyls.

III. 5-mg Scale Thiocarbonylation of 2 (Catalyst Screen)

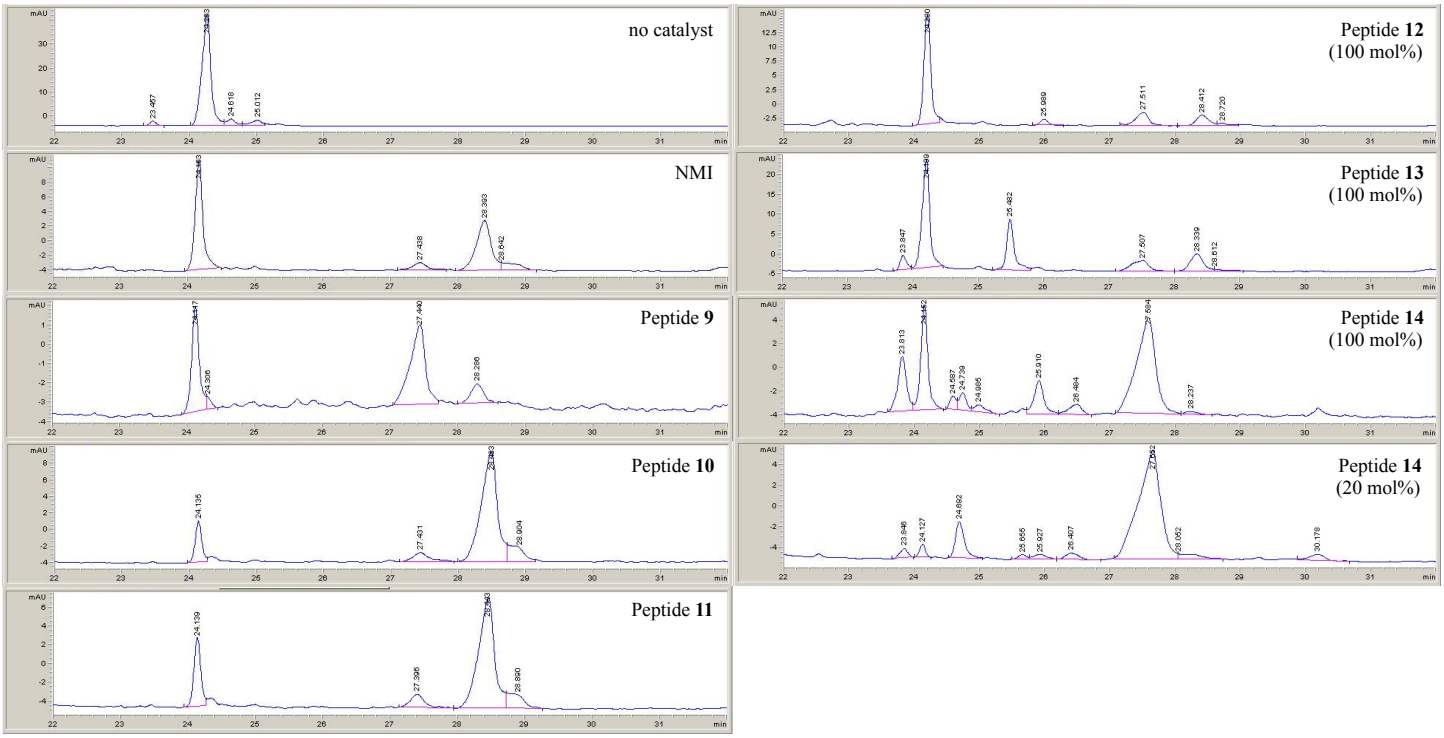


A 2-mL HPLC vial containing a micro-stir bar was oven-dried and allowed to cool to room temperature in a desiccator. To this was added the peptide catalyst (0.56 μmol), which was then dissolved in CH_2Cl_2 (70 μL). Protected vancomycin (**2**) (40 mM in THF, 70 μL, 2.8 μmol) and PEMP (80 mM in CH_2Cl_2, 70 μL, 5.6 μmol) were added to give a homogeneous solution. PCTF (60 mM in CH_2Cl_2, 56 μL, 3.4 μmol) was added in three portions: 23 μL at 0 h and 5 h, then 10 μL at 10 h. At 24 h, the reaction was quenched with methanol (1.0 mL) and analyzed by HR-LC/MS (Method 2) and analytical HPLC (Method 3).

IV. 50-mg Scale Thiocarbonylation of 2 (Catalyst Validation)

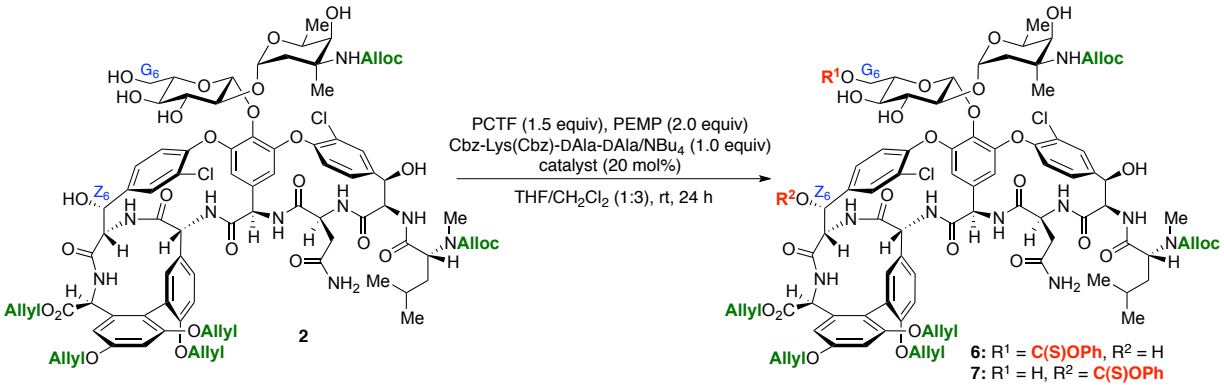


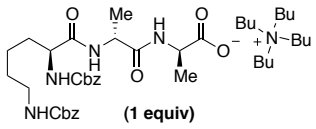
A 10-mL round-bottom flask containing a stir bar was flame-dried and allowed to cool to room temperature under vacuum. To the flask was added the peptide catalyst (5.6 μmol) and **2** (50 mg, 28 μmol), and the flask was fitted with a septum and placed under positive pressure of N_2. The solids were completely dissolved in THF (0.70 mL) before adding CH_2Cl_2 (2.0 mL) and PEMP (10 μL, 56 μmol) by syringe. A solution of PCTF (0.42 M in CH_2Cl_2, 0.10 mL, 42 μmol) was added over 10 h by syringe pump, and the reaction was allowed to stir an additional 14 h. An aliquot (20 μL) was taken for HR-LC/MS (Method 2) and HPLC (Method 3) analysis, and the reaction was quenched with methanol (2.0 mL) briefly before concentrating *in vacuo*. Product peaks were isolated by semi-preparative HPLC (Method 3) for identification by NMR.



HPLC Traces: Analytical HPLC Method 3, λ = 285 nm. Avg. t_R (**2**) = 24.2 min. Avg. t_R (**6**) = 27.5 min. Avg. t_R (**7**) = 28.4 min.

V. Competition Experiments with Cbz-Lys(Cbz)-DAla-DAla/NBu_4

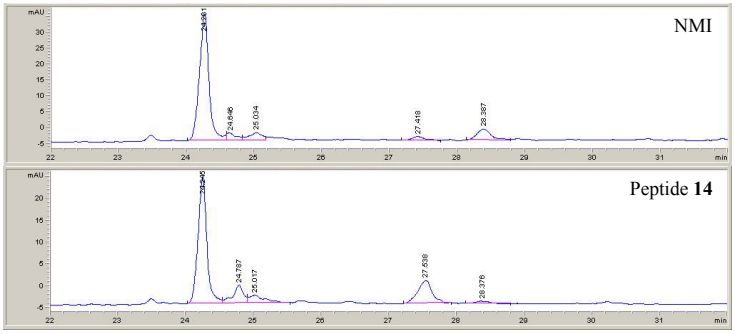




(1 equiv)

Catalyst	*without competitor* HPLC Conversion	$G_6 : Z_6$ Thionocarbonate	HPLC Conversion	$G_6 : Z_6$ Thionocarbonate
NMI	49%	1 : 5	16%	1 : 3
14	97%	27 : 1	27%	7 : 1

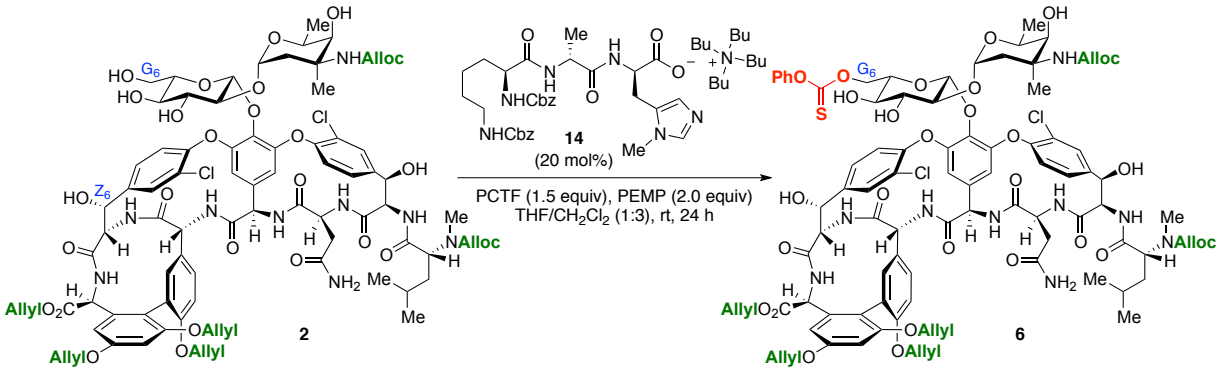
A 5-mL roundbottom flask containing a stir bar was flame-dried and allowed to cool to room temperature under vacuum. To the flask was added **2** (25 mg, 14 μmol), which was dissolved in THF (0.35 mL). To this was added Cbz-Lys(Cbz)-DAla-DAla/NBu_4 as a solution in CH_2Cl_2 (14 μmol in 0.20 mL), PEMP (5.1 μL, 28 μmol), catalyst as a solution in CH_2Cl_2 (2.8 μmol in 0.10 mL), and CH_2Cl_2 (700 μL). A solution of PCTF (0.21 M in CH_2Cl_2, 0.10 mL, 21 μmol) was added over 10 h by syringe pump, and the reaction was allowed to stir fro an additional 14 h. An aliquot (20 μL) was taken and diluted with 0.50 mL MeOH for HR-LC/MS (Method 2) and HPLC (Method 3) analysis.



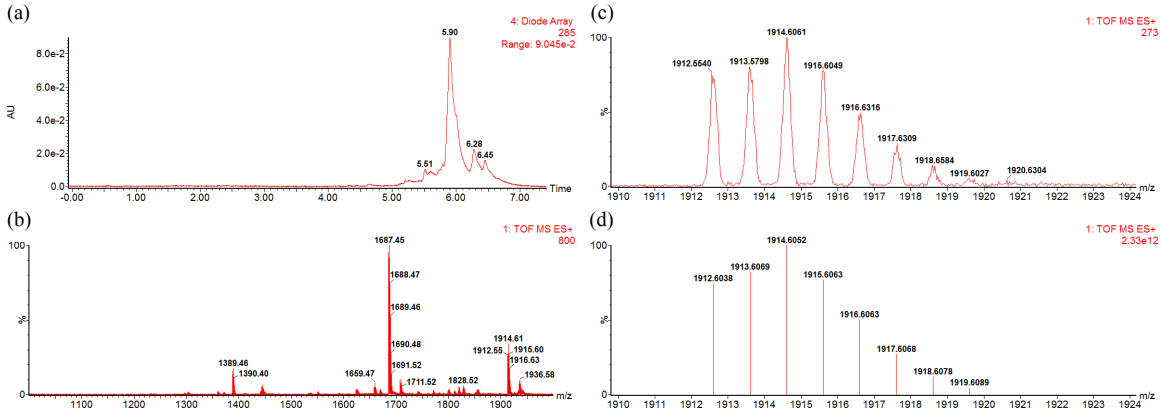
HPLC Traces: Analytical HPLC Method 3, λ = 285 nm. Avg. t_R (**2**) = 24.2 min. Avg. t_R (**6**) = 27.5 min. Avg. t_R (**7**) = 28.4 min.

VI. Synthesis of G_6-Deoxy-vancomycin (15)

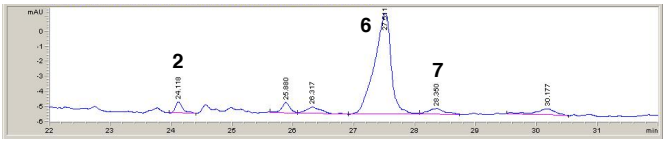
0.50-g Scale Thiocarbonylation of **2**



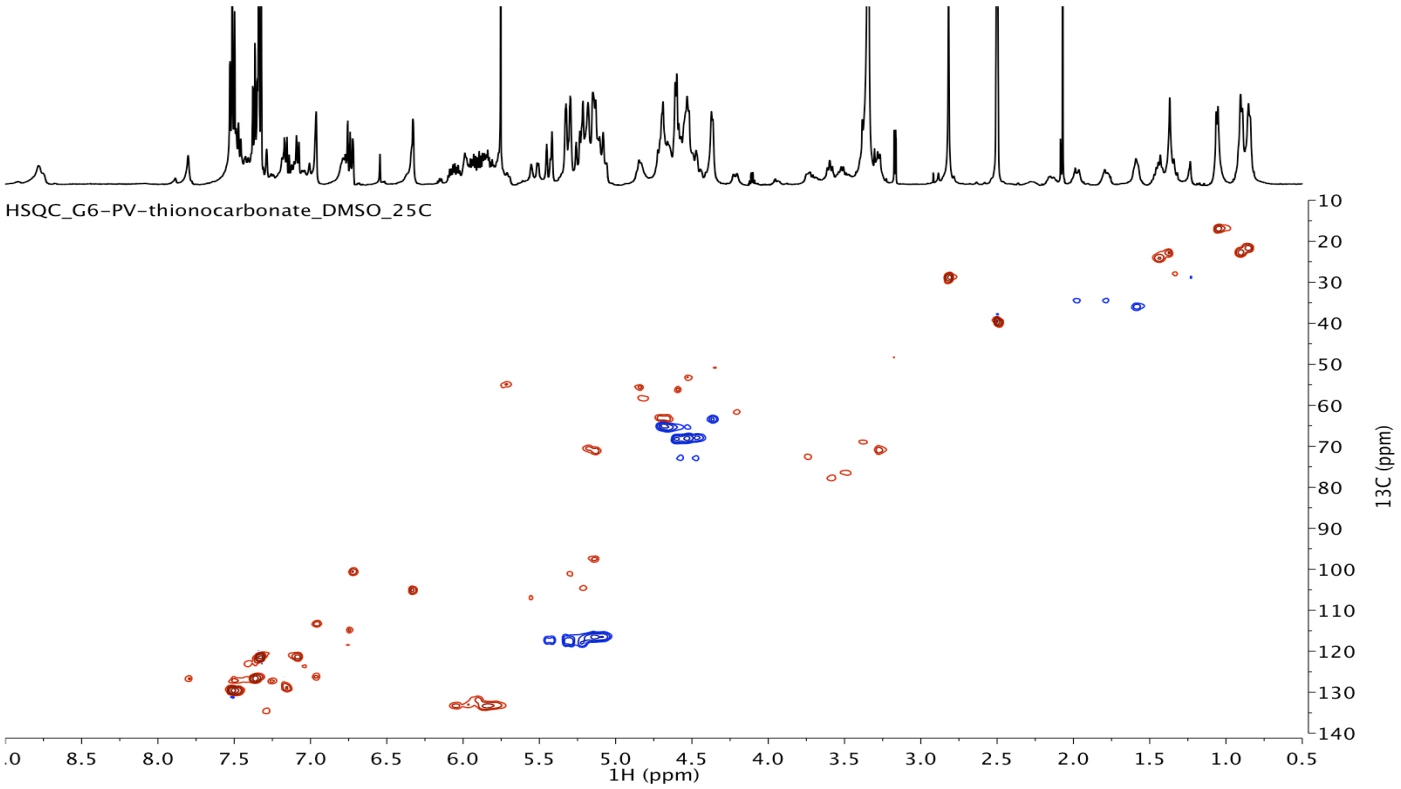
A 100-mL roundbottom flask containing a stir bar was flame-dried and allowed to cool to room temperature under vacuum. To the flask was added peptide catalyst **14** (49 mg, 56 μmol) and **2** (0.50 g, 0.28 mmol), and the flask was fitted with a septum and placed under positive pressure of N_2. The solids were completely dissolved in THF (7.0 mL) before adding CH_2Cl_2 (19 mL) and PEMP (0.10 mL) by syringe. A solution of PCTF (0.21 M in CH_2Cl_2, 2.0 mL, 0.42 mmol) was added over 10 h by syringe pump, and the reaction was allowed to stir for an additional 14 h. An aliquot (20 μL) was taken for HR-LC/MS (Method 2) and HPLC (Method 3) analysis, and the reaction was quenched with 2-propanol (20 mL). The solution was then concentrated *in vacuo* to a volume of ~20 mL and a white precipitate formed. The suspension was poured into water (100 mL) with vigorous stirring, and the tenuous solids were collected by vacuum filtration on a Whatman GF/F glass microfiber filter (0.7 μm). After drying on the filter for 10 min, the filtrate flask was replaced with a clean flask, and the filtered solids were dissolved in acetone and collected as the filtrate. The acetone filtrate was concentrated *in vacuo* to get a yellow/off-white solid (0.61 g), which was carried forward as the crude thionocarbonate **6**.



HR-LC/MS Data from Crude 6: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 5.90 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{93}H_{103}Cl_2N_9O_{29}S + H]^+$.

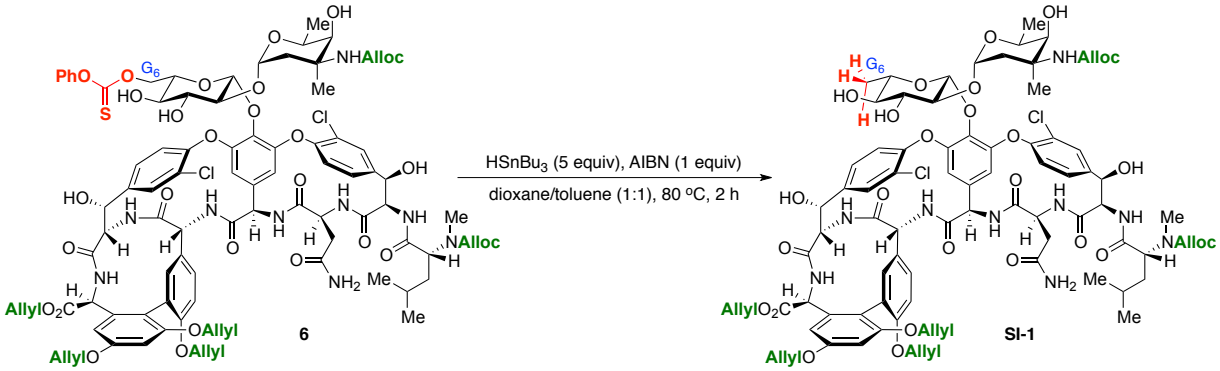


HPLC Trace of Crude 6: From manuscript Figure 8, entry 2. Analytical HPLC Method 3, λ = 285 nm, t_R (**6**) = 27.5 min.

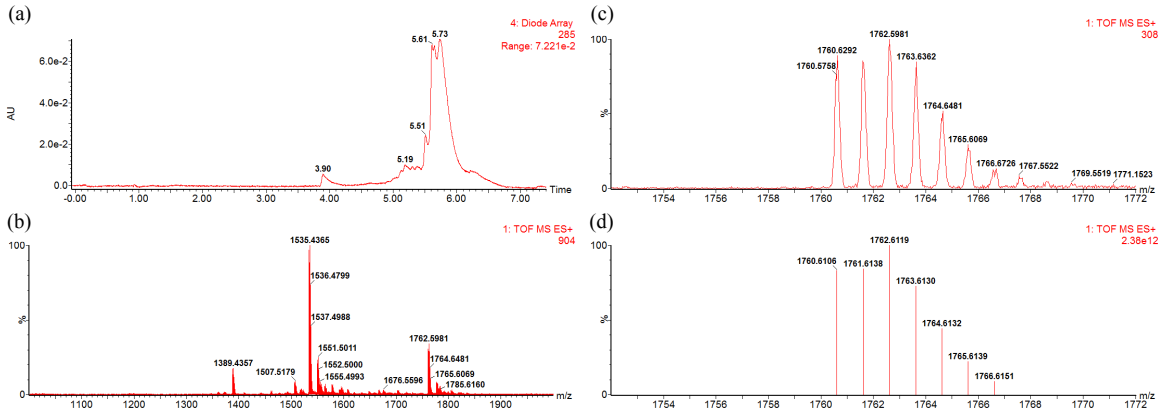


Full HSQC Spectrum of 6: Expanded from manuscript Figure 4. DMSO-d_6, 25 °C, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Blue phase = methylenes. Red phase = methines and methyls.

Deoxygenation of **6**[3]



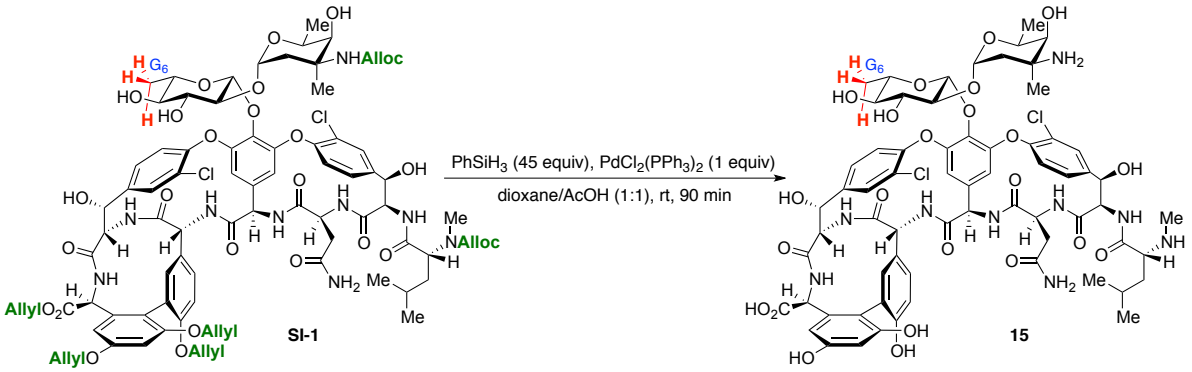
To a flame-dried 100-mL roundbottom flask and stir bar were added AIBN (46 mg, 0.28 mmol), toluene (9.4 mL), dioxane (4.4 mL), and $HSnBu_3$ (380 µL, 1.4 mmol) and the flask was placed under positive pressure of N_2. Crude **6** (0.61 g) was dissolved in dioxane (5 mL) and pulled into a syringe. The reaction flask containing AIBN and $HSnBu_3$ was placed in an oil bath at 80 °C, and the solution of **6** was added by syringe pump over 20 min. The reaction mixture was stirred an additional 30 min before removing it from the oil bath. HR-LC/MS (Method 2) indicated that no starting material remained, and the solution was poured into MeOH/H_2O (95:5, 200 mL) and washed with hexanes (3 × 200 mL). The MeOH/H_2O layer was concentrated *in vacuo* to yield an off-white solid (**SI-1**) (0.62 g), which was carried forward as the crude product.



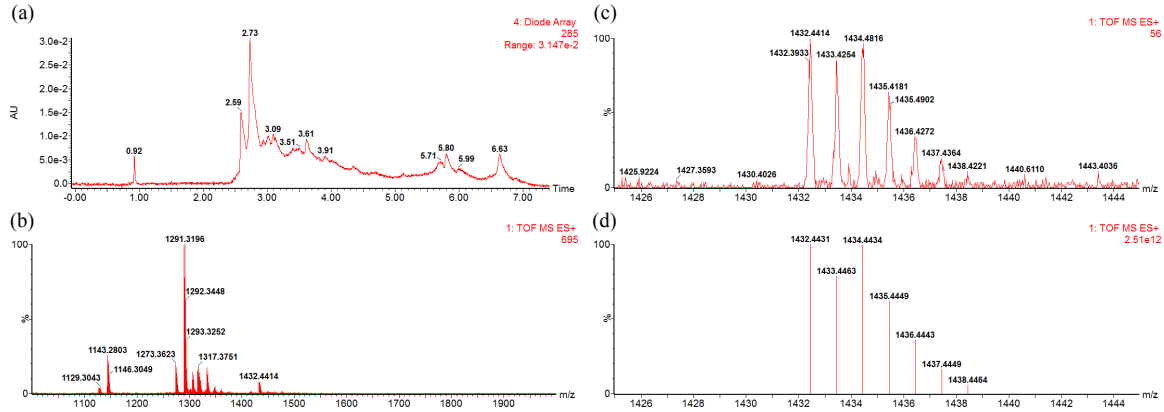
HR-LC/MS Data from Crude SI-1: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 5.61 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{86}H_{99}Cl_2N_9O_{27} + H]^+$.

***Note:** MS data of the peak at 5.73 min suggest this is a cyclic thionocarbonate due to the displacement of phenol by a nearby glucose hydroxyl group.

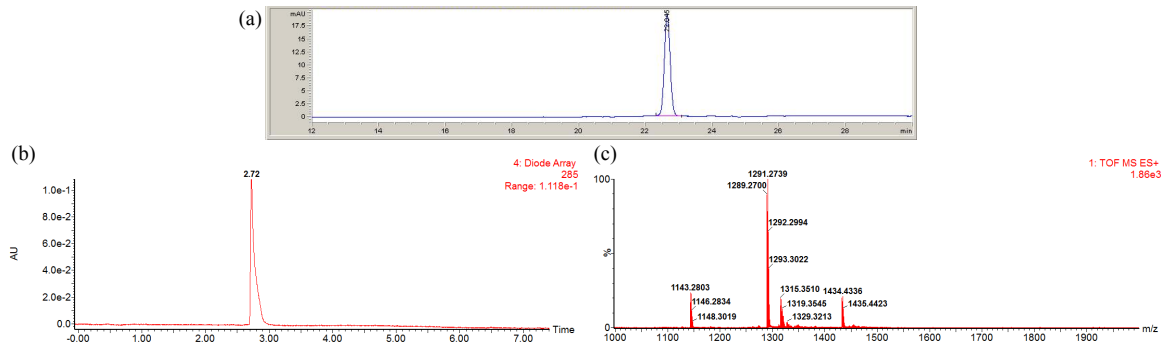
Allyl/Alloc Deprotection of **SI-1**[4]



Crude **SI-1** (0.62 g), in an oven-dried 100-mL two-neck flask, was dissolved in dioxane (12 mL) before adding acetic acid (12 mL). A line of dry N_2 was placed on one neck of the flask, while the second neck was capped with a glass stopper. $PhSiH_3$ (5 × 0.30 mL, 13 mmol total) and $PdCl_2(PPh_3)_2$ (5 × 40 mg, 0.28 mmol total) were added portionwise every 10 min for a total of five portions each. After 10 min from the final portion, HR-LC/MS (Method 2) indicated the reaction was nearly complete. The mixture was poured into water (200 mL), and was allowed to stir for 10 min at room temperature. The thick brown mixture was passed through a plug containing charcoal between two layers of Celite. The plug was rinsed with MeCN/H_2O (1:4 containing 0.1% formic acid, 500 mL) to get a partially clarified solution, which was concentrated *in vacuo*. The crude product was partially purified by MPLC (SNAP-C18 60-g column with a 12-g samplet; solvent A = 0.1% formic acid/H_2O, solvent B = 0.1% formic acid/MeCN, flow = 45 mL/min, λ = 210 nm; held at 0% B for 2.5 CV, ramped to 20% B over 15 CV, held at 20% B for 2.5 CV; **15** eluted at 10-12 CV) before purification by preparative HPLC (Method 5). G_6-Deoxy-vancomycin (**15**) was obtained as a colorless solid (33 mg, 0.023 mmol, 8% yield from 500 mg of **2**).



HR-LC/MS Data from Crude 15: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 2.73 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{66}H_{75}Cl_2N_9O_{23} + H]^+$.

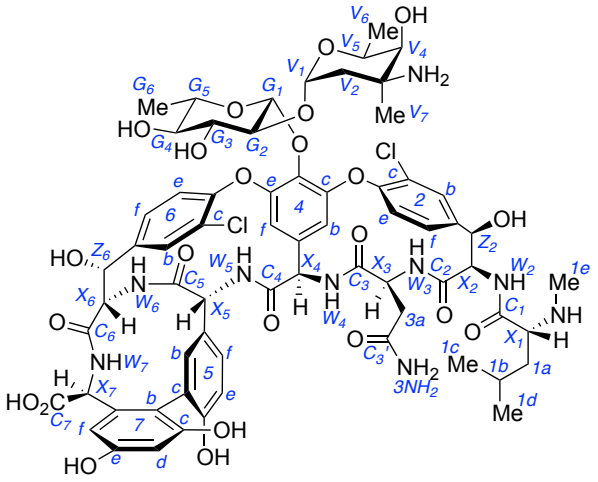


Purified 15: (a) Analytical HPLC Method 4, λ = 285 nm, t_R (**15**) = 22.6 min. (b) HR-LC/MS diode array trace. (c) Mass spectrum of the peak at 2.72 min.

Tabulated NMR Data for 15:

^{1}H NMR (500 MHz, DMSO-d_6)		^{13}C NMR (126 MHz, DMSO-d_6)			
W_5	8.62 (br s, 1H)	**C_1**	**173.1**	G_4	75.2
W_7	8.44 (br d, J = 4.5 Hz, 1H)	C_7	172.8	G_5	71.6
W_4	8.27 (br s, 1H)	C_3	171.1	Z_6	71.6
W_2	7.99 (br s, 1H)	**C_3'**	**170.8**	Z_2	71.2
6b	7.86 (s, 1H)	C_4	169.7	V_4	70.7
2f	7.52 (d, J = 8.0 Hz, 1H)	**C_5**	**169.2**	V_5	63.2
6f	7.46 (d, J = 8.4 Hz, 1H)	C_6	167.6	X_1	61.9
$3NH_2$[a], 6e, 2b	7.36 (br m, 3H)	**C_2**	**167.1**	X_6	61.9
2e	7.23 (d, J = 8.2 Hz, 1H)	7e	157.2	X_2	58.3
5b	7.17 (s, 1H)	**7c**	**156.5**	X_7	57.0
$3NH_2$[b]	6.89 (br s, 1H)	5d	155.1	X_4	55.0
5f	6.77 (d, J = 8.7 Hz, 1H)	**4c**	**152.5**	V_3	54.0
5e	6.71 (d, J = 8.5 Hz, 1H)	4e	151.5	X_5	53.8
W_6, W_3	6.69-6.62 (m, 2H)	2d	149.8	X_3	51.0
7d	6.38 (s, 1H)	6d	148.3	1a	40.7
7f	6.28 (s, 1H)	6a	142.6	3a	37.6
X_4	5.75 (d, J = 7.9 Hz, 1H)	2a	139.7	V_2	33.4
4b	5.52 (s, 1H)	7a	136.6	1e	33.3
V_1, 4f	5.21 (s, 2H)	5b	135.8	1b	24.1
G_1, Z_2	5.16 (m, 2H)	4a	134.8	1c/d	22.8
Z_6	5.11 (s, 1H)	4d	132.0	1c/d	22.6
X_2	4.90 (br s, 1H)	2b	128.8	V_7	22.4
V_5	4.70 (q, J = 6.3 Hz, 1H)	6b	127.3	G_6	17.8
X_5, X_7	4.47-4.40 (m, 2H)	6f	127.3	V_6	16.9
X_3	4.37 (br s, 1H)	2c	127.1		
X_6	4.19 (d, J = 11.4 Hz, 1H)	2f	127.1		
G_2	3.55 (t, J = 8.4 Hz, 1H)	5a	126.3		
G_3	3.39 (t, J = 9.0 Hz, 1H)	6c	126.1		
G_5	3.35-3.29 (m, 1H)	5f	125.5		
X_1	3.23 (br m, 1H)	2e	124.4		
V_4	3.18 (s, 1H)	6e	123.5		
G_4	2.98 (t, J = 9.0 Hz, 1H)	5c	121.8		
3a[a], 1e	2.37 (s, 4H)	7b	118.0		
3a[b]	2.15 (dd, J = 15.4, 4.8 Hz, 1H)	5e	116.2		
V_{2eq}	1.91 (d, J = 10.3 Hz, 1H)	4b	106.8		
V_{2ax}, 1b	1.77-1.64 (m, 2H)	7f	106.0		
1a	1.58-1.39 (m, 2H)	4f	104.5		
V_7	1.26 (s, 3H)	7d	102.2		
G_6	1.15 (d, J = 5.8 Hz, 3H)	G_1	101.4		
V_6	1.07 (d, J = 6.1 Hz, 3H)	V_1	96.9		
1c/d	0.90 (d, J = 6.4 Hz, 3H)	G_2	78.6		
1c/d	0.85 (d, J = 6.4 Hz, 3H)	G_3	76.9		

Blue values were assigned by analogy to **1** due to a lack of signal in the HMBC spectrum.



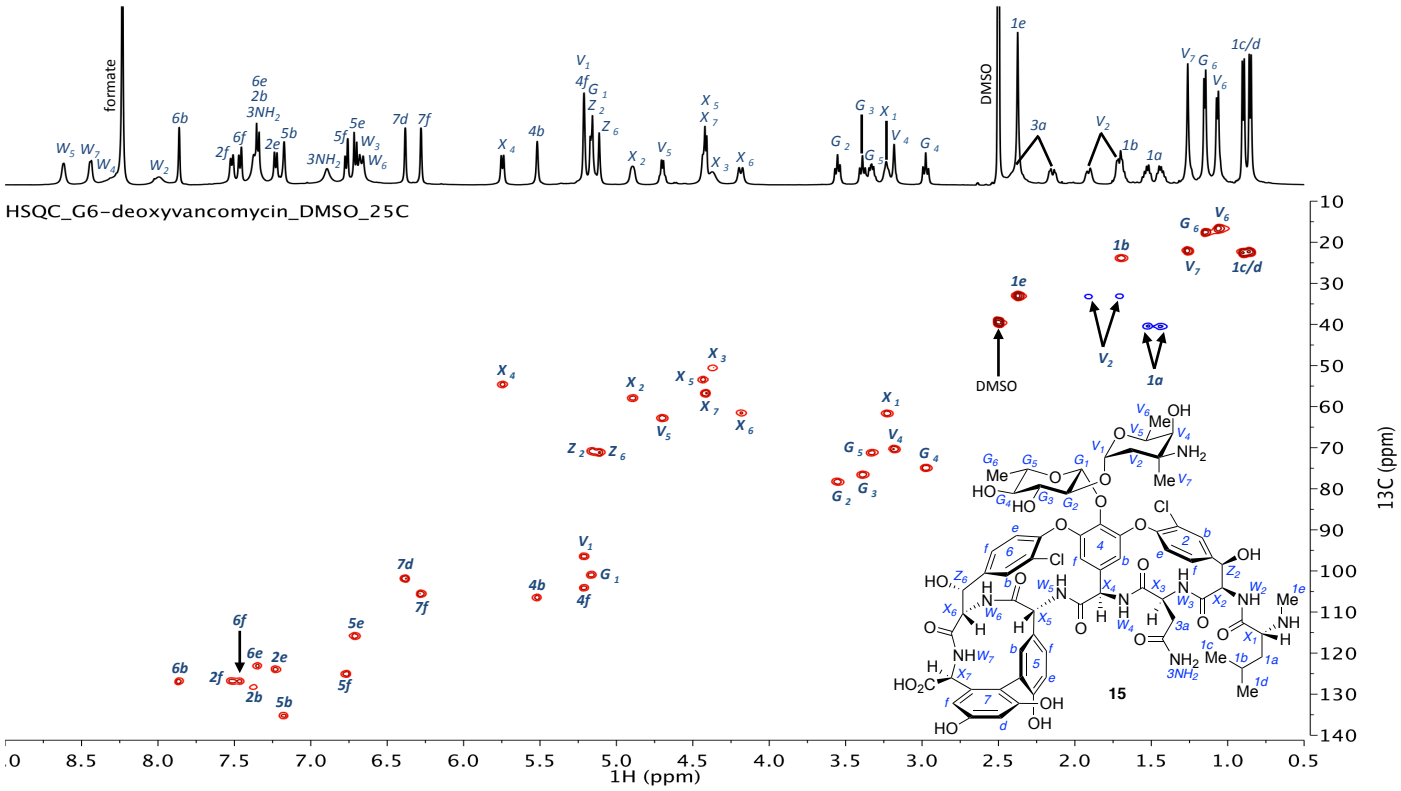
G_6-Deoxy-vancomycin (15)

Comparison of Chemical Shifts for 15 vs. 1:[5]

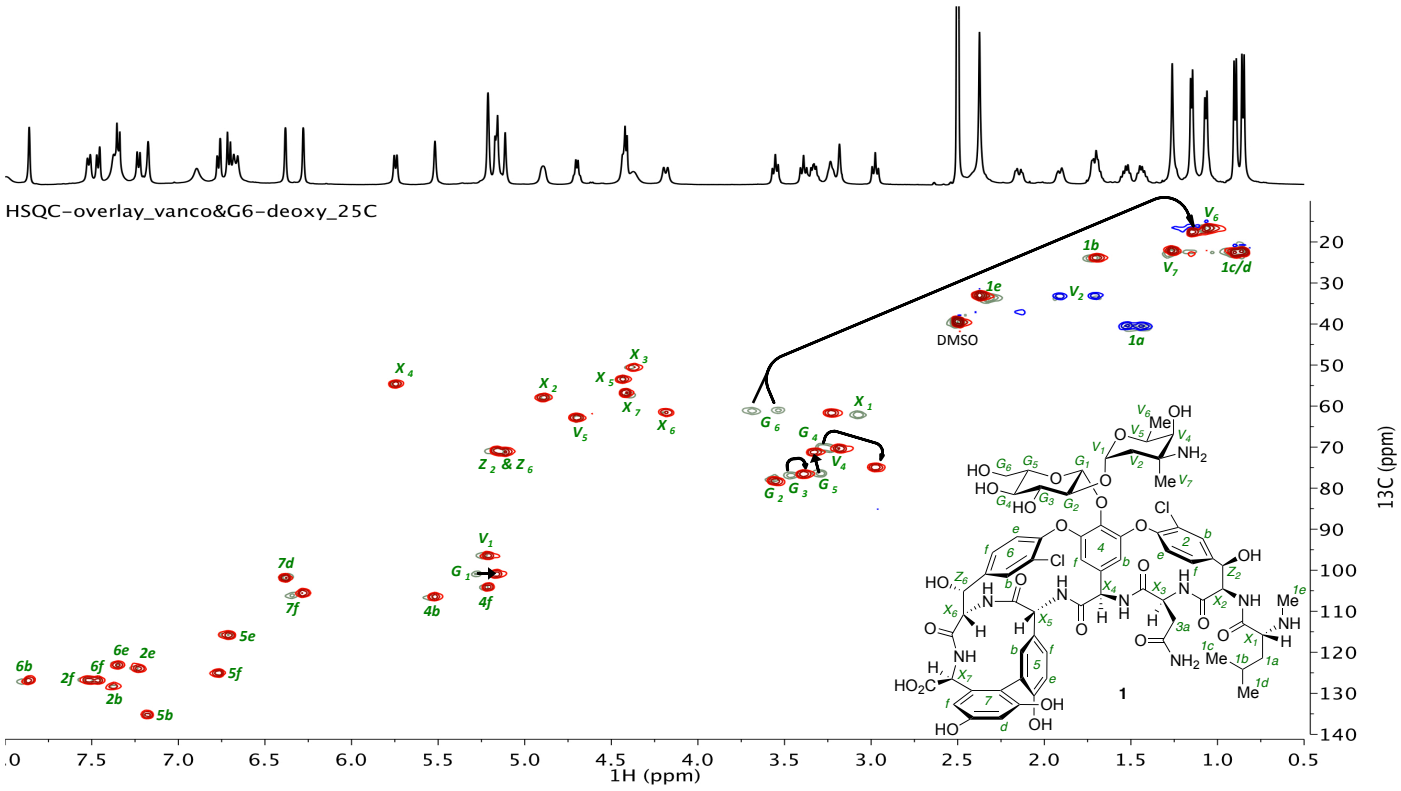
^{1}H NMR (DMSO-d_6)			^{13}C NMR (DMSO-d_6)					
	15	**1**		**15**	**1**		**15**	**1**
W_5	8.62	8.64	**C_1**	**173.1**	**173.4**	X_7	57.0	56.7
W_7	8.44	8.48	C_7	172.8	172.5	X_4	55.0	54.9
W_4	8.27	8.25	C_3	171.1	171.1	V_3	54.0	53.9
W_2	7.99	7.93	**C_3'**	**170.8**	**170.6**	X_5	53.8	53.7
6b	7.86	7.86	C_4	169.7	169.5	X_3	51.0	51.0
2f	7.52	7.52	**C_5**	**169.2**	**169.1**	1a	40.7	40.7
6f	7.46	7.47	C_6	167.6	167.6	3a	37.6	37.2
$3NH_2$	7.36	7.39	**C_2**	**167.1**	**167.1**	V_2	33.4	33.3
2b	7.36	7.39	7e	157.2	157.1	1e	33.3	33.2
6e	7.36	7.34	**7c**	**156.5**	**156.4**	1b	24.1	24.1
2e	7.23	7.26	5d	155.1	155.0	1c/d	22.8	22.9
5b	7.17	7.18	**4c**	**152.5**	**152.1**	1c/d	22.6	22.5
$3NH_2'$	6.89	6.92	4e	151.5	151.3	V_7	22.4	22.2
5f	6.77	6.77	2d	149.8	149.8	**G_6**	**17.8**	**61.2**
5e	6.71	6.72	6d	148.3	148.3	V_6	16.9	16.8
W_6	6.65	6.67	6a	142.6	142.4			
W_3	6.65	6.62	2a	139.7	139.8			
7d	6.38	6.42	7a	136.6	136.2			
7f	6.28	6.26	5b	135.8	135.6			
X_4	5.75	5.75	4a	134.8	134.5			
4b	5.52	5.55	4d	132.0	131.9			
V_1	5.21	5.24	2b	128.8	128.6			
4f	5.21	5.21	6b	127.3	127.3			
G_1	**5.16**	**5.27**	6f	127.3	127.3			
Z_2	5.16	5.16	2c	127.1	127.1			
Z_6	5.11	5.13	2f	127.1	127.2			
X_2	4.90	4.88	5a	126.3	126.2			
V_5	4.70	4.68	6c	126.1	126.2			
X_5	4.44	4.43	5f	125.5	125.4			
X_7	4.44	4.42	2e	124.4	124.2			
X_3	4.37	4.35	6e	123.5	123.3			
X_6	4.19	4.19	5c	121.8	121.6			
G_2	3.55	3.59	7b	118.0	118.0			
G_3	**3.39**	**3.50**	5e	116.2	116.2			
G_5	3.33	3.31	4b	106.8	107.1			
X_1	3.23	3.31	7f	106.0	105.8			
V_4	3.18	3.23	4f	104.5	104.6			
G_4	**2.98**	**3.31**	7d	102.2	102.3			
3a	2.37	2.42	G_1	101.4	101.2			
1e	2.37	2.37	V_1	96.9	97.6			
3a′	2.15	2.14	G_2	78.6	78.0			
V_2	1.91	1.90	G_3	76.9	77.0			
V_2'	1.74	1.75	**G_4**	**75.2**	**70.2**			
1b	1.74	1.72	**G_5**	**71.6**	**76.7**			
1a	1.53	1.51	Z_6	71.6	71.5			
1a′	1.44	1.47	Z_2	71.2	71.1			
V_7	1.26	1.32	V_4	70.7	70.7			
G_6	**1.15**	**3.68, 3.50**	V_5	63.2	63.1			
V_6	1.07	1.07	X_1	61.9	61.8			
1c/d	0.90	0.91	X_6	61.9	61.9			
1c/d	0.85	0.86	X_2	58.3	58.3			

Red values represent a change of ≥0.1 ppm for ^{1}H peaks or ≥1.0 ppm for ^{13}C peaks. **Blue values** were assigned by analogy to **1** due to a lack of signal in the HMBC spectrum.

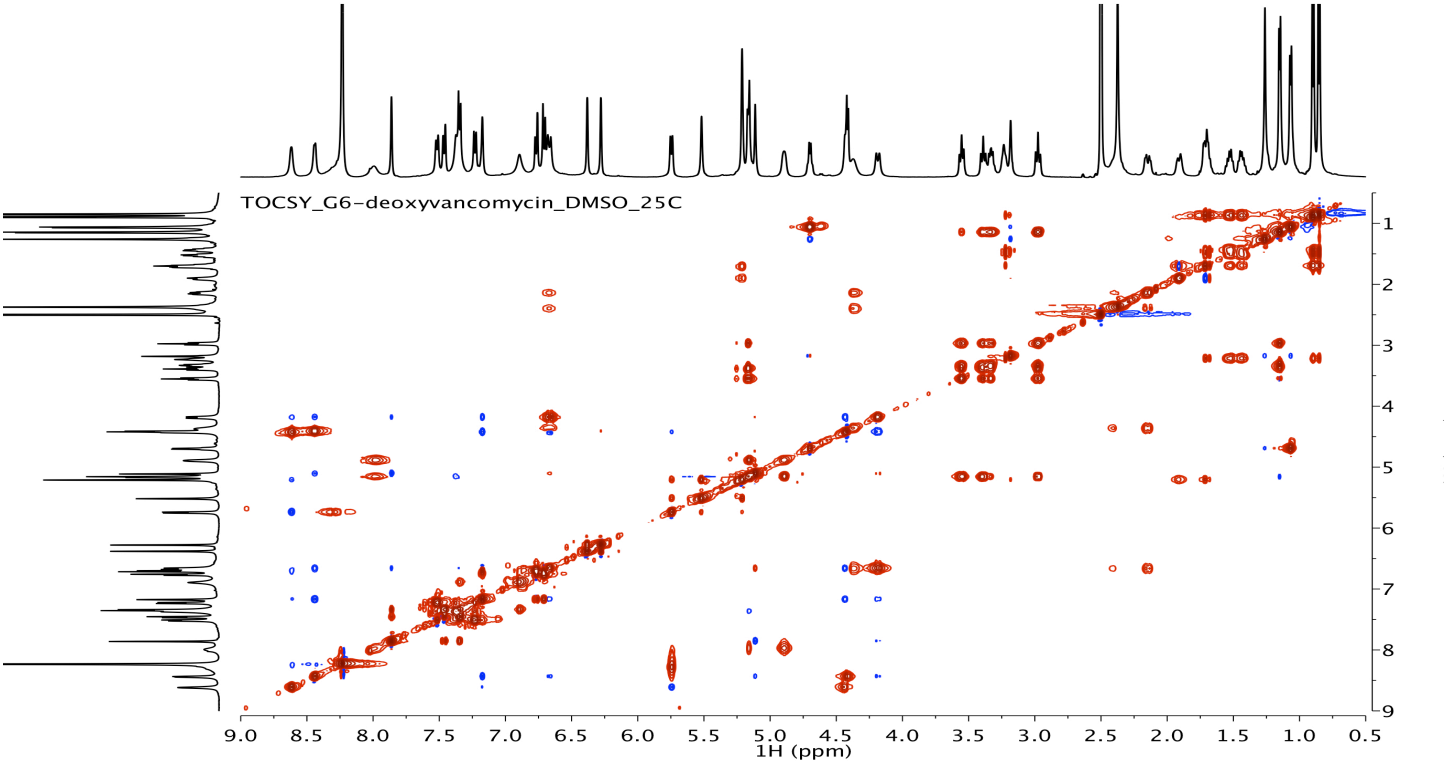
NMR Spectra of **15**



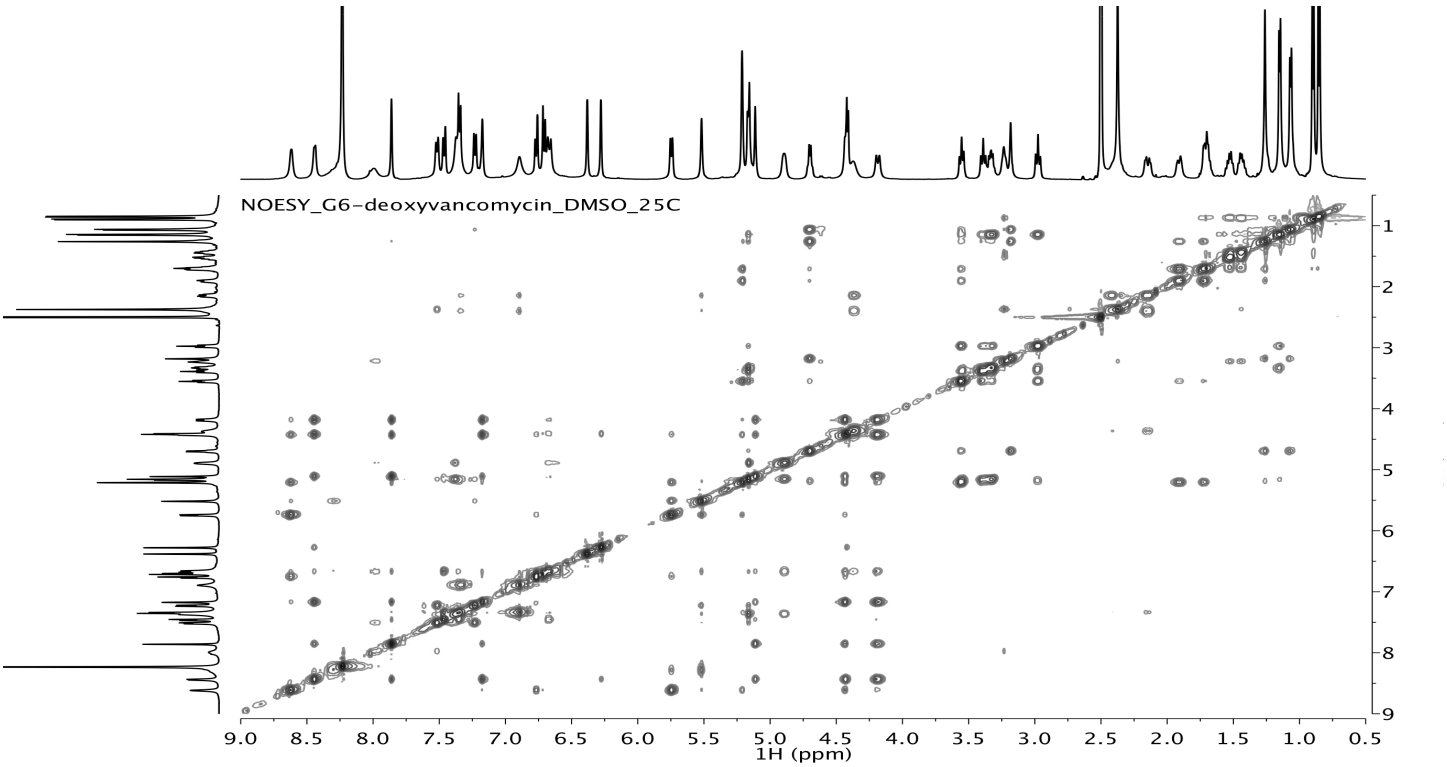
HSQC Spectrum of 15: DMSO-d_6 (20 mg/mL), 25 °C, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Blue phase = methylenes. Red phase = methines and methyls.



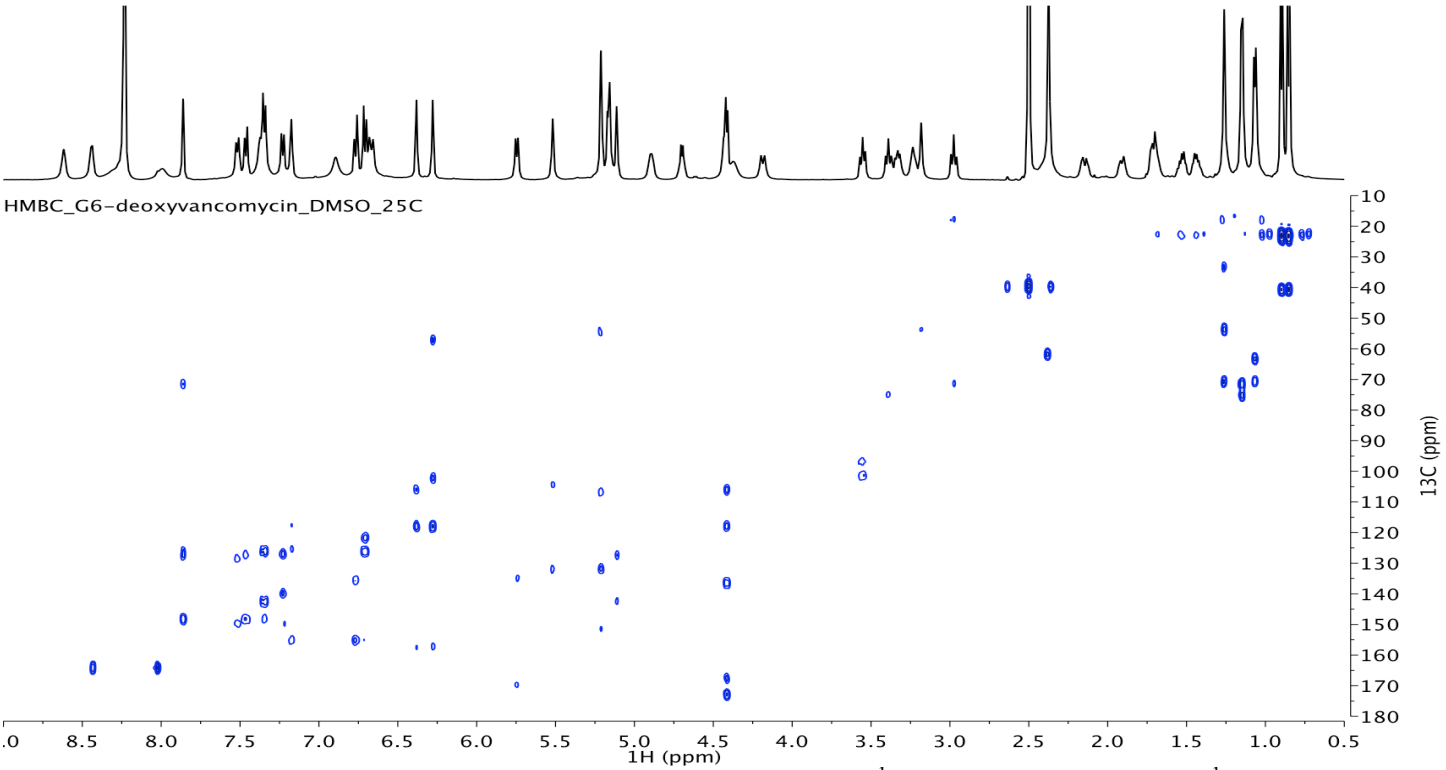
HSQC Spectrum of 1 with HSQC Spectrum of 15 Overlaid: DMSO-d_6 (20 mg/mL), 25 °C, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Green = vancomycin (**1**), labels refer to these peaks. Red/blue = overlay of G_6-deoxy-vancomycin (**15**), arrows indicate the changes from vancomycin to G_6-deoxyvancomycin. ^{1}H NMR spectrum of **15** shown on top.



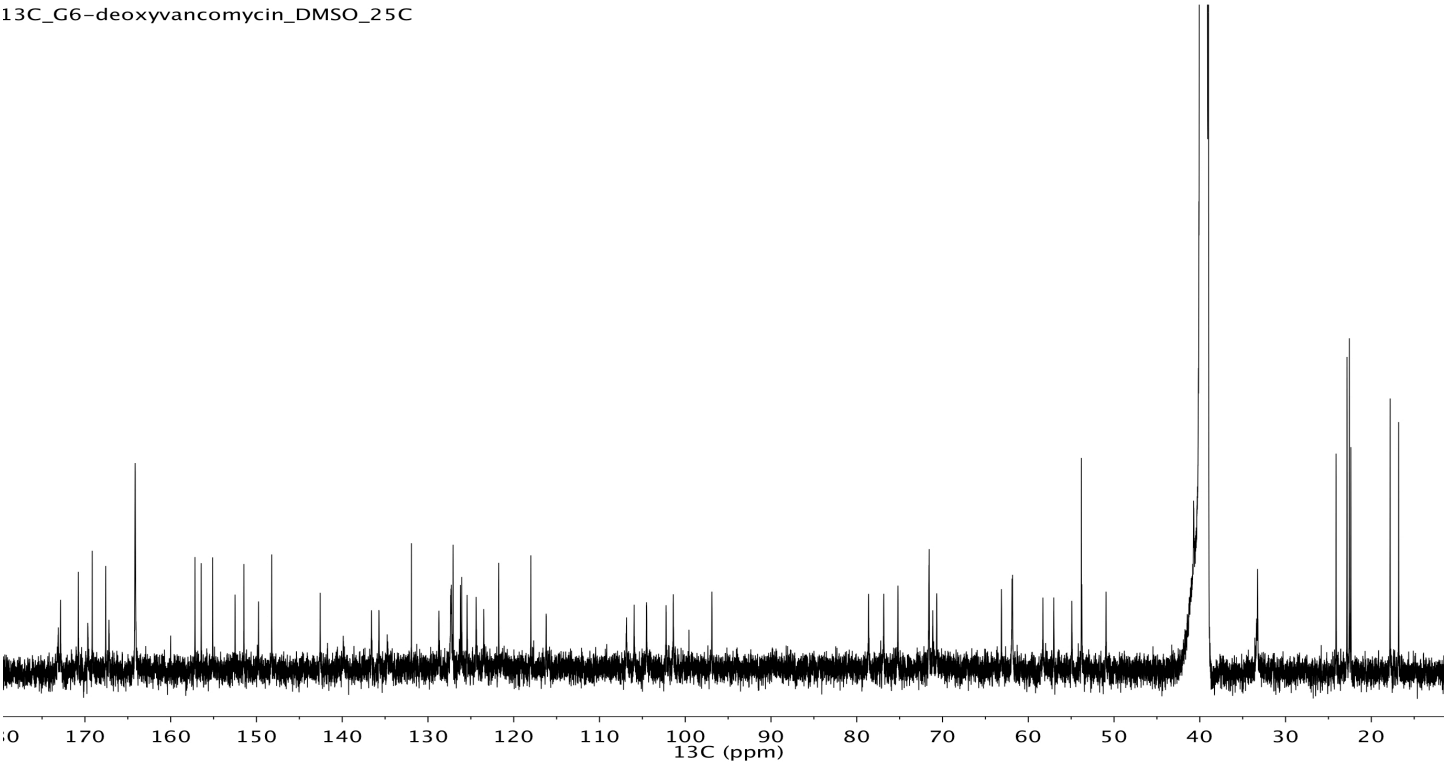
TOCSY Spectrum of 15: DMSO-d_6 (20 mg/mL), degassed, 25 °C, ^{1}H (500 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{1}H chemical shift. Red = TOCSY signals. Blue = Contaminant NOE signals. Mixing time = 80 ms.



NOESY Spectrum of 15: DMSO-d_6 (20 mg/mL), degassed, 25 °C, ^{1}H (500 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{1}H chemical shift. Mixing time = 300 ms.



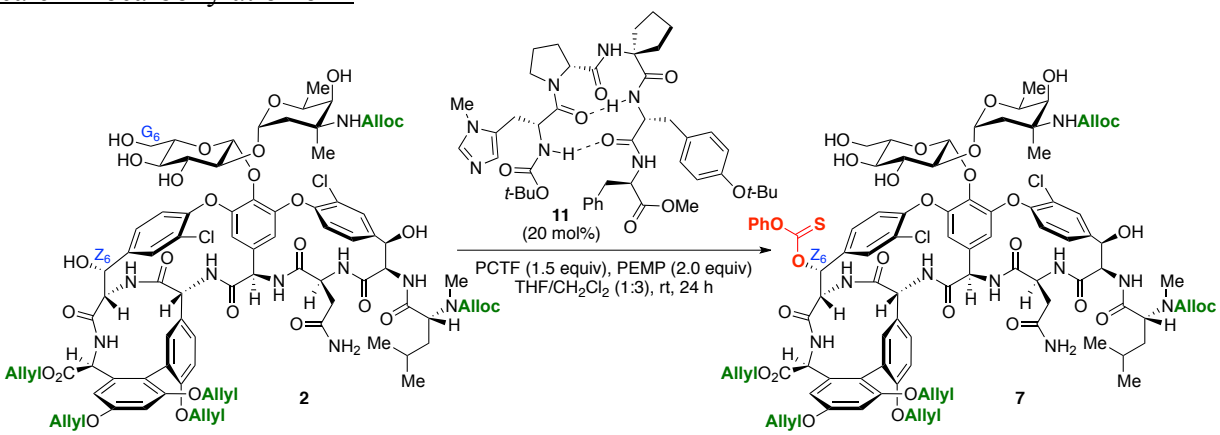
HMBC Spectrum of 15: DMSO-d_6 (20 mg/mL), degassed, 25 °C, ^{1}H (500 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift.



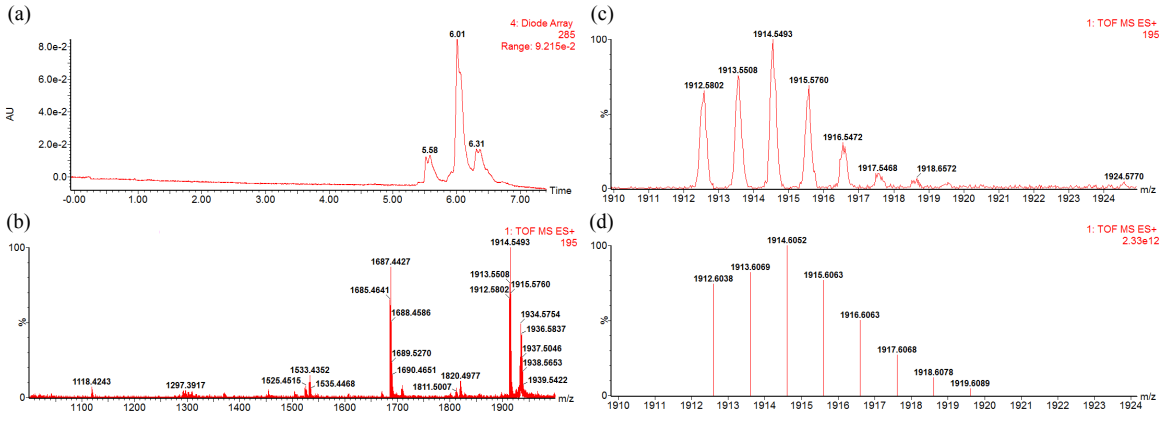
^{13}C NMR Spectrum for 15: DMSO-d_6 (20 mg/mL), 126 MHz, 25 °C.

VII. Synthesis of Z_6-Deoxy-vancomycin (16)

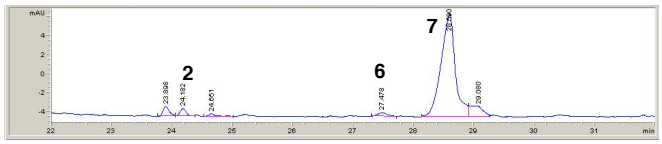
0.50-g Scale Thiocarbonylation of **2**



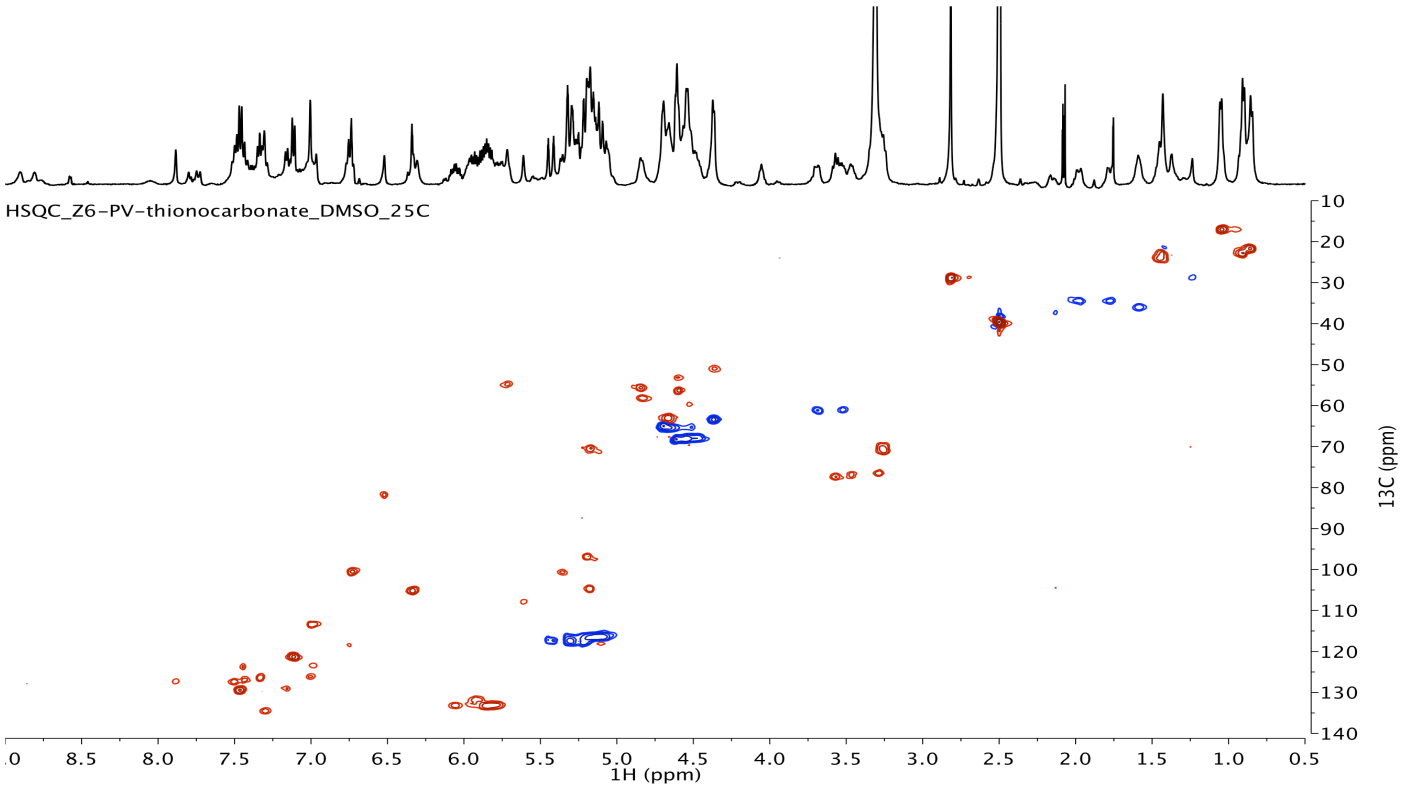
A 100-mL round bottom flask containing a stir bar was flame-dried and allowed to cool to room temperature under vacuum. To the flask was added peptide catalyst **11** (48 mg, 56 μmol) and **2** (0.50 g, 0.28 mmol), and the flask was fitted with a septum and placed under positive pressure of N_2. The solids were completely dissolved in THF (7.0 mL) before adding CH_2Cl_2 (20 mL) and PEMP (0.10 mL) by syringe. A solution of PCTF (0.42 M in CH_2Cl_2, 1.0 mL, 0.42 mmol) was added over 10 h by syringe pump, and the reaction was allowed to stir for an additional 14 h. An aliquot (20 μL) was taken for HR-LC/MS (Method 2) and HPLC (Method 3) analysis, and the reaction was quenched with 2-propanol (20 mL). The solution was then concentrated *in vacuo* to a volume of ~20 mL and a white precipitate formed. The suspension was poured into water (100 mL) with vigorous stirring, and the tenuous solids were collected by vacuum filtration on a Whatman GF/F glass microfiber filter (0.7 μm). After drying on the filter for 10 min, the filtrate flask was replaced with a clean flask, and the filtered solids were dissolved in acetone and collected as the filtrate. The acetone filtrate was concentrated *in vacuo* to get a yellow/off-white solid (0.51 g), which was carried forward as crude thionocarbonate **7**.



HR-LC/MS Data from Crude 7: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 6.01 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{93}H_{103}Cl_2N_9O_{29}S + H]^+$.

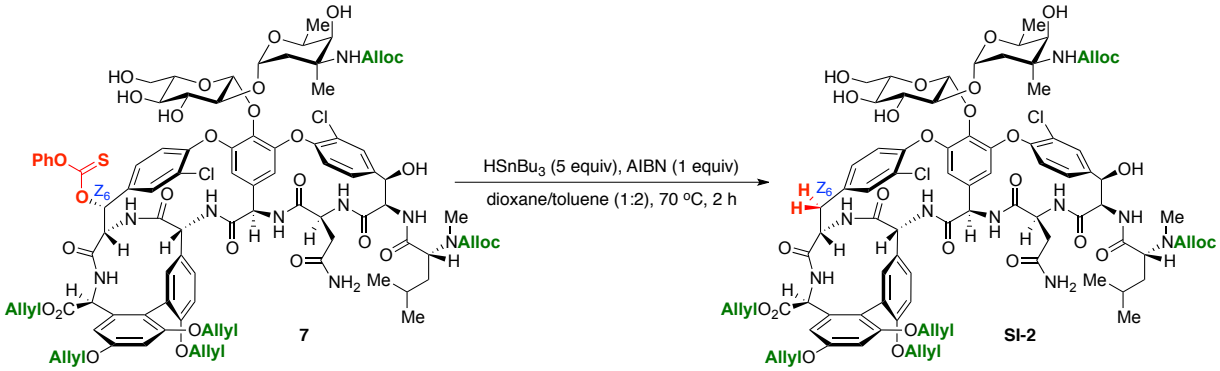


HPLC Trace of Crude 7: manuscript Figure 8, entry 1. Analytical HPLC Method 3, λ = 285 nm, t_R (**7**) = 28.6 min.

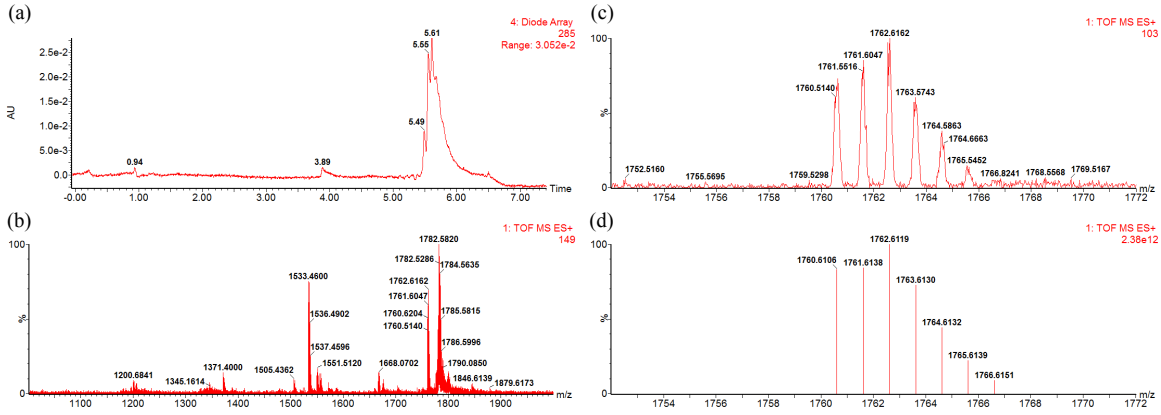


Full HSQC Spectrum of 6: Expanded from manuscript Figure 4. DMSO-d_6, 25 °C, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Blue phase = methylenes. Red phase = methines and methyls.

Deoxygenation of **7**



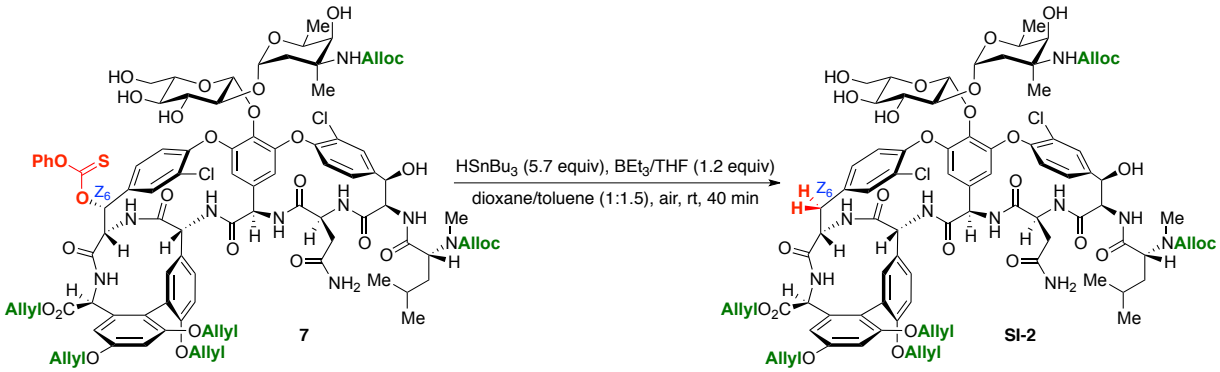
To a 100-mL roundbottom flask containing crude thionocarbonate **7** (0.51 g) was added AIBN (46 mg, 0.28 mmol), and the solids were dissolved in dioxane (9.4 mL). Toluene (19 mL) was added to give a slightly cloudy solution, and the flask was purged with N_2 and then kept under positive pressure of N_2. The flask was placed in an oil bath at 70 °C, and slow addition of neat $HSnBu_3$ (0.38 mL, 1.4 mmol; added over 3-5 min) was started immediately. After two hours at 70 °C, the flask was removed from the oil bath, and HR-LC/MS (Method 2) indicated that no starting material remained. The solution was poured into MeOH/H_2O (95:5, 200 mL) and washed with hexanes (3 × 200 mL). The MeOH/H_2O layer was concentrated *in vacuo* to yield a tan solid (**SI-2**) (0.61 g), which was carried forward as the crude product.



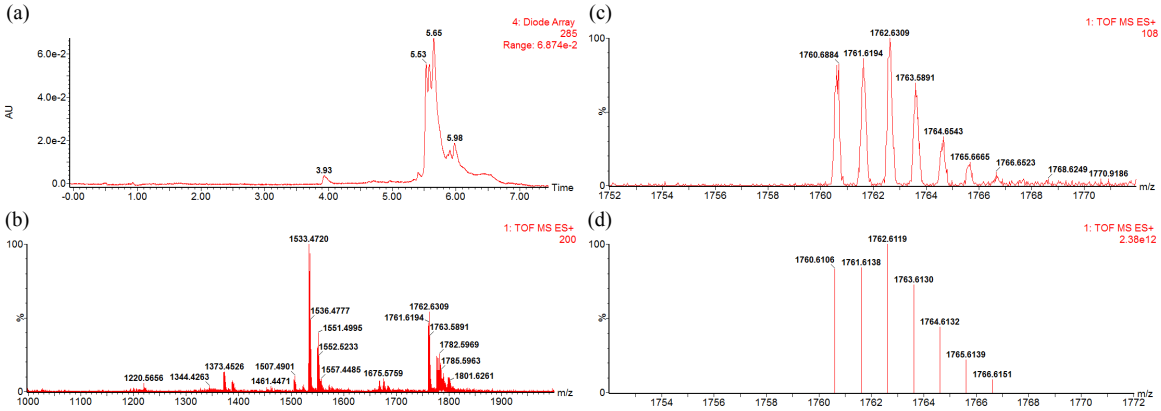
HR-LC/MS Data from Crude SI-2: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 5.61 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{86}H_{99}Cl_2N_9O_{27} + H]^+$.

***Note:** MS data of the peak at 5.55 min matches that of **2**, indicating some hydrolysis during the deoxygenation.

Alternative Deoxygenation of **7** with BEt_3/Air as the Radical Initiator[6]



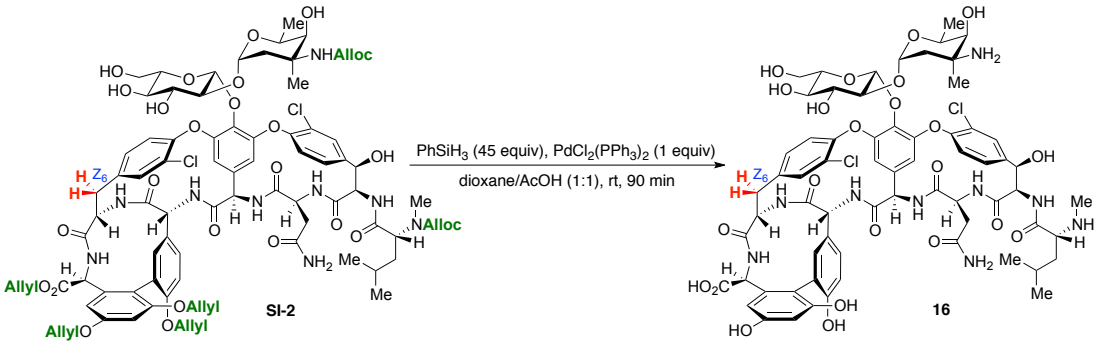
To a flame-dried 50-mL roundbottom flask and stir bar was added crude **7** (0.25 g, estimated 0.13 mmol), and the flask was fitted with a septum and kept under positive pressure of dry air (balloon). The substrate was dissolved in dioxane (5.2 mL) before adding touene (7.8 mL). $HSnBu_3$ (5 × 40 μL, 0.74 mmol total) and BEt_3 (1.0 M in THF, 5 × 50 μL, 0.15 mmol) were added portionwise every 5 min for a total of five portions each. After 20 min from the final portion, HR-LC/MS (Method 2) indicated the reaction was complete. The reaction mixture was poured into MeOH/H_2O (95:5, 100 mL) and washed with hexanes (3 × 100 mL). The MeOH/H_2O layer was concentrated *in vacuo* to give crude **SI-2** as an off-white solid (0.25 g). The crude product was carried forward to the deprotection.



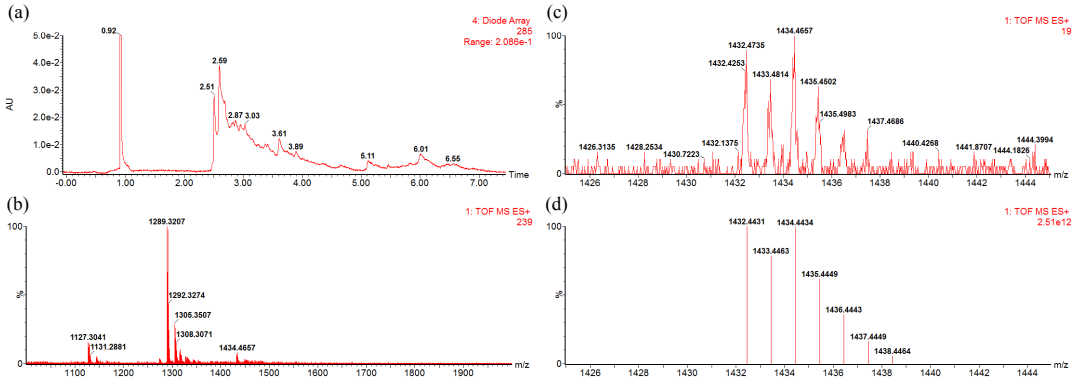
HR-LC/MS Data from Crude SI-2: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 5.65 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{86}H_{99}Cl_2N_9O_{27} + H]^+$.

***Note:** MS data of the peak at 5.53 min matches that of **2**, indicating some hydrolysis during the deoxygenation. MS data of the unlabeled peak between 5.53 min and 5.65 min matches that of **SI-2**, suggesting two or more isomers of **SI-2** may be present prior to deprotection.

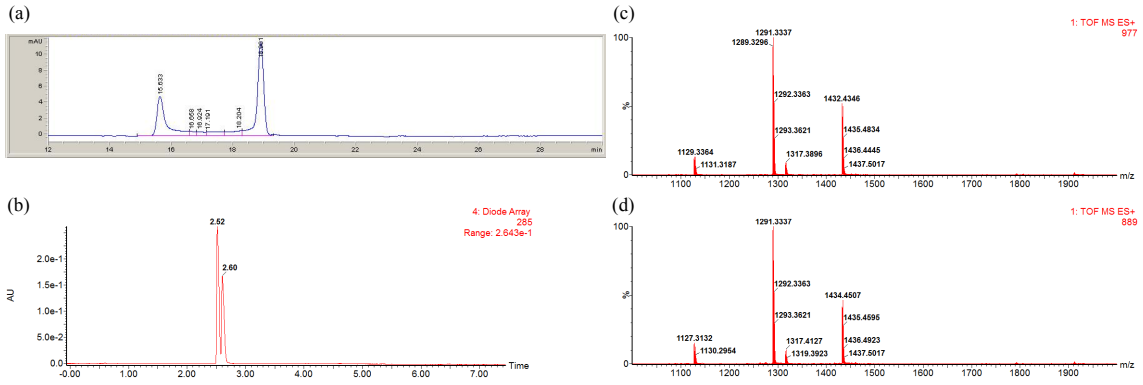
Allyl/Alloc Deprotection of **SI-2**



Crude **SI-2** (0.61 g), in an oven-dried 100-mL two-neck flask, was dissolved in dioxane (12 mL) before adding acetic acid (12 mL). A line of dry N_2 was placed on one neck of the flask, while the second neck was capped with a glass stopper. $PhSiH_3$ (5 × 0.30 mL, 13 mmol total) and $PdCl_2(PPh_3)_2$ (5 × 40 mg, 0.28 mmol total) were added portionwise every 10 min for a total of five portions each. After 10 min from the final portion, HR-LC/MS (Method 2) indicated the reaction was nearly complete. The mixture was poured into water (200 mL), and was allowed to stir for 90 min at room temperature. The solution was transferred to a separatory funnel and washed with hexanes (200 mL). The thick brown aqueous layer was passed through a plug containing charcoal between two layers of Celite. The plug was rinsed with MeCN/H_2O (1:4, 100 mL) to get a partially clarified solution, which was concentrated *in vacuo*. The crude product was partially purified by MPLC (SNAP-C18 60-g column with a 12-g samplet; solvent A = 0.1% formic acid/H_2O, solvent B = 0.1% formic acid/MeCN, flow = 45 mL/min, λ = 210 nm; held at 0% B for 2.5 CV, ramped to 12% B over 15 CV, held at 12% B for 2.5 CV; **16** eluted at 8-11 CV) before purification by preparative HPLC (Method 6). Z_6-Deoxy-vancomycin (**16**) was obtained as a colorless solid (64 mg, 45 μmol, 16% yield from 500 mg of **2**).

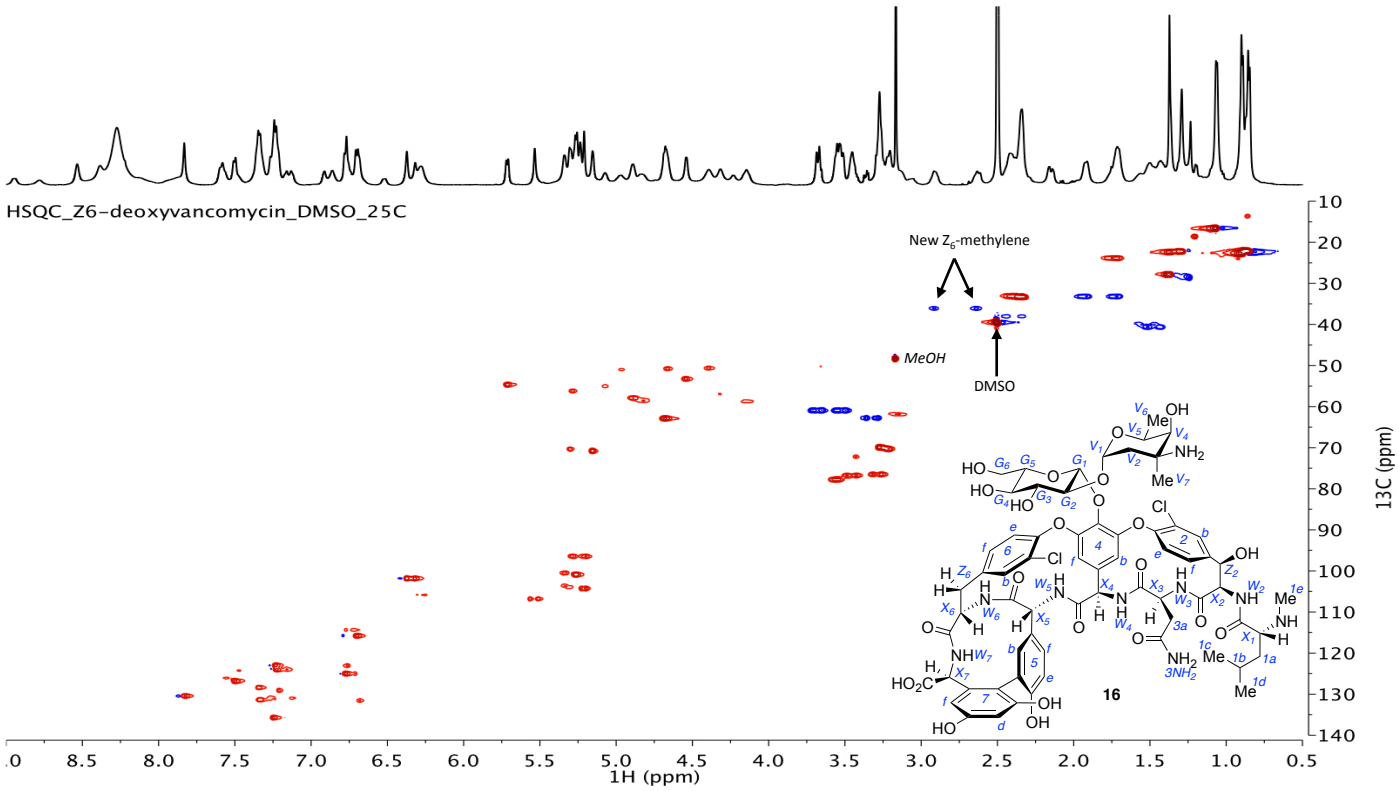


HR-LC/MS Data from Crude 16: (a) Diode array trace at 285 nm. (b) Mass spectrum of the peak at 2.59 min. (c) Isotope pattern of product peak $[M + H]^+$. (d) Expected isotope pattern of $[C_{66}H_{75}Cl_2N_9O_{23} + H]^+$.

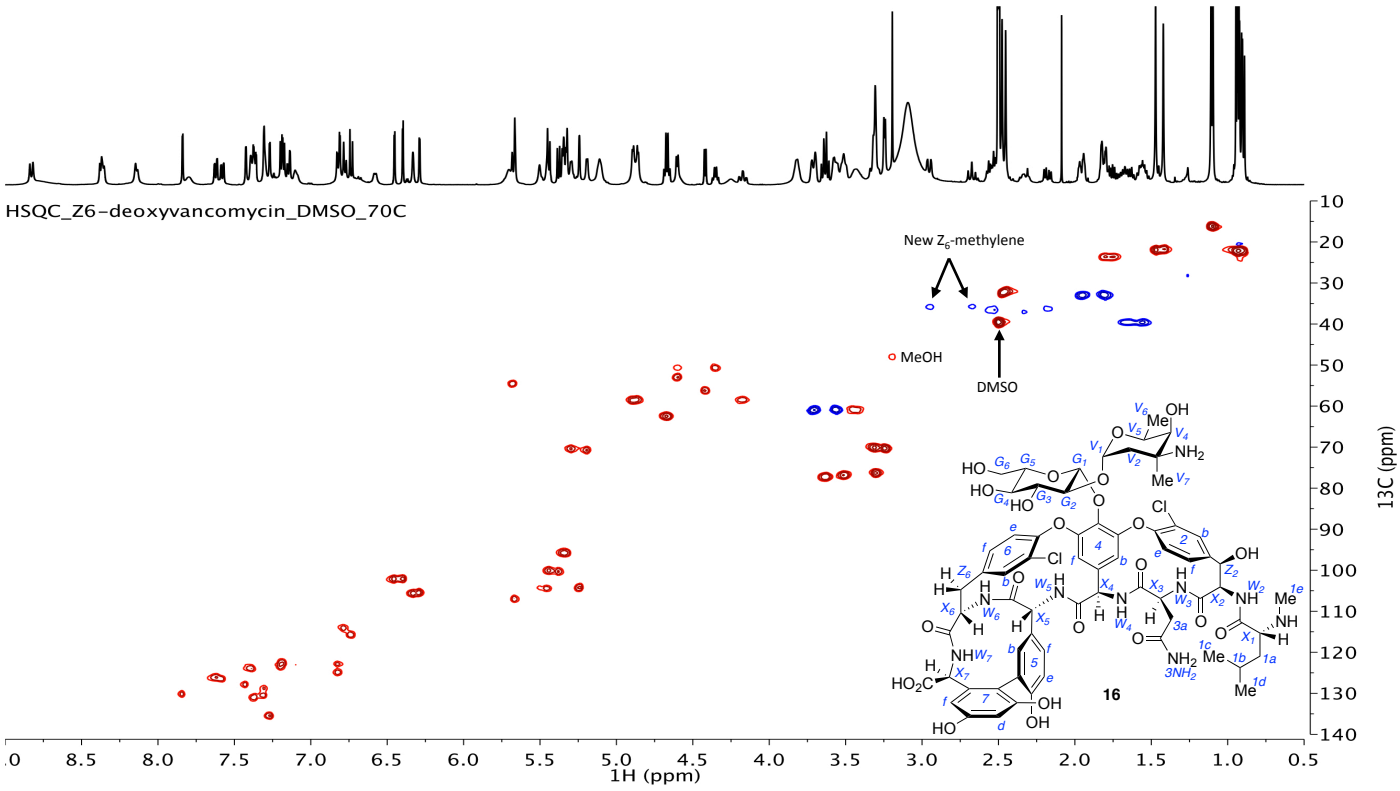


Purified 16: (a) Analytical HPLC Method 4, λ = 285 nm, t_R (**16**) = 15.6 min and 18.9 min. (b) HR-LC/MS diode array trace. (c) Mass spectrum of the peak at 2.52 min. (d) Mass spectrum of the peak at 2.60 min.

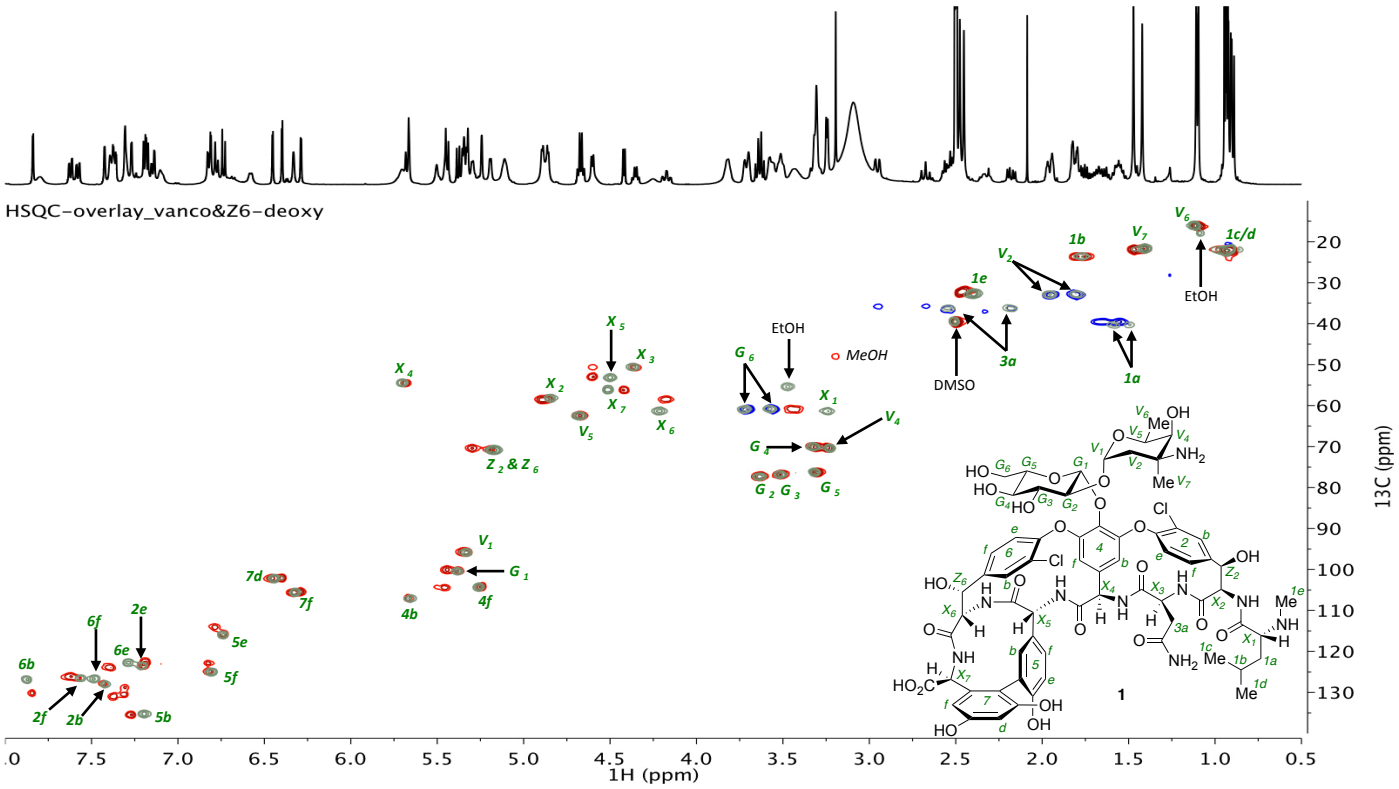
NMR Spectra of **16**



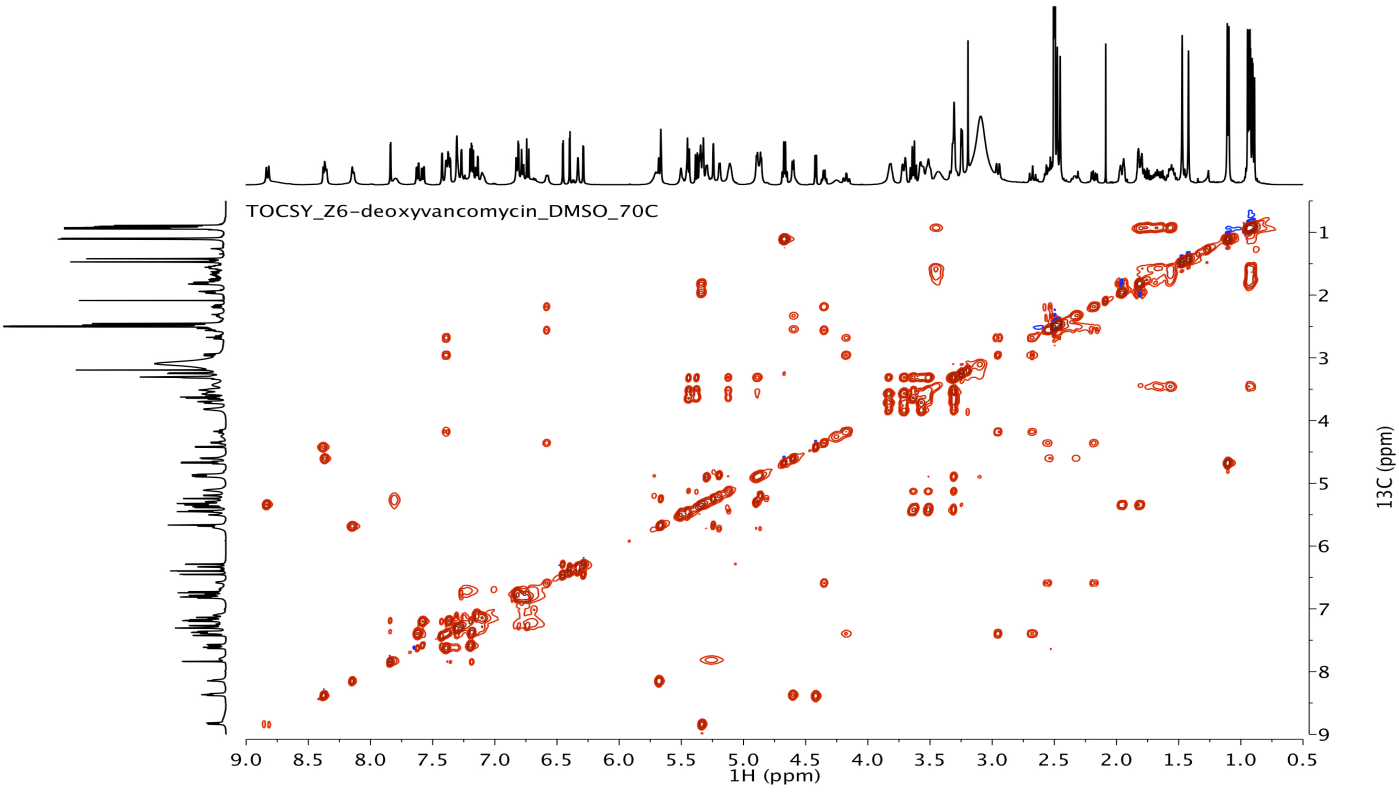
HSQC Spectrum of 16 (25 °C): DMSO-d_6 (20 mg/mL), ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Blue phase = methylenes. Red phase = methines and methyls.



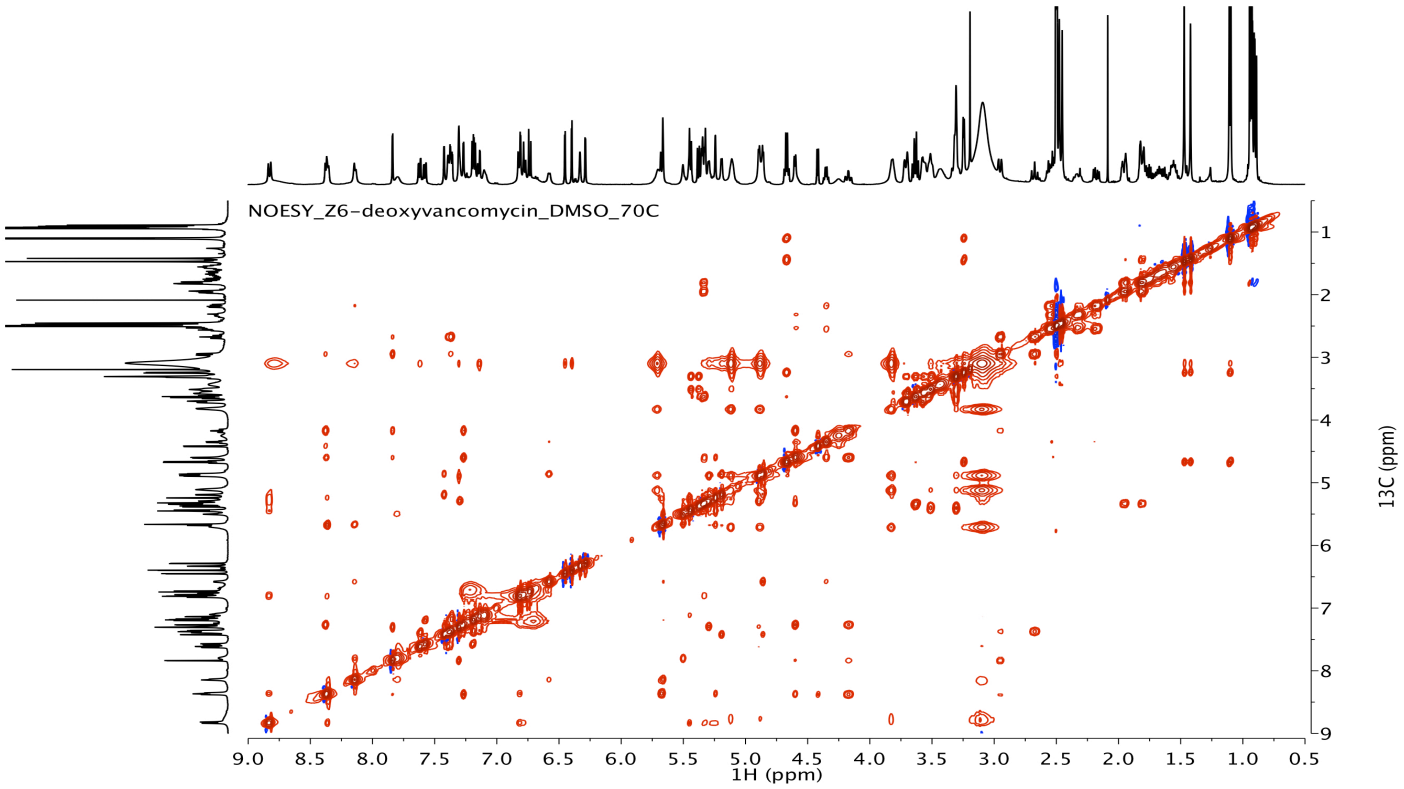
HSQC Spectrum of 16 (70 °C): DMSO-d_6 (20 mg/mL), degassed, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Blue phase = methylenes. Red phase = methines and methyls. At 70 °C, many peaks sharpen; however, there still exists significant doubling of peaks.



HSQC Spectrum of 1 with HSQC Spectrum of 16 Overlaid (70 °C): DMSO-d_6 (20 mg/mL), degassed, ^{1}H (500 MHz), ^{13}C (126 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{13}C chemical shift. Green = vancomycin (**1**), labels refer to these peaks. Red/blue = overlay of Z_6-deoxy-vancomycin (**16**). ^{1}H NMR spectrum of **16** shown on top.

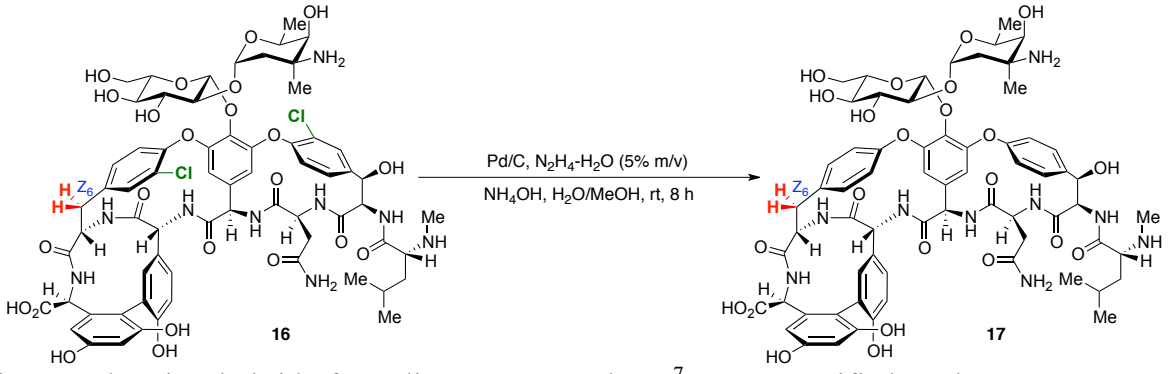


TOCSY Spectrum of 16 (70 °C): DMSO-d_6 (20 mg/mL), degassed, ^{1}H (500 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{1}H chemical shift. Mixing time = 80 ms.

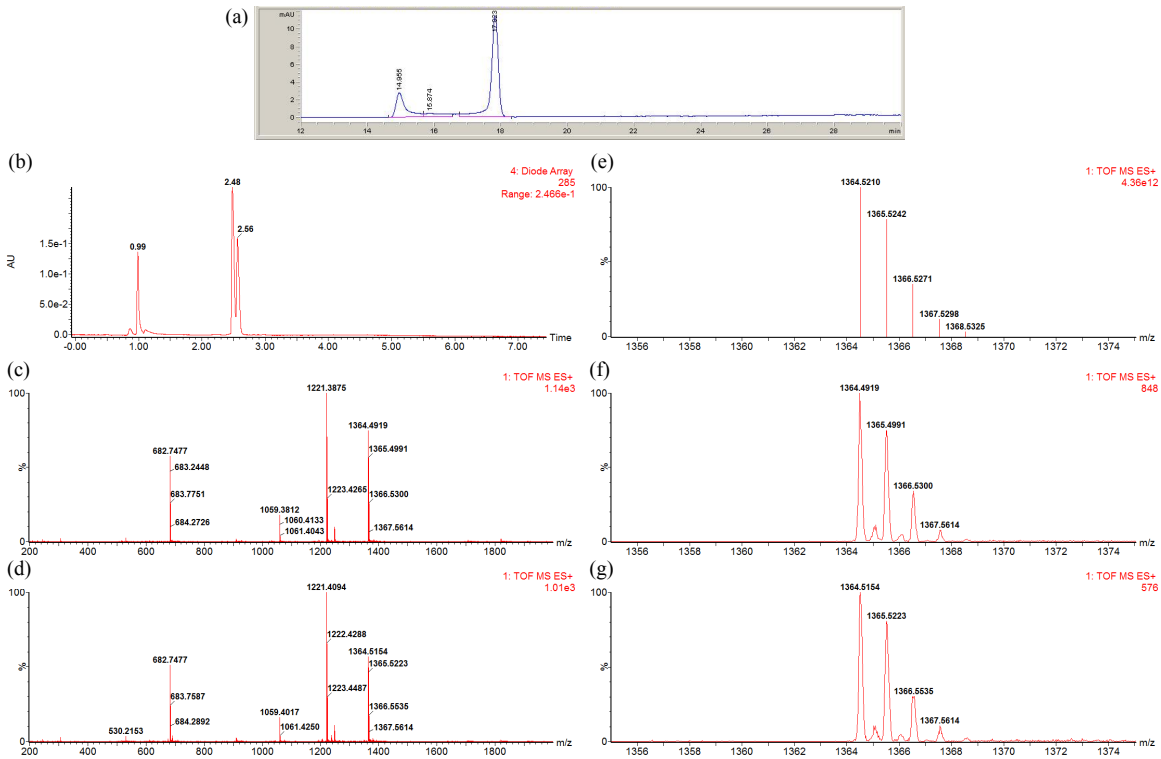


NOESY Spectrum of 16 (70 °C): DMSO-d_6 (20 mg/mL), degassed, ^{1}H (500 MHz). X-axis = ^{1}H chemical shift. Y-axis = ^{1}H chemical shift. Mixing time = 350 ms.

VIII. Synthesis of Didechloro-Z_6-deoxy-vancomycin (17)



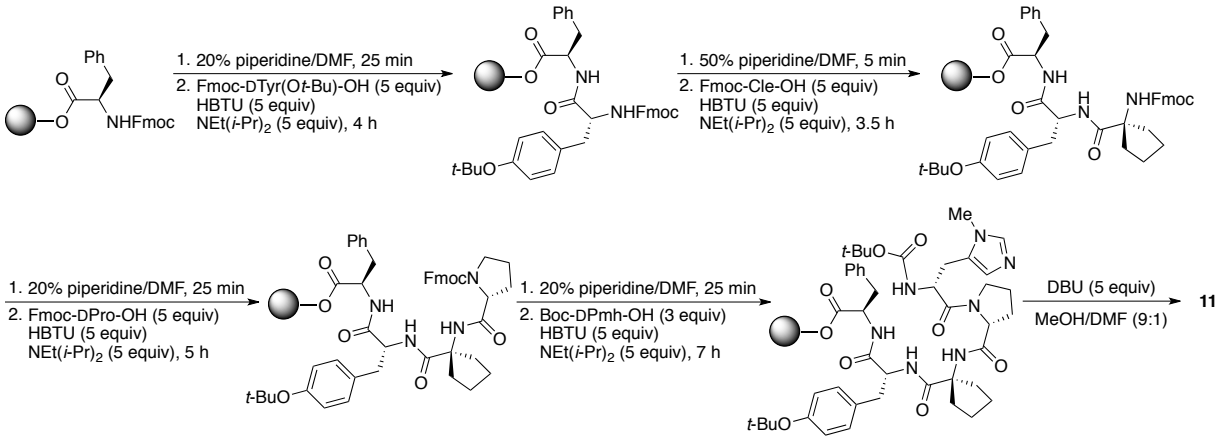
This procedure is a hybrid of two literature precedents.[7] HPLC-purified Z_6-deoxy-vancomycin (**16**) (16 mg, 11 μmol) was added to a 25-mL roundbottom flask with a stir bar. The solid was dissolved in H_2O (2.0 mL) before adding NH_4OH (concentrated, ~14 M, 0.70 mL) and 10% Pd/C (32 mg, 30 μmol). The flask was sealed with a septum, a vacuum/N_2 line was inserted, and the flask was briefly evacuated and backfilled with N_2 (× 3). The inside walls of the flask were rinsed with N_2-purged MeOH (1.0 mL), and hydrazine hydrate (150 mg, 0.15 mL) was added dropwise to the reaction mixture with vigorous stirring. The solution was stirred for 8 h at room temperature, at which time HR-LC/MS (Method 2) indicated the reaction was complete. The mixture was filtered through a cotton plug, and the solids were rinsed with H_2O (4 mL). The resulting clear and colorless solution was neutralized with AcOH and concentrated *in vacuo*. The crude product was purified by preparative HPLC (Method 7) to yield **17** as a tan solid (4.3 mg, 3.3 μmol, 28% yield).



Crude 17: (a) Analytical HPLC Method 4, λ = 285 nm, t_R (**17**) = 15.0 min and 17.9 min. (b) HR-LC/MS diode array trace at 285 nm. (c) Mass spectrum of the peak at 2.48 min. (d) Mass spectrum of the peak at 2.56 min. (e) Expected isotope pattern of $[C_{66}H_{77}Cl_2N_9O_{23} + H]^+$. (f) Isotope pattern of product peak $[M + H]^+$ for the peak at 2.48 min. (g) Isotope pattern of product peak $[M + H]^+$ for the peak at 2.56 min.

IX. Solid-Phase Synthesis of Peptides 9-11

This procedure describes the general synthetic strategy used for making nearly all of the peptides in the initial catalyst screen, including peptides **9**, **10**, and **11**. The synthesis and characterization of peptides **9** and ***ent*-10** have been reported previously by Sculimbrene *et al*.[8] The synthesis of **11** is described in detail below.



Swelling

Pre-loaded Fmoc-D-Phe-Wang resin (0.81 mmol/g, 1.49 g, 1.21 mmol) was swelled in CH_2Cl_2 in a fritted 20-mL reactor for 3 h. The solvent was removed by vacuum filtration.

Cycle 1

Typical Fmoc-Deprotection. A solution of 20% piperidine/DMF was added to the reactor, and the reactor was capped and placed on a spinner for 25 min. The solvent was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Typical Peptide Coupling. The Fmoc-D-Tyr(O*t*-Bu)-OH (2.77 g, 6.03 mmol) and HBTU (2.29 g, 6.03 mmol) were added to the dry deprotected resin in a 20-mL fritted reactor. DMF was added to nearly fill the reactor, then NEt(*i*-Pr)$_2$ (1.10 mL, 6.03 mmol) was added. The reactor was capped and placed on a spinner for 3-5 h. The reaction solution was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Cycle 2

****Rapid Fmoc-Deprotection.**** This method was used after the first coupling to minimized self-cleavage by diketopiperazine formation. A solution of 50% piperidine/DMF was added to the reactor, and the reactor was capped and placed on a spinner for 5 min. The solvent was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Typical Peptide Coupling. The Fmoc-Cle-OH (2.12 g, 6.03 mmol) and HBTU (2.29 g, 6.03 mmol) were added to the dry deprotected resin in a 20-mL fritted reactor. DMF was added to nearly fill the reactor, then NEt(*i*-Pr)$_2$ (1.10 mL, 6.03 mmol) was added. The reactor was capped and placed on a spinner for 3-5 h. The reaction solution was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Cycle 3

Typical Fmoc-Deprotection. A solution of 20% piperidine/DMF was added to the reactor, and the reactor was capped and placed on a spinner for 25 min. The solvent was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Typical Peptide Coupling. The Fmoc-D-Pro-OH (2.03 g, 6.03 mmol) and HBTU (2.29 g, 6.03 mmol) were added to the dry deprotected resin in a 20-mL fritted reactor. DMF was added to nearly fill the reactor, then NEt(*i*-Pr)$_2$ (1.10 mL, 6.03 mmol) was added. The reactor was capped and placed on a spinner for 3-5 h. The reaction solution was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Before Cycle 4, the resin was dried under vacuum, and a portion was removed for the synthesis of alternative peptides. The majority of the resin (1.63 g, ~0.980 mmol) was taken to Cycle 4.

Cycle 4

Typical Fmoc-Deprotection. A solution of 20% piperidine/DMF was added to the reactor, and the reactor was capped and placed on a spinner for 25 min. The solvent was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

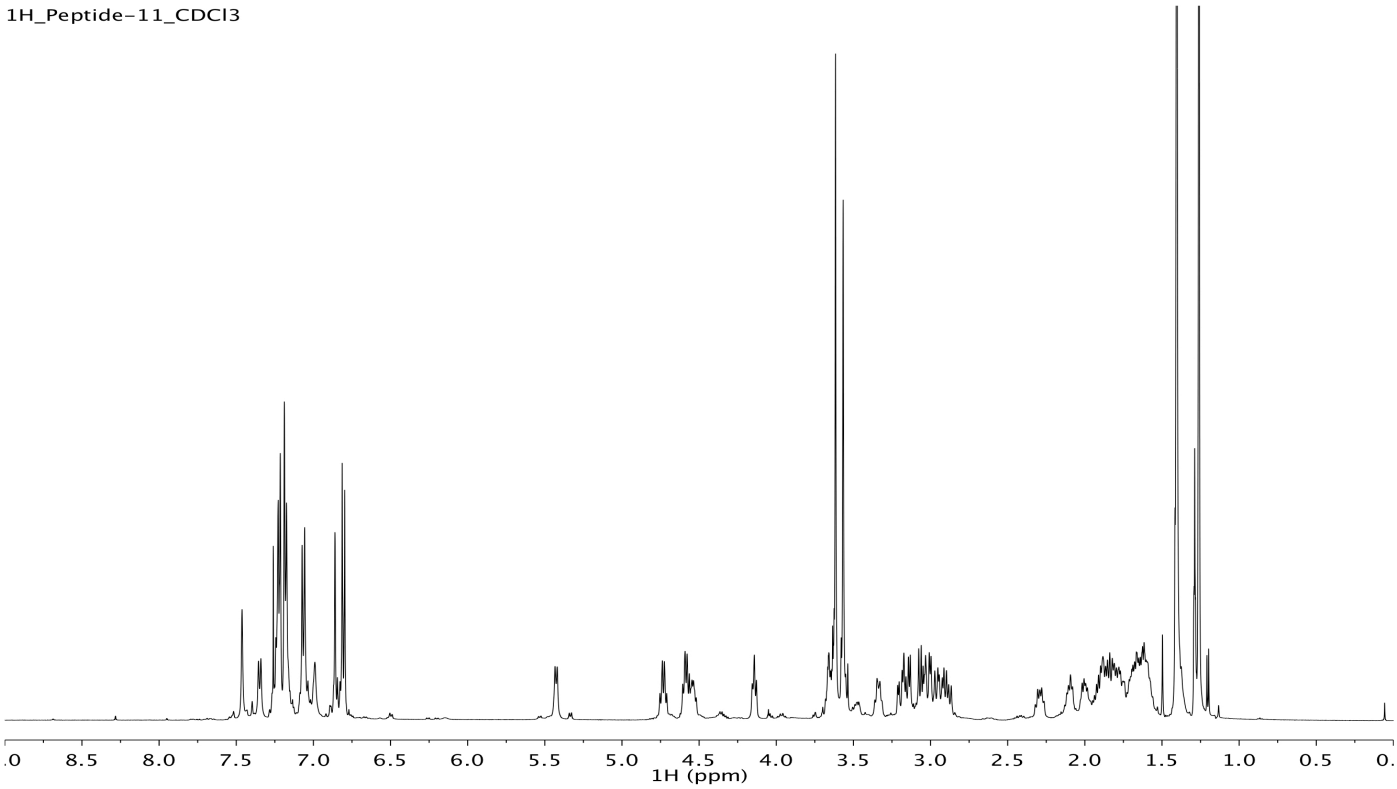
Pmh/DPmh Coupling. The Boc-DPmh-OH (0.795 g, 2.96 mmol) and HBTU (1.87 g, 4.90 mmol) were added to the dry deprotected resin in a 20-mL fritted reactor. DMF was added to nearly fill the reactor, then $NEt(i\text{-}Pr)_2$ (0.860 mL, 4.90 mmol) was added. The reactor was capped and placed on a spinner for 7 h. The reaction solution was removed by vacuum filtration, and the resin was washed with MeOH and CH_2Cl_2 (3 × each).

Cleavage

The fritted 20-mL reactor was nearly filled with MeOH/DMF (9:1), and DBU (0.733 mL, 4.90 mmol) was added. The reactor was capped and placed on a spinner for 3 h. The cleaved peptide was emptied into a 250-mL 2-neck roundbottom flask by vacuum filtration. The resin was washed with alternating CH_2Cl_2 and MeOH (3 × each), each time the solvent was emptied into the same 2-neck flask. A stir bar was added to the flask, and the solution was cooled to 0 °C. Acetic acid (0.280 mL, 4.90 mmol) was added dropwise to the stirring solution to quench the remaining DBU. The solution was then transferred to a 1-neck roundbottom and concentrated *in vacuo*.

Purification

The product was purified by **MPLC:** SNAP-C18 60-g column with a 12-g samplet; solvent A = H_2O, solvent B = MeOH, flow = 40 mL/min, λ = 210 nm; held at 40% B for 1.5 CV, ramped to 100% B over 12 CV, held at 100% B for 3 CV; peptide **11** eluted at 11-13 CV. The fractions containing **11** were concentrated *in vacuo* to yield a colorless solid (557 mg, 0.649 mmol, 66% yield). Peptide **11** appears to exist as rotameric mixtures (~8:1 major:minor); the following ^{1}H NMR data correspond to the major rotamer. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 7.46 (s, 1H; *DPmh-2-CH$_{imid}$*), 7.35 (d, *J* = 7.7 Hz, 1H; *DPhe-NH*), 7.23 (m, 3H; *DPhe-CH$_{aryl}$, DTyr-NH*), 7.18 (m, 3H; *DPhe-CH$_{aryl}$*), 7.06 (d, *J* = 8.3 Hz, 2H; *DTyr-2,6-CH$_{aryl}$*), 6.99 (br s, 1H; *Cle-NH*), 6.86 (s, 1H; *DPmh-4-CH$_{imid}$*), 6.81 (d, *J* = 8.4 Hz, 1H; *DTyr-3,5-CH$_{aryl}$*), 5.43 (d, *J* = 7.0 Hz, 1H; *DPmh-NH*), 4.73 (dt, *J* = 7.7, 7.7 Hz, 1H; *DPhe-α-CH*), 4.58 (dt, *J* = 7.3, 7.3 Hz, 1H; *DPmh-α-CH*), 4.53 (m, 1H; *DTyr-α-CH*), 4.14 (t, *J* = 6.8 Hz, 1H; *DPro-α-CH*), 3.66 (m, 1H; *DPro-δ-CH*), 3.63 (s, 3 H; *DPhe-OCH$_3$*), 3.57 (s, 3H; *DPmh-NCH$_3$*), 3.35 (m, 1H; *DPro-δ-CH*), 3.23 – 2.85 (m, 6H; *DPhe-β-CH$_2$, DPmh-β- CH$_2$, DTyr-β- CH$_2$*), 2.29 (m, 1H; *Cle-β-CH*), 2.09 (m, 1H; *DPro-β-CH*), 2.00 (m, 1H; *DPro-γ-CH*), 1.95 – 1.73 (m, 5H; *DPro-β-CH, DPro-γ-CH, Cle-β-CH, Cle-γ-CH$_2$*), 1.73 – 1.55 (m, 4H; *Cle-β-CH$_2$, Cle-γ-CH$_2$*), 1.40 (s, 9H; *Boc-O(CH$_3$)$_3$*), 1.26 (s, 9H; *Tyr-O(CH$_3$)$_3$*). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 173.5, 171.8, 171.7, 171.4, 171.2, 155.3, 153.8, 138.3, 136.8, 132.7, 129.5, 129.2, 128.3, 127.4, 126.8, 126.6, 124.1, 80.5, 78.2, 67.1, 62.0, 54.8, 53.7, 52.3, 52.1, 47.6, 37.5, 37.2, 36.9, 36.1, 31.6, 28.7, 28.4, 28.3, 28.2, 26.6, 25.4, 24.4. **LC/MS** (ESI, Method 1) exact mass calculated for the *C*-terminal carboxylic acid $[C_{46}H_{63}N_7O_9\ H]^+$ is *m/z* = 858.48, found *m/z* = 858.47 (t_R = 1.44 min).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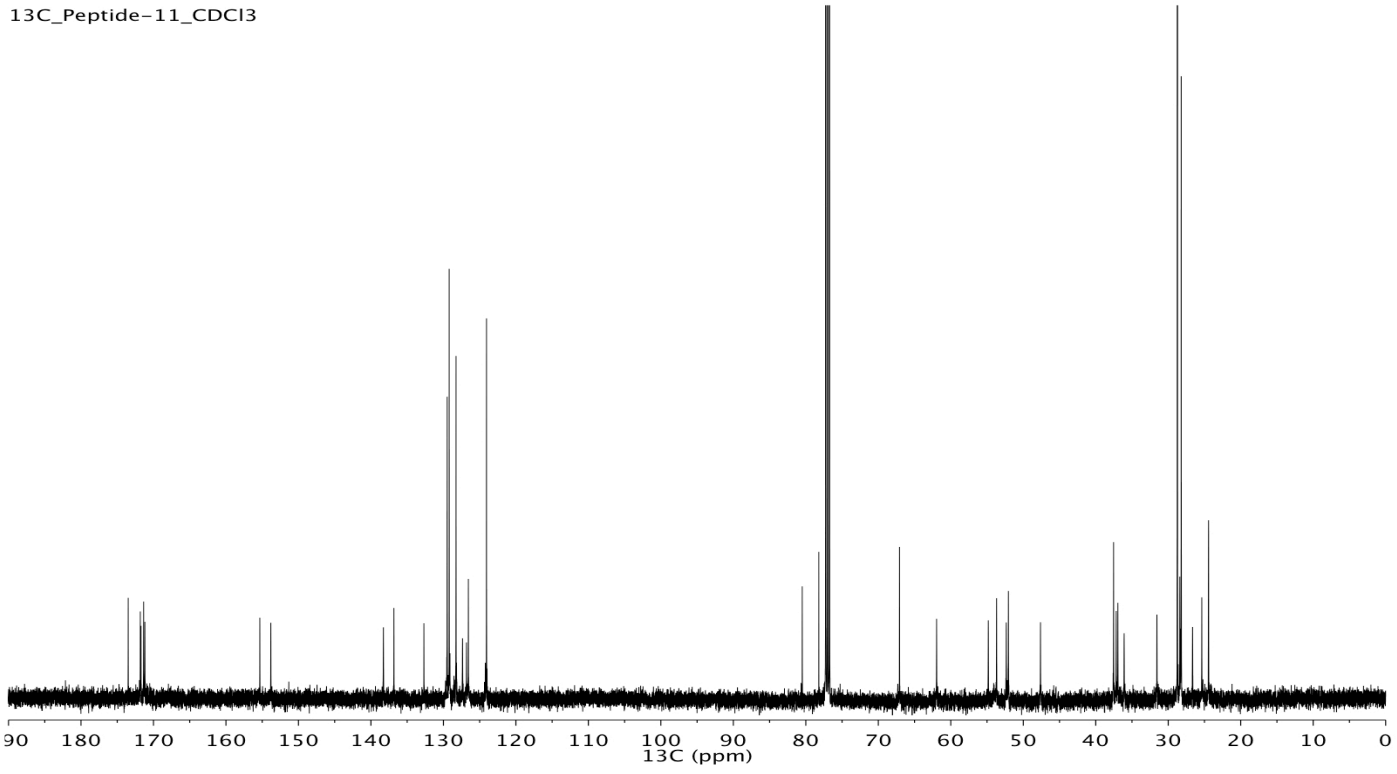
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
1H (ppm)


13C_Peptide-11_CDCl3
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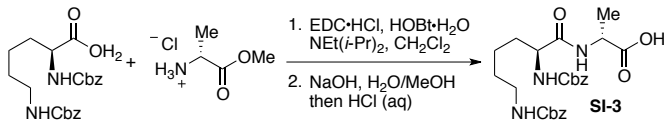
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
13C (ppm)

X. Synthesis of Peptides 12-14 from SI-3

These procedures describe the general synthetic strategy used for making peptide catalysts based on the Lys-DAla-DAla motif. The syntheses of **12**, **13**, **14**, and their common precursor, **SI-3**, are described in detail below.

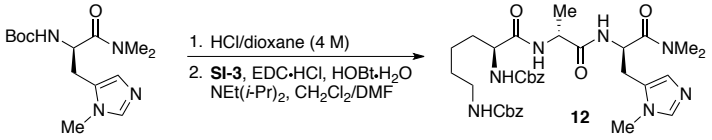
Synthesis of Cbz-Lys(Cbz)-DAla-OH (**SI-3**)



Cbz-Lys(Cbz)-OH (4.15 g, 10.0 mmol), H-DAla-OMe hydrochloride (1.40 g, 10.0 mmol), EDC•HCl (2.11 g, 11.0 mmol), and HOBt•H_2O (1.68 g, 11.0 mmol) were added to a 250-mL roundbottom flask with a stir bar. The solids were suspended in CH_2Cl_2 (50.0 mL) before adding NEt(*i*-Pr)$_2$ (2.09 mL, 12.0 mmol). The solids dissolved within a few minutes, and the mixture was stirred for 8 h. The reaction mixture was transferred to a separatory funnel where it was washed with aqueous citric acid (10%, 50 mL) and saturated aqueous $NaHCO_3$ (50 mL). An emulsion formed at this step, and the layers were allowed to separate over 2-3 h. The organic layer was then washed with saturated aqueous NaCl (50 mL), dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The resulting sticky foam was placed under vacuum overnight to yield a crunchy colorless solid (4.0 g, 8.01 mmol, 80% yield).

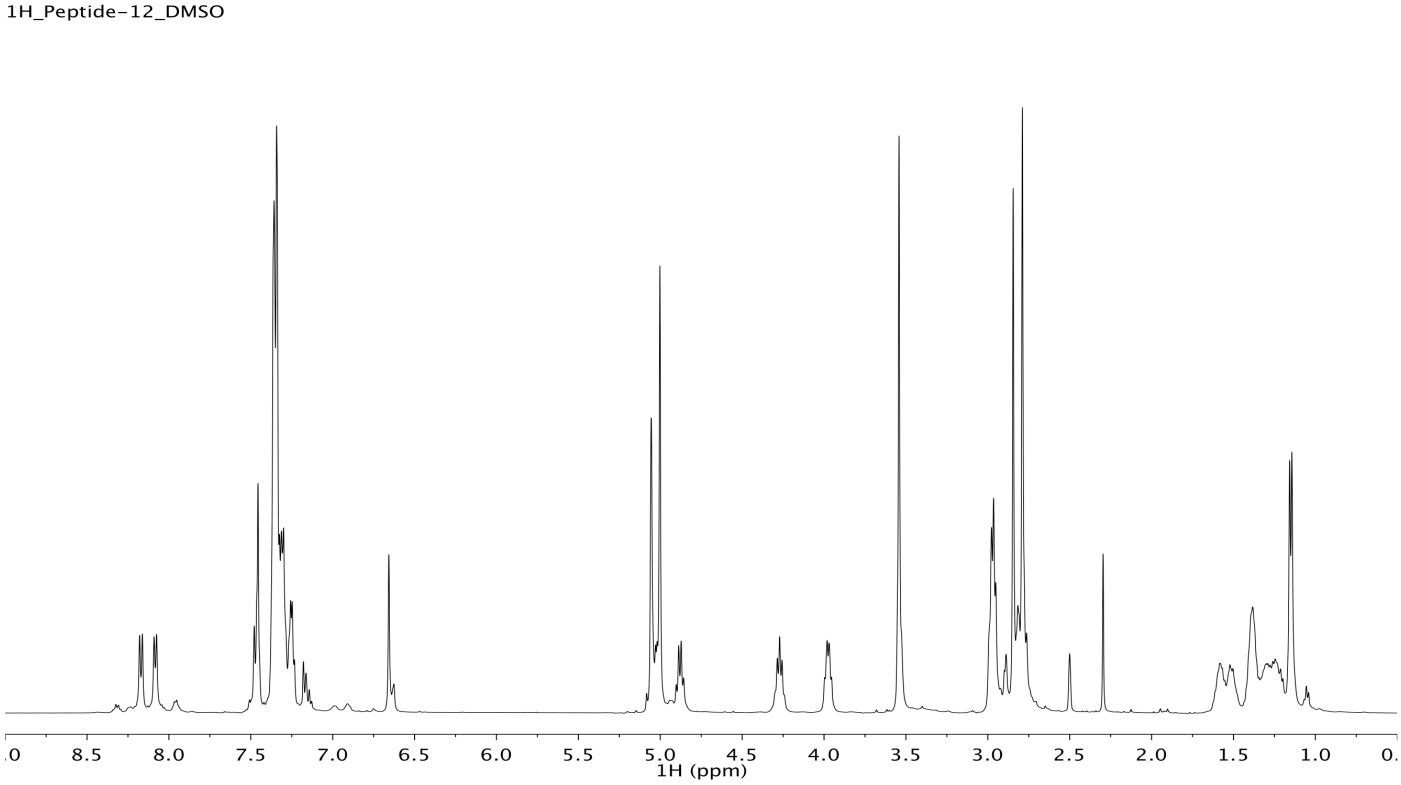
A portion of this methyl ester (3.56 g, 7.12 mmol) was added to a 100-mL roundbottom flask with a stir bar and was suspended in MeOH (37.5 mL). Aqueous NaOH (2.0 M, 7.50 mL) was added slowly to the stirring suspension at room temperature. After 70 min, the solution was clear and TLC (5% AcOH/EtOAc) indicated that no ester remained (ester R_f = 0.85, **SI-3** R_f = 0.44). The stir bar was removed and the MeOH was evaporated *in vacuo*, leaving an aqueous solution. Aqueous HCl (1.0 M) was slowly added and a white precipitate began to form. Once the solution was at or below pH 4, the solids were extracted into EtOAc (3 × 50 mL), and the combined organic phases were dried over $MgSO_4$, filtered, and concentrated to yield **SI-3** as a white solid (3.03 g, 6.24 mmol, 88% yield). The product was used for subsequent steps without purification. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.0 (br s, 1 H; *Lys-α-NH*), 7.3 (m, 11H; *Cbz-CH$_{aryl}$*, *DAla-α-NH*), 7.2 (t, *J* = 5.5 Hz, 1H; *Lys-ε-NH*), 5.0 (d, *J* = 11.3 Hz, 4H; *Cbz-CH$_2$*), 4.1 (m, 1H; *DAla-α-H*), 4.0 (m, 1H; *Lys-α-H*), 3.0 (dt, *J* = 6.5, 6.5 Hz, 2H; *Lys-ε-CH$_2$*), 1.6 (m, 1H; *Lys-β-CH*), 1.5 (m, 1H; *Lys-β-CH*), 1.4 (m, 1H; *Lys-δ-CH*), 1.2 (m, 5H; *Lys-γ-CH$_2$*, *DAla-β-CH$_3$*). **LC/MS** (ESI, Method 1) exact mass for $[C_{25}H_{31}N_3O_7\ H]^+$ is *m/z* = 486.22, found *m/z* = 486.16 (t_R = 1.36 min).

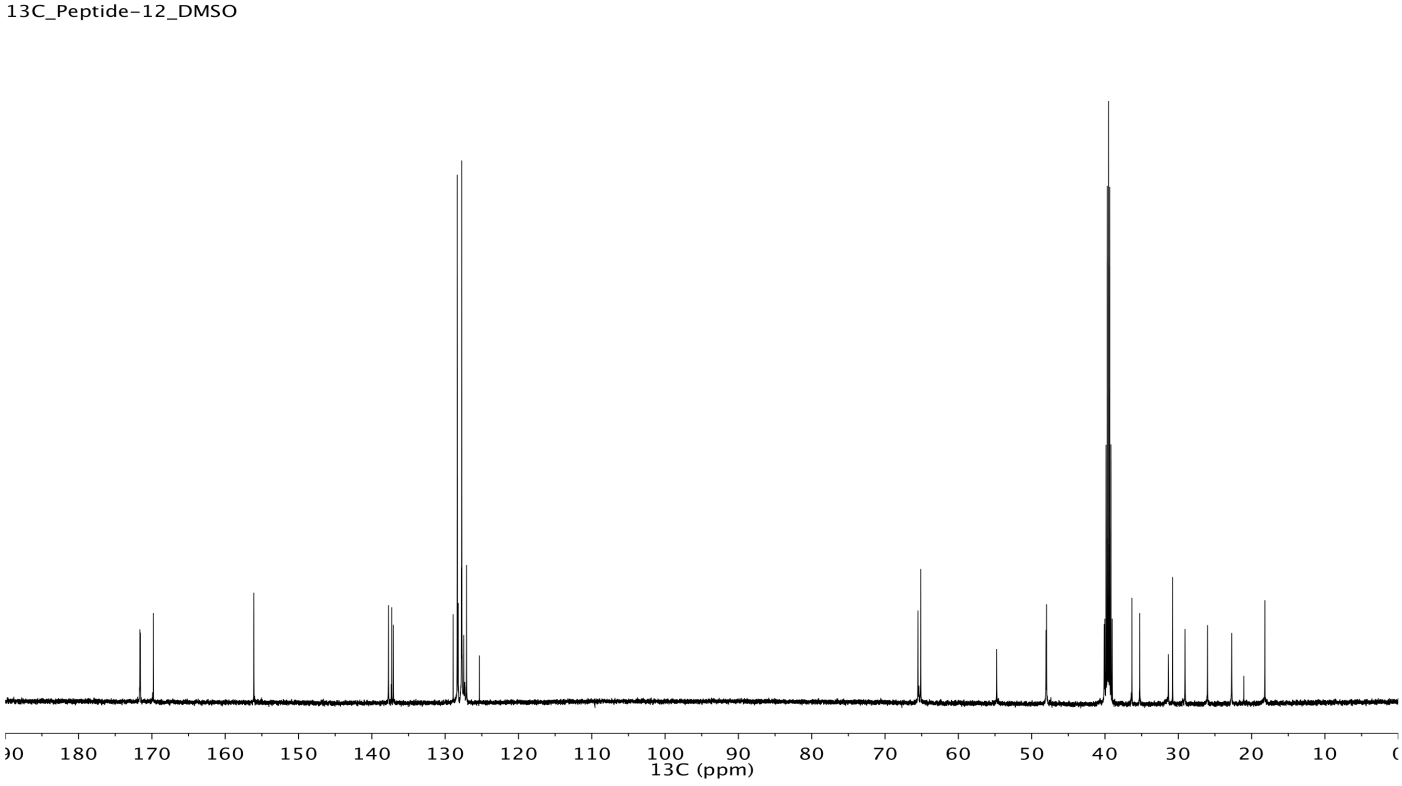
Synthesis of Peptide **12**



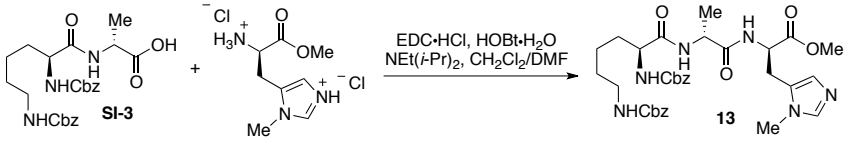
Boc-DPmh-NMe_2 (322 mg, 1.09 mmol) was added to a 25-mL roundbottom flask with a stir bar, and was dissolved in HCl/dioxane (4.0 M, 3.0 mL). The solution was stirred for 30 min, during which time a white precipitate formed. The HCl and dioxane were removed by passing dry N_2 through the headspace of the flask into an exit tube that led to an aqueous solution of $NaHCO_3$. After 2-3 h, the flask was transferred to a vacuum pump to dry for an additional 1 h. Dipeptide **SI-3** (580 mg, 1.20 mmol) was added, and the solids were suspended in DMF (2.0 mL) and CH_2Cl_2 (2.0 mL). NEt(*i*-Pr)$_2$ (0.284 mL, 1.63 mmol), HOBt•H_2O (200 mg, 1.30 mmol), and EDC•HCl (250 mg, 1.30 mmol) were added and rinsed from the flask walls with CH_2Cl_2 (1.5 mL). The chunky suspension eventually became a slightly cloudy solution, and after 14 h, LC/MS indicated that the reaction was complete. The solution was poured into saturated aqueous $NaHCO_3$ (50 mL) and extracted with CH_2Cl_2 (3 × 30 mL). The combined organic layers were dried over Na_2SO_4, and concentrated *in vacuo* to get the product and residual DMF. The product was purified by **MPLC:** SNAP-C18 60-g column with a 12-g

samplet; solvent A = H_2O, solvent B = MeOH, flow = 40 mL/min, λ = 210 nm; held at 10% B for 3 CV, ramped to 70% B over 15 CV, held at 70% B for 3 CV; peptide **12** eluted at 16-20 CV. The fractions containing **12** were concentrated *in vacuo* to yield a colorless solid (503 mg, 0.758 mmol, 70% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.17 (d, J = 8.3 Hz, 1H; *ᴅPmh-NH*), 8.08 (d, J = 7.4 Hz, 1H; *ᴅAla-NH*), 7.53 – 7.41 (m, 2H; *Lys-α-NH,* ; *ᴅPmh-2-CH$_{imid}$*), 7.41 – 7.28 (m, 10H; *Cbz-CH$_{aryl}$*), 7.28 – 7.22 (m, 1H; *Lys-ε-NH*), 6.66 (s, 1H; *ᴅPmh-4-CH$_{imid}$*), 5.10 – 4.98 (m, 4H; *Cbz-CH$_2$*), 4.88 (dt, J = 7.5, 7.5 Hz, 1H; *ᴅPmh-α-CH*), 4.33 – 4.22 (m, 1H; *ᴅAla-α-CH*), 3.98 (dt, J = 7.4, 7.4 Hz, 1H; *Lys-α-CH*), 3.54 (s, 3H; *ᴅPmh-NCH$_3$*), 3.03 – 2.92 (m, 3H; *ᴅPmh-β-CH, Lys-ε-CH$_2$*), 2.91 – 2.72 (m, 7H; *ᴅPmh-β-CH*, *N(CH$_3$)$_2$*), 1.65 – 1.45 (m, 2H; *Lys-β-CH$_2$*), 1.45 – 1.35 (m, 2H; *Lys-δ-CH$_2$*), 1.35 – 1.19 (m, 2H; *Lys-γ-CH$_2$*), 1.15 (d, J = 6.9 Hz, 3H; *ᴅAla-β-CH$_3$*). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.6, 171.6, 169.8, 156.1, 137.7, 137.3, 137.1, 128.9, 128.4, 128.2, 127.8, 127.7, 127.5, 127.1, 125.3, 65.5, 65.1, 54.8, 48.1, 48.0, 40.1, 36.3, 35.3, 31.4, 30.8, 29.1, 26.0, 22.7, 21.1, 18.2. **LC/MS** (ESI, Method 1) exact mass for $[C_{34}H_{45}N_7O_7\ H]^+$ is m/z = 664.35, found m/z = 664.25 (t_R = 1.04 min).



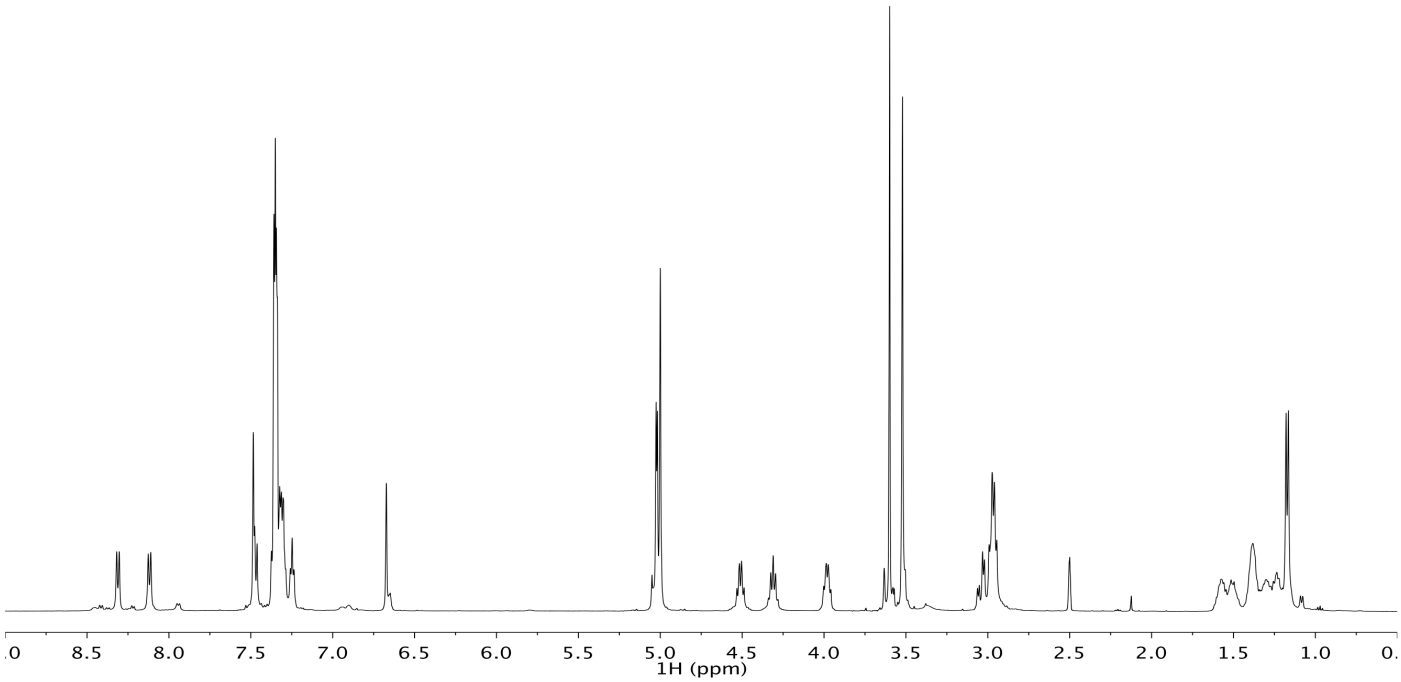


Synthesis of Peptide **13**

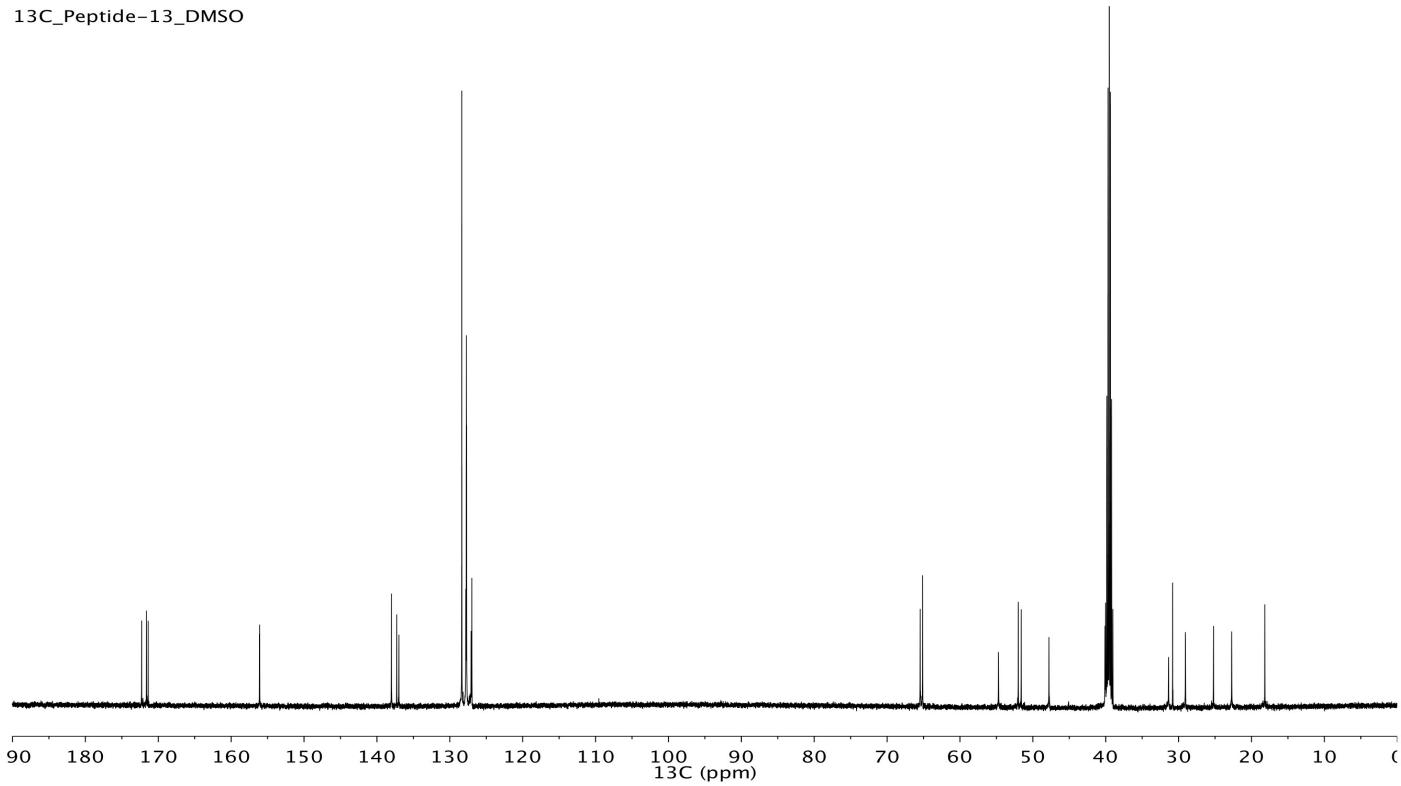


H-DPmh-OMe dihydrochloride (256 mg, 1.00 mmol), dipeptide **SI-3** (486 mg, 1.00 mmol), and HOBt•H_2O (184 mg, 1.20 mmol) were added to a flame-dried 10-mL roundbottom flask with a stir bar. DMF (2.0 mL), CH_2Cl_2 (2.5 mL), and NEt(*i*-Pr)$_2$ (0.523 mL, 3.00 mmol) were added followed by EDC•HCl (230 mg, 1.20 mmol) and additional CH_2Cl_2 (1.0 mL). The solids gradually dissolved and the solution was stirred at room temperature for 15 h. The mixture was poured into half-saturated aqueous $NaHCO_3$ (50 mL), and the product was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic layers were concentrated and purified by **MPLC:** SNAP-C18 60-g column with a 12-g samplet; solvent A = H_2O, solvent B = MeOH, flow = 50 mL/min, λ = 210 nm; held at 20% B for 1.5 CV, ramped to 100% B over 17 CV, held at 100% B for 3 CV; peptide **13** eluted at 13-15 CV. The fractions containing **13** were concentrated *in vacuo* to yield a colorless solid (481 mg, 0.739 mmol, 74% yield). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.31 (d, *J* = 7.7 Hz, 1H; *DPmh-NH*), 8.12 (d, *J* = 7.7 Hz, 1H; *DAla-NH*), 7.50 – 7.44 (m, 2H; *DPmh-2-CH$_{imid}$, Lys-α-NH*), 7.39 – 7.27 (m, 10H; *Cbz-CH$_{aryl}$*), 7.25 (t, *J* = 5.5 Hz, 1H; *Lys-ε-NH*), 6.67 (s, 1H; *DPmh-4-CH$_{imid}$*), 5.04 – 4.99 (m, 4H; *Cbz-CH$_2$*), 4.51 (dt, *J* = 7.7, 7.7 Hz, 1H; *DPmh-α-CH*), 4.37 – 4.26 (m, 1H; *DAla-α-CH*), 3.98 (dt, *J* = 7.8, 7.8 Hz, 1H; *Lys-α-CH*), 3.60 (s, 3H; *OCH$_3$*), 3.52 (s, 3H; *DPmh-NCH$_3$*), 3.08 – 2.91 (m, 4H; *DPmh-β-CH$_2$,* ; *Lys-ε-CH$_2$*), 1.65 – 1.45 (m, 2H; *Lys-β-CH$_2$*), 1.44 – 1.35 (m, 2H; *Lys-δ-CH$_2$*), 1.33 – 1.20 (m, 2H; *Lys-γ-CH$_2$*), 1.17 (d, *J* = 7.0 Hz, 3H; *DAla-β-CH$_3$*). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.3, 171.6, 171.4, 156.1, 156.1, 138.0, 137.3, 137.0, 128.4, 127.8, 127.7, 127.7, 127.1, 1267.0, 65.5, 65.1, 54.7, 52.0, 51.6, 47.8, 40.1, 31.4, 30.8, 29.1, 25.2, 22.7, 18.2. **LC/MS** (ESI, Method 1) exact mass for $[C_{33}H_{42}N_6O_8\ H]^+$ is *m/z* = 651.31, found *m/z* = 651.25 (t_R = 1.08 min).

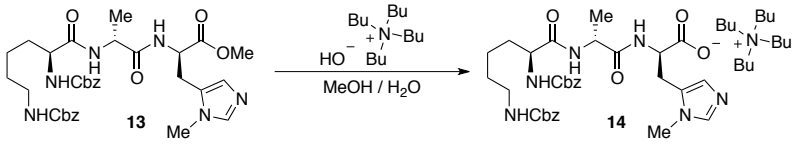
1H_Peptide-13_DMSO



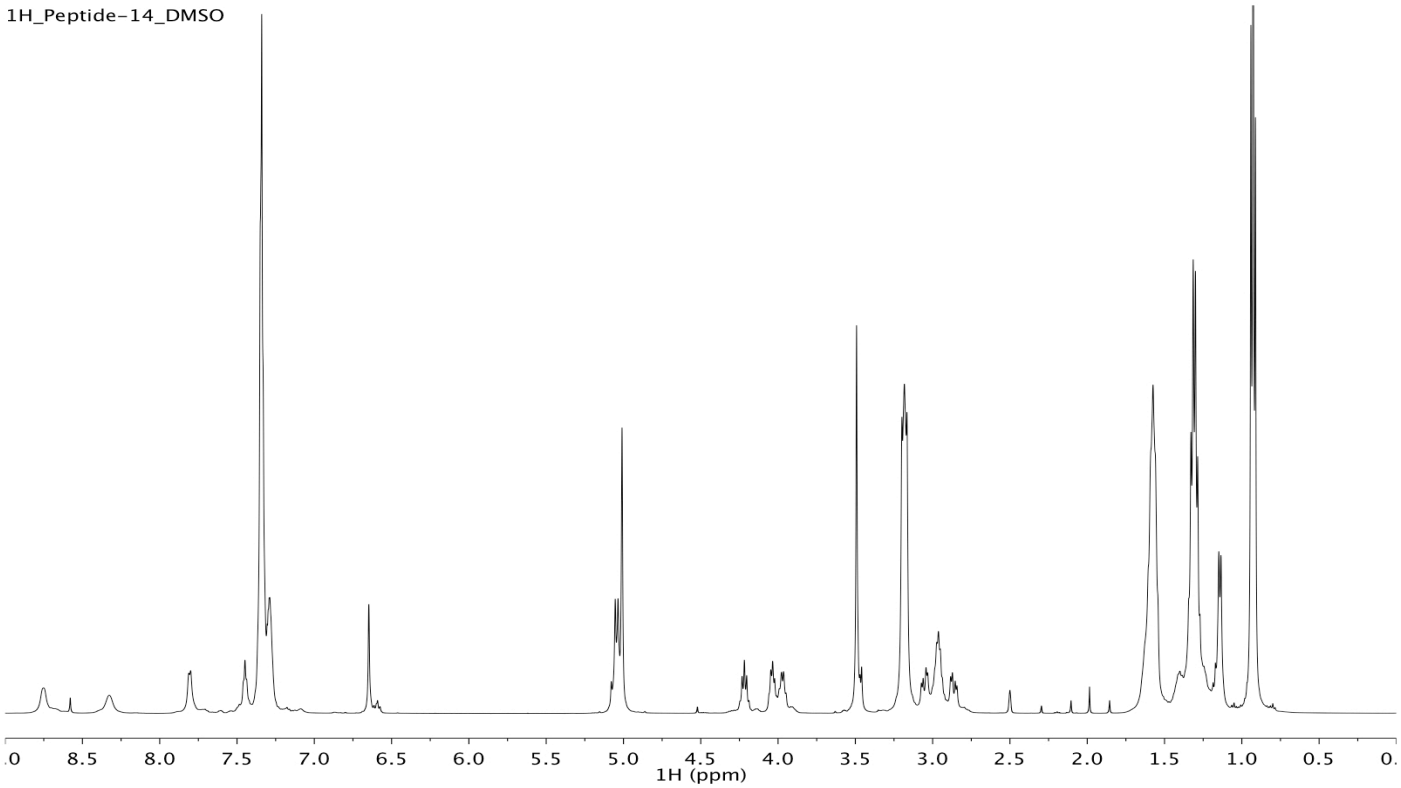
13C_Peptide-13_DMSO

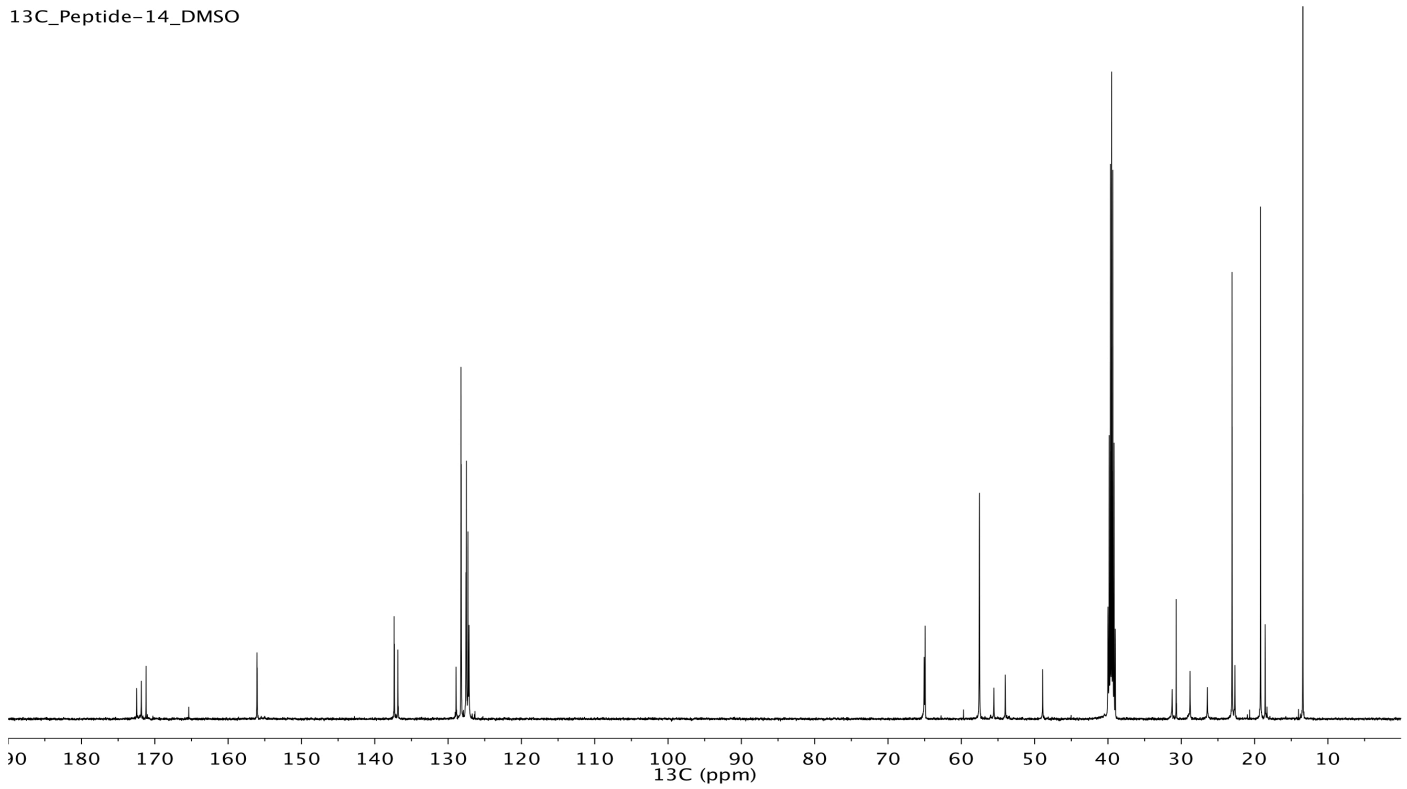


Synthesis of Peptide **14**



Peptide **13** (100 mg, 0.154 mmol) was added to 1-dram vial with a stir bar and was completely dissolved in MeOH (0.614 mL), which required some sonication to break up chunks. To this was added H_2O (0.154 mL), and the solution became cloudy. A solution of tetrabutylammonium hydroxide (1.0 M in MeOH, 0.154 mL, 0.154 mmol) was added dropwise to the mixture at room temperature, and the cloudiness dissipated within a few minutes. After 2.5 h, LC/MS indicated the reaction was complete, and the solution was transferred to a 15-mL centrifuge tube. The reaction vial was rinsed with H_2O (2 × 1.0 mL), and these rinses were also added to the centrifuge tube. The MeOH was removed under reduced pressure and the remaining aqueous solution was frozen and lyophilized to yield an off-white foam (137 mg, quantitative yield). The peptide was used without further purification. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.75 (s, 1H; *DAla-NH*), 8.33 (s, 1H; *Lys-α-NH*), 7.81 (d, J = 5.8, 1H; *DPmh-NH*), 7.45 (t, J = 4.9, 1H; *Lys-ε-NH*), 7.34 (s, 10H; *Cbz-CH_{aryl}*), 7.31-7.24 (m, 1H; *DPmh-2-CH_{imid}*), 6.65 (s, 1H; *DPmh-4-CH_{imid}*), 5.09-4.99 (m, 4H; *Cbz-CH_2*), 4.26-4.18 (m, 1H; *DAla-α-CH*), 4.03 (dt, J = 6.3, 6.3, 1H; *DPmh-α-CH*), 3.97 (dt, J = 7.5, 7.5, 1H; *Lys-α-CH*), 3.49 (s, 3H; *DPmh-NCH_3*), 3.27-3.11 (m, 8H; *TBA-α-CH_2*), 3.05 (dd, J = 14.6, 5.3, 1H; *DPmh-β-CH_2*), 3.02-2.92 (m, 2H; *Lys-ε-CH_2*), 2.86 (dd, J = 14.6, 5.6, 1H; *DPmh-β-CH_2*), 1.67-1.50 (m, 10H; *TBA-β-CH_2, Lys-β-CH_2*), 1.46-1.20 (m, 12H; *TBA-γ-CH_2, Lys-γ-CH_2, Lys-δ-CH_2*), 1.14 (d, J = 6.9, 3H; *DAla-β-CH_3*), 0.93 (t, J = 7.3, 12H; *TBA-δ-CH_3*). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.5, 171.8, 171.2, 165.4, 156.1, 156.0, 137.4, 137.3, 136.8, 128.9, 128.2, 128.2, 127.6, 127.5, 127.3, 127.1, 65.1, 64.9, 59.7, 57.5, 55.6, 54.0, 48.9, 31.2, 30.7, 28.8, 26.4, 23.1, 22.7, 19.2, 18.6, 13.4. **LC/MS** (ESI, Method 1) exact mass calculated for the *C*-terminal carboxylic acid $[C_{32}H_{40}N_6O_8\ H]^+$ is m/z = 637.30, found m/z = 637.20 (t_R = 1.01 min).





XI. Additional Minimum Inhibitory Concentration (MIC) Data

MIC Data Including the Positive Controls Teicoplanin and Linezolid

Compound	MSSA ATCC 29213	MRSA ATCC 43300	VSE ATCC 29212	VRE (VanB) ATCC 51299	VRE (VanA) MMX 486
vancomycin (1)	0.5	1.0	2.0	16	>64
15	0.5	1.0	2.0	16	>64
16	2.0	4.0	8.0	>64	>64
teicoplanin	0.5	0.5	0.12	0.25	>32
linezolid	4.0	2.0	2.0	2.0	2.0

MSSA = methicillin-susceptible *S. aureus*; **MRSA** = methicillin-resistant *S. aureus*; **VSE** = vancomycin-susceptible enterococci (*E. faecalis*); **VRE (VanB)** = vancomycin-resistant enterococci (*E. faecalis*, teicoplanin susceptible); **VRE (VanA)** = vancomycin-resistant enterococcus (*E. faecalis*, teicoplanin resistant). **ATCC #:** American Type Culture Collection isolate. **MMX #:** Micromyx, LLC isolate.

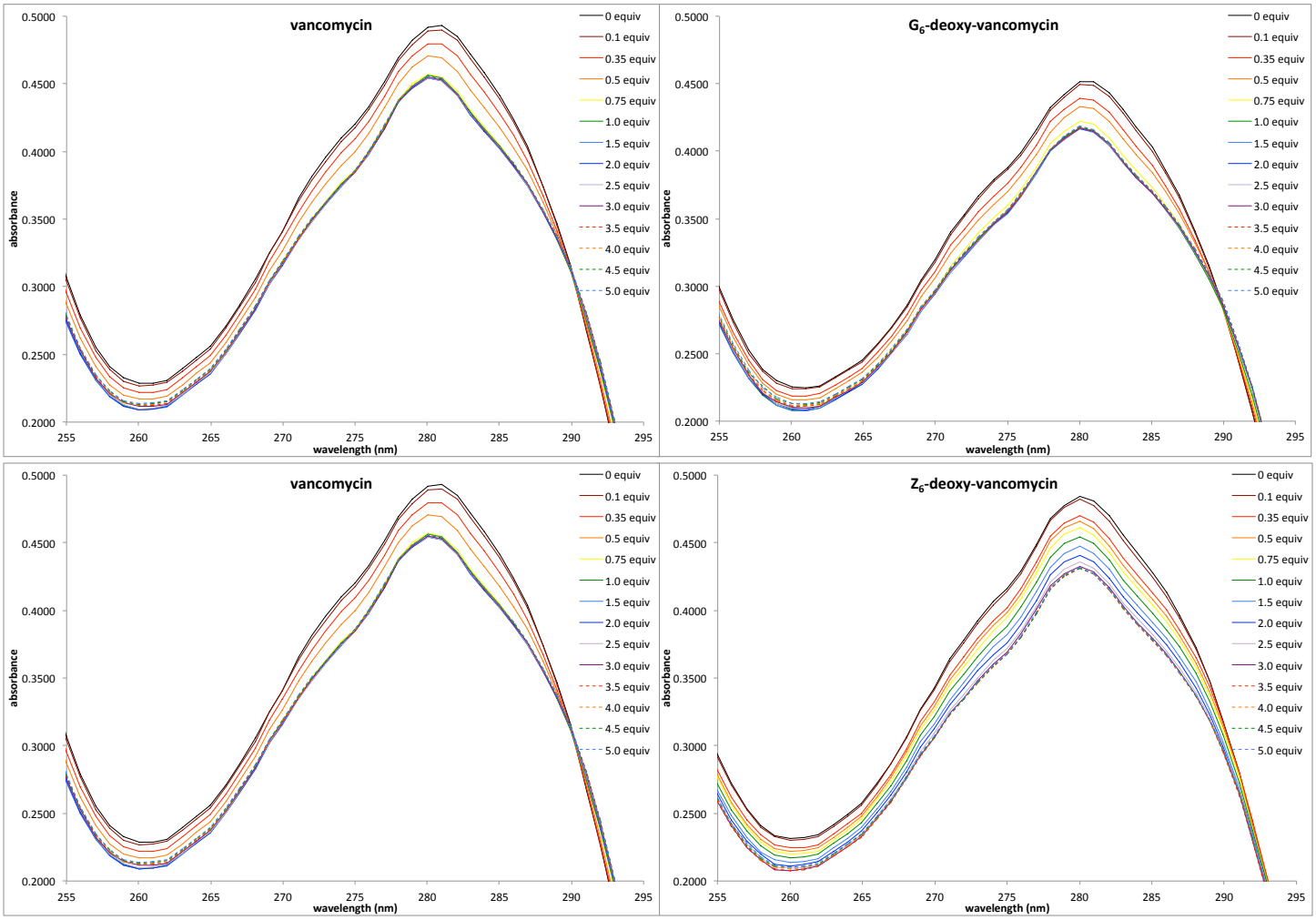
XII. UV-Difference Assay and Binding Data[9]

Spectra were collected on a CARY 50 Bio UV-Visible Spectrophotometer at 25 °C ± 0.1 °C in a quartz cuvette (1 cm). Stock solutions of antibiotic (77 mM) and the peptide Ac-Lys(Ac)-DAla-DAla-OH (46 mM) were prepared in 0.02 M sodium citrate buffer (pH = 5.1).

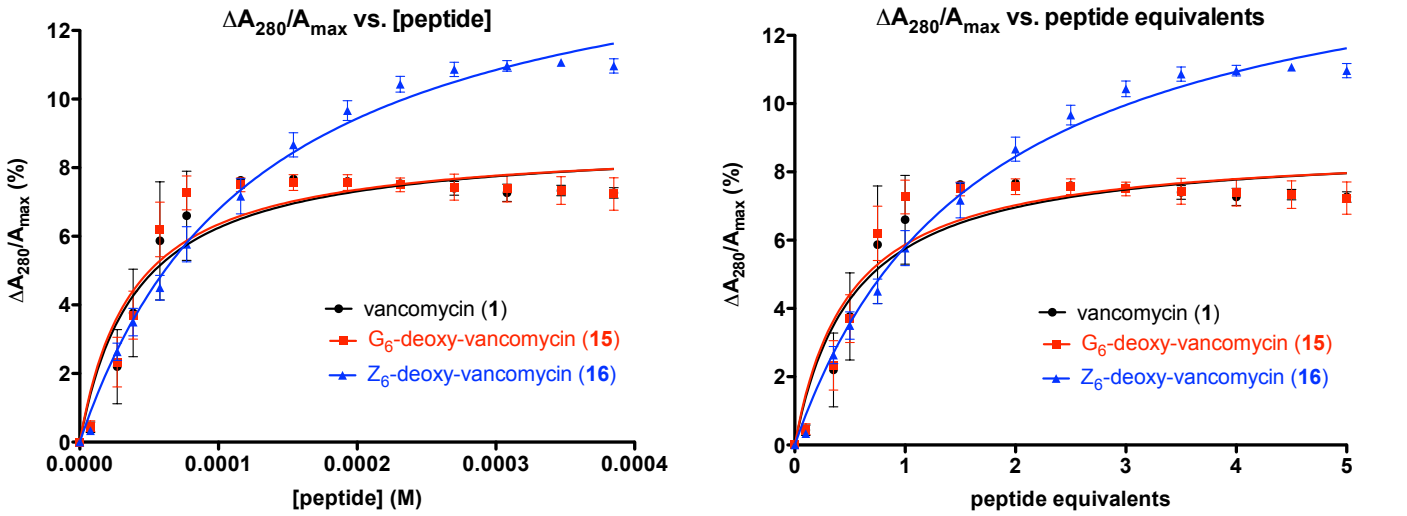
The following procedure was executed in triplicate for each compound (**1**, **15**, and **16**). The spectrophotometer was zeroed to a solution of citrate buffer (1.0 mL) over wavelengths 350 nm to 200 nm. A separate cuvette containing the antibiotic solution (1.0 mL) was scanned to determine the wavelength of maximum absorbance (λ_{max}, 280 nm) before addition of peptide. Solutions of peptide were incrementally added to the cuvette and allowed to stand for 3 min in the spectrophotometer before each scan. Examples of titration spectra for each

antibiotic are shown below. The change in absorbance (ΔA, $A_{no\ peptide} - A_{peptide}$) at 280 nm was calculated for each [peptide], and these values were plotted as a percentage of A_{max} vs. [peptide] and vs. peptide equivalents (see below).

Titration UV-Spectra for **1**, **15**, and **16**



ΔA_{280} as a Percentage of A_{max} Plotted vs. Peptide Concentration and Peptide Equivalents



XIII. References

[1] Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standards—Eighth Edition. CLSI document M07-A8. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2009.
[2] (a) Griffith, B. R.; Krepel, C.; Blanchard, S.; Ahmed, A.; Edmiston, C. E.; Thorson, J. S. *J. Am. Chem. Soc.* **2007**, *129*, 8150-8155. (b) Thompson, C.; Ge, M.; Kahne, D. *J. Am. Chem. Soc.* **1999**, *121*, 1237-1244.
[3] Barton, D. H. R.; McCombie, S. W. *J. Chem. Soc. Perkin Trans. 1* **1975**, 1574-1585.
[4] (a) Dessolin, M.; Guillerez, M.-G.; Thieriet, N.; Guibé, F.; Loffet, A. *Tetrahedron Lett.* **1995**, *36*, 5741-5744. (b) Guibé, F.; Dangles, O.; Balavoine, G. *Tetrahedron Lett.* **1986**, *27*, 2365-2368. Also see ref. 2b.
[5] Proton and carbon values for vancomycin: Pearce, C. M.; Williams, D. H. *J. Chem. Soc. Perkin Trans. 2* **1995**, 153-157.
[6] Nozaki, K.; Oshima, K.; Utimoto, K. *Tetrahedron Lett.* **1988**, *29*, 6125-6126.
[7] (a) Hoke, J. B.; Gramiccioni, G. A.; Balko, E. N. *Appl. Catal. B* **1992**, 285-296. (b) Harris, C. M.; Kannan, R.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1985**, *107*, 6652-6658.
[8] (a) Sculimbrene, B. R.; Morgan, A. J.; Miller, S. J. *J. Am. Chem. Soc.* **2002**, *124*, 11653-11656. (b) Sculimbrene, B. R.; Miller, S. J. *J. Am. Chem. Soc.* **2001**, *123*, 10125-10126.
[9] Xie, J.; Pierce, J. G.; James, R. C.; Okano, A.; Boger, D. L. *J. Am. Chem. Soc.* **2011**, *133*, 13946-13949.