SUPPLEMENTAL INFORMATION

Macrolide-Peptide Conjugates as Probes of the Path of Travel of the Nascent Peptides through the Ribosome

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[‡]This manuscript is dedicated to the memory of Derek B. Benicewicz

Compound Synthesis

General

Clarithromycin **1** was purchased from Greenfield Chemical. Carbamoylimidazolide ketolide **5** was synthesized according to literature protocol.[27, 30] All other reagents were purchased from Sigma–Aldrich and used without further purification. Analtech silica gel plates (60 F_{254}) and Analtech preparative TLC plates (UV 254, 2000 µm) were used for analytical TLC and prep TLC purification respectively. UV light was used to examine the spots. 200-400 Mesh silica was used in column chromatography. NMR spectra were recorded on a Varian-Gemini 400 magnetic resonance spectrometer. ^{1}H NMR spectra are recorded in parts per million (ppm) relative to the peak of $CDCl_3$, (7.24 ppm), CD_3OD (3.31 ppm), or DMSO-d_6 (2.49 ppm). ^{13}C spectra were recorded relative to the central peak of the $CDCl_3$ triplet (77.0 ppm), CD_3OD (49.0 ppm), or the DMSO-d_6 septet (39.7 ppm), and were recorded with complete hetero-decoupling. Multiplicities are described using the abbreviation s, singlet; d, doublet, t, triplet; q, quartet; m, multiplet; and app, apparent. Mass spectra were recorded at the Georgia Institute of Technology mass spectrometry facility in Atlanta.

C3 Azido-Ketolide (7a)

To a solution of **5** (1.55 g, 2.2 mmol), dissolved in ACN/H_2O (5 mL: 0.5 mL), was added **6a** (0.85 g, 8.5 mmol) and the mixture was allowed to stir at 55^{o}C for 12 h. After cooling to room temperature, the mixture was poured into DCM containing 0.5*M* NaH_2PO_4 (300 mL: 200 mL). The aqueous layer was extracted with DCM (1 x 300 mL). The combined organic layers were washed with brine (300 mL) followed by drying over Na_2SO_4. Solvent was evaporated to yield yellow oil which was dissolved in MeOH (25 mL) and stirred at room temperature for 30 h. Solvent was evaporated and the off-white solid was purified by column chromatography (silica, EtOAc/hexanes/triethyl amine 18:1:0.1) to furnish **7a** as a white foam (1.39 g, 90%). ^{1}H NMR ($CDCl_3$, 400 MHz) 4.87 – 4.81 (m, 1H), 4.17 (dd, *J* = 20.5, 8.0 Hz, 2H), 3.79 (q, *J* = 6.7 Hz, 1H), 3.71 – 3.51 (m, 2H), 3.48 (s, 1H), 3.47 – 3.41 (m, 1H), 3.31 – 3.22 (m, 2H), 3.11 – 2.98 (m, 3H), 2.57 (s, 2H), 2.56 (m, 2H), 2.47 – 2.38 (m, 2H), 2.31 (m, 1H), 2.21 (s, 6H), 1.86 – 1.74 (m, 3H), 1.65 – 1.59 (m, 2H), 1.54 – 1.48 (m, 2H), 1.40 (s, 3H), 1.26 – 1.15 (m, 15H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.77 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$, 101 MHz) δ 215.8, 203.3, 170.2, 169.4, 156.8, 103.6, 82.0, 79.2, 70.0, 69.2, 65.5, 60.2, 53.3, 49.4, 49.0, 47.3, 44.6, 41.0, 40.0, 39.9, 39.1, 38.6, 36.6, 28.5, 28.0, 26.5, 22.8, 21.9, 20.9, 19.9, 18.0, 14.1, 13.5, 10.1. MS (MALDI) calc for $[C_{34}H_{57}N_5O_{10} + H]^+$ 696.4224, found 696.4204.

C4 Azido-Ketolide (7b)

The reaction of **5** (1.53 g, 2.17 mmol) with **6b** (0.88 g, 7.7 mmol) as described for the synthesis of **7a**, followed by column chromatography (silica, EtOAc/MeOH 10:1 → 6:1 gradient), gave **7b** as white solid (0.563 g, 58% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.84 (dd, *J* = 10.7, 2.3 Hz, 1H), 4.17 (dd, *J* = 18.3, 8.1 Hz, 2H), 3.77 (q, *J* = 6.8 Hz, 1H), 3.72 – 3.55 (m, 1H), 3.48 (s, 1H), 3.47 – 3.42 (m, 1H), 3.37 (s, 1H), 3.21 (m, 2H), 3.13 – 2.91 (m, 3H), 2.79 – 2.71 (m, 2H), 2.57 (s, 3H), 2.36 (m, 2H), 2.18 (s, 6H), 1.83 – 1.75 (m, 1H), 1.63 – 1.41 (m, 7H), 1.41 – 1.35 (m, 2H), 1.28 (m, 4H), 1.21 (m, 4H), 1.17 – 1.10 (m, 5H), 1.08 (d, *J* = 7.0 Hz, 3H), 0.92 (d, *J* = 6.9

Hz, 3H), 0.76 (t, J = 7.4 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 216.1, 203.7, 169.5, 157.1, 103.9, 82.1, 79.5, 70.2, 69.5, 65.7, 60.3, 51.1, 51.0, 50.9, 50.7, 50.5, 47.6, 44.8, 42.8, 40.2, 39.4, 38.9, 28.1, 26.2, 26.1, 24.2, 22.2, 21.1, 19.7, 18.3, 15.8, 14.6, 14.3, 13.8, 10.3. MS (MALDI) calc for $[C_{35}H_{59}N_5O_{10} + H]^+$ 710.4340, found 710.4373.

Peptolide (12a)

A solution of DMSO (2 mL) and DIPEA (0.05 mL) was deoxygenated by bubbling Argon through for 5 min. To this, protected NLS peptide **8** (150.0 mg, 0.08 mmol) and **7a** (160.9 mg, 0.23 mmol) were added, followed by CuI (13.6 mg, 0.07 mmol). The reaction was stirred at room temperature for 3 h with constant Argon blowing across the surface. The reaction was quenched by addition of NH_4OH/NH_4Cl (1:4, 20 mL) and extracted with 20% isopropyl alcohol (IPA) in DCM (3 x 20 mL). The combined organic layers were washed once more with NH_4OH/NH_4Cl (1:4, 40 mL) and concentrated *in vacuo* to afford a crude white solid. This crude product was purified over preparative TLC (DCM/MeOH/IPA/NH_4OH 9:1:0.1:0.1) affording a white solid (72.2 mg).

The white solid obtained above was dissolved in TFA/TIPS/phenol (90:5:5, 2 mL) and stirred at room temperature for 2 h. The reaction was quenched by addition of diethyl ether (8 mL) leading to a white precipitate. The solution was vortexed for 1 min before pelleting through centrifugation (5,000 rpm, 5 min) followed by removal of the supernatant. The pellet was washed with ether (8 mL) twice more. The final pellet was dissolved in deionized water (4 mL), ether (4 mL) was added, and the mixture was shaken. After removing the ether layer, this washing was repeated twice more. The final aqueous layer was flash frozen in dry ice/acetone and lyophilized overnight to give **12a** as fluffy, white solid (59.5 mg, 39%). ^{1}H NMR (D_2O, 400 MHz) δ 7.88 (s, 1H), 7.50 – 7.40 (m, 1H), 7.31 (s, 1H), 4.64 (t, 1H), 4.55 – 4.38 (m, 4H), 4.28 – 4.22 (m, 8H), 4.11 (s, 3H), 4.05 (s, 2H), 4.01 – 3.83 (m, 5H), 3.80 (s, 1H), 3.73 – 3.50 (m, 11H), 3.17 – 2.79 (m, 20H) 2.57 (m, 1H), 2.38 (s, 2H), 2.32 – 2.23 (m, 1H), 2.13 (d, J = 11.5 Hz, 2H), 2.01 (dd, J = 10.9, 5.5 Hz, 3H), 1.90 (m, 1H), 1.85 – 1.61 (m, 26H), 1.58 (s, 5H), 1.41 (s, 9H), 1.33 (t, 7H), 1.27 (s, 3H), 1.25 – 1.20 (m, 3H), 1.21 – 1.13 (m, 5H), 0.98 (d, J = 6.2 Hz, 3H), 0.91 (m, 7H), 0.84 (m, 3H). MS (MALDI) calc for $[C_{87}H_{153}N_{25}O_{22} + H]^+$ 1901.1700, found 1901.1670.

Peptolide (12b)

The reaction of protected NLS peptide **8** (154.1 mg, 0.08 mmol), **7b** (167.9 mg, 0.24 mmol) and CuI (14.1 mg, 0.0740 mmol) in DMSO (2 mL) and DIPEA (0.05 mL) afforded protected analog of **12b** as white solid (56.0 mg) after purification over preparative TLC (DCM/MeOH/IPA/NH_4OH 6:0.5:0.5:0.1). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 2 mL) and product isolation as described for **12a** gave **12b** as fluffy, white solid (45.9 mg, 30%). ^{1}H NMR (D_2O, 400 MHz) δ 7.82 (s, 1H), 4.64 (dd, J = 8.5, 5.5 Hz, 1H), 4.49 – 4.36 (m, 8H), 4.31 – 4.18 (m, 9H), 4.12 – 4.06 (m, 4H), 4.03 (s, 3H), 3.95 (s, 1H), 3.89 (d, J = 11.8 Hz, 3H), 3.85 (s, 3H), 3.82 – 3.75 (m, 1H), 3.71 (s, 1H), 3.60 – 3.48 (m, 8H), 3.34 – 3.23 (m, 4H), 3.16 (t, J = 7.1 Hz, 3H), 3.11 (d, J = 8.9 Hz, 2H), 3.02 – 2.93 (m, 7H), 2.82 (s, 6H), 2.61 – 2.53 (m, 1H), 2.37 (s, 2H), 2.25 (s, 2H), 2.12 (d, J = 10.8 Hz, 1H), 2.06 – 1.94 (m, 4H), 1.87 – 1.58 (m, 25H), 1.56 (s, 4H), 1.42 (m, 10H), 1.32 (m, 6H), 1.28 (m, 3H), 1.20 (dd, J = 15.1, 7.5 Hz, 5H), 1.14 (d, J = 8.4 Hz, 1H), 0.97 (d, J = 6.7 Hz, 3H), 0.90 (t, J = 7.2 Hz, 3H), 0.81 (t, J = 7.3 Hz, 3H). MS (MALDI) calc for $[C_{88}H_{155}N_{25}O_{22} + H]^+$ 1915.1857, found 1915.1798.

Peptolide (12c)
The reaction of protected reversed-NLS peptide **9** (18.5 mg, 9.66 µmol), **7a** (21.0 mg, 30.2 µmol) and CuI (16.1 mg, 0.08 mmol) in DMSO (1 mL) and DIPEA (0.05 mL) afforded protected analog of **12c** as white solid (15.9 mg) after purification over preparative TLC (DCM/MeOH/NH_4OH 6:1:0.1). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 0.5 mL) and product isolation as described for **12a** gave **12c** as fluffy, white solid (7.6 mg, 40%). ^{1}H NMR (D_2O, 400 MHz) δ 7.82 (s, 1H), 7.41 (d, *J* = 7.7 Hz, 1H), 7.28 (d, *J* = 7.6 Hz, 1H), 4.46 (m, 3H), 4.42 – 4.35 (m, 1H), 4.34 – 4.21 (m, 6H), 4.21 – 4.15 (m, 1H), 4.15 – 4.04 (m, 3H), 4.02 (s, 1H), 3.97 – 3.90 (m, 8H), 3.84 – 3.71 (m, 1H), 3.59 (t, *J* = 6.5 Hz, 2H), 3.53 (dt, *J* = 8.9, 6.1 Hz, 2H), 3.49 – 3.41 (m, 3H), 3.31 (d, *J* = 2.0 Hz, 1H), 3.30 – 3.20 (m, 2H), 3.16 (m, 2H), 2.96 (dd, *J* = 11.4, 6.6 Hz, 6H), 2.88 (s, 3H), 2.76 (s, 3H), 2.72 (t, *J* = 7.2 Hz, 3H), 2.52 (m, 1H), 2.34 (t, *J* = 7.3 Hz, 2H), 2.29 (s, 3H), 2.25 – 2.07 (m, 3H), 2.07 – 2.01 (m, 1H), 2.01 – 1.92 (m, 4H), 1.87 – 1.68 (m, 14H), 1.68 – 1.58 (m, 14H), 1.56 (s, 6H), 1.39 (m, 10H), 1.31 – 1.11 (m, 18H), 0.96 (d, *J* = 7.0 Hz, 3H), 0.94 – 0.88 (m, 7H), 0.82 (t, *J* = 7.0 Hz, 3H). MS (MALDI) calc for $[C_{90}H_{157}N_{25}O_{23} + H]^+$ 1957.1962, found 1957.1907.

Peptolide (12d)
The reaction of protected reversed-NLS peptide **9** (21.8 mg, 0.01 mmol), **7b** (24.2 mg, 0.03 mmol), and CuI (13.7 mg, 0.07 mmol) in DMSO (0.5 mL), THF (0.5 mL) and DIPEA (0.1 mL, 0.57 mmol) at 55^{o}C for 4 h afforded protected analog of **12d** as white solid (15.5 mg) after purification over preparative TLC (EtOAc/MeOH/NH_4OH 2.5:1:0.1). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 0.5 mL) for 2.5 h and product isolation as described for **12a** gave **12d** as fluffy, white solid (9.4 mg, 48%). ^{1}H NMR (D_2O, 400 MHz) δ 7.72 (s, 1H), 4.71 (m, 1H), 4.44 – 4.39 (m, 5H), 4.35 – 4.15 (m, 7H), 4.20 – 4.08 (m, 4H), 4.01 (s, 1H), 3.96 (m, 3H), 3.93 (s, 1H), 3.90 (d, *J* = 7.9 Hz, 4H), 3.78 (s, 1H), 3.72 – 3.63 (m, 1H), 3.59 – 3.47 (m, 7H), 3.25 (dd, *J* = 14.8, 7.3 Hz, 1H), 3.16 (t, *J* = 6.6 Hz, 2H), 2.96 (m, 8H), 2.87 (s, 3H), 2.76 (s, 4H), 2.60 – 2.49 (m, 1H), 2.35 (s, 4H), 2.30 – 2.19 (m, 1H), 2.11 (d, *J* = 11.8 Hz, 1H), 2.06 – 1.92 (m, 4H), 1.92 – 1.83 (m, 3H), 1.72 1.57 (m, 24H), 1.55 (s, 5H), 1.40 (s, 11H), 1.32 – 1.23 (m, 9H), 1.20 (d, *J* = 7.2 Hz, 3H), 1.15 – 1.09 (m, 6H), 0.95 - 0.91 (m, 6H), 0.78 (t, *J* = 7.1 Hz, 3H). MS (MALDI) calc for $[C_{91}H_{159}N_{25}O_{23} + H]^+$ 1971.2119, found 1971.2101.

Peptolide (12e)
The reaction of protected plicatamide peptide **10** (23.7 mg, 0.01 mmol), **7a** (23.2 mg, 0.03 mmol) and CuI (13.7 mg, 0.07 mmol) in DMSO (1 mL) and DIPEA (0.05 mL, 0.29 mmol) afforded protected analog of **12e** as white solid (18.4 mg) after purification over preparative TLC (DCM/MeOH/NH_4OH 8:1:0.1). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 0.5 mL) and product isolation as described for **12a** gave **12e** as fluffy, white solid (9.7 mg, 44%). ^{1}H NMR (D_2O, 400 MHz) δ 8.60 (s, 2H), 8.55 (s, 1H), 7.83 (d, *J* = 12.7 Hz, 1H), 7.57 – 7.17 (m, 18H), 7.17 – 7.04 (m, 3H), 6.83 (d, *J* = 7.2 Hz, 2H), 4.69 – 4.32 (m, 18H), 4.21 (m, 6H), 3.93 (m, 1H), 3.81 (m, 1H), 3.58 (m, 1H), 3.51 (s, 2H), 3.46 (s, 1H), 3.27 (d, *J* = 6.9 Hz, 3H), 3.23 – 2.96 (m, 13H), 2.91 (s, 6H), 2.81 (s, 3H), 2.57 (m, 2H), 2.39 (s, 3H), 2.13 (m, 4H), 1.93 (s, 1H), 1.77 (m, 3H), 1.62 – 1.51 (m, 3H), 1.35 – 1.22 (m, 10H), 1.19 (dd, *J* = 7.0, 4.4 Hz, 3H), 1.14 – 1.00 (m, 1H), 1.00 – 0.90 (m, 4H), 0.90 – 0.73 (m, 5H). HRMS (ESI) calc for $[C_{101}H_{136}N_{22}O_{21} + 3H]^{3+}$ 665.3490, found 665.3485.

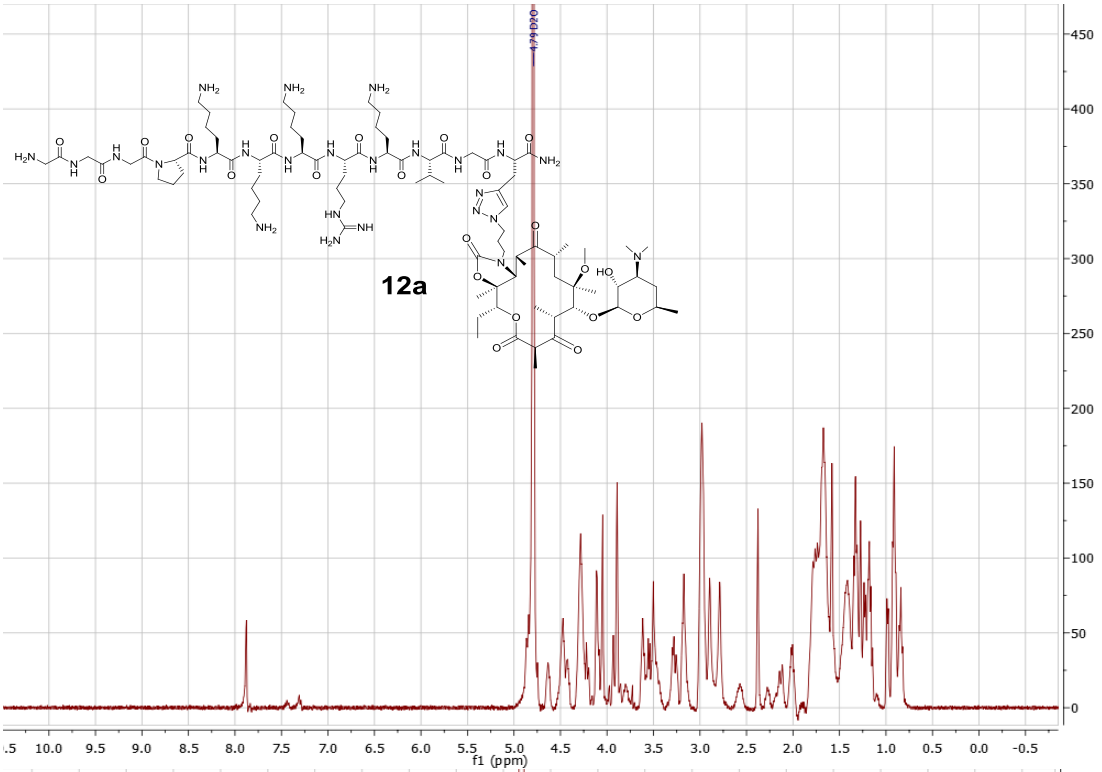
Peptolide (12f)
The reaction of protected plicatamide peptide **10** (15.8 mg, 0.0072 mmol), **7b** (24.5 mg, 0.03 mmol) and CuI (14.1 mg, 0.07 mmol) in DMSO (0.5 mL), THF (0.5 mL) and DIPEA (0.1 mL, 0.57 mmol) afforded protected analog of **12f** as white solid (12.5 mg) after purification over preparative TLC (DCM/MeOH/NH_4OH 10:1:0.1). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 0.5 mL) and product isolation as described for **12a** gave **12f** as fluffy, white solid (6.1 mg, 42%). ^{1}H NMR (D_2O, 400 MHz) δ 8.56 (d, *J* = 5.7 Hz, 2H), 8.51 (s, 1H), 7.77 (s, 1H), 7.41 – 6.98 (m, 25H), 6.78 (d, *J* = 7.3 Hz, 2H), 4.66 – 4.48 (m, 8H), 4.48 – 4.39 (m, 3H), 4.39 – 4.25 (m, 3H), 4.25 – 4.15 (m, 3H), 4.12 (dd, *J* = 8.9, 5.3 Hz, 1H), 3.88 (d, *J* = 17.8 Hz, 1H), 3.76 (d, *J* = 16.1 Hz, 2H), 3.53 (m, 3H), 3.46 (s, 3H), 3.26 (m, 3H), 3.19 – 3.10 (m, 4H), 3.08 (m, 3H), 3.03 – 2.91 (m, 6H), 2.82 (m, 10H), 2.61 – 2.49 (m, 1H), 2.36 (s, 2H), 2.10 (d, *J* = 10.4 Hz, 1H), 1.75 (m, 3H), 1.54 (s, 6H), 1.45 – 1.33 (m, 5H), 1.30 (m, 5H), 1.26 (d, *J* = 7.6 Hz, 3H), 1.21 (d, *J* = 7.1 Hz, 3H), 1.15 (m, 8H), 0.93 (d, *J* = 6.9 Hz, 2H), 0.89 (d, *J* = 5.4 Hz, 3H), 0.83 (d, *J* = 5.7 Hz, 3H), 0.77 (t, *J* = 7.1 Hz, 3H). MS (MALDI) calc for $[C_{102}H_{138}N_{22}O_{21} + H]^+$ 2008.0485, found 2008.0705.

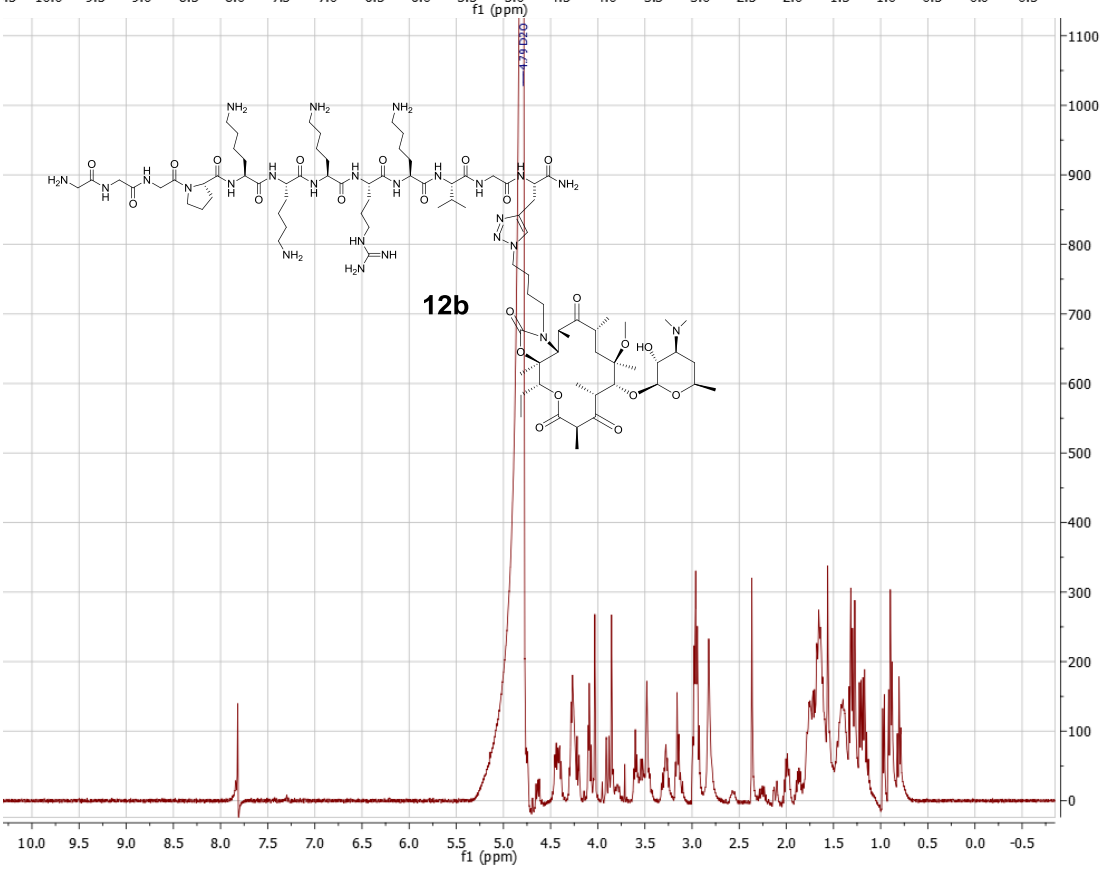
Peptolide (12g)
A reaction mixture containing protected RGD peptide **11** (151.2 mg, 0.0529 mmol), **7a** (74.4 mg, 0.11 mmol) and CuI (101.3 mg, 0.53 mmol) in DMSO (0.8 mL), THF (0.8 mL) and DIPEA (0.36 mL) was stirred at rt for 18 h. To the reaction was added 20% IPA in DCM (50 mL) and the mixture washed with NH_4OH/NH_4Cl (1:4, 3 x 30 mL) followed by brine (30 mL). The organic layer was dried over $MgSO_4$, and concentrated *in vacuo* to afford a crude white solid which was purified over preparative TLC (DCM/MeOH/NH_4OH 8:1:0.1) to afford protected analog of **12g** as white solid (86.4 mg). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 2 mL) and product isolation as described for **12a** gave **12g** as fluffy, white solid (60.6 mg, 50%). ^{1}H NMR (D_2O, 400 MHz) δ 7.89 – 7.79 (m, 2H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.29 (dd, *J* = 8.8, 7.5 Hz, 1H), 4.97 (t, *J* = 7.0 Hz, 1H), 4.71 (m, 2H), 4.61 (dd, *J* = 8.6, 5.6 Hz, 2H), 4.45 (m, 4H), 4.39 (m, 2H), 4.28 (m, 4H), 4.25 – 4.23 (m, 1H), 4.20 (t, *J* = 7.3 Hz, 2H), 4.08 (t, *J* = 6.5 Hz, 2H), 4.02 (s, 3H), 3.92 (m, 1H), 3.85 (s, 1H), 3.77 (m, 4H), 3.54 (m, 4H), 3.50 – 3.40 (m, 5H), 3.27 (m, 4H), 3.24 – 3.12 (m, 10H), 2.97 – 2.79 (m, 10H), 2.77 (s, 3H), 2.37 (s, 2H), 2.26 (m, 1H), 2.06 – 1.97 (m, 3H), 1.97 – 1.83 (m, 9H), 1.83 – 1.74 (m, 6H), 1.73 – 1.60 (m, 12H), 1.56 (s, 7H), 1.31 (m, 7H), 1.19 (m, 7H), 1.15 (m, 6H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.83 (t, *J* = 7.4 Hz, 3H). MS (MALDI) calc for $[C_{94}H_{155}N_{33}O_{33} + H]^+$ 2275.1543, found 2275.1490.

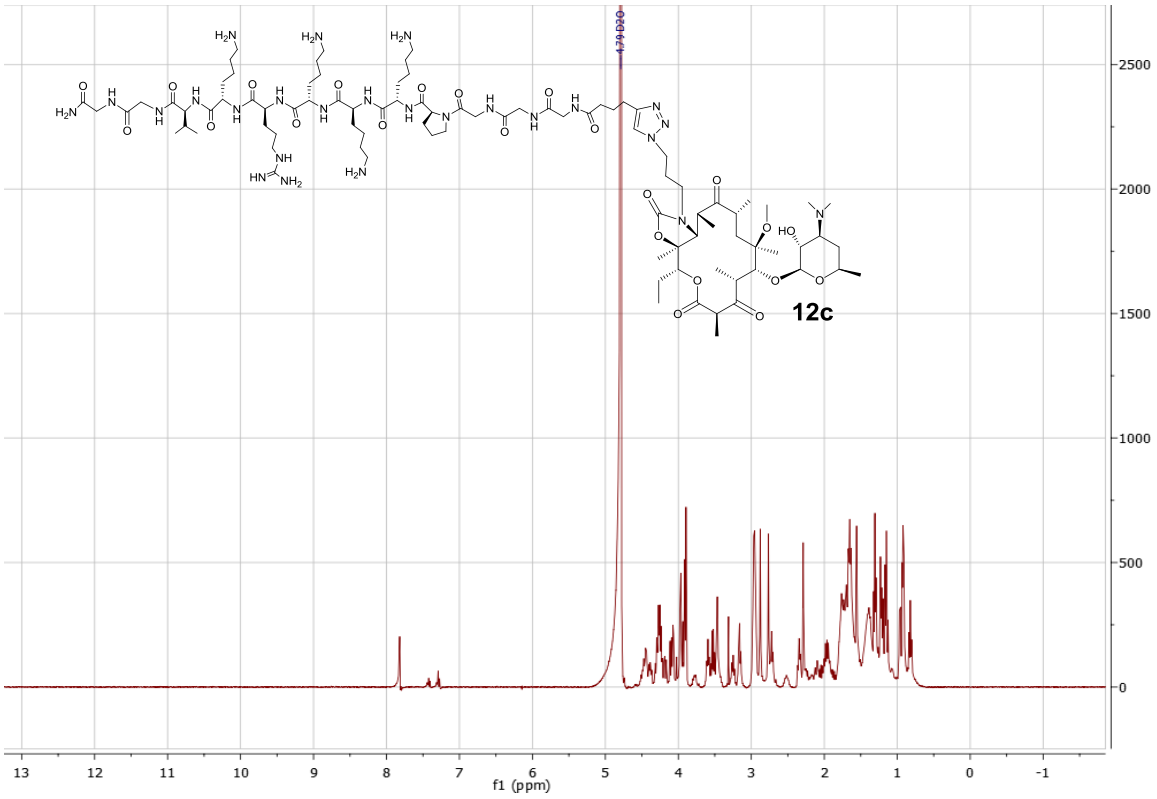
Peptolide (12h)
A reaction mixture containing protected RGD peptide **11** (99.7 mg, 0.03 mmol), **7b** (55.2 mg, 0.08 mmol) and CuI (67.3 mg, 0.35 mmol) in DMSO (0.75 mL), THF (0.75 mL) and DIPEA (0.24 mL) was stirred at rt for 18 h. To the reaction was added EtOAc (30 mL) and the mixture washed with NH_4OH/NH_4Cl (1:4, 3 x 30 mL) followed by brine (30 mL). The organic layer was dried over $MgSO_4$, and concentrated *in vacuo* to afford a crude white solid which was purified over preparative TLC (DCM/MeOH/NH_4OH 10:1:0.1) to afford protected analog of **12h** as white solid (35.5 mg). Subsequent functional group deprotection in TFA/TIPS/Phenol (90:5:5, 2 mL) and product isolation as described for **12a** gave **12h** as fluffy, white solid (18.7 mg, 27%)). ^{1}H NMR (D_2O, 400 MHz) δ 7.82 (s, 1H), 7.80 (s, 1H), 7.42 (t, *J* = 7.9 Hz, 1H), 7.29 (dd, *J* = 8.7,

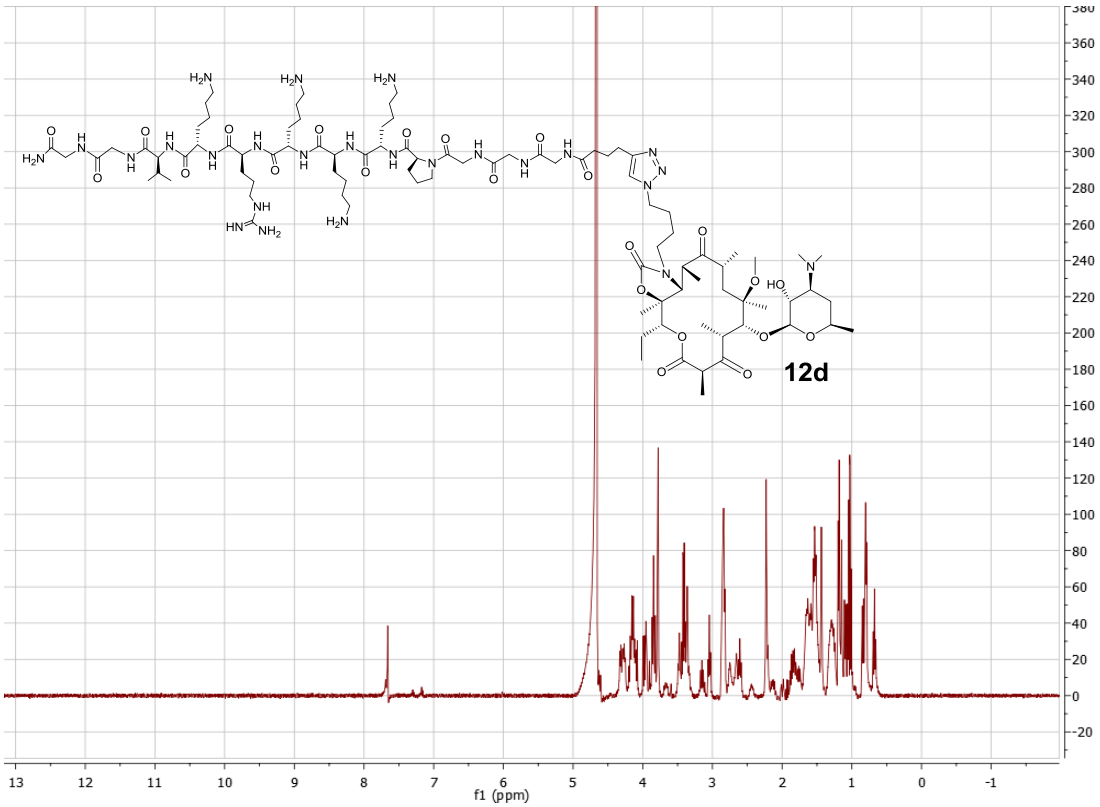
7.5 Hz, 1H), 4.96 (t, *J* = 7.1 Hz, 1H), 4.73 – 4.67 (m, 4H), 4.62 (dd, *J* = 9.0, 5.3 Hz, 1H), 4.48 – 4.33 (m, 5H), 4.33 – 4.26 (m, 3H), 4.26 – 4.17 (m, 3H), 4.07 (t, *J* = 6.6 Hz, 1H), 4.01 (s, 3H), 3.92 (s, 1H), 3.88 – 3.72 (m, 8H), 3.53 (, 3H), 3.50 – 3.42 (m, 4H), 3.36 – 3.25 (m, 4H), 3.25 – 3.09 (m, 10H), 2.95 – 2.89 (m, 1H), 2.88 (s, 5H), 2.87 – 2.82 (m, 5H), 2.81 (m, 1H), 2.77 (s, 3H), 2.68 (dd, *J* = 16.9, 7.5 Hz, 2H), 2.57 (m, 1H), 2.38 (s, 3H), 2.31 – 2.20 (m, 1H), 2.12 (d, *J* = 12.9 Hz, 1H), 2.00 (d, *J* = 6.0 Hz, 3H), 1.97 – 1.83 (m, 10H), 1.83 – 1.73 (m, 7H), 1.65 (m, 13H), 1.56 (s, 4H), 1.35 – 1.29 (m, 6H), 1.27 (s, 3H), 1.20 (m, 7H), 1.15 (m, 4H), 0.97 (d, *J* = 6.9 Hz, 4H), 0.80 (t, *J* = 7.4 Hz, 3H). MS (MALDI) calc for $[C_{95}H_{157}N_{33}O_{33} + H]^+$ 2289.1700, found 2289.1697.

1H-NMR of Peptolides






12c
f1 (ppm)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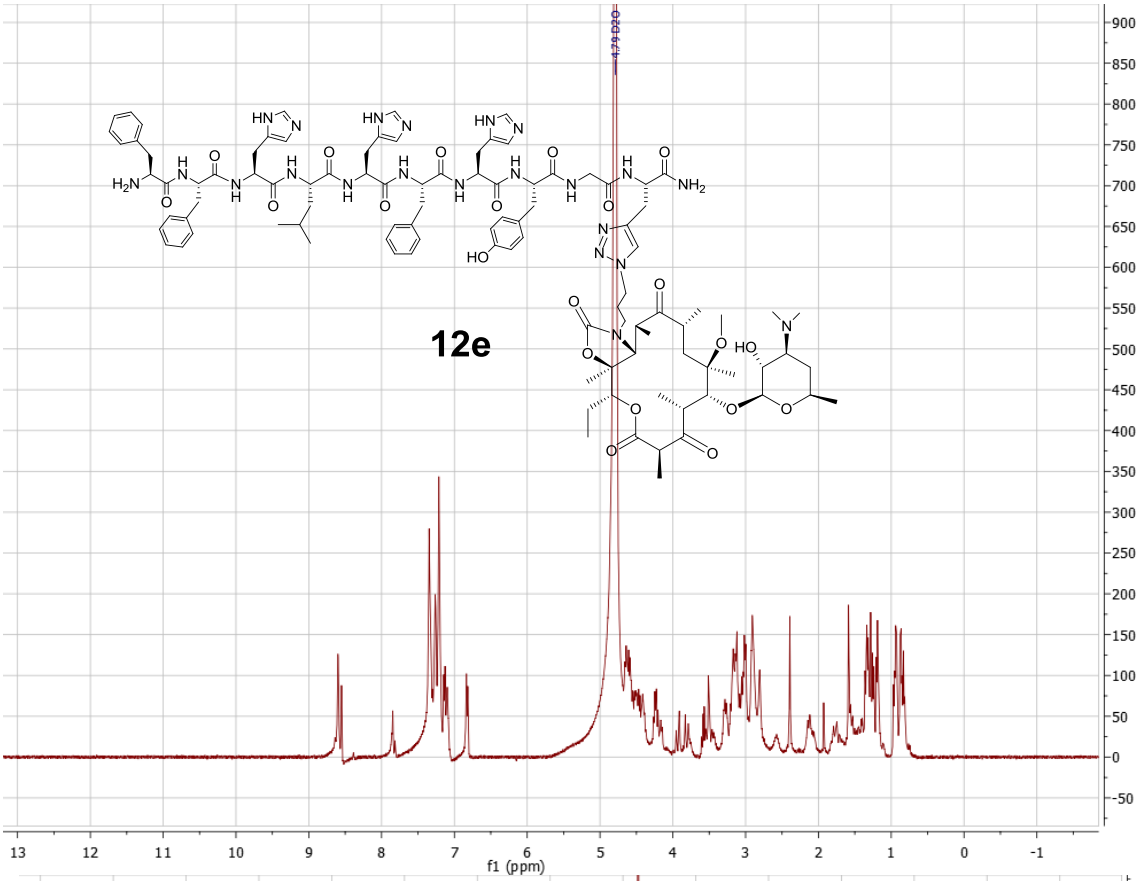
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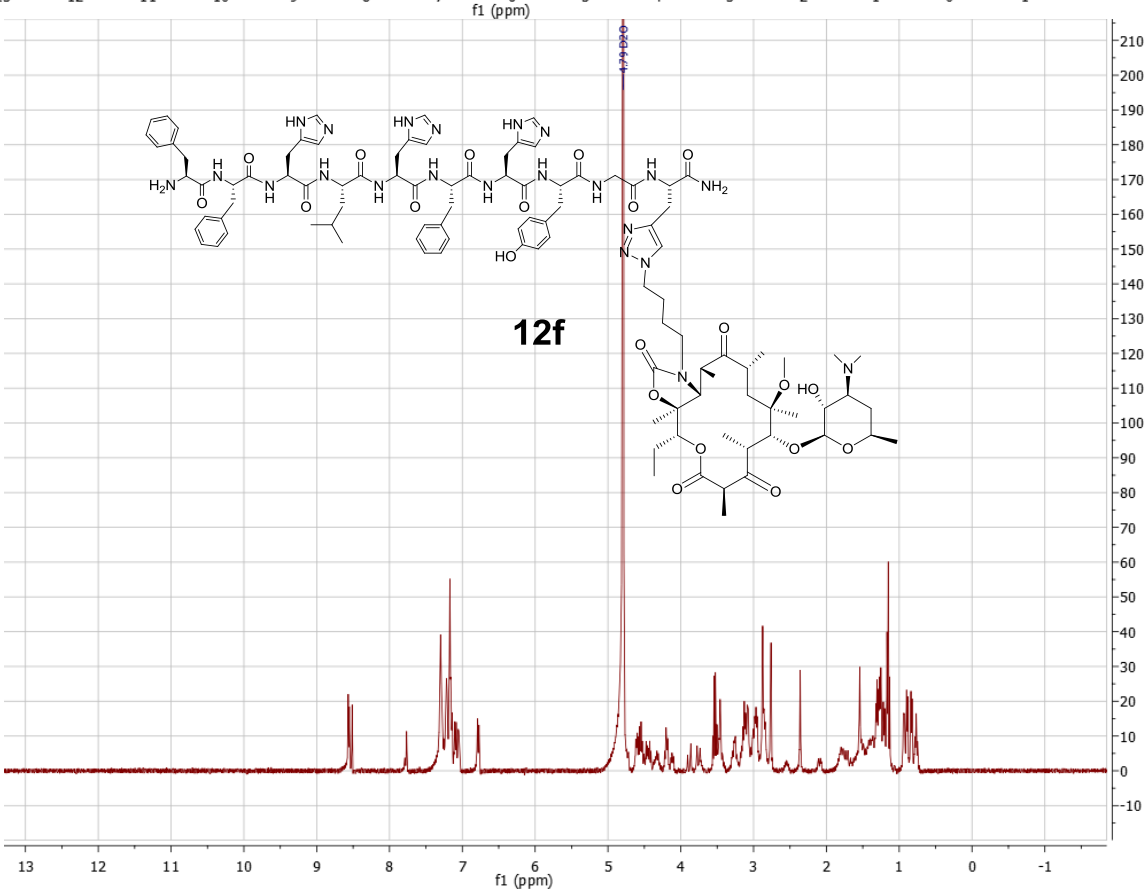
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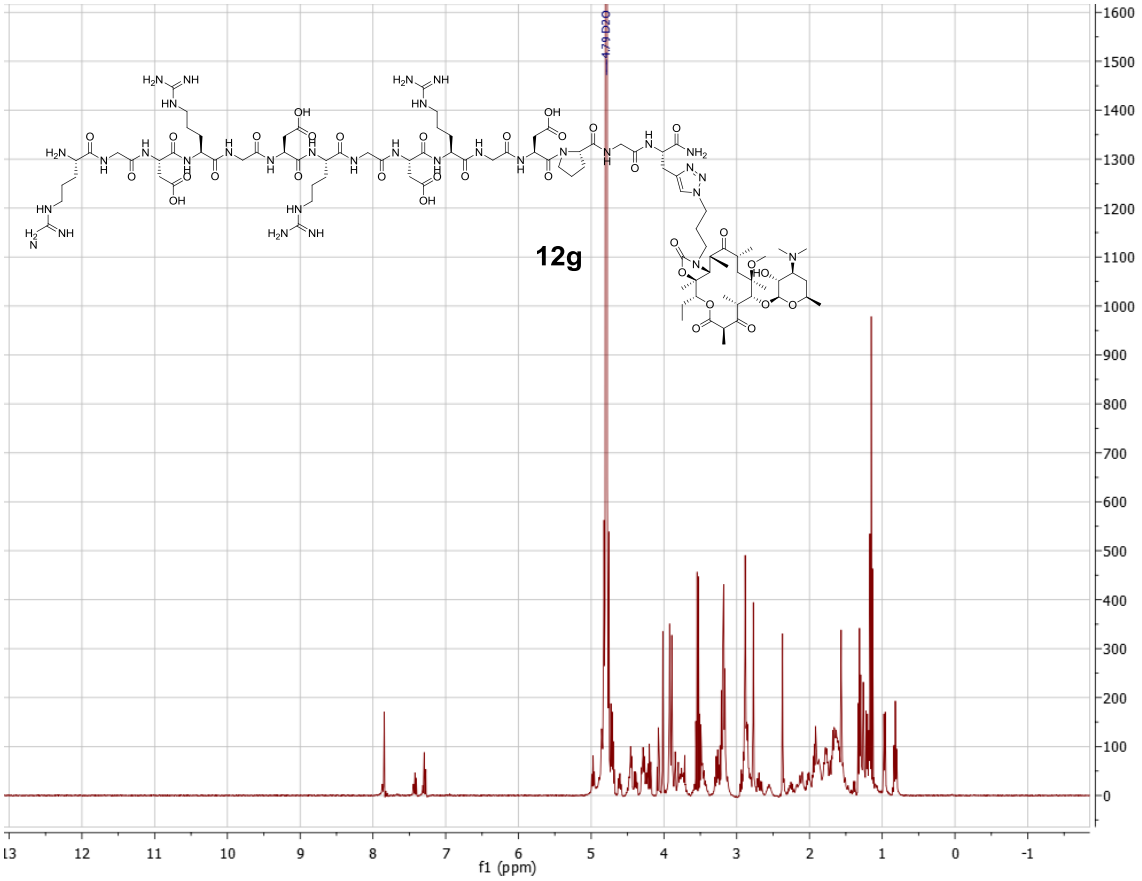
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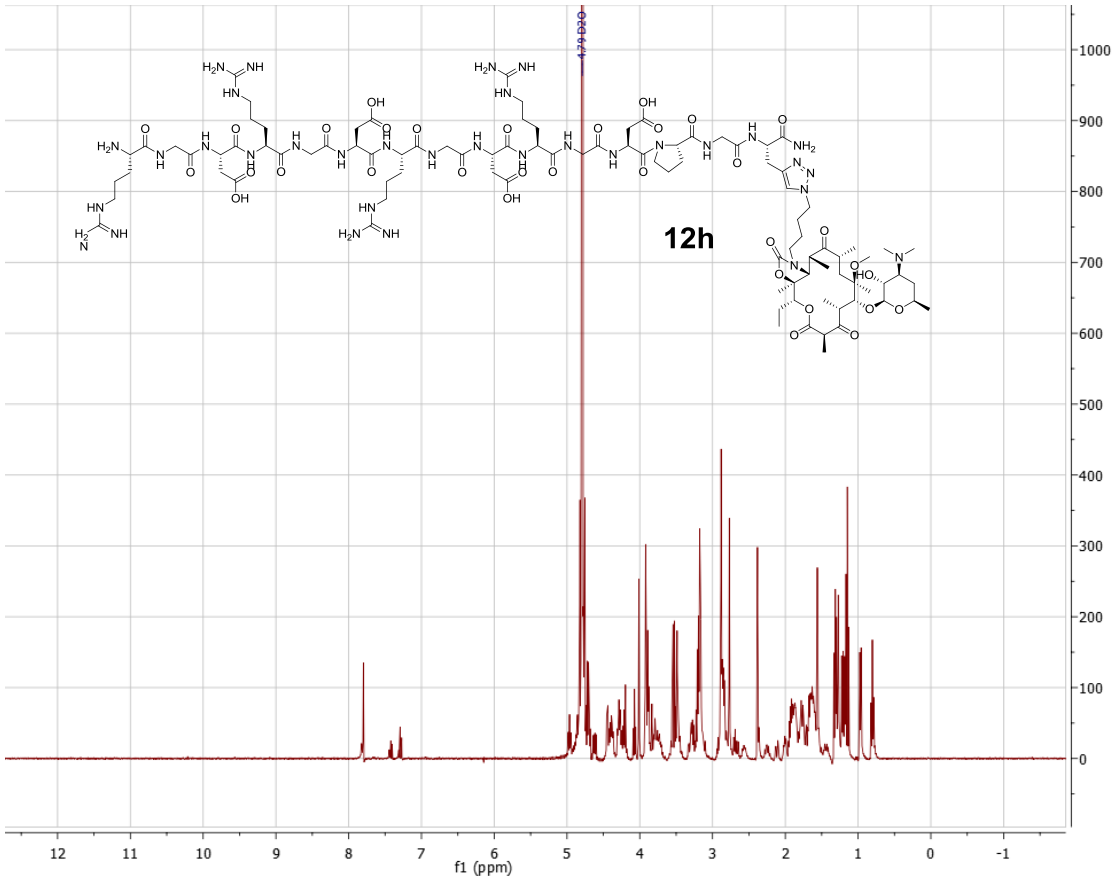
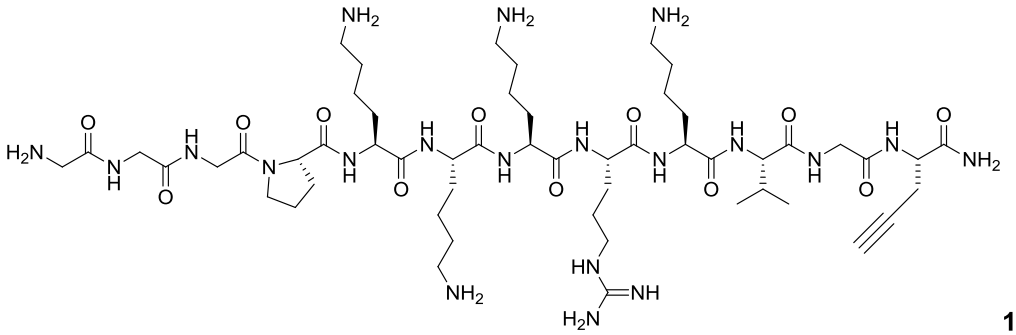

12h
f1 (ppm)



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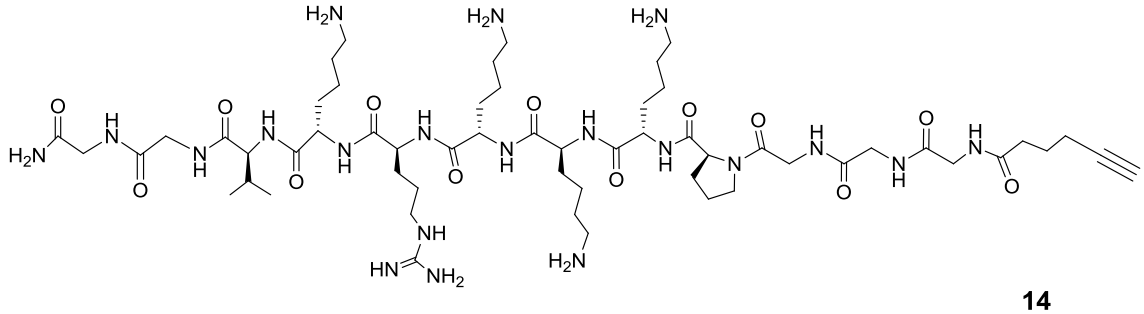

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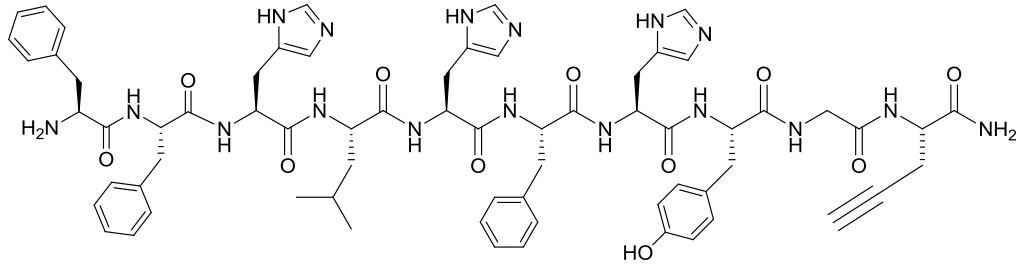

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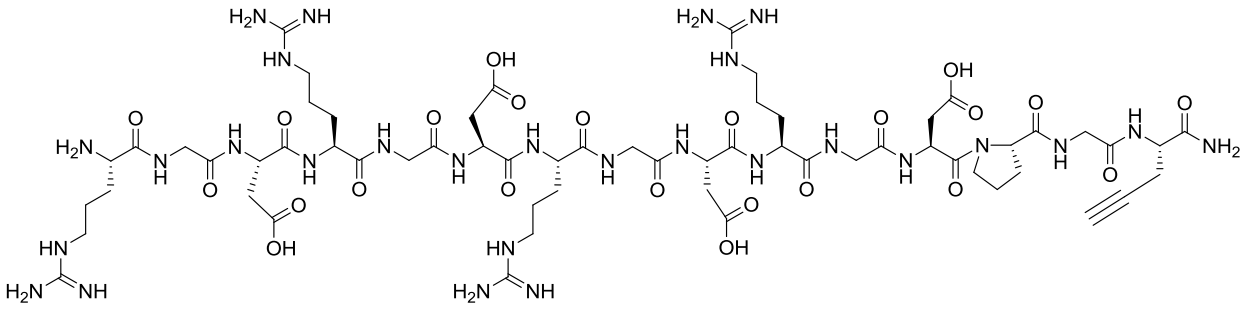

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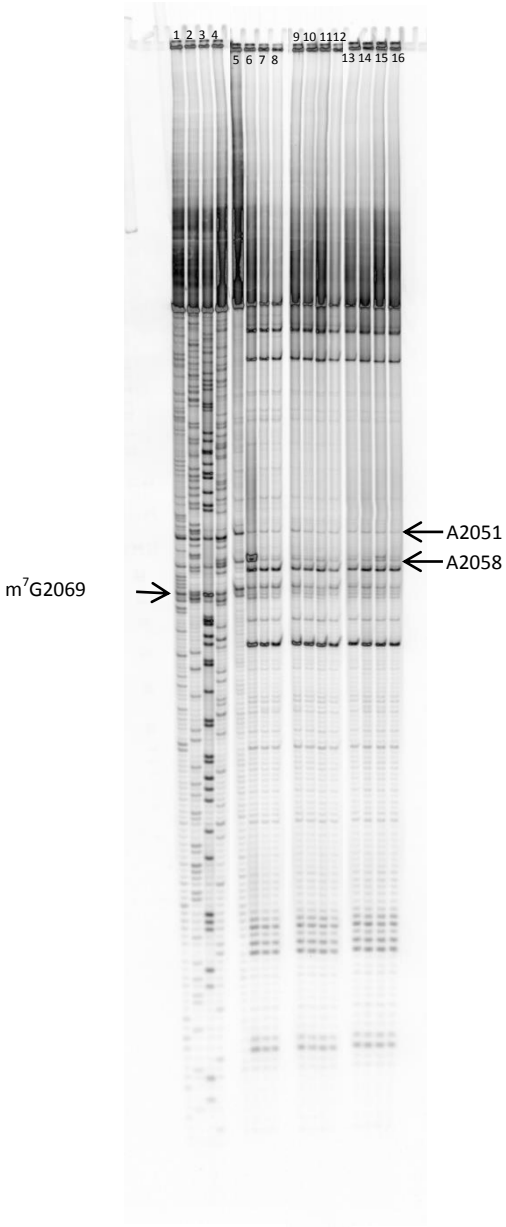
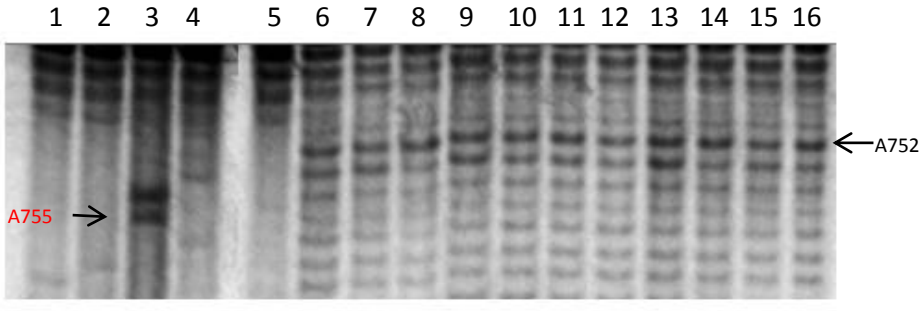
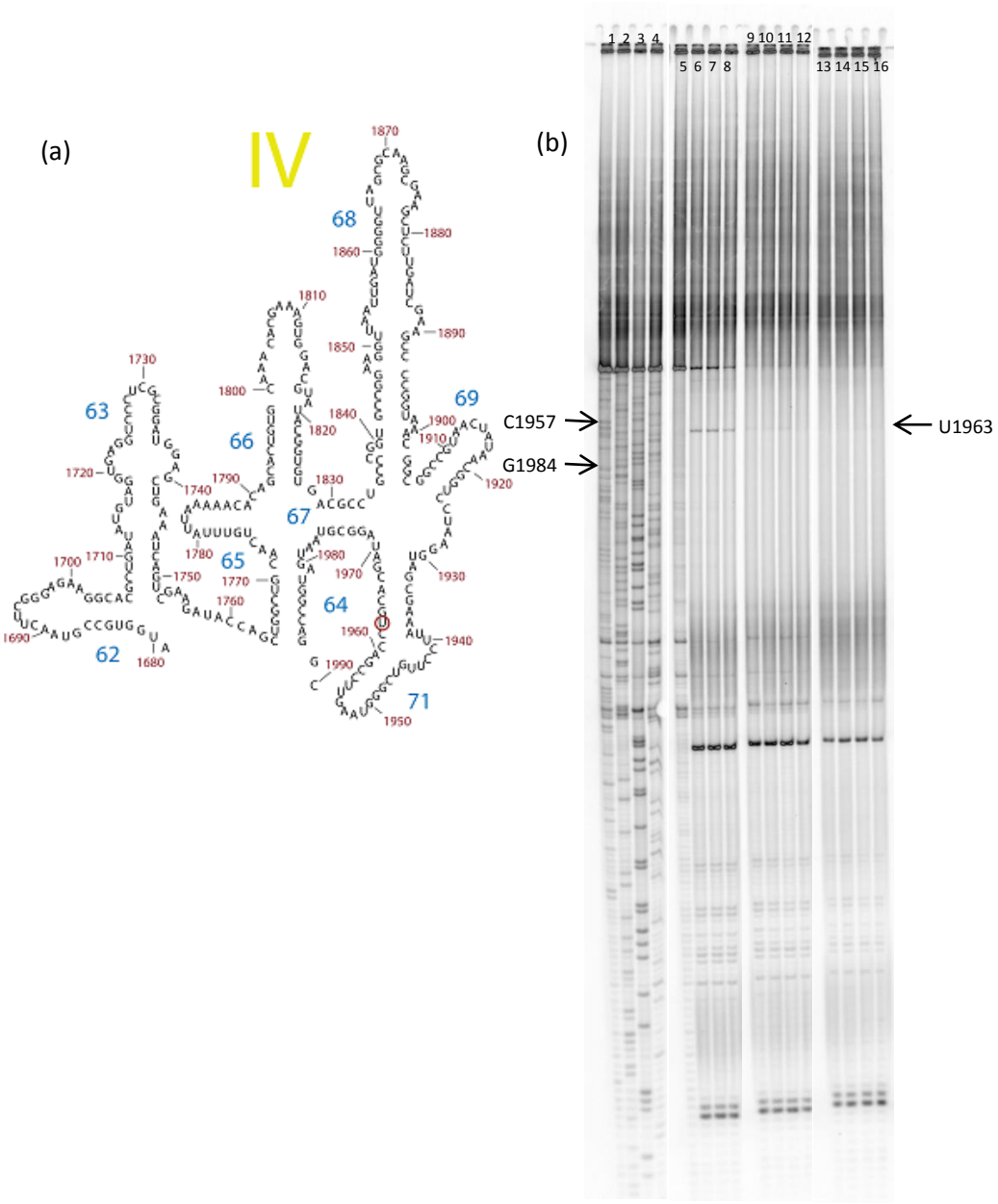
SI Fig. S1. Structures of deprotected peptides 13-16.



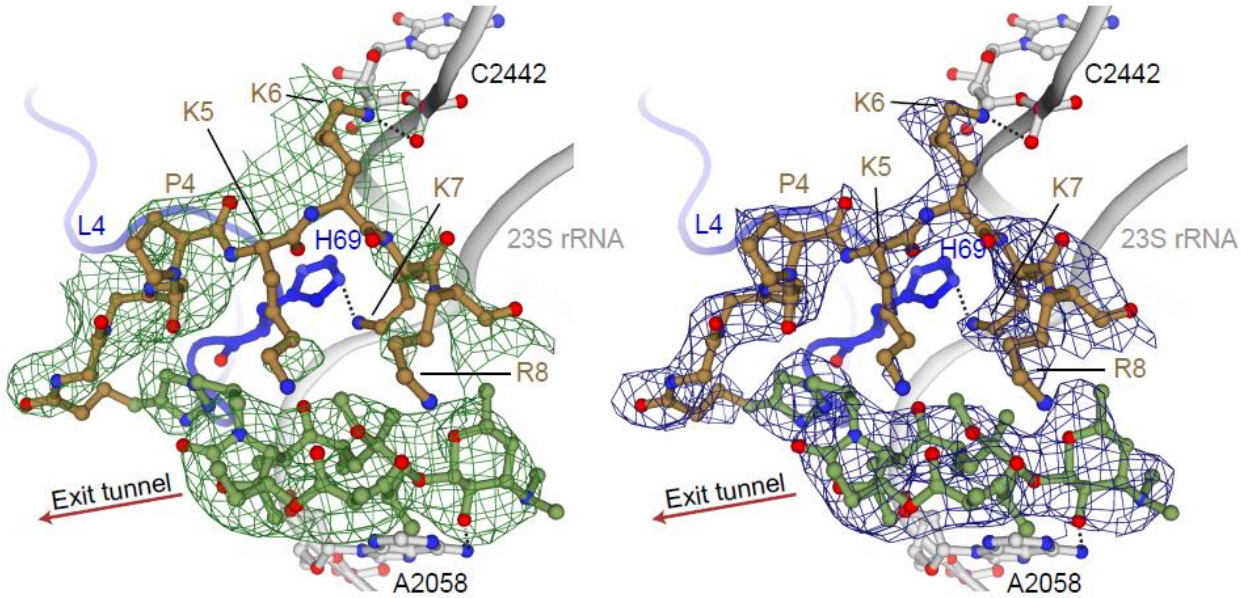
SI Fig. S2. Full gel image of DMS modification containing A2058, the macrolide binding site. DMS footprinting of 23S rRNA. Dideoxy sequencing lanes G, C, A and T (lanes 1-4) followed by unmodified rRNA (lane 5), DMS modified 23S rRNA (lane 6), DMS modified 23S rRNA in presence of clarithromycin and intermediate 7b (lanes 7-8, respectively), 23S rRNA DMS modified in the presence of 12a-h (lanes 9-16, respectively).



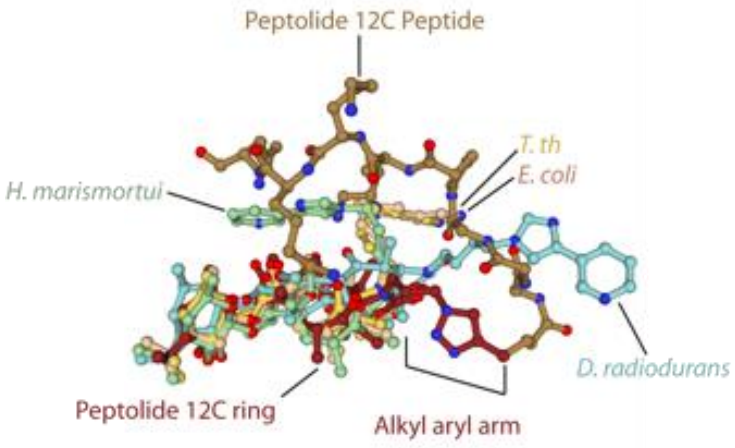
SI Fig. S3. Zoomed gel image of DMS modification showing section containing A752. Dideoxy sequencing lanes G,C,A and T (lanes 1-4) followed by unmodified rRNA (lane 5), DMS modified 23S rRNA (lane 6), DMS modified 23S rRNA in presence of clarithromycin and 7b intermediate (lanes 7-8, respectively), 23S rRNA DMS modified in the presence of 12a-h (lanes 9-16, respectively).



SI Fig. S4. (a) 2D structure of Domain IV containing H71 showing the location of U1963.[30] (b) Full gel image of CMCT modification containing U1963, the reported site of peptolide interaction. Dideoxy sequencing lanes G,C,A and T (lanes 1-4) followed by unmodified rRNA (lane 5), CMCT modified 23S rRNA (lane 6), CMCT modified 23S rRNA in presence of clarithromycin and intermediate 7b (lanes 7 and 8, respectively), 23S rRNA CMCT modified in the presence of 12a-h (lanes 9-16, respectively).



SI Fig S5. Electron density maps for peptolide 12c bound to the 70S ribosome. (Left) Unbiased F_o-F_c electron density map (contoured at 3 σ) for peptolide 12c bound to the macrolide binding pocket shows strong peaks for the macrolactone ring (green) and portions of the peptide tail (gold). All side chains of the peptolides are visible except for Lys5. The peptolide is stabilized through the formation of hydrogen bonds with 23S rRNA residue C2442 (grey) and His69 of ribosomal protein L4 (blue). (Right) The $2F_o$-F_c electron density map after refinement for peptolide 12C (contoured at 1.5 σ).



SI Fig S6. Superposition of peptolide 12c and TEL bound to the macrolide binding pocket of the 50S subunit. The ketolide macrocyclic ring of peptolide 12c (brown) binds in the same orientation as TEL when bound to *T. thermophilus* (yellow, PDB ID 3OI3), *E. coli* (beige, PDB ID 3OAT), *H. marismortui* (green, PDB ID 1YIJ) and *D. radiodurnans* (cyan, PDB ID 1P9X). The flexible alkyl-aryl arm of peptolide 12c adopts a similar conformation as observed when TEL is bound to D. radiodurans, however the peptide tail folds back over the top of the ketolide macrocyclic ring.