SUPPORTING INFORMATION

A peptidomimetic ligand targeting the chromodomain of MPP8 reveals HRP2's association with the HUSH complex

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S50 LC-MS and NMR Characterization

Supplemental Figures and Tables

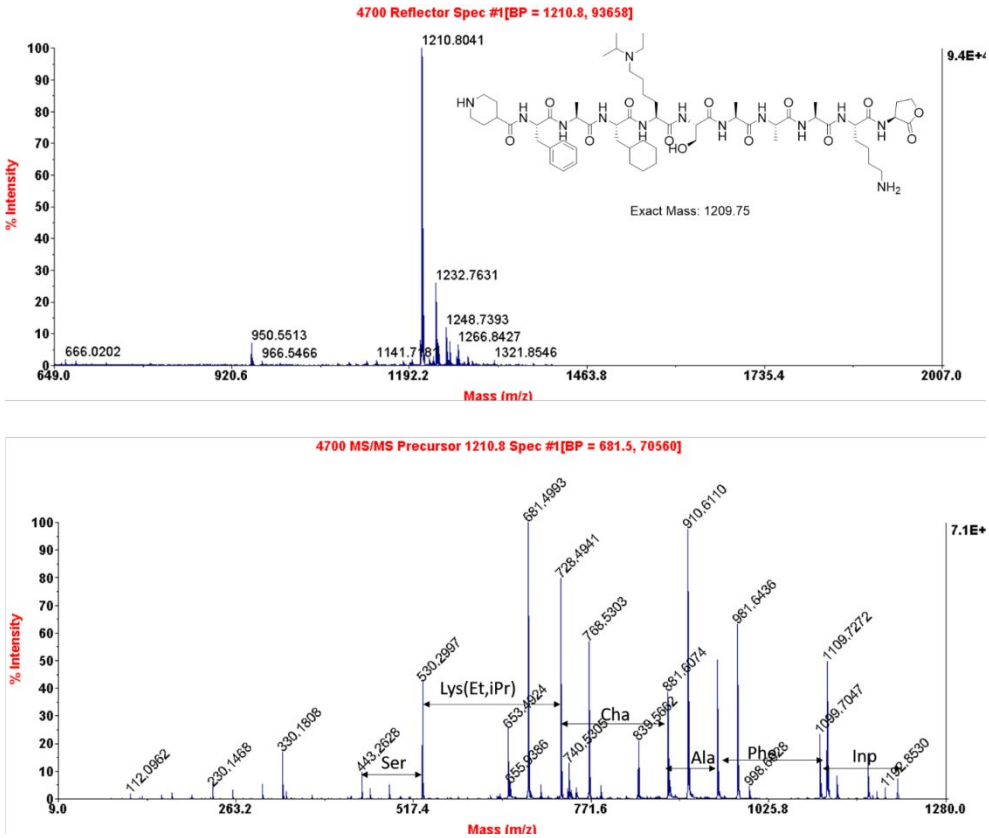


Figure S1. Representative example of deconvolution of a hit peptide from the OBOC library screen via Maldi-TOF/TOF mass spectrometry.

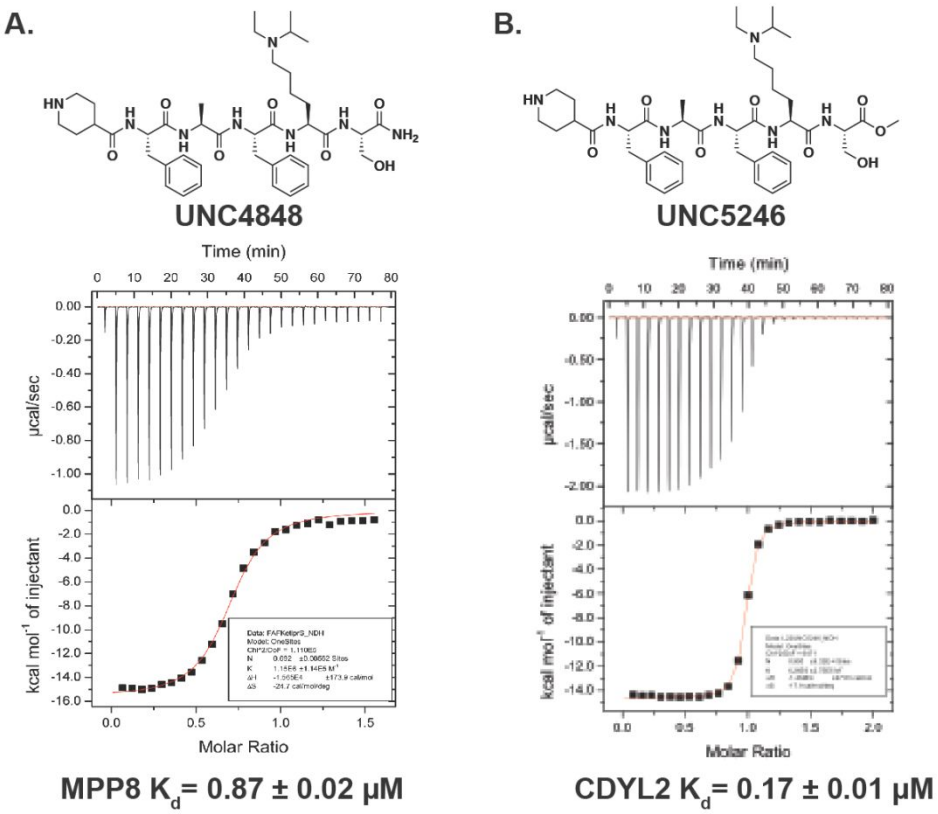


Figure S2. A. UNC4848 binds the MPP8 chromodomain with similar affinity as UNC5246 as determined by ITC. B. UNC5246 binds CDYL2 about 4-fold more potently than MPP8.

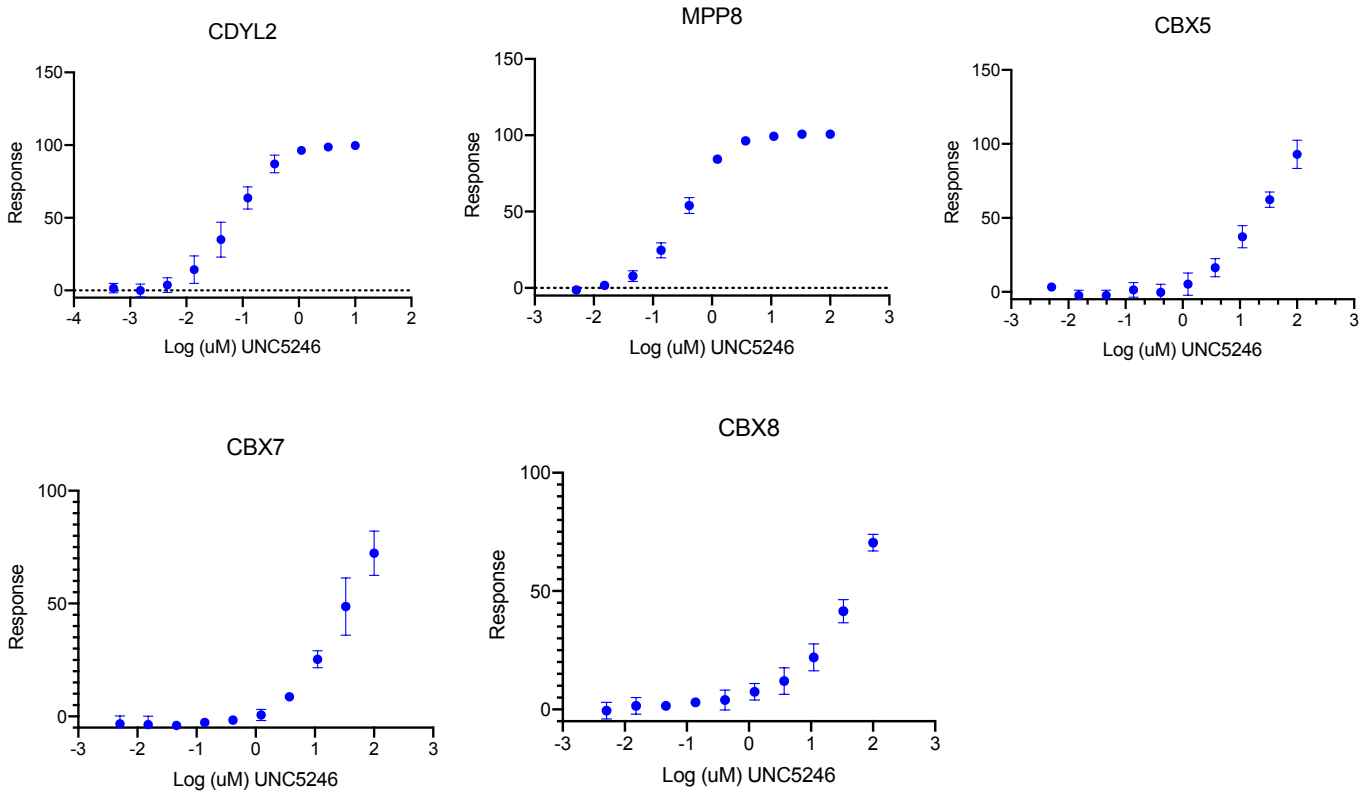


Figure S3. Representative UNC5246 TR-FRET curves with the chromodomains of CDYL2, MPP8, CBX5, CBX7, and CBX8.

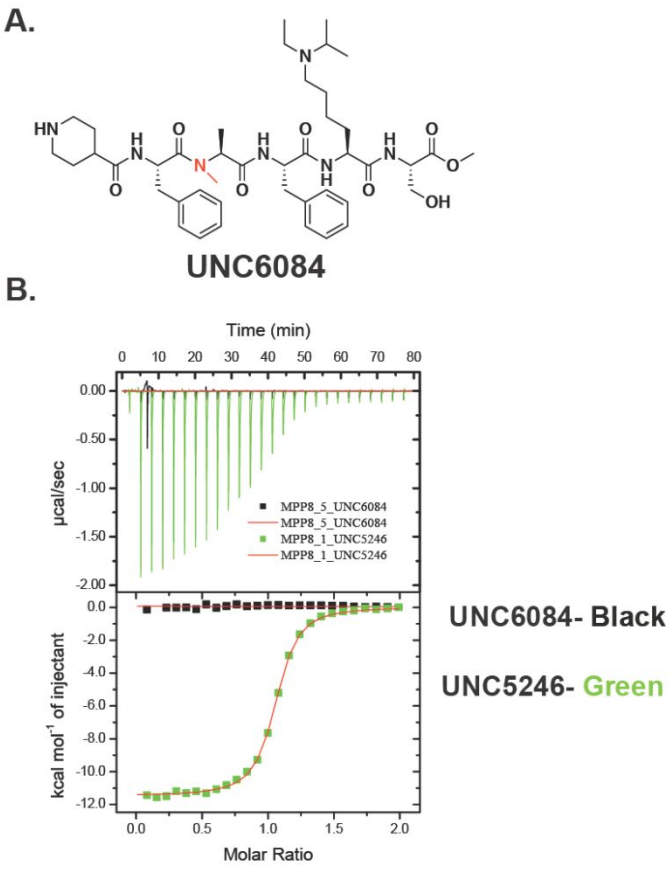


Figure S4. A. Structure of UNC6084, which contains methylation of a single backbone amide (red). B. ITC curves comparing UNC5246 (green) and UNC6084 (black) binding to MPP8. UNC6084 has no measurable affinity for MPP8.

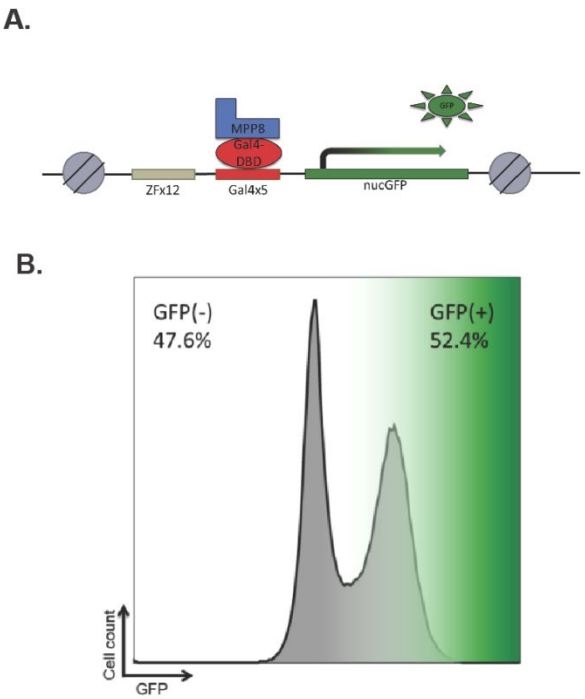


Figure S5. A. Schematic of the gene locus engineered to contain a Gal4x5 sequence upstream of GFP. Recruitment of a Gal4-MPP8 fusion protein leads to repression of GFP expression. B. Flow Assisted Cell Sorting (FACS) showing the distribution of GFP-negative (repressed) or GFP-positive (no repression) cell populations upon recruitment of Gal4-MPP8. The GFP-negative cells were collected and used for chemoproteomic analysis.

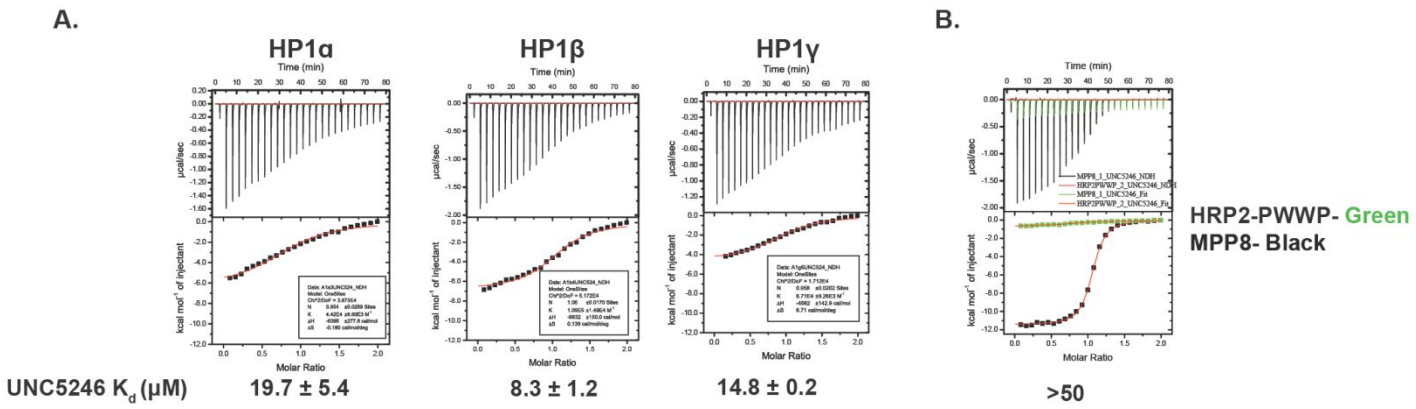


Figure S6 A. ITC data and calculated dissociation constants for UNC5246 with the various HP1 isoforms demonstrating relatively weak binding in each case. B. UNC5246 shows no measurable binding to the PWWP domain of HRP2 (green) by ITC.

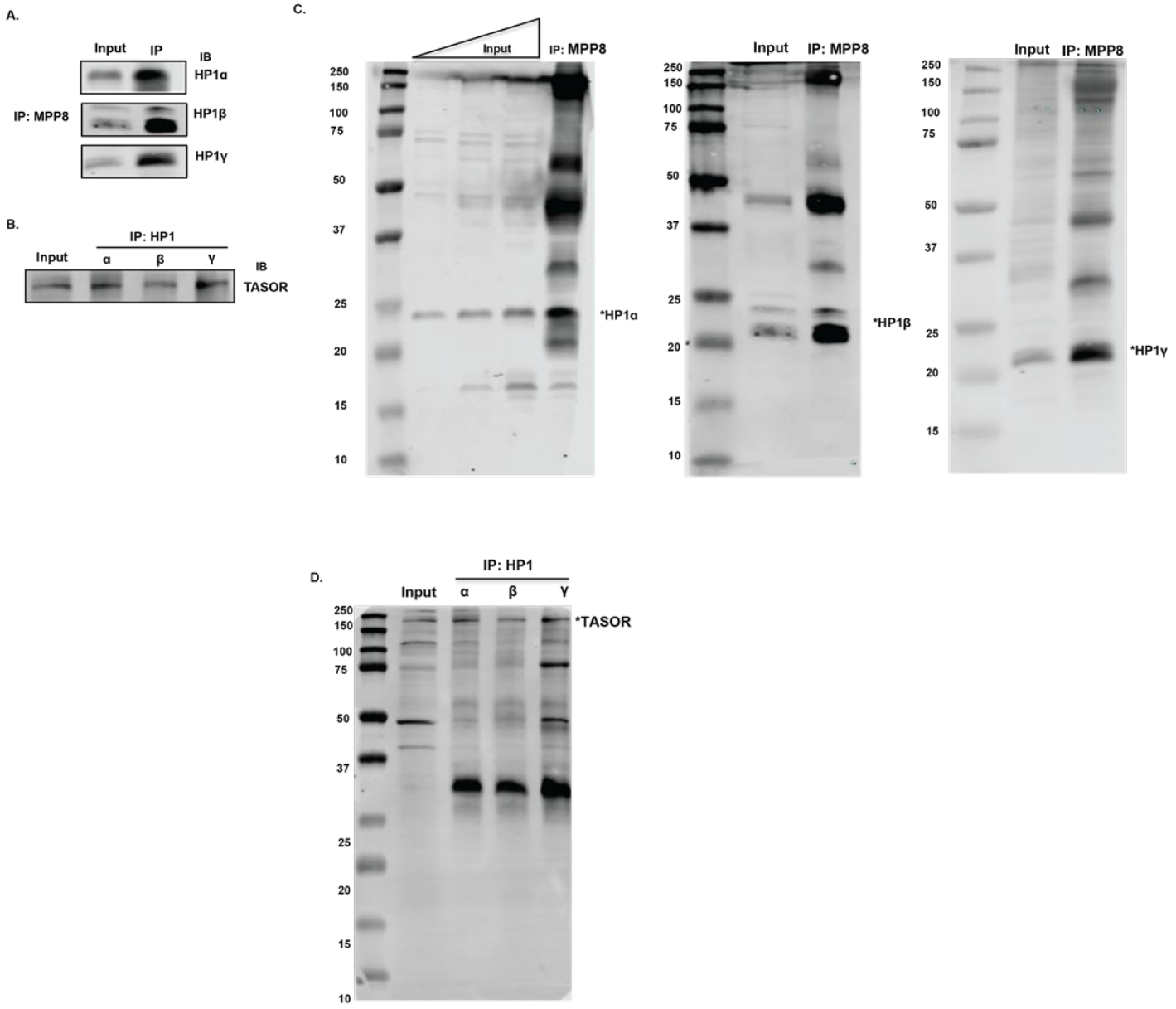


Figure S7. A. Reverse co-IP of Figure 3E demonstrating that immunoprecipitation of MPP8 results in co-immunoprecipitation of all three HP1 isoforms. B. Similarly to MPP8, TASOR co-immunoprecipitates with all three HP1 isoforms. C. Full western blots associated with Fig. S7A. D. Full western blot associated with Fig. S7B.

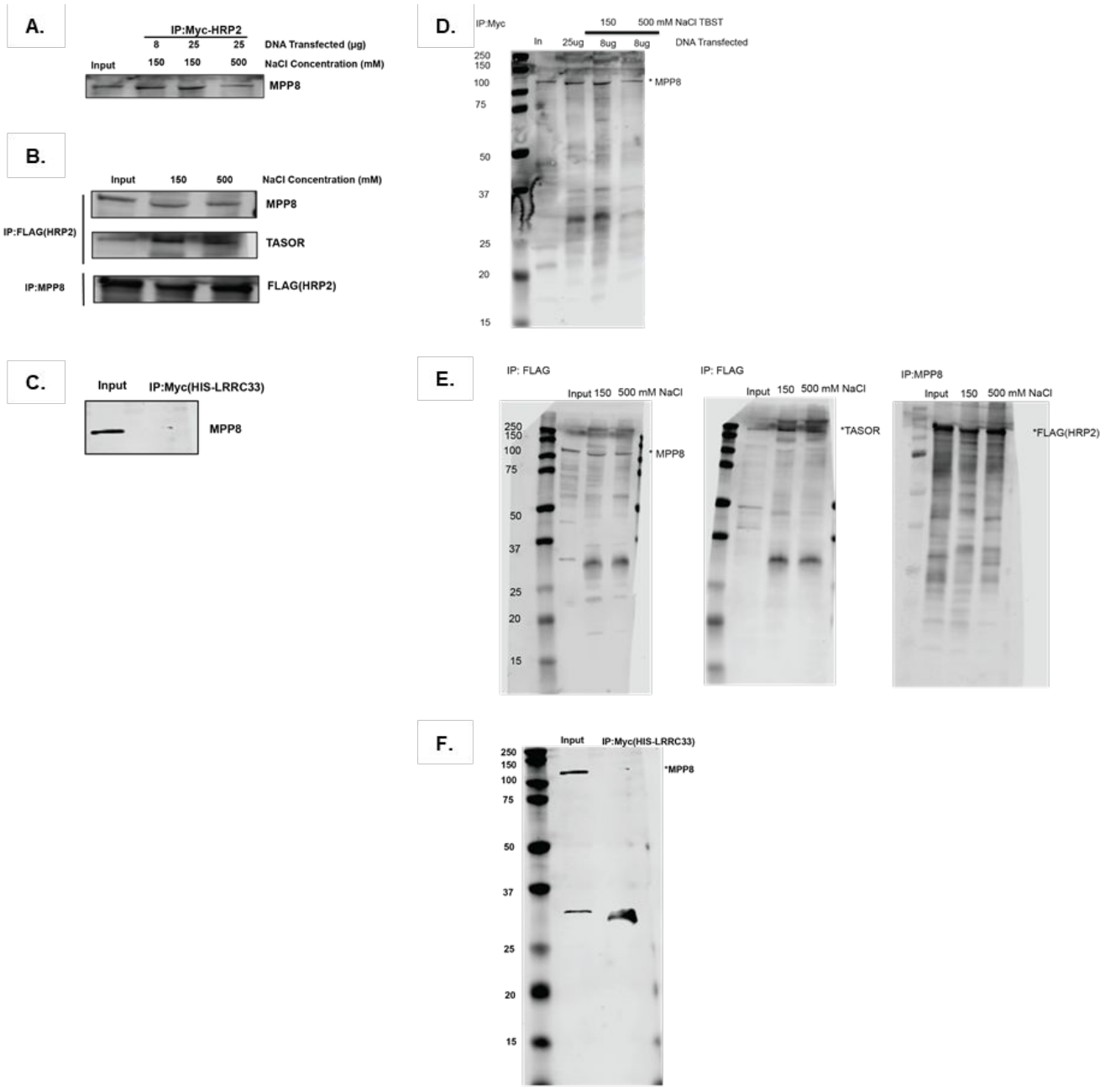


Figure S8. A. Myc-HRP2 co-immunoprecipitates with MPP8 in cell lysates generated from cells with varying Myc-HRP2 transfection levels (8 ug versus 25 ug of DNA) and with variable salt concentrations (150 mM versus 500 mM NaCl). B. Flag-HRP2 co-immunoprecipitates with MPP8 and TASOR similarly to Myc-HRP2 and does so at 150 mM and 500 mM NaCl concentrations. C. Overexpressed Myc(His-LRRC33) does not co-immunoprecipitate with MPP8. D. Full western blot associated with Fig. S8A. E. Full western blots associated with Fig. S8B. F. Full western blot associated with Fig. S8C.

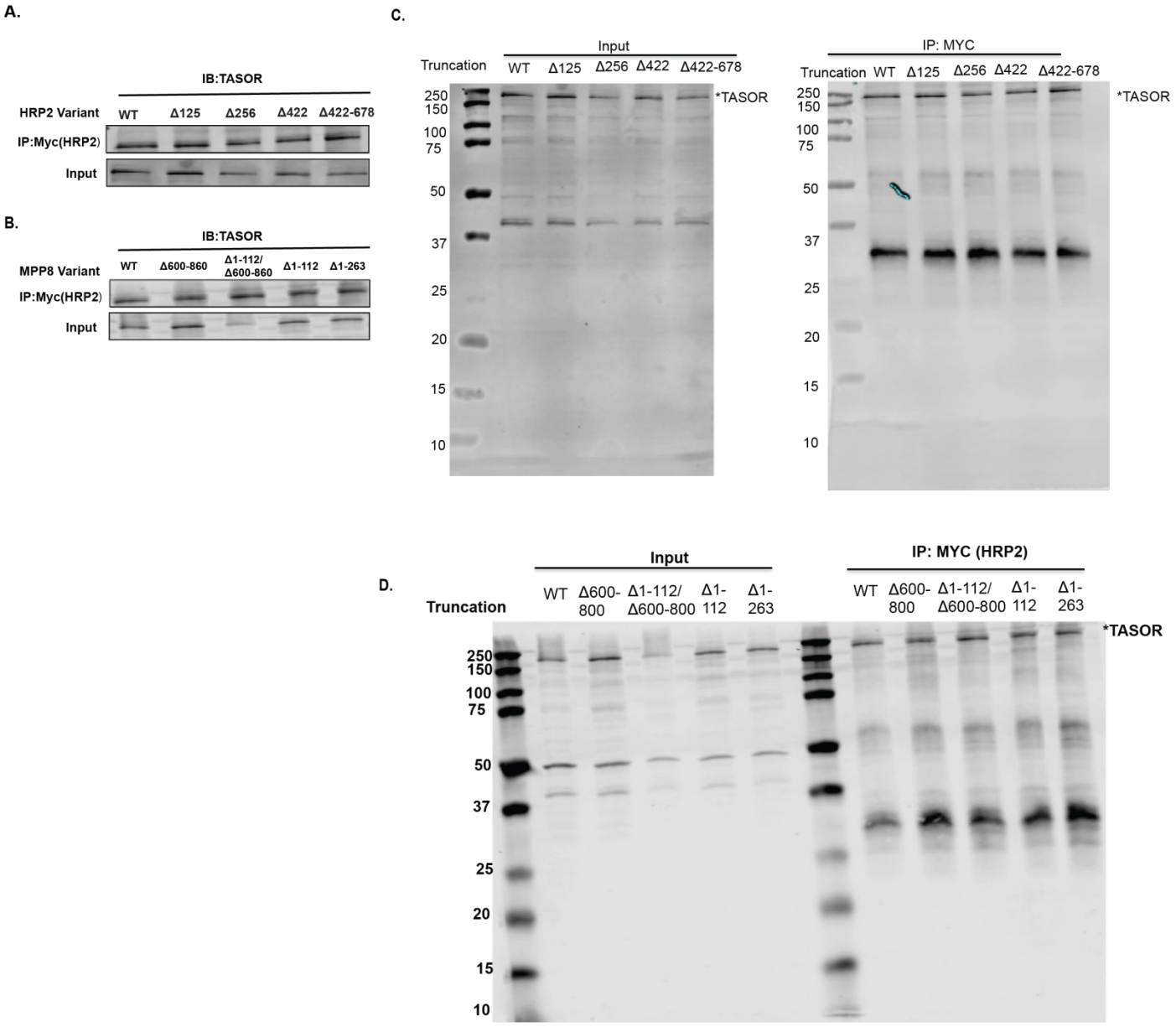


Figure S9. A. Myc-HRP2 truncation constructs expressed in 293T cells followed by IP-Western blot analysis demonstrating association between HRP2 truncations and TASOR. B. Flag-MPP8 truncation constructs expressed in 293T cells followed by IP-Western blot analysis demonstrating association between HRP2 and TASOR. C. Full western blots associated with Fig. S9A. D. Full western blots associated with Fig. S9B.

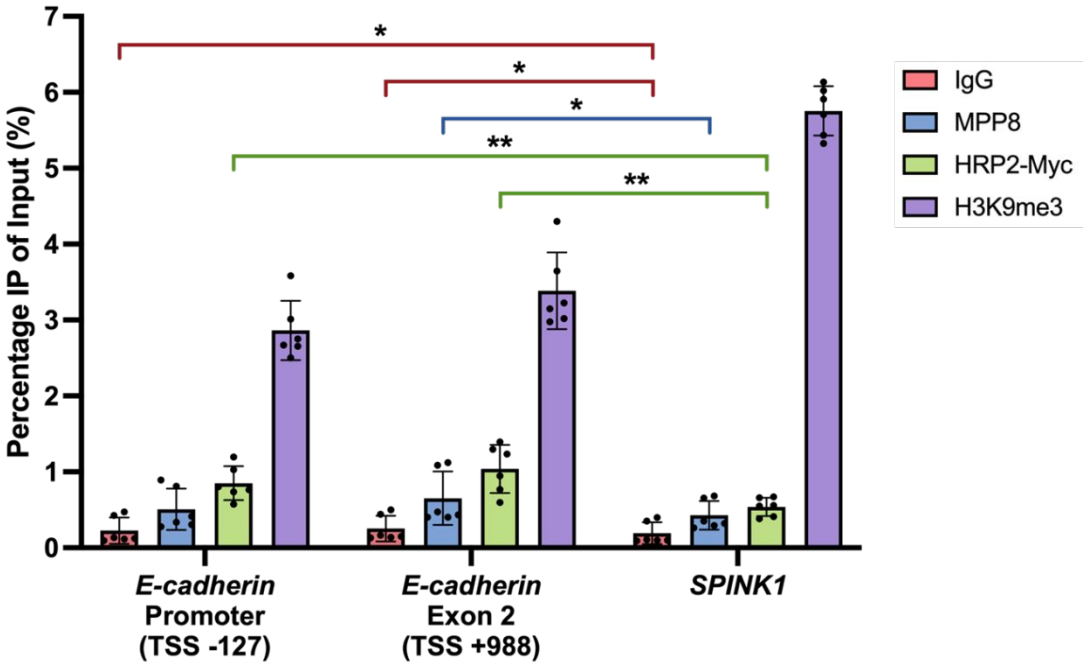


Figure S10. ChIP-qPCR analysis of MPP8, HRP2, and H3K9me3 enrichment at *E-cadherin* compared to *SPINK1*, a locus at which H3K9me3 deposition is directed by Polycomb Repressive Complex 2 (PRC2) in MDA-MB-231 cells. Significant decreases in MPP8 and HRP2 enrichment at *SPINK1* suggest that localization of HRP2 to heterochromatin is specific to its association with MPP8. Statistical significance was calculated using paired t-test (n = 6; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$).

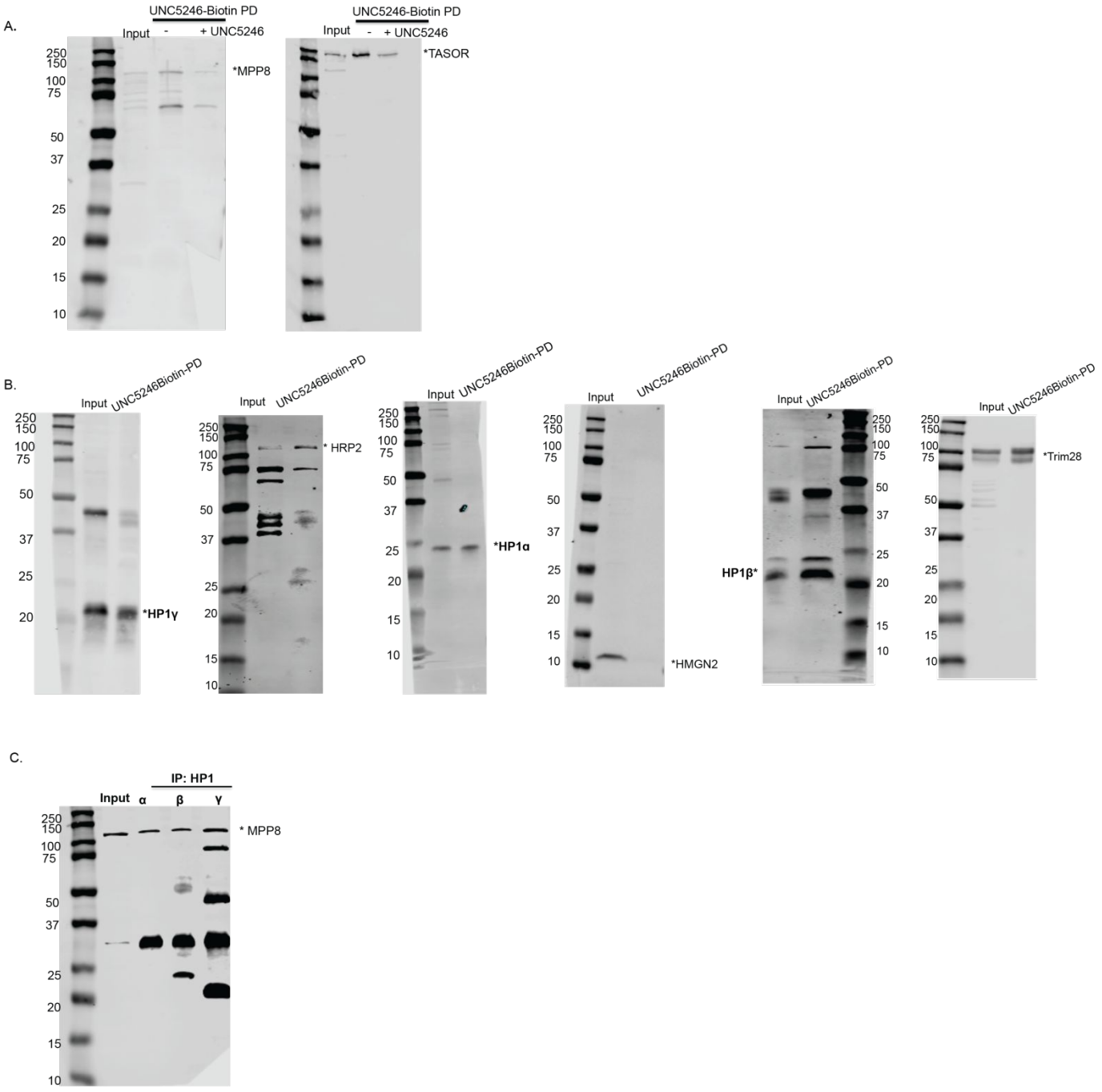


Figure S11. A) Full western blots associated with Fig. 3A. B) Full western blots associated with Fig. 3D. C) Full western blot associated with Fig. 3E.

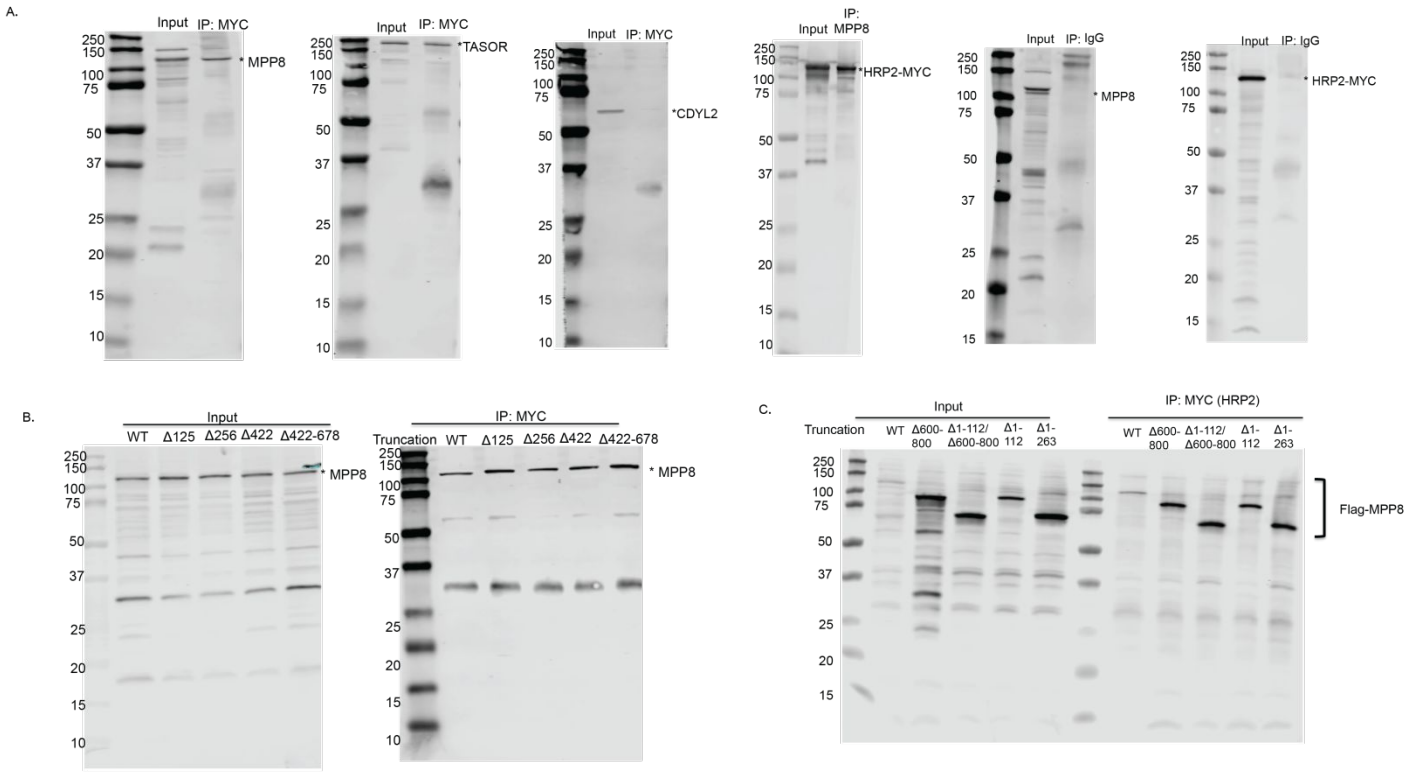


Figure S12. A) Full western blots associated with Fig. 4A. B) Full western blot associated with Fig. 4B. C) Full western blot associated with Fig. 4C.

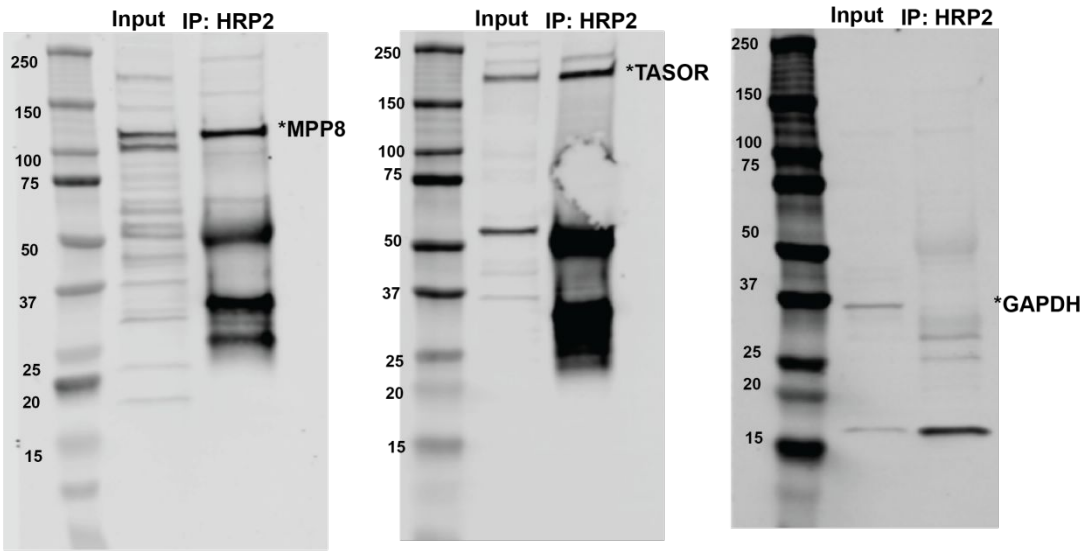


Figure S13. Full western blots associated with Fig. 5A.

Supplementary Table 1. Data collection and refinement statistics.

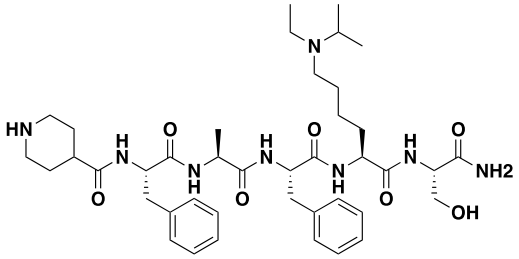
	PBD ID 7M5U
Data collection	
Space group	P 63 2 2
Cell dimensions	
a, b, c (Å)	87.123, 87.123, 57.802
α, β, γ (°)	90.00°, 90.00°, 120.00°
Resolution (Å)	2.02 Å
R_{merge}	0.044 (1.86)
R_{pim}	0.017 (0.63)
$I / \sigma I$	28.8 (1.66)
Completeness (%)	99.8 (100.0)
Redundancy	13.8 (14.5)
Refinement	
Resolution (Å)	2.02 Å
No. reflections	8912
R_{work} / R_{free}	21.6%/22.8%
No. atoms	550
Protein	488
Ligand/ion	57
Water	5
B-factors	
Protein	75.2
Ligand/ion	80.5
Water	65.3
R.m.s. deviations	
Bond lengths (Å)	0.008
Bond angles (°)	0.995

1 crystal used for diffraction data collection.
Values in parentheses are for highest-resolution shell.

General Chemistry Procedures. All LC-MS were obtained on an Agilent 6110 Series LCMS with a UV detector set to 220 and 254 nm and a single quadrupole mass spectrometer. LCMS samples were run on an analytical Agilent Eclipse Plus 4.6 x 50mm, 1.8 µm, C18 column at room temperature with mobile phases A (H_2O + 0.1% acetic acid) and B (MeOH + 0.1% acetic acid or MeCN + 0.1% acetic acid). Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AV at 400 MHz for proton (^{1}H NMR); chemical shifts are reported in ppm (δ) relative to residual protons in deuterated solvent peaks. Normal phase column chromatography was performed with a Teledyne Isco CombiFlash®Rf using silica RediSep®Rf columns with the UV detector set to 220 nm and 254 nm. A linear mobile phase of A (DCM) and B (MeOH) up to between 15-25% B was used to purify all Boc-protected intermediates. Reverse phase column chromatography was performed with a Teledyne Isco CombiFlash®Rf 200 using C18 RediSep®Rf Gold columns with the UV detector set to 220 nm and 254 nm. Mobile phases of A (H_2O + 0.1% TFA) and B (MeOH) were used with default gradients (10 to 100% B). Preparative HPLC was performed using an Agilent Prep 1200 series with the UV detector set to 220 nm and 254 nm. Samples were injected onto a Phenomenex Luna 250 75 x 30 mm, 5 µm, C18 column at room temperature. Mobile phases of A (H_2O + 0.1% TFA) and B (MeOH or MeCN) were used with a flow rate of 40 mL/min. A general gradient of 0-15 minutes increasing from 10 to 100% B, followed by a 100% B flush for another 5 minutes. Small variations in this purification method were made as needed to achieve ideal separation for each compound. Analytical LCMS (at 220 nm) and NMR were used to establish the purity of targeted compounds. All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by ^{1}HNMR and LC-MS.

Solid Phase Peptide Synthesis. Peptide resynthesis was conducted on Fmoc Rink amide MBHA resin (50 mg per peptide, Anaspec). The resin was initially swollen in DCM followed by DMF (10

minutes each). Fmoc protecting groups were removed in a solution of 2.5% 1,8-diazabicycloundec7-ene and 2.5% pyrrolidine in DMF for 10 min. The resin was filtered and washed twice with DMF, methanol, DMF, and DCM before adding the next amino acid for coupling. Standard Fmoc synthesis protocols were used. Briefly, Fmoc-protected amino acids (15 eq) were mixed for 5 minutes with HBTU (15 eq), HOAt (15 eq), and DIPEA (30 eq) in 5 mL of DMF and 3 mL of dichloromethane (DCM). The solution was then added to the resin and left on a shaker at room temperature for 1 hr. The resin was filtered and washed twice with DCM, DMF, methanol, and DMF again. Ethyl-isopropyl lysine was prepared as described previously [1]. Following installation of the capping residue, the resin was rinsed 10 times with DCM. Cleavage cocktail (95% trifluoroacetic acid, 2.5% triisopropylsilane, and 2.5% water) was added to the resin, the mixture was left on the shaker for 2 hours, and the filtrate was collected. The resin was rinsed twice with DCM and filtrates were pooled and then concentrated under vacuum. The mixture was dissolved in water and cold ether was added. Two aqueous extractions followed. The aqueous layers were combined then concentrated under vacuum. Preparative HPLC was performed using an Agilent Prep 1200 series with the UV detector set to 220 nm. Samples were dissolved in a 1 mL 1:1 water: acetonitrile mixture and injected onto a Phenomenex Luna 75 x 30 mm (5 µm) C18 column at room temperature. Mobile phases of A (H2O + 0.1% TFA) and B (MeCN) were used with a flow rate of 30 mL/min. Products peaks were identified via LC-MS, concentrated under vacuum, and lyophilized to a white powder.

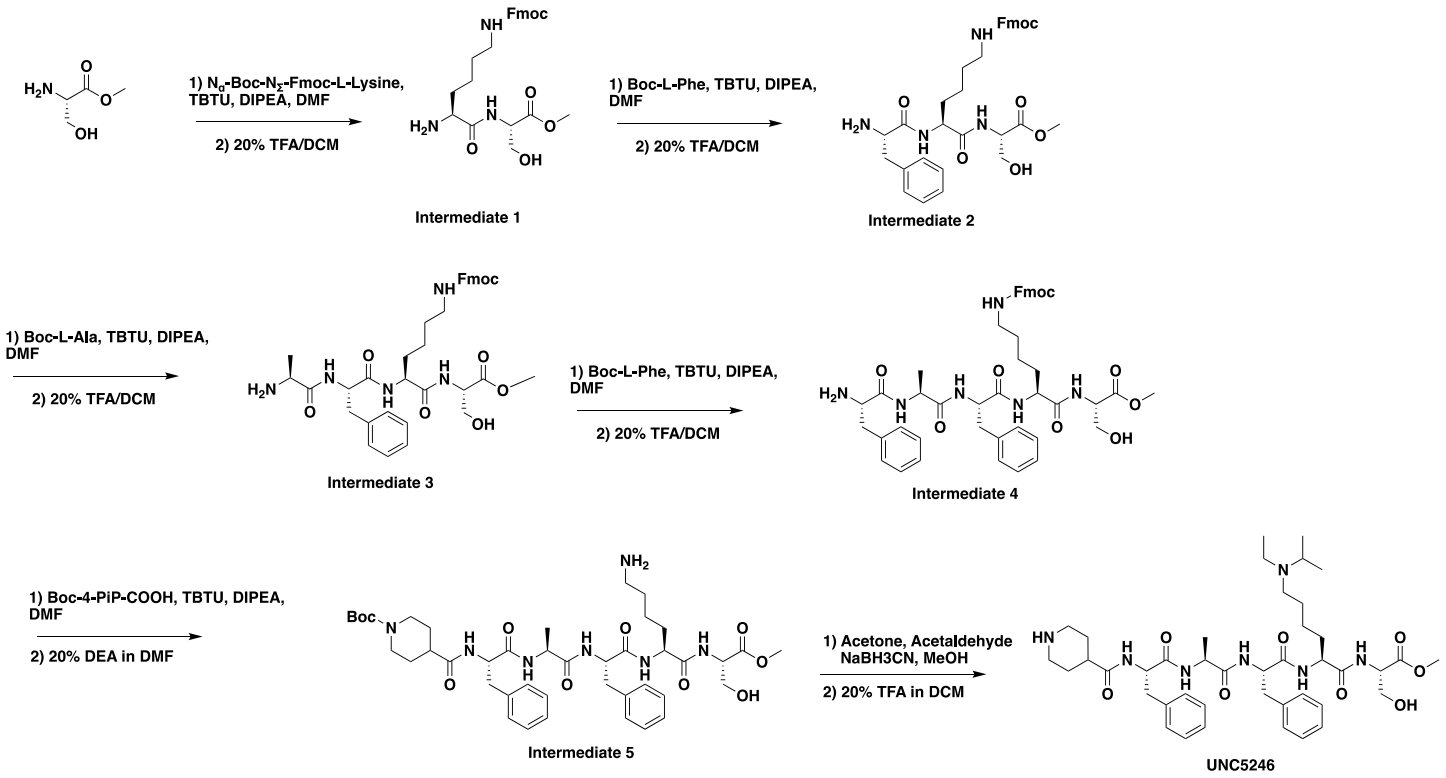


UNC4848

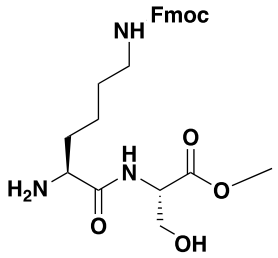
***N*-((8*S*,11*S*,14*S*,17*S*)-8-(((*S*)-1-amino-3-hydroxy-1-oxopropan-2-yl)carbamoyl)-11-benzyl-3-ethyl-2,14-dimethyl-10,13,16-trioxo-18-phenyl-3,9,12,15-tetraazaoctadecan-17-yl)piperidine-4-**

carboxamide. UNC4848 was prepared via solid phase peptide synthesis with standard Fmoc-Amino Acids, Fmoc-Lys(et,ipr), and 1-(*tert*-butoxycarbonyl)piperidine-4-carboxylic acid for the N-terminal cap, all coupled while on solid support. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.31 – 7.17 (m, 10H), 4.62 (dd, *J* = 10.2, 4.9 Hz, 1H), 4.53 (dd, *J* = 9.0, 5.2 Hz, 1H), 4.41 – 4.35 (m, 2H), 3.87 – 3.77 (m, 1H), 3.72 – 3.64 (m, 2H), 3.41 – 3.34 (m, 1H), 3.28 – 2.89 (m, 10H), 2.86 – 2.78 (m, 1H), 2.58 – 2.49 (m, 1H), 1.96 – 1.41 (m, 11H), 1.36 – 1.27 (m, 12H). MS (ESI+): 390.3 $[M+2H]^{2+}$. t_R = 2.82 min.

Synthesis of UNC5246-Biotin. UNC5246-Biotin was prepared by solid phase peptide synthesis as described above with some modifications. Upon Fmoc deprotection of the resin, bromoacetic acid (1 M) and N,N-diisopropylcarbodiimide (1 M) were first added for 30 minutes followed by 7 DMF washes. Next, propargylamine (1 M) in DMF was added for 2 hours. The remaining amino acids were installed by standard solid phase coupling and deprotection conditions as described above, starting with Fmoc-β-alanine to serve as a short spacer. Upon cleavage and purification of the alkynyl peptide, biotin-PEG5-azide (1 eq, 1.40 μmol, Click Chemistry Tools) in 1:1 DMF:water (2 mL), copper sulfate (3 eq, 4.2 μmol), and ascorbic acid (6 eq, 8.4 μmol) were added to the peptide (1.32 mg, 1.40 μmol). The mixture was stirred overnight at room temperature and concentrated under vacuum. Crude mixtures were redissolved in 1:1 water:acetonitrile (1 mL), filtered, and purified via HPLC. Product fractions as determined by LCMS were pooled and concentrated under vacuum. The desired product was redissolved in water and lyophilized to a white powder (1.5 mg, 73%). MS (ESI+): 739.4 $[M+2H]^{2+}$. t_R = 3.67 min.



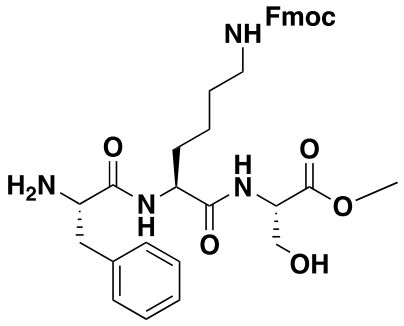
Supplemental Scheme 1. Synthesis of UNC5246.



Intermediate 1

methyl N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-*L*-lysyl-*L*-serinate. To a round bottom flask was added N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(tert-butoxycarbonyl)-L-lysine (1 g, 1.1 Eq, 2 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (0.7 g, 1.2 Eq, 2 mmol) followed by the addition of N,N-dimethylformamide (20 mL) and DIPEA (0.8 g, 1.2 mL, 3 Eq, 6 mmol). The mixture stirred for 15 minutes followed by the addition of methyl N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-(tert-butoxycarbonyl)-L-lysyl-L-serinate (850 mg, 1.0 eq, 2 mmol,). The reaction was stirred overnight followed by the addition of 100 mL of ethyl acetate and washed 3X with brine and the organic phase was concentrated under reduced pressure and purified by flash

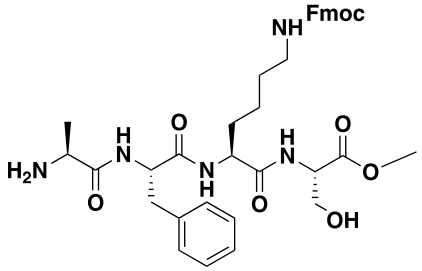
chromatography (DCM/MeOH). To the obtained product was added 20% TFA in DCM and the reaction was stirred overnight at room temperature followed by purification by reverse phase flash chromatography (H_2O + 0.1% TFA: MeOH) to yield the title compound as a TFA salt (0.96 g; 97%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.77 (d, J = 7.7 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.42 – 7.18 (m, 4H), 4.58 (t, 1H), 4.39 – 4.29 (m, 2H), 4.20 – 4.13 (m, 1H), 3.93 (dd, J = 11.2, 5.2 Hz, 2H), 3.83 (dd, J = 11.3, 3.9 Hz, 1H), 3.70 (s, 3H), 3.13 (t, J = 6.7 Hz, 2H), 2.02 – 1.71 (m, 2H), 1.64 – 1.30 (m, 4H).MSI (ESI): 470 $[M+H]^+$ t_R = 4.14 min.



Intermediate 2

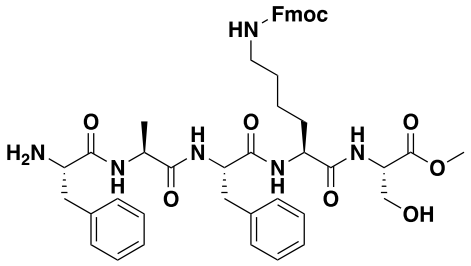
methyl N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^2-(*L*-phenylalanyl)-*L*-lysyl-*L*-serinate. To a round bottom flask was added (tert-butoxycarbonyl)-L-phenylalanine **(**336 mg, 0.9 Eq, 1.26 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (496 mg, 1.1 Eq, 1.55 mmol) followed by the addition of N,N-dimethylformamide (20 mL) and DIPEA (600 mg, 0.81 mL, 3 Eq, 4.65 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 1** (820 mg, 1.0 eq 1.41 mmol,). The reaction was stirred overnight followed by the addition of 100 mL of ethyl acetate and washed 3X with brine and the organic phase concentrated under reduced pressure and purified by flash chromatography (DCM:MeOH). To the obtained product was added 20% TFA in DCM and the reaction was stirred overnight at room temperature followed by purification by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a TFA salt (0.93 g; 93%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.5 Hz, 2H), 7.48 – 7.15 (m, 9H), 4.56 – 4.38 (m, 2H), 4.34 (d, J = 6.8 Hz, 2H), 4.23 – 4.07 (m, 2H), 3.93 (dd, J = 11.3,

4.8 Hz, 1H), 3.81 (dd, *J* = 11.3, 4.0 Hz, 1H), 3.72 (s, 3H), 3.29 – 3.24 (m, 1H), 3.11 (t, *J* = 6.7 Hz, 2H), 3.02 (dd, *J* = 14.3, 8.5 Hz, 1H), 1.96 – 1.62 (m, 2H), 1.57 – 1.38 (m, 4H).MSI (ESI): 617.3 $[M+H]^+$. t_R =4.50 min.



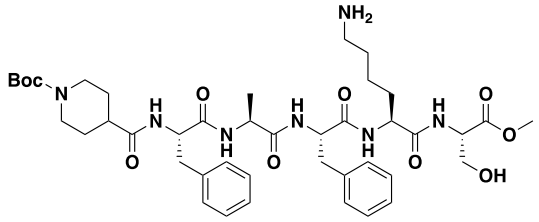
Intermediate 3

methyl N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^2-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. To a round bottom flask was added (tert-butoxycarbonyl)-L-alanine **(**466 mg, 1.0 Eq, 2.46 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (1.1 g, 1.4 Eq, 3.45 mmol) followed by the addition of N,N-dimethylformamide (25 mL) and DIPEA (892 mg, 1.2 mL, 3 Eq, 3.45 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 2** (1.80 g, 1.0 eq 2.46 mmol,). The reaction was stirred overnight followed by the addition of 100 mL of ethyl acetate and washed 3X with brine and the organic phase concentrated under reduced pressure and purified by flash chromatography (DCM:MeOH). To the obtained product was added 20% TFA in DCM and the reaction was stirred overnight at room temperature followed by purification by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a TFA salt (1.5 g; 89%) ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.79 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.32 – 7.13 (m, 7H), 4.75 – 4.61 (m, 1H), 4.57 – 4.44 (m, 1H), 4.42 – 4.27 (m, 3H), 4.21 – 4.10 (m, 1H), 3.91 (dd, *J* = 11.3, 4.8 Hz, 1H), 3.79 (dd, *J* = 11.3, 4.0 Hz, 2H), 3.72 (s, 3H), 3.27 – 3.04 (m, 3H), 3.02 – 2.62 (m, 1H), 1.91 – 1.61 (m, 2H), 1.54 – 1.23 (m, 7H). MSI (ESI): 688.4 $[M+H]^+$. T_R= 5.11 min.



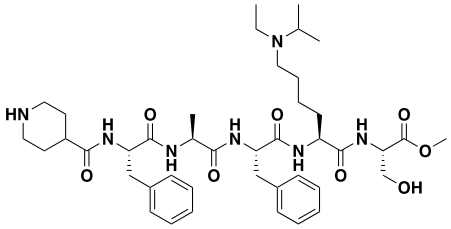
Intermediate 4

methyl N^6-(((9*H*-fluoren-9-yl)methoxy)carbonyl)-N^2-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. To a round bottom flask was added (tert-butoxycarbonyl)-L-phenylalanine **(**347 mg, 1.0 Eq, 1.31 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (589 mg, 1.4 Eq, 1.83 mmol) followed by the addition of N,N-dimethylformamide (25 mL) and DIPEA (474 mg, 0.64 mL, 3 Eq, 3.66 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 3** (1.05 g, 1.0 eq 1.31 mmol,). The reaction was stirred overnight followed by the addition of 100 mL of ethyl acetate and washed 3X with brine and the organic phase concentrated under reduced pressure and purified by flash chromatography (DCM:MeOH). To the obtained product was added 20% TFA in DCM and the reaction was stirred overnight at room temperature followed by purification by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a TFA salt (1.01 g; 92%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 2H), 7.33 – 7.12 (m, 12H), 4.68 – 4.54 (m, 1H), 4.50 (t, *J* = 4.4 Hz, 1H), 4.44 – 4.29 (m, 4H), 4.17 (t, *J* = 6.9 Hz, 1H), 4.09 – 4.01 (m, 1H), 3.90 (dd, *J* = 11.3, 4.7 Hz, 1H), 3.79 (dd, *J* = 11.3, 4.1 Hz, 1H), 3.71 (s, 3H), 3.25 – 3.07 (m, 4H), 3.05 – 2.84 (m, 2H), 1.91 – 1.77 (m, 1H), 1.75 – 1.64 (m, 1H), 1.58 – 1.36 (m, 4H), 1.31 (d, *J* = 7.1 Hz, 3H). MSI (ESI): 835.30 $[M+H]^+$. T_R= 5.65 min.



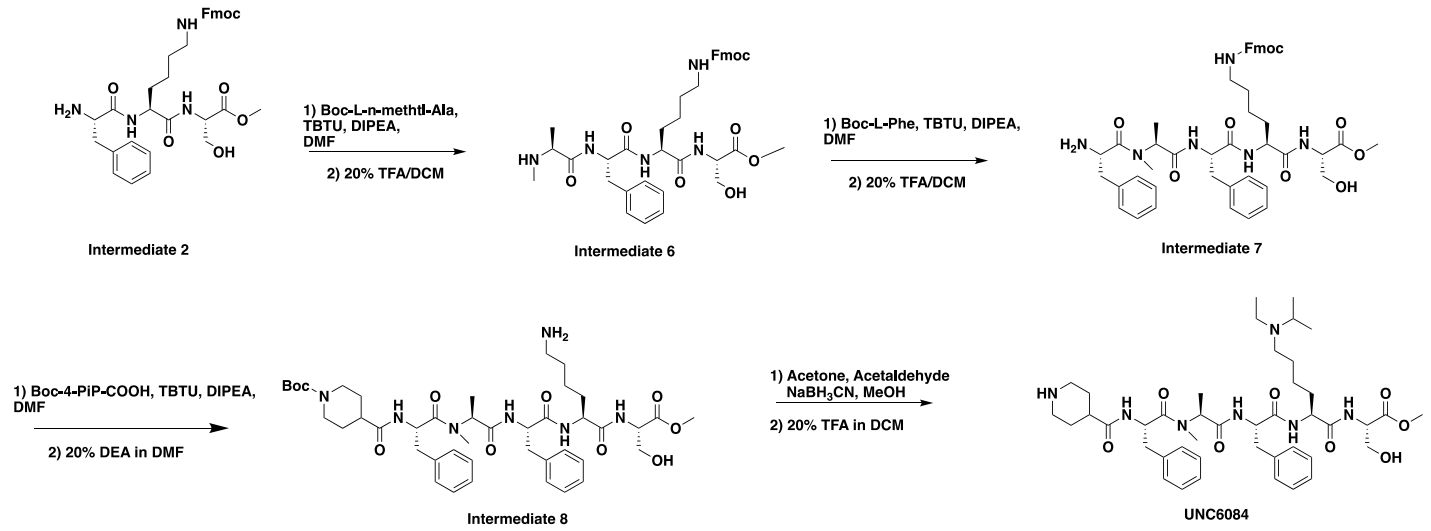
Intermediate 5

tert-butyl 4-(((4S,7S,10S,13S,16S)-7-(4-aminobutyl)-10-benzyl-4-(hydroxymethyl)-13-methyl-3,6,9,12,15-pentaoxo-17-phenyl-2-oxa-5,8,11,14-tetraazaheptadecan-16-yl)carbamoyl)piperidine-1-carboxylate. To a round bottom flask was added 1-(tert-butoxycarbonyl)piperidine-4-carboxylic acid (36.92 mg, 1.0 Eq, 0.16 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (67.21 mg, 1.3 Eq, 0.21 mmol) followed by the addition of N,N-dimethylformamide (5 mL) and DIPEA (62 mg, 0.108 mL, 3 Eq, 0.48 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 4** (152 mg, 1.0 eq 0.16 mmol,). The reaction was stirred overnight and solvent removed under reduced pressure. To the crude oil was added 20% diethylamine in DMF and the reaction stirred for 4 hours and the solvent was removed under reduced pressure. The crude mixture was purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a TFA salt (110 mg; 83%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.29 – 7.16 (m, 10H), 4.59 – 4.52 (m, 2H), 4.49 (t, J = 4.2 Hz, 1H), 4.46 – 4.40 (m, 1H), 4.23 (q, J = 7.2 Hz, 1H), 4.05 – 3.89 (m, 3H), 3.80 (dd, J = 11.3, 3.9 Hz, 1H), 3.73 (s, 3H), 3.19 (dd, J = 14.1, 5.2 Hz, 1H), 3.09 (dd, J = 14.0, 5.2 Hz, 1H), 3.00 (dd, J = 14.1, 9.2 Hz, 1H), 2.92 (t, J = 7.5 Hz, 2H), 2.84 (dd, J = 14.0, 9.9 Hz, 1H), 2.75 (d, J = 18.0 Hz, 2H), 2.36 (tt, J = 11.5, 3.8 Hz, 1H), 1.95 – 1.83 (m, 1H), 1.78 – 1.62 (m, 4H), 1.43 (s, 13H), 1.39 – 1.28 (m, 1H), 1.25 (d, J = 7.1 Hz, 3H). MSI (ESI): 824.4 $[M+H]^+$, 846 $[M+Na]^+$. T_R= 4.72 min.

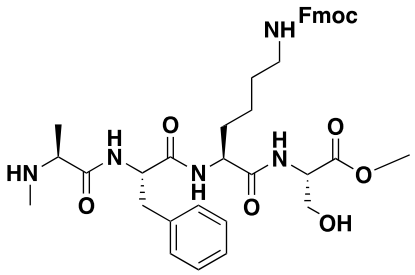


UNC5246

methyl N^6-ethyl-N^6-isopropyl-N^2-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate (UNC5246). Intermediate 5 (19.5 mg, 23.7 umol, 1 eq) was dissolved in methanol (5 mL) followed by the addition of acetone (1.37 mg, 1.75 uL, 23.7 umol 1 eq) and sodium cyanoborohydride (3 mg, 2.97 umol, 2 eq). The reaction was monitored for completion by LC/MS afterwhich acetaldehyde (2 mg, 2.5 uL, 2.97, umol, 2 eq) and 1 additional equivalent of sodium cyanoborohydride. Upon completion, the solvent was removed under reduced pressure and the crude mixture dissolved in 20% TFA in DCM. The mixture stirred for 2 hours followed by concentration under vacuum, and the crude oil was purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (19 mg, 85%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.28 – 7.14 (m, 10H), 4.68 – 4.58 (m, 2H), 4.54 – 4.44 (m, 2H), 4.30 (q, J = 7.1 Hz, 1H), 3.93 (dd, J = 11.4, 4.8 Hz, 1H), 3.81 (dd, J = 11.4, 3.9 Hz, 1H), 3.73 (s, 3H), 3.67 (qd, J = 6.6, 1.5 Hz, 1H), 3.40 – 3.35 (m, 1H), 3.26 – 3.06 (m, 6H), 3.04 – 2.89 (m, 4H), 2.86 – 2.78 (m, 1H), 2.58 – 2.48 (m, 1H), 1.96 – 1.66 (m, 7H), 1.59 (dtd, J = 14.8, 11.3, 4.1 Hz, 1H), 1.45 (p, J = 7.9 Hz, 2H), 1.35 – 1.28 (m, 9H), 1.26 (d, J = 7.1 Hz, 3H).MSI (ESI): 397.8 $[M+2H]^{+2}$, 794.4$[M+H]^+$. T_R= 3.78 min.



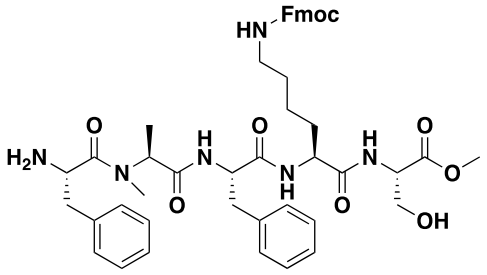
Supplemental Scheme 2. Synthesis of UNC6084.



Intermediate 6

methyl N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-methyl-L-alanyl-L-phenylalanyl-L-lysyl-L-serinate. To a round bottom flask was added (tert-butoxycarbonyl)-N-methyl-L-alanine **(**145 mg, 0.9 Eq, 0.7 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (280 mg, 1.1 Eq, 0.9 mmol) followed by the addition of N,N-dimethylformamide (5 mL) and DIPEA (340 mg, 0.46 mL, 3 Eq, 2.1 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 2** (580 mg, 1.0 eq 0.8 mmol,). The reaction was stirred overnight followed by the addition of 100 mL of ethyl acetate and washed 3X with brine and the organic phase concentrated under reduced pressure and purified by flash chromatography (DCM:MeOH). To the obtained product was added 20% TFA in DCM and the reaction was stirred overnight at room temperature followed by purification by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title

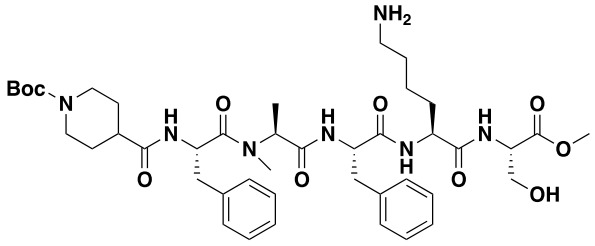
compound as a TFA salt (350 mg; 63%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.78 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.4 Hz, 2H), 7.42 – 7.15 (m, 9H), 4.83 – 4.76 (m, 1H), 4.51 (t, J = 4.3 Hz, 1H), 4.44 – 4.38 (m, 1H), 4.34 (d, J = 6.9 Hz, 2H), 4.18 (t, J = 6.9 Hz, 1H), 3.91 (dd, J = 11.3, 4.7 Hz, 1H), 3.80 (dd, J = 11.3, 4.0 Hz, 1H), 3.72 (s, 3H), 3.68 (t, J = 7.0 Hz, 1H), 3.24 (dd, J = 14.1, 4.7 Hz, 1H), 3.17 – 3.07 (m, 2H), 2.91 – 2.83 (m, 1H), 2.30 (s, 3H), 1.86 (dq, J = 13.8, 7.2 Hz, 1H), 1.71 (h, J = 8.2 Hz, 1H), 1.56 – 1.48 (m, 2H), 1.47 – 1.37 (m, 5H). MSI (ESI): 702 $[M+H]^+$ t_R = 4.57 min.



Intermediate 7

methyl N6-(((9H-fluoren-9-yl)methoxy)carbonyl)-N2-N-(L-phenylalanyl)-N-methyl-L-alanyl-L-phenylalanyl-L-lysyl-L-serinate. To a round bottom flask was added (tert-butoxycarbonyl)-L-phenylalanine **(**235 mg, 0.9 eq, 0.9 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (415 mg, 1.4 Eq, 1.3 mmol) followed by the addition of N,N-dimethylformamide (10 mL) and DIPEA (500 mg, 0.43 mL, 3 Eq, 3 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 6** (780 mg, 1.0 eq 1 mmol,). The reaction was stirred overnight followed by the addition of 100 mL of ethyl acetate and washed 3X with brine and the organic phase concentrated under reduced pressure and purified by flash chromatography (DCM:MeOH). To the obtained product was added 20% TFA in DCM and the reaction was stirred overnight at room temperature followed by purification by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a TFA salt (600 mg; 69%). ^{1}H NMR (400 MHz, Methanol-d_4) 1H NMR (400 MHz, Methanol-d4) δ 7.78 (d, J = 7.5 Hz, 2H), 7.62 (d, J = 7.4 Hz, 2H), 7.42 – 7.10 (m, 14H), 4.76 – 4.37 (m, 5H), 4.36 – 4.28 (m, 2H), 4.17 (t, J = 6.7 Hz, 1H), 3.90 (dd, J =

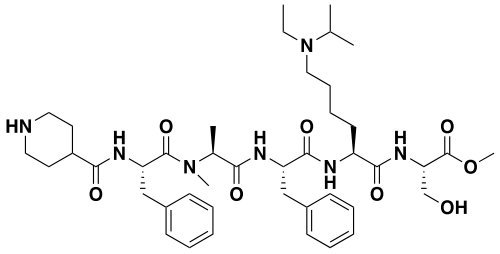
11.3, 4.8 Hz, 1H), 3.84 – 3.77 (m, 1H), 3.71 (d, *J* = 1.2 Hz, 3H), 3.29 – 3.19 (m, 1H), 3.19 – 3.08 (m, 2H), 3.08 – 2.85 (m, 3H), 2.63 (s, 3H), 1.93 – 1.82 (m, 1H), 1.81 – 1.68 (m, 1H), 1.60 – 1.49 (m, 2H), 1.48 – 1.35 (m, 2H), 1.28 (d, *J* = 7.1 Hz, 3H). MSI (ESI): 849 $[M+H]^+$ t_R = 5.07 min.



Intermediate 8

Intermediate 8 tert-butyl 4-(((4S,7S,10S,13S,16S)-7-(4-aminobutyl)-10-benzyl-4-(hydroxymethyl)-13,14-dimethyl-3,6,9,12,15-pentaoxo-17-phenyl-2-oxa-5,8,11,14-tetraazaheptadecan-16-yl)carbamoyl)piperidine-1-carboxylate. To a round bottom flask was added 1-(tert-butoxycarbonyl)piperidine-4-carboxylic acid **(**48 mg, 1.0 Eq, 0.2 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (96 mg, 1.4 eq, 0.3 mmol) followed by the addition of N,N-dimethylformamide (3 mL) and DIPEA (80 mg, 0.18 mL, 3 Eq, 0.6 mmol). The mixture stirred for 15 minutes followed by the addition of **Intermediate 7** (152 mg, 1.0 eq 0.16 mmol,). The reaction was stirred overnight and solvent removed under reduced pressure. To the crude oil was added 20% diethylamine in DMF and the reaction stirred for 4 hours and the solvent was removed under reduced pressure and purified by reverse phase chromatography (H_2O +0.1% TFA: MeOH) to yield title compound as a white powder TFA salt (120 mg, 76%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.35 – 7.13 (m, 10H), 5.08 – 5.00 (m, 1H), 4.63 – 4.54 (m, 1H), 4.54 – 4.41 (m, 3H), 4.08 (d, *J* = 13.4 Hz, 1H), 3.96 – 3.90 (m, 1H), 3.87 – 3.79 (m, 1H), 3.76 (d, *J* = 1.3 Hz, 3H), 3.50 – 3.41 (m, 1H), 3.26 – 3.16 (m, 1H), 3.09 – 3.00 (m, 2H), 2.98 – 2.92 (m, 2H), 2.84 (dt, *J* = 13.7, 4.6 Hz, 1H), 2.70 (dd, *J* = 6.0, 0.8 Hz, 1H), 2.59 – 2.52 (m, 1H), 2.39 (tt, *J* = 11.6, 3.8 Hz, 1H), 2.08 (d, *J* = 14.2 Hz, 3H), 1.90 (td, *J* = 8.2, 4.5 Hz, 2H), 1.84 – 1.75 (m, 2H), 1.74 – 1.58 (m, 4H), 1.50 (p, *J* = 7.3

Hz, 2H), 1.42 (d, *J* = 9.6 Hz, 7H), 1.26 – 1.21* (m, 2H), 0.28* (dd, *J* = 9.7, 6.7 Hz, 2H).MSI (ESI): 839.4 $[M+H]^+$. t_R = 4.90 min. *Split Peaks due to N-methyl alanine rotamer.

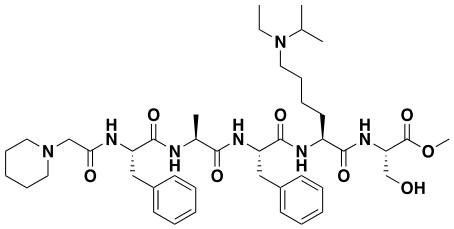


UNC6084

methyl N^6-ethyl-N^6-isopropyl-N^2-*N*-methyl-*N*-((piperidine-4-carbonyl)-*L*-phenylalanyl)-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. Intermediate 8 (70 mg, 0.08 mmol, 1 eq) was dissolved in methanol (5 mL) followed by the addition of acetone (14 mg 18 uL 3 eq 0.24 mmol) and sodium cyanoborohydride (21 mg, 0.3 mmol, 4 eq) and the reaction stirred overnight and was monitored for completion by LC-MS upon which acetaldehyde (15 mg, 19 uL, 4 eq, 0.32 mmol) was added along with 1 eq. of sodium cyanoborohydride and the reaction allowed to proceed until completion as monitored by LC-MS. Upon completion, the reaction was concentrated under reduced pressure and the resulting oil dissolved in 20% TFA in DCM. The mixture was allowed to stir overnight followed by concentration under vacuum and purification via reverse phase chromatography (H_2O + 0.1% TFA: MeOH) to yield the title compound as a TFA salt (16 mg, 35% over 2 steps).^{1}H NMR (400 MHz, Methanol-d_4) δ 7.32 – 7.15 (m, 10H), 5.06 – 4.93 (m, 1H), 4.59 – 4.41 (m, 4H), 3.98 – 3.89 (m, 1H), 3.82 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.75 (s, 3H), 3.70 (q, *J* = 6.7 Hz, 1H), 3.50 – 3.34 (m, 2H), 3.27 – 3.21 (m, 1H), 3.17 (dd, *J* = 13.8, 4.9 Hz, 2H), 3.11 – 2.91 (m, 6H), 2.74 – 2.64 (m, 2H), 2.08 (s, 3H), 2.05 – 1.69 (m, 7H), 1.59 – 1.44 (m, 2H), 1.38 – 1.30 (m, 9H), 1.24* (d, *J* = 7.0 Hz, 1H), 0.28* (d, *J* = 6.8 Hz, 2H). MSI (ESI): 404. 9 $[M+2H]^{+2}$, 808.5 $[M+H]^+$. t_R = 3.57 min. *Split peaks due to N-methyl alanine rotamer.

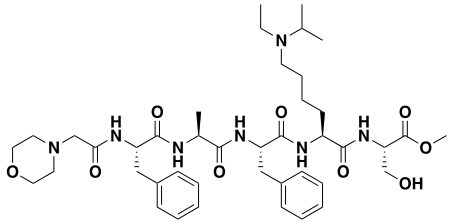
.

Compounds from Table 1



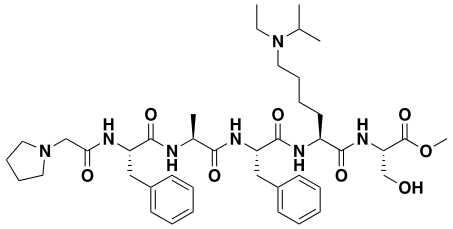
UNC6215

methyl N^6-ethyl-N^6-isopropyl-N^2-(2-(piperidin-1-yl)acetyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. 2-(piperidin-1-yl)acetic acid (4.5 mg 0.03 mmol, 1 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (14 mg, 0.4 mmol, 1.4 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (11.5 mg, 15.5 uL, 0.9 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (30 mg, 0.03 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH (5 mL) and acetone (6 mg, 7.5 uL, 0.09 mmol, 3 eq) and sodium cyanoborohydride (6 mg, 0.09 mmol, 3 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (3 mg, 4 uL, 0.06 mmol, 2 eq) and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound (14 mg, 55% across 3 steps) as a white powder TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.34 – 7.11 (m, 10H), 4.73 (dd, J = 10.3, 4.7 Hz, 1H), 4.59 – 4.40 (m, 3H), 4.28 (q, J = 7.1 Hz, 1H), 3.97 – 3.78 (m, 3H), 3.74 (s, 3H), 3.71 – 3.65 (m, 1H), 3.42 (d, J = 12.3 Hz, 1H), 3.28 – 2.86 (m, 9H), 2.83 – 2.71 (m, 2H), 1.99 – 1.60 (m, 10H), 1.46 (p, J = 7.8 Hz, 3H), 1.38 – 1.26 (m, 12H). MSI (ESI) Found: 404.8 $[M+2H]^{2+}$. t_R = 5.27 min.



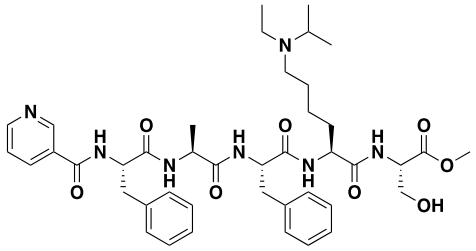
UNC6214

methyl N^6-ethyl-N^6-isopropyl-N^2-(2-morpholinoacetyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. 2-morpholinoacetic acid (5 mg 0.03 mmol, 1 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (14 mg, 0.4 mmol, 1.4 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (11.5 mg, 15.5 uL, 0.9 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (30 mg, 0.03 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH (5 mL) and acetone (6 mg, 7.5 uL, 0.09 mmol, 3 eq) and sodium cyanoborohydride (6 mg, 0.09 mmol, 3 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (3 mg, 4 uL, 0.06 mmol, 2 eq) and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound (18 mg, 70% across 3 steps) as a white powder TFA salt. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.36 – 7.12 (m, 10H), 4.71 (dd, *J* = 10.3, 4.7 Hz, 1H), 4.60 – 4.46 (m, 2H), 4.46 – 4.40 (m, 1H), 4.32 – 4.23 (m, 1H), 3.98 – 3.63 (m, 12H), 3.28 – 2.96 (m, 11H), 2.79 (dd, *J* = 14.0, 10.3 Hz, 1H), 1.97 – 1.69 (m, 4H), 1.53 – 1.44 (m, 2H), 1.39 – 1.24 (m, 12H). MSI (ESI): 405.6 $[M+2H]^{2+}$. t_R= 5.81 min.



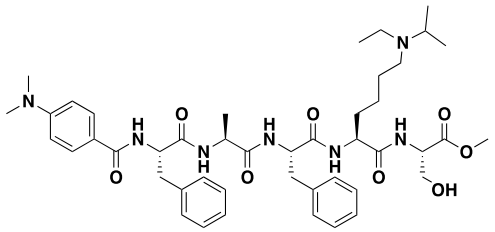
UNC6113

methyl N^6-ethyl-N^6-isopropyl-N^2-(2-(pyrrolidin-1-yl)acetyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. 2-(pyrrolidin-1-yl)acetic acid (7.0 mg 0.05 mmol, 1.2 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (22 mg, 0.7 mmol, 1.4 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (18 mg, 24 uL, 0.2 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (47 mg, 0.05 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH (5 mL) and acetone (9 mg, 12 uL, 0.16 mmol, 3 eq) and sodium cyanoborohydride (14 mg, 0.2 mmol, 4 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (7 mg, 15 uL, 0.2 mmol, 3 eq) and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound (30 mg, 60% across 3 steps) as a white powder TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.36 – 7.13 (m, 10H), 4.76 – 4.65 (m, 1H), 4.58 – 4.47 (m, 2H), 4.47 – 4.40 (m, 1H), 4.33 – 4.21 (m, 1H), 4.07 – 3.77 (m, 4H), 3.74 (s, 3H), 3.73 – 3.66 (m, 1H), 3.61 (s, 1H), 3.46 (d, J = 17.0 Hz, 1H), 3.29 – 2.92 (m, 6H), 2.90 – 2.73 (m, 2H), 2.16 – 1.64 (m, 10H), 1.54 – 1.43 (m, 2H), 1.38 – 1.28 (m, 12H). MSI (ESI): 397.8 $[M+2H]^{2+}$. t_R= 3.46 min.



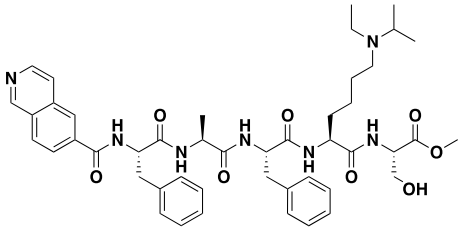
UNC6220

methyl N^6-ethyl-N^6-isopropyl-N^2-nicotinoyl-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. Nicotinic acid (3.0 mg 0.02 mmol, 1 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (8mg, 0.02 mmol, 1 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (9 mg, 6 uL, 0.06 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (20 mg, 0.02 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH (5 mL) and acetone (7 mg, 9 uL, 0.1 mmol, 5 eq) and sodium cyanoborohydride (3 mg, 0.05 mmol, 2 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (3.2 mg, 4 uL, 0.07 mmol, 3 eq) and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound (12 mg, 64% across 3 steps) as a white powder TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.91 (s, 1H), 8.72 (dd, *J* = 5.1, 1.6 Hz, 1H), 8.31 – 8.25 (m, 1H), 7.69 – 7.60 (m, 1H), 7.31 – 7.15 (m, 10H), 4.82 – 4.75 (m, 1H), 4.58 – 4.40 (m, 2H), 4.25 (q, *J* = 7.1 Hz, 1H), 3.92 (dd, *J* = 11.2, 4.8 Hz, 2H), 3.79 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.73 (s, 3H), 3.70 – 3.65 (m, 1H), 3.28 – 3.09 (m, 5H), 3.07 – 2.96 (m, 3H), 1.98 – 1.67 (m, 4H), 1.53 – 1.43 (m, 2H), 1.37 – 1.27 (m, 12H). MSI (ESI): 394.8 $[M+2H]^{2+}$. t_R= 6.93 min.



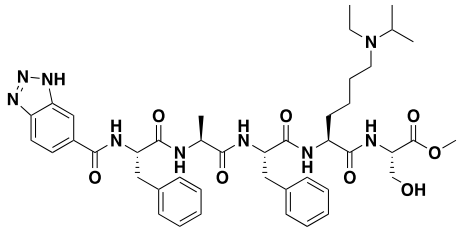
UNC6139

methyl N^2-(4-(dimethylamino)benzoyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. 4-(dimethylamino)benzoic acid (15.0 mg 0.05 mmol, 0.9 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (27 mg, 0.08 mmol, 1.4 eq) were dissolved in DMF (2 mL) followed by the addition of DIPEA (11 mg, 15 uL, 0.08 mmol, 1.4 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (50 mg, 0.06 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine (15 mg, 0.02 mmol, 1 eq) was dissolved in MeOH (5 mL) and acetone (3 mg, 4 uL, 0.05 mmol, 3 eq) and sodium cyanoborohydride (3.4 mg, 0.06 mmol, 3 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (1 mg, 1.1 uL, 0.02 mmol, 1 eq) and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound (8 mg, 40% across 3 steps. ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.75 – 7.67 (m, 2H), 7.42 – 7.04 (m, 10H), 6.75 (d, J = 9.0 Hz, 2H), 4.71 – 4.39 (m, 4H), 4.24 – 4.10 (m, 1H), 3.96 – 3.75 (m, 2H), 3.73 (s, 3H), 3.62 (p, J = 6.5 Hz, 1H), 3.28 – 2.86 (m, 14H), 1.99 – 1.38 (m, 6H), 1.37 – 1.16 (m, 12H). MSI(ESI): 415.8 $[M+2H]^{2+}$, 831 $[M+H]^+$. t_R= 4.69 min.



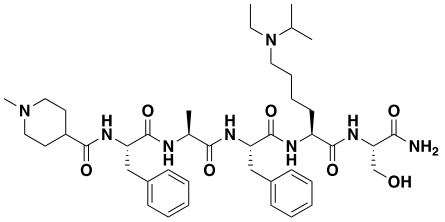
UNC6142

methyl N^6-ethyl-N^6-isopropyl-N^2-(isoquinoline-6-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. iloquinoline-6-carboxylic acid (7.3 mg 0.04 mmol, 1 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (19 mg, 0.06 mmol, 1.4 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (15 mg, 20 uL, 0.1 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (35 mg, 0.04 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine (10 mg, 0.01 mmol) was dissolved in MeOH (5 mL) and acetone (2 mg, 3 uL, 0.04 mmol, 3 eq) and sodium cyanoborohydride (3 mg, 0.02 mmol, 2 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (1.7 mg, 2 uL, 0.04 mmol, 3 eq) and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH) to yield the title compound (4 mg, 13% across 3 steps) as a white powder TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 9.68 (s, 1H), 8.62 (d, *J* = 6.3 Hz, 1H), 8.48 (d, *J* = 1.5 Hz, 1H), 8.43 (s, 1H), 8.35 (d, *J* = 6.3 Hz, 1H), 8.14 (dd, *J* = 8.7, 1.6 Hz, 1H), 7.60 – 6.82 (m, 10H), 4.73 – 4.42 (m, 3H), 4.38 – 4.17 (m, 1H), 3.91 (dd, *J* = 11.3, 4.6 Hz, 1H), 3.79 (dd, *J* = 11.4, 3.9 Hz, 1H), 3.73 (s, 3H), 3.71 – 3.65 (m, 1H), 3.27 – 2.91 (m, 9H), 2.04 – 1.70 (m, 4H), 1.55 – 1.39 (m, 2H), 1.34 – 1.25 (m, 12H). MSI (ESI): 419.8 $[M+2H]^{2+}$. t_R= 4.49 min.



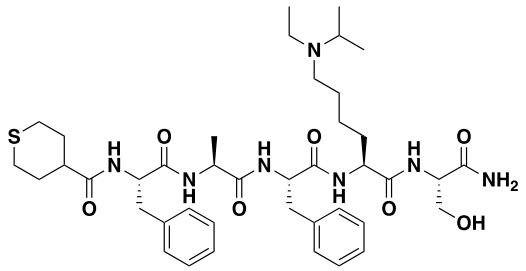
UNC6257

methyl N^2-(1*H*-benzo[*d*][1,2,3]triazole-6-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. 1H-benzo[d][1,2,3]triazole-5-carboxylic acid (8.0 mg, 0.05 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (15 mg, 0.07 mmol, 1.4 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (18 mg, 24 uL, 0.15 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (40 mg, 0.05 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH (5 mL) and acetone (9mg, 10 uL, 0.15 mmol, 3 eq) and sodium cyanoborohydride (9 mg, 0.14 mmol, 3 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (6 mg, 7 uL, 0.14 mmol, 3 eq) was added and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound (18 mg, 45% across 3 steps) as a white powder TFA salt. ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.34 (s, 1H), 7.86 (d, J = 1.5 Hz, 2H), 7.44 – 7.04 (m, 10H), 4.79 – 4.73 (m, 1H), 4.57 – 4.42 (m, 3H), 4.23 (q, J = 7.1 Hz, 1H), 3.90 (dd, J = 11.3, 4.6 Hz, 1H), 3.78 (dd, J = 11.4, 3.9 Hz, 1H), 3.72 (s, 3H), 3.63 (dt, J = 20.5, 6.9 Hz, 1H), 3.29 – 2.97 (m, 8H), 1.95 – 1.65 (m, 4H), 1.47 (q, J = 7.9 Hz, 2H), 1.41 – 1.21 (m, 12H). MSI (ESI): Found: 414.8 $[M+H]^{2+}$. t_R= 4.43 min.



UNC5430

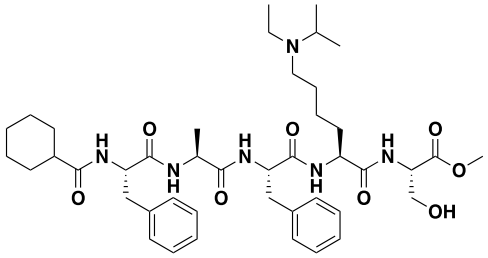
methyl N6-ethyl-N6-isopropyl-N2-(1-methylpiperidine-4-carbonyl)-L-phenylalanyl-L-alanyl-L-phenylalanyl-L-lysyl-L-serinate. UNC5430 was prepared via solid phase peptide synthesis with standard Fmoc-Amino Acids, Fmoc-Lys(et,ipr), and 1-methylpiperidine-4-carboxylic acid, all coupled on solid support. ^{1}H NMR (400 MHz, Methanol-*d*4) ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.32 – 7.16 (m, 10H), 4.63 – 4.49 (m, 2H), 4.42 – 4.35 (m, 2H), 4.28 – 4.20 (m, 1H), 3.87 – 3.75 (m, 2H), 3.72 – 3.61 (m, 1H), 3.55 – 3.41 (m, 2H), 3.28 – 3.07 (m, 5H), 3.07 – 2.87 (m, 4H), 2.86 – 2.74 (m, 4H), 2.53 – 2.41 (m, 1H), 2.04 – 1.58 (m, 9H), 1.52 – 1.39 (m, 2H), 1.36 – 1.29 (m, 9H), 1.29 – 1.25 (d, *J* = 7.2 Hz, 3H).). MSI (ESI): 794.4 $[M+H]^+$. t_R= 2.28 min.



UNC5432

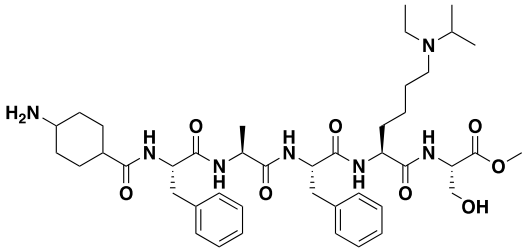
methyl *N*^{6}-ethyl-*N*^{6}-isopropyl-*N*^{2}-(tetrahydro-2*H*-thiopyran-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. UNC542 was prepared via solid phase peptide synthesis with standard Fmoc-Amino Acids, Fmoc-Lys(et,ipr), and tetrahydro-2*H*-thiopyran-4-carboxylic acid, all coupled on solid support. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.31 – 7.18 (m, 10H), 4.63 – 4.49 (m, 2H), 4.48 – 4.35 (m, 2H), 4.27 – 4.16 (m, 1H), 3.87 – 3.75 (m, 2H), 3.71 – 3.63 (m, 1H), 3.25 – 2.98 (m, 7H), 2.89 – 2.80 (m, 1H), 2.69 – 2.46 (m, 4H), 2.25 (tt, *J* = 11.5, 3.2 Hz, 1H), 2.03 – 1.86 (m, 2H),

1.83 – 1.52 (m, 6H), 1.50 – 1.41 (m, 2H), 1.36 – 1.22 (m, 12H). MSI (ESI): 796.4 $[M+H]^+$. t_R= 3.16 min.



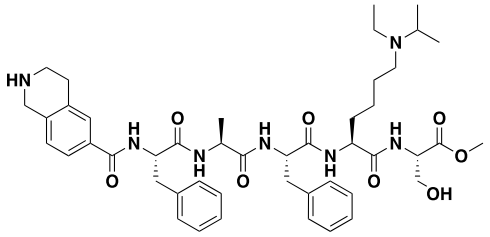
UNC5247

methyl N^2-(cyclohexanecarbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. UNC5247 was synthesized by solid phase peptide synthesis with the following changes. 2-chlorotrityl chloride resin was used and the first amino acid coupled was Fmoc-Lys(et,ipr) followed by standard Fmoc-amino acids and cyclohexanecarboxylic acid as the N-terminal cap residue. Following cleavage from resin (10% acetic acid, 10% trifluoroethanol, 80% DCM), the C-terminal carboxylic acid intermediate was purified by HPLC Chromatography (water + 0.1 trifluoroacetic acid; acetonitrile). H-Ser(tBu)-OMe was pretreated with DIPEA in DMF while the carboxylic acid peptide intermediate was preactivated with 1.2 equiv HBTU and 2 equiv DIPEA in DMF (2 mL) for 3 min. The H-Ser(tBu)-OMe solution was added and the reaction was left to stir overnight followed by concentration of the reaction. The crude mixture and was then dissolved in 50:50 TFA:DCM for 4 hours at RT followed by HPLC chromatography (water + 0.1 trifluoroacetic acid; acetonitrile). The product was concentrated and dried to obtain title compound as a TFA salt. (ESI): 793.5 $[M+H]^+$. t_R= 4.64 min.



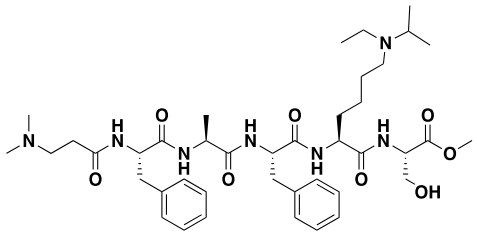
UNC6108

methyl N^2-(4-aminocyclohexane-1-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. 4-((tert-butoxycarbonyl)amino)cyclohexane-1-carboxylic acid (24 mg, 0.1 mmol, 0.9 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (50 mg, 0.16 mmol, 1.4 eq) were dissolved in DMF (5 mL) followed by the addition of DIPEA (40 mg, 54 uL, 0.32 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (93 mg, 0.05 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH, acetone (19 mg, 24 uL. 0.32 mmol, 3 eq) and sodium cyanoborohydride (27 mg, 0.43 mmol, 4 eq.) were added and the mixture stirred overnight. Following confirmation of completion by LC-MS, acetaldehyde (14 mg, 18 uL, 0.32 mmol, 3 eq) was added and the reaction proceeded for 2 hours. Following confirmation of completion by LC-MS, the mixture was concentrated under vacuum. The crude mixture was dissolved in 20% TFA in DCM and stirred for 2 hours followed by concentration under vacuum and purification by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (22 mg, 25% across 4 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.39 – 7.11 (m, 10H), 4.65 – 4.41 (m, 4H), 4.26 (td, J = 7.1, 5.0 Hz, 1H), 3.94 (dd, J = 11.3, 4.7 Hz, 1H), 3.81 (dd, J = 11.3, 3.9 Hz, 1H), 3.74 (s, 3H), 3.74 – 3.64 (m, 1H), 3.29 – 2.95 (m, 8H), 2.87 – 2.76 (m, 1H), 2.51 – 2.34 (m, 1H), 1.97 – 1.42 (m, 14H), 1.36 – 1.29 (m, 9H), 1.28 (d, J = 7.1 Hz, 3H). MS (ESI): 404.9$[M+2H]^{2+}$, 808.5 $[M+H]^+$. t_R= 3.42 min.



UNC6263

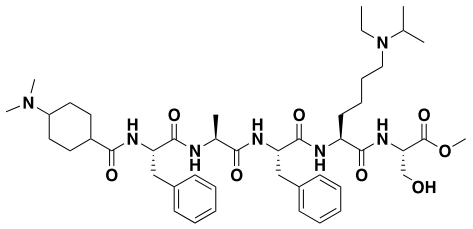
methyl N^6-ethyl-N^6-isopropyl-N^2-(1,2,3,4-tetrahydroisoquinoline-6-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. 2-(*tert*-butoxycarbonyl)-1,2,3,4-tetrahydroisoquinoline-6-carboxylic acid (8.5 mg, 0.03 mmol) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (13 mg, 0.04 mmol, 1.3 eq) were dissolved in DMF (1 mL) followed by the addition of DIPEA (10 mg, 14 uL, 0.08 mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (29 mg, 0.03 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH (5 mL) and acetone (6 mg, 7 uL, 0.1 mmol, 3 eq) and sodium cyanoborohydride (7 mg, 0.1 mmol, 3 eq) and the reaction proceeded overnight. Following confirmation of completion by LC-MS, acetaldehyde (4 mg, 5 uL, 0.1 mmol, 3 eq) was added and the reaction proceeded for 2 hours and was then concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield a boc-protected intermediate that was dissolved in 20% TFA in DCM for 2 hours, concentrated, and purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (11 mg, 48% across 4 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.65 – 7.58 (m, 2H), 7.35 – 7.11 (m, 11H), 4.79 – 4.69 (m, 1H), 4.57 – 4.40 (m, 3H), 4.38 (s, 2H), 4.24 (q, J = 7.1 Hz, 1H), 3.90 (dd, J = 11.3, 4.7 Hz, 1H), 3.78 (dd, J = 11.3, 3.9 Hz, 1H), 3.71 (s, 3H), 3.71 – 3.58 (m, 1H), 3.49 (t, J = 6.4 Hz, 2H), 3.27 – 2.93 (m, 10H), 1.95 – 1.59 (m, 4H), 1.46 (q, J = 7.8 Hz, 2H), 1.38 – 1.28 (m, 9H), 1.25 (d, J = 7.2 Hz, 3H). MSI (ESI): Found 421.8 $[M+2H]^{2+}$. t_R= 6.48 min.



UNC6231

methyl N^2-(3-(dimethylamino)propanoyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. 3-((tert-butoxycarbonyl)amino)propanoic acid (9.0 mg, 0.05 mmol, 0.9eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (24 mg, 0.07 mmol, 1.4 eq) were dissolved in DMF (2 mL) followed by the addition of DIPEA (20 mg, 26 uL, 0.15mmol, 3 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (50 mg, 0.03 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated amine was dissolved in methanol (10 mL) and acetone (9 mg, 12 uL, 0.16 mmol, 3 eq) and sodium cyanoborohydride (10 mg, 0.16 mmol, 3 eq) was added and the reaction proceeded overnight followed by the addition of acetaldehyde (7 mg, 9 uL, 0.16 mmol, 3 eq) was added and the reaction proceeded for 2 hours. Following confirmation of completion by LC-MS, the mixture was concentrated under vacuum. The crude mixture was dissolved in 20% TFA in DCM and stirred for 2 hours followed by concentration under vacuum and was purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH). The free amine was dissolved in MeOH (5 mL) followed by the addition of formaldehyde (2 mg, 2 eq, 7 uL of 30% solution, 0.03 mmol) and sodium cyanoborohydride (5 mg, 0.07 mmol, 4 eq) and stirred for 2 hours, concentrated under vacuum and purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (6 mg 15% across 5 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.41 – 7.16 (m, 10H), 4.64 – 4.39 (m, 4H), 4.31-4.23 (m, 1H), 3.93 (dd, J = 11.3, 4.7 Hz, 1H), 3.80 (dd, J = 11.3, 3.8 Hz, 1H), 3.74 (s, 3H), 3.69 (p, J = 6.6 Hz, 1H), 3.29 – 2.96 (m, 9H), 2.89 – 2.53

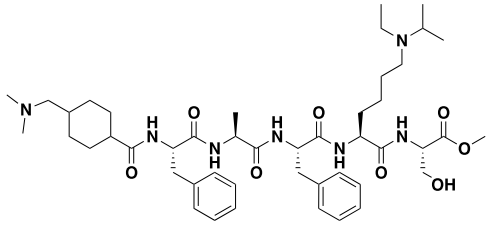
(m, 9H), 2.04-1.65 (m,, 4H), 1.47 (p, J = 7.5 Hz, 2H), 1.40 – 1.21 (m, 12H). MS (ESI): Found: 391.8 $[M+2H]^{2+}$. t_R= 4.77 min.



UNC6109

methyl N^2-(4-(dimethylamino)cyclohexane-1-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. 4-((tert-butoxycarbonyl)amino)cyclohexane-1-carboxylic acid (24 mg, 0.1 mmol, 0.9 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (50 mg, 0.16 mmol, 1.4 eq) were dissolved in DMF (5 mL) followed by the addition of DIPEA (40 mg, 54 uL, 0.32 mmol, 3 eq) and the mixture stirred for 15 minutes. . **Intermediate 4** (93 mg, 0.05 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine was dissolved in MeOH and acetone (19 mg, 24 uL. 0.32 mmol, 3 eq) and sodium cyanoborohydride (27 mg, 0.43 mmol, 4 eq.) were added and the mixture stirred overnight. Following confirmation of completion by LC-MS, acetaldehyde (14 mg, 18 uL, 0.32 mmol, 3 eq) was added and the reaction proceeded for 2 hours. Following confirmation of completion by LC-MS, the mixture was concentrated under vacuum. The crude mixture was dissolved in 20% TFA in DCM and stirred for 2 hours followed by concentration under vacuum and was purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH). The free amine (15 mg 0.02 mmol, 1 eq) was dissolved in MeOH (5 mL) followed by the addition of formaldehyde (3 mg, 5 eq, 10 uL of 30% solution, 0.09 mmol) and sodium cyanoborohydride (5 mg, 0.07 mmol, 4 eq) and stirred for 2 hours, concentrated under vacuum and

purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (7 mg, 8% across 5 steps. ^{1}H NMR (400 MHz, Methanol-*d*4) δ 7.32 – 7.16 (m, 10H), 4.70 – 4.39 (m, 4H), 4.33 – 4.21 (q, *J* = 7.4 Hz, 1H), 3.98 – 3.88 (m, 1H), 3.86 – 3.78 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.75 – 3.72 (s, 3H), 3.72 – 3.64 (m, 1H), 3.27 – 2.97 (m, 8H), 2.80 – 2.75 (m, 1H), 2.74 – 2.71 (s, 6H), 2.53 – 2.47 (m, 1H), 2.11 – 1.84 (m, 3H), 1.84 – 1.69 (m, 4H), 1.65 – 1.42 (m, 3H), 1.37 – 1.30 (m, 10H), 1.31 – 1.27 (d, *J* = 7.1 Hz, 3H). MS (ESI): Found: 418.9 $[M+2H]^{2+}$. t_R= 3.45 min.

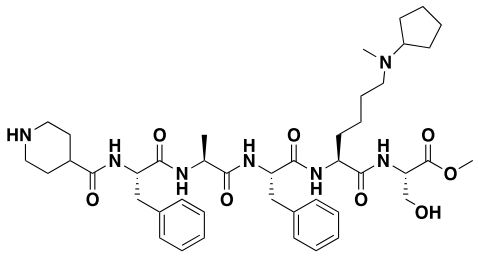


UNC6141

methyl N^2-(4-((dimethylamino)methyl)cyclohexane-1-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-ethyl-N^6-isopropyl-*L*-lysyl-*L*-serinate. 4-(((*tert*-butoxycarbonyl)amino)methyl)cyclohexane-1-carboxylic acid (15 mg, 0.06 mmol, 1 eq) and 2-(1H-benzo[d][1,2,3]triazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (27 mg, 0.08 mmol, 1.4 eq) were dissolved in DMF (2 mL) followed by the addition of DIPEA (11 mg, 15 uL, 0.08 mmol, 1.5 eq) and the mixture stirred for 15 minutes. **Intermediate 4** (50 mg, 0.06 mmol 1 eq) was added and the reaction stirred overnight at room temperature. The mixture was concentrated under reduced pressure and dissolved in 20% DEA in DMF and stirred at room temperature for 2 hours. The mixture was concentrated under reduced pressure and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH). The isolated free amine (15 mg, 0.02 mmol) was dissolved in methanol (5 mL) and acetone (3.1 mg, 5 uL, 0.05 mmol, 3 eq) and sodium cyanoborohydride (3.4 mg, 0.05 mmol, 3 eq) were added and the reaction proceeded overnight followed by the addition of acetaldehyde (1 mg, 2 uL, 0.02 mmol, 1 eq) and the reaction proceeded for 2 hours. Following confirmation of completion

by LC-MS, the mixture was concentrated under vacuum. The crude mixture was dissolved in 20% TFA in DCM and stirred for 2 hours followed by concentration under vacuum and purification by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH). The free amine (5 mg 0.005 mmol, 1 eq) was dissolved in MeOH (1 mL) followed by the addition of formaldehyde (2 mg, 5 eq, 9 uL of 30% solution, 0.02 mmol) and sodium cyanoborohydride (1 mg, 0.02 mmol, 4 eq) and stirred for 2 hours, concentrated under vacuum and purified by reverse phase flash chromatography(H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (3.5 mg 15% across 5 steps.) ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.34 – 7.16 (m, 10H), 4.62 – 4.41 (m, 4H), 4.31 – 4.22 (m, 1H), 3.96 – 3.92 (m, 1H), 3.85 – 3.79 (m, 1H), 3.74 (s, 3H), 3.73 – 3.66 (m, 1H), 3.28 – 2.90 (m, 8H), 2.86 (s, 6H), 2.25 – 2.06 (m, 1H), 1.96 – 1.87 (m, 1H), 1.85 – 1.67 (m, 6H), 1.65-1.52 (m, 1H), 1.53-1.41 (m, 4H), 1.38-1.30 (m, 12H), 1.27 (d, J = 7.1 Hz, 3H), 1.13 – 1.00 (m, 2H). MS (ESI): 425.8 $[M+2H]^{2+}$. t_R= 3.59 min.

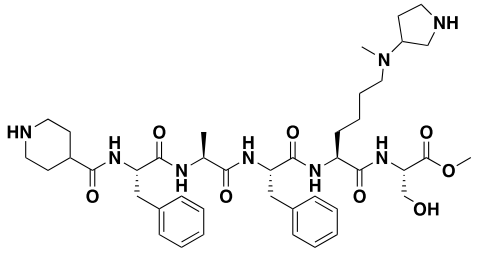
Compounds from Table 2



UNC6216

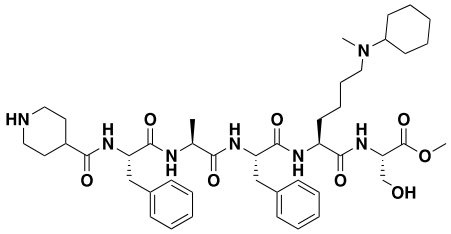
methyl *N*^{6}-cyclopentyl-*N*^{6}-methyl-*N*^{2}-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. Intermediate 5 (10 mg, 0.012 mmol, 1 eq) was dissolved in MeOH (5 mL)and cyclopentanone was added (2.0 mg, 2 uL, 0.024 mmol, 2 eq) followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, formaldehyde (1 mg, 3 uL of 30% solution, 0.024 mmol, 2 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was

concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (6 mg, 60% across 2 steps).^{1}H NMR (400 MHz, Methanol-d_4) δ 7.32 – 7.08 (m, 10H), 4.70 – 4.37 (m, 4H), 4.30 – 4.22 (m, 1H), 3.93 (dd, *J* = 11.3, 4.7 Hz, 1H), 3.81 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.74 (s, 3H), 3.67 – 3.57 (m, 1H), 3.43 – 3.34 (m, 1H), 3.28 – 2.90 (m, 8H), 2.84 (s, 3H), 2.83 – 2.77 (m, 1H), 2.56 – 2.47 (m, 1H), 2.24 – 2.08 (m, 2H), 1.97 – 1.39 (m, 16H), 1.28 (d, *J* = 7.1 Hz, 3H). MS (ESI) Found: 403.8[M+2H]$^{2+}$. t_R= 5.13 min.



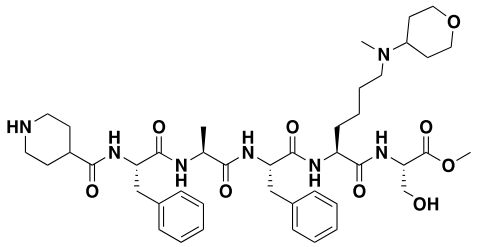
UNC6262

methyl *N*^{6}-methyl-*N*^{2}-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*N*^{6}-(pyrrolidin-3-yl)-*L*-lysyl-*L*-serinate. Intermediate 5 (10 mg, 0.012 mmol, 1 eq) was dissolved in MeOH (5 mL) and tert-butyl 3-oxopyrrolidine-1-carboxylate was added (4.5 mg, 0.024 mmol, 2 eq) followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, formaldehyde (1 mg, 3 uL of 30% solution, 0.024 mmol, 2 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (8 mg, 80% across 2 steps)^{1}H NMR (400 MHz, Methanol-d_4) δ 7.33 – 7.11 (m, 10H), 4.66 – 4.38 (m, 4H), 4.23 (q, *J* = 7.1 Hz, 1H), 4.18-4.07 (q, *J* = 7.9 Hz, 1H), 3.92 (dd, *J* = 11.3, 4.6 Hz, 1H), 3.84 – 3.74 (m, 2H), 3.72 (s, 3H), 3.65 – 3.53 (m, 2H), 3.36 (d, *J* = 12.2 Hz, 2H), 3.24 – 3.00 (m, 7H), 2.96 – 2.85 (m, 5H), 2.81 (dd, *J* = 14.1, 10.2 Hz, 1H), 2.59 – 2.45 (m, 2H), 2.36 – 2.22 (m, 1H), 1.96 – 1.39 (m, 10), 1.33 – 1.22 (m, 5). MS (ESI) Found: 404.3 [M+2H]$^{2+}$. t_R= 2.76 min.



UNC6217

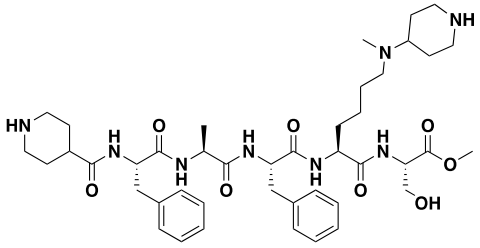
methyl N^6-cyclohexyl-N^6-methyl-N^2-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. Intermediate 5 (10 mg, 0.012 mmol, 1 eq) was dissolved in MeOH (5 mL) and cyclohexanone (3.6 mg, 0.036 mmol, 3 eq) followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, formaldehyde (1 mg, 3 uL of 30% solution, 0.024 mmol, 2 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (7 mg, 70% across 2 steps)^{1}H NMR (400 MHz, Methanol-d_4) δ 7.36 – 7.10 (m, 10H), 4.73 – 4.42 (m, 4H), 4.33 – 4.15 (m, 1H), 3.93 (dd, *J* = 11.3, 4.6 Hz, 1H), 3.81 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.74 (s, 3H), 3.42 – 3.34 (m, 1H), 3.29 – 2.87 (m, 10H), 2.85 – 2.75 (m, 4H), 2.58 – 2.46 (m, 1H), 2.10 – 1.88 (m, 6H), 1.95 – 1.67 (m, 6H), 1.63 – 1.36 (m, 6H), 1.31-1.15 (m, 4H). MS (ESI) Found: 410.8 $[M+H]^{2+}$. t_R= 5.60 min.



UNC6218

methyl N^6-methyl-N^2-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-N^6-(tetrahydro-2*H*-pyran-4-yl)-*L*-lysyl-*L*-serinate. Intermediate 5 (10 mg, 0.012 mmol, 1 eq) was

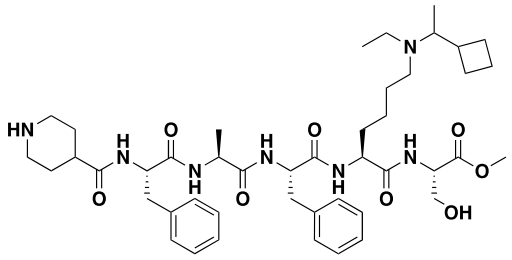
dissolved in MeOH (5 mL) and tetrahydro-4H-pyran-4-one (3.6 mg, 3.4 uL, 0.036 mmol, 3 eq) followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, formaldehyde (1 mg, 3 uL of 30% solution, 0.024 mmol, 2 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (6 mg, 60% across 2 steps)^{1}H NMR (400 MHz, Methanol-d_4) δ 7.33 – 7.19 (m, 10H), 4.68 – 4.38 (m, 4H), 4.26 (t, J = 6.9 Hz, 1H), 4.00-3.90 (m, , 2H), 3.94 (dd, J = 11.2, 4.6 Hz, 1H), 3.80 (dd, J = 11.4, 3.9 Hz, 1H), 3.74 (s, 3H), 3.54 – 3.36 (m, 4H), 3.26 – 2.87 (m, 8H), 2.83 (s, 3H), 2.83-2.76 (m, 4H) 2.57 – 2.44 (m, 1H), 2.02 – 1.43 (m, 14H), 1.28 (d, J = 7.1 Hz, 3H). MS (ESI) Found: 411.8 $[M+2H]^{2+}$. t_R= 5.30 min.



UNC6127

methyl N6-methyl-N6-(piperidin-4-yl)-N2-(piperidine-4-carbonyl)-L-phenylalanyl-L-alanyl-L-phenylalanyl-L-lysyl-L-serinate. Intermediate 5 (20 mg, 0.024 mmol, 1 eq) was dissolved in MeOH (5 mL) and tert-butyl 4-oxopiperidine-1-carboxylate (24 mg mg, 0.12 mmol, 5 eq) followed by sodium cyanoborohydride (5 mg, 0.72 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, formaldehyde (2 mg, 6 uL of 30% solution, 0.048 mmol, 2 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (10 mg, 50% across 2 steps).^{1}H NMR

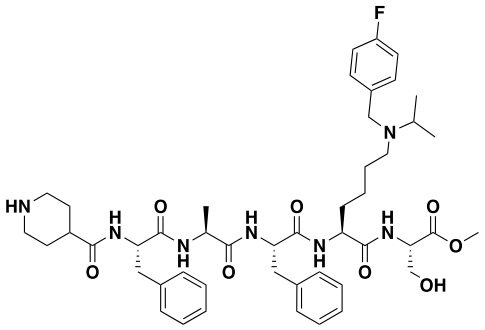
(400 MHz, Methanol-d_4) δ 7.35 – 7.10 (m, 10H), 4.68 – 4.38 (m, 4H), 4.32 – 4.18 (q, *J* = 7.1 Hz, 1H), 3.99 – 3.89 (dd, *J* = 11.3, 4.6 Hz, 1H), 3.88 – 3.77 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.77 – 3.70 (s, 3H), 3.77 – 3.55 (m, 3H), 3.41 – 3.35 (dt, *J* = 12.8, 4.0 Hz, 1H), 3.29 – 2.89 (m, 7H), 2.91 – 2.81 (s, 3H), 2.88 – 2.74 (m, 1H), 2.58 – 2.42 (m, 1H), 2.37 – 2.20 (m, 2H), 2.00 – 1.90 (m, 4H), 1.84 – 1.69 (m, 5H), 1.64 – 1.57 (td, *J* = 11.2, 3.9 Hz, 1H), 1.51 – 1.43 (q, *J* = 7.6 Hz, 2H), 1.33 – 1.23 (d, *J* = 7.1 Hz, 3H). MS (ESI) Found: 411.4 $[M+2H]^{2+}$. t_R= 5.30 min.



UNC6254

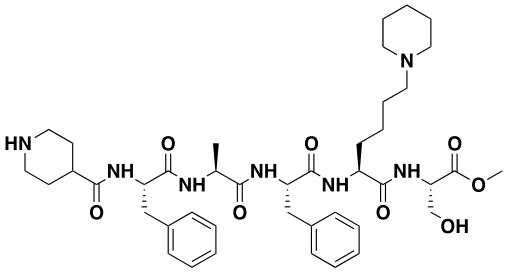
methyl N^6-(1-cyclobutylethyl)-N^6-ethyl-N^2-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. Intermediate 5 (10 mg, 0.012 mmol, 1 eq) was dissolved in MeOH (5 mL) and 1-cyclobutylethan-1-one (3.6 mg, 0.036 mmol, 3 eq) followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, formaldehyde (1 mg, 3 uL of 30% solution, 0.024 mmol, 2 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (6 mg, 60% across 2 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.31 – 7.14 (m, 10H), 4.68 – 4.38 (m, 4H), 4.30 – 4.21 (m, 1H), 3.97 – 3.89 (dd, *J* = 11.3, 4.7 Hz, 1H), 3.86 – 3.77 (dd, *J* = 11.3, 3.8 Hz, 1H), 3.77 – 3.72 (s, 3H), 3.55 – 3.45 (m, 1H), 3.42 – 3.33 (m, 1H), 3.26 – 3.14 (m, 4H), 3.13 – 2.90 (m, 6H), 2.84 – 2.76 (m, 1H), 2.74 – 2.63 (m, 1H), 2.58 – 2.47 (m, 1H), 2.14 – 2.02 (m, 1H), 1.97 – 1.87 (dd, *J* = 17.4, 7.3 Hz, 4H), 1.85 – 1.67

(m, 6H), 1.66 – 1.51 (m, 1H), 1.51 – 1.41 (d, *J* = 9.2 Hz, 2H), 1.38 – 1.30 (t, *J* = 7.3 Hz, 3H), 1.30 – 1.24 (m, 3H), 1.24 – 1.19 (m, 3H). MS (ESI) Found: 417.8 $[M+2H]^{+2}$. t_R= 6.70 min.



UNC6251

methyl *N*^{6}-(4-fluorobenzyl)-*N*^{6}-isopropyl-*N*^{2}-(piperidine-4-carbonyl)-*L*-phenylalanyl-*L*-alanyl-*L*-phenylalanyl-*L*-lysyl-*L*-serinate. Intermediate 5 (10 mg, 0.012 mmol, 1 eq) was dissolved in MeOH (5 mL) and acetone (1.4 mg, 0.024 mmol, 2 eq) was added followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. Upon confirmation of completion by LC-MS, 4-fluorobenzaldehyde (4.5 mg, 0.036 mmol, 3 eq) was added and the reaction stirred for 2 hours. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (5 mg, 50% across 2 steps)^{1}H NMR (400 MHz, Methanol-d_4) δ 7.66 – 7.48 (m, 2H), 7.36 – 7.09 (m, 13H), 4.65 – 4.55 (m, 1H), 4.55 – 4.44 (m, 2H), 4.45 – 4.35 (m, 2H), 4.32 – 4.15 (m, 2H), 3.95 – 3.89 (dd, *J* = 11.3, 4.7 Hz, 1H), 3.83 – 3.77 (dd, *J* = 11.3, 3.9 Hz, 1H), 3.74 – 3.71 (s, 3H), 3.71 – 3.64 (m, 1H), 3.42 – 3.34 (dt, *J* = 12.7, 4.0 Hz, 1H), 3.28 – 2.88 (m, 8H), 2.84 – 2.76 (m, 1H), 2.57 – 2.47 (m, 1H), 1.95 – 1.51 (m, 8H), 1.45 – 1.33 (m, 8H), 1.30 – 1.24 (m, 3H). MS (ESI) Found: 437.8 $[M+H]^{2+}$, 874.3 $[M+H]^+$. t_R= 5.74 min.

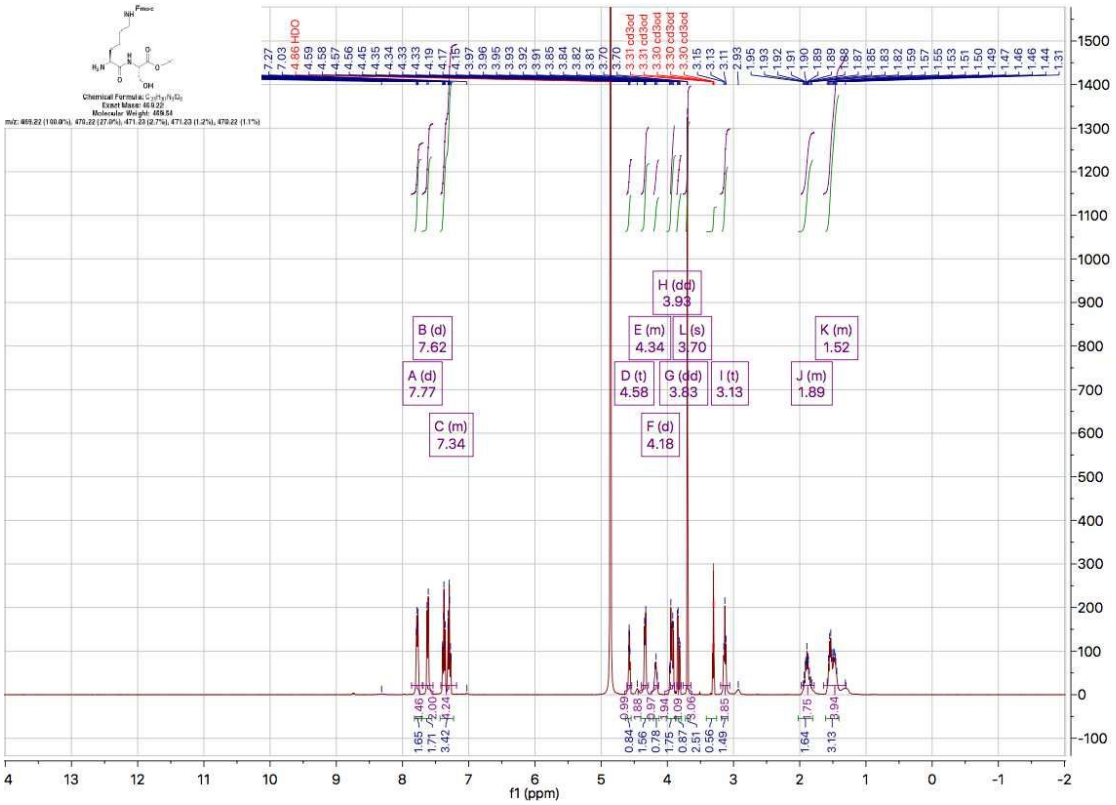


UNC6474

methyl ((*S*)-2-((*S*)-3-phenyl-2-((*S*)-2-((*S*)-3-phenyl-2-(piperidine-4-carboxamido)propanamido)propanamido)propanamido)-6-(piperidin-1-yl)hexanoyl)-*L*-serinate. **Intermediate 5** (13 mg, 0.016 mmol, 1 eq) was dissolved in MeOH (5 mL) and glutaraldehyde (3.2 mg, 3 uL 0.016 mmol, 1 eq) was added followed by sodium cyanoborohydride (2.5 mg, 0.036 mmol, 3 eq.) and the reaction stirred overnight. The mixture was concentrated under reduced pressure and the crude material was dissolved directly in 20% TFA in DCM and stirred overnight. The mixture was concentrated under vacuum and purified by reverse phase flash chromatography (H_2O +i0.1% TFA: MeOH) to yield the title compound as a white powder TFA salt (8 mg, 60% across 2 steps). ^{1}H NMR (400 MHz, Methanol-d_4) δ 7.33 – 7.16 (m, 10H), 4.65 – 4.58 (m, 1H), 4.58 – 4.47 (m, 2H), 4.47 – 4.39 (m, 1H), 4.32 – 4.19 (m, 1H), 3.96 – 3.89 (dd, J = 11.3, 4.7 Hz, 1H), 3.84 – 3.77 (dd, J = 11.3, 3.9 Hz, 1H), 3.75 – 3.70 (s, 3H), 3.56 – 3.47 (m, 2H), 3.41 – 3.34 (m, 1H), 3.28 – 2.77 (m, 11H), 2.59 – 2.46 (m, 1H), 1.97 – 1.65 (m, 11H), 1.65 – 1.38 (m, 4H), 1.31 – 1.24 (d, J = 7.1 Hz, 3H). MS (ESI): Found 396.8 $[M+2H]^{2+}$. t_R= 5.49 min.

NMR and LC-MS Spectra

Figure S12. Intermediate 1 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



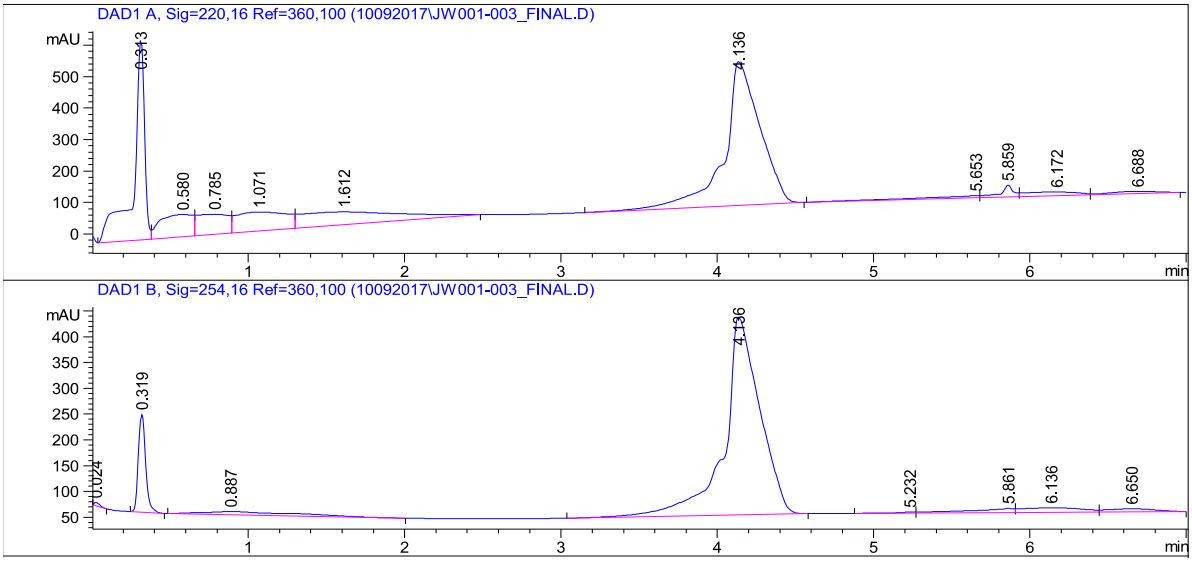
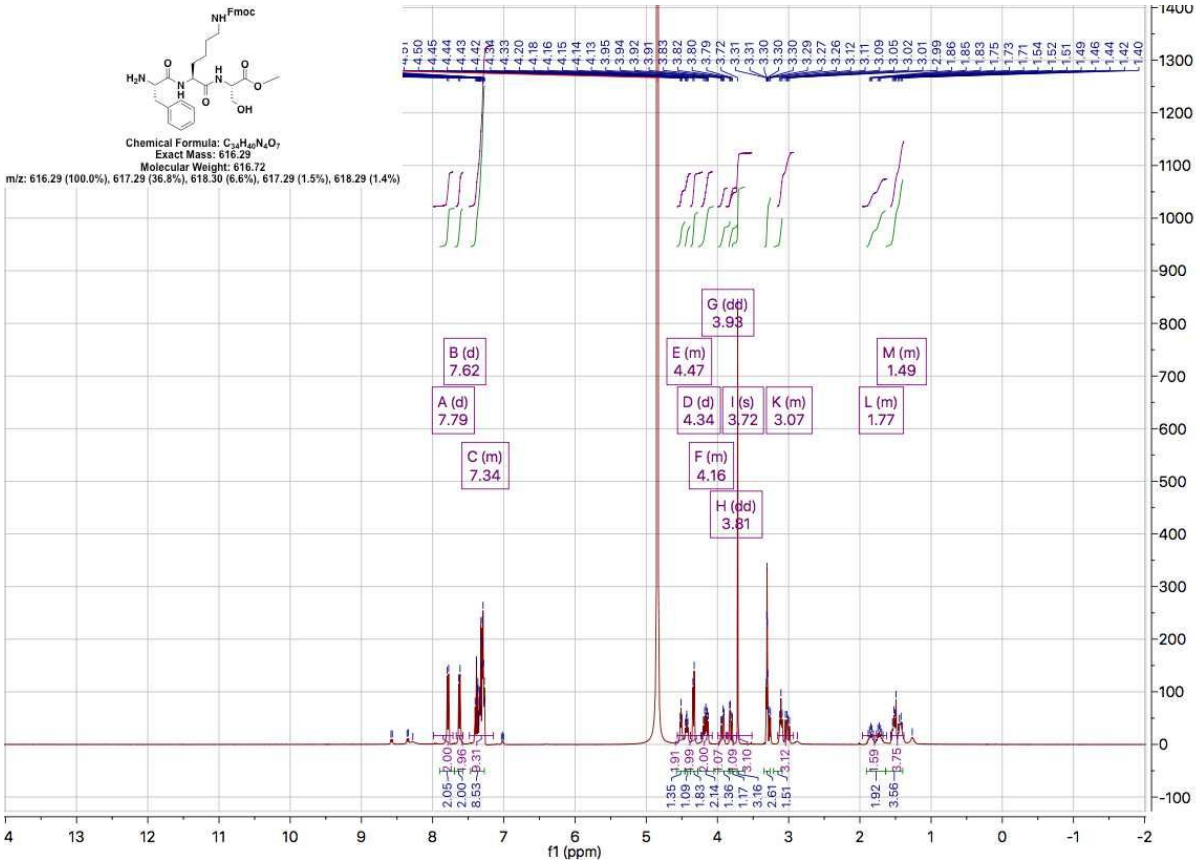


Figure S13. Intermediate 2 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



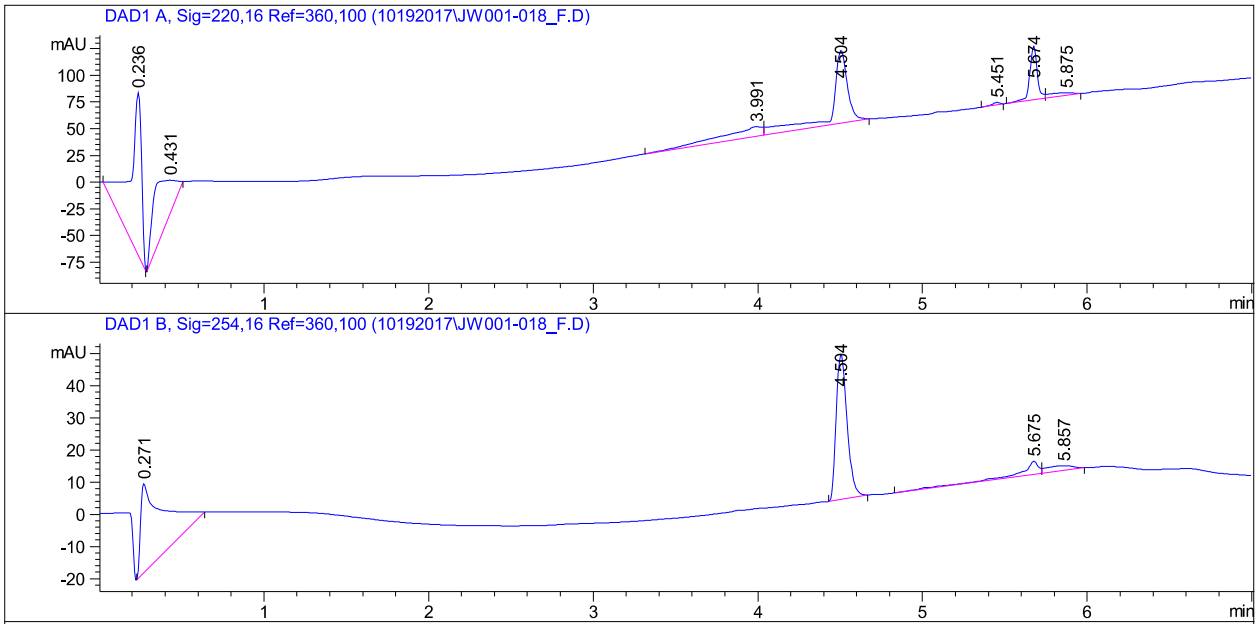
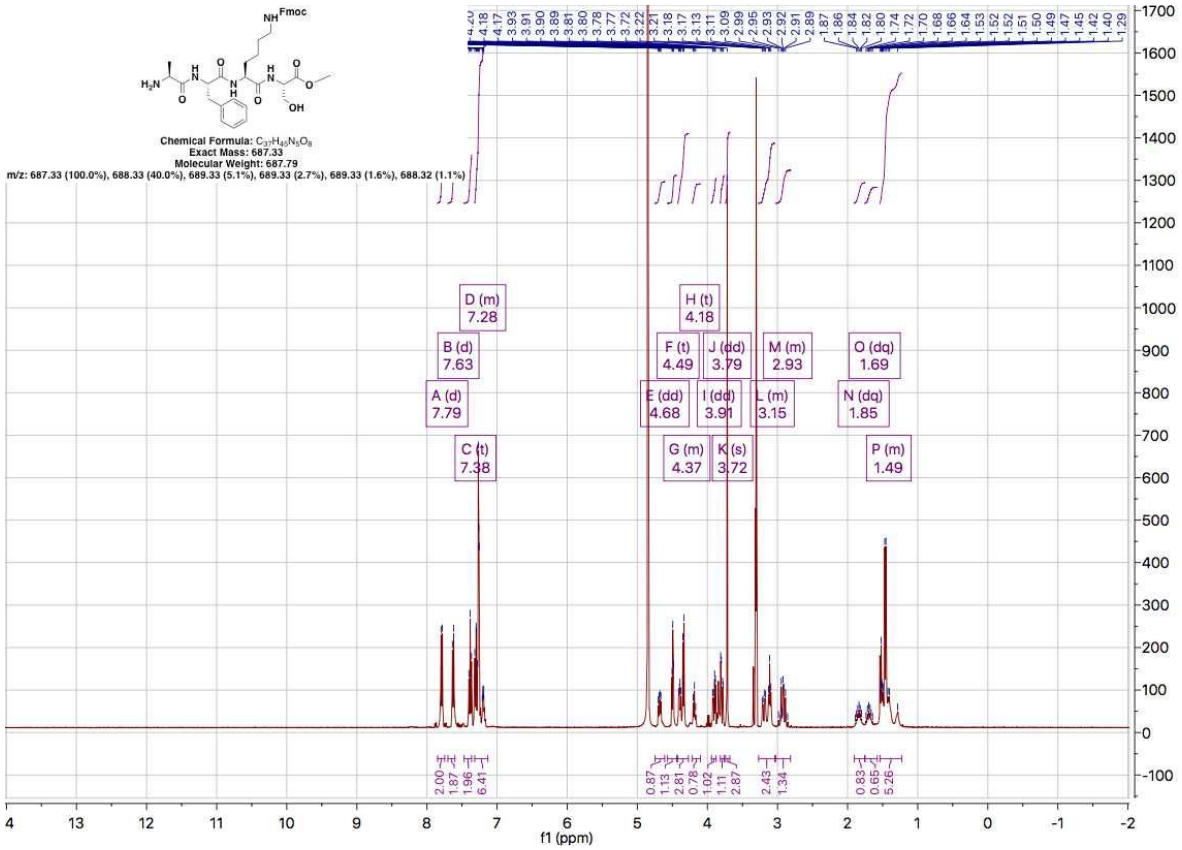


Figure S14. Intermediate 3 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



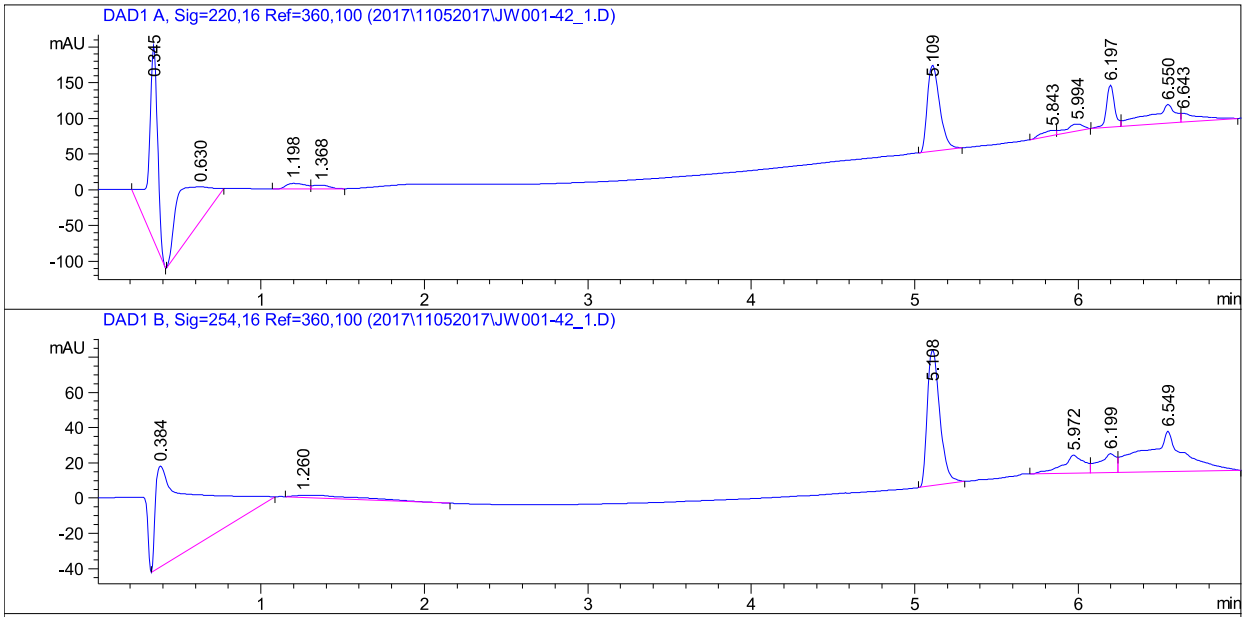
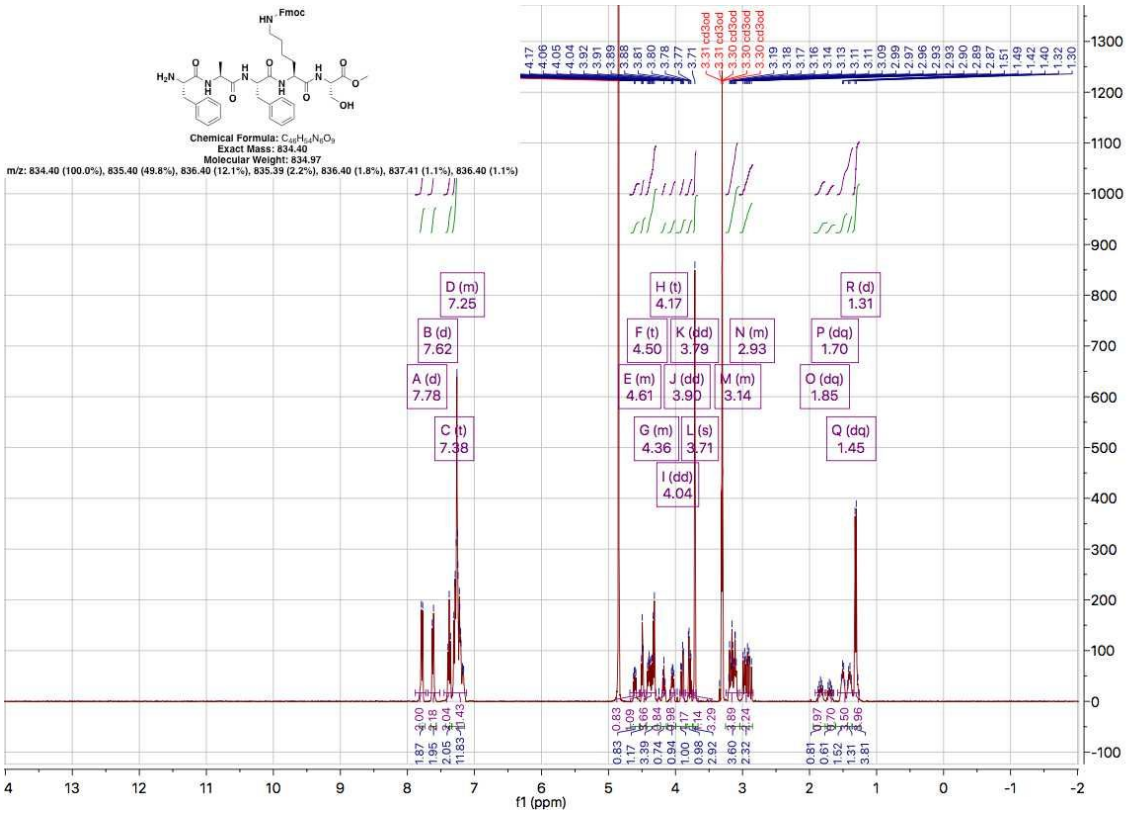


Figure S15. Intermediate 4 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



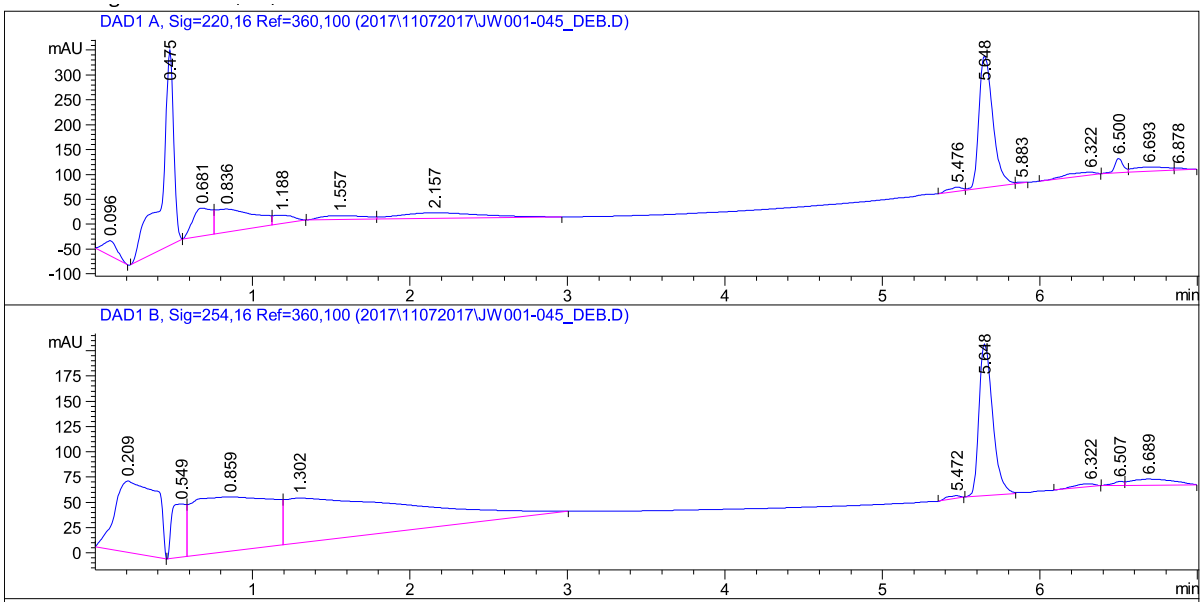
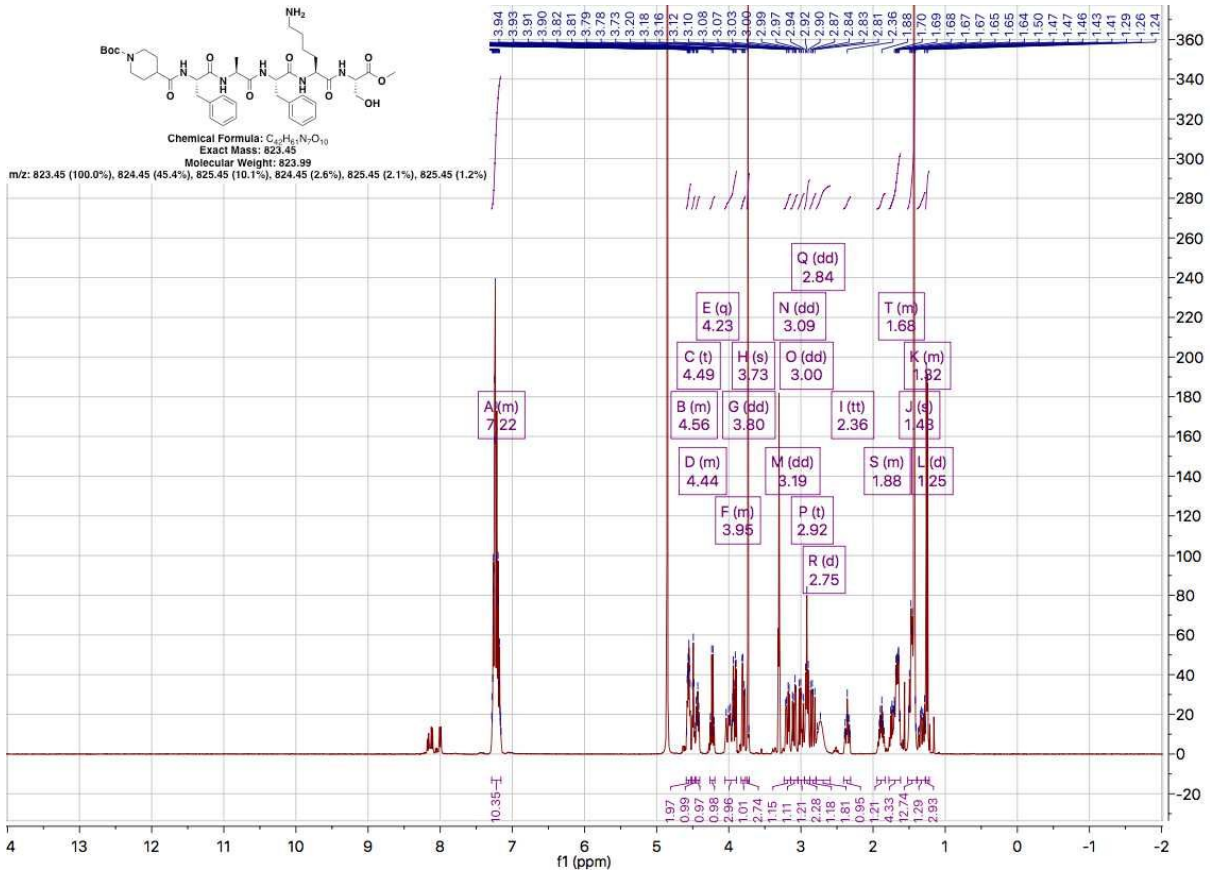


Figure S16. Intermediate 5 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



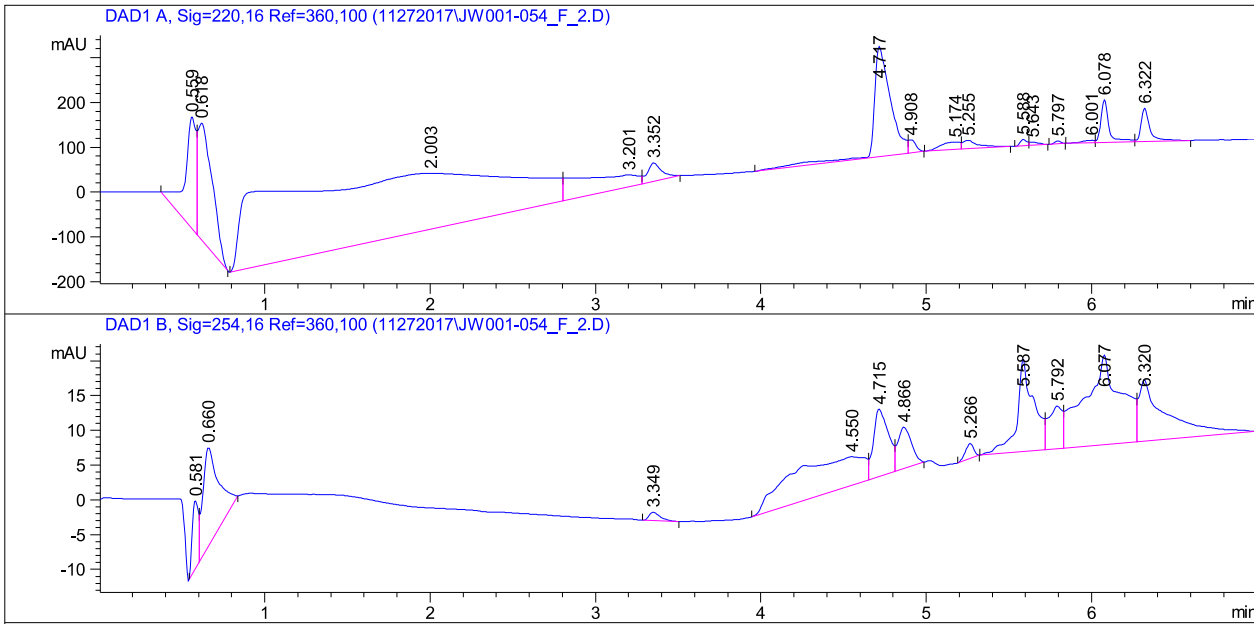
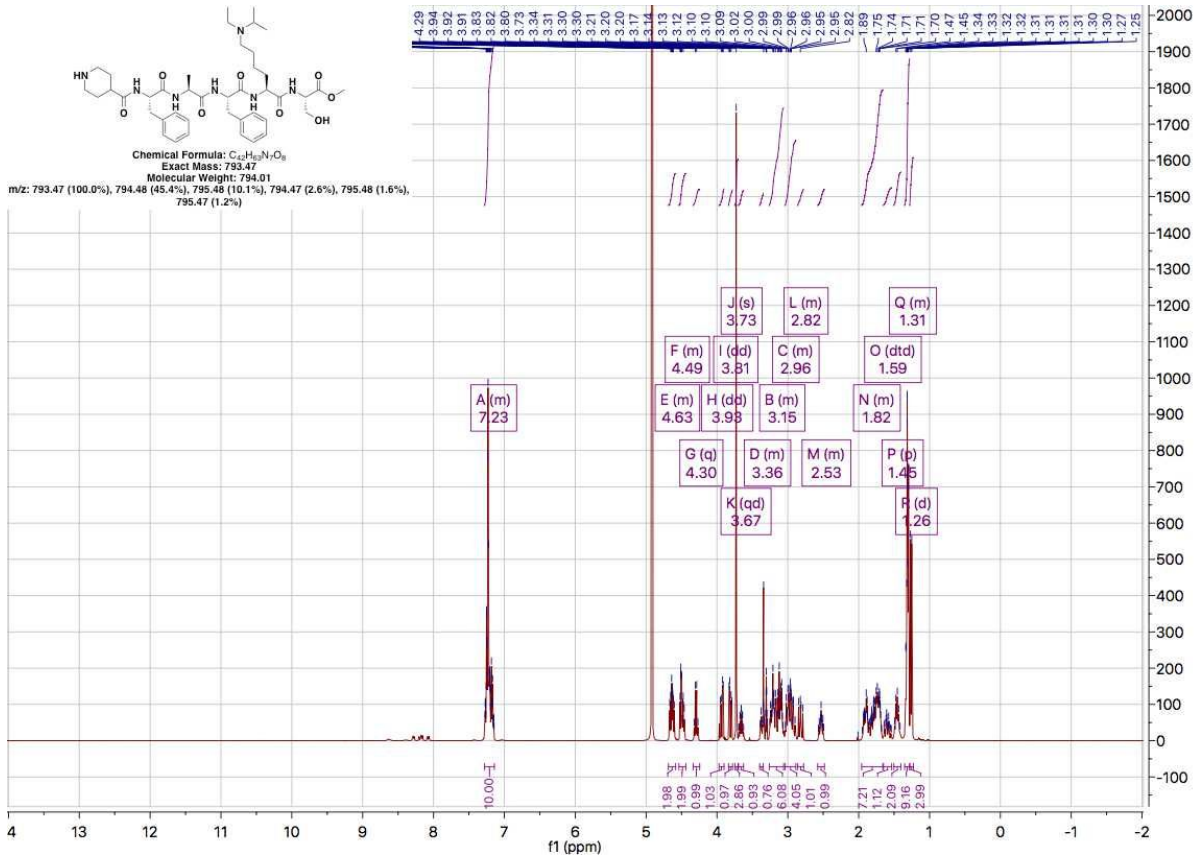
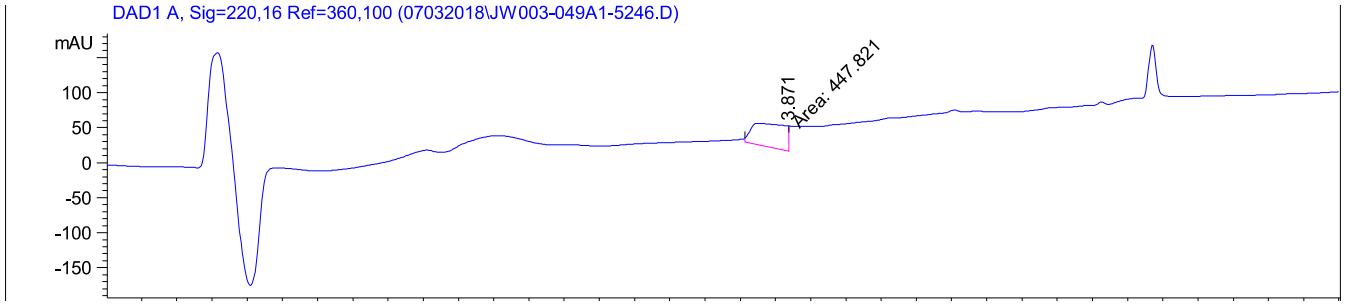
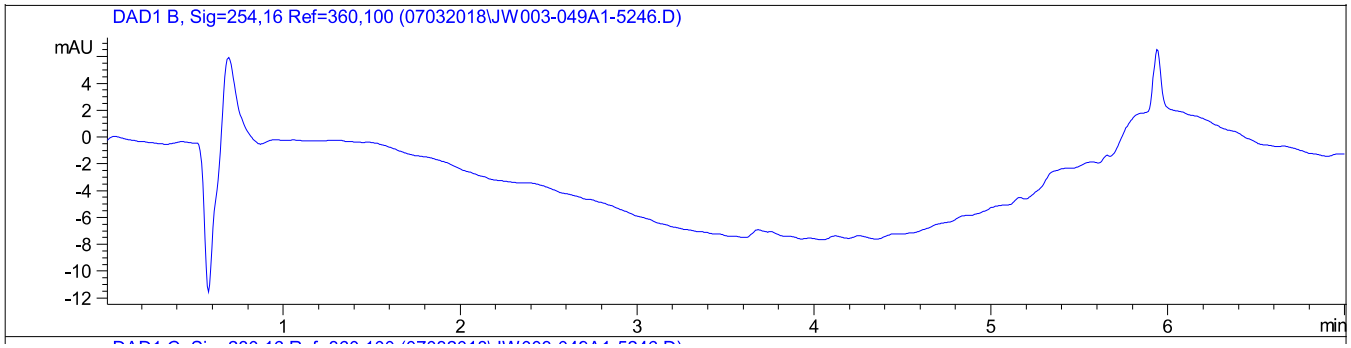


Figure S17. UNC5246 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM and TIC







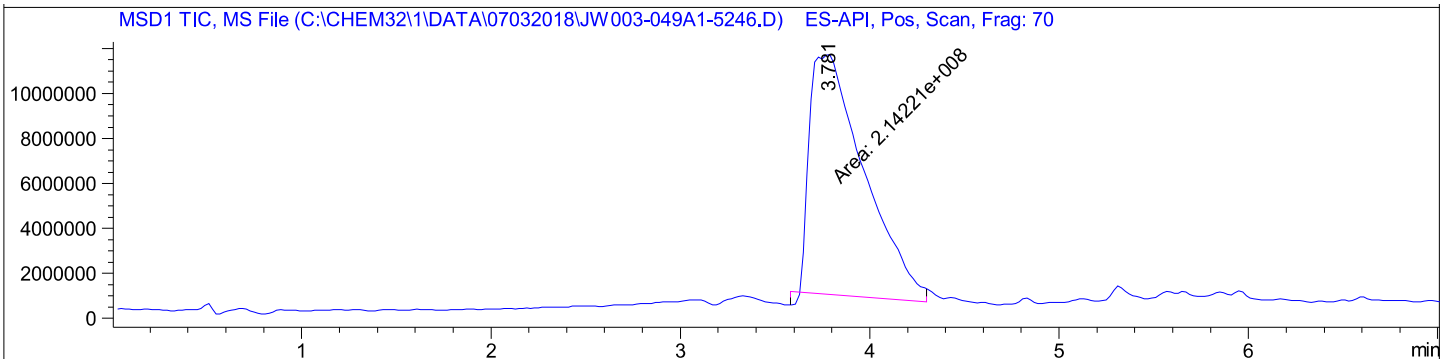
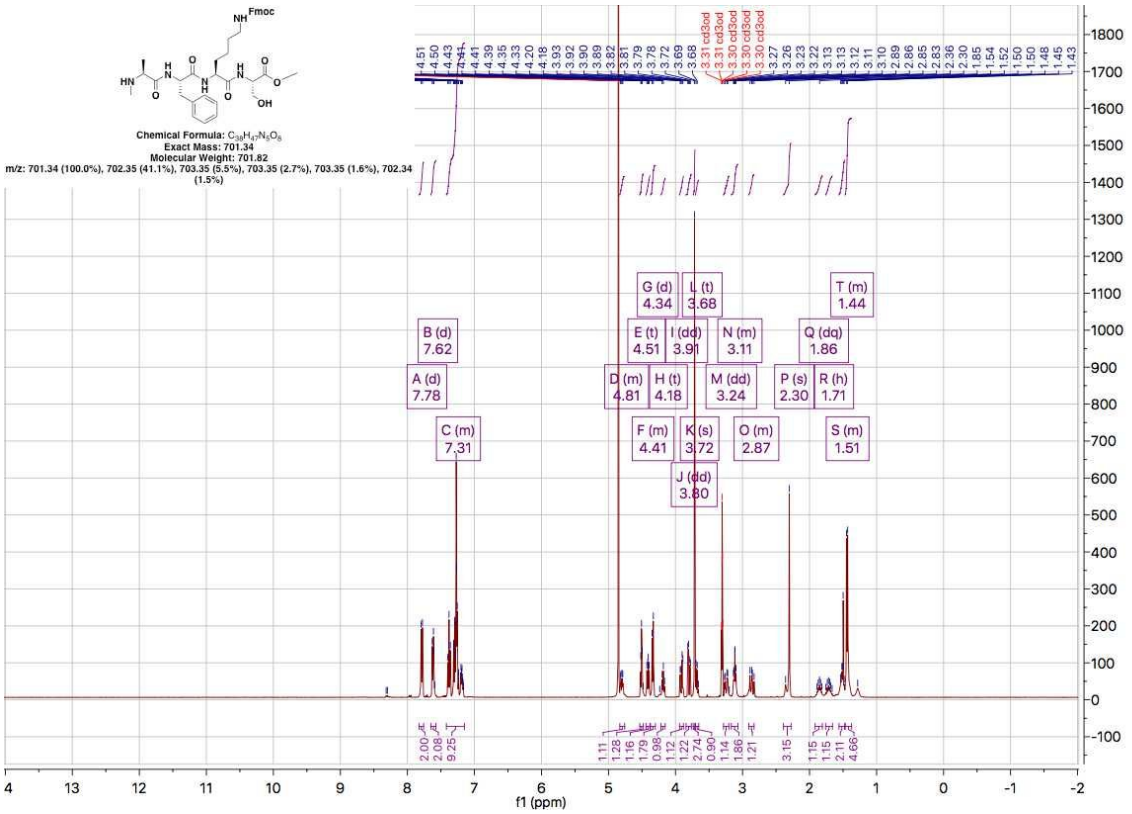

MSD1 TIC, MS File (C:\CHEM32\1\DATA\07032018\JW003-049A1-5246.D) ES-API, Pos, Scan, Frag: 70
10000000
8000000
6000000
4000000
2000000
0
3.781
Area: 2.14221e+008
1
2
3
4
5
6
min

Figure S18. Intermediate 6 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



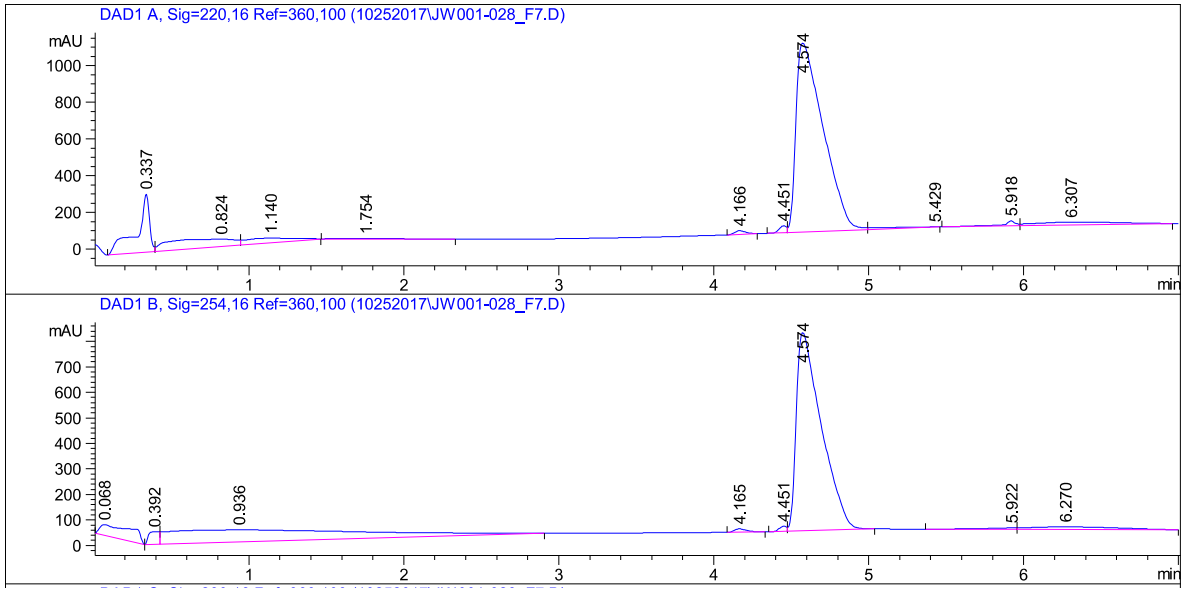
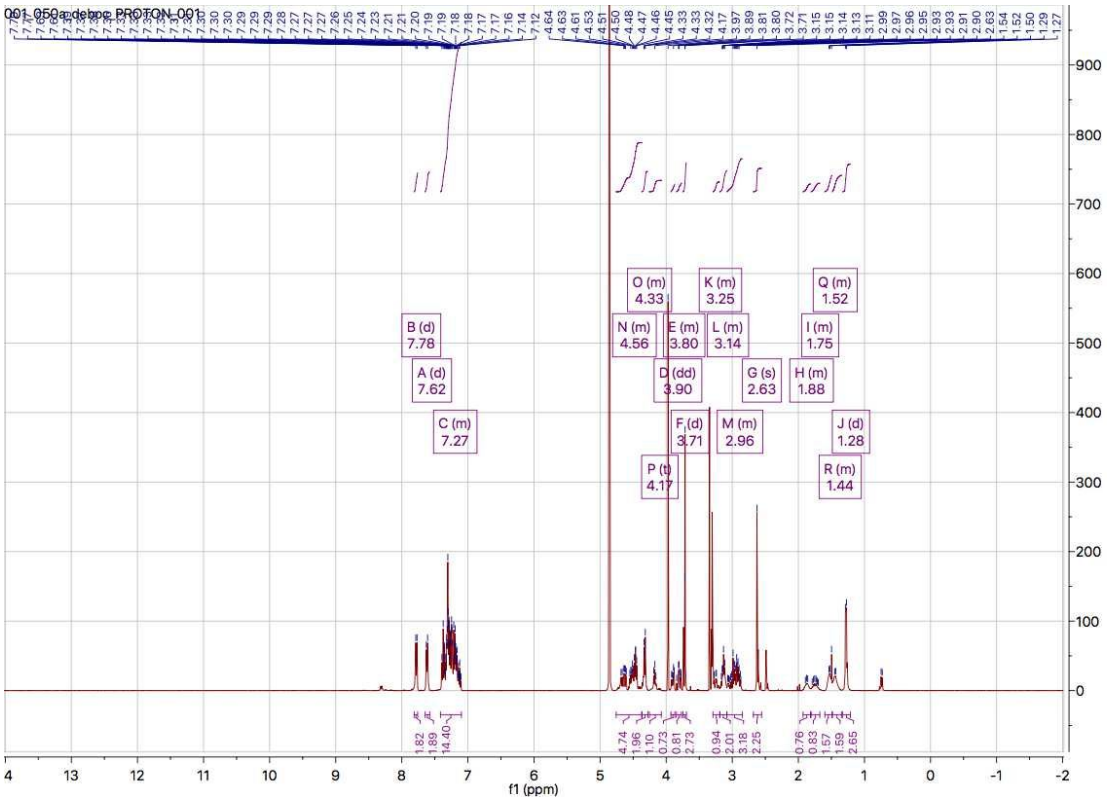


Figure S19. Intermediate 7 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



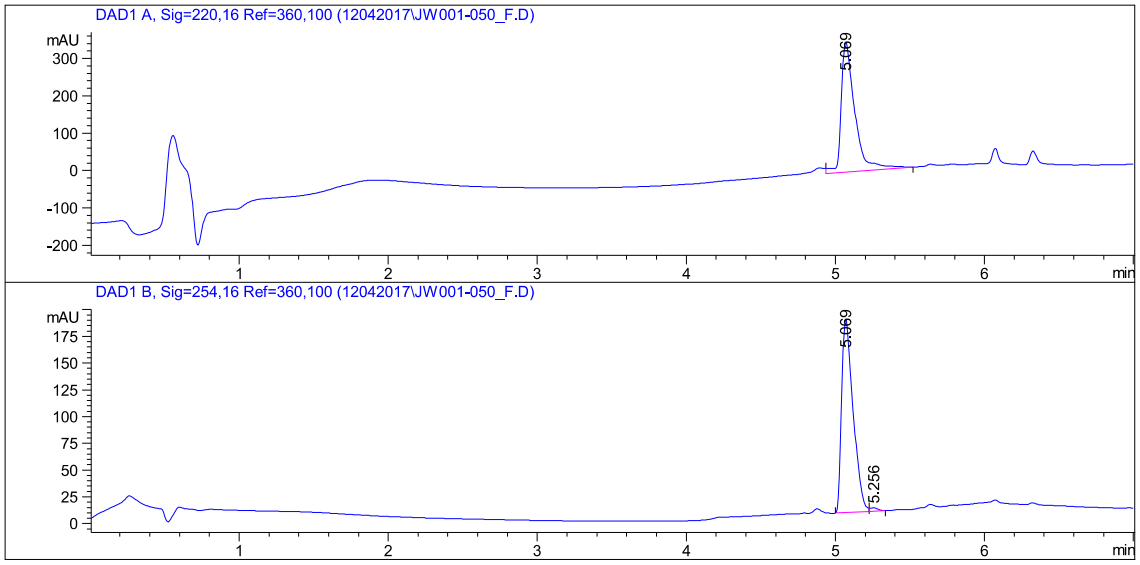
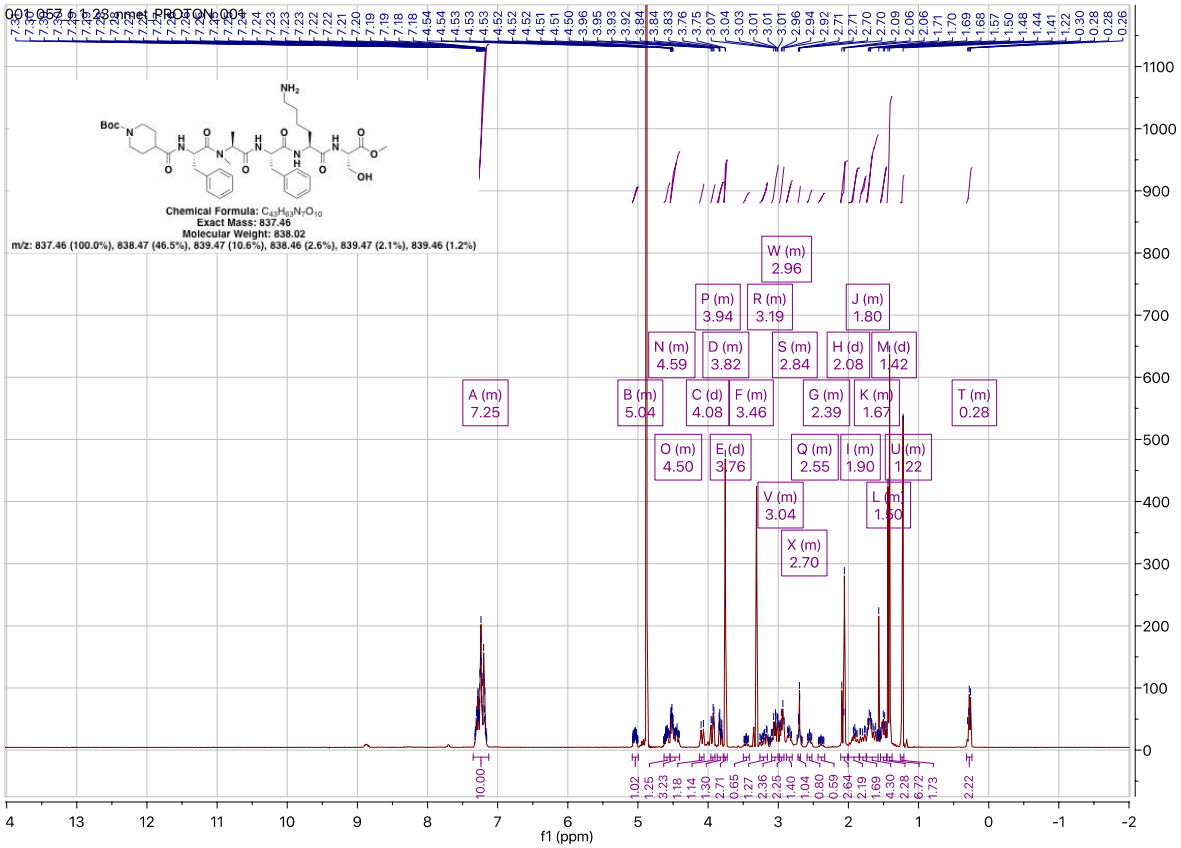


Figure S20. Intermediate 8 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



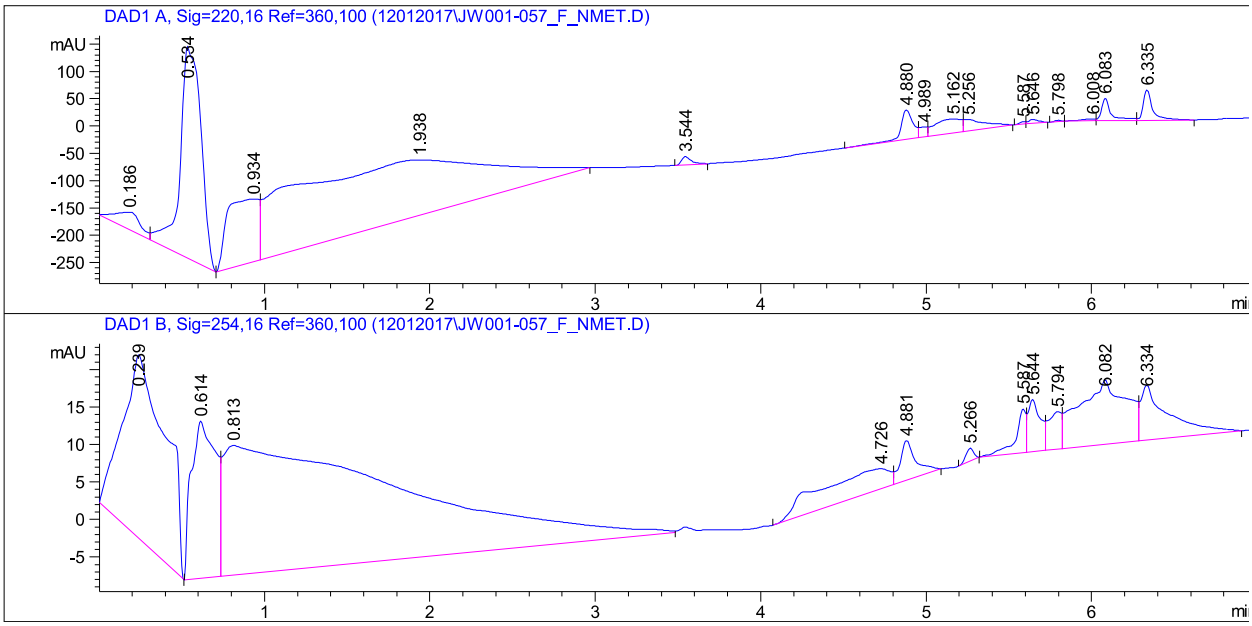
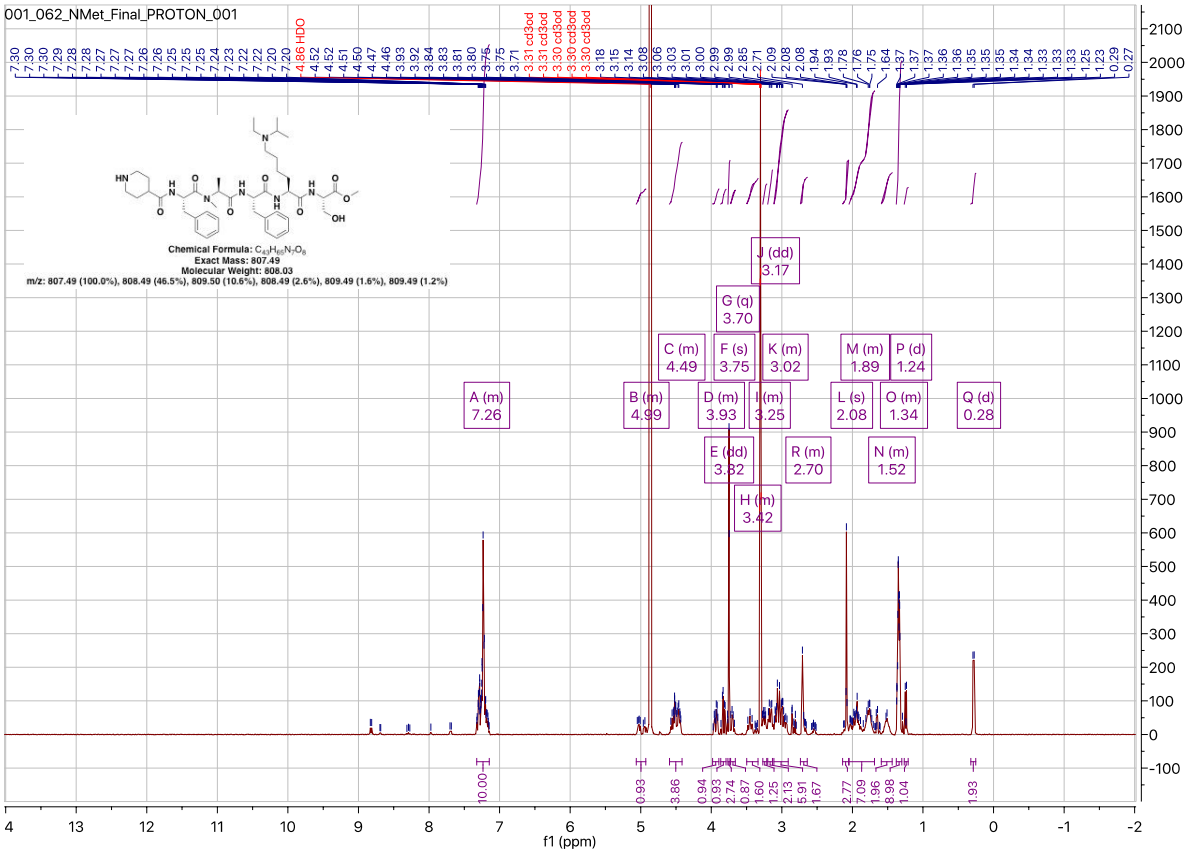


Figure S21. UNC6084 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



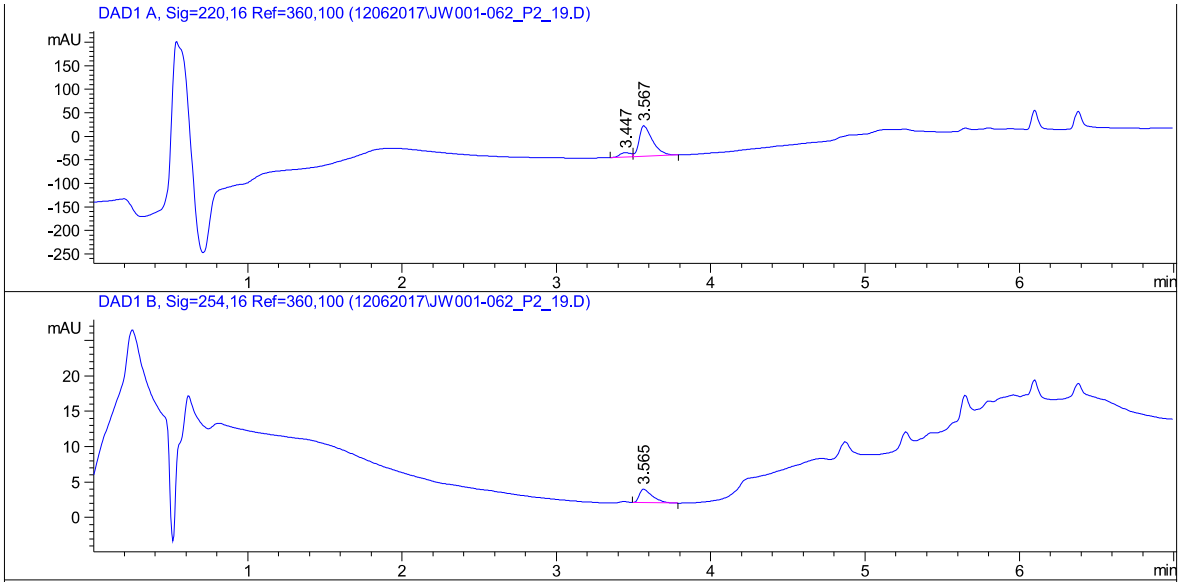


Figure S22. UNC5247 LC-MS Spectra at 220 and 254 nM.

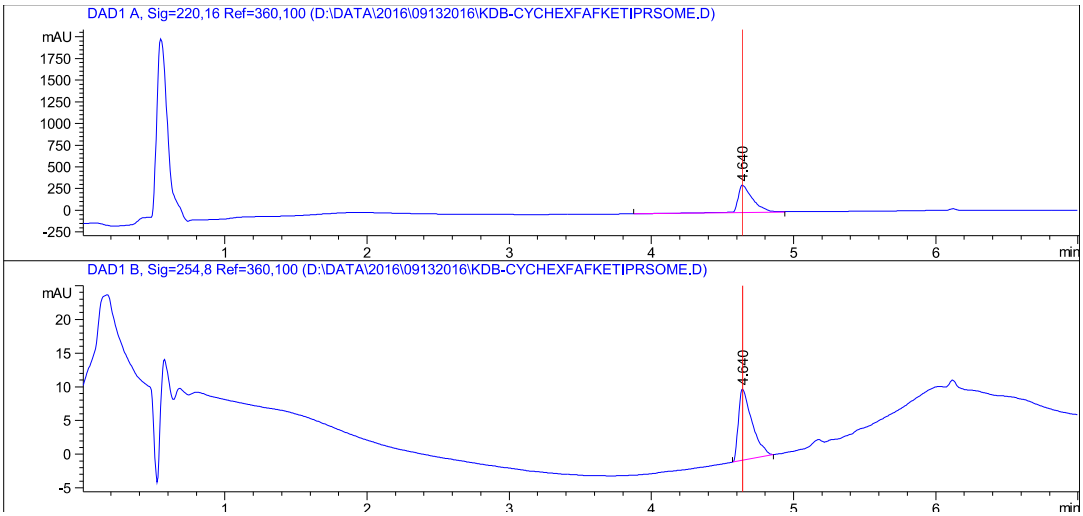
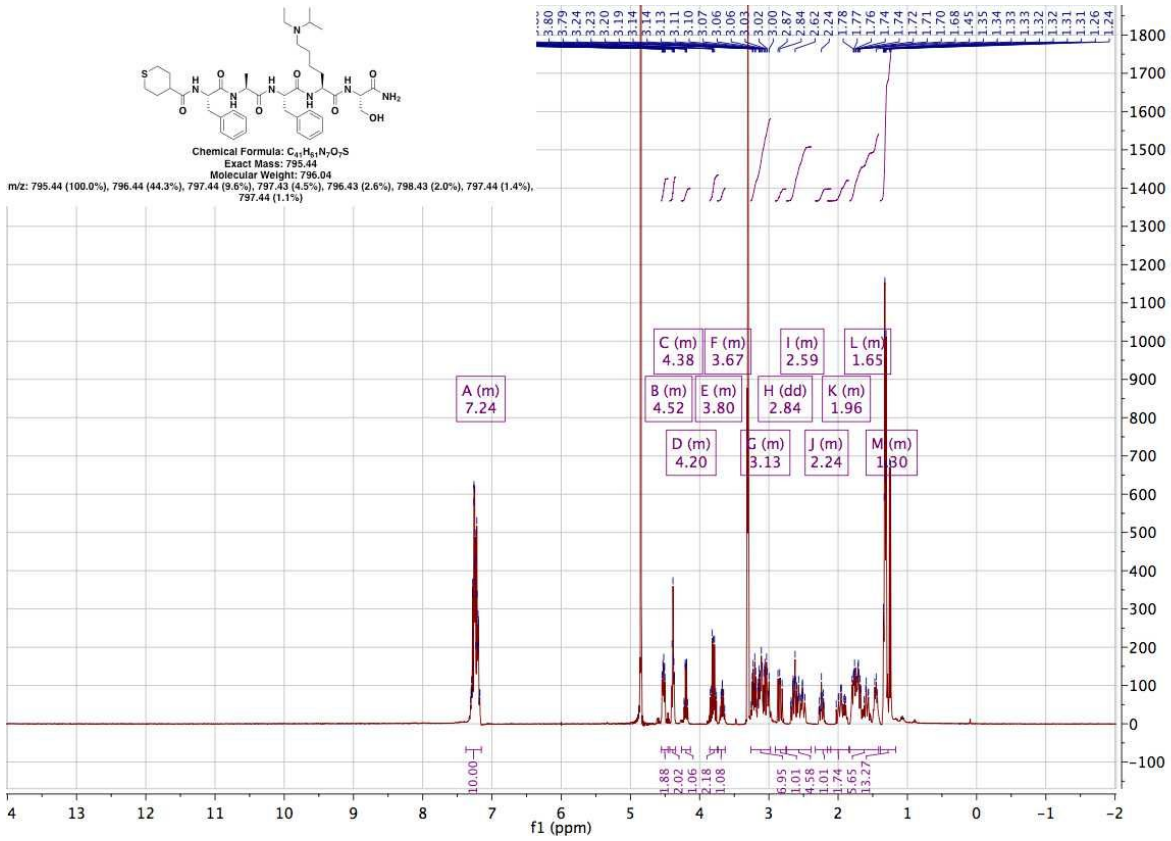


Figure S23. UNC5432 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



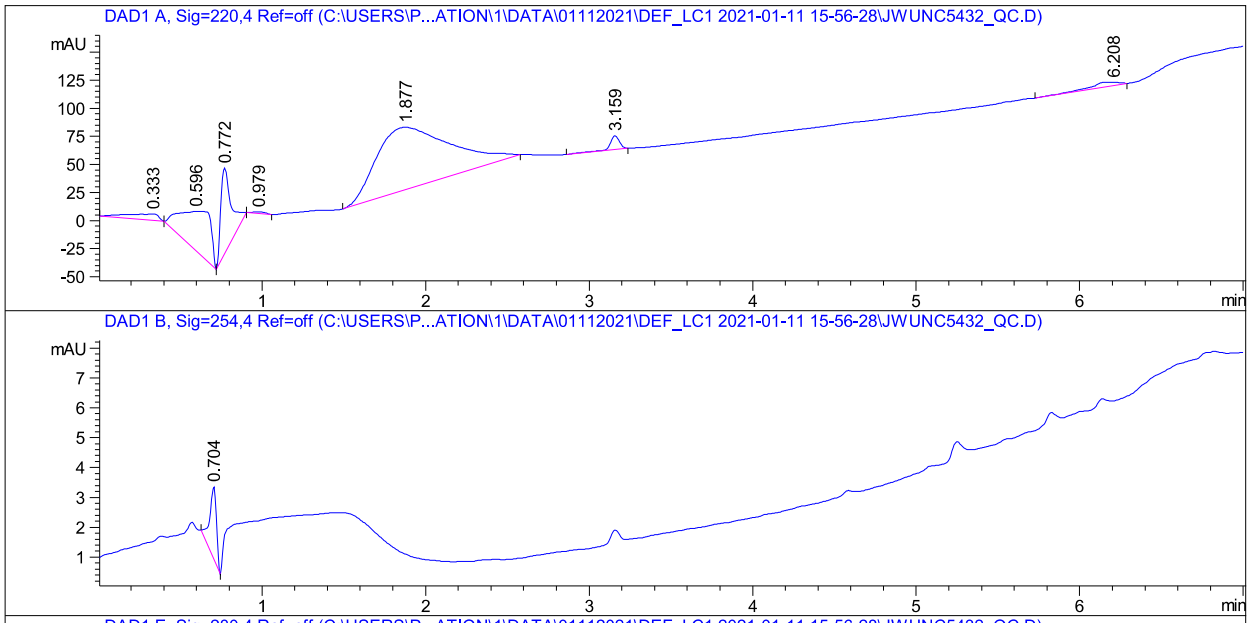
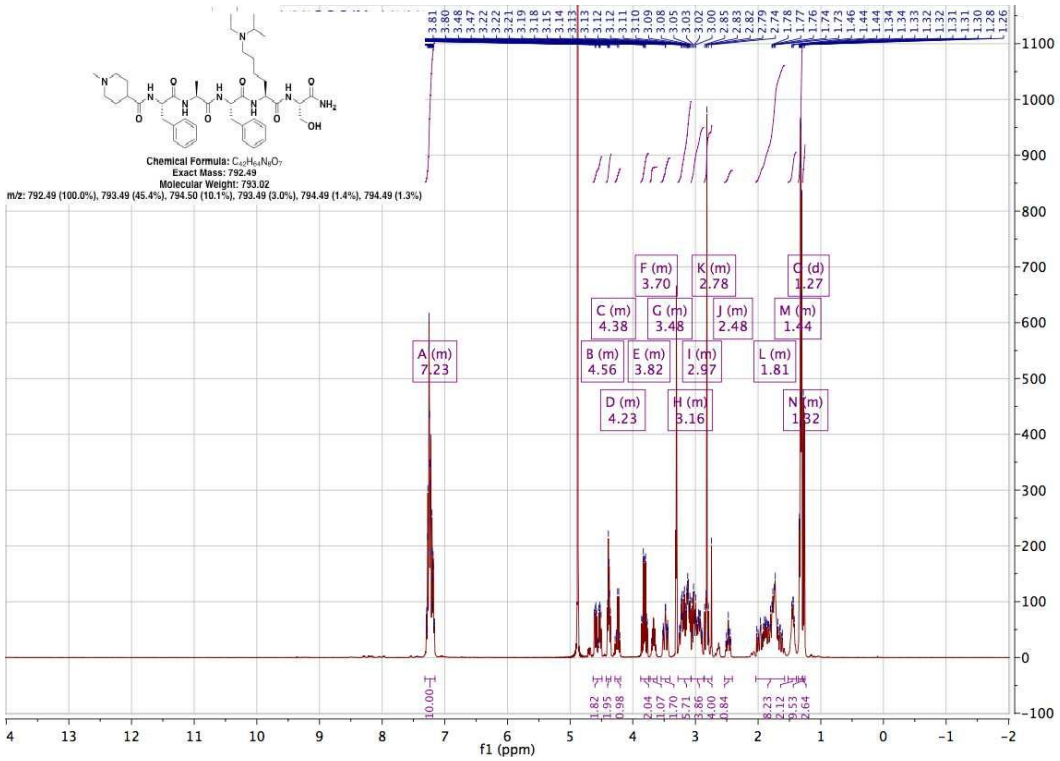


Figure S24. UNC5430 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



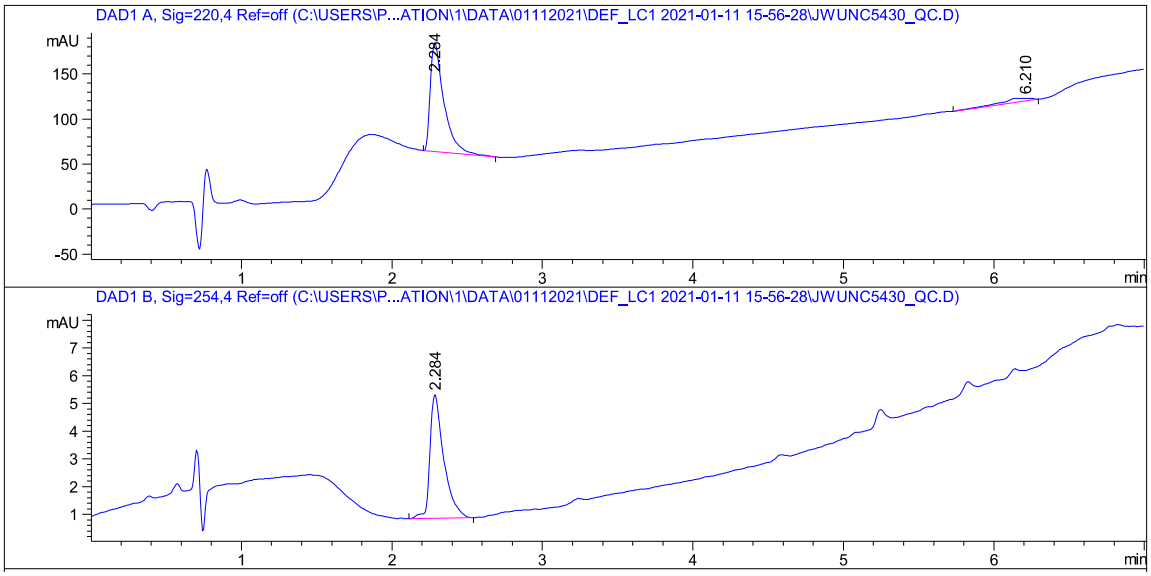
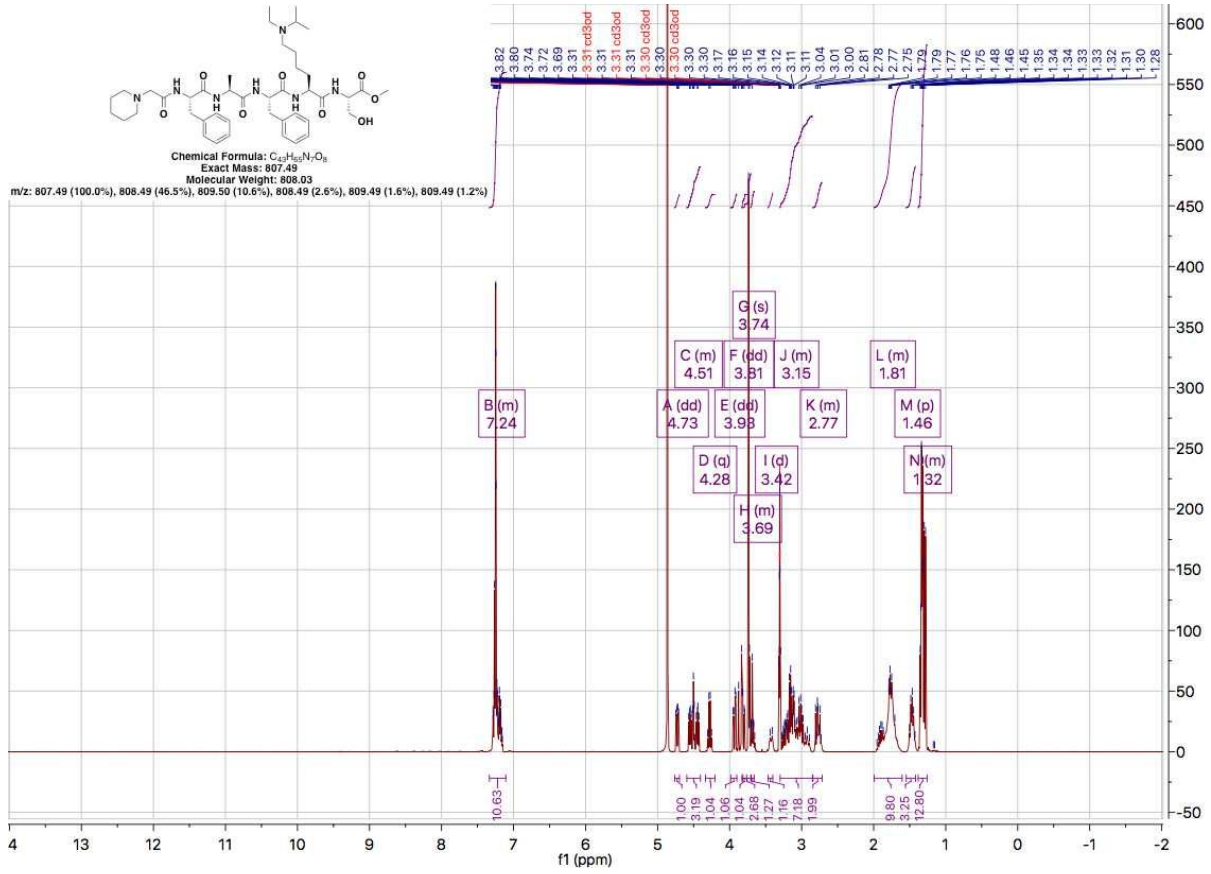


Figure S25. UNC6215 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



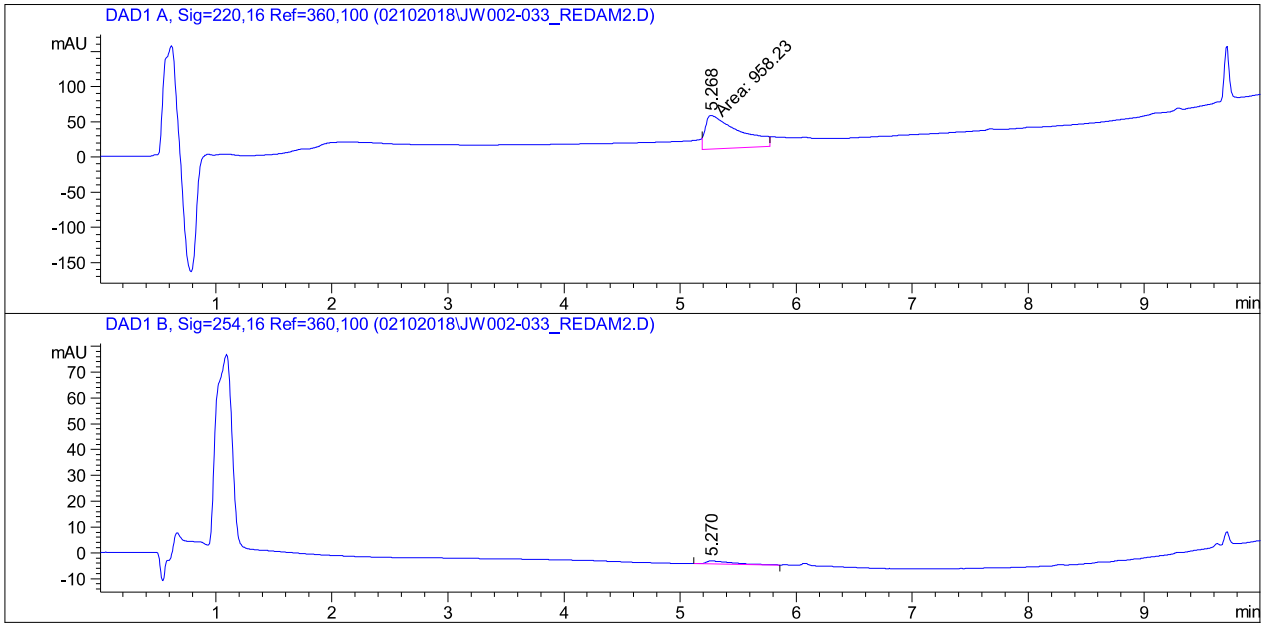
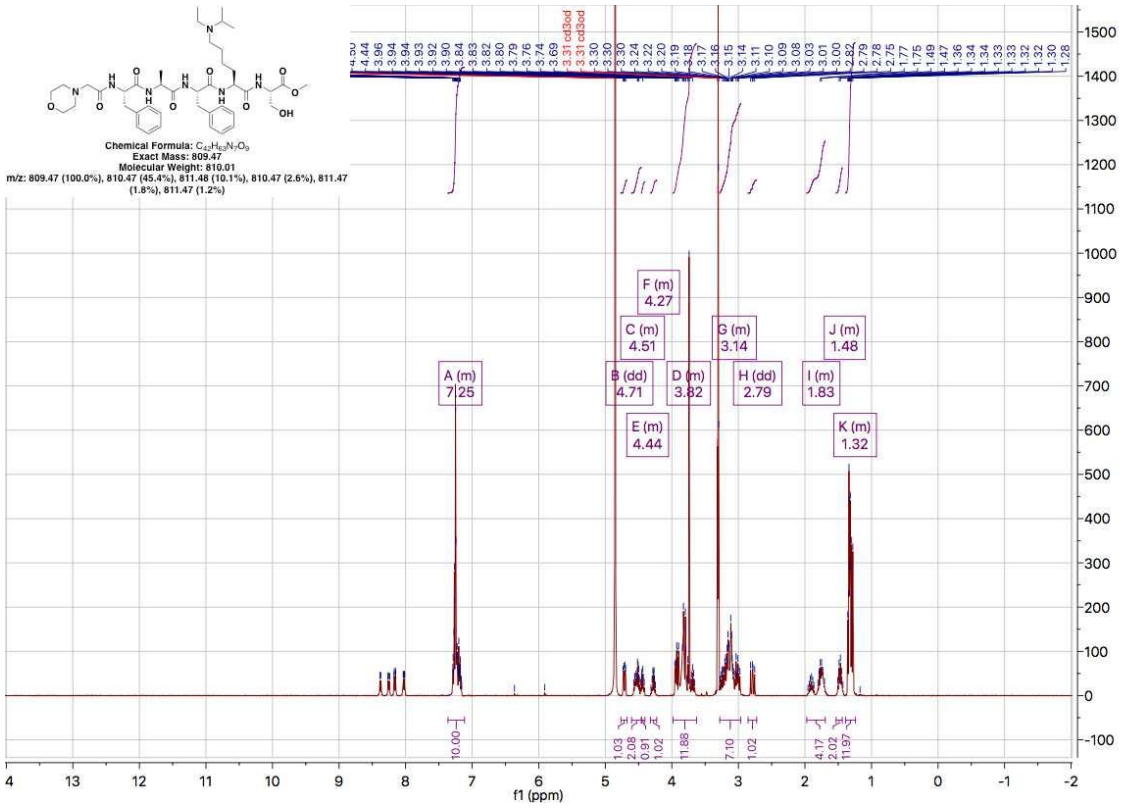


Figure S26. UNC6214 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



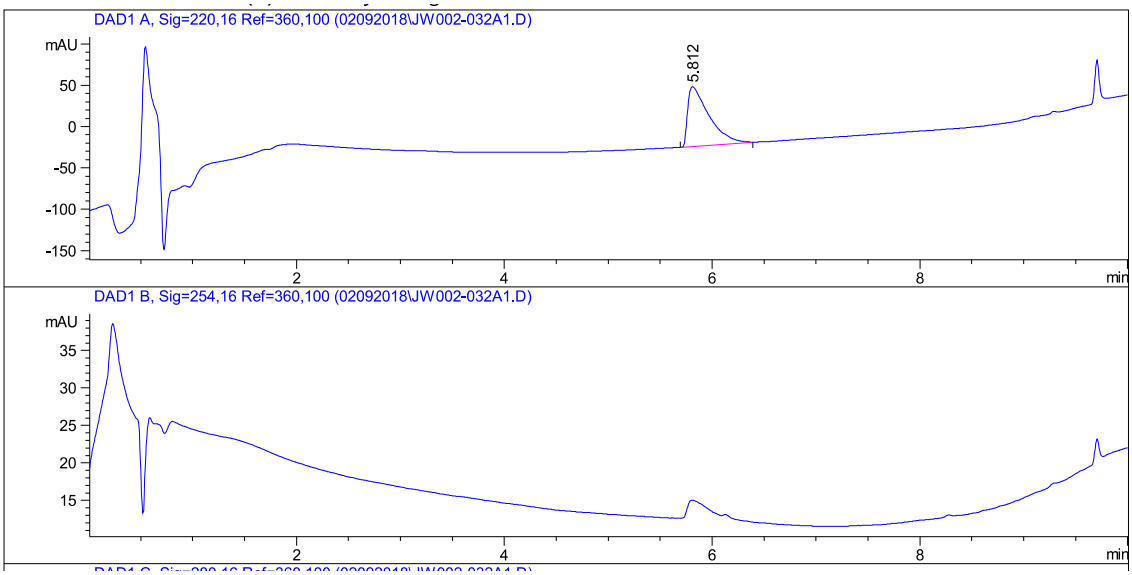
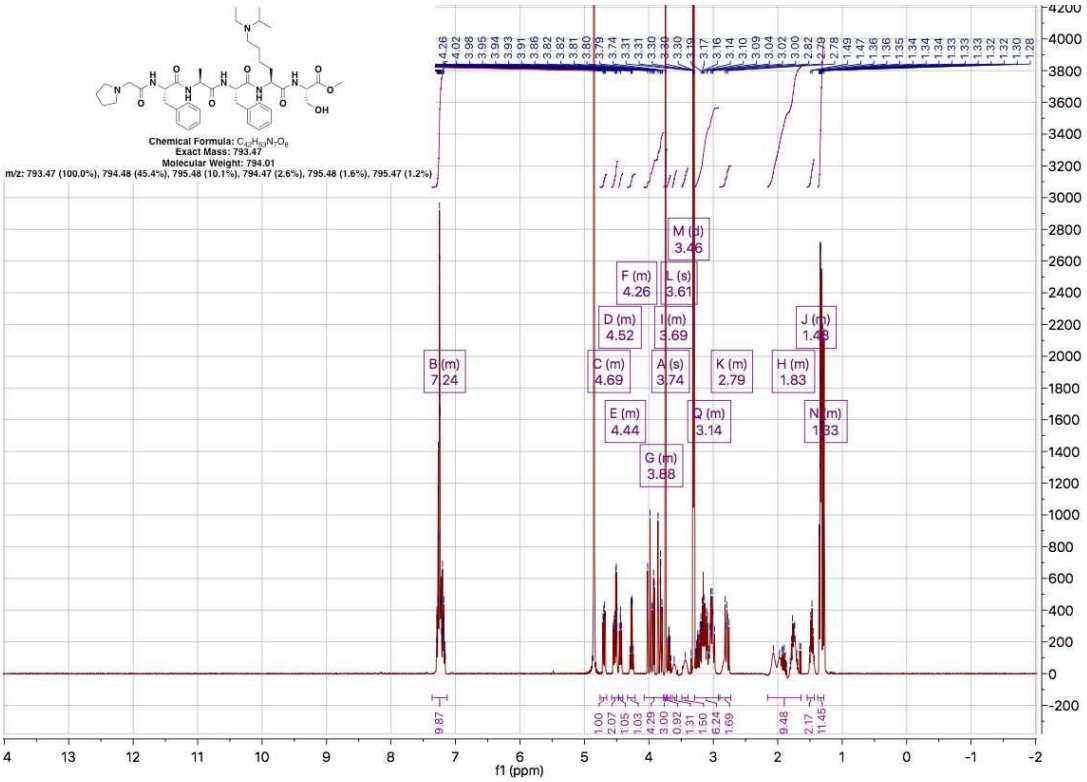


Figure S27. UNC6113 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



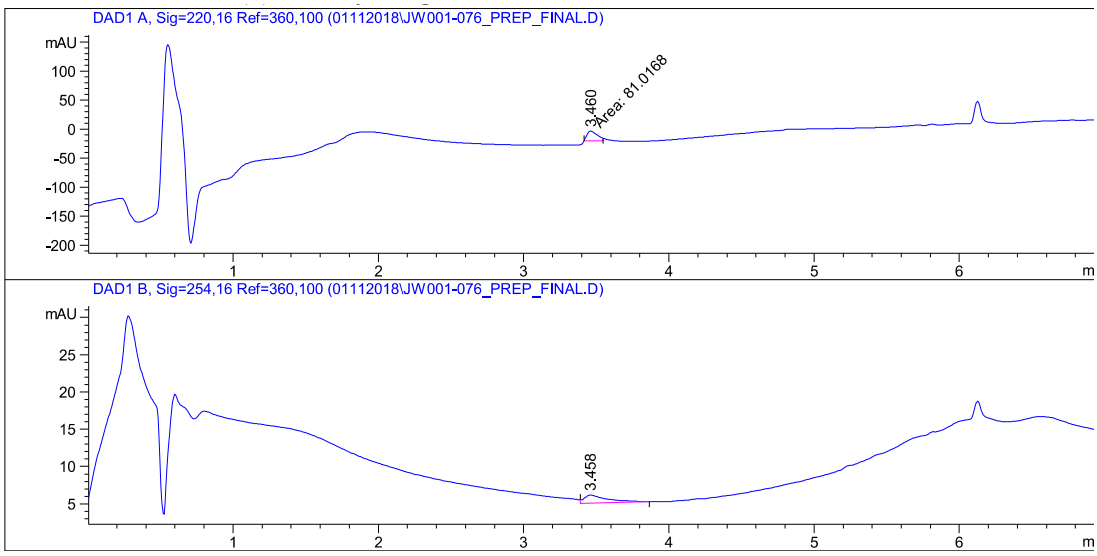
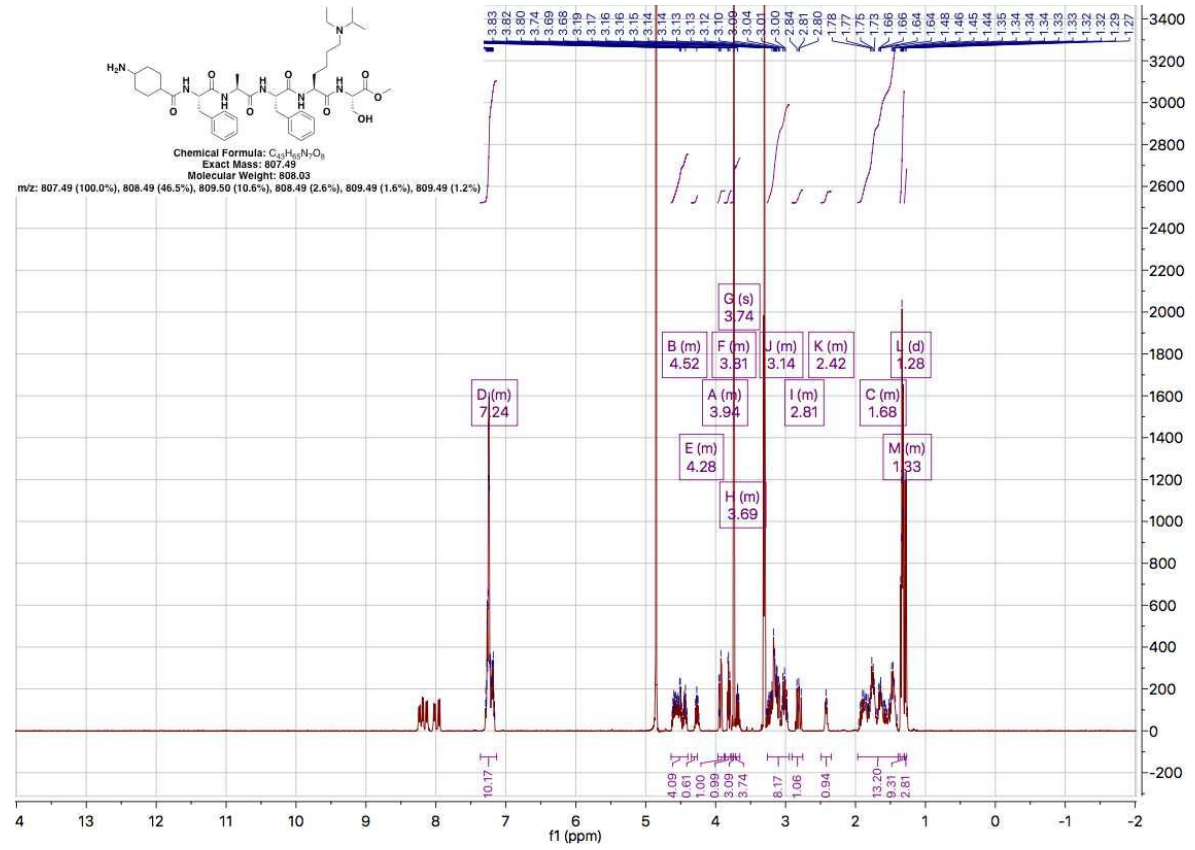


Figure S28. UNC6108 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



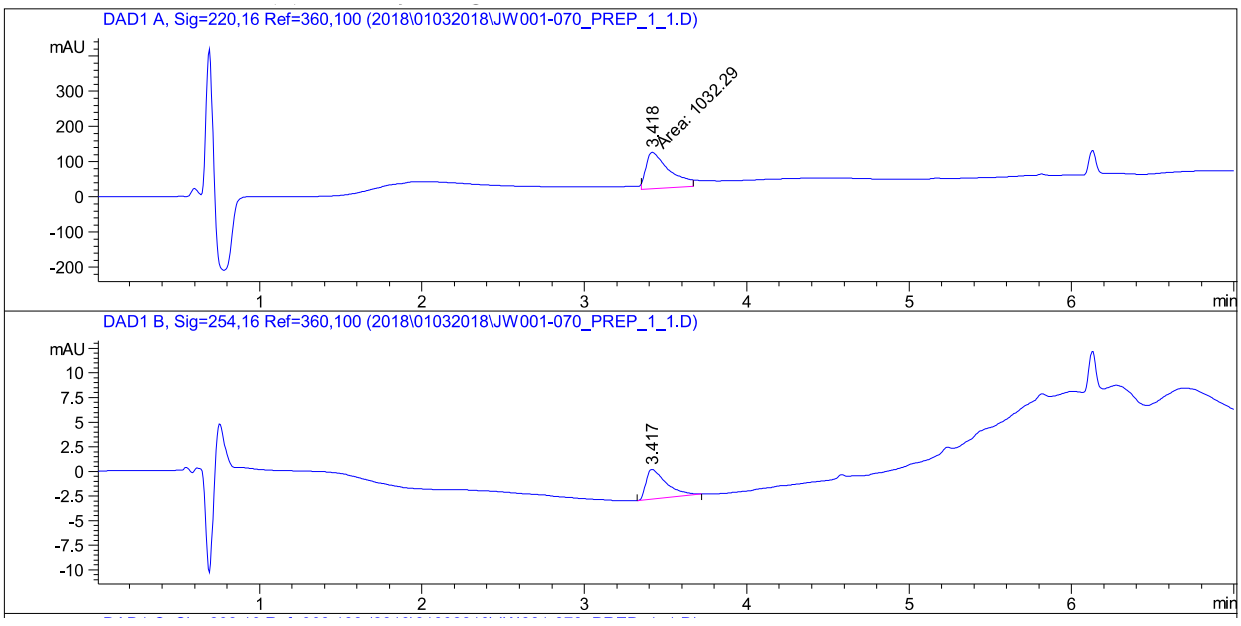
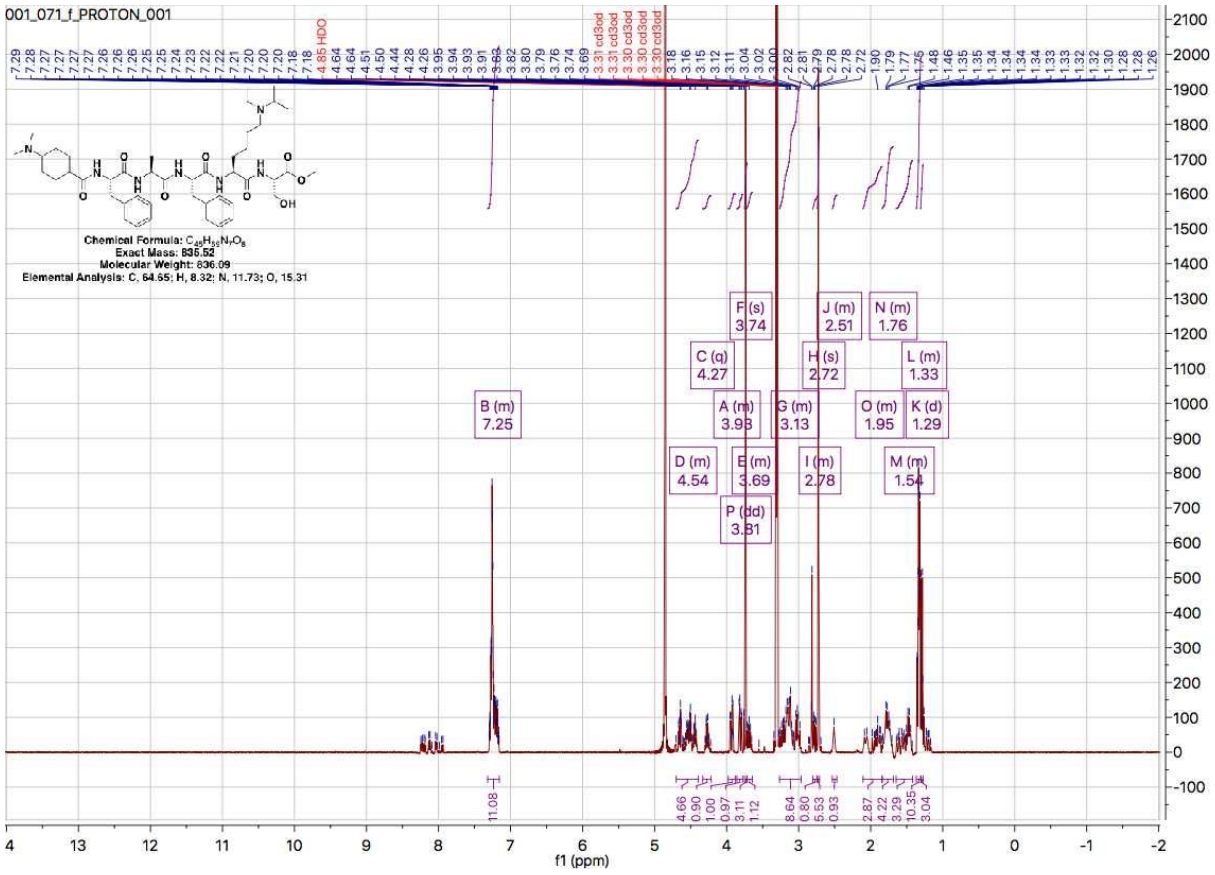


Figure S29. UNC6109 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



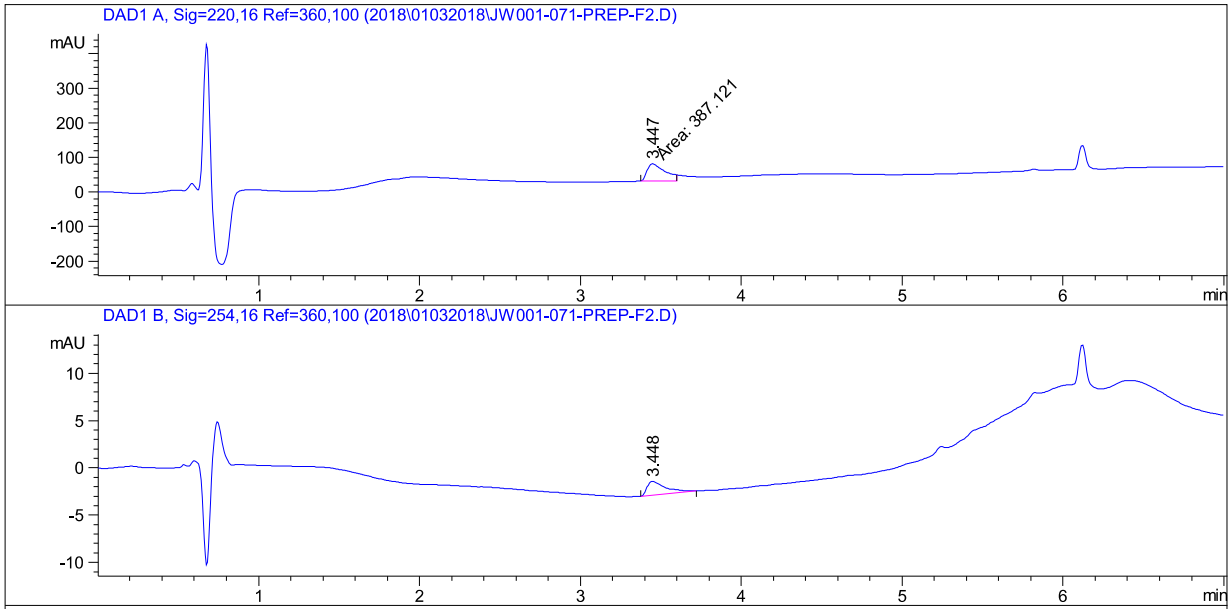
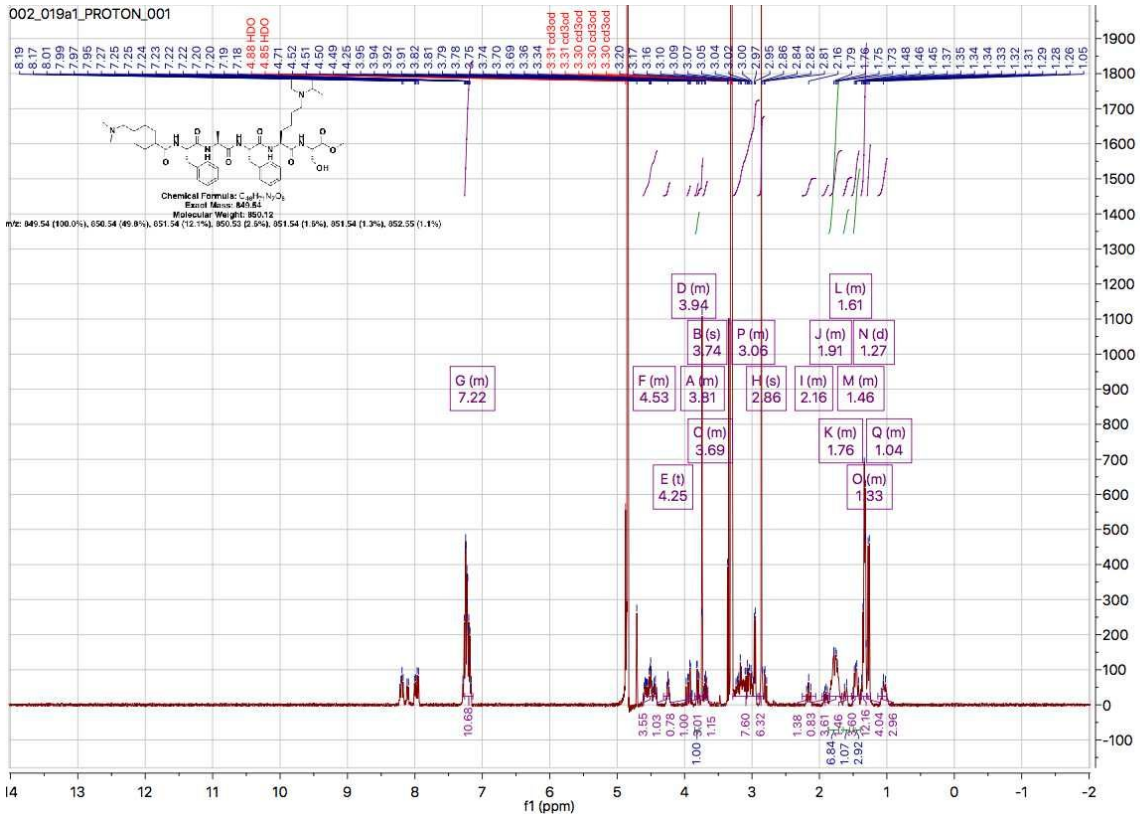


Figure S30. UNC6141 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



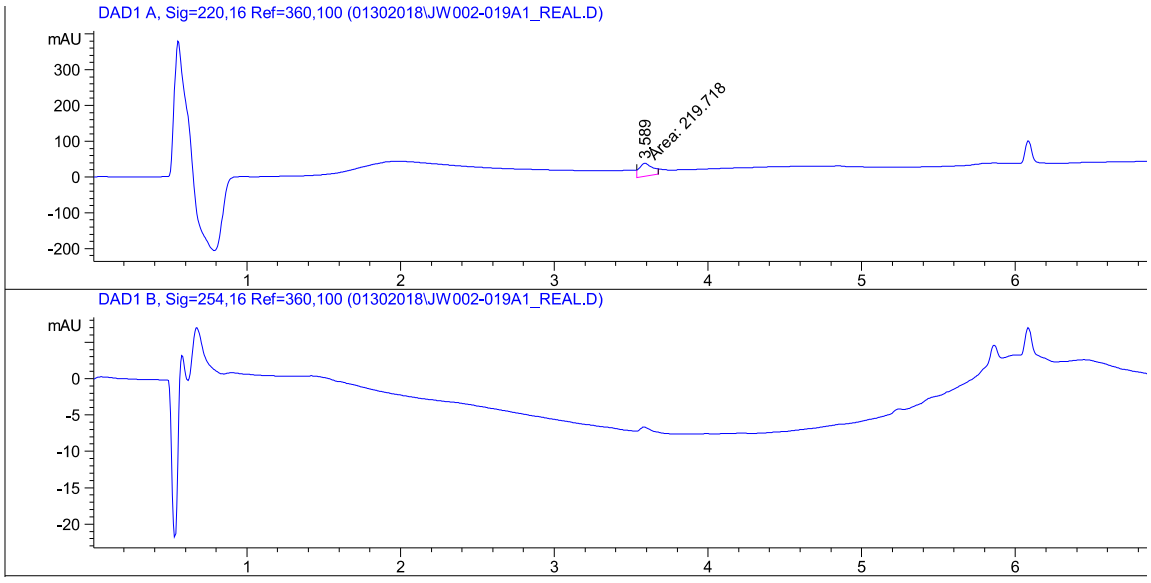
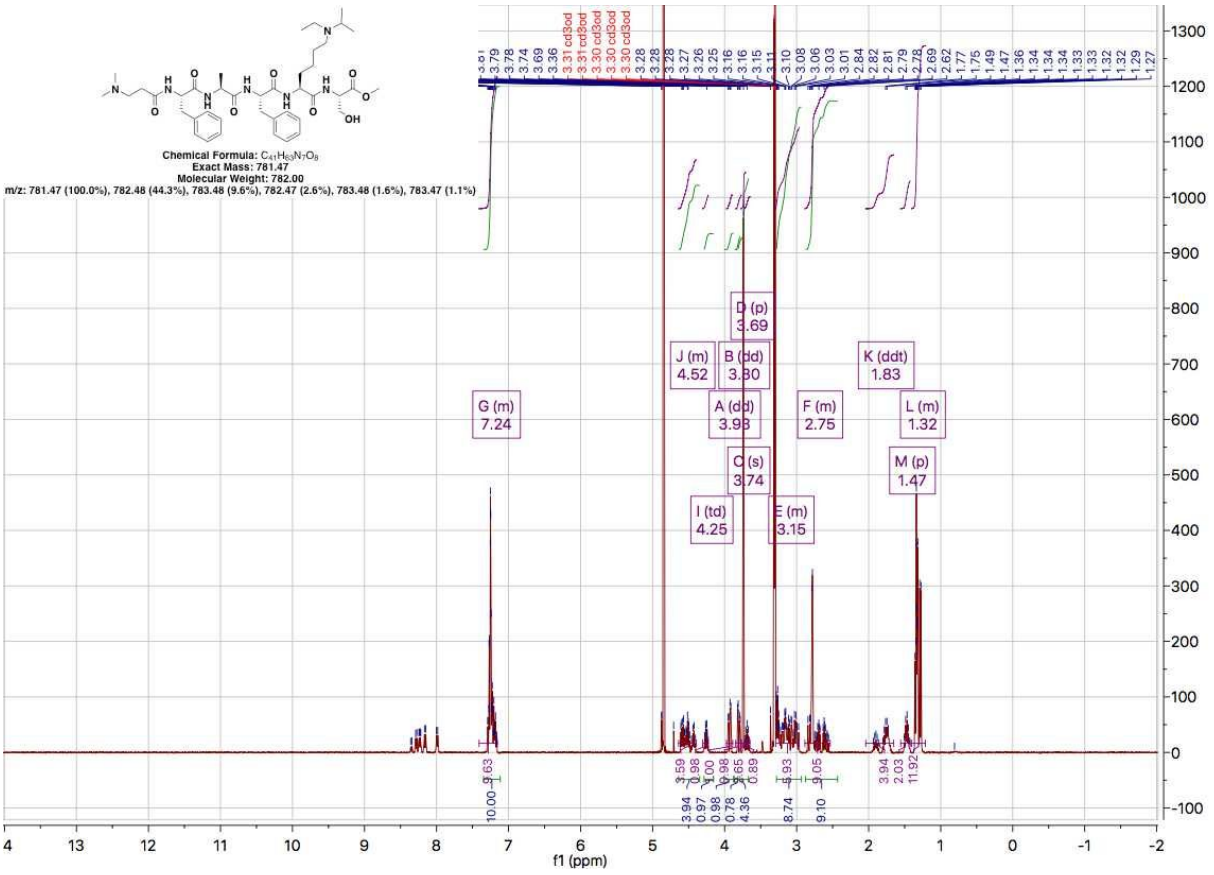


Figure S31. UNC6231 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



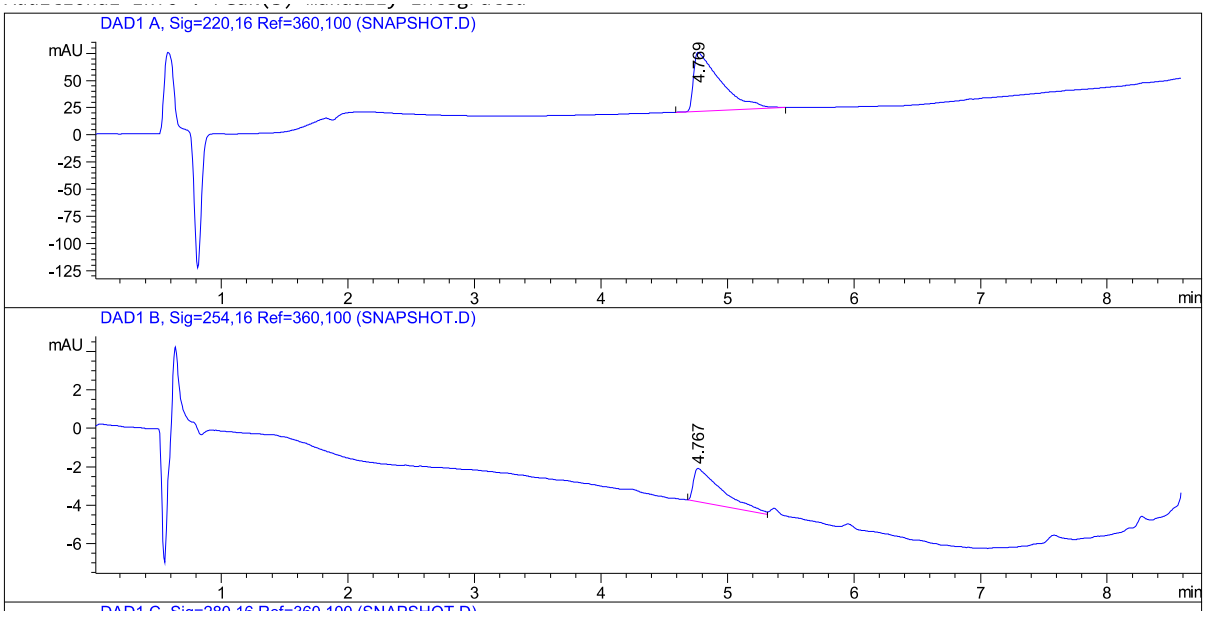
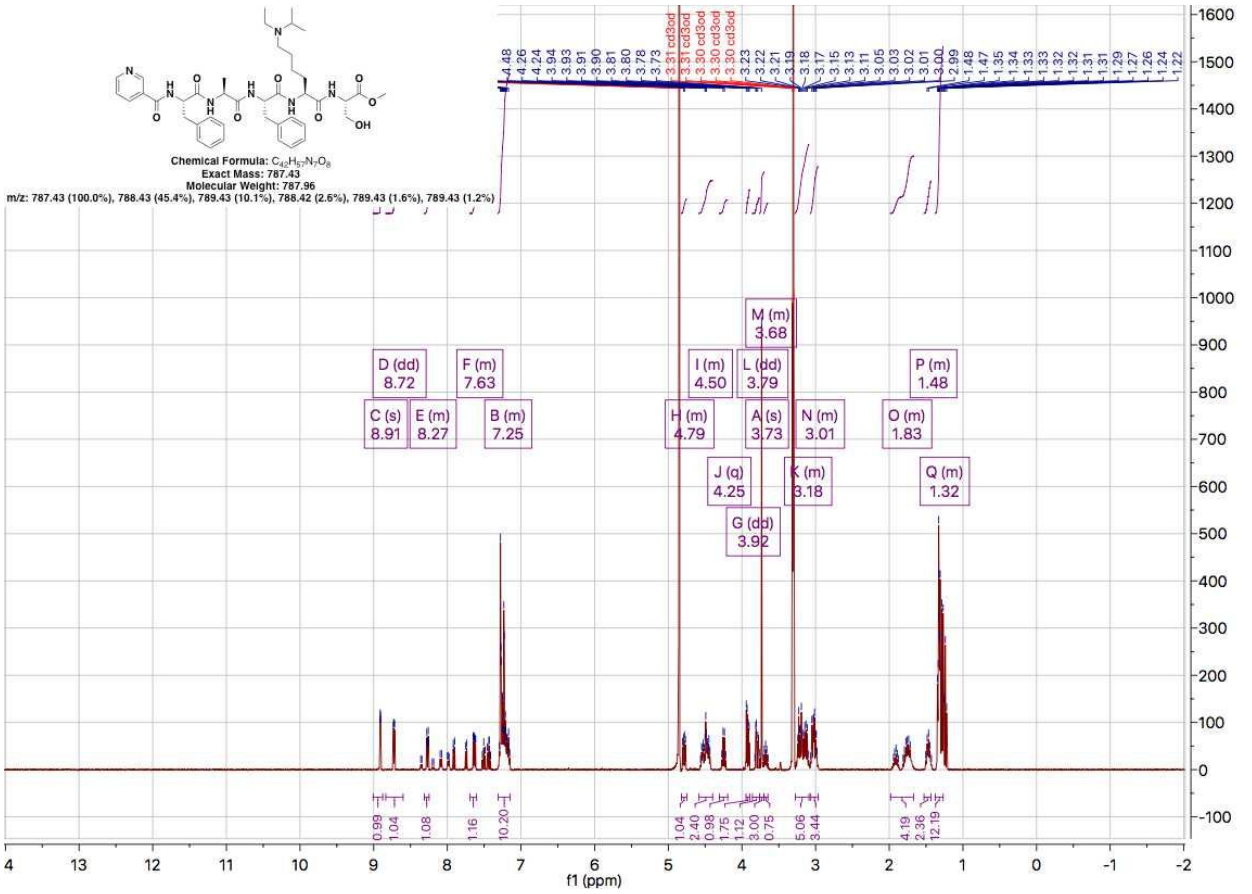


Figure S32. UNC6220 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



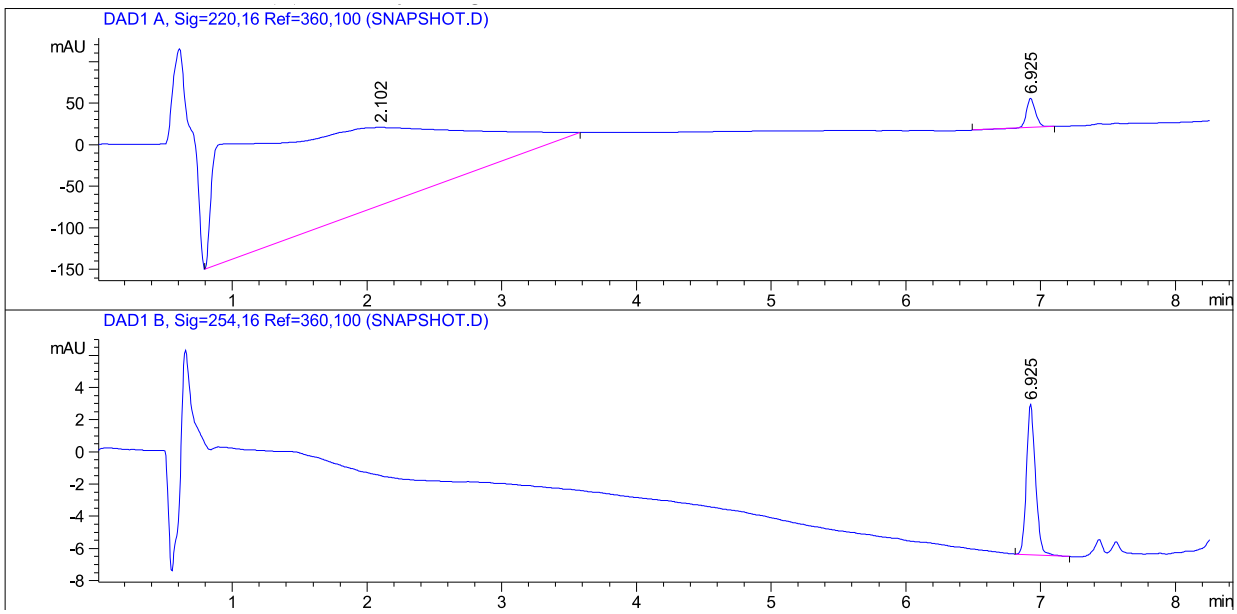
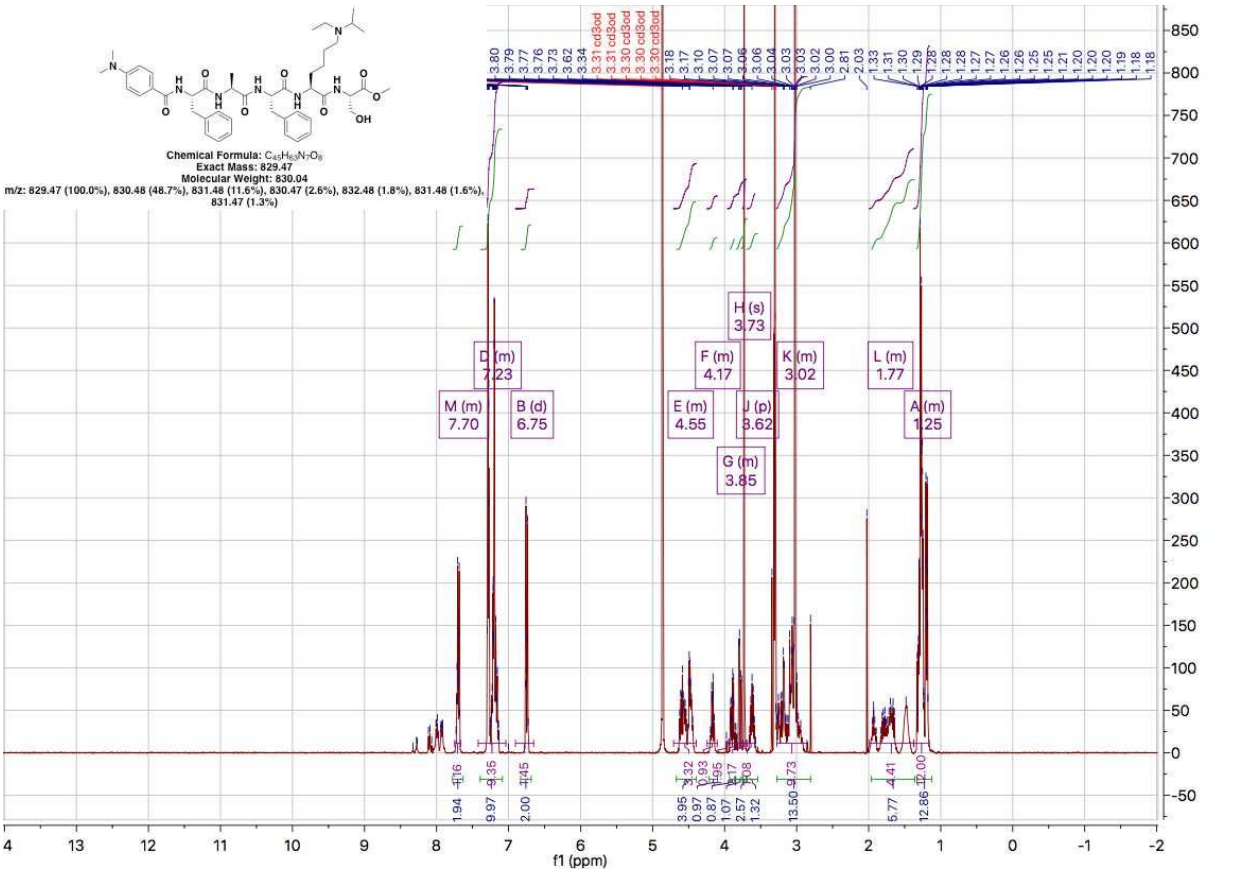


Figure S33. UNC6139 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



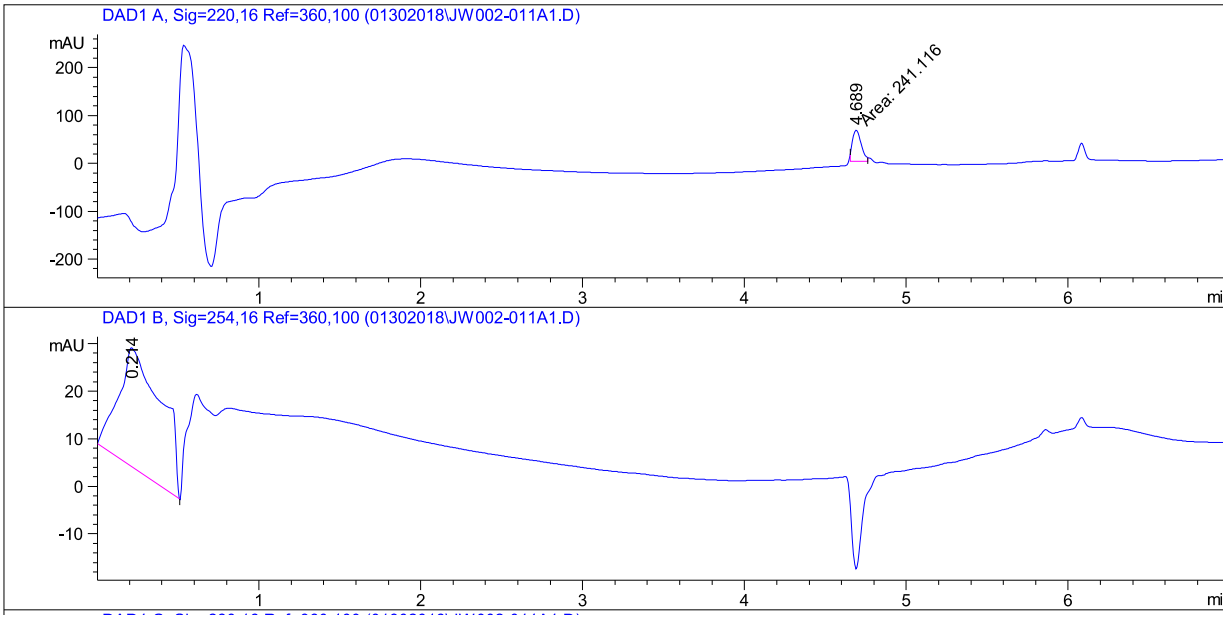
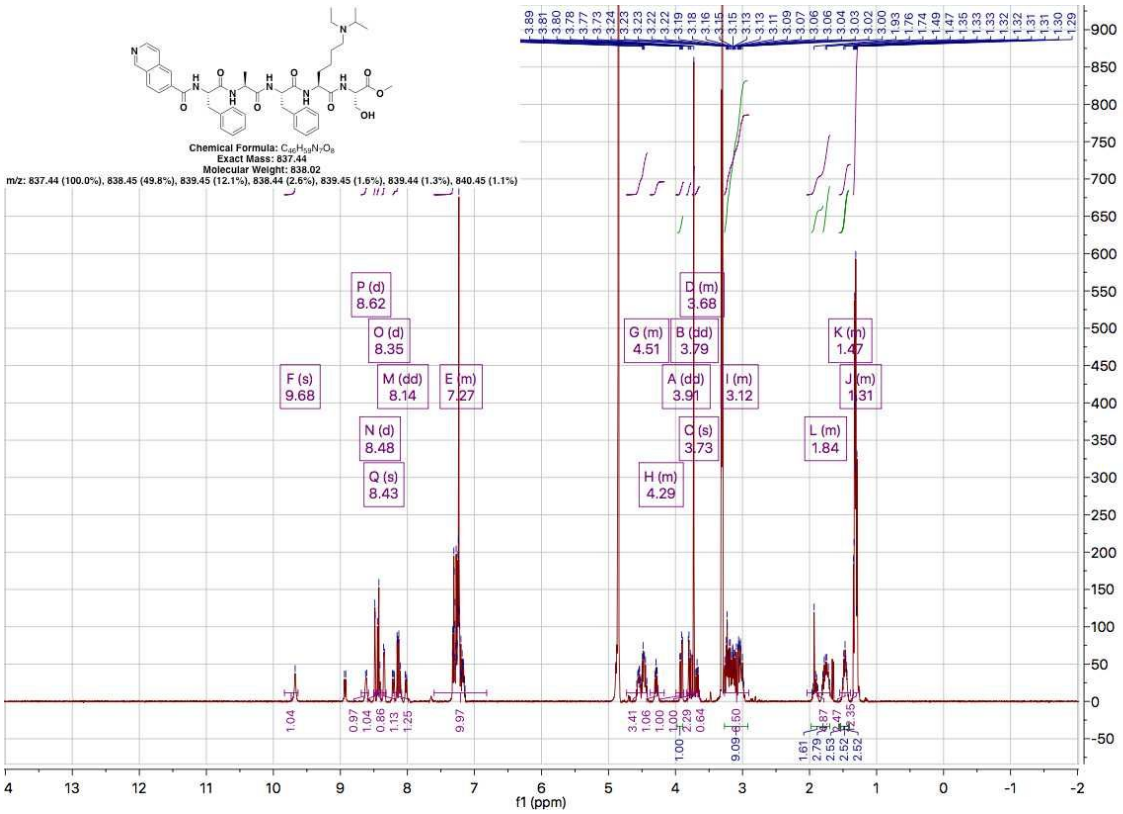


Figure S34. UNC6142 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



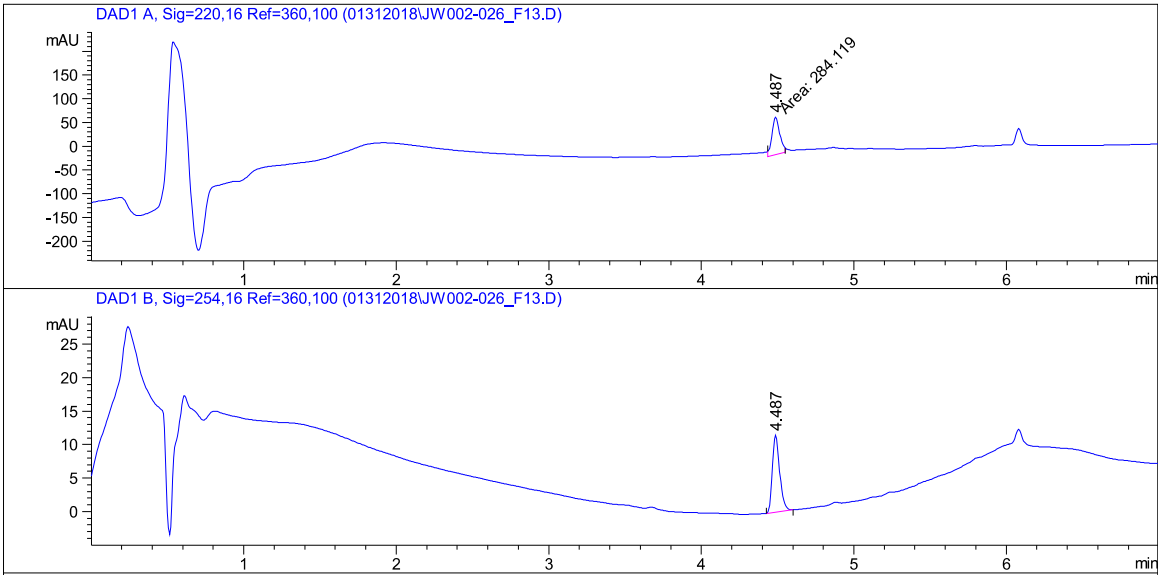
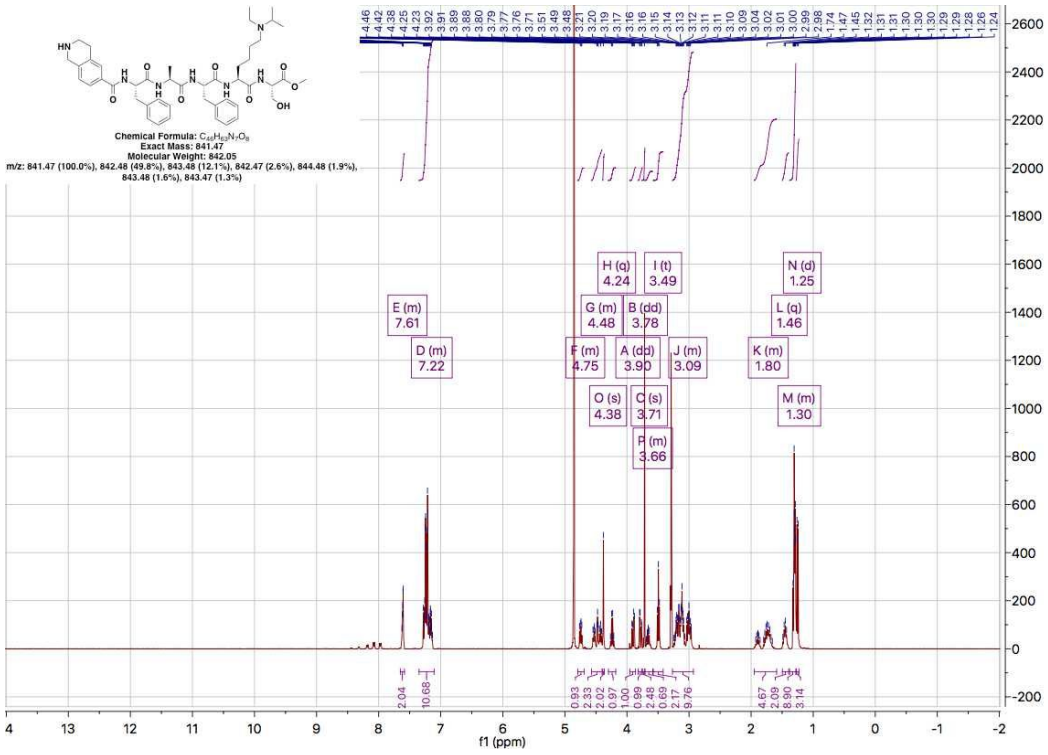
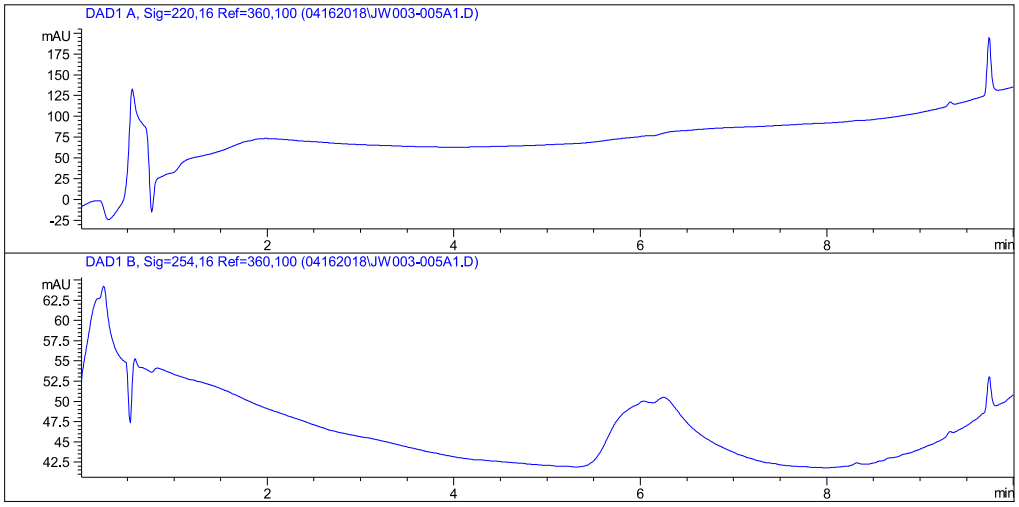


Figure S35. UNC6263 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM and TIC





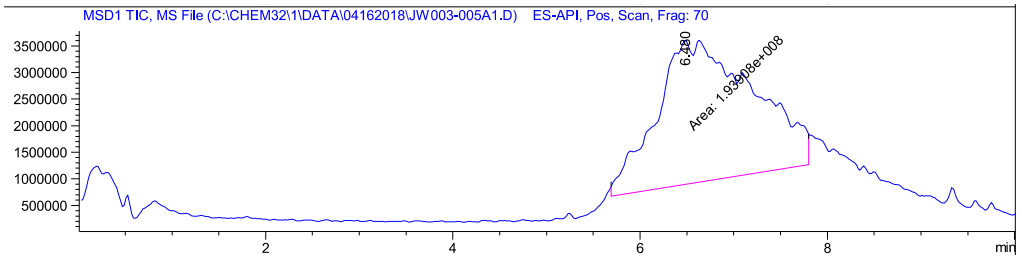
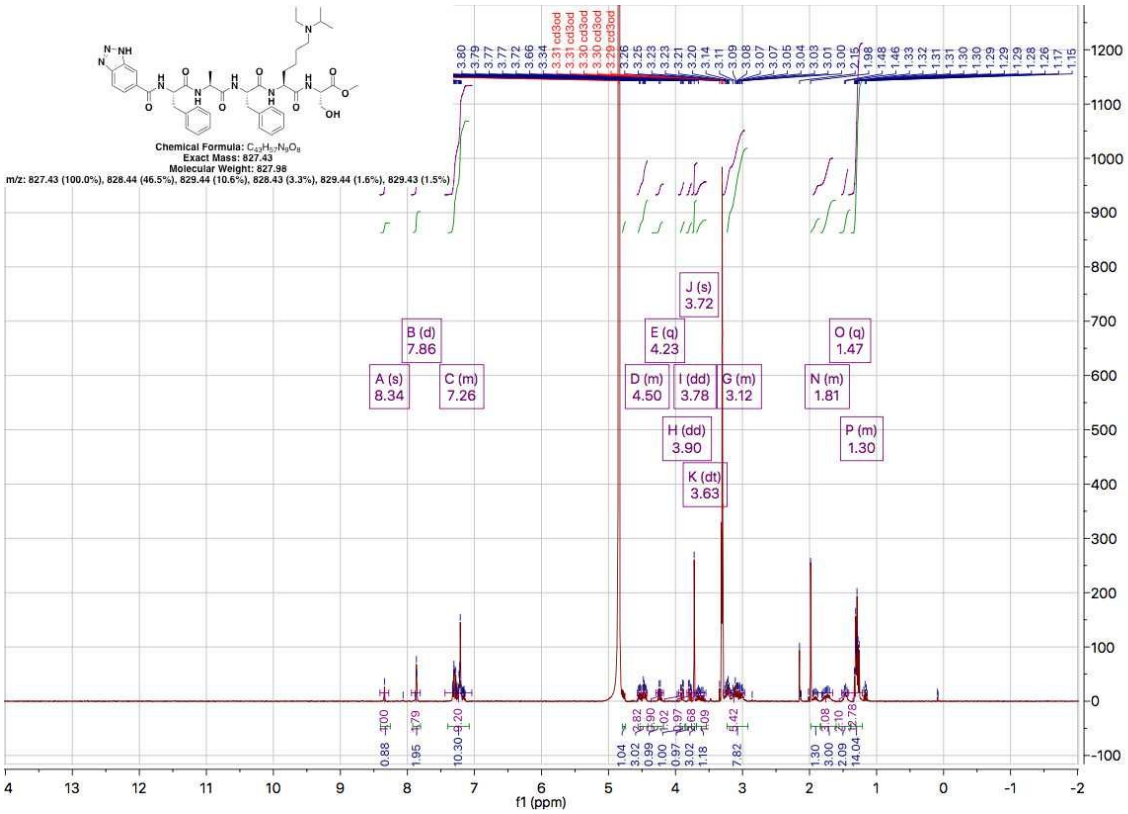


Figure S36. UNC6257 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



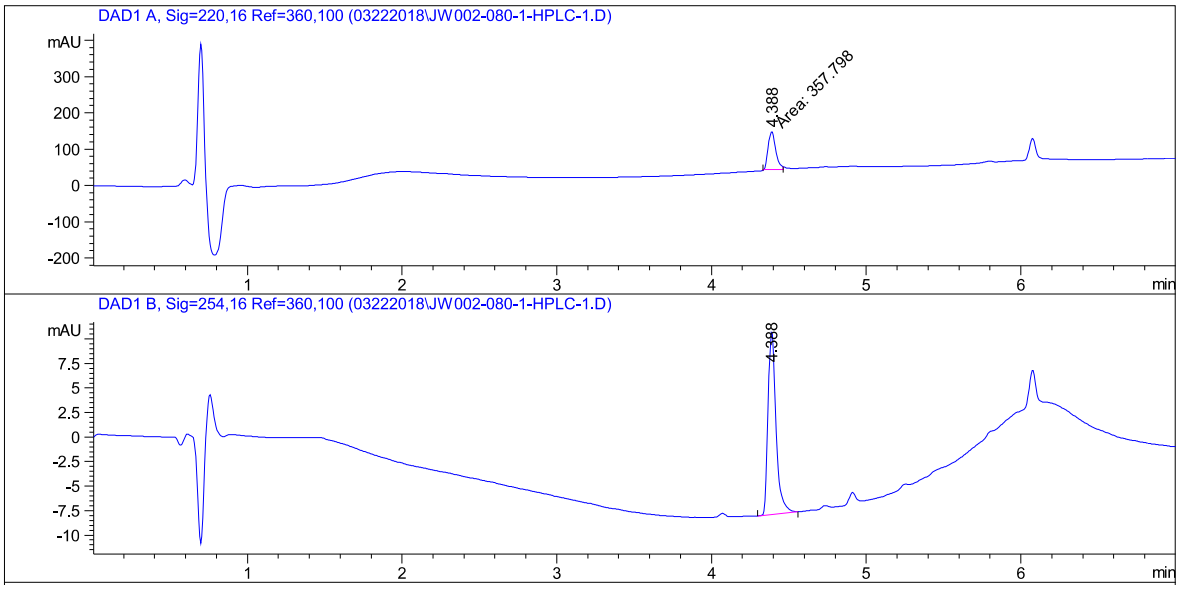
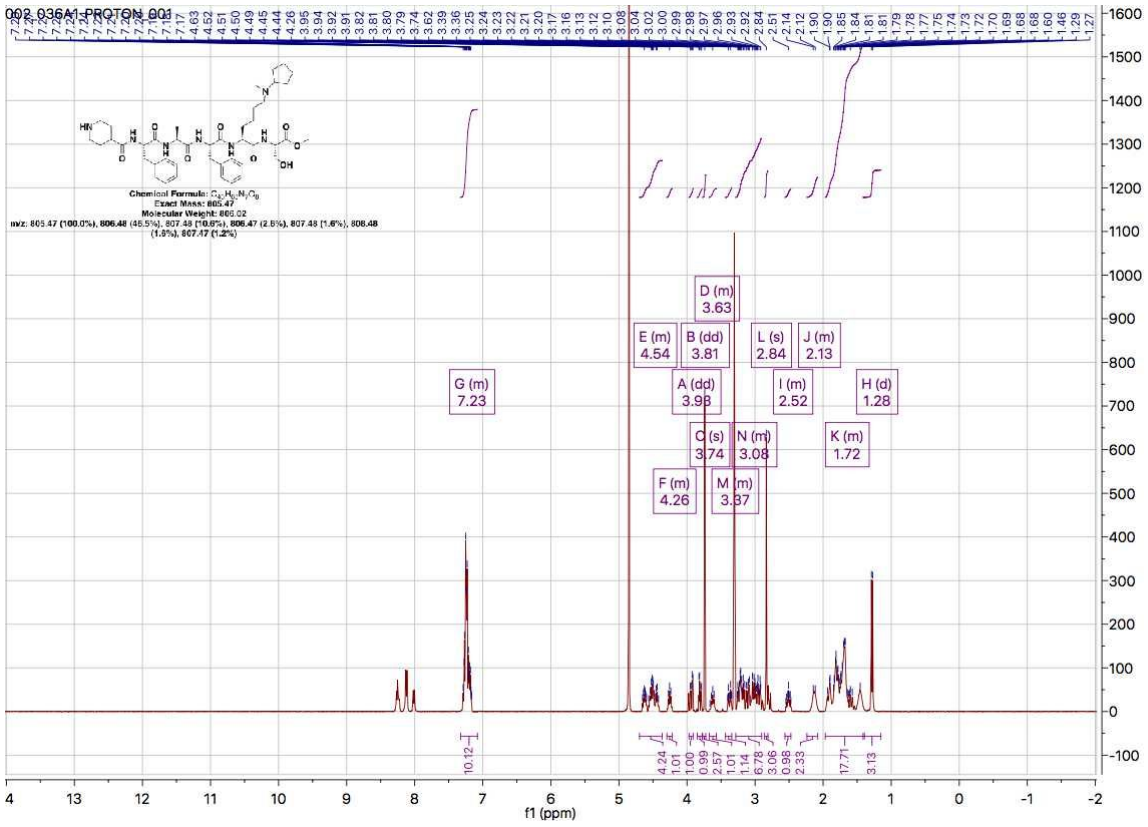


Figure S37. UNC6216 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



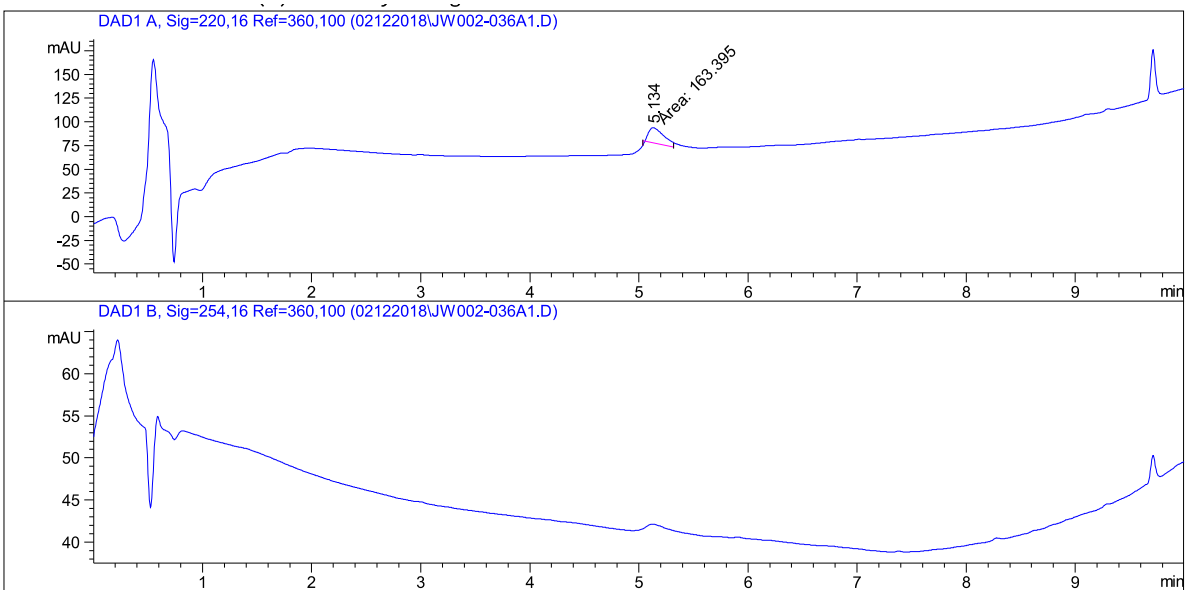
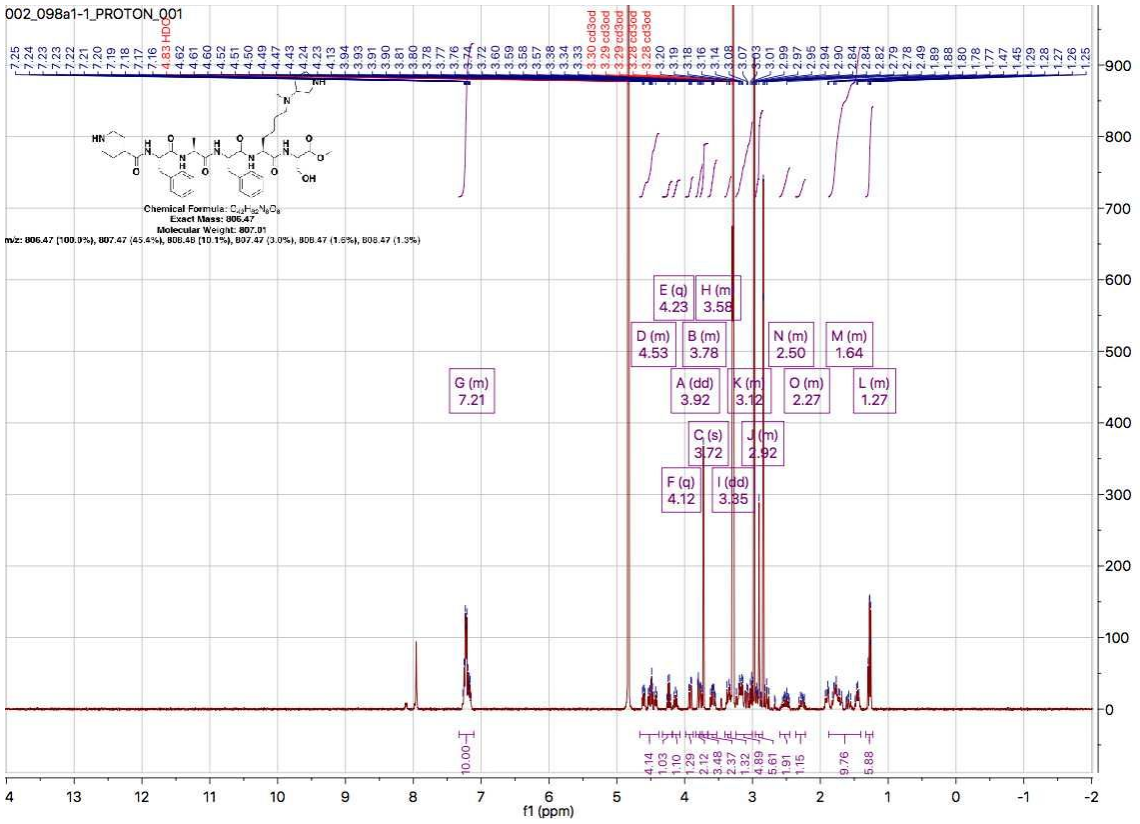


Figure S38. UNC6262 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



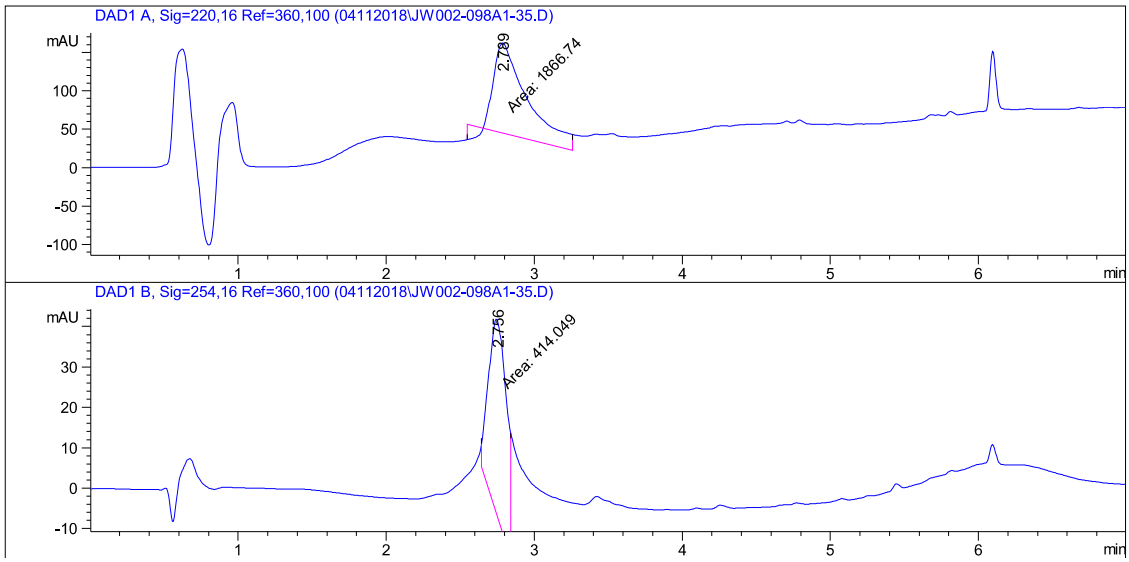
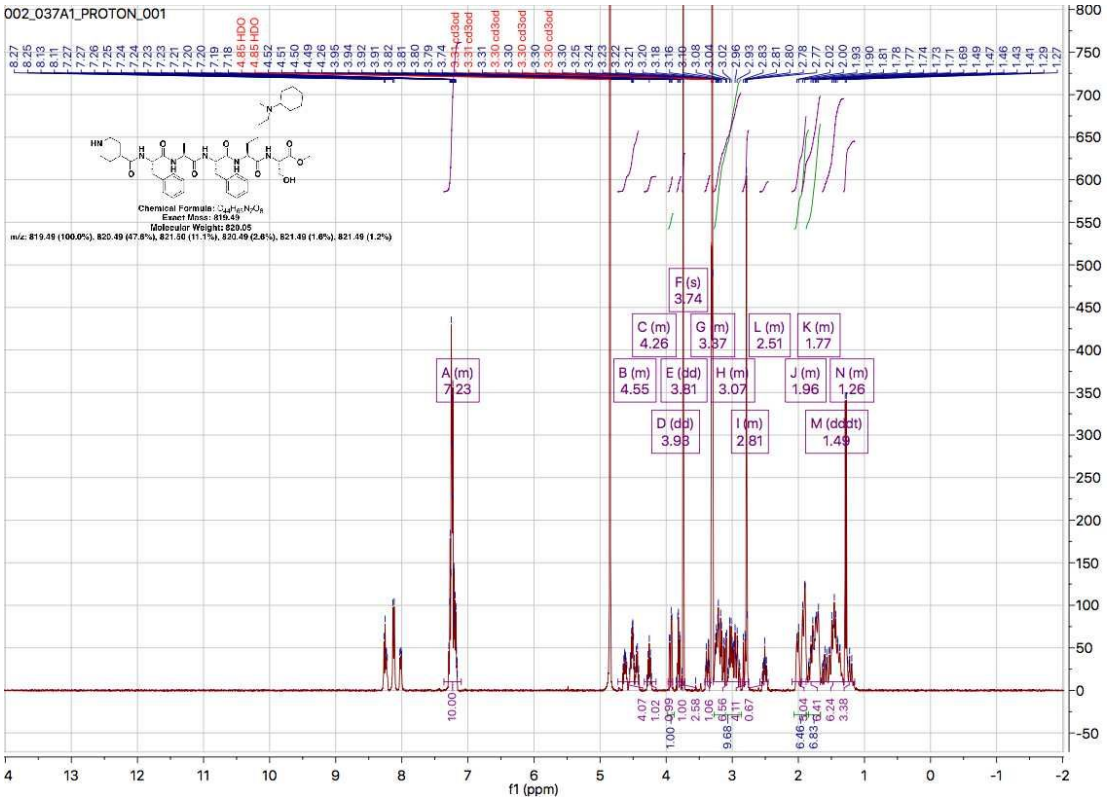


Figure S39. UNC6217 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



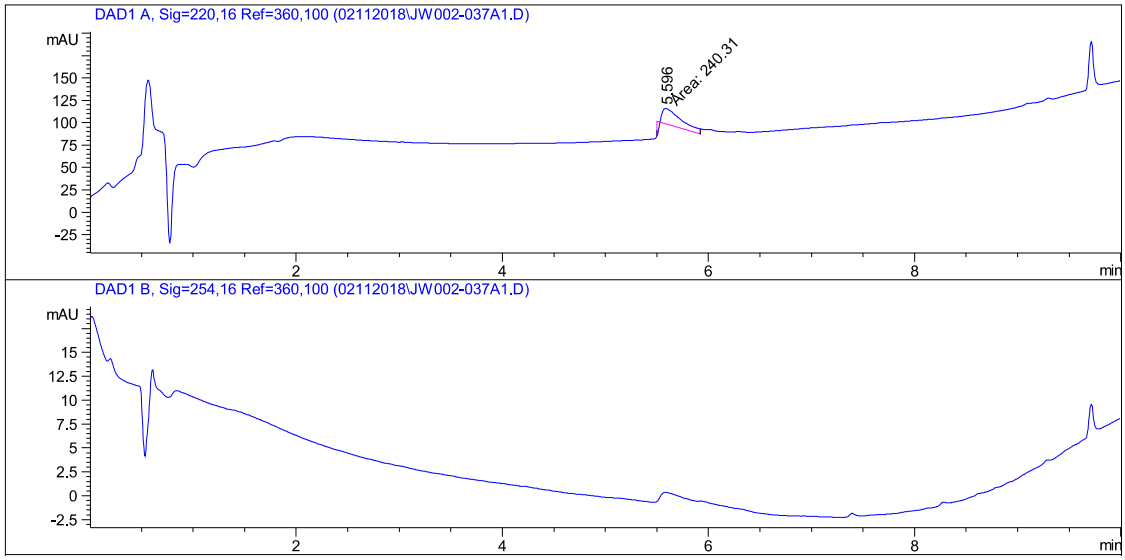
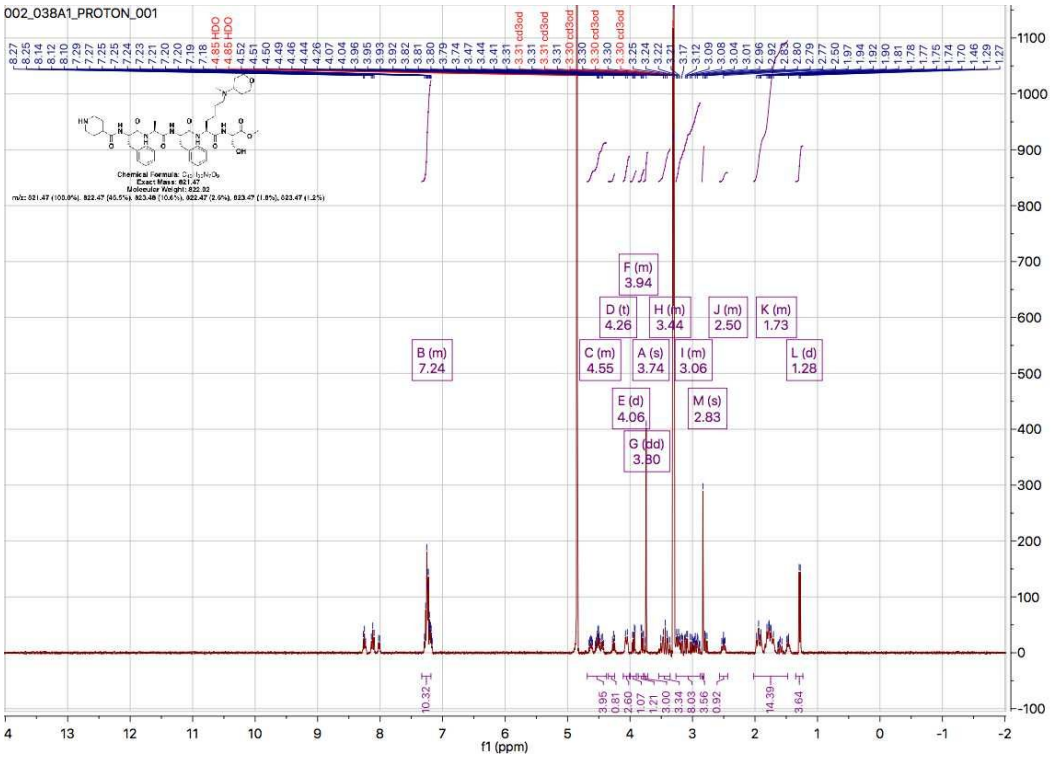
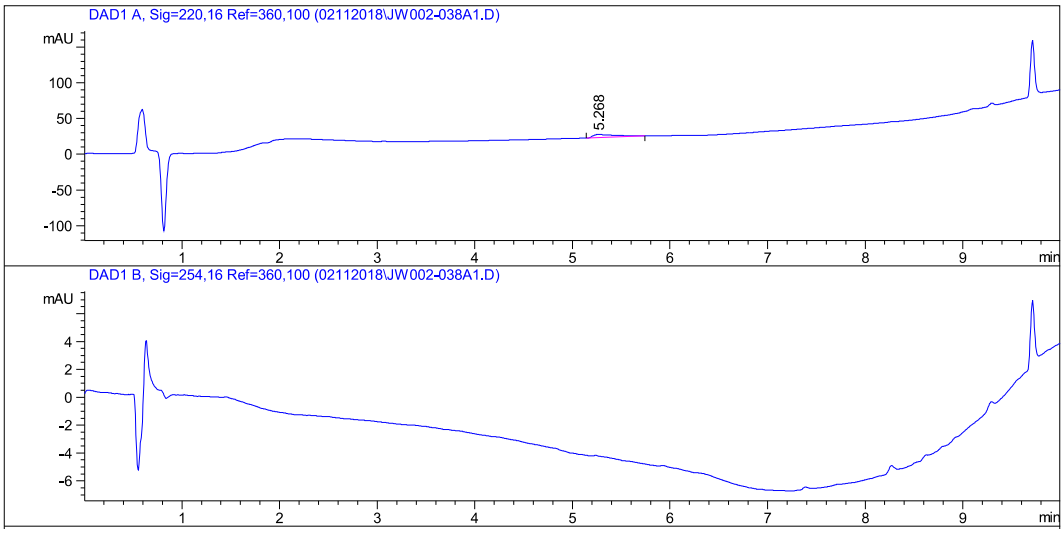


Figure S40. UNC6218 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM and MS Spectra.





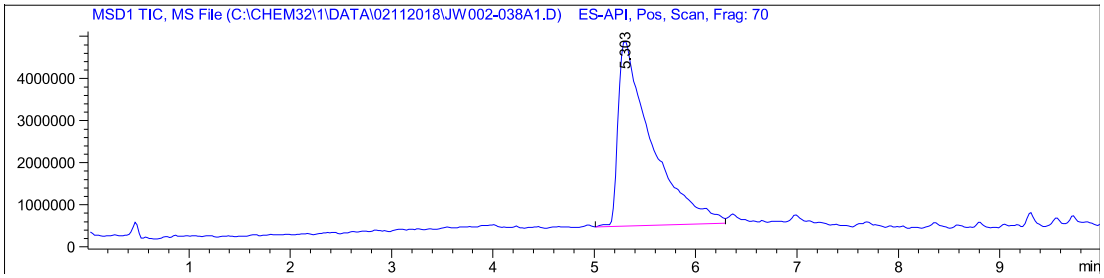
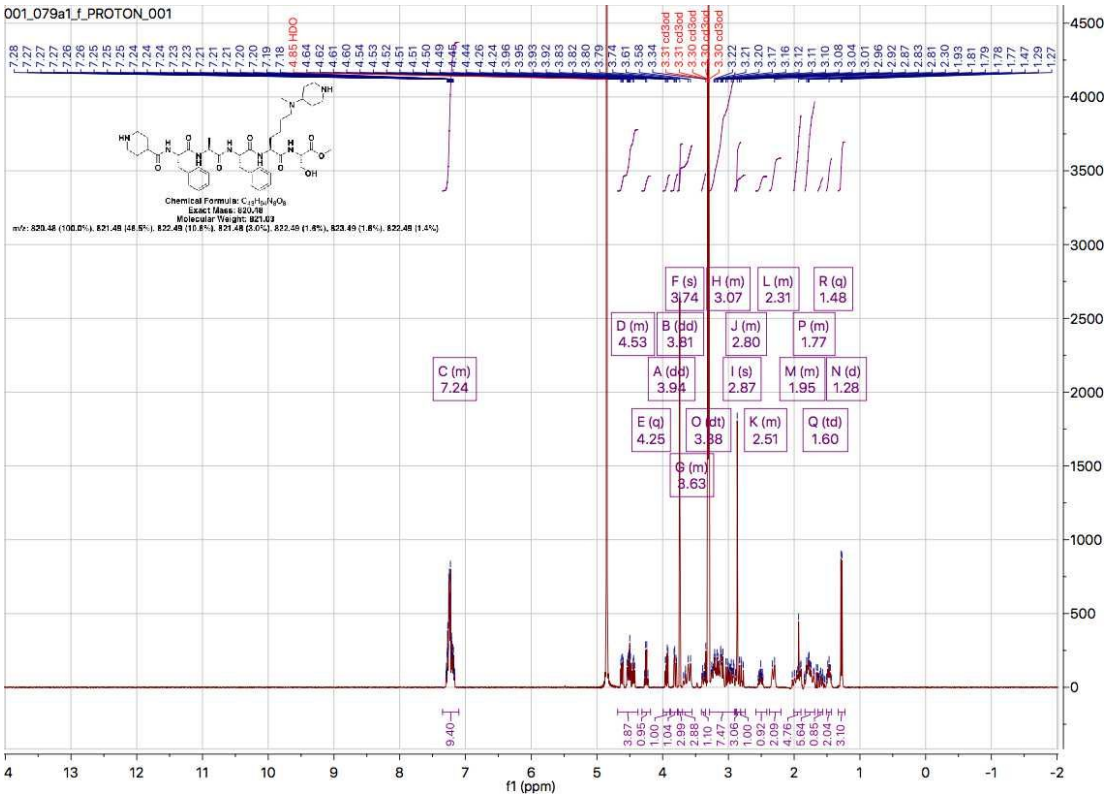


Figure S41. UNC6127 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



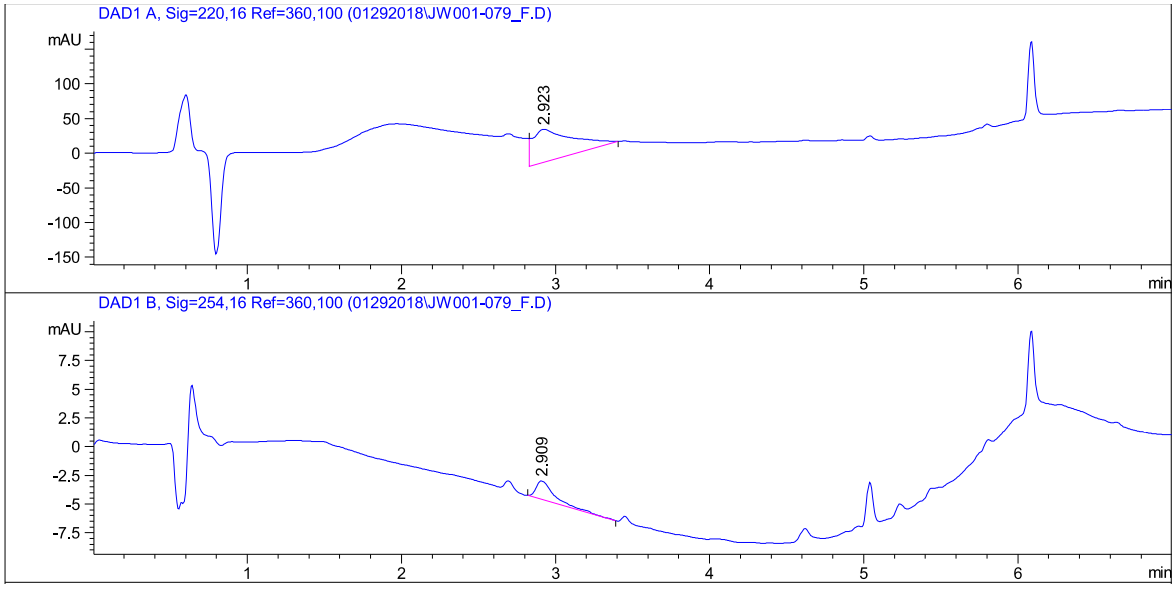
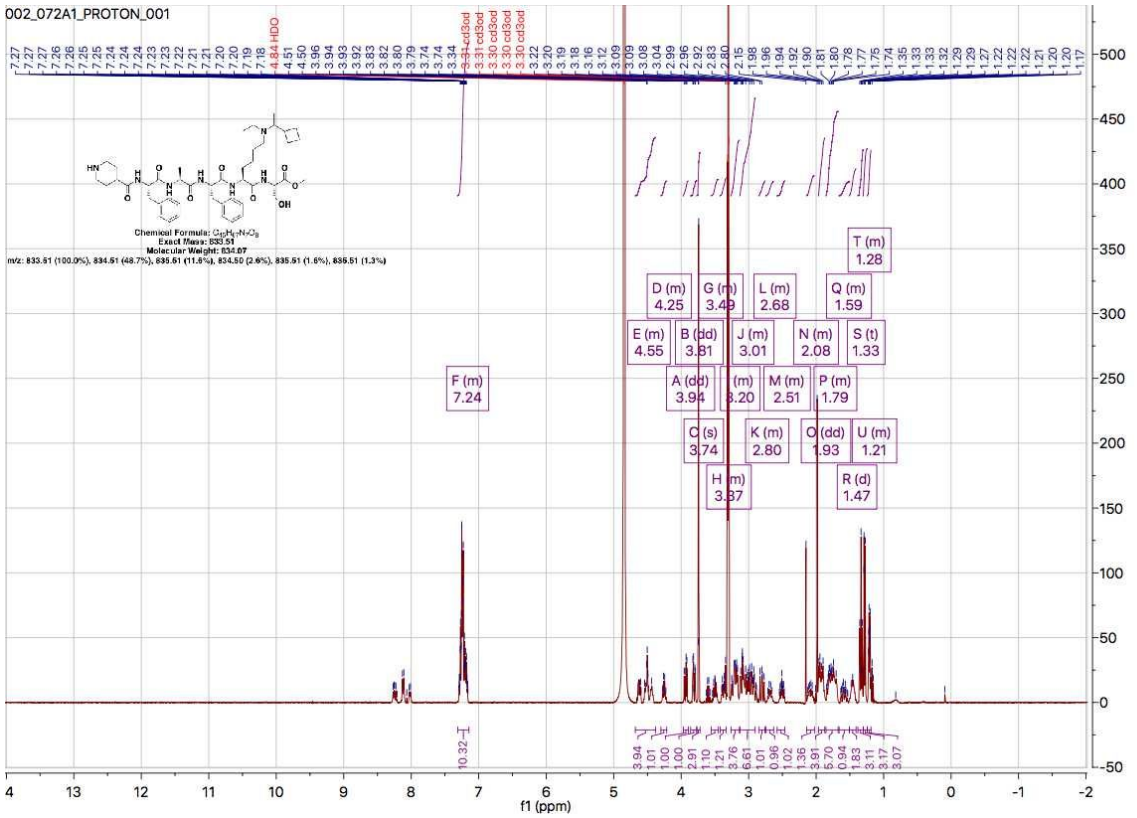
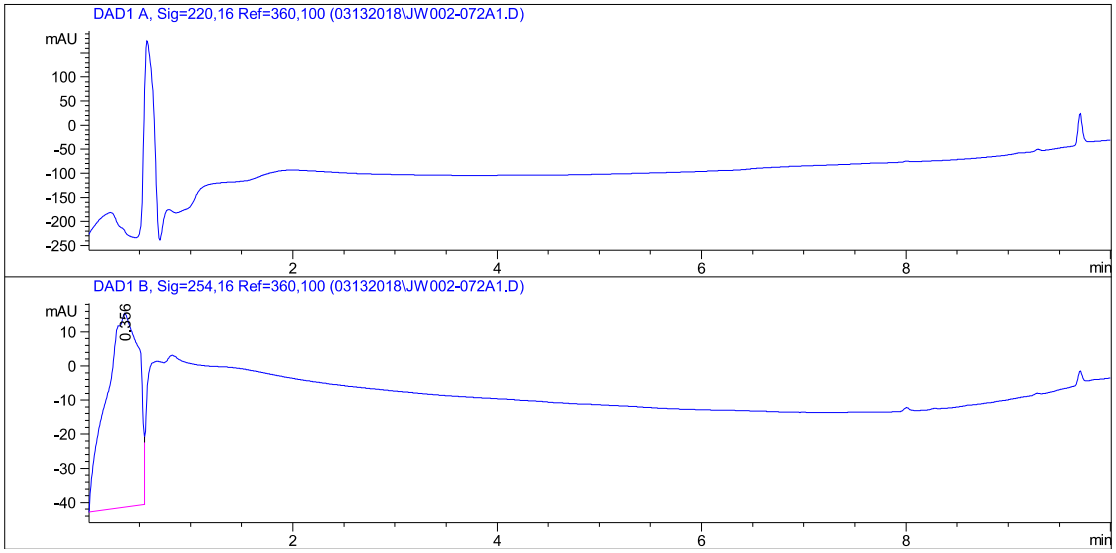


Figure S42. UNC6254 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM and MS Spectra.





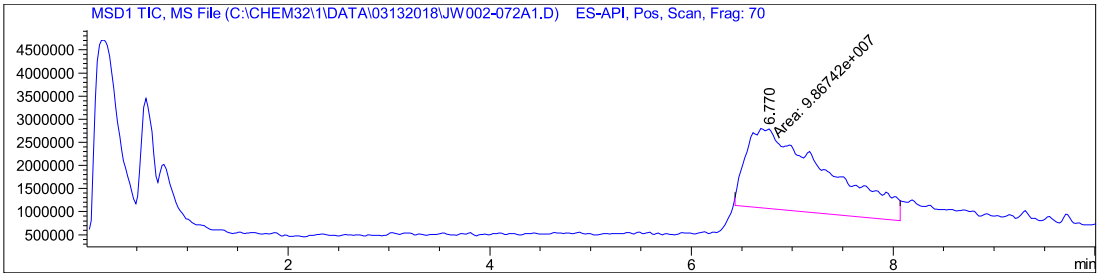
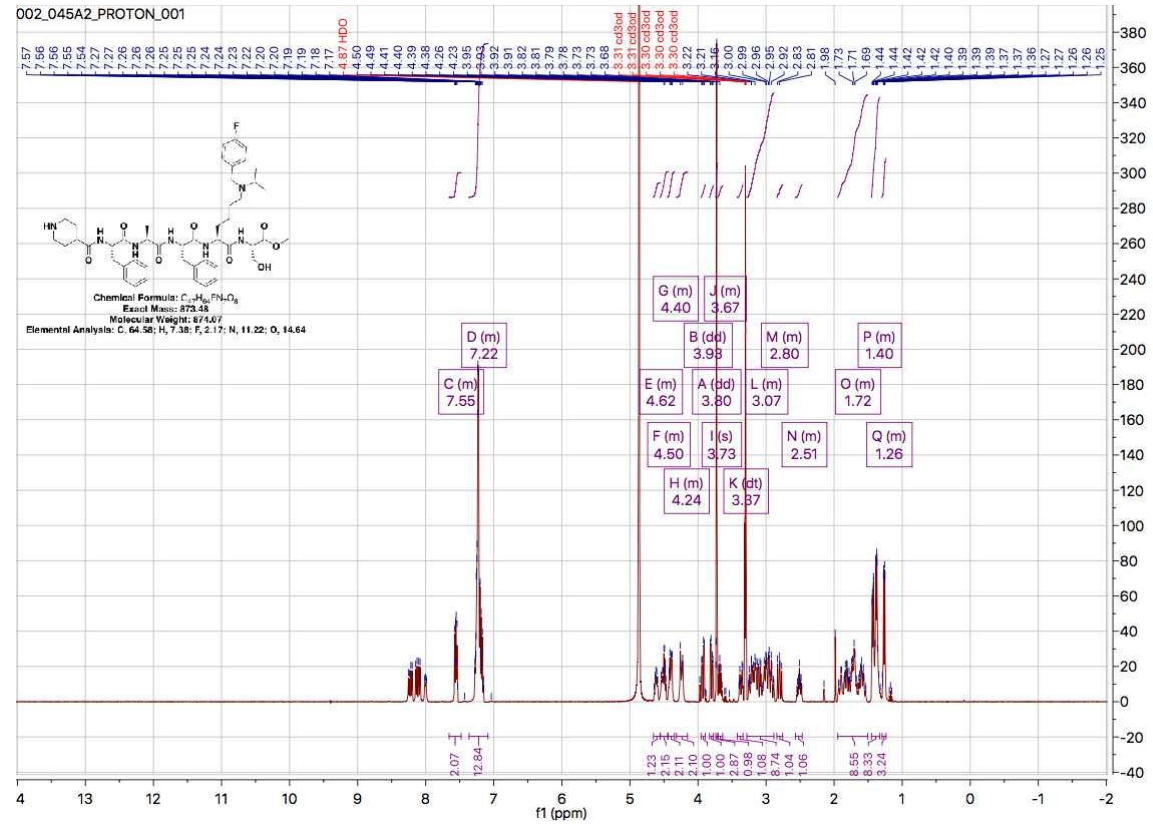


Figure S43. UNC6251 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM.



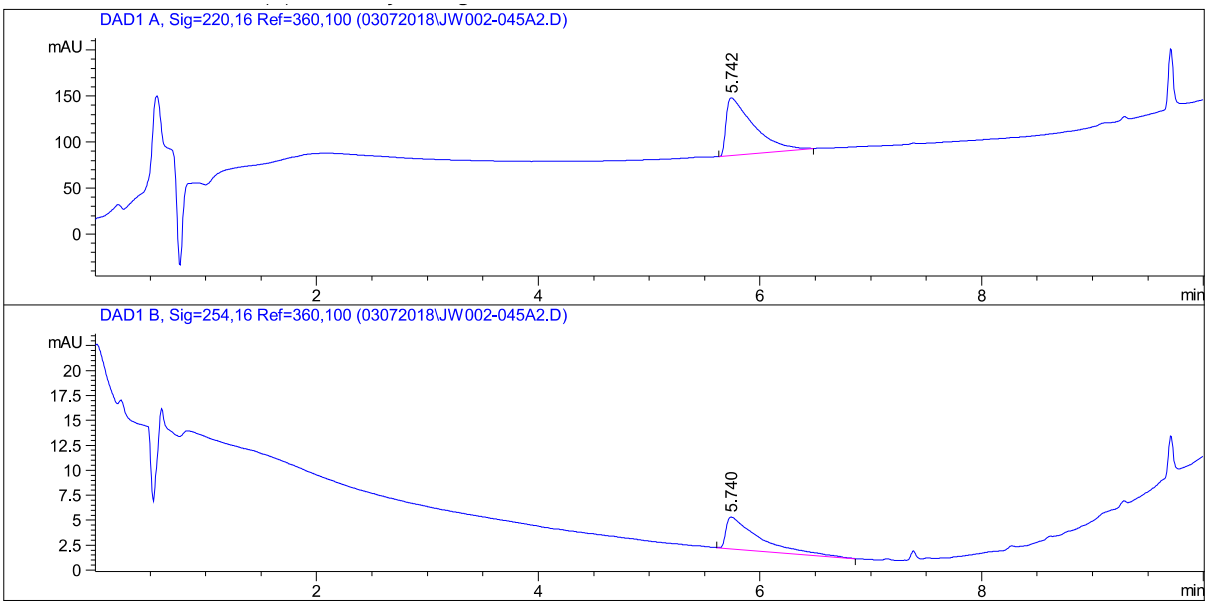
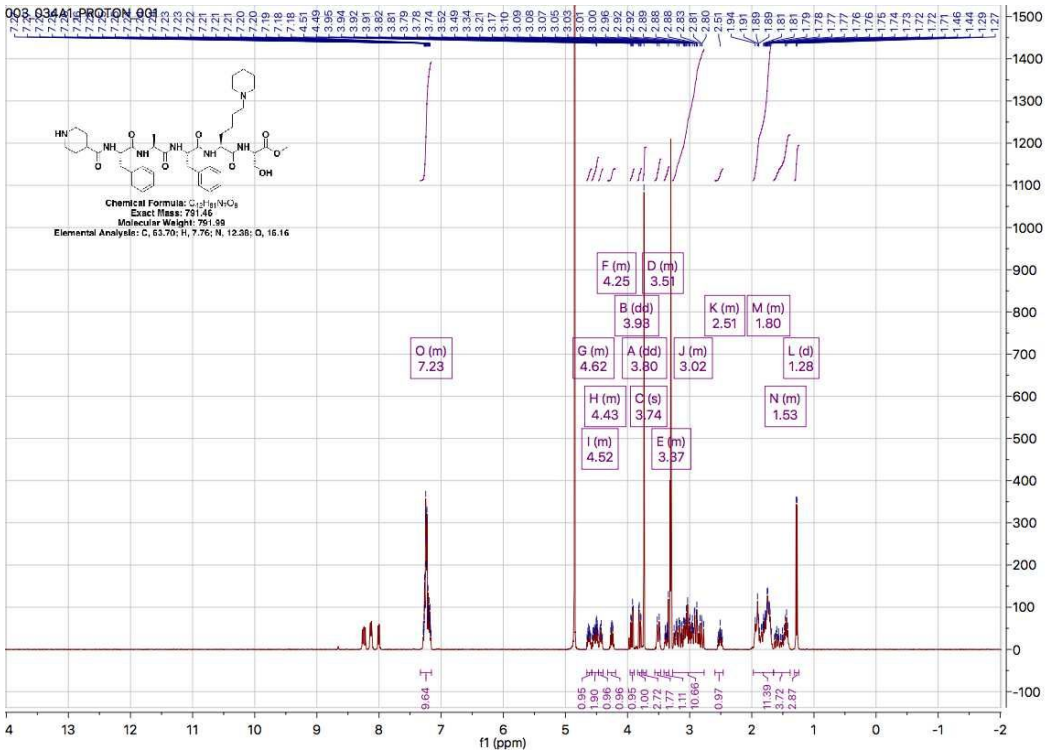
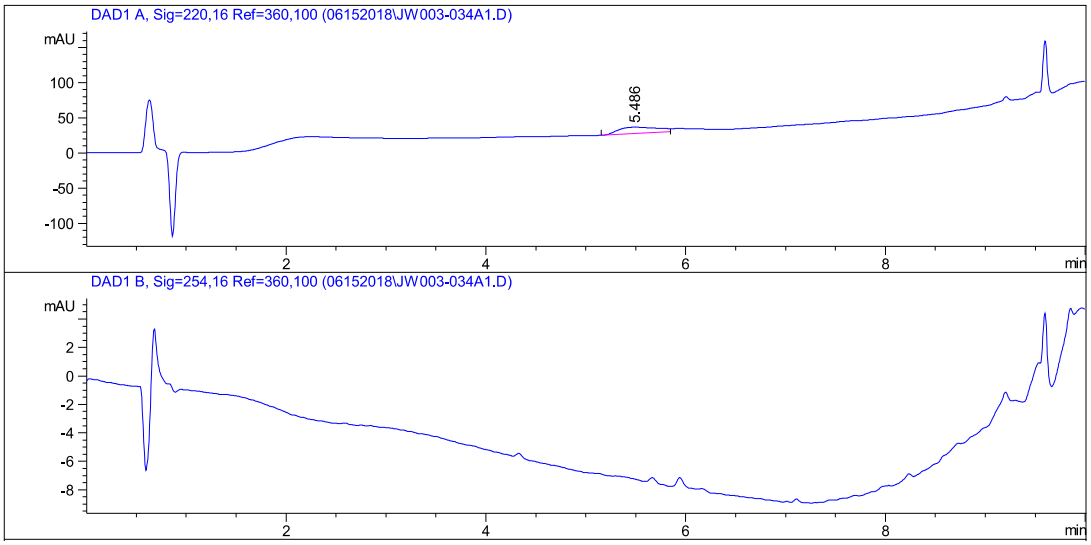


Figure S44. UNC6474 ^{1}H NMR Spectra and LC-MS Spectra at 220 and 254 nM and MS Spectra.





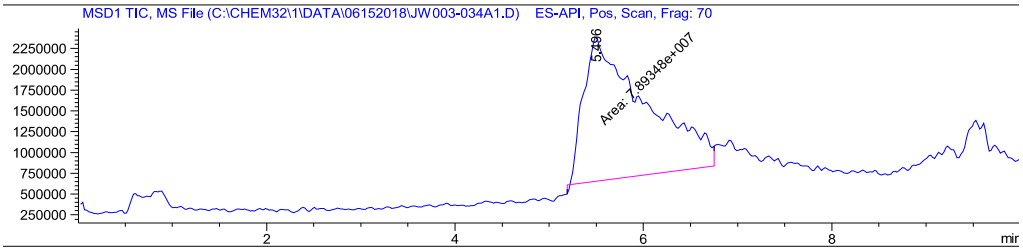


Figure S45. LC-MS Spectra of UNC5246-Biotin at 220 and 254 nM.

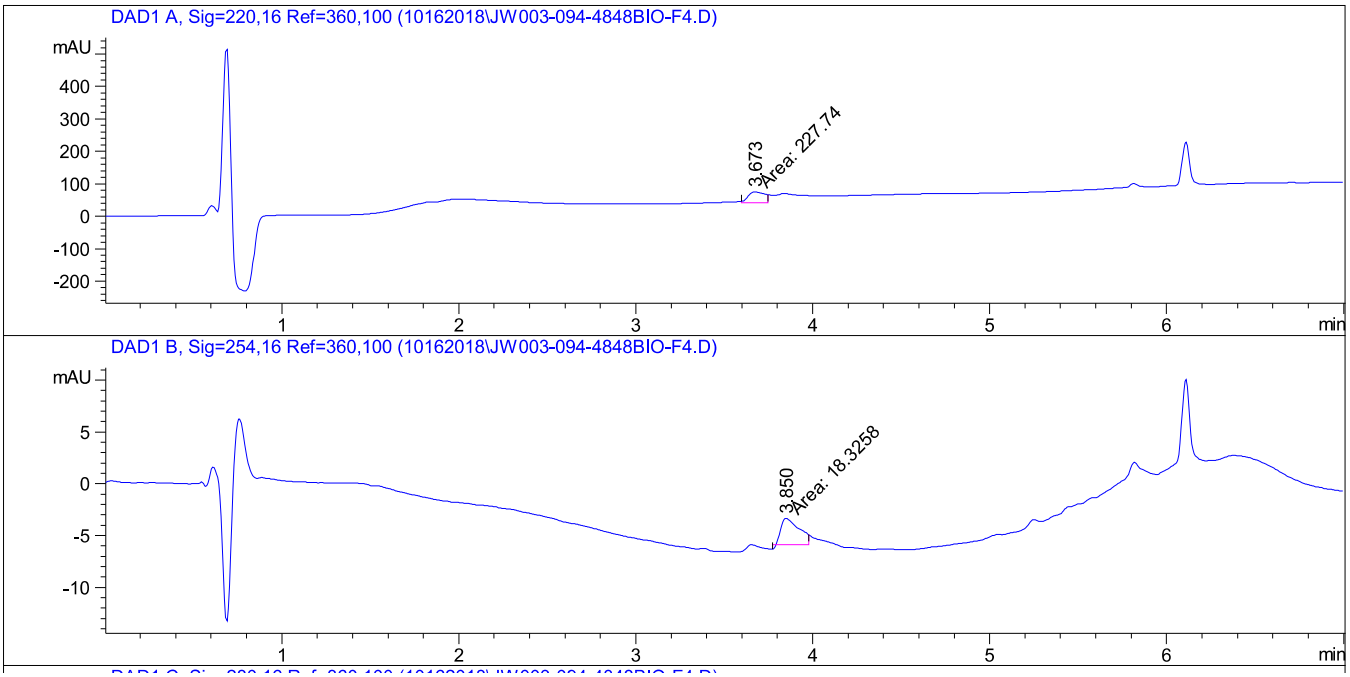
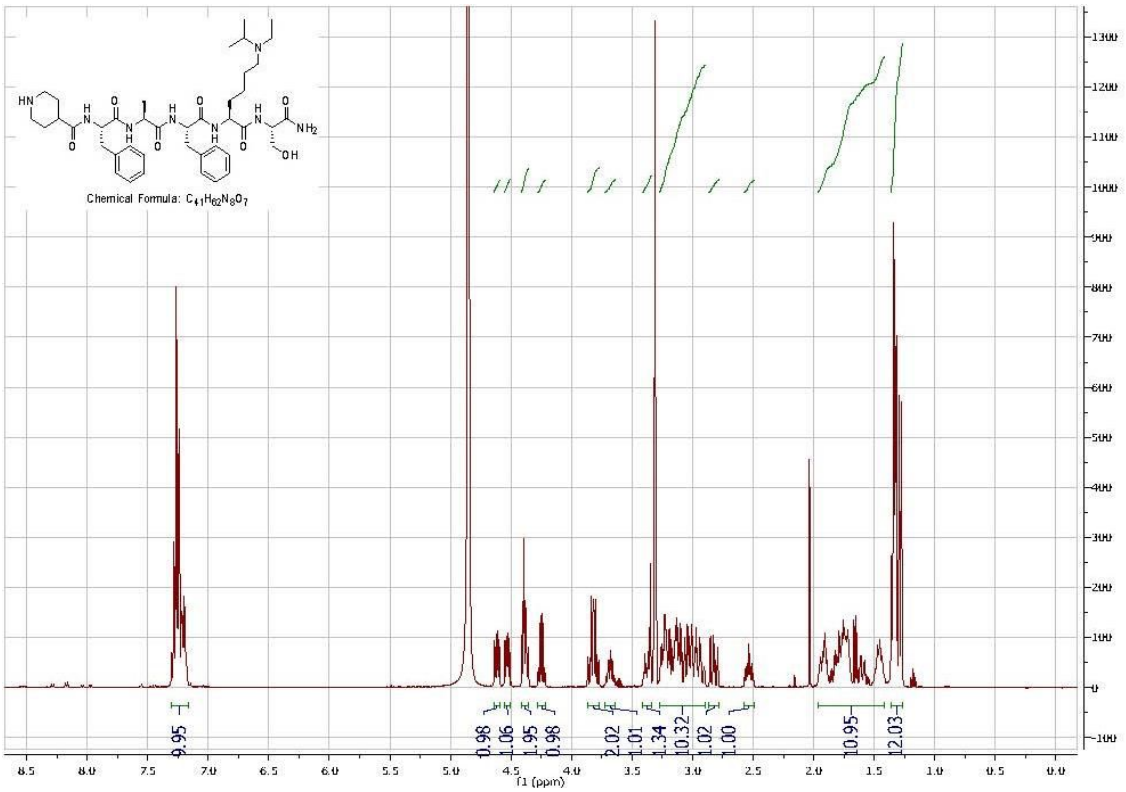
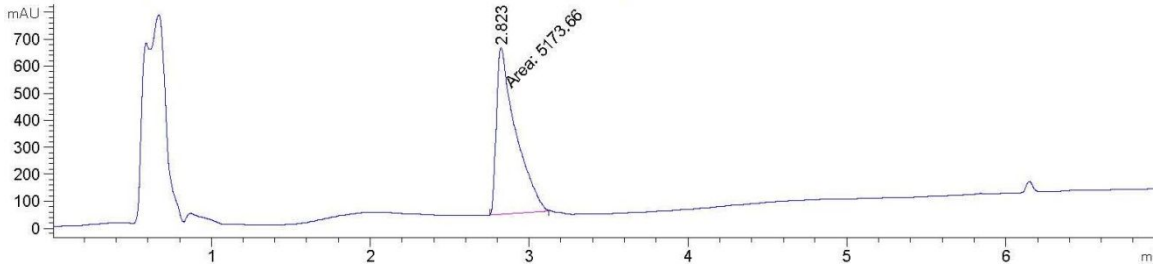


Figure S46. ^{1}H NMR Spectra and LC-MS Spectra of UNC4848 at 220 nM.





1. Barnash, K. D., Lamb, K. N., Stuckey, J. I., Norris, J. L., Cholensky, S. H., Kireev, D. B., Frye, S. V., and James, L. I. Chromodomain Ligand Optimization via Target-Class Directed Combinatorial Repurposing. ACS Chemical Biology 2016, 11, 2475-2483, DOI: 10.1021/acschembio.6b00415