SUPPORTING INFORMATION

Rational Design of Selective and Bioactive Inhibitors of the *Mycobacterium tuberculosis* Proteasome

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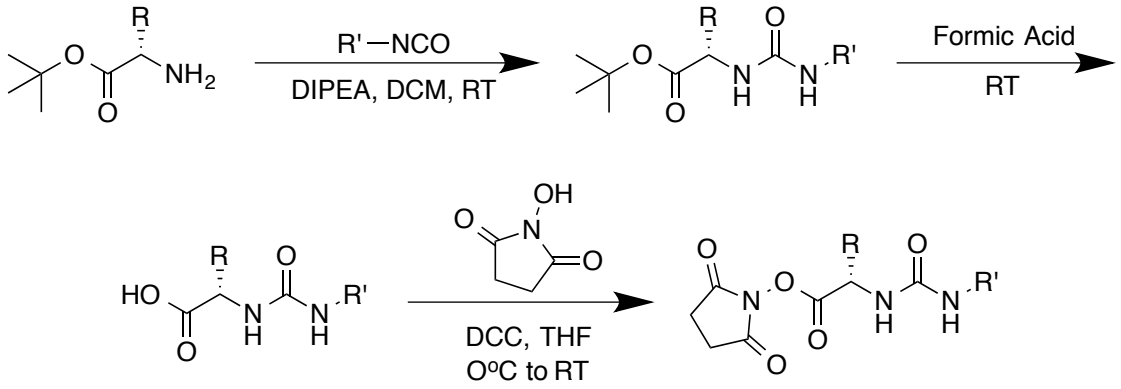
General Methods

All reactions were carried out in oven-dried glassware with dry solvent under an atmosphere of nitrogen unless indicated otherwise. Dry solvents were obtained using a solid-state solvent purification system provided at the Brown University Chemistry Department. Thin-layer chromatography was performed using pre-coated silica gel 60 plates and visualized by UV fluorescence quenching and stained with $KMnO_4$. Flash chromatography was performed using ZEOprep 60 silica gel (pore diameter 6 nm, particle size 40-63 µm). Yields refer to chromatographically pure compounds. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance III Ultra-Shield 400 MHz spectrometer for all intermediates and all final inhibitor analogs were recorded on a Bruker Avance III HD Ascend 600 MHz spectrometer. All chemical shifts are reported on a δ scale relative to the appropriate solvent peak. Multiplicities are described as follows: s (singlet), d (doublet), dd (doublet of doublets), t (triplet), q (quartet), quin (quintet), sext (sextet), m (multiplet). All high-resolution mass spectra were recorded by Brown University staff using a Jeol JMS-600H spectrometer and an Agilent Technologies 6530 Accurate-Mass Q-TOF LC/MS.

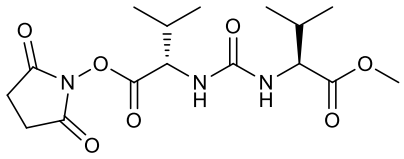
General Synthesis of Dipeptide Ureas



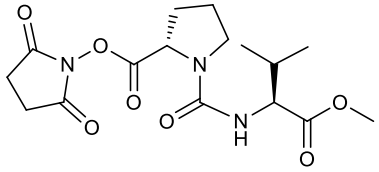
Supplementary Scheme 1. Synthesis of Dipeptide Ureas.

The appropriate tert-butyl amino acid (0.5 mmol) is dissolved in 1 mL of DCM. To the solution was added the isocyanate, or acid chloride, (0.5 mmol), followed by the slow addition of DIPEA (174.2 µL, 1.0 mmol). The reaction mixture was stirred for 16 hours at room temperature. Then, the reaction was evaporated to dryness and diluted in 50 mL of ethyl acetate. The solution was washed with 1 M HCl (3 x 25 mL), water (1 x 25 mL), and brine. The organic layer was collected, dried with sodium sulfate and filtered. After concentrating, the urea was dissolved in formic acid (1131.9 µL, 30 mmol) and a few drops of water were added before stirring for 16 hours. The reaction was concentrated and toluene was added to azeotropically remove water. This resulting solid was dissolved in 2 mL of THF and placed into an ice bath. To the solution was added *N*-hydroxysuccinimide (63.3 mg, 0.55 mmol). A solution of DCC (123.8 mg, 0.6 mmol) in 1 mL THF was then slowly added and stirred for 16 hours at room temperature. The reaction was filtered through silica eluting with ethyl acetate. After concentrating, ethyl acetate was added and placed into a freezer for 15-20 minutes, then filtered again through silica before

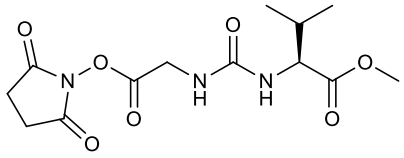
evaporating to dryness. (Note: The synthesis of the N,N-diethyl asparagine dipeptide urea was performed according to literature[1]).



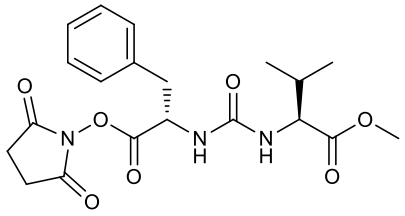
(S)-2,5-dioxopyrrolidin-1-yl 2-(3-((S)-1-methoxy-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate: Yield: 87%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 5.69-5.63 (dd, 2H), 4.83-4.79 (dd, 1H), 4.48-4.45 (dd, 1H), 3.75 (s, 3H), 2.84 (s, 4H), 2.31-2.23 (m, 1H), 2.15-2.07 (m, 1H), 1.03-1.02 (d, 3H), 1.00-0.98 (d, 3H), 0.95-0.93 (d, 3H), 0.87-0.86 (d, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 174.50, 168.87, 168.72, 156.96, 58.04, 56.39, 52.30, 31.79, 31.47, 25.60, 18.96, 18.69, 17.74, 17.35; HRMS (*m/z*): $[M+H]^+$ calcd., 372.1771; found, 372.1758.



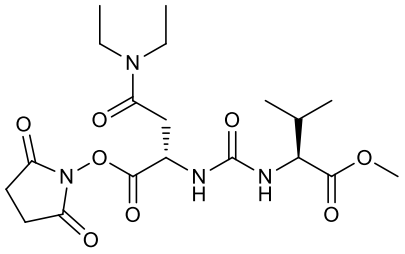
(S)-2,5-dioxopyrrolidin-1-yl 2-(3-((S)-1-methoxy-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate: Yield: 91 %; NMR ^{1}H (400 MHz, $CDCl_3$): δ 4.97-4.95 (d, 1H), 4.79-4.77 (dd, 1H), 4.44-4.41 (dd, 1H), 3.72 (s, 3H), 3.57-3.52 (m, 1H), 3.48-3.42 (m, 1H), 2.81 (s, 4H), 2.33-2.30 (m, 2H), 2.19-2.09 (m, 3H), 0.94-0.93 (d, 3H), 0.91-0.89 (d, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 173.64, 172.43, 168.91, 168.39, 155.91, 58.28, 57.25, 52.15, 45.66, 31.25, 29.95, 25.60, 25.44, 24.45, 18.90, 17.89; HRMS (*m/z*): $[M+H]^+$ calcd., 370.1614; found, 370.1607.



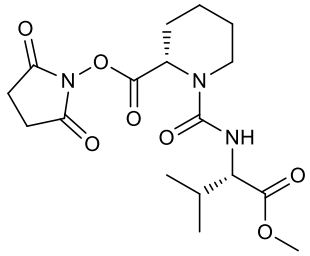
(S)-methyl 2-(3-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)ureido)-3-methylbutanoate: Yield: 95%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 5.78-5.76 (d, 1H), 5.71-5.68 (t, 1H), 4.45-4.43 (dd, 1H), 4.39-4.37 (d, 2H), 3.74 (s, 3H), 2.85 (s, 4H), 2.18-2.09 (m, 1H), 0.97-0.95 (d, 3H), 0.90-0.88 (d, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 173.98, 169.14, 167.14, 157.28, 58.14, 52.17, 39.92, 31.31, 25.60, 19.00, 17.69; HRMS (*m/z*): $[M+H]^+$ calcd., 330.1301; found, 330.1299.



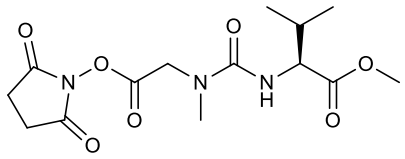
(S)-methyl 2-(3-((S)-1-((2,5-dioxopyrrolidin-1-yl)oxy)-1-oxo-3-phenylpropan-2-yl)ureido)-3-methylbutanoate: Yield: 75%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.30-7.29 (m, 5H), 5.71-5.68 (d, 1H), 5.47-5.44 (d, 1H), 5.26-5.21 (m, 1H), 4.48-4.45 (dd, 1H), 3.60 (s, 3H), 3.26-3.25 (d, 2H), 2.84 (s, 4H), 2.12-2.04 (m, 1H), 0.93-0.92 (d, 3H), 0.85-0.83 (d, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 174.29, 172.24, 168.85, 168.21, 157.43, 134.83, 129.99, 128.55, 127.26, 57.91, 52.30, 52.06, 38.27, 31.47, 25.60, 25.39, 18.92, 17.67. HRMS (*m/z*): $[M+Na]^+$ calcd., 442.1590; found, 442.1584.



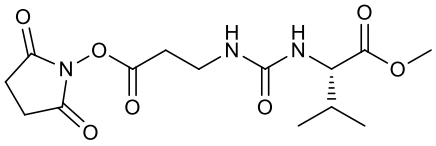
(S)-2,5-dioxopyrrolidin-1-yl 4-(diethylamino)-2-(3-((S)-1-methoxy-3-methyl-1-oxobutan-2-yl)ureido)-4-oxobutanoate: Yield: 88%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 6.50-6.48 (d, 1H), 5.97-5.95 (d, 1H), 5.24-5.21 (m, 1H), 4.42-4.38 (dd, 1H), 3.73 (s, 3H), 3.41-3.19 (m, 4H), 2.84-2.78 (m, 2H), 2.73 (s, 4H), 2.19-2.11 (m, 1H), 1.22-1.18 (m, 3H), 1.16-1.13 (m, 3H), 0.99-0.98 (d, 3H), 0.94-0.92 (d, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 173.52, 172.86, 169.43, 168.22, 157.20, 58.17, 52.04, 48.17, 42.40, 40.86, 36.42, 31.11, 25.54, 19.07, 17.67, 13.68, 12.75; HRMS (*m/z*): $[M+H]^+$ calcd., 443.2142; found, 443.2136.



(S)-2,5-dioxopyrrolidin-1-yl 1-(((S)-1-methoxy-3-methyl-1-oxobutan-2-yl)carbamoyl)piperidine-2-carboxylate: Yield: 62%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 5.18-5.16 (d, 1H), 4.34-4.30 (m, 1H), 4.17-4.10 (m, 1H), 3.82-3.79 (m, 1H), 3.71 (s, 3H), 3.65-3.59 (m, 1H), 2.82 (s, 4H), 2.34-2.20 (dd, 1H), 2.14-2.08 (m, 1H), 2.01-1.90 (dd, 1H), 1.85-1.67 (m, 2H), 1.54-1.42 (m, 2H), 0.93-0.87 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 173.59, 172.74, 169.23, 157.51, 58.55, 57.21, 52.37, 42.30, 31.31, 25.60, 25.46, 24.97, 20.89, 18.84, 17.97; HRMS (*m/z*): $[M+H]^+$ calcd., 384.1771; found, 384.1775.

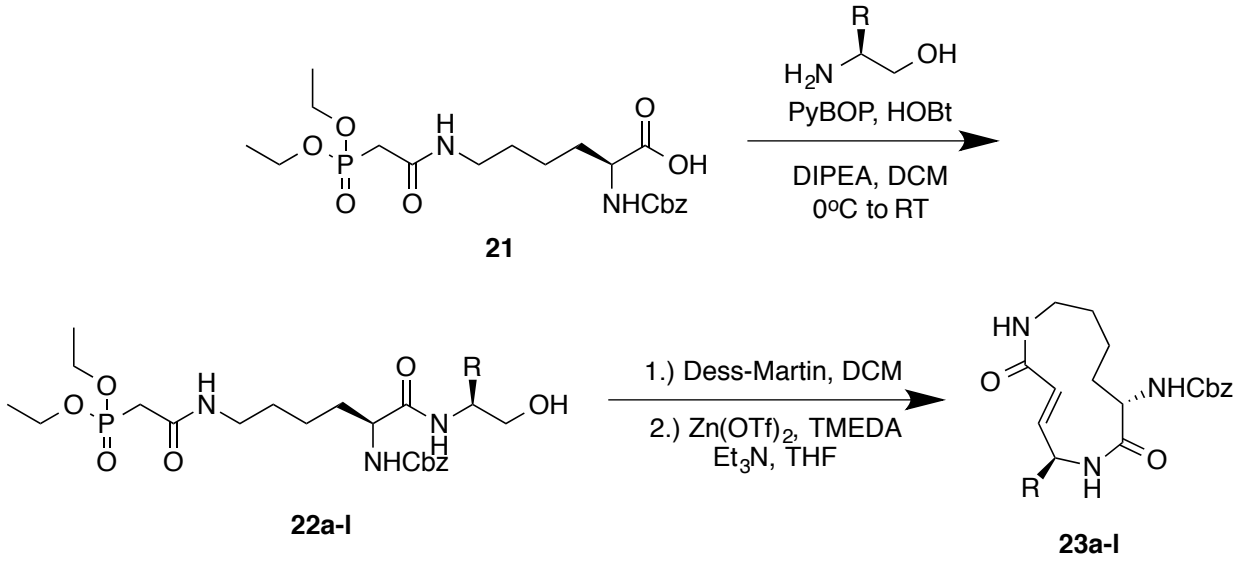


(S)-methyl 2-(3-(2-((2,5-dioxopyrrolidin-1-yl)oxy)-2-oxoethyl)-3-methylureido)-3-methylbutanoate: Yield: 70%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 5.20-5.18 (d, 1H), 4.39 (m, 2H), 4.34-4.32 (m, 1H), 3.65 (s, 3H), 2.97 (s, 3H), 2.76 (s, 4H), 2.07-2.03 (m, 1H), 0.87-0.83 (dd, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 173.41, 169.06, 165.87, 157.40, 58.68, 52.04, 48.01, 34.84, 31.20, 25.54, 25.36, 18.85, 17.89; HRMS (*m/z*): $[M+H]^+$ calcd., 344.145; found, 344.1448.



(S)-methyl 2-(3-(3-((2,5-dioxopyrrolidin-1-yl)oxy)-3-oxopropyl)ureido)-3-methylbutanoate; Yield: 86%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 5.49-5.46 (t, 1H), 5.31-5.28 (d, 1H), 4.40-4.37 (dd, 1H), 3.72 (s, 3H), 3.61-3.56 (q, 2H), 2.88-2.82 (m, 6H), 2.14-2.05 (m, 1H), 0.95-0.93 (d, 3H), 0.88-0.86 (d, 3H); ^{13}C (100 MHz, $CDCl_3$): δ 173.79, 169.54, 167.92, 157.59, 57.98, 52.03, 47.15, 35.85, 32.44, 31.28, 25.64, 25.61, 19.01, 17.70; HRMS (*m/z*): $[M+H]^+$ calcd., 344.1458; found, 344.1448.

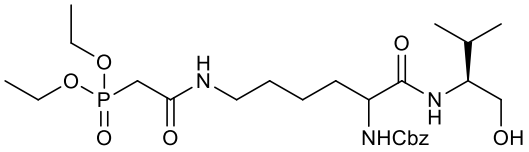
General Synthesis of Syringolin B Macrocyclic Precursors[2]



Supplementary Scheme 2. Synthesis of Intermediates **23a-l**.

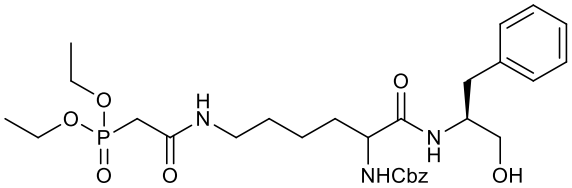
Intermediate **22a-l**:

N^2-((benzyloxy)carbonyl)-N^6-(2-(diethoxyphosphoryl)acetyl)-*L*-lysine (**21**) was synthesized as reported in literature.[2] **21** (458.4 mg, 1.0 mmol) was dissolved in 5 mL of DCM and placed into an ice bath. HOBt (183.7 mg, 1.2 mmol) and PyBOP (624.5 mg, 1.2 mmol) were added to the mixture, followed by the appropriate amino alcohol (1.1 mmol), which was prepared according to literature.[3, 4] After stirring for 5 minutes in the ice bath, DIPEA (261.3 µL, 1.5 mmol) was slowly added before stirring at room temperature for 16 hours. Then, the reaction was evaporated and redissolved in 100 mL of ethyl acetate. The solution was washed with 1 M HCl (3 x 50 mL), water (1 x 50 mL) and brine. The organic layer was collected, dried with sodium sulfate and evaporated before TLC and flash chromatography. TLC conditions – Acetone:DCM, 3:1 v/v.

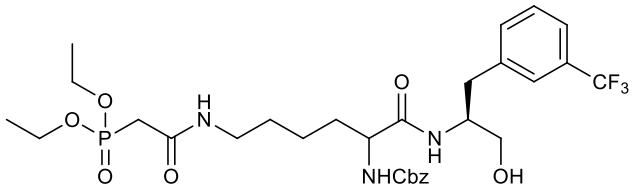


benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-hydroxy-3-methylbutan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22a**): Yield: 55%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.36-7.34 (m, 5H), 6.97-6.94 (t, 1H), 6.66-6.64 (d, 1H), 5.89-5.87 (d, 1H), 5.10 (s, 2H), 4.19-4.09 (m, 5H), 3.75-3.63 (m, 3H), 3.30-3.26 (m, 2H), 3.14-3.12 (sext, 1H), 2.87 (s, 1H), 2.82 (s, 1H), 1.89-1.79 (m, 3H), 1.76-1.69 (m, 1H), 1.56-1.52 (m, 2H), 1.46-1.41 (m, 2H), 1.35-1.32 (t, 6H), 0.96-0.91 (dd, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.97, 164.23, 156.21, 136.35, 128.50, 128.13, 128.02, 66.86, 63.04,

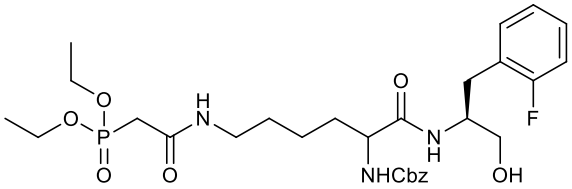
62.98, 62.93, 62.79, 57.05, 46.30, 38.99, 29.07, 28.53, 26.42, 22.18, 19.45, 19.06, 16.34, 16.28; HRMS (*m/z*): $[M+Na]^+$ calcd., 566.2607; found, 566.2625.



benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-hydroxy-3-phenylpropan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22b**): Yield: 66%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.38-7.29 (m, 5H), 7.24-7.16 (m, 6H), 6.11-6.09 (d, 1H), 5.10-5.04 (s, 2H), 4.18-4.13 (m, 2H), 4.12-4.05 (q, 4H), 3.64-3.62 (m, 1H), 3.53-3.49 (m, 1H), 3.21-3.19 (m, 2H), 2.89-2.82 (m, 4H), 1.76-1.72 (m, 1H), 1.67-1.62 (m, 1H), 1.47-1.46 (m, 2H), 1.36-1.34 (m, 1H), 1.31-1.28 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 172.00, 164.45, 156.27, 138.09, 136.38, 129.32, 128.51, 128.40, 128.13, 127.99, 126.38, 66.83, 62.98, 62.92, 62.85, 55.07, 52.93, 39.18, 37.02, 35.79, 34.47, 32.26, 28.56, 22.29, 16.34, 16.28; HRMS (*m/z*): $[M+Na]^+$ calcd., 614.2607; found, 614.2628.

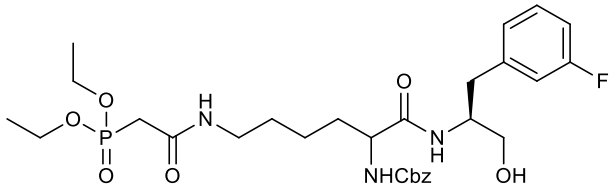


benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-hydroxy-3-(3-(trifluoromethyl)phenyl)propan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22c**): Yield: 67%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.49-7.36 (m, 4H), 7.32-7.23 (m, 6H), 7.19-7.17 (t, 1H), 6.14-6.13 (d, 1H), 5.06-5.00 (s, 2H), 4.17-4.11 (m, 2H), 4.12-4.05 (q, 4H), 3.61-3.60 (m, 1H), 3.51-3.47 (m, 1H), 3.20-3.15 (m, 2H), 2.92-2.87 (m, 2H), 2.85-2.81 (d, 1H), 1.74-1.68 (m, 1H), 1.65-1.57 (m, 1H), 1.48-1.42 (m, 2H), 1.35-1.29 (m, 2H), 1.27-1.25 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 172.17, 164.53, 156.25, 139.36, 136.35, 132.83, 128.79, 128.46, 128.08, 127.92, 126.00, 123.17, 66.76, 62.88, 62.82, 62.75, 55.13, 52.71, 46.30, 39.13, 36.66, 35.73, 34.43, 32.31, 28.51, 26.39, 16.27, 16.21; HRMS (*m/z*): $[M+Na]^+$ calcd., 682.2481; found, 682.2462.

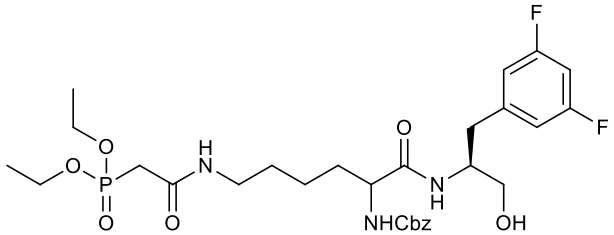


benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-(2-fluorophenyl)-3-hydroxypropan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22d**): Yield: 60%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.85-7.83 (d, 1H), 7.68-7.67 (d, 1H), 7.38-7.30 (m, 5H), 7.25-7.23 (t, 1H), 7.19-7.16 (q, 1H), 7.05-7.02 (t, 1H), 7.01-6.98 (t, 1H), 6.93-6.92 (t, 1H), 6.85-6.84 (d, 1H), 5.80-5.79 (d, 1H), 5.10 (s, 2H), 4.25-

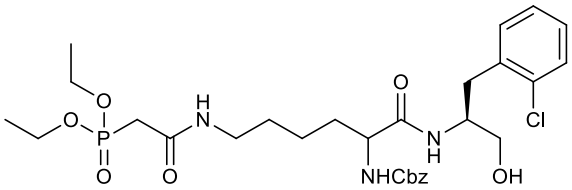
4.22 (m, 1H), 4.15-4.08 (m, 5H), 3.72-3.71 (d, 1H), 3.56-3.53 (dd, 1H), 3.28-3.22 (m, 2H), 2.92-2.90 (d, 2H), 2.86 (s, 1H), 2.83 (s, 1H), 1.80-1.76 (m, 1H), 1.68-1.63 (m, 1H), 1.53-1.49 (m, 2H), 1.40-1.35 (m, 2H), 1.34-1.32 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.70, 164.23, 160.49, 156.13, 136.37, 131.60, 128.51, 128.27, 128.13, 128.00, 127.92, 126.00, 124.86, 124.10, 115.27, 66.86, 63.40, 63.03, 62.99, 62.93, 55.02, 52.07, 46.29, 39.04, 35.58, 34.71, 32.18, 30.25, 29.51, 28.51, 22.10, 16.30, 16.27; HRMS (*m/z*): $[M+H]^+$ calcd., 610.2694; found, 610.2691.



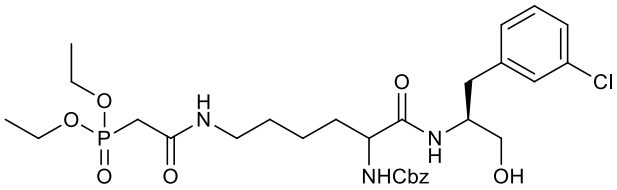
benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-(2-fluorophenyl)-3-hydroxypropan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22e**): Yield: 48%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.33-7.32 (m, 1H), 7.30-7.25 (m, 5H), 7.18-7.13 (m, 2H), 6.97-6.96 (d, 1H), 6.93-6.92 (d, 1H), 6.84-6.81 (t, 1H), 6.15-6.14 (d, 1H), 5.07-5.01 (q, 2H), 4.15-4.11 (m, 2H), 4.09-4.05 (q, 4H), 3.60-3.59 (d, 1H), 3.51-3.47 (m, 1H), 3.19-3.15 (m, 2H), 2.87-2.78 (m, 4H), 1.74-1.69 (m, 1H), 1.64-1.60 (m, 1H), 1.47-1.41 (m, 2H), 1.35-1.30 (m, 2H), 1.28-1.25 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 172.07, 164.45, 163.53, 161.91, 156.25, 140.88, 136.38, 129.78, 129.72, 128.45, 128.06, 127.93, 126.03, 125.00, 116.23, 113.22, 66.77, 62.90, 62.85, 62.81, 55.09, 52.70, 46.29, 39.17, 36.66, 35.54, 34.66, 32.21, 28.55, 26.36, 26.31, 22.33, 16.27, 16.24; HRMS (*m/z*): $[M+Na]^+$ calcd., 632.2513; found, 632.2538.



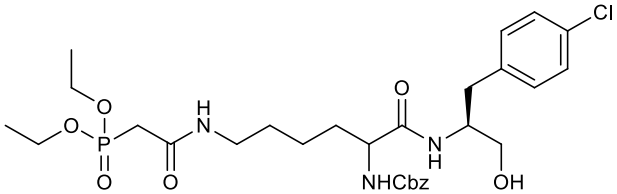
benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-(3,5-difluorophenyl)-3-hydroxypropan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22f**): Yield: 73%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.36-7.31 (m, 5H), 7.19-7.17 (m, 1H), 7.00-6.97 (d, 1H), 6.80-6.79 (d, 2H), 6.67-6.62 (t, 1H), 5.92-5.90 (d, 1H), 5.09-5.08 (d, 2H), 4.15-4.09 (m, 6H), 3.68-3.65 (d, 1H), 3.54-3.50 (m, 1H), 3.32-3.19 (m, 2H), 2.88-2.82 (m, 4H), 1.79-1.74 (m, 2H), 1.69-1.63 (m, 1H), 1.52-1.48 (m, 2H), 1.41-1.38 (m, 2H), 1.35-1.31 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.82, 164.37, 164.33, 161.73, 156.21, 142.19, 136.31, 128.51, 128.16, 128.01, 112.29, 112.04, 101.89, 66.90, 63.03, 62.96, 62.74, 55.02, 52.47, 46.33, 38.99, 36.73, 35.80, 34.49, 32.27, 28.48, 26.43, 26.35, 22.24, 16.33, 16.27; HRMS (*m/z*): $[M+Na]^+$ calcd., 650.2419; found, 650.2436.



benzyl (1-(((S)-1-(2-chlorophenyl)-3-hydroxypropan-2-yl)amino)-6-(2-(diethoxyphosphoryl)acetamido)-1-oxohexan-2-yl)carbamate (**22g**): Yield: 80%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.36-7.31 (m, 6H), 7.30-7.28 (t, 2H), 7.15-7.12 (m, 2H), 7.03-7.01 (m, 2H), 5.85-5.84 (d, 1H), 5.09 (s, 2H), 4.32-4.28 (m, 1H), 4.15-4.08 (m, 5H), 3.73-3.71 (d, 1H), 3.59-3.55 (dd, 1H), 3.27-3.23 (m, 2H), 3.02-3.00 (d, 2H), 2.88 (s, 1H), 2.83 (s, 1H), 1.79-1.74 (m, 2H), 1.69-1.62 (m, 1H), 1.52-1.46 (m, 2H), 1.41-1.36 (m, 2H), 1.34-1.31 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.70, 164.31, 164.28, 156.14, 136.35, 135.91, 134.28, 131.52, 129.44, 128.52, 128.15, 128.00, 126.82, 66.84, 63.44, 63.04, 62.96, 62.90, 55.02, 51.80, 46.27, 39.04, 35.80, 34.61, 32.25, 28.50, 26.42, 26.34, 22.11, 16.35, 16.28; HRMS (*m/z*): $[M+H]^+$ calcd., 626.2398; found, 626.2415.

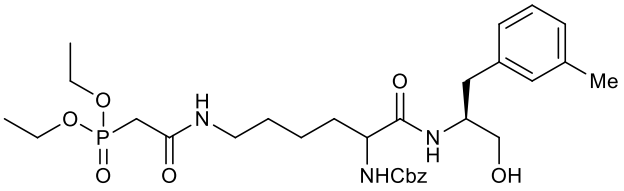


benzyl (1-(((S)-1-(3-chlorophenyl)-3-hydroxypropan-2-yl)amino)-6-(2-(diethoxyphosphoryl)acetamido)-1-oxohexan-2-yl)carbamate (**22h**): Yield: 66%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.39-7.33 (m, 5H), 7.25-7.19 (m, 3H), 7.15-7.12 (m, 1H), 6.89-6.84 (m, 2H), 5.81-5.79 (d, 1H), 5.11 (s, 2H), 4.18-4.07 (m, 6H), 3.70-3.67 (d, 1H), 3.53-3.49 (dd, 1H), 3.36-3.30 (m, 1H), 3.28-3.21 (m, 1H), 2.88-2.83 (m, 4H), 1.83-1.77 (m, 1H), 1.72-1.64 (m, 1H), 1.57-1.49 (m, 2H), 1.45-1.38 (m, 2H), 1.36-1.32 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.65, 164.25, 156.15, 140.17, 136.33, 134.13, 129.71, 129.44, 128.33, 128.17, 128.05, 127.52, 126.64, 66.90, 63.08, 63.01, 62.78, 55.06, 52.65, 38.95, 35.83, 34.53, 32.26, 28.49, 22.15, 16.35, 16.29; HRMS (*m/z*): $[M+Na]^+$ calcd., 648.2218; found, 648.2236.

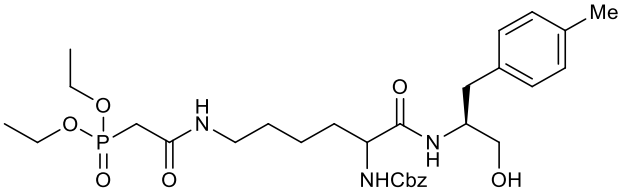


benzyl (1-(((S)-1-(4-chlorophenyl)-3-hydroxypropan-2-yl)amino)-6-(2-(diethoxyphosphoryl)acetamido)-1-oxohexan-2-yl)carbamate (**22i**): Yield: 94%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.75-7.73 (d, 1H), 7.62-7.60 (d, 1H), 7.37-7.31 (m, 5H), 7.25-7.19 (m, 2H), 7.18-7.14 (m, 3H), 7.11-7.09 (t, 1H), 5.92-5.90 (d, 1H), 5.08-5.07 (s, 2H), 4.15-4.07 (m, 6H), 3.68-3.58 (m, 1H), 3.52-3.48 (dd, 1H), 3.37-3.20 (m, 2H), 2.89-2.80 (m, 4H), 1.77-1.69 (m, 1H), 1.68-1.61 (m, 1H), 1.51-1.45 (m, 2H), 1.39-1.35 (m, 2H), 1.34-1.29 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.83,

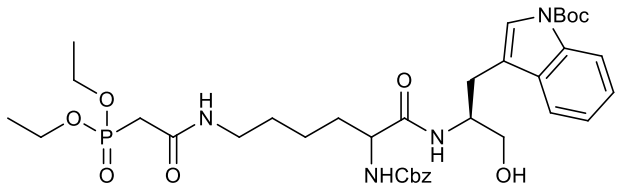
164.33, 156.22, 142.83, 136.61, 136.28, 132.15, 130.72, 126.88, 128.53, 128.48, 128.17, 128.00, 125.33, 124.64, 66.90, 63.07, 62.97, 62.82, 55.03 52.78, 46.27, 39.00, 36.33, 34.46, 32.30, 28.45, 22.21, 16.34, 16.27; HRMS (*m/z*): $[M+H]^+$ calcd., 626.2398; found, 626.2395.



benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-hydroxy-3-(m-tolyl)propan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22j**): Yield:63%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.35-7.28 (m, 5H), 7.20-7.10 (m, 3H), 7.05-6.97 (m, 3H), 6.12-6.10 (d, 1H), 5.07 (s, 2H), 4.18-4.04 (m, 6H), 3.63-3.61 (d, 1H), 3.53-3.48 (dd, 1H), 3.24-3.16 (m, 2H), 2.89-2.77 (m, 4H), 2.28 (s, 3H), 1.79-1.70 (m, 1H), 1.68-1.63 (m, 1H), 1.51-1.41 (m, 2H), 1.38-1.32 (m, 2H), 1.30-1.27 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 172.02, 164.56, 156.24, 138.00, 137.92, 136.38, 130.07, 128.49, 128.26, 128.11, 127.97, 127.12, 126.32, 66.80, 63.08, 62.95, 62.90, 62.83, 55.11, 52.99, 46.27, 39.13, 36.91, 32.31, 28.58, 26.42, 21.34, 16.33, 16.26; HRMS (*m/z*): $[M+H]^+$ calcd., 606.2944; found, 606.2937.



benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-hydroxy-3-(p-tolyl)propan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22k**): Yield: 66%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 7.34-7.26 (m, 6H), 7.10-7.08 (d, 2H), 7.04-7.02 (m, 2H), 6.18-6.16 (d, 1H), 5.06 (s, 2H), 4.18-4.04 (m, 6H), 3.62-3.60 (d, 1H), 3.51-3.47 (dd, 1H), 3.19-3.18 (d, 2H), 2.87-2.78 (m, 4H), 2.26 (s, 3H), 1.78-1.70 (m, 1H), 1.67-1.62 (m, 1H), 1.50-1.42 (m, 2H), 1.38-1.32 (m, 2H), 1.30-1.26 (t, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.99, 164.44, 156.25, 136.40, 135.72, 135.00, 129.19, 129.06, 128.48, 128.08, 127.94, 66.76, 62.92, 62.85, 62.76, 55.06, 53.02, 46.32, 39.19, 36.56, 32.32, 28.57, 26.42, 21.01, 16.34, 16.27; HRMS (*m/z*): $[M+H]^+$ calcd., 606.2944; found, 606.2952.

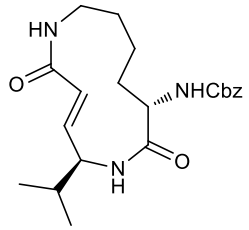


benzyl (6-(2-(diethoxyphosphoryl)acetamido)-1-(((S)-1-hydroxy-3-(1H-indol-3-yl)propan-2-yl)amino)-1-oxohexan-2-yl)carbamate (**22l**): Yield: 75%; NMR ^{1}H (400 MHz, $CDCl_3$): δ 8.13-8.11 (m, 1H), 7.71-7.69 (d, 1H), 7.48 (s, 1H), 7.38-7.28 (m, 6H), 7.27-7.23 (t, 1H), 6.93-6.91 (d, 1H), 6.86-6.84 (d, 1H), 5.84-5.82 (d, 1H), 5.10 (s, 2H), 4.36-4.26 (m, 1H), 4.19-4.07 (m, 5H), 3.74-3.71 (d, 1H), 3.61-3.57 (m, 1H), 3.35-3.31 (m, 1H), 3.26-3.20 (m, 1H), 3.00-2.94 (m, 1H), 2.86 (s, 1H), 2.81 (s, 1H), 1.85-1.77 (m, 2H), 1.75-1.65 (m, 10H), 1.56-1.52 (m, 2H), 1.46-1.38 (m, 2H), 1.35-1.28 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 171.75, 164.23, 156.12, 149.76, 136.35, 130.65, 128.50, 128.13, 128.03, 124.42, 123.89, 122.61, 119.31, 116.93, 115.18, 83.57, 66.88, 63.06, 62.99, 62.98, 55.09, 51.45, 46.30, 39.00, 32.43, 28.47, 28.22, 26.55, 22.17, 16.32, 16.26; HRMS (*m/z*): $[M+H]^+$ calcd., 731.3421; found, 731.3408.

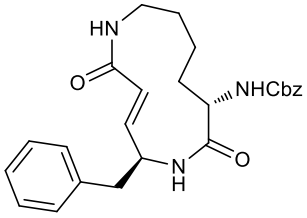
General Procedure for Horner-Emmons-Wadsworth Macrocyclization

Macrocycles **23a-l**:

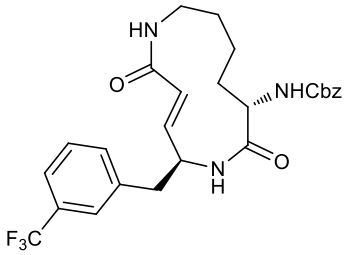
Individually, intermediates **22a-l** (0.9 mmol) were dissolved in 10 mL of DCM and Dess-Martin periodinane (420 mg, 0.99 mmol) was added. After stirring at room temperature for 90 minutes, the reaction was diluted in 100 mL of ethyl acetate. The organic solution was then washed with 1:1 mixture of saturated sodium bicarbonate and 2% $Na_2S_2O_3$ (2 x 50 mL). The organic phase was collected, dried, filtered and concentrated, which was used without further purification. TMEDA (162 µL, 1.08 mmol) and TEA (502 µL, 3.6 mmol) was added to a solution of $Zn(OTf)_2$ (720 mg, 1.98 mmol) in 200 mL of THF. The solution was stirred for 15-20 minutes before adding oxidized **22a-l** in 100 mL of THF to a pressure-equalizing addition funnel. The solution in the funnel was added drop-wise to the reaction over 2 hours. The reaction was stirred for 20 hours at room temperature before concentrating and redissolving in 100 mL of ethyl acetate. Then, the solution was washed with 1 M HCl (1 x 50 mL), water (1 x 50 mL) and brine. Once the organic phase was collected, dried, filtered and concentrated. The resulting oil was purified via flash chromatography, yielding a white or yellow solid. TLC Conditions – DCM:Acetone, 1:1 v/v. (Note: Pure macrocycle can be achieved in the case of yellow macrocycles by redissolving in a small amount of MeOH and placing the solution into the freezer for 15 minutes, followed by filtration.)



benzyl ((5S,8S,E)-5-isopropyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23a**): Yield: 58%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.25-8.23 (d, 1H), 7.41-7.31 (m, 6H), 7.12-7.11 (d, 1H), 6.80-6.76 (dd, 1H), 6.23-6.20 (d, 1H), 5.01 (s, 2H), 4.41-4.40 (m, 1H), 4.14-4.10 (q, 1H), 3.30-3.24 (m, 1H), 2.95-2.93 (d, 1H), 2.15-2.10 (m, 1H), 1.79-1.75 (m, 1H), 1.58-1.53 (m, 1H), 1.43-1.41 (m, 2H), 1.27-1.23 (m, 1H), 0.97-0.96 (d, 3H), 0.93-0.92 (d, 3H); ^{13}C (100 MHz, DMSO-d_6): δ 171.64, 166.25, 155.94, 145.40, 137.52, 128.80, 128.24, 128.16, 120.24, 65.78, 56.44, 53.64, 46.19, 40.50, 38.37, 31.65, 30.75, 30.65, 20.39, 19.76, 17.69; HRMS (*m/z*): $[M+H]^+$ calcd., 388.2236; found, 388.2235.

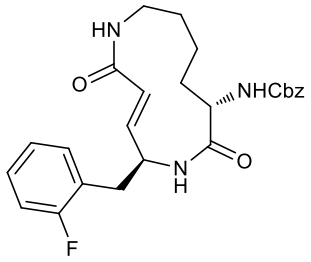


benzyl ((5S,8S,E)-5-benzyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23b**): Yield: 20%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.44-8.43 (d, 1H), 7.41-7.22 (m, 12H), 7.08-7.07 (d, 1H), 6.77-6.74 (dd, 1H), 6.35-6.32 (d, 1H), 5.01-4.97 (s, 2H), 4.61 (m, 1H), 4.33-4.32 (m, 1H), 2.99-2.97 (m, 1H), 2.92-2.89 (dd, 1H), 2.76-2.72 (dd, 1H), 2.22-2.17 (t, 1H), 1.60-1.56 (m, 1H), 1.45-1.43 (m, 2H), 1.28-1.19 (m, 2H), 0.93-0.91 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.63, 166.20, 155.92, 145.90, 138.84, 137.46, 129.37, 128.78, 128.21, 128.14, 126.85, 119.92, 65.81, 53.73, 52.18, 40.55, 38.87, 38.35, 30.76, 30.56, 17.66; HRMS (*m/z*): $[M+Na]^+$ calcd., 458.2056; found, 458.2056.

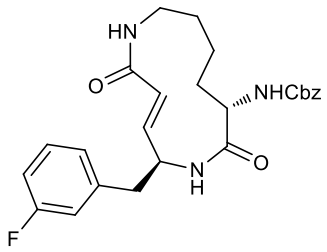


benzyl ((5S,8S,E)-2,7-dioxo-5-(3-(trifluoromethyl)benzyl)-1,6-diazacyclododec-3-en-8-yl)carbamate (**23c**): Yield: 25%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.47-8.46 (d, 1H), 7.71 (s, 1H), 7.65-7.55 (m, 3H), 7.43-7.41 (t, 1H), 7.36-7.30 (m, 5H), 7.10-7.09 (d, 1H), 6.82-6.78 (dd, 1H), 6.36-6.33 (d, 1H), 4.98 (s, 2H), 4.63 (m, 1H), 4.31 (m, 1H), 3.30-3.25 (m, 1H), 3.07-3.04 (dd, 1H), 2.99-2.97 (m, 1H), 2.79-2.75 (dd, 1H), 2.20-2.16 (t, 1H), 1.60-1.58 (m, 1H), 1.45-1.43 (m, 2H),

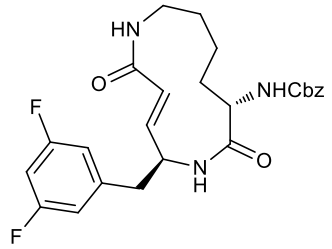
1.29-1.25 (m, 1H), 0.92-0.90 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.63, 166.20, 155.92, 145.90, 138.84, 137.46, 129.37, 128.78, 128.21, 128.14, 126.85, 119.92, 65.81, 53.73, 52.18, 40.55, 38.87, 38.35, 30.76, 30.56, 17.66; HRMS (*m/z*): $[M+Na]^+$ calcd., 526.1930; found, 526.1948.



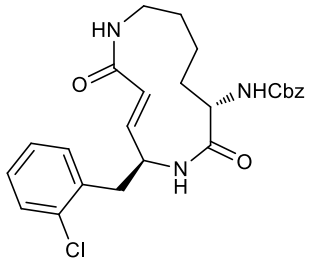
benzyl ((5S,8S,E)-5-(2-fluorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23d**): Yield: 22%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.46-8.45 (d, 1H), 7.41-7.38 (m, 2H), 7.36-7.33 (m, 4H), 7.32-7.29 (m, 2H), 7.20 -7.17 (m, 2H), 7.11-7.10 (d, 1H), 6.71-6.67 (dd, 1H), 6.37-6.35 (d, 1H), 5.00 (s, 2H), 4.66 (m, 1H), 4.30 (m, 1H), 3.30-3.28 (m, 1H), 2.98-2.94 (m, 2H), 2.84-2.80 (dd, 1H), 2.22-2.21 (t, 1H), 1.60-1.56 (m, 1H), 1.45-1.43 (m, 2H), 1.29-1.24 (m, 1H), 0.94-0.88 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.72, 166.15, 160.23, 155.94, 145.04, 137.46, 131.77, 128.78, 128.22, 128.15, 124.88, 120.49, 115.75, 65.82, 53.80, 50.68, 40.55, 38.36, 32.07, 30.76, 30.44, 17.72; HRMS (*m/z*): $[M+H]^+$ calcd., 454.2142; found, 454.2138.



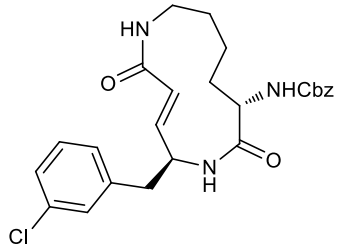
benzyl ((5S,8S,E)-5-(3-fluorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23e**): Yield: 39%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.44-8.43 (d, 1H), 7.42-7.40 (t, 1H), 7.37-7.33 (m, 4H), 7.32-7.30 (m, 1H), 7.18 -7.15 (m, 2H), 7.11-7.10 (d, 1H), 7.07-7.05 (t, 1H), 6.78-6.75 (dd, 1H), 6.34-6.32 (d, 1H), 4.99 (s, 2H), 4.61 (m, 1H), 4.32-4.31 (m, 1H), 3.32-3.28 (m, 1H), 2.99-2.93 (m, 2H), 2.74-2.70 (dd, 1H), 2.20-2.16 (t, 1H), 1.61-1.56 (m, 1H), 1.46-1.41 (m, 2H), 1.29-1.22 (m, 1H), 0.94-0.88 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.69, 166.16, 161.78, 155.93, 145.65, 137.45, 130.64, 128.78, 128.22, 128.15, 125.55, 120.06, 116.10, 113.76, 65.82, 53.74, 51.99, 40.54, 38.36, 30.75, 30.54, 17.66; HRMS (*m/z*): $[M+Na]^+$ calcd., 476.1962; found, 476.1978.



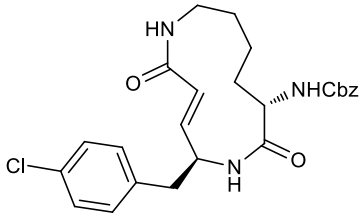
benzyl ((5S,8S,E)-5-(3,5-difluorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23f**): Yield: 30%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.43-8.42 (d, 1H), 7.43-7.41 (t, 1H), 7.36-7.33 (m, 4H), 7.32-7.29 (m, 1H), 7.13-7.10 (m, 1H), 7.09-7.05 (m, 3H), 6.78-6.75 (dd, 1H), 6.33-6.31 (d, 1H), 4.99 (s, 2H), 4.61 (m, 1H), 4.32-4.31 (m, 1H), 3.32-3.28 (m, 1H), 3.12-3.06 (m, 2H), 2.99-2.95 (m, 2H), 2.72-2.69 (dd, 1H), 2.18-2.14 (t, 1H), 1.61-1.57 (m, 1H), 1.46-1.42 (m, 2H), 1.29-1.23 (m, 1H), 0.92-0.88 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.76, 166.13, 163.55, 161.92, 155.95, 145.39, 135.46, 130.33, 128.79, 128.23, 128.16, 120.22, 112.71, 112.54, 102.45, 65.82, 59.61, 53.75, 40.53, 38.36, 30.73, 30.51, 17.67; HRMS (*m/z*): $[M+Na]^+$ calcd., 494.1867; found, 494.1889.



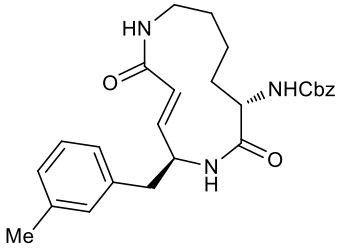
benzyl ((5S,8S,E)-5-(2-chlorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23g**): Yield: 22%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.51-8.50 (d, 1H), 7.47-7.43 (m, 2H), 7.42-7.40 (d, 1H), 7.37-7.33 (m, 4H), 7.32-7.29 (m, 2H), 7.19-7.18 (d, 1H), 6.71-6.68 (dd, 1H), 6.38-6.35 (d, 1H), 4.99 (s, 2H), 4.70 (m, 1H), 4.32-4.30 (m, 1H), 3.32-3.29 (m, 1H), 3.05-3.02 (dd, 1H), 2.99-2.97 (m, 1H), 2.89-2.85 (dd, 1H), 2.23-2.18 (t, 1H), 1.60-1.55 (t, 1H), 1.46-1.43 (m, 2H), 1.29-1.25 (m, 1H), 1.19-1.15 (m, 1H), 0.92-0.88 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.79, 166.07, 155.98, 144.98, 137.45, 135.97, 133.57, 131.73, 129.83, 128.80, 128.24, 128.17, 127.75, 120.44, 65.81, 53.79, 50.05, 46.19, 40.50, 36.48, 30.75, 30.46, 17.70; HRMS (*m/z*): $[M+H]^+$ calcd., 470.1847; found, 470.1858.



benzyl ((5S,8S,E)-5-(3-chlorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23h**): Yield: 28%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.43-8.42 (d, 1H), 7.41 (s, 1H), 7.37-7.33 (m, 5H), 7.32-7.28 (d, 3H), 7.09-7.08 (d, 1H), 6.79-6.76 (dd, 1H), 6.34-6.31 (d, 1H), 4.99 (s, 2H), 4.62-4.58 (m, 1H), 4.34-4.30 (m, 1H), 3.31-3.29 (m, 1H), 3.12-3.08 (m, 1H), 2.99-2.93 (m, 2H), 2.71-2.67 (dd, 1H), 2.20-2.16 (t, 1H), 1.61-1.57 (t, 1H), 1.47-1.43 (m, 2H), 1.29-1.25 (m, 1H), 1.19-1.15 (m, 1H), 0.94-0.88 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.71, 166.09, 155.92, 145.64, 141.51, 137.45, 133.34, 130.61, 129.32, 128.78, 128.21, 128.15, 126.89, 120.08, 65.82, 53.72, 51.97, 46.24, 40.55, 38.23, 30.74, 30.54, 17.65; HRMS (*m/z*): $[M+Na]^+$ calcd., 492.1666; found, 492.1651.

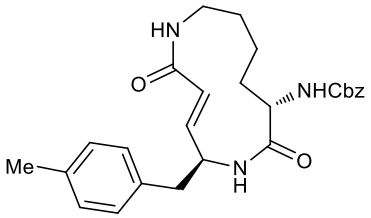


benzyl ((5S,8S,E)-5-(4-chlorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23i**): Yield: 13%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.44-8.42 (d, 1H), 7.44-7.42 (t, 1H), 7.39-7.36 (m, 3H), 7.35-7.30 (m, 5H), 7.14-7.13 (d, 1H), 6.77-6.74 (dd, 1H), 6.33-6.30 (d, 1H), 4.99 (s, 2H), 4.60-4.56 (m, 1H), 4.32-4.29 (m, 1H), 3.31-3.27 (m, 1H), 2.99-2.95 (m, 1H), 2.93-2.90 (dd, 1H), 2.71-2.67 (dd, 1H), 2.20-2.16 (t, 1H), 1.59-1.55 (t, 1H), 1.46-1.42 (m, 2H), 1.28-1.24 (m, 1H), 0.93-0.86 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.72, 166.14, 155.94, 145.74, 137.95, 137.45, 131.52, 131.28, 128.80, 128.72, 128.24, 128.17, 119.99, 65.81, 53.72, 52.10, 49.06, 40.50, 38.30, 30.75, 26.42, 17.64; HRMS (*m/z*): $[M+H]^+$ calcd., 470.1847; found, 470.1850.

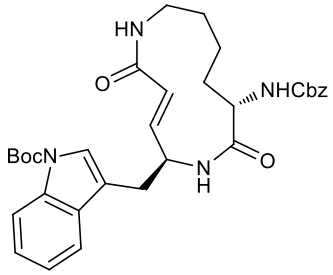


benzyl ((5S,8S,E)-5-(3-methylbenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23j**): Yield: 31%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.43-8.42 (d, 1H), 7.42-7.40 (t, 1H), 7.37-7.29 (m, 5H), 7.21-7.19 (t, 1H), 7.13-7.11 (m, 2H), 7.10-7.08 (d, 1H), 7.05-7.04 (d, 1H), 6.77-6.73 (dd, 1H),

6.33-6.30 (d, 1H), 4.99 (s, 2H), 4.61-4.57 (m, 1H), 4.32-4.30 (m, 1H), 3.32-3.27 (m, 1H), 2.99-2.96 (m, 1H), 2.87-2.84 (dd, 1H), 2.70-2.66 (dd, 1H), 2.30 (s, 3H), 2.21-2.17 (t, 1H), 1.59-1.55 (t, 1H), 1.45-1.42 (m, 2H), 1.28-1.24 (t, 1H), 0.93-0.87 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.65, 166.18, 155.94, 146.04, 138.73, 137.80, 137.45, 130.04, 128.80, 128.68, 128.23, 128.16, 127.54, 126.42, 119.76, 65.79, 53.72, 52.21, 40.50, 38.83, 38.31, 30.74, 30.54, 21.51, 17.64; HRMS (*m/z*): $[M+H]^+$ calcd., 450.2393; found, 450.2398.

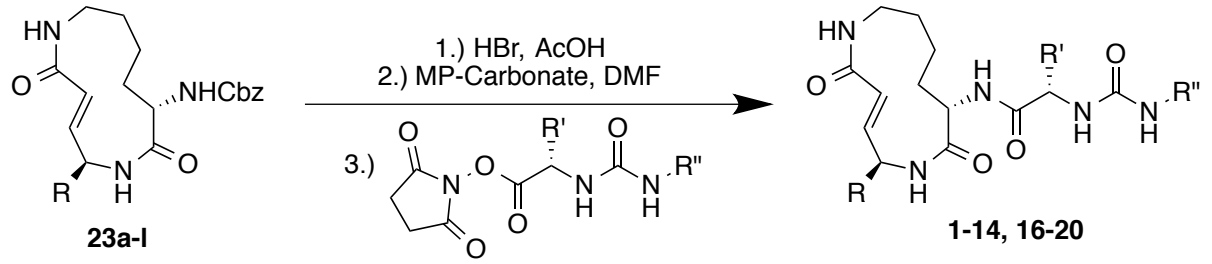


benzyl ((5S,8S,E)-5-(4-methylbenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamate (**23k**): Yield: 20%; NMR ^{1}H (400 MHz, DMSO-d_6): δ 8.42-8.40 (d, 1H), 7.42-7.40 (t, 1H), 7.37-7.29 (m, 5H), 7.19-7.10 (d, 2H), 7.13-7.11 (m, 3H), 6.75-6.72 (dd, 1H), 6.32-6.30 (d, 1H), 4.99 (s, 2H), 4.57-4.55 (m, 1H), 4.32-4.30 (m, 1H), 3.32-3.27 (m, 1H), 2.98-2.96 (m, 1H), 2.86-2.83 (dd, 1H), 2.70-2.66 (dd, 1H), 2.28 (s, 3H), 2.21-2.17 (t, 1H), 1.58-1.54 (t, 1H), 1.45-1.42 (m, 2H), 1.28-1.24 (t, 1H), 0.93-0.87 (q, 1H); ^{13}C (100 MHz, DMSO-d_6): δ 171.62, 166.18, 155.94, 146.03, 137.46, 135.83, 135.72, 129.37, 129.25, 128.80, 128.23, 128.15, 119.75, 65.79, 53.71, 52.31, 40.50, 38.48, 38.29, 30.76, 30.55, 21.14, 17.63; HRMS (*m/z*): $[M+H]^+$ calcd., 450.2393; found, 450.2392.



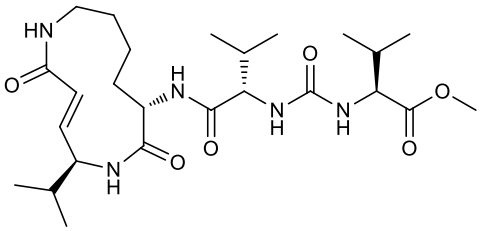
tert-butyl 3-(((2S,11S,E)-11-(((benzyloxy)carbonyl)amino)-5,12-dioxo-1,6-diazacyclododec-3-en-2-yl)methyl)-1H-indole-1-carboxylate (**23l**): Yield: 29%; NMR ^{1}H (400 MHz, MeOD-d_4): δ 8.14-8.12 (d, 1H), 7.65-7.63 (d, 1H), 7.55 (s, 1H), 7.36-7.26 (m, 6H), 7.24-7.7.16 (m, 1H), 7.06-7.03 (dd, 1H), 6.51-6.49 (d, 1H), 5.07 (s, 2H), 4.99-4.94 (m, 1H), 4.49-4.47 (m, 1H), 3.57-3.52 (t, 1H), 3.16-3.10 (m, 2H), 3.00-2.96 (dd, 1H), 2.22-2.17 (t, 1H), 1.87-1.83 (m, 1H), 1.70-1.63 (m, 10H), 1.52-1.46 (m, 1H), 1.45-1.40 (m, 1H), 1.18-1.14 (m, 1H); ^{13}C (100 MHz, MeOD-d_4): δ 172.34, 168.13, 156.52, 149.59, 146.50, 136.72, 128.04, 127.60, 127.47, 124.15, 123.51, 122.33, 119.06, 118.59, 116.45, 114.78, 83.44, 66.24, 53.73, 50.33, 48.16, 38.37, 30.03, 29.89, 28.20, 26.99, 17.35; HRMS (*m/z*): $[M+H]^+$ calcd., 575.2870; found, 575.2863.

General Synthesis of Syringolin Proteasome Inhibitor Analogs[2]

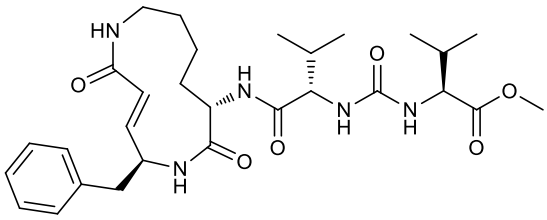


Supplementary Scheme 3. Synthesis of Proteasome inhibitors **1-14, 16-20**.

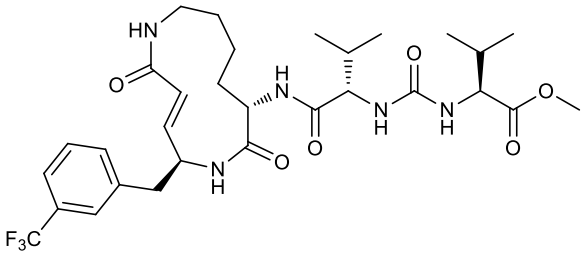
Proteasome inhibitor analogs **1-20**: Each macrocycle (**23a-l**) (0.05 mmol) was first deprotected by dissolving in 250 µL of acetic acid, followed by addition of a 33% HBr solution in acetic acid (753 µL, 4.3 mmol). After stirring for 45 minutes at room temperature, the solution was concentrated, dissolved in 500 µL of DMF, and placed into an ice bath. MP-Carbonate resin (133 mg, 0.4 mmol) was then added and the solution was stirred at room temperature for 20 minutes. Then, the dipeptide urea NHS-ester (0.1 mmol) was added to the reaction and was stirred at room temperature for 18 hours. The resin was then filtered, washing with THF, and concentrated before purification via flash chromatography using a 5%-10% MeOH in DCM gradient. TLC conditions – DCM:MeOH, 10:1 v/v.



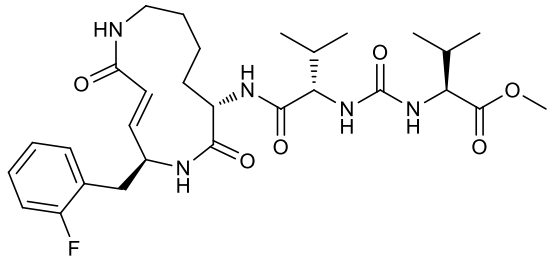
Syringolin B Methyl Ester (**1**): Yield: 51%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.93-7.92 (d, 1H), 7.07-7.04 (dd, 1H), 6.59-6.58 (d, 1H), 6.49-6.48 (d, 1H), 6.40-6.37 (d, 1H), 4.71-4.69 (m, 1H), 4.25-4.22 (m, 2H), 4.13-4.11 (dd, 1H), 3.73 (s, 3H), 3.53-3.48 (t, 1H), 3.15-3.12 (m, 1H), 2.14-2.08 (m, 3H), 1.91-1.83 (m, 2H), 1.67-1.63 (m, 1H), 1.46-1.38 (m, 2H), 1.16-1.14 (m, 1H), 1.08-1.07 (d, 3H), 1.05-1.04 (d, 3H), 0.99-0.97 (t, 6H), 0.94-0.93 (d, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.28, 172.69, 171.67, 168.27, 159.09, 146.61, 119.06, 58.85, 58.23, 56.86, 52.10, 50.97, 48.44, 38.43, 31.94, 30.73, 29.89, 29.80, 19.14, 18.42, 18.37, 18.18, 17.46, 16.62, 16.49; HRMS (*m/z*): $[M+H]^+$ calcd., 510.3292; found, 510.3287.



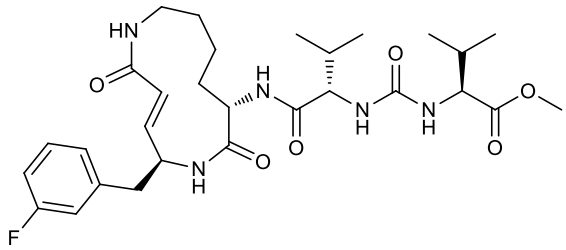
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-benzyl-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**2**): Yield: 60%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.91-7.90 (d, 1H), 7.33-7.29 (m, 4H), 7.25-7.23 (t, 1H), 7.00-6.97 (dd, 1H), 6.59-6.57 (d, 1H), 6.49-6.46 (d, 2H), 4.81 (m, 1H), 4.64 (m, 1H), 4.22-4.19 (dd, 1H), 4.10-4.08 (m, 1H), 3.72 (s, 3H), 3.57-3.52 (t, 1H), 3.16-3.13 (d, 1H), 3.00-2.98 (m, 1H), 2.87-2.85 (m, 1H), 2.19-2.07 (m, 3H), 1.93-1.88 (m, 1H), 1.66 (m, 1H), 1.49-1.39 (m, 2H), 1.19-1.13 (m, 1H), 1.00-0.99 (m, 1H), 0.96-0.95 (d, 6H), 0.93-0.90 (t, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.26, 172.68, 171.52, 168.08, 159.06, 146.61, 137.55, 128.56, 128.21, 126.38, 118.88, 58.94, 58.23, 52.22, 50.95, 48.17, 38.61, 38.37, 30.72, 30.62, 29.89, 29.72, 18.40, 18.15, 17.39, 16.62, 16.48; HRMS (*m/z*): $[M+H]^+$ calcd., 556.3130; found, 556.3127.



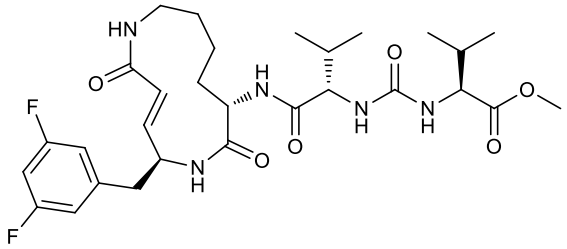
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-2,7-dioxo-5-(3-(trifluoromethyl)benzyl)-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**3**): Yield: 49%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.91-7.90 (d, 1H), 7.64 (s, 1H), 7.60-7.59 (d, 1H), 7.57-7.51 (m, 3H), 7.03-7.00 (dd, 1H), 6.59-6.57 (d, 1H), 6.50-6.46 (d, 1H), 4.86-4.85 (m, 1H), 4.62 (m, 1H), 4.20-4.19 (dd, 1H), 4.09-4.07 (m, 1H), 3.72 (s, 3H), 3.57-3.52 (t, 1H), 3.16-3.12 (d, 2H), 2.91-2.85 (m, 1H), 2.18-2.07 (m, 3H), 1.94-1.92 (m, 1H), 1.68-1.66 (m, 1H), 1.48-1.39 (m, 2H), 1.19-1.13 (m, 1H), 0.95-0.94 (d, 6H), 0.92-0.89 (t, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.25, 172.70, 171.66, 167.98, 159.01, 146.11, 139.11, 132.36, 128.99, 125.36, 123.19, 119.25, 58.94, 58.13, 52.01, 50.94, 48.16, 38.37, 38.01, 30.71, 30.61, 29.88, 29.68, 18.37, 18.13, 17.39, 16.59, 16.47; HRMS (*m/z*): $[M+Na]^+$ calcd., 648.2985; found, 648.2968.



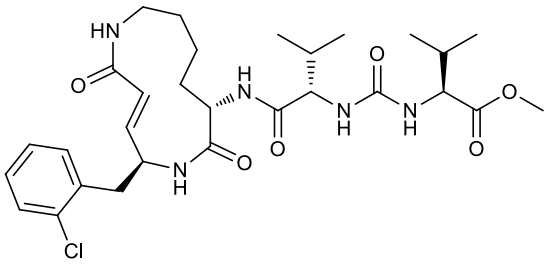
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-(2-fluorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**4**): Yield: 52%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.92-7.91 (d, 1H), 7.36-7.34 (t, 1H), 7.31-7.28 (q, 1H), 7.16-7.14 (t, 1H), 7.11-7.08 (t, 1H), 6.97-6.93 (dd, 1H), 6.60-6.59 (d, 1H), 6.51-6.48 (d, 2H), 4.87-4.84 (m, 1H), 4.62 (m, 1H), 4.21-4.19 (dd, 1H), 4.09-4.06 (m, 1H), 3.72 (s, 3H), 3.57-3.53 (t, 1H), 3.16-3.13 (d, 1H), 3.06-3.04 (dd, 1H), 2.94-2.90 (m, 1H), 2.21-2.16 (m, 1H), 2.14-2.09 (m, 2H), 1.93-1.89 (m, 1H), 1.69-1.64 (m, 1H), 1.49-1.40 (m, 2H), 1.18-1.12 (m, 1H), 0.97-0.95 (dd, 6H), 0.93-0.91 (d, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.25, 172.73, 171.55, 167.98, 160.36, 159.07, 145.98, 130.92, 128.56, 124.08, 119.22, 114.99, 114.84, 59.02, 58.19, 52.16, 50.96, 48.44, 48.16, 38.34, 31.76, 30.73, 29.89, 29.61, 18.40, 18.14, 17.37, 16.57, 16.46; HRMS (*m/z*): $[M+H]^+$ calcd., 576.3197; found, 576.3190.



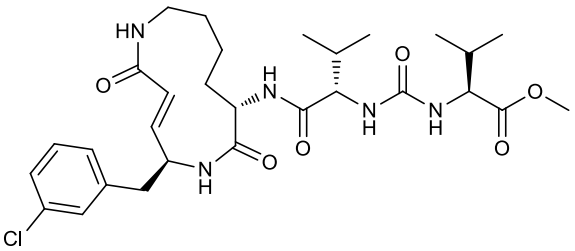
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-(3-fluorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**5**): Yield: 56%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.97-7.96 (d, 1H), 7.35-7.31 (q, 1H), 7.13-7.12 (d, 1H), 7.08-7.06 (d, 1H), 7.02-6.99 (d, 1H), 6.98-6.96 (d, 1H), 6.60-6.58 (d, 1H), 6.49-6.46 (m, 2H), 4.84-4.81 (m, 1H), 4.65 (m, 1H), 4.22-4.19 (dd, 1H), 4.13-4.10 (t, 1H), 3.72 (s, 3H), 3.56-3.52 (t, 1H), 3.16-3.13 (d, 1H), 3.05-3.02 (dd, 1H), 2.88-2.80 (m, 1H), 2.19-2.13 (m, 1H), 2.12-2.06 (m, 2H), 1.92-1.87 (m, 1H), 1.69-1.64 (m, 1H), 1.48-1.39 (m, 2H), 1.18-1.11 (q, 1H), 0.96-0.94 (dd, 6H), 0.92-0.89 (dd, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.29, 172.72, 171.62, 168.00, 163.73, 159.09, 146.31, 140.46, 129.91, 129.85, 124.47, 119.07, 115.41, 113.03, 58.93, 58.24, 52.01, 50.96, 38.37, 38.10, 30.74, 29.88, 29.73, 18.42, 18.16, 17.41, 16.62, 16.48; HRMS (*m/z*): $[M+Na]^+$ calcd., 598.3017; found, 598.3038.



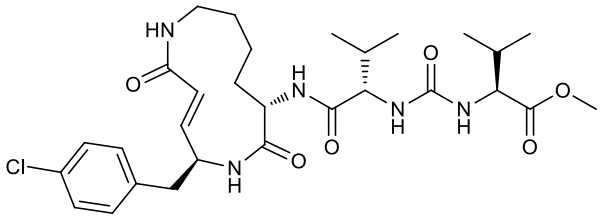
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-(3,5-difluorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**6**): Yield: 40%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.90-7.89 (d, 1H), 7.01-7.00 (d, 1H), 6.99-6.98 (d, 1H), 6.96-6.92 (d, 2H), 6.84-6.81 (t, 1H), 6.57-6.56 (d, 1H), 6.49-6.45 (m, 2H), 4.82-4.80 (m, 1H), 4.64-4.60 (m, 1H), 4.22-4.19 (dd, 1H), 4.09-4.07 (t, 1H), 3.72 (s, 3H), 3.55-3.51 (t, 1H), 3.16-3.14 (d, 1H), 3.08-3.04 (dd, 1H), 2.85-2.80 (m, 1H), 2.18-2.08 (m, 3H), 1.94-1.89 (m, 1H), 1.68-1.64 (m, 1H), 1.49-1.39 (m, 2H), 1.18-1.13 (q, 1H), 0.96-0.95 (dd, 6H), 0.93-0.91 (dd, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.27, 172.72, 171.66, 168.00, 163.99, 162.26, 159.01, 145.85, 119.40, 111.59, 111.42, 101.55, 59.00, 58.18, 52.14, 50.94, 48.16, 38.41, 37.89, 30.73, 29.87, 18.37, 18.12, 17.44, 16.61, 16.50; HRMS (*m/z*): $[M+Na]^+$ calcd., 616.2923; found, 616.2941.



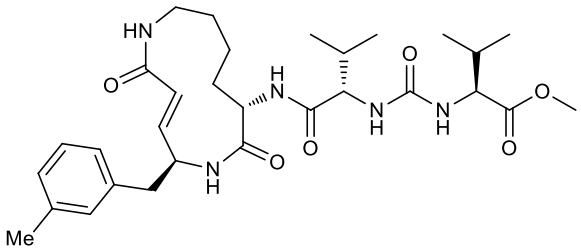
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-(2-chlorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**7**): Yield: 53%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.43-7.42 (dd, 1H), 7.38-7.36 (dd, 1H), 7.30-7.26 (m, 2H), 7.16-7.13 (d, 1H), 6.96-6.93 (dd, 1H), 6.52-6.49 (d, 1H), 4.65-4.63 (t, 1H), 4.21-4.20 (d, 1H), 4.08-4.07 (d, 1H), 3.72 (s, 3H), 3.58-3.52 (m, 1H), 3.17-3.14 (dd, 2H), 3.01-2.97 (dd, 1H), 2.21-2.14 (m, 1H), 2.13-2.08 (m, 2H), 1.94-1.88 (m, 1H), 1.68-1.64 (m, 1H), 1.50-1.40 (m, 2H), 1.18-1.12 (q, 1H), 0.97-0.94 (dd, 6H), 0.92-0.91 (d, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.24, 172.73, 171.55, 167.95, 159.03, 145.89, 135.00, 133.69, 130.96, 129.32, 128.97, 128.78, 128.27, 126.87, 119.25, 58.95, 58.11, 52.16, 50.97, 50.25, 38.35, 36.22, 30.72, 29.88, 18.42, 18.16, 17.39, 16.58, 16.48; HRMS (*m/z*): $[M+H]^+$ calcd., 592.2902; found, 592.2899.



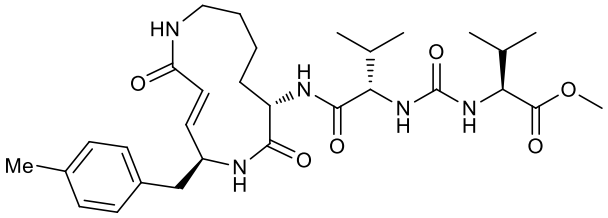
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-(2-chlorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**8**): Yield: 66%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.35 (s, 1H), 7.32-7.30 (m, 1H), 7.27-7.25 (m, 2H), 7.02-6.98 (dd, 1H), 6.58-6.57 (d, 1H), 6.49-6.46 (d, 1H), 4.83-4.80 (quin, 1H), 4.64-4.62 (m, 1H), 4.21-4.19 (m, 1H), 4.09-4.07 (m, 1H), 3.72 (s, 3H), 3.56-3.52 (t, 1H), 3.16-3.14 (d, 2H), 3.05-3.02 (dd, 1H), 2.83-2.79 (dd, 1H), 2.18-2.07 (m, 3H), 1.94-1.88 (m, 1H), 1.70-1.64 (m, 1H), 1.48-1.39 (m, 2H), 1.18-1.12 (q, 1H), 0.97-0.95 (dd, 6H), 0.92-0.90 (t, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.25, 172.69, 171.61, 168.00, 159.01, 146.20, 140.03, 133.94, 129.69, 128.69, 126.99, 126.52, 119.15, 58.96, 58.14, 52.11, 52.02, 50.95, 48.16, 38.37, 37.99, 30.73, 29.88, 29.68, 18.40, 18.15, 17.40, 16.61, 16.49; HRMS (*m/z*): $[M+Na]^+$ calcd., 614.2721; found, 614.2736.



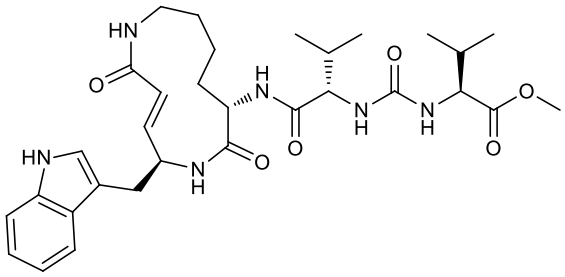
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-(4-chlorobenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**9**): Yield: 58%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 8.00 (s, 1H), 7.94-7.93 (d, 1H), 7.33-7.29 (m, 4H), 6.99-6.96 (dd, 1H), 6.60-6.59 (d, 1H), 6.48-6.46 (d, 2H), 4.80-4.77 (quin, 1H), 4.63-4.61 (m, 1H), 4.21-4.19 (dd, 1H), 4.09-4.07 (t, 1H), 3.72 (s, 3H), 3.56-3.51 (t, 1H), 3.16-3.13 (d, 2H), 3.01-2.98 (dd, 1H), 2.85-2.81 (dd, 1H), 2.72-2.68 (m, 1H), 2.18-2.07 (m, 3H), 1.92-1.88 (m, 1H), 1.70-1.64 (m, 1H), 1.48-1.39 (m, 2H), 1.34-1.30 (m, 2H), 1.18-1.12 (m, 1H), 0.96-0.95 (d, 6H), 0.92-0.90 (t, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.27, 171.61, 168.01, 163.45, 159.11, 146.30, 136.44, 132.20, 130.21, 128.24, 119.10, 58.21, 52.08, 50.96, 48.16, 38.37, 37.75, 30.73, 29.87, 29.66, 18.40, 18.16, 17.42, 16.59, 16.47; HRMS (*m/z*): $[M+H]^+$ calcd., 592.2902; found, 592.2889.



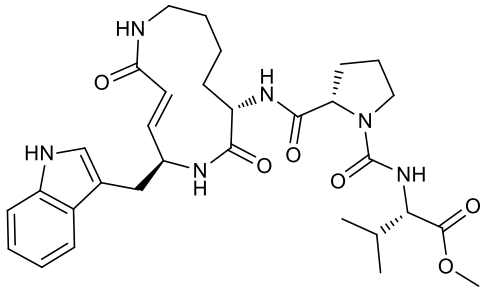
(S)-methyl 3-methyl-2-(3-((S)-3-methyl-1-(((5S,8S,E)-5-(3-methylbenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)ureido)butanoate (**10**): Yield: 58%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.94-7.92 (d, 1H), 7.20-7.18 (t, 1H), 7.12 (s, 1H), 7.09-7.07 (d, 1H), 7.06-7.05 (d, 1H), 7.00-6.97 (dd, 1H), 6.60-6.58 (d, 1H), 6.49-6.45 (m, 2H), 4.81-4.78 (m, 1H), 4.66-4.62 (m, 1H), 4.22-4.19 (m, 1H), 4.11-4.09 (m, 1H), 3.72 (s, 3H), 3.57-3.52 (t, 1H), 3.16-3.13 (d, 1H), 2.97-2.93 (dd, 1H), 2.85-2.80 (dd, 1H), 2.33 (s, 3H), 2.19-2.05 (m, 3H), 1.94-1.87 (m, 1H), 1.68-1.64 (m, 1H), 1.48-1.39 (m, 3H), 1.34-1.31 (m, 1H), 1.18-1.12 (q, 1H), 0.97-0.95 (d, 6H), 0.92-0.90 (t, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.27, 172.77, 171.55, 168.08, 159.10, 146.74, 137.89, 137.42, 129.25, 128.10, 127.06, 125.60, 118.76, 59.01, 58.23, 52.29, 52.19, 50.96, 48.17, 38.59, 38.35, 30.74, 29.89, 29.73, 20.06, 18.42, 18.16, 17.37, 16.62, 16.47; HRMS (*m/z*): $[M+H]^+$ calcd., 572.3443; found, 572.3440.



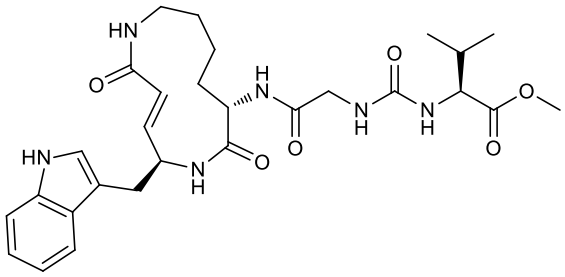
(S)-methyl 3-methyl-2-(3-((S)-3-methyl-1-(((5S,8S,E)-5-(4-methylbenzyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-1-oxobutan-2-yl)ureido)butanoate (**11**): Yield: 49%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.92-7.91 (d, 1H), 7.18-7.17 (d, 2H), 7.13-7.12 (d, 2H), 6.99-6.96 (dd, 1H), 6.59-6.58 (d, 1H), 6.49-6.45 (m, 2H), 4.79-4.75 (m, 1H), 4.65-4.63 (m, 1H), 4.21-4.19 (m, 1H), 4.10-4.08 (m, 1H), 3.72 (s, 3H), 3.56-3.52 (t, 1H), 3.15-3.13 (d, 1H), 2.96-2.92 (dd, 1H), 2.85-2.80 (dd, 1H), 2.32 (s, 3H), 2.19-2.08 (m, 3H), 1.92-1.88 (m, 1H), 1.68-1.64 (m, 1H), 1.49-1.39 (m, 3H), 1.34-1.31 (m, 1H), 1.18-1.12 (q, 1H), 0.96-0.95 (d, 6H), 0.92-0.90 (t, 6H); ^{13}C (150 MHz, MeOD-d_4): δ 173.27, 172.77, 171.50, 168.08, 159.07, 146.74, 136.02, 134.40, 128.80, 128.45, 118.76, 59.04, 58.23, 52.33, 52.21, 50.95, 48.16, 38.35, 38.23, 30.73, 29.89, 29.71, 19.68, 18.40, 18.16, 17.37, 16.61, 16.47; HRMS (*m/z*): $[M+H]^+$ calcd., 572.3443; found, 572.3444.



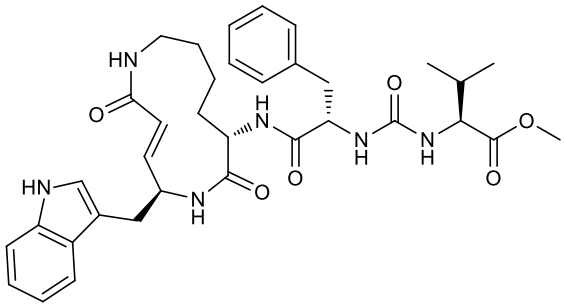
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**12**): Yield: 27%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.93-7.92 (d, 1H), 7.59-7.57 (d, 1H), 7.36-7.35 (d, 1H), 7.14 (s, 1H), 7.12-7.10 (t, 1H), 7.06-7.02 (m, 2H), 6.59-6.58 (d, 1H), 6.50-6.48 (m, 2H), 4.67-4.65 (m, 1H), 4.21-4.19 (dd, 1H), 4.11-4.08 (t, 1H), 3.72 (s, 3H), 3.57-3.52 (t, 1H), 3.15-3.11 (m, 2H), 3.07-3.04 (m, 1H), 2.19-2.14 (m, 1H), 2.12-2.06 (m, 2H), 1.93-1.89 (m, 1H), 1.68-1.64 (m, 1H), 1.48-1.38 (m, 2H), 1.21-1.15 (q, 1H), 0.97-0.96 (d, 3H), 0.94-0.93 (d, 3H), 0.92-0.91 (d, 3H), 0.91-0.89 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.28, 172.74, 171.50, 168.25, 159.10, 147.33, 136.74, 127.11, 122.72, 121.05, 118.41, 117.77, 110.91, 110.13, 59.00, 58.15, 52.19, 51.32, 50.95, 48.17, 38.39, 30.73, 29.89, 28.83, 18.41, 18.13, 17.43, 16.60, 16.50; HRMS (*m/z*): $[M+H]^+$ calcd., 597.3395; found, 597.3393.



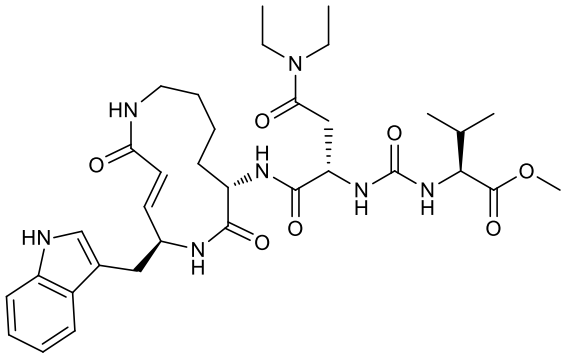
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-methyl-1-oxobutan-2-yl)ureido)-3-methylbutanoate (**13**): Yield: 20%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 8.63-8.62 (d, 1H), 8.00 (s, 1H), 7.89-7.88 (d, 1H), 7.59-7.57 (d, 1H), 7.36-7.35 (d, 1H), 7.15 (s, 1H), 7.12-7.10 (t, 1H), 7.07-7.02 (m, 2H), 6.50-6.48 (d, 1H), 6.05-6.03 (d, 1H), 4.64-4.60 (m, 1H), 4.37-4.35 (dd, 1H), 4.32-4.30 (d, 1H), 4.17-4.14 (t, 1H), 3.73 (s, 3H), 3.58-3.44 (m, 2H), 3.16-3.12 (m, 2H), 3.05-3.04 (d, 1H), 2.21-2.04 (m, 3H), 1.70-1.66 (m, 2H), 1.48-1.41 (m, 3H), 1.37-1.31 (m, 2H), 1.19-1.13 (m, 1H), 1.01-0.98 (d, 3H), 0.97-0.93 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.41, 173.28, 171.66, 168.23, 157.33, 147.35, 136.74, 127.08, 122.75, 121.04, 118.40, 117.75, 110.90, 110.17, 67.46, 60.22, 59.26, 52.26, 51.39, 50.93, 48.16, 46.10, 38.35, 30.44, 29.92, 29.72, 29.57, 28.78, 25.09, 24.15, 18.12, 17.62, 17.35; HRMS (*m/z*): $[M+H]^+$ calcd., 595.3239; found, 595.3240.



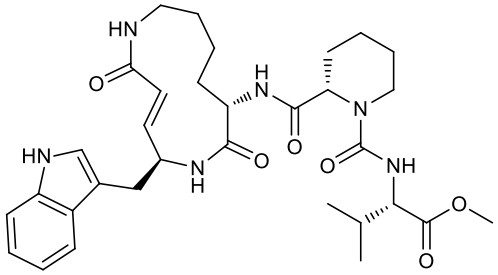
(S)-methyl 2-(3-(2-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-2-oxoethyl)ureido)-3-methylbutanoate (**14**): Yield: 57%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.59-7.57 (d, 1H), 7.36-7.35 (d, 1H), 7.15 (s, 1H), 7.12-7.10 (t, 1H), 7.07-7.02 (m, 2H), 6.68-6.67 (d, 1H), 6.50-6.47 (d, 1H), 4.68-4.66 (m, 1H), 4.21-4.18 (m, 1H), 3.80 (s, 2H), 3.70 (s, 3H), 3.58-3.53 (t, 1H), 3.16-3.12 (m, 2H), 3.07-3.04 (dd, 1H), 2.22-2.10 (m, 2H), 1.94-1.86 (m, 1H), 1.72-1.62 (m, 1H), 1.48-1.39 (m, 2H), 1.17-1.11 (m, 1H), 0.97-0.95 (d, 3H), 0.93-0.92 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.19, 171.54, 170.70, 165.95, 157.13, 147.35, 136.73, 127.08, 126.36, 125.25, 122.71, 121.05, 118.40, 117.76, 110.90, 110.15, 60.13, 58.39, 52.02, 51.00, 48.16, 44.94, 42.93, 38.35, 30.57, 29.88, 28.79, 24.86, 18.12, 17.11, 16.73; HRMS (*m/z*): $[M+H]^+$ calcd., 555.2931; found, 555.2921.



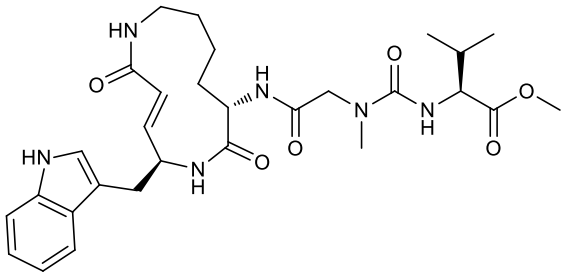
(S)-methyl 2-(3-(2-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-2-oxoethyl)ureido)-3-methylbutanoate (**16**): Yield: 70%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.60-7.59 (d, 1H), 7.37-7.36 (d, 1H), 7.21-7.18 (m, 3H), 7.15 (s, 1H), 7.12-7.10 (m, 2H), 7.07-7.02 (m, 2H), 6.68-6.67 (d, 1H), 6.47-6.44 (d, 1H), 4.60-4.58 (m, 1H), 4.48-4.45 (m, 1H), 4.17-4.14 (m, 1H), 3.70 (s, 3H), 3.56-3.51 (t, 1H), 3.16-3.12 (m, 2H), 3.03-2.99 (m, 3H), 2.13-2.04 (m, 2H), 1.90-1.86 (m, 1H), 1.62-1.59 (m, 1H), 1.41-1.36 (m, 2H), 1.24-1.21 (m, 1H), 1.08-1.02 (m, 1H), 0.91-0.90 (d, 3H), 0.88-0.87 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.16, 172.24, 171.64, 168.93, 158.45, 147.26, 136.77, 136.61, 129.01, 128.07, 127.08, 126.41, 122.72, 121.06, 118.41, 117.79, 110.93, 110.23, 60.13, 58.09, 55.03, 52.08, 51.36, 50.95, 48.16, 38.35, 37.73, 30.70, 29.92, 28.74, 24.86, 18.08, 17.05, 16.60; HRMS (*m/z*): $[M+H]^+$ calcd., 645.3401; found, 645.3392.



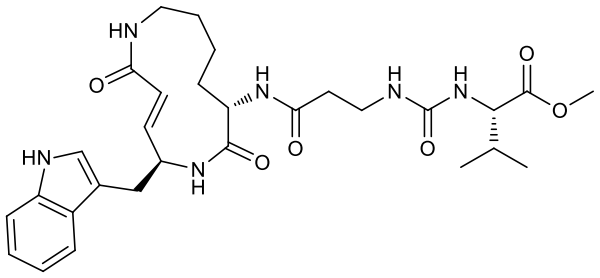
(S)-methyl 2-(3-((S)-1-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-4-(diethylamino)-1,4-dioxobutan-2-yl)ureido)-3-methylbutanoate (**17**): Yield: 62%; NMR ^{1}H (600 MHz, MeOD-d_4); δ 7.82-7.80 (d, 1H), 7.59-7.57 (d, 1H), 7.36-7.35 (d, 1H), 7.14 (s, 1H), 7.12-7.10 (m, 2H), 7.07-7.02 (m, 2H), 6.88-6.87 (d, 1H), 6.50-6.47 (d, 1H), 4.61-4.58 (m, 2H), 4.22-4.20 (m, 1H), 3.71 (s, 3H), 3.58-3.53 (t, 1H), 3.35-3.27 (m, 4H – Interference from Solvent Peak), 3.16-3.12 (m, 2H), 3.04-3.00 (dd, 2H), 2.75-2.72 (dd, 1H), 2.14-2.09 (m, 2H), 1.99-1.94 (m, 1H), 1.70-1.64 (m, 1H), 1.57-1.53 (m, 1H), 1.42-1.38 (t, 1H), 1.20-1.12 (m, 4H), 1.09-1.08 (t, 3H), 0.96-0.95 (d, 3H), 0.92-0.91 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.24, 172.15, 171.35, 170.06, 168.20, 158.48, 147.33, 136.73, 127.08, 122.70, 121.05, 118.40, 117.77, 110.90, 110.19, 58.27, 52.99, 51.38, 50.95, 50.59, 48.16, 41.97, 40.07, 38.36, 34.66, 30.68, 29.94, 29.52, 28.76, 18.13, 16.71, 13.05, 12.85, 11.86; HRMS (*m/z*): $[M+H]^+$ calcd., 668.3772; found, 668.3761.



(S)-methyl 2-((S)-2-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)carbamoyl)piperidine-1-carboxamido)-3-methylbutanoate (**18**): Yield: 62%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.59-7.58 (d, 1H), 7.36-7.35 (d, 1H), 7.15 (s, 1H), 7.12-7.10 (m, 2H), 7.07-7.02 (m, 2H), 6.50-6.47 (d, 1H), 4.74-4.71 (m, 1H), 4.64-4.63 (m, 1H), 4.17-4.11 (m, 1H), 3.88-3.86 (d, 1H), 3.67 (s, 3H), 3.58-3.54 (t, 1H), 3.16-3.12 (m, 3H), 3.07-3.03 (m, 1H), 2.23-2.14 (m, 1H), 2.13-2.07 (m, 1H), 1.99-1.91 (m, 1H), 1.86-1.78 (m, 1H), 1.70-1.64 (m, 4H), 1.53-1.39 (m, 5H), 1.20-1.14 (m, 1H), 0.98-0.96 (d, 3H), 0.95-0.94 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.58, 172.10, 171.61, 168.22, 158.24, 147.34, 136.73, 127.09, 122.74, 121.05, 118.41, 117.77, 110.90, 110.16, 59.88, 54.37, 52.33, 51.39, 50.88, 48.16, 42.27, 38.34, 30.25, 29.92, 29.68, 28.79, 26.33, 24.48, 19.96, 18.20, 17.86, 17.47; HRMS (*m/z*): $[M+H]^+$ calcd., 609.3401; found, 609.3384.

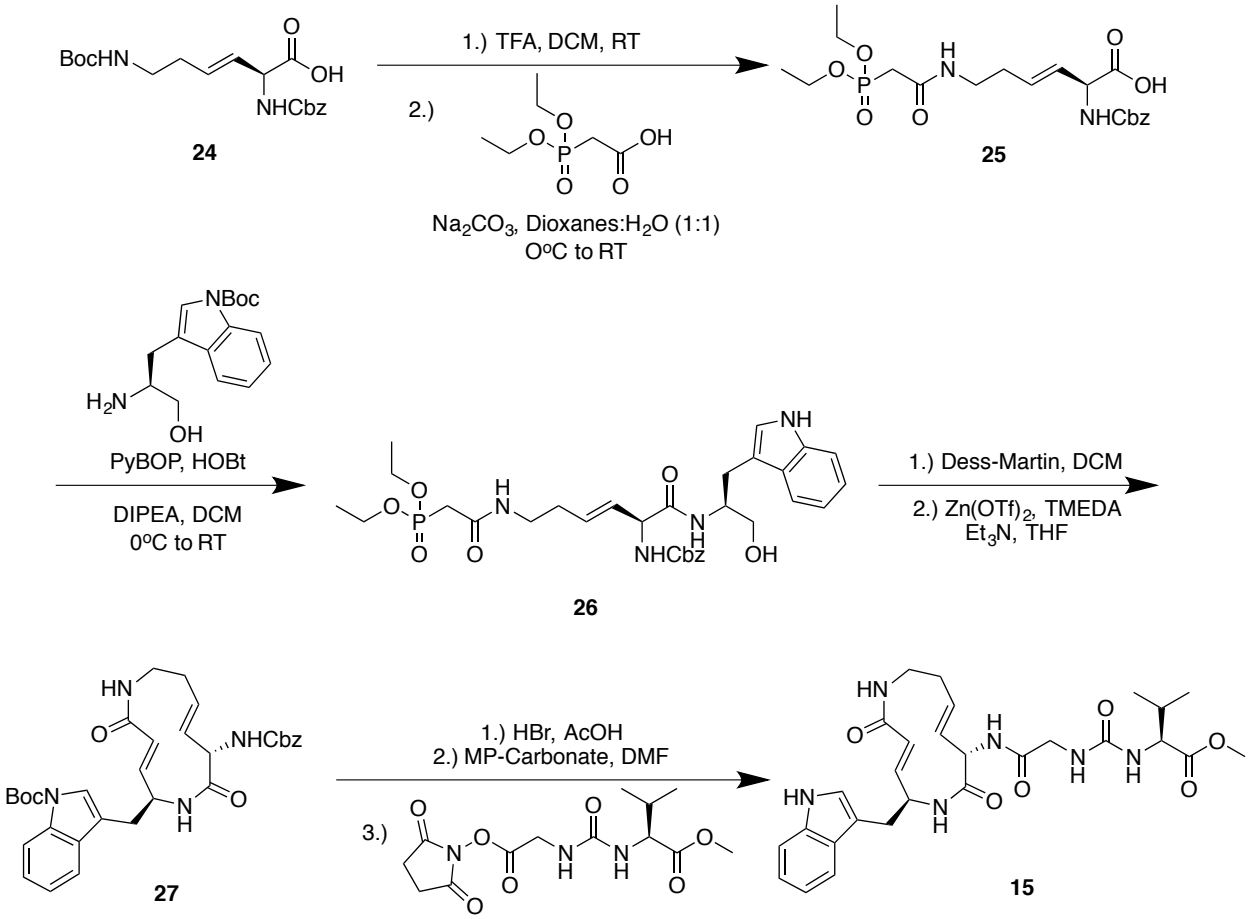


(S)-methyl 2-(3-(3-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-oxopropyl)ureido)-3-methylbutanoate (**19**): Yield: 31%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.60-7.58 (d, 1H), 7.37-7.35 (d, 1H), 7.17 (s, 1H), 7.13-7.10 (m, 1H), 7.06-7.02 (m, 2H), 6.51-6.48 (d, 1H), 6.37-6.32 (dd, 1H), 4.69-4.64 (m, 1H), 4.17-4.12 (m, 1H), 3.73 (s, 3H), 3.58-3.54 (t, 1H), 3.47-3.44 (m, 1H), 3.37 (s, 2H), 3.16-3.12 (m, 2H), 3.08-3.04 (m, 1H), 2.71 (s, 3H), 2.52-2.47 (m, 1H), 2.43-2.39 (m, 1H), 2.24-2.20 (m, 1H), 2.12-2.08 (m, 1H), 1.85-1.79 (m, 1H), 1.70-1.64 (m, 1H), 1.55-1.42 (m, 2H), 1.34-1.31 (m, 2H), 1.22-1.14 (q, 1H), 0.98-0.97 (d, 3H), 0.96-0.95 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.87, 172.86, 172.04, 170.44, 159.14, 147.40, 136.74, 127.11, 122.79, 121.06, 118.42, 117.78, 110.93, 110.20, 59.73, 53.40, 51.68, 51.00, 48.44, 38.33, 35.68, 35.17, 34.71, 30.41, 29.95, 28.79, 24.64, 18.17, 17.75; HRMS (*m/z*): $[M+H]^+$ calcd., 569.3088; found, 569.3077.



(S)-methyl 2-(3-(3-(((5S,8S,E)-5-((1H-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododec-3-en-8-yl)amino)-3-oxopropyl)ureido)-3-methylbutanoate (**20**): Yield: 54%; NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.59-7.58 (d, 1H), 7.36-7.35 (d, 1H), 7.16 (s, 1H), 7.12-7.10 (m, 1H), 7.06-7.02 (m, 2H), 6.51-6.48 (d, 1H), 6.36-6.34 (d, 1H), 4.67-4.64 (m, 1H), 4.19-4.17 (m, 1H), 3.69 (s, 3H), 3.58-3.53 (t, 1H), 3.44-3.40 (m, 1H), 3.36-3.35 (m, 1H), 3.16-3.12 (m, 2H), 3.08-3.04 (m, 1H), 2.44-2.42 (t, 2H), 2.23-2.20 (m, 1H), 2.11-2.05 (sext, 1H), 1.87-1.81 (m, 1H), 1.70-1.64 (m, 1H), 1.55-1.51 (m, 1H), 1.46-1.41 (m, 1H), 1.34-1.31 (m, 2H), 1.22-1.16 (q, 1H), 0.94-0.93 (d, 3H), 0.89-0.88 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.45, 173.34, 172.06, 168.27, 159.24, 147.38, 136.73, 127.10, 122.75, 121.05, 118.41, 117.76, 110.91, 110.14, 58.22, 52.40, 51.36, 50.94, 48.44, 38.32, 36.03, 35.88, 30.63, 29.95, 29.67, 28.82, 18.12, 17.43, 16.71; HRMS (*m/z*): $[M+H]^+$ calcd., 569.3088; found, 569.3079.

Synthesis of Syringolin A Proteasome Inhibitor Analog 15.[2, 5]



Supplementary Scheme 4. Synthesis of Syringolin A Analog (**15**).

benzyl ((*S*,*E*)-6-(2-(diethoxyphosphoryl)acetamido)-1-(((*S*)-1-hydroxy-3-(1*H*-indol-3-yl)propan-2-yl)amino)-1-oxohex-3-en-2-yl)carbamate (**26**): **24** was synthesized as reported in the literature.[5] First, **24** (1513.7 mg, 4.0 mmol) was dissolved in a 40% solution of TFA in DCM (8.0 mL, 4.0 mmol). The reaction was stirred for 45-60 minutes or until TLC indicated full consumption of starting material (TLC Conditions – DCM:MeOH, 10:1 v/v). The solution was then concentrated to dryness and used without purification. The material was then dissolved in 5 mL of a 1:1 Dioxane:Water mixture and was placed in an ice bath. To the reaction was added Na_2CO_3 (1695.8 mg, 16.0 mmol) and was stirred for 30 minutes at 0°C. Then, the phosphonohydroxysuccinate ester, as synthesized from literature[2], was dissolved in 2.5 mL of dioxane and added to the reaction, followed by 2.5 mL of water. The resulting mixture was stirred for 16 hours while warming to room temperature. Impurities from the reaction were then separated by extracting with ethyl acetate (2 x 50 mL). The aqueous layer was then acidified to pH = 2 with 1 M HCl and then extracted again with ethyl acetate (3 x 50 mL). The organic layers were combined and washed with water (1 x 50 mL) and brine (1 x 50 mL). After drying and filtering, the organic layer was concentrated and resulting **25** used without further purification.

25(232.8 mg, 0.51 mmol) was dissolved in 5 mL of DCM and placed into an ice bath. HOBt (93.7 mg, 0.61 mmol) and PyBOP (318.5 mg, 0.61 mmol) was added to the mixture, followed by N_{in}-Boc tryptophanol (160.7 mg, 0.56 mmol), which was prepared according to literature.[4] After stirring for 5 minutes in the ice bath, DIPEA (133.2 µL, 0.77 mmol) was slowly added before stirring at room temperature for 16 hours. Then, the reaction was evaporated and redissolved in 100 mL of ethyl acetate. The solution was then washed with 1 M HCl (3 x 50 mL), water (1 x 50 mL) and brine. The organic layer was collected, dried with sodium sulfate and evaporated before TLC and flash chromatography afforded **26**. TLC conditions – Acetone:DCM, 3:1 v/v. Yield: 60%. NMR ^{1}H (400 MHz, $CDCl_3$): δ 8.13-8.11 (m, 1H), 7.74-7.68 (m, 1H), 7.48 (s, 1H), 7.38-7.25 (m, 7H), 7.19-7.13 (m, 1H), 6.98-6.96 (m, 1H), 6.34-6.32 (m, 1H), 5.78-5.70 (m, 1H), 5.60-5.57 (m, 1H), 5.10 (s, 2H), 4.72-4.70 (m, 1H), 4.33-4.31 (m, 1H), 4.11-4.07 (m, 4H), 3.74-3.71 (m, 1H), 3.65-3.59 (m, 1H), 3.39-3.35 (m, 1H), 3.31-3.24 (m, 1H), 3.02-2.96 (m, 2H), 2.85 (s, 1H), 2.80 (s, 1H), 1.67 (s, 9H), 1.33-1.27 (m, 6H); ^{13}C (100 MHz, $CDCl_3$): δ 170.13, 164.15, 149.76, 136.37, 131.71, 130.67, 129.23, 128.48, 128.10, 128.05, 125.73, 124.42, 123.86, 122.60, 119.30, 117.02, 115.19, 110.90, 83.60, 66.87, 63.08, 63.01, 56.89, 51.79, 46.30, 38.78, 32.08, 28.22, 26.44, 16.28; HRMS (*m/z*): $[M+H]^+$ calcd., 729.3265; found, 729.3257.

tert-butyl 3-(((3*E*,9*E*)-11-(((benzyloxy)carbonyl)amino)-5,12-dioxo-1,6-diazacyclododeca-3,9-dien-2-yl)methyl)-1*H*-indole-1-carboxylate (**27**): **26** was dissolved in 2 mL of DCM and Dess-Martin periodinane (90 mg, 0.21 mmol) was added. After stirring at room temperature for 90 minutes, the reaction was diluted in 100 mL of ethyl acetate. The organic solution was then washed with 1:1 mixture of saturated sodium bicarbonate and 2% $Na_2S_2O_3$ (2 x 50 mL). The organic phase was collected, dried, filtered and concentrated, which was used without further purification. TMEDA (35 µL, 0.23 mmol) and TEA (108 µL, 0.77 mmol) was added to a solution of $Zn(OTf)_2$ (155 mg, 0.42 mmol) in 60 mL of THF. The solution was stirred for 15-20 minutes before adding oxidized **26** in 40 mL of THF to a pressure-equalizing addition funnel. The solution in the funnel was added drop-wise to the reaction over 2 hours. The reaction was stirred for 20 hours at room temperature before concentrating and redissolving in 100 mL of ethyl acetate. Then, the solution was washed with 1 M HCl (1 x 50 mL), water (1 x 50 mL) and brine. Once the organic phase was collected, dried, filtered and concentrated, the resulting oil was purified via flash chromatography. Yield: 8%. NMR ^{1}H (400 MHz, MeOD-d_4): δ 8.52-8.51 (d, 1H), 8.13-8.12 (d, 1H), 7.62-7.61 (d, 1H), 7.55 (s, 1H), 7.36-7.24 (m, 7H), 7.13-7.07 (dd, 1H), 7.00-6.97 (dd, 1H), 6.39-6.36 (d, 1H), 5.75-5.70 (m, 1H), 5.64-5.60 (m, 1H), 5.09-5.04 (dd, 2H), 4.73-4.72 (d, 1H), 3.09-3.06 (m, 1H), 3.00-2.96 (dd, 1H), 2.37-2.34 (m, 1H), 2.21-2.16 (m, 1H), 1.68-1.62 (s, 9H); ^{13}C (100 MHz, MeOD-d_4): δ 170.76, 168.54, 156.69, 149.58, 145.02, 136.69, 135.51, 134.46, 130.12, 128.05, 127.59, 127.44, 125.01, 124.13, 123.53, 122.34, 120.68, 118.62, 116.42, 114.77, 83.40, 66.28, 55.62, 49.79, 48.47, 43.13, 34.62, 28.74, 27.01; HRMS (*m/z*): $[M+H]^+$ calcd., 573.2713; found, 573.2706.

(2*S*)-methyl 2-(3-(2-(((3*E*,9*E*)-5-((1*H*-indol-3-yl)methyl)-2,7-dioxo-1,6-diazacyclododeca-3,9-dien-8-yl)amino)-2-oxoethyl)ureido)-3-methylbutanoate (**15**): Macrocycle **27** (30 mg, 0.052 mmol) was first deprotected by dissolving in 300 µL of acetic acid, followed by addition of a 33% HBr solution in acetic acid (800 µL, 4.5 mmol). After stirring for 45 minutes at room temperature, the solution was concentrated, dissolved in 500 µL of DMF, and placed into an ice bath. MP-

Carbonate resin (140 mg, 0.42 mmol) was then added and the solution was stirred at room temperature for 20 minutes. Then, the dipeptide urea NHS-ester (35 mg, 0.10 mmol) was added to the reaction and was stirred at room temperature for 18 hours. The resin was then filtered, washing with THF, and concentrated before purification via flash chromatography using a 5%-10% MeOH in DCM gradient to yield a yellow solid. TLC conditions – DCM:MeOH, 10:1 v/v. Yield: 25%. NMR ^{1}H (600 MHz, MeOD-d_4): δ 7.58-7.57 (d, 1H), 7.36-7.34 (d, 1H), 7.15 (s, 1H), 7.12-7.09 (t, 1H), 7.04-7.02 (t, 1H), 7.00-6.96 (dd, 1H), 6.65-6.64 (d, 1H), 6.38-6.36 (d, 1H), 5.77-5.72 (m, 1H), 5.70-5.66 (m, 1H), 4.21-4.19 (dd, 1H), 3.87-3.78 (dd, 2H), 3.71 (s, 3H), 3.40-3.39 (t, 2H), 3.13-3.05 (m, 2H), 2.39-2.36 (m, 1H), 2.24-2.18 (m, 2H), 2.15-2.08 (m, 2H), 0.97-0.96 (d, 3H), 0.94-0.93 (d, 3H); ^{13}C (150 MHz, MeOD-d_4): δ 173.33, 170.89, 170.45, 168.69, 159.17, 145.87, 136.71, 128.48, 127.15, 125.13, 122.76, 121.02, 118.38, 117.77, 110.87, 110.09, 58.38, 50.99, 48.16, 43.20, 42.60, 34.60, 30.62, 29.24, 24.83, 18.10, 16.73; HRMS (*m/z*): $[M+H]^+$ calcd., 553.2775; found, 553.2777.

Protein Purification

The *Mycobacterium tuberculosis* 20S peptidase was expressed in BL21 (DE3) RIL cells by induction with 1 mM IPTG at 37°C at an OD_{600} of 1 for 5h and then purified from inclusion bodies according to previously established protocols.[6] Proteins were buffer exchanged to HBS buffer (20 mM HEPES-KOH, pH 7.5, 50 mM NaCl, 5 mM $MgCl_2$), flash-frozen in liquid nitrogen and stored at -80°C. Protein concentration was determined by UV-Vis absorption using calculated extinction coefficients and is reported as 20S αββα equivalents.

20S Inhibition Kinetics[7,8]

In vitro 20S peptidase inhibition assays were recorded in a 384-well plate at a SpectraMax M5 (Molecular devises) plate-reader. Therefore, 5 nM human 20S (R&D) was incubated in buffer R (50 mM HEPES-KOH, pH 7.5, 0.5 mM EDTA) with 100 µM of the fluorogenic peptide substrates Suc-LLVY-AMC or Boc-LRR-AMC. Reactions were started by adding various amounts of inhibitors (5 nM to 500 µM) to a final volume of 30 µL and peptide hydrolysis was continuously monitored for 90 min by changes in fluorescence (excitation 380 nm; emission 440 nm) at 37°C. Peptide hydrolysis by 20 nM *Mtb* 20S was measured as described above in buffer X (20 mM HEPES-KOH, pH 7.5, 0.5 mM EDTA, 5 mM $MgCl_2$). The k_{obs} values were determined by fitting the raw data to equation (1). The slopes of the plots of k_{obs} versus I gave an apparent value of k_{obs}/I, also referred to as k_{inact}/K_i for an irreversible inhibitor, which was then corrected by equation (2) to compensate for the effect of substrate competition, where *app* is the apparent value at different inhibitor concentrations.

Equation 1

$$P = v_s t + \frac{(v_0 - v_s)}{k_{obs}} [1 - e^{(-k_{obs} t)}]$$

Equation 2

$$\frac{kobs}{I} = \frac{\left(\frac{kobs}{I}\right) app}{1 + S/Km}$$

Mtb Nitric-Oxide Stress Assay

The *Mtb* Erdman strain was grown overnight at 37 °C in Sauton's medium until log phase, washed twice, and resuspended with Sauton's medium at pH 5.5 with or without 0.5 mM $NaNO_2$. The washed cells were then dispensed into a 96-well plate that contained 0-50 µM of compounds 13^{sat}, 13, and 14. After an 11-day incubation at 37 °C, the cultures from each well were plated and the colony forming units observed after 19 days were determined. Values are averages ± SD for experiments performed in triplicate.

References

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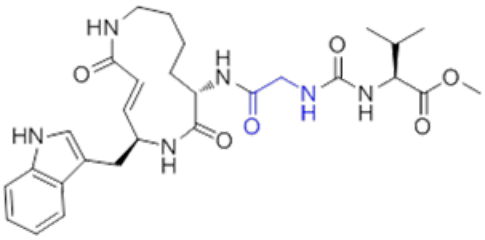
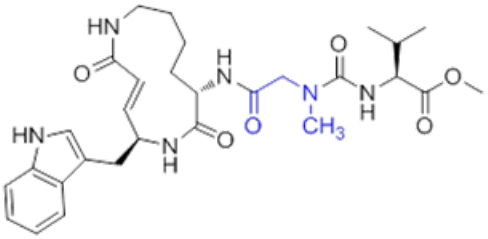
2. Pirrung, M. C., Biswas, G. & Ibarra-Rivera, T. R. Total Synthesis of Syringolin A and B. *Org. Lett.* **12**, 2402-2405 (2010).
3. McKennon, M. J., Meyers, A. I., Drauz, K. & Schwarm, M. A convenient reduction of amino acids and their derivatives. *J. Org. Chem.* **58**, 3568-3571 (1993).
4. Marson, C. M. *et al*. Discovery of Potent, Isoform-Selective Inhibitors of Histone Deacetylase Containing Chiral Heterocyclic Capping Groups and a N-(2-Aminophenyl)benzamide Binding Unit. *J. Med. Chem.* **56**, 6156-6174 (2013).
5. Dai, C. & Stephenson, C. R. J. Total Synthesis of Syringolin A. *Org. Lett.* **12**, 3453-3455 (2010).
6. Striebel, F., Hunkeler, M., Summer, H. & Weber-Ban, E. The mycobacterial Mpa-proteasome unfolds and degrades pupylated substrates by engaging Pup's N-terminus. *EMBO J* **29**, 1262-71 (2010).
7. Fenteany, G. et al. Inhibition of proteasome activities and subunit-specific amino-terminal threonine modification by lactacytsin. *Science* **1995**, *268*, 726-731.
8. Lin, G. et al. Inhibitors for mycobacteria versus human proteasomes. *Nature* **2011**, *461*, 621-626.

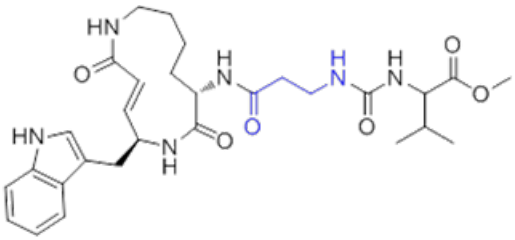
Analogs of Compound 14, the Most Selective Inhibitor of *Mtb* 20S. In place of the glycine residue, compound 19 includes a sarcosine and compound 20 has a β-alanine.



IC_{50} Mtb20S = 0.144 µM
IC_{50} Hs20S = 6.562 µM
IC_{50} Hs20S/IC_{50} Mtb20S = 45.57

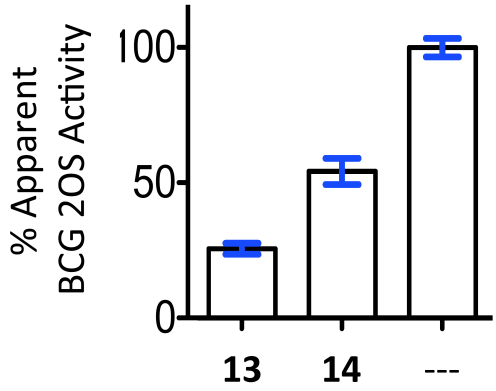


IC_{50} Mtb20S = 89.57 µM
IC_{50} Hs20S = 102.8 µM
IC_{50} Hs20S/IC_{50} Mtb20S = 1.13



IC_{50} Mtb20S = 17.25 µM
IC_{50} Hs20S = 16.76 µM
IC_{50} Hs20S/IC_{50} Mtb20S = 0.97

Evaluation of *Mtb* 20S Inhibitors in a *M. tuberculosis* Surrogate.



BCG cultures were incubated with 20 µM concentrations of compounds 13 or 14, or a buffer control for 4 h prior to lysis. 20S activity in lysates was inferred from the extent to which the fluorogenic Suc-LLVY-AMC substrate was cleaved. Values ± SD are averages of three experiments.

50 µL 10 mM Compound stock in DMSO were added to 25 mL of BCG (OD580nm 0.62) and incubated for 4 hours at 37˚C. Cells were then collected by centrifugation and washed with PBS + 0.02% Tyloxapol (detergent). Cell pellets were resuspend in 1 mL of the same buffer and transferred to a bead-beating tube. The cells were further washed with PBS only 1 mL once, and resuspended in 0.5 mL 50 mM HEPES, 0.5 mM EDTA, pH7.5, 0.5 mM PMSF. The cell suspensions were added 0.5 mL of Zirconium beads, and were lysed on a bead-beating instrument for 3 x 5000 rpm, 25 second each time. The tubes were cooled on ice for 5 minutes between each round. Cell free lysates were collected after centrifugation at 14,000 rpm for 15 minutes at 4˚C. The lysates were aliquot and flash frozen in liquid nitrogen and store at -80˚C.

40 µg of lysates were used in the 20S activity assay. Lysates were spot at the bottom of the wells of a 96-well plate. Buffer containing 100 µM Ac-NTW-AMC were added into each well. The reaction progresses in each were recorded at Ex 360nm, Em 460 in a plate reader. Data (average of three experiments) was plotted as shown above.

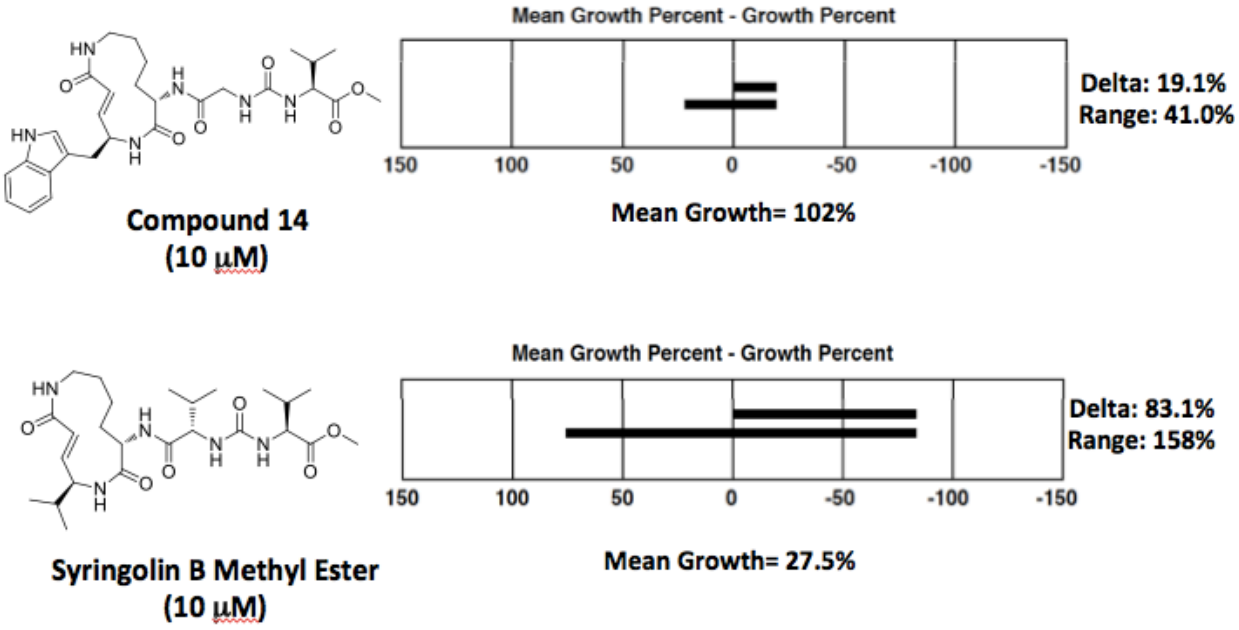
Kinetic Data for Calculating Second-Order Rate Constants

Human 20S				
Compound	k_{in} (s^{-1})	K_i (μM)	k_{in}/K_i (M^{-1} s^{-1})	Normalized k_{in}/K_i (M^{-1} s^{-1})
1	0.0005502	2.12	259.5283019	781.40
2	0.0004723	0.7434	635.3241862	1912.87
3	0.000472	7.575	62.31023102	187.61
4	0.0004789	0.9062	528.4705363	1591.15
5	0.0004856	0.5916	820.8248817	2471.39
6	0.0004995	0.9519	524.7399937	1579.92
7	0.0005163	2.113	244.3445338	735.69
8	0.0004887	0.9631	507.4239435	1527.78
9	0.0004988	1.237	403.2336297	1214.08
10	0.0004954	2.347	211.0779719	635.53
11	0.0005002	1.14	438.7719298	1321.08
12	0.0005141	1.711	300.4675628	904.67
13	0.00064	810	0.790123457	2.38
14	0.00064	141.4	4.526166902	13.63
15	0.0004527	19.06	23.75131165	71.51
16	0.0005508	0.3852	1429.906542	4305.25
17	0.0004581	12.34	37.12317666	111.77
18	0.0004584	191.4	2.394984326	7.21

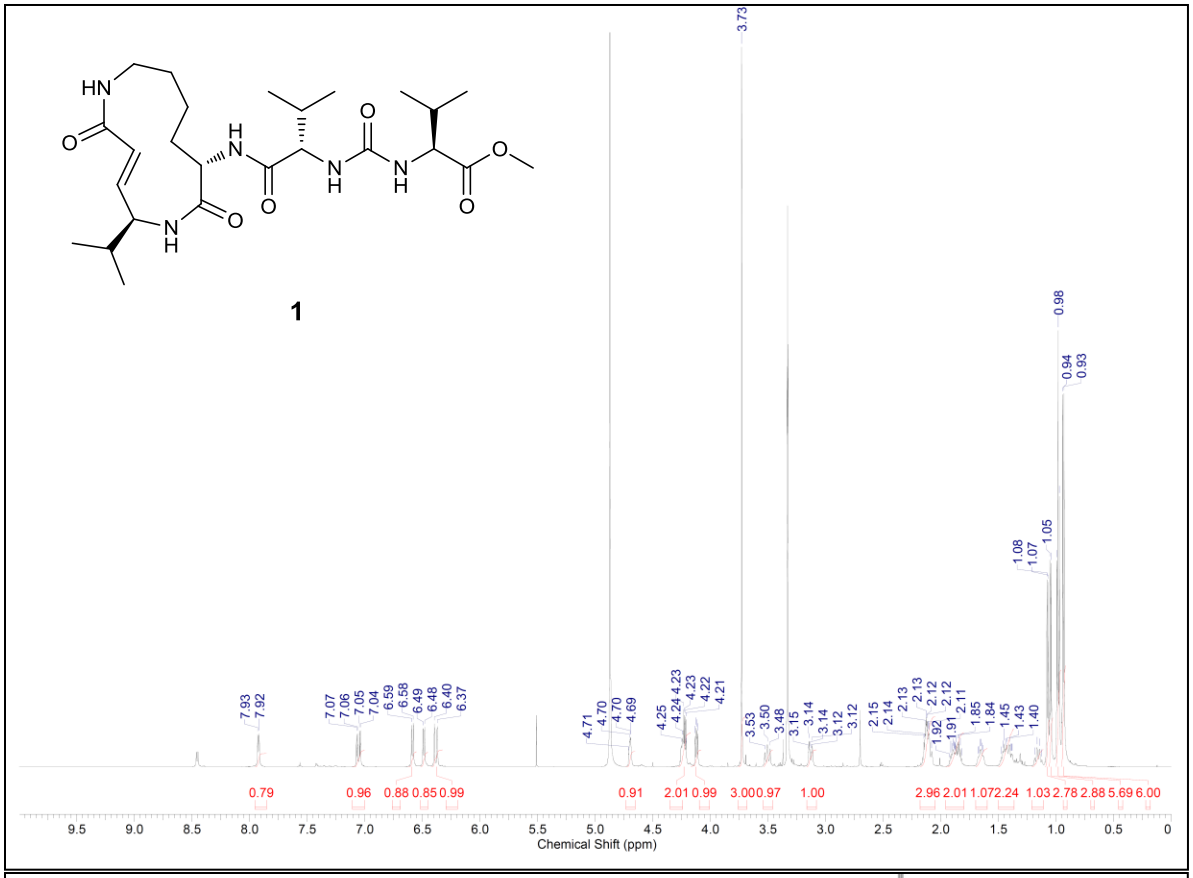
Mtb 20S				
Compound	k_{in} (s^{-1})	K_i (μM)	k_{in}/K_i (M^{-1} s^{-1})	Normalized k_{in}/K_i (M^{-1} s^{-1})
1	0.00045	318.8	1.411543287	4.60
2	0.0003895	14.79	26.33536173	85.80
3	0.0002773	53.66	5.167722698	16.84
4	0.0003555	9.431	37.69483618	122.80
5	0.0003748	7.769	48.24301712	157.17
6	0.0003918	7.429	52.73926504	171.82
7	0.0003535	6.816	51.86326291	168.96
8	0.000524	13.88	37.75216138	122.99
9	0.0005922	19.18	30.87591241	100.59
10	0.0004667	16.97	27.50147319	89.60
11	0.0004675	10.84	43.12730627	140.50
12	0.0004965	2.331	212.998713	693.92
13	0.0006257	25.65	24.39376218	79.47
14	0.0006598	2.122	310.933082	1012.97
15	0.0004547	0.816	557.2303922	1815.37
16	0.000507	0.7298	694.7108797	2263.26
17	0.0004231	0.559	756.8872987	2465.82
18	0.0003971	38.65	10.27425614	33.47

Mtb 20S/Human 20S	
Compound	Normalized[8] k_{in}/K_i
1	0.00588
2	0.0448
3	0.0897
4	0.0771
5	0.0636
6	0.109
7	0.230
8	0.0805
9	0.0829
10	0.141
11	0.106
12	0.767
13	33.41
14	74.33
15	25.39
16	0.526
17	22.06
18	4.64

Mean Growth of Syringolin B Methyl Ester and Compound 14 from NCI 60-Cell Screen



Aggregate effects of syringolin B methyl ester and compound 14 on the growth of the NCI panel of 60 human-cancer cell lines. Compounds were tested at 10 µM.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O
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0.93
1.08
1.07
1.05
7.93
7.92
7.07
7.06
7.05
7.04
6.59
6.58
6.49
6.48
6.40
6.37
4.71
4.70
4.70
4.69
4.25
4.24
4.23
4.23
4.22
4.21
3.53
3.50
3.48
3.15
3.14
3.14
3.12
3.12
2.15
2.14
2.13
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1.92
1.91
1.85
1.84
1.45
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1.40
0.79
0.96
0.88
0.85
0.99
0.91
2.01
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3.00
0.97
1.00
2.96
2.01
1.07
2.24
1.03
2.78
2.88
5.69
6.00
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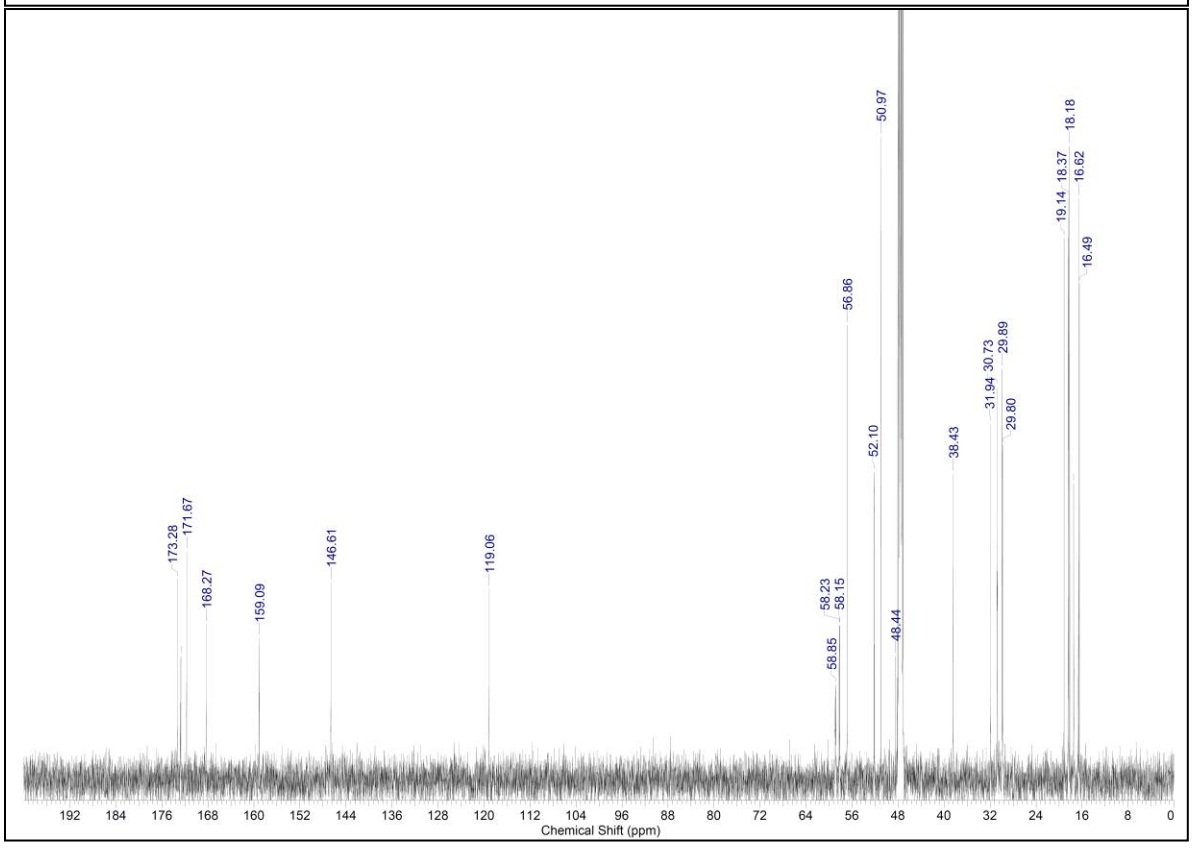
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31.94
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29.89
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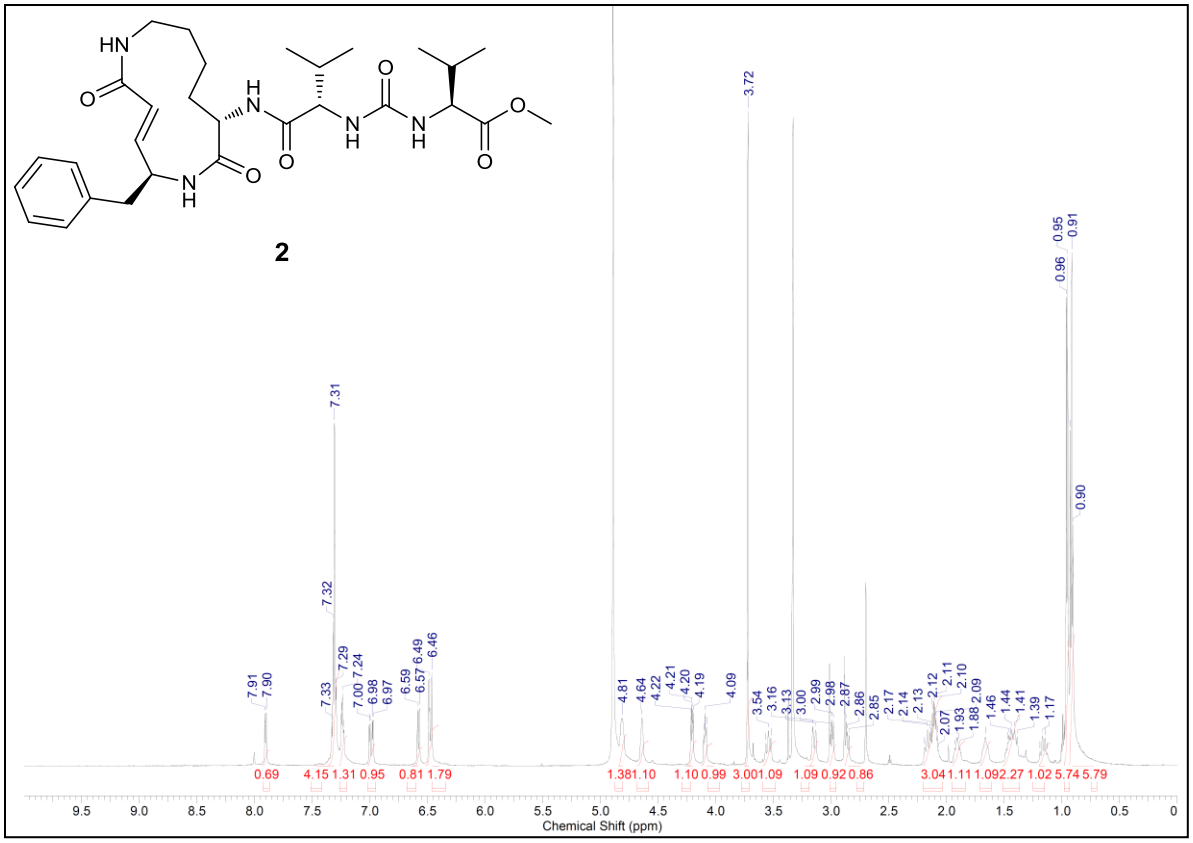
Chemical Shift (ppm)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0.90
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7.32
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7.33
7.29
7.24
7.00
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6.97
6.59
6.57
6.49
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4.64
4.22
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2.14
2.13
2.12
2.11
2.10
2.09
2.07
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1.88
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1.09
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0.92
0.86
3.04
1.11
1.09
2.27
1.02
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5.79
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8.5
8.0
7.5
7.0
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6.0
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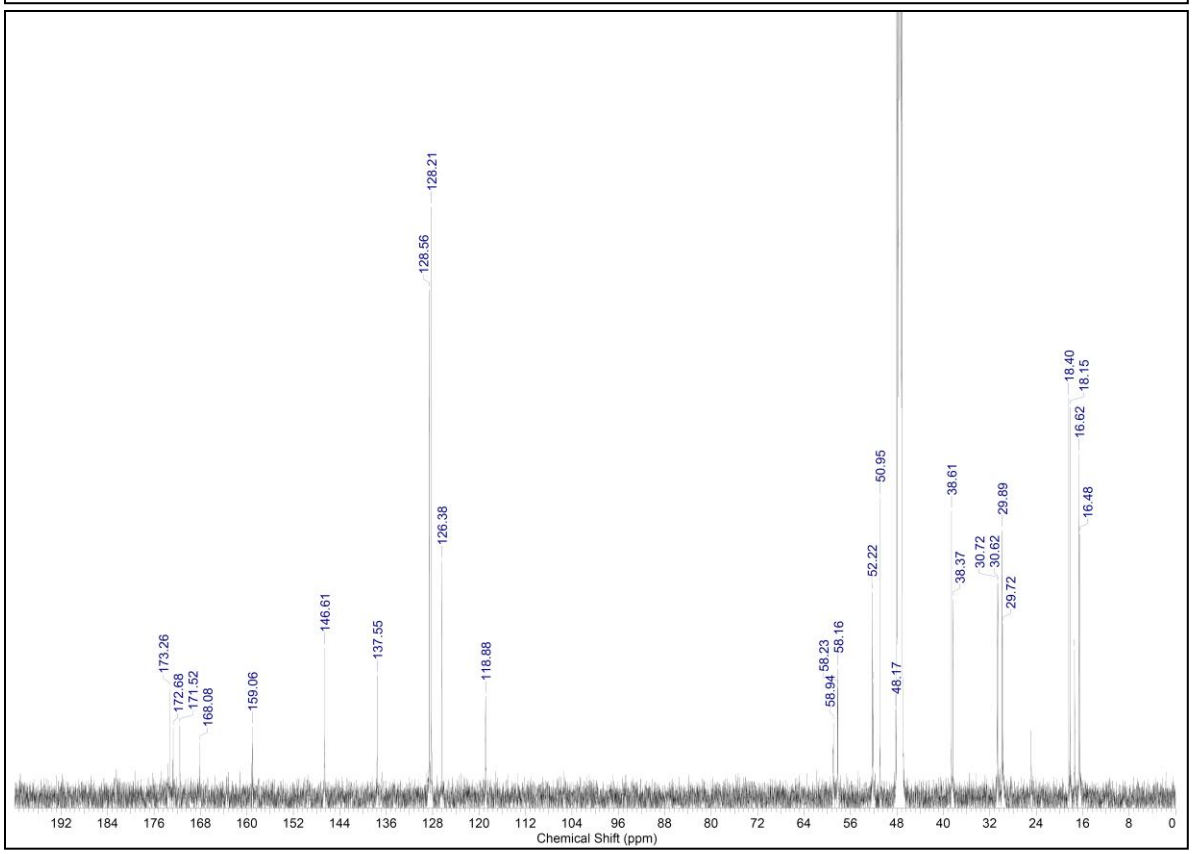
Chemical Shift (ppm)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16.48
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168
160
152
144
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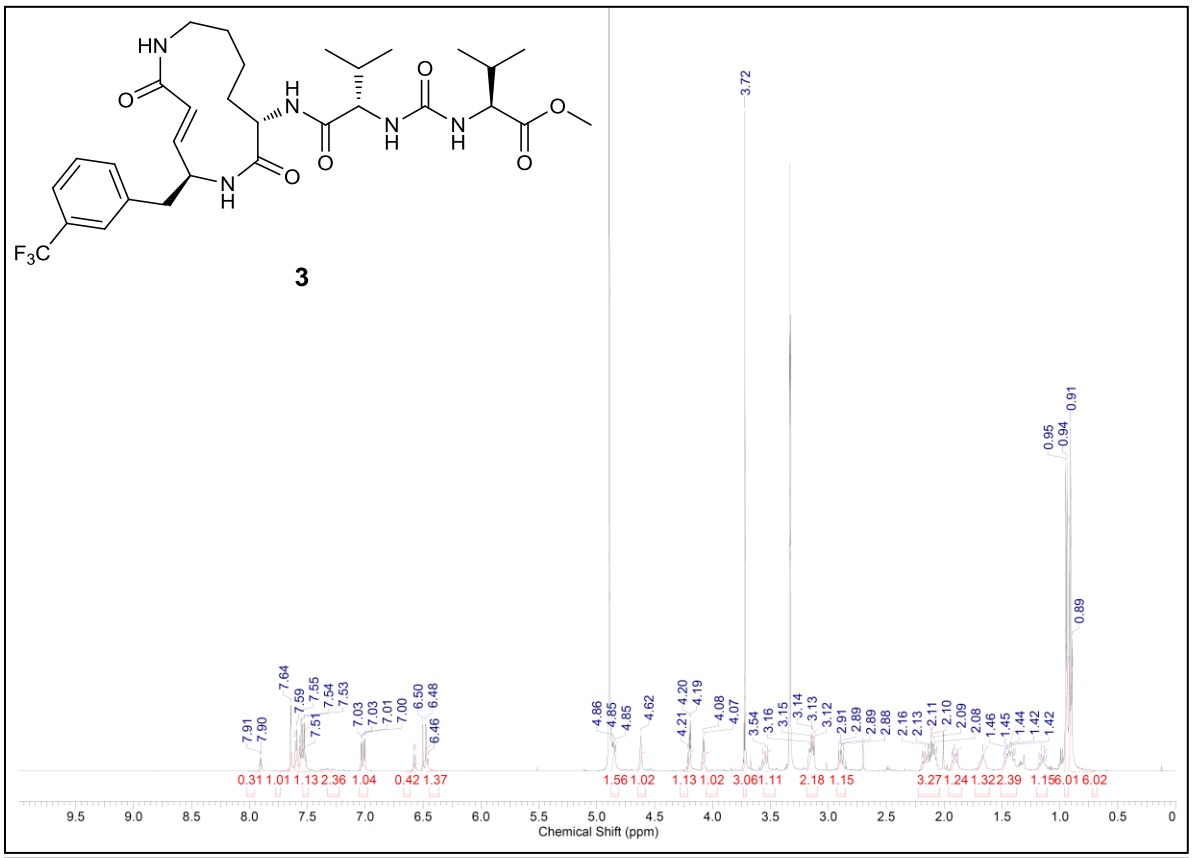
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7.03
7.03
7.01
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3.27 1.24 1.32 2.39 1.15 6.01 6.02
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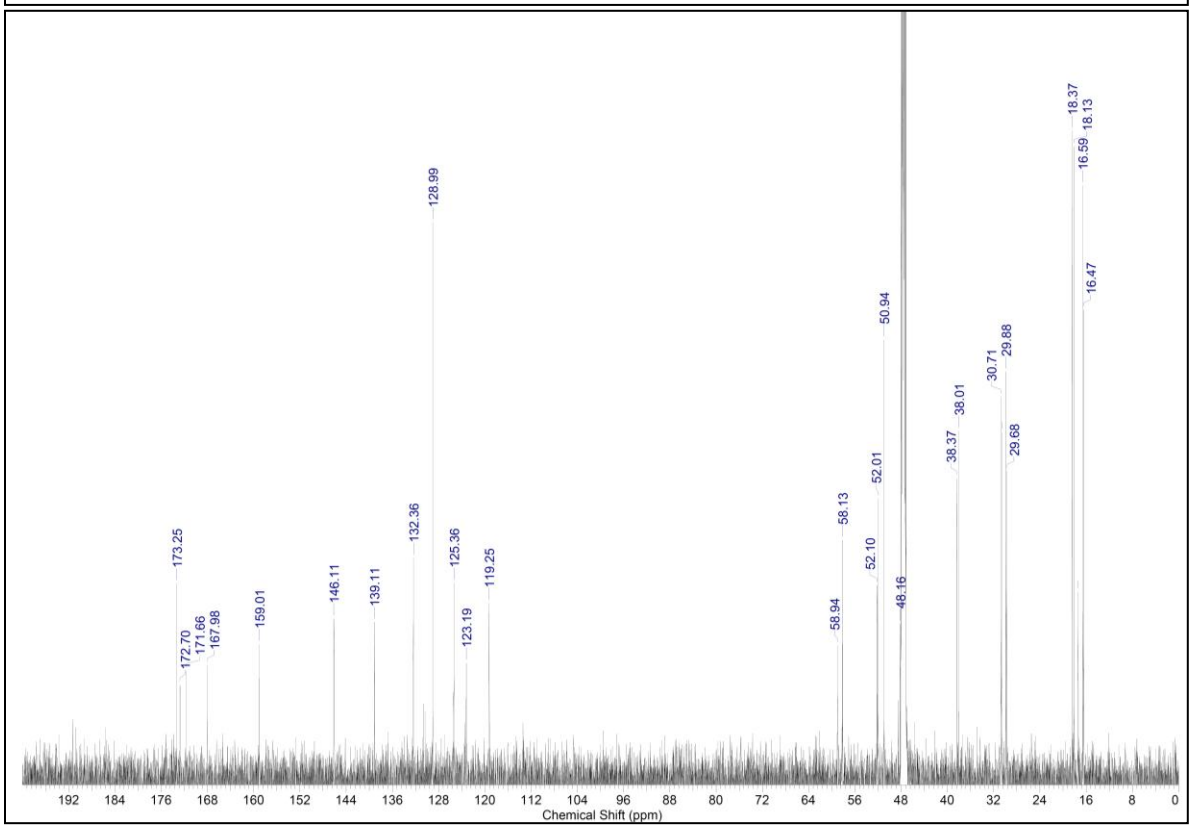
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50.94
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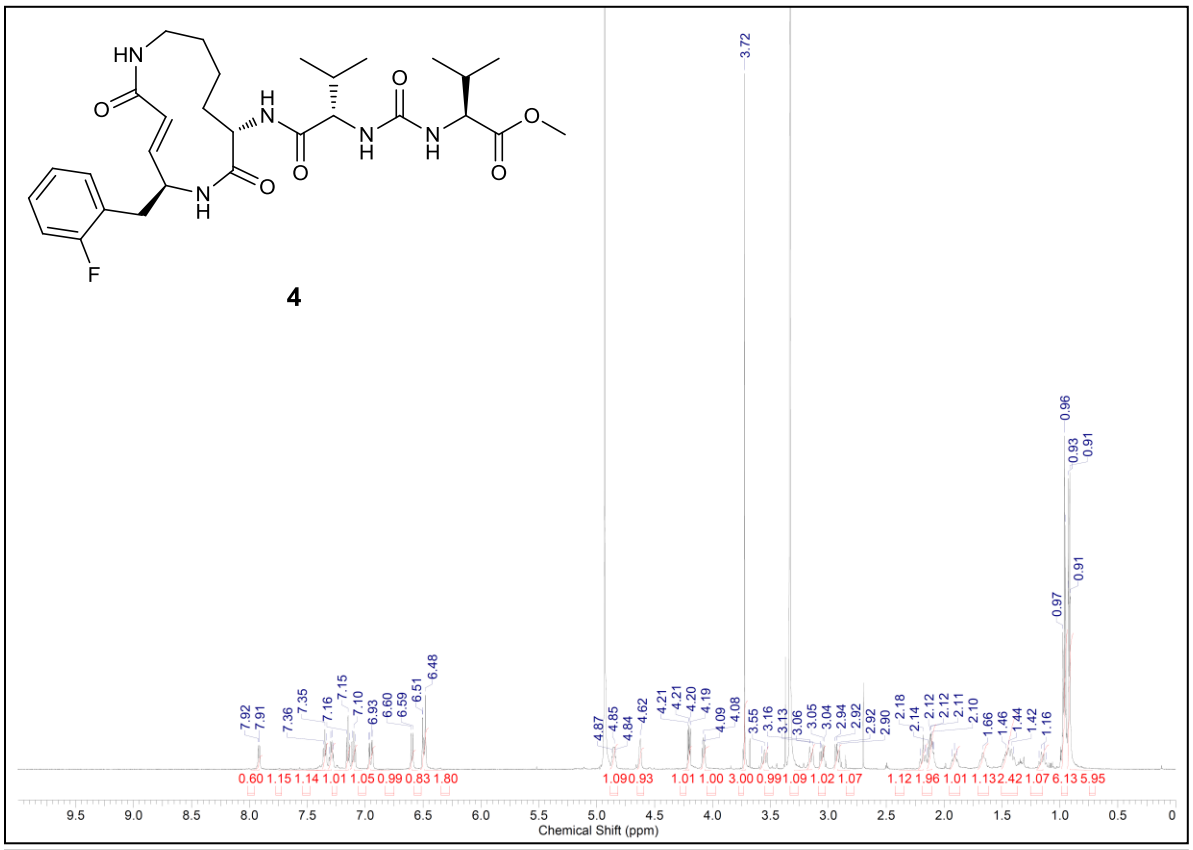
Chemical Shift (ppm)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0.97
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7.35
7.16
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7.10
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6.60
6.59
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6.48
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4.85
4.84
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4.20
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4.09
4.08
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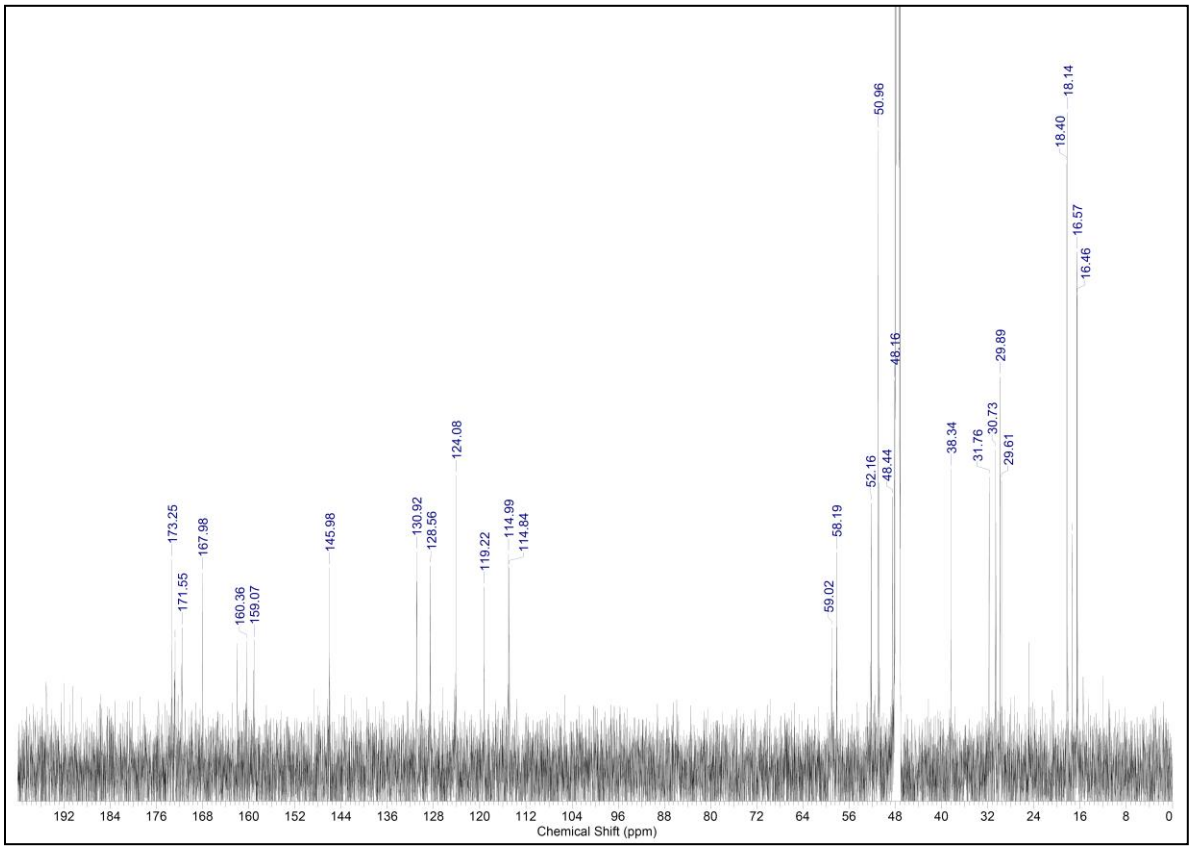
3.13
3.06
3.05
3.04
2.94
2.92
2.92
2.90
2.18
2.14
2.12
2.12
2.11
2.10
1.66
1.46
1.44
1.42
1.16
0.60 1.15 1.14 1.01 1.05 0.99 0.83 1.80
1.09 0.93 1.01 1.00 3.00 0.99 1.09 1.02 1.07
1.12 1.96 1.01 1.13 2.42 1.07 6.13 5.95
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)

4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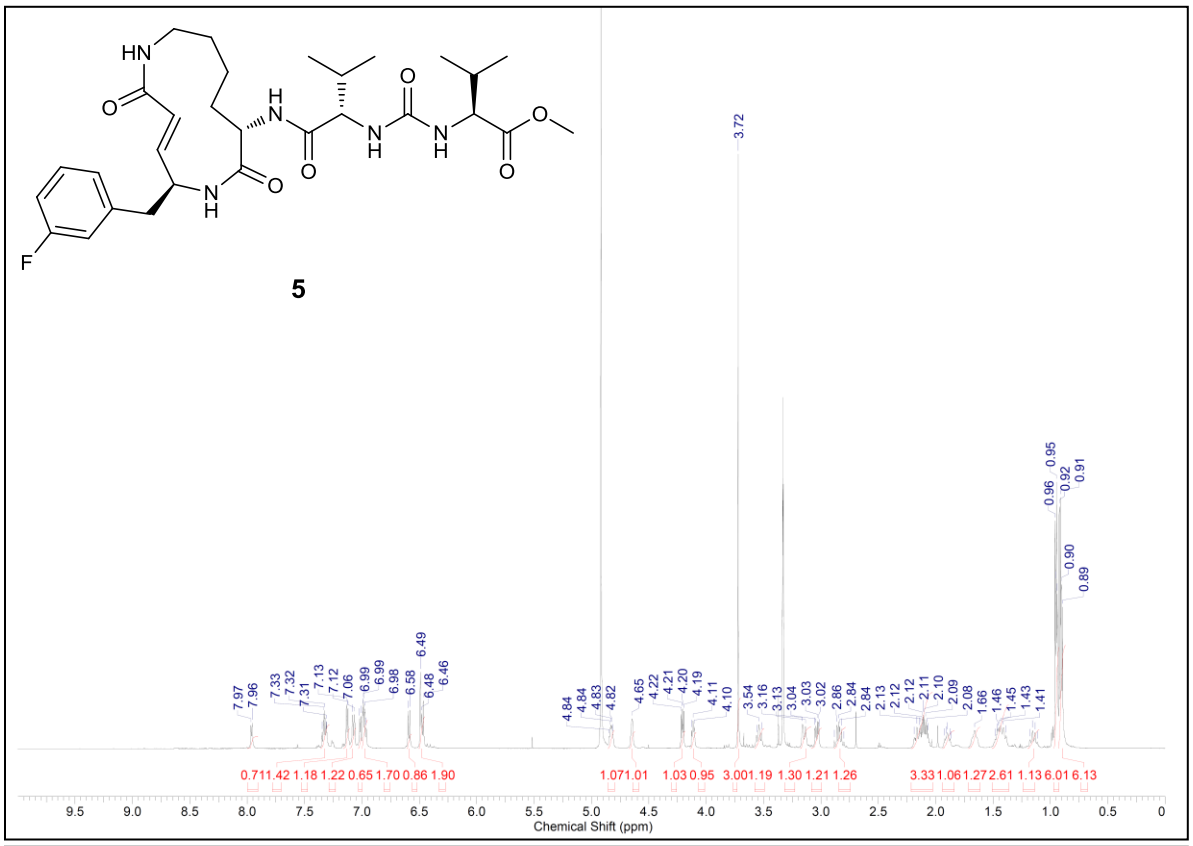
Chemical Shift (ppm)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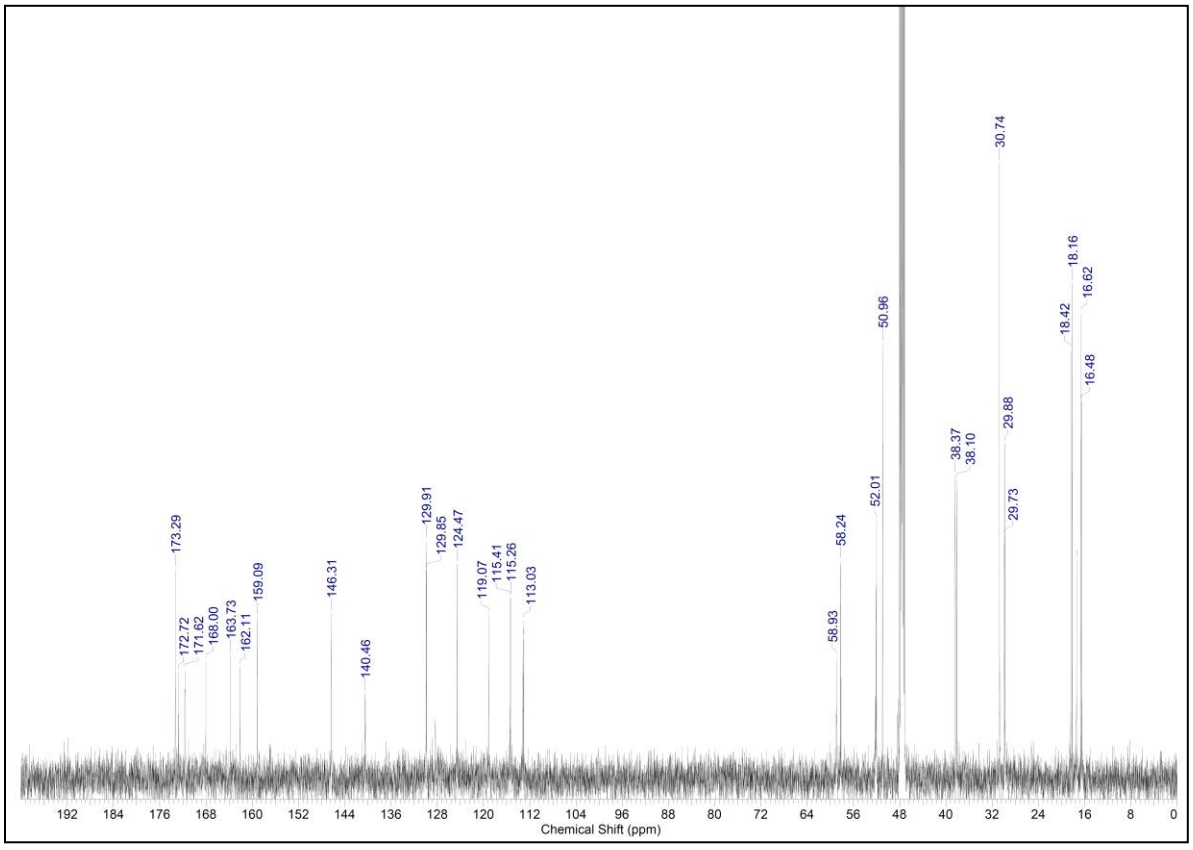
1.07 1.01 1.03 0.95 3.00 1.19 1.30 1.21 1.26
3.33 1.06 1.27 2.61 1.13 6.01 6.13
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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115.41
115.26
113.03
58.93
58.24
52.01
50.96
38.37
38.10
30.74
29.88
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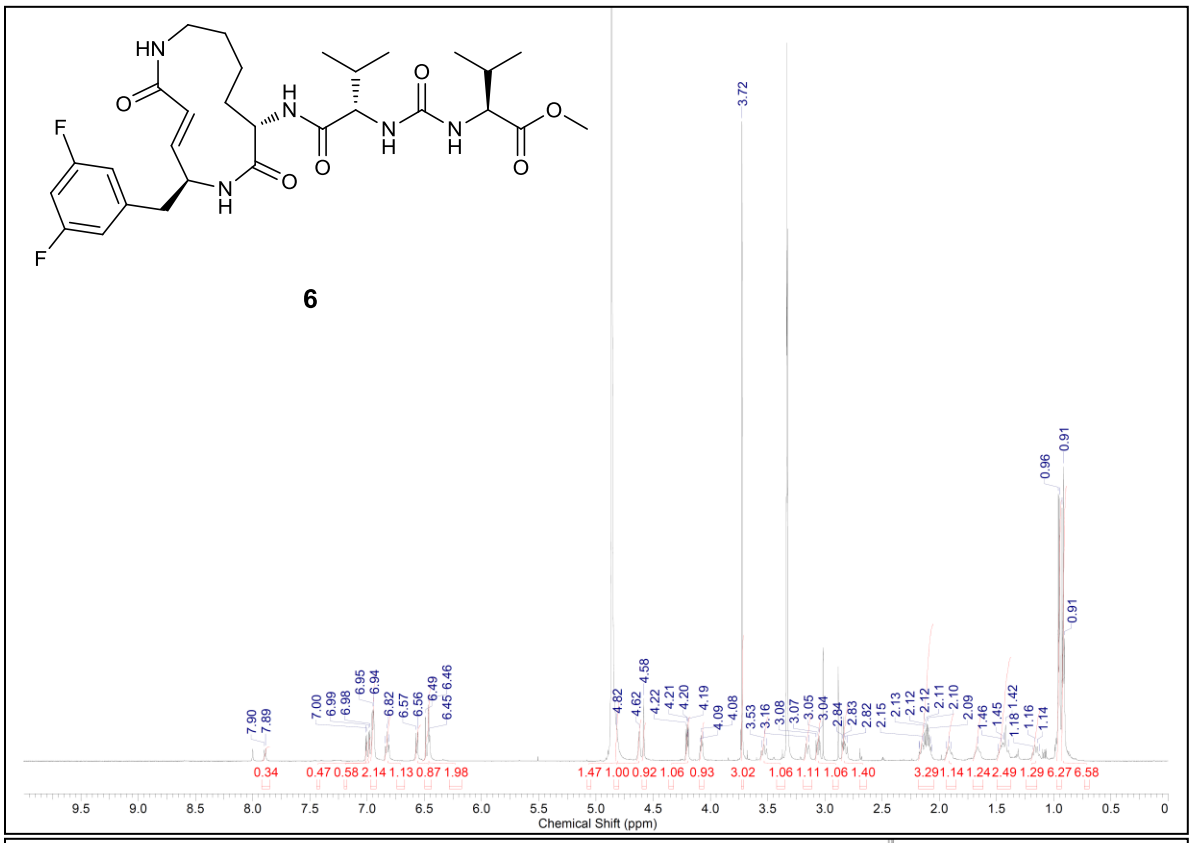
Chemical Shift (ppm)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H
N
O
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H
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O
F
F
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H
O
O
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0.91
0.91
7.90
7.89
7.00
6.99
6.98
6.95
6.94
6.82
6.57
6.56
6.49
6.45
6.46
4.82
4.62
4.58
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2.82
2.15
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1.45
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1.98
1.47
1.00
0.92
1.06
0.93
3.02
1.06
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1.06
1.40
3.29
1.14
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8.0
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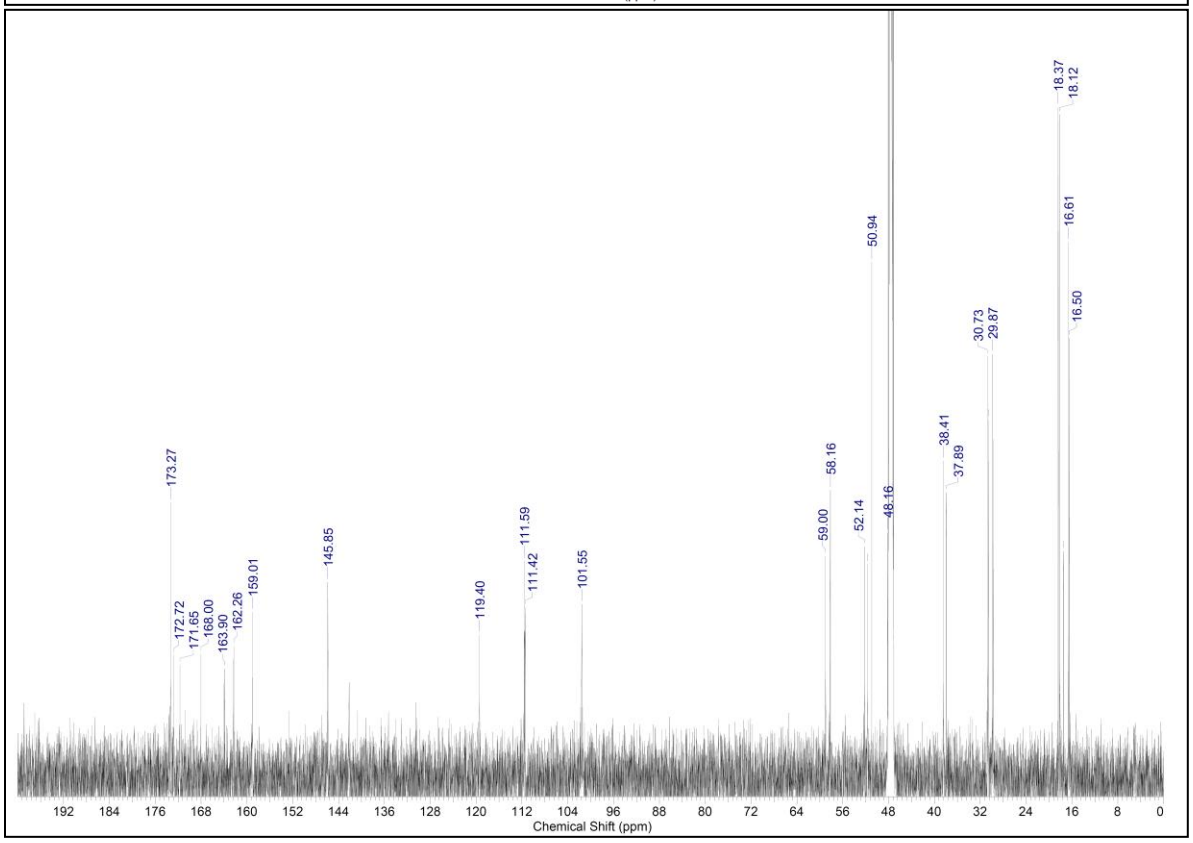
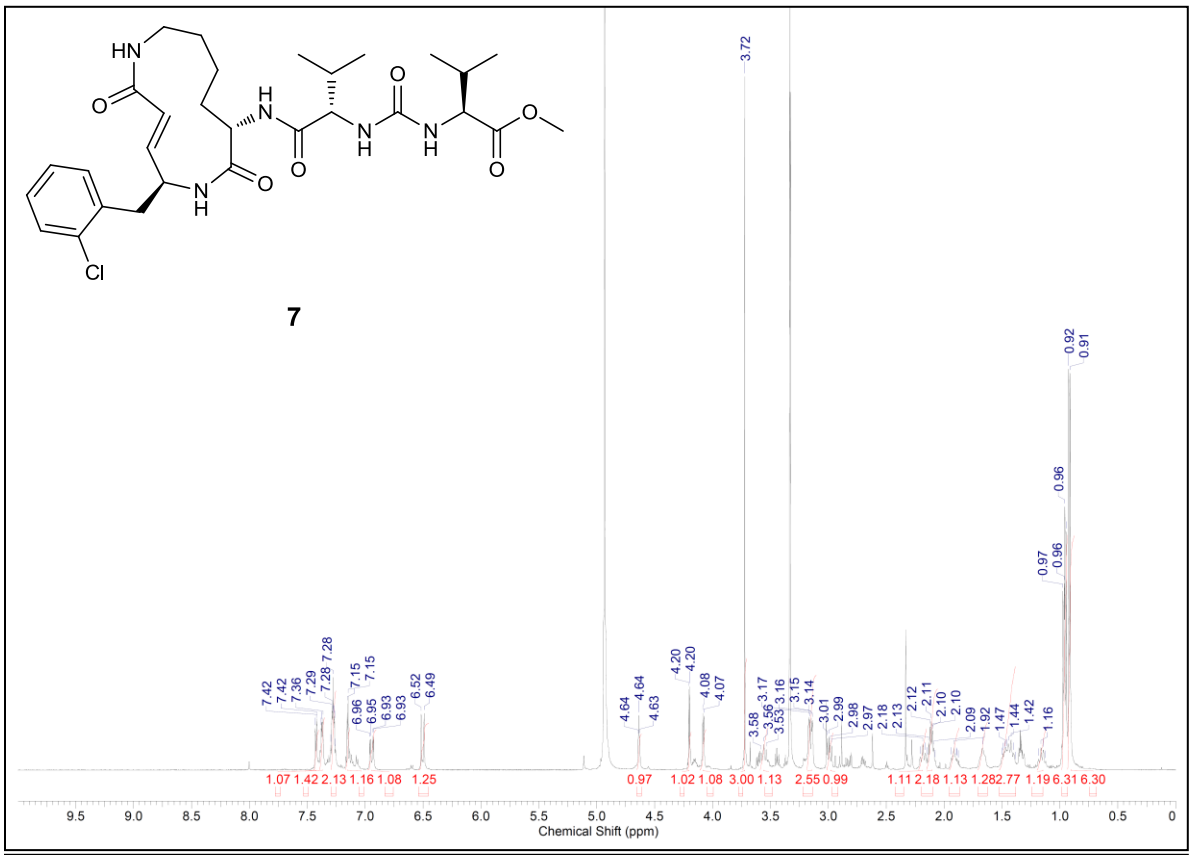
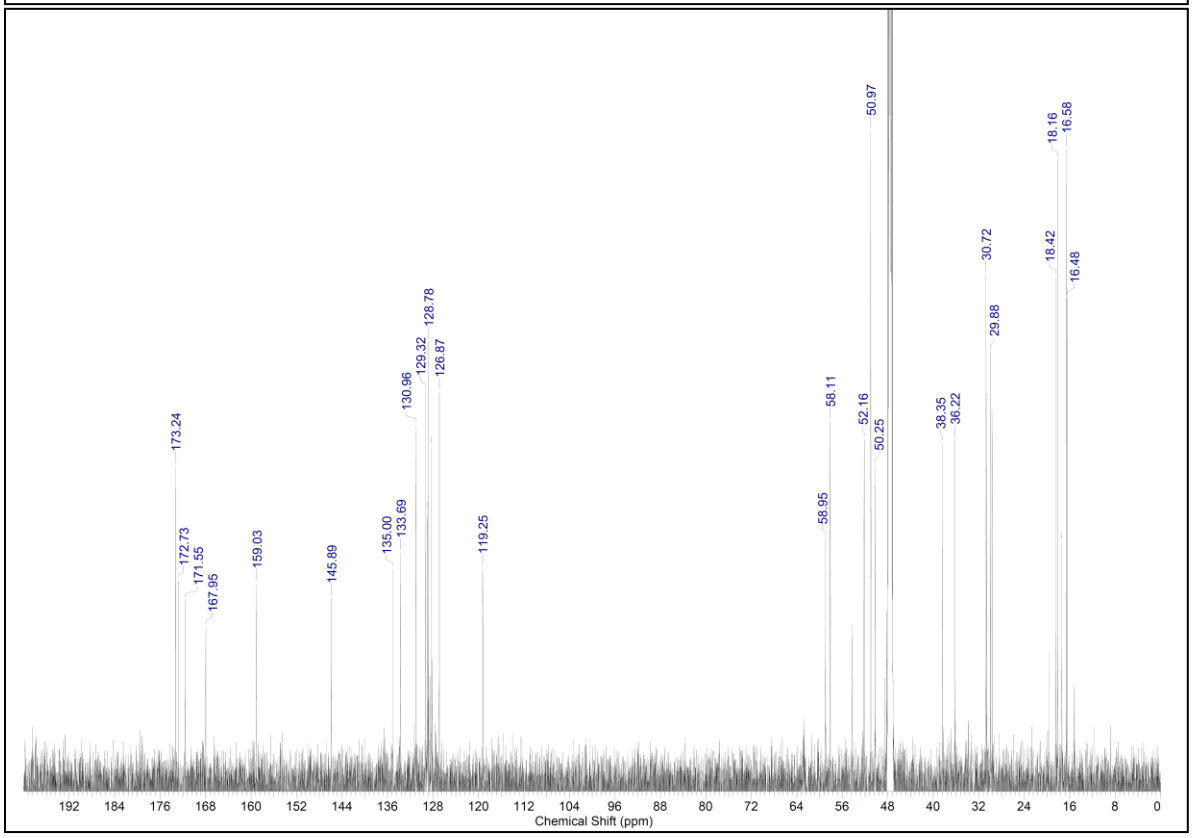
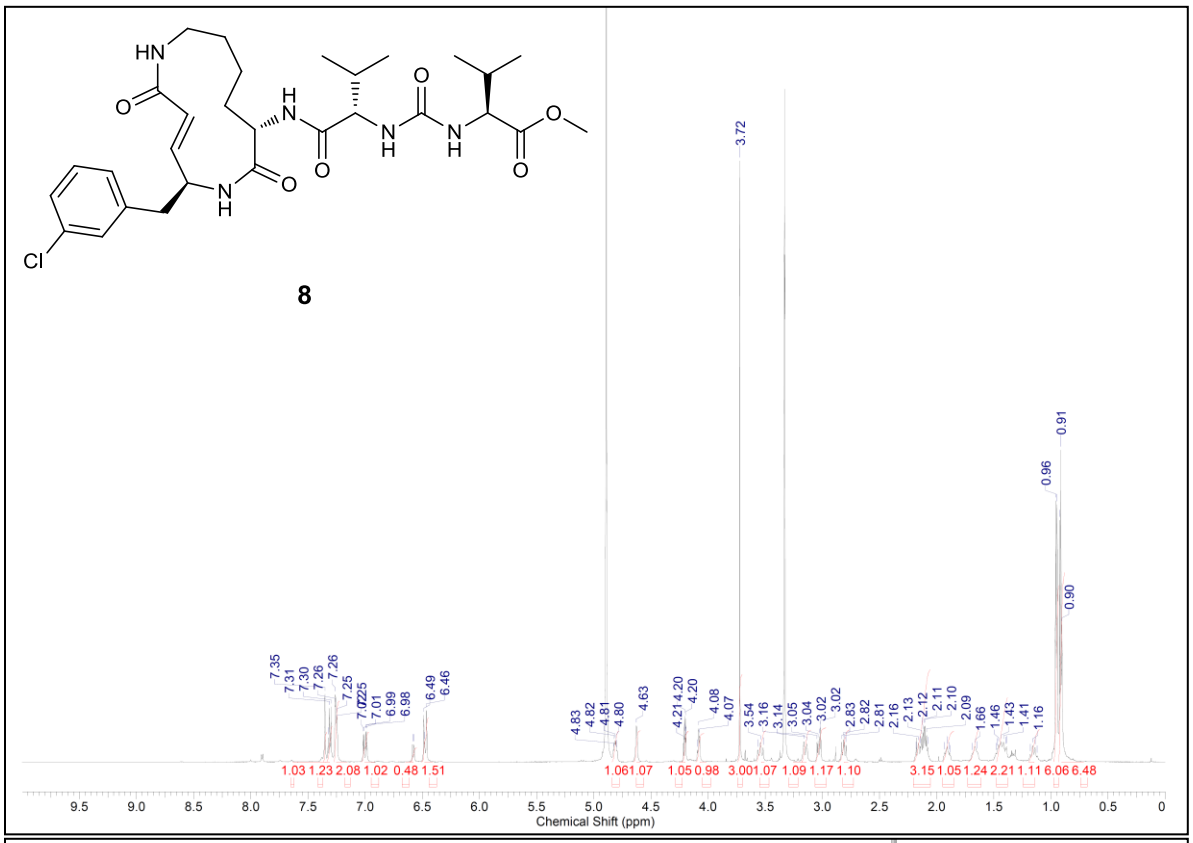
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

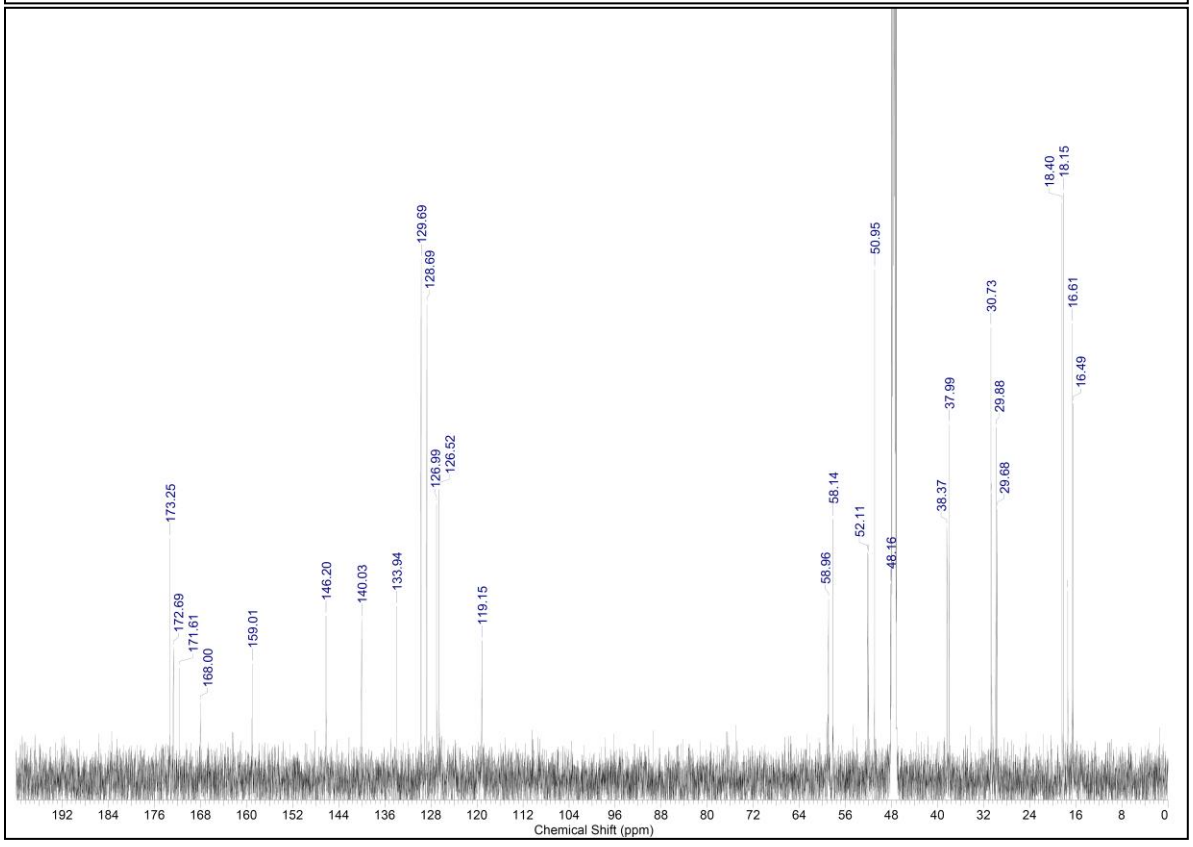

18.37
18.12
16.61
16.50
50.94
30.73
29.87
38.41
37.89
58.16
48.16
59.00
52.14
173.27
145.85
111.59
111.42
101.55
119.40
172.72
171.65
168.00
163.90
162.26
159.01
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

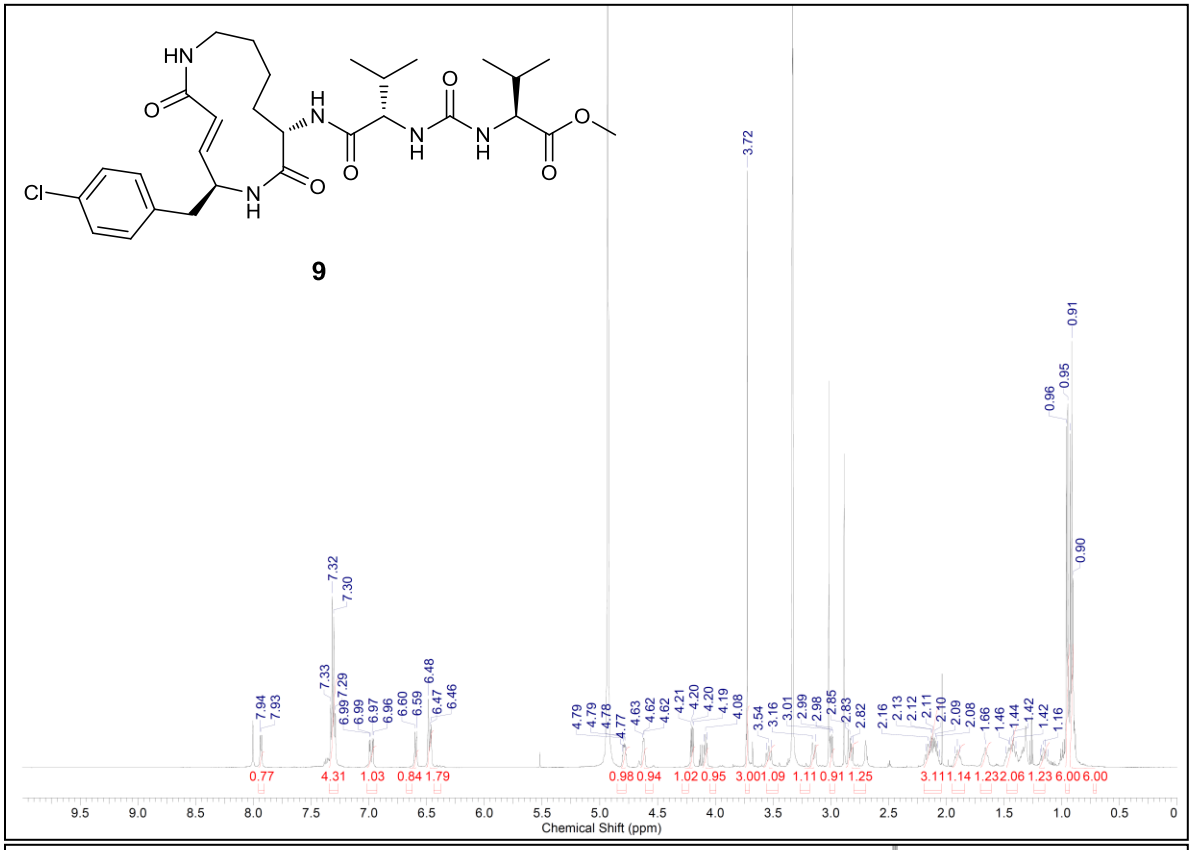

HN
O
H
N
O
N
H
O
N
H
O
O
N
H
O
O
Cl
7
Chemical Shift (ppm)

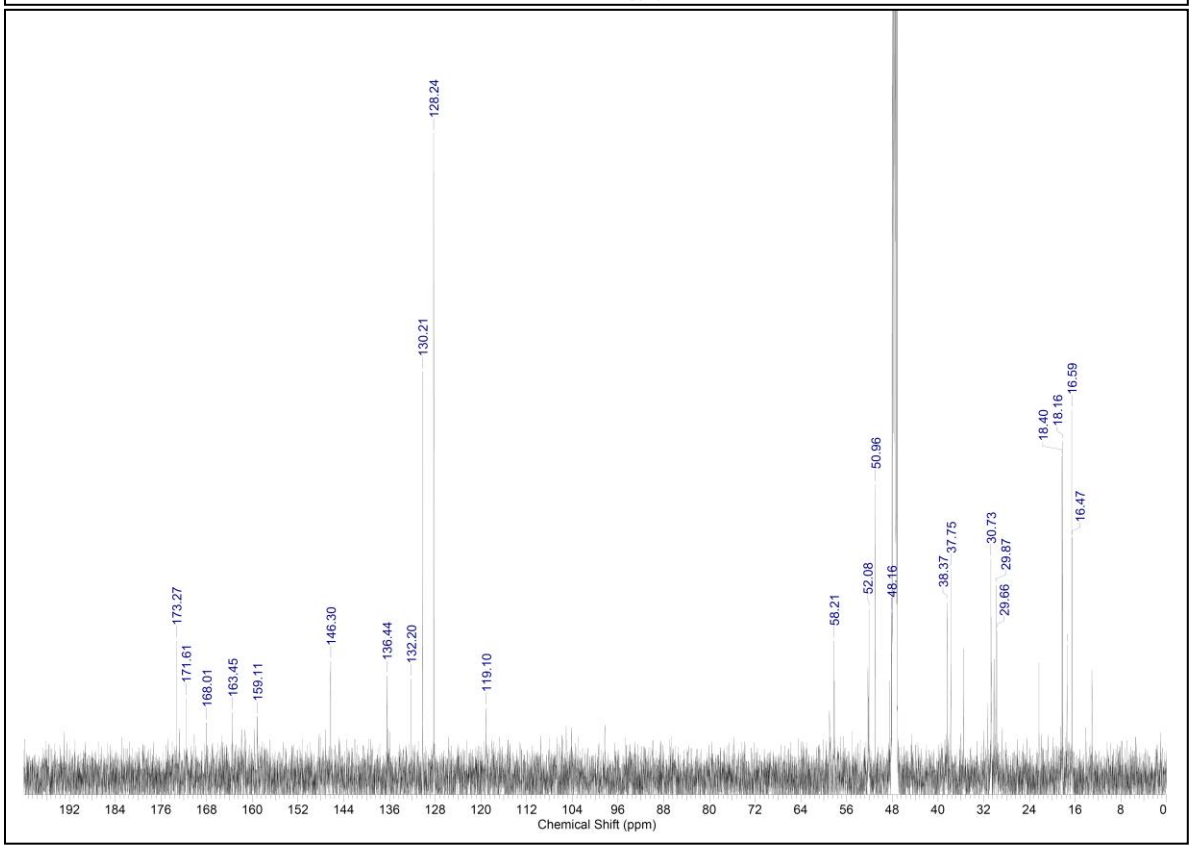

Chemical Shift (ppm)

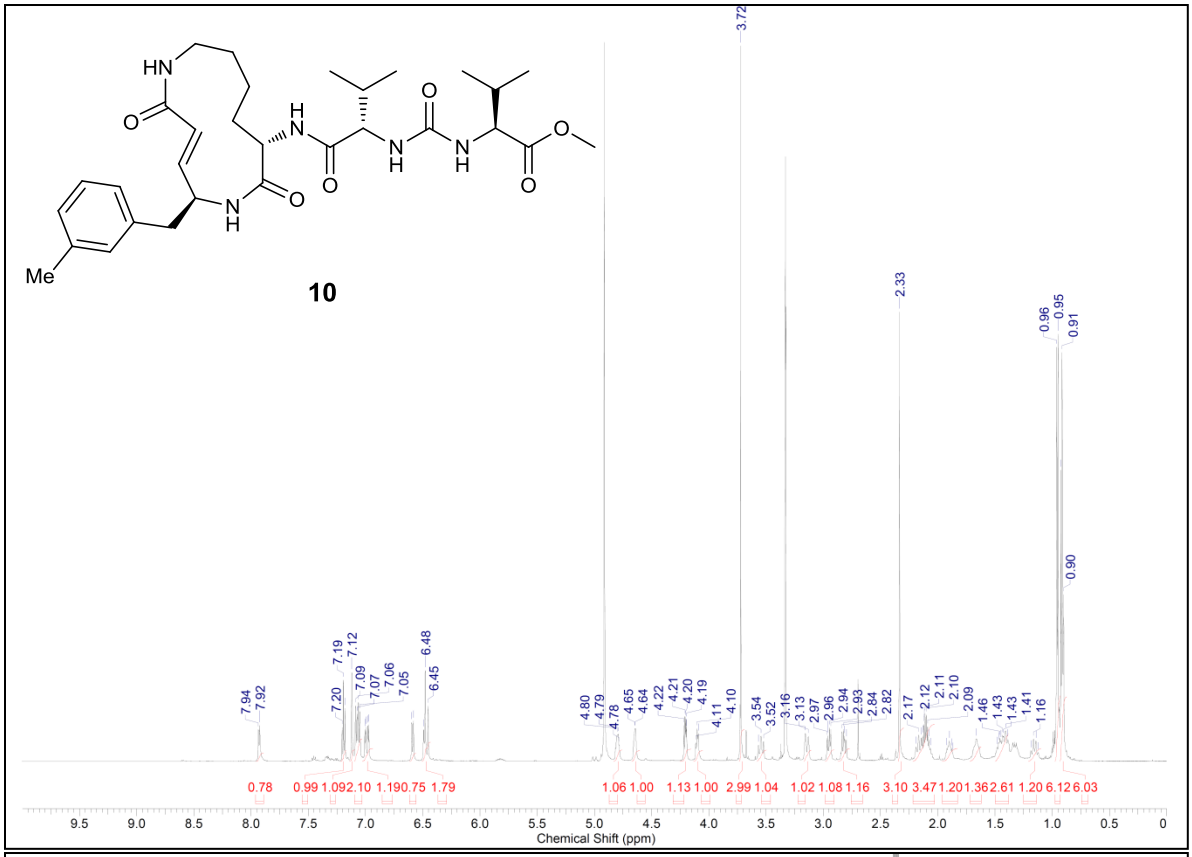

HN
O
N
H
O
N
H
O
N
H
O
O
O
N
H
O
Cl
3.72
0.96
0.91
0.90
7.35
7.31
7.30
7.26
7.26
7.25
7.01
6.99
6.98
6.49
6.46
4.83
4.82
4.81
4.80
4.63
4.21
4.20
4.20
4.08
4.07
3.54
3.16
3.14
3.05
3.04
3.02
3.02
2.83
2.82
2.81
2.16
2.13
2.12
2.11
2.10
2.09
1.66
1.46
1.43
1.41
1.16
1.03 1.23 2.08 1.02 0.48 1.51
1.06 1.07
1.05 0.98
3.00 1.07
1.09 1.17 1.10
3.15 1.05 1.24 2.21 1.11 6.06 6.48
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)

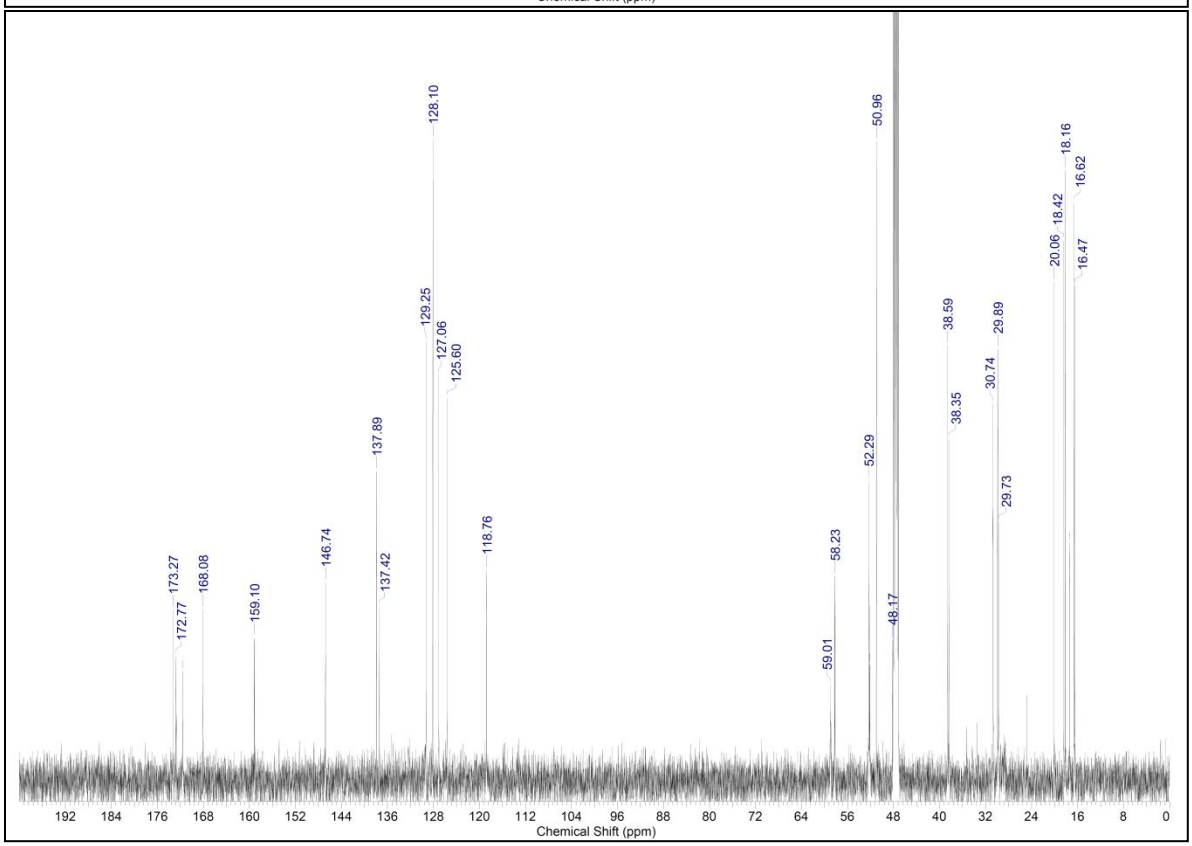
8

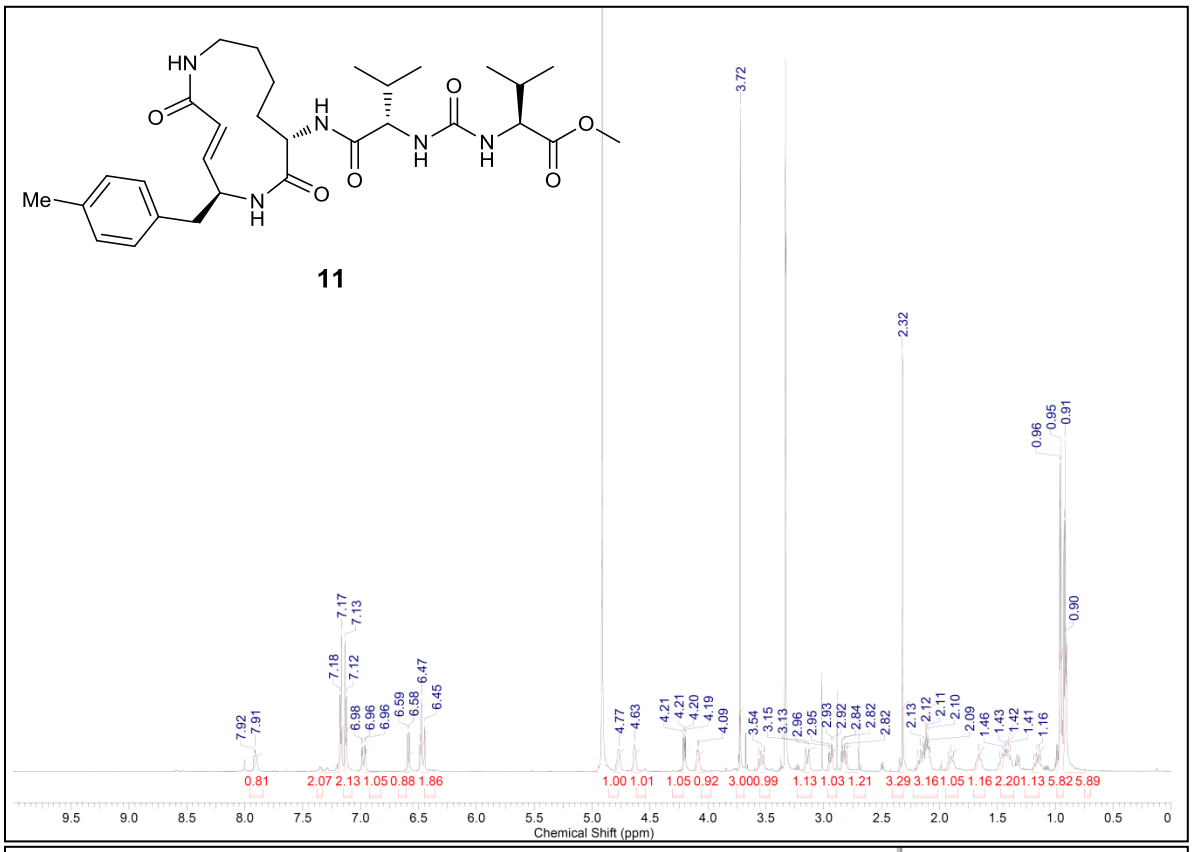

173.25
172.69
171.61
168.00
159.01
146.20
140.03
133.94
129.69
128.69
126.99
126.52
119.15
58.96
58.14
52.11
48.16
50.95
38.37
37.99
30.73
29.88
29.68
18.40
18.15
16.61
16.49
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)

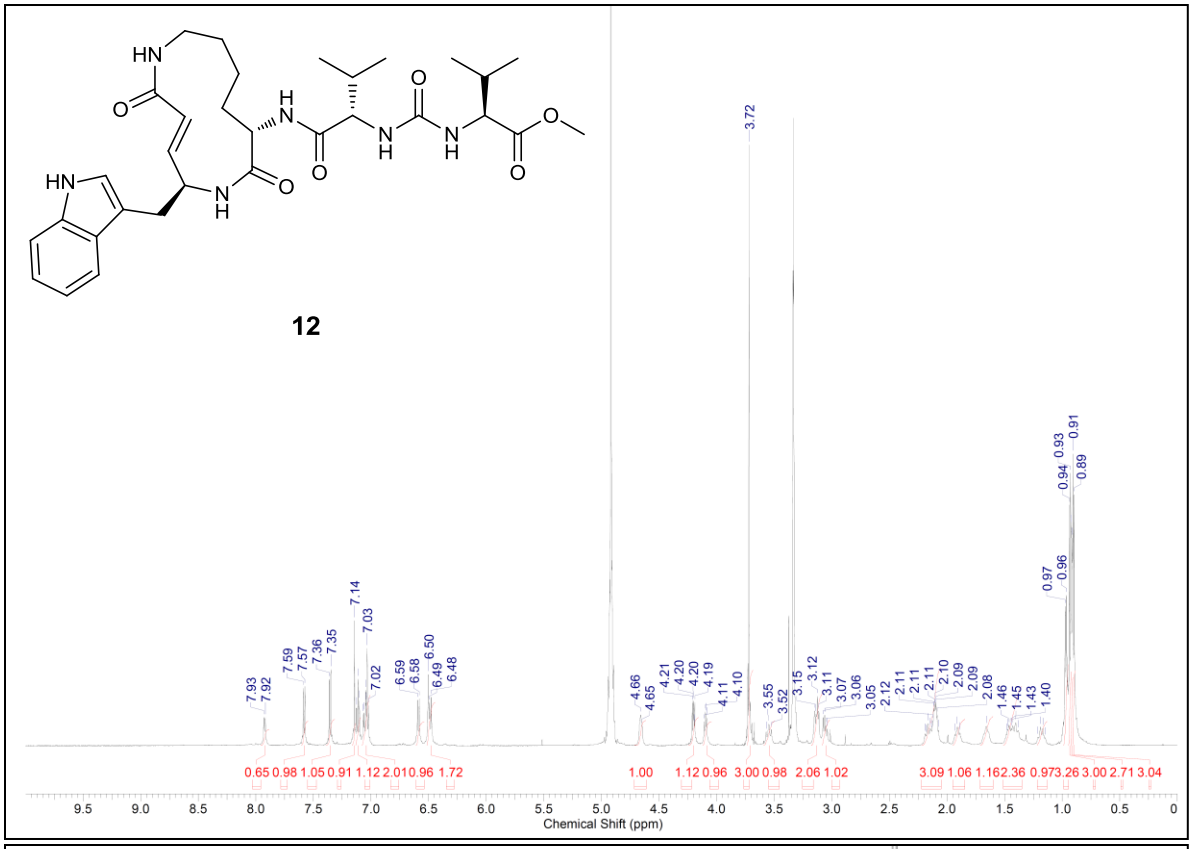

HN
O
H
N
O
N
H
N
H
O
O
O
O
Cl
N
H
O
9
3.72
0.91
0.96
0.95
0.90
7.32
7.30
7.33
7.29
6.99
6.99
6.97
6.96
6.60
6.59
6.48
6.47
6.46
7.94
7.93
4.79
4.79
4.78
4.77
4.63
4.62
4.62
4.21
4.20
4.20
4.19
4.08
3.54
3.16
3.01
2.99
2.98
2.85
2.83
2.82
2.16
2.13
2.12
2.11
2.10
2.09
2.08
1.66
1.46
1.44
1.42
1.42
1.16
0.77
4.31
1.03
0.84
1.79
0.98
0.94
1.02
0.95
3.00
1.09
1.11
0.91
1.25
3.11
1.14
1.23
2.06
1.23
6.00
6.00
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

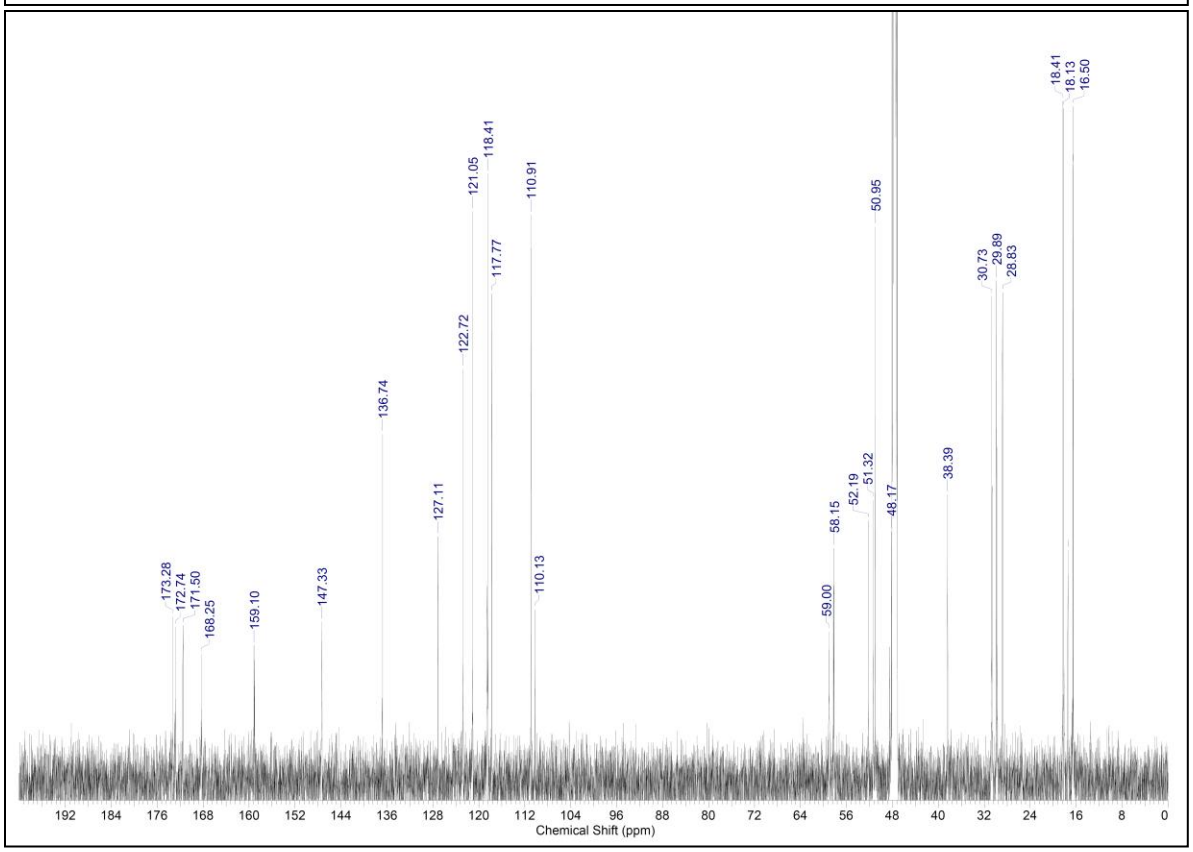

128.24
130.21
50.96
18.40
18.16
16.59
16.47
30.73
29.87
37.75
38.37
29.66
48.16
52.08
58.21
173.27
171.61
168.01
163.45
159.11
146.30
136.44
132.20
119.10
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

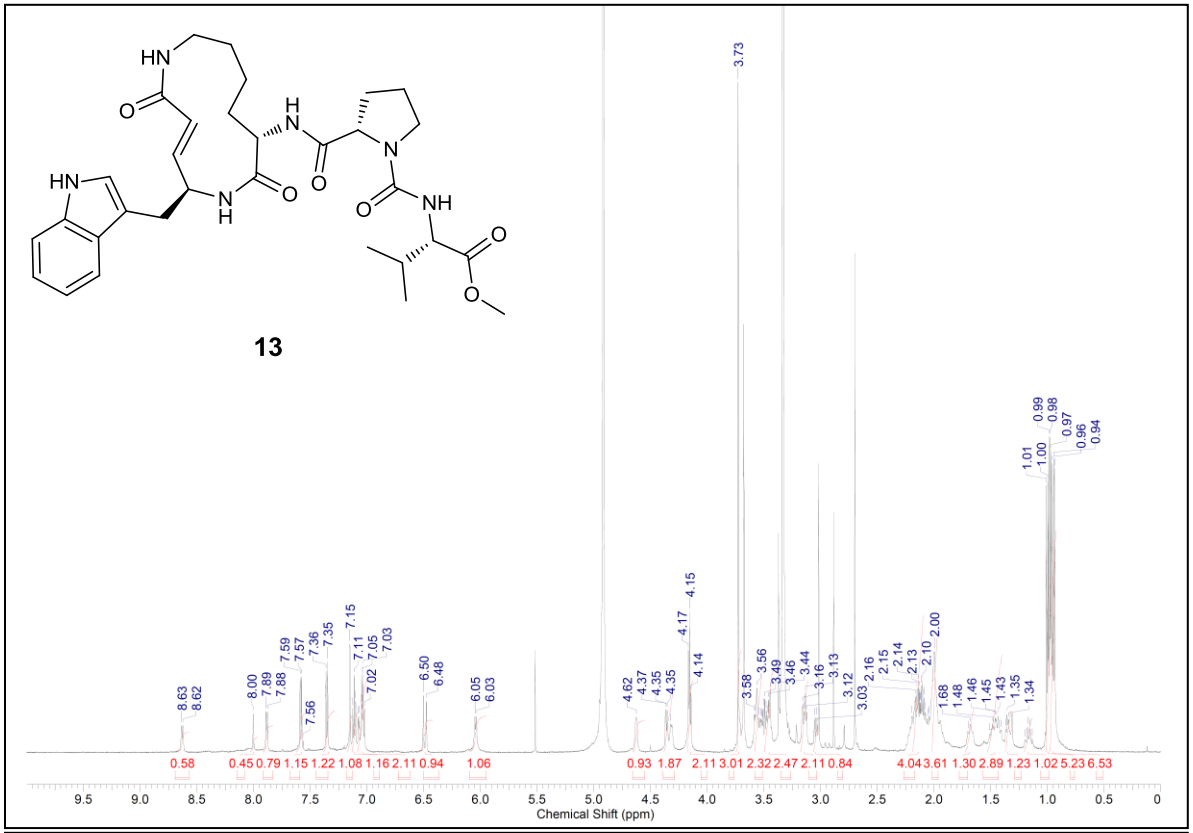

HN
O
H
N
O
O
N
H
N
H
O
O
N
H
O
O
Me
10
3.72
2.33
0.96
0.95
0.91
0.90
7.94
7.92
7.20
7.19
7.12
7.09
7.07
7.06
7.05
6.48
6.45
4.80
4.79
4.78
4.65
4.64
4.22
4.21
4.20
4.19
4.11
4.10
3.54
3.52
3.16
3.13
2.97
2.96
2.94
2.93
2.84
2.82
2.17
2.12
2.11
2.10
2.09
1.46
1.43
1.41
1.16
0.78
0.99
1.09
2.10
1.19
0.75
1.79
1.06
1.00
1.13
1.00
2.99
1.04
1.02
1.08
1.16
3.10
3.47
1.20
1.36
2.61
1.20
6.12
6.03
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

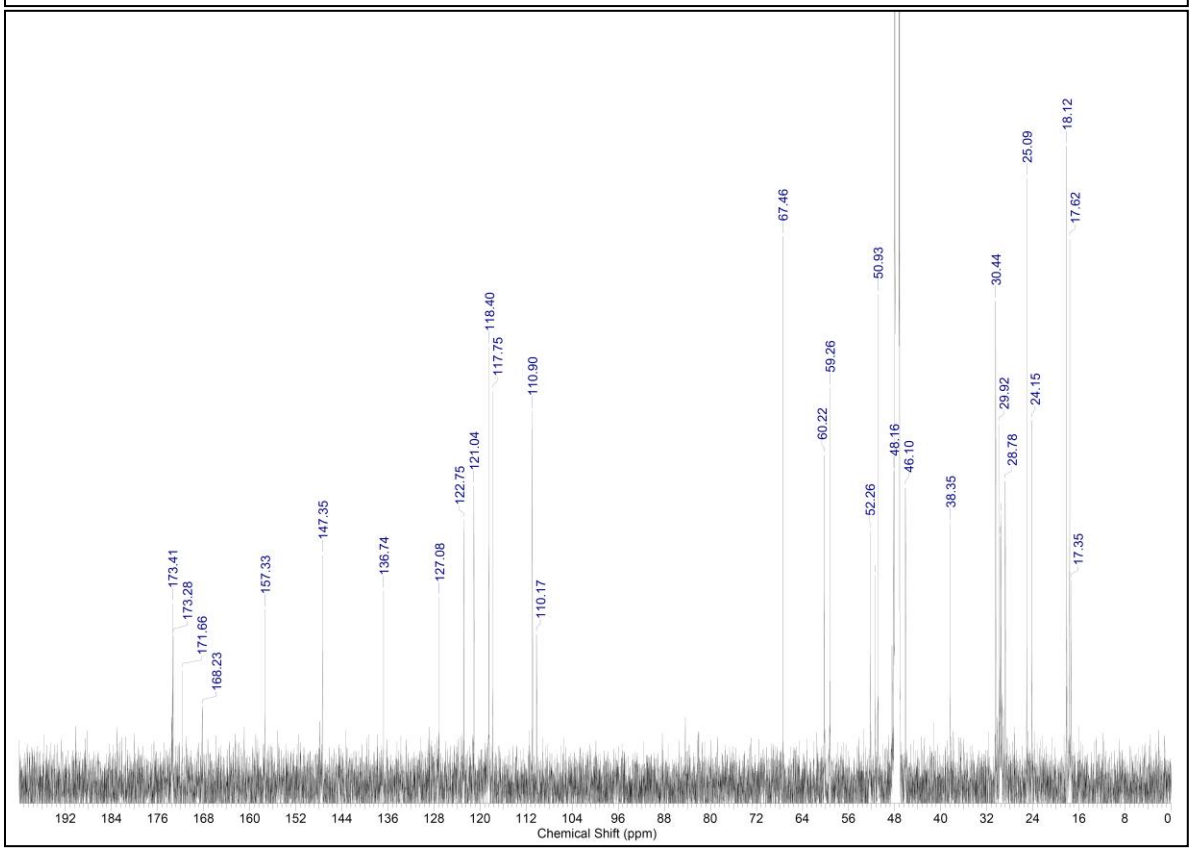

128.10
50.96
18.16
16.62
20.06
18.42
16.47
129.25
127.06
125.60
38.59
29.89
137.89
30.74
38.35
52.29
29.73
146.74
118.76
58.23
137.42
173.27
168.08
172.77
159.10
59.01
48.17
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

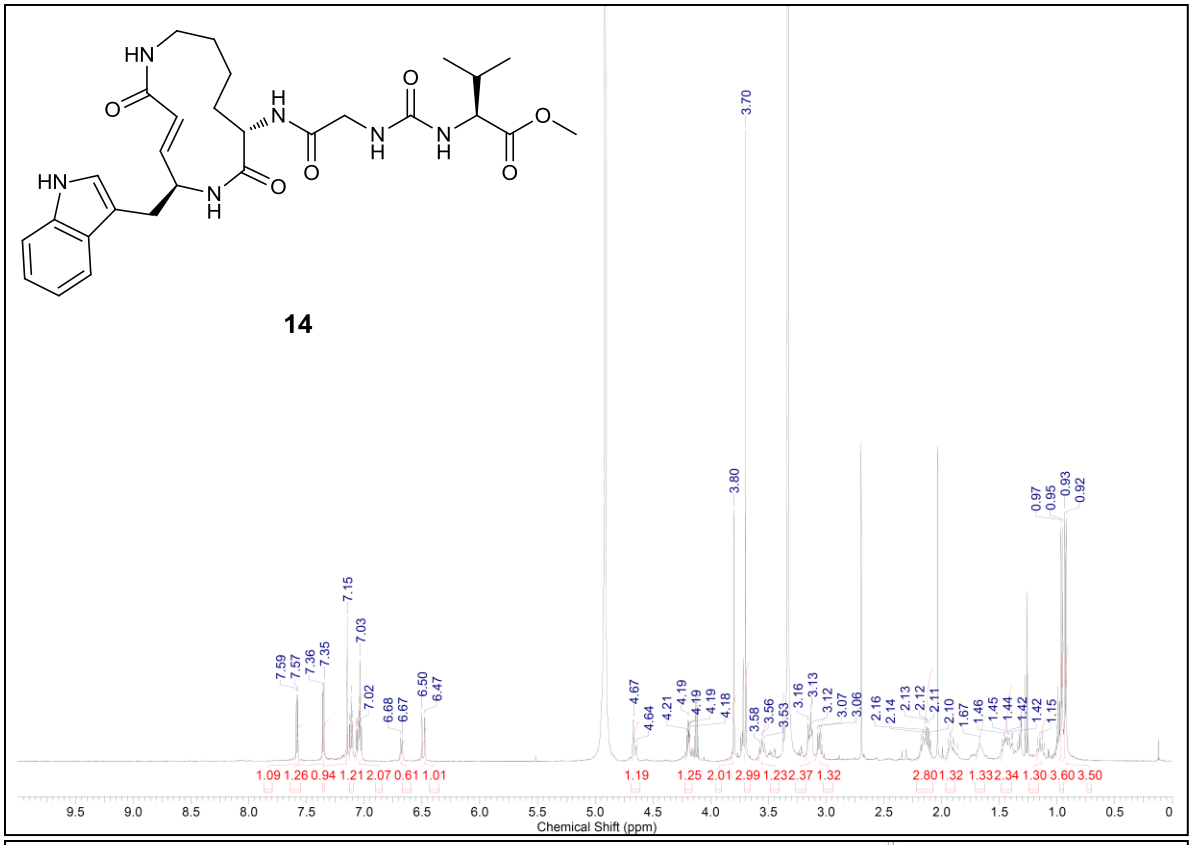

HN
O
H
N
O
N
H
N
H
O
O
O
Me
N
H
O
11
3.72
2.32
0.96
0.95
0.91
0.90
7.17
7.13
7.18
7.12
6.47
6.98
6.96
6.96
6.59
6.58
6.45
7.92
7.91
4.77
4.63
4.21
4.21
4.20
4.19
4.09
3.54
3.15
3.13
2.96
2.95
2.93
2.92
2.84
2.82
2.82
2.13
2.12
2.11
2.10
2.09
1.46
1.43
1.42
1.41
1.16
0.81
2.07 2.13 1.05 0.88 1.86
1.00 1.01 1.05 0.92 3.00 0.99 1.13 1.03 1.21 3.29 3.16 1.05 1.16 2.20 1.13 5.82 5.89
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)
128.80
128.45
173.27
171.50
168.08
159.07
146.74
136.02
134.40
118.76
59.04
58.23
52.33
50.95
48.16
38.35
38.23
30.73
29.89
29.71
19.68
18.16
16.61
16.47
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)

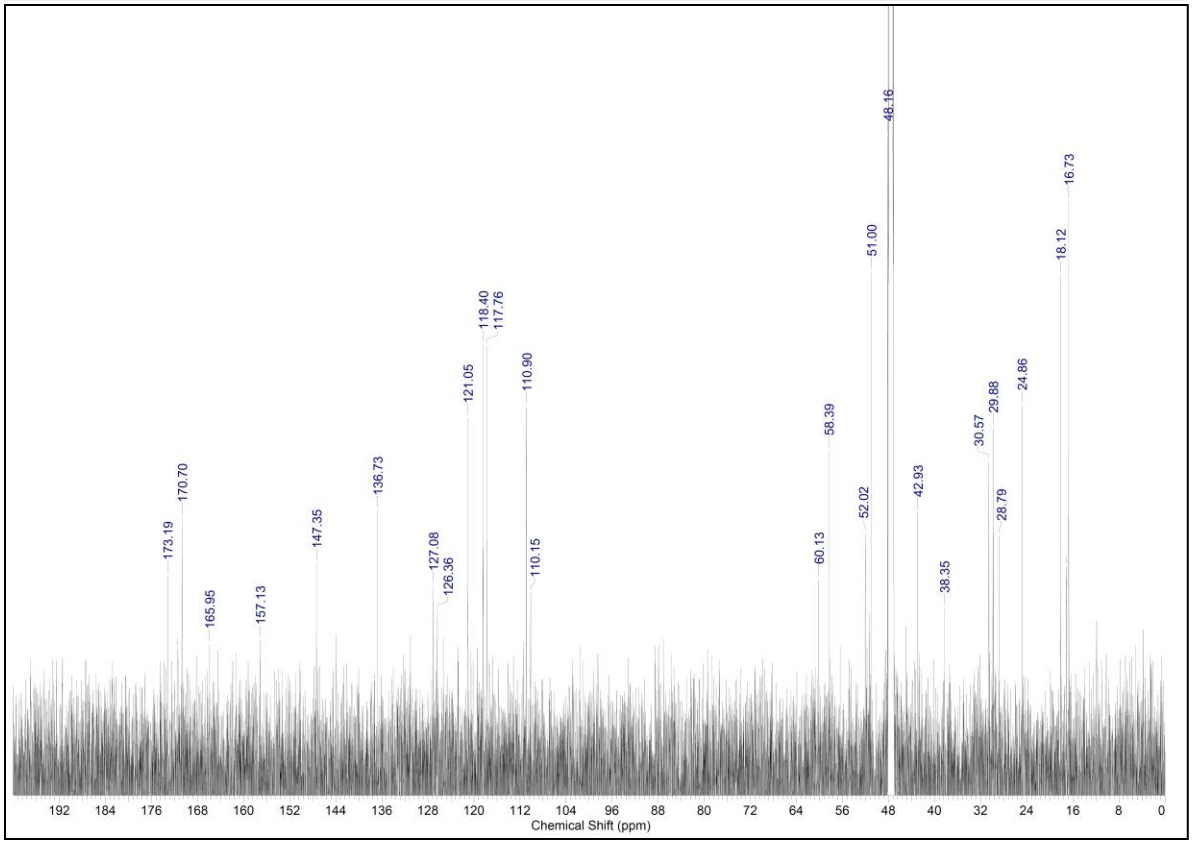

12
7.93
7.92
7.59
7.57
7.36
7.35
7.14
7.03
7.02
6.59
6.58
6.50
6.49
6.48
4.66
4.65
4.21
4.20
4.20
4.19
4.11
4.10
3.72
3.55
3.52
3.15
3.12
3.11
3.07
3.06
3.05
2.12
2.11
2.11
2.11
2.10
2.09
2.09
2.08
1.46
1.45
1.43
1.40
0.97
0.96
0.94
0.93
0.91
0.89
0.65 0.98 1.05 0.91 1.12 2.01 0.96 1.72
1.00
1.12 0.96 3.00 0.98 2.06 1.02
3.09 1.06 1.16 2.36 0.97 3.26 3.00 2.71 3.04
Chemical Shift (ppm)

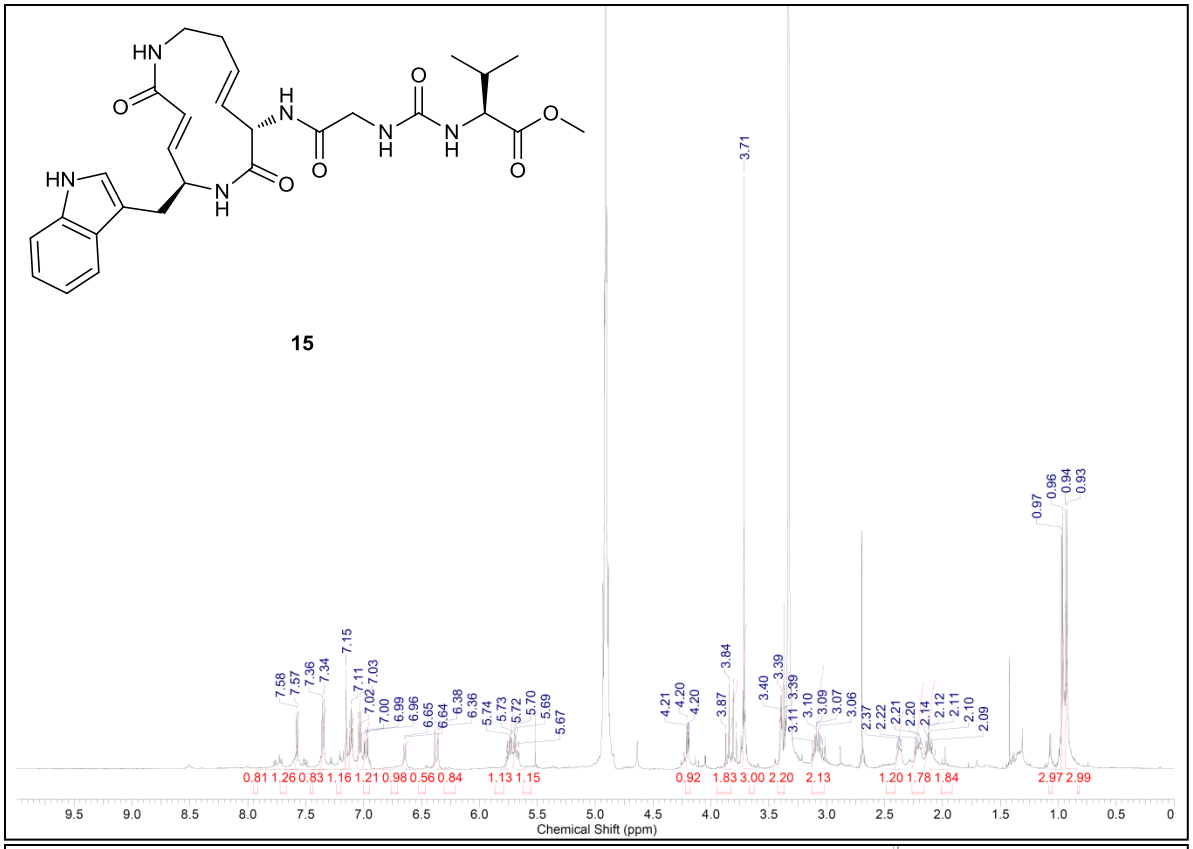

173.28
172.74
171.50
168.25
159.10
147.33
136.74
127.11
122.72
121.05
118.41
117.77
110.91
110.13
59.00
58.15
52.19
51.32
50.95
48.17
38.39
30.73
29.89
28.83
18.41
18.13
16.50
Chemical Shift (ppm)

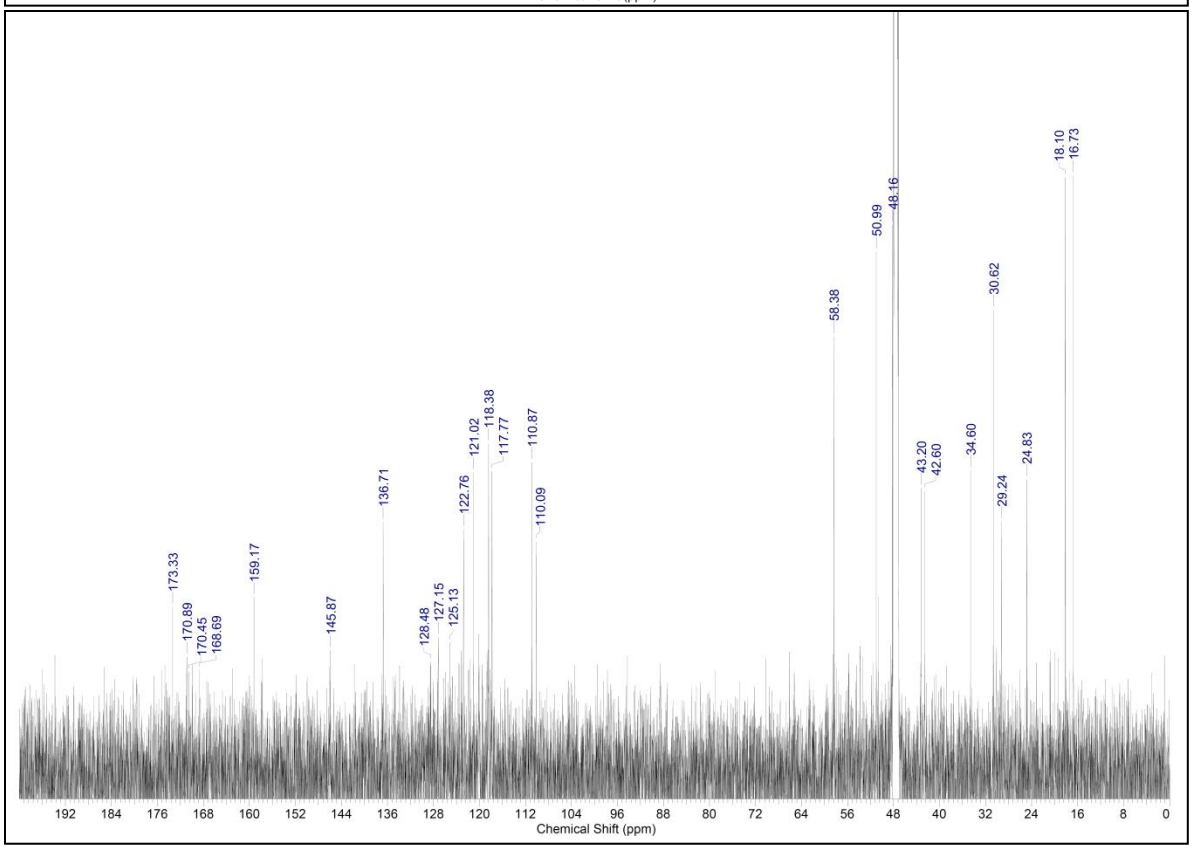

HN
O
H
N
O
HN
N
H
O
O
N
O
NH
O
O
13
Chemical Shift (ppm)

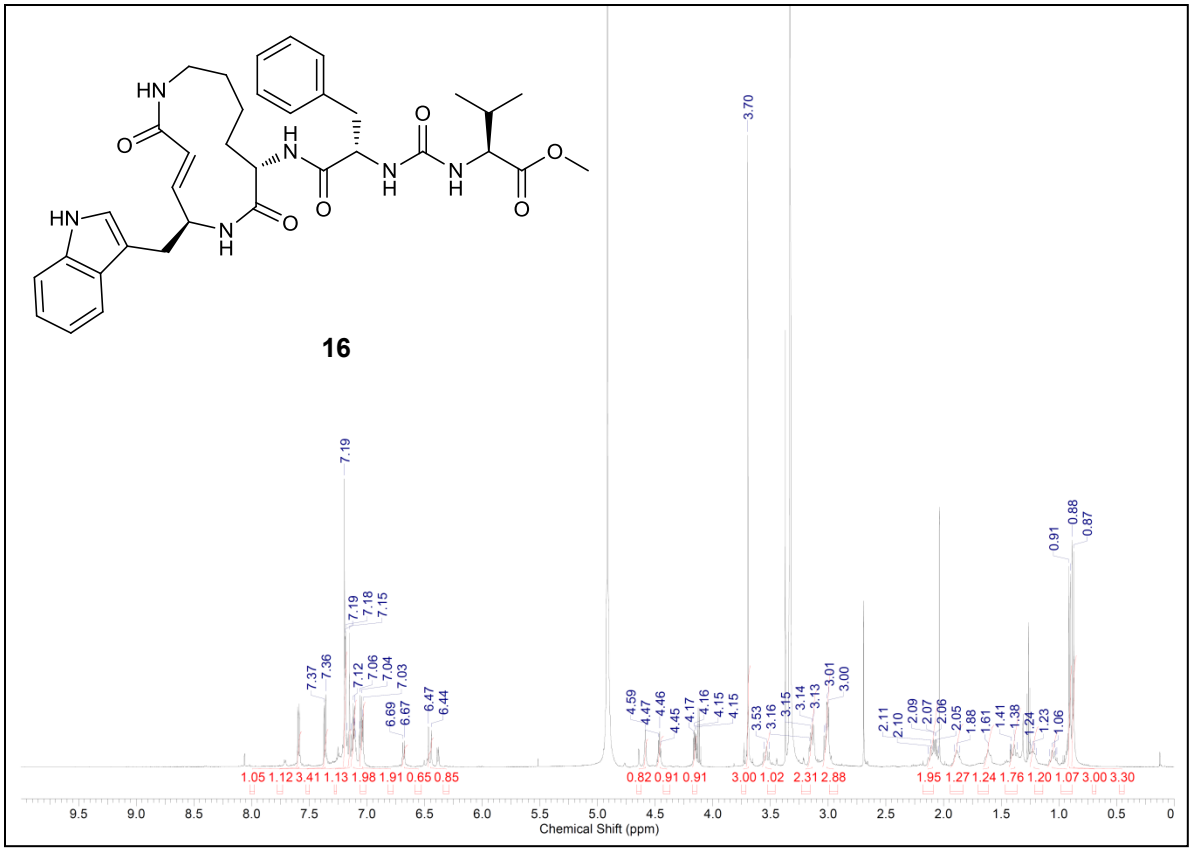

Chemical Shift (ppm)

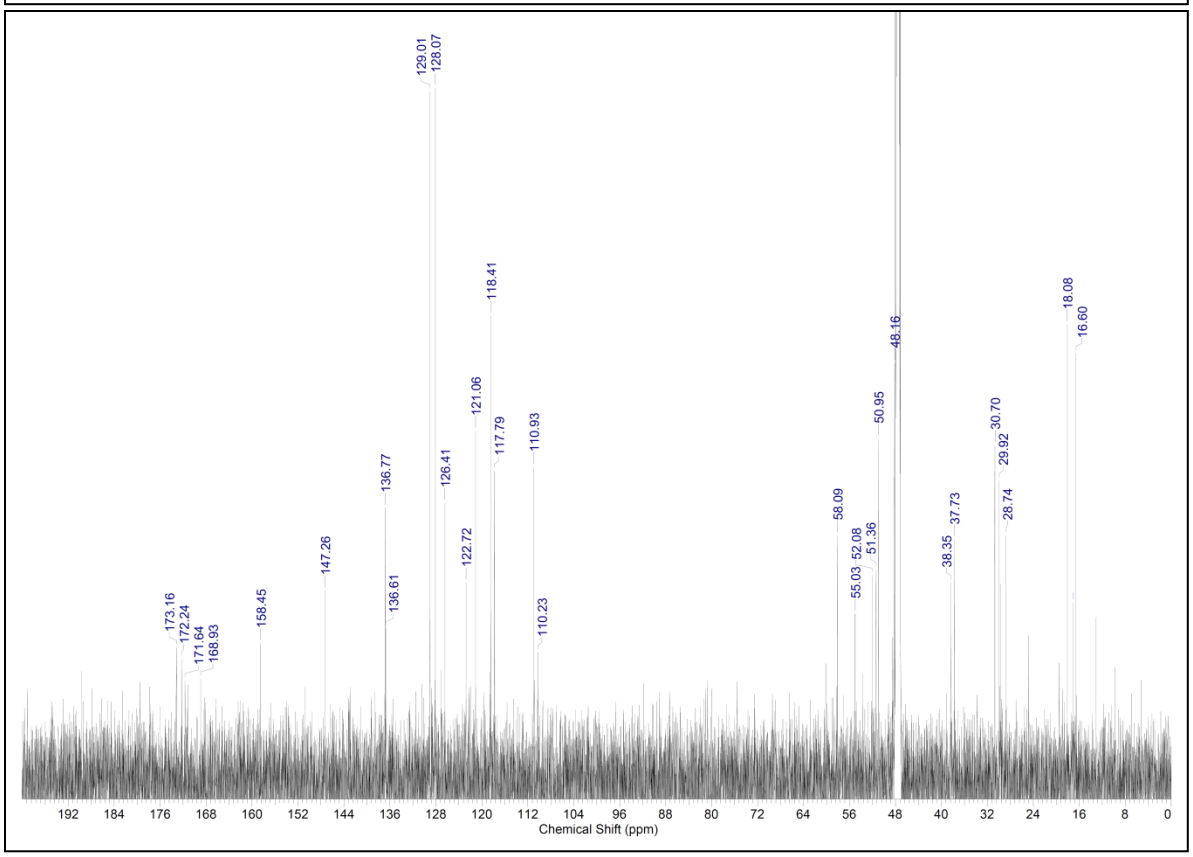

HN
O
H
N
O
N
H
O
N
H
O
O
HN
N
H
O
O
14
7.59
7.57
7.36
7.35
7.15
7.03
7.02
6.68
6.67
6.50
6.47
4.67
4.64
4.21
4.19
4.19
4.19
4.18
3.80
3.70
3.58
3.56
3.53
3.16
3.13
3.12
3.07
3.06
2.16
2.14
2.13
2.12
2.11
2.10
1.67
1.46
1.45
1.44
1.42
1.42
1.15
0.97
0.95
0.93
0.92
1.09 1.26 0.94 1.21 2.07 0.61 1.01
1.19
1.25 2.01 2.99 1.23 2.37 1.32
2.80 1.32 1.33 2.34 1.30 3.60 3.50
Chemical Shift (ppm)

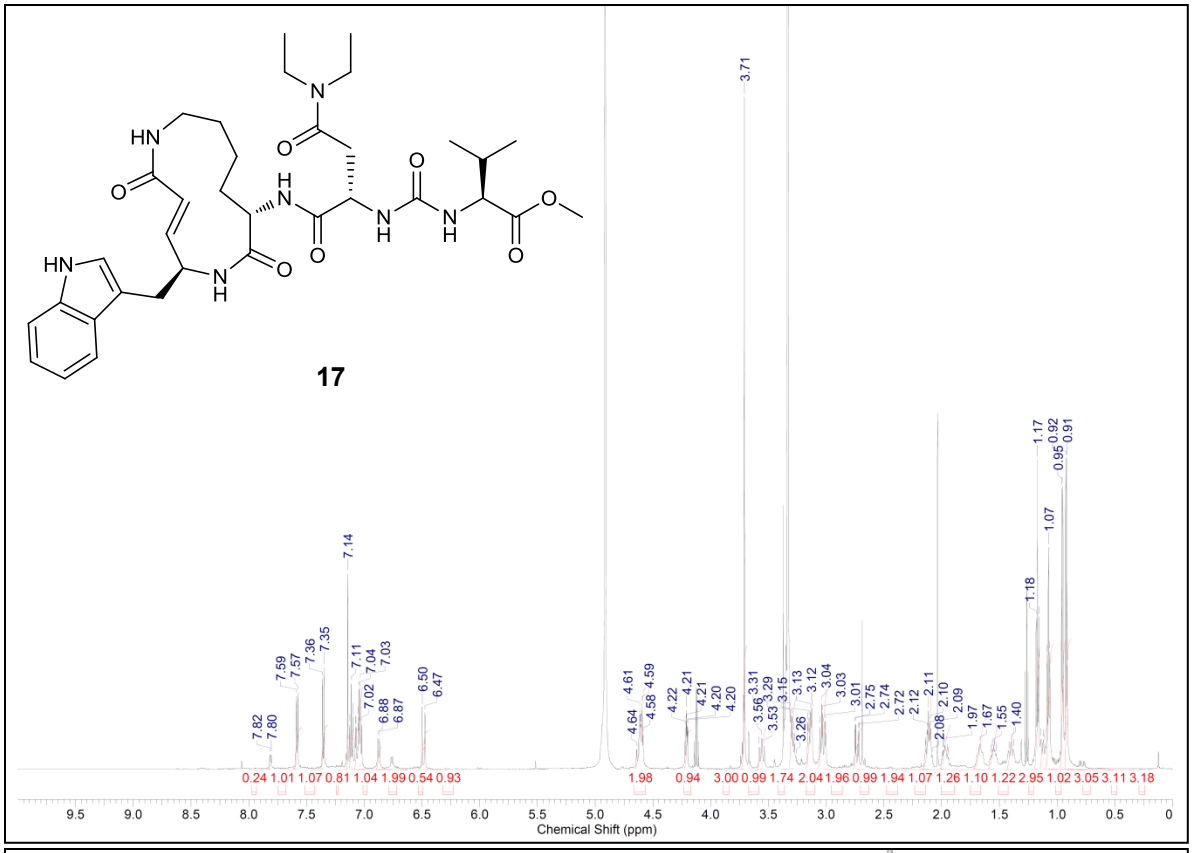

173.19
170.70
165.95
157.13
147.35
136.73
127.08
126.36
121.05
118.40
117.76
110.90
110.15
60.13
58.39
52.02
51.00
48.16
42.93
38.35
30.57
29.88
28.79
24.86
18.12
16.73
Chemical Shift (ppm)

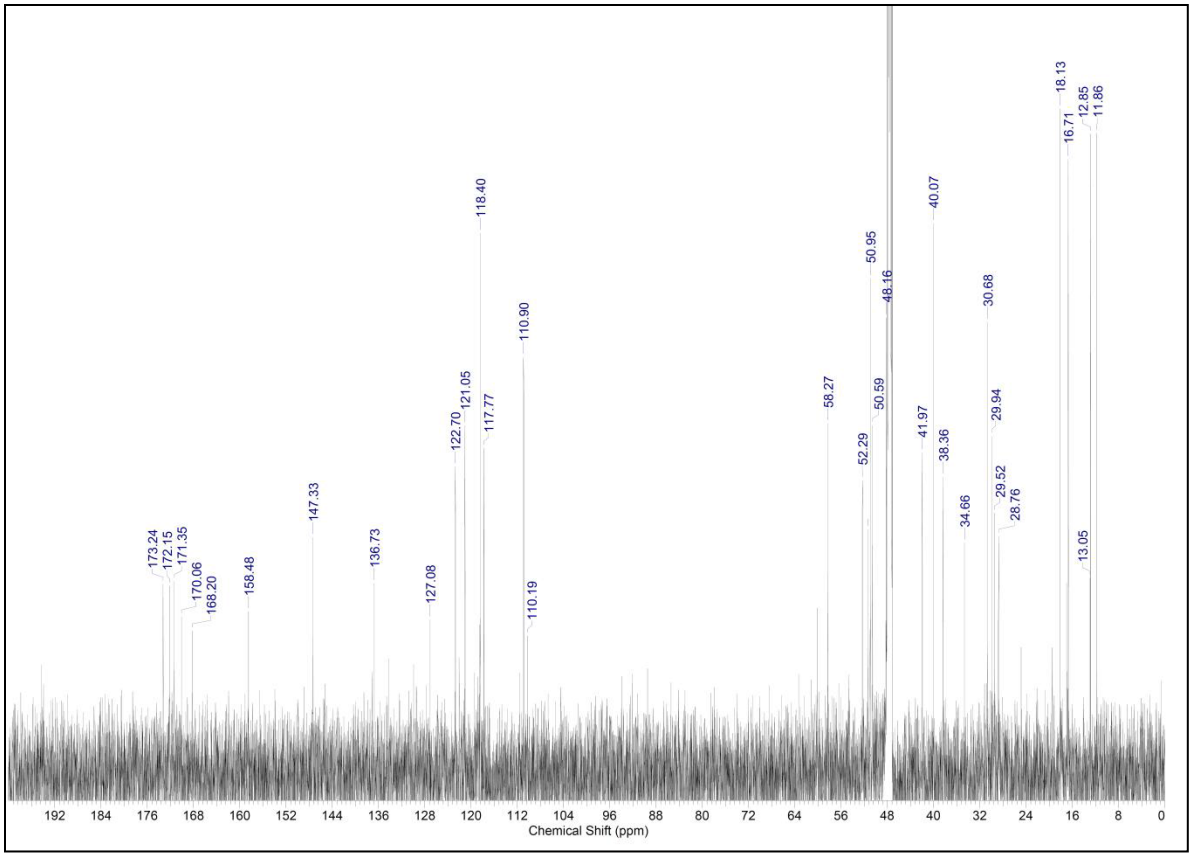

HN
O
HN
N
H
O
N
H
O
N
H
O
O
O
N
H
15
3.71
7.58
7.57
7.36
7.34
7.15
7.11
7.03
7.02
7.00
6.99
6.96
6.65
6.64
6.38
6.36
5.74
5.73
5.72
5.70
5.69
5.67
4.21
4.20
4.20
3.87
3.84
3.40
3.39
3.39
3.11
3.10
3.09
3.07
3.06
2.37
2.22
2.21
2.20
2.14
2.12
2.11
2.10
2.09
0.97
0.96
0.94
0.93
0.81 1.26 0.83 1.16 1.21 0.98 0.56 0.84
1.13 1.15
0.92 1.83 3.00 2.20 2.13
1.20 1.78 1.84
2.97 2.99
Chemical Shift (ppm)

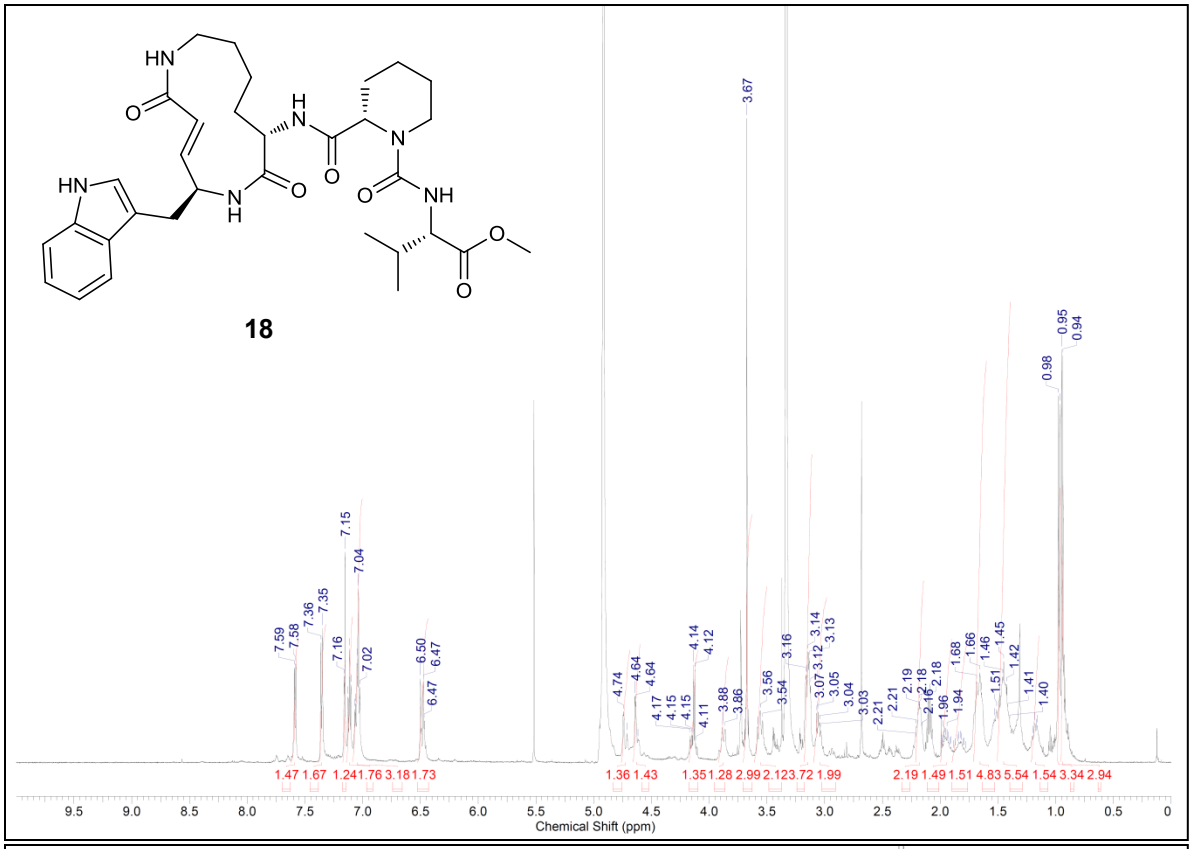

173.33
170.89
170.45
168.69
159.17
145.87
136.71
128.48
127.15
125.13
122.76
121.02
118.38
117.77
110.87
110.09
58.38
50.99
48.16
43.20
42.60
34.60
30.62
29.24
24.83
18.10
16.73
Chemical Shift (ppm)


16
7.19
3.70
0.91
0.88
0.87
7.37
7.36
7.12
7.19
7.18
7.15
7.06
7.04
7.03
6.69
6.67
6.47
6.44
4.59
4.47
4.46
4.45
4.17
4.16
4.15
3.53
3.16
3.15
3.14
3.13
3.01
3.00
2.11
2.10
2.09
2.07
2.06
2.05
1.88
1.61
1.41
1.38
1.24
1.23
1.06
1.05 1.12 3.41 1.13 1.98 1.91 0.65 0.85
0.82 0.91 0.91
3.00 1.02 2.31 2.88
1.95 1.27 1.24 1.76 1.20 1.07 3.00 3.30
Chemical Shift (ppm)


129.01
128.07
118.41
121.06
117.79
110.93
126.41
136.77
122.72
147.26
136.61
158.45
110.23
173.16
172.24
171.64
169.93
58.09
55.03
52.08
51.36
50.95
48.16
38.35
37.73
30.70
29.92
28.74
18.08
16.60
Chemical Shift (ppm)


17
Chemical Shift (ppm)


Chemical Shift (ppm)


HN
O
H
N
O
N
O
O
NH
O
HN
N
H
O
18
3.67
0.98
0.95
0.94
7.15
7.04
7.59
7.58
7.36
7.35
7.16
7.02
6.50
6.47
6.47
4.74
4.64
4.64
4.14
4.12
4.17
4.15
4.15
4.11
3.88
3.86
3.56
3.54
3.16
3.07
3.12
3.14
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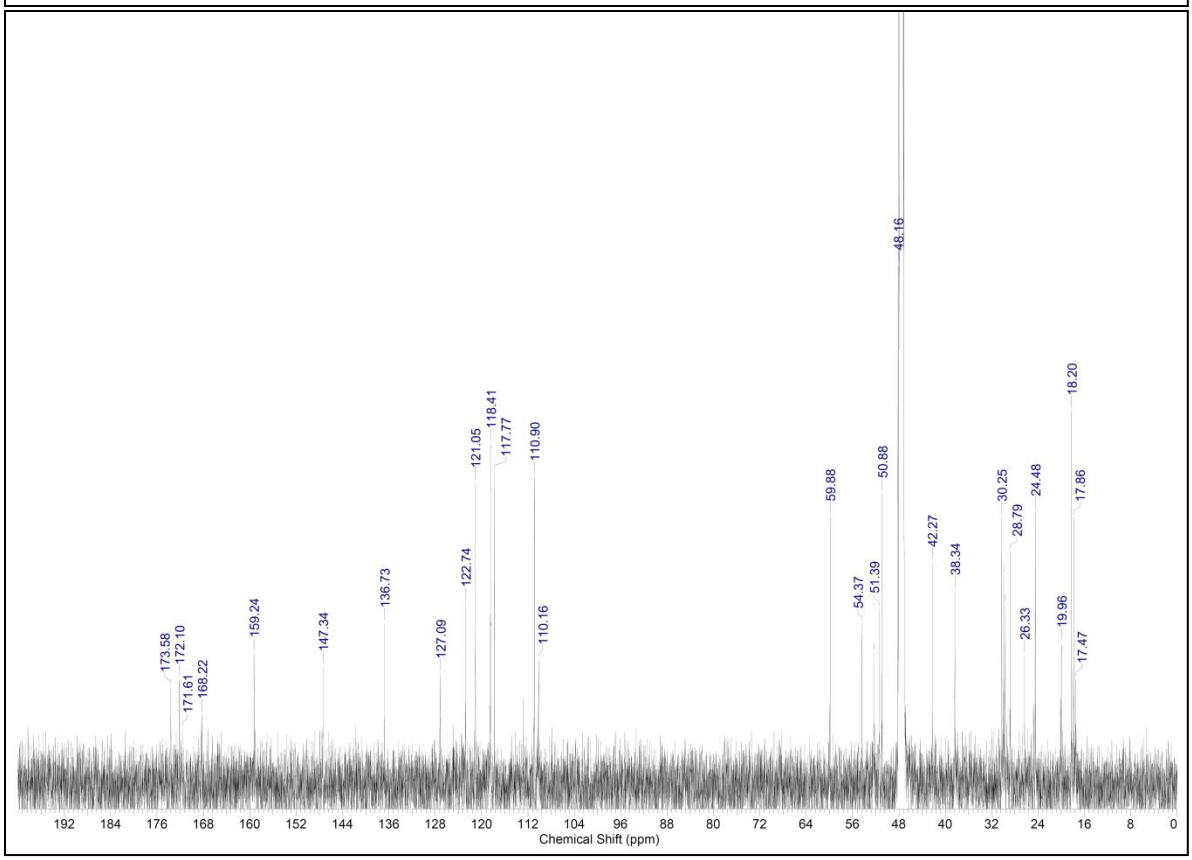
3.05
3.04
3.03
2.21
2.21
2.19
2.18
2.16
2.18
1.96
1.94
1.68
1.66
1.46
1.51
1.45
1.42
1.41
1.40
1.47 1.67 1.24 1.76 3.18 1.73
1.36 1.43
1.35 1.28 2.99 2.12 3.72 1.99
2.19 1.49 1.51 4.83 5.54 1.54 3.34 2.94
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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42.27
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38.34
122.74
136.73
159.24
147.34
127.09
110.16
54.37
51.39
26.33
19.96
17.47
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172.10
171.61
168.22
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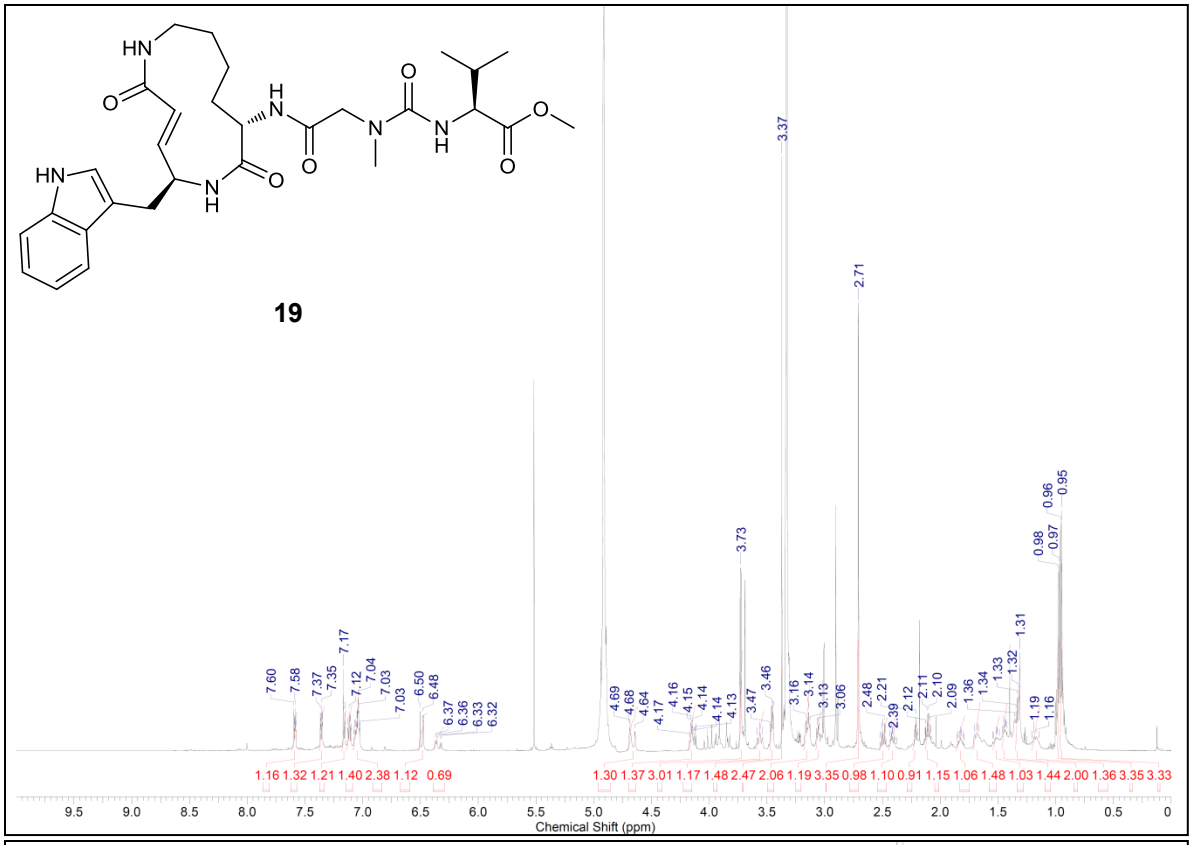
Chemical Shift (ppm)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HN
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19
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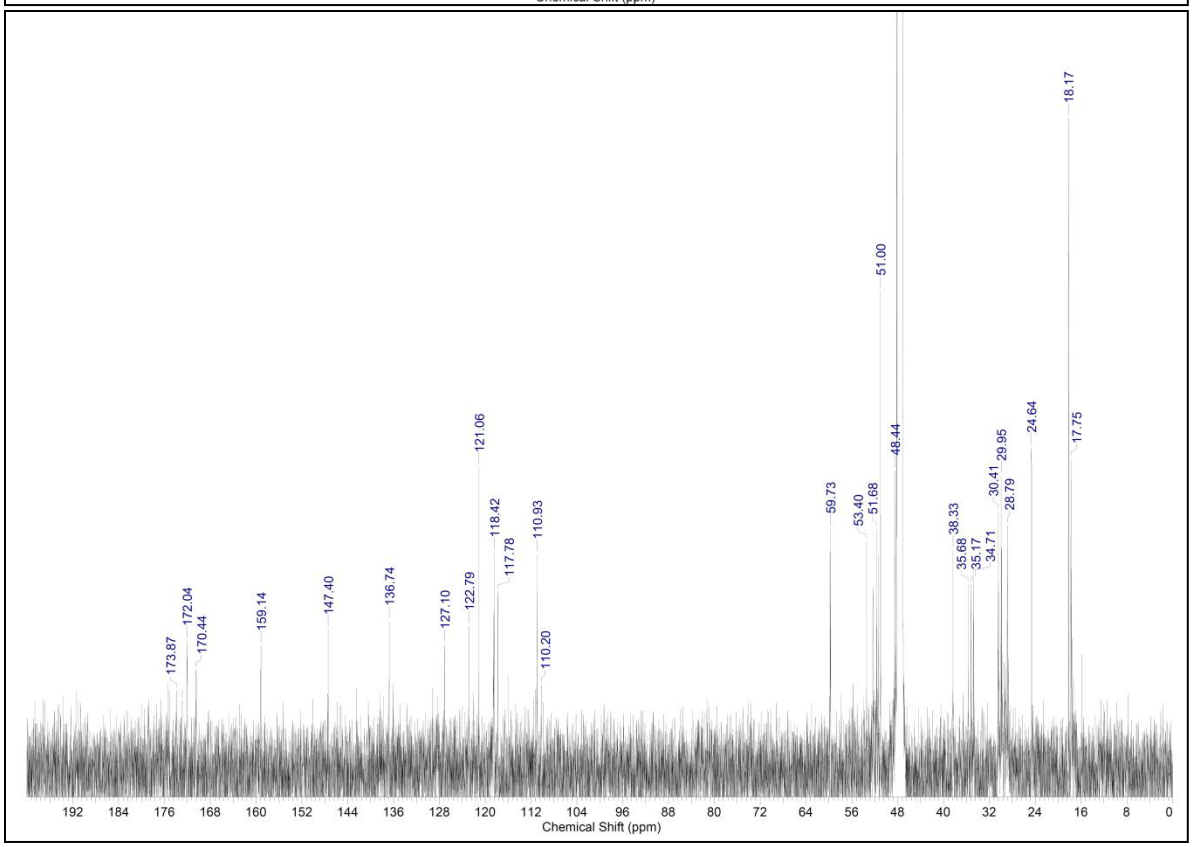
7.35
7.17
7.12
7.04
7.03
7.03
6.50
6.48
6.37
6.36
6.33
6.32
4.69
4.68
4.64
4.17
4.16
4.15
4.14
4.14
4.13
3.73
3.47
3.46
3.37
3.16
3.14
3.13
3.06
2.71
2.48
2.21
2.39
2.12
2.11
2.10
2.09
1.36
1.34
1.33
1.32
1.31
1.19
1.16
0.98
0.97
0.96
0.95
1.16 1.32 1.21 1.40 2.38 1.12 0.69
1.30 1.37 3.01 1.17 1.48 2.47 2.06 1.19 3.35 0.98 1.10 0.91 1.15 1.06 1.48 1.03 1.44 2.00 1.36 3.35 3.33
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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59.73
53.40
51.68
51.00
48.44
38.33
35.68
35.17
34.71
30.41
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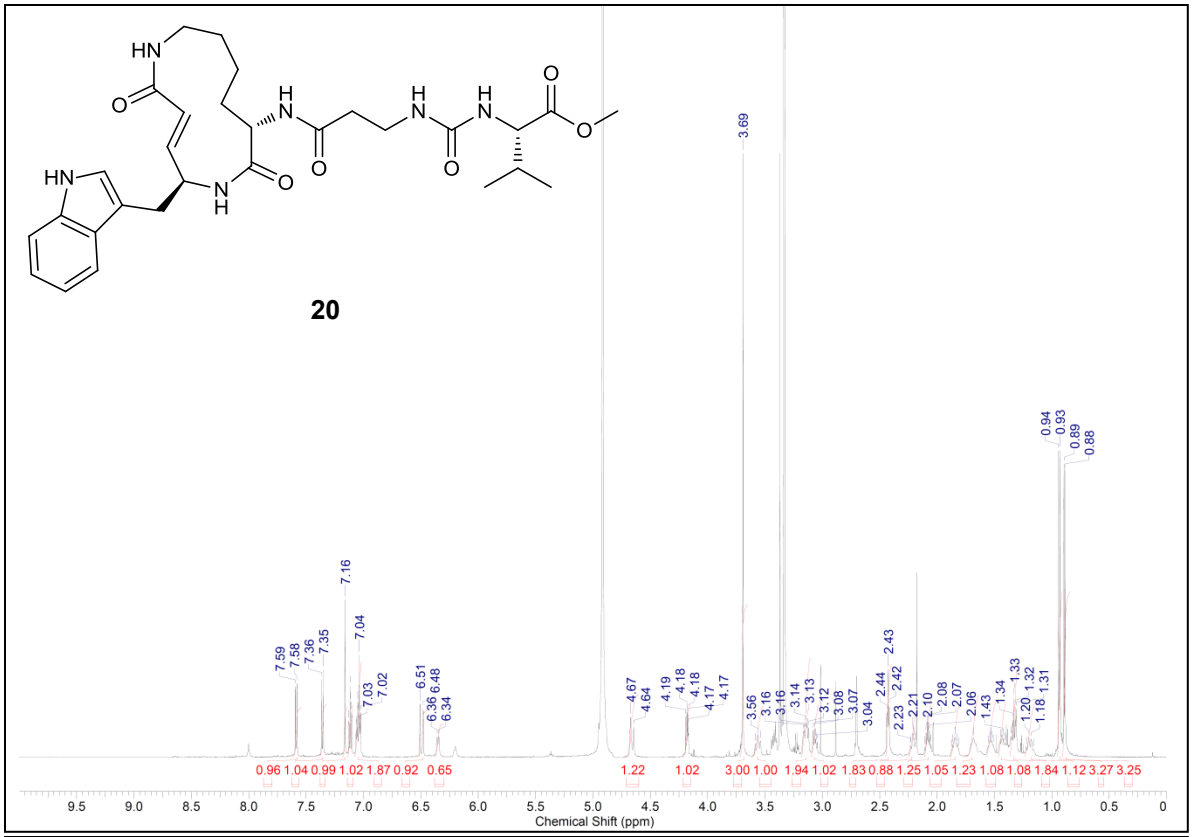
Chemical Shift (ppm)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N
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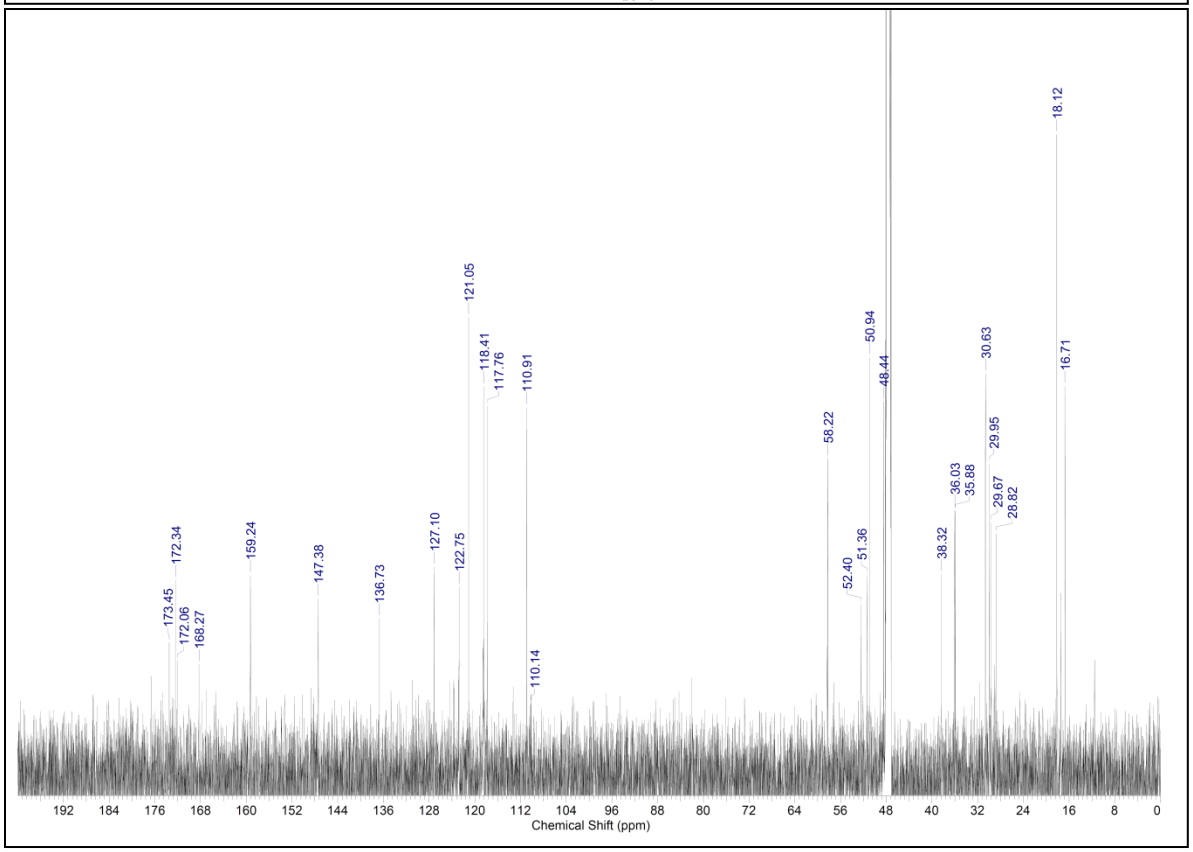
O
O
20
7.59
7.58
7.36
7.35
7.16
7.04
7.03
7.02
6.51
6.48
6.36
6.34
4.67
4.64
4.19
4.18
4.18
4.17
4.17
3.69
3.56
3.16
3.16
3.14
3.13
3.12
3.08
3.07
3.04
2.44
2.43
2.42
2.23
2.21
2.10
2.08
2.07
2.06
1.43
1.34
1.33
1.32
1.31
1.20
1.18
0.94
0.93
0.89
0.88
0.96 1.04 0.99 1.02 1.87 0.92 0.65
1.22
1.02
3.00 1.00 1.94 1.02 1.83 0.88 1.25 1.05 1.23 1.08 1.08 1.84 1.12 3.27 3.25
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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172.06
168.27
159.24
147.38
136.73
127.10
122.75
121.05
118.41
117.76
110.91
110.14
58.22
52.40
51.36
50.94
48.44
38.32
36.03
35.88
30.63
29.95
29.67
28.82
18.12
16.71
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)