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Supporting Information

Development of the Safe and Broad-Spectrum Aldehyde and Ketoamide Mpro inhibitors Derived from the Constrained α, γ-AA Peptide Scaffold

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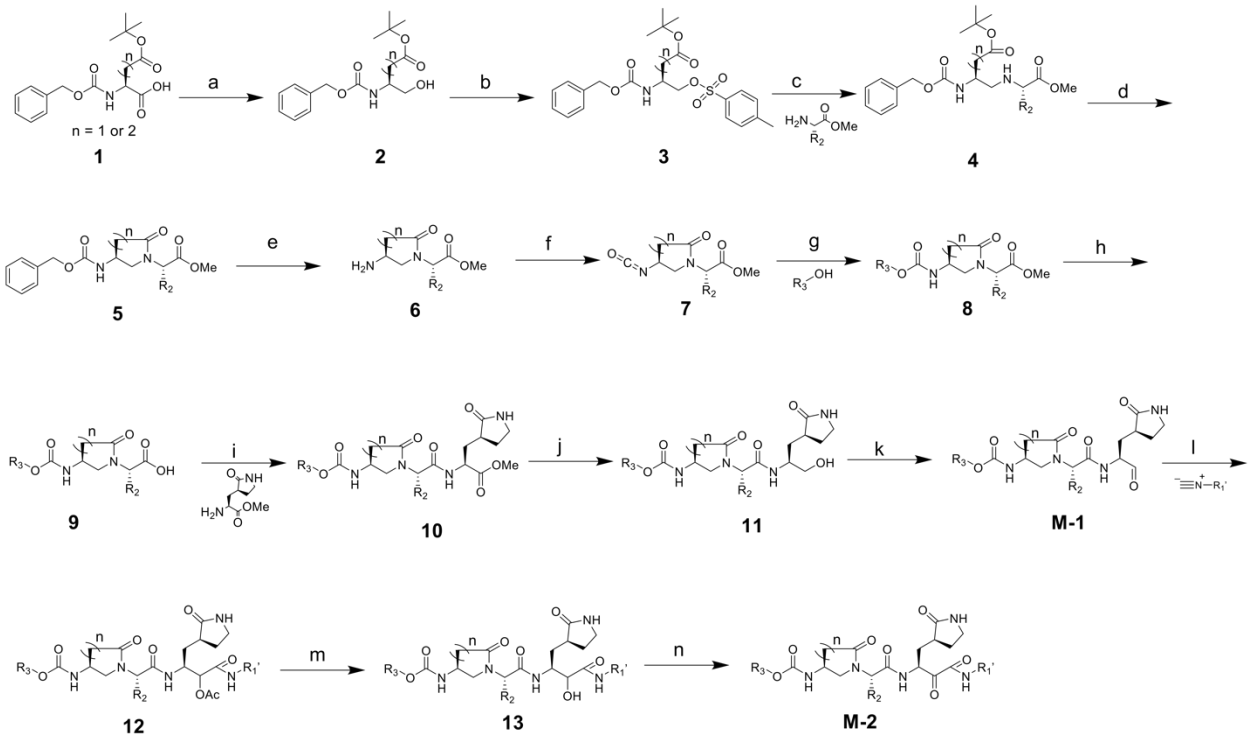


Scheme 1. Synthesis of α, γ-AApeptide based aldehydes and ketoamides. Reaction conditions: (a) isobutyl chloroformate, NMM, $NaBH_4$, THF; (b) TosCl, TEA, DCM; (c) K_2CO_3, NaI, ACN; (d) TFA, DCM, HOBT, DIC, DIPEA, DMF; (e) Pd/C, EA, MeOH; (f) trichloromethyl chloroformate, dioxane; (g) R_3-OH, TEA, ACN; (h) LiOH, THF/H_2O; (i) 10, HATU, DMF; (j) $NaBH_4$, MeOH; (k) Dess-Martin periodinane, $NaHCO_3$, DCM; (l) isocyanide, AcOH, DCM; (m) LiOH, THF/H2O; (n) DMP, $NaHCO_3$, DCM

Chemical synthesis of α, γ-AApeptide based aldehydes and ketoamides. General procedure: Reagents were utilized without being purified after being purchased from commercial sources. On silica gel with a mesh size ranging from 230 to 430, flash column chromatography was carried out.

F-254 (0.2mm thickness) was used for analytic thin-layer chromatography (TLC). ^{1}H NMR, ^{13}C NMR spectra were recorded on Bruker Avance NEO-600 MHz spectrometers. Chemical shifts were reported relative to $CDCl_3$ (δ 7.26 ppm) for ^{1}H and $CDCl_3$ (δ 77.00 ppm) for ^{13}C. the mass of each compound was confirmed by high-resolution mass spectrometry detected by Agilent 6220 using electrospray ionization time-of-flight (ESI-QTOF). All the compounds were purified by Waters Breeze 2 HPLC system. The purity of all compounds tested biologically was ≥ 95%.

The synthesis of compound 2: To a solution of Cbz protected glutamic acid or asparic acid (5.0 g, 15.47mmol) in THF (100mL) under argon was added isobutyl chloroformate (2.1 mL, 16.24 mmol) and 4-Methylmorpholine (18.7 mL, 17.017 mml). The mixture was stirred at -20 ℃ for 20 min. The resulting white suspension was t hen filtered off and the filtrate was added by the solution of $NaBH_4$ in water at -20 ^{o}C.The reaction was left overnight and then 1 M $KHSO_4$ solution was added. The resulting aqueous layer was extracted by EtOAc. The organic layer were combined, and washed two times with 1 M $KHSO_4$, two times with saturated $NaHCO_3$ solution, one time with brine, dried over Na_2SO_4 and then concentraed to give the oil. The residue was purified by flash chromatography to afforded compound **2** as the light-yellow oil.

The synthesis of compound 3: Compound **2** (4 g, 12.4 mmol) was dissolved in CH_2Cl_2 first, and the triethanolamine (6 mL, 62 mmol) was added. The resulting solution was cooled to 0 ^{o}C using an ice bath. Next, 4-Toluenesulfonyl chloride (3.54 g, 18.6 mmol) was added in one portion. The reaction was quenched after 12 h with HCl and extracted with CH_2Cl_2. The organic layer was dried with Na_2SO_4 and then concentraed to get dark-yellow oil. The crude was purified by column to give yellow oil.

The synthesis of compound 4: To an oven dried flask equipped with a stir bar and reflux condenser, L-Leucinate hydrochloride (4.6 g, 25.2 mmol) or L-Phenylalanine-hydrochloride (5.4

g, 25.2 mmol), sodium iodide (1.56 g, 10.5 mmol), potassium carbonate (10.1g, 73.5mmol) in acetonitrile were added under nitrogen. Before adding compound **3** dropwise, the resulting mixture was stirred for one hour and allowed to heat to 90 ^{o}C. The reaction was allowed to mix for 12 h. After that, the reaction was cooled and diluted by CH_2Cl_2. The suspension was flitered to remove solid precipitate and the remaining supermatant was concentrated by vacuo. The crude oil was purified by column to get white solid.

The synthesis of compound 5: The compound **4** (2 g, 4.4 mmol) was dissolved in 30 mL of CH_2Cl_2 first, and then 30 mL trifluoroacetic acid was added. The reaction was stirred at room temprature for 1.5 h and concentrated in vacuo to get the oil-like intermediate. This intermidiate (2 g, 5.07 mmol) was dissloved in N,N-Dimethylmethanamide (DMF), DIPEA (8 mL, 50.7 mmol) was added inside and the mixture was cooled to 0 ℃. HOBT (1.56 g, 10.14 mmol) and DIC (1.6 mL, 5.07 mmol) were added to the solotuion and then stirred at room temprature for overnight. The reaction micture was then quenched by 1 M HCl solution and extracted by EtOAc for two times. The combined organic layer was wahed by saturated $NaHCO_3$ solution and 1 M HCl for two times. The organic layer was dried by Na_2SO_4 and concentrated by vacuo. The crude oil was purified by flash column to get white solid.

The synthesis of compound 6: To a solution of compound **5** (2 g, 5.3 mmol) in 1:1 MeOH/EtOAc was added 10 % palladium on carbon (0.2 g). The resultant heterogeneous solution was put under an atmosphere of hydrogen and was stirred overnight. After that, the mixture was filtered and the filtrate was concentrated in vacuo to afford the colored crude which was then purified by flash column to get the white solid.

The synthesis of compound 7: The compound **6** (1 g, 4.13 mmol) was palced in an flask and dried overnight on the vacuum pump. Then the flask was flushed with nitrogen, and dried dioxane was

added followed by trichloromethyl chloroformate (1.23 g, 6.2 mmol), the reaction was refluxed for 10 h. The solvent was removed on the rotary evaporator first, and the residue was vacum distilled to yield pure compound **7** as the colorless oil. The crude can be used to the next step without further purification.

The synthesis of compound 8: A solution of alcohol (0.53 g, 3.7 mmol) in dry ACN was treated with triethylamine (10.3 mL, 7.4 mmol), the compound **7** (1 g, 3.7 mmol) was added. The resluting mixture was refluxed for 2 h with stirring and then allowed to cool to room temprature. The solvent was removed by vacum and the residue was dissloved inside of EtOAc. The organic layer was washed with 1 M HCl and brine and then dried with anhydrous Na_2SO_4, filtered, and concentrated to afford a crude product as the yellow-colored oil. The purification of the crude was performed by flash column to get the white solid.

The synthesis of compound 9: To a solution of compound **8** (1 g, 2.43 mmol) in 1:1 H_2O/THF, the LiOH (0.26 g, 6.08 mmol) was added. The reaction was stirred overnight at room temporature. The mixture was made acidic with 1 M HCl solution and extracted with EtOAc for three times. The organic layer was washed with brine, dried and filtered. The crude product was used in the next step without further purification.

The synthesis of compound 10: HATU (0.6 g, 1.6 mmol) was added to the solution of compound **9** (0.4 g, 1.06 mmol) in DMF under 0 ^oC, the mixture was stirred for 10 minutes, and then DIPEA (0.9 mL, 5.3 mmol) and Methyl (S)-2-((tert-butoxycarbonyl)amino)-3-((S)-2-oxopyrrolidin-3-yl)propanoate (0.24 g, 1.06 mmol) were added. The reaction was stirred at room temprature overnight. The reaction mixture was then quenched with 1M HCl and extracted with EtOAc for two times. The organic layer was washed with 1 M HCl, saturated $NaHCO_3$ solution and brine

sequentially, dried by Na_2SO_4 and concentrated by vacuo to get the yellow-color oil. The resultant residue was purified by flash column to get the white solid.

The synthesis of the compound 11: The compound **10** (0.5 g, 0.9 mmol) was dissloved in anhydrous CH_2Cl_2 first, a solution of $LiBH_4$ in anhydrous THF was added at 0 ℃. The resulting reaction solution was stirred at the same temperature for 3 h. Then a solution of saturated NH_4Cl was added dropwise to quench the reaction. The organic layer was washed with brine for two times and evaporate to dryness by the rotavapor to get the oil product. The crude was used in the next step without further purification.

The synthesis of compound M-1: The compound **11** (0.2 g, 0.4 mmol) was dissolved in CH_2Cl_2, then the Dess-martin periodinane (0.19 g, 0.44 mmol) and $NaHCO_3$ (0.04 g, 0.44 mmol) were added, and the reaction mixture was stirred at room temperature overnight. Then the reaction was quenched with saturated $NaHCO_3$ solution. The organic layer was concentrated and purified by Waters HPLC system to get the white solid.

The synthesis of compound 12: To a solution of compound **M-1** (0.1 g, 0.2 mmol) in CH_2Cl_2, acetic acid (0.017 mL, 0.3 mmol) and isocyanide (0.3 g, 0.24 mmol) or cyclopropyl cyanide (0.16 g, 0.24 mmol) were added successively. The reaction was stirred at room temperature for 24 h and diluted with DCM and poured into brine. After two layers were separated, the organic layer was concentrated and purified by the flash column for the further use.

The synthesis of compound 13: 1 M NaOH solution was added to a solution of compound **12** in MeOH (20mL). The reaction was stirred at 20 ℃ for 1h until no reactant left. Then the solvent was removed by vacuo. The residue was dissolved in EtOAc and washed with 1M HCl solution. The organic phase was dried and concentrated to dryness to generate the product as white solid, which can be used directly in the next step.

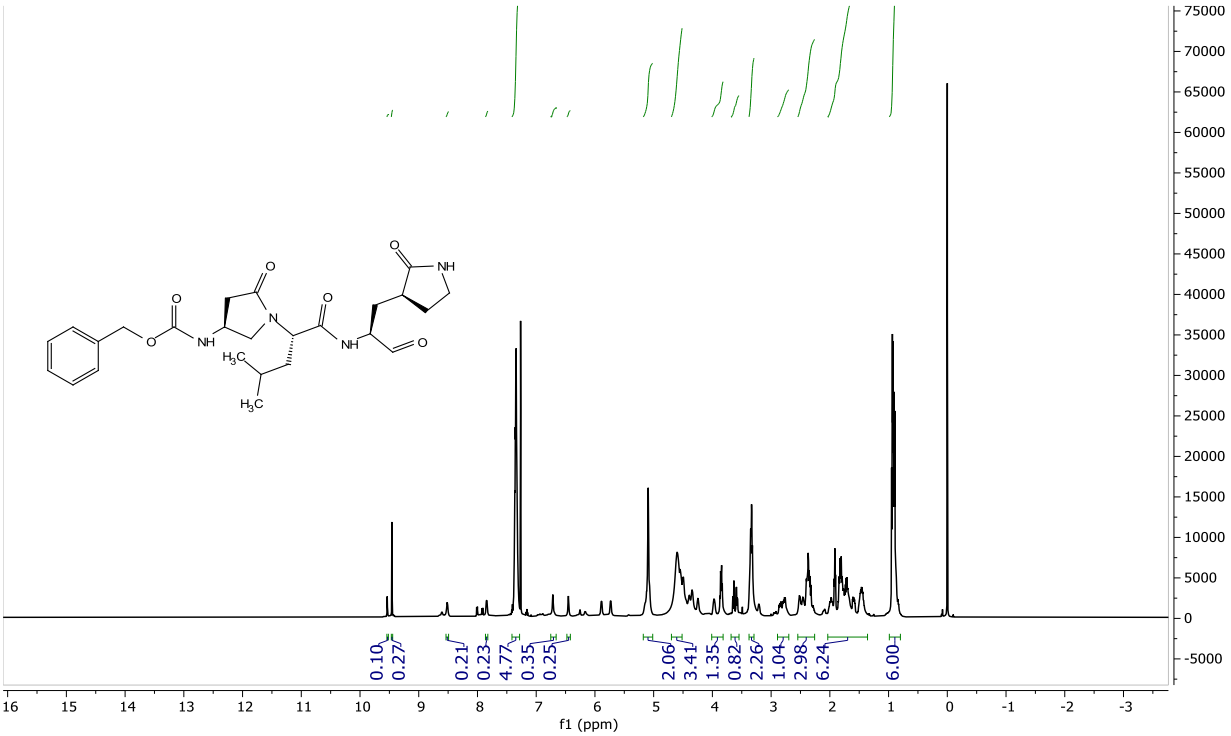
The synthesis of compound M-2: Compound **13** (0.1 g, 1.57 mmol) was dissolved in CH_2Cl_2, then the Dess-martin periodinane (0.08 g, 1.88 mmol) and $NaHCO_3$ (0.19 g, 0.44 mmol) were added, and the reaction mixture was stirred at room temperature overnight. Then the reaction was quenched with saturated $NaHCO_3$ solution. The organic layer was concentrated and purified by Waters HPLC system to get the white solid.

Table S1. Crystallographic statistics

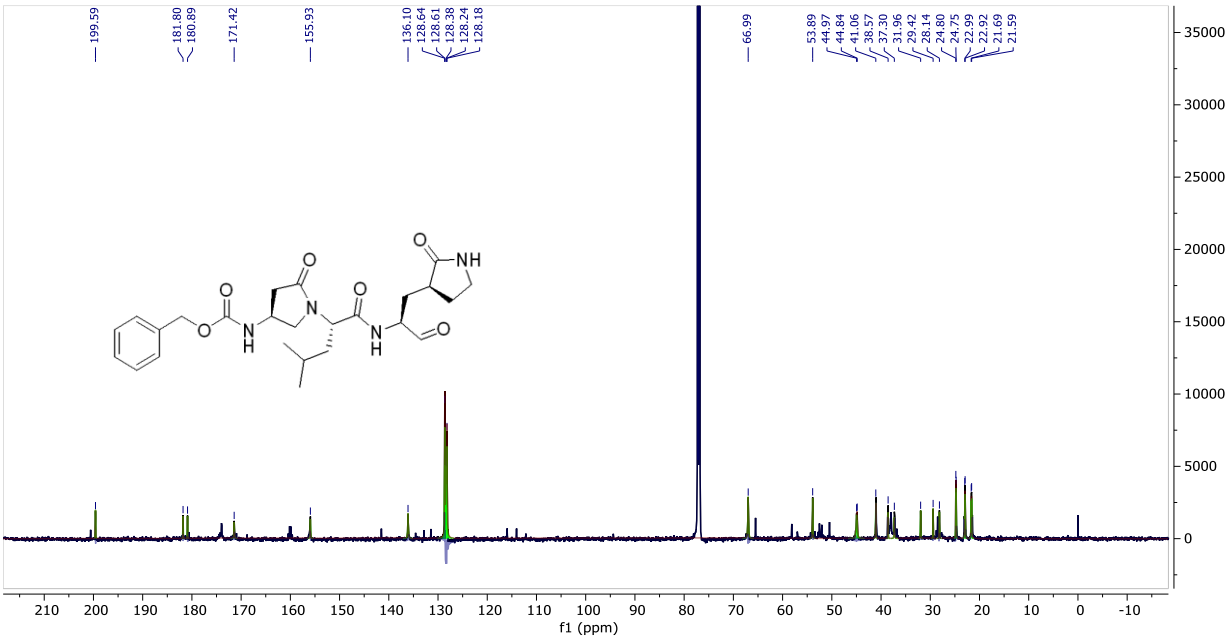
PDB ID	**8DZB**	**8DZC**
Protein	SARS-CoV2 M^{pro}	SARS-CoV2 M^{pro}
Inhibitor	11	17
Data Collection		
Space Group	I 1 2 1	I 1 2 1
Cell Dimensions		
a, b, c (Å)	45.78, 52.55, 113.54	45.75, 53.29, 113.23
α, β, γ (°)	90.00, 100.23, 90.00	90.00, 101.28, 90.00
Resolution (Å)	47.55 - 1.85	48.04 – 2.20
	(1.89 - 1.85)	(2.27 – 2.20)
R_{merge}	0.083 (0.407)	0.056 (0.164)
<I>/σ<I>	9.8 (3.7)	15.7 (6.7)
Completeness (%)	94.7 (97.2)	97.8 (98.7)
Redundancy	3.7 (3.7)	4.4 (4.4)
Refinement		
Resolution (Å)	39.42 - 1.85	48.04 - 2.20
	(1.916 - 1.85)	(2.279 - 2.20)
No. reflections/free	21486 / 1077	13371/ 668
R_{work}/R_{free}	0.187 / 0.211	0.214 / 0.237
Clashscore	2.99	5.10
No. Atoms		
Overall	2471	2423
Protein	2340	2351
Ligand/Ion	47	50
Water	84	22
B-Factors ($Å^2$)		
Overall	21.09	39.10
Protein	21.00	38.92
Ligand/Ion	22.93	52.04
Solvent	22.52	28.94
RMS Deviations		
Bond Lengths (Å)	0.015	0.014
Bond Angles (°)	1.88	1.88
Ramachandran Favored (%)	98.01	96.35
Ramachandran Allowed (%)	1.66	3.32
Ramachandran Outliers (%)	0.33	0.33
Rotameric Outliers (%)	1.15	3.83

HPLC trace, ^{1}H-NMR and ^{13}C-NMR of the inhibitors

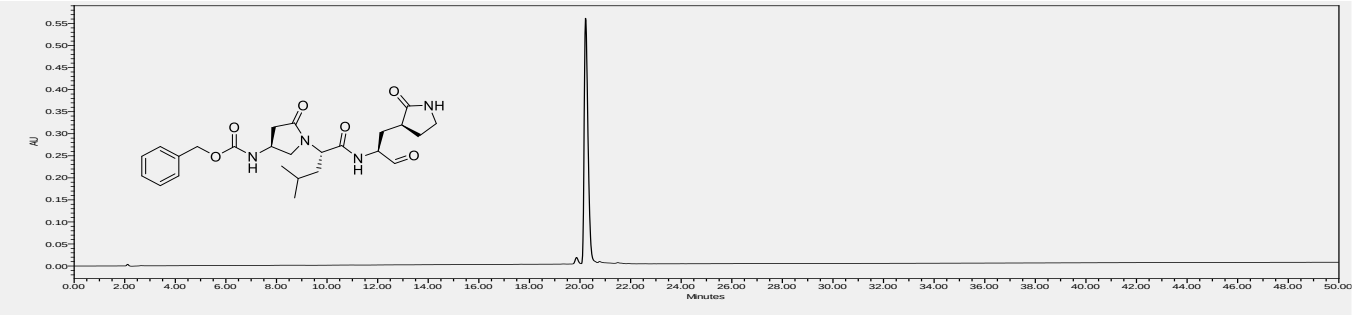
Compound M-1-1, ^{1}H NMR (600 MHz, $CDCl_3$)



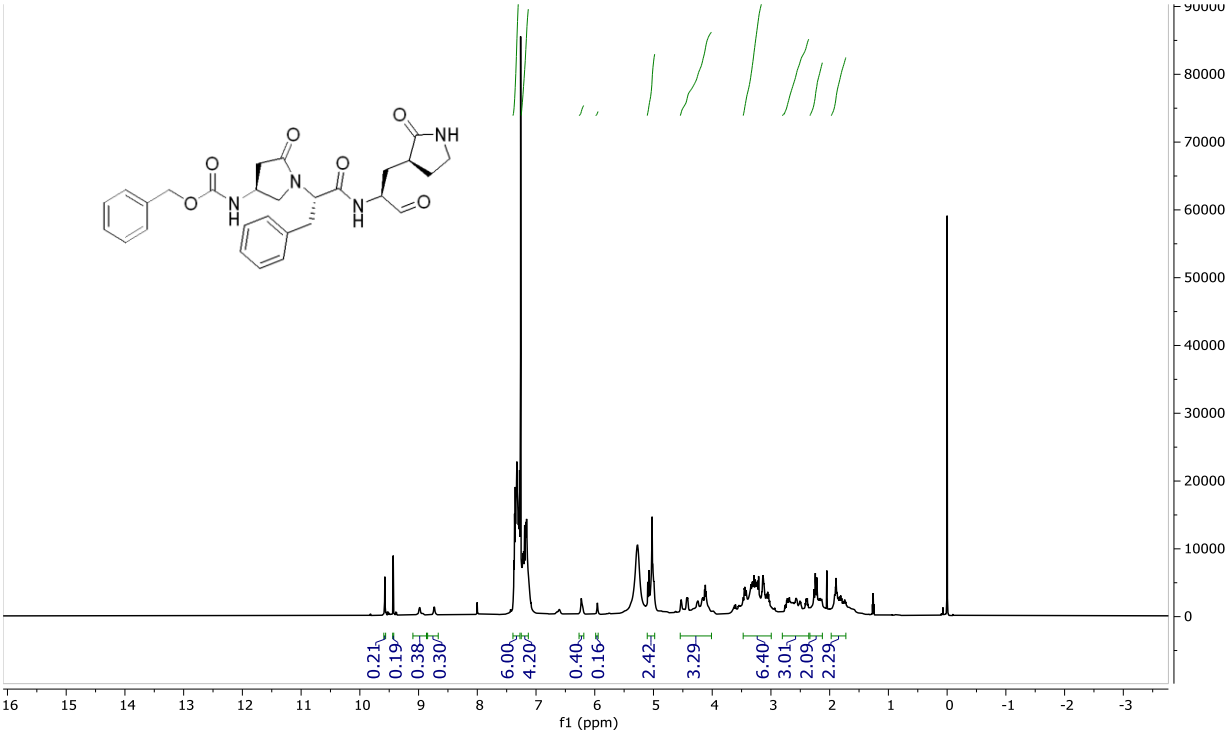
Compound M-1-1, ^{13}C NMR (150 MHz, $CDCl_3$)



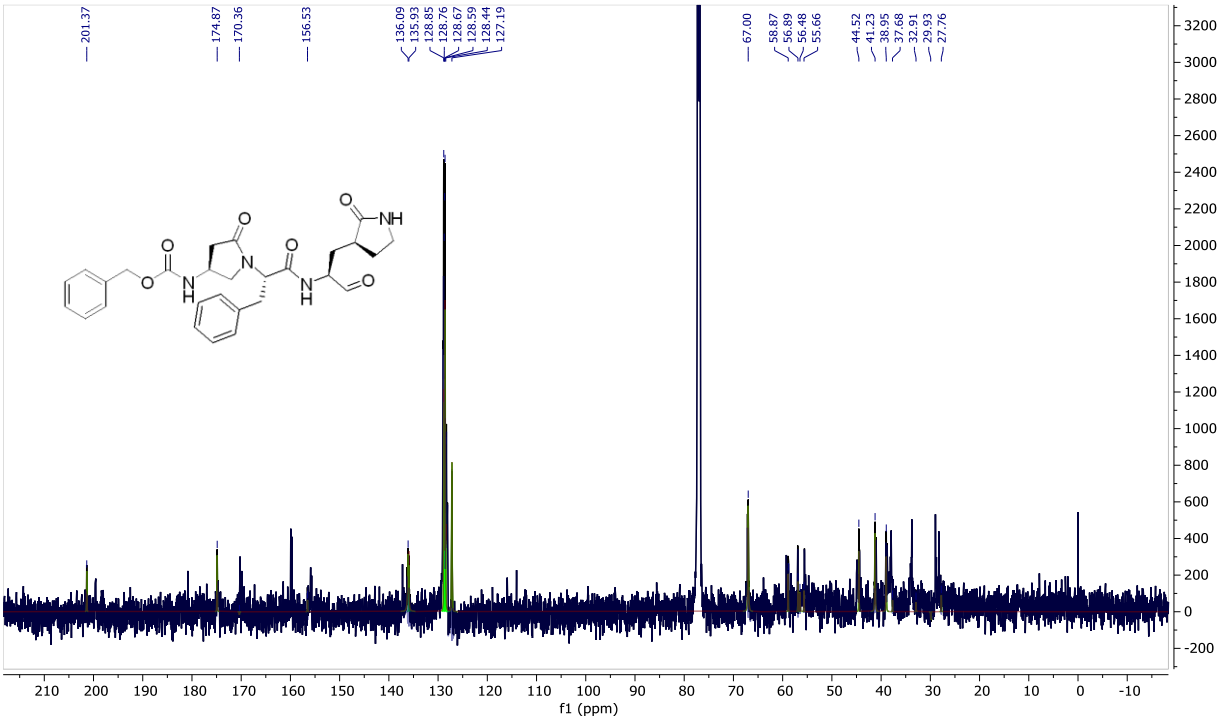
Compound M-1-1: HPLC Spectrum



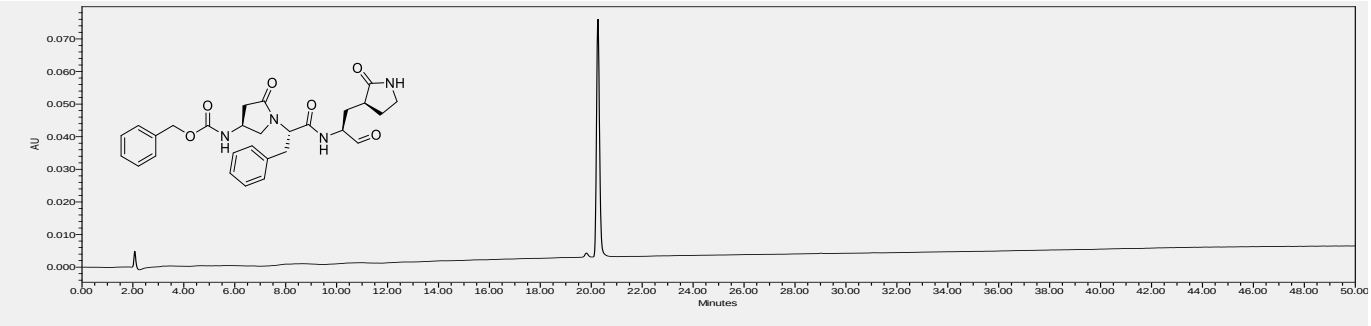
Compound M-1-2, ^{1}H NMR (600 MHz, $CDCl_3$)



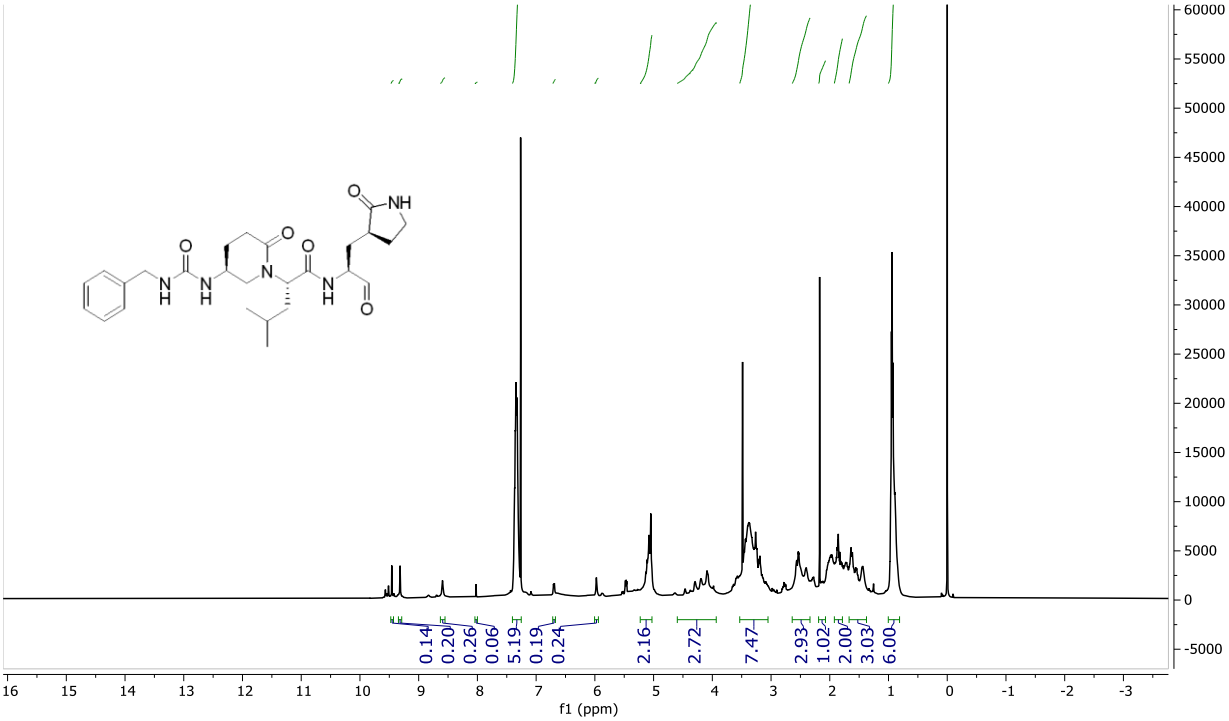
Compound M-1-2, ^{13}C NMR (150 MHz, $CDCl_3$)



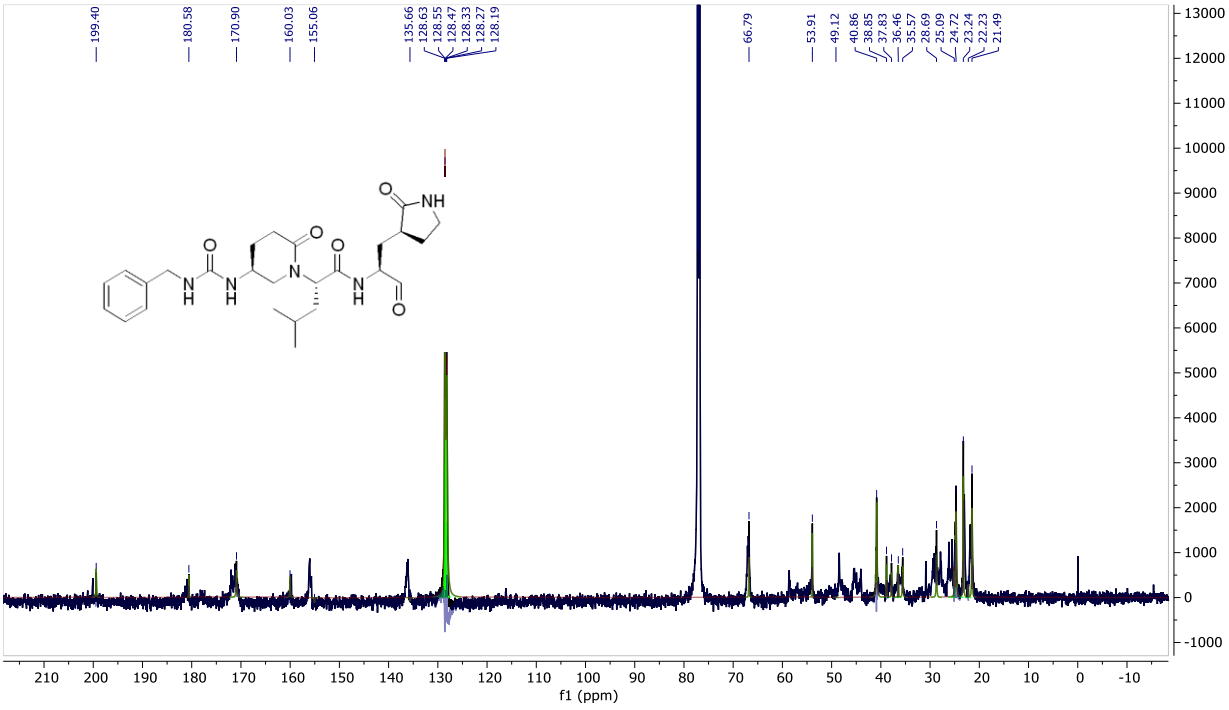
Compound M-1-2: HPLC Spectrum



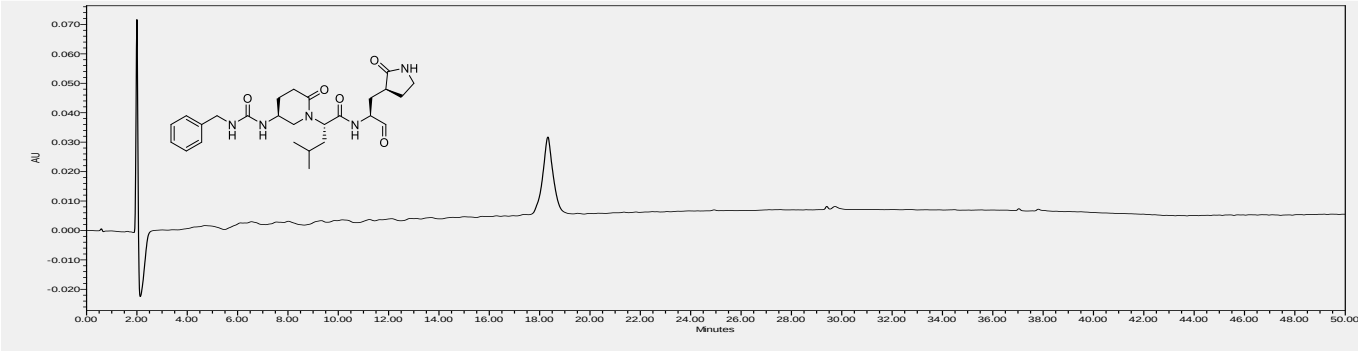
Compound M-1-3, ^{1}H NMR (600 MHz, $CDCl_3$)



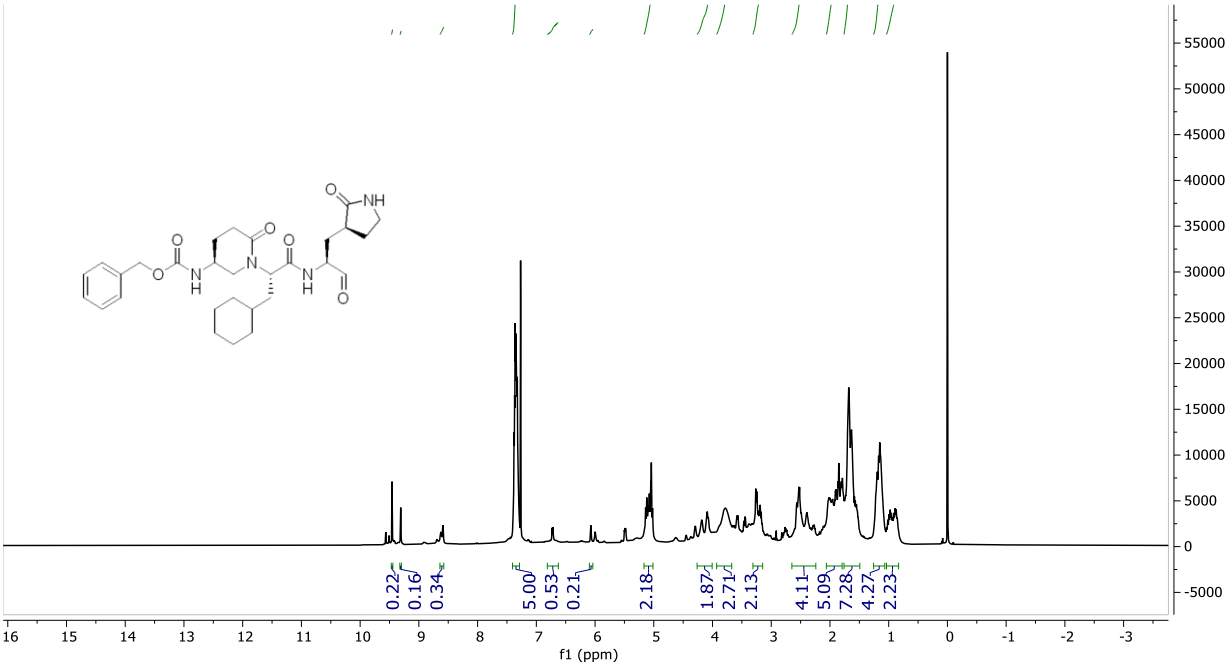
Compound M-1-3, ^{13}C NMR (150 MHz, $CDCl_3$)



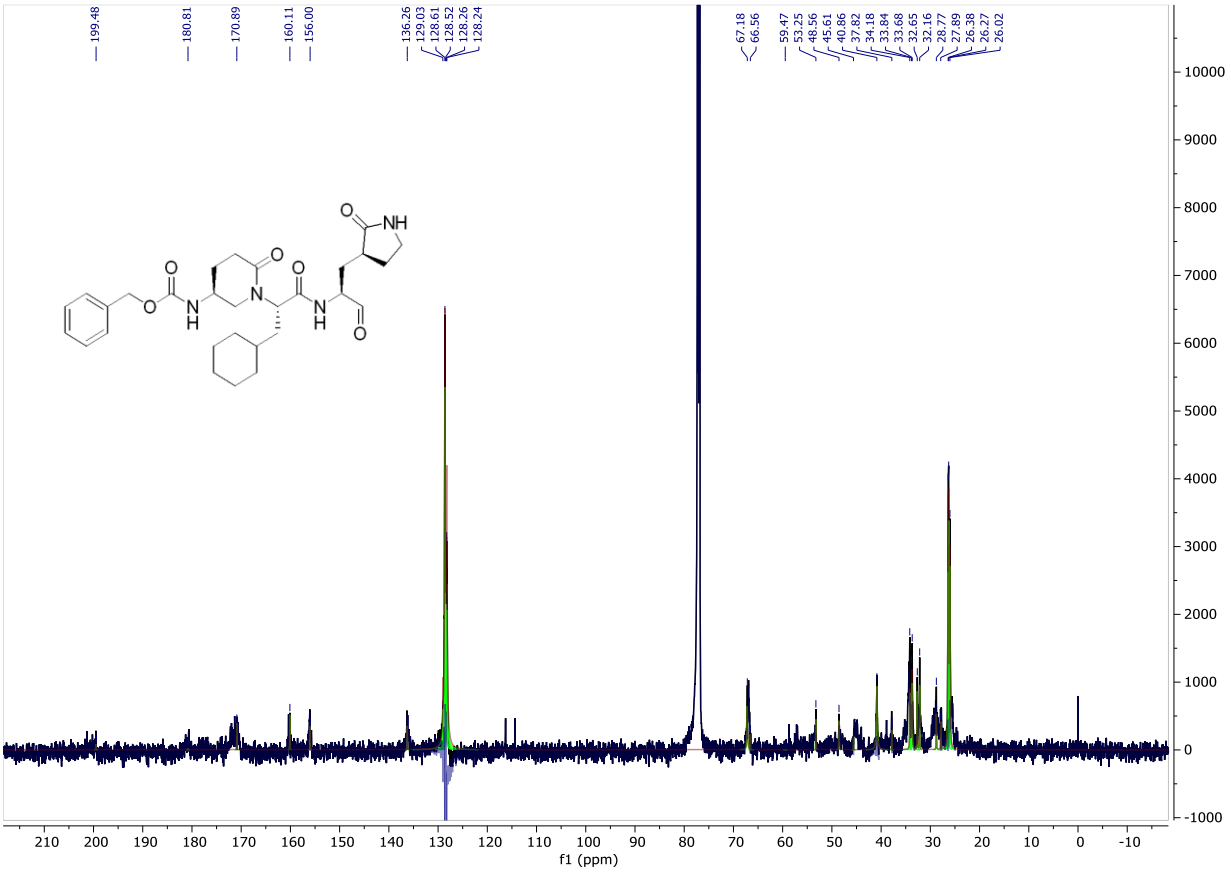
Compound M-1-3: HPLC Spectrum



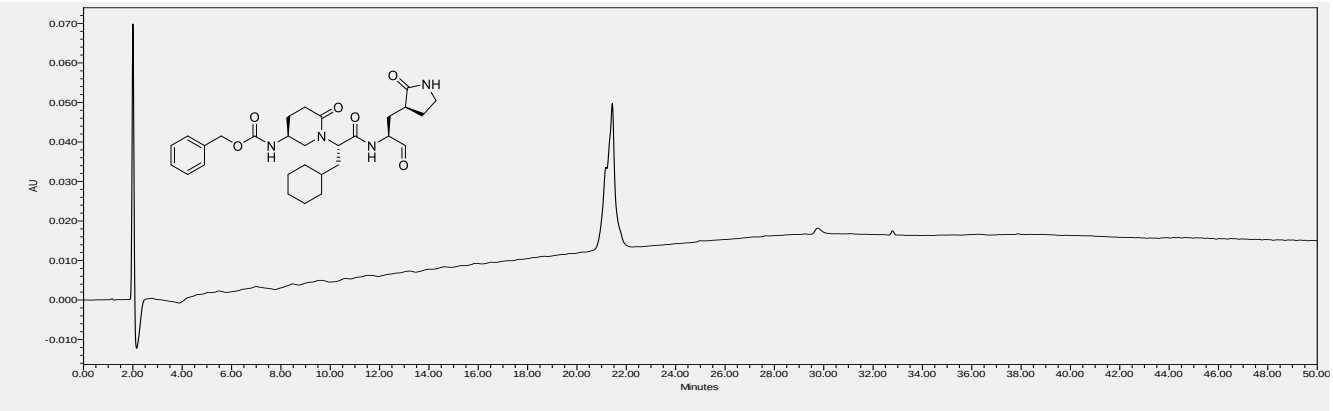
Compound M-1-4, ^{1}H NMR (600 MHz, $CDCl_3$)



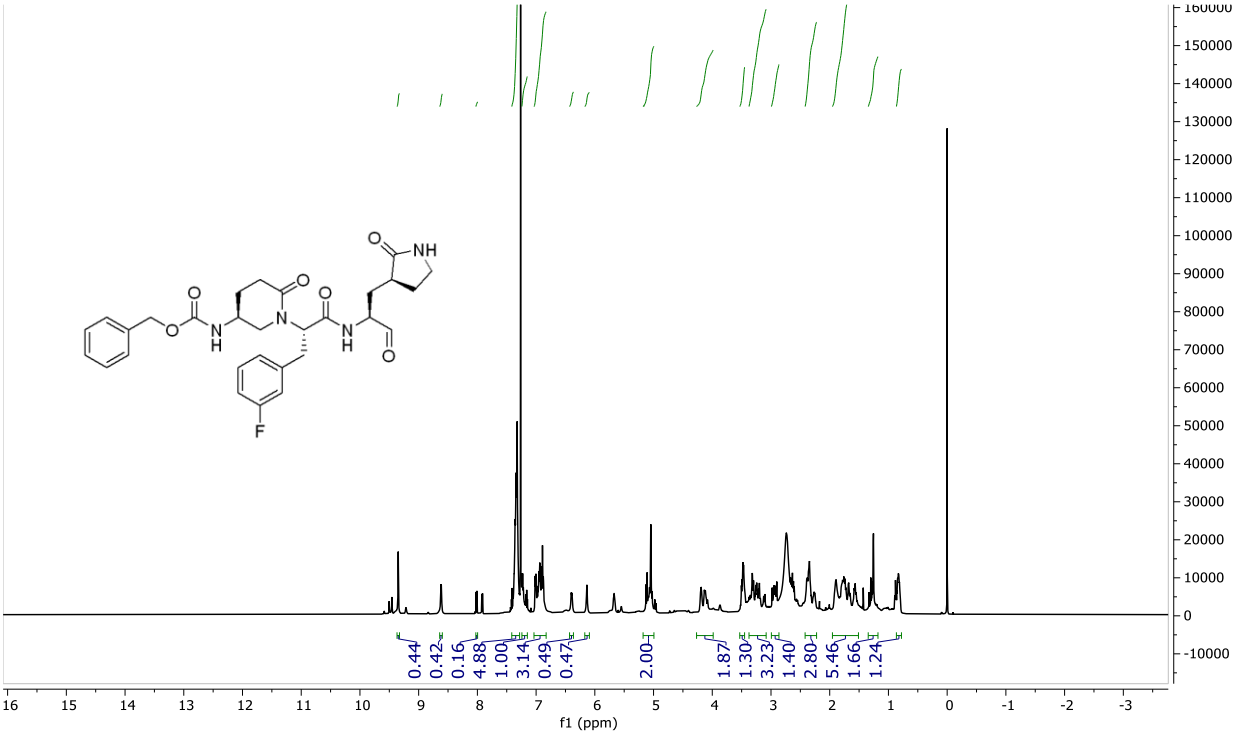
Compound M-1-4, ^{13}C NMR (150 MHz, $CDCl_3$)



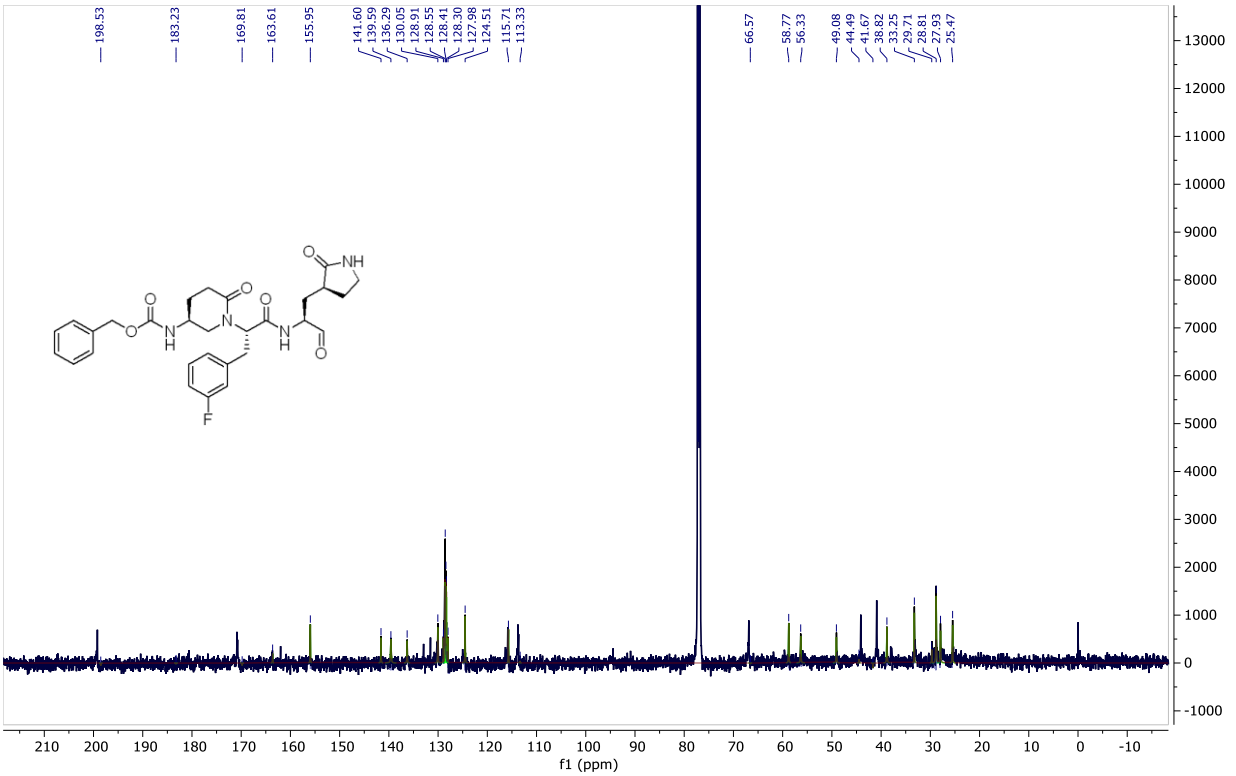
Compound M-1-4: HPLC Spectrum



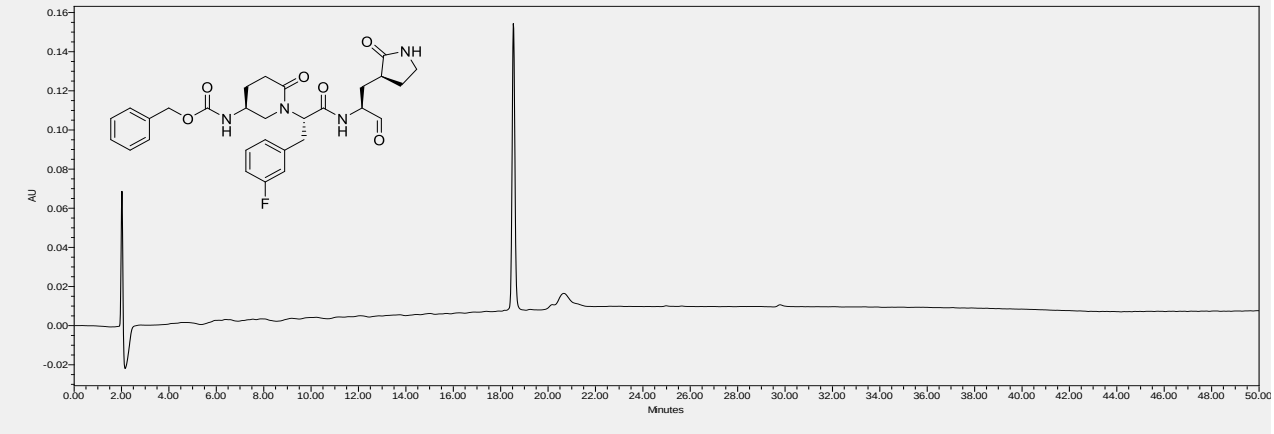
Compound M-1-5, ^{1}H NMR (600 MHz, $CDCl_3$)



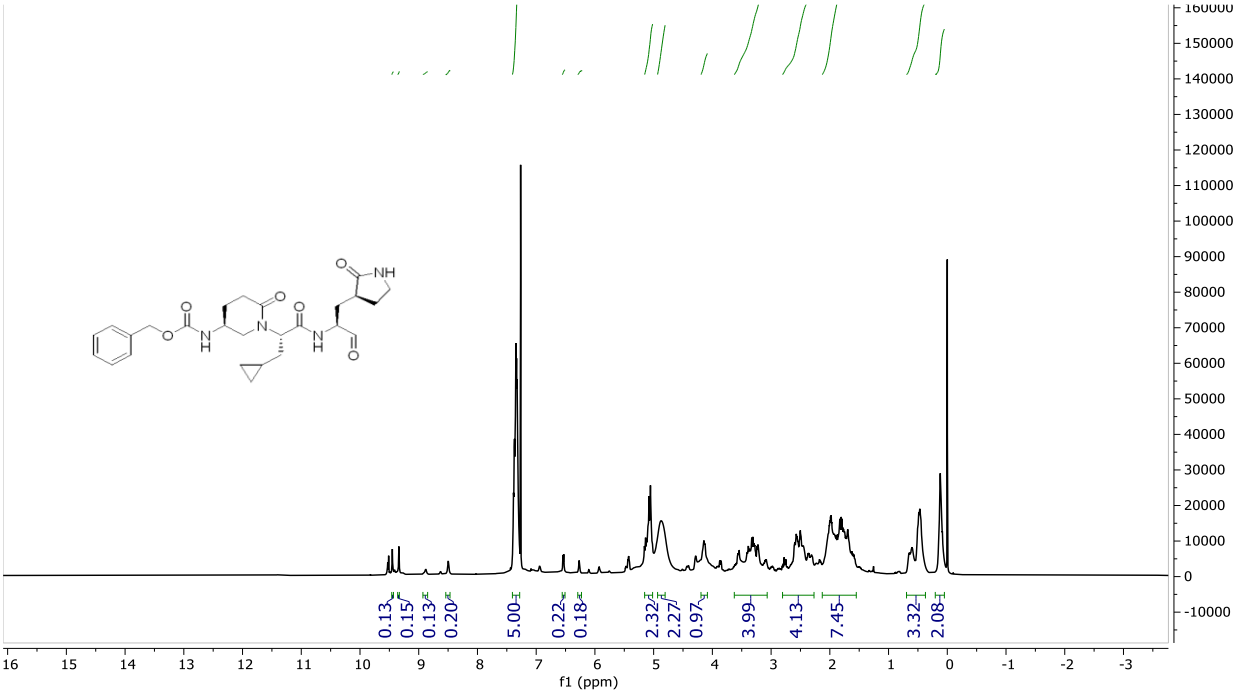
Compound M-1-5, ^{13}C NMR (150 MHz, $CDCl_3$)



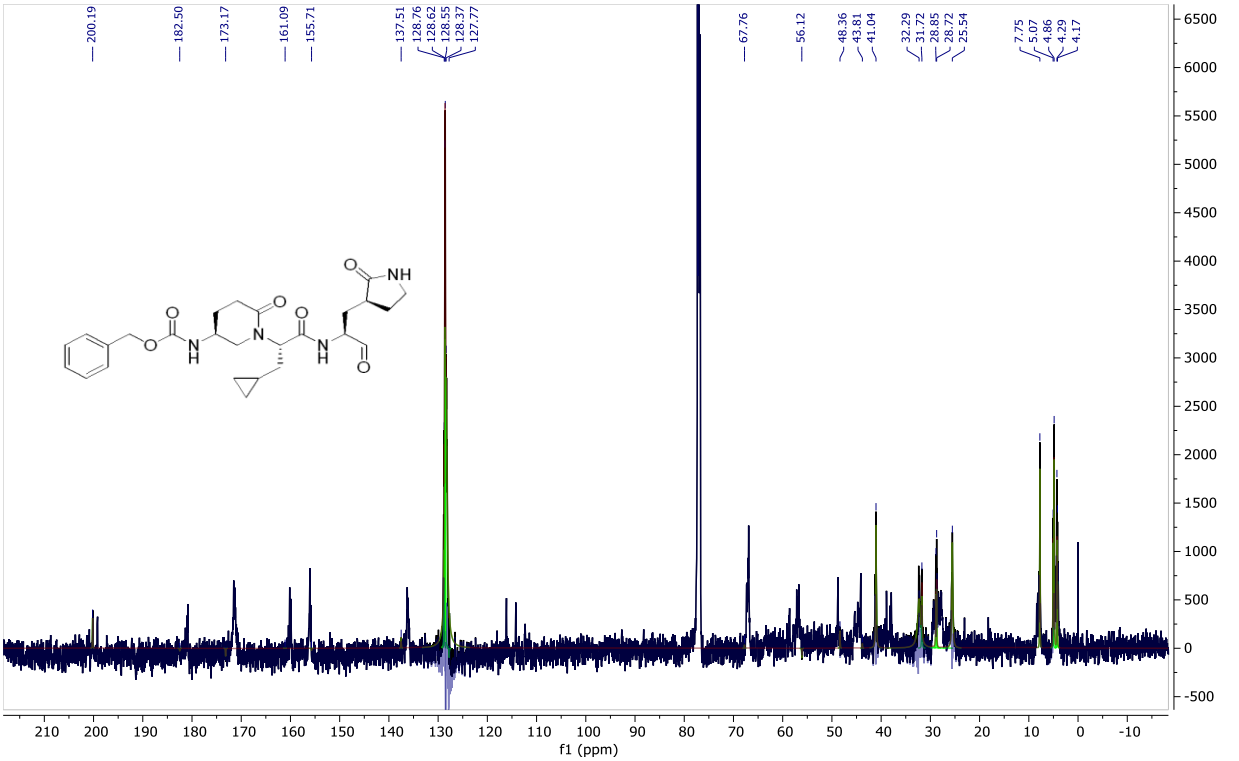
Compound M-1-5: HPLC Spectrum



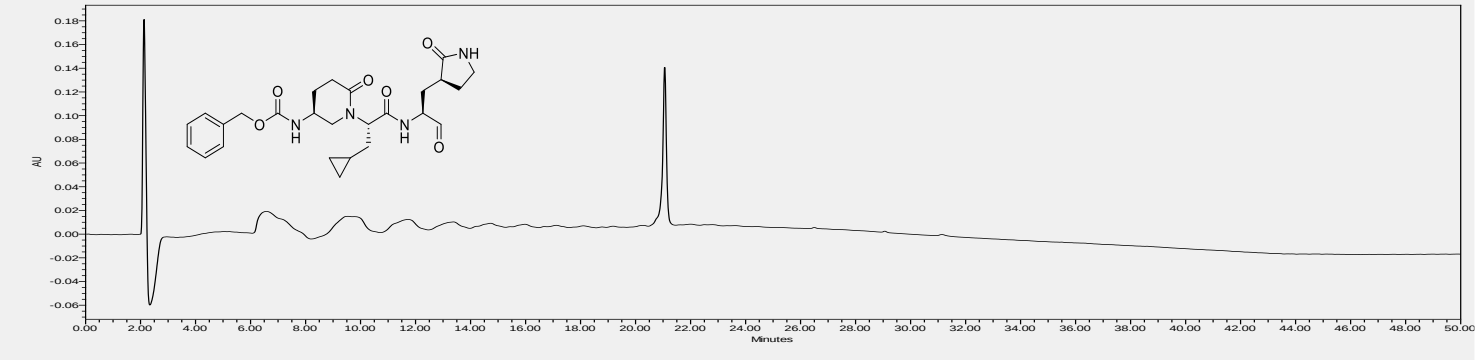
Compound M-1-6, ^{1}H NMR (600 MHz, $CDCl_3$)



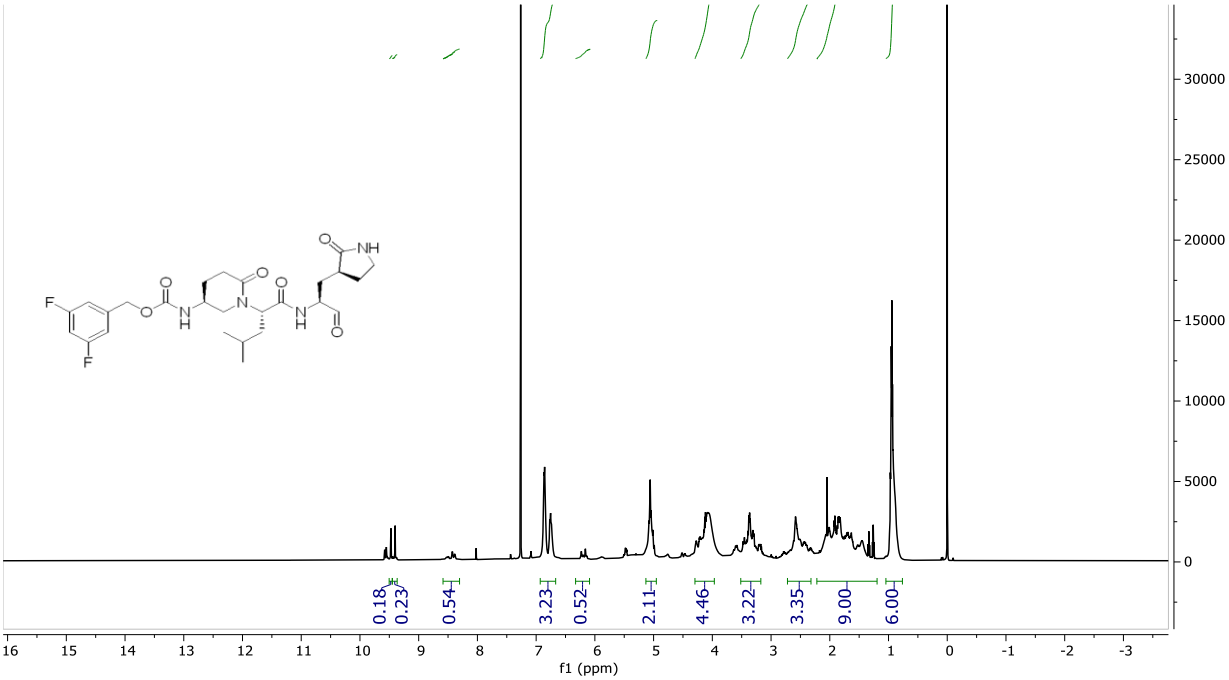
Compound M-1-6, ^{13}C NMR (150 MHz, $CDCl_3$)



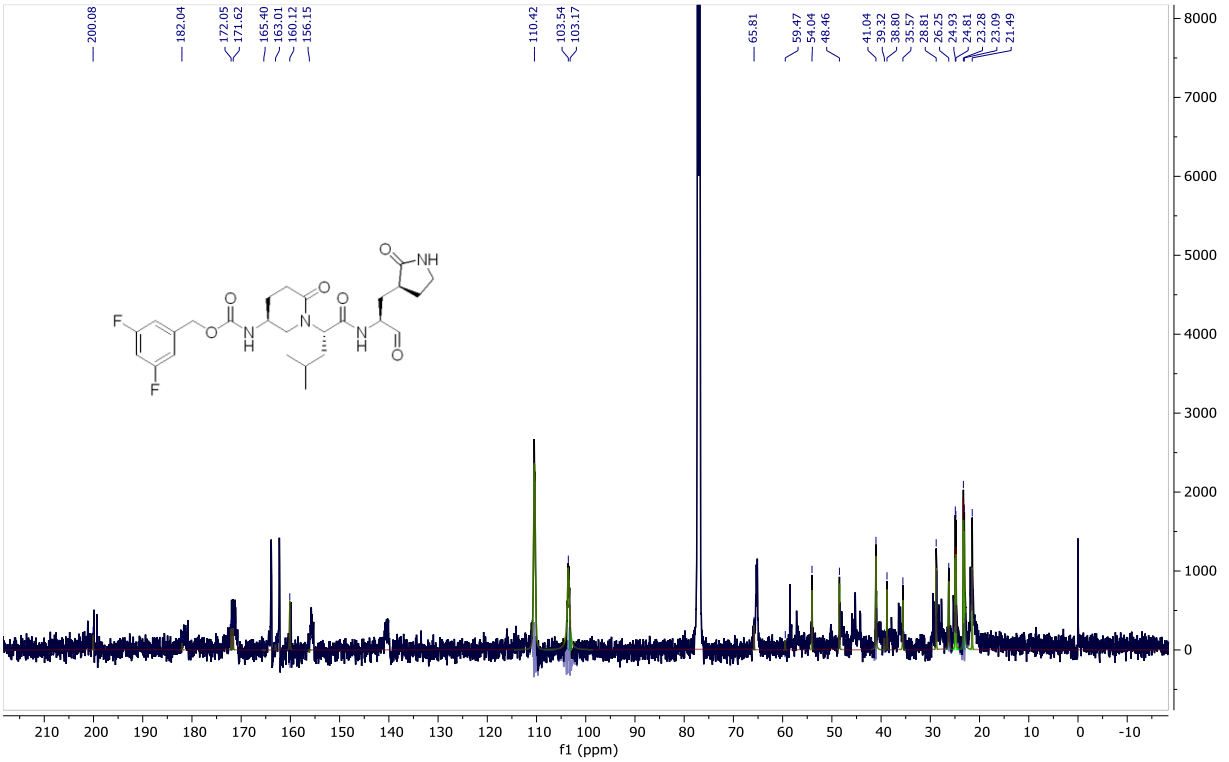
Compound M-1-6: HPLC Spectrum



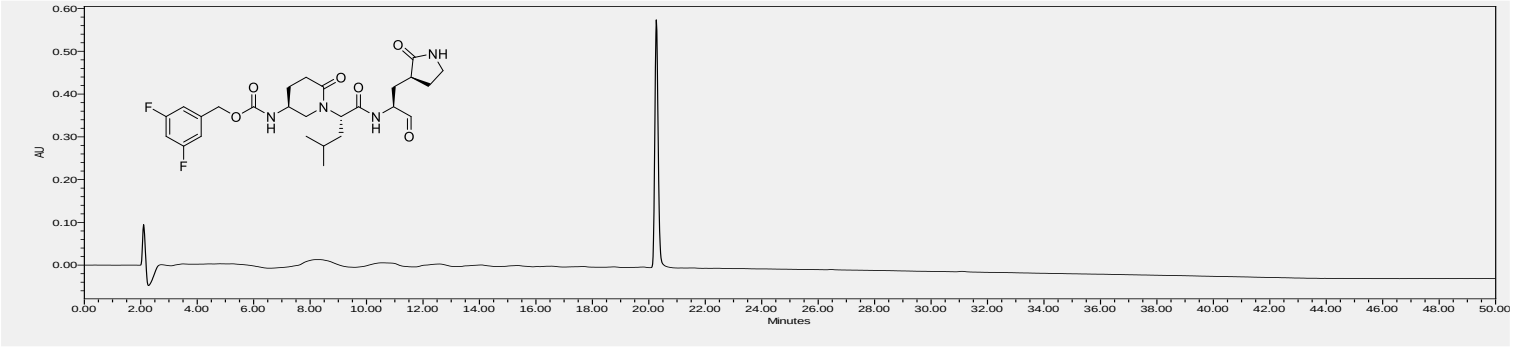
Compound M-1-7, ^{1}H NMR (600 MHz, $CDCl_3$)



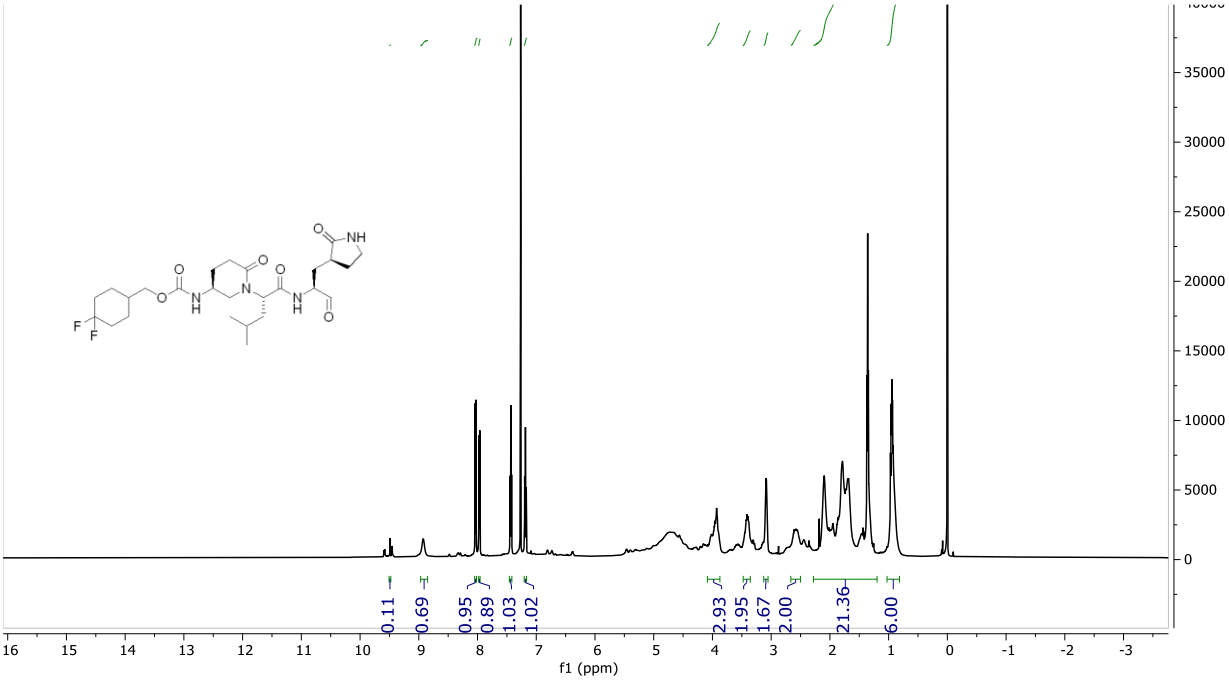
Compound M-1-7, ^{13}C NMR (150 MHz, $CDCl_3$)



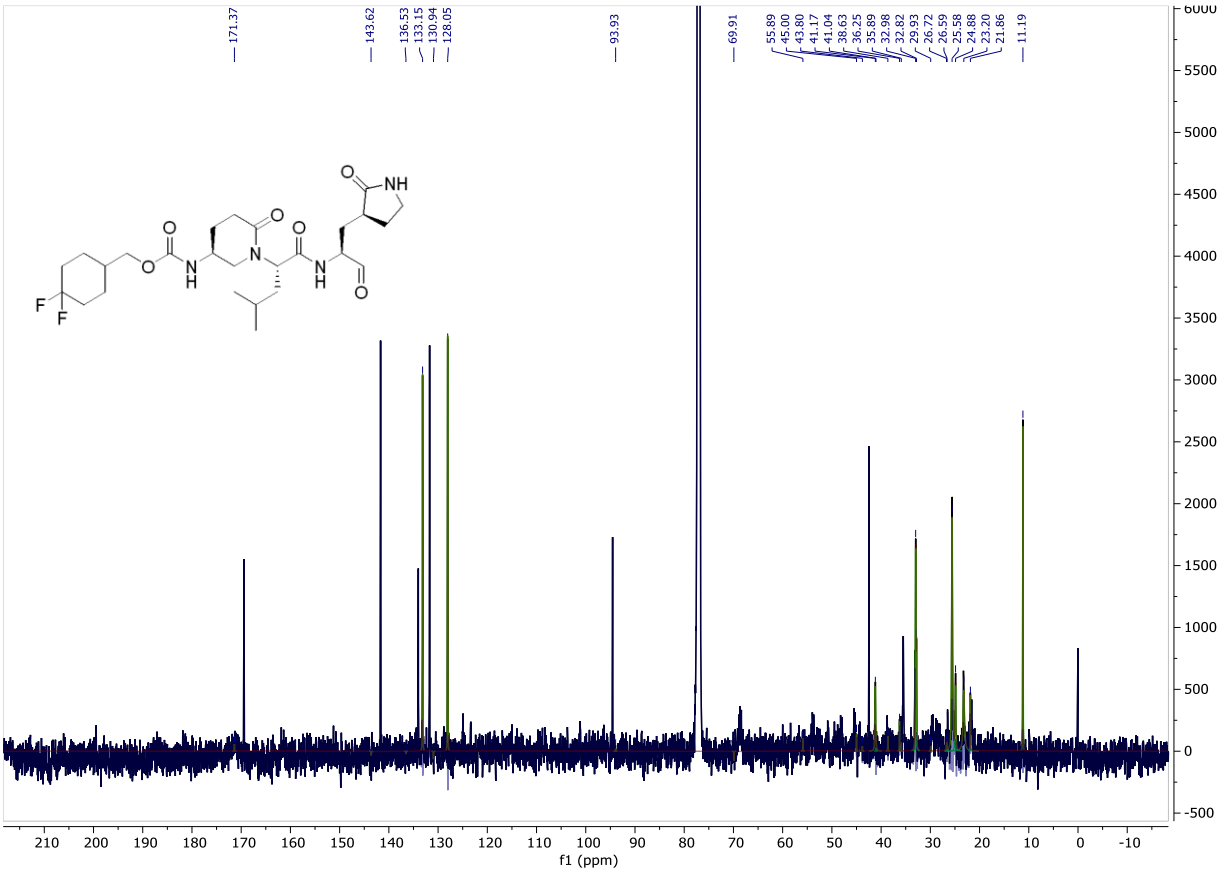
Compound M-1-7: HPLC Spectrum



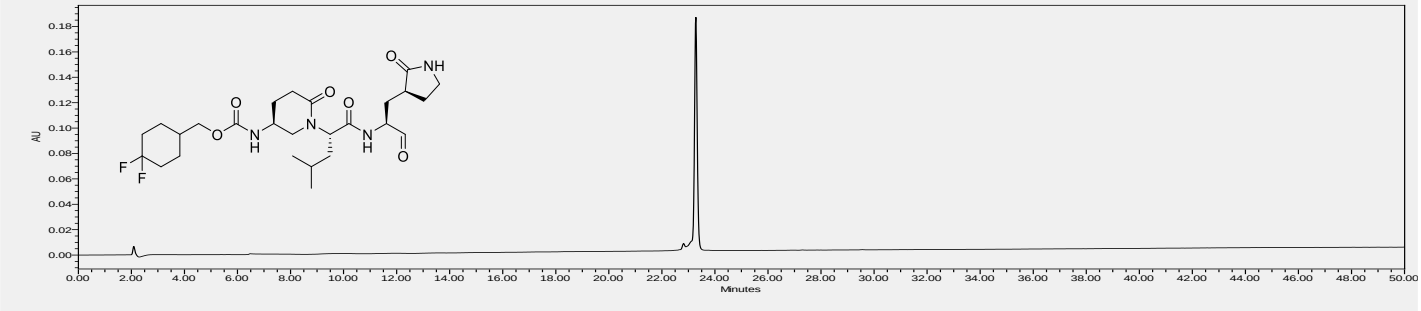
Compound M-1-8, ^{1}H NMR (600 MHz, $CDCl_3$)



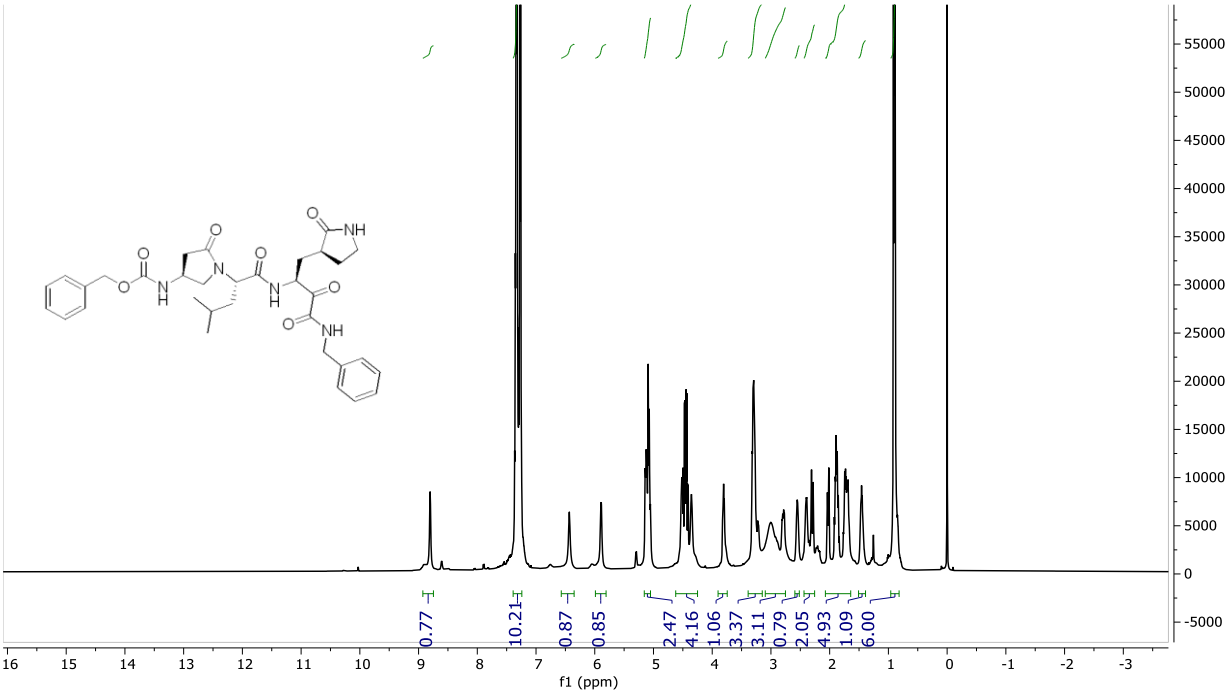
Compound M-1-8, ^{13}C NMR (150 MHz, $CDCl_3$)



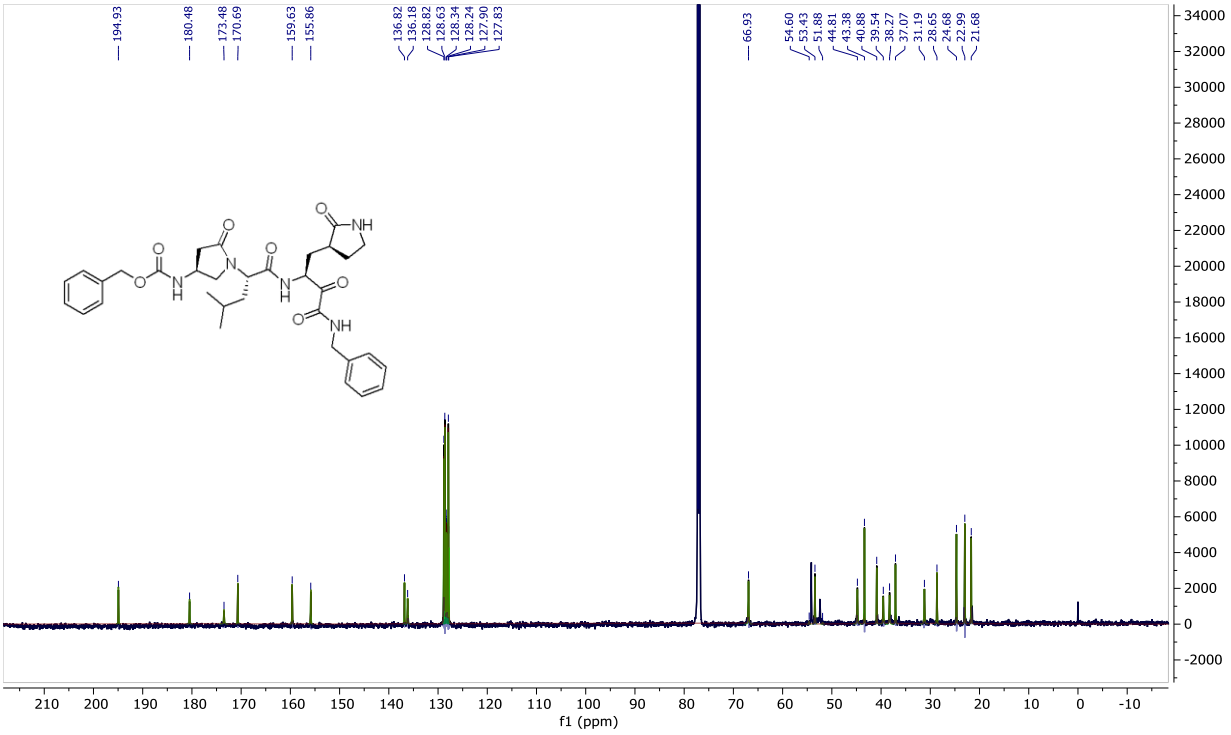
Compound M-1-8: HPLC Spectrum



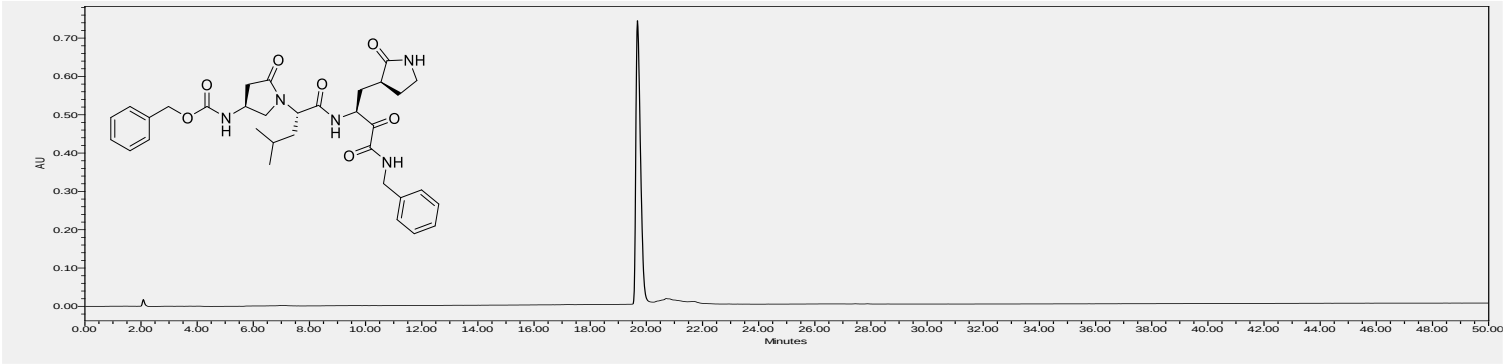
Compound M-2-1, ^{1}H NMR (600 MHz, $CDCl_3$)



Compound M-2-1, ^{13}C NMR (150 MHz, $CDCl_3$)



Compound M-2-1: HPLC Spectrum



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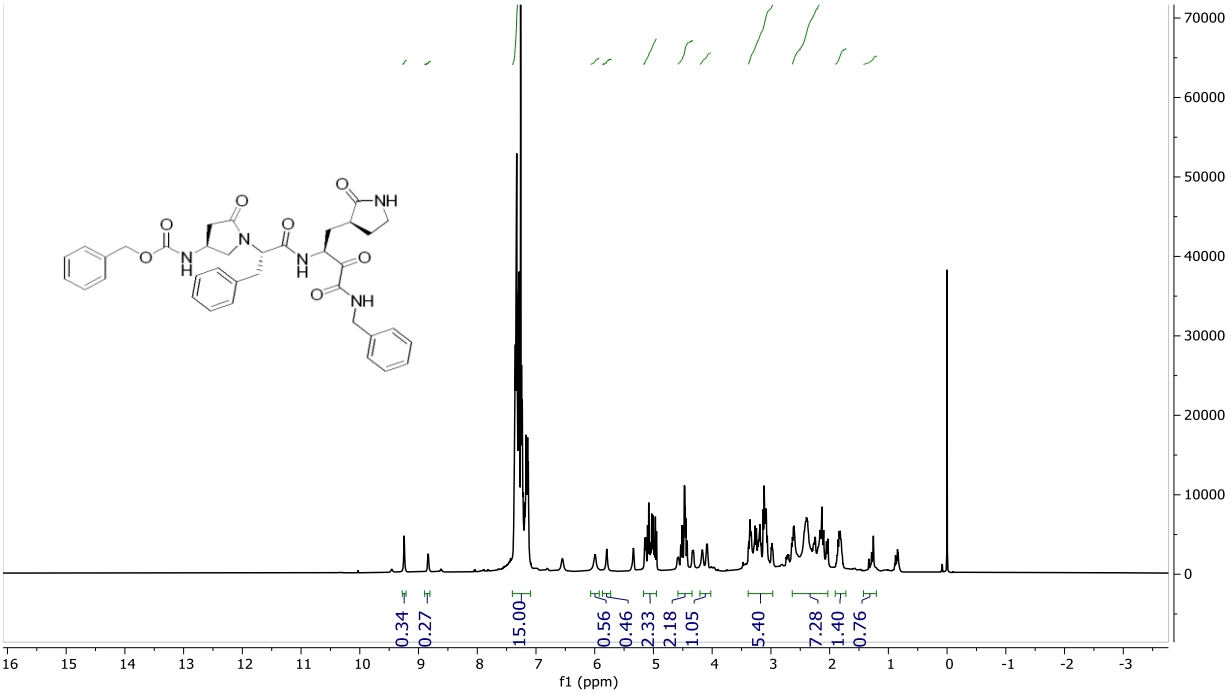
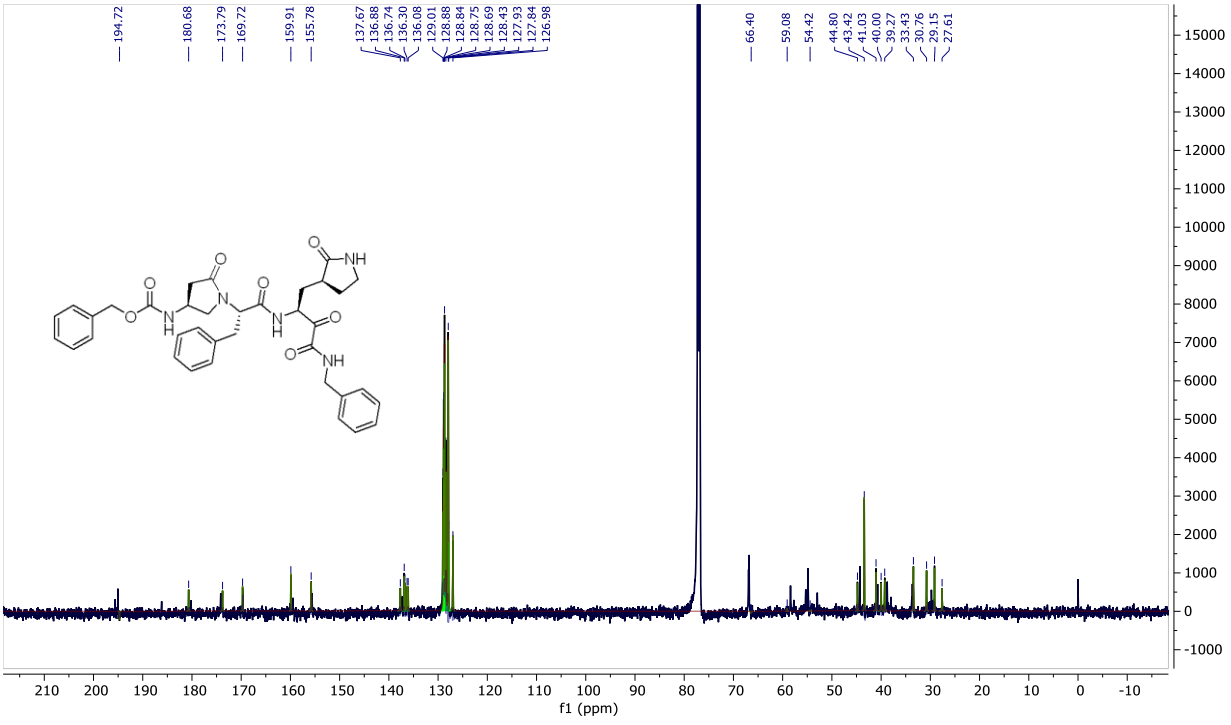
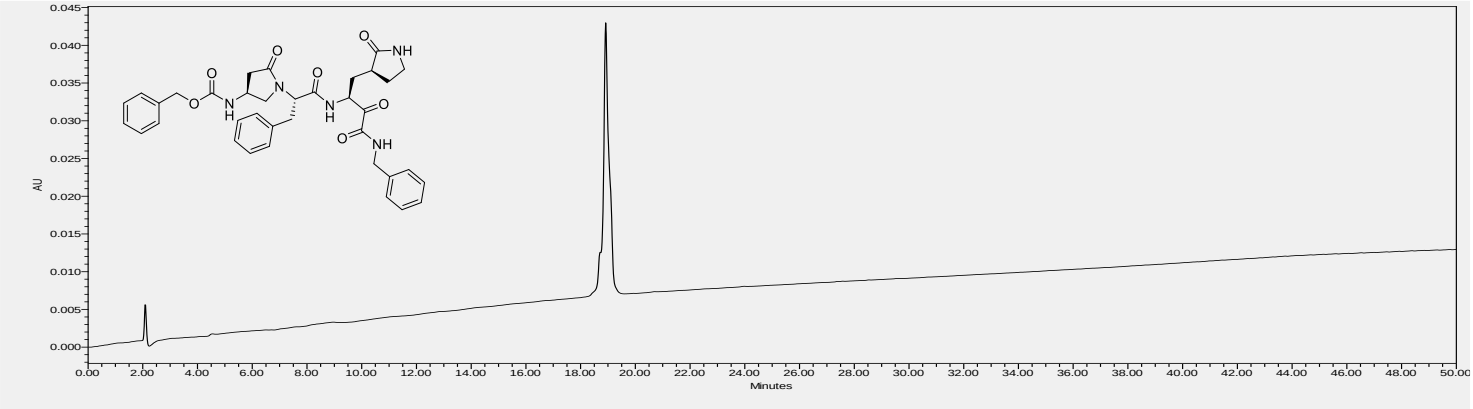


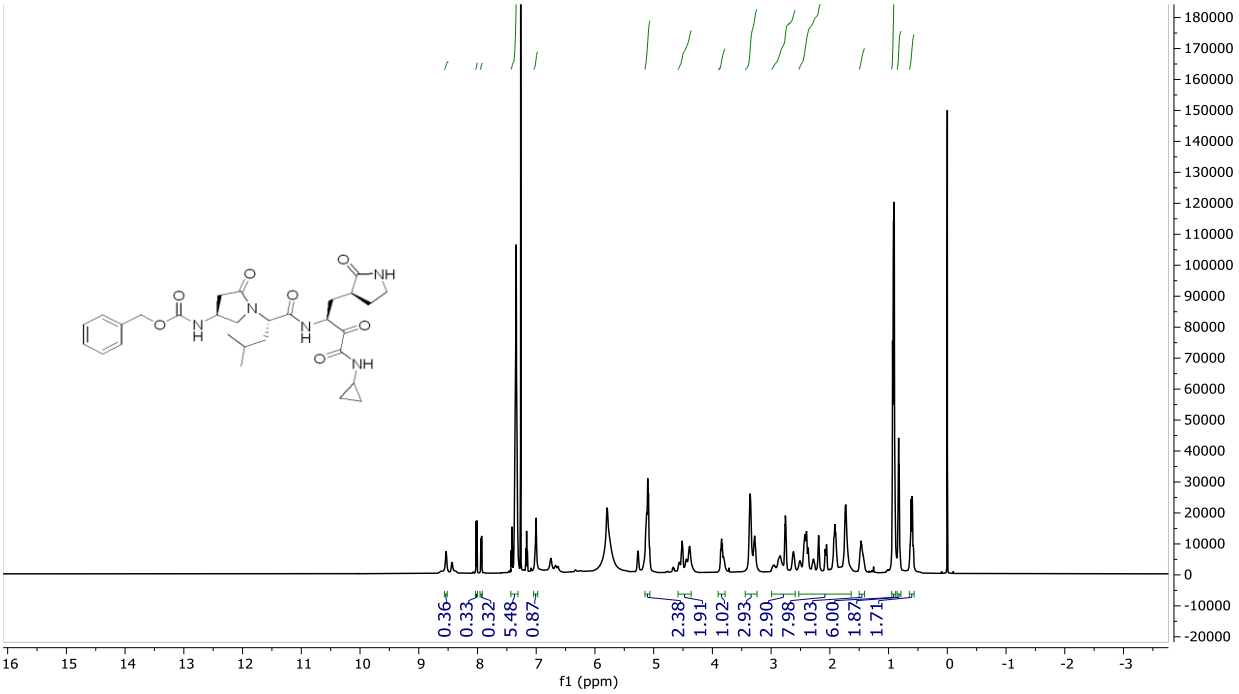
Compound M-2-2, ^{13}C NMR (150 MHz, $CDCl_3$)



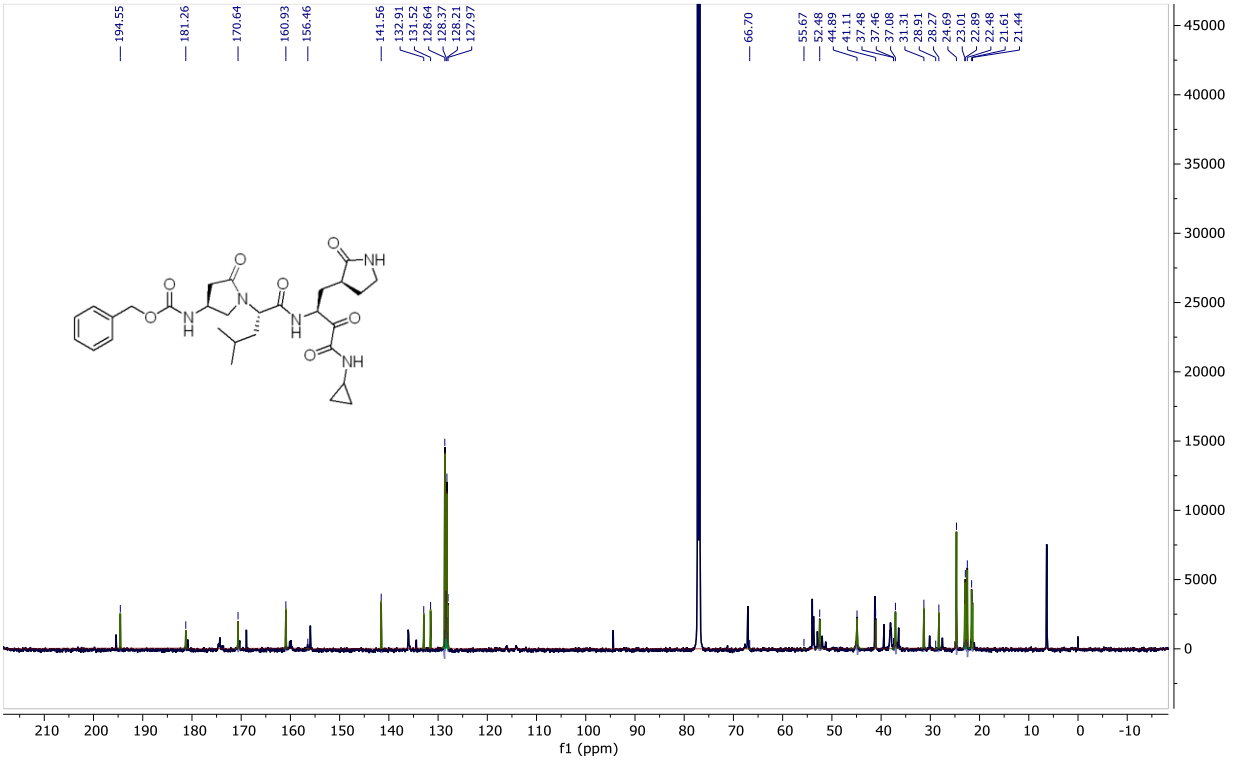
Compound M-2-2: HPLC Spectrum



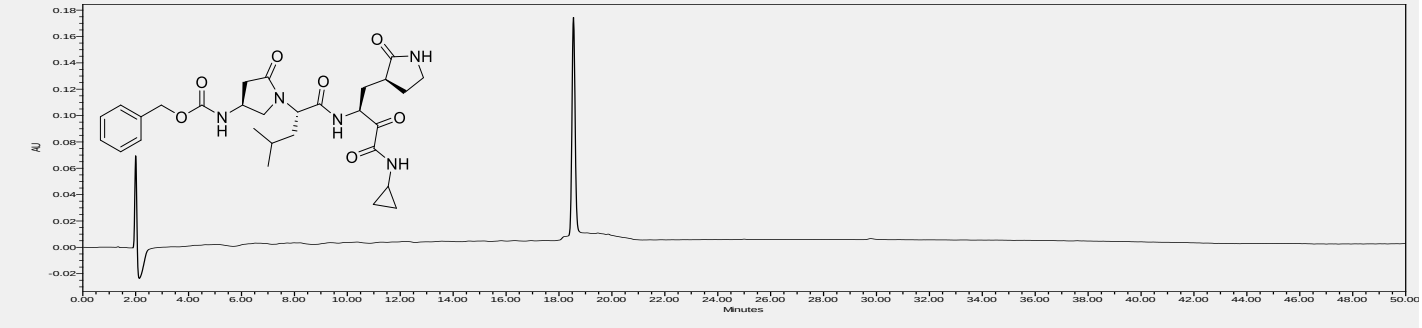
Compound M-2-3, ^{1}H NMR (600 MHz, $CDCl_3$)



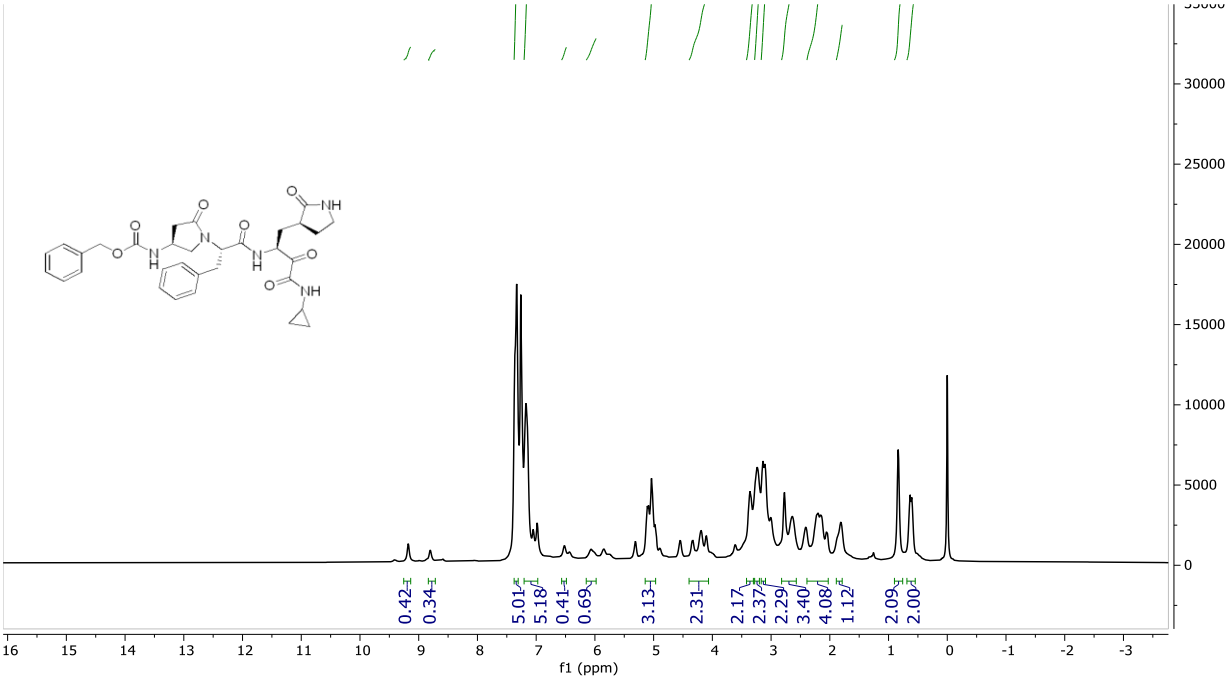
Compound M-2-3, ^{13}C NMR (150 MHz, $CDCl_3$)



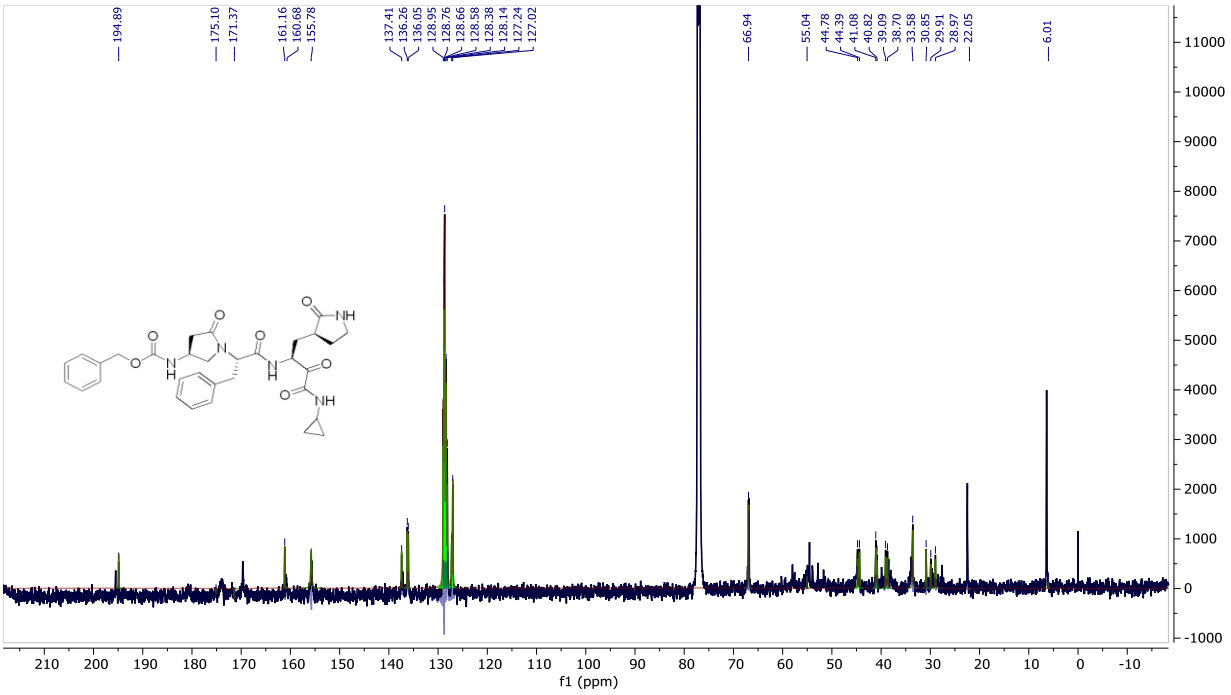
Compound M-2-3: HPLC Spectrum



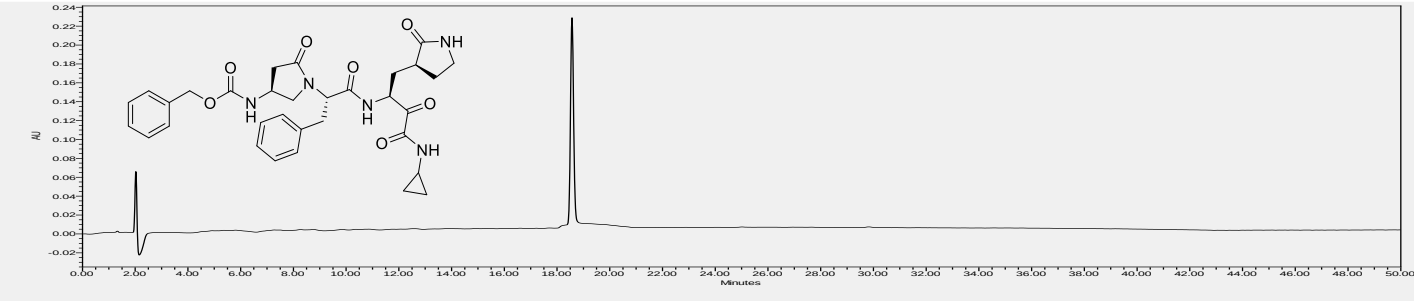
Compound M-2-4, ^{1}H NMR (600 MHz, $CDCl_3$)



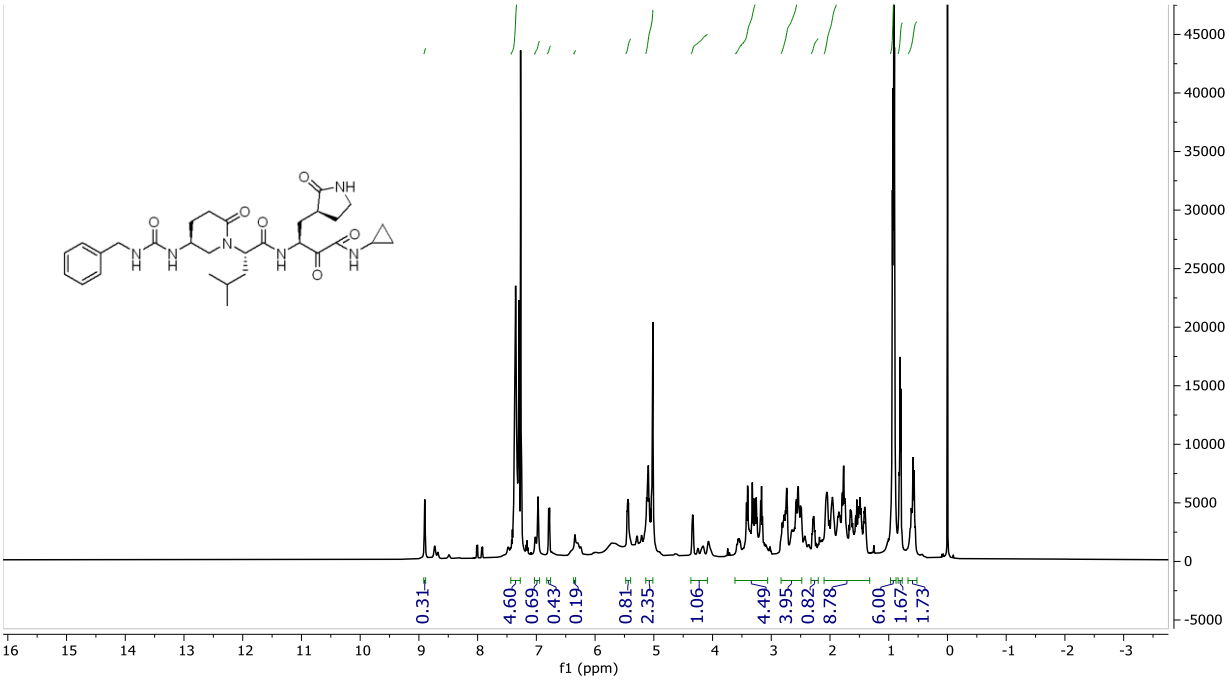
Compound M-2-4, ^{13}C NMR (150 MHz, $CDCl_3$)



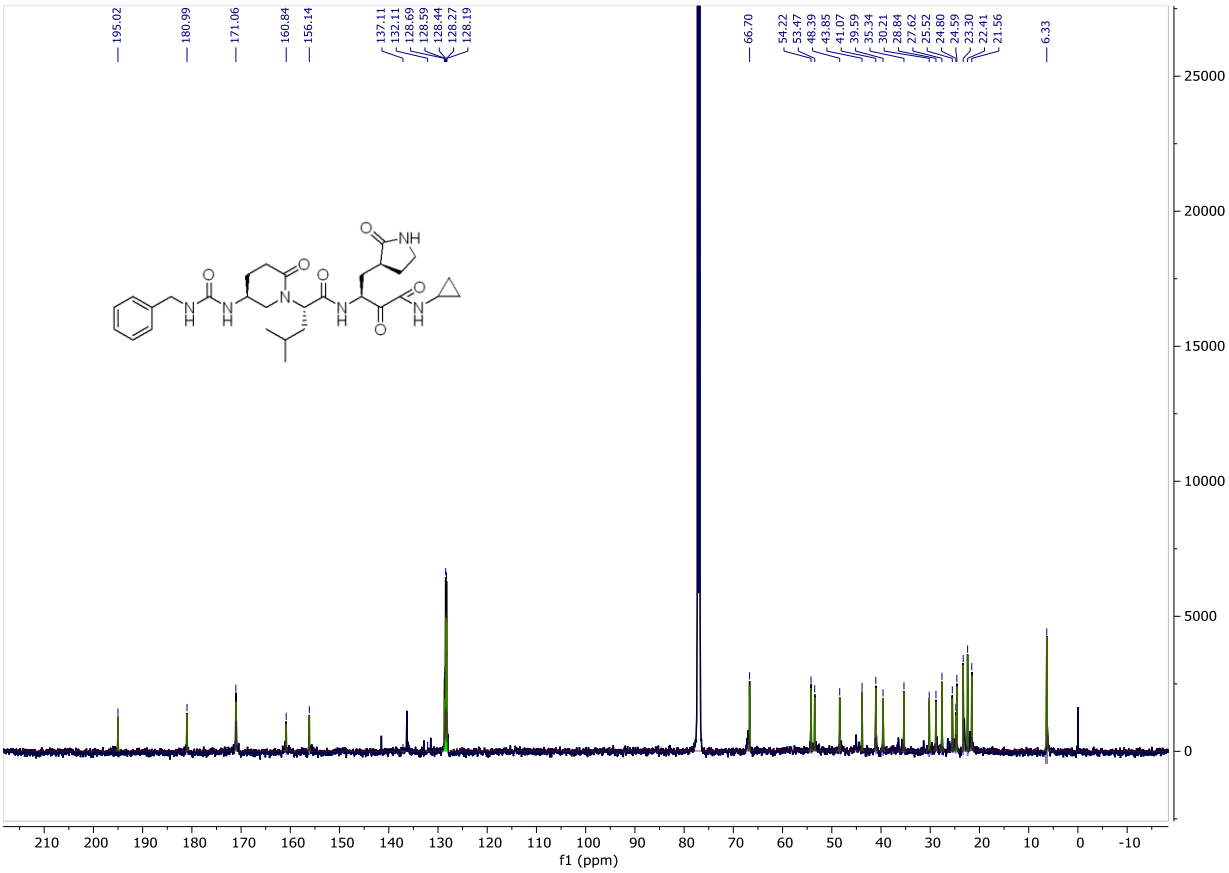
Compound M-2-4: HPLC Spectrum



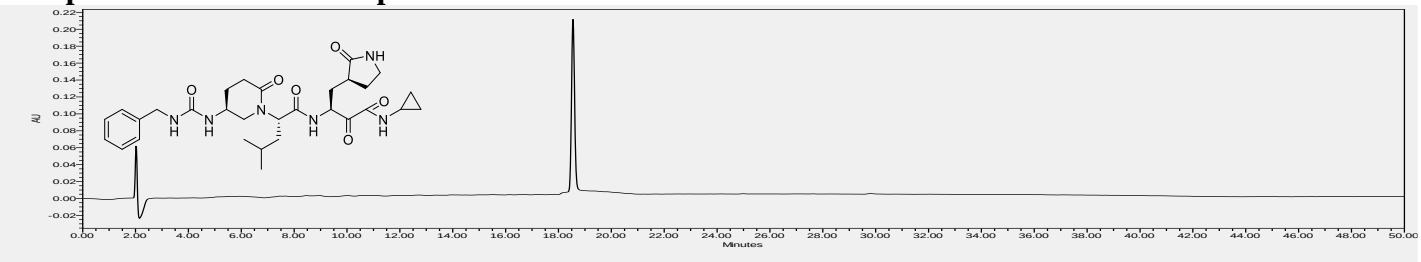
Compound M-2-5, ^{1}H NMR (600 MHz, $CDCl_3$)



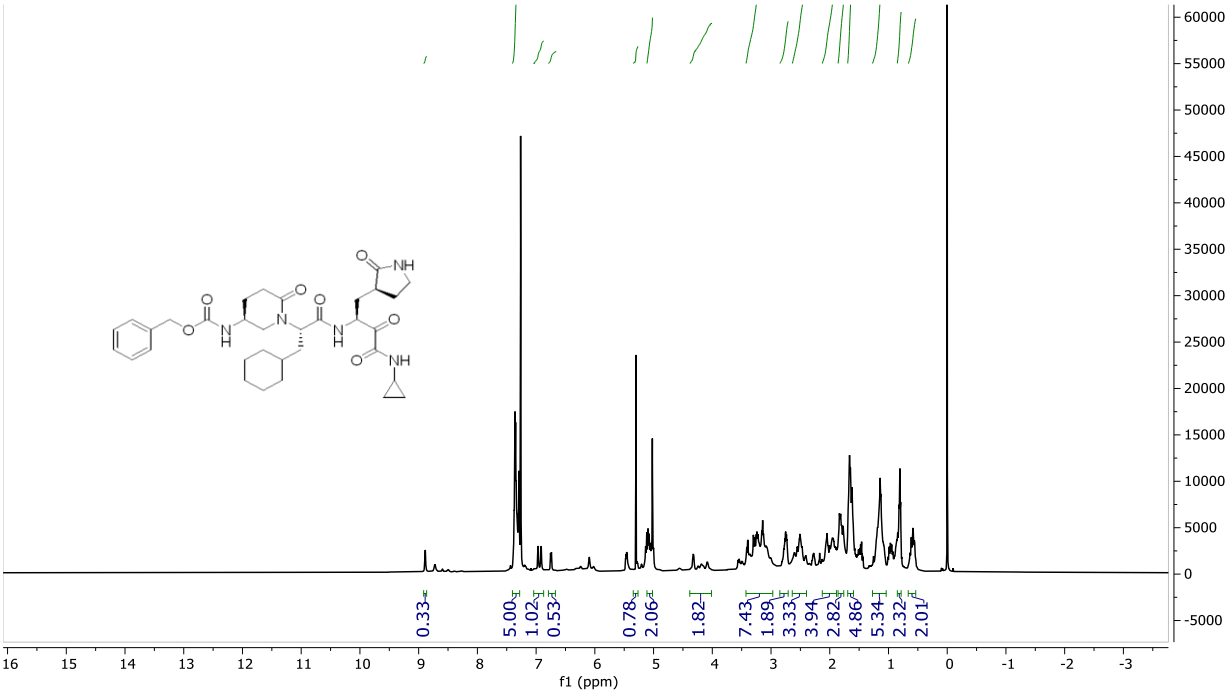
Compound M-2-5, ^{13}C NMR (150 MHz, $CDCl_3$)



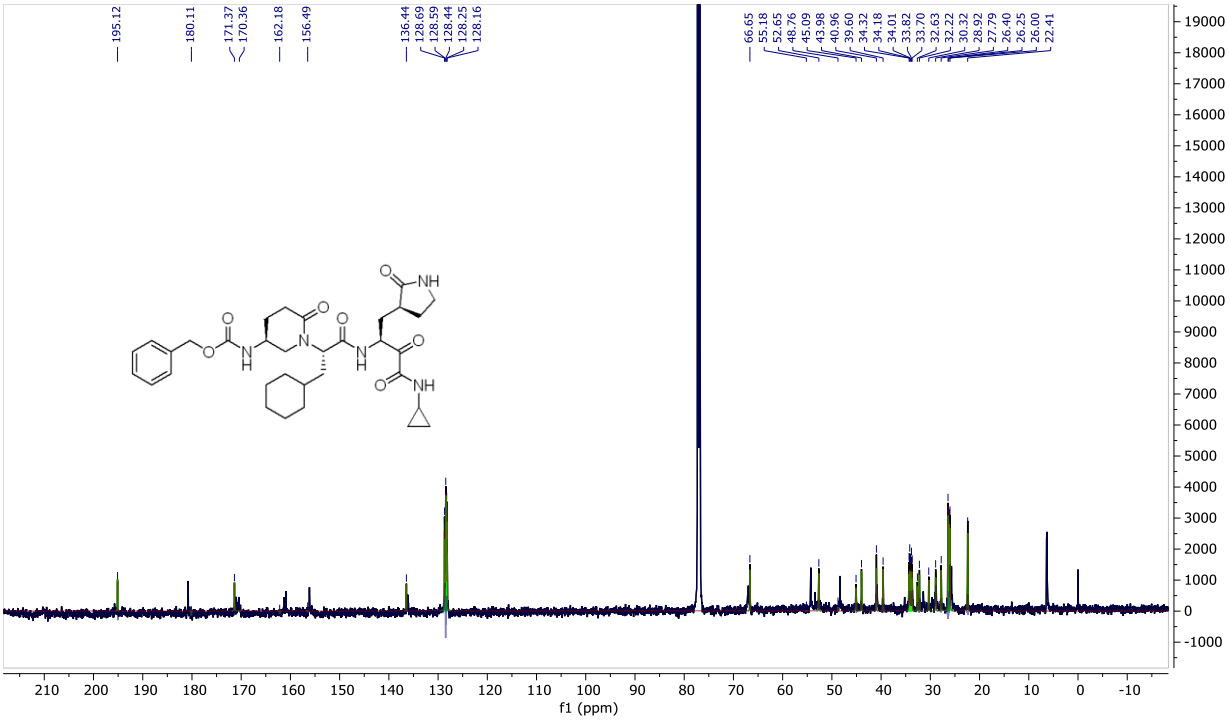
Compound M-2-5: HPLC Spectrum



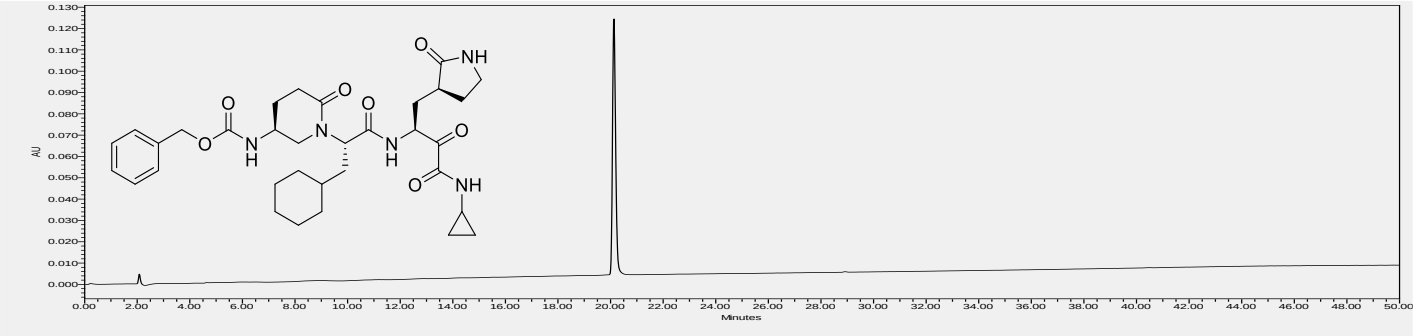
Compound M-2-6, ^{1}H NMR (600 MHz, $CDCl_3$)



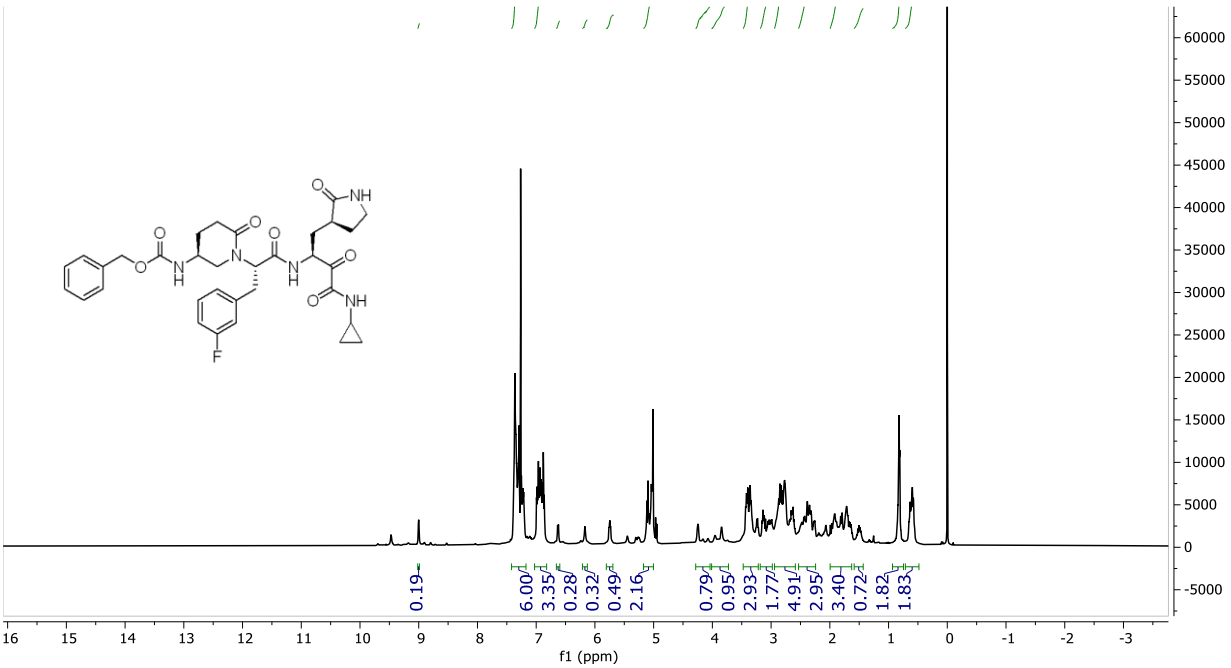
Compound M-2-6, ^{13}C NMR (150 MHz, $CDCl_3$)



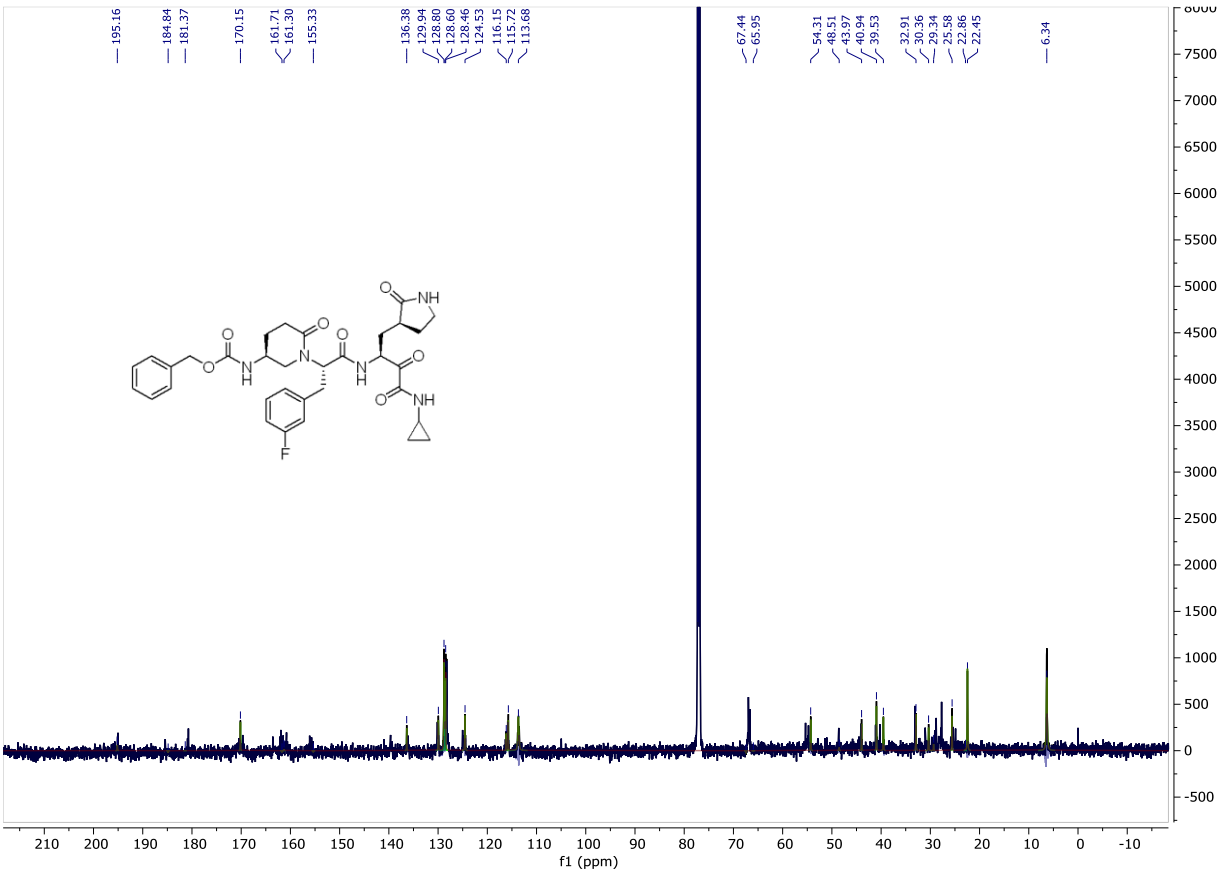
Compound M-2-6: HPLC Spectrum



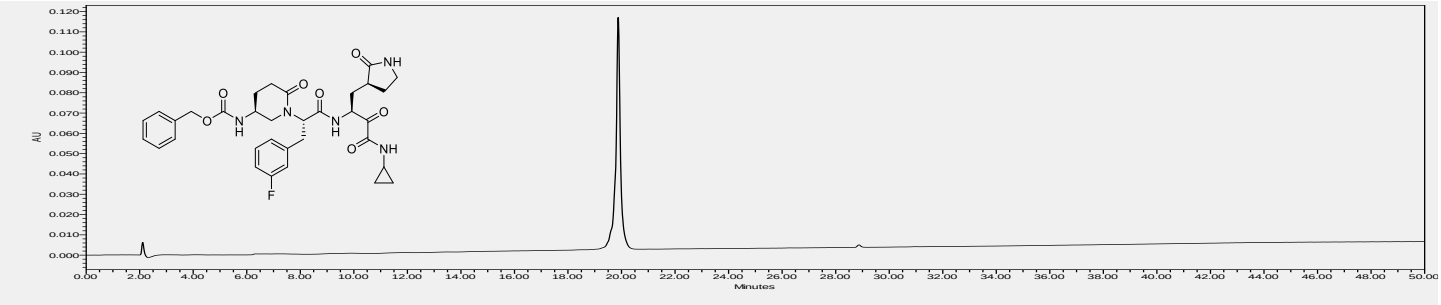
Compound M-2-7, ^{1}H NMR (600 MHz, $CDCl_3$)



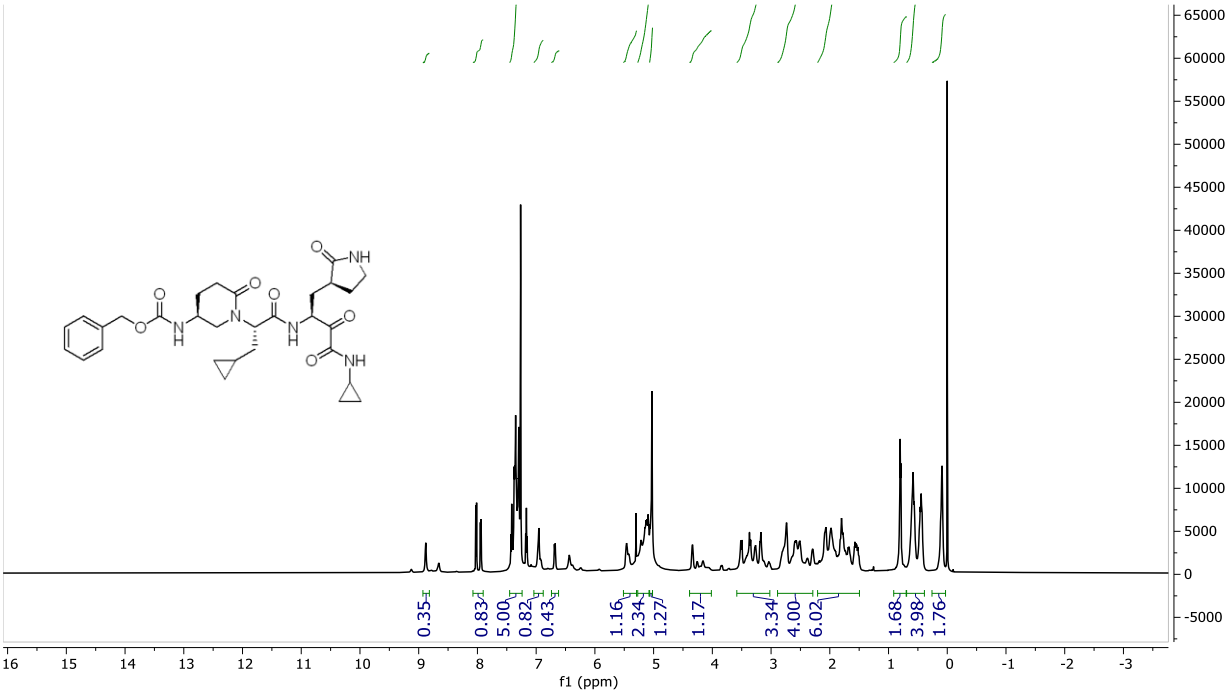
Compound M-2-7, ^{13}C NMR (150 MHz, $CDCl_3$)



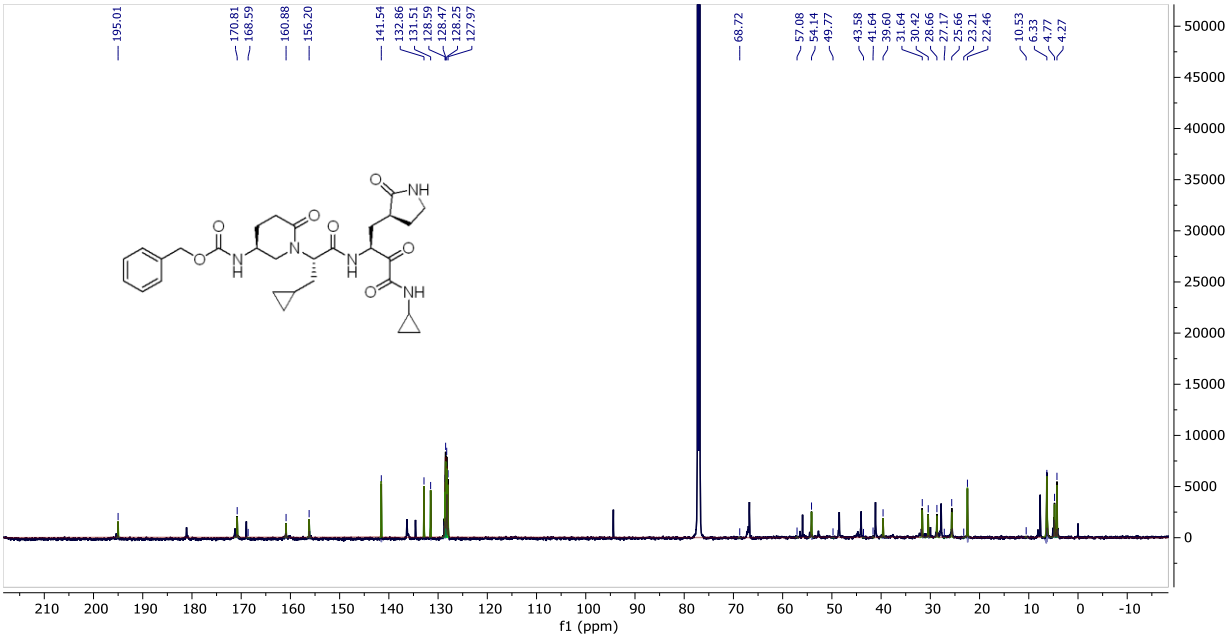
Compound M-2-7: HPLC Spectrum



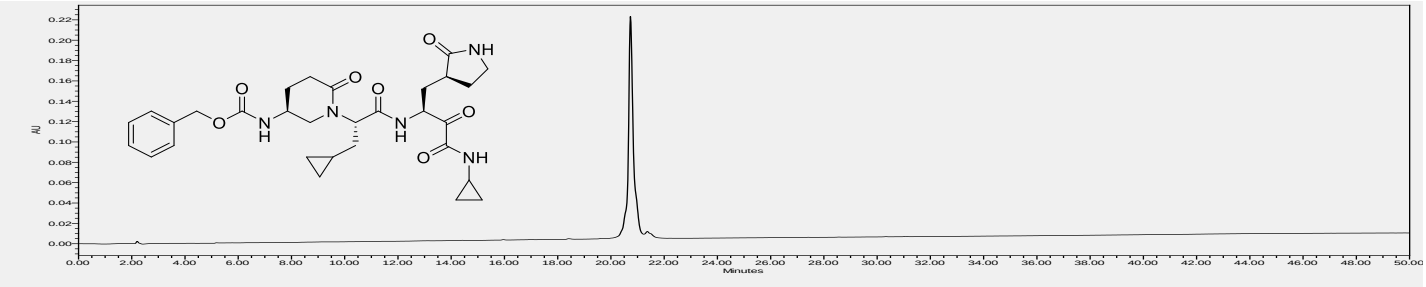
Compound M-2-8, ^{1}H NMR (600 MHz, $CDCl_3$)



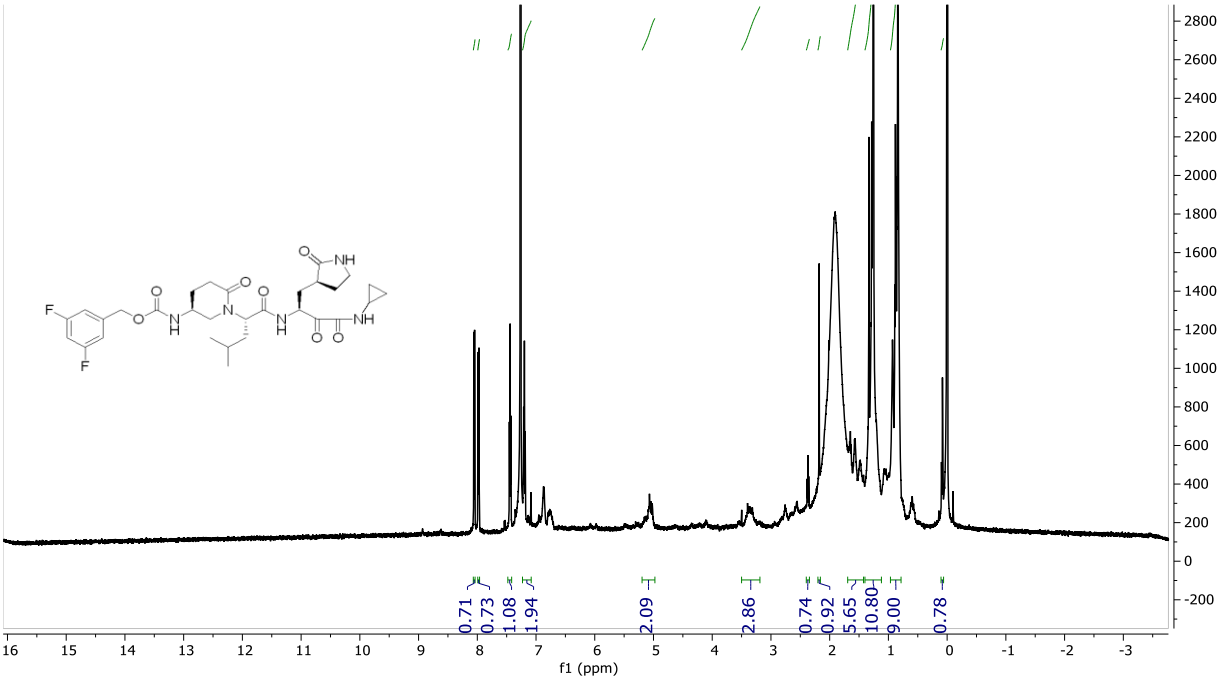
Compound M-2-8, ^{13}C NMR (150 MHz, $CDCl_3$)



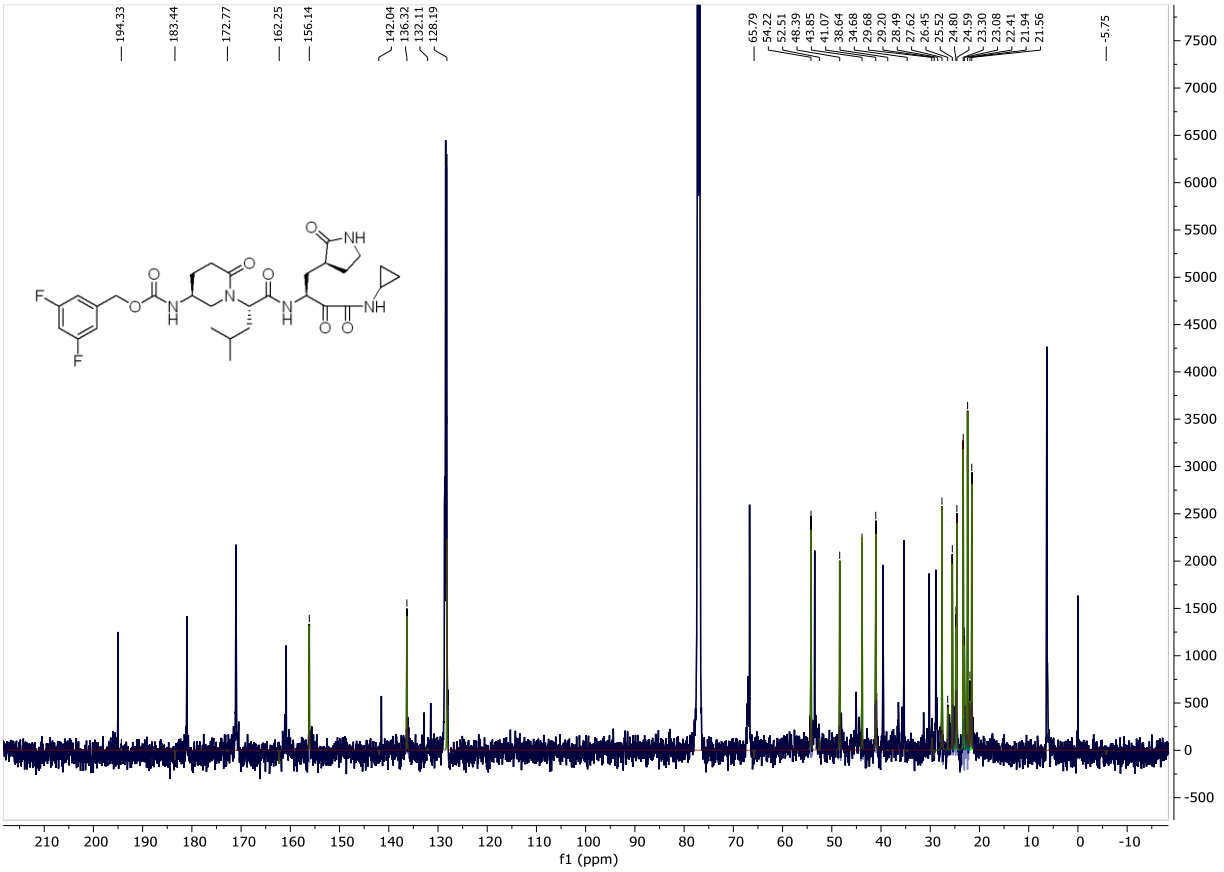
Compound M-2-8: HPLC Spectrum



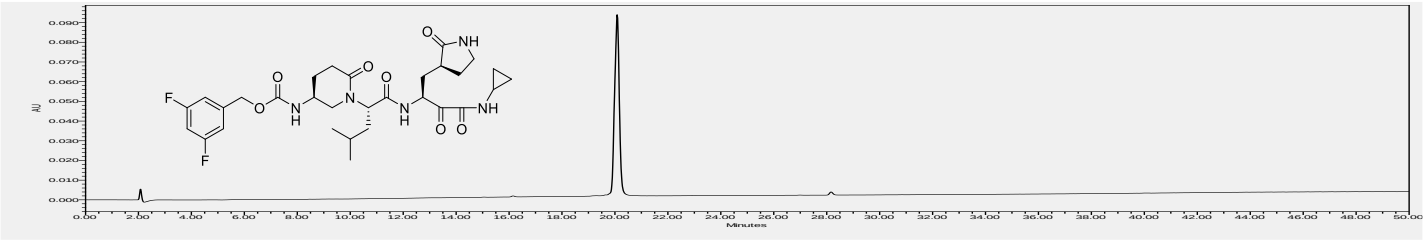
Compound M-2-9, ^{1}H NMR (600 MHz, $CDCl_3$)



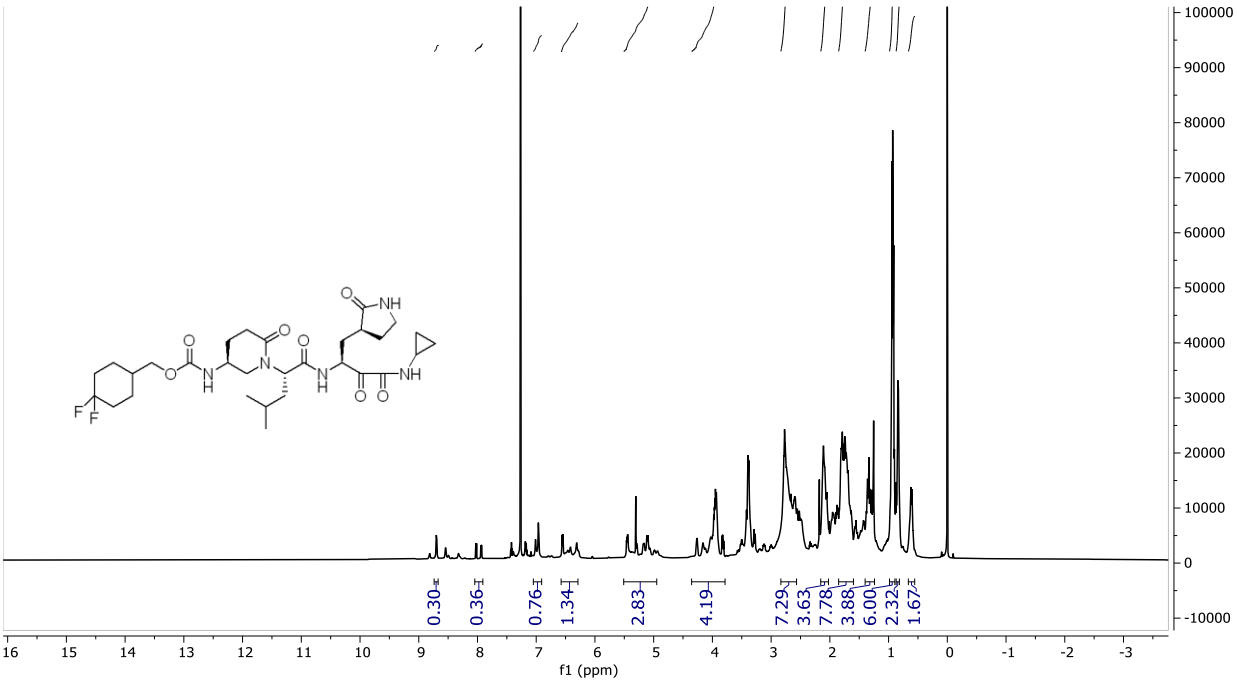
Compound M-2-9, ^{13}C NMR (150 MHz, $CDCl_3$)



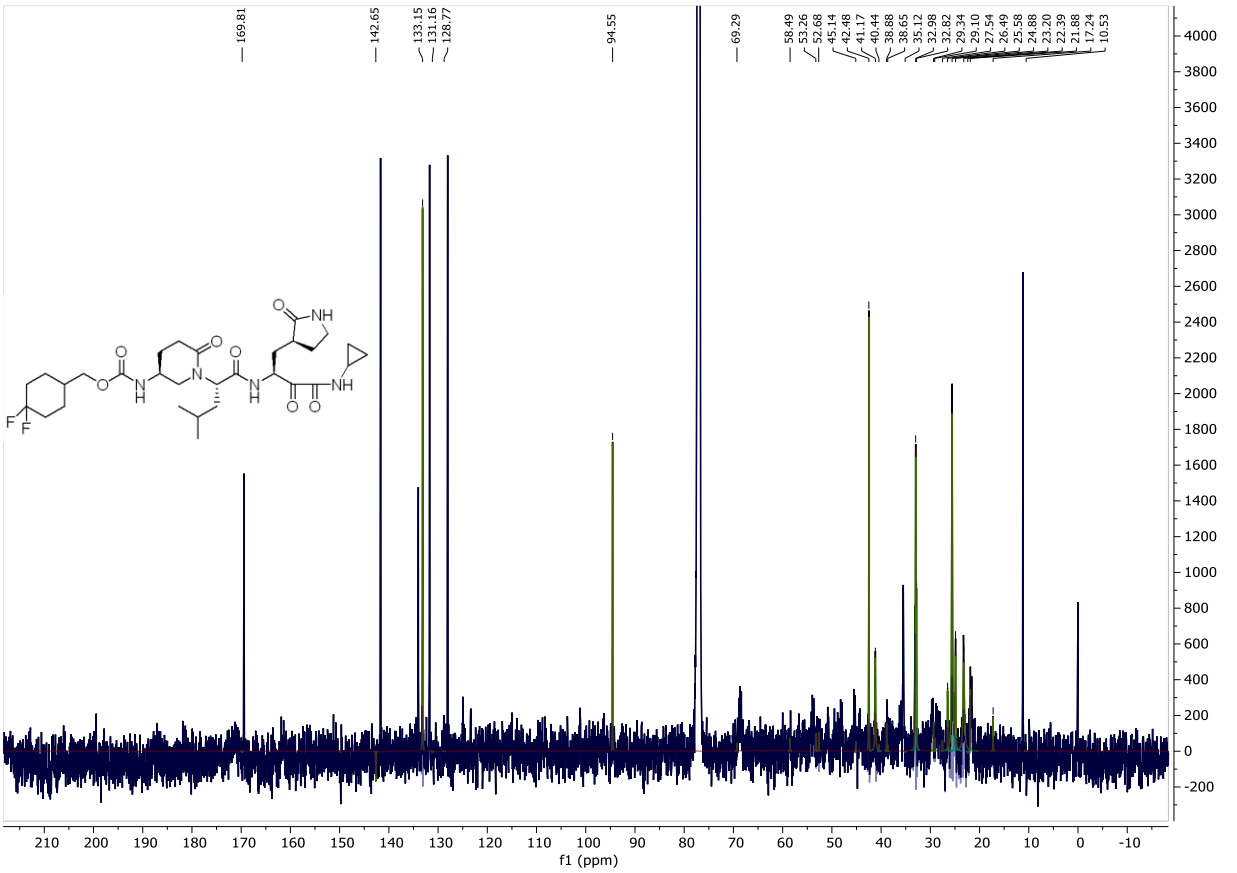
Compound M-2-9: HPLC Spectrum



Compound M-2-10, ^{1}H NMR (600 MHz, $CDCl_3$)



Compound M-2-10, ^{13}C NMR (150 MHz, $CDCl_3$)



Compound M-2-10: HPLC Spectrum