Supporting Information

Peptidomimetic nitrile warheads as SARS-CoV-2 3CL protease inhibitors

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Experimental Content

SARS-CoV-2 3CL^{pro} FRET assay and 18a reversibility study

Protein expression, purification, enzyme kinetics and FRET substrate synthesis are described in detail in reference 29 [W. Vuong *et al*. *Nat. Commun*. 2020, **11**, 4282 e1–8.]

The inhibitory effect of compounds was assessed at concentration range between 100 to 0.00001 μM with triplicate experiments being performed for each data point. The plot of percent of initial activity versus logarithm of compound concentration was fit using GraphPad Prism software (GraphPad 9.1.0) to determine the IC_{50} value. The value was presented as a mean ± standard error.

Reversibility study of **18a**.

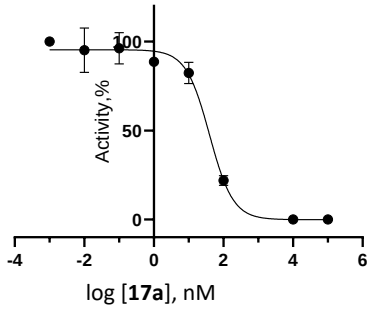
Table S1: Results for percent activity over time as **18a** is dialyzed away.

Time, h	**Percent Activity**	
	SARS-CoV-2 3CL^{pro}	**SARS-CoV-2 3CL^{pro} + 18a**
0	**100**	**0**
1.5	**100**	**0**
4	**105**	**11**
7	**100**	**17**
20	**82**	**29**
45	**70**	**40**
72	**30**	**45**

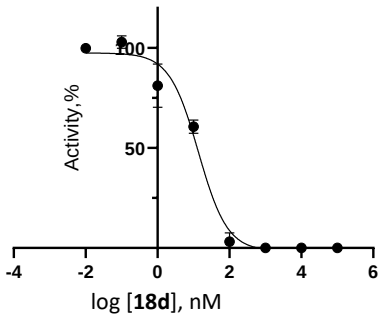
Reversibility of inhibition of SARS-CoV-2 3CL^{pro} by **18a** was assessed by dialysis method. The enzyme in a final concentration of 2 μM was incubated with 20 μM of **18a** inhibitor for 30 min at RT to allow for full inhibition. Then the protein sample was put on dialysis against 2 L of 50 mM Tris–HCl, pH 7.8, 150 mM NaCl, 5% glycerol, 1 mM DTT buffer at RT in a 6–8 kDa MWCO dialysis membrane (Fisher Scientific, Canada). The dialysis buffer was changed every 24 hours. Assessing the activity of apo-protease upon dialysis using the same conditions, including the dialysis buffer and the protein concentration was used as a control for enzyme stability over time. The aliquots of protease samples were taken out at specific time points for activity measurements. The data is represented as a plot of percent of initial protease activity at a zero time point versus incubation time.

SARS-CoV-2 3CL^{pro} IC_{50} curves for 17 - 19

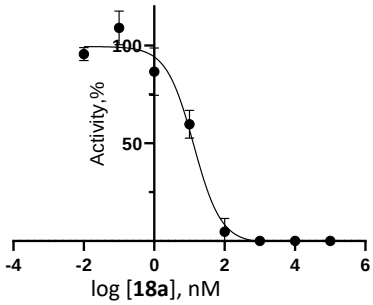
17a: IC_{50} = 40±8 nM



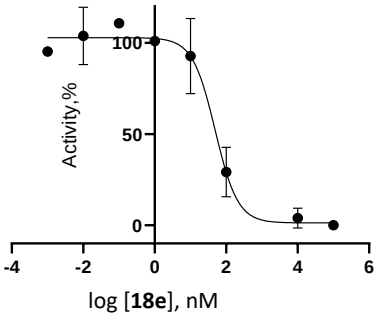
18d: IC_{50} = 13.7±1.5 nM



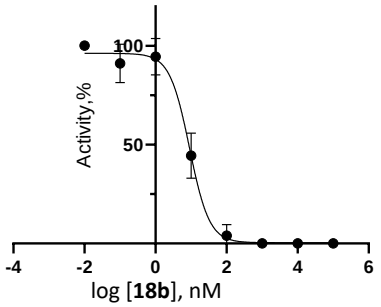
18a: IC_{50} = 13±3 nM



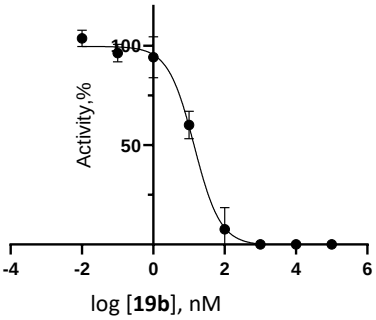
18e: IC_{50} = 49±10 nM



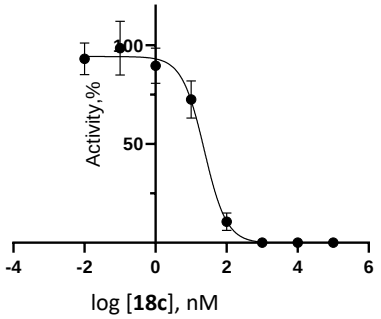
18b: IC_{50} = 9.1±2.5 nM



19b: IC_{50} = 14±2 nM



18c: IC_{50} = 24±4 nM



Cathepsin B, S and L assays

Human CatS Inhibition. Commercial CatS (CTSS, EC 3.4.22.27) inhibitor fluorometric assay kit (ab185437) from Abcam® (UK) and accompanying instructions and protocols were utilized. A 96-well white plate with flat bottom and Perkin Elmer's EnSpire multimode plate reader were used. Blank, enzyme and inhibitor (supplied) controls were tested simultaneously along with the inhibitors in triplicate. Test inhibitors were dissolved in and diluted in DMSO and the DMSO final concentration was 1%. Compounds were screened at 1 µM and for IC_{50} determination at least six different concentrations were tested in triplicate. Protocols from Abcam® were followed with the exception that incubation was 30 min at room temperature instead of 15 min. All test inhibitors and controls were run in triplicates for each concentration. Slopes of enzyme control (EC) and Slope of test inhibitors (S) were calculated (linear regression) and % relative inhibition was determined as in kit instructions. IC_{50} values were calculated using Prism 9 for Windows 64-bit (V 9.1.0) by GraphPad Software and standard error is reported.

Human CatB Inhibition. Commercial Cathepsin B (CTSB, EC 3.4.22.1) inhibitor fluorometric assay kit (K147-100) from BioVision® (USA) and accompanying instructions and protocols were utilized. Performed identical to CatS and BioVision® protocols were followed except a 30 min incubation period instead of 15 min was utilized.

Human CatL Inhibition. The inhibititory studies were performed using commercial Cathepsin L (CTSB, EC 3.4.22.1) inhibitor fluorometric assay kit (ab 197012) from Abcam® (UK). Inhibitors were dissolved and diluted in DMSO and the final DMSO concentration in the assay was 1%. Compounds were screened at 1 µM and for IC_{50} determinations at least six different concentrations were tested in triplicate. The activity measurements were performed according to the kit manual.

SARS-CoV-2 PRA Antiviral and Cytotoxicity

Determination of EC_{50} by plaque reduction assay. SARS-CoV-2/CANADA/VIDO 01/2020 was a kind gift from Darryl Falzarano (University of Saskatchewan). Vero (Female green monkey kidney) E6 cells were infected with an MOI of 0.0001 pfu/cell in infection medium consisting of Dulbecco's Modified Eagle's medium supplemented with 1× non-essential amino acids (Gibco), 10 mM HEPES, 2% fetal bovine serum, 50 IU/mL penicillin, 50 IU/mL streptomycin, and different doses of the compound. After 1 h, the infecting medium was removed and monolayers were overlaid with growth media (MEM supplemented with 10 mM HEPES) containing 1.2% Avicel RC-591 (DuPont), containing the relevant dose the compound. After 48 h, cells were fixed in 10% formaldehyde, and stained using 0.5% (w/v) crystal violet. Plaques were counted and data were plotted as % inhibition vs the log10 [compound] using Prism (GraphPad). EC_{50} values were determined using a non-linear regression analysis. Each concentration was done in triplicate. Error bars indicate standard deviation. For experiments where CP-100356 (CP) was added all procedures were the same except that the media contained 0.5 μM of CP.

Measuring cytotoxicity in A549 and Vero E6 cells. Cell viability was measured using the CellTiter-Glo luminescent cell viability assay (Promega) and the Cell Counting Kit-8 (CCK-8) assay (Sigma). A549 (male human lung epithelial) cells and Vero E6 cell lines were seeded at 1×10^4 cells/well in 96-well plates and incubated overnight before treatment. Compounds were solubilized in DMSO then diluted in culture media to a final DMSO concentration of 0.5% and final compound concentrations of 50 μM and 200 μM. Cells were incubated in the presence of compounds for 24 hours before addition of the kit substrates and measurement of luminescence (CellTiter-Glo) or absorbance at 450 nm (CCK-8) according to manufacturer's instructions. The percentage of viable cells was calculated relative to cells treated with solvent alone (0.5% DMSO). Results were plotted as the mean of quadruplicate wells ±SD from one experiment. CC_{50} values were either greater than the concentration tested or interpolated using GraphPad Prism software.

Crystallographic Procedures for SARS-CoV-2 3CL^{pro} – 17a Cocrystal Structure

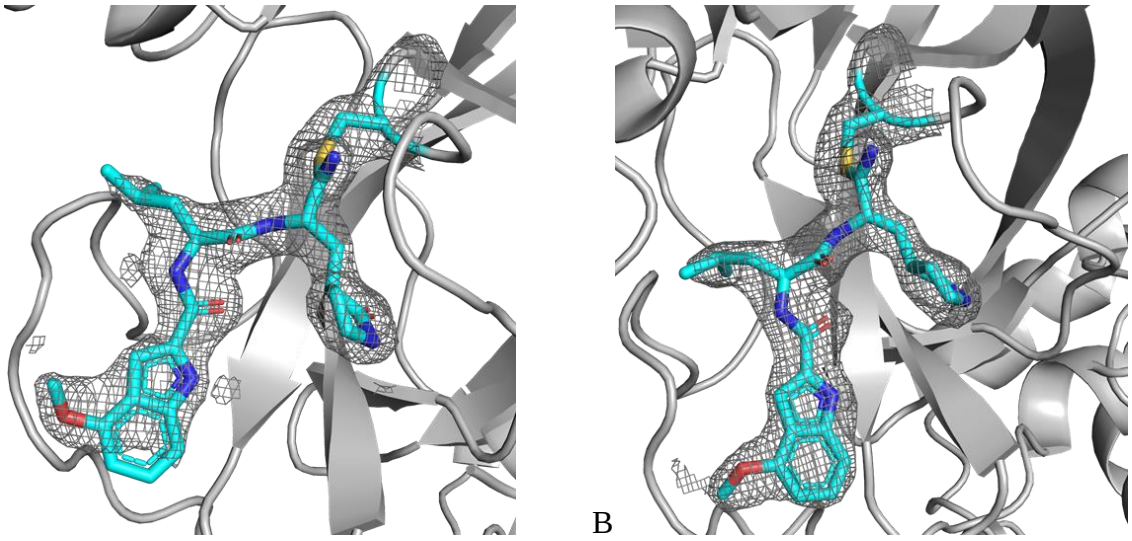
Crystallization. For crystallization, purified SARS-CoV-2 3CL^{pro} was dialyzed against buffer containing 10 mM NaCl and 5 mM Tris-HCl (pH 8.0) overnight at 4 °C, and concentrated with a Millipore centrifugal filter (10 kDaMW cut-off) to 9 mg/mL. Protein was incubated with five molar excess of inhibitor at 4 °C for 2 h prior to crystallization. For SARS-CoV-2 Mpro, the protein was subjected to the PACT crystallization screen (Molecular Dimensions), with hits identified in several conditions for both inhibitors. For Apo- SARS-CoV-2 3CL^{pro} the best crystals were observed with hanging drop trays at room temperature at a ratio of 1:1 with mother liquor 0.2 M sodium sulfate, 0.1 M Bis-Tris propane (pH 6.5), and 20% w/v PEG 3350. The SARS-CoV-2 3CL^{pro} with inhibitors crystallized with mother liquid containing 0.2 M sodium chloride 0.1 M 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.0), and 20% w/v PEG 6000. Prior to freezing, crystals were incubated with 15% glycerol as a cryoprotectant. Crystals were initially screened on our 007 MicroMax (Rigaku Inc) home source with crystal screening and final data collection at Stanford Synchrotron Radiation Lightsource (SSRL), beamline 12–2 with Blu-Ice using the Web-Ice interface.

Blu-Ice: McPhillips, T. M., McPhillips, S. E., Chiu, H. J., Cohen, A. E., Deacon, A. M., Ellis, P. J., Garman, E., Gonzalez, A., Sauter, N. K., Phizackerley, R. P., Soltis, S. M., Kuhn, P. Blu-Ice and the Distributed Control System: software for data acquisition and instrument control at macromolecular crystallography beamlines *J. Synchrotron Rad.* 2002, **9**, 401-406.

Web-Ice: Gonzalez, A., Moorhead, P., McPhillips, S. E., Song, J., Sharp, K., Taylor, J. R., Adams, P. D., Sauter, N. K. & Soltis, S. M. *Web-Ice:* Integrated Data Collection and Analysis for Macromolecular Crystallography *J. Appl. Cryst.* 2008, **41**, 176-184.

Diffraction Data Collection, Phase Determination, Model Building, and Refinement. X-ray diffraction data sets for SARS-CoV-2 3CL^{pro} and inhibitor complex were collected at 100 K in a cold nitrogen stream using beamlines at 12-1 at Stanford Synchrotron Radiation Lightsource (SSRL) California, USA, of wavelength 0.979460, equipped with Eiger 16 M Pixel Array Detector and Canadian Light Source, CLSI BEAMLINE 08B1-1 coupled with PILATUS 6M. About 10 data sets were collected for the inhibitor, all of them processed and the best one selected based on data statistics. XDS and Scala were used for processing of the data sets. The diffraction data set of compound **17a** with SARS-CoV-2 3CL^{pro} was processed at a resolution of 1.95 Å, in an orthorhombic space group P 2_1 (Table S2). The structure was determined by molecular replacement with the crystal structure of the free enzyme of the SARS-CoV-2 3CL^{pro} (PDB entry 6WTM) as a search model, using the Phaser program from Phenix, version v1.18.1-3855). Ligand was fit using Coot manually for the inhibitor in the density of pre-calculated map from Phenix refinement. Refinement of the structure was performed with phenix.refine in Phenix software. The structure refinement yielded final R_{work} and R_{free} of reasonable values. Statistics of diffraction, data processing and model refinement are given in the supplementary materials (Table S2). The model was inspected with Ramachandran plots and all showed good stereochemistry. Final model 7R7H.pdb was displayed using PyMOL molecular graphics software (Version 2.0 Schrödinger, LLC).

Electron Density of Active Sites and Overlay of Two active Sites at Different Angles



A B

Figure S1: Ligand interacts covalently with the active site cysteine145 of SARS-CoV-2 $3CL^{pro}$ making a thioimidate bond (protomer A and B, respectively). Electron density at 1σ is shown in gray mesh of both the chains in asymmetric unit (SARS-CoV-2 $3CL^{pro}$ – **17a**).

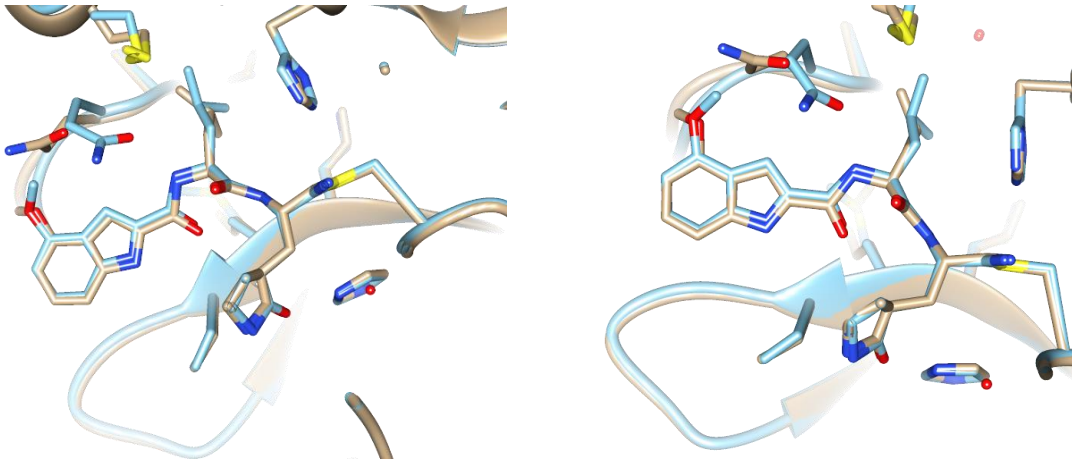


Figure S2: Superposition of the two subunits at different angles focusing on ligand binding site (SARS-CoV-2 $3CL^{pro}$ – **17a**).

Crystallographic Data for SARS-CoV-2 3CL^{pro} – 17a Cocrystal Structure

Table S2: Data collection and refinement statistics (molecular replacement)

	SARS-CoV-2 3CL^{pro}
PDB entry	7R7H
Data collection	
Space group	P 2_1
Cell dimensions	
a, b, c (Å)	45.46 53.79 115.46
α, β, γ (°)	90 101.091 90
Resolution (Å)	34.34 - 2.15 (2.227 – 2.15)
Observations	97778 (10184)
R_{merge}	0.0716 (0.8237)
I / σI	11.87 (2.45)
Completeness (%)	96.99 (98.24)
Redundancy	3.34
CC1/2	0.998 (0.755)
Refinement	
Resolution (Å)	34.34 - 2.15
No. reflections	29152
R_{work} / R_{free}	20.42/ 26.15
Number of non-hydrogen atoms	4920
Protein	4740
Ligand/ion	126
Water	116
B-factors	
Protein	51.87
Ligand/ion	50.28
Water	46.55
R.M.S. deviations	
Bond lengths (Å)	0.014
Bond angles (°)	1.46

*Values in parentheses are for highest-resolution shell. Each data was collected from single crystal

Chemistry

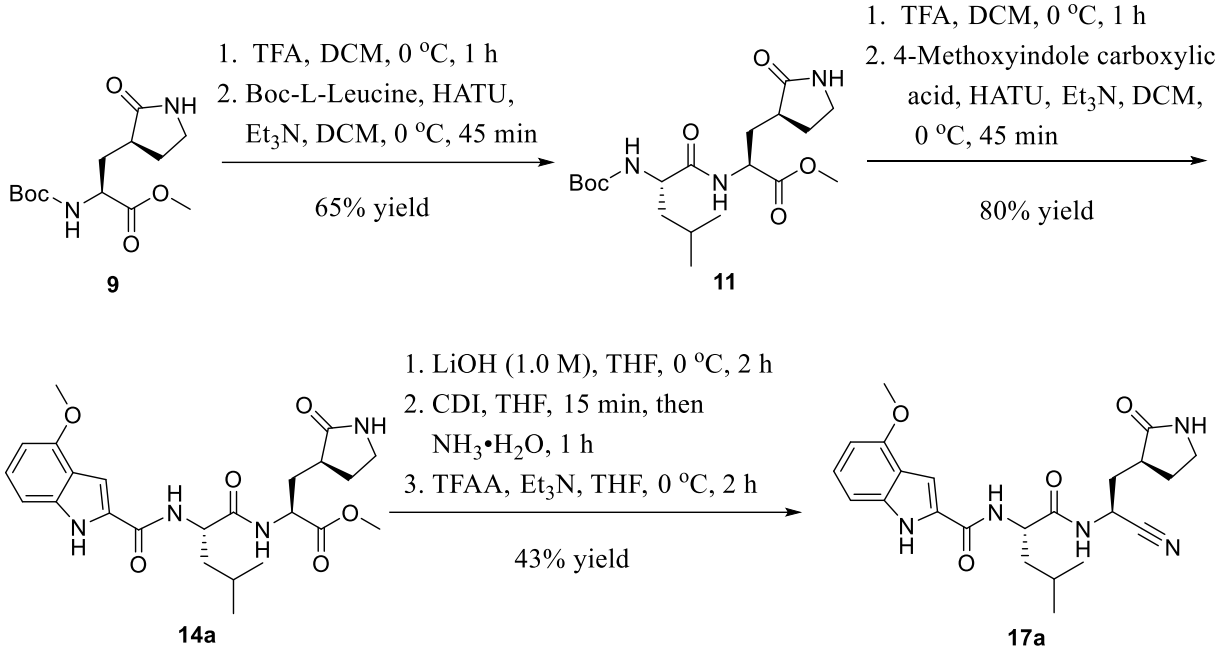
General. All reagents and solvents were used as purchased from commercial sources. Moisture sensitive reactions were carried out under a nitrogen atmosphere in oven-dried glassware. Reactions were stirred and at ambient temperature, unless otherwise stated. Reaction progress was monitored by TLC [pre-coated silica gel aluminum plates containing a fluorescent indicator (F-254)] with UV (254 nm) or staining (ninhydrin or potassium permanganate) or alternatively by LC/MS. ^{1}HNMR, ^{13}CNMR and ^{19}FNMR spectra were recorded at ambient temperature, unless indicate otherwise, with a Bruker Avance III 600 MHz NMR spectrometer equipped with a Bruker's 5 mm PABBO probe. DEPTQ was run for ^{13}CNMR. Chemical shifts are reported in ppm downfield from tetramethylsilane using residual solvent signals as internal reference for ^{1}HNMR and ^{13}CNMR. For ^{19}FNMR chemical shifts are reported relative to $CFCl_3$. NMR spectra were processed utilizing ACD/Spectrus processor (v2016.1.1, ACD/Labs Inc.) and Bruker TopSpin 4.0.6. Silica gel column purification was performed on a Biotage Isolera® system with either Biotage or Silicycle cartridges. The IR (infrared spectra) were taken from a Thermo Nicolet 8700 main bench with an attached Continuum FTIR microscope. The samples were analyzed in solid form on a silicon wafer substrate using the FTIR microscope. The sample and background spectra were acquired using 32 co-added scans for each at 4 wavenumber resolution. The absorption of nitriles was reported in cm^{-1}. The LC/MS system used for monitoring the progress of reactions and assessing the purity (absorbance at 254 nm) consisted of Dionex ULTIMATE 3000 uHPLC module and Thermo Scientific LTQ XL mass-spectrometer with electrospray ionization and Ion-Trap type of detector (alternating positive-negative mode). Separation was performed with Thermo Scientific™ Accucore™ aQ C18 Polar Endcapped LC column (100 mm × 2.1 mm; particle size 2.6 μm, 80 Å). The column was maintained at 30 °C. Commercial HPLC-grade methanol and domestic 'millipore (Milli-Q)' filtered water used for chromatography were modified by adding 0.1% (v/v) of formic acid. The eluent was delivered with constant flow rate of 0.4 mL/min, column was equilibrated for 5 min with the corresponding eluent prior to injection of the sample (1 to 3 μL). The gradient was methanol – water, 45 to 95% in 5.25 min, followed by 5 min of isocratic 95% methanol – water. Purity of the final compounds were all greater than 95% as determined by UV (254 nm), and the corresponding m/z had the correct M+H and/or M-H signal with the appropriate isotope pattern. High Resolution Mass Spectrometry (HRMS) work was done on an LTQ Orbitrap XL (Thermo Scientific) operated in positive mode. Compound **4** was prepared in reference 29. Compound **7** was prepared according to reference 15. Compound **9** and **10** were prepared as described in reference 27.

Reference 15: W. Vuong, *et al*. *Eur. J. Med Chem.* 2021, **222**, 113584.

Reference 27: Y. Zhai, *et al*. *J. Med. Chem.* 2015, **58**, 9414−9420.

Reference 29: W. Vuong *et al*. *Nat. Commun.* 2020, **11**, 4282 e1–8.

Synthesis of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-methoxy-1H-indole-2-carboxamide, 17a.

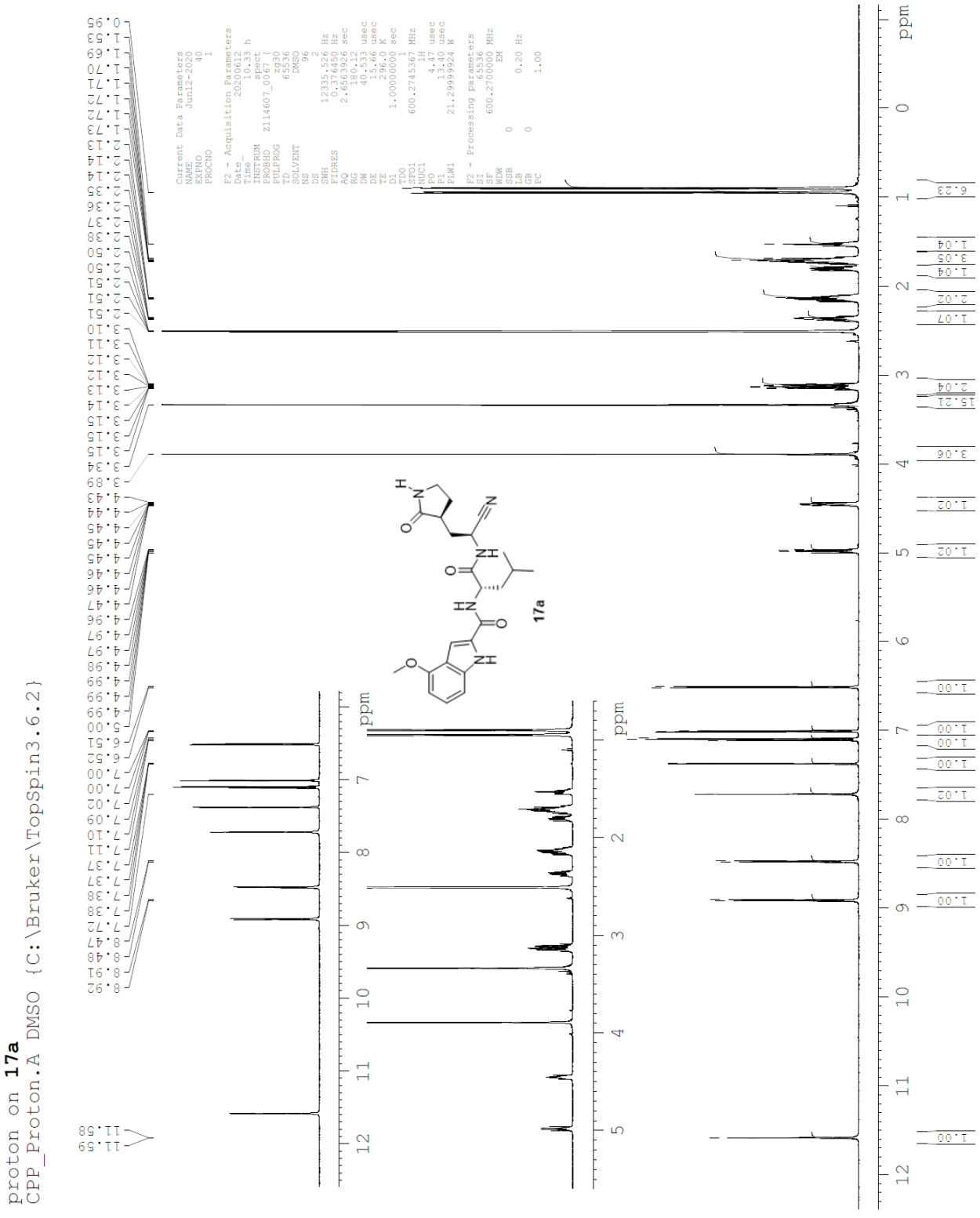


Preparation of methyl *N*-(*tert*-butoxycarbonyl)-L-leucyl-3-[(3*S*)-2-oxopyrrolidin-3-yl]-L-alaninate, **11**. Following a procedure in reference 36 [A.M. Priora *et al*. *Bioorg. Med. Chem. Lett.* 2013, **23**, 6317 – 6320] with minor modifications. To a solution of methyl *N*-(*tert*-butoxycarbonyl)-3-[(3*S*)-2-oxopyrrolidin-3-yl]-L-alaninate **9** (220 mg, 0.768 mmol) in DCM (5 mL) was cooled using an ice-water bath and then TFA (5 mL) was added. After 1 h, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 2 × 20 mL), and dried under reduced pressure for 1 h, afforded a white solid. To the solid was added DCM (10 mL) and Boc-L-Leucine (187 mg, 0.809 mmol) and after cooling in an ice-bath HATU (456 mg, 1.20 mmol) was added, followed by dropwise addition of Et_3N (0.536 mL, 3.85 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 20 mL) was added and the resulting mixture was extracted with DCM (2 × 15 mL). The combined organic layer was washed with saturated brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **11** (200 mg, 65 % yield) as a white solid. This material was consistent with that reported in reference 36.

Preparation of methyl *N*-(4-methoxy-1*H*-indole-2-carbonyl)-L-leucyl-3-[(3*S*)-2-oxopyrrolidin-3-yl]-L-alaninate, **14a**. To a solution of **11** (200 mg, 0.500 mmol) in DCM (5 mL) cooled in an ice-water bath was added TFA (5 mL). After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 2 × 20 mL), and dried under reduced pressure for 1 h to afford a white solid. Anhydrous DCM (10 mL) and 4-methoxy-1*H*-indole-2-carboxylic acid (105 mg, 0.549 mmol) were added to the solid and the solution was cooled in an ice bath after which HATU (304 mg, 0.800 mmol) was added followed by dropwise addition of Et_3N (0.209 mL, 1.50 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 20 mL) was added and the resulting mixture was extracted with DCM (2 × 15 mL). The combined organic layer was washed with

saturated brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **14a (**189 mg, 80% yield) as a white solid. LC/MS: 4.77 min retention time, 96.9% pure; ESI-MS: 473 $[M + H]^+$.

Preparation of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopyrrolidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, **17a**. To a solution of **14a** (189 mg, 0.400 mmol) in THF (5 mL) cooled in an ice bath was added a 1.0 M LiOH aqueous solution (4 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated and dried under reduced pressure. To the residue was added anhydrous THF (10 mL) and CDI (78.1 mg, 0.482 mmol). After 15 min, NH_3 (aq., 28%, 0.200 mL, 1.46 mmol) was added. After 2 h, water (5 mL) was added. The mixture was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the intermediate (90.0 mg) as a white sticky solid. To the intermediate (90.0 mg, 0.197 mmol) in anhydrous THF (5 mL) cooled in an ice bath was added Et_3N (82.9 µL, 0.595 mmol), followed by slow addition of TFAA (84.2 mg, 0.401 mmol). The progress of the reaction was monitored by TLC, once the intermediate disappeared after 2 h, water (5 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **17a** (75 mg, 43% yield for 3 steps) as a white solid. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.58 (d, *J* = 2.3 Hz, 1H), 8.91 (d, *J* = 7.9 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 7.72 (s, 1H), 7.37 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.09 (dd, *J* = 7.1, 8.1 Hz. 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.51 (d, *J* = 7.5 Hz, 1H), 5.01 – 4.95 (m, 1H), 4.49 – 4.41 (m, 1H), 3.89 (s, 3H), 3.18 – 3.08 (m, 2H), 2.41 – 2.33 (m, 1H), 2.19 – 2.09 (m, 2H), 1.84 – 1.76 (m, 1H), 1.76 – 1.65 (m, 3H), 1.57 – 1.49 (m, 1H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.90 (d, *J* = 6.8 Hz, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 177.5, 172.5, 161.2, 153.6, 137.8, 129.7, 124.5, 119.6, 118.1, 105.4, 101.3, 99.2, 55.1, 51.4, 40.0, 39.5, 38.3, 37.1, 33.4, 27.0, 24.4, 23.0, 21.3; LC/MS: 4.56 min retention time, 96.0% pure; ESI-MS: 440 $[M + H]^+$; IR: 2245 cm^{-1}; HRMS (ESI+) calcd for $C_{23}H_{29}N_5O_4$+H 440.2298, found 440.2309.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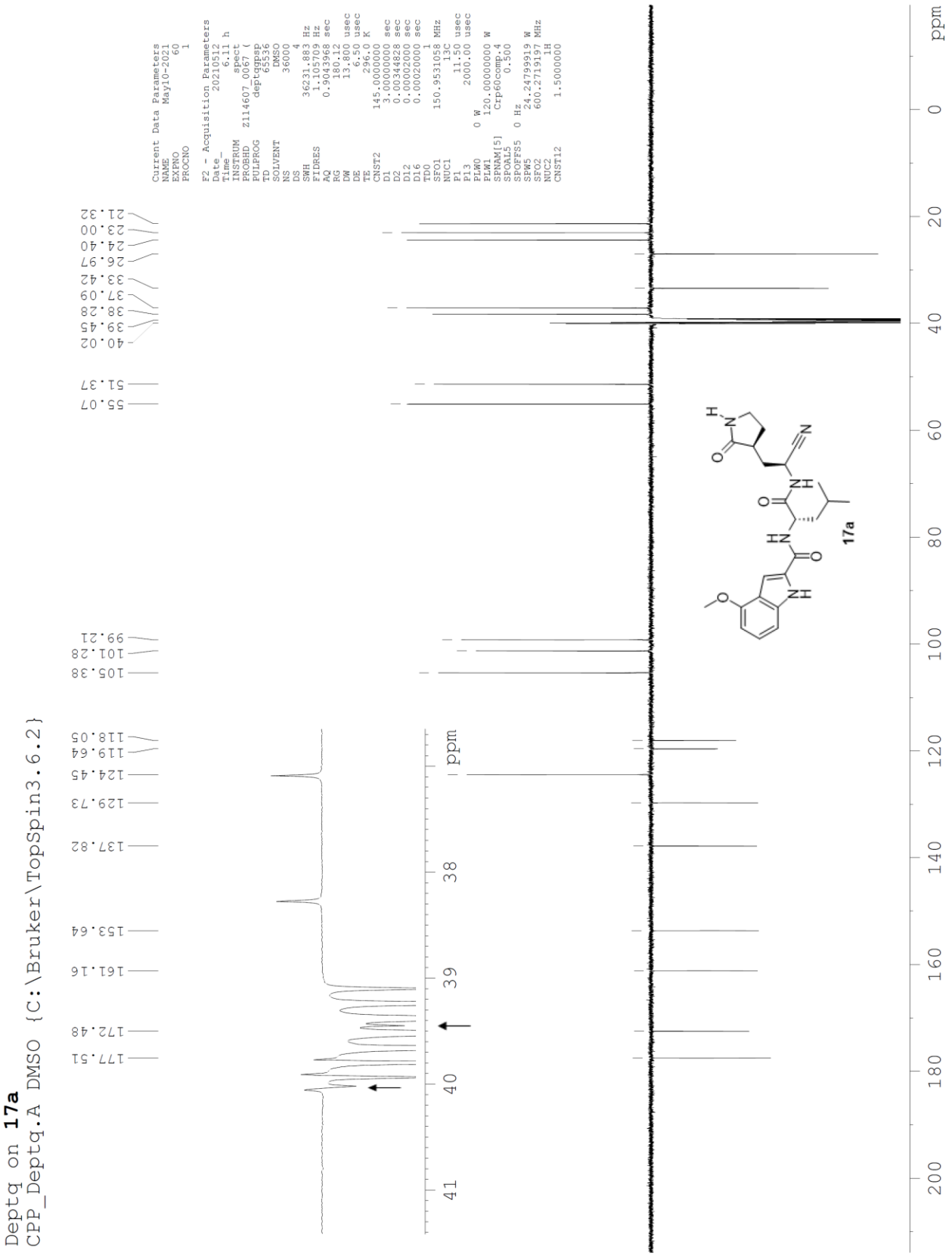
17a
ppm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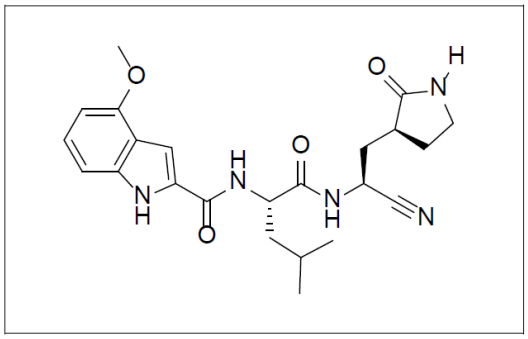
TD 65536
SOLVENT DMSO
NS 36000
DS 4
SWH 36231.883 Hz
FIDRES 1.105709 Hz
AQ 0.9043968 sec
RG 180.12
DW 13.800 usec
DE 6.50 usec
TE 296.0 K
CNST2 145.0000000
D1 3.00000000 sec
D2 0.00344828 sec
D12 0.00002000 sec
D16 0.00020001 sec
TD0 1
SFO1 150.9531058 MHz
NUC1 13C
P1 11.50 usec
P13 2000.00 usec
PLW0 0 W 120.00000000 W
PLW1 Crp60comp.4
SPNAM[5] 0.500
SPOAL5 0 Hz
SPOFFS5 24.24799919 W
SPW5 600.27719197 MHz
SFO2 1H
NUC2 1.5000000
CNST12
21.32
23.00
24.40
26.97
33.42
37.09
38.28
39.45
40.02
51.37
55.07
99.21
101.28
105.38
118.05
119.64
124.45
129.73
137.82
153.64
161.16
172.48
177.51
17a
ppm
ppm

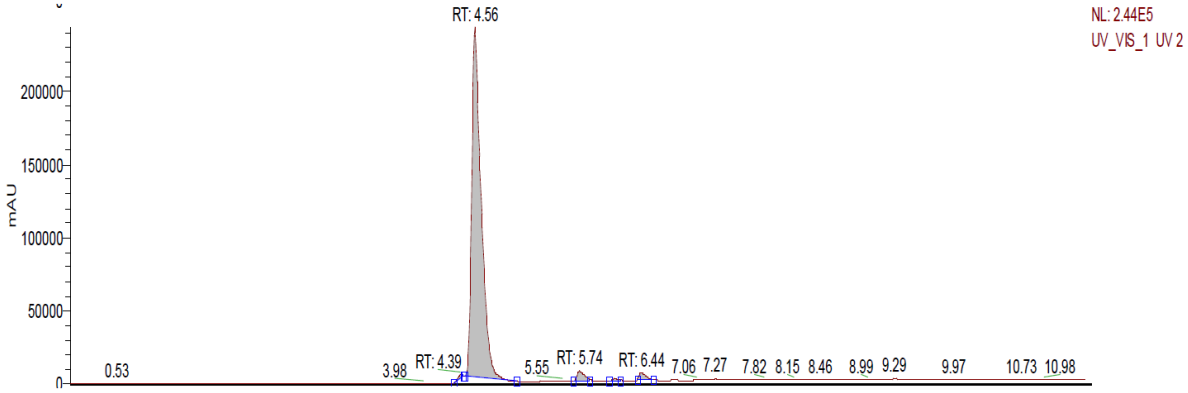
LC-MS Analysis of COMPOUND ____17a_____



Chromatogram

Column: Accucore aQ, 100 x 2.1 mm; particle size 2.6 µm;
Flow rate: 0.4 mL/min;
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

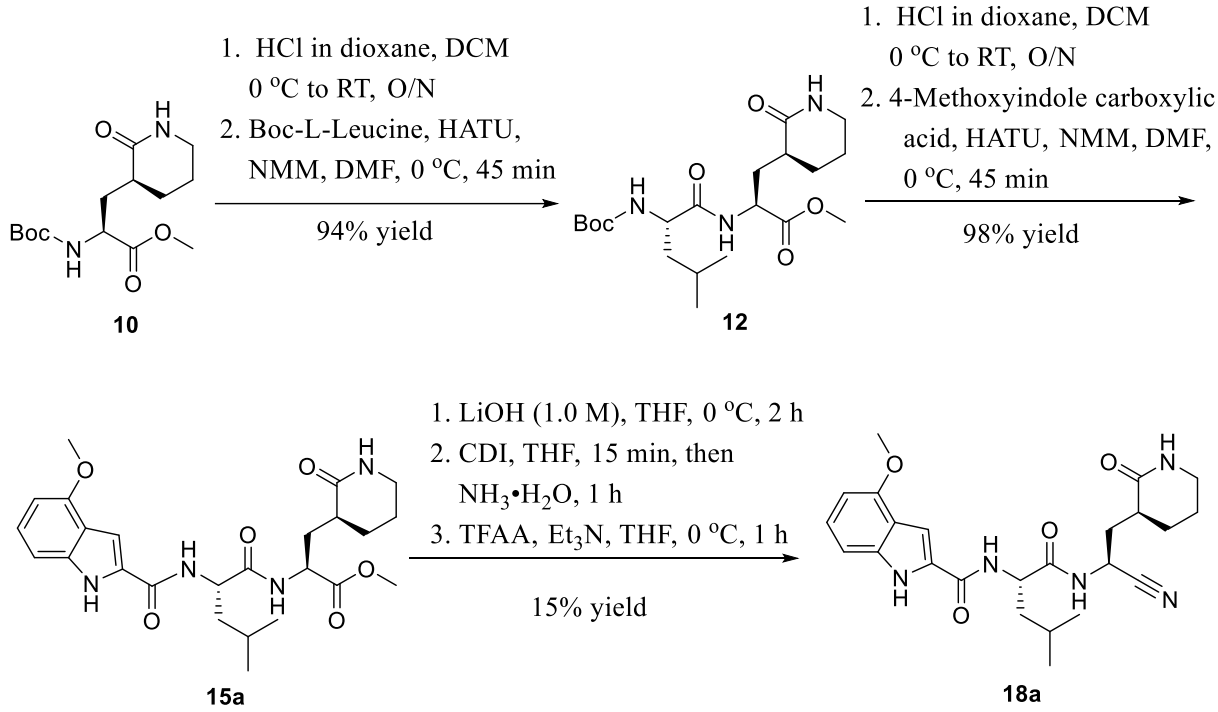
Detection: UV @ 254nm (UV2)



PEAK LIST
RT: 0.00 - 11.51
Number of detected peaks: 5 (blank subtracted)

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
4.39	4.32	4.43	7331.910	0.40	2390.143	0.94
4.56	4.45	5.03	1769923.172	**95.99**	238844.546	94.15
5.74	5.67	5.86	35772.351	1.94	6321.103	2.49
6.14	6.08	6.20	5285.470	0.29	1300.005	0.51
6.44	6.39	6.57	25495.752	1.38	4822.158	1.90

Synthesis of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, 18a.



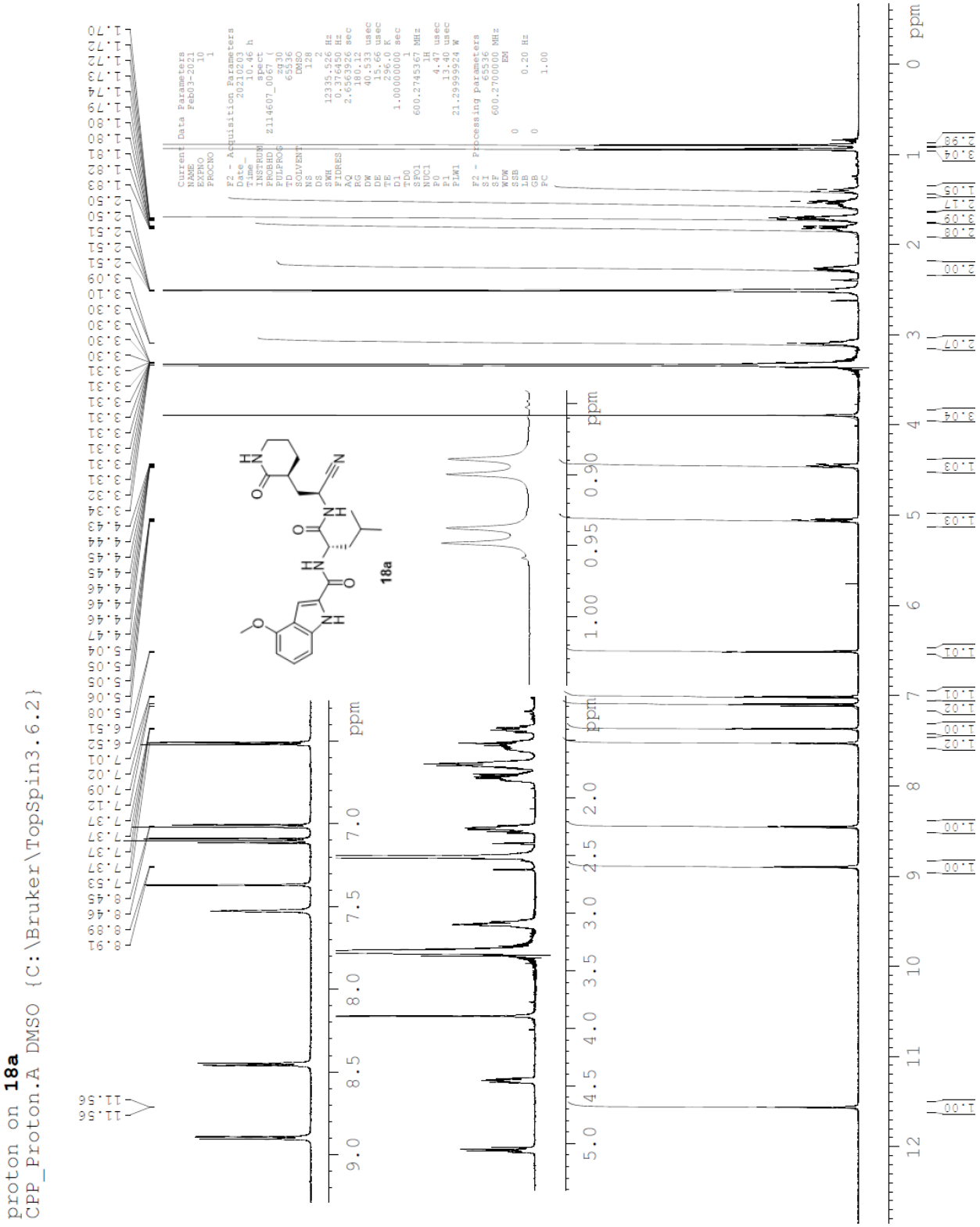
Preparation of methyl *N*-(*tert*-butoxycarbonyl)-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **12**. Prepared as described in reference 37 [Y. Zhai et al. *Eur. J. Med. Chem.* 2016, **124**, 559]. To a solution of methyl *N*-(*tert*-butoxycarbonyl)-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate **10** (501 mg, 1.67 mmol) in DCM (10 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (10 mL) was added. After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. Co-evaporated with DCM/ether (1:1, 3 × 20 mL), and drying under reduced pressure for 1 h afforded the hydrochloride salt as a white solid. This material was used without further purification in the next step. To a solution of hydrochloride salt and *N*-(*tert*-butoxycarbonyl)-L-leucine (425 mg, 1.84 mmol) in anhydrous DMF (20 mL) cooled in an ice bath was added HATU (700 mg, 1.84 mmol), followed by addition of NMM (0.551 mL, 5.01 mmol) dropwise. After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 20 mL) was added and the resulting mixture was extracted with EtOAc (3 × 50 mL). The combined organic layer was washed with saturated brine solution (20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **12** (650 mg, 94% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 8.31 (d, *J* = 8.1 Hz, 1H), 7.42 (s, 1H), 6.85 (d, *J* = 8.0 Hz, 1H), 4.41 – 4.31 (m, 1H), 3.98 – 3.87 (m, 1H), 3.60 (s, 3H), 3.13 – 3.04 (m, 2H), 2.28 – 2.13 (m, 2H), 1.89 – 1.80 (m, 1H), 1.77 – 1.68 (m, 1H), 1.69 – 1.56 (m, 2H), 1.56 – 1.48 (m, 1H), 1.36 (s, 9H), 1.42 – 1.28 (m, 3H), 0.87 (d, *J* = 6.6 Hz, 3H), 0.84 (d, *J* = 6.6 Hz, 3H).

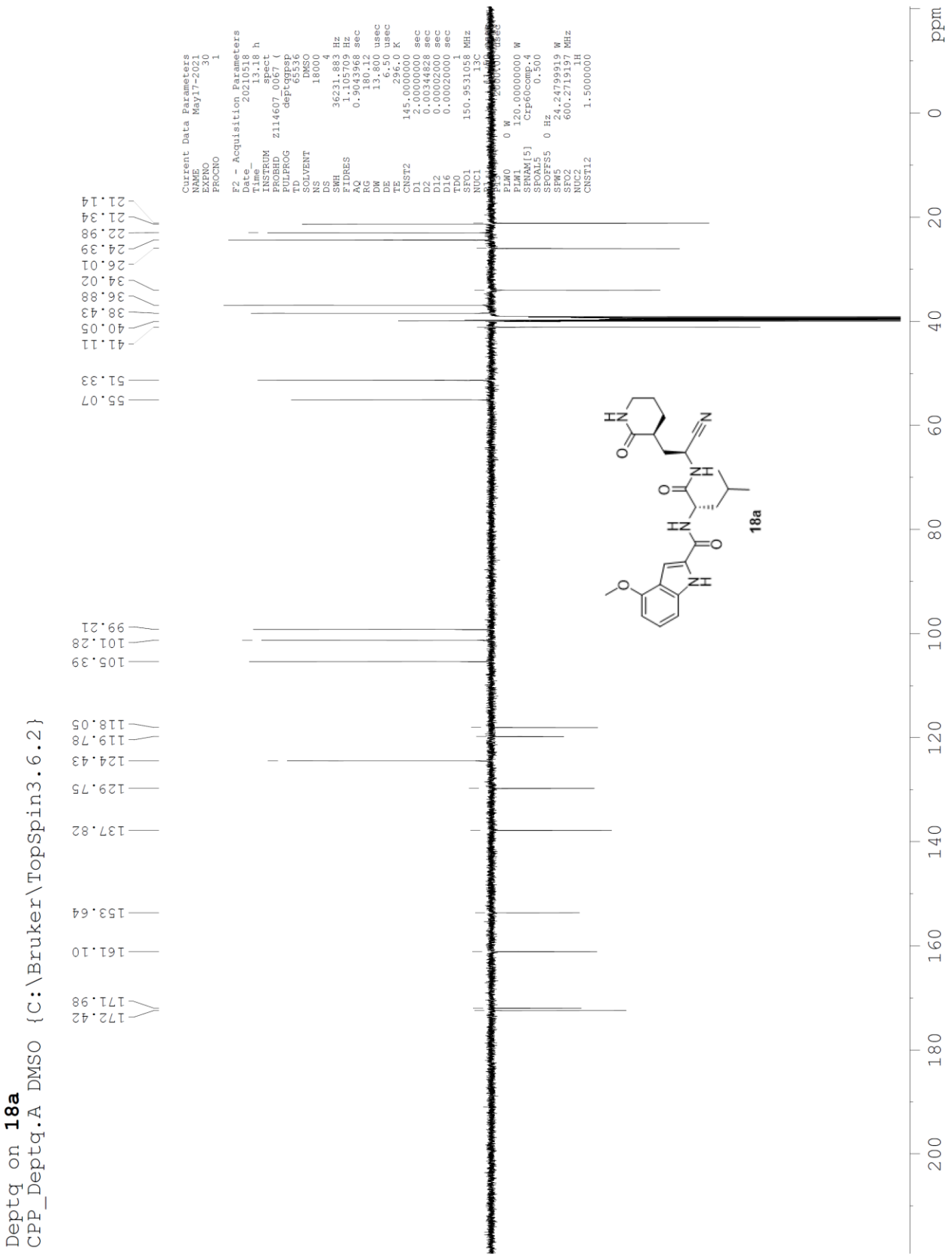
Preparation of methyl *N*-(4-methoxy-1*H*-indole-2-carbonyl)-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **15a**. A solution of **12** (200 mg, 0.484 mmol) in DCM (5 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (5 mL) was added. After 30 min, the ice bath

was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and dried under reduced pressure produced a white solid. The resulting residue was dissolved in in anhydrous DMF (20 mL) and the solution cooled in an ice-bath. To the solution was added 4-methoxy-1H-indole-2-carboxylic acid (101 mg, 0.528 mmol), HATU (202 mg, 0.531 mmol), and then dropwise NMM (0.162 mL, 1.44 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 20 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with saturated brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **15a** (231 mg, 98% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.54 (d, *J* = 2.1 Hz, 1H), 8.52 (d, *J* = 8.0 Hz, 1H), 8.35 (d, *J* = 8.1 Hz, 1H), 7.43 (s, 1H), 7.34 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.09 (dd, *J* = 7.7, 8.2 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.50 (d, *J* = 7.7 Hz, 1H), 4.55 – 4.49 (m, 1H), 4.43 – 4.37 (m, 1H), 3.88 (s, 3H), 3.60 (s, 3H), 3.14 – 3.03 (m, 2H), 2.30 – 2.20 (m, 2H), 1.87 – 1.80 (m, 1H), 1.75 – 1.62 (m, 4H), 1.58 – 1.49 (m, 2H), 1.38 – 1.30 (m, 1H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H). LC/MS: 4.95 min retention time, 96.5% pure; ESI-MS 487 $[M+H]^+$.

Preparation of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, **18a**. To a solution of **15a** (200 mg, 0.411 mmol) in THF (5 mL) in an ice bath was added slowly a 1.0 M LiOH aqueous solution (5 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was dried under reduced pressure. To the residue was added anhydrous THF (10 mL) and then CDI (100 mg, 0.617 mmol). The mixture was stirred for 15 min before NH_3 (aq., 28%, 0.260 mL, 1.89 mmol) was added. The reaction mixture was then stirred for 1 h before it was quenched with water (10 mL). The mixture was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the amide intermediate (116 mg) as a white solid. To this amide (115 mg, 0.243 mmol) in anhydrous THF (10 mL) was added Et_3N (0.10 mL, 0.729 mmol) at 0 ºC, followed by addition of TFAA (102 mg, 0.486 mmol) slowly at this temperature. The progress of the reaction was monitored by TLC and once the intermediate disappeared after 1h, water (10 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 5 % MeOH in $CHCl_3$, which generated **18a** (28 mg, 15% yield for 3 steps) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.55 (d, *J* = 2.1 Hz, 1H), 8.89 (d, *J* = 8.1 Hz, 1H), 8.45 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.36 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.09 (dd, *J* = 7.7, 8.2 Hz, 1H), 7.00 (d, *J* = 8.2 Hz, 1H), 6.51 (d, *J* = 7.7 Hz, 1H), 5.08 – 5.01 (m, 1H), 4.48 – 4.41 (m, 1H), 3.88 (s, 3H), 3.13 – 3.03 (m, 2H), 2.31 – 2.20 (m, 2H),1.87 – 1.76 (m, 2H), 1.75 – 1.64 (m, 3H), 1.60 – 1.46 (m, 2H), 1.44 – 1.34 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 172.4, 172.0, 161.1, 153.6, 137.8, 129.8, 124.4, 119.8, 118.0, 105.4, 101.3, 99.2, 55.1, 51.3, 41.1, 40.1, 38.4, 36.9, 34.0, 26.0, 24.4, 23.0, 21.3, 21.1; LC/MS: 4.75 min retention

time, 97.4% pure; ESI-MS: 454 $[M + H]^+$; IR: 2244 cm^{-1}; HRMS (ESI+) calcd for $C_{24}H_{31}N_5O_4$+H 454.2454, found 454.2470.

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PROCNO 1
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161.10
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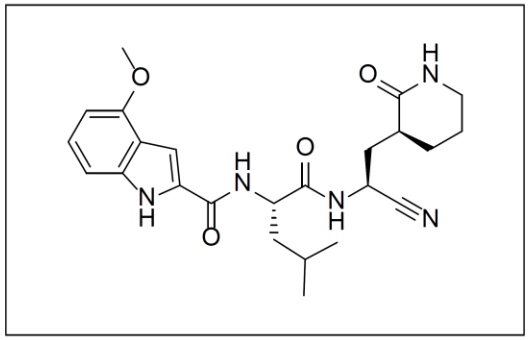
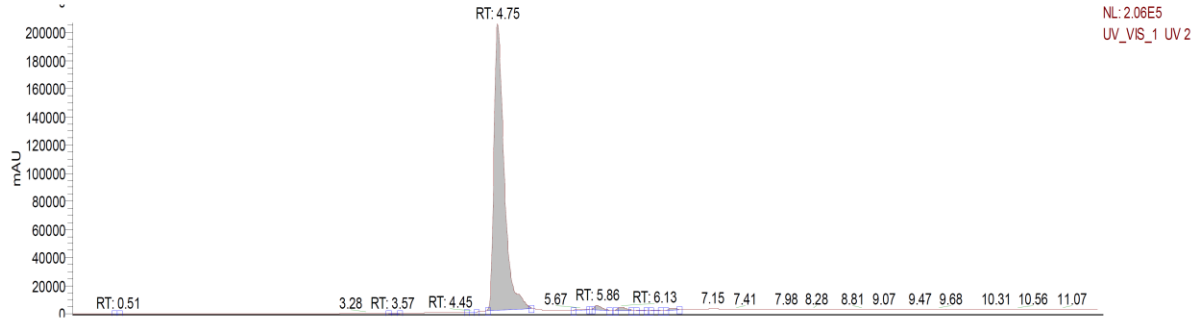
LC-MS Analysis of COMPOUND ____18a_____



Chromatogram

Column: Accucore aQ, 100 x 2.1 mm; particle size 2.6 µm;
Flow rate: 0.4 mL/min;
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

Detection: UV @ 254nm (UV2)



PEAK LIST
RT: 0.00 - 11.50
Number of detected peaks: 10 (blank subtracted)

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
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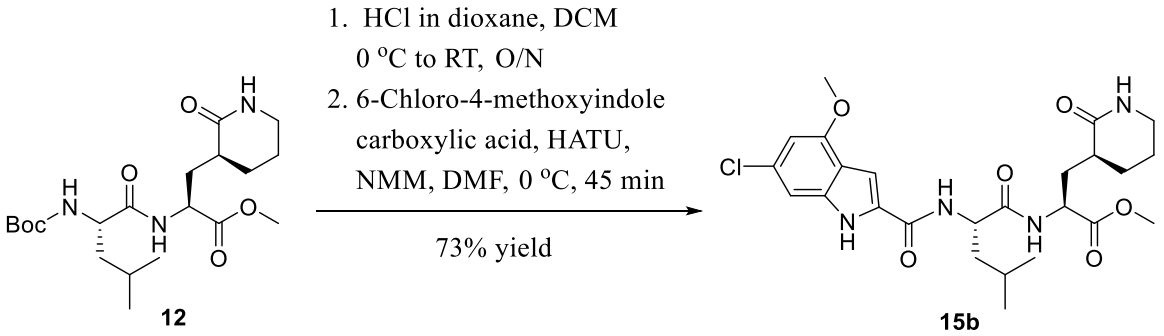
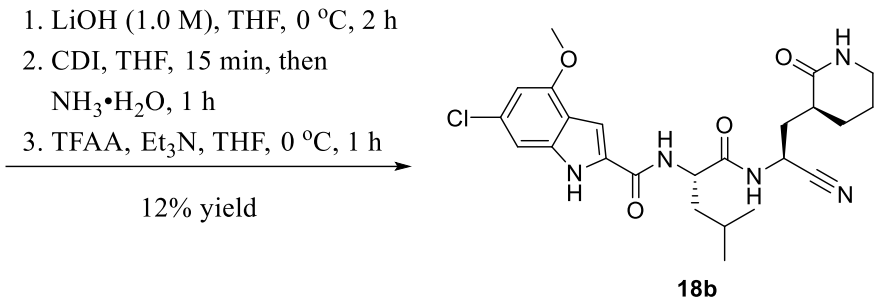
3.57	3.53	3.65	238.823	0.01	54.930	0.03
4.45	4.40	4.51	217.034	0.01	55.885	0.03
4.75	4.63	5.13	1611975.806	**97.41**	203687.002	96.31
5.65	5.59	5.77	4041.556	0.24	616.850	0.29
5.86	5.80	5.99	17807.242	1.08	3027.265	1.43
6.13	6.06	6.24	12437.192	0.75	2247.530	1.06
6.35	6.30	6.41	441.834	0.03	114.395	0.05
6.51	6.46	6.57	582.710	0.04	153.174	0.07
6.70	6.63	6.78	6621.058	0.40	1335.073	0.63

Synthesis of 6-chloro-*N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, 18b.

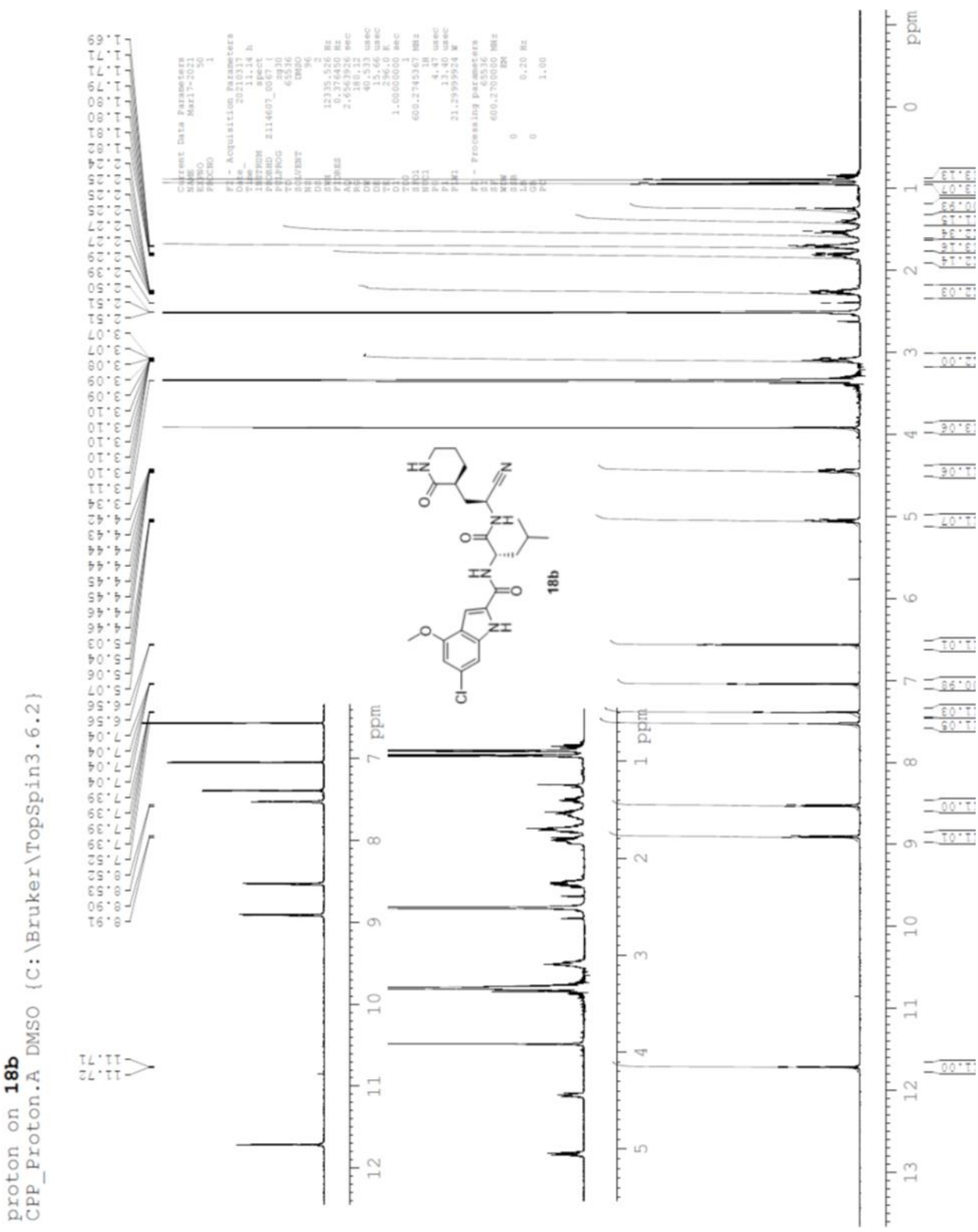




Preparation of methyl *N*-(6-chloro-4-methoxy-1*H*-indole-2-carbonyl)-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **15b**. A solution of **12** (201 mg, 0.486 mmol) in DCM (5 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (5 mL) was added. After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and dried under reduced pressure for 1 h. To the residue was added anhydrous DMF (10 mL) and 6-chloro-4-methoxy-1*H*-indole-2-carboxylic acid (120 mg, 0.532 mmol) and the solution was cooled in an ice bath. To the cooled solution was added HATU (202 mg, 0.531 mmol), followed by dropwise addition of NMM (0.160 mL, 1.46 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **15b** (185 mg, 73% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.69 (d, *J* = 2.1 Hz, 1H), 8.54 (d, *J* = 8.0 Hz, 1H), 8.42 (d, *J* = 8.1 Hz, 1H), 7.43 (s, 1H), 7.36 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.03 (dd, *J* = 0.8, 1.6 Hz, 1H), 6.55 (d, *J* = 1.6 Hz, 1H), 4.54 – 4.48 (m, 1H), 4.42 – 4.36 (m, 1H), 3.91 (s, 3H), 3.60 (s, 3H), 3.13 – 3.03 (m, 2H), 2.29 – 2.20 (m, 2H), 1.87 – 1.80 (m, 1H), 1.75 – 1.62 (m, 4H), 1.57 – 1.46 (m, 2H), 1.38 – 1.29 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H). LC/MS: 6.01 min retention time, 92.0% pure; ESI-MS: 521 $[M + H]^+$.

Preparation of 6-chloro-*N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, **18b**. To a solution of **15b** (140 mg, 0.270 mmol) in THF (5 mL) cooled in an ice bath was slowly added a 1.0 M LiOH aqueous solution (5 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over

anhydrous Na_2SO_4, filtered, and concentrated and dried under reduced pressure. To the residue was added THF (10 mL) and CDI (88.2 mg, 0.544 mmol). After 15 min, NH_3 (aq., 28%, 0.201 mL, 1.46 mmol) was added. After 1 h, water (10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the intermediate (108 mg) as a white solid. To this intermediate (106 mg, 0.238 mmol) in anhydrous THF (10 mL) cooled in an ice bath was added Et_3N (88.0 µL, 0.631 mmol), followed by slow addition of TFAA (88.4 mg, 0.421 mmol). The progress of the reaction was monitored by TLC and once the intermediate disappeared after 1 h, water (10 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 5 % MeOH in $CHCl_3$, which generated **18b** (16.3 mg, 12% yield for 3 steps) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.71 (d, *J* = 2.1 Hz, 1H), 8.90 (d, *J* = 8.1 Hz, 1H), 8.52 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.38 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.04 (dd, *J* = 0.9, 1.5 Hz, 1H), 6.56 (d, *J* = 1.5 Hz, 1H), 5.07 – 5.01 (m, 1H), 4.47 – 4.41 (m, 1H), 3.91 (s, 3H), 3.14 – 3.03 (m, 2H), 2.30 – 2.20 (m, 2H), 1.86 – 1.75 (m, 2H), 1.74 – 1.63 (m, 3H), 1.60 – 1.46 (m, 2H), 1.44 – 1.34 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.5 Hz, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 172.3, 172.0, 160.8, 154.1, 137.3, 130.5, 128.7, 119.8, 116.8, 105.0, 101.3, 100.8, 55.6, 51.4, 41.1, 40.0, 38.4, 36.9, 34.0, 26.0, 24.4, 23.0, 21.3, 21.1; LC/MS: 5.87 min retention time, 96.3% pure; ESI-MS: 488 $[M + H]^+$; IR: 2244 cm^{-1}; HRMS (ESI+) calcd for $C_{24}H_{30}ClN_5O_4$+H 488.2065, found 488.2079.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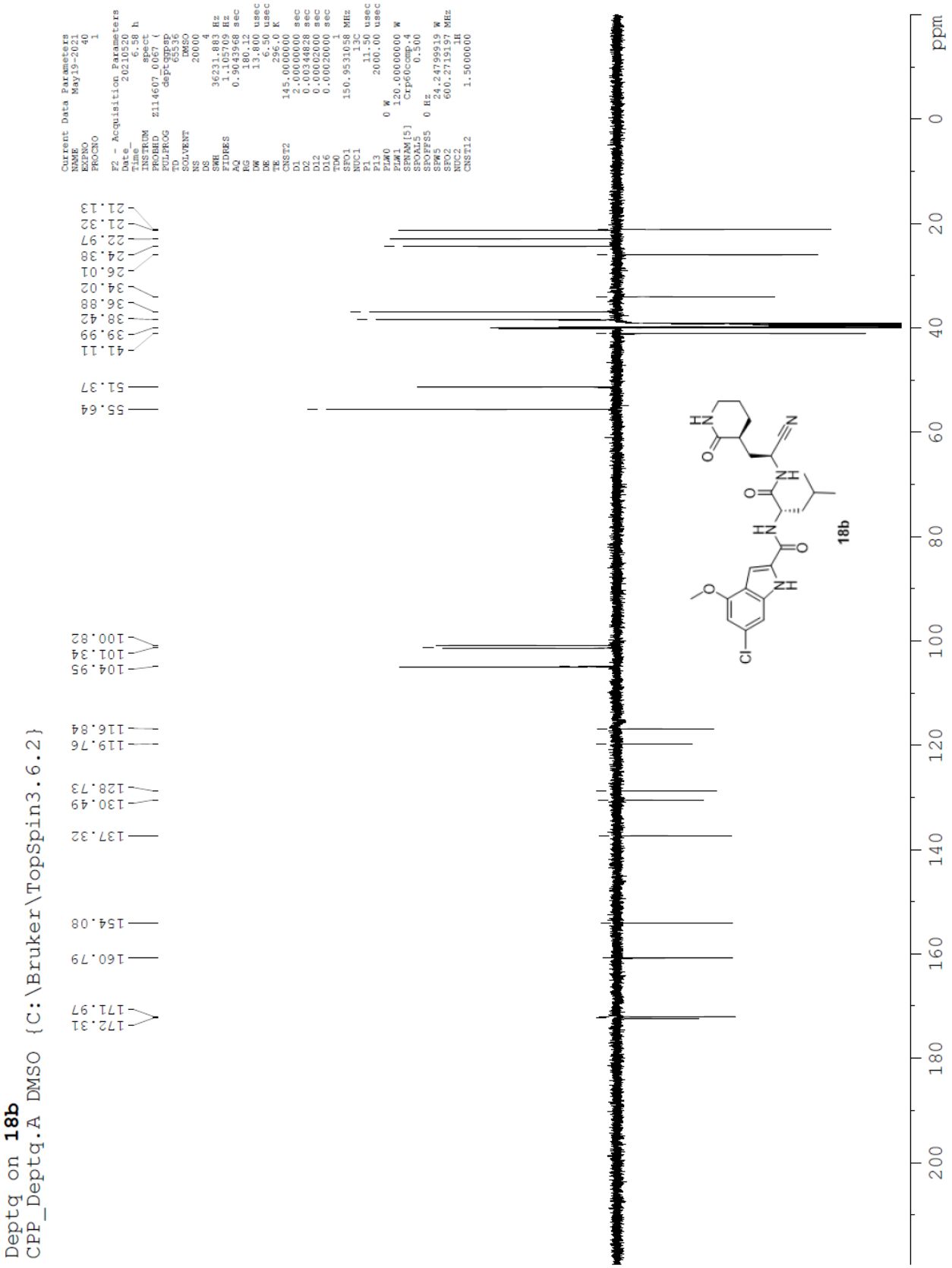
18b
ppm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SWH
FIDRES
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RG
DW
DE
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D2
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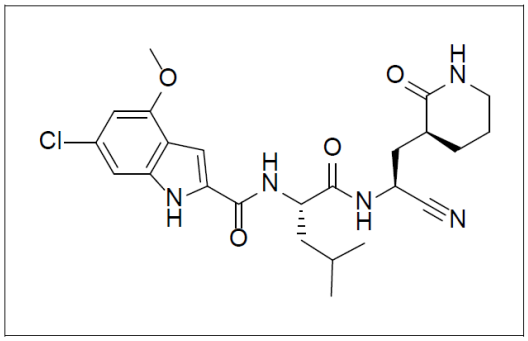
18b
ppm

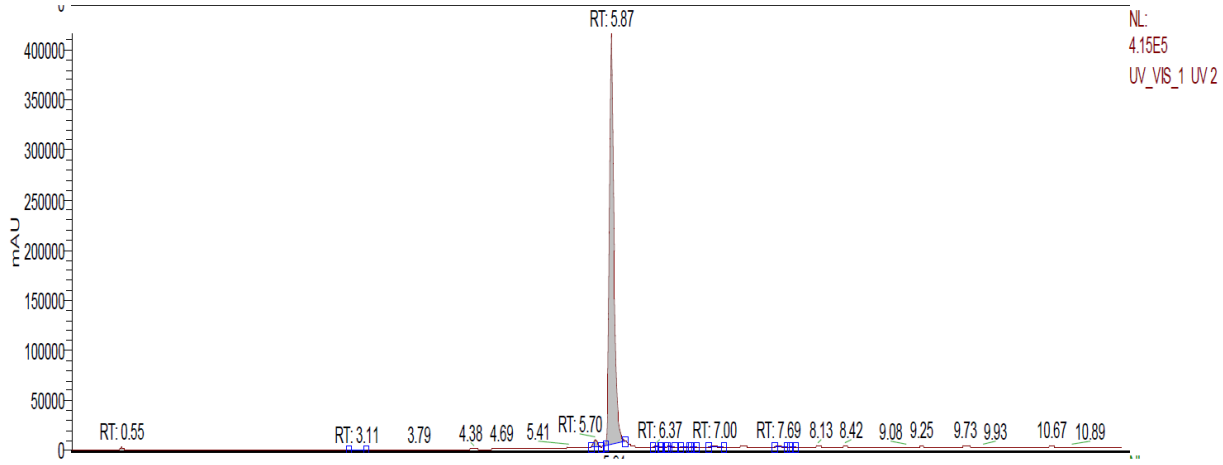
LC-MS Analysis of COMPOUND ____18b_____



Chromatogram

Column: Accucore aQ, 100 x 2.1 mm; particle size 2.6 µm;
Flow rate: 0.4 mL/min;
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

Detection: UV @ 254nm (UV2)



PEAK LIST
RT: 0.00 – 11.50
Number of detected peaks: 12 (blank substracted)

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
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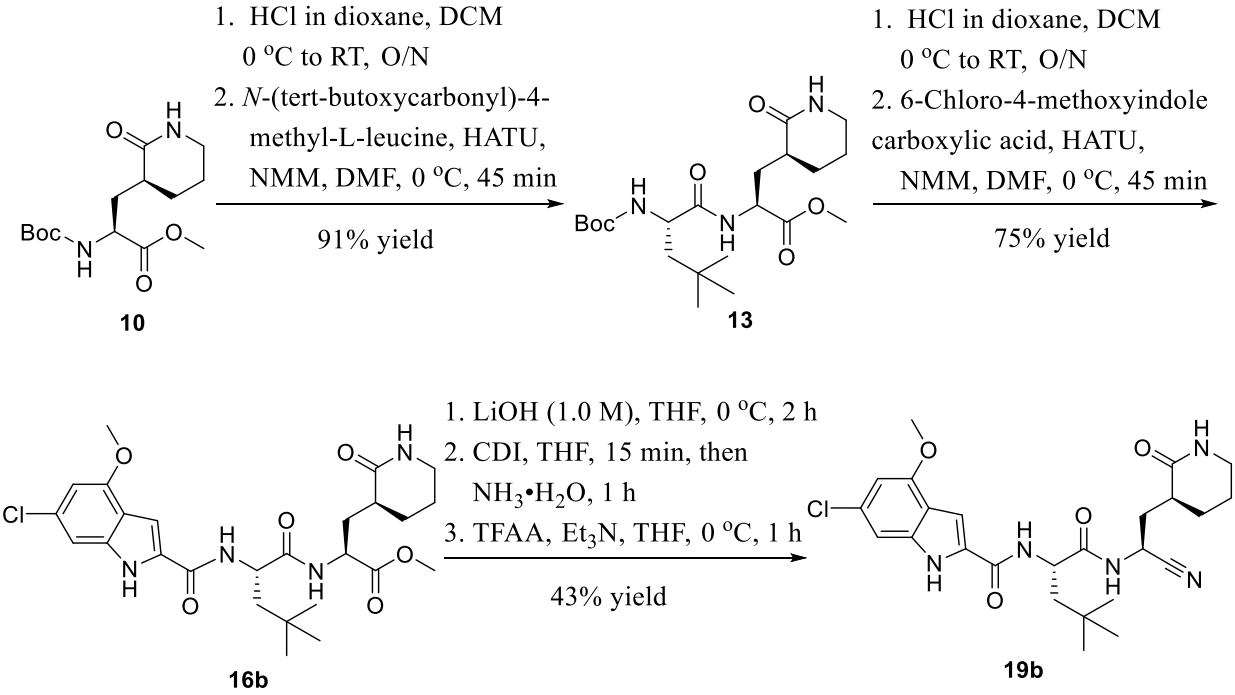
3.11	3.01	3.20	5492.846	0.37	948.335	0.22
5.70	5.65	5.76	22415.050	1.50	7724.318	1.81
5.87	5.81	6.03	1440244.184	**96.30**	408659.429	95.90
6.37	6.33	6.41	3548.234	0.24	1530.765	0.36
6.44	6.42	6.47	551.838	0.04	326.179	0.08
6.51	6.48	6.56	2490.933	0.17	1000.251	0.23
6.67	6.63	6.72	949.563	0.06	407.979	0.10
6.77	6.73	6.80	1097.436	0.07	436.215	0.10
7.00	6.93	7.09	5061.788	0.34	1158.115	0.27
7.69	7.65	7.79	1540.191	0.10	344.185	0.08
7.84	7.81	7.88	577.799	0.04	224.831	0.05

Synthesis of 6-chloro-*N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4,4-dimethyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, 19b



Preparation of methyl *N*-(*tert*-butoxycarbonyl)-4-methyl-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **13**. To a solution of **10** (200 mg, 0.667 mmol) in DCM (5 mL) cooled using an ice-water bath was adeded 4 M HCl in 1,4-dioxane (5 mL). After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and dried under reduced pressure afforded 157 mg of a white solid. To the solid was added *N*-(*tert*-butoxycarbonyl)-4-methyl-L-leucine (182 mg, 0.742 mmol) and anhydrous DMF (10 mL) and the resulting solution was cooled in an ice bath. Then HATU (282 mg, 0.741 mmol) was added, followed by dropwise addition of NMM (0.220 mL, 2.01 mmol) dropwise. After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with saturated brine solution (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **13** (260 mg, 91% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 8.22 (d, *J* = 8.0 Hz, 1H), 7.43 (s, 1H), 6.87 (d, *J* = 8.5 Hz, 1H), 4.38 – 4.32 (m, 1H), 3.99 (td, *J* = 8.5, 3.8 Hz, 1H), 3.59 (s, 3H), 3.14 – 3.04 (m, 2H), 2.28 – 2.15 (m, 2H), 1.88 – 1.80 (m, 1H), 1.77 – 1.69 (m, 1H), 1.67 – 1.61 (m, 1H), 1.56 – 1.41 (m, 3H), 1.37 (s, 9H), 1.36 – 1.31 (m, 1H), 0.89 (s, 9H).

Preparation of methyl *N*-(6-chloro-4-methoxy-1*H*-indole-2-carbonyl)-4-methyl-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **16b**. A solution of **13** (186 mg, 0.435 mmol) in DCM (5 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (5 mL) was added. After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and drying under reduced pressure. To the residue was added 6-chloro-4-methoxy-1*H*-indole-2-carboxylic acid

(108 mg, 0.480 mmol) and anhydrous DMF (10 mL) and the solution was cooled in an ice bath. Then HATU (183 mg, 0.481 mmol) was added, followed by dropwise addition of NMM (0.150 mL, 1.32 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in EtOAc, which generated **16b** (175 mg, 75% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.70 (d, *J* = 2.2 Hz, 1H), 8.48 (d, *J* = 7.9 Hz, 1H), 8.45 (d, *J* = 8.5 Hz, 1H), 7.43 (s, 1H), 7.34 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.04 (dd, *J* = 0.8, 1.6 Hz, 1H), 6.55 (d, *J* = 1.6 Hz, 1H), 4.57 (td, *J* = 8.9, 3.4 Hz, 1H), 4.41 – 4.34 (m, 1H), 3.90 (s, 3H), 3.59 (s, 3H), 3.10 – 3.02 (m, 2H), 2.29 – 2.17 (m, 2H), 1.85 – 1.78 (m, 1H), 1.77 – 1.71 (m, 1H), 1.70 – 1.63 (m, 3H), 1.54 – 1.44 (m, 1H), 1.38 – 1.28 (m, 1H), 0.93 (s, 9H). LC/MS: 6.28 min retention time, 98.9% pure; ESI-MS 535 $[M+H]^+$.

Preparation of 6-chloro-*N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4,4-dimethyl-1-oxopentan-2-yl]-4-methoxy-1*H*-indole-2-carboxamide, **19b**. To a solution of **16b** (150 mg, 0.280 mmol) in THF (5 mL) in an ice bath was added a 1.0 M LiOH aqueous solution (5 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated and dried under reduced pressure. To the residue was added THF (10 mL) and CDI (91.0 mg, 0.561 mmol). After 15 min, NH_3 (aq., 28%, 0.20 mL, 1.46 mmol) was added. After 1 h, water (10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the intermediate (124 mg) as a white solid. To this solid (124 mg, 0.238 mmol) dissolved in anhydrous THF (10 mL) and cooled in an ice bath was added Et_3N (0.101 mL, 0.714 mmol), followed by slow addition of TFAA (101 mg, 0.480 mmol). The progress of the reaction was carefully monitored by TLC and once the intermediate disappeared after 1h, water (10 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 5 % MeOH in $CHCl_3$, which generated **19b** (59.4 mg, 43% yield over 3 steps) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.71 (d, *J* = 2.2 Hz, 1H), 8.88 (d, *J* = 8.0 Hz, 1H), 8.53 (d, *J* = 8.0 Hz, 1H), 7.52 (s, 1H), 7.35 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.04 (dd, *J* = 0.8, 1.6 Hz, 1H), 6.55 (d, *J* = 1.6 Hz, 1H), 5.07 – 5.00 (m, 1H), 4.49 (td, *J* = 8.6, 3.6 Hz, 1H), 3.91 (s, 3H), 3.12 – 3.01 (m, 2H), 2.30 – 2.20 (m, 2H), 1.84 – 1.73 (m, 3H), 1.71 – 1.63 (m, 2H), 1.57 – 1.48 (m, 1H), 1.42 – 1.33 (m, 1H), 0.93 (s, 9H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 172.5, 172.0, 160.4, 154.1, 137.3, 130.7, 128.7, 119.6, 116.8, 105.0, 101.3, 100.8, 55.6, 50.6, 44.2, 41.1, 38.5, 36.9, 33.9, 30.3, 29.5 (3C), 26.0, 21.1; LC/MS: 6.05 min retention time, 99.7% pure; ESI-MS: 502 $[M + H]^+$; IR: 2245 cm^{-1}; HRMS (ESI+) calcd for $C_{25}H_{32}ClN_5O_4$+H 502.2221, found 502.2249.

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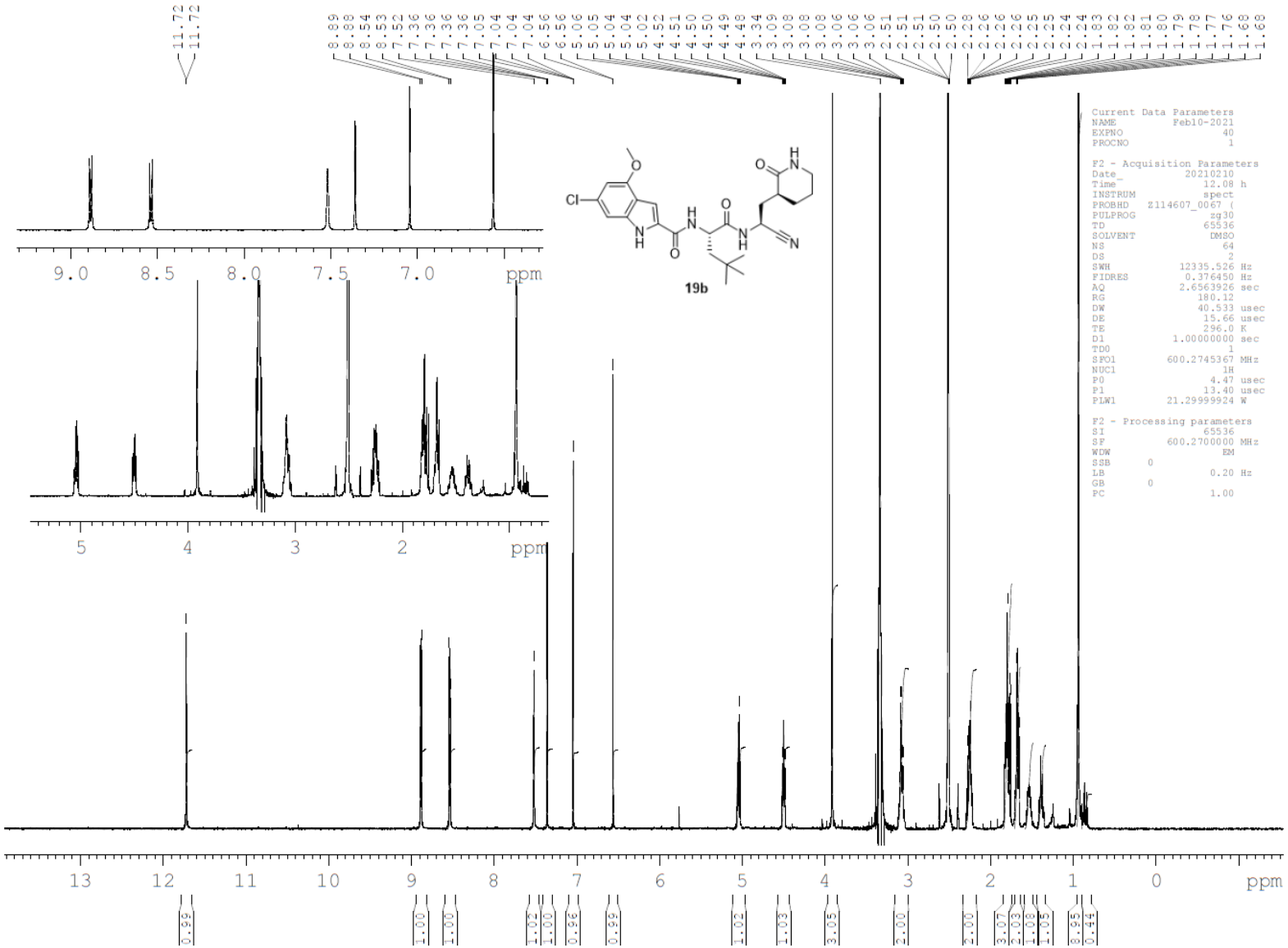

Deptq on **19b**
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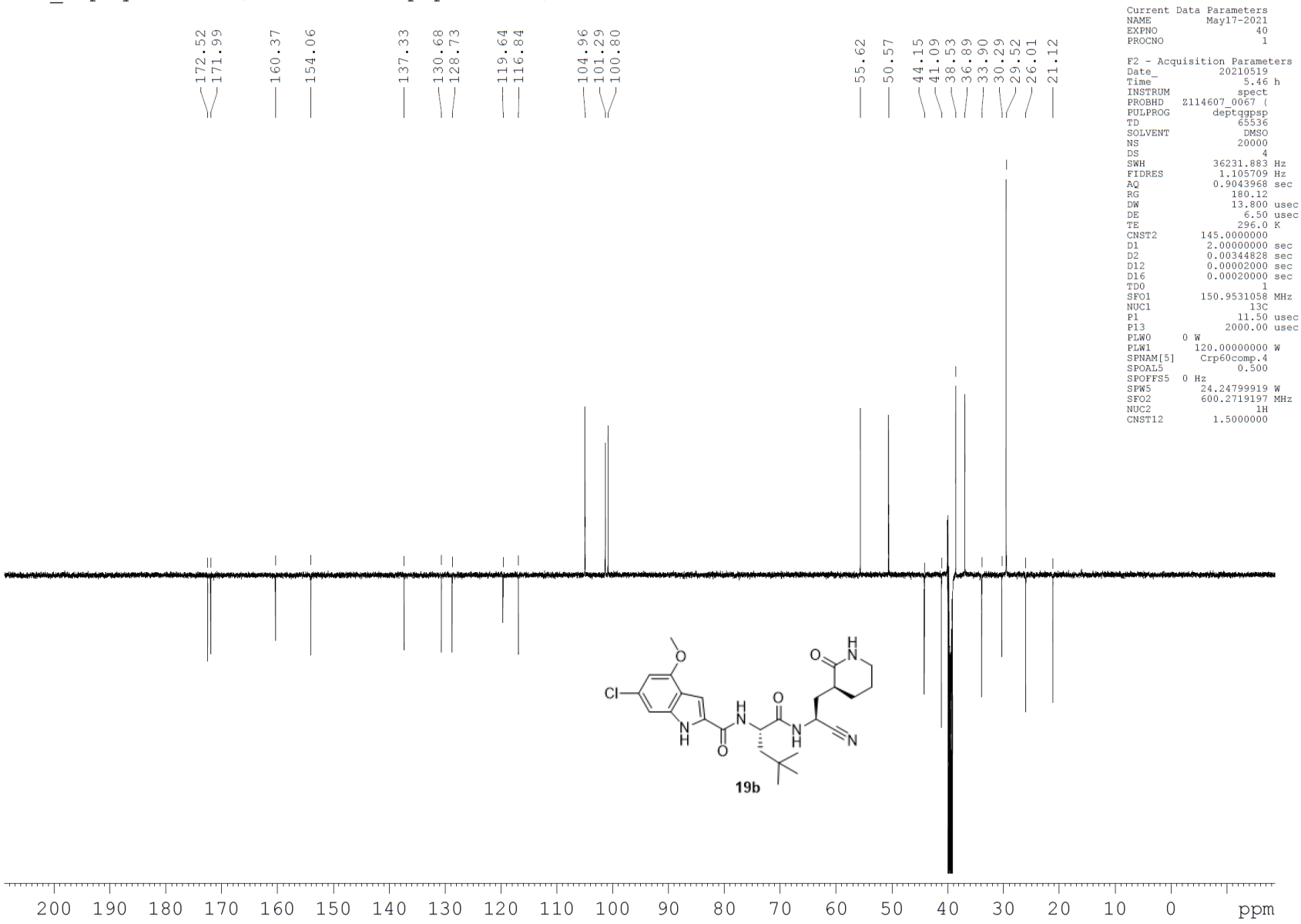
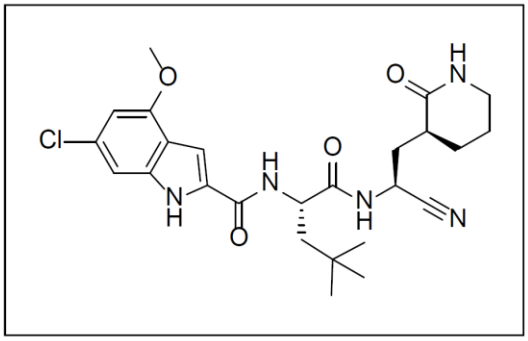


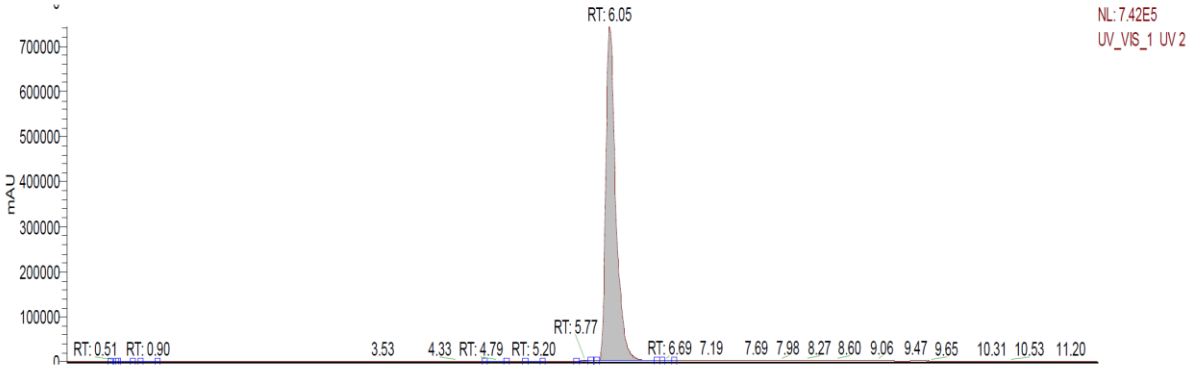
LC-MS Analysis of COMPOUND ____19b_____



Chromatogram

Column: Accucore aQ, 100 x 2.1 mm; particle size 2.6 µm;
Flow rate: 0.4 mL/min;
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

Detection: UV @ 254nm (UV2)



PEAK LIST
RT: 0.00 - 11.49
Number of detected peaks: 8 (blank substracted)

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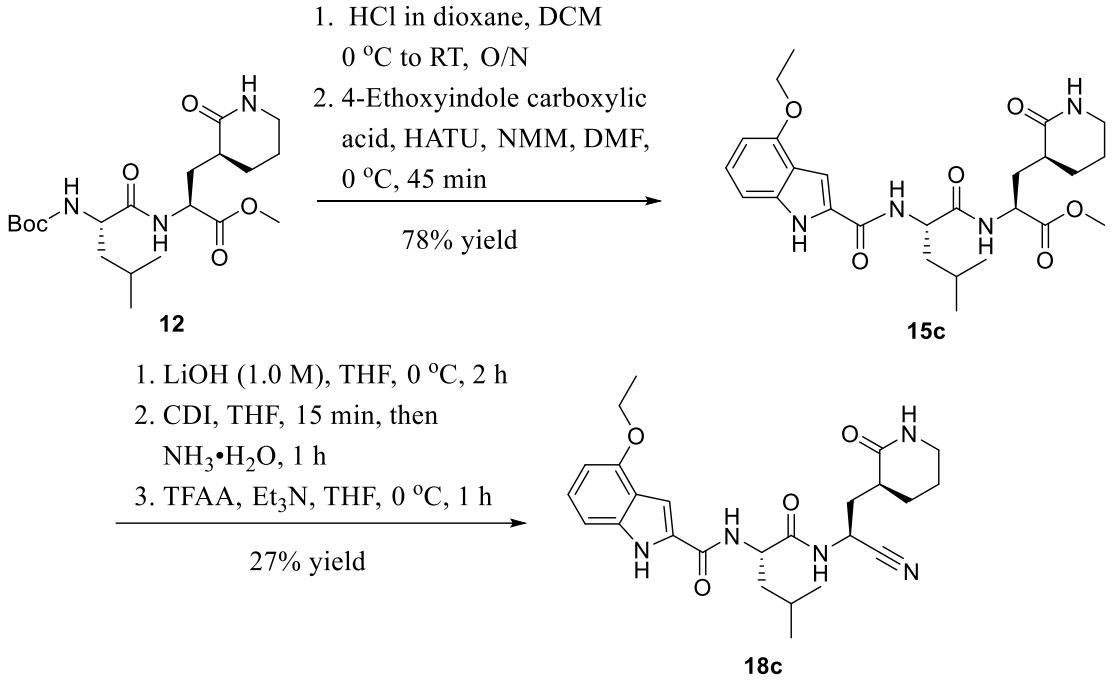
0.51	0.48	0.55	455.222	0.01	203.055	0.03
0.65	0.57	0.73	1418.496	0.02	226.929	0.03
0.90	0.81	1.01	2609.488	0.04	460.817	0.06
4.79	4.66	4.90	3579.752	0.06	376.214	0.05
5.20	5.11	5.30	2790.584	0.05	392.832	0.05
5.77	5.68	5.84	1571.060	0.03	254.667	0.03
6.05	5.91	6.58	5810454.544	**99.74**	739745.943	99.66
6.69	6.63	6.77	2880.800	0.05	612.670	0.08

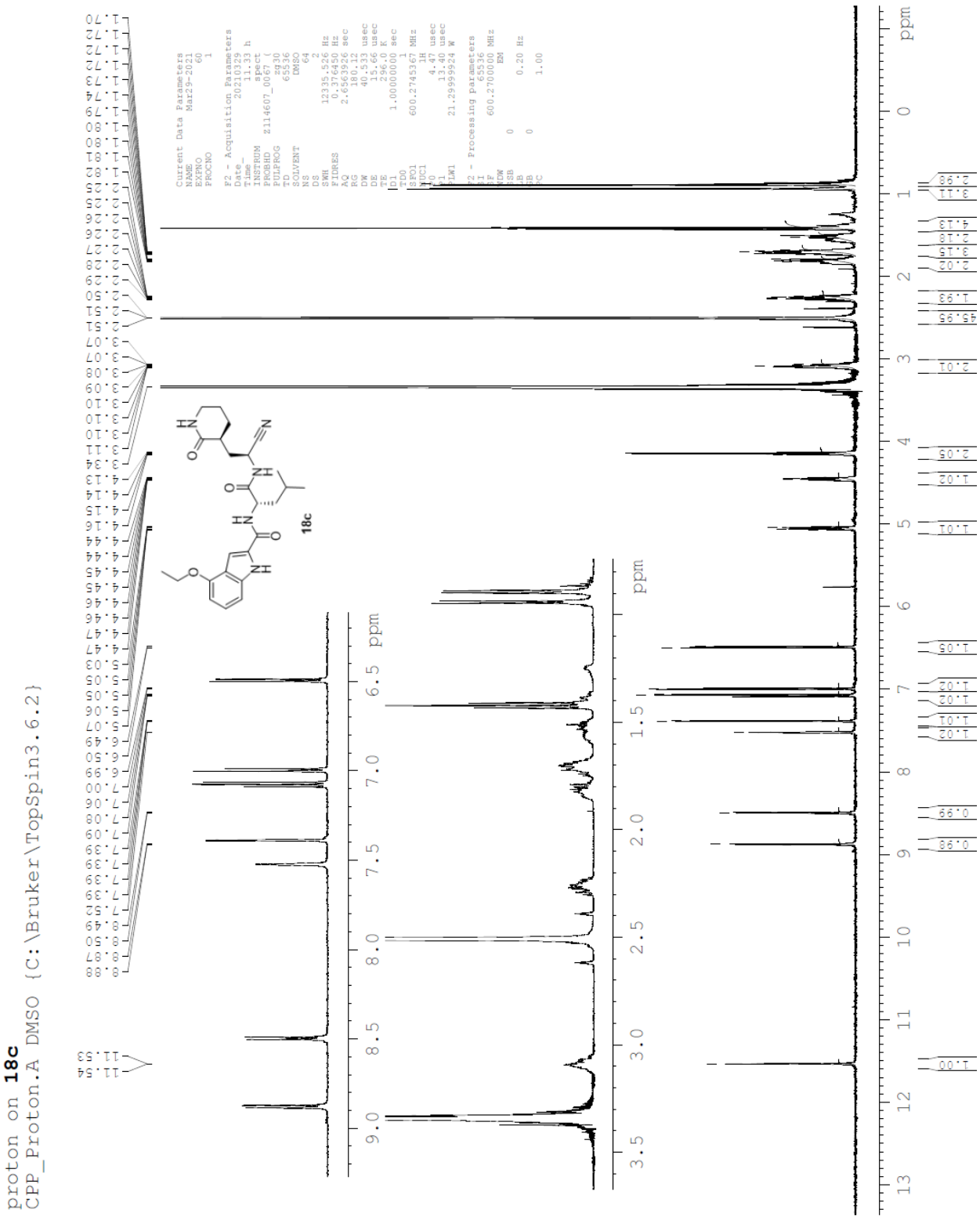
Synthesis of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-ethoxy-1*H*-indole-2-carboxamide, 18c.



Preparation of methyl *N*-(4-ethoxy-1*H*-indole-2-carbonyl)-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **15c**. A solution of **12** (175 mg, 0.423 mmol) in DCM (5 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (5 mL) was added. After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and dried under reduced pressure for 1 h. To the residue was added anhydrous DMF (10 mL) and 4-ethoxy-1*H*-indole-2-carboxylic acid (94.4 mg, 0.460 mmol) and the solution was cooled in an ice bath upon which HATU (175 mg, 0.459 mmol) was added, followed by dropwise addition of NMM (0.140 mL, 1.26 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in EtOAc, which generated **15c** (165 mg, 78% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.51 (d, *J* = 2.1 Hz, 1H), 8.49 (d, *J* = 8.0 Hz, 1H), 8.39 (d, *J* = 8.2 Hz, 1H), 7.43 (s, 1H), 7.36 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.06 (dd, *J* = 7.7, 8.3 Hz, 1H), 6.99 (d, *J* = 8.3 Hz, 1H), 6.48 (d, *J* = 7.6 Hz, 1H), 4.55 – 4.49 (m, 1H), 4.43 – 4.37 (m, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 3.60 (s, 3H), 3.13 – 3.03 (m, 2H), 2.30 – 2.20 (m, 2H), 1.87 – 1.80 (m, 1H), 1.73 – 1.63 (m, 4H), 1.57 – 1.50 (m, 2H), 1.41 (t, *J* = 7.0 Hz, 3H), 1.38 – 1.30 (m, 1H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H). LC/MS: 5.71 min retention time, 98.8% pure; ESI-MS: 501 [M + H]+.

Preparation of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-ethoxy-1*H*-indole-2-carboxamide, **18c**. To a solution of **15c** (145 mg, 0.290 mmol) in THF (5 mL) cooled in an ice bath was slowly added a 1.0 M LiOH aqueous solution (5 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25

mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated and dried under reduced pressure. To residue was added THF (10 mL) and CDI (94.0 mg, 0.580 mmol). After 15 min, NH_3 (aq., 28%, 0.220 mL, 1.60 mmol) was added. After 1 h, water (10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the intermediate (121 mg) as a white solid. To this intermediate (120 mg, 0.247 mmol) in anhydrous THF (10 mL) cooled in an ice bath was added Et_3N (0.101 mL, 0.741 mmol), followed by slow addition of TFAA (104 mg, 0.494 mmol). After 1 h, water (10 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 5 % MeOH in $CHCl_3$, which generated **18c** (36.8 mg, 27% yield for 3 steps) as a light yellow solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.53 (d, *J* = 2.1 Hz, 1H), 8.87 (d, *J* = 8.0 Hz, 1H), 8.49 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.38 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.07 (dd, *J* = 7.8, 8.2 Hz, 1H), 6.99 (d, *J* = 8.2 Hz, 1H), 6.48 (d, *J* = 7.6 Hz, 1H), 5.08 – 5.01 (m, 1H), 4.48 – 4.41 (m, 1H), 4.14 (q, *J* = 7.0 Hz, 2H), 3.13 – 3.03 (m, 2H), 2.32 – 2.20 (m, 2H), 1.86 – 1.76 (m, 2H), 1.75 – 1.64 (m, 3H), 1.60 – 1.46 (m, 2H), 1.41 (t, *J* = 7.0 Hz, 3H), 1.44 – 1.34 (m, 1H), 0.93 (d, *J* = 6.5 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H); ^{13}CNMR (151 MHz, DMSO-d_6) δ 172.4, 172.0, 161.1, 152.9, 137.9, 129.7, 124.4, 119.8, 118.2, 105.3, 101.3, 100.0, 62.9, 51.3, 41.1, 40.0, 38.4, 36.9, 34.0, 26.0, 24.4, 23.0, 21.3, 21.1, 14.9; LC/MS: 5.57 min retention time, 95.9% pure; ESI-MS: 468 $[M + H]^+$; IR: 2247 cm^{-1}; HRMS (ESI+) calcd for $C_{25}H_{33}N_5O_4$+H 468.2611, found 468.2629.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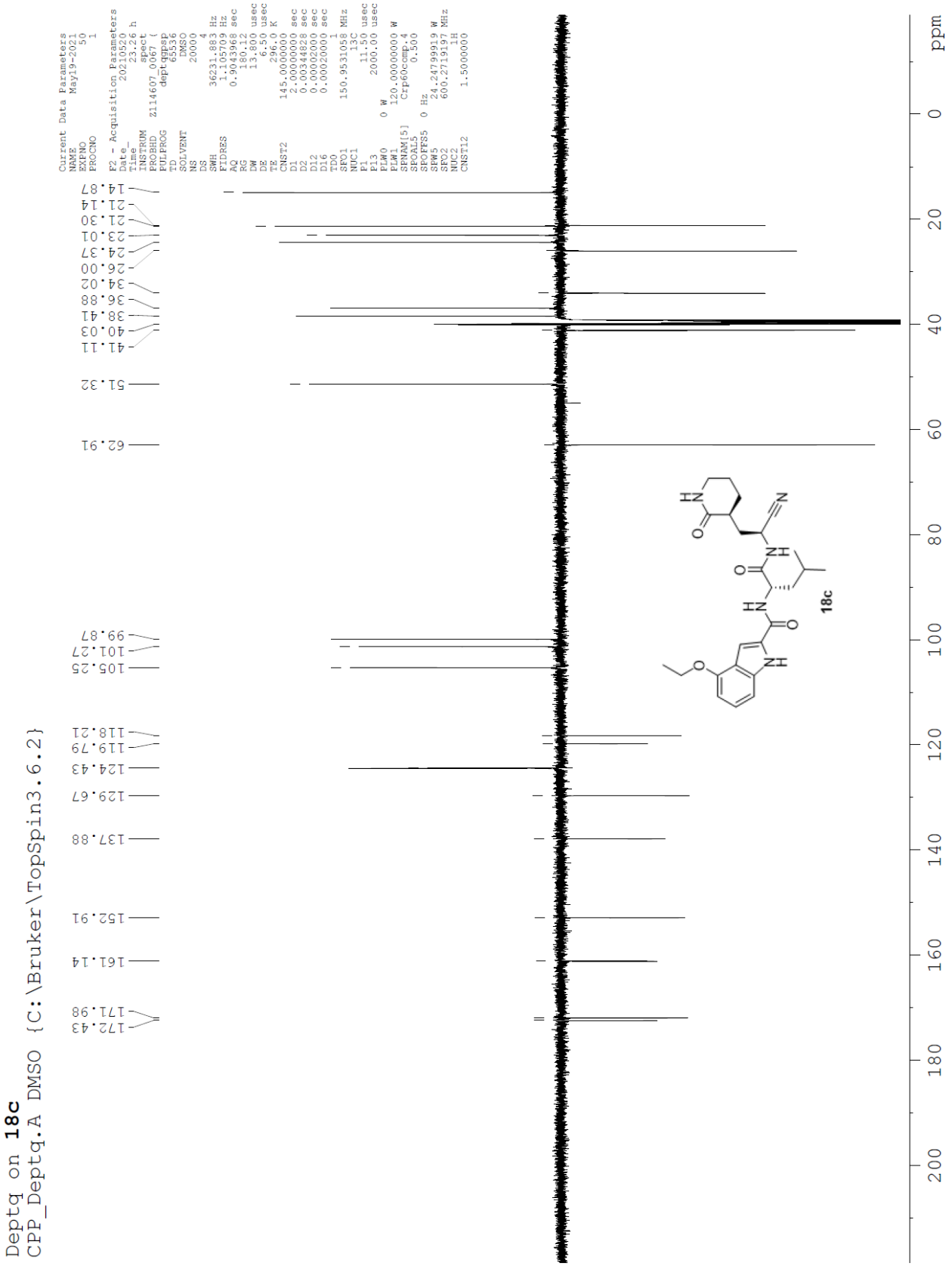
18c
ppm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SWH
FIDRES
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RG
DW
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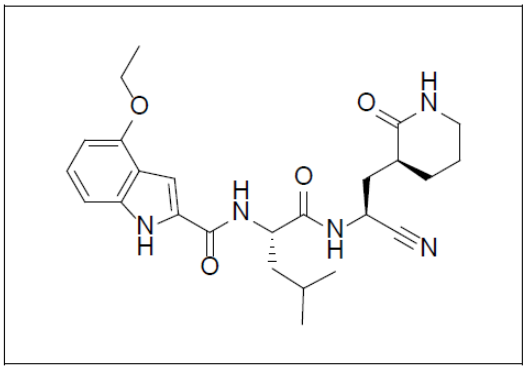
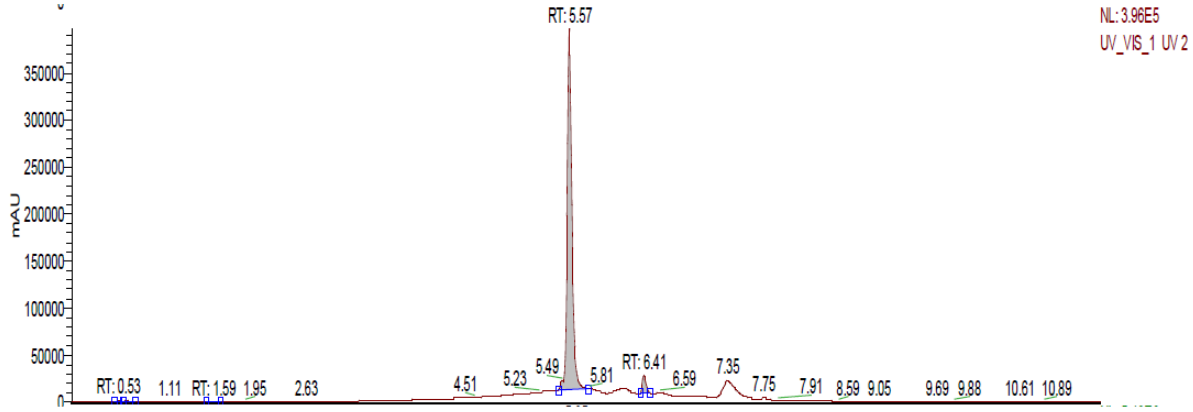
LC-MS Analysis of COMPOUND ____18c_____



Chromatogram

Column: Accucore aQ, 100 x 2.1 mm; particle size 2.6 µm;
Flow rate: 0.4 mL/min;
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

Detection: UV @ 254nm (UV2)



PEAK LIST
RT: 0.00 – 11.50
Number of detected peaks: 5 (blank substracted)

Apex RT	Start RT	End RT	Area	%Area	Height	%Height
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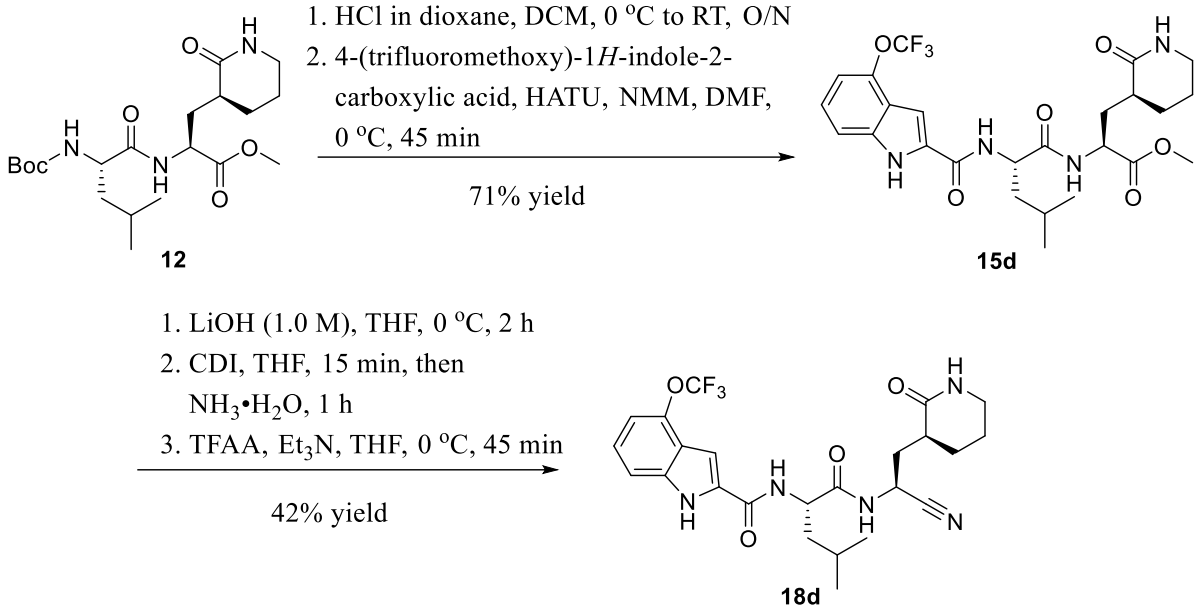
0.62	0.59	0.71	3511.596	0.24	888.890	0.22
1.59	1.51	1.67	1494.742	0.10	262.263	0.07
5.57	5.45	5.79	1411001.246	**95.86**	382081.447	94.78
6.41	6.37	6.47	52304.788	3.55	18280.640	4.53

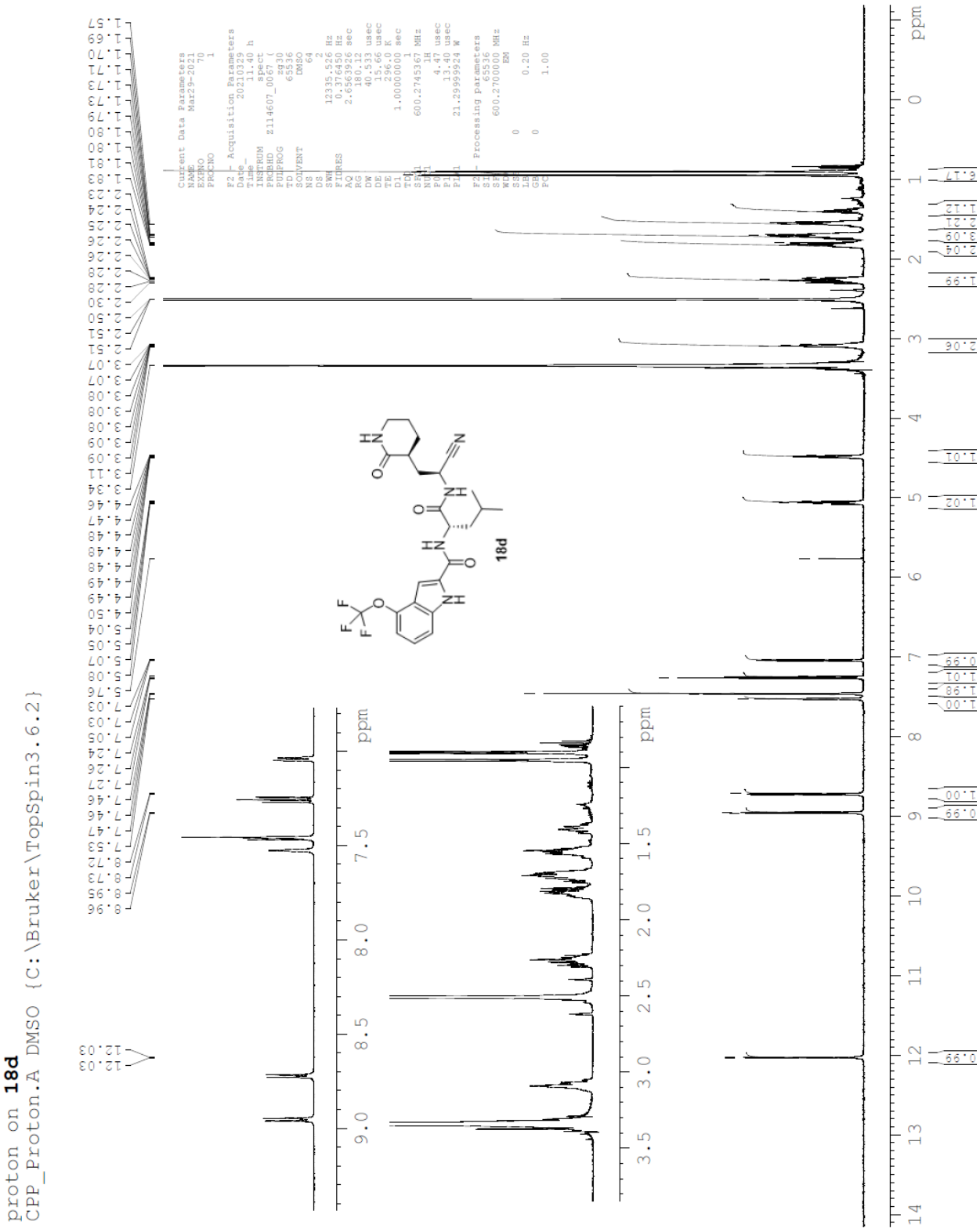
Synthesis of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-(trifluoromethoxy)-1*H*-indole-2-carboxamide, 18d.



Preparation of methyl *N*-[4-(trifluoromethoxy)-1*H*-indole-2-carbonyl]-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate, **15d**. A solution of **12** (177 mg, 0.428 mmol) in DCM (5 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (5 mL) was added. After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and dried under reduced pressure for 1 h. To the residue was added anhydrous DMF (10 mL) and 4-(trifluoromethoxy)-1*H*-indole-2-carboxylic acid (116 mg, 0.471 mmol) and the solution was cooled in an ice bath, after which HATU (179 mg, 0.471 mmol) was added followed by dropwise addition of NMM (0.141 mL, 1.28 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **15d** (165 mg, 71% yield) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 12.00 (d, *J* = 1.8 Hz, 1H), 8.62 (d, *J* = 8.1 Hz, 1H), 8.60 (d, *J* = 8.0 Hz, 1H), 7.47 – 7.43 (m, 2H), 7.43 (s, 1H),7.24 (dd, *J* = 7.8, 8.0 Hz, 1H), 7.05 – 7.01 (m, 1H), 4.59 – 4.52 (m, 1H), 4.43 – 4.36 (m, 1H), 3.61 (s, 3H), 3.12 – 3.04 (m, 2H), 2.30 – 2.21 (m, 2H), 1.87 – 1.80 (m, 1H), 1.75 – 1.64 (m, 4H), 1.60 – 1.48 (m, 2H), 1.38 – 1.30 (m, 1H), 0.94 (d, *J* = 6.5 Hz, 3H), 0.90 (d, *J* = 6.5 Hz, 3H). LC/MS: 6.40 min retention time, 98.3% pure; ESI-MS: 541 [M + H]+.

Preparation of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-4-(trifluoromethoxy)-1*H*-indole-2-carboxamide, **18d**. To a solution of **15d** (161 mg, 0.298 mmol) in THF (5 mL) cooled in an ice bath was slowly added a 1.0 M LiOH aqueous solution (5 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over

anhydrous Na_2SO_4, filtered, and concentrated and dried under reduced pressure. To the residue was added THF (10 mL) and CDI (96.6 mg, 0.596 mmol). After 15 min, NH_3 (aq., 28%, 0.202 mL, 1.46 mmol) was added. After 1 h, water (10 mL) was added. The resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the intermediate (127 mg) as a white solid. To this intermediate (127 mg, 0.242 mmol) in anhydrous THF (10 mL) cooled in an ice bath was added Et_3N (0.101 mL, 0.726 mmol), followed by slow addition of a solution of TFAA (102 mg, 0.484 mmol) in anhydrous THF (2 mL). The progress of the reaction was monitored by TLC, and once the intermediate disappeared after 45min, water (10 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 5 % MeOH in $CHCl_3$, which generated **18d** (58.2 mg, 42% yield for 3 steps) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 12.02 (d, *J* = 1.8 Hz, 1H), 8.95 (d, *J* = 8.1 Hz, 1H), 8.71 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.47 – 7.44 (m, 2H), 7.25 (dd, *J* = 7.8, 8.3 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 5.08 – 5.02 (m, 1H), 4.51 – 4.44 (m, 1H), 3.14 – 3.02 (m, 2H), 2.31 – 2.21 (m, 2H),1.87 – 1.76 (m, 2H), 1.76 – 1.64 (m, 3H), 1.61 – 1.50 (m, 2H), 1.44 – 1.34 (m, 1H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H); ^{19}FNMR (565 MHz, DMSO-d_6) δ -56.44 (s, 3F); ^{13}CNMR (151 MHz, DMSO-d6) δ 172.2, 172.0, 160.6, 141.7, 138.3, 132.3, 123.7, 121.4, 120.2 (q, *J* = 123.5 Hz), 119.7, 112.0, 111.2, 99.6, 51.4, 41.1, 40.0, 38.4, 36.9, 34.0, 26.0, 24.4, 23.0, 21.3, 21.1; LC/MS: 6.29 min retention time, 99.0% pure; ESI-MS: 508 $[M + H]^+$; IR: 2246 cm^{-1}; HRMS (ESI+) calcd for $C_{24}H_{28}F_3N_5O_4$+H 508.2172, found 508.2195.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18d

19F on **18d**
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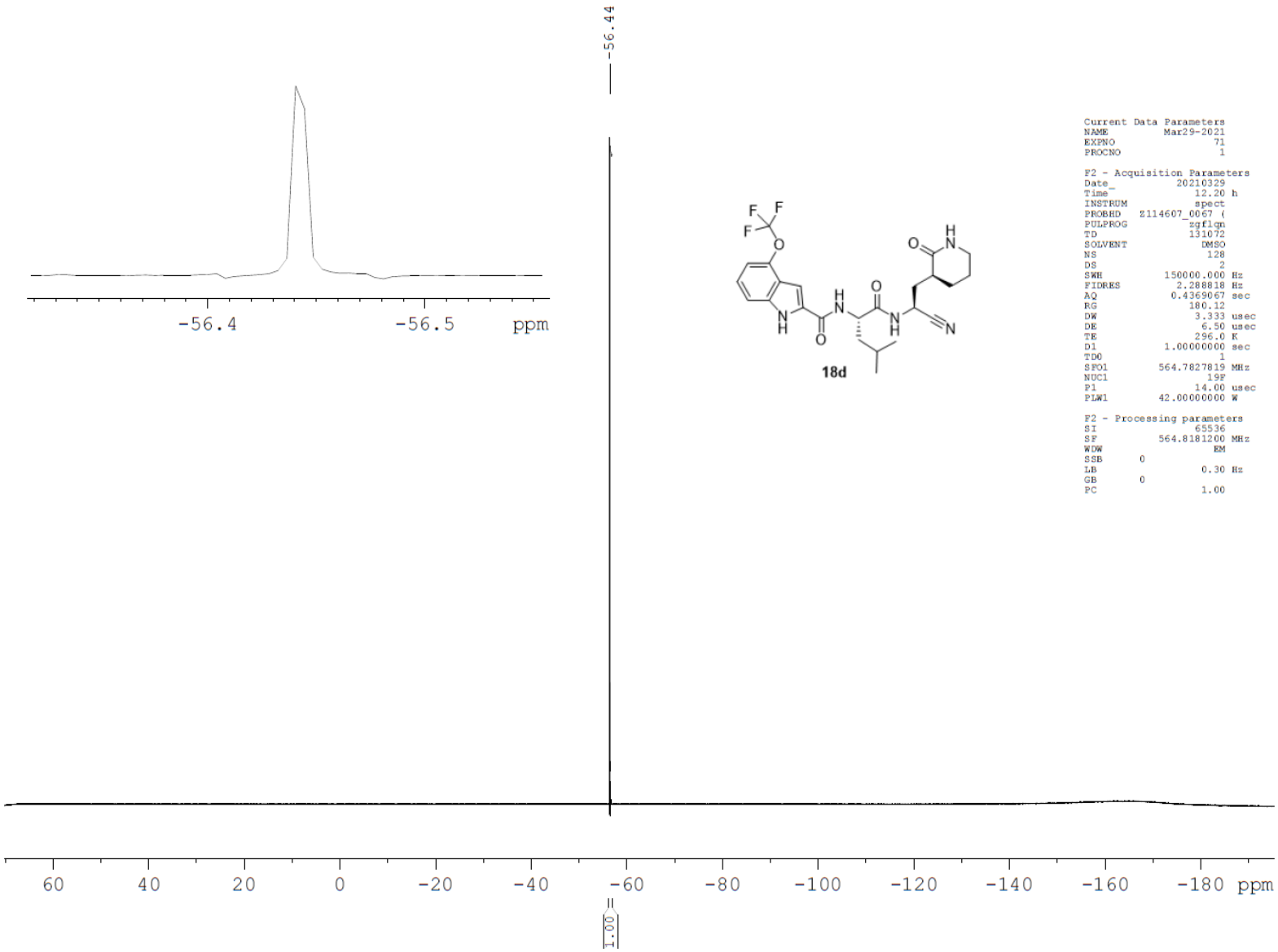


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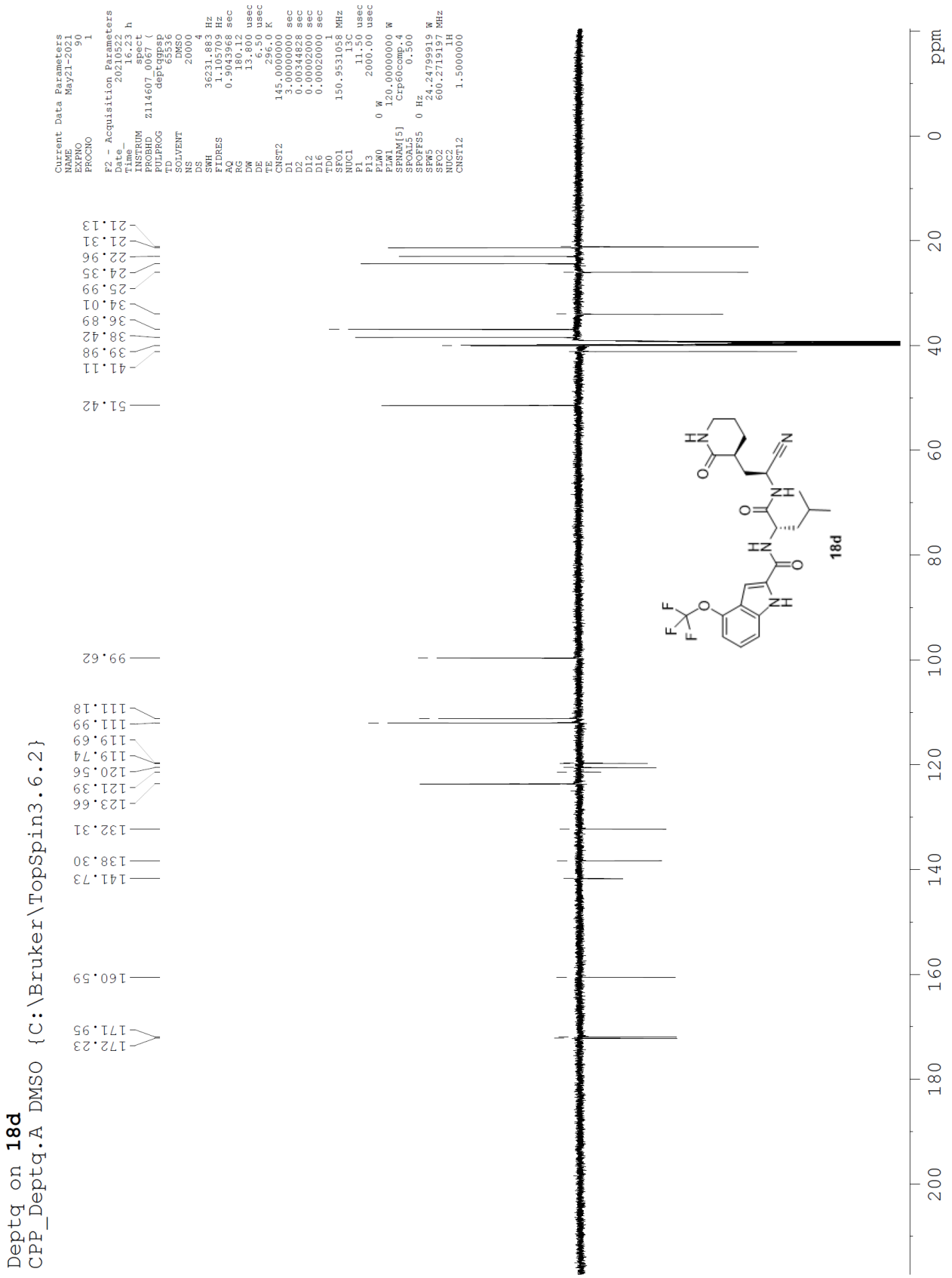
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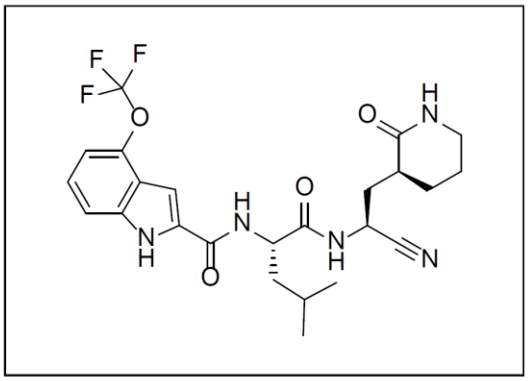
LC-MS Analysis of COMPOUND ____18d_____



Chromatogram

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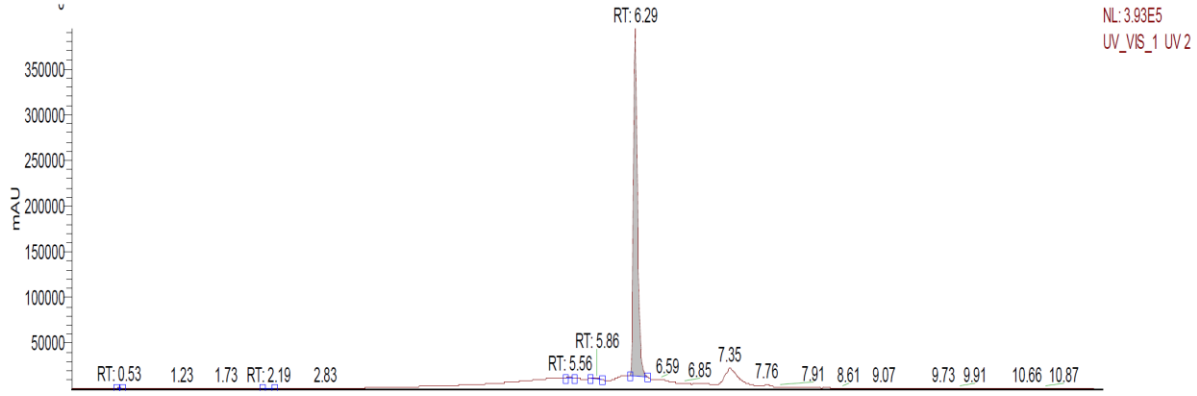
Flow rate: 0.4 mL/min;
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

Detection: UV @ 254nm (UV2)



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Number of detected peaks: 5 (blank substracted)

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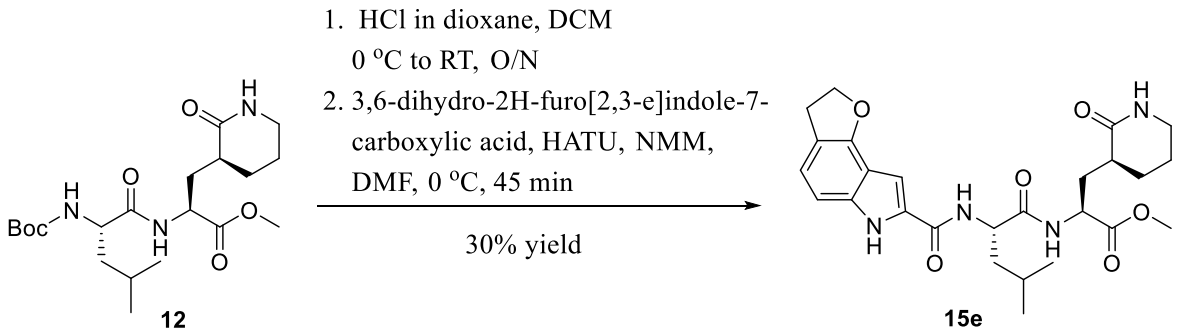
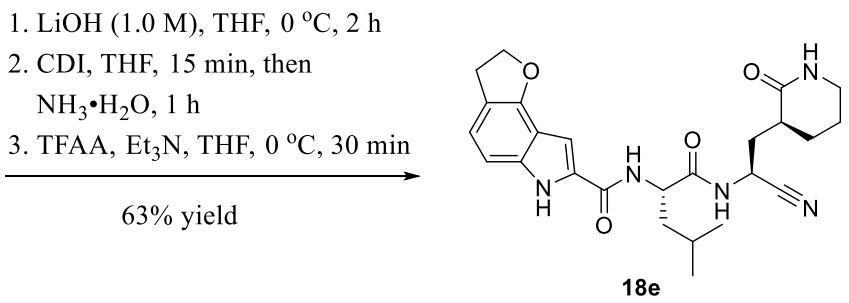
5.86	5.79	5.92	4600.566	0.37	908.503	0.24
6.29	6.23	6.43	1222421.942	**99.01**	378764.295	99.01

Synthesis of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-3,6-dihydro-2*H*-furo[2,3-*e*]indole-7-carboxamide, 18e.

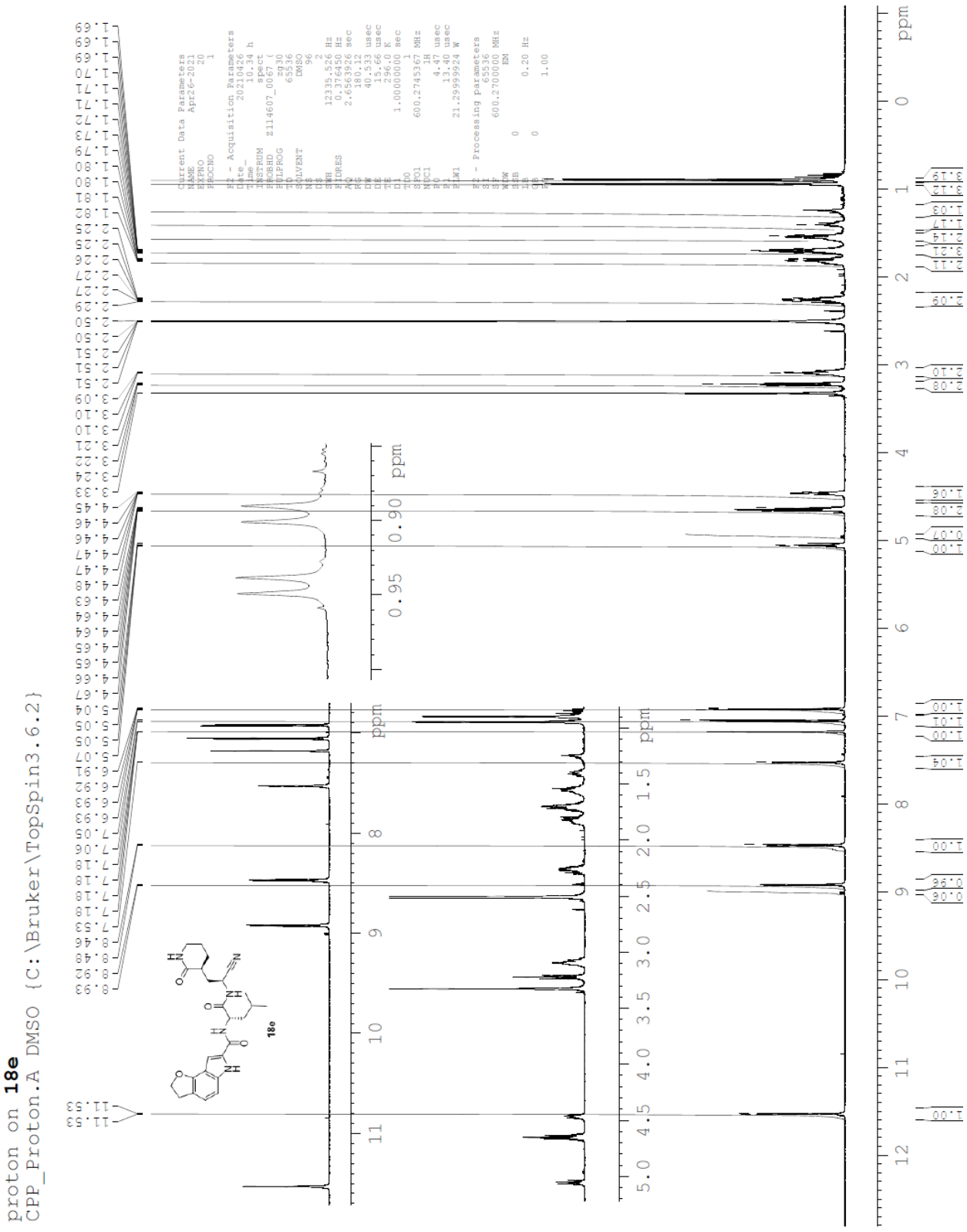




Preparation of methyl *N*-(3,6-dihydro-2*H*-furo[2,3-*e*]indole-7-carbonyl)-L-leucyl-3-[(3*S*)-2-oxopiperidin-3-yl]-L-alaninate **15e**. A solution of **12** (178 mg, 0.430 mmol) in DCM (5 mL) was cooled using an ice-water bath and then 4 M HCl in 1,4-dioxane (5 mL) was added. After 30 min, the ice bath was removed. After overnight, the mixture was concentrated under reduced pressure. The residue was co-evaporated with DCM/ether (1:1, 3 × 20 mL), and dried under reduced pressure for 1 h. To the residue was added anhydrous DMF (10 mL) and 3,6-dihydro-2*H*-furo[2,3-*e*]indole-7-carboxylic acid (78.9 mg, 0.388 mmol) [prepared using procedures in Velázquez, F. *et al Org. Lett.* **2012**, *14* (2), 556–559 and Patent US2005/0026987] and the solution was cooled in an ice bath after which HATU (221mg, 0.582 mmol) was added followed by dropwise addition of NMM (0.130 mL, 1.17 mmol). After 45 min, an ice/saturated aqueous $NaHCO_3$ mixture (1:1, 10 mL) was added and the resulting mixture was extracted with EtOAc (3 × 25 mL). The combined organic layer was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated **15e** (65 mg, 30% yield) as a white solid. LC/MS: 5.11 min retention time, 89.7% pure; ESI-MS: 499 $[M + H]^+$.

Preparation of *N*-[(2*S*)-1-({(1*S*)-1-cyano-2-[(3*S*)-2-oxopiperidin-3-yl]ethyl}amino)-4-methyl-1-oxopentan-2-yl]-3,6-dihydro-2*H*-furo[2,3-*e*]indole-7-carboxamide, **18e**. To a solution of **15e** (65.0 mg, 0.130 mmol) in THF (4 mL) cooled in an ice bath was added a 1.0 M LiOH aqueous solution (4 mL). After 2 h, the pH of the reaction mixture was adjusting to 3 by adding 1.0 M HCl aqueous solution. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated and dried under reduced pressure. To the residue was added THF (10 mL) and CDI (42.2 mg, 0.260 mmol). After 15 min, NH_3 (aq., 28%, 0.100

mL, 0.728 mmol) was added. After 1 h, water (10 mL) was added and the mixture was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification was accomplished by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in $CHCl_3$, which generated the intermediate (49.2 mg) as a white solid. To this intermediate (49.2 mg, 0.101 mmol) was added THF (5 mL) and the solution was cooled in an icebath. Then Et_3N (42.0 µL, 0.303 mmol) was added followed by slow addition of a solution of TFAA (42.4 mg, 0.202 mmol) in anhydrous THF (2 mL). The progress of the reaction was monitored by TLC, and once the intermediate disappeared after 30 min, water (10 mL) was added. The two layers were separated and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic phase was washed with brine (10 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by column chromatography on silica gel with a gradient of 0 to 10 % MeOH in EtOAc, which generated **18e** (38.2 mg, 63% yield for 3 steps) as a white solid. ^{1}HNMR (600 MHz, DMSO-d_6) δ 11.52 (d, *J* = 2.1 Hz, 1H), 8.91 (d, *J* = 8.1 Hz, 1H), 8.46 (d, *J* = 7.8 Hz, 1H), 7.52 (s, 1H), 7.17 (dd, *J* = 0.8, 2.3 Hz, 1H), 7.05 (d, *J* = 8.2 Hz, 1H), 6.91 (dd, *J* = 0.8, 8.1 Hz, 1H), 5.08 – 5.02 (m, 1H), 4.69 – 4.60 (m, 2H), 4.50 – 4.41 (m, 1H), 3.22 (t, *J* = 8.7 Hz, 2H), 3.13 – 3.03 (m, 2H), 2.32 – 2.20 (m, 2H), 1.87 – 1.75 (m, 2H), 1.74 – 1.64 (m, 3H), 1.61 – 1.47 (m, 2H), 1.44 – 1.35 (m, 1H), 0.94 (d, *J* = 6.4 Hz, 3H), 0.89 (d, *J* = 6.5 Hz, 3H); ^{13}CNMR (151 MHz, DMSO-d6) δ 172.4, 172.0, 161.0, 152.5, 138.2, 130.7, 120.5, 119.8, 114.3, 113.1, 104.1, 99.7, 71.6, 51.3, 41.1, 40.1, 38.5, 36.9, 34.0, 29.4, 26.0, 24.4, 23.0, 21.4, 21.1; LC/MS: 4.93 min retention time, 95.4% pure; ESI-MS: 466 [M + H]$^+$; IR: 2247 cm^{-1}; HRMS (ESI+) calcd for $C_{25}H_{31}N_5O_4$+H 466.2454, found 466.2467.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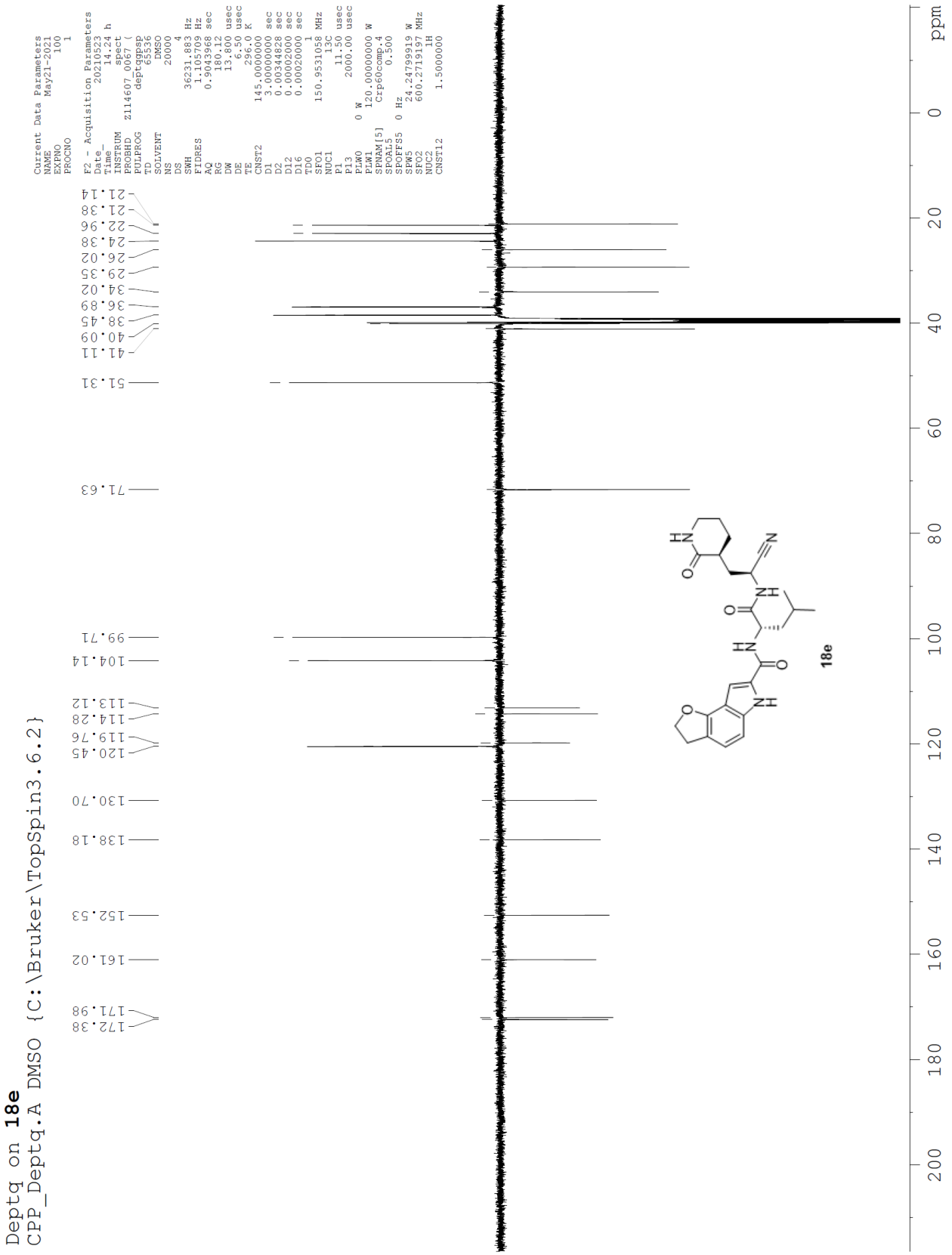
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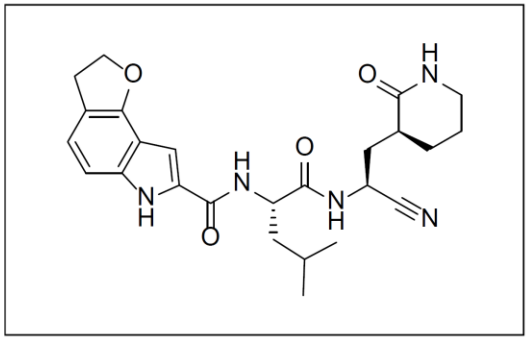
LC-MS Analysis of COMPOUND ___18e_____



Chromatogram

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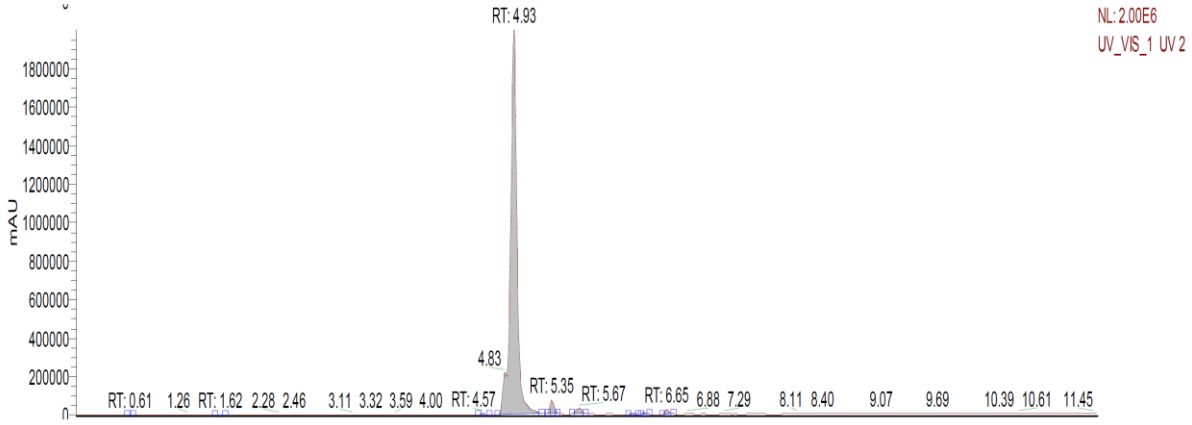
Eluent: Gradient of Methanol-Water, 45% to 95% in 5 min followed by 5 min of isocratic MeOH – water 95%;
Injection volume: 2µL;

Detection: UV @ 254nm (UV2)



PEAK LIST
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Number of detected peaks: 9 (blank substracted)

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4.93	4.74	5.24	10452771.646	**95.43**	1994216.975	93.20
5.35	5.31	5.41	215616.340	1.97	68378.258	3.20
5.67	5.58	5.73	132550.279	1.21	28126.736	1.31
6.25	6.21	6.33	20217.424	0.18	6182.662	0.29
6.39	6.35	6.45	21957.376	0.20	8026.646	0.38
6.65	6.59	6.72	78087.819	0.71	25803.143	1.21