

## Supporting Information

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## 1. Materials and General Methods

Commercially available Fmoc-amino acids were acquired from Astatech, Chem-Impex, Combi-Blocks, and Enamine, and 2-chlorotrityl chloride polystyrene resin was obtained from Bachem. Coupling reagents were obtained from AK Scientific, Alfa Aesar, Acros Organics, Chem-Impex, and Sigma-Aldrich. Custom building blocks were synthesized by Wuxi and Pharmaron as described below. Discovery scale linear peptide sequences were synthesized on a Biotage Syro II peptide synthesizer (~50  $\mu$ mol-scale per reaction vial) and cyclization was performed in solution assuming 100% yield of 50  $\mu$ mol reaction. Site-specific on-resin N-alkylation was performed via Mitsunobu chemistry<sup>1</sup> further optimized to enable robust automation. Peptide coupling efficiency was monitored using analytical UPLC. Analytical UPLC was performed on an Acquity UPLC with a single quad QDa mass detector using an Acquity UPLC BEH C18 1.7 $\mu$ m 2.1x50mm column. Peptide purification was performed on a prep Waters HPLC system equipped with a Waters 2767 Sample Manager, Waters 1525 Binary HPLC Pump, Waters 2545 Binary Gradient Module, Waters SFO System Fluidics Organizer, 515 HPLC Pump, Waters QDA, a Waters 2998 Photodiode Array Detector and a XBridge BEH C18 OBD Prep Column, 130Å, 5  $\mu$ m, 19 mm X 150 mm. The solvent system generally used for the linear peptide purification is solvent A: water with 0.1% TFA and solvent B: acetonitrile with 0.1% TFA. The solvent system generally used for final cyclic peptide purification is solvent A: water with 0.1% FA and solvent B: acetonitrile with 0.1% FA. Proton (<sup>1</sup>H) and Carbon (<sup>13</sup>C) were recorded on a Bruker Advance 400 MHz or Bruker Advance 500 MHz instrument using Methanol-*d*<sub>4</sub>, DMSO-*d*<sub>6</sub>, or Acetonitrile-*d*<sub>3</sub> as solvent. Chemical shifts are reported in parts per million (ppm) ( $\delta$  relative to residual solvent peak for <sup>1</sup>H and <sup>13</sup>C).

## 2. Synthesis of Peptide Probe and Inhibitors

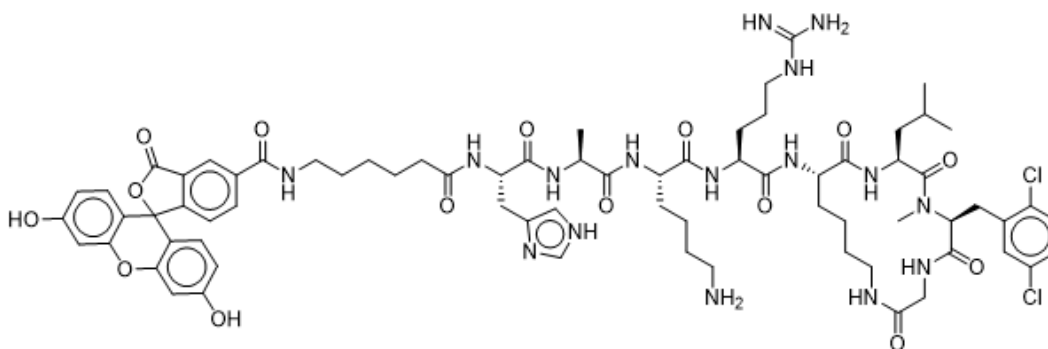
### 2.1. Mass and purity data for Cyclic Peptides

Supplementary Table 1. Characterization data for Cyclic Peptides

Compound	Mcalc.	[M+H] <sup>+</sup> exp.	50 $\mu$ mol-scale synthesis yield (mg)	Purity (%) <sup>*</sup>
CIR7-2706	1492.63	1493.80	0.7	> 99
CIRc-019	1000.42	1000.45	20.4	> 99
CIRc-001	930.42	930.55	6.4	> 99
CIRc-028	976.41	976.35	3.3	> 99
CIRc-004	994.38	994.37	6.5	> 99
CIRc-018	1029.60	515.69 (M/2Z)	22.6	95
CIRc-005	994.38	994.50	6.8	> 99
CIRc-014	961.43	962.30	N/A	> 95

<sup>\*</sup>Retention time and UV-based purity were determined under analytical HPLC conditions at 220 nM

### 2.2. Synthesis of Fluorescent Probe CIR7-2706 for Biochemical Assays



The fluorescent probe was synthesized via solid phase peptide synthesis followed by cyclization, fluorescent labeling, and deprotection in solution.

To load Fmoc-Glycine onto 100 mg of CTC resin, Fmoc-Glycine, CAS#29022-11-5, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The solution was dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride resin and was agitated for 2 hours at room temperature. The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of Fmoc-L-2,5-dichlorophenylalanine-OH, CAS#1260614-80-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

To N-Methylate the amine of 25ClF, 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin and then was agitated at 40 to 45°C for 10 to 15 minutes. The mixture was drained, then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Dry methanol (MeOH), (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv.) was added to the resin and the mixture was agitated at 45°C for 30 minutes. The mixture was drained and the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. The nosyl group was then deprotected. A solution of 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and the mixture was agitated at 45°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection was repeated twice.

Fmoc-L-Leucine-OH, CAS# 35661-60-0 (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. The process was repeated a second time to ensure complete coupling. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Lysine(Mtt)-OH, CAS#167393-62-6, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Arginine(Pbf)-OH, CAS#154445-77-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

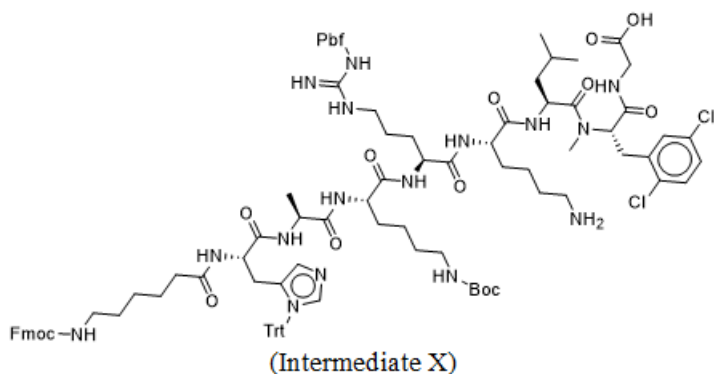
Fmoc-L-Lysine(Boc)-OH, CAS#71989-26-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Alanine-OH, CAS#35661-39-3, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Histidine(Trt)-OH, CAS#109425-51-6, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

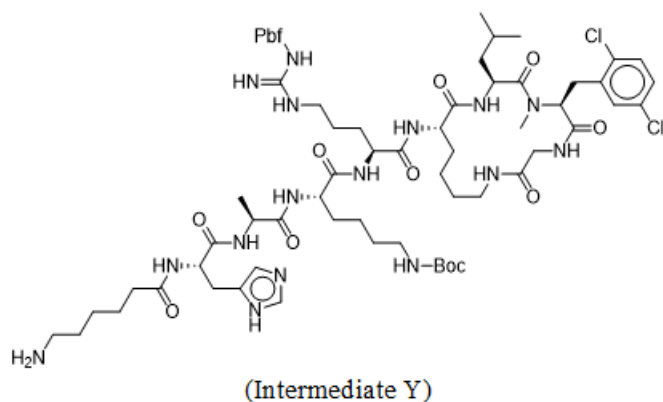
Fmoc-6-aminohexanoic acid, CAS#88574-06-5, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times.

To cleave peptide from CTC resin and simultaneously deprotect the Mtt protecting group, approximately 2 mL of a solution of 24% HFIP, 2% TIPS, in DCM was added to the polystyrene resin in a solid phase reaction vessel. The contents were shaken for 1 hour. The cleavage solution was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional 2 mL of DCM and the wash was collected in the conical vial. The solution was evaporated in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid and the purified fractions were pooled and lyophilized to yield white powder of intermediate X ( $M/z$  observed = 1968.65  $[M+H]^+$ ).



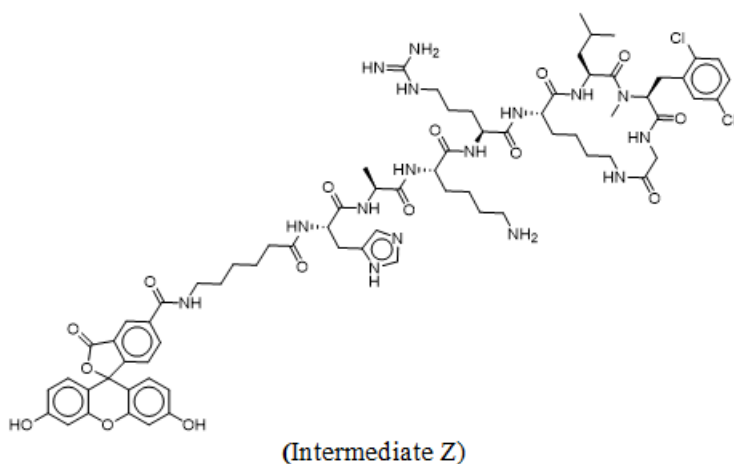
(Intermediate X)

The linear intermediate X (~15 mg) was cyclized using a medium volume, T3P solution cyclization method. The deprotected and purified linear product was transferred to a 50 mL conical vial and dissolved in 1 mL NMP followed by the addition of DIEA (0.5 mL) and DCM (35 mL). T3P (3 eqv) was added to the solution and the reaction pH was adjusted to pH 9 via dropwise addition of DIEA. The closed conical vial was agitated at room temperature for 2 hours at 150 rotations per minute. The solution was concentrated at 45°C under reduced pressure in a Genevac system. The Fmoc group was then removed with the addition of a 10% of KOH/Water solution (5 mL) heated at 70°C for 30 minutes. The resulting LCMS trace revealed that the trityl group had been unexpectedly removed during the cyclization and Fmoc-deprotection steps. The cyclic peptide was then purified via reverse phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The purified fractions were pooled and lyophilized to yield intermediate Y ( $M/z$  observed = 1485.94  $[M+Z]^+$ ).



(Intermediate Y)

The probe was fluorescently labeled via a peptide coupling in solution. A solution of 5-carboxyfluorescein (CAS#76823-03-5, FAM) (4 equiv.), EDC (4 equiv.), HOAt (3.9 equiv.) and DIEA (8 equiv.) in 1.0 mL of anhydrous DCM was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes. Intermediate Y was added to the coupling solution, and the reaction was agitated at room temperature until starting material was not observed by LCMS, resulting in the formation of Intermediate Z ( $M/z$  observed = 1844.29  $[M+Z]^+$ ).

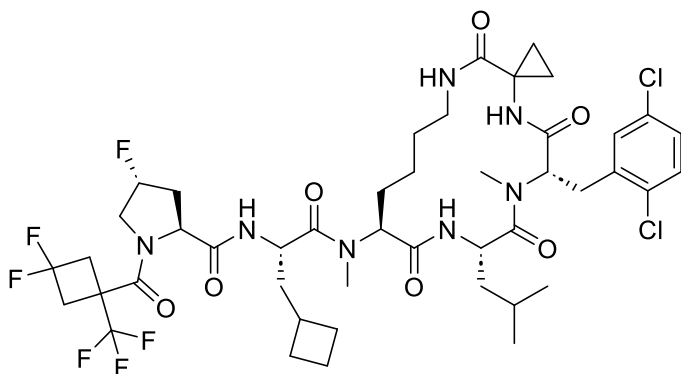


(Intermediate Z)

The Boc and Pbf protecting groups were removed from the cyclic intermediate Z by dissolving the cyclic peptide in a 1 mL solution of 90% TFA, 5% TIPS, 5% DCM and agitating for 1 hour. The reaction was monitored by LCMS for the disappearance of starting material. Upon completion, the reaction was concentrated. The crude material was co-evaporated with DCE (5 mL x 2), and then purified via reverse phase-HPLC to yield fluorescent probe (FAM probe) ( $M/z$  observed = 1492.14  $[M+Z]^+$ , 0.7 mg, 99% purity by UV)

## 2.3. Synthesis of Cyclin Inhibitors

### 2.3.1 Synthesis of CIRc-004



Fmoc-1-aminocyclopropane-1-carboxylic acid, CAS#126705-22-4, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The solution was dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride (CTC) resin and was agitated for 2 hours at room temperature. The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of Fmoc-L-2,5-dichlorophenylalanine-OH, CAS#1260614-80-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-alkylate the terminal amine. 1) Nosyl protection. A solution of 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 40 to 45°C for 10 to 15 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the

resin. Dry methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 45°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Leucine-OH, CAS# 35661-60-0, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The reaction was repeated a second time to ensure complete coupling. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Lysine(Boc)-OH, CAS# 71989-26-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-cyclobutylalanine-OH, CAS# 478183-62-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C to 45°C for 30 min. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated. To remove

Fmoc, A mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(2S,4R)-1-(3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carbonyl)-4-fluoropyrrolidine-2-carboxylic acid (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times.

To simultaneously cleave the peptide from CTC resin and deprotect the Boc-protected lysine sidechain, approximately 2 mL a solution of 20% TFA and 5% TIPS in DCM (2 mL) was added to the 100 mg of polystyrene resin in a solid phase reaction vessel. The contents of the vessel were shaken for one hour. The liquid phase of the reaction was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional DCM (2 mL) and the wash was collected in the conical vial. Toluene (2 mL) was added to the cleaved peptide solution and the solution was neutralized with triethylamine and concentrated under reduced atmosphere in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The purified fractions were pooled and lyophilized to yield white powder (LCMS m/z observed = 1012.32 [M+H]<sup>+</sup>).

The deprotected and purified linear peptide was transferred to a 50 mL conical vial and dissolved in 1 mL NMP followed by the addition of DIEA (0.5 mL) and DCM (35 mL). T3P (3 eqv) was added to the solution and the pH was adjusted to pH 9 via dropwise addition of DIEA. The closed conical vial was then shaken at room temperature for 2 hours at 150 rotations per minute. The conical vial was then uncapped and the solution was concentrated at 45°C under reduced pressure in a Genevac system. The evaporated crude material was then redissolved in acetonitrile and purified via reverse phase chromatography with 0.1% TFA and lyophilized to yield 6.5 mg of CIRc-004 as a white powder (m/z observed = 994.37 [M+Z]<sup>+</sup>).

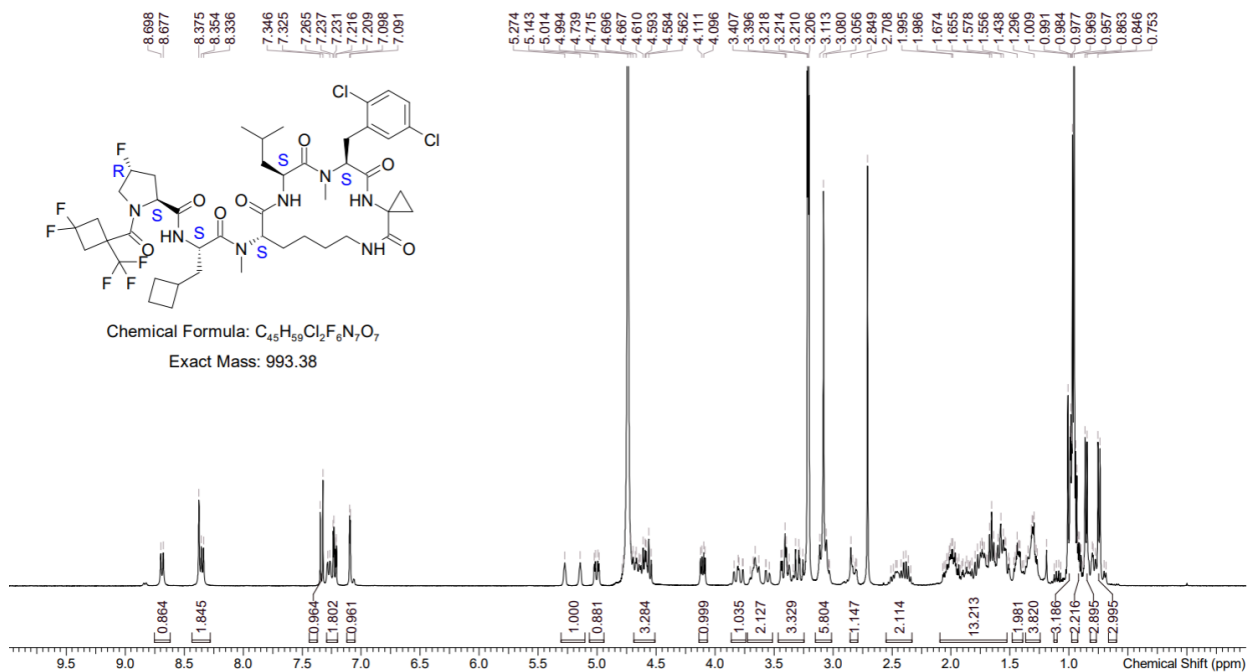
<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.75 - 8.62 (m, 1H), 8.43 - 8.28 (m, 2H), 7.34 (d, J = 8.4 Hz, 1H), 7.29 - 7.20 (m, 2H), 7.12 - 7.05 (m, 1H), 5.30 - 5.10 (m, 1H), 5.07 - 4.94 (m, 1H), 4.69 - 4.51 (m, 3H), 4.14 - 4.06 (m, 1H), 3.86 - 3.74 (m, 1H), 3.72 - 3.51 (m, 2H), 3.46 - 3.25 (m, 3H), 3.15 - 3.01 (m, 6H), 2.86 - 2.79 (m, 1H), 2.55 - 2.33 (m, 2H), 2.10 - 1.52 (m, 13H), 1.48 - 1.39 (m, 2H), 1.37 - 1.24 (m, 4H), 1.01 - 0.98 (m, 3H), 0.95 - 0.89 (m, 2H), 0.87 - 0.82 (m, 3H), 0.78 - 0.71 (m, 3H)

<sup>13</sup>C NMR (125 MHz, METHANOL-d<sub>4</sub>) δ : 173.75, 172.665, 172.604, 171.64, 171.519, 171.181, 137.547, 132.429, 132.395, 130.71, 128.513, 63.424, 59.705, 55.395, 48.781, 48.504, 48.436, 48.322, 48.25, 48.038, 47.825, 47.613, 47.4, 47.184, 46.971, 39.383, 39.151, 38.601, 38.434, 34.886, 34.154, 32.291, 31.949, 30.227, 28.003, 27.889, 27.783, 26.99, 24.752, 22.653, 22.517, 19.587, 17.861, 17.235, 16.981, 16.806, 16.609, 15.983, 12.871, 12.272, 11.638.

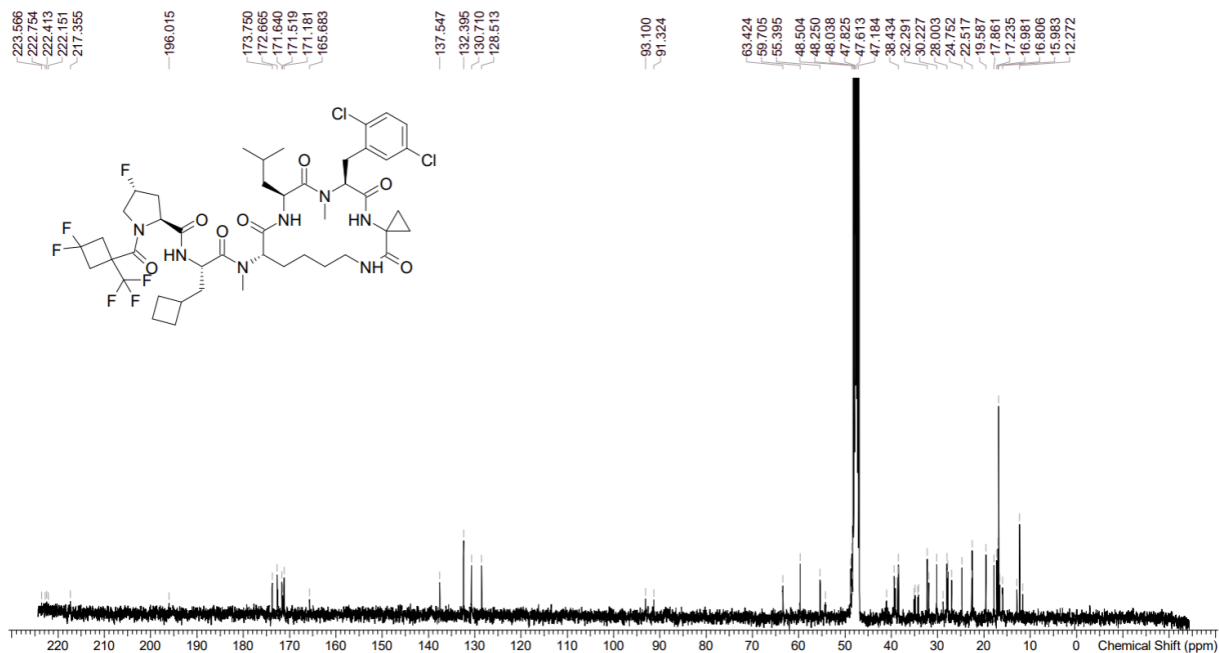
HRMS: [M+H] calc. 994.38, obs. 994.38

## Characterization of CIRc-004

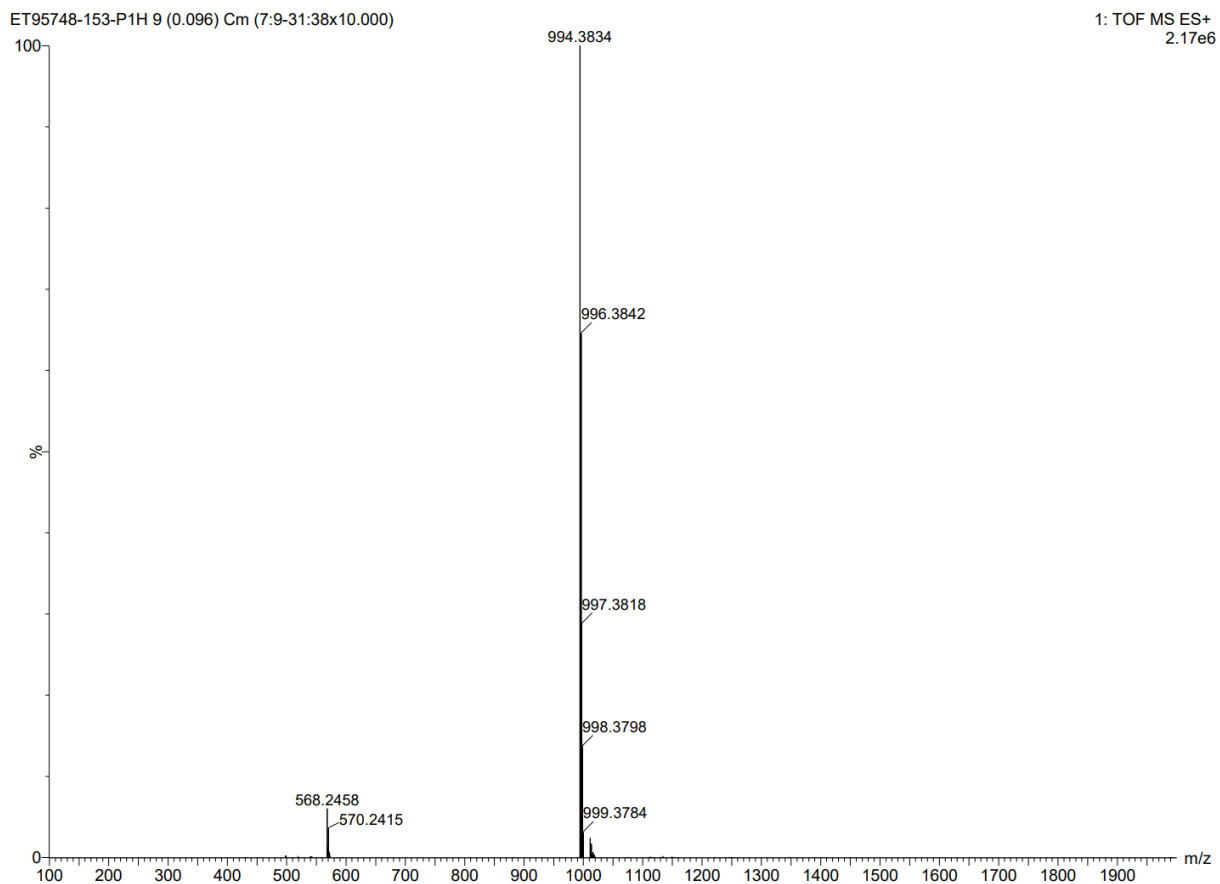
### <sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-004



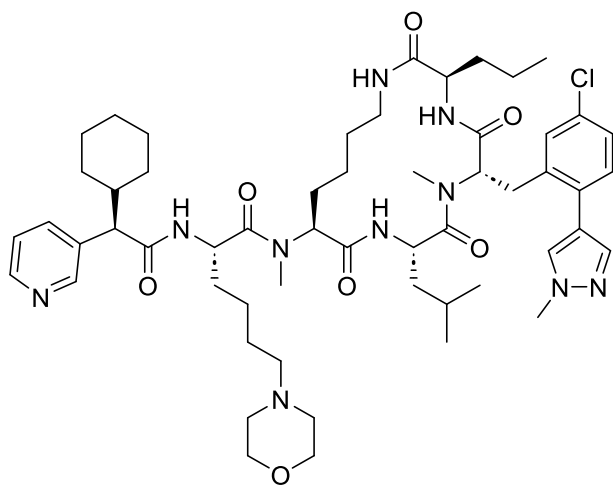
### <sup>13</sup>C NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-004



## HRMS [M+H]<sup>+</sup> of CIRc-004



### 2.3.2. Synthesis of CIRc-018



Fmoc-D-norvaline, CAS#144701-24-6, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The

solution was dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride (CTC) resin and was agitated for 2 hours at room temperature. The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoic acid (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Leucine-OH, CAS# 35661-60-0, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Lysine(Boc)-OH, CAS# 71989-26-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at

35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-morpholinohexanoic acid (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C to 45°C for 30 min. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated. To remove Fmoc, A mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(R)-2-cyclohexyl-2-(pyridin-3-yl)acetic acid (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared and adjusted to pH 9. The mixture was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling was repeated a second time.

To simultaneously cleave the peptide from CTC resin and deprotect the Boc-protected lysine sidechain, approximately 2 mL A solution of 20% TFA and 5% TIPS in DCM (2 mL) was added to the 100 mg of polystyrene resin in a solid phase reaction vessel. The contents of the vessel were shaken for one hour. The liquid phase of the reaction was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional DCM (2 mL) and the wash was collected in the conical vial. Toluene (2 mL) was added to the cleaved peptide solution and the solution was neutralized with triethylamine and concentrated under reduced atmosphere in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The purified fractions were pooled and lyophilized to yield white powder (LCMS m/z observed = 1047.6 [M+H]<sup>+</sup>).

The deprotected and purified linear peptide was transferred to a 50 mL conical vial and dissolved in 1 mL NMP followed by the addition of DIEA (0.5 mL) and DCM (35 mL). T3P (3 eqv) was added to the solution and the pH was adjusted to pH 9 via dropwise addition of DIEA. The closed conical vial was then shaken at room temperature for 2 hours at 150 rotations per minute. The conical vial was then uncapped and the solution was concentrated at 45°C under reduced pressure in a Genevac system. The evaporated crude material was then redissolved in acetonitrile and purified via reverse phase chromatography with 0.1% TFA and lyophilized to yield 22.6 mg of CIRc-018 as a white powder (m/z observed = 515.60 [M+2Z]<sup>+</sup>).

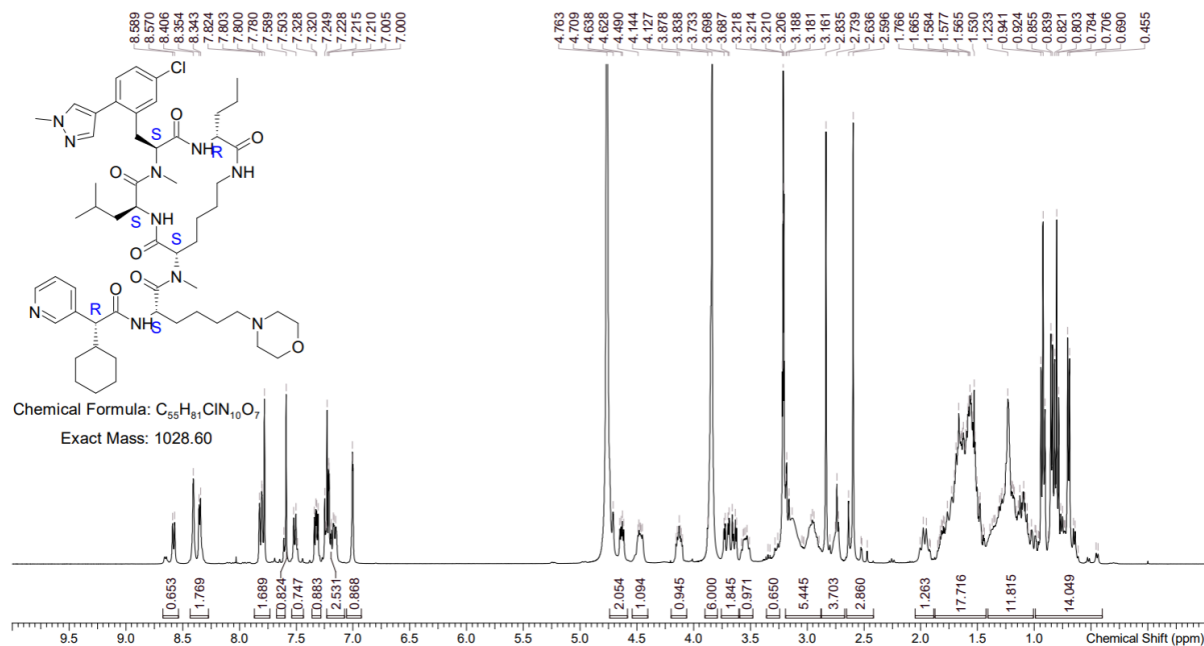
<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.68 - 8.54 (m, 1H), 8.44 - 8.27 (m, 2H), 7.87 - 7.73 (m, 2H), 7.62 - 7.55 (m, 1H), 7.54 - 7.44 (m, 1H), 7.36 - 7.28 (m, 1H), 7.27 - 7.11 (m, 3H), 7.06 - 6.93 (m, 1H), 4.71 (br s, 2H), 4.54 - 4.40 (m, 1H), 4.20 - 4.06 (m, 1H), 3.85 - 3.85 (m, 1H), 3.90 - 3.79 (m, 5H), 3.76 - 3.60 (m, 2H), 3.60 - 3.48 (m, 1H), 3.36 - 3.24 (m, 1H), 2.89 (br s, 5H), 2.88 - 2.80 (m, 2H), 2.82 - 2.67 (m, 2H), 2.65 - 2.42 (m, 3H), 2.05 - 1.89 (m, 1H), 1.87 - 1.43 (m, 18H), 1.41 - 1.01 (m, 12H), 0.99 - 0.40 (m, 14H)

<sup>13</sup>C NMR (125 MHz, METHANOL-d<sub>4</sub>) δ : 173.757, 173.186, 172.818, 171.875, 171.781, 170.518, 148.407, 146.977, 137.932, 137.199, 137.137, 135.754, 132.161, 131.446, 131.183, 129.72, 127.099, 123.997, 120.137, 63.978, 63.747, 56.713, 56.258, 55.626, 53.821, 51.589, 49.43, 49.209, 48.328, 48.256, 48.115, 48.043, 47.902, 47.83, 47.685, 47.617, 47.404, 47.191, 46.978, 40.037, 38.878, 38.597, 37.69, 32.364, 32.068, 31.768, 31.526, 30.862, 30.725, 30.382, 30.327, 30.194, 30.035, 28.417, 26.608, 25.904, 25.626, 25.525, 24.738, 22.881, 22.777, 22.513, 22.361, 19.884, 19.263, 16.862, 16.837, 16.786, 14.865, 14.771, 14.677, 14.601, 12.5.

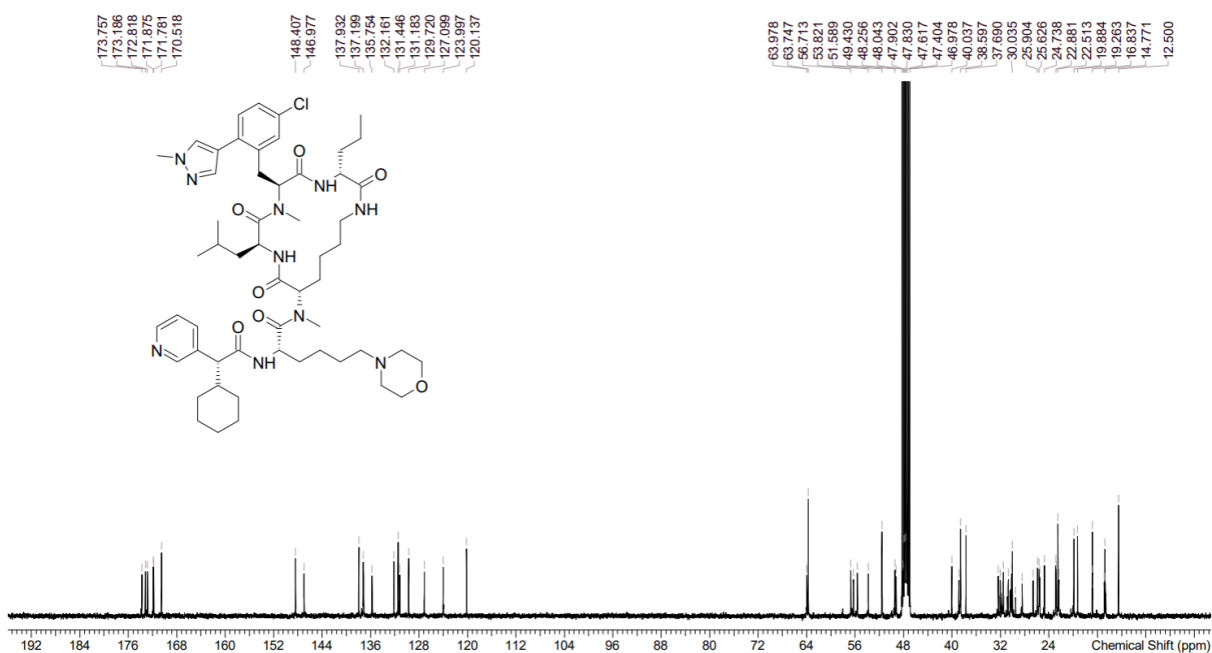
HRMS: [M+H]<sup>+</sup> calc. 129.60, obs. 129.60

## Characterization of CIRc-018

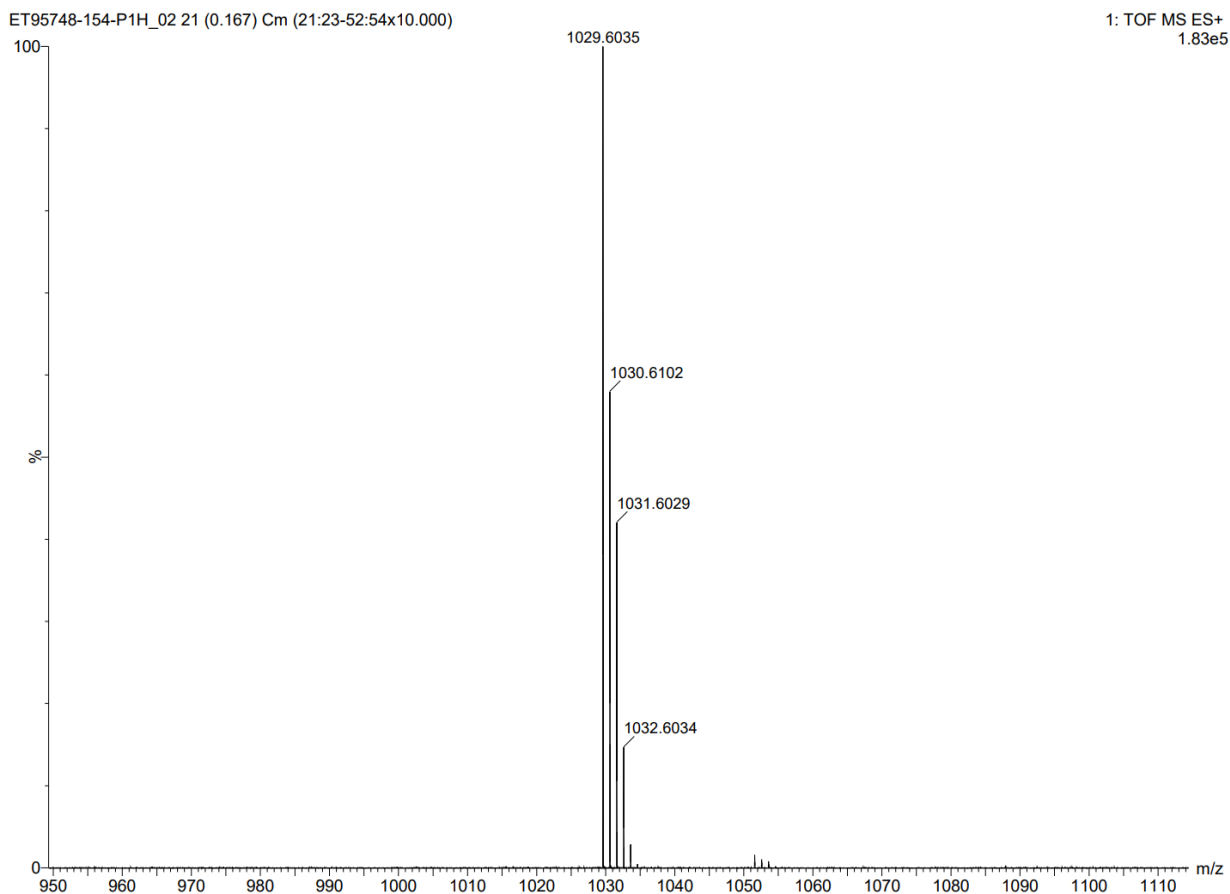
### <sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-018



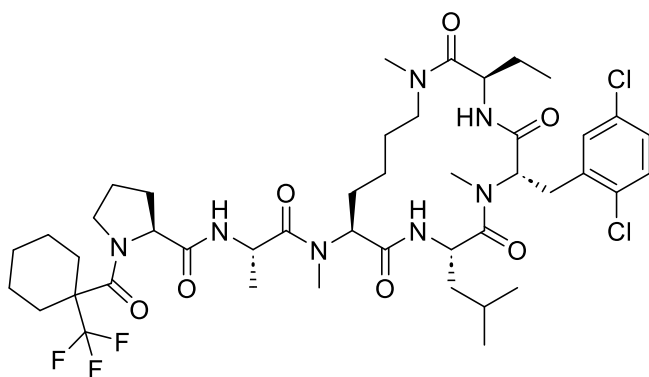
### <sup>13</sup>C NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-018



## HRMS [M+H]<sup>+</sup> of CIRc-018



### 2.3.3. Synthesis of CIRc-001



Fmoc-D-Abu, CAS#94744-50-0, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The solution was

dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride (CTC) resin and was agitated for 2 hours at room temperature. The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of Fmoc-L-2,5-dichlorophenylalanine-OH, CAS#1260614-80-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. A solution of 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 40 to 45°C for 10 to 15 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Dry methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 45°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Leucine-OH, CAS# 35661-60-0, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Lysine(Me,Boc)-OH, CAS# 2044709-77-3, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed

to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Ala-OH, CAS# 35661-39-3, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C to 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated a second time. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(1-(trifluoromethyl)cyclohexane-1-carbonyl)-L-proline, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

To simultaneously cleave the peptide from CTC resin and deprotect the Boc-protected lysine sidechain, approximately 2 mL A solution of 20% TFA and 5% TIPS in DCM (2 mL) was added to the 100 mg of polystyrene resin in a solid phase reaction vessel. The contents of the vessel were shaken for one hour. The liquid phase of the reaction was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional DCM (2 mL) and the wash was collected in the conical vial. Toluene (2 mL) was added to the

cleaved peptide solution and the solution was neutralized with triethylamine and concentrated under reduced atmosphere in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The purified fractions were pooled and lyophilized to yield white powder (LCMS m/z observed = 948.55 [M+H]<sup>+</sup>).

The linear peptide (~50 μmol linear synthesis yield) was dissolved in NMP (500 uL), DIEA (250 uL), and DCM (0.75 mL). T3P (31uL, 3eqv) is added, the solution is shaken and allowed to react for 1-10 minutes at room temperature. Cyclization progress was monitored via LCMS. The reaction mixture was directly injected and purified via reverse phase chromatography on an acetonitrile/water gradient with 0.1% TFA and lyophilized to yield 6.4 mg of CIRc-001 as a white powder (m/z observed = 930.55 [M+Z]<sup>+</sup>).

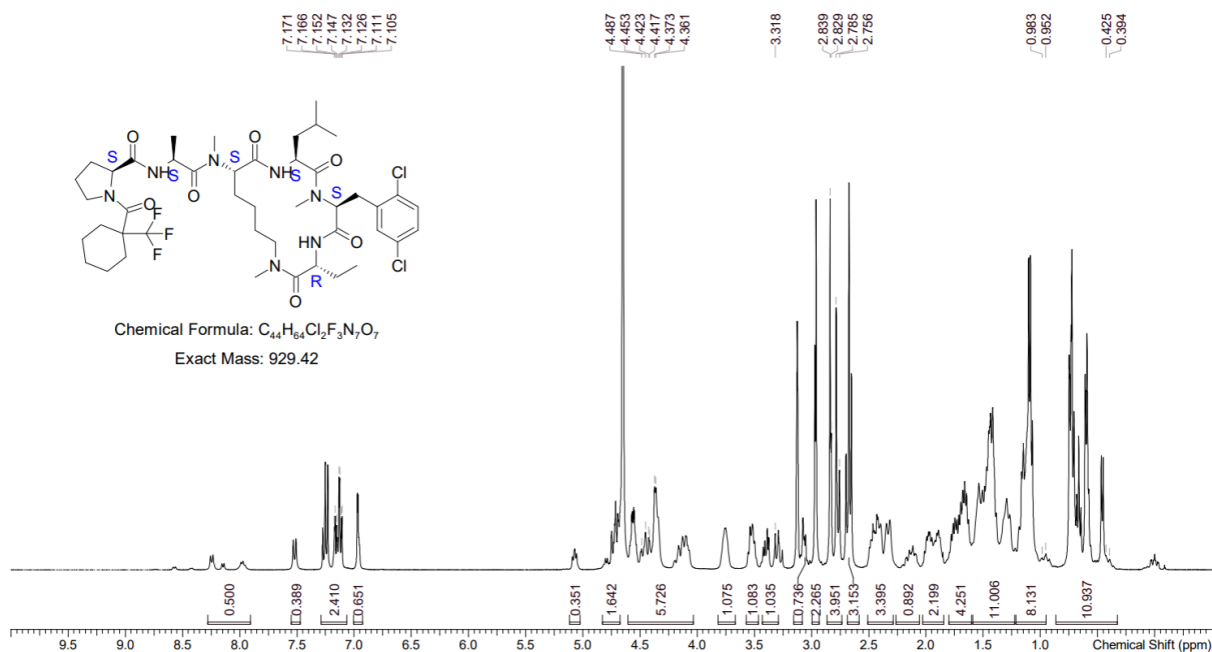
<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.28 - 7.91 (m, 1H), 7.55 - 7.47 (m, 1H), 7.19 - 7.08 (m, 1H), 7.29 - 7.06 (m, 1H), 7.01 - 6.93 (m, 1H), 5.12 - 5.02 (m, 1H), 4.83 - 4.68 (m, 2H), 4.61 - 4.32 (m, 1H), 4.49 - 4.03 (m, 3H), 3.82 - 3.67 (m, 1H), 3.57 - 3.47 (m, 1H), 3.32 (s, 1H), 3.10 - 3.02 (m, 1H), 3.00 - 2.93 (m, 2H), 2.87 - 2.74 (m, 4H), 2.72 - 2.62 (m, 3H), 2.51 - 2.29 (m, 3H), 2.26 - 2.06 (m, 1H), 2.03 - 1.84 (m, 2H), 1.80 - 1.60 (m, 4H), 1.59 - 1.22 (m, 11H), 0.97 (br d, J = 12.8 Hz, 8H), 0.41 (br d, J = 12.6 Hz, 11H)

<sup>13</sup>C NMR (125 MHz, METHANOL-d<sub>4</sub>) δ : 173.215, 173.131, 173.082, 172.896, 172.376, 171.667, 171.386, 171.003, 170.889, 170.634, 170.38, 169.174, 169.09, 167.732, 166.567, 137.798, 136.641, 132.949, 132.376, 132.315, 132.281, 132.144, 131.863, 131.131, 130.721, 130.672, 128.813, 128.384, 128.221, 125.405, 63.8, 63.382, 59.493, 58.609, 56.055, 55.995, 54.26, 54.029, 50.853, 50.762, 50.667, 48.664, 48.273, 48.189, 48.06, 47.977, 47.848, 47.768, 47.632, 47.419, 47.207, 46.994, 45.829, 45.598, 45.511, 45.237, 45.052, 41.819, 39.519, 38.62, 38.244, 33.854, 33.581, 32.686, 32.302, 31.912, 30.147, 30.052, 29.927, 28.857, 28.774, 28.182, 27.863, 26.47, 25.829, 25.423, 25.26, 24.949, 24.456, 24.201, 24.114, 22.653, 22.501, 22.217, 22.092, 21.78, 20.392, 20.054, 19.906, 19.834, 17.151, 16.005, 15.88, 9.32, 9.206.

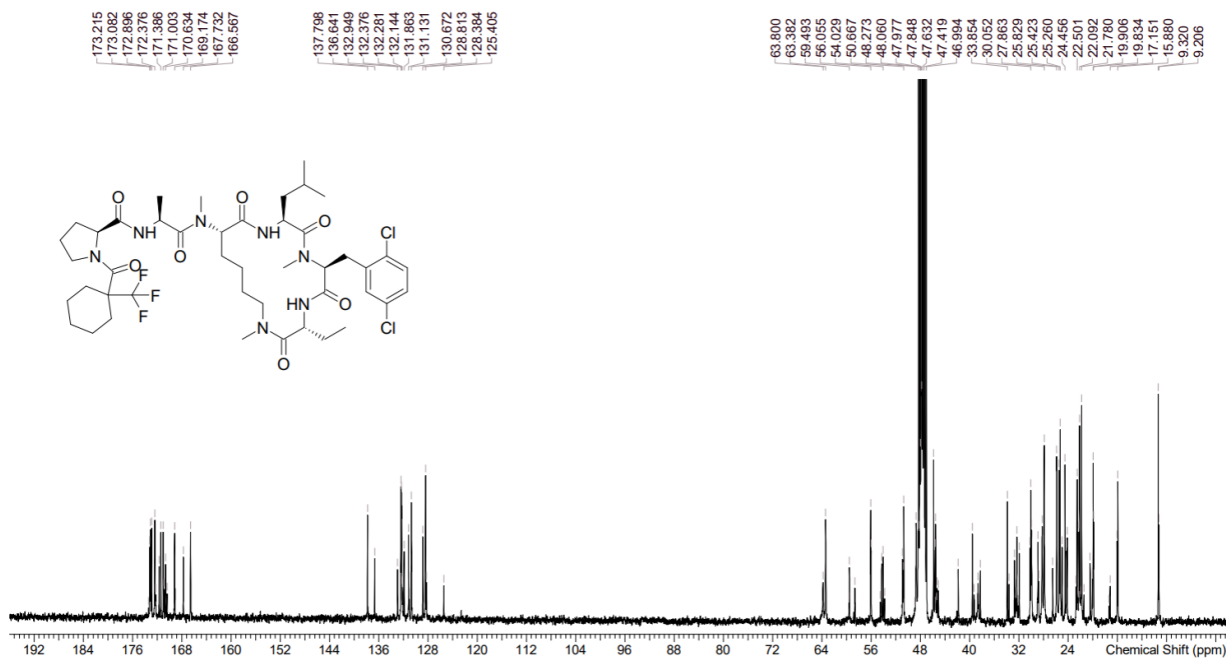
HRMS: [M+H] calc. 930.42, obs. 930.42

# Characterization of CIRc-001

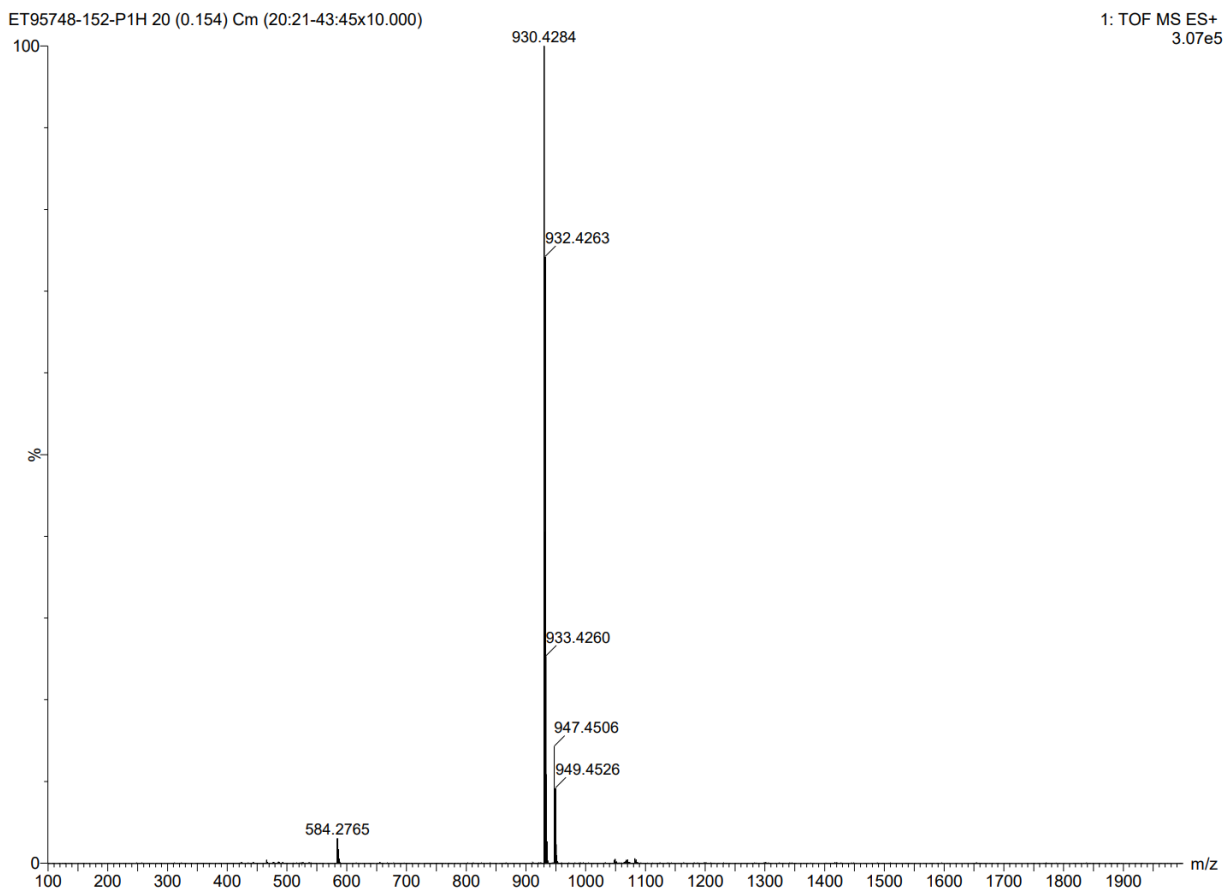
## <sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-001



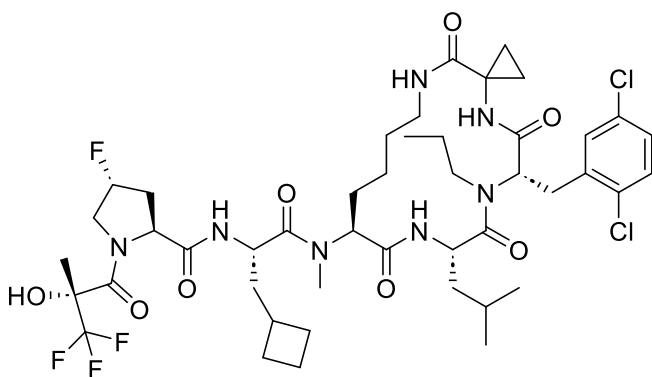
## <sup>13</sup>C NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-001



## HRMS [M+H]<sup>+</sup> of CIRc-001



### 2.3.4. Synthesis of CIRc-028



Fmoc-1-aminocyclopropane-1-carboxylic acid, CAS#126705-22-4, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The solution was dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride (CTC) resin and was agitated for 2 hours at room temperature.

The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of Fmoc-L-2,5-dichlorophenylalanine-OH, CAS#1260614-80-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-alkylate the terminal amine. 1) Nosyl protection. A solution of 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 40 to 45°C for 10 to 15 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Dry propanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv.) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 45°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Leucine-OH, CAS# 35661-60-0, (12 equiv.) was dissolved in 1.5 mL of anhydrous NMP:DCE (50:50). DIC (12 equiv.) was added to the Fmoc-Leucine-OH solution. The mixture was added to the resin and was agitated for 12 hours at room temperature. The slurry was drained and then the resin was washed with 1.0 mL of methanol four times and 1.0 mL of DMF three times. The coupling was repeated a second time. Following complete coupling, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Lysine(Dde)-OH, CAS# 150629-67-7, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv.) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0

mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv.) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Cyclobutylalanine-OH, CAS# 478183-62-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C to 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated a second time. To remove Fmoc, A mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-(2S,4R)-4-fluoro-1,2-pyrrolidinecarboxylate, CAS#203866-20-0, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(2R)-3,3,3-trifluoro-2-hydroxy-2-methylpropanoic acid, CAS# 44864-47-3, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared and adjusted to pH 9. The mixture was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling was repeated a second time.

To remove the Dde protecting group, 10% hydrazine monohydrate in 1.0 mL NMP was added to the resin and was agitated for 20 minutes at room temperature. The mixture was drained and then the resin was washed with 1.0 mL DMF three times.

To cleave peptide from CTC resin, approximately 2 mL of a solution of 24% HFIP, 2% TIPS, in DCM was added to the 100 mg of polystyrene resin in a solid phase reaction vessel. The contents were shaken for 1 hour. The cleavage solution was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional 2 mL of DCM and the wash was collected in the conical vial. The solution was evaporated in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The purified fractions were pooled and lyophilized to yield white powder (LCMS m/z observed = 994.44 [M+H]<sup>+</sup>).

The linear peptide (~50 µmol scale linear synthesis yield) was cyclized using a large volume, high dilution method. The linear peptide was transferred to a 500 mL round bottom flask with a stir bar, and dissolved in DCM (250 mL). DIEA (3 eqv) was added to the flask, followed by PyBop (3 eqv). The pH was adjusted to 9 with DIEA. The reaction was stirred at room temperature for 12 hours and monitored for reaction completion via LCMS. The solution was evaporated under reduced pressure and purified via reverse phase chromatography and lyophilized to yield 3.3 mg of CIRc-028 as a white powder (m/z observed = 976.35 [M+Z]<sup>+</sup>)

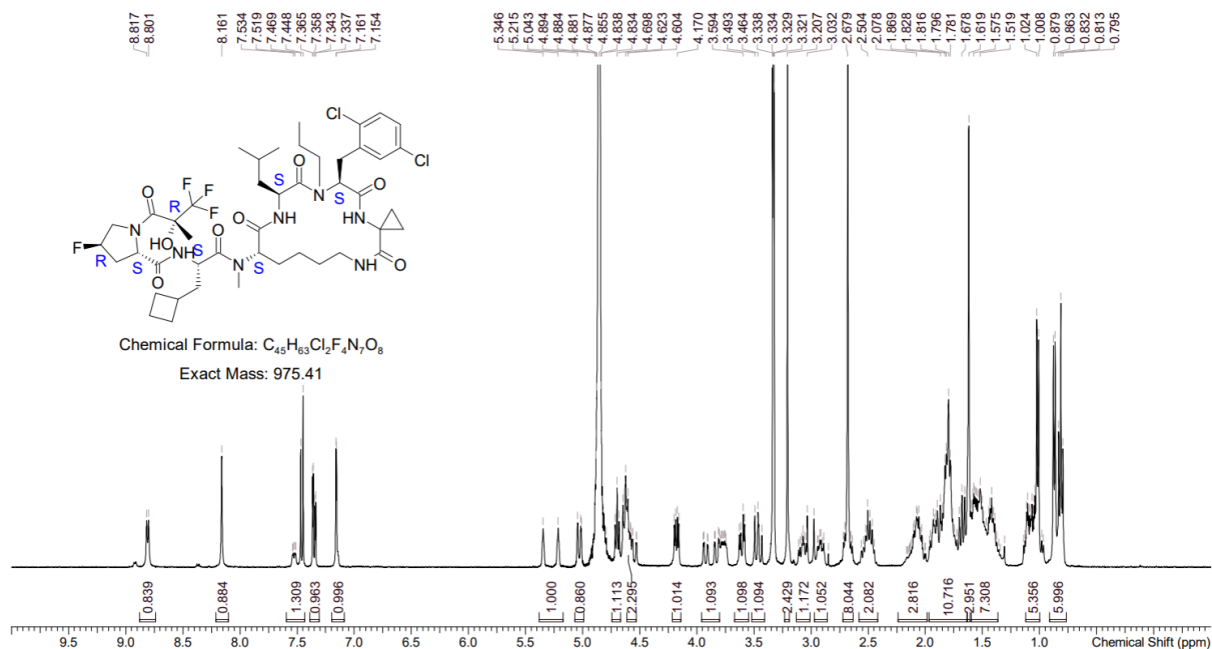
<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.88 - 8.74 (m, 1H), 8.21 - 8.10 (m, 1H), 7.59 - 7.43 (m, 1H), 7.39 - 7.31 (m, 1H), 7.20 - 7.09 (m, 1H), 5.38 - 5.17 (m, 1H), 5.07 - 4.99 (m, 1H), 4.74 - 4.67 (m, 1H), 4.56 (br d, J = 2.1 Hz, 2H), 4.21 - 4.14 (m, 1H), 3.82 (br dd, J = 2.8, 13.8 Hz, 1H), 3.67 - 3.55 (m, 1H), 3.52 - 3.41 (m, 1H), 3.23 - 3.19 (m, 2H), 3.13 - 3.01 (m, 1H), 2.97 - 2.86 (m, 1H), 2.72 - 2.63 (m, 8H), 2.58 - 2.42 (m, 2H), 2.24 - 1.98 (m, 3H), 1.97 - 1.64 (m, 11H), 1.62 (s, 3H), 1.60 - 1.37 (m, 7H), 1.12 - 1.00 (m, 5H), 0.91 - 0.77 (m, 6H)

<sup>13</sup>C (125 MHz, METHANOL-d<sub>4</sub>) δ : 174.333, 174.304, 172.607, 172.058, 171.978, 171.44, 168.515, 137.487, 132.403, 132.352, 132.305, 130.695, 128.546, 93.484, 91.726, 76.603, 61.361, 60.163, 55.541, 55.425, 55.382, 55.32, 55.31, 52.887, 49.211, 49.117, 48.481, 48.315, 48.243, 48.102, 47.889, 47.817, 47.672, 47.604, 47.497, 47.459, 47.055, 46.965, 39.949, 39.689, 39.031, 38.515, 34.594, 34.428, 34.377, 32.214, 31.932, 30.311, 29.372, 28.058, 27.827, 26.473, 24.703, 22.844, 22.584, 20.804, 20.049, 20.038, 19.691, 17.886, 16.117, 16.059, 9.902.

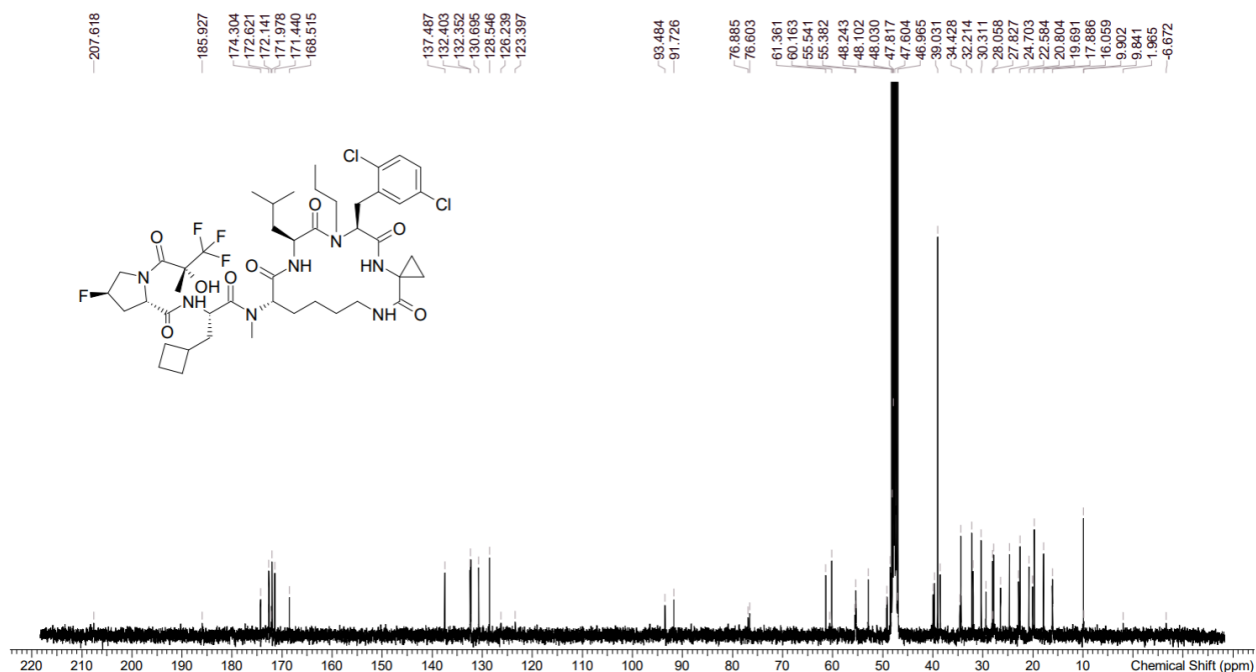
HRMS: [M+H] calc. 976.41, obs. 975.41

## Characterization of CIRc-028

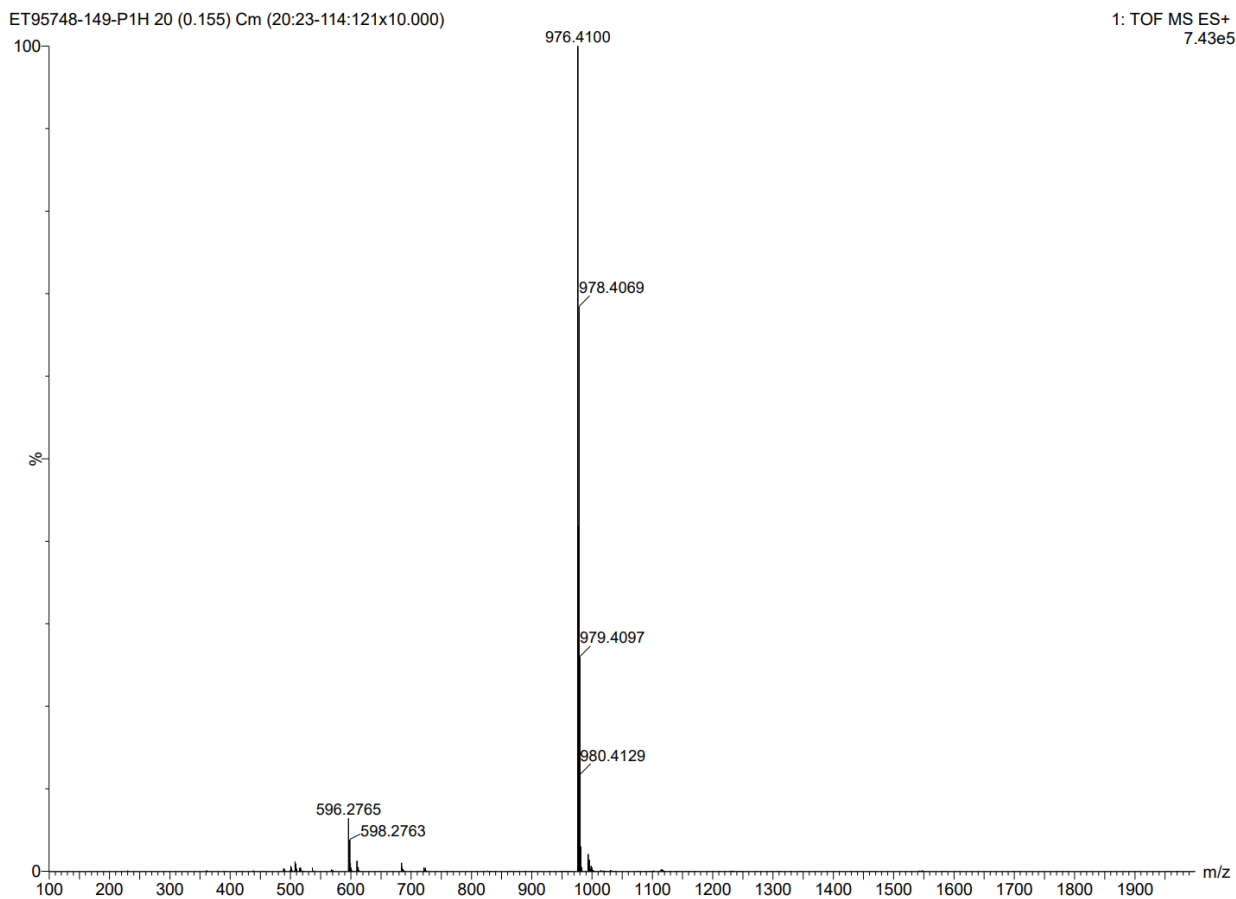
### <sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-028



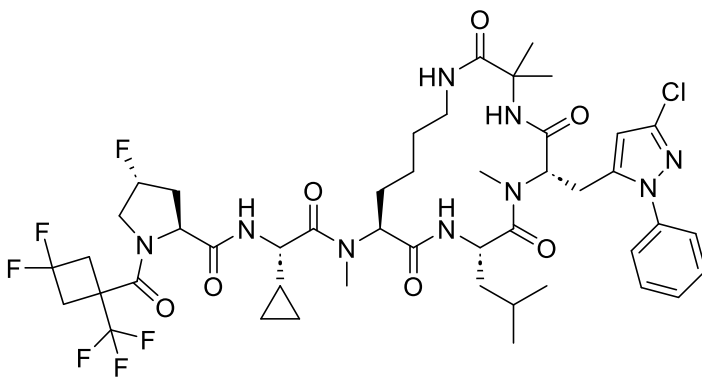
### <sup>13</sup>C NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-028



## HRMS [M+H]<sup>+</sup> of CIRc-028



### 2.3.5. Synthesis of CIRc-019



Fmoc-Aib-OH, CAS#94744-50-0, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The solution was

dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride (CTC) resin and was agitated for 2 hours at room temperature. The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoic acid (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-alkylate the terminal amine. 1) Nosyl protection. A solution of 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 40 to 45°C for 10 to 15 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Dry methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 45°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Leucine-OH, CAS# 35661-60-0, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated a second time. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Fmoc-L-Lysine(Boc)-OH, CAS# 71989-26-9, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to

pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

Fmoc-L-Cyclopropylalanine-OH, CAS# 1212257-18-5, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C to 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated a second time. To remove Fmoc, A mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(2S,4R)-1-(3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carbonyl)-4-fluoropyrrolidine-2-carboxylic acid (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times.

To simultaneously cleave the peptide from CTC resin and deprotect the Boc-protected lysine sidechain, approximately 2 mL of a solution of 20% TFA and 5% TIPS in DCM (2 mL) was added to the 100 mg of polystyrene resin in a solid phase reaction vessel. The contents of the vessel were shaken for one hour. The liquid phase of the reaction was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional DCM (2 mL) and the wash was collected in the conical vial. Toluene (2 mL) was added to the cleaved peptide solution and the solution was neutralized with triethylamine and concentrated under reduced atmosphere in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The

purified fractions were pooled and lyophilized to yield white powder (LCMS m/z observed = 1018.4 [M+H]<sup>+</sup>).

The deprotected and purified linear peptide was transferred to a 50 mL conical vial and dissolved in 1 mL NMP followed by the addition of DIEA (0.5 mL) and DCM (35 mL). T3P (3 eqv) was added to the solution and the pH was adjusted to pH 9 via dropwise addition of DIEA. The closed conical vial was then shaken at room temperature for 2 hours at 150 rotations per minute. The conical vial was then uncapped and the solution was concentrated at 45°C under reduced pressure in a Genevac system. The evaporated crude material was then redissolved in acetonitrile and purified via reverse phase chromatography with 0.1% TFA and lyophilized to yield 20.4 mg of CIRc-019 as a white powder (m/z observed = 1000.45 [M+Z]<sup>+</sup>)

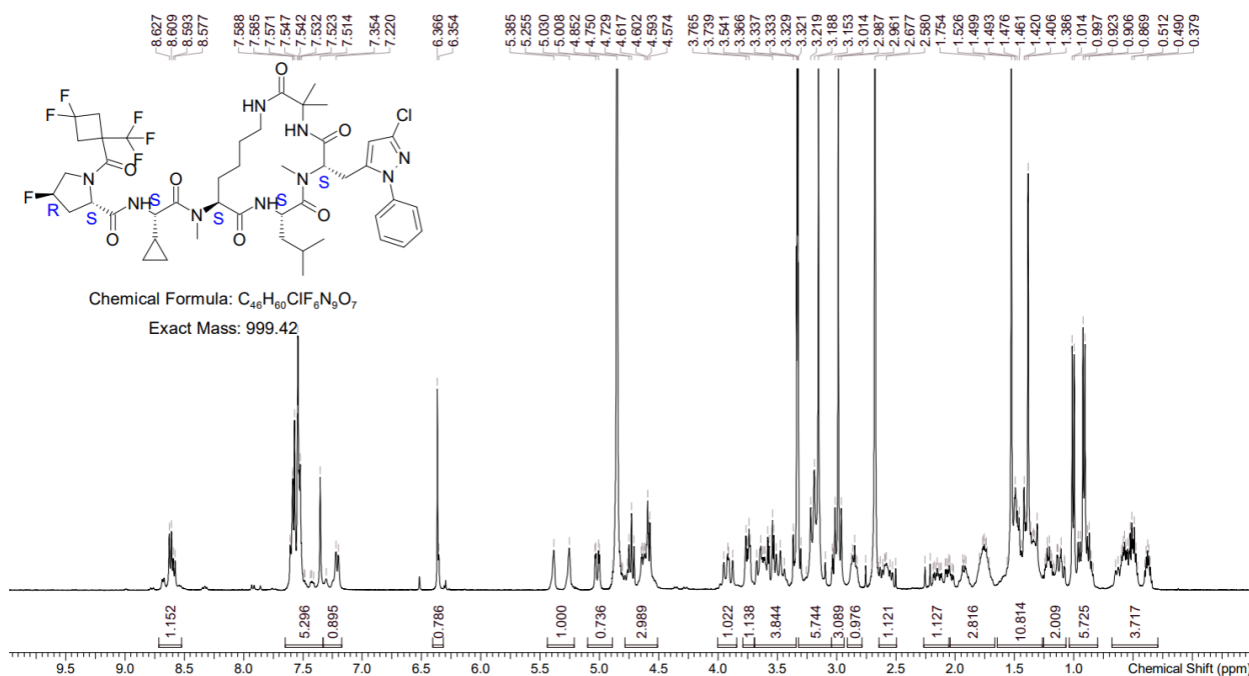
<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.71 - 8.52 (m, 1H), 7.35 (s, 4H), 7.33 - 7.17 (m, 1H), 6.41 - 6.32 (m, 1H), 5.44 - 5.21 (m, 1H), 5.10 - 4.89 (m, 1H), 4.78 - 4.51 (m, 3H), 4.00 - 3.84 (m, 1H), 3.79 - 3.69 (m, 1H), 3.69 - 3.34 (m, 4H), 3.32 - 3.04 (m, 6H), 3.04 - 2.94 (m, 3H), 2.91 - 2.79 (m, 1H), 2.64 - 2.50 (m, 1H), 2.25 (s, 1H), 2.04 - 1.67 (m, 3H), 1.64 - 1.26 (m, 7H), 1.25 - 1.06 (m, 2H), 1.04 - 0.80 (m, 6H), 0.68 - 0.29 (m, 4H)

<sup>13</sup>C NMR (125 MHz, METHANOL-d<sub>4</sub>) δ : 174.777, 173.553, 171.115, 171.072, 170.985, 170.87, 168.461, 168.386, 142.481, 139.726, 138.144, 129.048, 128.384, 124.802, 106.498, 92.672, 90.899, 63.582, 59.155, 57.216, 57.119, 54.905, 54.858, 51.785, 48.864, 47.915, 47.842, 47.701, 47.629, 47.485, 47.416, 47.203, 46.99, 46.777, 46.564, 38.645, 38.577, 38.158, 37.901, 34.626, 34.41, 29.777, 27.748, 26.715, 26.249, 24.357, 24.028, 22.024, 21.941, 21.75, 19.24, 11.383, 1.417, 0.929.

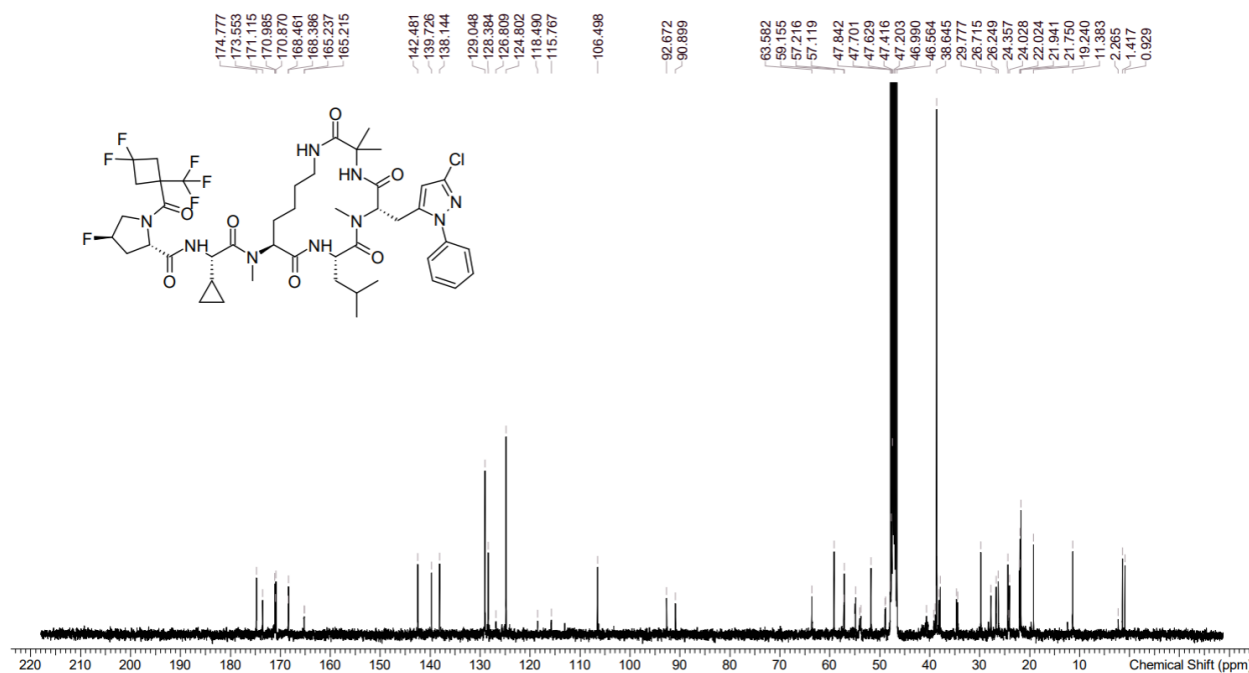
HRMS: [M+H] calc. 1000.42, obs. 1000.42

# Characterization of CIRc-019

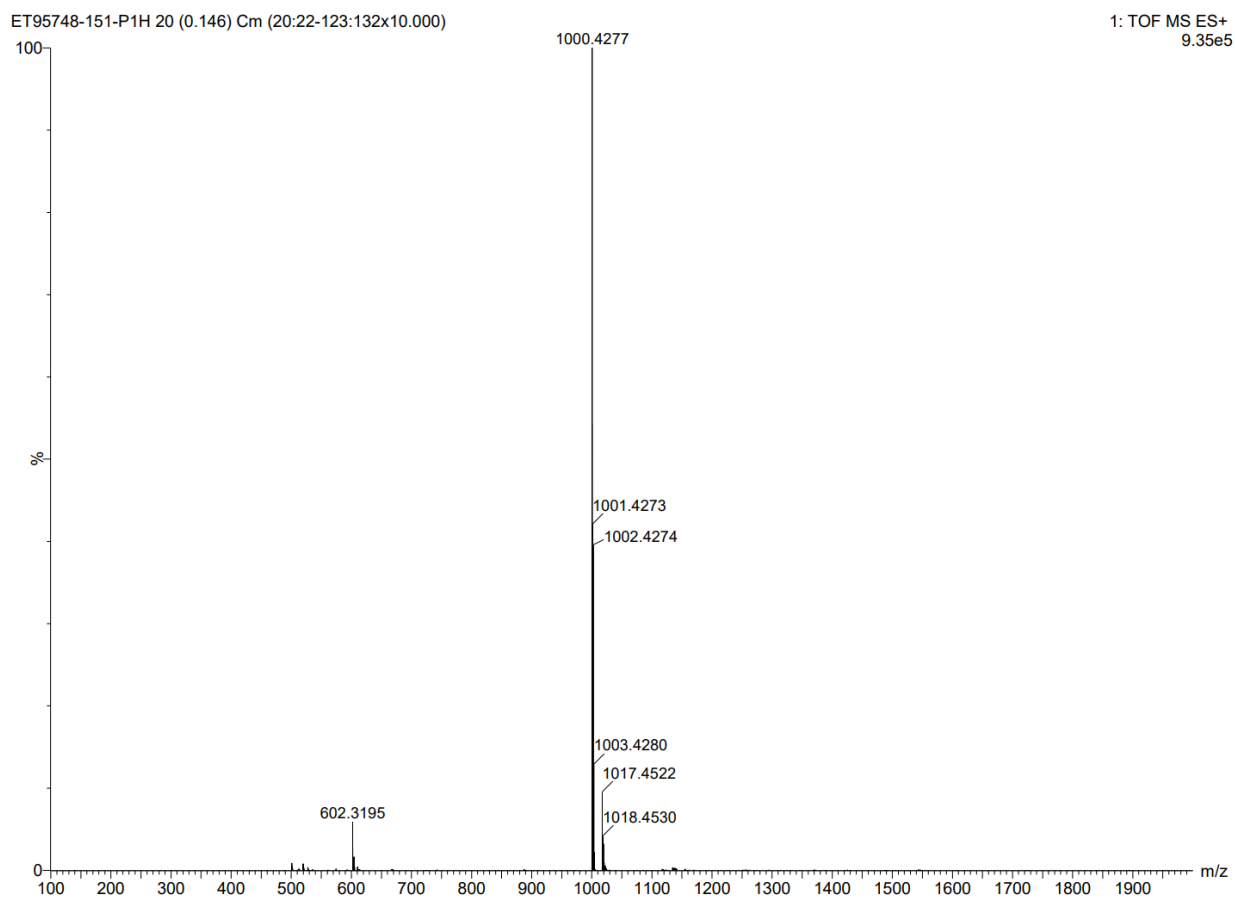
## <sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-019



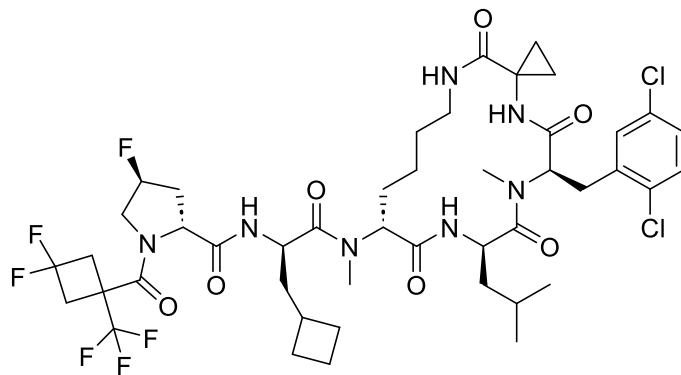
## <sup>13</sup>C NMR (400 MHz, METHANOL-d<sub>4</sub>) of CIRc-019



## HRMS [M+H] of CIRc-019



## 2.3.6. Synthesis of CIRc-005



Fmoc-1-aminocyclopropane-1-carboxylic acid, CAS#126705-22-4, (4 equiv.) was dissolved in 1.0 mL of anhydrous NMP. Neat DIEA (8 equiv.) was added to the Fmoc-amino acid solution. The solution was dispensed in a peptide reactor vessel containing 100 mg of 2-chlorotrityl chloride (CTC) resin and was agitated for 2 hours at room temperature. The amino acid solution was drained then the resin was washed with 1.0 mL DMF three times. Unreacted CTC resin was capped with 1.0 mL solution of methanol:DMF (50:50), and DIEA (8 equiv.) for 10 minutes at room temperature. The methanol solution was drained then the resin was washed with 1.0 mL DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

A solution of (R)-3-(2,5-dichloro-phenyl)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-propionic acid, CAS#1260596-66-4, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-alkylate the terminal amine. 1) Nosyl protection. A solution of 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 40 to 45°C for 10 to 15 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Dry methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 45°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

(2R)-2-([(9H-fluoren-9-yl)methoxy]carbonyl)amino)-4-methylpentanoic acid, CAS# 114360-54-2, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The reaction was repeated a second time to ensure complete coupling. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and

agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(2R)-6-[[tert-butoxy]carbonyl]amino}-2-([9H-fluoren-9-yl)methoxy]carbonyl]amino)hexanoic acid, CAS# 92122-45-7, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

Three steps are required to mono-methylate the terminal amine. 1) Nosyl protection. 2,6-lutidine (6 equiv.) dissolved in 0.5 mL of anhydrous DCE was added to the resin. 2-nitrobenzenesulfonyl chloride (5 equiv.) dissolved in 0.5 mL anhydrous toluene was added to the resin then was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous toluene three times. The method was repeated twice. 2) Alkylation via Mitsunobu conditions. Triphenylphosphine (10 equiv.) dissolved in 0.7 mL anhydrous toluene was added to the resin. Methanol (20 equiv.) was added to the resin. Azodicarboxylate (10 equiv) was added to the resin and was agitated at 45°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Alkylation was repeated twice. 3) Nosyl deprotection. 2-mercaptoethanol (5 equiv.) and 1,8-Diazabicyclo[5.4.0]undec-7-ene (5 equiv.) in 1.0 mL NMP was added to the resin and was agitated at 35°C for 10 minutes. The mixture was drained and then the resin was washed with 1.0 mL of anhydrous DMF three times. Deprotection of the nosyl group was repeated twice.

(2R)-3-cyclobutyl-2-([9H-fluoren-9-yl)methoxy]carbonyl]amino)propanoic acid CAS# 78183-63-0, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C to 45°C for 30 min. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling step was repeated. To remove Fmoc, A mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

(2R,4S)-1-([9H-fluoren-9-yl)methoxy]carbonyl]-4-fluoropyrrolidine-2-carboxylic acid, CAS# 913820-87-8, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared. The mixture was allowed to pre-activate at room temperature for 5 minutes, and then was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. To remove Fmoc, a mixture of piperidine:DMF (20:80, 1 mL) was added to the resin and agitated for 10 to 15 minutes at room temperature. The piperidine solution was drained and then the resin was washed with 1.0 mL DMF three times.

3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carboxylic acid, CAS# 2167095-52-3, (4 equiv.), HATU (4 equiv.), and DIEA (8 equiv) in 1.0 mL of anhydrous NMP was prepared and adjusted to pH 9. The mixture was added to the resin and agitated at 35°C for 30 minutes. The mixture was drained and then the resin was washed with 1.0 mL of DMF three times. The coupling was repeated a second time.

To simultaneously cleave the peptide from CTC resin and deprotect the Boc-protected lysine sidechain, approximately 2 mL a solution of 20% TFA and 5% TIPS in DCM (2 mL) was added to the 100 mg of polystyrene resin in a solid phase reaction vessel. The contents of the vessel were shaken for one hour. The liquid phase of the reaction was filtered into a 50 mL conical vial. The cleaved resin was washed with an additional DCM (2 mL) and the wash was collected in the conical vial. Toluene (2 mL) was added to the cleaved peptide solution and the solution was neutralized with triethylamine and concentrated under reduced atmosphere in a Genevac. The linear peptide was purified via reverse-phase HPLC using an Acetonitrile/Water gradient with 0.05% formic acid. The purified fractions were pooled and lyophilized to yield white powder (LCMS m/z observed = 1012.32 [M+H]<sup>+</sup>).

The deprotected and purified linear peptide was transferred to a 50 mL conical vial and dissolved in 1 mL NMP followed by the addition of DIEA (0.5 mL) and DCM (35 mL). T3P (3 eqv) was added to the solution and the pH was adjusted to pH 9 via dropwise addition of DIEA. The closed conical vial was then shaken at room temperature for 2 hours at 150 rotations per minute. The conical vial was then uncapped and the solution was concentrated at 45°C under reduced pressure in a Genevac system. The evaporated crude material was then redissolved in acetonitrile and purified via reverse phase chromatography with 0.1% TFA and lyophilized to yield 6.8 mg of CIRc-005 as a white powder (m/z observed = 994.50 [M+Z]<sup>+</sup>).

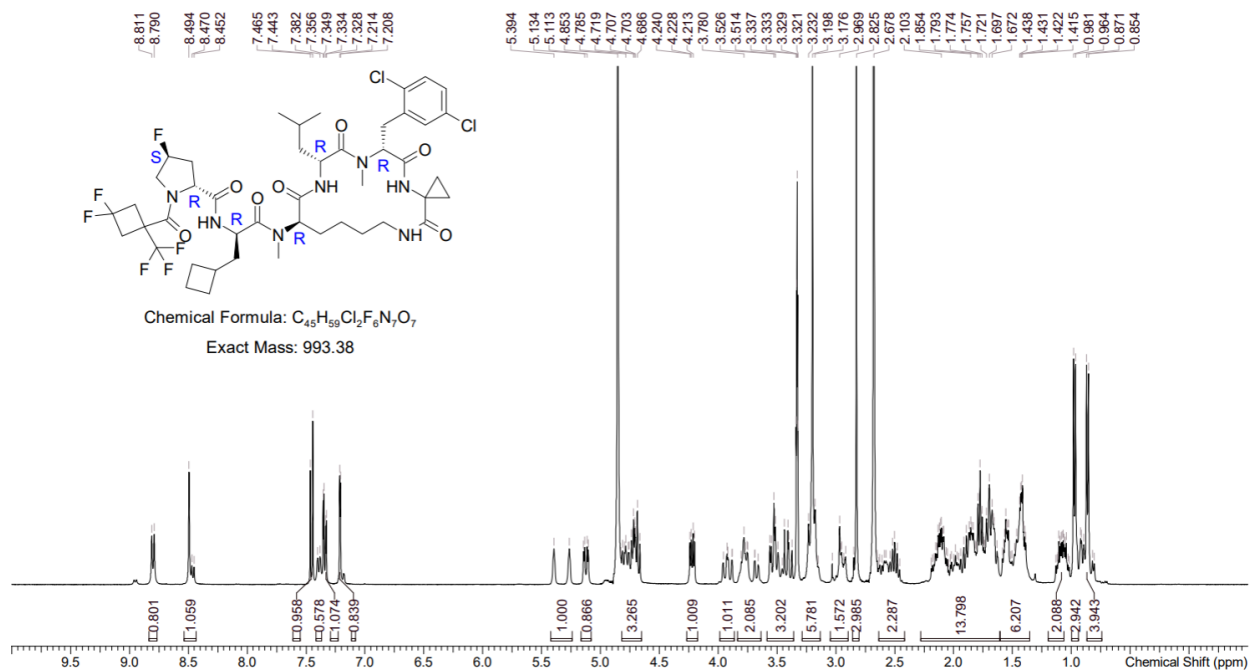
<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.80 (br d, J = 8.4 Hz, 1H), 8.54 - 8.43 (m, 1H), 7.49 - 7.43 (m, 1H), 7.39 (br d, J = 7.6 Hz, 1H), 7.37 - 7.30 (m, 1H), 7.21 (d, J = 2.5 Hz, 1H), 5.42 - 5.24 (m, 1H), 5.17 - 5.08 (m, 1H), 4.82 - 4.65 (m, 3H), 4.27 - 4.18 (m, 1H), 3.98 - 3.86 (m, 1H), 3.83 - 3.64 (m, 2H), 3.58 - 3.36 (m, 3H), 3.29 - 3.13 (m, 6H), 3.05 - 2.89 (m, 2H), 2.83 (s, 3H), 2.63 - 2.42 (m, 2H), 2.28 - 1.61 (m, 14H), 1.60 - 1.35 (m, 6H), 1.15 - 1.02 (m, 2H), 1.00 - 0.94 (m, 3H), 0.93 - 0.80 (m, 4H)

<sup>13</sup>C NMR (125 MHz, METHANOL-d<sub>4</sub>) δ : 173.74, 172.646, 172.574, 171.588, 171.368, 171.09, 137.548, 132.421, 132.374, 130.702, 128.499, 93.094, 91.318, 63.445, 59.7, 55.356, 48.709, 48.492, 48.326, 48.254, 48.109, 48.041, 47.896, 47.828, 47.615, 47.402, 47.189, 46.976, 39.349, 39.147, 39.053, 38.623, 38.439, 35.078, 34.861, 34.164, 32.283, 31.95, 30.224, 27.993, 27.91, 27.78, 26.982, 24.747, 22.649, 22.501, 19.601, 17.85, 16.575, 15.947.

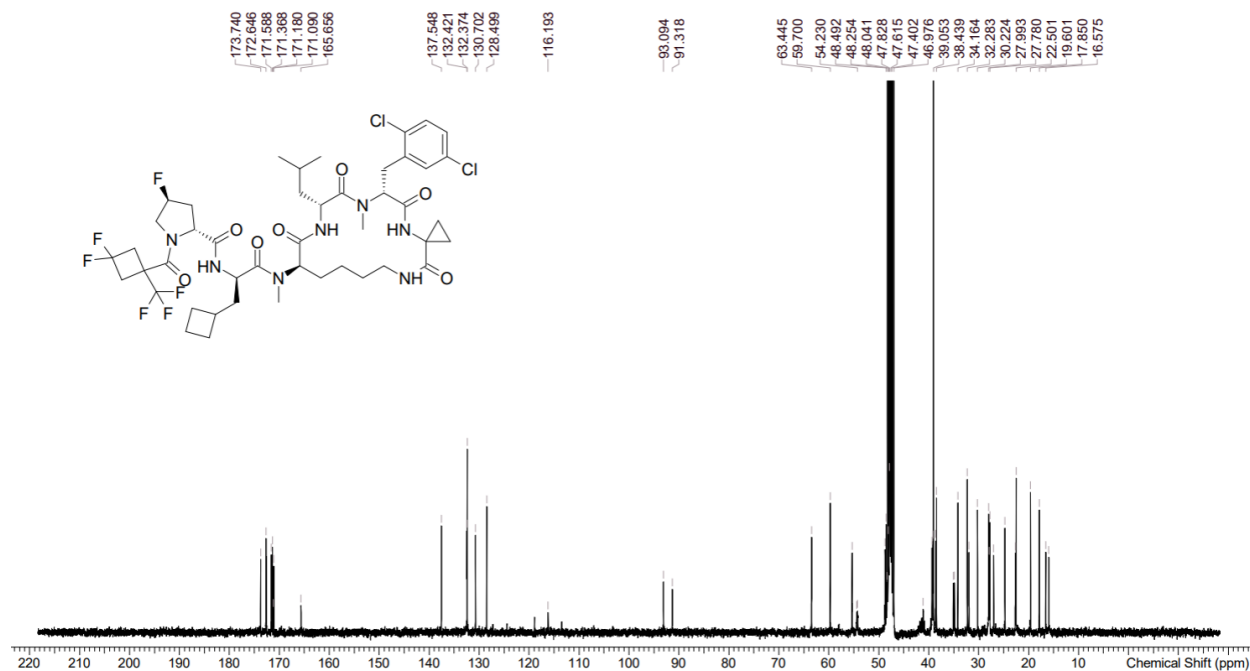
HRMS: [M+H] calc. 994.38, obs. 994.38

# Characterization of CIRc-005

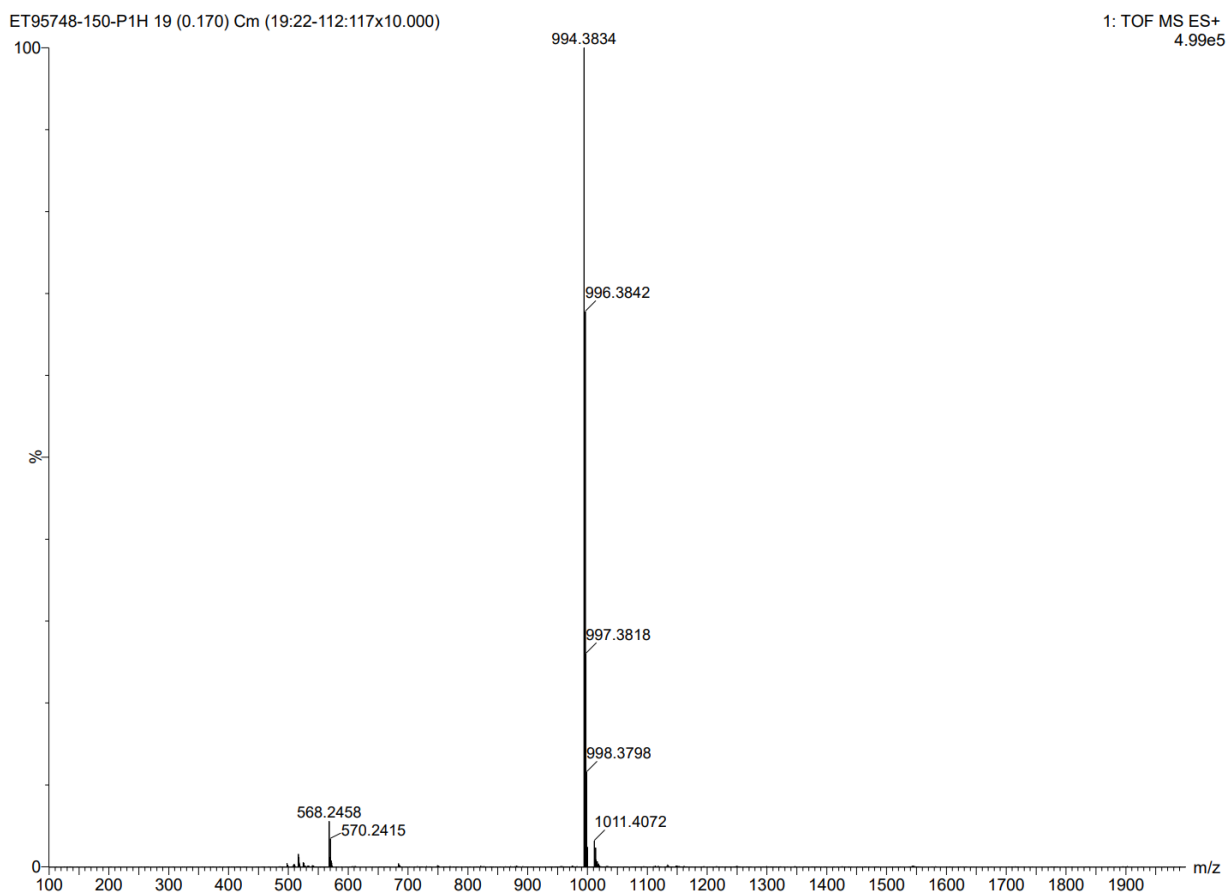
## <sup>1</sup>H NMR (400 MHz, METHANOL-d4) of CIRc-005



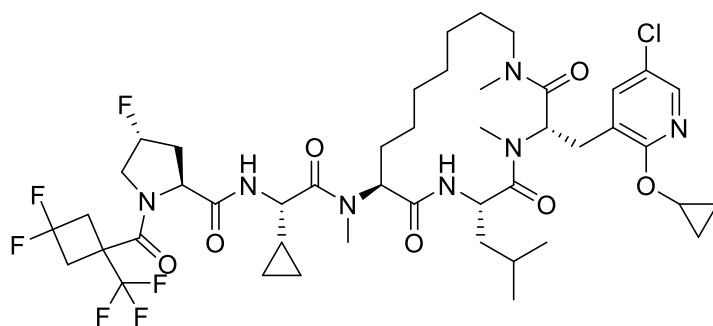
## <sup>13</sup>C NMR (400 MHz, METHANOL-d4) of CIRc-005



## HRMS [M+H] of CIRc-005



## 2.3.6. Synthesis of CIRc-014



Step 1: tert-butyl (S)-(3-(5-chloro-2-cyclopropoxypyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate



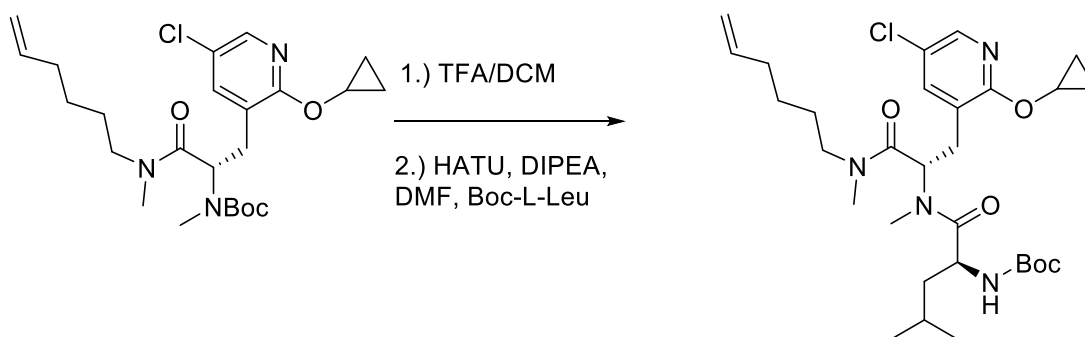
To a 500mL round-bottom flask were added (2S)-2-[(tert-butoxycarbonyl)(methyl)amino]-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoic acid (35 g, 94 mmol, 1 eq.) and HATU (46.4 g, 122.2 mmol, 1.3 eq.). The solids were dissolved in DMF (200 mL), and to the solution was added N-methylhex-5-en-1-amine (13.8 g, 122.2 mmol, 1.3 eq.) followed by DIPEA (48.5 g, 65.5 mL, 376 mmol, 4 eq.). The solution was confirmed basic by pH paper and stirred for 2 hours. The reaction mixture was diluted in water (400 mL) and extracted with ethyl acetate (300 mL) three times. The combined organics were washed with brine (300 mL), dried over anhydrous magnesium sulfate, and filtered over celite. The organic extract was concentrated under rotary evaporation to give a brown oil. The crude material was passed through a silica plug (30-40% ethyl acetate in hexanes) and the eluent was concentrated to give tert-butyl ((S)-1-(((S)-3-(5-chloro-2-cyclopropoxypyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl)(methyl)carbamate as a pale-yellow semi-pure oil (assumed 100% conversion) that was carried forward without further purification. A ~100mg sample was purified by flash chromatography (0 to 100% ethyl acetate in hexanes) for further analysis.

**LCMS:** (ESI, m/z):  $[M+H]^+ = 466.2$

**Spectrum:**

$^1\text{H}$  NMR (400 MHz, METHANOL- $d_4$ )  $\delta$ : 8.11 - 7.95 (m, 1H), 7.67 - 7.47 (m, 1H), 5.92 - 5.69 (m, 1H), 5.12 (dd,  $J = 3.3, 10.6$  Hz, 1H), 5.07 - 4.93 (m, 2H), 4.41 - 4.23 (m, 1H), 3.38 (m, 1H), 3.31 - 3.12 (m, 1H), 3.05 - 2.94 (m, 3H), 2.94 - 2.91 (m, 1H), 2.90 - 2.80 (m, 1H), 2.80 - 2.73 (m, 3H), 2.17 - 2.06 (m, 2H), 1.67 - 1.49 (m, 2H), 1.47 - 1.35 (m, 2H), 1.33 - 1.20 (m, 9H), 1.08 - 0.58 (m, 5H).

Step 2: Synthesis of tert-butyl ((S)-1-(((S)-3-(5-chloro-2-cyclopropoxypyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl)(methyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate



To a 500 mL flask containing (S)-3-(5-chloro-2-cyclopropoxy)pyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl(methyl)carbamate residue (94 mmol, 1 eq.) was added DCM (50 mL) and TFA (50 mL). The solution was allowed to sit for 1 hour and concentrated by rotary evaporation. The residue was suspended in toluene and concentrated three times, and then carried forward without purification.

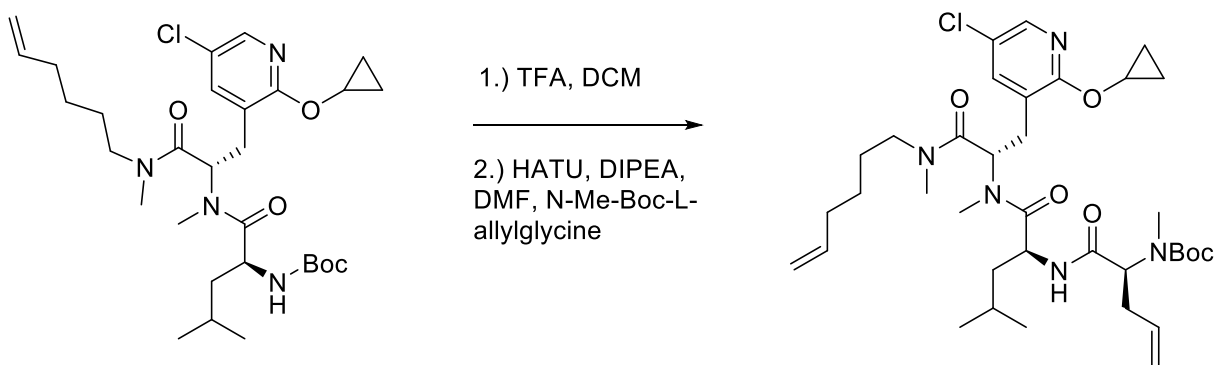
To the residue was added a solution of HATU (46.4 g, 122.2 mmol, 1.3 eq.), Boc-L-Leucine monohydrate (30.4 g, 122.2 mmol, 1.3 eq.), and DIPEA (48.5 g, 65.5 mL, 376 mmol, 4 eq.) in DMF (200 mL). The solution was checked by pH paper and DIPEA was added in 1 eq. increments until the solution was confirmed to be basic. The reaction was stirred for 2 hours, diluted with water (400 mL), and extracted with ethyl acetate (300 mL) three times. The combined organics were washed with brine (300 mL), dried over anhydrous magnesium sulfate, and filtered over celite. The organic extract was concentrated under rotary evaporation to give a yellow oil. The crude material was passed through a silica plug (30-40% ethyl acetate in hexanes) and the eluent was concentrated to give tert-butyl ((S)-1-(((S)-3-(5-chloro-2-cyclopropoxy)pyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl(methyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate as a yellow semi-pure oil (assumed 100% conversion) that was carried forward without further purification. A ~100mg sample was purified by flash chromatography (0 to 100% ethyl acetate in hexanes) for further analysis.

**LCMS:** (ESI, m/z):  $[M+H]^+ = 579.3$

**Spectrum:**

$^1\text{H}$  NMR (400 MHz, METHANOL- $d_4$ )  $\delta$  : 7.97 (d,  $J = 2.4$  Hz, 1H), 7.55 (dd,  $J = 8.4$  Hz, 4.2 Hz, 1H), 5.91 - 5.73 (m, 2H), 5.08 - 4.93 (m, 2H), 4.52 - 4.43 (m, 1H), 4.38 - 4.26 (m, 1H), 3.34 (br s, 2H), 3.08 (s, 4H), 3.02 - 2.95 (m, 1H), 2.96 (br s, 3H), 2.14 - 2.04 (m, 2H), 1.79 - 1.68 (m, 1H), 1.63 - 1.51 (m, 3H), 1.46 - 1.42 (m, 9H), 1.36 - 1.29 (m, 2H), 0.98 - 0.94 (m, 6H), 0.88 - 0.73 (m, 4H).

Step 3: Synthesis of tert-butyl ((S)-1-(((S)-1-(((S)-3-(5-chloro-2-cyclopropoxy)pyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl(methyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxopent-4-en-2-yl(methyl)carbamate



To a 500 mL flask containing tert-butyl ((S)-1-(((S)-3-(5-chloro-2-cyclopropoxy)pyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl(methyl)amino)-4-methyl-1-

oxopentan-2-yl)carbamate residue (94 mmol, 1 eq.) was added DCM (50 mL) and TFA (50 mL). The solution was allowed to sit for 1 hour and concentrated by rotary evaporation. The residue was suspended in toluene and concentrated three times, and then carried forward without purification.

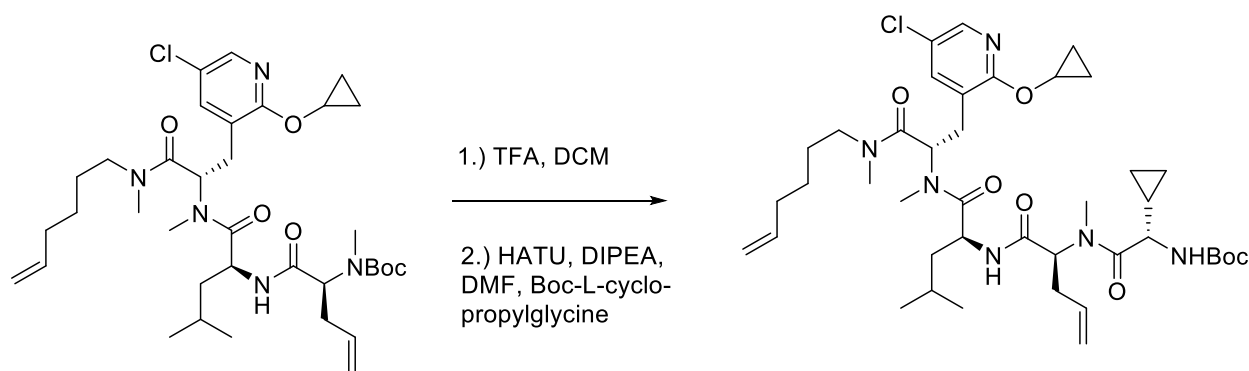
To the residue was added a solution of HATU (46.4 g, 122.2 mmol, 1.3 eq.), N-methyl-Boc-(S)-allylglycine (27.7 g, 122.2 mmol, 1.3 eq.), and DIPEA (48.5 g, 65.5 mL, 376 mmol, 4 eq.) in DMF (200 mL). The solution was checked by pH paper and DIPEA was added in 1 eq. increments until the solution was confirmed to be basic. The reaction was stirred for 2 hours, diluted with water (400 mL), and extracted with ethyl acetate (300 mL) three times. The combined organics were washed with brine (300 mL), dried over anhydrous magnesium sulfate, and filtered over celite. The organic extract was concentrated under rotary evaporation to give a dark yellow oil. The crude material was passed through a silica plug (30-40% ethyl acetate in hexanes) and the eluent was concentrated to give tert-butyl ((S)-1-(((S)-3-(5-chloro-2-cyclopropoxy)pyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl)(methyl)amino)-4-methyl-1-oxopentan-2-yl)carbamate as a yellow semi-pure oil (assumed 100% conversion) that was carried forward without further purification. A ~100mg sample was purified by flash chromatography (0 to 100% ethyl acetate in hexanes) for further analysis.

**LCMS:** (ESI, m/z):  $[M+H]^+ = 690.3$

**Spectrum:**

$^1\text{H}$  NMR (400 MHz, METHANOL- $d_4$ )  $\delta$  : 7.86 (d,  $J = 2.0$  Hz, 1H), 7.43 (dd,  $J = 10.8$  Hz, 2.4 Hz, 1H), 5.76 - 5.61 (m, 3H), 5.10 - 4.82 (m, 4H), 4.71 - 4.65 (m, 1H), 4.49 - 4.36 (m, 1H), 4.22 - 4.15 (m, 1H), 3.21-3.36 (m, 2H), 2.96 (s, 3H), 2.87 - 2.76 (m, 5H), 2.72 (s, 3H), 2.51 - 2.41 (m, 1H), 2.39 - 2.28 (m, 1H), 2.01 - 1.93 (m, 2H), 1.58 - 1.41 (m, 3H), 1.40 (br s, 1H), 1.37 - 1.35 (m, 9H), 1.26 - 1.17 (m, 3H), 0.79-0.87 (m, 6H), 0.77 - 0.64 (m, 4H).

Step 4: Synthesis of tert-butyl ((1S,4S,7S,10S)-4-allyl-10-((5-chloro-2-cyclopropoxy)pyridin-3-yl)methyl)-1-cyclopropyl-7-isobutyl-3,9,12-trimethyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazaoctadec-17-en-1-yl)carbamate



To a 500 mL flask containing tert-butyl ((S)-1-(((S)-1-(((S)-3-(5-chloro-2-cyclopropoxy)pyridin-3-yl)-1-(hex-5-en-1-yl(methyl)amino)-1-oxopropan-2-yl)(methyl)amino)-4-methyl-1-oxopentan-2-yl)amino)-1-oxopent-4-en-2-

yl)(methyl)carbamate residue (94 mmol, 1 eq.) was added DCM (50 mL) and TFA (50 mL). The solution was allowed to sit for 1 hour and concentrated by rotary evaporation. The residue was suspended in toluene and concentrated three times, and then carried forward without purification.

To the residue was added a solution of HATU (46.4 g, 122.2 mmol, 1.3 eq.), N-methyl-Boc-(S)-allylglycine (27.7 g, 122.2 mmol, 1.3 eq.), and DIPEA (48.5 g, 65.5 mL, 376 mmol, 4 eq.) in DMF (200 mL). The solution was checked by pH paper and DIPEA was added in 1 eq. increments until the solution was confirmed to be basic. The reaction was stirred for 2 hours, diluted with water (400 mL), and extracted with ethyl acetate (300 mL) three times. The combined organics were washed with brine (300 mL), dried over anhydrous magnesium sulfate, and filtered over celite. The organic extract was concentrated under rotary evaporation to give an orange oil. The crude material was purified by silica gel chromatography (0-100% ethyl acetate in hexanes) to give tert-butyl ((1S,4S,7S,10S)-4-allyl-10-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-1-cyclopropyl-7-isobutyl-3,9,12-trimethyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazaoctadec-17-en-1-yl)carbamate (70 g, 94% 7-step yield) as a light yellow oil.

**LCMS:** (ESI, m/z):  $[M+H]^+ = 787.45$

**Spectrum:**

$^1\text{H}$  NMR (400 MHz, METHANOL- $d_4$ )  $\delta$  = 7.92 - 7.81 (m, 1H), 7.47 - 7.40 (m, 1H), 5.79 - 5.49 (m, 3H), 5.17 - 4.82 (m, 5H), 4.67 (m, 1H), 4.21 - 3.93 (m, 2H), 3.38 - 3.23 (m, 1H), 3.19 - 3.01 (m, 1H), 3.00 - 2.93 (m, 5H), 2.87 - 2.72 (m, 5H), 2.68 (s, 3H), 2.03 - 1.93 (m, 2H), 1.47 (br s, 2H), 1.46 - 1.38 (m, 2H), 1.35 - 1.30 (m, 9H), 1.28 - 1.17 (m, 3H), 1.10 - 0.92 (m, 1H), 0.88 - 0.79 (m, 6H), 0.78 - 0.62 (m, 4H), 0.56 - 0.26 (m, 4H).

Step 5: Synthesis of tert-butyl ((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadec-11-en-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)carbamate



To a 2L three-necked flask was fitted a reflux condenser and two addition funnels. The system was evacuated and backfilled with argon and DCE (500 mL) was added. The solvent was degassed with argon for 15 minutes. Separately, tert-butyl ((1S,4S,7S,10S)-4-allyl-10-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-1-cyclopropyl-7-isobutyl-3,9,12-trimethyl-2,5,8,11-tetraoxo-3,6,9,12-tetraazaoctadec-17-en-1-yl)carbamate (17.5g,

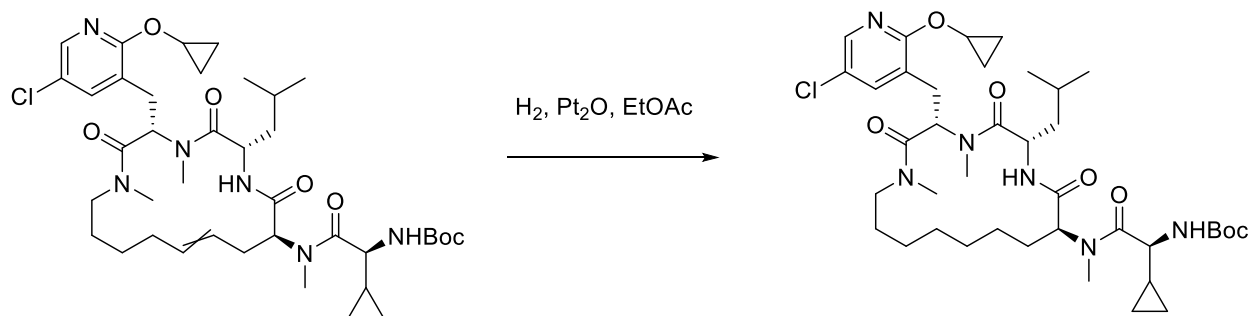
22.25 mmol, 1 eq.) was dissolved in DCE (250 mL) and the solution was degassed with argon for 15 minutes. This solution was added to one of the addition funnels. Separately, Hoyveda-Grubbs II M720 Umicore (1.67 g, 2.67 mmol, 12mol% catalyst loading) was dissolved in DCE (250 mL) and the solution was degassed with argon for 15 minutes. This solution was added into the second addition funnel. The flask was heated to 70 °C and the solutions were added dropwise simultaneously over 4 hours. The reaction was stirred for an additional 8 hours before being allowed to cool to room temperature. The solvent was evaporated by rotary evaporation and the residue was purified by silica gel chromatography (0-100% ethyl acetate in hexanes). This process was replicated 4 times in parallel reactions to yield tert-butyl ((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadec-11-en-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)carbamate (52 g, 77% yield) as a light-brown solid.

**LCMS:** (ESI, m/z):  $[M+H]^+ = 759.4$

**Spectrum:**

$^1\text{H}$  NMR (400 MHz, METHANOL- $d_4$ )  $\delta$  : 8.17 - 8.05 (m, 1H), 8.00 - 7.80 (m, 1H), 7.53 - 7.30 (m, 1H), 5.55 - 5.07 (m, 2H), 5.00 - 4.83 (m, 1H), 4.45 - 4.02 (m, 4H), 3.17 - 2.89 (m, 5H), 2.86 - 2.65 (m, 7H), 2.61 - 2.43 (m, 1H), 1.91 (s, 3H), 1.88 - 1.75 (m, 1H), 1.63 - 1.46 (m, 2H), 1.43 - 1.21 (m, 13H), 1.10-0.96 (m, 2H), 0.86 - 0.63 (m, 11H), 0.50 - 0.15 (m, 4H)

Step 6: Synthesis of tert-butyl ((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadecan-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)carbamate



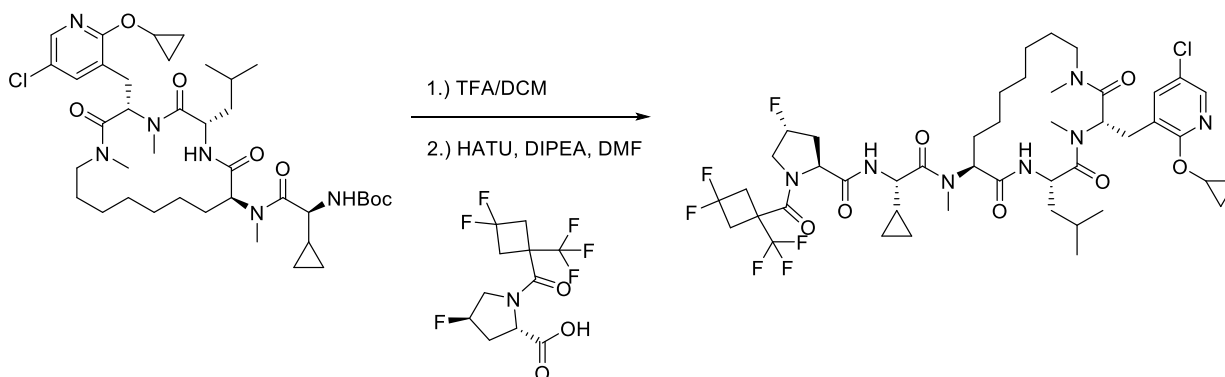
To a 1000 mL flask containing a solution of ((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadec-11-en-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)carbamate (52 g, 68.5 mmol) in ethyl acetate (500 mL) was added Adams' catalyst (777 mg, 3.4 mmol, 10mol% catalyst loading). The system was evacuated and backfilled with hydrogen gas ten times and allowed to stir at room temperature for 2 hours. The solution was filtered over celite to remove the catalyst and the solvent was removed by rotary evaporation to give tert-butyl ((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadecan-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)carbamate (52g, 68.5 mmol, quant. yield) as a light-brown solid, which was carried forward without further purification.

**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 761.4

**Spectrum:**

<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 7.85 - 7.78 (d, J = 2.4 Hz, 1H), 7.37 - 7.29 (d, J = 2.4 Hz, 1H), 4.19 - 4.01 (m, 4H), 2.98 - 2.79 (m, 5H), 2.78 - 2.67 (m, 7H), 2.54 - 2.43 (m, 1H), 1.48 - 1.29 (m, 4H), 1.26 - 1.20 (m, 13H), 1.18 - 1.08 (m, 5H), 0.96 - 0.82 (m, 2H), 0.79 - 0.67 (m, 5H), 0.53 - 0.64 (m, 7H), 0.33 - 0.12 (m, 3H), 0.02 (m, 1H).

Step 7: Synthesis of (2S,4R)-N-(((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-4-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadecan-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)-1-(3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carbonyl)-4-fluoropyrrolidine-2-carboxamide (**CIRc-014**)



To a 500 mL flask containing ((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-3-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadecan-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)carbamate (52g, 68.5 mmol, 1 eq.) was added DCM (50 mL) and TFA (50 mL). The solution was allowed to sit for 1 hour and concentrated by rotary evaporation. The residue was suspended in toluene and concentrated three times, and then carried forward without purification.

To the residue was added a solution of HATU (33.8 g, 89 mmol, 1.3 eq.), (2S,4R)-1-(3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carbonyl)-4-fluoropyrrolidine-2-carboxylic acid (28.3 g, 89 mmol, 1.3 eq.), and DIPEA (35.3 g, 47.72 mL, 273 mmol, 4 eq.) in DMF (200 mL). The solution was checked by pH paper and DIPEA was added in 1 eq. increments until the solution was confirmed to be basic. The reaction was stirred for 2 hours, diluted with water (400 mL), and extracted with ethyl acetate (300 mL) three times. The combined organics were washed with brine (300 mL), dried over anhydrous magnesium sulfate, and filtered over celite. The organic extract was concentrated under rotary evaporation to give a brown oil. The crude material was purified by reverse-phase HPLC (50-90% acetonitrile in water, 0.1% formic acid buffer) to give (2S,4R)-N-(((S)-2-(((3S,6S,9S)-3-((5-chloro-2-cyclopropoxypyridin-4-yl)methyl)-6-isobutyl-1,4-dimethyl-2,5,8-trioxo-1,4,7-triazacyclohexadecan-9-yl)(methyl)amino)-1-cyclopropyl-2-oxoethyl)-1-(3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carbonyl)-4-fluoropyrrolidine-2-carboxamide (24.3 g, 37% yield) as a white solid.

**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 962.3

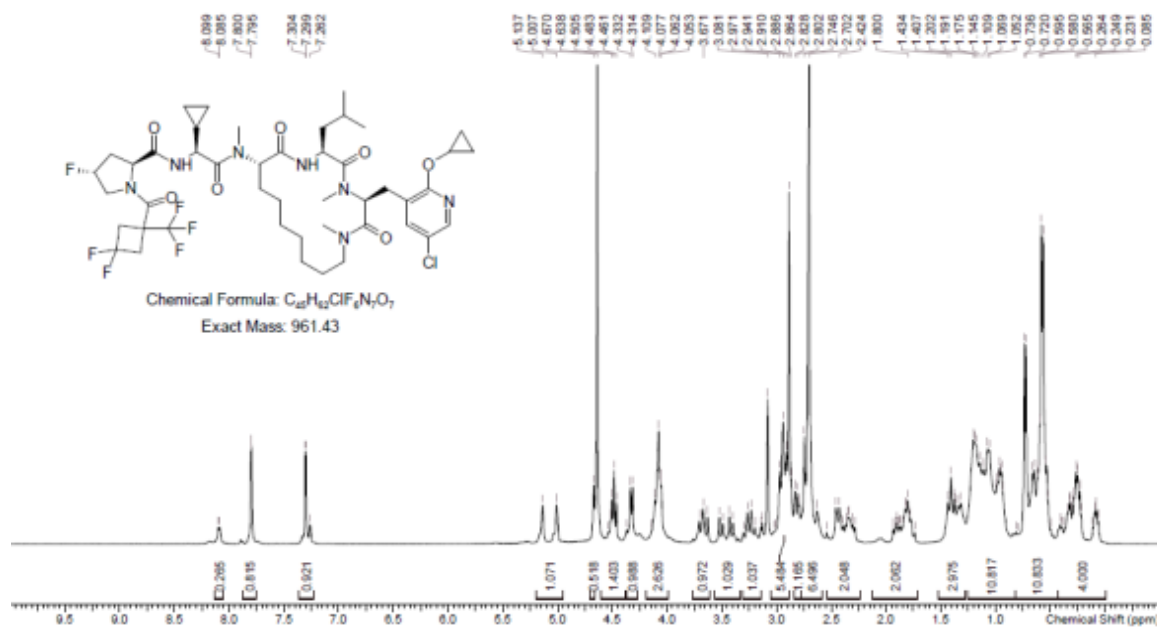
**Spectrum:**

<sup>1</sup>H NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 8.09 (d, J=2.4 Hz, 0.3H, active hydrogen that has not been completely exchanged), 7.79 (d, J=3.2 Hz, 1H), 7.30 (d, J=3.6 Hz, 1H), 5.08 (d, J=23.6 Hz, 1H), 4.67 (s, 1H), 4.37-4.60 (m, 2H), 4.35 - 4.28 (m, 1H), 4.26 - 4.01 (m, 3H), 3.78 - 3.59 (m, 1H), 3.57 - 3.35 (m, 1H), 3.32 - 3.12 (m, 1H), 3.06 - 2.85 (m, 6H), 2.85 - 2.78 (m, 1H), 2.77 - 2.53 (m, 7H), 2.50 - 2.23 (m, 2H), 2.09 - 1.70 (m, 2H), 1.49 - 1.27 (m, 3H), 1.27 - 0.86 (m, 11H), 0.48 (br s, 11H), 0.43 - 0.04 (m, 4H).

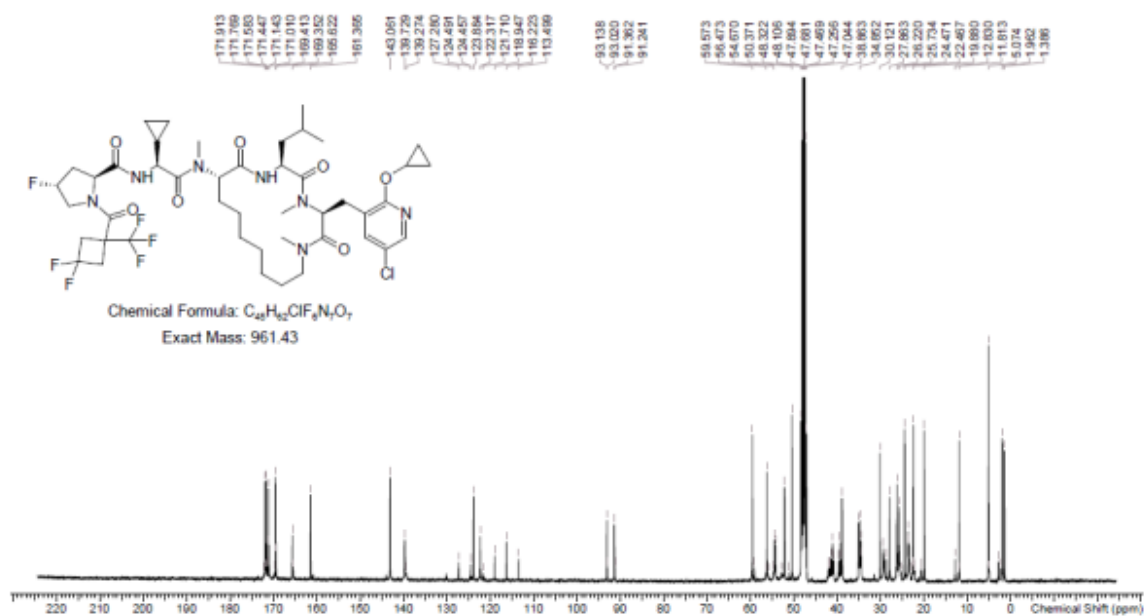
<sup>13</sup>C NMR (400 MHz, METHANOL-d<sub>4</sub>) δ : 172.088, 172.046, 171.913, 171.769, 171.583, 171.447, 171.143, 171.01, 169.671, 169.413, 169.352, 165.622, 165.413, 161.365, 143.061, 139.729, 139.274, 127.28, 127.242, 124.491, 124.457, 123.884, 122.317, 121.71, 118.947, 116.223, 113.499, 93.138, 93.02, 91.362, 91.241, 59.573, 59.079, 56.473, 56.203, 55.911, 54.67, 54.454, 54.253, 52.891, 52.174, 50.99, 50.371, 48.322, 47.894, 47.681, 47.469, 47.044, 42.031, 41.933, 41.891, 41.796, 41.743, 41.644, 41.602, 41.504, 41.314, 41.064, 40.809, 39.607, 39.345, 39.083, 38.863, 35.072, 34.981, 34.852, 34.765, 34.533, 34.446, 30.121, 29.373, 28.576, 27.863, 26.22, 25.734, 25.275, 24.471, 23.64, 23.439, 23.131, 22.577, 22.467, 22.278, 22.266, 22.133, 20.631, 20.547, 19.88, 12.83, 12.082, 11.813, 5.074, 4.918, 2.812, 2.228, 2.152, 1.962, 1.386

## Characterization of CIRc-014

# <sup>1</sup>H NMR (400 MHz, METHANOL-d4) of CIRc-014

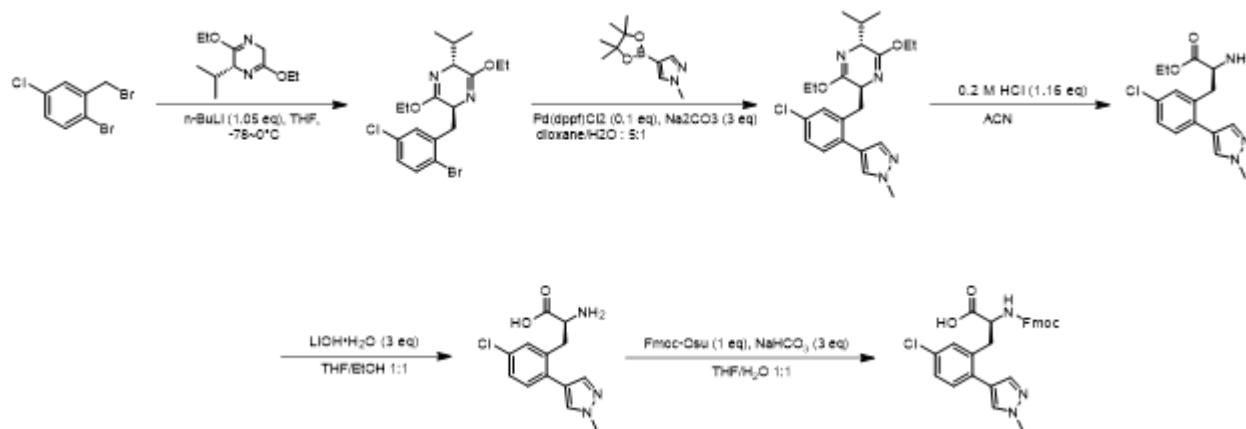


# <sup>13</sup>C NMR (400 MHz, METHANOL-d4) of CIRc-014

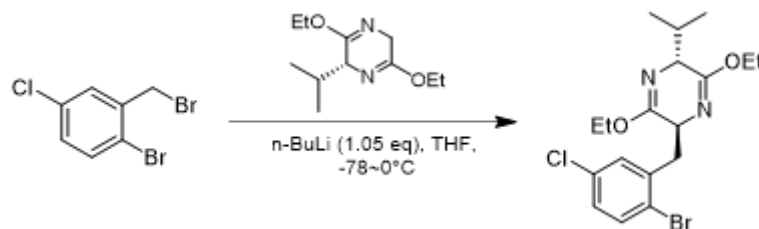


### 3. Preparation of Building Blocks Not Readily Available From Commercial Sources

#### 3.1 Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoic acid

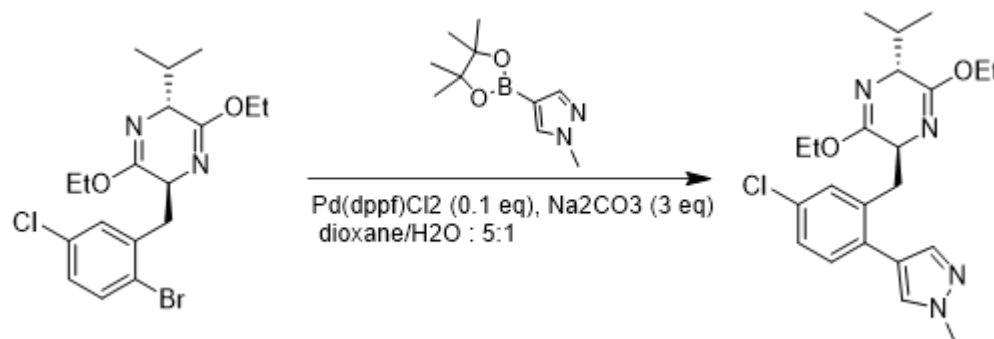


Step 1: Synthesis of (2S,5R)-2-(2-bromo-5-chlorobenzyl)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine



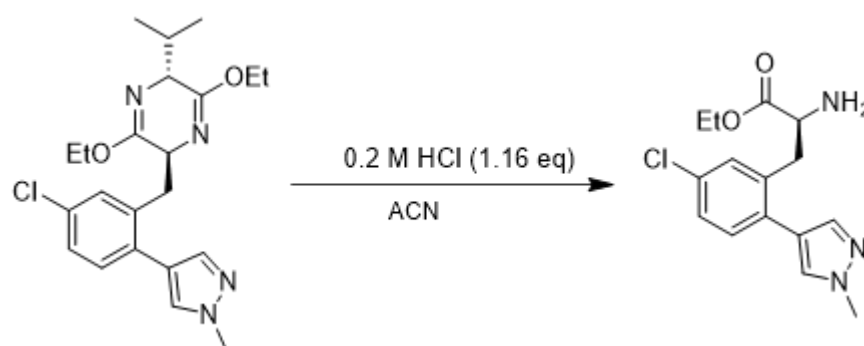
To a solution of compound (R)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (151 g, 711 mmol, 1.00 eq) in THF (1500 mL) was added n-BuLi (2.5 M, 285 mL, 1.00 eq) at -70 °C under N<sub>2</sub>, then to the above solution was added 1-bromo-2-(bromomethyl)-4-chlorobenzene (222.5 g, 782 mmol, 1.10 eq) in THF (220 mL) at -70 °C. The mixture was stirred at 25 °C for 12 hrs. TLC indicated completion of the reaction. The reaction was poured into sat.NH<sub>4</sub>Cl (aq.) 6000 mL, partitioned between water (6000) mL and THF (6000) mL. The organic phase was separated and dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether/Ethyl acetate = 100/0 to 1/1). The product (620 g, 1.48 mol, 104.10% yield, 99.3% purity) was obtained as a yellow gum.

Step 2: Synthesis of (2S,5R)-2-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)benzyl)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine



To a solution of (2S,5R)-2-(2-bromo-5-chlorobenzyl)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine (60.0 g, 144 mmol, 1.00 eq) and 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole (45.0 g, 216 mmol, 1.50 eq) in dioxane (600 mL) and H<sub>2</sub>O (120 mL) was added Na<sub>2</sub>CO<sub>3</sub> (45.9 g, 433 mmol, 3.00 eq) and Pd(dppf)Cl<sub>2</sub> (10.6 g, 14.4 mmol, 0.100 eq) was stirred at 80 °C for 2 hrs under N<sub>2</sub>. TLC (Petroleum ether: Ethyl acetate = 3:1) indicated starting material was consumed completely. The reaction mixture was diluted with H<sub>2</sub>O (600 mL) and extracted with EtOAc (500 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether/Ethyl acetate = 100/1 to 1/1). The desired compound (50.0 g, crude) was obtained as a yellow gum.

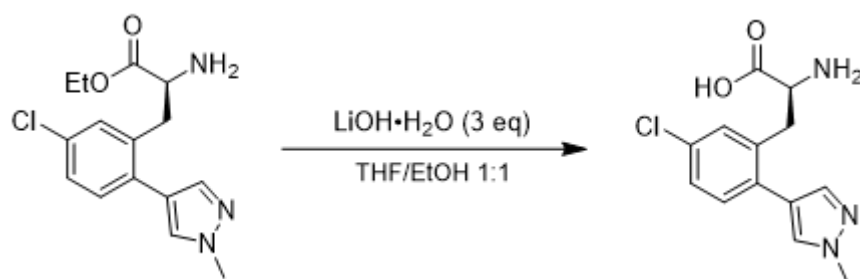
Step 3: Synthesis of ethyl (S)-2-amino-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoate



To a solution of (2S,5R)-2-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)benzyl)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine (50.0 g, 119 mmol, 1.00 eq) in ACN (750 mL) was added HCl (0.2 M, 695 mL, 1.16 eq) at 0 °C, then the mixture was stirred at 25 °C

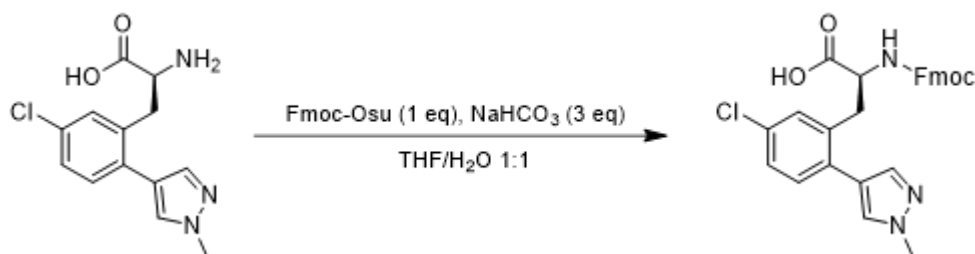
for 48 hrs. TLC (Petroleum ether: Ethyl acetate = 0:1) indicated starting material was consumed completely. The reaction mixture was concentrated under reduced pressure to remove CAN, and extracted with DCM (200 mL \* 2), then the water phase was basified with sat.aq. NaHCO<sub>3</sub> to pH = 7, and extracted with DCM (200 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The desired product (41.0 g, 114 mmol, 95.5% yield, 86% purity) was obtained as a brown gum

Step 4: Synthesis of (S)-2-amino-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoic acid



To a solution of ethyl (S)-2-amino-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoate (41.0 g, 114 mmol, 86% purity, 1.00 eq) in THF (200 mL) and EtOH (200 mL) was added LiOH·H<sub>2</sub>O (14.4 g, 344 mmol, 3.00 eq) at 0 °C, then was stirred at 25 °C for 12 hrs. LCMS showed starting material was consumed completely and one main peak with desired m/z was detected. The reaction mixture was concentrated under reduced pressure at 30 °C to remove half of solvent. It was added into a saturated solution of citric acid (500 mL) at 5-10 °C, to keep all the progress worked under acid condition. Some solid precipitated, then the suspension was filtered, the filter cake was washed with MTBE (500 mL). The filter cake was collected and dried in vacuum to obtain the desired product (30.0 g, crude) as white solid, which was used to the next step without further purification.

Step 5: Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoic acid



To a solution of (S)-2-amino-3-(5-chloro-2-(1-methyl-1H-pyrazol-4-yl)phenyl)propanoic acid (30.0 g, 107 mmol, 1.00 eq) in THF (900 mL) and H<sub>2</sub>O (900 mL) was added FMOC-OSU (36.2 g, 107 mmol, 1.00 eq) and NaHCO<sub>3</sub> (27.0 g, 322

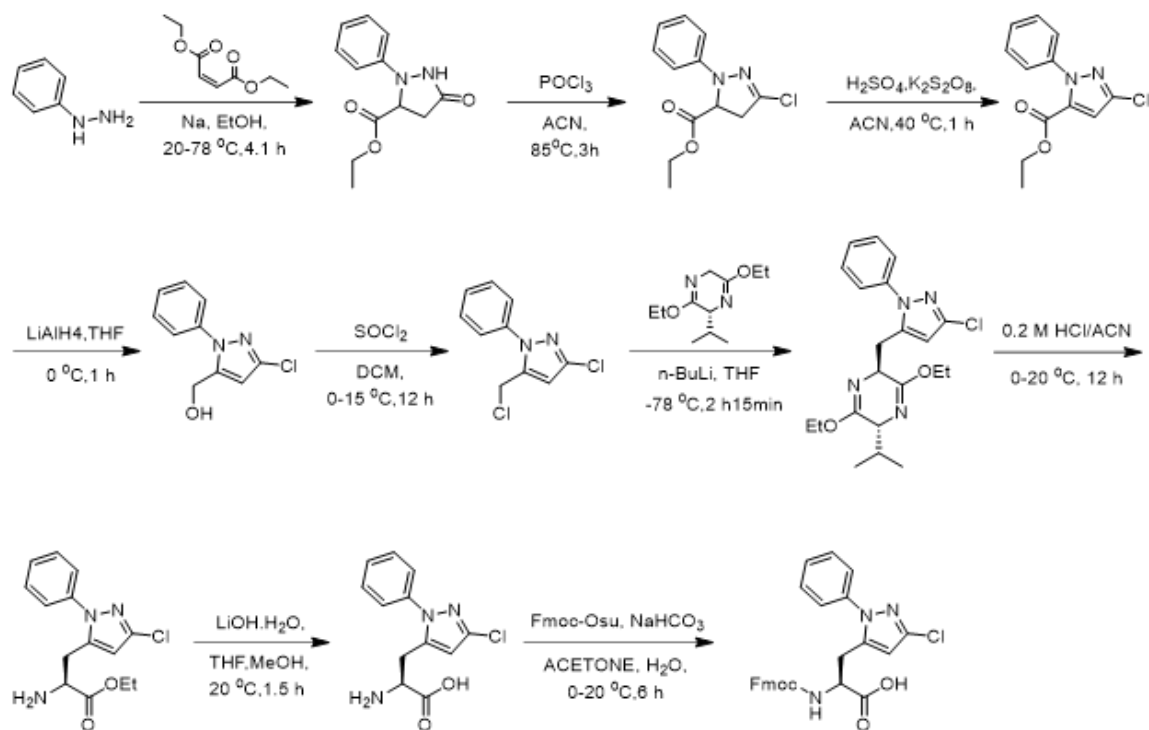
mmol, 12.5 mL, 3.00 eq) at 0 °C, then was stirred at 25 °C for 12 hrs. LCMS showed starting material was consumed completely and desired mass was detected. The reaction mixture was extracted with EA 2000 mL (1000 mL \* 2). The combined organic layers were dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by prep-HPLC (neutral condition; column: Welch Xtimate C18 250\*70mm#10um; mobile phase: [water( NH<sub>4</sub>HCO<sub>3</sub>)-ACN];B%: 16%-46%,20min). The final product (23.0 g, 44.7 mmol, 41.7% yield, 97.6% purity) was obtained as a white solid.

**<sup>1</sup>H NMR:** (400 MHz, CD<sub>3</sub>CN)

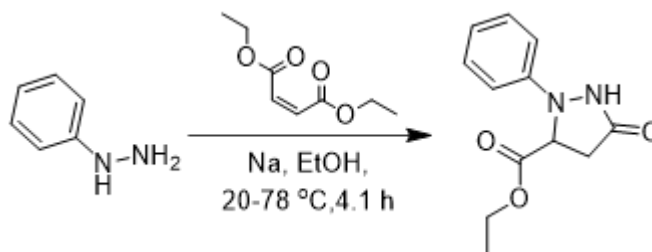
δ 7.60 - 7.84 (m, 2 H), 7.42 - 7.58 (m, 4 H), 7.32 - 7.35 (m, 7 H), 5.95 (s, 1 H), 4.17 - 4.29 (m, 4 H), 3.88 (s, 3 H), 3.37 - 3.38 (m, 1 H), 3.97 - 3.36 (m, 1 H).

**LCMS (ESI+):** m/z 502.15 (M+H); RT = 0.525 min)

### 3.2 Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoic acid

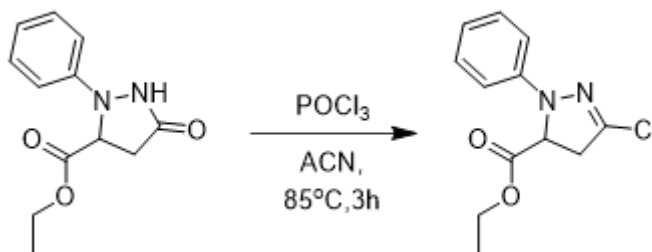


Step 1: Synthesis of ethyl 5-oxo-2-phenylpyrazolidine-3-carboxylate



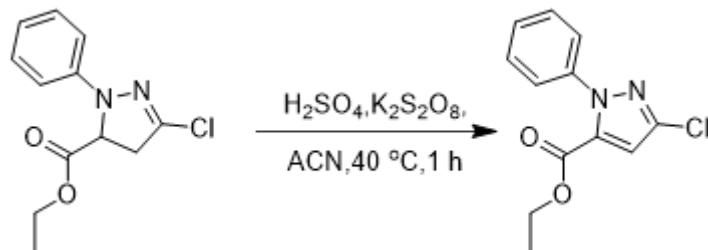
To EtOH (600 mL) was added sodium (7.02 g, 305.16 mmol, 7.23 mL, 1.1 eq) at 20 °C. After all sodium was reacted, the mixture was heated to 78 °C and phenylhydrazine (30 g, 277.42 mmol, 27.27 mL, 1 eq) was added, and stirred for 0.1 h, then diethyl (Z)-but-2-enedioate (52.54 g, 305.16 mmol, 49.10 mL, 1.1 eq) was added dropwise. The mixture was stirred at 78 °C for 4 h. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was cooled to 65 °C, and treated with AcOH until pH=6, then the reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted with H<sub>2</sub>O (500 mL) and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and the filtrate was concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether: Ethyl acetate = 20/1 to 1/2) to give the desired product (53.34 g, 227.70 mmol, 82.08% yield) as a yellow solid.

#### Step 2: Synthesis of ethyl 3-chloro-1-phenyl-4,5-dihydro-1H-pyrazole-5-carboxylate



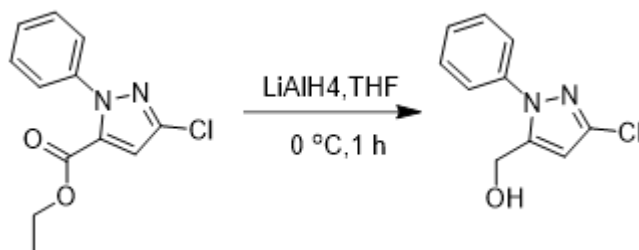
To a solution of ethyl 5-oxo-2-phenylpyrazolidine-3-carboxylate (48 g, 204.91 mmol, 1 eq) in ACN (1000 mL) was added POCl<sub>3</sub> (37.70 g, 245.89 mmol, 22.85 mL, 1.2 eq). The mixture was stirred at 85 °C for 3 hr. LC-MS showed consumption of the starting material and one main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was neutralized by sat. NaHCO<sub>3</sub> to pH = 8, then extracted with DCM. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether: Ethyl acetate = 100/1 to 80/1) to give the desired compound (39 g, 154.34 mmol, 75.32% yield) as a light yellow solid.

#### Step 3: Synthesis of ethyl 3-chloro-1-phenyl-1H-pyrazole-5-carboxylate



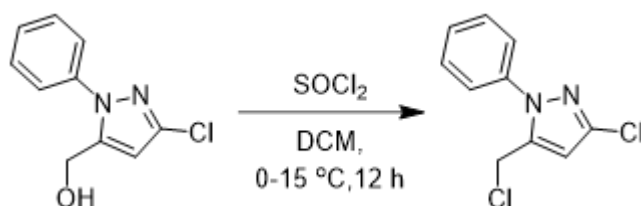
To a solution of ethyl 3-chloro-1-phenyl-4,5-dihydro-1H-pyrazole-5-carboxylate (22 g, 87.06 mmol, 1 eq) in ACN (500 mL) was added  $\text{H}_2\text{SO}_4$  (12.81 g, 130.59 mmol, 6.96 mL, 1.5 eq) and dipotassium;sulfonatoxy sulfate (28.24 g, 104.47 mmol, 20.92 mL, 1.2 eq). The mixture was stirred at  $40^\circ\text{C}$  for 1 hr. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was diluted with  $\text{H}_2\text{O}$ , and extracted with EtOAc. The combined organic layers were washed with brine, dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography ( $\text{SiO}_2$ , Petroleum ether: Ethyl acetate = 100/1 to 80/1) to give the desired product (5.6 g, 22.34 mmol, 25.66% yield) as a white solid.

#### Step 4: Synthesis of (3-chloro-1-phenyl-1H-pyrazol-5-yl)methanol



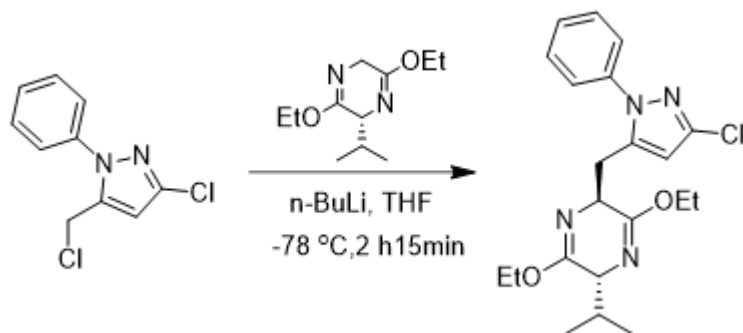
To a solution of ethyl 3-chloro-1-phenyl-1H-pyrazole-5-carboxylate (5.9 g, 23.54 mmol, 1 eq) in THF (60 mL) was added  $\text{LiAlH}_4$  (1.79 g, 47.07 mmol, 2 eq) at  $0^\circ\text{C}$ , then the reaction was stirred at  $0^\circ\text{C}$  for 1 hr. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was diluted with THF 20 mL, then was quenched by addition  $\text{H}_2\text{O}$  1.8 mL, 15%  $\text{NaOH}$  1.8 mL and  $\text{H}_2\text{O}$  5.4 mL. The mixture was added with  $\text{Na}_2\text{SO}_4$  and was stirred for 15 min, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography ( $\text{SiO}_2$ , Petroleum ether: Ethyl acetate = 20/1 to 3/1) to give the desired product (4.2 g, 20.13 mmol, 85.53% yield) as a light yellow solid.

#### Step 5: Synthesis of 3-chloro-5-(chloromethyl)-1-phenyl-1H-pyrazole



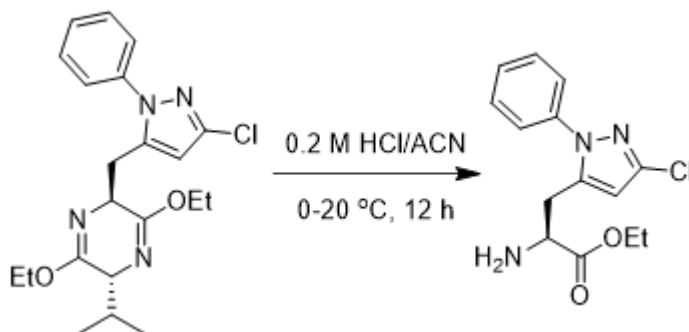
To a solution of (3-chloro-1-phenyl-1H-pyrazol-5-yl)methanol (4 g, 19.17 mmol, 1 eq) in DCM (40 mL) was added  $\text{SOCl}_2$  (22.81 g, 191.71 mmol, 13.91 mL, 10 eq) at 0 °C. The mixture was stirred at 15 °C for 12 h. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was concentrated to give a residue. The residue was purified by column chromatography ( $\text{SiO}_2$ , Petroleum ether: Ethyl acetate = 100/1 to 30/1) to give desired product (3.9 g, 17.17 mmol, 89.58% yield) as a light yellow solid.

Step 6: Synthesis of (2S,5R)-2-((3-chloro-1-phenyl-1H-pyrazol-5-yl)methyl)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine



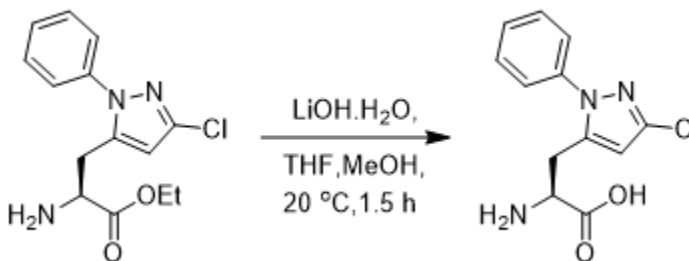
To a mixture of (2R)-3,6-diethoxy-2-isopropyl-2,5-dihydropyrazine (2.98 g, 14.05 mmol, 1.1 eq) in THF (30 mL) was added  $n\text{-BuLi}$  (2.5 M, 7.66 mL, 1.5 eq) at -78 °C under  $\text{N}_2$  atmosphere, then the mixture was stirred at -78 °C for 15 min, 3-chloro-5-(chloromethyl)-1-phenyl-1H-pyrazole (2.9 g, 12.77 mmol, 1 eq) in THF (30 mL) was added to the above mixture, then the mixture was stirred at -78 °C for another 2 hr under  $\text{N}_2$  atmosphere. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was quenched by added to sat.  $\text{NH}_4\text{Cl}$  at 0 °C, extracted with EtOAc 450 mL. The combined organic layers was dried over  $\text{Na}_2\text{SO}_4$ , filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography ( $\text{SiO}_2$ , Petroleum ether: Ethyl acetate = 50/1 to 5/1) to give the desired product (2.7 g, 6.70 mmol, 52.47% yield) as a light yellow oil.

Step 7: Synthesis of ethyl (S)-2-amino-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoate



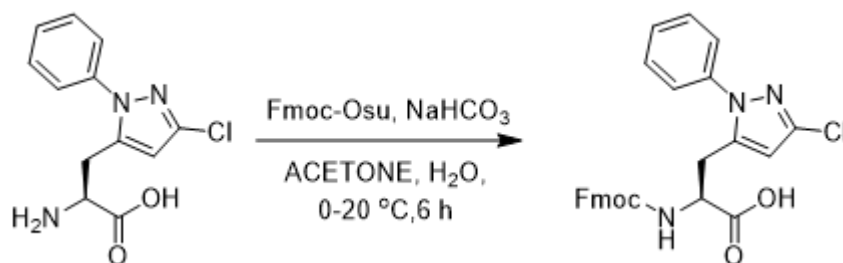
HCl (60 mL) (0.2M) was added a solution of (2S,5R)-2-((3-chloro-1-phenyl-1H-pyrazol-5-yl)methyl)-3,6-diethoxy-5-isopropyl-2,5-dihydropyrazine (2 g, 4.96 mmol, 1 eq) in ACN (60 mL) at 0 °C. The mixture was stirred at 20 °C for 12 hr. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was neutralized by sat. NaHCO<sub>3</sub> to pH = 8 at 0 °C, then the reaction was stirred for 5 min at 20 °C. The layers were separated, and the aqueous phase was extracted with DCM. The combined organic layers were washed with brine dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give the desired product (1.75 g, crude) as a yellow oil.

Step 8: Synthesis of (S)-2-amino-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoic acid



To a solution of ethyl (S)-2-amino-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoate (1.75 g, 5.96 mmol, 1 eq) in THF (10 mL) and MeOH (10 mL) was added LiOH.H<sub>2</sub>O (749.99 mg, 17.87 mmol, 3 eq). The mixture was stirred at 20 °C for 1.5 hr. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure at 25-30 °C to remove half of solvent. It was added into a saturated solution of citric acid at 0 °C, to keep all the progress worked under acid condition, no solid precipitated, then the suspension was concentrated under reduced pressure at 45 °C to give a residue, the residue was diluted with MeOH (100 mL). The resulting suspension in MeOH was filtered and the filtrate was collected and dried in vacuum to give the desired product (2 g, crude) as a light yellow solid.

Step 9: Synthesis of (S)-2-(((9H-fluoren-9-yl)methoxy)carbonyl)amino-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoic acid

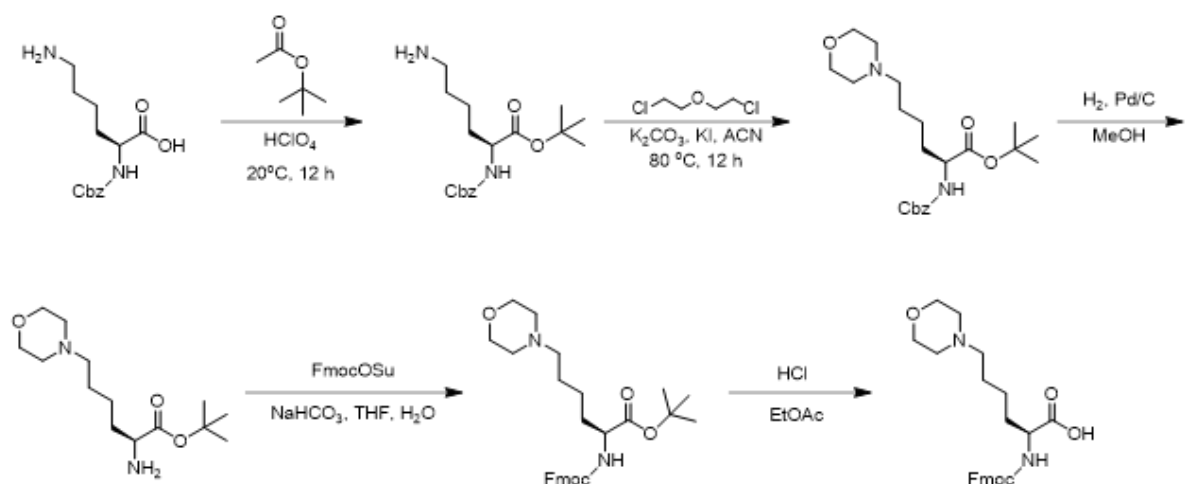


To a solution of (S)-2-amino-3-(3-chloro-1-phenyl-1H-pyrazol-5-yl)propanoic acid (1.8 g, 6.77 mmol, 1 eq) in ACETONE (18 mL) and H<sub>2</sub>O (18 mL) was added NaHCO<sub>3</sub> (1.71 g, 20.32 mmol, 790.44  $\mu$ L, 3 eq) and (2,5-dioxopyrrolidin-1-yl) 9H-fluoren-9-ylmethyl carbonate (2.06 g, 6.10 mmol, 0.9 eq) at 0 °C. The mixture was stirred at 20 °C for 6 hr. LC-MS showed starting material was consumed and one main peak with desired mass was detected. The reaction mixture was concentrated under reduced pressure at 30 °C to remove Acetone. Then the mixture was diluted with H<sub>2</sub>O and EtOAc, then sat.citric acid was added to above solution, adjust pH~2, extracted with EtOAc 150 mL. The combined organic layers were washed with brine, dried over Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated under reduced pressure to give a residue. The residue was purified by column chromatography (SiO<sub>2</sub>, Petroleum ether : Ethyl acetate = 10/1 to 0/1) to give the desired final product (1 g, 2.01 mmol, 29.70% yield, 98.16% purity) as a white solid.

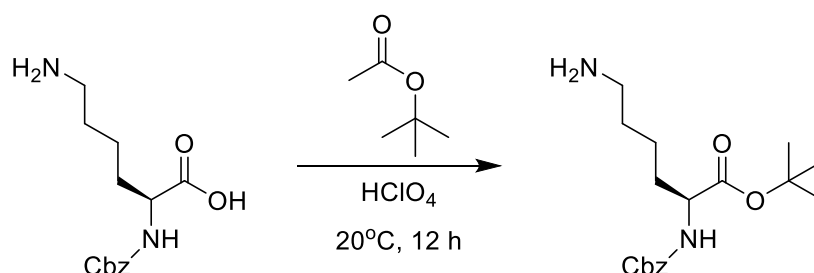
**LCMS** (ESI<sup>+</sup>): m/z 488.2 (M+H); RT = 2.61 min)

**Spectrum:** <sup>1</sup>H NMR (400 MHz, METHANOL-*d*<sub>4</sub>)  $\delta$  7.80 (d, J = 7.6 Hz, 2H), 7.62 (dd, J = 3.4, 7.2 Hz, 2H), 7.49-7.46 (m, 3H), 7.44-7.40 (m, 2H), 7.40-7.35 (d, 2H), 7.32-7.28 (t, 2H), 6.32 (s, 1H), 4.40-4.27 (m, 2H), 4.26 (d, J = 6.0 Hz, 1H), 4.22-4.16 (m, 1H), 3.27 (dd, J = 4.6, 15.6 Hz, 1H), 3.05 (dd, J = 9.2, 15.6 Hz, 1H)

### 3.3. Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-morpholinohexanoic acid

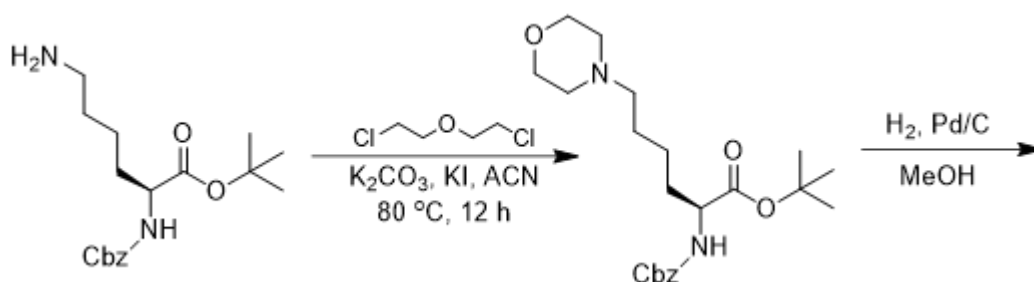


Step 1: Synthesis of tert-butyl ((benzyloxy)carbonyl)-L-lysinate



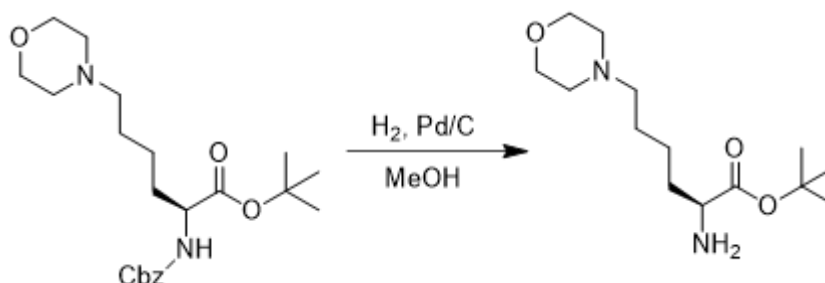
To a solution of (2S)-6-amino-2-(benzyloxycarbonylamino)hexanoic acid (40 g, 142.69 mmol) in tert-butyl acetate (1200 mL) was added HClO<sub>4</sub> (26.72 g, 186.19 mmol, 16.10 mL, 70% purity). The mixture was stirred at 20 °C for 16 hour. LC-MS showed ~30% of (2S)-6-amino-2-(benzyloxycarbonylamino) hexanoic acid remained. Several new peaks were shown on LC-MS and ~50% of desired compound was detected. The reaction mixture was quenched by addition Na<sub>2</sub>SO<sub>3</sub> solvent at 0°C, and then diluted with H<sub>2</sub>O and extracted with EtOAc. The combined organic layers were concentrated under reduced pressure to give desired product tert-butyl (2S)-6-amino-2-(benzyloxycarbonylamino)hexanoate (26 g, crude) was obtained as a yellow oil.

Step 2: Synthesis of tert-butyl (S)-2-(((benzyloxy)carbonyl)amino)-6-morpholinohexanoate



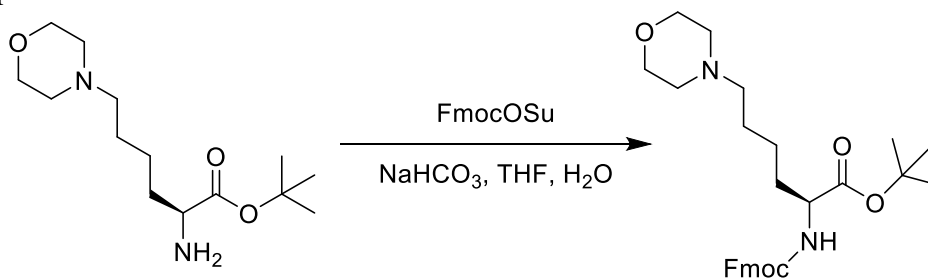
A mixture of tert-butyl (2S)-6-amino-2-(benzyloxycarbonylamino)hexanoate (48 g, 142.68 mmol), 1-chloro-2-(2-chloroethoxy)ethane (20.40 g, 142.68 mmol, 16.72 mL), K<sub>2</sub>CO<sub>3</sub> (59.16 g, 428.03 mmol, 3 eq), KI (4.74 g, 28.54 mmol, 0.2 eq) in ACN (480 mL) was degassed and purged with N<sub>2</sub> for 3 times, and then the mixture was stirred at 80 °C for 12 hour under N<sub>2</sub> atmosphere. LC-MS showed 60% of tert-butyl (2S)-6-amino-2-(benzyloxycarbonylamino)hexanoate remained. Several new peaks were shown on LC-MS and 40% of desired compound was detected. The reaction mixture was diluted with DCM 500 mL, then filtered and wash the filtrate three times with water 1500 ml (500mL \* 3), and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography to give desired product tert-butyl (2S)-2-(benzyloxycarbonylamino)-6-morpholino-hexanoate (43 g, 74.14% yield) as a yellow oil.

### Step 3: Synthesis of tert-butyl (S)-2-amino-6-morpholinohexanoate



To a solution of tert-butyl (2S)-2-(benzyloxycarbonylamino)-6-morpholinohexanoate (40 g, 98.40 mmol) in MeOH (2 L) was added Pd/C (7 g, 10% purity) under Ar. The suspension was degassed under vacuum and purged H<sub>2</sub> 3 times. The mixture was stirred under H<sub>2</sub> (15 psi) at 50 °C for 20 mins. TLC showed the material was remained and desired product was detected. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give desired product tert-butyl (2S)-2-amino-6-morpholinohexanoate (24 g, crude) as a yellow oil.

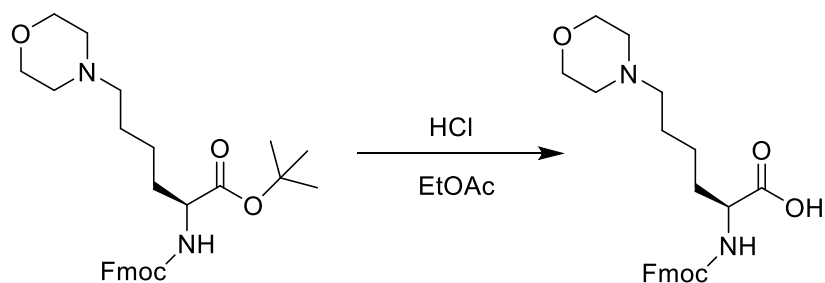
### Step 4: Synthesis of tert-butyl (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-morpholinohexanoate



To a solution of tert-butyl (2S)-2-amino-6-morpholinohexanoate (24 g, 88.11 mmol) in ACETONE (250 mL) and H<sub>2</sub>O (250 mL) was added NaHCO<sub>3</sub> (22.21 g, 264.33 mmol, 10.28 mL) and FmocOSu (29.72 g, 88.11 mmol) at 0 °C. The mixture was stirred

at 20 °C for 10 hours. LC-MS showed 23% of tert-butyl (2S)-2-amino-6-morpholino-hexanoate remained. Several new peaks were shown on LC-MS and 77% of desired compound was detected. The product is obtained by direct extraction and filtration of the reaction liquid. The solid was dissolved with 500 ml ethyl acetate and dried with anhydrous sodium sulfate, then filtered and concentrated under reduced pressure to give a residue. The residue was purified by flash silica gel chromatography to give desired product tert-butyl (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-6-morpholino-hexanoate (25 g, 57.36% yield) as a white solid.

Step 5: Synthesis of (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-morpholinohexanoic acid



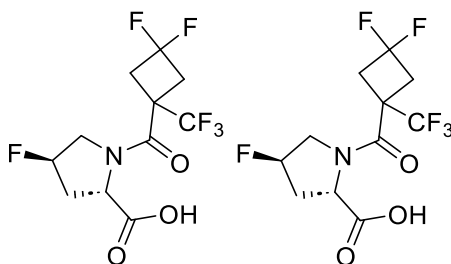
To a solution of tert-butyl (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-6-morpholino-hexanoate (25 g, 50.54 mmol) in HCl/EtOAc (250 mL). The mixture was stirred at 20 °C for 3 hour. LC-MS (ET55559-8-P1B1) showed tert-butyl (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-6-morpholino-hexanoate was consumed completely and one main peak with desired m/z or desired mass was detected. The reaction mixture was concentrated under reduced pressure to remove solvent. The residue was diluted and stirred with petroleum ether 200 ml and concentrate and then concentrated under reduced pressure to give a desired product (2S)-2-(9H-fluoren-9-ylmethoxycarbonylamino)-6-morpholino-hexanoic acid (22 g, HCl salt, 98% purity) as a white solid.

LCMS (ESI+): m/z 439.2 (M+H); RT = 0.64 min)

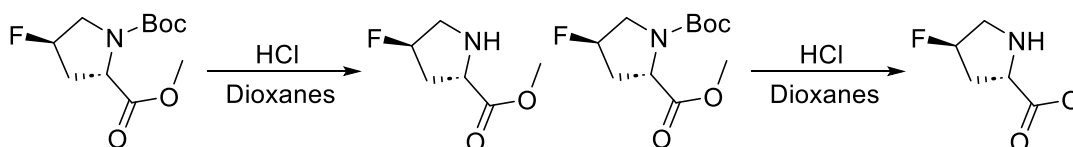
#### Spectrum:

$\delta$  ppm 1.25-1.44 (m, 2 H) 1.55-1.81 (m, 4 H) 2.90-3.09 (m, 4 H) 3.30-3.43 (m, 2 H) 3.71-3.98 (m, 5 H) 4.15-4.37 (m, 3 H) 7.27-7.37 (m, 2 H) 7.37-7.47 (m, 2 H) 7.73 (dd,  $J$  = 7.32, 4.0 Hz, 2 H) 7.89 (d,  $J$  = 7.4 Hz, 2 H) 10.66-11.40 (m, 1 H) 12.27-13.05 (m, 1 H).

### 3.4. Synthesis of (2S,4R)-1-(3,3-difluoro-1-(trifluoromethyl)cyclobutane-1-carbonyl)-4-fluoropyrrolidine-2-carboxylic acid

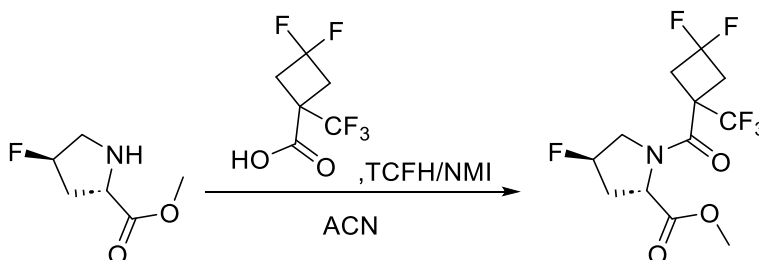


### Step1: Synthesis of methyl (2S,4R)-4-fluoropyrrolidine-2-carboxylate



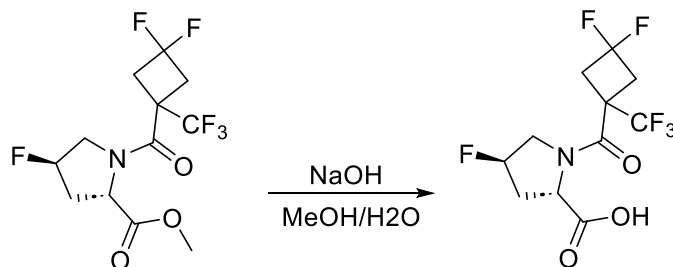
A solution of 1-tert-butyl 2-methyl (2S,4R)-4-fluoropyrrolidine-1,2-dicarboxylate (150 g, 101.106 mmol, 1 equiv) and HCl(gas) in 1,4-dioxane (758.29 mL, 505.530 mmol, 5 equiv) was stirred for 0.5h at r.t and then concentrated under vacuum to afford the desired product (110 g, P=80%, Yield=98.58%) as a white solid. The crude product was used in the next step directly without further purification.

### Step 2: Synthesis of methyl (2S,4R)-1-[3,3-difluoro-1-(trifluoromethyl)cyclobutanecarbonyl]-4-fluoropyrrolidine-2-carboxylate



To a stirred solution of methyl (2S,4R)-4-fluoropyrrolidine-2-carboxylate (110 g, 103.297 mmol, 1 equiv, 80%), 3,3-difluoro-1-(trifluoromethyl)cyclobutanecarboxylic acid (122.06 g, 103.297 mmol, 1 equiv) and TCFH (251.69 g, 154.945 mmol, 1.5 equiv) in ACN (1100 mL) was added NMI (245.51 g, 516.485 mmol, 5 equiv) dropwise in 45 minutes at 0°C. The mixture was slowly warmed up to room temperature and stirred overnight at r.t. The mixture was concentrated under vacuum at 28°C and diluted with EtOAc and washed with HCl. The aqueous layer was extracted again with EtOAc. The combined organic layers were washed with saturated NaHCO<sub>3</sub>. The combined organic layers were washed with brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration, the filtrate was concentrated under vacuum to afford the desired product (180 g, 81.29%) as a light yellow solid. The crude product was used in the next step directly without further purification.

Step 3: Synthesis of (2S,4R)-1-[3,3-difluoro-1-(trifluoromethyl)cyclobutanecarbonyl]-4-fluoropyrrolidine-2-carboxylic acid



To a stirred solution of methyl (2S,4R)-1-[3,3-difluoro-1-(trifluoromethyl)cyclobutanecarbonyl]-4-fluoropyrrolidine-2-carboxylate (180 g, 82.150 mmol, 1 equiv, 90%) in MeOH (1400 mL) was added dropwise NaOH (58.33 g, 246.450 mmol, 3 equiv) in H<sub>2</sub>O (400 mL) in 30 minutes at 0-20°C. The mixture was stirred for 2h at r.t. MeOH was evaporated out under vacuum. The residue was diluted with water and acidified with HCl(3N) at 0-20°C. Then the precipitated solids were collected by filtration and washed with water. The filtrate was added HCl(3N). The precipitated solids were collected by filtration and washed with water. The combined solids were dried in an oven for 48h at 40°C. This resulted in the desired final product (150.6472 g, 97.08%) as a white solid.

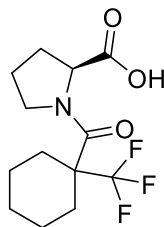
**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 319.95.

**Spectrum:**

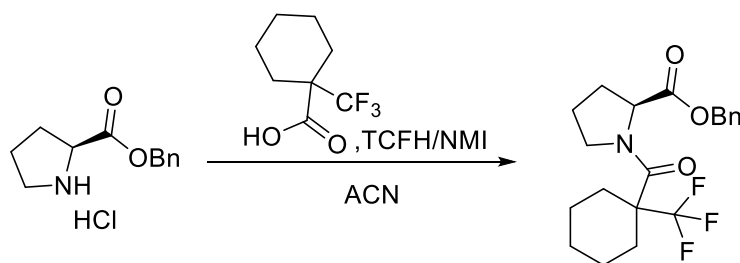
<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 2.11 (dddd, *J* = 40.2, 14.7, 8.8, 4.0 Hz, 1H), 2.53 – 2.65 (m, 1H), 3.10 (q, *J* = 14.1 Hz, 1H), 3.31 (tt, *J* = 10.5, 2.7 Hz, 3H), 3.58 – 3.88 (m, 2H), 4.49 (t, *J* = 8.7 Hz, 1H), 5.39 (dt, *J* = 52.5, 3.2 Hz, 1H), 12.88 (s, 1H).

SFC: enantiomeric excess = 100.0%

### 3.5. Synthesis of (2S)-1-[1-(trifluoromethyl)cyclohexanecarbonyl]pyrrolidine-2-carboxylic acid

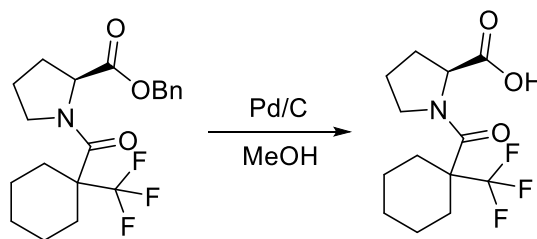


Step 1: Synthesis of benzyl (2S)-1-[1-(trifluoromethyl)cyclohexanecarbonyl]pyrrolidine-2-carboxylate



To a stirred solution/mixture of benzyl (2S)-pyrrolidine-2-carboxylate hydrochloride (3 g, 12.411mmol, 1.1equiv) and 1-(trifluoromethyl)cyclohexane-1-carboxylic acid (2014.13 mg, 10.267mmol, 0.91equiv) in ACN (40 mL, 38.048mmol) were added TCFH (6331.44 mg, 22.565mmol, 2equiv) and NMI (4631.92 mg, 56.414mmol, 5equiv) in portions at 0° under nitrogen atmosphere. The resulting mixture was stirred for additional 2h at room temperature. The resulting mixture was concentrated under vacuum. The residue was purified by reverse flash chromatography with the following conditions: column, C18 silica gel; mobile phase, ACN in water, 10% to 80% gradient in 25 min; detector, UV 254 nm. This resulted in the desired product as a white solid.

Step 2: Synthesis of (2S)-1-[1-(trifluoromethyl)cyclohexanecarbonyl]pyrrolidine-2-carboxylic acid



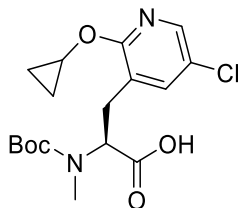
To a solution of benzyl (2S)-1-[1-(trifluoromethyl)cyclohexanecarbonyl]pyrrolidine-2-carboxylate (2 g, 5.216mmol, 1equiv) in 20mL MeOH was added Pd/C (749.42 mg, 7.042mmol, 0.36equiv) under nitrogen atmosphere in a 50mL 3-necked round-bottom flask. The mixture was hydrogenated at room temperature for 2h under hydrogen atmosphere using a hydrogen balloon, filtered through a Celite pad and concentrated under reduced pressure. This resulted in (5.615 g, 95.82%) as a white solid.

**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 294.00.

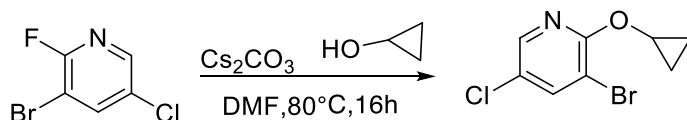
**Spectrum:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>): δ 12.43 (s, 1H), 4.35 (dd, J = 8.3, 6.3 Hz, 1H), 3.78 (ddd, J = 9.8, 7.1, 5.1 Hz, 1H), 3.61 (dt, J = 9.8, 7.1 Hz, 1H), 2.49 – 2.38 (m, 1H), 2.14 (ddt, J = 12.5, 8.3, 6.5 Hz, 1H), 2.08 – 1.82 (m, 2H), 1.80 – 1.10 (m, 10H).

### 3.6. (S)-2-((tert-butoxycarbonyl)amino)-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoate methyl ester



#### Step 1: 3-bromo-5-chloro-2-cyclopropoxypyridine



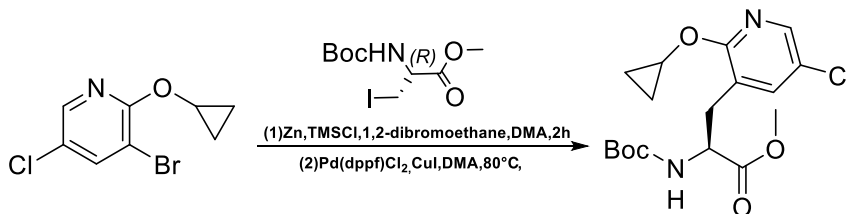
Into a 1000mL round-bottom flask were added 3-bromo-5-chloro-2-fluoropyridine (50 g, 237.609mmol, 1equiv) in DMF (400mL), Cs<sub>2</sub>CO<sub>3</sub> (232.97 g, 712.827mmol, 3equiv) was added at room temperature under nitrogen atmosphere. The mixture was stirred at room temperature for 50 min. cyclopropanol (9.94 g, 171.078mmol, 1.2equiv) was added dropwise over 2min at room temperature. The resulting mixture was stirred at 80°C for 16h. The reaction was quenched with ice-water (700mL) and extracted with EA (3 x 100mL). The organic layer combined and washed with brine (300 mL), dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE/EA (0~20%). This resulted in 3-bromo-5-chloro-2-cyclopropoxypyridine (40 g, 67.74%) as a colorless oil.

**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 249.95.

#### **Spectrum:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.76 (ddd, J = 5.7, 4.5, 3.1 Hz, 2H), 0.96 – 0.78 (m, 2H), 4.32 (tt, J = 6.4, 3.1 Hz, 1H), 8.17 (d, J = 2.5 Hz, 1H), 8.26 (d, J = 2.4 Hz, 1H).

#### Step 2: Synthesis of (S)-2-((tert-butoxycarbonyl)amino)-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoate methyl ester

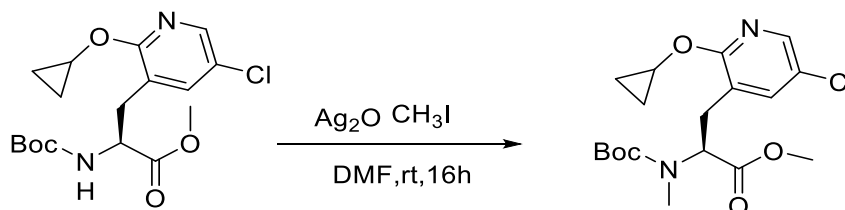


To a mixture of Zn (26.84 g, 410.463mmol, 1.7equiv) in DMA (135mL) was added 1,2-dibromoethane (6.80 g, 36.217mmol, 0.15equiv) in one portion under N<sub>2</sub>. Then chlorotrimethylsilane (2.62 g, 24.145mmol, 0.1equiv) was added slowly and the mixture was stirred for 30 min at 25°C. A solution of methyl 2-((tert-butoxycarbonyl)amino)-3-iodopropanoate (95.36 g, 289.739mmol, 1.2equiv) in DMA (135mL) was added dropwise slowly (60 min) to maintain temperature below 50°C, the

resulting mixture was stirred at rt for 2h and then added 1000mL 3-necked round-bottom flask a cannula to a solution of 3-bromo-5-chloro-2-cyclopropoxypyridine (60 g, 241.449mmol, 1equiv), Pd(dppf)Cl<sub>2</sub>.CH<sub>2</sub>Cl<sub>2</sub> (19.67 g, 24.14mmol, 0.1equiv) and CuI (9.20 g, 48.290mmol, 0.2equiv) in DMA(135ml) under N<sub>2</sub>, the color of the mixture turned brown, then the mixture was heated and stirred at 80°C for 2h under N<sub>2</sub>. The mixture was quenched with ice-water (700ml) and extracted with EA (3x500 ml). The organic layer was combined and washed with brine (300 ml), dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered and concentrated in vacuum to give the crude product. The crude product was run through a silica-plug (PE/EA 0 to 50%) to afford methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoate (30 g, 33.51%) as a white solid.

**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 371.10

Step 3: Synthesis of (S)-2-((tert-butoxycarbonyl)(methyl)amino)-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoate methyl ester



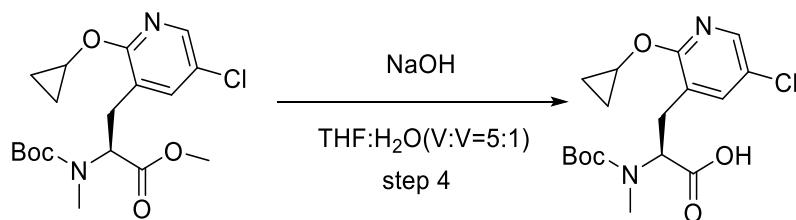
Into a 1000mL round-bottom flask were added methyl (2S)-2-[(tert-butoxycarbonyl)amino]-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoate (25 g, 67.416mmol, 1equiv) in DMF(400mL), Ag<sub>2</sub>O (78.11 g, 337.080mmol, 5equiv) was added at 0°C under nitrogen atmosphere. The mixture was stirred at 0°C for 30min, CH<sub>3</sub>I (95.69 g, 674.160mmol, 10equiv) was added dropwise over 2min at 0°C. The resulting mixture was stirred at room temperature for 16h. The reaction was quenched with ice-Water (500 mL) and extracted with EtOAc (3 x500mL). The organic layer combined and washed with brine (300 mL) and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. After filtration, the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography, eluted with PE / EA (7:1) to afford methyl (2S)-2-[(tert-butoxycarbonyl)(methyl)amino]-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoate (22 g, 84.79%) as a colorless oil.

**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 385.10.

**Spectrum:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.83 – 0.62(m, 2H), 1.19 (t, J = 7.1 Hz, 2H), 1.28 (s, 9H), 2.65 (d, J = 3.3 Hz, 3H), 2.95 (dd, J = 13.6, 10.4 Hz, 1H), 3.07(ddd, J = 14.2, 4.9, 1.9 Hz, 2H), 3.73 – 3.61 (m, 3H), 4.69 (dd, J = 10.5, 4.6 Hz, 1H), 8.12 (dd, J = 17.7, 2.6 Hz, 2H).

Step 4: (S)-2-((tert-butoxycarbonyl)(methyl)amino)-3-(5-chloro-2-cyclopropoxypyridin-3-yl)propanoic acid



Into a 1000mL round-bottom flask were added methyl (2S)-2-[(tert-butoxycarbonyl)(methyl)amino]-3-(5-chloro-2-cyclopropoxy)pyridin-3-ylpropanoate (20 g, 51.967mmol, 1equiv) in THF(250ml) and NaOH (10.39 g, 259.835mmol, 5equiv) in water(50ml) was added dropwise at 0 °C under nitrogen atmosphere. The resulting mixture was stirred at r.t for 2h. The solvent was removed by reduce pressure and the residue was purified by reverse flash chromatography with the following conditions: column, C18; mobile phase, ACN in water (0.5% FA), 0% to 100% gradient in 40 min; detector, UV 254 nm. This resulted in (2S)-2-[(tert-butoxycarbonyl)(methyl)amino]-3-(5-chloro-2-cyclopropoxy)pyridin-3-ylpropanoic acid (13.9495 g, 72.39%) as a yellow oil.

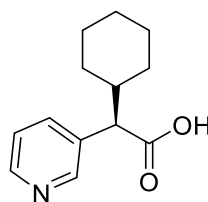
**LCMS:** (ESI, m/z): [M+H]<sup>+</sup> = 371.00.

**Spectrum:**

<sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 0.79 – 0.61 (m, 4H), 1.21 (s, 9H), 2.95 – 2.84 (m, 2H), 3.00 (s, 3H), 3.09 – 3.01 (m, 1H), 4.86 (dd, J = 11.3, 4.6 Hz, 1H), 7.67 (d, J = 2.6 Hz, 1H), 8.11 (dd, J = 18.4, 2.6 Hz, 1H).

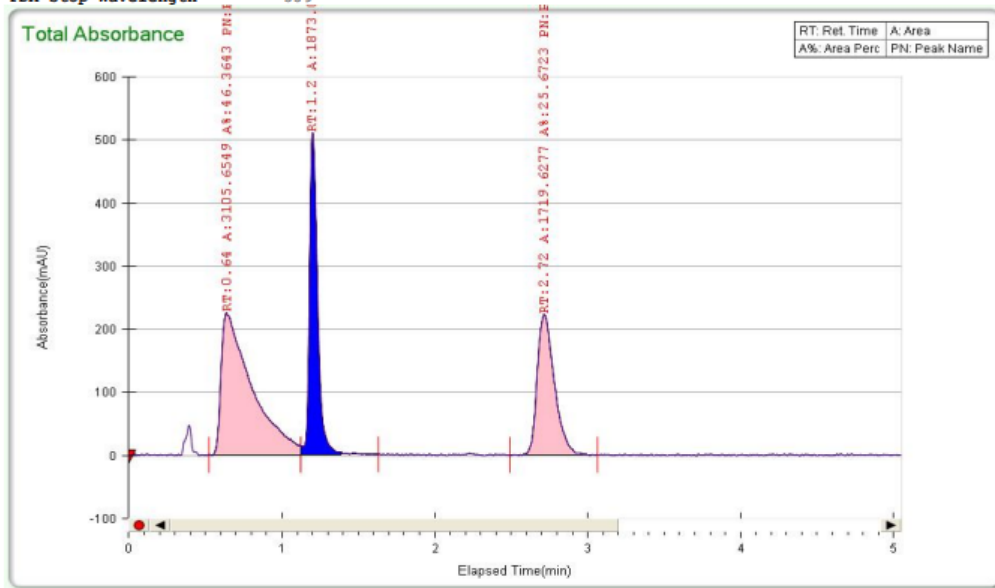
### 3.7. Chiral purification and determination of absolute stereochemistry of (R)-2-cyclohexyl-2-(pyridin-3-yl)acetic acid

#### 3.7.1 Supercritical fluid chromatography of CAS#1518459-54-5



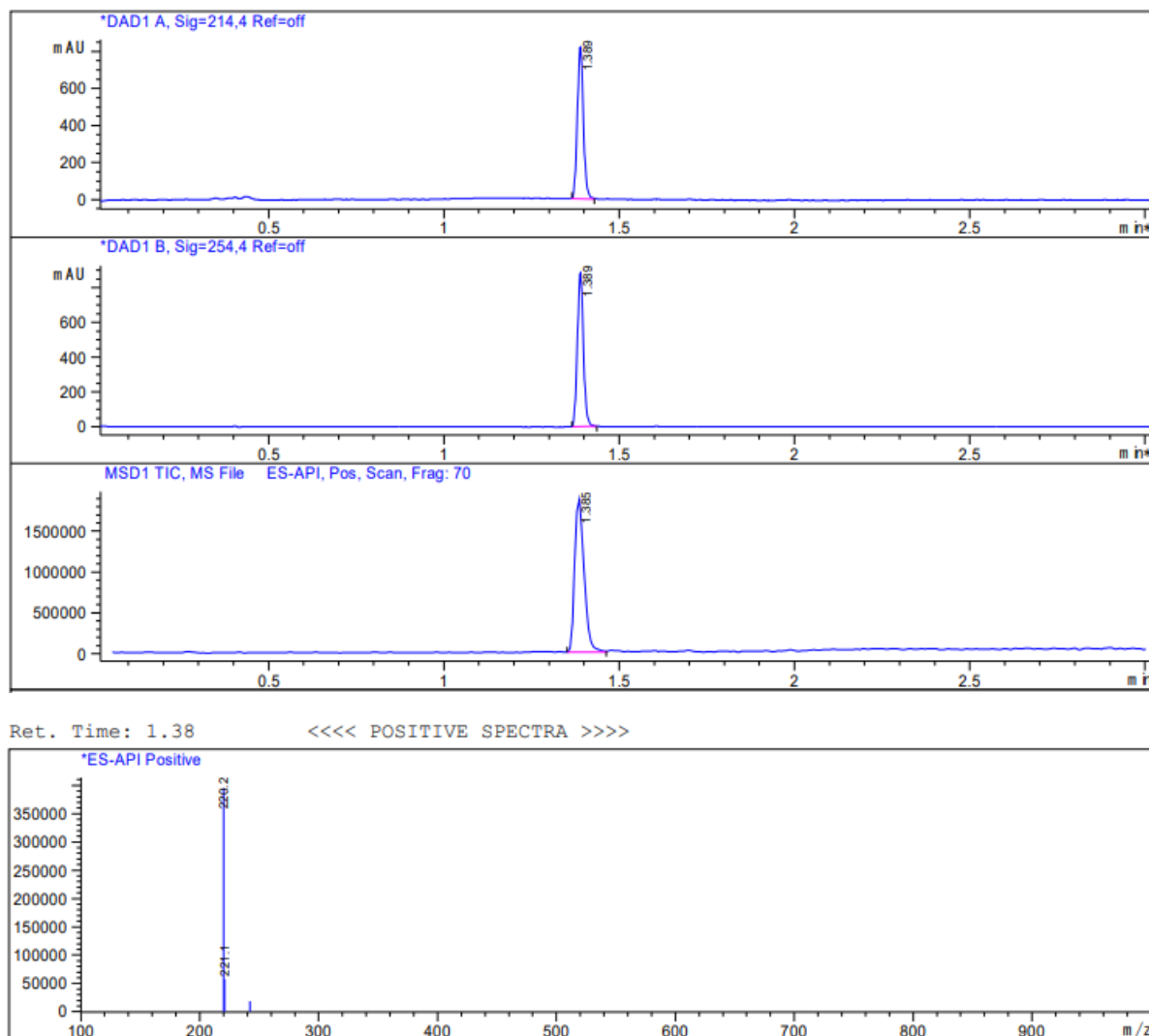
Chiral separation of racemic material CAS#1518459-54-5 was performed via SFC at 40 °C with a 0.2% Methanol ammonia co-solvent. Peak 3 was isolated.

Injection Info  
Injection Date Time Stamp 11/26/2018 6:29:38 PM  
Injection Volume 3  
Co-Solvent MeOH(0.2%Methanol Ammonia)  
Column AD-H 4.6\*100mm 5um  
Sample Acd0357  
Sample Well Pl: 4C  
Column Temperature 40  
CO2 Flow Rate 3.6  
Co-Solvent Flow Rate 0.4  
Co-Solvent % 10  
Total Flow 4  
Front Pressure 143  
Back Pressure 118  
Pressure Drop 25  
PDA Start Wavelength 214  
PDA Stop Wavelength 359



Peak Info				
Number	RT (min)	Area %	Area	Height
1	0.64	46.3643	3105.6549	225.1956
2	1.2	27.9634	1873.0907	510.3323
3	2.72	25.6723	1719.6277	221.744

### 3.7.2 LCMS data for Peak 3 isolated from racemic mixture of CAS#1518459-54-5



Analytical LCMS was performed on a SUNFIRE C18 4.6\*50 mm, 3.5  $\mu$ m column at 50°C. Mobile phase was water(0.05% TFA)/acetonitrile(0.05% TFA).

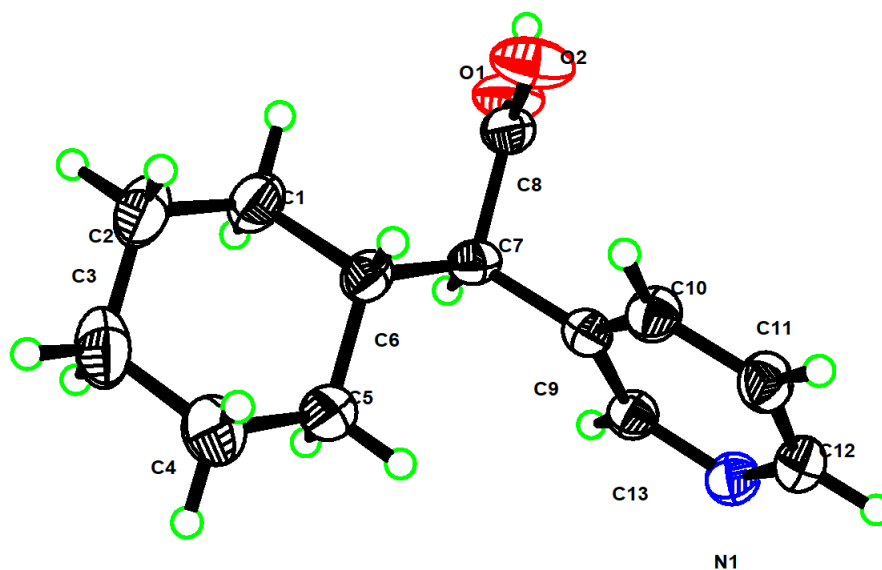
### 3.7.3 Crystallographic data for Peak 3 isolated from racemic mixture of CAS#1518459-54-5

#### **Instrument parameters:**

Light source: Cu target	X-ray: Cu-K $\alpha$ (=1.54178 Å)
Detector: CMOS surface detector	Resolution: 0.77 Å
Current and voltage: 45 kV, 1.2 mA	Exposure time: 30 s
Distance from surface detector to sample: 40 mm	Test temperature: 170(2)K

#### **Structural analysis and Refining process:**

After the integral reduction of the diffraction data by the SAINT program, empirical absorption correction of data is done by using the SADABS program, analysis of monocrystal structure with the direct method using SHELXT2014, and the structure is refined by least squares method. The hydrogen atom refinement process is obtained by isotropic calculation, hydrogen atoms on O and N are obtained by residual electron density, hydrogen atoms on C-H are obtained by calculating hydrogenation, and refined by adopting a riding model. The flack constant is 0.08. (8) through the structure diagram, and we can determine the absolute configuration. The C7 configuration in the structure is R.



(cu\_22020719\_0m\_sq)

#### Crystal data

$C_{13}H_{17}NO_2$	$D_x = 0.919 \text{ Mg m}^{-3}$
$M_r = 219.27$	Cu $K\alpha$ radiation, $\lambda = 1.54178 \text{ \AA}$
Hexagonal, $P6_3$	Cell parameters from 6038 reflections
$a = 22.0451 (6) \text{ \AA}$	$\theta = 2.3\text{--}74.7^\circ$
$c = 5.6513 (2) \text{ \AA}$	$\mu = 0.50 \text{ mm}^{-1}$
$V = 2378.50 (15) \text{ \AA}^3$	$T = 170 \text{ K}$
$Z = 6$	Block, colourless
$F(000) = 708$	$0.15 \times 0.05 \times 0.04 \text{ mm}$

#### Data collection

Bruker D8 VENTURE diffractometer	2940 reflections with $I > 2\sigma(I)$
$\phi$ and $\omega$ scans	$R_{\text{int}} = 0.041$
Absorption correction: multi-scan <i>SADABS2016/2</i> (Bruker,2016/2) was	$\theta_{\text{max}} = 74.7^\circ$ , $\theta_{\text{min}} = 2.3^\circ$

used for absorption correction. wR2(int) was 0.1468 before and 0.0605 after correction. The Ratio of minimum to maximum transmission is 0.8744. The $\lambda/2$ correction factor is Not present.	
$T_{\min} = 0.659$ , $T_{\max} = 0.754$	$h = -27 \text{®} 26$
15199 measured reflections	$k = -24 \text{®} 27$
3132 independent reflections	$l = -7 \text{®} 6$

### Refinement

Refinement on $F^2$	Hydrogen site location: inferred from neighbouring sites
Least-squares matrix: full	H-atom parameters constrained
$R[F^2 > 2\sigma(F^2)] = 0.029$	$w = 1/[\sigma^2(F_o^2) + (0.0364P)^2 + 0.1133P]$ where $P = (F_o^2 + 2F_c^2)/3$
$wR(F^2) = 0.078$	$(\Delta/\sigma)_{\max} = 0.010$
$S = 1.08$	$\Delta \rho_{\max} = 0.10 \text{ e } \text{\AA}^{-3}$
3132 reflections	$\Delta \rho_{\min} = -0.11 \text{ e } \text{\AA}^{-3}$
146 parameters	Absolute structure: Flack x determined using 1158 quotients $[(I^+)-(I^-)]/[(I^+)+(I^-)]$ (Parsons, Flack and Wagner, Acta Cryst. B69 (2013) 249-259).
1 restraint	Absolute structure parameter: 0.07 (13)

### Special details

<i>Geometry.</i> All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell esds are taken into account individually in the estimation of esds in distances, angles and torsion angles; correlations between esds in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell esds is used for estimating esds involving l.s. planes.
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### Fractional atomic coordinates and isotropic or equivalent isotropic displacement parameters ( $\text{\AA}^2$ ) for (cu\_22020719\_0m\_sq)

	$x$	$y$	$z$	$U_{\text{iso}}^*/U_{\text{eq}}$
O1	0.99572 (6)	0.69300 (7)	0.4760 (2)	0.0441 (3)

H1	1.024499	0.712818	0.365579	0.066*
O2	0.93387 (6)	0.61752 (8)	0.1912 (2)	0.0508 (3)
N1	0.74999 (7)	0.65291 (7)	0.6572 (3)	0.0365 (3)
C8	0.94079 (8)	0.63690 (8)	0.3949 (3)	0.0331 (3)
C9	0.82326 (8)	0.60687 (8)	0.5340 (3)	0.0295 (3)
C13	0.80371 (8)	0.64172 (8)	0.6926 (3)	0.0322 (3)
H13	0.829845	0.658703	0.834823	0.039*
C7	0.88542 (8)	0.59714 (8)	0.5836 (3)	0.0301 (3)
H7	0.905250	0.617768	0.741931	0.036*
C6	0.86658 (8)	0.51970 (8)	0.5823 (3)	0.0326 (3)
H6	0.848440	0.499782	0.421803	0.039*
C11	0.72957 (9)	0.59511 (9)	0.2858 (3)	0.0373 (3)
H11	0.702902	0.579476	0.144134	0.045*
C12	0.71425 (9)	0.63056 (8)	0.4557 (3)	0.0370 (4)
H12	0.676808	0.639297	0.427644	0.044*
C5	0.80993 (9)	0.47652 (9)	0.7656 (4)	0.0447 (4)
H5A	0.826956	0.495958	0.925499	0.054*
H5B	0.767503	0.479679	0.732369	0.054*
C1	0.93125 (9)	0.51306 (10)	0.6339 (4)	0.0446 (4)
H1A	0.968064	0.540360	0.515459	0.054*
H1B	0.949923	0.532763	0.792198	0.054*
C10	0.78442 (8)	0.58282 (8)	0.3256 (3)	0.0347 (3)
H10	0.795566	0.558159	0.211886	0.042*
C2	0.91329 (11)	0.43679 (11)	0.6259 (4)	0.0522 (5)
H2A	0.898133	0.418239	0.463896	0.063*
H2B	0.955605	0.433908	0.663973	0.063*
C3	0.85587 (12)	0.39261 (11)	0.7987 (5)	0.0580 (5)
H3A	0.873242	0.407197	0.962158	0.070*
H3B	0.842914	0.342904	0.780237	0.070*
C4	0.79128 (11)	0.39972 (10)	0.7597 (5)	0.0576 (6)
H4A	0.756256	0.373421	0.884220	0.069*
H4B	0.770060	0.379082	0.604673	0.069*

*Atomic displacement parameters ( $\text{\AA}^2$ ) for (cu\_22020719\_0m\_sq)*

	$U^{11}$	$U^{22}$	$U^{33}$	$U^{12}$	$U^{13}$	$U^{23}$
O1	0.0306 (6)	0.0508 (7)	0.0338 (6)	0.0074 (5)	0.0044 (5)	-0.0045 (6)
O2	0.0402 (6)	0.0608 (8)	0.0291 (6)	0.0085 (6)	0.0056 (5)	-0.0069 (6)
N1	0.0413 (7)	0.0413 (7)	0.0329 (7)	0.0251 (6)	0.0031 (6)	-0.0012 (6)
C8	0.0292 (7)	0.0399 (8)	0.0296 (8)	0.0169 (7)	0.0007 (6)	-0.0003 (6)
C9	0.0310 (7)	0.0291 (7)	0.0273 (8)	0.0143 (6)	0.0020 (6)	0.0016 (5)
C13	0.0346 (7)	0.0367 (7)	0.0271 (7)	0.0192 (6)	0.0004 (6)	-0.0015 (6)
C7	0.0266(7)	0.0367 (8)	0.0258 (7)	0.0148 (6)	0.0013 (6)	-0.0021 (6)
C6	0.0335 (7)	0.0384 (8)	0.0311 (8)	0.0219 (6)	-0.0004 (6)	-0.0030 (7)
C1 1	0.0400 (8)	0.0439 (8)	0.0314 (8)	0.0235 (7)	-0.0054 (7)	-0.0020 (7)
C1 2	0.0380 (8)	0.0398 (8)	0.0395 (9)	0.0241 (7)	0.0005 (7)	0.0020 (7)
C5	0.0433 (9)	0.0422 (9)	0.0532 (11)	0.0249 (7)	0.0114 (8)	0.0095 (8)
C1	0.0410 (9)	0.0531 (10)	0.0491 (11)	0.0306 (8)	0.0002 (8)	0.0002 (8)
C1 0	0.0389 (8)	0.0398 (8)	0.0300 (9)	0.0232 (7)	-0.0007 (7)	-0.0039 (6)
C2	0.0595 (11)	0.0593 (11)	0.0573 (12)	0.0443 (10)	-0.0039 (10)	-0.0022 (10)
C3	0.0814 (14)	0.0510 (11)	0.0574 (12)	0.0450 (11)	-0.0042 (12)	0.0035 (10)
C4	0.0589 (12)	0.0414 (10)	0.0741 (16)	0.0263 (9)	0.0113 (11)	0.0122 (10)

*Geometric parameters (Å, °) for (cu\_22020719\_0m\_sq)*

O1—H1	0.8400	C11—C10	1.384 (2)
O1—C8	1.3069 (19)	C12—H12	0.9500
O2—C8	1.211 (2)	C5—H5A	0.9900
N1—C13	1.340 (2)	C5—H5B	0.9900
N1—C12	1.331 (2)	C5—C4	1.530 (3)
C8—C7	1.525 (2)	C1—H1A	0.9900
C9—C13	1.382 (2)	C1—H1B	0.9900
C9—C7	1.515 (2)	C1—C2	1.523 (3)
C9—C10	1.395 (2)	C10—H10	0.9500
C13—H13	0.9500	C2—H2A	0.9900
C7—H7	1.0000	C2—H2B	0.9900
C7—C6	1.542 (2)	C2—C3	1.507 (3)
C6—H6	1.0000	C3—H3A	0.9900

C6—C5	1.533 (2)	C3—H3B	0.9900
C6—C1	1.532 (2)	C3—C4	1.524 (3)
C11—H11	0.9500	C4—H4A	0.9900
C11—C12	1.382 (2)	C4—H4B	0.9900
C8—O1—H1	109.5	H5A—C5—H5B	108.0
C12—N1—C13	118.26 (14)	C4—C5—C6	110.92 (16)
O1—C8—C7	113.30 (14)	C4—C5—H5A	109.5
O2—C8—O1	124.12 (16)	C4—C5—H5B	109.5
O2—C8—C7	122.59 (15)	C6—C1—H1A	109.4
C13—C9—C7	120.94 (14)	C6—C1—H1B	109.4
C13—C9—C10	117.12 (14)	H1A—C1—H1B	108.0
C10—C9—C7	121.93 (14)	C2—C1—C6	111.04 (15)
N1—C13—C9	123.75 (15)	C2—C1—H1A	109.4
N1—C13—H13	118.1	C2—C1—H1B	109.4
C9—C13—H13	118.1	C9—C10—H10	120.2
C8—C7—H7	108.8	C11—C10—C9	119.56 (15)
C8—C7—C6	109.15 (12)	C11—C10—H10	120.2
C9—C7—C8	108.06 (13)	C1—C2—H2A	109.3
C9—C7—H7	108.8	C1—C2—H2B	109.3
C9—C7—C6	113.20 (12)	H2A—C2—H2B	108.0
C6—C7—H7	108.8	C3—C2—C1	111.41 (17)
C7—C6—H6	108.5	C3—C2—H2A	109.3
C5—C6—C7	111.66 (13)	C3—C2—H2B	109.3
C5—C6—H6	108.5	C2—C3—H3A	109.3
C1—C6—C7	110.89 (13)	C2—C3—H3B	109.3
C1—C6—H6	108.5	C2—C3—C4	111.43 (18)
C1—C6—C5	108.68 (15)	H3A—C3—H3B	108.0
C12—C11—H11	120.6	C4—C3—H3A	109.3
C12—C11—C10	118.83 (16)	C4—C3—H3B	109.3
C10—C11—H11	120.6	C5—C4—H4A	109.4
N1—C12—C11	122.47 (14)	C5—C4—H4B	109.4
N1—C12—H12	118.8	C3—C4—C5	111.26 (17)
C11—C12—H12	118.8	C3—C4—H4A	109.4
C6—C5—H5A	109.5	C3—C4—H4B	109.4

C6—C5—H5B	109.5	H4A—C4—H4B	108.0
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## 4. References and notes

(1) Chatterjee, J.; Laufer, B.; Kessler, H. Synthesis of N-Methylated Cyclic Peptides. *Nat Protoc* **2012**, No. 3, 432–444. <https://doi.org/10.1038/nprot.2011.450>.