Targeting Nox2 via p47/phox-p22/phox inhibition with novel triproline mimetics

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SUPPORTING INFORMATION

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I. Experimental Section

I.1 General Methods

I.1.1 Peptides

Native p47 protein and modified p22 peptides for assays and other experiments were purchased from Genepep and Bachem. Mutated p47 was synthesized by Selvita.

I.1.2 Compounds I/II

Unless stated otherwise, all solvents and chemicals were laboratory or reagent grade and were purchased from commercial sources. All chemicals were used as received. Water was purified via Millipore filtration prior to use. All reactions were conducted under normal atmosphere. Heating of reactions was performed with Radleys heating blocks. Small quantities of liquid reagents were measured and added to reactions via syringe or autopipette. Unless otherwise noted, all filtrations were conducted as vacuum filtration through a sintered glass funnel (medium porosity). Solvent removal via concentration was performed on a rotary evaporator under reduced pressure. Reaction controls were performed by Agilent 1260 Infinity II UHPLC (MS) system. Flash and HILIC chromatography were performed on RediSep®Rf Gold columns using CombiFlash® NEXTGEN 300+ instrument. Reversed phase preparative chromatographic separation was achieved employing a 50 x 250 mm Gemini-NX 5µ C18 110A C18 column with gradient elution at 118 mL/min at rt. Mobile phase A: 10 mM aqueous NH_4HCO_3 solution; mobile phase B: acetonitrile. All synthesized compounds were dried under high vacuum (<1 mbar) before determination of chemical yields and spectroscopic characterization. High-resolution MS spectra were measured by Agilent 6230 TOF LC/MS spectrometer. All final compounds tested in biological assays were >95% pure as judged by HPLC and ^{1}H NMR analysis. The NMR experiments were carried out on a Bruker 500 MHz spectrometer equipped with a TCI cryoprobe probehead and processed using Topspin 3.5 software. The compounds were dissolved in DMSO-d_6 and the NMR experiments were measured at 300K. Chemical shifts are given on the δ-scale and are referenced to the solvent (DMSO-d_6: δ_C = 44.0 and δ_H = 2.50 ppm). Pulse programs of all experiments (^{1}H, DEPTQ, gs-HSQC, gs-HMBC (optimized for 10 Hz)) were taken from the Bruker software library. For 1D measurements, 64K data points were used to yield the FID. For 2D measurements, sweep width in F_2 was 4000 Hz; all data points (t_2 x t_1) were acquired with 2 K x 128. For F_1, linear prediction was applied to enhance the resolution.

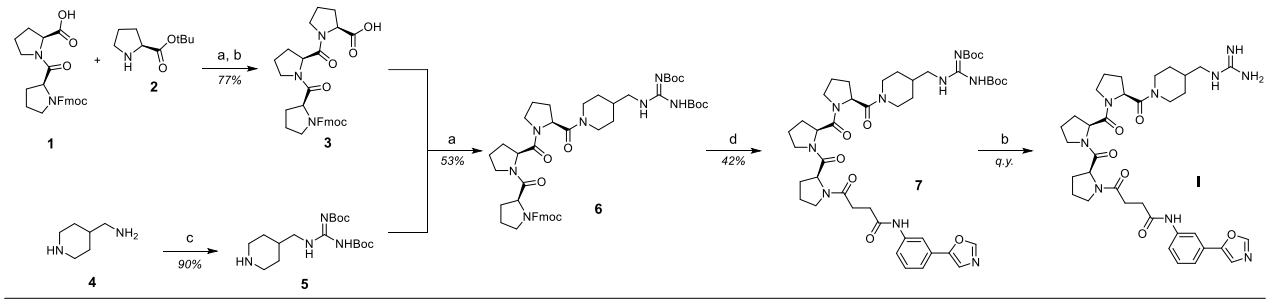
I.1.3 Compounds III/IV

Unless otherwise stated, all reactions were carried out in flame-dried glassware under a positive pressure of dry, oxygen free, argon and in dry solvents. Anhydrous solvents were distilled under a positive pressure of argon before use and dried by standard methods. THF, ether, CH_2Cl_2 and toluene were dried by the SDS (Solvent Delivery System). Dimethylformamide was purchased at Sigma-Aldrich with a Sure/Seal packaging. Commercial grade reagents were used without further purification. Silica column chromatography was performed on 230-400 mesh silica gel. Reactions were monitored by mass spectrometry with positive ionization or by thin-layer chromatography carried out on a 0.25 mm aluminium-backed plate. Visualisation was effected by UV light (254 nm) or by staining with potassium

permanganate solution, cerium ammonium molybdate, p-anisaldehyde or ninhydrin solution followed by heating. ^{1}H and ^{13}C NMR spectra were recorded on Brüker instruments with field strengths notated in the text at room temperature (298 K) with complete proton decoupling for nucleus other than ^{1}H unless otherwise stated. Chemical shifts are reported in parts per million (ppm) referenced from $CDCl_3$ (δ_H: 7.26 ppm and δ_C: 77.0 ppm) or MeOD (δ_H: 3.31 ppm and δ_C: 49.0 ppm). Coupling constants (*J*) are reported in Hertz (Hz). Multiplicities are given as multiplet (m), singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin.) and broad (br.). Optical rotations were determined on an Anton Paar MCP 300 polarimeter at 589 nm at 25 °C. Specific rotations are given in units of $deg \cdot mL \cdot g^{-1} \cdot dm^{-1}$. High resolution mass spectra (HRMS) and high performance liquid chromatography (HPLC) were performed by the "Centre régional de spectroscopie de masse de l'Université de Montréal" with electrospray ionisation (ESI) coupled to a quantitative time-of-flight (TOF) detector.

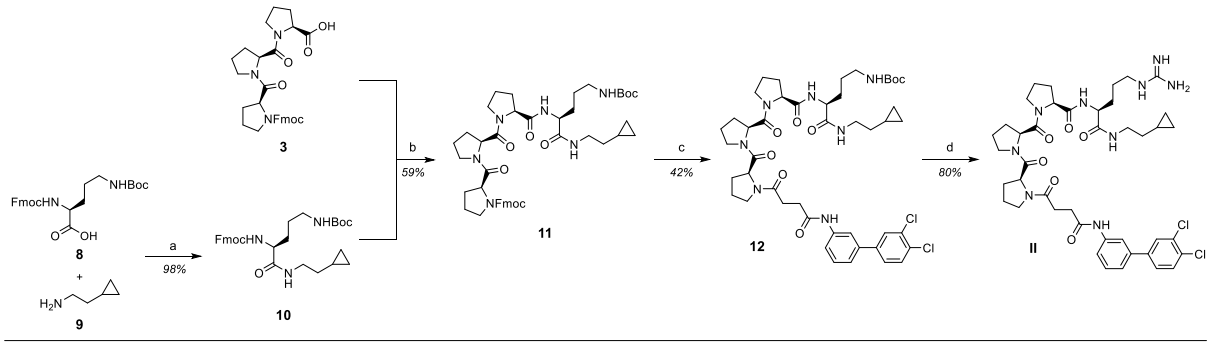
I.2 Synthetic Schemes

Scheme SI1. Synthesis of compounds **I**



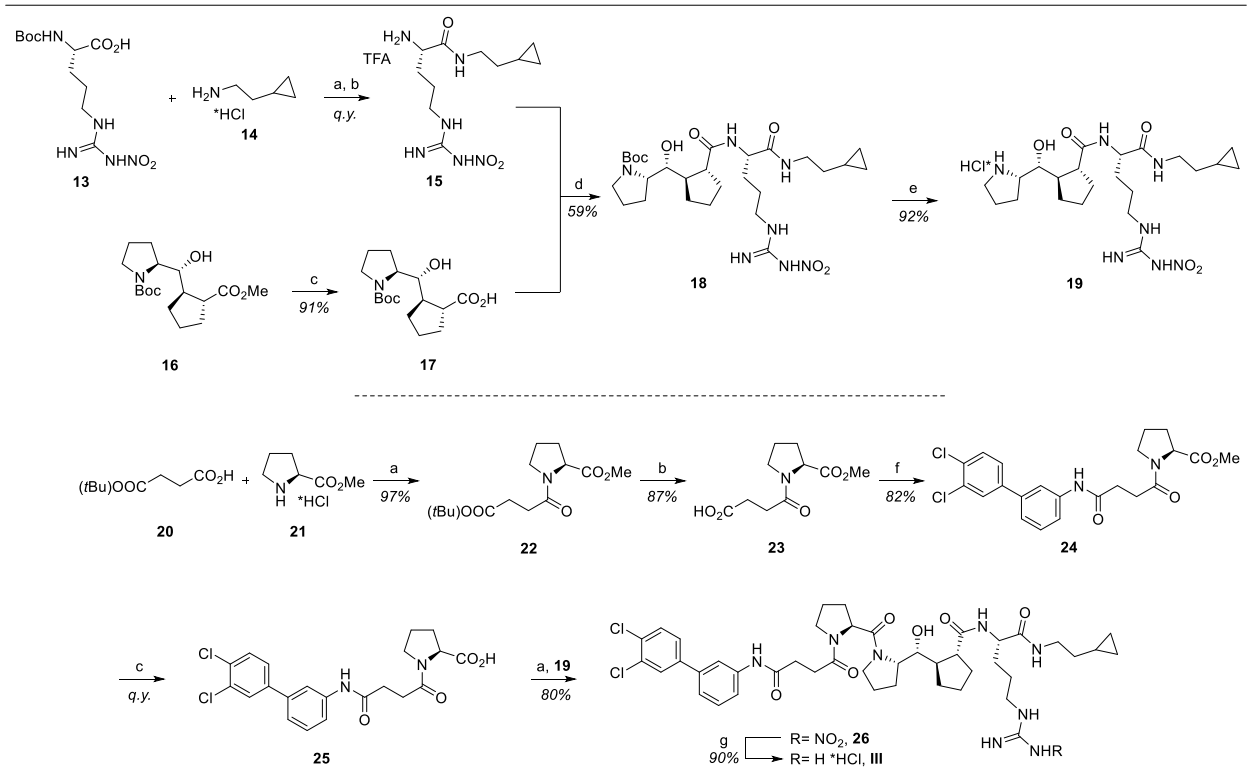
[a]Reagents and conditions: (a) TBTU, DIPEA, DMF, rt, 2h; (b) 1,2-DCE, TFA, 2h, reflux; (c) 1,3-di-Boc-2-methylisothiourea, CH_2Cl_2, 1h, rt; (d) DBU, DMF, 10 min, 50°C *then* succinic anhydride, DIPEA, 20 min, rt *then* TBTU, 3-oxazol-5-ylaniline, 2h, rt ;

Scheme SI2. Synthesis of compounds **II**



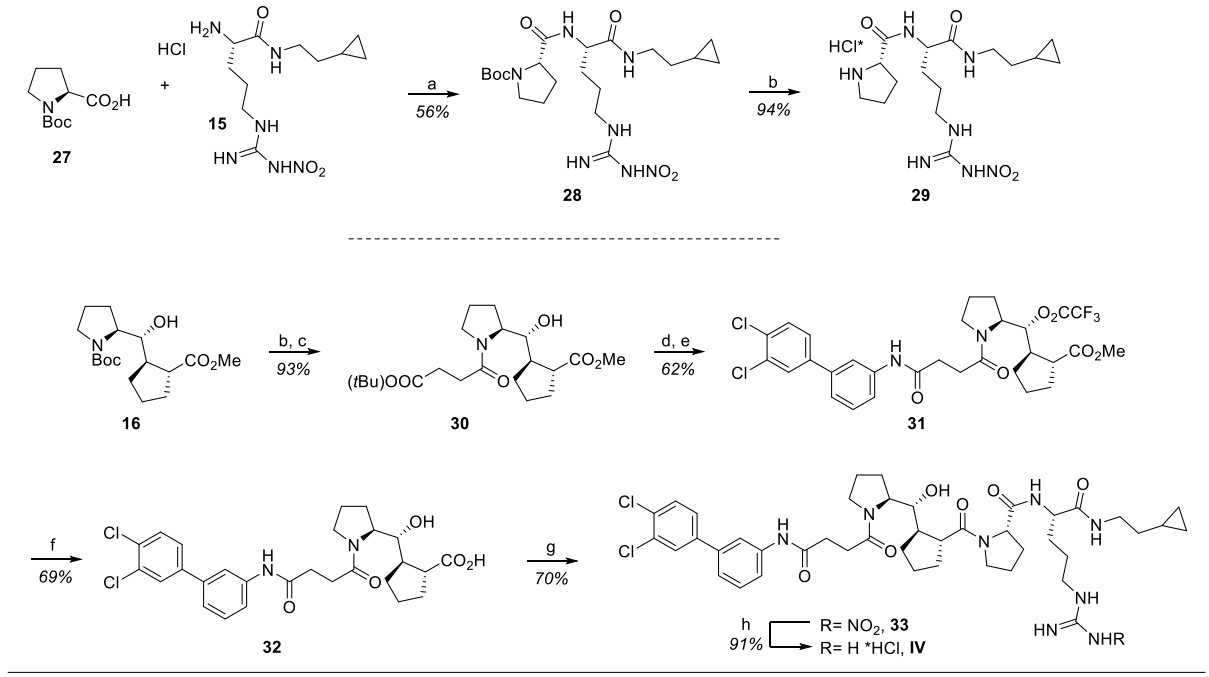
[a]Reagents and conditions: (a) TBTU, DIPEA, DMF, 1h, rt; (b) DBU, DMF, 50°C *then* TBTU, DIPEA, DMF, 1h, rt; (c) DBU, DMF, 10 min, 50°C *then* succinic anhydride, DIPEA, 20 min, rt *then* TBTU, 3-(3,4-dichlorophenyl)aniline, 2h, rt (d) HCl/1,4-dioxane, MeOH, 1h, rt *then* pyrazole-1-carboxamidine hydrochloride, DIPEA, DMA, 1h, 60 °C;

Scheme SI3. Synthesis of compounds **III**



(a) EDC, HOBt, DIPEA, CH_2Cl_2, rt, 16h; (b) TFA/CH_2Cl_2, rt, 2h; (c) 1:1 1M aq. LiOH/THF, rt, 1h; (d) DIPEA, PyBOP, CH_2Cl_2, rt, 16h; (e) HCl (4M), dioxane, rt, 30 min; (f) 3',4'-dichloro-[1,1'-biphenyl]-3-amine, HATU, DIPEA, CH_2Cl_2, rt, 7h; (g) H_2, 10% Pd/C, 1:1 MeOH/EtOAc, 6M aq. HCl (2 drops), rt, 4h.

Scheme SI4. Synthesis of compounds **IV**



(a) DIPEA, PyBOP, CH_2Cl_2, rt, 16h; (b) HCl (4M), dioxane, rt, 30 min; (c) **20**, EDC, HOBt, DIPEA, CH_2Cl_2, rt, 16h; (d) TFA/CH_2Cl_2, rt, 2h; (e) 3',4'-dichloro-[1,1'-biphenyl]-3-amine, HATU, DIPEA, CH_2Cl_2, rt, 7h; (f) LiOH, THF, rt, 1h; (g) **29**, EDC, HOBt, DIPEA, CH_2Cl_2, rt, 16h; (h) H2, 10% Pd/C, 1:1 MeOH/EtOAc, 6M aq. HCl (2 drops), rt, 4h.

I.3 Synthesis and Characterization of Peptidomimetics

I.3.1 General procedures

General Procedure A: Amide coupling

Carboxylic acid (1.0 eq), TBTU (1.05 - 1.1 eq) and DIPEA (1.1 – 1.2 eq) were combined in a DMF solution (1.25 - 5 mL/mmol acid). After stirring at rt for 10 min, the amine (1.0 – 1.2 eq) was added and the mixture was stirred for another 1h. Work-up and purification (unless otherwise specified): the reaction mixturewas filtered, injected to an RP C18 column and purified via preparative reversed phase chromatography. The appropriate fractions were combined, concentrated under reduced pressure to approximately 25 mL then extracted with CH_2Cl_2 (2 × 20 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to furnish the targeted amide product.

General Procedure B: Fmoc deprotection

Fmoc protected amine (1.0 eq) and DBU (0.1 eq) were combined in a DMF solution (1.0 – 5.5 mL/mmol acid). After 20 min to 1h of stirring at 50 °C the solution was used in the next amide coupling step.

General Procedure C: Sequential amide coupling

To a solution of amine (1.0 eq) and DIPEA (2.8 – 3.0 eq) in DMF, succinic anhydride (1.1 eq) was added and the reaction mixture was stirred for 20 min at rt. TBTU (1.2 - 1.6 eq) followed by the substituted aniline (1.3 - 1.6 eq, dissolved in 1 mL DMF) were added and the mixture was stirred for 2h. Work-up and purification: same as described in General Procedure A (unless otherwise specified).

General Procedure D: Boc deprotection 1

Boc-protected amine (1.0 eq) was dissolved in an appropriate solvent and the acid (12.5 – 95.0 eq) was added. The mixture was stirred for 0.5 - 1h at rt then concentrated under reduced pressure.

General procedure E: Ester hydrolysis

To the ester substrate (1.0 eq.) dissolved in THF (0.67 M) was added LiOH (1.5 eq., 1.0M in H_2O) at 0 °C. The reaction mixture was stirred at 0°C for 5 min then at rt for 1h. The mixture was diluted with water, washed with Et_2O, acidified (pH = 3) with 10% aq. citric acid and extracted twice with EtOAc. The pooled organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure.

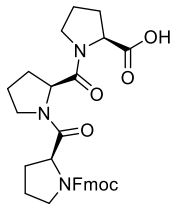
General procedure F: Boc deprotection 2

The protected compound was dissolved in 4M HCl/dioxane (10 eq.) and the solution was stirred at rt for 2h. The solvent was removed under reduced pressure.

General procedure G: *tert*-butyl ester deprotection

The protected compound was dissolved in CH_2Cl_2 (0.65 M). The solution was cooled at 0 °C and TFA (20 eq.) was added. The solution was stirred at rt for 4h then the solvent was removed under reduced pressure.

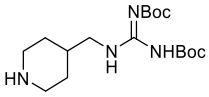
I.3.2 Synthesis of ***I***



(2S)-1-[(2S)-1-[(2S)-1-(9H-Fluoren-9-ylmethoxycarbonyl)pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carboxylic acid (3)

Performed according to **General Procedure A.** Carboxylic acid **1** (1.3 g, 3.00 mmol, 1.0 eq.), TBTU (1.0 g, 3.10 mmol, 1.03 eq.), DIPEA (0.6 mL, 3.45 mmol, 1.2 eq.) and tert-butyl (2S)-pyrrolidine-2-carboxylate **2** (0.600 g, 3.50 mmol, 1.2 eq.) were reacted in DMF (10 mL). Exception to the general procedure: the reaction was quenched by the addition of saturated aqueous solution of Na_2CO_3 then it was extracted with CH_2Cl_2 (2 × 50 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated to give a brown syrup which was dissolved in DCE (5 mL). TFA (3.0 mL, 39.20 mmol, 13 eq.) was added and the reaction mixture was refluxed for 2h. The reaction was cooled to rt, concentrated and the crude residue was purified by flash column chromatography over silica (eluent: CH_2Cl_2 /acetone 10-40%) to provide the title compound **3** as a white foam (1.2 g, 77%).

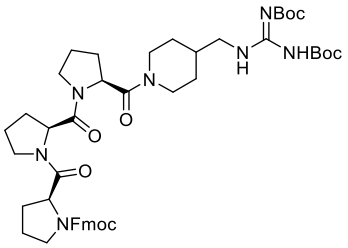
^{1}H NMR (500 MHz, DMSO-d6) δ 7.93-7.86 (m, 2H), 7.69-7.62 (m, 1H), 7.60+7.57 (2d, J=7.7 Hz, 1H). 7.46-7.28 (m, 4H), 4.65-4.11 (m, 6H), 3.71-3.07 (m, 6H), 2.24-1.58 (m, 12H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{30}H_{33}N_3O_6$ 532.2448, found 532.2439.



tert-Butyl (NE)-N-[(tert-butoxycarbonylamino)-(4-piperidylmethylamino)methylene]carbamate (5)

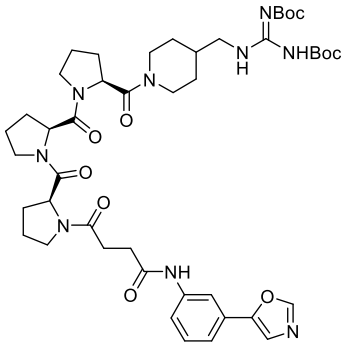
To the solution of **4** (1.8 g, 15.40 mmol, 1.5 eq.) in CH_2Cl_2 (40 mL) tert-butyl (NZ)-N-[(tert-butoxycarbonylamino)-methylsulfanyl-methylene]carbamate (2.9 g, 9.99 mmol, 1.0 eq.) was added over 5 minutes. The reaction was stirred for 1h then the volatiles were removed under reduced pressure and water (20 mL) was added to the residue. A white precipitate was formed and filtered off. The precipitate was dissolved in CH_2Cl_2 (50 mL), the organic solution was dried over $MgSO_4$ and concentrated to afford the title compound **5** as white crystals (3.2 g, 90%).

^{1}H NMR (500 MHz, DMSO-d6) δ 8.28 (t, J=5.5 Hz, 1H), 3.16 (t, J=5.5 Hz, 2H), 2.90 (brd, J=12.0 Hz, 2H), 2.39 (td, J=12.0, 2.2 Hz, 2H), 1.64-1.53 (m, 1H), 1.52 (d, J=11.8 Hz, 2H), 1.47 (s, 9H), 1.39 (s, 9H), 1.01 (qd, J=11.8, 3.8 Hz, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{17}H_{32}N_4O_4$ 357.2502, found 357.2491.



9H-Fluoren-9-ylmethyl (2S)-2-[(2S)-2-[(2S)-2-[4-[[[(E)-N,N'-bis(tertbutoxycarbonyl)carbamimidoyl]amino]methyl]piperidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carboxylate (6)
Following **General Procedure A,** carboxylic acid **3** (2.7 g, 5.00 mmol, 1.0 eq.), TBTU (1.8 g, 5.50 mmol, 1.1 eq.), DIPEA (1.1 mL, 6.25 mmol, 1.3 eq.) and amine **5** (2.1 g, 6.00 mmol, 1.2 eq.) were reacted in DMF (10.0 mL) to afford the title product **6** as a white foam (2.3 g, 53%).

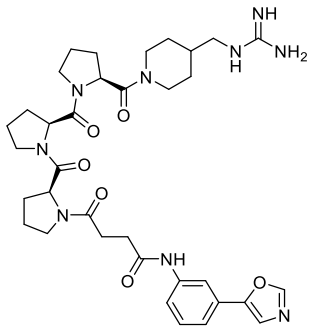
^{1}H NMR (500 MHz, DMSO-d6) δ 11.50 (s, 1H), 8.40-8.27 (m, 1H), 7.94-7.82 (m, 2H), 7.69-7.62 (m, 1H), 7.60+7.57 (2d, J=7.7 Hz, 1H), 7.45-7.28 (m, 4H), 4.88-4.10 (m, 8H), 4.04-3.87 (m, 1H), 3.72-3.35 (m, 4H), 3.32-3.09 (m, 4H), 3.05-2.87 (m, 1H), 2.21-2.00 (m, 3H), 1.97-1.55 (m, 12H), 1.47 (s, 9H), 1.39 (s, 9H) 1.19-0.87 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{47}H_{63}N_7O_9$ 870.4766, found 870.4746.



tert-Butyl (NE)-N-[(tert-butoxycarbonylamino)-[[1-[(2S)-1-[(2S)-1-[(2S)-1-[4-(3-oxazol-5-ylanilino)-4-oxo-butanoyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]-4-piperidyl]methylamino] methylene]carbamate (7)

Step I. Following **General Procedure B,** Fmoc-amine **6** (214.0 mg, 0.25 mmol, 1.0 eq.) and DBU (2.5 µL, 17 µmol, 0.07 eq.) were reacted in DMF (3.0 mL) for 20 min. Step II. Following **General Procedure C,** DIPEA (120.0 µL, 0.70 mmol, 2.8 eq.), succinic anhydride (27.0 mg, 0.27 mmol, 1.1 eq.), TBTU (95 mg, 0.30 mmol, 1.2 eq.) and 3-oxazol-5-ylaniline (98.0 mg, 0.39 mmol, 1.6 eq.) were added successively to the solution of amine prepared in Step I. After purification and work-up the title compound **7** was isolated as a white foam (102 mg, 42%).

^{1}H NMR (500 MHz, DMSO-d6) δ 11.52+11.50 (2s, 1H), 10.1 (s, 1H), 8.45 (s, 1 H), 8.37-8.30 (m, 1H), 8.05 (brs, 1H), 7.62 (s, 1H), 7.54-7.48 (m, 1H), 7.42-7.36 (m, 2H), 4.87-4.49 (m, 3H), 4.36-2.90 (m, 11H), 2.74-1.54 (m, 20H), 1.47 (s, 9H), 1.39 (s, 9H), 1.19-0.86 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{47}H_{63}N_7O_9$ 890.4776, found 890.4776.

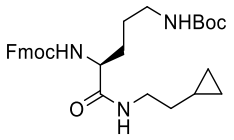


4-[(2S)-2-[(2S)-2-[(2S)-2-[4-(Guanidinomethyl)piperidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidin-1-yl]-N-(3-oxazol-5-ylphenyl)-4-oxo-butanamide trifluoroacetic acid salt (I)

Following **General Procedure D** deprotection step**,** Boc-guanidine **7** (100.0 mg, 0.11 mmol, 1.0 eq.) and TFA (0.8 mL, 10.40 mmol, 95.0 eq.) were reacted in DCE (2.0 mL) for 1h at 50 °C. Methanol (5.0 mL) was added and the solution was concentrated under reduced pressure at rt. The residue was dissolved in acetonitrile/water (1:2 mL) and freeze-dried to furnish the title compound **I** as a white foam (94.0 mg, quantitative yield).

^{1}H NMR (500 MHz, DMSO-d_6) δ 10.11 (s, 1H), 8.45 (s, 1H), 8.05 (bs, 1H), 7.62 (s, 1H), 7.59 (m, 1H), 7.54 – 7.49 (m, 1H), 7.39 (m, 2H), 7.25 – 6.68 (m, 4H), 4.86 – 4.51 (m, 3H), 4.34 – 4.24 (m, 1H), 4.00 (m, 1H), 3.79 – 3.58 (m, 2H), 3.56 – 3.32 (m, 4H), 3.02 (m, 2H), 2.72 – 2.63 (m, 1H), 2.60 – 2.55 (m, 1H), 2.55 – 2.52 (m, 3H), 2.20 – 1.59 (m, 14H), 1.17 – 0.88 (m, 2H).
^{13}C NMR (126 MHz, DMSO) δ 170.9, 169.5, 169.2, 169.2, 158.4, 158.2, 156.8, 150.5, 140.1, 129.6, 127.8, 122.0, 118.9, 118.8, 114.1, 57.9, 57.7, 57.4, 57.2, 56.4, 56.3, 46.7, 46.5, 46.5, 46.3, 45.6, 44.1, 41.1, 35.5, 31.3, 31.0, 29.5, 28.8, 28.7, 28.4, 27.9, 27.8, 27.4, 27.3, 27.1, 24.4, 24.3, 24.3, 24.1, 22.0.
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{35}H_{47}N_9O_6$ 690.3728, found 690.3725.
HPLC: 94%

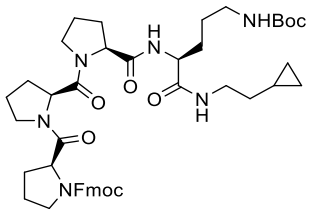
*I.3.3 Synthesis of **II***



9H-Fluoren-9-ylmethyl N-[(1S)-4-(tert-butoxycarbonylamino)-1-(2-cyclopropylethylcarbamoyl) butyl] carbamate (10)

Following **General Procedure A**, carboxylic acid **8** (5.0 g, 11.00 mmol, 1.0 eq.), TBTU (3.9 g, 12.10 mmol, 1.1 eq.), DIPEA (3.8 mL, 21.50 mmol, 2.0 eq.) and 2-cyclopropylethanamine **9** (1.2 mL, 13.00 mmol, 1.2 eq.) were reacted in DMF (20.0 mL) then the reaction mixture was poured into ice/water (300 mL). The precipitated product was collected by filtration, washed with water and dried in high vacuum at 40 °C to provide the title compound **10** as a white solid (5.6 g, 98%).

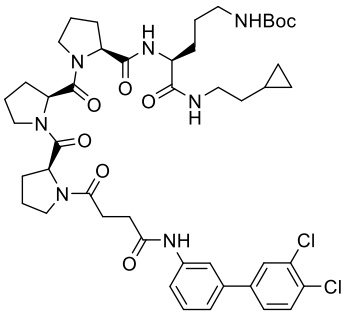
^{1}H NMR (500 MHz, DMSO-d6) δ 7.89 (dm, J=7.4 Hz, 2H), 7.84 (dm, J=7.4 Hz, 2H), 7.82-7.70 (m, 1H), 7.41 (td, J=7.4, 1.1 Hz, 2H), 7.34 (td, J=7.4, 1.1 Hz, 2H), 6.84-6.75 (m, 1H), 6.54-6.31 (br, 1H), 4.32-3.00 (m, 5H), 2.94-2.80 (m, 2H), 1.64-1.18 (m, 16H), 0.73-0.58 (m, 1H), 0.42-0.29 (m, 2H), 0.08- -0.05 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{30}H_{39}N_3O_5$ 522.2968, found 522.2961.



9H-Fluoren-9-ylmethyl (2S)-2-[(2S)-2-[(2S)-2-[[(1S)-4-(tert-butoxycarbonylamino)-1-(2-cyclopropylethylcarbamoyl) butyl]carbamoyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carbonyl]pyrrolidine-1-carboxylate (11)

Step I. Following **General Procedure B**, Fmoc-amine **10** (2.2 g, 4.26 mmol, 1.2 eq.) and DBU (65.0 µL, 0.43 mmol, 0.12 eq.) were reacted in DMF (10.0 mL) for 1h. Step II. Following **General Procedure A,** carboxylic acid **3** (1.9 g, 3.56 mmol, 1.0 eq.), TBTU (1.3 g, 3.91 mmol, 1.1 eq.), DIPEA (0.7 mL, 4.27 mmol, 1.2 eq.) and the solution of amine **10** prepared in Step I were reacted in DMF (10.0 mL) then the reaction mixture was concentrated to give a brown syrup. The crude product was dissolved in CH_2Cl_2 (50 mL) and washed with water (2 × 30 mL). The organic phase was dried over Na_2SO_4 and concentrated to provide the title compound **11** as a white foam which was used in the next step without further purification (1.7 g, 59%).

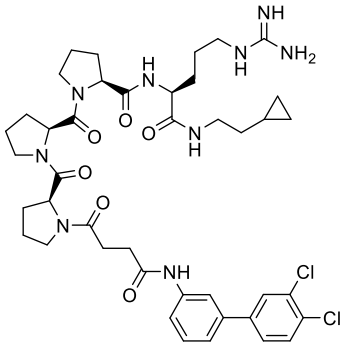
^{1}H NMR (500 MHz, DMSO-d6) δ 7.92-7.87 (m, 2H), 7.77-7.71 (m, 2H), 7.68-7.63 (m, 1H), 7.61-7.55 (m, 1H), 7.45-7.28 (m, 4H), 6.78-6.71 (m, 1H), 4.59-4.03 (m, 7H), 3.72-2.96 (m, 8H), 2.93-2.79 (m, 2H), 2.22-1.21 (m, 27H), 0.72-0.59 (m, 1H), 0.41-0.32 (m, 2H), 0.05- -0.05 (m, 2H);
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{45}H_{60}N_6O_8$ 813.4551, found 813.4542.



tert-Butyl N-[(4S)-5-(2-cyclopropylethylamino)-4-[[(2S)-1-[(2S)-1-[(2S)-1-[4-[3-(3,4-dichlorophenyl)anilino]-4-oxo-butanoyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]amino]-5-oxo-pentyl]carbamate (12)

Step I. Following **General Procedure B**, Fmoc-amine **11** (152.0 mg, 0.19 mmol, 1.0 eq.) and DBU (3.0 µL, 0.02 mmol, 0.1 eq.) were reacted in DMF (2.0 mL) for 20 min. Step II. Following **General Procedure C,** DIPEA (90.0 µL, 0.53 mmol, 2.8 eq.), succinic anhydride (21.0 mg, 0.21 mmol, 1.1 eq.), TBTU (72.0 mg, 0.22 mmol, 1.2 eq,) and 3-(3,4-dichlorophenyl)aniline hydrochloride (82.0 mg, 0.30 mmol, 1.6 eq.) were added successively to the solution of amine prepared in Step I. After purification and work-up the title compound **12** was isolated as a white foam (102.0 mg, 42%).

^{1}H NMR (500 MHz, DMSO-d6) δ 10.11-9.99 (s, 1H), 7.98-7.93 (m, 1H), 7.85-7.81 (m, 1H), 7.78-7.69 (m, 3H), 7.63-7.55 (m, 2H), 7.43-7.33 (m, 2H), 6.75 (t, J=5.7 Hz, 1H), 4.89-4.02 (m, 4H), 3.83-3.25 (m, 7H), 3.19-2.98 (m, 2H), 2.91-2.82 (m, 2H), 2.80-1.18 (m, 31H), 0.71-0.59 (m, 1H), 0.40-0.31 (m, 2H), 0.05- -0.04 (m, 2H);
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{46}H_{61}Cl_2N_7O_8$: 910.4037, found 910.4025.



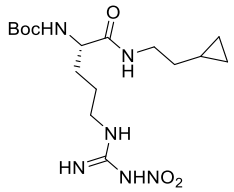
(2S)-N-[(1S)-1-(2-Cyclopropylethylcarbamoyl)-4-guanidino-butyl]-1-[(2S)-1-[(2S)-1-[4-[3-(3,4-dichlorophenyl) anilino]-4-oxo-butanoyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carbonyl]pyrrolidine-2-carboxamide trifluoroacetic acid salt (II)

Following **General Procedure D**, Boc-amine **12** (33.0 mg, 36.00 µmol, 1.0 eq.) and HCl (0.8 mL, 3.20 mmol, 4.0M in 1,4-dioxane, 90.0 eq) were reacted in 1,4-dioxane (3.0 mL) and methanol (1.0 mL) for 1h then concentrated under reduced pressure. The residue was then dissolved in DMA (1.0 mL), and DIPEA (50.0 µL, 0.30 mmol, 8.0 eq.) followed by pyrazole-1-carboxamidine hydrochloride (42.0 mg, 0.30 mmol, 8.0

eq.) were added. After stirring for 16 h at 60 °C the reaction mixture was concentrated under reduced pressure. The residue was dissolved in acetonitrile/water (2:2 mL) then directly injected onto a preconditioned 40 g silica column and purified via HILIC to afford compound **II** as a white powder (28.0 mg, 80%).

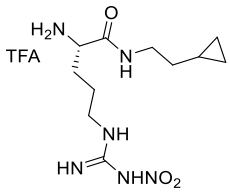
^{1}H NMR (500 MHz, DMSO-d_6) δ 10.09 (m, 1H), 7.96 (bs, 1H), 7.89 – 7.79 (m, 3H), 7.73 (d, *J* = 8.4 Hz, 1H), 7.59 (m, 2H), 7.48 (m, 1H), 7.42 – 7.35 (m, 2H), 7.01 (m, 4H), 4.65 – 4.50 (m, 1H), 4.30 (m, 1H), 4.14 – 4.10 (m, 1H), 3.70 – 3.32 (m, 7H), 3.18 – 3.01 (m, 4H), 2.72 – 2.52 (m, 3H), 2.20 – 1.83 (m, 9H), 1.81 – 1.63 (m, 5H), 1.55 – 1.36 (m, 3H), 1.29 (q, *J* = 7.0 Hz, 2H), 0.66 (m, 1H), 0.37 (dd, *J* = 8.0, 1.4 Hz, 2H), 0.01 (m, 2H).
^{13}C NMR (126 MHz, DMSO) δ 171.4, 170.8, 170.4, 169.6, 169.2, 156.6, 140.8, 140.1, 138.0, 131.1, 129.8, 129.7, 129.5, 129.5, 129.3, 129.1, 128.3, 126.8, 121.3, 118.7, 117.0, 72.3, 60.2, 59.4, 57.4, 57.2, 52.2, 46.6, 46.5, 33.9, 31.0, 29.0, 28.8, 28.4, 27.9, 27.7, 27.6, 25.0, 24.5, 24.4, 24.1, 22.0, 8.5, 4.1, 4.1, 0.1.
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{42}H_{55}Cl_2N_9O_6$ 852.3731, found 852.3716.
HPLC: 98%

*I.3.4 Synthesis of **III***



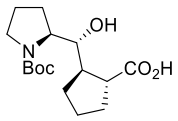
(*S*)-*tert*-Butyl (1-((2-cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)carbamate (13a)
2-Cyclopropylethanamine hydrochloride **14** (100.0 mg, 0.82 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (2.7 mL) and the solution was cooled to 0°C. BocArg(NO_2)OH **13** (328.0 mg, 0.99 mmol, 1.2 eq.) and DIPEA (501.0 µL, 2.88 mmol, 3.5 eq.) were added, followed by HOBt (133.0 mg, 0.99 mmol, 1.2 eq.) and EDC (378.0 mg, 1.97 mmol, 2.4 eq.). The reaction mixture was stirred at 0 °C for 10 min, then at rt for 14h. The reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 95:5 to 92.5:7.5 CH_2Cl_2/MeOH obtaining the title compound as a white foam (318.0 mg, q.y.).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.66 (bs, 1H), 8.10 – 7.42 (bs, 2H), 6.96 (bs, 1H), 5.64 (bs, 1H), 4.34 – 3.96 (m, 1H), 3.46 – 3.19 (m, 4H), 1.87 – 1.55 (m, 4H), 1.41 (m, 11H), 0.64 (m, 1H), 0.47 – 0.34 (m, 2H), 0.08 – -0.04 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{16}H_{30}N_6O_5$: 387.23504; found 387.23440. $[M+Na]^+$ calcd for $C_{16}H_{30}N_6O_5$: 409.21699; found 409.21668.



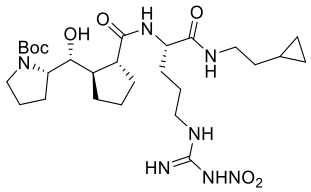
(*S*)-2-Amino-*N*-(2-cyclopropylethyl)-5-(3-nitroguanidino)pentanamide trifluoroacetate (15)
The title compound was obtained from **13a** (318.0 mg, 0.82 mmol, 1.0 eq.) following general procedure **G** as an orange foam (236.0 mg, q.y.).

^{1}H NMR (500 MHz, Methanol-d_4) δ 3.87 (m, 1H), 3.33 – 3.25 (m, 4H), 1.90 (m, 2H), 1.68 (m, 2H), 1.42 (m, 2H), 0.77 – 0.65 (m, 1H), 0.50 – 0.39 (m, 2H), 0.08 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{11}H_{22}N_6O_3$: 287.18262; found 287.18198. $[M+Na]^+$ calcd for $C_{11}H_{22}N_6O_3$: 309.16456; found 309.16415.



(1*R*,2*R*)-2-((*R*)-((*S*)-1-(*tert*-Butoxycarbonyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarboxylic acid (17)
The title compound was synthetized from **16**[1] (53.5 mg, 0.16 mmol, 1.0 eq.) following general procedure **E**. The reaction mixture was acidified with 10% aq. citric acid, then extracted twice with EtOAc. The pooled organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to afford **17** as a colourless oil (46.4 mg, 91%).

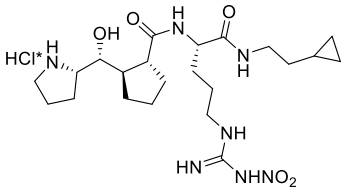
^{1}H NMR (400 MHz, Methanol-d_4) δ 3.94 – 3.77 (m, 2H), 3.47 (m, 1H), 3.27 (m, 1H), 2.71 (m, 1H), 2.27 – 2.15 (m, 1H), 2.09 – 1.79 (m, 6H), 1.71 (m, 3H), 1.47 (m, 10H).
HRMS (ESI-TOF) m/z: $[M+Na]^+$ calcd for $C_{16}H_{27}NO_5$: 336.17814; found 336.17916. $[M+K]^+$ calcd for $C_{16}H_{27}NO_5$: 352.15208; found 352.15194.



(*S*)-*tert*-Butyl 2-((*R*)-((1*R*,2*R*)-2-(((*S*)-1-((2-cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)carbamoyl)cyclopentyl)(hydroxy)methyl)pyrrolidine-1-carboxylate (18)
An amount of **17** (46.4 mg, 0.15 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (740.0 µL) and the solution was cooled to 0°C. **15** (71.1 mg, 0.18 mmol, 1.2 eq.) and DIPEA (111.0 µL, 0.64 mmol, 4.3 eq.) were added, followed by PyBOP (116.0 mg, 0.22 mmol, 1.5 eq.). The reaction mixture was stirred at 0°C for 10 min,

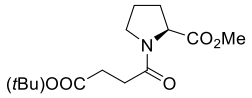
then at rt for 14h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 95:5 to 92:8 CH_2Cl_2/MeOH to afford **18** as a white foam (51.2 mg, 59%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.55 (bs, 1H), 7.67 (m, 2H), 7.22 (m, 1H), 4.56 (m, 1H), 3.91 (m, 2H), 3.47 (m, 2H), 3.29 – 3.17 (m, 4H), 2.67 (m, 1H), 2.06 – 1.56 (m, 14H), 1.43 (s, 11H), 1.35 (q, *J* = 7.1 Hz, 2H), 0.61 (m, 1H), 0.43 – 0.34 (m, 2H), 0.01 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{27}H_{47}N_7O_7$: 582.36097; found 582.36248. $[M+Na]^+$ calcd for $C_{27}H_{47}N_7O_7$: 604.34292; found 604.34452.



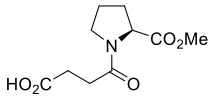
(1*R*,2*R*)-*N*-((*S*)-1-((2-Cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)-2-((*R*)-hydroxy((*S*)-pyrrolidin-2-yl)methyl)cyclopentanecarboxamide hydrochloride (19)
The title compound was obtained from **18** (51.2 mg, 88.00 µmol, 1.0 eq.) following general procedure **F** as a white foam (42.0 mg, 92%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.41 (m, 1H), 3.83 (dd, *J* = 9.5, 2.8 Hz, 1H), 3.69 (m, 1H), 3.40 – 3.26 (m, 6H), 2.77 (q, *J* = 8.1 Hz, 1H), 2.28 (p, *J* = 8.7 Hz, 1H), 2.13 – 1.96 (m, 5H), 1.81 (m, 9H), 1.44 – 1.40 (m, 3H), 0.73 (m, 1H), 0.51 – 0.41 (m, 2H), 0.09 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{22}H_{39}N_7O_5$: 482.30854; found 482.30957.



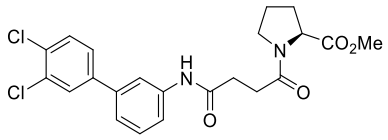
(*S*)-methyl 1-(4-(*tert*-Butoxy)-4-oxobutanoyl)pyrrolidine-2-carboxylate (22)
ProOMe*HCl **21** (151.0 mg, 0.90 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (3.0 mL) and the solution was cooled to 0 °C. Mono-*tert*-butyl succinate **20** (187.0 mg, 1.10 mmol, 1.2 eq.) and DIPEA (545.0 µL, 3.13 mmol, 3.5 eq.) were added, followed by HOBt (145.0 mg, 1.07 mmol, 1.2 eq.) and EDC (412.0 mg, 2.15 mmol, 2.4 eq.). The reaction mixture was stirred at 0°C for 10 min, then at rt for 14h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 1:1 to 4:6 hexanes/EtOAc obtaining the title compound as a colorless oil (247.0 mg, 97%).

^{1}H NMR (500 MHz, Chloroform-*d*, mixture of rotamers) δ 4.49 (dd, *J* = 8.7, 3.5 Hz, 1H), 3.75 (s, 0.6H), 3.70 (s, 2.4H), 3.68 – 3.59 (m, 1H), 3.55 (m, 1H), 2.69 – 2.47 (m, 4H), 2.27 (m, 0.4H), 2.17 (m, 1H), 2.11 – 1.94 (m, 2.3H), 1.89 (m, 0.3H), 1.43 (s, 9H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{14}H_{23}NO_5$ 286.16490; found 286.16513. $[M+Na]^+$ calcd for $C_{14}H_{23}NO_5$: 308.14684; found 308.14760.



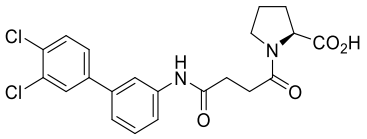
(*S*)-4-(2-(Methoxycarbonyl)pyrrolidin-1-yl)-4-oxobutanoic acid (23)
The title compound was obtained from **22** (229.0 mg, 0.80 mmol, 1.0 eq.) following general procedure **G** as a colorless oil (184.0 mg, 87%).

^{1}H NMR (500 MHz, Chloroform-*d*, mixture of rotamers) δ 8.44 (bs, 1H), 4.48 (m, 1H), 3.75 (s, 0.6H), 3.70 (s, 2.4H), 3.64 (m, 1H), 3.53 (m, 1H), 2.79 – 2.51 (m, 4H), 2.40 – 2.15 (m, 1.4H), 2.07 – 1.86 (m, 2.6H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{10}H_{15}NO_5$: 230.10230; found 230.10259. $[M+Na]^+$ calcd for $C_{10}H_{15}NO_5$: 252.08424; found 252.08483.



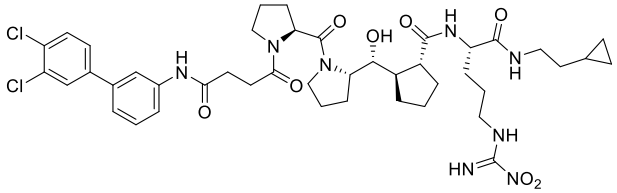
(*S*)-methyl 1-(4-((3',4'-Dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidine-2-carboxylate (24)
An amount of **23** (105.0 mg, 0.46 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (2.4 mL) and the solution was cooled to 0°C. 3',4'-dichloro-[1,1'-biphenyl]-3-amine (131.0 mg, 0.55 mmol, 1.2 eq.) and DIPEA (239.0 µL, 1.37 mmol, 3.0 eq.) were added, followed by HATU (264.0 mg, 0.69 mmol, 1.5 eq.). The reaction mixture was stirred at 0°C for 10 min, then at rt for 7h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 1:9 hexanes/EtOAc to afford **24** as a white foam (168.0 mg, 82%).

^{1}H NMR (500 MHz, Chloroform-*d*, mixture of rotamers) δ 8.97 (m, 1H), 7.78 (s, 0.14H), 7.75 (s, 0.86H), 7.62 (m, 1H), 7.47 – 7.42 (m, 2H), 7.36 (m, 1H), 7.32 – 7.27 (m, 1H), 7.19 – 7.13 (m, 1H), 4.50 (ddd, *J* = 12.6, 8.5, 3.1 Hz, 1H), 3.77 (s, 0.4H), 3.66 (s, 3.6H), 3.56 (dt, *J* = 9.8, 7.0 Hz, 1H), 2.88 – 2.56 (m, 3.86H), 2.52 – 2.44 (m, 0.14H), 2.33 – 2.25 (m, 0.14H), 2.19 (m, 0.86H), 2.12 – 2.04 (m, 1H), 2.03 – 1.97 (m, 1.7H), 1.90 (m, 0.3H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{22}H_{22}Cl_2N_2O_4$: 449.10294; found 449.10399. $[M+Na]^+$ calcd for $C_{22}H_{22}Cl_2N_2O_4$: 471.08488; found 471.08561.



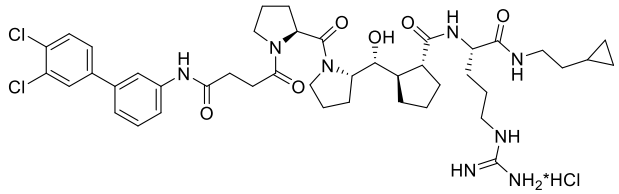
(*S*)-1-(4-((3',4'-Dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidine-2-carboxylic acid (25)
The title compound was obtained from **24** (102.0 mg, 0.23 mmol, 1.0 eq.) following general procedure **E**. The reaction mixture was acidified with 10% aq. citric acid, then extracted twice with EtOAc. The pooled organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure to afford **25** as a white foam (98.8 mg, q.y.).

^{1}H NMR (500 MHz, Chloroform-*d*, mixture of rotamers) δ 10.03 (bs, 1H), 9.32 (m, 0.2H), 9.22 (m, 0.8H), 7.72 (m, 1H), 7.52 (s, 1H), 7.42 (m, 1H), 7.34 (m, 1H), 7.18 (t, *J* = 7.9 Hz, 1H), 7.07 (m, 1H), 4.49 (dd, *J* = 8.3, 2.9 Hz, 0.16H), 4.40 (m, 0.84H), 3.56 (m, 1H), 3.41 (m, 1H), 2.89 – 2.56 (m, 4H), 2.14 – 1.77 (m, 4H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{21}H_{20}Cl_2N_2O_4$: 435.08729; found 435.08776. $[M+H]^+$ calcd for $C_{21}H_{20}Cl_2N_2O_4$: 457.06923; found 457.06938.



(1*R*,2*R*)-*N*-((*S*)-1-((2-Cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)-2-((*R*)-((*S*)-1-((*S*)-1-(4-((3',4'-dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidine-2-carbonyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarboxamide (26)
An amount of **25** (48.8 mg, 94.00 µmol, 1.0 eq.) and **19** (41.0 mg, 94.00 µmol, 1.0 eq.) were dissolved in CH_2Cl_2 (314 µL) and the solution was cooled to 0 °C. DIPEA (57.4 µL, 0.33 mmol, 3.5 eq.) was added, followed by HOBt (15.3 mg, 0.11 mmol, 1.2 eq.) and EDC (43.3 mg, 0.23 mmol, 2.4 eq.). The reaction mixture was stirred at 0°C for 10 min, then at rt for 14h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 93:7 CH_2Cl_2/MeOH to afford **26** as an amorphous solid (68.0 mg, 80%).

^{1}H NMR (500 MHz, Methanol-d_4, mixture of rotamers) δ 7.89 (m, 0.15H), 7.86 (m, 0.15H), 7.83 (m, 0.7H), 7.72 (t, *J* = 2.0 Hz, 0.3H), 7.71 (m, 0.7H), 7.56 – 7.45 (m, 3H), 7.35 (m, 1H), 7.28 (m, 1H), 4.91 (dd, *J* = 8.7, 3.1 Hz, 0.3H), 4.64 (m, 0.85H), 4.53 – 4.45 (m, 0.15H), 4.34 (m, 1H), 4.17 (m, 0.15H), 4.10 – 4.04 (m, 0.85H), 3.93 (m, 1H), 3.79 – 3.65 (m, 3H), 3.48 (m, 0.15H), 3.42 (m, 0.85H), 3.29 – 3.19 (m, 4H), 2.90 – 2.72 (m, 2H), 2.72 – 2.53 (m, 3H), 2.37 (m, 0.3H), 2.30 – 1.95 (m, 7.7H), 1.87 – 1.60 (m, 10H), 1.41 – 1.34 (m, 3H), 0.72 – 0.60 (m, 1H), 0.42 (m, 2H), 0.10 – -0.02 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{43}H_{57}Cl_2N_9O_8$: 898.37799; found 898.38163.



(1*R*,2*R*)-*N*-((*S*)-1-((2-Cyclopropylethyl)amino)-5-guanidino-1-oxopentan-2-yl)-2-((*R*)-((*S*)-1-((*S*)-1-(4-((3',4'-dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidine-2-carbonyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarboxamide hydrochloride (III)

10% Pd/C (33.0 mg, 31.00 µmol, 0.8 eq.) was added to a solution of **26** (33.0 mg, 37.00 µmol, 1.0 eq.) in 1:1 MeOH/EtOAc (0.02 M) and 6 M aq. HCl (2 drops), and the mixture was stirred under H_2 (atmospheric pressure) at rt for 4 h. The catalyst was removed by filtration and the solution was dried under reduced pressure to afford **III** as an amorphous solid (29.6 mg, 90%). The residue was purified by preparative HPLC (Atlantis dC18, 19x100m, 5µm - flow: 24 mL/min – gradient: A. H_2O (+0.1% formic acid)/ B. MeCN. 0min: 15%B, 10min: 75%B, 11.5min: 15%B, 15min: 15%B).

^{1}H NMR (700 MHz, Methanol-d_4) δ 8.55 (bs, 2H), 7.91 (m, 1H), 7.76 (d, *J* = 2.1 Hz, 1H), 7.59 (d, *J* = 8.4 Hz, 1H), 7.56 – 7.50 (m, 2H), 7.39 (m, 1H), 7.34 (m, 1H), 4.94 (dd, *J* = 8.7, 3.0 Hz, 0.2H), 4.71 – 4.61 (m, 0.8H), 4.34 – 4.26 (m, 1H), 4.20 – 4.07 (m, 1H), 3.93 (m, 1H), 3.85 – 3.58 (m, 3H), 3.57 – 3.42 (m, 1H), 3.24 (m, 3H), 3.19 – 3.13 (m, 1H), 2.91 – 2.73 (m, 2H), 2.71 – 2.54 (m, 3H), 2.25 (m, 1H), 2.20 – 1.99 (m, 6H), 1.90 – 1.75 (m, 6H), 1.67 (m, 5H), 1.46 (m, 1H), 1.39 (m, 2H), 0.71 – 0.64 (m, 1H), 0.47 – 0.40 (m, 2H), 0.05 (m, 2H).

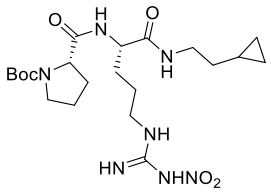
^{13}C NMR (176 MHz, MeOD) δ 179.2, 179.1, 173.9, 173.8, 173.4, 173.3, 173.3, 173.0, 172.9, 172.8, 172.7, 172.3, 170.2, 158.6, 158.6, 142.7, 142.6, 140.8, 140.8, 140.8, 140.5, 140.4, 133.8, 133.8, 132.5, 132.4, 132.0, 132.0, 130.6, 130.6, 129.8, 129.8, 127.8, 127.8, 123.5, 123.4, 120.7, 120.7, 120.6, 119.4, 119.4, 119.3, 78.5, 76.1, 75.9, 62.6, 62.4, 61.6, 60.8, 60.0, 59.9, 54.0, 54.0, 54.0, 51.4, 51.3, 51.3, 42.0, 40.7, 40.7, 35.5, 35.5, 32.6, 32.3, 32.2, 31.9, 31.4, 31.3, 31.2, 30.9, 30.8, 30.7, 30.4, 30.4, 30.3, 30.3, 30.3, 30.1, 30.0, 26.8, 26.8, 26.7, 26.5, 26.4, 26.4, 26.3, 26.2, 25.7, 25.7, 24.5, 24.4, 24.3, 23.6, 9.5, 9.4, 4.7, 4.7.

HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{43}H_{58}Cl_2N_8O_6$: 853.39291; found 853.39191. $[M+Na]^+$ calcd for $C_{43}H_{58}Cl_2N_8O_6$: 875.37486; found 875.37126.

HPLC: 99% with by-products:

- 94% (**III**) (96:4 mixture of isomer)
- 4% (**III**) as a mono-chlorinated analog
- 1% (**III**) as a dechlorinated analog

I.3.5 Synthesis of ***IV***

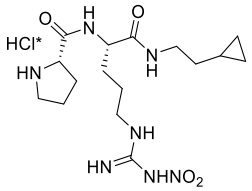


(*S*)-*tert*-Butyl 2-(((*S*)-1-((2-cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)carbamoyl)pyrrolidine-1-carboxylate (28)

BocProOH **27** (64.5 mg, 0.30 mmol, 1.2 eq.) was dissolved in CH_2Cl_2 (1.3 mL) and the solution was cooled to 0°C. **15** (100.0 mg, 0.25 mmol, 1.0 eq.) and DIPEA (187.0 µL, 1.07 mmol, 4.3 eq.) were added, followed by PyBOP (195.0 mg, 0.38 mmol, 1.5 eq.). The reaction mixture was stirred at 0°C for 10 min, then at rt for 14h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 97:3 to 92:8 CH_2Cl_2/MeOH to afford **28** as a white foam (68.4 mg, 56%).

^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.67 (m, 1H), 8.24 – 7.33 (m, 3.5H), 6.81 (m, 0.5H), 4.75 (bs, 0.25H), 4.56 (m, 0.75H), 4.33 (bs, 0.75H), 4.15 (bs, 0.25H), 3.52 – 3.33 (m, 3H), 3.30 – 3.08 (m, 3H), 2.10 (m, 1H), 2.04 – 1.53 (m, 7H), 1.41 (m, 9H), 1.37 – 1.30 (m, 2H), 0.62 (m, 1H), 0.39 (dd, *J* = 7.8, 5.4 Hz, 2H), 0.01 (m, 2H).

HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{21}H_{37}N_7O_6$: 484.28781; found 484.28746. $[M+Na]^+$ calcd for $C_{21}H_{37}N_7O_6$: 506.26975; found 506.27039.

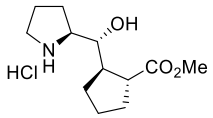


(*S*)-*N*-((*S*)-1-((2-Cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)pyrrolidine-2-carboxamide hydrochloride (29)

The title compound was obtained from **28** (52.0 mg, 0.11 mmol, 1.0 eq.) following general procedure **F** as a white powder (42.4 mg, 94%).

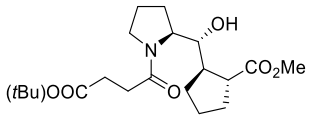
^{1}H NMR (500 MHz, Methanol-d_4) δ 4.38 (dd, *J* = 8.5, 5.3 Hz, 2H), 3.46 – 3.33 (m, 2H), 3.28 – 3.18 (m, 3H), 2.47 (h, *J* = 7.6 Hz, 1H), 2.06 (m, 3H), 1.93 – 1.65 (m, 4H), 1.40 – 1.36 (m, 2H), 0.75 – 0.65 (m, 1H), 0.46 – 0.39 (m, 2H), 0.06 (td, *J* = 4.9, 3.0 Hz, 2H).

HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{16}H_{29}N_7O_4$: 384.23538; found 384.23579. $[M+Na]^+$ calcd for $C_{16}H_{29}N_7O_4$: 406.21732; found 406.21743.



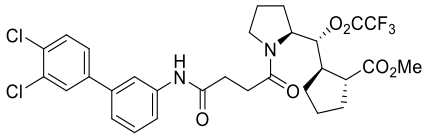
(1*R*,2*R*)-Methyl 2-((*R*)-hydroxy((*S*)-pyrrolidin-2-yl)methyl)cyclopentanecarboxylate hydrochloride (16a)
The title compound was obtained from **16**[1] (70.9 mg, 0.22 mmol, 1.0 eq.) following the general procedure **F** as a colorless oil (57.1 mg, q.y.).

^{1}H NMR (400 MHz, Methanol-d_4) δ 3.71 – 3.59 (m, 5H), 3.27 (m, 2H), 2.79 (dt, *J* = 9.3, 7.3 Hz, 1H), 2.38 – 2.27 (m, 1H), 2.12 – 1.94 (m, 5H), 1.91 – 1.66 (m, 4H), 1.37 (m, 1H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{12}H_{21}NO_3$: 228.15942; found 228.16012.



(1*R*,2*R*)-Methyl 2-((*R*)-((*S*)-1-(4-(*tert*-butoxy)-4-oxobutanoyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarboxylate (30)
An amount of **16a** (57.0 mg, 0.22 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (723.0 µL) and the solution was cooled to 0 °C. Mono-*tert*-butyl succinate **20** (45.4 mg, 0.26 mmol, 1.2 eq.) and DIPEA (132.0 µL, 0.76 mmol, 3.5 eq.) were added, followed by HOBt (35.2 mg, 0.26 mmol, 1.2 eq.) and EDC (99.8 mg, 0.52 mmol, 2.4 eq.). The reaction mixture was stirred at 0°C for 10 min, then at rt for 14h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 4:6 to 3:7 hexanes/EtOAc to afford **30** as a white solid (77.4 mg, 93%) which was recrystallized from 1:3 EtOAc/hexanes as colourless needles.

m.p. = 110.6-111.4 °C
^{1}H NMR (500 MHz, Chloroform-*d*) δ 4.11 (ddd, *J* = 8.1, 5.8, 1.9 Hz, 1H), 4.03 (dd, *J* = 9.4, 1.9 Hz, 1H), 3.66 (s, 3H), 3.54 (m, 1H), 3.44 (dt, *J* = 10.0, 7.3 Hz, 1H), 2.67 – 2.60 (m, 2H), 2.59 – 2.43 (m, 3H), 2.27 – 2.19 (q, *J* = 8.2 Hz, 1H), 2.02 (m, 2H), 1.96 – 1.84 (m, 2H), 1.83 – 1.72 (m, 3H), 1.70 – 1.62 (m, 2H), 1.49 (dq, *J* = 12.3, 8.5 Hz, 1H), 1.43 (s, 9H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{20}H_{33}NO_6$: 384.23806; found 384.24013. $[M+Na]^+$ calcd for $C_{20}H_{33}NO_6$: 406.22001, found 406.21972.



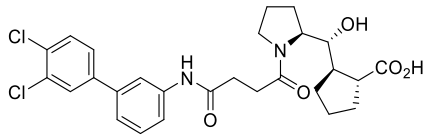
(1*R*,2*R*)-Methyl 2-((*R*)-((*S*)-1-(4-((3',4'-dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidin-2-yl)(trifluoroacetoxy)methyl)cyclopentanecarboxylate (31)

An amount of **30** (70.0 mg, 0.21 mmol, 1.0 eq.) was submitted to general procedure **G**. The crude contained a mixture of 4-((*S*)-2-((*R*)-hydroxy((1*R*,2*R*)-2-(methoxycarbonyl)cyclopentyl)methyl)pyrrolidin-1-yl)-4-oxobutanoic acid and 4-((S)-2-((R)-trifluoroacetoxy((1*R*,2*R*)-2-(methoxycarbonyl)cyclopentyl)methyl)pyrrolidin-1-yl)-4-oxobutanoic acid and was submitted to the next step without further purification.

The mixture of acids (0.21 mmol, 1.0 eq.) was dissolved in CH_2Cl_2 (1.1 mL) and the solution was cooled to 0°C. 3',4'-dichloro-[1,1'-biphenyl]-3-amine (61.1 mg, 0.26 mmol, 1.2 eq.) and DIPEA (112.0 µL, 0.64 mmol, 3.0 eq.) were added, followed by HATU (123.0 mg, 0.32 mmol, 1.5 eq.). The reaction mixture was stirred at 0°C for 10 min, then at rt for 7h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 2:8 hexanes/EtOAc to 100% EtOAc, and then 95:5 CH_2Cl_2/MeOH to afford **31** as a white foam (84.9 mg, 62%) and a fraction containing tetramethylurea byproduct in mixture with (1*R*,2*R*)-methyl 2-((*R*)-((*S*)-1-(4-((3',4'-dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarboxylate (17.0 mg, 14%, calculated by ^{1}H NMR).

^{1}H NMR (500 MHz, Methanol-*d*$_4$) δ 7.90 (t, *J* = 2.0 Hz, 1H), 7.75 (d, *J* = 2.1 Hz, 1H), 7.59 – 7.50 (m, 3H), 7.38 (t, *J* = 7.9 Hz, 1H), 7.32 (dt, *J* = 7.9, 1.3 Hz, 1H), 5.73 (dd, *J* = 10.6, 2.0 Hz, 0.9H), 5.60 – 5.53 (m, 0.1H), 4.30 (ddd, *J* = 7.7, 5.5, 1.9 Hz, 1H), 3.74 – 3.64 (m, 1H), 3.61 (m, 3H), 3.51 – 3.45 (m, 0.1H), 3.37 (ddd, *J* = 10.3, 7.4, 6.1 Hz, 1H), 2.81 – 2.46 (m, 6H), 2.12 – 2.04 (m, 2H), 2.04 – 1.90 (m, 4H), 1.79 – 1.72 (m, 3H), 1.63 – 1.54 (m, 1H).

HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{30}H_{31}Cl_2F_3N_2O_6$: 643.15840; found 643.15949. $[M+Na]^+$ calcd for $C_{30}H_{31}Cl_2F_3N_2O_6$: 665.14035; found 665.14106.

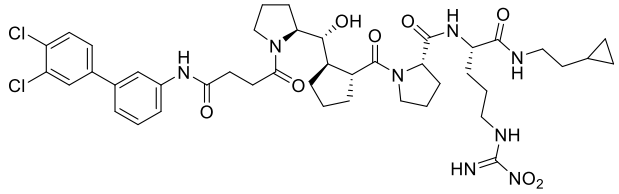


(1*R*,2*R*)-2-((*R*)-((*S*)-1-(4-((3',4'-Dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarboxylic acid (32)

An amount of **31** (86.0 mg, 0.13 mmol, 1.0 eq.) was dissolved in THF (389.0 µL) and LiOH (389.0 µL, 1.0M in H_2O, 3.0 eq.) was added at 0 °C. The reaction was stirred at 0°C for 5 min then at rt for 1h. The reaction mixture was acidified with 10% aq. citric acid, then extracted twice with EtOAc. The pooled organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The obtained crude was purified by column chromatography on SiO_2 gel with 95:5 CH_2Cl_2/MeOH to afford **32** as a colourless oil (48.0 mg, 69%).

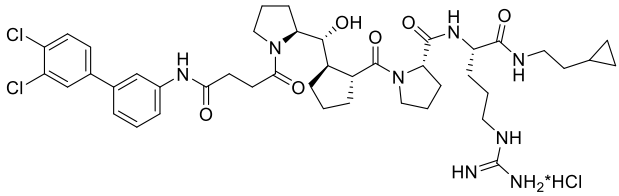
^{1}H NMR (500 MHz, Chloroform-*d*) δ 9.18 (s, 0.9H), 8.99 (s, 0.1H), 7.69 – 7.61 (m, 1H), 7.56 (d, *J* = 2.1 Hz, 0.1H), 7.52 (d, *J* = 2.2 Hz, 0.9H), 7.49 – 7.44 (m, 1H), 7.40 (d, *J* = 8.3 Hz, 1H), 7.27 (m, 0.5H), 7.25 (m, 0.5H), 7.23 (m, 1H), 7.13 (m, 1H), 4.15 – 4.06 (m, 1.8H), 3.97 (m, 0.1H), 3.84 (d, *J* = 10.2 Hz, 0.1H), 3.73 (m, 0.1H),

3.72 (m, 0.1H), 3.50 (m, 1.8.H), 3.29 (m, 0.1H), 2.91 – 2.73 (m, 1.8H), 2.67 (m, 1.2H), 2.57 (m, 1H), 2.46 (td, *J* = 8.7, 6.2 Hz, 1H), 2.08 – 1.92 (m, 4H), 1.88 – 1.65 (m, 3H), 1.59 (m, 1H), 1.49 (m, 2H), 1.26 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for for $C_{27}H_{30}Cl_2N_2O_5$: 533.16045; found 533.16090. $[M+Na]^+$ calcd for for $C_{27}H_{30}Cl_2N_2O_5$: 555.14240; found 555.14295.



(*S*)-*N*-((*S*)-1-((2-Cyclopropylethyl)amino)-5-(3-nitroguanidino)-1-oxopentan-2-yl)-1-((1*R*,2*R*)-2-((*R*)-((*S*)-1-(4-((3',4'-dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarbonyl)pyrrolidine-2-carboxamide (33)
An amount of **32** (47.8 mg, 90.00 µmol, 1.0 eq.) and **29** (37.6 mg, 90.00 µmol, 1.0 eq.) were dissolved in CH_2Cl_2 (299 µL) and the solution was cooled to 0 °C. DIPEA (54.6 µL, 0.31 mmol, 3.5 eq.) was added, followed by HOBt (14.5 mg, 0.11 mmol, 1.2 eq.) and EDC (41.2 mg, 0.22 mmol, 2.4 eq.). The reaction mixture was stirred at 0 °C for 10 min, then at rt for 14h. Reaction mixture was diluted with CH_2Cl_2, washed with 10% aq. citric acid, 10% aq. $NaHCO_3$, and brine. The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent removed under reduced pressure. The crude was purified by column chromatography on SiO_2 gel with 93:7 CH_2Cl_2/MeOH to afford **33** as an amorphous solid (56 mg, 70%).

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.89 (t, *J* = 1.9 Hz, 0.1H), 7.84 (m, 0.9H), 7.72 (m, 0.3H), 7.70 (d, *J* = 2.1 Hz, 0.7H), 7.56 – 7.46 (m, 3H), 7.39 – 7.33 (m, 1H), 7.30 (m, 1H), 4.51 (dd, *J* = 8.6, 2.5 Hz, 0.1H), 4.42 – 4.35 (m, 0.9H), 4.32 (dd, *J* = 8.8, 4.8 Hz, 1H), 4.12 – 4.05 (m, 1H), 3.98 (dd, *J* = 10.2, 1.9 Hz, 1H), 3.84 (m, 0.1H), 3.80 – 3.44 (m, 4H), 3.29 – 3.16 (m, 4H), 2.91 (q, *J* = 8.1 Hz, 0.1H), 2.86 – 2.63 (m, 4.9H), 2.33 – 2.24 (m, 1H), 2.16 – 1.59 (m, 17H), 1.45 – 1.34 (m, 3H), 0.74 – 0.62 (m, 1H), 0.48 – 0.37 (m, 2H), 0.04 (m, 2H).
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{43}H_{57}Cl_2N_9O_8$ 898.37799; found 898.37767. $[M+Na]^+$ calcd for $C_{43}H_{57}Cl_2N_9O_8$: 920.35994; found 920.35885.



(*S*)-*N*-((*S*)-1-((2-Cyclopropylethyl)amino)-5-guanidino-1-oxopentan-2-yl)-1-((1*R*,2*R*)-2-((*R*)-((*S*)-1-(4-((3',4'-dichloro-[1,1'-biphenyl]-3-yl)amino)-4-oxobutanoyl)pyrrolidin-2-yl)(hydroxy)methyl)cyclopentanecarbonyl)pyrrolidine-2-carboxamide hydrochloride (IV)
10% Pd/C (38.0 mg, 0.36 µmol, 0.9 eq.) was added to a solution of **33** (38.0 mg, 42.00 µmol, 1.0 eq.) in 1:1 MeOH/EtOAc and 6 M aq. HCl (2 drops), and the mixture was stirred under H_2 (atmospheric pressure) at rt for 4 h. The catalyst was removed by filtration and the solution was concentrated under reduced

pressure to afford **IV** as an amorphous solid (34.3 mg, 91%). The residue was purified by preparative HPLC (Atlantis dC18, 19x100m, 5µm - flow: 24 mL/min – gradient: A. H_2O (+0.1% formic acid)/ B. MeCN. 0min: 25%B, 10min: 65%B, 11.5min: 25%B, 15min: 25%B).

^{1}H NMR (500 MHz, Methanol-d_4) δ 7.93 – 7.82 (m, 1H), 7.76 (m, 1H), 7.67 – 7.48 (m, 3H), 7.40 (m, 1H), 7.35 (m, 1H), 4.38 (dd, *J* = 8.3, 4.4 Hz, 1H), 4.31 (dd, *J* = 8.8, 4.6 Hz, 1H), 4.09 (m, 1H), 4.00 (m, 0.8H), 3.86 (m, 0.2H), 3.81 – 3.58 (m, 3H), 3.51 (m, 1H), 3.28 – 3.15 (m, 4H), 2.99 – 2.61 (m, 5H), 2.33 – 2.25 (m, 1H), 2.19 – 1.79 (m, 11.5H), 1.68 (, 5.5H), 1.54 – 1.46 (m, 1H), 1.42 – 1.35 (m, 2H), 0.69 (m, 1H), 0.47 – 0.40 (m, 2H), 0.06 (d, *J* = 4.8 Hz, 2H).
^{13}C NMR (126 MHz, MeOD) δ 178.7, 174.9, 173.8, 173.5, 158.5, 142.5, 140.5, 140.3, 133.7, 132.4, 132.0, 130.6, 129.8, 127.9, 127.8, 123.6, 120.9, 119.6, 75.4, 62.7, 61.9, 54.1, 49.8, 49.7, 42.0, 40.7, 35.4, 32.7, 32.4, 31.9, 30.8, 30.7, 30.4, 30.2, 26.6, 26.3, 26.2, 25.9, 24.7, 23.6, 14.4, 9.4, 4.7, 4.7.
HRMS (ESI-TOF) m/z: $[M+H]^+$ calcd for $C_{43}H_{58}Cl_2N_8O_6$ 853.39291; found 853.39359. $[M+Na]^+$ calcd for $C_{43}H_{58}Cl_2N_8O_6$: 875.37486; found 875.37496.
HPLC: 95% with by-products:

- 92% (**IV**)
- 3% (**IV**) as a mono-chlorinated analog

I.4 Biological and Structural Data

I.4.1 Alanine Scan

The binding constant of the alanine modified peptides were determined by SPR. **ΔG_{bind}** is computed from **K_D**. **ΔΔG** is the binding free energy difference between the unmodified peptide and the a alanine derivatives.

Name	K_D	EC_{50}	ΔG_{bind} (kj/mol)	ΔΔG (kj/mol)	Sequence
Unmodified	2.14E-07	1.33E-07	-39.58	0	PPSNPPPRPPAEARKK
Ala(153)	2.35E-07	1.99E-07	-39.34	0.24	PPANPPPRPPAEARKK
Ala(154)	2.76E-07	3.23E-07	-38.93	0.66	PPSAPPPRPPAEARKK
Ala(155)	4.48E-06	1.18E-06	-31.74	7.84	PPSNAPPRPPAEARKK
Ala(156)	3.33E-05	2.33E-05	-26.57	13.01	PPSNPAPRPPAEARKK
Ala(157)	1.64E-06	1.47E-06	-34.33	5.25	PPSNPPARPPAEARKK
Ala(158)	1.99E-04	1.64E-04	-21.96	17.62	PPSNPPPAPPAEARKK
Ala(159)	1.02E-07	5.14E-08	-41.49	-1.91	PPSNPPPRAPAEARKK

Ala(160)	2.62E-06	1.52E-06	-33.12	6.46	PPSNPPPRPAAEARKK
Ala(161)	1.97E-07	8.40E-08	-39.79	-0.21	PPSNPPPRPPAAARKK
I	5.71E-06	1.91E-06	-29.91	-9.67	n/a
II	4.25E-08	6.69E-7	-42.06	2.48	n/a
III	3.12E-07	nd	-37.12	-2.46	n/a
IV	nd	nd	nd	nd	n/a

I.4.2 X-Ray

PDB access code: 7YXW

Protein-peptide complex was prepared by mixing the protein with 5x molar excess of the peptide followed by overnight incubation at 4 °C and subsequent filtration using Durapore PVDF 0.1 µm centrifugal filters (Millipore).

Crystallization screening was performed using sitting drop vapor diffusion technique, 96-well 3-drop (or 2-drop) conical crystallization plates, Crystal Gryphon Nanodispender (Art Robbins Instruments) and commercially available crystallization kits (Molecular Dimensions, Hampton Research). Typically 100 nL of the prepared complex was mixed with 100 nL of the reservoir solution and equilibrated against the reservoir solution at 20 or 4 °C. The initial hits were further optimized using grid screening approach by systematic variation of pH, salt concentration and/or precipitant concentration. The diffracting crystals were obtained in 0.1 M HEPES pH 7.5, 1.2 M sodium citrate tribasic dihydrate at 4 °C and 1 : 1 complex : reservoir solution ration. The crystals were cryoprotected in mother liquor supplemented with 0.3 M sodium malonate pH 7.0 and flash-frozen in liquid nitrogen. All crystallographic data were collected at 100 K at the SOLEIL Synchrotron (Gif-sur-Yvette, France).

Data were indexed and integrated using XDS and scaled and merged in Aimless from the CCP4 package. The crystal structure of protein-peptide complex was determined by molecular replacement with two separate ensembles as search models (Ensemble 1 = residues 157-199 and Ensemble 2 = residues 226-283, both derived from PDB entry 1ng2). The solution contained one complex per asymmetric unit and had R_{work}/R_{free} values of 0.37/0.43. Upon obtaining the MR solution, alternate cycles of model building and refinement were performed using WinCoot and Refmac5, respectively, with 5% randomly selected reflections to monitor Rfree.

I.4.3 Modelling

Schrödinger Glide and LiveDesign was used to perform docking.[2] In-house X-ray structure of p47-inhibitor complex was used to construct docking grid in the rigid Glide SP docking protocol without constrains. The ligands were prepared by LigPrep at pH 7.4+/- 2.0. The docking yielded 3 poses per ligand. For gas phase optimization all atom EHT forcefield was used, in MOE. Visualizations were prepared with MOE.[3]

I.4.4 Surface Plasmon Resonance

Binding assay to p47 was performed by Surface Plasmon Resonance (SPR) on a Biacore T200 (GE Healthcare) at 20°C using CM5 chip (GE Healthcare). h-p47 6His-Thr-Tev-(151-285)(N166D), clived form acquired from Selvita was immobilized covalently by amine coupling using standard wizard protocol, at a concentration of 23 µg/mL in sodium acetate pH 4 (GE Healthcare Life Sciences). The ligand is injected for 300s at 5µl/min to obtain about 400 immobilized RU. Amine blank is used as the reference flow cell. Binding of analytes to the ligand was monitored in real time with associations of 60 seconds and a dissociation of 300 seconds. Association (ka) and dissociation (kd) rates were fitted with a simple 1:1 kinetic interaction model using Biacore T200 Evaluation Software (GE Healthcare). The equilibrium constant (KD=kd/ka) was calculated from these fitted parameters. In the experiment, a half-log dilution of analytes was injected over p47 in Single Cycle Kinetics (SCK) with 5 increasing concentrations of 100nM, 300nM, 1µM, 3µM and 10µM per cycle. The direct binding assay (DBA) was performed with a flow rate of 50µL/min using a running buffer of Bis Tris 25mM pH 6.5, 150mM NaCl, 0.05% P20, 5% DMSO. Double subtraction was used by subtracting both reference flow cell and previous blank cycle (with running buffer). Solvent correction is applied to correct DMSO bulk effect.

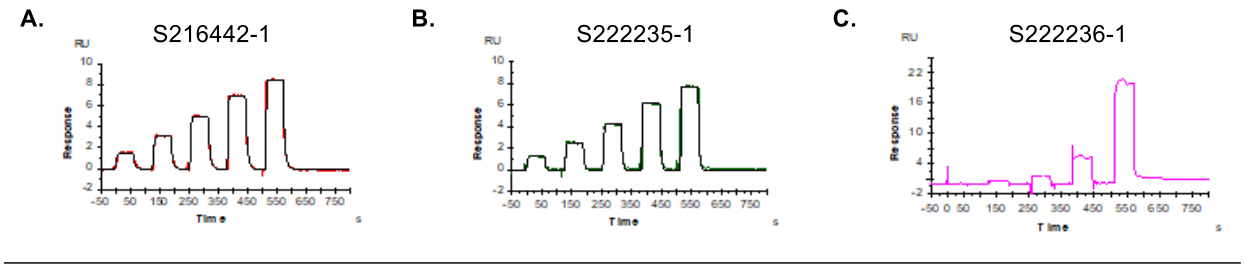


Figure SI1. Direct binding assay to p47 by SPR on Biacore T200 (GE Healthcare).

p47 was immobilized by amine coupling on a CM5 chip at a level of about 400 RU. Five concentrations of analytes were injected in a single kinetic cycle for 60s per concentration and with a dissociation of 300s at a flow rate of 50µl/min. All the experiments were performed in Bis Tris 25mM pH 6.5, 150mM NaCl, 0.05% P20, 5% DMSO as running buffer.

The analysis was double subtracted with a reference flow cell (blank immobilization) and the previous blank cycle with buffer, a solvent correction was applied. The fit used is a 1:1 kinetic interaction model with the Biacore T200 Evaluation Software. **A. II** (S216442-1) was used as the control (100% binding at Cmax). The range studied was 10nM, 30nM, 100nM, 300nM and 1µM. **B. III** (S222235-1) was injected at 30nM, 100nM, 300nM, 1µM and 3µM. It showed a binding similar to control. **C. IV** (S222236-1) was injected at 1µM, 3µM, 10µM, 30µM and 100µM. S222236-1 was not considered as a binder as it showed bad behavior at equilibrium and no saturation.

II. References and Notes

(1) Garsi, J.-B.; Aguiar, P. M.; Hanessian, S. Design of Pseudodiproline Dimers as Mimetics of Pro-Pro Units: Stereocontrolled Synthesis, Configurational Relevance, and Structural Properties. *J. Org. Chem.* **2021**. https://doi.org/10.1021/acs.joc.1c02061.

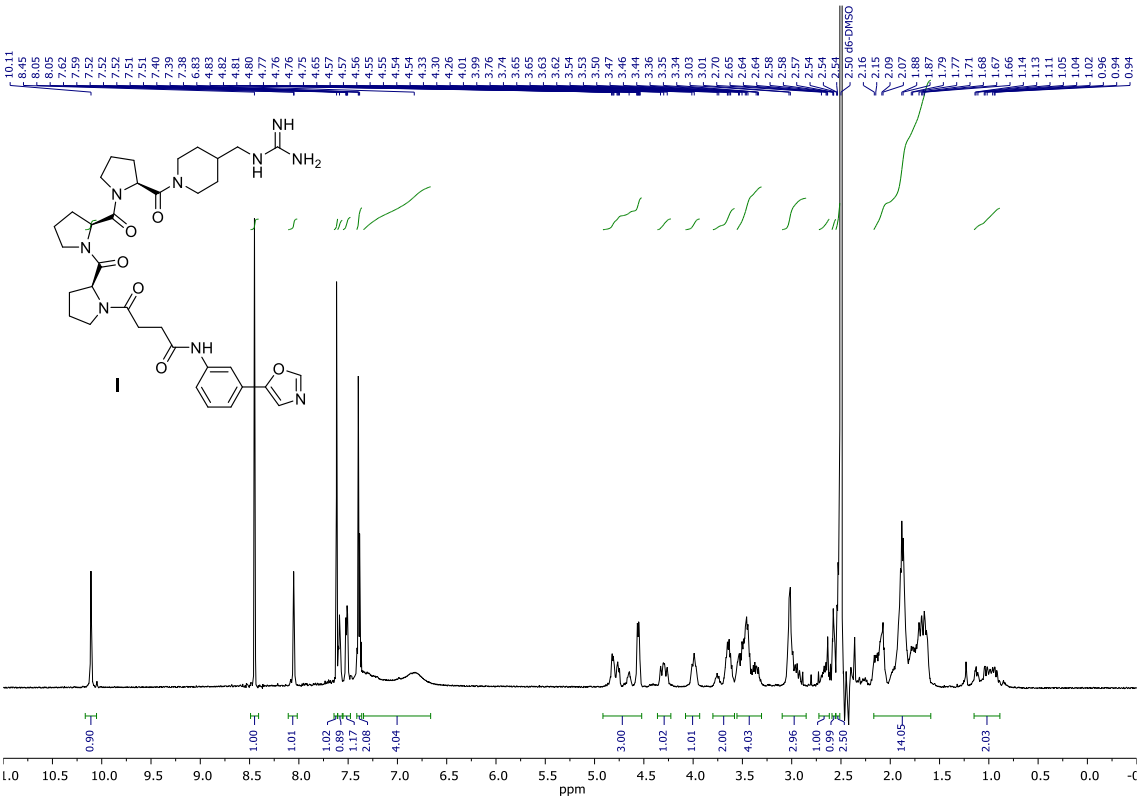
(2) Friesner, R. A.; Murphy, R. B.; Repasky, M. P.; Frye, L. L.; Greenwood, J. R.; Halgren, T. A.; Sanschagrin, P. C.; Mainz, D. T. Extra Precision Glide: Docking and Scoring Incorporating a Model

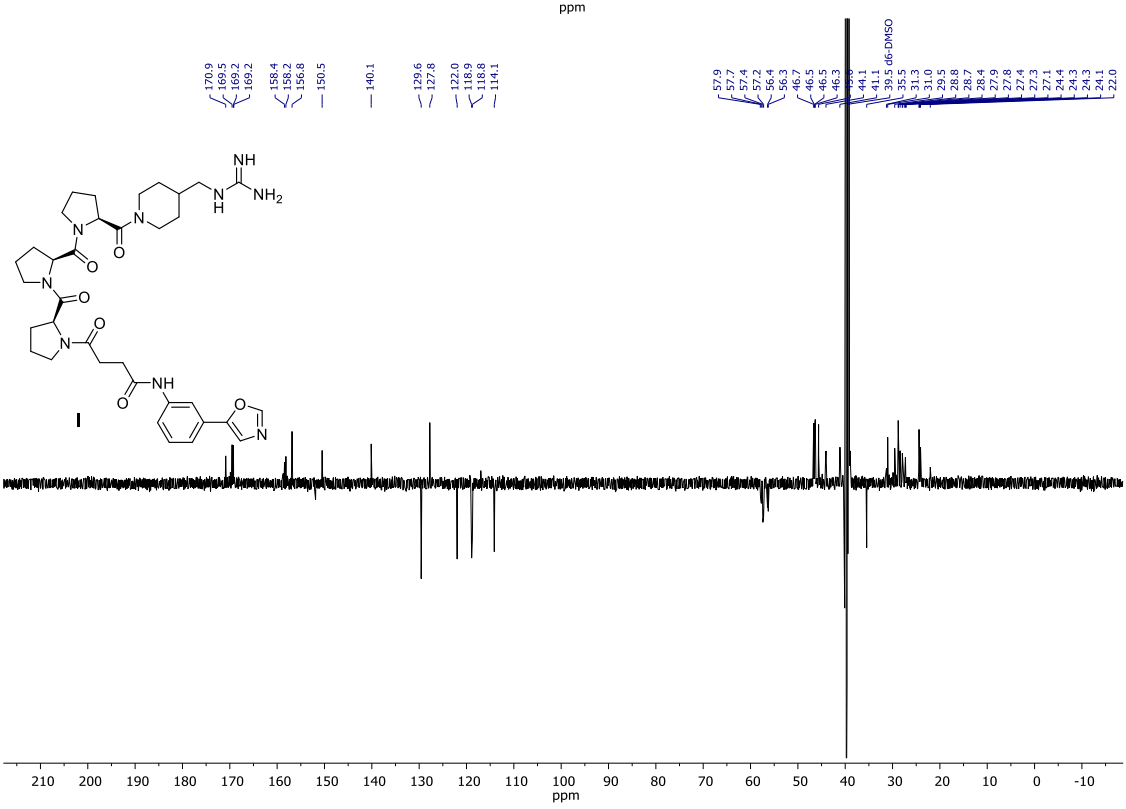
of Hydrophobic Enclosure for Protein-Ligand Complexes. *J. Med. Chem.* **2006**, *49*, 6177–6196. https://doi.org/10.1021/jm051256o.

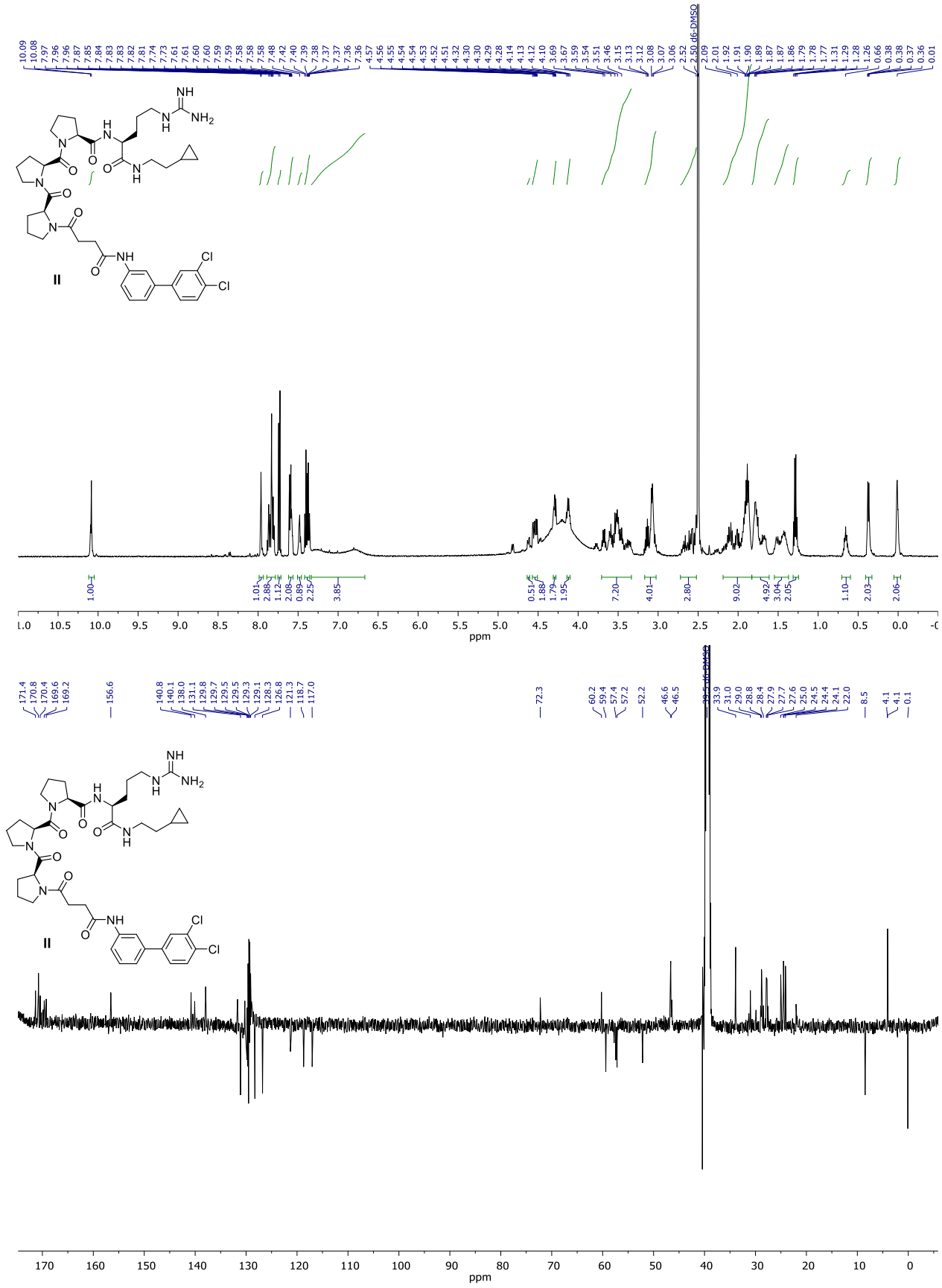
(3) Molecular Operating Environment (MOE), 2019.01; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2021.

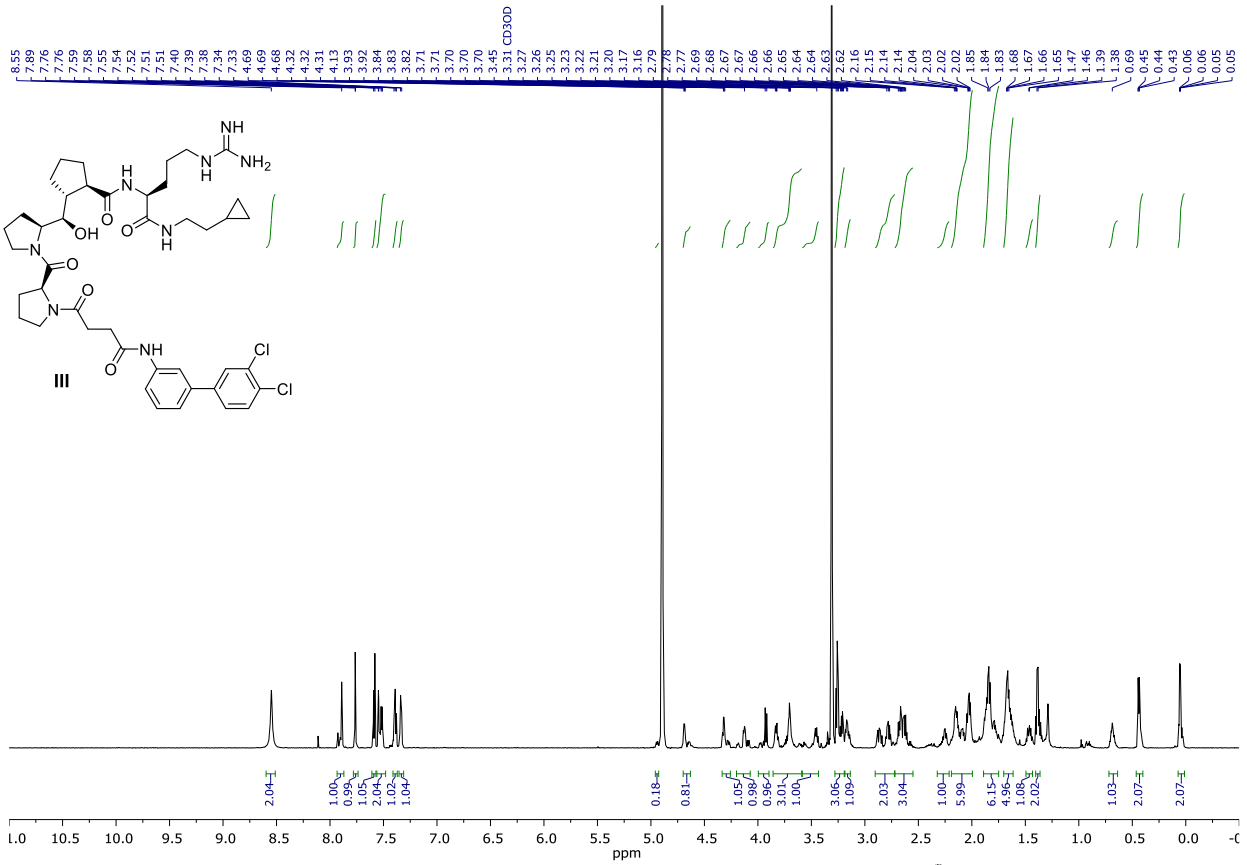
III. Associated Analytical Data

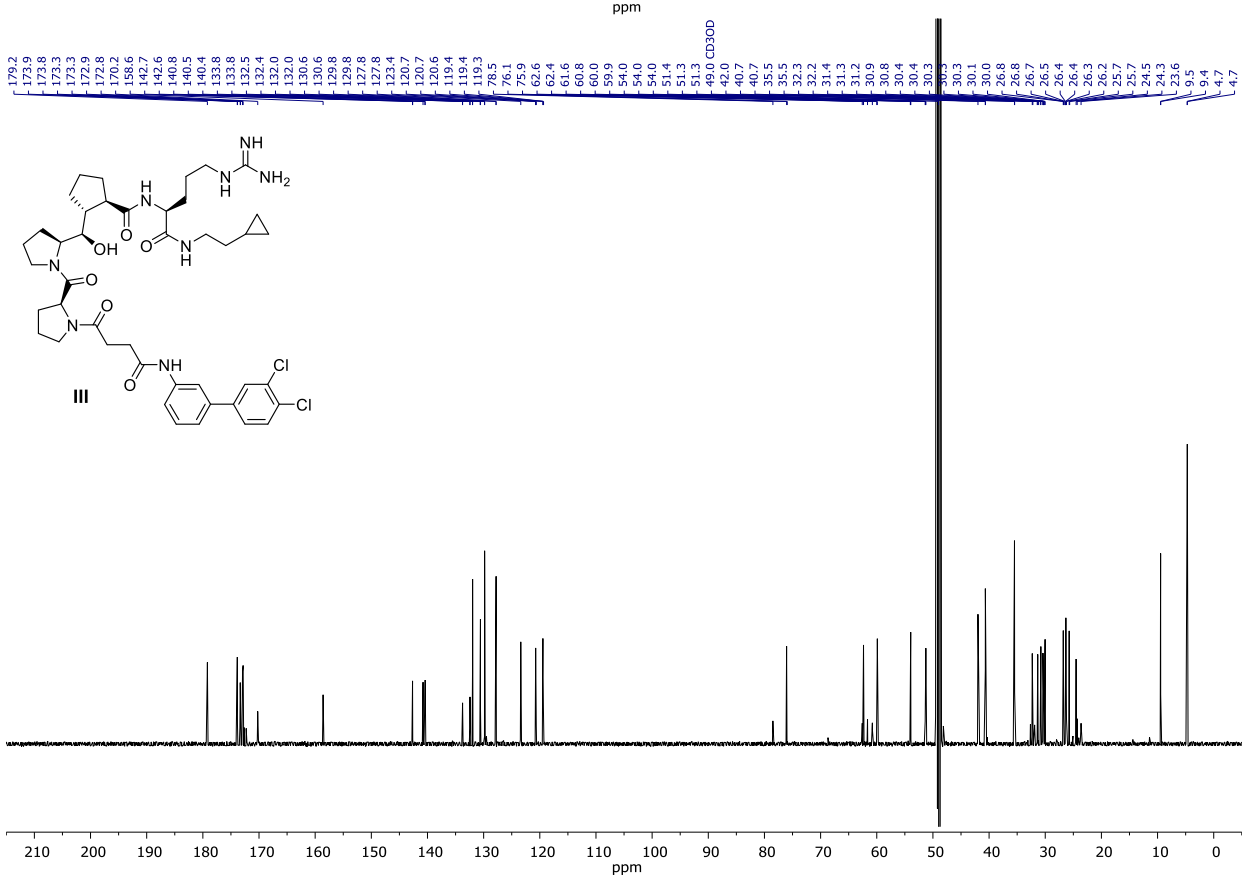
III.1 NMR Spectra of I – IV

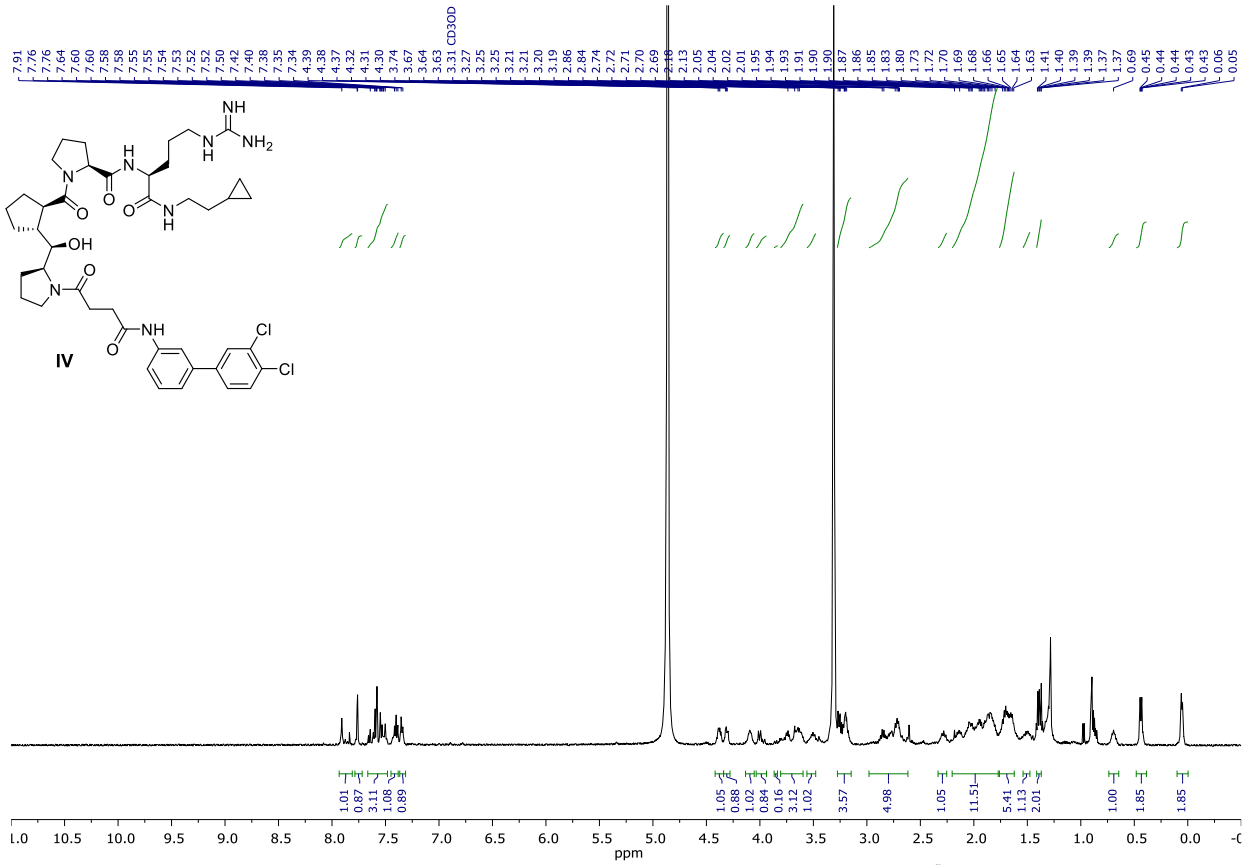


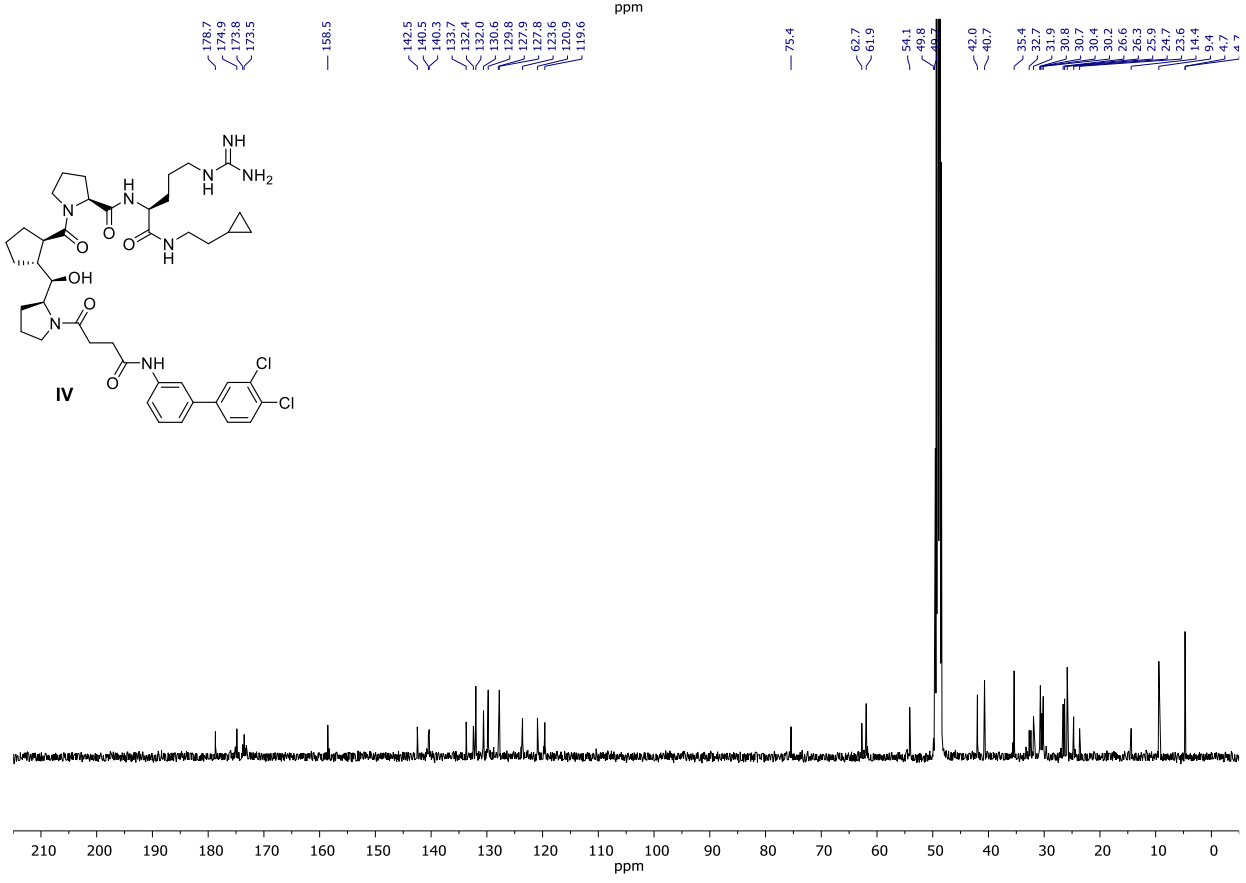



II
ppm
II
ppm


CD3OD
III
ppm

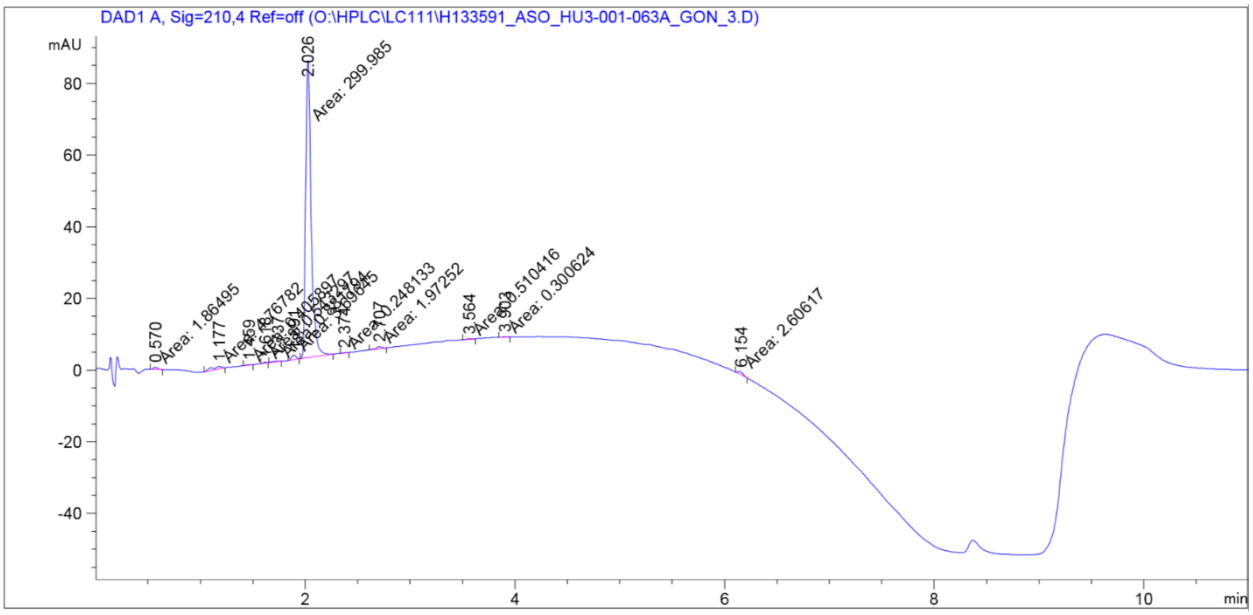

CD3OD
III
ppm


CD3OD
IV
ppm


IV
ppm

III.2 HPLC Traces of I-IV

I HPLC: Kinetex XB C18 2.1x50mm 2.6um.
Eluent: **A**: 100:5:0.05 H2O:ACN:TFA, **B**: 5:100:0.075 H2O:ACN:TFA, gradient: 0min_0%**B**, 7.5min_100%**B**, 8.5min_100%**B**, 8.6min_0%**B**, 10min_0%**B**. Flow rate = 1.0 mL/min, detection: UV = 210 nm.
tR = 2.03 min (94%, **I**)

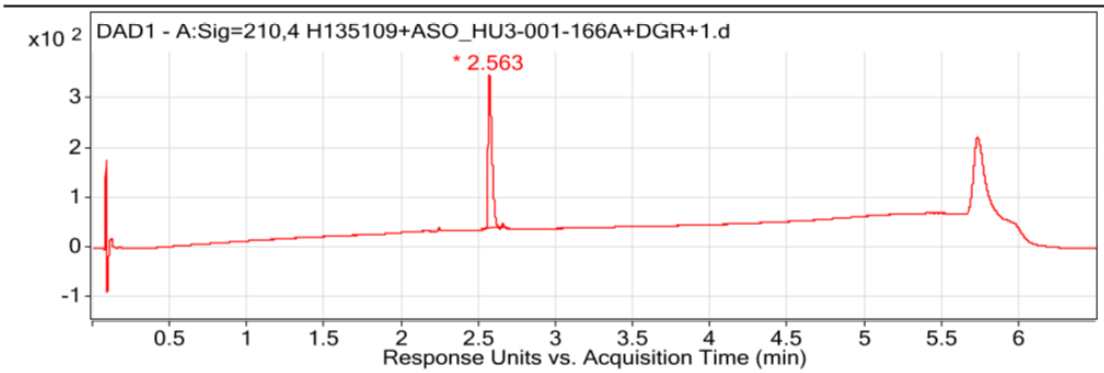


Peak #	RetTime [min]	Sig	Type	Area [mAU*s]	Height [mAU]	Area %
1	0.570	1	MM	1.86495	6.30526e-1	0.5869
2	1.177	1	MM	4.76782	6.83193e-1	1.5003
3	1.459	1	MM	4.05897e-1	1.45594e-1	0.1277
4	1.618	1	MM	5.43297e-1	2.47814e-1	0.1710
5	1.737	1	MM	8.81794e-1	1.33649e-1	0.2775
6	1.891	1	MM	3.69645	1.33579	1.1632
7	2.026	1	MM	299.98480	83.52110	94.3993
8	2.374	1	MM	2.48133e-1	8.82135e-2	0.0781
9	2.707	1	MM	1.97252	5.76373e-1	0.6207
10	3.564	1	MM	5.10416e-1	1.48588e-1	0.1606
11	3.903	1	MM	3.00624e-1	1.08682e-1	0.0946
12	6.154	1	MM	2.60617	8.03579e-1	0.8201
Totals :				317.78287	88.42311	

*** End of Report ***

II HPLC: Kinextex XB-C18 50 x 2.1 mm, 2.6 µm.
Eluent: **A**: 100:3:0.025 H_2O:IPA:NH_3HCO_2H, **B**: 95:5:3:0.025 MeCN:H_2O:IPA:NH_3HCO_2H, gradient: 0min_0%**B**, 4.5min_90%**B**, 5.5min_100%**B**, 5.6min_0%**B**, 6.5min_0%**B**. Flow rate = 0.7 mL/min, detection: UV = 210 nm.
tR = 2.56 min (98%, **II**)

User Chromatograms

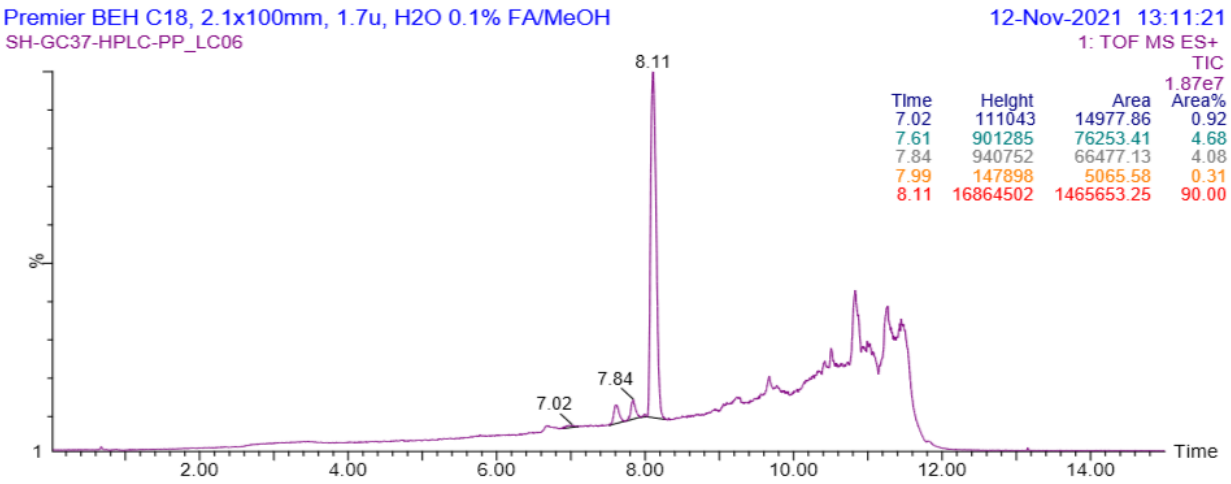


Integration Peak List

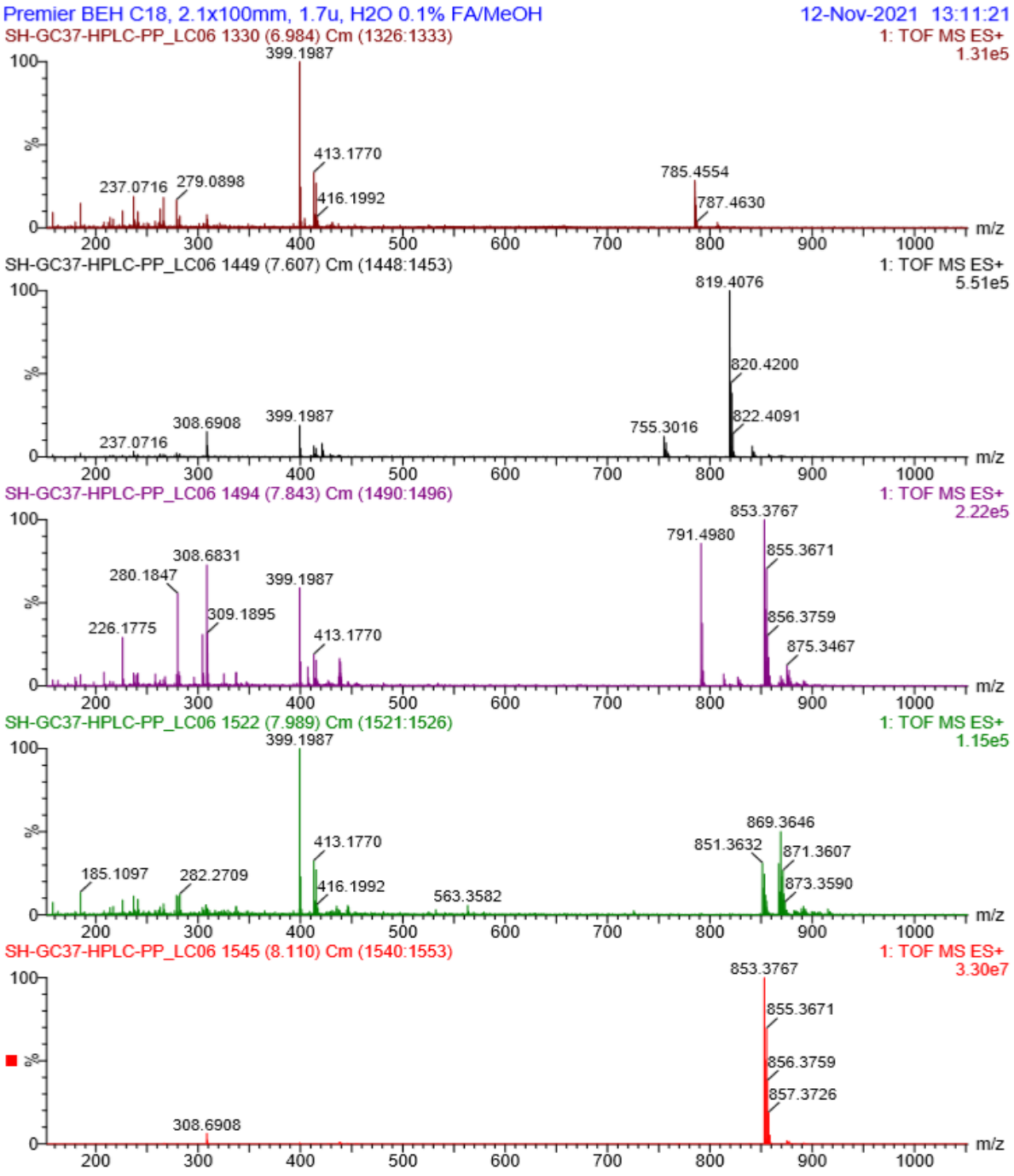
RT	Start	End	Height	Area	Area%
2.563	2.515	2.632	311.11	529.31	97.78
2.65	2.632	2.703	9.21	12.02	2.22

--- End Of Report ---

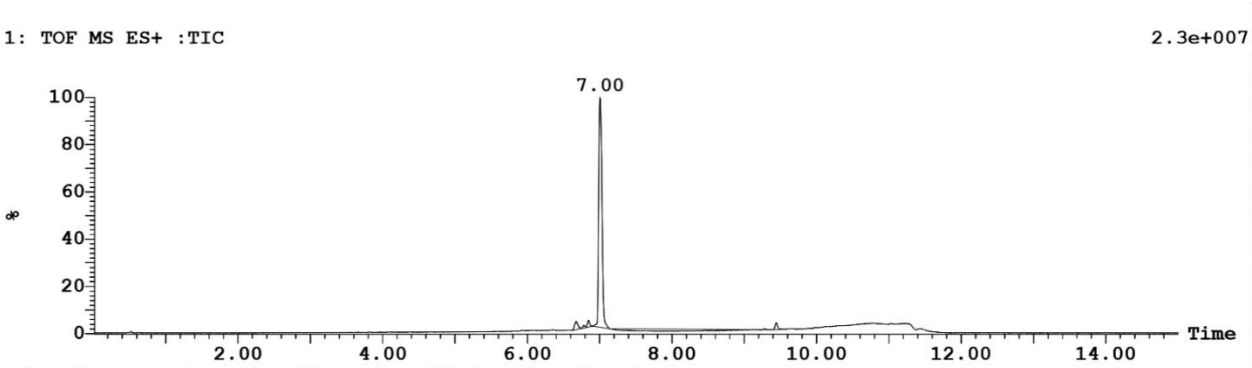
III HPLC: Premier BEH C18 2.1x100mm 1.7µm.
Eluent: H2O (0.1% formic acid)/MeOH = 30/70 to 90/10 gradient, flow rate = 0.5 mL/min, 15min, TIC+.
tR = 7.02 min (1%, dechlorinated byproduct), 7.61 min (5%, mono-chlorinated byproduct), 7.84 min (4%, **III** minor isomer), 8.11 min (90%, **III**)



MS spectrum

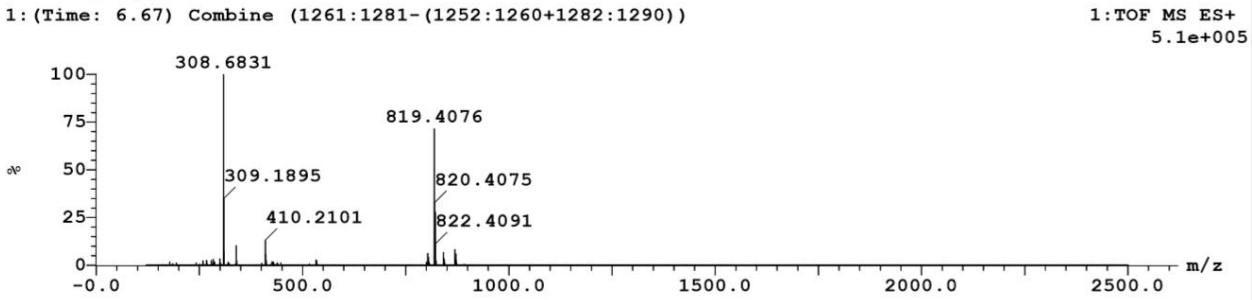

Premier BEH C18, 2.1x100mm, 1.7u, H2O 0.1% FA/MeOH
12-Nov-2021 13:11:21
SH-GC37-HPLC-PP_LC06 1330 (6.984) Cm (1326:1333)
1: TOF MS ES+
1.31e5
399.1987
413.1770
237.0716
279.0898
416.1992
785.4554
787.4630
SH-GC37-HPLC-PP_LC06 1449 (7.607) Cm (1448:1453)
1: TOF MS ES+
5.51e5
819.4076
820.4200
822.4091
308.6908
399.1987
237.0716
755.3016
SH-GC37-HPLC-PP_LC06 1494 (7.843) Cm (1490:1496)
1: TOF MS ES+
2.22e5
853.3767
791.4980
855.3671
308.6831
280.1847
399.1987
309.1895
856.3759
226.1775
413.1770
875.3467
SH-GC37-HPLC-PP_LC06 1522 (7.989) Cm (1521:1526)
1: TOF MS ES+
1.15e5
399.1987
869.3646
413.1770
851.3632
871.3607
185.1097
282.2709
416.1992
873.3590
563.3582
SH-GC37-HPLC-PP_LC06 1545 (8.110) Cm (1540:1553)
1: TOF MS ES+
3.30e7
853.3767
855.3671
856.3759
857.3726
308.6908
%
m/z
100
0
200
300
400
500
600
700
800
900
1000

IV HPLC: Premier BEH C18 2.1x100mm 1.7µm.
H2O (0.1% formic acid)/MeCN = 5/95 to 75/25 gradient, flow rate = 0.5 mL/min, 15min, TIC+.
tR = 6.85 min (3%, mono-chlorinated byproduct), 7.00 min (92%, **IV**)

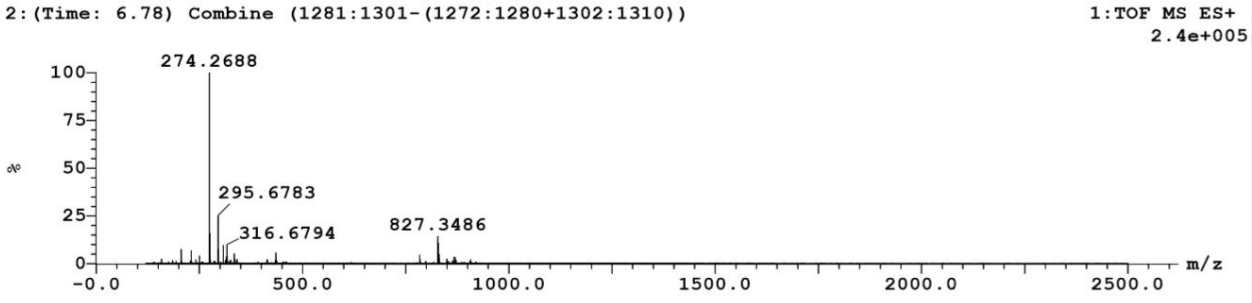


Peak Number	Time	AreaAbs	Area %Total	Mass Found
1	6.67	4e+004	3.24	
2	6.78	9e+003	0.70	
3	6.85	2e+004	1.73	
4	7.00	1e+006	92.25	
5	9.44	3e+004	2.09	

Peak ID	Time	Mass Found
1	6.67	



Peak ID	Time	Mass Found
2	6.78	



Peak ID	Time	Mass Found
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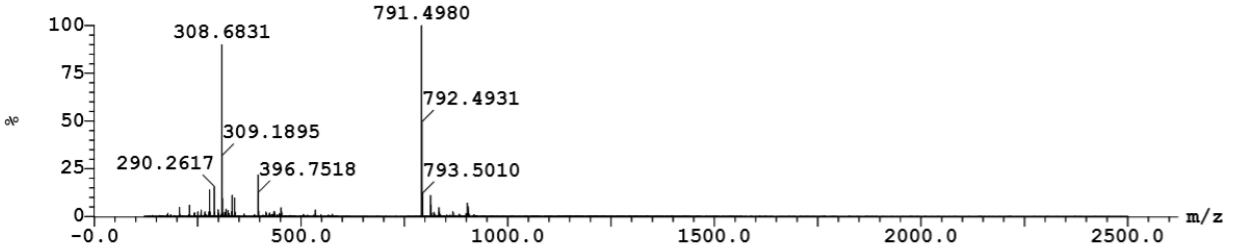
3:(Time: 6.85) Combine (1294:1314-(1285:1293+1315:1323))

1:TOF MS ES+
2.8e+005



Peak ID	Time	Mass Found
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4:(Time: 7.00) Combine (1324:1344-(1315:1323+1345:1353))

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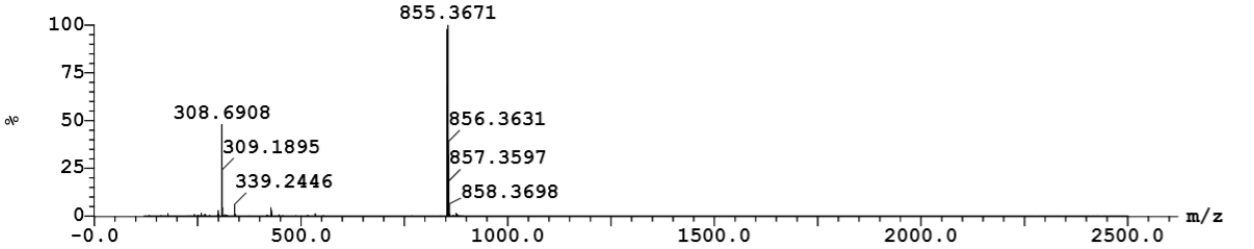
2.1e+007



Peak ID	Time	Mass Found
5	9.44	

5:(Time: 9.44) Combine (1789:1809-(1780:1788+1810:1818))

1:TOF MS ES+
4.7e+005