Design, Synthesis and Biological Evaluation of Allosteric Effectors That Enhance CO Release from Carboxyhemoglobin

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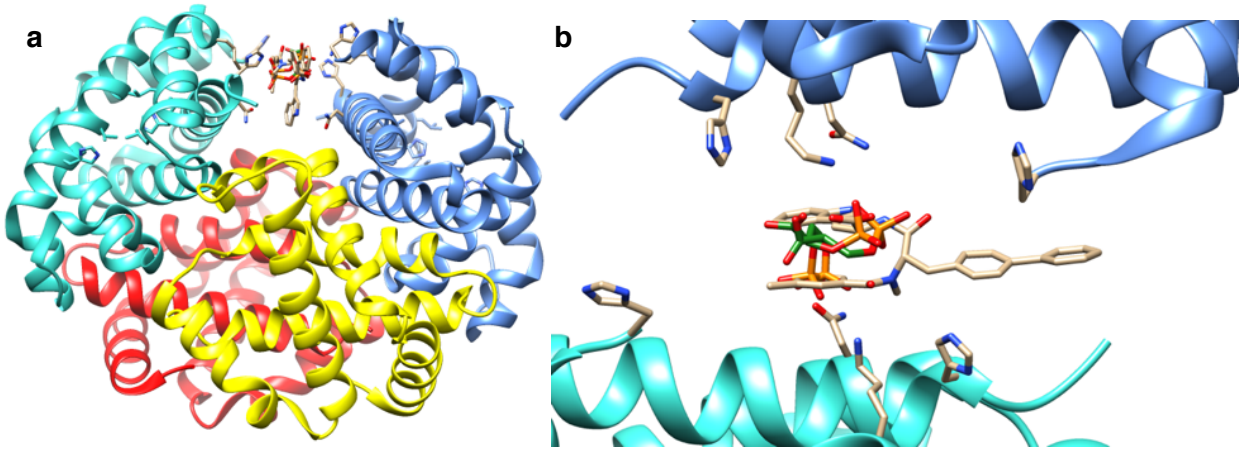
Supporting Information

X-RAY CRYSTALLOGRAPHY



Supplementary Figure S1. a) IRL 2500 **1** (tan) and 2,3-diphosphogylcerate (DPG, green) bound to deoxyHb. DPG shown bound in two conformations. Teal and blue are the β-subunits. Red and yellow are the α-subunits. b) A close-up view of DPG and **1** in the β-cleft.

Supplementary Table S1. Crystallographic data and refinement statistics for deoxyHb-DPG and **1** complex[a]

Data Collection	**DeoxyHb with Compound 1 and 2**
Space group	$P2_12_12$
Unit-cell *a, b, c* (Å)	95.8, 98.2, 65.3
Resolution (Å)	30 – 1.80 (1.86 – 1.80)
Unique reflections	53413 (5004)
Redundancy	6.6 (6.8)
Completeness (%)	92.4 (87.7)
Average I/σ(I)	30.3 (8.4)
R_{merge} (%)	3.9 (17.2)
Refinement[b]	
No. of reflections	53413 (5004)
Resolution (Å)	30 – 1.80 (1.86 – 1.80)
R_{work} (%; 95% of data)	20.3 (29.4)
R_{free} (%; 5% of data)	22.8 (32.4)
R.m.s.d. bonds (Å)	0.008
R.m.s.d. angles (°)	1.5
Dihedral angles	
Most favored (%)	93.5
Allowed (%)	6.5
Average B (Å^2) / atoms	
Protein	17.4/4370
Heme	14.8/172
Effector	41.9/73
Solvent	29.6/649
PDB ID code	4L7Y

[a]Numbers in parentheses are for the highest resolution shell. All positive reflections were used in the refinement. [b]$R_{merge} = \Sigma_{hkl}\Sigma_i|I_{hkli} - \langle I_{hkli}\rangle|/\Sigma_{hkl}\Sigma_i\langle I_{hkli}\rangle$. R_{free} calculated with 5% of reflections excluded throughout the refinement.

EXPERIMENTAL SECTION

Crystal Structure of Deoxygenated Hb in Complex with IRL 2500. IRL 2500 was purchased from Santa Cruz Biotechnology (Dallas, TX). To obtain unliganded hemoglobin structure in complex with IRL 2500, freshly prepared solution of IRL 2500 in DMSO was incubated with deoxygenated Hb (deoxyHb) (50 mg/mL) for 1h at a hemoglobin tetramer-compound molar ratio of 1:5, followed by crystallization with a low-salt precipitant (0.2 M sodium acetate trihydrate, 0.1 M sodium cacodylate trihydrate, pH 6.6 and 30% PEG 8000) using the batch method as previously described.[1] The crystals were washed in a cryo-protectant solution containing mother liquor and glycerol (3:1 ratio) prior to data collection. The complex crystallized in the orthorhombic space group $P2_12_12$ with typical cell dimensions a=96 Å, b=98 Å and c=65 Å with one functional tetramer per asymmetric unit.

Diffraction data were collected at 100 K with a Rigaku IV ++ image plate detector using a CuKα X-rays (λ = 1.54 Å) from a MicroMax-007 source fitted with Varimax Confocal optics (Rigaku, The Woodlands, TX). The datasets were processed with the d*trek software (Rigaku) and the CCP4 suite of programs.[2] X-ray data is summarized in Supplementary Table 1.

Initial phase for the deoxyHb- IRL 2500 complex structure was obtained by a molecular replacement method with the program CNS,[3] using human deoxyHb α1α1β2β2 tetramer structure (PDB code 2DN2) as a search model. Subsequent structure refinements were performed with the CNS program.[3] Model building and correction were carried out using the graphic program COOT.[4]

The initial electron density map of the deoxyHb-IRL 2500 complex obtained from refining the molecular model (without ligand or water) showed a very strong electron density at the beta β-cleft, exactly where 2,3-DPG is known to bind. IRL 2500 was modeled in the electron density.

However, refinement of the compound (either in one conformation or two alternate conformations) still resulted in significant unaccounted for positive difference density. IRL 2500 was then removed from the model, and following simulated annealing refinement, 2,3-DPG was modeled into the density at the β-cleft and refined in two alternate conformations as previously reported.[6] The model still resulted in a significant presence of positive difference density at the β-cleft site. At this stage, we assumed the density to be a mixture of IRL 2500 and 2,3-DPG, occupying the same position. IRL 2500 was fitted to the positive density and refined at 33% occupancy although some of the substituents, especially the biphenyl moiety lacked clear density, while 2,3-DPG was refined at 67%. The final refined model contains one hemoglobin tetramer, a bound 2,3-DPG molecule at the β-cleft in two alternate conformations, and a bound IRL 2500, also at the β-cleft in one conformation (Fig. S1). We note that if crystals are obtained at high-salt concentration (2 M phosphate buffer, pH 6.8), the ensuing structure does not show any bound ligand at the β-cleft, suggesting that high salt levels may interfere with the binding of allosteric effectors.

Measurement of COHb half-life. COHb was prepared by mixing oxyHb with CO saturated Dulbecco's phosphate buffered saline (DPBS). DPBS solution of CO was prepared by bubbling DPBS with 1% CO gas (balanced with nitrogen) for 30 min. IRL 1 was purchased from ChemPartner (Shanghai, China) and the other tested compounds were synthesized as described in a section below (Synthesis and Characterization). COHb and compounds (dissolved in DMSO) were mixed in test tubes and then dispensed to 384-well plates. The final concentration of Hb was 10 μM (as a tetramer) and DMSO was 5 vol% in DPBS. The final concentration of tested compound was 200 μM (20 fold excess or 20x) or 50 μM (5 fold excess or 5x).

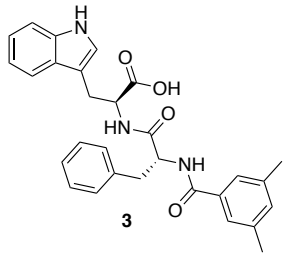
The prepared 384 well plate was placed in an inflatable polyethylene chamber (AtmosBag, Sigma) and the chamber was inflated with 1% CO (balanced with nitrogen). The plate was shaken at 25ºC using a plate shaker (SI-4000A, Scientific Industries) at 2000/min for 1 h to equilibrate CO gas with the COHb solution. The plate was then sealed with a plastic seal and centrifuged at 2000 rpm for 10 s. The plastic seal was removed and the plate was set into a plate reader (Multiskan Go, Thermo Scientific) in air.

The sample plate was shaken by the plate reader in air and absorbance of the sample was measured at 534 and 579 nm (A_{534} and A_{579}) using the plate reader before shaking and every 15 min of the shaking for 90 min. The fraction of COHb in a sample (COHb%) was determined by an equation (COHb% = -2.07 x (A_{579}/A_{534}) + 2.35). After shaking the plate for 90 min in air, the half-life of COHb was calculated by fitting the COHb% at all time points with a single exponential decay equation.

Hemolysis Assay. Human blood was collected under the approval by the institutional review board of Partners Human Research Committee. After obtaining informed consent from volunteers, blood was drawn from the volunteers into tubes coated with EDTA. The concentration of Hb tetramer in the collected blood was determined using a blood gas analyzer (ABL 800 FLEX, Radiometer Medical). The blood was diluted using HEMOX solution (TCS Scientific Corporation) containing N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid (TES, 30 mM), sodium chloride (135 mM), and potassium chloride (5 mM) in water (pH 7.4) and the diluted blood was mixed with DMSO and/or compound in DMSO. The final concentration of Hb tetramer and compound was 50 μM and 1 mM in 0.1 vol % DMSO respectively. The mixture was incubated at 37 °C for 60 min and then the mixture was centrifuged at 2,800 g for 20 min. After the centrifugation, the absorption of the supernatant was measured at wavelength of 415 nm and

compared to the absorption of 0.5 μM Hb ($A_{415ctrl}$, 0.5 μM of Hb tetramer corresponds to 1% of hemoglobin in total Hb). If $A_{415sample}$ was greater than $A_{415ctrl}$, we concluded that hemolysis (>1%) caused by the tested compound.

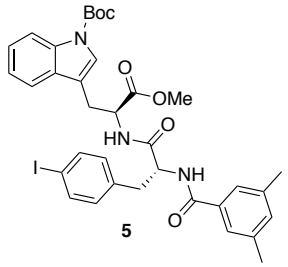
Synthesis and Characterization. Solvents used for extraction and purification were HPLC grade from Fisher. Unless otherwise indicated, all reactions were run under an inert atmosphere of argon. Merck pre-coated silica gel plates (250 mm, 60 F254) were used for analytical TLC. Spots were visualized using 254 nm ultraviolet light. Chromatographic purifications were performed on Sorbent Technologies silica gel (particle size 32-63 microns). ^{1}H and ^{13}C NMR spectra were recorded at 500 MHz and 125 MHz, in d6-DMSO, $CDCl_3$ and MeOD on a Bruker AM-500 or a DRX-500 spectrometer. Chemical shifts are reported relative to internal d6-DMSO (δ 2.54 for ^{1}H), $CDCl_3$ (δ 7.26 for ^{1}H), MeOD (δ 3.34 for ^{1}H). Infrared spectra were recorded as a solid using a Perkin-Elmer 1600 series Fourier transform spectrometer. UV/VIS spectra were obtained using a Varian-530. High resolution mass spectra were obtained at the University of Pennsylvania Mass Spectrometry Service Center on an Autospec high resolution double-focusing electrospray ionization/chemical ionization spectrometer with either DEC 11/73 or OPUS software data system. Melting points were obtained on a Thomas Hoover capillary melting point apparatus and are uncorrected. Purity was determined by ^{1}HNMR and ^{13}CNMR to be greater than or equivalent to 95%.



3

(3,5-Dimethylbenzoyl)-D-*phenylalanyl*-L-*tryptophan* (**3**). To (tert-butoxycarbonyl)-*D*-phenylalanine[3] (2.65 g, 10 mmol, 1 eq) was added tryptophan methyl ester hydrochloride[2] (2.54 g, 10 mmol, 1 eq), anhydrous N,N-diisopropylethylamine (9 mL, 50 mmol, 5 eq) and anhydrous acetonitrile (33 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (5.7 g, 15 mmol, 1.5 eq) was added. The reaction mixture was quenched with saturated ammonium chloride (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed twice with deionized water (50 mL), followed by brine (50 mL). The organic was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the crude product as an orange foam. This residue was purified through a plug of silica (20% EtOAc/Hexanes) to afford the dipeptide as a white foam (4.65 g, quant). To this dipeptide (100 mg, 0.2 mmol, 1 eq) in anhydrous dichloromethane (5 mL, 0.04 M), trifluoroacetic acid (160 µL, 2 mmol, 10 eq) was added dropwise at room temperature (20 °C). The reaction mixture was allowed to stir for 18 h. The reaction was then concentrated to dryness *in vacuo*. To the crude amine salt was added 3,5-dimethylbenzoic acid (33 mg, 0.2 mmol, 1 eq), anhydrous *N,N*-diisopropylethylamine (220 µL, 1.3 mmol, 6 eq) and anhydrous acetonitrile (0.7 mL, 0.3M). The reaction mixture was then cooled to 0 °C and HATU (120 mg, 0.3 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed twice with deionized water (10 mL), followed by brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as an

orange foam. This residue was purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the benzoyl amide as an off-white foam (88 mg, 82%). To a stirring solution of this amide (88 mg, 0.18 mmol, 1 eq) in a mixture of tetrahydrofuran, methanol and water (3:1:1, 2.5 mL, 0.067 M), lithium hydroxide monohydrate (30 mg, 0.7 mmol, 4 eq) was added portionwise. The reaction mixture was stirred at room temperature (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to afford a white solid (85.5 mg, 81%). m.p. = 110-120 °C. IR (solid) 2918, 1633, 1601, 1520, 1456 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.55 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 7.21 (s, 2H), 7.14 – 6.90 (m, 10H), 4.76 (p, J = 4.0 Hz, 1H), 3.52 – 3.43 (m, 1H), 3.37 – 3.30 (m, 1H), 3.18 (dd, J = 14.9, 8.1 Hz, 1H), 3.08 (dd, J = 14.0, 5.5 Hz, 1H), 2.84 (dt, J = 13.3, 5.3 Hz, 1H), 2.27 (t, J = 2.6 Hz, 6H). ^{13}C NMR (126 MHz, MeOD) δ 175.02, 173.30, 170.24, 139.31, 138.27, 138.05, 135.11, 134.20, 130.33, 129.27, 128.72, 127.62, 126.01, 124.63, 122.44, 119.94, 119.21, 112.32, 110.65, 66.87, 56.13, 38.80, 28.46, 21.24. HRMS (ESI) m/z calc'd for $C_{29}H_{29}N_3O_4$ $[M+H]^+$ 484.2236, found 484.2226.



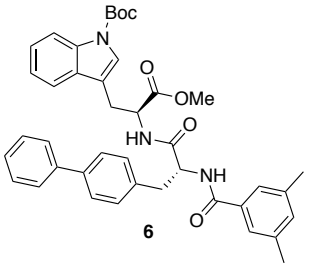
5

Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-iodophenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**5**). To tryptophan methyl ester hydrochloride (7.6 g, 30 mmol, 1 eq) and iodide **4**[11] (11.7 g, 30 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (100 mL, 0.3M) was added *N,N*-diisopropylethylamine (25 mL, 150 mmol, 5 eq). The reaction

mixture was then cooled to 0 °C and HATU (17 g, 45 mmol, 1.5 eq) was added. The reaction mixture was allowed to warm to room temperature (20 °C) for 18 h. The reaction mixture was poured over ice water (500 mL), filtered and dried *in vacuo* to afford the crude product as a purple solid. The crude product was then dissolved in anhydrous dichloromethane (150 mL, 0.2 M) and trifluoroacetic acid (20 mL, 300 mmol, 10 eq) was added. After stirring for 18 h at room temperature (20 °C), the reaction was quenched slowly with saturated sodium bicarbonate (200 mL). Layers were then partitioned and the aqueous was extracted a further two times with dichloromethane. The combined organic was washed with brine (100 mL) and dried over sodium sulfate. The reaction was concentrated *in vacuo* to yield the crude product as an orange foam. This residue was purified by flash chromatography (1-2% MeOH/CH_2Cl_2) to afford the amino ester as an off-white foam (10.5 g, 71% over two steps). This amino ester (1.15 g, 2.3 mmol, 1 eq) was added 3,5-dimethylbenzoic acid (350 mg, 2.3 mmol, 1 eq), anhydrous *N,N*-diisopropylethylamine (5 eq) and anhydrous *N,N*-dimethylformamide (0.3M) The reaction mixture was then cooled to 0 °C and HATU (1.5 eq) was added. The reaction mixture was allowed to warm to rt (20 °C) for 18 h. The reaction mixture was then poured over ice water. This precipitate was filtered to afford the indole as an off white solid (1.5 g, quant). To a stirring solution of indole (215 mg, 0.35 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (0.1 M), di-*tert*-butyl dicarbonate (1.3 eq) was added portionwise, followed by *N,N*-dimethylamino pyridine (0.1 eq) at 0 °C. Allowed to warm to rt (20 °C) for 18 h. The reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate two times. The organic layer was washed with water ten times, brine and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as a foam. This residue was purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the product as a white foam (250 mg, quant). IR (solid) 3277, 2977,

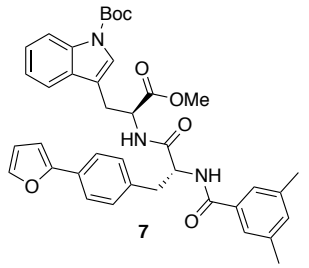
1732, 1634, 1602 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.53 – 7.41 (m, 4H), 7.38 – 7.29 (m, 1H), 7.27 (d, *J* = 4.6 Hz, 2H), 7.26 – 7.16 (m, 2H), 7.11 (s, 1H), 6.86 (d, *J* = 7.7 Hz, 1H), 6.76 (d, *J* = 8.1 Hz, 2H), 4.98 (q, *J* = 6.9 Hz, 1H), 4.88 (td, *J* = 7.4, 5.7 Hz, 1H), 3.62 (s, 3H), 3.19 (dd, *J* = 14.7, 5.7 Hz, 1H), 3.12 (dt, *J* = 14.2, 7.3 Hz, 2H), 3.02 (dd, *J* = 13.9, 6.2 Hz, 1H), 2.31 (s, 6H), 1.64 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.84, 170.87, 167.77, 149.63, 138.43, 137.64, 136.12, 133.68, 133.63, 131.50, 130.37, 124.94, 124.83, 124.33, 122.87, 118.84, 115.55, 115.13, 100.11, 92.58, 83.96, 54.26, 52.75, 52.62, 37.98, 28.33, 27.66, 21.34. HRMS (ESI) m/z calc'd for $C_{35}H_{38}IN_3O_6$ $[M+H]^+$ 724.1884, found 724.1882.

General Procedure for Suzuki Coupling. To a sealed vessel containing iodide (1 eq), borane (2 eq), [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (0.1 eq) and potassium acetate (5 eq) was added in anhydrous *N,N*-dimethylformamide (0.1 M) at rt (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was diluted with ethyl acetate and then quenched with saturated ammonium chloride. The organic layer was extracted and washed 4 times with deionized water, followed by brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as a brown foam. This residue was purified by flash chromatography to afford the carbamate as a foam.



6

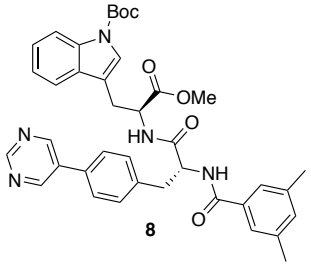
Tert-*butyl 3-((*S*)-2-((*R*)-3-([1,1'-biphenyl]-4-yl)-2-(3,5-dimethylbenzamido)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**6**). From **5** (200 mg, 0.28 mmol) and phenyl boronic acid (67 mg, 0.55 mmol), purified by flash chromatography (30% EtOAc/Hex) to afford the carbamate as an off-white foam (141 mg, 76%). IR (solid) 3276, 1732, 1635, 1602, 1537 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 8.11 (s, 1H), 7.50 (d, *J* = 8.2 Hz, 3H), 7.45 (s, 1H), 7.44 – 7.37 (m, 4H), 7.35 – 7.29 (m, 4H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.21 (t, *J* = 8.0 Hz, 1H), 7.18 (d, *J* = 8.1 Hz, 2H), 7.10 (s, 1H), 7.01 (d, *J* = 7.7 Hz, 1H), 5.10 (q, *J* = 7.0 Hz, 1H), 4.94 (dt, *J* = 8.0, 6.4 Hz, 1H), 3.60 (s, 3H), 3.28 – 3.19 (m, 2H), 3.15 (d, *J* = 6.4 Hz, 2H), 2.30 (s, 6H), 1.63 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.86, 171.21, 167.84, 149.66, 140.86, 139.96, 138.37, 135.65, 133.91, 133.53, 130.44, 129.97, 128.85, 127.36, 127.32, 127.11, 125.04, 124.77, 124.34, 122.82, 118.91, 115.52, 115.22, 83.84, 54.71, 52.71, 52.54, 38.31, 28.30, 27.77, 21.32. HRMS (ESI) m/z calc'd for $C_{41}H_{43}N_3O_6$ $[M+H]^+$ 674.3230, found 674.3233.



7

Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(furan-2-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**7**). From **5** (200 mg, 0.28 mmol) and 2-furyl

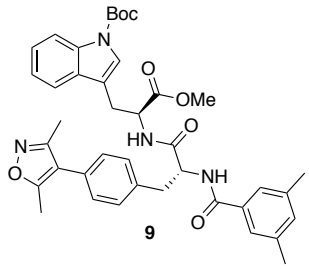
boronic acid (61 mg, 0.55 mmol), purified by flash chromatography (30% EtOAc/Hex) to afford the carbamate as an off-white foam (176 mg, 96%). IR (thin film) 3280, 2979, 1735, 1637, 1602 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.10 (s, 1H), 7.47 (d, *J* = 7.9 Hz, 3H), 7.45 – 7.39 (m, 2H), 7.35 – 7.27 (m, 3H), 7.20 (t, *J* = 7.7 Hz, 1H), 7.15 (d, *J* = 7.9 Hz, 1H), 7.13 – 7.02 (m, 3H), 6.91 (d, *J* = 7.6 Hz, 1H), 6.55 (d, *J* = 3.3 Hz, 1H), 6.43 (dd, *J* = 3.4, 1.8 Hz, 1H), 5.03 (q, *J* = 6.9 Hz, 1H), 4.95 – 4.82 (m, 1H), 3.59 (s, 3H), 3.22 – 3.07 (m, 4H), 2.28 (s, 6H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.85, 171.06, 167.75, 153.86, 149.63, 142.05, 138.37, 135.63, 135.47, 133.83, 133.53, 130.40, 129.82, 129.77, 124.98, 124.75, 124.29, 124.05, 122.81, 118.87, 115.50, 115.17, 111.75, 105.02, 83.83, 54.59, 52.71, 52.56, 38.37, 28.30, 27.72, 21.31. HRMS (ESI) m/z calc'd for $C_{39}H_{41}N_3O_7$ $[M+Na]^+$ 686.2842, found 686.2845.



8

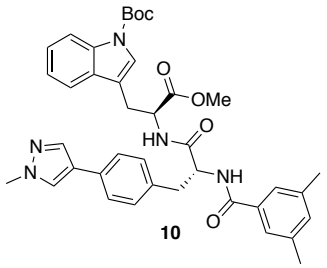
Tert-*butyl* *3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(pyrimidin-5-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H-*indole-1-carboxylate* (**8**). From **5** (200 mg, 0.28 mmol) and pyrimidin-5-ylboronic acid (68 mg, 0.55 mmol), purified by flash chromatography (50% EtOAc/Hex) to afford the carbamate as an off-white foam (146 mg, 78%). IR (solid) 1731, 1636, 1602, 1533, 1451 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 9.10 (s, 1H), 8.76 (d, J = 1.5 Hz, 2H), 8.04 (s, 1H), 7.71 (d, J = 8.1 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.45 (s, 1H), 7.28 (s, 3H), 7.26 – 7.23 (m, 2H), 7.19 (d, J = 7.6 Hz, 2H), 7.10 (d, J = 7.7 Hz, 2H), 7.05 (s, 1H), 5.13 (q, J = 6.9 Hz, 1H), 4.92 (q, J = 7.2 Hz, 1H), 3.59 (s, 3H), 3.33 – 3.05 (m, 4H), 2.24 (s, 6H), 1.56 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.99, 171.15, 167.77, 157.37, 154.75, 149.61, 138.32, 137.75,

135.42, 133.91, 133.74, 133.56, 132.69, 130.59, 130.35, 126.91, 124.99, 124.80, 124.39, 122.81, 118.88, 115.52, 115.29, 83.90, 54.40, 52.59, 38.25, 28.24, 27.70, 21.29. HRMS (ESI) m/z calc'd for $C_{39}H_{41}N_5O_6$ $[M+H]^+$ 676.3135, found 676.3132.

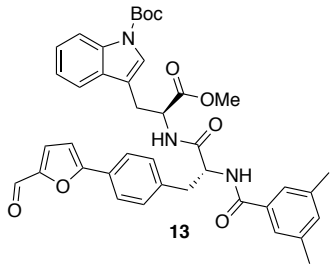


9

Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(3,5-dimethylisoxazol-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**9**). From **5** (200 mg, 0.28 mmol) and 3,5-dimethylisooxazole-4-boronic ester (123 mg, 0.55 mmol), purified by flash chromatography (30% EtOAc/Hex) to afford the carbamate as an off-white foam (144 mg, 75%). IR (solid) 3280, 2928, 1732, 1635, 1603 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.09 (s, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.43 (s, 1H), 7.28 (d, J = 9.7 Hz, 3H), 7.21 (t, J = 7.4 Hz, 1H), 7.13 (d, J = 8.9 Hz, 3H), 7.06 (d, J = 7.5 Hz, 2H), 6.99 (d, J = 7.9 Hz, 1H), 6.85 (d, J = 7.7 Hz, 1H), 5.00 (q, J = 7.0 Hz, 1H), 4.90 (q, J = 6.8 Hz, 1H), 3.63 (s, 3H), 3.23 – 3.10 (m, 4H), 2.32 (d, J = 7.1 Hz, 9H), 2.19 (s, 3H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.81, 170.90, 167.73, 165.23, 158.71, 149.61, 138.41, 135.90, 135.47, 133.82, 133.58, 130.37, 129.94, 129.26, 124.92, 124.82, 124.33, 122.83, 118.81, 116.31, 115.51, 115.03, 83.94, 54.50, 52.72, 52.59, 38.17, 28.28, 27.74, 21.31, 21.27, 11.64, 10.89. HRMS (ESI) m/z calc'd for $C_{40}H_{44}N_4O_7$ $[M+H]^+$ 693.3288, found 693.3295.



Tert-*butyl* *3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**10**). From **5** (200 mg, 0.28 mmol) and 1-methyl-4-pyrazole boronic ester (115 mg, 0.55 mmol), purified by flash chromatography (1% MeOH/CH_2Cl_2) to afford the carbamate as an off-white foam (149 mg, 80%). IR (solid) 1732, 1636, 1602, 1532, 1452 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.08 (s, 1H), 7.66 (s, 1H), 7.48 (s, 1H), 7.43 (d, J = 7.7 Hz, 1H), 7.37 (s, 1H), 7.31 – 7.23 (m, 5H), 7.19 (td, J = 7.5, 2.6 Hz, 1H), 7.08 (d, J = 8.7 Hz, 3H), 6.84 (t, J = 7.9 Hz, 2H), 4.98 – 4.85 (m, 2H), 3.89 (d, J = 2.2 Hz, 3H), 3.59 (d, J = 2.0 Hz, 3H), 3.12 (ddd, J = 13.9, 6.8, 4.2 Hz, 4H), 2.29 (s, 6H), 1.63 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.78, 171.01, 167.68, 149.63, 138.37, 136.77, 135.46, 134.52, 133.85, 133.51, 131.50, 130.39, 129.94, 126.99, 125.76, 124.96, 124.73, 124.23, 122.93, 122.78, 118.83, 115.47, 115.03, 83.88, 54.74, 52.55, 39.16, 38.29, 28.30, 27.72, 24.99, 21.30. HRMS (ESI) m/z calc'd for $C_{39}H_{43}N_5O_6$ $[M+H]^+$ 678.3292, found 678.3291.

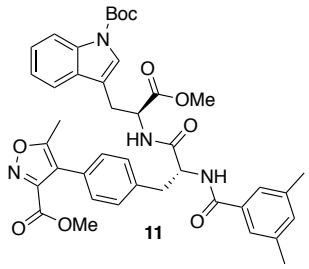


Tert-*butyl* *3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(5-formylfuran-2-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**13**). From **5** (200 mg, 0.28 mmol) and (5-formylfuran-2-yl)boronic acid (77 mg, 0.55 mmol), purified by flash

chromatography (40% EtOAc/Hex) to afford the aldehyde as an off-white foam (145 mg, 76%). IR (solid) 1732, 1670, 1636, 1603, 1530 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 9.60 (s, 1H), 8.08 (d, J = 7.8 Hz, 1H), 7.58 (d, J = 7.9 Hz, 2H), 7.46 (d, J = 7.8 Hz, 1H), 7.40 (s, 1H), 7.33 – 7.24 (m, 4H), 7.20 (t, J = 7.5 Hz, 1H), 7.13 (d, J = 8.0 Hz, 2H), 7.09 (s, 1H), 7.02 (s, 1H), 6.85 (s, 1H), 6.72 (d, J = 3.6 Hz, 1H), 4.99 (s, 1H), 4.89 (q, J = 6.7 Hz, 1H), 3.61 (s, 3H), 3.24 – 3.08 (m, 4H), 2.29 (s, 6H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 177.29, 171.77, 170.81, 167.71, 159.28, 152.09, 149.60, 138.43, 135.47, 133.69, 133.62, 130.36, 130.07, 127.84, 125.56, 124.91, 124.79, 124.22, 122.81, 118.78, 115.51, 115.00, 107.77, 83.93, 54.46, 53.55, 52.62, 38.41, 28.30, 27.70, 21.30. HRMS (ESI) m/z calc'd for $C_{40}H_{41}N_3O_8$ $[M+H]^+$ 692.2972, found 692.2979.

General Procedure for Isoxazole Iodination, Borylation, Suzuki Coupling. 1) To a flask containing isoxazole (1 eq) and *N*-iodosuccinamide (1.2 eq), trifluoroacetic acid (0.18 M) was added at room temperature (20 °C). After stirring for 18 h, the reaction mixture was concentrated *in vacuo*, diluted with water and extracted with diethyl ether. The organic layer was then washed with 1N NaOH, 5% $NaHSO_4$ and brine. The organic was then dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford the iodide as a solid. To a flask containing iodide (1 eq) and bis(triphenylphosphine)palladium(II) dichloride (0.05 eq) in degassed anhydrous dioxane (0.53 M) was added pinacol borane (1.5 eq) followed by anhydrous triethylamine (3 eq) at rt (20 °C). The reaction mixture was then heated to reflux for one hour. The reaction mixture was then cooled to room temperature and concentrated *in vacuo* to afford the crude product as a yellow oil. This was taken up in diethyl ether, run through Celite, concentrated *in vacuo* and triturated with hexane. The hexane was concentrated *in vacuo* to yield the borane as a solid. 2) To a sealed vessel containing this borane (2 eq), iodide (1 eq), [1,1′-

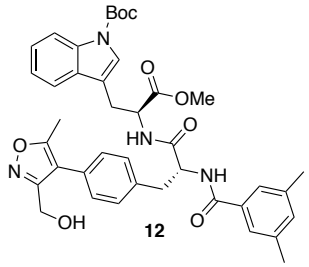
bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (0.1 eq) and potassium acetate (5 eq) was added in anhydrous *N,N*-dimethylformamide (0.1 M) at rt (20 °C). The reaction mixture was then heated to 80 °C for 18 h. The reaction mixture was diluted with ethyl acetate and then quenched with saturated ammonium chloride. The organic layer was extracted and washed 4 times with deionized water, followed by brine. The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness *in vacuo* to afford the crude product as a brown foam. This residue was purified by flash chromatography to afford the carbamate as a foam.



11

*Methyl 4-(4-((*R*)-3-(((*S*)-3-(1-(tert-butoxycarbonyl)-1*H*-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)amino)-2-(3,5-dimethylbenzamido)-3-oxopropyl)phenyl)-5-methylisoxazole-3-carboxylate* (**11**). From methyl 5-methylisoxazole-3-carboxylate[12] (212 mg, 1.5 mmol) concentrated to afford the iodide as an off-white solid (410 mg, quant). This iodide (410 mg, 1.5 mmol, 1 eq) was borylated to afforded the borane as a yellow solid (410 mg, quant). For part 2, **5** (200 mg, 0.28 mmol) and borane from part 1 (145 mg, 0.55 mmol) were coupled and purified by flash chromatography (20-40% EtOAc/Hex) to afford the carbamate as an off-white foam (63 mg, 32%). IR (solid) 3278, 2924, 1733, 1635, 1602 cm^{-1}. ^1H NMR (500 MHz, Chloroform-d) δ 8.07 (s, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.40 (s, 1H), 7.28 (d, J = 6.3 Hz, 3H), 7.17 (qd, J = 7.8, 4.1 Hz, 5H), 7.11 (s, 1H), 6.83 (t, J = 7.3 Hz, 2H), 4.96 (q, J = 7.1 Hz, 1H), 4.88 (q, J = 6.7 Hz, 1H), 3.78 (s, 3H), 3.60 (s, 3H), 3.18 (dd, J = 6.9, 4.0 Hz, 2H), 3.14 (d, J = 6.4 Hz, 2H), 2.35 (s, 3H), 2.31 (s, 6H),

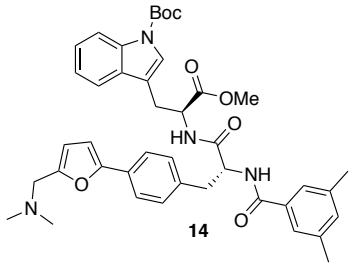
1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.80, 170.87, 168.37, 167.73, 160.74, 153.84, 149.63, 138.41, 136.68, 135.46, 133.81, 133.56, 130.41, 130.15, 129.50, 127.43, 124.94, 124.76, 124.30, 122.78, 118.83, 117.31, 115.47, 115.06, 83.92, 54.57, 52.76, 52.56, 38.23, 28.30, 27.72, 21.30, 11.52. HRMS (ESI) m/z calc'd for $C_{41}H_{44}N_4O_9$ $[M+Na]^+$ 759.3006, found 759.3018.



12

Tert-*butyl 3-((*S*)-2-((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(3-(hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**12**). From (5-methylisoxazol-3-yl)methanol[13] (500 mg, 4.4 mmol) purified by flash chromatography (10% EtOAc/Hexanes) to afford the iodide as an off-white solid (773 mg, 73%). To a flask containing this iodide (500 mg, 2.1 mmol, 1 eq) in anhydrous *N,N*-dimethylformamide (2.1 mL, 1 M), imidazole (356 mg, 5.2 mmol, 2.5 eq) was added, followed by *tert*-butyldimethylsilyl chloride (380 mg, 2.5 mmol, 1.2 eq) at room temperature (20 °C). After stirring for two hours, the reaction mixture was quenched with saturated ammonium chloride (20 mL) and extracted two times with ethyl acetate (20 mL). The combined organic was then washed four times with water (20 mL) and brine (20 mL). The organic was then dried over anhydrous sodium sulfate and concentrated *in vacuo* to afford a crude oil. This oil purified by flash chromatography (10% EtOAc/Hexanes) to afford the silyl iodide as a clear oily solid (710 mg, 96%). This iodide (353 mg, 1 mmol) was borylated to afford the borane as a yellow solid which was carried forward without further purification. For part 2, **5** (200 mg, 0.28 mmol) and the borane from part 1 (194 mg, 0.55 mmol) were coupled and purified by flash chromatography (40-50% EtOAc/Hex) to afford the carbamate

as an off-white foam (59 mg, 30% over 2 steps). IR (thin film) 3289, 2925, 1733, 1637, 1603 cm^{-1}. ^{1}H NMR (500 MHz, Chloroform-d) δ 8.08 (s, 1H), 7.47 (d, J = 7.8 Hz, 1H), 7.41 (s, 1H), 7.29 (s, 3H), 7.20 (t, J = 7.5 Hz, 1H), 7.16 (d, J = 7.9 Hz, 2H), 7.11 (d, J = 8.4 Hz, 3H), 7.04 (d, J = 7.8 Hz, 1H), 6.94 (d, J = 7.8 Hz, 1H), 4.99 (q, J = 7.0 Hz, 1H), 4.88 (q, J = 6.4 Hz, 1H), 4.61 (d, J = 4.5 Hz, 2H), 3.61 (s, 3H), 3.20 – 3.08 (m, 4H), 2.86 (s, 1H), 2.34 (s, 3H), 2.29 (s, 6H), 1.60 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.96, 170.98, 167.77, 166.08, 161.58, 149.66, 138.40, 136.27, 135.49, 133.77, 133.58, 130.39, 130.03, 129.28, 128.47, 124.94, 124.82, 124.34, 122.84, 118.84, 115.65, 115.49, 115.09, 83.99, 55.94, 54.51, 52.76, 52.64, 38.27, 28.27, 27.68, 21.30, 11.64. HRMS (ESI) m/z calc'd for $C_{40}H_{44}N_4O_8$ $[M+H]^+$ 709.3237, found 709.3250.

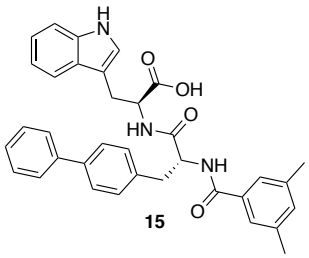


14

Tert-*butyl 3-((*S*)-2-((*R*)-3-(4-(5-((dimethylamino)methyl)furan-2-yl)phenyl)-2-(3,5-dimethylbenzamido)propanamido)-3-methoxy-3-oxopropyl)-1*H*-indole-1-carboxylate* (**14**). To a flask containing aldehyde **13** (70 mg, 0.1 mmol, 1 eq), dimethylamine hydrochloride (27 mg, 0.3 mmol, 3 eq) and sodium triacetoxyborohydride (26 mg, 0.12 mmol, 1.15 eq) in dichloromethane (560 μL, 0.2 M) at room temperature (20 °C), triethylamine (40 μL, 0.26 mmol, 2.4 eq) was added. After four hours, the reaction mixture was diluted with ethyl acetate and then quenched with water (10 mL). The organic layer was extracted and washed with brine (10 mL). The organic layer was then dried over anhydrous sodium sulfate and concentrated to dryness in vacuo to afford the carbamate as an orange foam (68 mg, 93%). IR (solid) 3268, 2934, 1733, 1636, 1602 cm^{-1}. ^{1}H

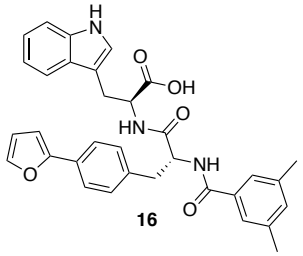
NMR (500 MHz, Chloroform-d) δ 8.09 (d, J = 7.8 Hz, 1H), 7.49 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 7.8 Hz, 1H), 7.41 (s, 1H), 7.32 – 7.24 (m, 3H), 7.19 (t, J = 7.6 Hz, 1H), 7.14 – 7.05 (m, 3H), 6.91 (d, J = 7.9 Hz, 1H), 6.79 (d, J = 7.6 Hz, 1H), 6.50 (d, J = 3.2 Hz, 1H), 6.24 (d, J = 3.2 Hz, 1H), 4.95 (q, J = 6.9 Hz, 1H), 4.92 – 4.83 (m, 1H), 3.59 (s, 3H), 3.51 (s, 2H), 3.13 (t, J = 7.1 Hz, 4H), 2.28 (d, J = 5.2 Hz, 12H), 1.62 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.78, 170.97, 167.74, 153.31, 152.05, 149.61, 138.37, 135.50, 135.40, 133.83, 133.52, 130.38, 129.86, 129.71, 124.94, 124.73, 124.28, 124.02, 122.80, 118.83, 115.47, 115.06, 110.77, 105.67, 83.83, 55.93, 54.61, 52.69, 52.54, 45.03, 38.26, 28.30, 27.73, 21.30.

General Procedure for Boc Deprotection and Saponification. To a stirring solution of carbamate (1 eq) in anhydrous dichloromethane (0.2 M), trifluoroacetic acid (20 eq) was added dropwise at rt (20 °C). After stirring for 18 h, the reaction mixture was quenched with saturated sodium bicarbonate and partitioned. The organic layer was washed with brine and dried over anhydrous sodium sulfate. The organic layer was then concentrated to dryness *in vacuo* to afford the crude product as a foam. This residue was purified by flash chromatography to afford the ester as a foam. This foam was then dissolved in a mixture of tetrahydrofuran, methanol and water (3:1:1, 0.067 M) and lithium hydroxide monohydrate (10 eq) was added portionwise. The reaction mixture was stirred at rt (20 °C) for 18 h. The reaction mixture was then concentrated to remove the organic solvents. 5% $NaHSO_4$ was added until a solid precipitated. The solid was collected and washed with water. Product was dried to afford a solid.



*((*R*)-3-([1,1'-biphenyl]-4-yl)-2-(3,5-dimethylbenzamido)propanoyl)*-L-*tryptophan* (**15**).

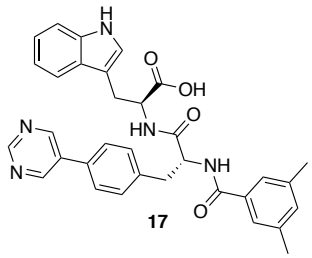
Carbamate **6** (161 mg, 0.23 mmol, 1 eq) was deprotected and purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (61 mg, 0.1 mmol). This ester was saponified to afford the product as an off-white solid (59 mg, 45% over 2 steps). m.p. = 145-150 °C. IR (solid) 3288, 2920, 1723, 1634, 1600 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.59 (d, *J* = 8.0 Hz, 1H), 7.49 (d, *J* = 7.1 Hz, 2H), 7.37 (t, *J* = 7.8 Hz, 2H), 7.33 – 7.27 (m, 4H), 7.23 (s, 2H), 7.14 (s, 1H), 7.08 (s, 1H), 7.06 (ddd, *J* = 8.1, 7.0, 1.2 Hz, 1H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.97 (t, *J* = 7.8 Hz, 1H), 4.92 – 4.86 (m, 1H), 4.78 (dd, *J* = 8.1, 4.9 Hz, 1H), 3.37 (dd, *J* = 14.8, 4.8 Hz, 1H), 3.21 (dd, *J* = 14.7, 8.1 Hz, 1H), 3.15 (dd, *J* = 13.9, 5.4 Hz, 1H), 2.89 (dd, *J* = 13.9, 8.2 Hz, 1H), 2.29 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.63, 173.06, 170.27, 142.13, 140.77, 139.37, 138.11, 137.37, 135.19, 134.22, 130.87, 129.74, 128.12, 127.80, 126.10, 126.02, 124.67, 122.43, 119.96, 119.34, 112.33, 110.92, 101.39, 56.04, 54.91, 38.50, 28.61, 21.24. HRMS (ESI) m/z calc'd for $C_{35}H_{33}N_3O_4$ $[M+H]^+$ 560.2549, found 560.2552.



16

*((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(furan-2-yl)phenyl)propanoyl)*-L-*tryptophan* (**16**).

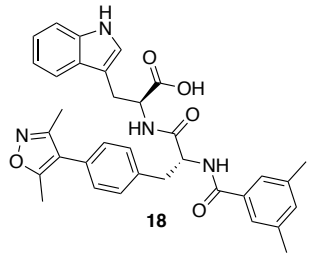
Carbamate **7** (176 mg, 0.27 mmol) was deprotected and purified by flash chromatography (50% EtOAc/Hexanes) to afford the ester as an off-white foam (61 mg, 0.1 mmol). This ester was saponified to afford the product as an off-white solid (63 mg, 43% over 2 steps). m.p. = 168-170 °C. IR (solid) 2920, 1720, 1633, 1600, 1516 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 7.56 (d, J = 8.0 Hz, 1H), 7.48 (s, 1H), 7.38 (d, J = 8.0 Hz, 2H), 7.30 (d, J = 8.1 Hz, 1H), 7.21 (d, J = 7.8 Hz, 3H), 7.11 (s, 1H), 7.07 (q, J = 7.8, 5.9 Hz, 2H), 6.96 (dd, J = 11.2, 7.6 Hz, 3H), 6.61 (d, J = 3.1 Hz, 1H), 6.44 (s, 1H), 4.90 – 4.83 (m, 1H), 4.77 (dd, J = 8.2, 4.8 Hz, 1H), 3.36 (dd, J = 14.8, 4.9 Hz, 1H), 3.19 (dd, J = 14.7, 8.3 Hz, 1H), 3.08 (dd, J = 13.9, 5.5 Hz, 1H), 2.88 – 2.79 (m, 1H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 173.97, 171.90, 169.01, 153.92, 141.89, 138.10, 136.98, 136.17, 133.86, 132.97, 129.48, 127.53, 124.76, 123.57, 123.41, 123.34, 121.22, 118.75, 118.02, 111.34, 111.17, 109.58, 104.47, 54.69, 53.42, 37.34, 27.27, 19.98. HRMS (ESI) m/z calc'd for $C_{33}H_{31}N_3O_5$ $[M+Na]^+$ 572.2156, found 572.2166.



17

*((*R*)-2-(3,5-dimethylbenzamido)-3-(4-(pyrimidin-5-yl)phenyl)propanoyl)*-L-*tryptophan* (**17**)

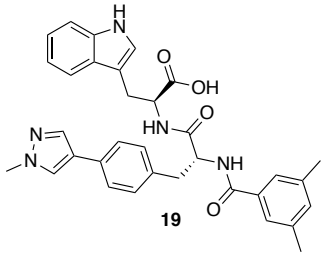
Carbamate **8** (100 mg, 0.15 mmol) was purified by flash chromatography (4% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (51.4 mg, 0.09 mmol). This ester was saponified to afford the product as an off-white solid (47 mg, 56% over 2 steps). m.p. = 175-180 °C. IR (solid) 3301, 2926, 2467, 1722, 1634 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 9.05 (s, 1H), 8.87 (s, 2H), 8.09 (dd, *J* = 34.1, 7.9 Hz, 1H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.34 – 7.21 (m, 5H), 7.14 – 7.01 (m, 5H), 6.96 (t, *J* = 7.5 Hz, 1H), 4.96 – 4.88 (m, 1H), 4.80 – 4.73 (m, 1H), 3.37 (dd, *J* = 14.8, 4.6 Hz, 1H), 3.17 (ddd, *J* = 19.6, 14.3, 7.0 Hz, 2H), 2.90 (dd, *J* = 13.8, 8.0 Hz, 1H), 2.27 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 174.14, 171.62, 168.82, 156.35, 154.53, 138.40, 138.14, 136.85, 133.81, 133.01, 132.04, 130.25, 127.57, 126.52, 124.84, 124.75, 123.40, 121.19, 118.71, 118.09, 111.11, 109.71, 54.58, 53.54, 37.29, 27.31, 19.97. HRMS (ESI) m/z calc'd for $C_{33}H_{31}N_5O_4$ $[M+H]^+$ 562.2454, found 562.2451.



18

*((*R*)-2-(3,5-Dimethylbenzamido)-3-(4-(3,5-dimethylisoxazol-4-yl)phenyl)propanoyl)*-L-*tryptophan* (**18**). Carbamate **9** (100 mg, 0.14 mmol) was deprotected and purified by flash

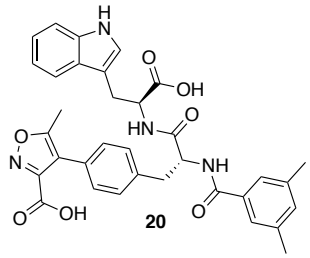
chromatography (2% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (60.3 mg, 0.1 mmol, 70%). This ester was saponified to afford the product as an off-white solid (58.9 mg, 70% over two steps). m.p. = 140-150 °C. IR (solid) 3302, 2923, 1726, 1635, 1602 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 10.33 (s, 1H), 8.22 – 8.01 (m, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.29 (d, J = 8.2 Hz, 1H), 7.25 (s, 2H), 7.17 – 7.10 (m, 1H), 7.10 – 7.01 (m, 2H), 6.97 (s, 5H), 4.78 (s, 1H), 3.36 (dd, J = 15.5, 4.2 Hz, 1H), 3.15 (ddd, J = 20.9, 14.5, 7.1 Hz, 2H), 2.87 (dd, J = 13.9, 7.7 Hz, 1H), 2.28 (s, 9H), 2.12 (s, 3H). ^{13}C NMR (126 MHz, MeOD) δ 173.91, 171.74, 168.86, 165.43, 158.66, 138.12, 136.51, 133.01, 129.70, 128.73, 128.34, 127.47, 124.76, 123.62, 123.46, 121.22, 118.72, 118.03, 116.35, 111.17, 111.11, 109.61, 54.60, 53.34, 37.34, 27.32, 19.99, 10.11, 9.41. HRMS (ESI) m/z calc'd for $C_{34}H_{34}N_4O_5$ $[M+Na]^+$ 601.2421, found 601.2429.



19

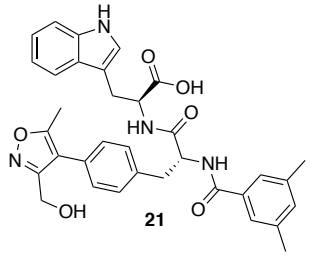
*((*R*)-2-(3,5-Dimethylbenzamido)-3-(4-(1-methyl-1*H*-pyrazol-4-yl)phenyl)propanoyl)*-L-*tryptophan* (**19**). Carbamate **10** (100 mg, 0.14 mmol) was deprotected and purified by flash chromatography (3% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (62.4 mg, 0.11 mmol, 73%). This ester was saponified to afford the product as an off-white solid (60.9 mg, 70% over two steps). m.p. = 132-145 °C. IR (solid) 3300, 2922, 1726, 1643, 1602 cm^{-1}. ^1H NMR (500 MHz, Methanol-d_4) δ 7.77 (s, 1H), 7.67 (s, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.30 (d, J = 7.8 Hz, 1H), 7.26 – 7.15 (m, 4H), 7.12 (s, 1H), 7.10 – 7.00 (m, 3H), 6.97 (t, J = 7.6 Hz, 1H), 6.91 (d, J = 7.8 Hz, 2H), 4.86 – 4.82 (m, 1H), 4.77 (dd, J = 8.3, 4.9 Hz, 1H), 3.86 (s, 3H), 3.34 (dd, J = 14.8, 5.0 Hz, 1H), 3.18 (dd, J = 14.7, 8.2 Hz, 1H), 3.07 (dd, J = 13.8, 5.4 Hz, 1H), 2.83 (dd, J = 13.8, 8.3

Hz, 1H), 2.28 (s, 6H). ^{13}C NMR (126 MHz, MeOD) δ 173.87, 171.92, 168.93, 138.11, 136.84, 135.95, 135.03, 132.97, 130.87, 129.60, 127.64, 127.49, 124.96, 124.81, 124.76, 123.40, 123.00, 121.18, 118.70, 118.03, 111.11, 109.50, 54.79, 53.30, 37.61, 37.26, 27.25, 19.97. HRMS (ESI) m/z calc'd for $C_{33}H_{33}N_5O_4$ $[M+Na]^+$ 586.2430, found 586.2430.



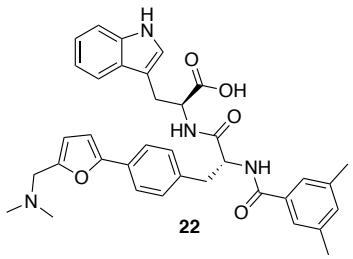
20

*4-(4-((*R*)-3-(((*S*)-1-Carboxy-2-(1H-indol-3-yl)ethyl)amino)-2-(3,5-dimethylbenzamido)-3-oxopropyl)phenyl)-5-methylisoxazole-3-carboxylic acid* (**20**). Carbamate **11** (63 mg, 0.09 mmol) was deprotected and purified by flash chromatography (2% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (49.3 mg, 0.08 mmol). This ester was precipitated to afford an off-white solid (45.7 mg, 88% over 2 steps). m.p. = 130-142 °C. IR (solid) 3331, 2924, 1722, 1634, 1601 cm^{-1}. ^{1}H NMR (500 MHz, Methanol-d_4) δ 8.09 (dd, J = 38.3, 8.0 Hz, 1H), 7.55 (d, J = 7.9 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 7.25 (s, 2H), 7.13 (s, 1H), 7.07 – 7.01 (m, 4H), 7.01 – 6.90 (m, 3H), 4.79 (td, J = 8.1, 4.9 Hz, 2H), 3.35 (dd, J = 14.7, 4.9 Hz, 1H), 3.18 (dd, J = 14.7, 8.3 Hz, 1H), 3.11 (dd, J = 13.9, 5.7 Hz, 1H), 2.89 (dd, J = 13.8, 7.9 Hz, 1H), 2.28 (s, 9H). ^{13}C NMR (126 MHz, MeOD) δ 173.77, 171.95, 171.87, 169.03, 168.26, 161.91, 138.14, 136.86, 136.83, 133.89, 132.99, 129.44, 129.12, 127.46, 127.27, 124.78, 123.43, 121.23, 118.72, 118.00, 116.76, 111.13, 109.48, 54.69, 53.33, 53.24, 37.30, 27.28, 19.99, 10.05. HRMS (ESI) m/z calc'd for $C_{34}H_{32}N_4O_7$ $[M+H]^+$ 609.2349, found 609.2372.



21

*((*R*)-2-(3,5-Dimethylbenzamido)-3-(4-(3-(hydroxymethyl)-5-methylisoxazol-4-yl)phenyl)propanoyl)*-L-*tryptophan* (**21**). Carbamate **12** (58 mg, 0.08 mmol) was deprotected and purified by preparative thin layer chromatography (4% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (15.6 mg, 0.03 mmol). This ester was saponified to afford the product as an off-white solid (24.3 mg, 35% over 2 steps). m.p. = 160-175 °C. IR (solid) 3302, 2923, 1640, 1602, 1516 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.58 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 8.0 Hz, 1H), 7.22 (s, 2H), 7.13 (d, *J* = 8.1 Hz, 3H), 7.07 (s, 1H), 7.02 (t, *J* = 8.3 Hz, 3H), 6.94 (t, *J* = 7.5 Hz, 1H), 4.90 (dd, *J* = 8.3, 5.4 Hz, 1H), 4.75 (dd, *J* = 7.8, 4.8 Hz, 1H), 4.52 (s, 2H), 3.36 (dd, *J* = 14.7, 4.8 Hz, 1H), 3.25 – 3.14 (m, 2H), 2.89 (dd, *J* = 13.8, 8.3 Hz, 1H), 2.33 (s, 3H), 2.30 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 171.26, 168.68, 165.75, 162.51, 161.20, 139.53, 137.85, 136.51, 133.66, 132.71, 129.37, 128.62, 127.78, 127.43, 124.51, 123.14, 120.84, 118.41, 117.88, 115.54, 110.72, 109.61, 54.49, 54.25, 53.89, 37.12, 27.23, 19.74, 9.92. HRMS (ESI) m/z calc'd for $C_{34}H_{34}N_4O_6$ $[M+H]^+$ 595.2557, found 595.2542.

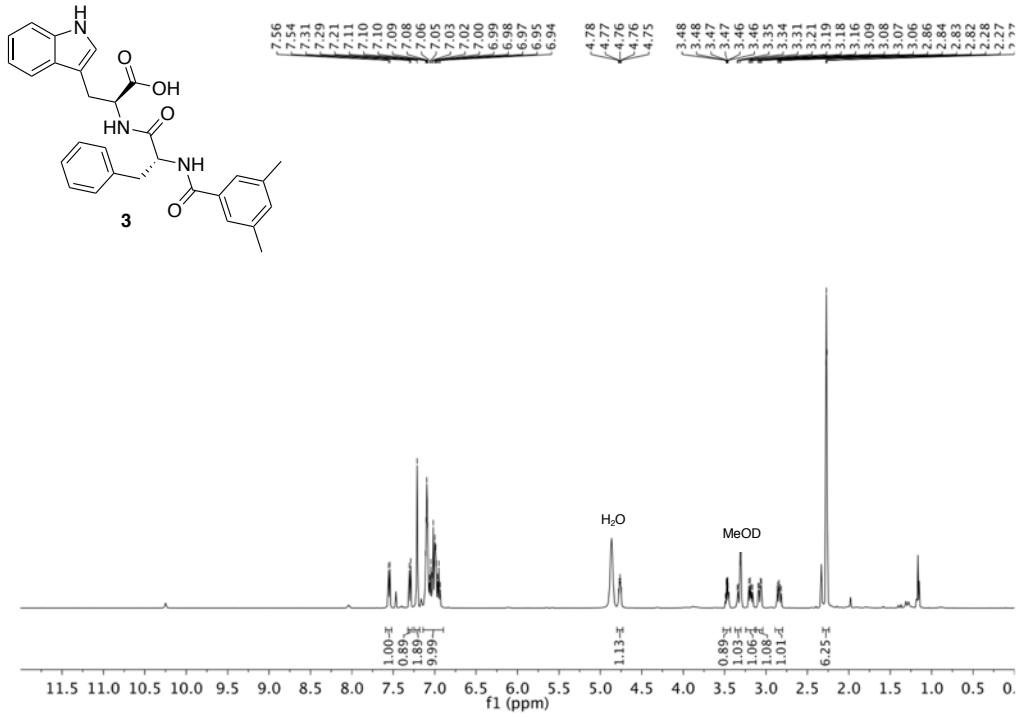


22

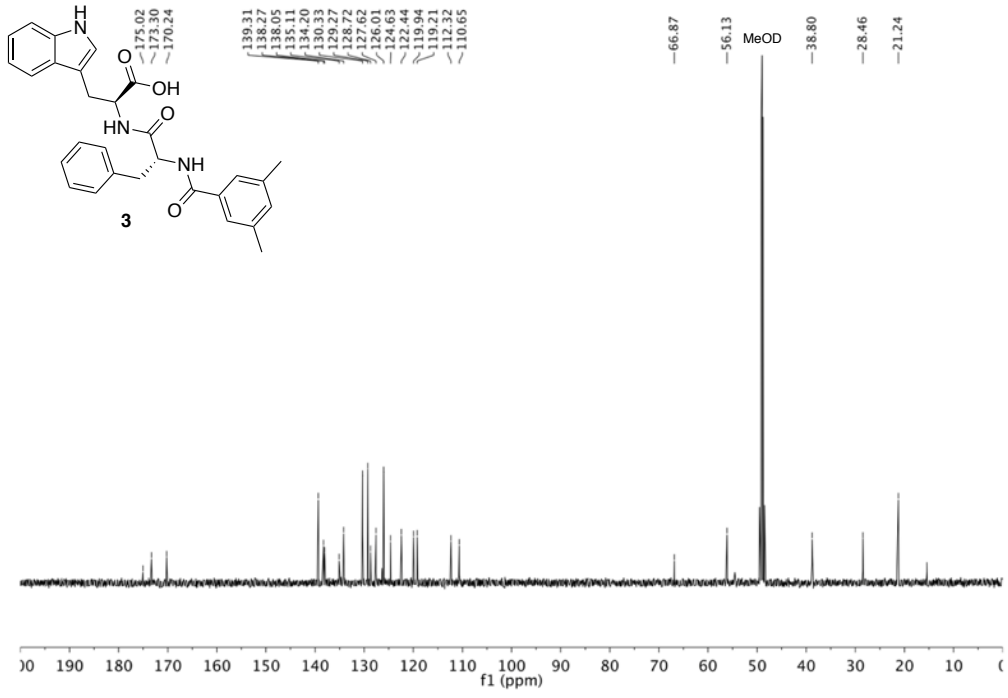
*((*R*)-3-(4-(5-((dimethylamino)methyl)furan-2-yl)phenyl)-2-(3,5-dimethylbenzamido)propanoyl)*-L-*tryptophan* (**22**). Carbamate **14** (67 mg, 0.09 mmol) was

deprotected and purified by preparative thin layer chromatography (4% MeOH/CH_2Cl_2) to afford the ester as an off-white foam (33 mg, 0.05 mmol). This ester was saponified to afford the product as off-white solid (33 mg, 59% over 2 steps). m.p. = 152-169 °C. IR (solid) 3288, 2922, 1644, 1602, 1498 cm^{-1}. ^1H NMR (500 MHz, Chloroform-*d*) δ 7.61 – 7.57 (m, 1H), 7.42 (d, *J* = 8.0 Hz, 2H), 7.26 (d, *J* = 8.0 Hz, 1H), 7.15 (s, 2H), 7.08 (d, *J* = 11.2 Hz, 2H), 7.01 (dd, *J* = 17.7, 7.8 Hz, 3H), 6.94 (t, *J* = 7.4 Hz, 1H), 6.68 (s, 2H), 4.70 (dd, *J* = 7.1, 4.8 Hz, 1H), 4.29 – 4.19 (m, 2H), 3.35 (dd, *J* = 14.6, 5.0 Hz, 1H), 3.21 (ddd, *J* = 23.2, 14.3, 6.1 Hz, 2H), 2.87 (dd, *J* = 13.9, 8.8 Hz, 1H), 2.72 (s, 6H), 2.24 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 175.75, 171.06, 171.03, 155.78, 137.75, 137.35, 136.48, 133.53, 132.64, 129.42, 128.17, 127.65, 124.50, 123.43, 123.14, 120.77, 118.35, 118.00, 115.52, 115.41, 110.73, 109.82, 105.35, 54.58, 52.68, 41.41, 41.30, 37.05, 27.27, 19.71. HRMS (ESI) m/z calc'd for $C_{36}H_{38}N_4O_5$ $[M+H]^+$ 607.2920, found 607.2913.

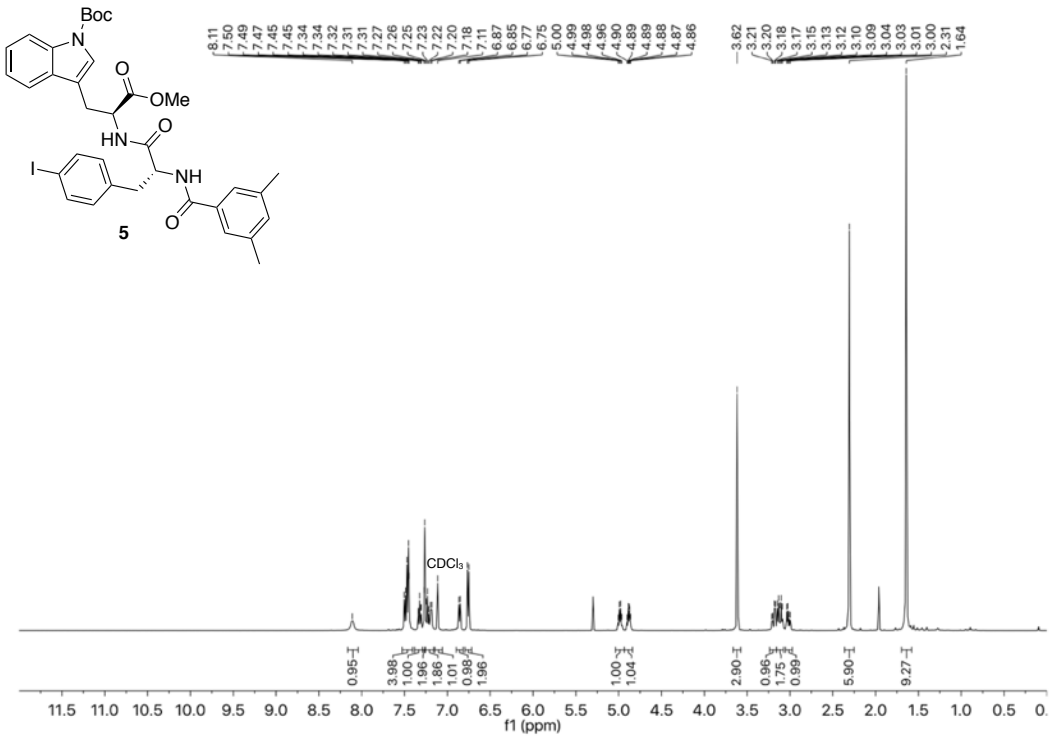
NMR SPECTRA



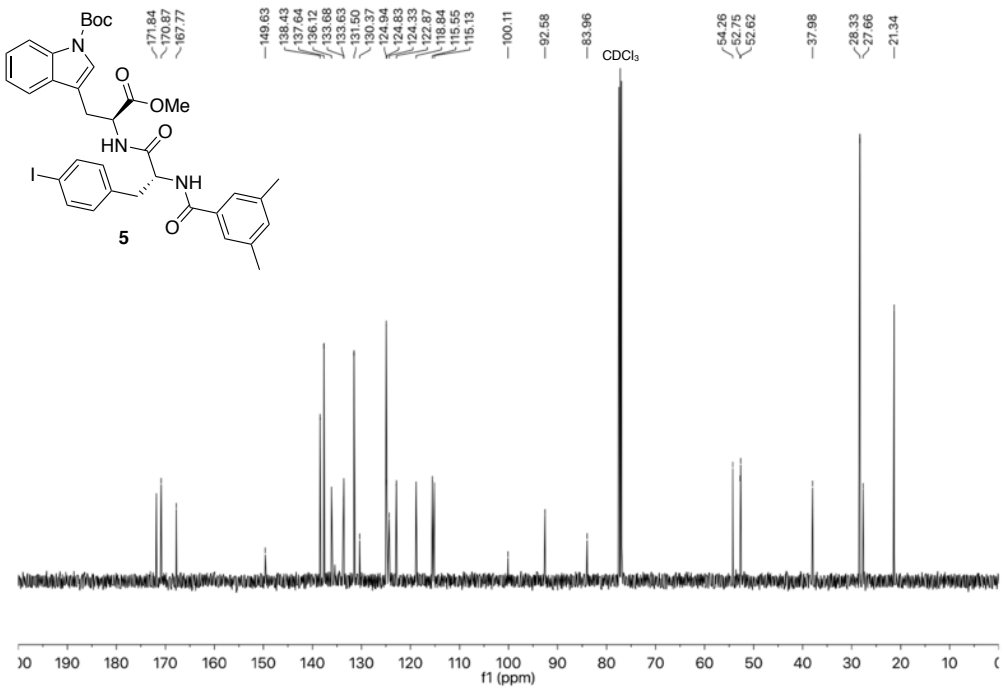
^{1}H NMR of **3**



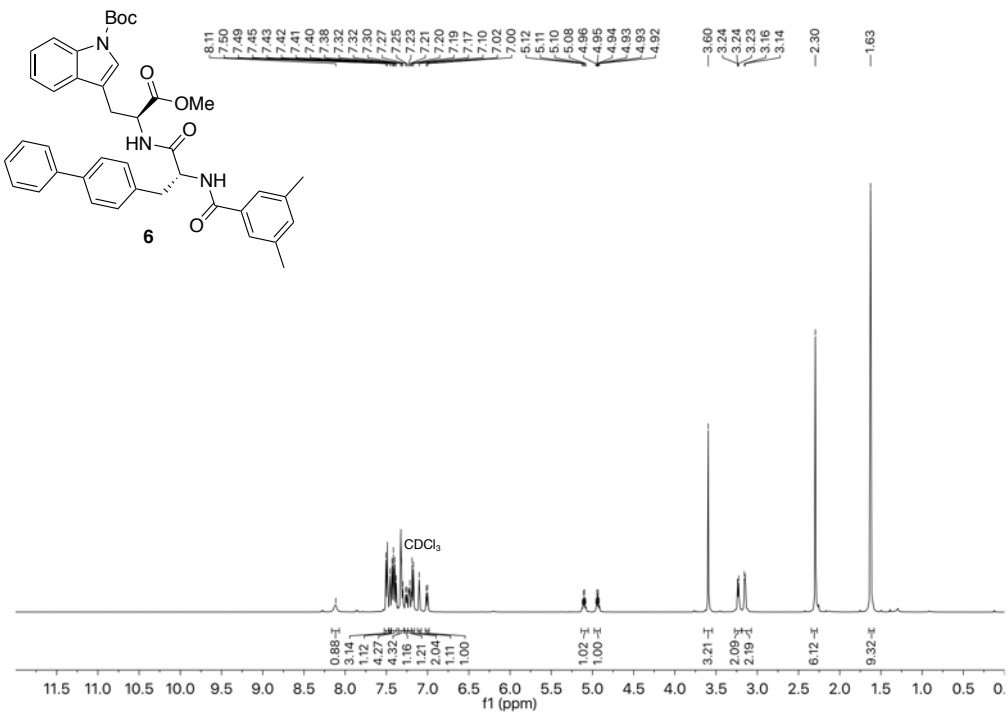
^{13}C NMR of **3**



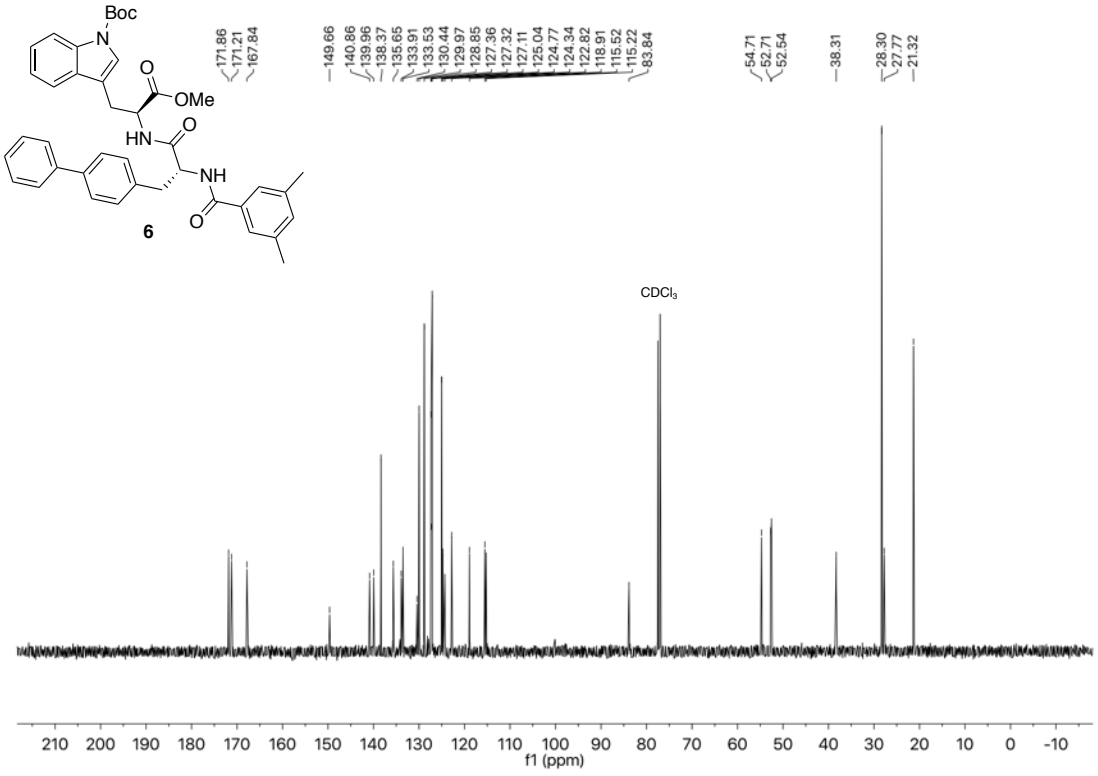
^{1}H NMR of **5**



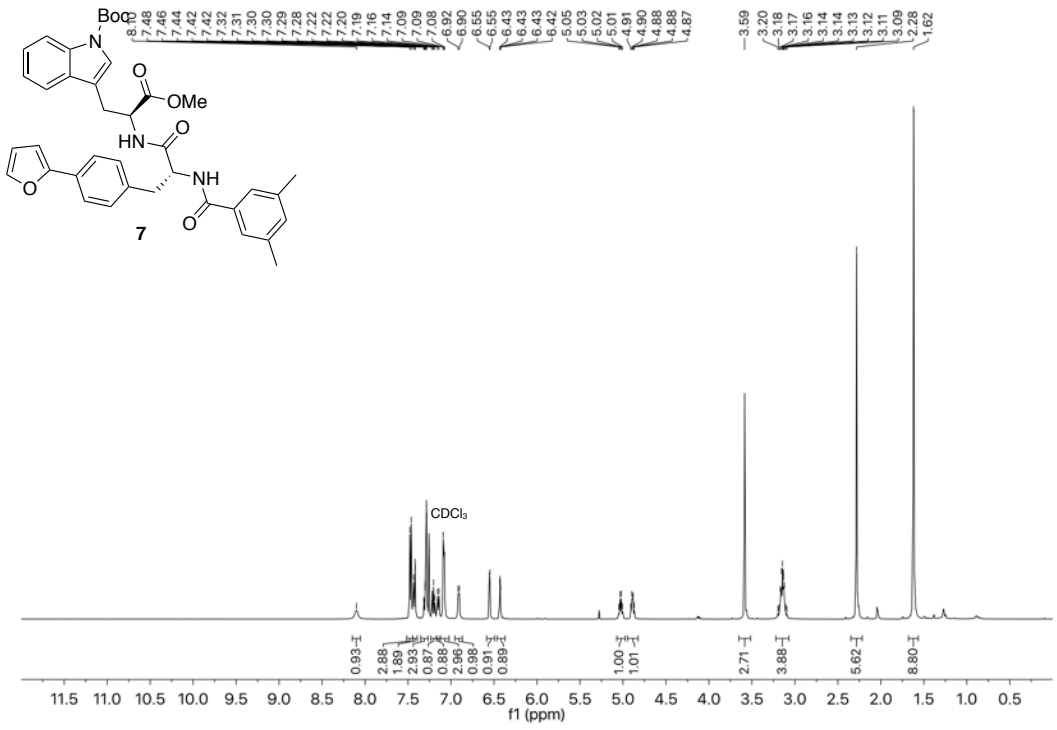
^{13}C NMR of **5**



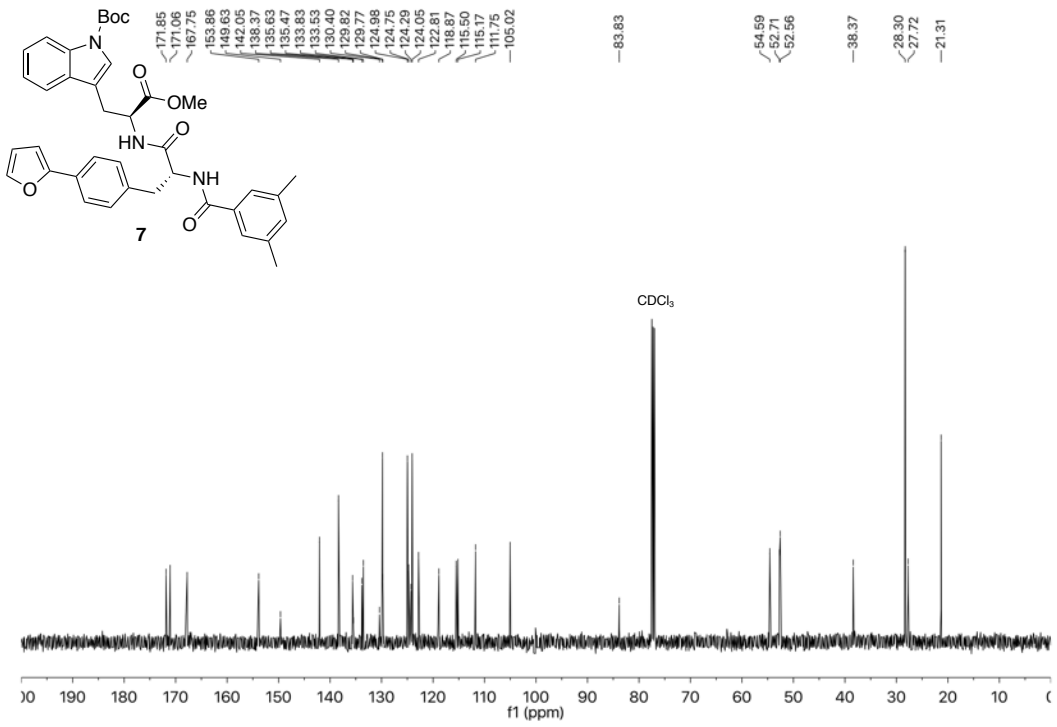
^{1}H NMR of **6**



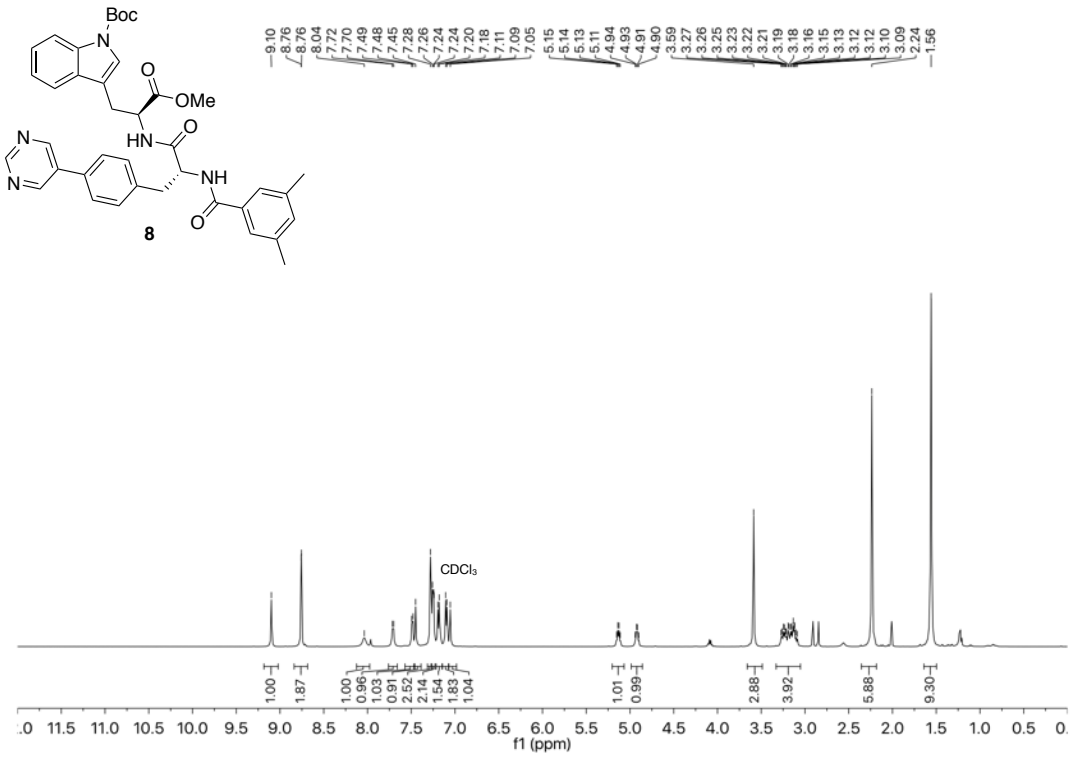
^{13}C NMR of **6**



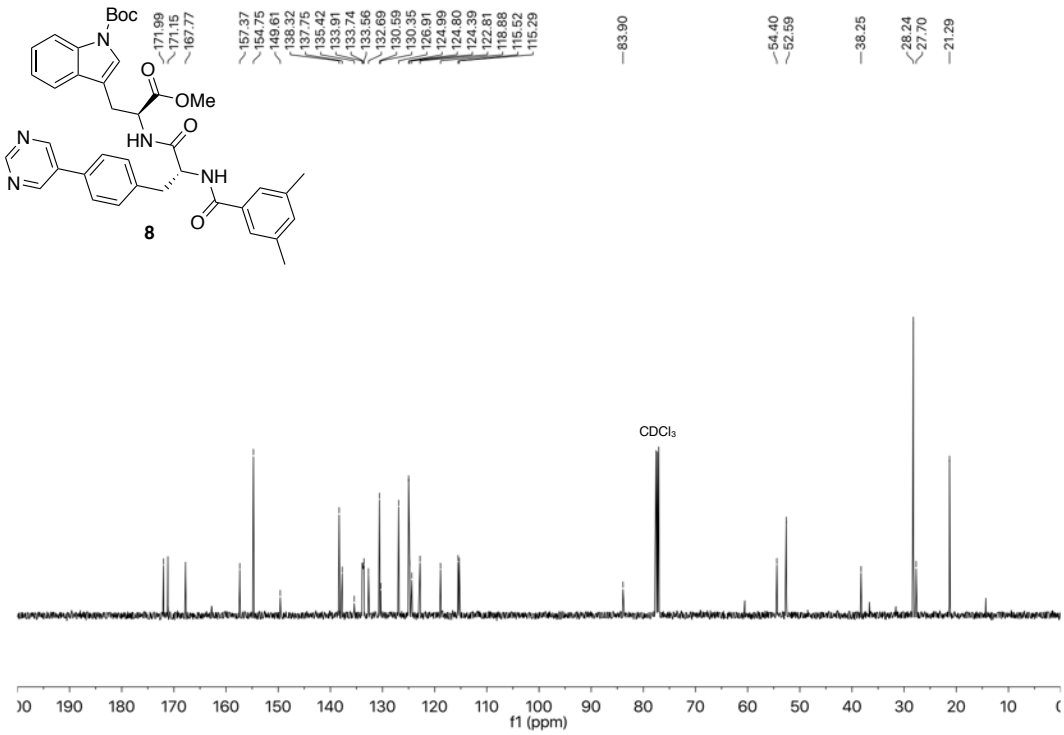
^{1}H NMR of **7**



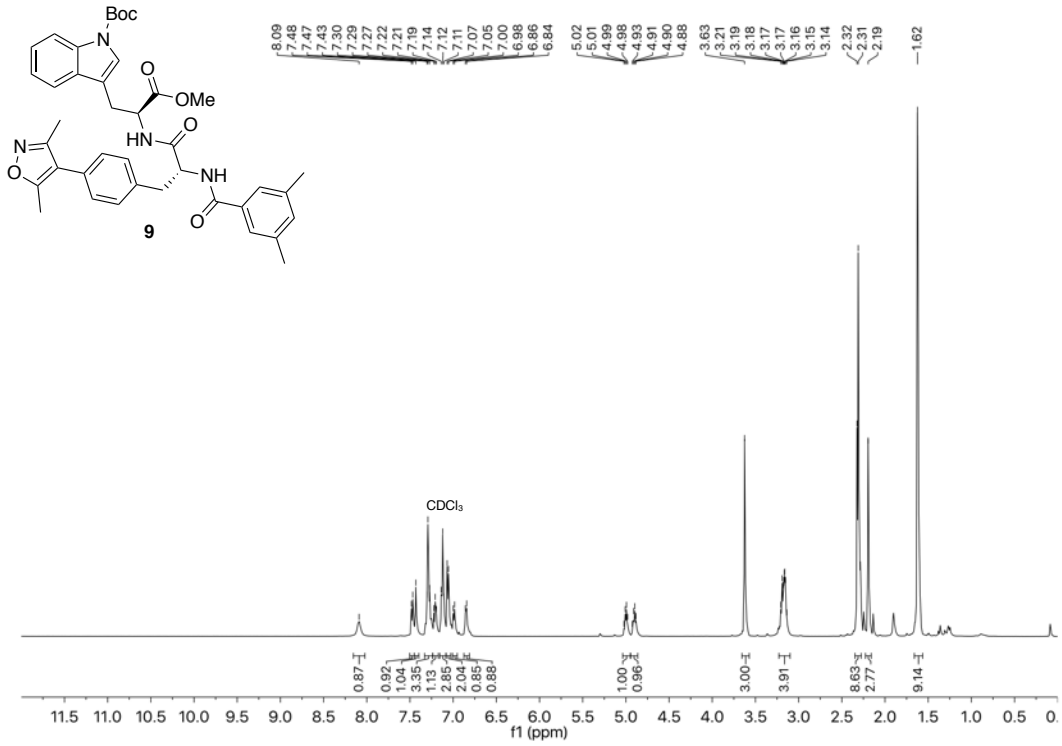
^{13}C NMR of **7**



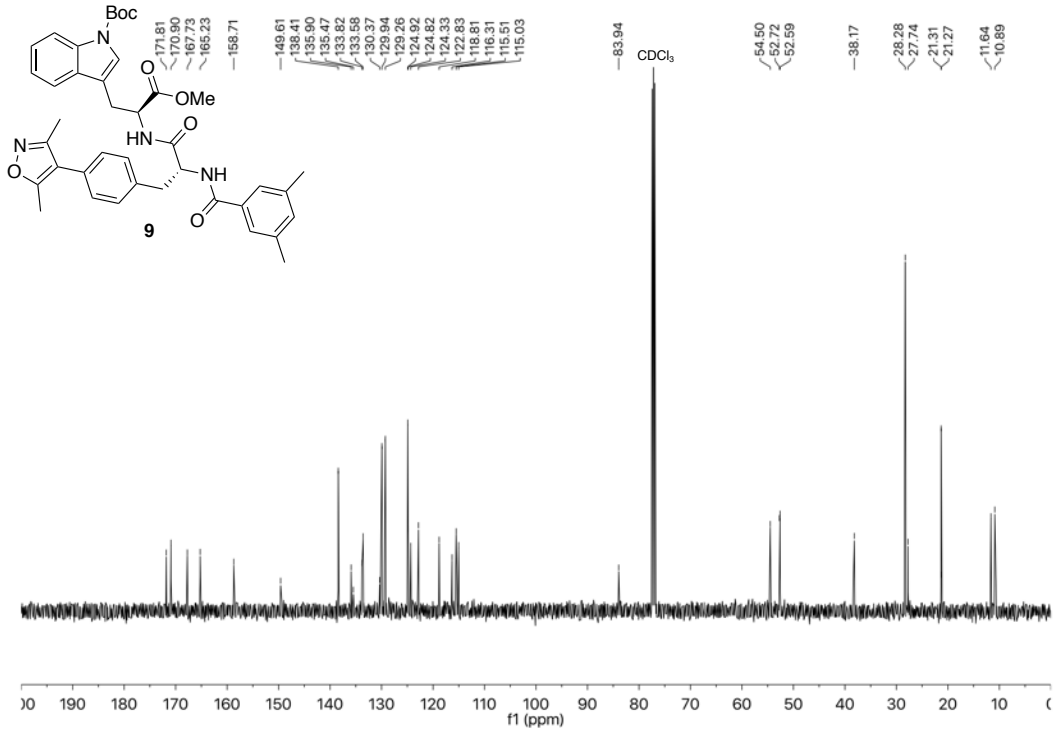
^{1}H NMR of **8**



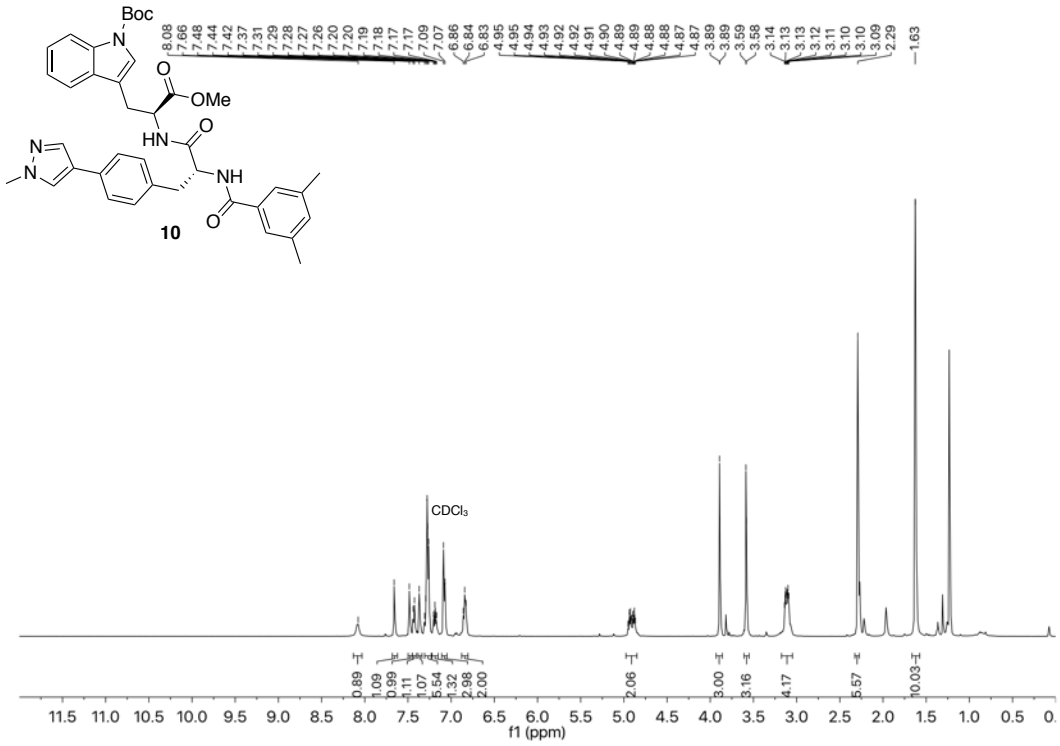
^{13}C NMR of **8**



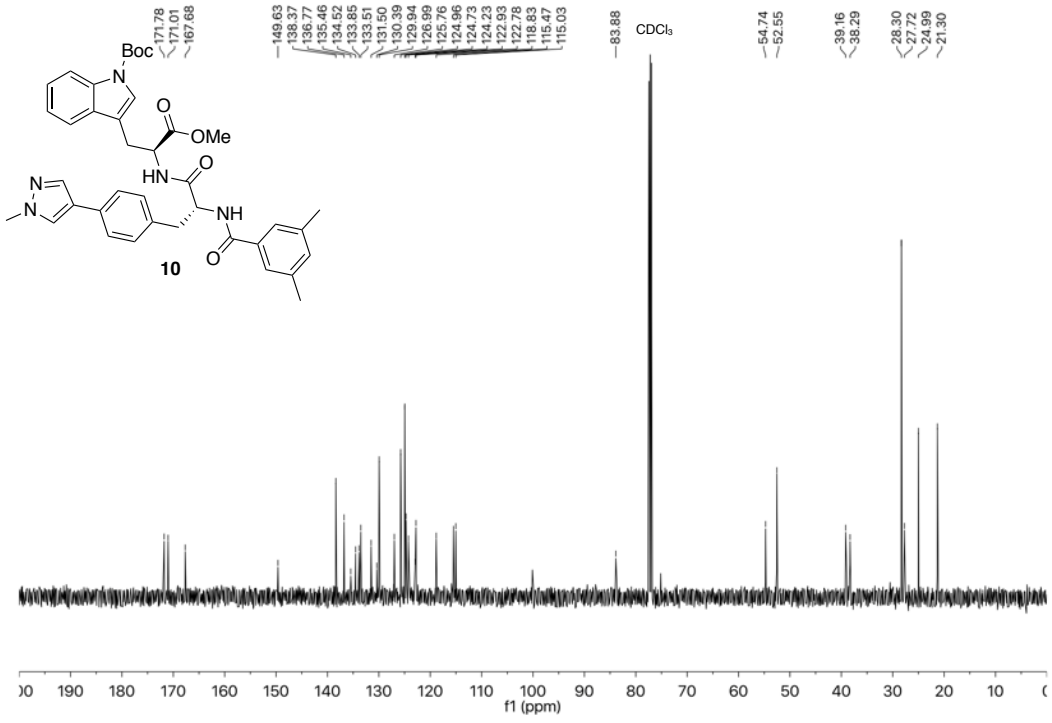
^{1}H NMR of **9**



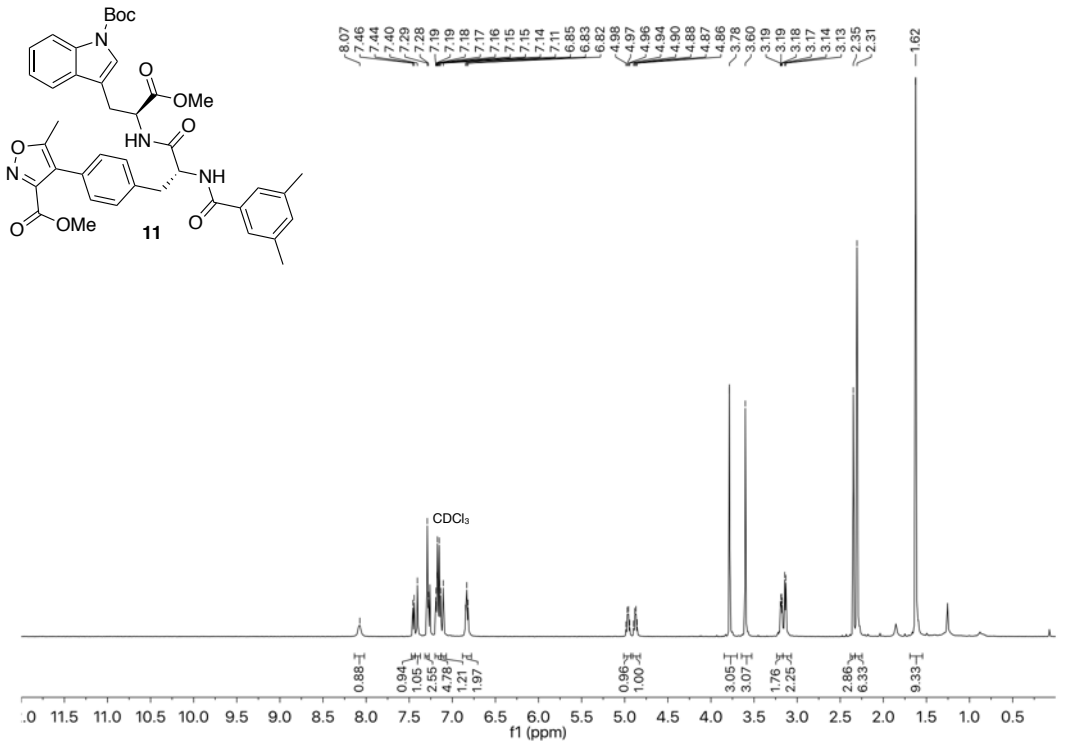
^{13}C NMR of **9**



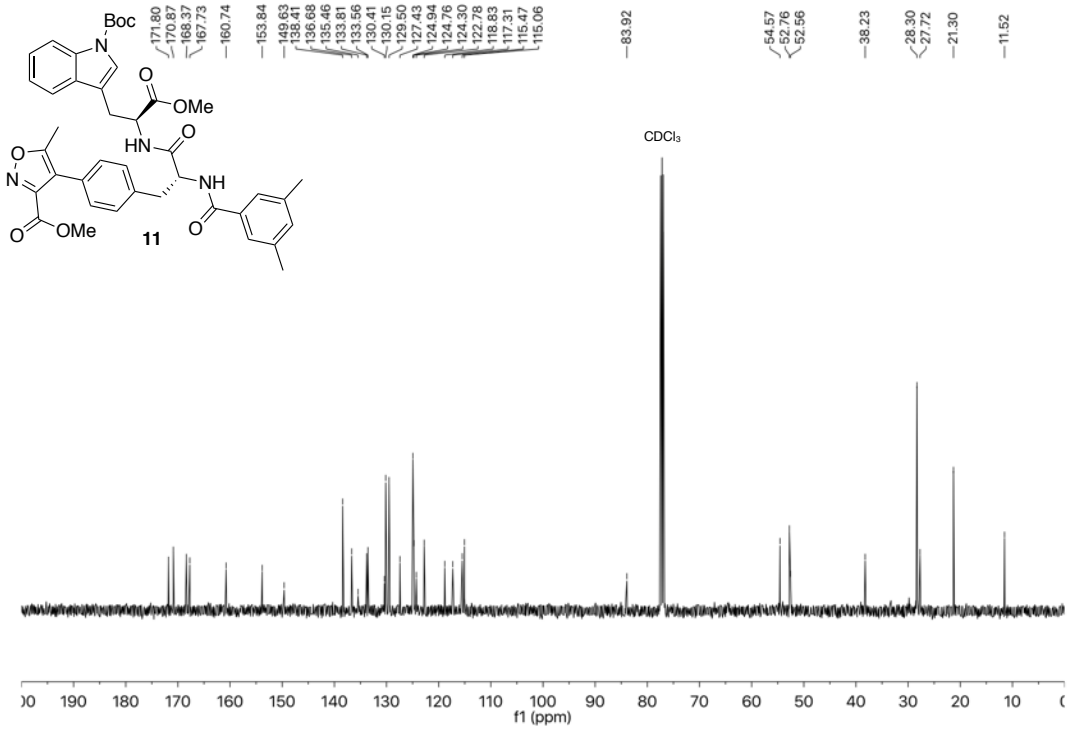
^{1}H NMR of **10**



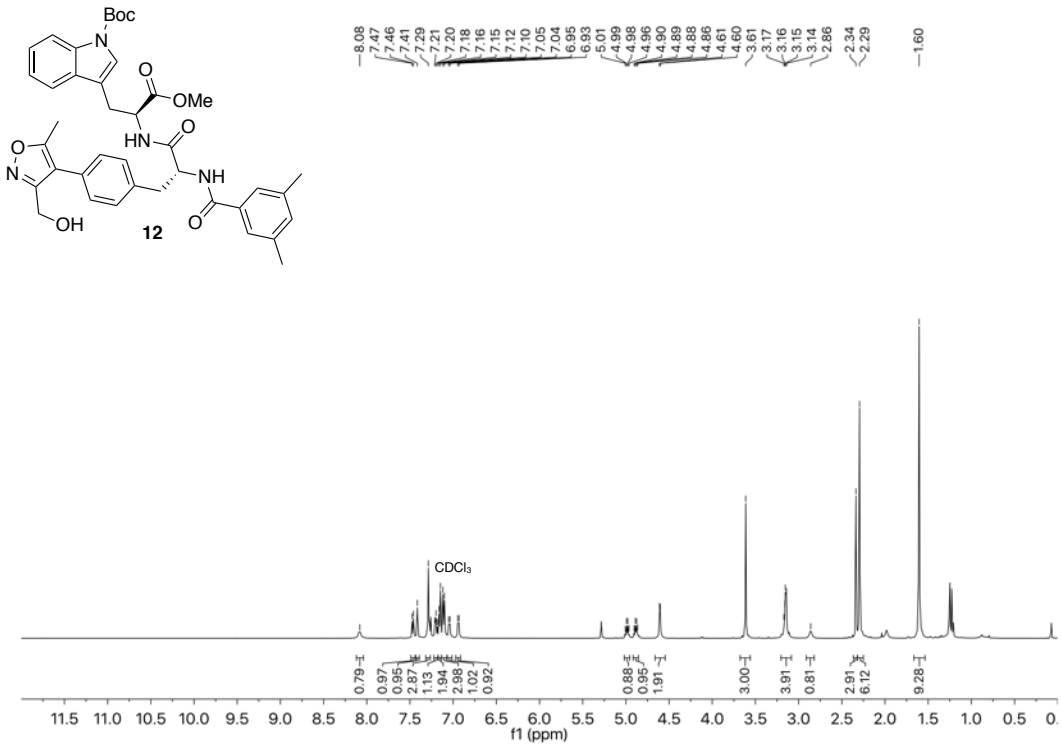
^{13}C NMR of **10**



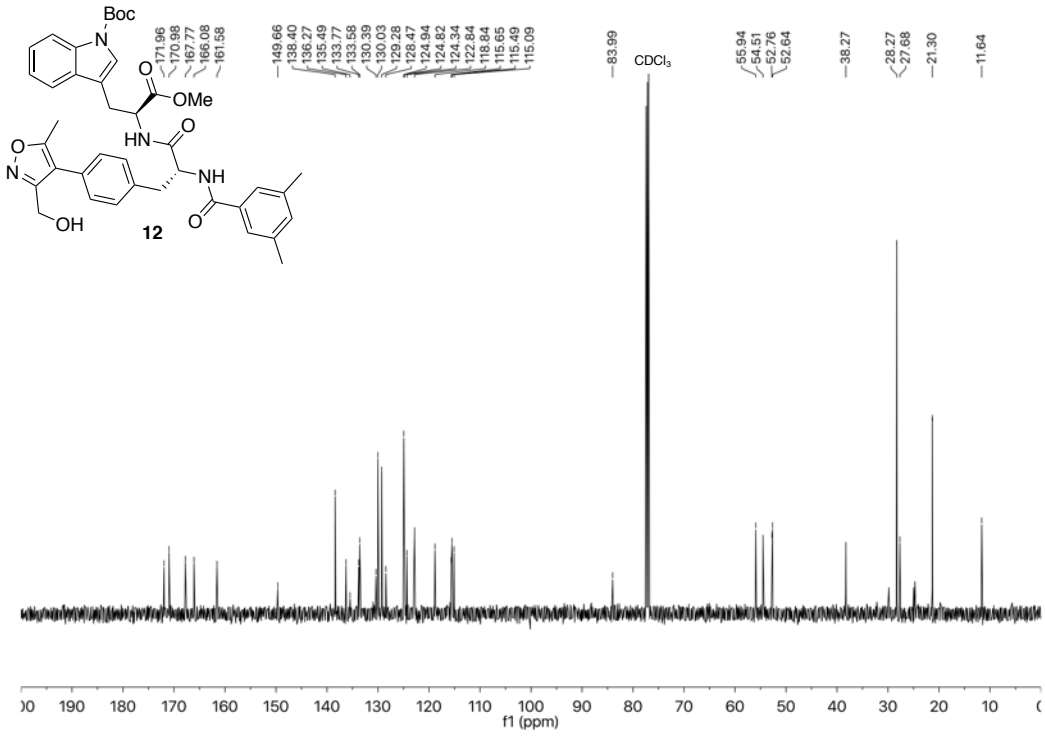
^{1}H NMR of **11**



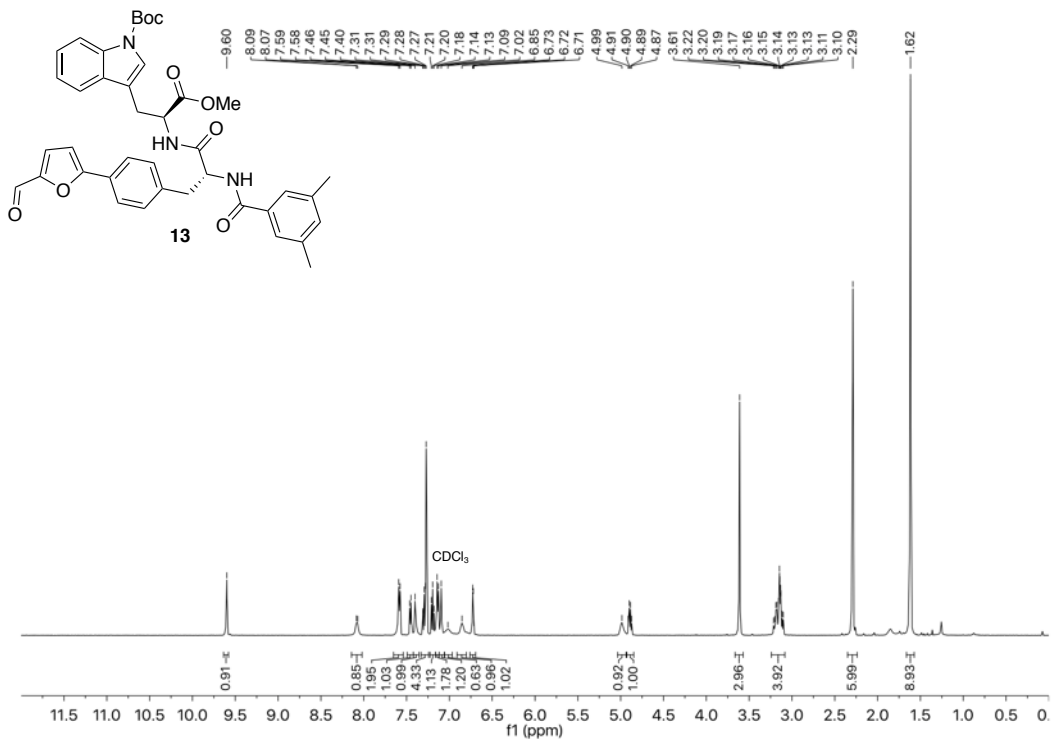
^{13}C NMR of **11**



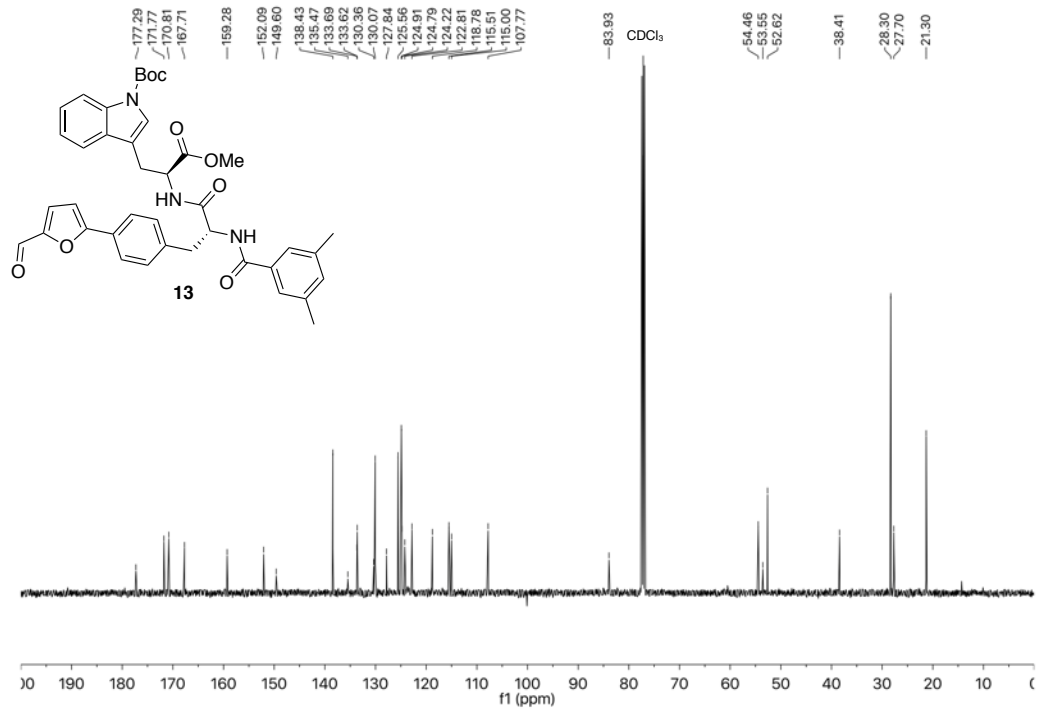
^{1}H NMR of **12**



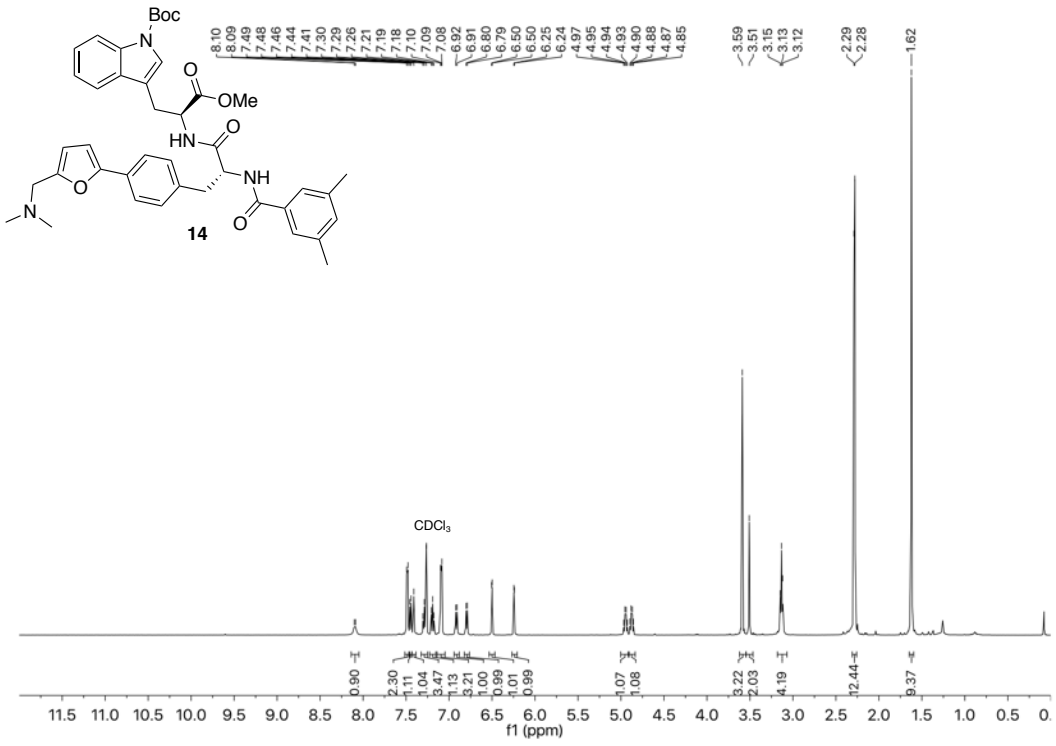
^{13}C NMR of **12**



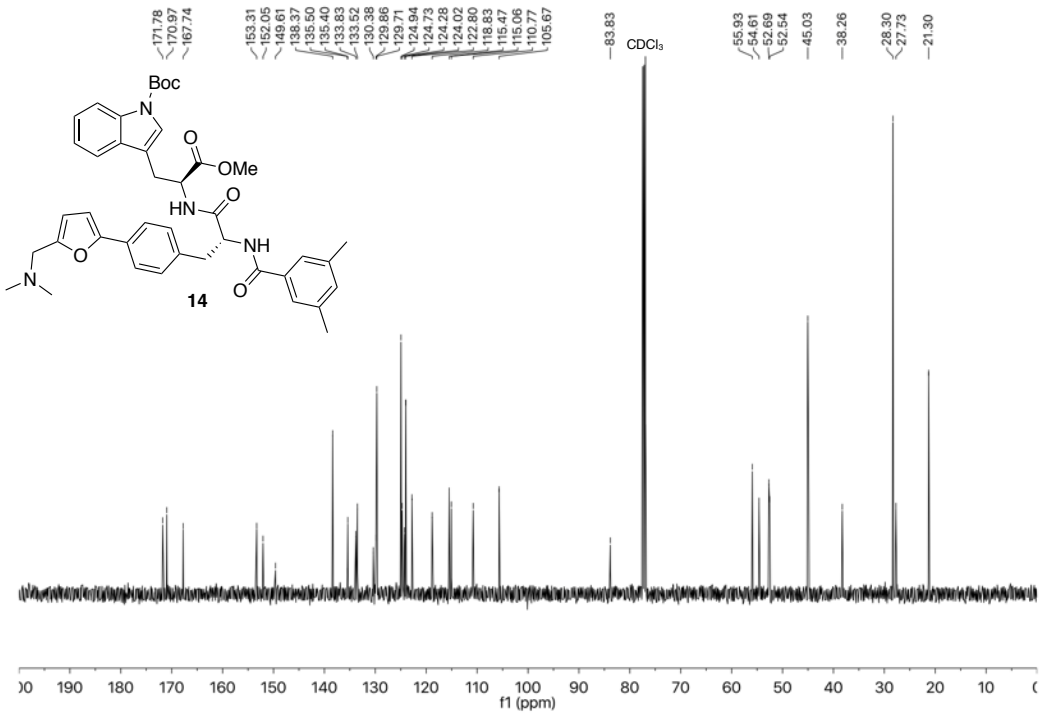
^{1}H NMR of **13**



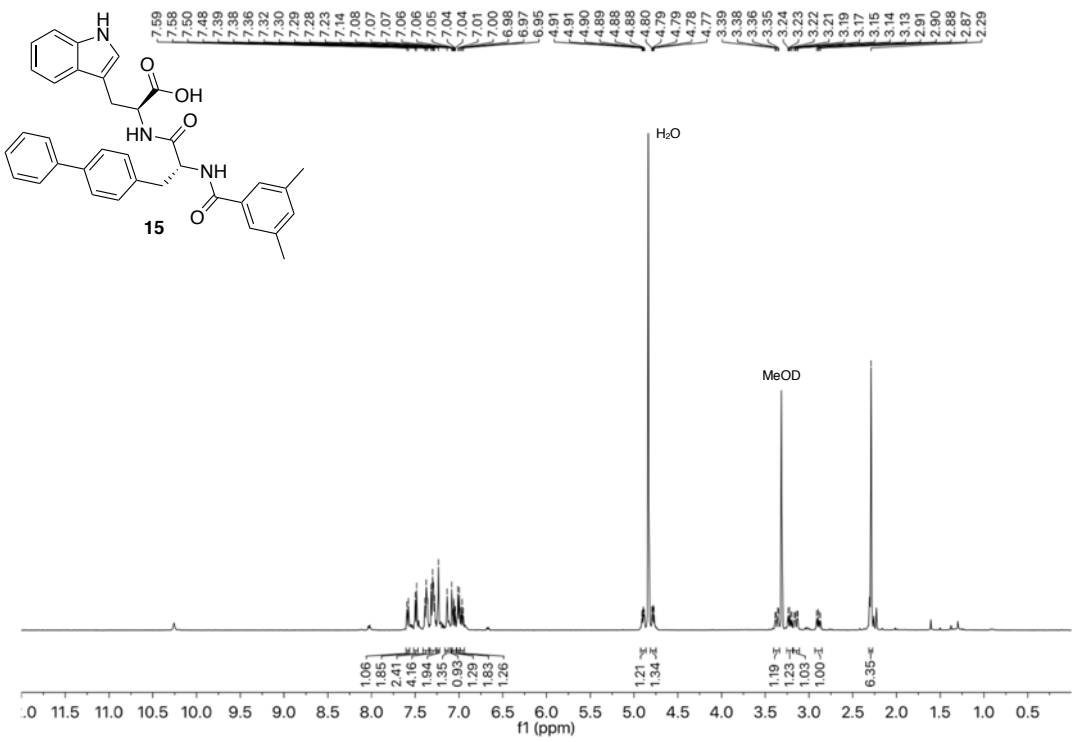
^{13}C NMR of **13**



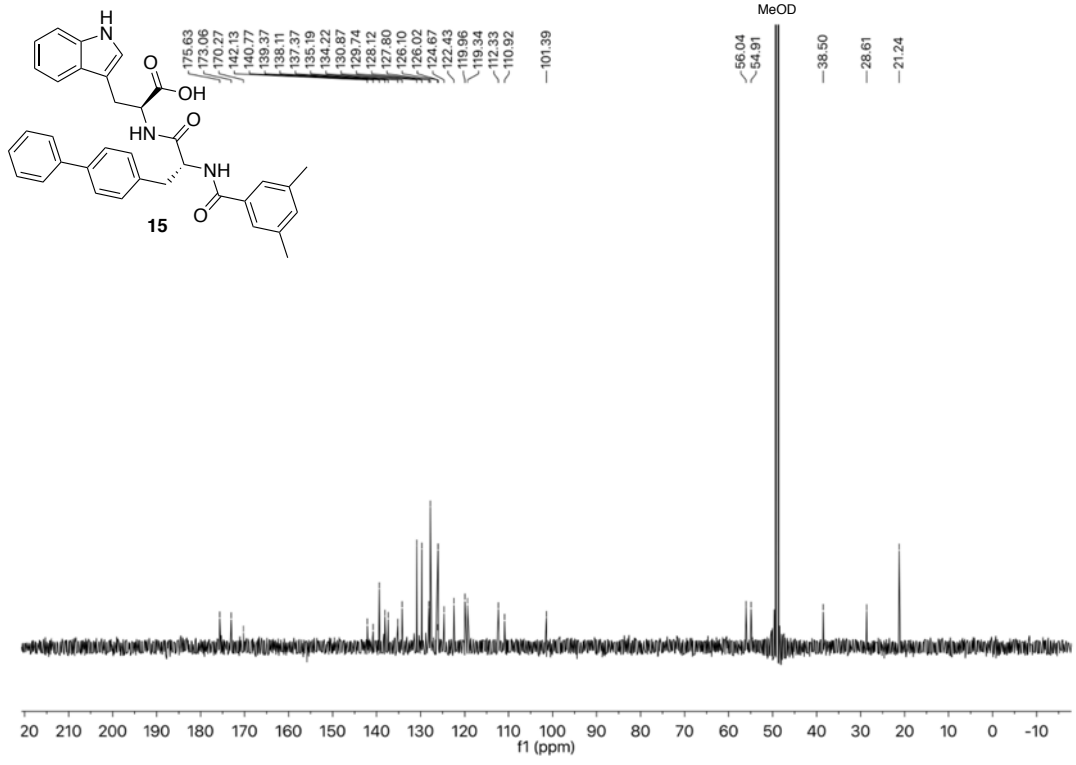
^{1}H NMR of **14**



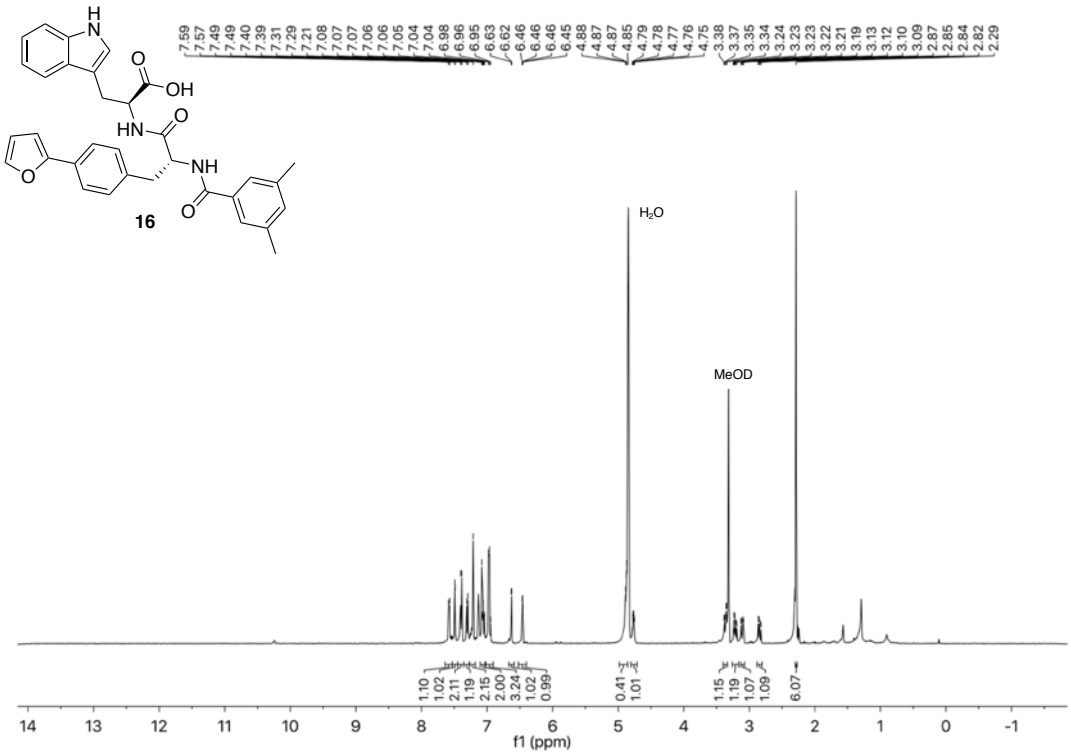
^{13}C NMR of **14**



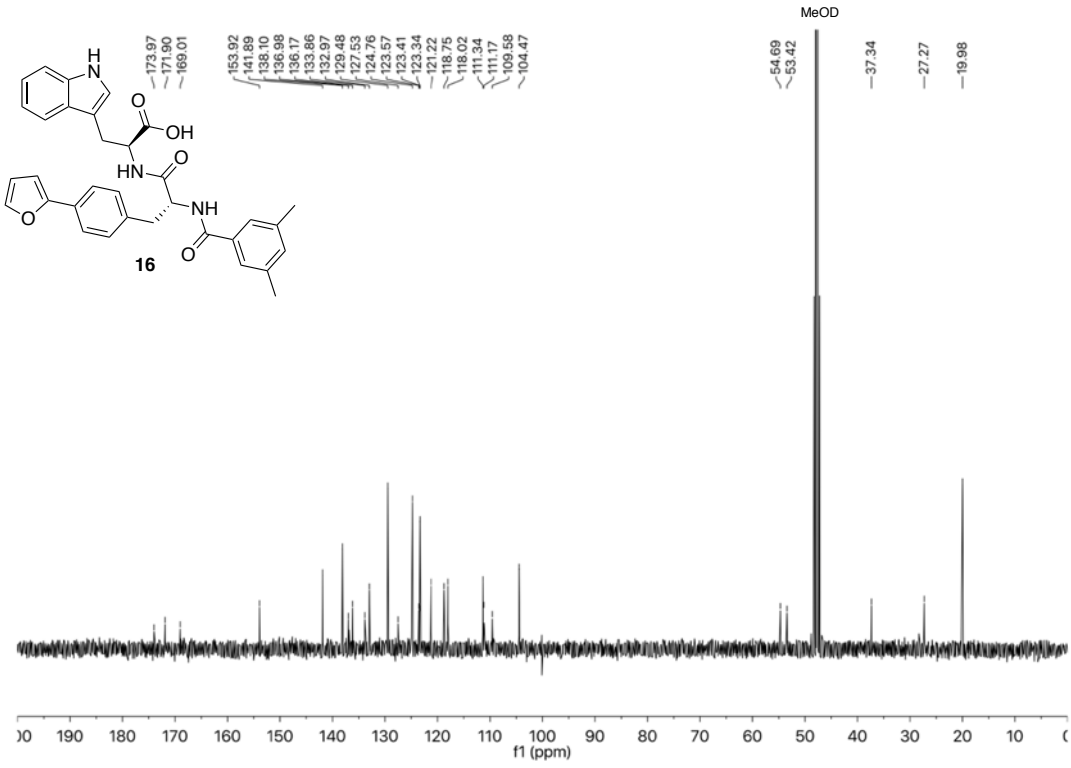
^{1}H NMR of **15**



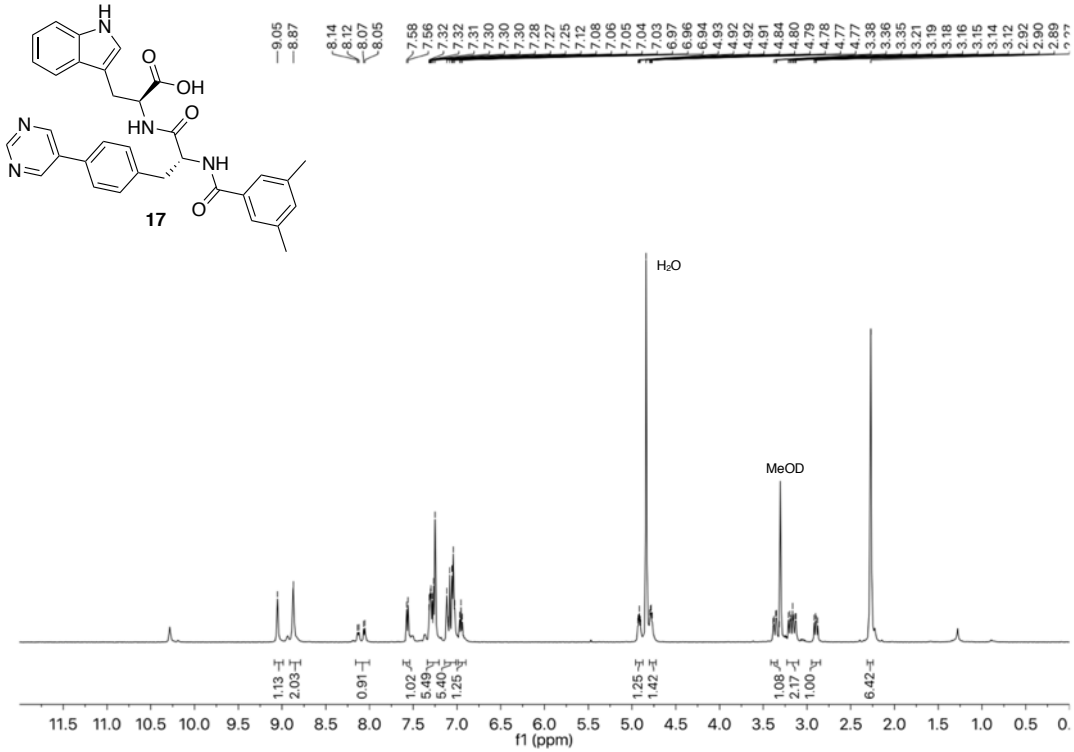
^{13}C NMR of **15**



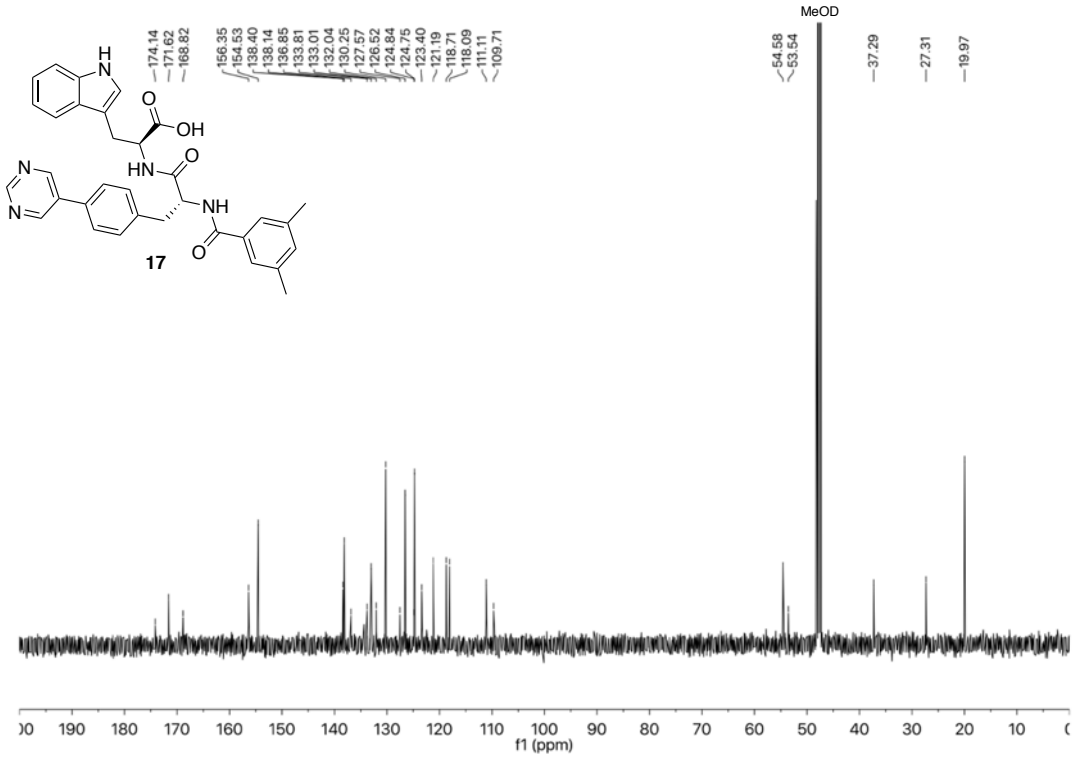
^{1}H NMR of **16**



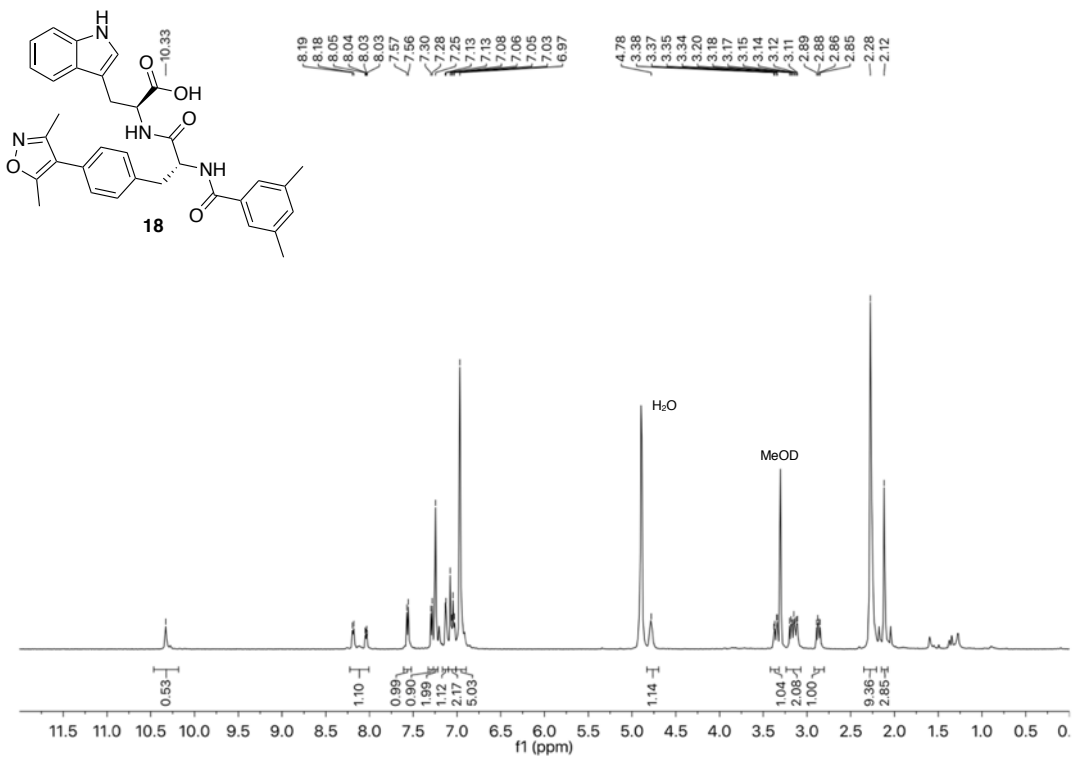
^{13}C NMR of **16**



^{1}H NMR of **17**



^{13}C NMR of **17**



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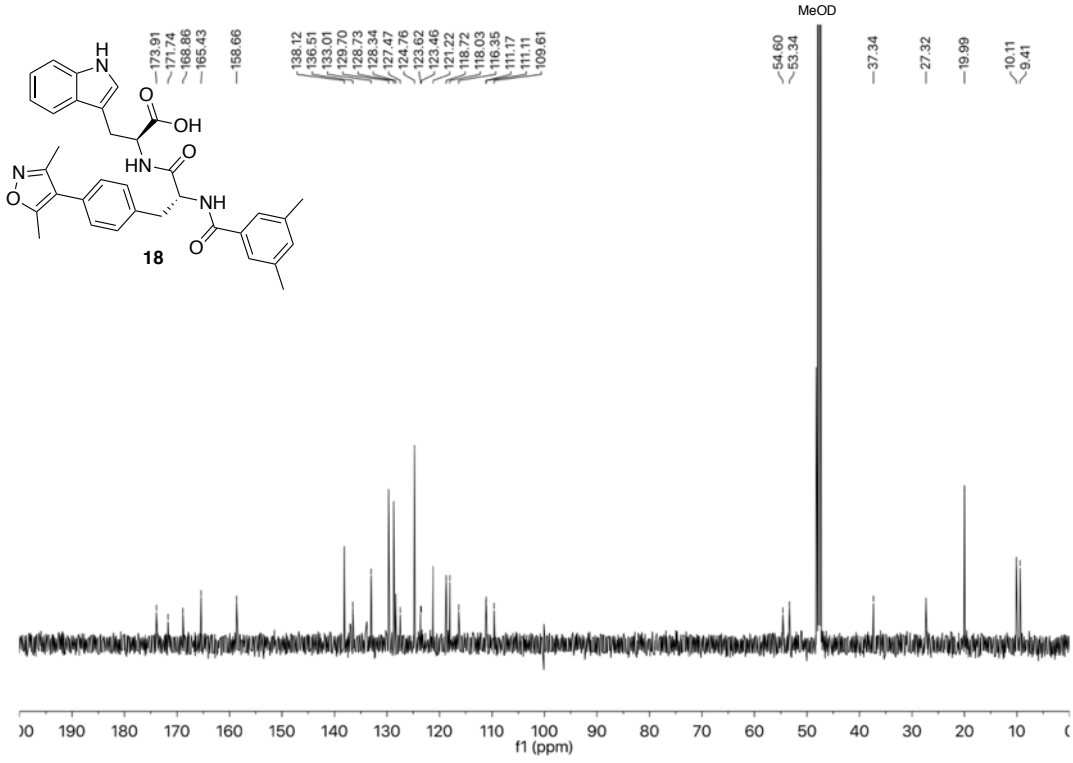

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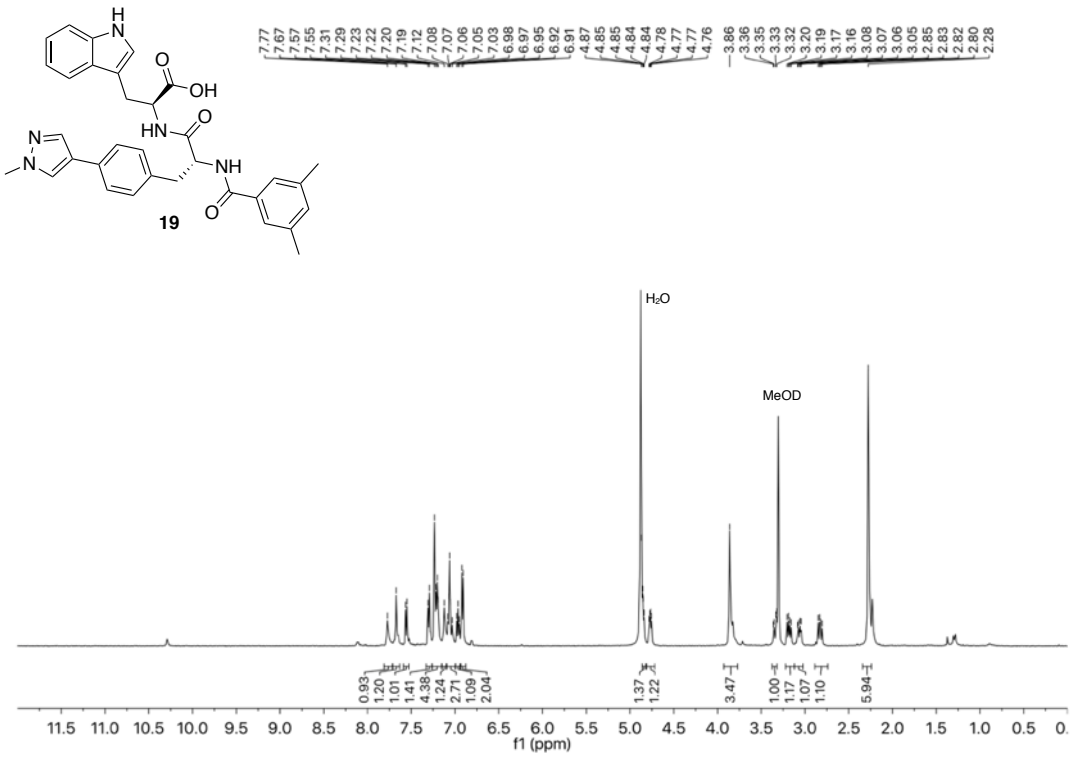
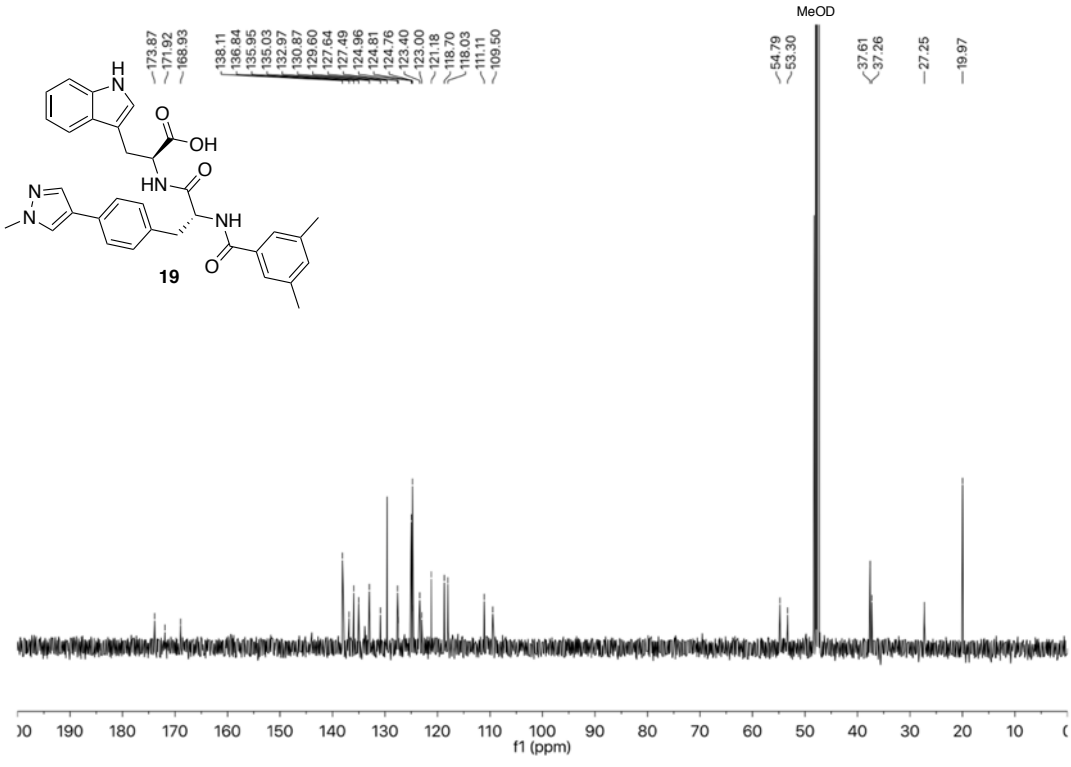
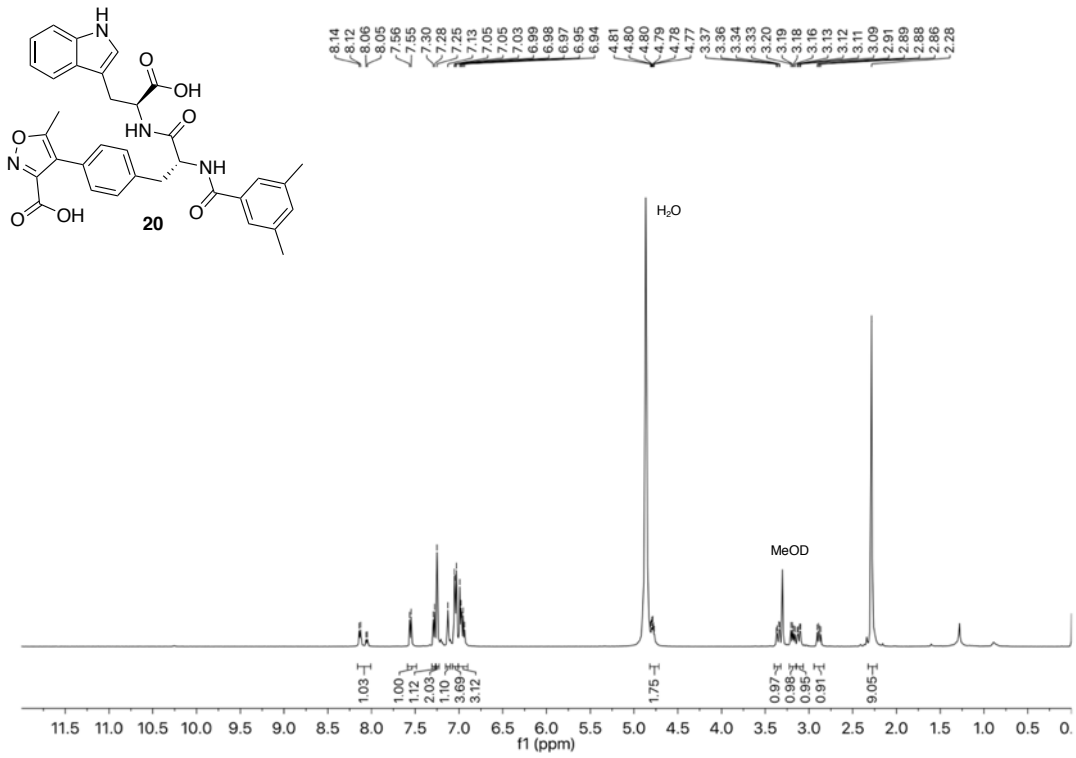


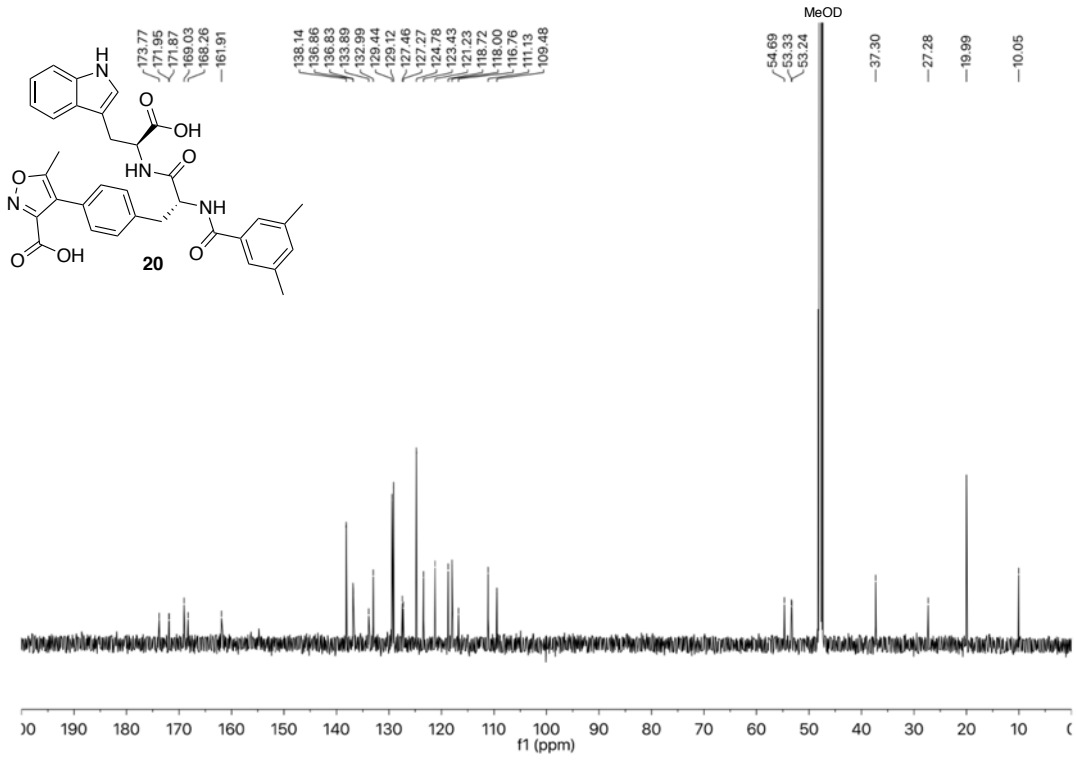
^{1}H NMR of **19**



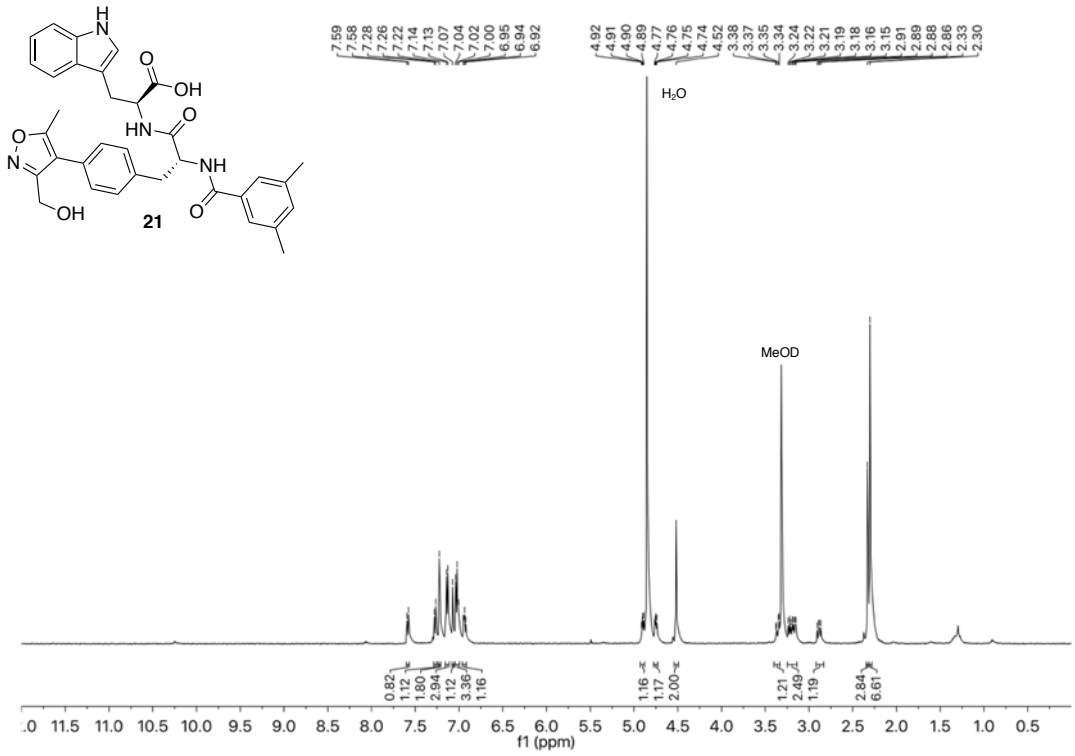
^{13}C NMR of **19**



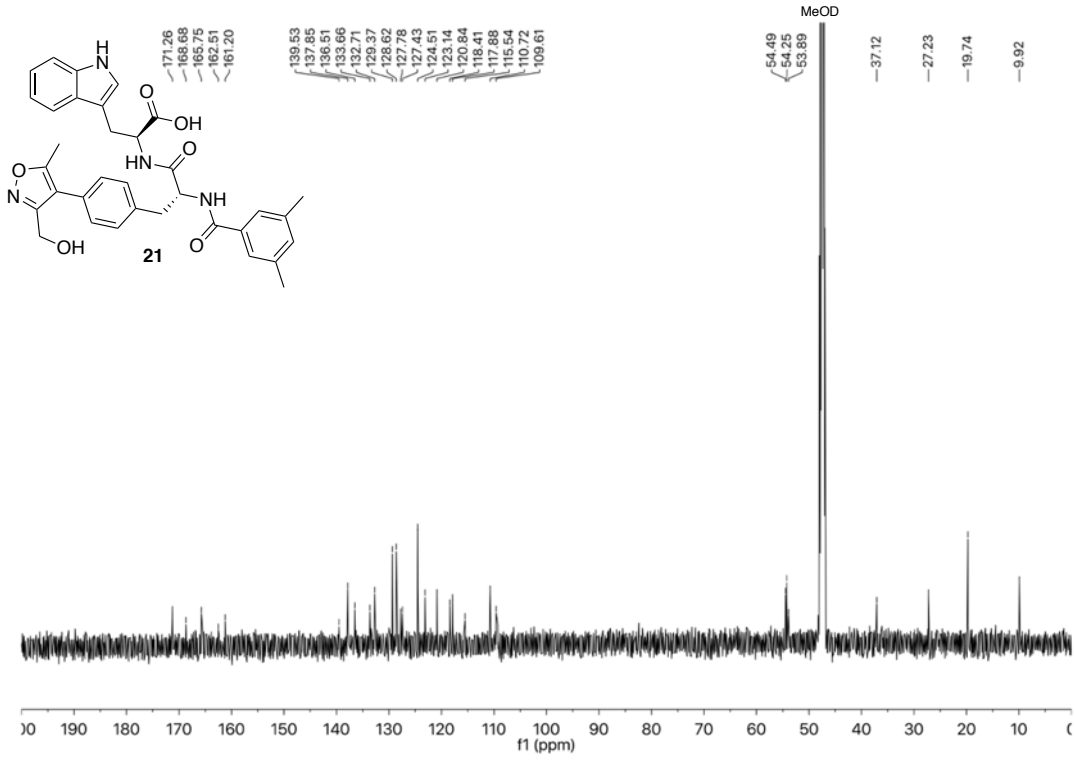
^{1}H NMR of **20**



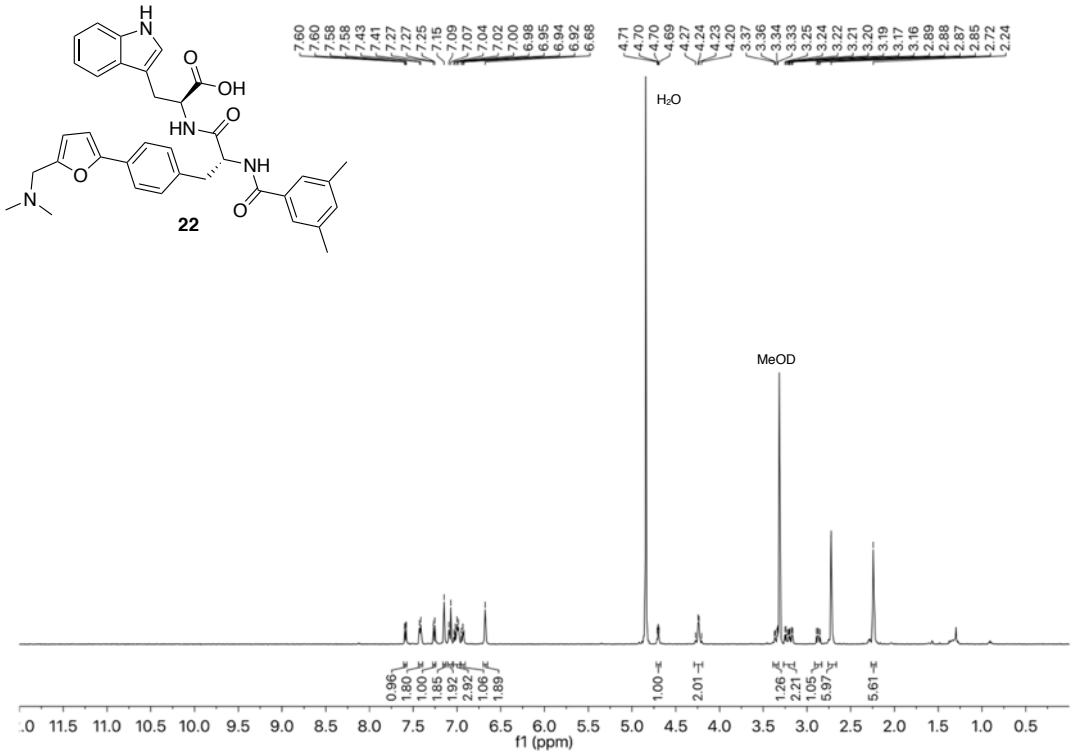
^{13}C NMR of **20**



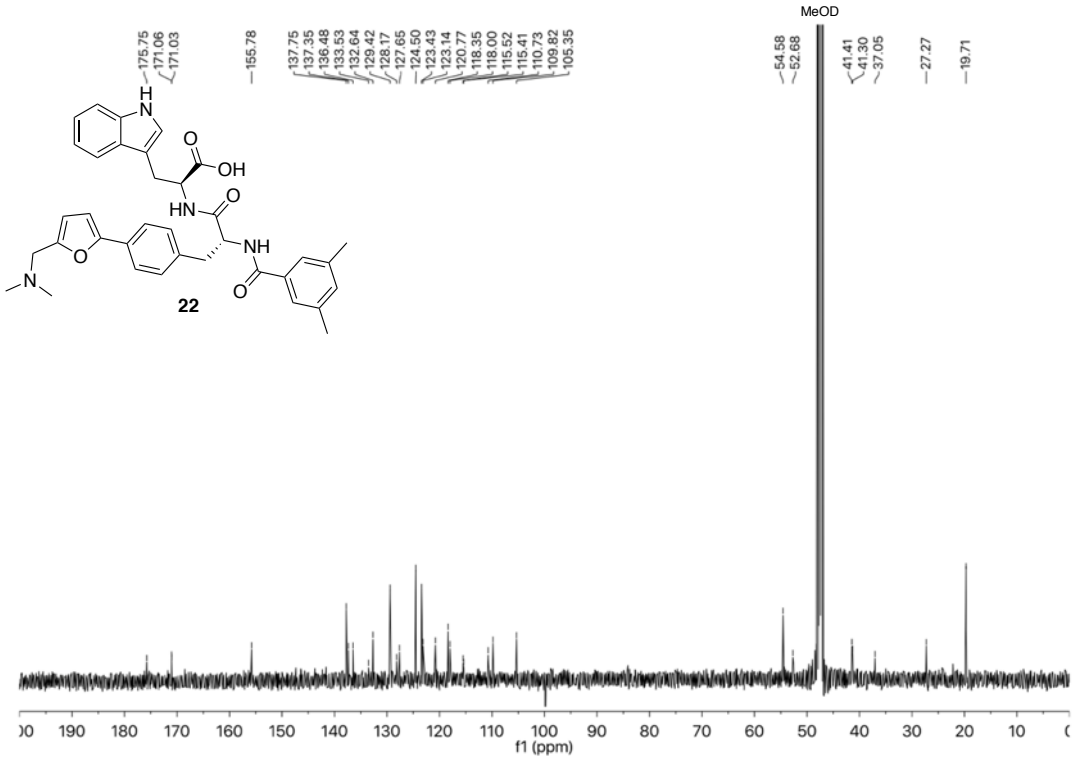
^{1}H NMR of **21**



^{13}C NMR of **21**



^{1}H NMR of **22**



^{13}C NMR of **22**

REFERENCES

(1) Safo, M. K.; Abraham, D. J. X-Ray Crystallography of Hemoglobins. In *Methods in Molecular Medicine: Hemoglobin Disorders. Molecular Methods and Protocols*; 2003; p 1-20.

(2) Winn, M. D.; Ballard, C. C.; Cowtan, K. D.; Dodson, E. J.; Emsley, P.; Evans, P. R.; Keegan, R. M.; Krissinel, E. B.; Leslie, A. G. W.; McCoy, A.; McNicholas, S. J.; Murshudov, G. N.; Pannu, N. S.; Potterton, E. A.; Powell, H. R.; Read, R. J.; Vagin, A.; Wilson, K. S. Overview of the CCP4 Suite and Current Developments. *Acta Crystallogr*. **2011**, *D67*, 235–242.

(3) Brunger, A. T.; Adams, P. D.; Clore, G. M.; DeLano, W. L.; Gros, P.; Grosse-Kunstleve, R. W.; Jiang, J.-S.; Kuszewski, J.; Nilges, M.; Pannu, N. S.; Read, R. J.; Rice, L. M.; Simonson, T.; Warren, G. L. Crystallography & NMR System: A New Software Suite for Macromolecular Structure Determination. *Acta Crystallogr*. **1998**, *D54*, 905–921.

(4) Emsley, P.; Lohkamp, B.; Scott, W. G.; Cowtan, K. Features and Development of Coot. *Acta Crystallogr*. **2010**, *66*, 486–501.

(5) Jahani, F.; Tahbakhsh, M.; Golchoubian, H.; Khaksar, S. Guanidine Hydrochloride as an Organocatalyst for N-Boc Protection of Amino Groups. *Tetrahedron Lett*. **2011**, *52* (12), 1260-1264.

(6) Williams, T. J.; Reay, A. J.; Whitwood, A. C.; Fairlamb, I. J. S. A Mild and Selective Pd-Mediated Methodology for the Synthesis of Highly Fluorescent-2-Arylated Tryptophans and Tryptophan-Containing Peptides: A Catalytic Role for Pd^0 Nanoparticles. *Chem. Commun*. **2014**, *50* (23), 3052-3054.

(7) Pyun, D. K.; Lee, C. H.; Ha, H.-J.; Park, C. S.; Chang, J.-W.; Lee, W. K. Synthesis of Substituted-(L)-Tryptophanols from an Enantiomerically Pure Aziridine-2-Methanol. *Org. Lett.* **2001**, *3* (26), 4197–4199.

(8) Rannoux, C.; Roussi, F.; Retailleau, P.; Gueritte, F. Direct and Selective Functionalization of Tetrahydro-β-Carboline at Position 4. *Org. Lett.* **2010**, *12* (6), 1240–1243.

(9) Okumura, H. S.; Philmus, B.; Portmann, C.; Hemscheidt, T. K. Homotyrosine-Containing Cyanopeptolins 880 and 960 and Anabaenopeptins 908 and 915 from Planktothrix Agardhii CYA 126/8. *J. Nat. Prod.* **2009**, *72* (1), 172–176.

(10) Technology, N.I. of A.I.S. and. O-Acetoxybenzoic Acid. *Spectral Database for Organic Compounds*. http://sdbs.db.aist.go.jp/sdbs/ (accessed May 29, 2017).

(11) Ritzen, A.; Basu, B.; Chattopadhyay, S. K.; Dossa, F.; Frejd, T. Synthesis of Optically Active Arylene Bis-Analine Derivatives Carrying Orthogonal Protecting Groups. *Tetrahedron: Asymmetry* **1998**, *9* (3), 503–512.

(12) Pasunooti, K. K.; Banerjee, B.; Yap, T.; Jiang, Y.; Liu, C.-F. Auxiliary-Directed Pd-Catalyzed γ-C(sp3)–H Bond Activation of α-Aminobutanoic Acid Derivatives. *Org. Lett.* **2015**, *17* (24), 6094–6097.

(13) Shakya, G.; Rivera Jr, H.; Lee, D. J.; Jaremko, M. J.; La Clair, J. J.; Fox, D. T.; Haushalter, R. W.; Schaub, A. J.; Bruegger, J.; Barajas, J. F.; White, A. R.; Kaur, P.; Gwozdziowski, E. R.; Wong, F.; Tsai, S.-C.; Burkart, M. D. Modeling Linear and Cyclic PKS Intermediates through Atom Replacement. *J. Am. Chem. Soc.* **2014**, *136* (48), 16792–16799.