Chemoselective, Regioselective, and Positionally Selective Fluorogenic Stapling of Unprotected Peptides for Cellular Uptake and Direct Cell Imaging

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Experimental Procedures

Materials

Unless otherwise stated, chemicals were purchased from Ambeed, Sigma Aldrich, TCI America, and Acros Organics and were used without further purification. Standard L-Fmoc-AA-OH and coupling reagents were purchased from Gyros Protein Technologies, Aapptec Peptides, and Ambeed. Fmoc-Rink-Amide-MBHA resin (0.70 mmol/g) was purchased from Iris Biotech GmbH and used in all Solid Phase Peptide Synthesis (SPPS) outlined in this supplementary material. HPLC-grade solvents (THF, DCM, DMF, and MeCN) were used unless otherwise stated.

NMR Spectroscopy:
^{1}H and ^{13}C and ^{19}F NMR spectra for 2-arylketobenzaldehydes compounds and their intermediates (**1-6**) were acquired on Bruker Avance 300 Spectrometer equipped with a BACS-120 autosampler and Bruker Avance 400inv Spectrometer. Field strengths for samples are reported in the text and NMR data.
^{1}H and ^{13}C NMR spectra for peptide **7aa** were acquired on Bruker Avance 400inv Spectrometer, equipped with VT module and Bruker 5mm BBI probe. Details of this experiment are outlined in the text.
^{1}H and ^{13}C NMR spectra for peptide **S4** were acquired on Bruker Avance 600 Spectrometers equipped with xyz-gradient TCI cryoprobe. All NMR data were processed using MestReNova v14.2.0

Mass Spectrometry:

Low-Resolution mass spectra (LRMS) were obtained on Waters ZQ equipped with ESCI ion source. Waters 2695 HPLC was used to deliver the samples. All samples were dissolved in MeOH (for polar organic compounds) and MeCN/H_2O (0.1% formic acid) for peptides. High-Resolution mass spectra (HRMS) were obtained on Agilent 6545 QTOF LCMS and samples were dissolved in MeCN/H_2O (0.1% formic acid).

UV Spectrophotometry:

Peptide quantification and calibration plots were obtained via UV spectrophotometry on Carry 5000 UV-Vis-NIR spectrophotometer. Linear and Grubbs-stapled peptides (**11-20**) were quantified at 280nm ε = 7100 cm^{-1} M^{-1} and the corresponding FIICk peptides were quantified at 365nm (ε = 14300 cm^{-1} M^{-1}). All samples were dissolved in 1:1 MeCN/H_2O (v/v).

Analytical Reverse-Phase HPLC:

Agilent 1100 HPLC system was used for analytical reinjections of purified material as outlined in the text. This instrument was outfitted with G1379A Degasser, G1311A Quat Pump, G1313A Autosampler, G1316A COLCOM, G1315B DAD, G1364C Analytical Fraction Collector

Preparative Reverse-Phase HPLC:

All peptide purification was done on Agilent 1260 Infinity II Preparative LC system using Agilent Prep 100Å C18, (21.2 x 50 mm, 5 µm) or Phenomenex Aeris PEPTIDE XB-C18 Axia packed (21.2 x 50 mm, 5µm), with optimized solvents and methods detailed in each dataset. Agilent 1260 Infinity II instrument is equipped with the following modules: G1379A Degasser, G1311A Quat Pump, G1313A Autosampler, G1316A COLCOM, G1315B DAD, G1364C Analytical Fraction Collector. HPLC 2 was outfitted with the following modules: G7161A Prep Pump, G7157A Prep Autosampler, G7115A DAD, G1364E Fraction Collector.

HPLC-MS/MS:

LC-MS/MS analysis was carried out using an Agilent 6546 LC/Q-TOF. Reverse-phased LC was carried out using an Agilent Eclipse plus C18 1.8µm, 2.1x50 mm column with a flow rate of 0.400 mL/min an an injection volume of 5 µL. Deionized water and acetonitrile containing 0.1 % formic acid was used as eluent A and B, respectively. A gradient profile of 5%-95% over 15 minutes was used as the analytical LC method. MS-MS data was acquired with three different collision energies at 50, 75, and 100 eV.

Circular Dichroism (CD):
CD spectra were acquired on Jasco J-815 CD spectrophotometer and a quartz cuvette of path length 1mm. Peptides were dissolved in H_2O (20% TFE) to a concentration of 50µM and all measurements were done with the following parameters: 190-260nm, step resolution 0.5nm, scanning speed of 1000nm/min, 10 accumulations, 1s response time, and 1nm bandwidth. α-helical content of each peptide was calculated by dividing the mean residue ellipticity $[\theta]222_{obs}$ by the reported value for model helical polylysine $[\theta]_{222}$ = -37400 deg cm^2 $dmol^{-1}$.[1]

Quantum Yield

Absolute photoluminescence quantum yields were determined using an Edinburgh Instruments FS5 spectrofluorometer equipped with an SC–30 Integrating Sphere Module, with optical densities less than 0.1 and we report the values in the manuscript to +/- 5% based on the error of the integrating sphere.

General Cell Culturing Protocol

All cell culturing media and supplements were purchased from Gibco and all cell culturing plastics were purchased from Corning or Falcon, unless otherwise stated. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. All experiments were conducted in a laminar flow cabinet under sterile conditions. DMSO used in cell-based experiments was purified by filtration through a 0.2 mm filter. To revive cells, a 1 mL cryotube of frozen cell stock (culture media + 10% DMSO) was gently thawed in a water bath and diluted with 12 mL of fresh media in a T-75 flask. After 24 hours, media was replaced with fresh media. Jurkat T-cell leukemia cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 10K U/mL penicillin, 10K mg/mL streptomycin, and 2 mM L-glutamine. DLD-1 cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 10K U/mL penicillin, and 10K mg/mL streptomycin. When cells reached a confluence level of 85-95% for adherent cells, or $2x10^6$ viable cells/mL for suspension cells, the cells were subcultured. Adherent cells were treated with 0.25% trypsin containing 1.3 mM EDTA for ten minutes in the incubator. Once cells were detached from the flask, 1-2 mL of media was added to quench the trypsin, and the cell suspension was transferred to a 10 mL falcon tube and centrifuged for 5 minutes at 8000 rpm. The supernatant was discarded, and the pellet of cells was resuspended in fresh media, diluted as required, and transferred to new T-25 culture flasks. Suspension cells were directly transferred to a 10 mL falcon tube and centrifuged for 5 minutes at 8000 rpm. The supernatant was discarded, and the pellet of cells was resuspended in fresh media, diluted as required, and transferred to new T-75 culture flasks. Cell viability was assessed by counting cells with a hemacytometer after treatment with Trypan blue.

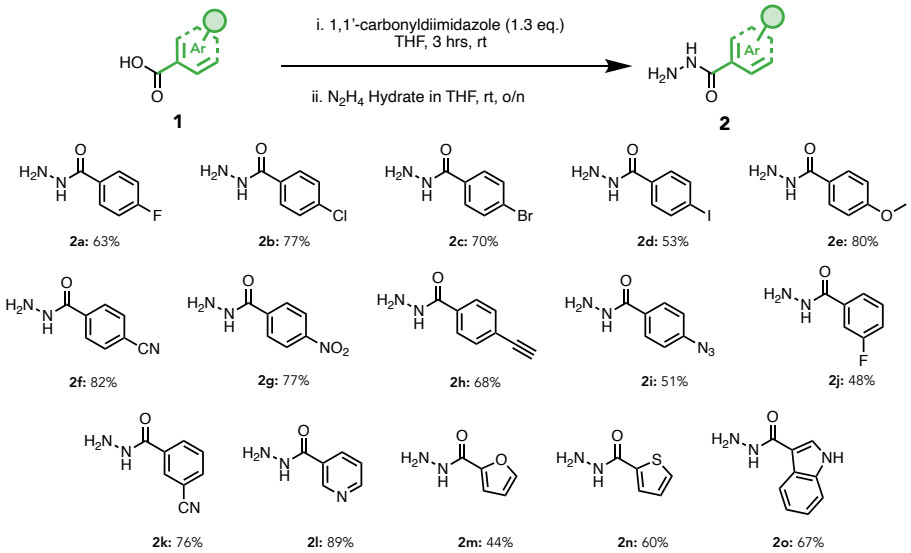
Cell Viability Assays

To assay cell viability, Jurkat cells were suspended in media lacking FBS (serum-free media) and seeded in 96 well plates ($1x10^4$ cells in 25 µL/well). Peptides **19** (negative control), **19a** (RCM, positive control), and **19b** (FIICk) were lyophilized and dissolved in serum-free media with 1% DMSO and further diluted to the appropriate concentrations. The plated cells were treated with 25 µL of the serial dilutions of the peptides of interest in triplicate and incubated at 37°C for 2 hours, at which point 50 µL of media containing 20% FBS was added to each well (serum replacement) (final well volume of 100 µL, 10% FBS). The cells were then incubated for 24 hours. To each well, 10 µL of MTS labeling reagent (Abcam) was added and the cells were incubated for 4 hours. The absorbance of the wells was measured at 490 nm using a Microplate Reader Multi-Mode FilterMax F5 and the IC_{50} values were determined by nonlinear regression analysis with GraphPad Prism software.

Results and Discussion

Synthesis of 2-ketoarylketobenzaldehyde library

Synthesis of benzhydrazides 2:

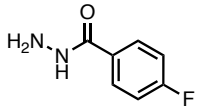


Scheme S1: Benzohydrazides **2** synthesis and substrate scope

General procedure for the synthesis of benzohydrazides (2)[2]:

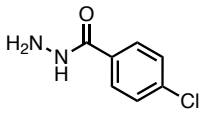
In a 100mL RBF, carboxylic acid **1** (1 eq.) was dissolved in THF (0.5M) and to this mixture was added 1,1'-carbonyldiimidazole (1.3 eq.). After stirring at room temperature for 3h, this imidazolyl solution was added dropwise to hydrazine hydrate (50% in water, v/v) (3 eq.) in THF and left to stir overnight at room temperature. After completion of the reaction, a bilayer was observed, and the top THF phase was decanted into a clean RBF and evaporated under reduced pressure to afford damp solids. This crude product was then recrystallized in EtOH:H_2O mixture to give pure benzohydrazides **2.** This general procedure was applied to all benzohydrazides **2a-2o**.

4-fluorobenzohydrazide (2a)



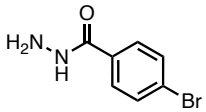
2a was prepared following the general procedure for the synthesis of benzohydrazides, using 4-fluorobenzoic acid (5.00g, 35.7mmol, 1 eq.) and 1,1'-carbonyldiimidazole (7.52g, 46.4mmol, 1.3 eq.) stirred in 45mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6.7mL hydrazine hydrate (50%, v/v) in 30mL THF. Purification by recrystallization in EtOH afforded **2a** as long needle-like white crystals (3.47g, 63%).
^{1}H NMR (300 MHz, DMSO) δ 9.79 (s, 1H), 7.95 – 7.82 (m, 2H), 7.35 – 7.21 (m, 2H), 4.47 (s, 2H).

4-chlorobenzohydrazide (2b)



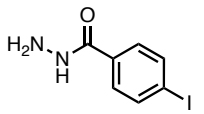
2b was prepared following the general procedure for the synthesis of benzohydrazides, using 4-chlorobenzoic acid (5.00g, 31.9mmol, 1 eq.) and 1,1'-carbonyldiimidazole (6.73g, 41.5mmol, 1.3 eq.) stirred in 40mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6mL hydrazine hydrate (50%, v/v) in 26mL THF. Purification by recrystallization in EtOH afforded **2b** as granular light yellow crystals (4.20g, 77%).
^{1}H NMR (300 MHz, DMSO) δ 9.85 (s, 1H), 7.90 – 7.75 (m, 2H), 7.58 – 7.45 (m, 2H), 4.50 (s, 2H).

4-bromobenzohydrazide (2c)



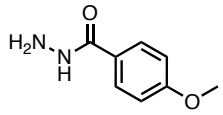
2c was prepared following the general procedure for the synthesis of benzohydrazides, using 4-bromobenzoic acid (5.00g, 24.9mmol, 1 eq.) and 1,1'-carbonyldiimidazole (5.24g, 32.3mmol, 1.3 eq.) stirred in 30mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 4.7mL hydrazine hydrate (50%, v/v) in 21mL THF. Purification by recrystallization in EtOH afforded **2c** as small white crystals (3.75g, 70%).
^{1}H NMR (300 MHz, DMSO) δ 9.85 (s, 1H), 7.81 – 7.71 (m, 2H), 7.71 – 7.62 (m, 2H).

4-iodobenzohydrazide (2d)



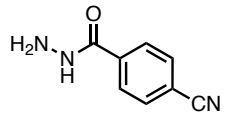
2d was prepared following the general procedure for the synthesis of benzohydrazides, using 4-iodobenzoic acid (5.00g, 20.2mmol, 1 eq.) and 1,1'-carbonyldiimidazole (4.25g, 26.2mmol, 1.3 eq.) stirred in 25mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 3.8mL hydrazine hydrate (50%, v/v) in 17mL THF. Purification by recrystallization in EtOH afforded **2d** as short needle-like crystals (2.65g,
^{1}H NMR (300 MHz, DMSO) δ 9.83 (s, 1H), 7.90 – 7.71 (m, 2H), 7.68 – 7.48 (m, 2H), 4.49 (s, 2H).

4-methoxybenzohydrazide (2e)



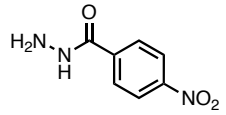
2e was prepared following the general procedure for the synthesis of benzohydrazides, using 4-methoxybenzoic acid (5.00g, 23.9mmol, 1 eq.) and 1,1'-carbonyldiimidazole (6.93g, 42.7mmol, 1.3 eq.) stirred in 30mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6.3mL hydrazine hydrate (50%, v/v) in 30mL THF. Purification by recrystallization in EtOH afforded **2e** as flaky white crystals (4.37g, 80%).
^{1}H NMR (300 MHz, DMSO) δ 9.61 (s, 1H), 7.85 – 7.73 (m, 2H), 7.02 – 6.92 (m, 2H), 4.41 (s, 2H), 3.79 (s, 3H).

4-cyanobenzohydrazide (2f)



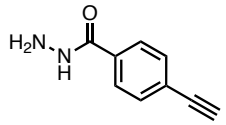
2f was prepared following the general procedure for the synthesis of benzohydrazides, using 4-cyanobenzoic acid (5.00g, 33.9mmol, 1 eq.) and 1,1'-carbonyldiimidazole (7.16g, 44.2mmol, 1.3 eq.) stirred in 42mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6.54mL hydrazine hydrate (50%, v/v) in 30mL THF. Purification by recrystallization in EtOH afforded **2f** as fluffy pale orange crystals (4.49g, 82%).
^{1}H NMR (300 MHz, DMSO) δ 10.04 (s, 1H), 8.06 – 7.74 (m, 4H), 4.58 (s, 2H).

4-nitrobenzoyhydrazide (2g)



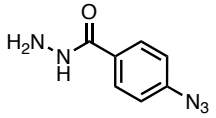
2g was prepared following the general procedure for the synthesis of benzohydrazides, using 4-nitrobenzoic acid (5.00g, 29.9mmol, 1 eq.) and 1,1'-carbonyldiimidazole (6.31g, 38.9mmol, 1.3 eq.) stirred in 38mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 5.7mL hydrazine hydrate (50%, v/v) in 26mL THF. Purification by recrystallization in EtOH afforded **2g** as yellow crystals (4.17g, 77%).
^{1}H NMR (300 MHz, DMSO) δ 10.12 (s, 1H), 8.34 – 8.25 (m, 2H), 8.10 – 7.99 (m, 2H), 4.63 (s, 2H).

4-ethynylbenzohydrazide (2h)



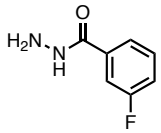
2h was prepared following the general procedure for the synthesis of benzohydrazides, using 4-ethynylbenzoic acid (5.00g, 34.2mmol, 1 eq.) and 1,1'-carbonyldiimidazole (7.21g, 44.54mmol, 1.3 eq.) stirred in 43mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6.6mL hydrazine hydrate (50%, v/v) in 30mL THF. Purification by recrystallization in EtOH:H_2O, followed by Et_2O rinse, afforded **2h** as white crystals (3.73g, 68%).
^{1}H NMR (300 MHz, DMSO) δ 9.86 (s, 1H), 7.89 – 7.76 (m, 2H), 7.59 – 7.50 (m, 2H), 4.52 (s, 2H), 4.35 (s, 1H).

4-azidobenzohydrazide (2i)



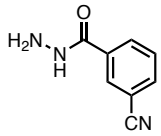
2i was prepared following the general procedure for the synthesis of benzohydrazides, using 4-azidobenzoic acid (5.00g, 30.7mmol, 1 eq.) and 1,1'-carbonyldiimidazole (6.46g, 39.8mmol, 1.3 eq.) stirried in 38mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwosie to a stirring mixture of 6mL hydrazine hydrate (50%, v/v) in 27mL THF. Purification by recrystallization in EtOH and DMSO afforded **2i** as fluffy pale pink crystals (2.77g, 51%).
^{1}H NMR (300 MHz, DMSO) δ 9.78 (s, 1H), 8.05 – 7.71 (m, 2H), 7.44 – 6.96 (m, 2H), 4.47 (s, 2H).

3-fluorobenzohydrazide (2j)



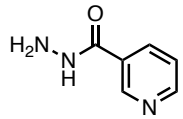
2j was prepared following the general procedure for the synthesis of benzohydrazides, using 3-fluorobenzoic acid (5.00g, 35.7mmol, 1 eq.) and 1,1'-carbonyldiimidazole (7.52g, 46.4mmol, 1.3 eq.) stirred in 45mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6.7mL hydrazine hydrate (50%, v/v) in 30mL THF. Purification by recrystallization in EtOH afforded **2j** as short light-beige crystals (2.64g, 48%).
^{1}H NMR (300 MHz, DMSO) δ 9.87 (s, 1H), 7.72 – 7.56 (m, 2H), 7.51 (td, *J* = 8.0, 5.8 Hz, 1H), 7.36 (tdd, *J* = 8.4, 2.7, 1.0 Hz, 1H), 4.46 (s, 2H).

3-cyanobenzohydrazide (2k)



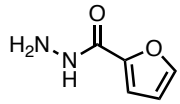
2k was prepared following the general procedure for the synthesis of benzohydrazides, using 3-cyanobenzoic acid (5.00g, 33.9mmol, 1 eq.) and 1,1'-carbonyldiimidazole (7.16g, 44.2mmol, 1.3 eq.) stirred in 42mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 6.54mL hydrazine hydrate (50%, v/v) in 30mL THF. Purification by recrystallization in EtOH and DMSO afforded **2k** as yellow-orange crystals (4.16g, 76%).
^{1}H NMR (300 MHz, DMSO) δ 9.98 (s, 1H), 8.20 (dt, *J* = 1.7, 0.9 Hz, 1H), 8.16 – 8.09 (m, 1H), 7.99 (dt, *J* = 7.8, 1.4 Hz, 1H), 7.74 – 7.62 (m, 1H), 4.57 (d, *J* = 3.2 Hz, 2H).

Nicotinohydrazide (2l)



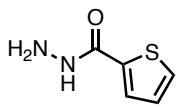
2l was prepared following the general procedure for the synthesis of benzohydrazide, using nicotinic acid (5.00g, 40.6mmol, 1 eq.) and 1,1'-carbonyldiimidazole (8.56g, 52.8mmol, 1.3 eq.) stirred in 50mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 7.8mL hydrazine hydrate (50%, v/v) in 35mL THF. Purification by recrystallization in EtOH afforded **2l** as fluffy white needle-like crystals (4.95g, 89%).
^{1}H NMR (300 MHz, DMSO) δ 9.95 (s, 1H), 8.96 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.69 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.15 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.49 (ddd, *J* = 8.0, 4.8, 0.9 Hz, 1H), 4.56 (s, 2H).

Furan-2-carbohydrazide (2m)



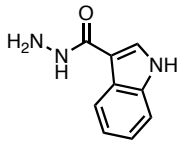
2m was prepared following the general procedure for the synthesis of benzohydrazide, using furan-2-carboxylic acid (2.00g, 17.8mmol, 1 eq.) and 1,1'-carbonyldiimidazole (3.76g, 23.2mmol, 1.3 eq.) stirred in 22mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 3.5mL hydrazine hydrate (50%, v/v) in 16mL THF. Purification by recrystallization in EtOH:H_2O afforded **2m** as small white flaky crystals (0.86g, 44%).
^{1}H NMR (300 MHz, DMSO) δ 9.62 (s, 1H), 7.80 (dd, *J* = 1.8, 0.8 Hz, 1H), 7.07 (dd, *J* = 3.4, 0.9 Hz, 1H), 6.59 (dd, *J* = 3.4, 1.8 Hz, 1H), 4.41 (s, 2H).

Thiophene-2-carbohydrazide (2n)



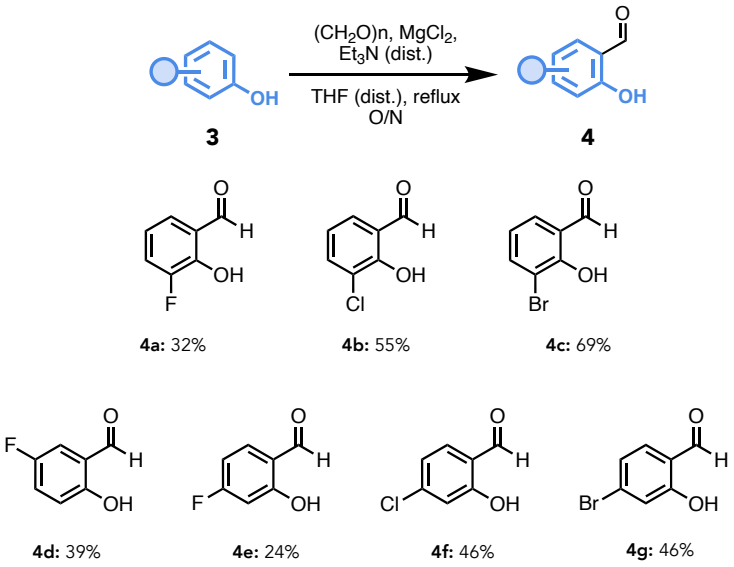
2n was prepared following the general procedure for the synthesis of benzohydrazide, using thiophene-2-carboxylic acid (2.00g, 15.6mmol, 1 eq.) and 1,1'-carbonyldiimidazole (3.29g, 20.3mmol, 1.3 eq.) stirred in 20mL THF. This imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 3mL hydrazine hydrate (50%, v/v) in 15mL THF. Purification by recrystallization in EtOH:H_2O afforded **2n** as shiny white granules (1.33g, 60%).
^{1}H NMR (300 MHz, DMSO) δ 9.74 (s, 1H), 7.72 (ddd, *J* = 9.3, 4.4, 1.1 Hz, 2H), 7.12 (dd, *J* = 5.0, 3.7 Hz, 1H), 4.44 (s, 2H).

1H-indole-3-carbohydrazide (2o)



2o was prepared following the general procedure for the synthesis of benzohydrazide, using 1H-indole-3-carboxylic acid (2.00g, 12.4mmol, 1 eq.) and 1,1'-carbonyldiimidazole (2.62g, 16.1mmol, 1.3 eq.) stirred in 16mL THF. Ths imidazoyl solution was then transferred into an addition funnel and added dropwise to a stirring mixture of 2.3mL hydrazine hydrate (50%, v/v) in 10mL THF. Purification by recrystallization in EtOH:H_2O afforded **2o** as yellow powder (0.73g, 67%).
^{1}H NMR (300 MHz, DMSO) δ 11.51 (s, 1H), 9.14 (s, 1H), 8.17 – 8.08 (m, 1H), 7.96 (d, *J* = 2.9 Hz, 1H), 7.42 (dt, *J* = 8.1, 0.9 Hz, 1H), 7.18 – 7.05 (m, 2H), 4.32 (s, 2H)

Synthesis of salicylaldehyde derivatives:

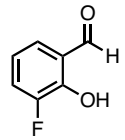


Scheme S2: Synthesis and scope of salicylaldehyde derivatives **4**

General procedure for the synthesis of salicylaldehyde derivatives (4)[3]:

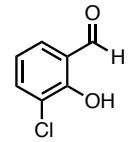
A two-neck 250-mL RBF, equipped with a condenser and a stir bar, was charged with $MgCl_2$ (1.5 eq.) and flame dried and purged with Ar. To this RBF was added the corresponding commercially available phenol derivatives **3** (1 eq.), Et_3N (distilled, CaH_2) (2.5 eq.) and THF (distilled, Na) to afford a 0.37M reaction. Solution was stirred at room temperature for 20 minutes, and to it was added p-formaldehyde (6.75 eq.). The reaction was heated to reflux and stirred overnight, after which it was cooled to 0ºC the following day and acidified to pH 1 with 1M HCl. The resulting aqueous mixture was extracted successively with EtOAc, dried over $MgSO_4$, filtered, and evaporated under reduced pressure. The resulting crude product was purified in acetone:hexanes (1:9, v/v) to afford pure salicylaldehyde derivatives **4**.

3-fluoro-2-hydroxybenzaldehyde (4a)



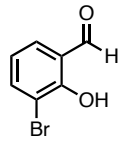
4a was synthesized according to the general procedure of salicylaldehyde derivatives, using 2-fluorophenol (3.00g, 26.8mmol, 1 eq.), Et_3N (9.33mL, 67.0mmol, 2.5 eq.), $MgCl_2$ (3.83g, 40.2mmol, 1.5 eq.), and p-formaldehyde (5.43g, 181mmol, 6.75 eq.) in 75mL distilled THF. Purification via flash column chromatography in 1:9 acetone/hexanes afforded **4a** as white solids (1.2g, 32%).
$^1H\{^{19}F\}$ NMR (300 MHz, DMSO) δ 10.94 (s, 1H), 10.28 (s, 1H), 7.53 (dd, *J*= 3, 7.9 Hz, 1H), 7.49 (dd), *J*= 3, 7.9 Hz, 1H), 6.95 (t, *J* = 7.9 Hz, 1H) ppm
$^{19}F\{^1H\}$ NMR (282 MHz, DMSO) δ -135.53 ppm.

3-chloro-2-hydroxybenzaldehyde (4b)



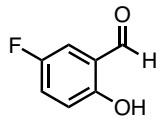
4b was synthesized according to the general procedure of salicylaldehyde derivatives, using 2-chlorophenol (3.00g, 23.3mmol, 1 eq.), Et_3N (8.13mL, 58.3mmol, 2.5 eq.), $MgCl_2$ (3.33g, 35.0mmol, 1.5 eq.), and p-formaldehyde (4.72g, 157mmol, 6.75 eq.) in 63mL distilled THF. Purification via flash column chromatography in 1:9 acetone/hexanes afforded **4b** as white solids (2.00g, 55).
^1H NMR (300 MHz, DMSO) δ = 11.11 (s, 1H), 10.13 (s, 1H), 7.72 (ddd, *J* = 3, 3, 7.5 Hz, 2H), 7.05 (t, *J* = 7.5 Hz, 1H) ppm.

3-bromo-2-hydroxybenzaldehyde (4c)



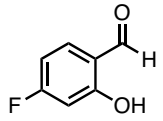
4c was synthesized according to the general procedure for salicylaldehyde derivatives, using 2-bromophenol (3.00g, 17.3mmol, 1 eq.), Et_3N (6.04mL, 43.4mmol, 2.5 eq.), $MgCl_2$ (2.48g, 26.0mmol, 1.5 eq.) and p-formaldehyde (3.91g, 117mmol, 6.75 eq.) in 47mL distilled THF. Purification via flash column chromatography in 2:8 acetone/hexanes afforded **4c** as yellow solids (2.39g, 69%).
^1H NMR (300 MHz, DMSO) δ = 11.26 (s, 1H), 10.06 (s, 1H), 7.89 (dd, *J* = 3.0, 7.5 Hz, 1H), 7.77 (dd, *J* = 3.0, 7.5 Hz, 1H), 7.02 (t, *J* = 7.5 Hz, 1H) ppm

5-fluoro-2-hydroxybenzaldehyde (4d)



4d was synthesized according to the general procedure for salicylaldehyde derivatives, using 3-fluorophenol (3.00g, 26.8mmol, 1 eq.), Et_3N (9.33mL, 67.0mmol, 2.5 eq.), $MgCl_2$ (3.83g, 40.2mmol, 1.5 eq.), and p-formaldehyde (5.43g, 181mmol, 6.75 eq.) in 75mL distilled THF. Purification via flash column chromatography in 1:9 acetone/hexanes afforded **4d** as white solids (1.46g, 39%).
^1H NMR (300 MHz, DMSO) δ 10.74 (s, 1H), 10.25 (d, *J* = 2.8 Hz, 1H), 7.46 – 7.30 (m, 2H), 7.02 (dd, *J* = 8.9, 4.3 Hz, 1H).
$^{19}F\{^1H\}$ NMR (282 MHz, DMSO) δ -124.62 ppm.

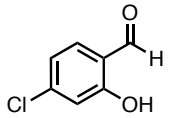
4-fluoro-2-hydroxybenzaldehyde (4e)



4e was synthesized according to the general procedure for salicylaldehyde derivatives, using 4-fluorophenol (3.00g, 26.8mmol, 1 eq.), Et_3N (9.33mL, 67.0mmol, 2.5 eq.), $MgCl_2$ (3.83g, 40.2mmol, 1.5 eq.), and p-formaldehyde (5.43g, 181mmol, 6.75 eq.) in 75mL distilled THF. Purification via flash column chromatography in 1:9 acetone/hexanes afforded **4e** as white solids (0.90g, 39%).

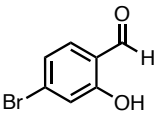
^{1}H NMR (300 MHz, DMSO) δ 10.70 (s, 1H), 10.25 (s, 1H), 7.42(d, *J* = 4.0 Hz, 1H), 7.38 (dd, *J* = 4.0 Hz, 8.9 Hz, 1H), 7.03 (d, *J* = 8.9 Hz, 1H).
^{19}F{^{1}H} NMR (282 MHz, DMSO) δ -124.62 ppm.

4-chloro-2-hydroxybenzaldehyde (4f)



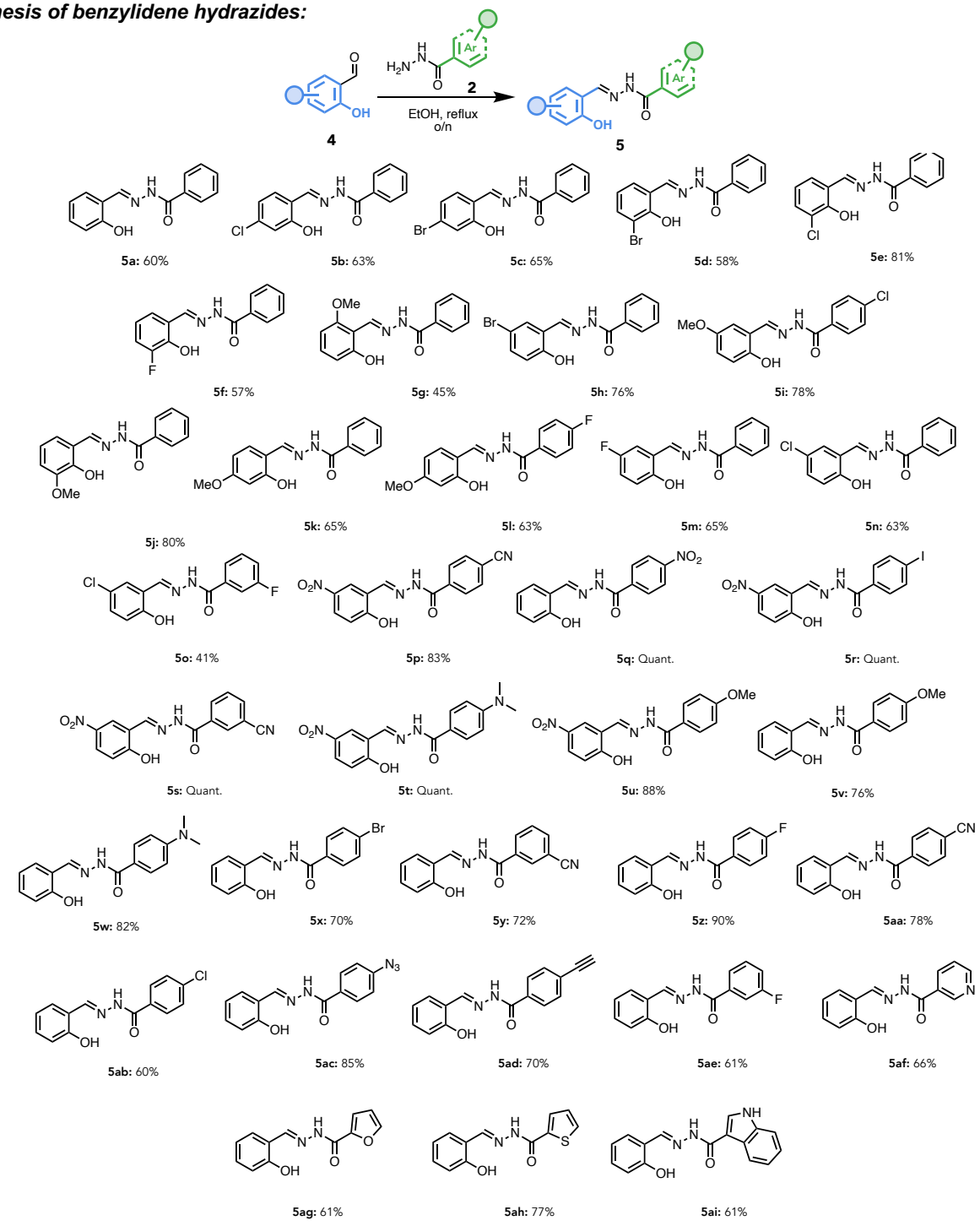
4f was synthesized according to the general procedure of salicylaldehyde derivatives, using 3-chlorophenol (3.00g, 23.3mmol, 1 eq.), Et_3N (8.13mL, 58.3mmol, 2.5 eq.), $MgCl_2$ (3.33g, 35.0mmol, 1.5 eq.), and p-formaldehyde (4.72g, 157mmol, 6.75 eq.) in 63mL distilled THF. Purification via flash column chromatography in 1:9 acetone/hexanes afforded **4f** as pale yellow solids (1.68g, 46%).
^{1}H NMR (300 MHz, DMSO) δ = 11.15 (s. 1H), 10.23 (s, 1H), 7.66 (d, *J* = 9 Hz, 1H), 7.05 (d, *J* = 3 Hz, 1H), 7.01 (dd, *J* = 3 Hz, 9 Hz) ppm.

4-bromo-2-hydroxybenzaldehyde (4g)



4g was synthesized according to the general procedure for salicylaldehyde derivatives, using 3-bromophenol (3.00g, 17.3mmol, 1 eq.), Et_3N (6.04mL, 43.4mmol, 2.5 eq.), $MgCl_2$ (2.48g, 26.0mmol, 1.5 eq.) and p-formaldehyde (3.91g, 117mmol, 6.75 eq.) in 47mL distilled THF. Purification via flash column chromatography in 1:9 acetone/hexanes afforded **4g** as white solids (1.60g, 46%).
^{1}H NMR (300 MHz, DMSO) δ = 11.10 (s, 1H), 10.22 (s, 1H), 7.56 (d, *J* = 9 Hz, 1H), 7.19 (d, *J* = 3 Hz, 1H), 7.14 (dd, *J* = 3, 9 Hz, 1H) ppm

Synthesis of benzylidene hydrazides:

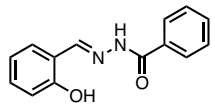


Scheme S3: Synthesis and scope of benzylidene hydrazides **5**:

General procedure for the synthesis of benzylidene hydrazides (5)[4]:

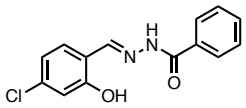
To a 100mL-RBF equipped with a stir bar was added the corresponding salicylaldehyde (1 eq.), benzohydrazide (1 eq.) and EtOH (0.2M reaction). The solution was brought to reflux and stirred overnight, after which it was passively cooled to room temperature. The reaction mixture was concentrated *in vacuo* and the resulting crude product was recrystallized in EtOH:H_2O mixture. Crystals were filtered and rinsed with diethyl ether to pure product **5.**

***N*'-(2-hydroxybenzylidene)benzohydrazide (5a)**



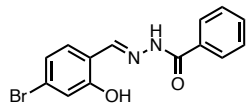
5a was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (4.5mmol, 1eq.) and benzhydrazide (4.5mmol, 1eq.), affording **5a** as small white crystals in 60% yield (0.65g).
^{1}H NMR (400 MHz, DMSO) δ 12.11 (s, 1H), 11.30 (s, 1H), 8.65 (s, 1H), 7.98 – 7.91 (m, 2H), 7.66 – 7.50 (m, 4H), 7.31 (ddd, *J* = 8.6, 7.2, 1.7 Hz, 1H), 6.98 – 6.89 (m, 2H).

***N*'-(4-chloro-2-hydroxybenzylidene)benzohydrazide (5b)**



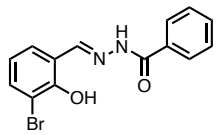
5b was synthesized following the general procedure for benzylidene hydrazide using 4-chloro-2-hydroxybenzaldehyde **4f** (1.0g, 6.38mmol, 1 eq.) and benzhydrazide (0.86g, 6.38mmol, 1 eq.), affording **5b** as pale yellow powder in 63% yield (1.10g).
^{1}H NMR (300 MHz, DMSO) δ 12.14 (s, 1H), 11.57 (s, 1H), 8.64 (s, 1H), 7.97 – 7.89 (m, 2H), 7.68 – 7.46 (m, 3H), 6.99 (d, *J* = 8.1 Hz, 2H).

***N*'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5c)**



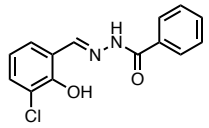
5c was synthesized following the general procedure for benzylidene hydrazide using 4-bromo-2-hydroxybenzaldehyde **4g** (1.0g, 4.97mmol, 1 eq.) and benzhydrazide (0.67g, 4.97mmol, 1 eq.), affording **5c** as yellow solids (1.03g, 65% yield).
^{1}H NMR (300 MHz, DMSO) δ 12.12 (s, 1H), 11.57 (s, 1H) 8.63 (s, 1H), 7.93 (d, *J* = 7.5 Hz, 2H), 7.57 (p, *J* = 7.1 Hz, 4H), 7.17 – 7.07 (m, 2H).

***N*'-(3-bromo-2-hydroxybenzylidene)benzohydrazide (5d)**



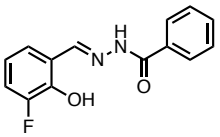
5d was synthesized following the general procedure for benzylidene hydrazide using 3-bromo-2-hydroxybenzaldehyde **4c** (1.0g, 4.97mmol, 1 eq.) and benzhydrazide (0.67g, 4.97mmol, 1 eq.), affording **5d** as yellow crystals (0.92g, 58%)
^{1}H NMR (300 MHz, DMSO) δ 8.59 (s, 1H), 8.01 – 7.91 (m, 2H), 7.70 – 7.46 (m, 5H), 6.92 (t, *J* = 7.8 Hz, 1H).

***N*'-(3-chloro-2-hydroxybenzylidene)benzohydrazide (5e)**



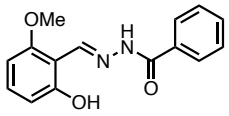
5e was synthesized following the general procedure for benzylidene hydrazide using 3-chloro-2-hydroxybenzaldehyde **4b** (1.0g, 6.39mmol, 1 eq.) and benzhydrazide (0.87g, 6.39mmol, 1 eq.), affording **5e** as white crystals (1.39g, 80%).
^{1}H NMR (300 MHz, DMSO) δ 8.62 (s, 1H), 8.01 – 7.91 (m, 2H), 7.68 – 7.53 (m, 3H), 7.49 (d, *J* = 7.8 Hz, 2H), 6.97 (t, *J* = 7.8 Hz, 1H).

***N*'-(3-fluoro-2-hydroxybenzylidene)benzohydrazide (5f)**



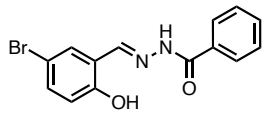
5f was synthesized following the general procedure for benzylidene hydrazide using 3-fluoro-2-hydroxybenzaldehyde **4a** (0.64g, 4.59mmol, 1 eq.) and benzhydrazide (0.62g, 4.59mmol, 1 eq.), affording **5f** as white needle-like crystals (0.68g, 57%).
^{1}H NMR (300 MHz, DMSO) δ 12.23 (s, 1H), 11.64 (s, 1H), 8.66 (s, 1H), 8.04 – 7.87 (m, 2H), 7.71 – 7.49 (m, 3H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.29 (ddd, *J* = 11.2, 8.1, 1.5 Hz, 1H), 6.92 (td, *J* = 8.0, 4.8 Hz, 1H).
^{19}F NMR (282 MHz, DMSO) δ -136.97.

***N*'-(2-hydroxy-6-methoxybenzylidene)benzohydrazide (5g)**



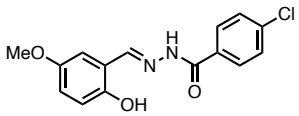
5g was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-6-methoxybenzaldehyde (1.0g, 6.57mmol, 1 eq.) and benzhydrazide (0.89g, 6.57mmol, 1 eq.) affording **5g** as white crystals (0.79g, 45%).
^{1}H NMR (300 MHz, DMSO) δ 12.23 (s, 0H), 12.19 (s, 1H), 8.97 (s, 1H), 7.99 – 7.91 (m, 2H), 7.58 (ddd, *J* = 14.5, 7.8, 6.2 Hz, 3H), 7.28 (t, *J* = 8.3 Hz, 1H), 6.56 (dd, *J* = 8.3, 6.7 Hz, 2H), 3.86 (s, 3H).

N'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5h)



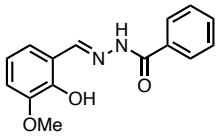
5h was synthesized following the general procedure for benzylidene hydrazide using 5-bromo-2-hydroxybenzaldehyde (1.0g, 4.97mmol, 1 eq.) and benzhydrazide (0.67g, 4.87mmol, 1 eq.) affording **5h** as bright yellow crystals (1.21g, 76%)
^{1}H NMR (300 MHz, DMSO) δ 12.18 (s, 1H), 11.30 (s, 1H), 8.62 (s, 1H), 7.94 (d, *J* = 7.3 Hz, 2H), 7.80 (d, *J* = 2.5 Hz, 1H), 7.61 (d, *J* = 7.1 Hz, 1H), 7.54 (t, *J* = 7.3 Hz, 2H), 7.43 (dd, *J* = 8.8, 2.5 Hz, 1H), 6.91 (d, *J* = 8.8 Hz, 1H).

4-chloro-N'-(2-hydroxy-5-methoxybenzylidene)benzohydrazide (5i)



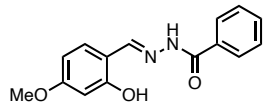
5i was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-5-methoxybenzaldehyde (1.0g, 6.57mmol, 1 eq.) and 4-chlorobenzohydrazide **2b** (1.12g, 6.57mmol, 1 eq.) affording **5i** as pale yellow crystals (1.57g, 78%).
^{1}H NMR (300 MHz, DMSO) δ 11.56 (s, 1H), 10.66 (s, 1H), 8.55 (s, 1H), 8.32 (s, 1H), 7.95 (d, *J* = 8.5 Hz, 1H), 7.63 (d, *J* = 8.5 Hz, 1H), 7.44 (d, *J* = 8.5 Hz, 1H), 6.58 – 6.41 (m, 3H), 3.75 (s, 3H).

N'-(2-hydroxy-3-methoxybenzylidene)benzohydrazide (5j)



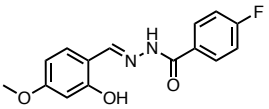
5j was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-3-methoxybenzaldehyde (2.0g, 13.1mmol, 1 eq.) and benzhydrazide (1.79g, 13.1mmol, 1 eq.) affording **5j** as pale yellow crystals (2.8g, 80%).
^{1}H NMR (300 MHz, DMSO) δ 12.08 (s, 1H), 10.99 (s, 1H), 8.66 (s, 1H), 8.01 – 7.88 (m, 2H), 7.57 (ddd, *J* = 14.3, 7.9, 6.1 Hz, 3H), 7.15 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.04 (dd, *J* = 8.1, 1.5 Hz, 1H), 6.87 (t, *J* = 7.9 Hz, 1H), 3.82 (s, 3H).

N'-(2-hydroxy-2-methoxybenzylidine)benzohydrazide (5k)



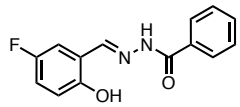
5k was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-4-methoxybenzaldehyde (1.0g. 6.57mmol, 1 eq.) and benzhydrazide (0.89g, 6.57mmol, 1 eq.) affording **5k** as pale yellow granular crystals (1.16g, 65%).
^{1}H NMR (300 MHz, DMSO) δ 12.00 (s, 1H), 11.64 (s, 1H), 8.55 (s, 1H), 7.93 (dt, *J* = 6.9, 1.5 Hz, 2H), 7.56 (ddd, *J* = 14.3, 7.9, 6.1 Hz, 3H), 7.43 (d, *J* = 8.4 Hz, 1H), 6.58 – 6.47 (m, 2H), 3.78 (s, 3H).

4-fluoro-N'-(2hydroxy-2-methoxybenzylidene)benzohydrazide (5l)



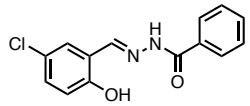
5l was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-4-methoxybenzaldehyde (0.987g, 6.49mmol, 1 eq.) and 4-fluoro-benzhydrazide **2a** (1.0g, 6.49mmol, 1 eq.) affording **5l** as thin needle-like crystals (1.19g, 63%).
^{1}H NMR (300 MHz, DMSO) δ 12.01 (s, 1H), 11.59 (s, 1H), 8.54 (s, 1H), 8.06 – 7.94 (m, 2H), 7.49 – 7.30 (m, 3H), 6.58 – 6.43 (m, 2H), 3.78 (s, 3H).
^{19}F NMR (282 MHz, DMSO) δ -108.64.

N'-(5-fluoro-2-hydroxybenzylidene)benzohydrazide (5m)



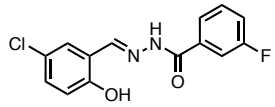
5m was synthesized following the general procedure for benzylidene hydrazide using 5-fluoro-2-hydroxybenzaldehyde **4d** (0.91g, 6.44mmol, 1 eq.) and benzhydrazide (0.88g, 6.44mmol, 1 eq.) affording **5m** as small white crystals (1.19g, 65%).
^{1}H NMR (300 MHz, DMSO) δ 12.12 (s, 1H), 11.06 (s, 1H), 8.64 (s, 1H), 7.98 – 7.89 (m, 2H), 7.57 (dt, *J* = 14.4, 7.0 Hz, 3H), 7.43 (dd, *J* = 9.4, 3.2 Hz, 1H), 7.15 (td, *J* = 8.6, 3.2 Hz, 1H), 6.94 (dd, *J* = 9.0, 4.7 Hz, 1H).
^{19}F NMR (282 MHz, DMSO) δ -125.11.

N'(5-chloro-2-hydroxybenzylidene)benzohydrazide (5n)



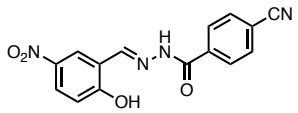
5n was synthesized following the general procedure for benzylidene hydrazide using 5-chloro-2-hydroxybenzaldehyde (1.10g, 7.02mmol, 1 eq.) and benzhydrazide (0.95g, 7.02mmol, 1 eq.), affording **5n** as white crystals (1.17g, 63%).
^{1}H NMR (300 MHz, DMSO) δ 12.10 (s, 1H), 11.36 (s, 1H), 8.63 (s, 1H), 7.99 – 7.90 (m, 2H), 7.67 (d, *J* = 2.7 Hz, 1H), 7.57 (dt, *J* = 14.7, 7.1 Hz, 3H), 7.32 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 1H).

***N*'-(5-chloro-2-hydroxybenzylidene)-3-fluorobenzohydrazide (5o)**



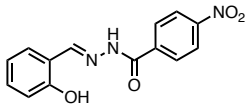
5o was synthesized following the general procedure for benzylidene hydrazide using 5-chloro-2-hydroxybenzaldehyde (1.02g, 6.51mmol, 1 eq.) and 3-fluorobenzhydrazide (1.0g, 6.51mmol, 1 eq.), affording **5o** as white solids (0.78g, 41%).
^{1}H NMR (300 MHz, DMSO) δ 12.22 (s, 1H), 11.19 (s, 1H), 8.63 (s, 1H), 7.84 – 7.69 (m, 2H), 7.69 (d, *J* = 2.7 Hz, 1H), 7.61 (td, *J* = 8.0, 5.8 Hz, 1H), 7.54 – 7.41 (m, 1H), 7.33 (dd, *J* = 8.8, 2.7 Hz, 1H), 6.96 (d, *J* = 8.8 Hz, 1H).

4-cyano-*N*'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide (5p)



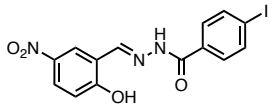
5p was synthesized following the general procedure for benzylidene hydrazide using 5-nitro-2-hydroxybenzaldehyde (1.04g, 6.2mmol, 1 eq.) and 4-cyanobenzhydrazide **2f** (1.0g, 6.2mmol, 1 eq.) affording **5p** as bright yellow powder (1.69g, 83%).
^{1}H NMR (300 MHz, DMSO) δ 8.74 (s, 1H), 8.60 (d, *J* = 2.9 Hz, 1H), 8.17 (dd, *J* = 9.1, 2.9 Hz, 1H), 8.06 (q, *J* = 8.5 Hz, 4H), 7.10 (d, *J* = 9.1 Hz, 1H).

N'-(2-hydroxybenzylidene)-4-nitrobenzohydrazide (5q)



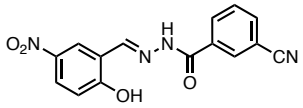
5q was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.26g, 11.26mmol, 1 eq.) and 4-nitrobenzohydrazide **2g** (2.04g, 11.26mmol, 1 eq.). Crude material was triturated in Et_2O and filtered to obtain **5q** as yellow powder (2.00g, 93%).
^{1}H NMR (300 MHz, DMSO) δ 12.35 (s, 1H), 11.11 (s, 1H), 8.69 (s, 1H), 8.44 – 8.36 (m, 2H), 8.23 – 8.13 (m, 2H), 7.61 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.32 (ddd, *J* = 8.5, 7.3, 1.7 Hz, 1H), 6.93 (dd, *J* = 8.0, 7.0 Hz, 2H).

N'-(2-hydroxy-5-nitrobenzylidene)-4-iodobenzohydrazide (5r)



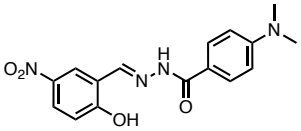
5r was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-5-nitrobenzaldehyde (0.64g, 3.82mmol, 1 eq.) and 4-iodobenzohydrazide **2d** (1.0g, 3.82mmol, 1 eq.), affording **5r** as pale yellow powder in quantitative yield (1.50g).
^{1}H NMR (300 MHz, DMSO) δ 8.74 (s, 1H), 8.60 (d, *J* = 2.9 Hz, 1H), 8.18 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.95 (d, *J* = 8.2 Hz, 2H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.12 (d, *J* = 9.1 Hz, 1H).

***N*'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide (5s)**



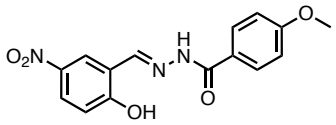
5s was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-5-nitrobenzaldehyde (1.04g, 6.21mmol, 1 eq.) and 3-cyanobenzohydrazide **2k** (1.0g, 6.21mmol, 1 eq.), affording **5s** as yellow precipitate in quantitative yield (2.10g).
^{1}H NMR (300 MHz, DMSO) δ 8.74 (s, 1H), 8.62 (d, *J* = 2.9 Hz, 1H), 8.38 (d, *J* = 2.1 Hz, 1H), 8.25 (d, *J* = 8.0 Hz, 1H), 8.18 (dd, *J* = 9.1, 2.9 Hz, 1H), 8.09 (d, *J* = 7.7 Hz, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 9.1 Hz, 1H)

4-(dimethylamino)-N'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide (5t)



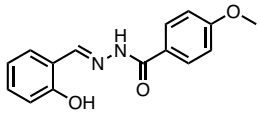
5t was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-5-nitrobenzaldehyde (2.0g, 11.97mmol, 1 eq.) and 4-(dimethylamino)benzohydrazide (2.14g, 11.97mmol, 1 eq.), affording **5t** as bright yellow solids in quantitative yield.
^{1}H NMR (300 MHz, DMSO) δ 12.02 (s, 1H), 8.68 (s, 1H), 8.55 (d, *J* = 2.9 Hz, 1H), 8.15 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.89 – 7.81 (m, 2H), 7.10 (d, *J* = 9.1 Hz, 1H), 6.82 – 6.73 (m, 2H), 3.01 (s, 6H).

N'-(2-hydroxy-5-nitrobenzylidene)-4-methoxybenzohydrazide (5u)



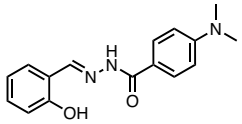
5u was synthesized following the general procedure for benzylidene hydrazide using 2-hydroxy-5-nitrobenzaldehyde (2.0g, 6.34mmol, 1 eq.) and 4-methoxybenzohydrazide **2e** (1.05g, 6.34mmol, 1 eq.), affording **5u** as yellow solids (3.32g, 88%).
^{1}H NMR (300 MHz, DMSO) δ 12.17 (s, 1H), 8.72 (s, 1H), 8.58 (s, 1H), 8.17 (dd, *J* = 9.1, 2.9 Hz, 1H), 7.95 (d, *J* = 8.6 Hz, 2H), 7.14 – 7.05 (m, 3H), 3.85 (s, 3H).

***N*'-(2-hydroxybenzylidene)-4-methoxybenzohydrazide (5v)**



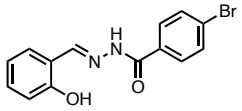
5v was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (3.0g, 24.6mmol, 1 eq.) and 4-methoxybenzohydraizide **2e** (4.08g, 24.6mmol, 1 eq.), affording **5v** as shiny granular crystals (5.04g, 76%).
^{1}H NMR (300 MHz, DMSO) δ 12.00 (s, 1H), 11.37 (s, 1H), 8.62 (s, 1H), 7.93 (d, *J* = 8.7 Hz, 2H), 7.57 – 7.48 (m, 1H), 7.30 (td, *J* = 7.7, 1.7 Hz, 1H), 7.13 – 7.03 (m, 2H), 6.93 (d, *J* = 7.9 Hz, 2H), 3.84 (s, 3H).

4-(dimethylamino)-N'-(2-hydroxybenzylidene)benzohydrazide (5w)



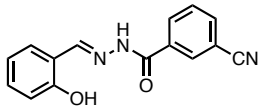
5w was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (2.0g, 16.4mmol, 1 eq.) and 4-(dimethylamino)benzohydrazide (2.94g, 16.4mmol, 1 eq.), affording **5w** as yellow crystals (3.80g, 82%).
^{1}H NMR (300 MHz, DMSO) δ 11.82 (s, 1H), 11.53 (s, 1H), 8.58 (s, 1H), 7.83 (d, *J* = 8.9 Hz, 2H), 7.51 – 7.46 (m, 1H), 7.33 – 7.24 (m, 1H), 6.97 – 6.87 (m, 2H), 6.77 (d, *J* = 9.0 Hz, 2H), 3.01 (s, 6H).

4-bromo-N'-(2-hydroxybenzylidene)benzohydrazide (5x)



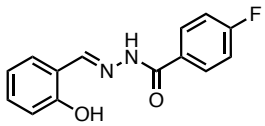
5x was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.50g, 12.3mmol, 1 eq.) and 4-bromobenzohydrazide **2c** (2.64g, 12.3mmol, 1 eq.) without recrystallization. Beige-brown precipitate was filtered and dried, affording **5x** (2.74g, 70%).
^{1}H NMR (300 MHz, DMSO) δ 12.16 (s, 1H), 11.20 (s, 1H), 8.64 (s, 1H), 7.89 (d, *J* = 8.5 Hz, 2H), 7.77 (d, *J* = 8.5 Hz, 2H), 7.60 – 7.53 (m, 1H), 7.31 (td, *J* = 7.8, 1.7 Hz, 1H), 6.94 (d, *J* = 7.8 Hz, 2H).

3-cyano-N'-(2-hydroxybenzylidene)benzohydrazide (5y)



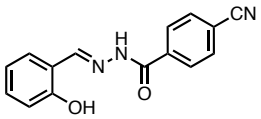
5y was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (0.69g, 6.21mmol, 1 eq.) and 3-cyanobenzohydrazide **2k** (1.0g, 6.21mmol, 1 eq.) without recrystallization. Crude material was triturated in Et_2O, filtered, and dried under reduced pressure to afford **5y** as beige powder (1.19g, 72%).
^{1}H NMR (300 MHz, DMSO) δ 12.22 (s, 1H), 11.09 (s, 1H), 8.66 (s, 1H), 8.37 (t, *J* = 1.7 Hz, 1H), 8.24 (d, *J* = 7.9 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.64 – 7.56 (m, 1H), 7.32 (td, *J* = 7.7, 1.7 Hz, 1H), 6.93 (t, *J* = 7.7 Hz, 2H).

4-fluoro-*N*'-(2-hydroxybenzylidene)benzohydrazide (5z)



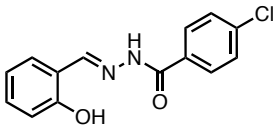
5z was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.60g, 14.3mmol, 1 eq.) and 4-fluorobenzohydrazide **2a** (2.20g, 14.3mmol, 1 eq.), affording **5z** as white crystals (3.31g, 90%)
^{1}H NMR (300 MHz, DMSO) δ 12.12 (s, 1H), 11.25 (s, 1H), 8.64 (s, 1H), 8.08 – 7.95 (m, 2H), 7.56 (dd, *J* = 7.7, 1.7 Hz, 1H), 7.45 – 7.24 (m, 3H), 6.93 (t, *J* = 8.0 Hz, 2H).

4-cyano-N'-(2-hydroxybenzylidene)benzohydrazide (5aa)



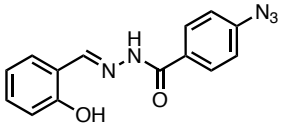
5aa was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (0.69g, 6.21mmol, 1 eq.) and 4-cyanobenzohydrazide **2f** (1.0g, 6.21mmol, 1 eq.) without recrystallization. Suspension was filtered and rinsed with Et_2O, affording **5aa** as pale yellow solids (1.29g, 78%).
^{1}H NMR (300 MHz, DMSO) δ 11.12 (s, 1H), 8.67 (s, 1H), 8.14 – 8.01 (m, 4H), 7.59 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.32 (ddd, *J* = 8.5, 7.3, 1.7 Hz, 1H), 6.93 (dd, *J* = 8.1, 7.0 Hz, 2H).

4-chloro-N'-(2-hydroxybenzylidene)benzohydrazide (5ab)



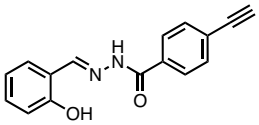
5ab was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.50g, 12.3mmol, 1 eq.) and 4-chlorobenzohydrazide **2a** (2.10g, 12.3mmol, 1 eq.) without recrystallization. Pale yellow precipitate was filtered and dried, affording **5ab** (2.02g, 60%).
^{1}H NMR (300 MHz, DMSO) δ 12.08 (s, 1H), 11.27 (s, 1H), 8.65 (s, 1H), 7.97 (d, *J* = 8.1 Hz, 2H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.31 (t, *J* = 7.8 Hz, 1H), 6.94 (d, *J* = 8.4 Hz, 2H).

4-azido-N'-(2-hydroxybenzylidene)benzohydrazide (5ac)



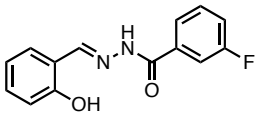
5ac was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (0.65g, 5.32mmol 1 eq.) and 4-azidobenzohydrazide **2i** (0.94g, 5.32mmol, 1 eq.), affording **5ac** as orange crystals (1.03g, 69%).
^{1}H NMR (300 MHz, DMSO) δ 12.11 (s, 1H), 11.27 (s, 1H), 8.63 (s, 1H), 8.07 – 7.93 (m, 2H), 7.54 (d, *J* = 7.4 Hz, 1H), 7.41 – 7.19 (m, 3H), 6.93 (d, *J* = 7.9 Hz, 2H).

4-ethynyl-N'-(2-hydroxybenzylidene)benzohydrazide (5ad)



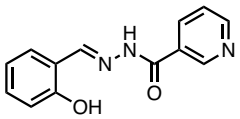
5ad was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (0.45g, 3.64mmol, 1 eq.) and 4-ethynylbenzohydrazide **2h** (0.58g, 3.64mmol, 1 eq.) without further recrystallization. Crude material was triturated in Et_2O, filtered, and dried, affording **5ad** as yellow powder (0.68g, 71%).
^{1}H NMR (300 MHz, DMSO) δ 12.17 (s, 1H), 11.23 (s, 1H), 8.65 (s, 1H), 8.00 – 7.91 (m, 2H), 7.65 (d, *J* = 8.2 Hz, 2H), 7.56 (dd, *J* = 7.8, 1.7 Hz, 1H), 7.31 (ddd, *J* = 8.6, 7.3, 1.7 Hz, 1H), 6.93 (t, *J* = 7.5 Hz, 2H), 4.44 (s, 1H).

3-fluoro-N'-(2-hydroxybenzylidene)benzohydrazide (5ae)



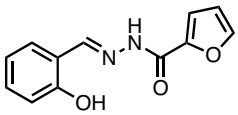
5ae was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.19g, 9.74mmol, 1 eq.) and 3-fluorobenzohydrazide **2j** (1.50g, 9.74mmol, 1 eq.), affording **5ae** as white crystals (1.08g, 43%).
^{1}H NMR (300 MHz, DMSO) δ 12.13 (s, 1H), 11.19 (s, 1H), 8.65 (s, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.75 (s, 1H), 7.64 – 7.53 (m, 2H), 7.50 – 7.43 (m, 1H), 7.35 – 7.26 (m, 1H), 6.93 (t, *J* = 7.6 Hz, 2H).
^{19}F NMR (282 MHz, DMSO) δ -112.40.

N'-(2-hydroxybenzylidene)nicotinohydrazide (5af)



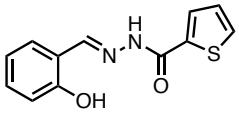
5af was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.23g, 11.0mmol, 1 eq.) and nicotinohydrazide **2l** (1.51g, 11.0mmol, 1 eq.), affording **5af** as yellow flaky crystals (1.72g, 66%).
^{1}H NMR (400 MHz, DMSO) δ 12.24 (s, 1H), 11.14 (s, 1H), 9.09 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.78 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.65 (s, 1H), 8.28 (dt, *J* = 8.0, 2.0 Hz, 1H), 7.62 – 7.55 (m, 2H), 7.32 (ddd, *J* = 8.5, 7.3, 1.7 Hz, 1H), 6.98 – 6.89 (m, 2H).

N'-(2-hydroxybenzylidene)furan-2-carbohydrazide (5ag)



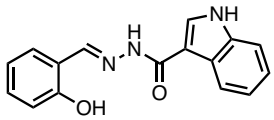
5ag was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.33g, 10.89mmol, 1 eq.) and furan-2-carbohydrazide **2m** (1.37g, 10.89mmol, 1 eq.), affording **5ag** as small white crystals (0.98g, 39%).
^{1}H NMR (300 MHz, DMSO) δ 12.11 (s, 1H), 11.14 (s, 1H), 8.64 (s, 1H), 7.97 (dd, *J* = 1.7, 0.8 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.35 – 7.24 (m, 2H), 6.91 (dd, *J* = 8.1, 6.7 Hz, 2H), 6.72 (dd, *J* = 3.5, 1.7 Hz, 1H).

N'-(2-hydroxybenzylidene)thiophene-2-carbohydrazide (5ah)



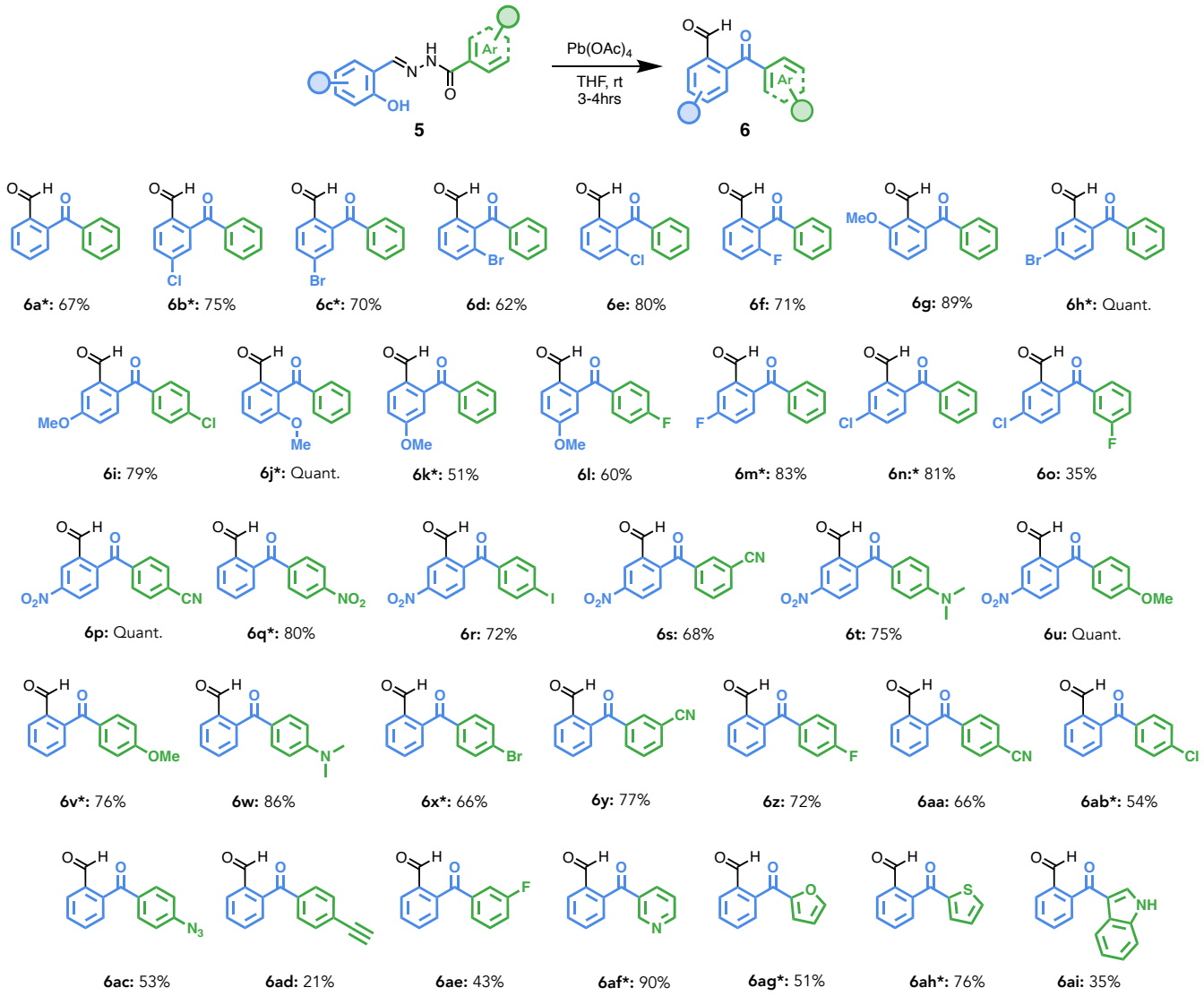
5ah was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (0.92g, 7.53mmol, 1 eq.) and thiophene-2-carbohydrazide **2n** (1.07g, 7.53mmol, 1 eq.), affording **5ah** as pale yellow powder (1.41g, 76%), isolated with minor impurities.
^{1}H NMR (300 MHz, DMSO) δ 12.11 (s, 1H), 11.12 (s, 1H), 8.63 (s, 1H), 7.91 (dd, *J* = 9.9, 4.5 Hz, 3H), 7.24 (t, *J* = 4.6 Hz, 2H), 6.93 (d, *J* = 7.8 Hz, 3H).

***N*'-(2-hydroxybenzylidene)-1*H*-indole-3-carbohydrazide (5ai)**



5ai was synthesized following the general procedure for benzylidene hydrazide using salicylaldehyde (1.09g, 8.90mmol, 1 eq.) and 1H-indole-3-carbohydrazide **2o** (1.56g, 8.90mmol, 1 eq.), affording **5ai** as white flaky crystals (1.66g, 67%).
^{1}H NMR (300 MHz, DMSO) δ 11.78 (s, 1H), 11.68 (s, 1H), 11.49 (s, 1H), 8.51 (s, 1H), 8.29 – 8.12 (m, 2H), 7.58 – 7.45 (m, 2H), 7.31 – 7.12 (m, 3H), 6.92 (t, *J* = 7.7 Hz, 2H).

Synthesis of 2-arylketobenzaldehydes

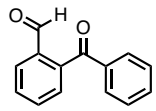


Scheme S4: Synthesis and scope of aryl-ketobenzaldehydes **6**

General procedure for the synthesis of 2-ketobenzaldehyde (6)[4]:

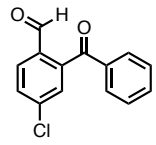
To a 100mL-RBF equipped with a stir bar was added the corresponding starting material **5** (1 eq.) and THF (0.2M reaction) and solution was stirred rapidly at room temperature. To this stirring mixture was added $Pb(OAc)_4$ (1.1 eq.) in portions. This addition was accompanied by a colour change and mild bubbling. Vigorous stirring was maintained for 4 hours at room temperature and then filtered through a celite sandwich to remove solid by-products. The resulting filtrate was concentrated under reduced pressure to afford crude material, after which it was purified by trituration in Et_2O or flash column chromatography in EtOAc/Hexanes, unless stated otherwise.

2-benzoylbenzaldehyde (6a)



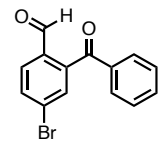
6a was synthesized according to the general procedure for 2-ketobenzaldehydes using X (0.65g, 2.70mmol, 1 eq.) and $Pb(OAc)_4$ (1.32g, 2.97mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 1:9 EtOAc/Hexanes (v/v), affording **6a** as pale yellow solids (0.37g, 67%).
^{1}H NMR (300 MHz, DMSO) δ 9.96 (s, 1H), 8.18 – 8.03 (m, 1H), 7.89 – 7.74 (m, 2H), 7.73 – 7.58 (m, 3H), 7.57 – 7.46 (m, 3H).
^{13}C NMR (75 MHz, DMSO) δ 196.3, 192.2, 140.1, 136.5, 134.8, 133.9, 133.5, 132.0, 130.6, 129.3, 128.75, 128.3, 40.4, 40.1, 39.8, 39.7, 39.5, 39.2, 39.0, 38.7.
LRMS (ESI-MeOH, m/z):
Methyl hemiacetal ($[C_{15}H_{14}O_3]+Na)^+$ calc. 265.1, found 265.2

2-benzoyl-4-chlorobenzaldehyde (6b)



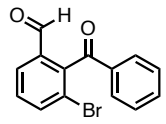
6b was synthesized according to the general procedure for 2-ketobenzaldehydes using X (1.10g, 4.00mmol, 1 eq.) and $Pb(OAc)_4$ (1.95g, 4.4mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 2:8 EtOAc/Hexanes (v/v), affording **6b** as yellow-brown powder (0.73g, 75%).
^{1}H NMR (300 MHz, DMSO) δ 9.91 (s, 1H), 8.13 (d, *J* = 8.3 Hz, 1H), 7.90 (dd, *J* = 8.3, 2.1 Hz, 1H), 7.72 – 7.63 (m, 4H), 7.52 (td, *J* = 7.0, 1.5 Hz, 2H).
^{13}C NMR (75 MHz, DMSO) δ 194.7, 191.2, 141.7, 139.0, 136.0, 134.2, 133.7, 133.1, 130.4, 129.2, 128.8, 128.0.
LRMS (ESI-MeOH, m/z):
2-ketoarylbenzaldehyde ($[C_{14}H_9ClO_2]+H)^+$ calc. 244.0, found 244.3

2-benzoyl-4-bromobenzaldehyde (6c)



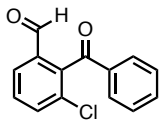
6c was synthesized according to the general procedure for 2-ketobenzaldehydes using X (1.00g, 3.1mmol, 1 eq.) and $Pb(OAc)_4$ (1.51g, 3.41mmol, 1.1 eq). Crude material was then purified via flash column chromatography, eluted in 2:8 EtOAc/Hexanes (v/v), affording **6c** as beige powder (0.67g, 75%)
^{1}H NMR (300 MHz, DMSO) δ 9.90 (s, 1H), 8.04 (d, *J* = 1.2 Hz, 2H), 7.79 (s, 1H), 7.72 – 7.62 (m, 3H), 7.53 (tt, *J* = 6.9, 1.0 Hz, 2H).
^{13}C NMR (75 MHz, DMSO) δ 194.6, 191.4, 141.7, 136.1, 134.1, 133.7, 133.4, 130.7, 129.2, 128.8, 128.1.
LRMS (ESI-MeOH, m/z):
2-ketoarylbenzaldehyde ($[C_{14}H_{19}BrO_2]+H)^+$ calc. 288.9, found 289.0

2-benzoyl-3-bromobenzaldehyde (6d)



6d was synthesized according to the general procedure for 2-ketobenzaldehydes using X (0.92g, 2.88mmol, 1 eq.) and $Pb(OAc)_4$ (1.40g, 3.17mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6d** as brown powder (0.52g, 62%).
^{1}H NMR (300 MHz, DMSO) δ 9.86 (s, 1H), 8.18 (dd, *J* = 7.6, 1.1 Hz, 1H), 8.09 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.76 (t, *J* = 7.8 Hz, 1H), 7.66 (ddd, *J* = 7.2, 3.4, 2.0 Hz, 3H), 7.56 – 7.47 (m, 2H).
^{13}C NMR (75 MHz, DMSO) δ 194.6, 191.5, 139.7, 138.1, 135.8, 135.5, 133.8, 133.3, 131.6, 129.0, 128.5, 119.9.
LRMS (ESI-MeOH, m/z): Methyl hemiacetal ($[C_{15}H_{13}BrO_3]+Na)^+$, calc. 343.0, found 343.3

2-benzoyl-3-chlorobenzaldehyde (6e)



6e was synthesized according to the general procedure for 2-ketobenzaldehydes using X (1.00g, 3.64mmol, 1 eq.) and $Pb(OAc)_4$ (1.78g, 4.00mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6e** as yellow-beige powder (0.71g, 80%)

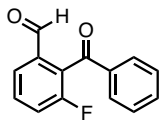
^{1}H NMR (300 MHz, DMSO) δ 9.91 (s, 1H), 8.16 (dd, *J* = 7.5, 1.2 Hz, 1H), 7.95 (dd, *J* = 8.1, 1.2 Hz, 1H), 7.85 (t, *J* = 7.8 Hz, 1H), 7.67 (ddd, *J* = 7.0, 3.2, 1.9 Hz, 3H), 7.63 – 7.46 (m, 2H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 194.6, 190.1, 139.8, 136.7, 136.2, 135.6, 134.3, 132.5, 131.2, 131.2, 129.3, 129.3.

LRMS (ESI-MeOH, m/z):

2-ketoarylbenzaldehyde ($[C_{14}H_9ClO_2]+H)^+$ calc. 245.0, found 245.1

2-benzoyl-3-fluoro-benzaldehyde (6f)



6f was synthesized according to the general procedure for 2-ketobenzaldehydes using X (0.62g, 2.40mmol, 1 eq.) and $Pb(OAc)_4$ (1.17g, 2.64mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 2:8 EtOAc/Hexanes (v/v), affording **6f** as pale-yellow solids (0.39g, 71%).

^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.91 (s, 1H), 7.85 – 7.73 (m, 3H), 7.70 (t, *J* = 7.7 Hz, 1H), 7.66 – 7.59 (m, 1H), 7.53 – 7.43 (m, 3H).

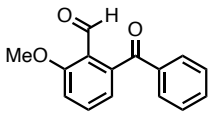
^{19}F NMR (282 MHz, CD_2Cl_2) δ -115.41.

^{13}C NMR (75 MHz, CD_2Cl_2) δ 190.2, 190.1, 137.3, 136.9, 134.4, 132.0 (d, *J* = 8.1 Hz), 129.4, 129.2, 128.2 (d, *J* = 2.9 Hz), 122.3, 121.9.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal ($[C_{15}H_{13}FO_3]+Na)^+$ calc. 283.1, found 283.3

2-benzoyl-6-methoxybenzaldehyde (6g)



6g was synthesized according to the general procedure for 2-ketobenzaldehydes using X (0.79g, 2.92mmol, 1 eq.) and $Pb(OAc)_4$ (1.43g, 3.22mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes, affording **6g** as sand-coloured powder (0.62g, 89%).

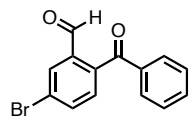
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.38 (d, *J* = 0.8 Hz, 1H), 7.75 – 7.62 (m, 3H), 7.60 – 7.51 (m, 1H), 7.47 – 7.37 (m, 2H), 7.18 (d, *J* = 8.5 Hz, 1H), 6.90 (d, *J* = 7.5 Hz, 1H), 4.00 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 197.4, 189.2, 162.5, 142.7, 137.3, 136.0, 133.4, 129.3, 128.8, 123.5, 119.9, 113.2, 56.5.

LRMS (ESI-MeOH, m/z):

2-ketoarylbenzaldehyde ($[C_{15}H_{12}O_3]+H)^+$ calc. 241.1, found 241.2

2-benzoyl-5-bromobenzaldehyde (6h)



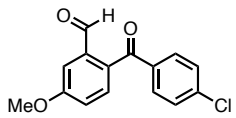
6h was synthesized according to the general procedure for 2-ketobenzaldehydes using X (1.20g, 3.76mmol, 1 eq.) and $Pb(OAc)_4$ (1.83g, 4.14mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6h** as brown waxy solids (1.1g, quantitative).

^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.95 (s, 1H), 8.15 (d, *J* = 2.0 Hz, 1H), 7.84 (dd, *J* = 8.1, 2.0 Hz, 1H), 7.81 – 7.74 (m, 2H), 7.68 – 7.61 (m, 1H), 7.56 – 7.45 (m, 2H), 7.42 (d, *J* = 8.1 Hz, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.6, 189.7, 140.2, 137.5, 137.1, 136.5, 134.2, 133.2, 131.1, 130.2, 129.1, 125.6, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z): 2-ketoarylbenzaldehyde ($[C_{14}H_9O_2]+H)^+$ calc. 288.9, found 290.0

2-(4-chlorobenzoyl)-5-methoxybenzaldehyde (6i)



6i was synthesized according to the general procedure for 2-ketobenzaldehydes using X (1.43g, 4.65mmol, 1 eq.) and $Pb(OAc)_4$ (2.27g, 5.11mmol, 1.1 eq.). Crude material was then purified via flash column chromatography, eluted in 1:1 EtOAc/Hexanes (v/v), affording **6i** as yellow solids (1.01g, 79%).

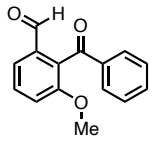
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.80 (s, 1H), 7.94 (d, *J* = 8.6 Hz, 1H), 7.75 – 7.64 (m, 2H), 7.47 – 7.37 (m, 2H), 7.16 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.93 (d, *J* = 2.5 Hz, 1H), 3.90 (s, 4H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.5, 189.6, 164.3, 143.1, 140.1, 135.7, 134.4, 131.2, 129.3, 128.4, 128.2, 115.5, 114.4, 56.4, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal ($[C_{16}H_{15}ClO_4]+Na)^+$ calc. 329.1, found 329.4

2-benzoyl-3-methoxybenzaldehyde (6j)



6j was synthesized according to the general procedure for 2-ketobenzaldehydes using X (3.12g, 11.5mmol, 1 eq.) and $Pb(OAc)_4$ (5.63g, 12.7mmol, 1.1 eq.). Crude material was then triturated in Et2O and further purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6j** as yellow waxy solids in quantitative yield.

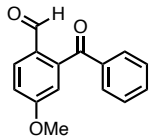
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.87 (s, 1H), 7.82 – 7.73 (m, 2H), 7.70 – 7.53 (m, 3H), 7.45 (dd, *J* = 8.4, 7.0 Hz, 2H), 7.32 (dd, *J* = 8.2, 1.1 Hz, 1H), 3.76 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.8, 191.1, 157.4, 137.8, 135.7, 133.7, 131.2, 130.2, 129.2, 129.0, 124.2, 117.5, 56.6, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

2-ketoarylbenzaldehyde ($[C_{15}H_{12}O_3]+H)^+$ calc. 241.1, found 241.1

2-benzoyl-4-methoxybenzaldehyde (6k)



6k was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.16g, 4.29mmol, 1 eq.) and $Pb(OAc)_4$ (2.09g, 4.72mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6k** as pale yellow solids (0.56g, 51%).

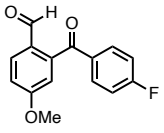
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.82 (s, 1H), 7.96 (d, *J* = 8.6 Hz, 1H), 7.82 – 7.74 (m, 2H), 7.65 – 7.57 (m, 1H), 7.52 – 7.42 (m, 2H), 7.16 (dd, *J* = 8.6, 2.5 Hz, 1H), 6.95 (d, *J* = 2.5 Hz, 1H), 3.90 (s, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 196.6, 189.5, 164.2, 143.9, 137.3, 133.9, 133.8, 129.9, 129.1, 129.0, 128.8, 128.5, 115.5, 114.4, 56.3.

LRMS (ESI-MeOH, m/z):

2-ketoarylbenzaldehyde ($[C_{15}H_{12}O_3]+H)^+$ calc. 241.1, found 241.2

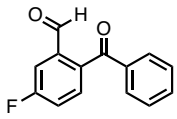
2-(4-fluorobenzoyl)-4-methoxybenzaldehyde (6l)



6l was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.19g, 4.11mmol, 1 eq.) and $Pb(OAc)_4$ (2.00g, 4.52mmol, 1.1 eq.), affording crude material as a thick oil-like amber substance, which was then purified via flash column chromatography and eluted in 3:7 EtOAc/Hexanes (v/v) to yield **6l** as chunky yellow solids (0.64g, 60%).

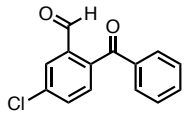
^{1}H NMR (300 MHz, DMSO) δ 9.81 – 9.76 (m, 1H), 8.07 (d, *J* = 8.6 Hz, 1H), 7.78 – 7.68 (m, 2H), 7.38 – 7.27 (m, 3H), 7.06 (d, *J* = 2.5 Hz, 1H), 3.90 (s, 3H).
^{13}C NMR (75 MHz, DMSO) δ 194.5, 190.4, 166.8, 163.5 (d, *J* = 12.7 Hz), 142.2, 135.4, 133.2 (d, *J* = 2.7 Hz), 132.0 (d, *J* = 9.8 Hz), 127.4, 115.8 (d, *J* = 22.1 Hz), 115.1, 113.8, 56.1.
^{19}F NMR (282 MHz, DMSO) δ -105.90.
LRMS (ESI-MeOH, m/z):
2-ketoarylbenzaldehyde ($[C_{15}H_{11}FO_3]+Na)^+$ calc. 281.1, found 281.3

2-benzoyl-5-fluorobenzaldehyde (6m)



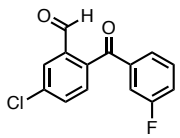
6m was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.19g, 4.61mmol, 1 eq.) and $Pb(OAc)_4$ (2.25g, 5.07mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 2:8 EtOAc/Hexanes (v/v), affording **6m** as brown solids (0.87g, 83%).
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.00 (d, *J* = 2.2 Hz, 1H), 7.82 – 7.77 (m, 2H), 7.71 (dd, *J* = 8.7, 2.7 Hz, 1H), 7.68 – 7.62 (m, 1H), 7.58 (dd, *J* = 8.5, 5.1 Hz, 1H), 7.55 – 7.47 (m, 2H), 7.40 (td, *J* = 8.2, 2.7 Hz, 1H).
^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.4, 189.8 (d, *J* = 1.7 Hz), 165.9, 162.6, 138.8 (d, *J* = 6.7 Hz), 137.8, 137.4, 134.1, 132.3 (d, *J* = 8.3 Hz), 130.4, 129.1, 120.4 (d, *J* = 22.1 Hz), 116.5 (d, *J* = 22.9 Hz).
^{19}F NMR (282 MHz, DMSO) δ -109.04.
LRMS (ESI-MeOH, m/z):
Methyl hemiacetal ($[C_{15}H_{13}FO_3]+H)^+$ calc. 261.1, found 261.1

2-benzoyl-5-chlorobenzaldehyde (6n)



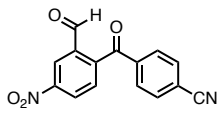
6n was prepared according to the general procedure for 2-ketobenzaldehydes using (1.17g, 4.26mmol, 1 eq.) and $Pb(OAc)_4$ (2.08g, 4.68mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6n** yellow powder (0.84g, 81%).
^{1}H NMR (300 MHz, DMSO) δ 9.93 (s, 1H), 8.16 (d, *J* = 2.2 Hz, 1H), 7.90 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.69 (tt, *J* = 8.7, 1.3 Hz, 3H), 7.62 – 7.48 (m, 3H).
^{13}C NMR (75 MHz, DMSO) δ 195.3, 191.2, 138.5, 136.6, 136.2, 135.3, 133.7, 133.5, 131.4, 130.3, 129.3, 128.8.
LRMS (ESI-MeOH, m/z):
2-arylketobenzaldehyde ($[C_{15}H_{13}FO_3]+H)^+$ calc. 245.0, found 245.1

5-chloro-2-(3-fluorobenzoyl)benzaldehyde (6o)



6o was prepared according to the general procedure for 2-ketobenzaldehydes using X (0.78g, 2.66mmol, 1 eq.) and $Pb(OAc)_4$ (1.30g, 2.93mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6o** as waxy brown-yellow solids (0.24g, 35%)
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.96 (s, 1H), 7.99 (d, *J* = 2.2 Hz, 1H), 7.70 (dd, *J* = 8.1, 2.2 Hz, 1H), 7.55 – 7.43 (m, 5H), 7.39 – 7.29 (m, 1H).
^{13}C NMR (75 MHz, CD_2Cl_2) δ 194.4, 189.8, 164.8, 161.5, 139.2 (d, *J* = 6.4 Hz), 139.0, 137.4, 133.7, 131.4 – 129.9 (m), 126.2 (d, *J* = 3.0 Hz), 121.1 (d, *J* = 21.6 Hz), 116.5 (d, *J* = 22.5 Hz).
LRMS (ESI-MeOH, m/z):
Methyl hemiacetal ($[C_{15}H_{12}ClFO_3]+H)^+$ calc. 295.0, found 295.3

4-(2-formly-4-nitrobenzoyl)benzonitrile (6p)



6p was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.69g, 5.44mmol, 1 eq.) and $Pb(OAc)_4$ (2.66g, 5.99mmol, 1.1 eq.). Crude material was triturated in Et_2O and filtered, affording **6p** as fine pale-orange powder in quantitative yield (1.50g).

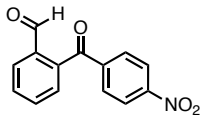
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.03 (d, *J* = 0.6 Hz, 1H), 8.83 (d, *J* = 2.3 Hz, 1H), 8.58 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.87 – 7.75 (m, 4H), 7.70 (d, *J* = 8.3 Hz, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 194.0, 189.1, 149.6, 145.1, 139.2, 136.6, 133.1, 130.3, 130.1, 128.7, 126.8, 118.1, 117.6, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde $([C_{15}H_8N_2O_4]+H)^+$ calc. 281.1, found 282.2

2-(4-nitrobenzoyl)benzaldehyde (6q)



6q was prepared according to the general procedure for 2-ketobenzaldehydes using X (2.00g, 7.01mmol, 1 eq.) and $Pb(OAc)_4$ (3.42g, 7.71mmol, 1.1 eq.). Crude material was triturated in Et_2O and hexanes and filtered, affording **6q** as pale pink solids (1.43g, 80%).

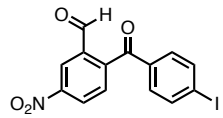
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.98 – 9.94 (m, 1H), 8.32 – 8.20 (m, 2H), 8.07 – 7.98 (m, 1H), 7.94 – 7.87 (m, 2H), 7.84 – 7.74 (m, 2H), 7.54 – 7.46 (m, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.6, 191.3, 150.7, 142.0, 139.8, 135.6, 134.4, 132.5, 131.5, 130.7, 128.9, 124.1.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde $([C_{14}H_9NO_4]+H)^+$ calc. 256.1, found 256.4

2-(4-iodobenzoyl)-5-nitrobenzaldehyde (6r)



6r was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.50g, 3.65mmol, 1 eq.) and $Pb(OAc)_4$ (1.78g, 4.01mmol, 1.1 eq.). Crude material was triturated in Et_2O and filtered, affording **6r** as red-brown powder (1.00g, 72%).

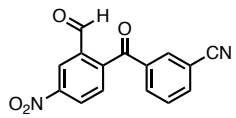
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.02 (s, 1H), 8.80 (d, *J* = 2.3 Hz, 1H), 8.53 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.92 – 7.84 (m, 2H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.50 – 7.42 (m, 2H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 194.3, 188.9, 149.3, 145.9, 138.6, 136.6, 135.6, 131.1, 130.3, 128.3, 126.0, 103.0, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde $([C_{14}H_8INO_4]+Na)^+$ calc. 403.9, found 404.2

3-(2-formyl-4-nitrobenzoyl)benzonitrile (6s)



6s was prepared according to the general procedure for 2-ketobenzaldehydes using X (2.10g, 6.77mmol, 1 eq.) and $Pb(OAc)_4$ (3.30g, 7.44mmol, 1.1 eq.). Crude material was triturated in Et_2O and filtered, affording **6s** as yellow powder (1.29g, 68%)

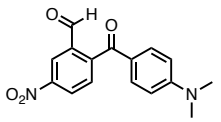
^{1}H NMR (300 MHz, DMSO) δ 10.06 (s, 1H), 8.98 (d, *J* = 2.3 Hz, 1H), 8.64 (dd, *J* = 8.3, 2.3 Hz, 1H), 8.15 (dt, *J* = 7.7, 1.4 Hz, 1H), 8.06 (dd, *J* = 7.8, 1.4 Hz, 2H), 7.86 (d, *J* = 8.3 Hz, 1H), 7.81 – 7.71 (m, 1H).

^{13}C NMR (75 MHz, DMSO) δ 193.8, 191.4, 148.6, 143.8, 137.1, 136.5, 135.7, 133.1, 132.8, 130.3, 129.7, 128.7, 127.8, 117.9, 112.1.

LRMS (ESI-MeOH, m/z):

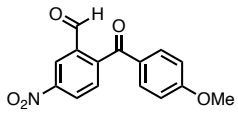
2-arylketobenzaldehyde $([C_{15}H_8N_2O_4]+Na)^+$ calc. 303.1, found 303.1

2-(4-(dimethylamino)benzoyl)-5-nitrobenzaldehyde (6t)



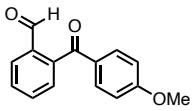
6t was prepared according to the general procedure for 2-ketobenzaldehydes using X (3.21g, 9.78mmol, 1 eq.) and $Pb(OAc)_4$ (4.77g, 10.75mmol, 1.1 eq.). Crude material was triturated in Et_2O and filtered, affording **6t** as bright orange powder (2.19g, 75%)
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.02 (s, 1H), 8.79 (d, *J* = 2.3 Hz, 1H), 8.47 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.69 (d, *J* = 8.3 Hz, 1H), 7.66 – 7.62 (m, 2H), 6.73 – 6.63 (m, 2H), 3.08 (s, 6H).
^{13}C NMR (75 MHz, CD_2Cl_2) δ 191.7, 189.0, 154.8, 148.9, 136.2, 132.8, 130.4, 127.8, 124.2, 124.0, 111.2, 54.6, 54.2, 53.8, 53.5, 53.1, 40.2.
LRMS (ESI-MeOH, m/z):
2-arylketobenzaldehyde ($[C_{16}H_{14}N_2O_4]+Na)^+$ calc. 321.1, found 321.2

2-(4-methoxybenzoyl)-5-nitrobenzaldehyde (6u)



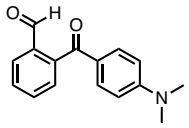
6u was prepared according to the general procedure for 2-ketobenzaldehydes using X (3.32g, 10.53mmol, 1 eq.) and $Pb(OAc)_4$ (5.13g, 11.58mmol, 1.1 eq.). Crude material was triturated in Et_2O and hexanes and filtered, affording **6u** as bright yellow powder in quantitative yield (2.94g).
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.03 (s, 1H), 8.82 – 8.79 (m, 1H), 8.51 (dd, *J* = 8.3, 2.3 Hz, 1H), 7.78 – 7.72 (m, 2H), 7.70 (d, *J* = 8.3 Hz, 1H), 7.03 – 6.93 (m, 2H), 3.89 (s, 3H).
^{13}C NMR (75 MHz, CD_2Cl_2) δ 193.2, 188.8, 165.2, 149.2, 147.5, 136.5, 132.7, 130.4, 129.5, 128.1, 125.0, 114.6, 56.1, 54.6, 54.2, 53.8, 53.5, 53.1.
LRMS (ESI-MeOH, m/z):
2-arylketobenzaldehyde ($[C_{15}H_{11}NO_5]+H)^+$ calc. 286.1, found 286.0

2-(4-methoxybenzoyl)benzaldehyde (6v)



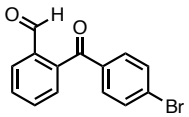
6v was prepared according to the general procedure for 2-ketobenzaldehydes using X (3.38g, 12.52mmol, 1 eq.) and $Pb(OAc)_4$ (5.54g, 13.76mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes, affording **6v** as pale yellow chunky solids (2.28g, 76%).
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.99 (s, 1H), 8.04 – 7.96 (m, 1H), 7.79 – 7.73 (m, 2H), 7.72 – 7.66 (m, 2H), 7.53 – 7.45 (m, 1H), 6.99 – 6.90 (m, 2H), 3.87 (s, 3H).
^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.2, 191.1, 164.5, 142.2, 135.5, 133.7, 132.6, 130.6, 130.5, 130.4, 129.0, 114.2, 56.0.
LRMS (ESI-MeOH, m/z):
Methyl hemiacetal ($[C_{16}H_{16}O_4]+H)^+$ calc. 273.1, found 273.1

2-(4-(dimethylamino)benzoyl)benzaldehyde (6w)



6w was prepared according to the general procedure for 2-ketobenzaldehydes using X (3.80g, 13.41mmol, 1 eq.) and $Pb(OAc)_4$ (6.54g, 14.75mmol, 1.1 eq.). Crude material was purified via column chromatography, eluted in 1:1 EtOAc/Hexanes (v/v), affording **6w** as bright yellow chunky solids (2.92g, 86%).
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.99 (s, 1H), 8.02 – 7.95 (m, 1H), 7.72 – 7.55 (m, 4H), 7.48 (dd, *J* = 6.9, 1.9 Hz, 1H), 6.71 – 6.61 (m, 2H), 3.06 (s, 6H).
^{13}C NMR (75 MHz, CD_2Cl_2) δ 194.1, 191.2, 154.3, 143.6, 135.4, 133.6, 132.6, 130.1, 129.2, 128.9, 125.2, 111.0, 54.6, 54.2, 53.8, 53.5, 53.1, 40.2.
LRMS (ESI-MeOH, m/z): 2-arylketobenzaldehyde ($[C_{16}H_{15}NO_2]+H)^+$ calc. 254.1, found 254.2

2-(4-bromobenzoyl)benzaldehyde (6x)



6x was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.54g, 4.83mmol, 1 eq.) and $Pb(OAc)_4$ (2.35g, 5.31mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes, affording **6x** as dark brown solids (0.93g, 66%).

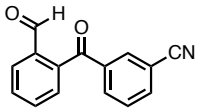
^{1}H NMR (300 MHz, CD2Cl2) δ 9.98 (s, 1H), 8.03 – 7.96 (m, 1H), 7.75 – 7.70 (m, 2H), 7.66 – 7.60 (m, 4H), 7.50 – 7.43 (m, 1H).

^{13}C NMR (75 MHz, CD2Cl2) δ 195.9, 191.2, 140.8, 136.3, 135.6, 134.0, 132.3, 131.5, 131.1, 129.0, 129.0, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal $([C_{15}H_{13}BrO_3]+H)^+$ calc. 321.0, found 321.0

3-(2-formylbenzoyl)benzonitrile (6y)



6y was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.19g, 4.48mmol, 1 eq.) and $Pb(OAc)_4$ (2.35g, 5.31mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 1:1 EtOAc/Hexanes (v/v), affording **6y** as orange solids (0.82g, 77%).

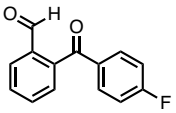
^{1}H NMR (300 MHz, DMSO) δ 9.94 (s, 1H), 8.15 – 8.07 (m, 2H), 8.04 – 8.00 (m, 1H), 7.96 (dt, *J* = 8.0, 1.4 Hz, 1H), 7.84 (dd, *J* = 5.6, 3.2 Hz, 2H), 7.72 (t, *J* = 7.8 Hz, 1H), 7.55 (dd, *J* = 5.5, 3.2 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 195.0, 192.5, 138.7, 137.3, 136.6, 134.8, 134.2, 133.4, 132.7, 132.5, 131.0, 130.2, 128.3, 118.0, 112.0, 40.3, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal $([C_{16}H_{13}NO_3]+Na)^+$ calc. 290.1, found 290.1

2-(4-fluorobenzoyl)benzaldehyde (6z)



6z was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.92g, 7.44mmol, 1 eq.) and $Pb(OAc)_4$ (3.63g, 8.18mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 100% DCM, affording **6z** as white flaky solids (1.22g, 72%).

^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.99 (s, 1H), 8.05 – 7.97 (m, 1H), 7.85 – 7.77 (m, 2H), 7.77 – 7.67 (m, 2H), 7.53 – 7.44 (m, 1H), 7.21 – 7.10 (m, 2H).

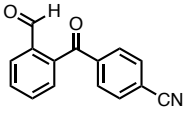
^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.3, 191.1, 168.0, 164.7, 141.2, 135.6, 133.9, 132.8 (d, *J* = 9.6 Hz), 131.1 (d, *J* = 21.8 Hz), 128.9, 116.3, 116.0.

^{19}F NMR (282 MHz, CD_2Cl_2) δ -105.13.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal $([C_{16}H_{13}NO_3]+H)^+$ calc. 261.1, found 261.0

4-(2-formylbenzoyl)benzonitrile (6aa)



6aa was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.29g, 4.86mmol, 1 eq.) and $Pb(OAc)_4$ (2.37g, 5.35mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 1:1 EtOAc/hexanes (v/v), affording **6aa** as light brown solids (0.75g, 66%).

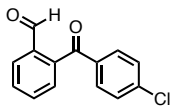
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.97 (s, 1H), 8.06 – 7.97 (m, 1H), 7.87 – 7.80 (m, 2H), 7.80 – 7.72 (m, 5H), 7.47 (dd, *J* = 5.5, 3.3 Hz, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.8, 191.3, 140.4, 139.9, 135.7, 134.3, 132.9, 132.3, 131.4, 130.1, 128.9, 118.3, 116.9.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal $([C_{16}H_{13}NO_3]+H)^+$ calc. 268.1, found 268.1

2-(4-chlorobenzoyl)benzaldehyde (6ab)



6ab was prepared according to the general procedure for 2-ketobenzaldehydes using X (2.02g, 7.34mmol, 1eq.) and $Pb(OAc)_4$ (3.58g, 8.07mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 2:8 EtOAc/Hexanes, affording **6ab** as amber crystalline solids (0.97g, 54%).

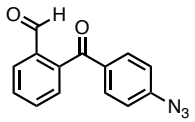
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.98 (s, 1H), 8.05 – 7.96 (m, 1H), 7.72 (dt, *J* = 6.6, 2.3 Hz, 4H), 7.52 – 7.41 (m, 3H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.7, 191.1, 140.8, 140.2, 135.9, 135.6, 134.0, 131.4, 131.4, 131.1, 129.3, 128.9, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal ($[C_{15}H_{13}ClO_3]+H)^+$ calc. 299.1, found 299.1

2-(4-azidobenzoyl)benzaldehyde (6ac)



6ac was prepared according to the general procedure for 2-ketobenzaldehydes using X (2.76g, 9.81mmol, 1 eq.) and $Pb(OAc)_4$ (4.78g, 10.79mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes, affording **6ac** as waxy bright yellow solids (1.30g, 53%).

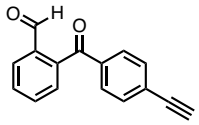
^{1}H NMR (300 MHz, DMSO) δ 9.95 (s, 1H), 8.16 – 8.04 (m, 1H), 7.89 – 7.75 (m, 2H), 7.75 – 7.65 (m, 2H), 7.58 – 7.47 (m, 1H), 7.29 – 7.19 (m, 2H).

^{13}C NMR (75 MHz, DMSO) δ 194.9, 192.2, 144.7, 140.0, 134.6, 134.0, 133.2, 132.1, 131.3, 130.6, 128.2, 119.4, 40.3, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde ($[C_{14}H_9N_3O_2]+H)^+$ calc. 252.1, found 252.2

2-(4-ethynylbenzoyl)benzaldehyde (6ad)



6ad was prepared according to the general procedure for 2-ketobenzaldehydes using X (0.68g, 2.67mmol, 1 eq.) and $Pb(OAc)_4$ (1.25g, 2.83mmol, 1.1 eq.). Crude material was purified via flash column chromatography, eluted in 3:7 EtOAc/Hexanes (v/v), affording **6ad** as pale yellow clumped solids (0.13g, 21%)

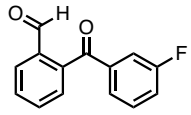
^{1}H NMR (300 MHz, CD_2Cl_2) δ 9.99 (s, 1H), 8.07 – 7.95 (m, 1H), 7.81 – 7.66 (m, 4H), 7.72 – 7.54 (m, 2H), 7.54 – 7.42 (m, 1H), 3.35 (s, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 196.0, 191.2, 141.0, 137.2, 135.7, 134.0, 132.6, 131.4, 131.1, 129.9, 129.0, 127.5, 82.9, 81.0, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde ($[C_{16}H_{10}O_2]+H)^+$ calc. 235.1, found 235.3

2-(3-fluorobenzoyl)benzaldehyde (6ae)



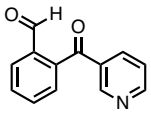
6ae was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.08g, 4.18mmol, 1 eq.) and $Pb(OAc)_4$ (2.04g, 4.60mmol, 1.1 eq.). Crude material was triturated in a mixture of Et_2O and hexanes, to afford **6ae** as orange solids (0.41g, 43%).

^{1}H NMR (300 MHz, DMSO) δ 9.96 (s, 1H), 8.12 (dd, *J* = 5.8, 3.1 Hz, 1H), 7.90 – 7.78 (m, 2H), 7.55 (h, *J* = 6.9 Hz, 3H), 7.49 – 7.38 (m, 2H). **^{13}C NMR** (75 MHz, DMSO) δ 195.2, 192.4, 163.7, 160.4, 139.3, 138.8, 138.7, 134.7, 134.1, 132.4, 131.1, 131.0, 130.8, 128.2, 125.7, 125.6, 120.6, 120.3, 115.2, 114.9, 40.4, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7.

^{19}F NMR (282 MHz, DMSO) δ -112.14.

LRMS (ESI-MeOH, m/z): Methyl hemiacetal ($[C_{15}H_{13}FO_3]+Na)^+$ calc. 283.1, found 283.1

2-nicotinoylbenzaldehyde (6af)



6af was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.73g, 7.15mmol, 1 eq.) and $Pb(OAc)_4$ (3.49g, 7.87mmol, 1.1 eq.). Crude material was purified via flash column chromatography in 2:98 Et_3N/DCM (v/v), affording **6af** as fine beige powder (1.35g, 90%).

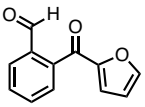
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.00 (s, 1H), 8.84 (dd, *J* = 2.3, 0.9 Hz, 1H), 8.77 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.16 – 8.10 (m, 1H), 8.05 – 7.98 (m, 1H), 7.80 – 7.72 (m, 2H), 7.54 – 7.48 (m, 1H), 7.45 (ddd, *J* = 8.0, 4.9, 0.9 Hz, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 195.8, 191.3, 153.9, 151.2, 140.1, 136.8, 135.7, 134.2, 132.9, 132.0, 131.4, 129.0, 124.0.

LRMS (ESI-MeOH, m/z):

Methyl hemiacetal ($[C_{15}H_{13}FO_3]+H)^+$ calc. 244.1, found 244.1

2-(furan-2-carbonyl)benzaldehyde (6ag)



6ag was prepared according to the general procedure for 2-ketobenzaldehydes using X (0.98g, 4.26mmol, 1 eq.) and $Pb(OAc)_4$ (2.08g, 4.68mmol, 1.1 eq.). Crude material was purified via flash column chromatography in 3:7 EtOAc/hexanes, affording **6ag** as light yellow solids (0.44, 51%).

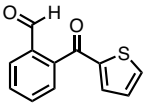
^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.05 (s, 1H), 8.03 – 7.97 (m, 1H), 7.74 – 7.63 (m, 4H), 7.07 (dd, *J* = 3.6, 0.8 Hz, 1H), 6.61 (dd, *J* = 3.6, 1.7 Hz, 1H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 191.1, 183.1, 152.9, 148.3, 140.5, 136.0, 133.7, 131.5, 130.2, 129.3, 121.4, 113.0, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde ($[C_{12}H_8O_3]+Na)^+$ calc. 223.0, found 223.0

2-(thiophene-2-carbonyl)benzadehyde (6ah)



6ah was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.40g, 5.68mmol, 1 eq.) and and $Pb(OAc)_4$ (2.77g, 6.25mmol, 1.1 eq.). Crude material was purified via flash column chromatography in 3:7 EtOAc/hexanes, affording **6ah** as amber-brown solids (0.93, 76%).

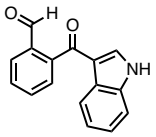
^{1}H NMR (300 MHz, DMSO) δ 9.99 (s, 1H), 8.13 (dd, *J* = 4.9, 1.2 Hz, 1H), 8.09 – 8.05 (m, 1H), 7.86 – 7.78 (m, 2H), 7.71 – 7.64 (m, 1H), 7.40 (dd, *J* = 3.8, 1.2 Hz, 1H), 7.22 (dd, *J* = 4.9, 3.8 Hz, 1H).

^{13}C NMR (75 MHz, DMSO) δ 191.8, 188.1, 143.5, 139.8, 136.3, 135.8, 134.6, 133.9, 131.3, 131.0, 128.9, 128.4, 40.4, 40.1, 39.8, 39.5, 39.2, 39.0, 38.7.

LRMS (ESI-MeOH, m/z):

2-arylketobenzaldehyde ($[C_{12}H_8O_2S]+H)^+$ calc. 217.0, found 217.1

2-(1H-indole-3-carbonyl)benzaldehyde (6ai)



6ai was prepared according to the general procedure for 2-ketobenzaldehydes using X (1.66g, 5.94mmol, 1 eq.) and $Pb(OAc)_4$ (2.90g, 6.54mmol, 1.1 eq.). Crude material was purified via flash column chromatography in 3:7 EtOAc/Hexanes, affording **6ai** as dark blue-green powder (0.52g, 35%).

^{1}H NMR (300 MHz, CD_2Cl_2) δ 10.12 (s, 1H), 9.03 (s, 1H), 8.35 (ddt, *J* = 6.2, 3.7, 0.8 Hz, 1H), 8.06 – 7.99 (m, 1H), 7.75 – 7.62 (m, 3H), 7.52 – 7.43 (m, 2H), 7.39 – 7.30 (m, 2H).

^{13}C NMR (75 MHz, CD_2Cl_2) δ 191.4, 144.2, 137.0, 135.5, 135.3, 133.7, 130.5, 129.0, 129.0, 126.0, 124.6, 123.5, 122.5, 119.0, 112.1, 54.6, 54.2, 53.8, 53.5, 53.1.

LRMS (ESI-MeOH, m/z): 2-arylketobenzaldehyde ($[C_{16}H_{11}NO_2]+H)^+$ calc. 250.1, found 250.3

Optimization of FIICk reaction of 2-arylketobenzaldehyde

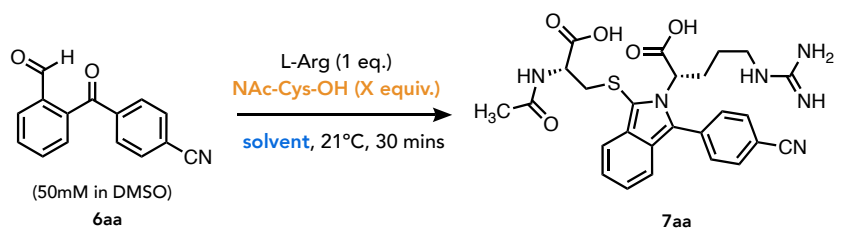
We conducted preliminary FIICk reactions with 2-arylketobenzaldehyde **6aa** using N-Ac-Cys and L-Arg as model substrates to optimize the formation of the desired disubstituted isoindole. L-Arg was chosen as the amine source to aid solubility in aqueous media and for its minimal interference with the isoindole's UV absorbance profile. Experimental protocols for entries 1-5 (Table 1) proceed as follow:

To a 15-mL conical tube containing 5mL Na borate buffer (pH 9) was added **6aa** (15µmol, 1 eq., prepared as 50mM solution in DMSO), NAc-Cys (X eq., prepared as 50mM solution), and L-Arg (15µmol, 1 eq., prepared as 50mM solution in H_2O). Then, solvent additive (2mL) was added into the conical tube and the reaction was vortexed for 30 seconds and left to sit at room temperature for 30 minutes. An aliquot of 900µL was obtained and this crude reaction mixture was then injected on preparative reverse-phase HPLC. Under optimized conditions, this reaction was repeated to accumulate enough material for NMR acquisition. Collected peak was not fluorescent as observed under 365nm hand-held UV lamp. Lyophilized product is bright yellow in colour.

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 50x21.2mm

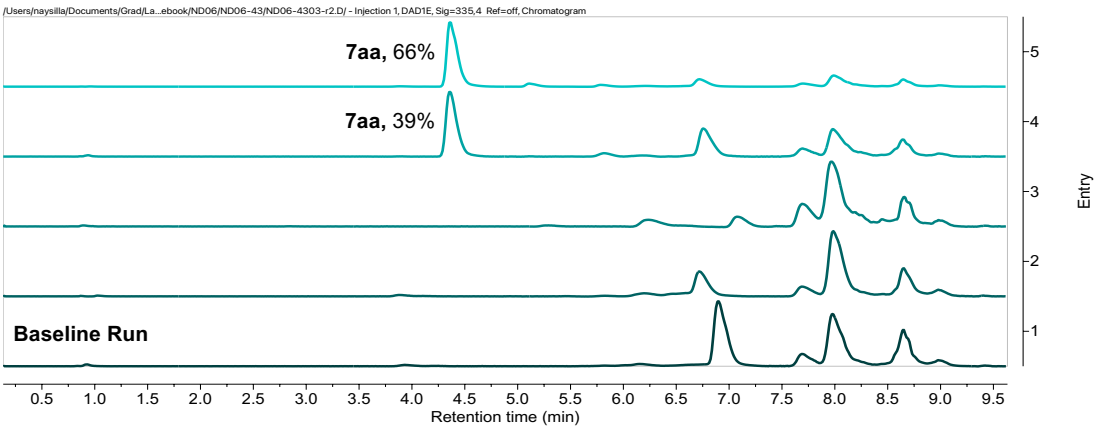
Time (min)	A%	B%	Flow (mL/min)
0.00	95	5	15
8.00	0	100	
10.00	0	100	

Table 1: Screening of conditions for the conversion of **6aa** to isoindole **7aa**



Entry	NAc-Cys-OH	L-Arg	Additive:Solvent	Conversion [%] to 7aa[b]
1 (Baseline)[a]	1 eq. (50mM in water)	1 eq.	Na borate buffer pH 9	0%
2	3 eq. (50mM in water)	1 eq.	Na borate buffer pH 9	0%
3	1 eq. (50mM in water)	1 eq.	MeCN:Na borate buffer pH 9 (25:75 v/v)	0%
4	1 eq. (50mM in H_2O 0.1% formic acid)	1 eq.	DMSO:Na borate buffer pH 9 (25:75 v/v)	39%
5	**1 eq. (50mM in H_2O 0.1% formic acid)**	**1 eq.**	**EtOH:Na borate buffer pH 9 (25:75 v/v)**	**66%**

[a] Baseline run was obtained by injecting the crude reaction mixture immediately after all reagents were added, amounting to <2 minutes of reaction time.
[b] % conversion was determined by HPLC peak integration of **7aa** relative to peaks observed in the baseline run (entry 1) observed at 335 nm.



NMR analysis and discussion for 7aa

NMR spectra of **7aa** were obtained at variable temperatures, initially cooled to -50ºC to observe aliphatic proton signals that were otherwise not well-resolved at room temperature. However, we see the opposite effect on the aromatic region, where signals were increasingly resolved as sample was warmed to +25ºC at 10ºC increments. As a result, we use both sets of data to analyze the structure of **7aa.**

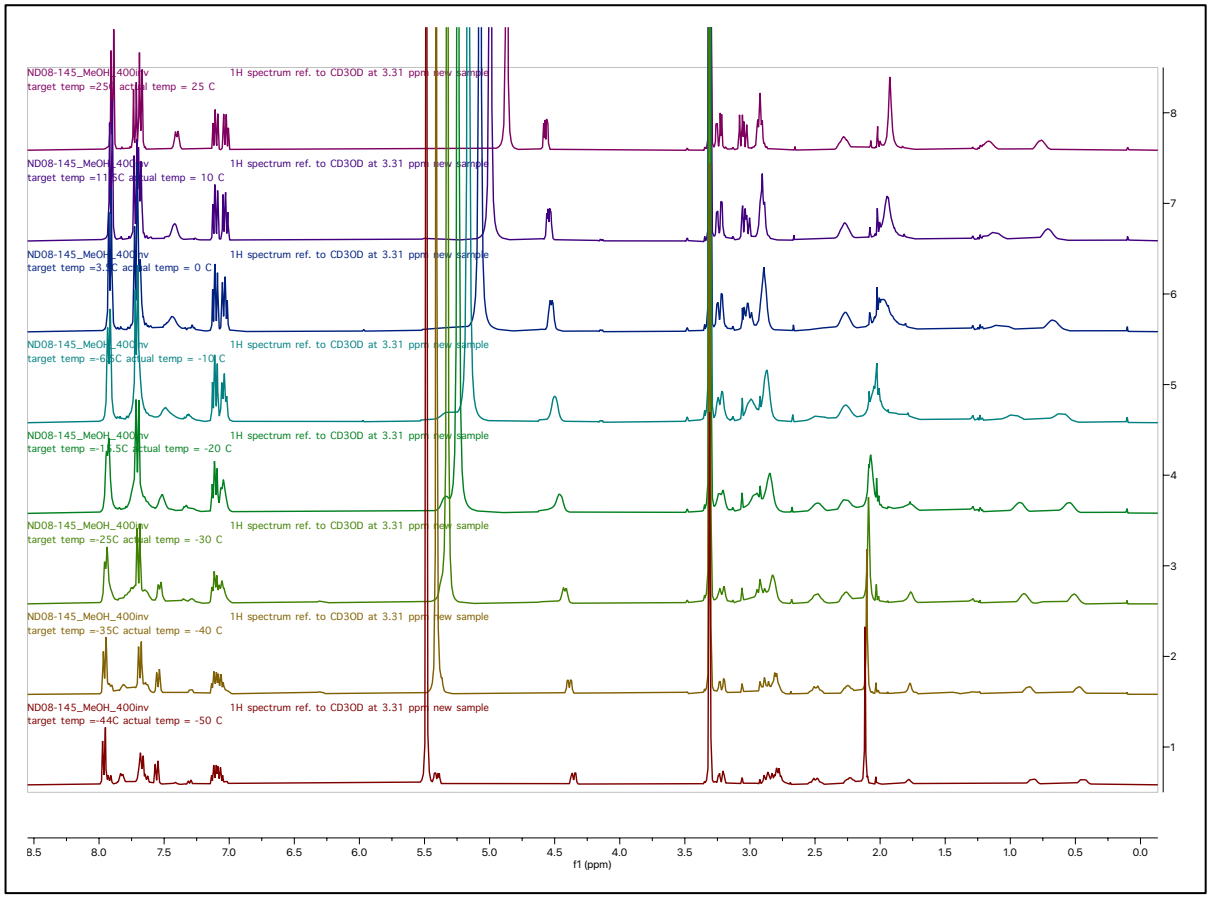


Figure S1: Overlay of ^{1}H NMR spectra of **7aa**, decreasing temperature from top to bottom.

Using the -50ºC data set to determine the isoindole linkage, we turn to the aliphatic region to identify the proton signals found in N-Ac-Cys and Arg. Here we were able to see four sets of methylene -CH2- signals (in red), which corresponds to the proposed structure of **7aa** and its accompanying H-integration in the 1D NMR.

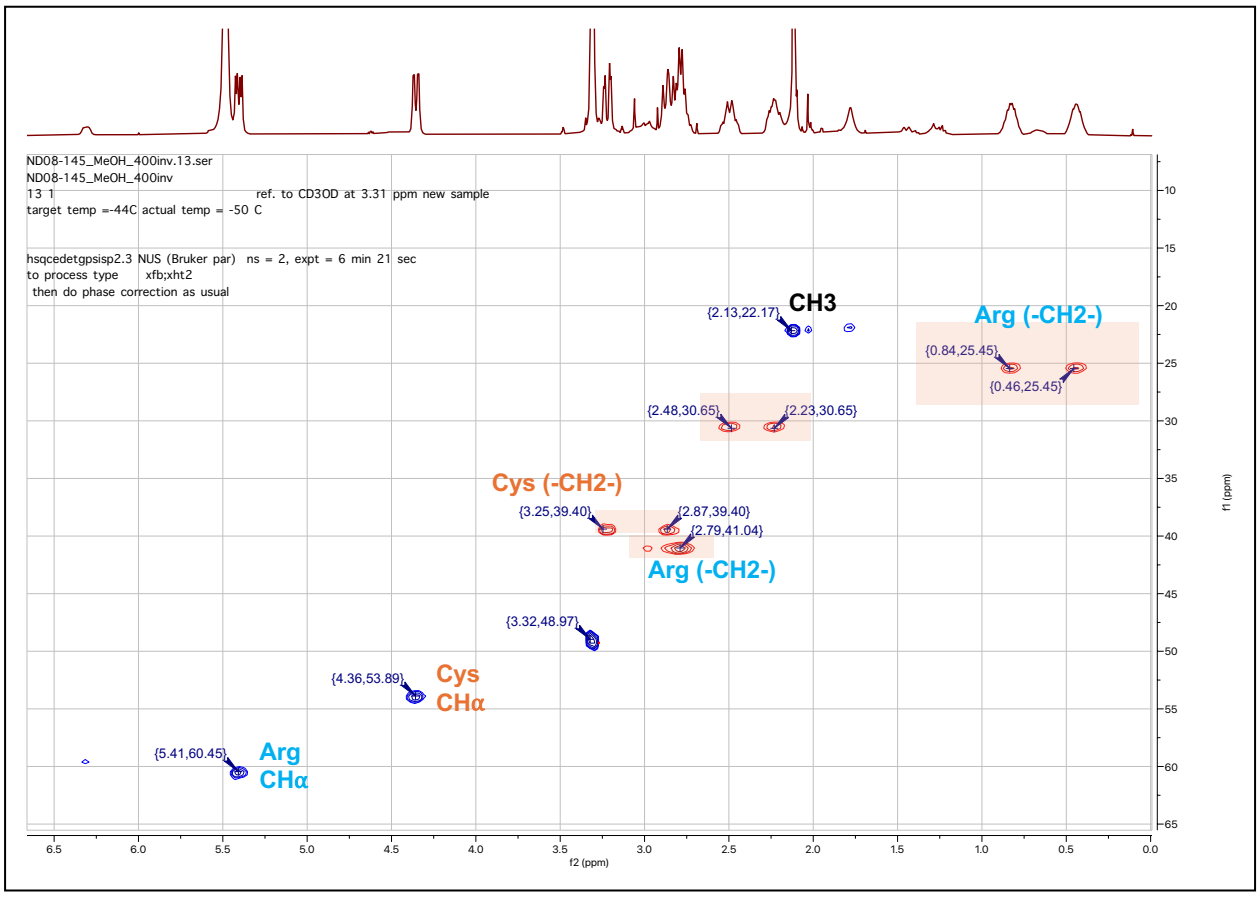


Figure S2: HSQC of **7aa** referenced to MeOD (3.31ppm) obtained at -50ºC. In red; -CH2- protons. In blue; CH/CH3 protons.

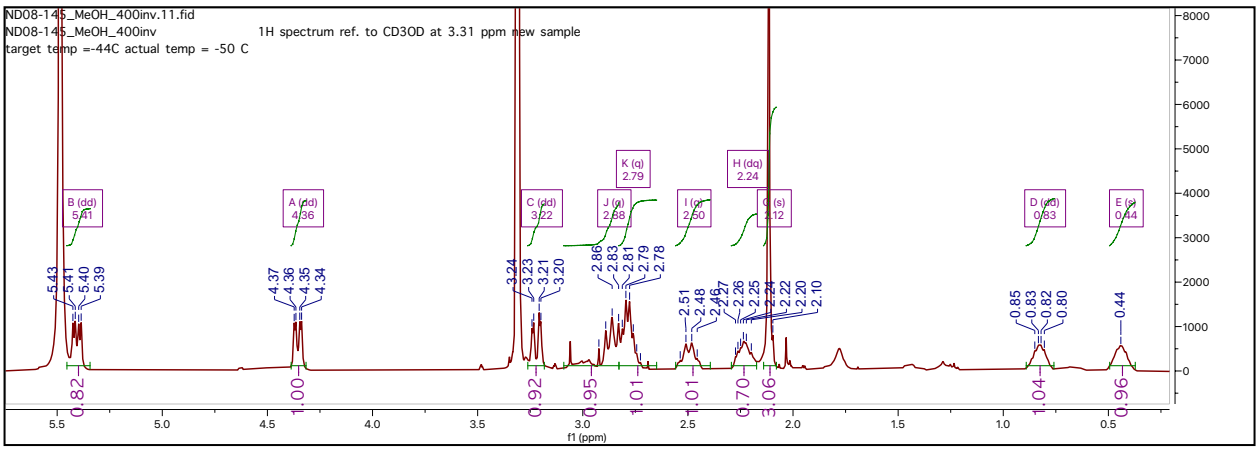


Figure S3: ^{1}H NMR of **7aa** taken at -50ºC, showing the aliphatic proton region from 0-5.5ppm.

Then, key HMBC signals of CHα of Arg and CH2 of NAc-Cys was shown to correlate to the same aromatic carbon of the isoindole (111.15ppm) confirming the proposed linkage between Cys-thiol and N-terminus of L-Arg.

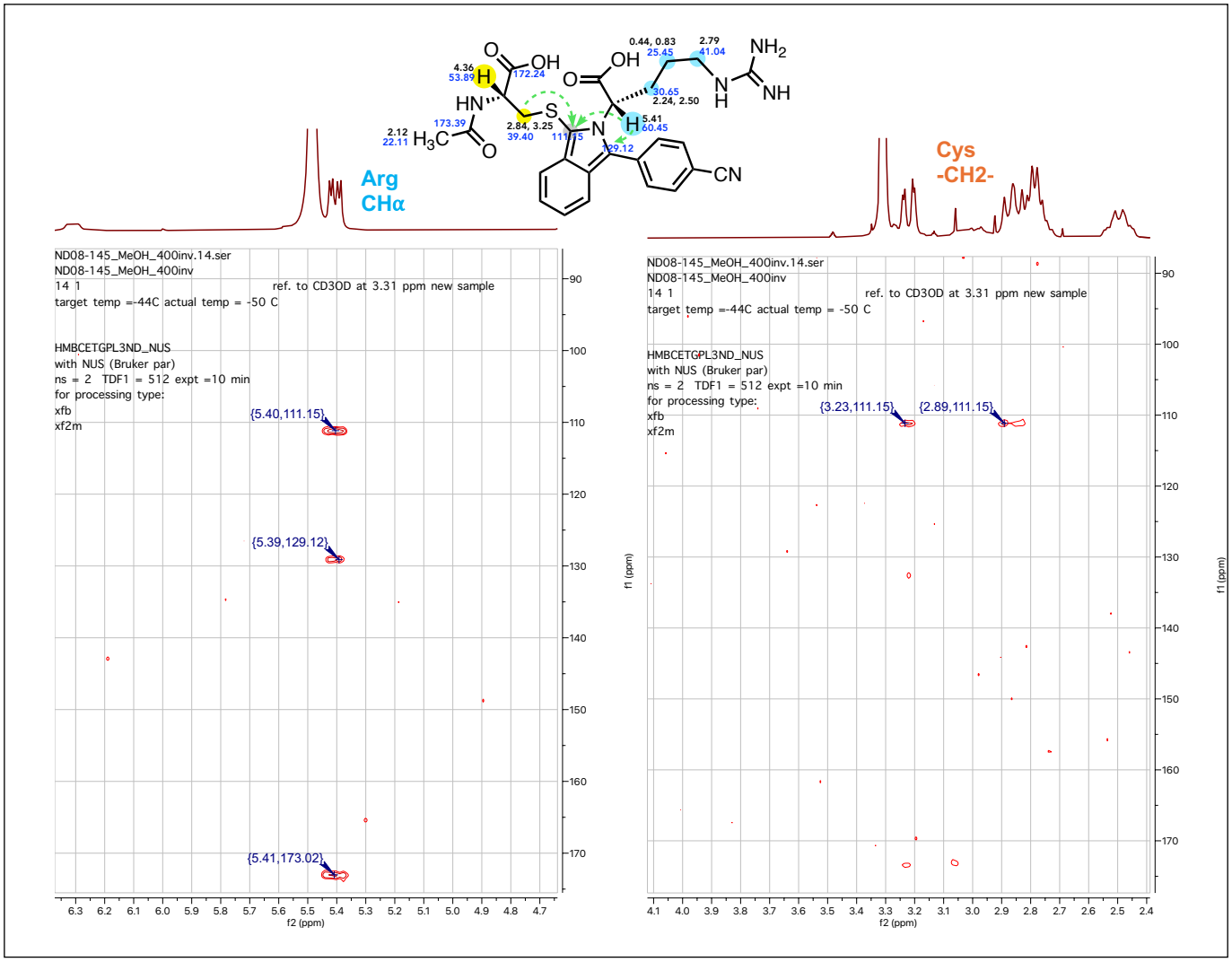


Figure S4: Key HMBC signals showing the isoindole scaffold forming between Cys-Thiol and N-terminal of Arg.

Then, aliphatic protons of Arg were assigned via analysis of COSY spectrum obtained at -50ºC.

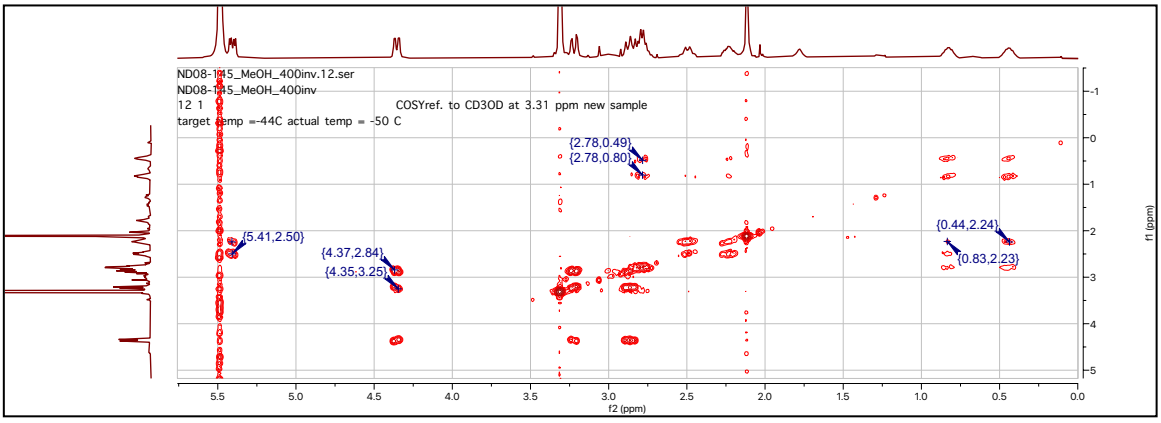


Figure S5: Key COSY signals showing the aliphatic J^3 correlations of Cys-Thiol and N-terminal of Arg.

Then, aromatic region analysis was done on +25ºC data set given the improved signal resolution. The same sample was slowly warmed up to room temperature from -50ºC.

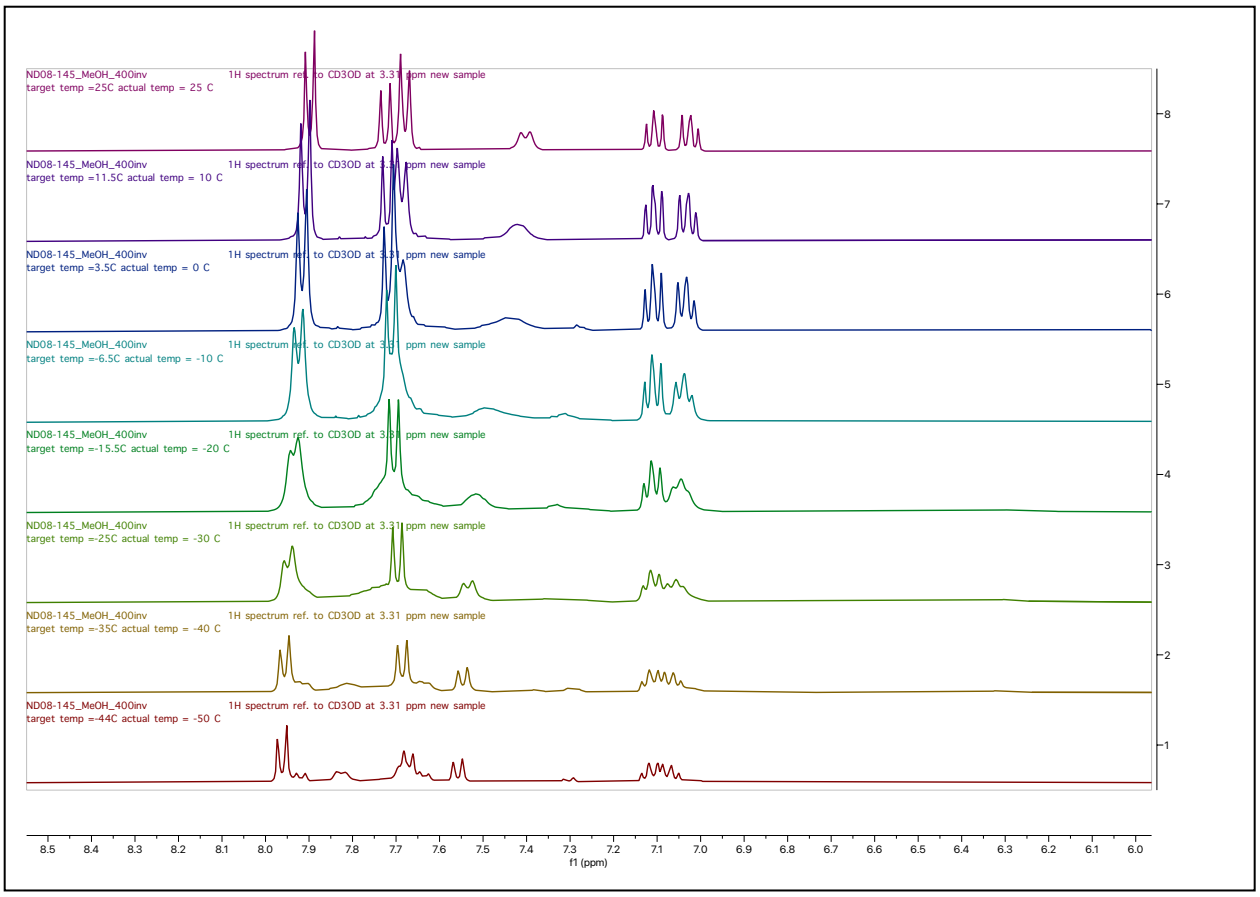


Figure S6: Overlay of ^{1}H NMR spectra of **7aa**, focusing on the aromatic region, showing increased signal resolution as a function of increasing temperature, going from bottom-top.

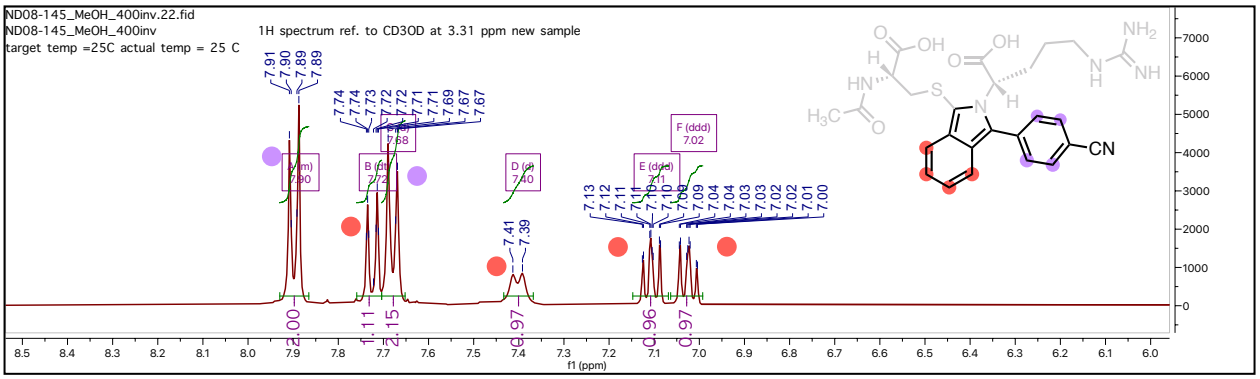


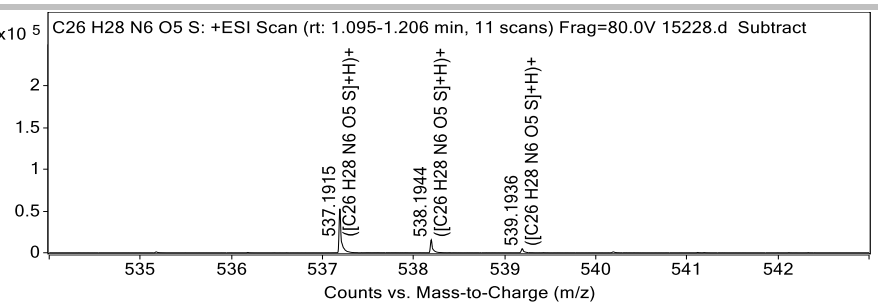
Figure S7: Integration of 1H NMR spectrum obtained at 25ºC showing 8 aromatic protons.

Table S2: ^{1}H NMR assignment for compound **7aa**. Carbon chemical shifts assignment was determined from HSQC and HMBC. Signals were assigned from two different data sets that originates from the same sample taken at different temperatures as indicated.

Residue	Proton	Shift (ppm)	Coupling constant (J, Hz), Integration	Carbon	Shift (ppm)
Cys				COOH C=O (Ac)	172.2[a] 173.4[a]
	HCα	4.36[a]	dd, *J* = 10.7, 3.4 Hz, 1H	Cα	52.8[a]
	HCβ	2.84[a] 3.25[a]	q, *J* = 12.7 Hz, 1H dd, *J* = 13.7, 3.3 Hz, 1H	Cβ	39.4[a]
	CH3	2.12[a]	s, 3H	CH3	22.1[a]
Arg				C=O C=NH	173.0[a] 156.8[b]
	HCα	5.41[a]	dd, *J* = 11.1, 4.6 Hz, 1H	Cα	60.4[a]
	HCβ	2.24[a] 2.50[a]	dq, *J* = 15.1, 7.0 Hz, 1H q, *J* = 10.6 Hz, 1H	Cβ	30.6[a]
	HCɣ	0.44[a] 0.83[a]	m, 1H m, 1H	Cɣ	25.4[a]
	HCδ	2.79-2.99[a]	m, 2H (overlapping with impurities)	Cδ	41.0[a]
Isoindole	-	-	-	C1	111.1[a]
	-	-	-	C2	130.4[a]
	H3	7.67[a] 7.73[b]	m, 1H (overlapping with H10/10') dt, *J* = 8.6, 1.0 Hz, 1H	C3	119.8[a] 118.9[b]
	H4	7.12[a] 7.12[b]	m, 1H (overlapping with H5) ddd, *J* = 8.6, 6.5, 0.9 Hz, 1H	C4	125.0[a] 123.1[b]
	H5	7.08[a] 7.03[b]	m, 1H (overlapping with H4) ddd, *J* = 8.5, 6.5, 1.0 Hz, 1H	C5	124.1[a] 122.8[b]
	H6	7.55[a] 7.41[b]	d, *J* = 8.6 Hz, 1H s, 1H	C6	120.1[a] 118.7[b]
	-	-	-	C7	123.9[a]
	-	-	-	C8	129.1[a]
	-	-	-	C9	119.5[a] 117.9[b]
	H10/10'	7.69[b]	d, *J* = 8.0 Hz, 2H	C10/10'	130.6[b]
		7.83,7.69[a]	d, *J* = 7.7 Hz, 1H, m, 1H (overlapping with H3). Split into two proton signals at -50ºC.		131.8[a] 131.5[a]
	H11/11'	7.96[a] 7.9a[b]	m, 1H d, *J* = 8.1 Hz, 2H	C11/11'	134.0[a] 132.5[b]
	-	-	-	C12	111.2[a] 111.1[b]
	-	-	-	C(N)	136.9[a] 126.4[b]

[a] T = -50ºC
[b] T = +25ºC

HRMS of **7aa**

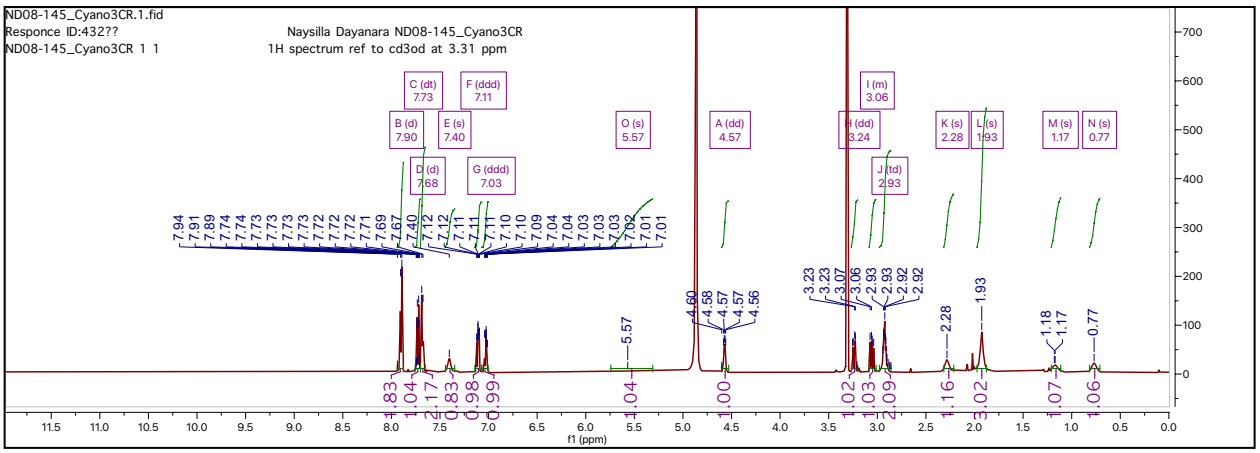


Calculated $([C_{26}H_{28}N_6O_5S]+H)^+ = 537.1920$

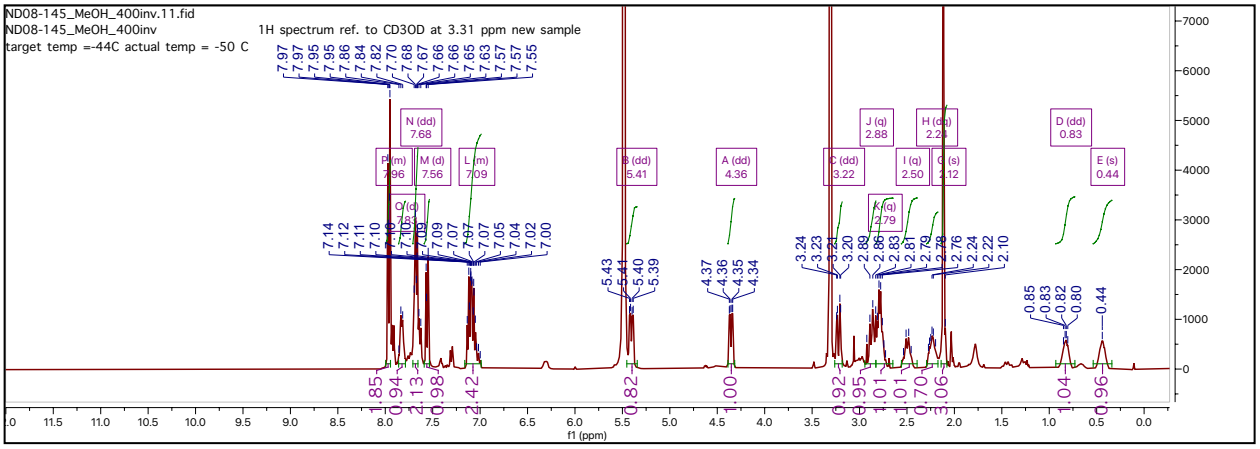
Found $([C_{26}H_{28}N_6O_5S]+H)^+ = 537.1915$

Full NMR data set:

^{1}H NMR (+25ºC)



^{1}H NMR (-50ºC)



Chemical shifts and multiplicity at -50ºC:

^{1}H NMR (400 MHz, MeOD) δ 8.00 – 7.94 (m, 2H), 7.83 (d, *J* = 7.7 Hz, 1H), 7.68 (dd, *J* = 8.7, 5.0 Hz, 2H), 7.56 (d, *J* = 8.6 Hz, 1H), 7.16 – 6.99 (m, 2H), 5.41 (dd, *J* = 11.1, 4.6 Hz, 1H), 4.36 (dd, *J* = 10.7, 3.4 Hz, 1H), 3.22 (dd, *J* = 13.7, 3.3 Hz, 1H), 2.88 (q, *J* = 12.7 Hz, 1H), 2.79 (q, *J* = 6.9 Hz, 1H), 2.50 (q, *J* = 10.6 Hz, 1H), 2.24 (dq, *J* = 15.1, 7.0 Hz, 1H), 2.12 (s, 3H), 0.83 (dd, *J* = 11.6, 6.6 Hz, 1H), 0.44 (s, 1H).

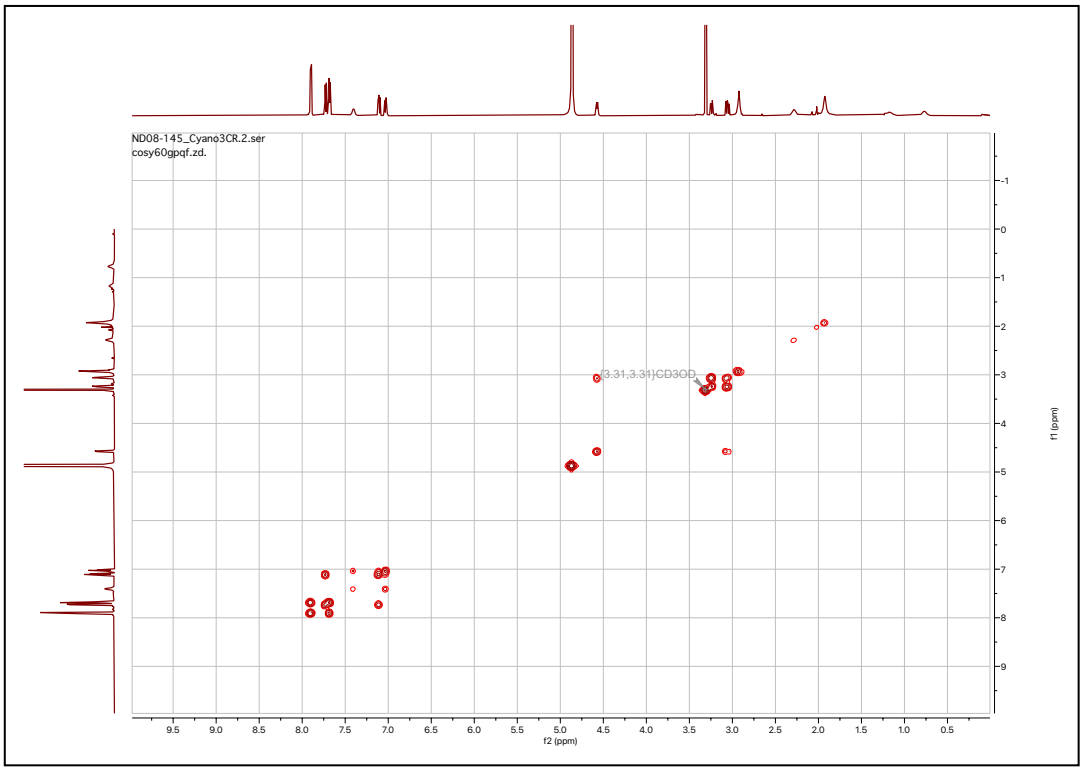


Figure S8A: COSY spectrum obtained at +25ºC

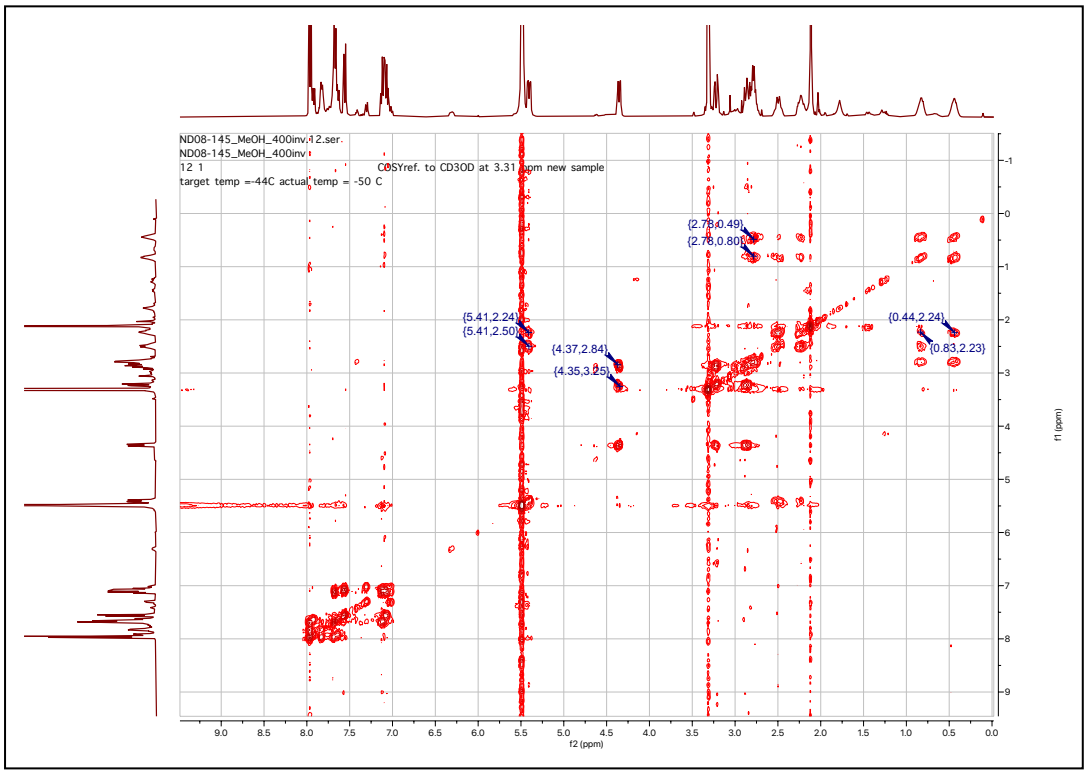


Figure S8B: COSY spectrum obtained at -50ºC

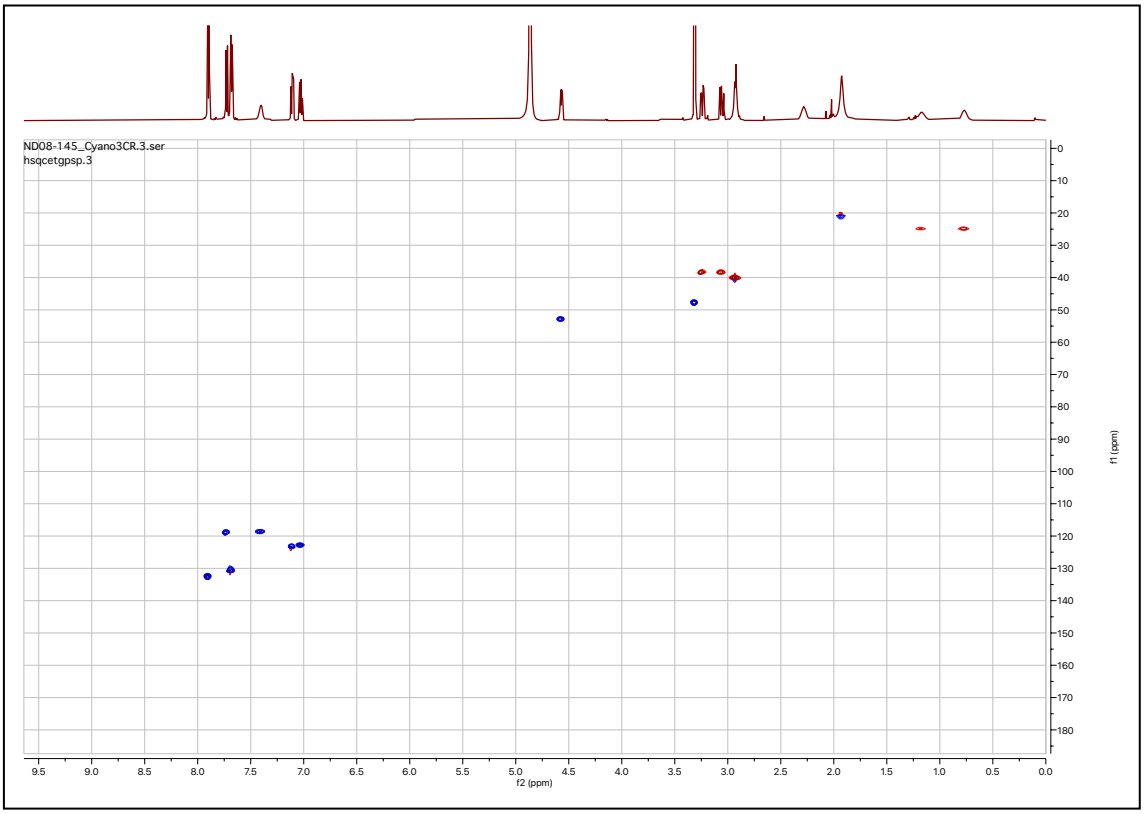


Figure S9A: HSQC spectrum obtained at +25ºC

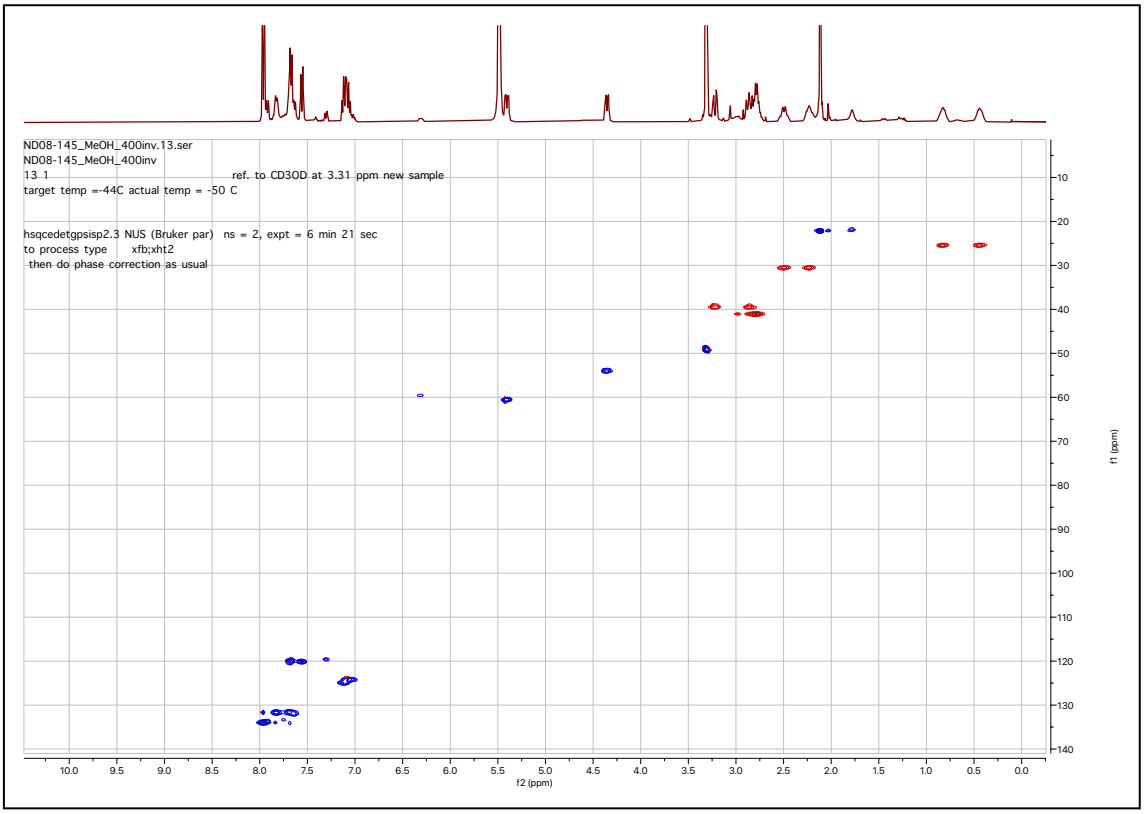


Figure S9B: HSQC spectrum obtained at -50ºC

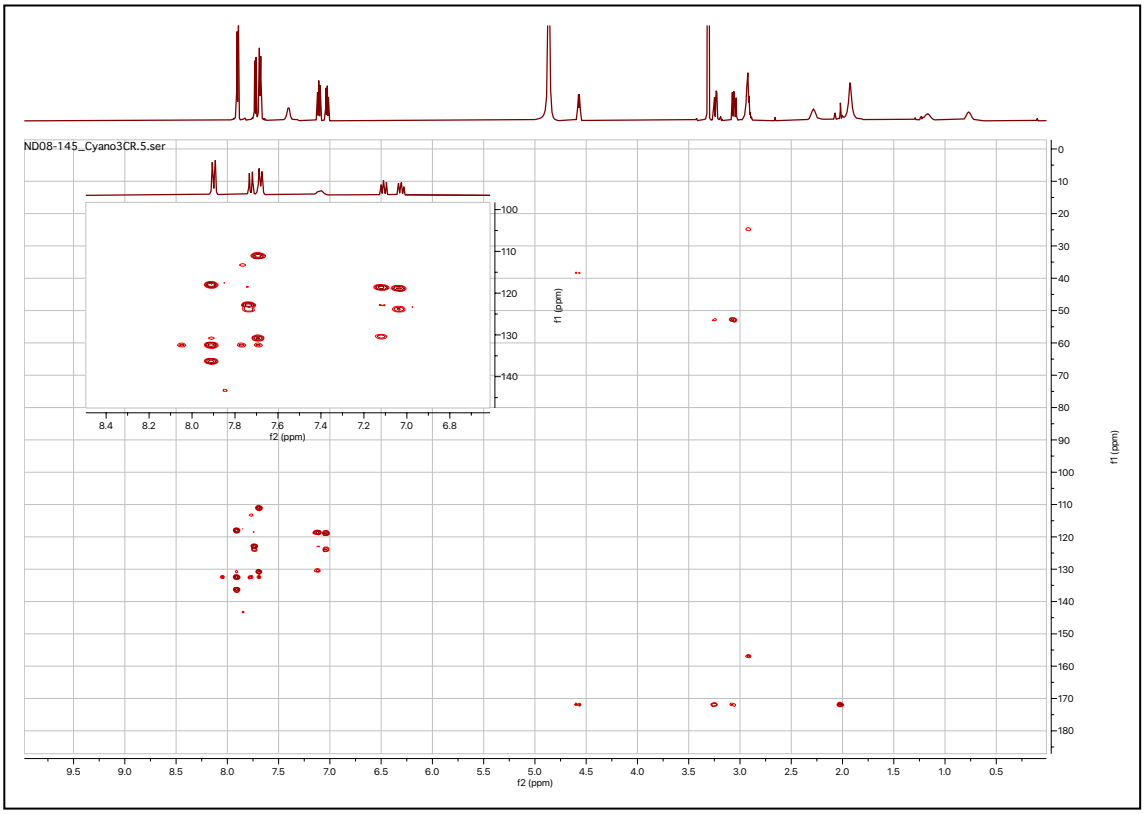


Figure S10A: HMBC spectrum obtained at +25ºC

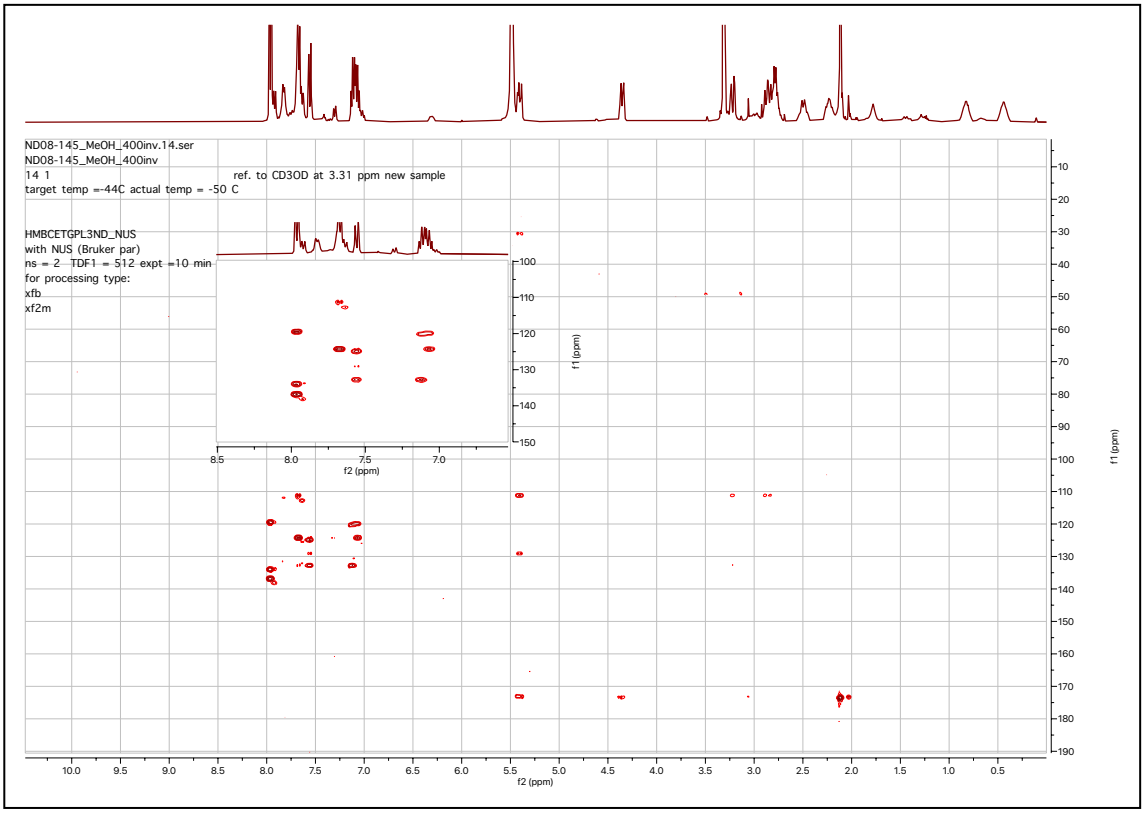
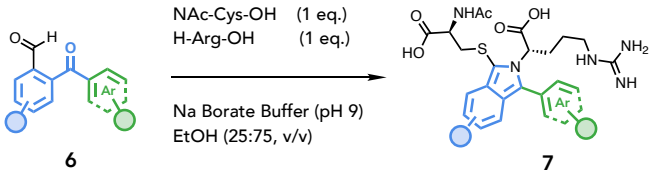


Figure S10B: HMBC spectrum obtained at -50ºC

General protocol for the synthesis and photophysical determination of disubstituted isoindoles 7a-7ai

Under optimized conditions, we synthesized disubstituted isoindoles **7a-7ai** for the determination of their unique set of photophysical properties. Similarly, NAc-Cys-OH and L-Arg were used as model substrates. Reaction time was also extended from 30 minutes to 1-2 hours to maximize the percent conversion of **6** to **7**.

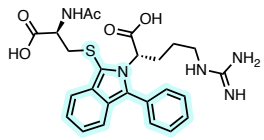


6 **7**

To a 50mL RBF containing 10mL Na borate buffer (pH 9) was added **6** (50µmol, 1 eq., prepared as 50mM solution in DMSO), NAc-Cys (50µmol, 1 eq, prepared as 50mM solution in H_2O 0.1% formic acid), and L-Arg (50µmol, 1 eq., prepared as 50mM solution in H_2O). Then, EtOH (4mL) was added into the RBF and the reaction was stirred for 1 hour at room temperature. This crude reaction mixture was purified by preparative reverse-phase HPLC (see each data set for specific purification method).

Purified fractions were observed under hand-held UV lamp (365nm), and if fluorescent, were pooled and lyophilized and directly measured for quantum yields using a spectrofluorometer equipped with an integrating sphere (+/- 5%). After successful QY determination, all fractions containing the product was pooled and lyophilized to afford **7** as fluffy powder. To ensure that the isolated product was free of salts, they were crudely analysed by ^1H NMR (in MeOD). If salts were present, the compound was subjected to de-salting treatment by C18 SepPak and lyophilized again. The solid product was weighed and dissolved in H_2O:MeCN (1:1, v/v) to afford a stock solution of known concentration, and 5 standard solutions (3mL each) of appropriate absorbance were prepared. These solutions were used for calibration where its extinction coefficient was determined at the respective wavelength of maximum absorbance. Non-fluorescent products were not measured for extinction coefficient and labeled NA in this text and the manuscript.

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7a)



Chemical Formula: $C_{25}H_{29}N_5O_5S$
Exact Mass: 511.189

7a was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6a** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7a** as fluffy white powder (20µmol, 10.2mg, 40%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x, 21.2mm

Time (min)	A%	B%	Flow (mL/min)
0.00	95	15	15
10.00	0	100	

Figure S11A: Crude injection of FIICk reaction with **6a** to afford **7a.** Shown here the LC traces at 290, 230, and 360nm and the corresponding UV excitation profile and LRMS data.

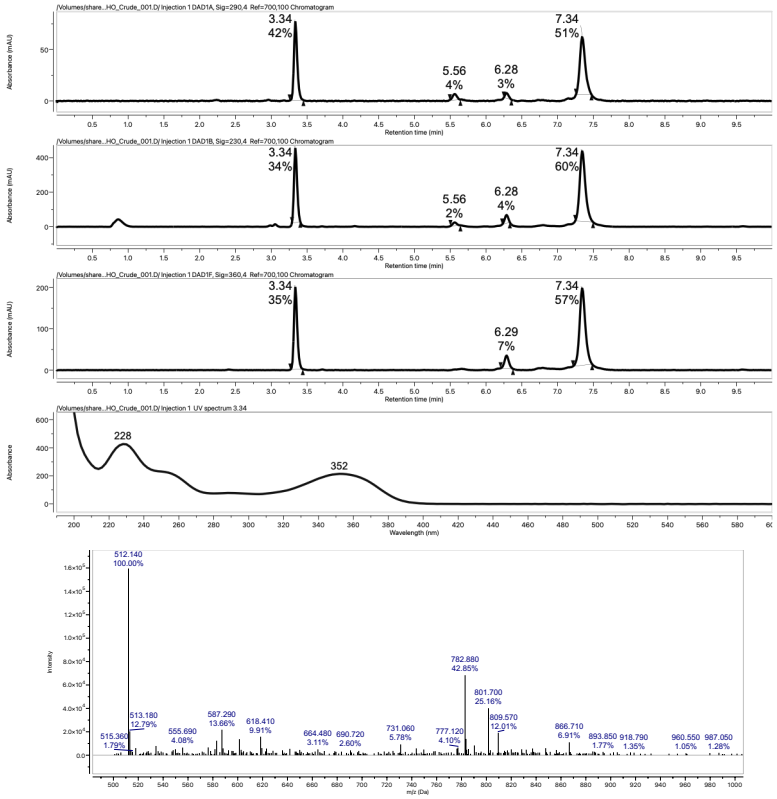
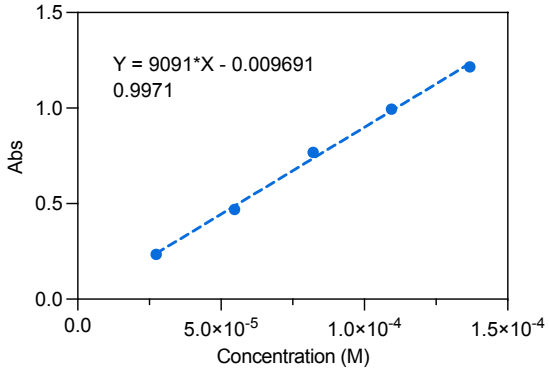
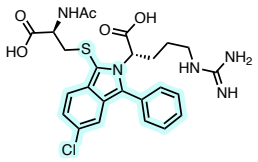


Figure S11B: *Left:* Standard concentration and the corresponding absorbance detected at 356nm. *Right*: Calibration plot of **7a**

Concentration (M)	Abs (λ=352nm)
2.74E-05	0.23441
5.47E-05	0.46984
8.21E-05	0.76863
1.09E-04	0.99427
1.37E-04	1.2161
Extinction Coefficient: 9100 cm^{-1} M^{-1}	
Quantum Yield: 9%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-chloro-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7b)



Chemical Formula: $C_{25}H_{28}ClN_5O_5S$
Exact Mass: 545.150

7b was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6b** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7b** as fluffy pale yellow powder (23.5µmol, 12.8mg, 47%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x, 21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	15
6.00	0	100	

Figure S12A: Crude injection of FIICk reaction with **6b** to afford **7b.** Shown here the LC traces at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.

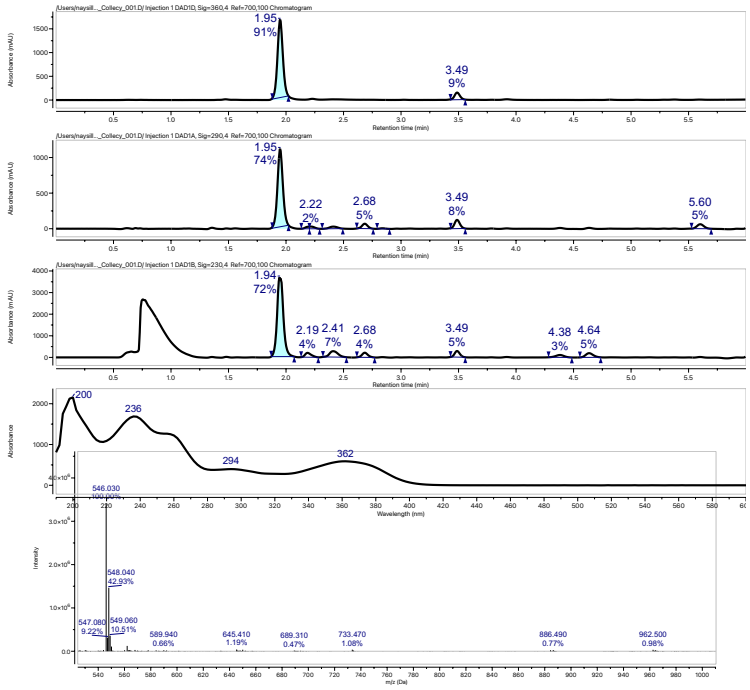
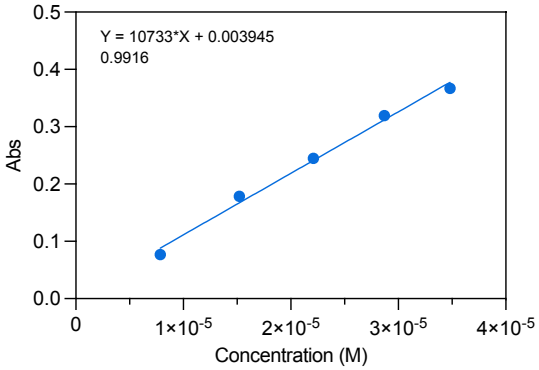
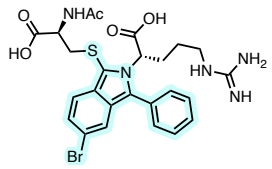


Figure S12B: *Left:* Standard concentration and the corresponding absorbance detected at 362nm. *Right*: Calibration plot of **7b**

Concentration (M)	Abs (λ=362nm)
7.86E-06	0.07667
1.52E-05	0.178546
2.21E-05	0.24468
2.87E-05	0.31924
3.48E-05	0.36687
Extinction Coefficient: 10700 cm^{-1} M^{-1}	
Quantum Yield: 13%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-bromo-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7c)



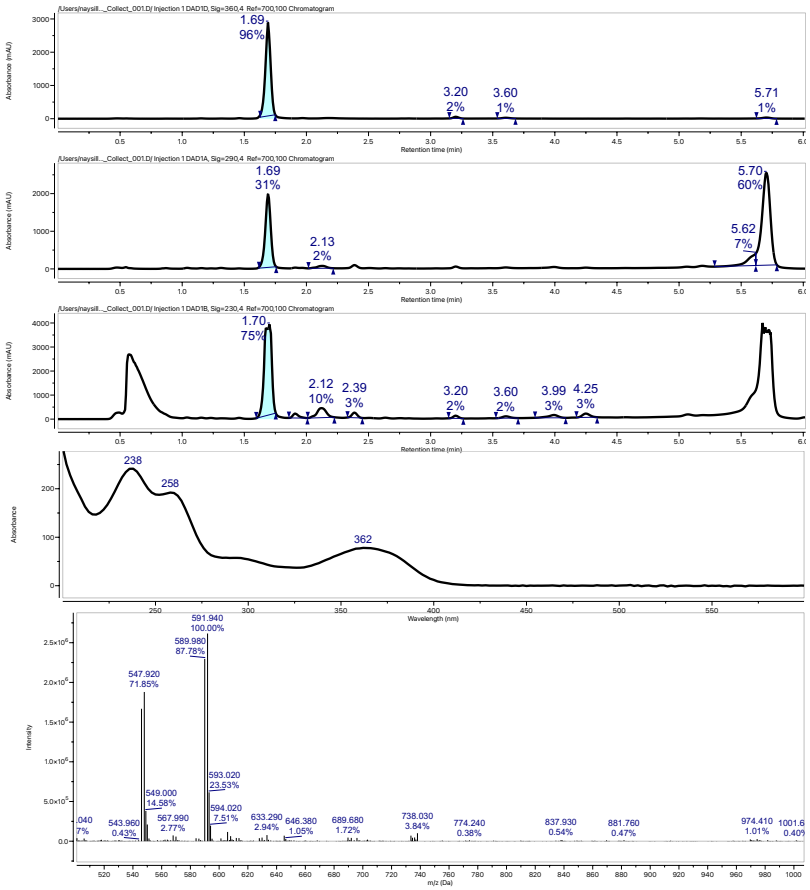
Chemical Formula: $C_{25}H_{28}BrN_5O_5S$
Exact Mass: 589.099

7c was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6c** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7c** as fluffy pale orange powder (12.5µmol, 7.4mg, 25%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

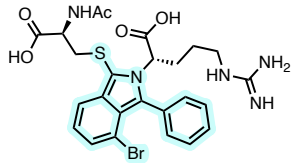
Figure S13: Crude injection of FIICk reaction with **6c** to afford **7c**. Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is weakly fluorescent.
Quantum yield: < 5%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-4-bromo-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7d)



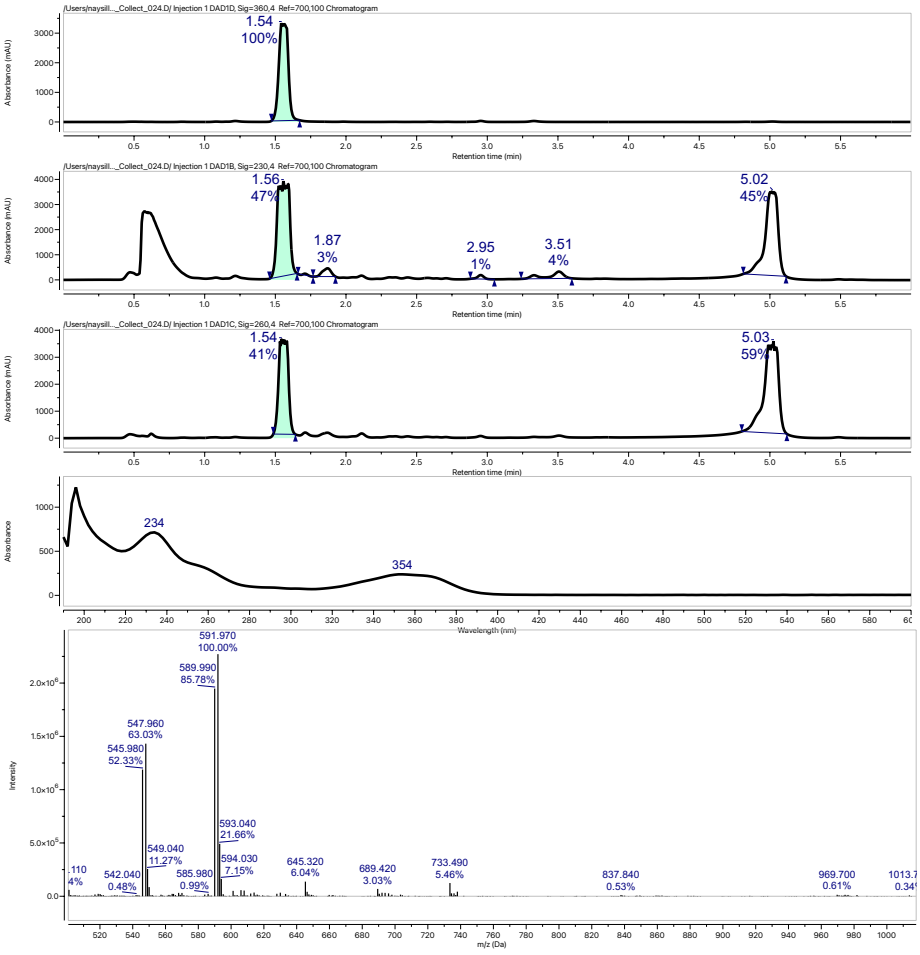
Chemical Formula: $C_{25}H_{28}BrN_5O_5S$
Exact Mass: 589.099

7d was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6d** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7d** as pale orange powder (10.5µmol, 6.2mg, 21%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

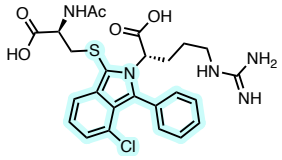
Figure S14: Crude injection of FIICk reaction with **6d** to afford **7d.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data.



Collected peak is weakly fluorescent.
Quantum yield: < 5%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-4-chloro-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7e)



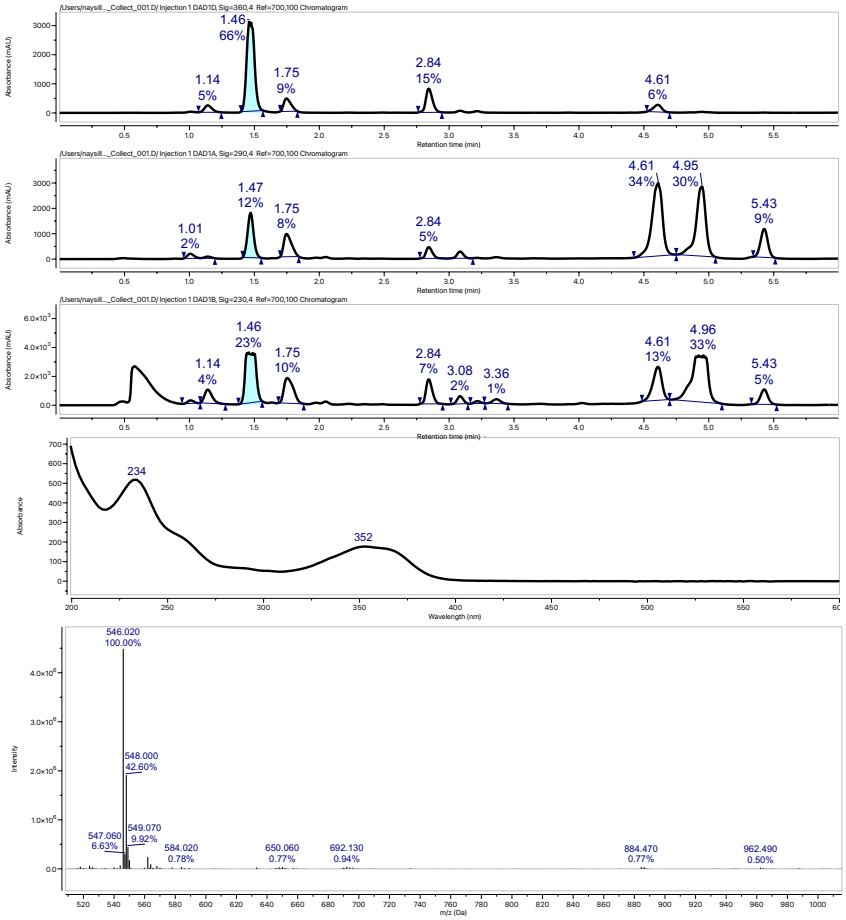
Chemical Formula: $C_{25}H_{28}ClN_5O_5S$
Exact Mass: 545.150

7e was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6e** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded 7e as white powder (10.4µmol, 5.7mg, 21%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

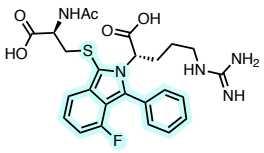
Figure S15: Crude injection of FIICk reaction with **6e** to afford **7e.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is weakly fluorescent.
Quantum yield: < 5%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-4-fluoro-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7f)



Chemical Formula: $C_{25}H_{28}FN_5O_5S$
Exact Mass: 529.180

7f was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6f** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7f** as fluffy white powder (27.3µmol, 14mg, 55%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S16A: Crude injection of FIICk reaction with **6f** to afford **7f.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.

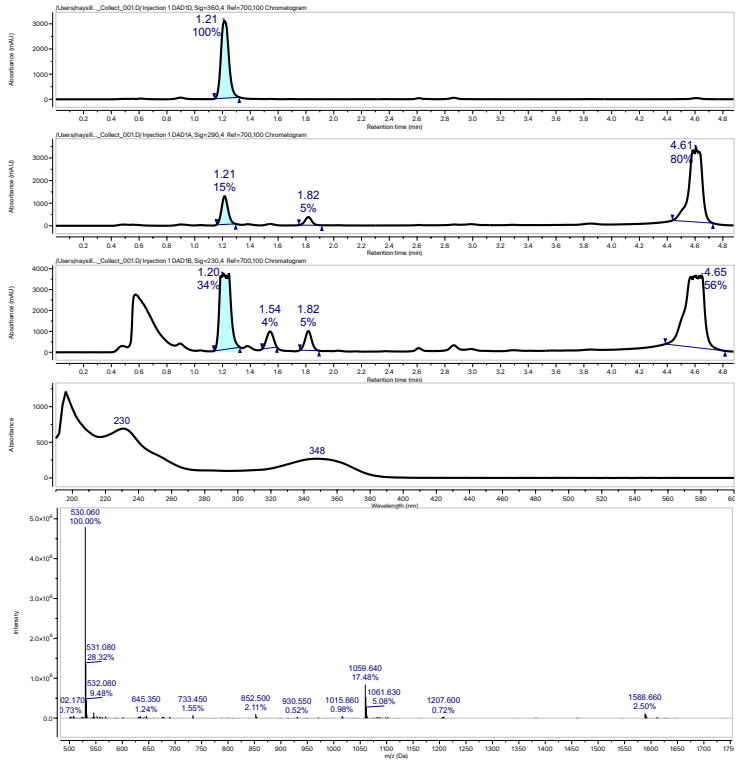
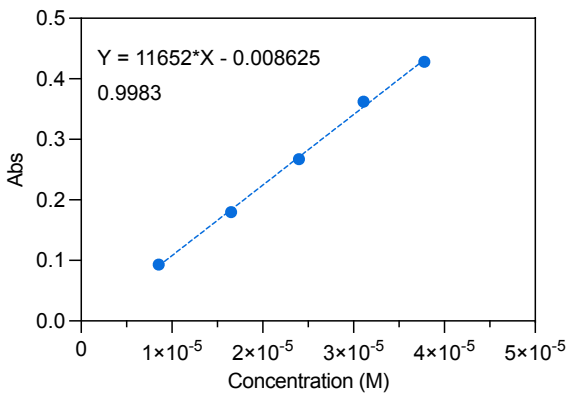
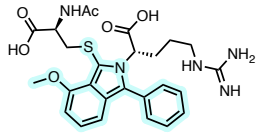


Figure S16B: *Left:* Standard concentration and the corresponding absorbance detected at 346nm. *Right*: Calibration plot of **7f**

Concentration (M)	Abs (λ=346nm)
8.53E-06	0.0933
1.65E-05	0.17989
2.40E-05	0.26722
3.11E-05	0.36235
3.78E-05	0.42819
Extinction Coefficient: 11600 cm^{-1} M^{-1}	
Quantum Yield: 8%	



(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-4-methoxy-1-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7g)



Chemical Formula: $C_{26}H_{31}N_5O_6S$
Exact Mass: 541.200

7g was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6g** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7g** as white powder (23.6µmol, 12.8mg, 47%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S17A: Crude injection of FIICk reaction with **6g** to afford **7g.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.

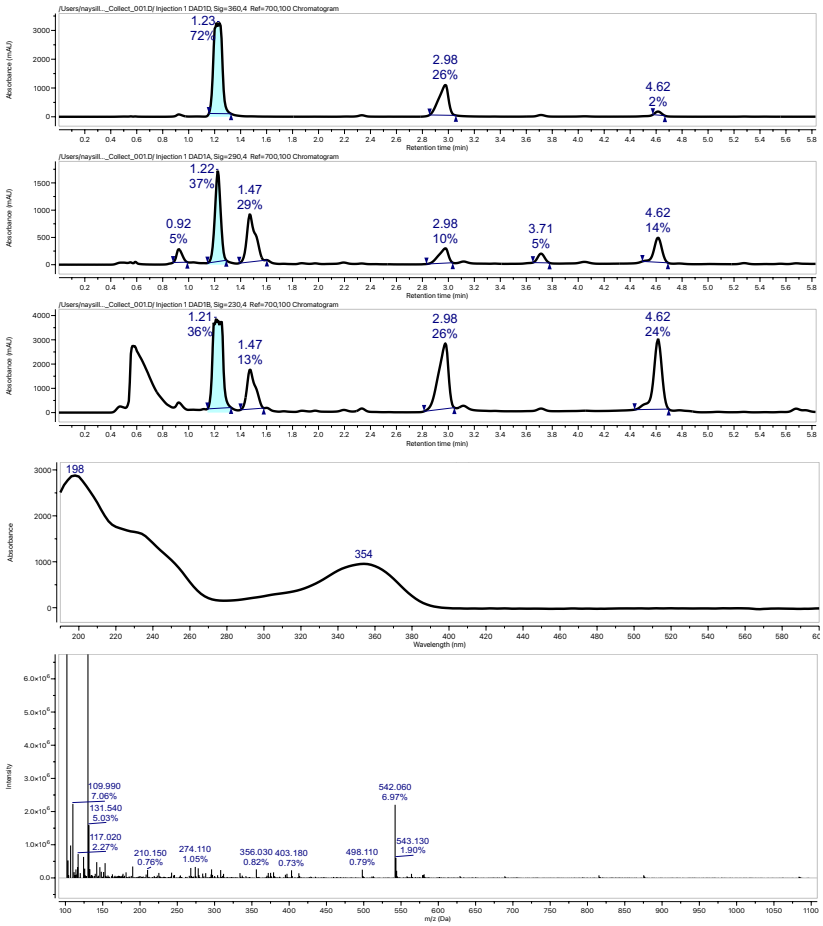
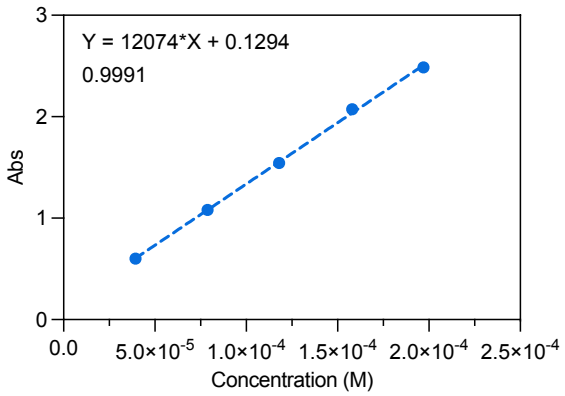
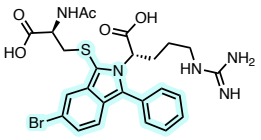


Figure S17B: *Left:* Standard concentration and the corresponding absorbance detected at 354nm. *Right*: Calibration plot of **7f**

Concentration (M)	Abs (λ=354nm)
3.94E-05	0.60187
7.88E-05	1.08006
1.18E-04	1.54306
1.58E-04	2.07467
1.97E-04	2.48535
Extinction Coefficient: 12000 cm^{-1} M^{-1}	
Quantum Yield: 9%	



(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-bromo-1-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7h)



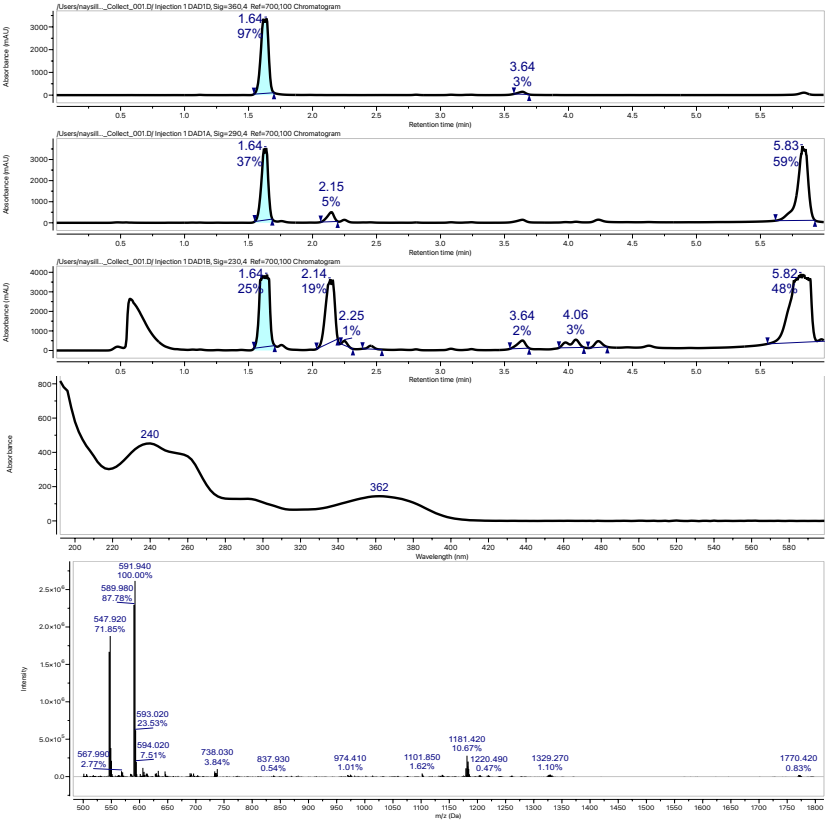
Chemical Formula: $C_{25}H_{28}BrN_5O_5S$
Exact Mass: 589.099

7h was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6h** (50µmol, 1mL of 50mM solution in DMSO). After 2 hours sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7h** as white powder (20µmol, 12mg, 40%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

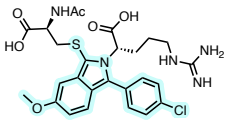
Figure S18: Crude injection of FIICk reaction with **6h** to afford **7h.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is weakly fluorescent.
Quantum yield: < 5%

Extinction coefficient: N/A

(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-1-(4-chlorophenyl)-5-methoxy-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7i)



Chemical Formula: $C_{26}H_{30}ClN_5O_6S$
Exact Mass: 575.161

7i was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6i** (50µmol, 1mL of 50mM solution in DMSO). After 2 hours sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7i** as fluffy white powder (23.1µmol, 13mg, 46%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S19A: Crude injection of FIICk reaction with **6i** to afford **7i.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.

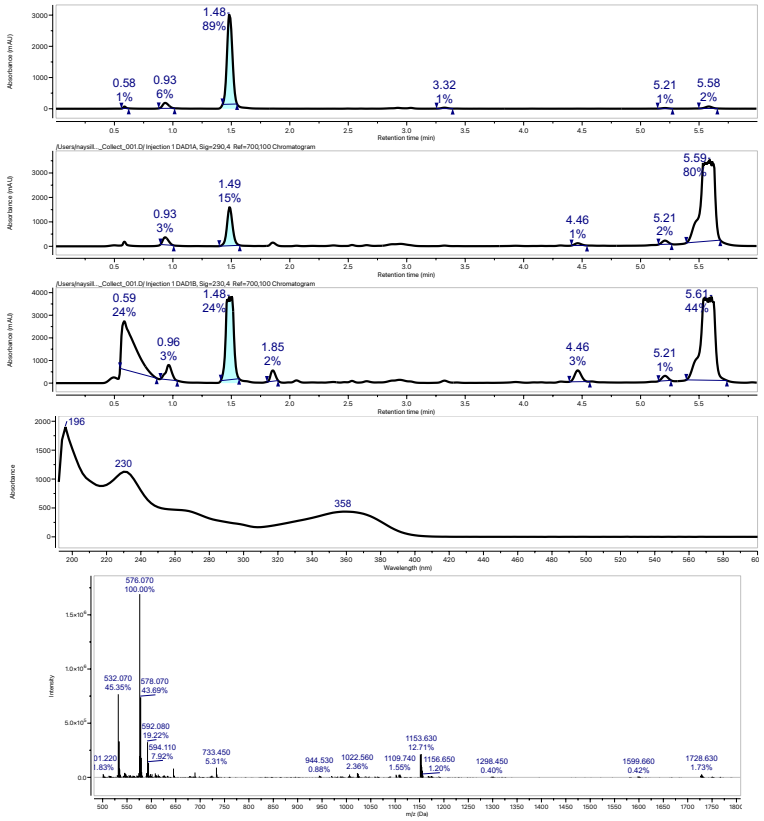
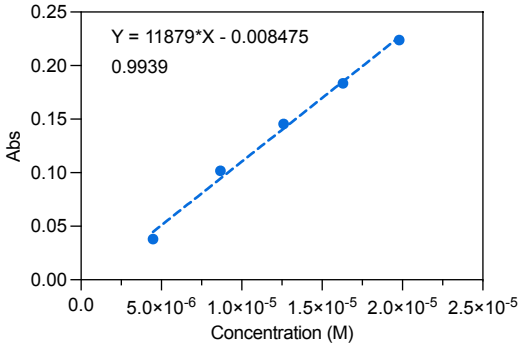
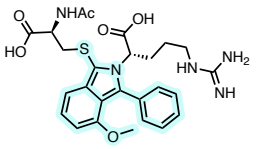


Figure S19B: *Left:* Standard concentration and the corresponding absorbance detected at 358nm. *Right*: Calibration plot of **7i**

Concentration (M)	Abs (λ=358nm)
4.48E-06	0.03796
8.68E-06	0.10176
1.26E-05	0.14544
1.63E-05	0.18347
1.98E-05	0.22382
Extinction Coefficient: 11900 cm^{-1} M^{-1}	
Quantum Yield: 10%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-4-methoxy-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7j)



Chemical Formula: $C_{26}H_{31}N_5O_6S$
Exact Mass: 541.200

7j was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6j** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7j** as fluffy white powder (19µmol, 11mg, 38%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S20A: Crude injection of FIICk reaction with **6j** to afford **7j.** Shown here the LC trace at 360, 290, and 230nm and the corresponding

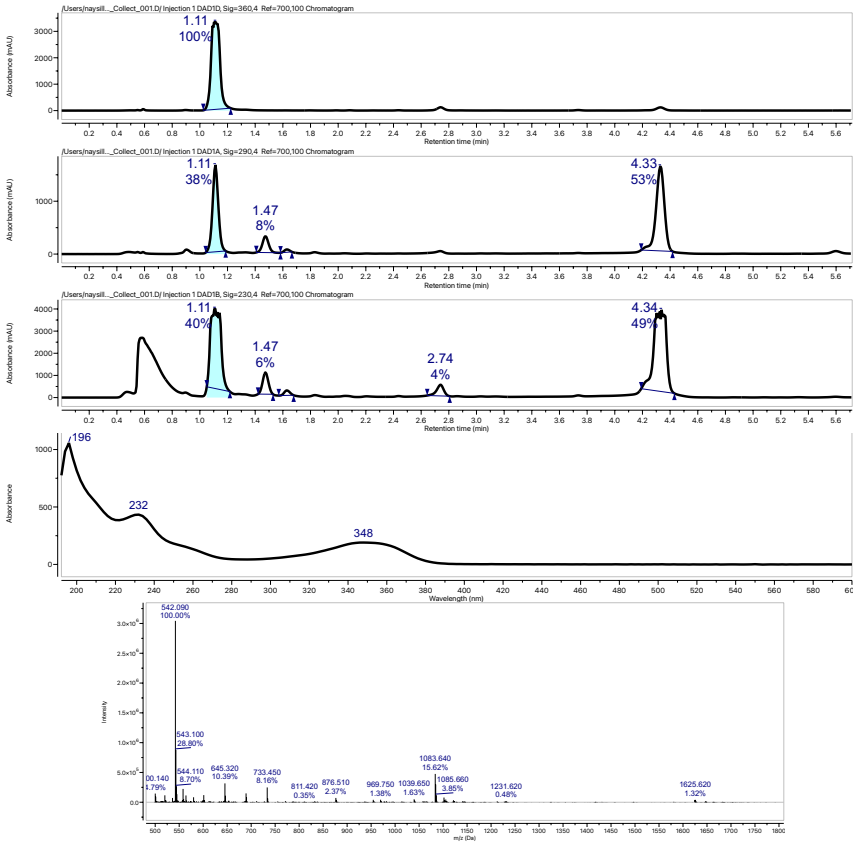
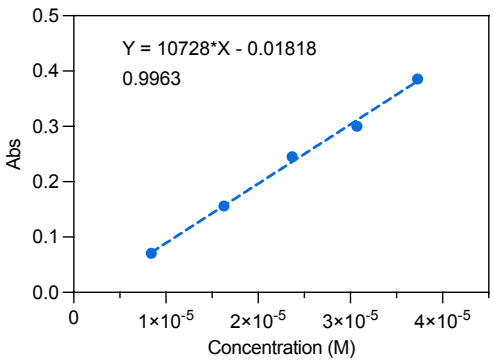
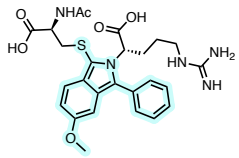


Figure S20B: *Left:* Standard concentration and the corresponding absorbance detected at 348nm. *Right*: Calibration plot of **7j**

Concentration (M)	Abs (λ=348nm)
8.41E-06	0.07059
1.63E-05	0.15616
2.37E-05	0.24535
3.07E-05	0.30021
3.73E-05	0.38571
Extinction Coefficient: 10700 cm^{-1} M^{-1}	
Quantum Yield: 7%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-methoxy-3-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7k)



Chemical Formula: $C_{26}H_{31}N_5O_6S$
Exact Mass: 541.200

7k was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6k** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7k** as fluffy white powder, subject to desalting treatment with C18 SepPak (2.0g) (14.2µmol, 7.7mg, 28%).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

Figure S21A: Crude injection of FIICk reaction with **6k** to afford **7k.** Shown here the LC trace at 360, 260, and 230nm and the corresponding UV excitation profile and LRMS data.

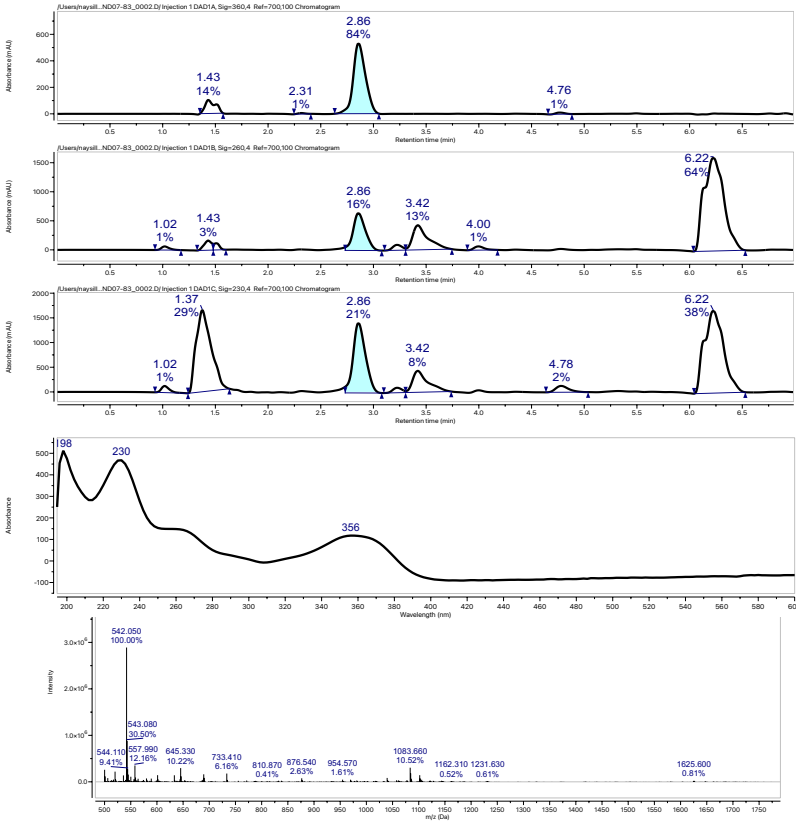
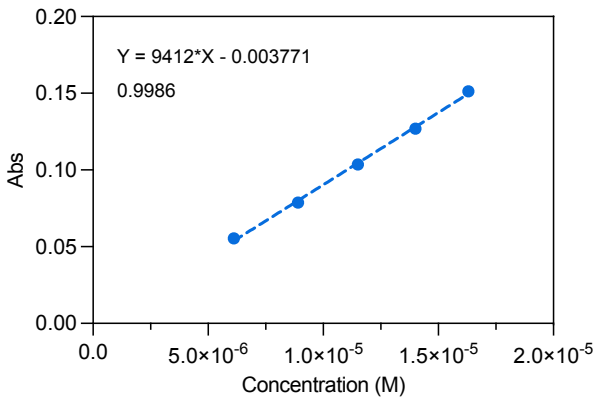
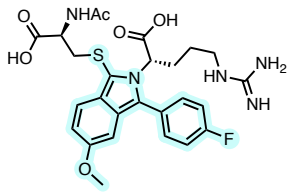


Figure S21B: *Left:* Standard concentration and the corresponding absorbance detected at 356m. *Right*: Calibration plot of **7k**

Concentration (M)	Abs (λ=356nm)
6.12E-06	0.05535
8.90E-06	0.07879
1.15E-05	0.10363
1.40E-05	0.12689
1.63E-05	0.15127
Extinction Coefficient: 9400cm^{-1} M^{-1}	
Quantum Yield: 16%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-fluorophenyl)-5-methoxy-2*H*-isoindol-2-yl)-5 guanidino pentanoic acid (7l)



Chemical Formula: $C_{26}H_{30}FN_5O_6S$
Exact Mass: 559.190

7l was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6l** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7l** as fluffy white powder (16.7µmol, 9.4mg, 34%)

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S22A: Crude injection of FIICk reaction with **6k** to afford **7k.** Shown here the LC trace at 360, 260, and 230nm and the corresponding UV excitation profile and LRMS data.

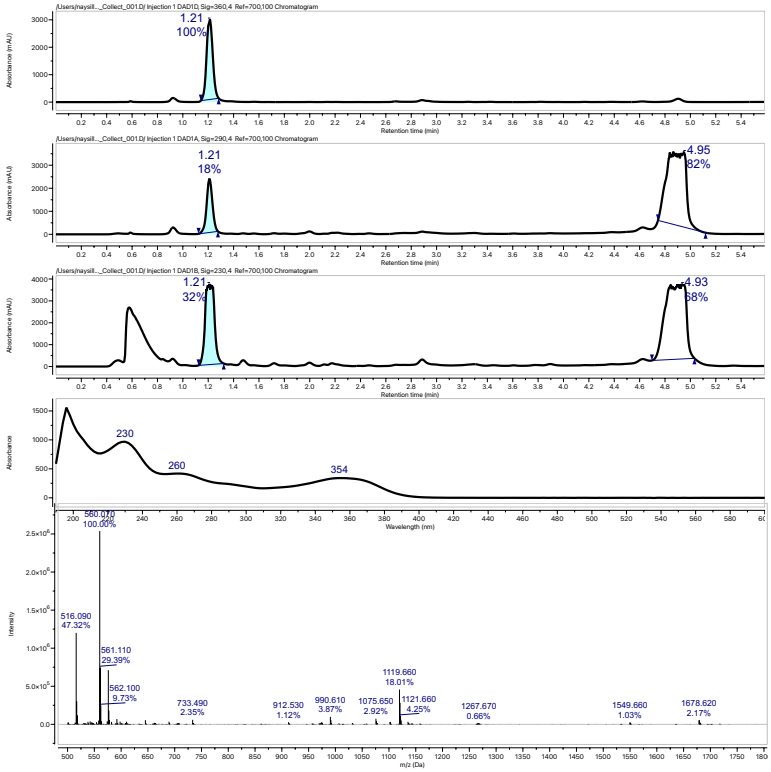
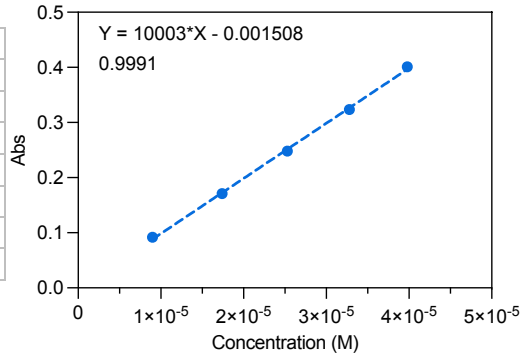
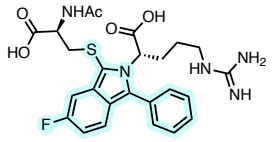


Figure S22B: *Left:* Standard concentration and the corresponding absorbance detected at 354m. *Right*: Calibration plot of **7l**

Concentration (M)	Abs (λ=354nm)
6.12E-06	0.05535
8.90E-06	0.07879
1.15E-05	0.10363
1.40E-05	0.12689
1.63E-05	0.15127
Extinction Coefficient: 10000cm^{-1} M^{-1}	
Quantum Yield: 24%	



(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-fluoro-1-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7m)



Chemical Formula: $C_{25}H_{28}FN_5O_5S$
Exact Mass: 529.180

7m was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6m** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7m** as fluffy white powder (22µmol, 12mg, 44%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S23A: Crude injection of FIICk reaction with **6m** to afford **7m.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS data.

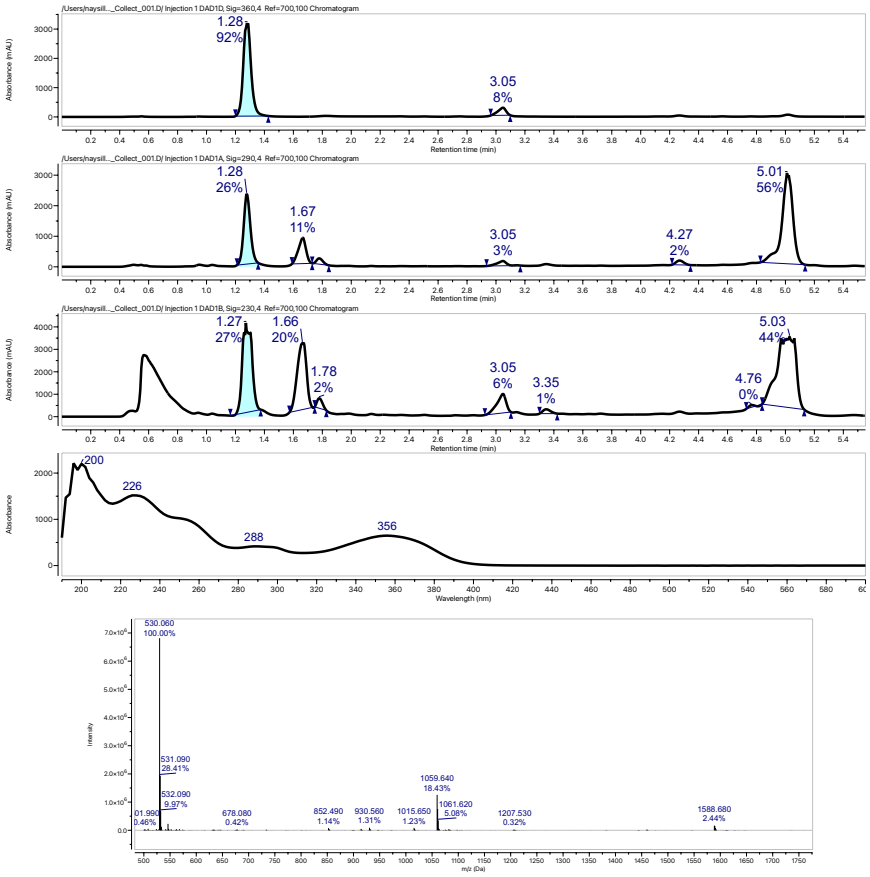
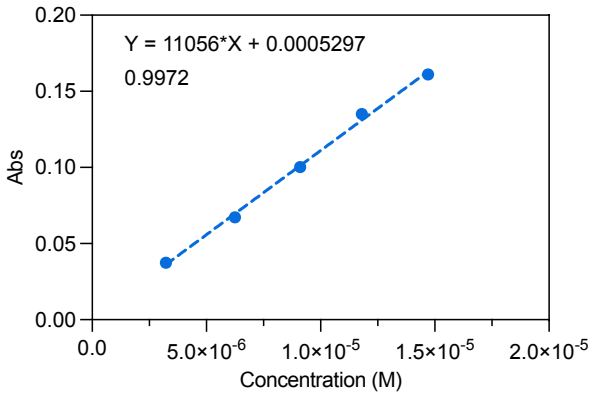
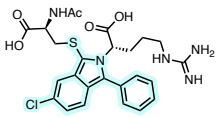


Figure S23B: Left: Standard concentration and the corresponding absorbance detected at 356m. Right: Calibration plot of **7m**

Concentration (M)	Abs (λ=356nm)
3.23E-06	0.03734
6.25E-06	0.06731
9.10E-06	0.10028
1.18E-05	0.13507
1.47E-05	0.16106
Extinction Coefficient: 11100cm^{-1} M^{-1}	
Quantum Yield: 45%	



(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-chloro-1-phenyl-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7n)



Chemical Formula: $C_{25}H_{28}ClN_5O_5S$
Exact Mass: 545.150

7n was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6n** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7n** as white powder (28.3µmol, 15mg, 57%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S24A: Crude injection of FIICk reaction with **6n** to afford **7n.** Shown here the LC trace at 360, 290, and 230nm and the corresponding UV excitation profile and LRMS dat

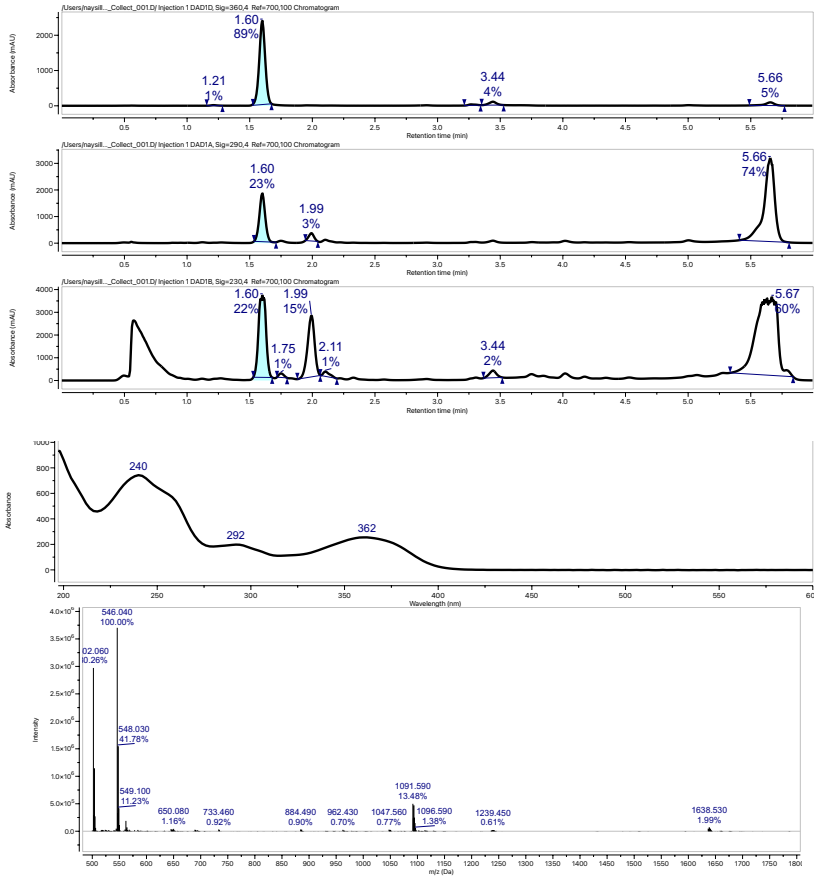
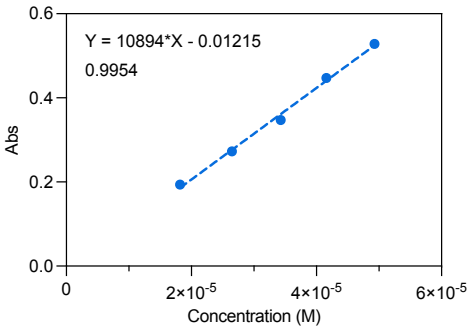
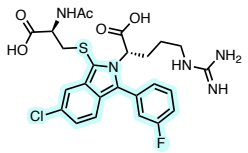


Figure S24B: *Left:* Standard concentration and the corresponding absorbance detected at 362m. *Right*: Calibration plot of **7n**

Concentration (M)	Abs (λ=362nm)
1.82E-05	0.19398
2.65E-05	0.27299
3.43E-05	0.34732
4.16E-05	0.44736
4.93E-05	0.52842
Extinction Coefficient: 10900 cm^{-1} M^{-1}	
Quantum Yield: 10%	



(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-5-chloro-1-(3-fluorophenyl)-2*H*-isoindol-2-yl)-5-guanidino pentanoic acid (7o)



Chemical Formula: $C_{25}H_{27}ClFN_5O_5S$
Exact Mass: 563.141

7o was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6o** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. Lyophilization of purified fractions afforded **7o** as white powder (26.4µmol, 15mg, 53%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S25A: Crude injection of FIICk reaction with **6o** to afford **7o.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data.

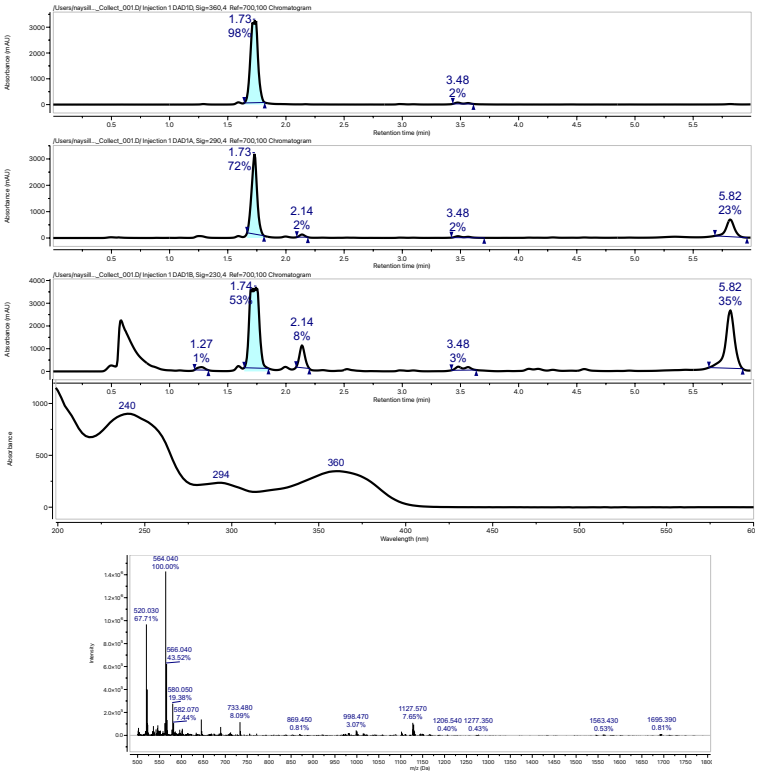
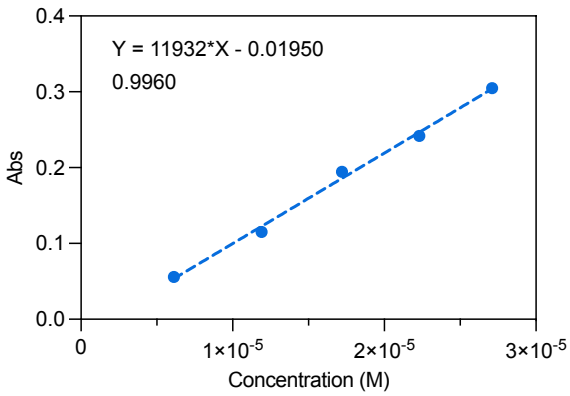
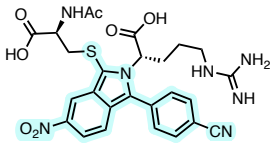


Figure S25B: *Left:* Standard concentration and the corresponding absorbance detected at 360m. *Right*: Calibration plot of **7o**

Concentration (M)	Abs (λ=360nm)
6.12E-06	0.0557
1.19E-05	0.11532
1.72E-05	0.19447
2.23E-05	0.24179
2.71E-05	0.30493
Extinction Coefficient: 11900 cm^{-1} M^{-1}	
Quantum Yield: 28%	



(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-1-(4-cyanophenyl)-5-nitro-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7p)



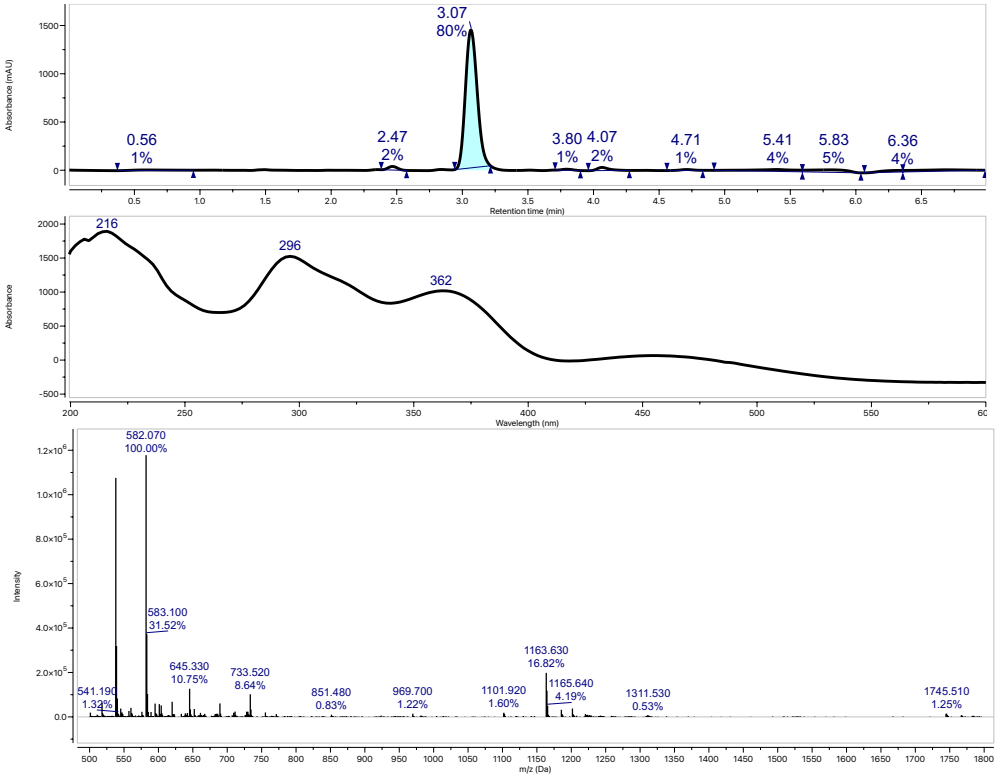
Chemical Formula: $C_{26}H_{27}N_7O_7S$
Exact Mass: 581.169

7p was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6p** (50µmol, 1mL of 50mM solution in DMSO). This reaction started to precipitate upon acidification, as such, it was purified under basic conditions. Lyophilization of purified fractions afforded **7p** as deep red powder subject to desalting treatment with C18 SepPak (2.0g) (13.1µmol, 7.6mg, 26%).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

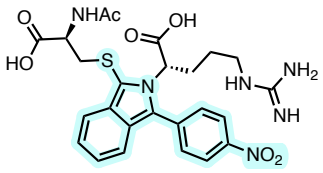
Figure S26: Crude injection of FIICk reaction with **6o** to afford **7o.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-nitrophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7q)



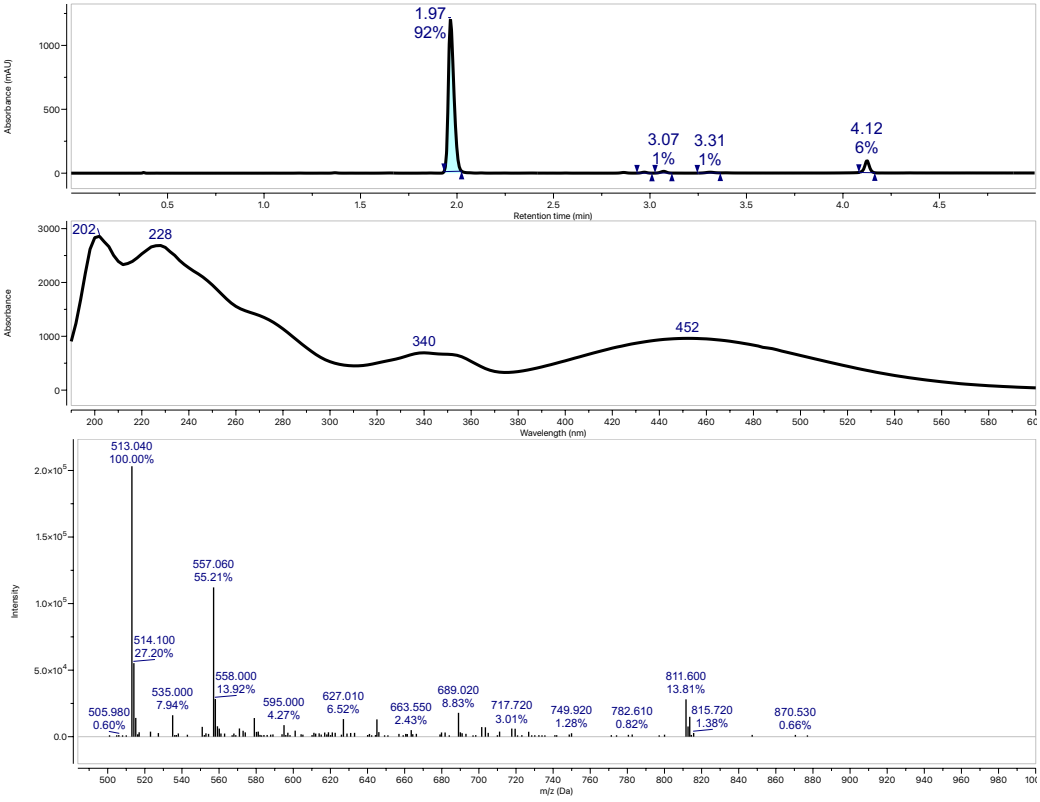
Chemical Formula: $C_{25}H_{28}N_6O_7S$
Exact Mass: 556.174

7q was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6q** (50µmol, 1mL of 50mM solution in DMSO). This reaction started to precipitate upon acidification, as such, it was purified under basic conditions. Lyophilization of purified fractions afforded **7q** as deep orange powder subject to desalting treatment with C18 SepPak (2.0g) (12µmol, 6.7mg, 24%)

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

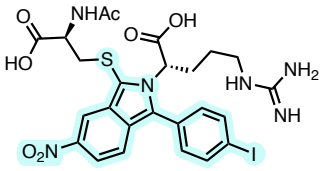
Figure S27: Crude injection of FIICk reaction with **6o** to afford **7o.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data. On the right, a picture of purified **7o** dissolved in 1:1 H_2O/MeCN. **Right;** Picture of purified compound **7o** following lyophilization, dissolved in 1:1 MeCN/H_2O.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-1-(4-iodophenyl)-5-nitro-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7r)



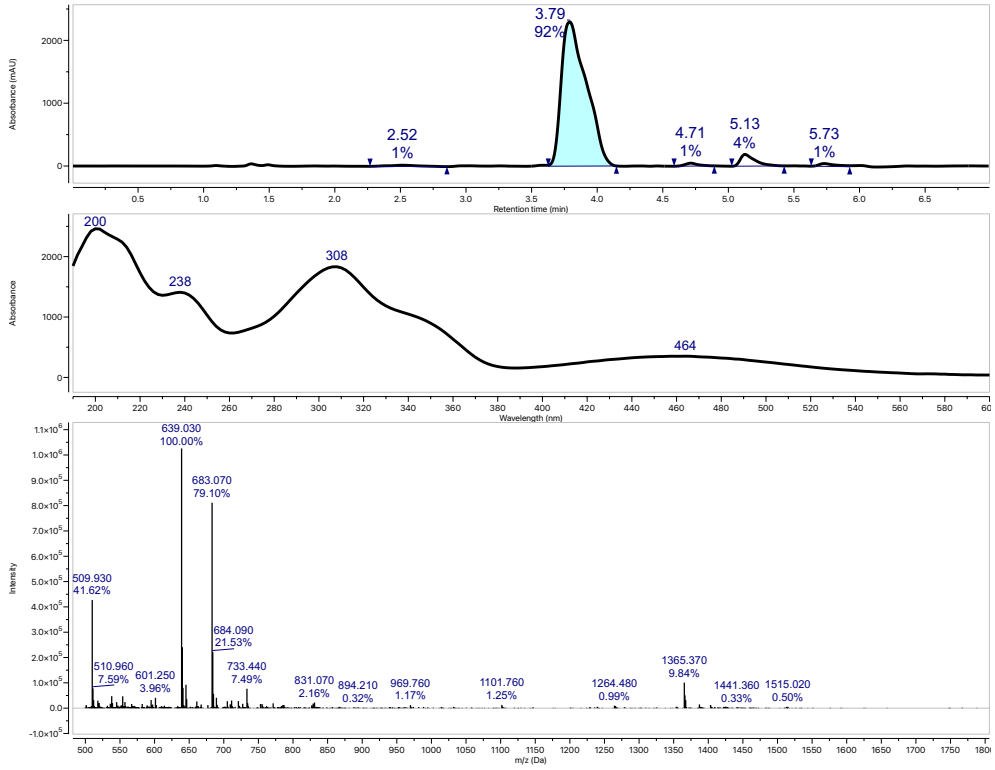
Chemical Formula: $C_{25}H_{27}IN_6O_7S$
Exact Mass: 682.071

7r was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6r** (50µmol, 1mL of 50mM solution in DMSO). This reaction started to precipitate upon acidification, as such, it was purified under basic conditions. Lyophilization of purified fractions afforded **7r** as orange-brown powder subject to desalting treatment with C18 SepPak (2.0g) (11.1µmol, 7.6mg, 22%)

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

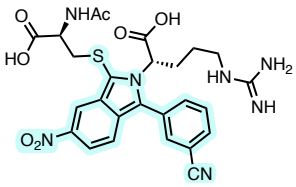
Figure S28: Crude injection of FIICk reaction with **6r** to afford **7r**. Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-1-(3-cyanophenyl)-5-nitro-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7s)



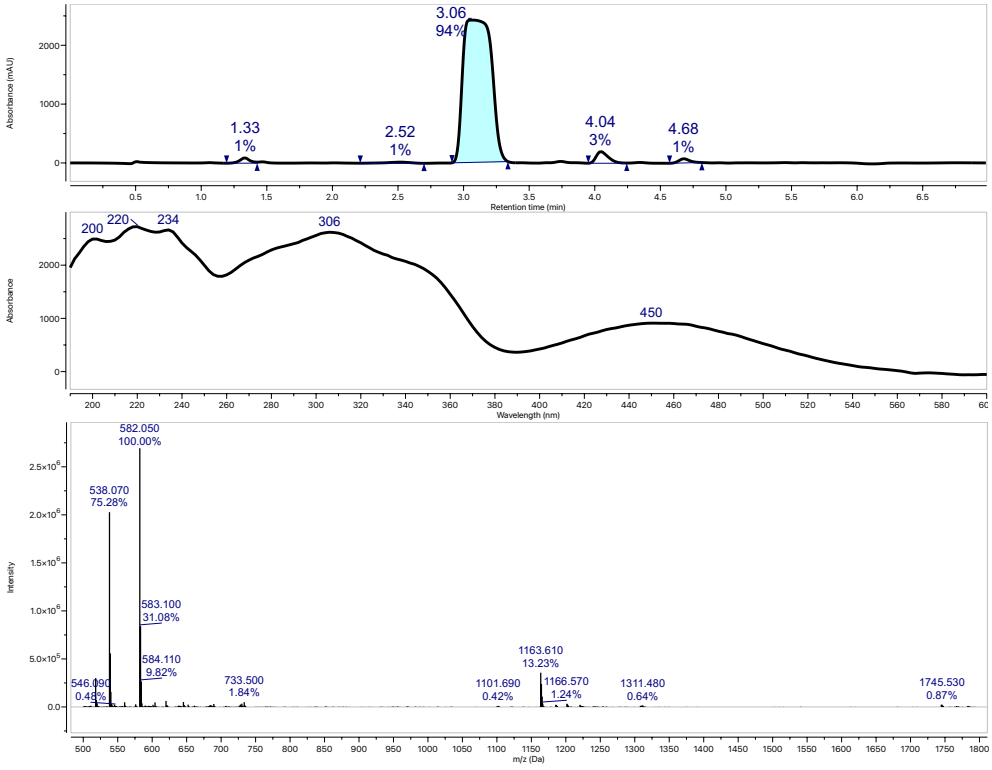
Chemical Formula: $C_{26}H_{27}N_7O_7S$
Exact Mass: 581.169

7s was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6s** (50µmol, 1mL of 50mM solution in DMSO). This reaction started to precipitate upon acidification, as such, it was purified under basic conditions. Lyophilization of purified fractions afforded **7s** as dark pink powder subject to desalting treatment with C18 SepPak (2.0g) (14.6µmol, 8.5mg, 29%)

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

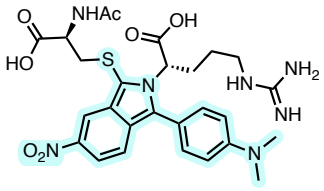
Figure S29: Crude injection of FIICk reaction with **6s** to afford **7s.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-1-(4-(dimethylamino)phenyl)-5-nitro-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7t)



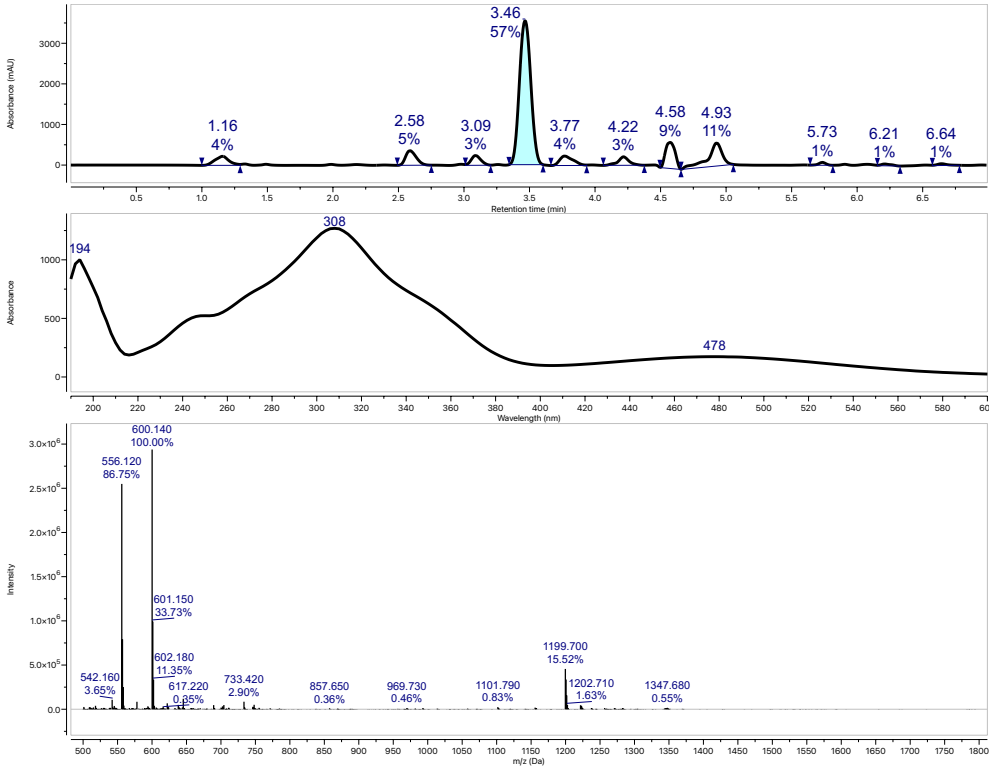
Chemical Formula: $C_{27}H_{33}N_7O_7S$
Exact Mass: 599.216

7t was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6t** (50µmol, 1mL of 50mM solution in DMSO). This reaction started to precipitate upon acidification, as such, it was purified under basic conditions. Lyophilization of purified fractions afforded **7t** as red-brown powder subject to desalting treatment with C18 SepPak (2.0g) (13.3µmol, 8mg, 27%).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

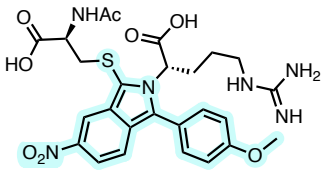
Figure S30: Crude injection of FIICk reaction with **6t** to afford **7t.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(3-(((*R*)-2-acetamido-2-carboxyethyl)thio)-1-(4-methoxyphenyl)-5-nitro-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7u)



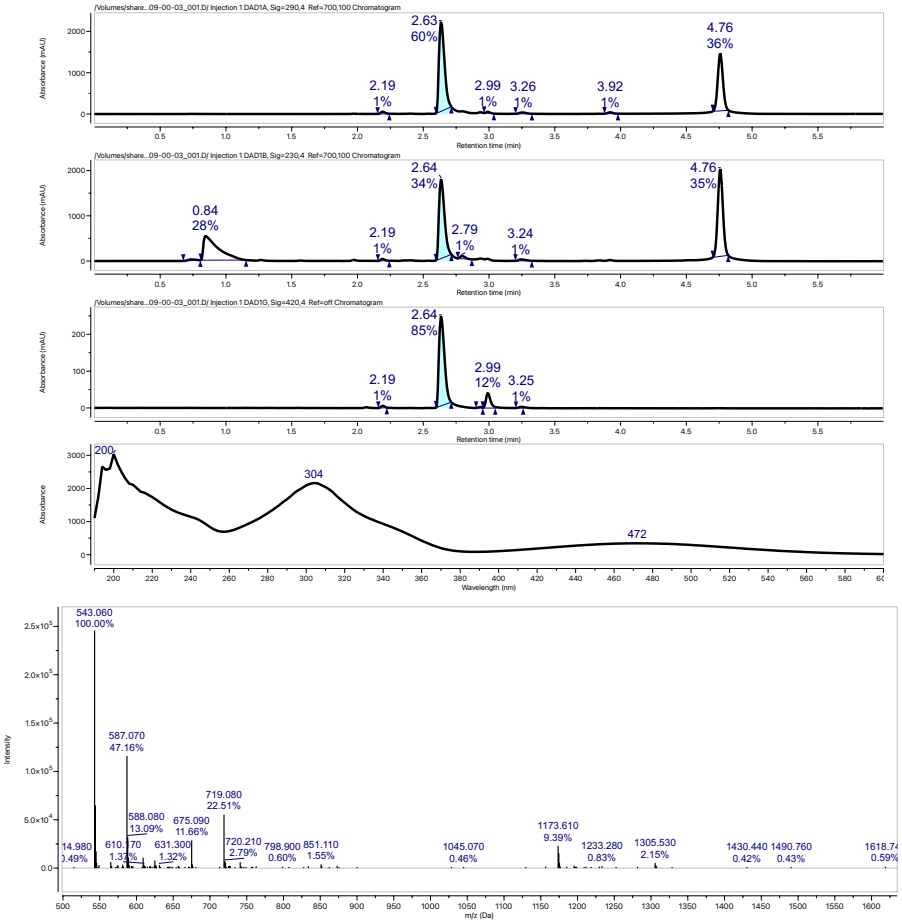
Chemical Formula: $C_{26}H_{30}N_6O_8S$
Exact Mass: 586.185

7u was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6u** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7u** as orange powder subject to desalting treatment with C18 SepPak (2.0g) (10.5µmol, 6.2mg, 21%).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	95	5	15
6	50	100	

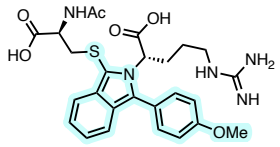
Figure S31: Crude injection of FIICk reaction with **6u** to afford **7u.** Shown here the LC trace at 290, 230, 360nm and the corresponding UV excitation profile and LRMS data.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-methoxyphenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7v)



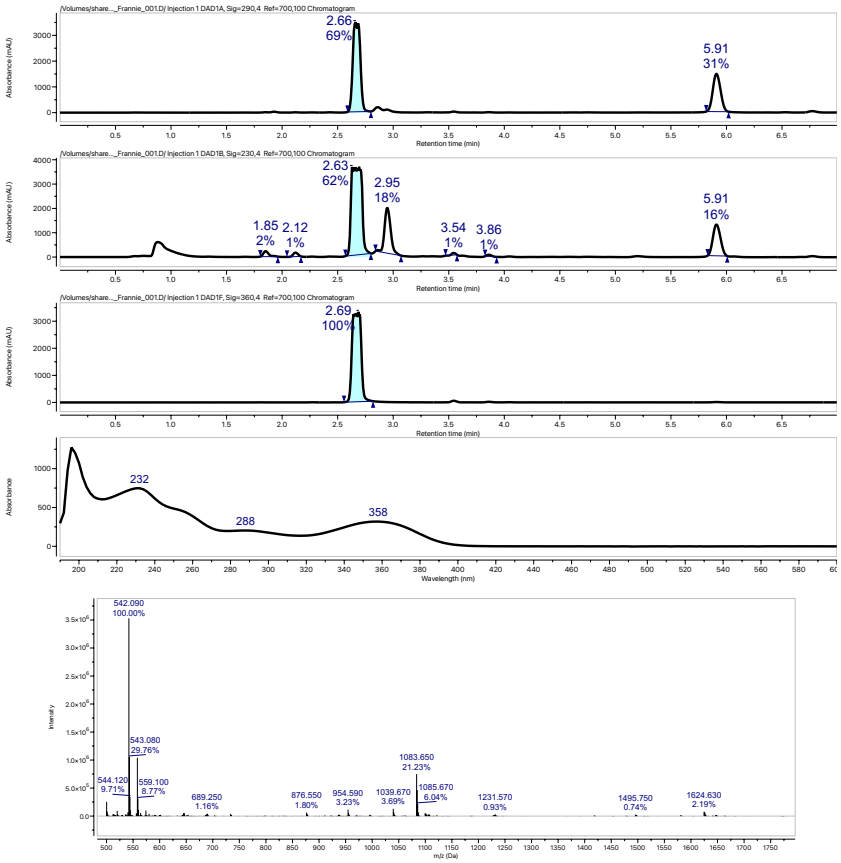
Chemical Formula: $C_{26}H_{31}N_5O_6S$
Exact Mass: 541.200

7v was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6v** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7v** as white powder (15.2µmol, 8.3mg, 30%)

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	20
7	0	100	

Figure S32A: Crude injection of FIICk reaction with **6v** to afford **7v.** Shown here the LC trace at 360nm and the corresponding UV excitation profile and LRMS data. **Right;** purified and lyophilized compound **7v** dissolved in 1:1 MeCN:H_2O.



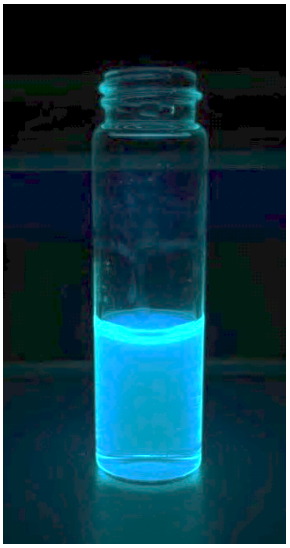
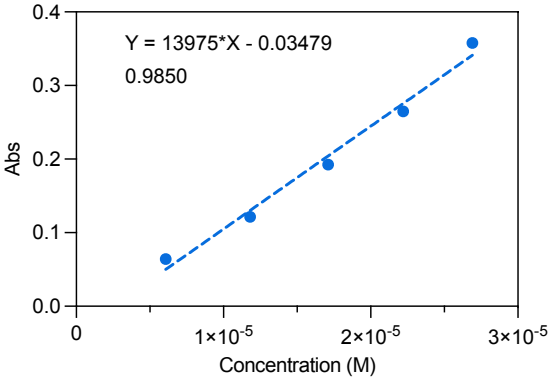
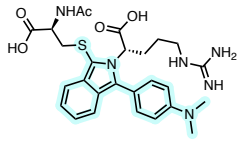


Figure S32B: *Left:* Standard concentration and the corresponding absorbance detected at 358m. *Right*: Calibration plot of **7v**

Concentration (M)	Abs (λ=358nm)
6.07E-06	0.06414
1.18E-05	0.12151
1.71E-05	0.19263
2.22E-05	0.26471
2.69E-05	0.35795
Extinction Coefficient: 14000 cm^{-1} M^{-1}	
Quantum Yield: 46%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-(dimethylamino)phenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7w)



Chemical Formula: $C_{27}H_{34}N_6O_5S$
Exact Mass: 554.231

7w was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6w** (50µmol, 1mL of 50mM solution in DMSO). Reaction was left to sit at room temperature for 1 hour. Lyophilization of purified fractions afforded **7w** as pale yellow powder (7.5µmol, 4.2mg, 15%). This reaction was repeated to obtain enough material for calibration curve and characterization.

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	20
10	0	100	

Figure S33A: Crude injection of FIICk reaction with **6w** to afford **7w.** Shown here the LC trace at 360. 290nm, 230nm and the corresponding UV excitation profile and LRMS data. **Right;** purified and lyophilized **7w** dissolved in 1:1 MeCN/H_2O.

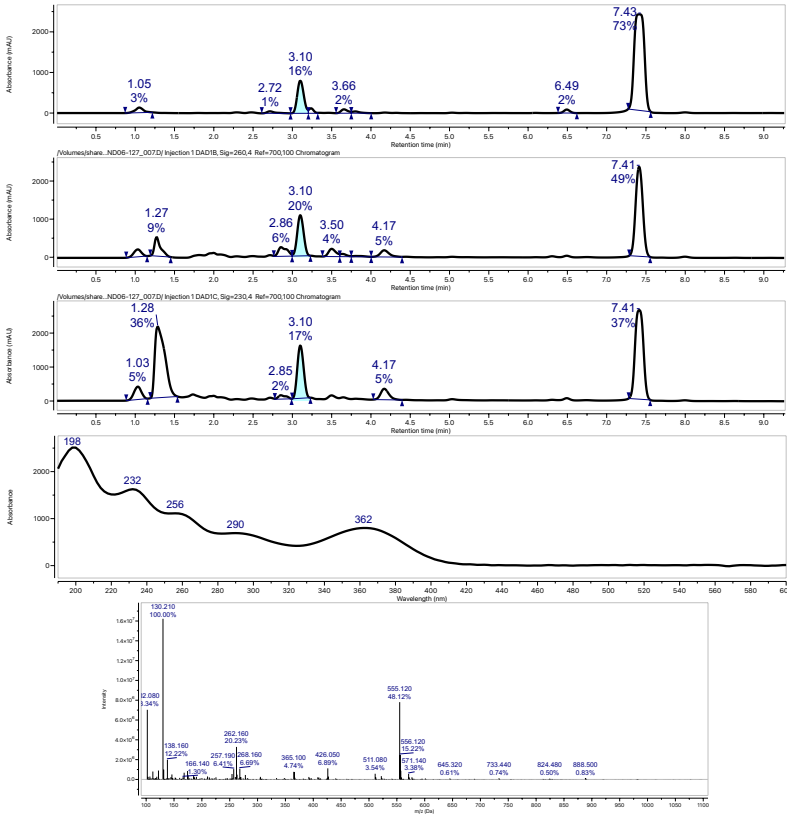
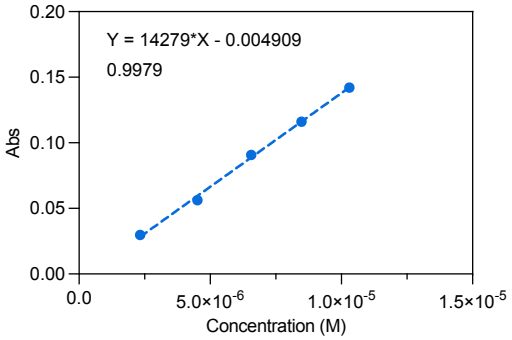
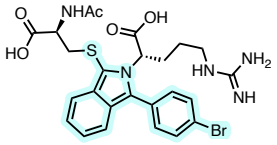


Figure S33B: *Left:* Standard concentration and the corresponding absorbance detected at 362m. *Right*: Calibration plot of **7w.**

Concentration (M)	Abs (λ=362nm)
2.33E-06	0.02983
4.51E-06	0.05622
6.56E-06	0.09071
8.48E-06	0.11615
1.03E-05	0.14205
Extinction Coefficient: 14300 cm^{-1} M^{-1}	
Quantum Yield: 46%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-bromophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7x)



Chemical Formula: $C_{25}H_{28}BrN_5O_5S$
Exact Mass: 589.099

7x was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6x** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7x** as pale orange powder (17.4µmol, 10.3mg, 35%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
10	0	100	

Figure S34A: Crude injection of FIICk reaction with **6x** to afford **7x**. Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

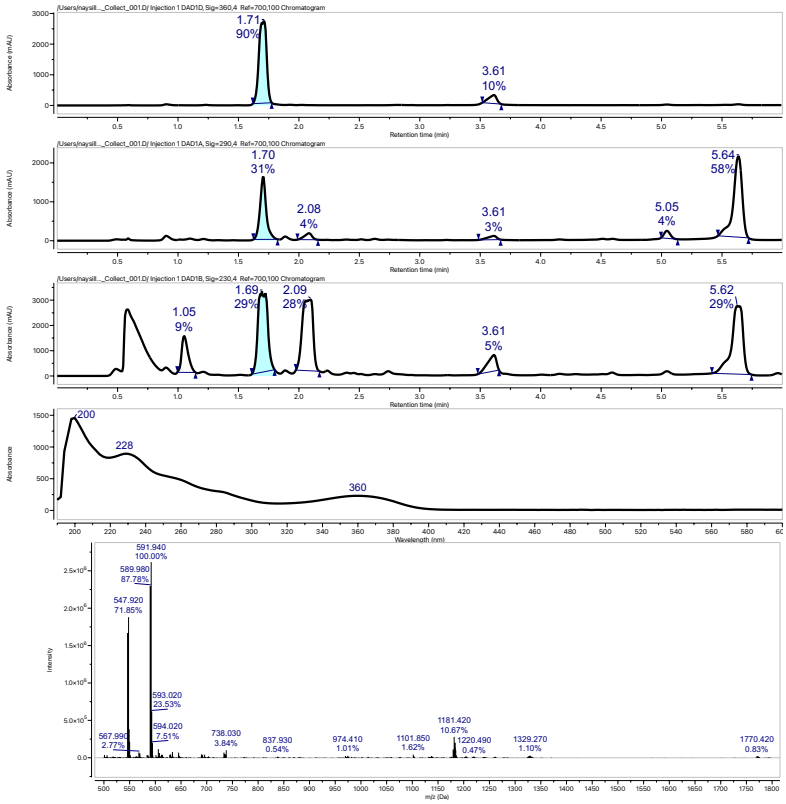
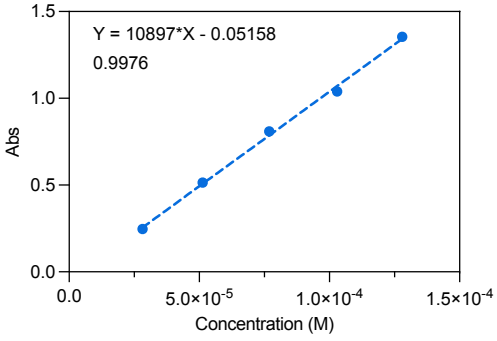
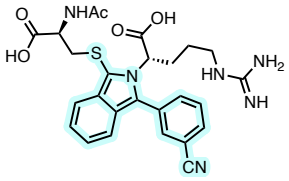


Figure S34B: Left: Standard concentration and the corresponding absorbance detected at 362m. Right: Calibration plot of **7x**

Concentration (M)	Abs (λ=360nm)
2.82E-05	0.24651
5.13E-05	0.51468
7.69E-05	0.809
1.03E-04	1.03917
1.28E-04	1.35426
Extinction Coefficient: 10900 cm^{-1} M^{-1}	
Quantum Yield: 8%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(3-cyanophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7y)



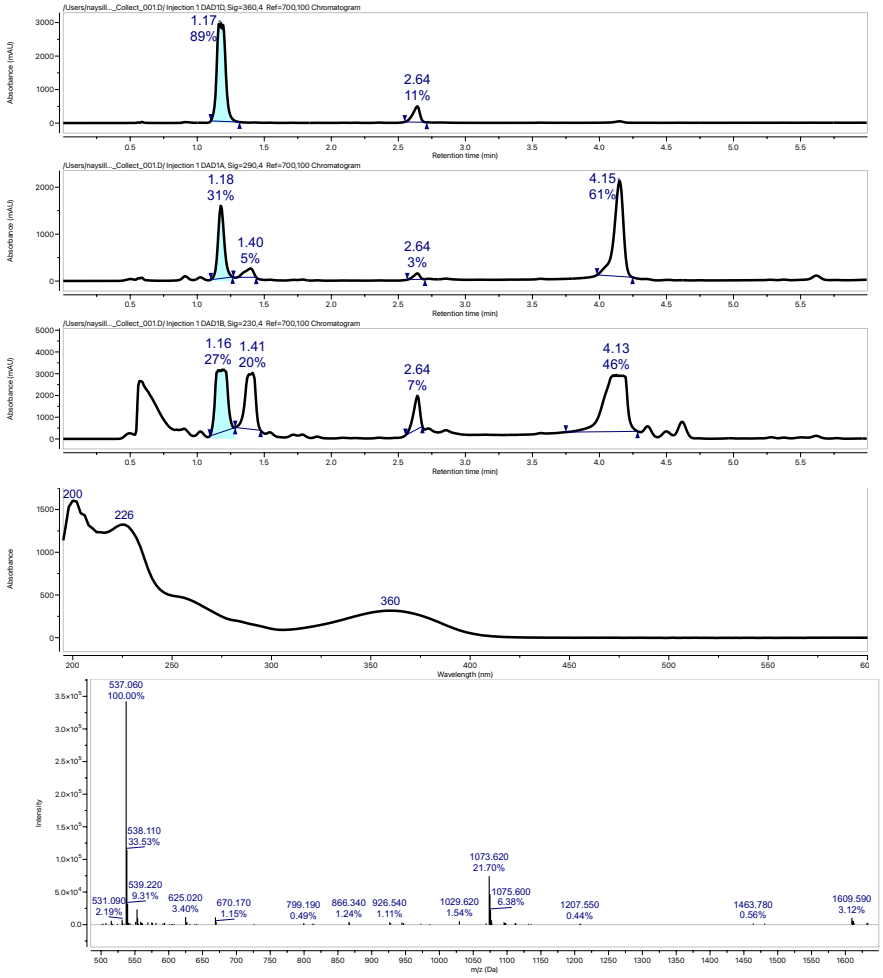
Chemical Formula: $C_{26}H_{28}N_6O_5S$
Exact Mass: 536.184

7y was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6y** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. (19µmol, 10.3mg, 38%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

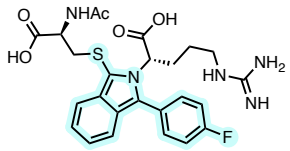
Figure S35: Crude injection of FIICk reaction with **6y** to afford **7y.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-fluorophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7z)



Chemical Formula: $C_{25}H_{28}FN_5O_5S$
Exact Mass: 529.180

7z was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6z** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system. (21µmol, 11mg, 42%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S36: Crude injection of FIICk reaction with **6z** to afford **7z**. Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

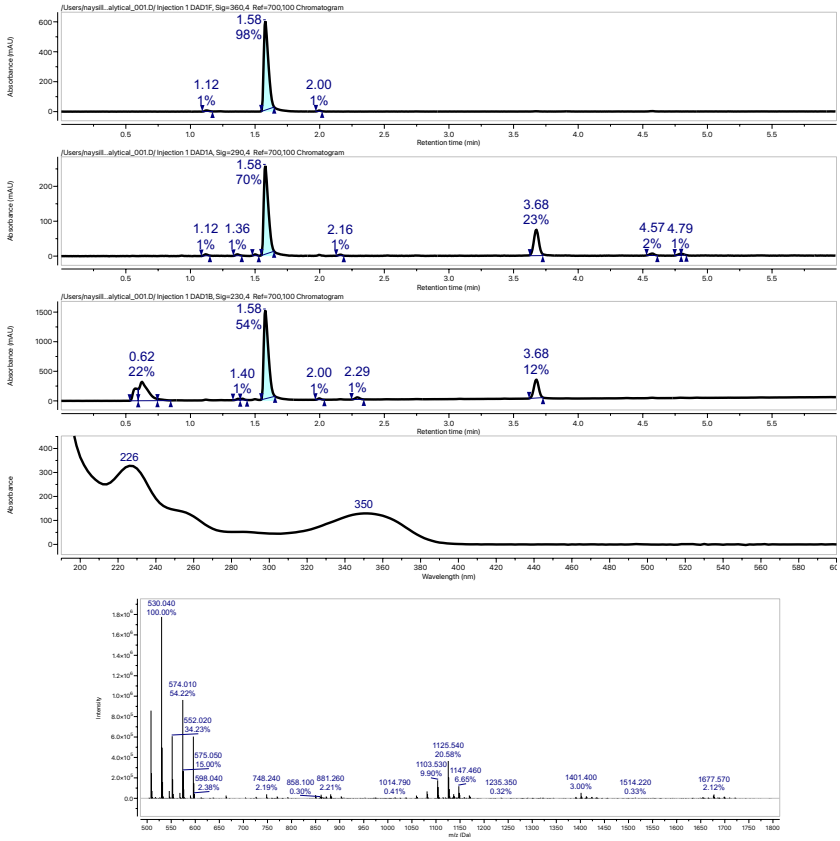
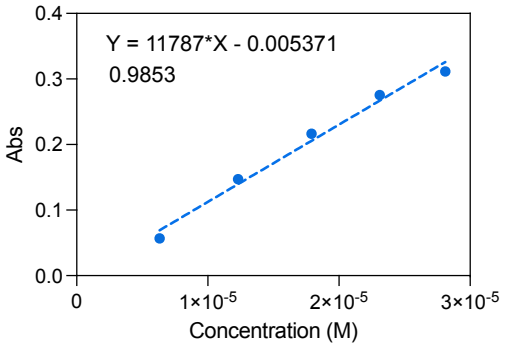
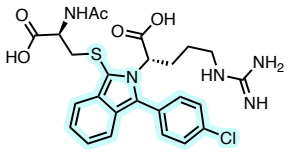


Figure S36B: *Left:* Standard concentration and the corresponding absorbance detected at 350m. *Right*: Calibration plot of **7z.**

Concentration (M)	Abs (λ=350nm)
6.33E-06	0.0567
1.23E-05	0.1472
1.79E-05	0.2164
2.31E-05	0.2755
2.81E-05	0.3114
Extinction Coefficient: 11800 cm^{-1} M^{-1}	
Quantum Yield: 14%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-chlorophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ab)



Chemical Formula: $C_{25}H_{28}ClN_5O_5S$
Exact Mass: 545.150

7x was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6x** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system (19.5µmol, 10.6mg, 39%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S38A: Crude injection of FIICk reaction with **6ab** to afford **7ab.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

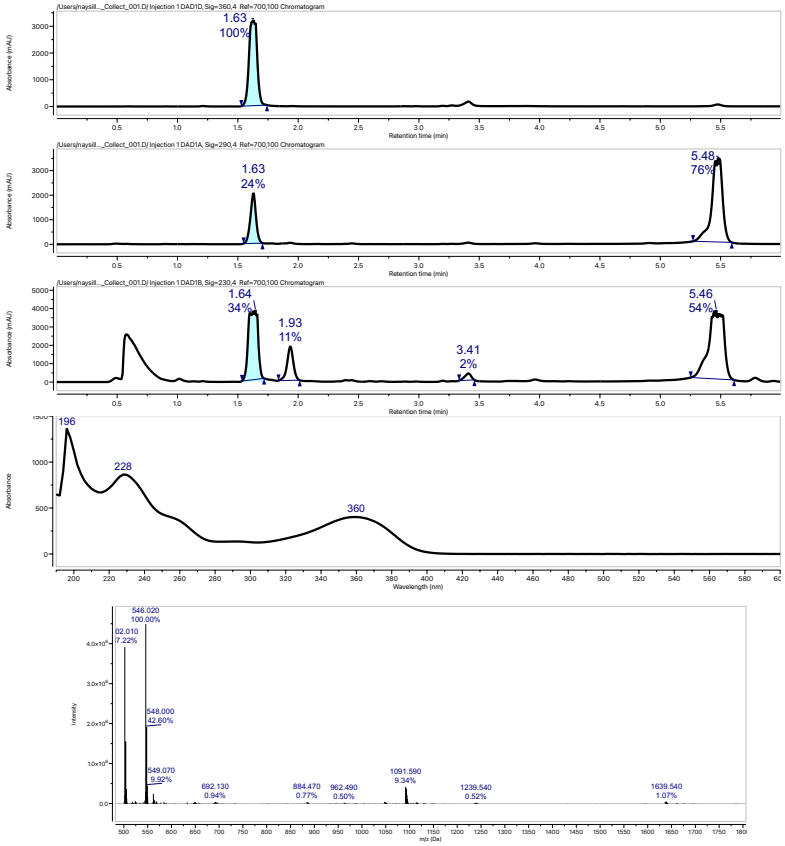
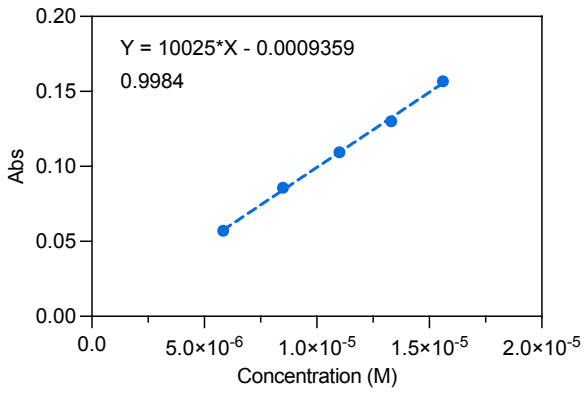
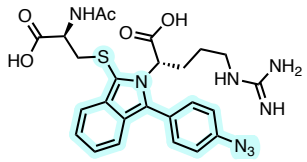


Figure S38B: Left: Standard concentration and the corresponding absorbance detected at 350m. Right: Calibration plot of **7ab**.

Concentration (M)	Abs (λ=360nm)
5.84E-06	0.05704
8.49E-06	0.0857
1.10E-05	0.10934
1.33E-05	0.13011
1.56E-05	0.15679
Extinction Coefficient: 10000 cm^{-1} M^{-1}	
Quantum Yield: 8%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-azidophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ac)



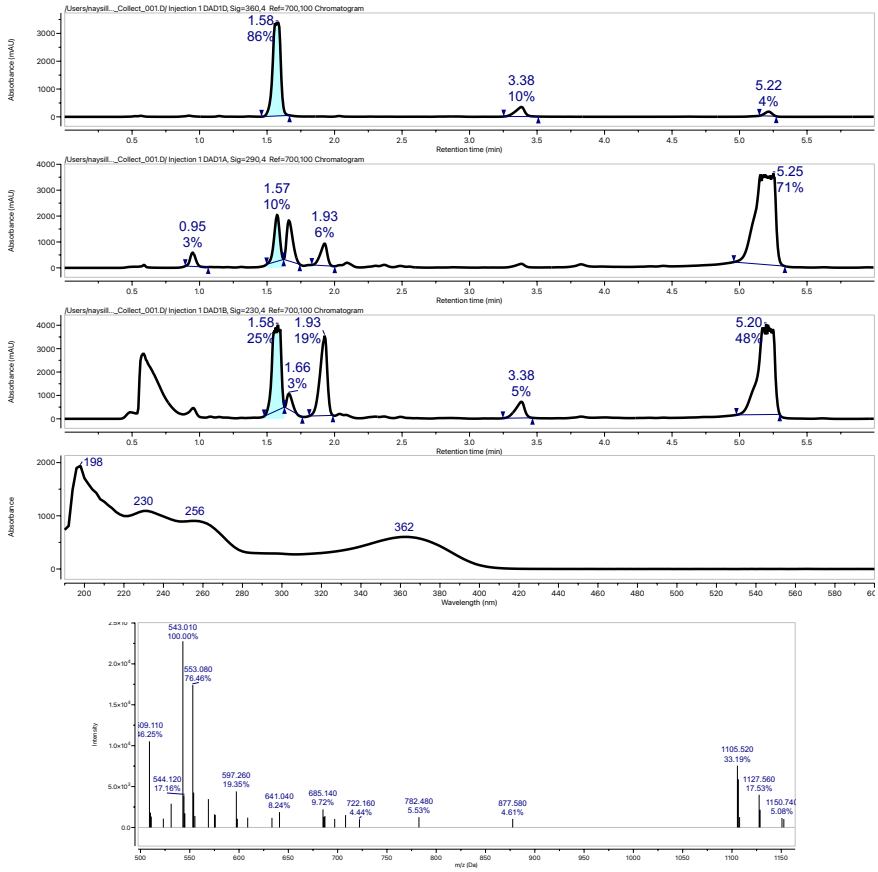
Chemical Formula: $C_{25}H_{28}N_8O_5S$
Exact Mass: 552.190

7ac was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6ac** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7ac** as white powder. (14µmol, 7.7mg, 28%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

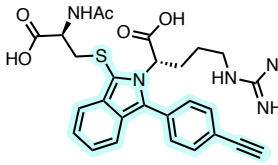
Figure S39: Crude injection of FIICk reaction with **6ac** to afford **7ac.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is weakly fluorescent.
Quantum yield: < 5%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(4-ethynylphenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ad)



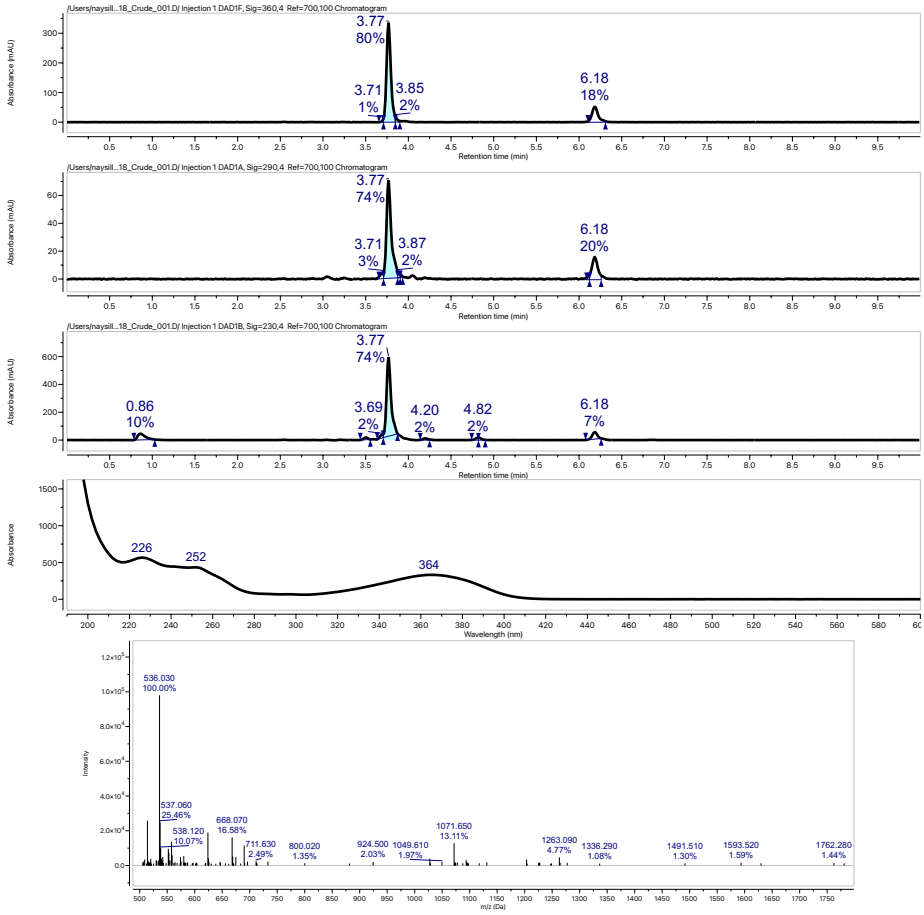
Chemical Formula: $C_{27}H_{29}N_5O_5S$
Exact Mass: 535.189

7ae was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6ae** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system (23µmol, 12.2mg, 46%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	20
10	0	100	

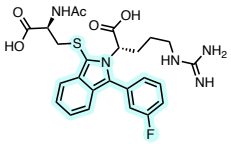
Figure S40: Crude injection of FIICk reaction with **6ad** to afford **7ad.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is weakly fluorescent.
Quantum yield: < 5%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(3-fluorophenyl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ae)



Chemical Formula: $C_{25}H_{28}FN_5O_5S$
Exact Mass: 529.180

7ae was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6ae** (50µmol, 1mL of 50mM solution in DMSO). After 1 hour sitting at room temperature, reaction was acidified to pH 3 with formic acid and purified with 0.1% formic acid system (15.5µmol, 8.2mg, 31%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S41A: Crude injection of FIICk reaction with **6ae** to afford **7ae.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

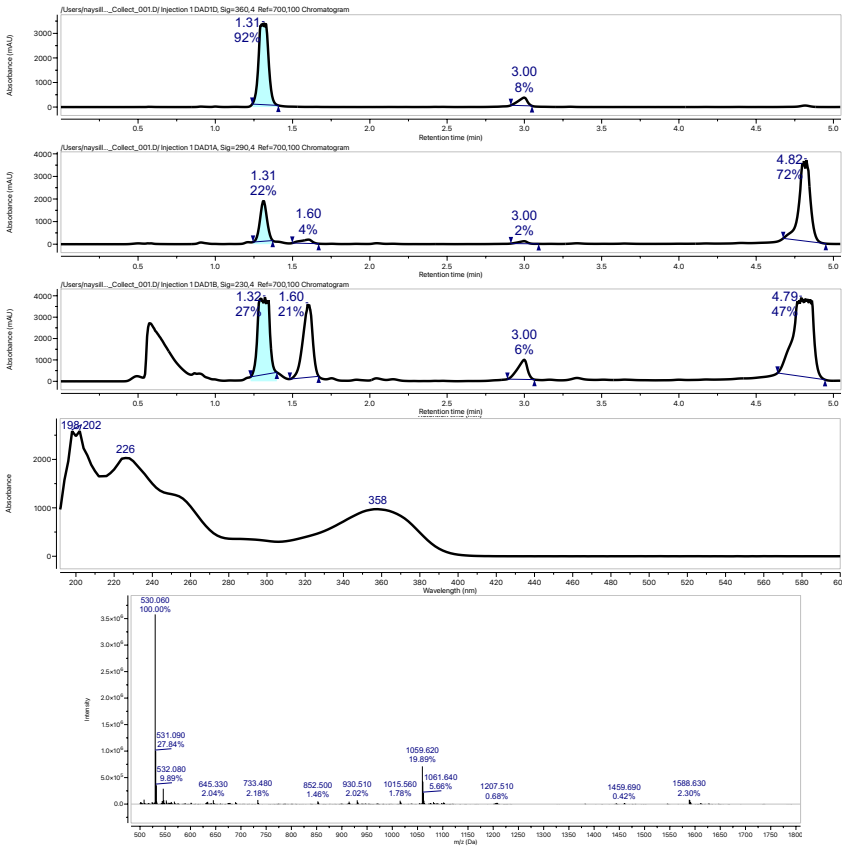
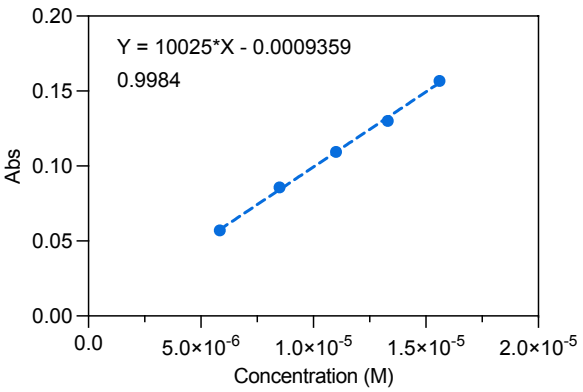
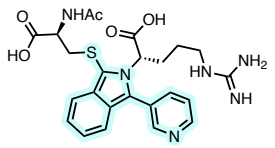


Figure S41B: *Left:* Standard concentration and the corresponding absorbance detected at 358m. *Right*: Calibration plot of **7ae.**

Concentration (M)	Abs (λ=358nm)
5.91E-06	0.09046
1.14E-05	0.15012
1.67E-05	0.24721
2.15E-05	0.31974
2.62E-05	0.36507
Extinction Coefficient: 14200 cm^{-1} M^{-1}	
Quantum Yield: 14%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(pyridin-3-yl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7af)



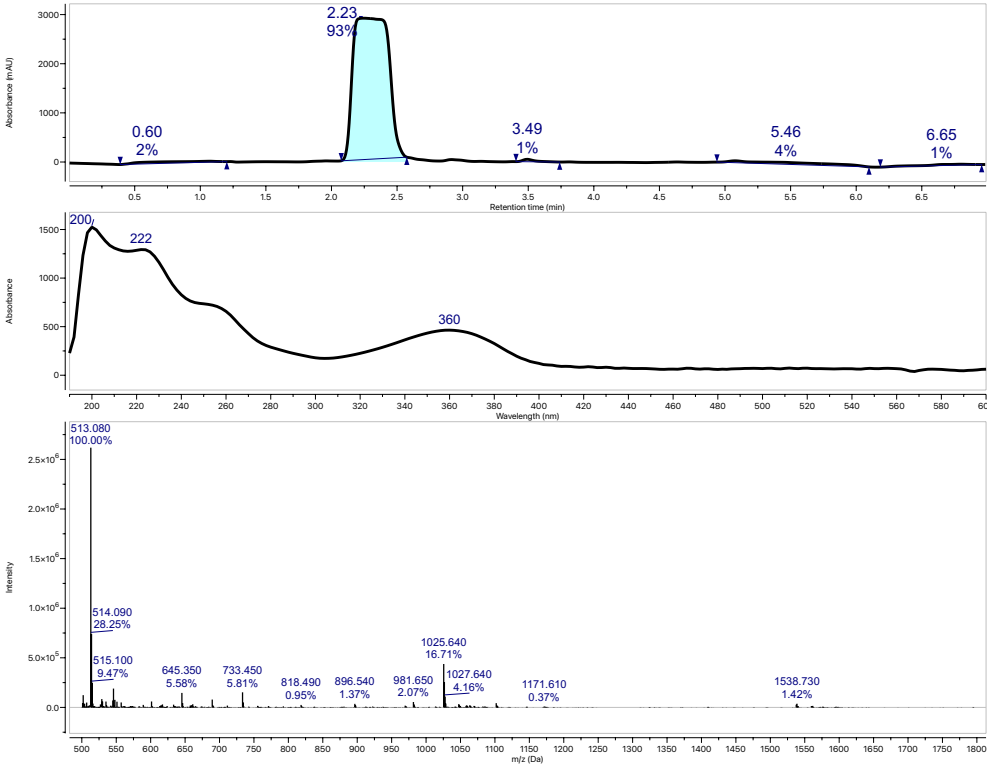
Chemical Formula: $C_{24}H_{28}N_6O_5S$
Exact Mass: 512.184

7af was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6af** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7af** as white powder subject to desalting treatment with C18 SepPak (2.0g) (5.5µmol, 2.8mg, 11%).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

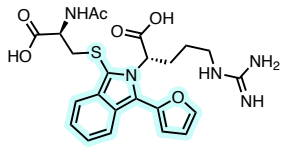
Figure S42: Crude injection of FIICk reaction with **6ae** to afford **7ae.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.



Collected peak is not fluorescent.
Quantum yield: 0%

Extinction coefficient: N/A

(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(furan-2-yl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ag)



Chemical Formula: $C_{23}H_{27}N_5O_6S$
Exact Mass: 501.168

7ag was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6ag** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7ag** as white powder subject to desalting treatment with C18 SepPak (2.0g) (9.5µmmol, 4.7mg, 19%).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

Figure S43A: Crude injection of FIICk reaction with **6ag** to afford **7ag.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

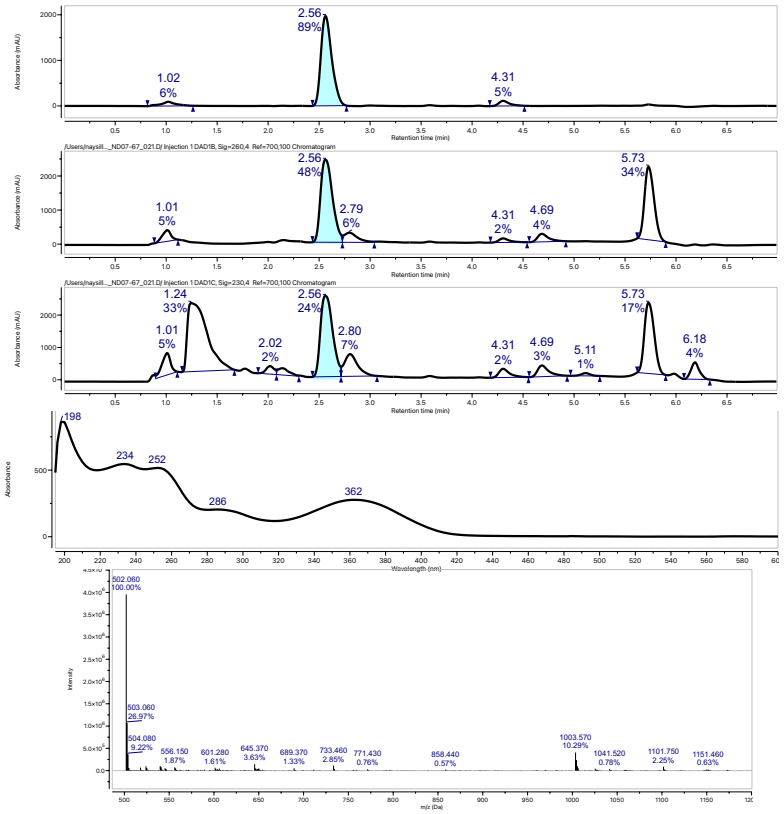
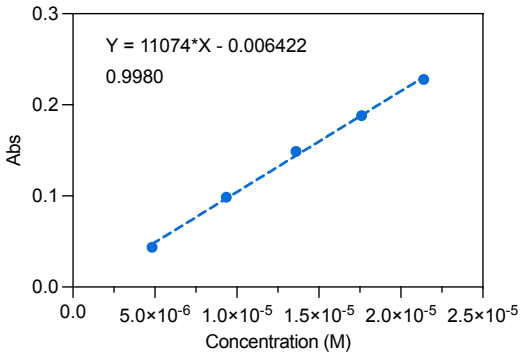
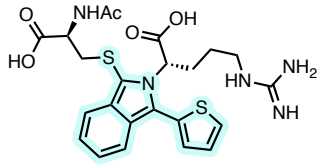


Figure S43B: Left: Standard concentration and the corresponding absorbance detected at 358m. Right: Calibration plot of **7ag**.

Concentration (M)	Abs (λ=362nm)
4.82E-06	0.04374
9.35E-06	0.09855
1.36E-05	0.14894
1.76E-05	0.18818
2.14E-05	0.22787
Extinction Coefficient: 11100 cm^{-1} M^{-1}	
Quantum Yield: 15%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(thiophen-2-yl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ah)



Chemical Formula: $C_{23}H_{27}N_5O_5S_2$
Exact Mass: 517.145

7ah was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6ah** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7ah** as white powder subject to desalting treatment with C18 SepPak (2.0g) (11µmol, 5.7mg, 22%)

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 250x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	40
4.30	50	50	
5.00	0	100	
7.00	0	100	

Figure S44A: Crude injection of FIICk reaction with **6ah** to afford **7ah.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

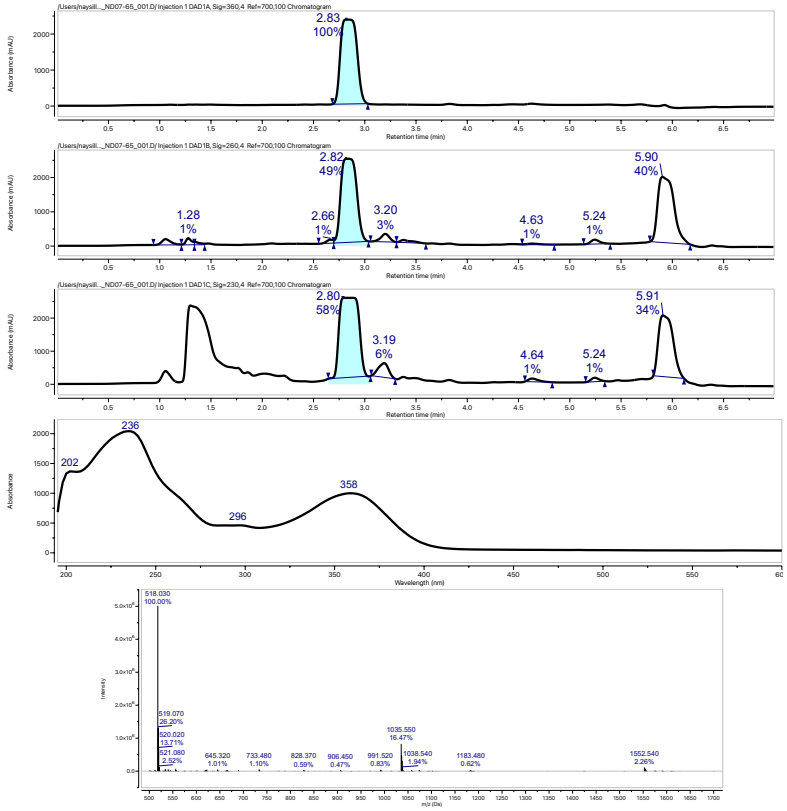
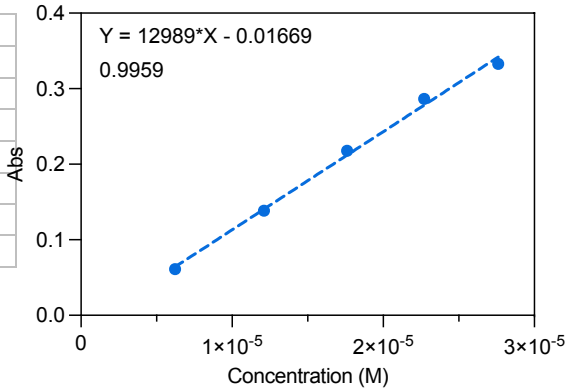
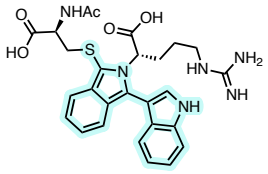


Figure S44B: *Left:* Standard concentration and the corresponding absorbance detected at 358m. *Right*: Calibration plot of **7ah.**

Concentration (M)	Abs (λ=358nm)
6.23E-06	0.06102
1.21E-05	0.13853
1.76E-05	0.21774
2.27E-05	0.28648
2.76E-05	0.33284
Extinction Coefficient: 13000 cm^{-1} M^{-1}	
Quantum Yield: 9%	



(*S*)-2-(1-(((*R*)-2-acetamido-2-carboxyethyl)thio)-3-(1*H*-indol-3-yl)-2*H*-isoindol-2-yl)-5-guanidinopentanoic acid (7ai)



Chemical Formula: $C_{27}H_{30}N_6O_5S$
Exact Mass: 550.1998

7ai was prepared according to the general protocol for the synthesis of disubstituted isoindole, starting from **6ai** (50µmol, 1mL of 50mM solution in DMSO). Lyophilization of purified fractions afforded **7ai** as murky yellow powder (21µmol, 11.6mg, 42%).

Solvent A : H_2O (0.1% formic acid)
Solvent B : MeCN (0.1% formic acid)
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

Figure S45A: Crude injection of FIICk reaction with **6ai** to afford **7ai.** Shown here the LC trace at 360, 290nm, 230nm and the corresponding UV excitation profile and LRMS data.

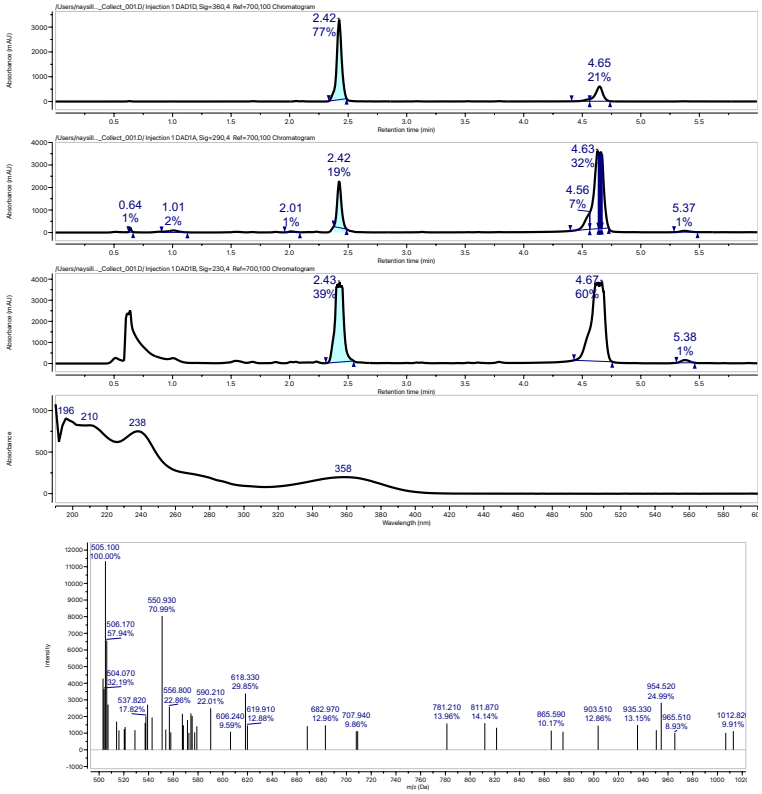
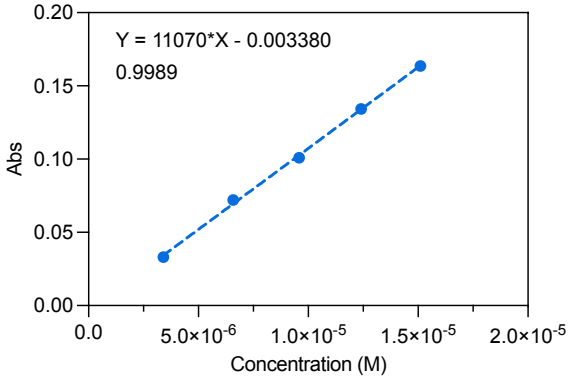


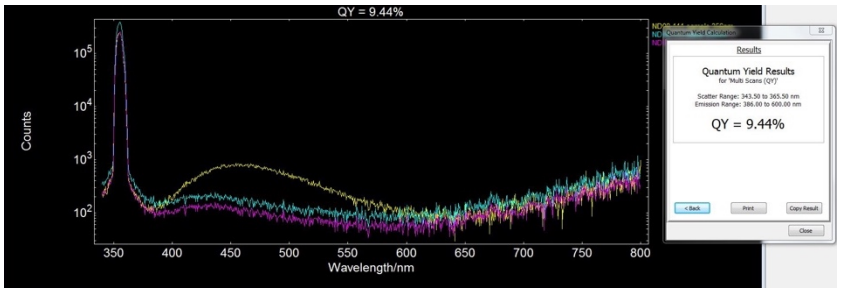
Figure S45B: *Left:* Standard concentration and the corresponding absorbance detected at 358m. *Right*: Calibration plot of **7ai.**

Concentration (M)	Abs (λ=358nm)
3.40E-06	0.03301
6.58E-06	0.07215
9.58E-06	0.10096
1.24E-05	0.13433
1.51E-05	0.16362
Extinction Coefficient: 11000 cm^{-1} M^{-1}	
Quantum Yield: 18%	

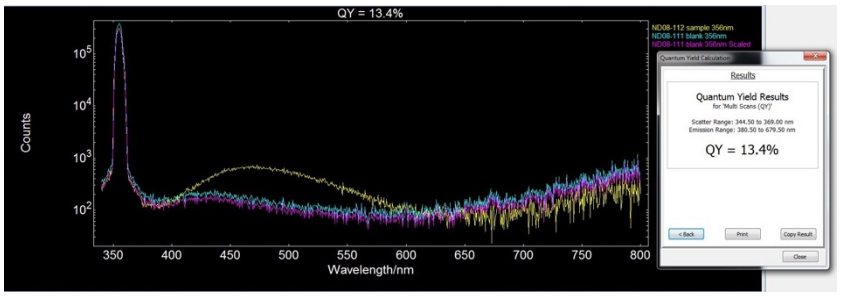


Quantum Yield Data

Compound	**7a**
λ excitation (nm)	356
λ emission (nm)	460
QY	9%



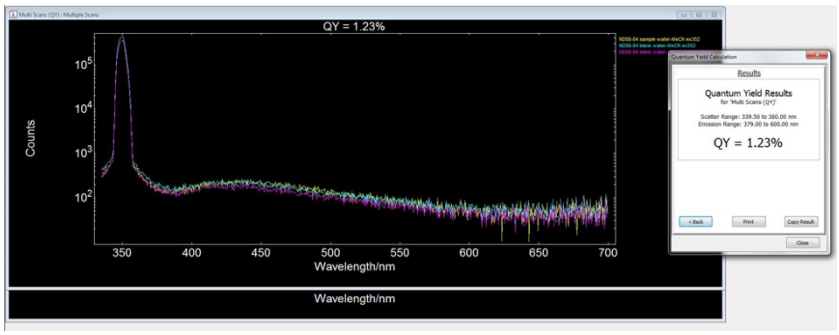
Compound	**7b**
λ excitation (nm)	362
λ emission (nm)	470
QY	13%



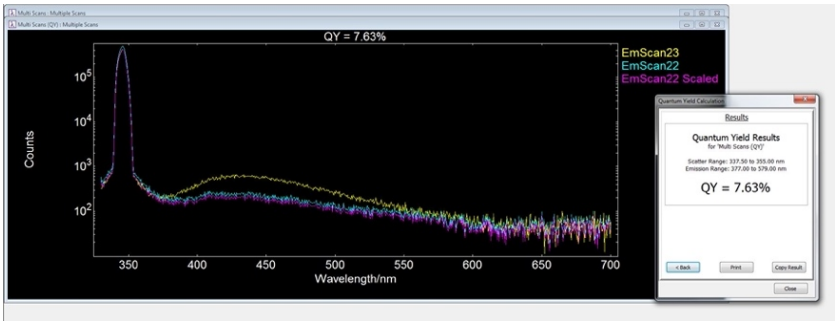
Compound	**7c**
λ excitation (nm)	362
λ emission (nm)	-
QY	0%

Compound	**7d**
λ excitation (nm)	352
λ emission (nm)	-
QY	0%

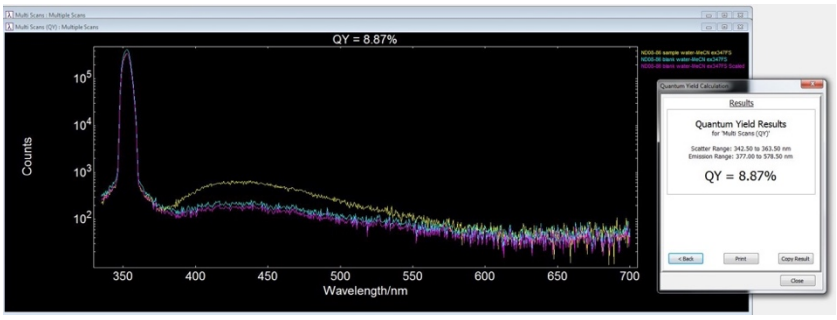
Compound	**7e**
λ excitation (nm)	351
λ emission (nm)	-
QY	<2%



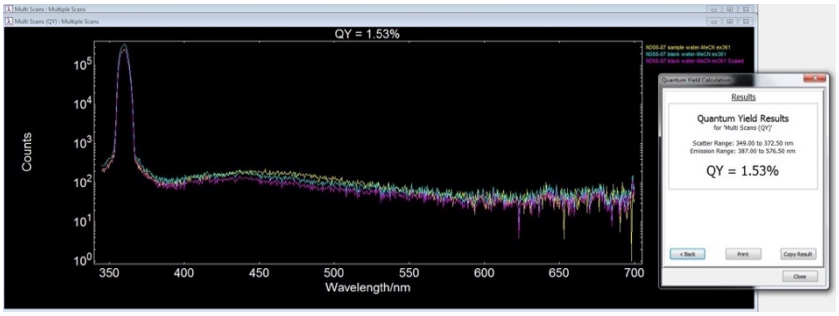
Compound	**7f**
λ excitation (nm)	346
λ emission (nm)	437
QY	8%



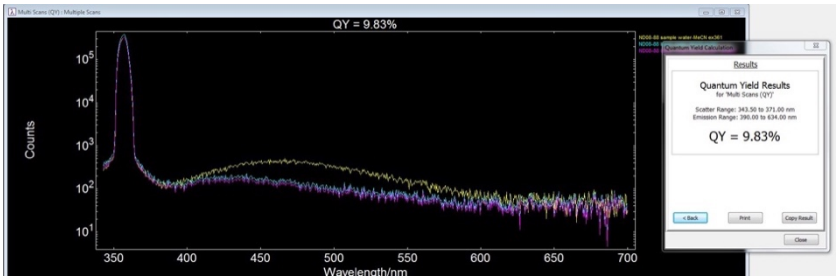
Compound	**7g**
λ excitation (nm)	352
λ emission (nm)	420
QY	9%



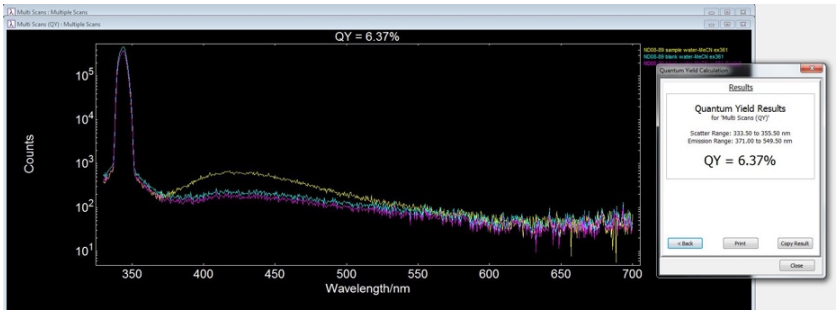
Compound	**7h**
λ excitation (nm)	362
λ emission (nm)	-
QY	<2 %



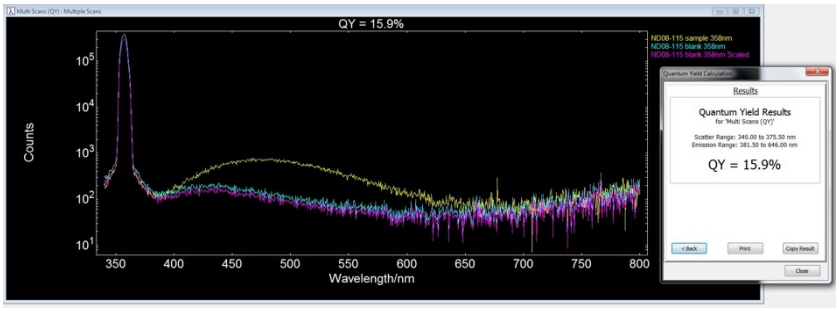
Compound	**7i**
λ excitation (nm)	358
λ emission (nm)	462
QY	10%



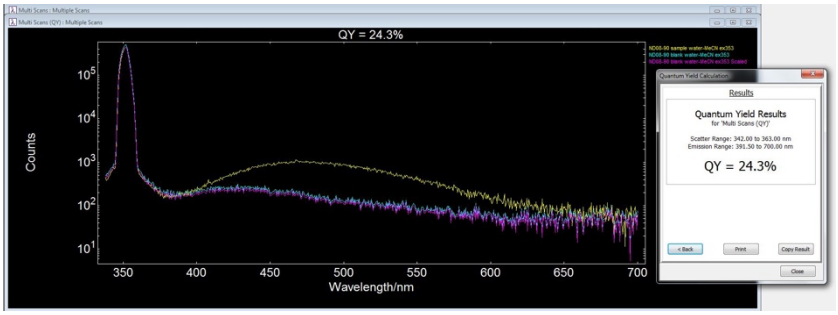
Compound	**7j**
λ excitation (nm)	348
λ emission (nm)	429
QY	6%



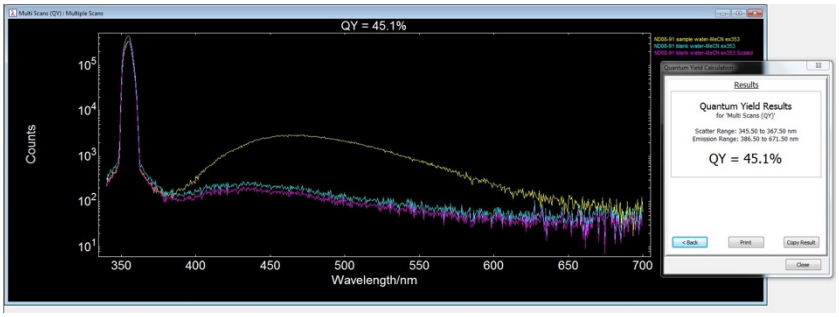
Compound	**7k**
λ excitation (nm)	358
λ emission (nm)	469
QY	16%



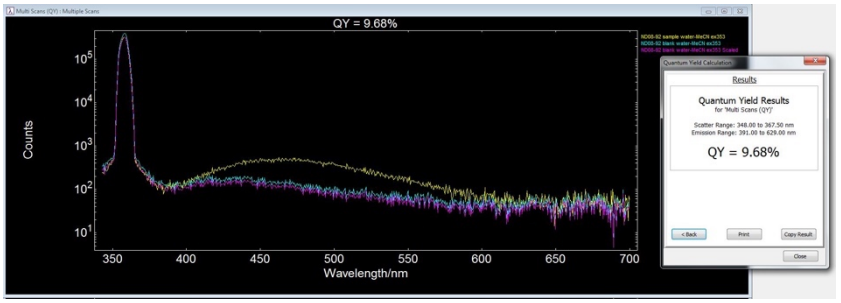
Compound	**7l**
λ excitation (nm)	354
λ emission (nm)	468
QY	24%



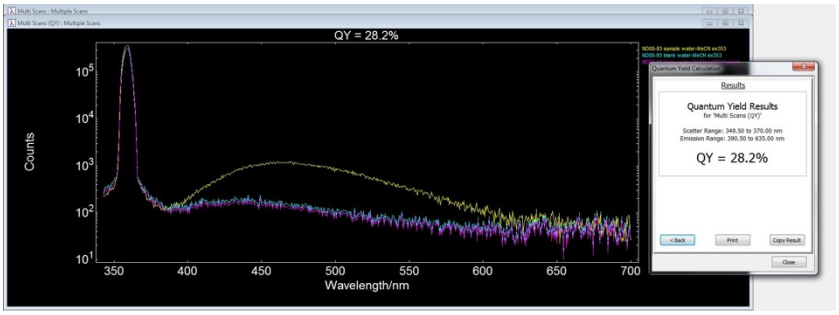
Compound	**7m**
λ excitation (nm)	356
λ emission (nm)	423
QY	45%



Compound	**7n**
λ excitation (nm)	360
λ emission (nm)	464
QY	10%



Compound	**7o**
λ excitation (nm)	360
λ emission (nm)	463
QY	28%



Compound	**7p**
λ excitation (nm)	362, 454
λ emission (nm)	-
QY	0%

Compound	**7q**
λ excitation (nm)	452
λ emission (nm)	-
QY	0%

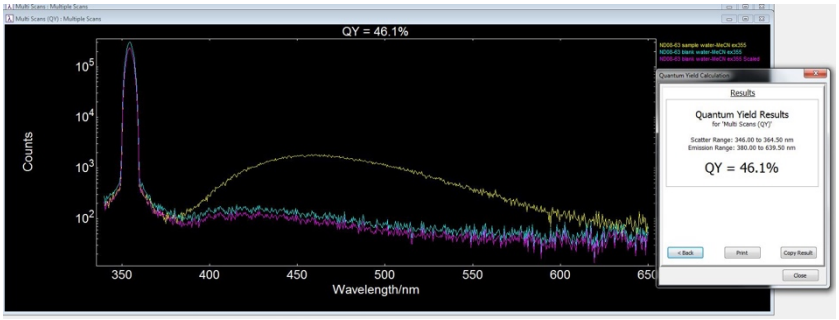
Compound	**7r**
λ excitation (nm)	308, 464
λ emission (nm)	-
QY	0%

Compound	**7s**
λ excitation (nm)	306, 450
λ emission (nm)	-
QY	0%

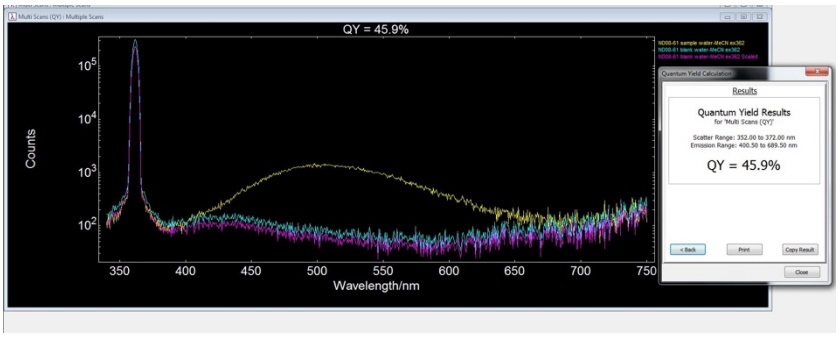
Compound	**7t**
λ excitation (nm)	308, 478
λ emission (nm)	-
QY	0%

Compound	**7u**
λ excitation (nm)	304, 472
λ emission (nm)	-
QY	0%

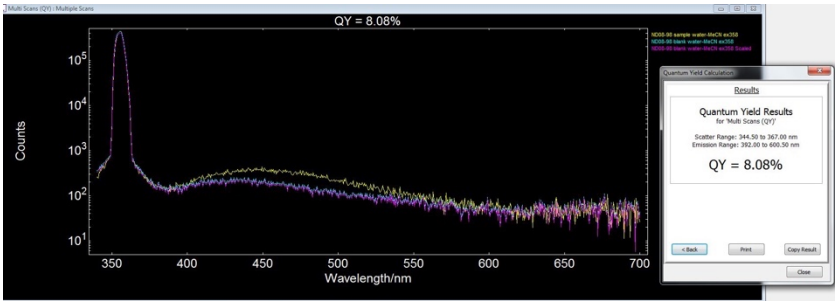
Compound	**7v**
λ excitation (nm)	360
λ emission (nm)	464
QY	46%



Compound	**7w**
λ excitation (nm)	364
λ emission (nm)	494
QY	46%

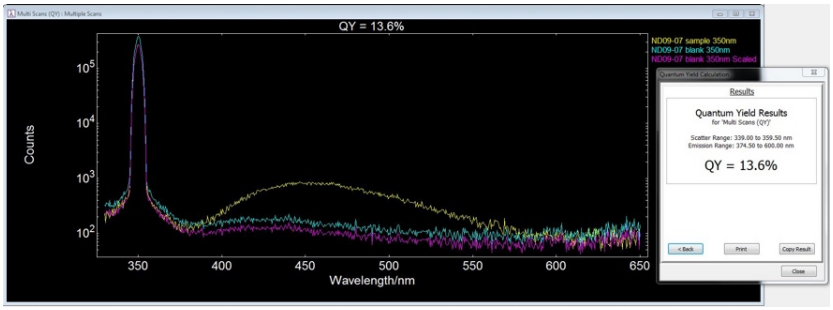


Compound	**7x**
λ excitation (nm)	358
λ emission (nm)	450
QY	8%



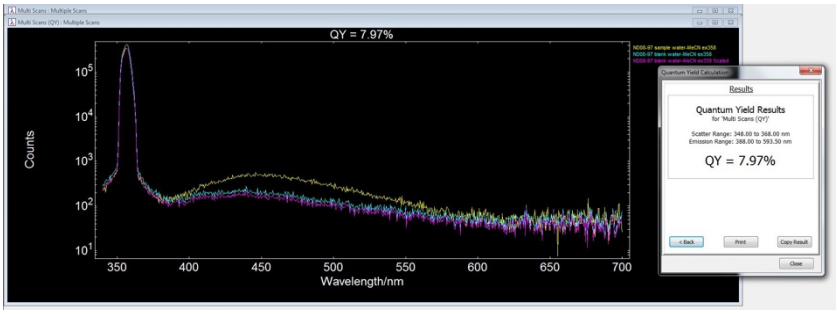
Compound	**7y**
λ excitation (nm)	360
λ emission (nm)	-
QY	0%

Compound	**7z**
λ excitation (nm)	350
λ emission (nm)	450
QY	14

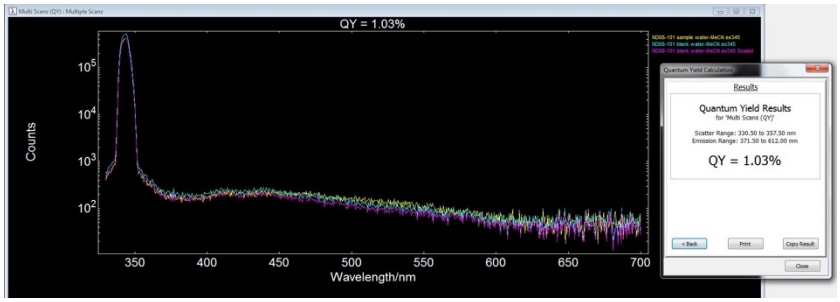


Compound	**7aa**
λ excitation (nm)	388
λ emission (nm)	-
QY	0%

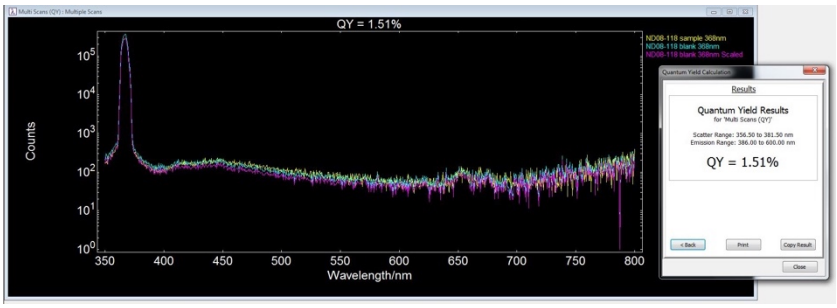
Compound	**7ab**
λ excitation (nm)	360
λ emission (nm)	452
QY	8%



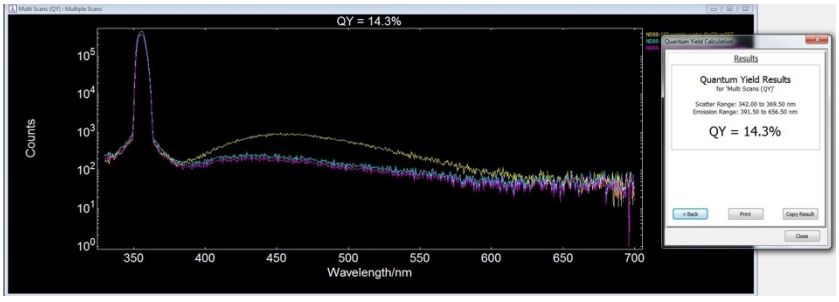
Compound	**7ac**
λ excitation (nm)	355
λ emission (nm)	-
QY	< 2%



Compound	**7ad**
λ excitation (nm)	366
λ emission (nm)	-
QY	2%

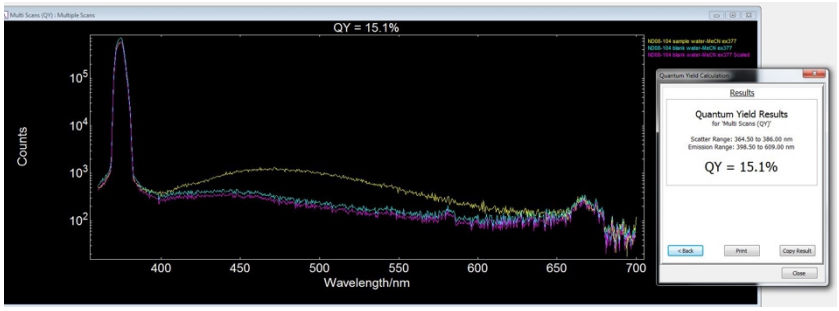


Compound	**7ae**
λ excitation (nm)	358
λ emission (nm)	456
QY	14%

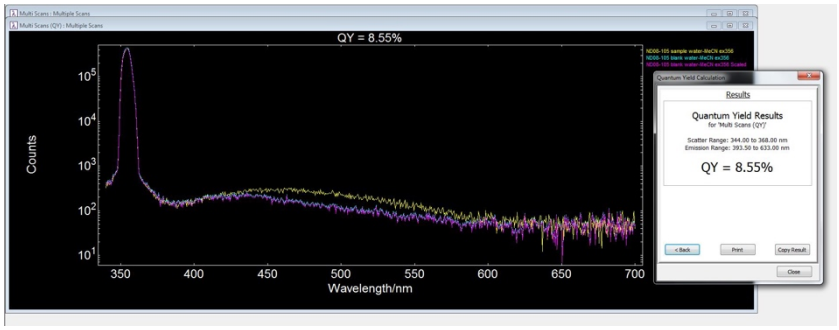


Compound	**7af**
λ excitation (nm)	360
λ emission (nm)	-
QY	0%

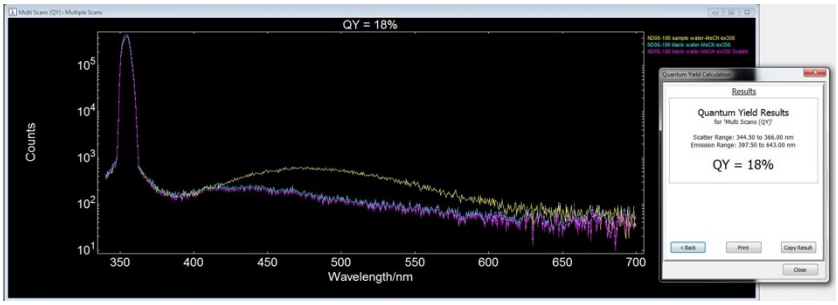
Compound	**7ag**
λ excitation (nm)	362
λ emission (nm)	470
QY	15%



Compound	**7ah**
λ excitation (nm)	358
λ emission (nm)	470
QY	9%

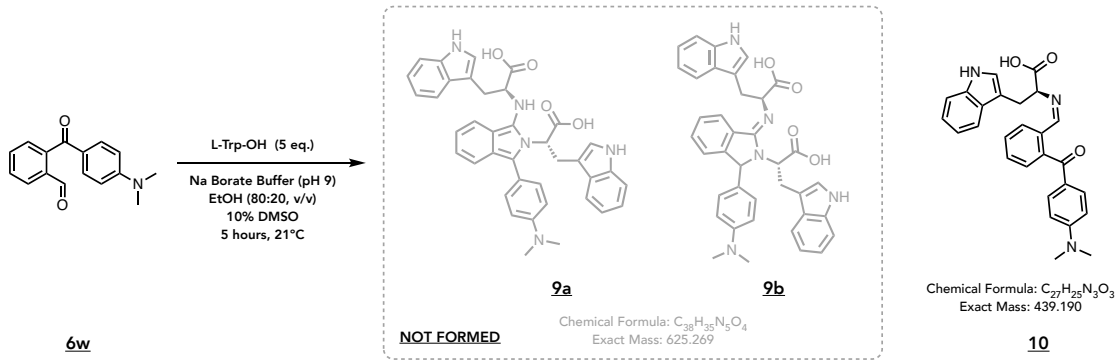


Compound	**7ai**
λ excitation (nm)	360
λ emission (nm)	462
QY	18%



Intermolecular Chemoselectivity Studies

Reaction of 6w with excess L-Trp-OH

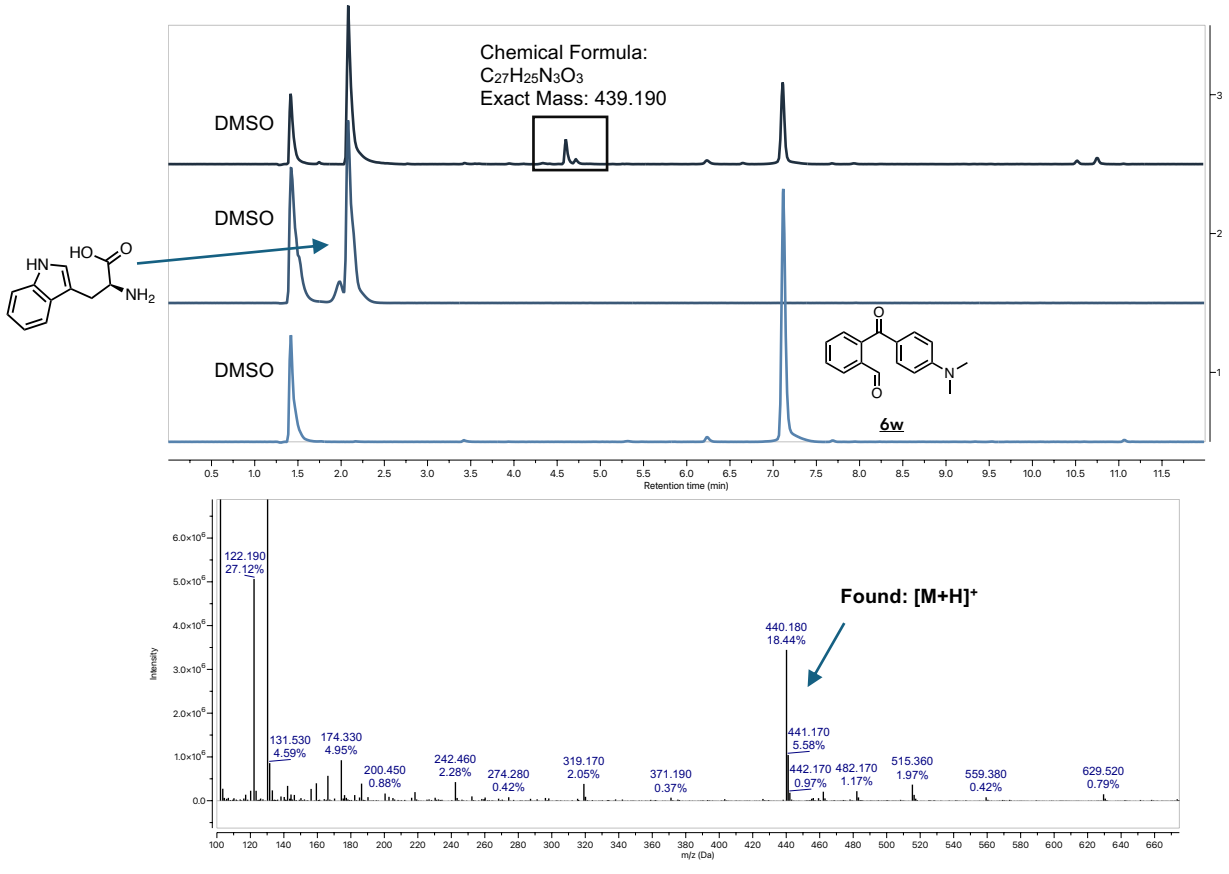


6w was prepared as a 50mM solution in DMSO, and a 1mL aliquot (50µmol, 1 eq.) was dispensed into a stirring mixture of L-Trp-OH (51mg, 250µmol, 5 eq.) in 9mL Na Borate buffer:EtOH (80:20, v/v), resulting in a clear yellow solution. This mixture was stirred at room temperature for 5 hours, after which a 200µL aliquot was injected directly for HPLC analysis and purification (Entry 1). Two blank runs for each reaction component were prepared in parallel and analyzed by HPLC, where L-Trp-OH (5 eq.) was dissolved in 1mL DMSO, and Na Borate buffer:EtOH (80:20, v/v) resulting in Entry 2, and **6w** (1 eq.) was dissolved in Na Borate buffer:EtOH (80:20, v/v) resulting in Entry 3.

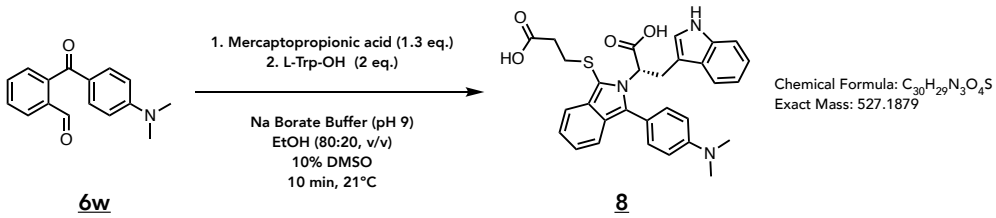
Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	15
12	0	100	

Figure S46: UV trace and corresponding MS from HPLC analysis of the crude reaction mixture (entry 3), and blank runs of L-Trp (entry 2) and **6w** (entry 1). Chromatogram observed at 230nm. Peak at the solvent front is DMSO as observed at 230nm



Reaction of 6w with mercaptopropionic acid followed by excess L-Trp-OH

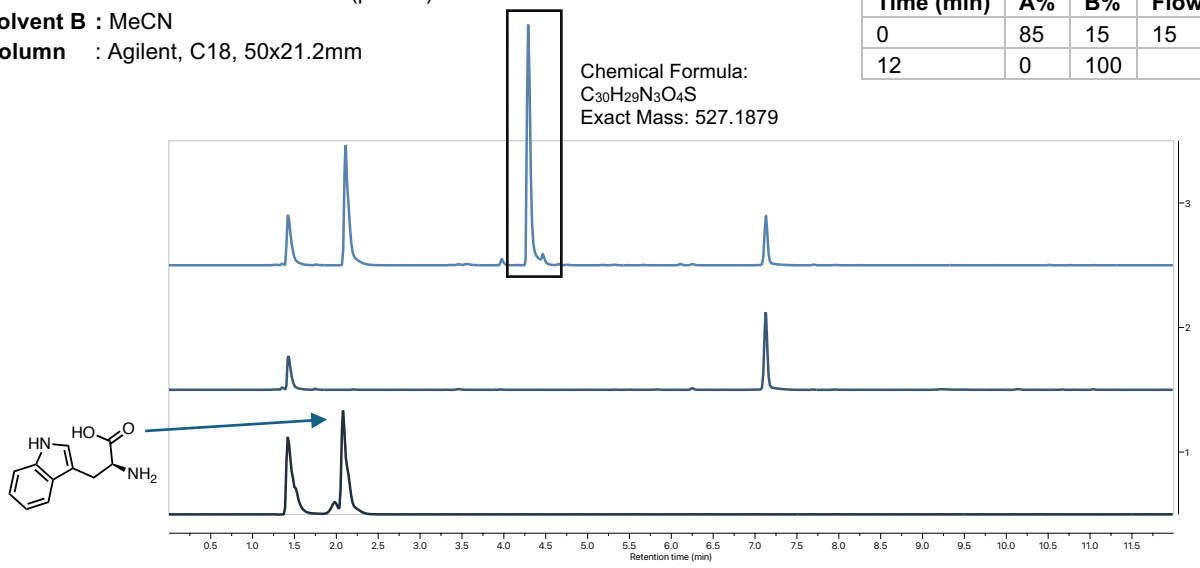


6w **8**

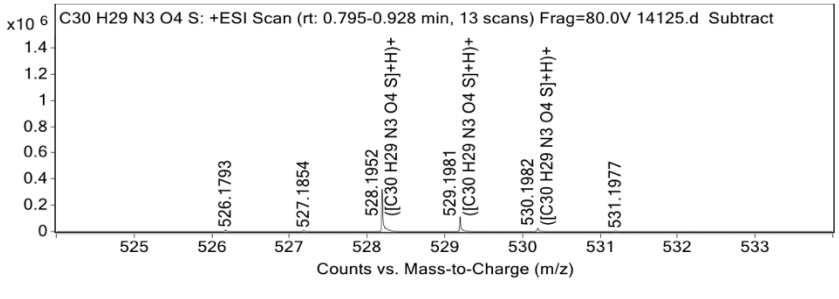
6w was prepared as a 50mM solution in DMSO, and a 1mL aliquot (50µmol, 1 eq.) was dispensed into a stirring mixture of mercaptopropionic acid (5.6µL, 65µmol, 1.3 eq.) in 9mL Na Borate buffer:EtOH (80:20, v/v), resulting in a clear yellow solution. Observed with a hand-held UV lamp (365nm), this mixture was not fluorescent. To this solution was added L-Trp-OH (20mg, 100µmol, 2.0 eq.) as solids, and upon stirring at room temperature for 1 minute, fluorescence started to appear and gradually became brighter. After 10 minutes stirring at room temperature, a 200µL aliquot was injected and analyzed by HPLC (Entry 3). Two blank runs for each reaction component were prepared in parallel and analyzed by HPLC, where **6w** (1 eq.) was dissolved in Na Borate buffer:EtOH (80:20, v/v) resulting in Entry 2, and L-Trp-OH (2 eq.) was dissolved in 1mL DMSO, and Na Borate buffer:EtOH (80:20, v/v) resulting in Entry 1.

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	15
12	0	100	



Calc. $[C_{30}H_{29}N_3O_4S+H]^+$ = 528.1957
Found $[C_{30}H_{29}N_3O_4S+H]^+$ = 528.1952



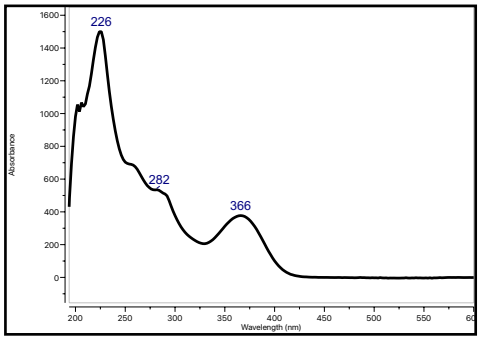
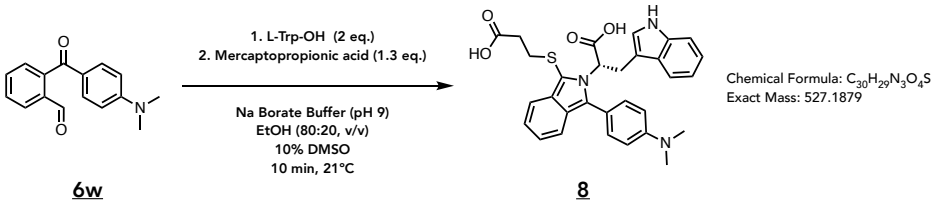


Figure S47: UV trace and corresponding HRMS from HPLC analysis of the crude reaction mixture (entry 3). Blank runs of **6w** (entry 2) and L-Trp (entry 1). Chromatogram observed at 230nm. Peak at the solvent front is DMSO as observed at 230nm. Insert: UV excitation profile of **8**.

Reaction of 6w with excess L-Trp-OH followed by mercaptopropionic acid



6w **8**

6w was prepared as a 50mM solution in DMSO, and a 1mL aliquot (50µmol, 1 eq.) was dispensed into a stirring mixture of L-Trp-OH (20mg, 100µmol, 2.0 eq.) in 9mL Na Borate buffer:EtOH (80:20, v/v), resulting in a clear yellow solution. This mixture was stirred at room temperature for 1 hour, after which a 200µL aliquot of this solution was directly injected and analyzed by HPLC (Entry 3). Then, mercaptopropionic acid (5.6µL, 65µmol, 1.3 eq.) was subsequently added, where fluorescence was observed via hand-held UV lamp (365nm) after stirring for 2 minutes. Another 200µL aliquot was again injected and analyzed by HPLC after reacting for 10 minutes (Entry 4). Finally, solution was left to stir for an additional hour and full conversion to **8** was observed (Entry 5).

Solvent A : 40mM NH_4OH/HCOOH (pH 8-9)
Solvent B : MeCN
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	85	15	15
12	0	100	

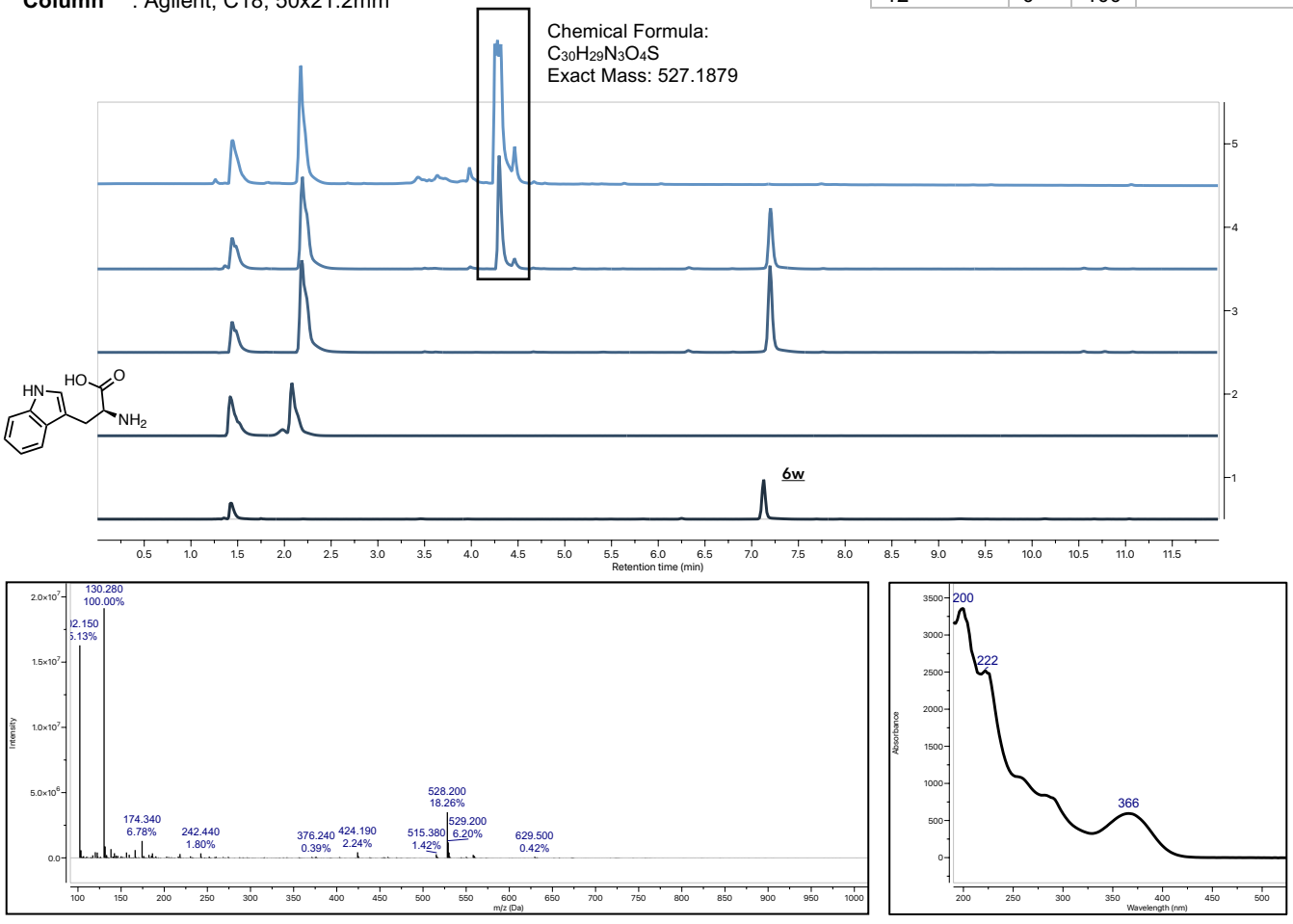


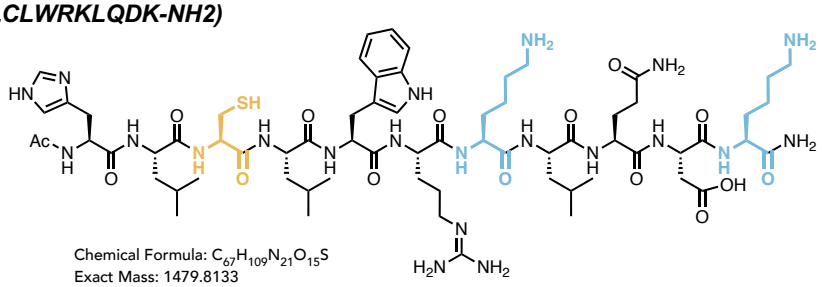
Figure S48: UV trace and corresponding LRMS from HPLC analysis of the crude reaction mixture (entry 5). Blank runs of L-Trp (entry 2) and **6w** (entry 1). Chromatogram observed at 230nm. Peak at the solvent front is DMSO as observed at 230nm Insert: UV excitation profile of **8**.

Synthesis of linear peptides 11-21

General Procedure for Solid Phase Peptide Synthesis:

Linear peptides were synthesized on Gyros Protein Technologies, PurePepTM Chorus on Fmoc-Rink-Amide MBHA resin (Loading: 0.35mmol/g). Fmoc-AA-OH solutions were prepared as 0.3M solutions in 0.3M Oxyma/DMF and stored as stock solution to be used in multiple syntheses. All amino acid couplings were carried out with Fmoc-AA-OH (7.5 equiv.), Oxyma (7.5 equiv.), and DIC (15 eq.) in DMF, under N_2 flow at 50ºC for 5 minutes, while agitated at 350 RPM. Following this linear peptide assembly, N-terminus acetylation was done with a mixture of Ac_2O:collidine:EtOAc (1:2:2, v/v/v), mixed under N_2 flow at room temperature for 20 minutes. Global deprotection and resin cleavage was done in TFA:TIPS:H_2O (95:2.5:2.5, v/v/v) at room temperature for 3 hours, while resin is mixed under N_2 flow and gently agitated at 150 RPM. Crude peptide was then obtained by triturating the TFA mixture, achieved by adding this solution dropwise into cold diethyl ether. Precipitate was centrifuged and washed successively with fresh diethyl ether, repeated three times, then dissolved in 1:1 H2O/MeCN, and lyophilized. Dry crude peptide was then purified via preparative HPLC using optimized methods detailed in each dataset. Unless otherwise stated, all peptides were purified with H_2O and MeCN 0.1% TFA as the mobile phases. Purified material was then lyophilized and quantified via UV spectroscopy, where linear peptides **11-20** were quantified at 280nm ε = 7100 cm^{-1} M^{-1} and FIICk peptides were quantified at 365nm (ε = 14300 cm^{-1} M^{-1}).

Synthesis of 11 (Ac-HLCLWRKLQDK-NH2)



Linear peptide **11** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **11** (22µmol, 55%), to be used for subsequent FIICk reaction.

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	80	20	25
7	55	45	
8	0	100	

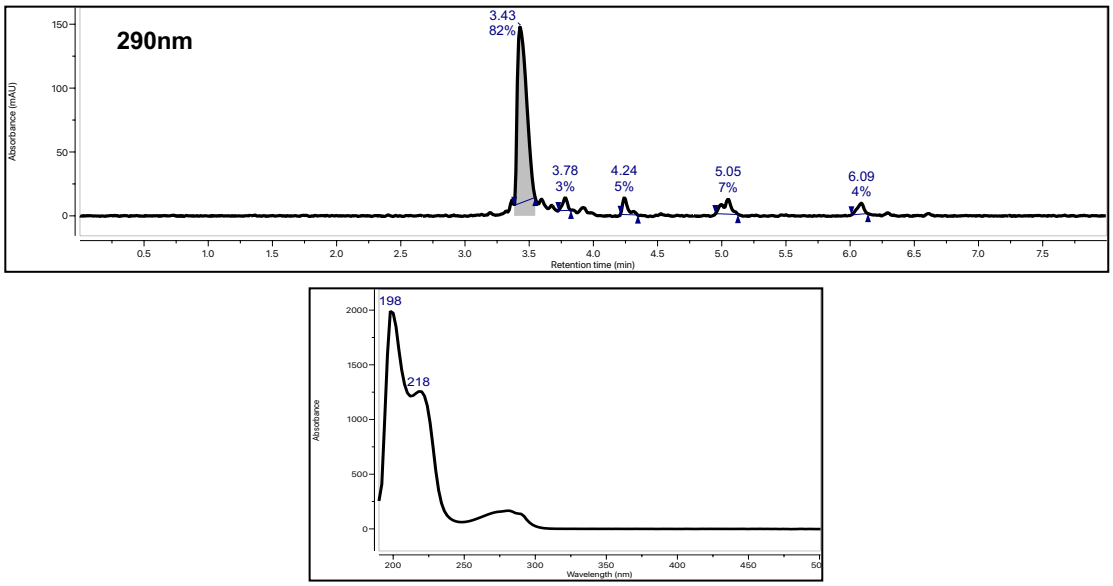
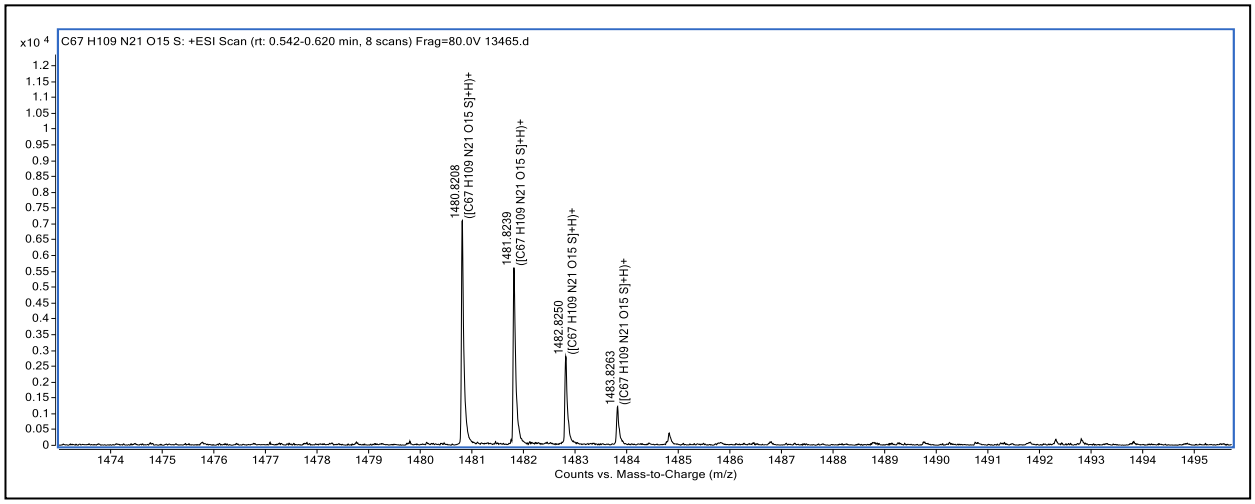


Figure S49A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile.



HRMS of **11**.
Calculated $([C_{67}H_{109}N_{21}O_{15}S]+H)^+ = 1480.8233$
Found $([C_{67}H_{109}N_{21}O_{15}S]+H)^+ = 1480.8208$

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

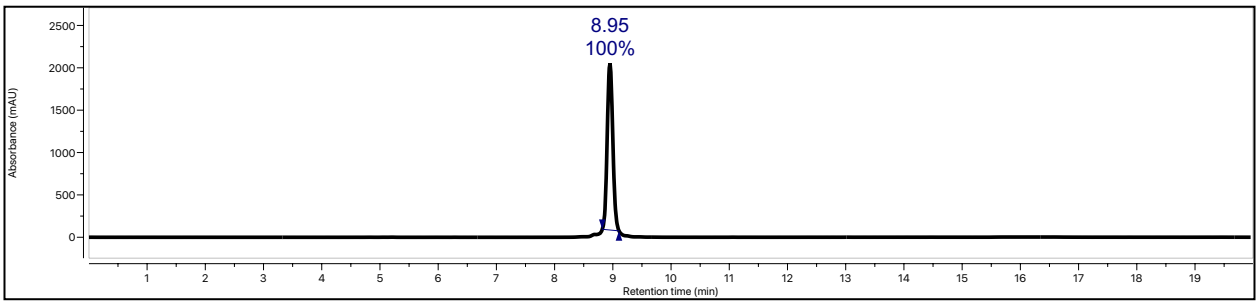
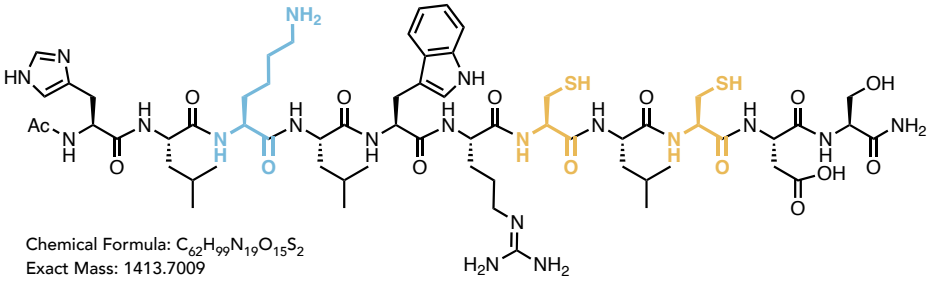


Figure S49B: LC trace of purified compound 11. Observed at 230nm.

Synthesis of 12 (Ac-HLKLWRCLCDS-NH_2)

Linear peptide **12** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **12** (20µmol, 50%), to be used for subsequent FIICk reaction.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	80	20	30
11	60	40	
12	0	100	

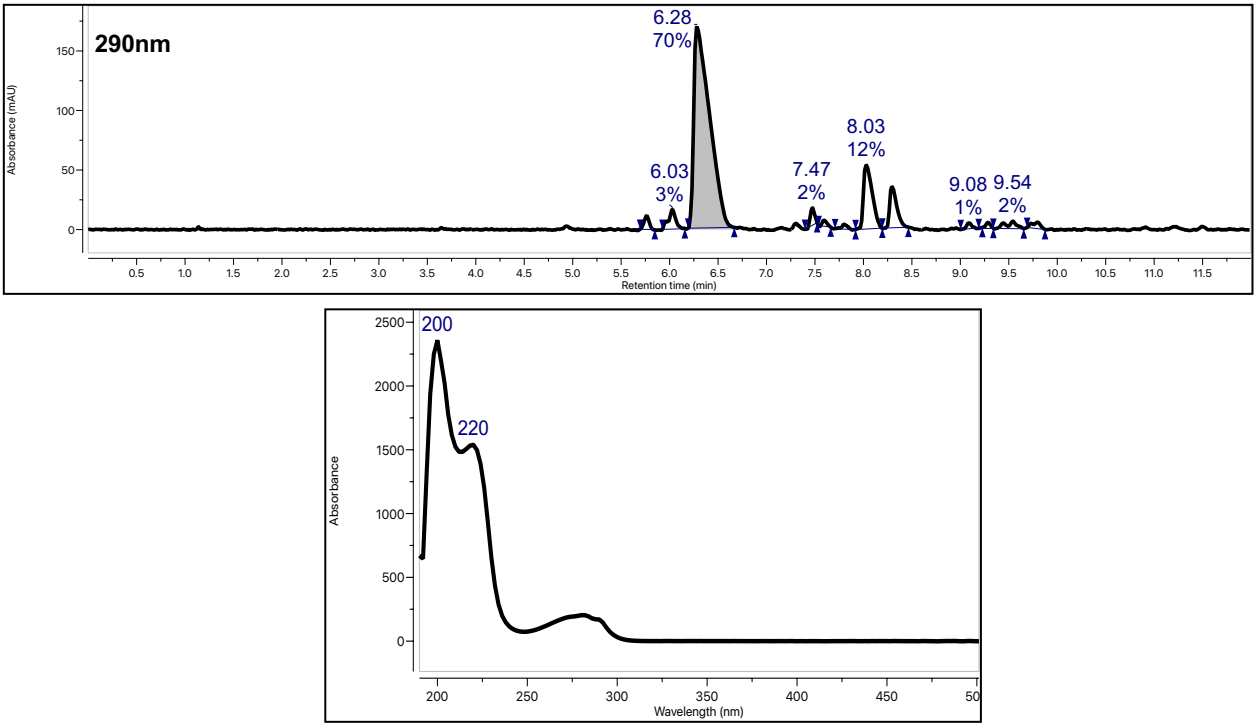


Figure S50A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile.

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

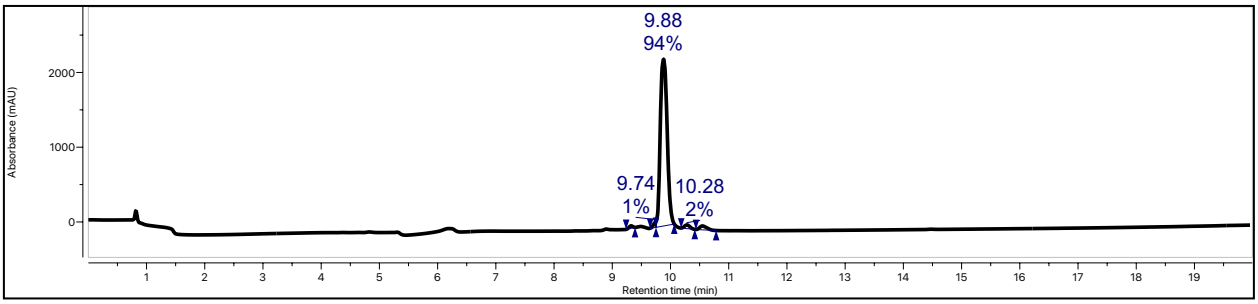
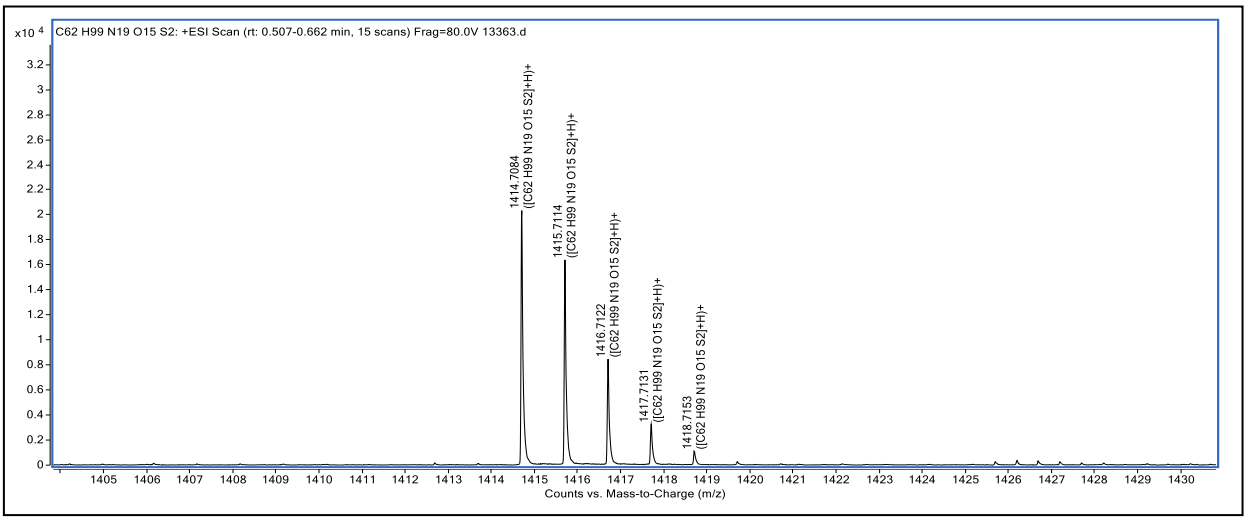


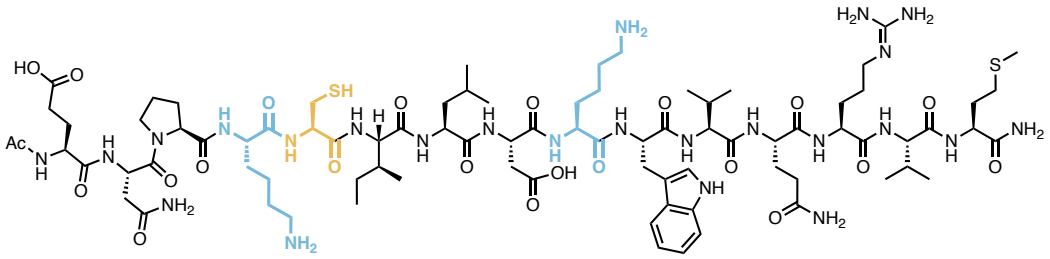
Figure S50B: LC trace of purified compound **12**. Observed at 230nm



HRMS of **12**
Calculated $([C_{62}H_{99}N_{19}O_{15}S_2]+H)^+ = 1414.7088$
Found $([C_{62}H_{99}N_{19}O_{15}S_2]+H)^+ = 1414.7084$

Synthesis of 13 (Ac-ENPKCILDKWVQRVM-NH$_2$)

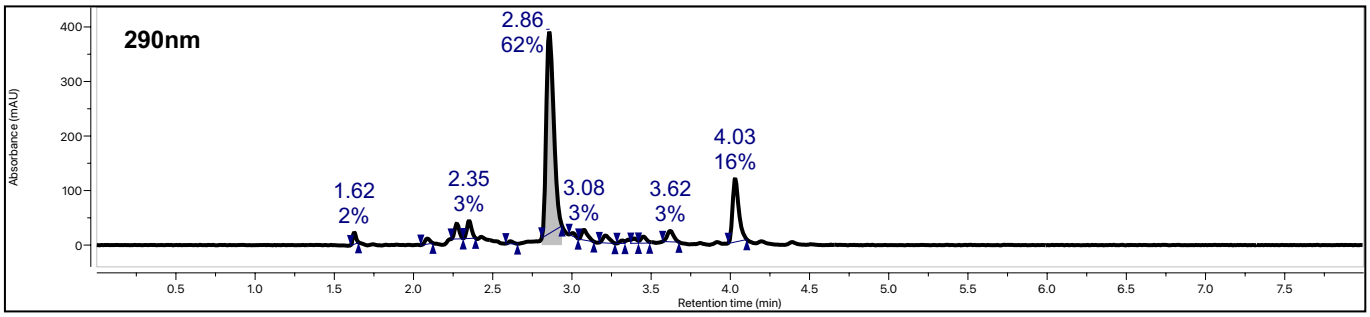
Linear peptide **13** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **13** (13µmol, 32%), to be used for subsequent FIICk reaction.



Chemical Formula: $C_{84}H_{138}N_{24}O_{22}S_2$
Exact Mass: 1898.9859

Time (min)	A%	B%	Flow (mL/min)
0	70	30	20
10	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)



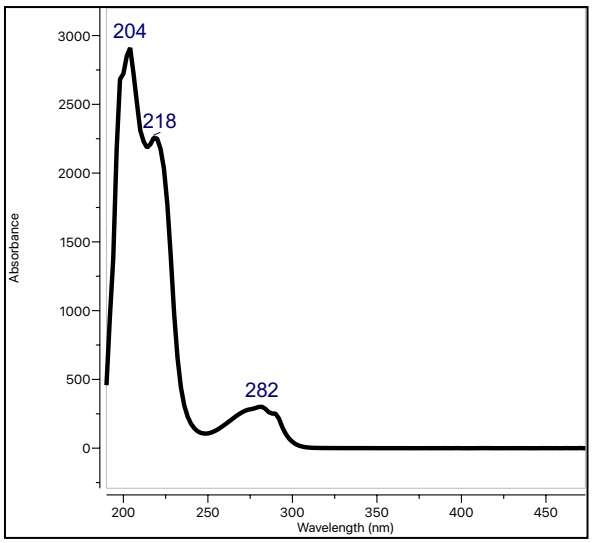


Figure S51A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile.

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

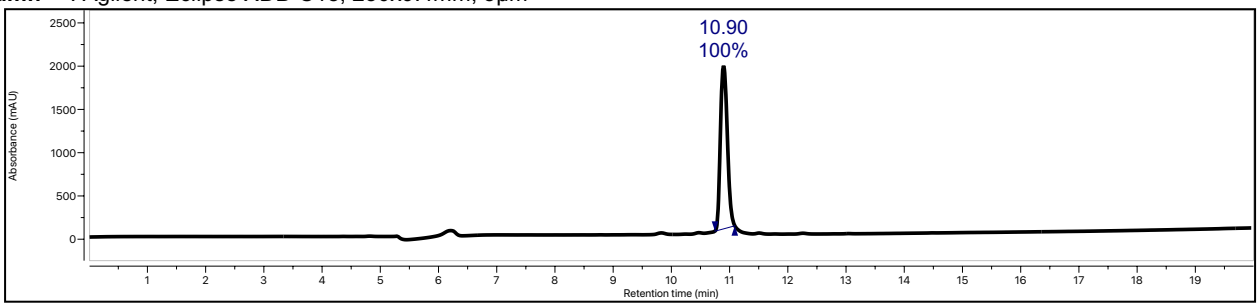
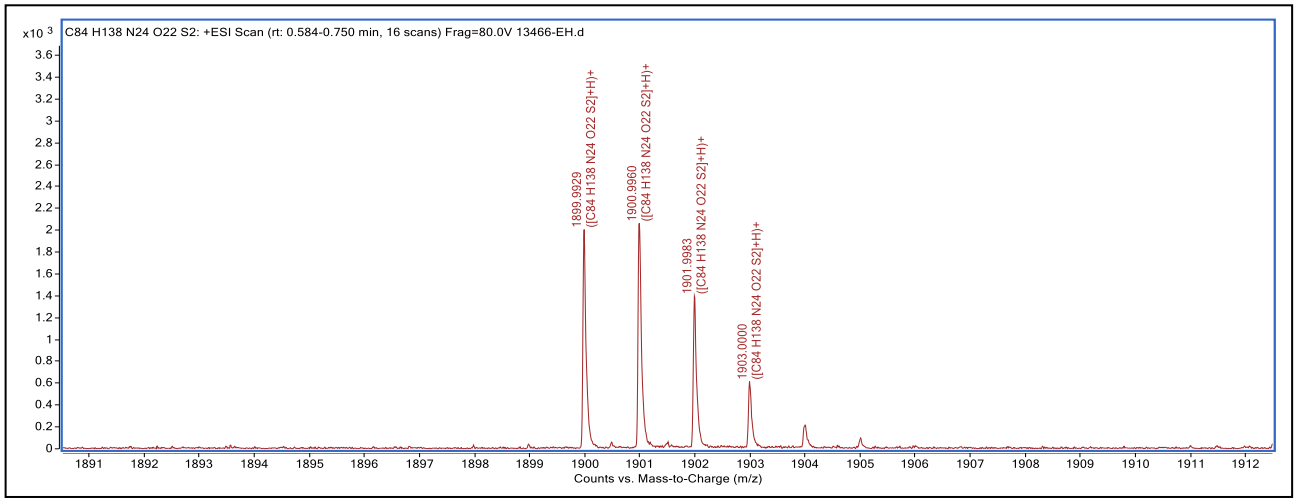


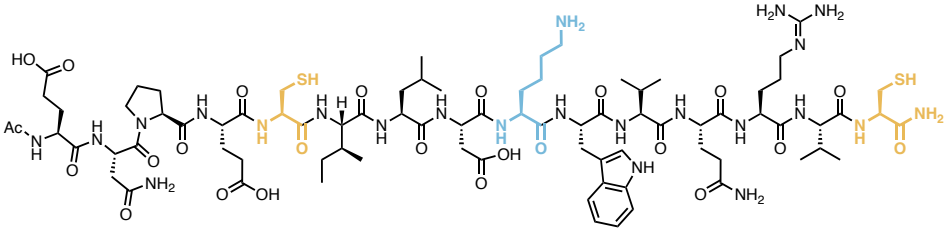
Figure S51B: LC trace of purified compound **13**. Observed at 230nm.



HRMS of **13**
Calculated $([C_{84}H_{138}N_{24}O_{22}S_2]+H)^+ = 1899.9937$
Found $([C_{84}H_{138}N_{24}O_{22}S_2]+H)^+ = 1899.9929$

Synthesis of 14 (Ac-ENPECILDKWVQRVC-NH_2)

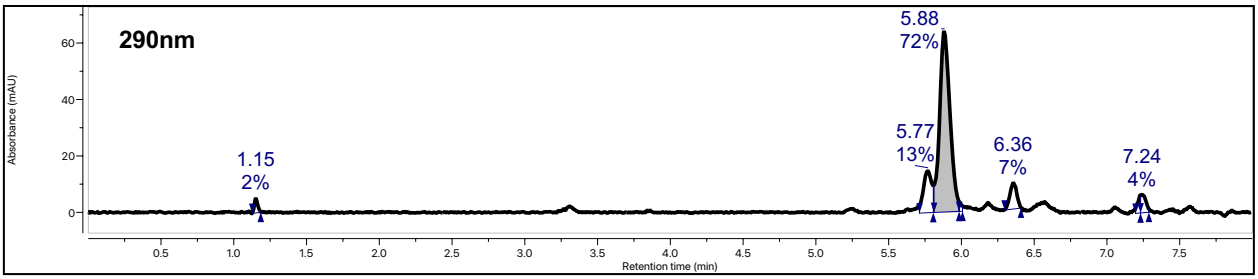
Linear peptide **14** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **14** (11µmol, 27%), to be used for subsequent FIICk reaction.



Chemical Formula: $C_{81}H_{129}N_{23}O_{24}S_2$
Exact Mass: 1871.9022

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	70	30	30
7	55	45	
8	0	100	



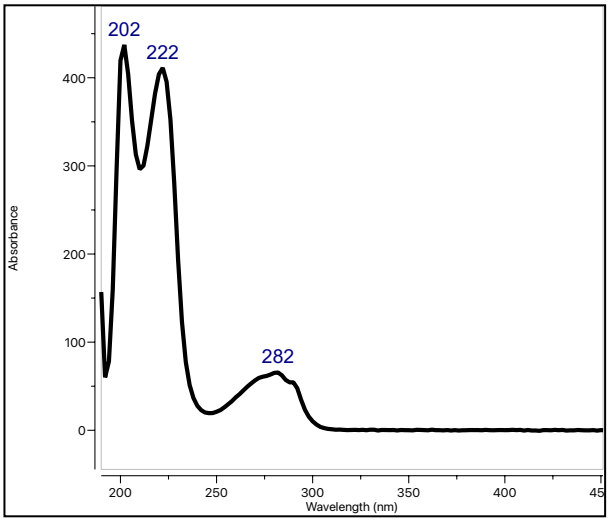


Figure S52A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile.

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

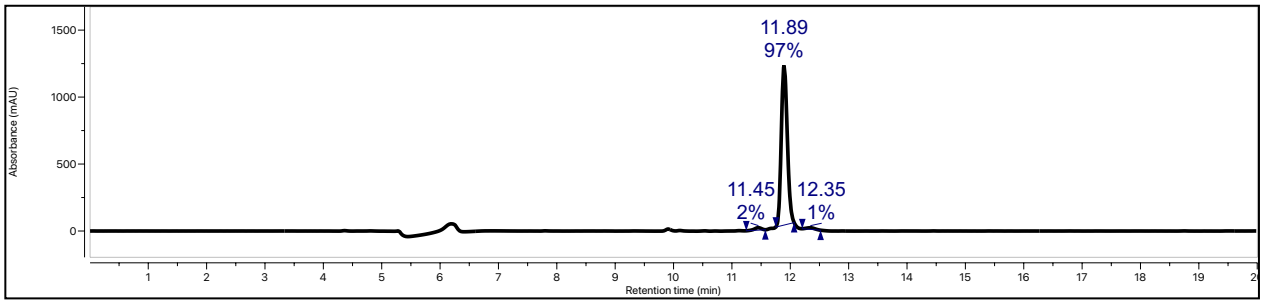
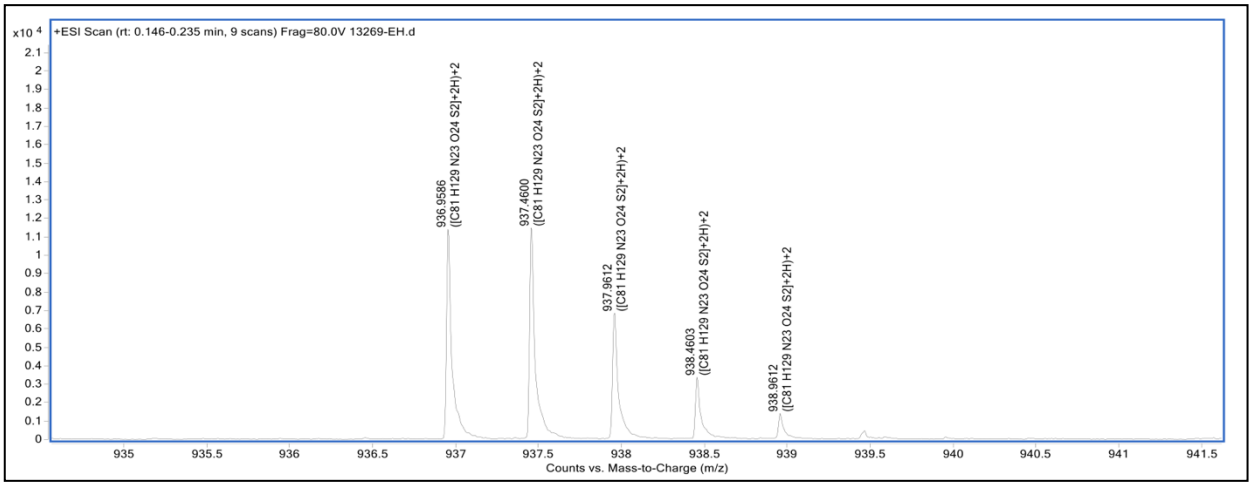


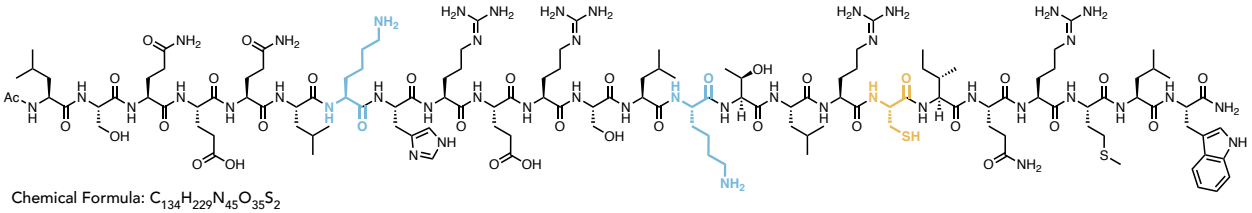
Figure S52B: LC trace of purified compound **14**. Observed at 230nm.



HRMS of **14**
Calculated $([C_{81}H_{129}N_{23}O_{24}S_2]+2H)/2 = 936.9589$
Found $([C_{81}H_{129}N_{23}O_{24}S_2]+2H)/2 = 936.9586$

Synthesis of 15 (Ac-LSQEQLKHRERSLKTLRCIQRMLW-NH$_2$)

Linear peptide **15** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **14** (7µmol, 18%), to be used for subsequent FIICk reaction.



Chemical Formula: $C_{134}H_{229}N_{45}O_{35}S_2$
Exact Mass: 3092.6964

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	70	30	25
6	50	50	
7	0	100	

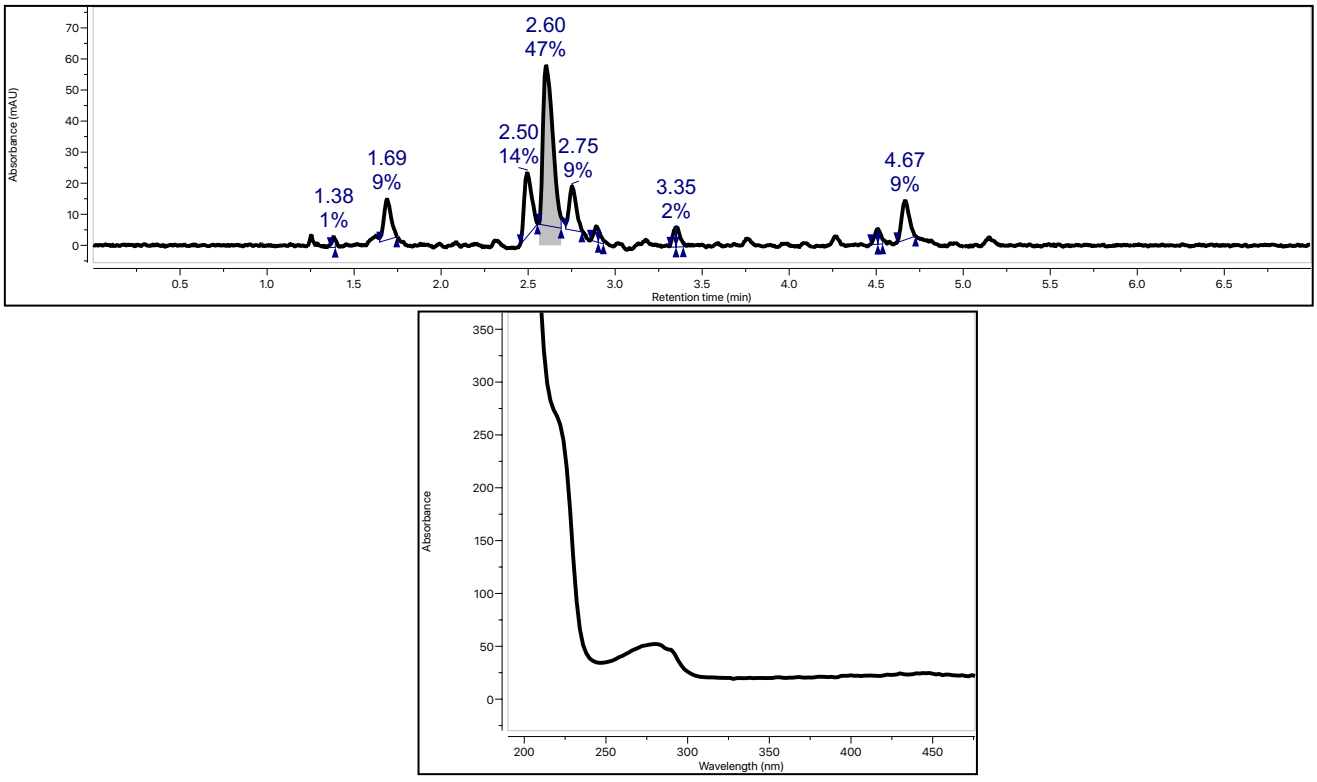


Figure S53A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

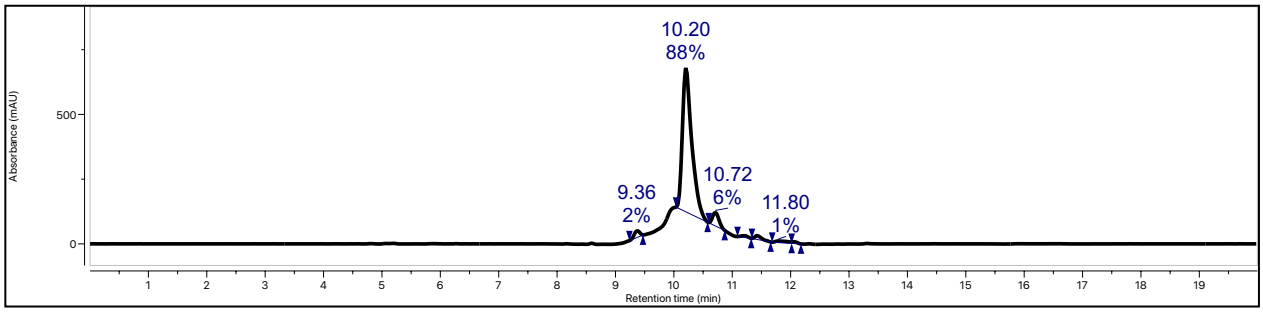
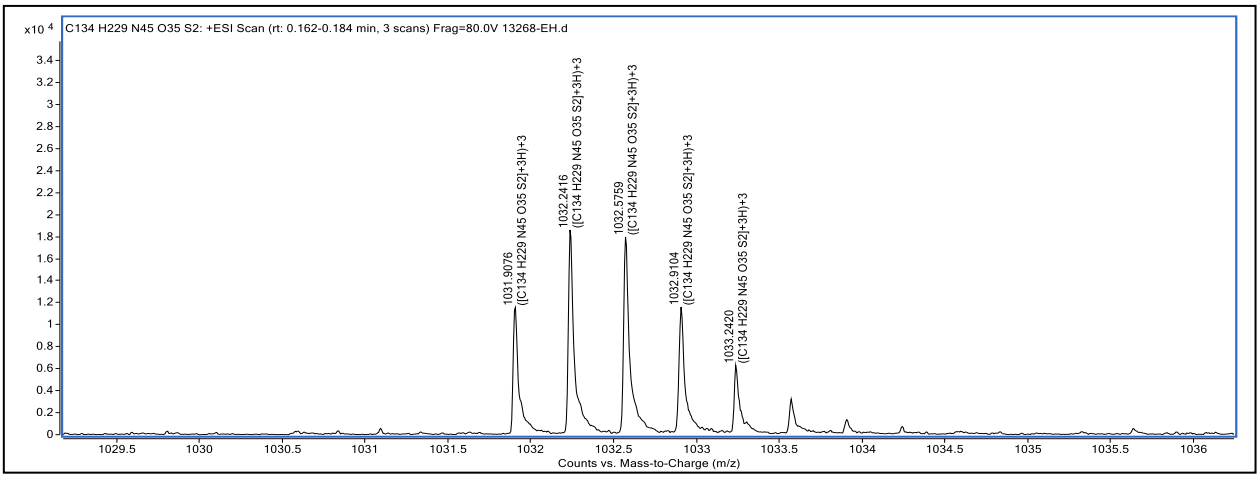


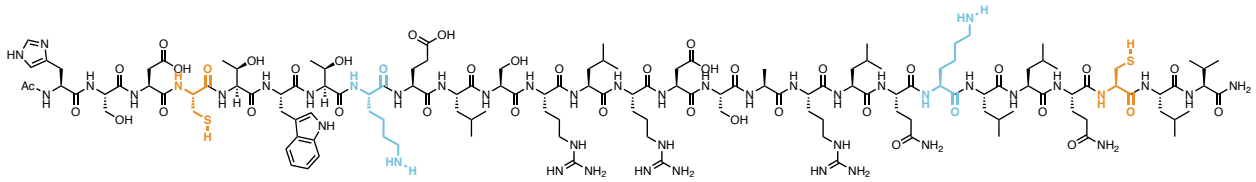
Figure S53B: LC trace of purified **15.** Observed at 230nm.



HRMS of **15**
Calculated $([C_{134}H_{229}N_{45}O_{35}S_2]+3H)/3 = 1031.9066$
Found $([C_{134}H_{229}N_{45}O_{35}S_2]+3H)/3 = 1031.9076$

Synthesis of 16 (Ac-LSQEQLKHRERSLKTLRCIQRMLW-NH$_2$)

Linear peptide **16** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **16** (5.4µmol, 13%), to be used for subsequent FIICk reaction.



Chemical Formula: $C_{139}H_{234}N_{44}O_{41}S_2$
Exact Mass: 3239.7020

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	70	30	25
6	50	50	
7	0	100	

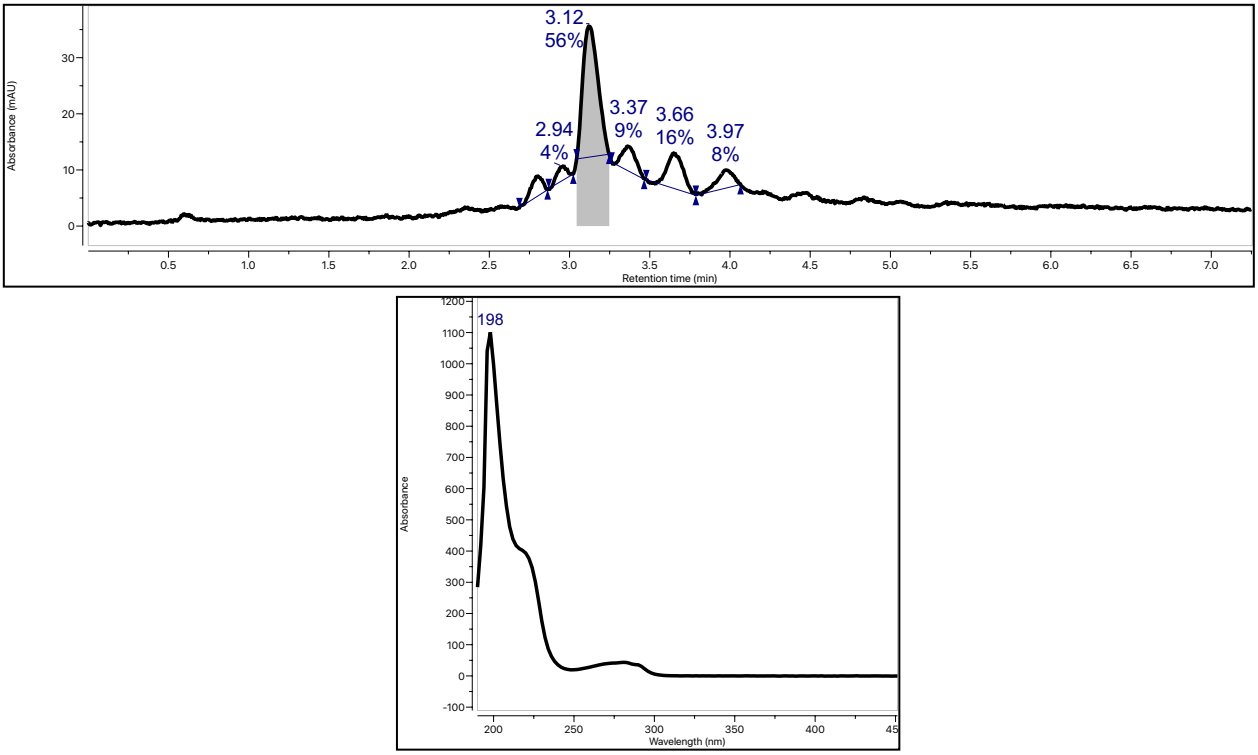
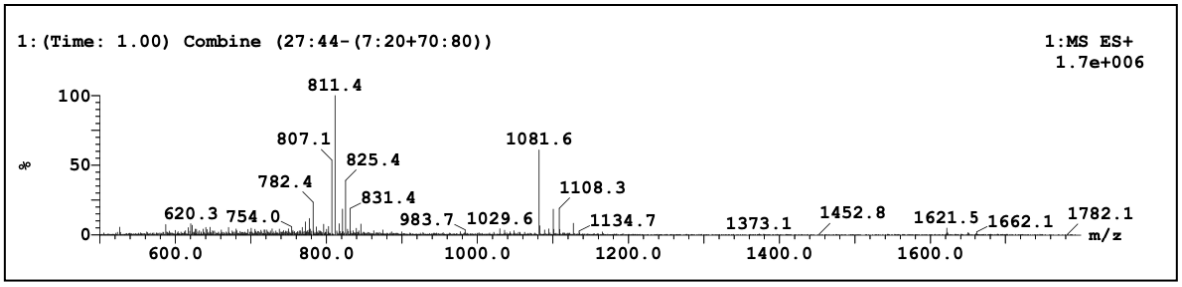


Figure S54A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile and HRMS of the major peak (t=3.12min).



LRMS of **16**
Calculated $([C_{139}H_{234}N_{44}O_{41}S_2]+3H)/3 = 1080.9$
Found $([C_{139}H_{234}N_{44}O_{41}S_2]+3H)/3 = 1081.6$

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

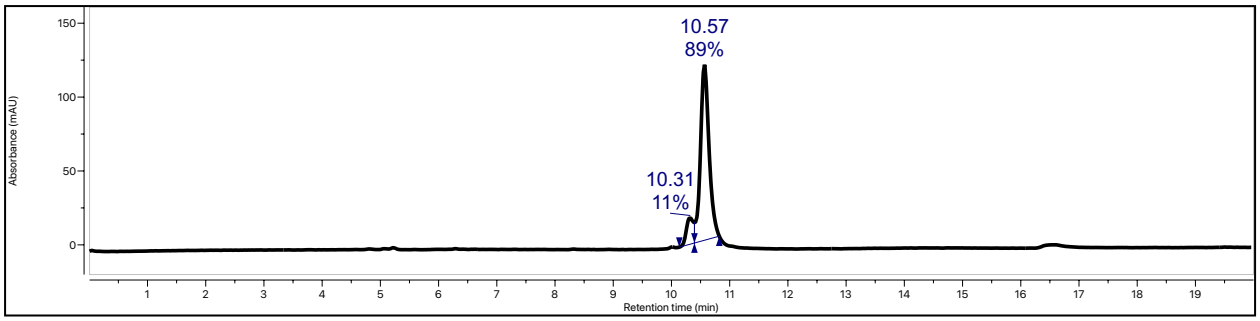
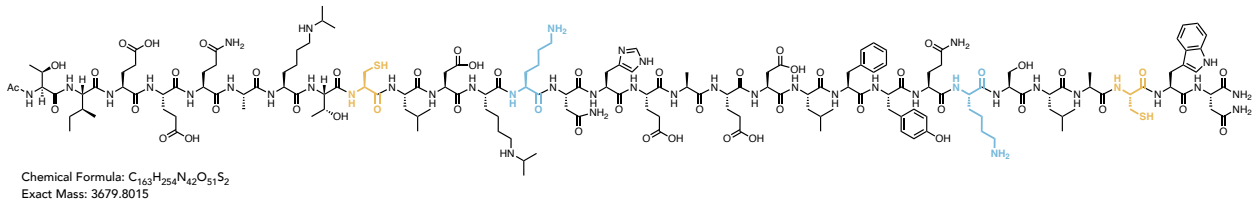


Figure S54B: LC trace of purified **16.** Observed at 230nm.

Synthesis of 17 (Ac-TIEEQAkTCLDkKNHEAEDLFYQKSLACWN-NH_2)

Linear peptide **17** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **17** (8.5µmol, 21%), to be used for subsequent FIICk reaction.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	95	5	30
3.50	65	35	
5.00	0	100	

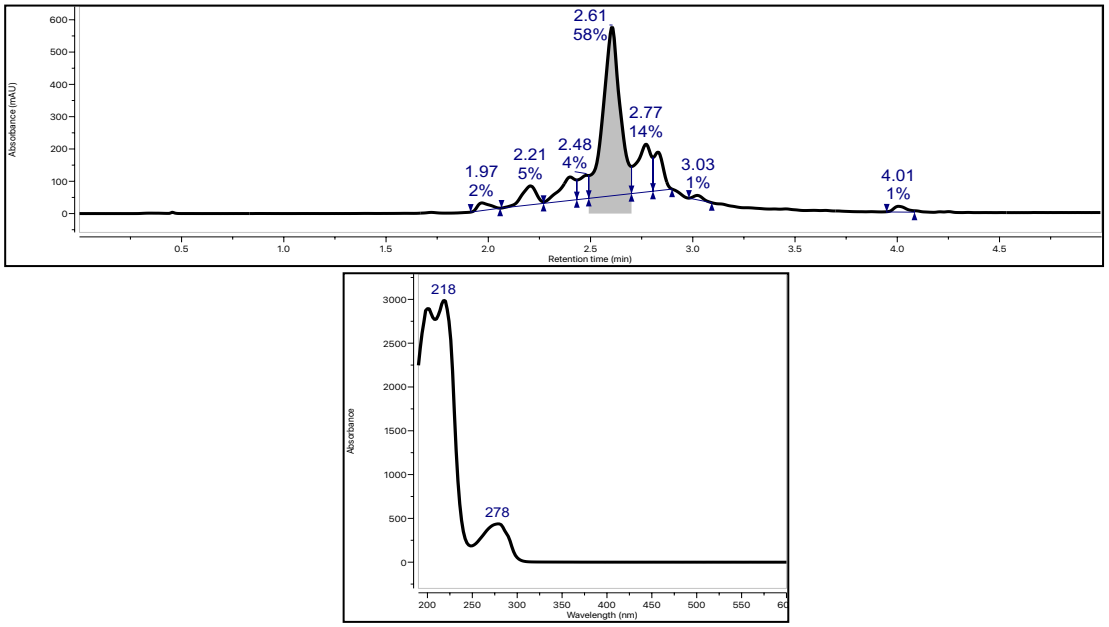


Figure S55A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

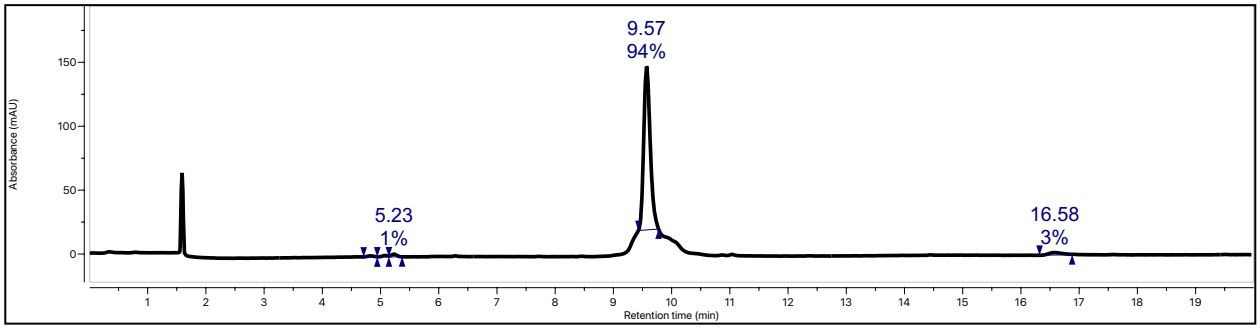
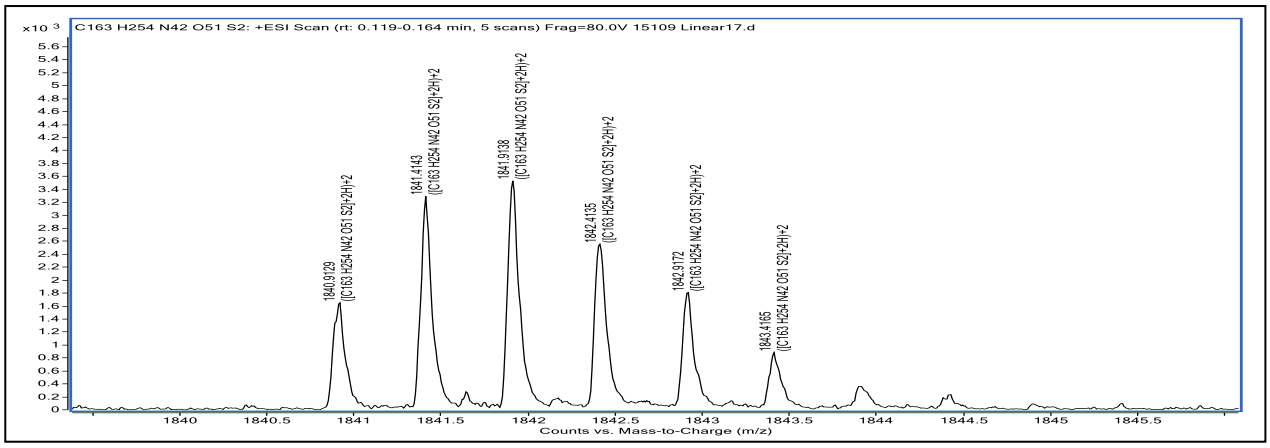


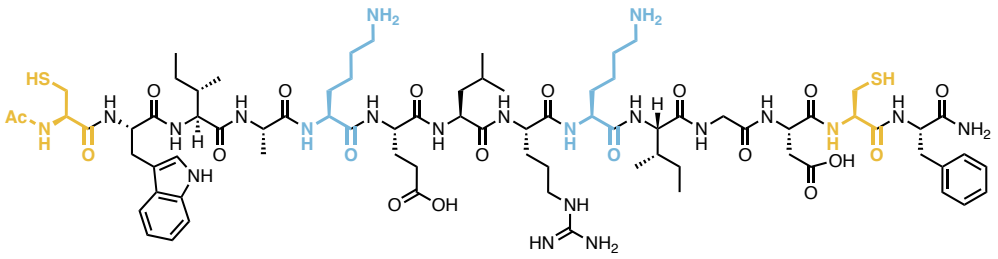
Figure S55B: LC trace of purified **17.** Observed at 230nm.



HRMS of **17**
Calculated $([C_{163}H_{254}N_{42}O_{51}S_2]+2H)/2 = 1840.9108$
Found $([C_{163}H_{254}N_{42}O_{51}S_2]+2H)/2 = 1840.9129$

Synthesis of 18 (Ac-CWIAKELRKIGDCF-NH_2)

Linear peptide **18** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **18** (14µmol, 35%), to be used for subsequent FIICk reaction.



Chemical Formula: $C_{78}H_{123}N_{21}O_{19}S_2$
Exact Mass: 1721.8746

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	70	30	25
6	50	50	
7	0	100	

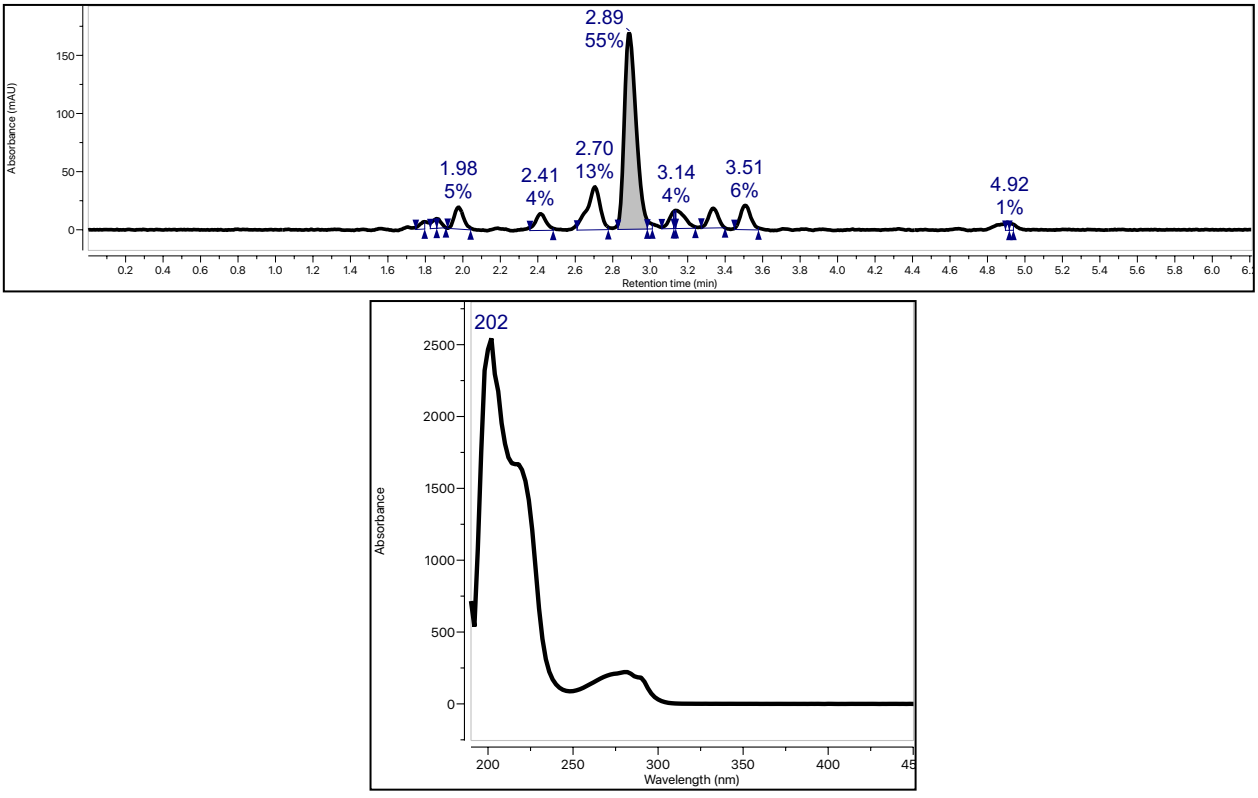


Figure S56A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile.

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

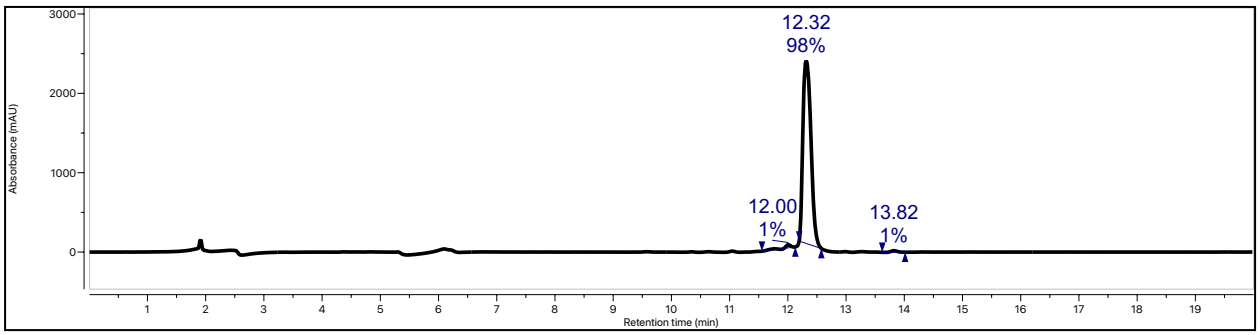
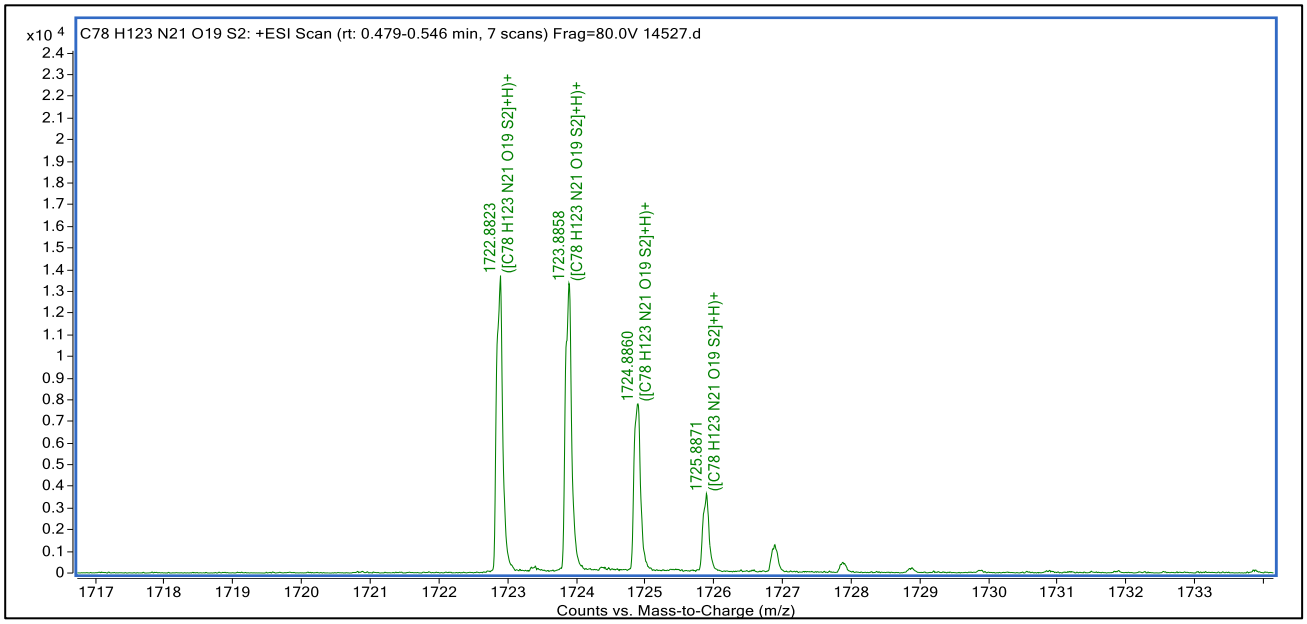


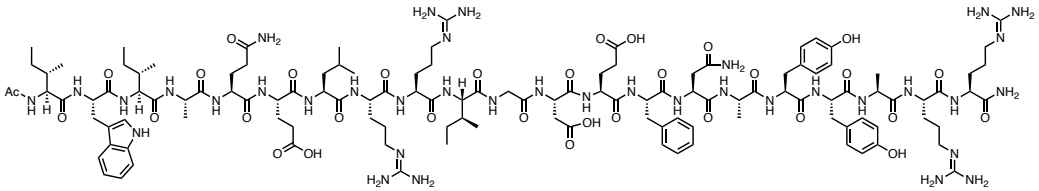
Figure S56B: LC trace of purified **18**. Observed at 230nm.



HRMS of **18**
Calculated $([C_{78}H_{123}N_{21}O_{19}S_2]+H)^+ = 1722.8824$
Found $([C_{78}H_{123}N_{21}O_{19}S_2]+H)^+ = 1722.8823$

Synthesis of 20 Negative Control (Ac-IWIAQELRRIGDEFNAYYARR-NH_2)

Linear peptide **20** was synthesized according to the general procedure for solid phase peptide synthesis, using 224mg Rink Amide MBHA resin (80μmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **20** (63mg, 23.5μmol, 30%), which served as the negative control for Jurkat cell viability assay.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm, 5μm

Time (min)	A%	B%	Flow (mL/min)
0	60	40	20
10	0	100	

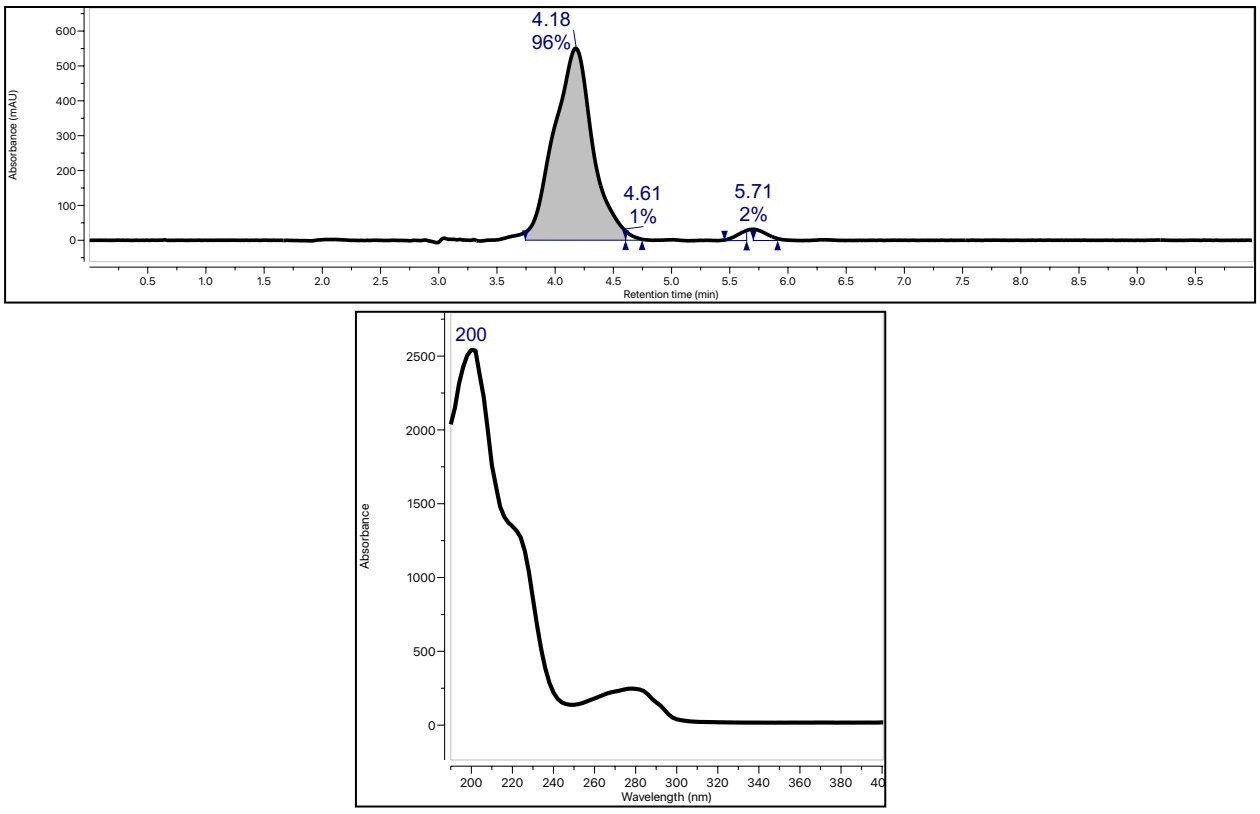


Figure S57A: Crude HPLC trace of **19** observed at 290nm and the corresponding UV excitation profile.

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	90	10	2
15	40	60	
20	0	100	
25	90	10	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

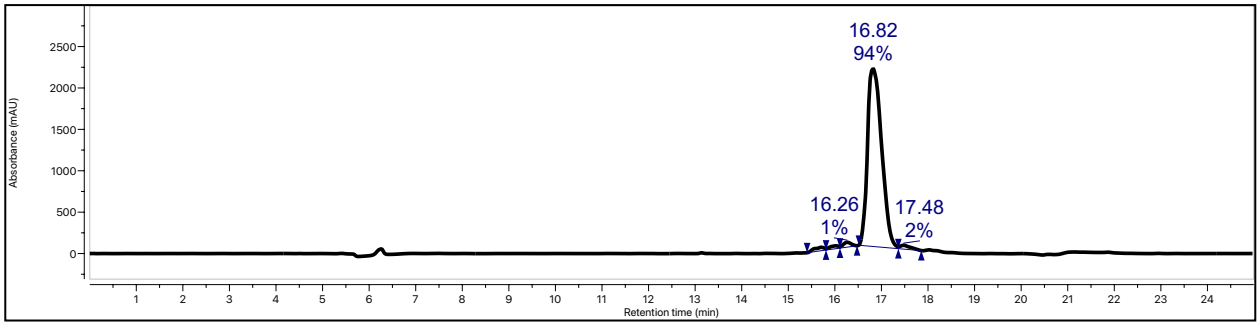
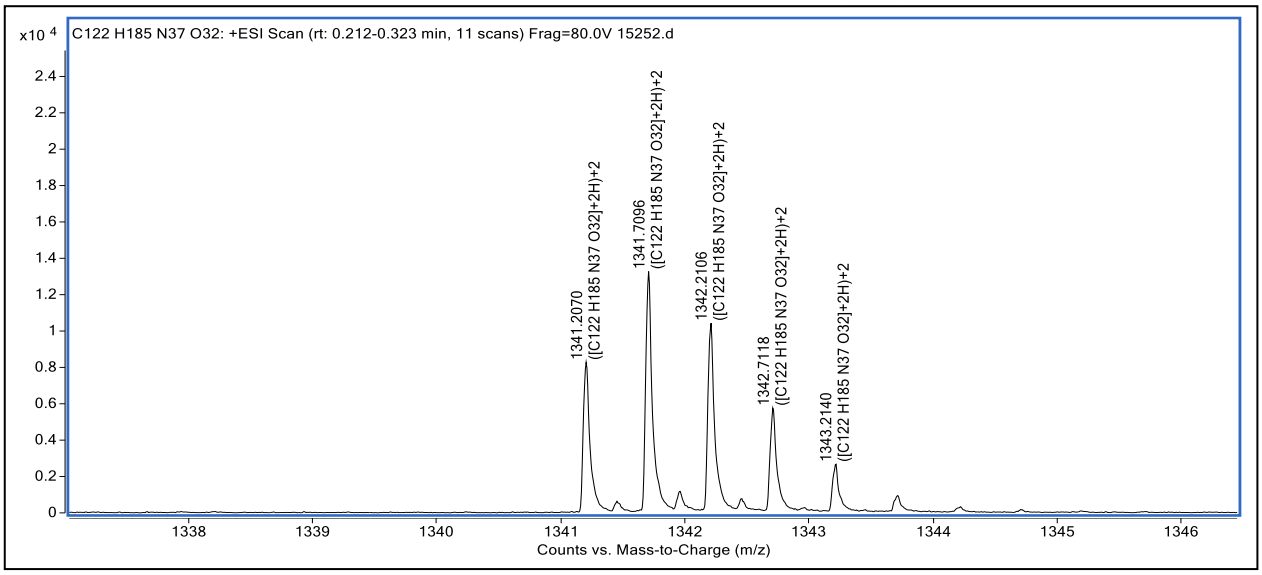


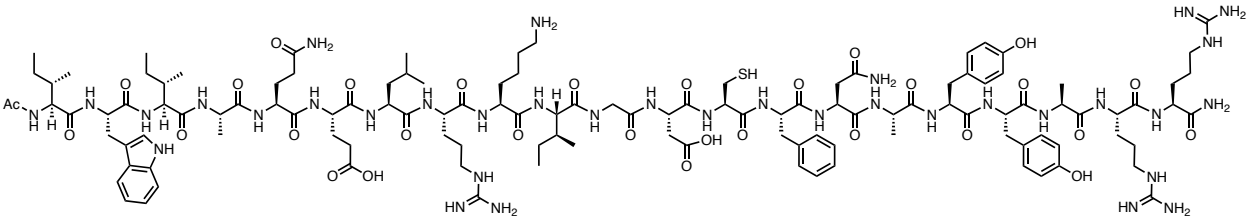
Figure S57B: LC trace of purified **20 (Negative Control)**. Observed at 230nm



HRMS of **20 (Negative Control)**
Calculated $([C_{122}H_{185}N_{37}O_{32}S]+2H)/2 = 1314.2071$
Found $([C_{122}H_{185}N_{37}O_{32}S]+2H)/2 = 1314.2070$

Synthesis of 20' (Ac-IWIAQELRKIGDCFNAYYARR-NH$_2$)

Linear peptide **20'** (FIICk precursor) was synthesized according to the general procedure for solid phase peptide synthesis, using 224mg Rink Amide MBHA resin (80µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **20'** (15.2µmol, 19%), which was then subsequently treated with **6w** to obtain a FIICk-stapled helix.



Chemical Formula: $C_{120}H_{183}N_{35}O_{30}S$
Exact Mass: 2626.35908
Molecular Weight: 2628.05900

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	60	40	20
10	0	100	

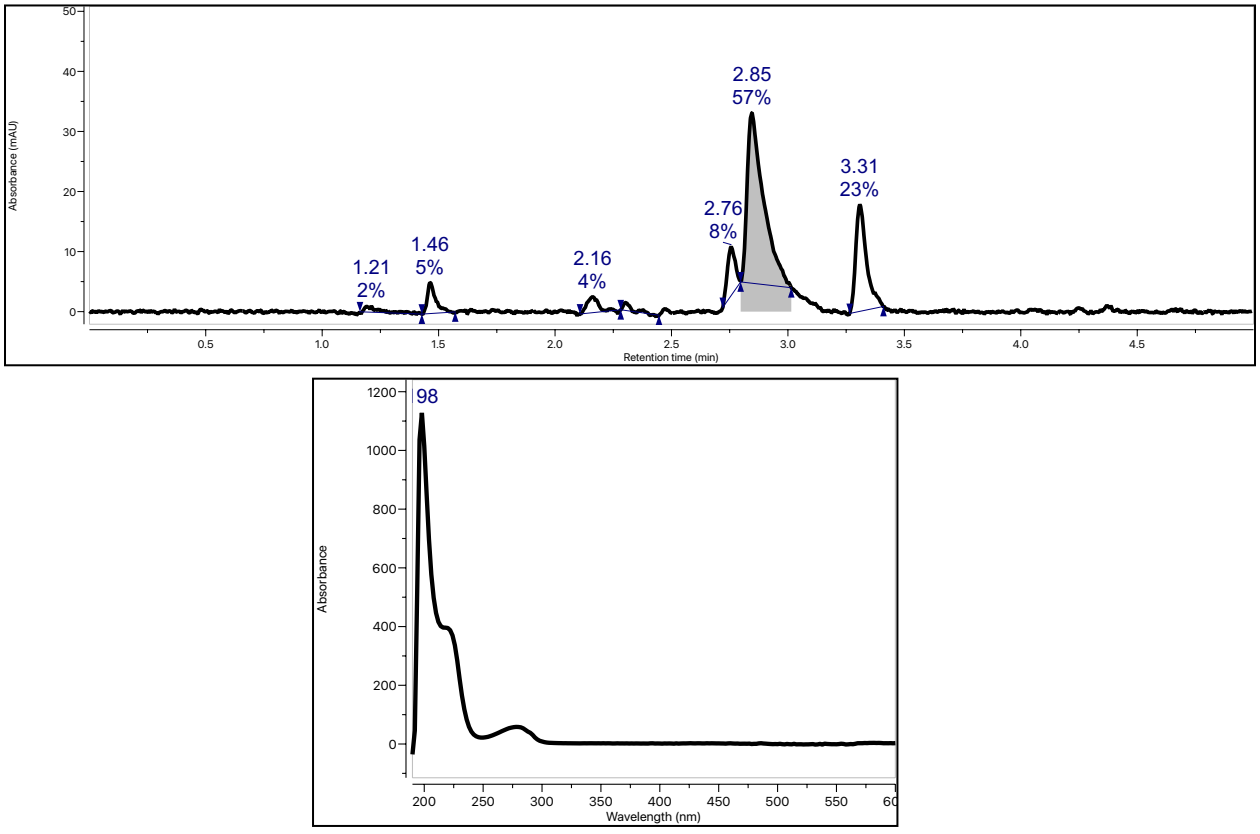


Figure S58A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile.

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	90	10	2
15	40	60	
20	0	100	
25	90	10	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

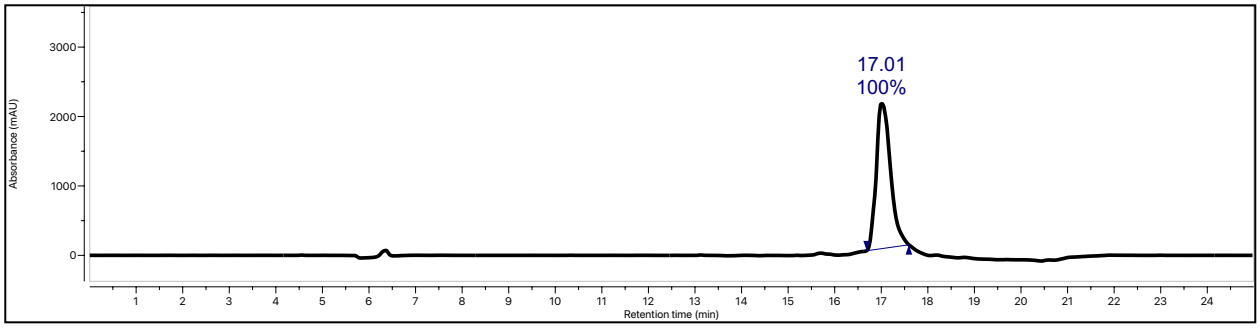
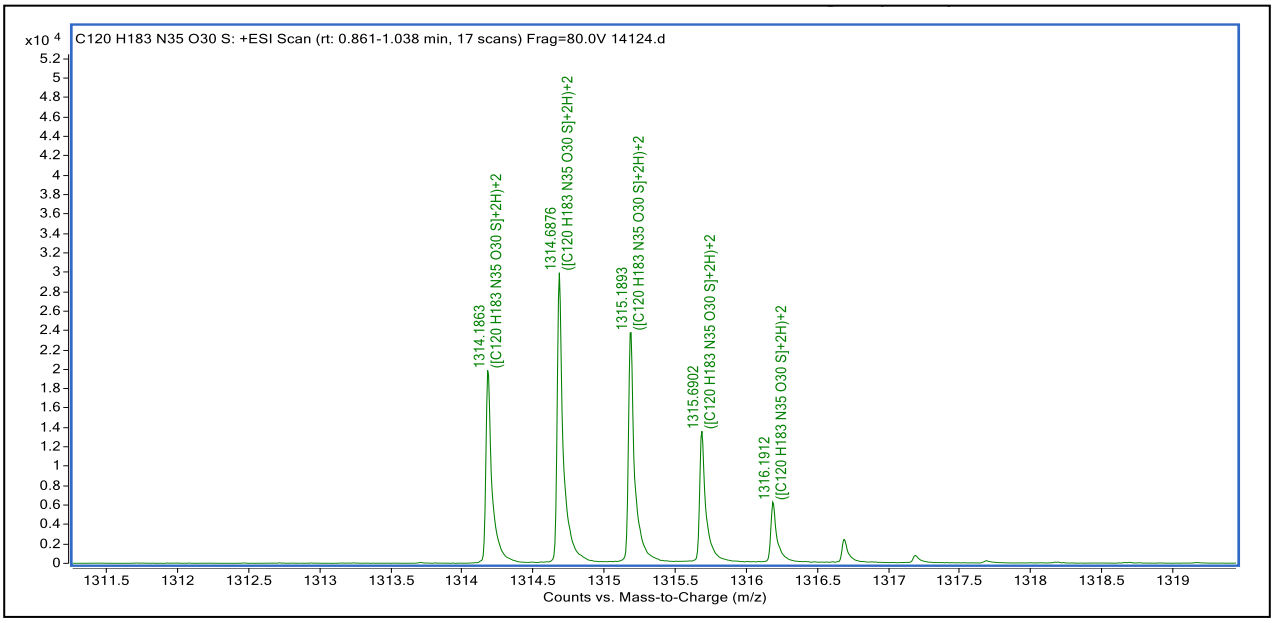


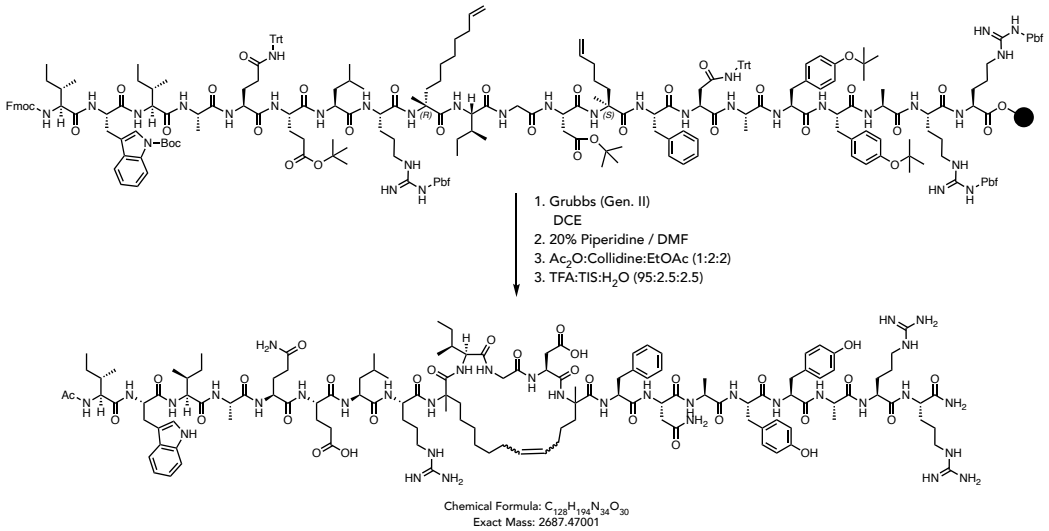
Figure S58B: LC trace of purified **20'**. Observed at 230nm



HRMS of **20'**
Calculated $([C_{120}H_{183}N_{35}O_{30}S]+2H)/2 = 1314.1873$
Found $([C_{120}H_{183}N_{35}O_{30}S]+2H)/2 = 1314.1863$

Synthesis of 20a (Ac-IWIAQELRR8IGDS5FNAYYARR-NH$_2$)

Linear peptide **20a** was synthesized according to the general procedure for solid phase peptide synthesis, using 224mg Rink Amide MBHA resin (80µmol scale synthesis). Following completion of SPPS, to the reaction vessel containing the resin-bound peptide was added Grubbs Gen. II catalyst (16mg) and 5mL DCE. This mixture was agitated at 350RPM, under N_2 flow, at 50ºC, for 2 hours. Completion of RCM reaction was then determined via test-cleavage of the peptide, and the Grubbs RCM reaction was repeated two more times. Once the unstapled starting material was fully consumed, the resin-bound peptide was subjected to Fmoc cleavage (20% piperidine/DMF), acetylated (Ac_2O:Collidine:EtOAc), and cleaved from the resin (TFA:TIS:H_2O) following standard protocol. Trituration and HPLC purification of crude material resulted in the isolation of **20a** as white powder (10.8mg, 4µmol, 5%). This peptide served as the positive control for Jurkat cell viability assay.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	60	40	20
10	0	100	

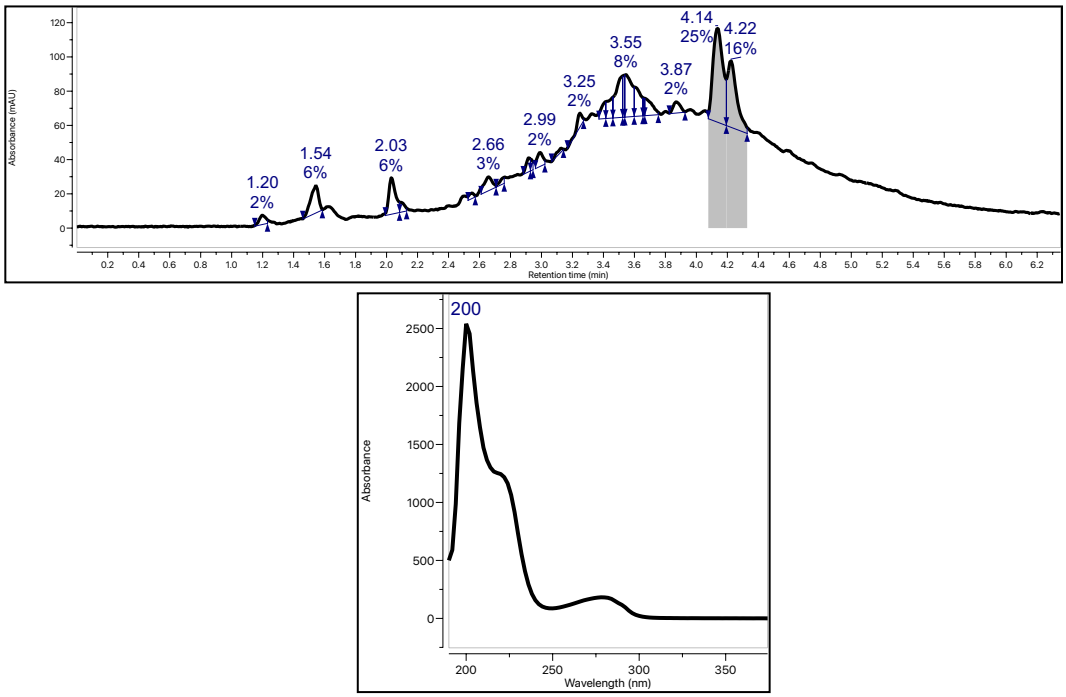


Figure S59A: Prep-HPLC purification of crude **20a,** peaks were collected as time slices due to challenging purification. Peaks highlighted in grey were found to have the desired mass. Observed at 290nm.

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	60	50	2
20	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

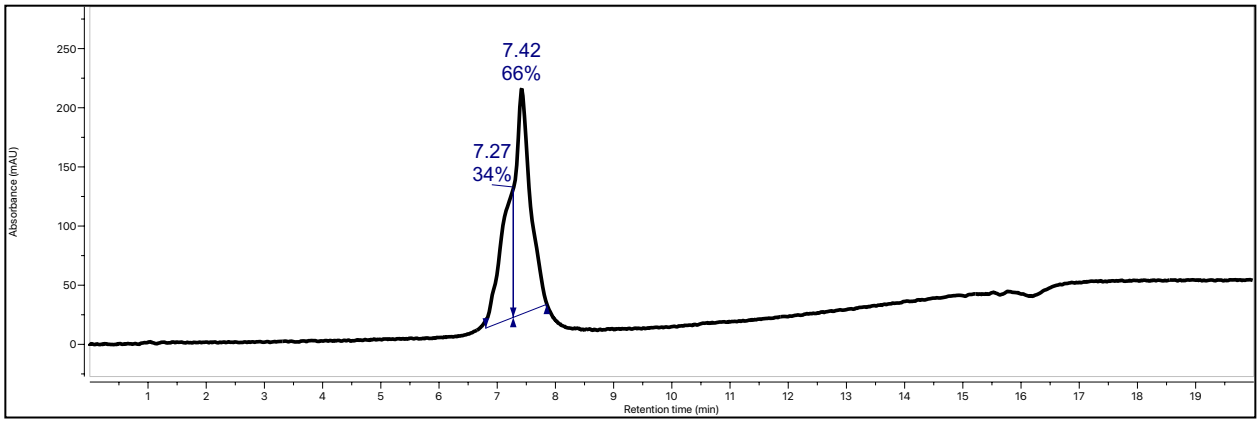
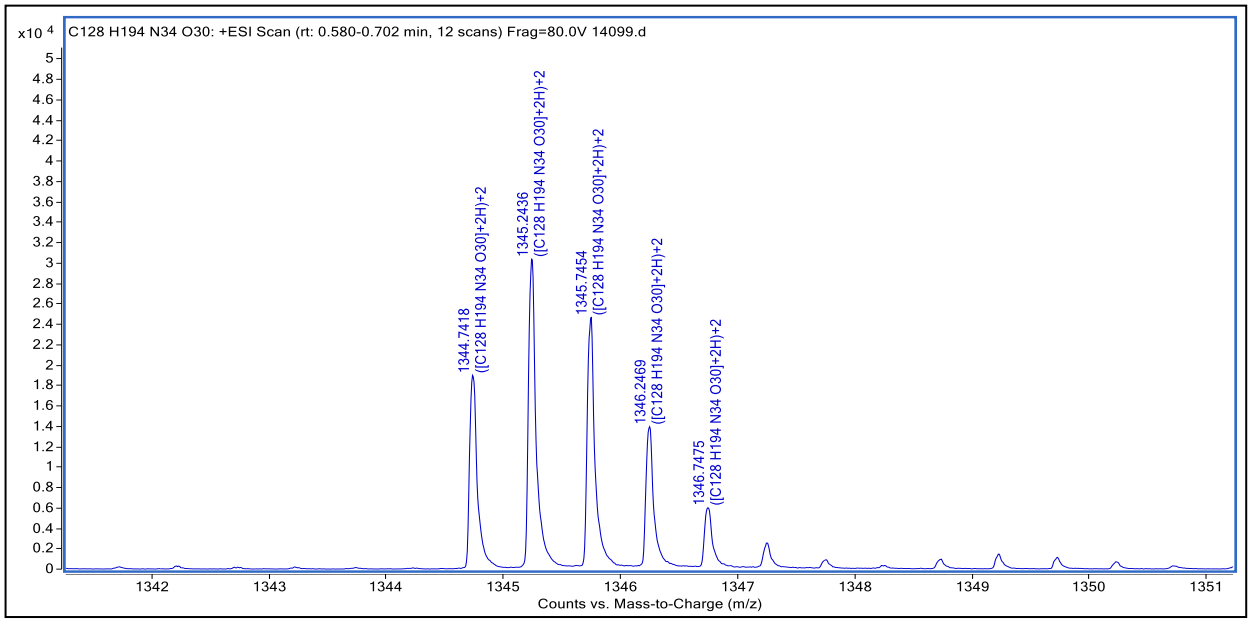


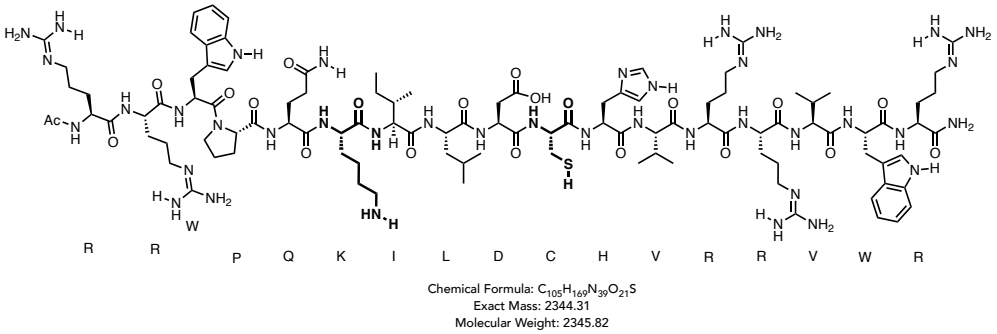
Figure S59B: LC trace of purified compound **20a.** Observed at 230nm.



HRMS of **20a.**
Calculated $([C_{128}H_{194}N_{34}O_{30}]+2H)/2 = 1344.7428$
Found $([C_{128}H_{194}N_{34}O_{30}]+2H)/2 = 1344.7418$

Synthesis of 21 (Ac-RRPQKILDCHVRRVWR-NH2)

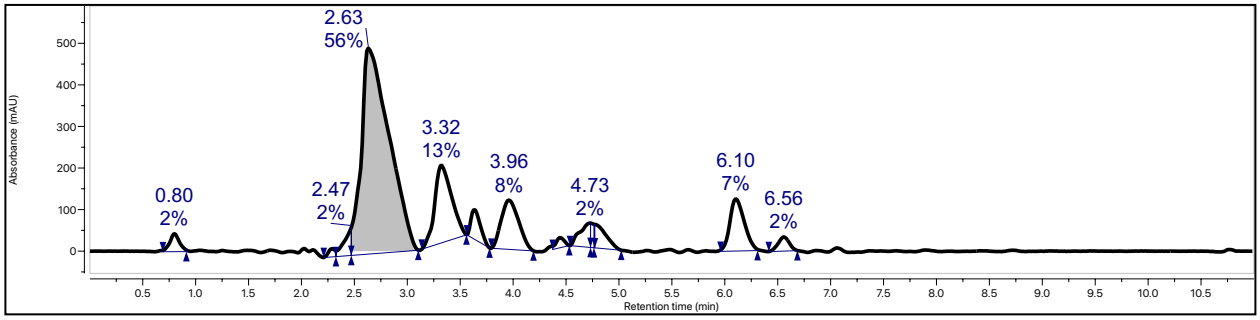
Linear peptide **21** (FIICk peptide precursor) was synthesized according to the general procedure for solid phase peptide synthesis, using 224mg Rink Amide MBHA resin (80µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **21** (24.3µmol, 30%), which was then subsequently treated with **6w** to obtain a FIICk-stapled helix.



Chemical Formula: $C_{105}H_{169}N_{39}O_{21}S$
Exact Mass: 2344.31
Molecular Weight: 2345.82

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	60	40	20
10	0	100	



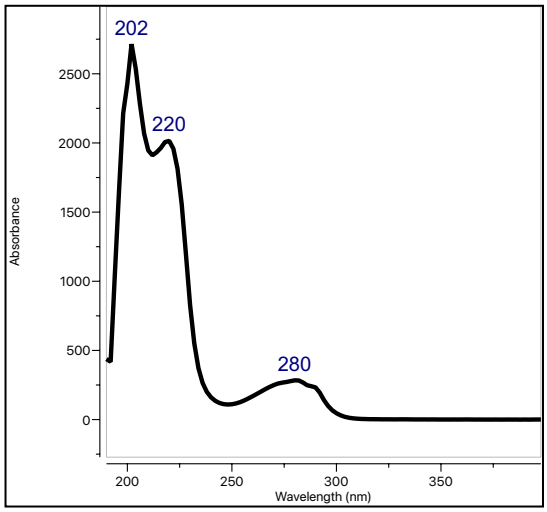


Figure S60A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile and LRMS of the major peak (t=2.63min).

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	2
15	40	60	
20	0	100	
25	90	10	

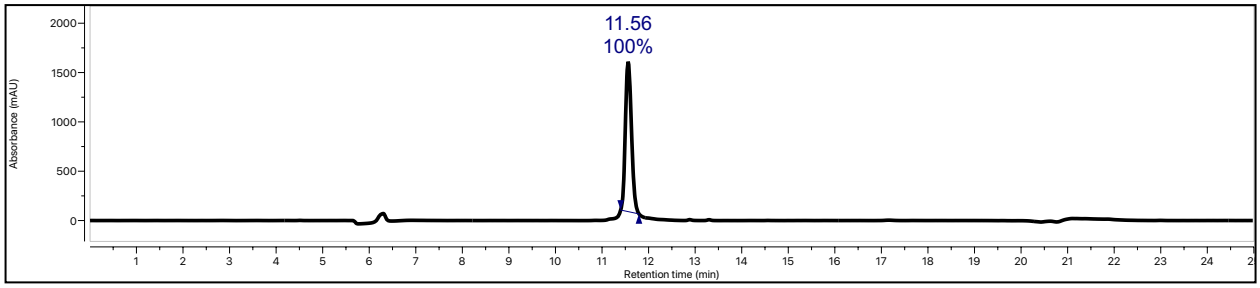
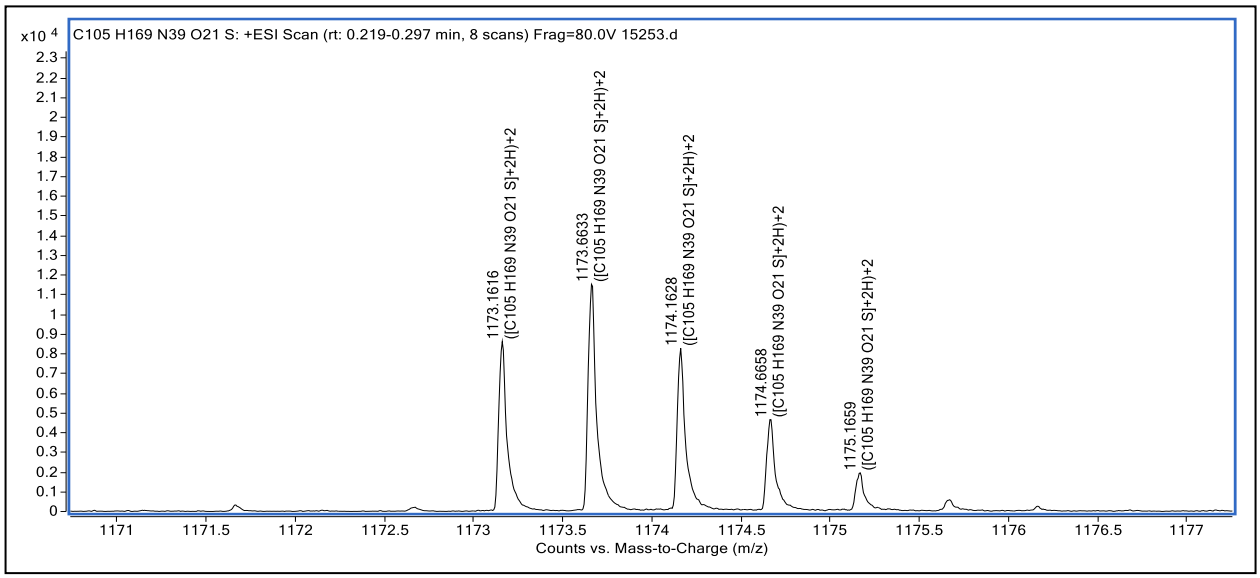


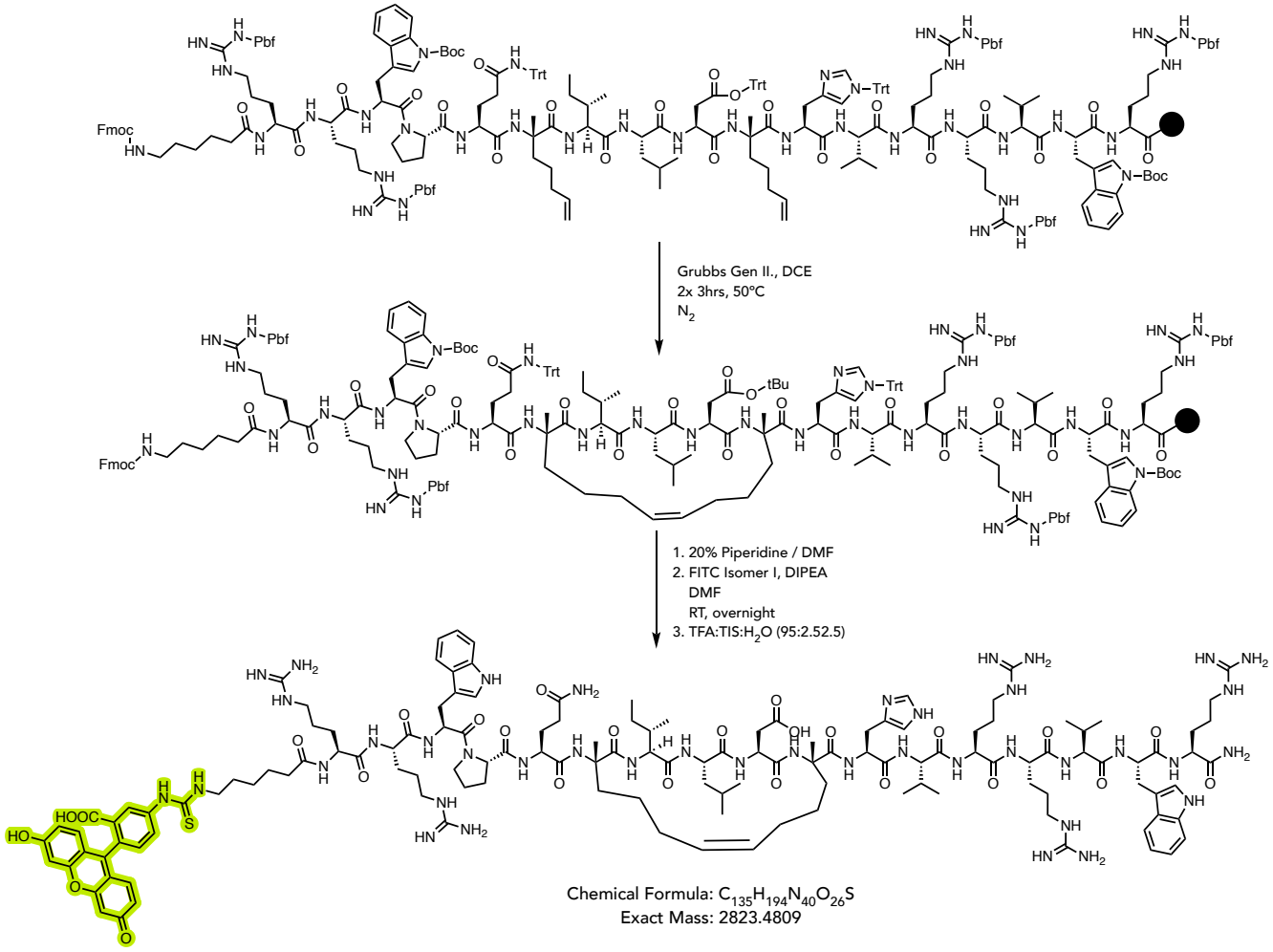
Figure S60B: LC trace of purified **21.** Observed at 230nm



HRMS of **21**
Calculated $([C_{105}H_{169}N_{39}O_{21}S]+2H)/2 = 1173.1616$
Found $([C_{105}H_{169}N_{39}O_{21}S]+2H)/2 = 1173.1616$

Synthesis of 21a (FITC-Ahx-RRPQS$_5$ILDS$_5$HVRRVWR-NH$_2$)

Linear peptide **21a** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40µmol scale synthesis). Following completion of SPPS, to the reaction vessel containing the resin-bound peptide was added Grubbs Gen. II catalyst (7.8mg) and 3mL DCE. This mixture was agitated at 350RPM, under N_2 flow, at 50ºC, for 2 hours. Completion of RCM reaction was then determined via test-cleavage of the peptide, and the Grubbs RCM reaction was repeated two more times. Once the unstapled starting material was fully consumed, the resin-bound peptide was subjected to Fmoc cleavage (20% piperidine/DMF), allowing the terminal Ahx residue to be coupled to FITC Isomer I (80mg) with DIPEA (70µL) in DMF (3mL). Reaction vessel was agitated at 150 RPM, under N_2 flow, at room temperature, for 12 hours. The FITC-labeled peptide was cleaved from the resin (TFA:TIS:H_2O, 95:2.5:2.5) following standard protocol. Trituration in $Et_2O$and HPLC purification of crude material resulted in the isolation of **21a** as bright yellow powder (30mg, 10.6µmol, 26%). This peptide served as the positive control for DLD1 cell permeability assay.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	75	25	20
10	40	60	
12	0	100	

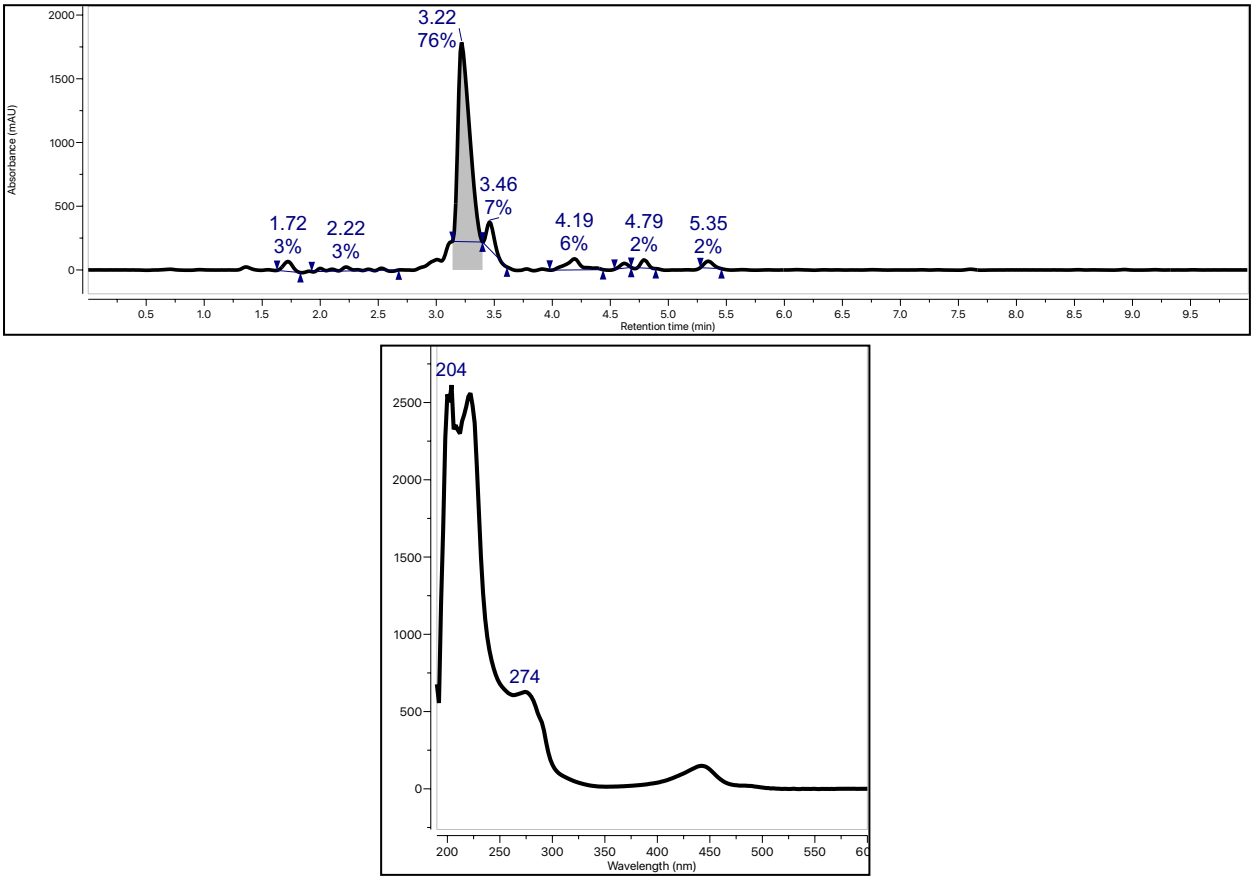


Figure S61A: Crude HPLC trace observed at 290nm and the corresponding UV excitation profile

<u>Reinjections of purified material:</u>

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	70	40	2
20	0	100	

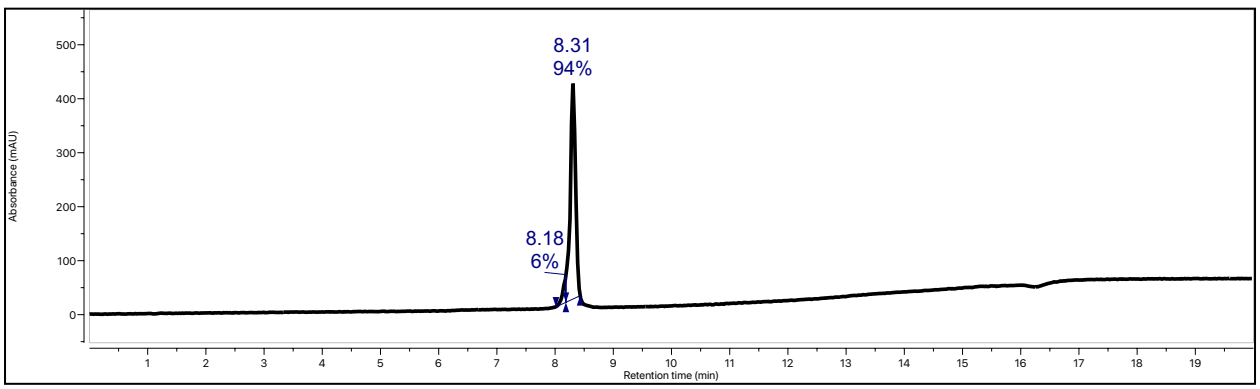
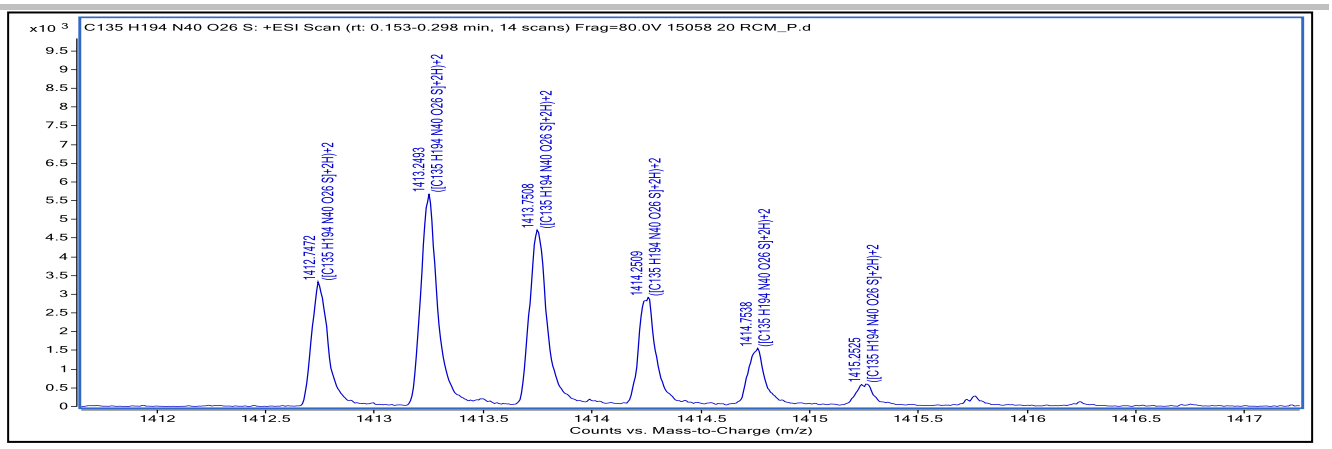


Figure S61B: LC trace of purified compound **21a**. Observed at 230nm.



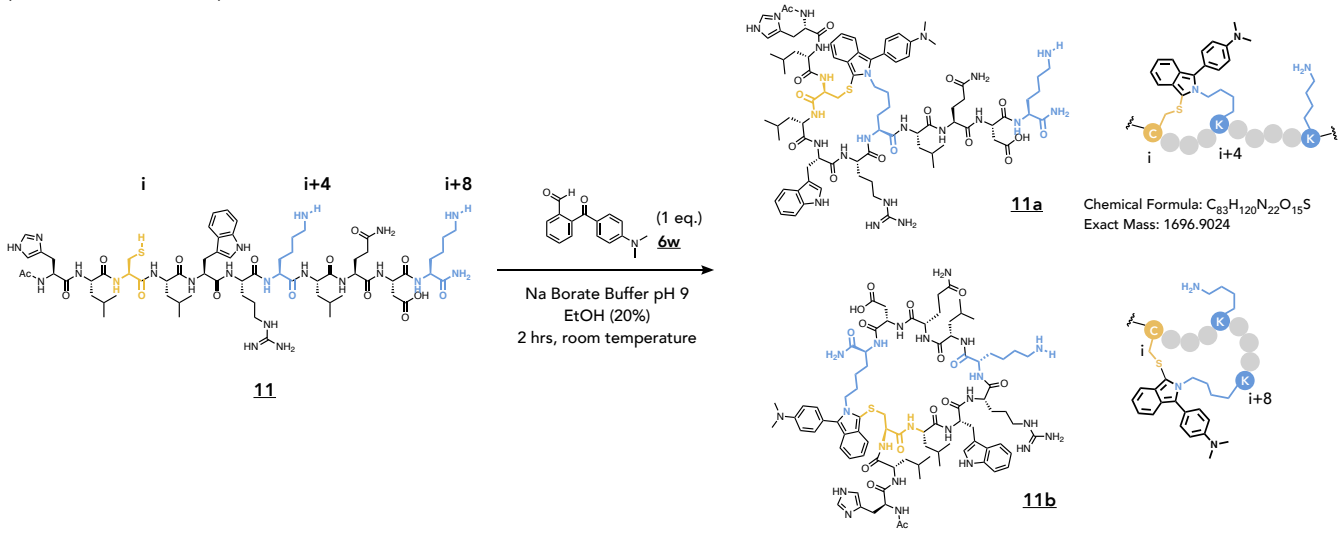
HRMS of **21a**

Calculated $([C_{135}H_{194}N_{40}O_{26}S]+2H)/2 = 1412.7483$

Found $([C_{135}H_{194}N_{40}O_{26}S]+2H)/2 = 1412.7472$

Synthesis and characterization of 11a and 11b:

Purified **11** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **11** (2µmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200µL), and to this mixture was added **6w** (50mM solution in DMSO, 40µL, 1 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 2 hours, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear yellow solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm), where ε = 14300 cm^{-1} M^{-1}, affording **11a** (421nmol, 21% isolated) and **11b** (82nmol, 4% isolated).



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	90	10	30
14	40	60	
17	0	100	

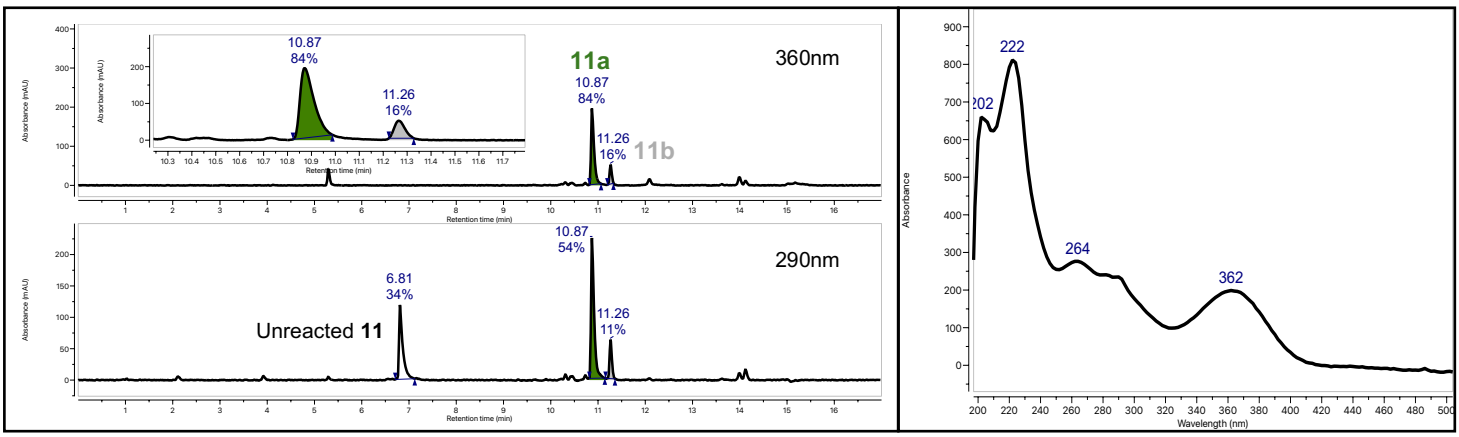


Figure S62: Prep-HPLC traces of crude reaction mixture of peptide **11** with **6w.**

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
20	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

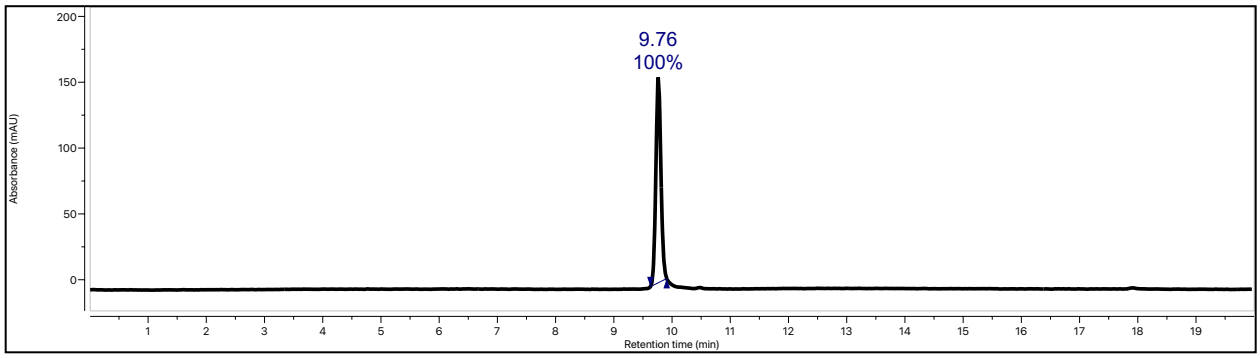


Figure S63A: LC trace of purified compound 11a. Observed at 230nm

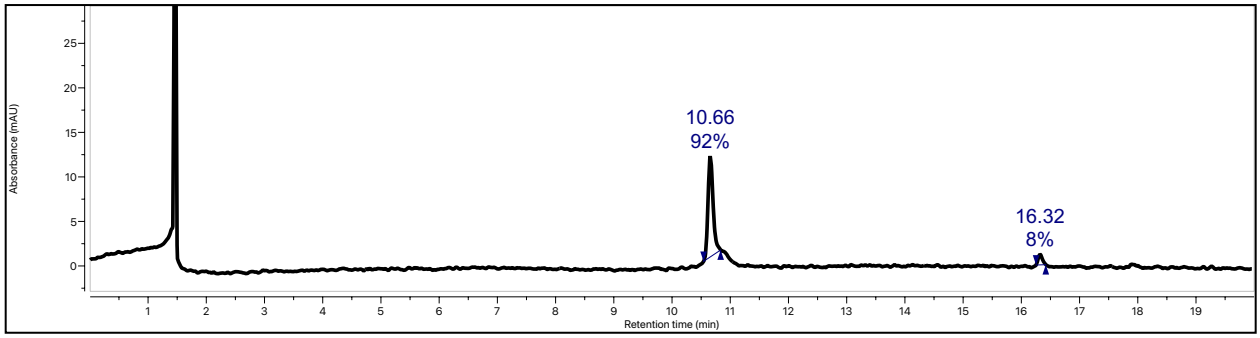
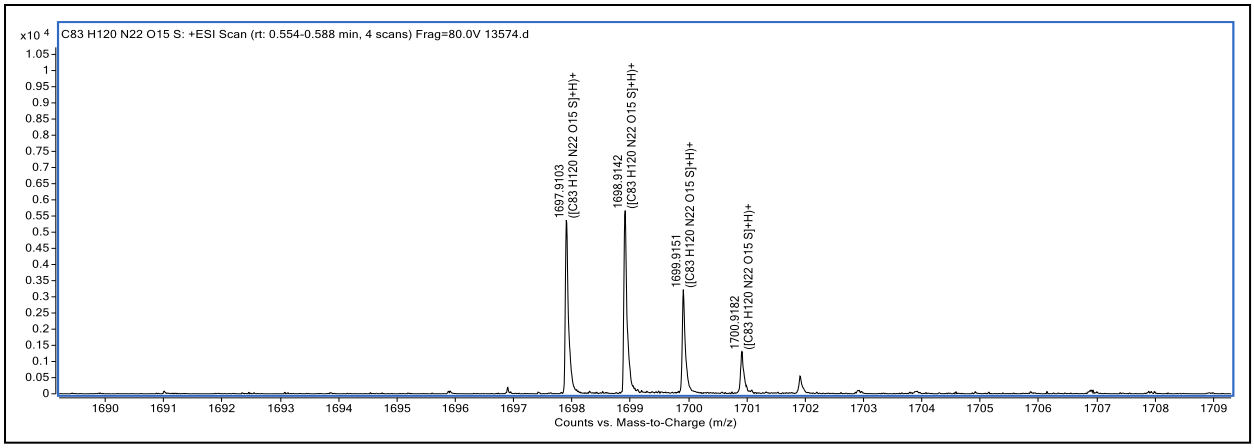
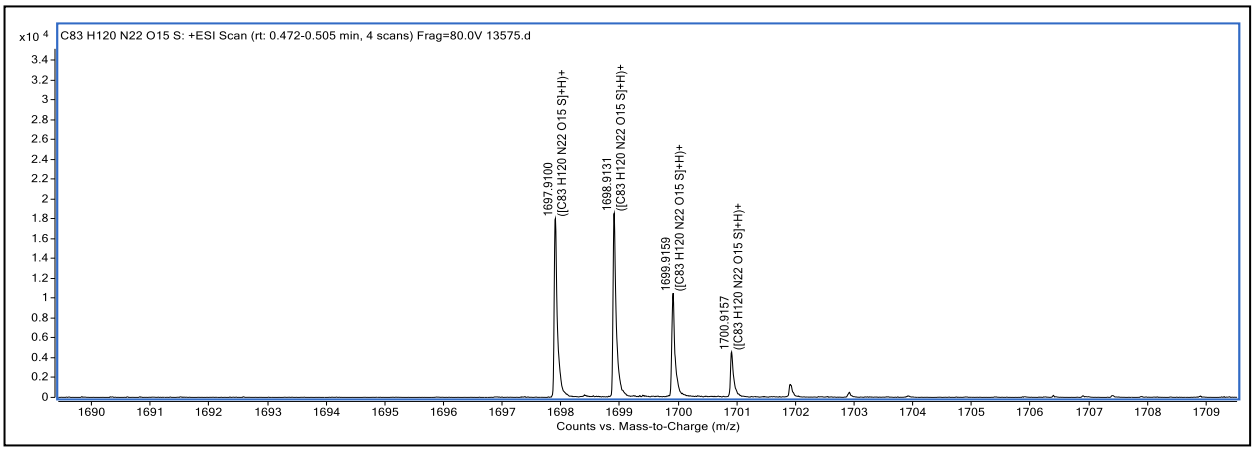


Figure S63B: LC trace of purified compound **11b**. Observed at 230nm.



HRMS of **11a**
Calculated $([C_{83}H_{120}N_{22}O_{15}S]+H)^+ = 1697.9102$
Found $([C_{83}H_{120}N_{22}O_{15}S]+H)^+ = 1697.9103$

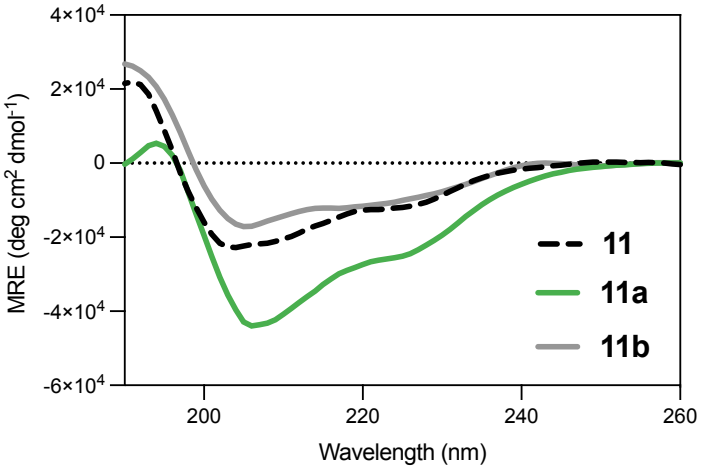


HRMS of **11b**

Calculated $([C_{83}H_{120}N_{22}O_{15}S]+H)^+ = 1697.9102$

Found $([C_{83}H_{120}N_{22}O_{15}S]+H)^+ = 1697.9100$

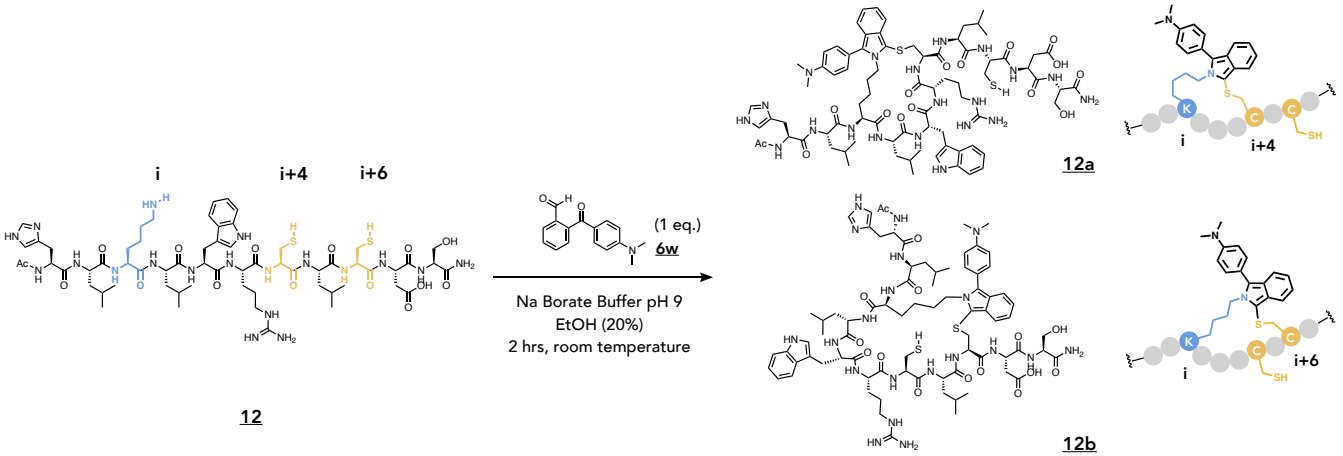
Figure S63C: Circular dichroism of **11, 11a, 11b,** as 50μM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
11	-12500	33
11a	-26200	70
11b	-11200	30

Synthesis and characterization of 12a and 12b

Purified **12** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **12** (2µmol) was dissolved in EtOH (200µL), followed by Na borate buffer pH 9 (1mL), and to this mixture was added **6w** (50mM solution in DMSO, 80µL, 2 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 1 hour, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear pale yellow solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) where ε = 14300 cm^{-1} M^{-1}, affording **12a** (350nmol, 18% isolated) and **12b** (107nmol, 5% isolated). This synthesis was repeated to obtain enough material for NMR characterization of **12a**.



Chemical Formula: $C_{78}H_{110}N_{20}O_{15}S_2$
Exact Mass: 1630.7901
Molecular Weight: 1631.9830

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFmA
Column : Agilent, C18, 5 µm, 50x21.2mm.

Time (min)	A%	B%	Flow (mL/min)
0	90	10	20
14	40	60	
17	0	100	

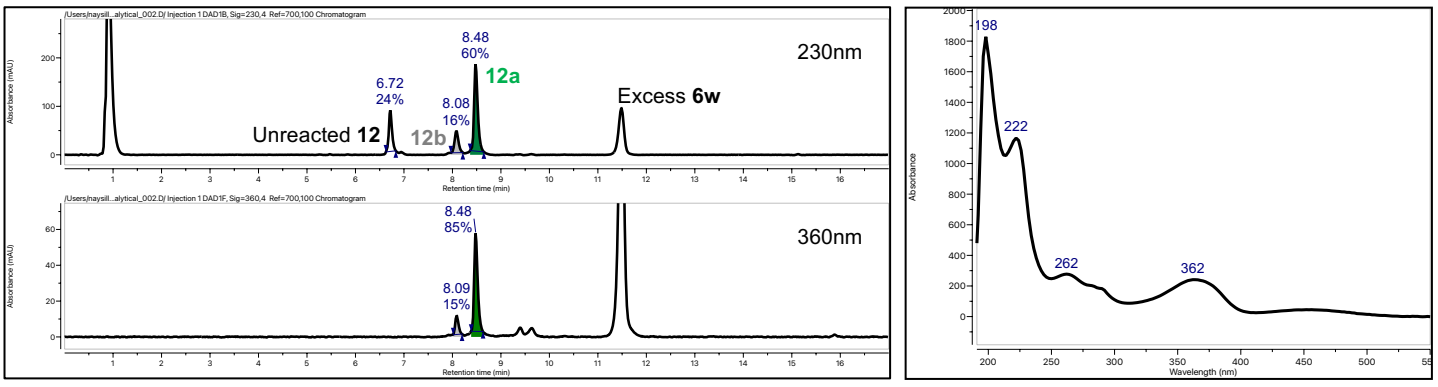


Figure S64: Prep-HPLC traces of crude reaction mixture of peptide **12** with **6w.**

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	2
25	0	100	

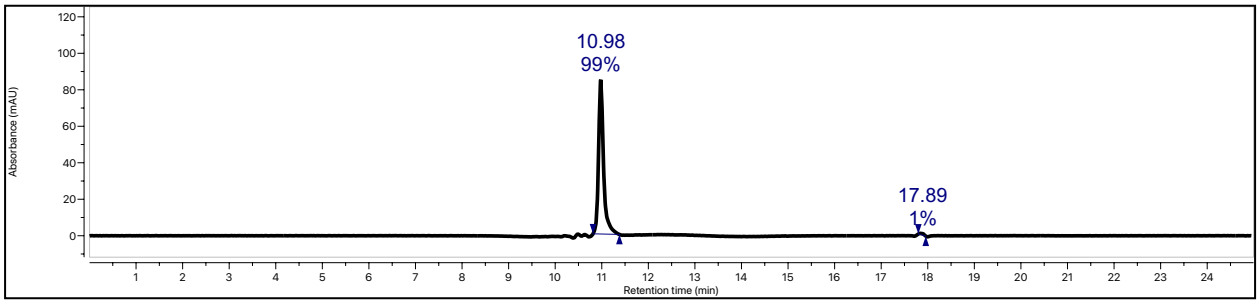


Figure S65A: LC trace of purified compound **12a**. Observed at 230nm.

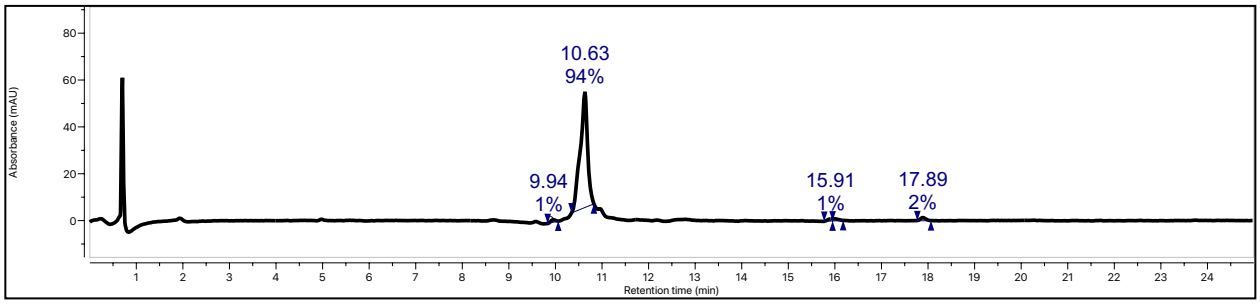
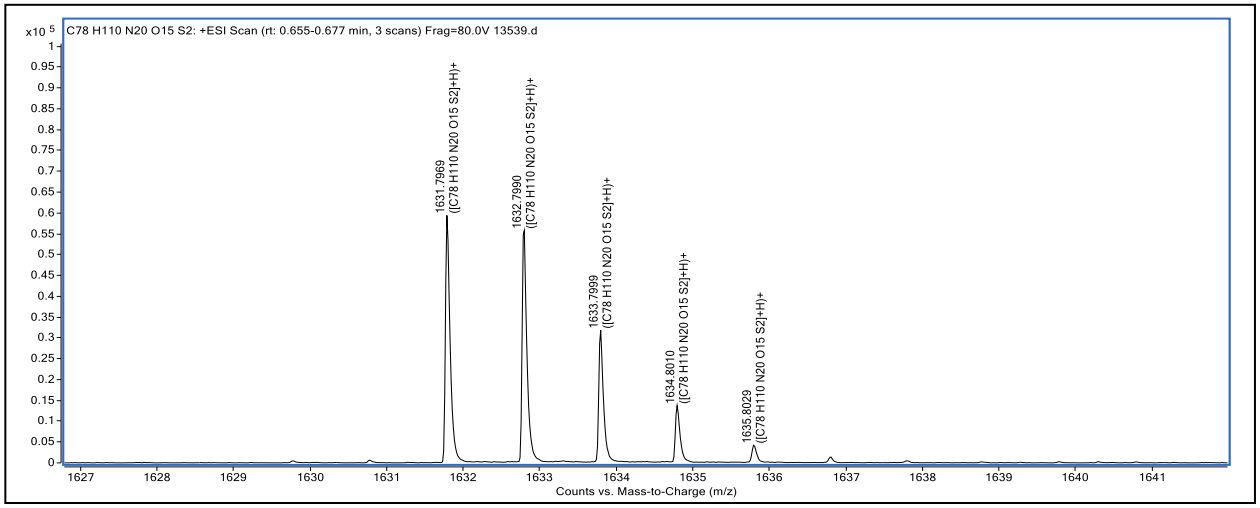
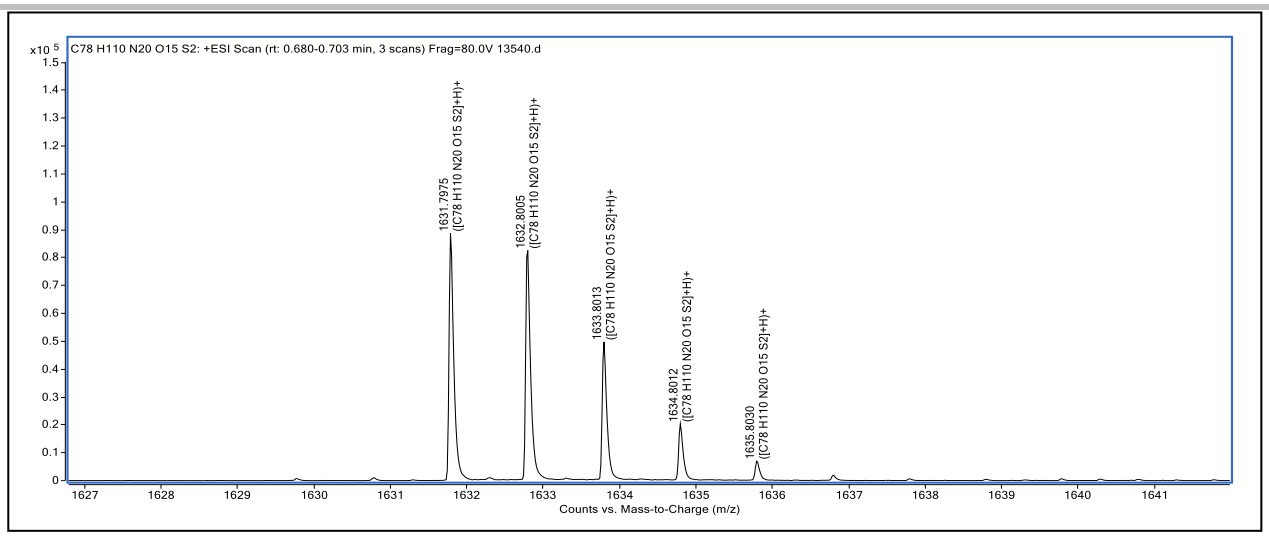


Figure S65B: LC trace of purified compound **12b.** Observed at 230nm.



HRMS of **12a**
Calculated $([C_{78}H_{110}N_{20}O_{15}S_2]+H)^+ = 1631.7979$
Found $([C_{78}H_{110}N_{20}O_{15}S_2]+H)^+ = 1631.7969$

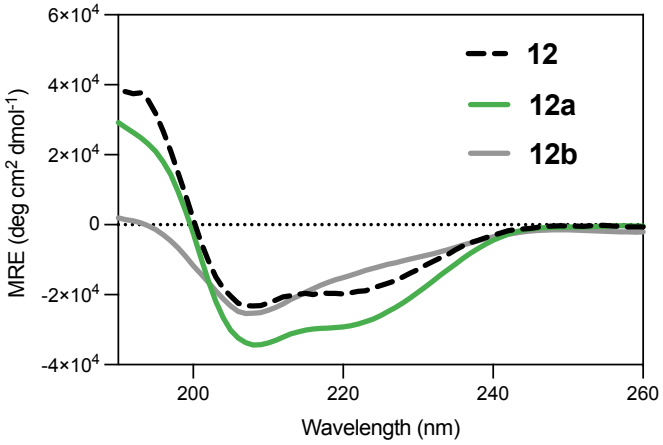


HRMS of **12b**

Calculated $([C_{78}H_{110}N_{20}O_{15}S_2]+H)^+ = 1631.7979$

Found $([C_{78}H_{110}N_{20}O_{15}S_2]+H)^+ = 1631.7975$

Figure S65C: Circular dichroism of **12, 12a, 12b,** as 50μM solutions in TFE/H_2O (20:80)

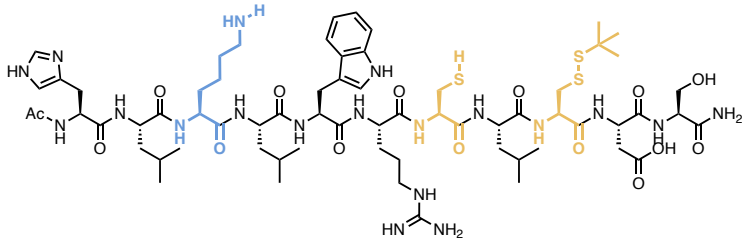


Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
12	-19000	51
12a	-28400	76
12b	-13700	36

Confirming positional selectivity of 12b

Orthogonal protecting group strategy:

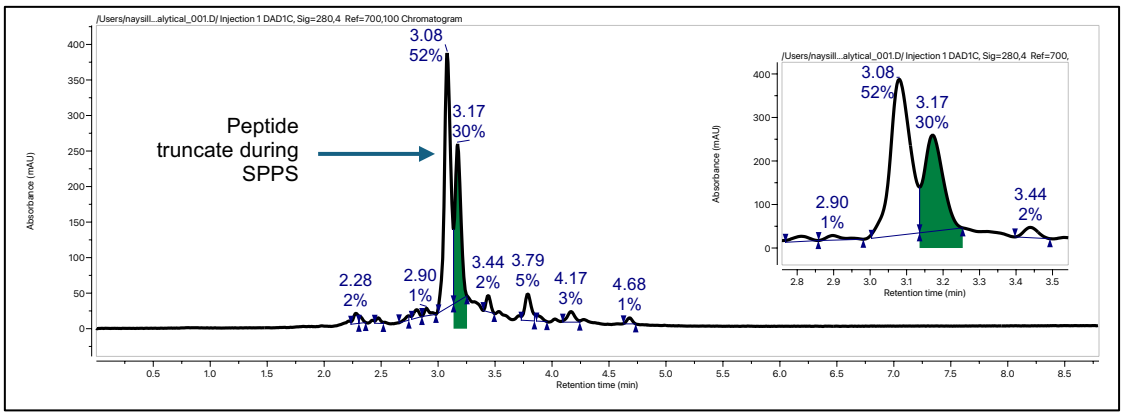
Linear peptide **12c** was synthesized according to the general procedure for solid phase peptide synthesis, using 200mg Rink Amide MBHA resin (80µmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **12c** (16µmol, 20%), to be used for subsequent FIICk reaction.

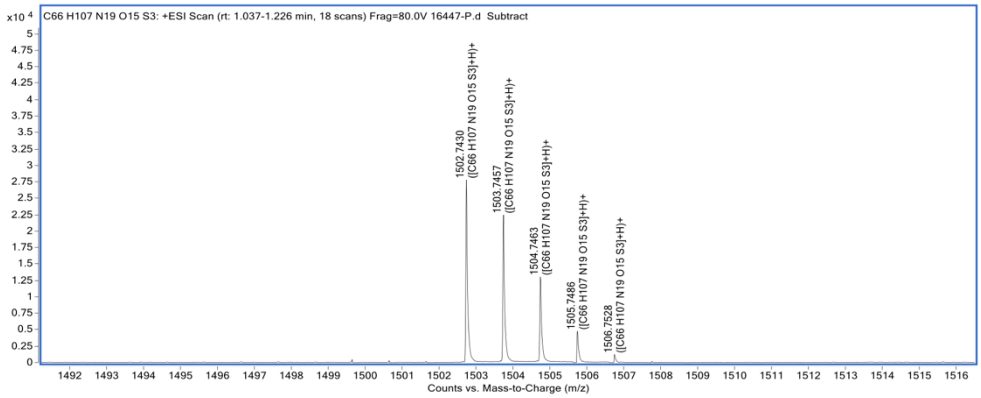


Chemical Formula: $C_{66}H_{107}N_{19}O_{15}S_3$
Exact Mass: 1501.7356
Molecular Weight: 1502.8800

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	15
10	0	100	

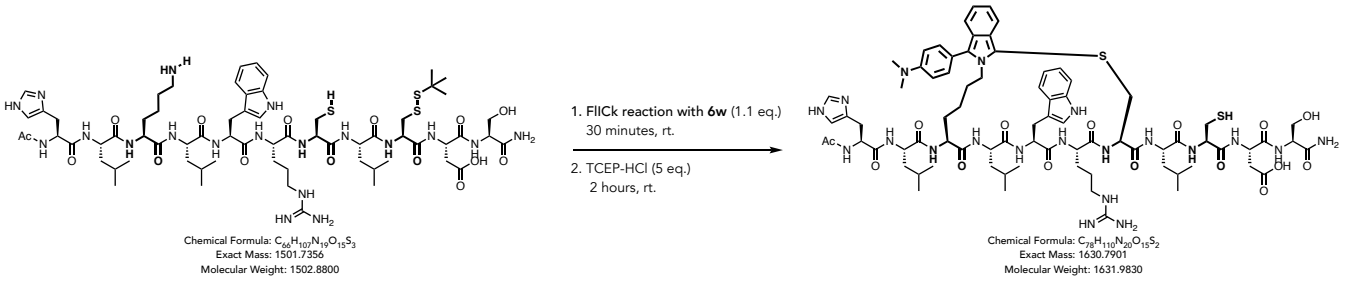




HRMS of **12c**
Calculated $([C_{66}H_{107}N_{19}O_{15}S_3]+H)^+ = 1502.7434$
Found $([C_{66}H_{107}N_{19}O_{15}S_3]+H)^+ = 1502.7430$

Figure S65D: Crude HPLC trace observed at 280nm and the corresponding UV excitation profile and HRMS of the isolated peak (t=3.17min)

Purified **12c** were partitioned into 15mL-falcon tubes in 8μmol portions (1 eq.) and lyophilized to afford dry white powder. **12c** (8μmol) was dissolved in Na borate buffer pH 9 (4mL), EtOH (1000μL), and to this mixture was added **6w** (50mM solution in DMSO, 176μL, 1.1 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 30 minutes, an aliquot (1μmol) of the reaction was quenched and acidified to pH 3 with formic acid and analysed by HPLC to determine reaction completion, affording intermediate **12c'** with a singly protected Cys. This same reaction mixture was then treated with TCEP-HCl (5 eq.) and left to sit in the same falcon tube for 2 hours at room temperature. Following this disulfide reduction, reaction was acidified with formic acid and purified by prep-HPLC to afford pure **12d** with the method outlined below.



12c **12d**

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	15
14	40	60	
17	0	100	

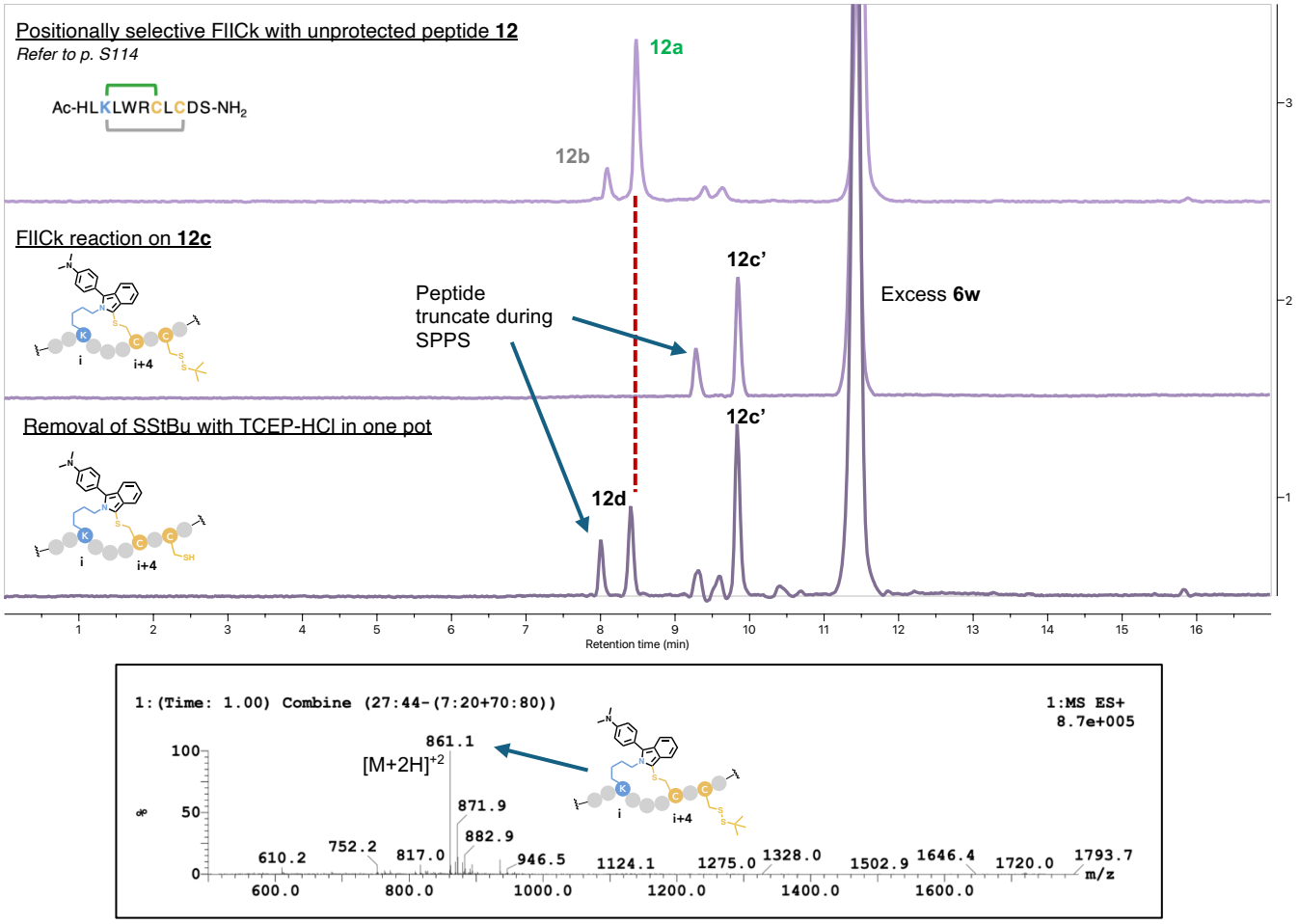


Figure S65E: Overlaid prep-HPLC traces of reaction mixture of peptide **12**, **12c** with **6w** following one-pot disulfide reduction with TCEP treatment. **12c'** was isolated and analyzed my LRMS (shown).

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse C18, 5 µm, 250x4.9mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	2
20	0	100	

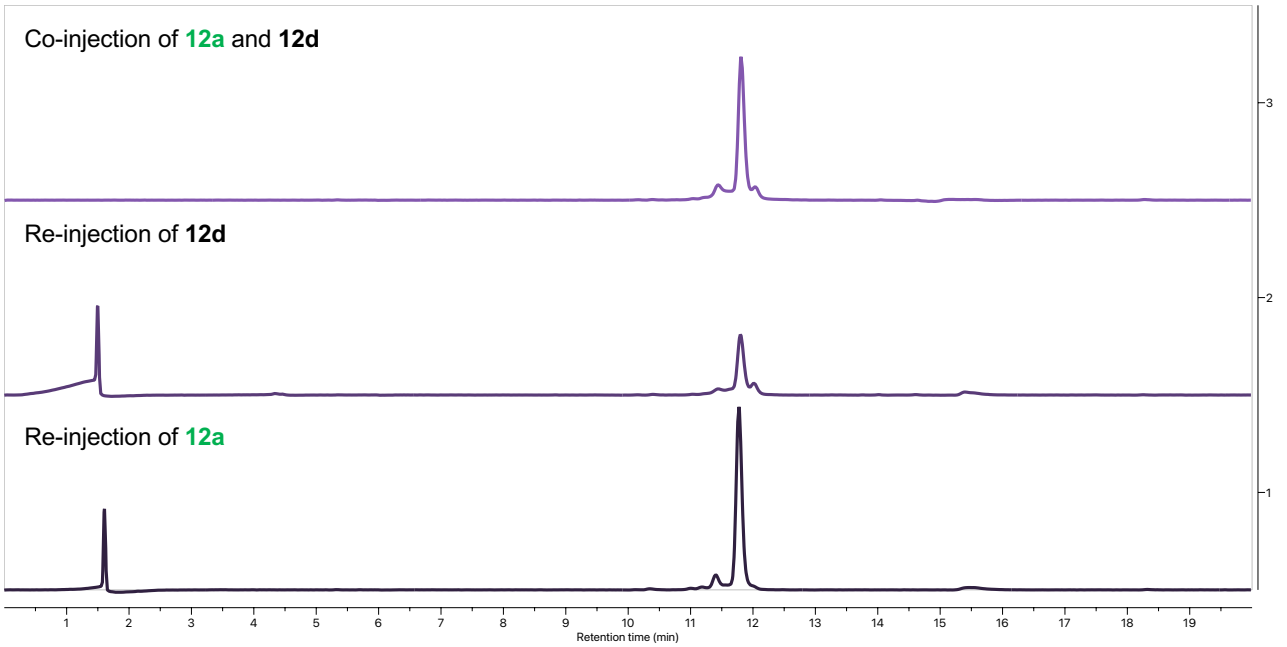


Figure S65F: Overlaid analytical HPLC traces observed at 230nm, confirming the identity of **12a** and **12d**.

Crude MS/MS analysis:

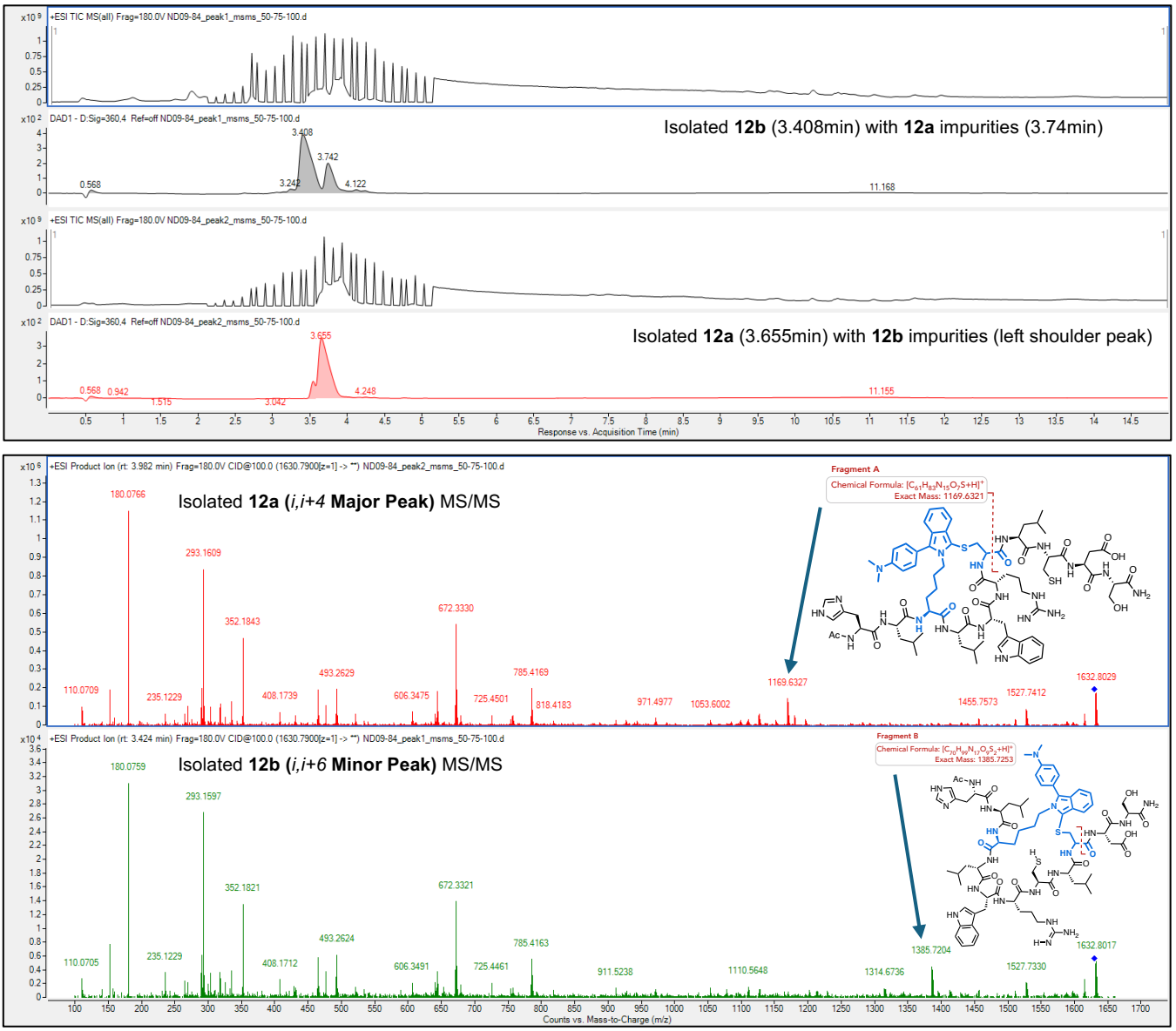
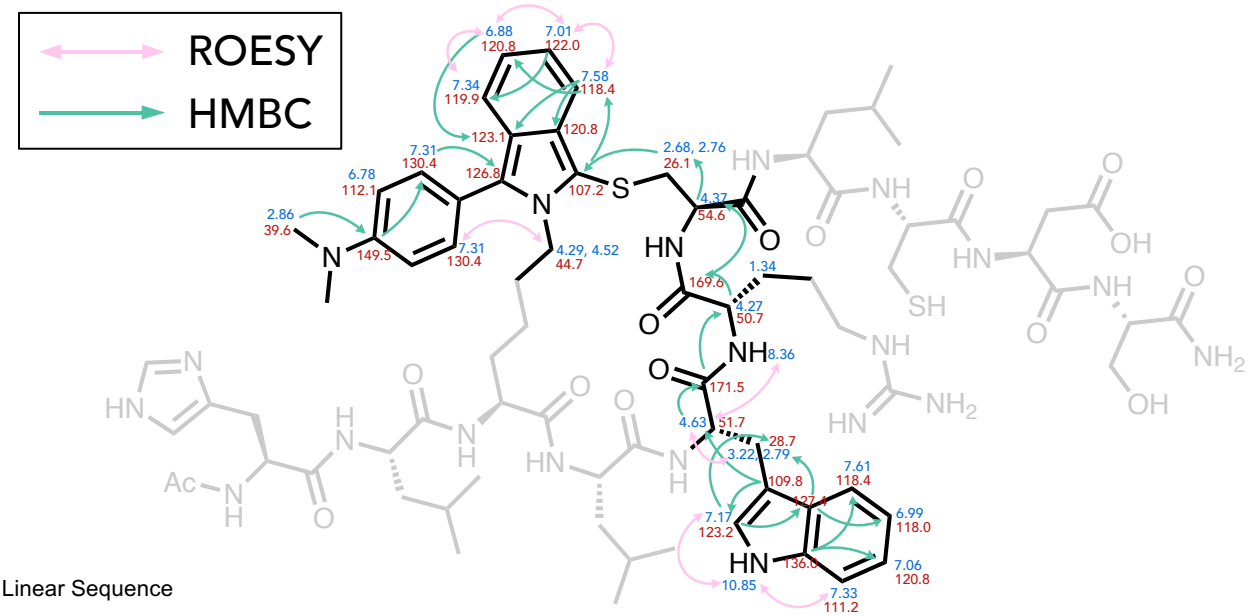


Figure S65G: MS/MS fragmentation spectrum of isolated peptides **12a** (major isomer) and **12b** (minor isomer) acquired using HPLC-QTOF with tandem mass spectrometer. Collision-induced dissociation (CID) high resolution MS data acquired with 100 eV collision energy, using positive-mode electrospray ionization (ESI+).

NMR analysis and discussion of isolated major isomer 12a:



Linear Sequence

Ac– HLKLWRCLCDS–NH$_2$
1234567891 0 1

Positional selectivity of the stapling reaction between peptide **12** and **6w** was further corroborated by an orthogonal protecting group strategy (p. **S118**), crude MS/MS fragmentation analysis, (p. **S120**), and NMR analysis of the isolated major isomer **12a** as follow.

The key peptide skeleton that confirmed the positional selectivity of the FIICk staple was carefully mapped starting from the distinctive 1H-indole proton signal of Trp5. Using this as the starting point, we are able to observe a sequence of 1H and 13C correlations that lead to -CH2- protons of Cys7 (*i,i+4* linkage with Lys3) exhibiting correlation in HMBC to one single aromatic carbon on the isoindole scaffold. In the minor isomer, Cys7 would not show correlation to any aromatic carbons.

Aromatic NH on Trp5 shows correlation in COSY to C2 of the indole ring {7.18, 123.24}. From this signal, shown in the red square below, we trace a chain of correlations on HMBC to three aromatic carbons (109.80, 127.42, and 136.03). One of these signals belong to C3 of the indole ring and it further correlates to -CH2- protons on Trp5 (3.25ppm, 2.79ppm.)

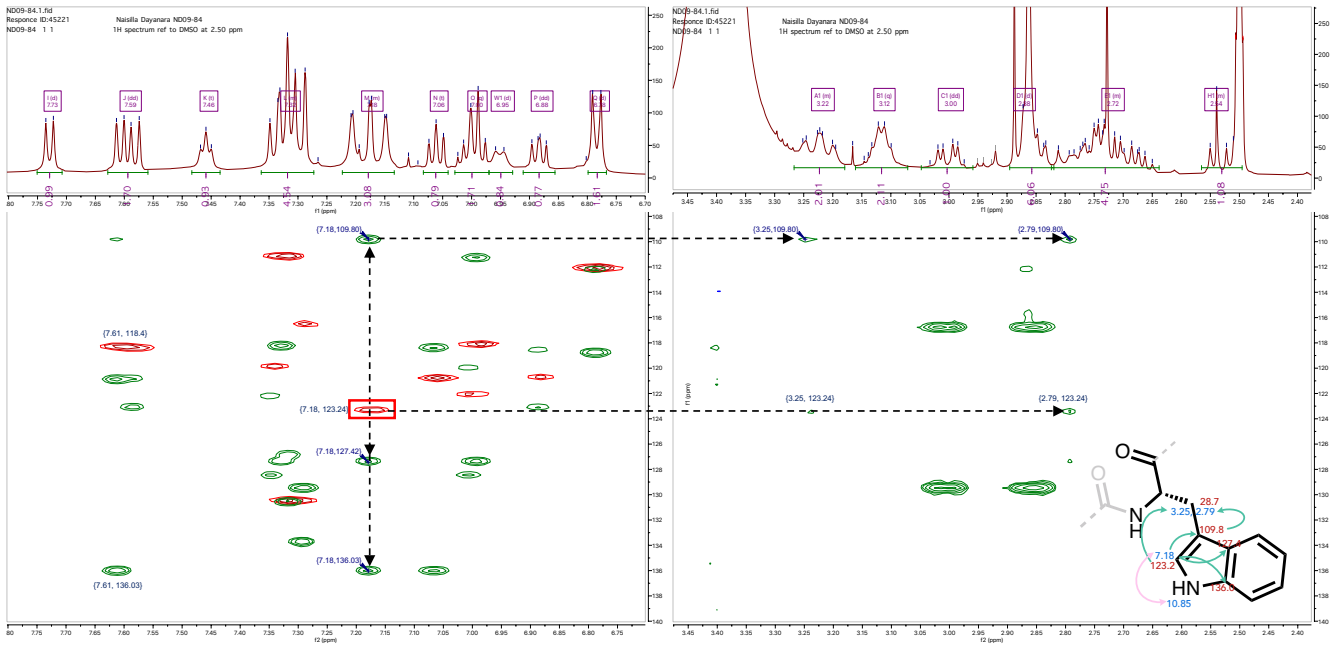


Figure S65H: A subset of HMBC and HSQC spectra showing a sequence of signals that elucidates the Trp5 starting point.

These -CH2- signals (3.22pm and 2.79ppm) take us further down the peptidic backbone via correlation on HMBC between its corresponding ^{13}C signal (28.7ppm, observed in HSQC) and CHα of Trp5 (4.63ppm). This CHα subsequently shows linkage to two ^{13}C=O signals at 163.63ppm and 171.ppm. Through process of elimination, we determine that the ^{13}C signal at 171.48ppm corresponds to the carbonyl of Trp5, thus allowing us to proceed further down the peptide backbone towards Arg6.

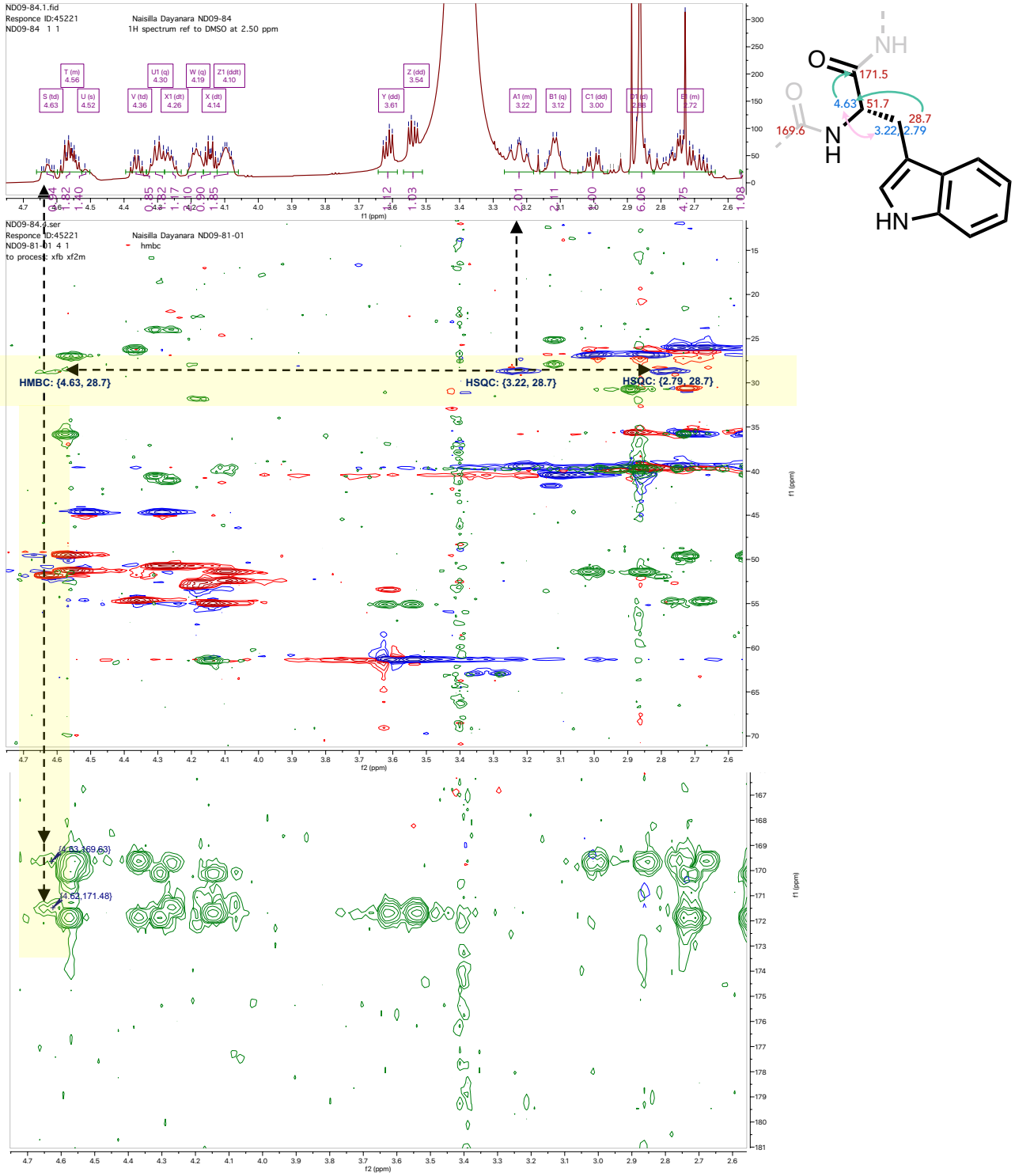


Figure S65I: A subset of HMBC and HSQC spectra showing a sequence of signals that links Trp5 to Arg6

This carbonyl signal (171.48ppm) shows additional correlation in HMBC with the adjacent Arg6 (CHα - 4.27ppm), which we then trace further to its own ^{13}C=O (169.63ppm). This allows us to finally reach CHα of Cys7, where we found correlation in HMBC between 169.63ppm and **4.37ppm.**

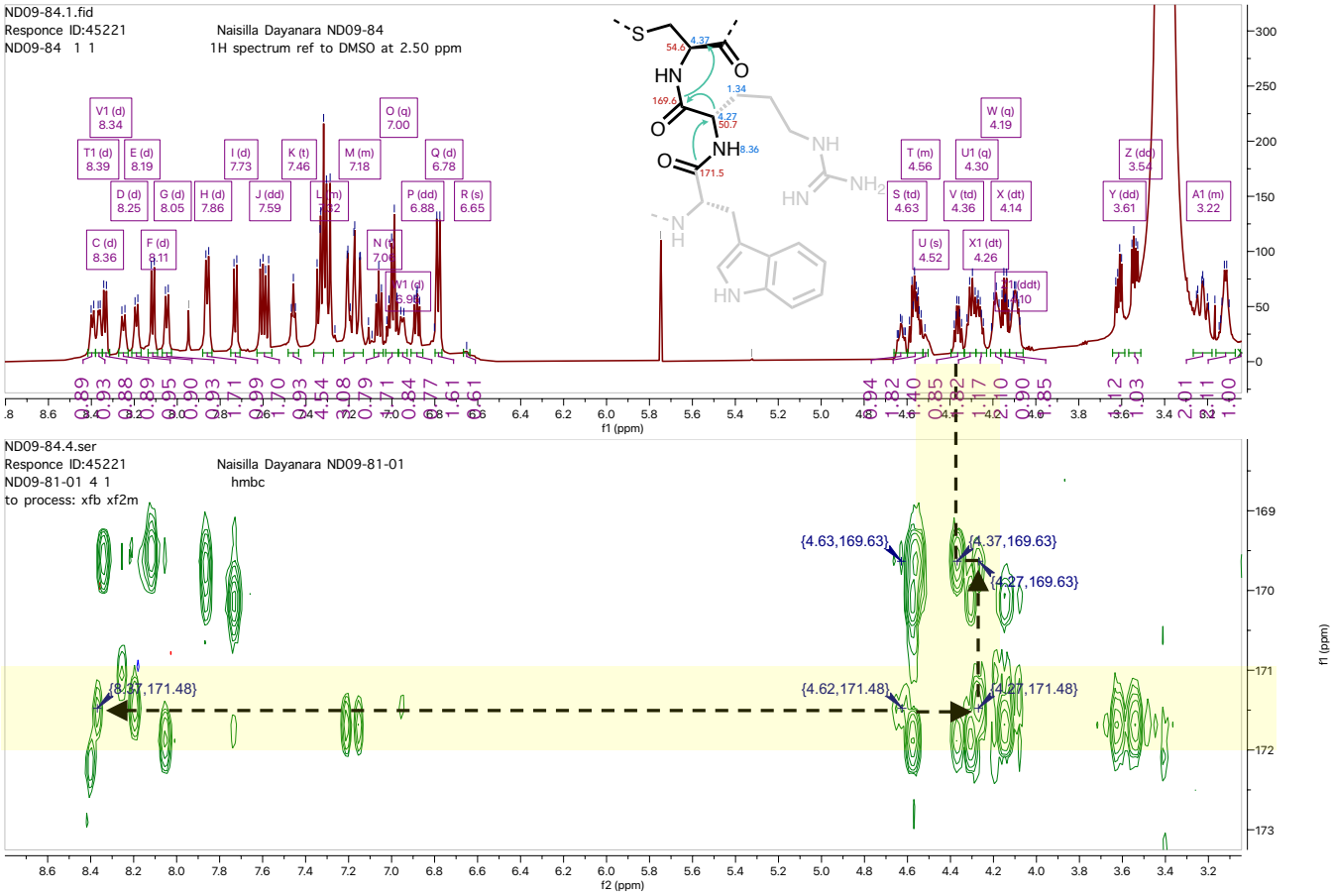


Figure S65J: A subset of HMBC spectrum showing signals that link the CHα of Arg6 (4.27ppm) to CHα of Cys7 (4.37ppm).

Arriving at CHα of Cys7 (4.37ppm), we observed correlations in COSY and HMBC (shown here) to the key -CH2- signals that would link this amino acid residue to the isoindole linkage. 4.37ppm shows HMBC to ^{13}C at 26.2ppm, where in the corresponding HSQC confirms the presence of -CH2- protons (2.68 ppm, 2.76ppm). These -CH2- of Cys7 additionally shows HMBC to ^{13}C 54.68ppm, tracing back to its own CHα at 4.37ppm.

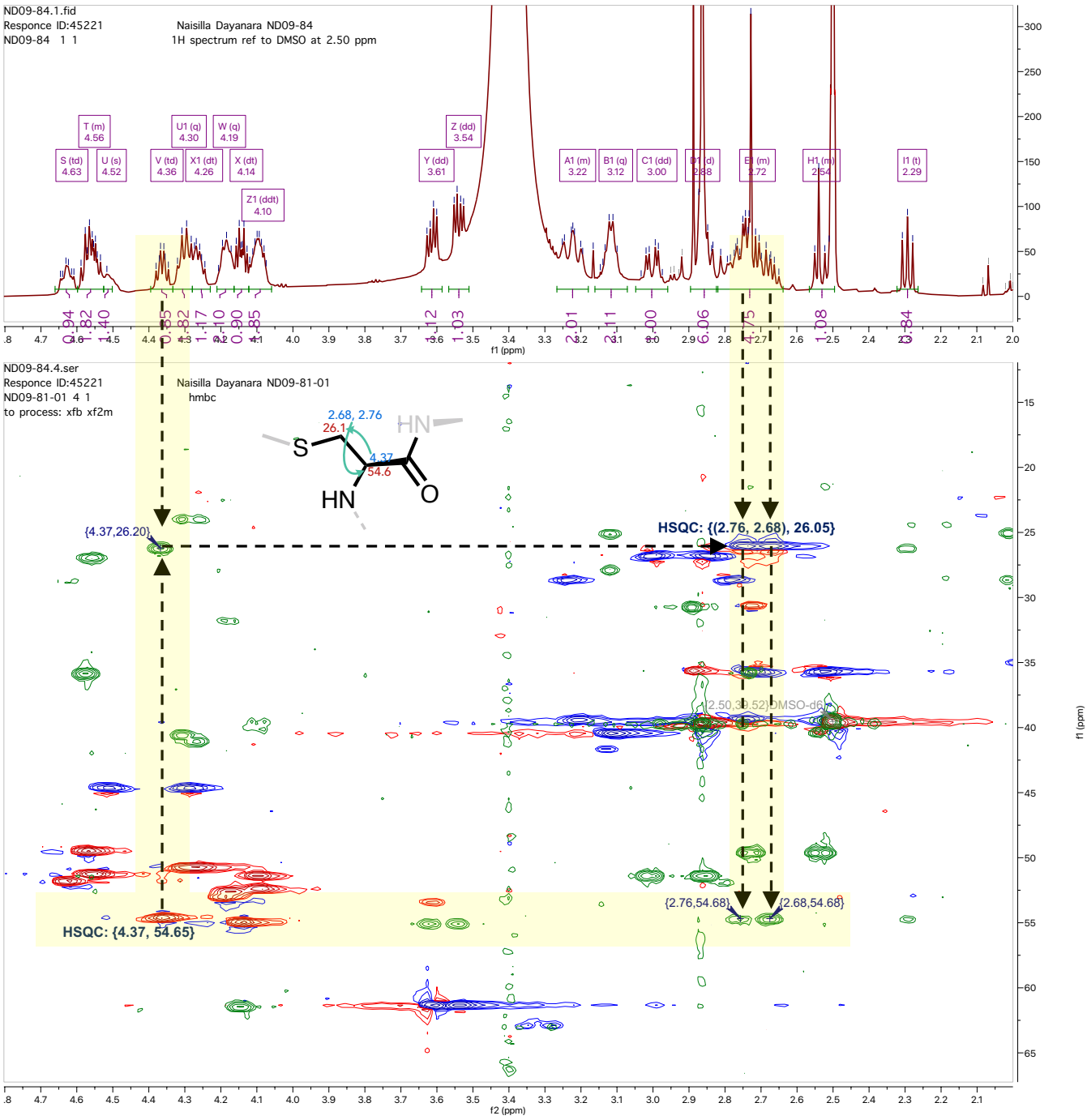


Figure S65K: A subset of HMBC and HSQC spectra elucidating the 1H and 13C nuclei found in Cys7

After successfully mapping the signals in Cys7, we were able to find the key -CH2- HMBC signal that links this residue to the aromatic structure. More importantly, there is only one HMBC signal between 2.76ppm (Cys7, -CH2-) and the aromatic region. Furthermore, the carbon signal at 107.14ppm doesn't show a corresponding signal in HSQC, indicating its quaternary nature. The isoindole structure is also confirmed, where the carbon at 107.14ppm shows a single HMBC signal to 7.58ppm. This proton allows us to elaborate the rest of the isoindole structure, showing HMBC with three aromatic carbons: 120.8, 120.8 (overlapping), and 123.1ppm.

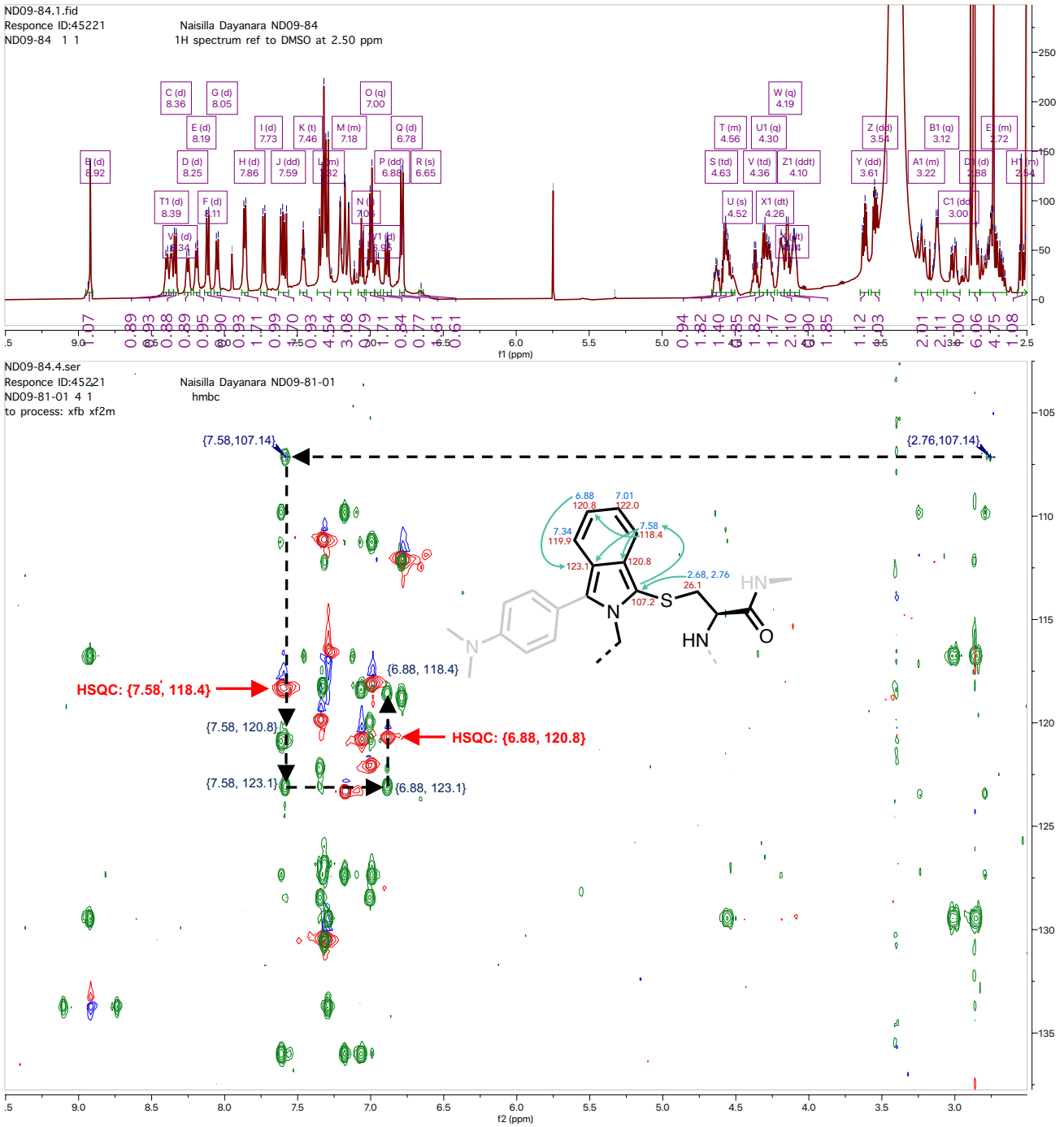
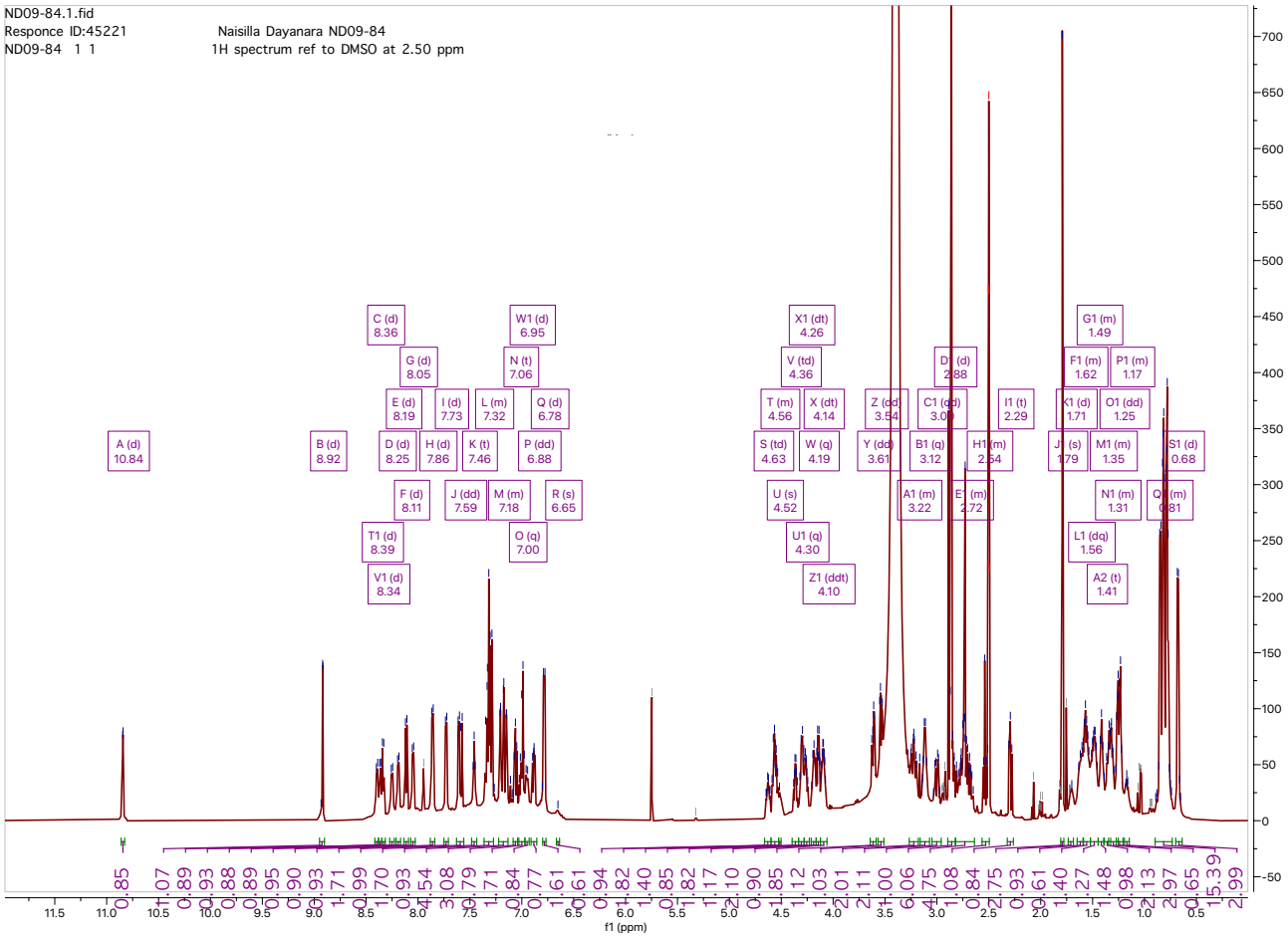


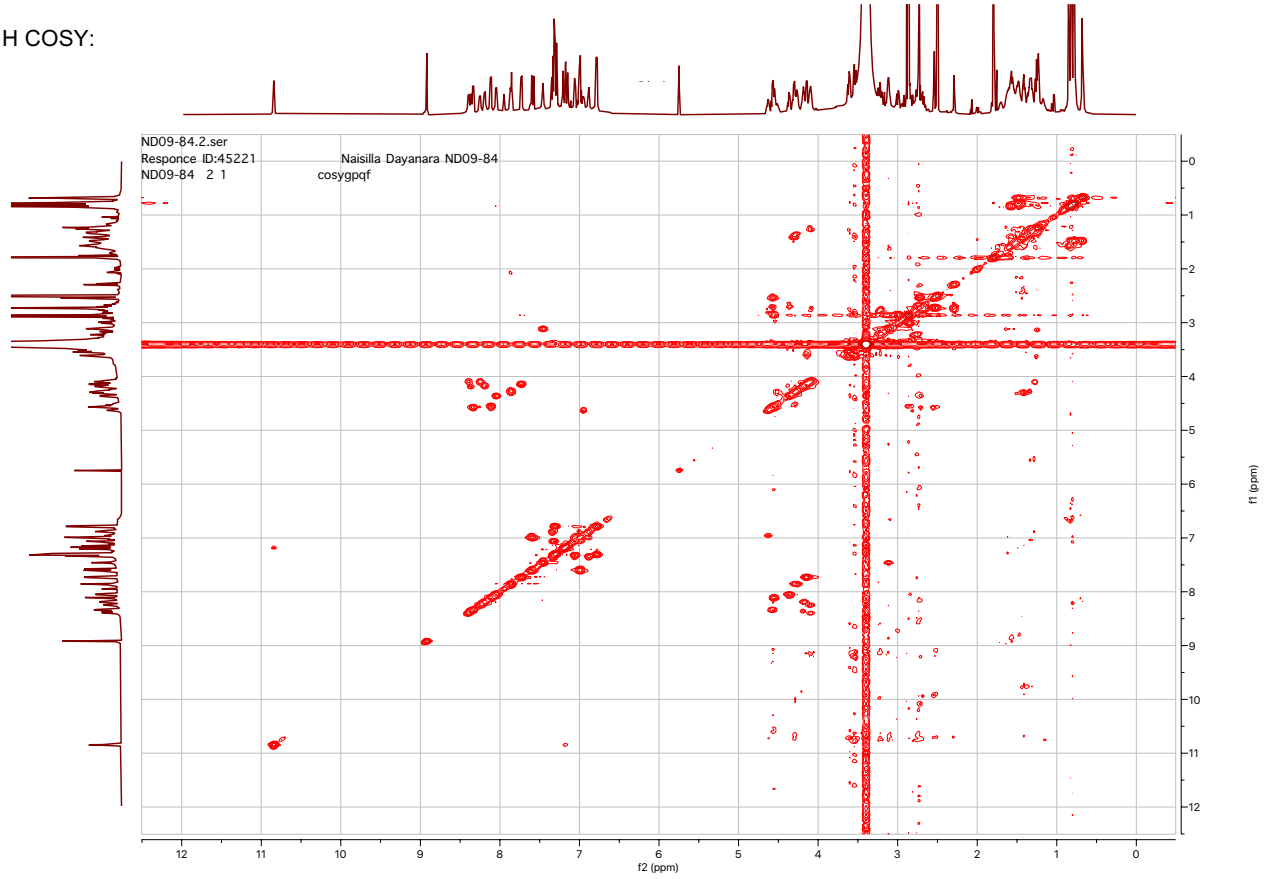
Figure S65L: A subset of HMBC and HSQC spectra showing the sequence of signals that links Cys7 (-CH2-, 2.76ppm) to the aromatic region found in isoindole.

Full NMR data set:

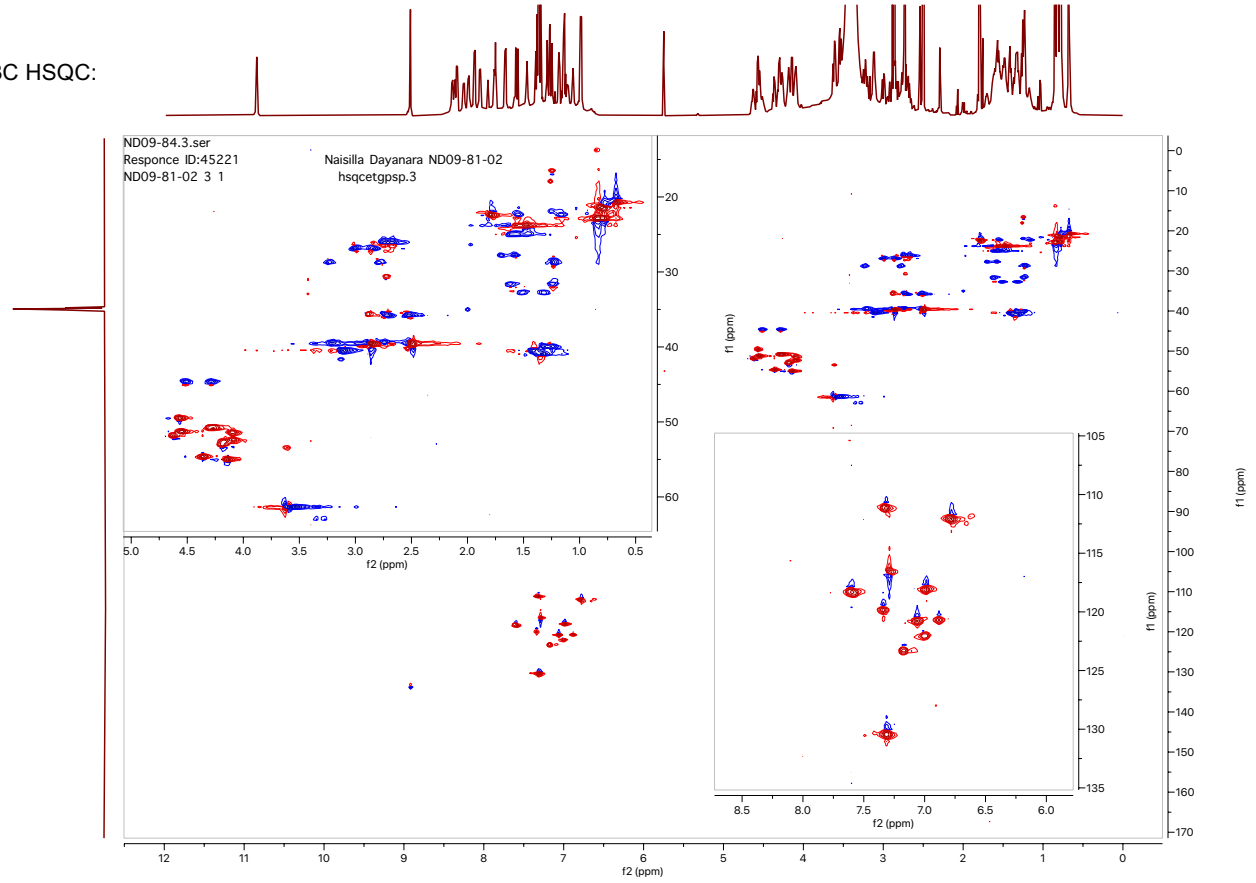


^{1}H NMR (600 MHz, DMSO) δ 10.84 (d, *J* = 2.4 Hz, 1H), 8.92 (d, *J* = 1.3 Hz, 1H), 8.39 (d, *J* = 7.4 Hz, 1H), 8.36 (d, *J* = 5.6 Hz, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.25 (d, *J* = 8.5 Hz, 1H), 8.19 (d, *J* = 7.8 Hz, 1H), 8.11 (d, *J* = 8.3 Hz, 1H), 8.05 (d, *J* = 7.8 Hz, 1H), 7.86 (d, *J* = 7.9 Hz, 2H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.59 (dd, *J* = 15.4, 8.2 Hz, 2H), 7.46 (t, *J* = 5.6 Hz, 1H), 7.36 – 7.27 (m, 5H), 7.22 – 7.13 (m, 3H), 7.06 (t, *J* = 7.5 Hz, 1H), 7.00 (q, *J* = 7.5 Hz, 2H), 6.95 (d, *J* = 8.7 Hz, 1H), 6.88 (dd, *J* = 8.5, 6.4 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 6.65 (s, 1H), 4.63 (td, *J* = 9.1, 3.8 Hz, 1H), 4.60 – 4.53 (m, 2H), 4.52 (s, 1H), 4.36 (td, *J* = 7.5, 5.3 Hz, 1H), 4.30 (q, *J* = 7.5 Hz, 2H), 4.26 (dt, *J* = 8.7, 4.2 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 2H), 4.14 (dt, *J* = 7.8, 5.1 Hz, 1H), 4.10 (ddt, *J* = 11.5, 7.8, 4.3 Hz, 2H), 3.61 (dd, *J* = 10.9, 5.4 Hz, 1H), 3.54 (dd, *J* = 10.8, 4.9 Hz, 1H), 3.27 – 3.18 (m, 2H), 3.12 (q, *J* = 6.8 Hz, 2H), 3.00 (dd, *J* = 15.3, 5.2 Hz, 1H), 2.88 (d, *J* = 14.7 Hz, 6H), 2.82 – 2.64 (m, 5H), 2.57 – 2.50 (m, 1H), 2.29 (t, *J* = 8.6 Hz, 1H), 1.79 (s, 3H), 1.71 (d, *J* = 11.4 Hz, 1H), 1.65 – 1.59 (m, 1H), 1.56 (dq, *J* = 12.9, 6.3 Hz, 1H), 1.52 – 1.45 (m, 1H), 1.41 (t, *J* = 7.3 Hz, 1H), 1.39 – 1.33 (m, 1H), 1.35 – 1.25 (m, 2H), 1.25 (dd, *J* = 11.1, 4.5 Hz, 3H), 1.19 – 1.15 (m, 1H), 0.90 – 0.73 (m, 15H), 0.68 (d, *J* = 6.4 Hz, 3H).

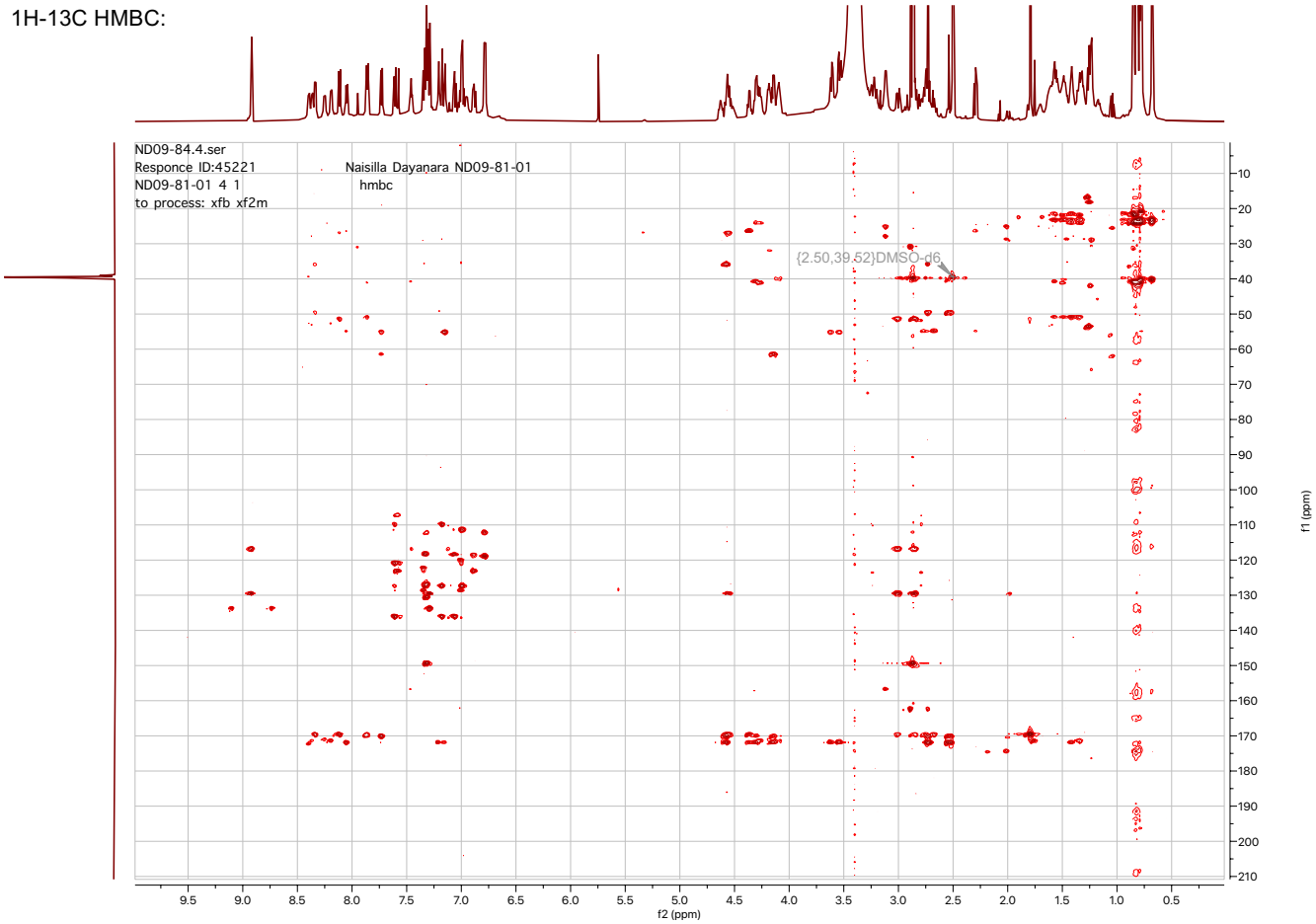
1H-1H COSY:



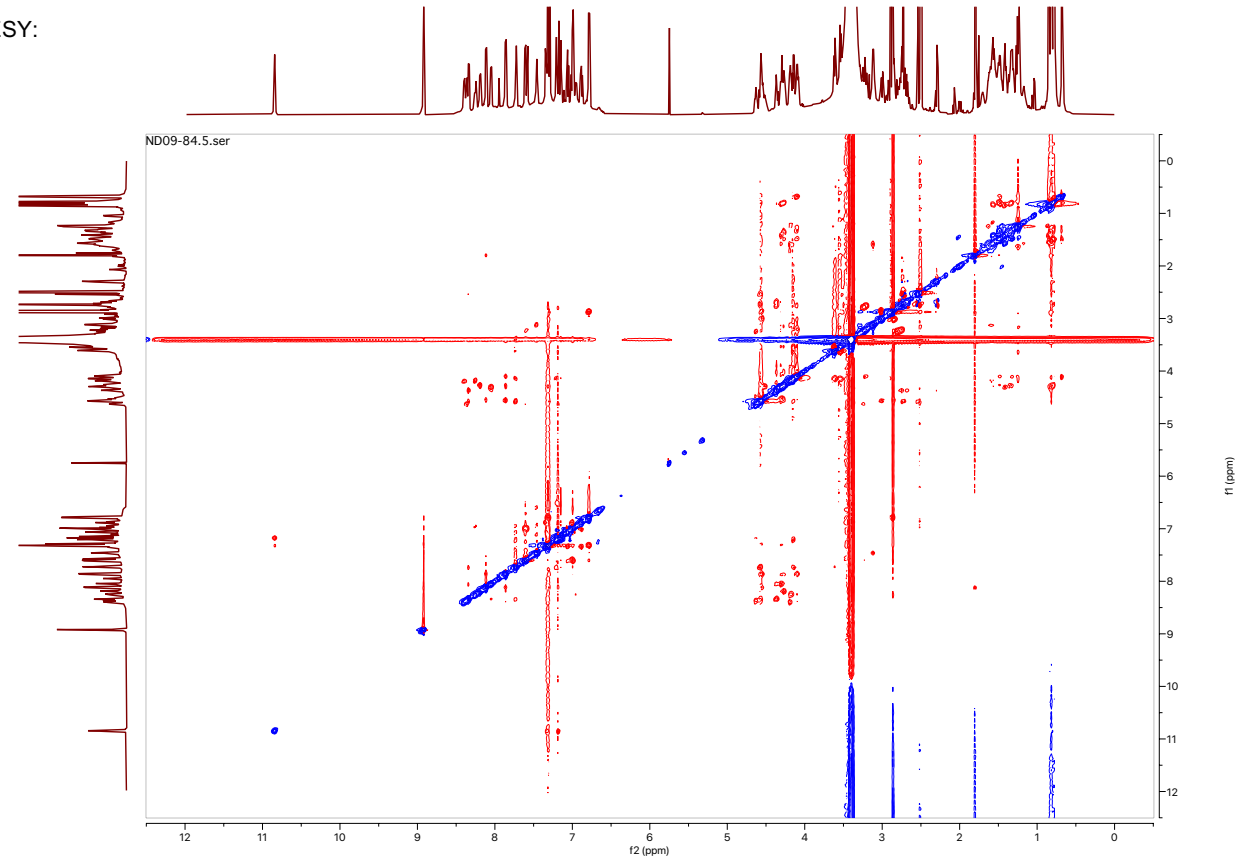
1H-13C HSQC:



1H-13C HMBC:

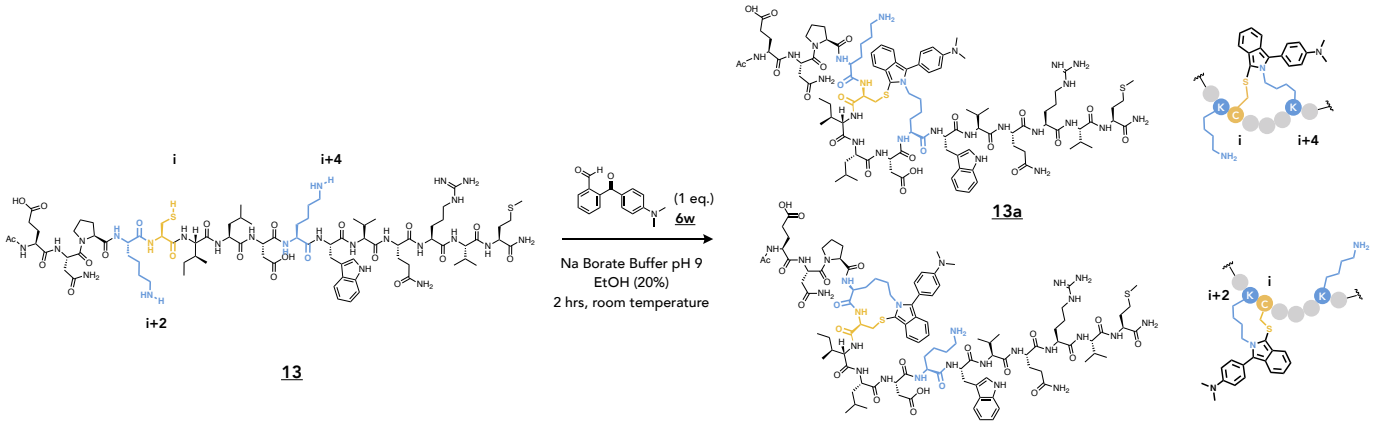


ROESY:



Synthesis and characterization of 13a and 13b

Purified **13** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **13** (2µmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200µL), and to this mixture was added **6w** (50mM solution in DMSO, 40µL, 1 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 1 hour, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **13a** (385nmol, 19% isolated) and **13b** (30nmol, 1% isolated). This reaction was repeated to obtain enough material for characterization.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Chemical Formula: $C_{100}H_{149}N_{25}O_{22}S_2$
Exact Mass: 2116.0750

Time (min)	A%	B%	Flow (mL/min)
0	85	15	20
10	0	100	

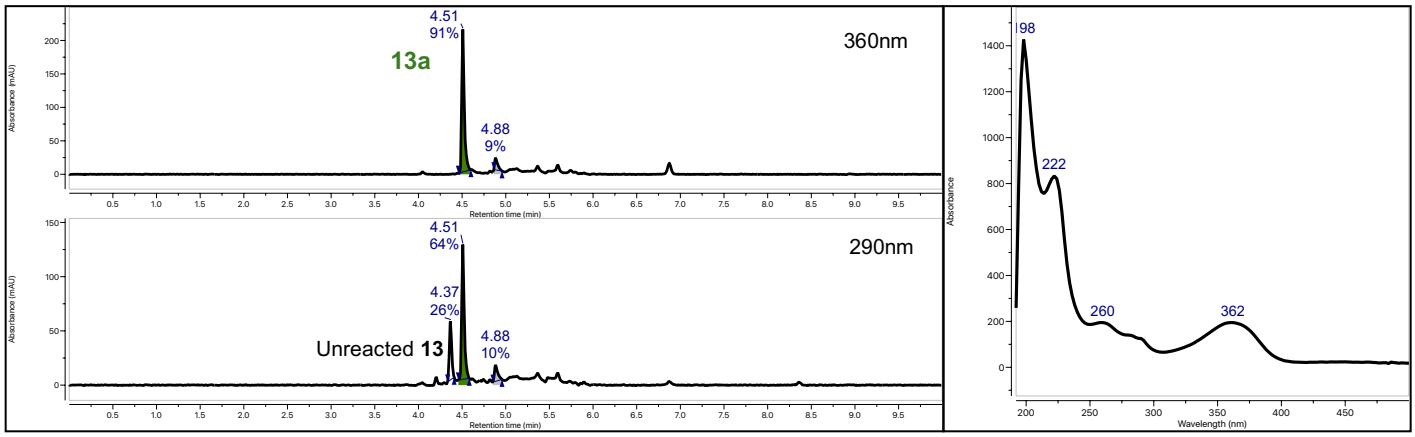


Figure S66: Prep-HPLC traces of crude reaction mixture of peptide **13** with **6w.**

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9,4mm. 5µm

Time (min)	A%	B%	Flow (mL/min)
0	80	30	2
20	0	100	

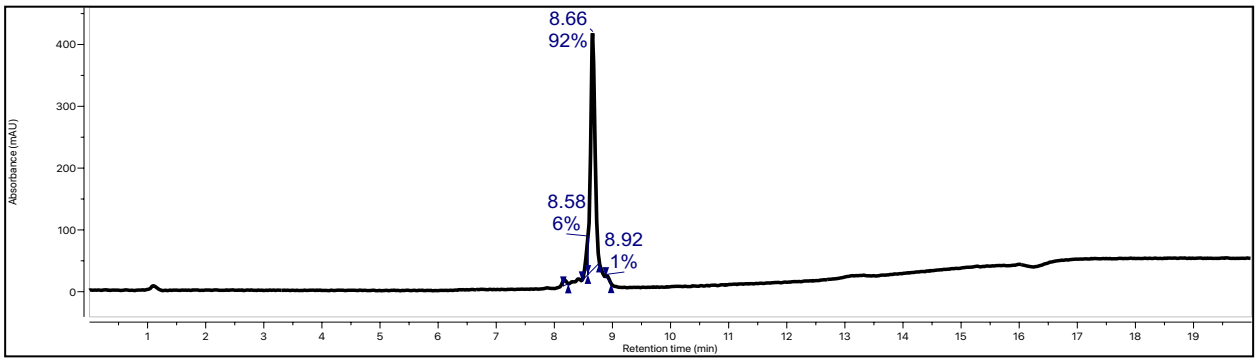


Figure S67A: LC trace of purified compound **13a**. Observed at 230nm.

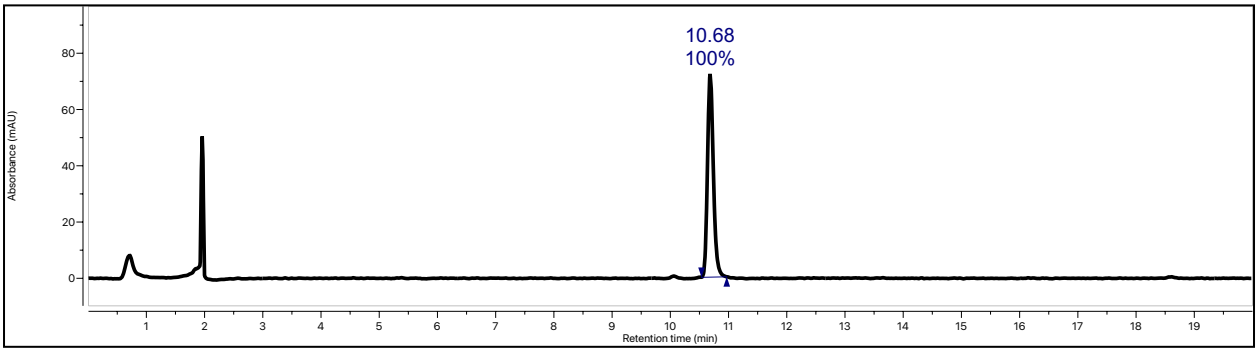
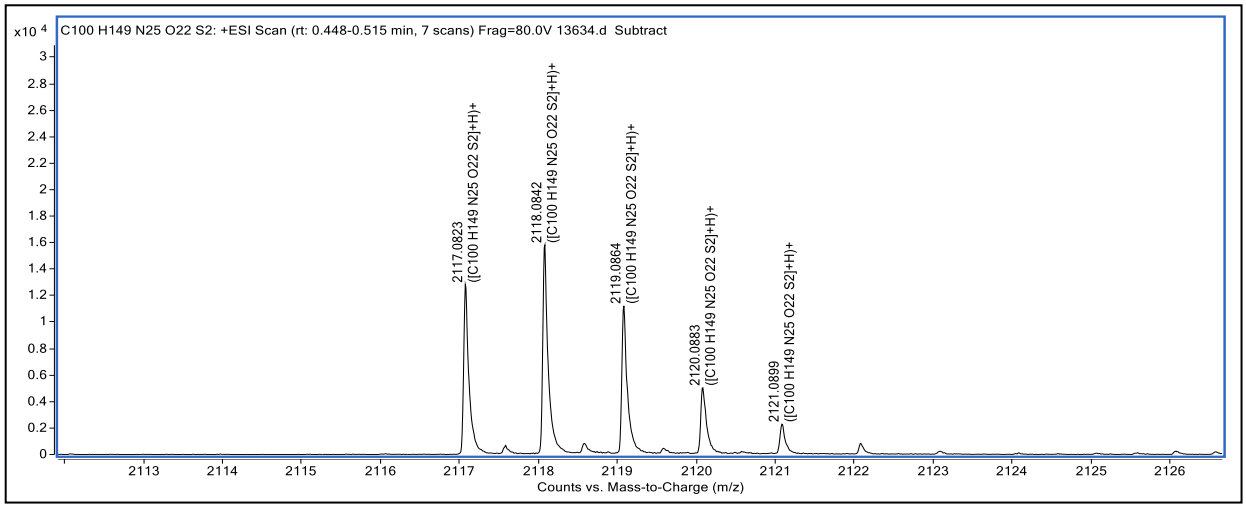
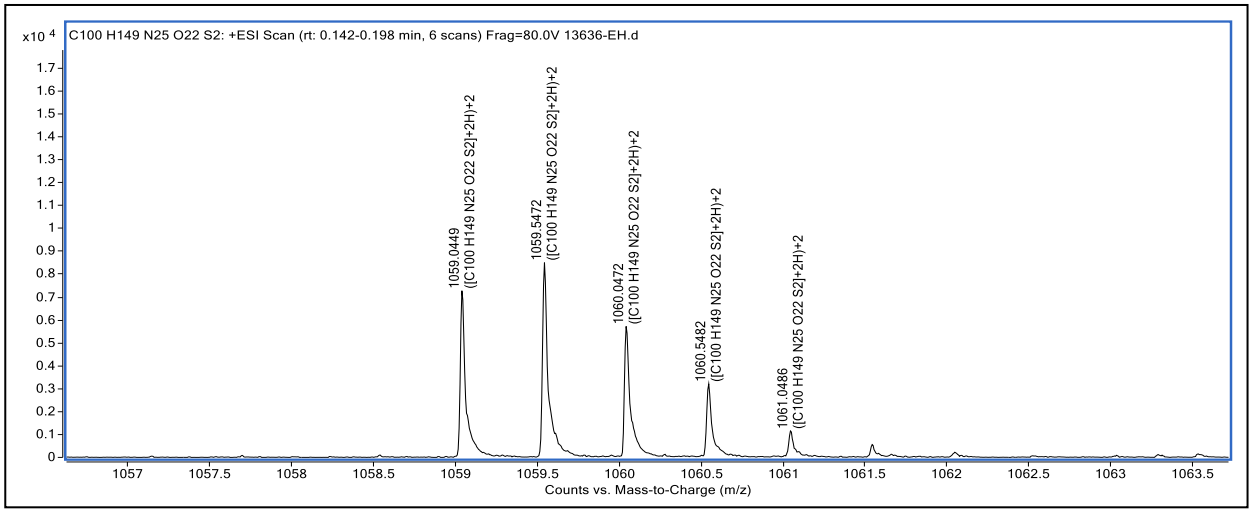


Figure S67B: LC trace of purified compound **13b**. Observed at 230nm.



HRMS of **13a**
Calculated $([C_{100}H_{149}N_{25}O_{22}S_2]+H)^+ = 2117.0829$
Found $([C_{100}H_{149}N_{25}O_{22}S_2]+H)^+ = 2117.0823$

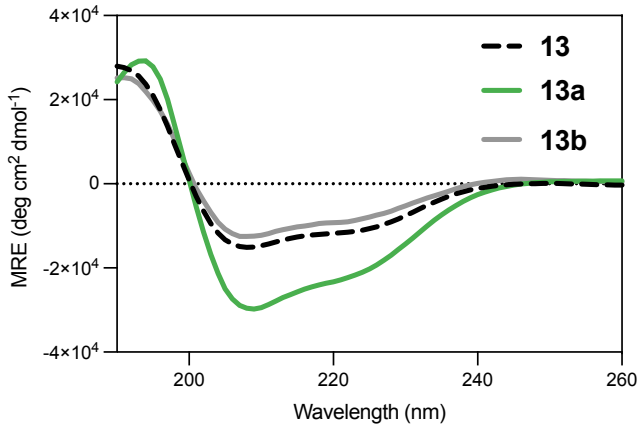


HRMS of **13b**

Calculated $([C_{100}H_{149}N_{25}O_{22}S_2]+2H)/2 = 1059.0453$

Found $([C_{100}H_{149}N_{25}O_{22}S_2]+2H)/2 = 1059.0449$

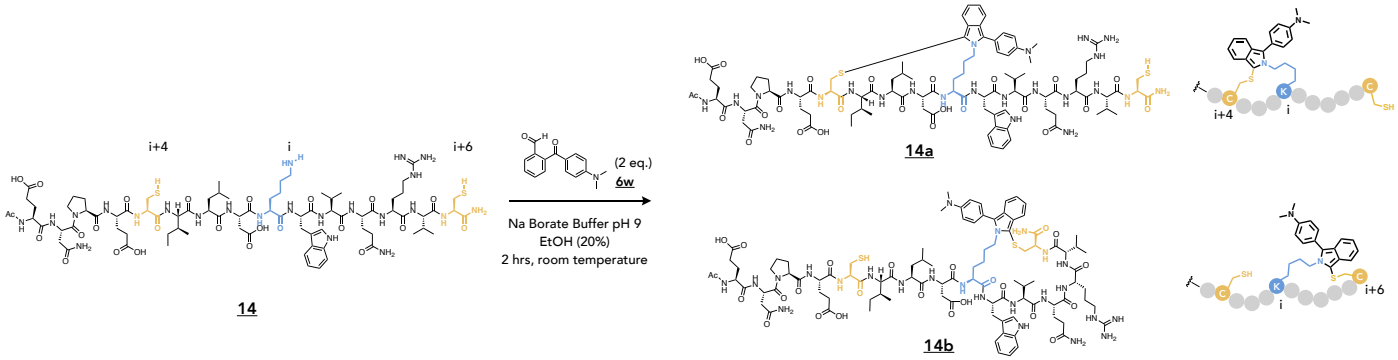
Figure S67C: Circular dichroism of **13, 13a, 13b,** as 50μM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
13	-11500	31
13a	-22300	60
13b	-9000	24

Synthesis and characterization of 14a and 14b

Purified **14** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **14** (2µmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200µL), and to this mixture was added **6w** (50mM solution in DMSO, 80µL, 2 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 1 hour, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **14a** (412nmol, 21% isolated) and **14b** (51nmol, 3% isolated).



Chemical Formula: $C_{97}H_{140}N_{24}O_{24}S_2$
Exact Mass: 2088.9914

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	80	20	30
15	40	60	
17	0	100	

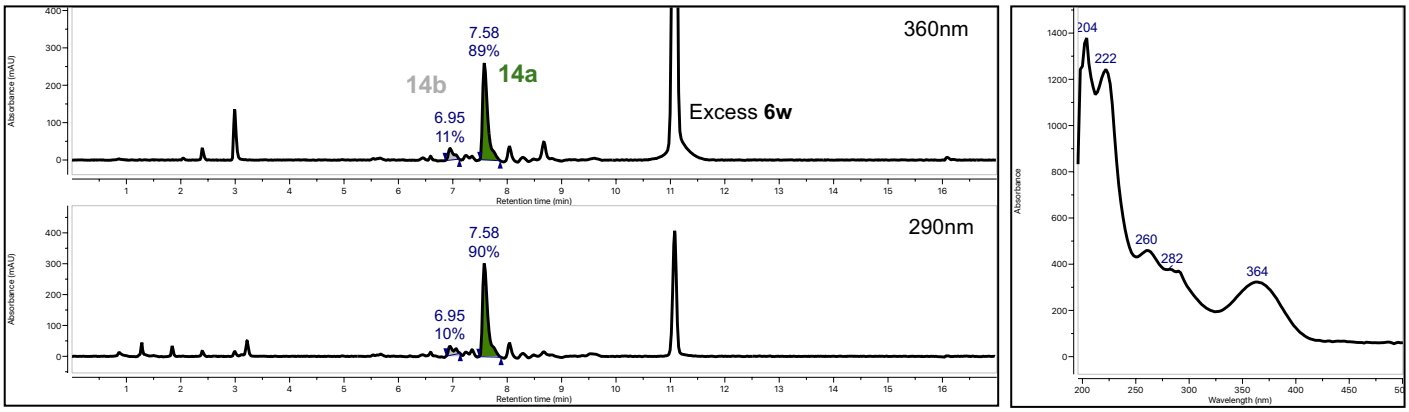


Figure S68: Prep-HPLC traces of reaction mixture of peptide **14** with **6w**

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	70	30	2
20	0	100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

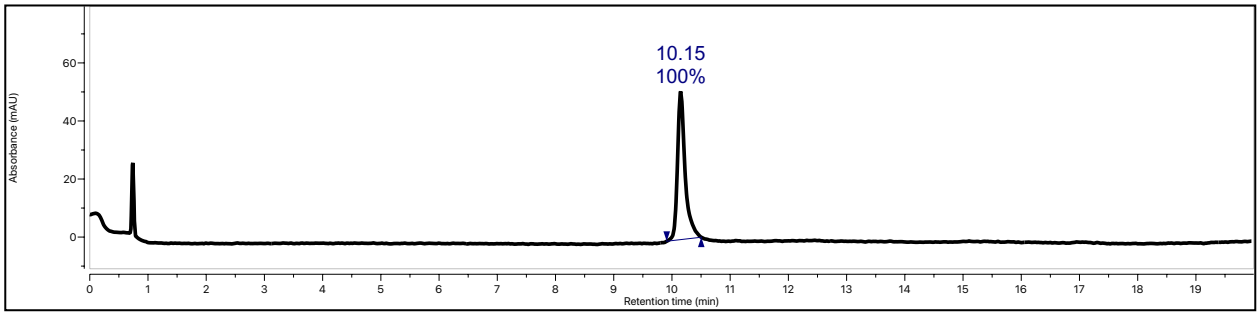


Figure S68A: LC trace of purified compound **14a.** Observed at 230nm.

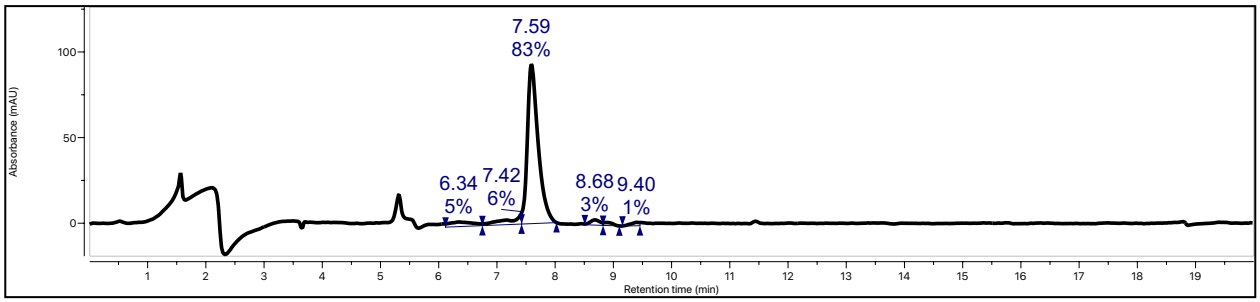
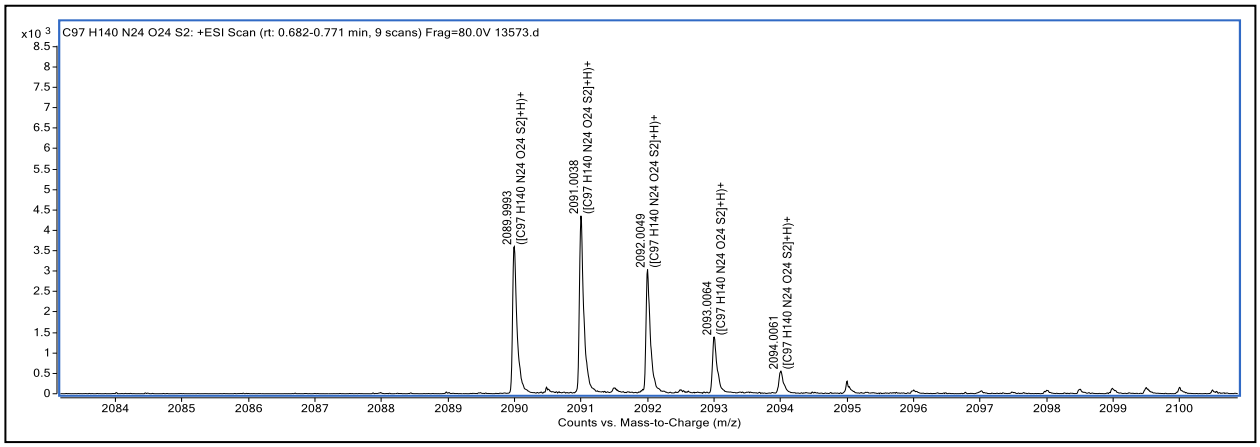
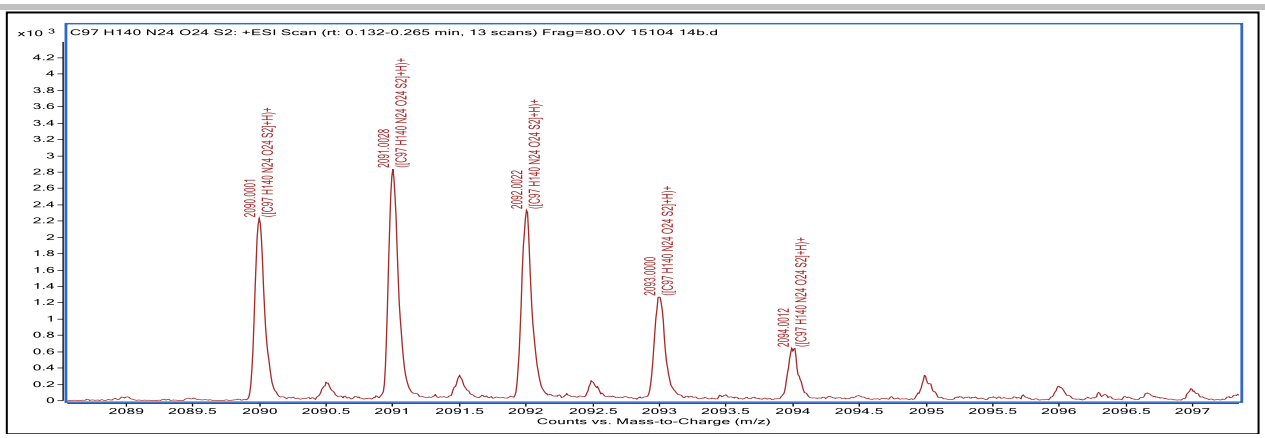


Figure S68B: LC trace of purified compound **14b.** Observed at 230nm.



HRMS of **14a**
Calculated $([C_{97}H_{140}N_{24}O_{24}S_2]+H)^+ = 2089.9992$
Found $([C_{97}H_{140}N_{24}O_{24}S_2]+H)^+ = 2089.9993$

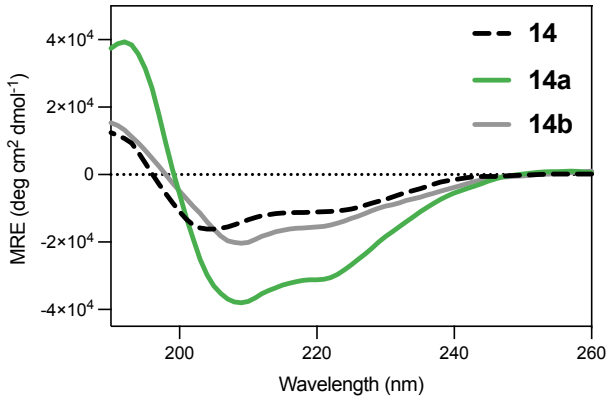


HRMS of **14b**

Calculated $([C_{97}H_{140}N_{24}O_{24}S_2]+H)^+ = 2089.9992$

Found $([C_{97}H_{140}N_{24}O_{24}S_2]+H)^+ = 2090.0001$

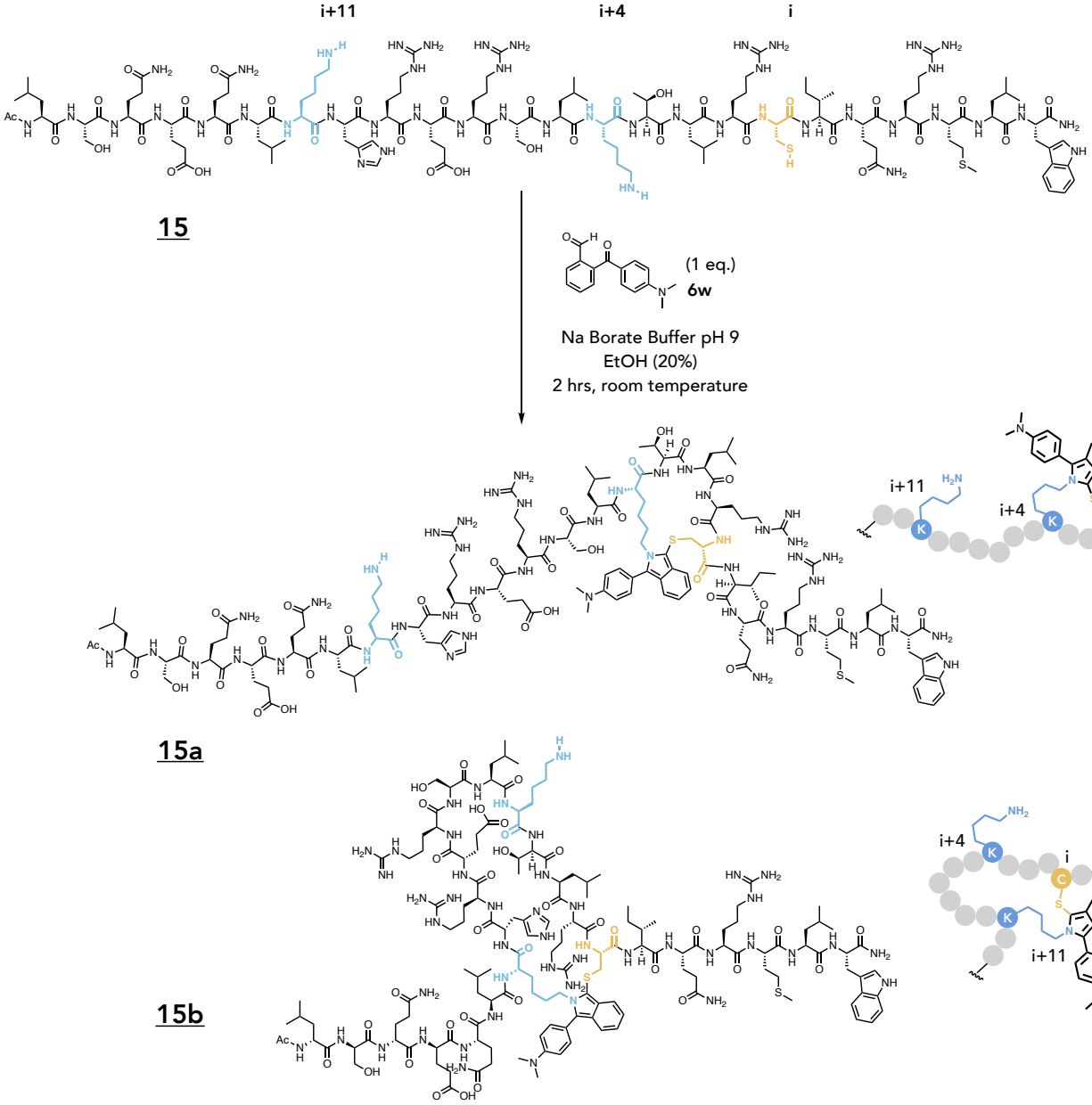
Figure S68C: Circular dichroism of **14**, **14a**, **14b**, as 50μM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
14	-10900	30
14a	-30500	80
14b	-15000	40

Synthesis and characterization of 15a and 15b

Purified **15** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **15** (2µmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200µL), and to this mixture was added **6w** (50mM solution in DMSO, 80µL, 2 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 2 hours, solution became cloudy and the reaction was then quenched and acidified to pH 3 with formic acid, to then afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **15a** (175nmol, 9% isolated) and **15b** (30nmol, 2% isolated). This reaction was repeated with several 2µmol portions of **15** to accumulate enough material for characterization. Low isolated yield largely caused by the nature of the peptide being strongly retained in our chosen column.



Chemical Formula: $C_{150}H_{240}N_{46}O_{35}S_2$
Exact Mass: 3309.7856

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Phenomenex Aeris PEPTIDE XB-C18 Axia Packed (21.2 x 150 mm, 5µm)

Time (min)	A%	B%	Flow (mL/min)
0	80	20	25
10	40	60	
12	0	100	

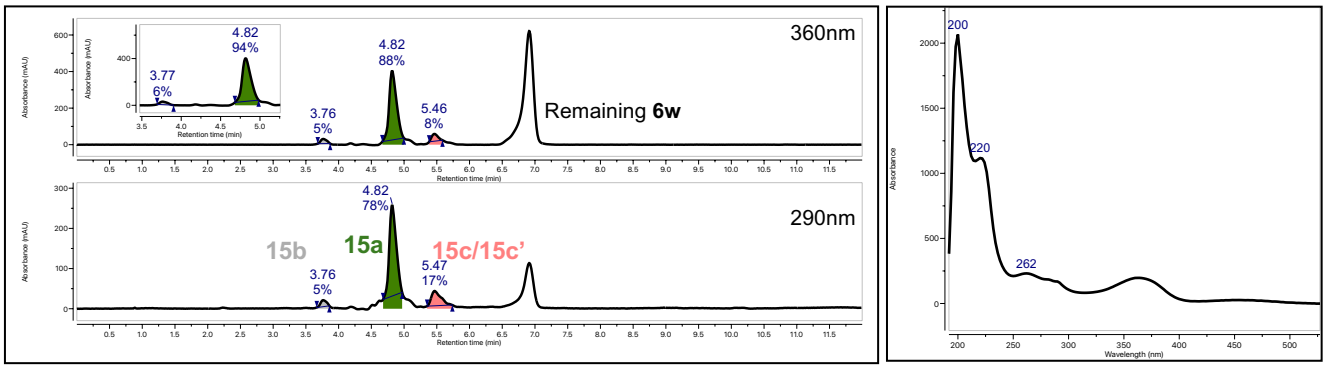
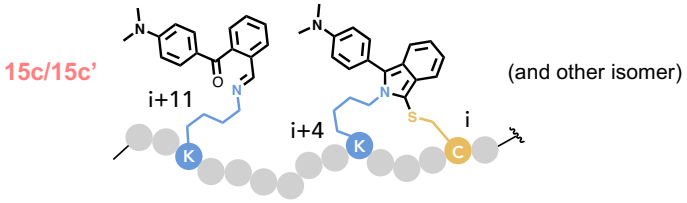


Figure S69: Prep-HPLC traces of reaction mixture of peptide **15** with **6w. 15c/15c'** was determined by HRMS to be the product of over-reaction with **6w**, and regioisomers for this is not resolved.



Chemical Formula: $C_{166}H_{253}N_{47}O_{36}S_2$
Exact Mass: 3544.8853

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	90	30	2
20	0	100	

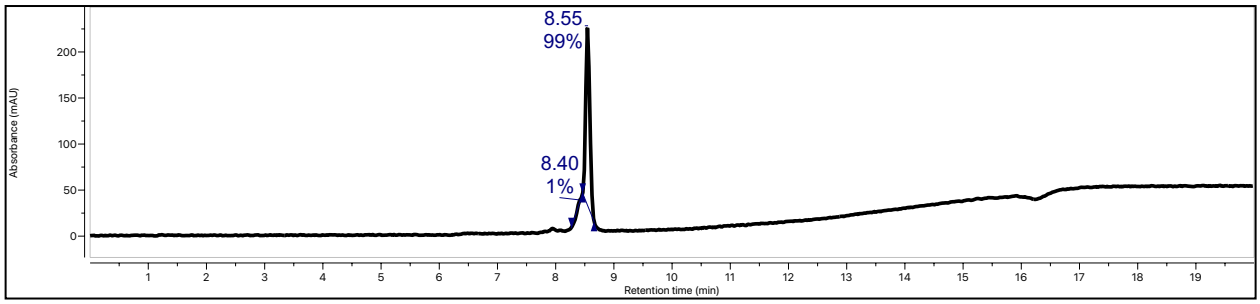


Figure 70A: LC trace of purified compound **15a**. Observed at 230nm.

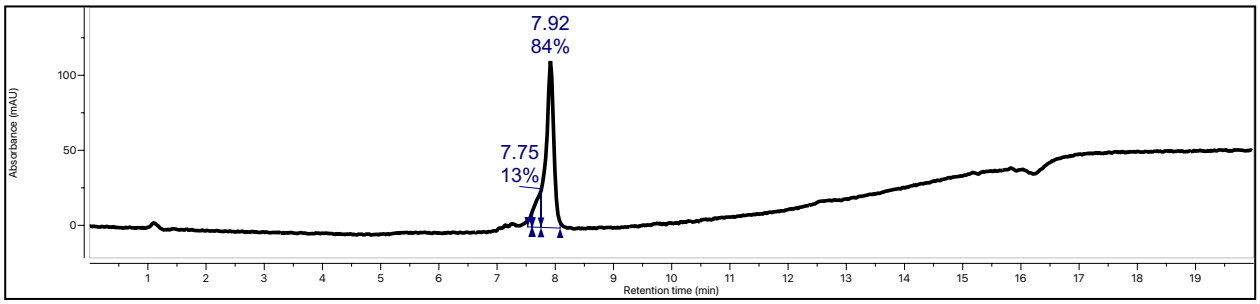
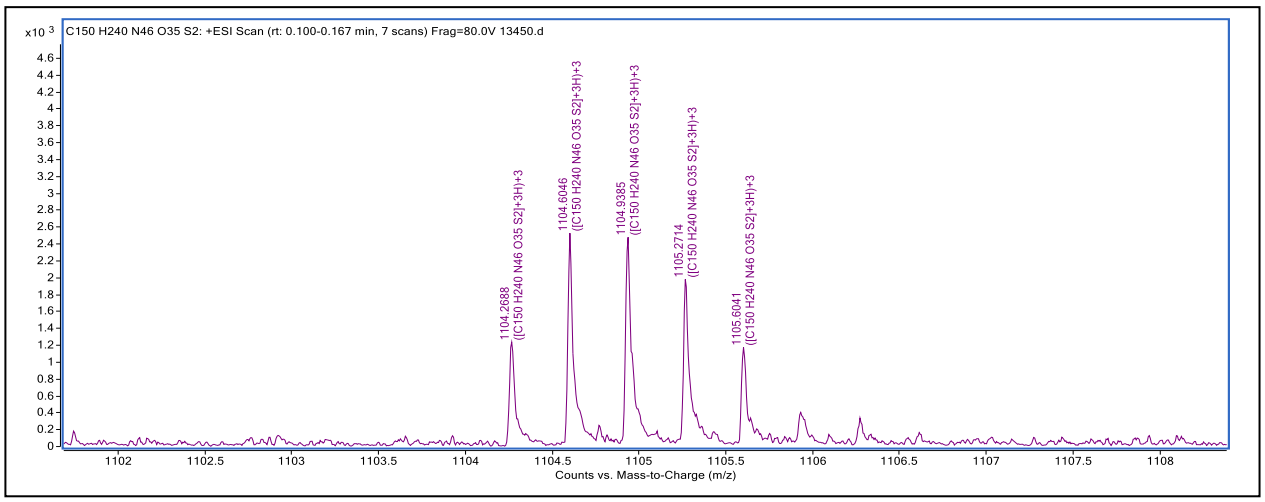


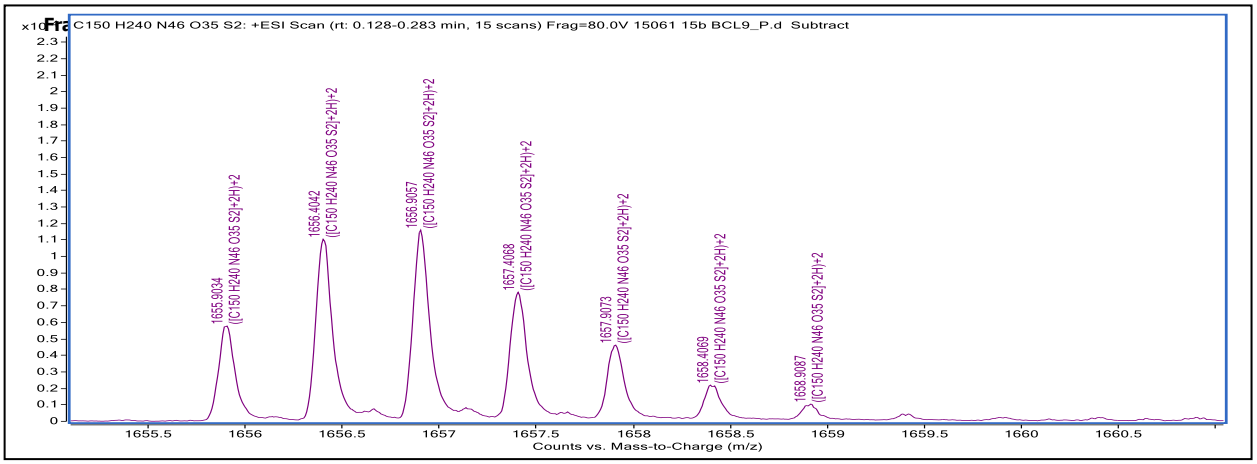
Figure 70B: LC trace of purified compound **15b.** Observed at 230m



HRMS of **15a**

Calculated $([C_{150}H_{240}N_{46}O_{35}S_2]+3H)/3 = 1104.2697$

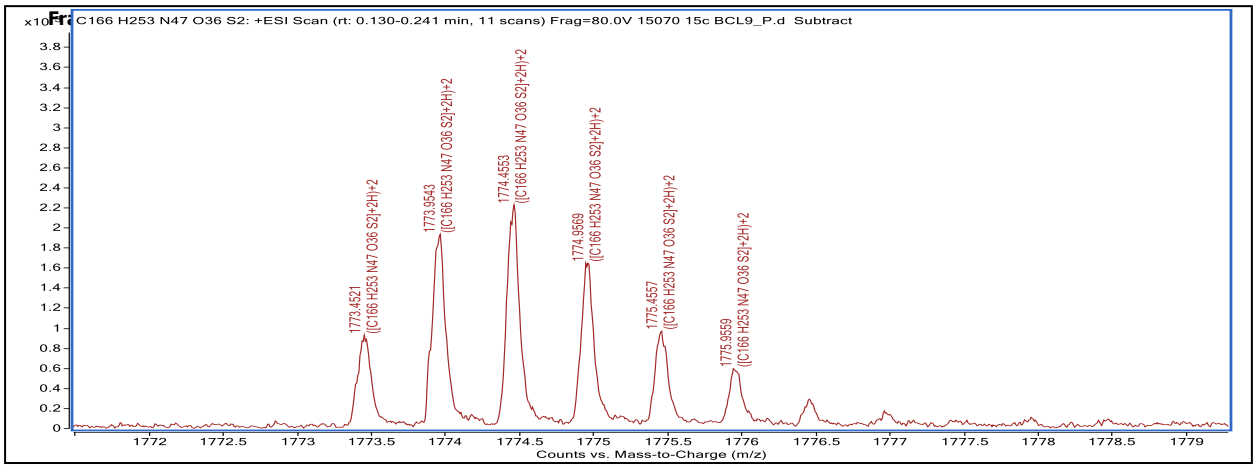
Found $([C_{150}H_{240}N_{46}O_{35}S_2]+3H)/3 = 1104.2688$



HRMS of **15b**

Calculated $([C_{150}H_{240}N_{46}O_{35}S_2]+2H)/2 = 1655.9028$

Found $([C_{150}H_{240}N_{46}O_{35}S_2]+2H)/2 = 1655.9034$

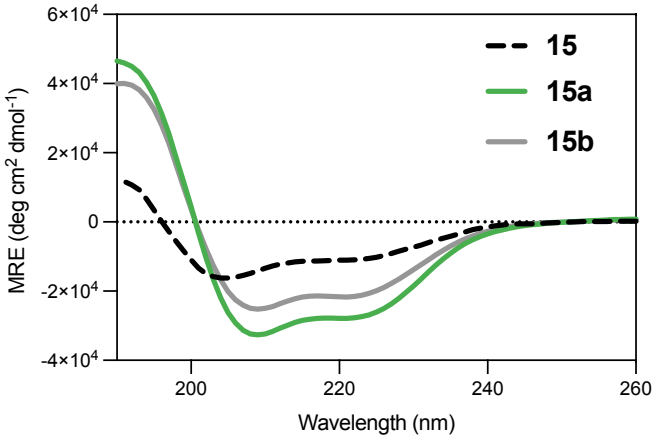


HRMS of **15c/15c'**

Calculated $([C_{166}H_{253}N_{47}O_{36}S_2]+2H)/2 = 1773.4526$

Found $([C_{166}H_{253}N_{47}O_{36}S_2]+2H)/2 = 1773.4521$

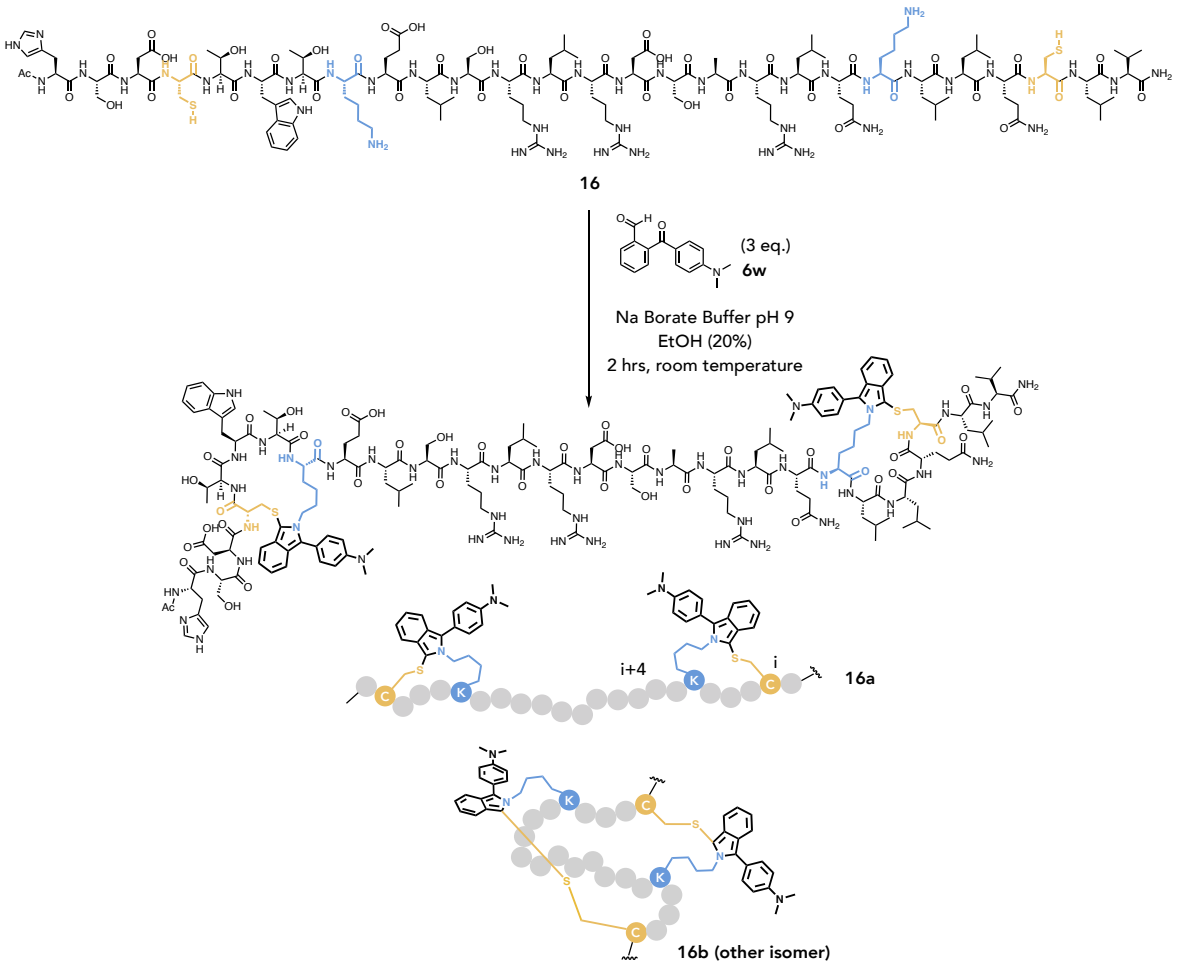
Figure 70C Circular dichroism of **15, 15a, 15b,** as 50μM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
15	-10900	29
15a	-27700	74
15b	-21500	58

Synthesis and characterization of 16a and 16b

Purified **16** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **16** (2µmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200µL), and to this mixture was added **6w** (50mM solution in DMSO, 120µL, 3 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 2 hours, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **16a** (212nmol, 11% isolated) and **16b** (14nmol, <1% isolated). This reaction was repeated to obtain enough material for characterization.



Chemical Formula: $C_{171}H_{256}N_{46}O_{41}S_2$
Exact Mass: 3673.8803

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	25
10	40	60	
12	0	100	

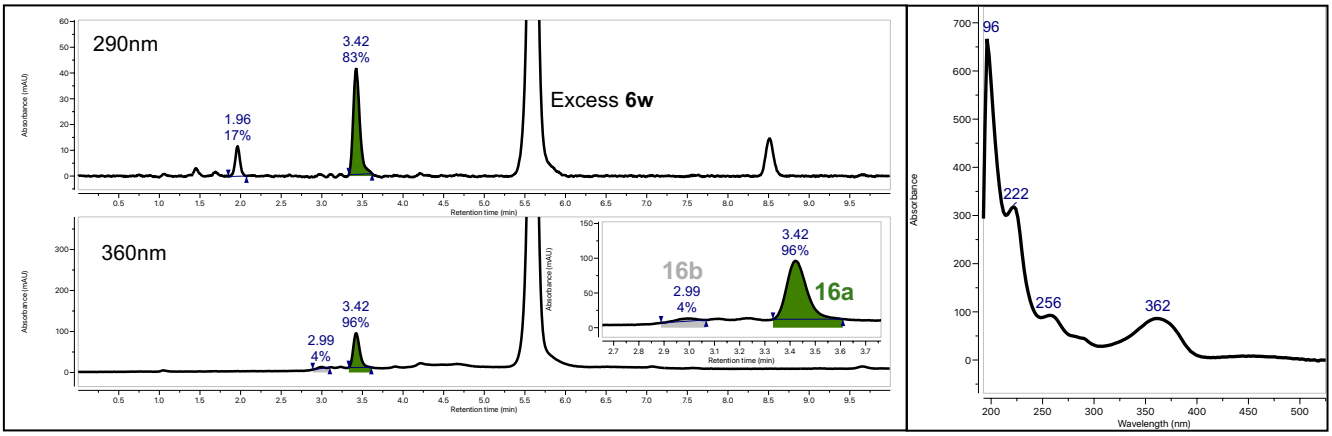


Figure S71: Prep-HPLC traces of reaction mixture of peptide **16** with **6w**

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9,4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	90	40	2
20	0	100	

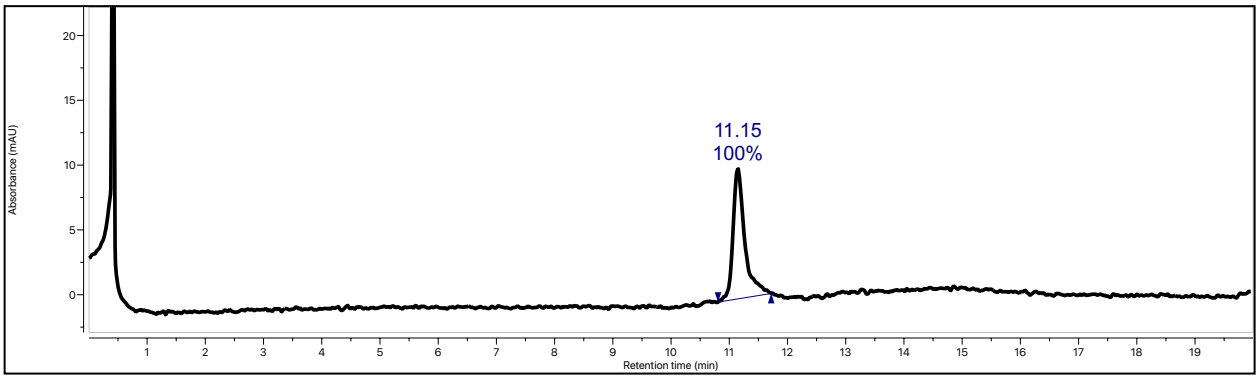


Figure S72A: LC trace of purified compound **16a**. Observed at 230nm.

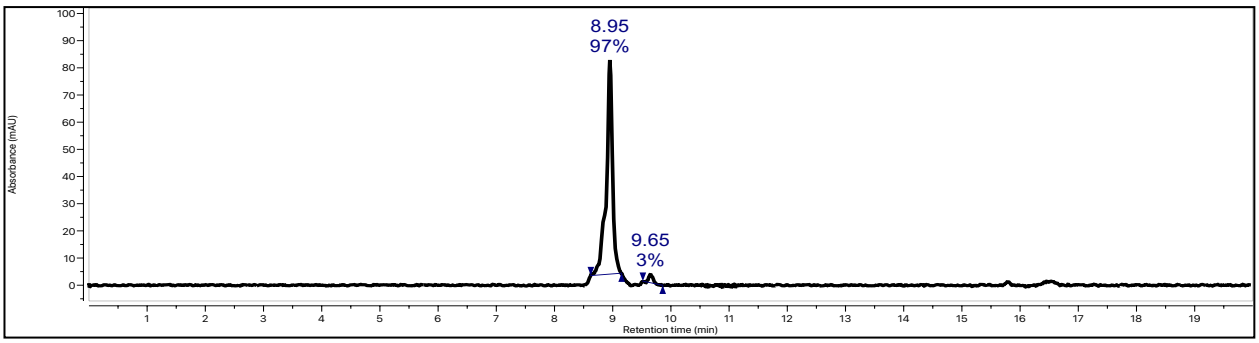
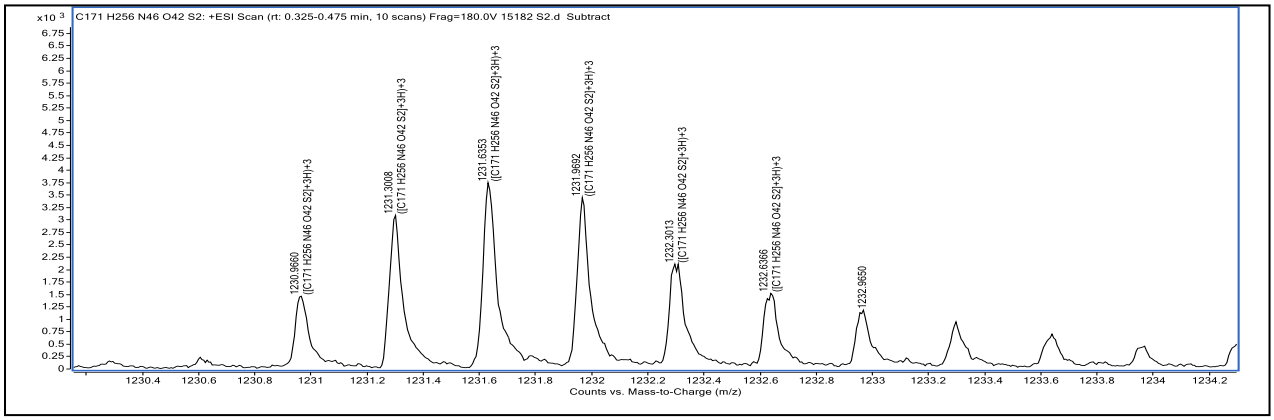


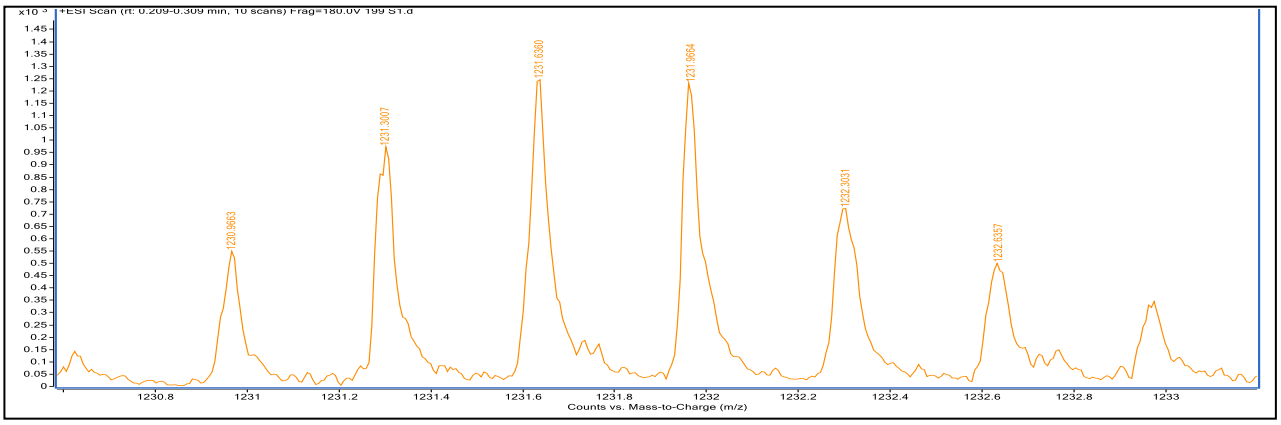
Figure S72B: LC trace of purified compound **16b**. Observed at 230nm.



HRMS of **16a**

Calculated $([C_{171}H_{256}N_{46}42_6S_2]+3H)/3= 1230.9662$

Found $([C_{171}H_{256}N_{46}42_6S_2]+3H)/3 = 1230.9660$

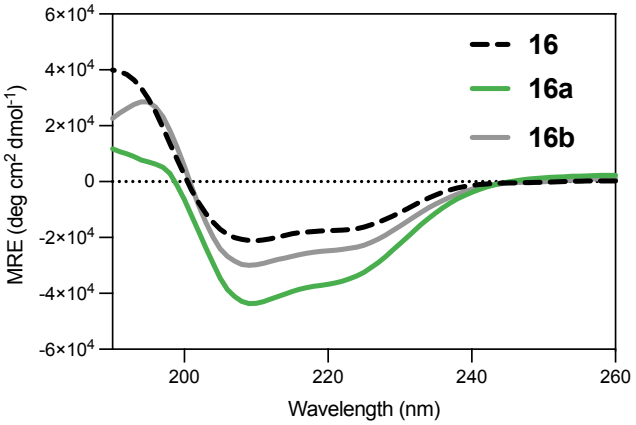


HRMS of **16b**

Calculated $([C_{171}H_{256}N_{46}42_6S_2]+3H)/3 = 1230.9662$

Found $([C_{171}H_{256}N_{46}42_6S_2]+3H)/3 = 1230.9663$

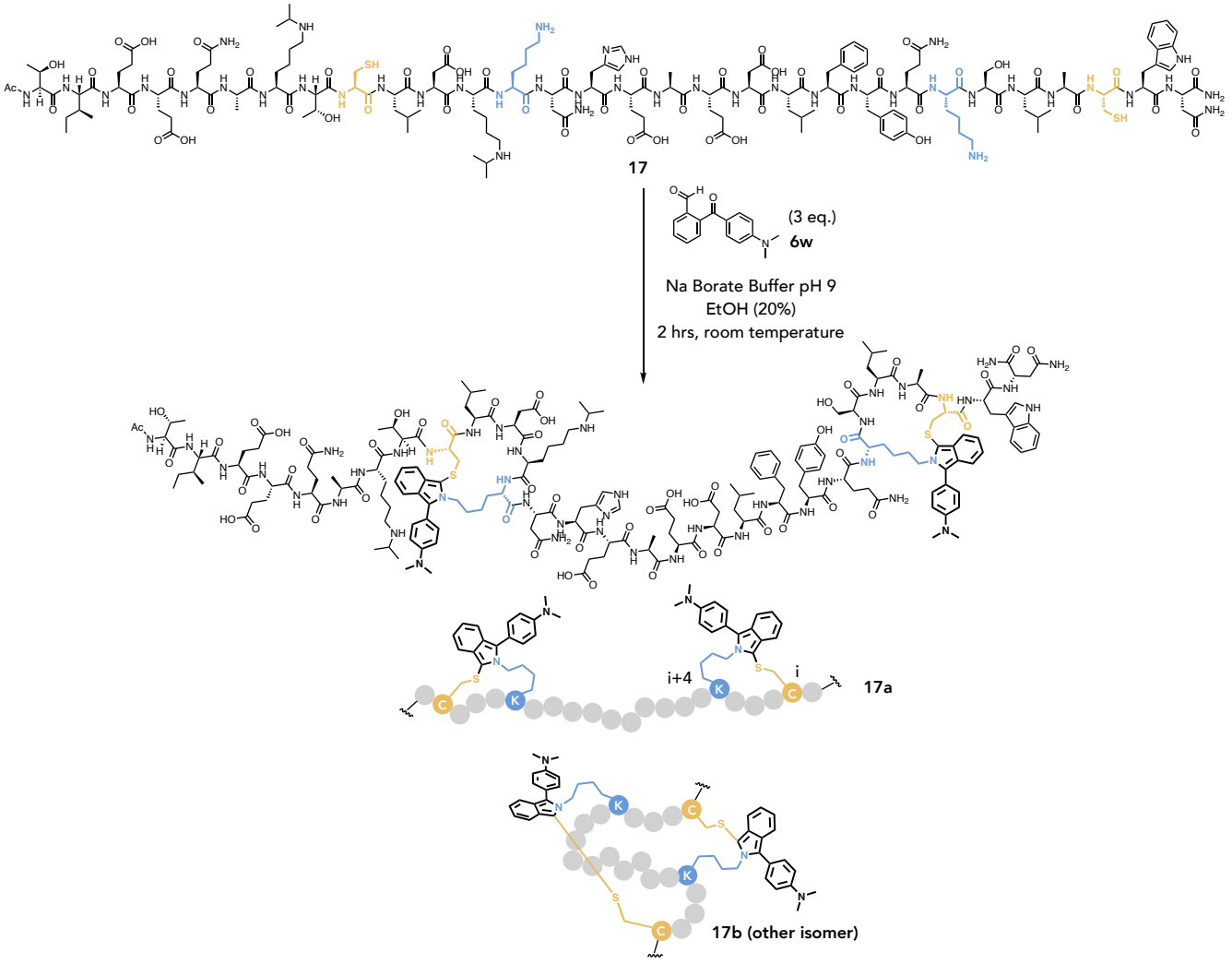
Figure S72C: Circular dichroism of **16, 16a, 16b,** as 50µM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 dmol^{-1})	% Helicity
16	-17400	47
16a	-35600	95
16b	-24200	65

Synthesis and characterization of 17a and 17b

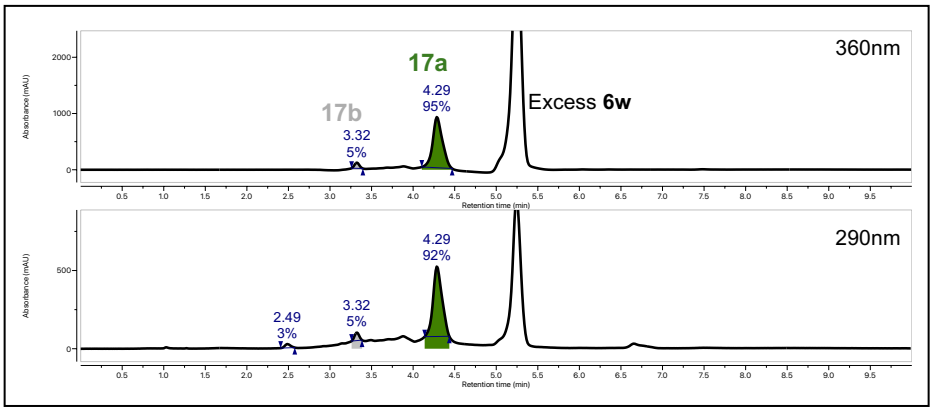
Purified **17** were partitioned into 15mL-falcon tubes in 2μmol portions (1 eq.) and lyophilized to afford dry white powder. **17** (2μmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200μL), and to this mixture was added **6w** (50mM solution in DMSO, 120μL, 3 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was equalitatively monitored by hand-held UV lamp (365nm). After 2 hours, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **17a** (378nmol, 19% isolated) and **17b** (25nmol, 1% isolated). This reaction was repeated to obtain enough material for characterization.



Chemical Formula: $C_{195}H_{276}N_{44}O_{51}S_2$
Exact Mass: 4113.9798

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	15
10	0	100	



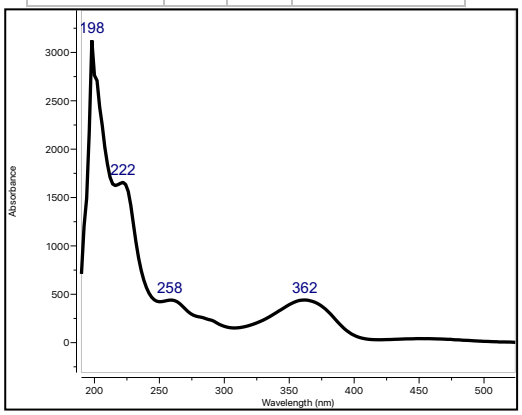


Figure S73: Prep-HPLC traces of reaction mixture of peptide **17** with **6w**

Reinjections of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

Time (min)	A%	B%	Flow (mL/min)
0	90	30	2
20		100	

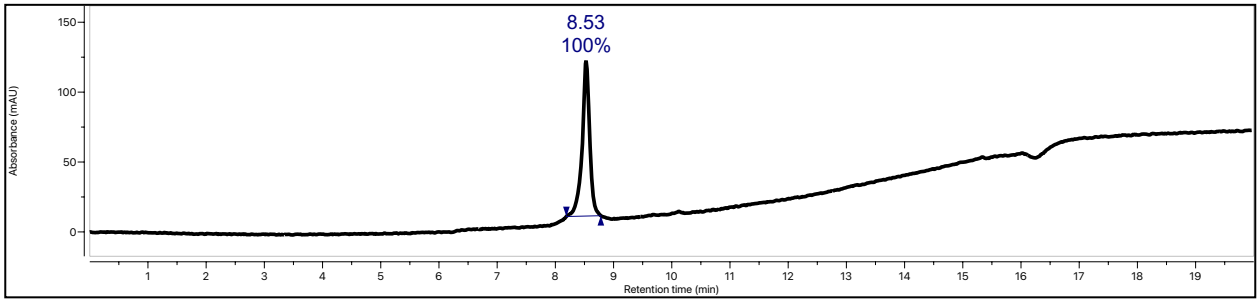


Figure S74A: LC trace of purified compound **17a**. Observed at 230nm.

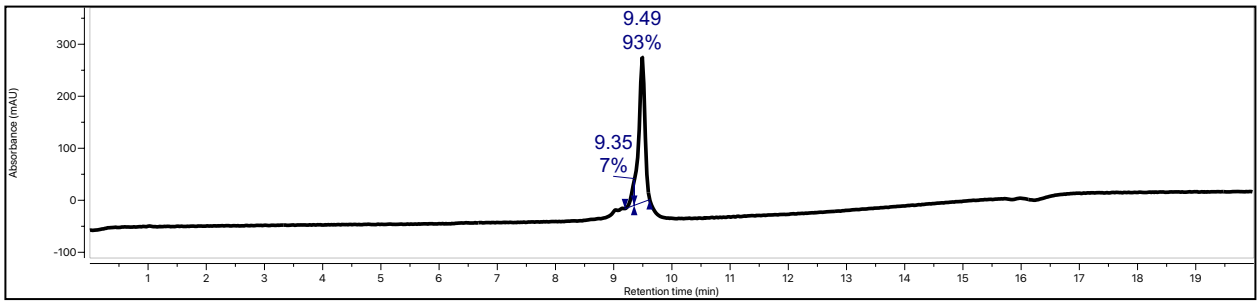
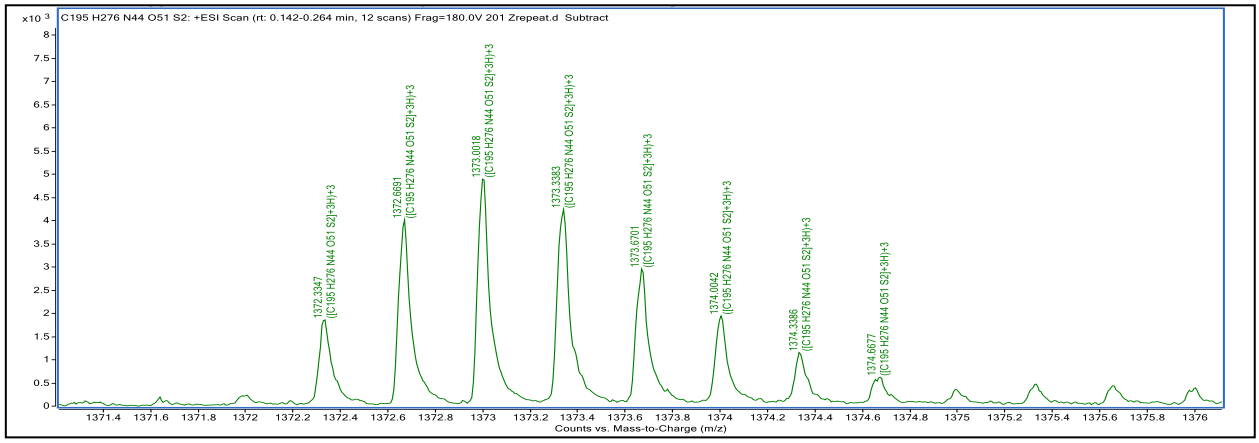


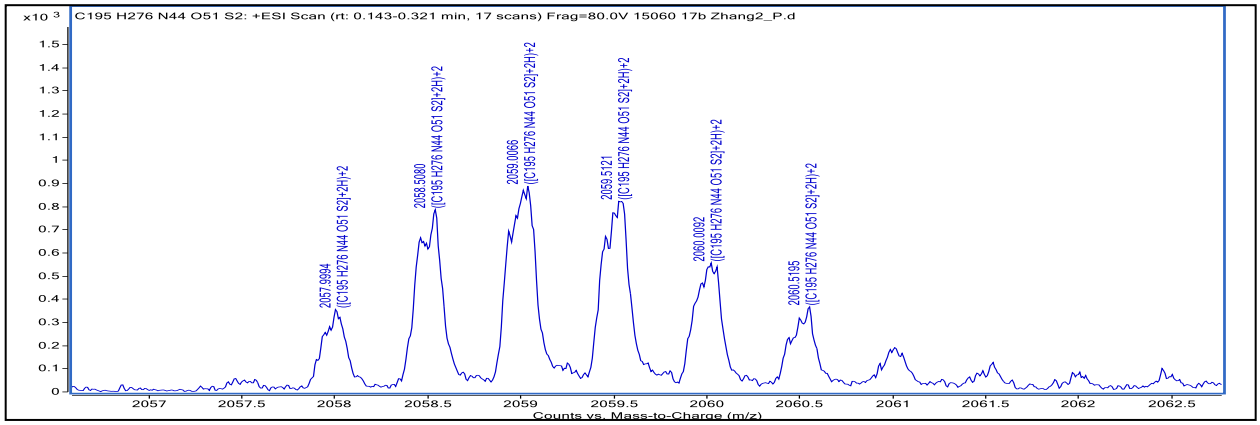
Figure S74B: LC trace of purified compound **17b**. Observed at 230nm.



HRMS of **17a**

Calculated $([C_{195}H_{276}N_{44}O_{51}S_2]+3H)/3 = 1372.3344$

Found $([C_{195}H_{276}N_{44}O_{51}S_2]+3H)/3 = 1372.3347$

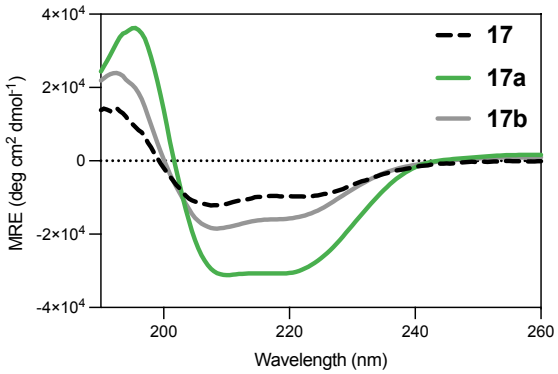


HRMS of **17b**

Calculated $([C_{195}H_{276}N_{44}O_{51}S_2]+2H)/2 = 2057.9999$

Found $([C_{195}H_{276}N_{44}O_{51}S_2]+2H)/2 = 2057.9994$

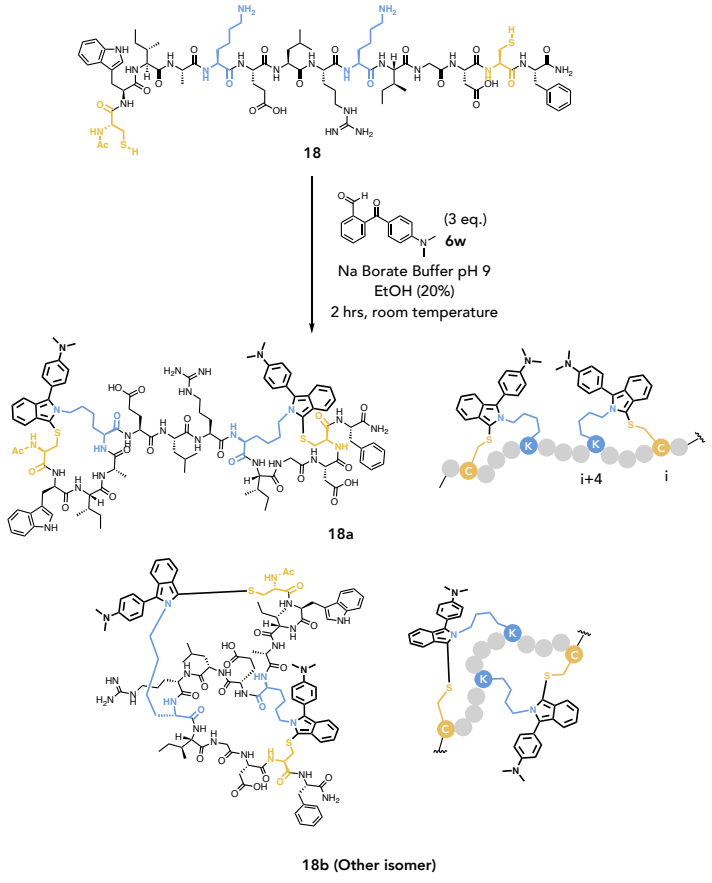
Figure S74C Circular dichroism of **17, 17a, 17b,** as 50μM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
17	-9700	26
17a	-29700	79
17b	-15100	40

Synthesis and characterization of 18a and 18b

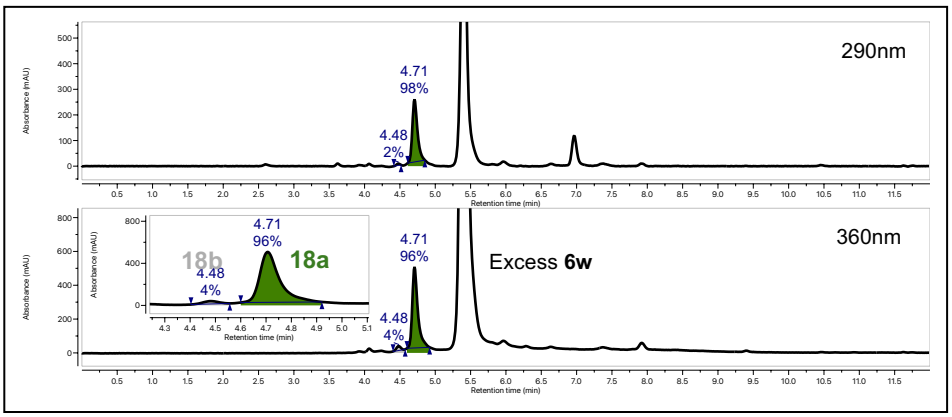
Purified **18** were partitioned into 15mL-falcon tubes in 2μmol portions (1 eq.) and lyophilized to afford dry white powder. **18** (2μmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200μL), and to this mixture was added **6w** (50mM solution in DMSO, 120μL, 3 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 2 hours, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **18a** (290nmol, 15% isolated) and **18b** (18nmol, <1% isolated). This reaction was repeated to obtain enough material for characterization.



Chemical Formula: $C_{110}H_{145}N_{23}O_{19}S_2$
Exact Mass: 2156.0529

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	65	35	15
10	0	100	



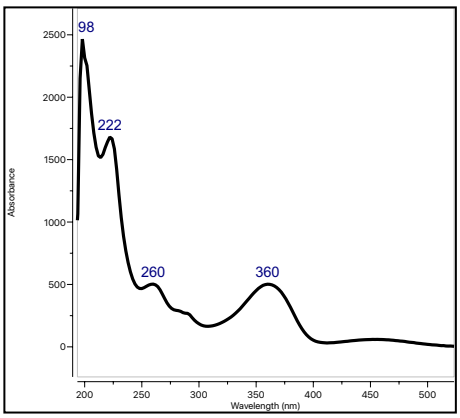


Figure S75: Prep-HPLC traces of reaction mixture of peptide **18** with **6w**

Reinjections of purified material:

Time (min)	A%	B%	Flow (mL/min)
0	65	35	2
20		100	

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, Eclipse XDB-C18, 250x9.4mm, 5µm

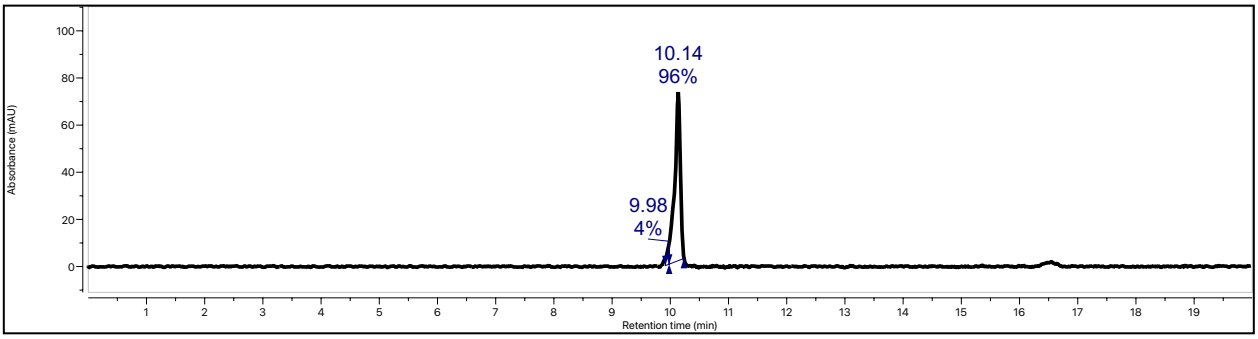


Figure S76A: LC trace of purified compound **18a**. Observed at 230nm.

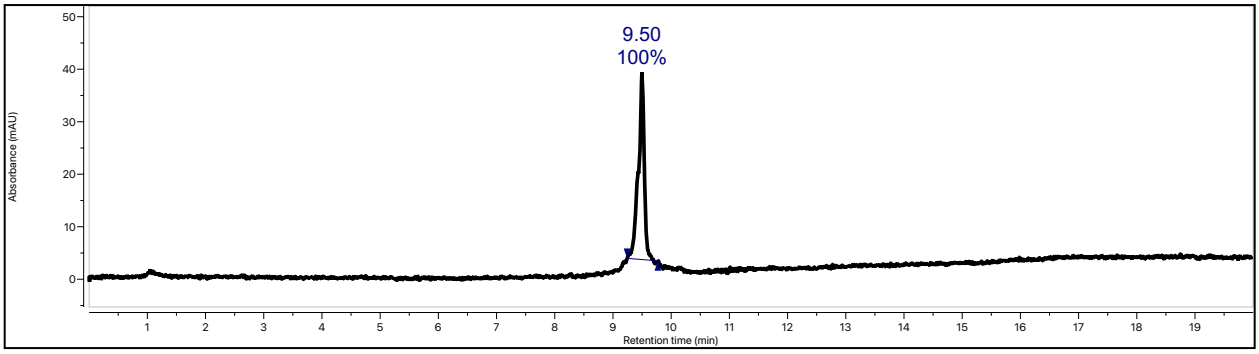
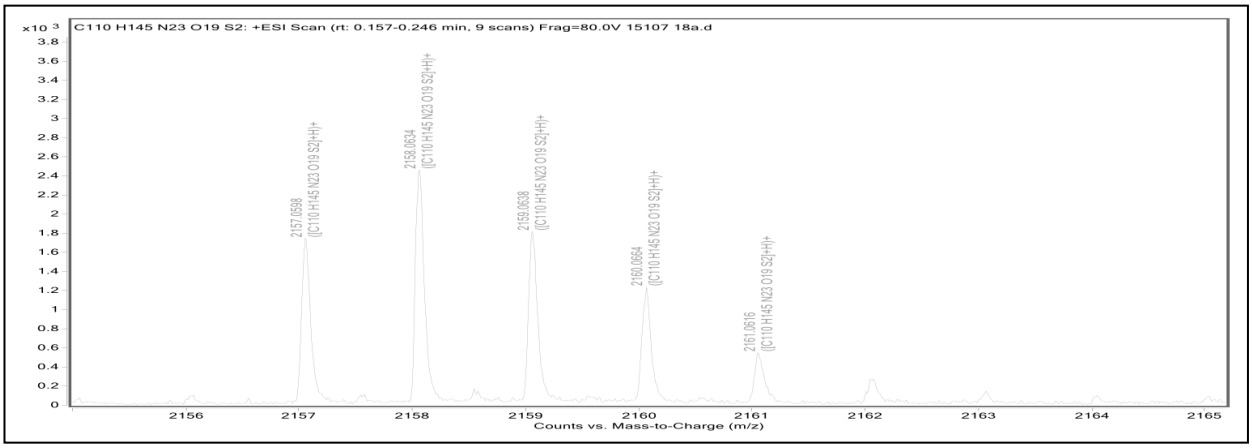
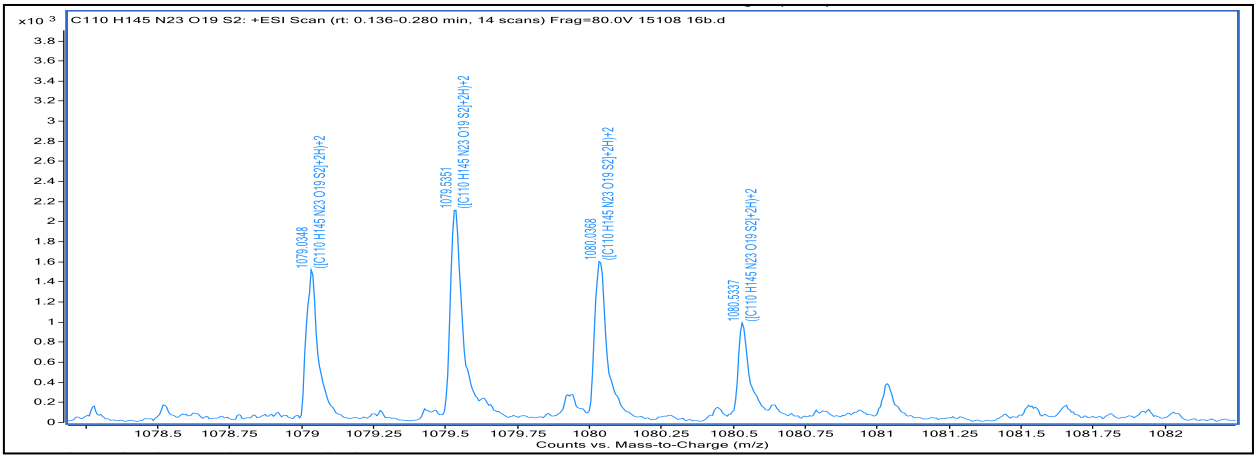


Figure S76B: LC trace of purified compound **18b**. Observed at 230nm.



HRMS of **18a**
Calculated $([C_{110}H_{145}N_{23}O_{19}S_2]+H)^+ = 2157.0607$
Found $([C_{110}H_{145}N_{23}O_{19}S_2]+H)^+ = 2157.0598$

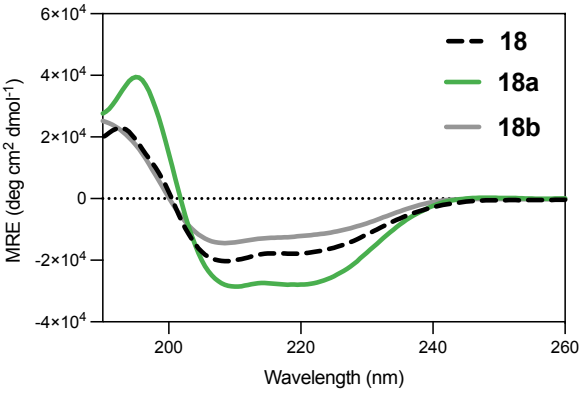


HRMS of **18b**

Calculated $([C_{110}H_{145}N_{23}O_{19}S_2]+2H)/2 = 1079.0343$

Found $([C_{110}H_{145}N_{23}O_{19}S_2]+2H)/2 = 1079.0348$

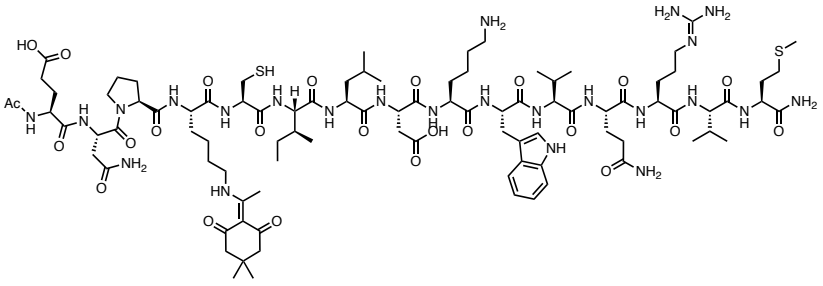
Figure S76C Circular dichroism of **18, 18a, 18b,** as 50μM solutions in TFE/H_2O (20:80)



Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
18	-17300	46
18a	-27500	73
18b	-11700	31

Proving positional selectivity using orthogonal protecting group strategy:

Linear peptide **19** was synthesized according to the general procedure for solid phase peptide synthesis, using 114mg Rink Amide MBHA resin (40μmol scale synthesis). The resulting crude material was purified via preparative HPLC using the given parameters to afford pure **19** (13μmol, 33%), to be used for subsequent FIICk reaction.



Chemical Formula: $C_{94}H_{150}N_{24}O_{24}S_2$
Exact Mass: 2063.0696
Molecular Weight: 2064.4980

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

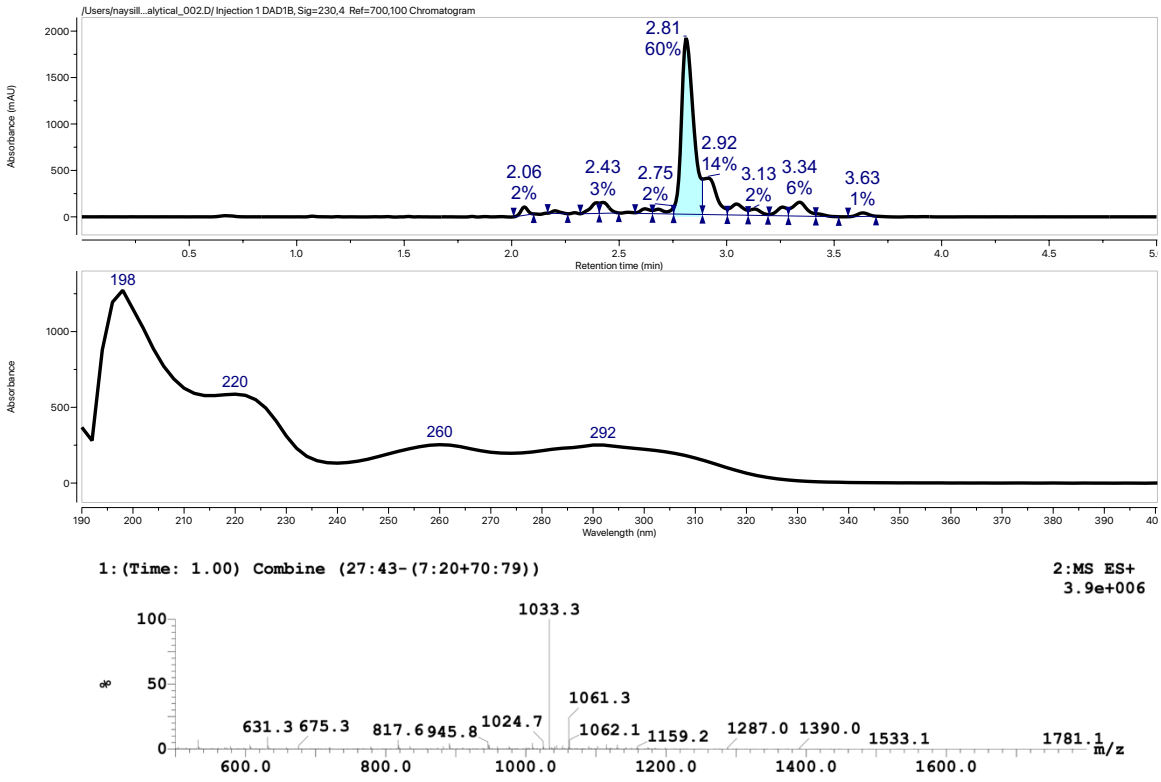
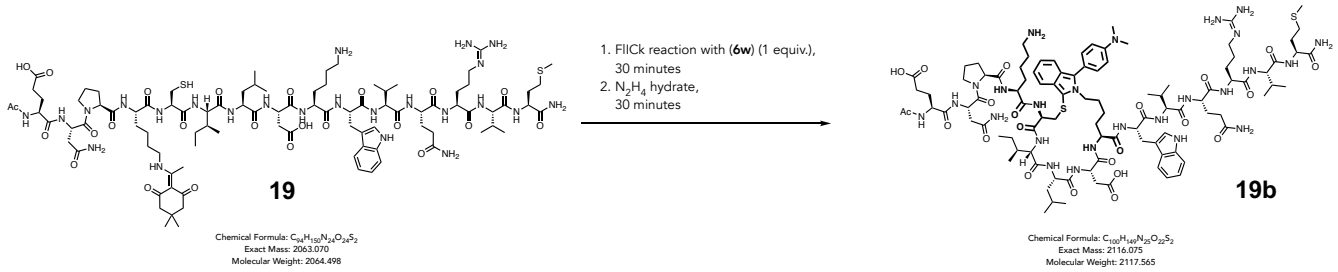


Figure S77: Crude HPLC trace observed at 230nm and the corresponding UV excitation profile and LRMS of the major peak (t=2.81min).

Purified **19** were partitioned into 15mL-falcon tubes in 2μmol portions (1 eq.) and lyophilized to afford dry white powder. **19** (2μmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200μL), and to this mixture was added **6w** (50mM solution in DMSO, 80μL, 2 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 30 minutes, an aliquot (200nmol) of the reaction was quenched and acidified to pH 3 with formic acid and analysed by HPLC to determine peak conversion. The remaining peptide in FIICk reaction mixture was then treated with N_2H_4 hydrate (200μL, 50%, v/v) and left to sit in the same falcon tube for 30 minutes at room temperature. Following this Dde-cleavage, reaction was acidified with formic acid and turned into a bright clear yellow solution. Purification by prep-HPLC afforded pure **19b**.



Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	20
6	0	100	

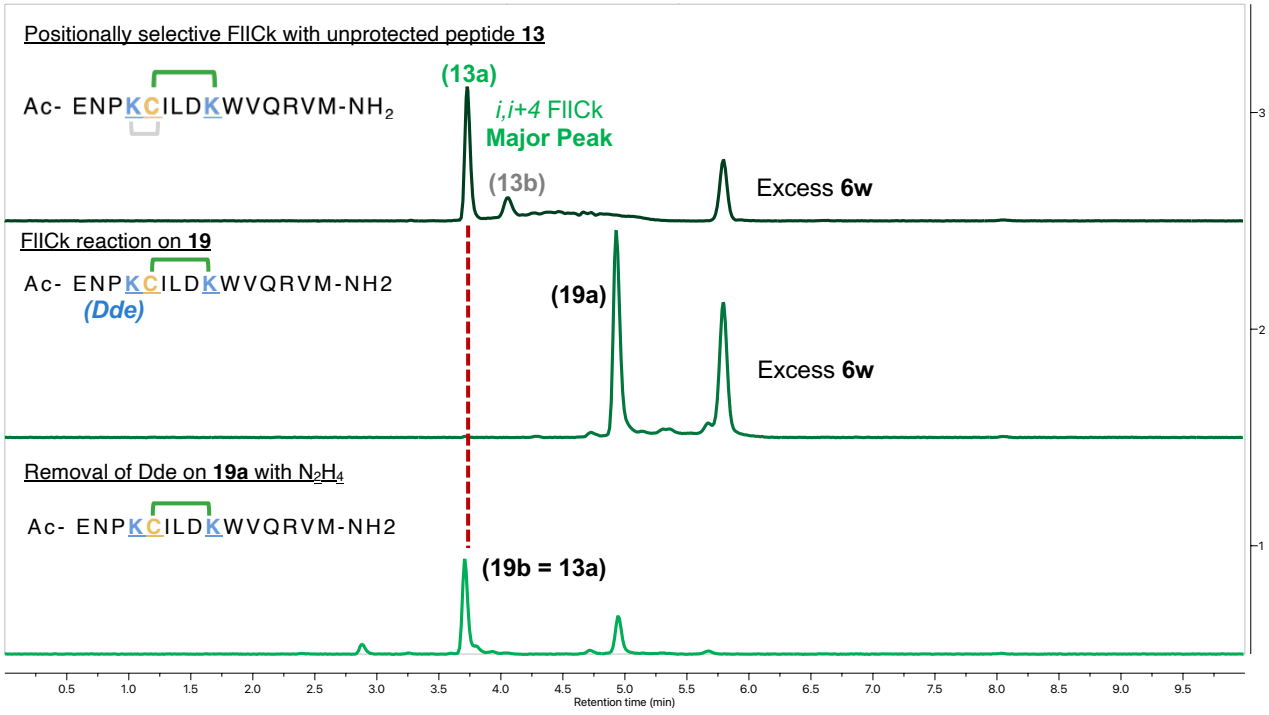


Figure S78A: Overlaid prep-HPLC traces of reaction mixture of peptide **13**, **19** with **6w** and following the one-pot Dde cleavage with N_2H_4 treatment.

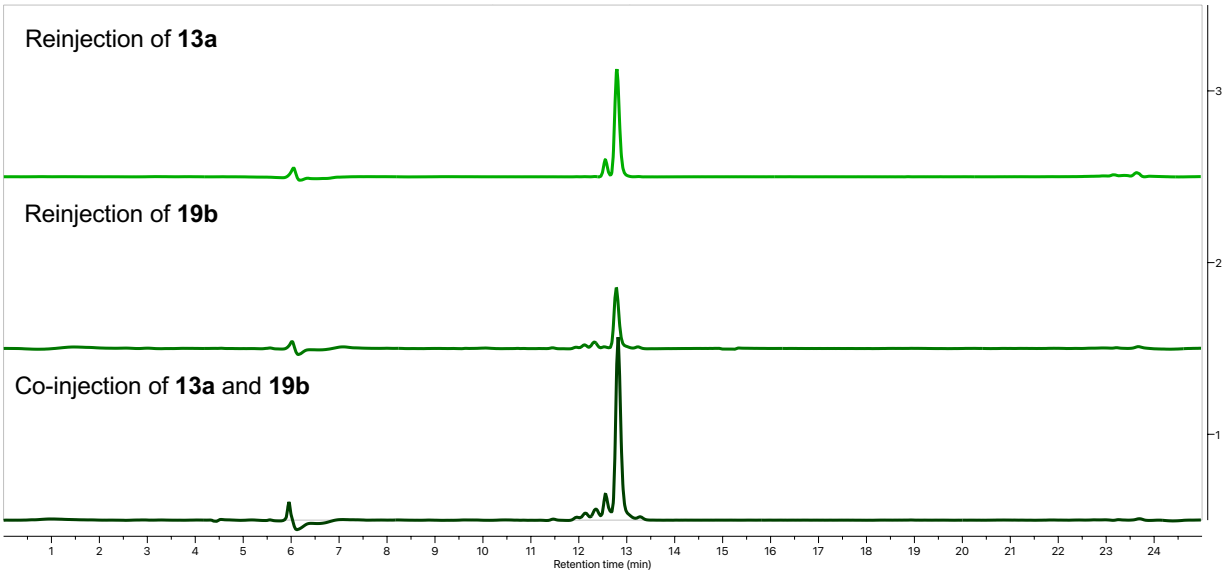
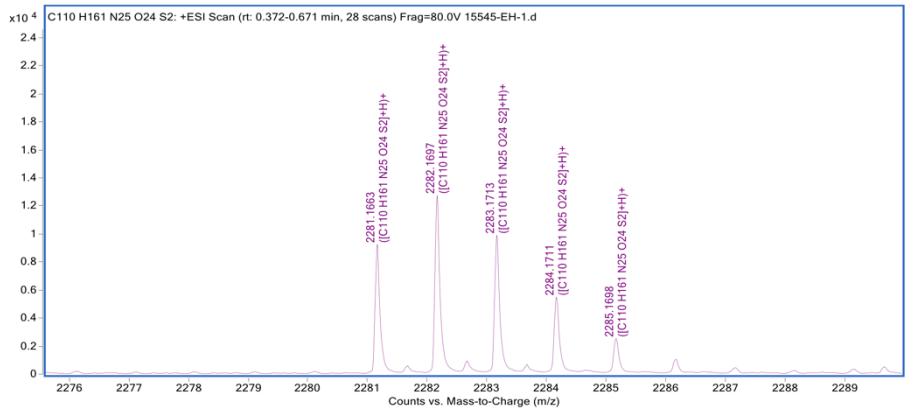


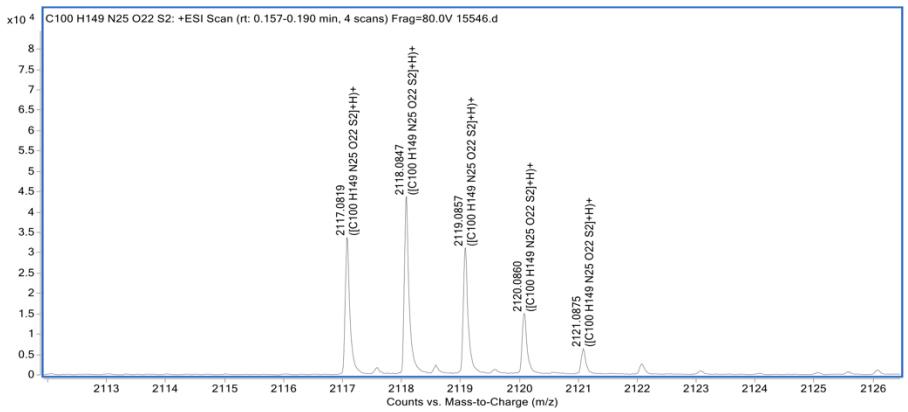
Figure S78B: Overlaid prep-HPLC traces observed at 230nm



HRMS of **19a**

Calculated $([C_{110}H_{161}N_{25}O_{24}S_2]+H) = 2281.1666$

Found $([C_{110}H_{161}N_{25}O_{24}S_2]+H) = 2281.1663$



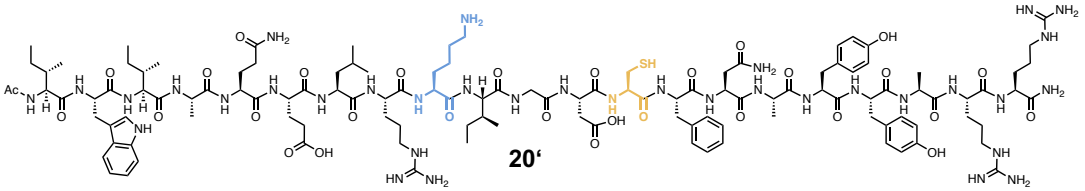
HRMS of **19b**

Calculated $([C_{100}H_{149}N_{25}O_{22}S_2]+H) = 2117.0829$

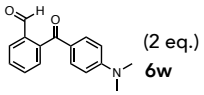
Found $([C_{100}H_{149}N_{25}O_{22}S_2]+H) = 2117.0819$

Synthesis and characterization of 20b

Purified **20'** were partitioned into 15mL-falcon tubes in 2µmol portions (1 eq.) and lyophilized to afford dry white powder. **20'** (2µmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200µL), and to this mixture was added **6w** (50mM solution in DMSO, 80µL, 2 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 2 hours, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **20b** as pale yellow powder (227nmol, 11% isolated). This peptide was tested against **20a** for Jurkat cell viability.

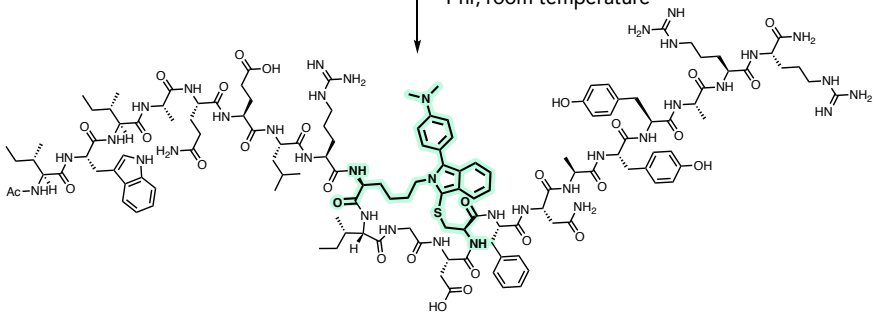


20'



(2 eq.)
6w

Na Borate Buffer pH 9
EtOH (20%)
1 hr, room temperature



20b

Chemical Formula: $C_{136}H_{194}N_{36}O_{30}S$
Exact Mass: 2843.4482

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	50	50	30
6	0	100	

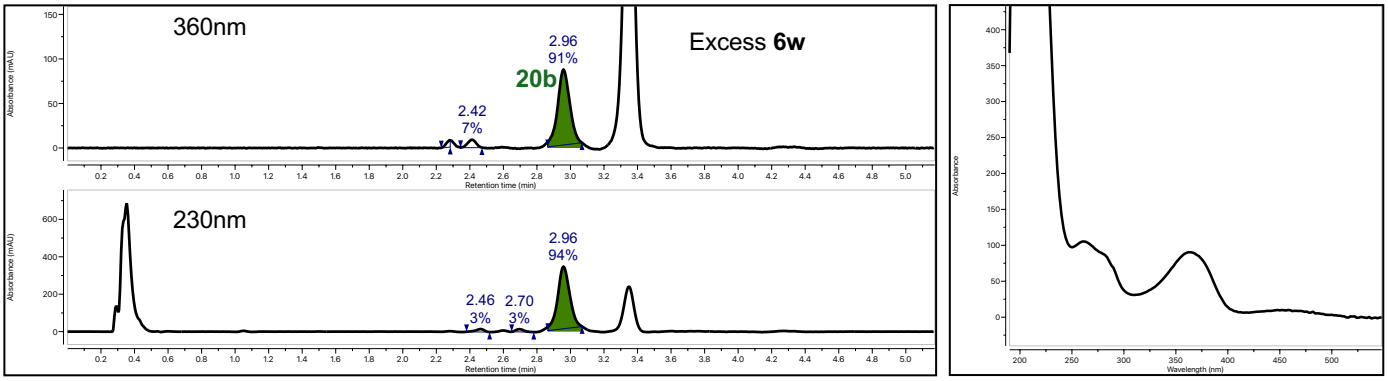


Figure S79: Prep-HPLC traces of reaction mixture of peptide **20'** with **6w**

Reinjection of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent Eclipse XDB-C18 250x9.4 mm

Time (min)	A%	B%	Flow (mL/min)
0	80	20	1
20	0	100	
30	80	20	

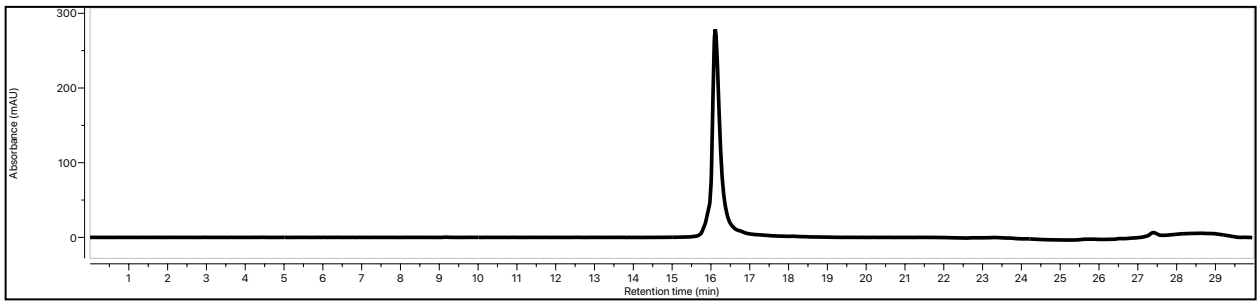
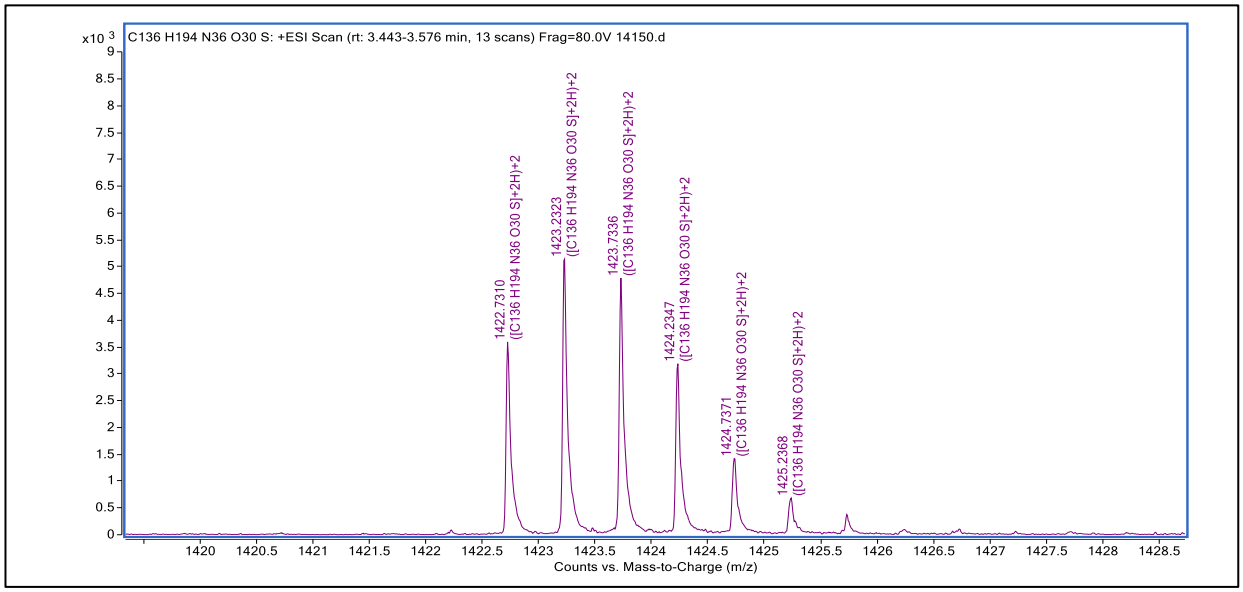
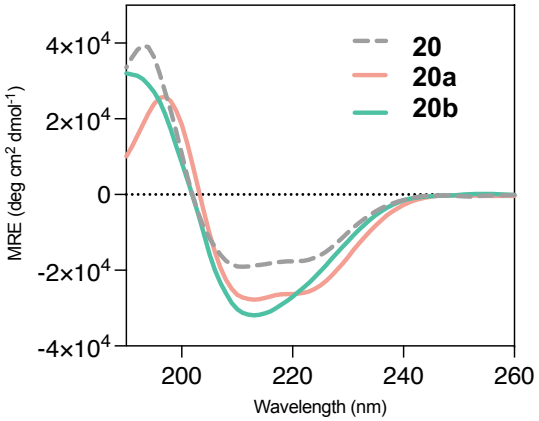


Figure S80A: LC trace of purified compound **20b**. Observed at 230nm



HRMS of **20b**
Calculated $([C_{136}H_{194}N_{36}O_{30}S]+2H)/2 = 1422.7319$
Found $([C_{136}H_{194}N_{36}O_{30}S]+2H)/2 = 1422.7310$

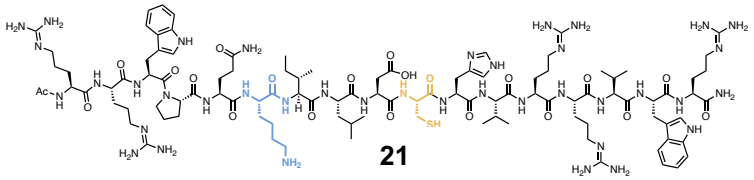
Figure S80B Circular dichroism of **20**, **20a**, **20b**, as 50μM solutions in TFE/H_2O (20:80)

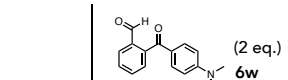


Compound	MRE (222nm) (deg cm^2 $dmol^{-1}$)	% Helicity
20	-17500	47
20a	-25900	69
20b	-24500	66

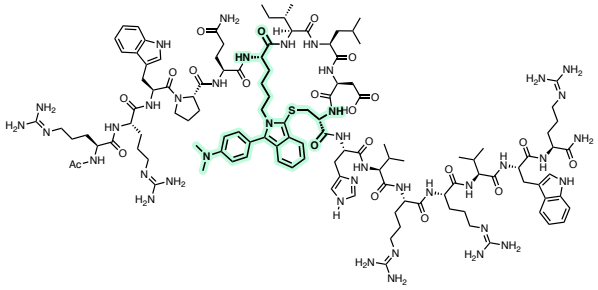
Synthesis and characterization of 21b

Purified **21** were partitioned into 15mL-falcon tubes in 2μmol portions (1 eq.) and lyophilized to afford dry white powder. **21** (2μmol) was dissolved in Na borate buffer pH 9 (1mL), EtOH (200μL), and to this mixture was added **6w** (50mM solution in DMSO, 80μL, 2 eq.). Reaction was left to sit at room temperature undisturbed and fluorescence was qualitatively monitored by hand-held UV lamp (365nm). After 2 hours, reaction was then quenched and acidified to pH 3 with formic acid, to afford a clear solution to be purified by preparative HPLC. Purified peptides were lyophilized and then quantified by UV absorbance, measured at the wavelength of maximum absorbance for the resulting isoindole (365nm) with ε = 14300 cm^{-1} M^{-1}, affording **21b** as pale-yellow powder (311nmol, 15% isolated). This peptide was tested against **21a** for DLD1 cell permeability studies.





Na Borate Buffer pH 9
EtOH (20%)
2 hrs, room temperature

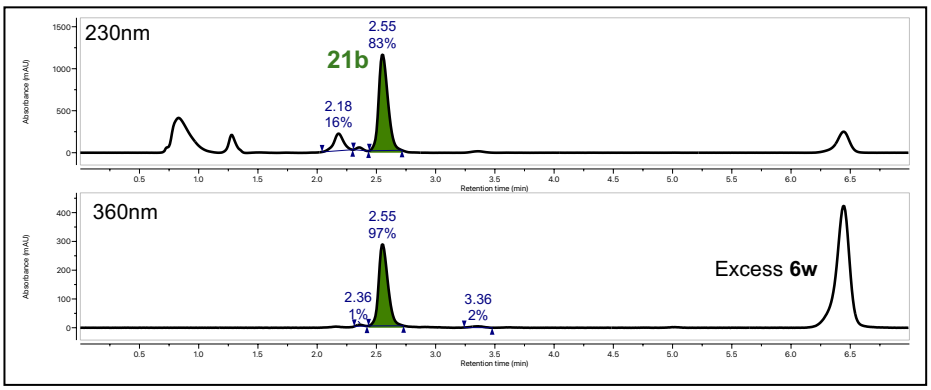


21b

Chemical Formula: $C_{121}H_{180}N_{40}O_{21}S$
Exact Mass: 2561.3967

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	25	15
6	50	50	
7	0	100	



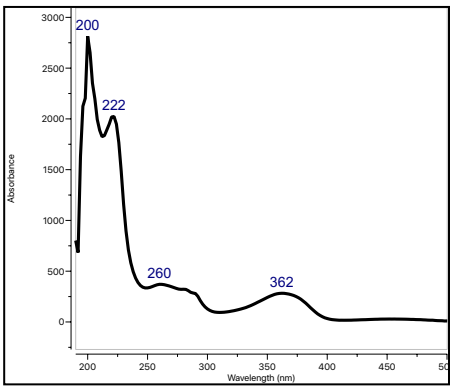


Figure S81: Prep-HPLC traces of reaction mixture of peptide **21** with **6w**

Reinjection of purified material:

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	80	25	15
6	50	50	
7	0	100	

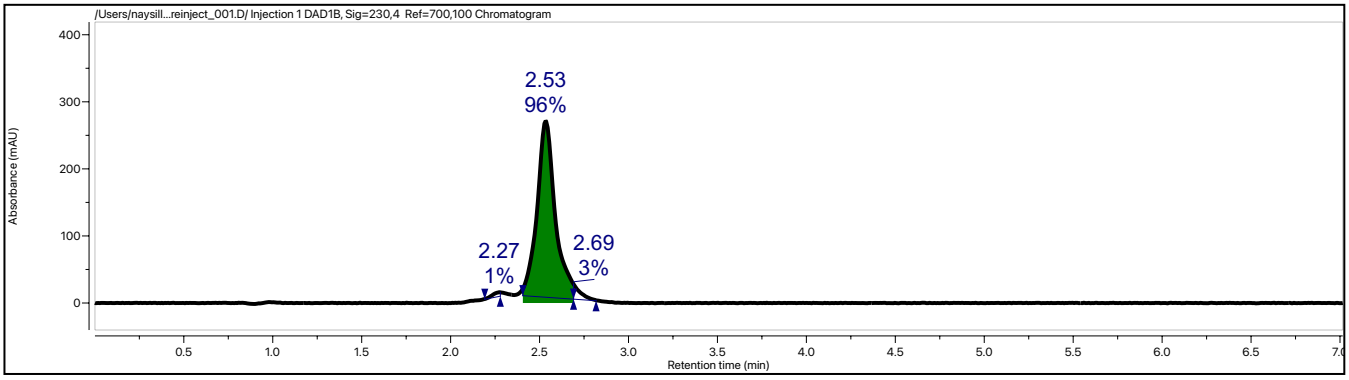
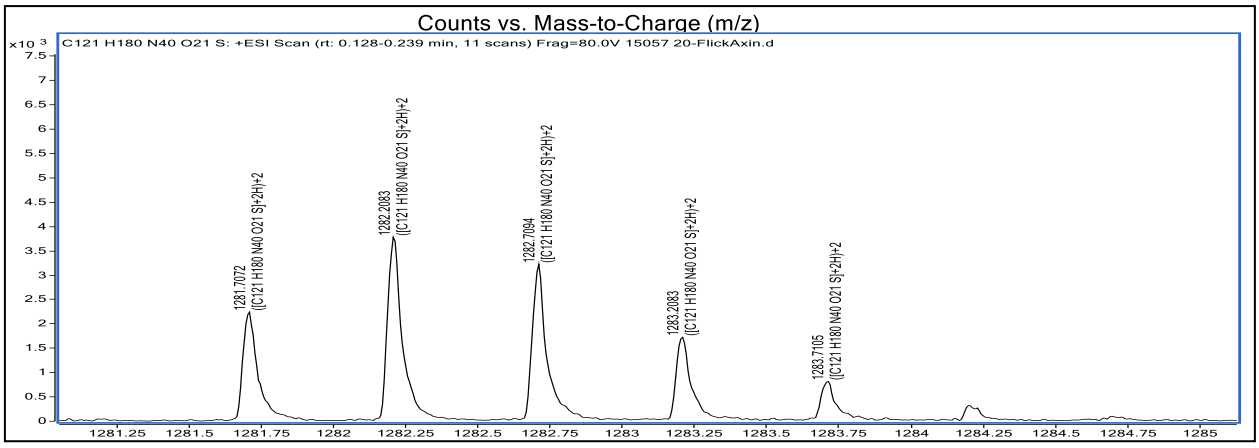


Figure S82: LC trace of purified compound **21b**, reinjected after storage of lyophilized product over 3 months at -20ºC.



HRMS of **21b**
Calculated $([C_{136}H_{194}N_{36}O_{30}S]+2H)/2 = 1281.7062$
Found $([C_{136}H_{194}N_{36}O_{30}S]+2H)/2 = 1281.7072$

Biological Assays

General Cell Culturing Protocol

All cell culturing media and supplements were purchased from Gibco and all cell culturing plastics were purchased from Corning or Falcon, unless otherwise stated. Cells were cultured at 37°C in a humidified incubator with 5% CO_2. All experiments were conducted in a laminar flow cabinet under sterile conditions. DMSO used in cell-based experiments was purified by filtration through a 0.2 mm filter. To revive cells, a 1 mL cryotube of frozen cell stock (culture media + 10% DMSO) was gently thawed in a water bath and diluted with 12 mL of fresh media in a T-75 flask. After 24 hours, media was replaced with fresh media. Jurkat T-cell leukemia cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 10K U/mL penicillin, 10K mg/mL streptomycin, and 2 mM L-glutamine. DLD-1 cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum (FBS), 10K U/mL penicillin, and 10K mg/mL streptomycin. When cells reached a confluence level of 85-95% for adherent cells, or $2x10^6$ viable cells/mL for suspension cells, the cells were subcultured. Adherent cells were treated with 0.25% trypsin containing 1.3 mM EDTA for ten minutes in the incubator. Once cells were detached from the flask, 1-2 mL of media was added to quench the trypsin, and the cell suspension was transferred to a 10 mL falcon tube and centrifuged for 5 minutes at 8000 rpm. The supernatant was discarded, and the pellet of cells was resuspended in fresh media, diluted as required, and transferred to new T-25 culture flasks. Suspension cells were directly transferred to a 10 mL falcon tube and centrifuged for 5 minutes at 8000 rpm. The supernatant was discarded, and the pellet of cells was resuspended in fresh media, diluted as required, and transferred to new T-75 culture flasks. Cell viability was assessed by counting cells with a hemacytometer after treatment with Trypan blue.

Cell Viability Assays

To assay cell viability, Jurkat cells were suspended in media lacking FBS (serum-free media) and seeded in 96 well plates ($1x10^4$ cells in 25 µL/well). Peptides **20** (negative control), **20a** (RCM, positive control), and **20b** (FIICk) were lyophilized and dissolved in serum-free media with 1% DMSO and further diluted to the appropriate concentrations. The plated cells were treated with 25 µL of the serial dilutions of the peptides of interest in triplicate and incubated at 37°C for 2 hours, at which point 50 µL of media containing 20% FBS was added to each well (serum replacement) (final well volume of 100 µL, 10% FBS). The cells were then incubated for 24 hours. To each well, 10 µL of MTS labeling reagent (Abcam) was added and the cells were incubated for 4 hours. The absorbance of the wells was measured at 490 nm using a Microplate Reader Multi-Mode FilterMax F5 and the IC_{50} values were determined by nonlinear regression analysis with GraphPad Prism software.

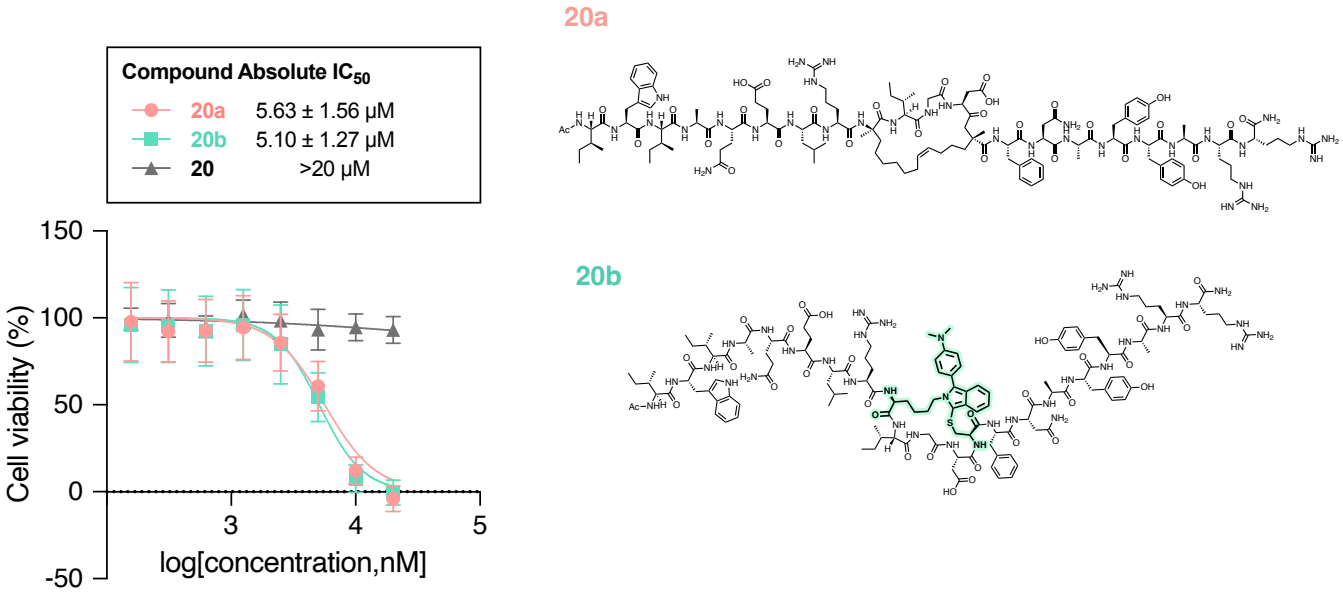
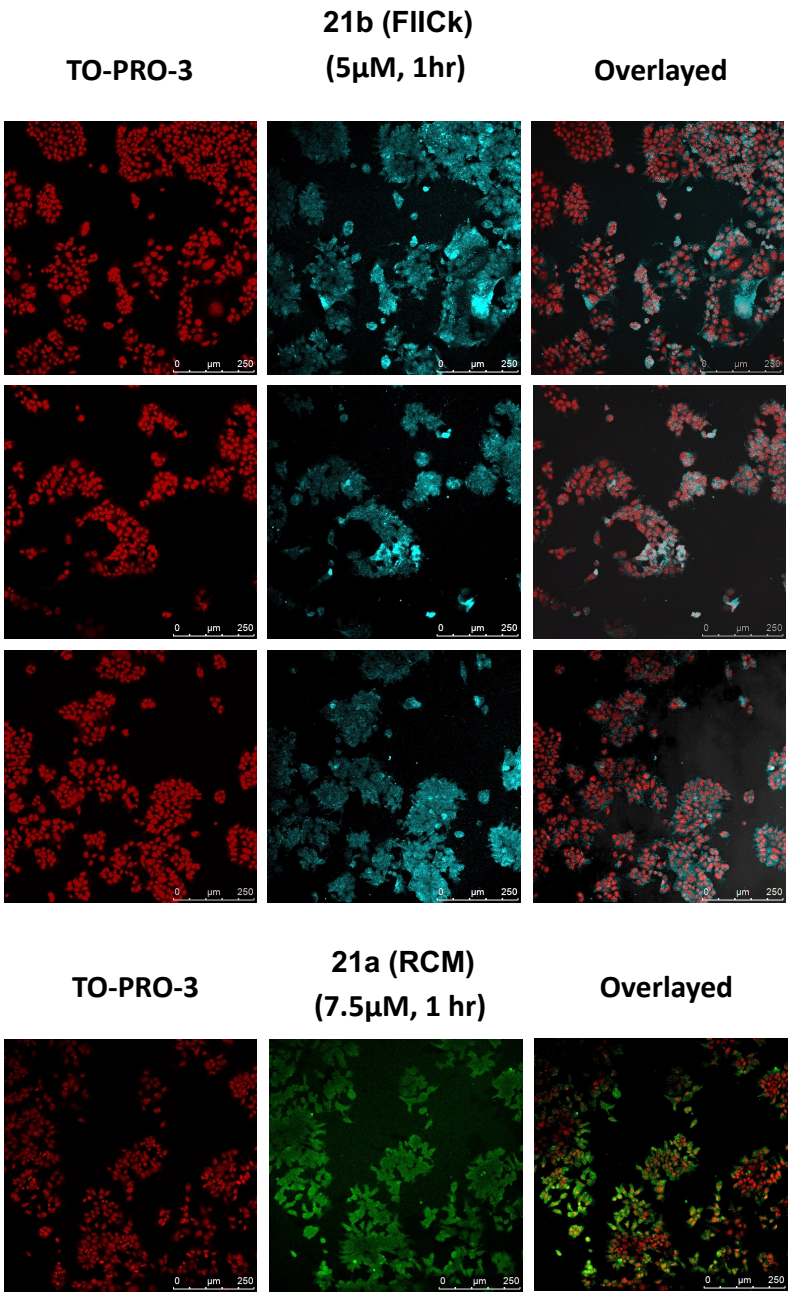


Figure S83 Viability of Jurkat cells upon treatment with **20**, **20a**, and **20b** for 24 hours.

Confocal Microscopy Imaging

To observe cell internalization, DLD-1 cells were seeded in 8-well chamber slides (Lab-Tek) ($1x10^5$ cells in 400 µL/well) and incubated overnight. Media was removed and wells were washed twice with 1x PBS. Fluorescent peptides **21a** (FITC, RCM positive control) and **21b** (FIICk) were lyophilized and dissolved in serum-free media with 0.5% DMSO, and cells were incubated with 400 µL of 7.5 µM (**21a)** or 5 µM (**20b**) for 1 hour. The peptide solutions were then removed, the wells were washed twice with PBS, and the cells were fixed with 4% (w/v) paraformaldehyde in PBS for 15 minutes at room temperature. The cells were then stained with 1 µM TO-PRO-3 in PBS for 30 minutes at room temperature, washed twice with PBS, and 1.5 coverslips were mounted with 1:9 PBS:glycerol mounting media. Confocal fluorescence microscopy was performed with a Leica SP5 X Laser Scanning Confocal Microscope (inverted), objectives HP PL APO 10x/0.40 CS, 20x/0.7 IMM CS, and 63x/1.4-0.6 oil CS. TO-PRO-3 was excited at 663 nm, FITC at 488 nm, and FIICk peptides at 405 nm. All images were recorded with the same parameters and instrument settings.

Figure S84 Analysis of cell permeability of **21a** (7.5µM, 1 hour) and **21b** (5µM, 1 hour) on DLD1 cells by confocal fluorescence microscopy. In red, TO-PRO-3 nuclear stain. In green, FITC (**21a**). In teal blue, isoindole FIICk peptide (**21b**).



Comparative modeling of FIICk-stapled helix with RCM-stapled helix

Molecular modeling was done on Molecular Operating Environment (MOE)

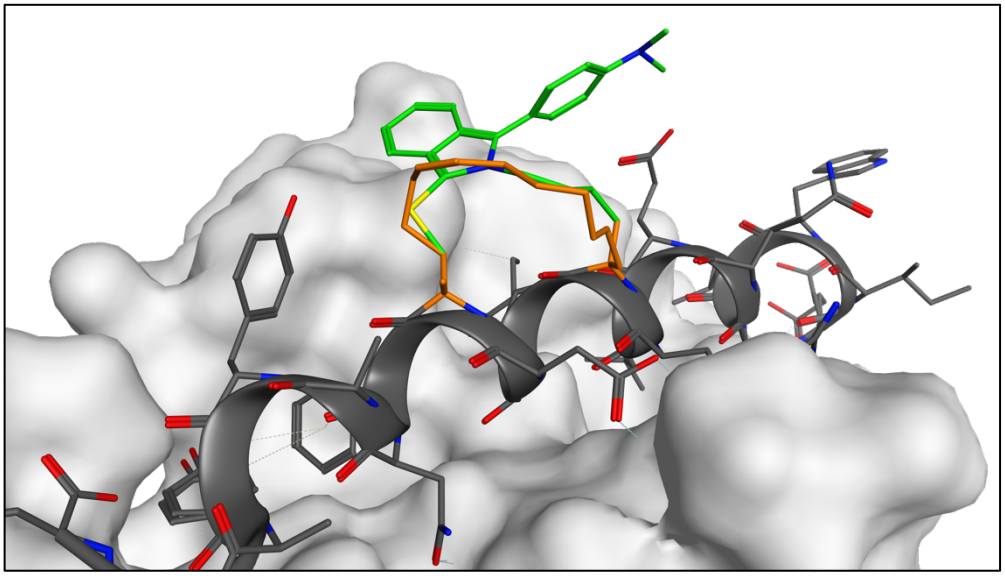


Figure S85 Modelling of RCM-stapled BIMBH3 mimic (**19a**, in orange), based on the crystal structure of MCL-1:BIMBH3 complex[5] (PDB ID: 2NL9) overlaid with a model of FIICk-stapled BIMBH3 mimic (**19b**, in green). **19b** was docked onto the receptor using **19a** as the template.

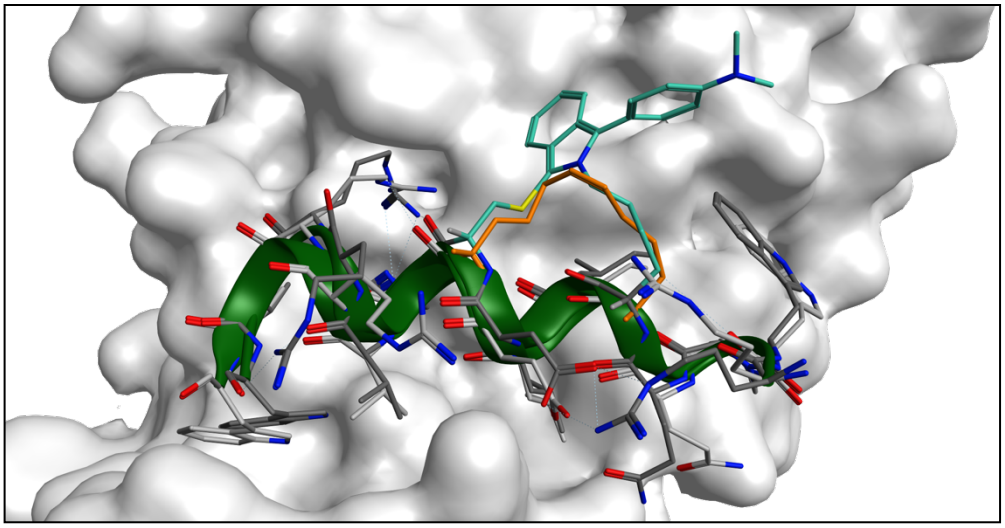
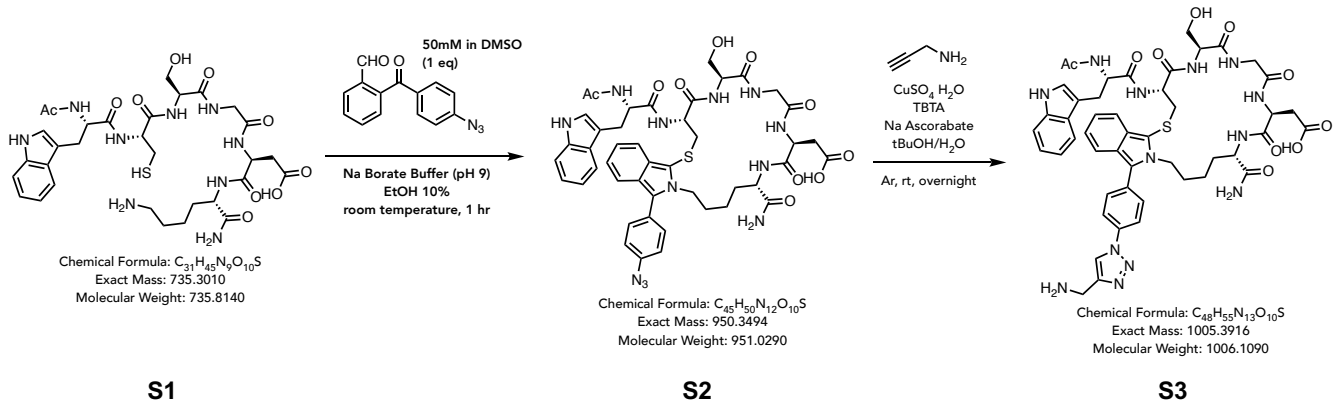


Figure S86 Structure of RCM-stapled Axin mimic (**20a**, in orange) in complex with beta-catenin (PDB ID: 4DJS)[6] overlaid with a model of FIICk-stapled Axin mimic (**20b**, in teal). **20b** was docked onto the receptor using **20a** as the template.

Exploring reactions on isoindole derivatives – Proof of concept of isoindole undergoing CuAAC reaction

Stapling aldehyde **6ac** (azido) was prepared as a 50mM solution in DMSO. An 60µL aliquot of this solution (3µmol, 1 eq.) was dispensed into a stirring mixture of monocycle **S1** (Ac-WCSGEK-NH_2) (3µmol, 1 eq.) in 2mL Na Borate buffer:EtOH (80:20, v/v). This mixture was stirred at room temperature for 30 minutes, quenched with formic acid to pH 3, and purified by HPLC with the parameters outlined below, to afford monocycle **S2** (1.47µmmol, 49%) as white lyophilized powder.

A 15mL conical tube was then charged with: 7.4µL solution of $CuSO_4$ $5H_2O$ in PBS buffer pH 7.4 (2 eq., 400mM), 14.7µL solution of TBTA in DMSO (2 eq., 200mM), and 22µL solution of sodium ascorbate in PBS buffer pH 7.4 (3 eq., 800mM). To this mixture was added 22µL solution of propargyl amine in DMSO (3 eq., 800mM) and lyophilized **S2** (1.47µmol, 1 eq.) dissolved in 600µL tBuOH:H_2O (1:1, v/v). This reaction cocktail was then stirred by bubbling under Argon at room temperature overnight, and then purified by HPLC with the parameters outlined below to afford monocycle **S3**



S1 **S2** **S3**

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	15
10	0	100	

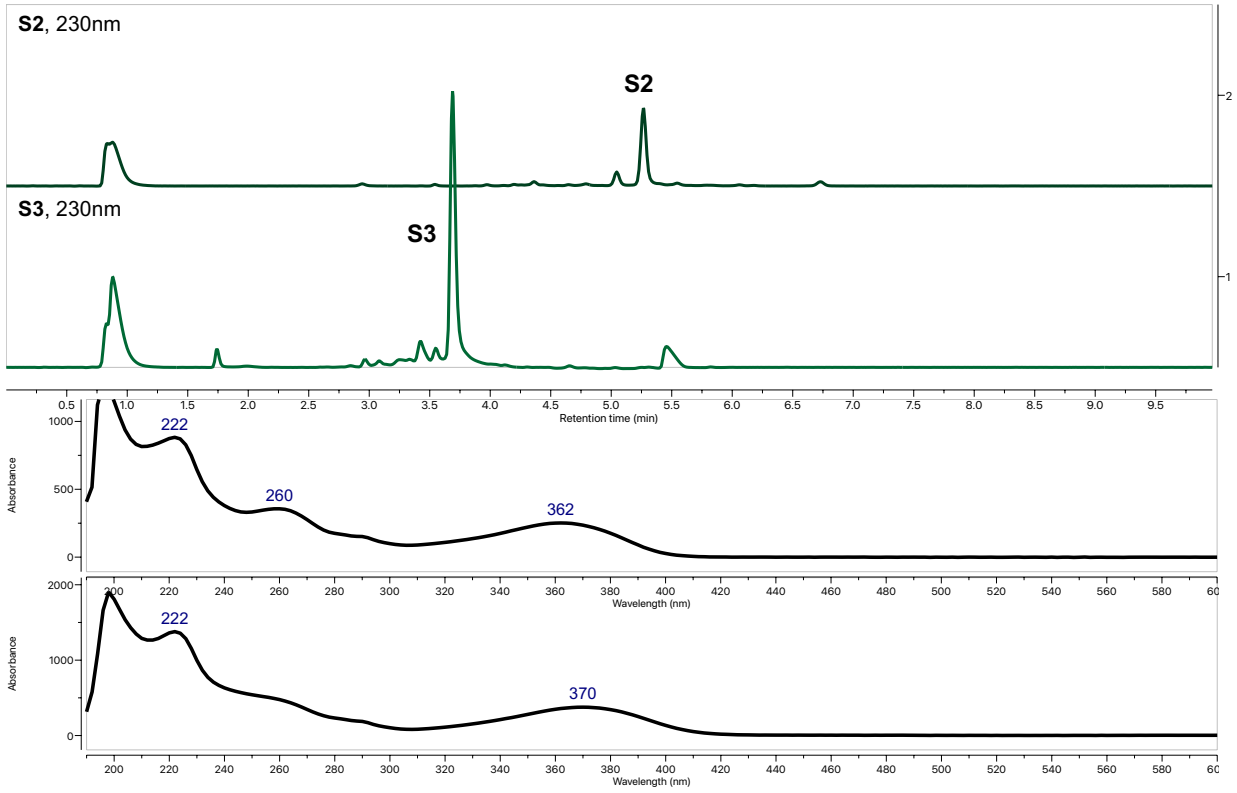
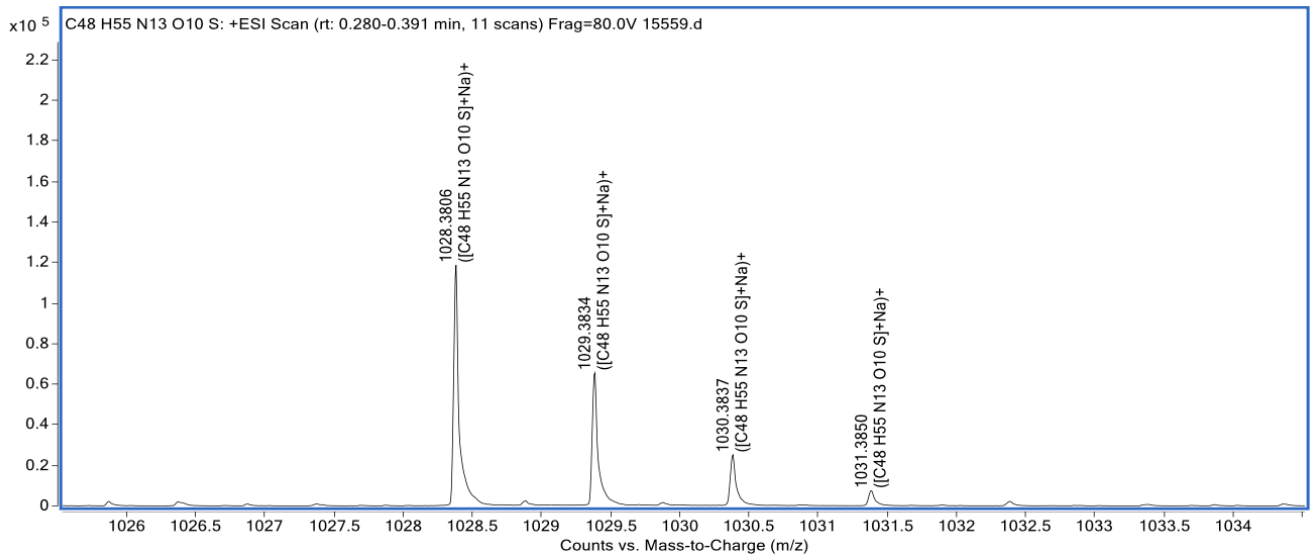


Figure S87: HPLC purification of **S2** and **S3** observed at 230nm and corresponding UV excitation profiles.



HRMS of **S3**

Calculated $([C_{48}H_{55}N_{13}O_{10}S]+Na)^+ = 1028.3808$

Found $([C_{48}H_{55}N_{13}O_{10}S]+Na)^+ = 1028.3806$

Reinjection of purified material:

Solvent A : H_2O 0.1% TFA

Solvent B : MeCN 0.1% TFA

Column : Agilent Eclipse XDB-C18 250 x 9.4 mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	2
20	0	100	
25	90	10	

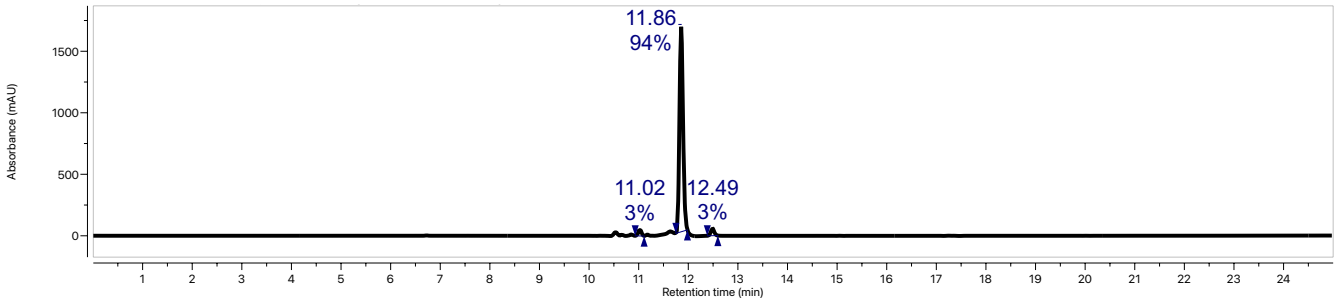
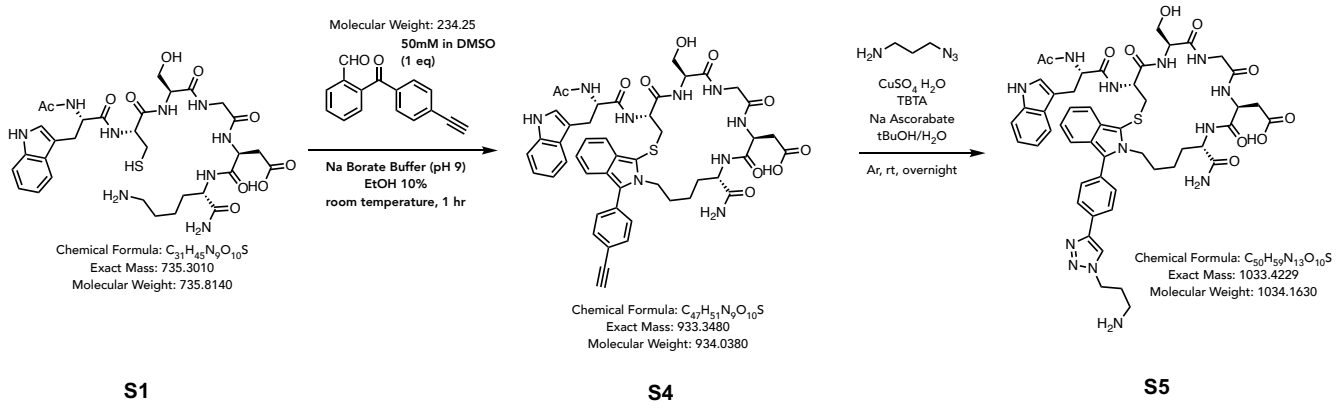


Figure S88: LC trace of purified **S3** observed at 230nm.

Stapling aldehyde **6ad** (alkynyl) was prepared as a 50mM solution in DMSO. An 120µL aliquot of this solution (6µmol, 1 eq.) was dispensed into a stirring mixture of monocycle **S1** (Ac-WCSGEK-NH_2) (6µmol, 1 eq.) in 4mL Na Borate buffer:EtOH (80:20, v/v). This mixture was stirred at room temperature for 30 minutes, quenched with formic acid to pH 3, and purified by HPLC with the parameters outlined below, to afford monocycle **S4** (3.13µmmol, 52%) as white lyophilized powder.

A 15mL conical tube was then charged with: 165µL solution of $CuSO_4$ $5H_2O$ in PBS buffer pH 7.4 (2 eq., 400mM), 32µL solution of TBTA in DMSO (2 eq., 200mM), and 12µL solution of sodium ascorbate in PBS buffer pH 7.4 (3 eq., 800mM). To this mixture was added 12µL solution of 3-azido-1-propanamine in DMSO (3 eq., 800mM) and lyophilized **S2** (1.47µmol, 1 eq.) dissolved in 600µL tBuOH:H_2O (1:1, v/v). This reaction cocktail was then stirred by bubbling under Argon at room temperature overnight, and then purified by HPLC with the parameters outlined below to afford monocycle **S5**



S1 **S4** **S5**

Solvent A : H_2O 0.1% TFA
Solvent B : MeCN 0.1% TFA
Column : Agilent, C18, 50x21.2mm

Time (min)	A%	B%	Flow (mL/min)
0	70	30	15
8	0	100	

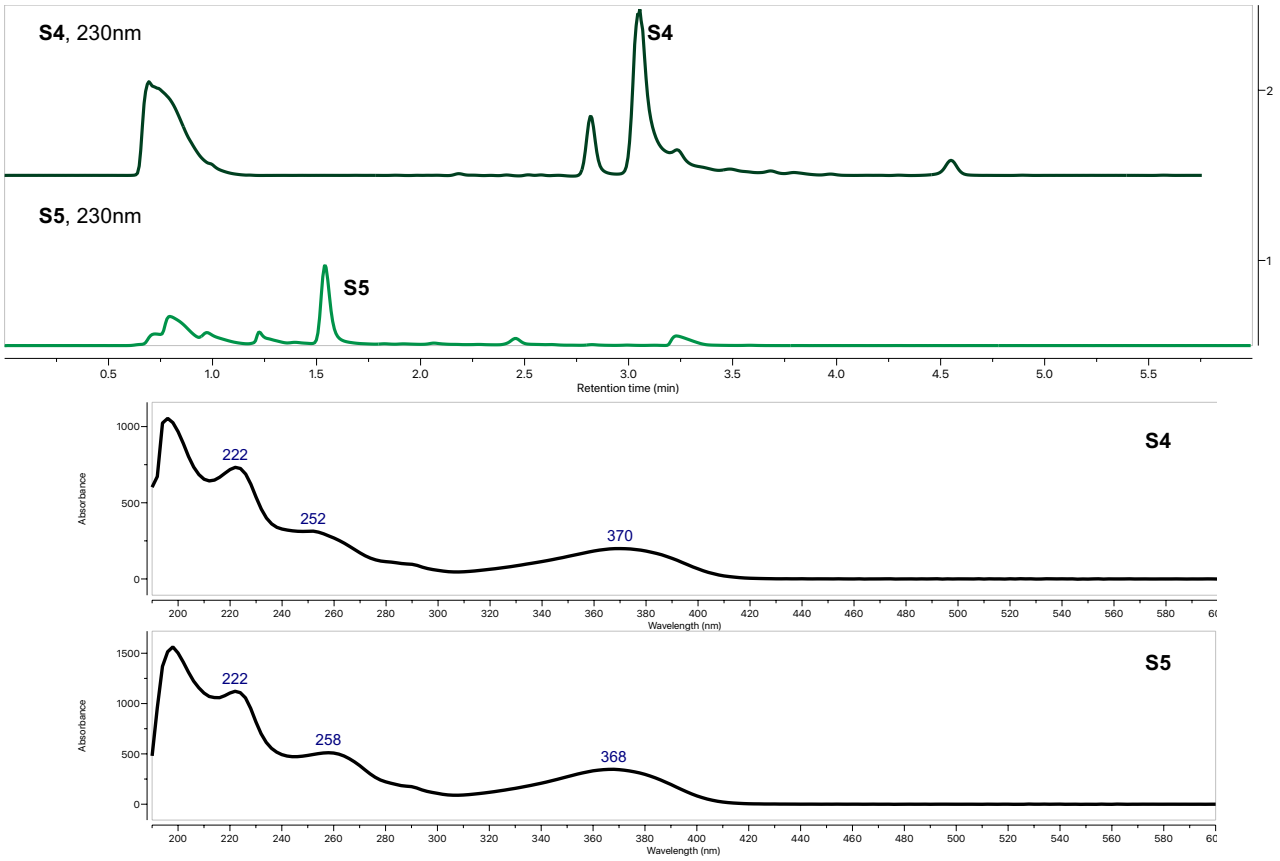
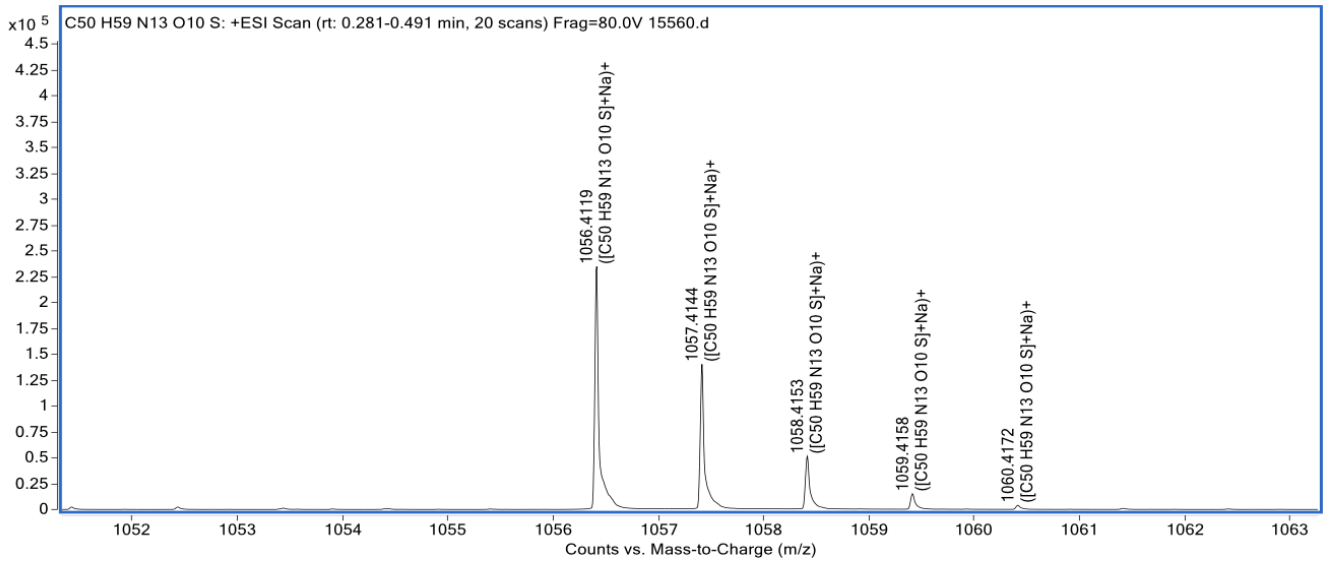


Figure S89: HPLC purification of **S4** and **S5** observed at 230nm and corresponding UV excitation profiles.



HRMS of **S5**

Calculated $([C_{50}H_{59}N_{13}O_{10}S]+Na)^+ = 1056.4126$

Found $([C_{50}H_{59}N_{13}O_{10}S]+Na)^+ = 1056.4119$

Reinjection of purified material:

Solvent A : H_2O 0.1% TFA

Solvent B : MeCN 0.1% TFA

Column : Agilent Eclipse XDB-C18 250 x 9.4 mm

Time (min)	A%	B%	Flow (mL/min)
0	90	10	2
20	0	100	
25	90	10	

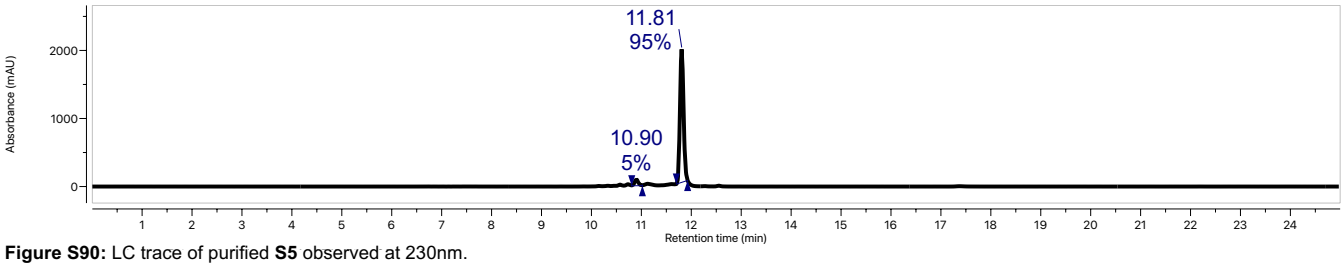


Figure S90: LC trace of purified **S5** observed at 230nm.

References

(1) Yang, J. T.; Wu, C.-S. C.; Martinez, H. M. [11] Calculation of protein conformation from circular dichroism. In *Methods in Enzymology*, Vol. 130; Academic Press, 1986; pp 208-269.

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(5) Czabotar, P. E.; Lee, E. F.; van Delft, M. F.; Day, C. L.; Smith, B. J.; Huang, D. C. S.; Fairlie, W. D.; Hinds, M. G.; Colman, P. M. Structural insights into the degradation of Mcl-1 induced by BH3 domains. *Proceedings of the National Academy of Sciences* **2007**, *104* (15), 6217-6222. DOI: doi:10.1073/pnas.0701297104.

(6) Dietrich, L.; Rathmer, B.; Ewan, K.; Bange, T.; Heinrichs, S.; Dale, T. C.; Schade, D.; Grossmann, T. N. Cell Permeable Stapled Peptide Inhibitor of Wnt Signaling that Targets beta-Catenin Protein-Protein Interactions. *Cell Chem Biol* **2017**, *24* (8), 958-968 e955. DOI: 10.1016/j.chembiol.2017.06.013

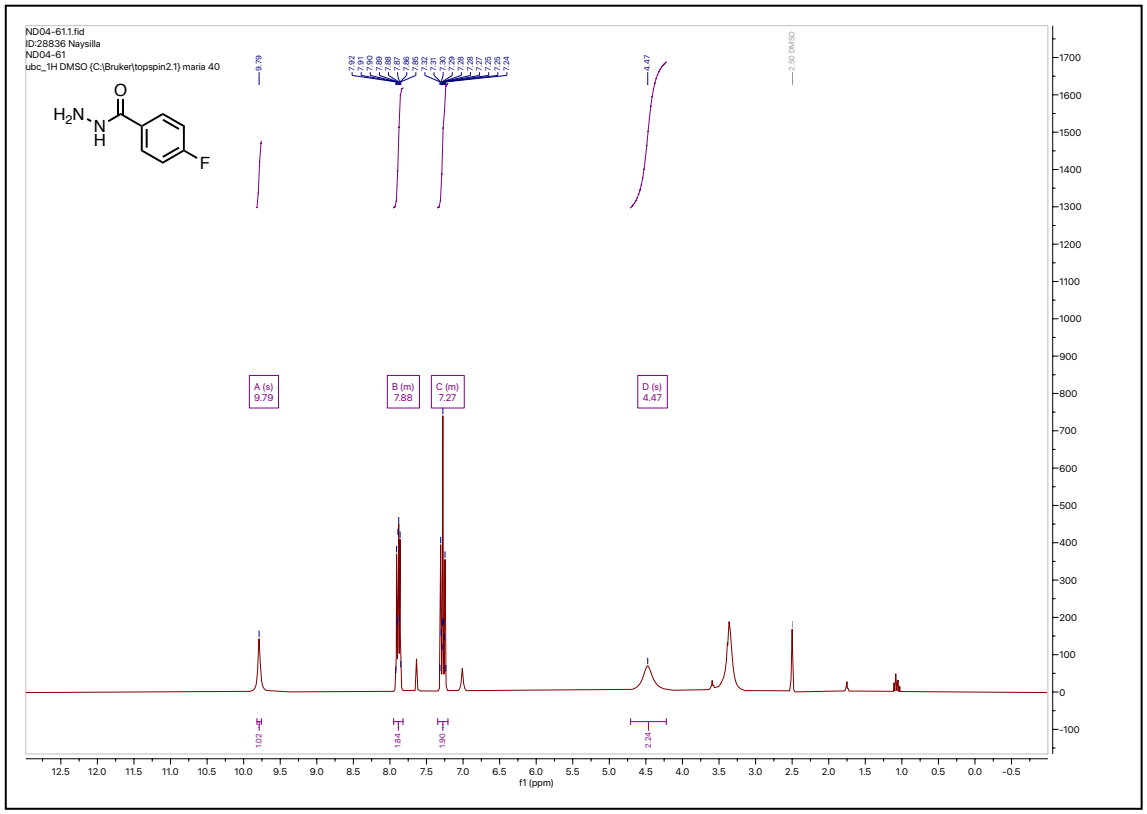
Author Contributions

Design, syntheses, experimental execution, UV spectroscopy, circular dichroism, and peptide characterization was conducted by Naysilla L. Dayanara who supervised undergraduate researcher Pascale Roome who contributed to the synthesis of 2-arylketobenzaldehyde compounds. Cell culturing, MTS assay, and confocal microscopy were performed by Juliette Froelich. Naysilla L. Dayanara worked on data curation, formal analysis, and full NMR spectroscopic assignments of isoindole-stapled peptides and amino acids. Writing of manuscript was a collaborative effort between Naysilla L. Dayanara and Dr. David M. Perrin. Funding acquisition was accomplished by Dr. David M. Perrin.

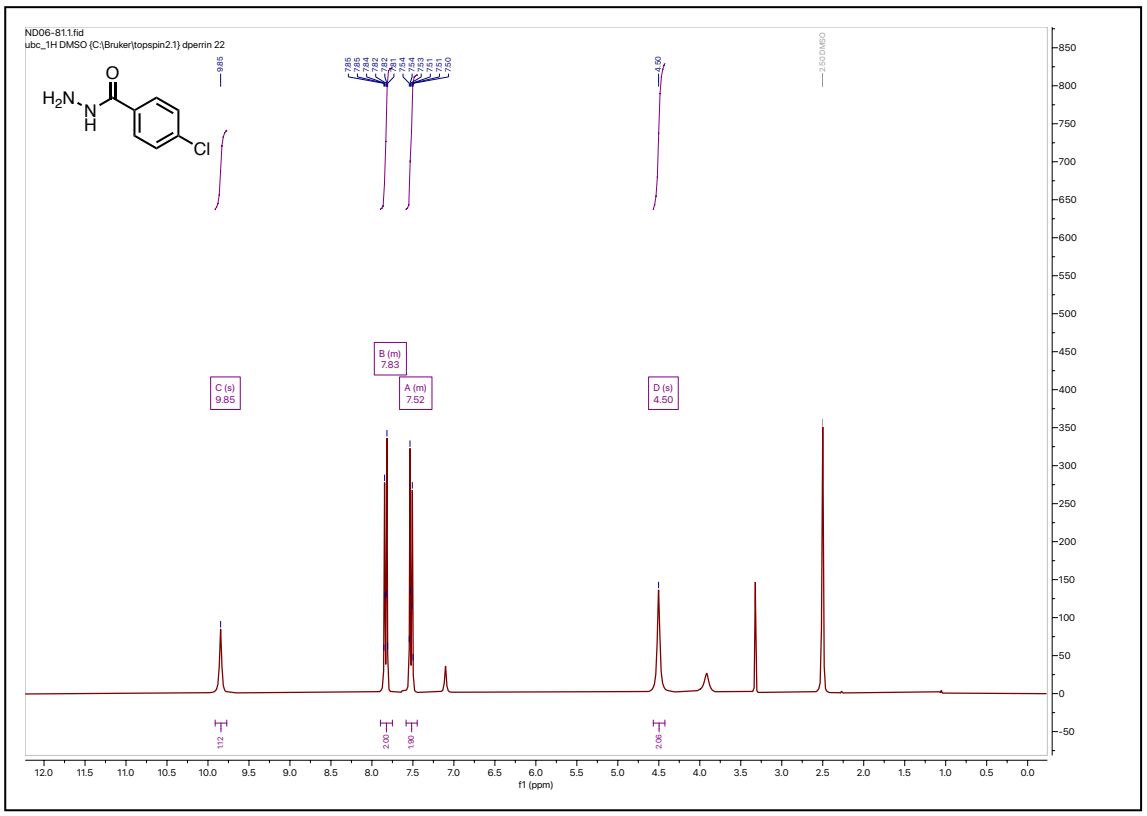
NMR Spectra

NMR Spectra of hydrazide derivatives (2):

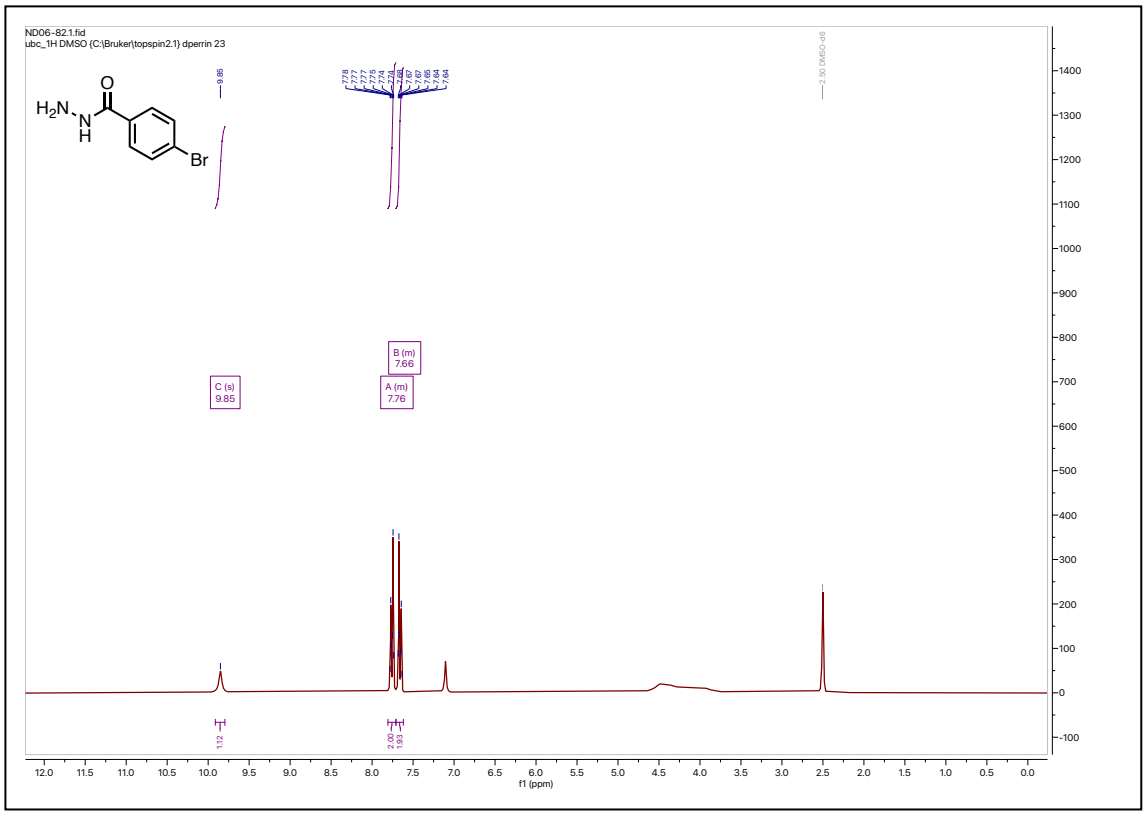
4-fluorobenzohydrazide (2a)



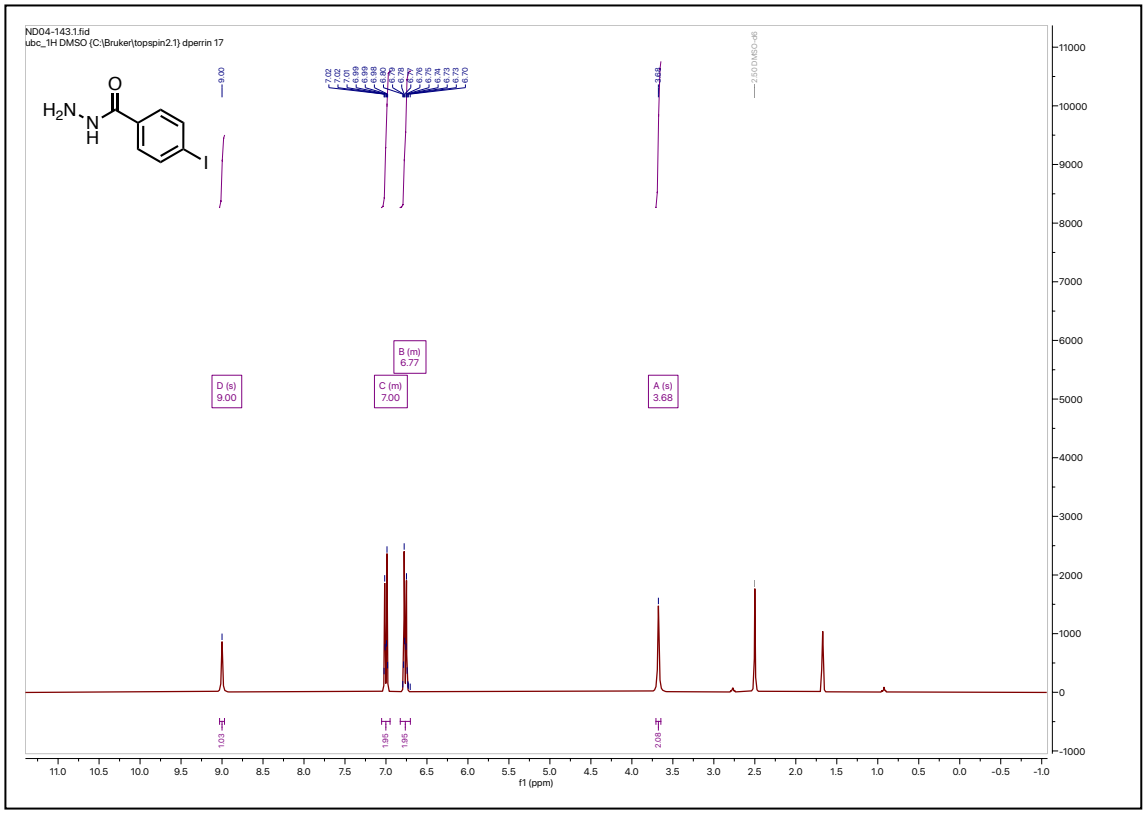
4-chlorobenzohydrazide (2b)



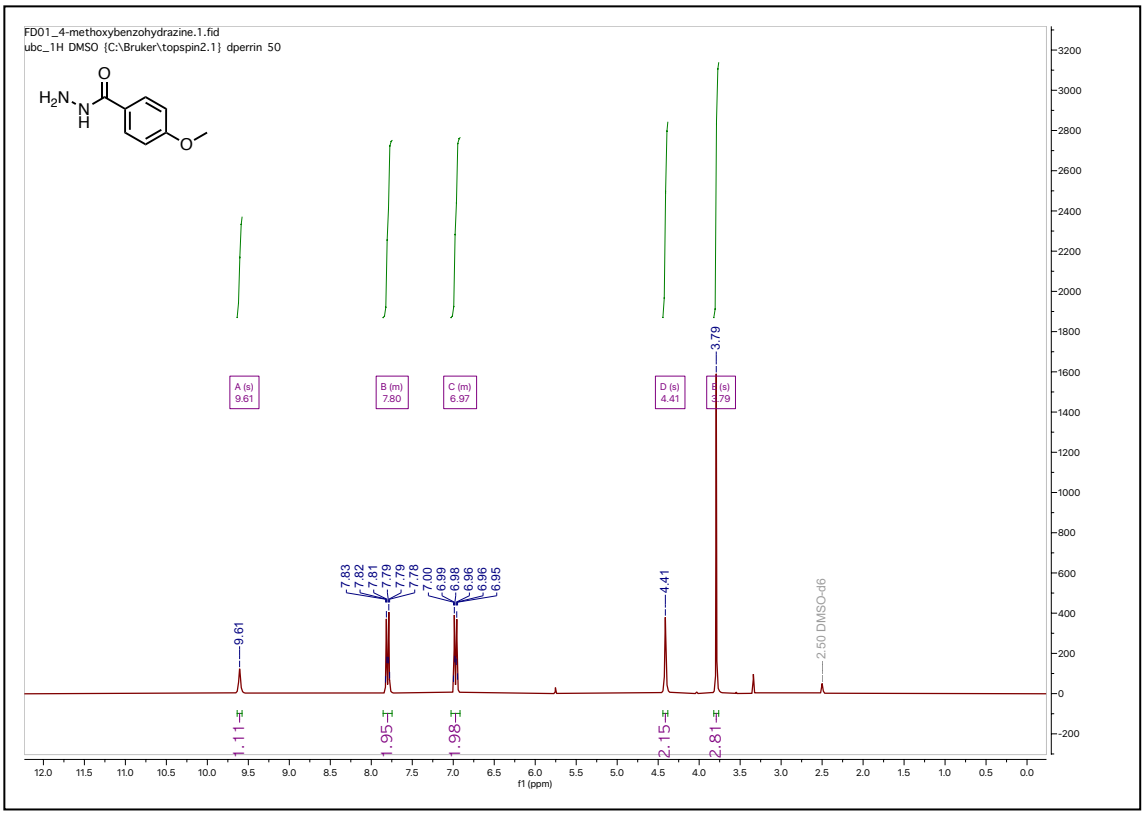
4-bromobenzohydrazide (2c)



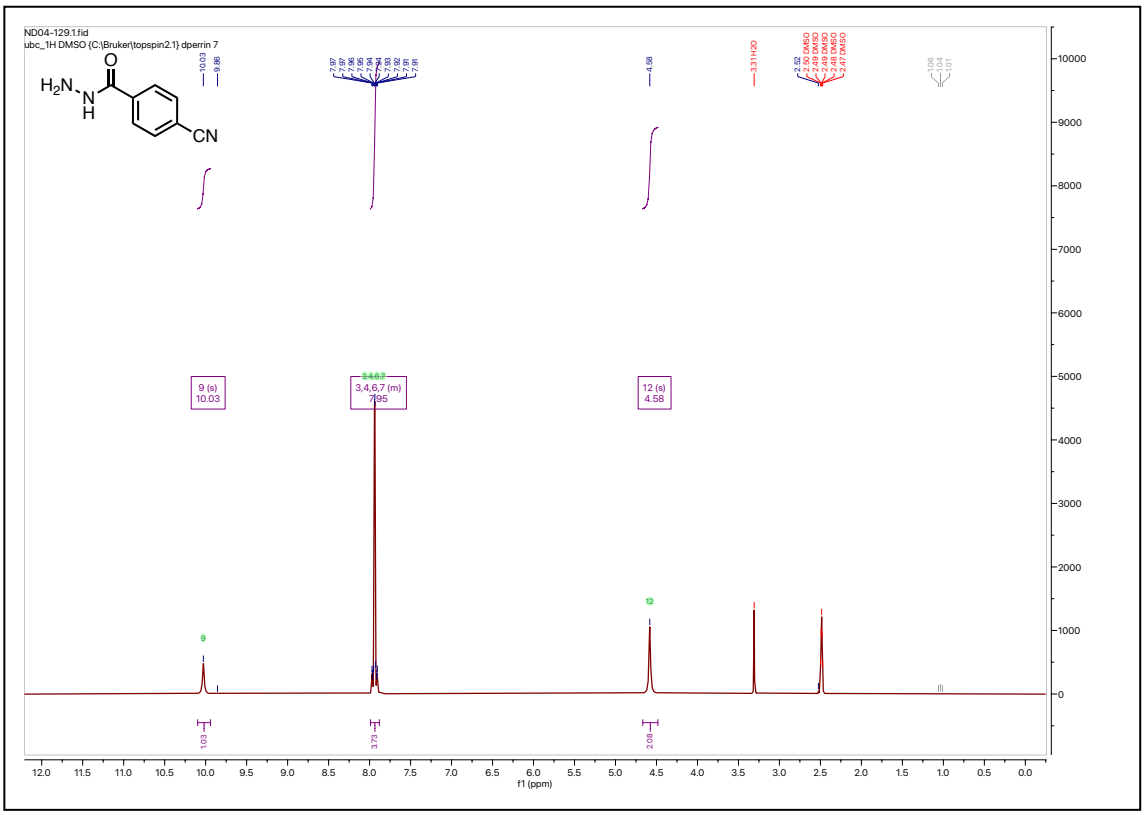
4-iodobenzohydrazide (2d)



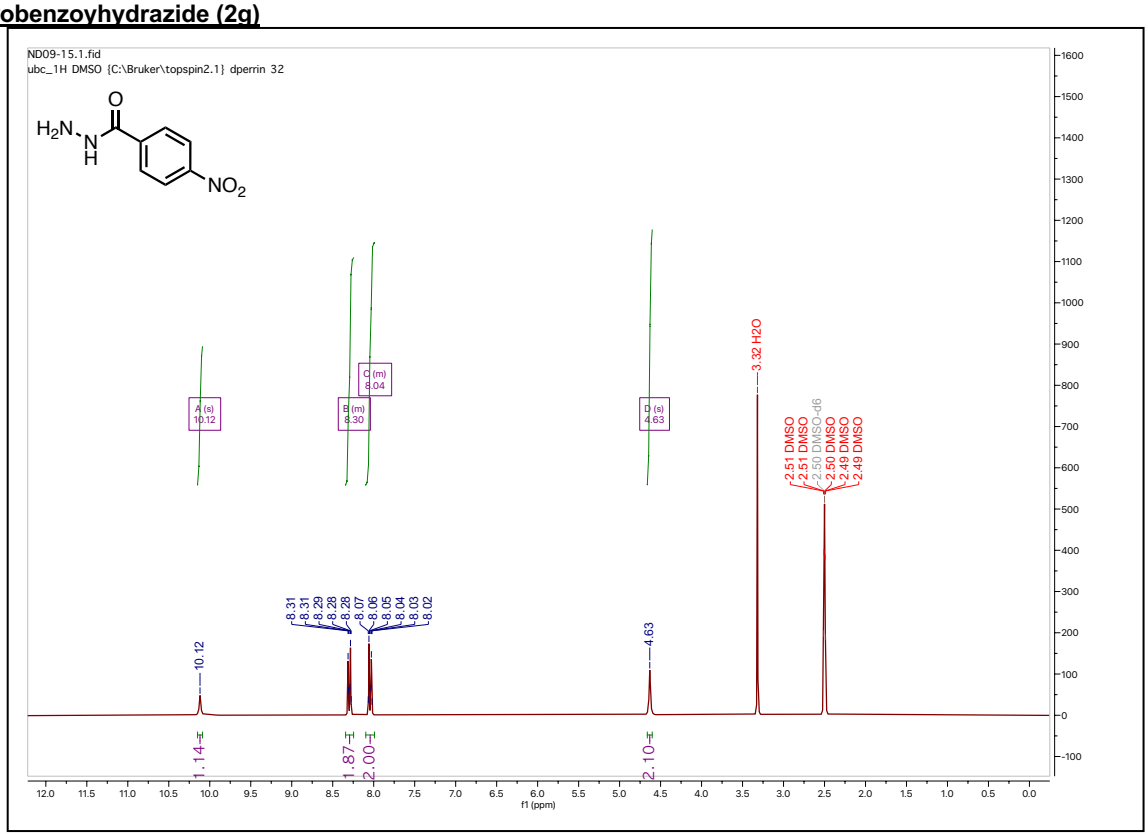
4-methoxybenzohydrazide (2e)



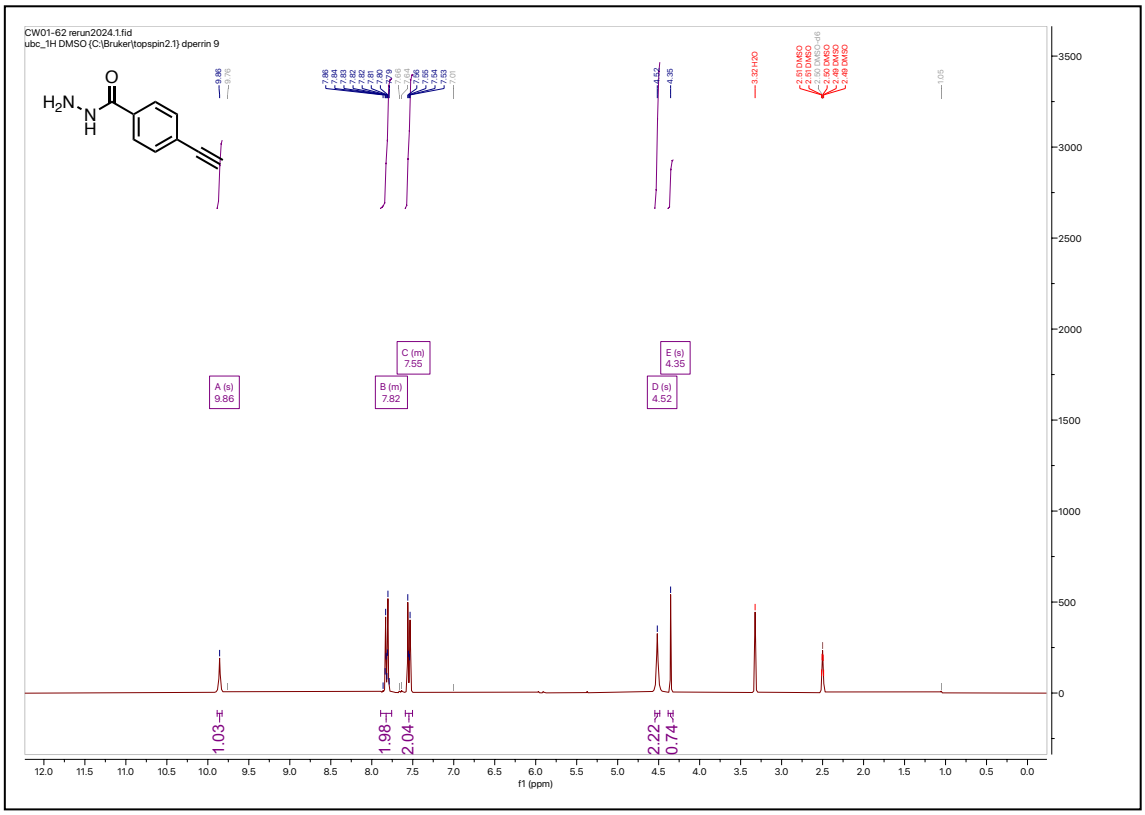
4-cyanobenzohydrazide (2f)



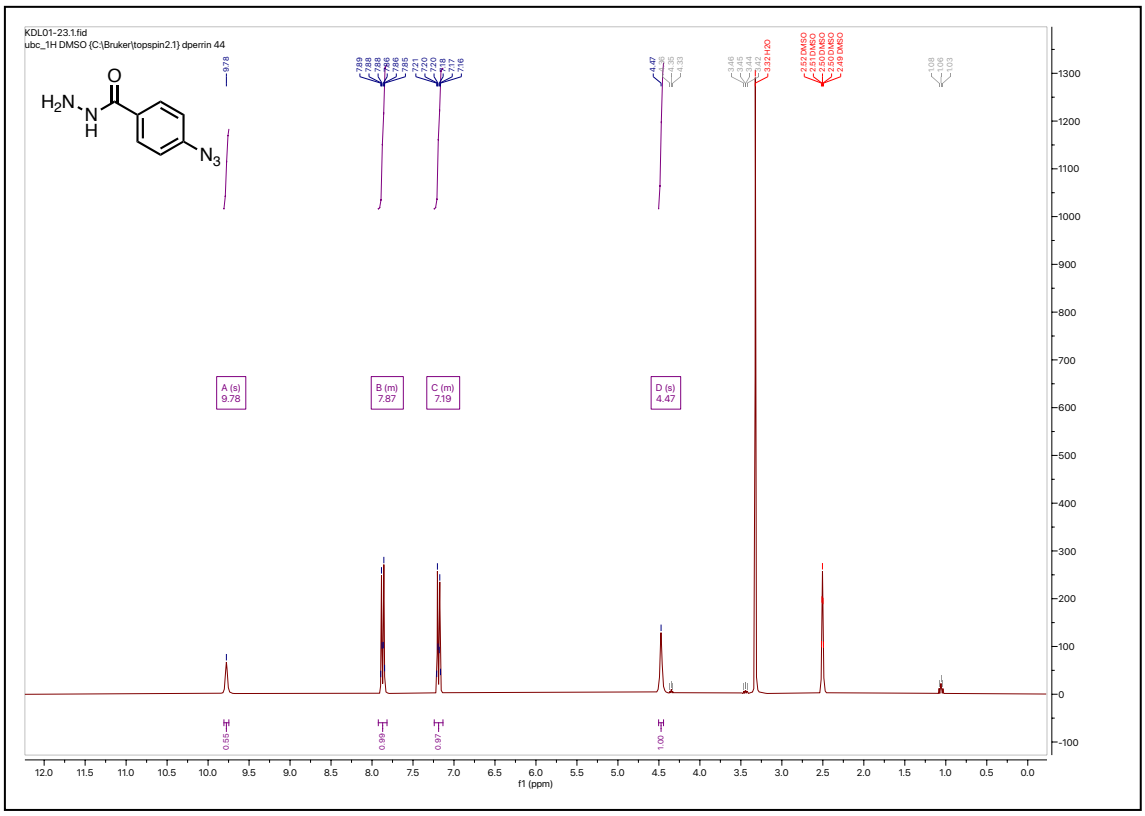
4-nitrobenzoyhydrazide (2g)



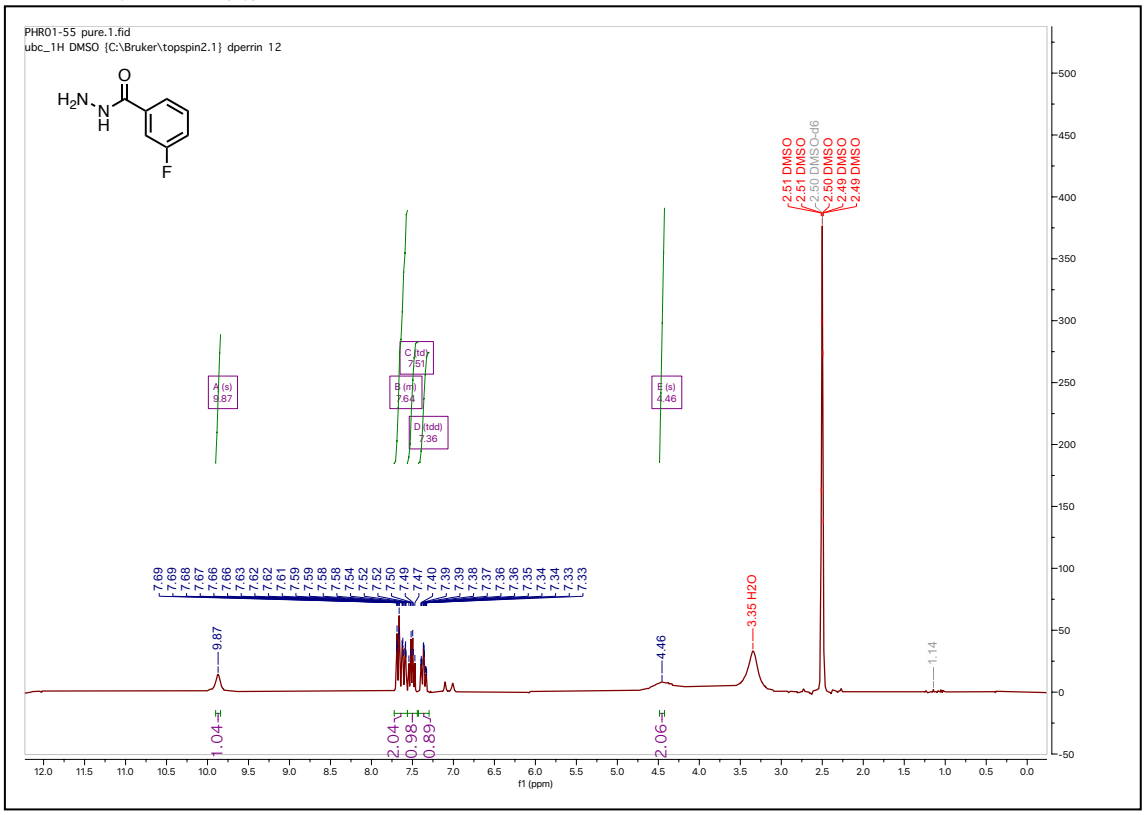
4-ethynylbenzohydrazide (2h)



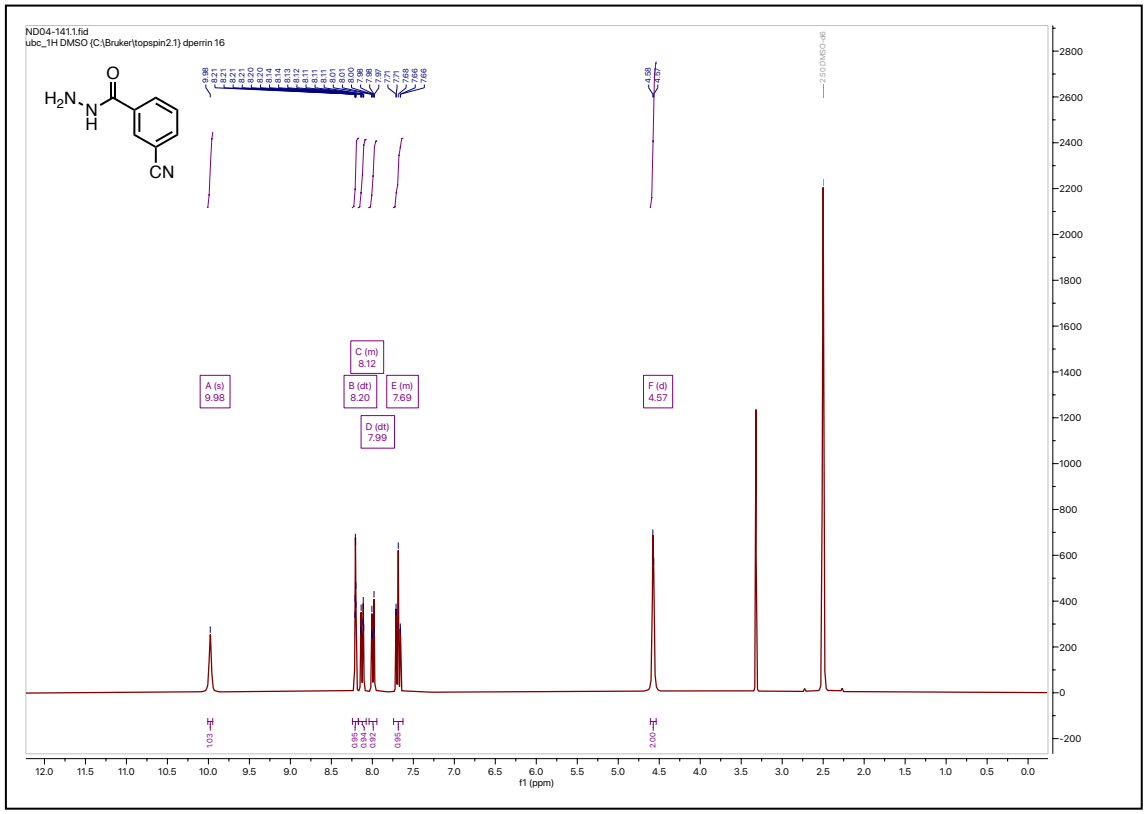
4-azidobenzohydrazide (2i)



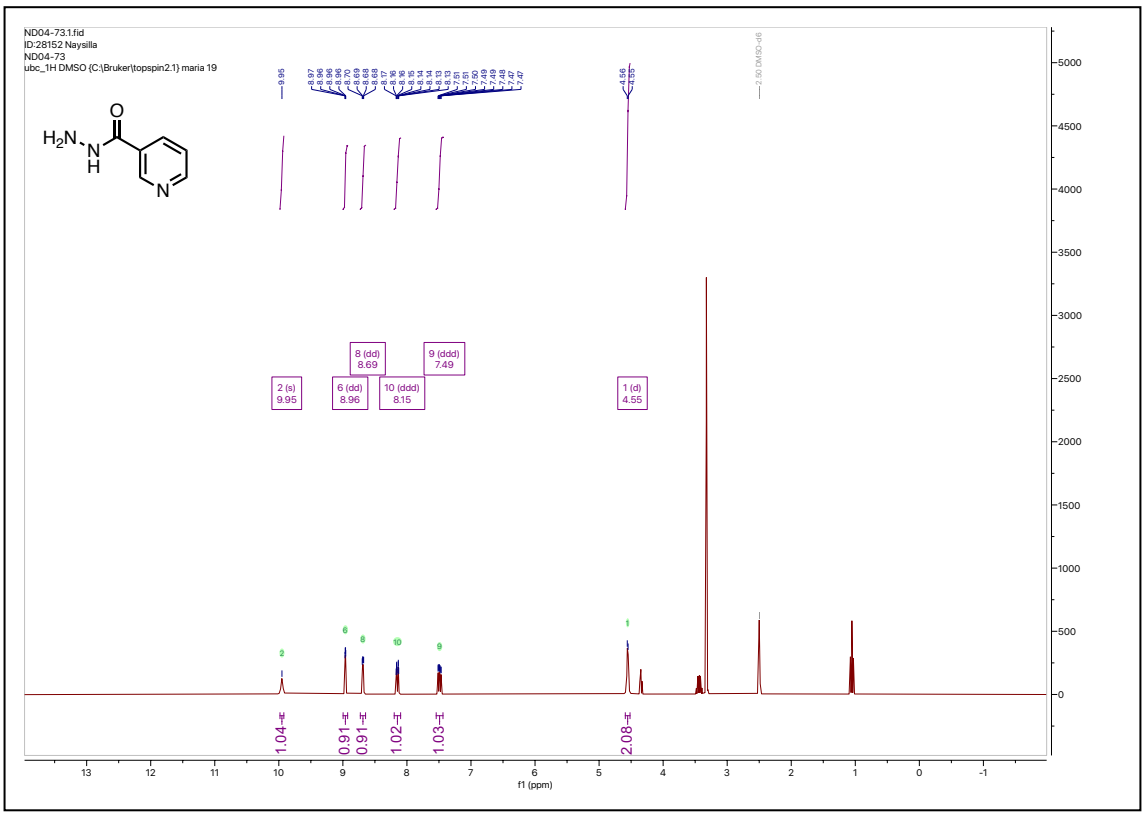
3-fluorobenzohydrazide (2j)



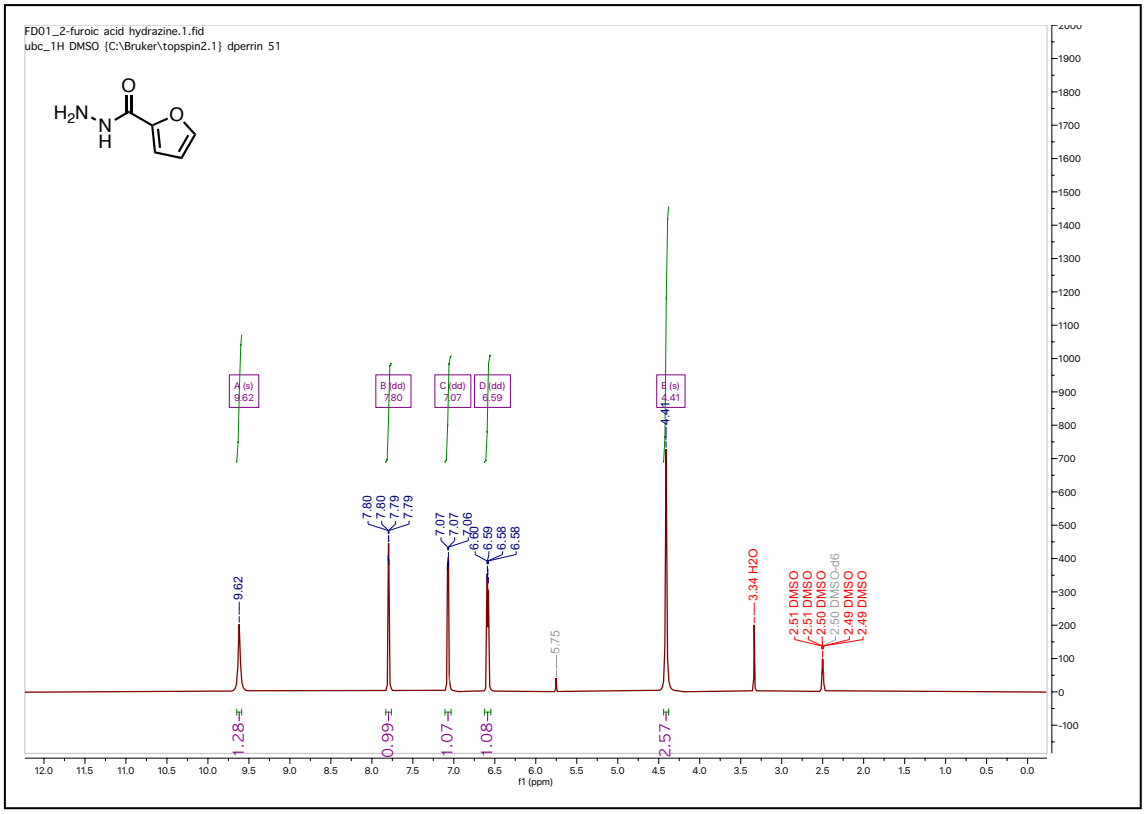
3-cyanobenzohydrazide (2k)



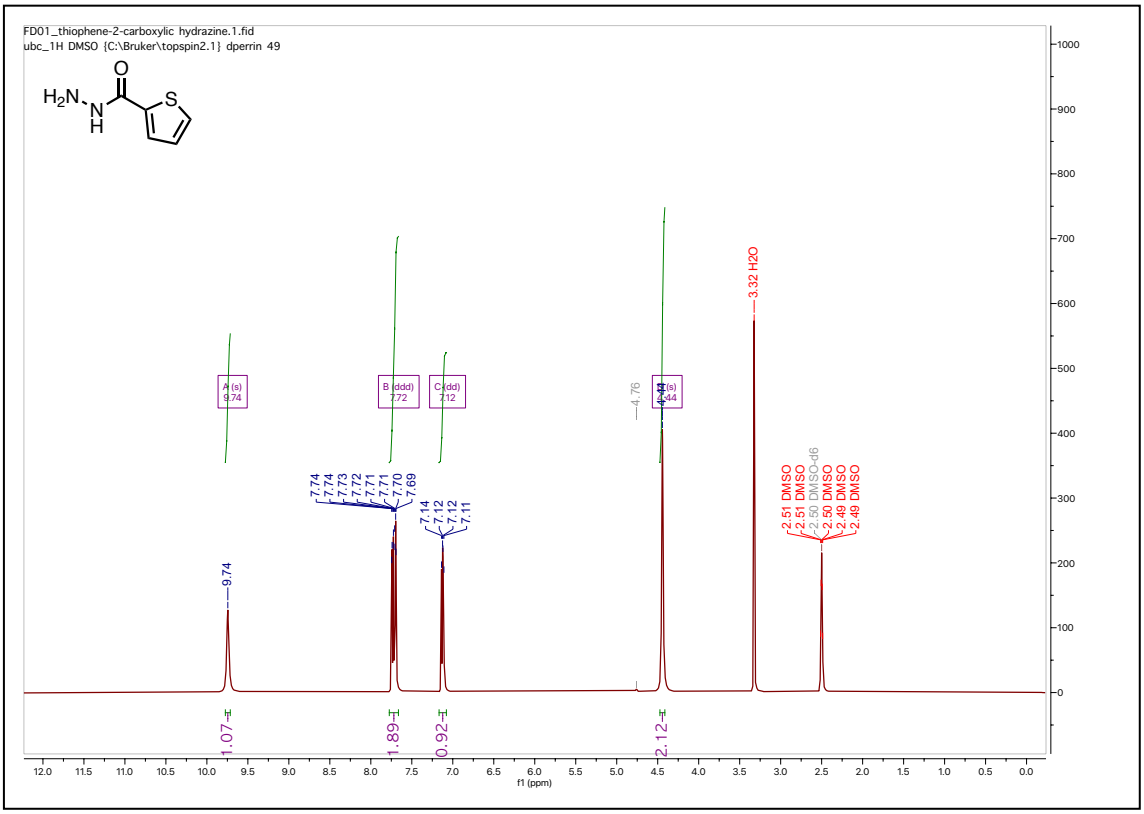
Nicotinohydrazide (2l)



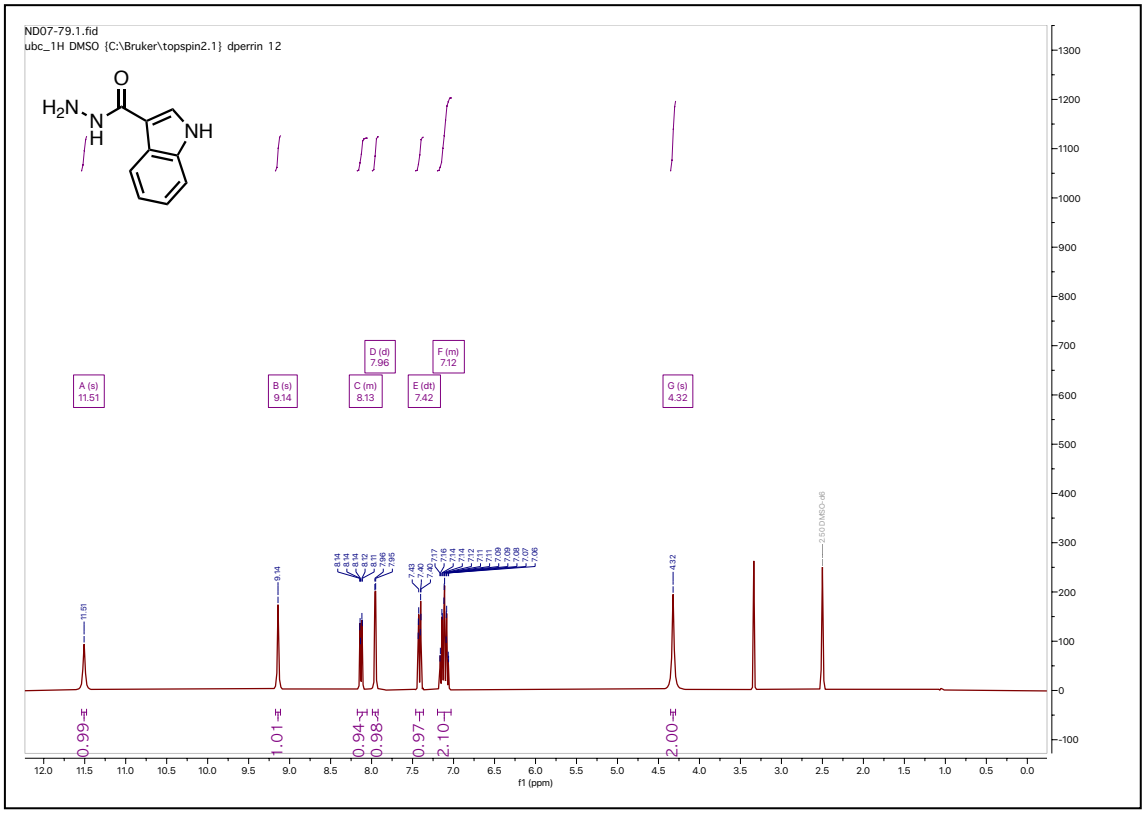
Furan-2-carbohydrazide (2m)



Thiophene-2-carbohydrazide (2n)

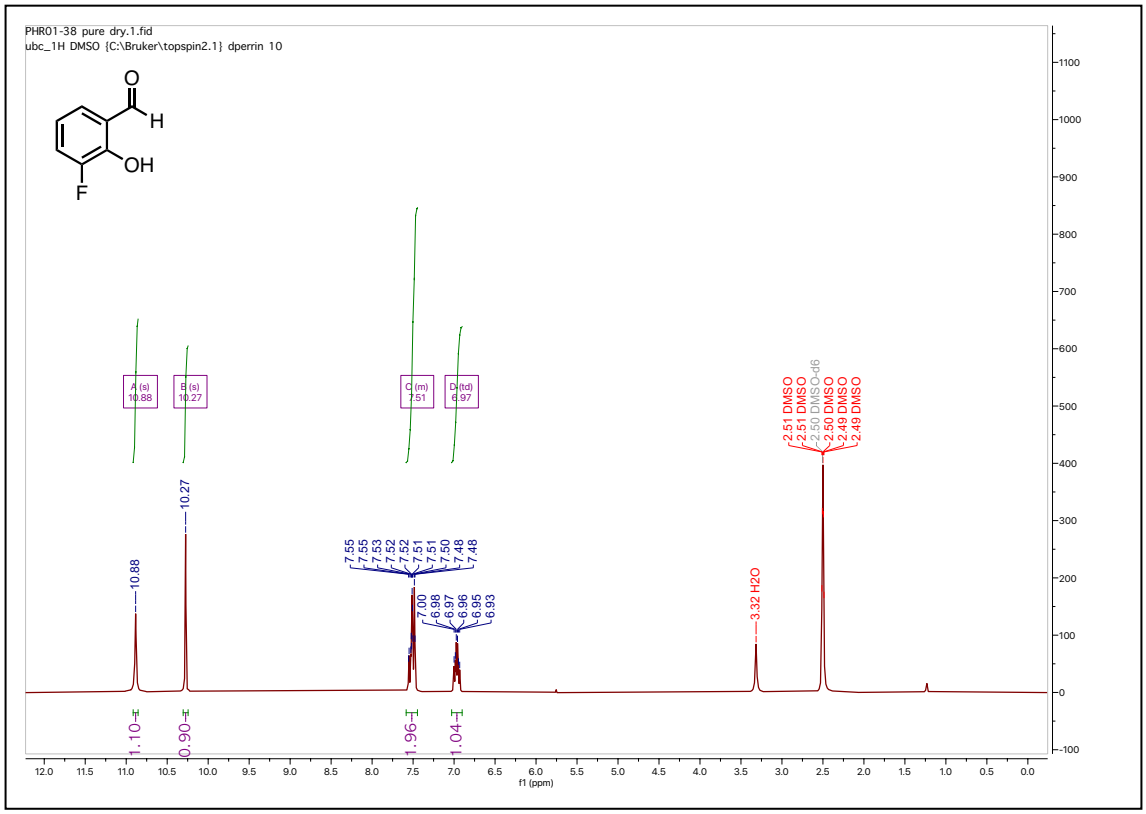


1H-indole-3-carbohydrazide (2o)

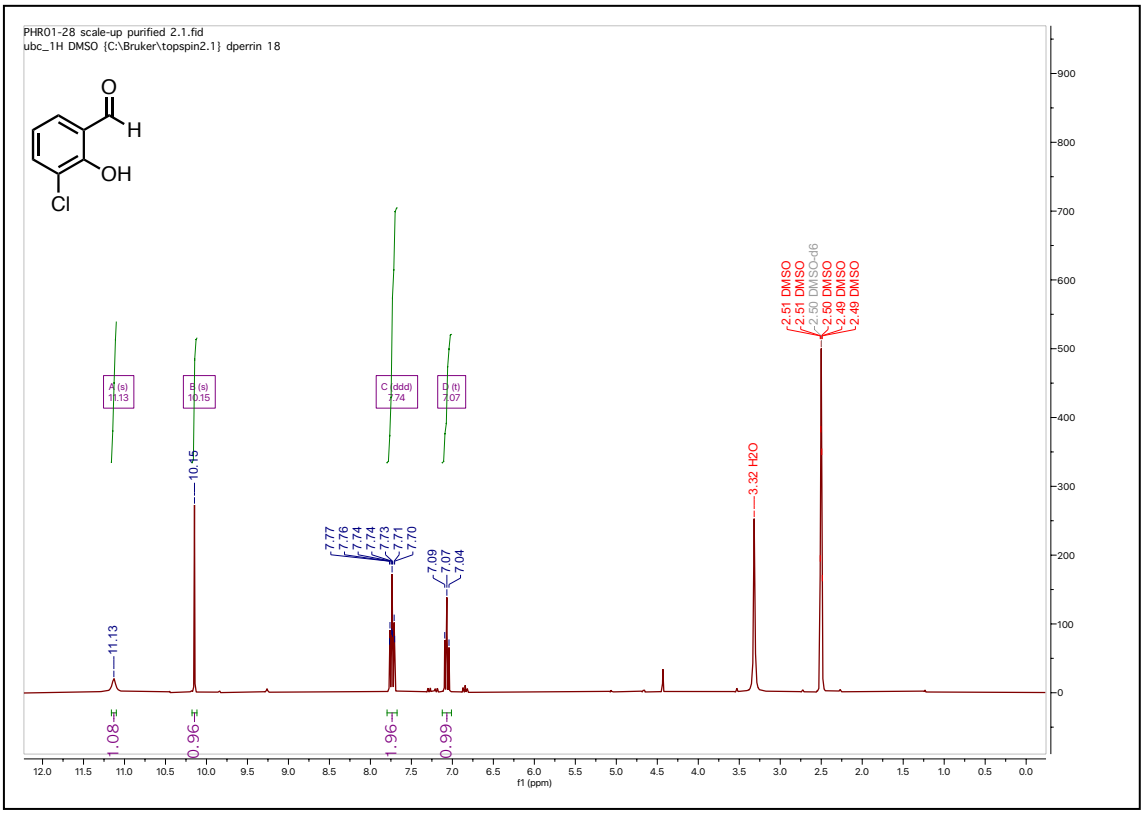


NMR Spectra of salicylaldehyde derivatives (4):

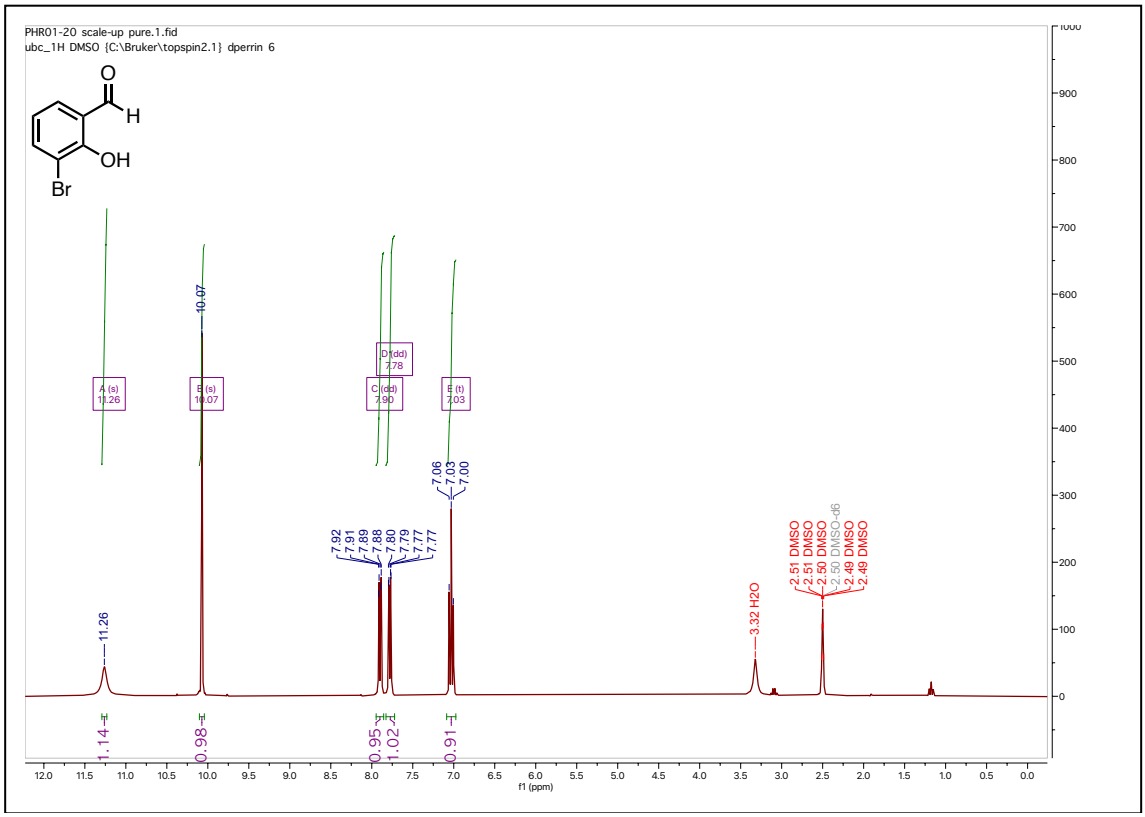
3-fluoro-2-hydroxybenzaldehyde (4a)



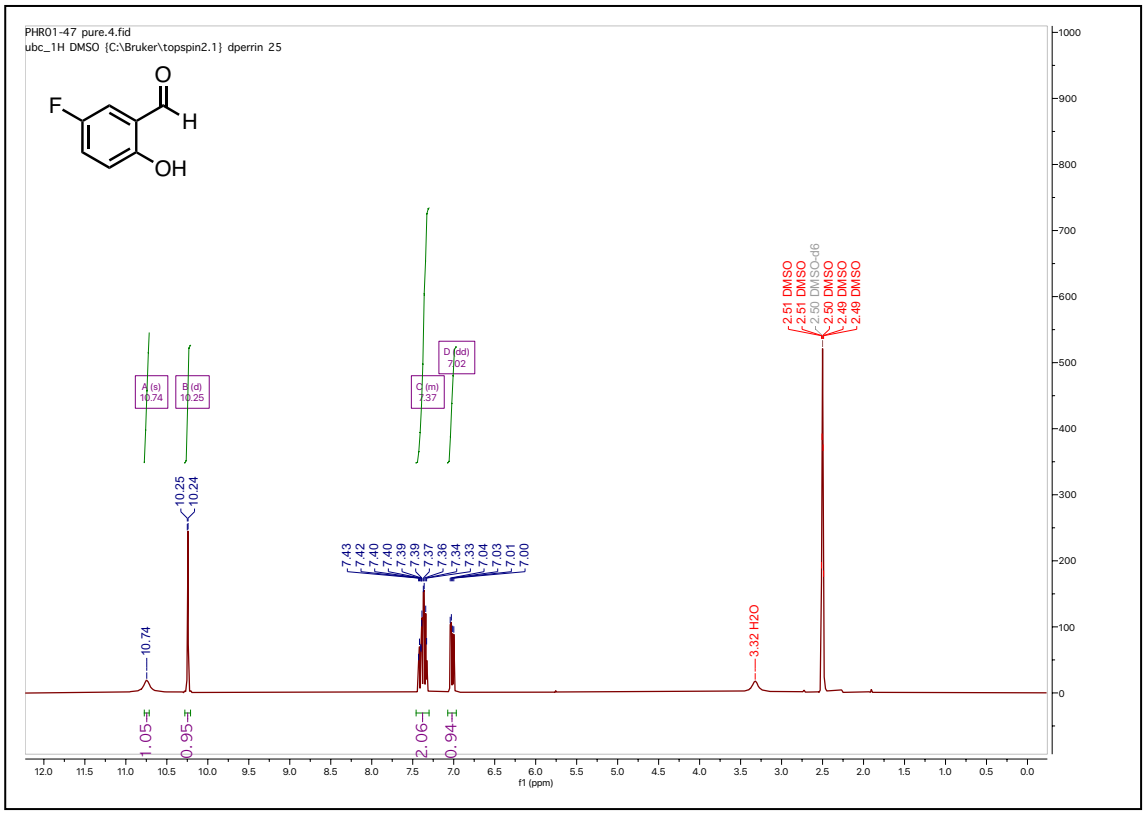
3-chloro-2-hydroxybenzaldehyde (4b)



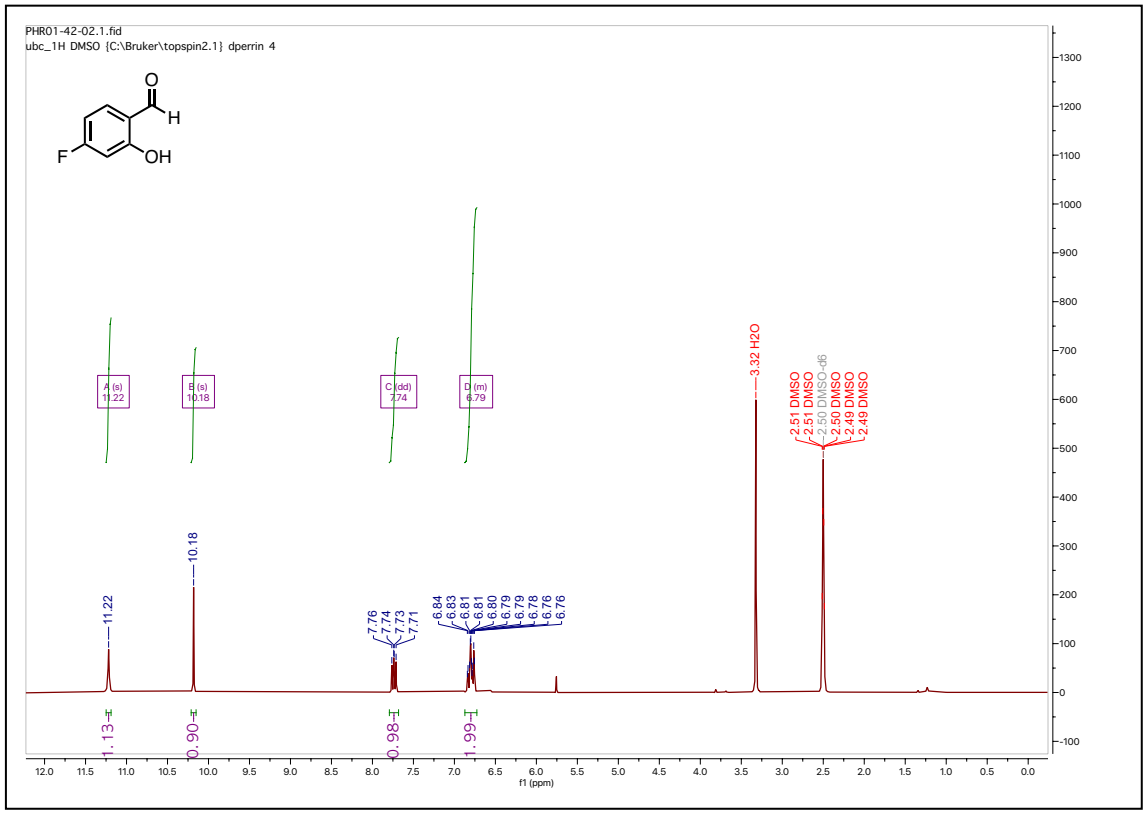
3-bromo-2-hydroxybenzaldehyde (4c)



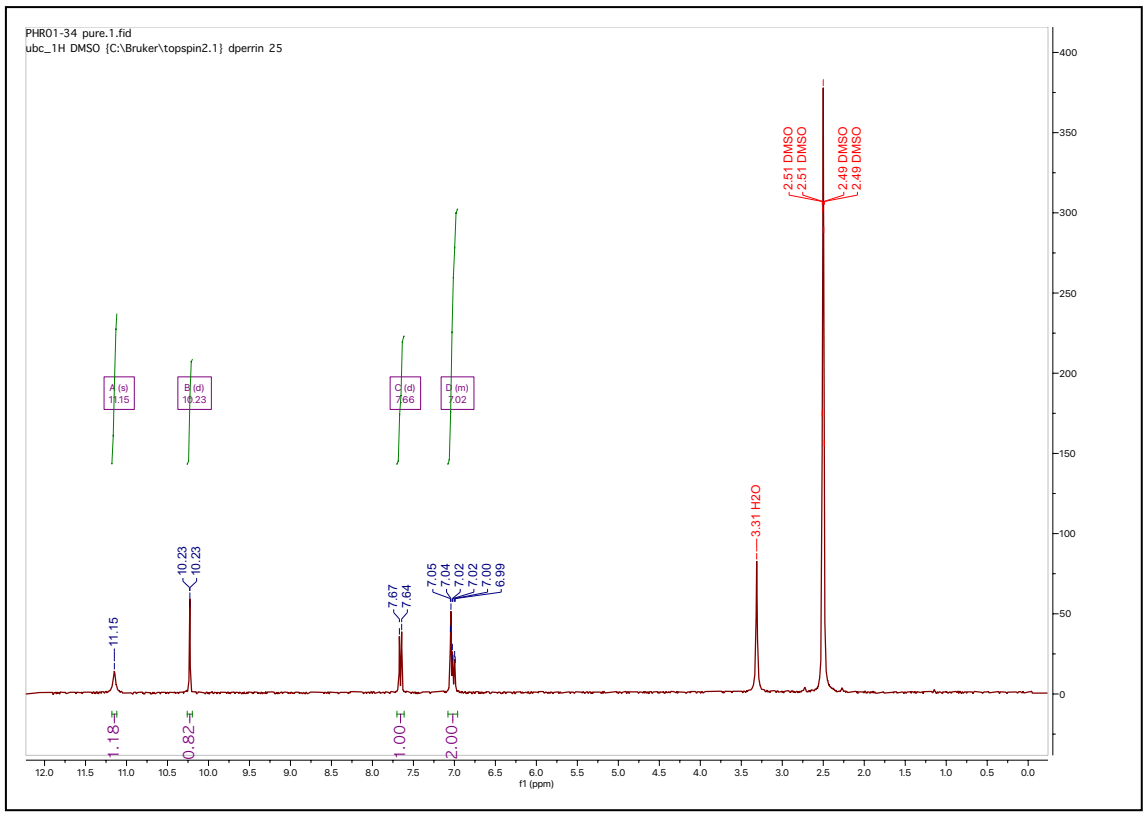
5-fluoro-2-hydroxybenzaldehyde (4d)



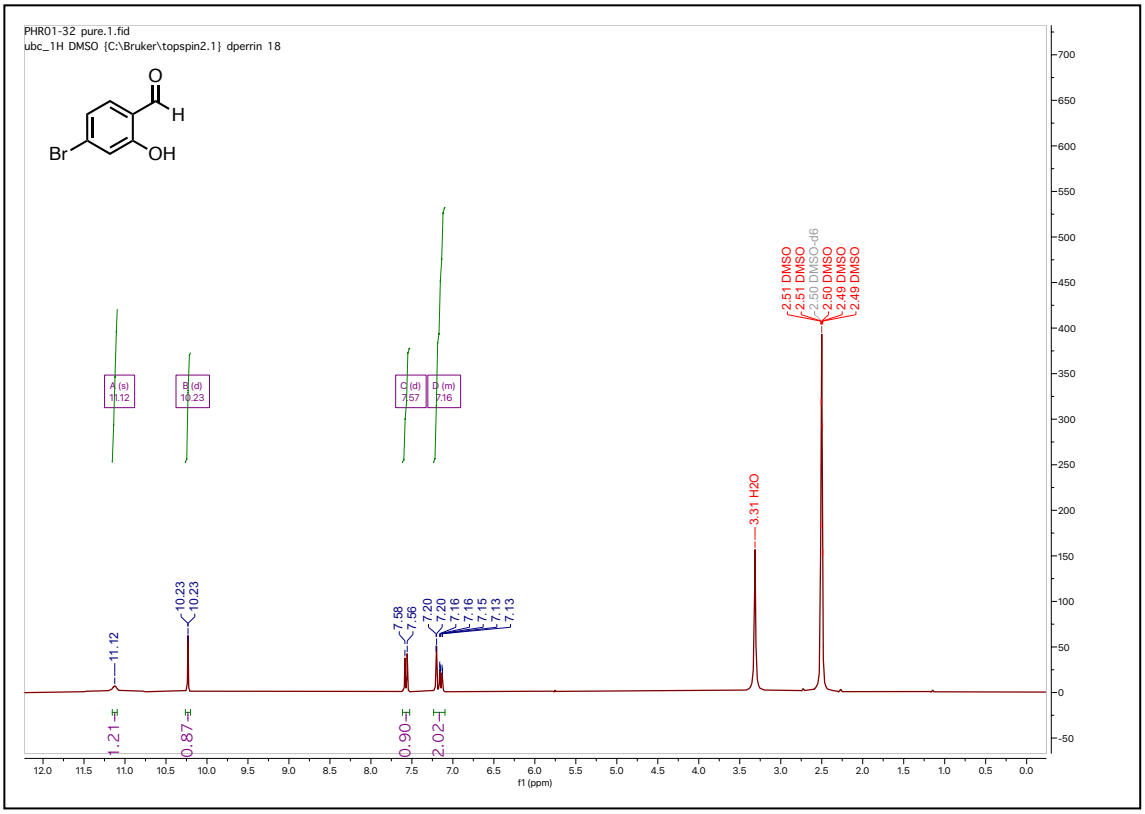
4-fluoro-2-hydroxybenzaldehyde (4e)



4-chloro-2-hydroxybenzaldehyde (4f)

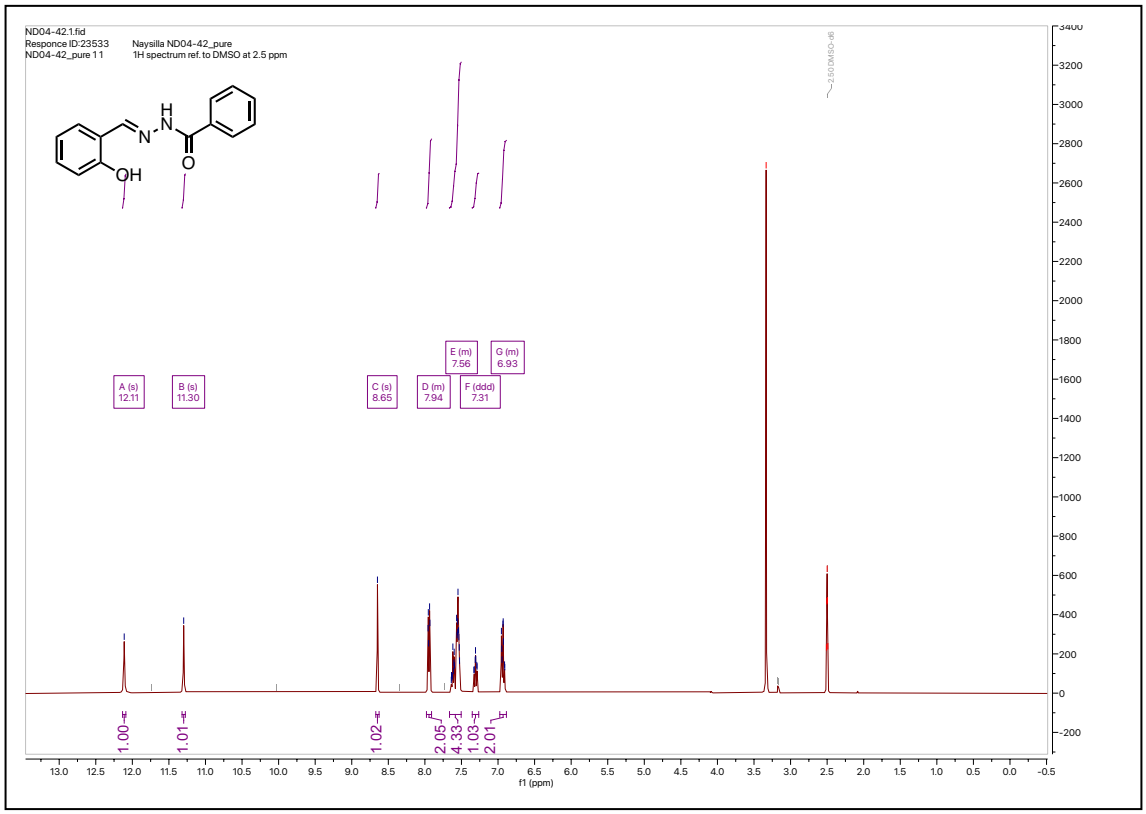


4-bromo-2-hydroxybenzaldehyde (4g)

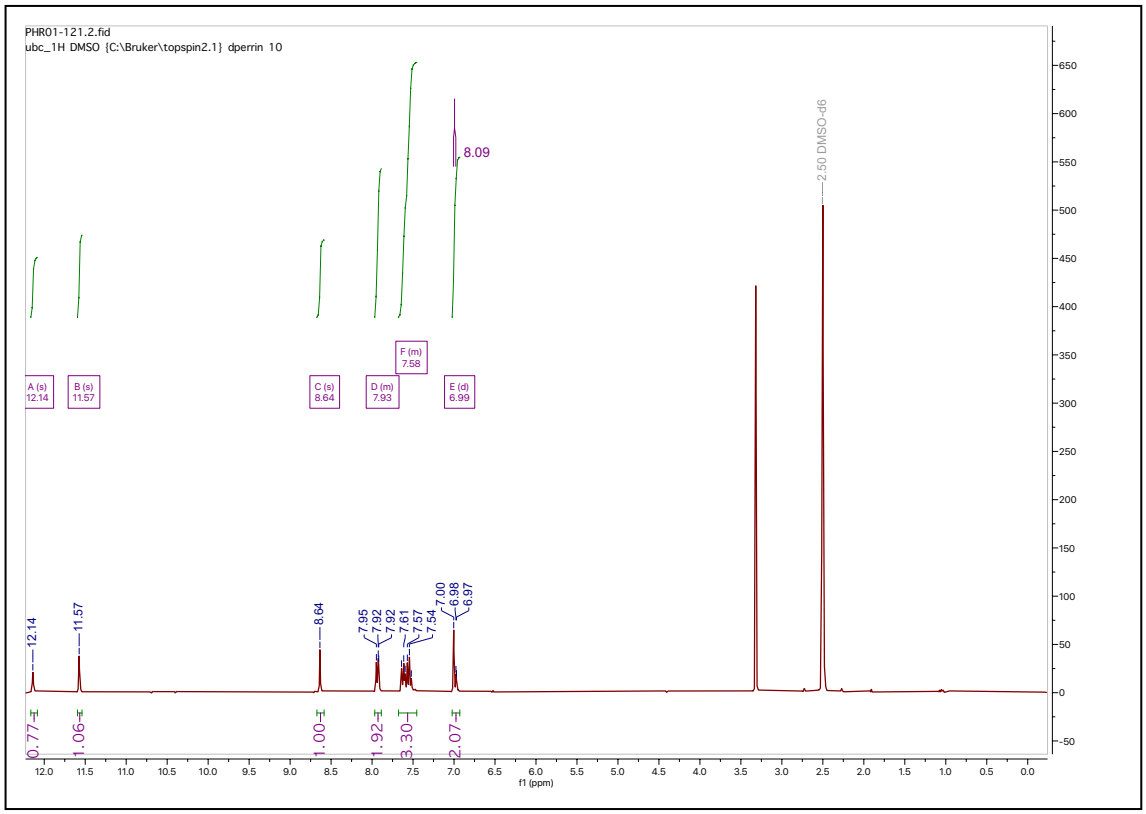


NMR Spectra of benzylidene benzohydrazides:

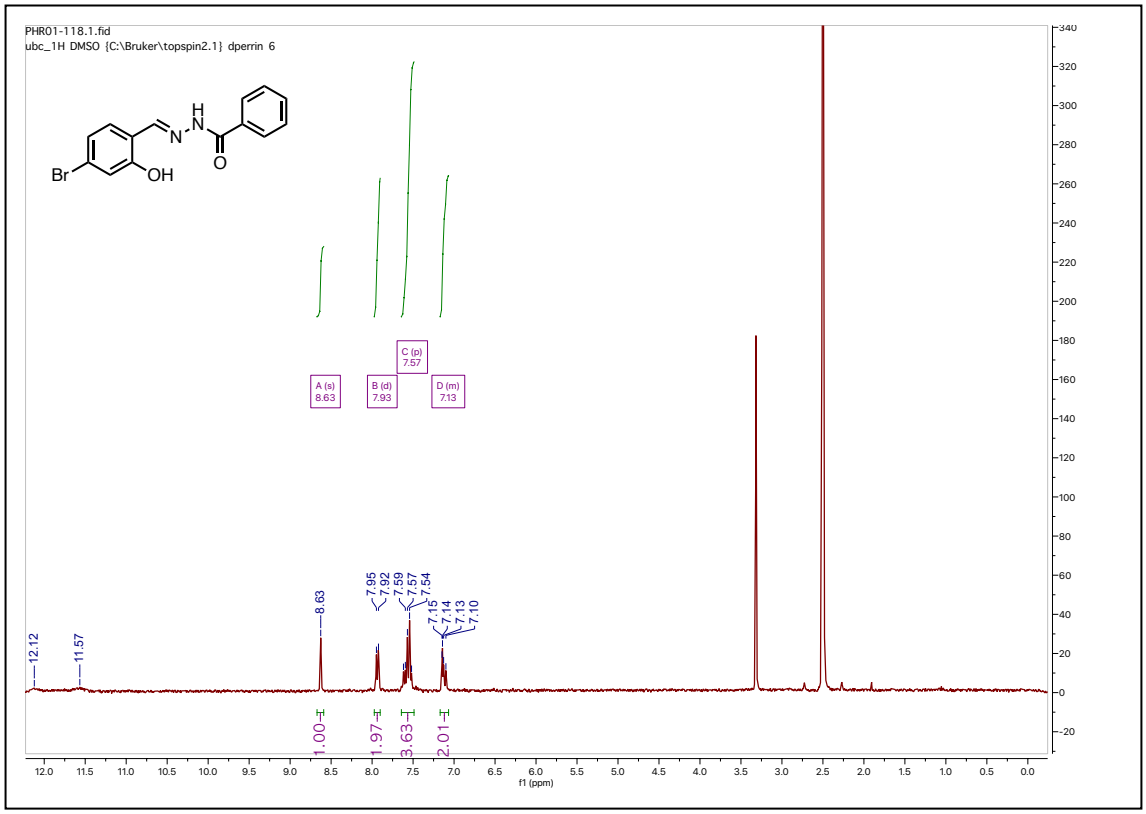
***N*'-(2-hydroxybenzylidene)benzohydrazide (5a)**



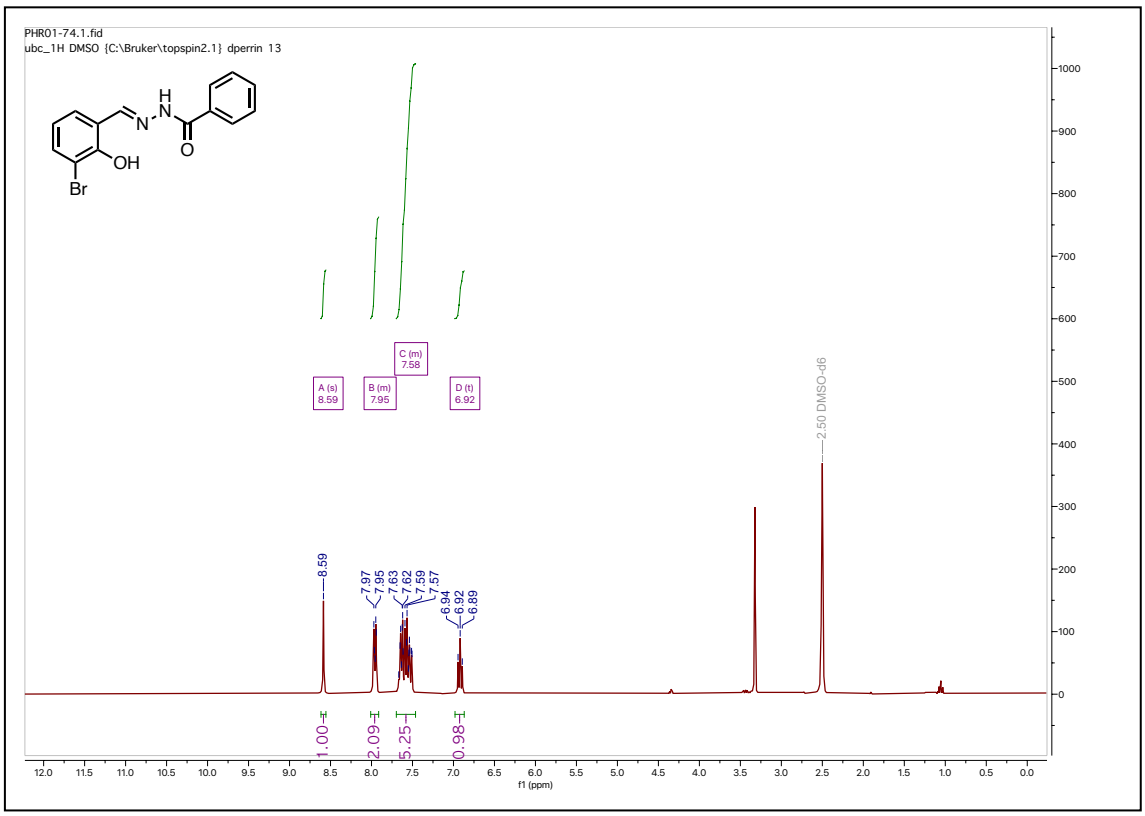
***N*'-(4-chloro-2-hydroxybenzylidene)benzohydrazide (5b)**



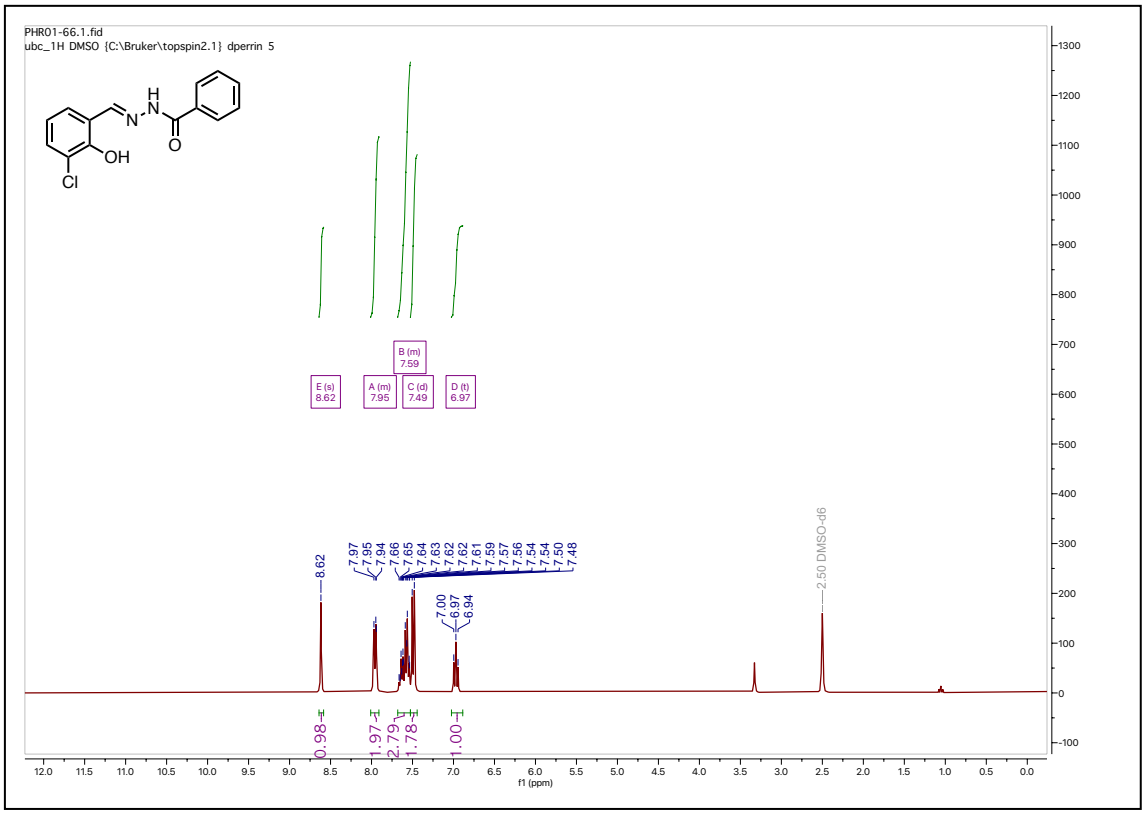
N'-(4-bromo-2-hydroxybenzylidene)benzohydrazide (5c)



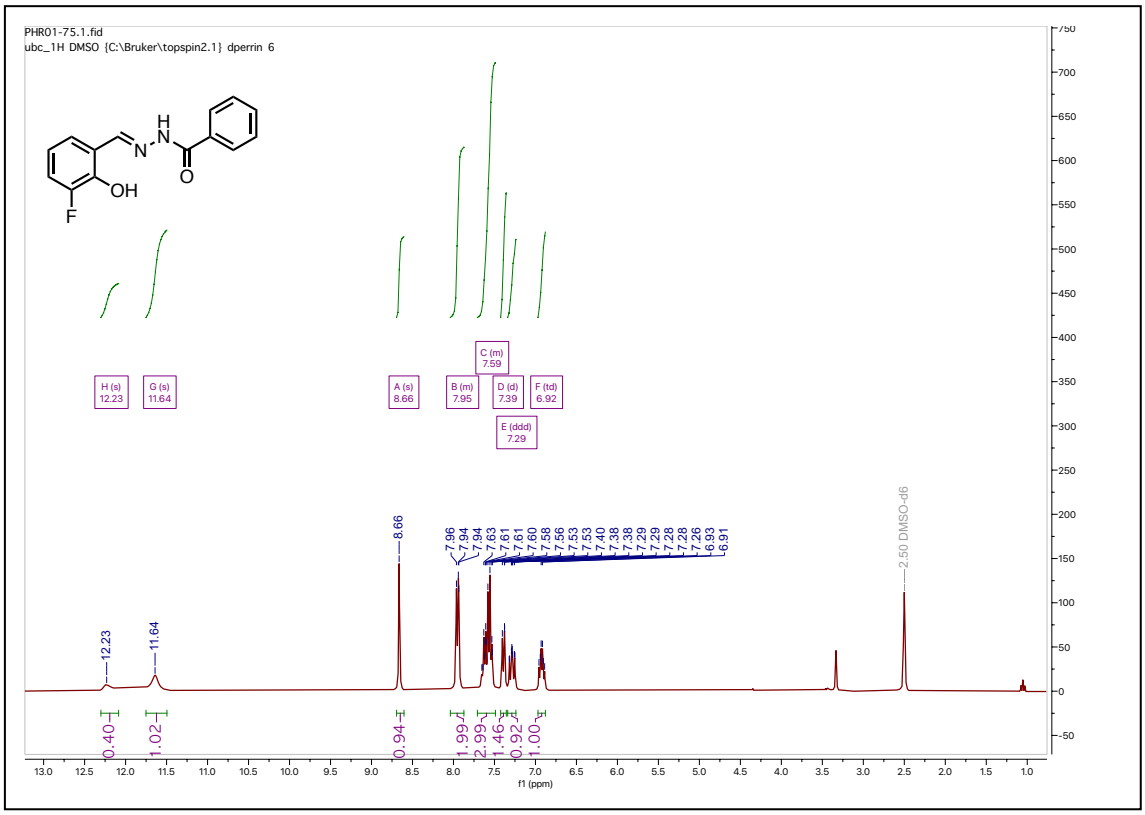
N'-(3-bromo-2-hydroxybenzylidene)benzohydrazide (5d)



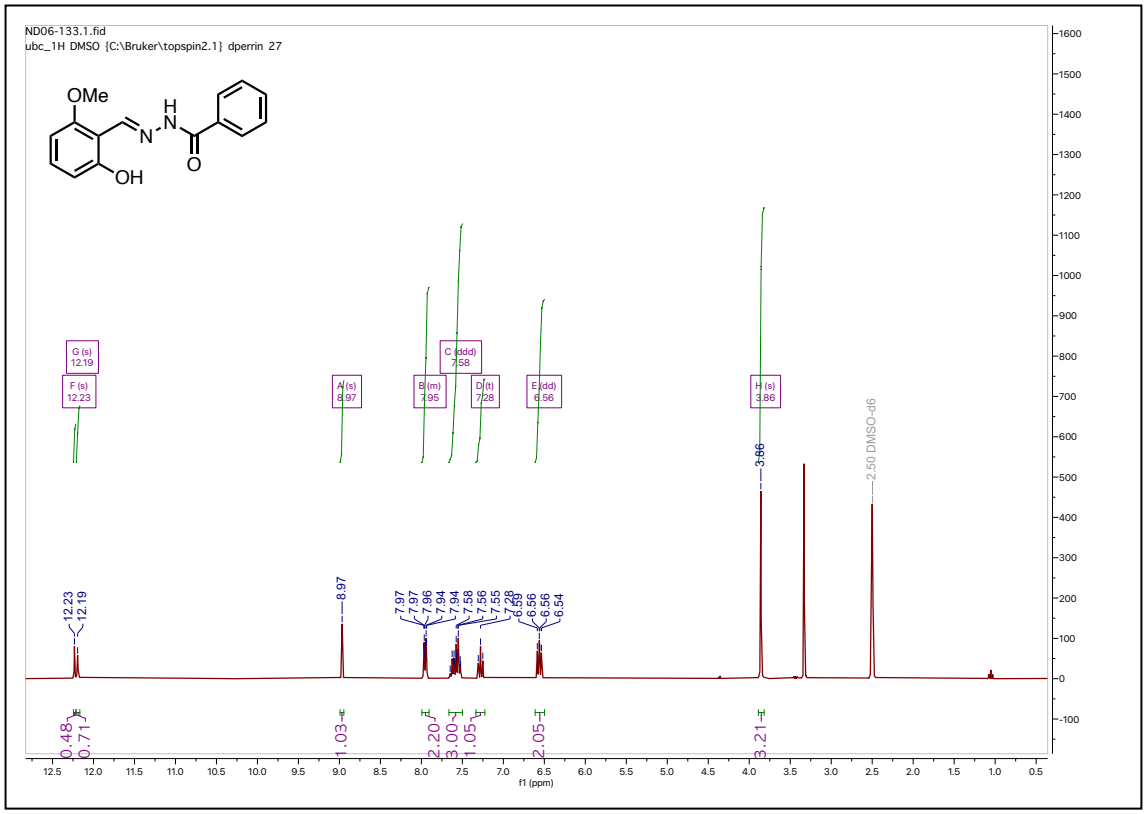
N'-(3-chloro-2-hydroxybenzylidene)benzohydrazide (5e)



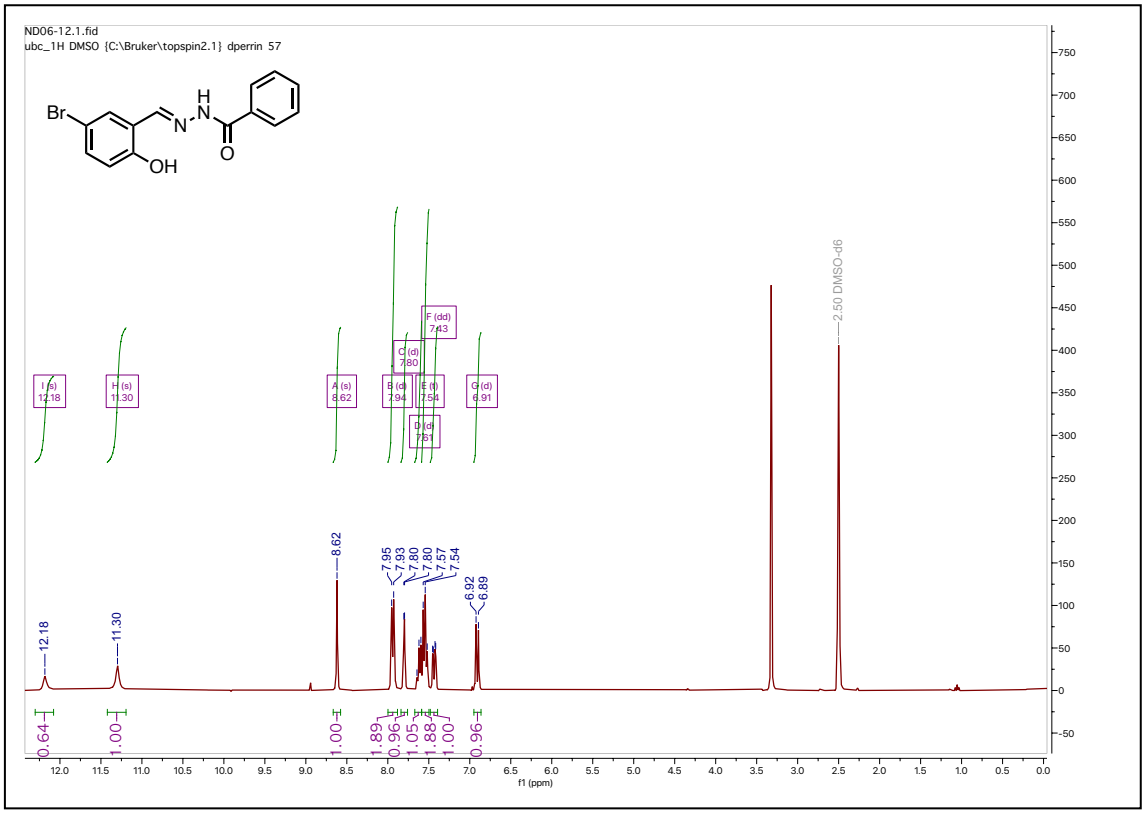
N'-(3-fluoro-2-hydroxybenzylidene)benzohydrazide (5f)



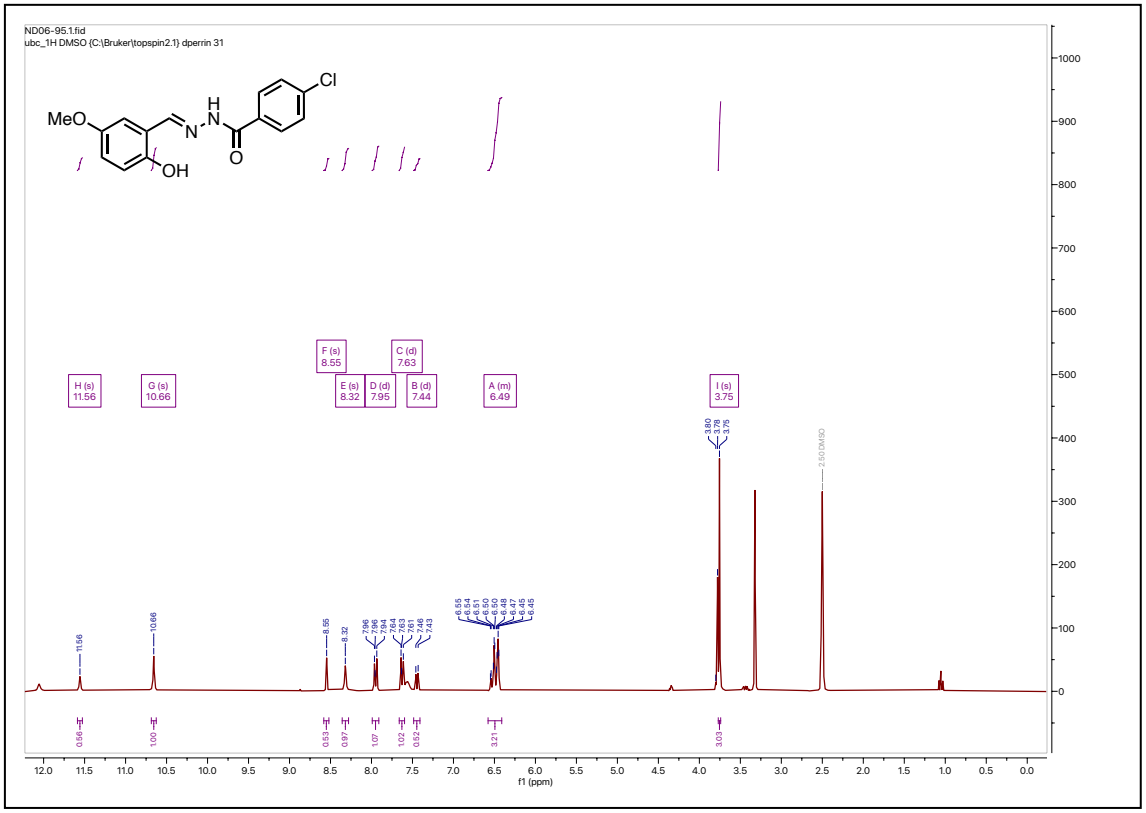
N'-(2-hydroxy-6-methoxybenzylidene)benzohydrazide (5g)



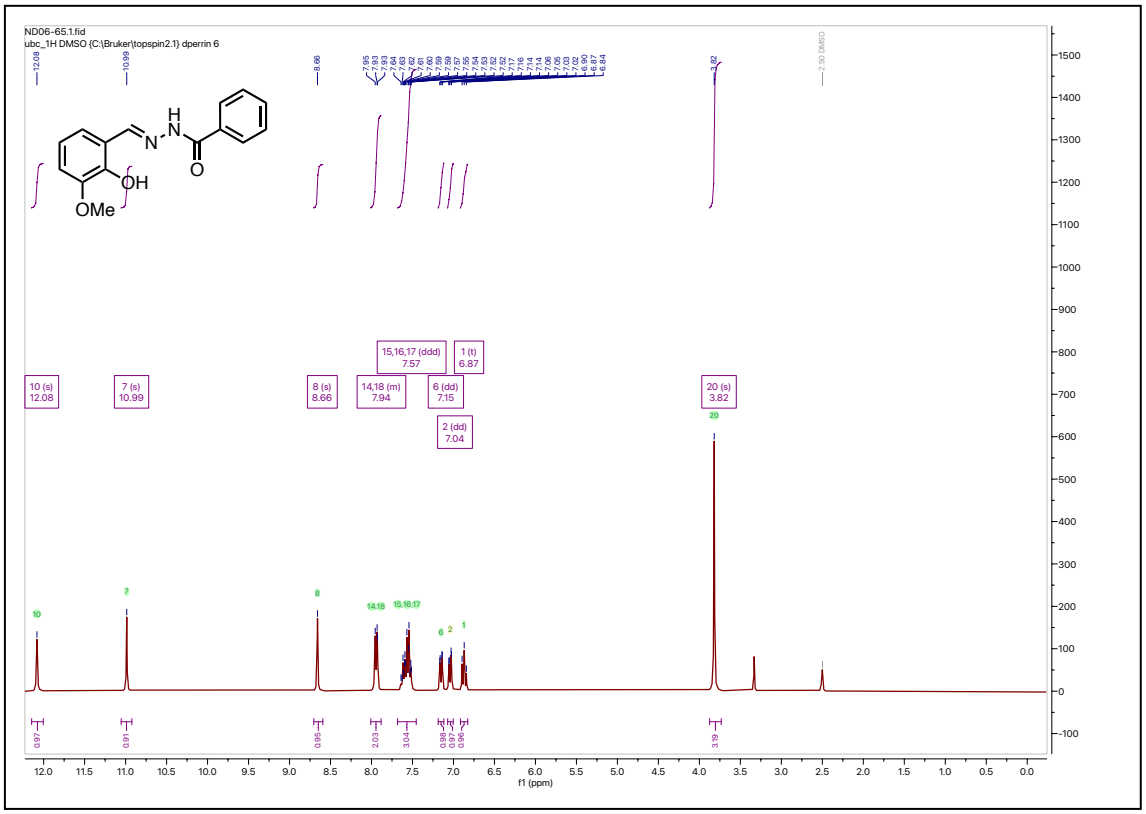
N'-(5-bromo-2-hydroxybenzylidene)benzohydrazide (5h)



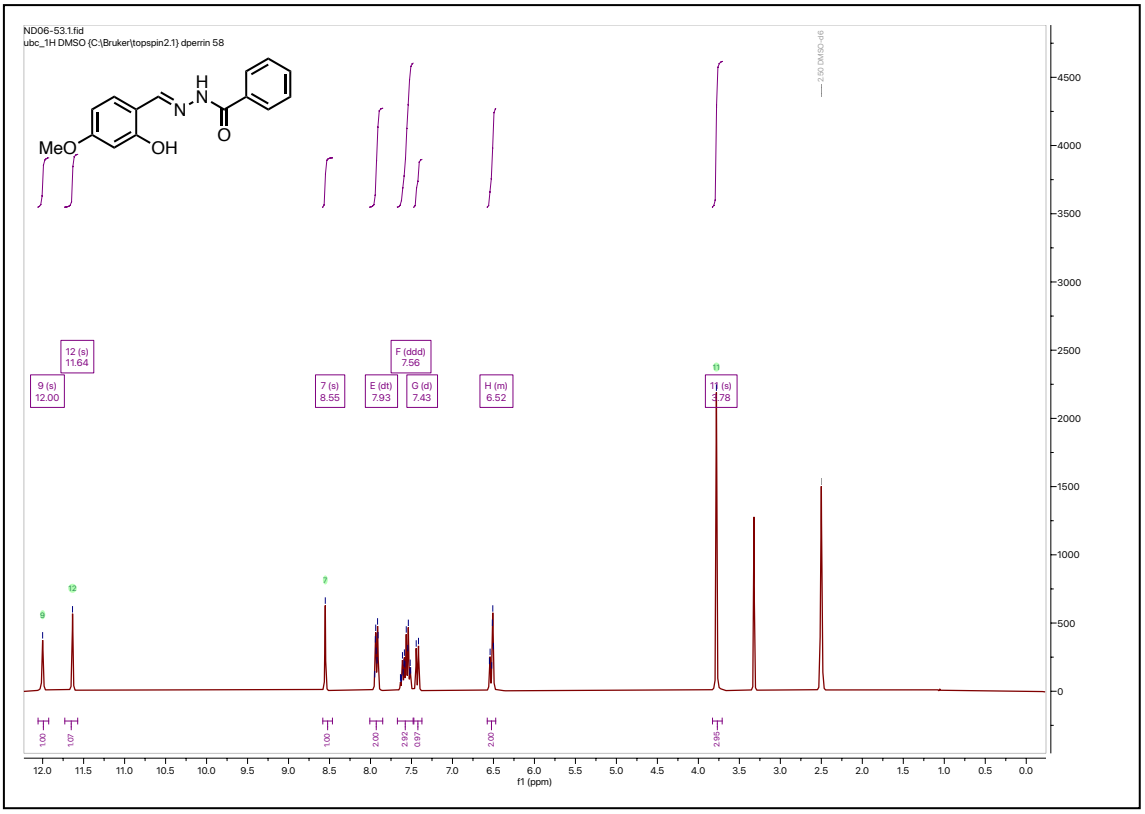
4-chloro-N'-(2-hydroxy-5-methoxybenzylidene)benzohydrazide (5i)



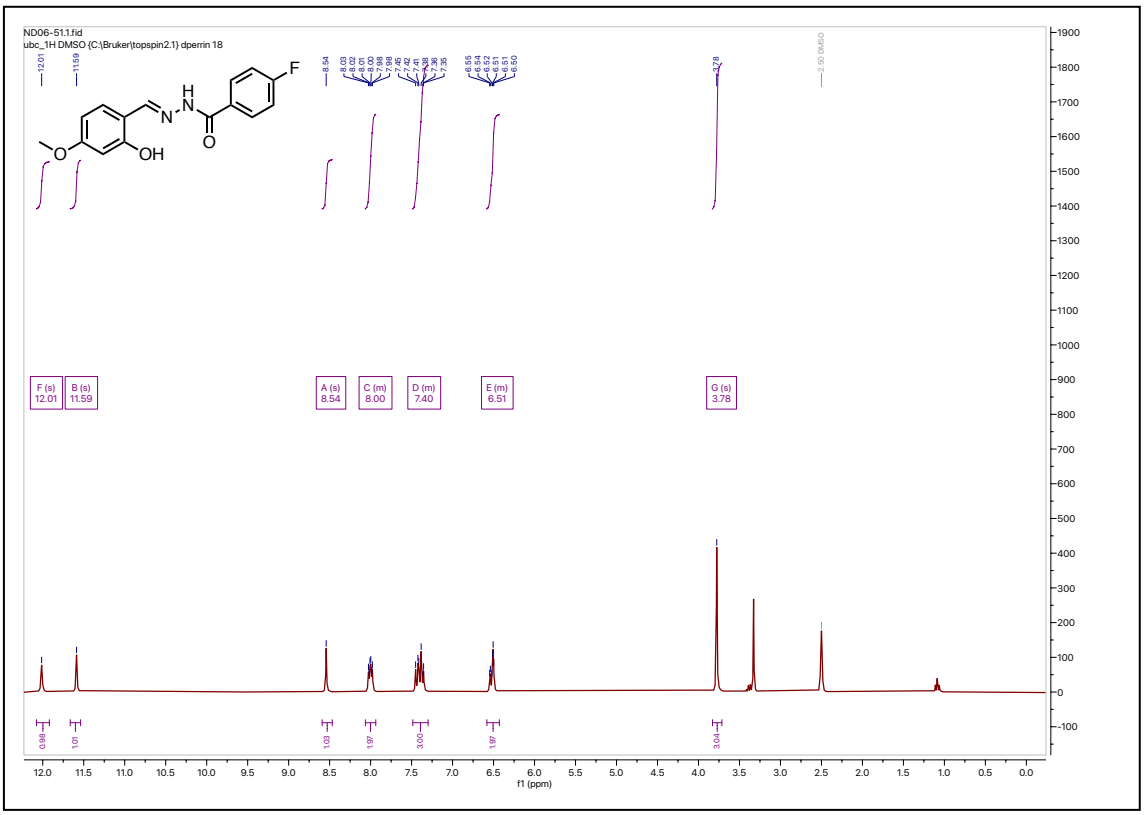
N'-(2-hydroxy-3-methoxybenzylidene)benzohydrazide (5j)



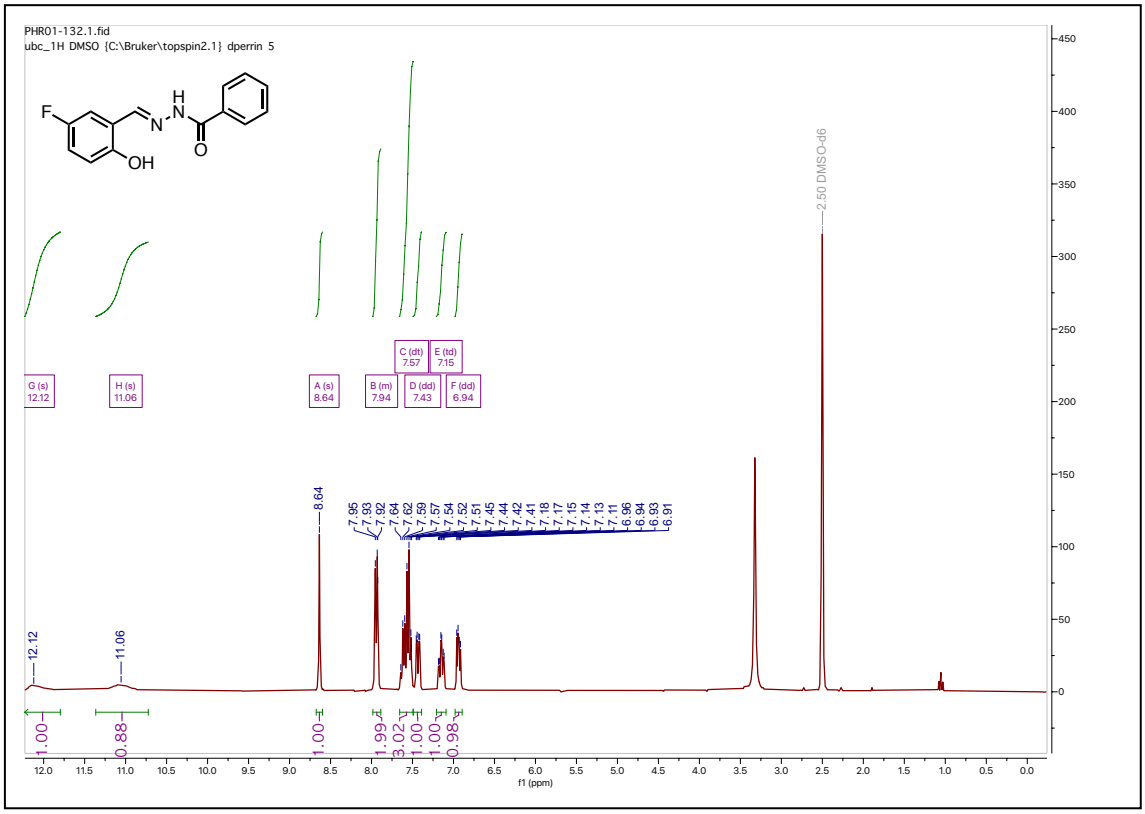
N’-(2-hydroxy-2-methoxybenzylidine)benzohydrazide (5k)



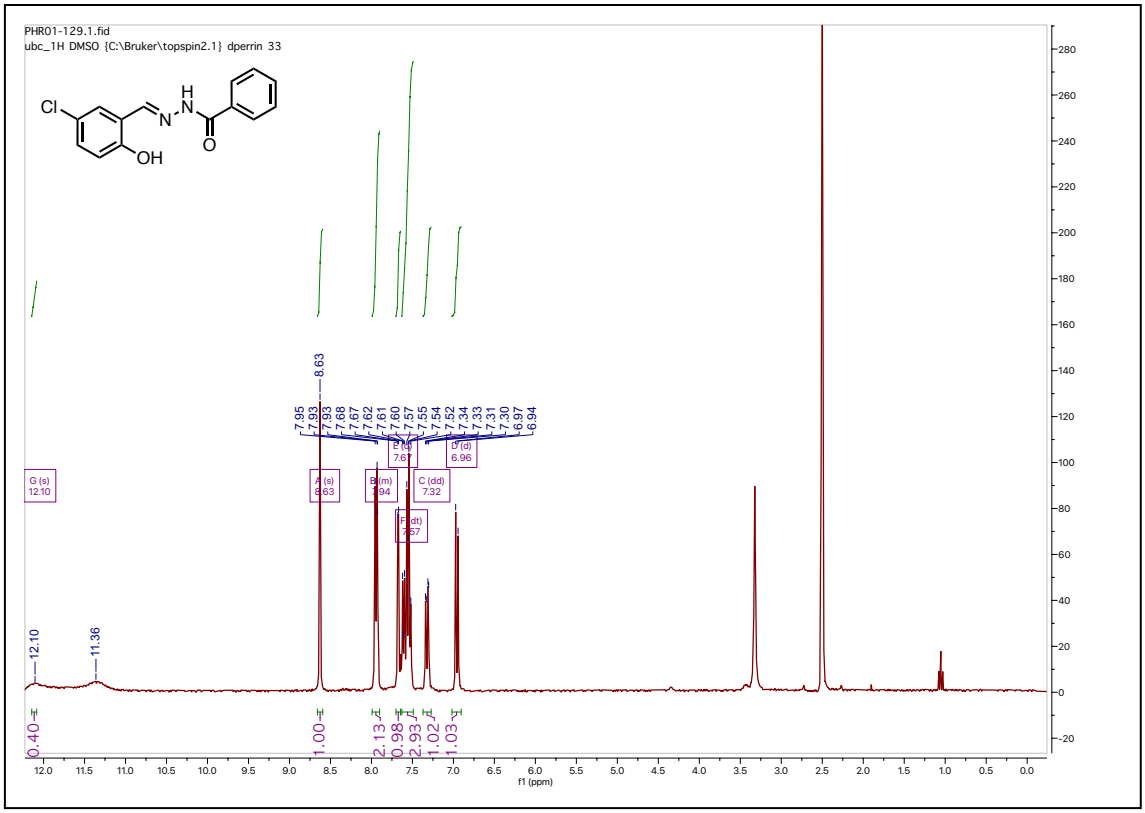
4-fluoro-N’-(2hydroxy-2-methoxybenzylidene)benzohydrazide (5l)



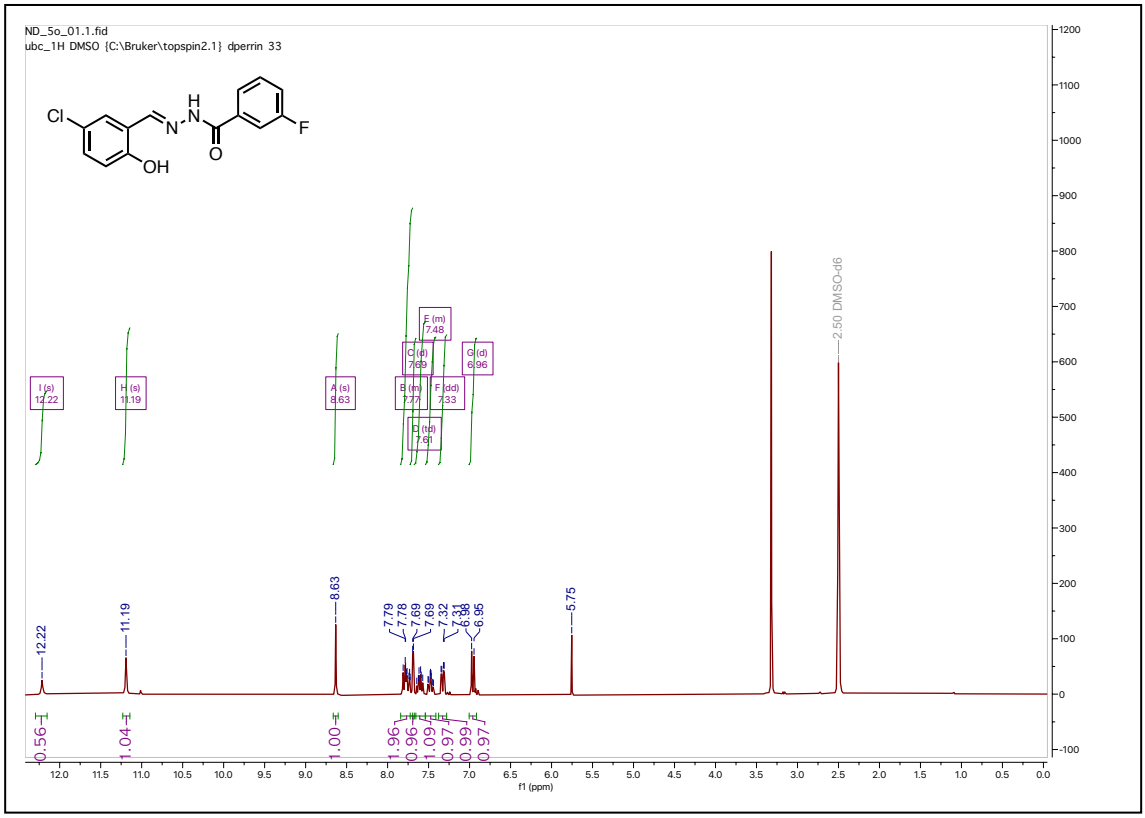
N’-(5-fluoro-2-hydroxybenzylidene)benzohydrazide (5m)



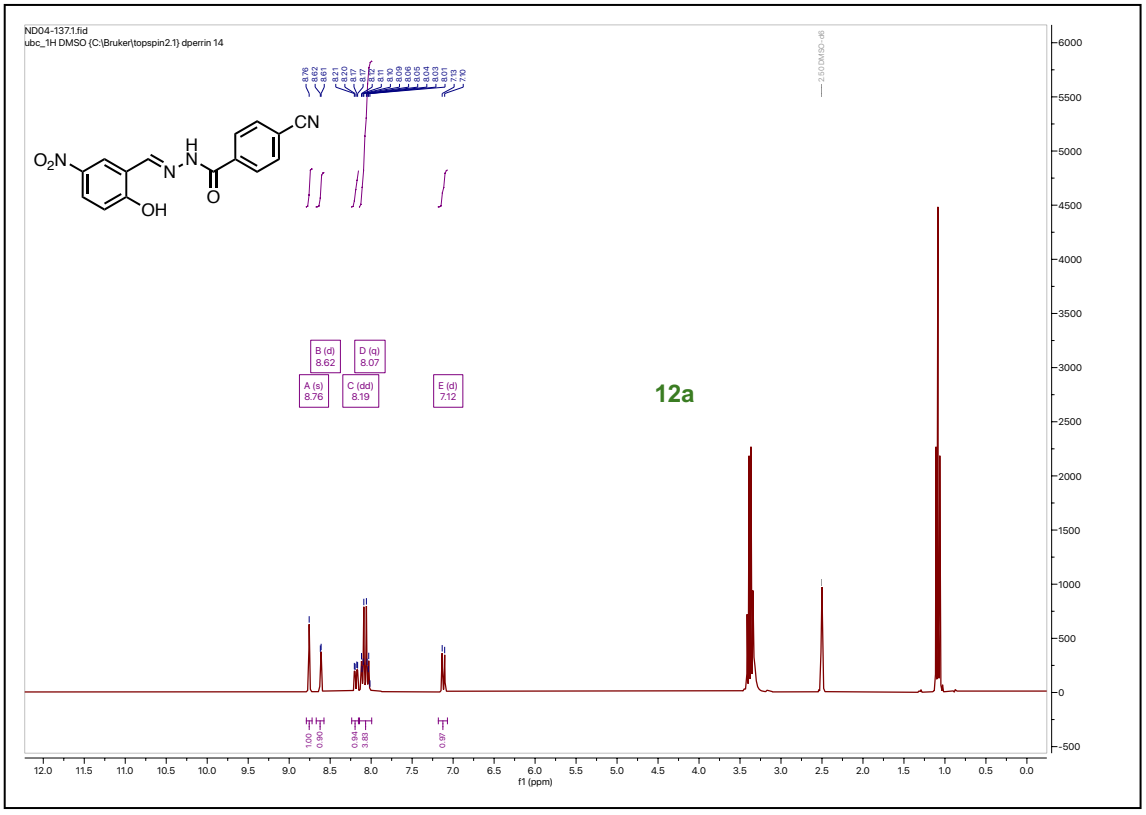
N’(5-chloro-2-hydroxybenzylidene)benzohydrazide (5n)



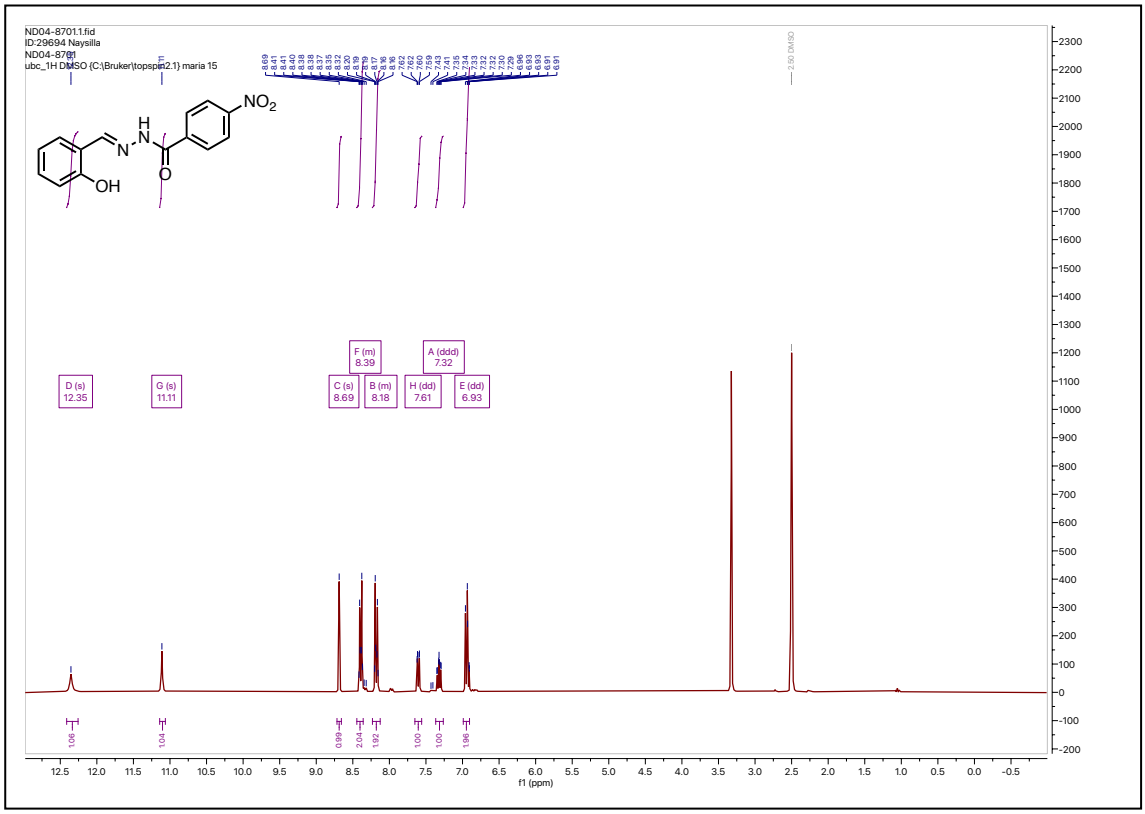
N'-(5-chloro-2-hydroxybenzylidene)-3-fluorobenzohydrazide (5o)



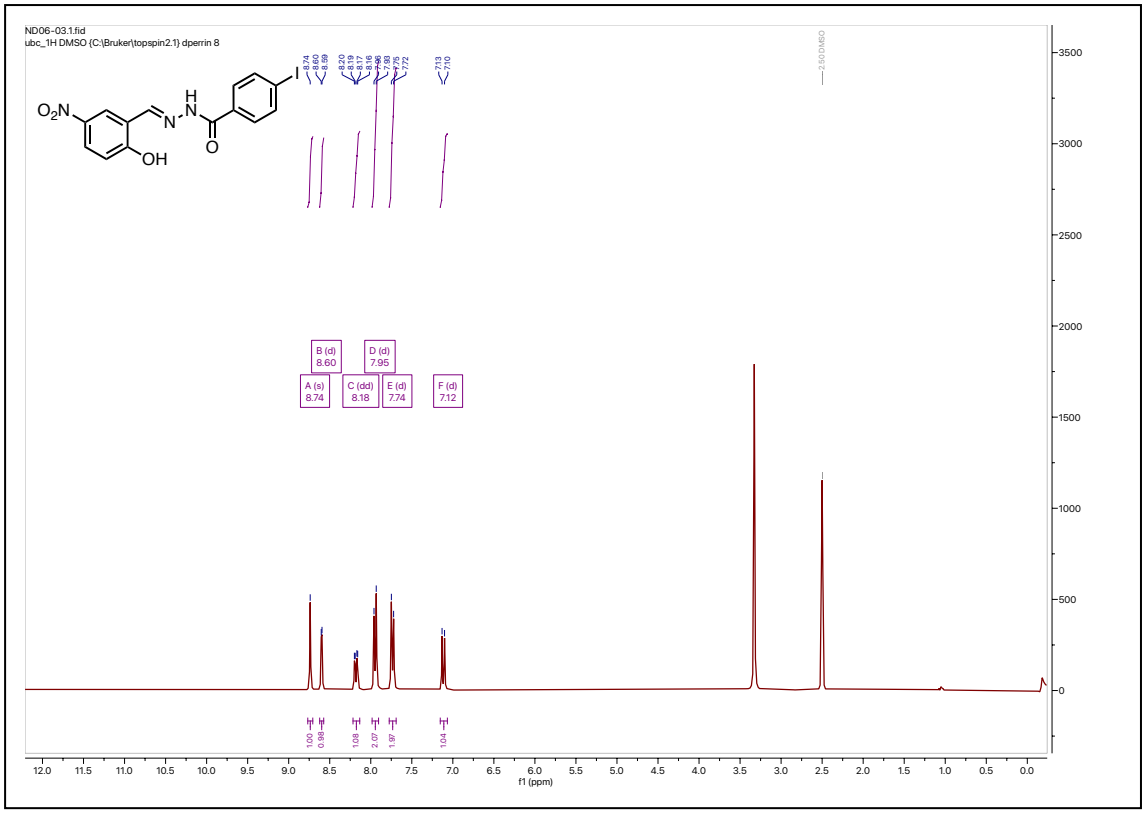
4-cyano-*N*'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide (5p)



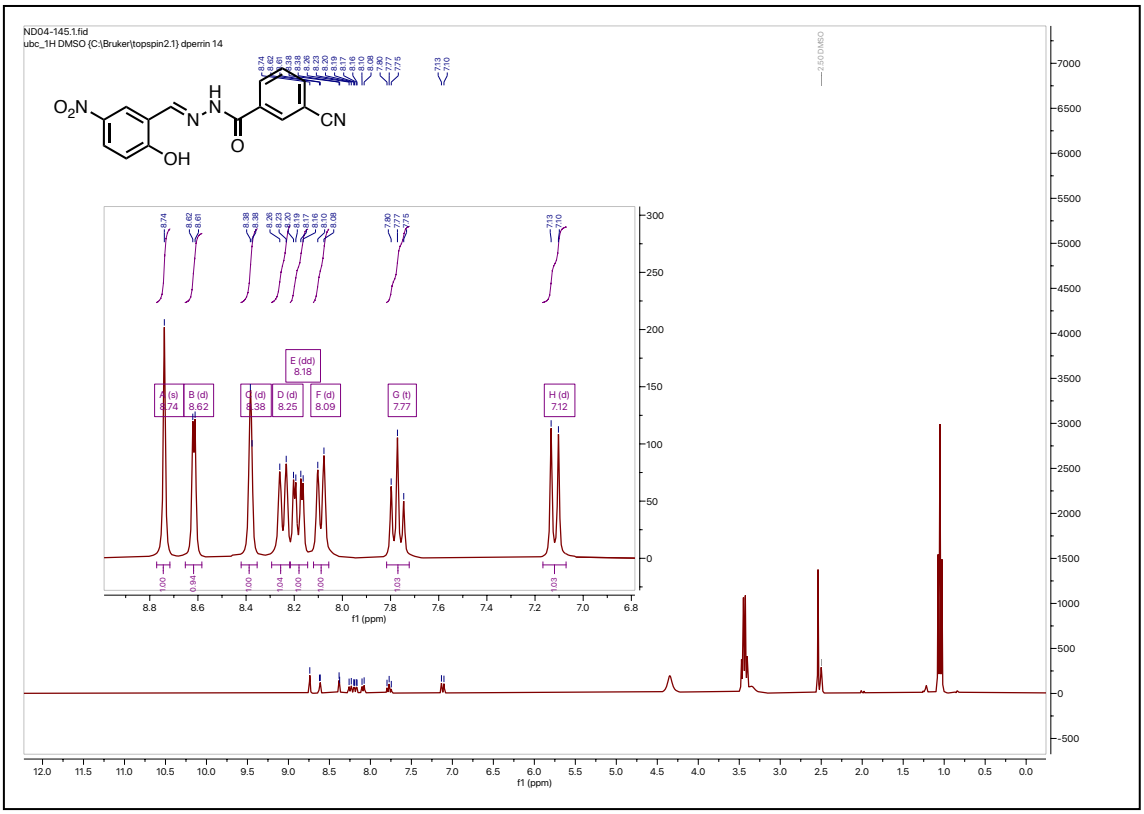
N’-(2-hydroxybenzylidene)-4-nitrobenzohydrazide (5q)



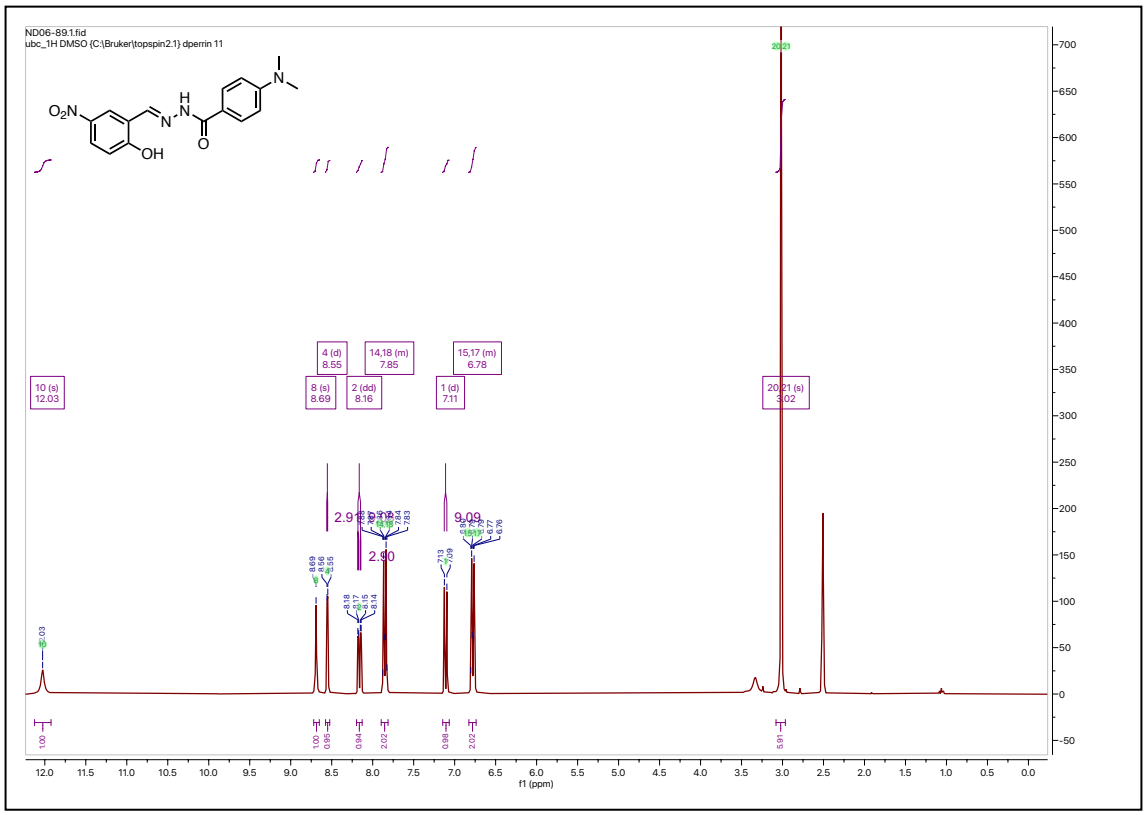
N’-(2-hydroxy-5-nitrobenzylidene)-4-iodobenzohydrazide (5r)



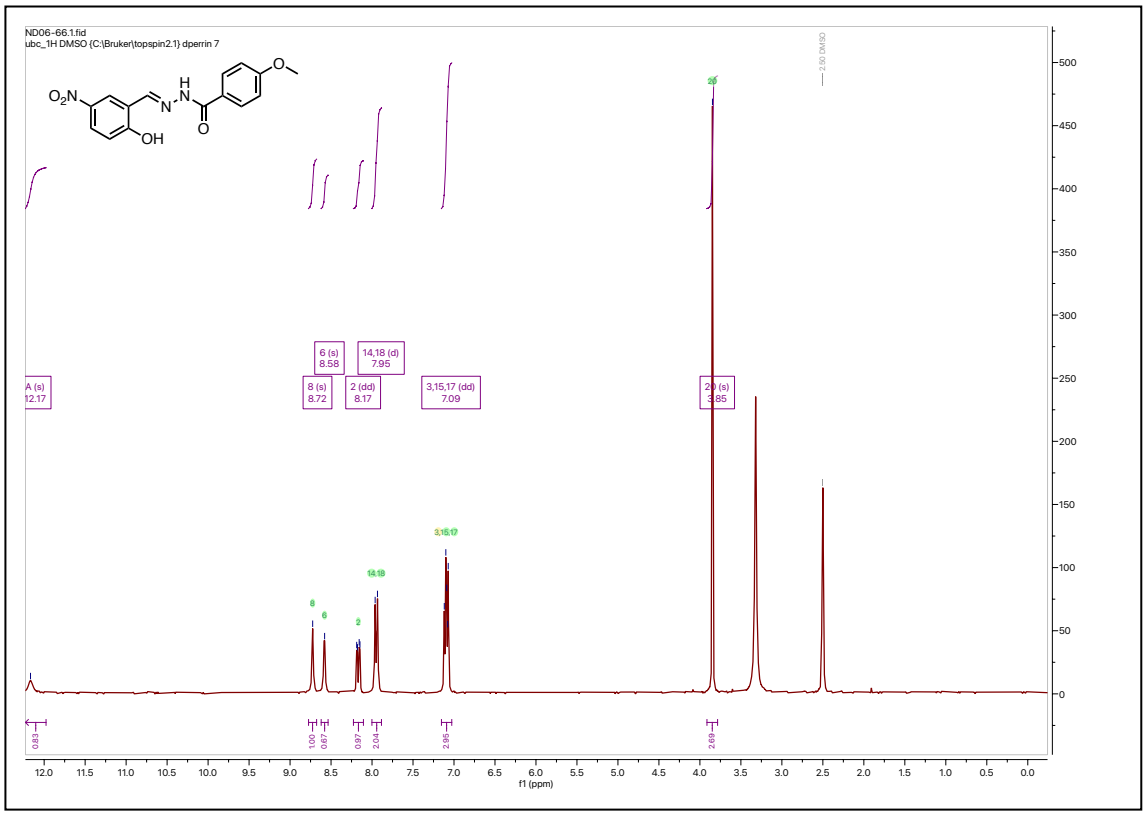
N'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide (5s)



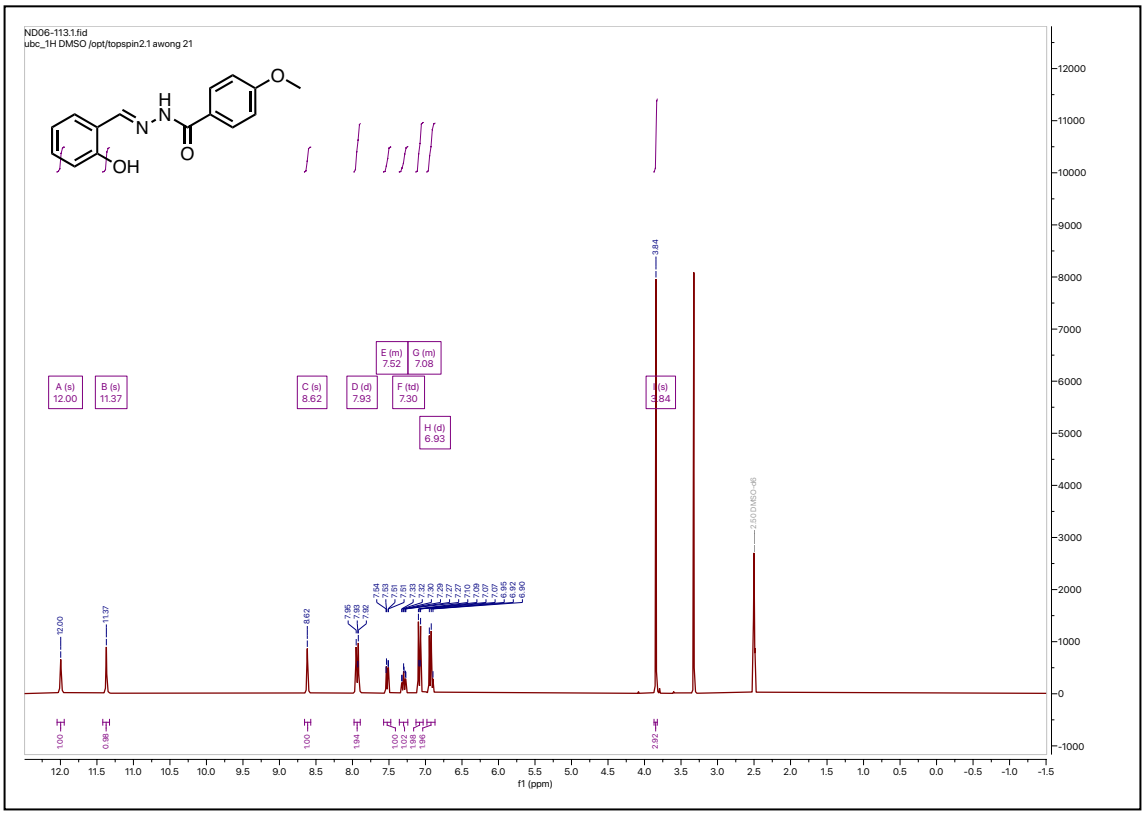
4-(dimethylamino)-N'-(2-hydroxy-5-nitrobenzylidene)benzohydrazide (5t)



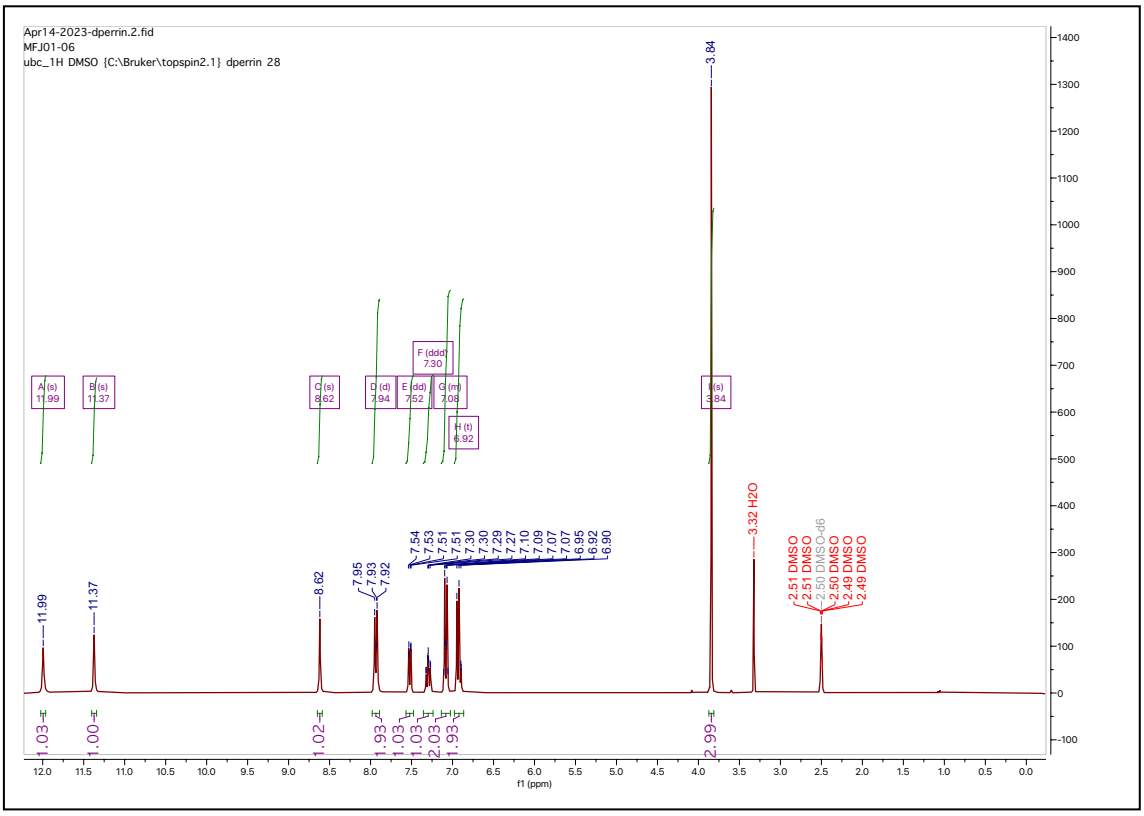
N'-(2-hydroxy-5-nitrobenzylidene)-4-methoxybenzohydrazide (5u)



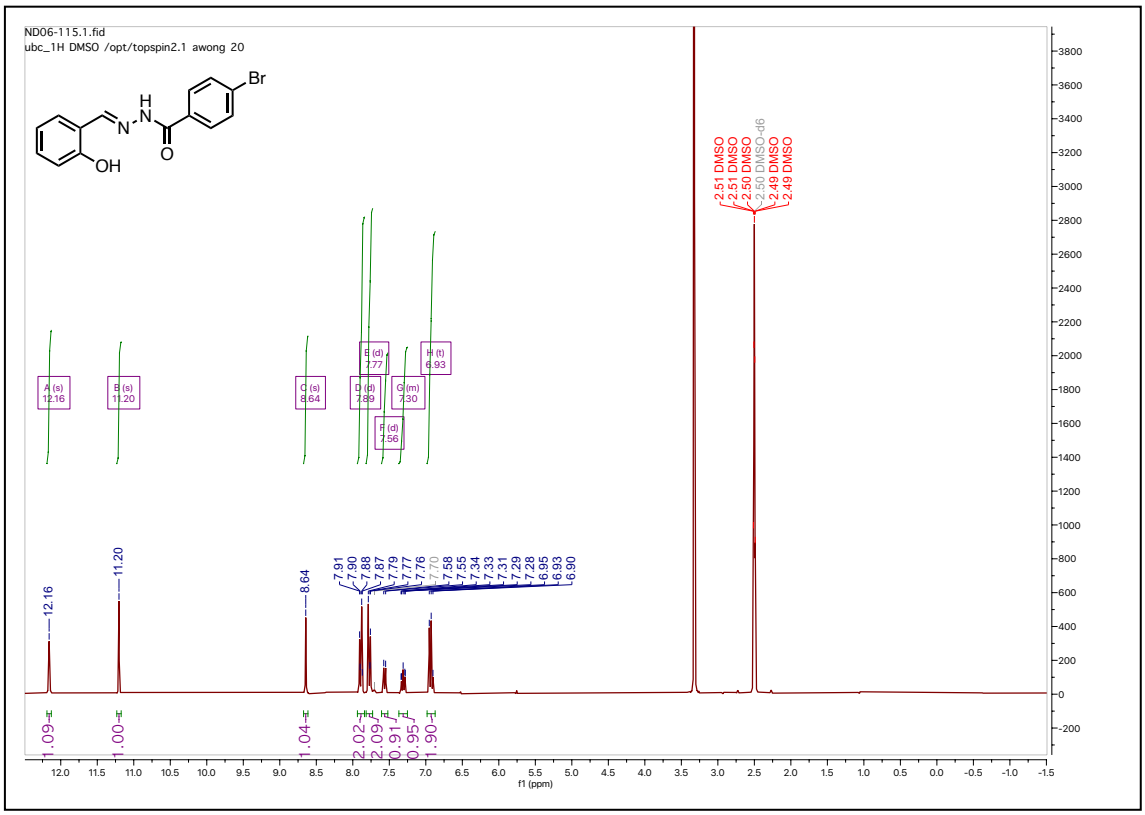
***N'*-(2-hydroxybenzylidene)-4-methoxybenzohydrazide (5v)**



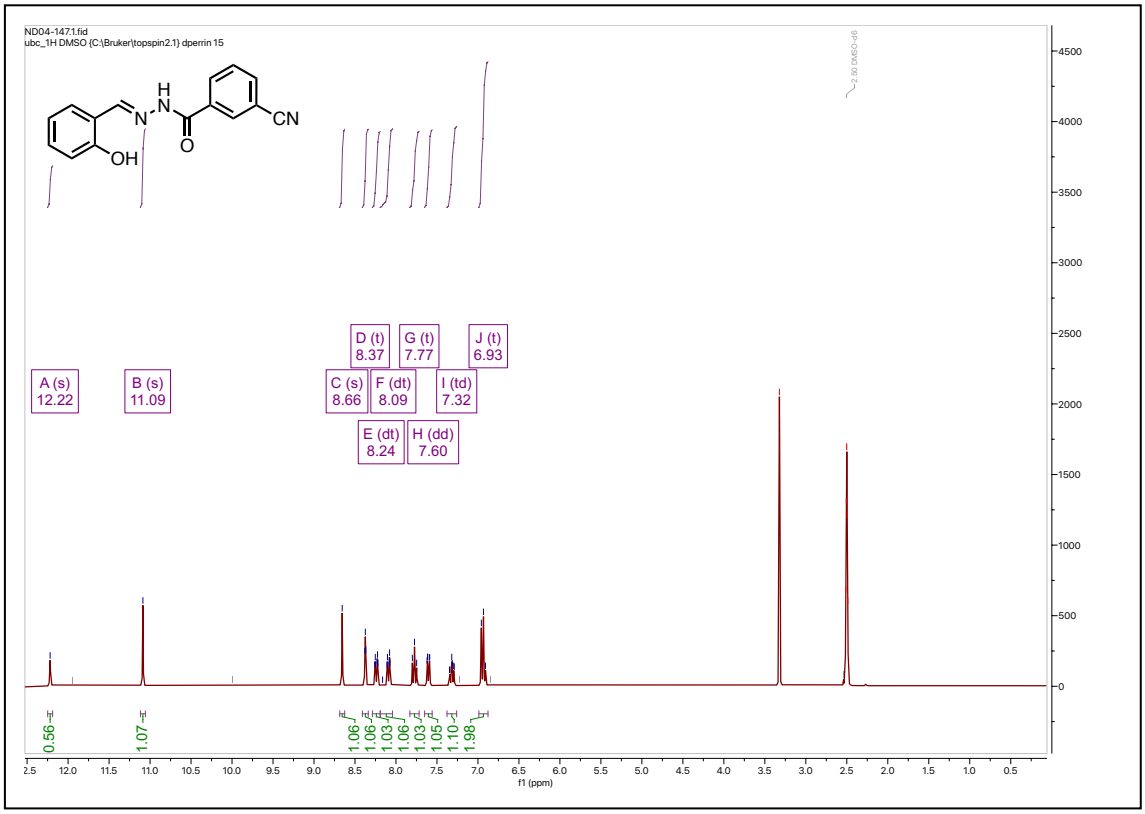
4-(dimethylamino)-N’-(2-hydroxybenzylidene)benzohydrazide (5w)



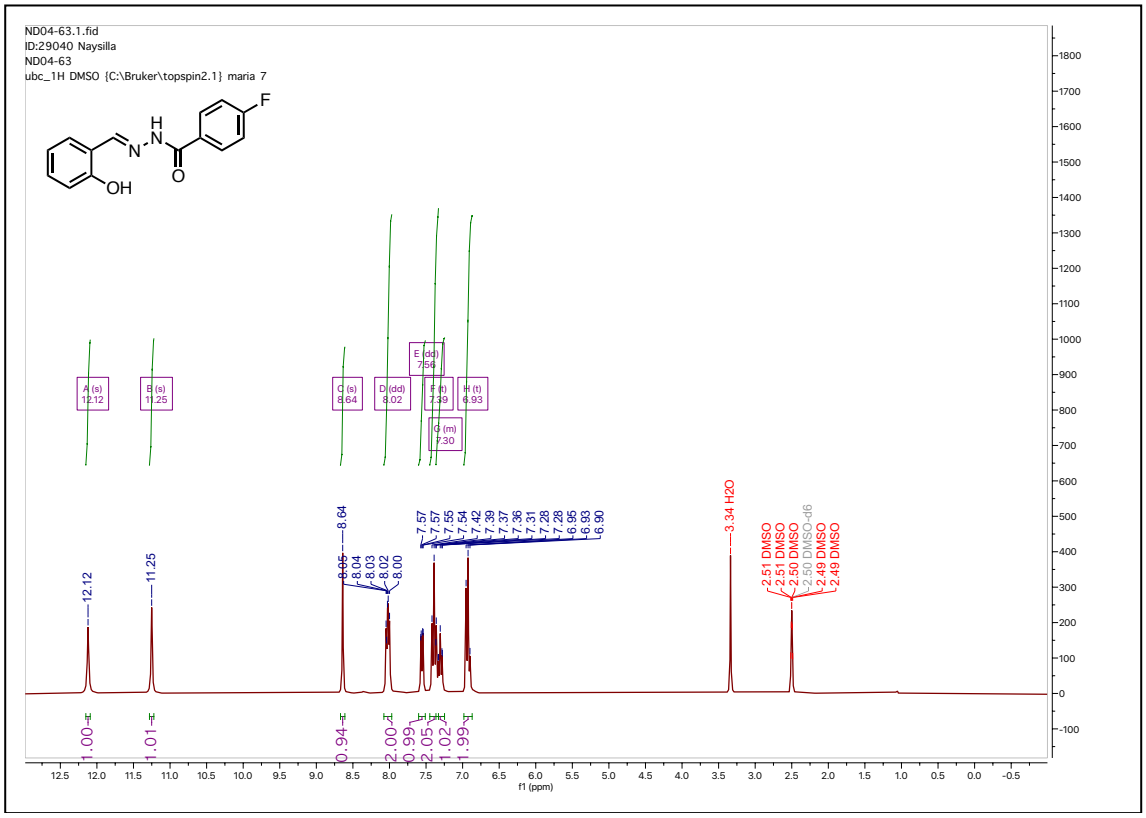
4-bromo-N’-(2-hydroxybenzylidene)benzohydrazide (5x)



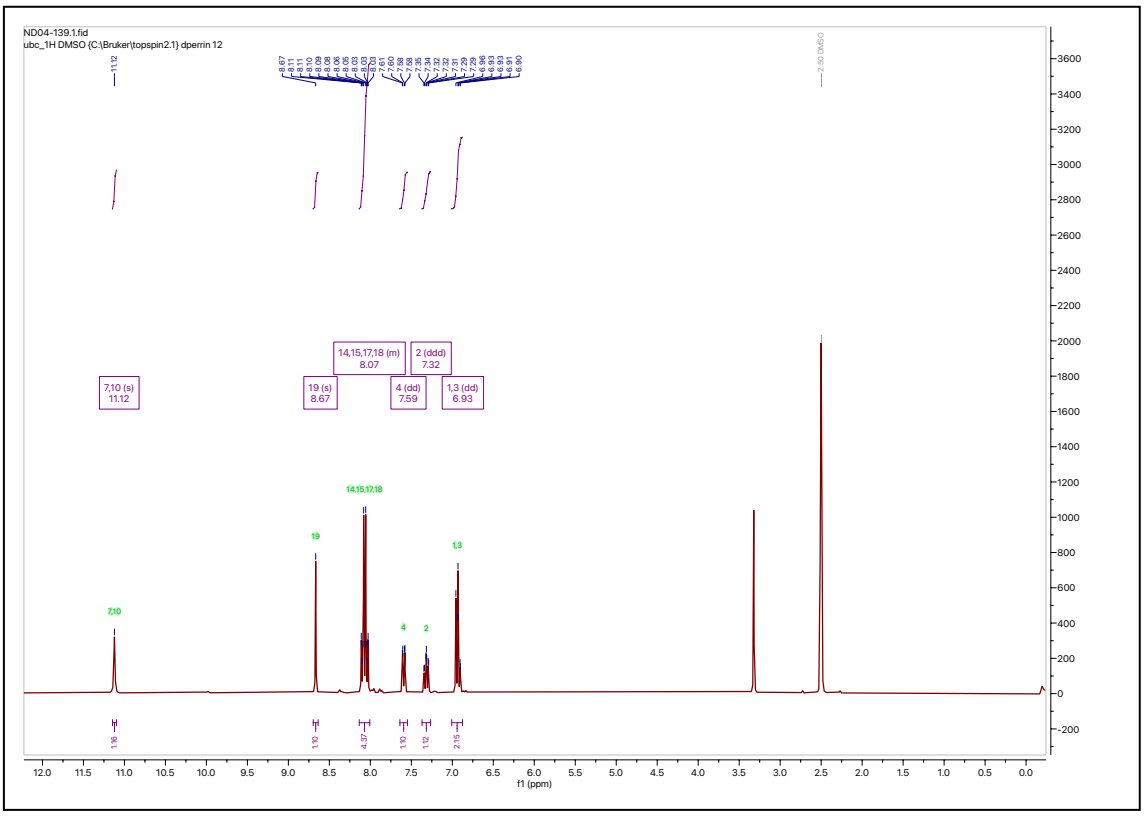
3-cyano-N'-(2-hydroxybenzylidene)benzohydrazide (5y)



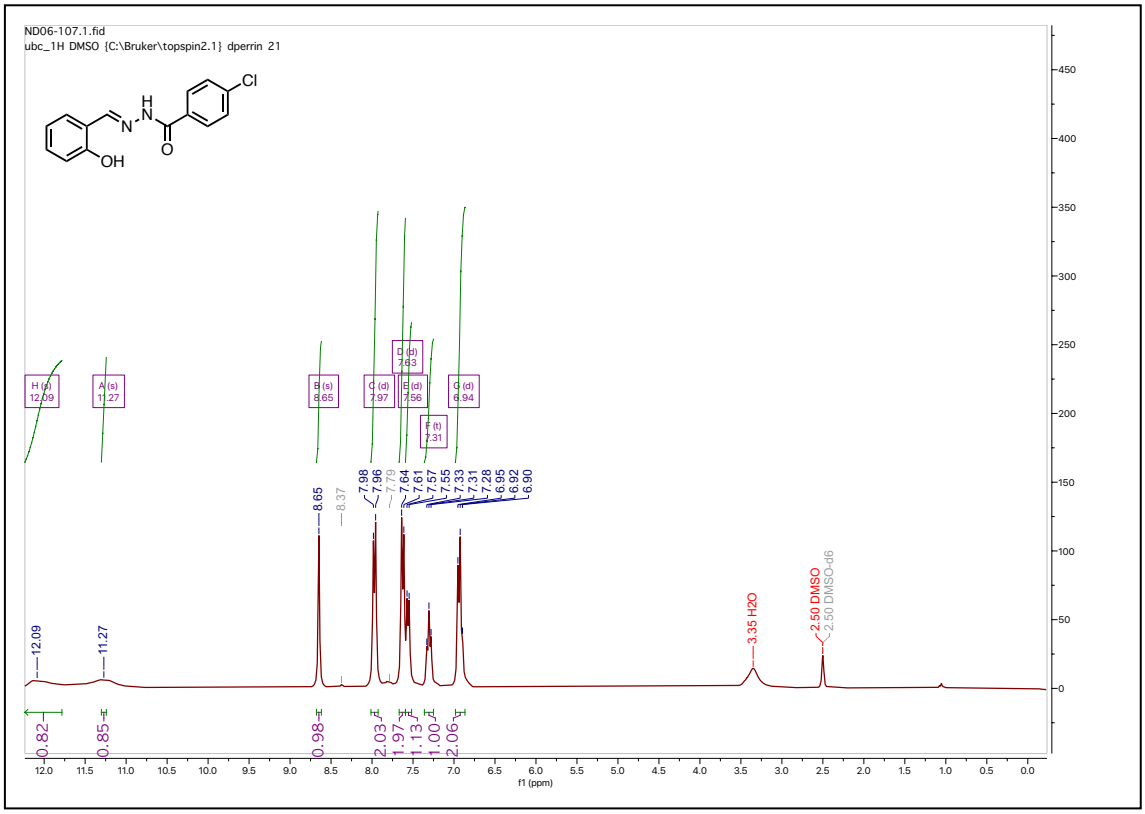
4-fluoro-*N*'-(2-hydroxybenzylidene)benzohydrazide (5z)



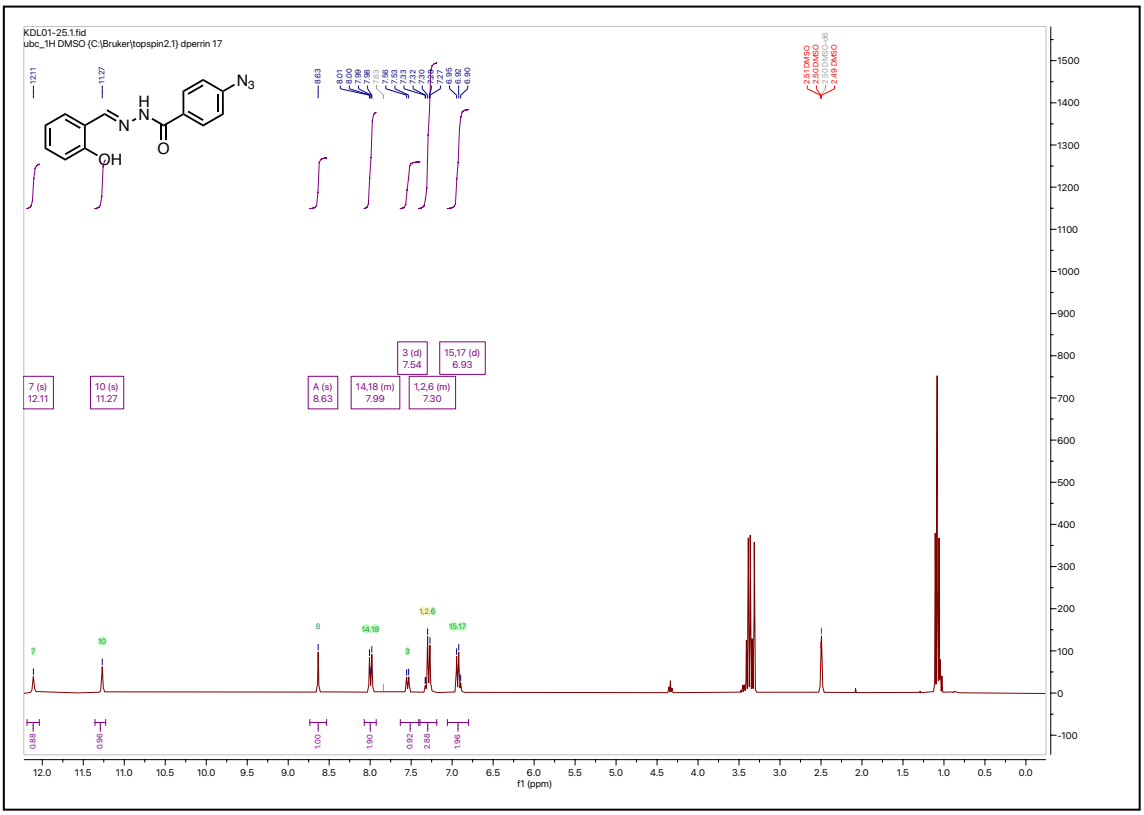
4-cyano-N'-(2-hydroxybenzylidene)benzohydrazide (5aa)



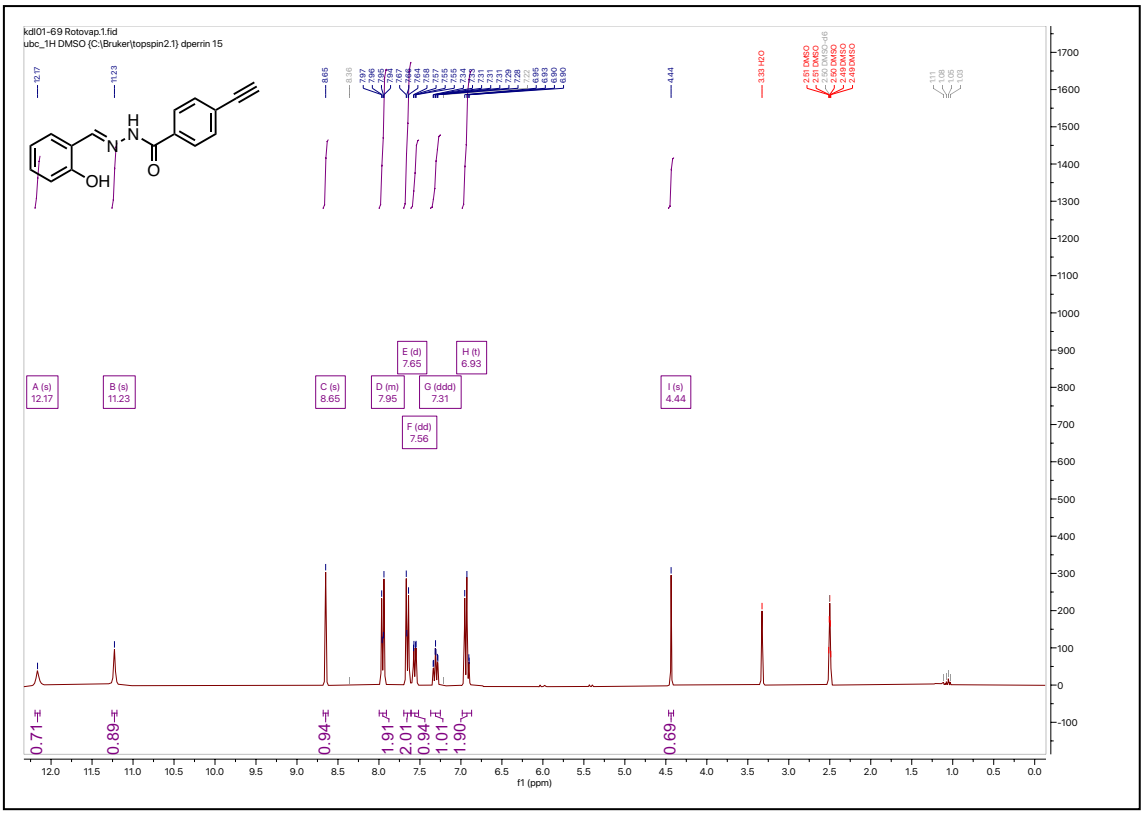
4-chloro-N'-(2-hydroxybenzylidene)benzohydrazide (5ab)



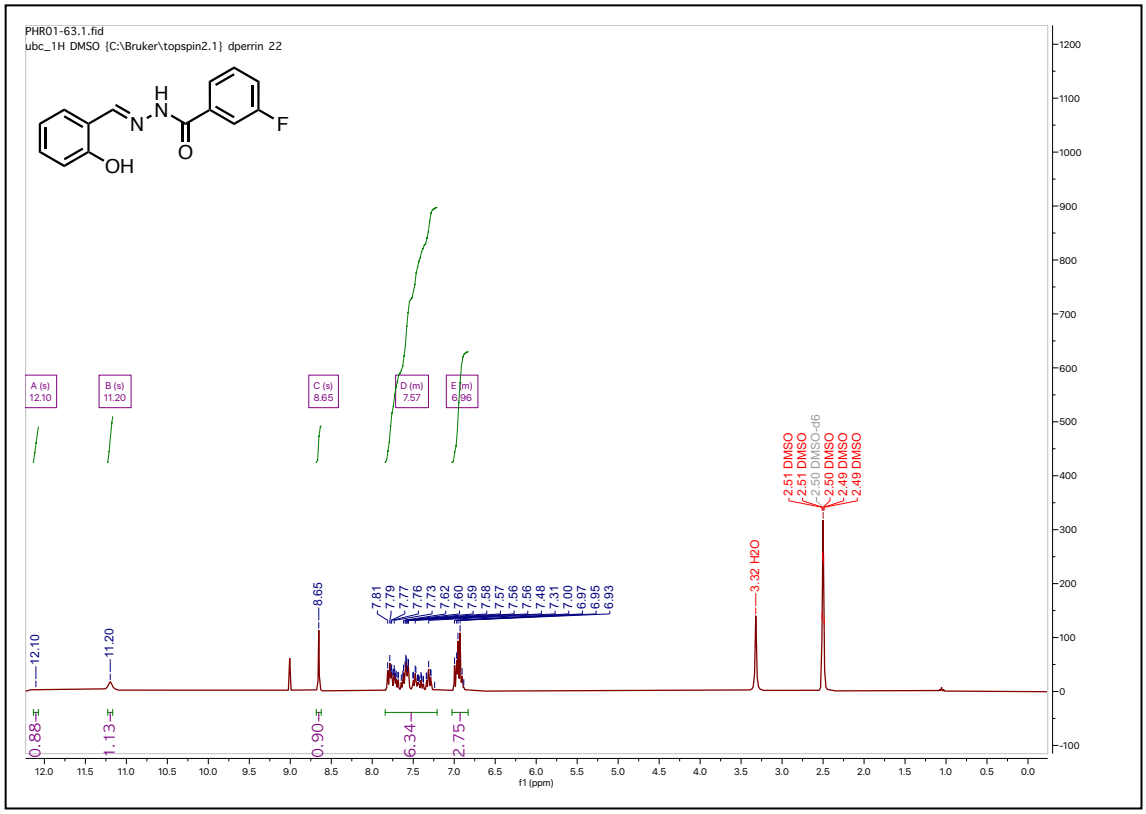
4-azido-N’-(2-hydroxybenzylidene)benzohydrazide (5ac)



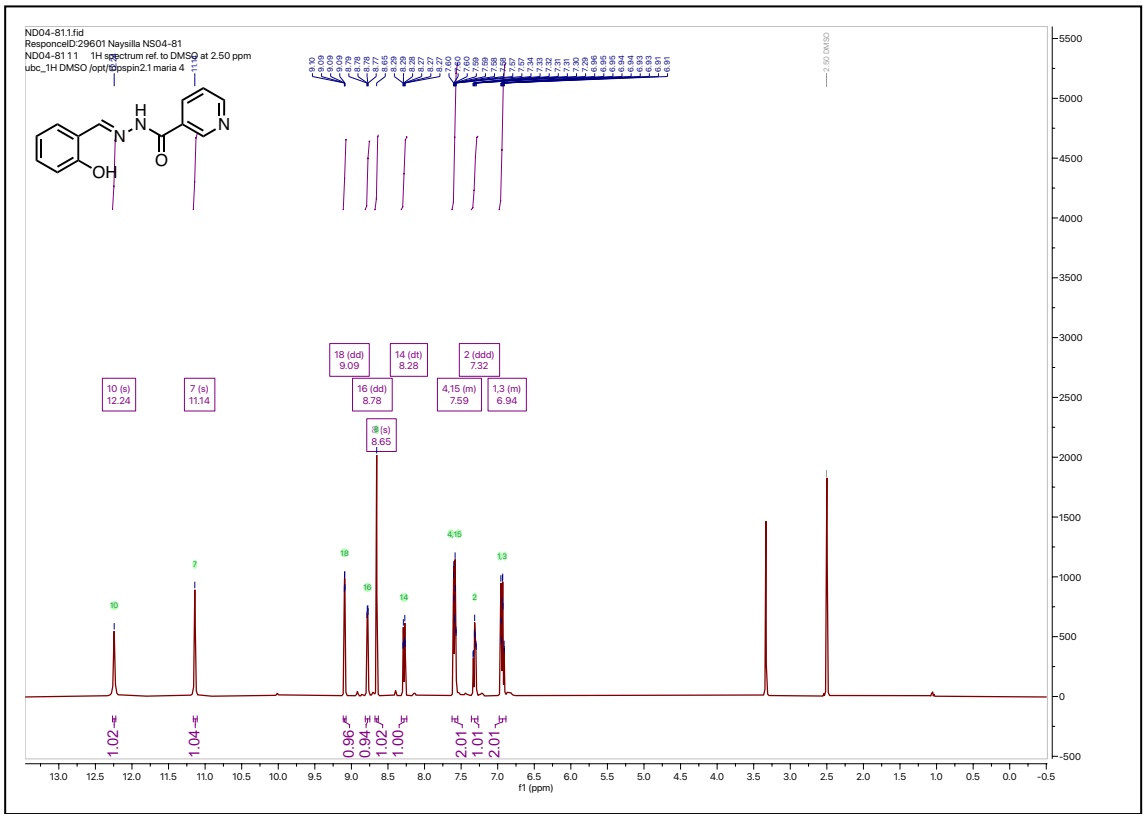
4-ethynyl-N’-(2-hydroxybenzylidene)benzohydrazide (5ad)



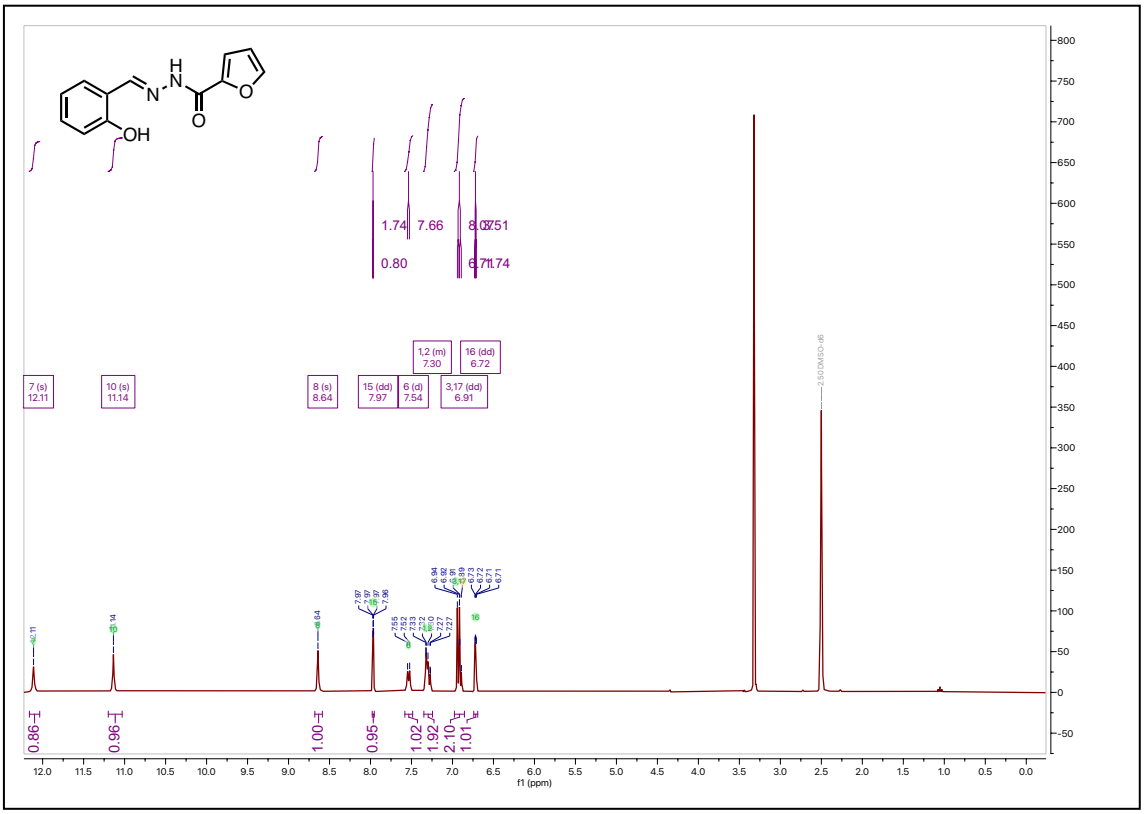
3-fluoro-N’-(2-hydroxybenzylidene)benzohydrazide (5ae)



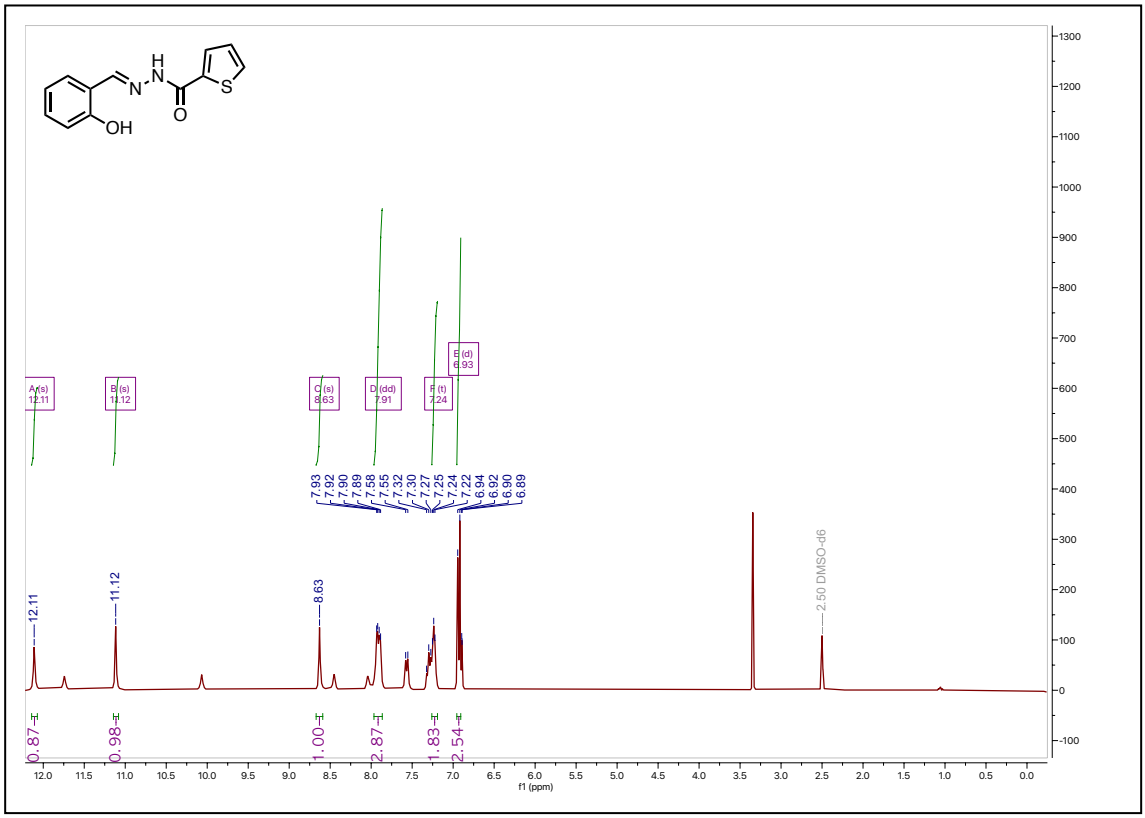
N’-(2-hydroxybenzylidene)nicotinohydrazide (5af)



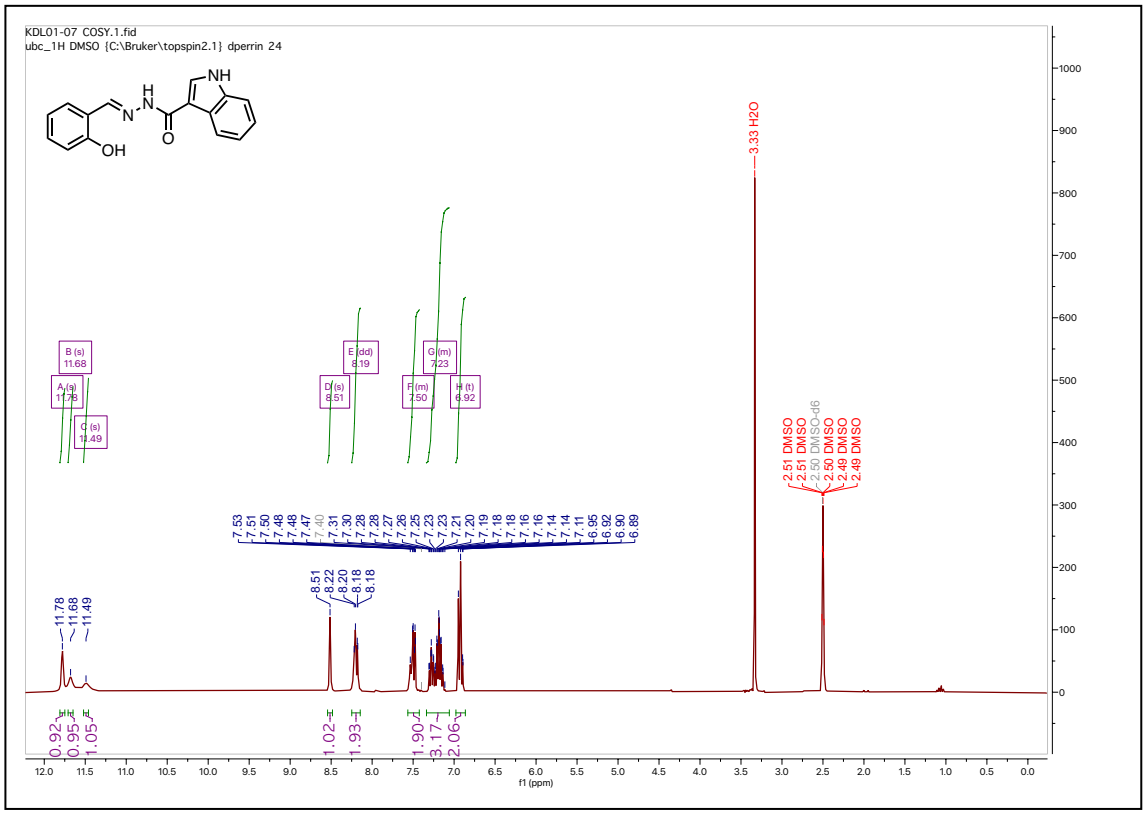
N’-(2-hydroxybenzylidene)furan-2-carbohydrazide (5ag)



N’-(2-hydroxybenzylidene)thiophene-2-carbohydrazide (5ah)

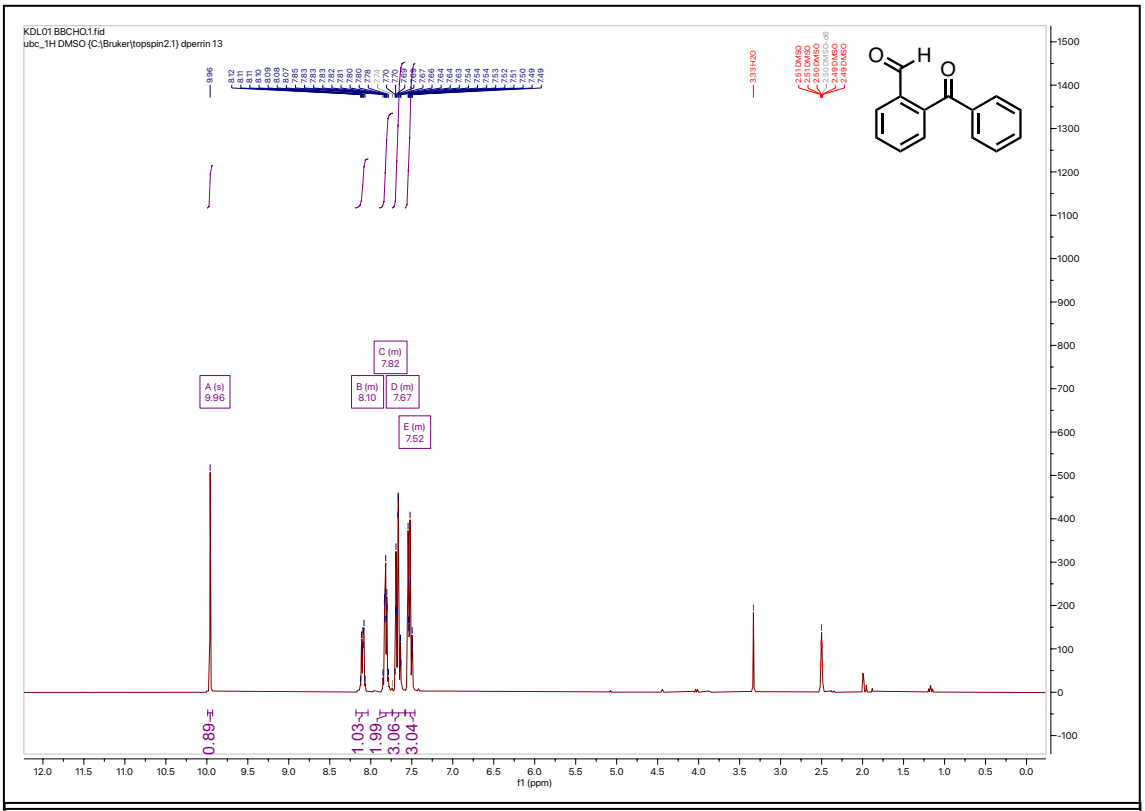


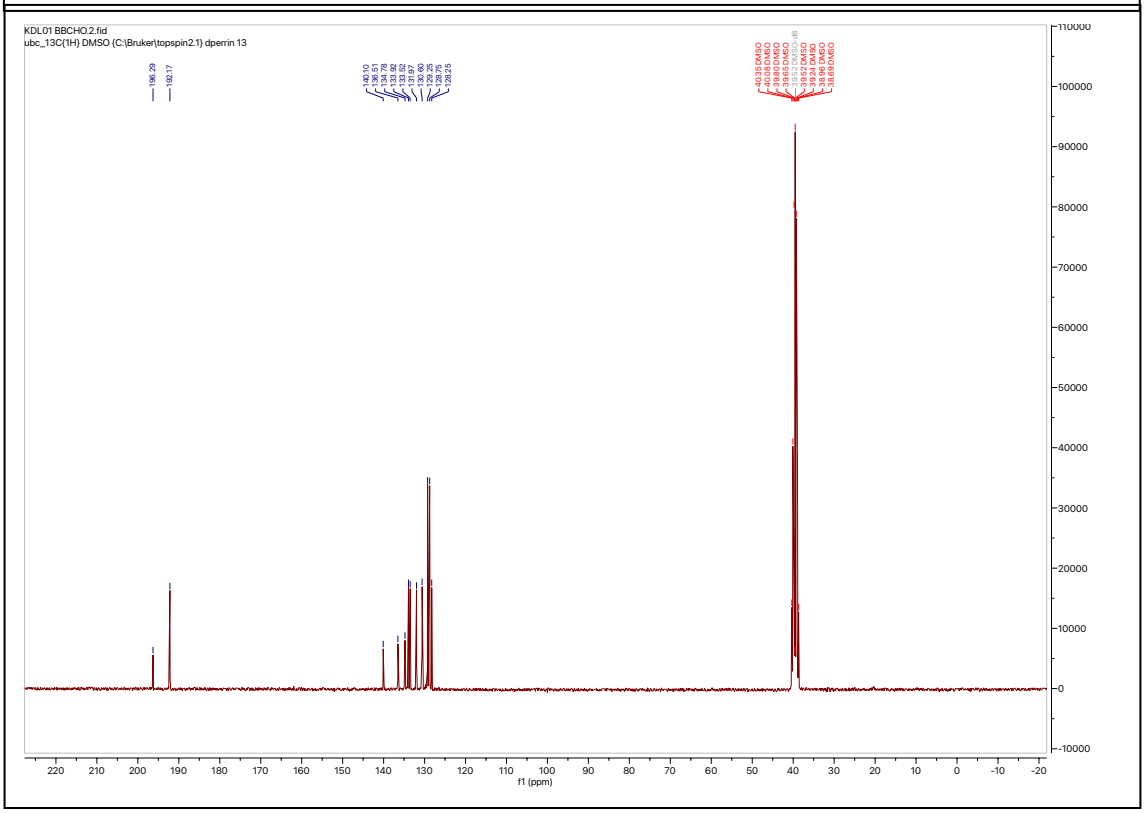
N'-(2-hydroxybenzylidene)-1*H*-indole-3-carbohydrazide (5ai)



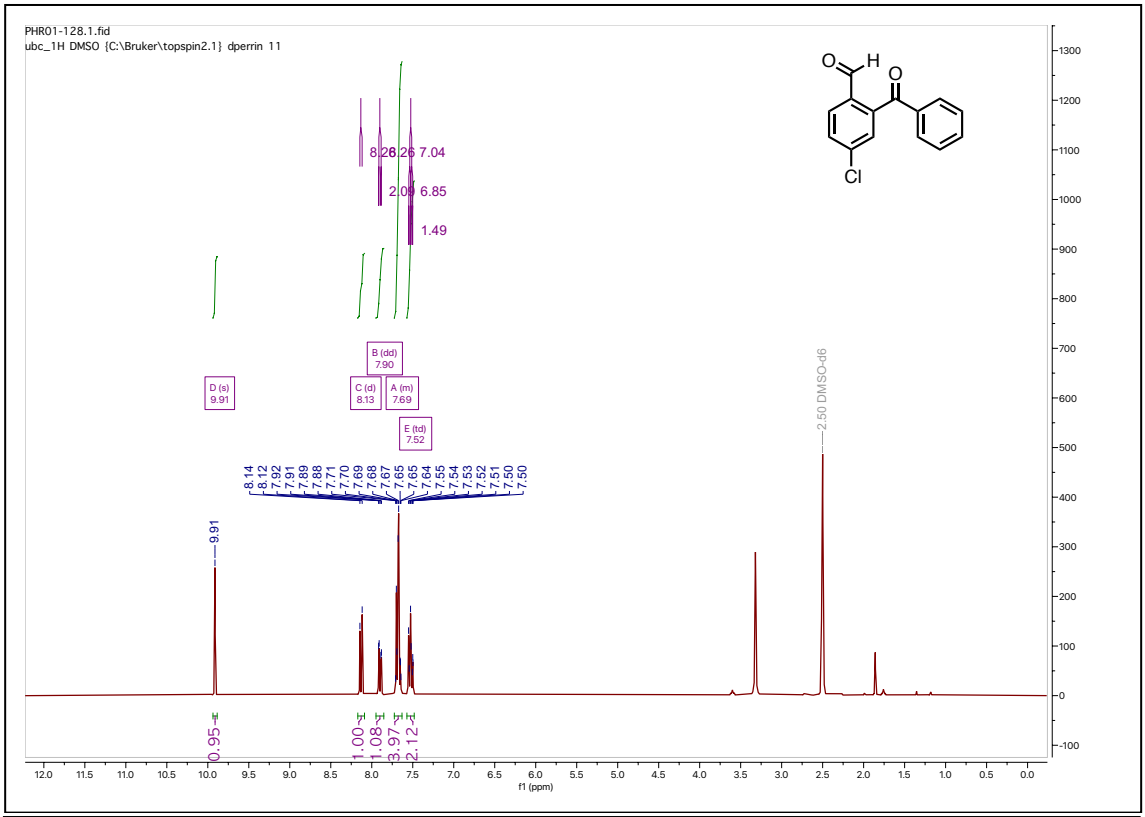
NMR Spectra of 2-ketobenzaldehyde derivatives (6):

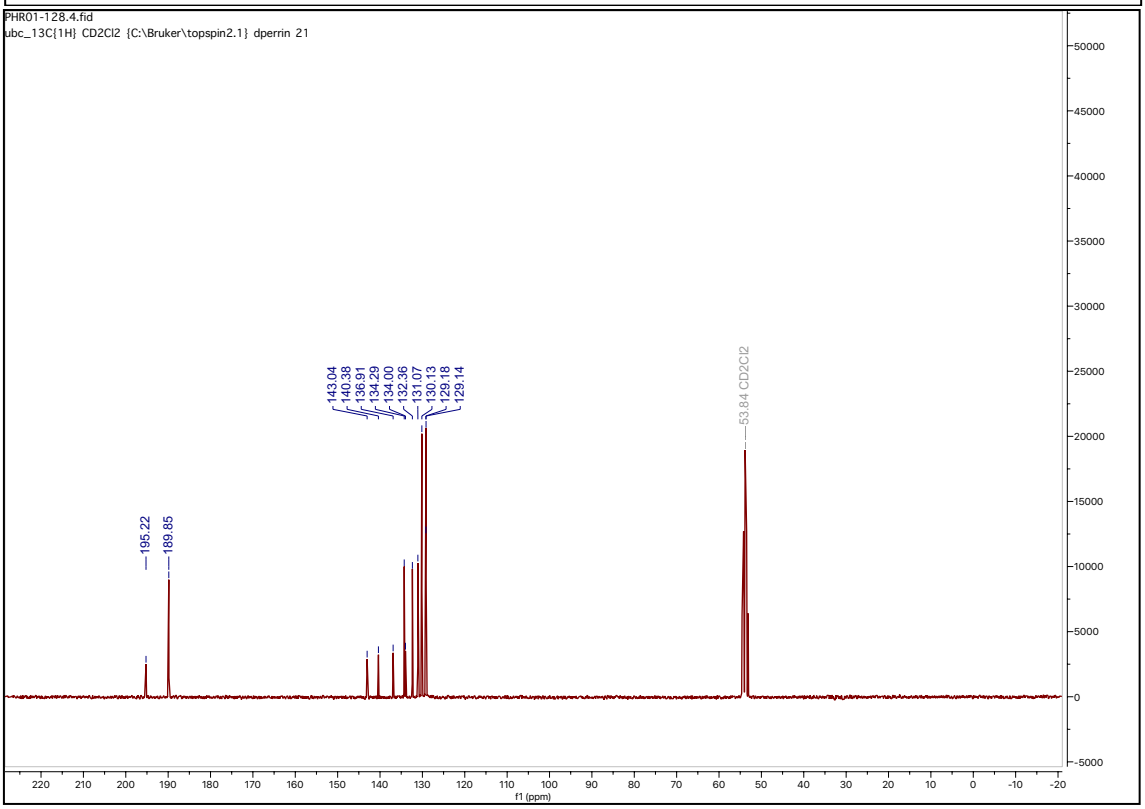
2-benzoylbenzaldehyde (6a)



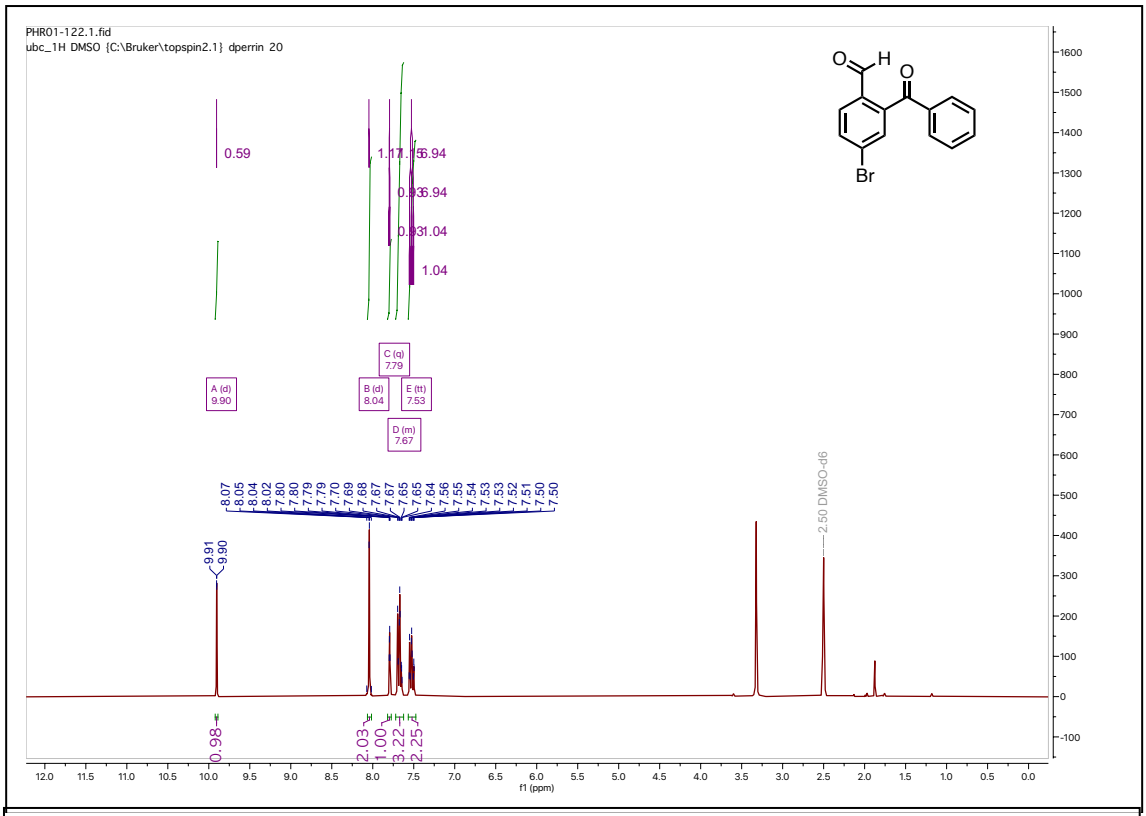


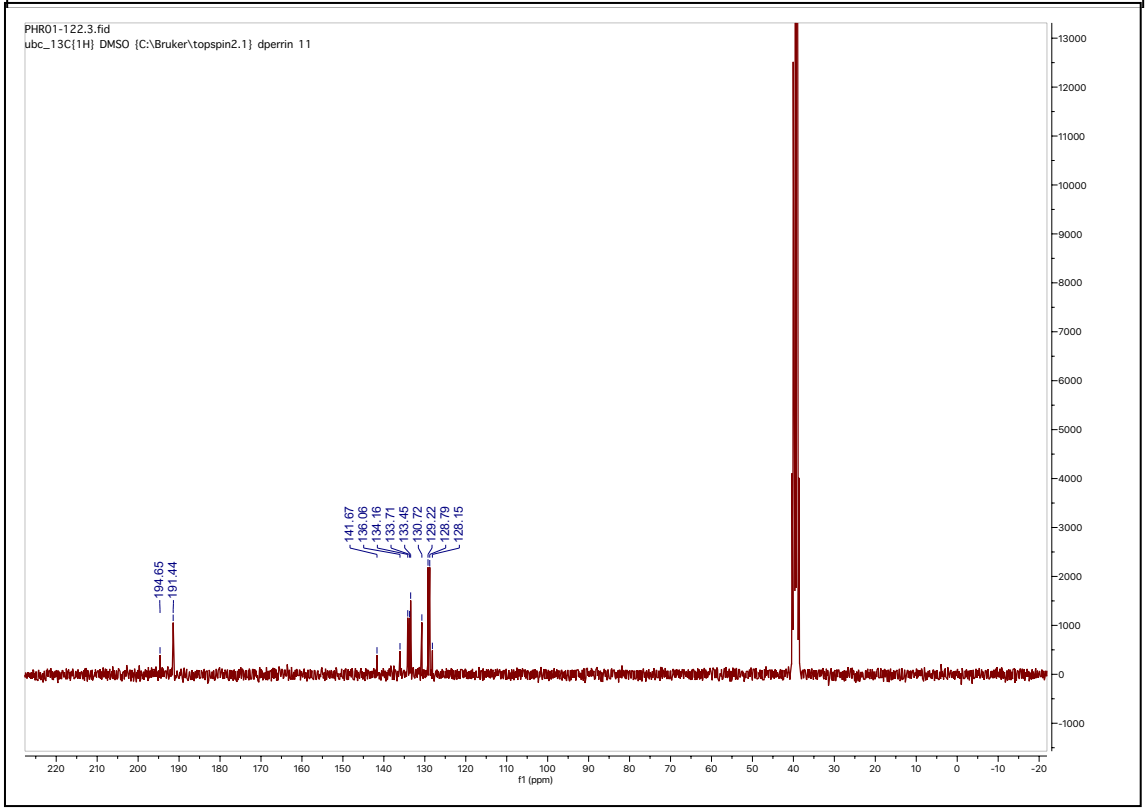
2-benzoyl-4-chlorobenzaldehyde (6b)



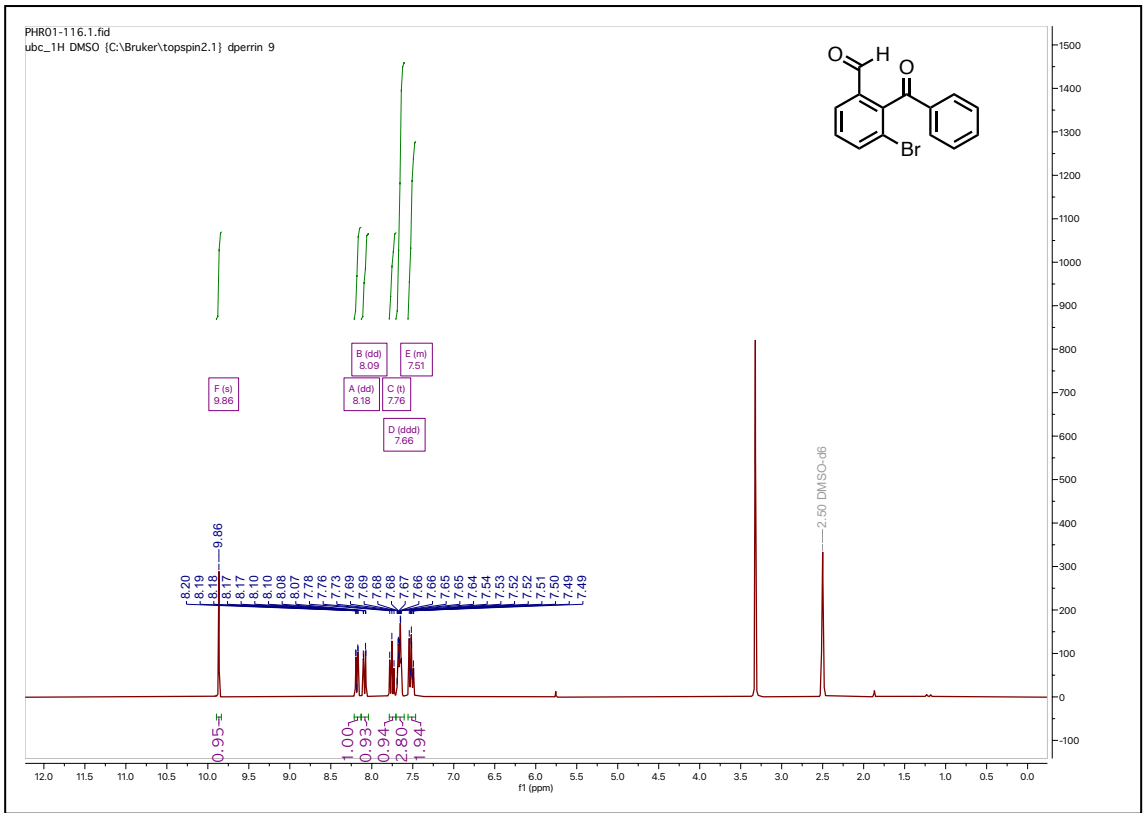


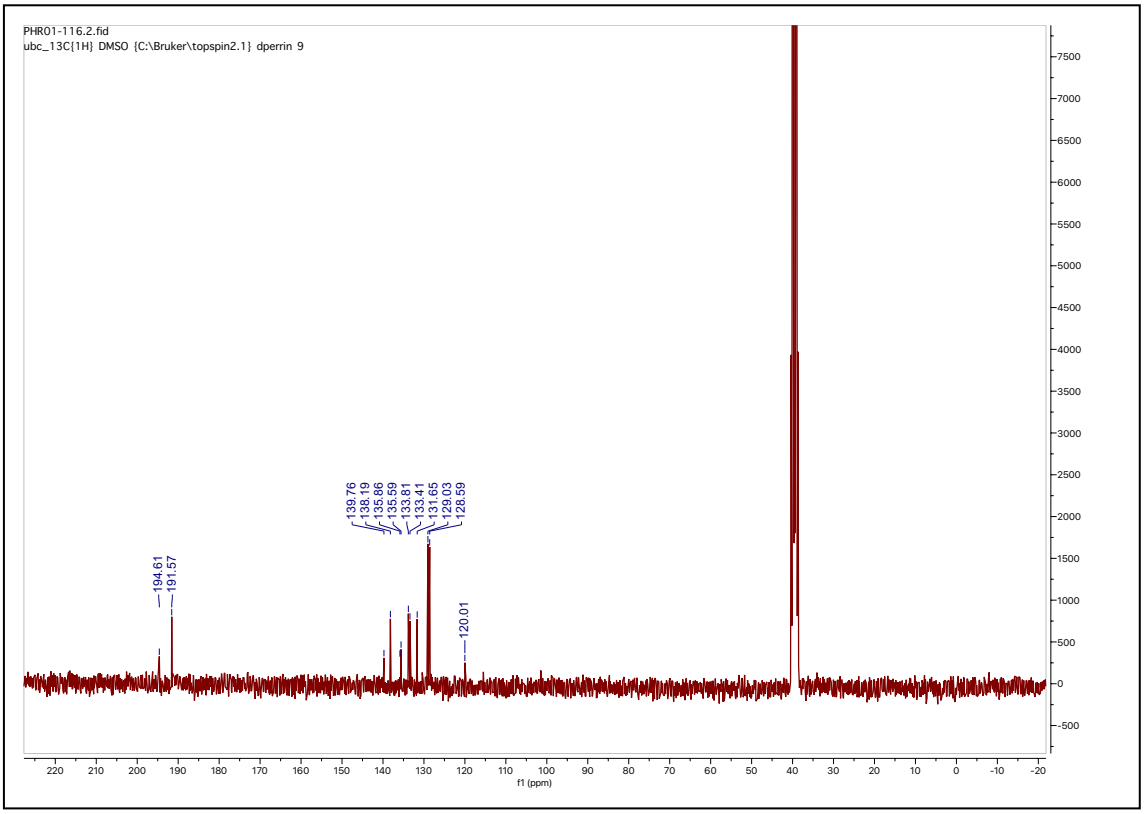
2-benzoyl-4-bromobenzaldehyde (6c)



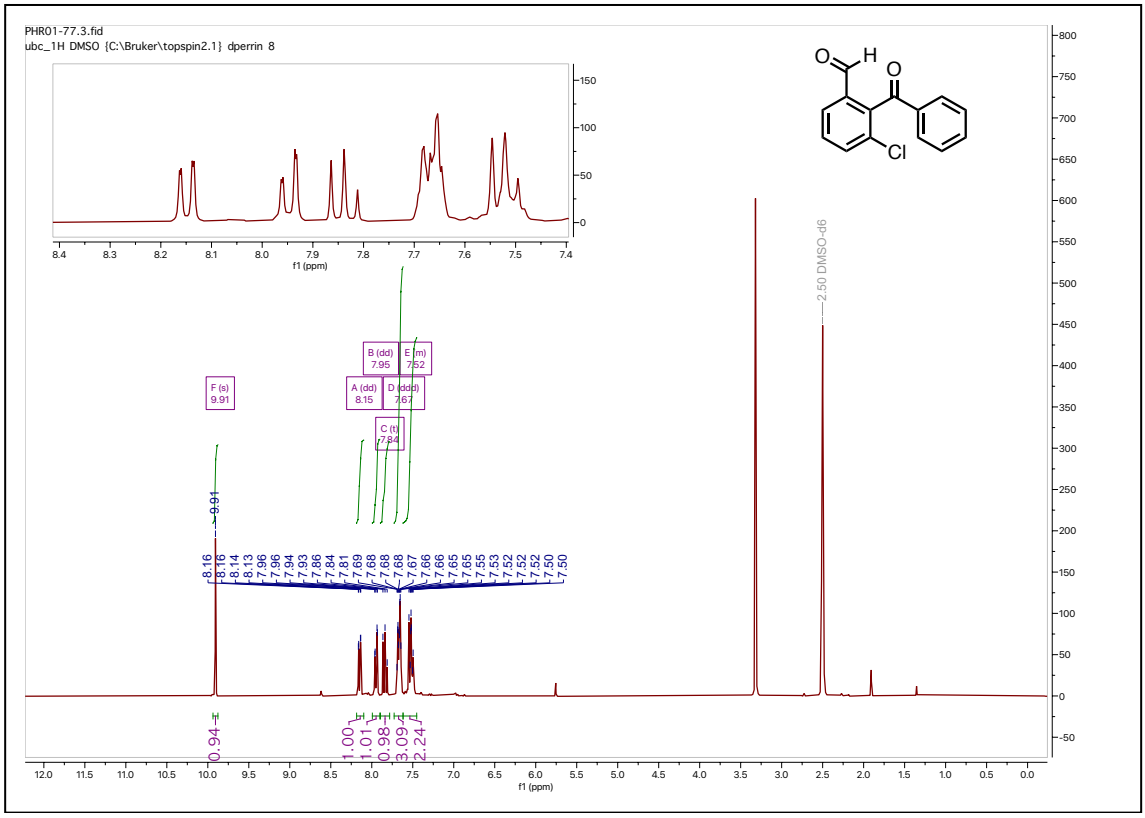


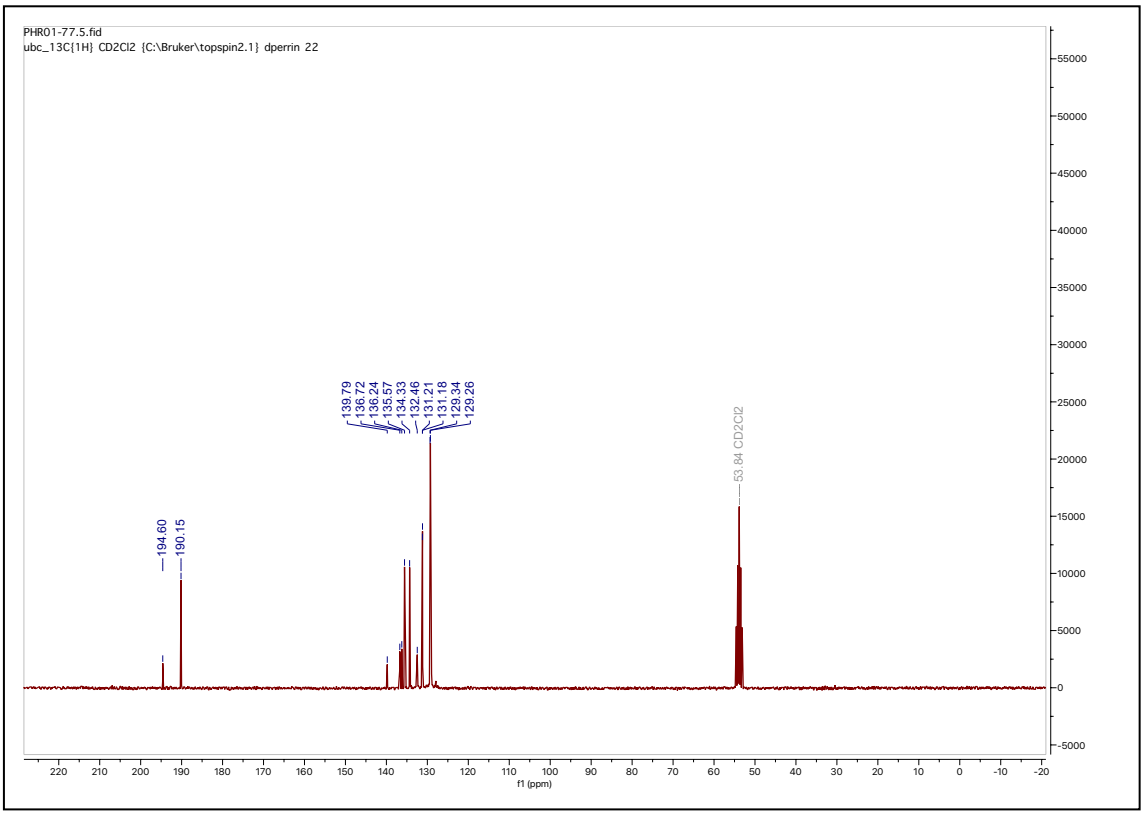
2-benzoyl-3-bromobenzaldehyde (6d)



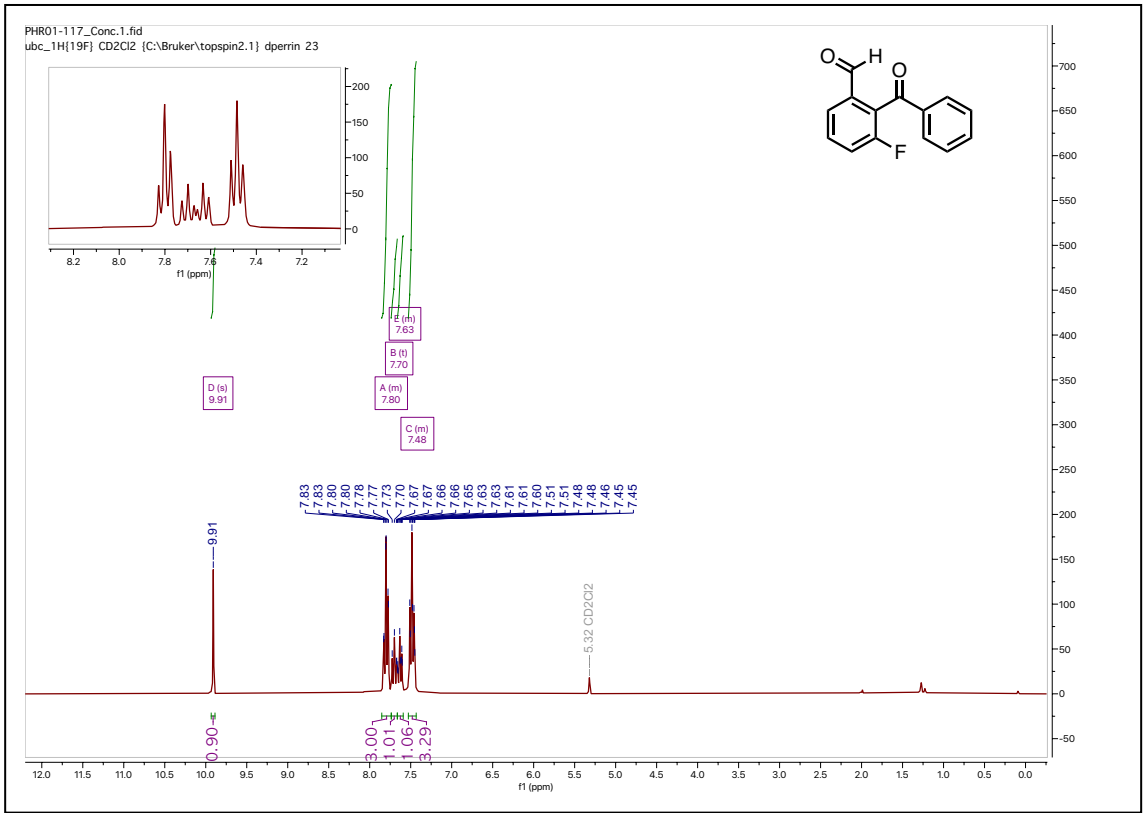


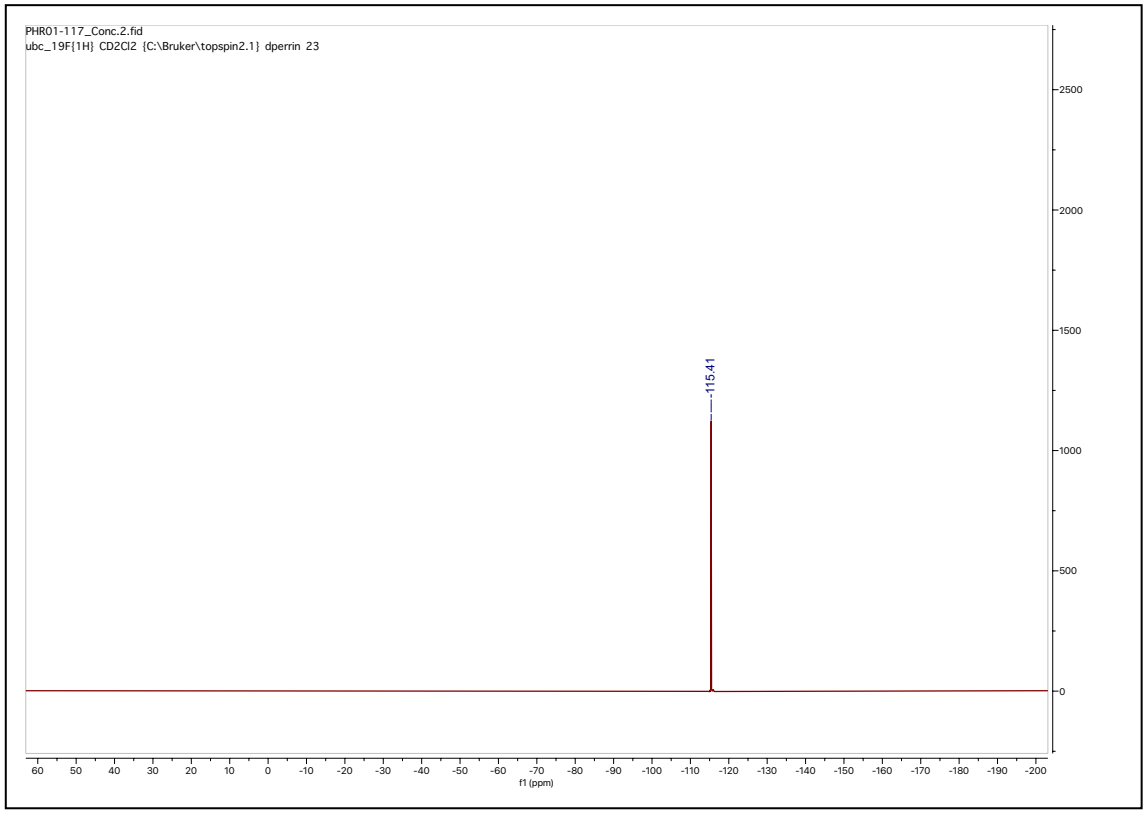
2-benzoyl-3-chlorobenzaldehyde (6e)

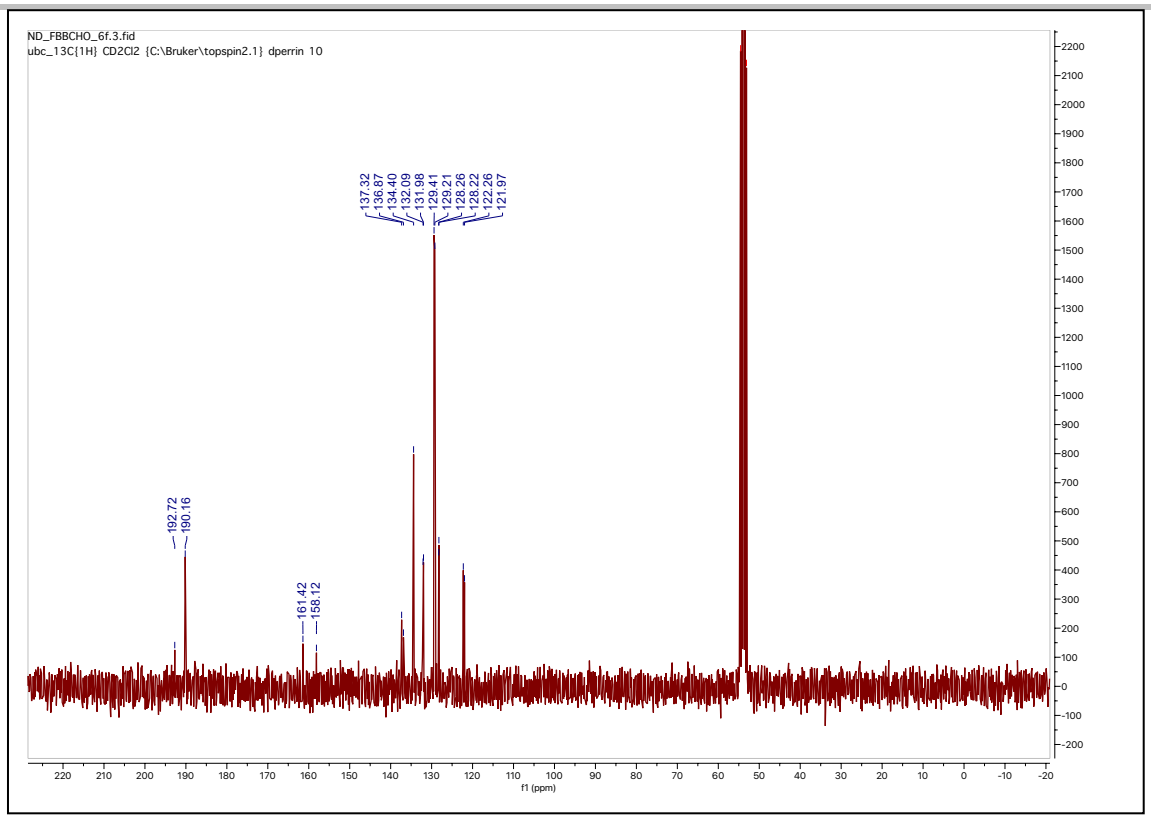




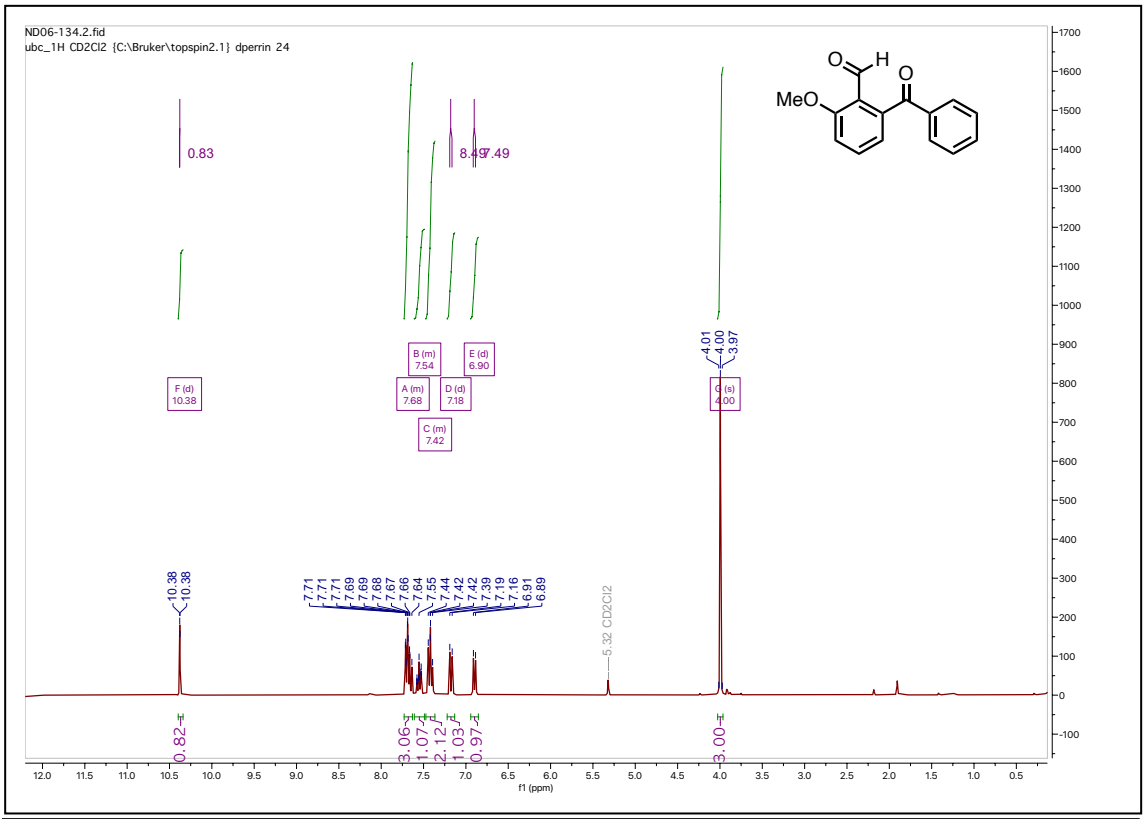
2-benzoyl-3-fluoro-benzaldehyde (6f)

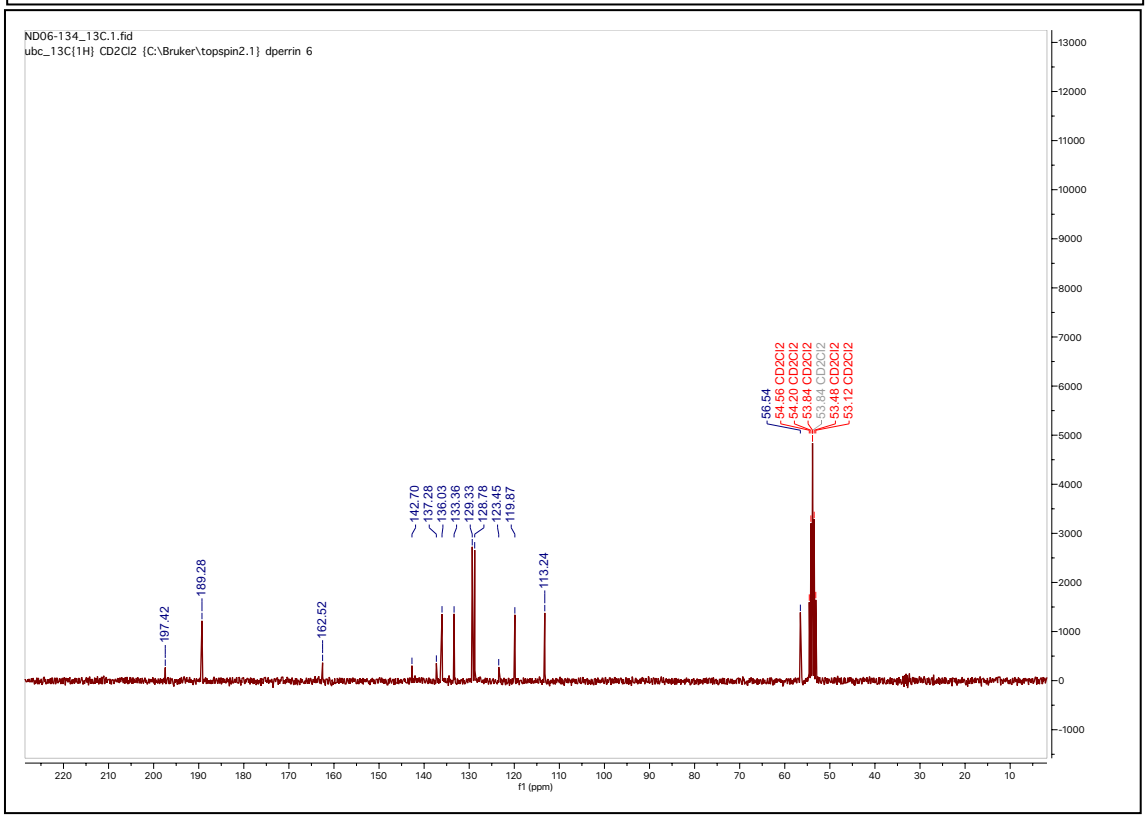




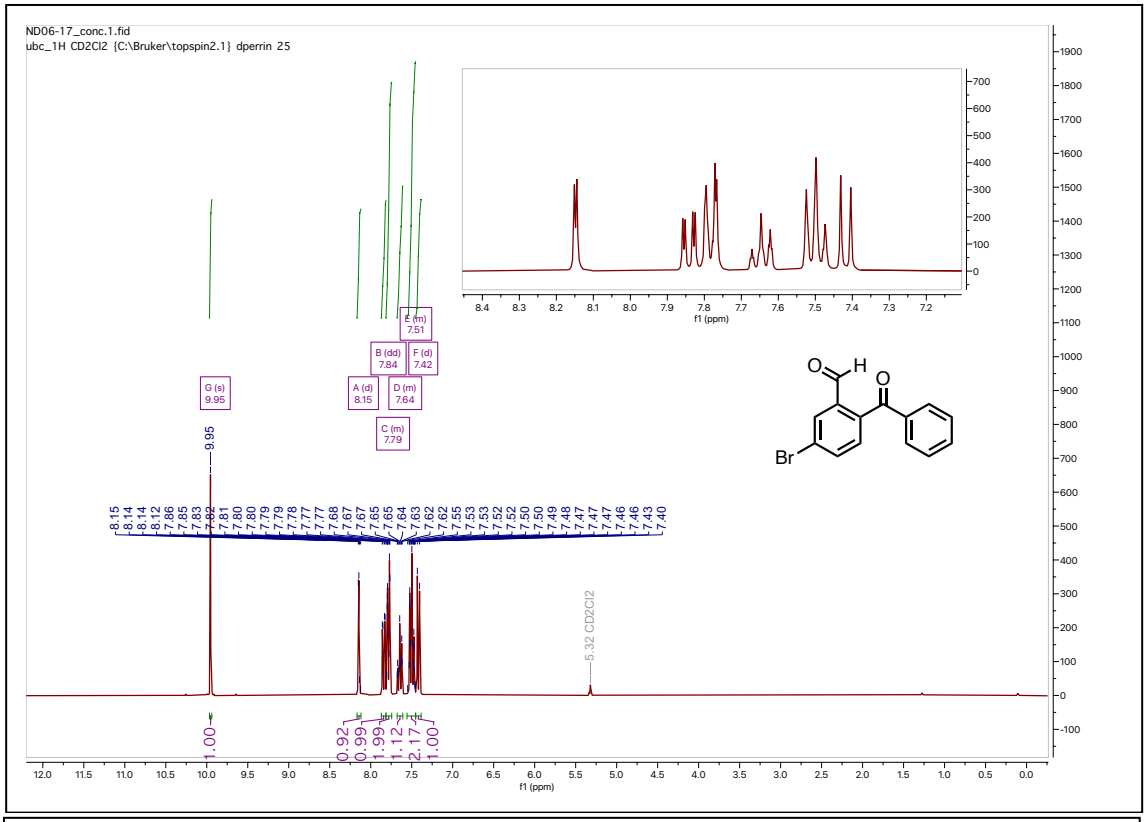

ND_FBBCHO_6f.3.fid
ubc_13C{1H} CD2Cl2 {C:\Bruker\topspin2.1} dperrin 10
137.32
136.87
134.40
132.09
131.98
129.41
129.21
128.26
128.22
122.26
121.97
192.72
190.16
161.42
158.12
f1 (ppm)

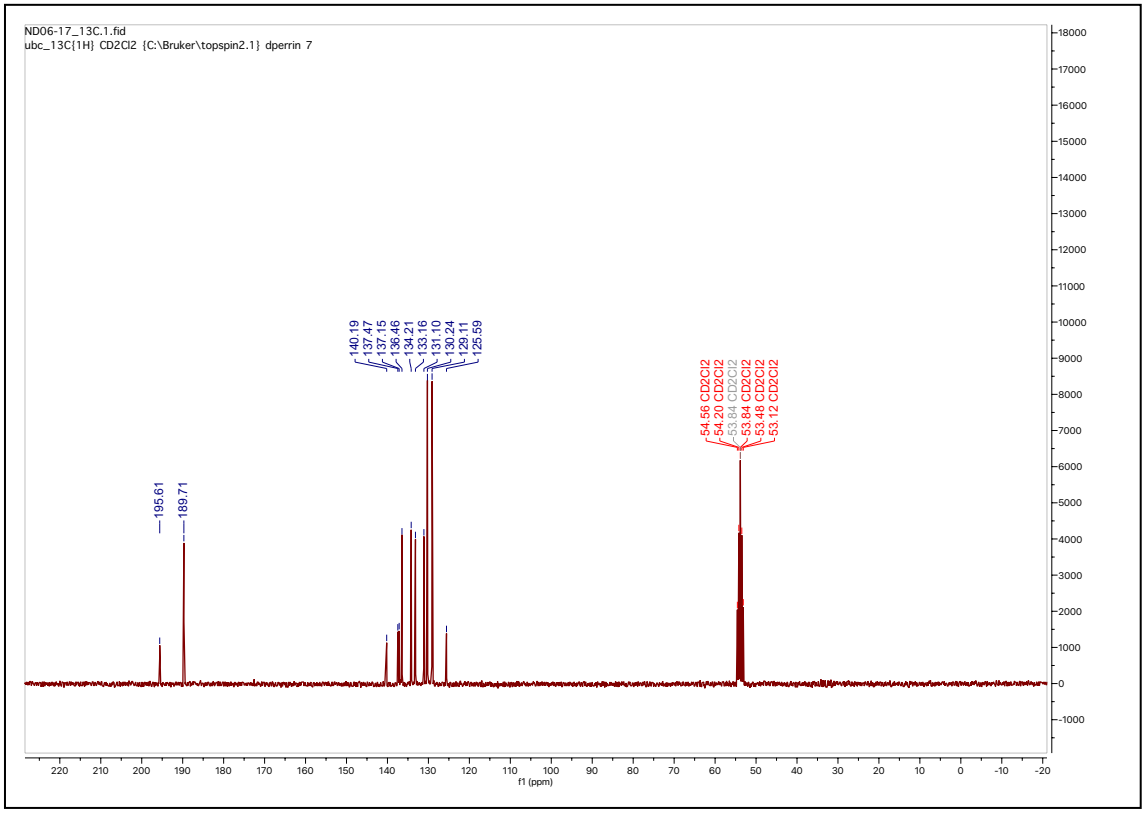
2-benzoyl-6-methoxybenzaldehyde (6g)



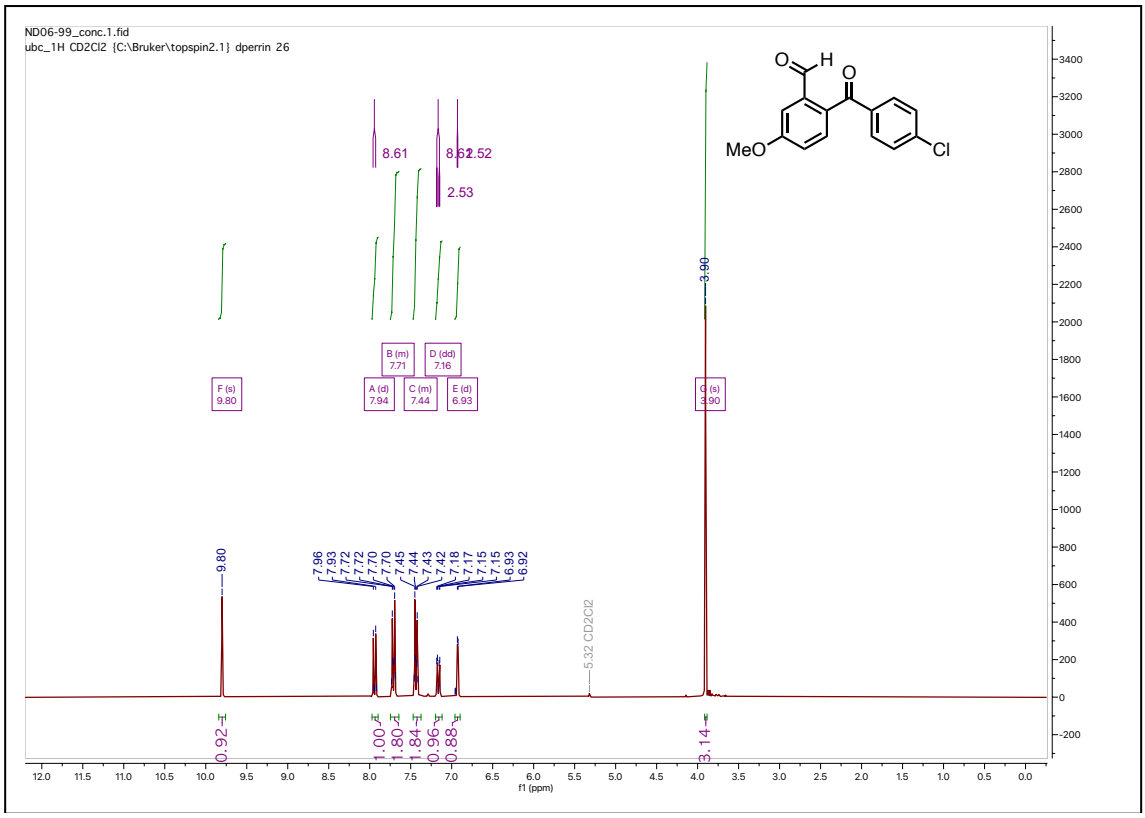


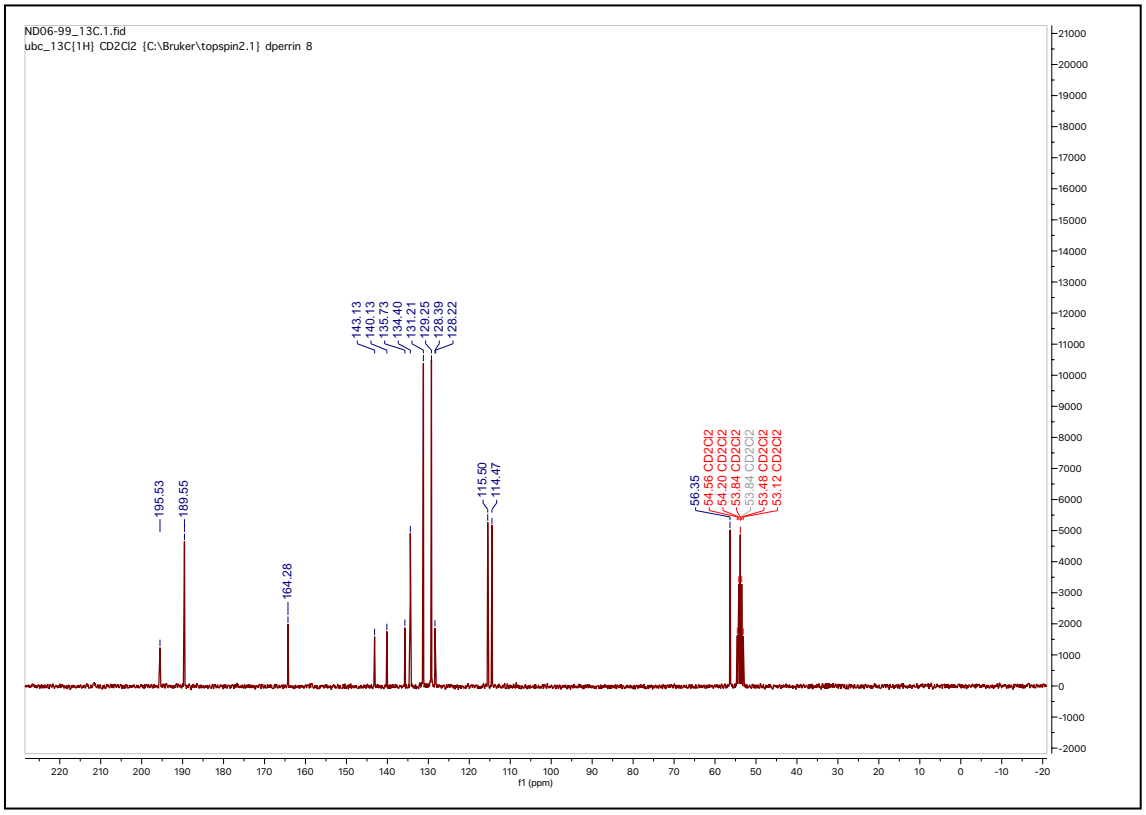
2-benzoyl-5-bromobenzaldehyde (6h)



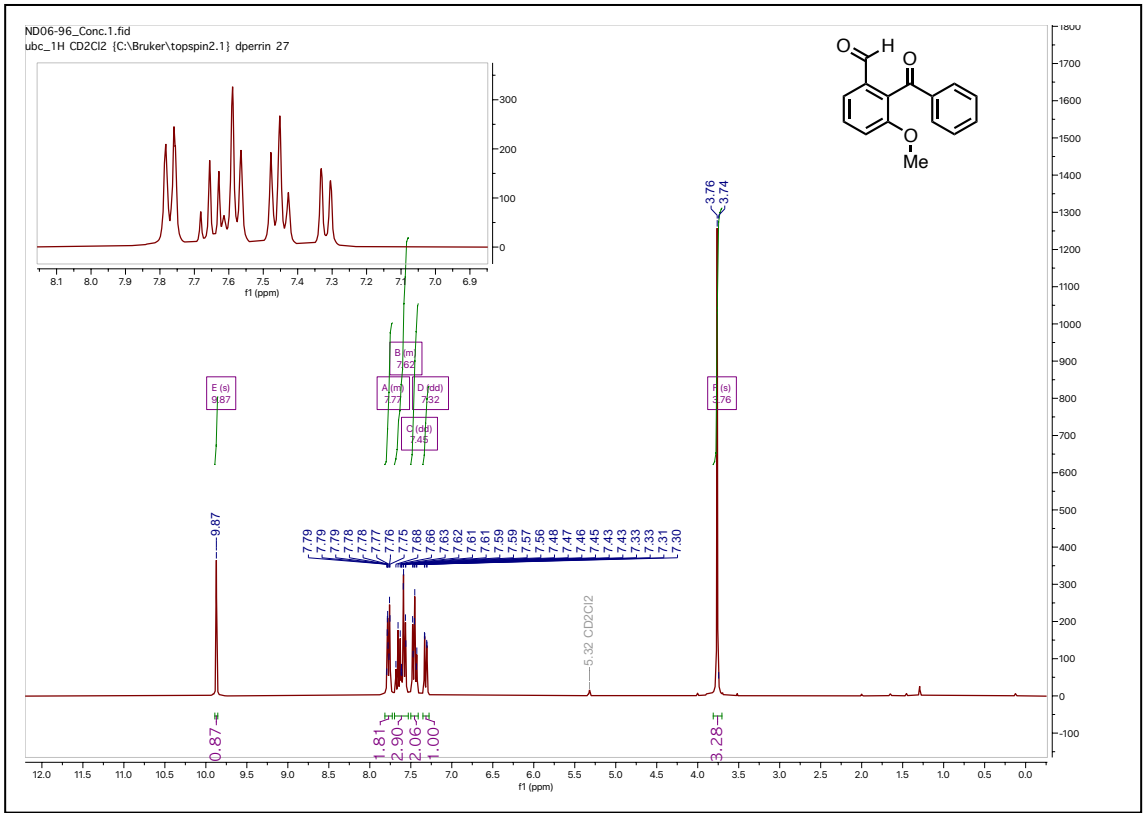


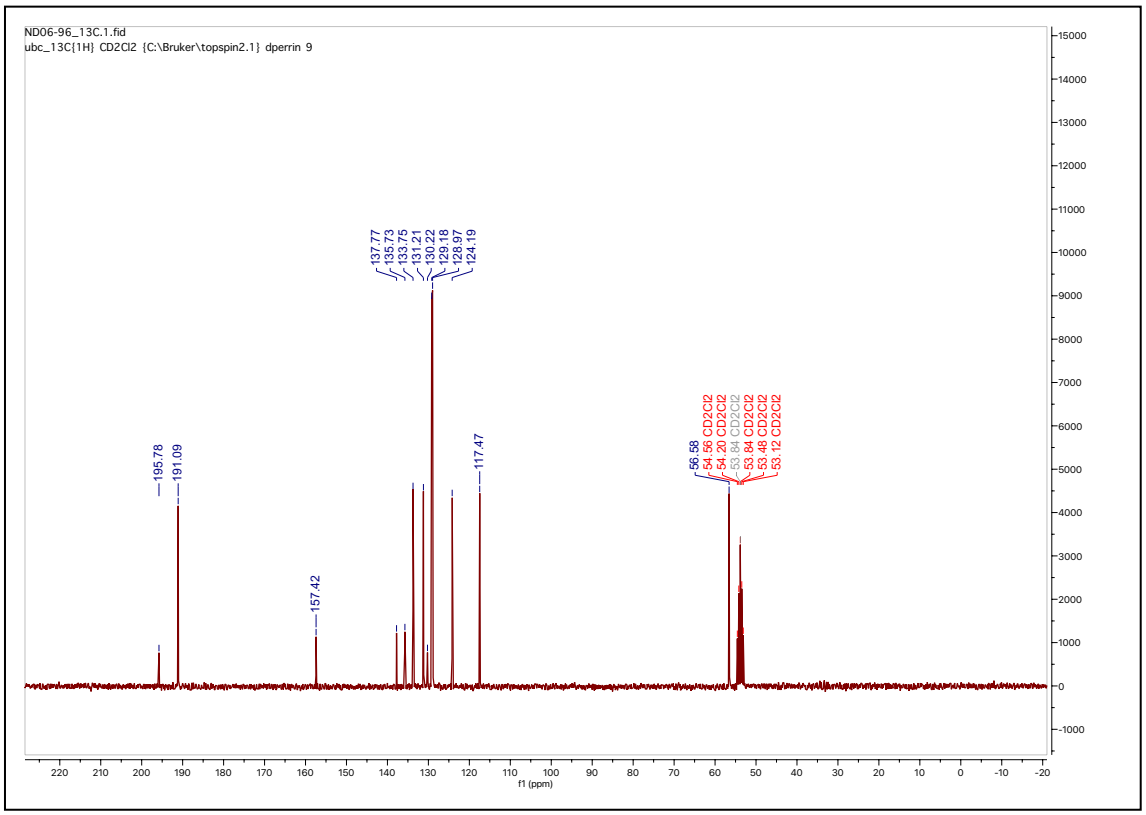
2-(4-chlorobenzoyl)-5-methoxybenzaldehyde (6i)



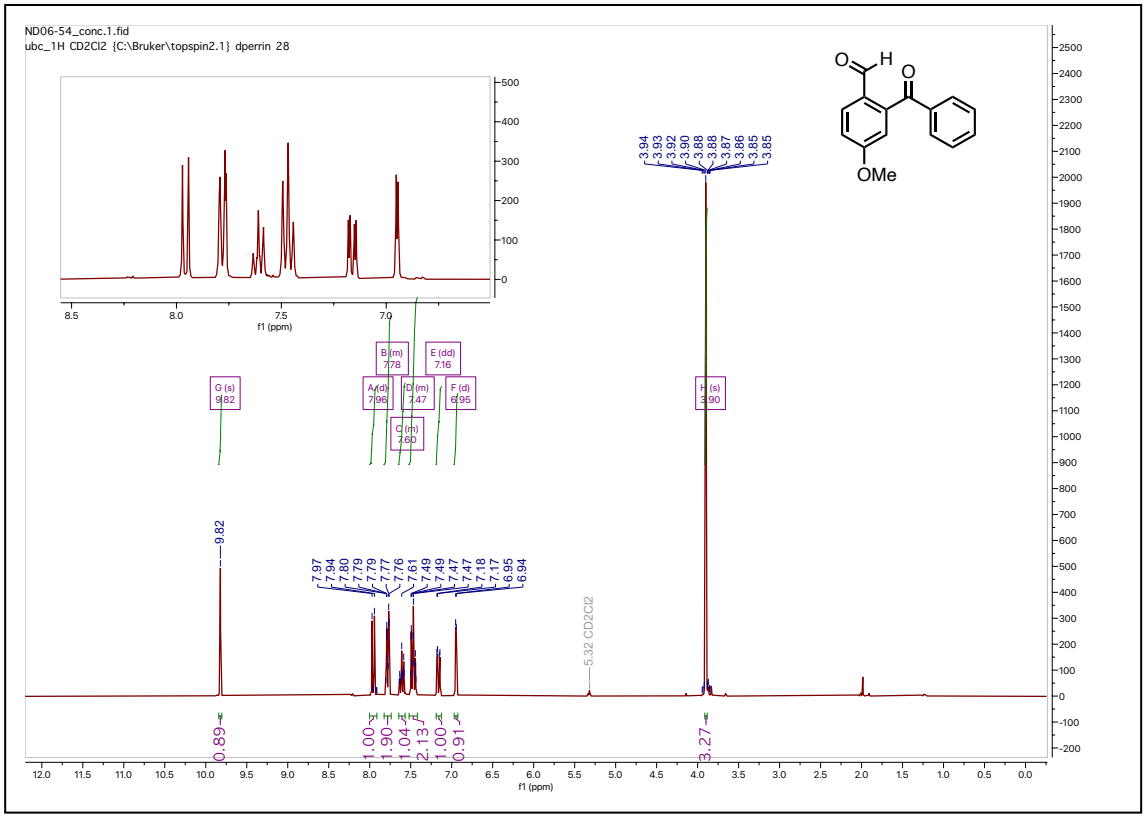


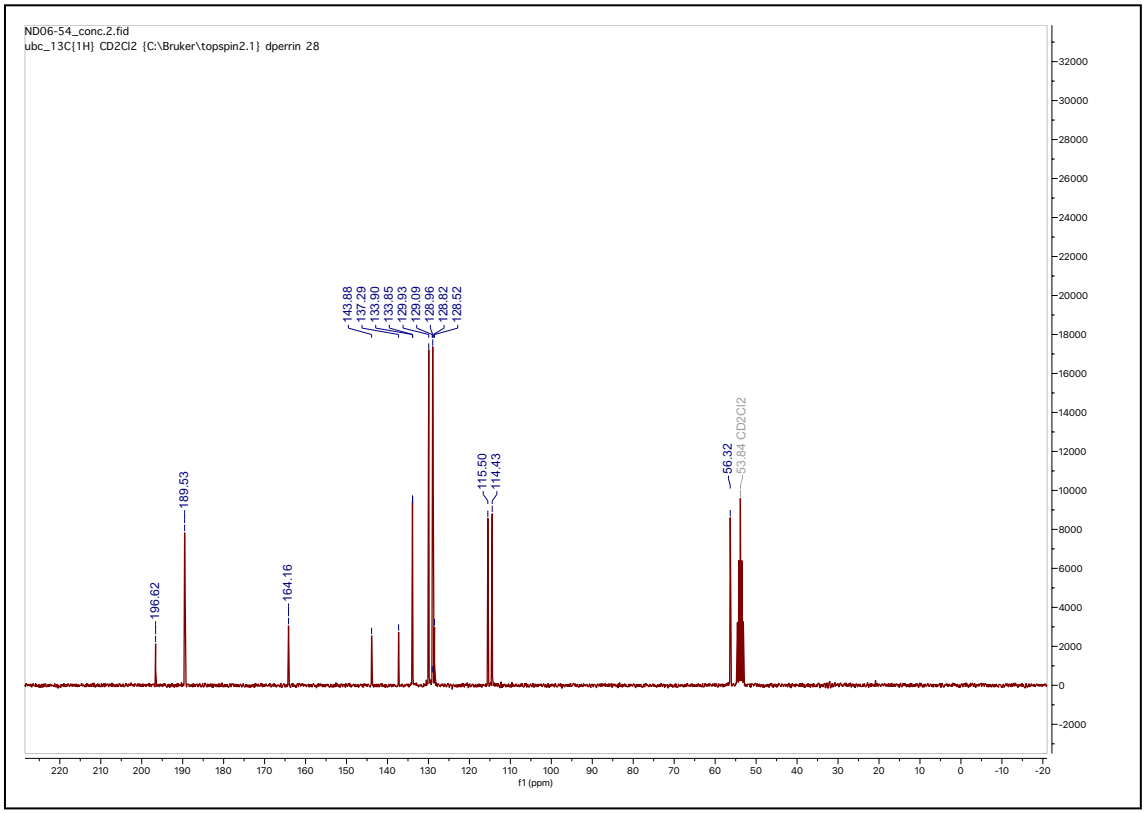
2-benzoyl-3-methoxybenzaldehyde (6j)



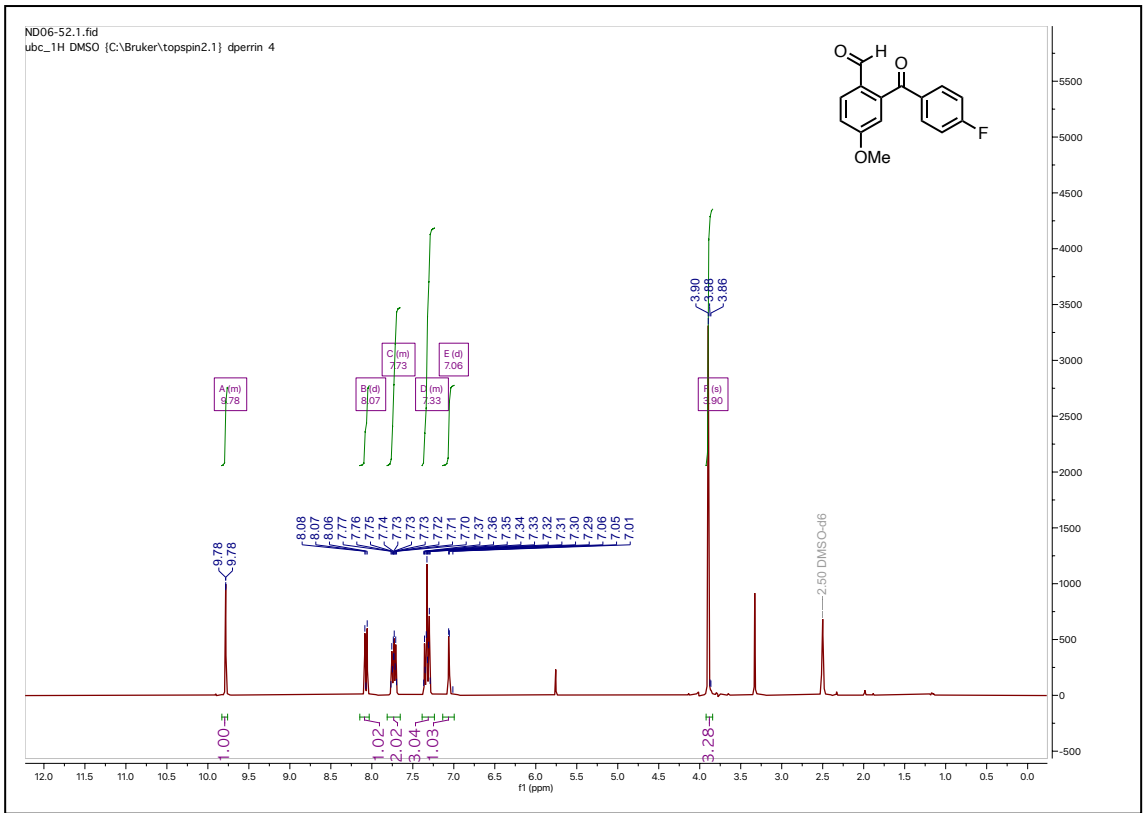


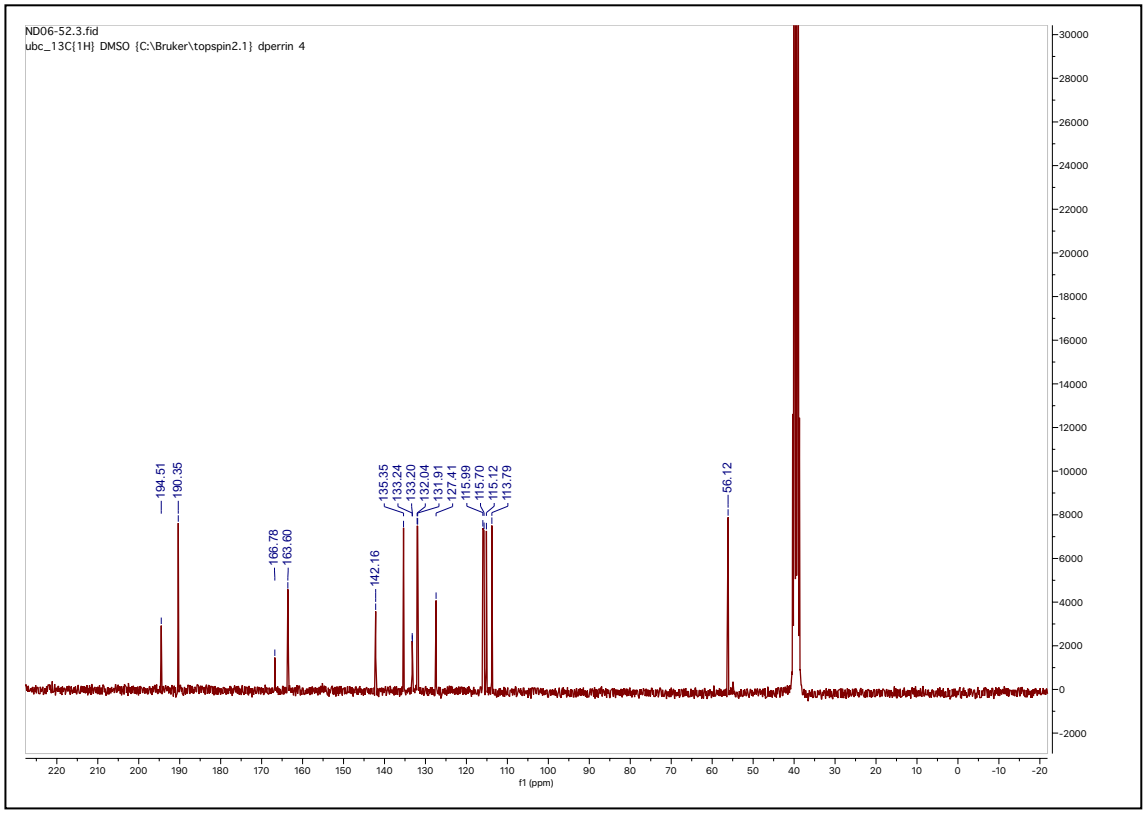
2-benzoyl-4-methoxybenzaldehyde (6k)

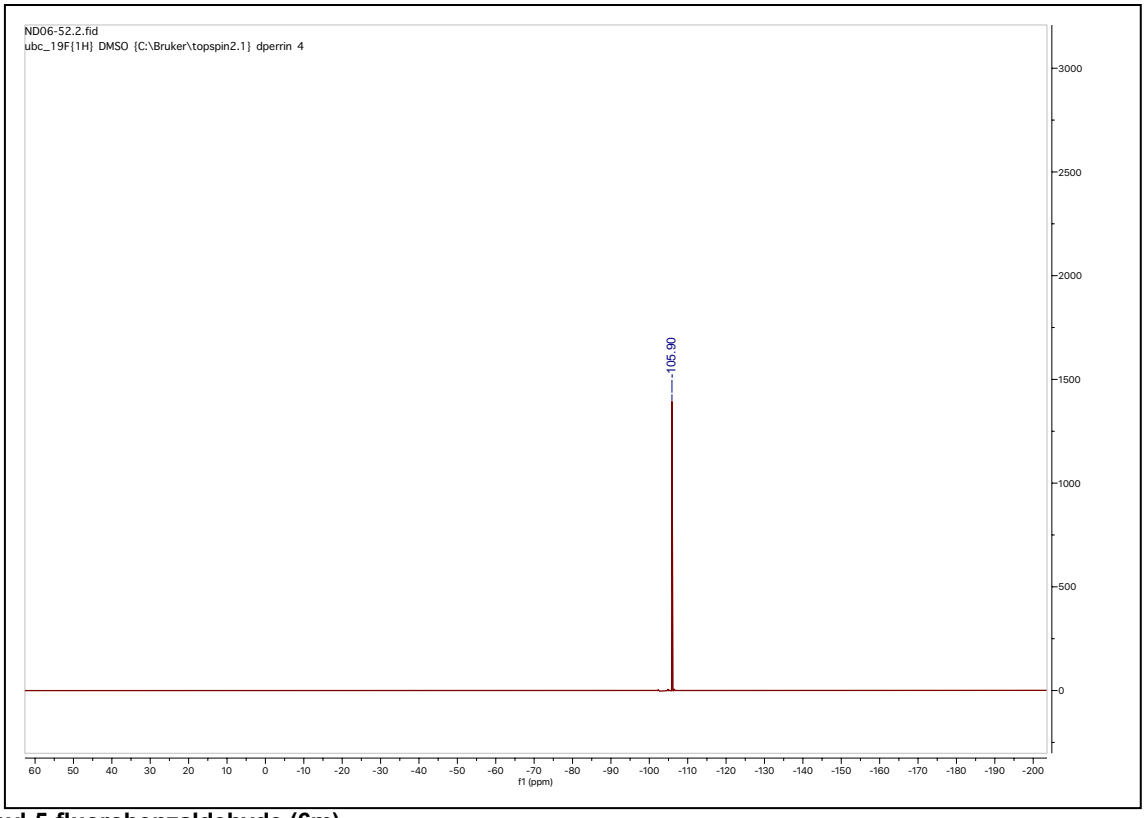




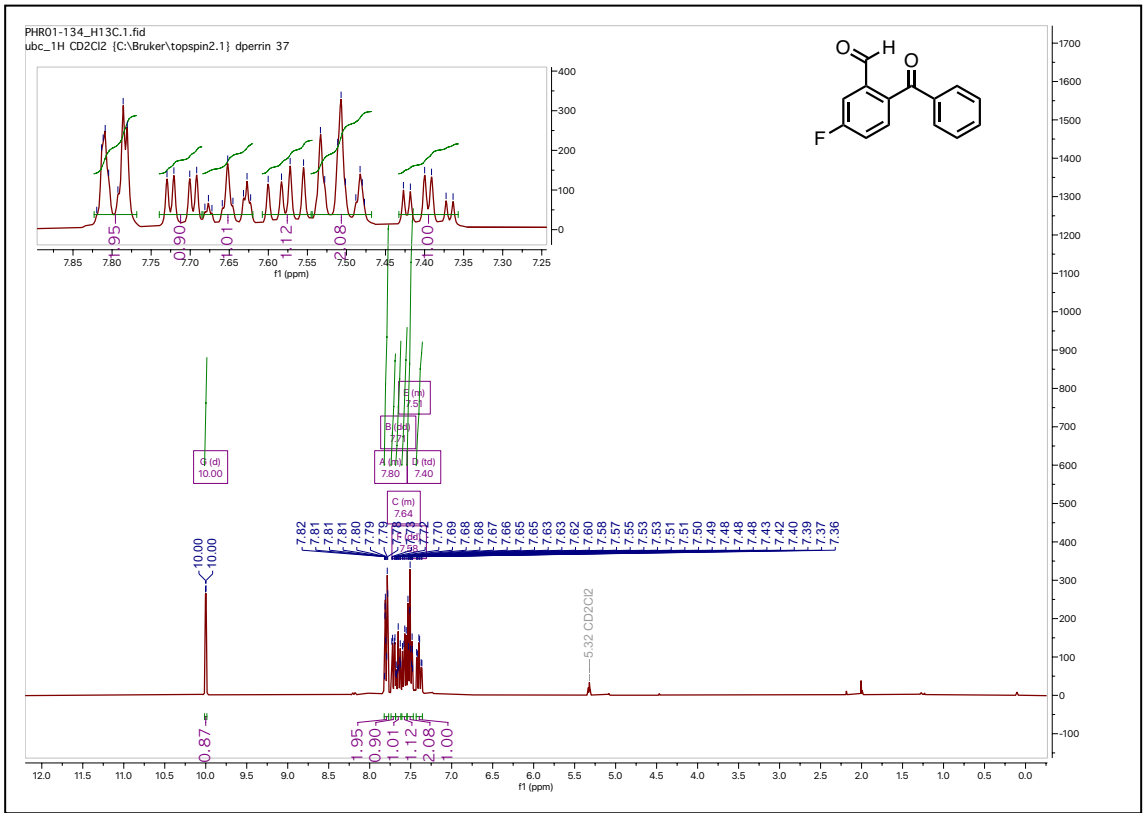
2-(4-fluorobenzoyl)-4-methoxybenzaldehyde (6l)

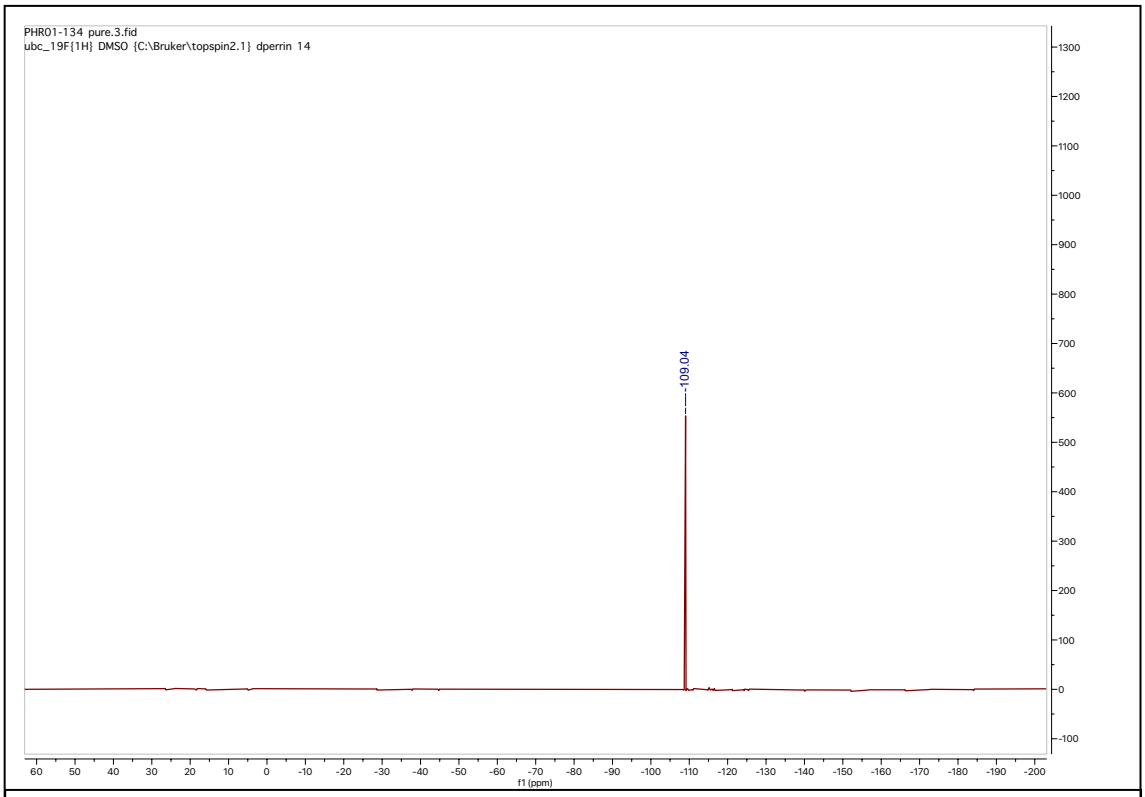






2-benzoyl-5-fluorobenzaldehyde (6m)

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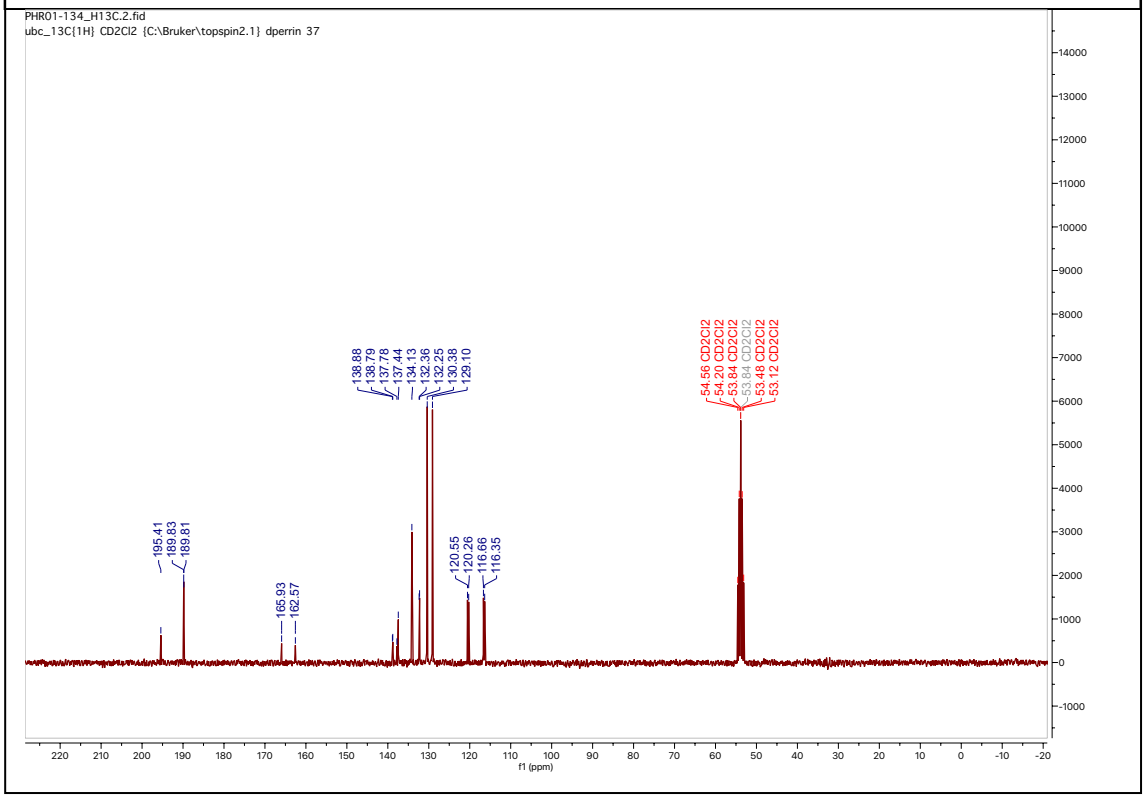
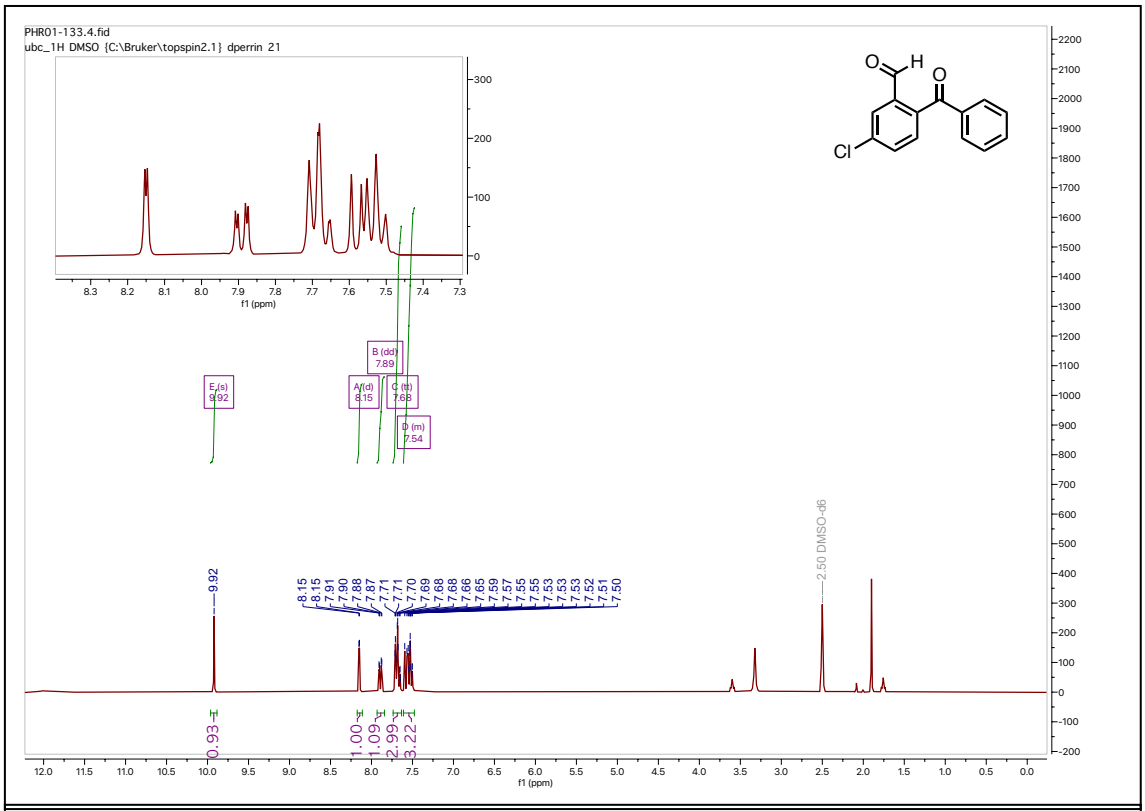
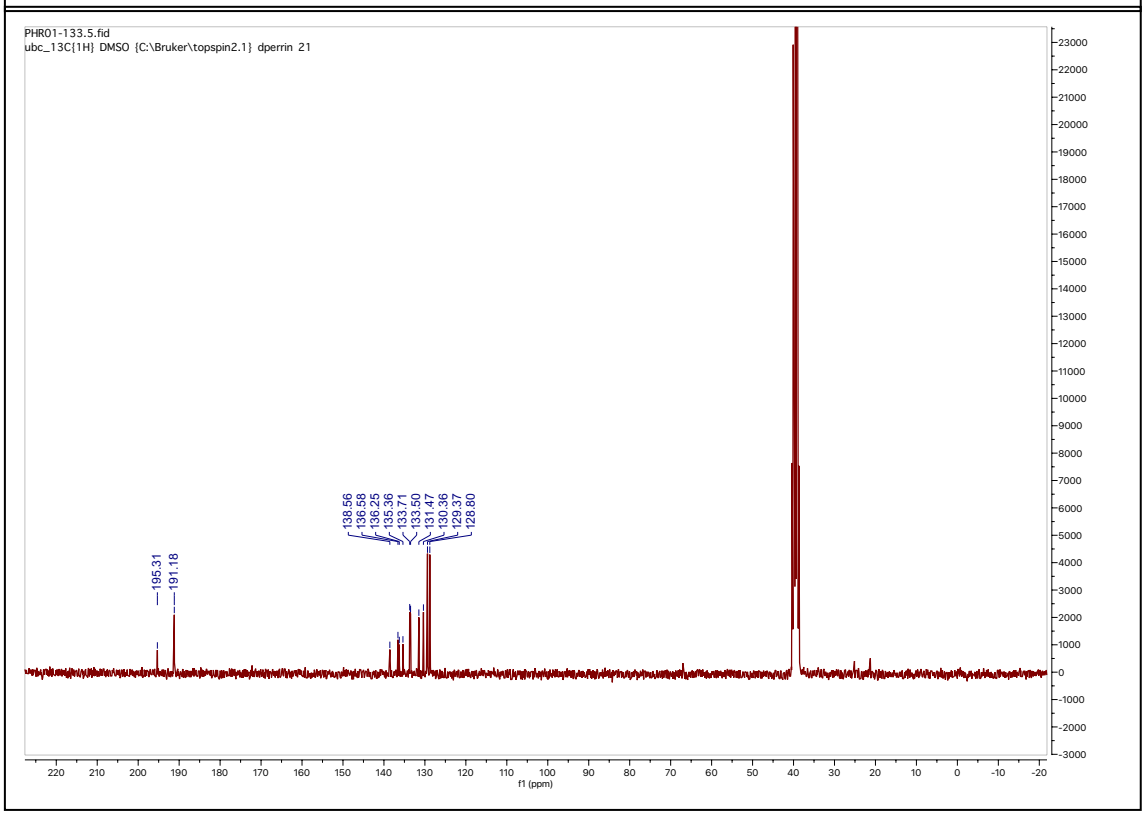
ubc_19F{1H} DMSO {C:\Bruker\topspin2.1} dperrin 14
109.04
f1 (ppm)

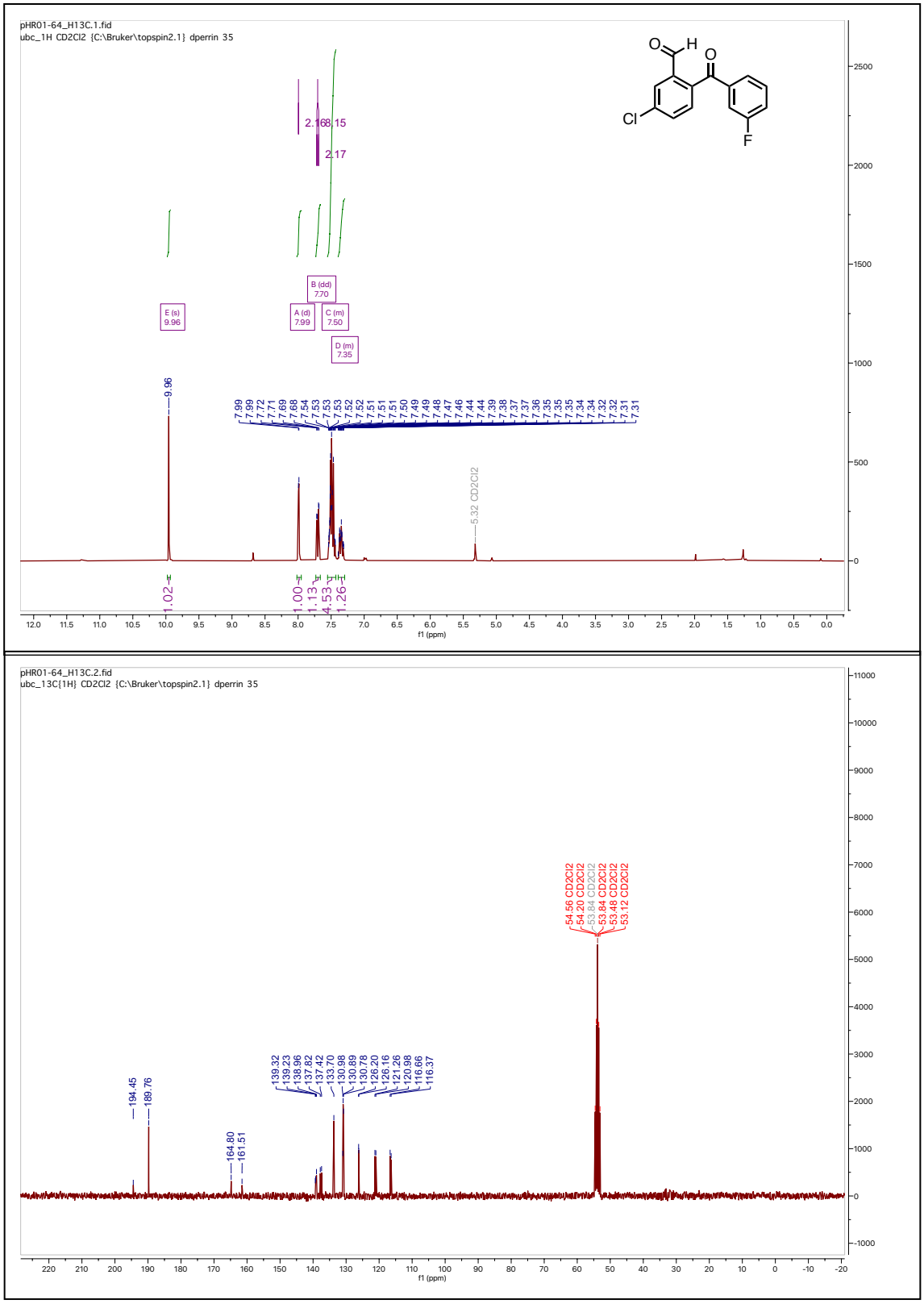

PHR01-134_H13C.2.fid
ubc_13C{1H} CD2Cl2 {C:\Bruker\topspin2.1} dperrin 37
195.41
189.83
189.81
165.93
162.57
138.88
138.79
137.78
137.44
134.13
132.36
132.25
130.38
129.10
120.55
120.26
116.66
116.35
54.56 CD2Cl2
54.20 CD2Cl2
53.84 CD2Cl2
53.48 CD2Cl2
53.12 CD2Cl2
f1 (ppm)

2-benzoyl-5-chlorobenzaldehyde (6n)

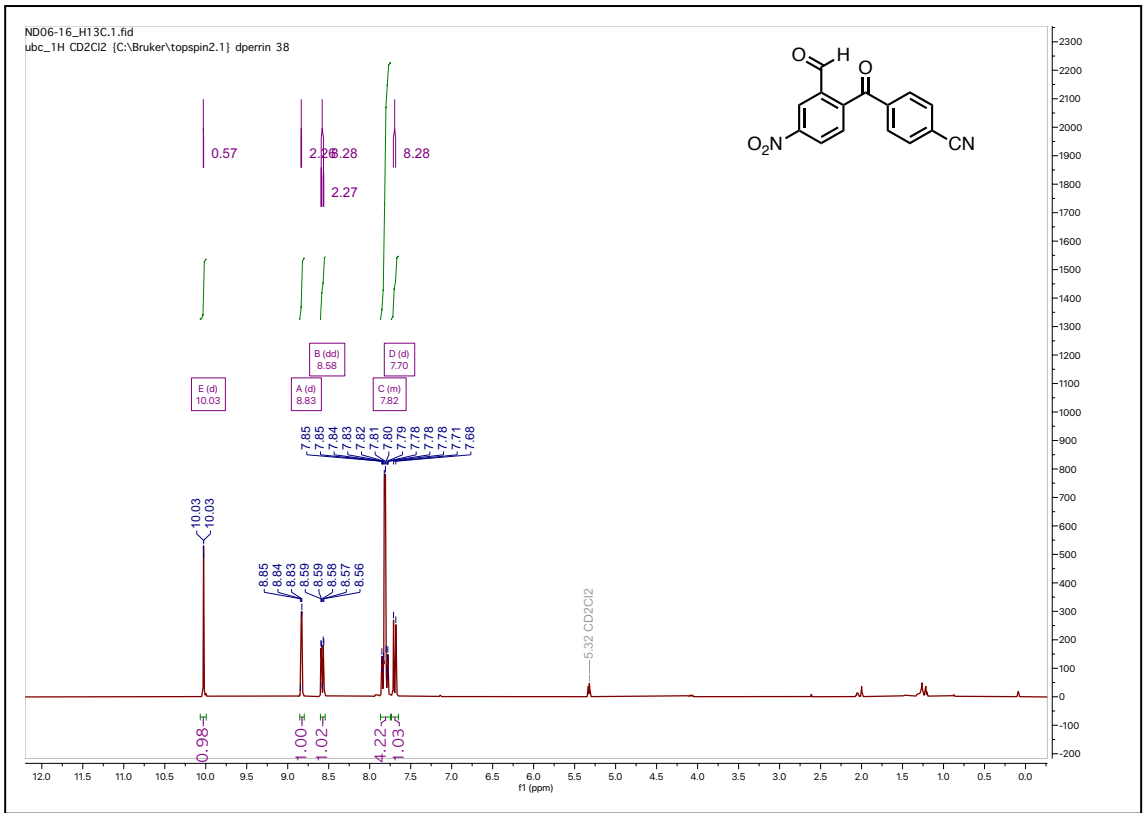


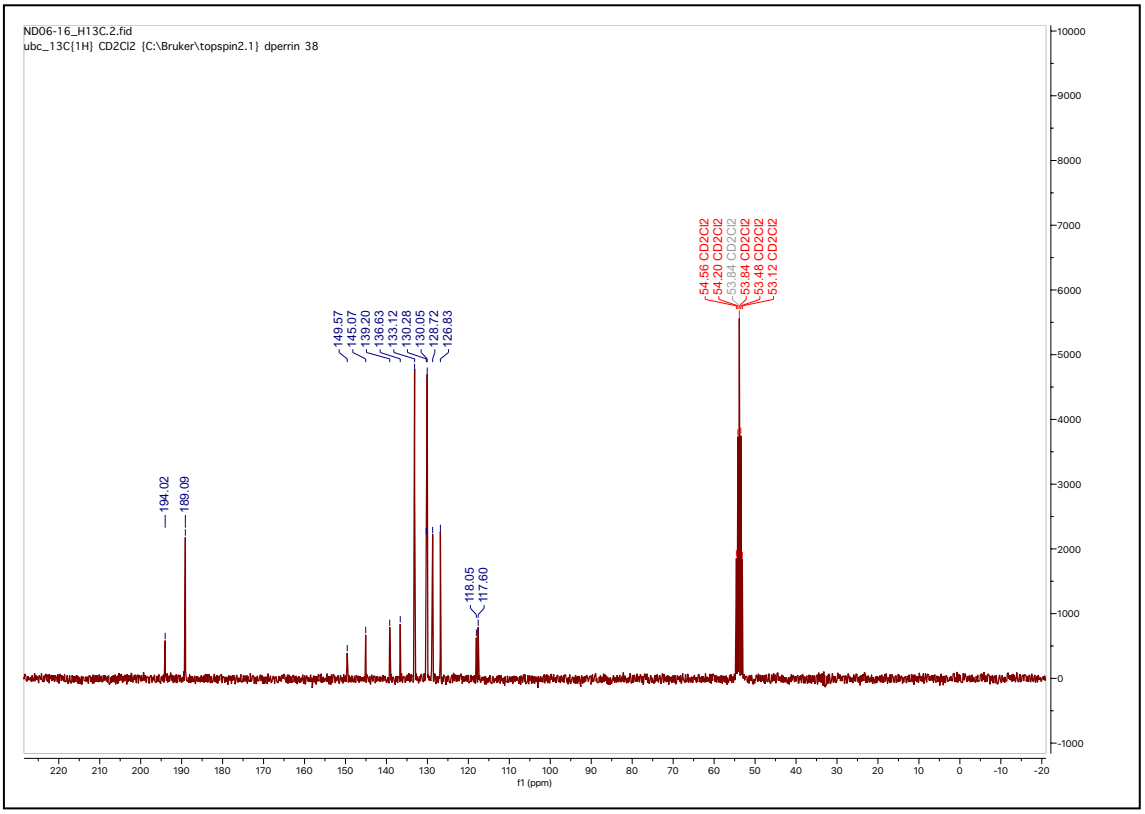


5-chloro-2-(3-fluorobenzoyl)benzaldehyde (6o)

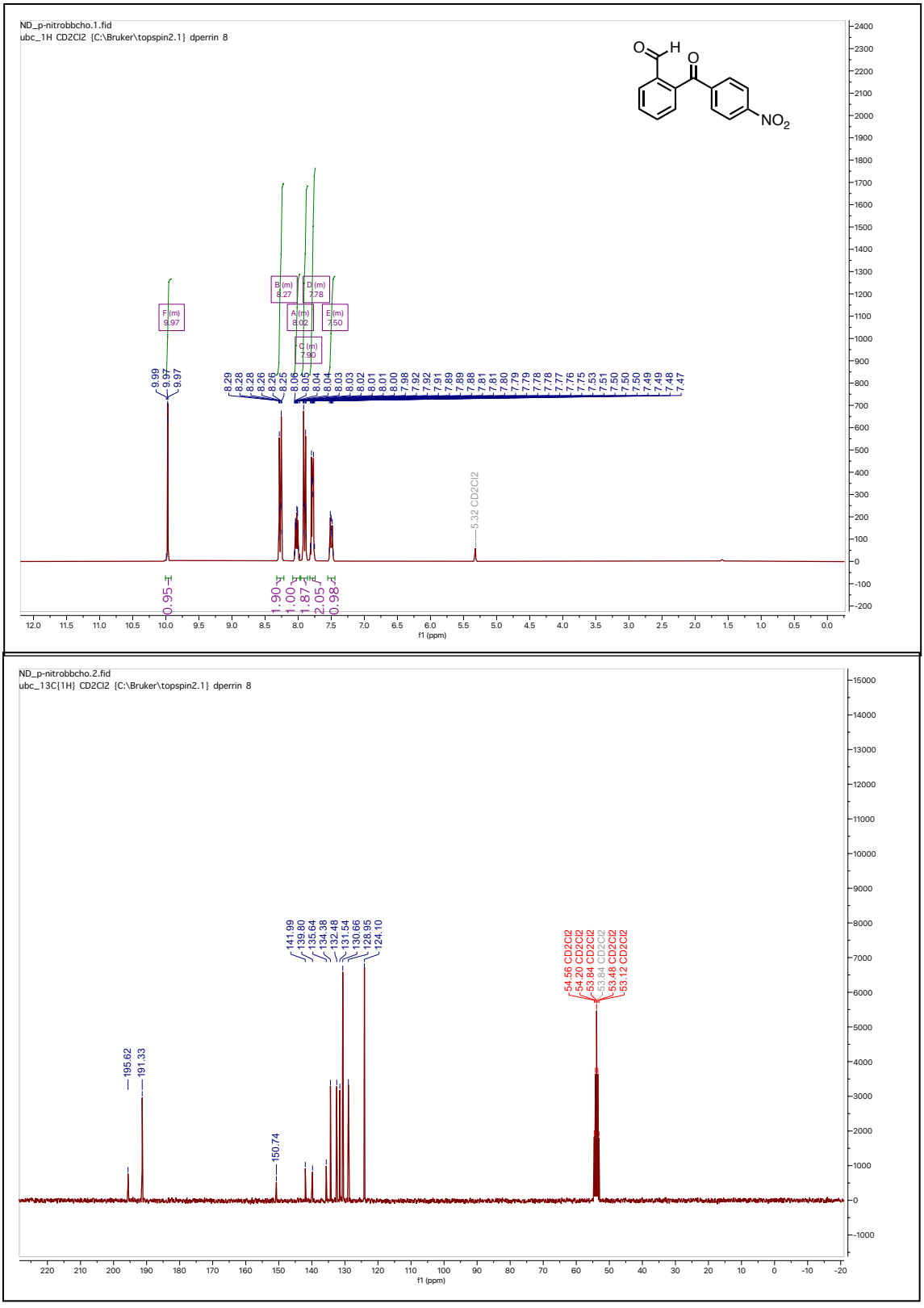


4-(2-formly-4-nitrobenzoyl)benzonitrile (6p)

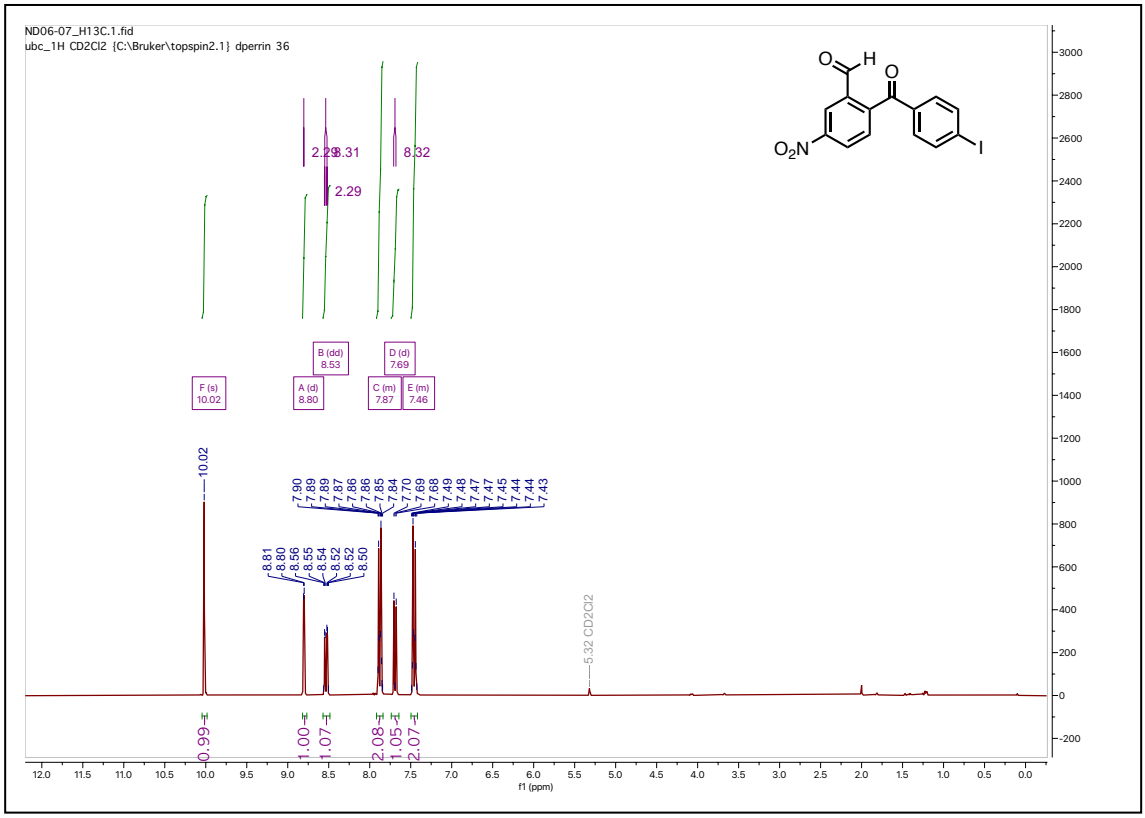


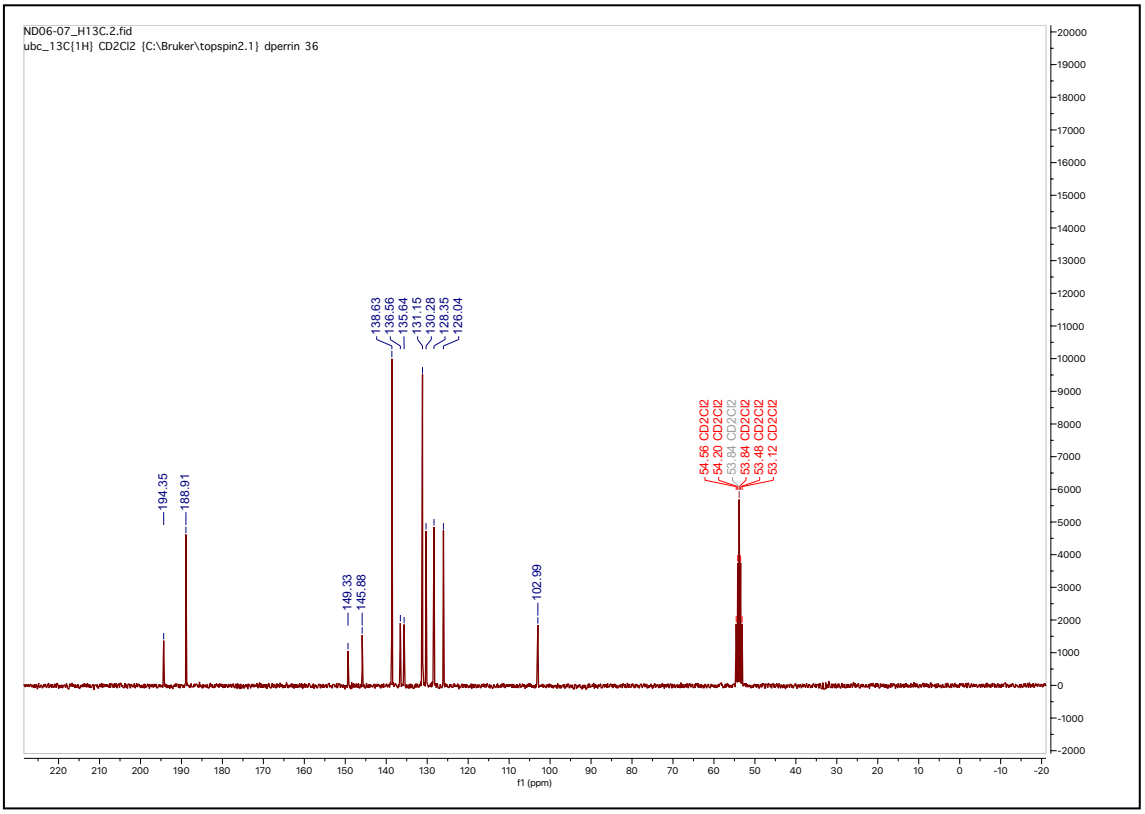


2-(4-nitrobenzoyl)benzaldehyde (6q)

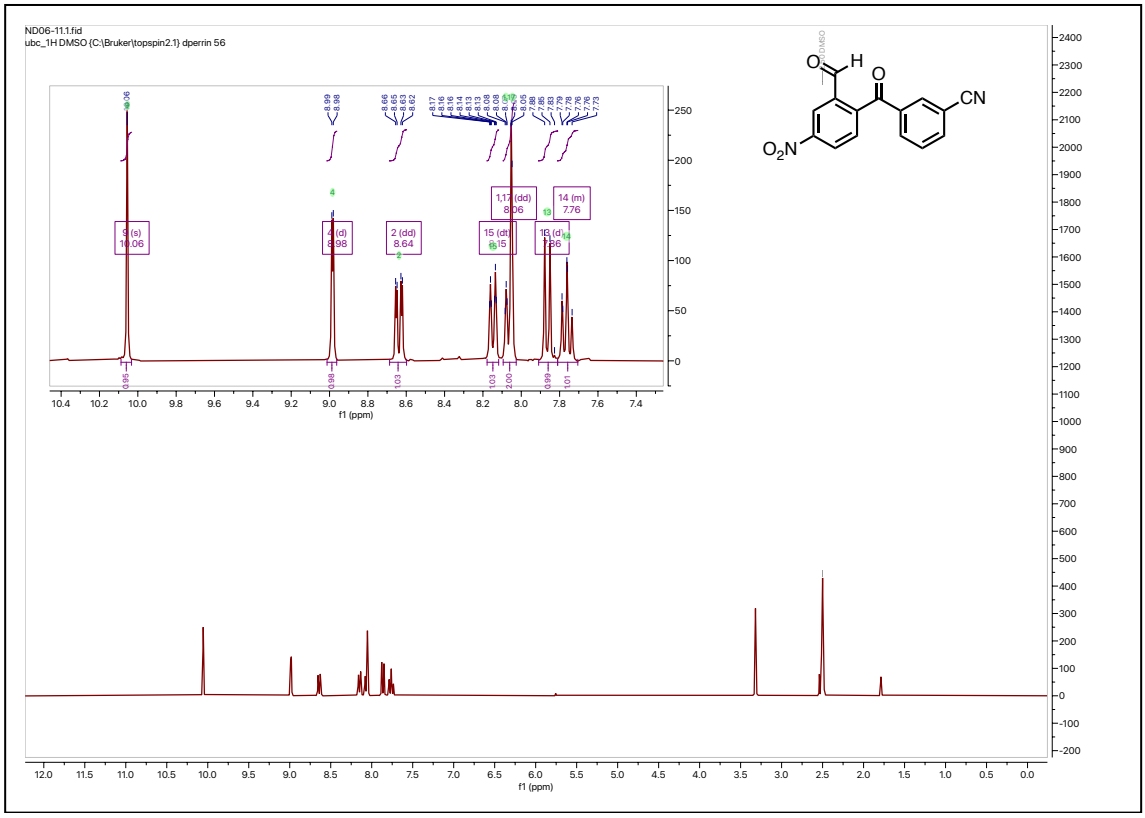


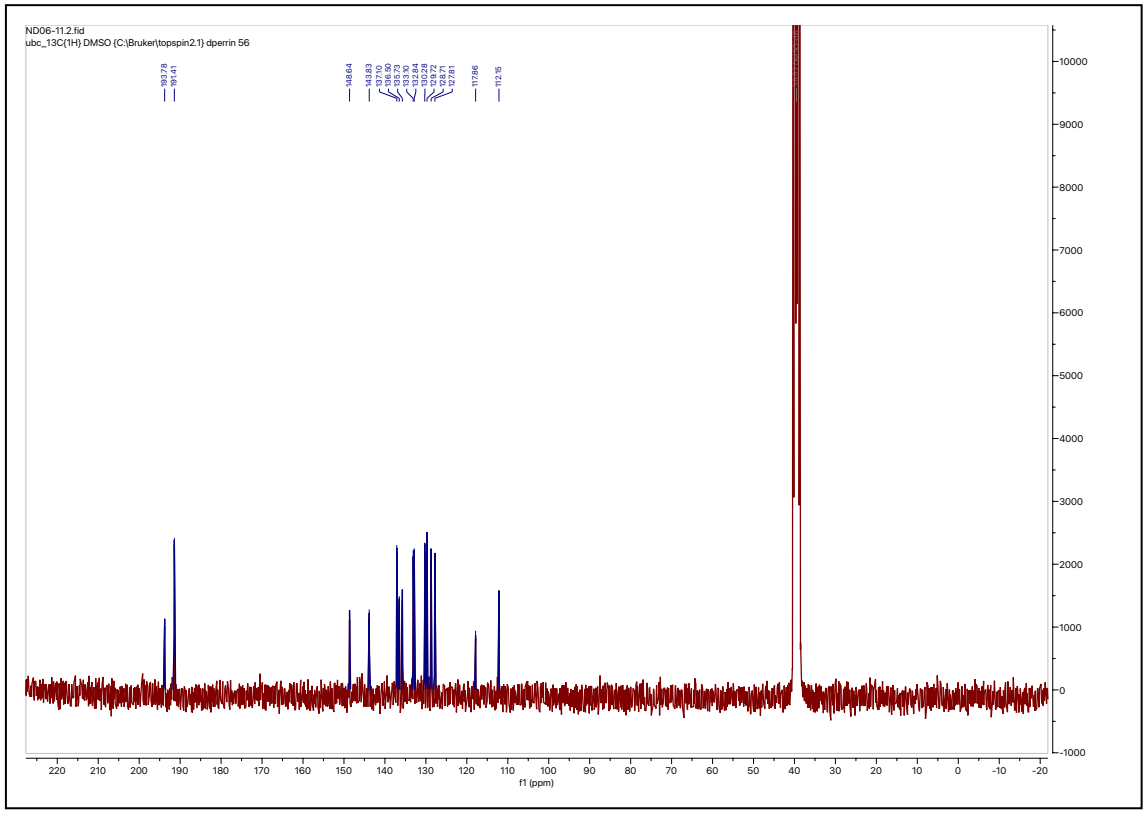
2-(4-iodobenzoyl)-5-nitrobenzaldehyde (6r)



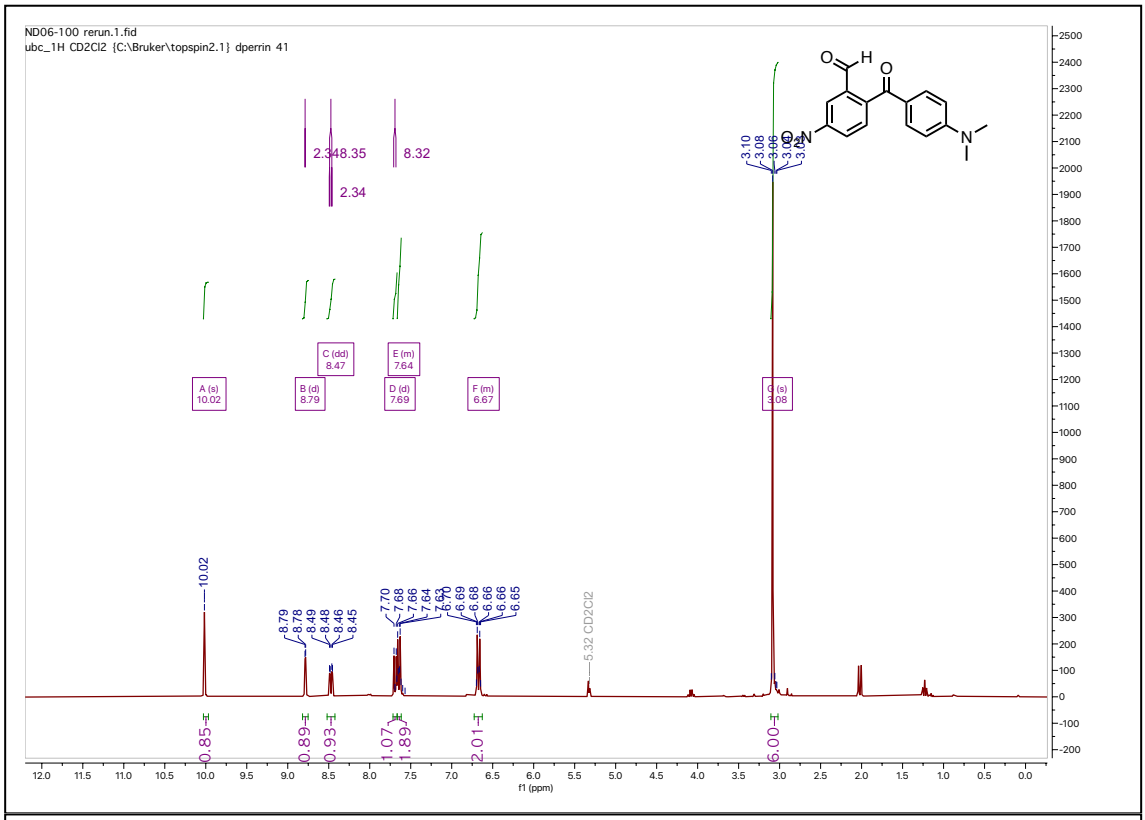


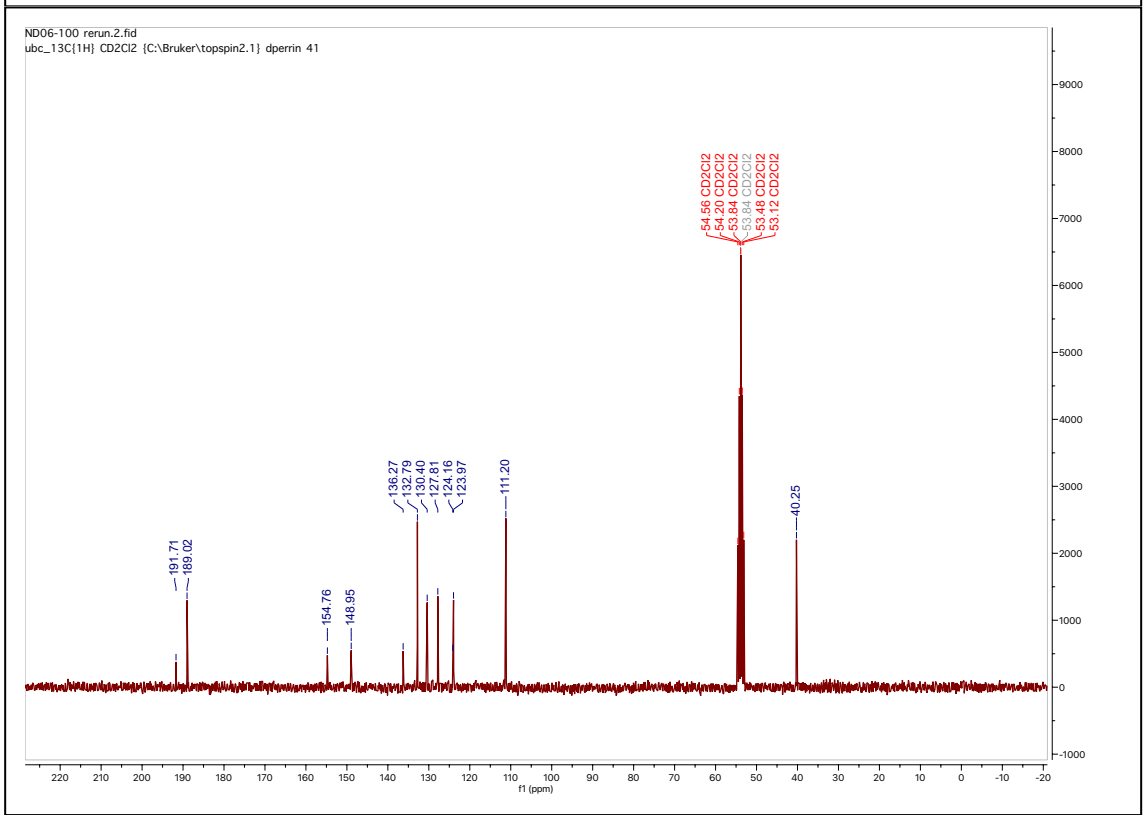
3-(2-formyl-4-nitrobenzoyl)benzonitrile (6s)



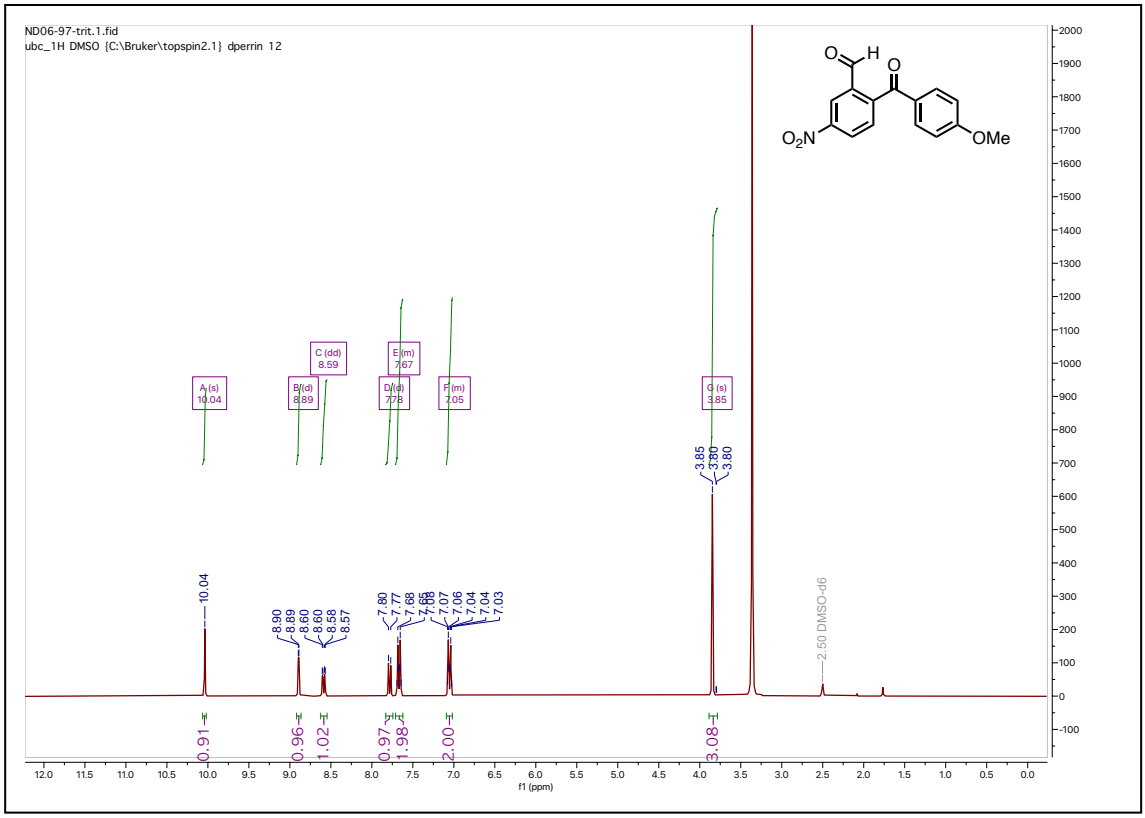


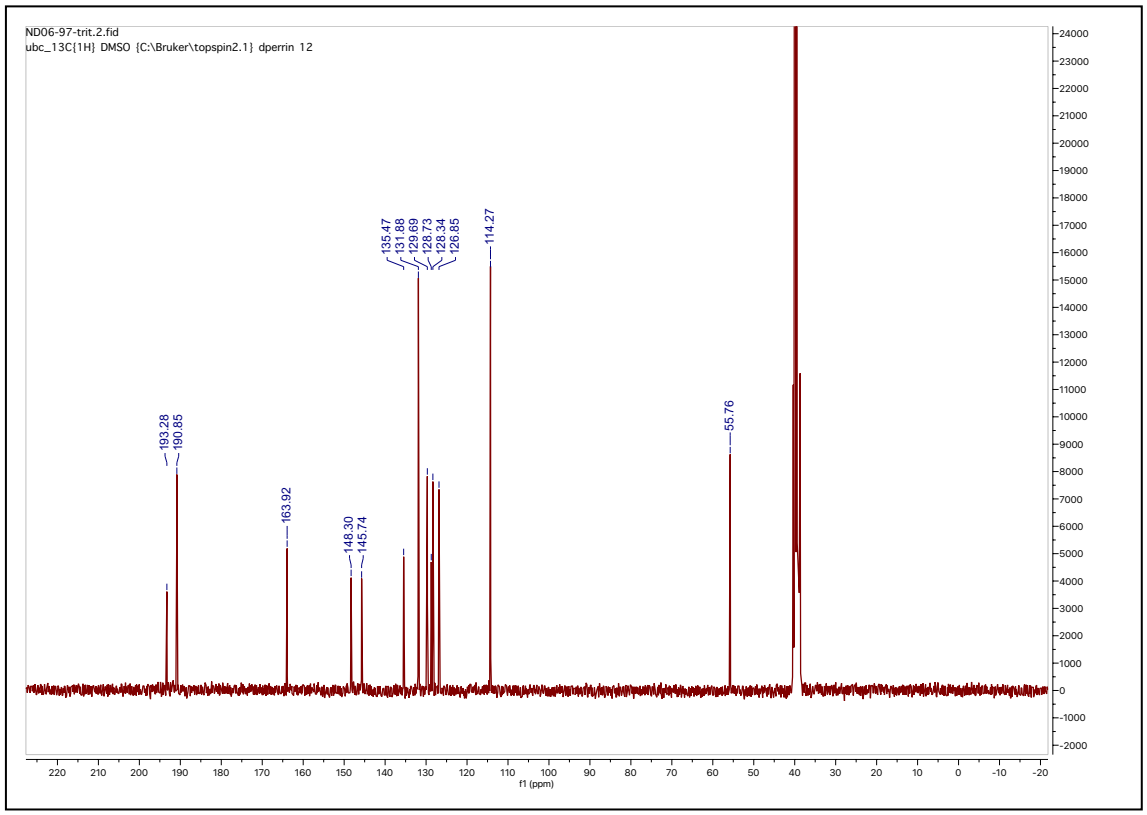
2-(4-(dimethylamino)benzoyl)-5-nitrobenzaldehyde (6t)



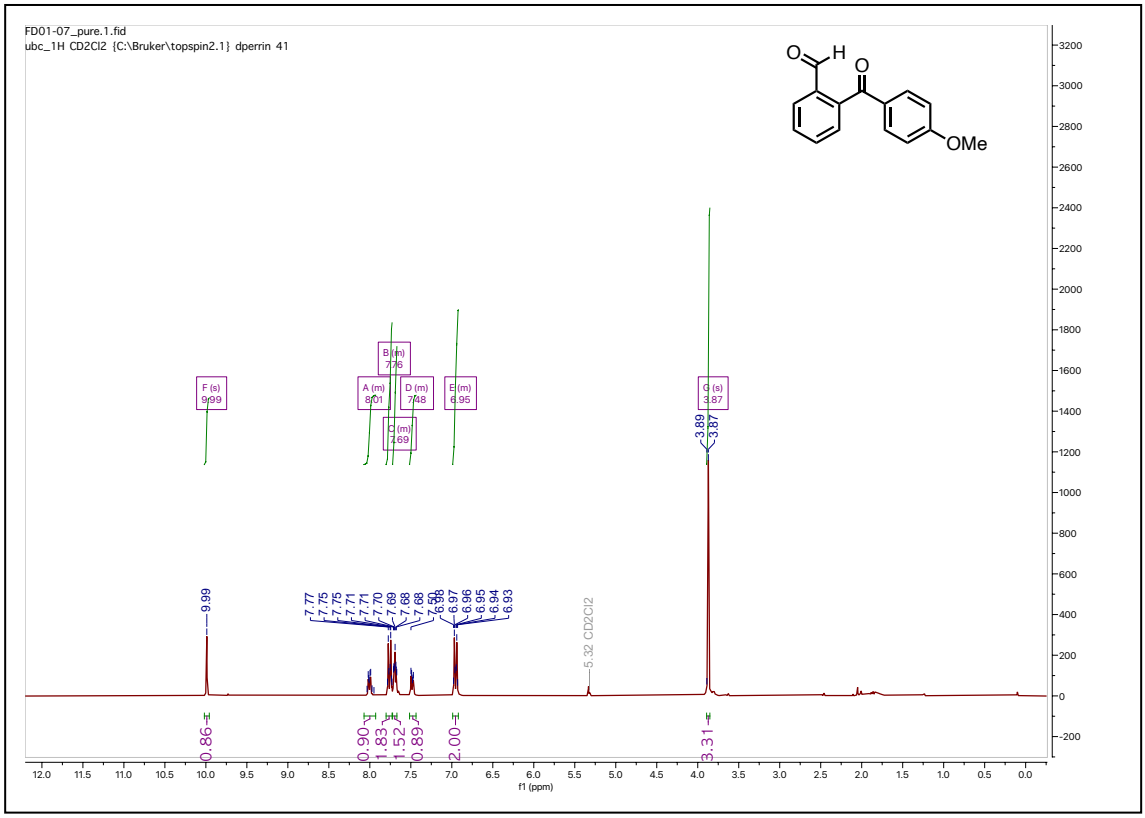


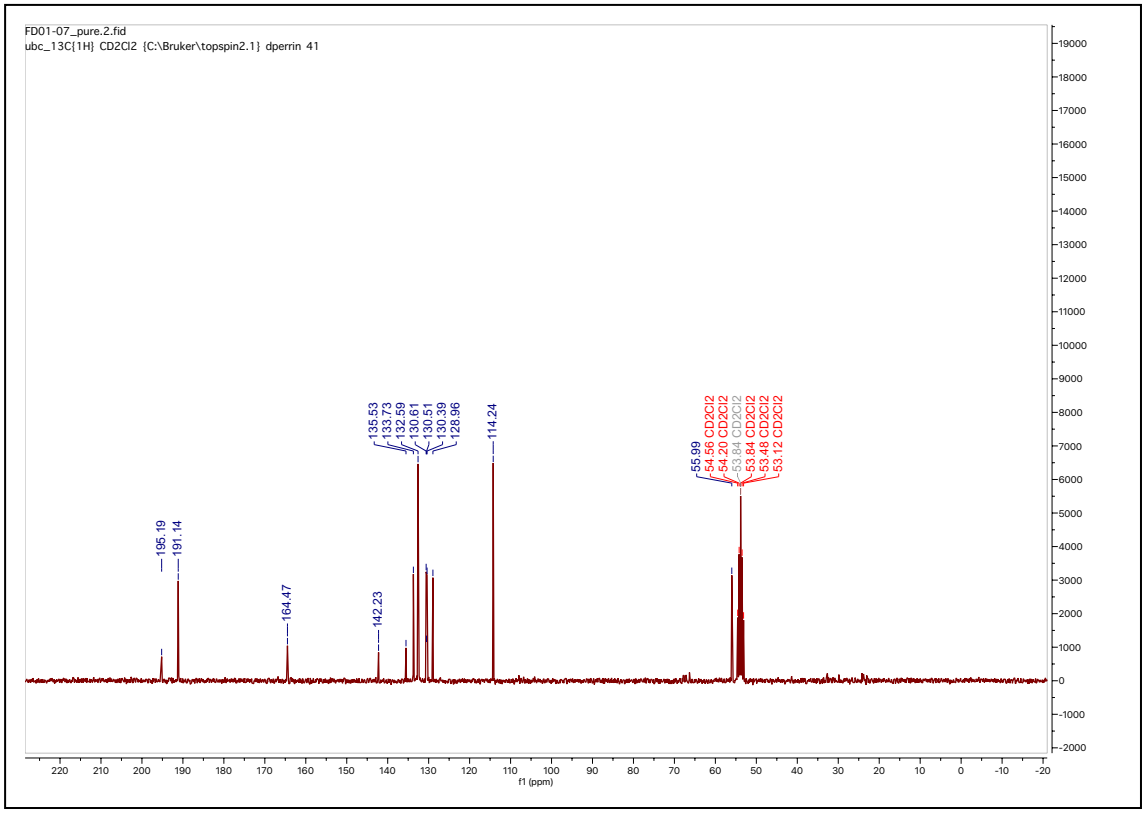
2-(4-methoxybenzoyl)-5-nitrobenzaldehyde (6u)



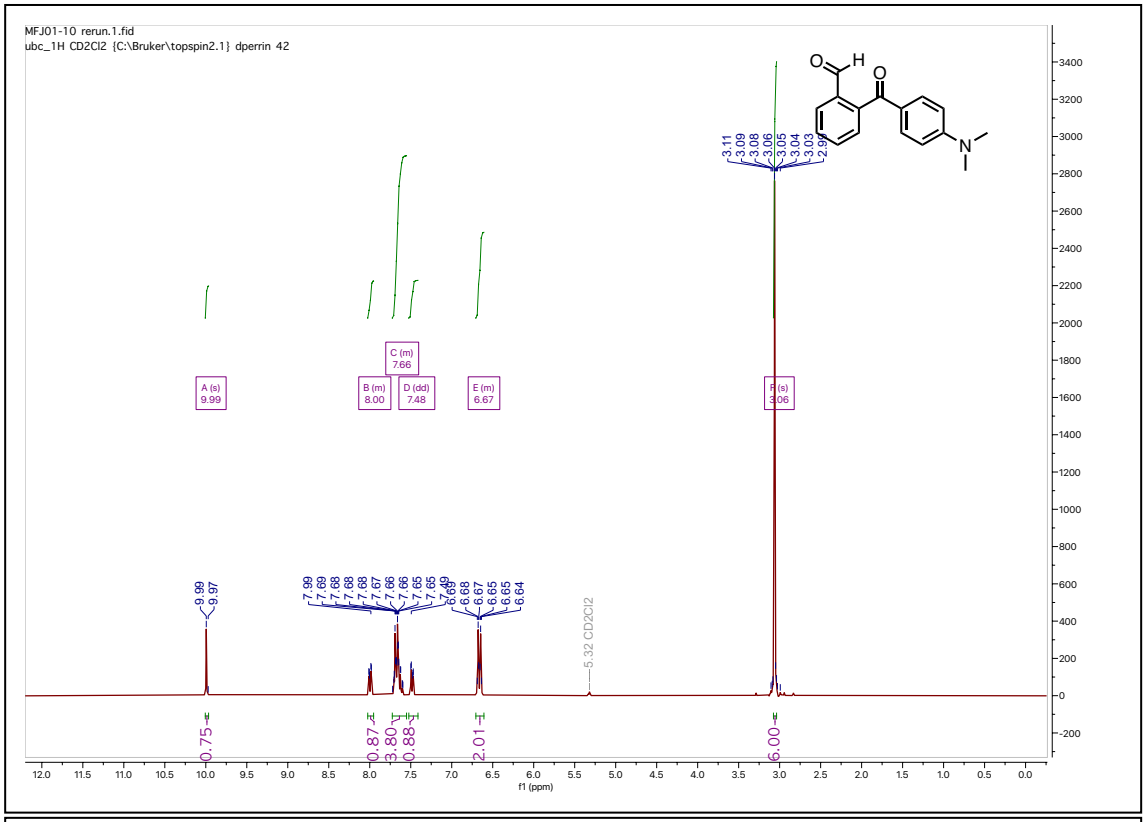


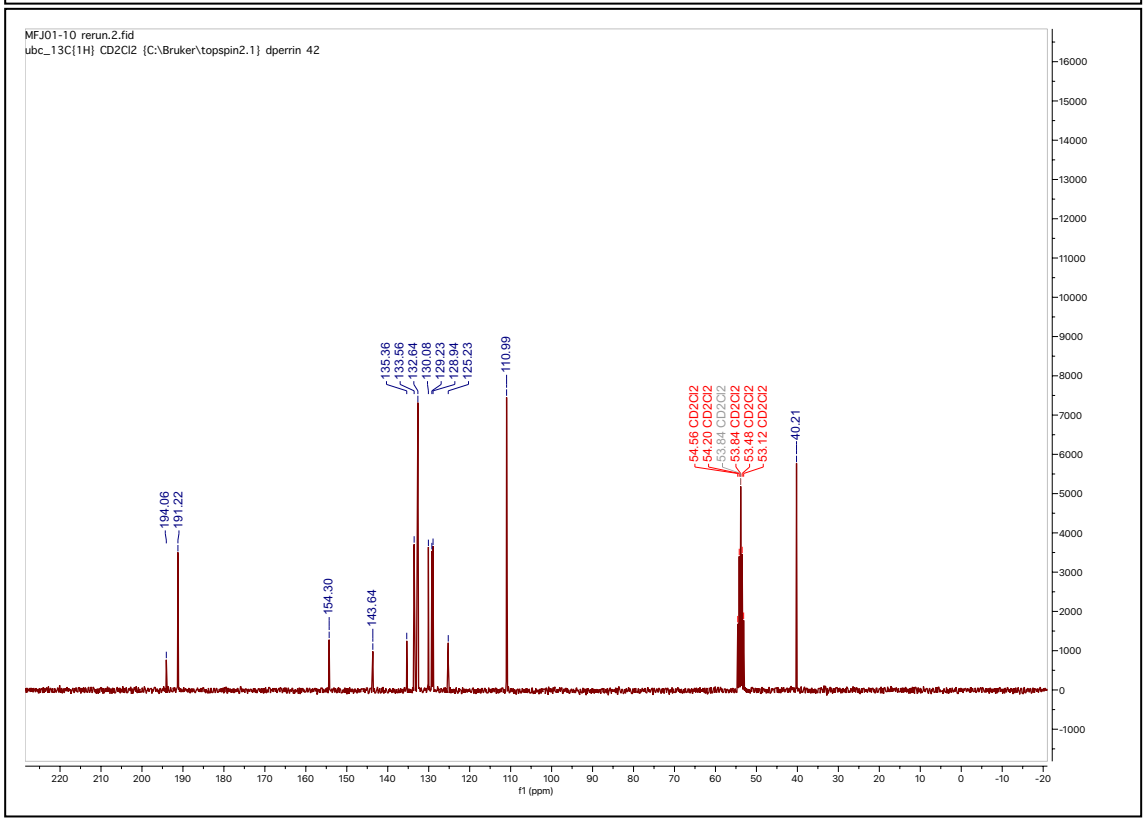
2-(4-methoxybenzoyl)benzaldehyde (6v)



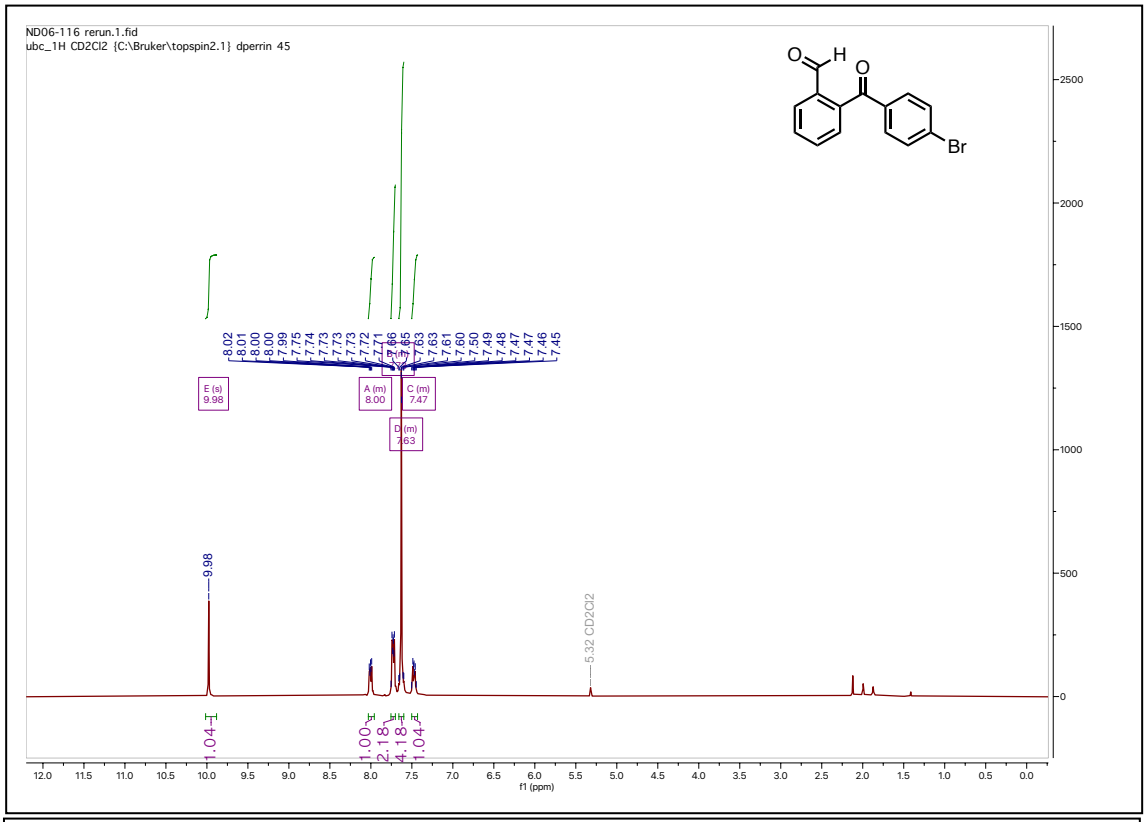


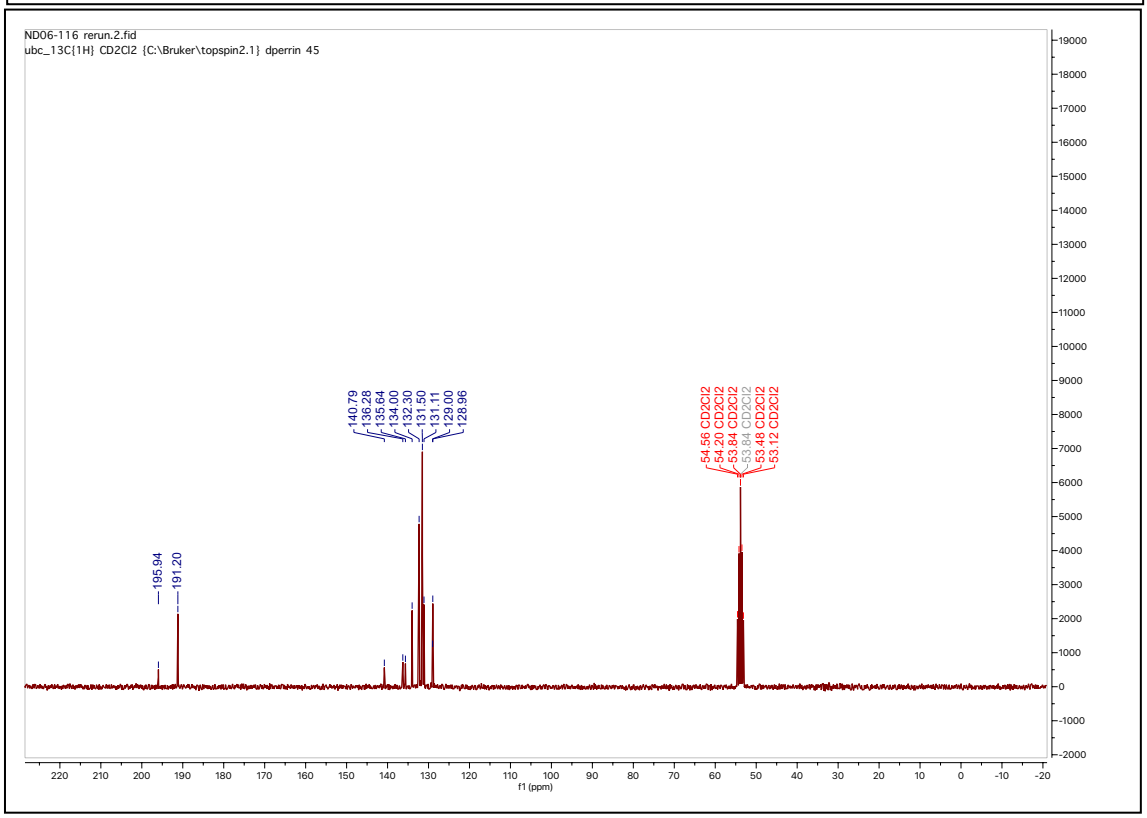
2-(4-(dimethylamino)benzoyl)benzaldehyde (6w)



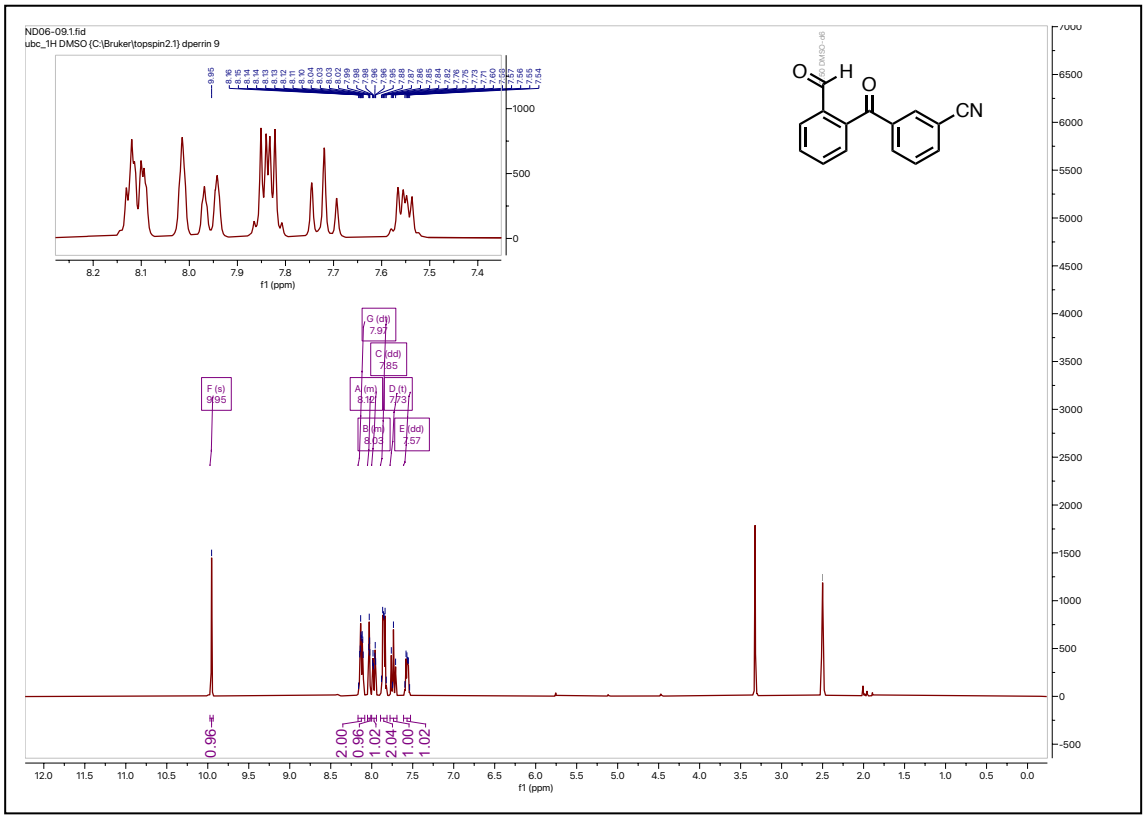


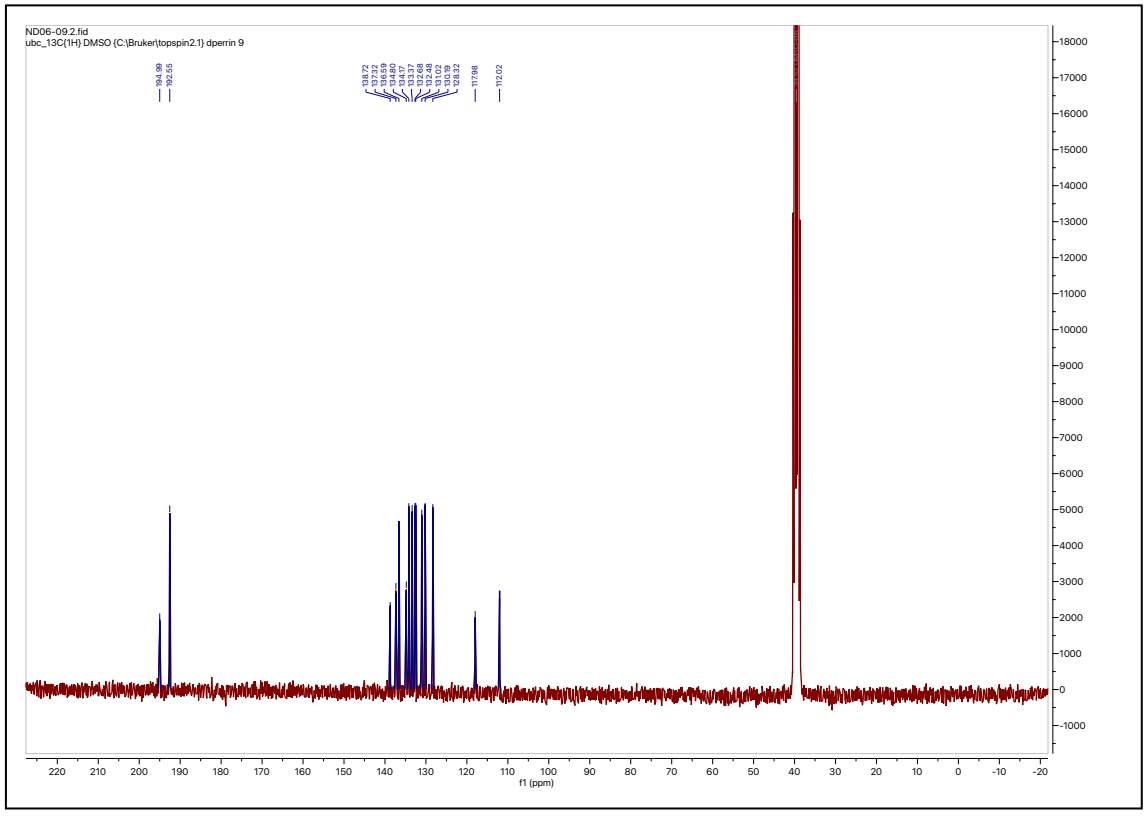
2-(4-bromobenzoyl)benzaldehyde (6x)



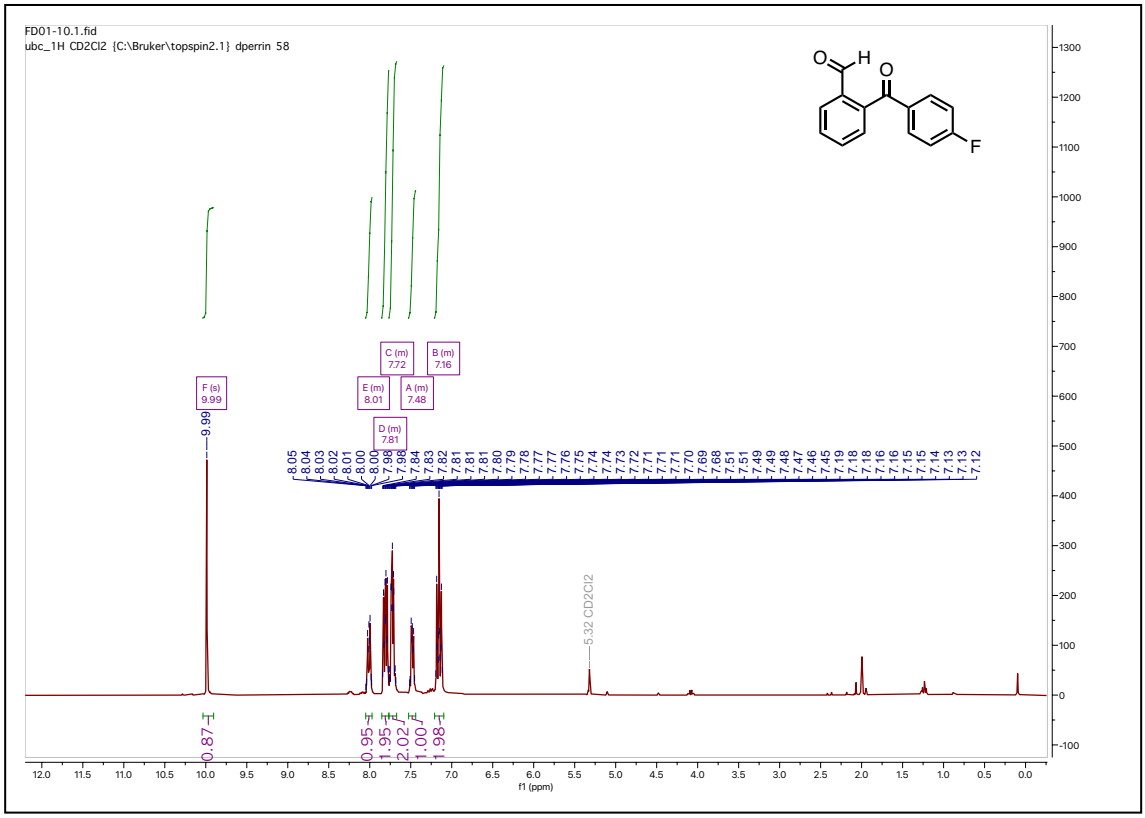


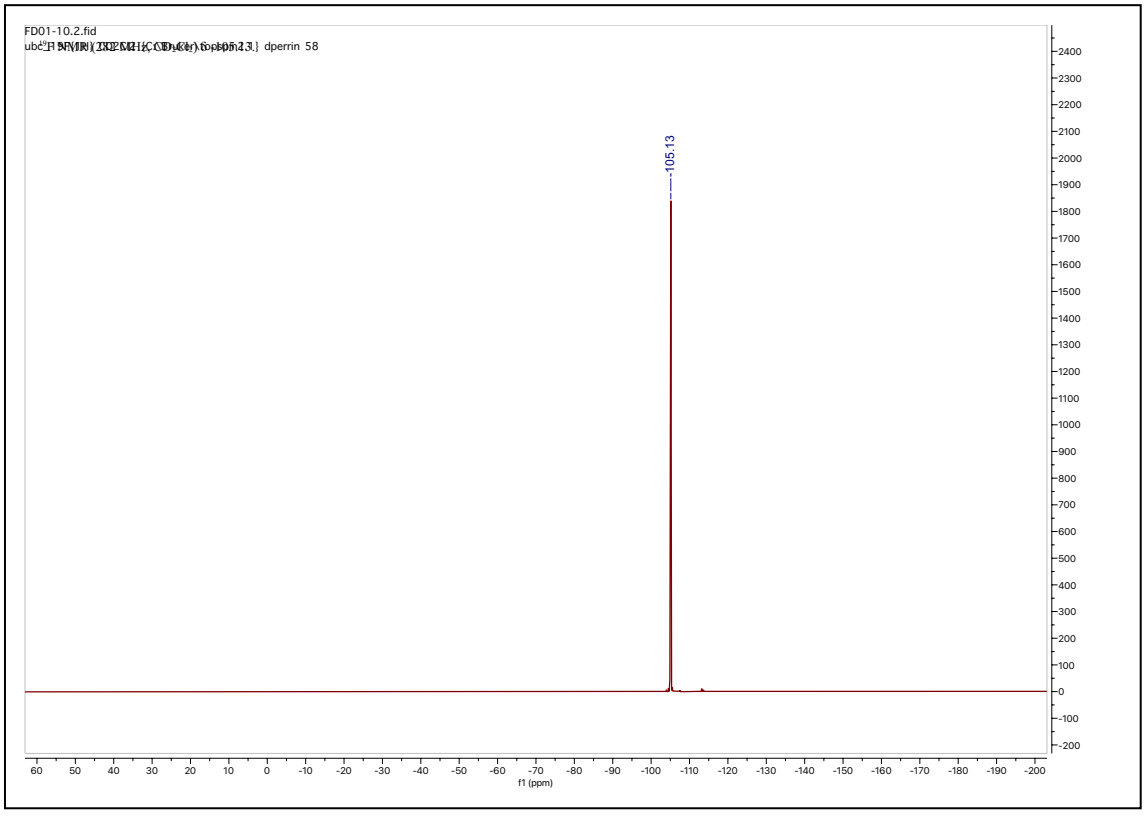
3-(2-formylbenzoyl)benzonitrile (6y)

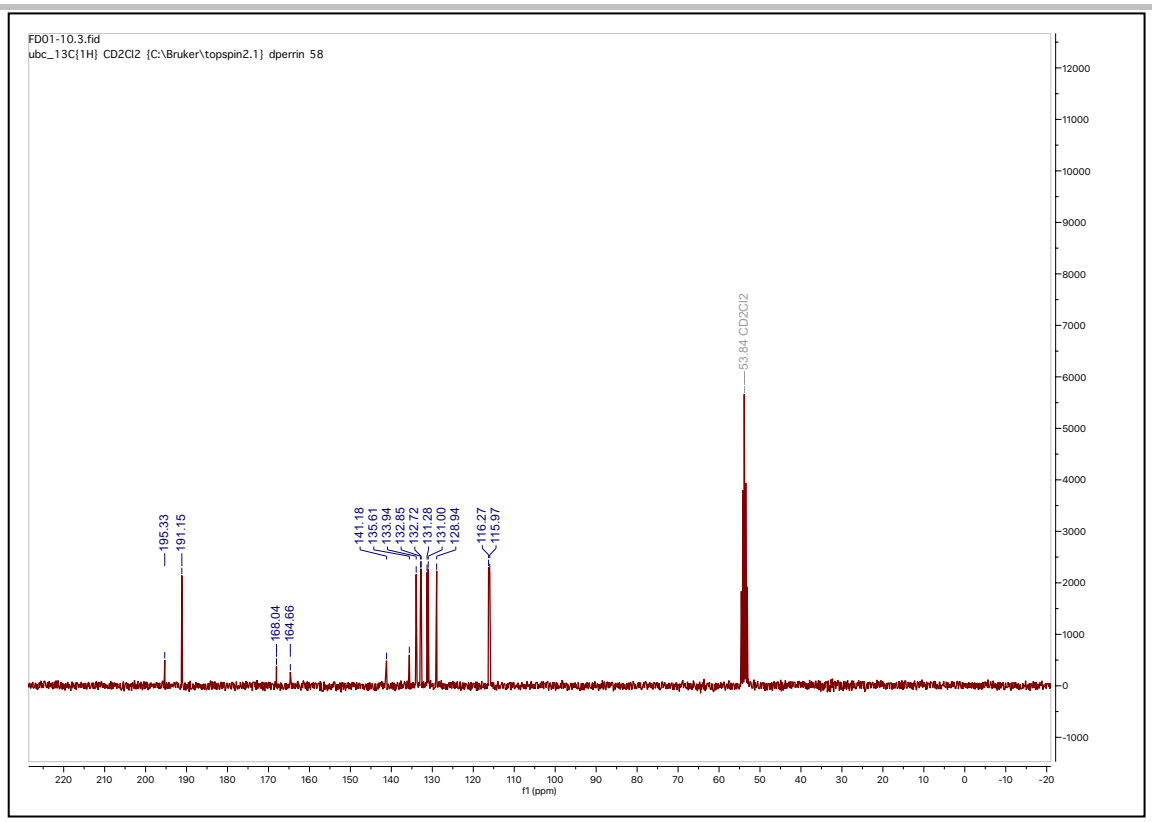




2-(4-fluorobenzoyl)benzaldehyde (6z)



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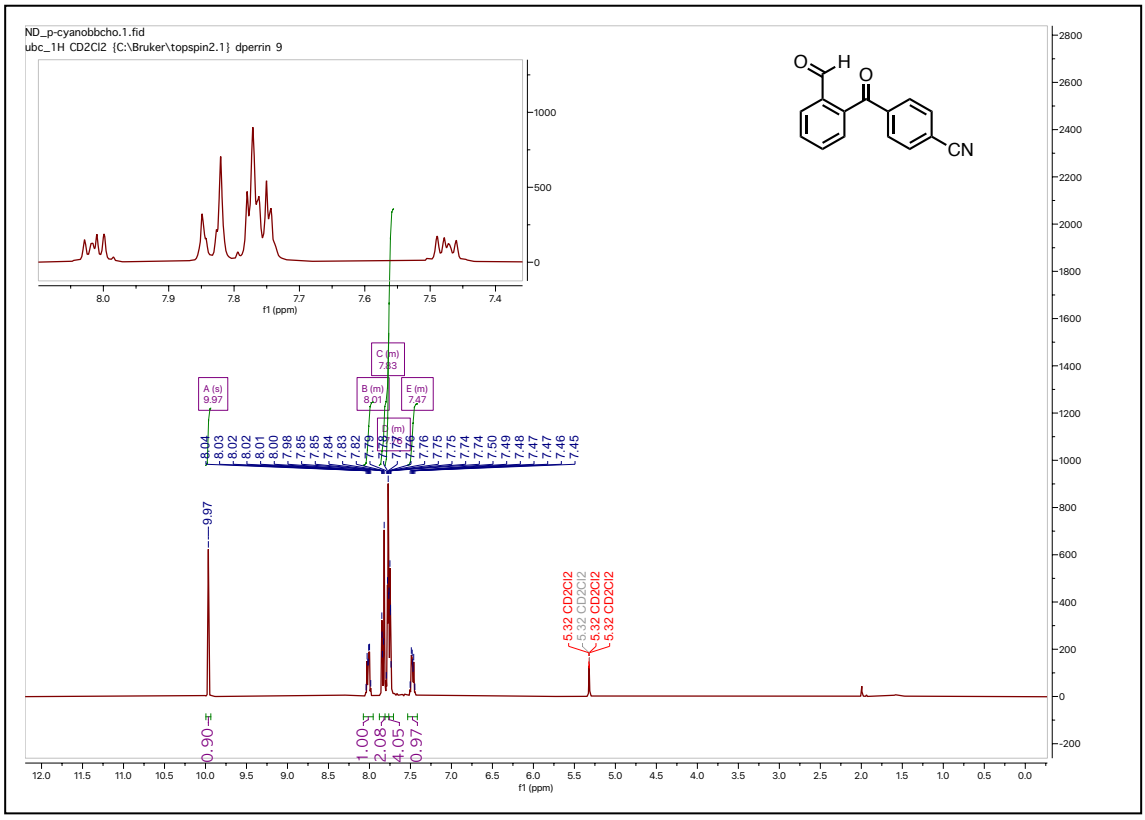
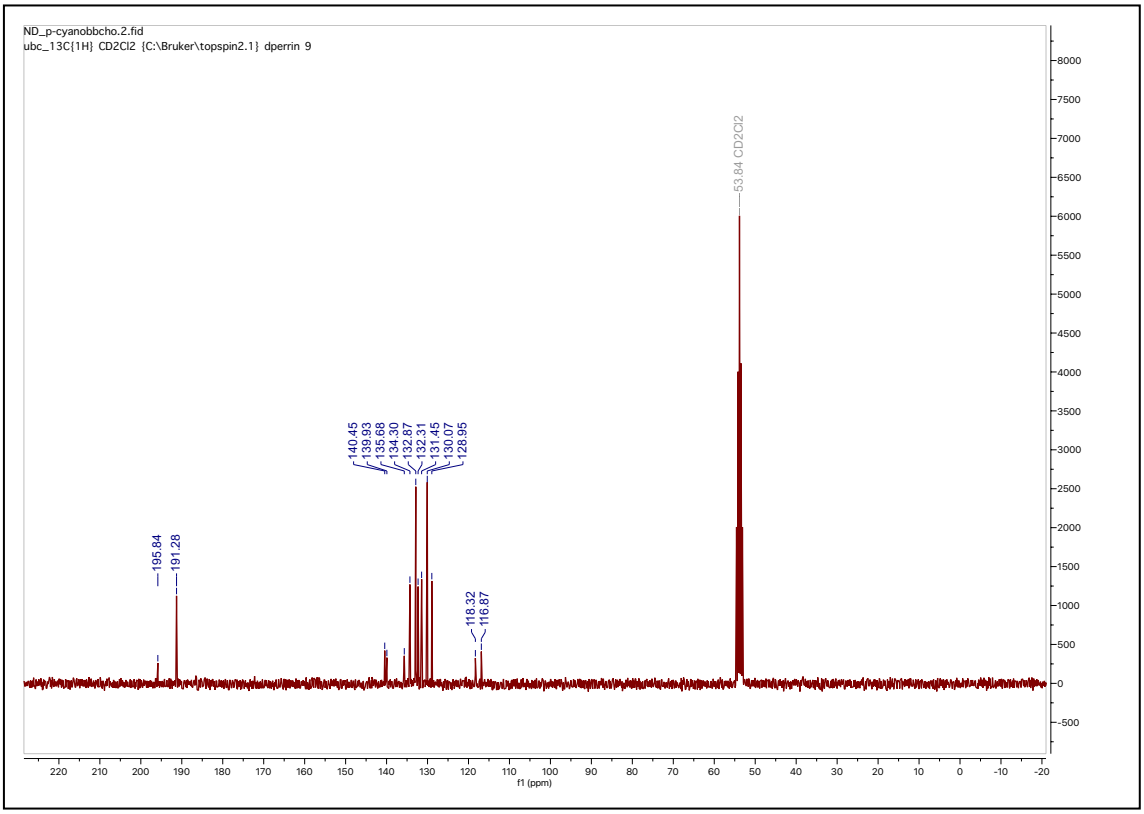
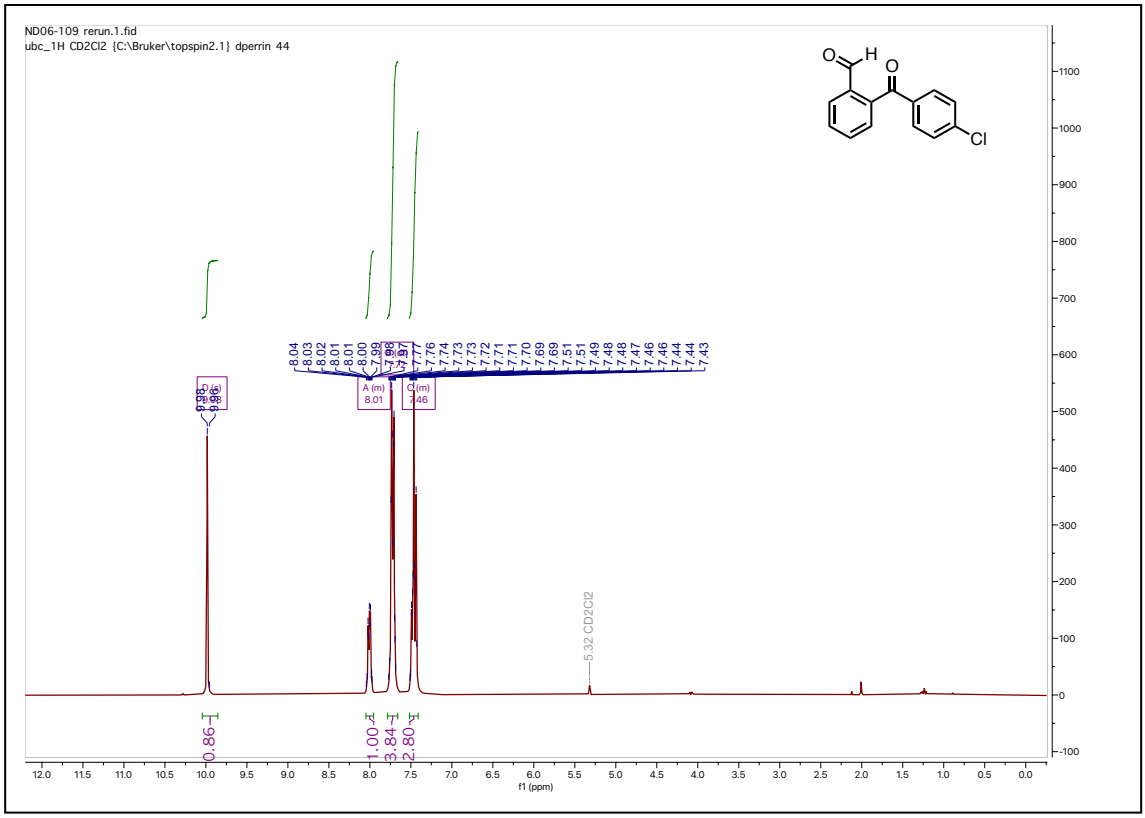
ubc_13C{1H} CD2Cl2 {C:\Bruker\topspin2.1} dperrin 58
195.33
191.15
168.04
164.66
141.18
135.61
133.94
132.85
132.72
131.28
131.00
128.94
116.27
115.97
53.84 CD2Cl2
f1 (ppm)

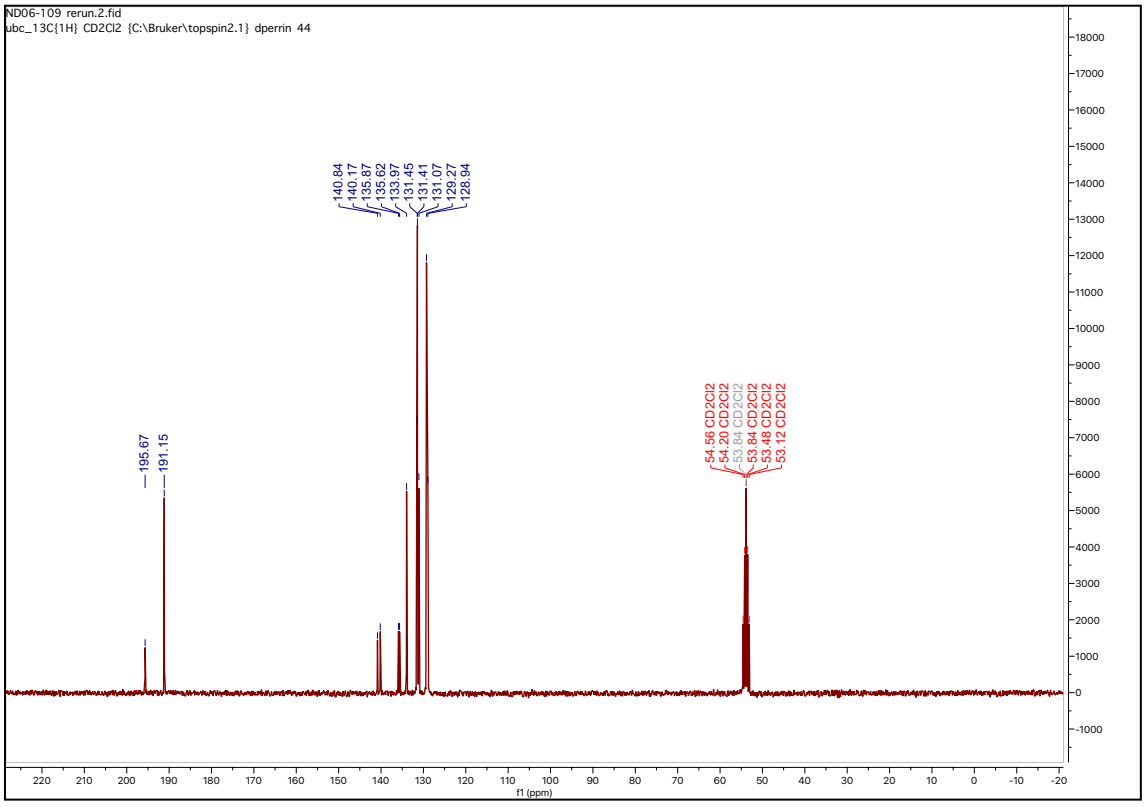
4-(2-formylbenzoyl)benzonitrile (6aa)



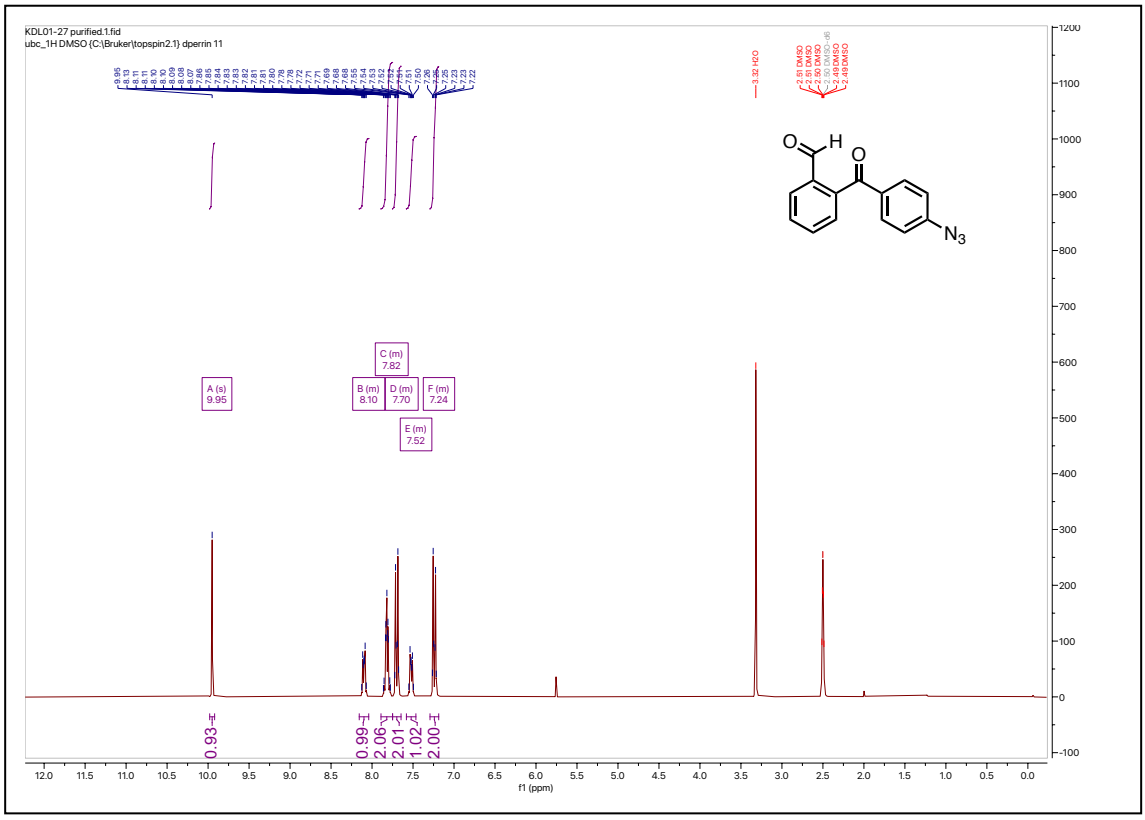


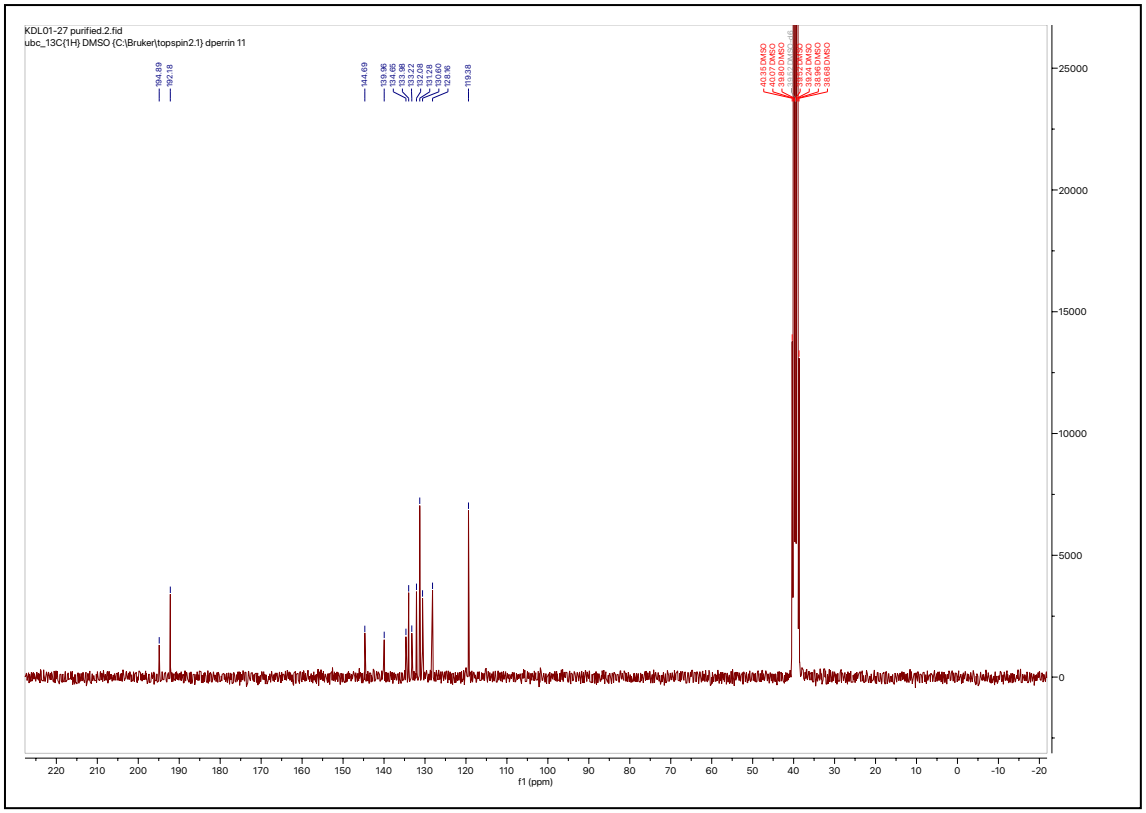
2-(4-chlorobenzoyl)benzaldehyde (6ab)



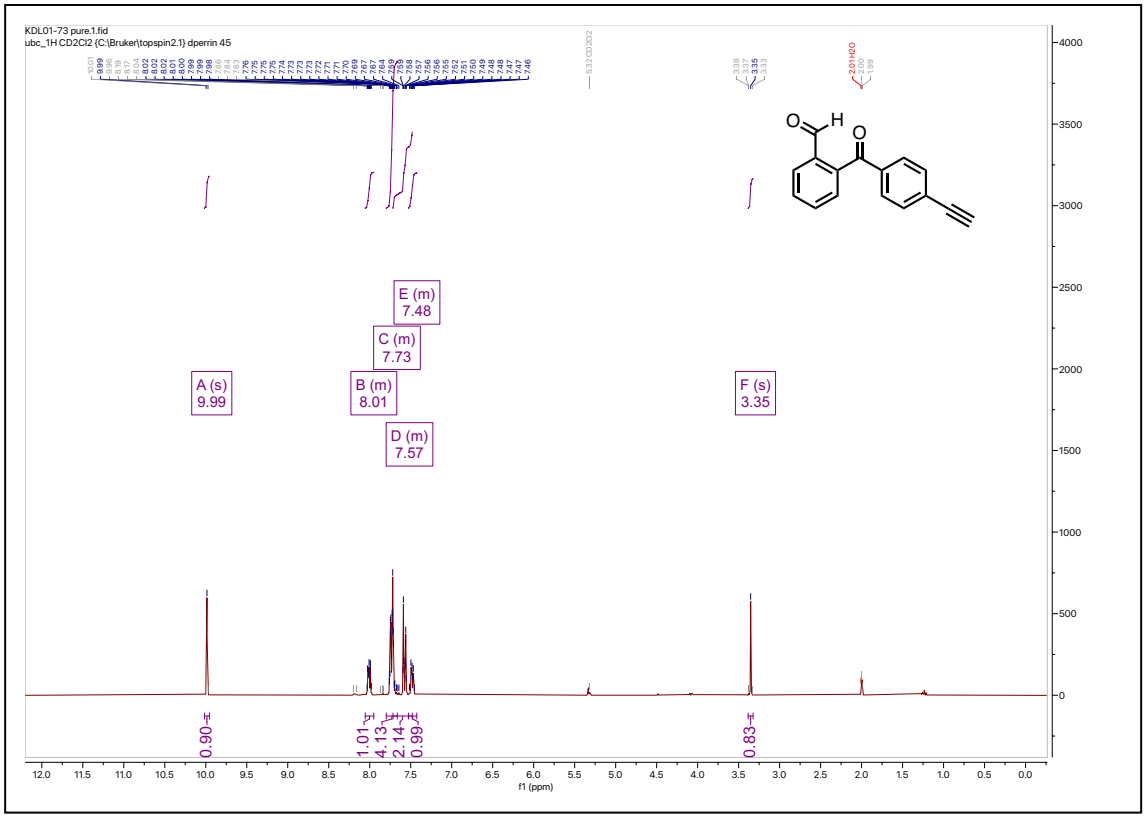


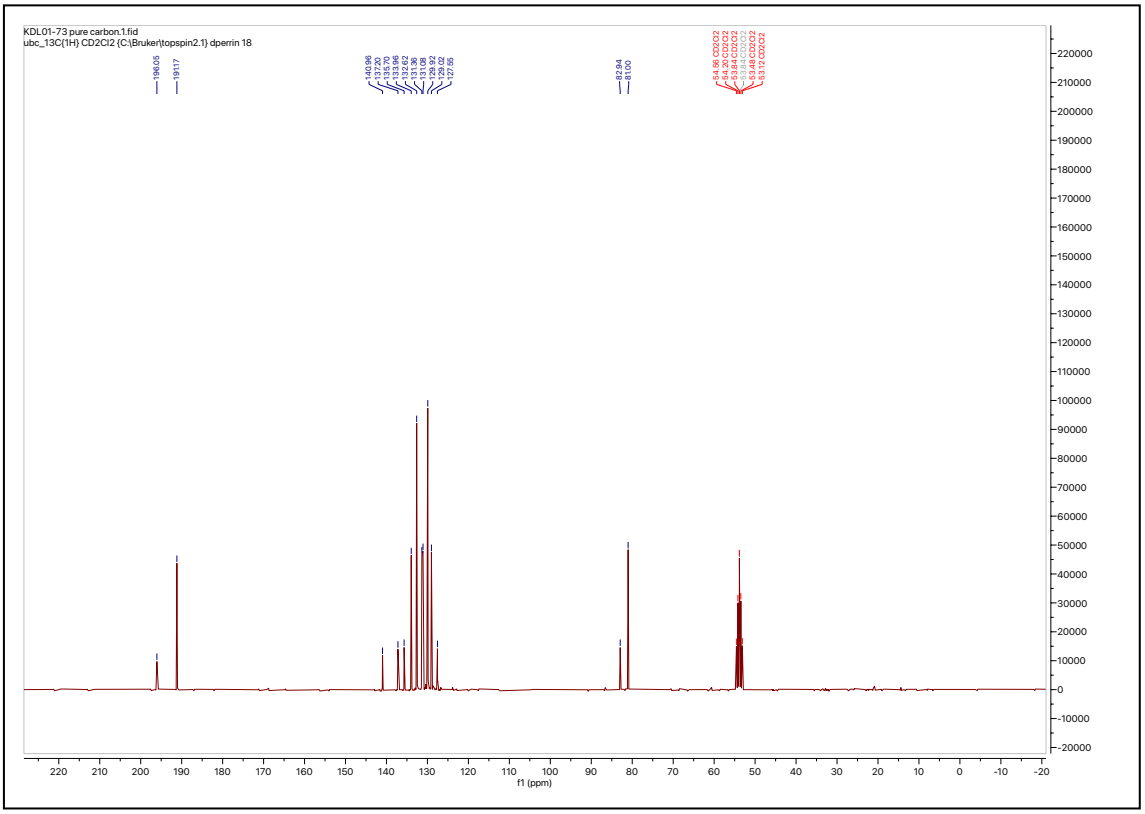
2-(4-azidobenzoyl)benzaldehyde (6ac)



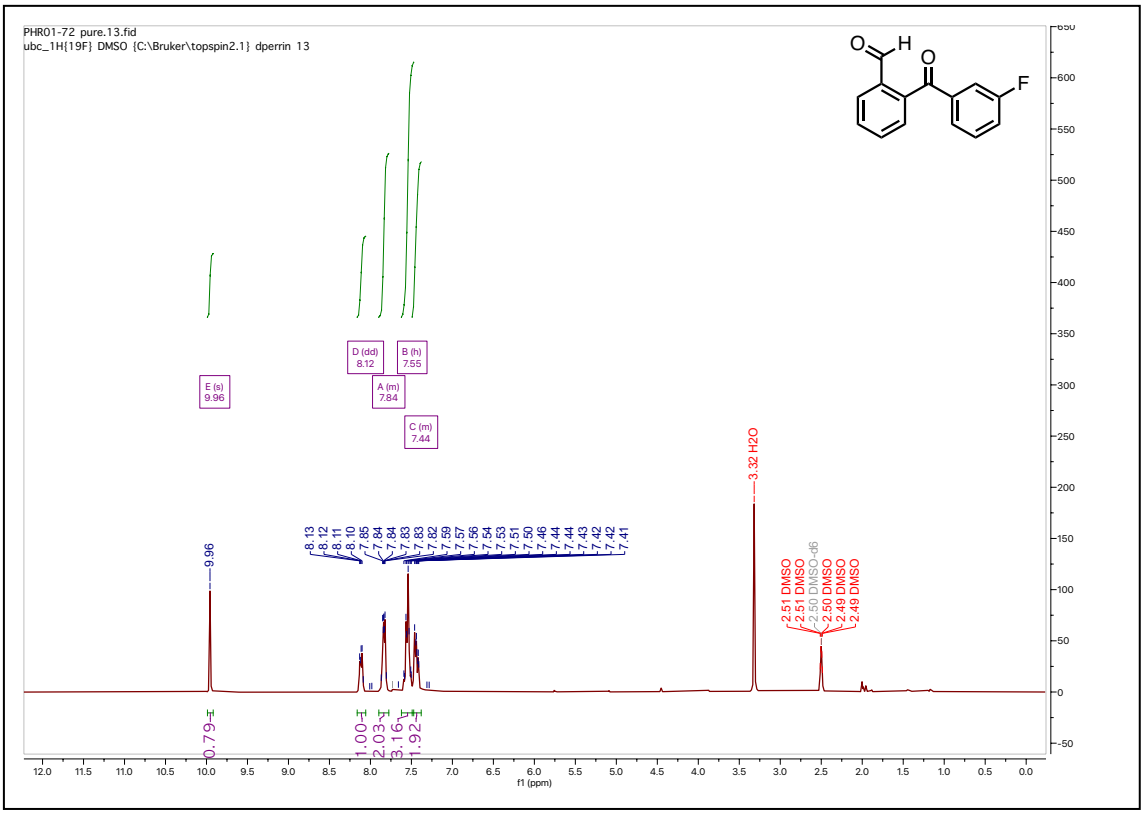


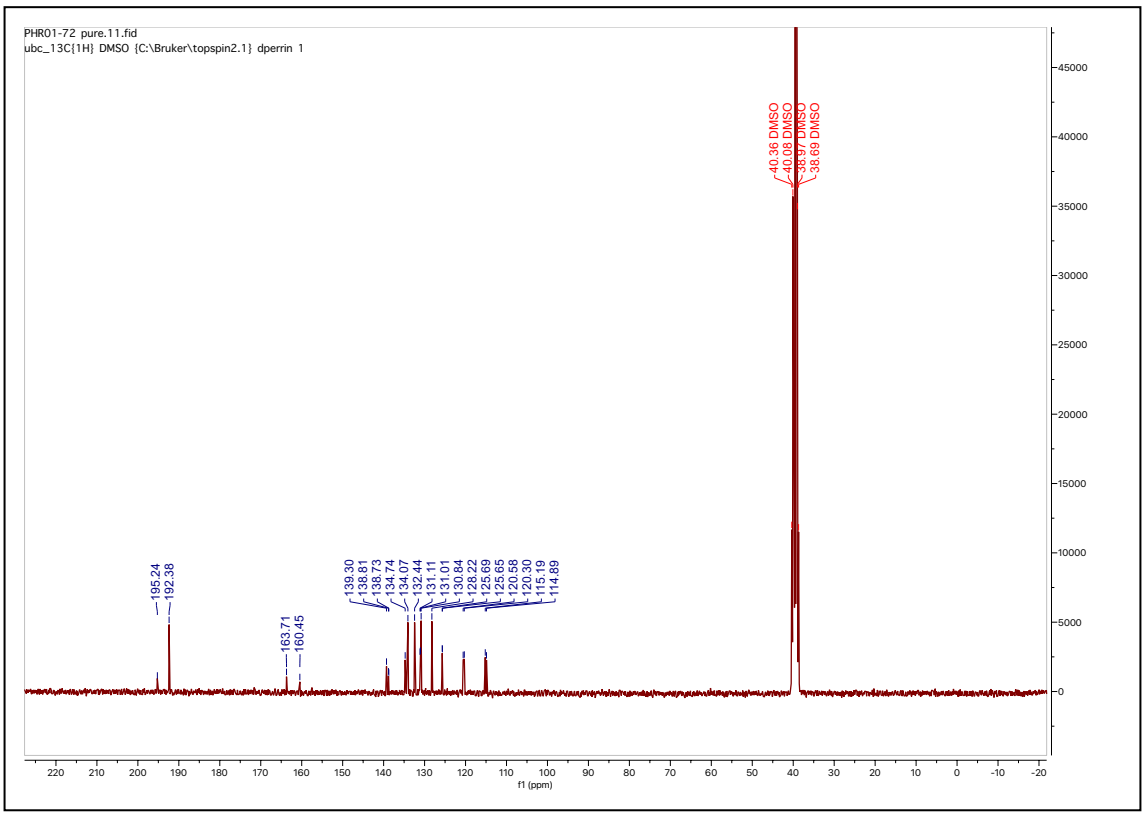
2-(4-ethynylbenzoyl)benzaldehyde (6ad)



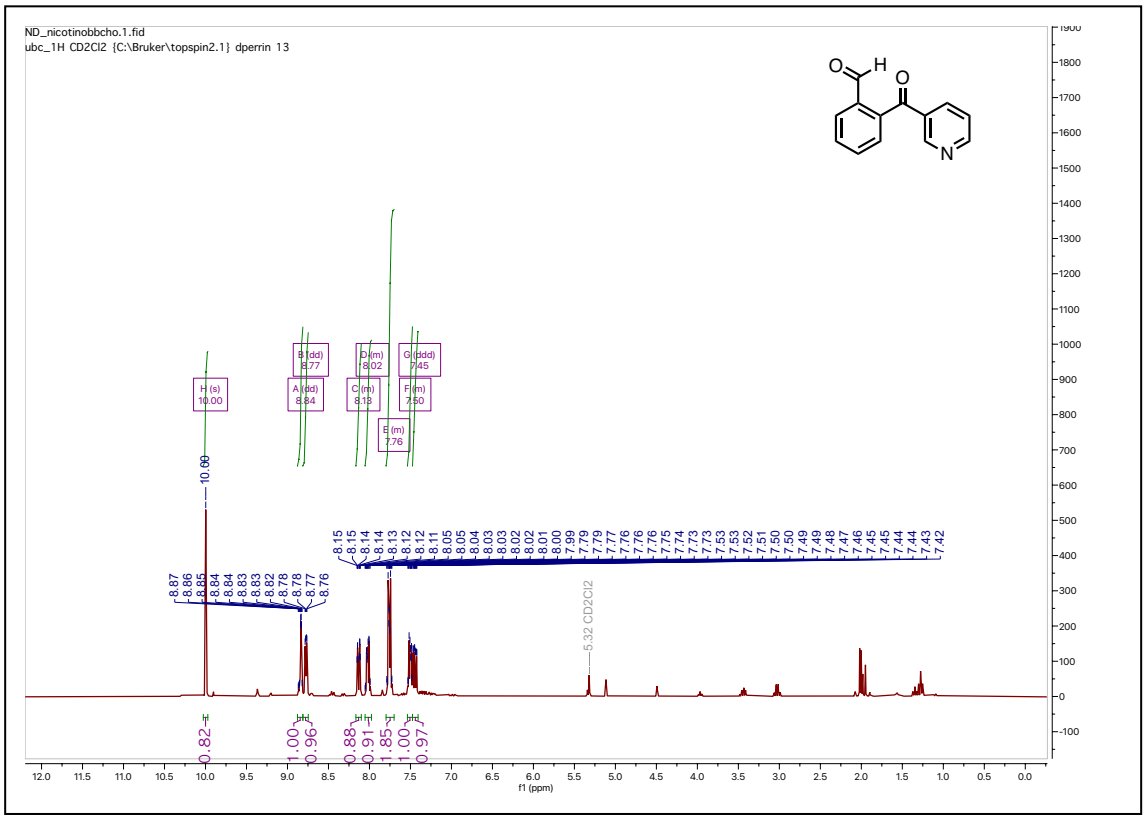


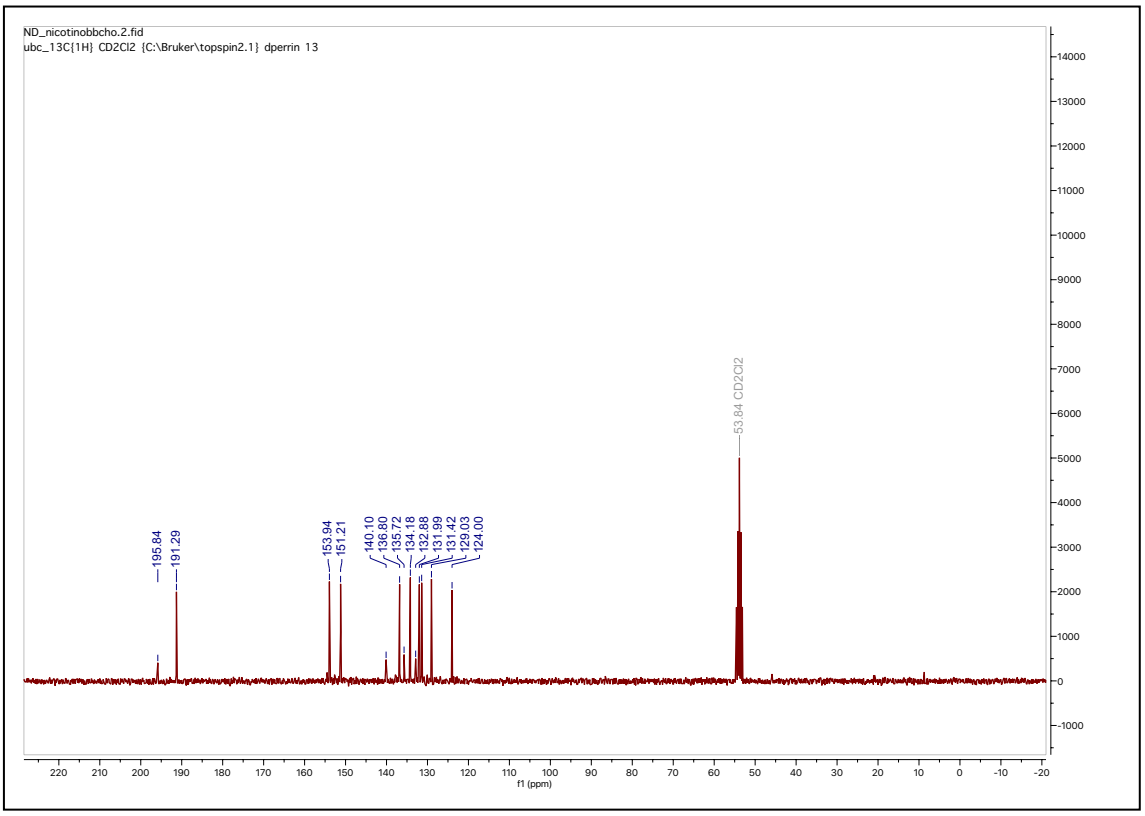
2-(3-fluorobenzoyl)benzaldehyde (6ae)



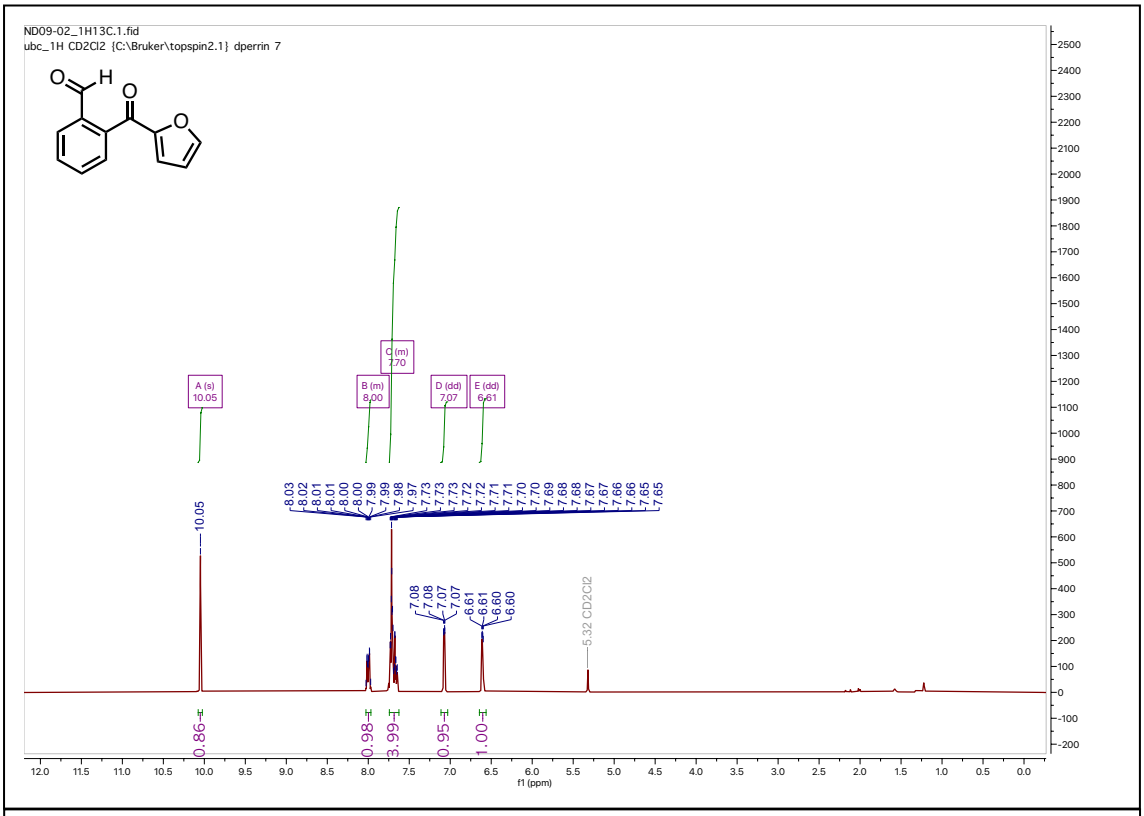


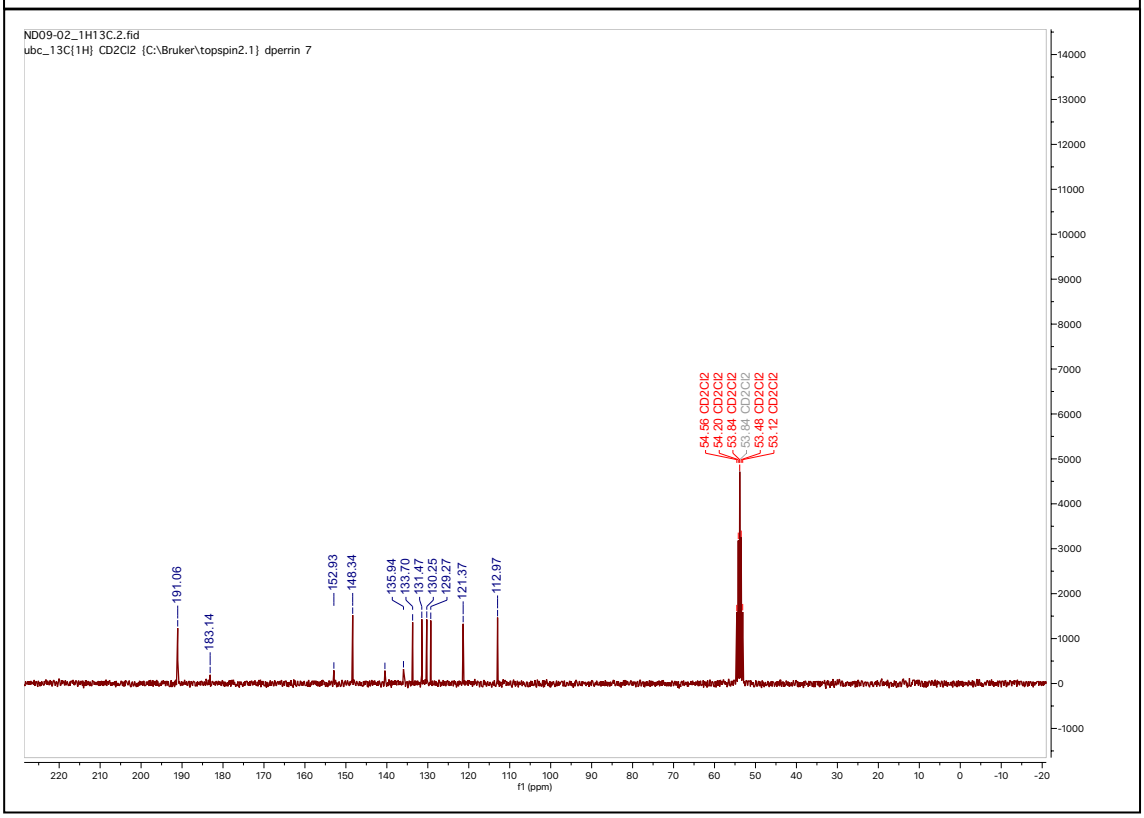
2-nicotinoylbenzaldehyde (6af)



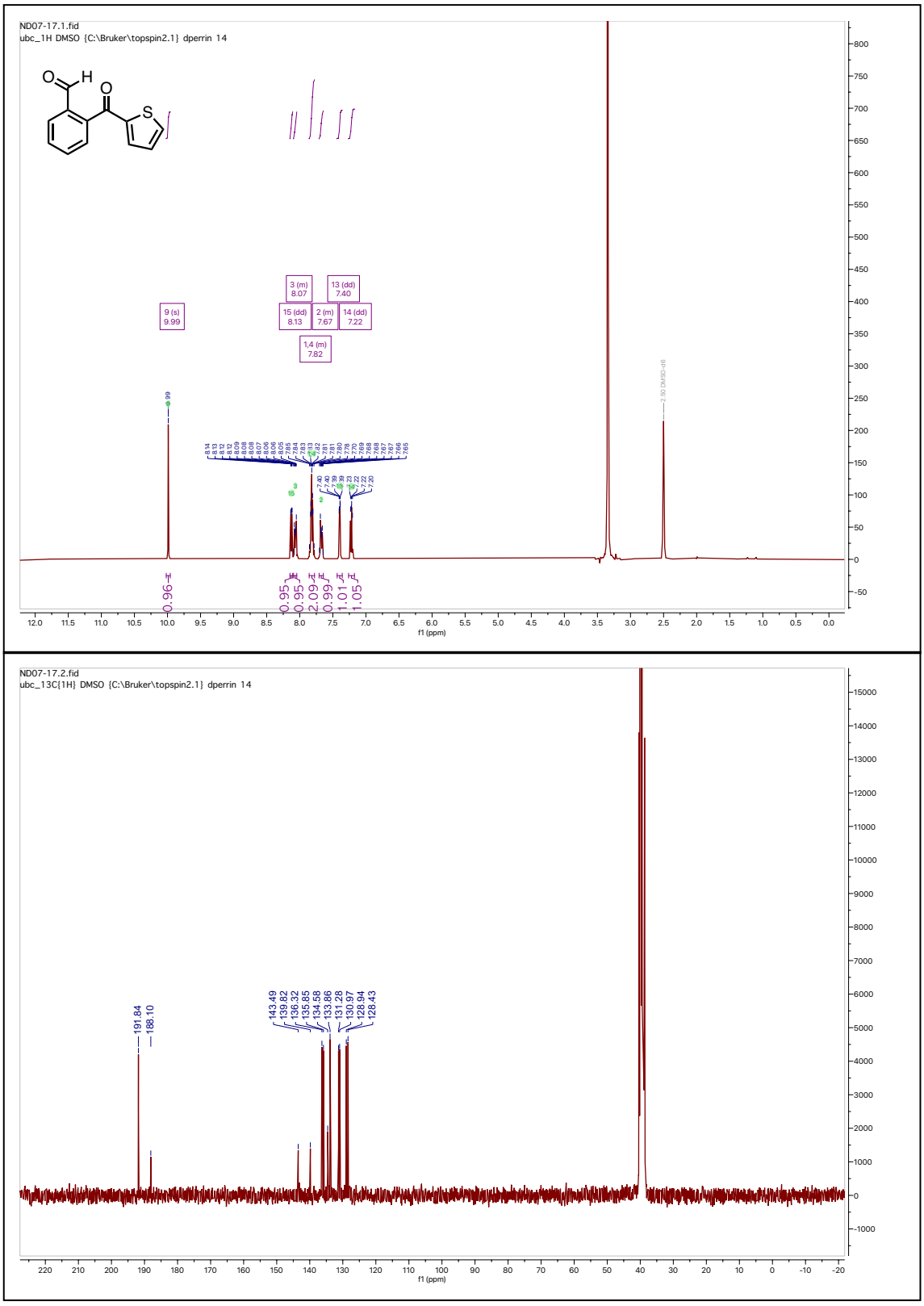


2-(furan-2-carbonyl)benzaldehyde (6ag)





2-(thiophene-2-carbonyl)benzadehyde (6ah)



2-(1H-indole-3-carbonyl)benzaldehyde (6ai)