ChemComm

SUPPLEMENTARY INFORMATION

One-pot, chemoselective desulfurative functionalization of cysteine containing peptides using pyridinium salts

Jeroen W. van den Heuvel, Esther Olaniran Håkansson, Bobo Skillinghaug, Luke R. Odell*

Department of Medicinal Chemistry, Uppsala Biomedical Center, Uppsala University, P. O. Box 574, SE-751 23 Uppsala, Sweden.
***email:** luke.odell@ilk.uu.se

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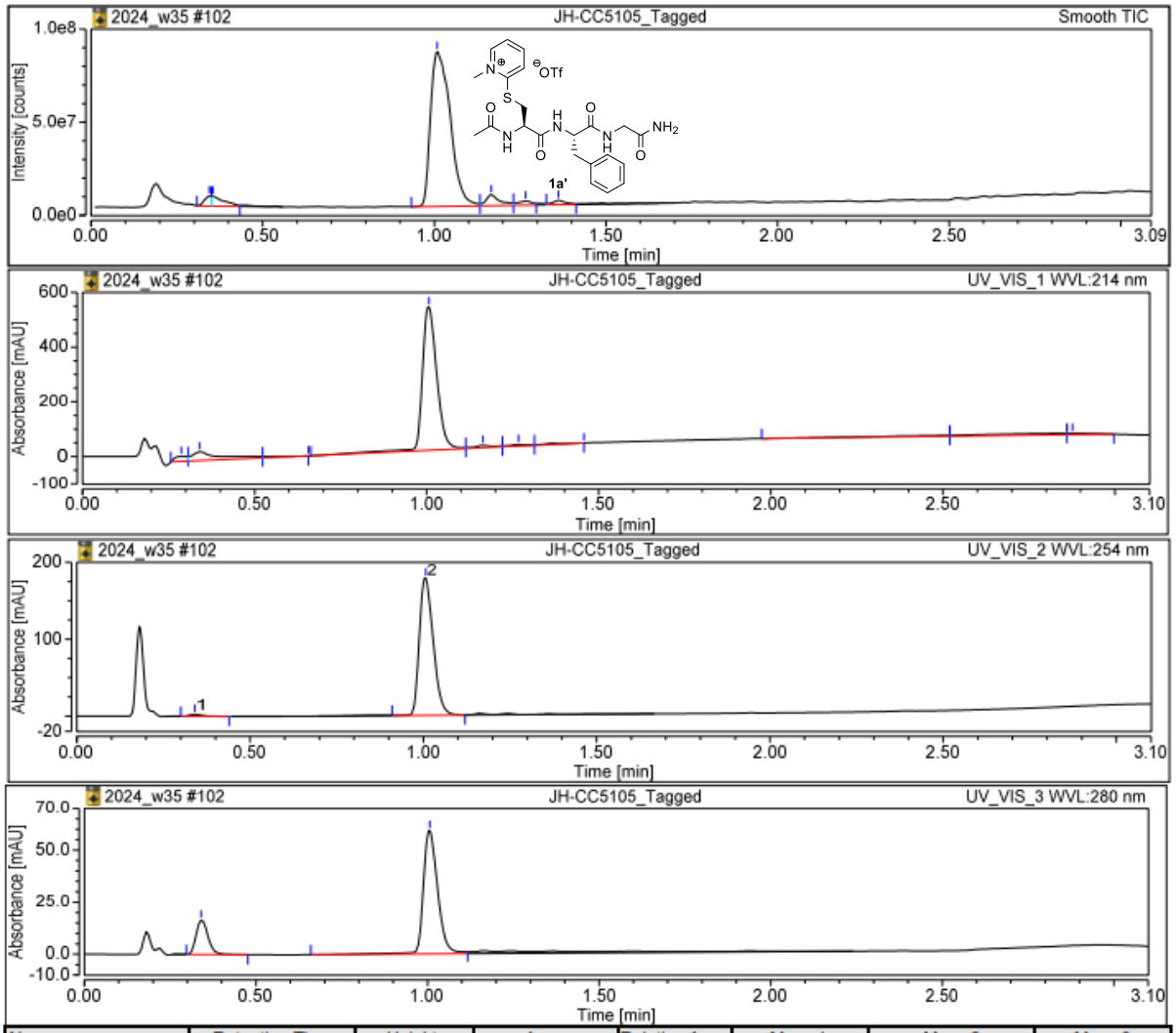
1. Supplementary figures and tables

Optimization of reactions conditions

Tagging of cysteine containing peptide 1a

Mixing **1a** and **2a** resulted in the formation of tagged intermediate **1a'** (see Figure S1).

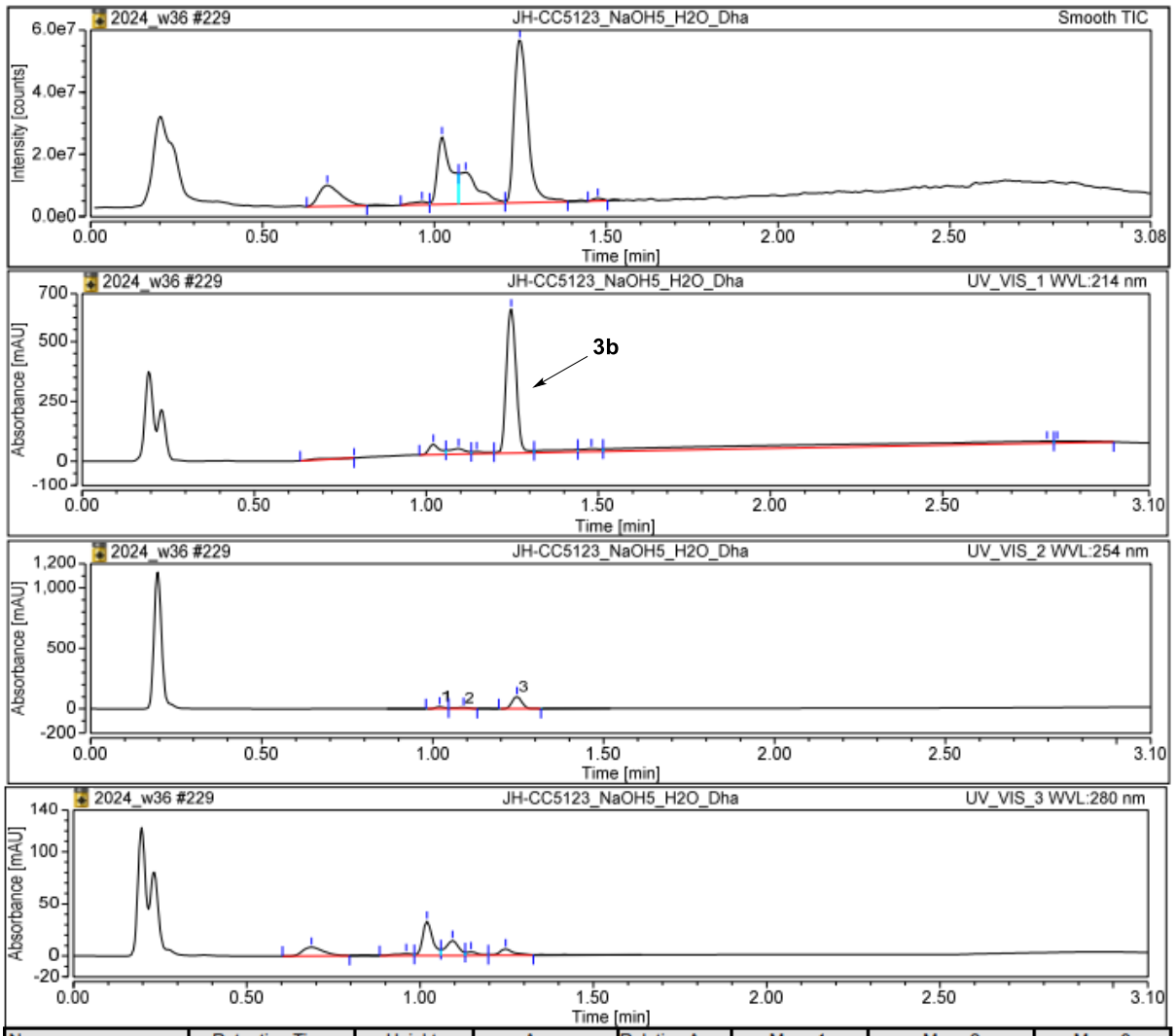


No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.340	2.554	0.116	1.38	110.1	111.3	101.2
2	1.007	178.700	8.283	98.62	459.2	460.3	461.3
Total:		**181.254**	**8.399**	**100.00**			

Figure S1 Analytical HPLC chromatograms of the pyridinium tagged intermediate **1a'** in DMF, 5-100% ACN + 0.05% formic acid in 3 minutes.

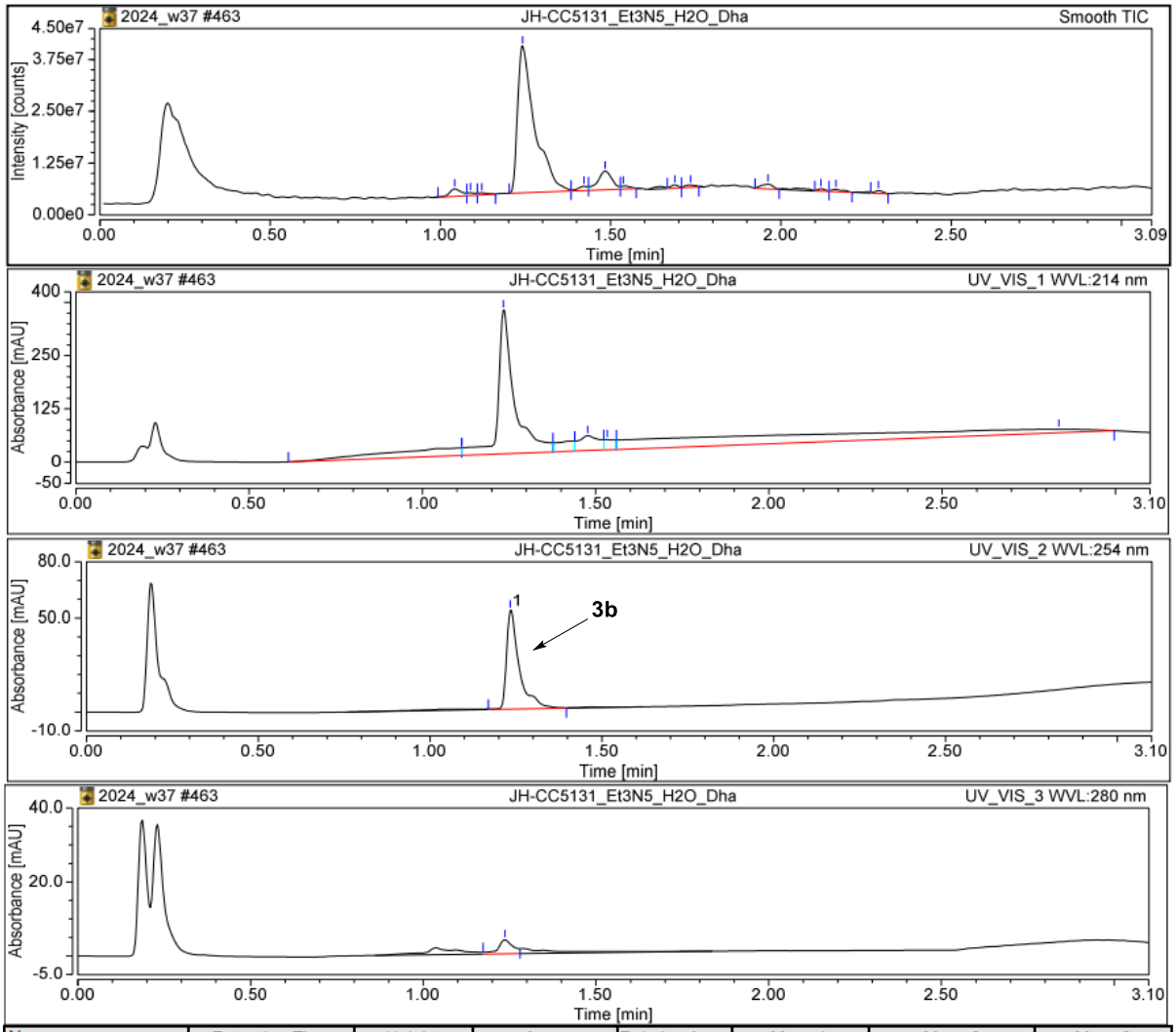
Elimination reaction of tagged cysteine containing peptide 1b'

Treatment of the tagged cysteine containing peptide **1b'** with base led to elimination to form the Dha product **3b**. Both NaOH (Figure S10) and Et_3N (Figure S11) could be employed.



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.020	14.782	0.464	11.02	383.2	384.3	101.3
2	1.090	8.474	0.513	12.17	425.2	236.2	426.3
3	1.247	98.385	3.237	76.81	334.2	223.2	356.2
Total:		**121.641**	**4.214**	**100.00**			

Figure S2 Analytical HPLC chromatograms of the reaction mixture of the synthesis of **3b** with NaOH (5 equiv.), 5-100% ACN + 0.05% formic acid in 3 minutes.



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.233	52.706	2.274	100.00	334.2	356.2	223.2
Total:		**52.706**	**2.274**	**100.00**			

Figure S3 Analytical HPLC chromatograms of the reaction mixture of the synthesis of **3b** with Et_3N (5 equiv.), 5-100% ACN + 0.05% formic acid in 3 minutes.

Experiments in deuterated water

Performing the reaction in deuterated buffer and analyzing the reaction mixture by ^{1}H NMR demonstrates robust formation of the Dha intermediate **3b** and subsequent reaction with piperidine to form **6b**.

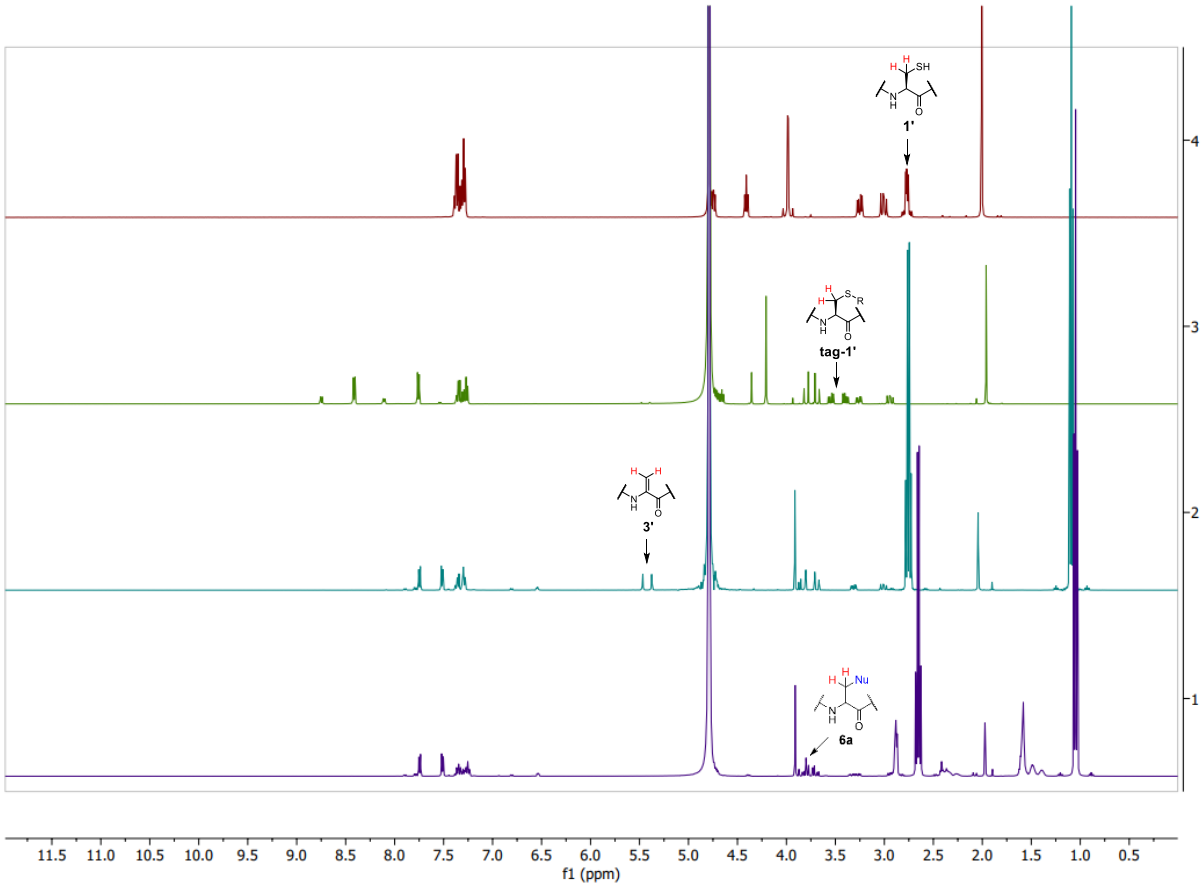
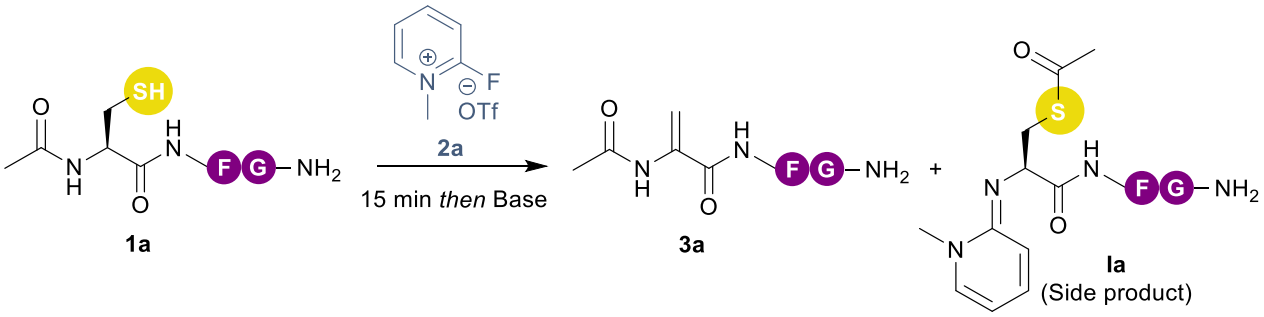


Figure S4 Stacked ^{1}H NMR (400 MHz) of compound **1b**, **1b'** (tagged intermediate), **3b** and **6a**. Reaction was performed in D_2O (50 mM PB, pH = 9.0).

Screening of bases

Although the generation of Dha product **3b** was successful, the conditions for the two-step process required optimization to prevent formation of side product **I**.

Table S1 Screening of bases in the formation of the Dha intermediate **3**.[a]

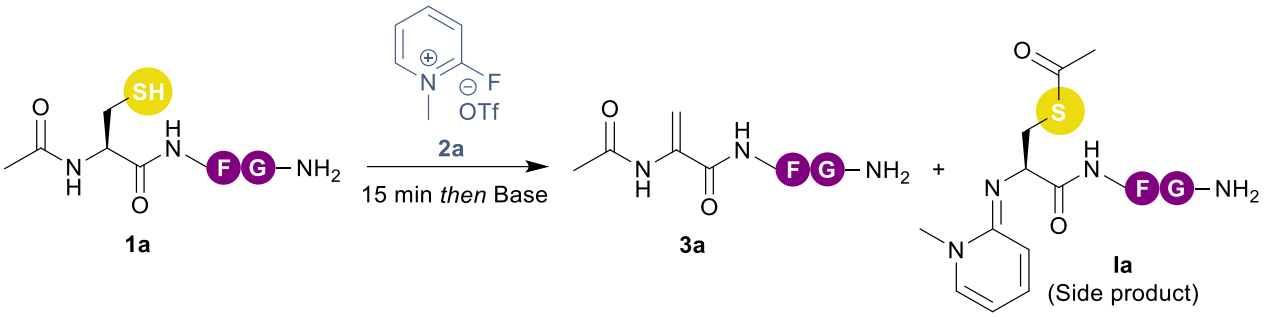


Entry	Solvent	Base	Temperature, Time	Conversion[b]
1	DMF	DIPEA	20 °C, 16 h	0%
2	DMF	DIPEA	60 °C, 16 h	0%
3	DMF	Et_3N	20 °C, 16 h	0%
4	DMF	Et_3N	60 °C, 16 h	40%
5	DMF	Pyridine	20 °C, 16 h	0%
6	DMF	Pyridine	60 °C, 16 h	0%
7	DMF	DMAP	20 °C, 16 h	0%
8	DMF	DMAP	60 °C, 16 h	83%
9	DMF	DBN	20 °C, <0.5 h	37%
10	DMF	NaOH	20 °C, <0.5 h	0%

[a] Reaction conditions: **1** (0.025 mmol) and **2** (0.03 mmol) for 15 min followed by base (0.03 mmol), solvent (0.2 mL). [b] Conversion to **3a** based on the ratio of pyridinium tagged **1a** and compound **3a** determined by HPLC-UV analysis.

Screening of solvents

Table S2 Screening of solvents in the formation of the Dha intermediate **3**.[a]



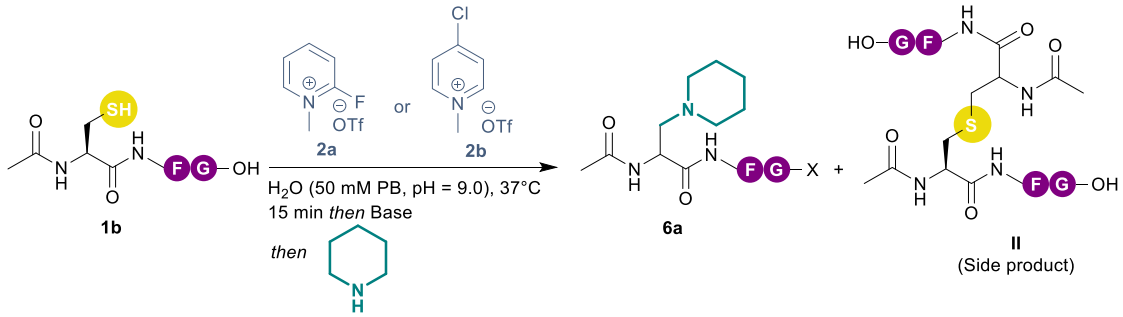
Entry	Solvent	Base	Temperature, Time	Conversion[b]
1	DMSO	DABCO	37 °C, 5 h	56%[c]
2	MeOH	DABCO	37 °C, 5 h	36%[d]
3	H_2O	DABCO	37 °C, 5 h	94%[d]
4	DMA	DABCO	37 °C, 5 h	78%
5	ACN	DABCO	37 °C, 2h	>99%[d]

[a] Reaction conditions: **1** (0.025 mmol) and **2** (0.03 mmol) for 15 min followed by base (0.03 mmol), solvent (0.2 mL).
[b] Conversion to **3a** based on the ratio of pyridinium tagged **1a** and compound **3a** determined by HPLC-UV analysis.
[c] Side-product formation was observed. [d] Precipitation was observed.

Investigation of tagging agents

The reaction was further optimized by exploring a different pyridinium salt as tagging agent (see Table S3). The use of **2b** resulted in a higher yield of **6a**.

Table S3 Optimization of tagging agent **2** and stoichiometry for the three-step one-pot synthesis of **6a**[a].



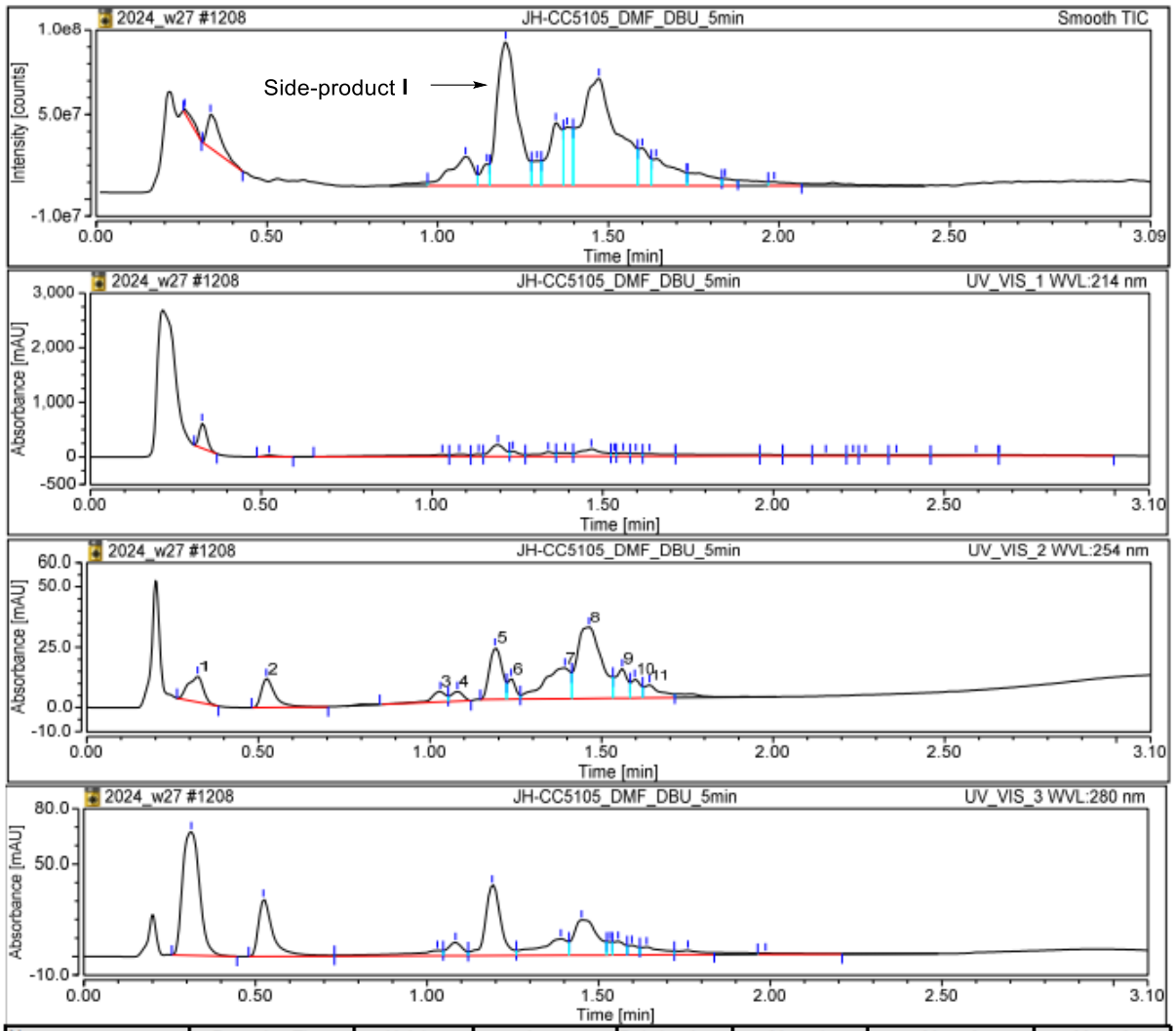
Entry	Pyridinium salt	Base (equiv.)	Yield 6a[b] (%)	Yield II (%)
1	**2a**	NaOH (10)	40	26
2	**2a**	NaOH (5)	42	23
3	**2b**	NaOH (5)	56	0
4	**2b**	Et_3N (5)	69	0

[a] Reaction conditions: 1 (0.1 mmol) and 2 or 5 (0.12 mmol) in H_2O (50 mM PB, pH = 9.0)(0.2 mL) for 15 min at 37°C, base, piperidine (0.3 mmol). [b] Isolated yield as TFA salt and a mixture of diastereomers.

Side-product I

During base-mediated elimination of the **2a**-tagged thiol group to form Dha intermediate **3**, competing formation of side product **I** was consistently observed (see Figure S5).

Analytical HPLC chromatograms of side product I



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.323	10.737	0.539	7.58	153.2	110.1	154.3
2	0.523	11.981	0.554	7.78	115.1	153.2	126.1
3	1.030	4.353	0.217	3.05	459.2	460.3	254.7
4	1.080	3.966	0.158	2.22	262.2	425.2	263.3
5	1.190	21.216	0.901	12.65	459.2	460.3	461.3
6	1.237	8.521	0.228	3.20	459.3	460.3	461.3
7	1.393	12.702	1.131	15.88	342.0	498.3	153.2
8	1.463	29.549	2.372	33.33	532.3	342.0	533.3
9	1.560	12.176	0.479	6.72	341.8	407.3	700.4
10	1.597	7.701	0.241	3.39	483.2	341.9	101.2
11	1.640	5.396	0.298	4.19	101.1	153.2	341.7
Total:		**128.299**	**7.118**	**100.00**			

Figure S5 Analytical HPLC chromatograms of side-product **I** in the reaction mixture in DMF, 5-100% ACN + 0.05% formic acid in 3 minutes.

NMR spectra for side product I

Side product **I** could be isolated and characterized by NMR.

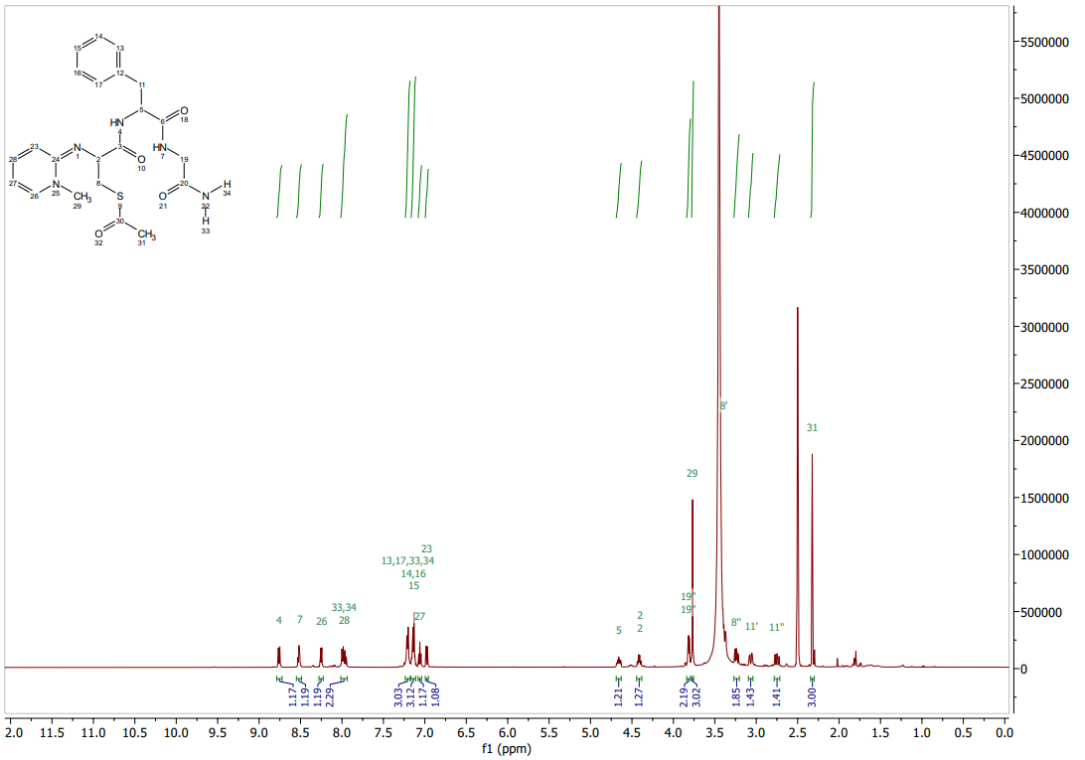


Figure S6 ^{1}H NMR (400 MHz) of side product **I**.

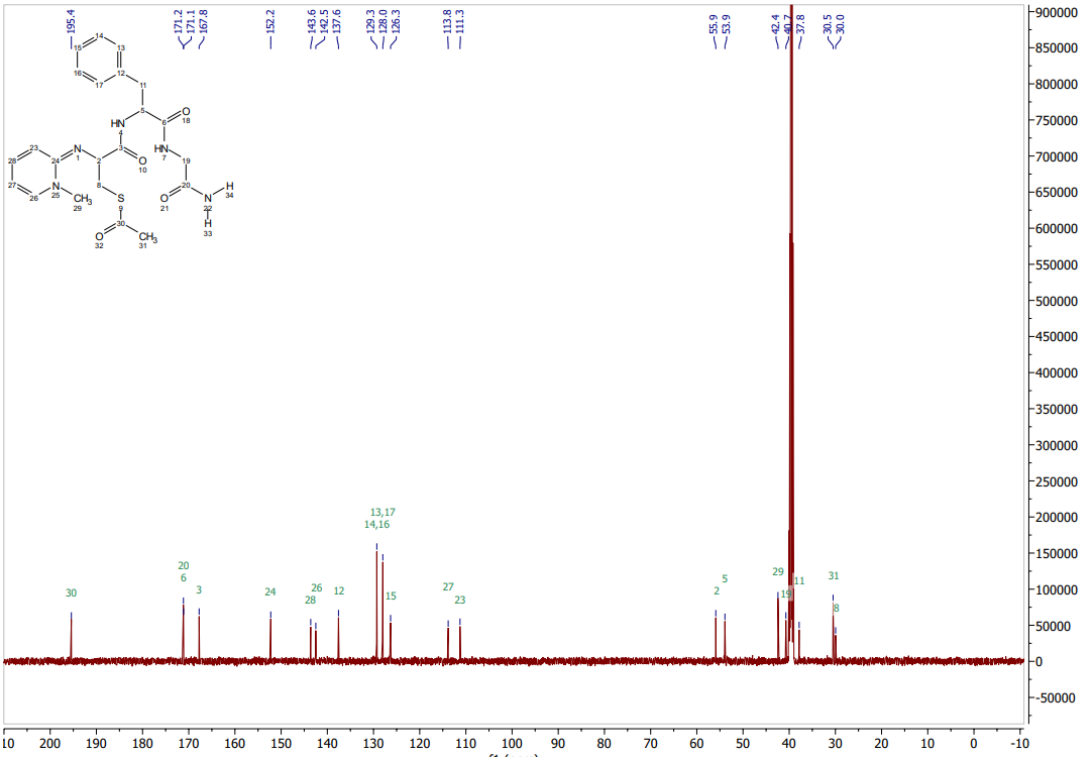
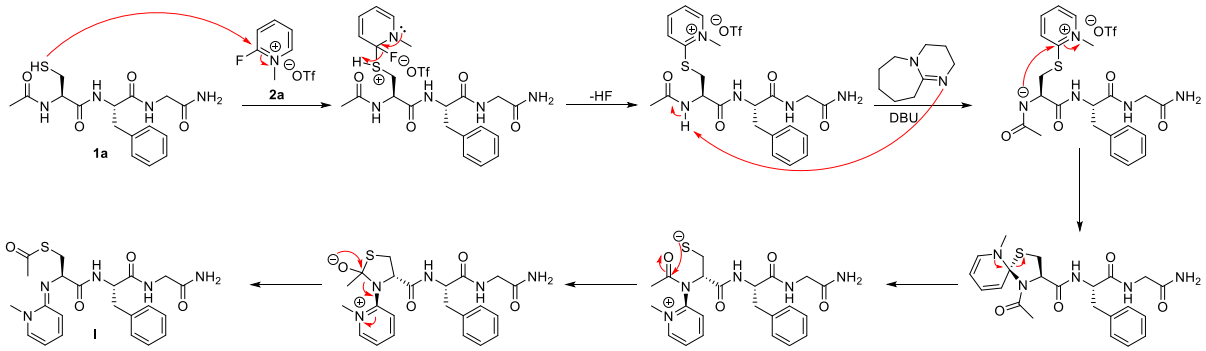


Figure S7 ^{13}C NMR (126 MHz) of side product **I**.

Proposed mechanism of the formation of side product I

Side product **I** likely forms through the proposed mechanism in Scheme S1.

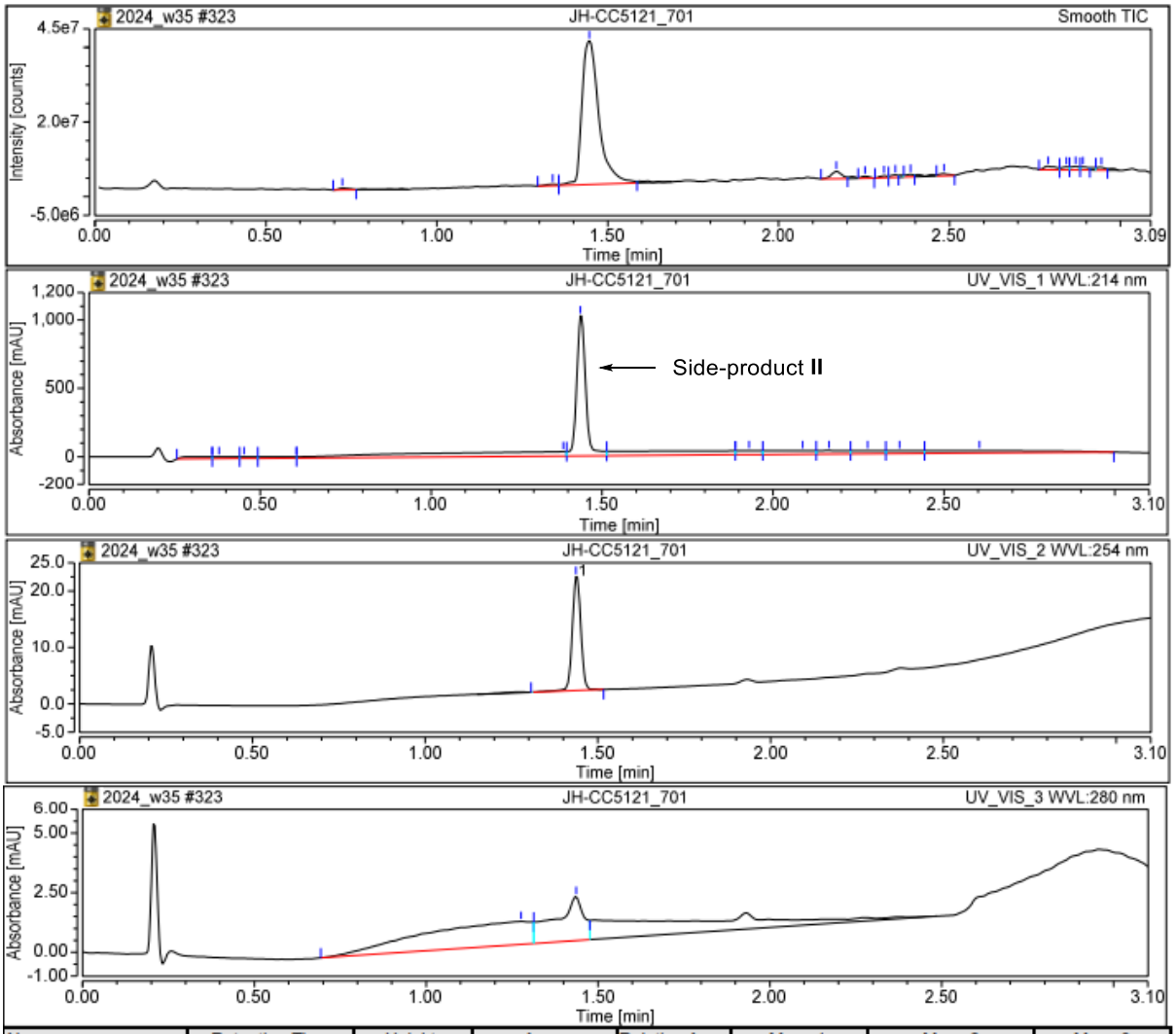


Scheme S1 Proposed mechanism for the formation of side-product **I**.

Side Product II

During two-step functionalization of **1a** with **2b** and piperidine, formation of side product **II** was observed. This side product could be isolated and characterized.

Analytical HPLC chromatograms for side product II



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.437	20.179	0.581	100.00	701.3	702.4	479.2
Total:		**20.179**	**0.581**	**100.00**			

Figure S8 Analytical HPLC chromatograms of purified side-product **II**, 5-100% ACN + 0.05% formic acid in 3 minutes.

NMR spectra for side product II

Side product **I** could be isolated and characterized by NMR.

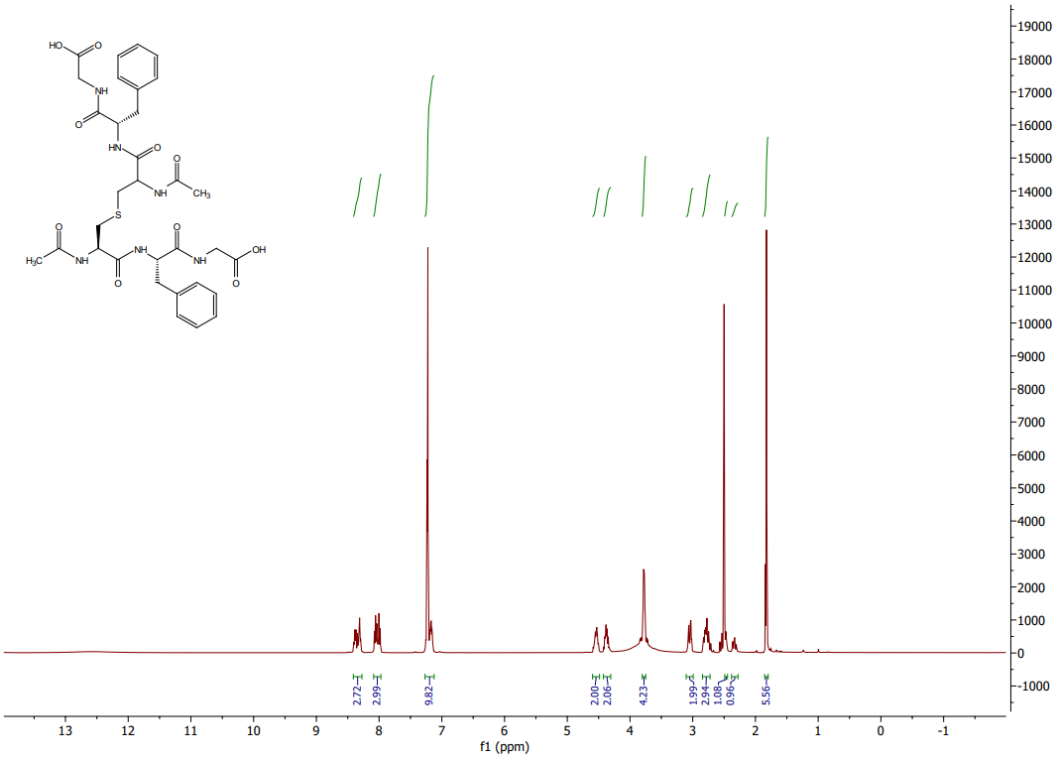


Figure S9 ^{1}H NMR (400 MHz) of side-product **II**.

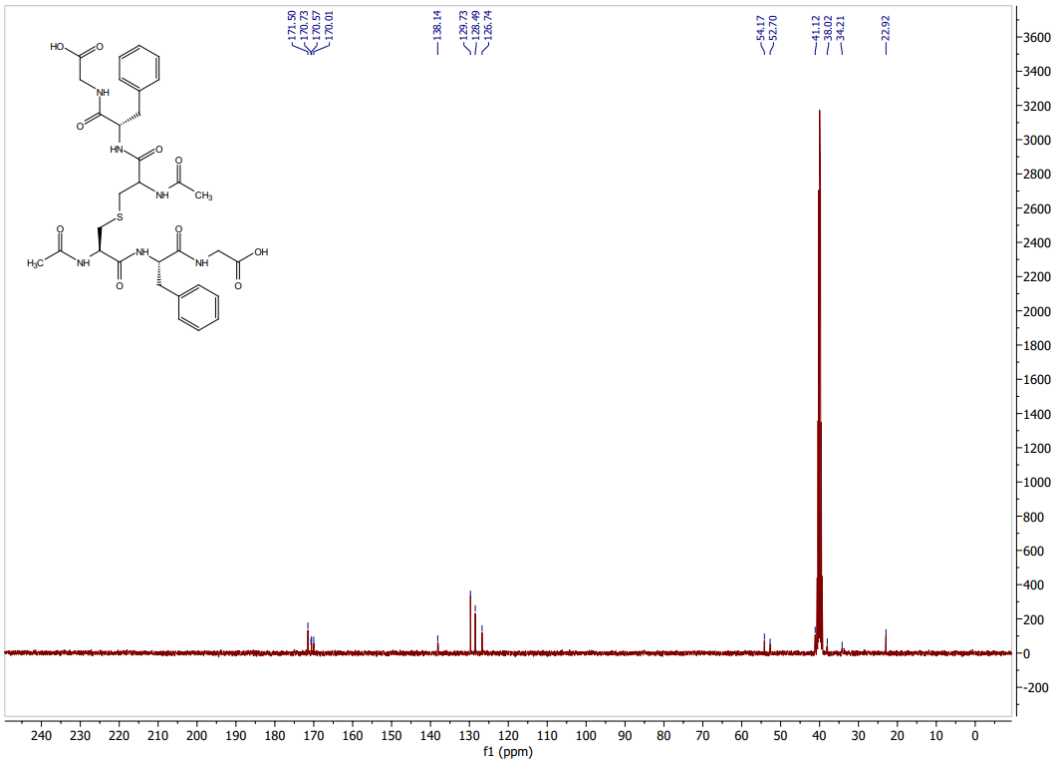
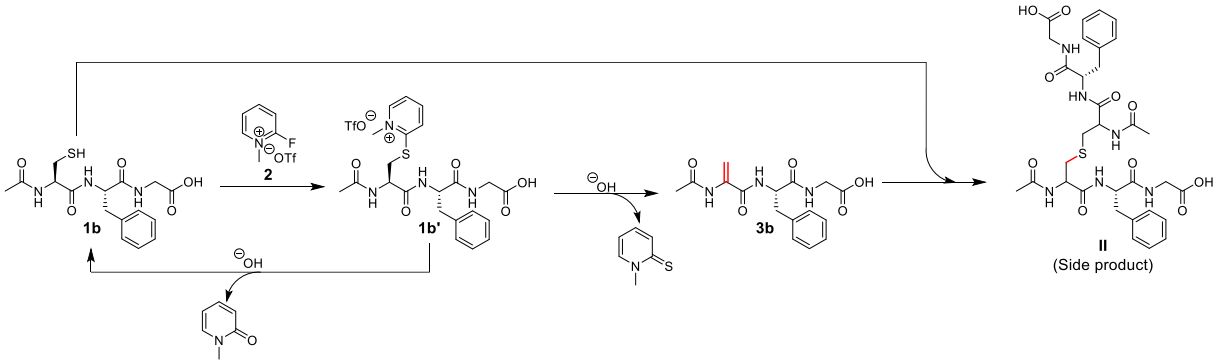


Figure S10 ^{13}C NMR (126 MHz) of side-product **II**.

Proposed mechanism for the formation of side product II

Side product **II** likely forms through the proposed mechanism in Scheme S2.

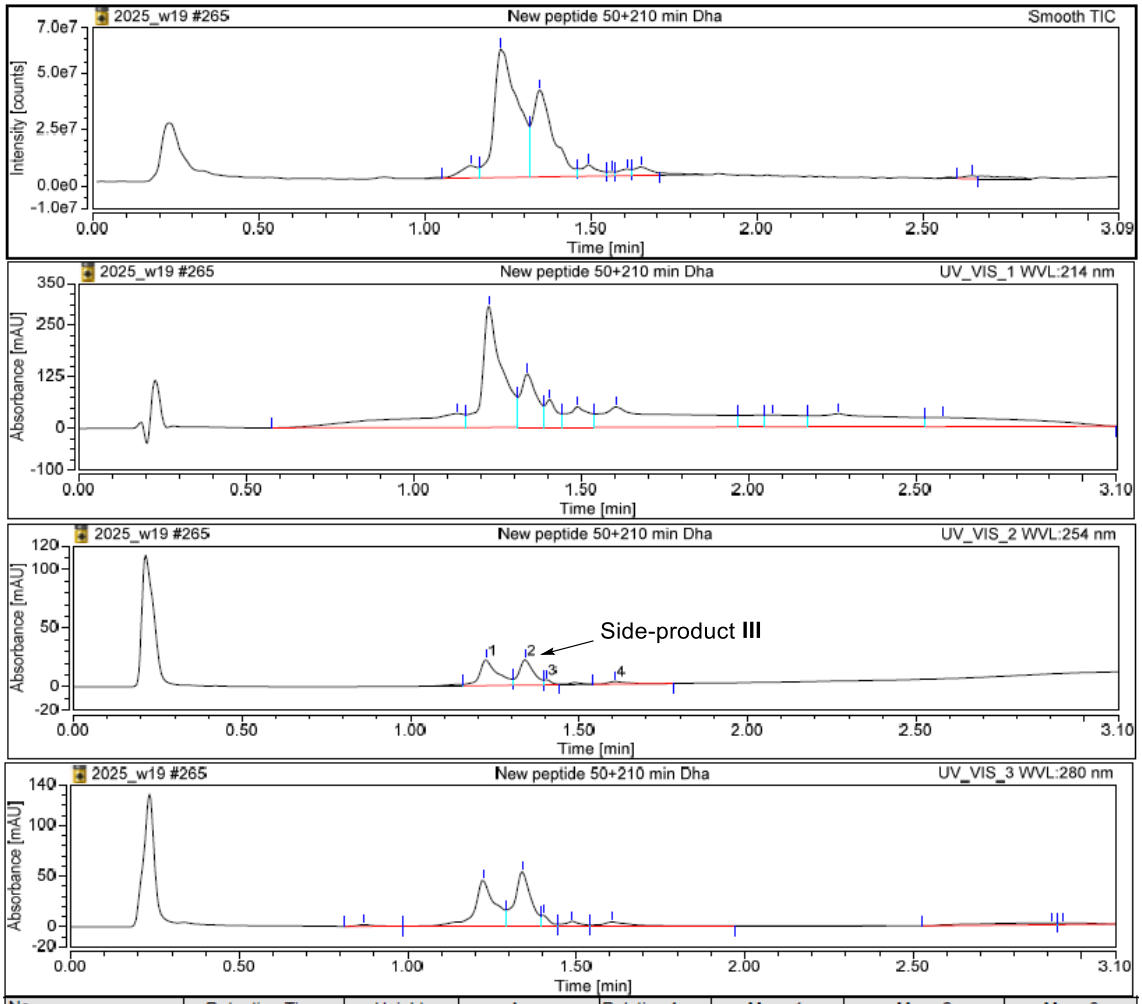


Scheme S2 Proposed mechanism for the formation of side-product **II**. After tagging of compound **1b** and elimination to Dha **3b**, **1b** and **3b** can then combine to form side-product **II**. **1b'** can also be detagged back to compound **1b**.

Side product III and IV

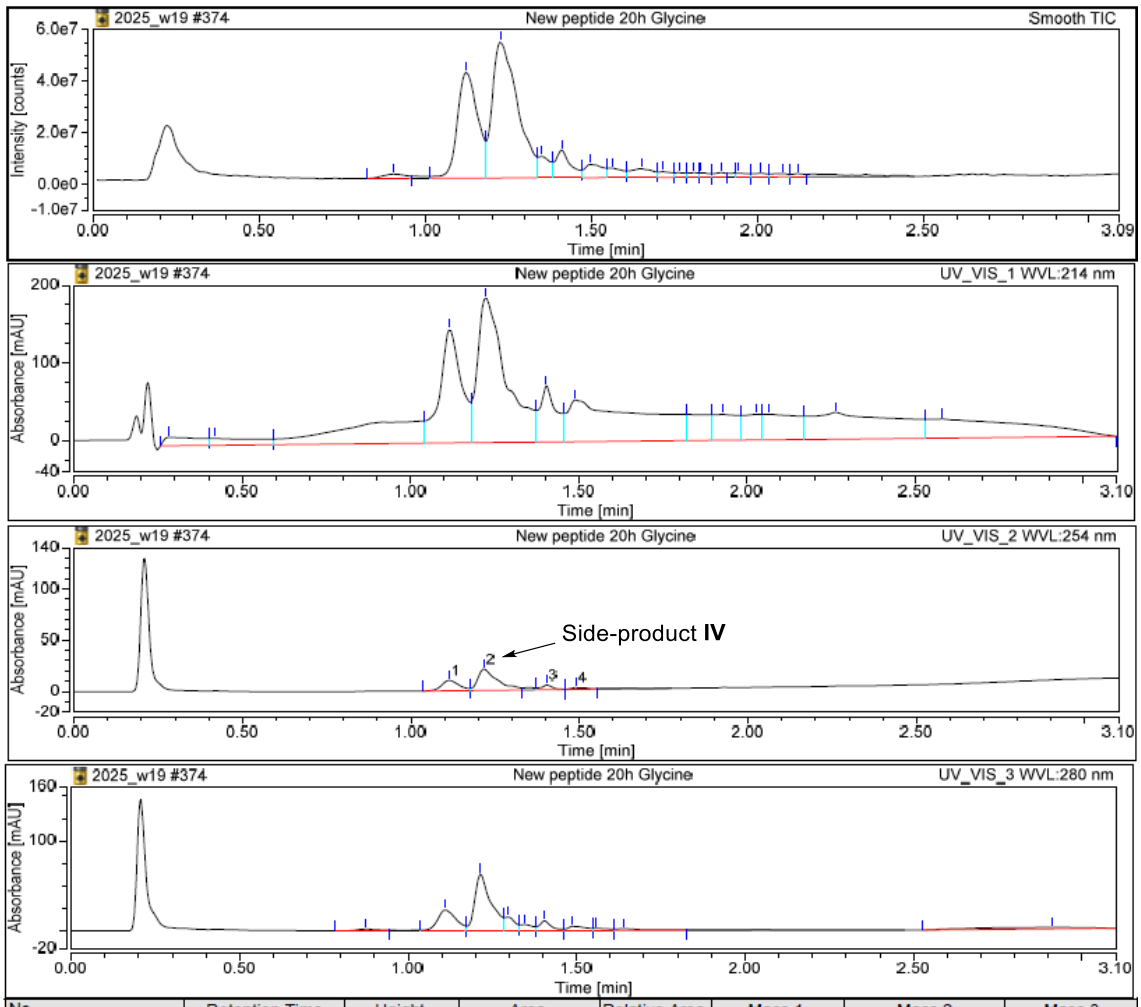
Side product **III** and **IV** were formed during elimination of the tagged intermediate corresponding to peptide **1d** and subsequent functionalization with glycine, respectively.

Analytical HPLC chromatograms of side products III and IV



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.223	21.926	1.352	50.27	480.9	482.4	960.4
2	1.340	21.648	1.063	39.53	526.4	102.4	480.6
3	1.403	4.459	0.120	4.46	526.5	102.4	399.3
4	1.607	2.311	0.154	5.74	102.4	101.3	571.6
Total:		**50.344**	**2.688**	**100.00**			

Figure S11 Analytical HPLC chromatograms of reaction mixture during Et_3N-mediated elimination containing side-product **III**, 5-100% ACN + 0.05% formic acid in 3 minutes.

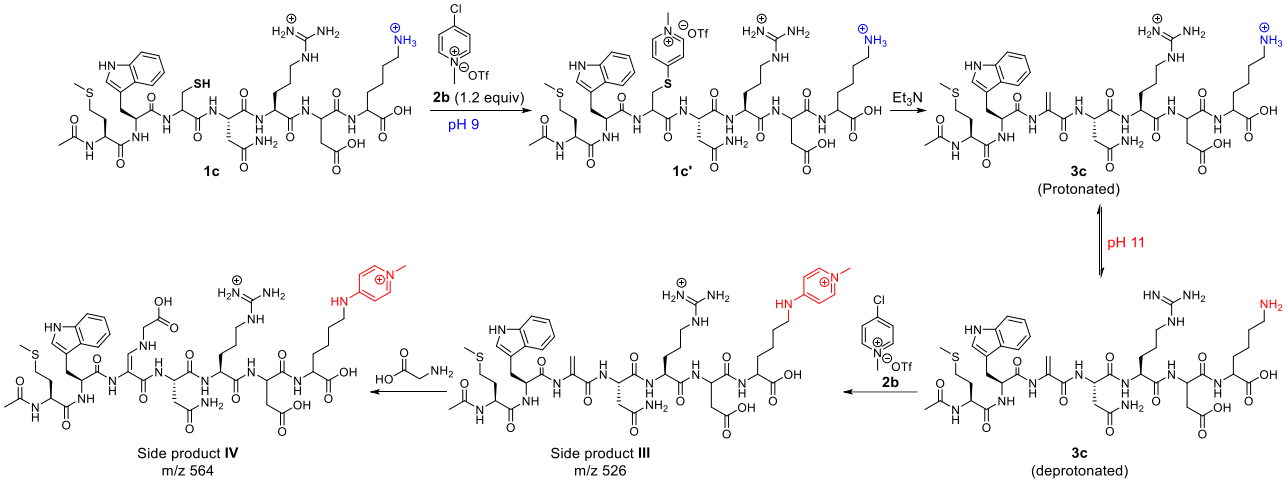


No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.113	10.012	0.603	26.55	518.4	346.0	431.7
2	1.217	20.904	1.386	61.04	564.0	480.9	376.1
3	1.403	4.583	0.179	7.88	526.2	102.5	377.1
4	1.490	1.746	0.103	4.52	102.4	101.4	378.2
Total:		**37.245**	**2.271**	**100.00**			

Figure S12 Analytical HPLC chromatograms of reaction mixture during Michael addition of glycine containing side-product **IV**, 5-100% ACN + 0.05% formic acid in 3 minutes.

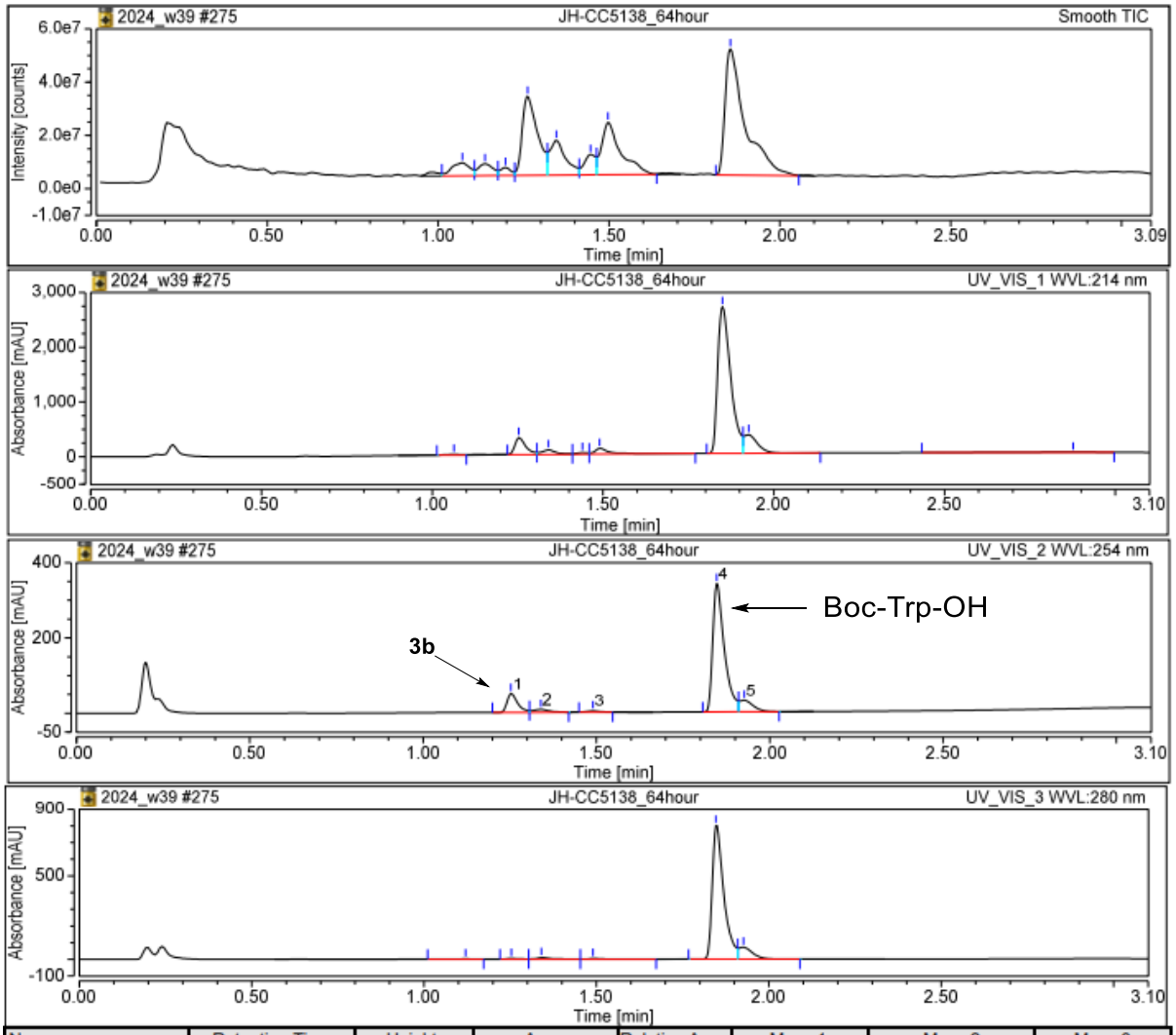
Proposed mechanism for the formation of side product III and IV

When peptide **1d** was used as the substrate, side product **III** with m/z 526 was observed by LC-MS during elimination of the tagged thiol group. This side product was not observed during the tagging reaction (at pH 9) and is likely formed by reaction of the free lysine residue with excess **2b** (Scheme S3). This side product can then undergo addition of glycine to form side product **IV**, which was observed by LCMS (m/z 564).



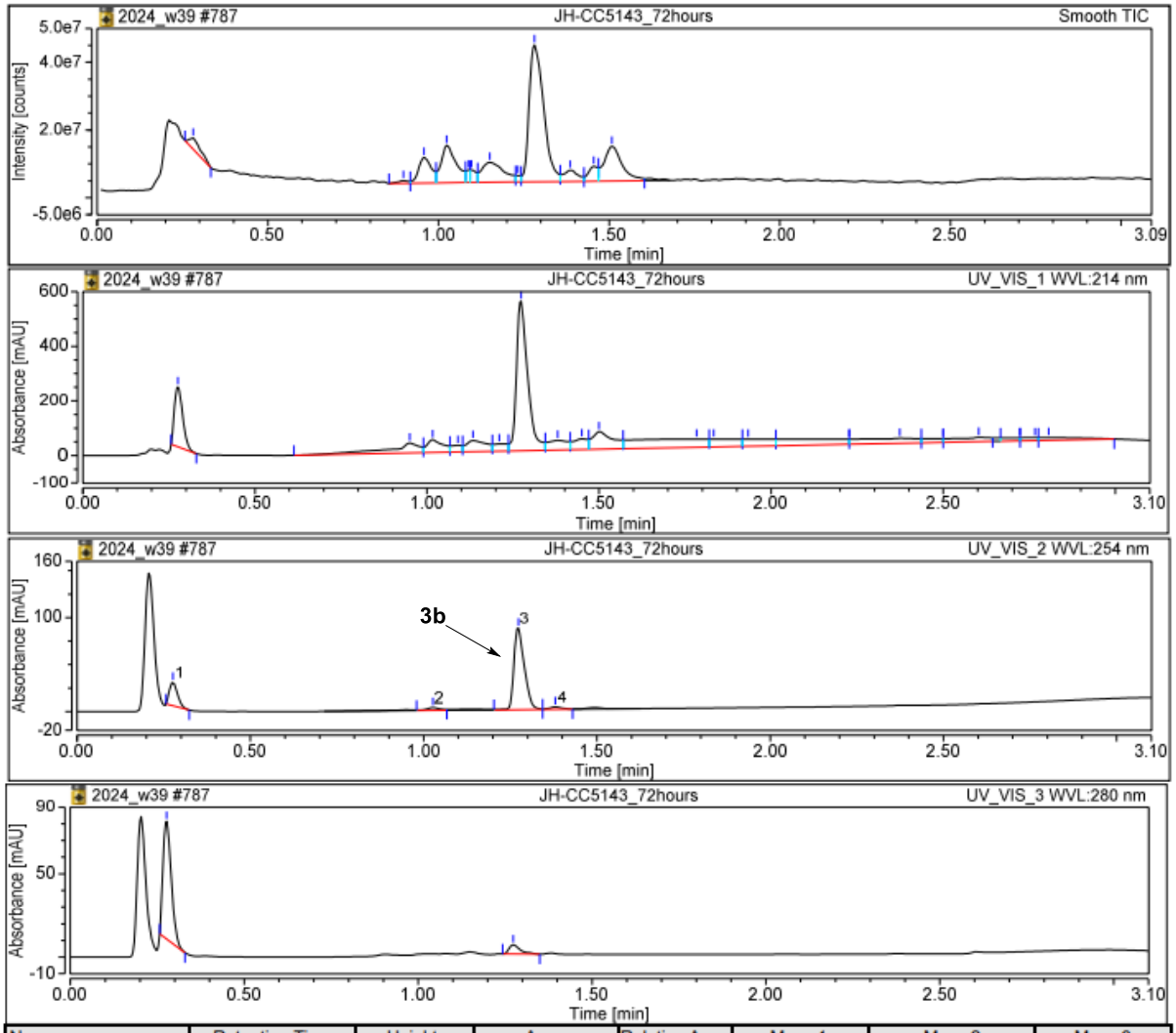
Scheme S3 *Proposed mechanism for the formation of side-product **III** and **IV**. At pH 11,* the free lysine redidue can react with excess **2b** to form side-product **III** *and subsequent addition of glycine forms side-product **IV**.*

Attempted functionalization with amino acids bearing poorly nucleophilic side chains



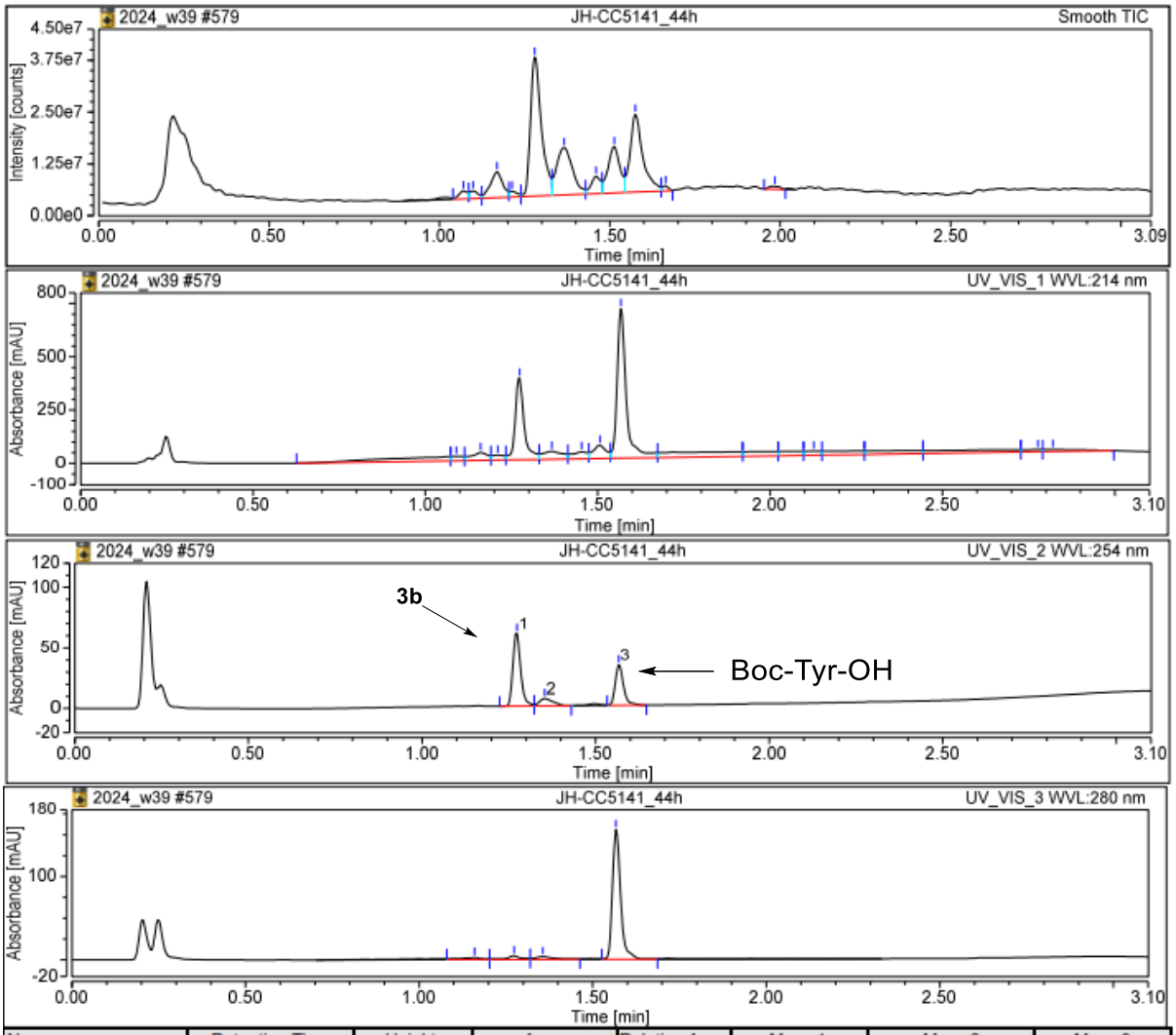
No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.253	50.791	1.788	10.54	334.3	223.2	356.2
2	1.340	8.021	0.442	2.60	538.4	539.4	102.3
3	1.490	4.037	0.178	1.05	567.3	479.3	102.3
4	1.847	341.221	13.142	77.42	205.4	249.2	204.9
5	1.927	30.869	1.424	8.39	249.2	205.2	246.2
Total:		**434.940**	**16.975**	**100.00**			

Figure S13 Analytical HPLC chromatograms after 64 hours of attempted functionalization of **3b** with tryptophan. No desired reaction was observed. 5-100% ACN + 0.05% formic acid in 3 minutes.



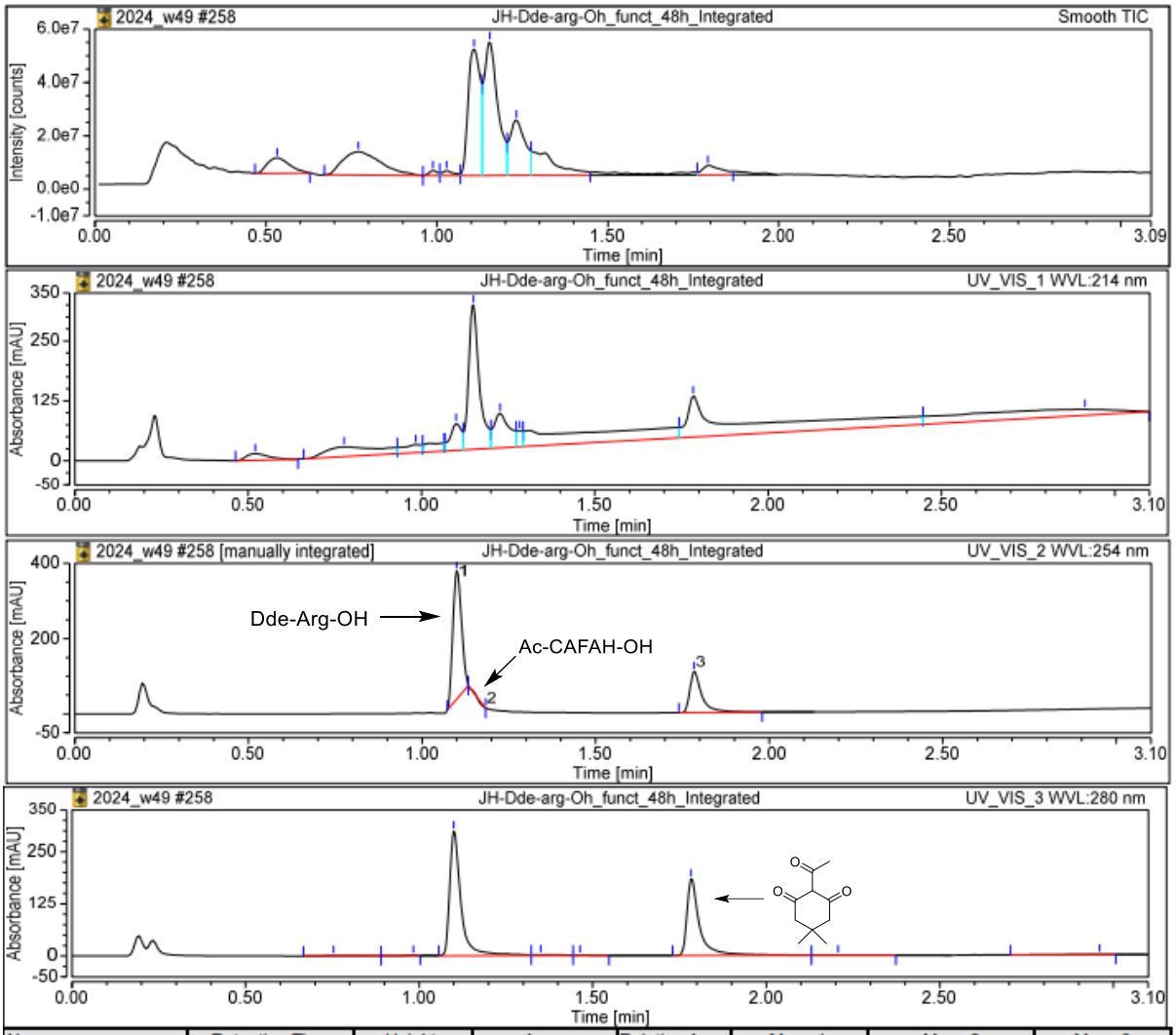
No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.277	25.093	0.683	17.26	102.4	126.2	101.8
2	1.027	2.994	0.138	3.48	439.3	102.3	440.4
3	1.273	86.821	3.019	76.30	334.3	356.3	223.5
4	1.380	2.681	0.117	2.96	102.3	101.3	350.2
Total:		**117.589**	**3.957**	**100.00**			

Figure S14 Analytical HPLC chromatograms after 72 hours of attempted functionalization of **3b** with serine. No desired reaction was observed. 5-100% ACN + 0.05% formic acid in 3 minutes.



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.273	59.954	1.569	56.87	334.2	223.2	356.2
2	1.353	5.984	0.277	10.04	373.3	102.3	374.3
3	1.567	33.449	0.913	33.09	182.2	223.2	102.2
Total:		**99.387**	**2.760**	**100.00**			

Figure S15 Analytical HPLC chromatograms after 44 hours of attempted functionalization of **3b** with tyrosine. No desired reaction was observed. 5-100% ACN + 0.05% formic acid in 3 minutes.



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.100	341.357	9.725	68.20	339.3	340.3	677.3
2	1.183	0.000	0.083	0.58	556.3	339.3	557.3
3	1.783	109.078	4.452	31.22	102.3	183.3	224.2
Total:		**450.435**	**14.260**	**100.00**			

Figure S16 Analytical HPLC chromatograms after 48 hours of attempted functionalization of Ac-CAFAH-OH (instead of **3b**) with arginine. No desired reaction was observed. 5-100% ACN + 0.05% formic acid in 3 minutes.

2. General Information

Chemicals and instruments

Peptides and pyridinium salts were synthesized according to procedures reported in sections 3 and 4. All other chemicals were commercially available from Sigma-Aldrich or Chemtronica and were used as received without any further purification. Reaction monitoring was done using TLC (silica gel 60 matrix F254 or Al_2O_3 60 matrix F254 neutral). Solvent evaporation was done using a Büchi Rotavapor R-200. Centrifugation was done using an IEC Centra-EC4 and freeze drying was done using a Flexi-Dry MP with Microprocessor control.

High performance liquid chromatography (HPLC) with Ultraviolet/Visible (UV) and mass spectrometry (MS) detectors was performed on a Dionex Ultimate 3000 equipped with a Surveyor MSQ plus mass spectrometer, 214, 254 and 280 nm UV-detector and Kinetex C18 column (2.6 µm, 50x3.0 mm). Mobile phases consisted of MeCN/water gradients (0.05% formic acid) at a flowrate of 1.5 mL/min.

Preparative LC-UV purification was done using an Agilent 1290 Infinity II equipped with a 214 and 254 nm UV-detector, auto-collector and Nucleodur C18 column (5.0 µm, 125x21 mm). Mobile phases consisted of MeCN/water gradients (0.1% TFA) at a flowrate of 25 mL/min.

Nuclear magnetic resonance (NMR) spectra were recorded using an Agilent MR400-DD2 spectrometer equipped with a OneNMR probe at 400 MHz (^{1}H), 101 MHz (^{13}C) or a Bruker Avance Neo spectrometer equipped with a TXO (CRPHe TR-^{13}C/^{15}N/^{1}H 5mm-Z) probe at 500 MHz (1H), 125 MHz (^{13}C). NMR spectra were analyzed and processed using MestReNova. Chemical shifts (δ) are reported in parts per million (ppm) using the residual solvent signal peaks of $CDCl_3$ (δ^H = 7.26), methanol-d_4 (δ^H = 3.31), D_2O (δ^H = 4.79) or DMSO-d_6 (δ^H = 2.50) as internal reference. Chemical shifts in ^{13}C NMR spectra are reported in parts per million (ppm) using the residual solvent signal peak for $CDCl_3$ (δ^C = 77.0), methanol-d_4 (δ^C = 49.0), D_2O or DMSO-d_6 (δ^C =39.5) as internal reference. Coupling constants (J) are given in Hz and multiplicity is reported as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

High resolution mass spectra (HRMS) were obtained using a Waters aquity UPLC I-class system with an injection volume of 10 uL and flow rate of 0.25 mL/min (50:50 ACN:Water). The system was equipped with a Waters LCT Premier mass spectrometer operating in ES+ or ES- mode, with a capillary voltage of 2kV, sample cone voltage of 30V, desolvation temperature of 350°C, source temperature of 120°C, con gas flow (N_2) of 10 l/h, desolvation gas flow (N_2) of 400 l/h and MCP voltage of 2.1 kV. HRMS measurement for compound **9** was conducted using a matrix-assisted laser-desorption ionization source with a Fourier transform ion cyclotron resonance mass analyzer.

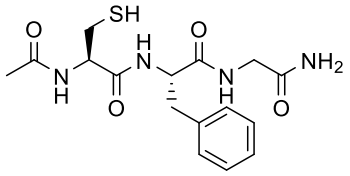
3. Synthesis of cysteine-containing peptides

Manual solid phase peptide synthesis (SPPS)

All SPPS was done using standard SPPS techniques. 10 mL solvent per gram of resin was used.

Swelling: 2-CTC resin (100-200 mesh, loading: 1.51 mmol/g) was swelled using DCM (2x) and DMF (2x). Activation of 2-CTC resin: Activation of the resin was done for 30 min by addition of $SOCl_2$ (1.2 equiv.) and pyridine (2.4 equiv.) in DCM (1x). The resin was washed with DCM (3x). Loading of the 2-CTC resin: AA_x-OH (2.5 equiv.) and DIPEA (5 equiv.) in DCM were added to the resin for 60 min. The resin was subsequently washed with DCM (2x) and DMF (2x). Standard AA_x coupling: To AA_x-OH (4 equiv.) and Oxyma (4 equiv.) in DMF was added DIC (4 equiv.). Pre-activation was performed for 2 min before the mixture was added to the resin for 60 min. The resin was then washed with DMF (2x). Standard Fmoc-deprotection: Piperidine in DMF (20v%) was added to the resin for 10 min (2x) followed by washing with DMF (6x). Acetylation: Acetylation was done using DIPEA (13.7 equiv.) and Ac_2O (12 equiv.) in DMF for 30 min. The resin was washed with DMF (2x) and DCM (2x). The resin was dried under a nitrogen flow before cleavage. Cleavage: The resin was added to a cleavage mixture (10 ml per gram of resin), consisting of TFA/TIS/H_2O/EDT (94/1/2.5/2.5). The mixture was stirred for 2 h. The mixture was filtered, and the solid residue was washed with TFA (2x). The organic layer was concentrated under vacuum.[1] Purification: The crude product was dissolved in an MeCN/H_2O mixture and filtered. The solution was injected on a preparative Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) system equipped with a UV detector.

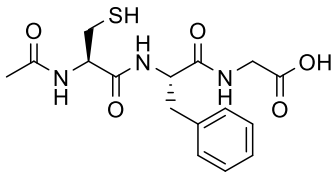
Synthesis of **1**a
(Ac-Cys-Phe-Gly-NH_2)



Peptide **1a** was prepared using the standard protocol for SPPS with Rink amide resin (3.04 mmol, 4 g., loading: 0.76 mmol/g, 1.0 equiv.) in a 50 mL SPPS tube. After cleavage and concentration, the crude product was purified by preparative RP-HPLC using a 15-26.5% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 5.5 minutes, then 26.5% isocratic elution for 7 minutes, followed by a 26.5-35% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 3 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **1a** as a white cloudy solid (630 mg, 57%). **^{1}H NMR** (400 MHz, Methanol-d4) δ 7.34 – 7.19 (m, 6H), 4.58 (dd, J = 8.9, 6.2 Hz, 1H), 4.41 (dd, J = 7.1, 5.7 Hz, 1H), 3.92 (d, J = 17.1 Hz, 1H), 3.69 (d, J = 17.0 Hz, 1H), 3.22 (dd, J = 13.9, 6.2 Hz, 1H), 3.00 (dd, J = 13.9, 8.9 Hz, 1H), 2.84 – 2.66 (m, 2H), 1.99 (s, 3H). **^{13}C NMR** (101 MHz, Methanol-d4) δ 172.8, 172.4, 171.3, 136.9, 128.9, 128.2, 126.5, 55.9, 55.2, 41.7, 36.7, 25.1, 21.1.

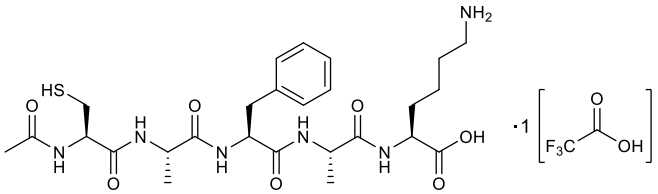
[1] Note: Due to EDT, evaporation must be done in a fume hood or well-ventilated area.

Synthesis of 1b
(Ac-Cys-Phe-Gly-OH)



Peptide **1b** was prepared using the standard protocol for SPPS with 2-CTC resin (9.06 mmol, 6 g., loading: 1.51 mmol/g, 1.0 equiv.) in a 50 mL SPPS tube. After cleavage and concentration, the crude product was purified by preparative RP-HPLC using a 15-26.5% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 5.5 minutes, then 26.5% isocratic elution for 7 minutes, followed by a 26.5-35% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 3 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **1b** as a white cloudy solid (1345 mg, 40%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.32 (t, J = 5.8 Hz, 1H), 8.06 (d, J = 8.3 Hz, 1H), 7.98 (d, J = 8.0 Hz, 1H), 7.24 (m , 4H), 7.18 (ddd, J = 8.6, 4.9, 3.7 Hz, 1H), 4.54 (ddd, J = 9.6, 8.3, 4.4 Hz, 1H), 4.33 (ddd, J = 8.0, 7.8, 5.3 Hz, 1H), 3.78 (dd, J = 5.8, 2.5 Hz, 2H), 3.05 (dd, J = 13.9, 4.4 Hz, 1H), 2.80 (dd, J = 13.9, 9.6 Hz, 1H), 2.70 (ddd, J = 13.7, 8.8, 5.3 Hz, 1H), 2.57 (ddd, J = 13.7, 8.0 Hz, 1H), 2.21 (t, J = 8.8 Hz, 1H), 1.84 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.2, 171.1, 169.8, 169.5, 137.7, 129.3, 128.1, 126.3, 55.0, 53.8, 40.7, 37.4, 26.1, 22.5. **ESI-HRMS (+)**: m/z calcd for $C_{16}H_{21}N_3O_5S$ $[M+H]^+$ 368.1280, found: 368.1279.

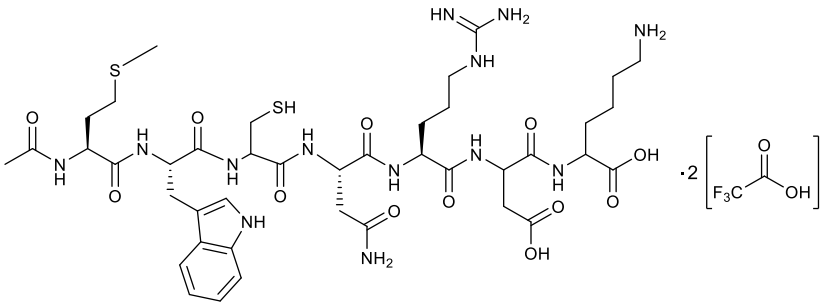
Synthesis of 1c
(Ac-CAFAK-OH)



Peptide **1c** was prepared using the standard protocol for SPPS with 2-CTC resin (100-200 mesh, 0.4 gr, loading: 1.51 mmol/g). After cleavage and concentration, the crude product was purified by preparative RP-HPLC using a 5-20% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 20% isocratic elution for 4 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **1c** as a white solid (258 mg, 61%). **^{1}H NMR (500 MHz, DMSO-d_6)** δ 8.17 (d, J = 7.2 Hz, 1H), 8.10 (m, 2H), 8.02 (d, J = 7.4 Hz, 1H), 7.89 (d, J = 8.2 Hz, 1H), 7.74 (t, J = 5.9 Hz, 3H), 7.23 (m, 4H), 7.18 (m, 1H), 4.48 (ddd, *J* = 8.6, 8.3, 3.9 Hz, 1H), 4.35 (ddd, *J* = 7.9, 7.7, 5.5 Hz, 1H), 4.30 (ddd, *J* = 7.7, 7.2, 6.7 Hz, 1H), 4.16 (m, 2H), 3.03 (dd, J = 14.0, 4.2 Hz, 1H), 2.77 (m, 3H), 2.70 (dd, *J* = 13.6, 3.2 Hz, 1H), 2.61 (dd, J = 13.6, 7.9 Hz, 1H), 2.32 (t, J = 8.5 Hz, 1H), 1.72 (m, 1H), 1.56 (m, 3H), 1.35 (m, 2H), 1.22 (d, J = 7.0 Hz, 3H), 1.14 (d, J = 7.2 Hz, 3H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 173.4, 172.2, 172.0, 170.5, 169.8, 169.6, 158.2 (q, ^{2}J(C-F), J = 31.3 Hz), 137.7, 129.3, 128.1, 126.3, 117.2 (q, ^{1}J(C-F), J = 299.5 Hz), 55.0, 53.6, 51.6, 48.6, 48.0, 38.7, 37.2, 30.5, 26.6, 26.2, 22.5, 22.4, 18.3, 17.9. **ESI-HRMS (+)**: m/z calcd for $C_{26}H_{41}N_6O_7S$ $[M+H]^+$ 581.2757, found: 581.2753.

Synthesis of 1d

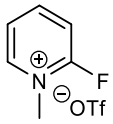
(Ac-MWCRNDK-OH)



Peptide **1d** was prepared using the standard protocol for SPPS with 2-CTC resin (0.604 mmol, 0.4 g., loading: 1.51 mmol/g, 1.0 equiv.) in a 50 mL SPPS tube. After cleavage and concentration, the crude product was purified by preparative RP-HPLC using a 5-20% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 20% isocratic elution for 4 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **1d** as a white solid (288 mg, 39%). **^{1}H NMR** (500 MHz, DMSO-d_6) δ 10.85 (d, *J* = 2.4 Hz, 1H), 8.31 (d, *J* = 7.3 Hz, 1H), 8.25 (d, *J* = 7.7 Hz, 1H), 8.15 – 8.05 (m, 4H), 7.81 – 7.78 (m, 4H), 7.64 (t, *J* = 5.7 Hz, 1H), 7.57 (d, *J* = 7.9 Hz, 1H), 7.54 (s, 1H), 7.32 (d, *J* = 8.1 Hz, 1H), 7.13 (d, *J* = 2.4 Hz, 1H), 7.07 – 7.02 (m, 2H), 6.97 (t, *J* = 7.4 Hz, 1H), 4.57 – 4.53 (m, 3H), 4.43 (q, *J* = 6.8 Hz, 1H), 4.26 (td, *J* = 7.9, 5.2 Hz, 1H), 4.17 – 4.14 (m, 2H), 3.16 (dd, *J* = 15.0, 4.5 Hz, 1H), 3.07 (q, *J* = 6.4 Hz, 2H), 3.00 (dd, *J* = 14.9, 9.2 Hz, 1H), 2.81 – 2.68 (m, 5H), 2.61 – 2.55 (m, 1H), 2.55 – 2.46 (m, 3H), 2.39 – 2.34 (m, 2H), 2.31 (t, *J* = 8.6 Hz, 1H), 1.98 (s, 3H), 1.82 (s, 3H), 1.80 – 1.76 (m, 1H), 1.76 – 1.65 (m, 3H), 1.64 – 1.57 (m, 1H), 1.57 – 1.51 (m, 5H), 1.32 (p, *J* = 7.9 Hz, 2H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 173.3, 171.9, 171.8, 171.7, 171.6, 171.3, 171.2, 170.7, 169.9, 169.7, 158.8 (q, ^{2}J(C-F) *J* = 34 Hz), 158.5, 156.9, 136.1, 127.4, 123.7, 121.0, 120.2, 118.5, 117.8 (q, ^{1}J(C-F), *J* = 296.2 Hz), 111.4, 109.9, 55.1, 53.5, 52.7, 52.2, 51.8, 50.0, 49.7, 40.6, 38.7, 36.9, 36.0, 31.6, 30.5, 29.6, 28.7, 27.1, 26.7, 26.3, 24.9, 22.5, 22.3, 14.7. **ESI-HRMS (+)**: m/z calcd for $C_{41}H_{64}N_{13}O_{12}S_2$ $[M+H]^+$ 994.6913, found: 994.4273.

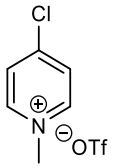
4. Synthesis of pyridinium salts[2]

Synthesis of 2a
(2-fluoro-N-methylpyridinium triflate)



To a 50 mL was added 2-fluoropyridine (0.97 gr, 0.86 ml, 10 mmol) and DCM (10 mL). MeOTf (1,36 ml, 1.2 eq, 12 mmol) was slowly added and the reaction was stirred for 1 hour. Isohexane (20 mL) was added and a white precipitate formed. The precipitate was filtrated and washed with EtO_2 (3x15 ml). The product was dried in a vacuum oven, yielding **2a** as a white solid (2.48 gr, 95%). **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.80 (ddd, J = 6.3, 4.7, 1.9 Hz, 1H), 8.64 (tdd, J = 7.7, 5.8, 1.9 Hz, 1H), 8.01 (ddd, J = 8.7, 4.7, 1.3 Hz, 1H), 7.87 (ddd, J = 7.7, 6.3, 1.3 Hz, 1H), 4.10 (d, J = 4.0 Hz, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 150.7, 144.6, 140.1, 123.9, 114.4, 41.6.

Synthesis of 2b
(4-chloro-N-methylpyridinium triflate)



To a 50 ml round bottom flask was added 4-fluoropyridine hydrochloride (1500 mg, 10 mmol) and dissolved in 0.5M $NaHCO_3$ (20 mL) and stirred for 15 minutes. The solution was extracted with EtOAc (2x20 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and reduced under vacuum. To the clear oil was added 25 mL of DCM and MeOTf (2,26 ml, 2 eq) was slowly added. The reaction was stirred for 1 hour which during a yellow precipitate formed. The precipitate was filtered and washed with Et_2O (3x10 mL). The solid was dried in a vacuum oven yielding **5** as yellow crystals (1.86 gr, 67%). **^{1}H NMR** (400 MHz, D_2O) δ 8.76 (dd, J = 5.3, 1.7 Hz, 2H), 8.12 (dd, J = 5.3, 1.7 Hz, 2H), 4.37 (s, 3H). **^{13}C NMR** (101 MHz, D_2O) δ 153.8, 145.9, 128.4, 119.6 (q, 317.3 Hz, C-F), 47.8.

[2] Note: **2a** and **2b** are hygroscopic and should be stored under nitrogen.

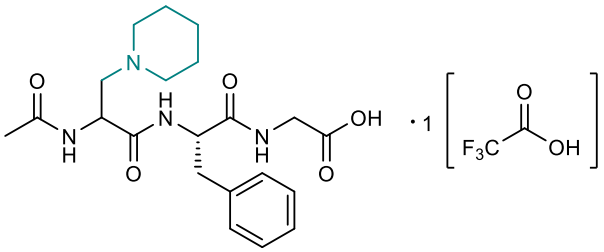
5. Desulfurative functionalization of cysteine-containing peptides

General method for peptide functionalization[3]

To an 8 mL reaction vial was added **1b** (Ac-Cys-Phe-Gly-OH, 36.7 mg, 0.1 mmol) and **2b** (4-chloro-1-methyl-pyridin-1-ium triflate, 33.4 mg, 1.2 equiv., 0.12 mmol). Sodium phosphate buffer (50 mM, pH 9.0, 2.5 mL) was added and the mixture was stirred at 37°C for 15 min while maintaining the pH-value at 9.0 by addition of 10 µL aliquots of 1M NaOH solution. Et_3N (0.07 mL, 5 equiv.) was added and the mixture was stirred at 37°C for 2.5 hours or until complete conversion was confirmed by LC-MS. The corresponding nucleophile (0.5 - 10 equiv.) was added and stirring continued for another 0.5 to 72 hours at 37°C while maintaining the pH-value at 11 by addition of 10 µL aliquots of 1M NaOH solution. The reaction mixture was filtered, and aliquots were directly purified by preparative Reversed-Phase High Performance Liquid Chromatography (RP-HPLC) with UV detection. Fractions corresponding to the product were pooled, frozen, and lyophilized (freeze dried). The functionalized peptide products were isolated as 1:1 mixtures of diastereomers, unless otherwise stated.

Synthesis of 6a

((2-Acetamido-3-(piperidin-1-yl)propanoyl)-L-phenylalanylglycine)

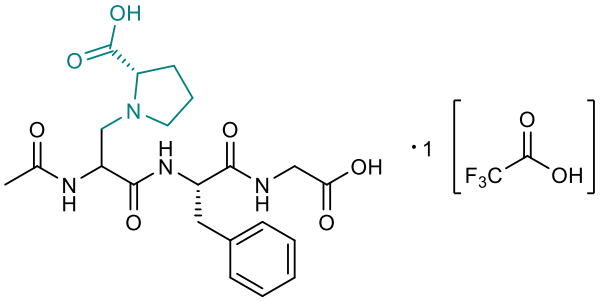


Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 2 hours. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 12 minutes. Lyophilization of fractions afforded **6a** (white solid, 36.6 mg, 69%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 9.32 (m, 1H), 8.55 (m, 1H), 8.29 (m, 1H), 7.26 (m, 4H), 7.20 (m, 1H), 4.78 (m, 1H), 4.60 (m, 1H), 3.80 (m, 2H), 3.37 (m, 2H), 3.10 (m, 1H), 2.87 (m, 2H), 2.77 (m, 2H), 1.87 (m, 3H), 1.76 (m, 2H), 1.64 (m, 4H), 1.37 (m, 1H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.1, 170.1, 168.4, 167.9, 158.5 (q, ^{2}J(C-F), J = 33.2 Hz), 137.4, 129.3, 128.2, 126.5, 116.7 (q, ^{1}J(C-F), J = 296.2 Hz), 56.8, 54.1, 52.0, 47.8, 40.8, 37.5, 22.7, 22.2, 21.1. **ESI-HRMS (+)**: m/z calcd for $C_{21}H_{30}N_4O_5$ $[M+H]^+$ 419.2294, found: 419.2289.

[3] Note: **2a** and **2b** are hygroscopic and should be stored under nitrogen.

Synthesis of 6b

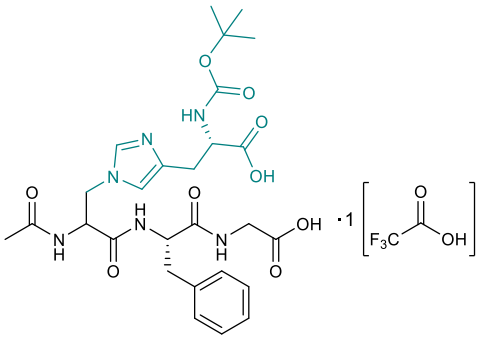
((2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)-L-proline)



Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 2 hours. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 12 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6b** (white solid, 36.4 mg, 64%) as a 3:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d6) δ 8.50 (m, 1H), 8.43 (m, 1H), 8.23 (m, 1H), 7.26 (m, 4H), 7.16 (m, 1H), 4.73 (m, 1H), 4.57 (m, 1H), 4.34 (m, 1H), 3.79 (m, 2H), 3.46 (m, 1H), 3.25 (m, 1H), 3.07 (m, 1H), 2.97 (dd, J = 13.2, 8.9 Hz, 1H), 2.85 (m, 1H), 2.75 (dd, J = 13.7, 10.3 Hz, 1H), 2.33 (m, 1H), 2.01 (m, 1H), 1.92 (m, 1H), 1.87 (m, 3H), 1.82 (m, 1H). **^{13}C NMR** (126 MHz, DMSO) δ 171.5, 171.3, 170.8, 170.4, 168.4, 158.7 (q, ^{2}J(C-F), J = 33.5 Hz), 138.1, 129.8, 128.6, 126.9, 117.0 (q, ^{1}J(C-F), J = 296.0 Hz), 66.5, 55.6, 54.2, 49.7, 41.1, 38.3, 28.0, 23.1, 22.8, 22.1. **ESI-HRMS (+)**: m/z calcd for $C_{21}H_{29}N_4O_7$ $[M+H]^+$ 449.2036, found: 449.2034.

Synthesis of 6c

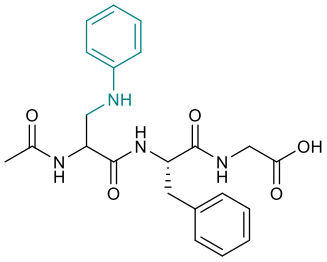
(N^t-(2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)-N^a-(tert-butoxycarbonyl)-L-histidine)



Prepared according to the general method for peptide functionalization using 10 equiv. of the nucleophile for 72 hours. Preparative RP-HPLC was performed using a 5-20% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 20% isocratic elution for 8 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6c** (white solid, 21.7 mg, 37%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.66 (m, 1H), 8.56 (m, 1H), 8.36 (m, 1H), 8.17 (m, 1H), 7.26 (m, 4H), , 7.20 (m, 1H), 4.79 (m, 1H), 4.61 (m, 1H), 4.34 (m, 1H), 4.19 (m, 1H), 4.12 (m, 1H), 3.86 (m, 1H), 3.82 (m, 2H), 3.08 (m, 2H), 2.95 (m, 1H), 2.76 (m, 1H), 1.77 (m, 3H), 1.35 (m, 9H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.6, 172.4, 171.3, 171.1, 169.8, 168.2, 167.6, 158.2 (q, ^{2}J(C-F), J = 33.5 Hz), 155.5, 137.7, 137.5, 135.4, 131.3, 130.3, 129.4, 128.2, 126.6, 78.5, 54.0, 52.7, 52.3, 51.7, 49.9, 40.7, 38.2, 28.1, 26.4, 22.4. **ESI-HRMS (+)**: m/z calcd for $C_{27}H_{37}N_6O_9$ $[M+H]^+$ 589.2622, found: 589.2607.

Synthesis of 6d

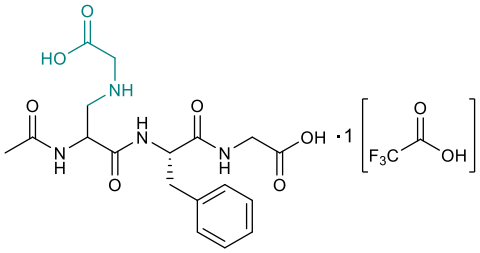
((2-Acetamido-3-(phenylamino)propanoyl)-*L*-phenylalanylglycine)



Prepared according to the general method for peptide functionalization using 10 equiv. of the nucleophile for 72 hours. Preparative RP-HPLC was performed using a 5-30% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by a 30-37% gradient over 8 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6d** (white solid, 10.3 mg, 24%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.36 (m, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.24 (m, 2H), 7.18 (m, 4H), 7.08 (m, 2H), 6.54 (m, 2H), 4.56 (ddd, J = 10.3, 8.6, 4.0 Hz, 1H), 4.42 (td, J = 8.0, 4.9 Hz, 1H), 3.78 (d, J = 5.8 Hz, 1H), 3.06 (dd, J = 13.8, 4.0 Hz, 1H), 3.00 (dd, J = 13.2, 4.9 Hz, 1H), 2.87 (dd, J = 13.1, 8.2 Hz, 1H), 2.77 (dd, J = 13.7, 10.3 Hz, 1H), 1.82 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.4, 171.1, 170.3, 169.7, 148.0, 137.8, 129.3, 128.9, 128.0, 126.3, 116.2, 112.4, 53.7, 52.4, 44.9, 40.7, 37.5, 22.6. **ESI-HRMS (+)**: m/z calcd for $C_{22}H_{27}N_4O_5$ $[M+H]^+$ 427.1981, found: 427.1989.

Synthesis of 6e

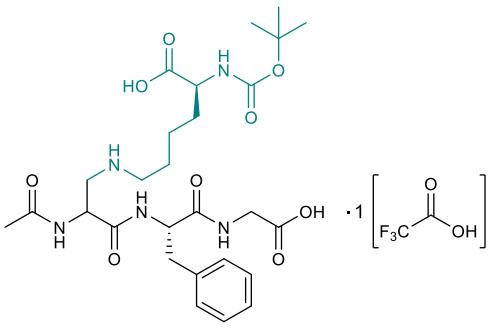
((2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)glycine)



Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 16 hours. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 12 minutes. Fractions corresponding to the two different diastereomers of the product were pooled separately, frozen, and lyophilized to afford **6e** (white solid, 45.6 mg, 87%) as a 1:1 mixture of diastereomers. **DS1: ^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.46 (t, J = 5.9 Hz, 1H), 8.34 (d, J = 8.7 Hz, 1H), 8.22 (d, J = 8.5 Hz, 1H), 7.26 (m, 4H), 7.18 (m, 1H), 4.65 (dd, J = 9.3, 3.2 Hz, 1H), 4.61 (d, J = 3.9 Hz, 1H), 4.57 (dd, J = 9.5, 3.5 Hz, 1H), 3.80 (m, 4H), 3.08 (m, 2H), 2.85 (dd, J = 12.9, 10.0 Hz, 1H), 2.77 (dd, J = 13.7, 10.2 Hz, 1H), 1.88 (s, 3H). **^{13}C NMR (**126 MHz, DMSO-d_6**)** δ 171.1, 171.0, 170.6, 168.1, 168.0, 158.3 (q, ^{2}J(C-F), J = 34.0 Hz), 137.6, 129.3, 128.1, 126.4, 116.4 (q, ^{1}J(C-F), J = 295.4 Hz), 53.8, 49.0, 48.2, 47.1, 40.7, 37.8, 22.9. **DS2: ^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.51 (t, J = 5.9 Hz, 1H), 8.22 (d, J = 8.3 Hz, 1H), 8.11 (d, J = 8.1 Hz, 1H), 7.24 (m, 4H), 7.18 (m, 1H), 4.66 (ddd, J = 8.7, 4.5 Hz, 1H), 4.53 (ddd, J = 8.7, 4.5 Hz, 1H), 3.87 (d, J = 2.5 Hz, 2H), 3.80 (d, J = 6.2 Hz, 2H), 3.27 (dd, J = 13.0, 4.6 Hz, 1H), 3.05 (m, 2H), 2.84 (dd, J = 13.9, 9.2 Hz, 1H), 1.86 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.1, 171.1, 170.39, 168.6, 168.10, 158.5 (q, ^{2}J(C-F), J = 34.1 Hz), 137.5, 129.4, 128.2, 126.5, 116.4 (q, ^{1}J(C-F), J = 295.0 Hz), 54.0, 49.1, 47.8, 47.4, 40.7, 37.4, 22.8. **ESI-HRMS (+)**: m/z calcd for $C_{18}H_{25}N_4O_7$ $[M+H]^+$ 409.1723, found: 409.1723.

Synthesis of 6f

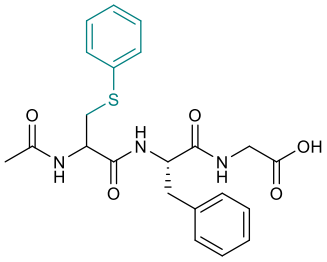
(N^6-(2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)-N^2-(tert-butoxycarbonyl)-L-lysine)



Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 16 hours. Preparative RP-HPLC was performed using a 5-25% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 25% isocratic elution for 6 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6f** (white solid, 34.6 mg, 50%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.52 (m, 1H), 8.41 (d, J = 8.9 Hz, 1H), 8.21 (dd, J = 8.5, 6.2 Hz, 1H), 7.24 (m, 4H), 7.19 (m, 1H), 7.09 (m, 1H), 4.61 (m, 2H), 4.55 (m, 1H), 3.85 (m, 1H), 3.80 (m, 2H), 3.07 (m, 1H), 2.88 (m, 2H), 2.78 (m, 2H), 2.68 (m, 1H), 1.87 (m, 3H), 1.66 (m, 1H), 1.55 (m, 3H), 1.38 (m, 9H), 1.35 (m, 2H), 1.30 (m, 1H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 174.2, 171.1, 170.4, 168.5, 168.0, 158.3 (q, J = 33.4 Hz), 155.7, 137.6, 129.4, 128.1, 126.4, 116.6 (q, J = 296.1 Hz), 78.1, 53.7, 53.3, 49.1, 47.9, 46.7, 40.7, 37.9, 30.2, 28.3, 24.7, 22.9, 22.7. **ESI-HRMS (+)**: m/z calcd for $C_{27}H_{41}N_5O_9$ $[M+H]^+$ 580.2983, found: 580.2982.

Synthesis of 6g

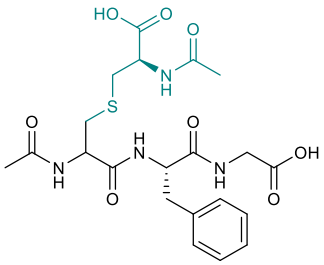
(N-Acetyl-S-phenylcysteinyl-L-phenylalanylglycine)



Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 3 hours. Preparative RP-HPLC was performed using a 5-15% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by a 15-55% gradient over 12 minutes. Fractions corresponding to the two different diastereomers of the product were pooled separately, frozen, and lyophilized to afford **6g** (white solid, 24.4 mg, 55%). **DS1: ^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.31 (dd, J = 5.8, 5.8 Hz, 1H), 8.16 (d, J = 8.3 Hz, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.32 (m, 4H), 7.23 (m, 4H), 7.20 (m, 1H), 7.18 (m, 1H), 4.53 (ddd, J = 13.1, 9.2, 4.3 Hz, 1H), 4.41 (ddd, J = 13.4, 8.5, 5.1 Hz, 1H), 3.78 (dd, J = 5.9, 3.5 Hz, 2H), 3.20 (dd, J = 13.3, 5.1 Hz, 1H), 3.05 (dd, J = 13.9, 4.3 Hz, 1H), 2.95 (dd, J = 13.3, 9.4 Hz, 1H), 2.80 (dd, J = 13.9, 9.4 Hz, 1H), 1.80 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.1, 171.1, 169.8, 169.5, 137.6, 135.9, 129.3, 129.1, 128.4, 128.1, 126.3, 126.0, 53.8, 52.0, 40.7, 37.5, 34.8, 22.5. **DS2: ^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.47 (d, J = 8.7 Hz, 1H), 8.39 (dd, J = 5.5 Hz, 1H), 8.16 (d, J = 8.2 Hz, 1H), 7.32 (m, 2H), 7.24 (m, 4H), 7.16 (m, 4H), 4.57 (ddd, J = 12.6, 10.3, 4.0 Hz, 1H), 4.43 (ddd, J = 13.5, 9.0, 5.1 Hz, 1H), 3.79 (d, J = 5.8 Hz, 2H), 3.06 (dd, J = 13.5, 4.0 Hz, 1H), 2.88 (dd, J = 13.2, 5.1 Hz, 1H), 2.75 (m, 2H), 1.80 (s, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.3, 171.1, 169.8, 169.5, 137.8, 136.0, 129.4, 129.0, 128.1, 128.0, 126.3, 125.8, 53.8, 52.1, 40.7, 37.6, 35.0, 22.5. **ESI-HRMS (+)**: m/z calcd for $C_{22}H_{25}N_3O_5S$ $[M+H]^+$ 444.1593, found: 444.1584.

Synthesis of 6h

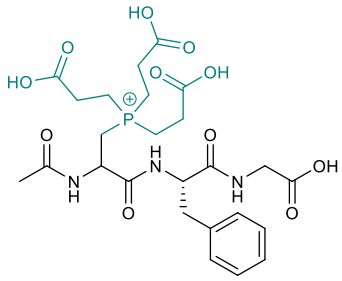
(S-(2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)-N-acetyl-L-cysteine)



Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 0.5 hours. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 12 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6h** (white solid, 36.1 mg, 73%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.40 (m, 1H), 8.18 (m, 1H), 8.17 (m, 1H), 8.04 (m, 1H), 7.23 (m, 4H), 7.17 (m, 1H), 4.55 (m, 1H), 4.41 (m, 1H), 4.32 (m, 1H), 3.78 (m, 2H), 3.05 (m, 1H), 2.90 (m, 1H), 2.81 (m, 1H), 2.72 (m, 1H), 2.45 (m, 1H), 1.86 (m, 3H), 1.82 (m, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.7, 171.8, 171.5, 170.5, 167.0, 169.8, 138.2, 129.8, 128.5, 126.7, 54.2, 52.6, 52.4, 41.1, 38.0, 34.8, 33.4, 22.9, 22.8. **ESI-HRMS (+)**: m/z calcd for $C_{21}H_{28}N_4O_8S$ $[M+H]^+$ 497.1703, found: 497.1706.

Synthesis of 6i

((2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)tris(2-carboxyethyl)phosphonium)[4]

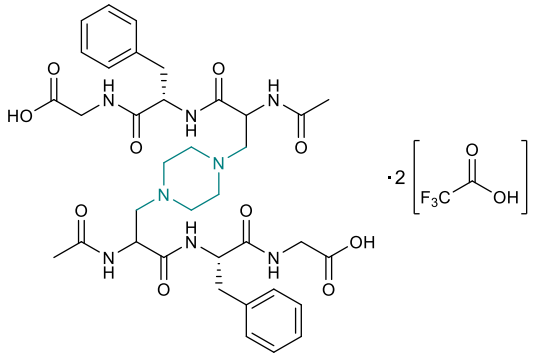


Prepared according to the general method for peptide functionalization using 3 equiv. of the nucleophile for 0.5 hours. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 12 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6i** (white solid, 48.3 mg, 83%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.55 (m, 1H), 8.50 (m, 1H), 8.50 (m, 1H), 7.23 (m, 5H), 4.73 (m, 1H), 4.60 (m, 1H), 3.79 (m, 2H), 3.07 (m, 1H), 2.76 (m, 2H), 2.60 (m, 7H), 2.49 (m, 6H), 1.83 (m, 3H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 172.6, 171.2, 170.9, 169.8, 169.1, 137.3, 129.3, 128.1, 126.4, 54.0, 46.2, 40.7, 38.2, 25.5, 22.5, 14.3, 13.9. **ESI-HRMS (+)**: m/z calcd for $C_{25}H_{35}N_3O_{11}P$ $[M+H]^+$ 584.2009, found: 584.1990.

1. Yap, S. Y. *et al.* Chemo- and regio-selective differential modification of native cysteines on an antibody via the use of dehydroalanine forming reagents. *Chem Sci* **15**, 8557–8568 (2024).

Synthesis of 6j

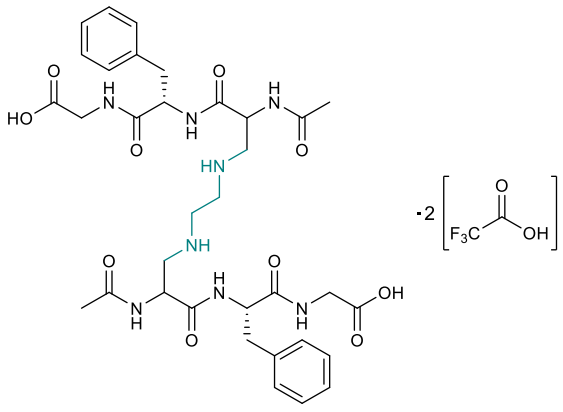
(2,2'-(((2S,2'S)-2,2'-((3,3'-(Piperazine-1,4-diyl)bis(2-acetamidopropanoyl))bis(azanediyl))bis(3-phenylpropanoyl))bis(azanediyl))diacetic acid)



Prepared according to the general method for peptide functionalization using 0.5 equiv. of the nucleophile for 24 hours. Preparative RP-HPLC was performed using a 5-20% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 20% isocratic elution for 6 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6j** (white solid, 30.0 mg, 61%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.50 (m, 3H), 8.23 (m, 1H), 8.14 (m, 2H), 7.25 (m, 8H), 7.20 (m, 2H), 4.59 (m, 4H), 3.80 (m, 4H), 3.07 (m, 4H), 2.93 (m, 4H), 2.83 (m, 4H), 2.74 (m, 3H), 2.55 (m, 1H), 1.85 (m, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.3, 171.2, 169.8, 169.1, 158.5 (q, ^{2}J(C-F), J = 35.1 Hz), 137.8, 137.5, 129.3, 128.1, 126.4, 116.1 (q, ^{1}J(C-F), J = 293.4 Hz), 57.3, 53.6, 48.7, 40.7, 38.0, 37.6, 22.7. **ESI-HRMS (+)**: m/z calcd for $C_{36}H_{49}N_8O_{10}$ $[M+H]^+$ 753.3572, found: 753.3591.

Synthesis of 6k

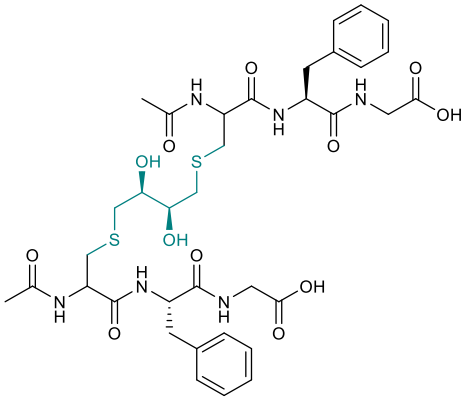
((5S,18S)-8,15-Diacetamido-5,18-dibenzyl-4,7,16,19-tetraoxo-3,6,10,13,17,20-hexaazadocosanedioic acid)



Prepared according to the general method for peptide functionalization using 0.5 equiv. of the nucleophile for 24 hours. Preparative RP-HPLC was performed using a 5-20% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 20% isocratic elution for 6 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6k** (white solid, 14.9 mg, 31%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.52 (m, 2H), 8.37 (m, 1H), 8.29 (m, 2H), 8.14 (m, 1H), 7.25 (m, 8H), 7.19 (m, 2H), 4.59 (m, 4H), 3.80 (m, 4H), 3.22 (m, 4H), 3.05 (m, 4H), 2.81 (m, 4H), 1.89 (m, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.0, 170.4, 168.4, 168.0, 158.6 (q, ^{2}J(C-F), J = 32.7 Hz), 137.4, 129.3, 128.1, 126.4, 116.8 (q, ^{1}J(C-F), J = 297.3 Hz), 53.8, 49.2, 48.0, 43.1, 40.7, 37.5, 22.8. **ESI-HRMS (+)**: m/z calcd for $C_{34}H_{47}N_8O_{10}$ $[M+H]^+$ 727.3436, found: 727.3415.

Synthesis of 6l

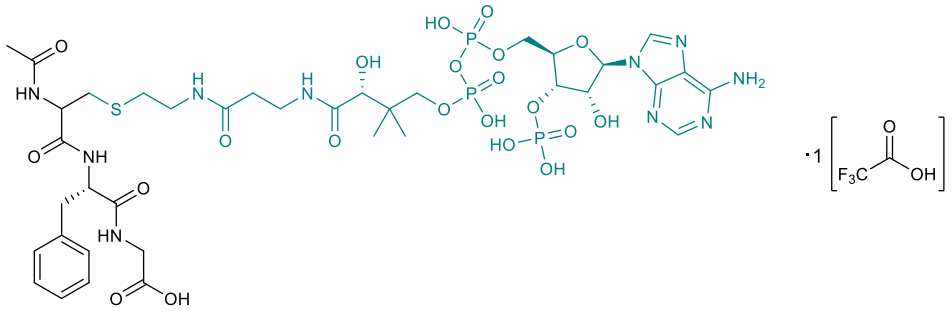
((5S,11R,12R,18S)-8,15-Diacetamido-5,18-dibenzyl-11,12-dihydroxy-4,7,16,19-tetraoxo-10,13-dithia-3,6,17,20-tetraazadocosanedioic acid)



Prepared according to the general method for peptide functionalization using 0.5 equiv. of the nucleophile for 0.5 hours. Preparative RP-HPLC was performed using a 5-26% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by 26% isocratic elution for 6 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6l** (white solid, 21.2 mg, 52%) as a 1:1 mixture of diastereomers. **^{1}H NMR** (500 MHz, DMSO-d_6) δ 8.39 (m, 2H), 8.30 (m, 1H), 8.06 (m, 3H), 7.24 (m, 8H), 7.18 (m, 2H), 4.53 (m, 2H), 4.40 (m, 2H), 3.78 (m, 4H), 3.51 (m, 2H), 3.05 (m, 2H), 2.76 (m, 3H), 2.59 (m, 5H), 2.45 (m, 1H), 2.32 (m, 1H), 1.82 (m, 6H). **^{13}C NMR** (126 MHz, DMSO-d_6) δ 171.4, 171.1, 170.3, 169.5, 137.6, 129.3, 128.1, 126.3, 71.8, 71.5, 53.8, 52.4, 37.5, 35.2, 34.4, 22.6. **ESI-HRMS (+)**: m/z calcd for $C_{36}H_{49}N_6O_{12}S_2$ $[M+H]^+$ 821.2850, found: 821.2844.

Synthesis of 6m

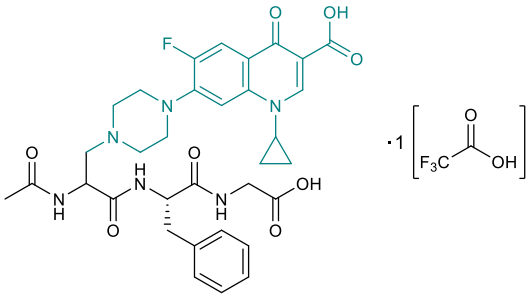
(N-Acetyl-S-(2-(3-((2R)-4-(((((((2R,3S,4R,5R)-5-(6-amino-9H-purin-9-yl)-4-hydroxy-3-(phosphonooxy)tetrahydrofuran-2-yl)methoxy)(hydroxy)phosphoryl)oxy)(hydroxy)phosphoryl)oxy)-2-hydroxy-3,3-dimethylbutanamido)propanamido)ethyl)cysteinyl-L-phenylalanylglycine)



To a 8 mL reaction vial was added **1b** (27.6 mg, 1.5 equiv., 0.075 mmol) and **2b** (22.2 mg, 1.6 equiv., 0.08 mmol). Sodium phosphate buffer (50 mM, pH = 9.0, 2.5 mL) was added and the mixture was stirred while maintaining the pH-value at 9.0 for 15 min at 37°C. Et_3N (0.042 mL, 6 equiv.) was added and the mixture was stirred for 2.5 hour at 37°C. coenzyme A (38.4 mg, 1 equiv., 0.05 mmol) was added and stirring was continued for 2 hours at 37°C. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 14 minutes. Lyophilization of fractions afforded **6m** (white solid, 36.6 mg, 88%) as a 1:1 mixture of diastereomers. **^{1}H NMR (500 MHz, DMSO-d_6)** δ 9.45 (s, 1H), 9.01 (s, 1H), 8.70 (s, 1H), 8.45 (s, 1H), 8.42 (m, 1H), 8.30 (dd, J = 5.9 Hz, 1H), 8.11 (d, J = 8.3 Hz, 1H), 8.04 (m, 2H), 7.77 (m, 1H), 7.25 (m, 1H), 7.23 (m, 3H), 7.17(m, 1H), 5.99 (d, J = 5.6 Hz, 1H), 4.78 (m, 1H), 4.72 (m, 1H), 4.53 (m, 1H), 4.43 (m, 1H), 4.38 (m, 1H), 4.22 (m, 2H), 3.91 (m, 1H), 3.78 (m, 2H), 3.73 (d, J = 3.4 Hz, 1H), 3.62 (dd, J = 9.6, 4.8 Hz, 1H), 3.32 (m, 1H), 3.24 (m, 1H), 3.16 (m, 2H), 3.05 (dt, J = 14.0, 4.6 Hz, 1H), 2.77 (m, 1H), 2.53 (m, 1H), 2.42 (m, 1H), 2.27 (m, 2H), 1.82 (m, 3H), 0.91 (d, J = 3.5 Hz, 3H), 0.77 (d, J = 2.3 Hz, 3H). **^{13}C NMR (126 MHz, DMSO-d_6)** δ 172.2, 171.5, 171.1, 170.7, 170.3, 169.6, 158.6 (q, ^{2}J(C-F), J = 36.8 Hz), 150.6, 148.5, 145.9, 141.7, 137.7, 129.3, 128.1, 126.4, 118.7, 115.6 (q, ^{1}J(C-F), J = 290.8 Hz), 87.4, 82.1, 74.1, 73.5, 73.0, 72.4, 65.2, 53.8, 52.3, 52.3, 40.8, 38.6, 38.3, 37.8, 35.2, 33.3, 31.0, 22.6, 21.1, 19.1. **MALDI-HRMS (-):** m/z calcd for $C_{37}H_{54}N_{10}O_{21}P_3S$ $[M-H]^-$ 1099.2399, found: 1099.2404

Synthesis of 6n

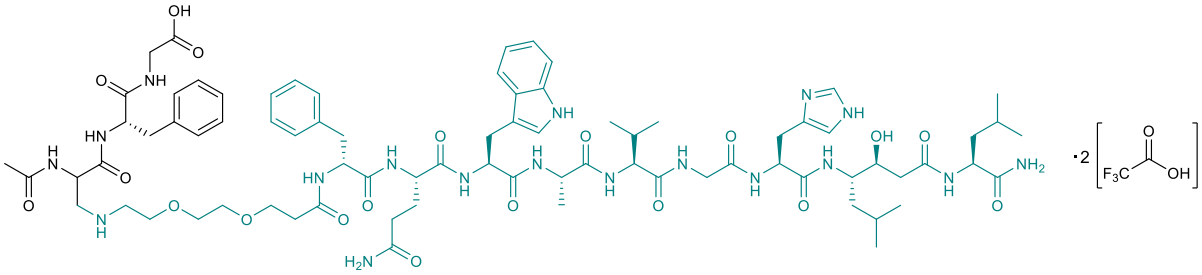
(7-(4-(2-Acetamido-3-(((S)-1-((carboxymethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-3-oxopropyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid)



To a 8 mL reaction vial were added **1b** (40.4 mg, 0.11 mmol) and **2b** (36.6 mg, 1.32 equiv., 0.132 mmol). Sodium phosphate buffer (50 mM, pH = 9.0, 3 mL) was added and the mixture was stirred while maintaining the pH-value at 9.0 for 15 min at 37°C. Et_3N (0.076 mL, 5 equiv.) was added and the mixture was stirred for 2.5 hour at 37°C. Ciprofloxacin (33.1 mg, 1.0 equiv.) was added and stirring was continued for 20 hours at 37°C. The reaction mixture was filtered and purification was performed by preparative RP-HPLC using a 5-10% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by a 10-50% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 8 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6n** (white solid, 55.3 mg, 71%) as a 1:1 mixture of diastereomers. **^{1}H NMR (500 MHz, DMSO-d$_6$)** δ 8.67 (m, 1H), 8.54 (m, 1H), 8.32 (m, 2H), 7.95 (m, 1H), 7.59 (m, 1H), 7.23 (m, 5H), 4.86 (m, 1H), 4.62 (m, 1H), 3.93 (m, 1H), 3.81 (m, 4H), 3.56 (m, 2H), 3.51 (s, 2H), 3.38 (m, 2H), 3.31 (m, 2H), 3.08 (m, 2H), 2.80 (m, 1H), 1.89 (m, 3H), 1.32 (m, 2H), 1.19 (m, 2H). **^{13}C NMR** (126 MHz, DMSO-d$_6$) δ 176.4, 171.1, 170.3, 168.3, 167.8, 165.9, 158.4 (q, ^{2}J(C-F), J = 34.9 Hz), 155.4, 153.9, 151.9, 148.3, 143.7, 139.1, 137.4, 129.5, 129.3, 128.2, 126.5, 119.5, 116.2 (q, ^{1}J(C-F), J = 294.0 Hz), 111.4, 108.1, 106.9, 56.8, 54.0, 47.8, 46.3, 45.4, 40.7, 37.5, 36.0, 22.8, 7.7. **ESI-HRMS (+)**: m/z calcd for $C_{33}H_{38}N_6O_8F$ $[M+H]^+$ 665.2735, found: 665.2725.

Synthesis of 6o

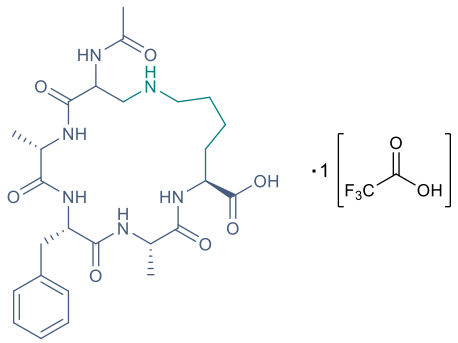
(((2R)-15-Acetamido-2-(((S)-5-amino-1-(((S)-1-(((S)-1-(((S)-1-((2-(((S)-1-(((3S,4S)-1-(((S)-1-amino-4-methyl-1-oxopentan-2-yl)amino)-3-hydroxy-6-methyl-1-oxoheptan-4-yl)amino)-3-(1H-imidazol-4-yl)-1-oxopropan-2-yl)amino)-2-oxoethyl)amino)-3-methyl-1-oxobutan-2-yl)amino)-1-oxopropan-2-yl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)amino)-1,5-dioxopentan-2-yl)carbamoyl)-4-oxo-1-phenyl-7,10-dioxa-3,13-diazahexadecan-16-oyl)-L-phenylalanylglycine)



To a 8 mL reaction vial were added **1b** (7.54 mg, 3.0 equiv., 0.021 mmol) and **2b** (6.83 mg, 3.6 equiv., 0.025 mmol). Sodium phosphate buffer (50 mM, pH = 9.0, 2.5 mL) was added and the mixture was stirred while maintaining the pH-value at 9.0 for 15 min at 37°C. Et_3N (0.07 mL, 5 equiv.) was added and the mixture was stirred for 2.5 hour at 37°C. $_2$HN-PEG2-RM26 (11 mg, 1.0 equiv., 0.007 mmol) was added and stirring was continued for 72 hours at 37°C. The reaction mixture was filtered and purification was performed by preparative RP-HPLC using a 5-30% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 2 minutes, followed by a 30-50% ACN (0.1% TFA) in H_2O (0.1% TFA) gradient over 8 minutes. Fractions corresponding to the product were pooled, frozen, and lyophilized to afford **6o** (white solid, 6.3 mg, 50%) as a 1:1 mixture of diastereomers. **LC-UVMS**: Retention time = 1.57 min using 5-100% ACN + 0.05% formic acid in 3 minutes. **ESI-HRMS (+)**: m/z calcd for $C_{78}H_{113}N_{18}O_{19}$ $[M+H]^+$ 1605.8429, found: 1605.8464.

Synthesis of 6p

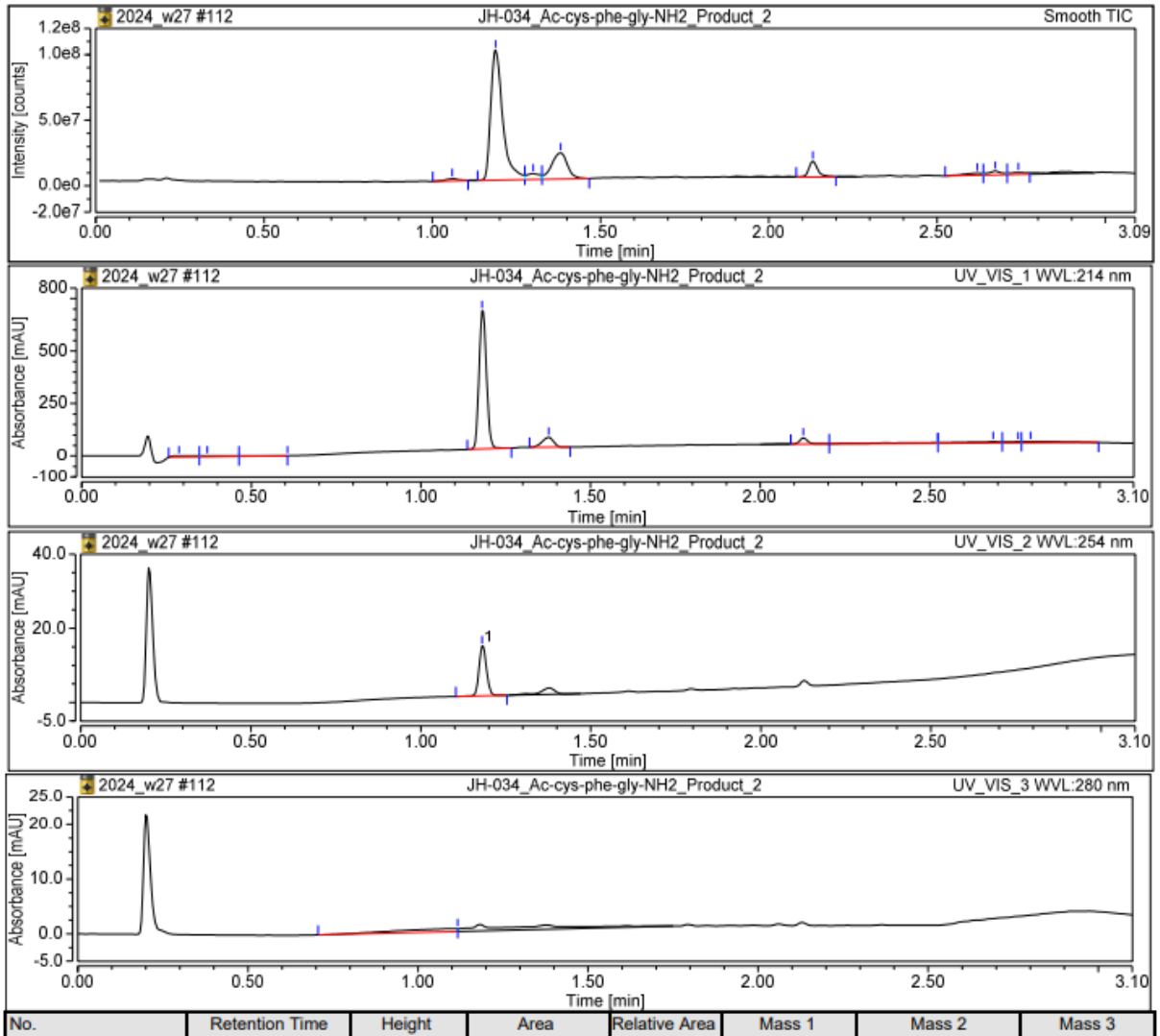
((3S,6S,9S,19S)-12-Acetamido-6-benzyl-3,9-dimethyl-2,5,8,11-tetraoxo-1,4,7,10,14-pentaazacyclononadecane-19-carboxylic acid)



To a 8 mL reaction vial was added **1c** (34.7 mg, 0.05 mmol) and **5** (16.7 mg, 1.2 equiv., 0.06 mmol). Sodium phosphate buffer (50 mM, pH = 9.0, 2 mL) was added and the mixture was stirred while maintaining the pH-value at 9.0 for 30 min at 37°C. Et_3N (0.035 mL, 5 equiv.) was added and the mixture was stirred for 72 hours at 37°C. Preparative RP-HPLC was performed using isocratic elution with 5% ACN (0.1% TFA) in H_2O (0.1% TFA) for 2 minutes, followed by a 5–40% gradient over 14 minutes. Lyophilization of fractions afforded **6p** (white solid, 12.2 mg, 37%) as a 7:1 mixture of diastereomers. **^{1}H NMR (500 MHz, DMSO-d$_6$)** δ 8.84 (d, J = 5.6 Hz, 1H), 8.33 (m, 2H), 8.27 (d, J = 7.9 Hz, 1H), 8.05 (m, 1H), 7.57 (d, J = 8.3 Hz, 1H), 7.26 (m, 4H), 7.20 (m, 1H), 4.69 (ddd, *J* = 11.9, 7.9, 6.2 Hz, 1H), 4.50 (ddd, J = 9.9, 8.3, 4.0 Hz, 1H), 4.31 (ddd, J = 10.3, 8.8, 3.0 Hz, 1H), 4.11 (ddd, *J* = 13.4, 12.6, 6.6 Hz, 1H), 3.92 (ddd, *J* = 14.4, 12.8, 7.0 Hz, 1H), 3.16 (m, 2H), 3.07 (dd, J = 14.3, 3.9 Hz, 1H), 2.92 (m, 1H), 2.84 (dd, J = 14.2, 9.9 Hz, 1H), 1.86 (s, 3H), 1.73 (m, 1H), 1.59 (m, 2H), 1.42 (m, 1H), 1.36 (m, 1H), 1.24 (d, J = 7.1 Hz, 3H), 1.14 (d, J = 7.4 Hz, 3H). **^{13}C NMR (126 MHz, DMSO-d$_6$)** δ 173.6, 172.3, 171.9, 171.4, 169.6, 168.7, 158.5 (q, ^{2}J(C-F), J = 33.8 Hz), 137.5, 129.2, 128.1, 126.4, 116.5 (q, ^{1}J(C-F), J = 295.1 Hz), 52.4, 51.7, 50.5, 49.0, 49.0, 48.4, 48.2, 37.3, 29.7, 24.4, 23.6, 22.6, 17.0, 16.6. **ESI-HRMS (+)**: m/z calcd for $C_{26}H_{39}N_6O_7$ $[M+H]^+$ 547.2880, found: 547.2881.

6. Analytical HPLC chromatograms

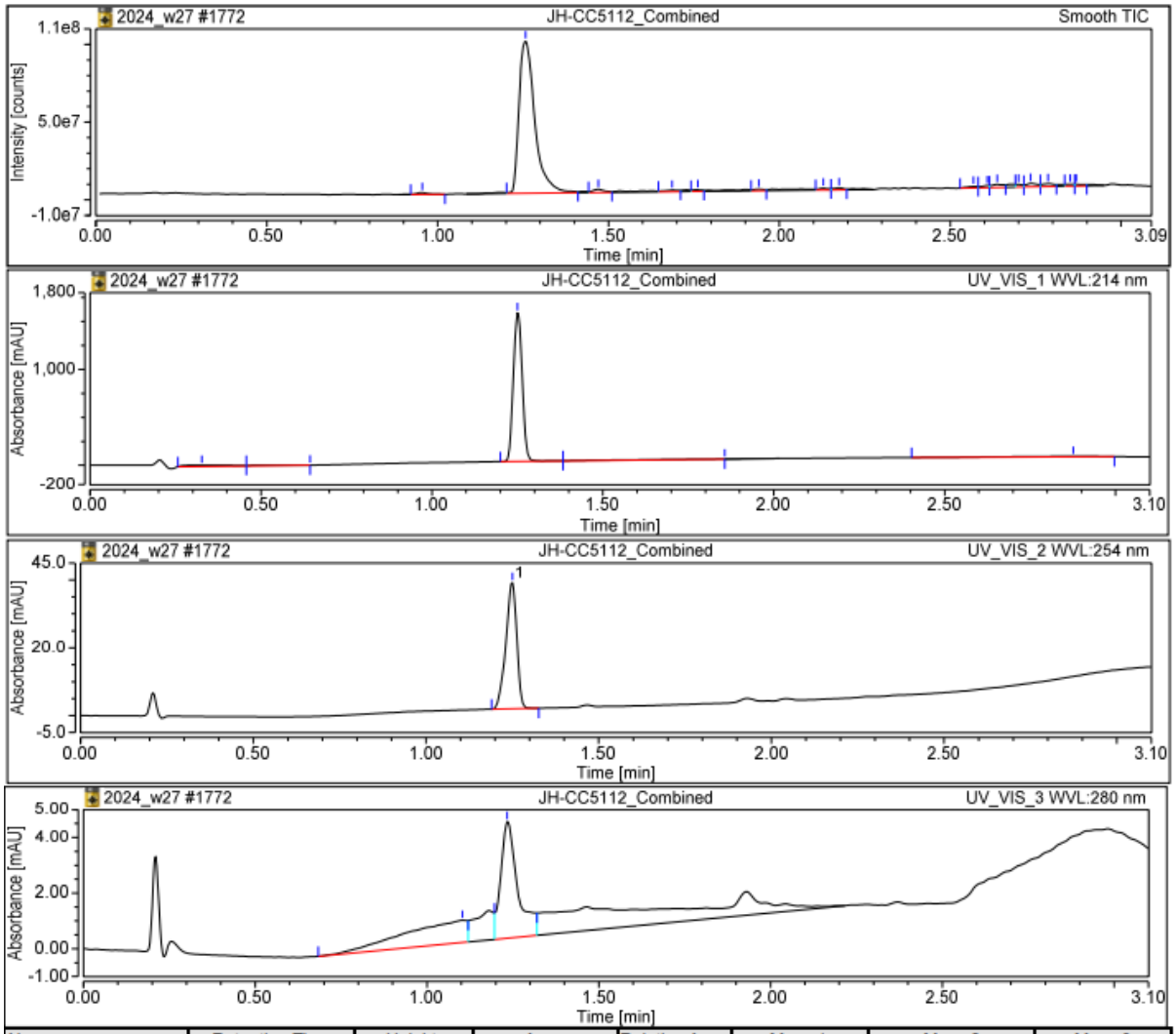
Compound 1a



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.180	13.473	0.345	100.00	367.2	389.2	350.2
Total:		**13.473**	**0.345**	**100.00**			

Figure S17 Analytical HPLC chromatograms of **1a**, 5-100% ACN + 0.05% formic acid in 3 minutes.

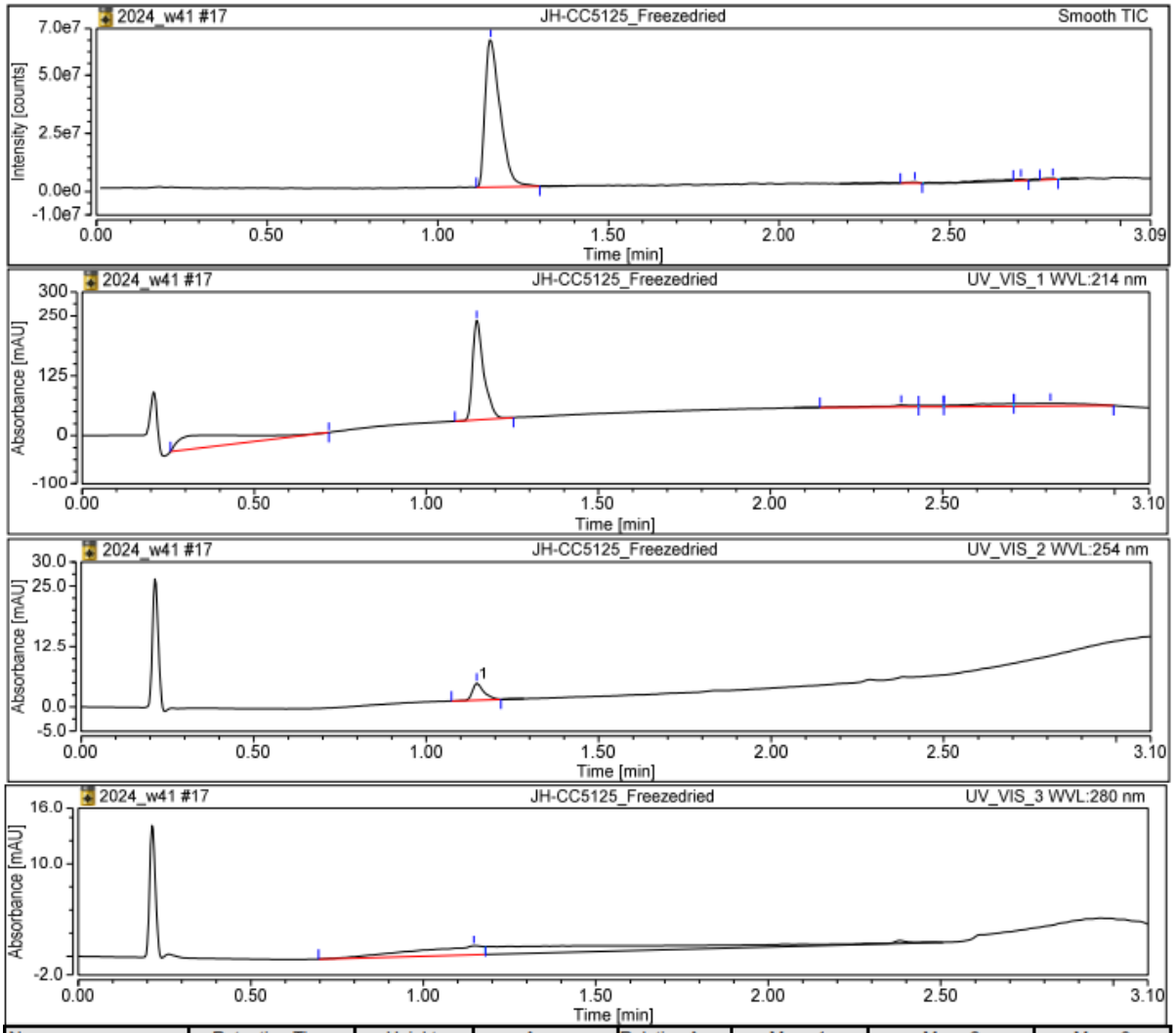
Compound 1b



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.250	37.232	1.361	100.00	368.2	223.2	369.2
Total:		**37.232**	**1.361**	**100.00**			

Figure S18 Analytical HPLC chromatograms of **1b**, 5-100% ACN + 0.05% formic acid in 3 minutes.

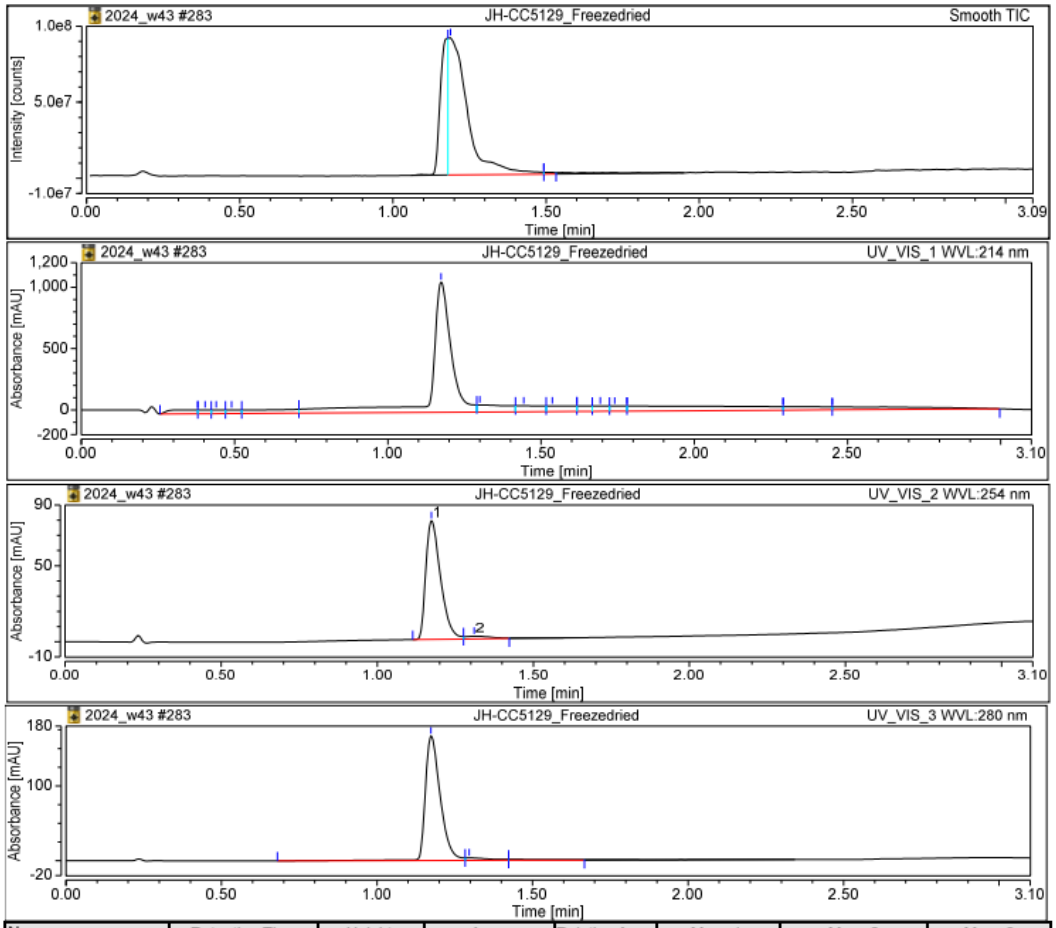
Compound 1c



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.147	3.522	0.134	100.00	581.4	582.5	583.5
Total:		**3.522**	**0.134**	**100.00**			

Figure S19 Analytical HPLC chromatograms of **1c**, 5-100% ACN + 0.05% formic acid in 3 minutes.

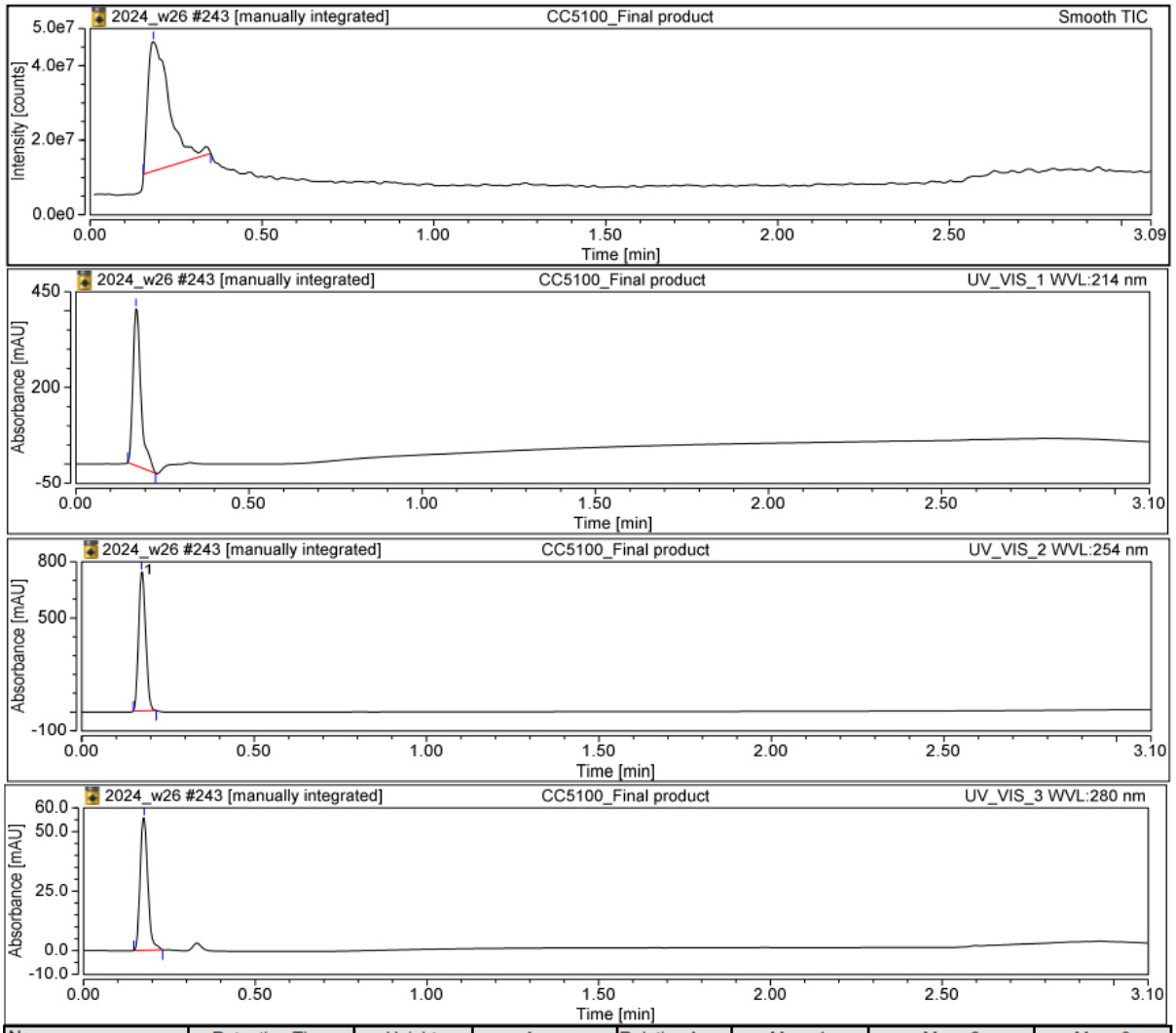
Compound 1d



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.173	78.137	4.260	96.14	498.1	994.6	995.6
2	1.310	1.789	0.171	3.86	498.2	497.6	101.2
Total:		**79.927**	**4.431**	**100.00**			

Figure S20 Analytical HPLC chromatograms of **1d**, 5-100% ACN + 0.05% formic acid in 3 minutes.

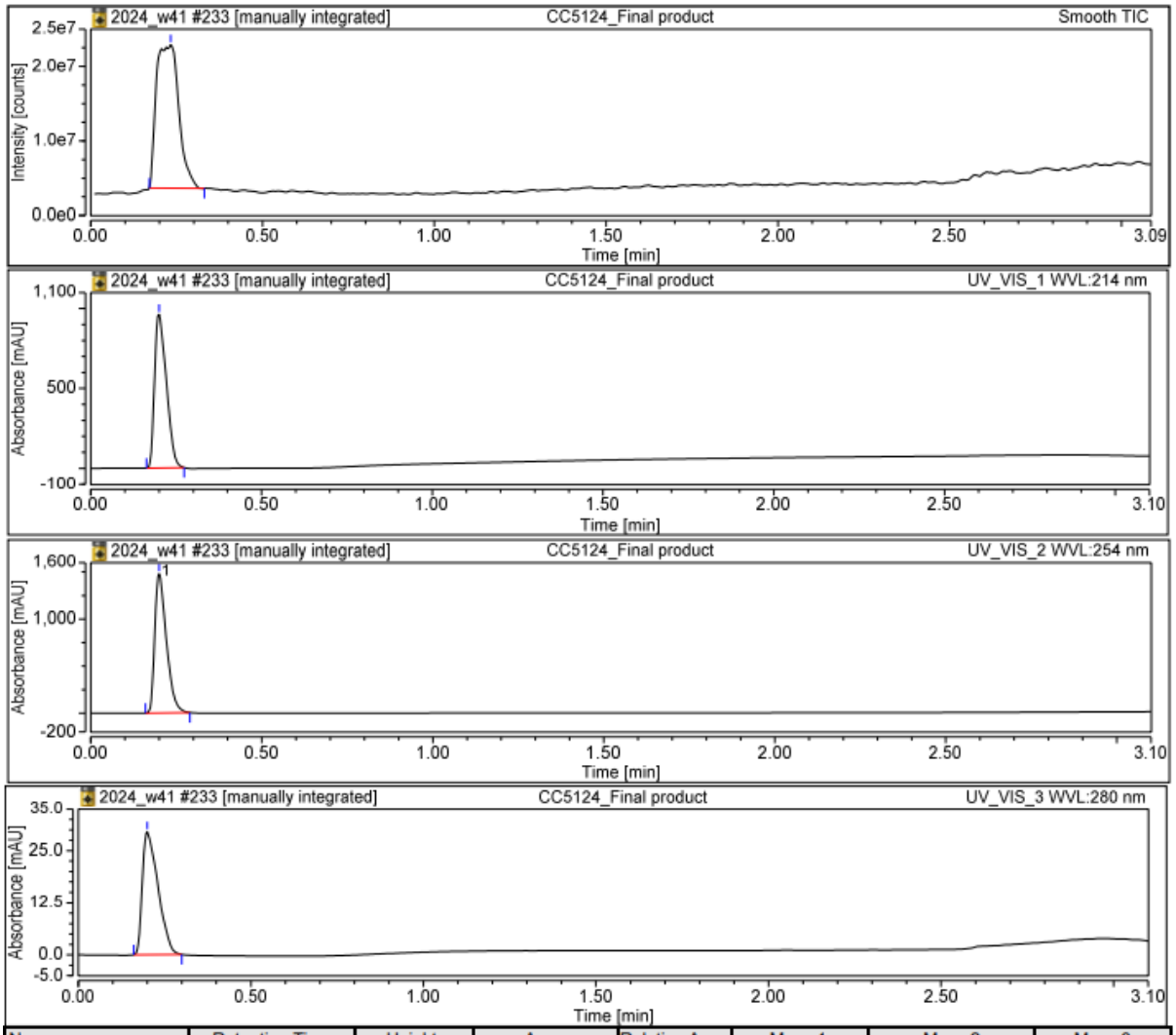
Compound 2a



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.173	738.075	17.684	100.00	112.1	113.2	110.1
Total:		**738.075**	**17.684**	**100.00**			

Figure S21 Analytical HPLC chromatograms of **2a**, 5-100% ACN + 0.05% formic acid in 3 minutes.

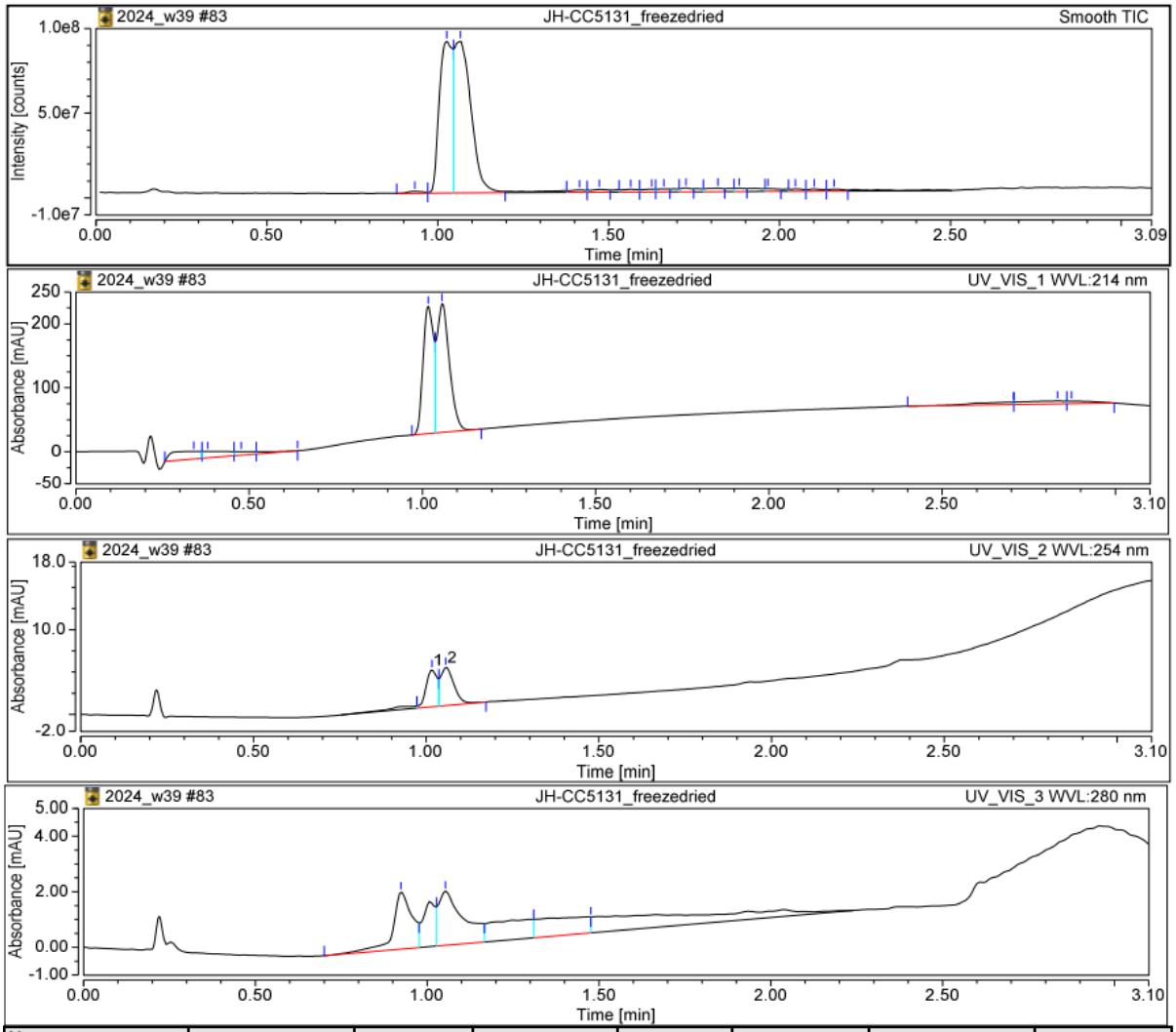
Compound 2b



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.200	1481.091	58.030	100.00	128.2	130.2	129.3
Total:		**1481.091**	**58.030**	**100.00**			

Figure S22 Analytical HPLC chromatograms of **2b**, 5-100% ACN + 0.05% formic acid in 3 minutes.

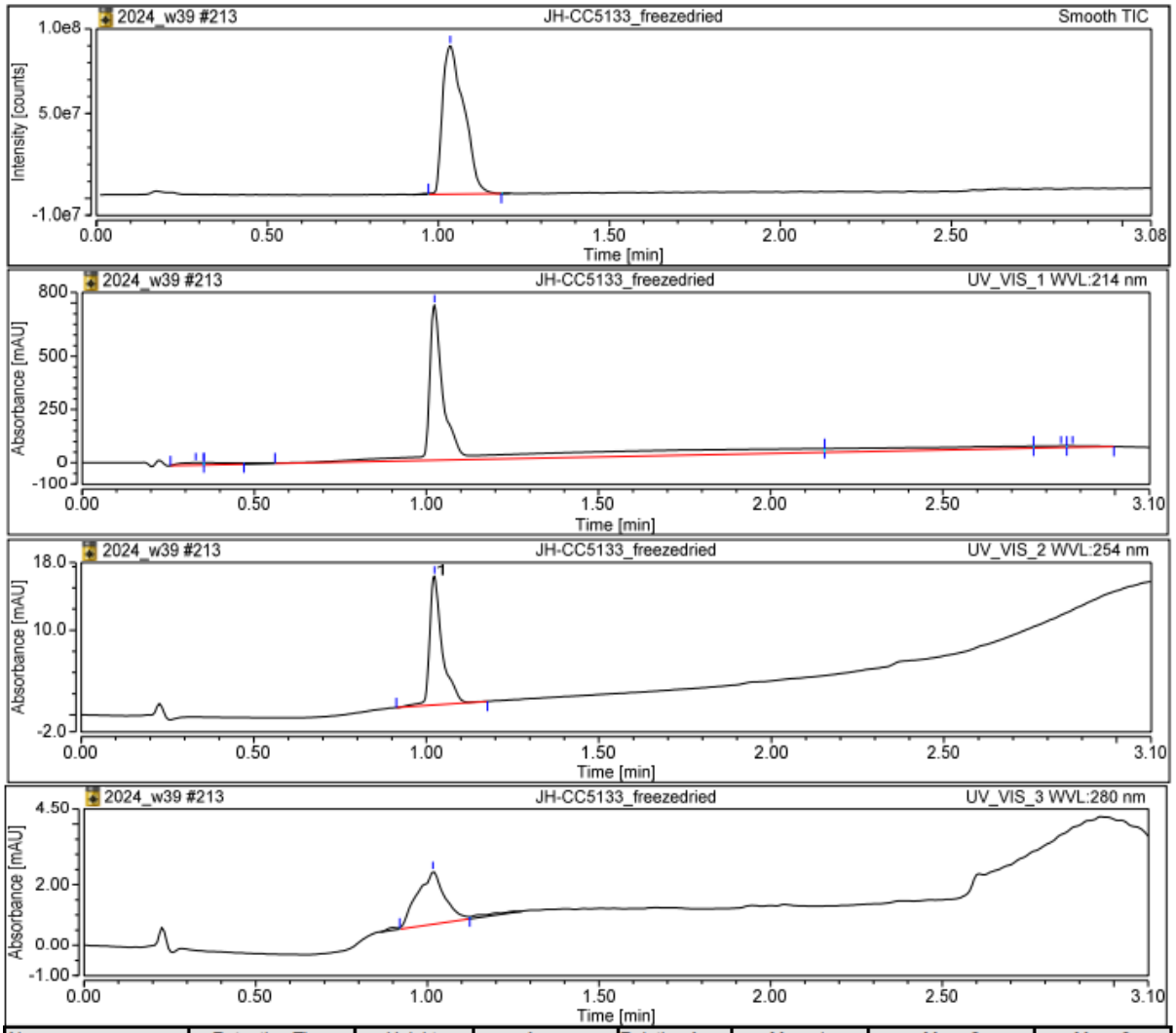
Compound 6a



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.017	4.386	0.160	44.09	419.4	420.4	421.4
2	1.057	4.522	0.203	55.91	419.4	420.4	421.4
Total:		**8.908**	**0.363**	**100.00**			

Figure S23 Analytical HPLC chromatograms of compound **6a**, 5-100% ACN + 0.05% formic acid in 3 minutes.

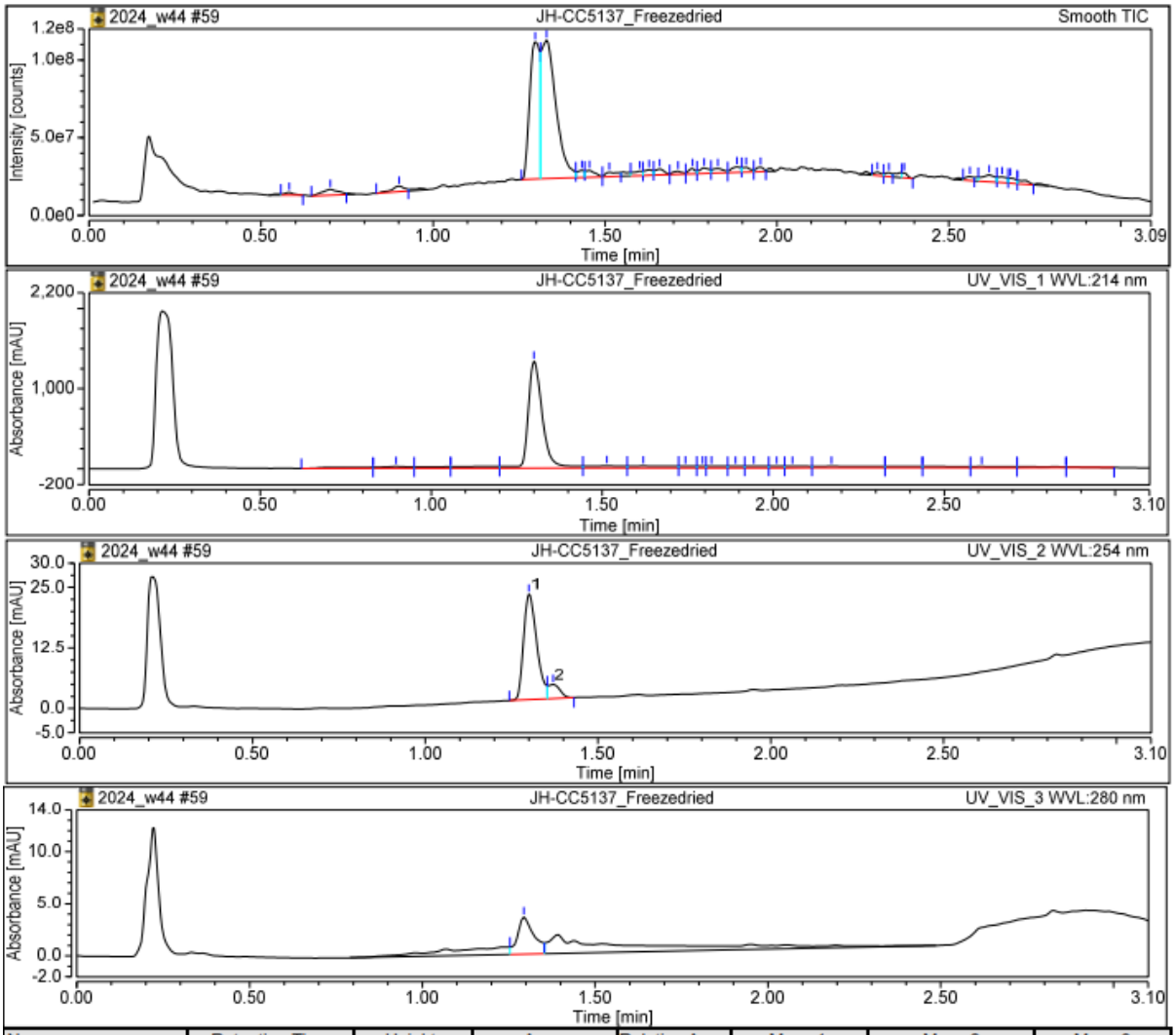
Compound 6b



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.023	15.219	0.649	100.00	449.3	450.4	451.4
Total:		**15.219**	**0.649**	**100.00**			

Figure S24 Analytical HPLC chromatograms of compound **6b**, 5-100% ACN + 0.05% formic acid in 3 minutes.

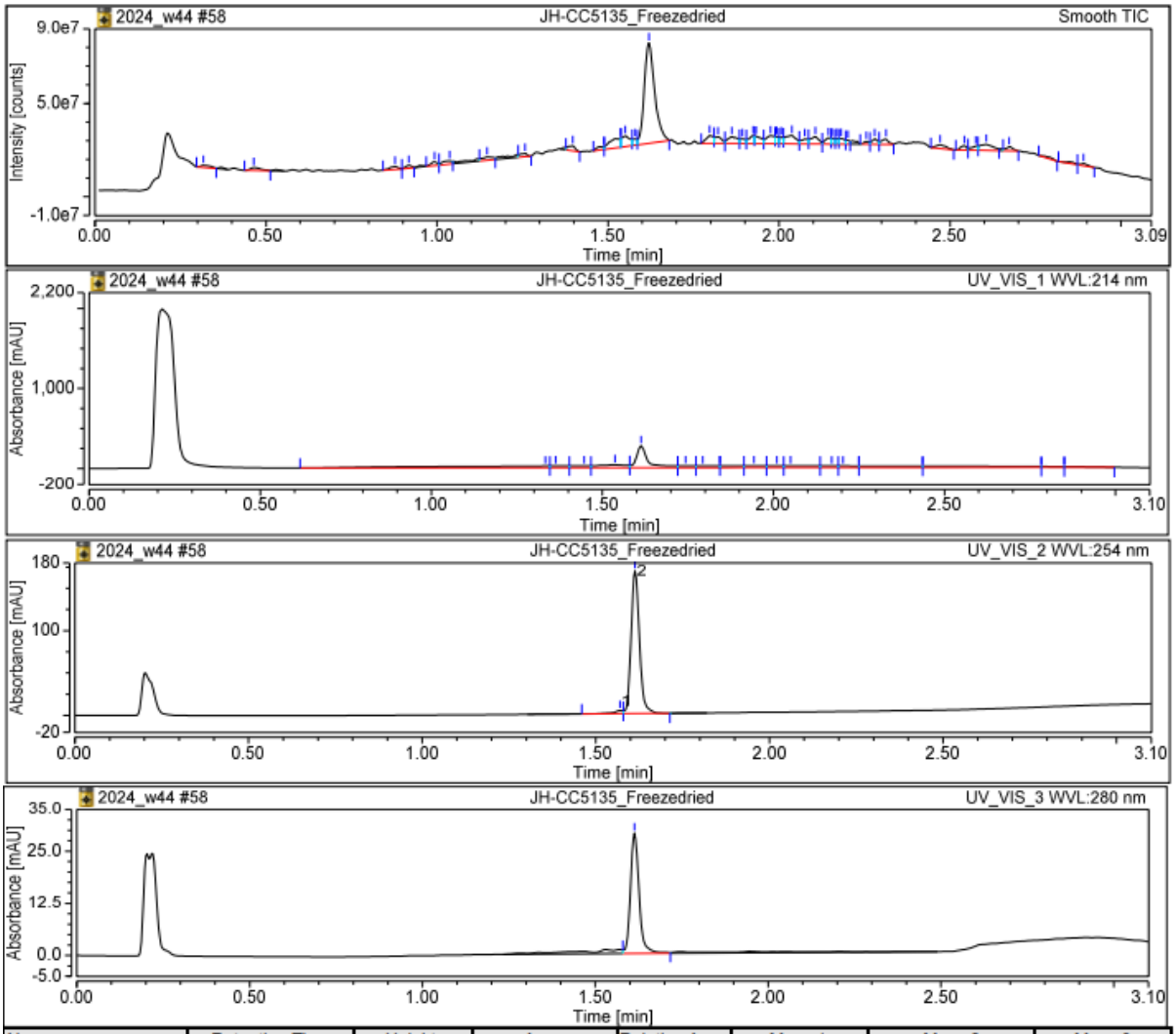
Compound 6c



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.300	21.758	0.957	89.43	589.3	590.4	591.4
2	1.370	2.865	0.113	10.57	589.3	126.1	590.4
Total:		**24.624**	**1.070**	**100.00**			

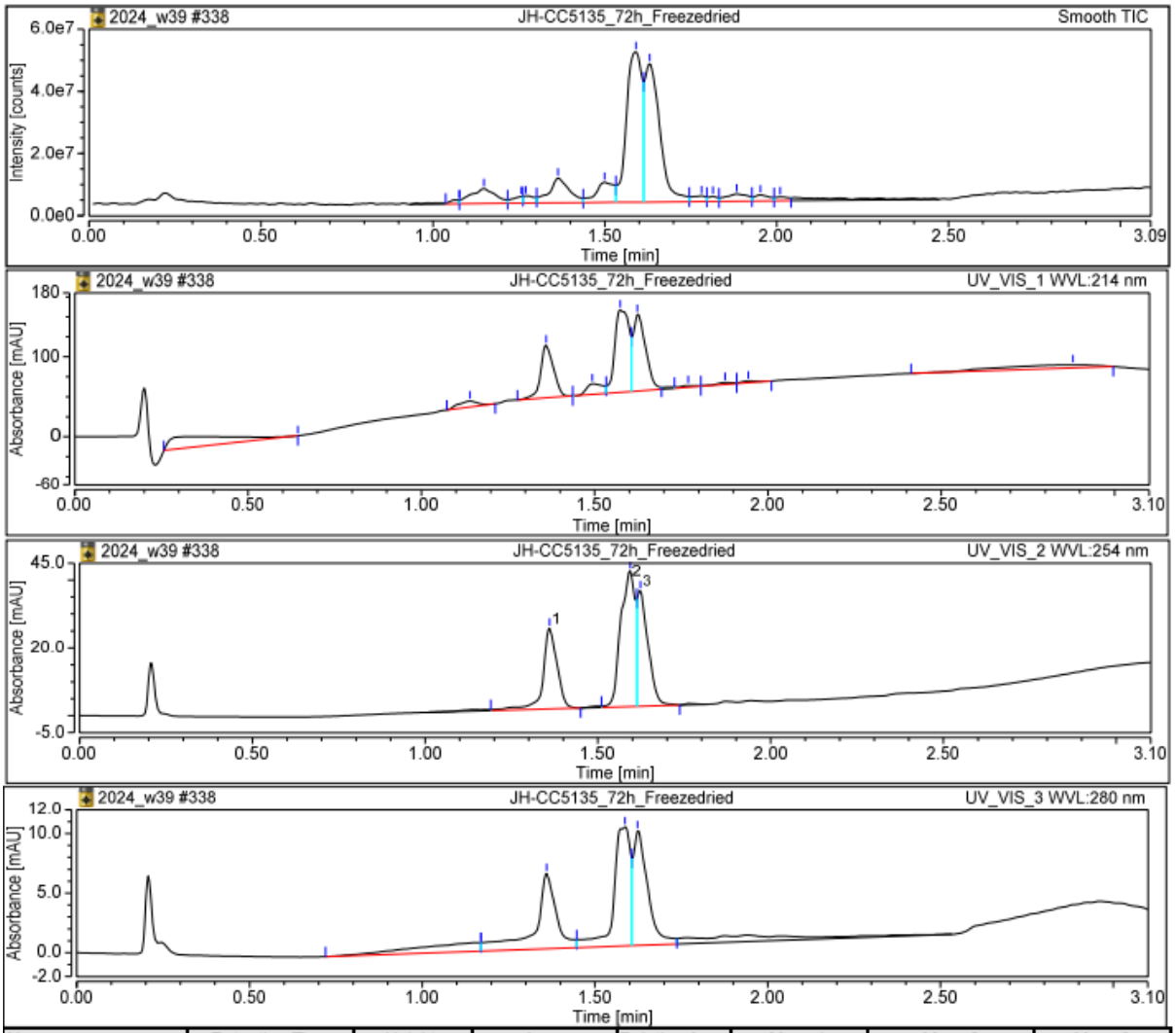
Figure S25 Analytical HPLC chromatograms of compound **6c**, 5-100% ACN + 0.05% formic acid in 3 minutes.

Compound 6d



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.570	3.807	0.121	2.51	126.1	427.3	169.1
2	1.613	168.445	4.712	97.49	427.2	428.3	126.4
Total:		**172.251**	**4.833**	**100.00**			

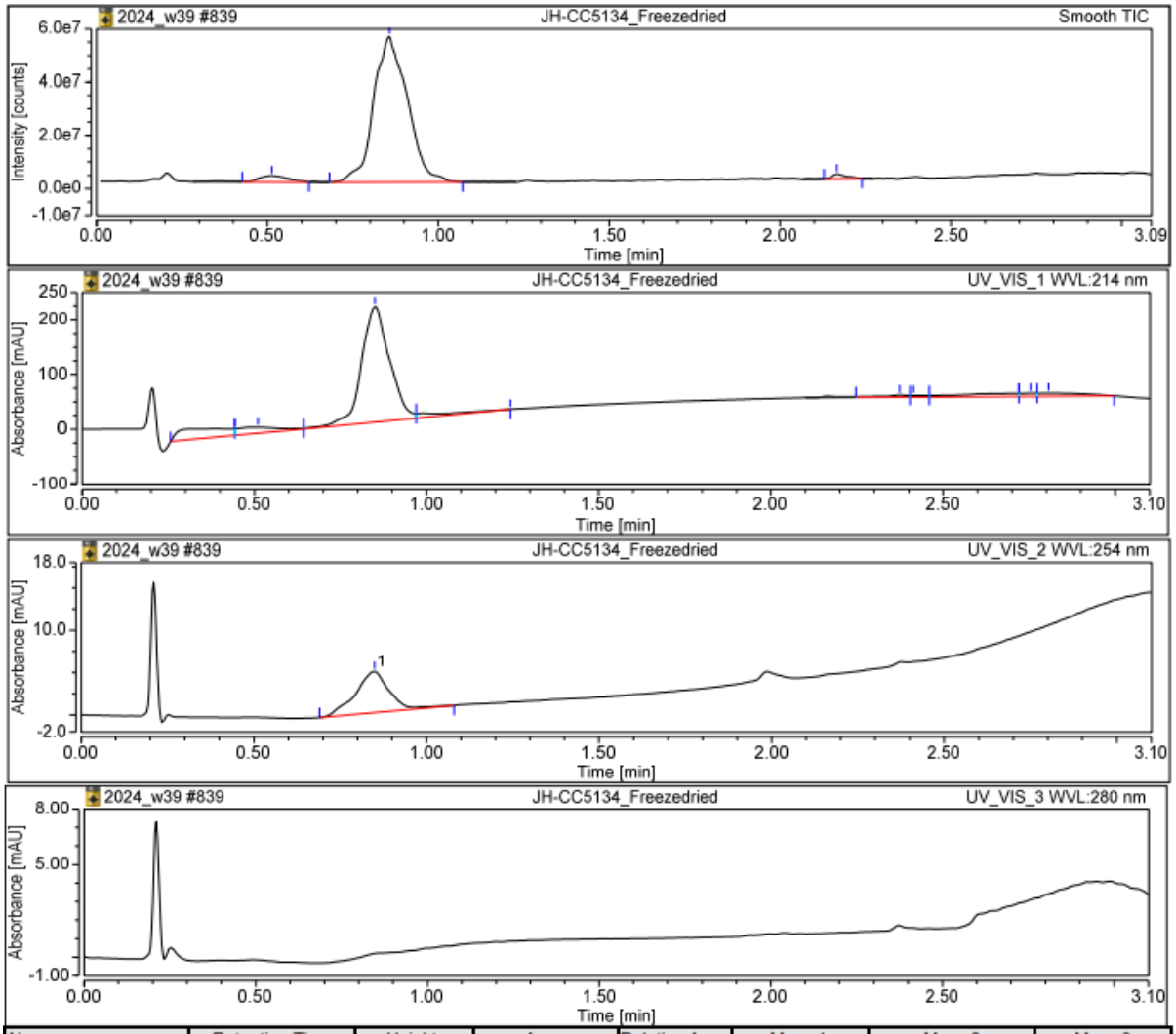
Figure S26 Analytical HPLC chromatograms of compound **6d**, 5-100% ACN + 0.05% formic acid in 3 minutes.



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.360	23.759	1.130	26.44	293.2	102.3	101.3
2	1.593	40.019	1.929	45.14	427.3	368.2	449.3
3	1.623	34.035	1.215	28.42	368.3	427.3	449.3
Total:		**97.814**	**4.274**	**100.00**			

Figure S27 Analytical HPLC chromatograms of compound **6d** after the first purification, 5-100% ACN + 0.05% formic acid in 3 minutes.

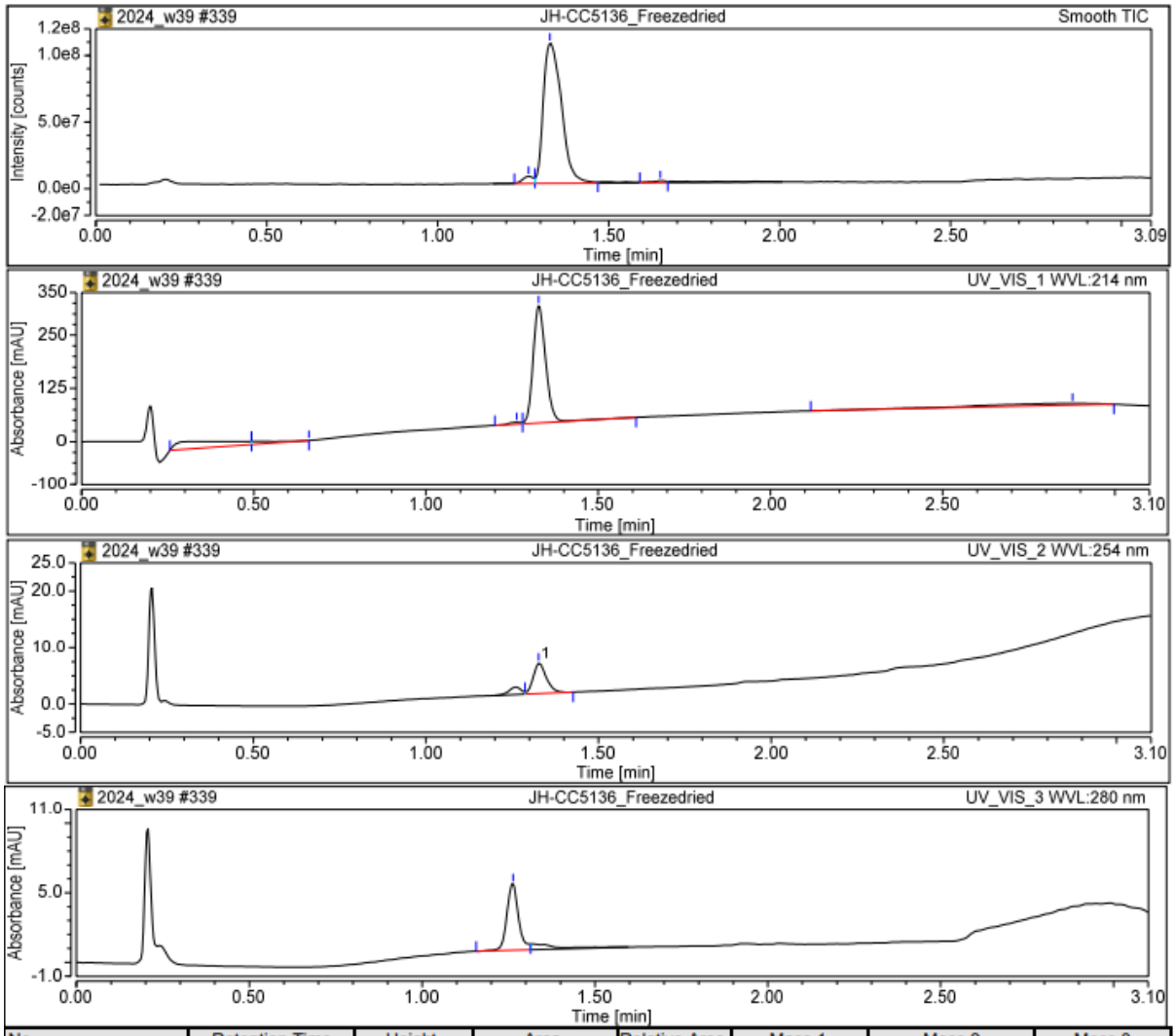
Compound 6e



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.850	4.874	0.546	100.00	409.3	410.3	391.3
Total:		**4.874**	**0.546**	**100.00**			

Figure S28 Analytical HPLC chromatograms of compound **6e**, 5-100% ACN + 0.05% formic acid in 3 minutes.

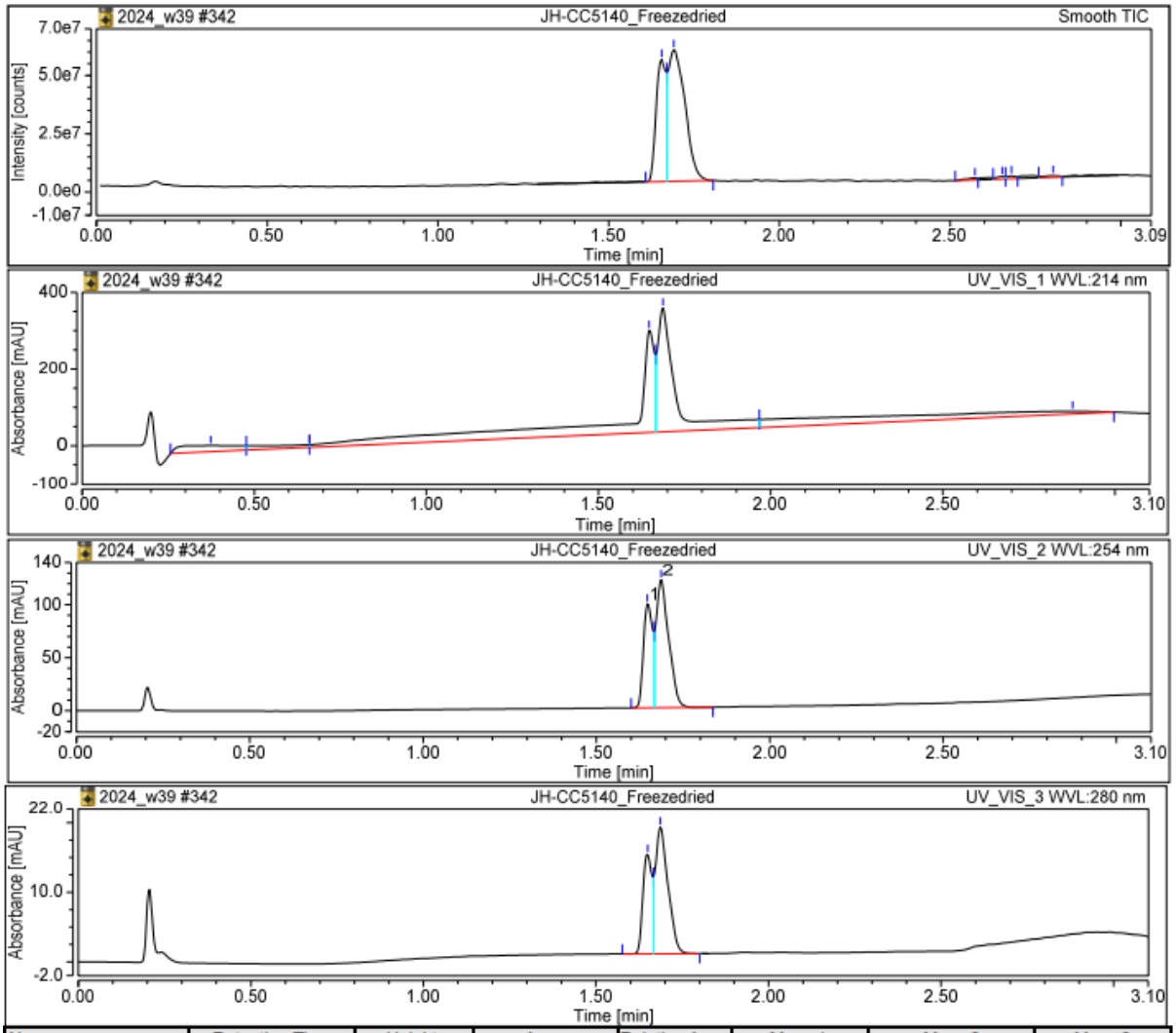
Compound 6f



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.327	5.411	0.239	100.00	580.4	581.4	582.5
Total:		**5.411**	**0.239**	**100.00**			

Figure S29 Analytical HPLC chromatograms of compound **6f**, 5-100% ACN + 0.05% formic acid in 3 minutes.

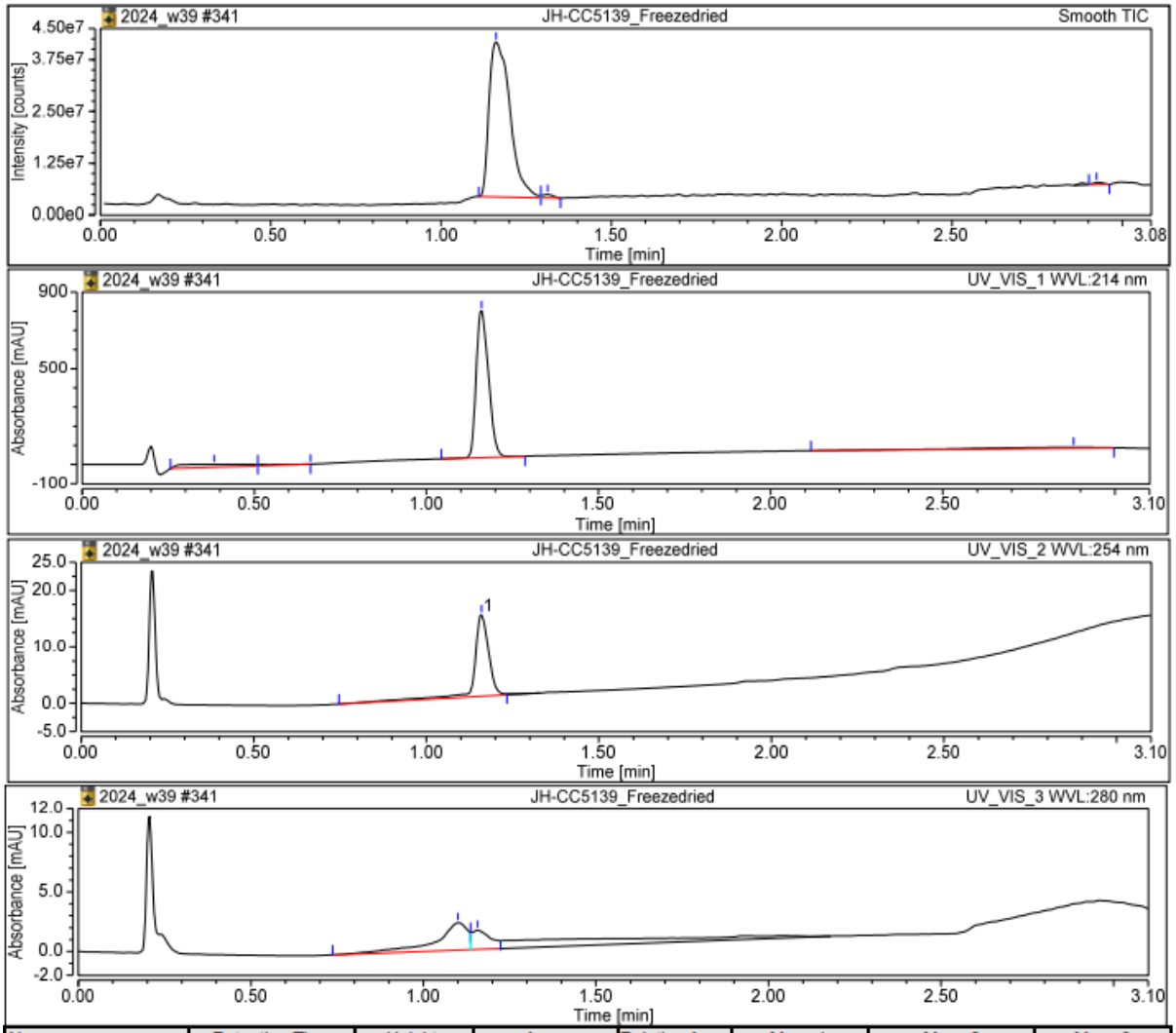
Compound 6g



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.647	98.027	2.966	36.91	444.3	466.2	445.3
2	1.687	120.756	5.070	63.09	444.3	466.2	445.4
Total:		**218.783**	**8.036**	**100.00**			

Figure S30 Analytical HPLC chromatograms of compound **6g**, 5-100% ACN + 0.05% formic acid in 3 minutes.

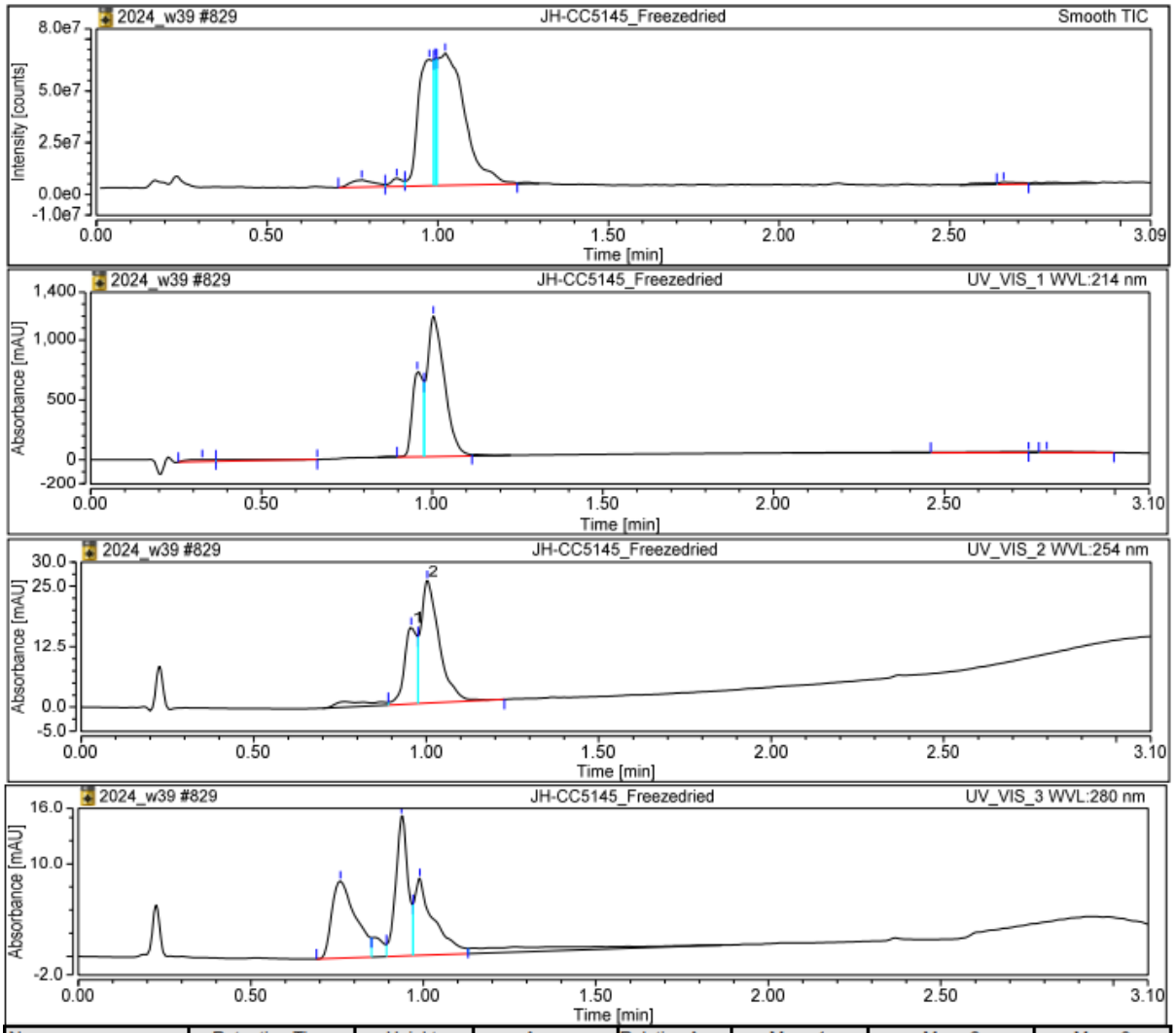
Compound 6h



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.160	14.403	0.686	100.00	497.3	498.3	223.2
Total:		**14.403**	**0.686**	**100.00**			

Figure S31 Analytical HPLC chromatograms of compound **6h**, 5-100% ACN + 0.05% formic acid in 3 minutes.

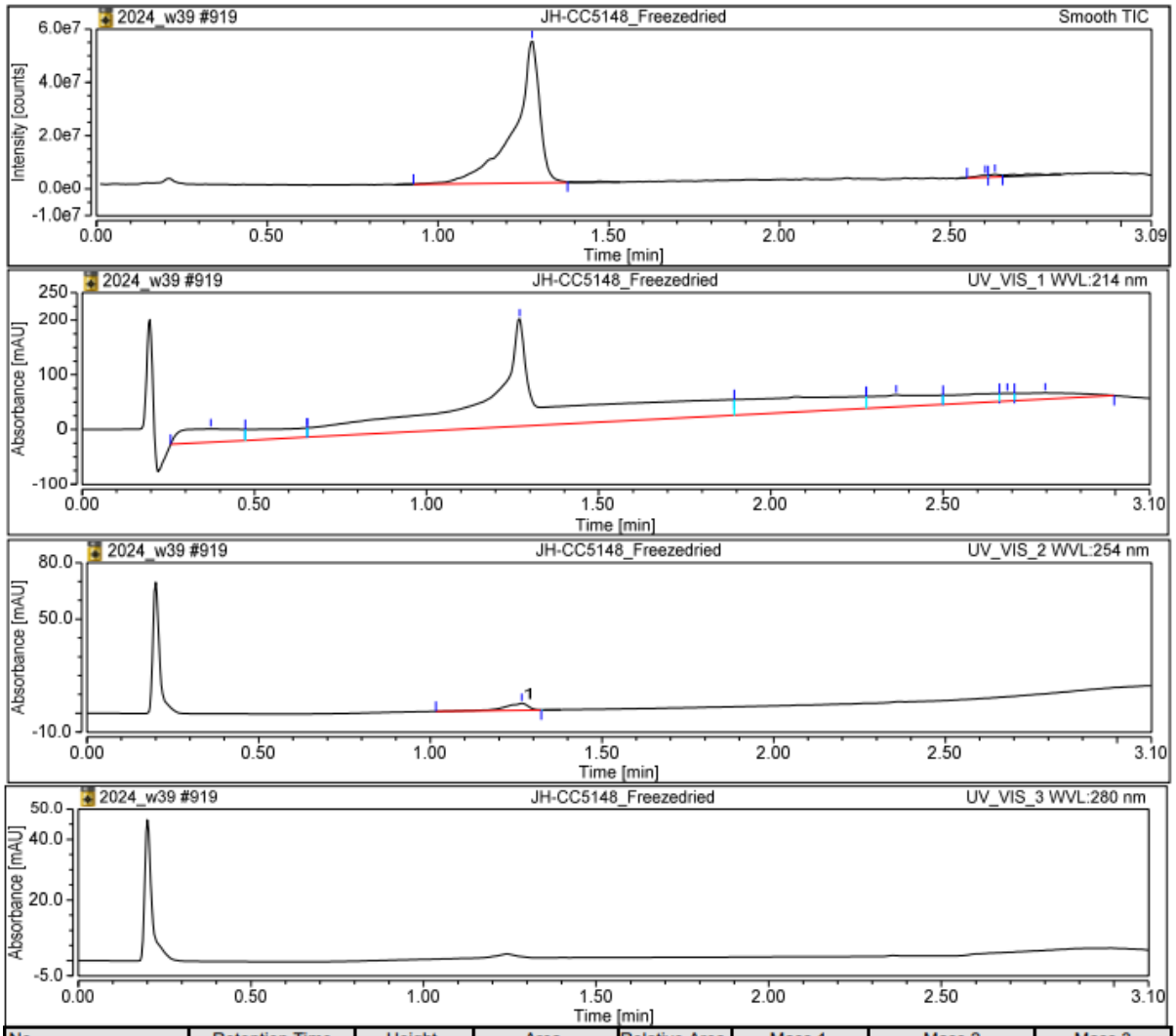
Compound 6i



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	0.957	15.853	0.666	30.08	584.4	585.4	586.4
2	1.003	25.357	1.548	69.92	584.4	585.4	586.4
Total:		**41.210**	**2.214**	**100.00**			

Figure S32 Analytical HPLC chromatograms of compound **6i**, 5-100% ACN + 0.05% formic acid in 3 minutes.

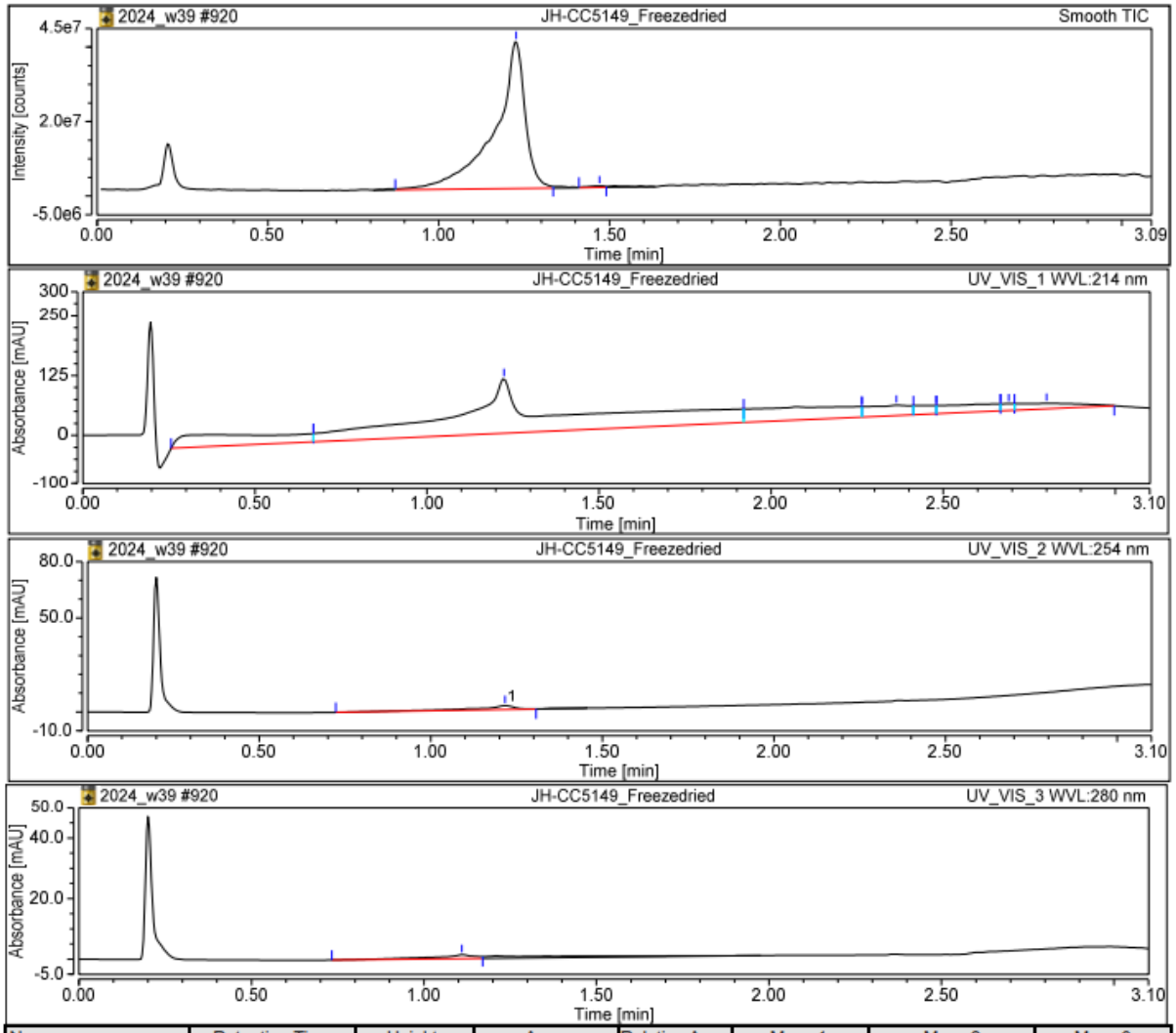
Compound 6j



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.267	3.699	0.289	100.00	753.5	754.5	755.6
Total:		**3.699**	**0.289**	**100.00**			

Figure S33 Analytical HPLC chromatograms of compound **6j**, 5-100% ACN + 0.05% formic acid in 3 minutes.

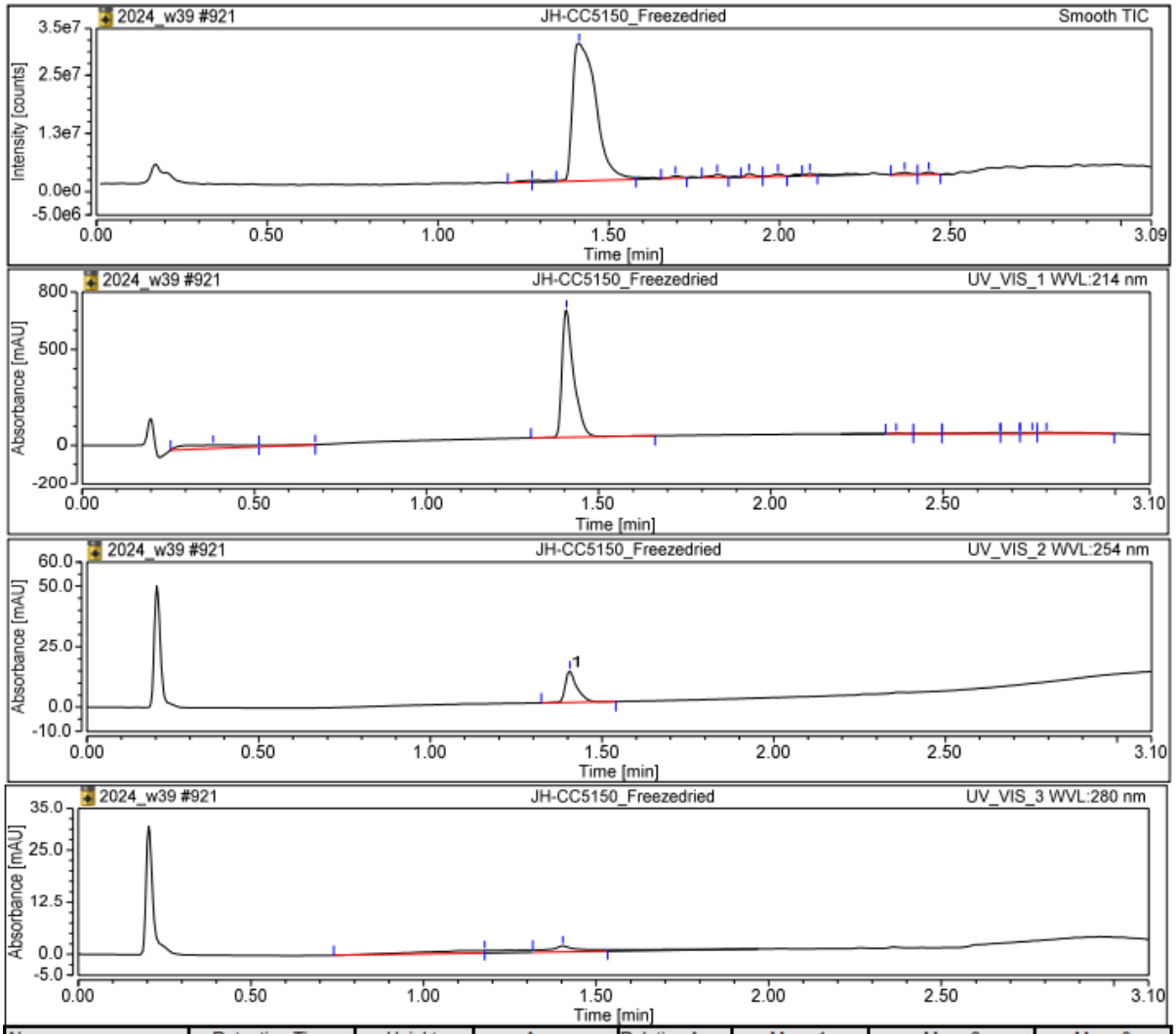
Compound 6k



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.217	2.135	0.290	100.00	727.5	728.5	729.6
Total:		**2.135**	**0.290**	**100.00**			

Figure S34 Analytical HPLC chromatograms of compound **6k**, 5-100% ACN + 0.05% formic acid in 3 minutes.

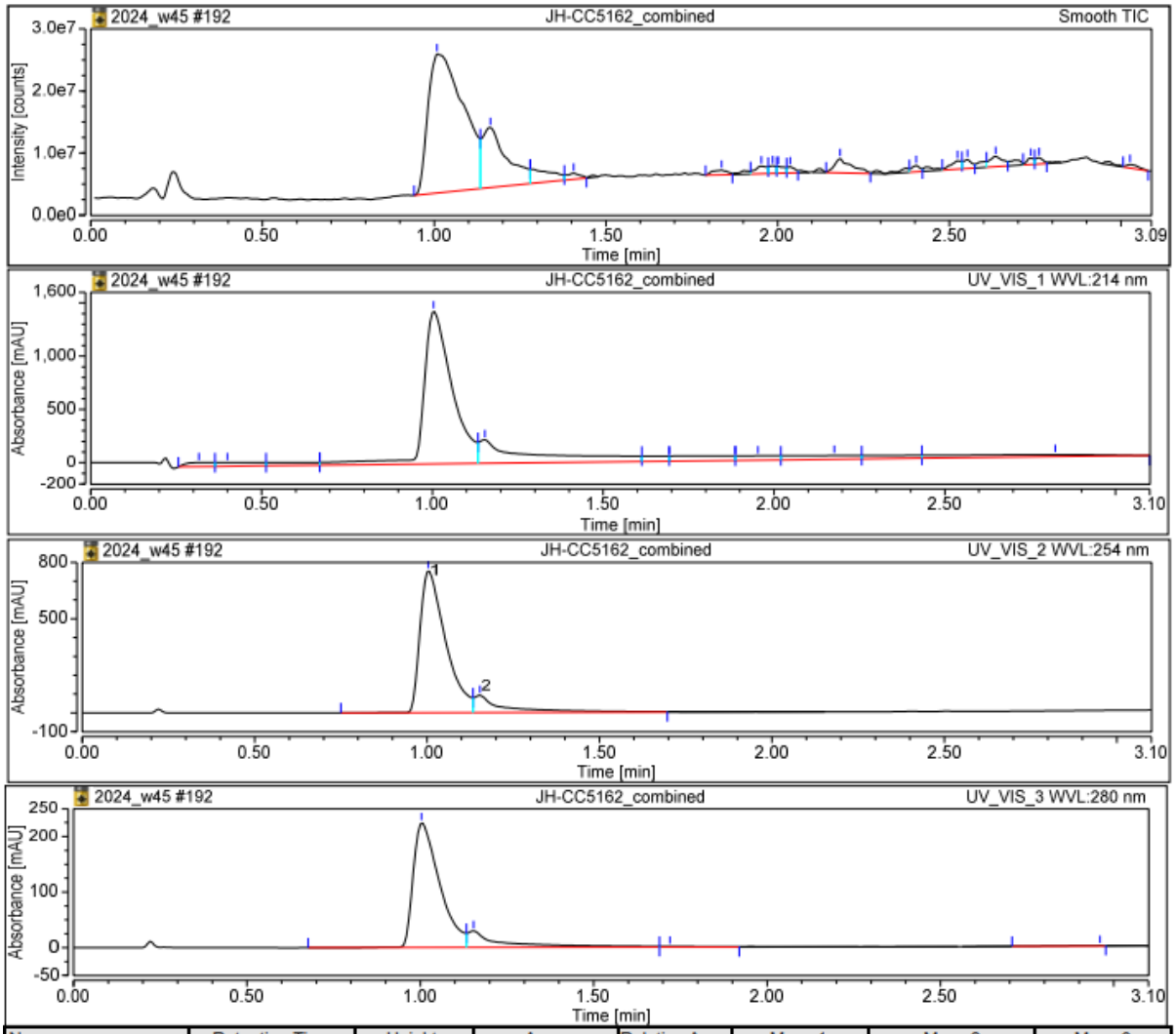
Compound 6l



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.407	12.938	0.510	100.00	821.4	822.4	823.5
Total:		**12.938**	**0.510**	**100.00**			

Figure S35 Analytical HPLC chromatograms of compound **6l**, 5-100% ACN + 0.05% formic acid in 3 minutes.

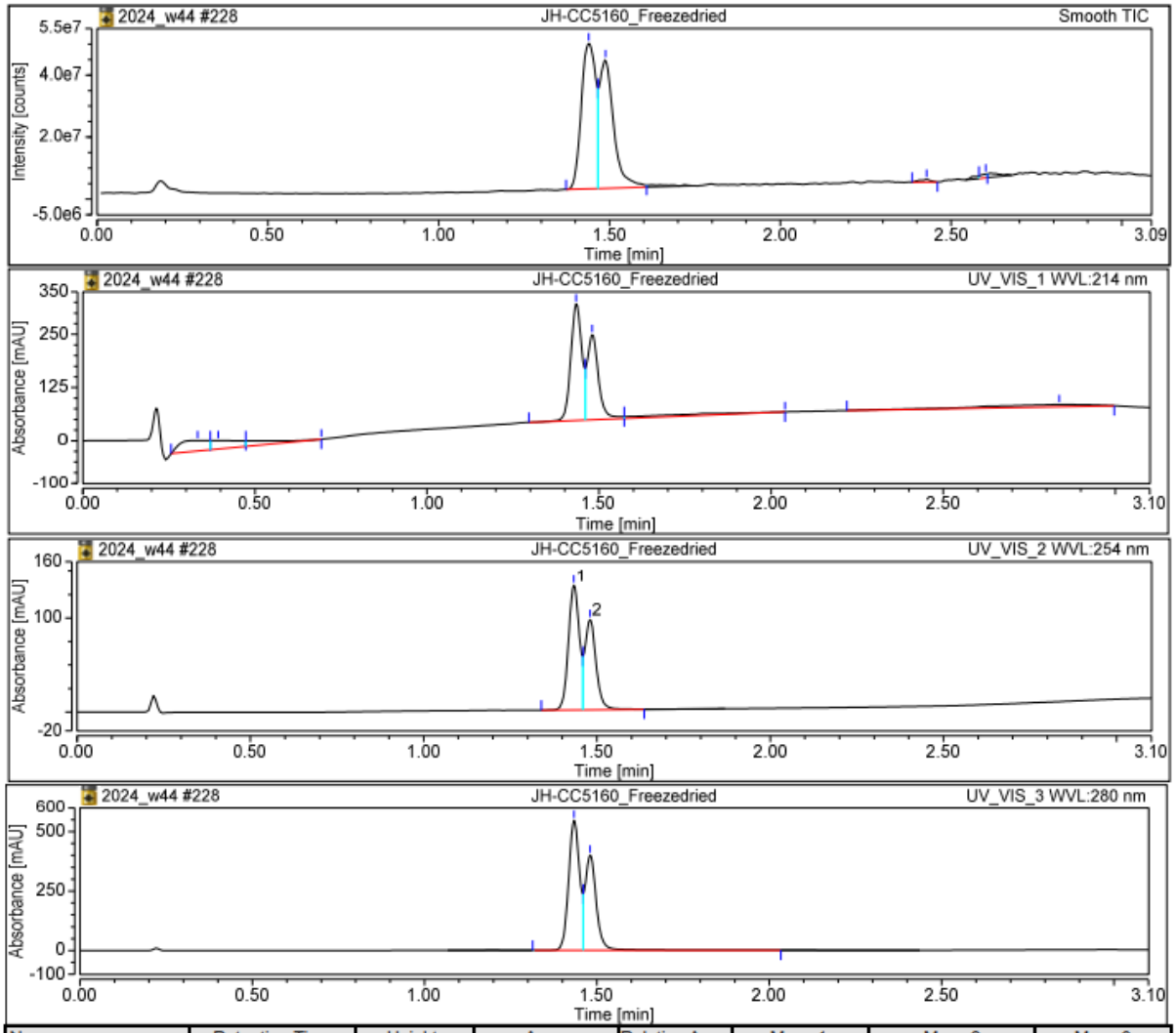
Compound 6m



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.003	755.099	64.333	86.77	551.8	1101.4	551.0
2	1.153	91.875	9.813	13.23	563.5	594.3	1101.4
Total:		**846.974**	**74.146**	**100.00**			

Figure S36 Analytical HPLC chromatograms of compound **6m**, 5-100% ACN + 0.05% formic acid in 3 minutes.

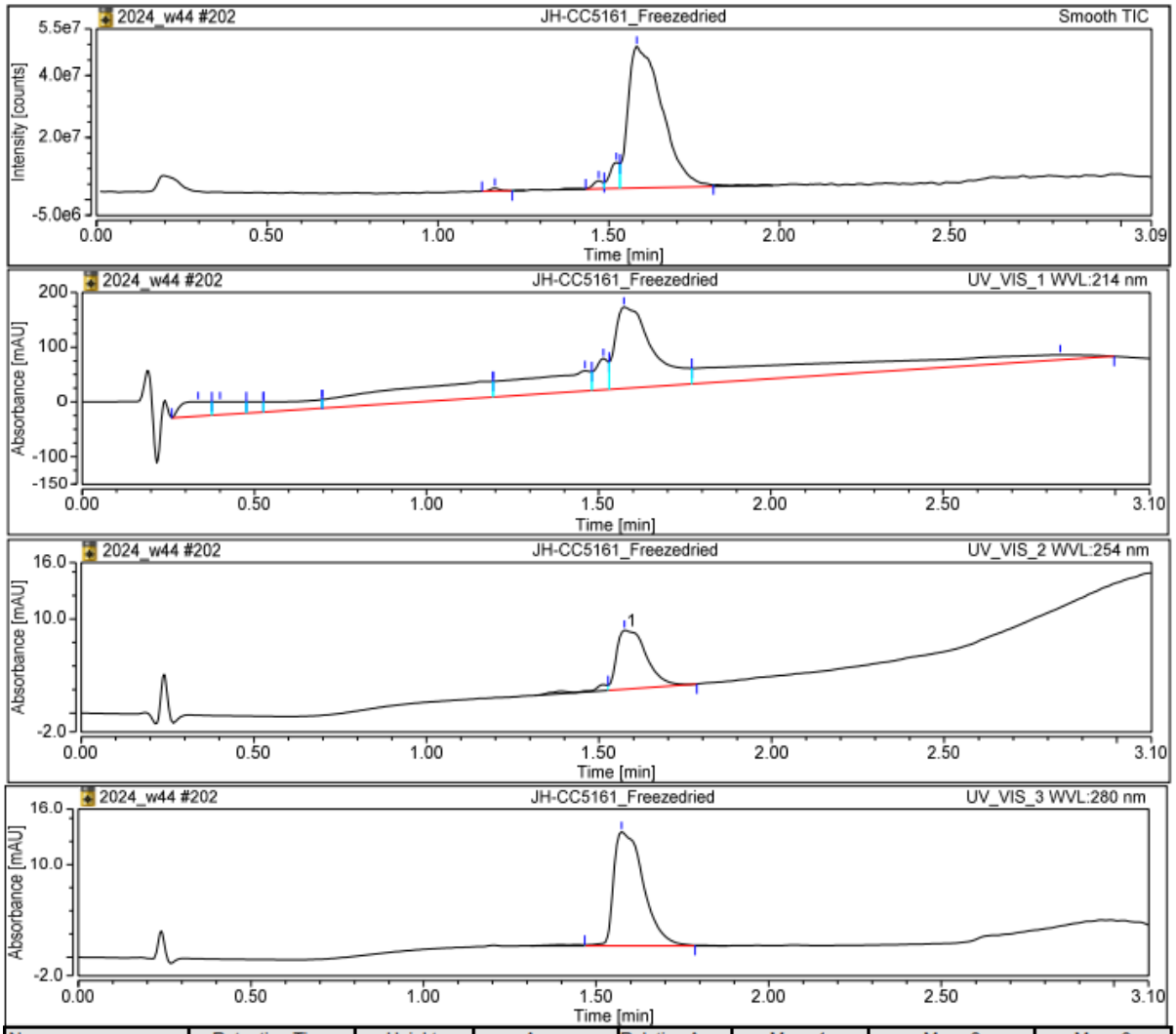
Compound 6n



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.433	132.811	4.874	57.74	665.3	423.2	666.4
2	1.480	96.008	3.567	42.26	665.3	666.3	423.2
Total:		**228.819**	**8.442**	**100.00**			

Figure S37 Analytical HPLC chromatograms of compound **6n**, 5-100% ACN + 0.05% formic acid in 3 minutes.

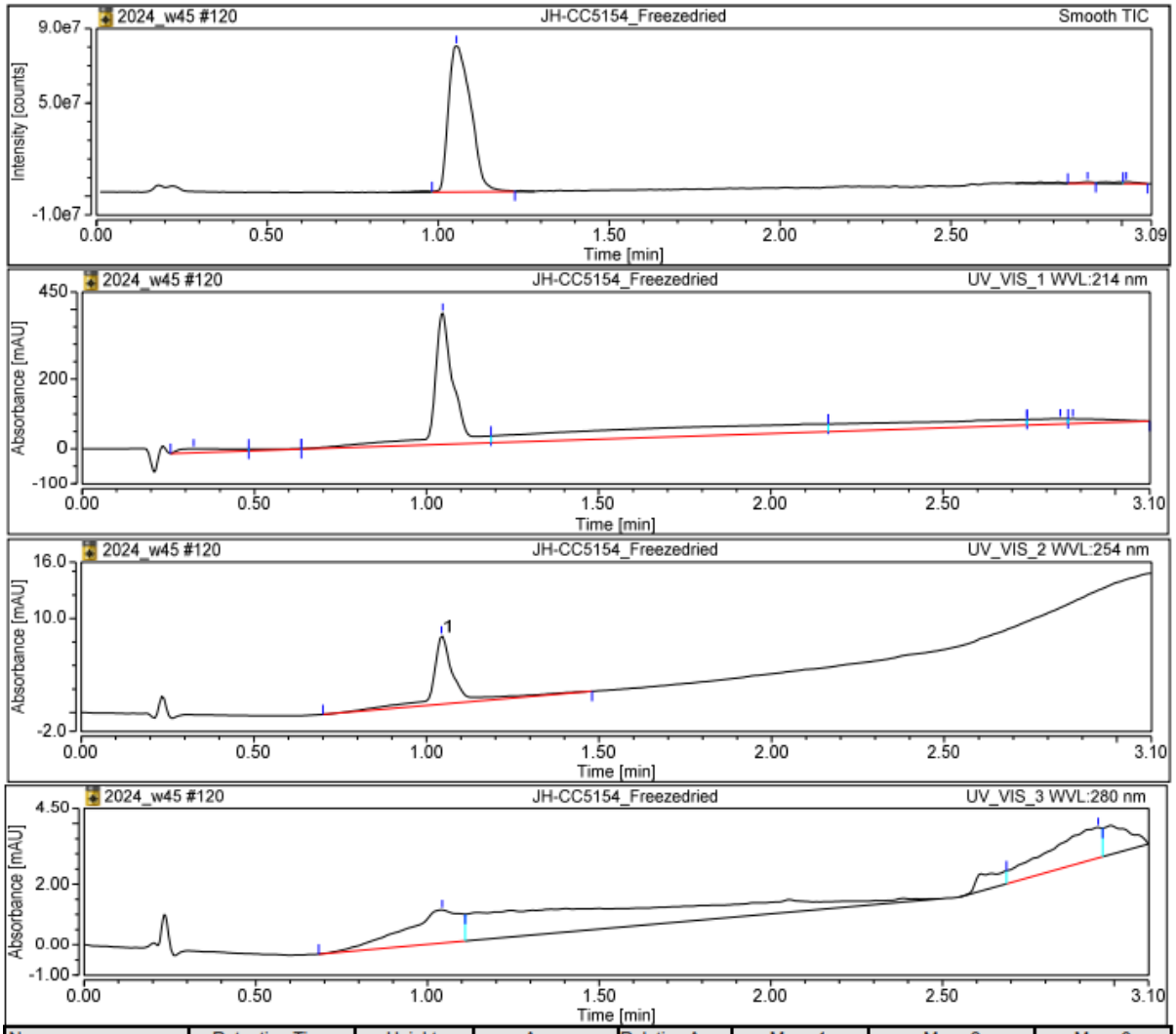
Compound 6o



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.573	6.258	0.617	100.00	804.2	803.3	101.1
Total:		**6.258**	**0.617**	**100.00**			

Figure S38 Analytical HPLC chromatograms of compound **6o**, 5-100% ACN + 0.05% formic acid in 3 minutes.

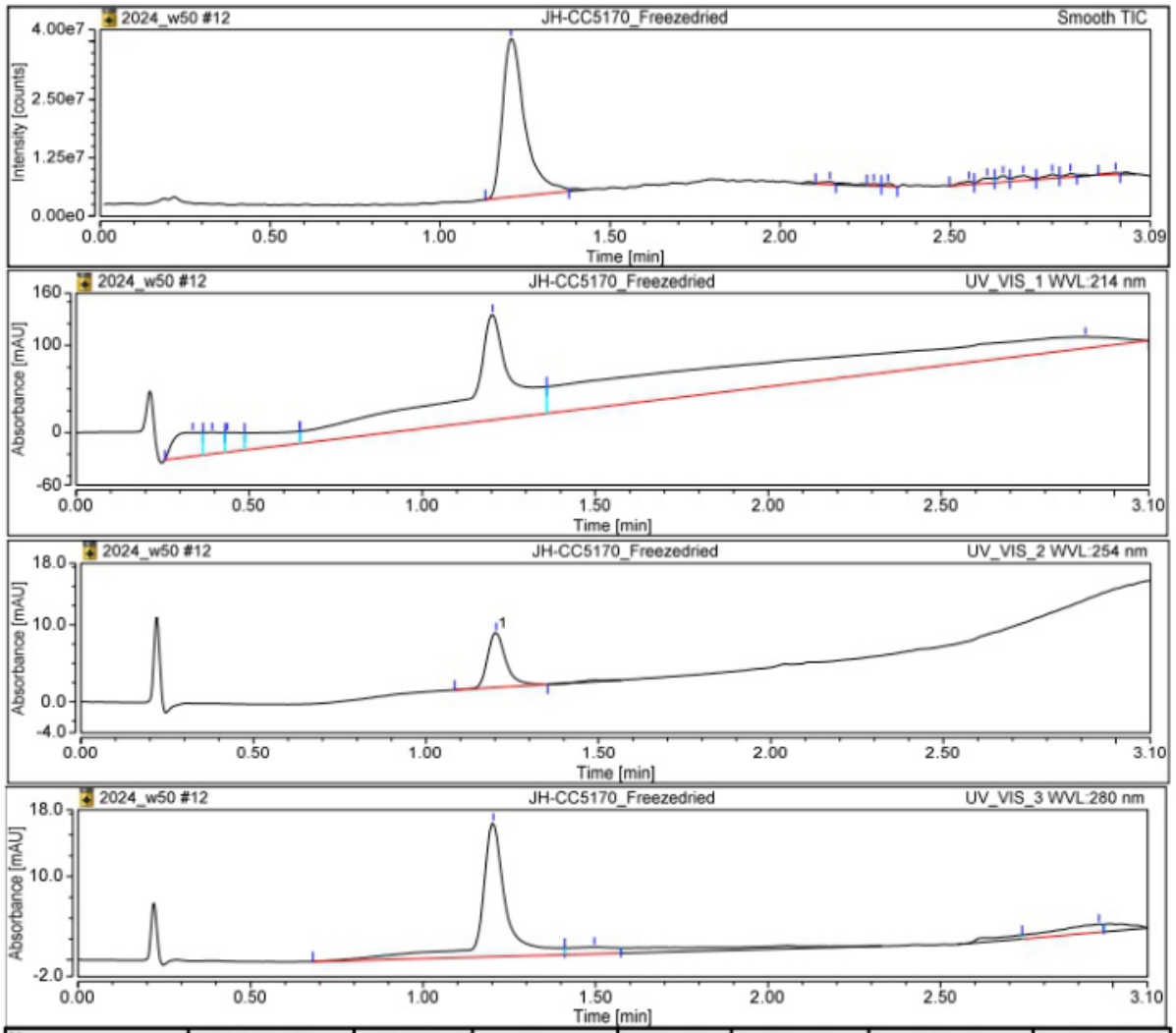
Compound 6p



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.043	7.252	0.532	100.00	547.4	548.4	529.4
Total:		**7.252**	**0.532**	**100.00**			

Figure S39 Analytical HPLC chromatograms of **6p**, 5-100% ACN + 0.05% formic acid in 3 minutes.

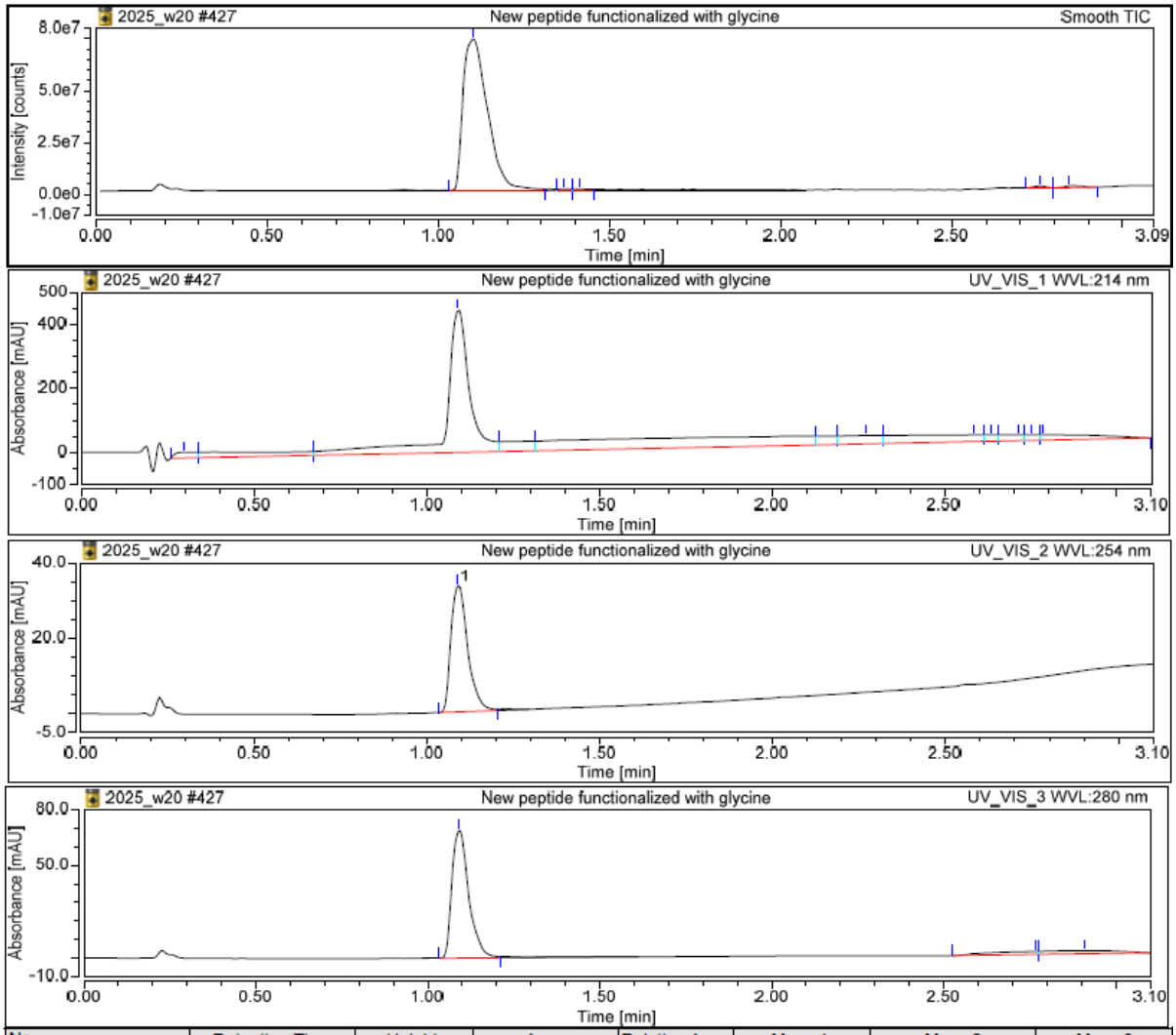
Compound 6q



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.203	7.115	0.441	100.00	524.0	349.7	363.4
Total:		**7.115**	**0.441**	**100.00**			

Figure S40 Analytical HPLC chromatograms of peptide **6q,** 5-100% ACN + 0.05% formic acid in 3 minutes.

Compound 6r



No.	Retention Time min	Height mAU	Area mAU*min	Relative Area %	Mass 1 m/z	Mass 2 m/z	Mass 3 m/z
1	1.090	33.527	1.875	100.00	518.4	346.0	519.9
Total:		**33.527**	**1.875**	**100.00**			

Figure S41 Analytical HPLC chromatograms **6r,** 5-100% ACN + 0.05% formic acid in 3 minutes.

7. NMR spectra

Compound 1a

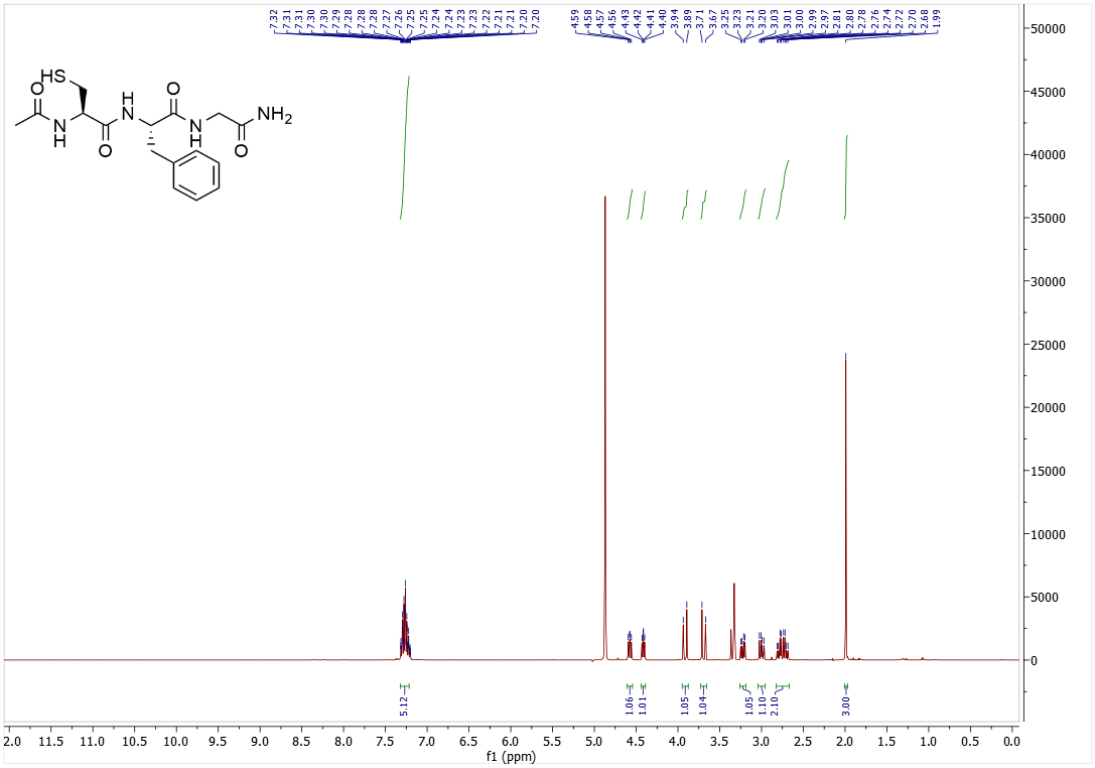


Figure S42 ^{1}H NMR (400 MHz) of **1a** (Ac-Cys-Phe-Gly-NH_2).

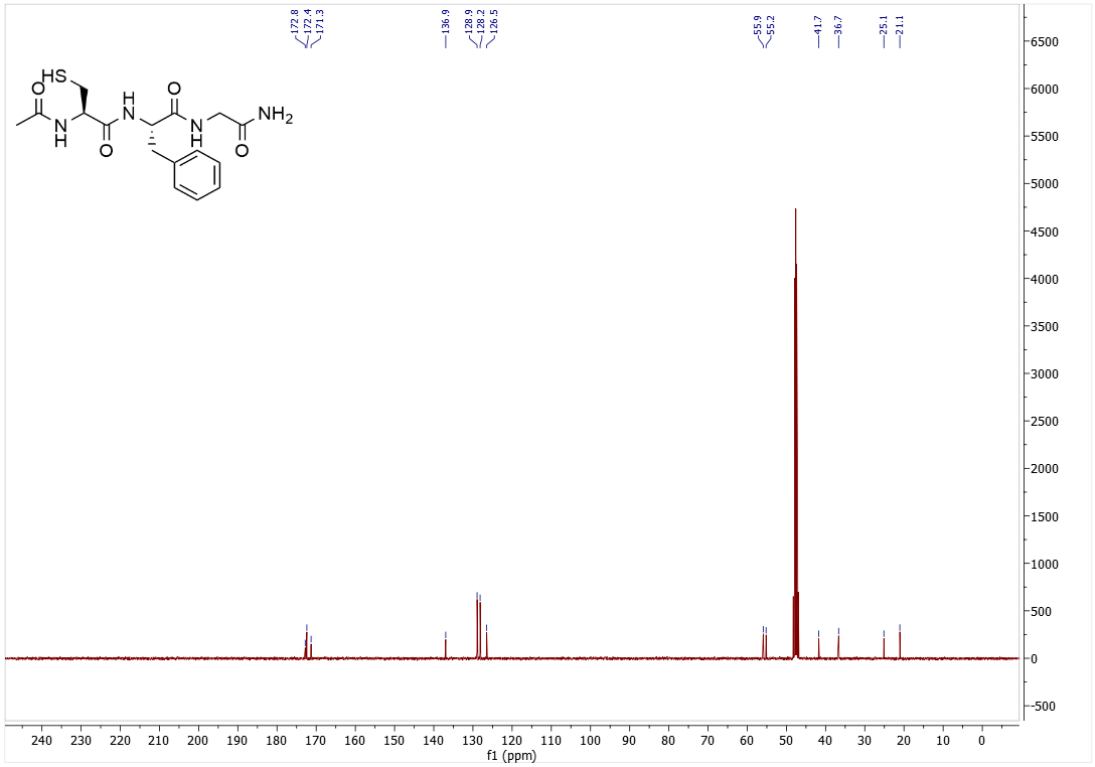


Figure S43 ^{13}C NMR (101 MHz) of **1a** (Ac-Cys-Phe-Gly-NH_2).

Compound 1b

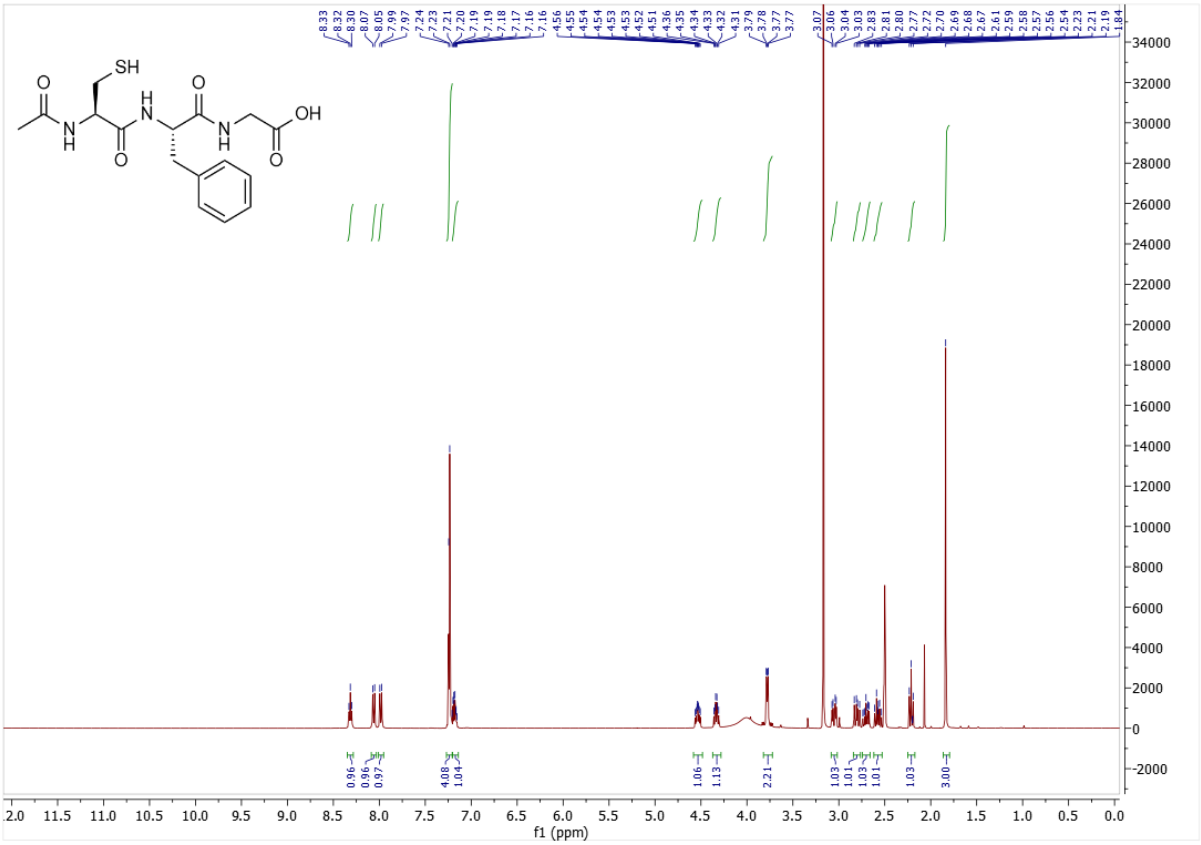


Figure S44 ^{1}H NMR (400 MHz) of **1b** (Ac-Cys-Phe-Gly-OH).

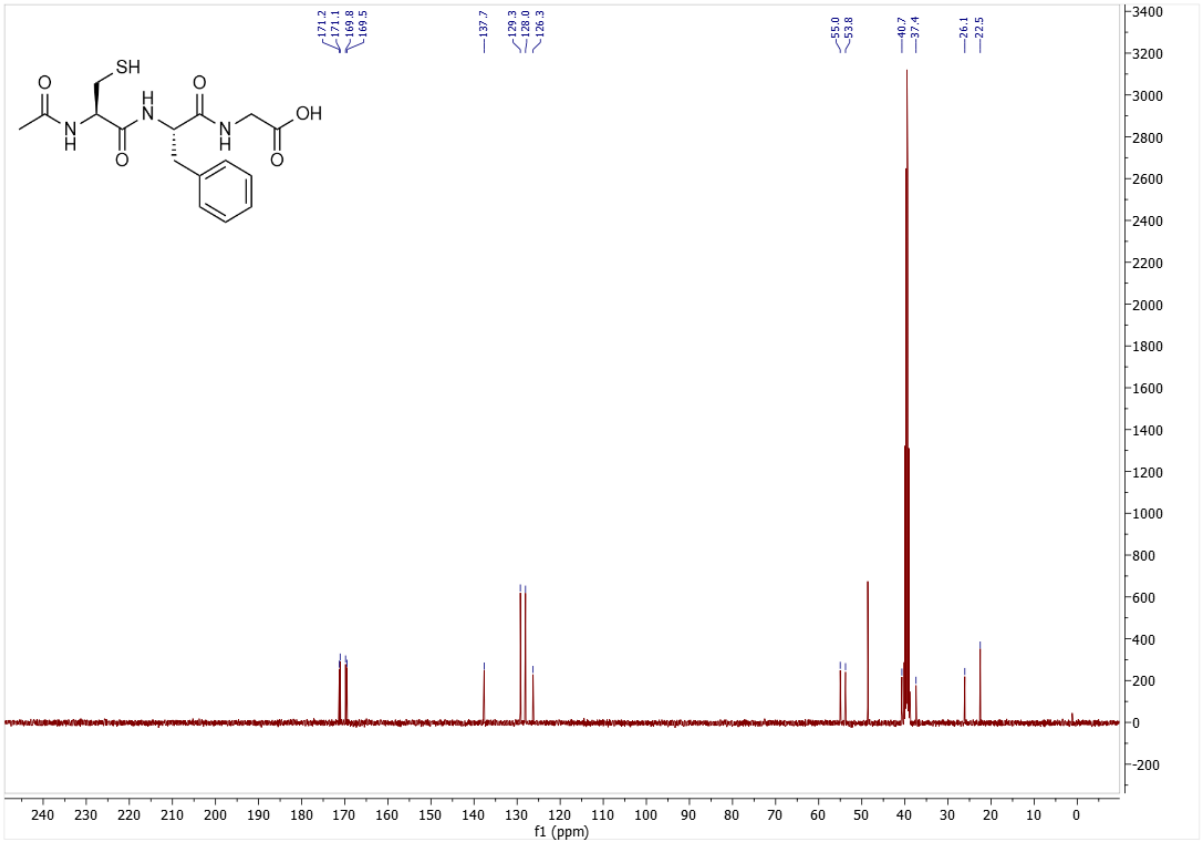


Figure S45 ^{13}C NMR (101 MHz) of **1b** (Ac-Cys-Phe-Gly-OH).

Compound 1c

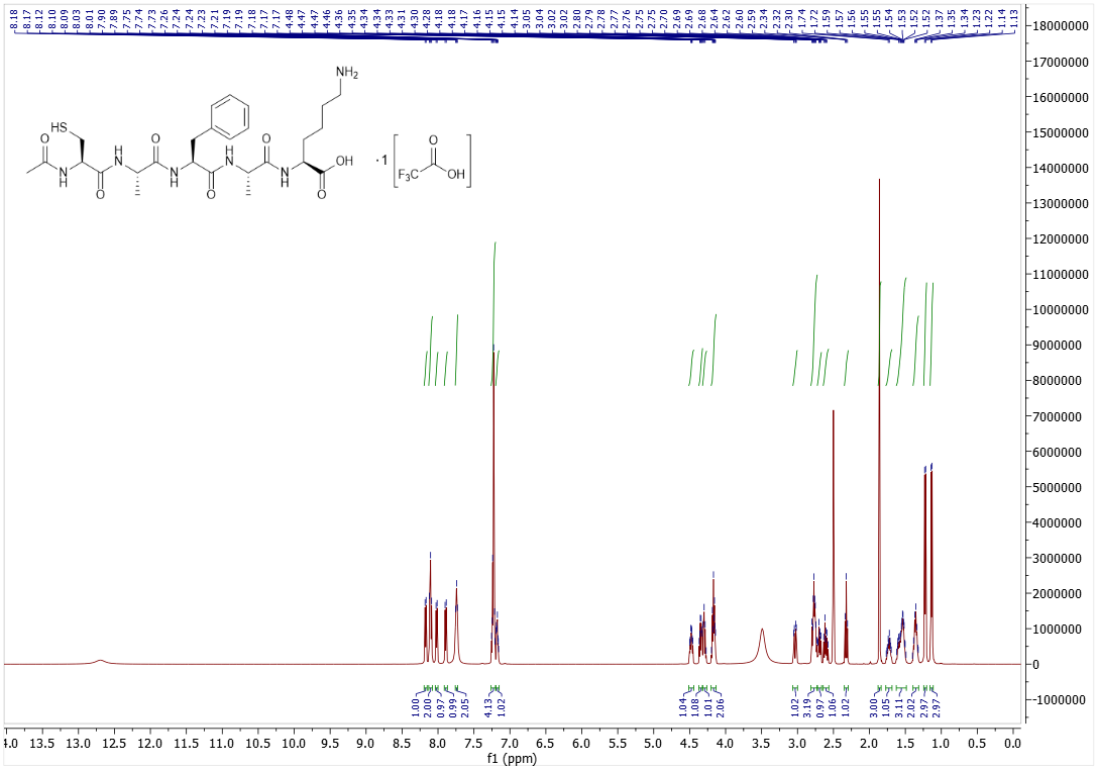


Figure S46 ^{1}H NMR (500 MHz) of compound **1c**.

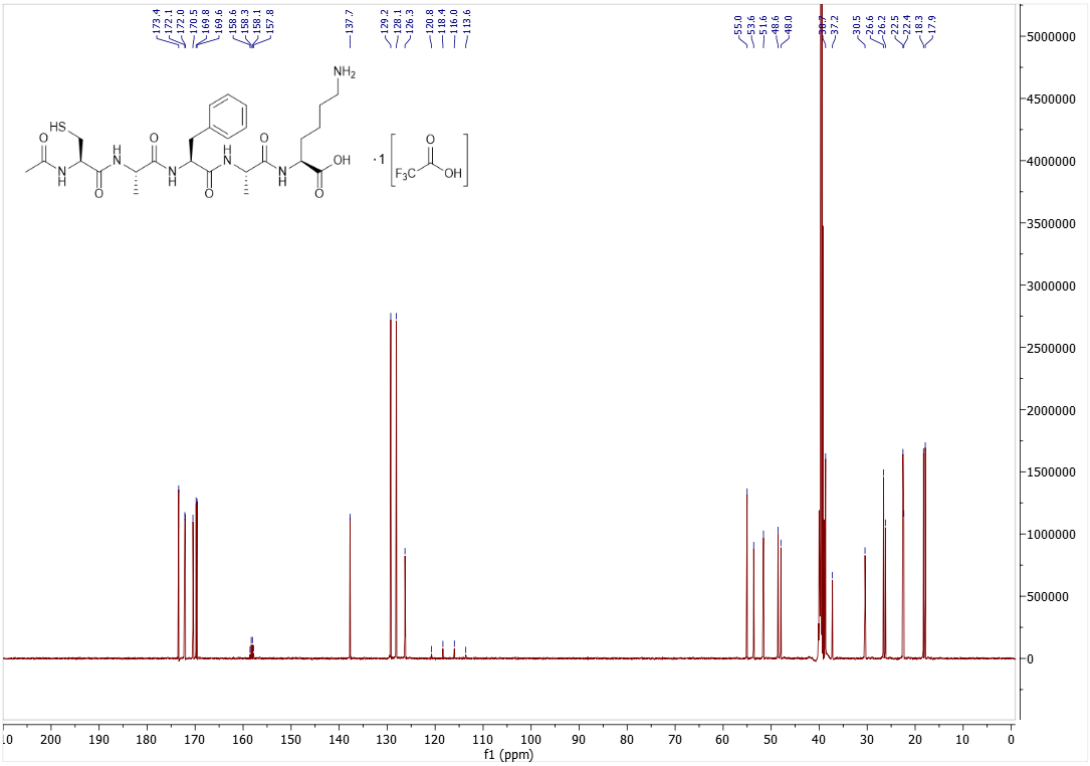


Figure S47 ^{13}C NMR (126 MHz) of compound **1c**.

Compound 1d

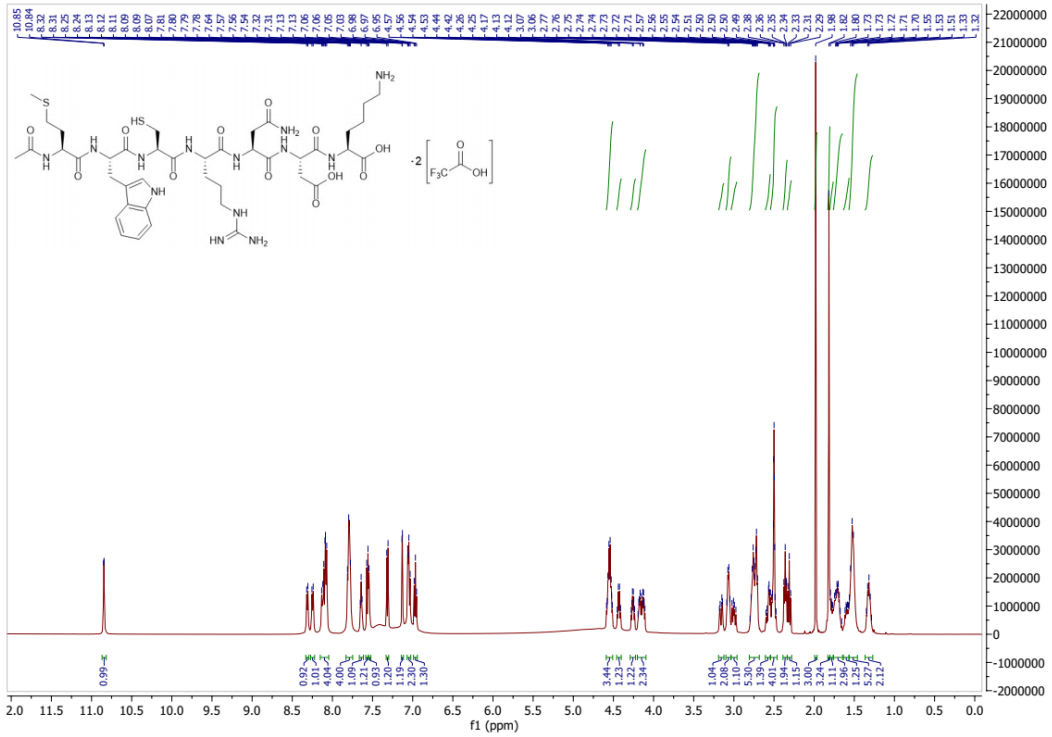


Figure S48 ^{1}H NMR (500 MHz) of compound **1d**.

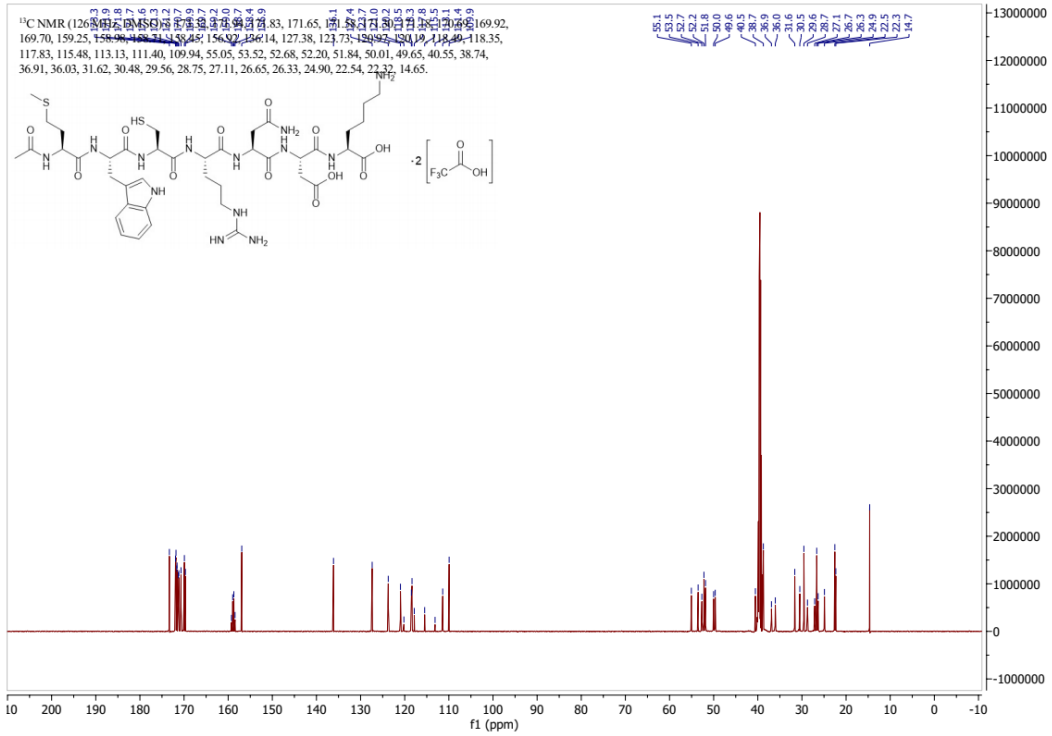


Figure S49 ^{13}C NMR (126 MHz) of compound **1d**.

Compound 2a

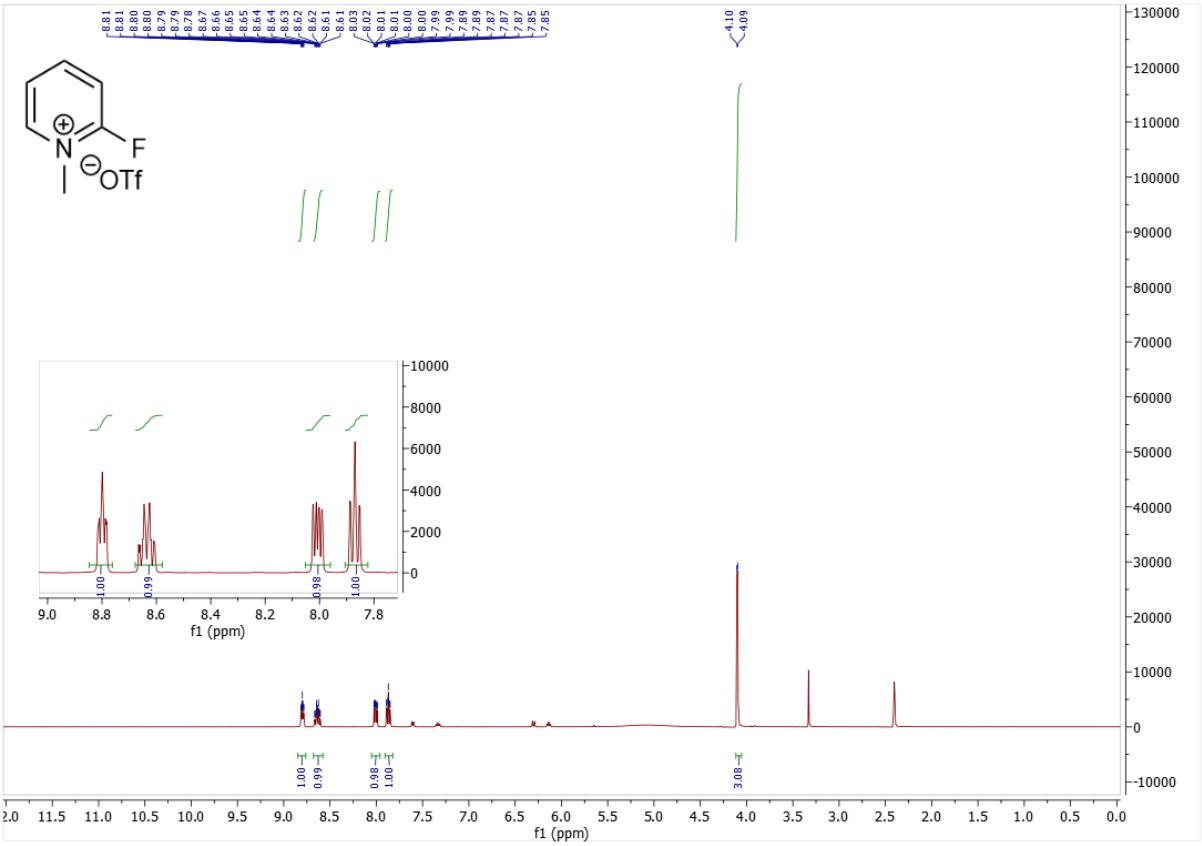


Figure S50 ^{1}H NMR (400 MHz) of **2a** (2-fluoro-N-methylpyridinium triflate).

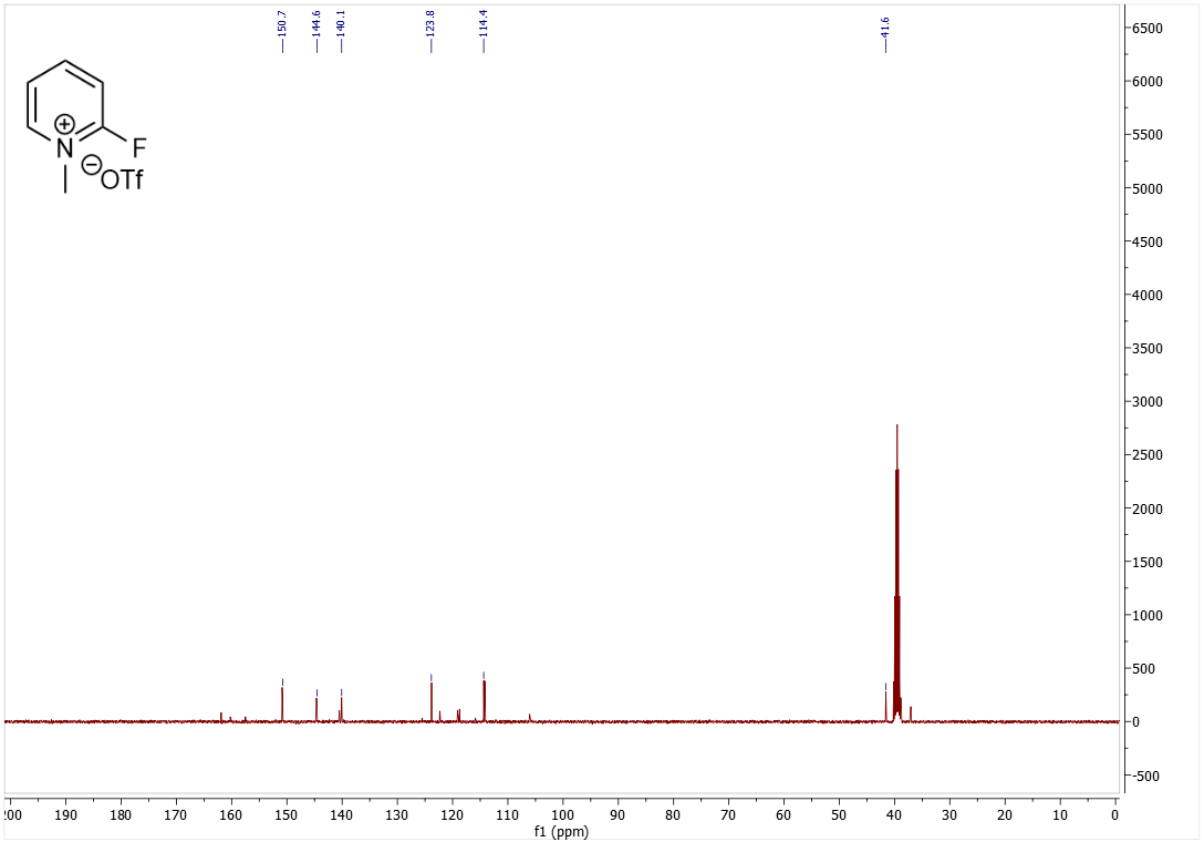


Figure S51 ^{13}C NMR (101 MHz) of **2a** (2-fluoro-N-methylpyridinium triflate).

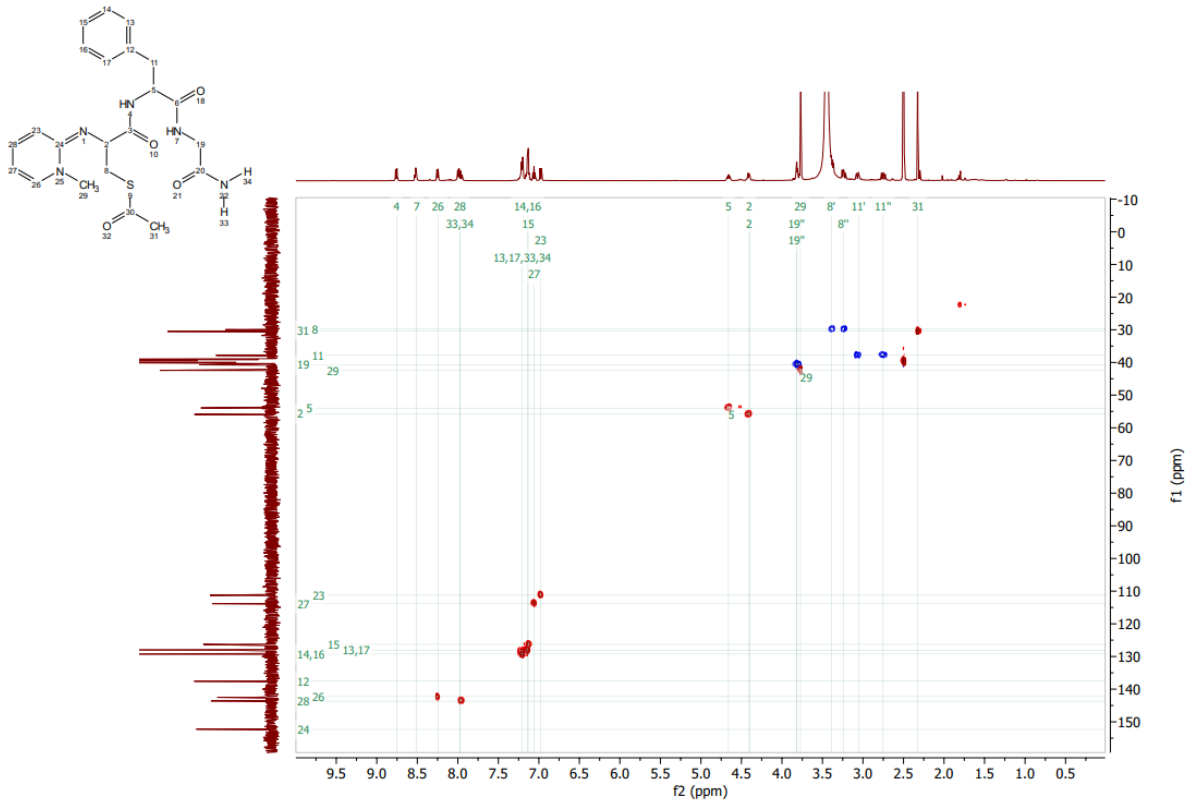


Figure S52 HSQC of side-product **I**.

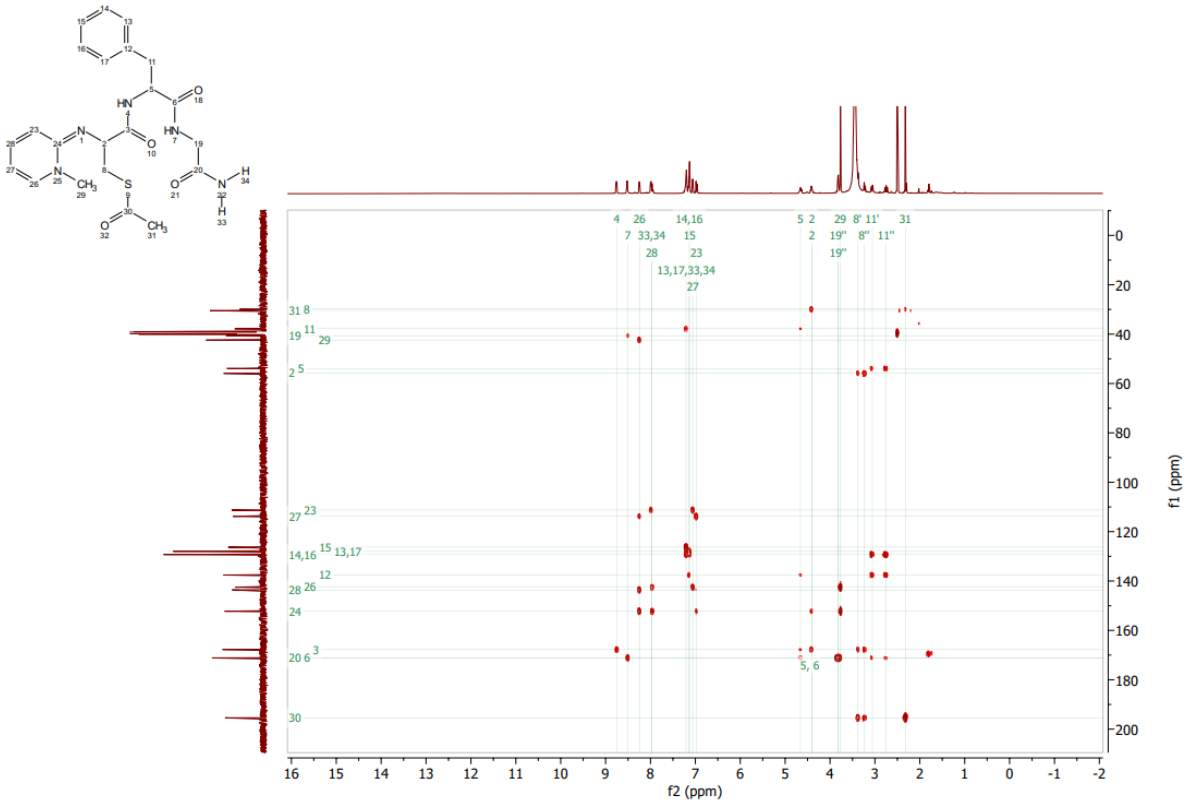


Figure S53 HMBC of side-product **I**.

Compound 2b

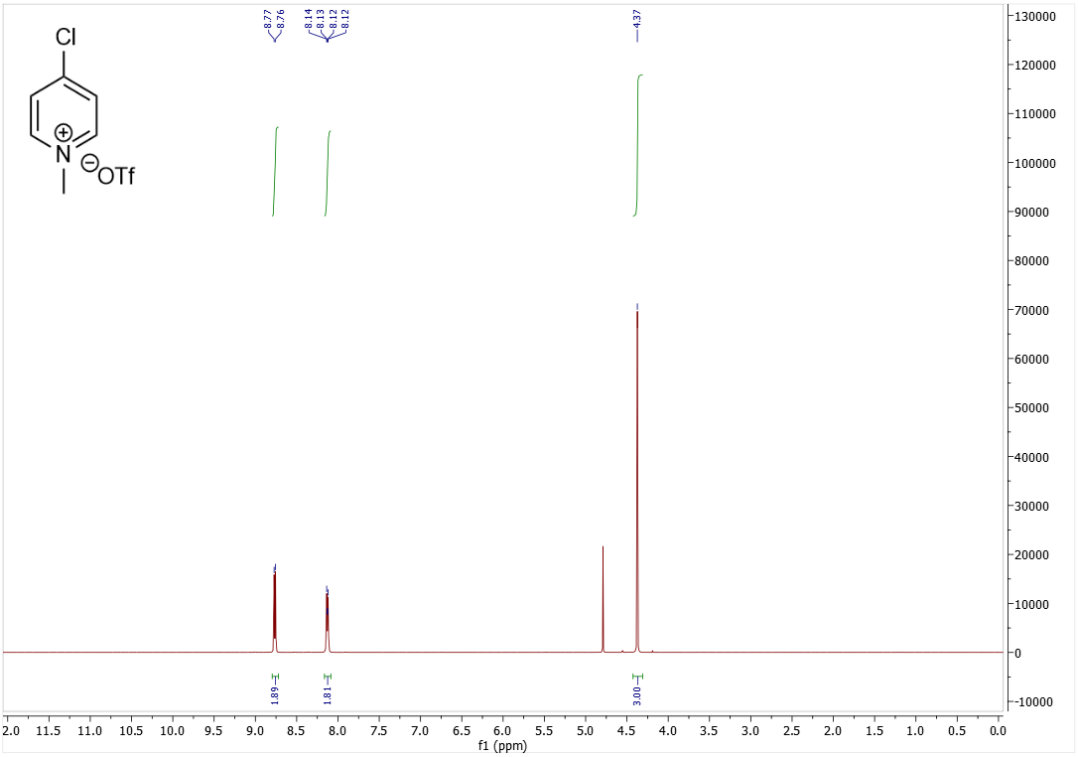


Figure S54 ^{1}H NMR (400 MHz) of **2b** (4-chloro-N-methylpyridinium triflate).

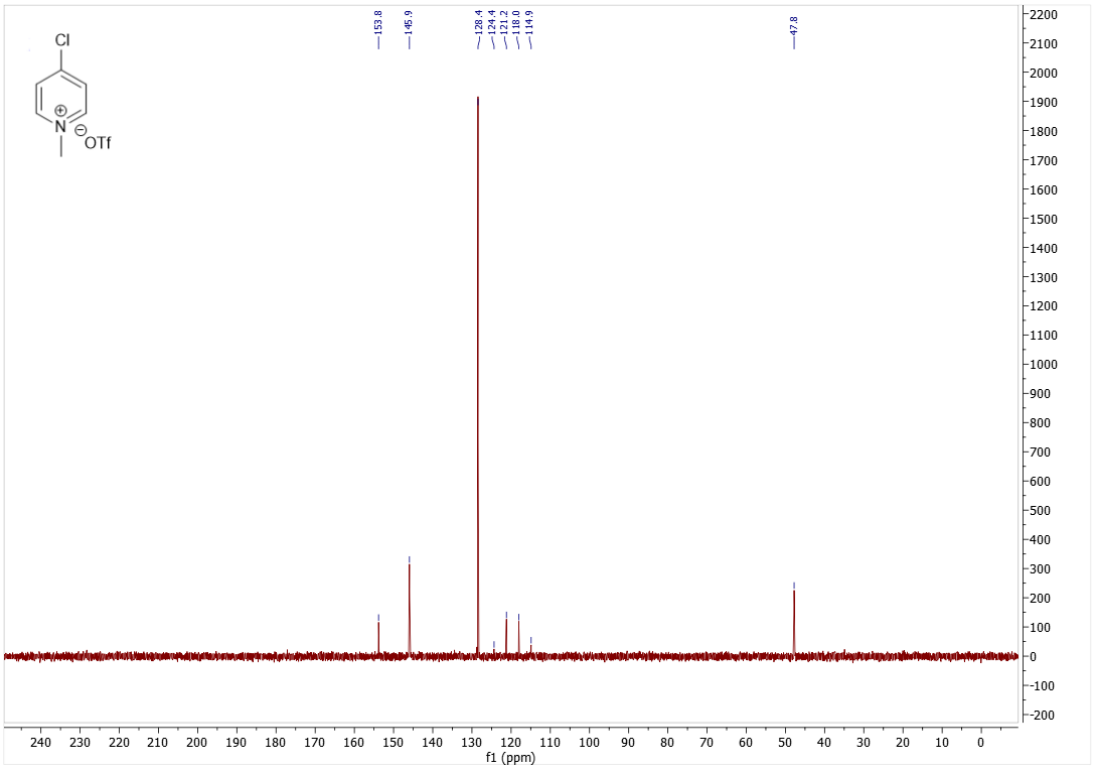


Figure S55 ^{1}H NMR (101 MHz) of **2b** (4-chloro-N-methylpyridinium triflate).

Compound 6a

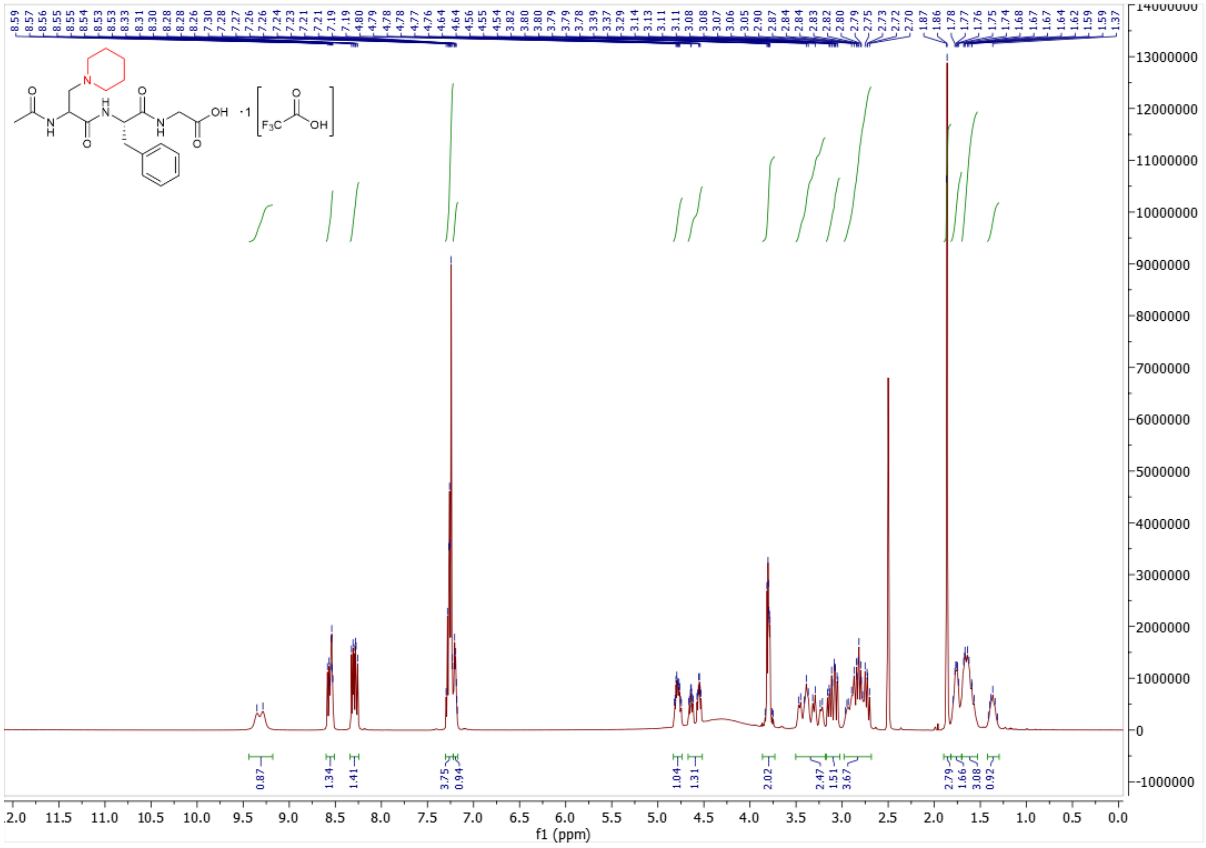


Figure S56 ^{1}H NMR (500 MHz) of compound **6a**.

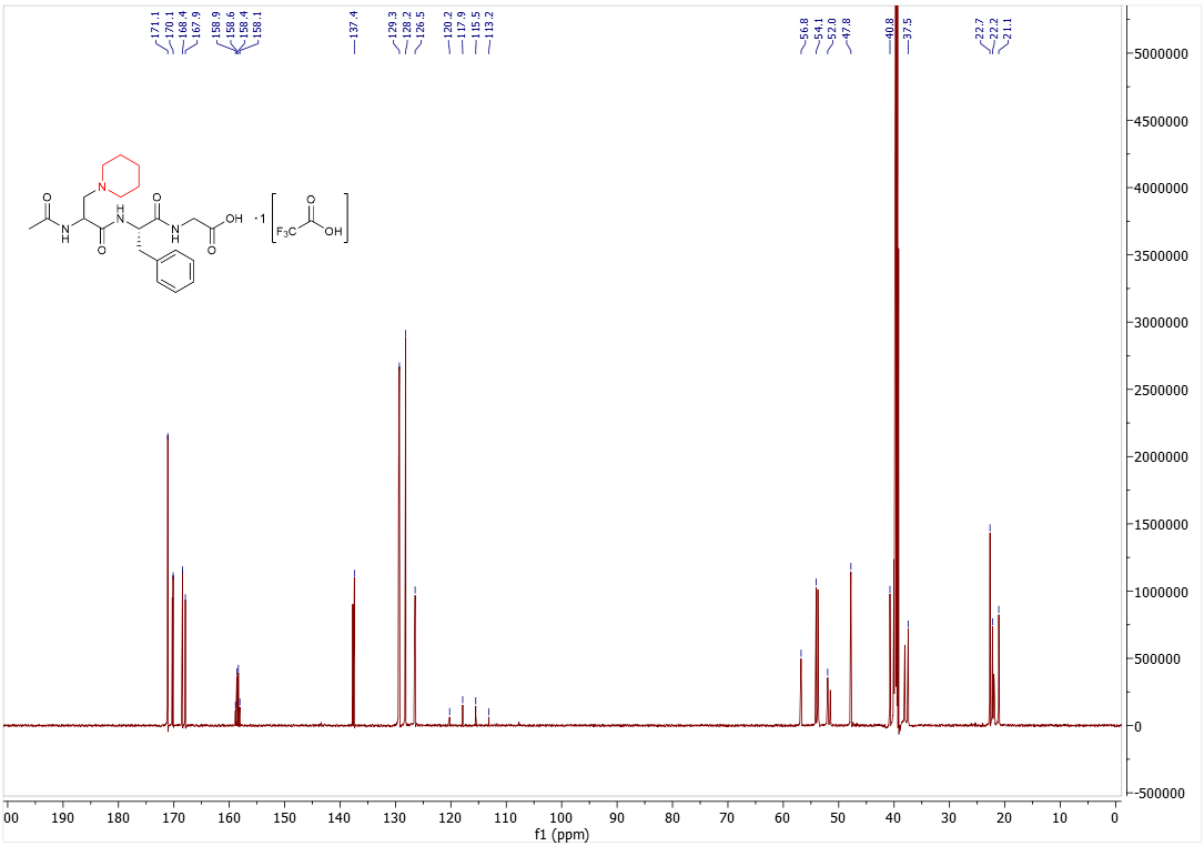


Figure S57 ^{13}C NMR (126 MHz) of compound **6a**.

Compound 6b

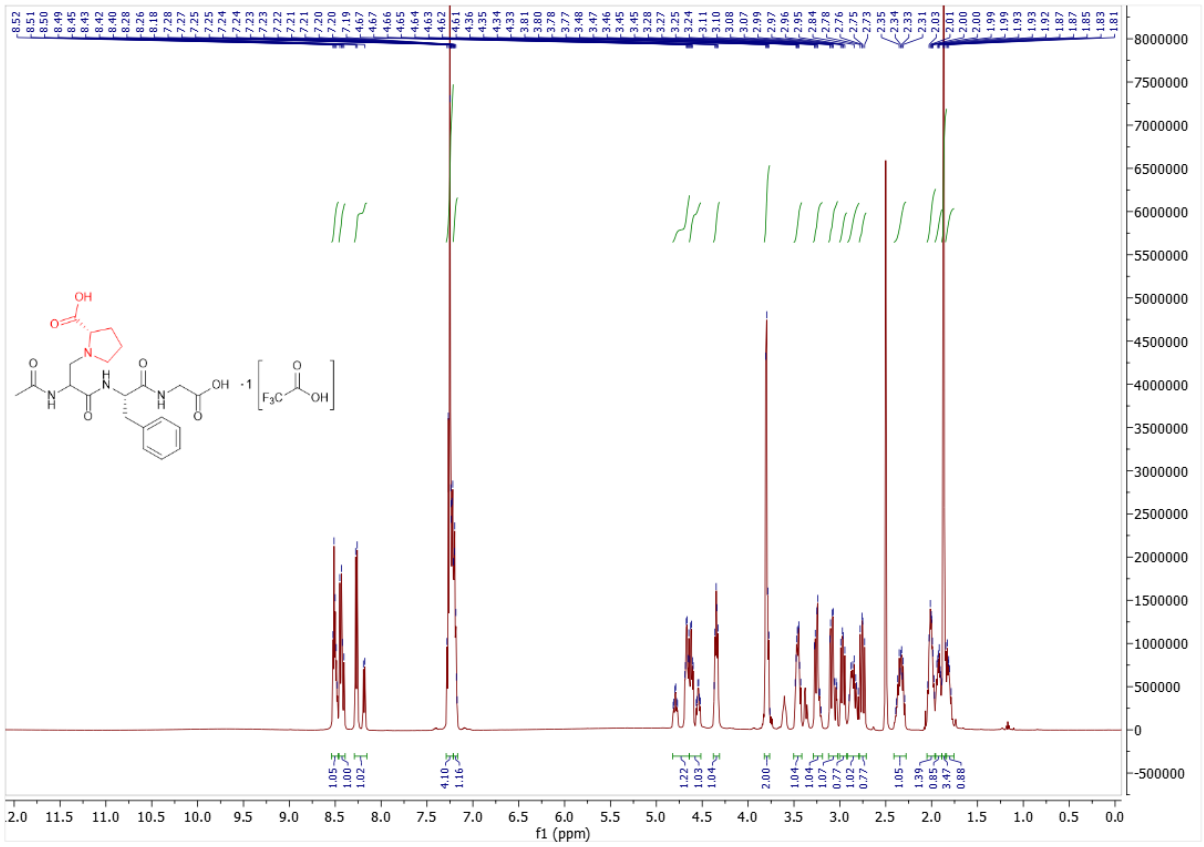


Figure S58 ^{1}H NMR (500 MHz) of compound **6b**.

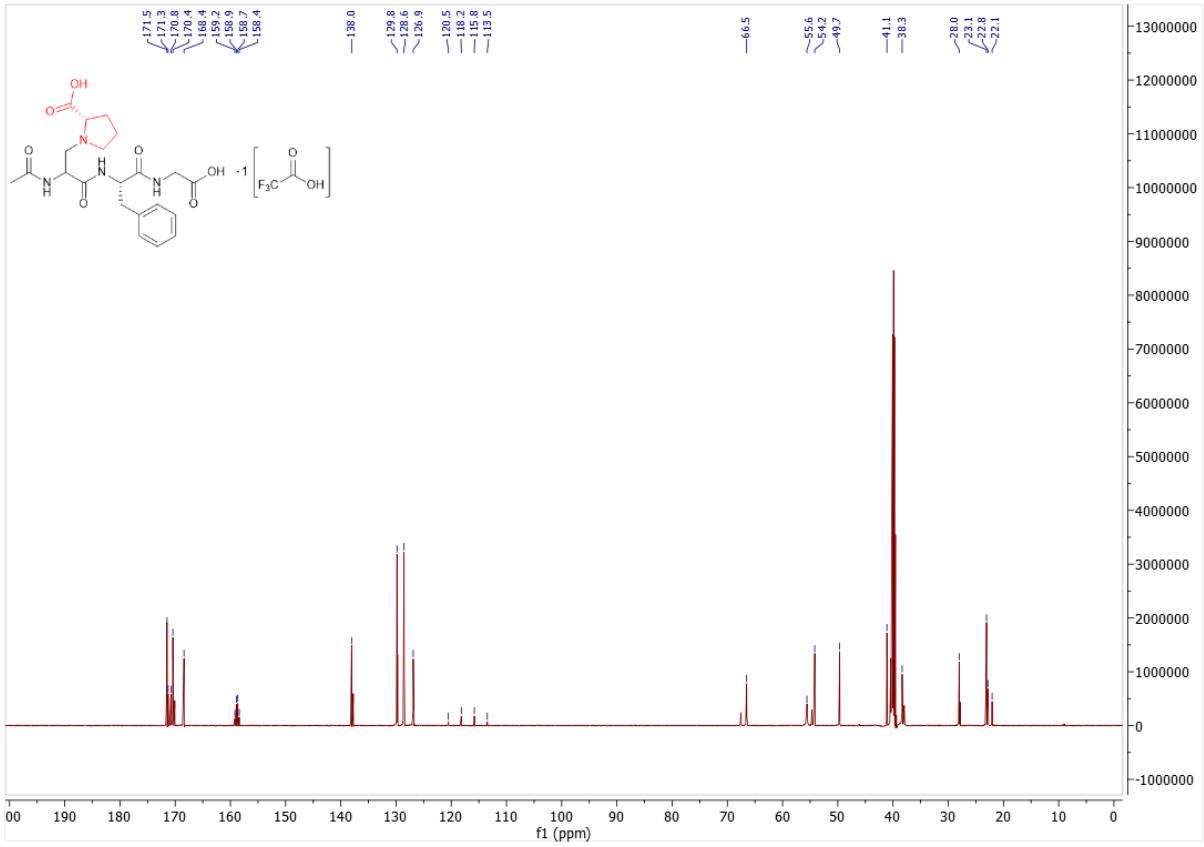


Figure S59 ^{13}C NMR (126 MHz) of compound **6b**.

Compound 6c

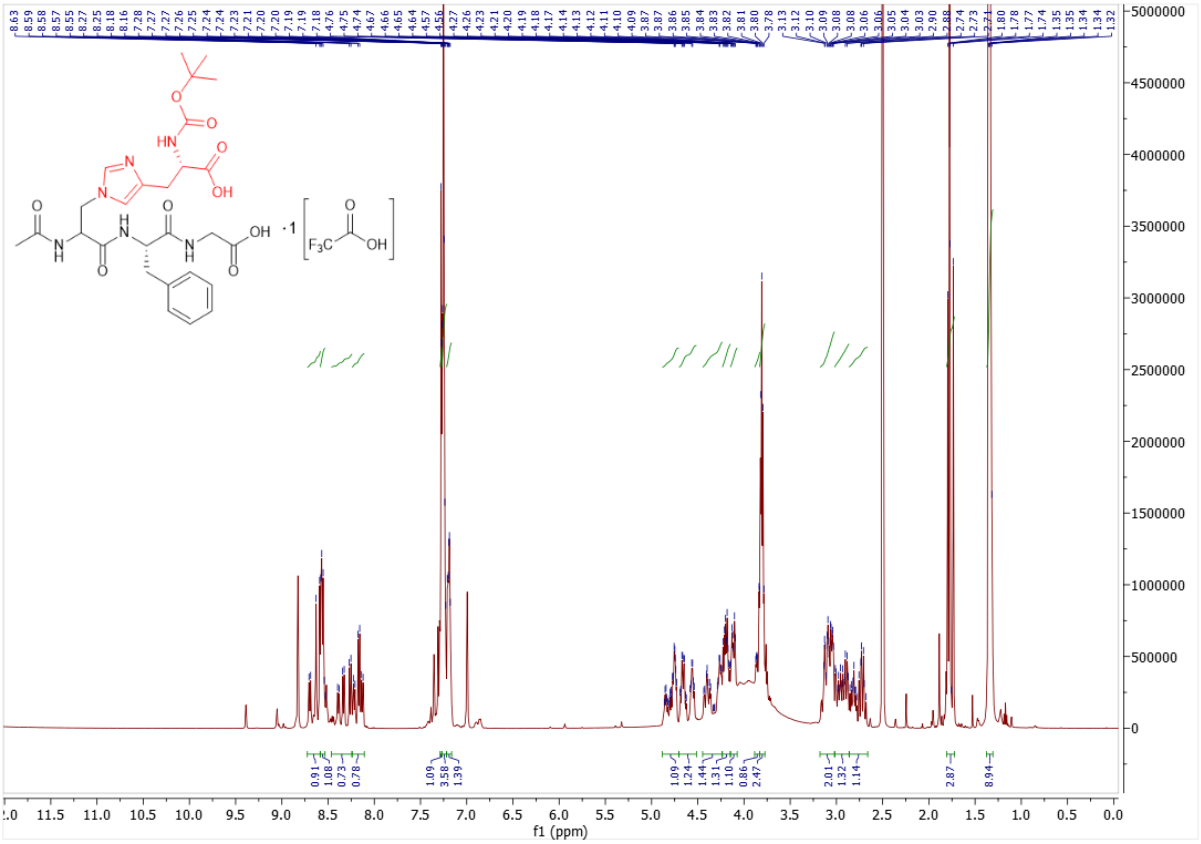


Figure S60 ^{1}H NMR (500 MHz) of compound **6c**.

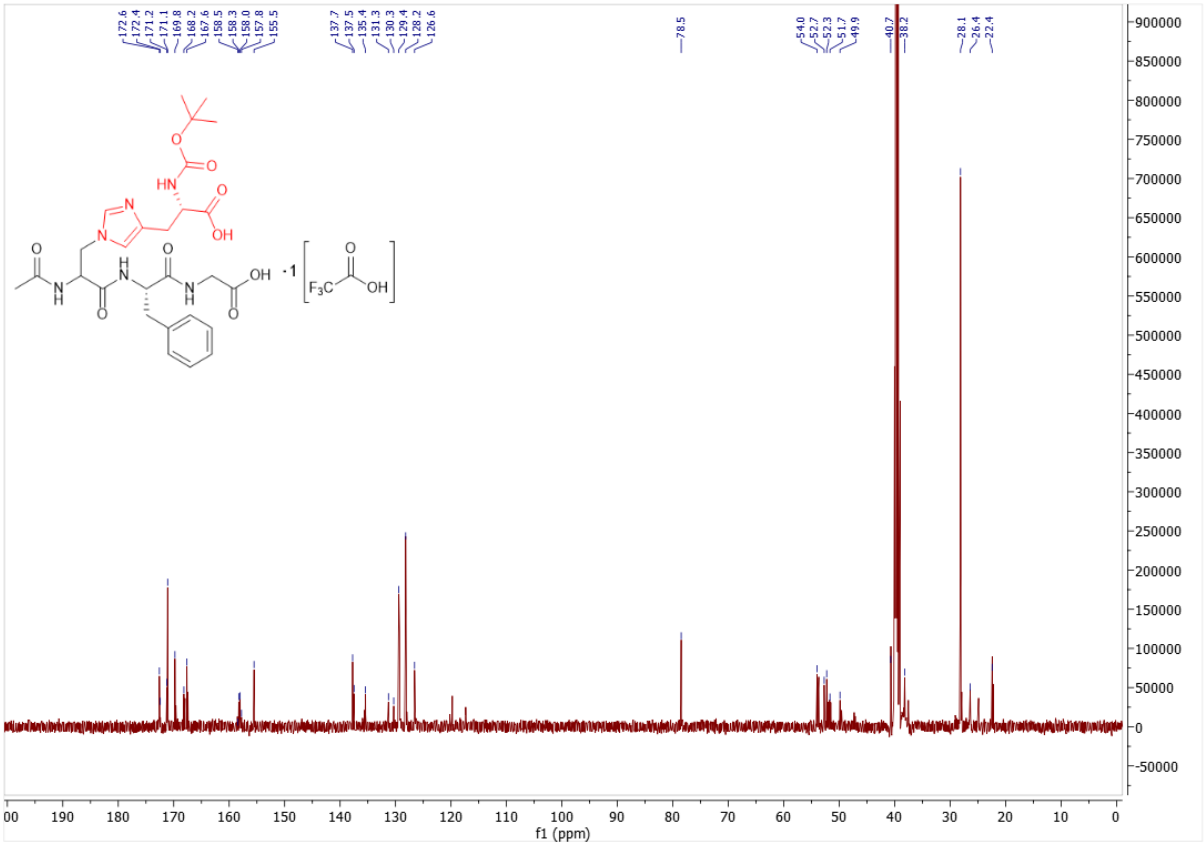


Figure S61 ^{13}C NMR (126 MHz) of compound **6c**.

Compound 6d

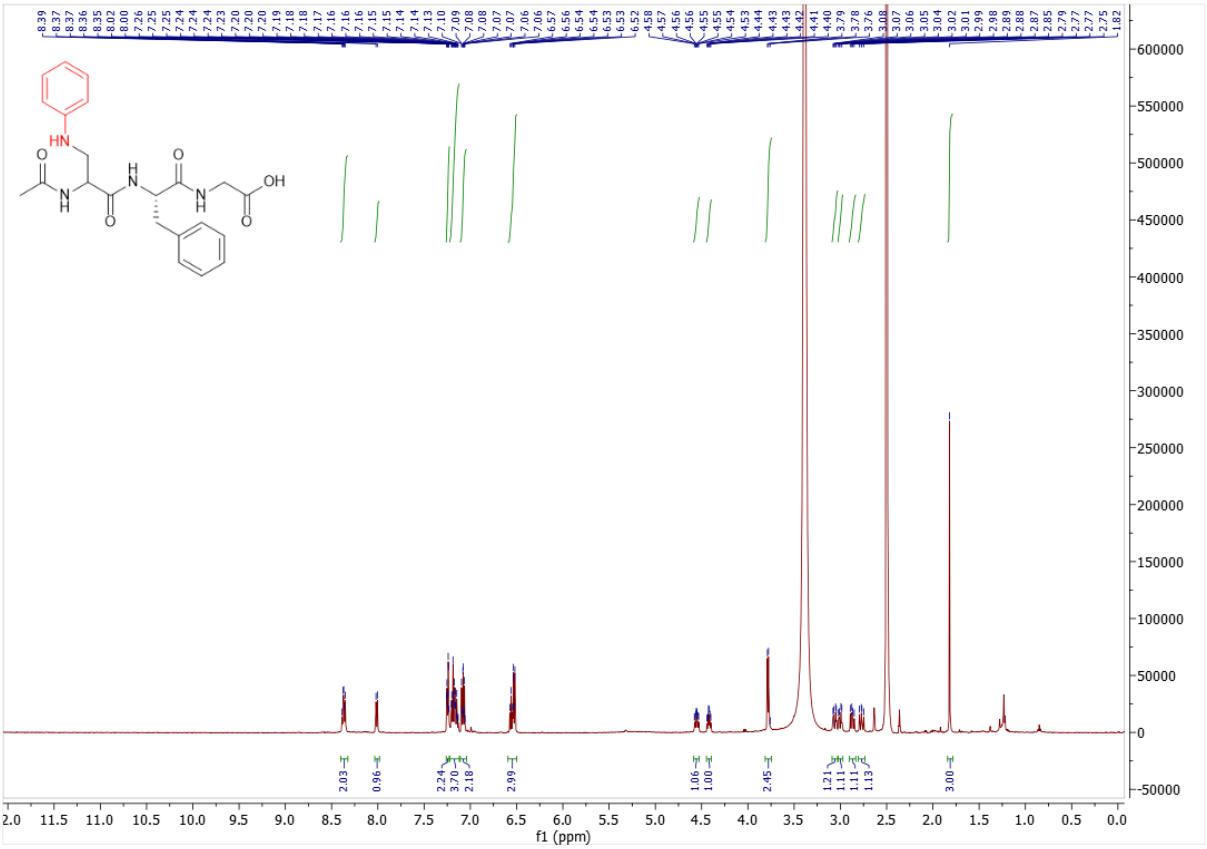


Figure S62 ^{1}H NMR (500 MHz) of compound **6d**.

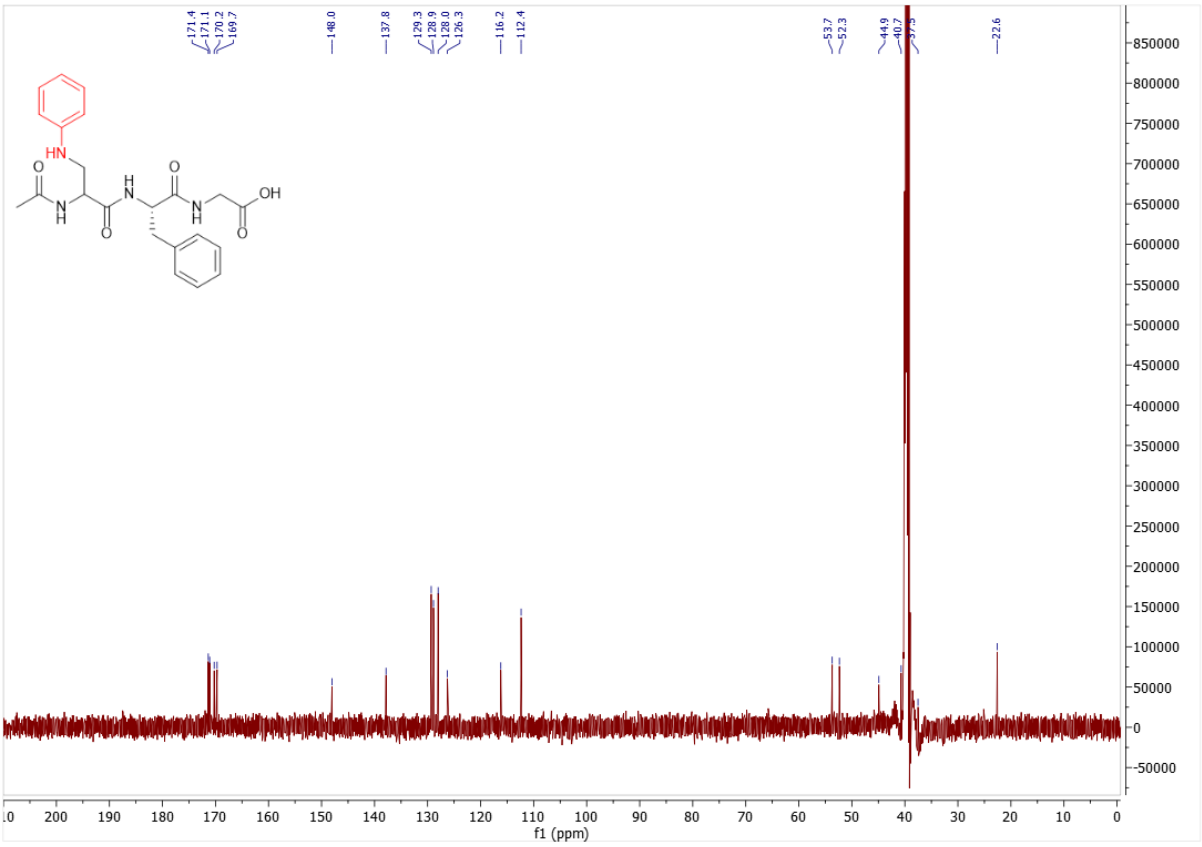


Figure S63 ^{13}C NMR (126 MHz) of compound **6d**.

Compound 6e (Diastereomer 1)

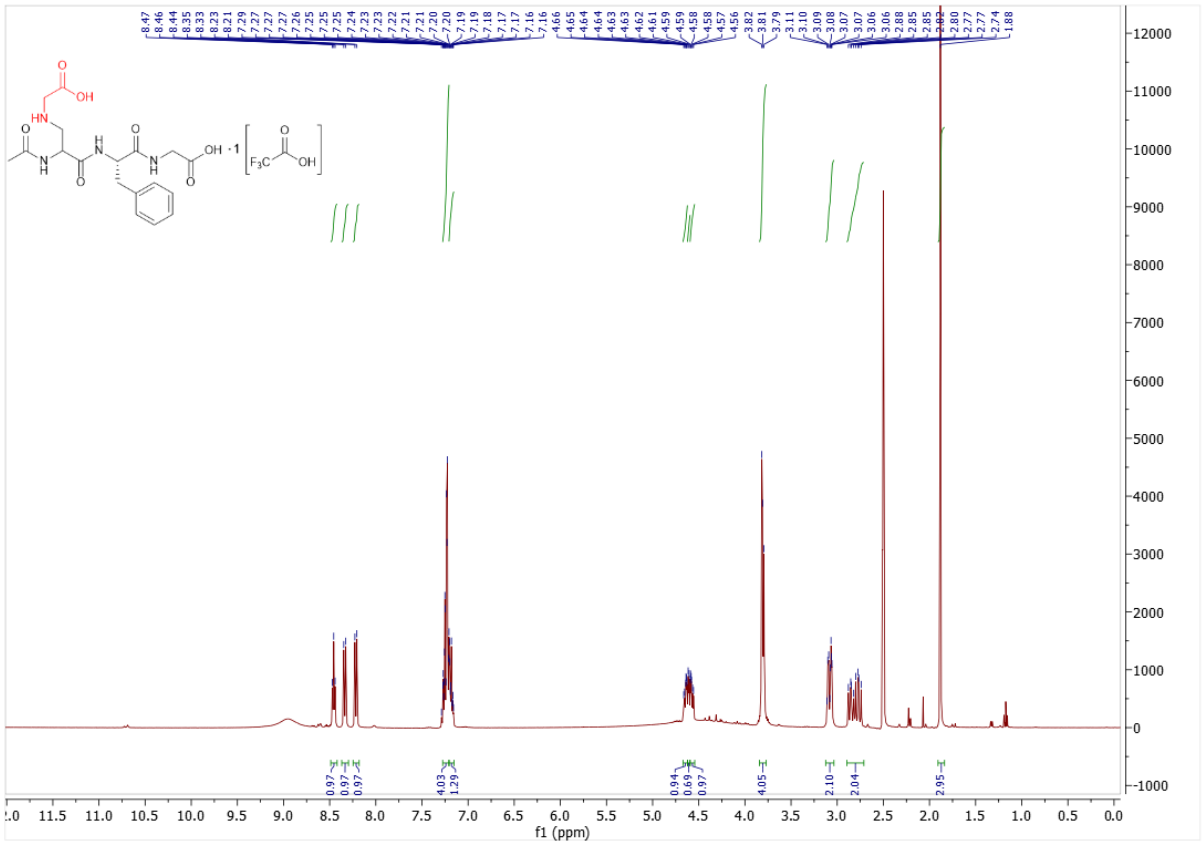


Figure S64 ^{1}H NMR (500 MHz) of diastereomer 1 of compound **6e**.

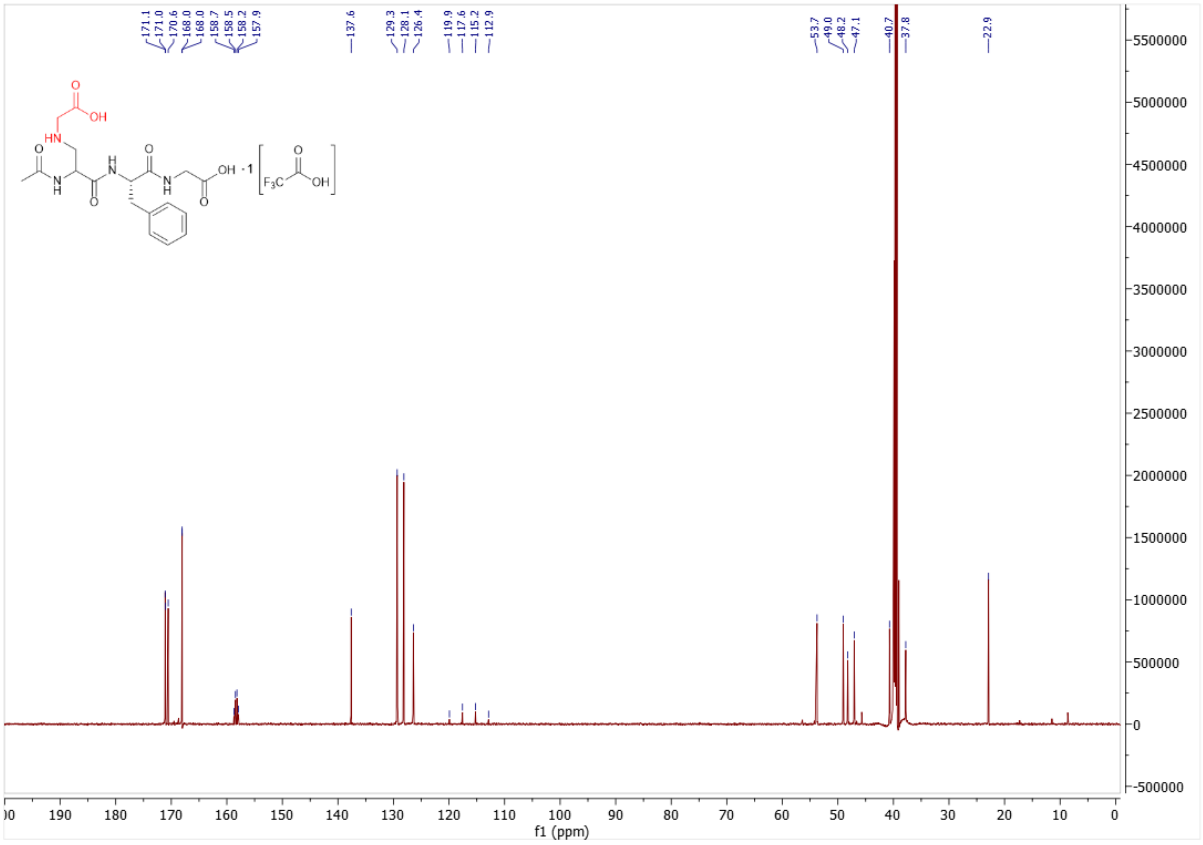


Figure S65 ^{13}C NMR (126 MHz) of diastereomer 1 of compound **6e**.

Compound 6e (Diastereomer 2)

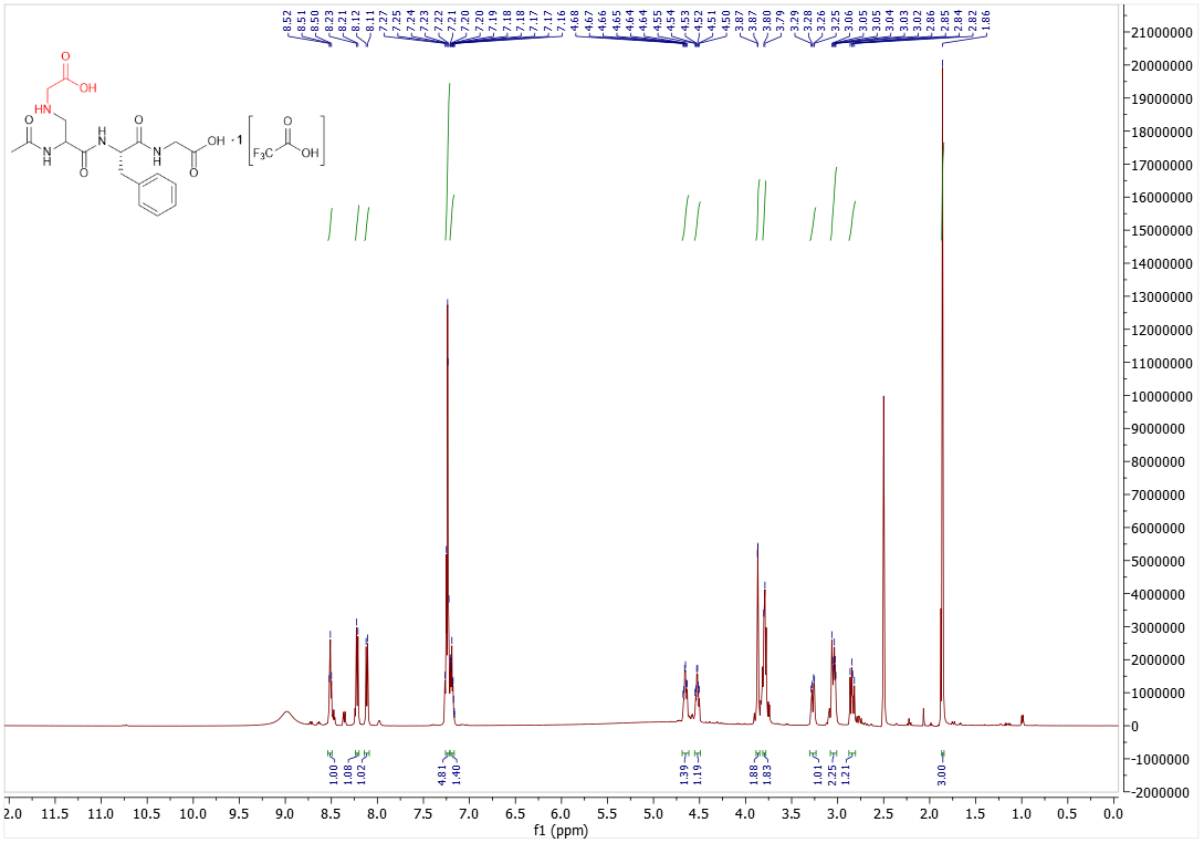


Figure S66 ^{1}H NMR (500 MHz) of diastereomer 2 of compound **6e**.

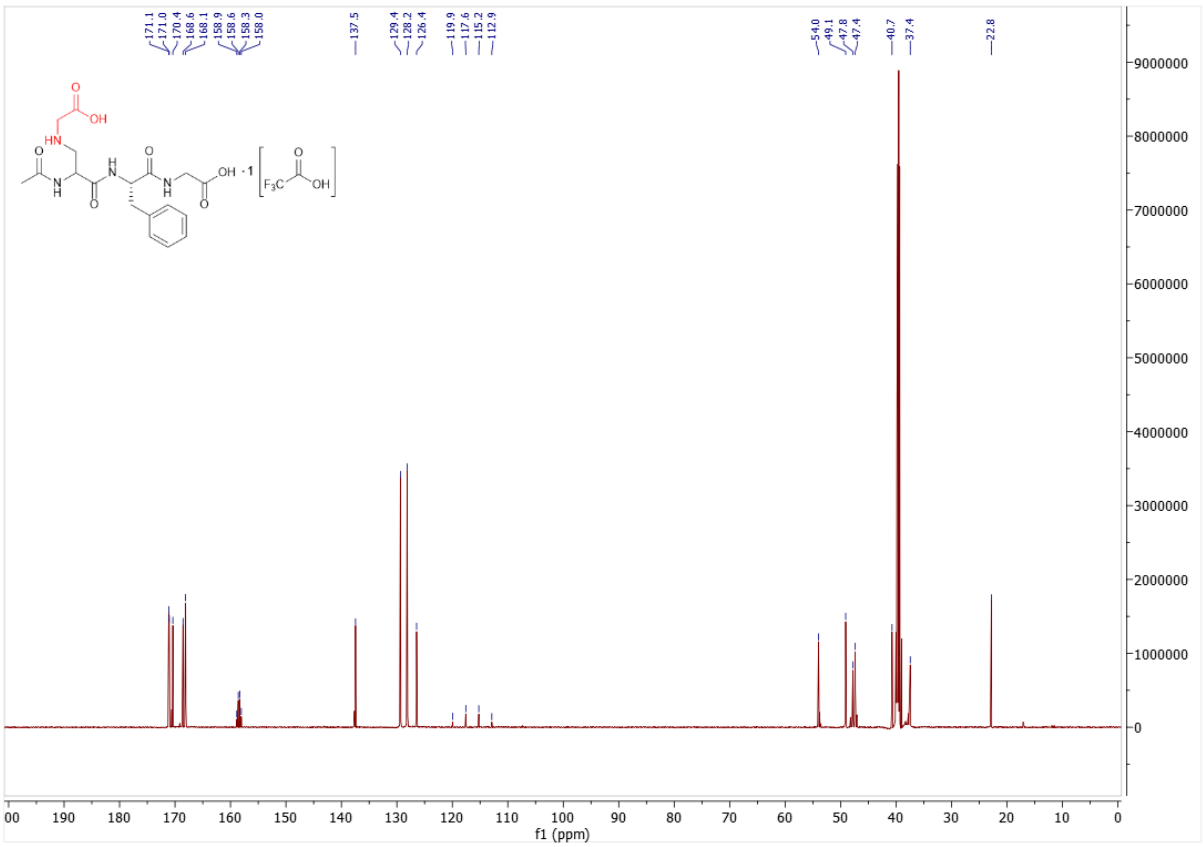


Figure S67 ^{13}C NMR (126 MHz) of diastereomer 2 of compound **6e**.

Compound 6f

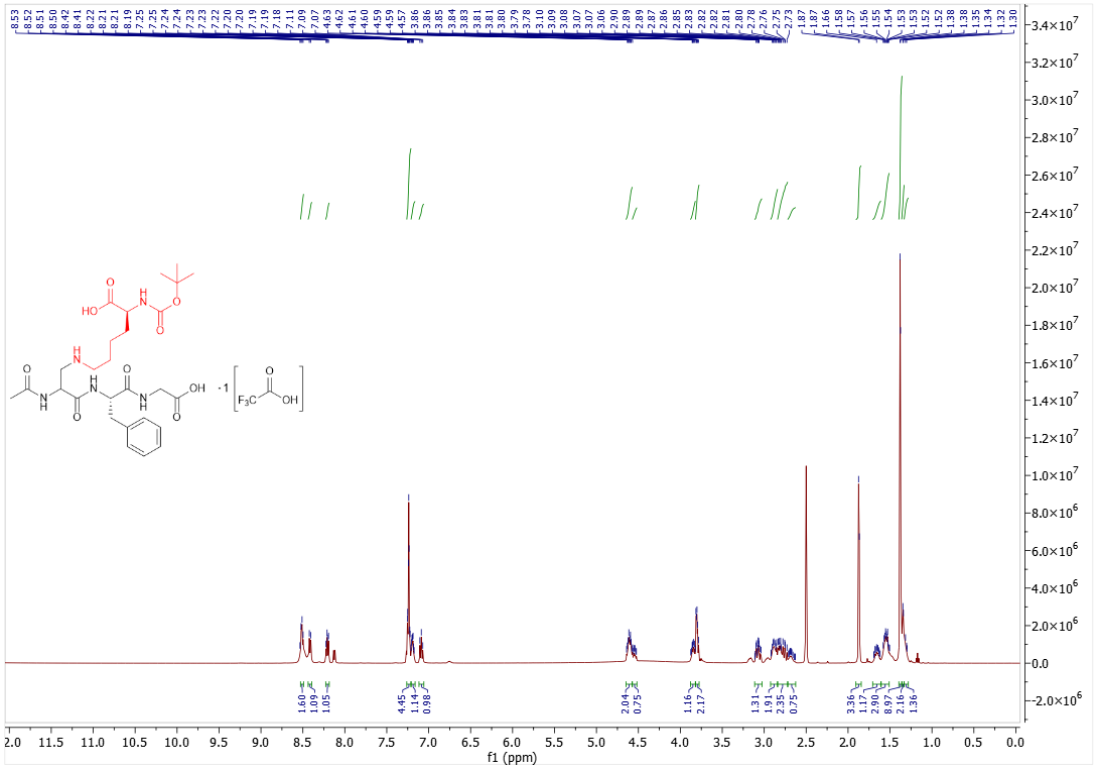


Figure S68 ^{1}H NMR (500 MHz) of compound **6f**.

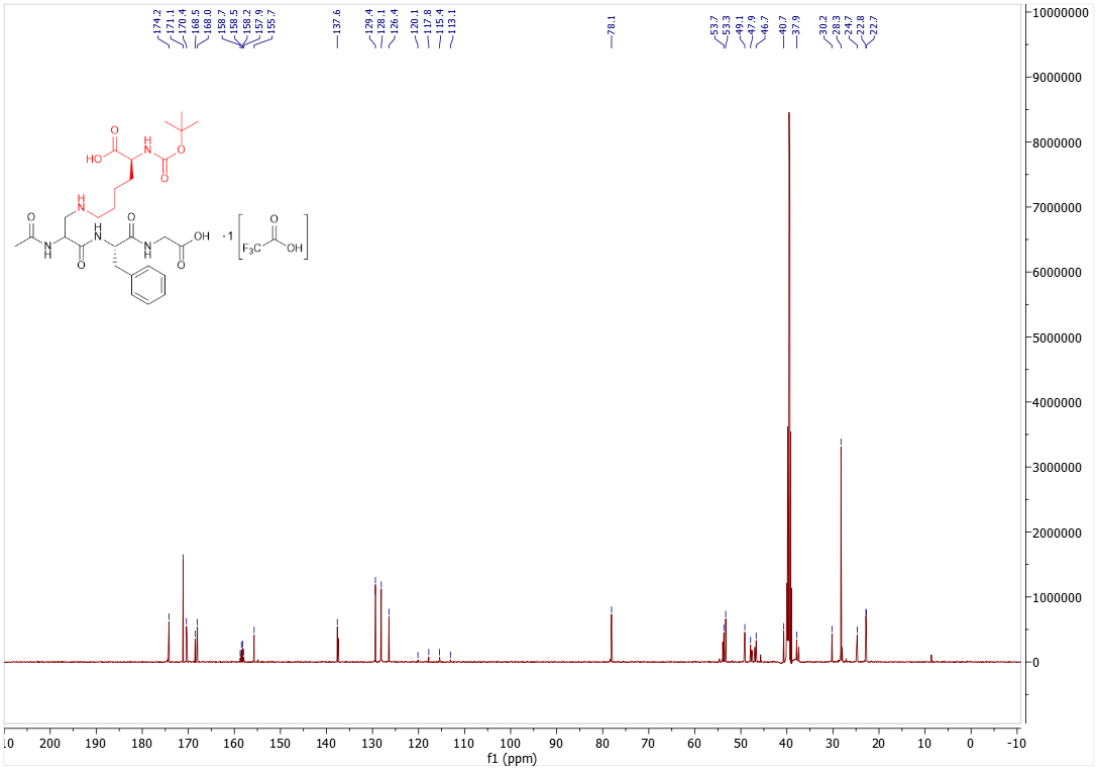


Figure S69 ^{13}C NMR (126 MHz) of compound **6f**.

Compound 6g (Diastereomer 1)

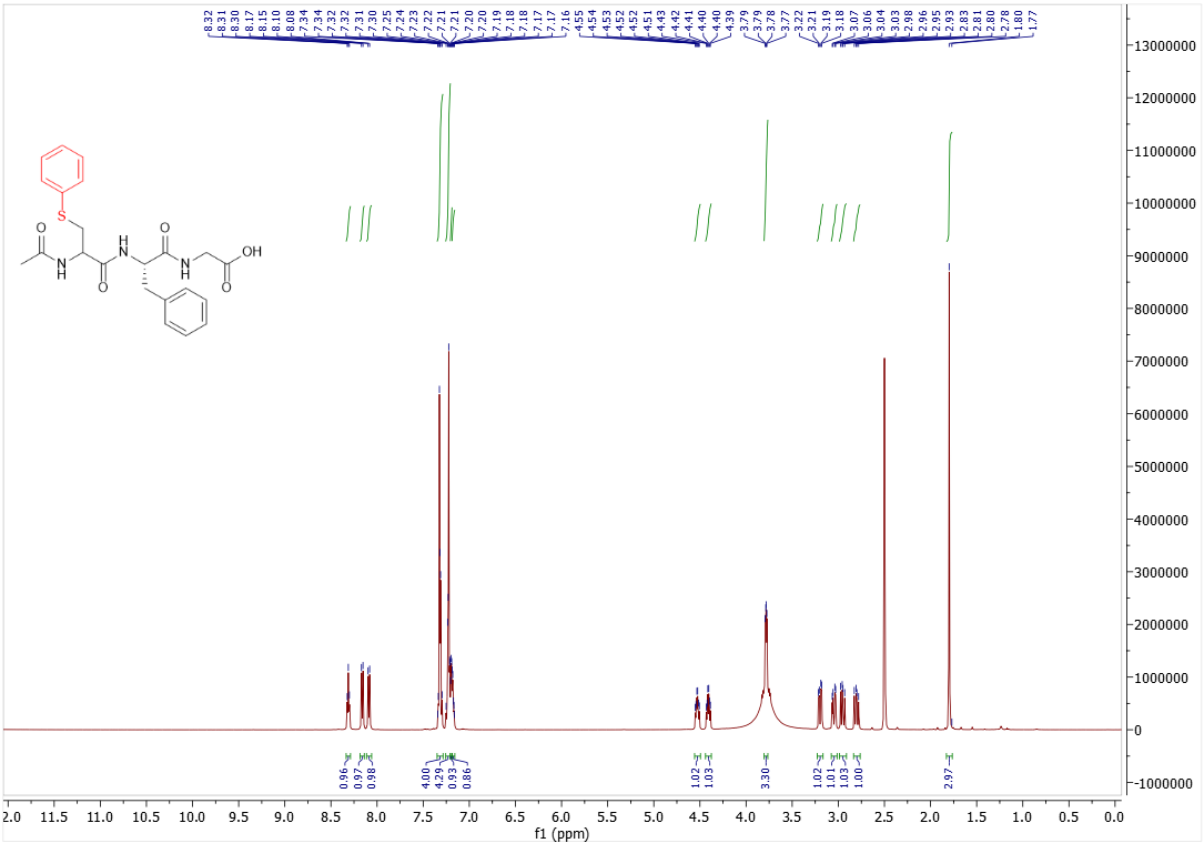


Figure S70 ^{1}H NMR (500 MHz) of diastereomer 1 of compound **6g**.

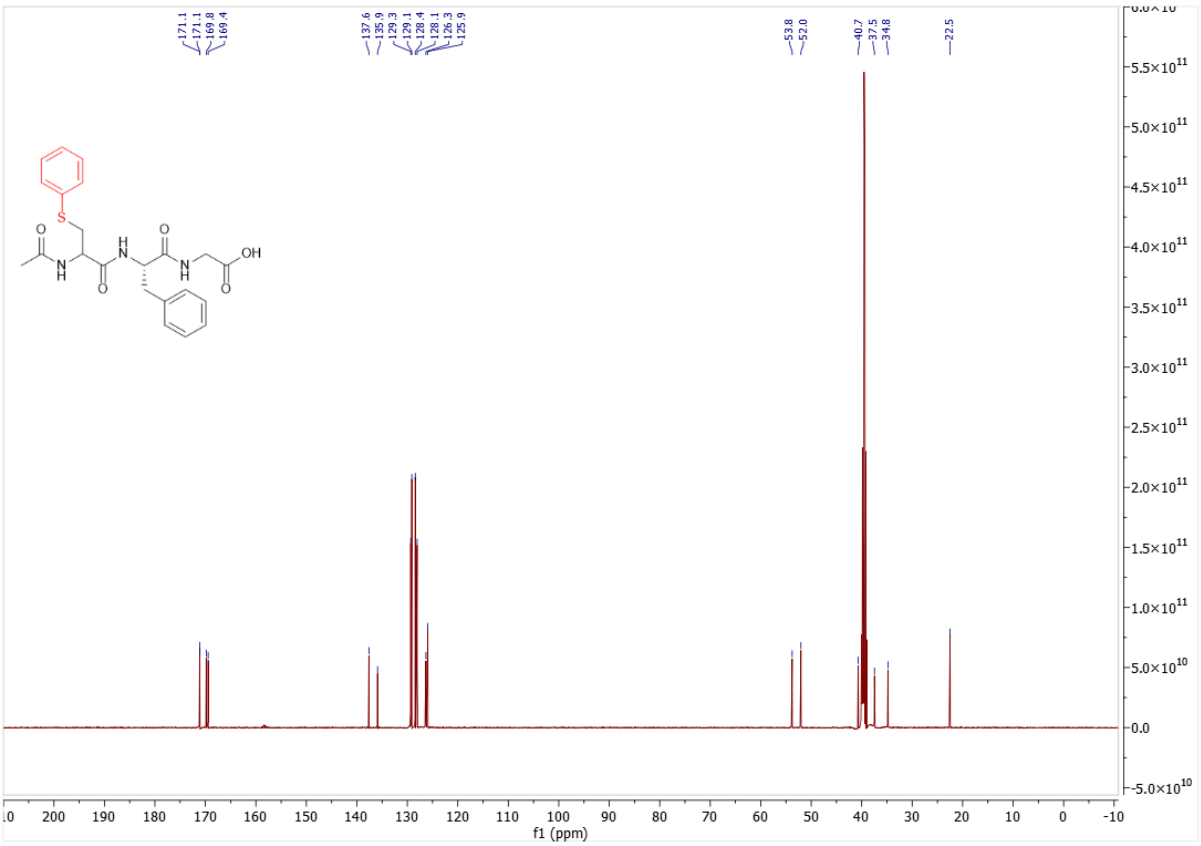


Figure S71 ^{13}C NMR (126 MHz) of diastereomer 1 of compound **6g**.

Compound 6g (Diastereomer 2)

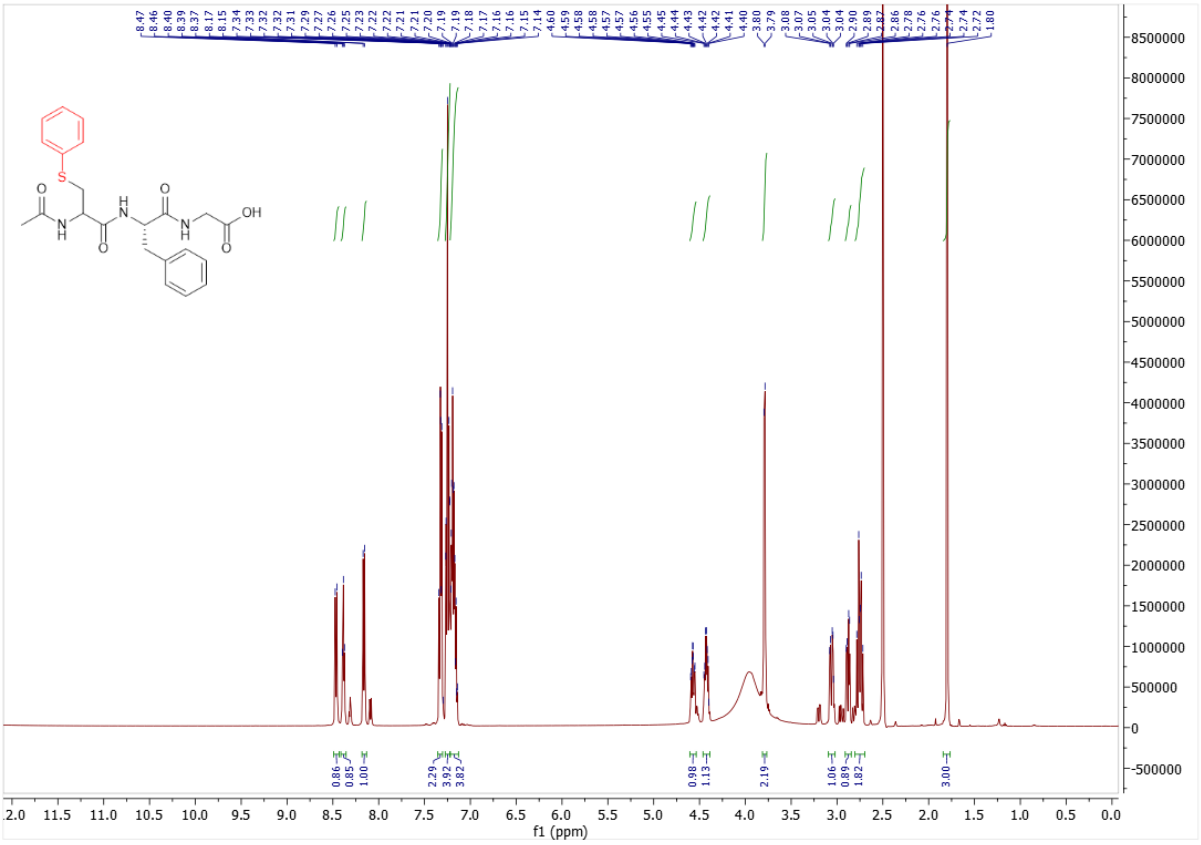


Figure S72 ^{1}H NMR (500 MHz) of diastereomer 2 of compound **6g**.

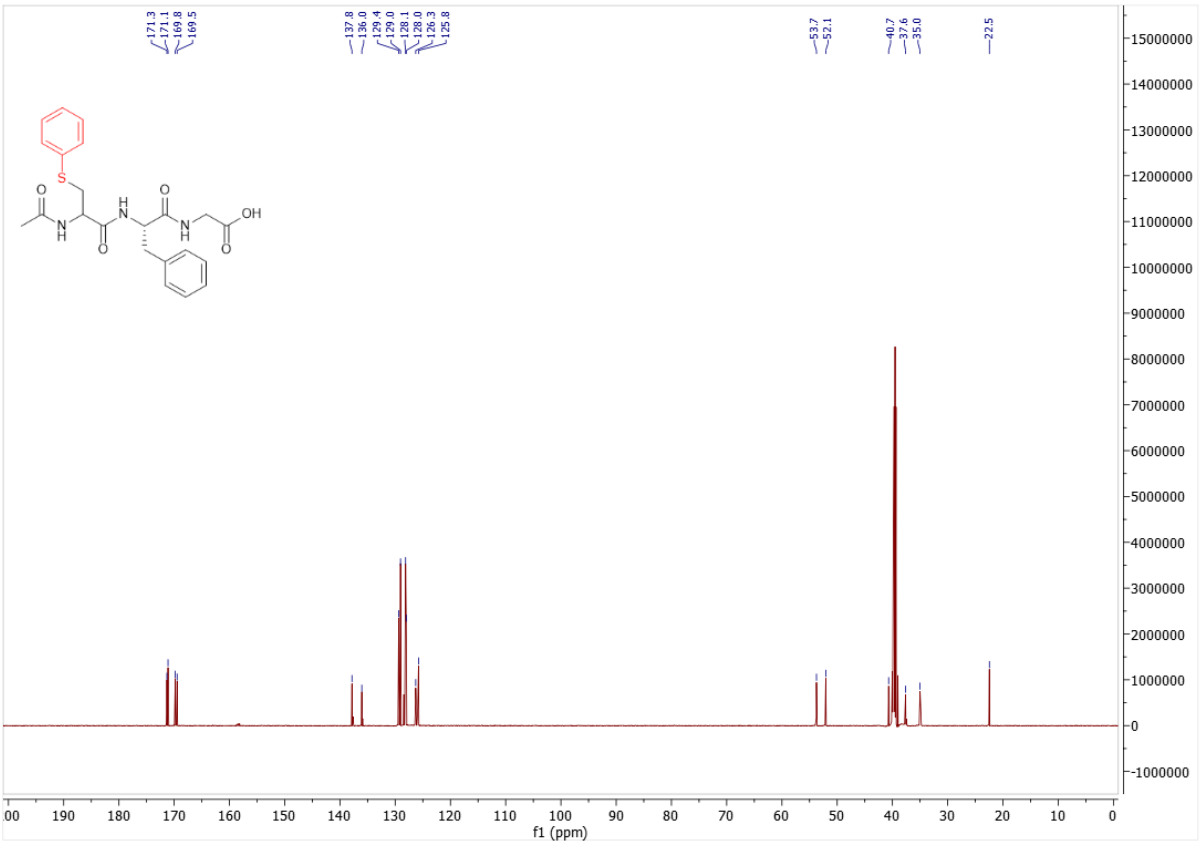


Figure S73 ^{13}C NMR (126 MHz) of diastereomer 2 of compound **6g**.

Compound 6h

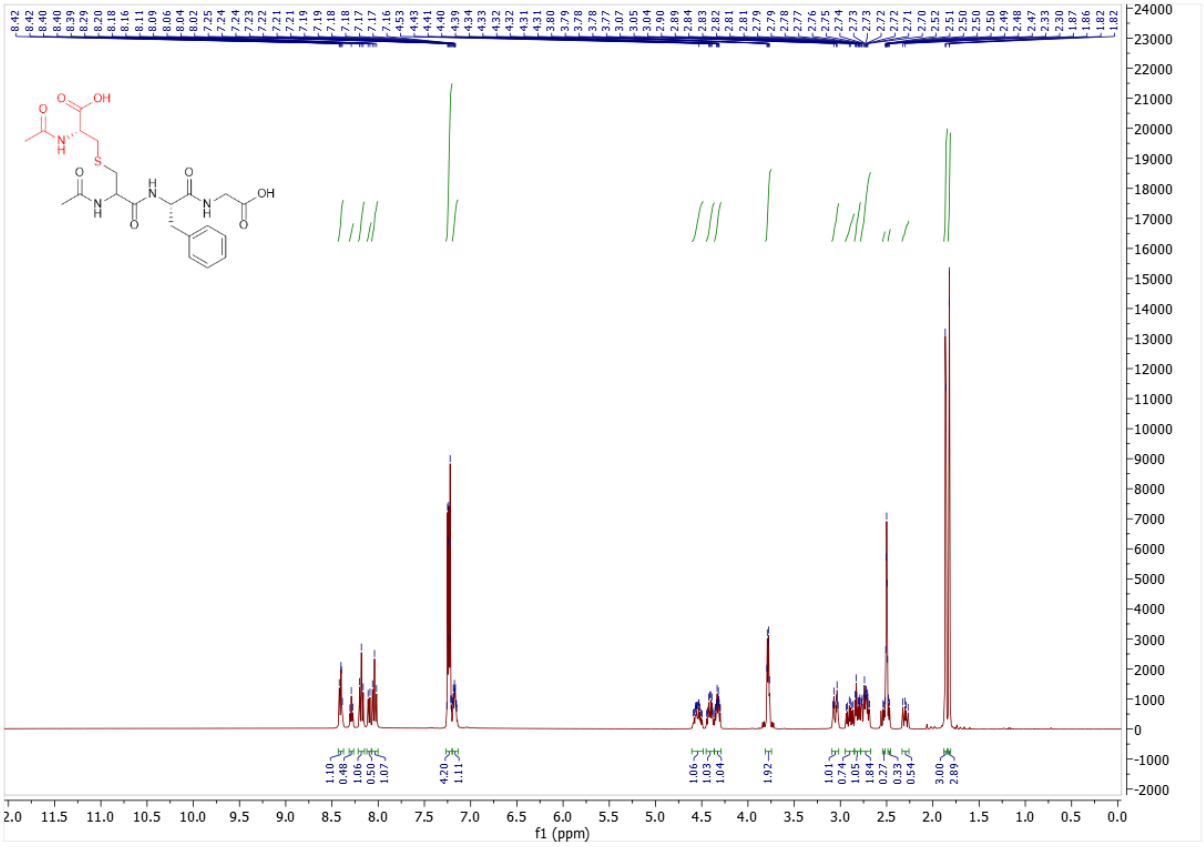


Figure S74 ^{1}H NMR (500 MHz) of compound **6h**.

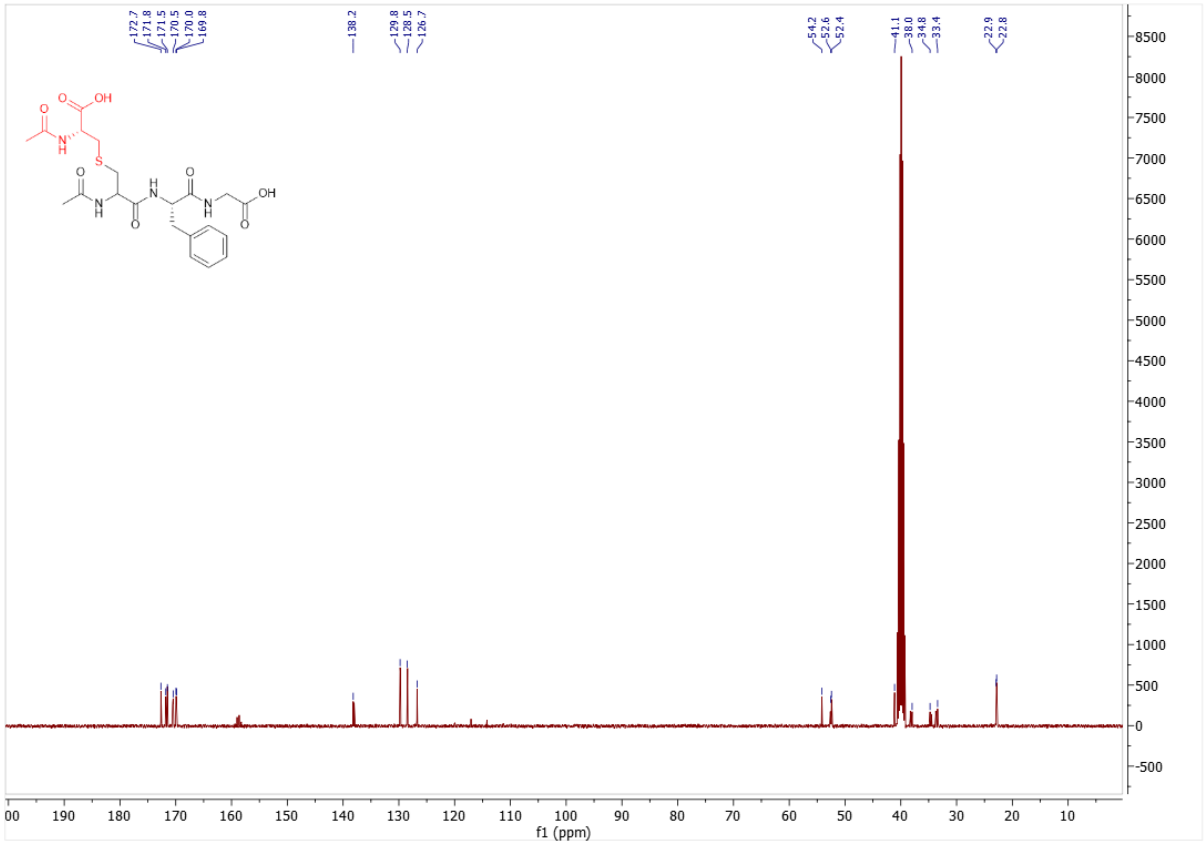


Figure S75 ^{13}C NMR (126 MHz) of compound **6h**.

Compound 6i

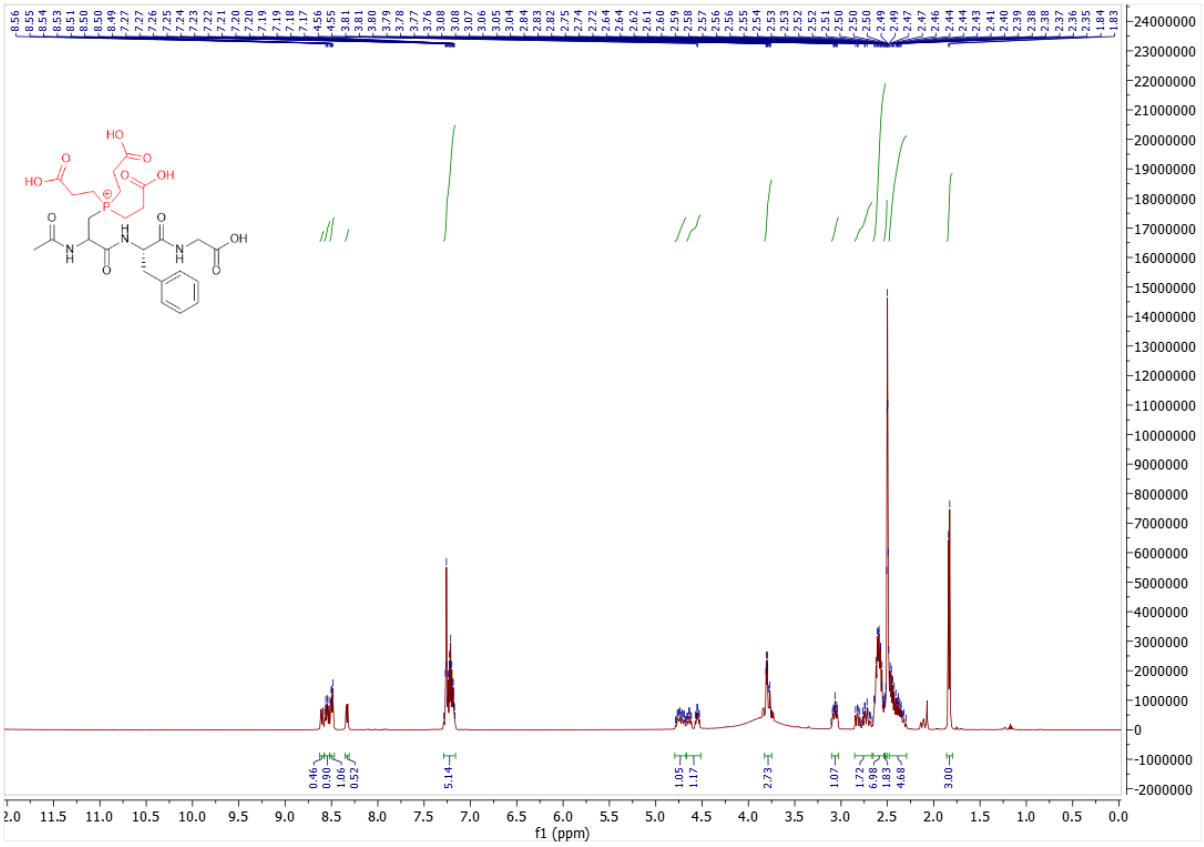


Figure S76 ^{1}H NMR (500 MHz) of compound **6i**.

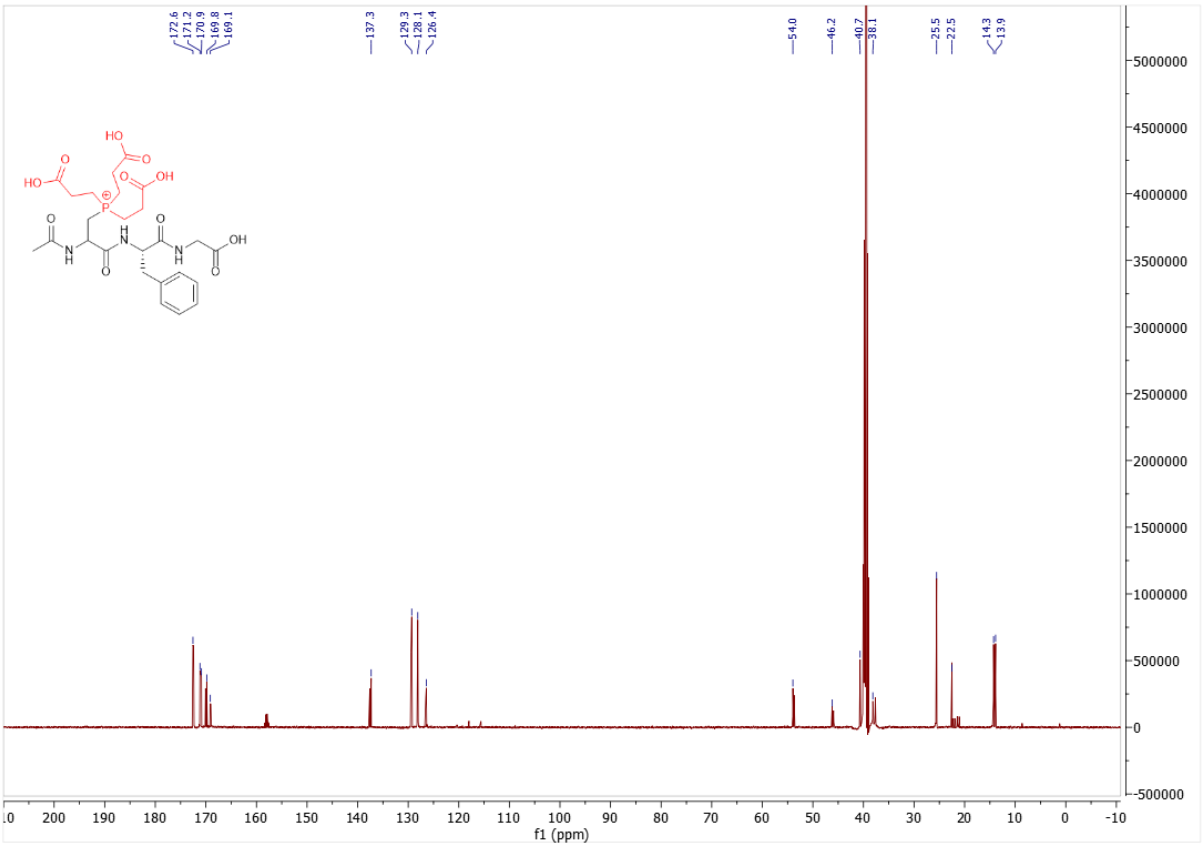


Figure S77 ^{13}C NMR (126 MHz) of compound **6i**.

Compound 6j

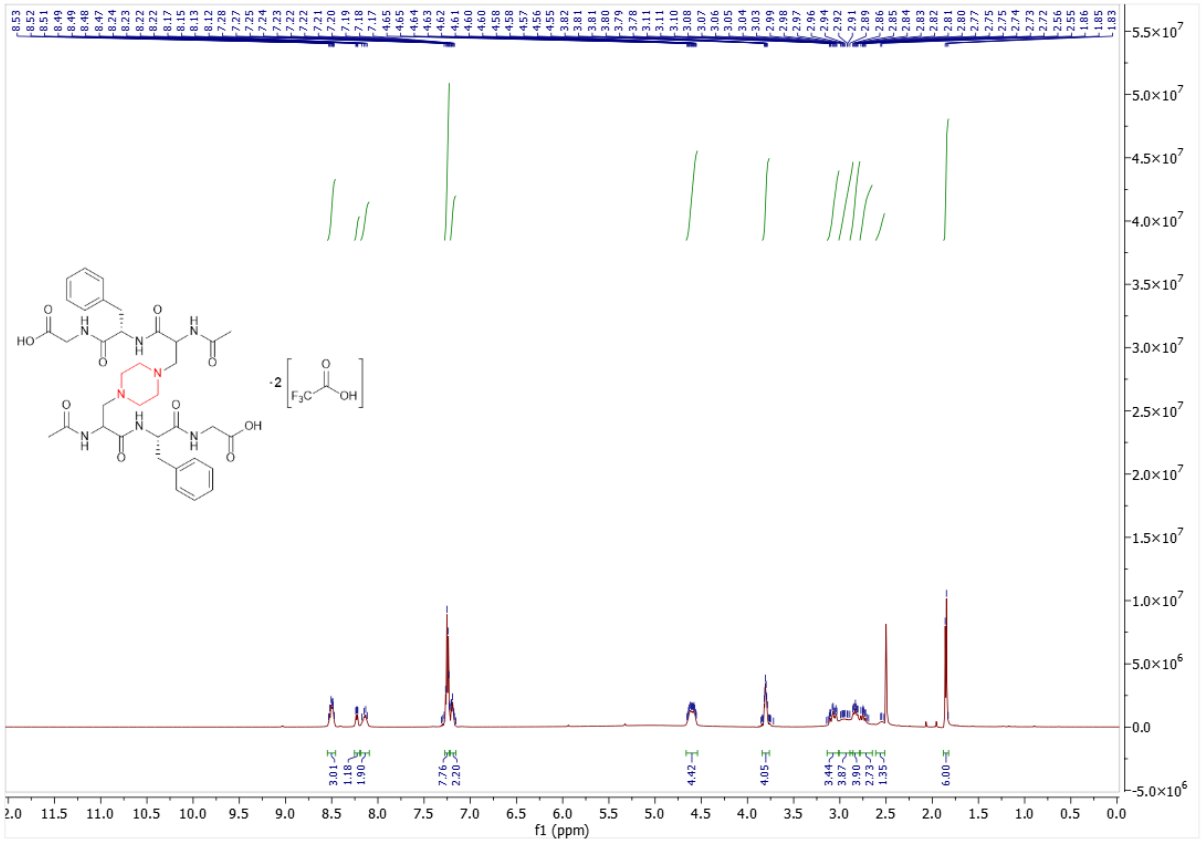


Figure S78 ^{1}H NMR (500 MHz) of compound **6j**.

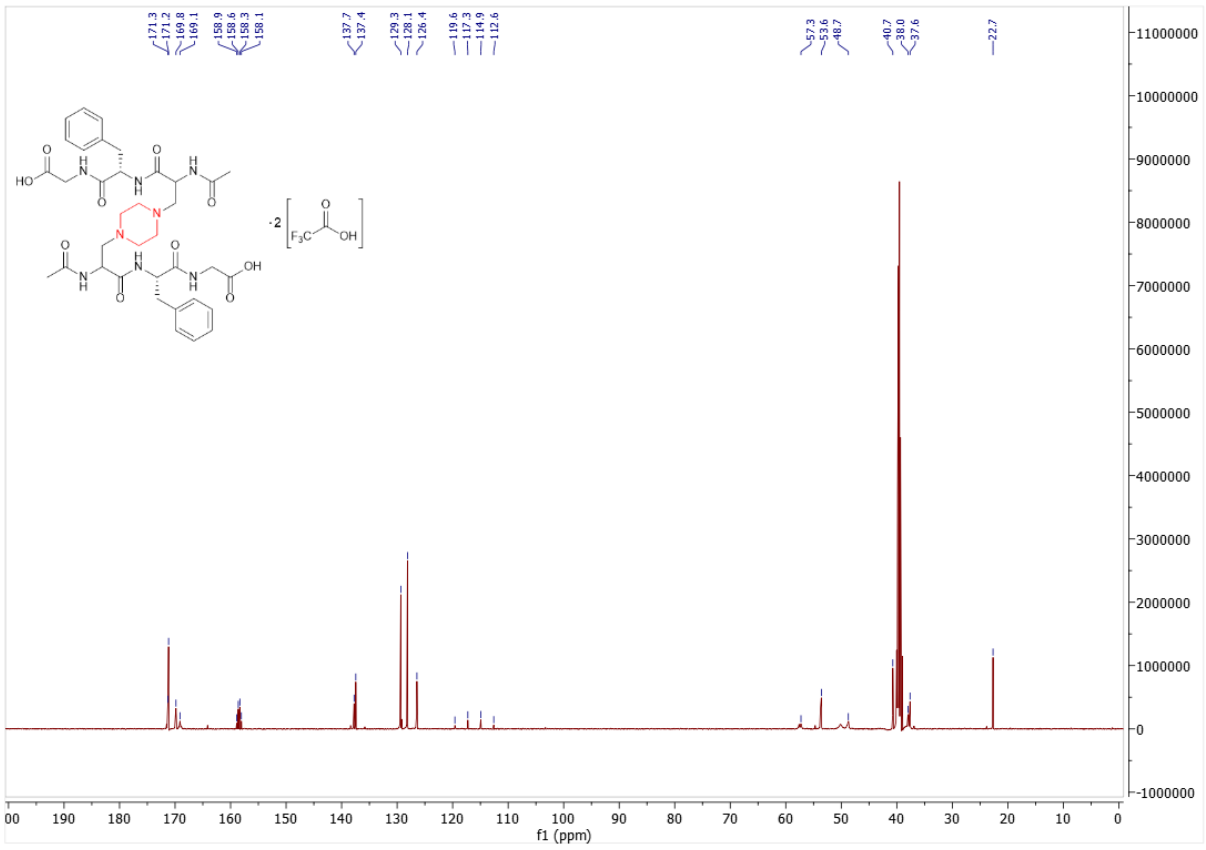


Figure S79 ^{13}C NMR (126 MHz) of compound **6j**.

Compound 6k

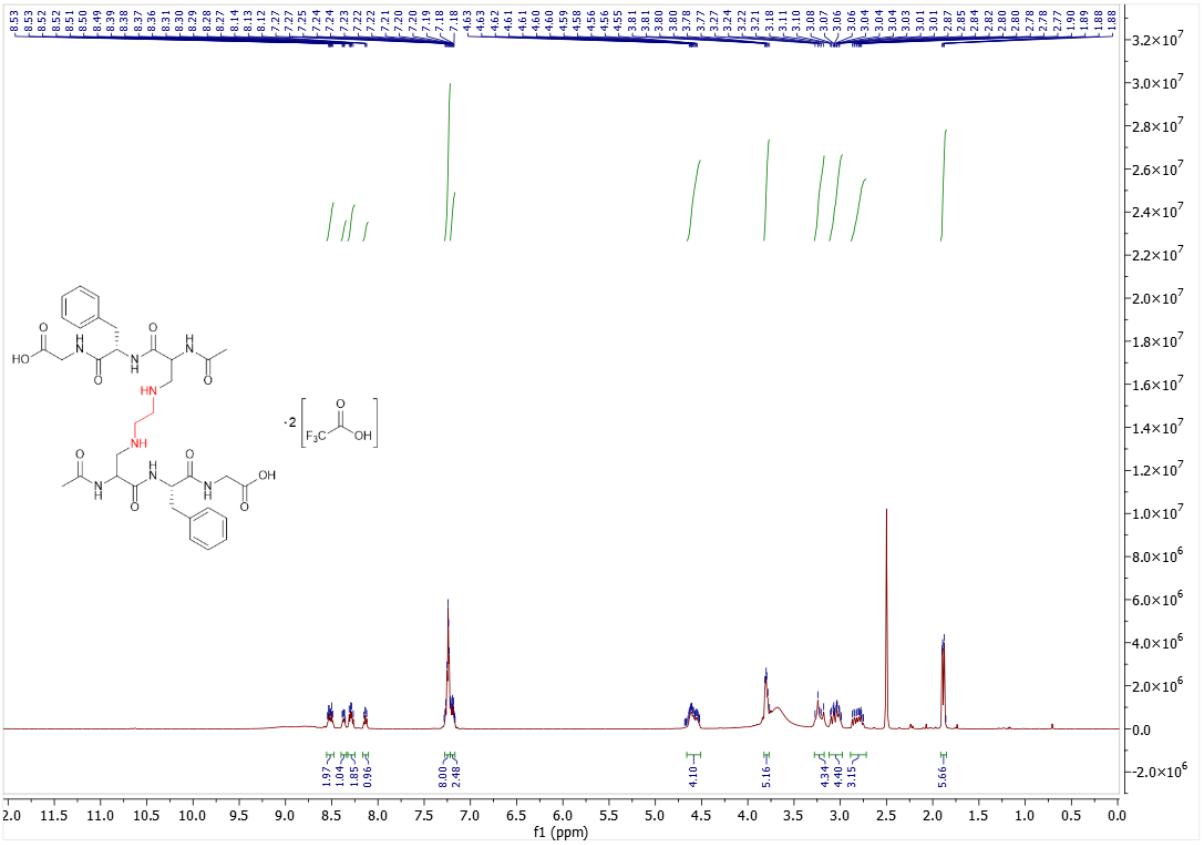


Figure S80 ^{1}H NMR (500 MHz) of compound **6k**.

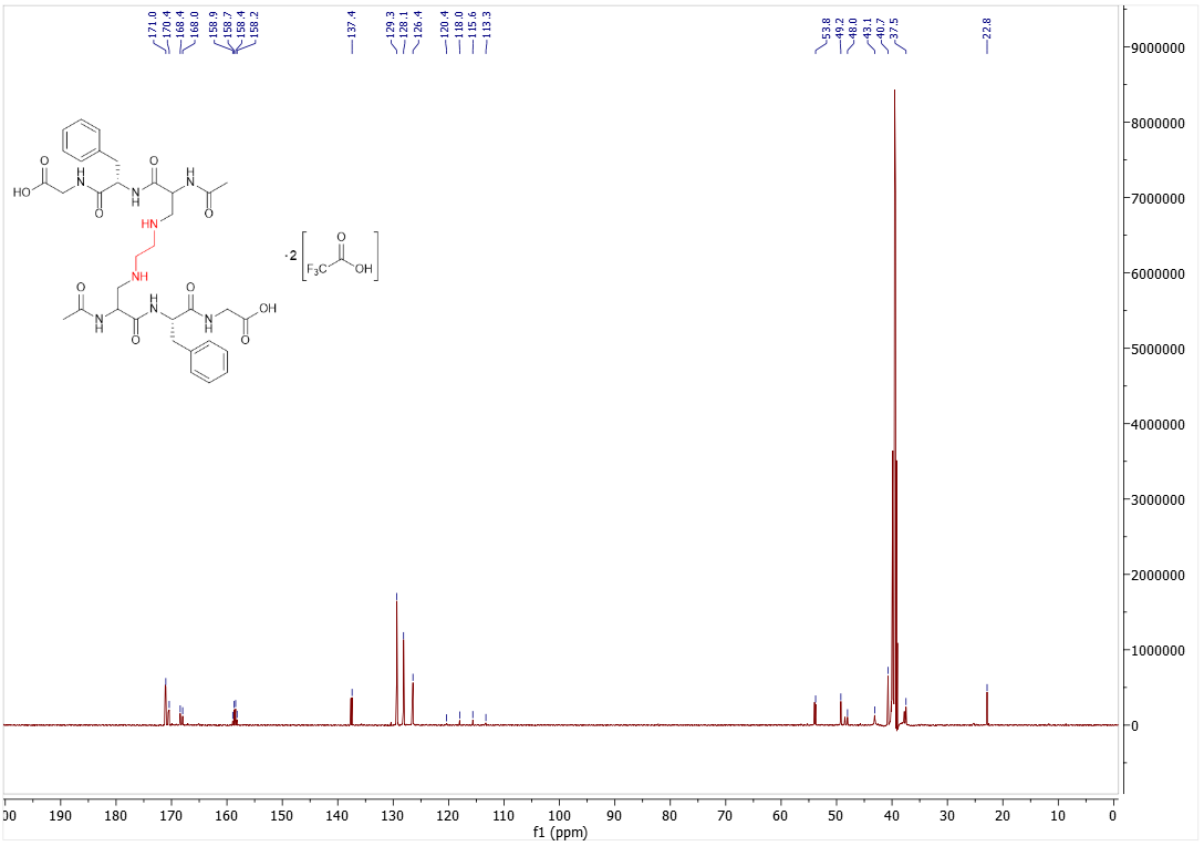


Figure S81 ^{13}C NMR (126 MHz) of compound **6k**.

Compound 6l

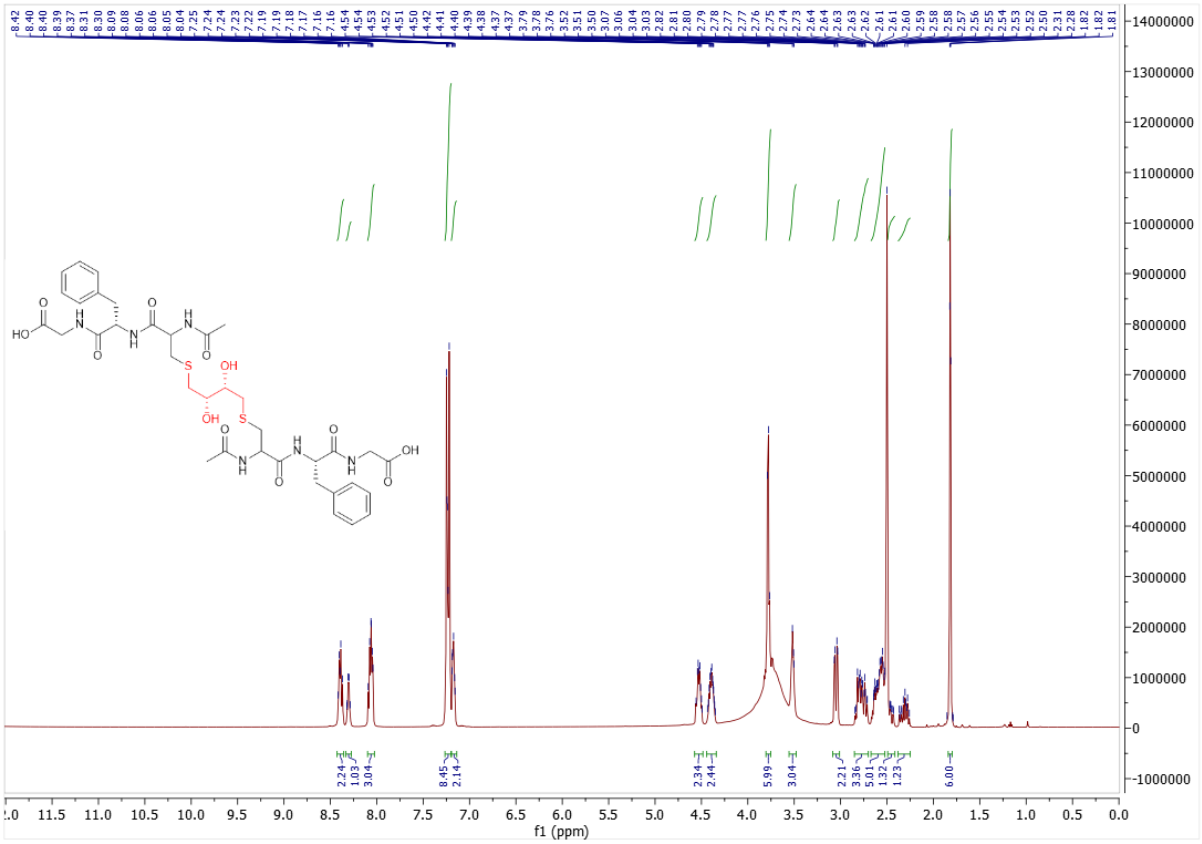


Figure S82 ^{1}H NMR (500 MHz) of compound **6l**.

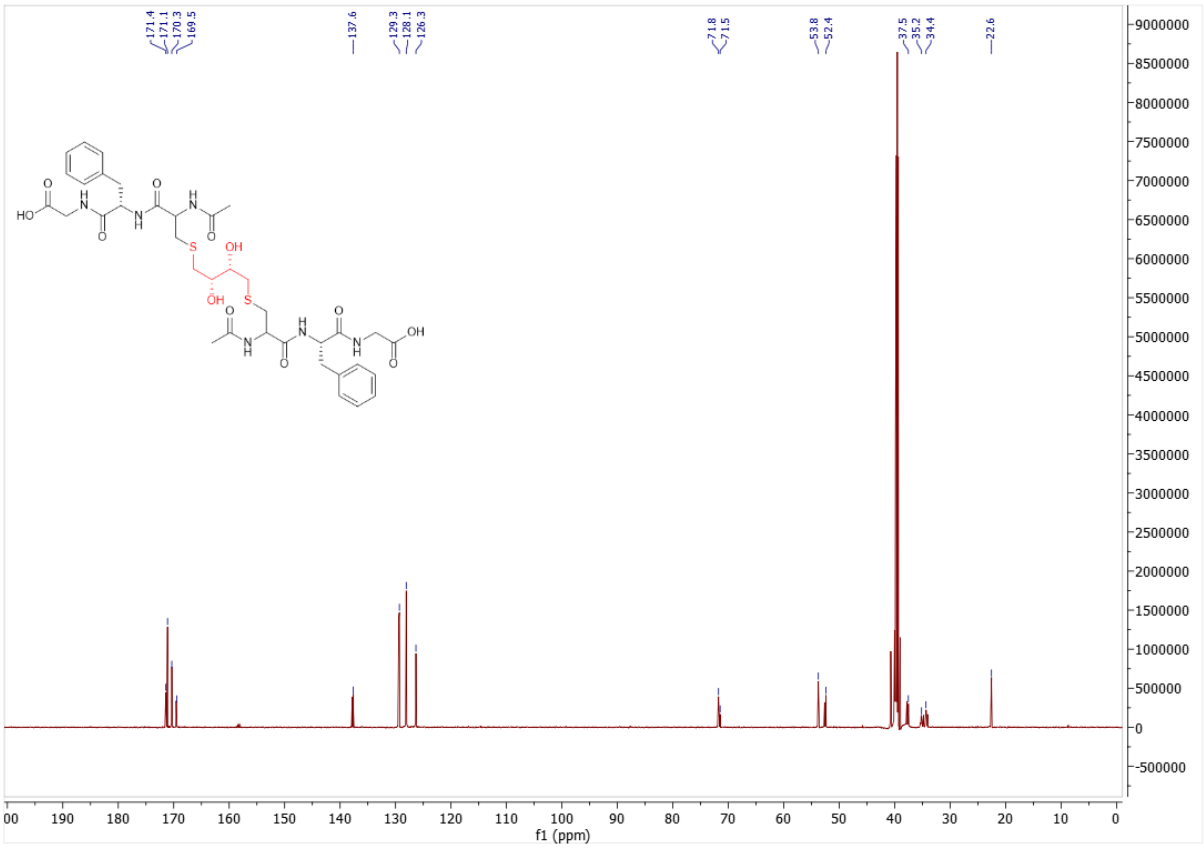


Figure S83 ^{13}C NMR (126 MHz) of compound **6l**.

Compound 6m

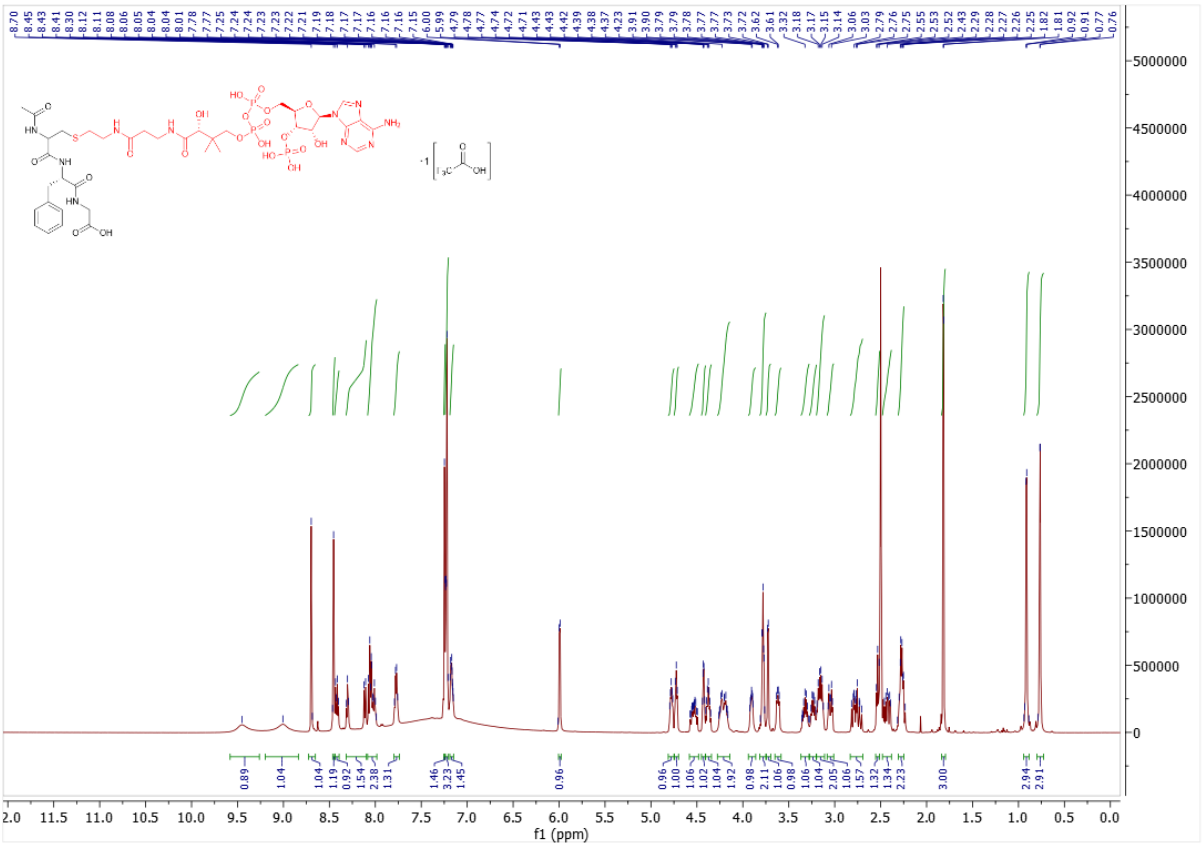


Figure S84 ^{1}H NMR (500 MHz) of compound **6m**.

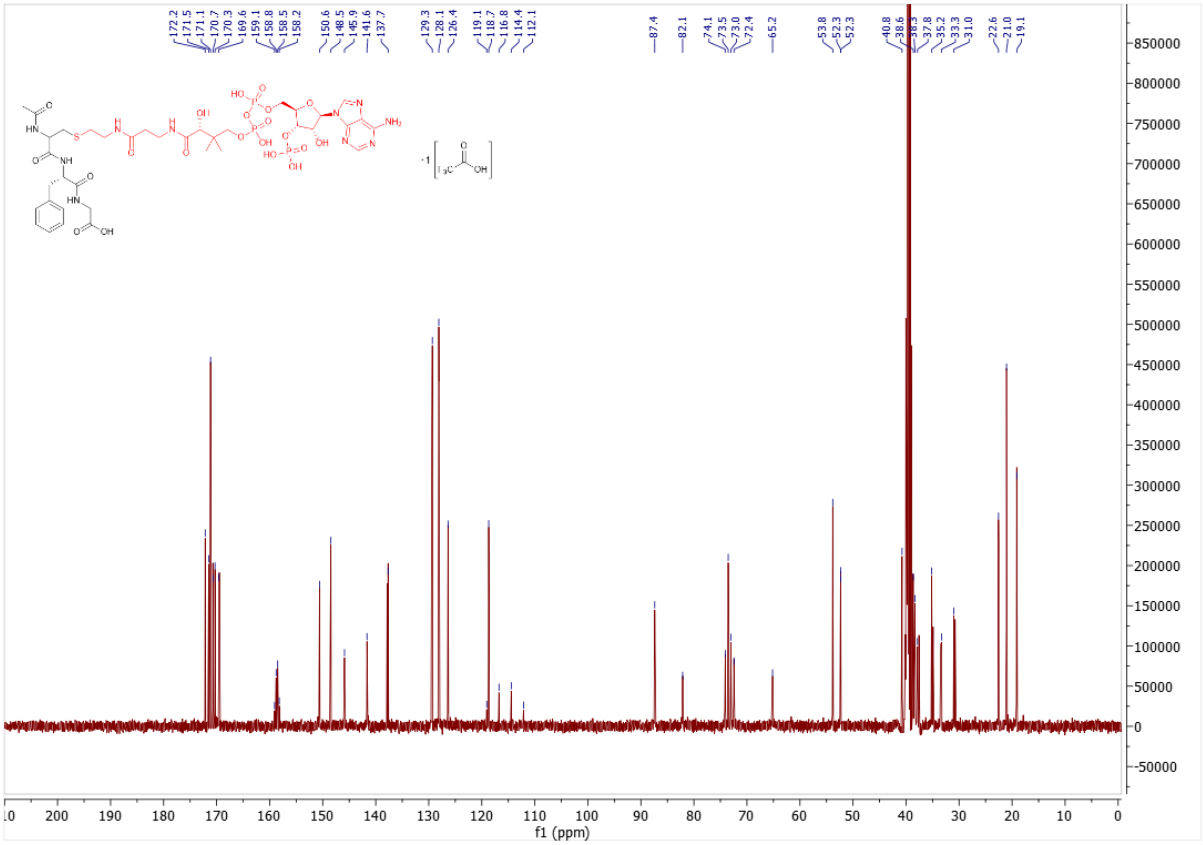


Figure S85 ^{13}C NMR (126 MHz) of compound **6m**.

Compound 6n

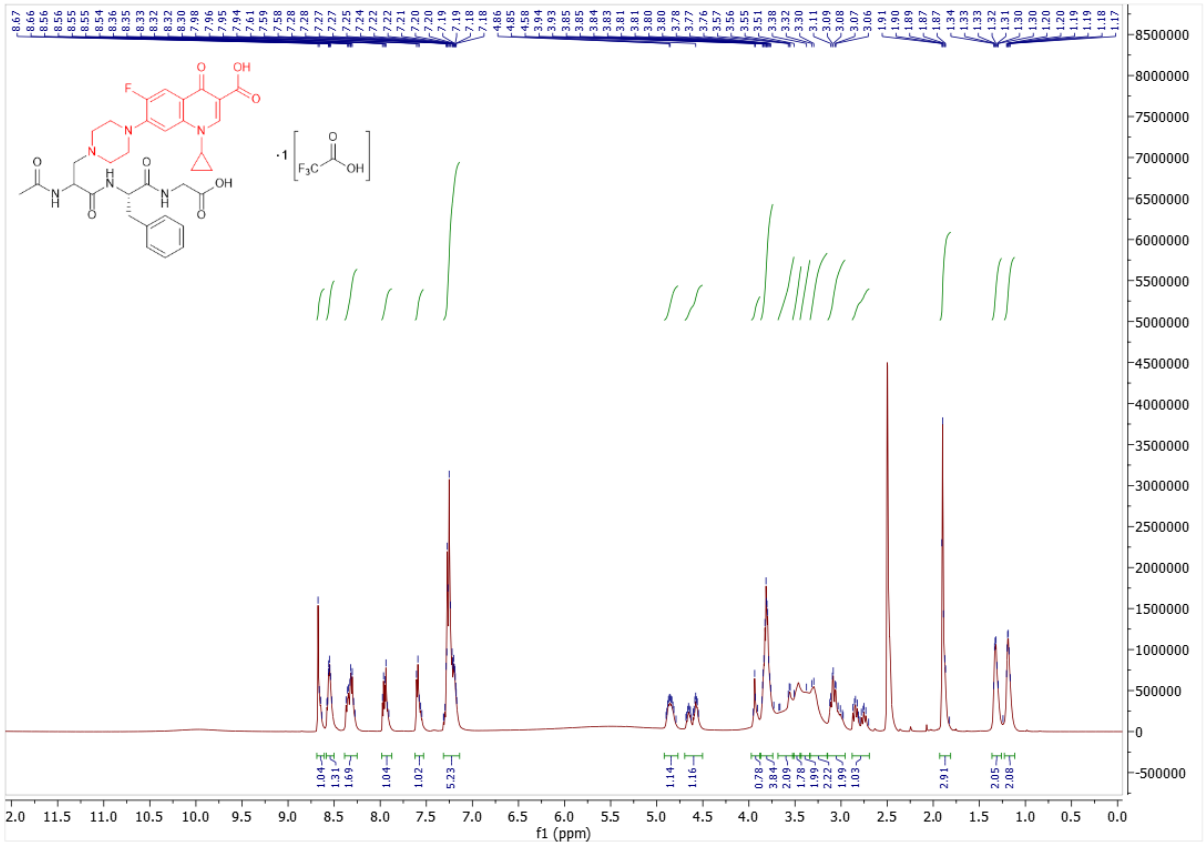


Figure S86 ^{1}H NMR (500 MHz) of compound **6n**.

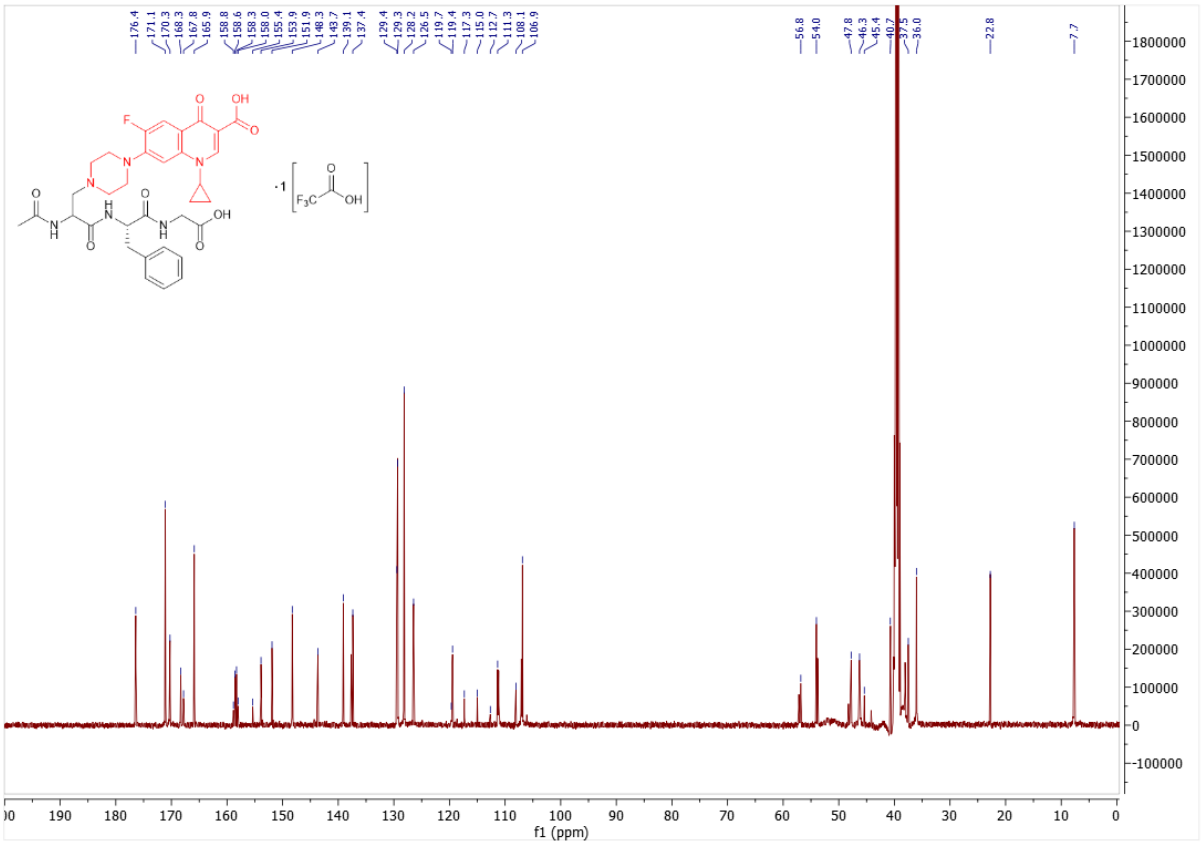


Figure S87 ^{13}C NMR (126 MHz) of compound **6n**.

Compound 6p

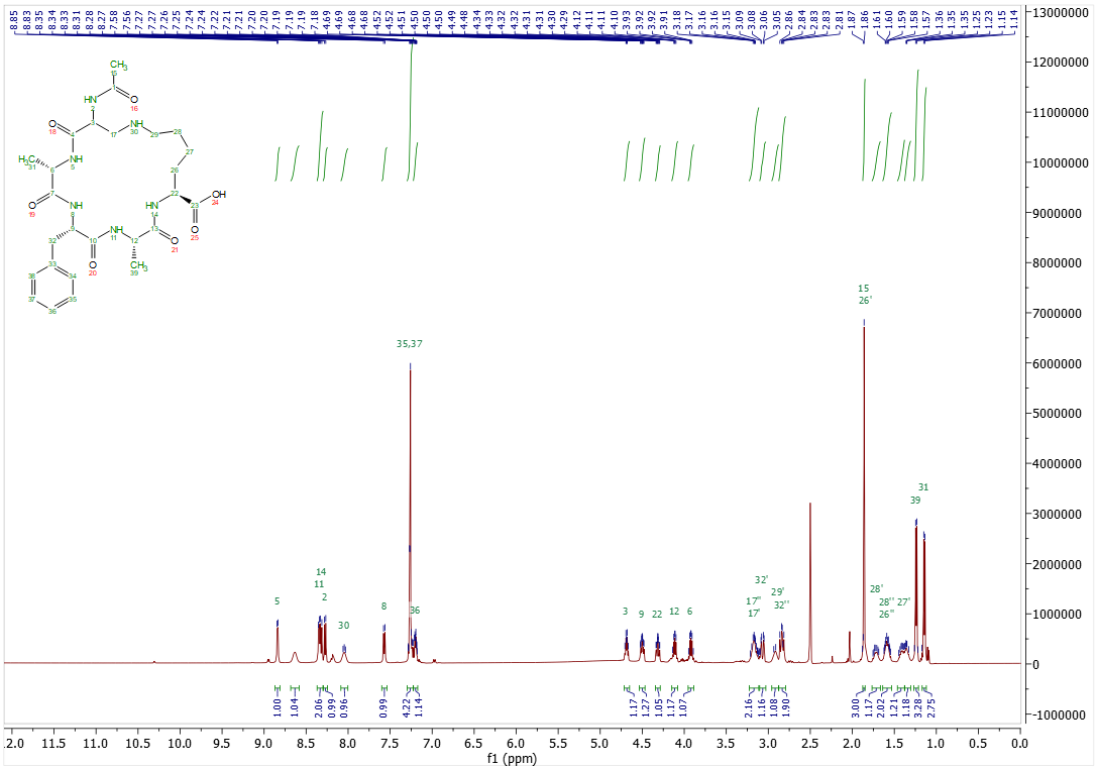


Figure S88 ^{1}H NMR (500 MHz) of compound **6p**. No protons corresponding to Dha are present.

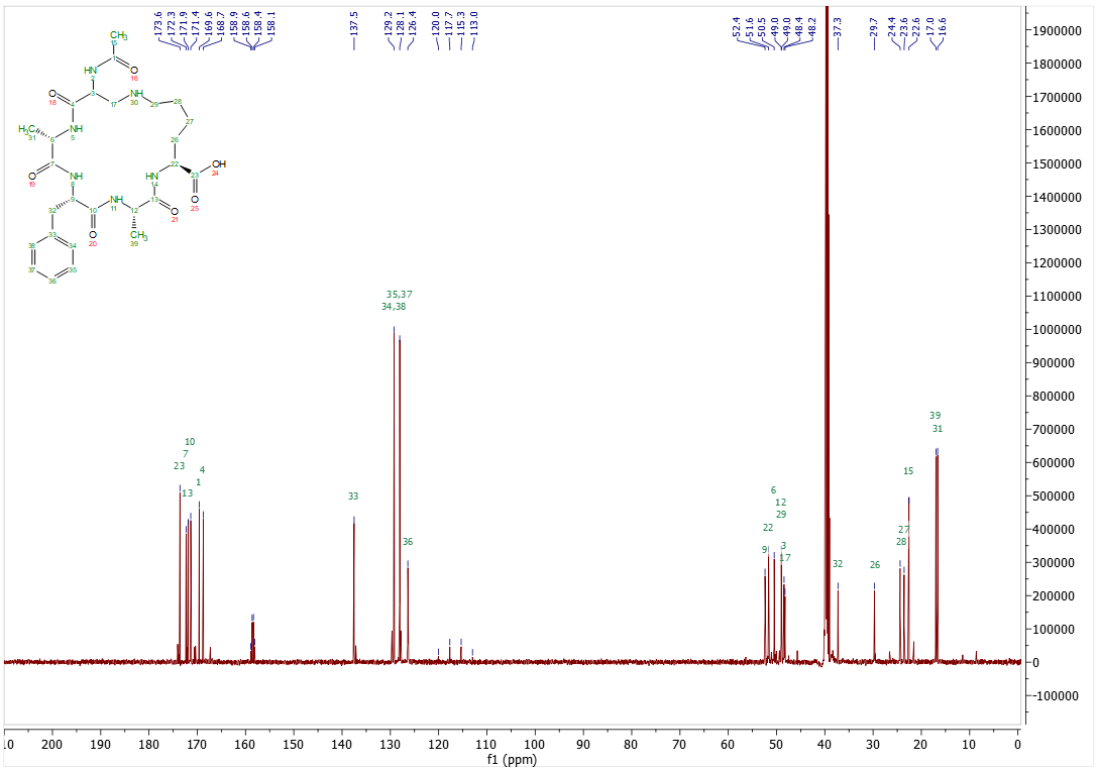


Figure S89 ^{13}C NMR (126 MHz) of compound **6p**.

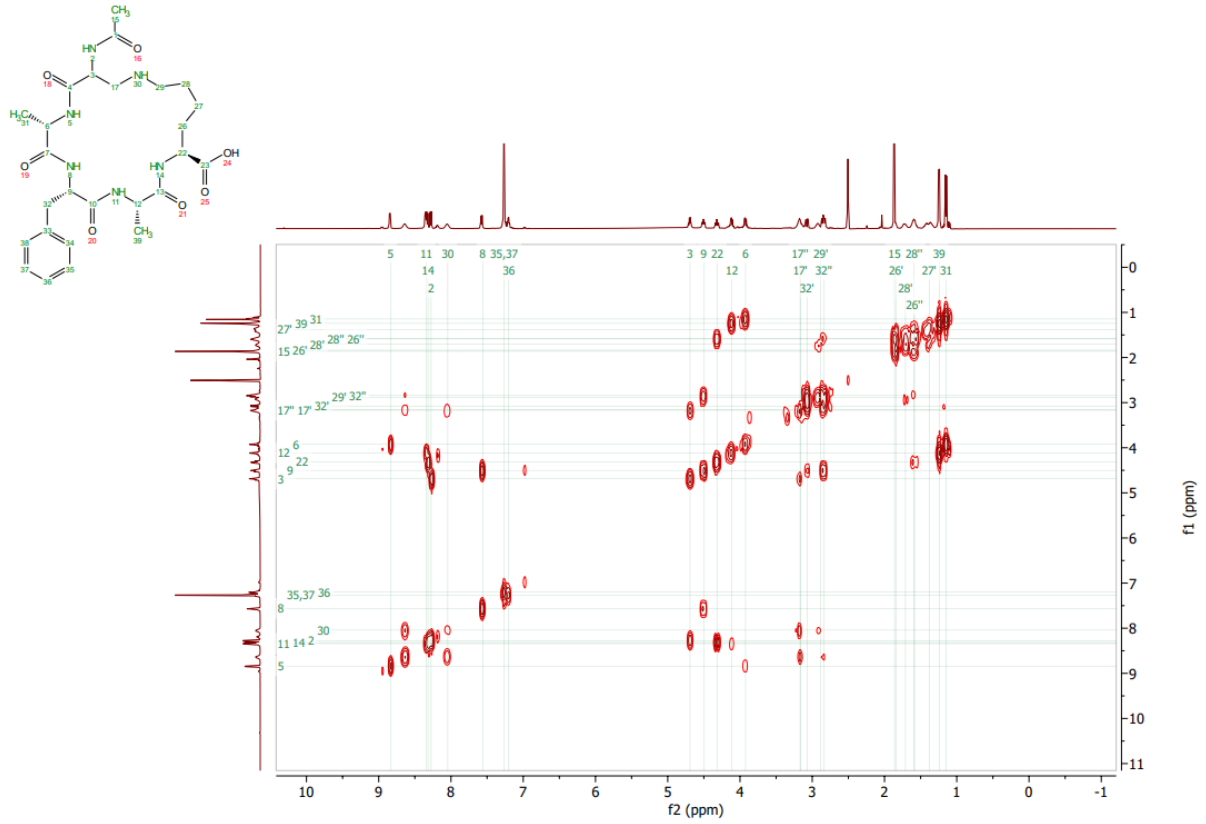


Figure S90 COSY of compound **6p**.

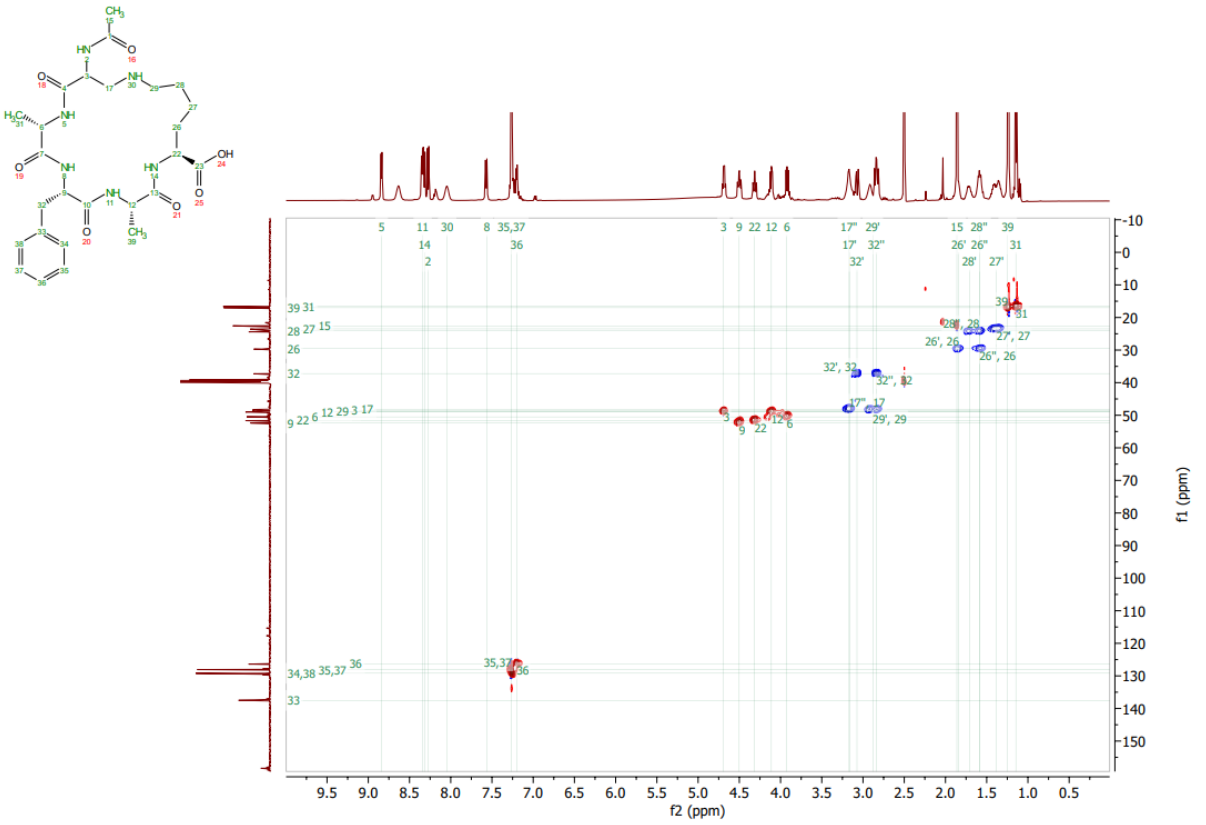


Figure S91 HSQC of compound **6p**.

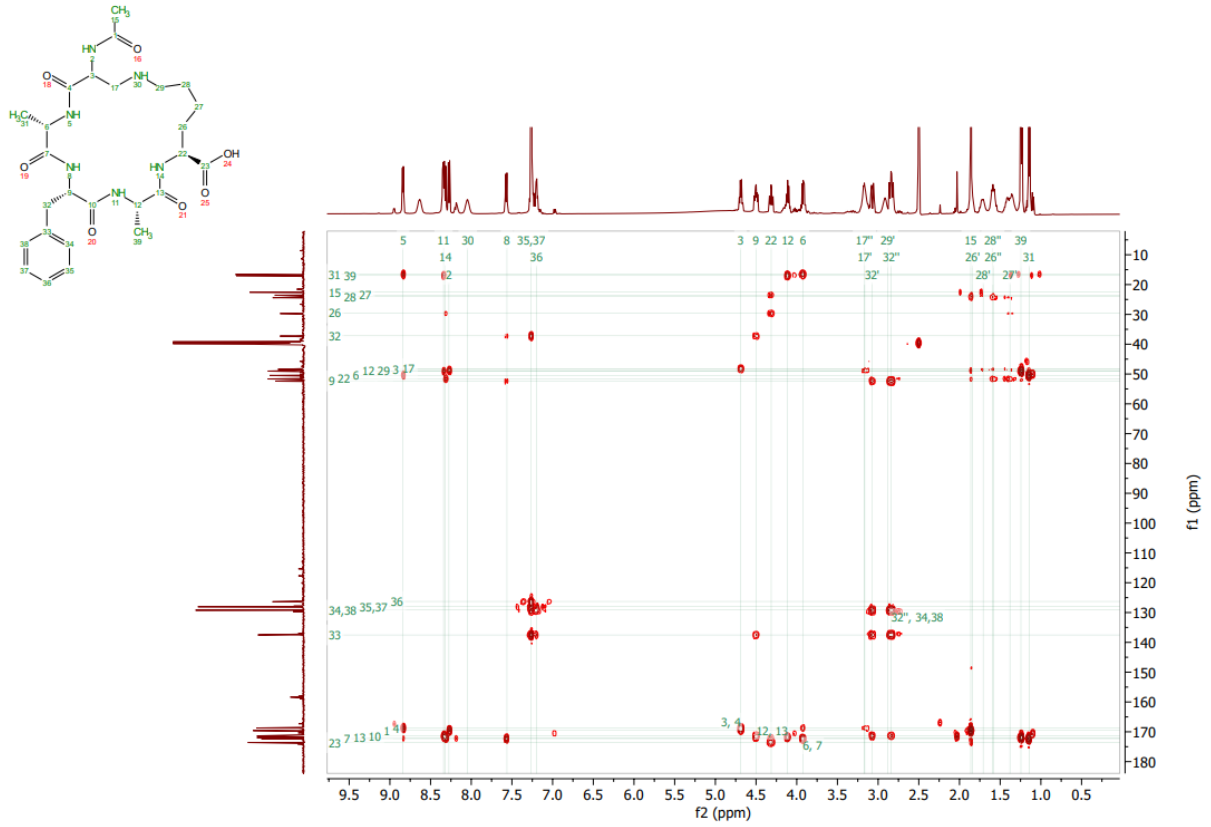


Figure S92 HMBC of compound **6p**.

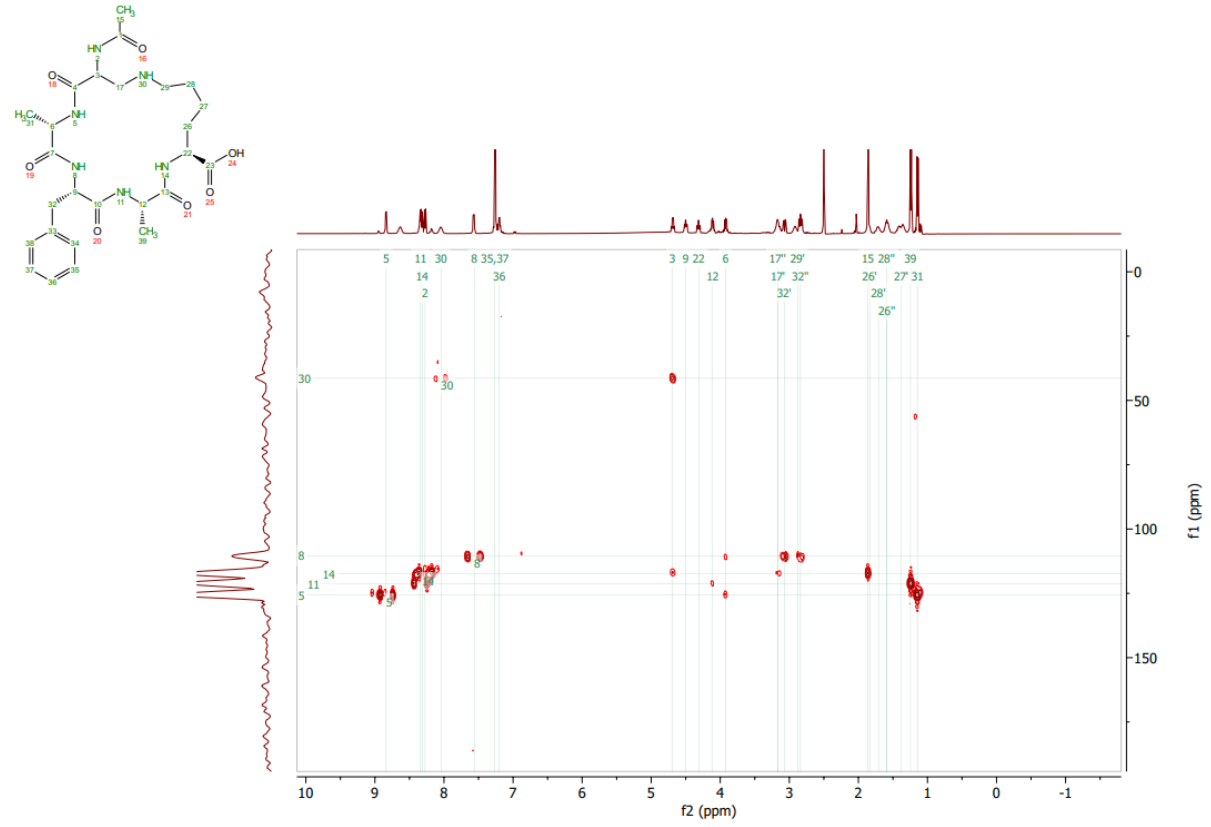


Figure S93 ^{15}N HMBC of compound **6p**, showing the important cross-peak between proton 3 and nitrogen 30.

8. High Resolution Mass Spectra (HRMS)

Compound 1b

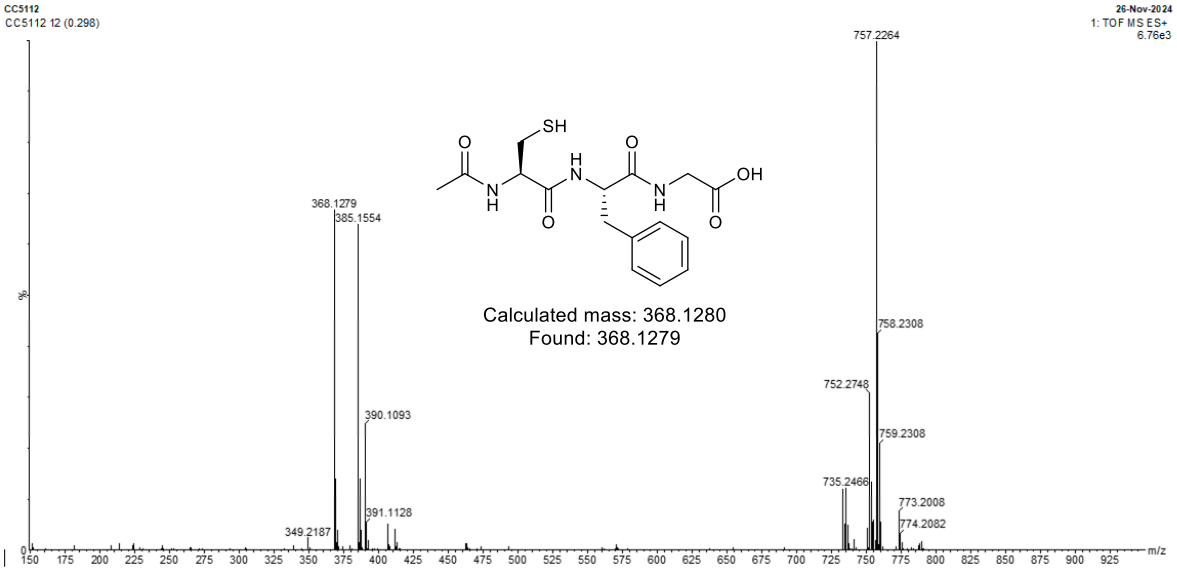


Figure S94 HRMS spectrum of compound **1b**. $[M+NH_4]^+$ 385 m/z, $[M+Na]^+$ 390 m/z and $[2M+Na]^+$ 757 m/z.

Compound 1c

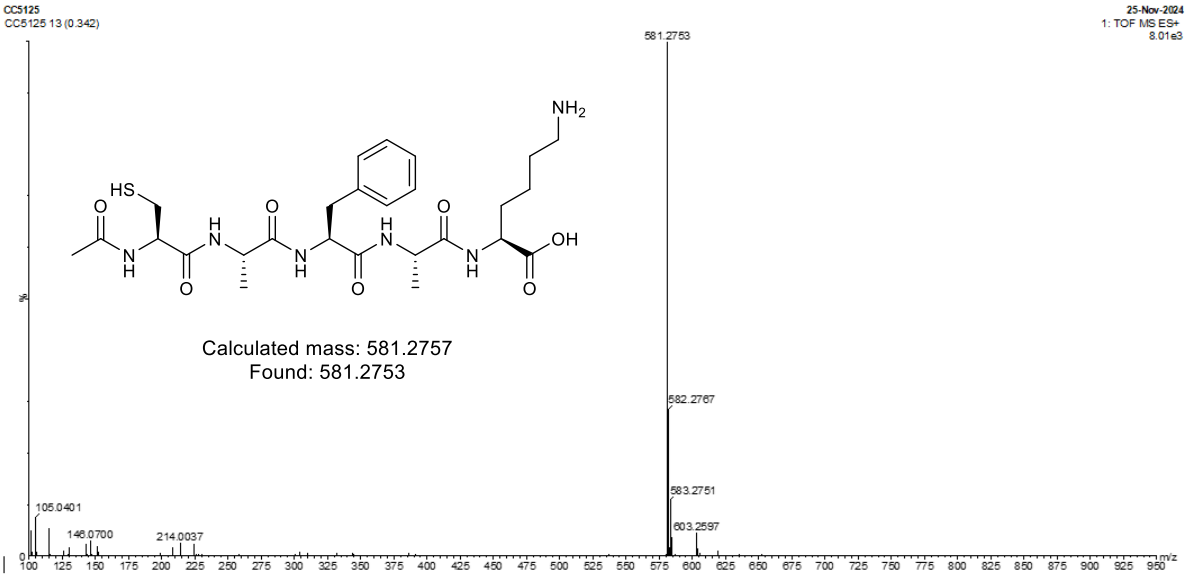


Figure S95 HRMS spectrum of compound **1c**.

Compound 1d

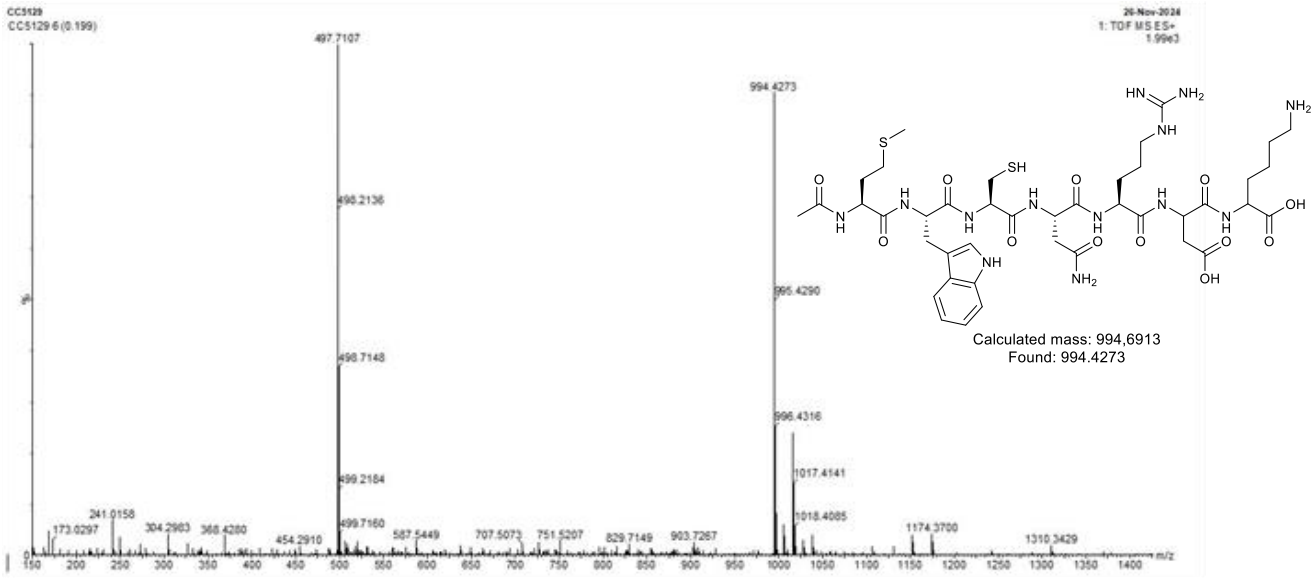


Figure S96 HRMS spectrum of compound **1d**.

Compound 6a

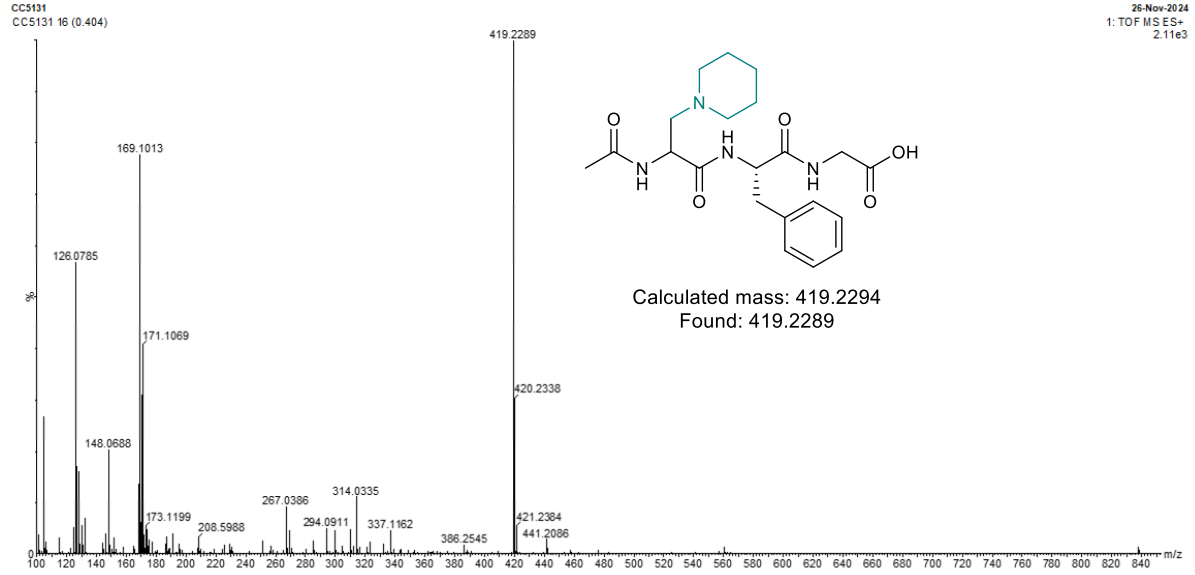


Figure S97 HRMS spectrum of compound **6a**.

Compound 6**b**

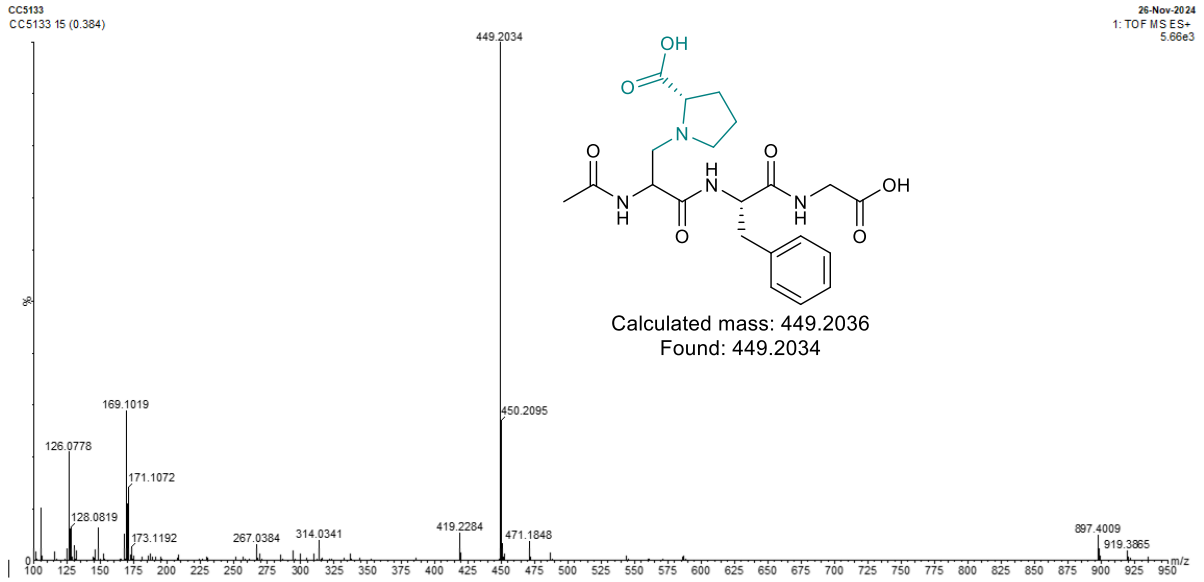


Figure S98 HRMS spectrum of compound **6b**.

Compound 6c

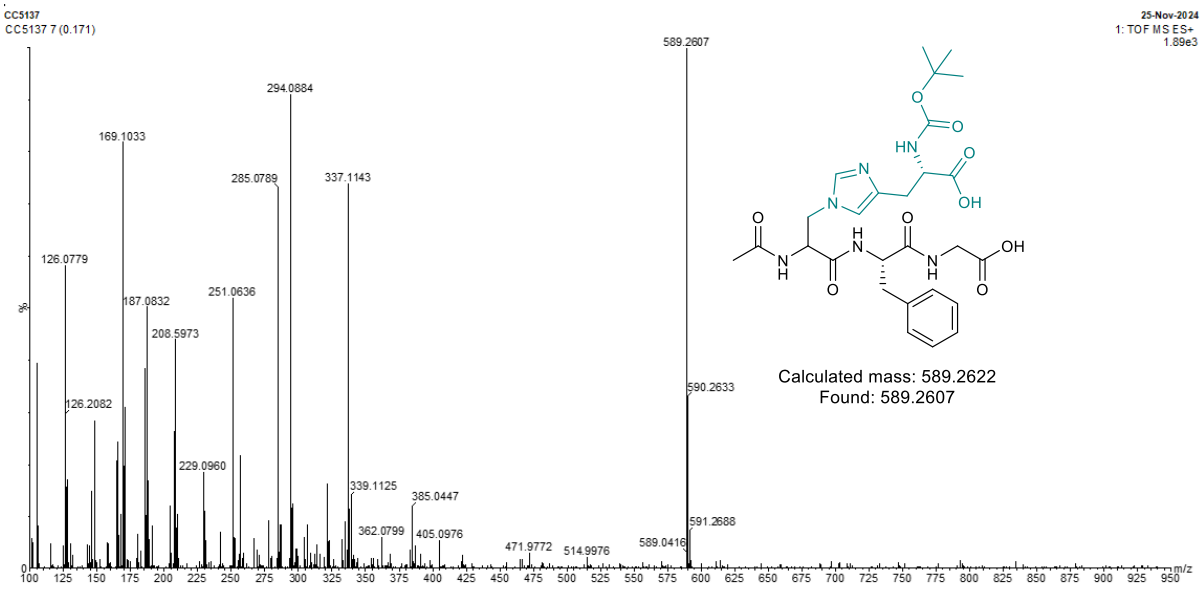


Figure S99 HRMS spectrum of compound **6c**.

Compound 6d

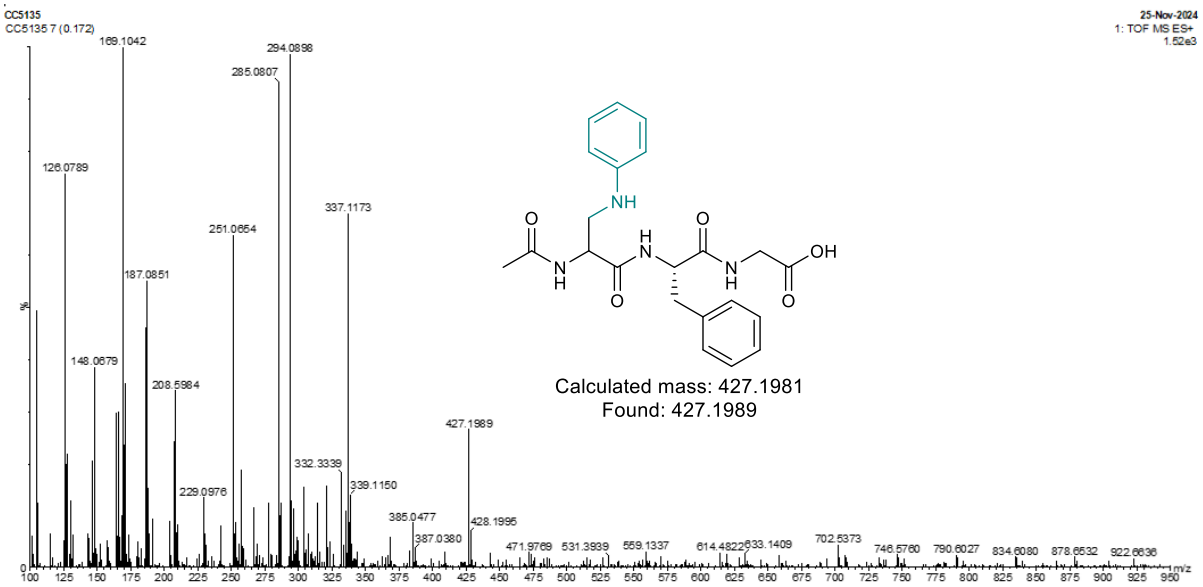


Figure S100 HRMS spectrum of compound **6d**.

Compound 6e

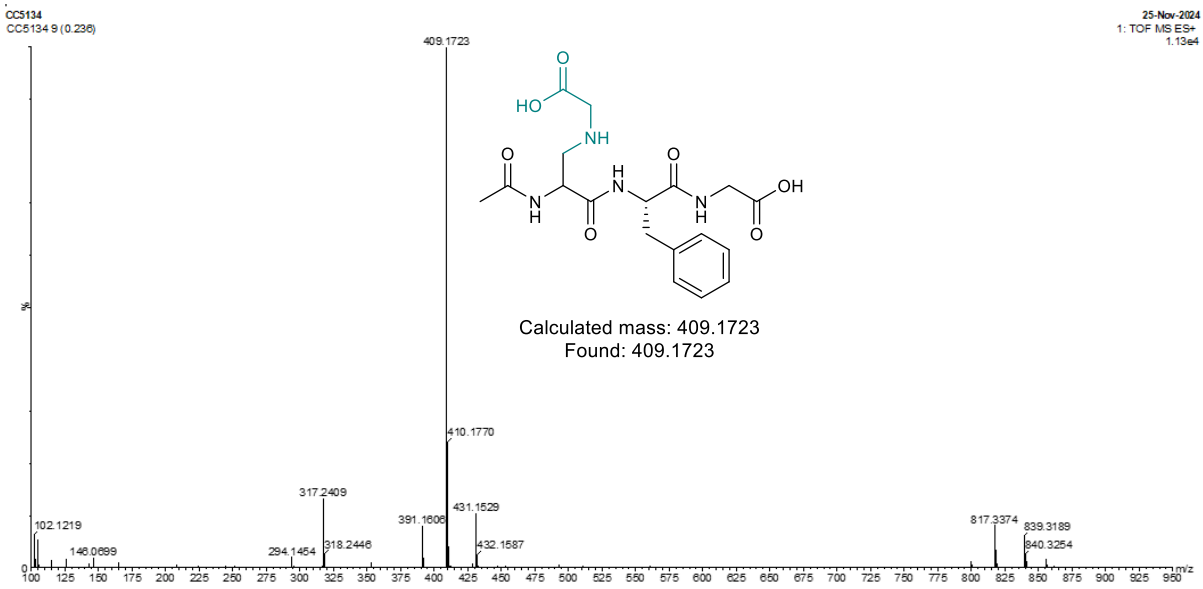


Figure S101 HRMS spectrum of compound **6e**.

Compound 6f

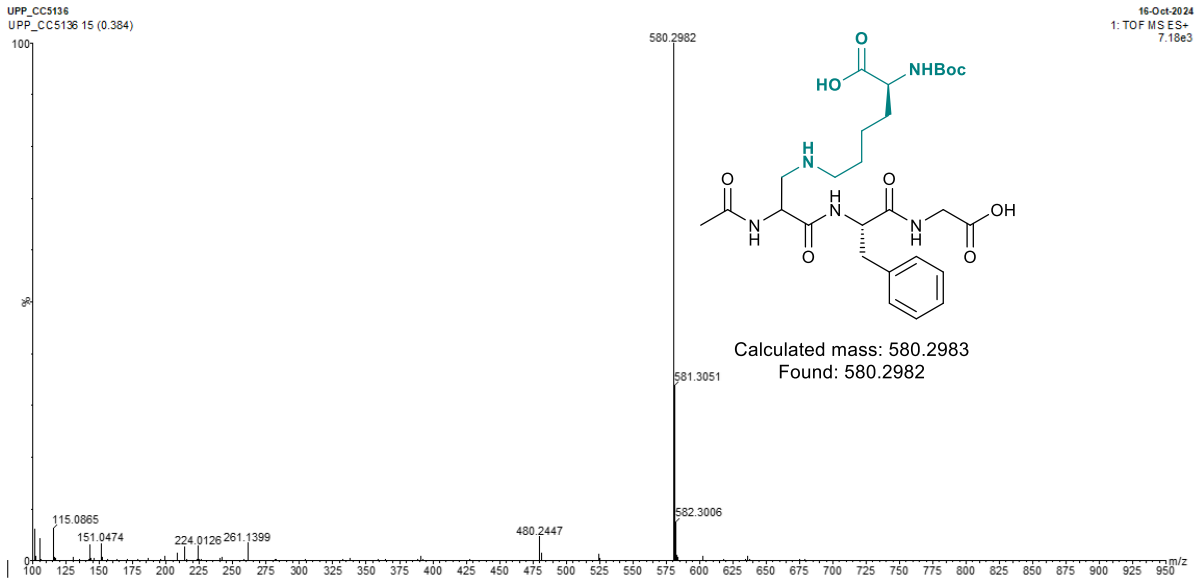


Figure S102 HRMS spectrum of compound **6f**. $[M+NH_4]^+$ 580 m/z.

Compound 6g

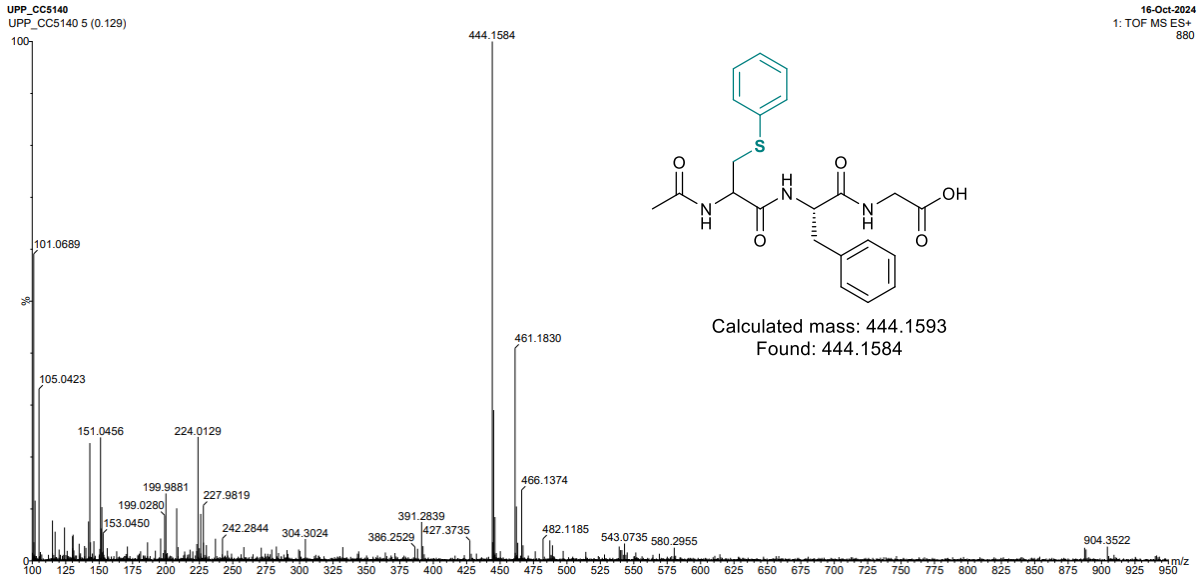


Figure S103 HRMS spectrum of compound **6g**. $[M+NH_4]^+$ 461 m/z.

Compound 6h

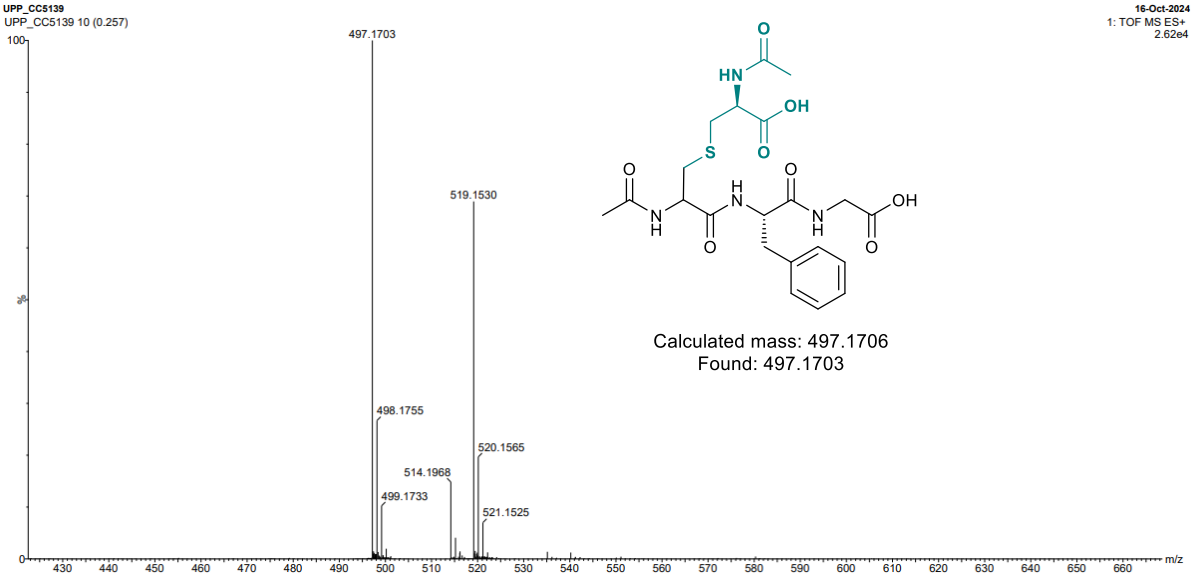


Figure S104 HRMS spectrum of compound **6h**. $[M+NH_4]^+$ 514 m/z and $[M+Na]^+$ 519 m/z.

Compound 6i

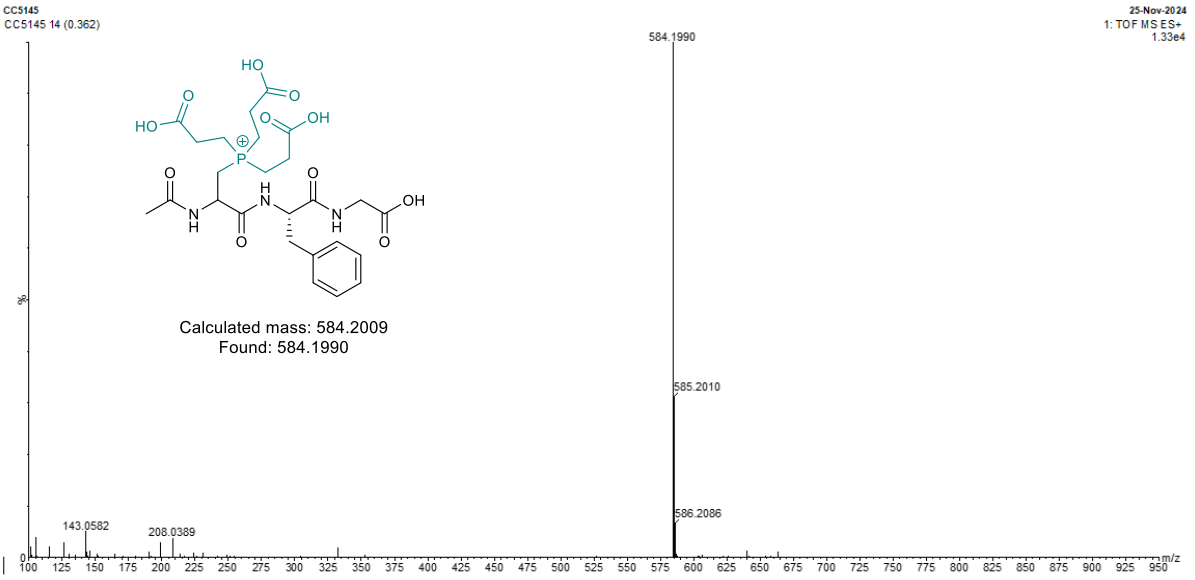


Figure S105 HRMS spectrum of compound **6i**.

Compound 6j

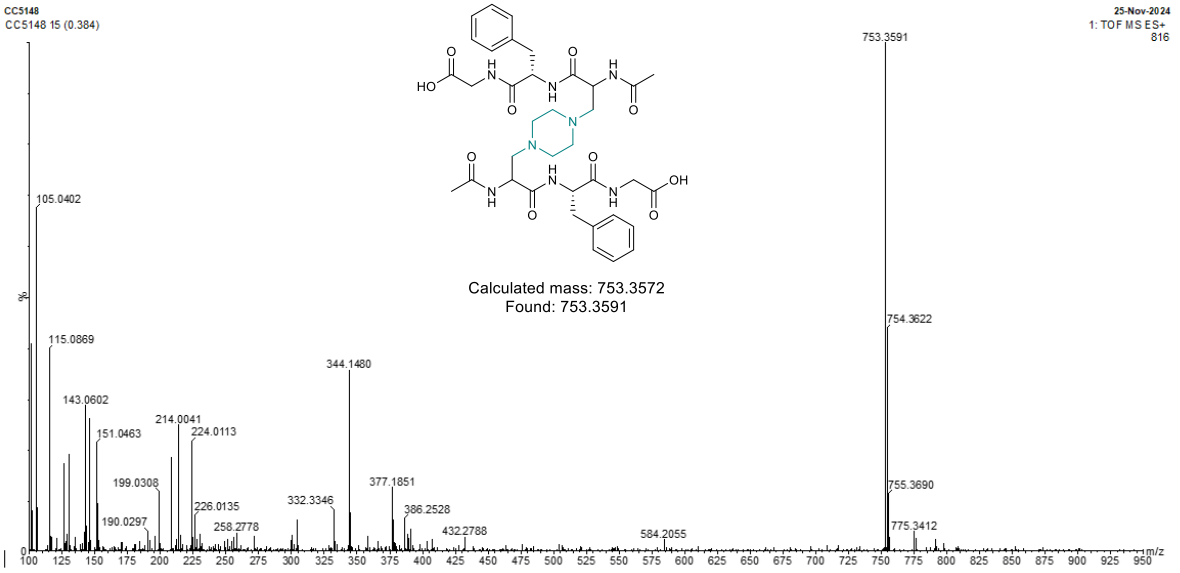


Figure S106 HRMS spectrum of compound **6j**.

Compound 6k

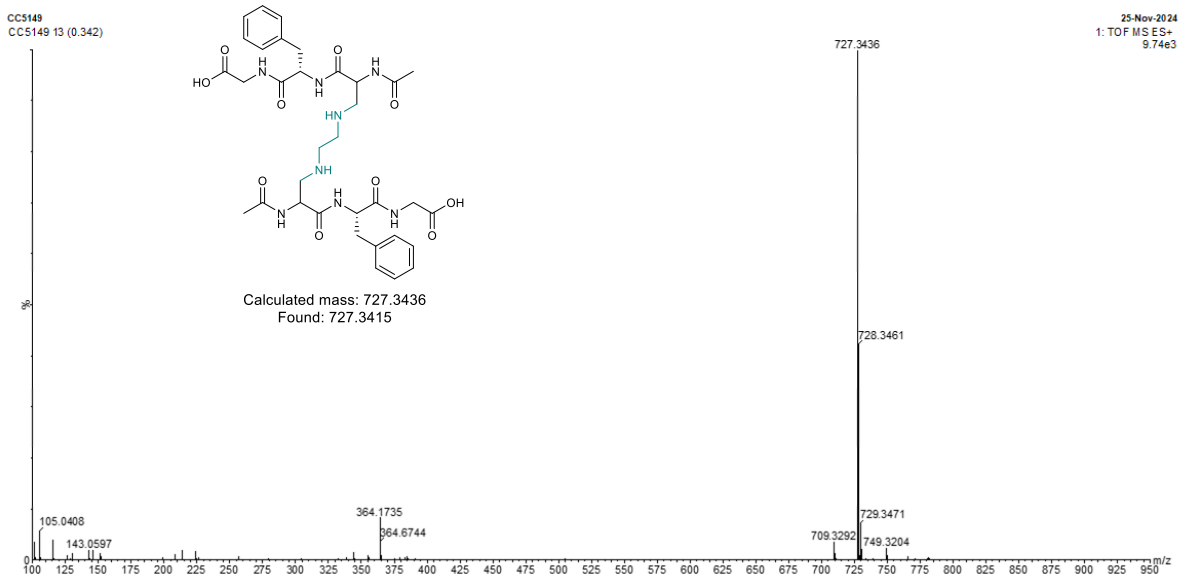


Figure S107 HRMS spectrum of compound **6k**.

Compound 6l

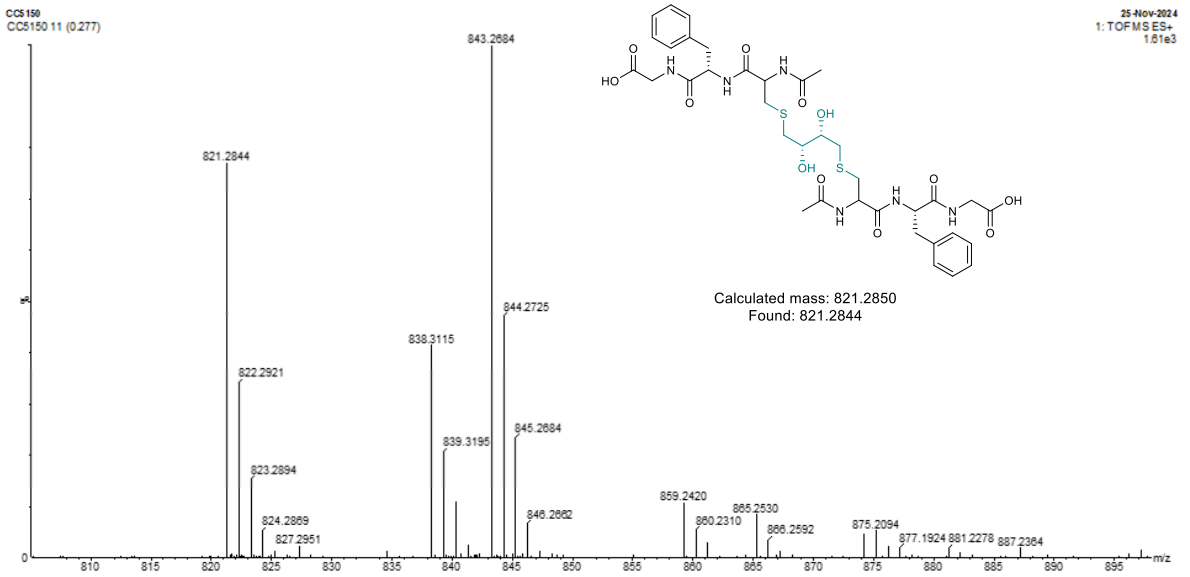


Figure S108 HRMS spectrum of compound **6l**. $[M+NH_4]^+$ 838 m/z and $[M+Na]^+$ 843 m/z.

Compound 6m

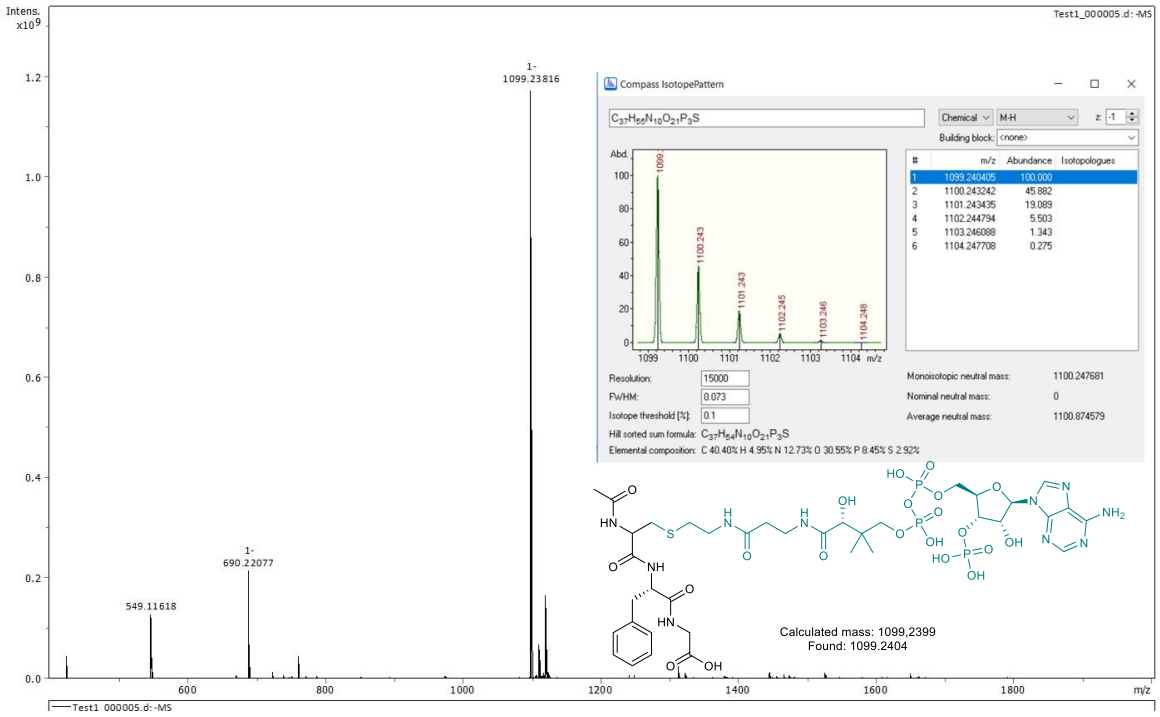


Figure S109 HRMS spectrum of compound **6m**. $[M-H]^-$ 1099.

Compound 6n

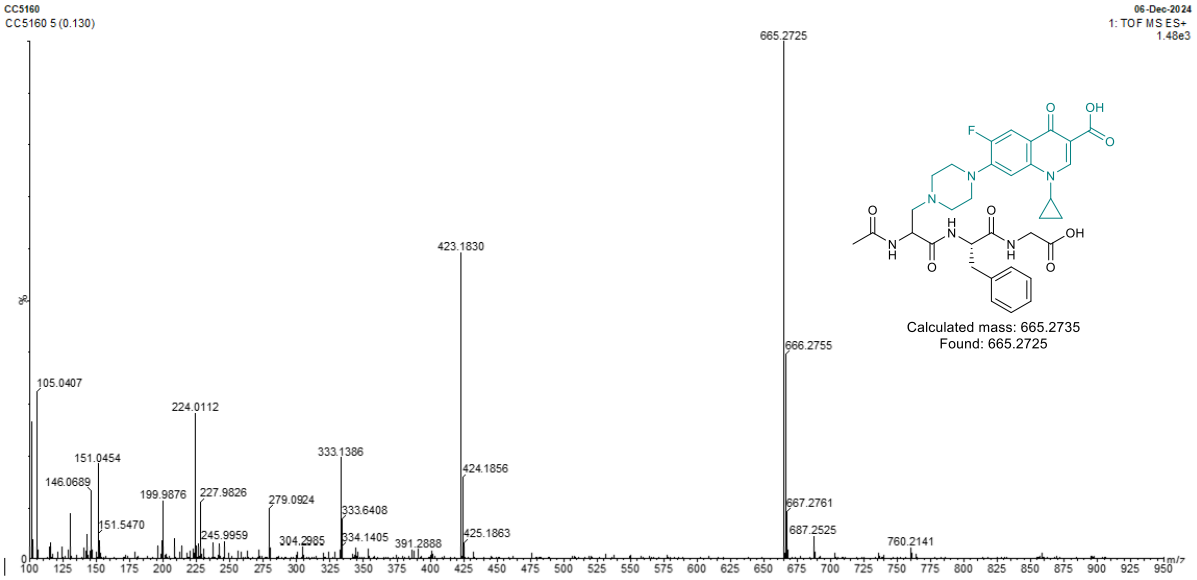


Figure S110 HRMS spectrum of compound **6n**.

Compound 6o

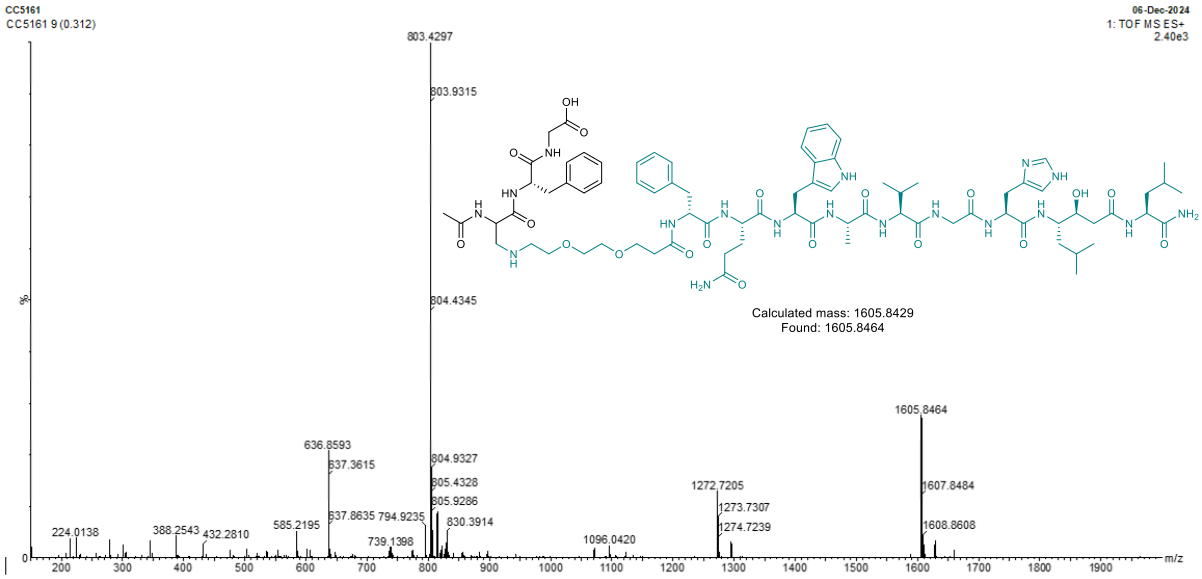


Figure S111 HRMS spectrum of compound **6o**. $[M+2H]^{2+}$ 803 m/z.

Compound 6p

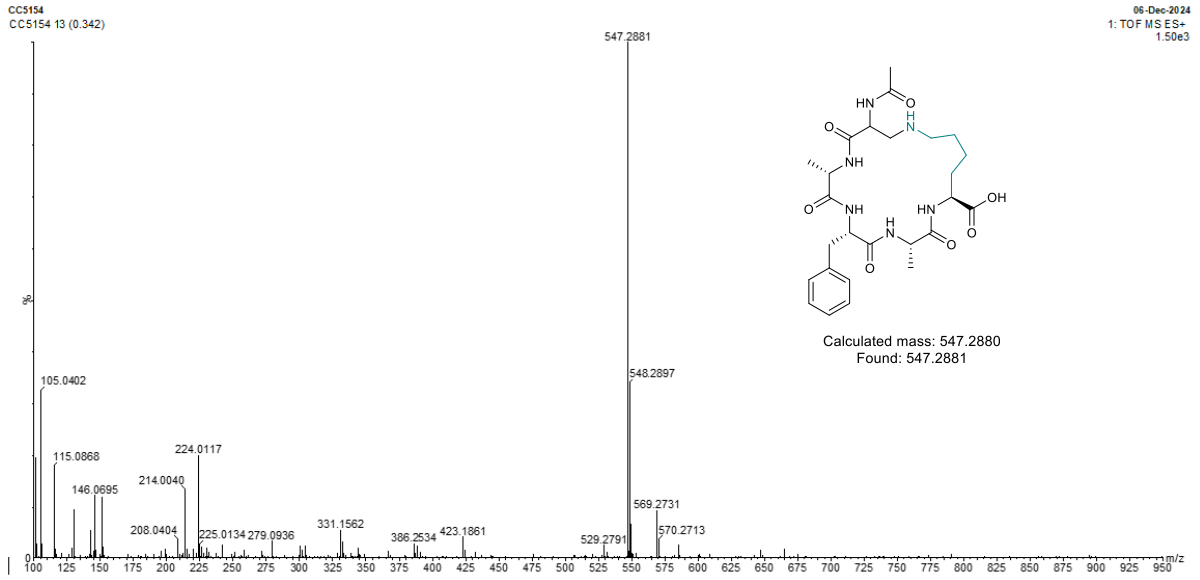


Figure S112 HRMS spectrum of compound **6p**.

Compound 6q

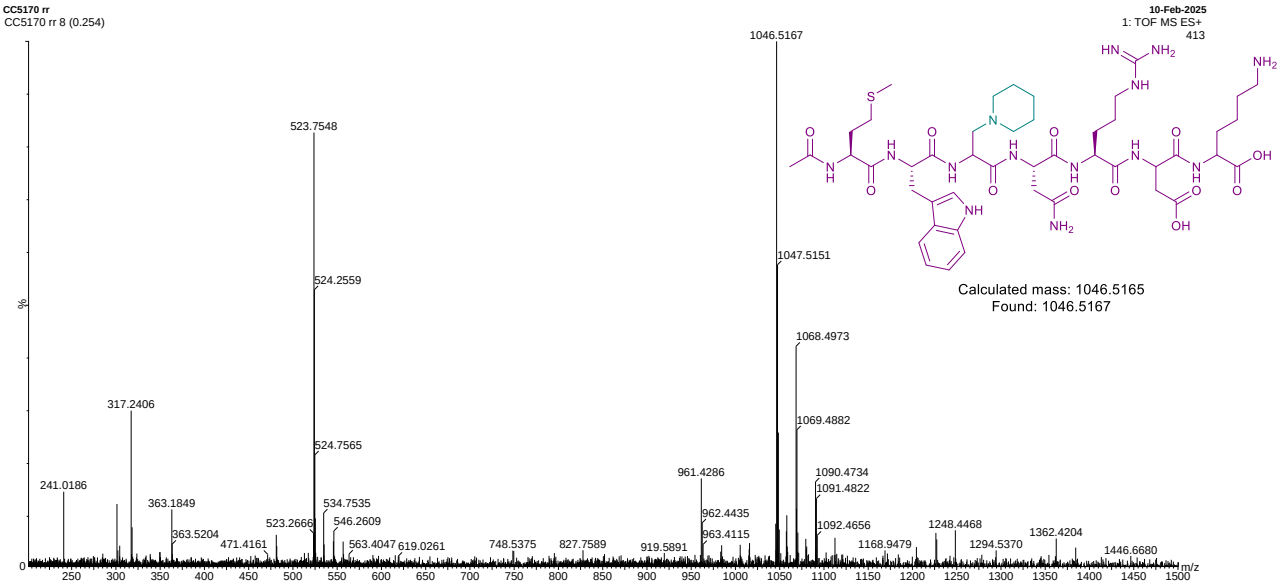


Figure S113 HRMS spectrum of compound **6q**.

Compound 6r

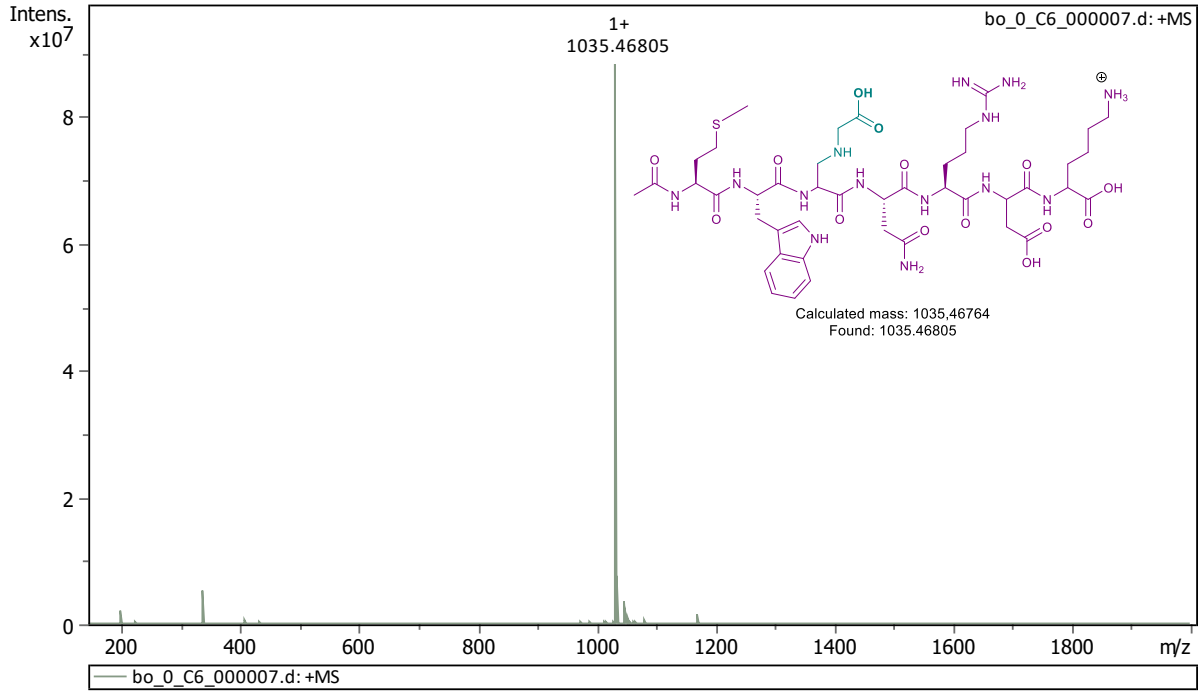


Figure S114 HRMS spectrum of compound **6r**.