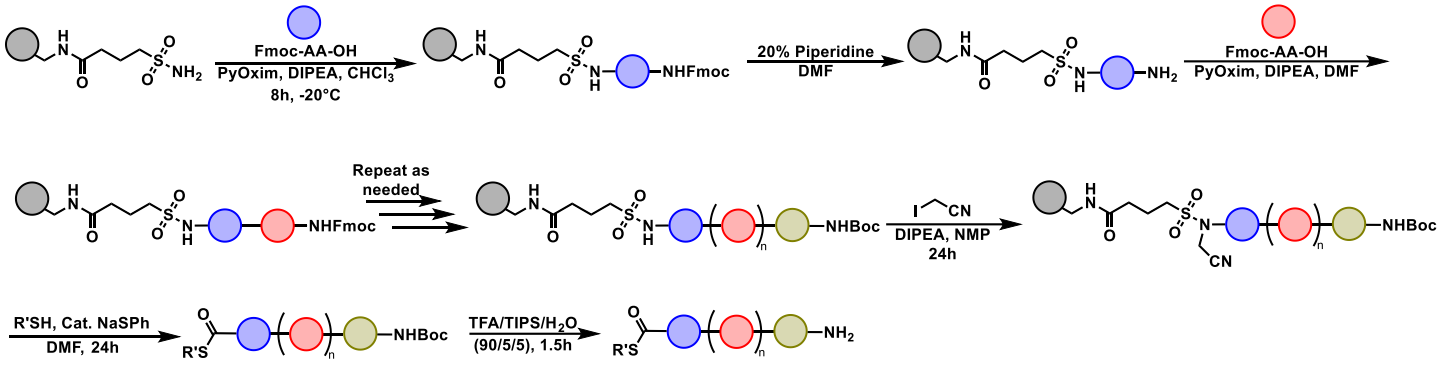
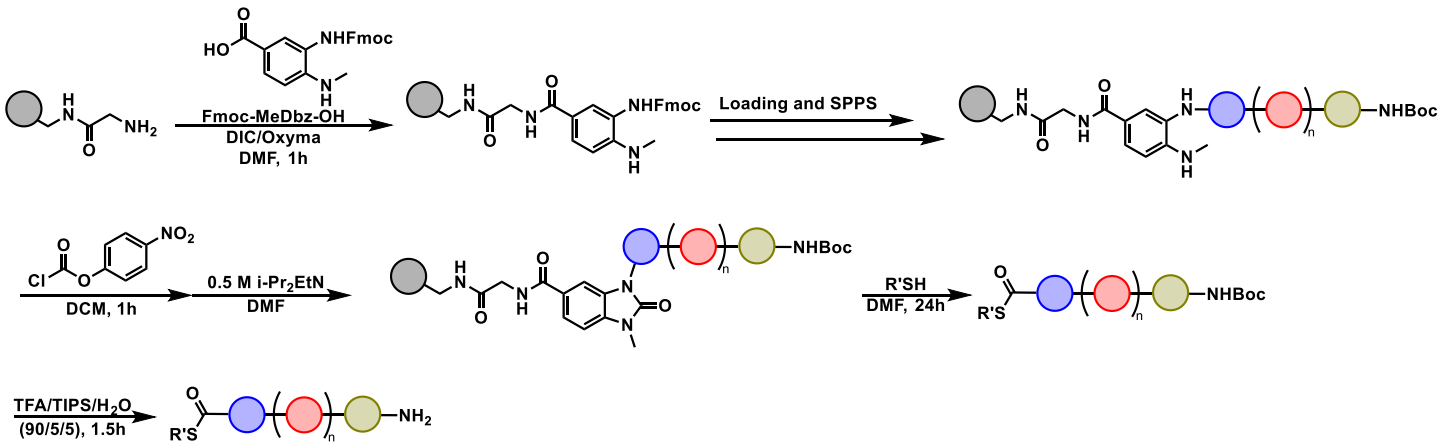
Supplementary Methods



Supplementary Scheme 1: Conventional peptide thioester synthesis at the sulfonamide "safety-catch" resin

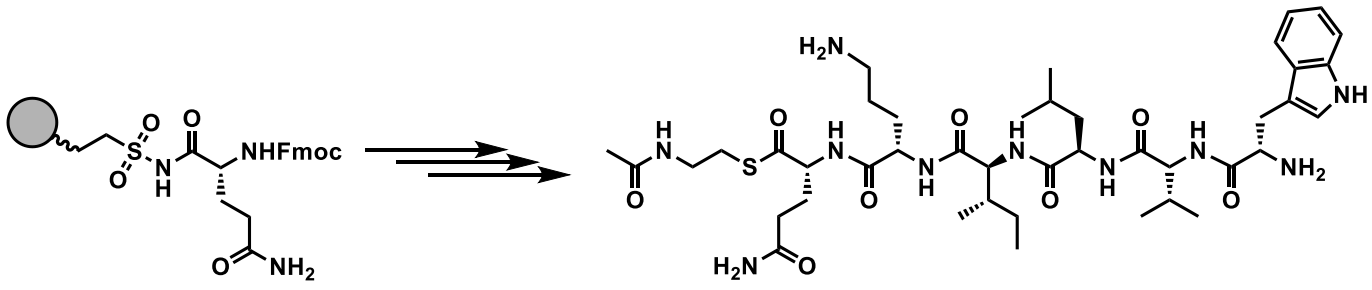


Supplementary Scheme 2: Conventional peptide thioester synthesis at the NDbz "safety-catch" resin

Peptide Thioesters Used in This Study

In this study, UPLC and/or analytical HPLC were utilized to assess the purity of the peptide prior to assay or purification by monitoring absorbance at 214 nm. The analytical HPLC analysis was performed on a Luna Omega 5 µm Polar C18 100 Å 150x4.6 mm (Phenomenex) column, while the UPLC analysis was carried out on a CORTECS T3 Column, 120Å, 1.6 µm, 2.1 mm X 50 mm (Waters) column. For purification, semi-preparative HPLC was employed using a Luna Omega 5 µm Polar C18 100 Å 150x21.2 mm (Phenomenex) column. The specific gradient and flow rate for each peptide can be found in their respective sections.

Synthesis of **1**

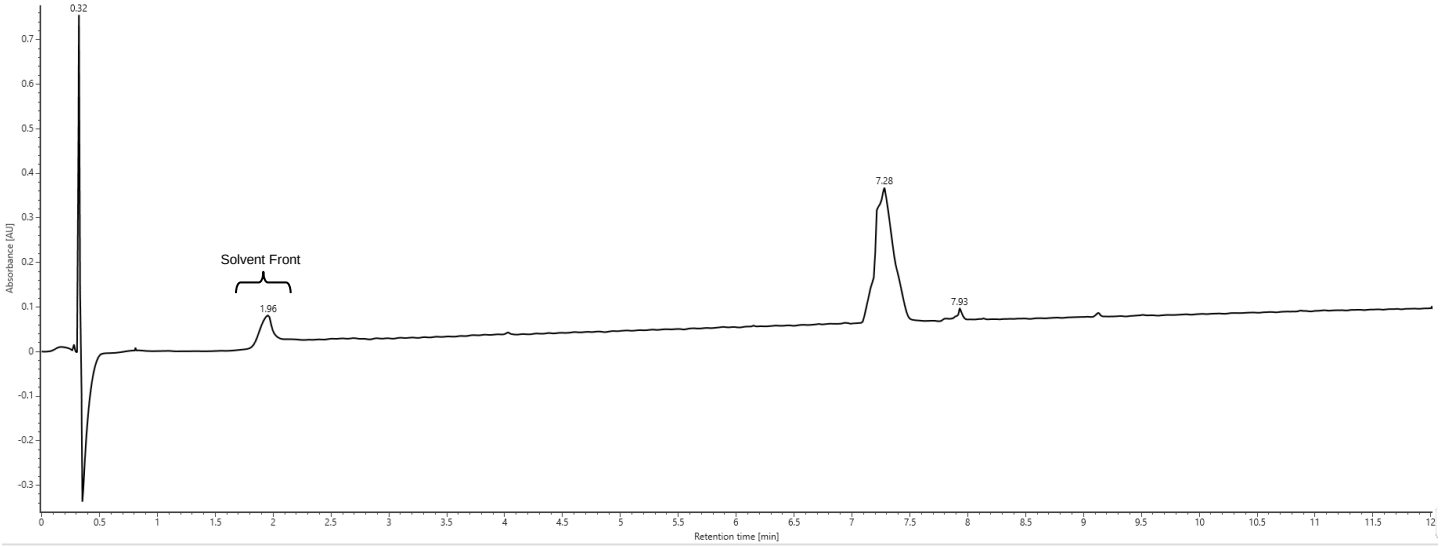


S1 **1**

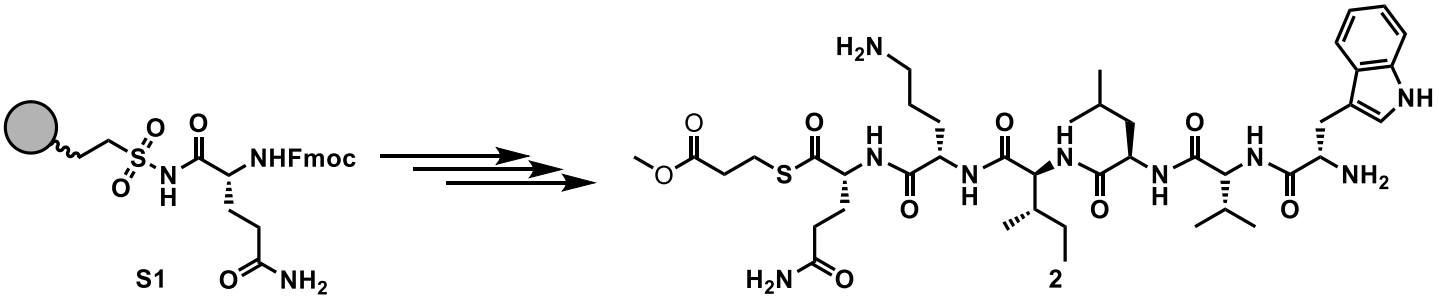
Peptide **1** was synthesized following the general protocol outlined above for the sulfonamide "safety-catch" resin. The synthesis was initiated from 4-sulfamylbuyryl resin preloaded with Fmoc-D-Gln(Trt)-OH **S1** (0.05 mmol)

using the following amino acids (Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, Boc-L-Trp(Boc)-OH) and N-Aceytlcysteamine as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (30.3 mg, 55% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 11.03 (s, 1H), 8.64 (q, *J* = 6.8 Hz, 2H), 8.20 (dd, *J* = 8.2, 8.2 Hz, 2H), 8.07 (t, *J* = 6.1 Hz, 1H), 7.91 – 7.68 (m, 6H), 7.36 (d, *J* = 7.5 Hz, 1H), 7.27 (s, 1H), 7.23 (s, 1H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.01 (t, *J* = 8.2 Hz, 1H), 6.81 (s, 1H), 4.40 (p, *J* = 5.4 Hz, 2H), 4.34 – 4.26 (m, 2H), 4.23 (t, *J* = 7.5 Hz, 1H), 4.15 (t, *J* = 5.6 Hz, 1H), 3.31 (q, *J* = 8.2 Hz, 1H), 3.25 (dd, *J* = 6.8, 5.4 Hz, 1H), 3.11 (q, *J* = 7.0 Hz, 2H), 3.02 (q, *J* = 9.6 Hz, 1H), 2.87 – 2.73 (m, 4H), 2.17 – 2.05 (m, 2H), 1.97 (h, *J* = 6.1 Hz, 1H), 1.92 (h, *J* = 5.4 Hz, 1H), 1.82 – 1.65 (m, 7H), 1.62 – 1.49 (m, 4H), 1.46 (t, *J* = 4.9 Hz, 2H), 1.42 – 1.35 (m, 1H), 1.05 (hept, *J* = 6.1 Hz, 1H), 0.87 (d, *J* = 7.5 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.79 (d, *J* = 5.6 Hz, 3H), 0.78 – 0.71 (m, 9H).
^{13}C NMR (201 MHz, DMSO) δ 200.6, 173.2, 171.7, 171.6, 170.8, 170.4, 169.3, 136.3, 127.1, 124.9, 121.1, 118.7, 118.3, 111.4, 107.2, 58.7, 57.6, 56.5, 52.8, 51.7, 51.2, 42.2, 41.1, 38.5, 38.1, 38.0, 37.3, 36.8, 30.9, 30.9, 29.1, 28.3, 27.6, 27.0, 24.2, 24.1, 23.5, 23.0, 22.6, 22.5, 21.5, 19.1, 17.8, 15.3, 10.9.
Mass spec: expected neutral mass for $C_{42}H_{68}N_{10}O_8S$ (Da): 872.4942, observed neutral mass (Da): 872.4967, mass error (ppm) 2.8.
UPLC Trace: Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and after the gradient. The crude peptide purity was determined to be 96%

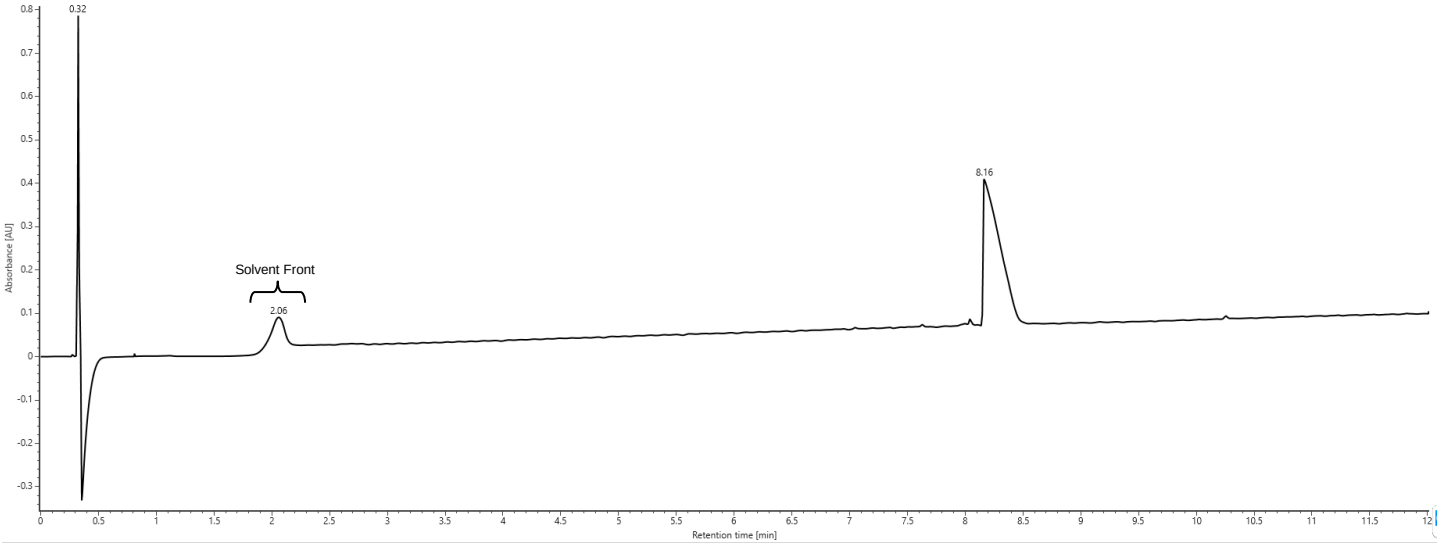


Synthesis of **2**

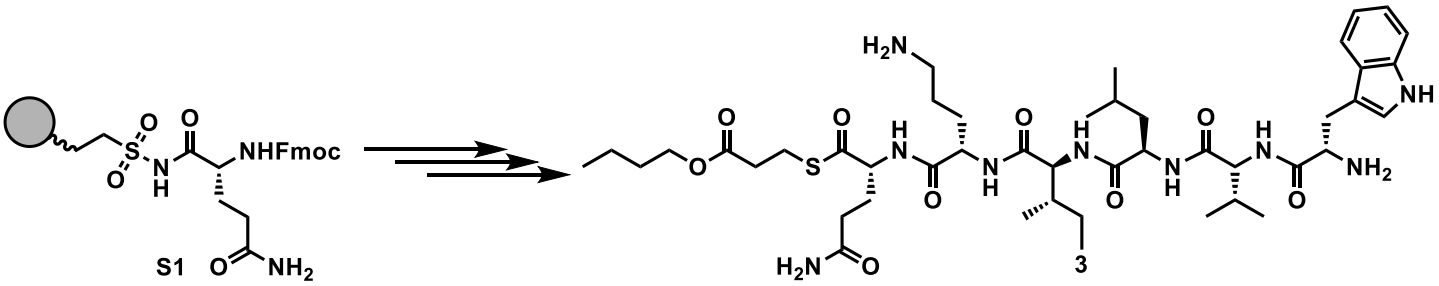


Peptide **2** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from 4-sulfamylbuyryl resin preloaded with Fmoc-D-Gln(Trt)-OH **S1** (0.05 mmol) using the following amino acids (Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, Boc-L-Trp(Boc)-OH) and methyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (27.2 mg, 49% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 11.03 (s, 1H), 8.64 (dd, *J* = 335.2, 4.7 Hz, 2H), 8.20 (dd, *J* = 9.1, 9.1 Hz, 2H), 7.83 (d, *J* = 9.1 Hz, 2H), 7.74 (d, *J* = 9.1 Hz, 6H), 7.34 (d, *J* = 7.5 Hz, 1H), 7.26 (d, *J* = 24.4 Hz, 2H), 7.09 (t, *J* = 7.5 Hz, 1H), 7.00 (t, *J* = 6.3 Hz, 1H), 6.81 (s, 1H), 4.40 (p, *J* = 7.9 Hz, 2H), 4.33 – 4.26 (m, 2H), 4.24 (t, *J* = 7.5 Hz, 1H), 4.14 (t, *J* = 9.1 Hz, 1H), 3.60 (s, 4H), 3.24 (dd, *J* = 6.3, 5.9 Hz, 1H), 3.04 – 2.92 (m, 4H), 2.79 (t, *J* = 9.4 Hz, 2H), 2.56 (q, *J* = 5.9 Hz, 2H), 2.11 (h, *J* = 5.9 Hz, 2H), 1.99 – 1.89 (m, 2H), 1.77 – 1.66 (m, 4H), 1.61 – 1.50 (m, 5H), 1.46 (t, *J* = 7.5 Hz, 3H), 1.42 – 1.35 (m, 1H), 1.05 (h, *J* = 4.7 Hz, 1H), 0.90 – 0.69 (m, 24H).
^{13}C NMR (201 MHz, DMSO) δ 200.6, 173.1, 171.7, 171.6, 171.6, 170.8, 170.4, 136.4, 127.1, 124.9, 121.1, 118.7, 118.3, 111.4, 107.3, 58.6, 57.6, 56.5, 52.8, 51.7, 51.6, 51.2, 41.1, 38.5, 36.8, 33.5, 30.9, 30.8, 29.1, 28.3, 26.9, 24.2, 24.1, 23.5, 23.2, 23.0, 21.5, 19.1, 17.8, 15.3, 10.9.
Mass spec: expected neutral mass for $C_{42}H_{67}N_9O_9S$ (Da): 873.4782, observed neutral mass (Da): 873.4809, mass error (ppm) 3.1.
UPLC Trace: Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and after the gradient. The crude peptide purity was determined to be 97%.



Synthesis of **3**



Peptide **3** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from 4-sulfamylbuyryl resin preloaded with Fmoc-D-Gln(Trt)-OH **S1** (0.05 mmol) using the following amino acids (Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, Boc-L-Trp(Boc)-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (22.3 mg, 39% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

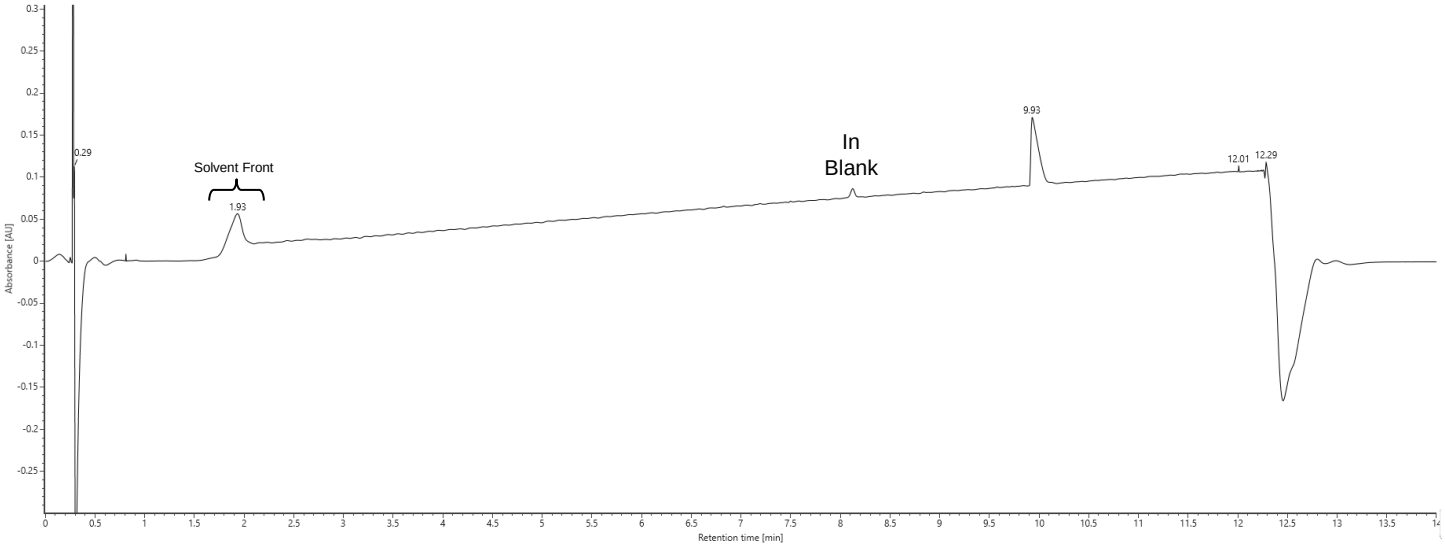
^{1}H NMR (800 MHz, DMSO) δ 10.87 (s, 1H), 8.66 (d, J = 8.4 Hz, 1H), 8.45 (s, 1H), 8.20 (dd, J = 6.2, 4.3 Hz, 3H), 7.98 (d, J = 7.3 Hz, 1H), 7.56 (d, J = 7.3 Hz, 1H), 7.38 (s, 1H), 7.33 (d, J = 7.3 Hz, 1H), 7.17 (s, 1H), 7.05 (t, J = 6.5 Hz, 1H), 6.97 (t, J = 6.2 Hz, 1H), 6.77 (s, 1H), 4.41 – 4.33 (m, 2H), 4.31 – 4.25 (m, 1H), 4.20 (t, J = 8.6 Hz, 1H), 4.14 (t, J = 6.5 Hz, 1H), 4.02 (t, J = 6.5 Hz, 2H), 3.60 (t, J = 5.4 Hz, 1H), 3.10 (dd, J = 6.5, 5.4 Hz, 1H), 3.00 – 2.90 (m, 2H), 2.74 – 2.65 (m, 3H), 2.54 (dd, J = 962.0, 6.2 Hz, 2H), 2.16 – 2.06 (m, 2H), 2.00 – 1.92 (m, 2H),

1.79 – 1.67 (m, 3H), 1.61 – 1.43 (m, 9H), 1.42 – 1.35 (m, 1H), 1.31 (dd, J = 181.8, 7.5 Hz, 2H), 1.07 (p, J = 8.4 Hz, 1H), 0.88 (t, J = 7.3 Hz, 7H), 0.83 (d, J = 7.3 Hz, 4H), 0.79 (q, J = 6.5 Hz, 8H), 0.77 (t, J = 2.2 Hz, 6H).

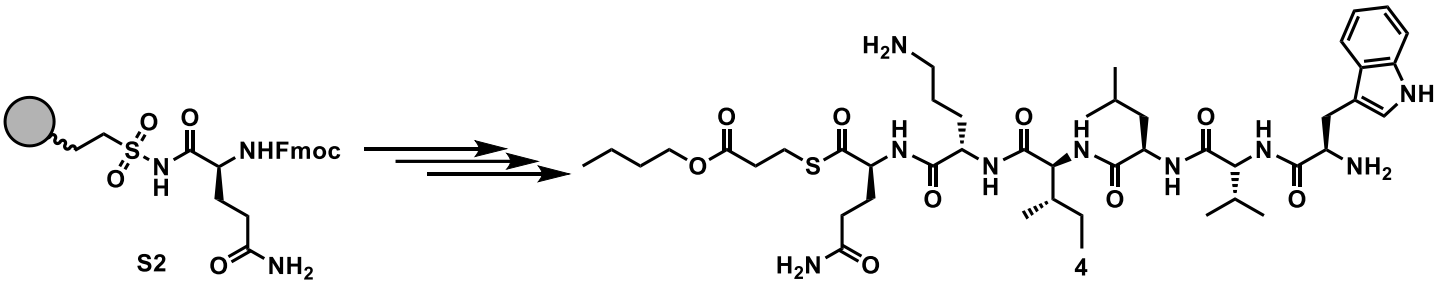
^{13}C NMR (201 MHz, DMSO) δ 201.15, 174.93, 173.48, 172.19, 171.49, 171.38, 171.21, 166.30, 136.66, 127.70, 124.16, 121.25, 118.82, 118.57, 111.70, 111.01, 70.17, 64.29, 59.08, 58.02, 57.04, 55.46, 52.10, 51.66, 41.26, 39.00, 36.73, 34.02, 31.23, 30.91, 30.51, 29.59, 27.27, 24.62, 23.62, 23.37, 21.89, 19.61, 18.98, 18.41, 15.74, 13.94, 11.24.

Mass spec: expected neutral mass for $C_{45}H_{73}N_9O_9S$ (Da): 915.5250, observed neutral mass (Da): 915.5249, mass error (ppm) 0.3.

UPLC Trace: Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and after the gradient. The crude peptide purity was determined to be 99%.



Synthesis of **4**



Peptide **4** was synthesized using the sulfonamide "safety-catch" resin following the general protocol described earlier. The synthesis was started from 4-sulfamylbuyryl resin preloaded with Fmoc-L-Gln(Trt)-OH **S2** (0.05 mmol) and utilized the following amino acids: Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, and Boc-D-Trp-OH, with Butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 55% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (8.1 mg, 16% yield).

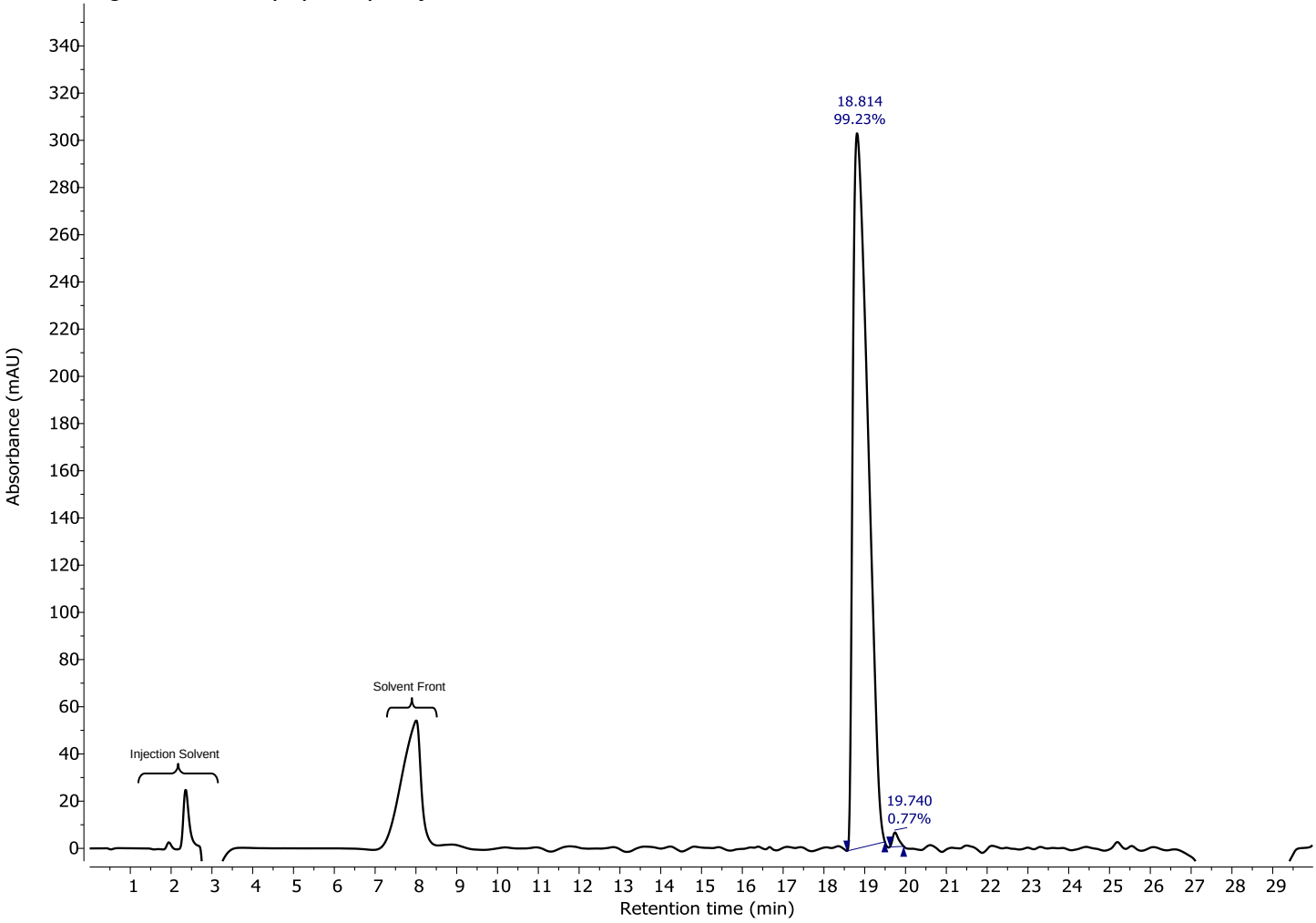
^{1}H NMR (800 MHz, DMSO) δ 10.91 (s, 1H), 8.72 (s, 1H), 8.45 (s, 2H), 8.34 – 7.97 (m, 6H), 7.56 (s, 1H), 7.33 (s, 3H), 7.19 (s, 2H), 7.06 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 6.9 Hz, 2H), 6.78 (s, 1H), 6.16 (t, *J* = 56.3 Hz, 2H), 4.33

(d, *J* = 42.5 Hz, 5H), 4.20 (dd, *J* = 136.6, 17.8 Hz, 4H), 4.02 (d, *J* = 5.7 Hz, 3H), 3.60 – 3.43 (m, 2H), 3.17 – 3.02 (m, 1H), 2.97 (d, *J* = 6.3 Hz, 3H), 2.74 (d, *J* = 6.9 Hz, 5H), 2.55 (d, *J* = 7.5 Hz, 3H), 2.13 (d, *J* = 7.7 Hz, 3H), 1.95 (s, 3H), 1.87 – 1.67 (m, 5H), 1.66 – 1.17 (m, 29H), 1.06 (s, 1H), 0.94 – 0.58 (m, 42H).

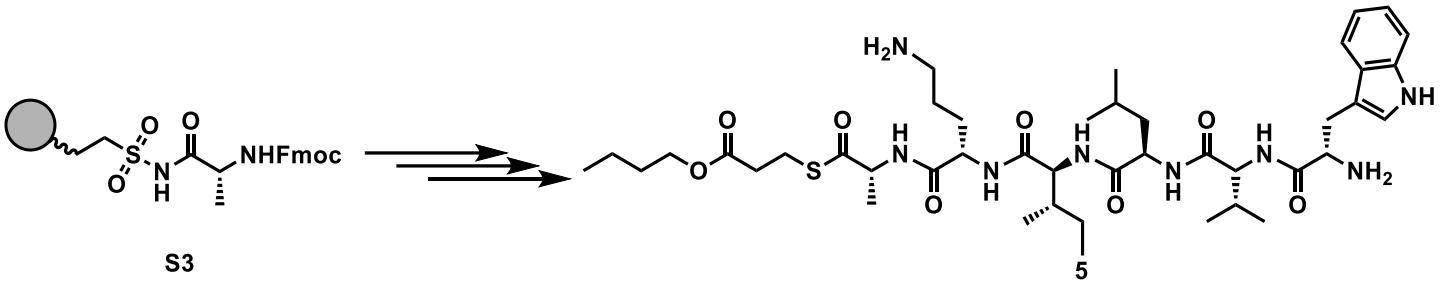
^{13}C NMR (201 MHz, DMSO) δ 200.8, 174.1, 173.2, 171.8, 171.7, 171.2, 171.0, 171.0, 166.0, 136.3, 118.2, 117.4, 116.2, 115.0, 111.3, 110.4, 63.9, 58.8, 57.2, 56.6, 55.1, 51.6, 51.4, 40.8, 38.2, 36.3, 33.6, 31.0, 30.8, 30.6, 30.1, 28.4, 27.1, 24.3, 24.2, 23.9, 23.2, 22.9, 21.5, 20.5, 20.4, 20.2, 19.2, 18.6, 17.9, 15.4, 13.5, 10.8.

Mass spec: expected neutral mass for $C_{45}H_{73}N_9O_9S$ (Da): 915.5251, observed neutral mass (Da): 915.5215, mass error (ppm) 6.0.

HPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The peptide purity was determined to be 99%.



Synthesis of **5**



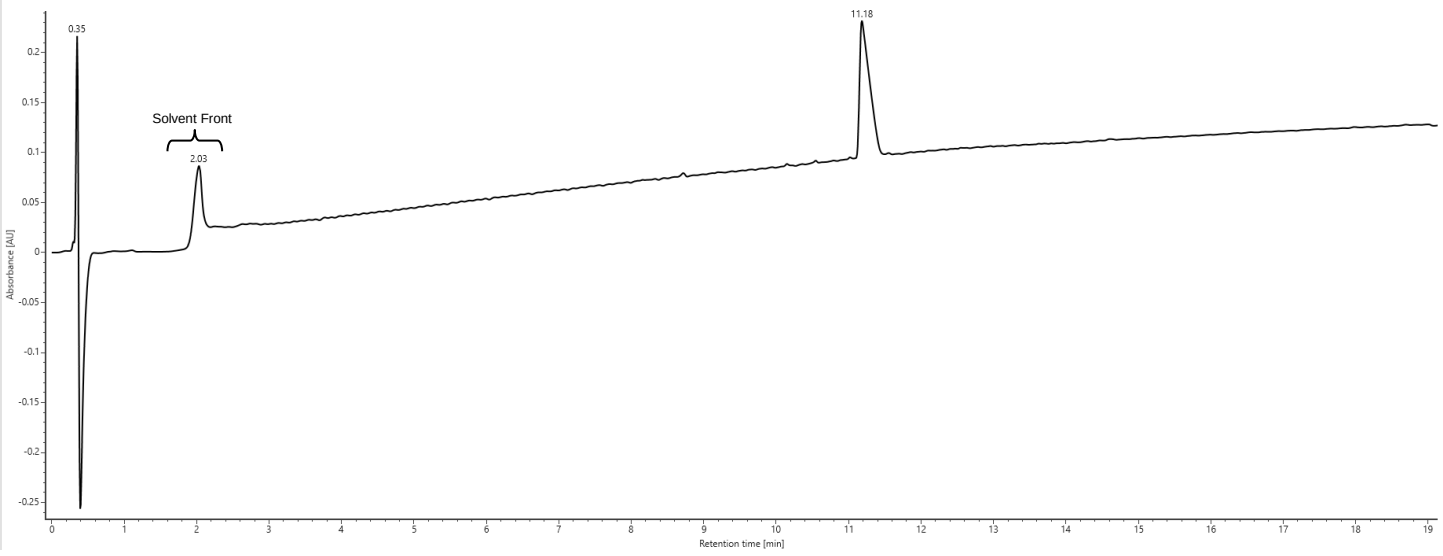
Peptide **5** was synthesized using the sulfonamide "safety-catch" resin according to the general protocol described earlier. The synthesis was initiated from 4-sulfamylbutryl resin preloaded with Fmoc-D-Ala-OH **S3** (0.05 mmol) and utilized the following amino acids: Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, and Boc-L-Trp(Boc)-OH, with butyl 3-mercaptopropionate used as the cleaving thiol. The crude peptide was obtained as an off-white solid (15.5 mg, 29% yield) and was analyzed via RP-UPLC. It was used in further enzyme assays without undergoing further purification.

^{1}H NMR (800 MHz, DMSO) δ 11.04 (s, 1H), 8.68 (dd, *J* = 36.9, 8.6 Hz, 2H), 8.21 (dd, *J* = 24.9, 7.9 Hz, 2H), 8.06 (bs, 3H), 7.85 (bs, 3H), 7.82 (d, *J* = 8.5 Hz, 2H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.37 (d, *J* = 7.9 Hz, 1H), 7.24 (s, 1H), 7.09 (t, *J* = 7.3 Hz, 1H), 7.01 (t, *J* = 7.2 Hz, 1H), 4.42 – 4.31 (m, 4H), 4.29 (d, *J* = 7.2 Hz, 1H), 4.25 (t, *J* = 7.6 Hz, 1H), 4.19 (s, 1H), 4.02 (t, *J* = 6.3 Hz, 2H), 3.35 (bs, 3H), 3.26 (dd, *J* = 14.3, 5.6 Hz, 1H), 3.05 (dd, *J* = 14.6, 8.9 Hz, 1H), 3.00 – 2.92 (m, 2H), 2.78 (s, 2H), 2.54 (t, *J* = 6.6 Hz, 2H), 2.50 (s, 2H), 1.93 (h, *J* = 6.9 Hz, 1H), 1.74 (s, 1H), 1.69 (s, 1H), 1.61 – 1.49 (m, 8H), 1.46 (t, *J* = 6.9 Hz, 2H), 1.38 (s, 1H), 1.32 (h, *J* = 7.3 Hz, 3H), 1.26 (d, *J* = 7.3 Hz, 4H), 1.05 (hept, *J* = 7.3 Hz, 1H), 0.88 (quint, *J* = 7.2, 4.3 Hz, 8H), 0.84 (d, *J* = 6.3 Hz, 4H), 0.81 – 0.71 (m, 16H).

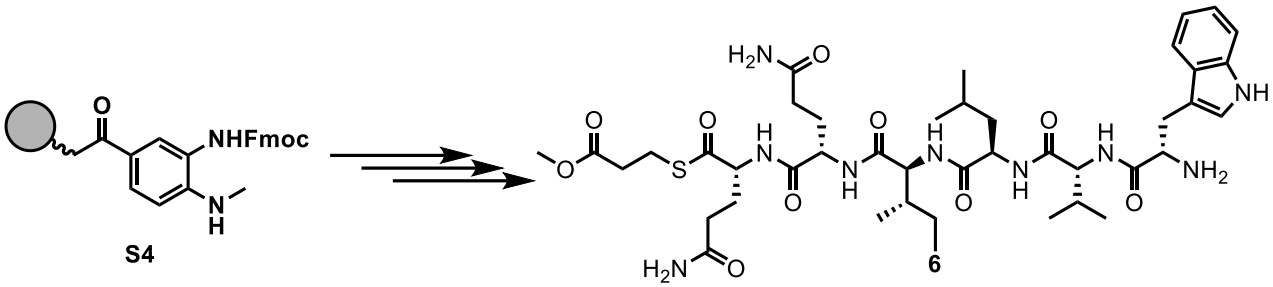
^{13}C NMR (201 MHz, DMSO) δ 201.2, 171.6, 171.3, 171.1, 170.8, 170.3, 168.7, 136.4, 127.0, 125.0, 121.1, 118.7, 118.3, 111.4, 106.9, 63.9, 57.6, 56.4, 54.7, 52.6, 51.7, 51.2, 41.0, 38.5, 36.9, 33.6, 31.0, 30.1, 29.0, 28.0, 24.2, 24.1, 23.5, 23.2, 23.0, 21.6, 19.1, 18.6, 17.8, 17.3, 15.3, 13.5, 10.9.

Mass spec: expected neutral mass for $C_{43}H_{70}N_8O_8S$ (Da): 858.5037, observed neutral mass (Da): 858.5063, mass error (ppm) 3.0.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 18 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and after the gradient. The crude peptide purity was determined to be 96%



Synthesis of **6**



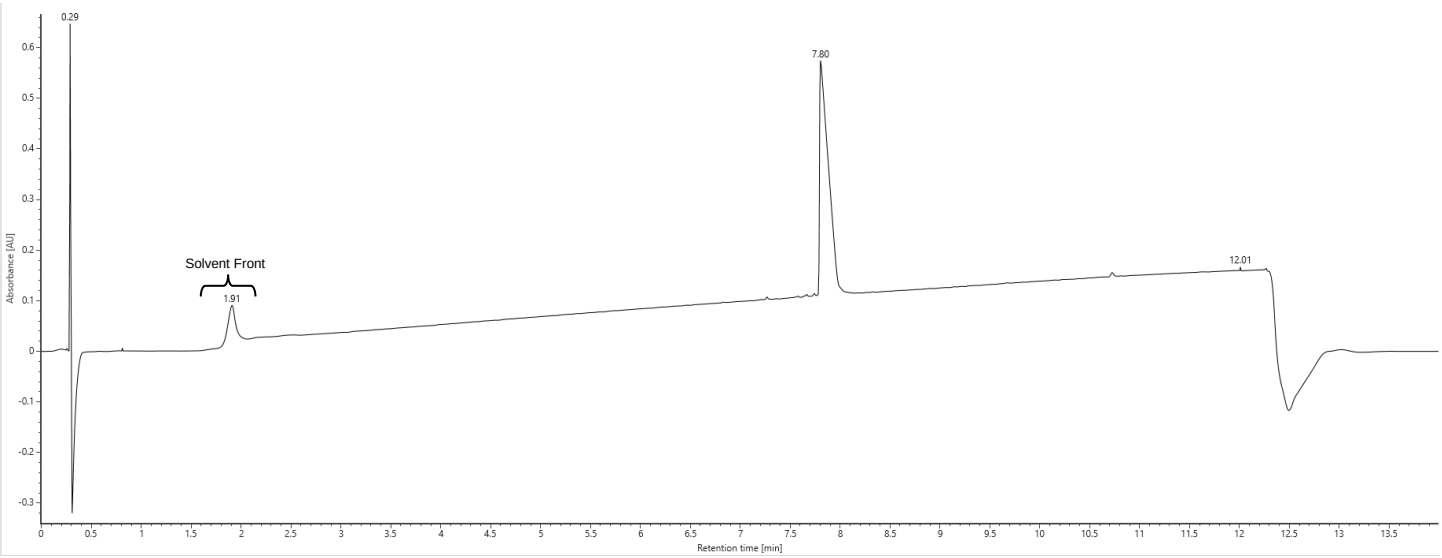
Peptide **6** was synthesized following the general protocol outlined above for the NDbz “safety-catch” resin. The synthesis was initiated from **S4** (0.15 mmol) using the following amino acids (Fmoc-L-Gln(Trt)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, Boc-L-Trp(Boc)-OH) and methyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (82.5 mg, 53% yield).

^{1}H NMR (800 MHz, DMSO) δ 11.04 (s, 1H), 8.68 (d, *J* = 8.4 Hz, 1H), 8.52 (d, *J* = 9.5 Hz, 1H), 8.20 (d, *J* = 8.4 Hz, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.07 (s, 3H), 7.81 (d, *J* = 8.4 Hz, 1H), 7.76 (d, *J* = 8.1 Hz, 1H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.26 (t, *J* = 25.5 Hz, 3H), 7.09 (dd, *J* = 50.4, 7.0 Hz, 1H), 7.01 (t, *J* = 8.1 Hz, 1H), 6.79 (d, *J* = 6.7 Hz, 2H), 4.40 (q, *J* = 7.0 Hz, 1H), 4.35 – 4.27 (m, 3H), 4.26 – 4.18 (m, 2H), 3.59 (s, 3H), 3.26 (dd, *J* = 5.3, 5.3 Hz, 1H), 3.06 (q, *J* = 6.7 Hz, 1H), 3.01 – 2.92 (m, 2H), 2.55 (t, *J* = 6.7 Hz, 2H), 2.17 – 2.03 (m, 4H), 2.01 – 1.91 (m, 2H), 1.89 – 1.83 (m, 1H), 1.82 – 1.70 (m, 3H), 1.58 (h, *J* = 8.1 Hz, 1H), 1.51 – 1.42 (m, 2H), 1.42 – 1.36 (m, 1H), 1.06 (h, *J* = 7.0 Hz, 1H), 0.87 (d, *J* = 8.1 Hz, 3H), 0.82 (d, *J* = 7.0 Hz, 3H), 0.82 – 0.70 (m, 13H).

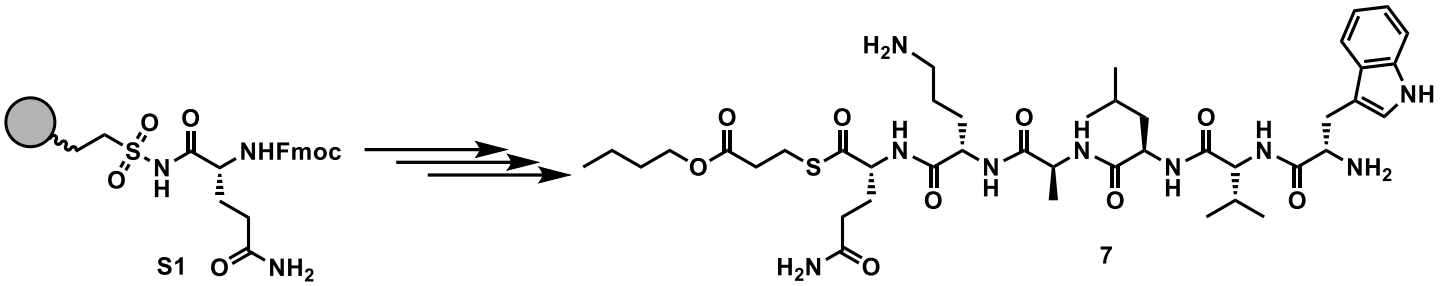
^{13}C NMR (201 MHz, DMSO) δ 201.07, 174.08, 173.57, 172.03, 171.94, 171.15, 170.66, 169.10, 136.74, 127.43, 125.41, 121.53, 119.09, 118.71, 111.81, 107.29, 59.07, 58.04, 56.90, 52.96, 52.68, 51.94, 51.65, 41.36, 36.99, 33.85, 31.83, 31.35, 31.20, 28.53, 28.36, 27.28, 24.58 (d, *J* = 14.8 Hz), 23.60, 23.29, 22.03, 19.44, 18.11, 15.69, 11.23.

Mass spec: expected neutral mass for $C_{45}H_{71}N_9O_{10}S$ (Da): 887.4575, observed neutral mass (Da): 887.4599, mass error (ppm) 2.7.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and after the gradient. The crude peptide purity was determined to be 99%



Synthesis of **7**



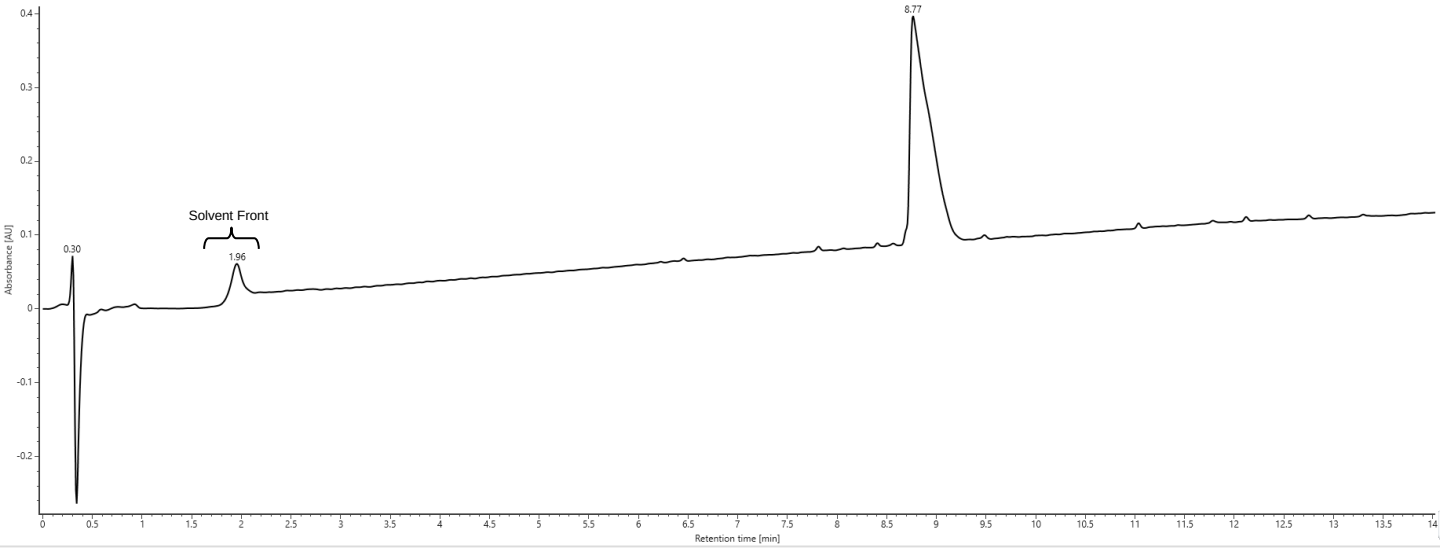
Peptide **7** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids (Boc-L-Trp(Boc)-OH, Fmoc-D-Val-OH, Fmoc-D-Leu-OH, Fmoc-L-Ala-OH, Fmoc-L-Orn(Boc)-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white sold (26.9 mg, 49% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 10.99 (s, 1H), 8.59 (d, *J* = 7.2 Hz, 1H), 8.49 (s, 1H), 8.16 (d, *J* = 8.6 Hz, 1H), 8.09 (d, *J* = 7.8 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.71 (d, *J* = 5.6 Hz, 1H), 7.35 (d, *J* = 8.8 Hz, 1H), 7.32 (d, *J* = 7.2 Hz, 1H), 7.25 (s, 1H), 7.21 (s, 1H), 7.08 (t, *J* = 5.6 Hz, 1H), 7.00 (t, *J* = 7.8 Hz, 1H), 6.80 (s, 1H), 4.38 – 4.27 (m, 4H), 4.23 (t, *J* = 5.4 Hz, 1H), 4.01 (t, *J* = 4.8 Hz, 3H), 3.20 (d, *J* = 10.2 Hz, 1H), 3.07 (s, 1H), 3.00 – 2.91 (m, 3H), 2.79 (t, *J* = 6.4 Hz, 2H), 2.55 (t, *J* = 6.2 Hz, 2H), 2.10 (hept, *J* = 7.2 Hz, 2H), 2.01 – 1.91 (m, 2H), 1.78 – 1.69 (m, 2H), 1.62 – 1.50 (m, 6H), 1.50 – 1.40 (m, 2H), 1.31 (h, *J* = 4.8 Hz, 2H), 1.18 (d, *J* = 6.2 Hz, 3H), 1.10 (s, 2H), 0.87 (t, *J* = 6.2 Hz, 6H), 0.84 (d, *J* = 7.0 Hz, 3H), 0.76 (dd, *J* = 8.6, 6.2 Hz, 6H).

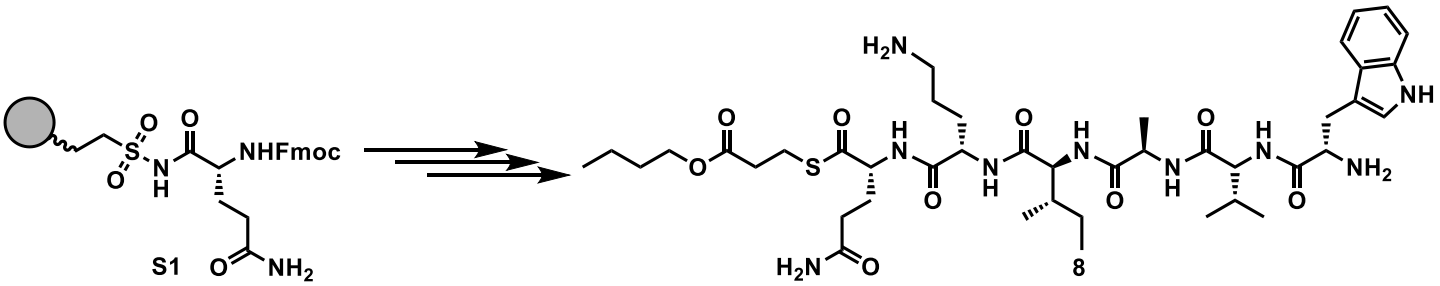
^{13}C NMR (201 MHz, DMSO) δ 201.0, 173.6, 172.4, 172.0, 171.9, 171.5, 171.0, 136.7, 127.5, 125.0, 121.5, 119.0, 118.7, 111.8, 64.3, 59.1, 58.1, 53.7, 52.2, 51.6, 49.1, 48.4, 41.1, 38.9, 34.0, 31.2, 31.2, 30.5, 29.3, 29.3, 27.3, 27.2, 24.6, 23.9, 23.6, 23.4, 21.9, 19.5, 19.0, 18.8, 18.2, 13.9.

Mass spec: expected neutral mass for $C_{42}H_{67}N_9O_9S$ (Da): 873.4782, observed neutral mass (Da): 873.4791, mass error (ppm) 1.0.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 97%.



Synthesis of **8**



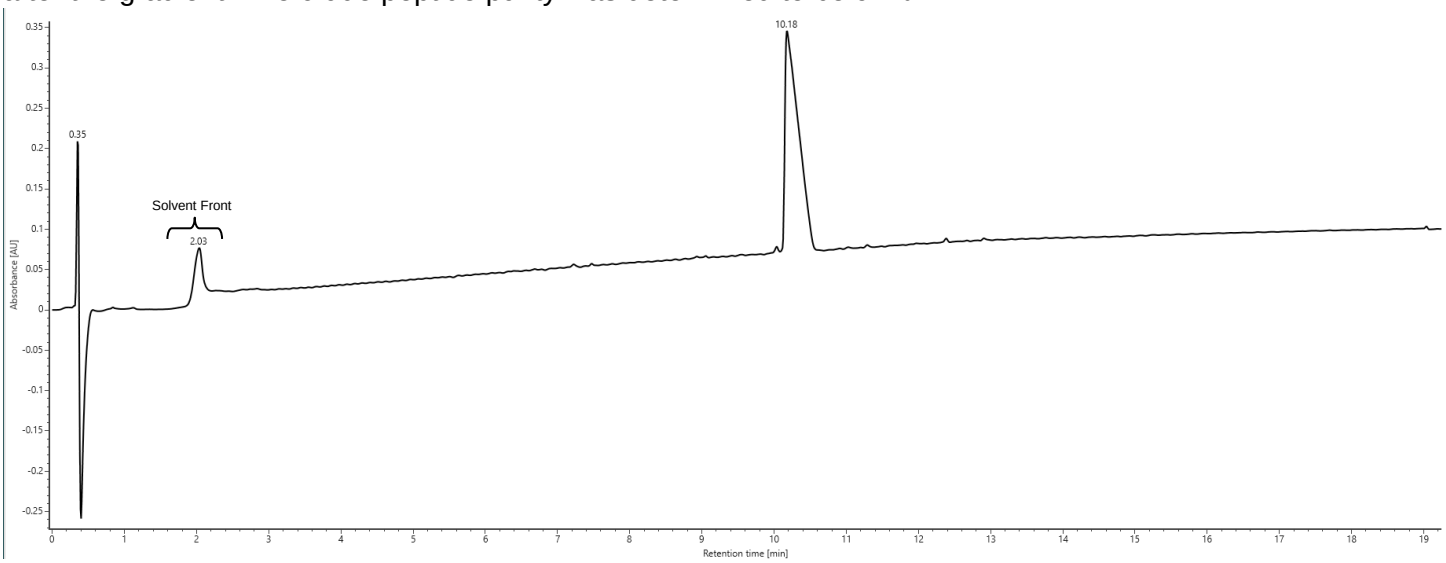
Peptide **8** was synthesized using the sulfonamide "safety-catch" resin according to the general protocol described earlier. The synthesis was initiated from **S1** (0.05 mmol) and utilized the following amino acids: Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Ala-OH, Fmoc-D-Val-OH, and Boc-L-Trp(Boc)-OH, with butyl 3-mercaptopropionate used as the cleaving thiol. The obtained crude peptide was obtained as an off-white solid (22.48 mg, 41% yield) and was analyzed via RP-UPLC. It was used in further enzyme assays without undergoing further purification.

^{1}H NMR (800 MHz, DMSO) δ 11.00 (s, 1H), 8.64 (d, *J* = 5.1 Hz, 1H), 8.54 (d, *J* = 5.9 Hz, 1H), 8.29 (d, *J* = 5.1 Hz, 1H), 8.18 (d, *J* = 7.5 Hz, 1H), 7.80 (d, *J* = 8.6 Hz, 1H), 7.71 (d, *J* = 6.9 Hz, 1H), 7.48 (s, 3H), 7.35 (d, *J* = 6.9 Hz, 1H), 7.22 (d, *J* = 30.9 Hz, 2H), 7.08 (t, *J* = 6.4 Hz, 1H), 7.00 (t, *J* = 8.1 Hz, 1H), 6.80 (s, 1H), 4.41 (p, *J* = 4.5 Hz, 1H), 4.35 (p, *J* = 5.1 Hz, 1H), 4.33 – 4.21 (m, 3H), 4.02 (t, *J* = 6.4 Hz, 3H), 3.22 (dd, *J* = 5.3, 3.5 Hz, 1H), 3.07 – 2.92 (m, 3H), 2.79 (t, *J* = 6.3 Hz, 2H), 2.57 – 2.52 (m, 2H), 2.23 – 2.07 (m, 2H), 1.97 (h, *J* = 5.1 Hz, 1H), 1.90 (h, *J* = 5.7 Hz, 1H), 1.78 – 1.64 (m, 3H), 1.60 – 1.46 (m, 5H), 1.41 – 1.35 (m, 1H), 1.32 (h, *J* = 7.5 Hz, 2H), 1.22 (d, *J* = 6.9 Hz, 3H), 1.05 (h, *J* = 7.5 Hz, 1H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.82 – 0.72 (m, 13H).

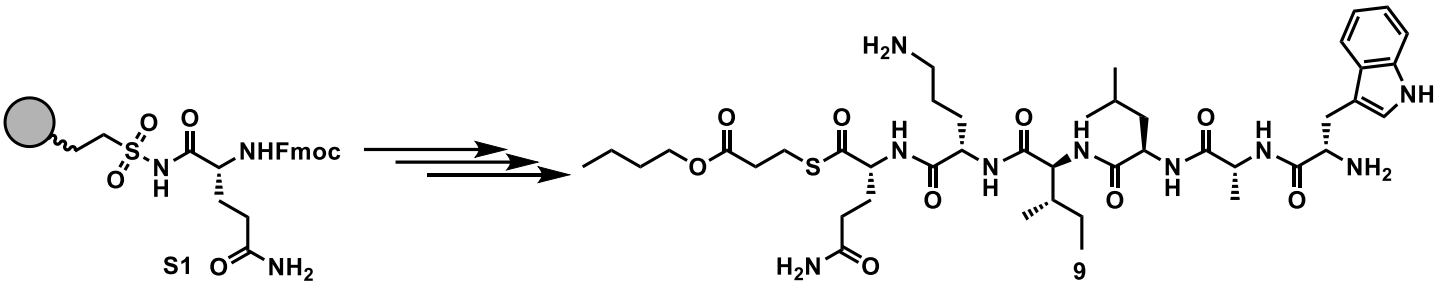
^{13}C NMR (201 MHz, DMSO) δ 200.6, 173.1, 171.8, 171.6, 171.1, 170.8, 170.4, 136.3, 127.1, 124.7, 121.1, 118.6, 118.3, 111.4, 107.8, 63.9, 58.7, 57.5, 56.4, 53.2, 51.6, 48.3, 38.5, 37.1, 33.7, 30.8, 30.8, 30.1, 29.2, 28.7, 26.9, 24.1, 23.6, 23.2, 19.1, 18.6, 17.9, 15.3, 13.5, 11.0.

Mass spec: expected neutral mass for $C_{42}H_{67}N_9O_9S$ (Da): 873.4782, observed neutral mass (Da): 873.4759, mass error (ppm) 2.7.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 18 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 97%.



Synthesis of **9**



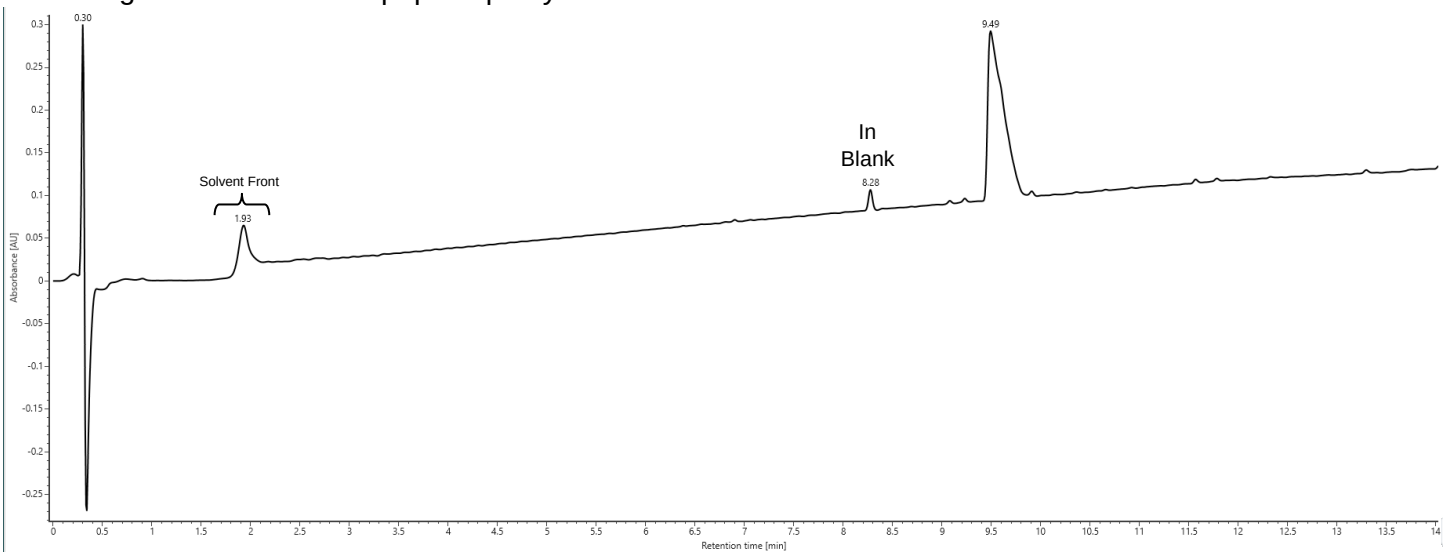
Peptide **9** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids (Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Ala-OH, Boc-L-Trp(Boc)-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off-white solid (21.4 mg, 41% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 10.98 (s, 1H), 8.61 (d, *J* = 6.8 Hz, 1H), 8.49 (d, *J* = 6.8 Hz, 1H), 8.15 (dd, *J* = 9.1, 7.9 Hz, 2H), 7.84 (d, *J* = 6.8 Hz, 1H), 7.61 (d, *J* = 8.4 Hz, 1H), 7.34 (d, *J* = 7.9 Hz, 1H), 7.25 (s, 2H), 7.19 (d, *J* = 1.8 Hz, 2H), 7.08 (t, *J* = 7.9 Hz, 2H), 6.99 (t, *J* = 6.2 Hz, 1H), 6.80 (s, 1H), 4.41 (q, *J* = 6.1 Hz, 1H), 4.36 – 4.26 (m, 3H), 4.23 (t, *J* = 6.7 Hz, 1H), 4.02 (t, *J* = 6.2 Hz, 2H), 3.87 (t, *J* = 6.2 Hz, 1H), 3.16 (dd, *J* = 6.2, 6.2 Hz, 1H), 3.01 – 2.90 (m, 3H), 2.79 (t, *J* = 7.3 Hz, 2H), 2.58 – 2.52 (m, 2H), 2.11 (hept, *J* = 8.5 Hz, 2H), 1.96 (h, *J* = 4.6 Hz, 1H), 1.79 – 1.64 (m, 3H), 1.61 – 1.50 (m, 7H), 1.49 – 1.42 (m, 3H), 1.42 – 1.35 (m, 1H), 1.32 (h, *J* = 6.8 Hz, 3H), 1.11 (d, *J* = 4.6 Hz, 5H), 1.05 (p, *J* = 6.1 Hz, 1H), 0.88 (t, *J* = 7.3 Hz, 7H), 0.83 (d, *J* = 5.6 Hz, 4H), 0.81 – 0.73 (m, 8H).

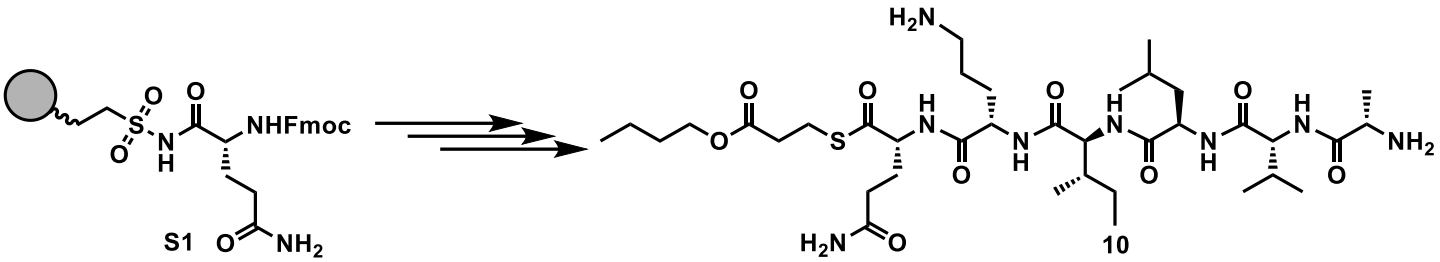
^{13}C NMR (201 MHz, DMSO) δ 200.6, 173.1, 172.0, 171.8, 171.6, 171.1, 170.8, 136.2, 127.2, 124.5, 121.0, 118.5, 118.3, 111.4, 64.7, 63.9, 58.7, 56.5, 53.5, 51.7, 51.3, 48.7, 48.2, 40.8, 38.5, 36.8, 33.6, 30.8, 30.1, 29.1, 26.9, 26.8, 24.3, 24.1, 23.6, 23.2, 23.0, 21.5, 18.8, 18.3, 17.6, 17.5, 15.3, 13.5, 10.9.

Mass spec: expected neutral mass for $C_{43}H_{69}N_9O_9S$ (Da): 887.4939, observed neutral mass (Da): 887.4965, mass error (ppm) 2.9.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 45% over 13 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 96%.



Synthesis of **10**



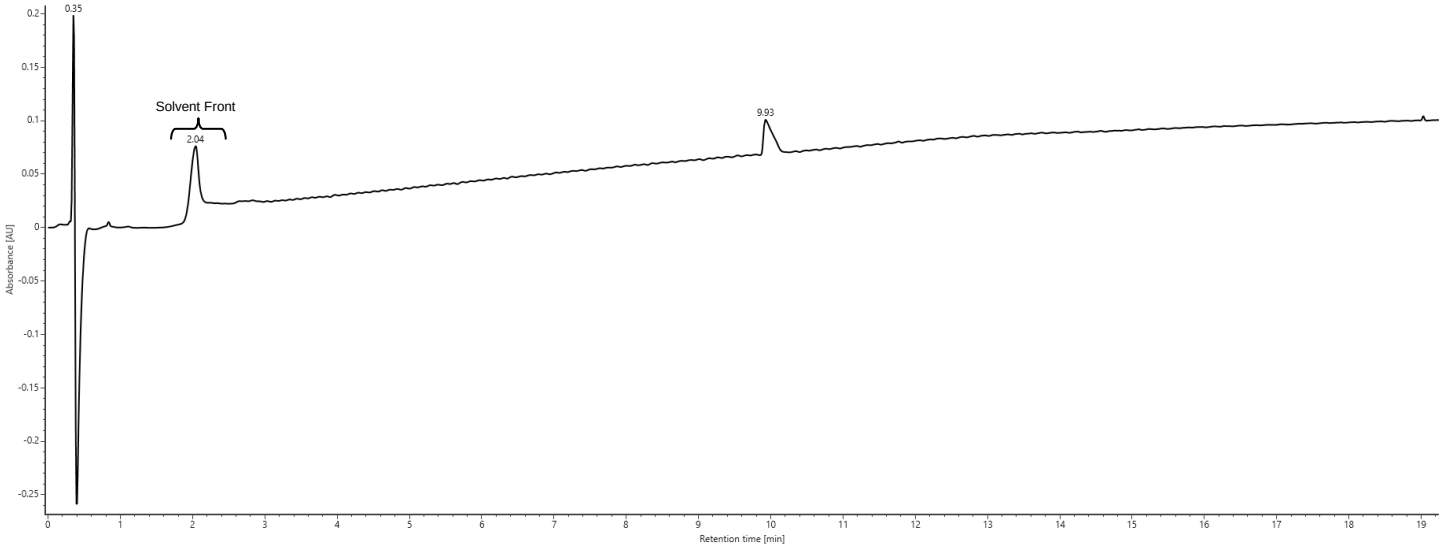
Peptide **10** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids (Fmoc-L-Orn(Boc)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-D-Val-OH, Boc-L-Ala-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (10.6 mg, 21% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 8.64 (d, *J* = 7.3 Hz, 1H), 8.47 (d, *J* = 8.5 Hz, 1H), 8.21 (d, *J* = 6.7 Hz, 1H), 8.15 (d, *J* = 7.3 Hz, 1H), 7.96 (s, 5H), 7.84 (d, *J* = 8.6 Hz, 2H), 7.25 (s, 1H), 6.80 (s, 1H), 4.39 (p, *J* = 4.1 Hz, 2H), 4.33 (q, *J* = 7.0 Hz, 1H), 4.29 (q, *J* = 5.7 Hz, 1H), 4.24 (t, *J* = 7.6 Hz, 1H), 4.02 (t, *J* = 6.7 Hz, 2H), 3.94 (q, *J* = 5.7 Hz, 1H), 3.00 – 2.91 (m, 2H), 2.79 (t, *J* = 6.1 Hz, 2H), 2.58 – 2.51 (m, 2H), 2.11 (h, *J* = 8.2 Hz, 2H), 2.03 – 1.92 (m, 2H), 1.77 – 1.66 (m, 3H), 1.60 – 1.49 (m, 7H), 1.45 (t, *J* = 6.0 Hz, 3H), 1.37 (d, *J* = 7.0 Hz, 5H), 1.32 (h, *J* = 8.0 Hz, 3H), 1.04 (hept, *J* = 7.6 Hz, 1H), 0.90 – 0.73 (m, 27H).

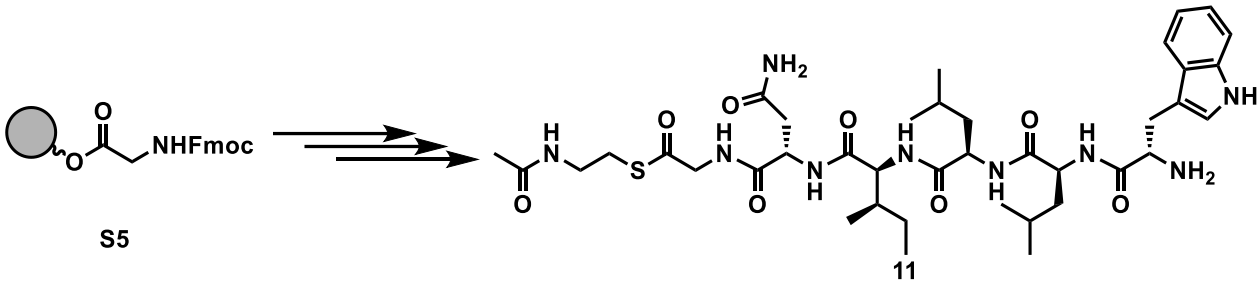
^{13}C NMR (201 MHz, DMSO) δ 200.6, 173.1, 171.6, 171.1, 170.8, 170.4, 169.6, 63.9, 58.7, 57.4, 56.4, 51.6, 51.2, 48.2, 41.0, 38.5, 36.8, 33.6, 31.1, 30.8, 30.1, 29.2, 26.9, 24.2, 24.1, 23.5, 23.2, 23.0, 21.5, 19.2, 18.6, 17.8, 17.7, 15.3, 13.5, 10.9

Mass spec: expected neutral mass for $C_{37}H_{68}N_8O_9S$ (Da): 800.4830, observed neutral mass (Da): 800.4830, mass error (ppm) 1.2.

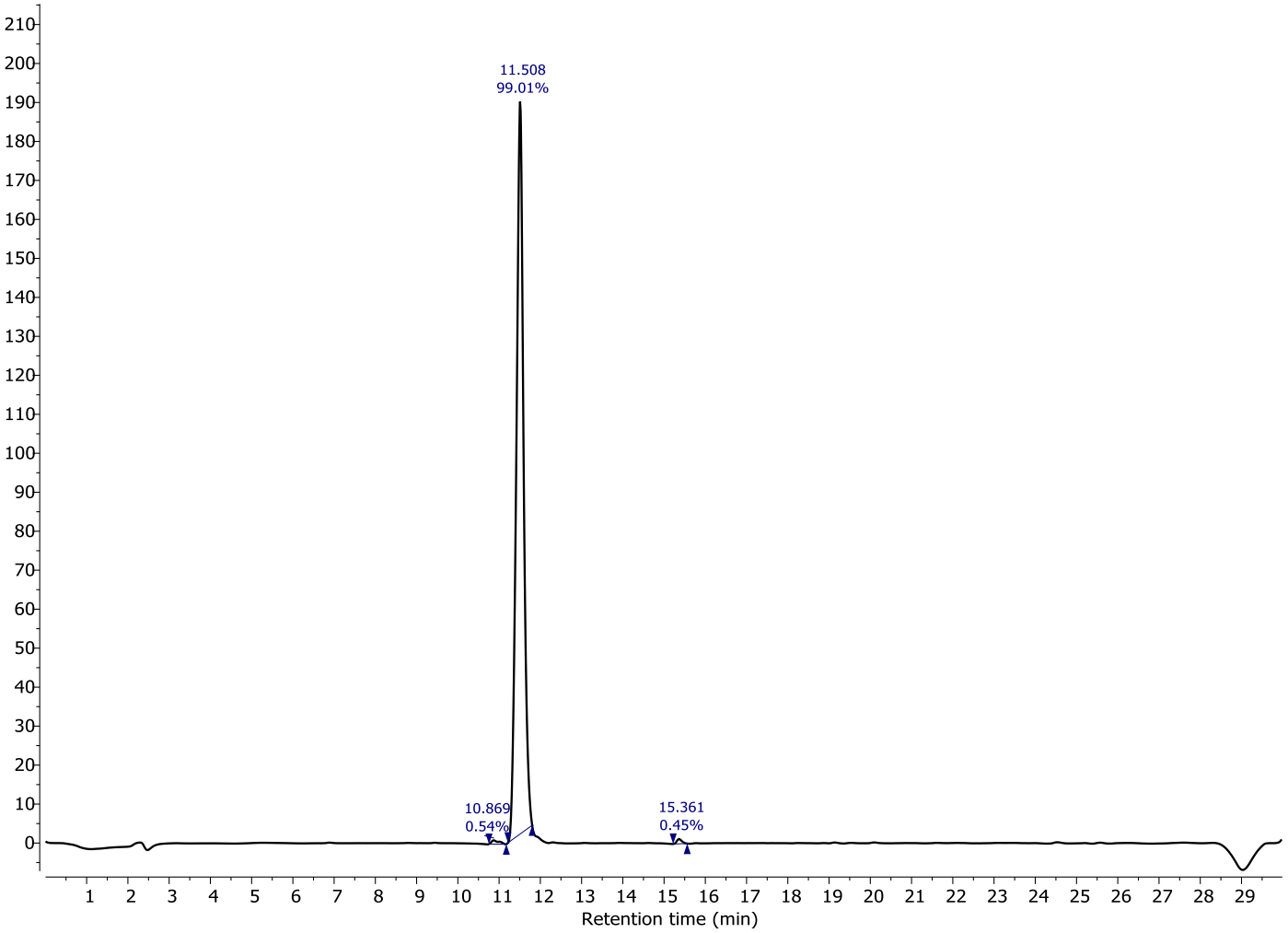
UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 5% to 60% over 18 minutes at a flow rate of 1 mL/min. The column was equilibrated with 5% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be >99%.



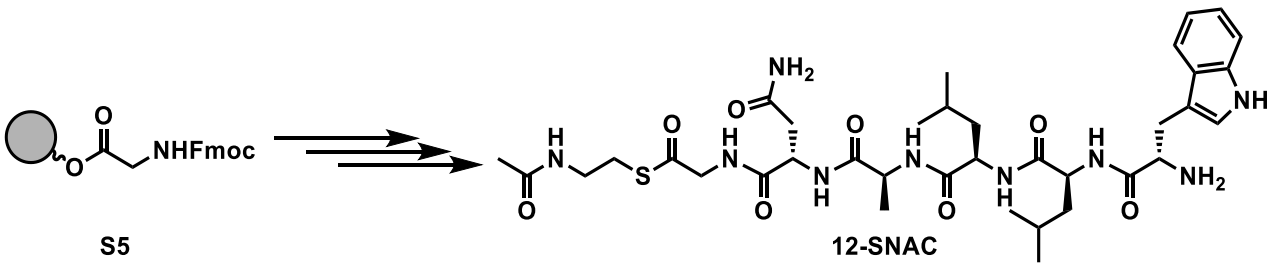
Synthesis of **11**



Peptide **11** was synthesized following a previously described method[1].Briefly, Fmoc-Gly-OH (3 eq) and i-Pr_2EtN (6 eq) were loaded onto 2-chlorotritylchloride (2-CTC) resin in DMF. After 2 hours, unreacted resin was capped with MeOH, and solid-phase peptide synthesis (SPPS) was initiated from **S5** (0.05 mmol) using Fmoc-L-Asn(Trt)-OH, Fmoc-L-Ile-OH, Fmoc-D-Leu-OH, Fmoc-L-Leu-OH, and Boc-L-Trp(Boc)-OH. To cleave the peptide from the 2-chlorotrityl resin, a solution of 25% hexafluoroisopropanol (HFIP) in DCM (3.0 mL) was added to the resin and shaken for 30 minutes at room temperature. The reaction mixture was filtered, and the filtrate was washed with DCM (10 mL), concentrated, redissolved in a mixture of acetonitrile-water, frozen, and lyophilized. The crude linear peptide was used in the next reaction without further purification. For the next step, the crude peptide was dissolved in DCM/DMF, and N-Aceytlcysteamine (10 equiv.), 2,6 Lutidine (5 equiv.), and HCTU (5 quiv.) were added. The reaction was stirred overnight at room temperature, quenched with 40 mL EtOAc, and washed with saturated aqueous NH_4Cl (2x 15 mL), saturated aqueous $NaHCO_3$ (2x 10 mL), and brine (3x 20 mL). The product was dried over sodium sulfate, filtered, and concentrated. A cleaving cocktail of TFA/TIPS = 90:10 (3 mL) was added to remove protecting groups, and the mixture was shaken for 90 minutes. The volatiles were removed by a stream of air, and the peptide crashed out with 25 mL -20°C Et_2O. The product was centrifuged at 3200 x g for 10 minutes at 4 °C, and the Et_2O layer was removed by decantation. This procedure was repeated twice. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.1% Formic acid (A) and acetonitrile+0.1% Formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 10% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 10% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (3.7 mg, 9% yield).
Mass spec: expected neutral mass for $C_{39}H_{61}N_9O_8S$ (Da): 815.4364, observed neutral mass (Da): 815.4375, mass error (ppm) 1.3.
HPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 5% to 60% over 18 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 5% mobile phase B for 1 minute before and 5 minutes after the gradient. The peptide purity was determined to be 99%.



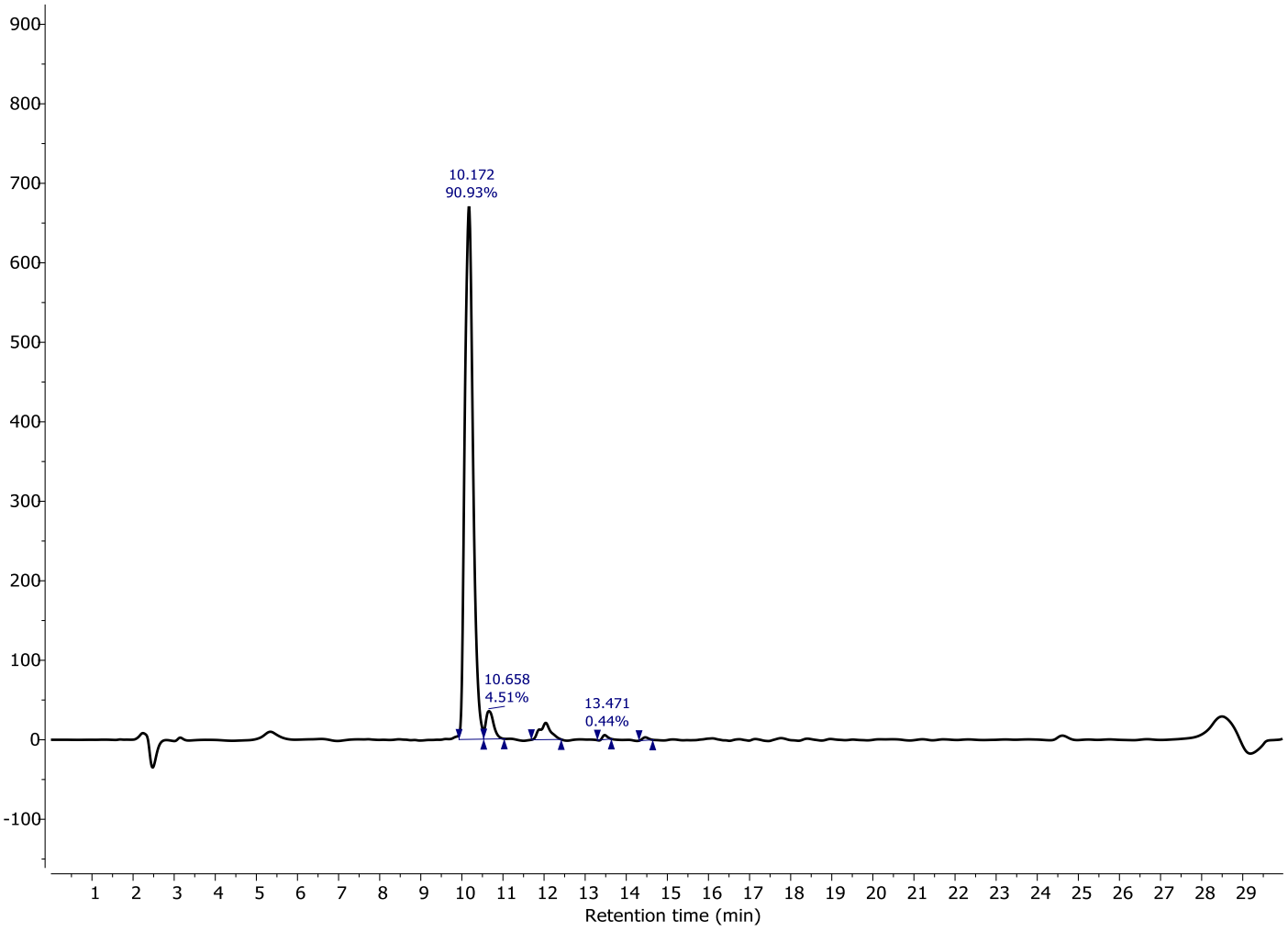
Synthesis of **12-SNAC**



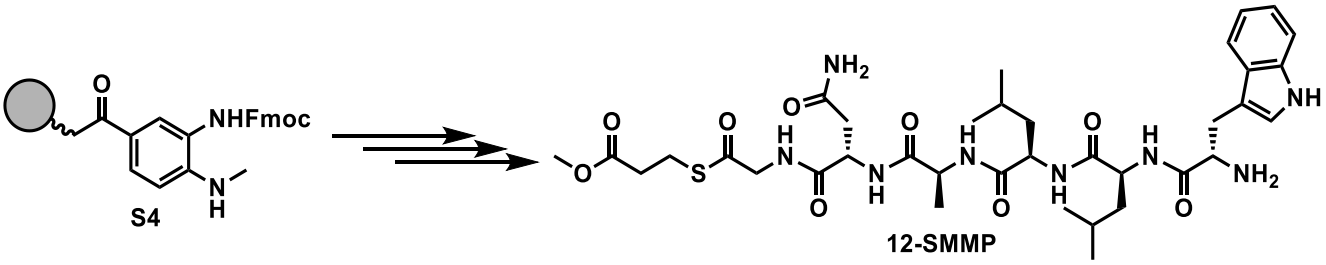
Peptide **12-SNAC** was synthesized following an identical protocol followed for Peptide 11 however Fmoc-L-Leu-OH was substituted with Fmoc-L-Ala-OH. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 10% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 10% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (2.1 mg, 5% yield).

Mass spec: expected neutral mass for $C_{36}H_{55}N_9O_8S$ (Da): 773.3894, observed neutral mass (Da): 773.3904, mass error (ppm) 1.2.

HPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 5% to 60% over 18 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 5% mobile phase B for 1 minute before and 5 minutes after the gradient. The peptide purity was determined to be 91%.



Synthesis of **12-SMMP**



Peptide **12-SMMP** was synthesized following the general protocol outlined above for the NDbz “safety-catch” resin. The synthesis was initiated from **S4** (0.10 mmol) using the following amino acids (Fmoc-Gly-OH, Fmoc-L-Asn(Trt)-OH, Fmoc-L-Ala-OH, Fmoc-D-Leu-OH, Fmoc-L-Leu-OH, and Boc-L-Trp(Boc)-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.05% Trifluoroacetic acid (A) and acetonitrile+0.05% Trifluoroacetic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (24.8 mg, 28% yield).

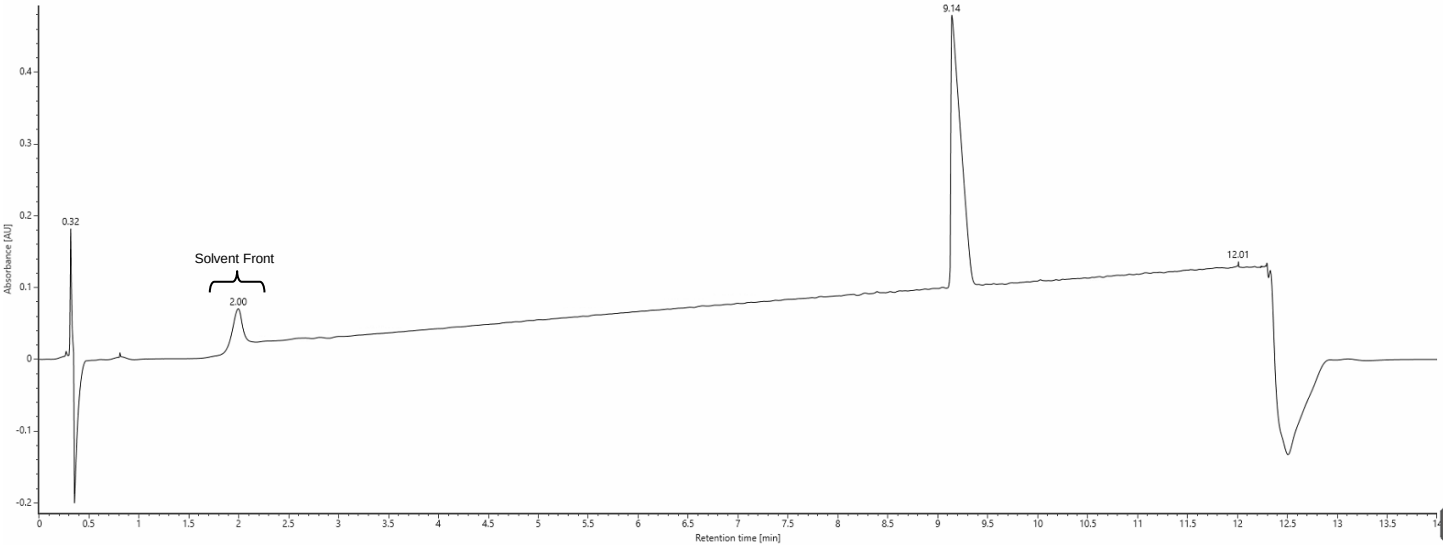
1H NMR (800 MHz, DMSO) δ 10.97 (s, 1H), 8.76 (d, J = 6.5 Hz, 1H), 8.42 – 8.32 (m, 2H), 8.21 (d, J = 8.5 Hz, 1H), 8.11 (d, J = 8.5 Hz, 1H), 8.02 (s, 3H), 7.73 (d, J = 7.4 Hz, 1H), 7.37 (t, J = 7.4 Hz, 2H), 7.19 (s, 1H), 7.10 (t, J = 7.4 Hz, 1H), 7.01 (t, J = 8.2 Hz, 1H), 6.90 (s, 1H), 4.62 (q, J = 6.2 Hz, 1H), 4.44 (q, J = 6.2 Hz, 1H), 4.39 –

4.34 (m, 1H), 4.30 (p, J = 6.2 Hz, 1H), 4.06 (s, 1H), 3.97 (dd, J = 7.4, 4.2 Hz, 1H), 3.89 (dd, J = 7.4, 5.3 Hz, 1H), 3.60 (s, 3H), 3.28 (dd, J = 5.1, 4.2 Hz, 1H), 3.04 – 2.94 (m, 3H), 2.60 – 2.53 (m, 3H), 2.46 (q, J = 8.5 Hz, 1H), 1.63 (h, 1H), 1.58 (hept, J = 5.3 Hz, 1H), 1.54 – 1.42 (m, 4H), 1.21 (d, J = 6.2 Hz, 3H), 0.93 (d, J = 6.2 Hz, 3H), 0.88 (q, J = 7.4 Hz, 6H), 0.82 (d, J = 5.1 Hz, 3H).

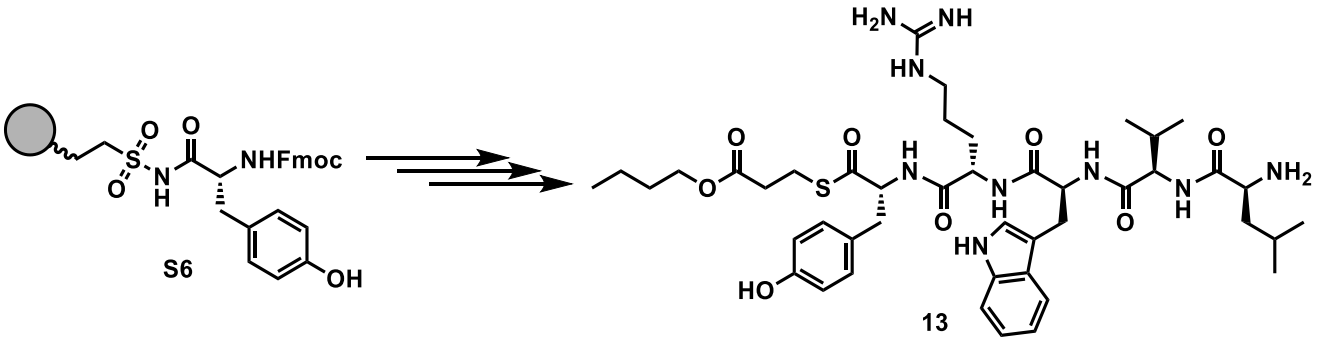
13C NMR (201 MHz, DMSO) δ 198.44, 172.11 (d, J = 24.3 Hz), 171.59, 168.82, 136.74, 127.41, 125.50, 121.54, 118.87 (d, J = 24.3 Hz), 111.91, 107.17, 52.88, 51.96, 51.43, 49.99, 49.33, 48.53, 41.54, 40.00, 39.89, 39.79, 37.17, 33.81, 27.75, 24.59 (d, J = 21.8 Hz), 23.48, 23.40, 23.33, 22.17, 21.72, 18.65.

Mass spec: expected neutral mass for $C_{36}H_{54}N_8O_9S$ (Da): 774.3734, observed neutral mass (Da): 774.3759, mass error (ppm) 3.2.

UPLC Trace Obtained using mobile phases of H2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The peptide purity was determined to be >99%.



Synthesis of **13**



Peptide **13** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S6** (0.05 mmol) using the following amino acids (Fmoc-L-Arg(Pbf)-OH, Fmoc-L-Trp(Boc)-OH, Fmoc-D-Val-OH, Boc-L-Leu-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (23.7 mg, 43% yield) analyzed via RP-HPLC and was used in further enzyme assays without further purification.

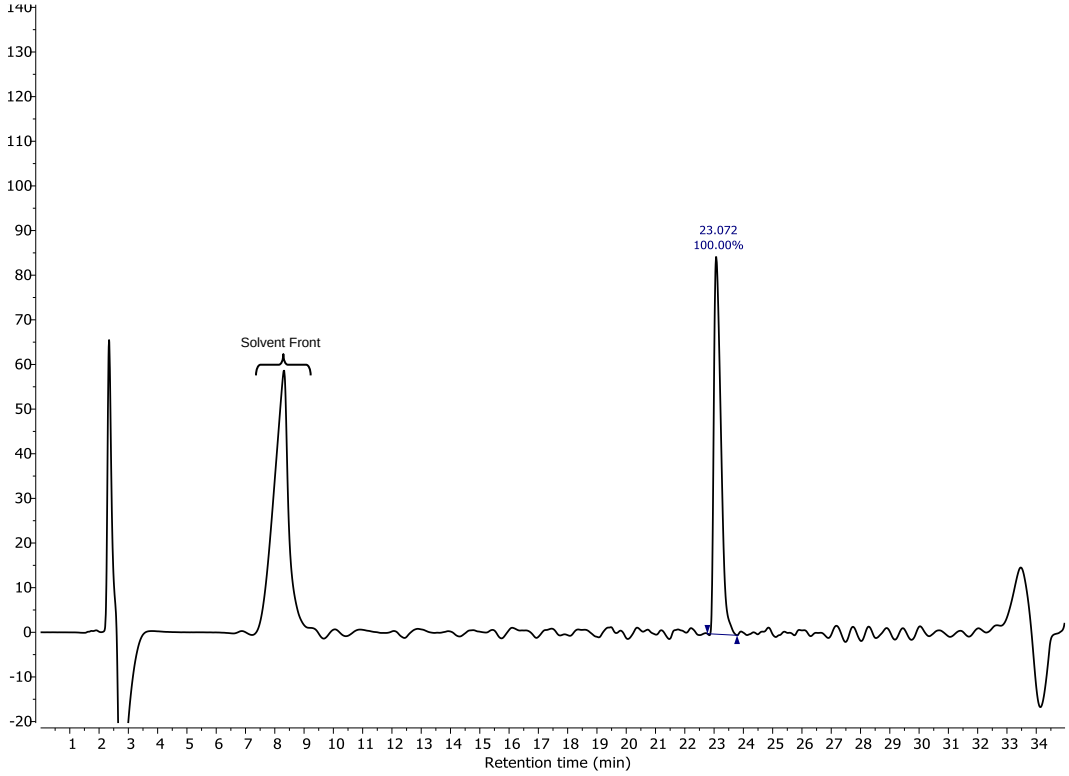
^{1}H NMR (800 MHz, DMSO) δ 10.82 (s, 1H), 9.37 (d, *J* = 528.2 Hz, 1H), 8.70 (d, *J* = 7.9 Hz, 1H), 8.46 (d, *J* = 7.5 Hz, 1H), 8.29 (d, *J* = 8.6 Hz, 1H), 8.19 (d, *J* = 8.1 Hz, 1H), 7.92 (s, 1H), 7.66 (dd, *J* = 8.1, 7.5 Hz, 3H), 7.42 – 7.22 (m, 4H), 7.14 (s, 1H), 7.03 – 6.82 (m, 4H), 6.65 (d, *J* = 8.1 Hz, 2H), 4.66 (d, *J* = 10.3 Hz, 1H), 4.37 (ddt, *J* = 8.5, 5.7, 5.1 Hz, 2H), 4.06 – 3.87 (m, 2H), 3.78 (s, 1H), 3.51 (s, 1H), 3.44 – 3.28 (m, 1H), 3.15 (d, *J* = 13.8 Hz, 1H), 3.07 – 2.91 (m, 5H), 2.87 (t, *J* = 13.2 Hz, 1H), 2.75 – 2.57 (m, 1H), 2.55 (t, *J* = 6.3 Hz, 1H), 1.71 –

1.58 (m, 2H), 1.56 – 1.37 (m, 8H), 1.37 – 1.03 (m, 5H), 0.85 (d, *J* = 5.7 Hz, 9H), 0.50 (d, *J* = 5.7 Hz, 2H), 0.42 (d, *J* = 6.9 Hz, 3H).

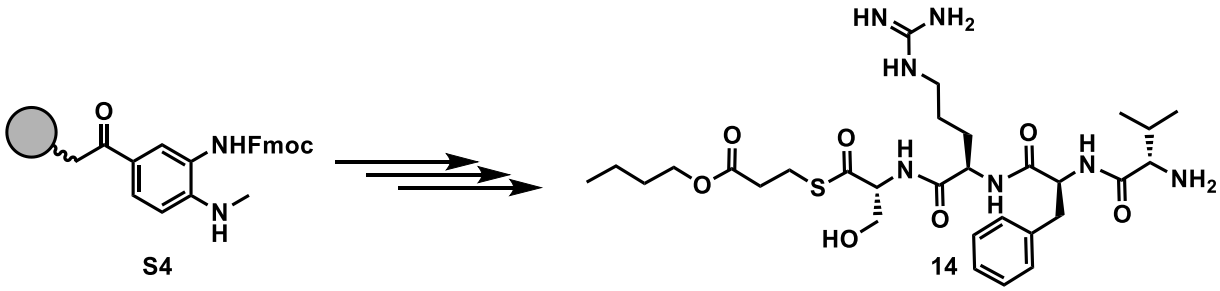
^{13}C NMR (201 MHz, DMSO) δ 200.6, 172.2, 172.0, 171.5, 170.6, 157.4, 156.5, 136.6, 130.5, 129.0, 128.1, 127.5, 127.3, 125.9, 124.5, 121.1, 118.3, 116.8, 116.6, 115.5, 111.5, 110.3, 70.1, 64.3, 61.4, 57.7, 53.5, 52.5, 51.5, 41.7, 40.6, 36.5, 33.9, 31.2, 30.5, 29.5, 28.5, 25.3, 24.1, 23.8, 23.1, 22.1, 19.3, 18.9, 17.6, 13.9.

Mass spec: expected neutral mass for $C_{44}H_{65}N_9O_8S$ (Da): 879.4677, observed neutral mass (Da): 879.4673, mass error (ppm) 0.4.

HPLC Trace Obtained using mobile phases of H2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 55% over 28 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The peptide purity was determined to be >99%.



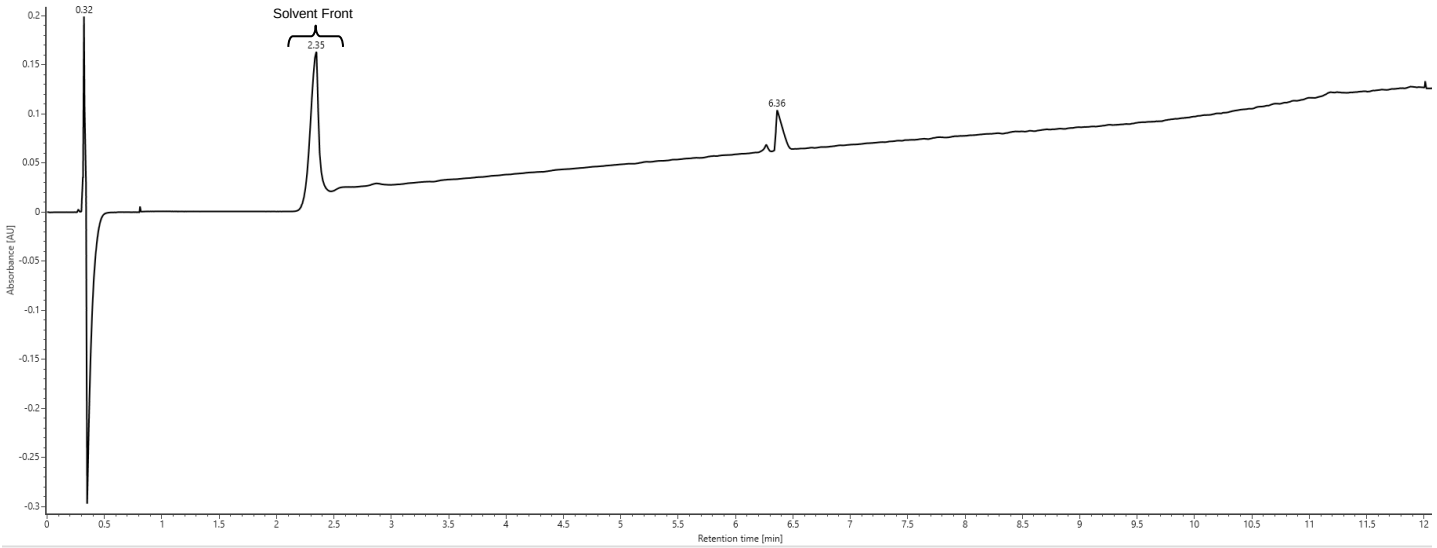
Synthesis of **14**



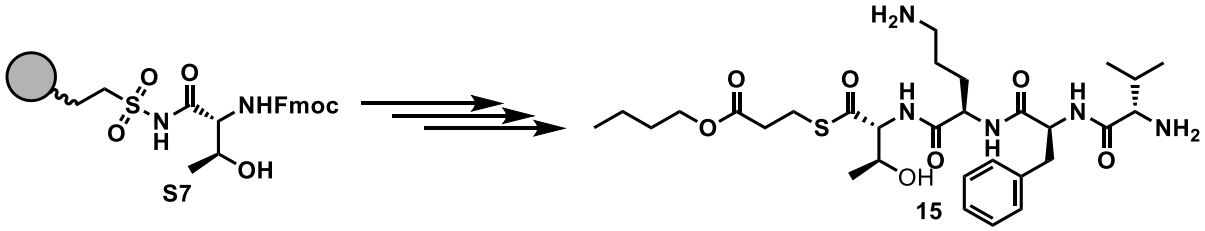
Peptide **14** was synthesized following the general protocol outlined above for the NDbz “safety-catch” resin. The synthesis was initiated from **S4** (0.10 mmol) using the following amino acids (Fmoc-D-Ser(tBu)-OH, Fmoc-D-Arg(Pbf)-OH, Fmoc-L-Phe-OH, and Boc-L-Val-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.05% trifluoroacetic acid (A) and acetonitrile+0.05% trifluoroacetic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (17.8 mg, 20% yield).

^{1}H NMR (800 MHz, DMSO) δ 8.66 (d, *J* = 7.0 Hz, 1H), 8.56 (d, *J* = 7.3 Hz, 1H), 8.33 (d, *J* = 7.3 Hz, 1H), 8.07 (s, 4H), 7.77 (s, 1H), 7.44 (s, 2H), 7.32 – 7.06 (m, 10H), 4.70 (p, *J* = 4.6 Hz, 1H), 4.41 (d, *J* = 38.4 Hz, 2H), 4.01 (dd, *J* = 220.7, 6.1 Hz, 3H), 3.72 (p, *J* = 8.2 Hz, 1H), 3.62 (s, 3H), 3.07 – 2.90 (m, 7H), 2.90 – 2.71 (m, 2H), 2.53 (t, *J* = 4.9 Hz, 2H), 2.08 (d, *J* = 4.9 Hz, 1H), 1.68 (d, *J* = 6.1 Hz, 1H), 1.53 (t, *J* = 7.0 Hz, 3H), 1.45 (p, *J* = 7.3 Hz, 1H), 1.31 (p, *J* = 8.2 Hz, 5H), 0.97 – 0.76 (m, 13H).
^{13}C NMR (201 MHz, DMSO) δ 199.9, 172.7, 171.6, 169.8, 169.7, 157.4, 138.7, 138.2, 129.7, 129.2, 128.6, 126.6, 70.2, 64.3, 62.8, 61.6, 60.7, 56.0, 55.4, 34.5, 34.1, 30.5, 24.7, 24.3, 23.6, 19.8, 19.0, 17.3, 17.0, 13.9.

Mass spec: expected neutral mass for $C_{30}H_{49}N_7O_7S$ (Da): 651.3414, observed neutral mass (Da): 651.3411, mass error (ppm) 0.5.
UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 90.3% the only impurity being epimerized peptide that could not be separated out.

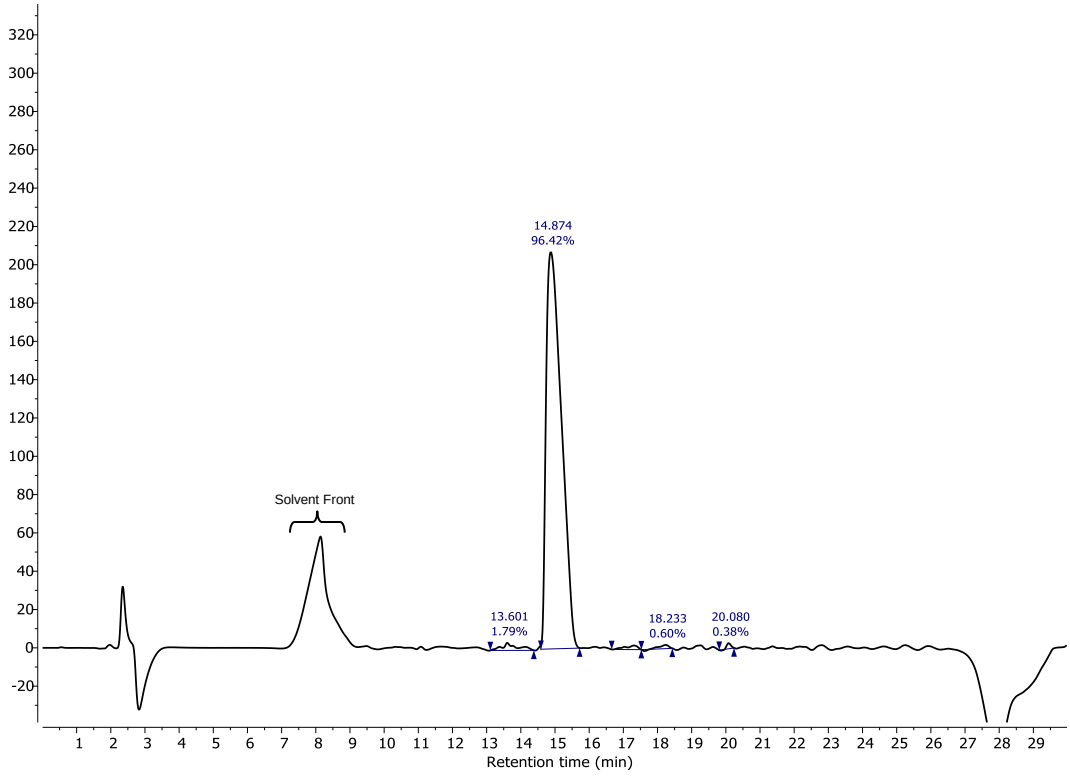


Synthesis of **15**

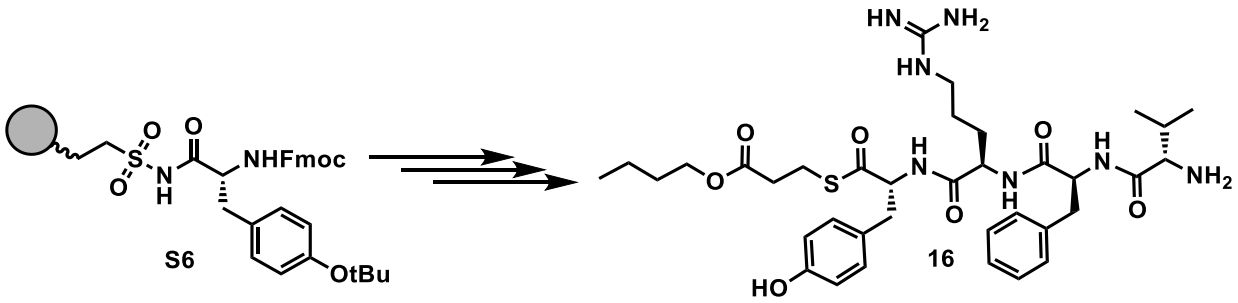


Peptide **15** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S7** (0.05 mmol) using the following amino acids (Fmoc-D-Orn(Boc)-OH, Fmoc-L-Phe-OH, Boc-L-Val-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (30.4 mg, 70% yield) analyzed via RP-HPLC and was used in further enzyme assays without further purification.
^{1}H NMR (800 MHz, DMSO) δ 8.63 (d, *J* = 8.3 Hz, 1H), 8.41 (dd, *J* = 9.1, 9.1 Hz, 2H), 7.96 (s, 6H), 7.34 – 7.23 (m, 4H), 7.19 (t, *J* = 6.8 Hz, 1H), 5.12 (s, 1H), 4.74 (q, *J* = 6.0 Hz, 1H), 4.54 (h, *J* = 6.8 Hz, 1H), 4.31 (dd, *J* = 3.1, 3.1 Hz, 1H), 4.22 (s, 1H), 4.02 (t, *J* = 6.0 Hz, 2H), 3.60 (d, *J* = 3.9 Hz, 1H), 3.02 – 2.92 (m, 3H), 2.85 (q, *J* = 8.3

Hz, 1H), 2.73 (h, *J* = 7.6 Hz, 2H), 2.55 (t, *J* = 6.0 Hz, 2H), 2.07 (h, *J* = 7.0 Hz, 1H), 1.81 – 1.74 (m, 1H), 1.56 – 1.42 (m, 5H), 1.32 (h, *J* = 6.8 Hz, 2H), 0.98 (d, *J* = 6.0 Hz, 3H), 0.91 – 0.86 (m, 6H), 0.84 (d, *J* = 6.0 Hz, 3H).
^{13}C NMR (201 MHz, DMSO) δ 200.6, 172.6, 171.6, 171.0, 168.2, 137.8, 129.6, 128.5, 126.8, 66.4, 64.9, 64.3, 57.4, 54.3, 51.6, 38.7, 38.4, 34.0, 30.5, 30.3, 29.0, 23.8, 23.7, 20.5, 19.0, 18.7, 17.6, 13.9.
Mass spec: expected neutral mass for $C_{30}H_{49}N_5O_7S$ (Da): 623.3352, observed neutral mass (Da): 623.3350, mass error (ppm) 0.4.
UPLC Trace Obtained using mobile phases of H2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The peptide purity was determined to be 96%



Synthesis of **16**



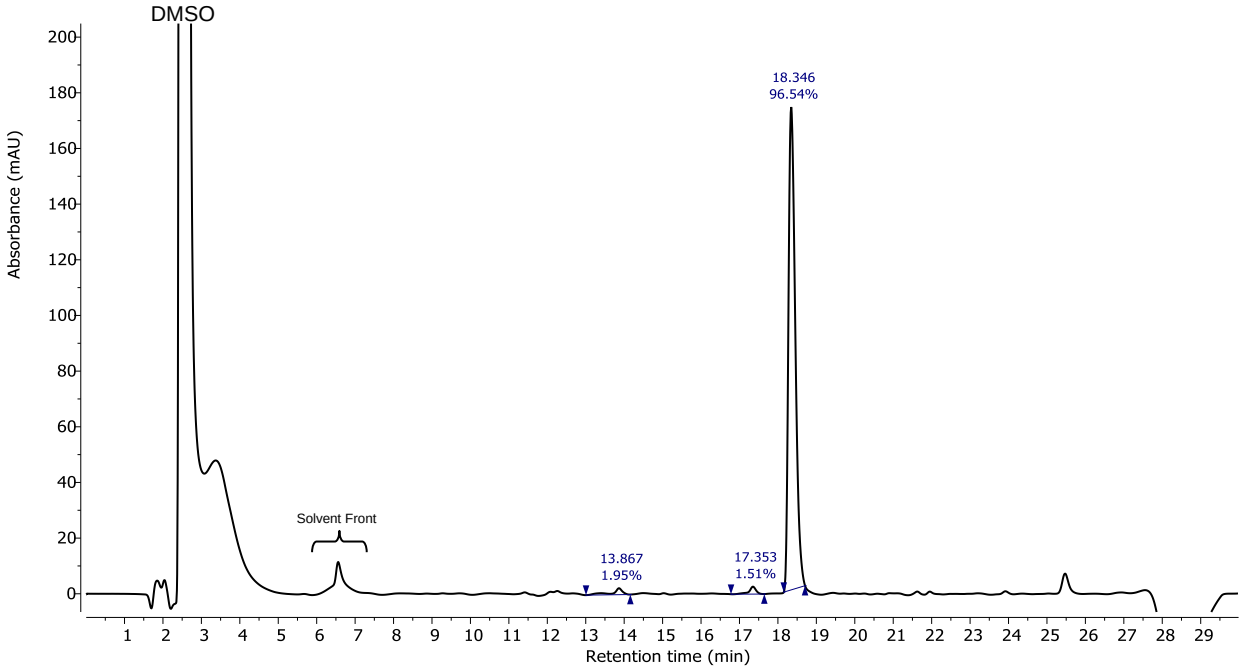
Peptide **16** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from 4-sulfamylbutyryl resin preloaded with Fmoc-D-Tyr(tBu)-OH **S6** (0.05 mmol) using the following amino acids (Fmoc-D-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Boc-L-Val-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (29.6 mg, 61% yield) analyzed via RP-UPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 9.32 (s, 1H), 8.68 (s, 1H), 8.54 (d, *J* = 4.5 Hz, 1H), 8.27 (s, 1H), 7.98 (s, 2H), 7.83 (s, 1H), 7.44 (s, 2H), 7.26 (d, *J* = 2.6 Hz, 6H), 7.19 (s, 2H), 6.99 (d, *J* = 5.2 Hz, 3H), 6.64 (d, *J* = 5.9 Hz, 2H), 4.68 (d, *J* = 7.1 Hz, 1H), 4.42 (s, 1H), 4.29 (s, 1H), 3.97 (t, *J* = 7.0 Hz, 2H), 3.58 (t, *J* = 4.0 Hz, 1H), 3.38 (s, 1H), 3.03 – 2.89 (m, 8H), 2.87 (t, *J* = 10.3 Hz, 1H), 2.81 (t, *J* = 10.3 Hz, 1H), 2.06 (d, *J* = 5.7 Hz, 1H), 1.64 (d, *J* = 7.1 Hz, 1H), 1.54 (t, *J* = 7.1 Hz, 3H), 1.43 (dd, *J* = 27.3, 6.4 Hz, 1H), 1.39 (s, 1H), 1.32 (q, *J* = 9.0 Hz, 3H), 1.23 (s, 3H), 0.92 – 0.82 (m, 12H).

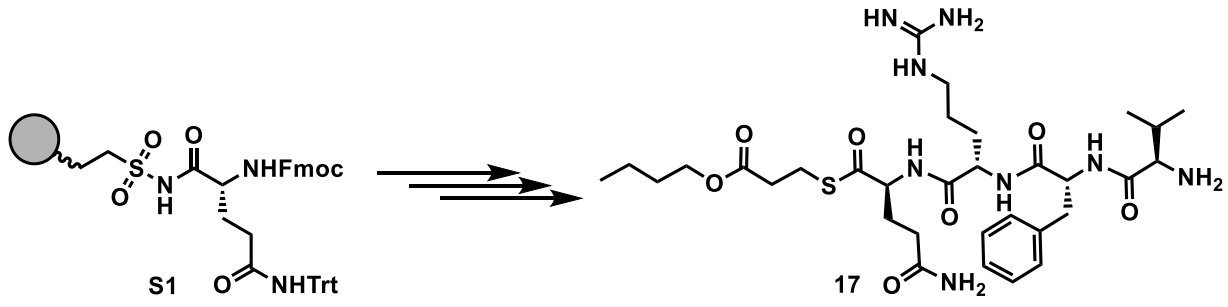
^{13}C NMR (201 MHz, DMSO) δ 200.5, 171.7, 171.5, 170.7, 168.5, 157.3, 156.5, 137.5, 130.3, 129.6, 128.5, 128.1, 127.1, 126.8, 125.8, 115.5, 64.3, 61.4, 57.6, 54.6, 52.1, 40.7, 38.2, 36.5, 34.0, 30.5, 30.4, 29.3, 25.1, 23.7, 19.0, 17.7, 13.9.

Mass spec: expected neutral mass for $C_{36}H_{53}N_7O_7S$ (Da): 727.3727, observed neutral mass (Da): 727.3748, mass error (ppm) 2.9.

HPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 21 minutes at a flow rate of 1 mL/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The crude peptide purity was determined to be 96%.



Synthesis of **17**



Peptide **17** was synthesized following the general protocol outlined above for the sulfonamide "safety-catch" resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids (Fmoc-D-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Boc-L-Val-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H2O+0.05% Trifluoroacetic acid (A)

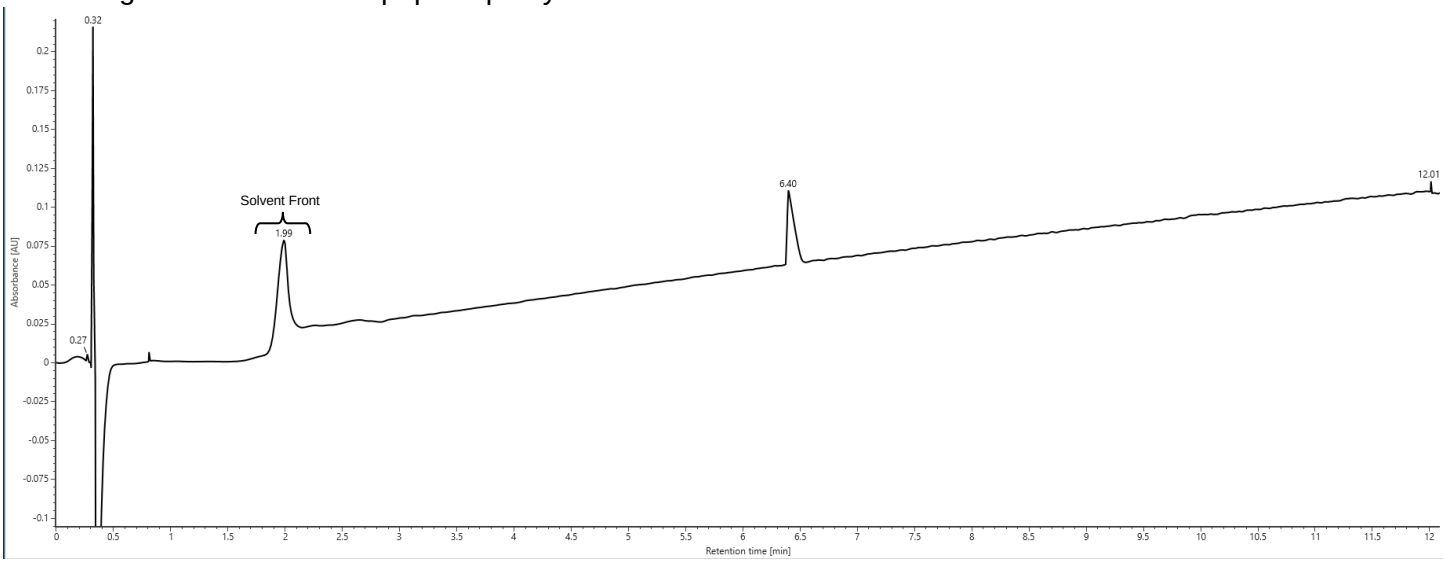
and acetonitrile+0.05% Trifluoroacetic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (7.8 mg, 17% yield).

^{1}H NMR (800 MHz, DMSO) δ 9.31 (s, 1H), 8.67 (s, 1H), 8.53 (s, 1H), 8.26 (s, 1H), 7.97 (bs, 3H), 7.81 (s, 1H), 7.43 (bs, 2H), 7.24 (d, *J* = 54.6 Hz, 11H), 6.99 (s, 3H), 6.63 (s, 3H), 4.66 (s, 1H), 4.41 (s, 1H), 4.28 (s, 1H), 4.01 (s, 3H), 3.57 (s, 1H), 3.36 (s, 1H), 3.01 – 2.74 (m, 13H), 2.03 (s, 1H), 1.62 (s, 1H), 1.52 (s, 3H), 1.48 – 1.18 (m, 9H), 0.87 (s, 15H).

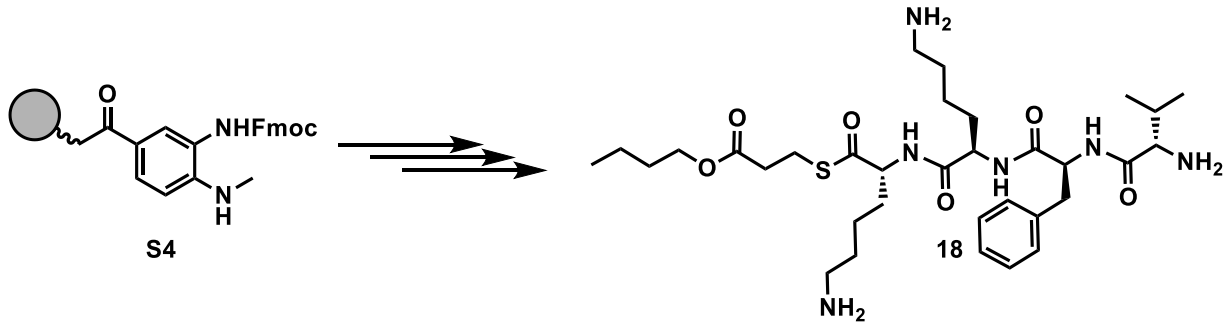
^{13}C NMR (201 MHz, DMSO) δ 200.5, 171.7, 171.5, 170.7, 168.5, 157.3, 156.5, 137.5, 130.3, 129.6, 128.5, 128.1, 127.1, 126.8, 125.8, 118.2, 116.7, 115.5, 114.2, 70.2, 65.1, 64.3, 64.0, 61.4, 57.6, 54.6, 52.1, 39.6, 38.2, 36.5, 34.0, 30.4, 29.3, 25.1, 19.0, 17.7, 13.9.

Mass spec: expected neutral mass for $C_{32}HC_{52}N_8O_7S$ (Da): 692.3679, observed neutral mass (Da): 692.3686, mass error (ppm) 0.9.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 98%.



Synthesis of **18**



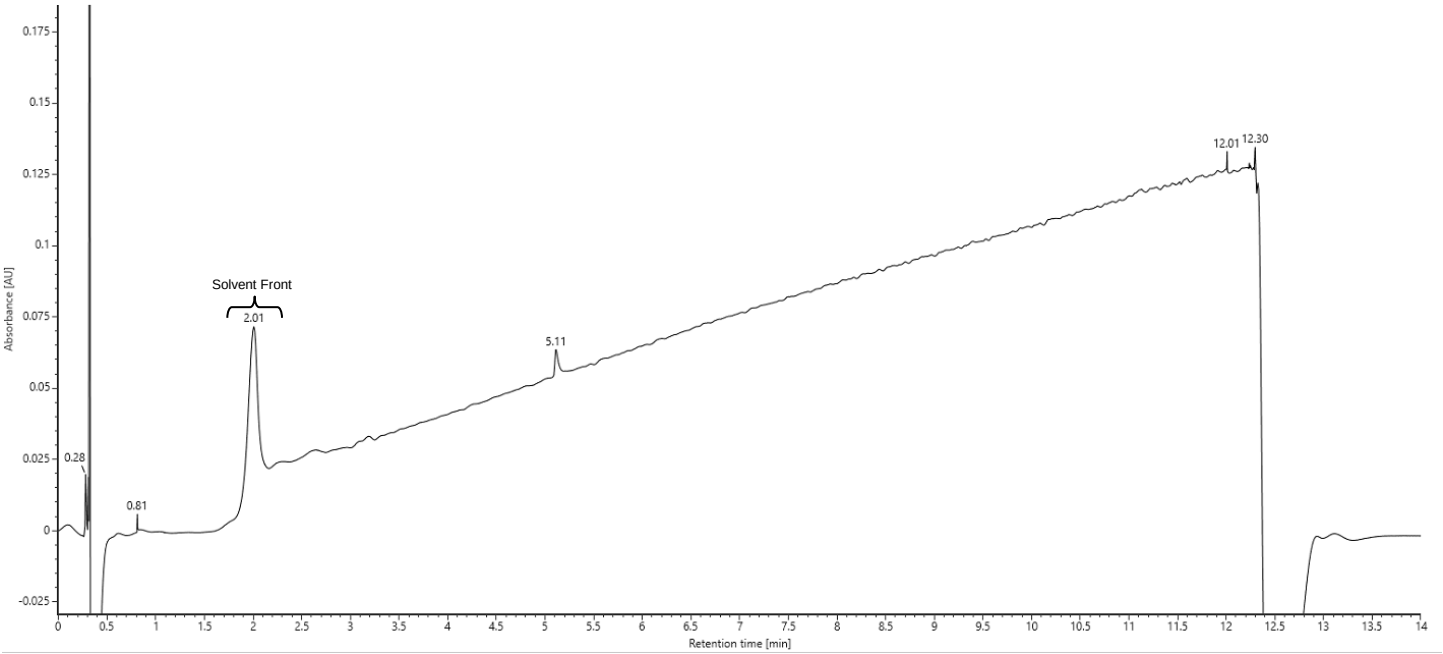
Peptide **14** was synthesized following the general protocol outlined above for the NDbz “safety-catch” resin. The synthesis was initiated from **S4** (0.15 mmol) using the following amino acids (Fmoc-D-Lys(Boc)-OH, Fmoc-D-Lys(Boc)-OH, Fmoc-L-Phe-OH, and Boc-L-Val-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.05% Trifluoroacetic acid (A) and acetonitrile+0.05% Trifluoroacetic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (2.31 mg, 2% yield).

1H NMR (800 MHz, DMSO) δ 8.64 (d, J = 8.0 Hz, 1H), 8.29 (dd, J = 8.9, 8.3 Hz, 1H), 8.03 (s, 2H), 7.92 – 7.65 (m, 6H), 7.27 (d, J = 5.6 Hz, 3H), 7.21 (s, 2H), 4.68 (p, J = 7.5 Hz, 1H), 4.30 – 4.19 (m, 1H), 4.13 (s, 1H), 4.02 (q, J = 6.2 Hz, 1H), 3.65 – 3.51 (m, 1H), 3.51 (d, J = 505.9 Hz, 1H), 2.99 – 2.89 (m, 2H), 2.87 (d, J = 253.5 Hz, 1H), 2.76 – 2.57 (m, 3H), 2.54 (d, J = 386.2 Hz, 1H), 2.04 (h, 1H), 1.65 – 1.60 (m, 1H), 1.60 – 1.55 (m, 1H), 1.55 – 1.47 (m, 4H), 1.43 (h, 3H), 1.37 – 1.12 (m, 6H), 1.09 – 0.94 (m, 2H), 0.92 – 0.75 (m, 9H).

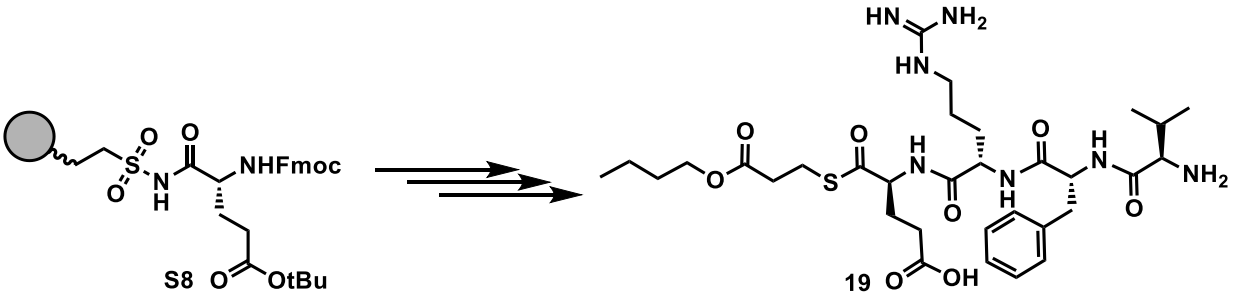
13C NMR (201 MHz, DMSO) δ 201.31, 158.55, 158.40, 137.52, 129.62, 128.52, 126.83, 118.40, 116.91, 70.17, 64.29, 59.40, 57.48, 54.47, 52.22, 39.07, 39.02, 38.85, 38.40, 34.02, 31.42, 30.76, 30.50, 30.33, 27.04, 26.83, 23.64, 22.50, 22.40, 18.97, 18.73, 17.79, 13.94

Mass spec: expected neutral mass for $C_{33}H_{56}N_6O_6S$ (Da): 664.3982, observed neutral mass (Da): 664.3973, mass error (ppm) 1.3.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient.



Synthesis of **19**



Using the general protocol described above for the sulfonamide "safety-catch" resin, Peptide **19** was synthesized by initiating the synthesis from **S9** (0.05 mmol) and coupling the following amino acids in the order of Fmoc-D-Arg(Pbf)-OH, Fmoc-L-Phe-OH, and Boc-L-Val-OH. Butyl 3-mercaptopropionate was used as the cleaving thiol. The crude peptide was obtained as an off-white solid with a yield of 36% (16.4 mg) and was analyzed via RP-HPLC. The peptide was used in further enzyme assays without additional purification.

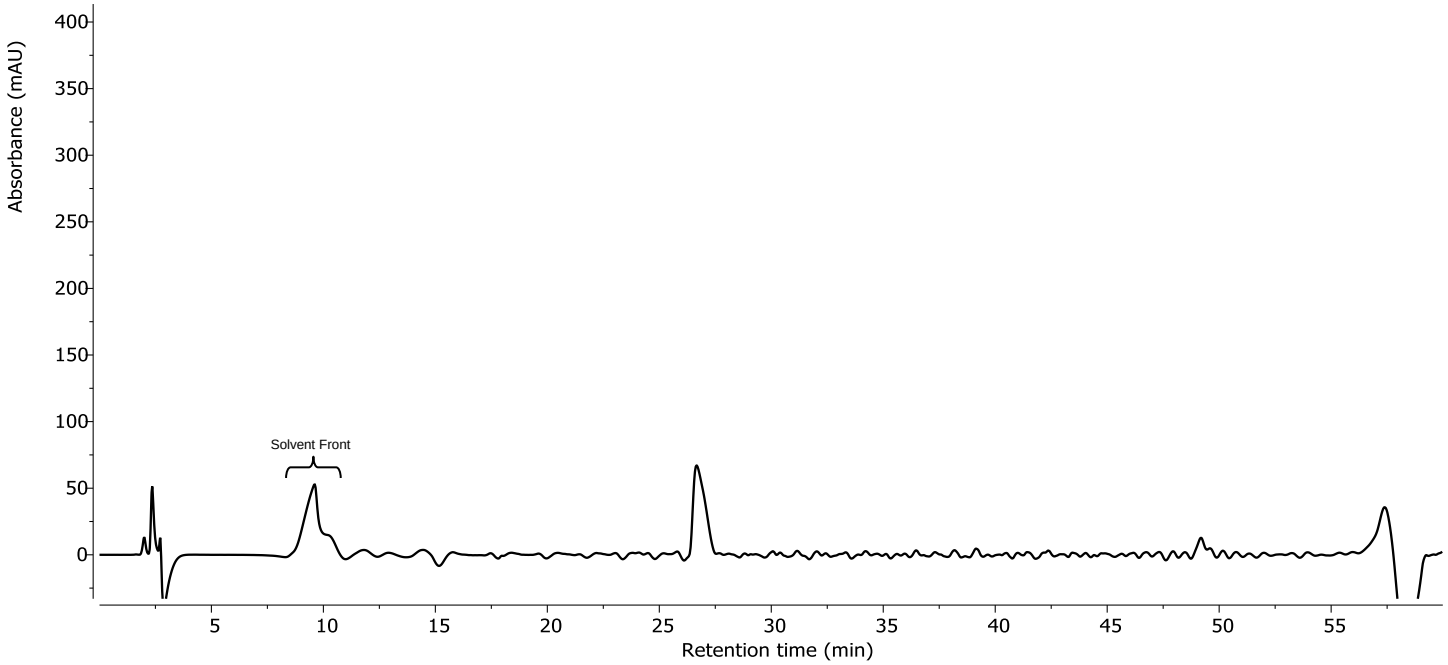
^{1}H NMR (800 MHz, DMSO) δ 8.66 (d, *J* = 7.9 Hz, 1H), 8.60 (d, *J* = 6.1 Hz, 1H), 8.33 (d, *J* = 8.2 Hz, 1H), 7.80 (s, 1H), 7.26 (t, *J* = 6.1 Hz, 5H), 7.21 – 7.18 (m, 1H), 7.15 (s, 1H), 4.67 (q, *J* = 7.9 Hz, 1H), 4.36 – 4.30 (m, 1H),

4.30 – 4.23 (m, 1H), 4.02 (t, *J* = 6.8 Hz, 2H), 3.58 – 3.46 (m, 1H), 3.07 – 2.90 (m, 5H), 2.87 (q, *J* = 8.2 Hz, 1H), 2.54 (t, *J* = 7.1 Hz, 2H), 2.31 – 2.20 (m, 2H), 2.05 – 1.92 (m, 2H), 1.82 – 1.74 (m, 1H), 1.74 – 1.66 (m, 1H), 1.53 (p, *J* = 6.1 Hz, 2H), 1.47 – 1.35 (m, 2H), 1.32 (hept, *J* = 7.5 Hz, 2H), 1.25 (p, *J* = 6.8 Hz, 2H), 0.90 – 0.86 (m, 6H), 0.84 (d, *J* = 6.7 Hz, 3H).

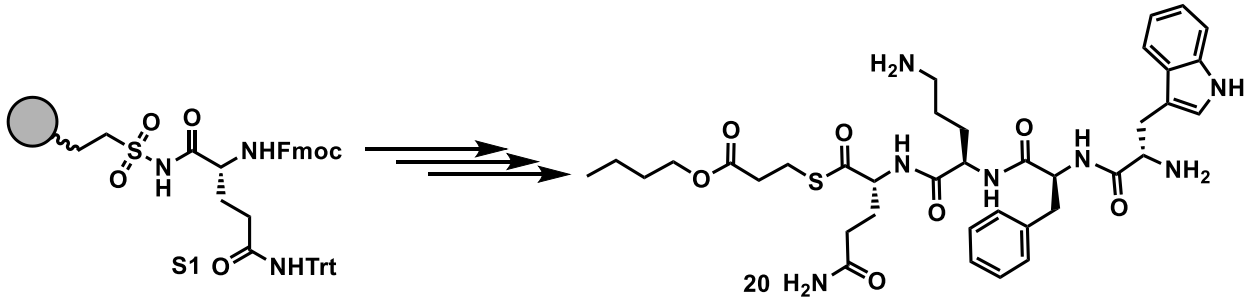
^{13}C NMR (201 MHz, DMSO) δ 200.6, 173.9, 171.7, 171.1, 170.4, 168.6, 156.9, 137.1, 129.2, 128.1, 126.4, 63.9, 58.6, 57.5, 54.2, 51.7, 40.3, 37.9, 33.6, 30.1, 30.1, 29.9, 28.6, 26.4, 24.8, 23.3, 18.6, 18.5, 17.3, 13.5.

Mass spec: expected neutral mass for $C_{32}H_{51}N_7O_8S$ (Da): 693.3520, observed neutral mass (Da): 693.3529, mass error (ppm) 1,3.

HPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 60% over 52 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The crude peptide purity was determined to be >99%.



Synthesis of **20**

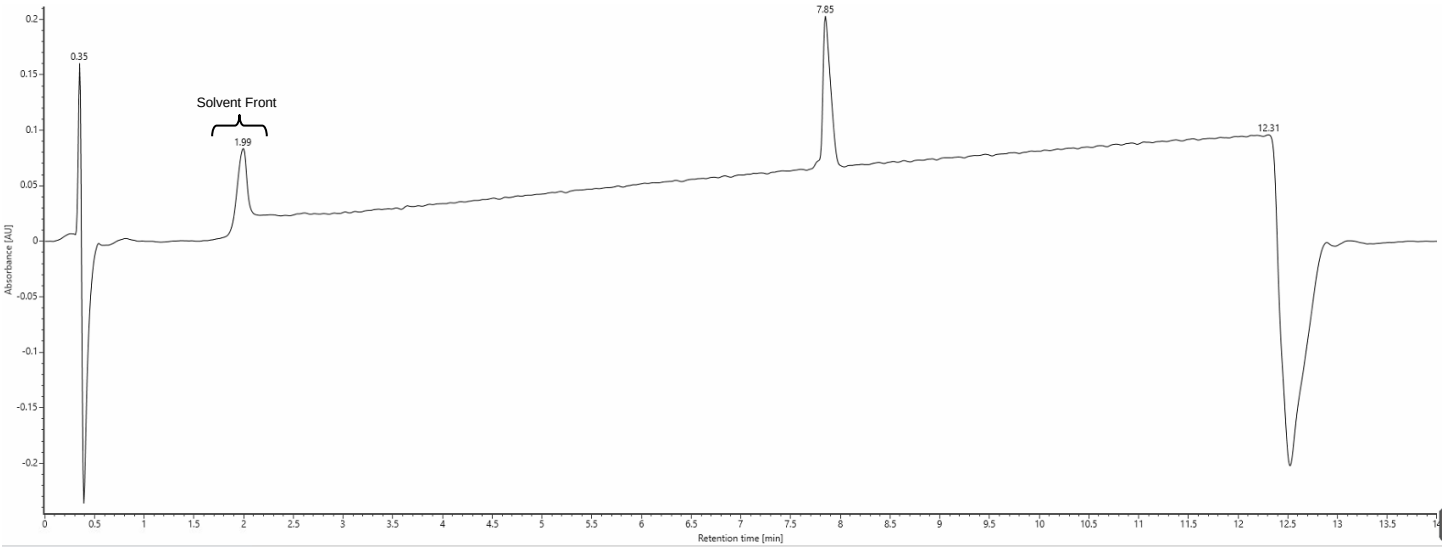


Peptide **20** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids Fmoc-D-Orn(Boc)-OH, Fmoc-L-Phe-OH, Boc-L-Trp(Boc)-OH and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.05% Formic Acid (A) and acetonitrile+0.05% Formic Acid (B). Samples were eluted using a gradient mode with mobile phase B ranging

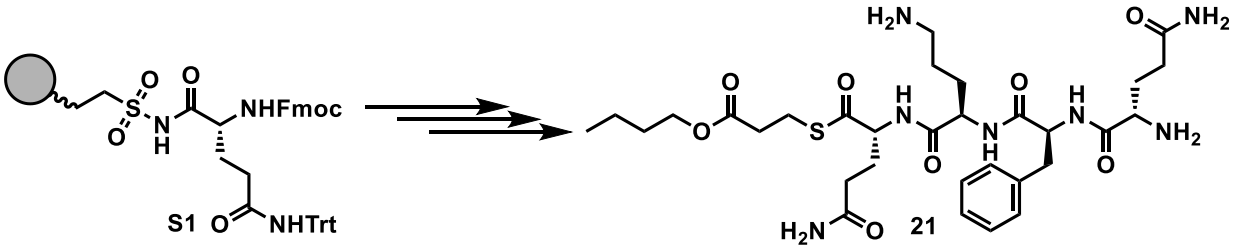
from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (3.5 mg, 8% yield).

Mass spec: expected neutral mass for $C_{37}H_{51}N_7O_7S$ (Da): 737.3571, observed neutral mass (Da): 737.3567, mass error (ppm) 0.5.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The peptide purity was determined to be 98%.



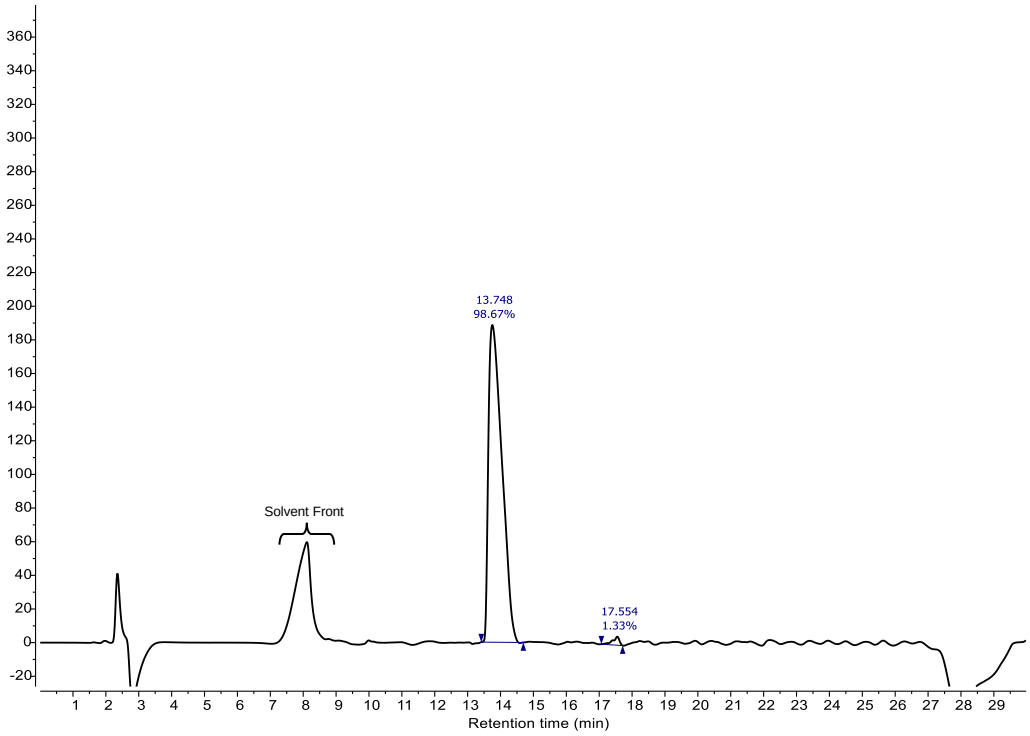
Synthesis of **21**



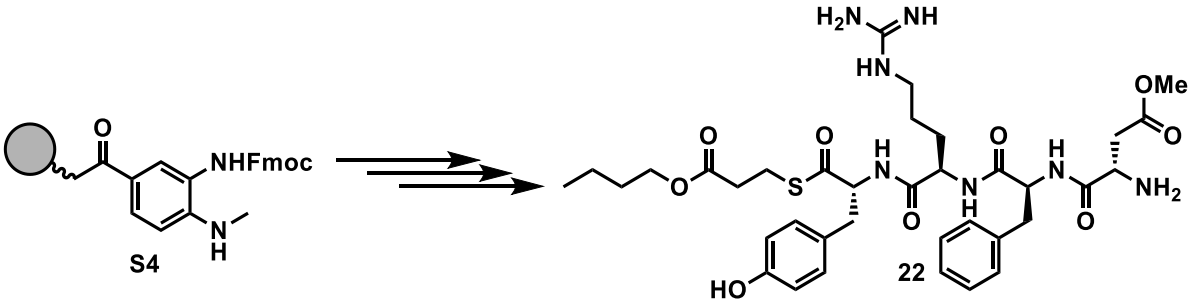
Peptide **21** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids Fmoc-D-Orn(Boc)-OH, Fmoc-L-Phe-OH, Boc-L-Gln(Trt)-OH and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was obtained as an off white sold (28.1 mg ,62% yield) analyzed via RP-HPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 8.79 (d, *J* = 8.4 Hz, 1H), 8.65 (d, *J* = 7.6 Hz, 1H), 8.54 (d, *J* = 8.4 Hz, 1H), 7.99 (s, 5H), 7.43 (s, 1H), 7.36 – 7.07 (m, 6H), 7.00 (s, 1H), 6.81 (s, 1H), 4.65 (q, *J* = 3.7 Hz, 1H), 4.37 (dt, *J* = 37.4, 5.5 Hz, 2H), 4.02 (t, *J* = 6.8 Hz, 2H), 3.77 (d, *J* = 5.5 Hz, 1H), 3.09 – 2.86 (m, 3H), 2.82 (dd, *J* = 195.6, 14.4 Hz, 1H), 2.74 (s, 2H), 2.55 (t, *J* = 6.8 Hz, 2H), 2.33 – 2.18 (m, 2H), 2.15 (s, 2H), 2.00 – 1.81 (m, 3H), 1.78 (q, *J* = 4.7 Hz, 2H), 1.53 (p, *J* = 7.0 Hz, 5H), 1.31 (p, *J* = 7.8 Hz, 2H), 1.10 (s, 1H), 0.88 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (201 MHz, DMSO) δ 200.8, 173.9, 173.4, 171.7, 171.2, 170.9, 168.6, 137.5, 129.3, 128.2, 126.5, 69.8, 63.9, 58.9, 54.5, 51.7, 51.6, 38.5, 37.8, 33.7, 30.8, 30.5, 30.2, 28.8, 27.2, 27.0, 26.9, 23.5, 23.3, 18.6, 13.6.
Mass spec: expected neutral mass for $C_{31}H_{49}N_7O_8S$ (Da): 679.3363, observed neutral mass (Da): 679.3366, mass error (ppm) 0.4.
HPLC Trace Obtained using mobile phases of H2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The peptide purity was determined to be 98%.



Synthesis of **22**



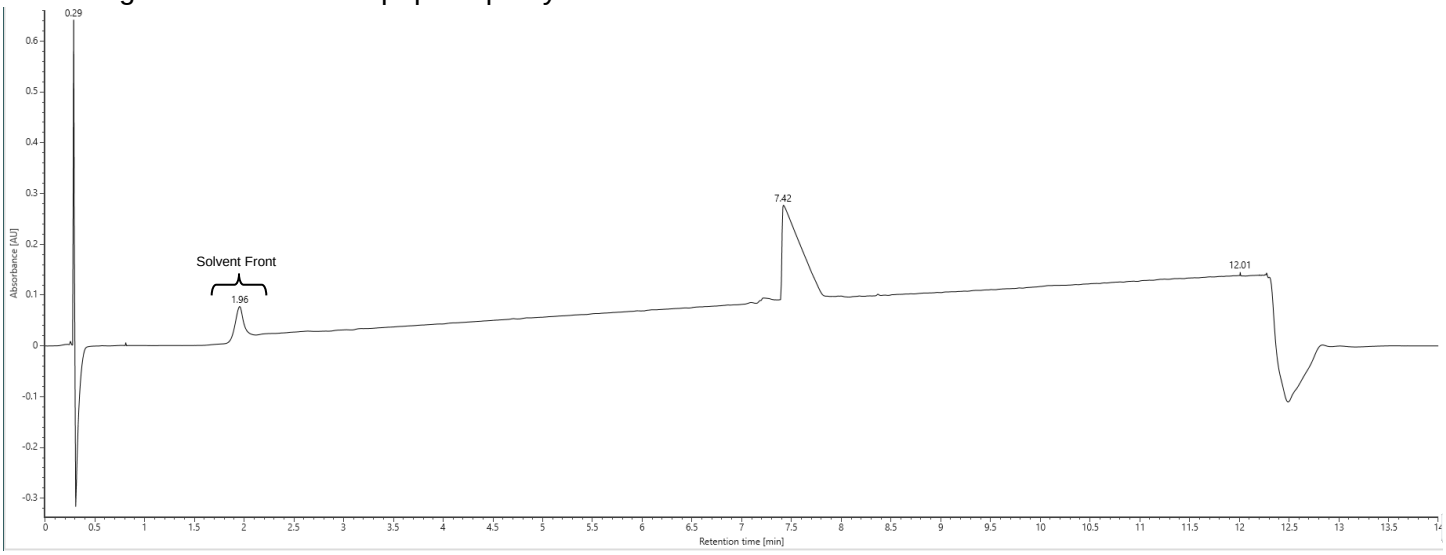
Peptide **22** was synthesized using the NDbz "safety-catch" resin. The synthesis was initiated from Fmoc-MeDbz-OH loaded resin **S4** (0.15 mmol) and involved the following amino acids: Fmoc-D-Tyr(tBu)-OH, Fmoc-D-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Boc-L-Asp(OMe)-OH. Butyl 3-mercaptopropionate was used as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of acetonitrile+0.05% TFA (B) and H_2O+0.05% TFA (A). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 5 minutes after and 1 minute before the gradient to yield an off-white solid (65.2 mg, 44% yield).
^{1}H NMR (800 MHz, DMSO) δ 9.32 (s, 1H), 8.73 (d, *J* = 8.7 Hz, 1H), 8.58 (d, *J* = 7.3 Hz, 1H), 8.30 (d, *J* = 8.8 Hz, 1H), 8.22 (s, 2H), 7.80 (t, *J* = 6.7 Hz, 1H), 7.44 (s, 1H), 7.28 – 7.24 (m, 4H), 7.21 – 7.18 (m, 1H), 7.00 (d, *J* = 8.1 Hz, 2H), 6.66 (d, *J* = 8.8 Hz, 2H), 4.65 (q, *J* = 8.1 Hz, 1H), 4.43 (q, *J* = 8.1 Hz, 1H), 4.32 (h, *J* = 5.1 Hz,

1H), 4.09 (dd, *J* = 4.5, 3.7 Hz, 1H), 4.02 (t, *J* = 6.7 Hz, 2H), 3.61 (s, 3H), 3.12 – 2.84 (m, 7H), 2.84 – 2.75 (m, 3H), 2.52 (t, *J* = 6.7 Hz, 2H), 1.73 – 1.64 (m, 1H), 1.58 (p, *J* = 7.3 Hz, 2H), 1.46 (h, *J* = 9.6 Hz, 1H), 1.36 – 1.21 (m, 4H), 0.88 (t, *J* = 6.7 Hz, 3H).

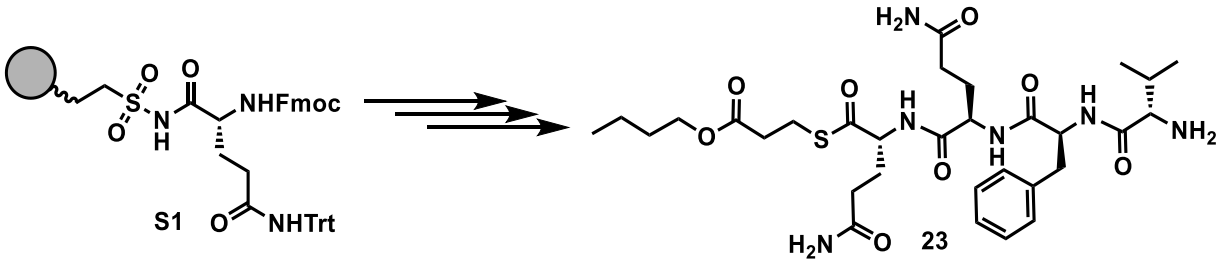
^{13}C NMR (201 MHz, DMSO) δ 200.53, 171.82, 171.53, 170.58, 170.01, 167.86, 157.27, 156.49, 137.57, 130.38, 129.58, 128.52, 127.08, 126.82, 115.48, 64.30, 61.45, 54.80, 52.41, 52.07, 49.04, 40.77, 38.20, 36.58, 35.81, 33.99, 30.51, 29.46, 25.16, 23.68, 18.97, 13.92.

Mass spec: expected neutral mass for $C_{36}H_{51}N_7O_9S$ (Da): 757.3469, observed neutral mass (Da): 757.3480, mass error (ppm) 1.5.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 96%.



Synthesis of **23**

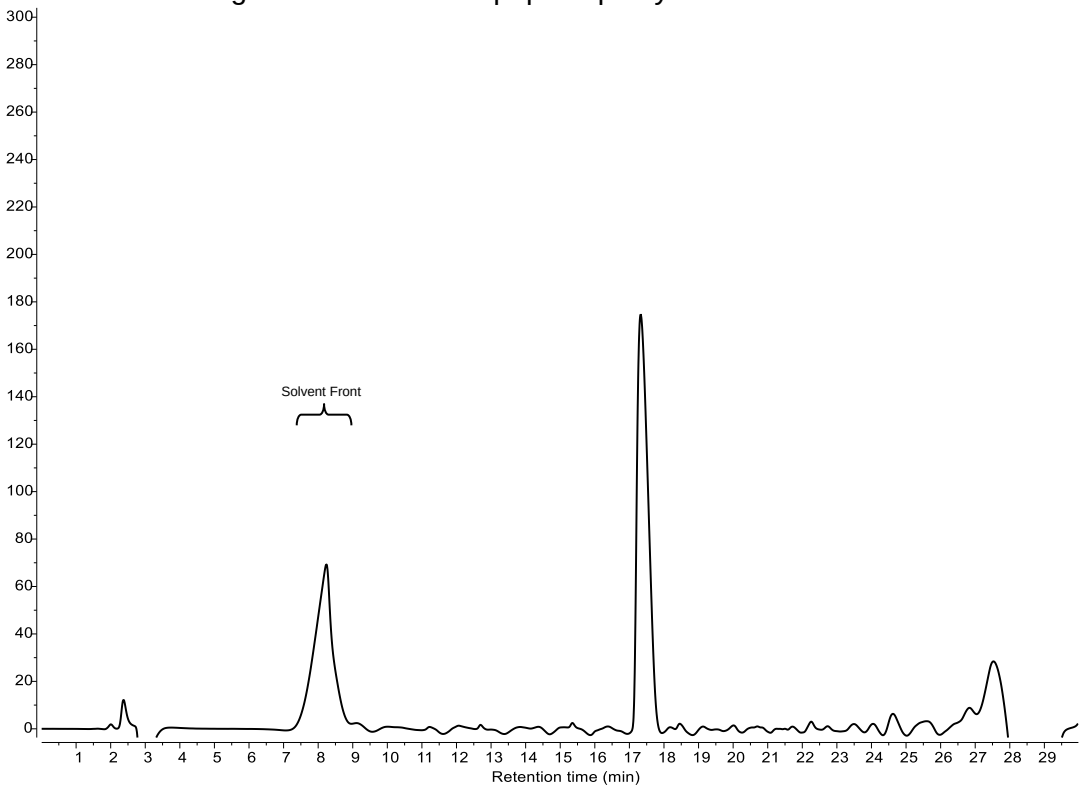


Peptide **23** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids Fmoc-D-Gln(Trt)-OH, Fmoc-L-Phe-OH, Boc-L-Val-OH and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was obtained as an off white sold (18.5 mg, 48% yield) analyzed via RP-HPLC and was used in further enzyme assays without further purification.

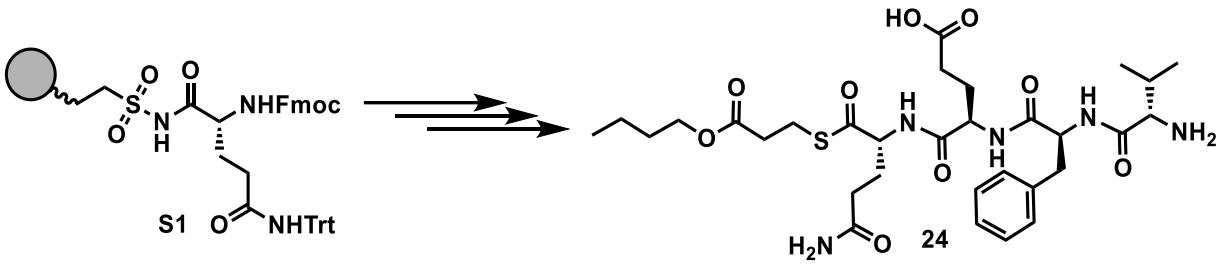
^{1}H NMR (800 MHz, DMSO) δ 8.65 (d, *J* = 7.8 Hz, 1H), 8.53 (d, *J* = 5.6 Hz, 1H), 8.36 (d, *J* = 7.0 Hz, 1H), 7.94 (s, 2H), 7.27 (t, *J* = 7.1 Hz, 5H), 7.16 (d, *J* = 29.0 Hz, 2H), 6.75 (d, *J* = 43.0 Hz, 2H), 4.64 (dd, *J* = 490.4, 4.0 Hz, 1H), 4.29 (s, 2H), 4.01 (t, *J* = 6.2 Hz, 2H), 3.59 (s, 1H), 3.50 (s, 1H), 3.35 (s, 1H), 3.07 – 2.93 (m, 3H), 2.87 (q, *J* = 7.0 Hz, 1H), 2.54 (t, *J* = 5.4 Hz, 2H), 2.20 – 2.02 (m, 3H), 1.94 (t, *J* = 16.4 Hz, 4H), 1.80 (s, 1H), 1.67 (t, *J* = 7.0 Hz, 1H), 1.54 (q, *J* = 7.8 Hz, 2H), 1.31 (p, *J* = 5.4 Hz, 2H), 0.99 – 0.64 (m, 10H).

^{13}C NMR (201 MHz, DMSO) δ 200.8, 173.6, 173.4, 171.7, 171.2, 170.4, 168.2, 137.3, 129.3, 128.2, 126.5, 69.8, 63.9, 58.9, 57.2, 54.4, 52.0, 37.7, 33.7, 31.3, 31.0, 30.1, 30.0, 27.5, 27.1, 23.3, 18.6, 18.5, 17.3, 13.6, 13.6.
Mass spec: expected neutral mass for $C_{31}H_{48}N_6O_8S$ (Da): 664.3254, observed neutral mass (Da): 664.3247, mass error (ppm) 1.1.

HPLC Trace Obtained using mobile phases of H2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient. The crude peptide purity was determined to be >99%



Synthesis of **24**

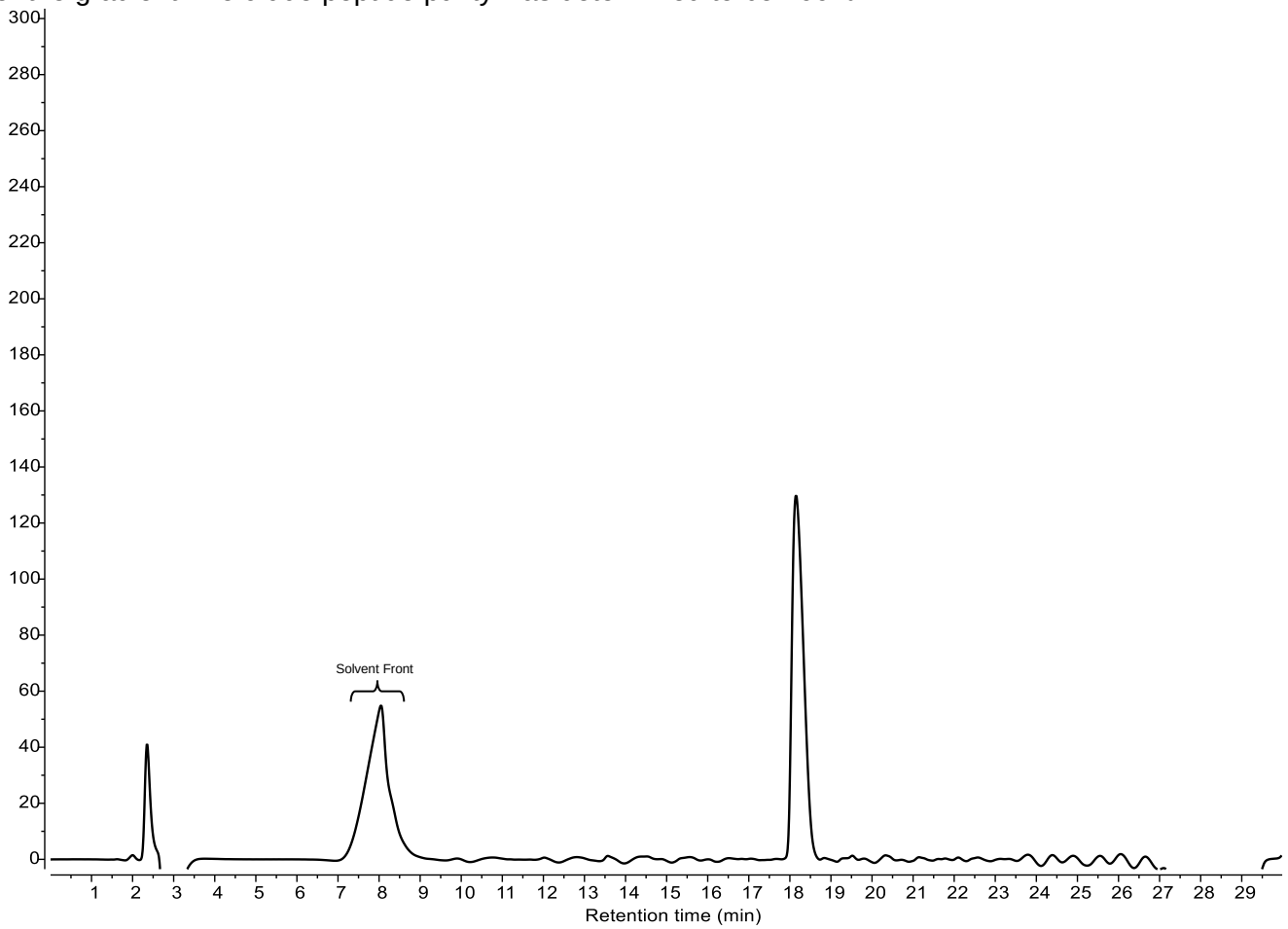


Peptide **24** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids Fmoc-D-Glu(tBu)-OH, Fmoc-L-Phe-OH, Boc-L-Val-OH and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was obtained as an off white sold (11.2 mg, 28% yield) analyzed via RP-HPLC and was used in further enzyme assays without further purification.

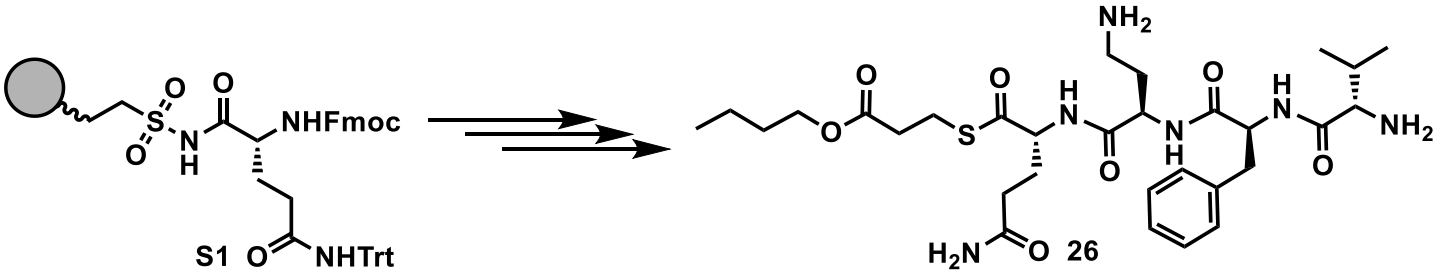
^{1}H NMR (800 MHz, DMSO) δ 8.64 (s, 1H), 8.51 (d, *J* = 48.7 Hz, 2H), 7.26 (d, *J* = 57.6 Hz, 6H), 6.82 (s, 1H), 4.61 (s, 1H), 4.30 (d, *J* = 19.3 Hz, 2H), 4.01 (d, *J* = 3.8 Hz, 2H), 3.55 (d, *J* = 31.7 Hz, 2H), 2.97 (d, *J* = 59.3 Hz, 4H), 2.54 (d, *J* = 3.6 Hz, 2H), 2.13 (s, 2H), 2.03 (s, 3H), 1.93 (s, 2H), 1.81 (s, 1H), 1.69 (s, 1H), 1.52 (t, *J* = 10.2 Hz, 4H), 1.31 (dd, *J* = 334.3, 5.3 Hz, 2H), 0.88 (d, *J* = 30.4 Hz, 9H).
^{13}C NMR (201 MHz, DMSO) δ 201.2, 174.4, 173.8, 171.9, 171.5, 170.7, 169.0, 137.6, 129.6, 128.5, 126.8, 70.2, 64.3, 59.3, 57.8, 54.8, 52.0, 38.0, 34.0, 31.4, 30.5, 30.4, 27.4, 27.2, 23.6, 19.0, 18.9, 17.6, 13.9.

Mass spec: expected neutral mass for $C_{31}H_{47}N_5O_9S$ (Da): 665.3094, observed neutral mass (Da): 665.3090, mass error (ppm) 0.7.

HPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 1 ml/min. The column was equilibrated with 0% mobile phase B for 3 minutes before and 5 minutes after the gradient The crude peptide purity was determined to be >99%



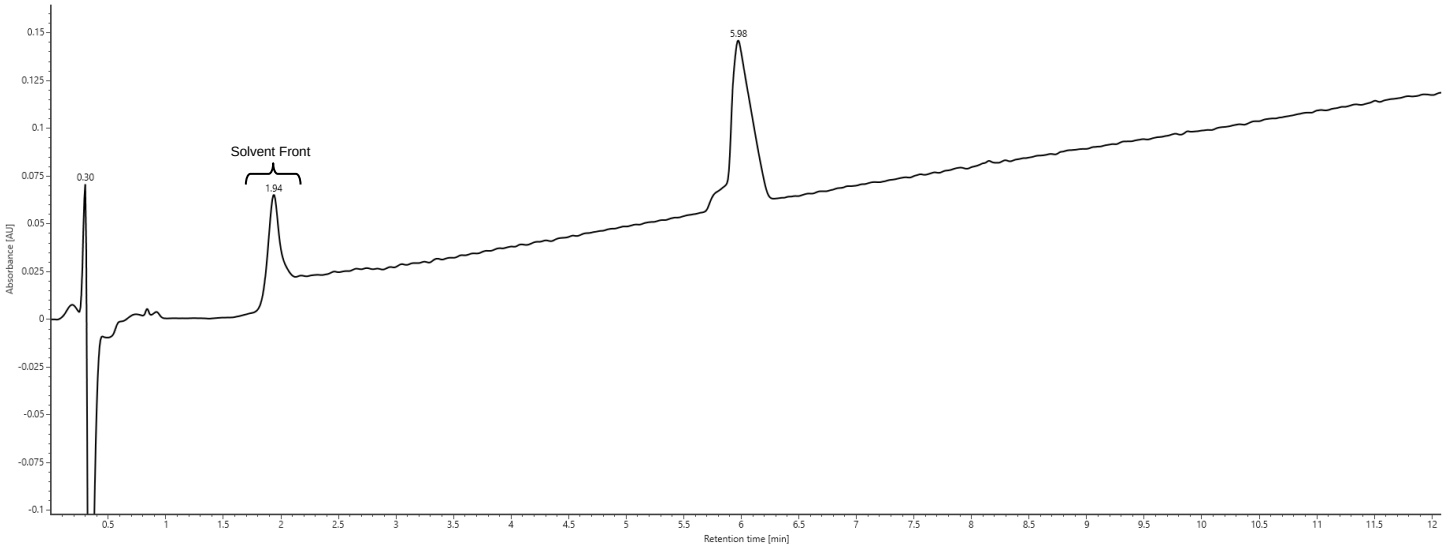
Synthesis of **25**



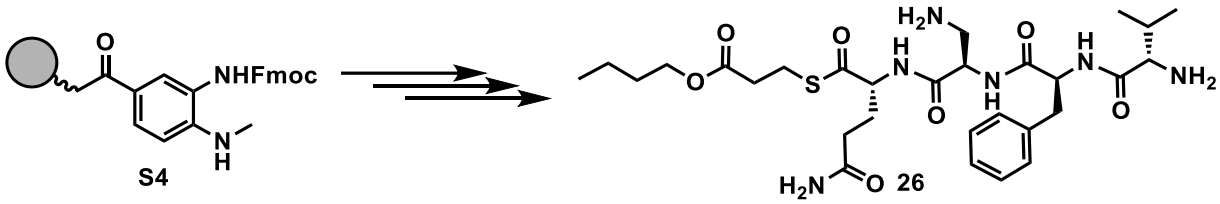
Peptide **25** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids (Fmoc-D-Dab(Boc)-OH, Fmoc-L-Phe-OH, Boc-L-Val-OH) and butyl 3-mercaptopropionate as the cleaving thiol. The obtained crude peptide was obtained as an off white solid (23.3 mg, 54% yield) analyzed via RP-HPLC and was used in further enzyme assays without further purification.

^{1}H NMR (800 MHz, DMSO) δ 8.75 (t, *J* = 6.5 Hz, 1H), 8.56 (t, *J* = 9.4 Hz, 2H), 7.98 (s, 5H), 7.34 – 7.11 (m, 6H), 6.83 (s, 1H), 4.61 (p, *J* = 6.8 Hz, 1H), 4.39 (p, *J* = 4.6 Hz, 1H), 4.29 (p, *J* = 3.6 Hz, 1H), 4.02 (q, *J* = 5.6 Hz, 2H),

3.61 (t, *J* = 2.7 Hz, 1H), 2.97 (d, *J* = 3.9 Hz, 3H), 2.91 – 2.82 (m, 1H), 2.66 – 2.57 (m, 1H), 2.57 – 2.51 (m, 2H), 2.21 – 2.08 (m, 2H), 2.06 (h, *J* = 4.8 Hz, 1H), 2.02 – 1.88 (m, 2H), 1.87 – 1.78 (m, 1H), 1.78 – 1.68 (m, 1H), 1.56 – 1.41 (m, 3H), 1.31 (h, *J* = 6.5 Hz, 2H), 1.06 (d, *J* = 3.9 Hz, 1H), 0.91 (t, *J* = 5.8 Hz, 2H), 0.88 (t, *J* = 6.8 Hz, 6H).
^{13}C NMR (201 MHz, DMSO) δ 200.5, 173.4, 171.1, 170.9, 170.7, 168.3, 137.1, 129.2, 128.2, 126.6, 64.0, 59.1, 57.2, 54.5, 50.0, 48.7, 37.5, 36.0, 33.6, 31.0, 30.1, 30.0, 29.6, 27.1, 26.8, 23.2, 18.6, 18.4, 17.4, 13.6.
Mass spec: expected neutral mass for $C_{30}H_{48}N_6O_7S$ (Da): 636.3305, observed neutral mass (Da): 636.3310, mass error (ppm) 0.8.
UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 99%.



Synthesis of **26**

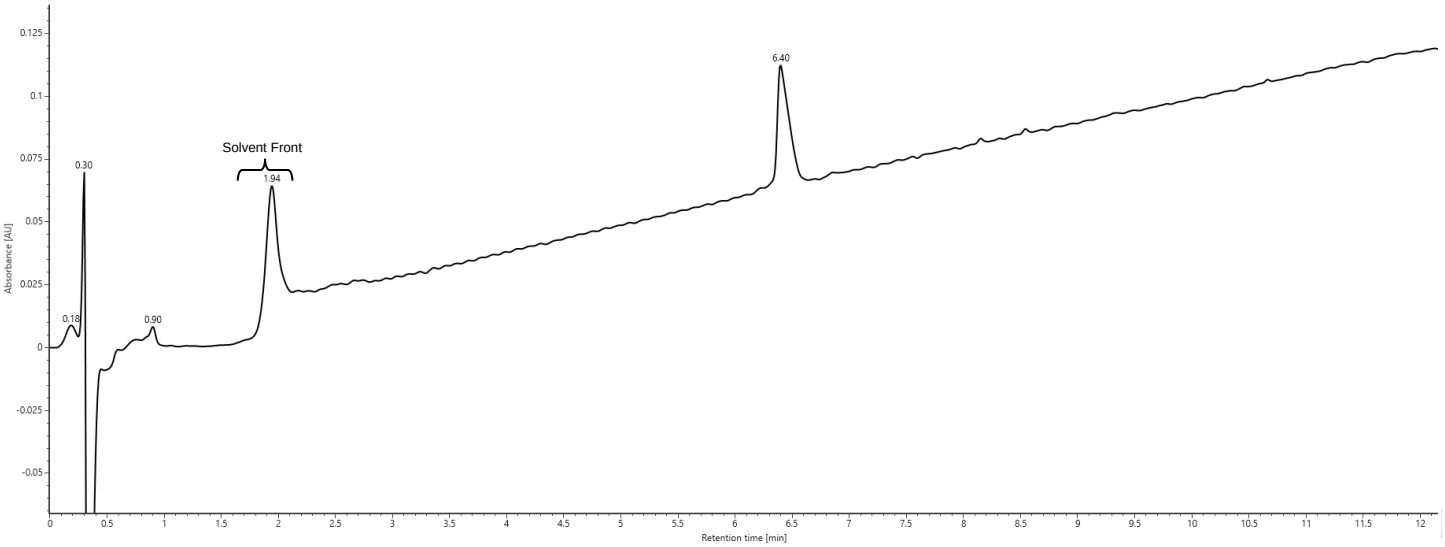


Peptide **26** was synthesized using the NDbz "safety-catch" resin. The synthesis was initiated from Fmoc-MeDbz-OH loaded resin **S4** (0.1 mmol) and involved the following amino acids: Fmoc-D-Gln(Trt)-OH, Fmoc-D-Dap(Boc)-OH, Fmoc-L-Phe-OH, and Boc-L-Val-OH. Butyl 3-mercaptopropionate was used as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (16.3 mg, 19% yield).

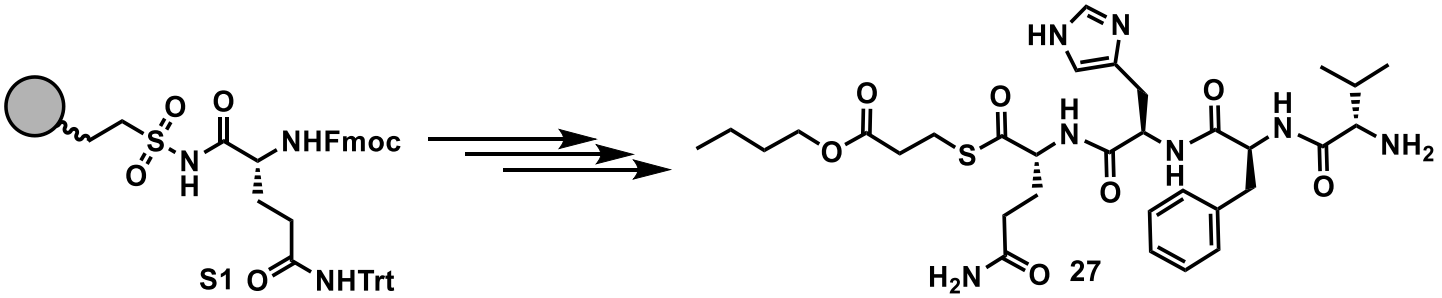
^{1}H NMR (800 MHz, DMSO) δ 8.87 (d, *J* = 6.6 Hz, 2H), 8.40 (d, *J* = 7.1 Hz, 1H), 8.08 (s, 6H), 7.34 – 7.25 (m, 4H), 7.22 (dd, *J* = 2056.0, 7.1 Hz, 1H), 6.89 (s, 1H), 4.63 (p, *J* = 6.1 Hz, 1H), 4.49 (h, *J* = 4.8 Hz, 1H), 4.32 – 4.21 (m, 1H), 4.02 (t, *J* = 6.6 Hz, 2H), 3.60 (s, 1H), 3.23 (dd, *J* = 5.2, 3.3 Hz, 2H), 3.01 – 2.90 (m, 2H), 2.90 – 2.75 (m, 2H), 2.60 – 2.52 (m, 1H), 2.24 – 1.81 (m, 5H), 1.53 (p, *J* = 8.7 Hz, 2H), 1.31 (h, *J* = 7.9 Hz, 2H), 0.95 – 0.68 (m, 9H).
^{13}C NMR (201 MHz, DMSO) δ 200.77, 174.16, 171.85, 171.60, 170.00, 169.25, 137.91, 129.55, 128.67, 126.95, 70.20, 64.39, 59.62, 57.47, 55.63, 50.52, 39.96, 36.69, 34.05, 31.40, 30.57, 30.47, 27.43, 23.67, 19.04, 18.86, 17.89, 13.99

Mass spec: expected neutral mass for $C_{29}H_{46}N_6O_7S$ (Da): 622.3148, observed neutral mass (Da): 622.3152, mass error (ppm) 0.5.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 96%.



Synthesis of **27**



The synthesis of Peptide **27** was initiated from **S1** (0.05 mmol) using the following amino acids in the order of Fmoc-D-His(Trt)-OH, Fmoc-L-Phe-OH, and Boc-L-Val-OH, with butyl 3-mercaptopropionate used as the cleaving thiol, following the general protocol outlined above for the sulfonamide 'safety-catch' resin The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (12.1 mg, 27%).

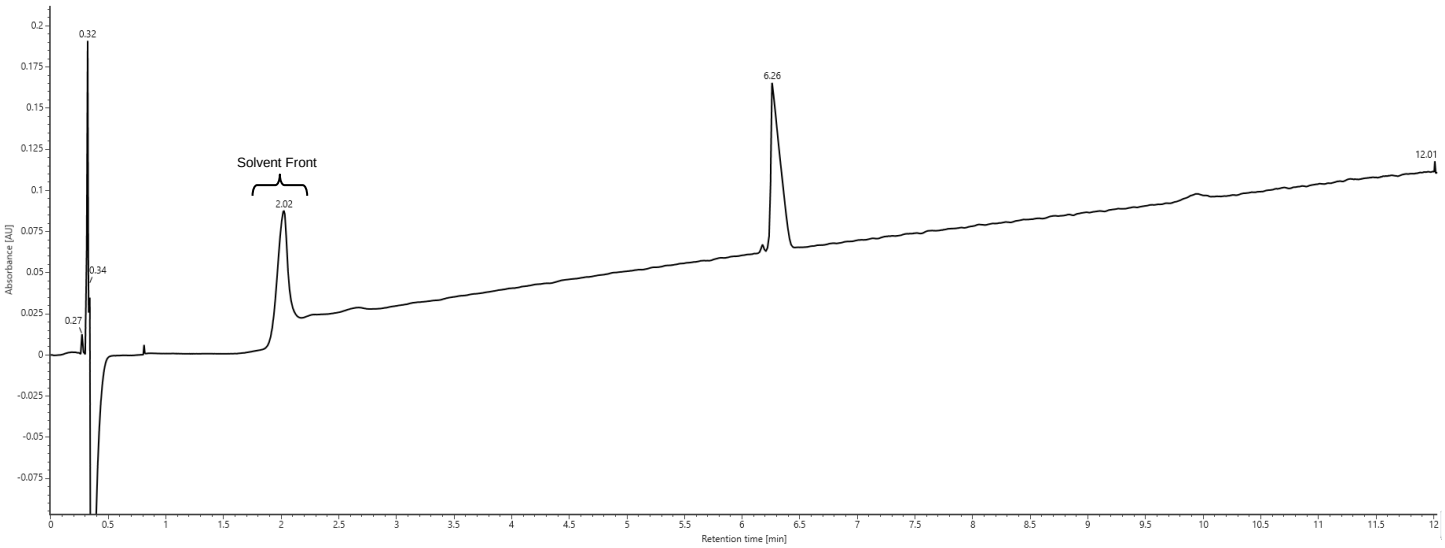
^{1}H NMR (800 MHz, DMSO) δ 8.96 (s, 1H), 8.65 (dd, *J* = 42.6, 6.3 Hz, 3H), 8.05 (s, 2H), 7.39 – 7.05 (m, 7H), 6.83 (s, 1H), 4.64 (dt, *J* = 66.4, 5.7 Hz, 2H), 4.30 (s, 1H), 4.01 (t, *J* = 6.2 Hz, 2H), 3.59 (s, 1H), 3.13 (d, *J* = 13.7 Hz, 1H), 2.95 (s, 2H), 2.84 (q, *J* = 7.4 Hz, 2H), 2.73 (t, *J* = 8.1 Hz, 1H), 2.54 (s, 4H), 2.23 – 2.00 (m, 3H), 2.00 –

1.94 (m, 1H), 1.91 – 1.73 (m, 1H), 1.49 (t, *J* = 7.4 Hz, 2H), 1.31 (q, *J* = 6.2 Hz, 2H), 0.90 (d, *J* = 5.7 Hz, 3H), 0.87 (s, 6H).

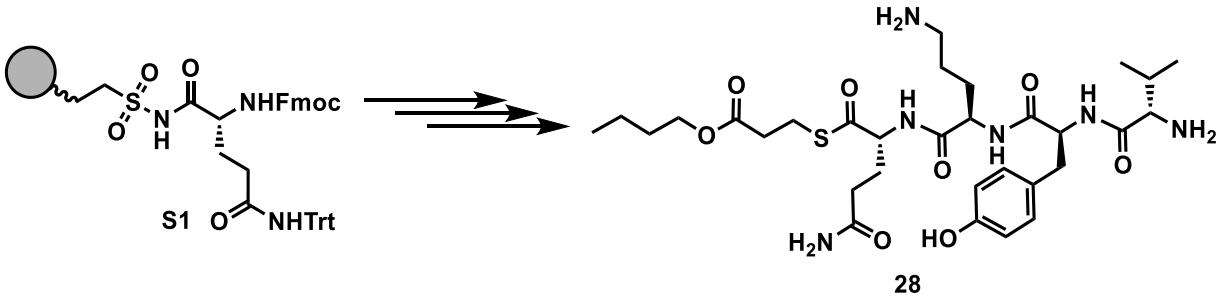
^{13}C NMR (201 MHz, DMSO) δ 200.84, 173.72, 171.51, 171.00, 170.69, 168.50, 137.47, 134.18, 129.51 (d, *J* = 16.1 Hz), 128.47, 126.84, 117.26, 64.30, 59.44, 57.43, 54.69, 51.58, 40.81, 37.71, 33.95, 31.29, 30.49, 30.33, 27.40, 27.15, 23.60, 18.95, 18.76, 17.71, 13.90.

Mass spec: expected neutral mass for $C_{32}H_{47}N_7O_7S$ (Da): 673.3257, observed neutral mass (Da): 673.3257, mass error (ppm) < 0.0.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 98%.



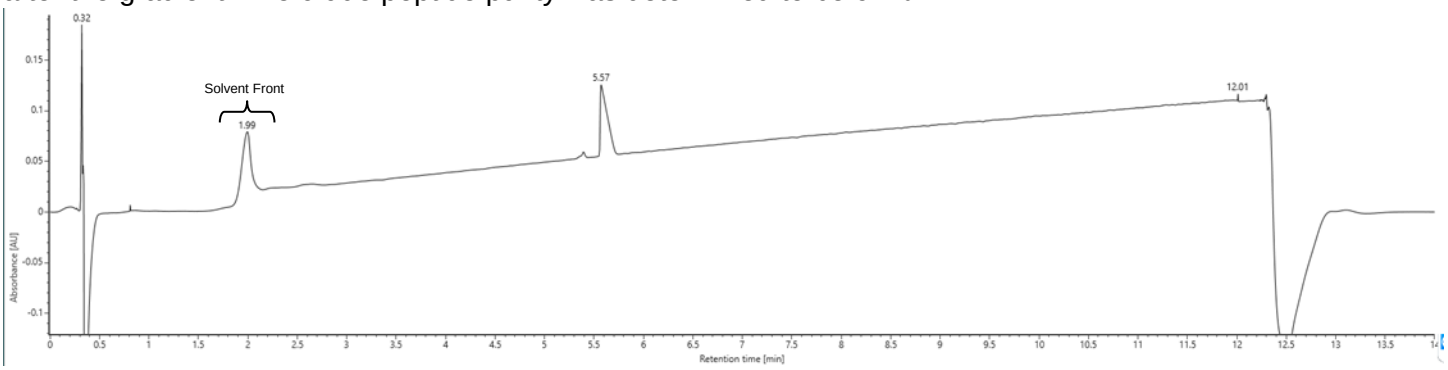
Synthesis of **28**



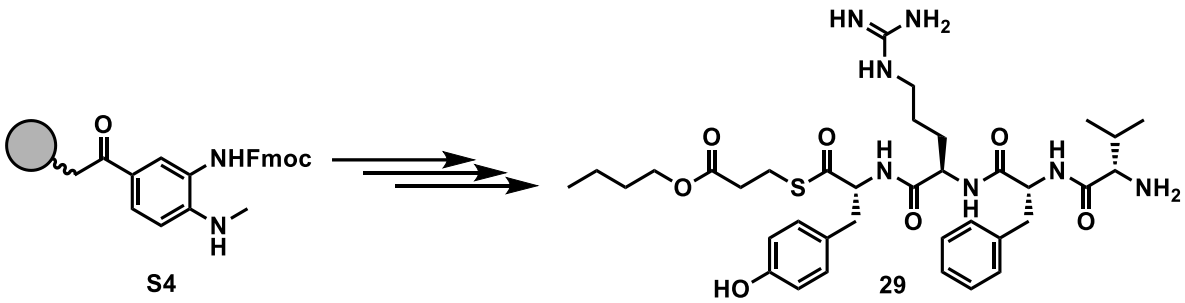
Peptide **28** was synthesized following the general protocol outlined above for the sulfonamide “safety-catch” resin. The synthesis was initiated from **S1** (0.05 mmol) using the following amino acids Fmoc-D-Orn(Boc)-OH, Fmoc-L-Tyr(tBu)-OH, Boc-L-Val-OH and butyl 3-mercaptopropionate as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of H_2O+0.05% TFA (A) and acetonitrile+0.05% TFA (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 5 minutes after the gradient to yield an off-white solid (13.0 mg, 29% yield).

^{1}H NMR (800 MHz, DMSO) δ 9.32 (s, 1H), 8.60 (d, *J* = 6.5 Hz, 1H), 8.52 (d, *J* = 4.7 Hz, 1H), 8.39 (d, *J* = 7.3 Hz, 1H), 8.04 (s, 3H), 7.83 (s, 3H), 7.26 (s, 1H), 7.08 (d, *J* = 7.1 Hz, 2H), 6.82 (s, 1H), 6.67 (d, *J* = 4.7 Hz, 2H), 4.58 (s, 1H), 4.31 (d, *J* = 42.2 Hz, 2H), 4.02 (s, 2H), 3.60 (s, 1H), 2.97 (s, 2H), 2.87 (d, *J* = 12.8 Hz, 1H), 2.54

(d, *J* = 33.4 Hz, 7H), 2.13 (d, *J* = 46.2 Hz, 3H), 1.94 (s, 1H), 1.80 (d, *J* = 39.9 Hz, 2H), 1.54 (s, 2H), 1.51 – 1.37 (m, 3H), 1.32 (d, *J* = 4.7 Hz, 2H), 1.01 – 0.60 (m, 9H).
^{13}C NMR (201 MHz, DMSO) δ 201.09, 173.72, 171.95, 171.56, 171.10, 168.41, 156.37, 130.58, 127.67, 115.34, 64.29, 59.28, 57.44, 55.11, 51.74, 40.81, 38.86, 37.31, 34.01, 31.34, 30.50, 30.36, 27.49, 23.78, 23.60, 18.97, 18.79, 17.68, 13.93.
Mass spec: expected neutral mass for $C_{31}H_{50}N_6O_8S$ (Da): 666.4311, observed neutral mass (Da): 666.3410, mass error (ppm) 0.1.
UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The crude peptide purity was determined to be 97%.

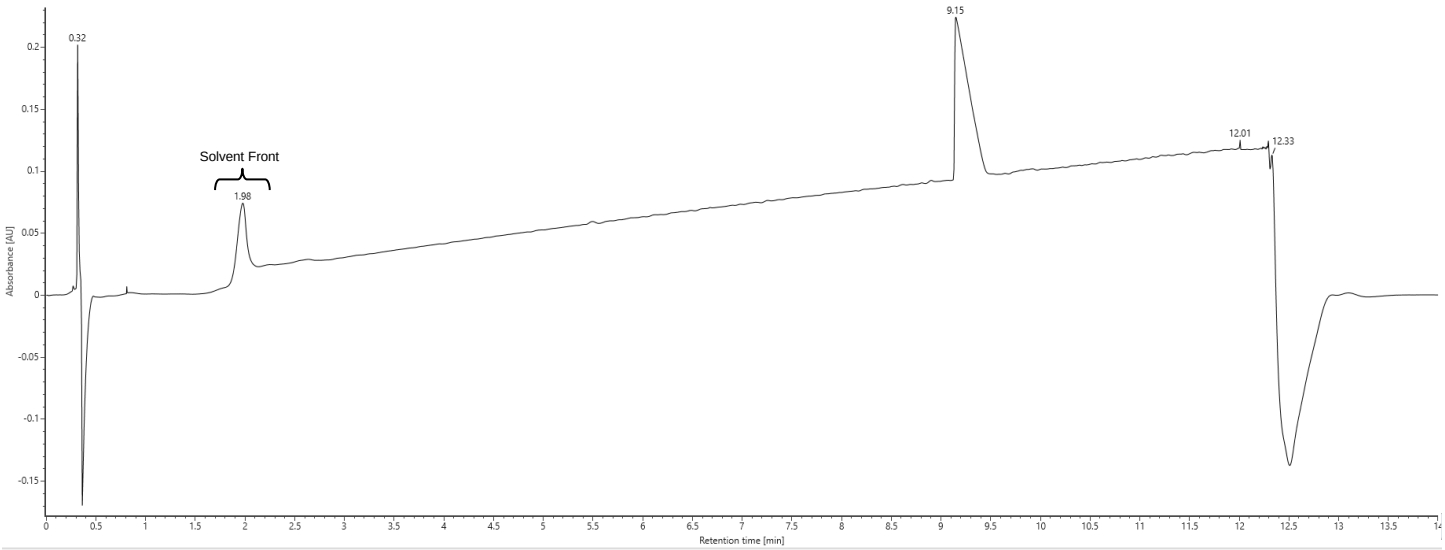


Synthesis of **29**

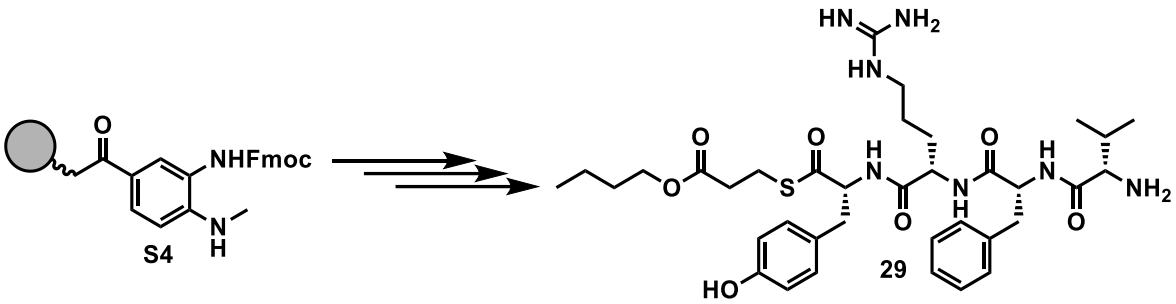


Peptide **29** was synthesized using the NDbz "safety-catch" resin. The synthesis was initiated from Fmoc-MeDbz-OH loaded resin **S4** (0.15 mmol) and involved the following amino acids: Fmoc-D-Tyr(tBu)-OH, Fmoc-D-Arg(Pbf)-OH, Fmoc-D-Phe-OH, Boc-L-Val-OH. Butyl 3-mercaptopropionate was used as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of acetonitrile+0.05% trifluoroacetic Acid (B) and H2O+0.05% trifluoroacetic Acid (A). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 mL/min. The column was equilibrated with 0% mobile phase B for 5 minutes after and 1 minute before the gradient to yield an off-white solid (42.4 mg, 30% yield).
^{1}H NMR (800 MHz, DMSO) δ 8.65 (q, *J* = 7.7 Hz, 2H), 8.49 (d, *J* = 7.7 Hz, 1H), 7.94 (d, *J* = 3.6 Hz, 3H), 7.90 (t, *J* = 5.4 Hz, 1H), 7.48 (s, 1H), 7.26 (d, *J* = 8.0 Hz, 2H), 7.23 (t, *J* = 8.0 Hz, 2H), 7.15 (t, *J* = 8.0 Hz, 2H), 7.01 (d, *J* = 8.1 Hz, 2H), 6.65 (d, *J* = 8.4 Hz, 2H), 4.78 (td, *J* = 5.7, 3.9 Hz, 1H), 4.52 – 4.47 (m, 1H), 4.36 (q, *J* = 8.1 Hz, 1H), 4.02 (t, *J* = 6.5 Hz, 2H), 3.59 (t, *J* = 5.4 Hz, 1H), 3.17 – 3.08 (m, 2H), 3.06 (dd, *J* = 3.1, 2.7 Hz, 1H), 3.02 – 2.93 (m, 3H), 2.81 (q, *J* = 9.5 Hz, 1H), 2.66 (t, *J* = 11.9 Hz, 1H), 2.54 (td, *J* = 3.1, 2.7 Hz, 4H), 1.81 (h, *J* = 5.7 Hz, 1H), 1.78 – 1.70 (m, 1H), 1.60 – 1.44 (m, 5H), 1.31 (h, *J* = 6.9 Hz, 2H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.65 (d, *J* = 6.9 Hz, 3H), 0.39 (d, *J* = 6.9 Hz, 3H).
^{13}C NMR (201 MHz, DMSO) δ 200.53, 171.95, 171.53, 171.26, 167.90, 157.31, 156.46, 137.88, 130.36, 129.55, 128.37, 127.14, 126.69, 115.44, 64.30, 61.25, 57.54, 54.04, 52.58, 40.81, 38.62, 36.44, 33.99, 30.50, 30.01, 29.47, 25.58, 23.74, 18.96, 18.55, 16.72, 13.92.
Mass spec: expected neutral mass for $C_{36}H_{53}N_7O_7S$ (Da): 727.3727, observed neutral mass (Da): 727.3740, mass error (ppm) 1.8.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 mL/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The peptides purity was determined to be 98%.



Synthesis of **30**



Peptide **30** was synthesized using the NDbz "safety-catch" resin. The synthesis was initiated from Fmoc-MeDbz-OH loaded resin **S4** (0.15 mmol) and involved the following amino acids: Fmoc-D-Tyr(tBu)-OH, Fmoc-L-Arg(Pbf)-OH, Fmoc-D-Phe-OH, Boc-L-Val-OH. Butyl 3-mercaptopropionate was used as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of acetonitrile+0.05% TFA (B) and H_2O+0.05% TFA (A). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 ml/min. The column was equilibrated with 0% mobile phase B for 5 minutes after and 1 minute before the gradient to yield an off-white solid (49.1 mg, 34% yield).

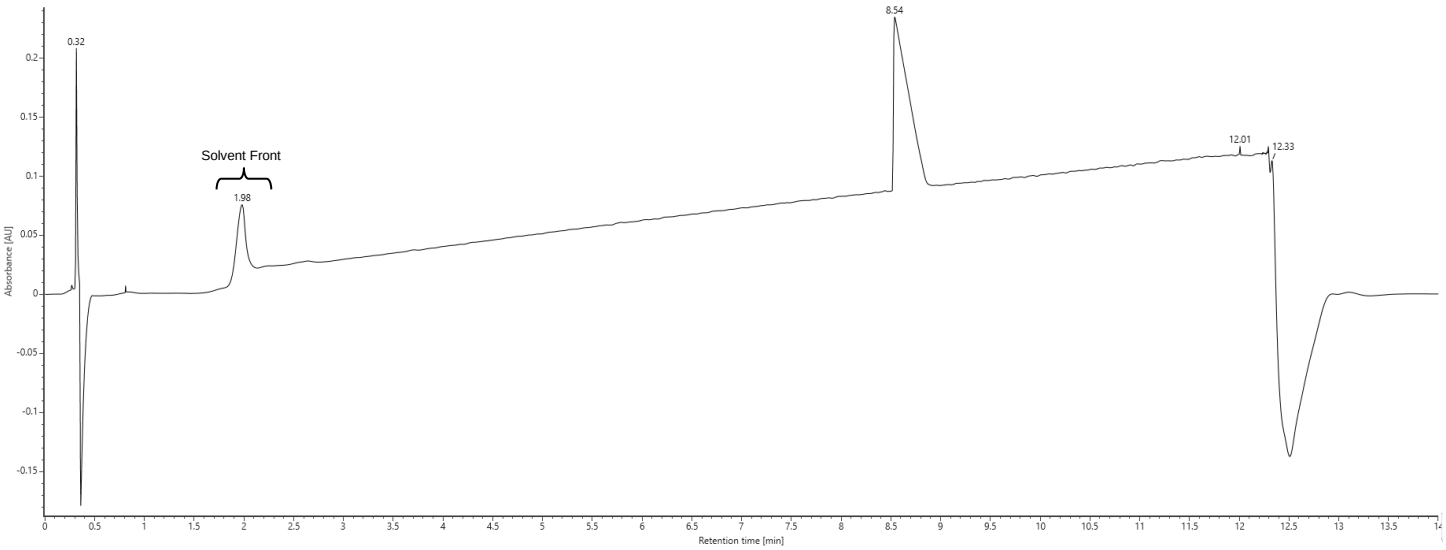
^{1}H NMR (800 MHz, DMSO) δ 8.71 (t, *J* = 8.8 Hz, 2H), 8.45 (d, *J* = 8.4 Hz, 1H), 7.94 (d, *J* = 4.2 Hz, 3H), 7.73 (t, *J* = 6.0 Hz, 1H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.23 (dd, *J* = 39.7, 7.3 Hz, 2H), 7.16 (t, *J* = 7.7 Hz, 1H), 7.01 (d, *J* = 8.3 Hz, 2H), 6.66 (d, *J* = 8.4 Hz, 2H), 4.93 – 4.86 (m, 1H), 4.54 – 4.49 (m, 1H), 4.40 (h, *J* = 4.5 Hz, 1H), 4.03 (t, *J* = 6.3 Hz, 2H), 3.59 (t, *J* = 5.2 Hz, 1H), 3.04 – 2.91 (m, 6H), 2.70 (t, *J* = 10.5 Hz, 2H), 2.61 – 2.52 (m, 2H), 1.81 (h, *J* = 2.4 Hz, 1H), 1.55 (p, *J* = 6.9 Hz, 2H), 1.43 – 1.37 (m, 1H), 1.32 (h, *J* = 7.7 Hz, 2H), 1.29 – 1.23 (m, 1H), 1.18 (p, *J* = 6.6 Hz, 2H), 0.88 (t, *J* = 6.9 Hz, 3H), 0.67 (d, *J* = 7.7 Hz, 3H), 0.42 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (201 MHz, DMSO) δ 200.35, 171.85, 171.59, 170.92, 167.92, 157.25, 156.52, 137.64, 130.54, 129.63, 128.36, 127.07, 126.73, 115.43, 64.35, 61.19, 57.56, 53.95, 51.88, 40.63, 39.29, 36.83, 33.94, 30.51, 30.13, 29.99, 24.96, 23.82, 18.98, 18.64, 16.82, 13.93.

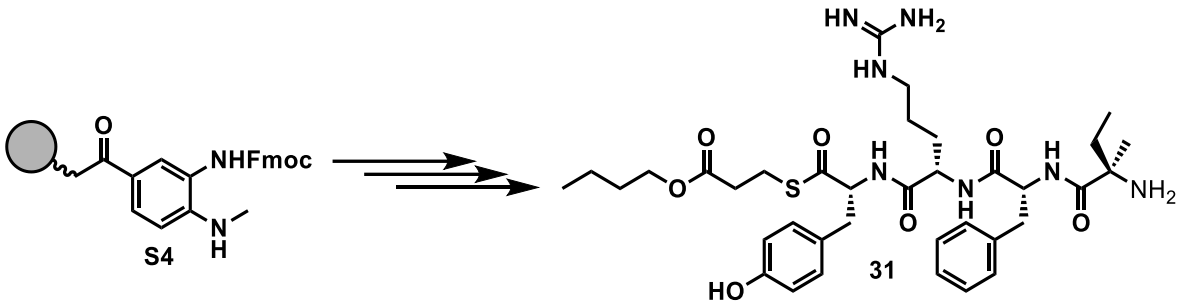
Mass spec: expected neutral mass for $C_{36}H_{53}N_7O_7S$ (Da): 727.3727, observed neutral mass (Da): 727.3733, mass error (ppm) 0.9.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a

flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The peptide purity was determined to be 99%.



Synthesis of **31**



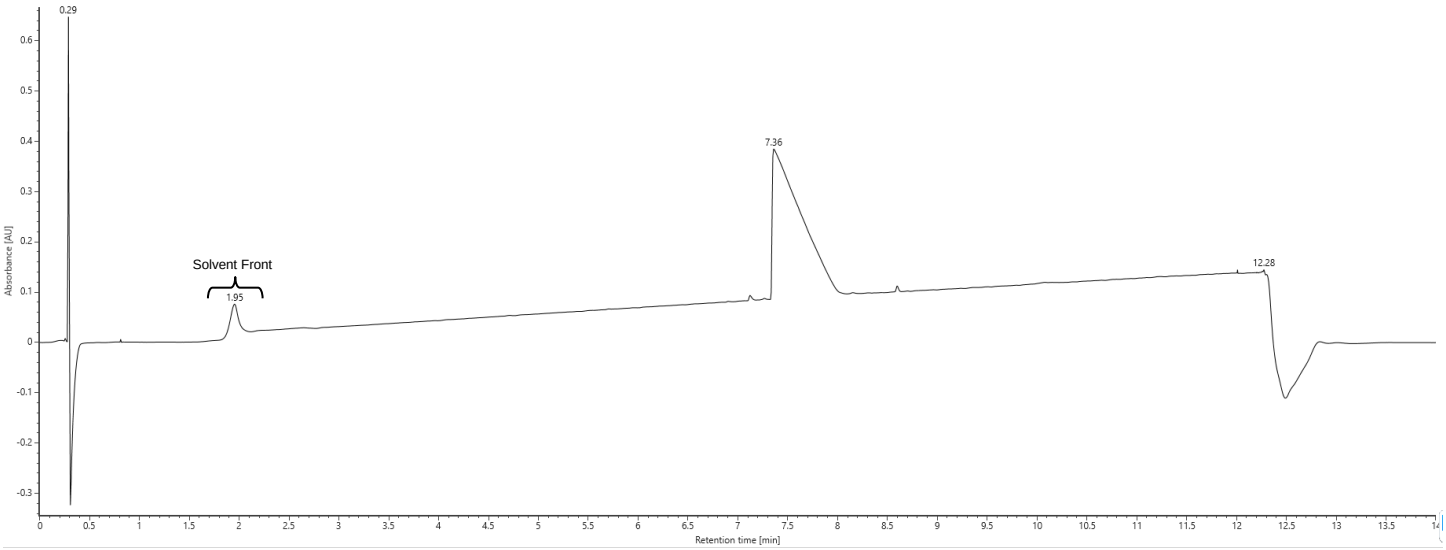
Peptide **31** was synthesized using the NDbz "safety-catch" resin. The synthesis was initiated from Fmoc-MeDbz-OH loaded resin **S4** (0.15 mmol) and involved the following amino acids: Fmoc-D-Tyr(tBu)-OH, Fmoc-D-Arg(Pbf)-OH, Fmoc-L-Phe-OH, Boc-D-isoVal-OH. Butyl 3-mercaptopropionate was used as the cleaving thiol. The crude peptide was purified via reverse-phase semi-preparative HPLC using mobile phases of acetonitrile+0.05% TFA (B) and H_2O+0.05% TFA (A). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 50% over 22 minutes at a flow rate of 20 ml/min. The column was equilibrated with 0% mobile phase B for 5 minutes after and 1 minute before the gradient to yield an off-white solid (65.7 mg, 46% yield).

^{1}H NMR (800 MHz, DMSO) δ 9.35 (s, 1H), 8.76 (d, *J* = 7.2 Hz, 1H), 8.39 (dd, *J* = 9.7, 9.1 Hz, 2H), 7.98 – 7.88 (m, 4H), 7.47 (s, 1H), 7.32 (d, *J* = 7.2 Hz, 2H), 7.23 (t, *J* = 7.2 Hz, 3H), 7.16 (t, *J* = 7.9 Hz, 1H), 7.02 (d, *J* = 7.9 Hz, 2H), 6.65 (d, *J* = 9.1 Hz, 2H), 4.80 (td, *J* = 3.9, 3.9 Hz, 1H), 4.49 – 4.42 (m, 2H), 4.03 (t, *J* = 6.5 Hz, 2H), 3.13 – 3.03 (m, 3H), 2.98 (t, *J* = 7.2 Hz, 2H), 2.92 (dd, *J* = 5.9, 5.2 Hz, 1H), 2.80 (h, *J* = 7.2 Hz, 2H), 2.53 (t, *J* = 6.5 Hz, 2H), 1.79 – 1.73 (m, 1H), 1.67 (h, *J* = 7.9 Hz, 1H), 1.52 (h, *J* = 6.5 Hz, 4H), 1.50 – 1.40 (m, 5H), 1.32 (h, *J* = 7.9 Hz, 2H), 0.88 (t, *J* = 7.9 Hz, 3H), 0.24 (t, *J* = 6.5 Hz, 3H).

^{13}C NMR (201 MHz, DMSO) δ 200.62, 171.94, 171.53, 171.19, 170.68, 157.33, 156.52, 138.22, 130.39, 129.59, 128.37, 127.08, 126.65, 115.51, 64.31, 61.46, 60.47, 54.73, 51.86, 40.78, 37.96, 36.57, 34.01, 30.51, 30.30, 29.93, 25.28, 23.70, 22.63, 18.97, 13.92, 7.45.

Mass spec: expected neutral mass for $C_{36}H_{53}N_7O_7S$ (Da): 727.3727, observed neutral mass (Da): 727.3774, mass error (ppm) 6.4.

UPLC Trace Obtained using mobile phases of H_2O+0.1% formic acid (A) and acetonitrile+0.1% formic acid (B). Samples were eluted using a gradient mode with mobile phase B ranging from 0% to 40% over 11 minutes at a flow rate of 0.5 ml/min. The column was equilibrated with 0% mobile phase B for 1 minute before and 2 minutes after the gradient. The peptide purity was determined to be 97%.


0.29
7.36
Solvent Front
1.95
12.28
Absorbance [AU]
Retention time [min]

Supplementary Table 1: Ulm16 total turnover assays. Enzymatic reactions were conducted in 20 mM Tris at pH 8.0 with 5% DMSO for 4 hours at 30°C using various concentrations of peptides and Ulm16 as indicated by [Peptide] and [Ulm16], respectively. The areas of the enzymatic products and starting material were determined by analyzing the UPLC UV Trace at 214 nm. Total turnover numbers were calculated using a formula outlined in the methods section. Red numbers indicate substrates that were neither cyclized nor hydrolyzed.

Substrate	[Ulm16]	[Peptide]	Area Cyclic Peptide	Area Peptide Thioester	Area Hydrolyzed Peptide	Area Hydrolyzed Dimer	Area Cyclic Dimer	Sum	TTN	Cyc/SM	Cyc/H
2	14 nM	320 µM	2684660	37204	*			2721764	22545	72	>20:1
6	17 nM	320 µM	855364	891865	30967			1778196	9055	1.0	27.6
11	70 nM	280 µM	903688	927094	*			1830782	1974	1.0	>20:1
12-SNAC	70 nM	280 µM	1614745	512012	*			2126758	3037	3.2	>20:1
12-SMMP	70 nM	320 µM	2242929	61585	*			2304514	4449	36	>20:1
13	17 nM	320 µM	2133202	97416	78504			2309122	17389	21.9	27.2
14	70 nM	280 µM	584777	138590	9594			732961	3191	4.2	61
15	70 nM	280 µM	620130	56328	2801			679259	3652	11	221.4
16	11.5 nM	280 µM	1922450	320576	45581	15733	9476	2313817	34679	6.0	42.2
17	45 nM	280 µM	426879	176018	73213			676110	3929	2.4	5.8
18	70 nM	280 µM	439897	7240	5536			452673	3887	60.8	79.5
19	70 nM	280 µM									
20	70 nM	280 µM	1214431	14538	79313			1308282	3713	83.5	15.3
21	70 nM	280 µM	54373	138754	53697			246824	881	0.4	1.0
22	17 nM	320 µM	697840	227450	53826			979116	13416	3.1	13.0
23	70 nM	280 µM	175012	21341	29731			226084	3096	8.2	5.9
24	70 nM	280 µM	357393	277874	39241			674508	2119	1.3	9.1
25	70 nM	280 µM	346723	32901	21140			400764	3461	10.5	16.4
26	70 nM	280 µM	243137	67031	112373			422541	2302	3.6	2.2
27	70 nM	280 µM	142639	404619	86311			633569	1401	0.4	1.7
28	45 nM	280 µM	138785	296882	N.D			435668	1982	0.5	-
29	17 nM	320 µM	1078614	444170	86863	28754	0	1638401	12392	2.4	12.4
30	17 nM	320 µM	178195	270391	262105	57793	7509	775992	4323	0.7	0.7
31	17 nM	320 µM									

Supplementary Table 2: Ulm16 Mutant total turnover assays. Enzymatic reactions were conducted in 20 mM Tris at pH 8.0 with 5% DMSO for 4 hours at 30°C using various concentrations of peptides and mutated Ulm16 as indicated by [Peptide] and [Ulm16], respectively. The areas of the enzymatic products and starting material were determined by analyzing the UPLC UV Trace at 214 nm. Total turnover numbers were calculated using a formula outlined in the methods section. In some cases, Tris from the reaction buffer was conjugated to the peptide and is indicated by 'Area Tris Peptide'. For Ulm16 R431A peptides 14 and 29 did not cyclize and the hydrolyzed peptide was used for TTN and are indicated with an asterisk.

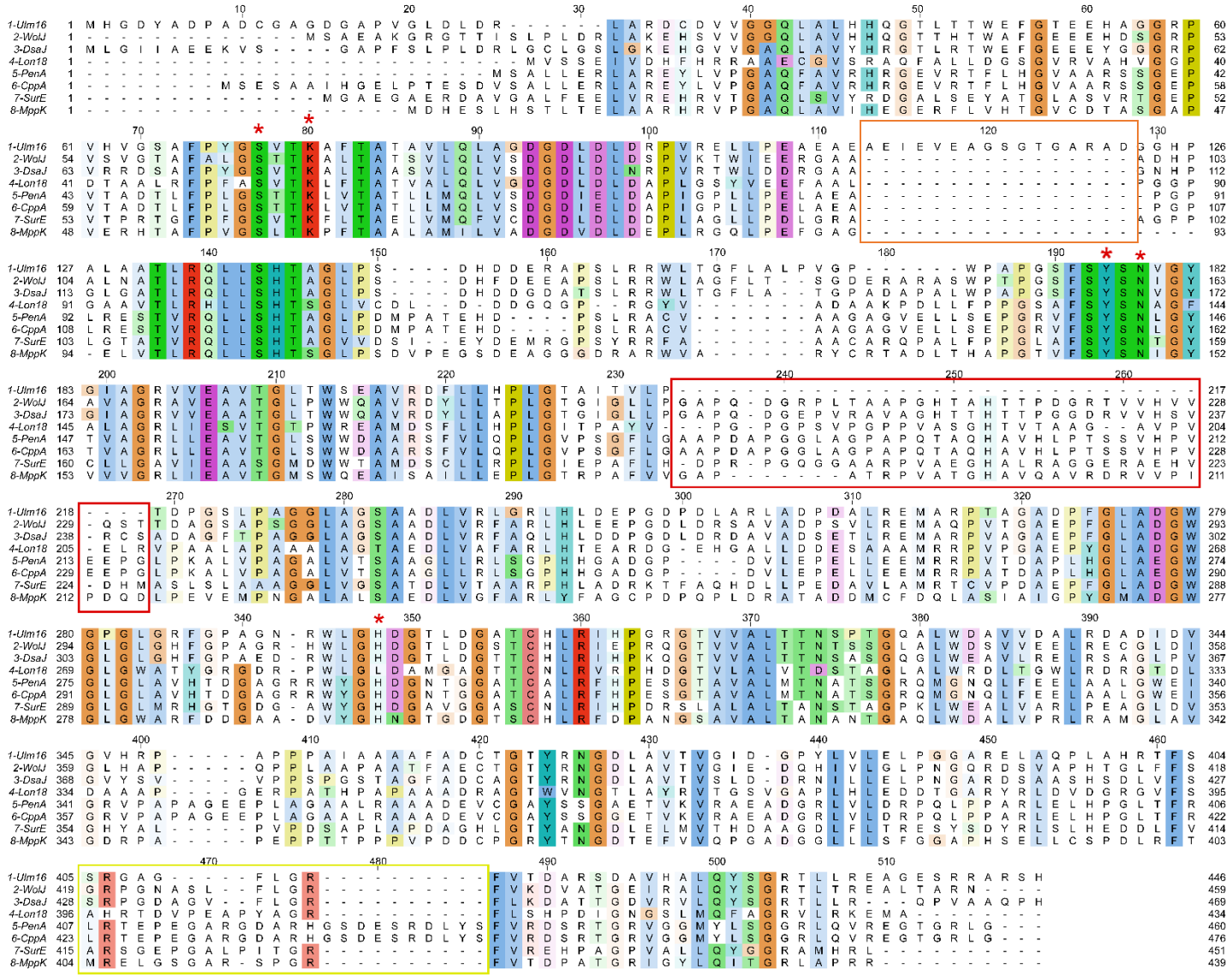
Substrate	[Ulm16]	Mutant	[Peptide]	Area Cyclic Peptide	Area Peptide Thioester	Area Hydrolyzed Peptide	Hydrolyzed Dimer	Area Tris Peptide	Sum	TTN	Cyc/SM	Cyc/H
12-MMP	70 nM	R431A	280 µM	76920	1297157	55670	6952	384405	1821105	169	0.1	1.4
12-MMP	70 nM	S429A	280 µM	2243756	463979	330492	-	-	3038226	2757	5	7
12-MMP	70 nM	Y428A	280 µM	337847	2114642	476929	-	-	2929418	431	0.2	0.7
12-MMP	500 nM	1-344	280 µM	34981	1914589	398195	118632	-	2466397	8	0.0	0.1
12-MMP	70 nM	L300G	280 µM	352754	1333933	73655	9596	-	1769938	797	0.3	4.8
12-MMP	70 nM	D297N	280 µM	639759	889575	25572	15839	-	1570745	1629	0.7	25.0
14	70 nM	R431A	280 µM	0	350874	40992	-	-	391866	391*	8.6*	-
14	70 nM	S429A	280 µM	421988	68621	15890	-	-	506499	3110	6	26.6
14	70 nM	Y428A	280 µM	265996	285678	14688	-	-	566362	1753	1	18.1
14	500 nM	1-344	280 µM				-	-				
14	70 nM	L300G	280 µM	92709	320930	14295	-	-	427935	800	0.3	6.5
14	70 nM	D297N	280 µM	46591	325366	13582	-	-	385539	451	0.1	3.4
16	32.5 nM	R431A	280 µM	206670	21343	451015	69447	-	748474	2379	9.7	0.5
16	32.5 nM	S429A	280 µM	1676219	16367	21117	-	-	1713703	8427	102	79
16	32.5 nM	Y428A	280 µM	1077523	510451	37788	26261	-	1652023	5619	2.1	28.5
16	500 nM	1-344	280 µM	32923	1428807	20947	-	-	1482676	12	0	1.6
16	32.5 nM	L300G	280 µM	676744	1097879	17546	-	-	1792168	3253	0.6	38.6
16	32.5 nM	D297N	280 µM	936327	819680	21794	-	-	1777802	4538	1.1	43
29	32.5 nM	R431A	280 µM	-	12064	506230	279574		797868	8485*	23.2*	-
29	32.5 nM	S429A	280 µM	1316148	7336	95074	44703	-	1463262	7749	179	14
29	32.5 nM	Y428A	280 µM	1139426	15322	99508	78596		1332852	7365	74	11.5
29	500 nM	1-344	280 µM	15295	546699	54107	-	-	616101	14	0	0.3
29	32.5 nM	L300G	280 µM	831196	626389	41292	-	-	1498876	4778	1.3	20.1
29	32.5 nM	D297N	280 µM	1013803	379441	111736	-	-	1504979	5804	2.7	9.1
30	32.5 nM	R431A	280 µM	17557	43403	535460	98024	313789	1008232	150	0.4	0.02
30	32.5 nM	S429A	280 µM	283097	12587	183397	206969	-	686050	3555	22.5	1.5
30	32.5 nM	Y428A	280 µM	295075	11015	280007	122283	-	708380	3589	26.8	1.1
30	500 nM	1-344	280 µM	22817	1153654	58062	-	-	1234532	10	0	0.4
30	32.5 nM	L300G	280 µM	501905	13196	246999	76568	-	838668	5156	38	2
30	32.5 nM	D297N	280 µM	127879	11739	380677	78431	74063	672789	1638	11	0.3

Supplementary Table 3: Crystallography Table

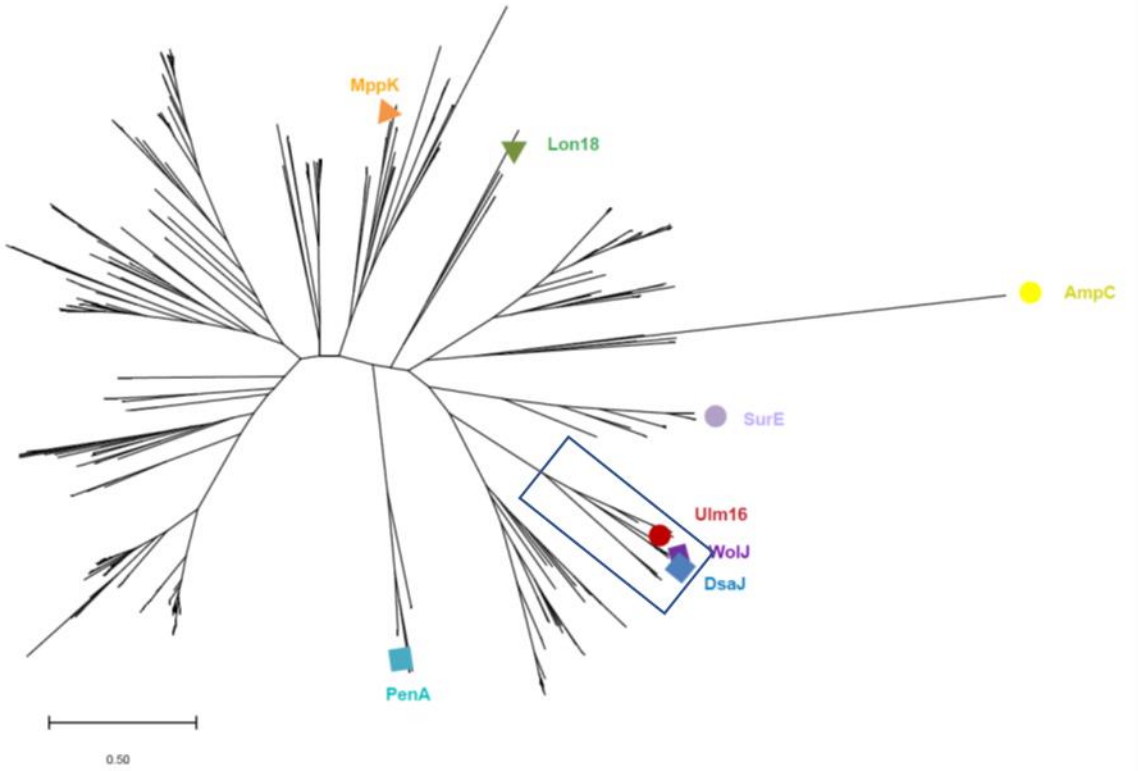
Ulm16 12-440 8FEK	
Data Collection	
Beamline	SSRL BL 12-2
Wavelength (Å)	0.98
Resolution range (Å)	32.8-2.058 (2.132-2.058)
Space group	P 1 21 1
Cell dimensions	
a, b, c (Å)	48.822, 67.871, 56.779
α, β, γ (˚)	90.0, 102.505, 90.0
Total reflections	1987820
Unique reflections	22120 (2171)
Redundancy (%)	5.9 (6.0)
Completeness (%)	97.74 (95.59)
Rmerge	0.141 (0.710)
Rmeas	0.156 (0.785)
Rpim	0.065 (0.328)
I/σ(I)	15.11 (4.31)
CC1/2	0.984 (0.505)
Wilson B (Å2)	28.42
Refinement	
Copies/A.S.U.	1
Resolution (Å)	2.07
Rwork / Rfree	0.186 / 0.219
No. nonhydrogen atoms	3176
Protein	2981
Water	195
B factors (Å2)	40.67
Protein	40.65
Water	40.92
R.m.s.d	
Bond lengths (Å)	0.004
Bond angles (˚)	0.66
Ramachandran (favored/allowed/outliers)	97.75, 2.25, 0
Rotamer outliers (%)	0.0
Clash score	3.07

Supplementary Table 4: Primers used for site directed mutagenesis

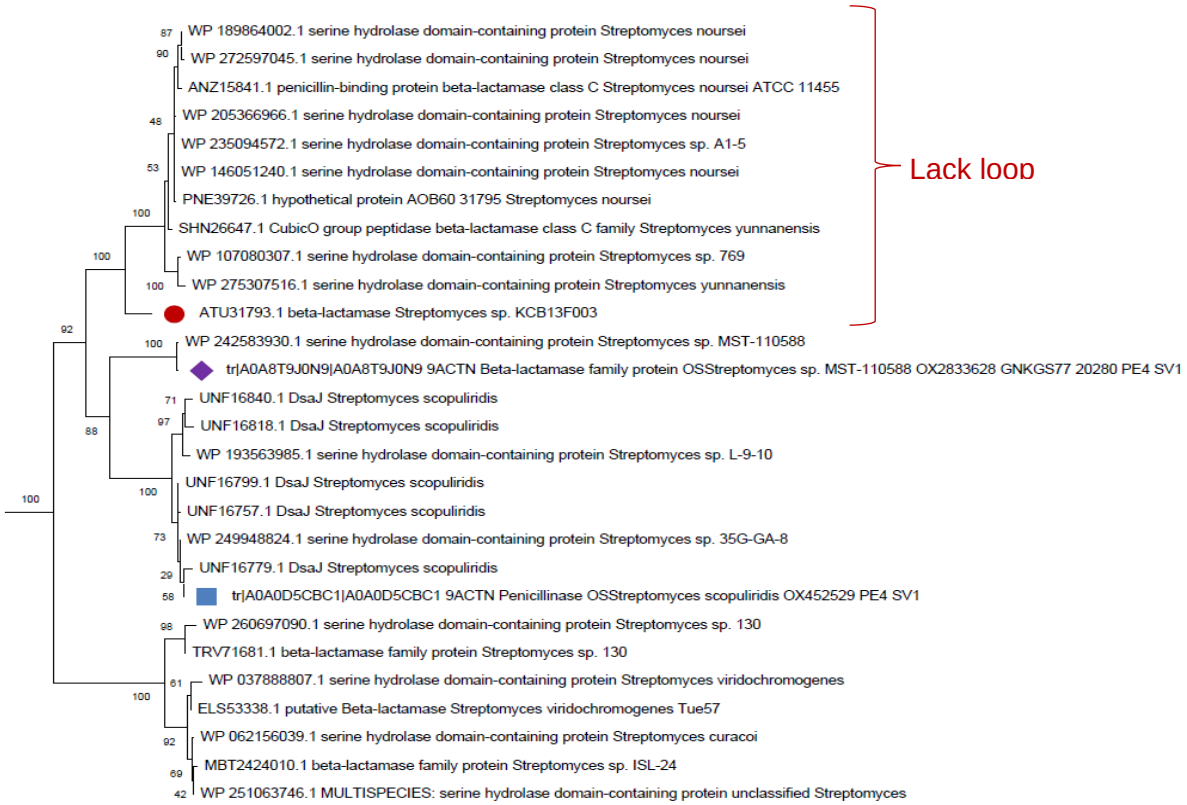
Primer Name	Oligonucleotide Sequence (5'-3')
BamHI-Ulm16 12 Fwd	CATCATGGATCCGGCGCCGGTGACGGCG
Ulm16 440-XhoI Rev	ATGATGATGCTCGAGTCAGGACTCTCCCGCTTCCCGC
pGEX6p1 Ulm16 FL Fwd	CAGGGGCCCCTGGGATCCGGCGCCGGTGACGGCG
pGEX6p1 Ulm16 FL Rev	GCGCGAGGCAGATCGTCAGGACTCTCCCGCTTCCCGC
5GEX Fwd	GGGCTGGCAAGCCACGTTTGGTG
3GEX Rev	CCGGGAGCTGCATGTGTCAGAGG
Ulm16 D297N Fwd	TGGCTCGGCCACAACGGCACCCTG
Ulm16 D297N Rev	CAGGGTGCCGTTGTGGCCGAGCCA
Ulm16 L300A Fwd	CCACGACGGCACCGCCGACGGCGCCACCTG
Ulm16 L300A Rev	CAGGTGGCGCCGTCGGCGGTGCCGTCGTGG
Ulm16 L300G Fwd	CCACGACGGCACCGGCGACGGCGCCACCTG
Ulm16 L300G Rev	CAGGTGGCGCCGTCGCCGGTGCCGTCGTGG
Ulm16 R431A Fwd	CTCCAGTACAGCGGAGCCACCCTCCTGCGGGAAG
Ulm16 R431A Rev	CTTCCCGCAGGAGGGTGGCTCCGCTGTACTGGAG
Ulm16 Y428A Fwd	CGCCCTCCAGGCCAGCGGACGCACCC
Ulm16 Y428A Rev	GGGTGCGTCCGCTGGCCTGGAGGGCG
Ulm16 S429A Fwd	CGCCCTCCAGTACGCCGGACGCACCCTCCT
Ulm16 S429A Rev	AGGAGGGTGCGTCCGGCGTACTGGAGGGCG



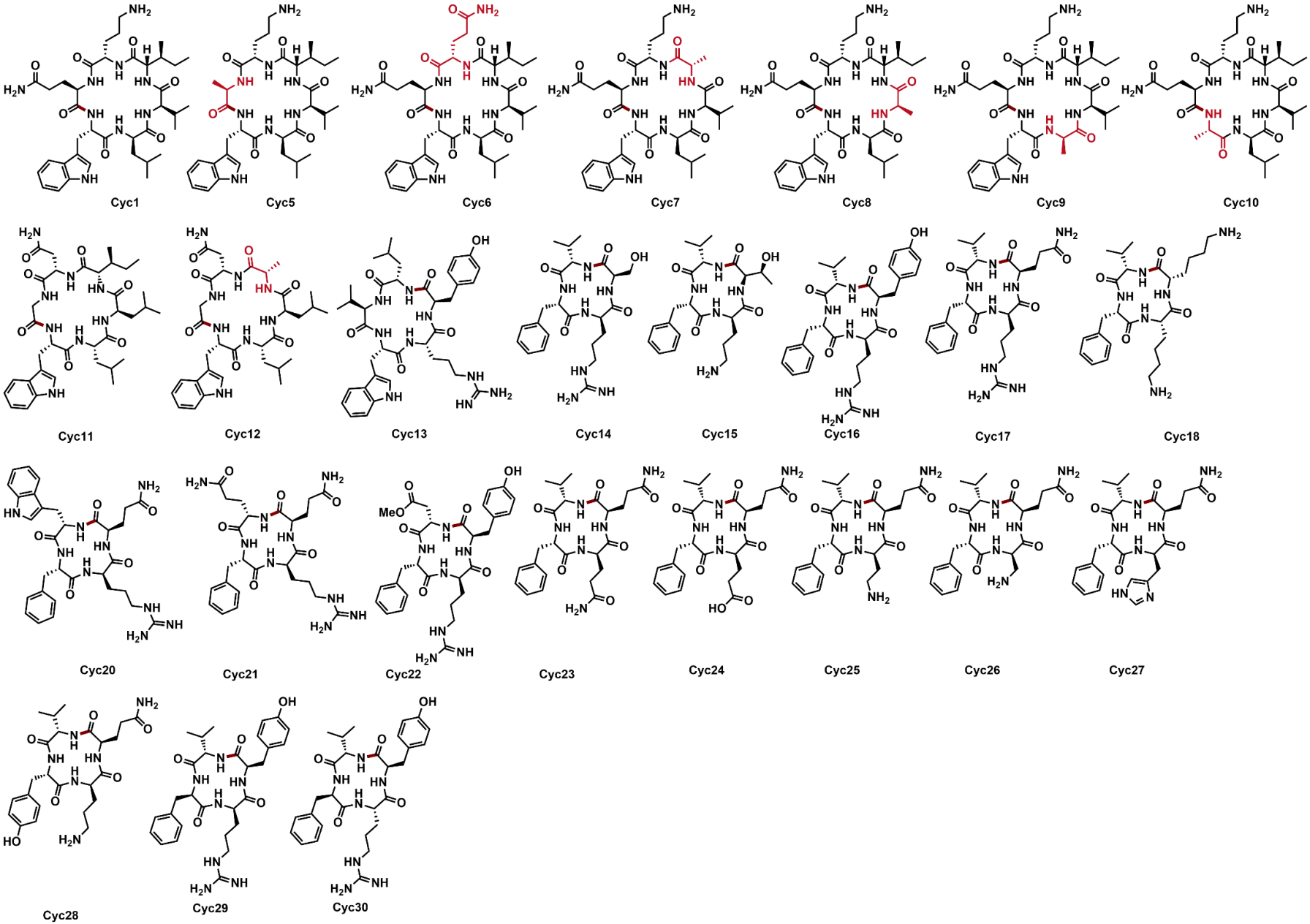
Supplementary Fig. 1: Multiple sequence alignment of predicted and validated PBP-TEs. Residues in the conserved catalytic tetrad are highlighted with a red star. The loop regions that were identified in the alpha fold structure are also highlighted in the multiple sequence alignment, with red being the loop region, which is truncated in Ulm16, yellow being the lipocalin domain loop, and orange being the insertion in Ulm16. Note: the numbering of the amino acids is off compared to the numbering in the paper due to the alignment. The orange loop is residues 113-128.



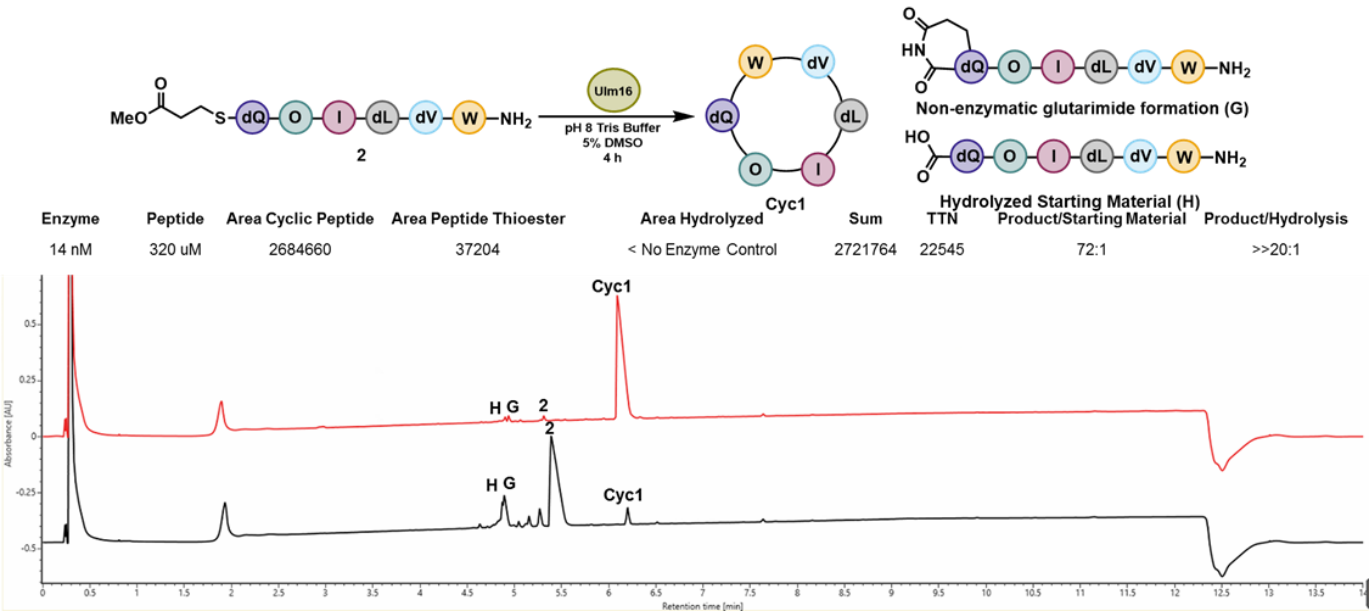
Supplementary Fig. 2 Phylogenetic tree of PBP-TEs. The top 500 hits to Ulm16 from NCBI along with the known PBP-TEs are shown. PBP-TEs belonging to known biosynthetic gene clusters are labeled in the figure. Including the outlier AmpC, which is a beta-lactamase from *E. coli.*



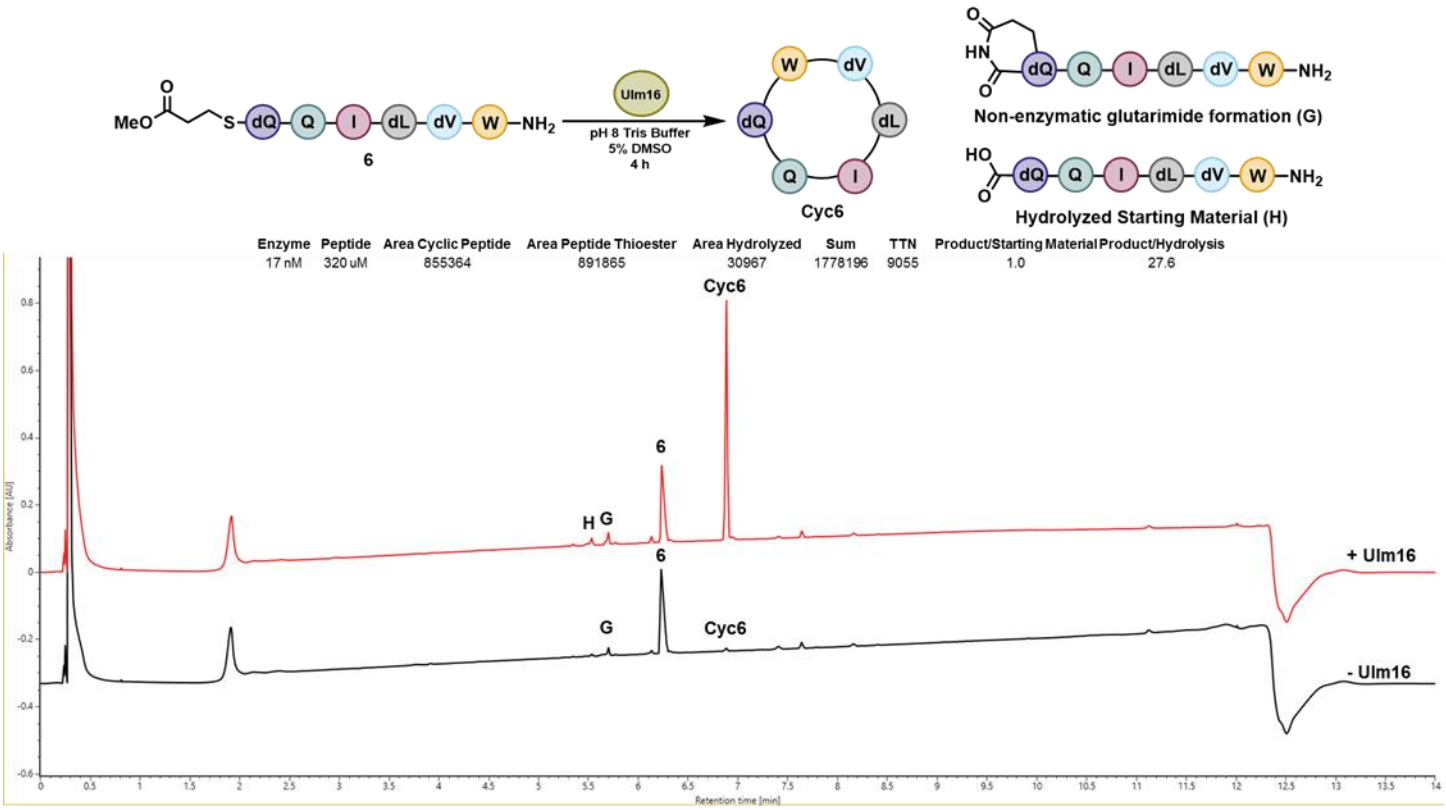
Supplementary Fig. 3: Zoom in of Phylogenetic tree the PBP-TEs most similar to Ulm16. Homologs to Ulm16 that lack the loop region are indicated. Numbers at nodes represent bootstrap values.



Supplementary Fig. 4: Cyclic peptides identified by MS/MS analysis. For cyclic peptides belonging to the Ulm16 alanine scan (Cyc 5-10) residues which differ from the original structure (Cyc1) are colored in red. For all peptides point of cyclization is colored in maroon. Actual MS/MS data can be found on pages S76-S104.

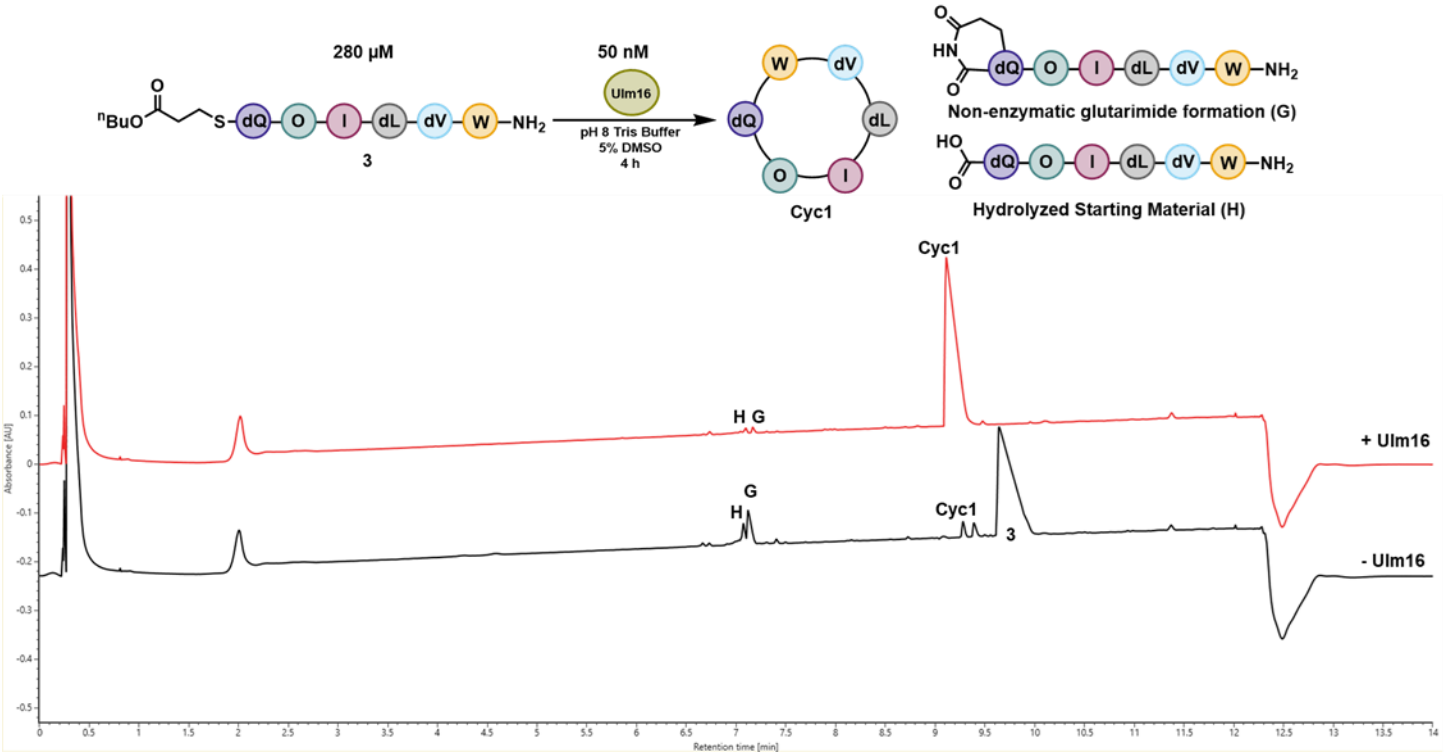


Supplementary Fig. 5: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with Ulm-SMMP (2). The enzyme and substrate concentrations are indicated in **Supplementary Table 1** and above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). The UPLC trace is representative of three individual replicates, while the TTN, product to hydrolysis/starting material ratios are averages of three replicates. UPLC was taken on a 0 to 70% gradient (See methods section for more details).

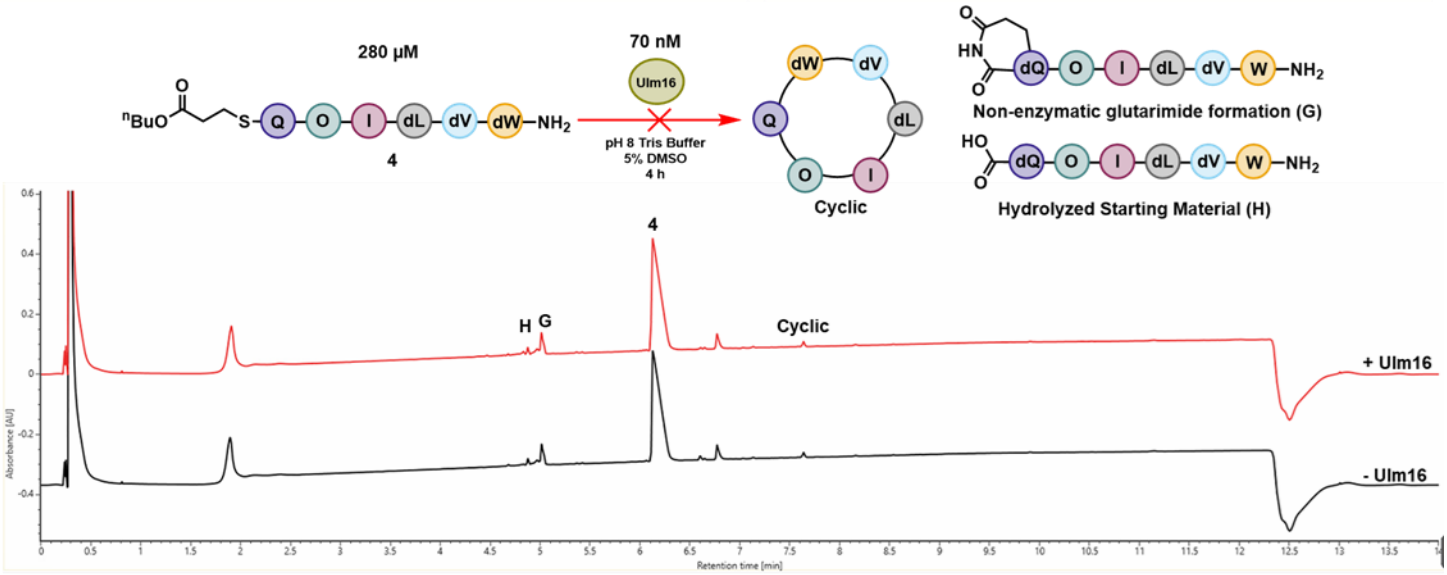


Supplementary Fig. 6: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with Ulm-SMMP where L-Orn replaced by L-Gln (6). The enzyme and substrate concentrations are indicated in **Supplementary Table 1** and above the trace. The substrates are labeled with their corresponding numbers from

the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). The UPLC trace is representative of three individual replicates, while the TTN, product to hydrolysis/starting material ratios are averages of three replicates. UPLC was taken on a 0 to 70% gradient (See methods section for more details).

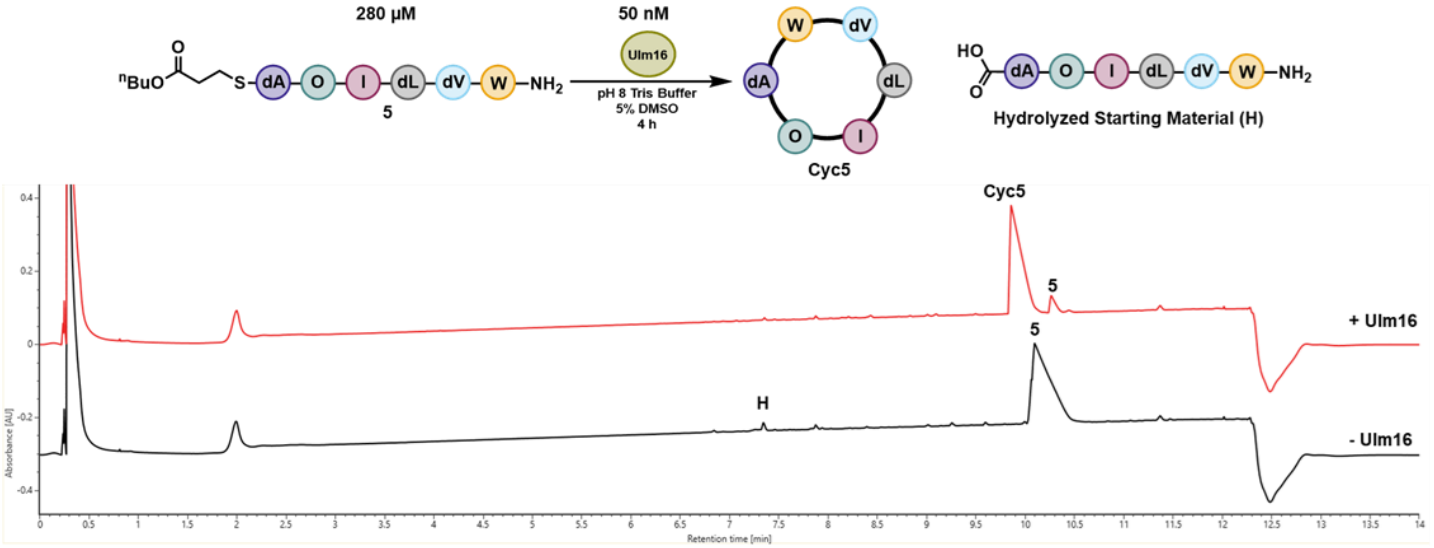


Supplementary Fig. 7: In vitro reaction of Ulm16 with Ulm-SBMP (3). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative of three individual replicates.

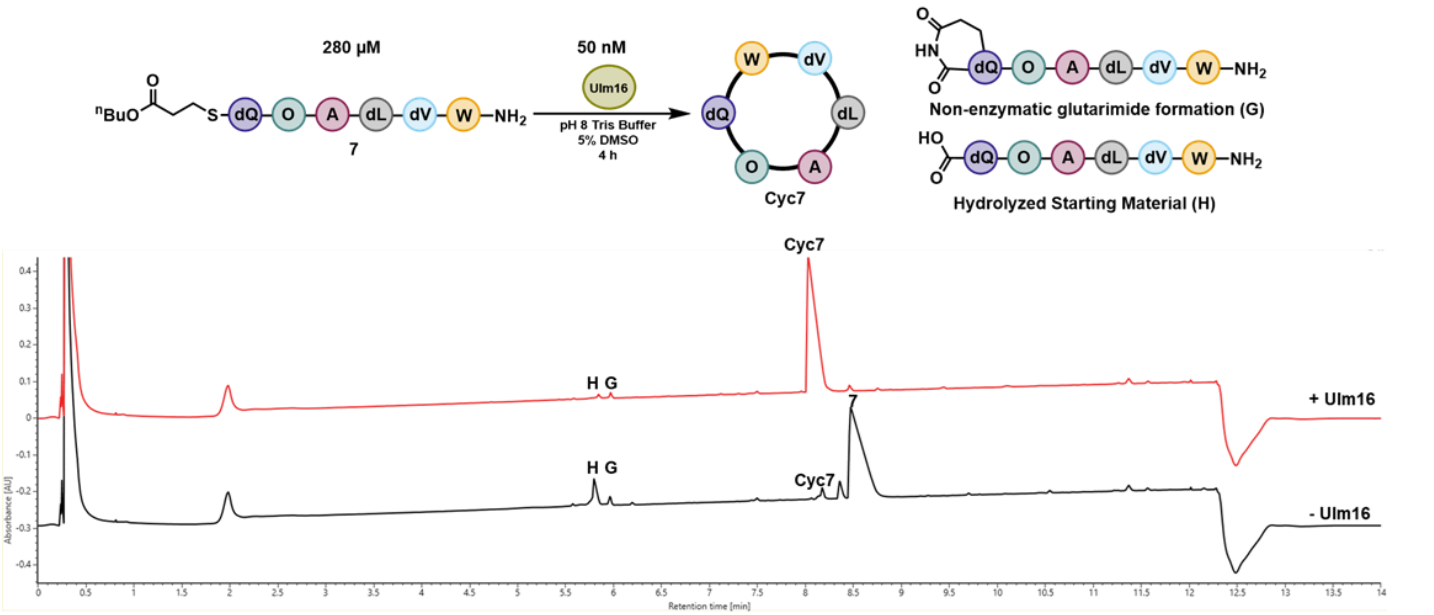


Supplementary Fig. 8: In vitro reaction of Ulm16 with Ulm-SBMP where D-Gln was replaced by L-Gln (4). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to

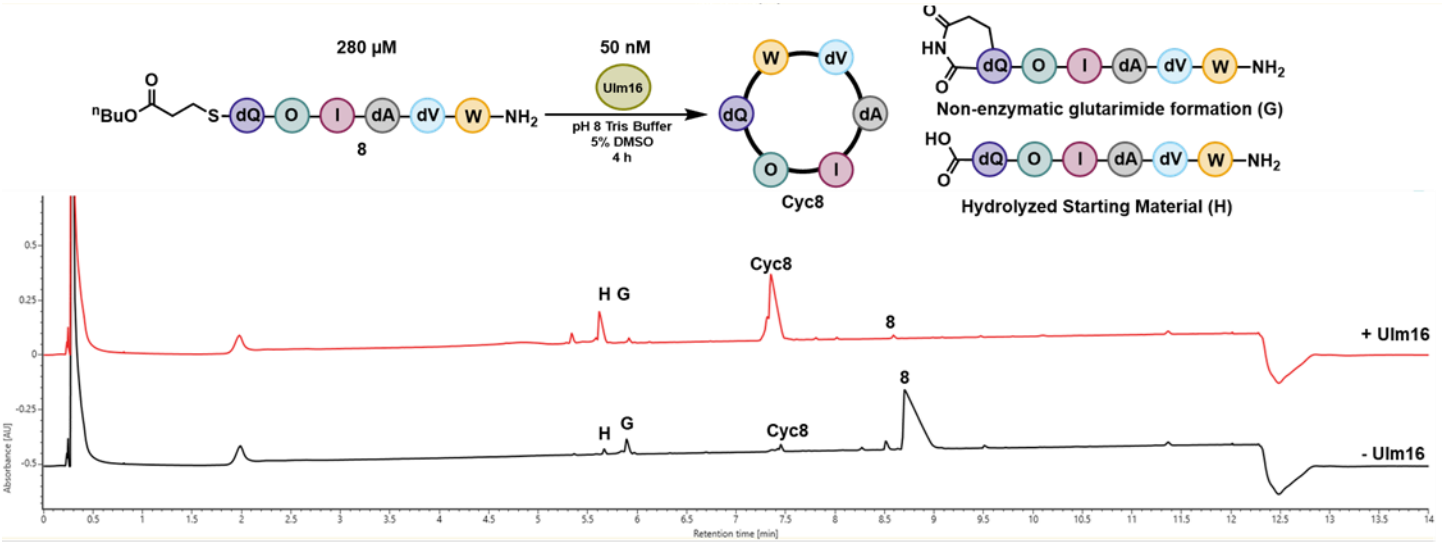
extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative of three individual replicates.



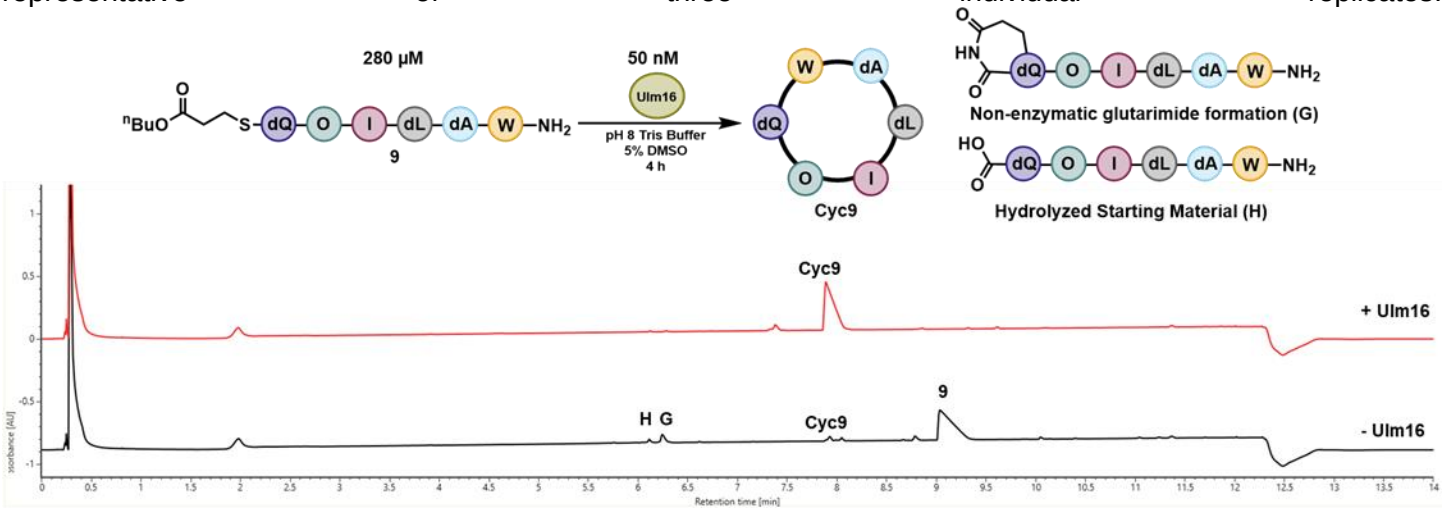
Supplementary Fig. 9: *In vitro* reaction of Ulm16 with Ulm-SBMP where D-Gln was replaced by D-Ala (5). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative of three individual replicates.



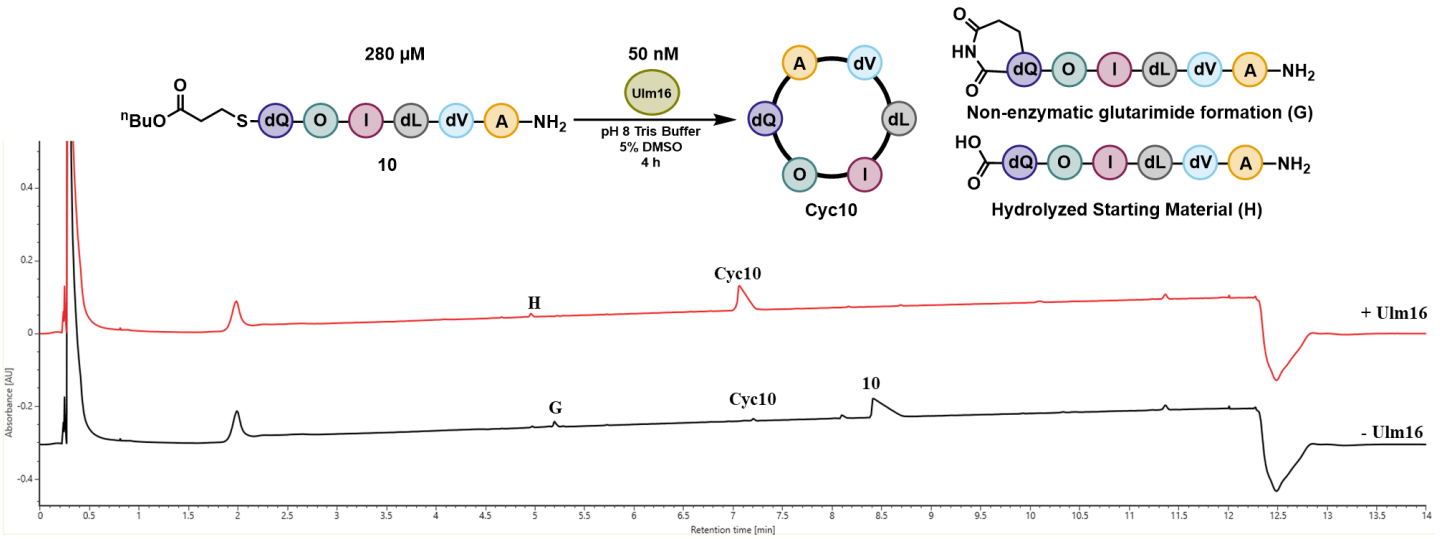
Supplementary Fig. 10: *In vitro* reaction of Ulm16 with Ulm-SBMP where L-Ile was replaced by L-Ala (7). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative of three individual replicates.



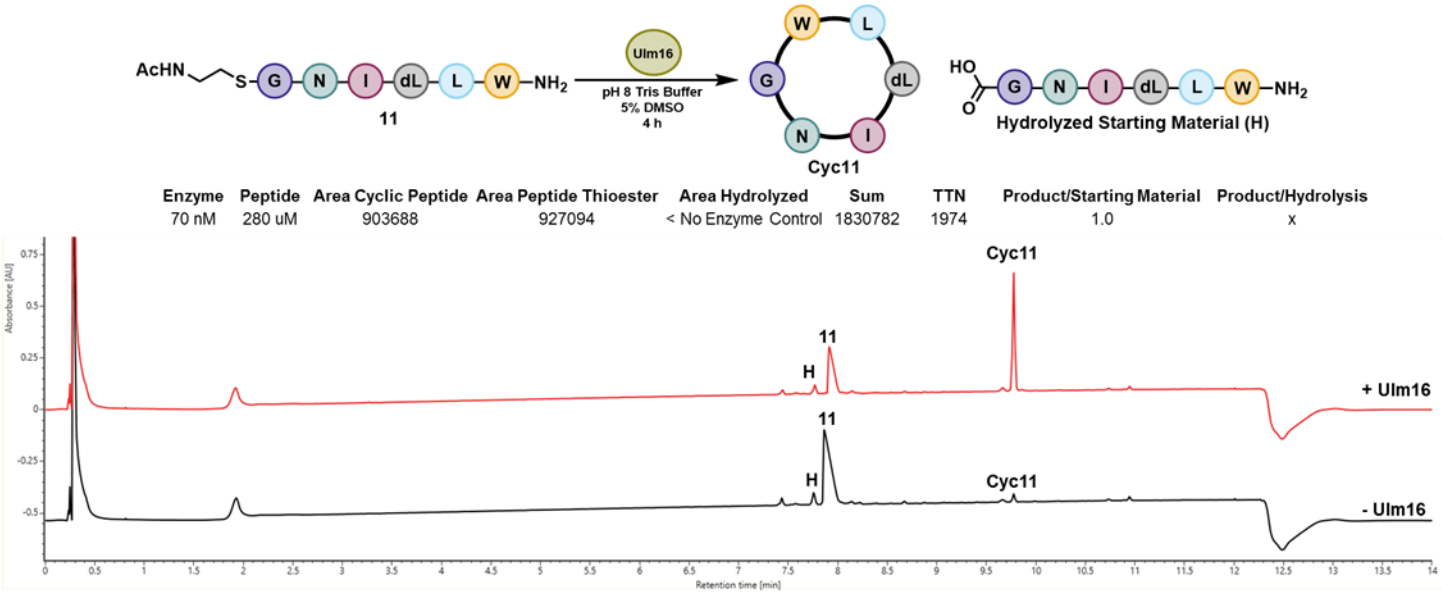
Supplementary Fig. 11: *In vitro* reaction of Ulm16 with Ulm-SBMP where D-Leu was replaced by D-Ala (8). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative of three individual replicates.



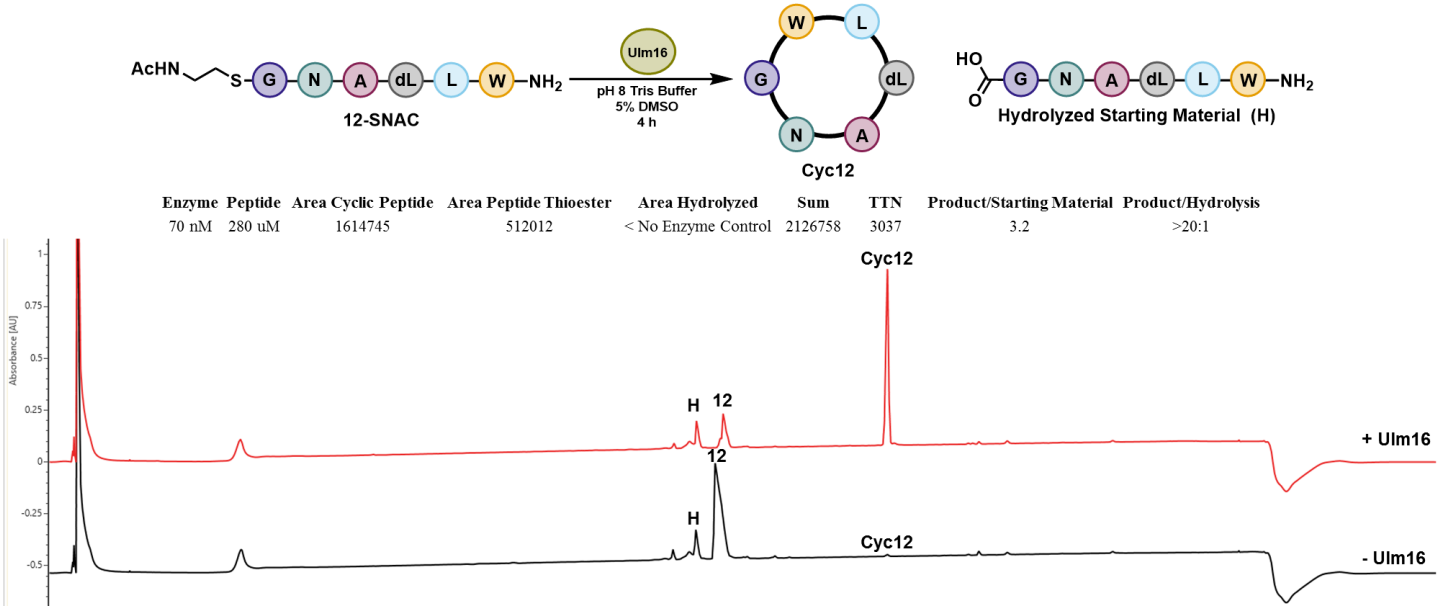
Supplementary Fig. 12: *In vitro* reaction of Ulm16 with Ulm-SBMP where D-Val was replaced by D-Ala (9). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative



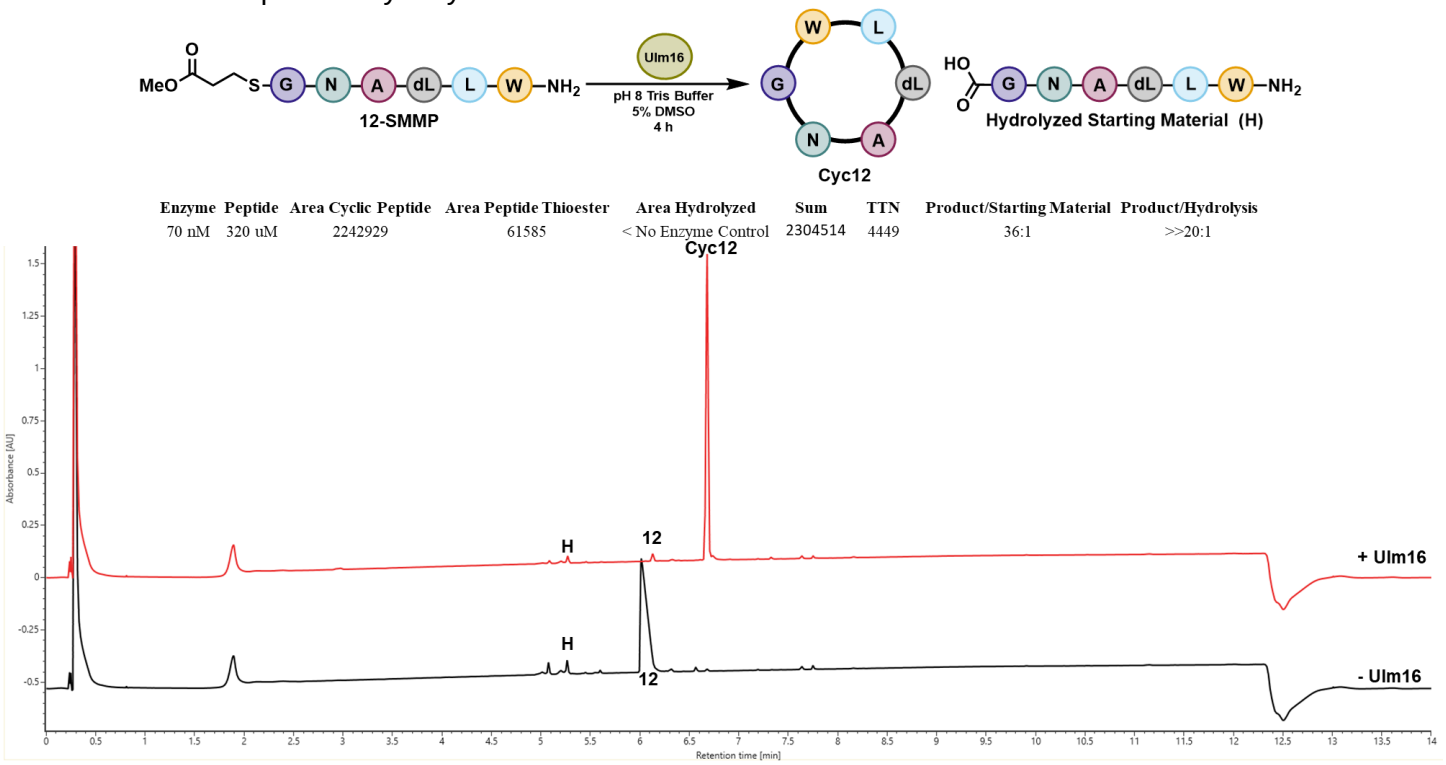
Supplementary Fig. 13: *In vitro* reaction of Ulm16 with Ulm-SBMP where L-Trp was replaced by L-Ala (10). Enzyme and substrate concentrations are indicated above each reaction in the figure. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'G' represents non-enzymatic glutaramide formation, which can occur due to extended incubation periods in the absence of enzyme (-Ulm16). All UPLC UV traces (214 nm) are representative



Supplementary Fig. 14: The UPLC UV trace (214 nm) displays the total turnover number (TTN) assay results of Ulm16 with substrate 11. The enzyme and substrate concentrations are noted in Supplementary Table 1 and above the trace, and the substrates are labeled with their corresponding numbers from the paper. The nomenclatures 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace represents three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates. It is important to note that in the case of these specific substrates, the no enzyme control (-Ulm16) showed more hydrolysis, and thus it was not possible to determine an exact product/hydrolysis ratio.

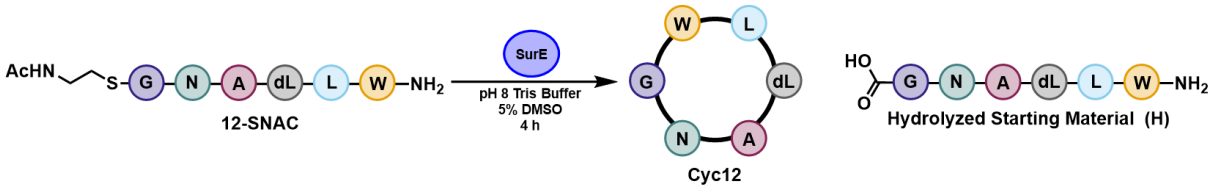


Supplementary Fig. 15: The UPLC UV trace (214 nm) displays the total turnover number (TTN) assay results of Ulm16 with substrate 12-SNAC. The enzyme and substrate concentrations are noted in Supplementary Table 1 and above the trace, and the substrates are labeled with their corresponding numbers from the paper. The nomenclatures 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace represents three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates. It is important to note that in the case of these specific substrates, the no enzyme control (-Ulm16) showed more hydrolysis, and thus it was not possible to determine an exact product/hydrolysis ratio.

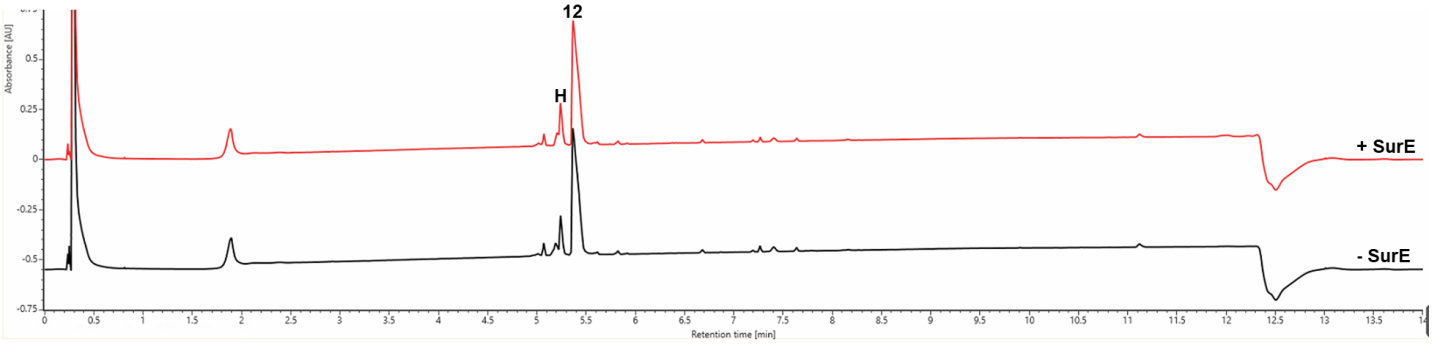


Supplementary Fig. 16: The UPLC UV trace (214 nm) displays the total turnover number (TTN) assay results of Ulm16 with substrate 12-SMMP. The enzyme and substrate concentrations are noted in Supplementary Table 1 and above the trace, and the substrates are labeled with their corresponding numbers from the paper. The nomenclatures 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds,

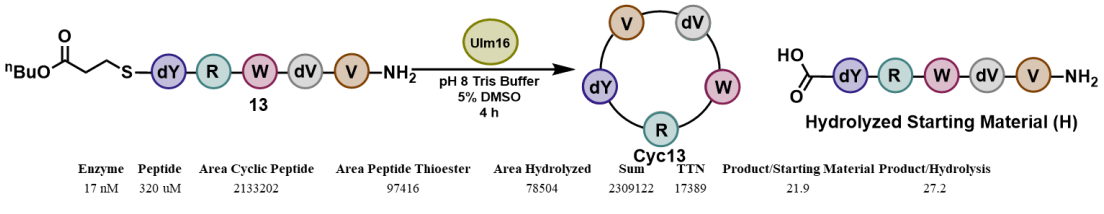
respectively. The UPLC trace represents three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates. It is important to note that in the case of these specific substrates, the no enzyme control (-Ulm16) showed more hydrolysis, and thus it was not possible to determine an exact product/hydrolysis ratio. The reaction was analyzed with a 0 to 70% (Acetontirile 0.1% Formic acid) gradient instead of the typical 0 to 40%



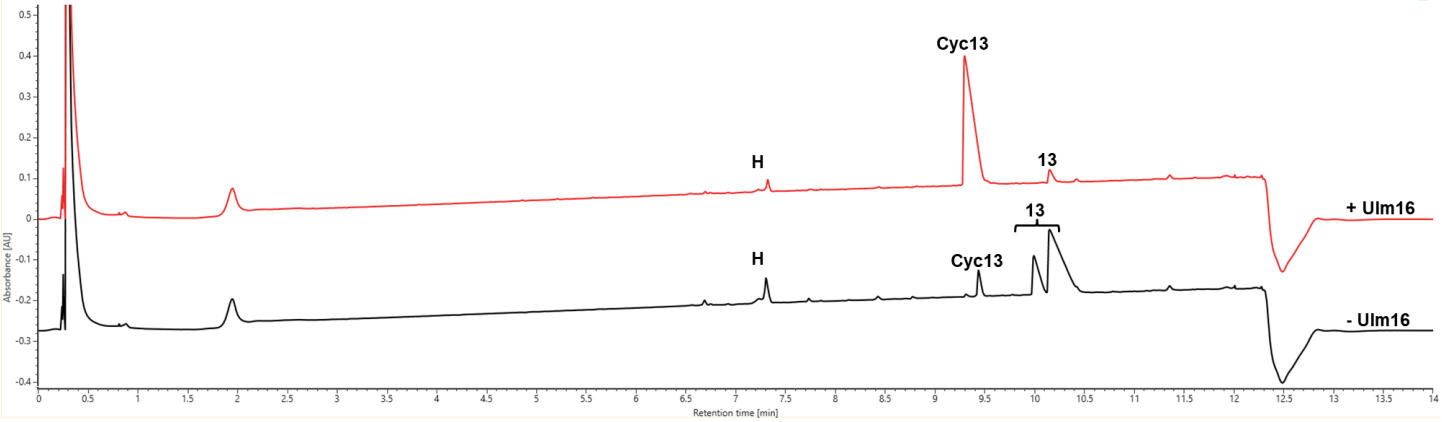
Enzyme	Peptide	Area Cyclic Peptide	Area Peptide Thioester	Area Hydrolyzed	Sum	TTN	Product/Starting Material	Product/Hydrolysis
250 nM	280 uM	-	-	-	3105769	-	-	-



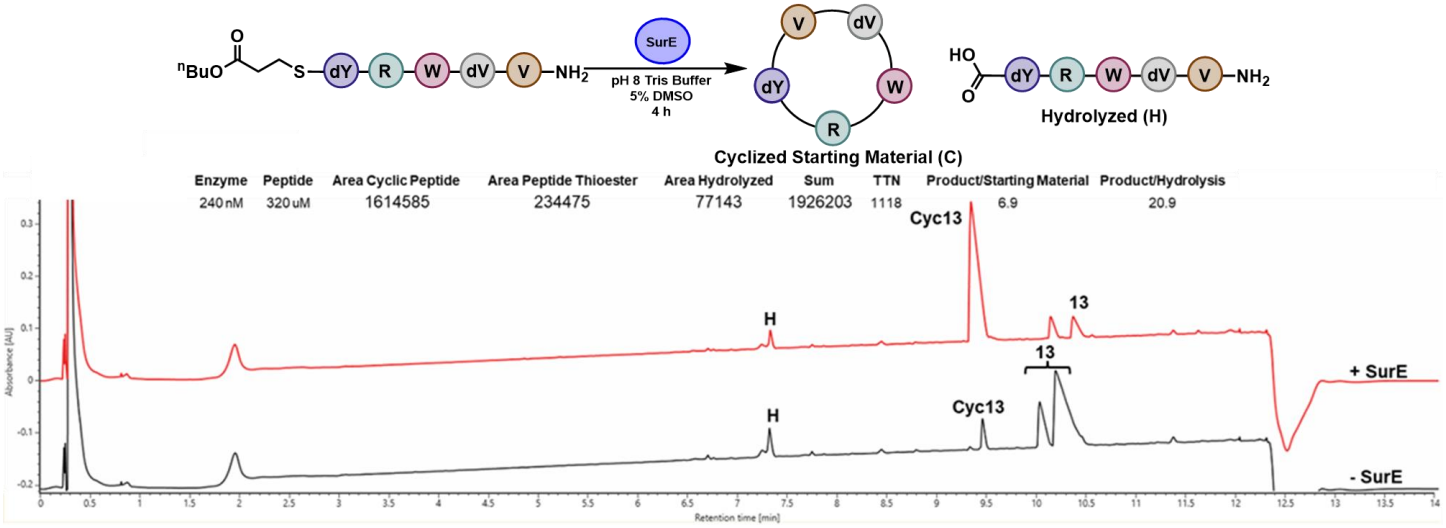
Supplementary Fig. 17: The UPLC UV trace (214 nm) displays the total turnover number (TTN) assay results of SurE with substrate 12-SNAC. The enzyme and substrate concentrations are noted in Supplementary Table 1 and above the trace, and the substrates are labeled with their corresponding numbers from the paper. The nomenclatures 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace represents three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates. It is important to note that in the case of these specific substrates, the no enzyme control (-Ulm16) showed more hydrolysis, and thus it was not possible to determine an exact product/hydrolysis ratio. No significant difference was observed between the +/- SurE Samples. The reaction was analyzed with a 0 to 70% (Acetontirile 0.1% Formic acid) gradient instead of the typical 0 to 40%



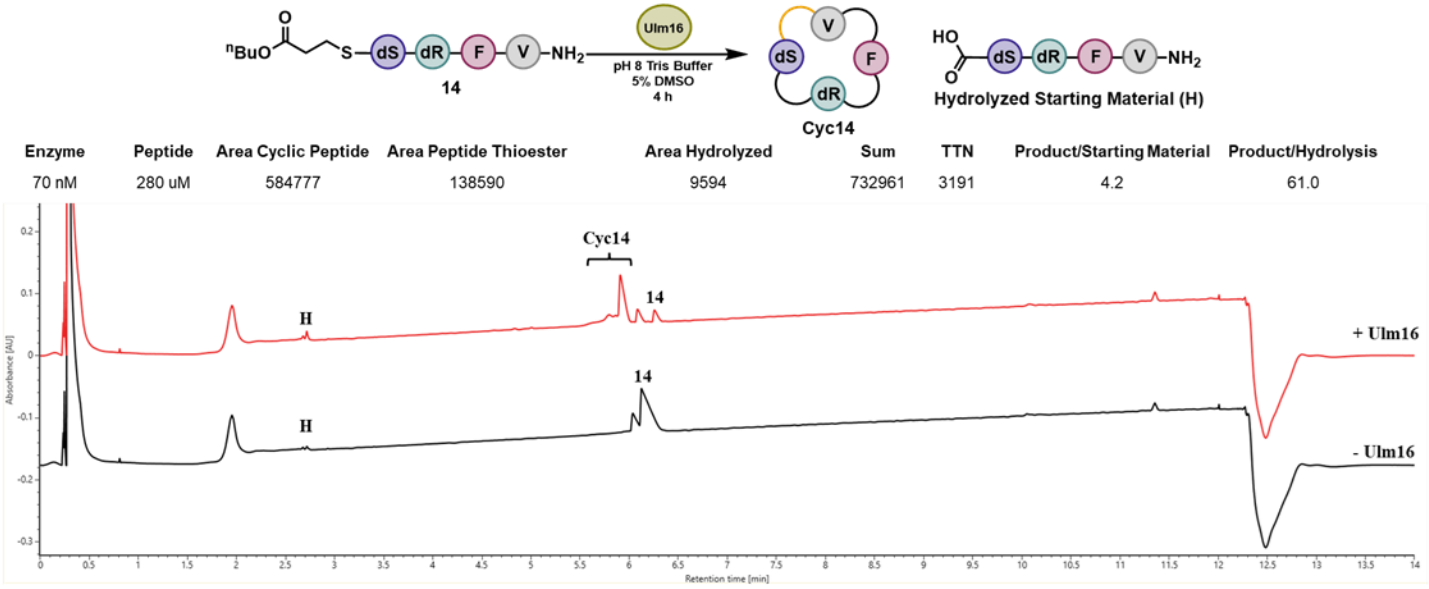
Enzyme	Peptide	Area Cyclic Peptide	Area Peptide Thioester	Area Hydrolyzed	Sum	TTN	Product/Starting Material	Product/Hydrolysis
17 nM	320 uM	2133202	97416	78504	2309122	17389	21.9	27.2



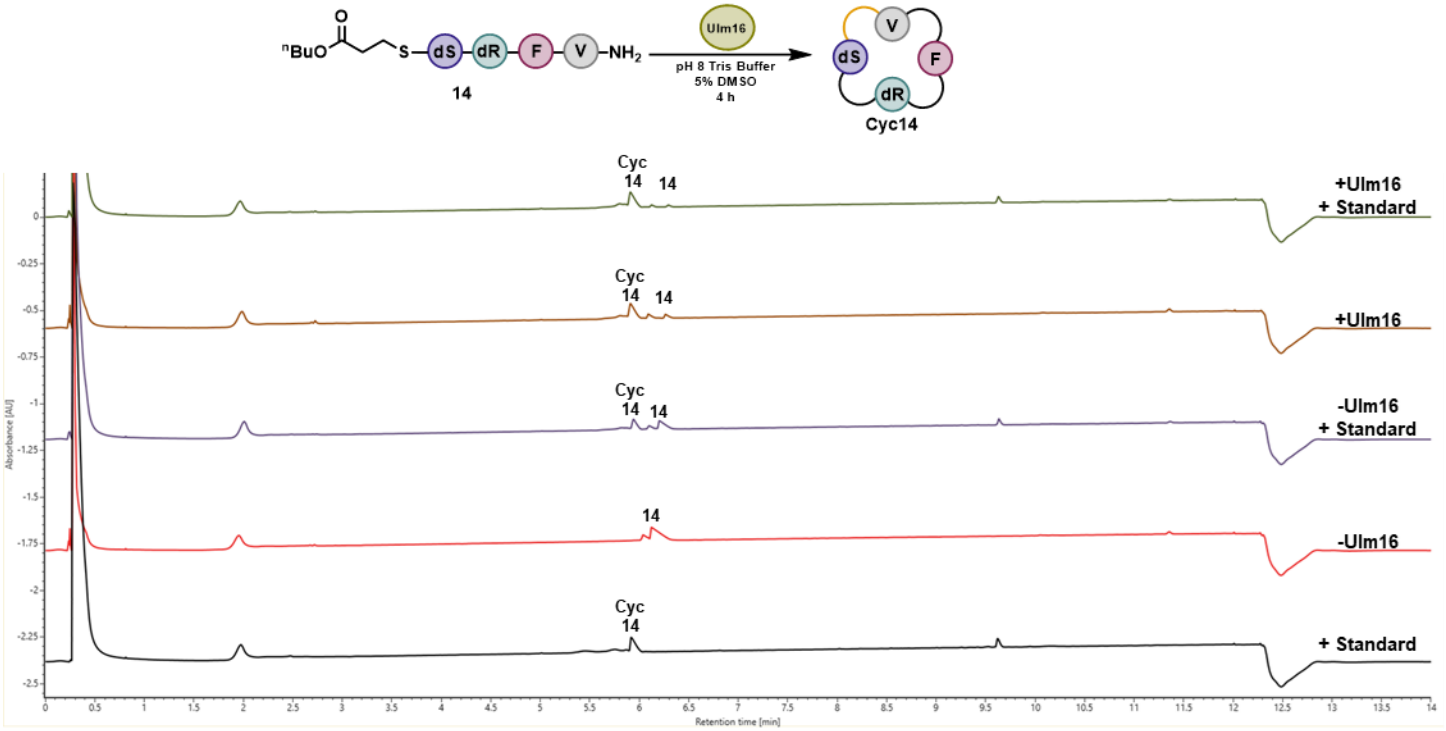
Supplementary Fig. 18: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with a substrate of a known PBP-TE PenA (13). The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



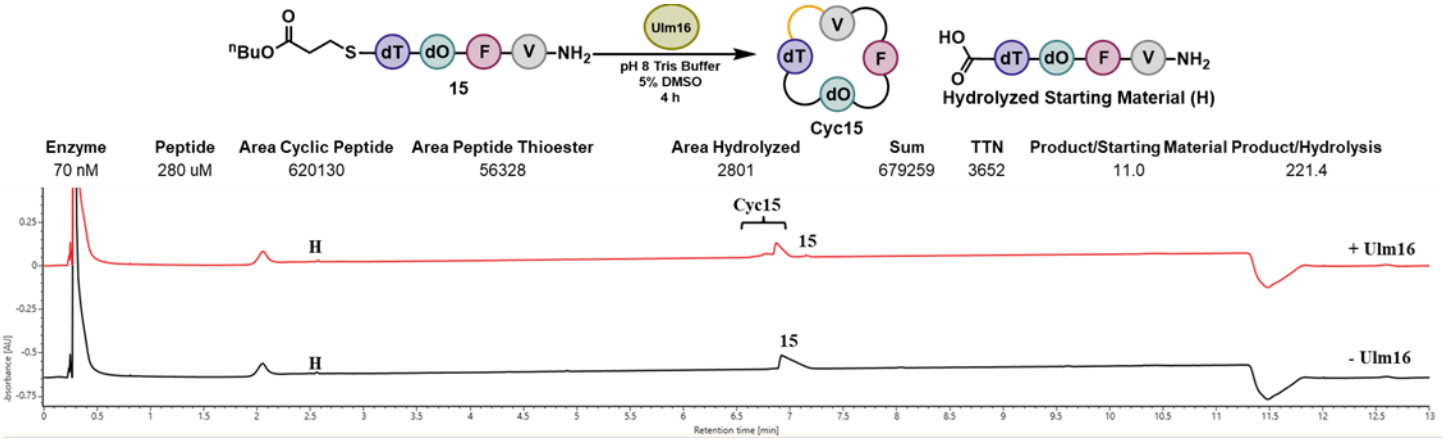
Supplementary Fig. 19: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of SurE with a substrate of a known PBP-TE PenA (13). The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



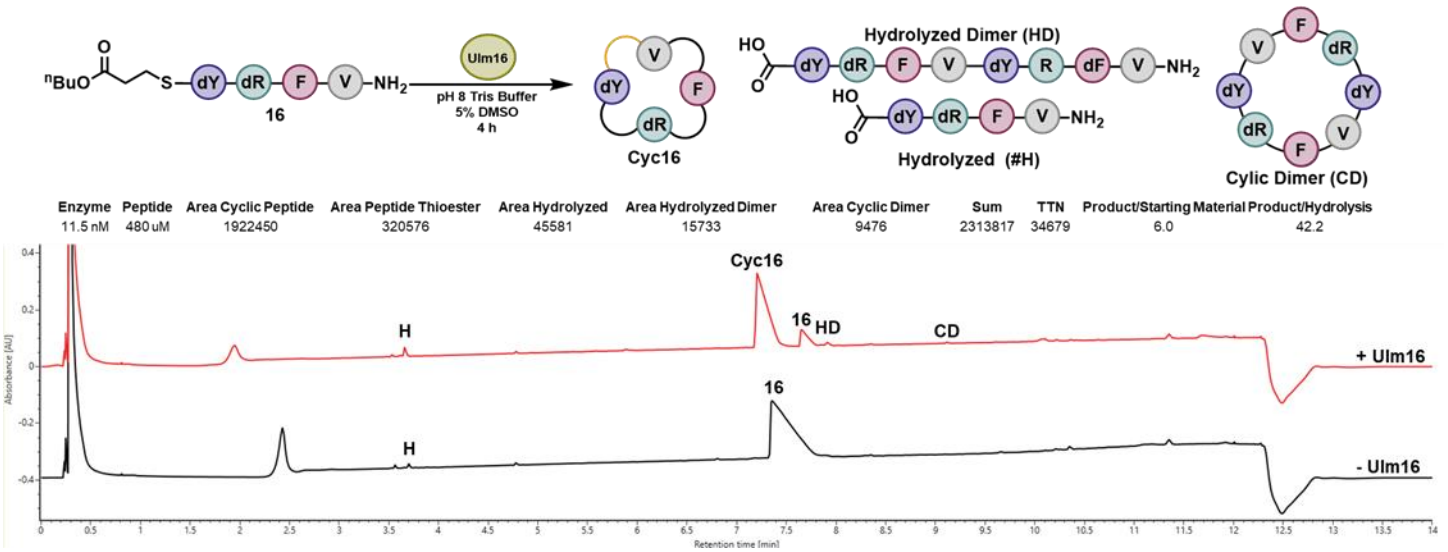
Supplementary Fig. 20: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrate 14. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'HD' and 'CD' represent hydrolyzed dimer and cyclic dimer, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



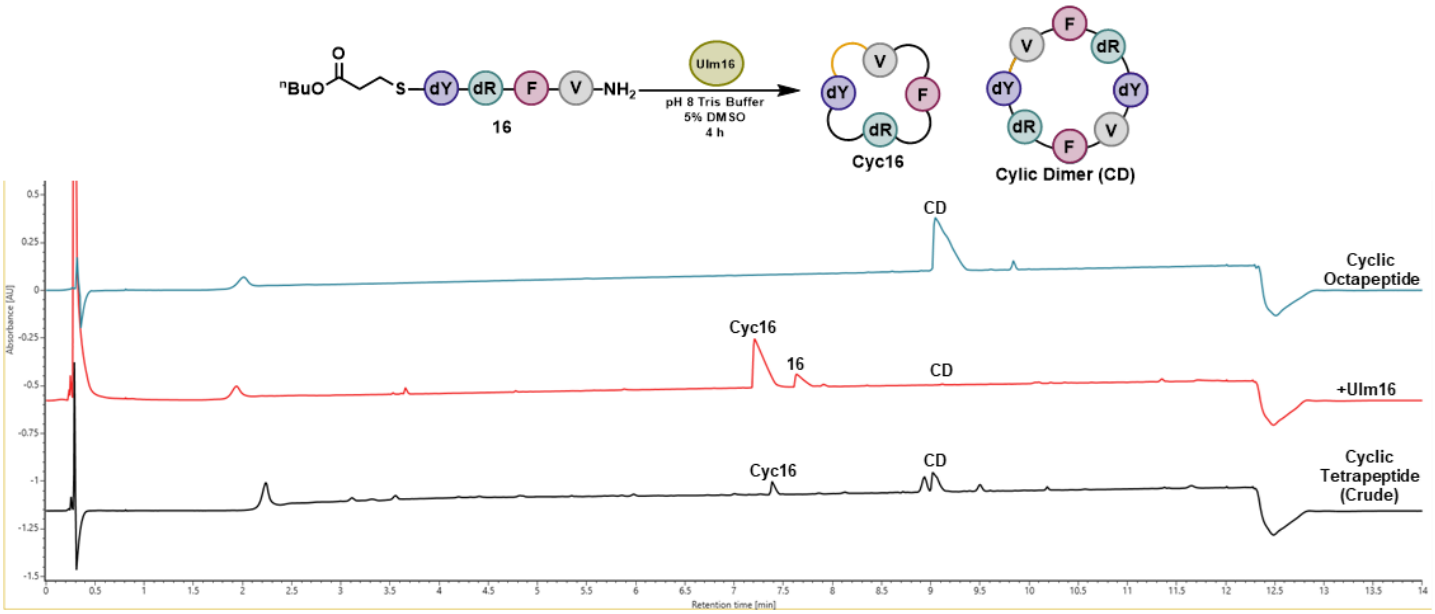
Supplementary Fig. 21: Spiking with synthetic standard to confirm cyclic tetrapeptide. UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with substrate 14, which was previously seen in Supplementary Fig. 26. To confirm the identity of the cyclic product, a purified cyclic standard from a previous publication[2] was used.



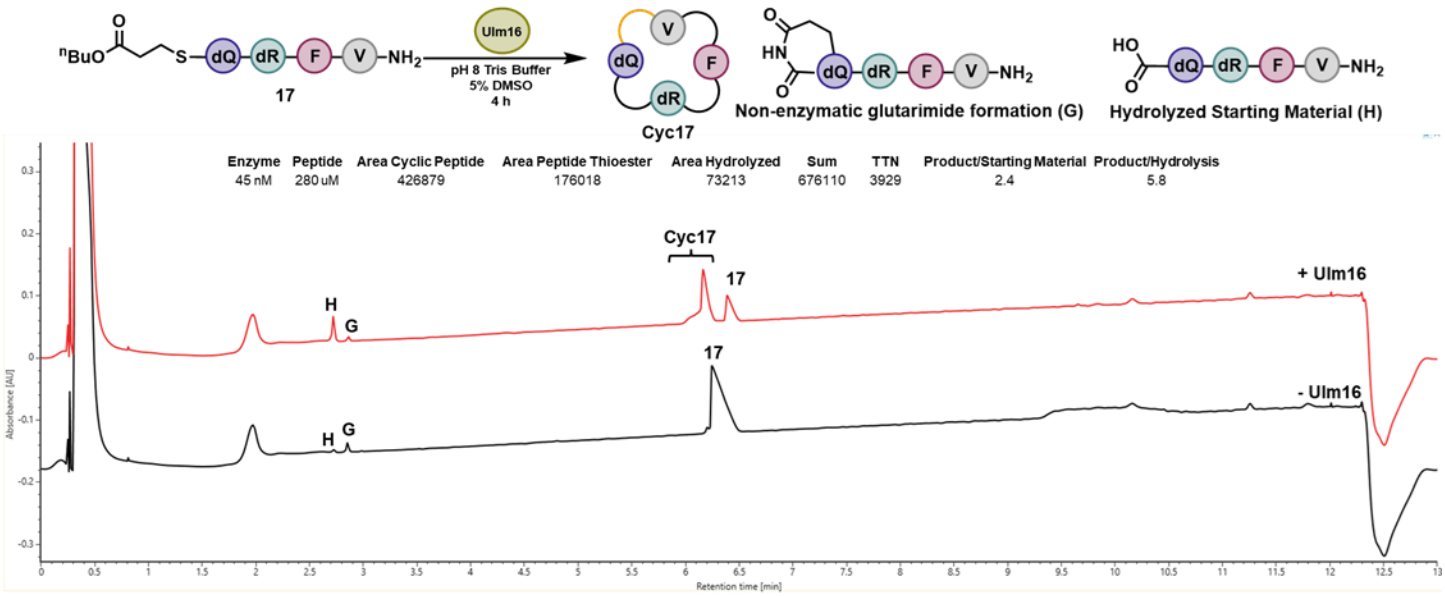
Supplementary Fig. 22: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrate 15. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'HD' and 'CD' represent hydrolyzed dimer and cyclic dimer, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



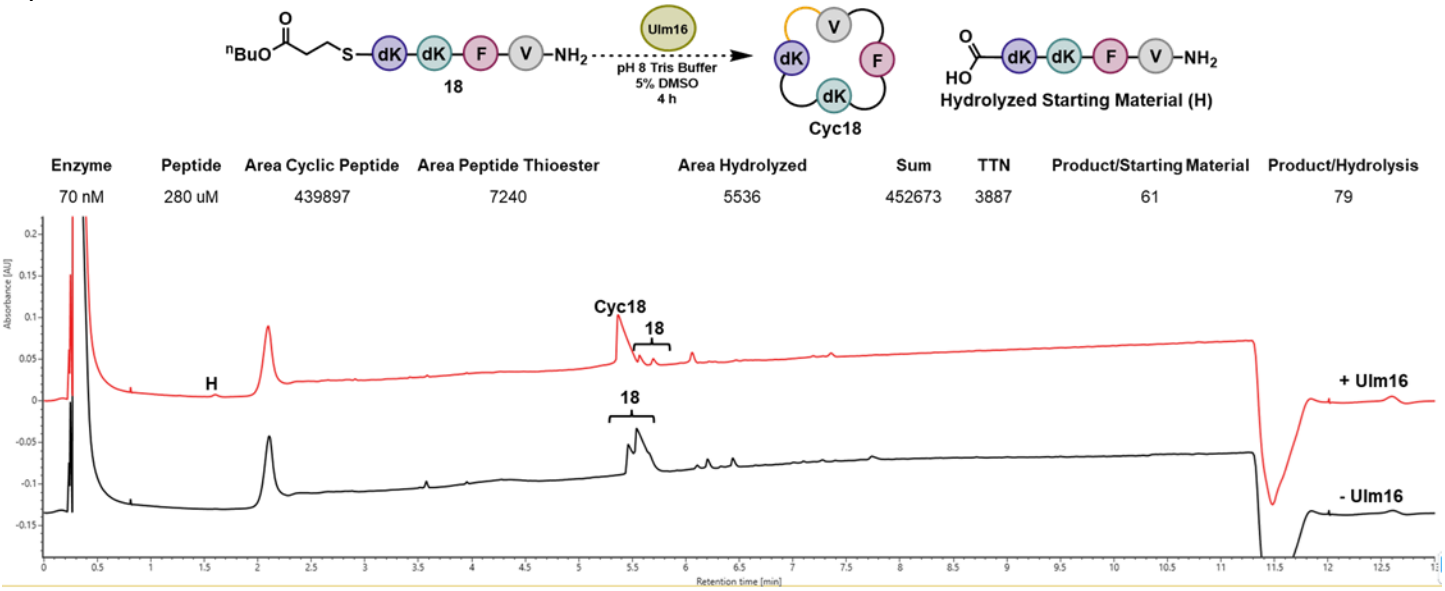
Supplementary Fig. 23: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrate 16. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. 'HD' and 'CD' represent hydrolyzed dimer and cyclic dimer, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



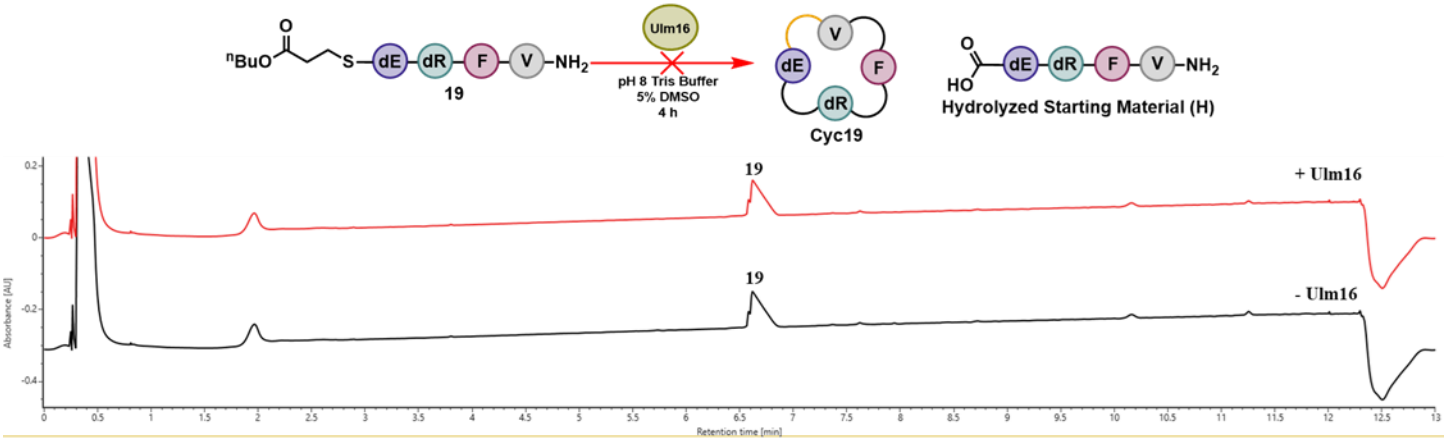
Supplementary Fig. 24: Spiking with synthetic standard to confirm cyclic tetrapeptide. UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with substrates 16, which was previously seen in Supplementary Fig. 27. To confirm the identity of the cyclic product, cyclic tetrapeptide standards and a cyclic octapeptide standard, which would be the head-to-tail cyclic dimerized product of substrate 16, were used. The cyclic tetrapeptide of substrate 16 matched in retention time but could not be isolated during purification (top panel). The cyclic octapeptide (CD) matched in retention time with a peak observed at ~9 minutes in the enzyme reaction, but not enough was produced to accurately determine a ratio.



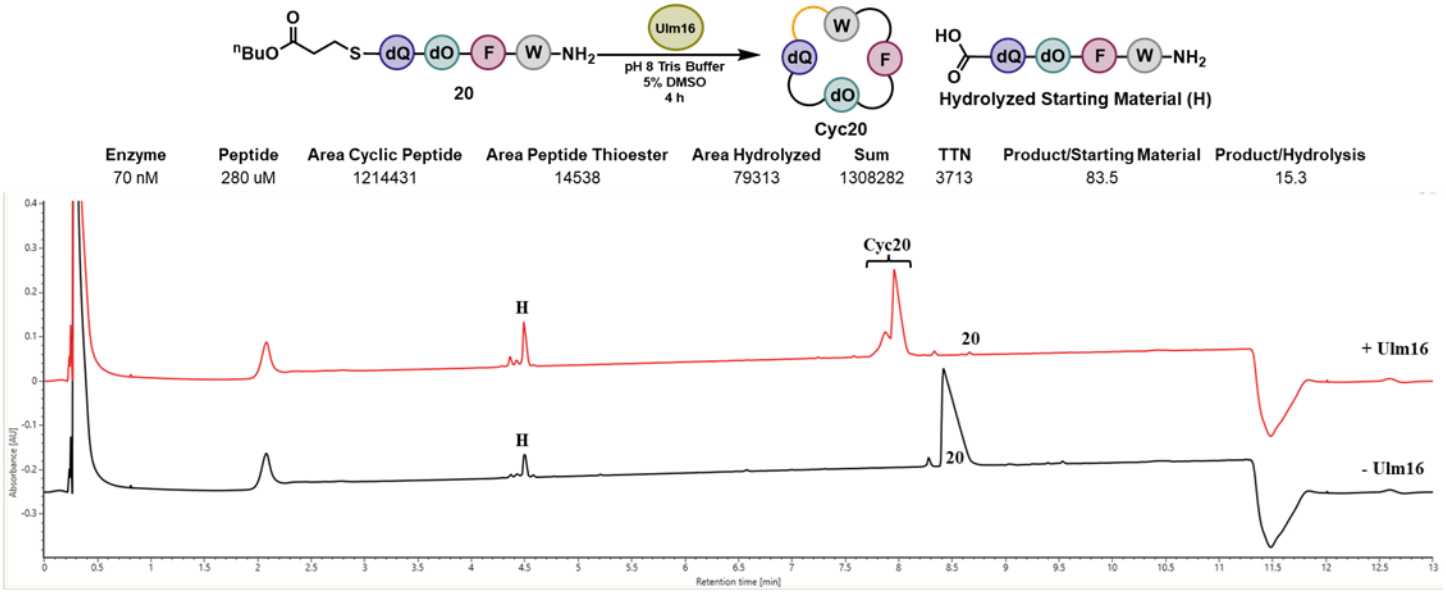
Supplementary Fig. 25: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrate 17. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



Supplementary Fig. 26: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrate 18. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.

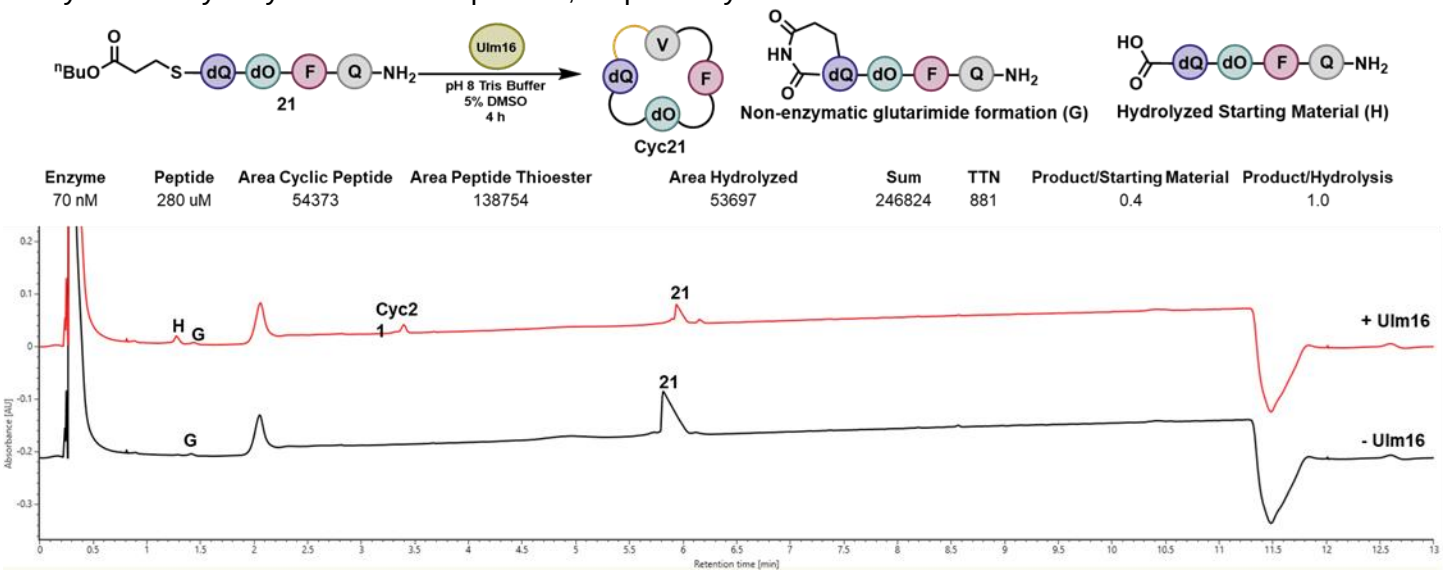


Supplementary Fig. 27: UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 19. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.

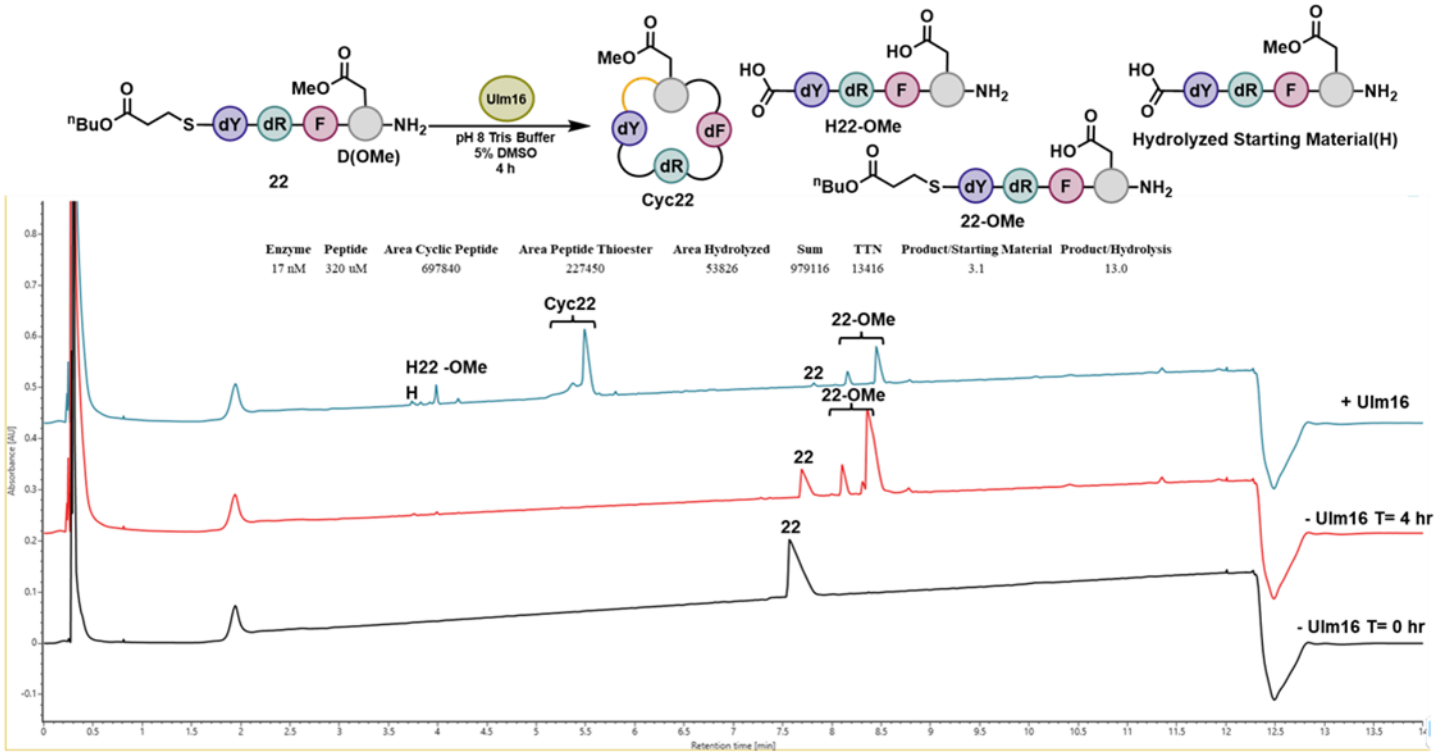


Enzyme	Peptide	Area Cyclic Peptide	Area Peptide Thioester	Area Hydrolyzed	Sum	TTN	Product/Starting Material	Product/Hydrolysis
70 nM	280 uM	1214431	14538	79313	1308282	3713	83.5	15.3

Supplementary Fig. 28: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 20. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively.

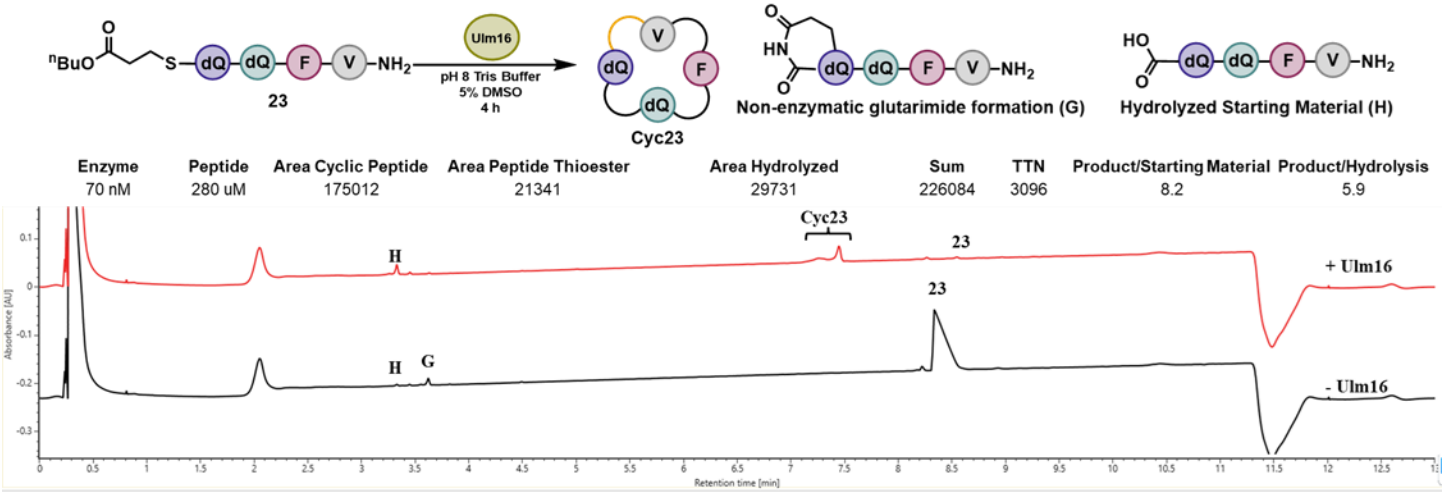


Supplementary Fig. 29: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 21. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively.

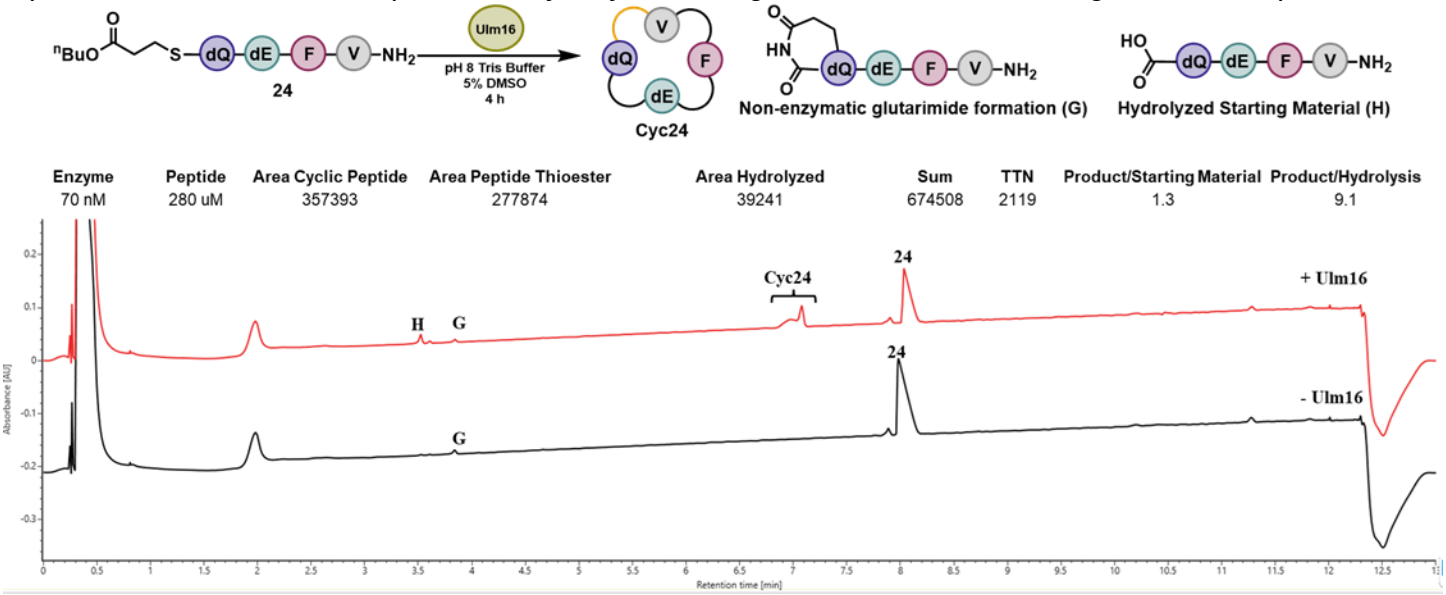


Supplementary Fig. 30: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 22. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. Rapid asparamide formation at the C-terminus resulted in both epimerization and deprotection of the N-Terminal aspartate. This phenomenon was also observed in an

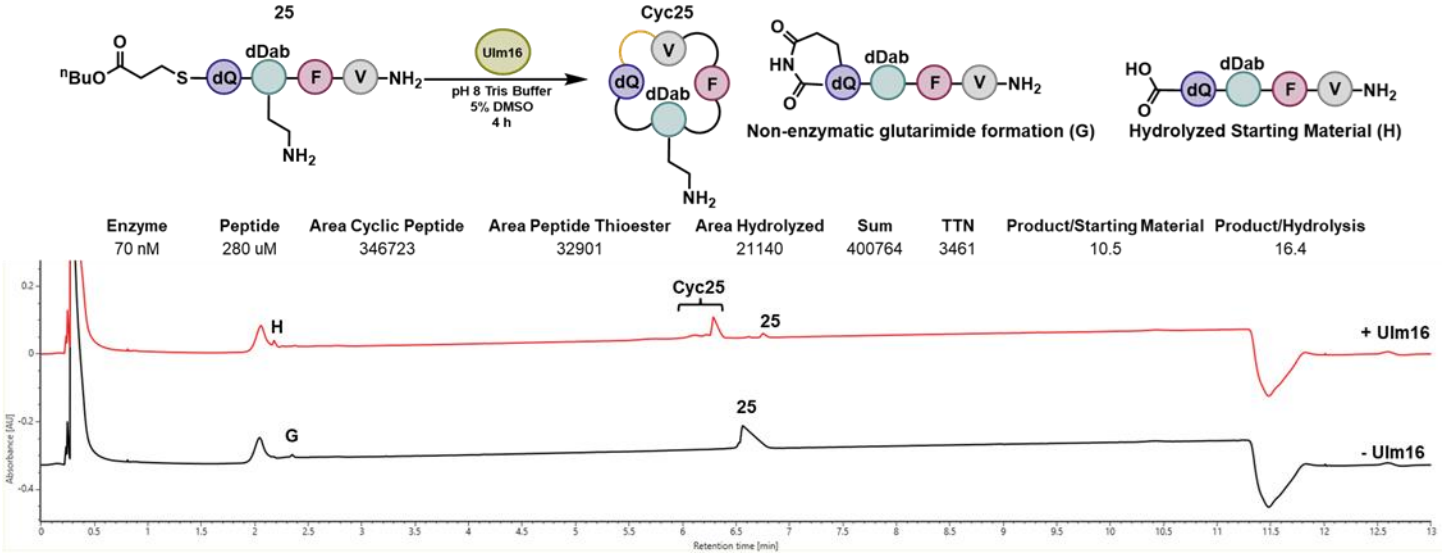
allyl glycine-protected variant (data not shown), but no cyclic product corresponding to the deprotected peptide with an N-Terminal aspartate was observed. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



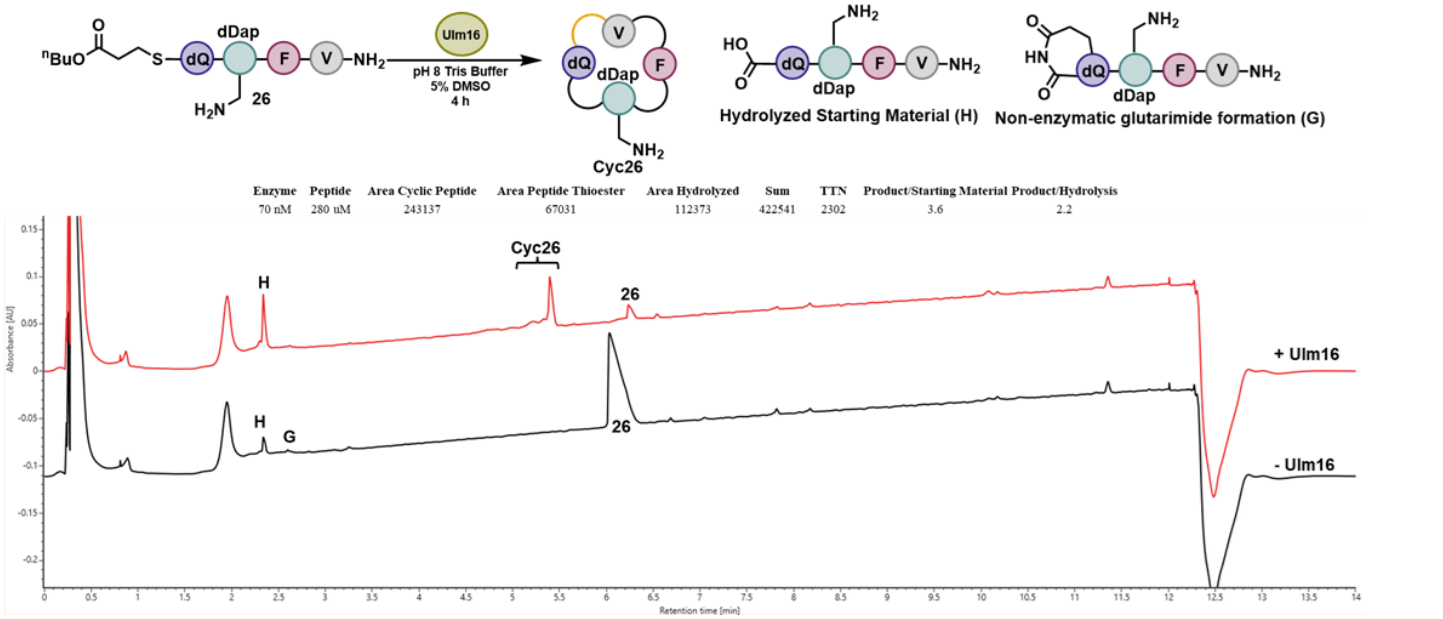
Supplementary Fig. 31: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 23. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



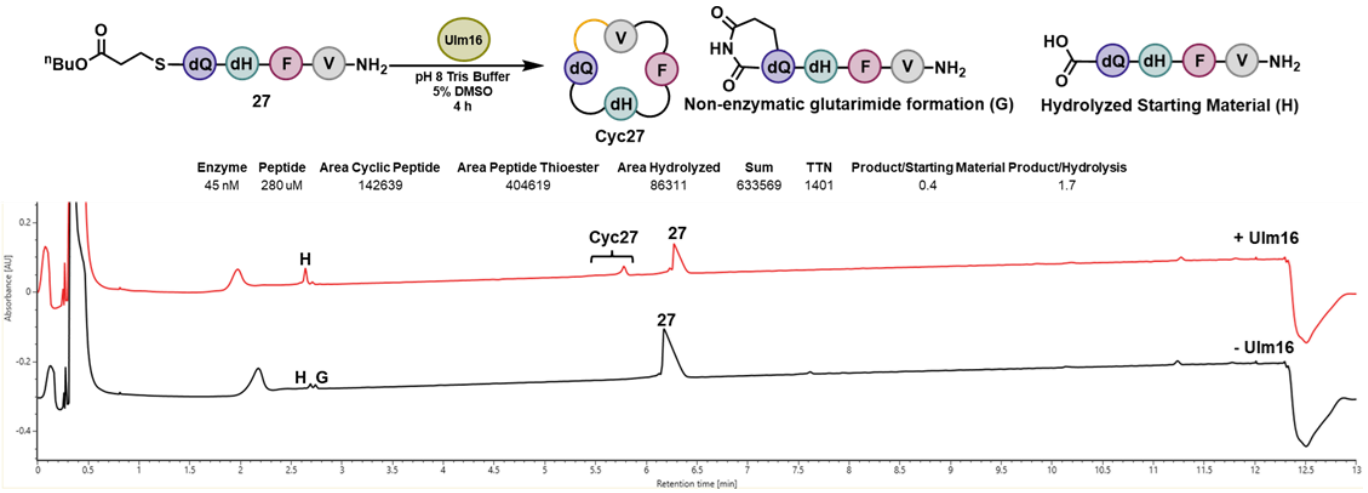
Supplementary Fig. 32: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 24. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



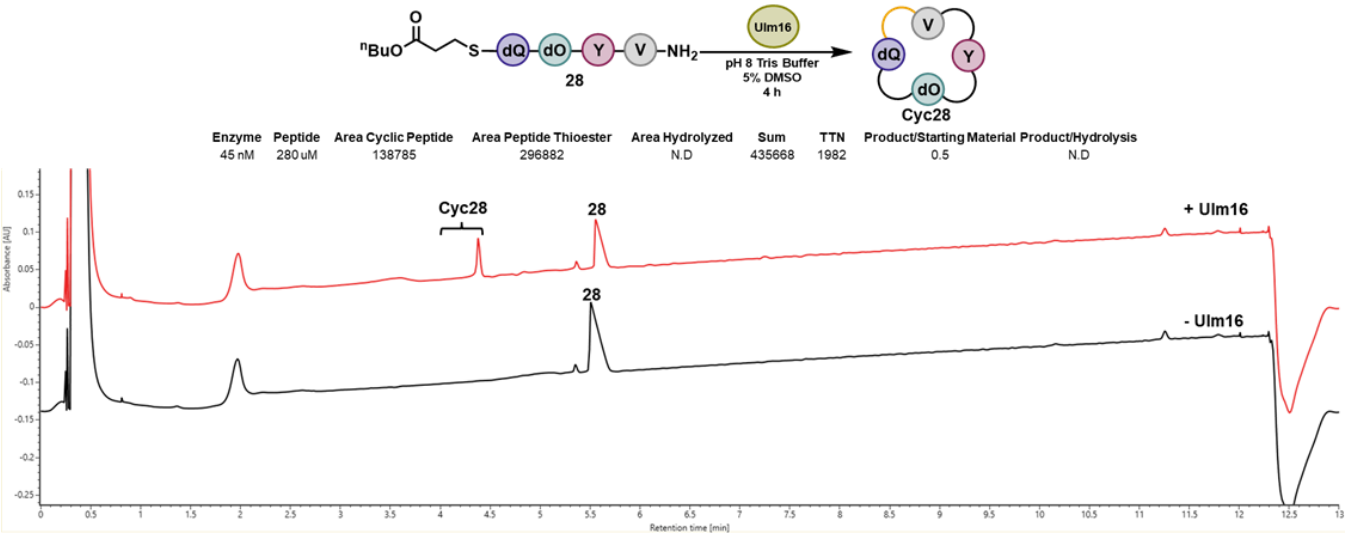
Sup Supplementary Fig. 33: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 25. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



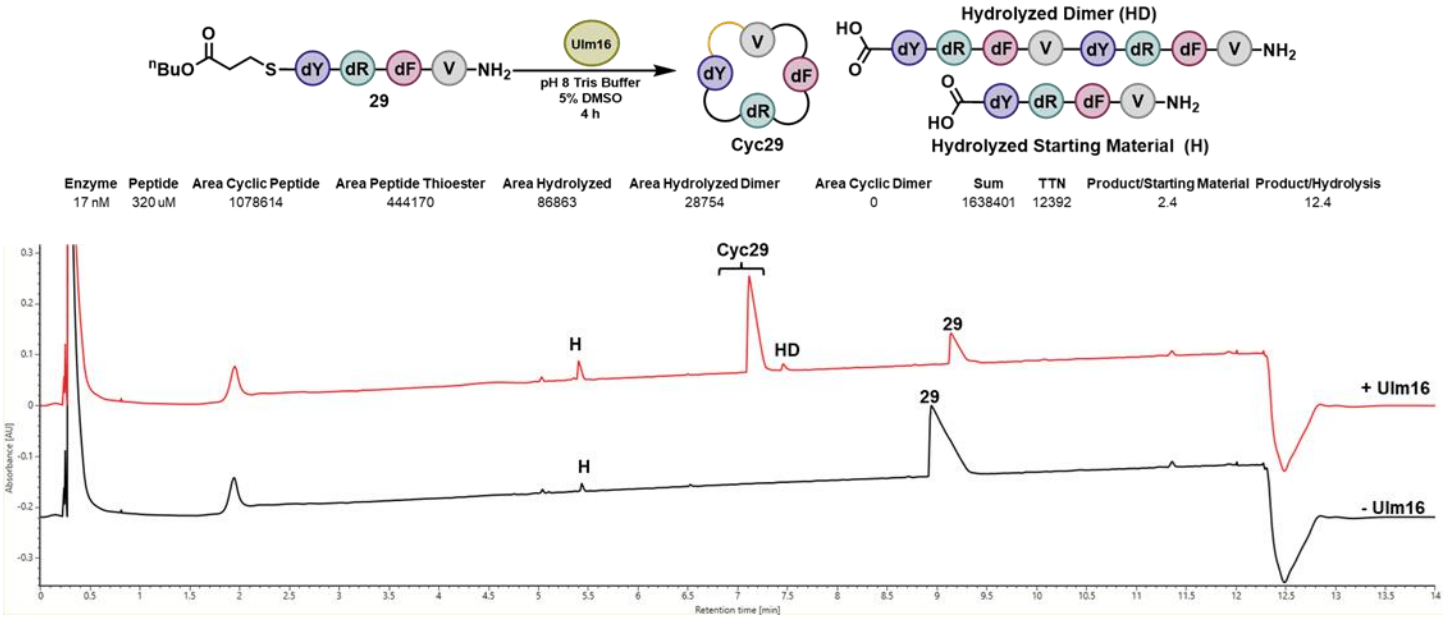
Supplementary Fig. 34: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 26. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



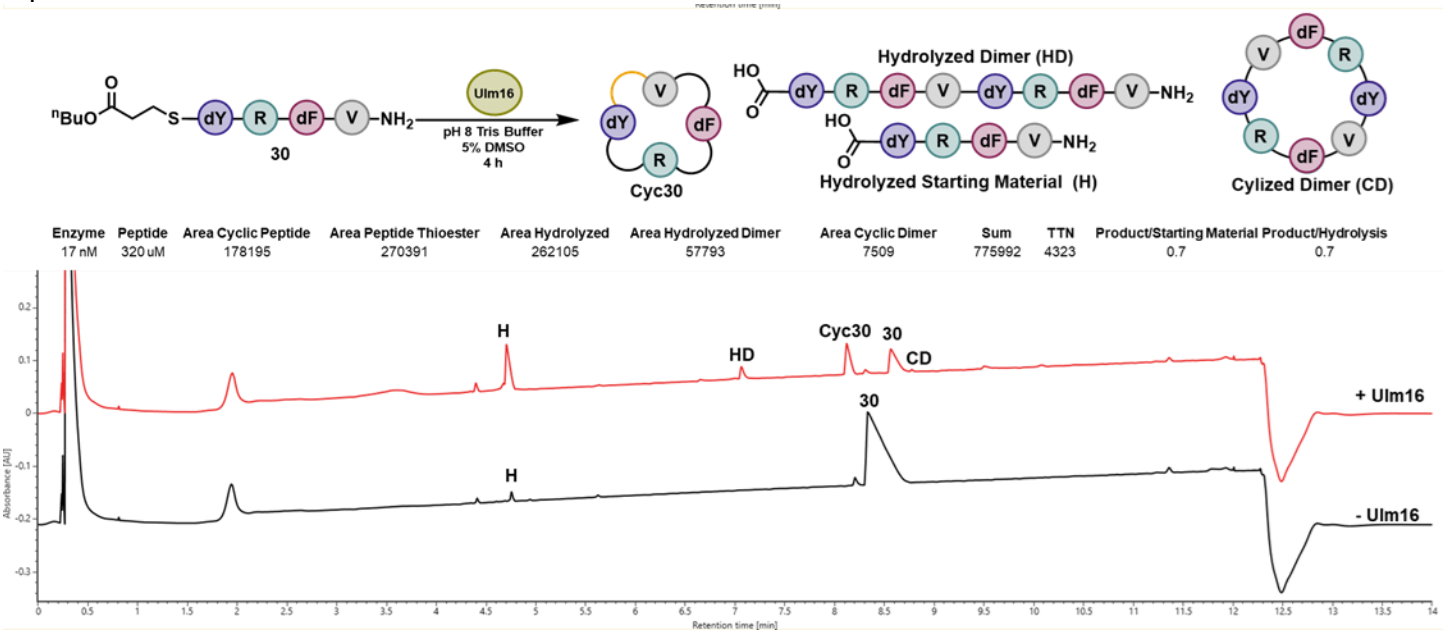
Supplementary Fig. 35: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 27. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



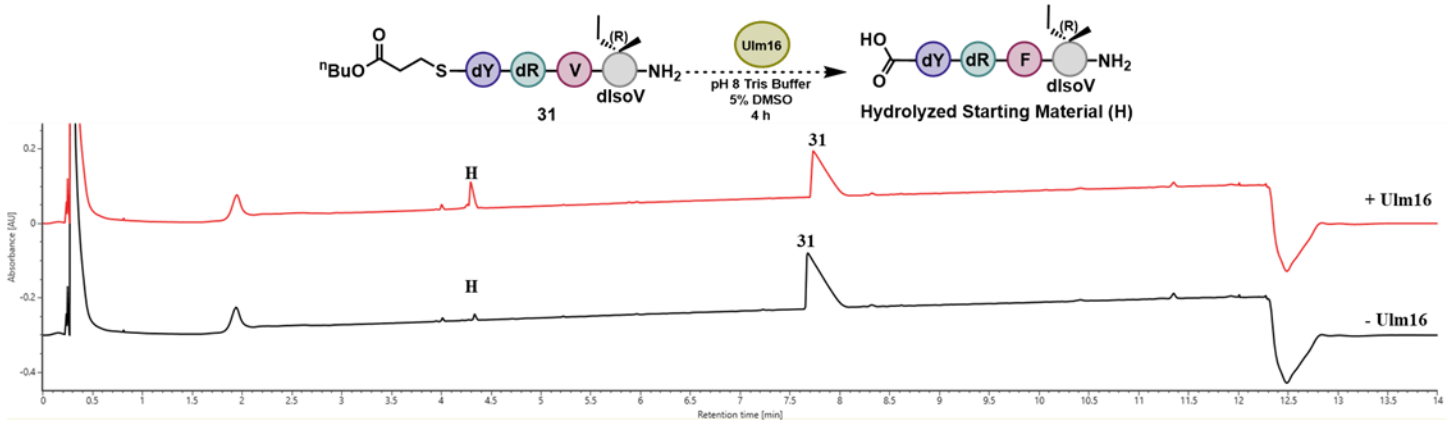
Supplementary Fig. 36: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 28. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



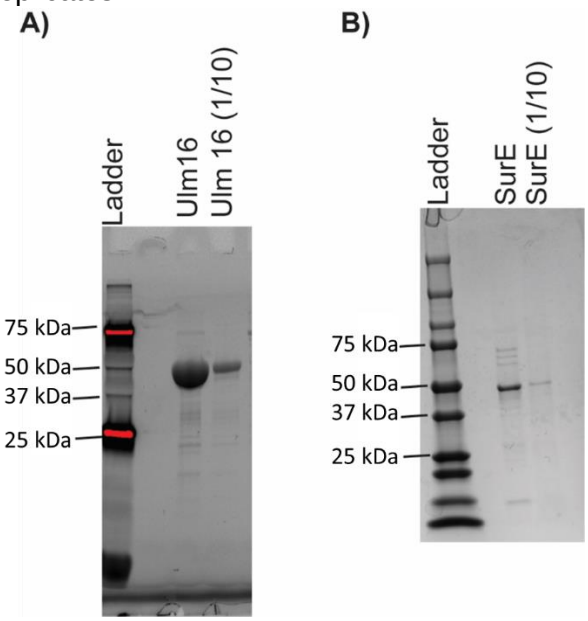
Supplementary Fig. 37: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 29. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. While CD and HD represent head-to-tail cyclized dimerized product and hydrolyzed dimerized product, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



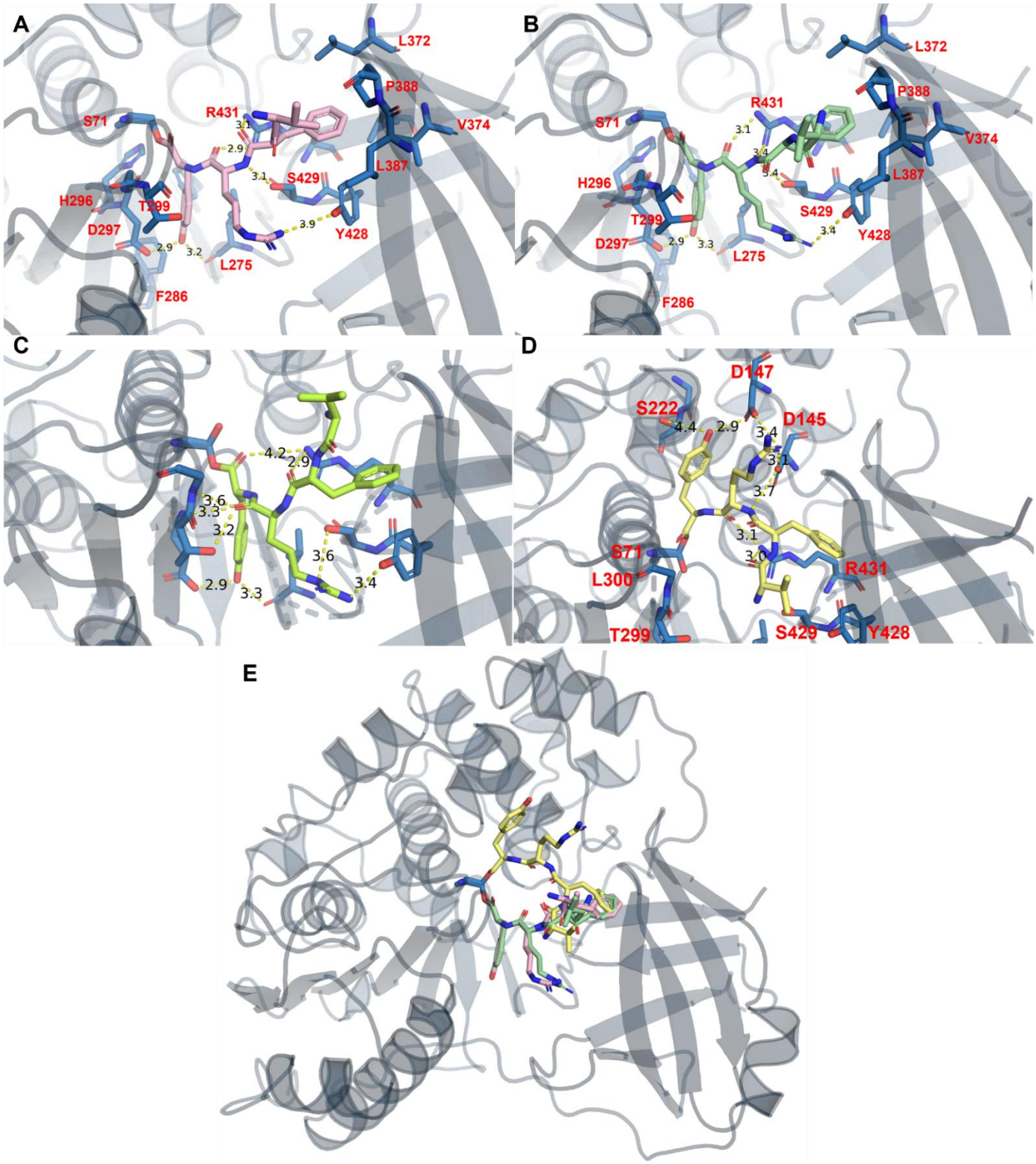
Supplementary Fig. 38: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 30. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. While CD and HD represent head-to-tail cyclized dimerized product and hydrolyzed dimerized product, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



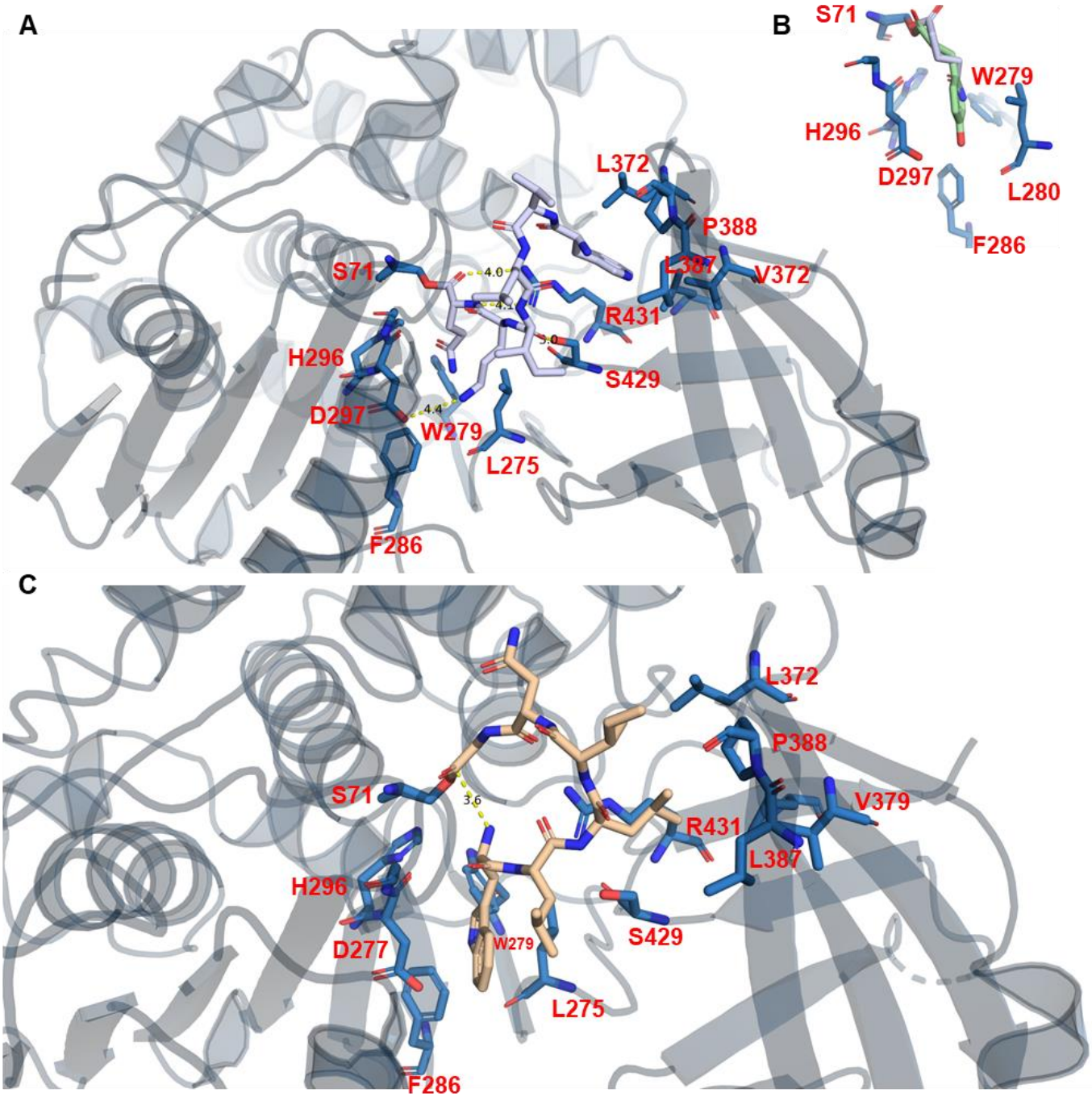
Supplementary Fig. 39: UPLC UV trace (214 nm) for the total turnover number (TTN) assay of Ulm16 with tetrapeptide substrates 31. The enzyme and substrate concentrations are indicated above the trace. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. While CD and HD represent head-to-tail cyclized dimerized product and hydrolyzed dimerized product, respectively. The UPLC trace is representative of three individual replicates, while the TTN and product to hydrolysis/starting material ratios are averages of three replicates.



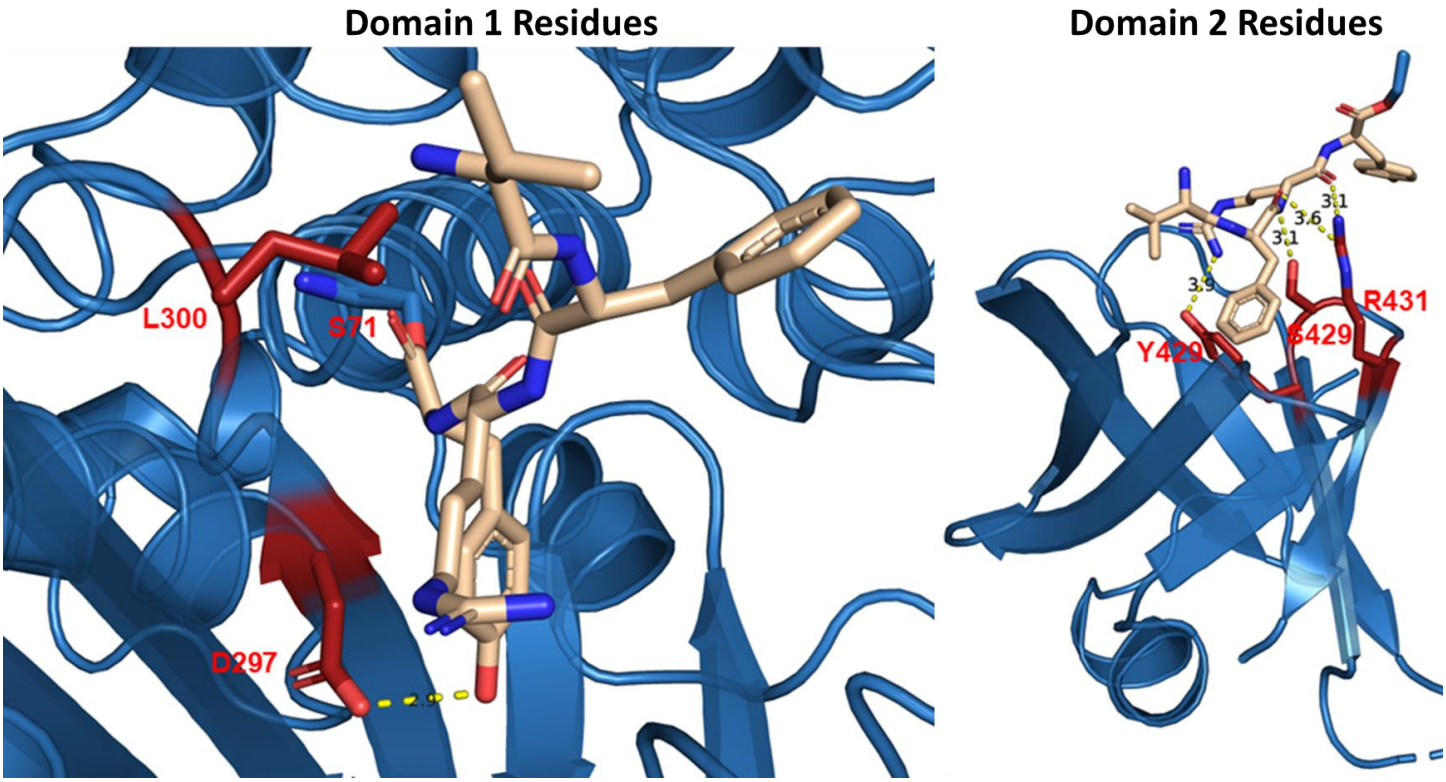
Supplementary Fig. 40: Expression of Ulm16 and SurE. A) Heterologous expression of Ulm16 and B) SurE. 1/10 indicates a 1/10 dilution of the initial protein stock used in the Ulm16 or SurE lane.



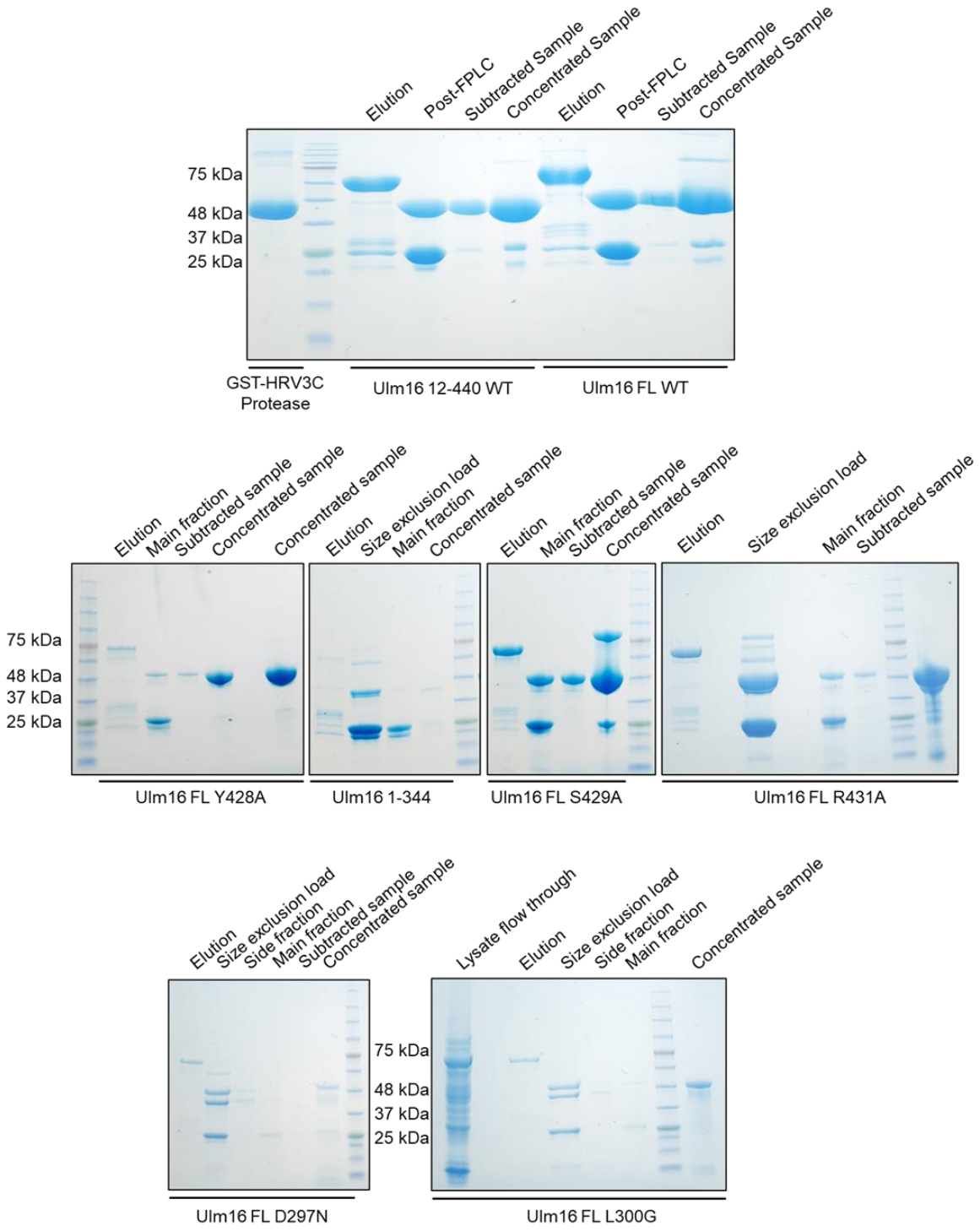
Supplementary Fig. 41: Covalent docking of tetrapeptides to the Ulm16 crystal structure. (A-D) Representative poses of peptide **29** (pink, A), peptide **16** (dark green, B), and peptide **30** (dark green or yellow, C-D) docked to Ulm16. Hydrogen bonding residues are labeled and shown in stick representation, while lipocalin domain residues involved in possible hydrophobic interactions are also displayed as sticks. Panels (A) and (B) show the lowest MMGBSA and dock score poses for peptides **29** and **16**, respectively. Panels (C) and (D) show the lowest dock score and MMGBSA score poses for peptide **30**, respectively. (E) Overlay of the lowest MMGBSA scoring poses for peptides **16**, **29**, and **30** in the Ulm16 active site, highlighting the different initial orientation of peptide **30**.



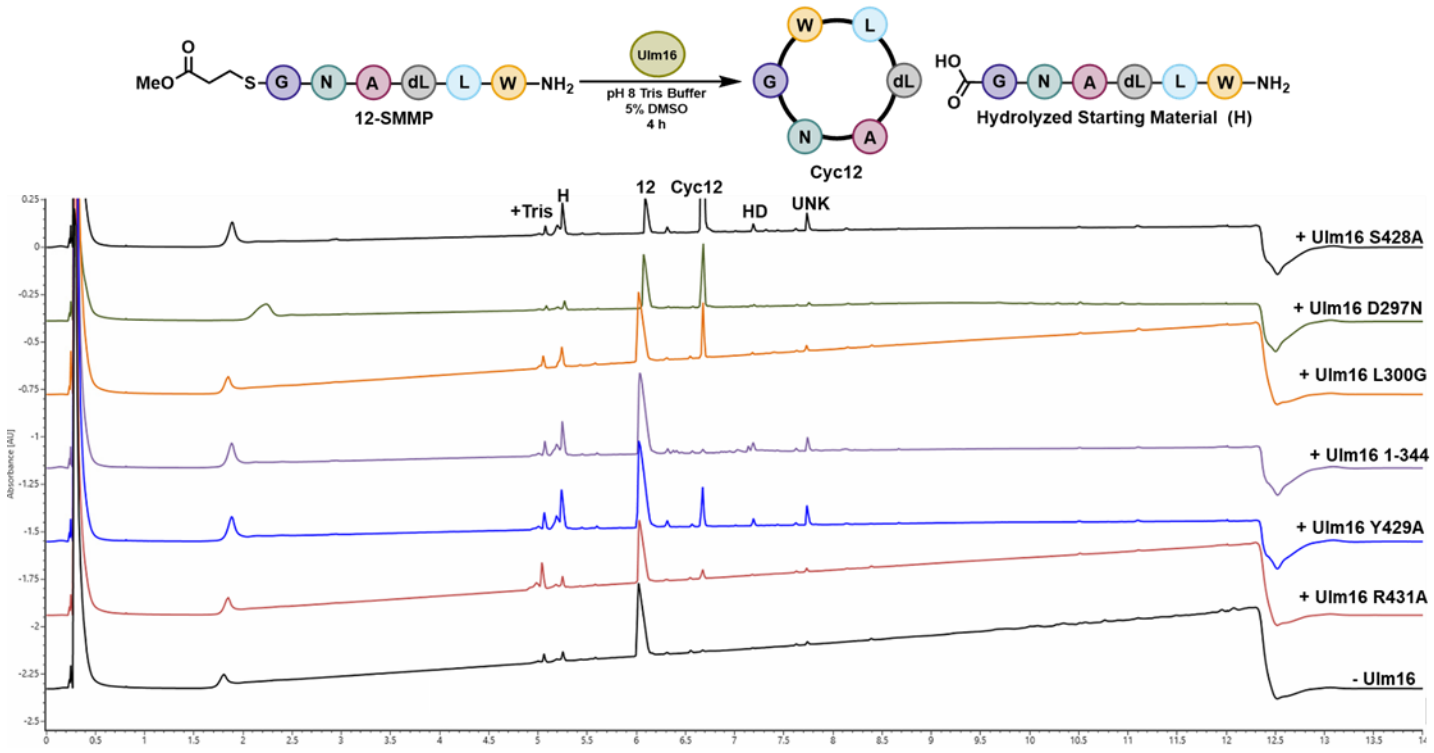
Supplementary Fig. 42: Covalent docking of hexapeptides to the Ulm16 crystal structure. (A-C) Representative poses of peptide 1 (light blue) and peptide 11 (tan) covalently docked to Ulm16. Hydrogen bonding residues are labeled and shown in stick representation, while lipocalin domain residues involved in possible hydrophobic interactions are also displayed as sticks. Panels (A) and (C) show the lowest MMGBSA and dock score poses for Peptides 1 and 2, respectively. Panel (B) shows the C-terminal D-Gln of the Ulm16 peptide overlayed with the C-terminal D-Tyrosine of peptide 16.



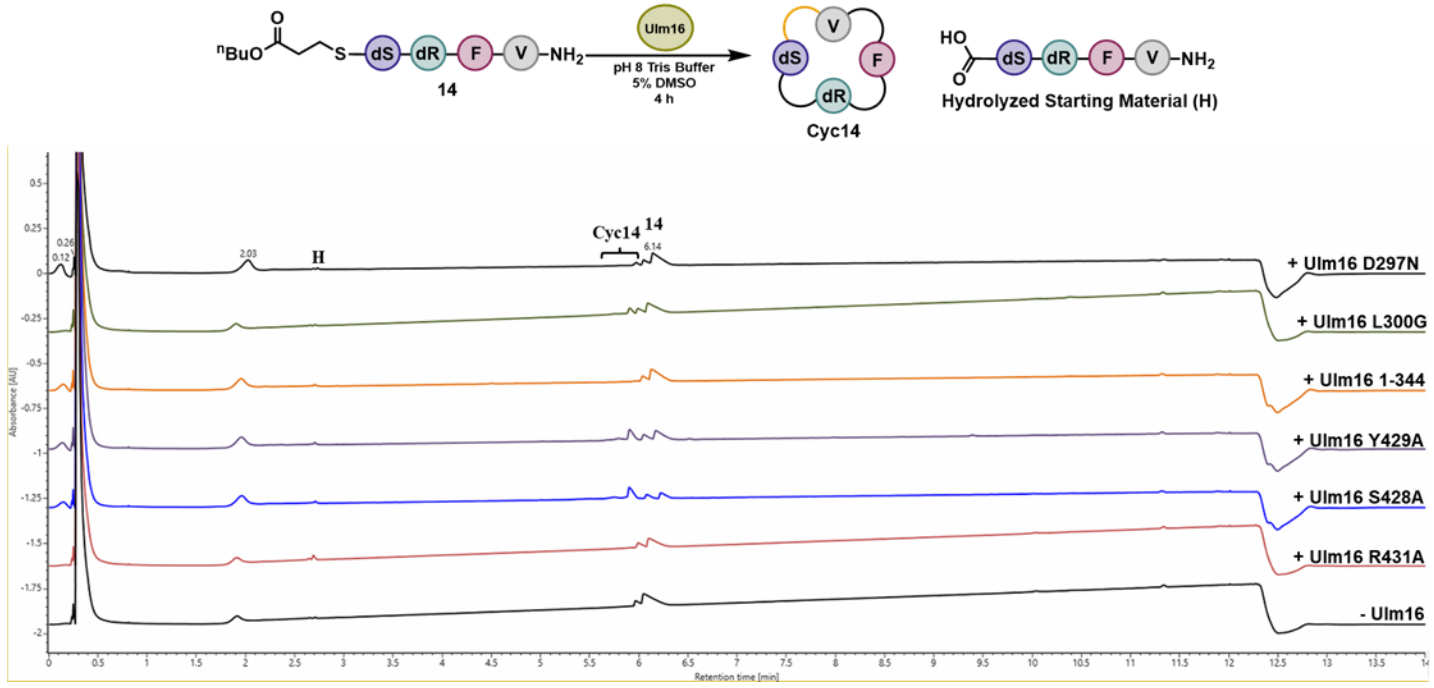
Supplementary Fig. 43: Residues mutated in SDM studies A) Close-up of mutated residues (L300 and D297) within Domain 1, highlighting their proximity and interactions with the covalently docked peptide 29. **B)** Close-up of mutated residues (Y428, S429, and R431) located in Domain 2, highlighting their proximity and interactions with the covalently docked peptide 29



Supplementary Fig. 44. Purification gels for GST tagged Ulm16 FL, Ulm16 FL mutants, and Ulm16 12-440. Purification gels as described in the following: HRV3C protease used to cleave GST-Ulm16 WT to GST and Ulm16 WT individually along with the purification of Ulm16 12-440 and FL constructs (topUlm16 FL mutants Y428A, 1-344, S429, R431A (middle); and Ulm16 D297N, L300G (bottom) . The fusion protein appears in the lysate elution at ~70 kDa and is cleaved by the HRV3C protease into fragments of Ulm16 (~45 kDa) and GST (two bands that appear near the 25 kDa marker). Tag subtraction and concentration lead to highly pure samples that are then used for assays.

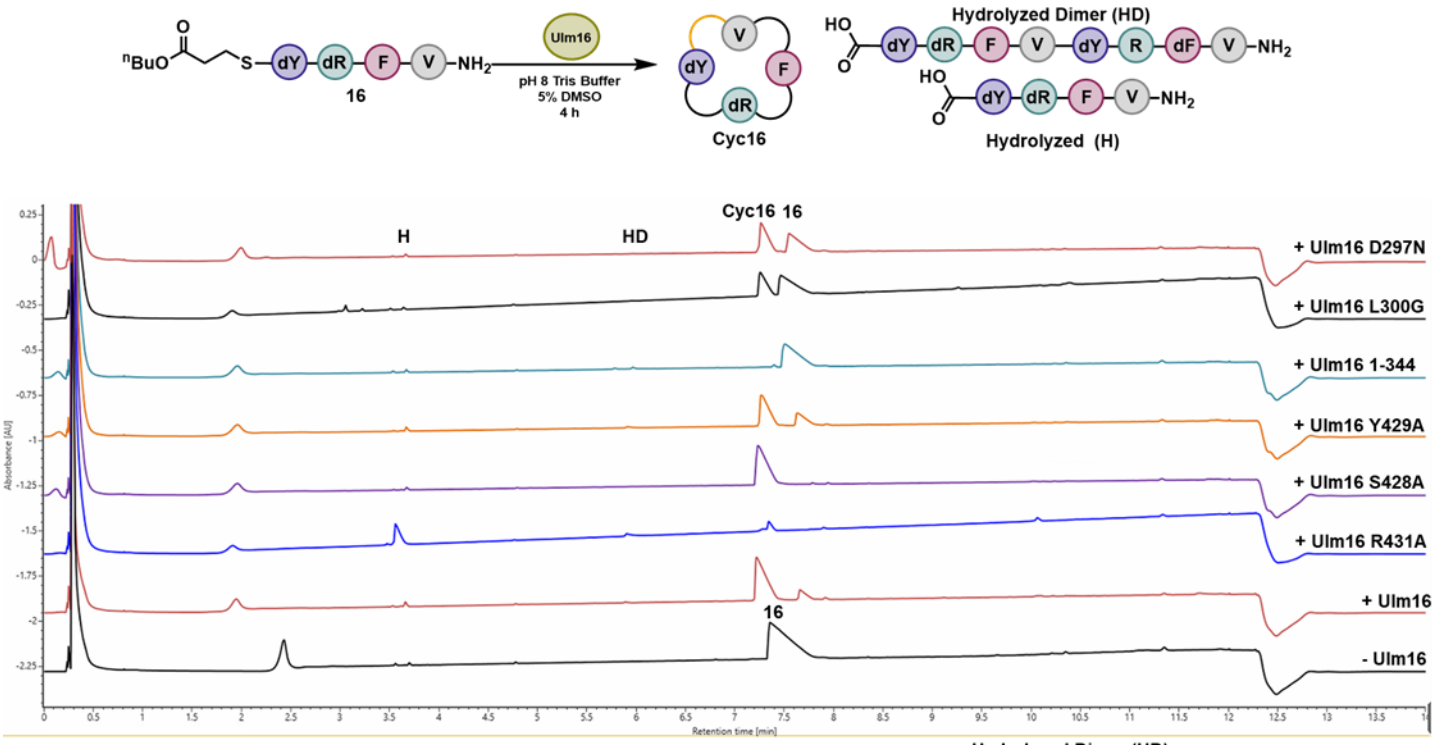


Supplementary Fig. 45. UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 mutant reactions with peptide 12-MMP. The enzyme and substrate concentrations can be found in Supplementary Table 2. Each UPLC trace is labeled with the Ulm16 Mutant tested. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. Other side reactions included dimerization followed by hydrolysis (HD) and addition of Tris to the C-terminus of the peptide (+Tris) The UPLC trace is representative of three individual replicates.

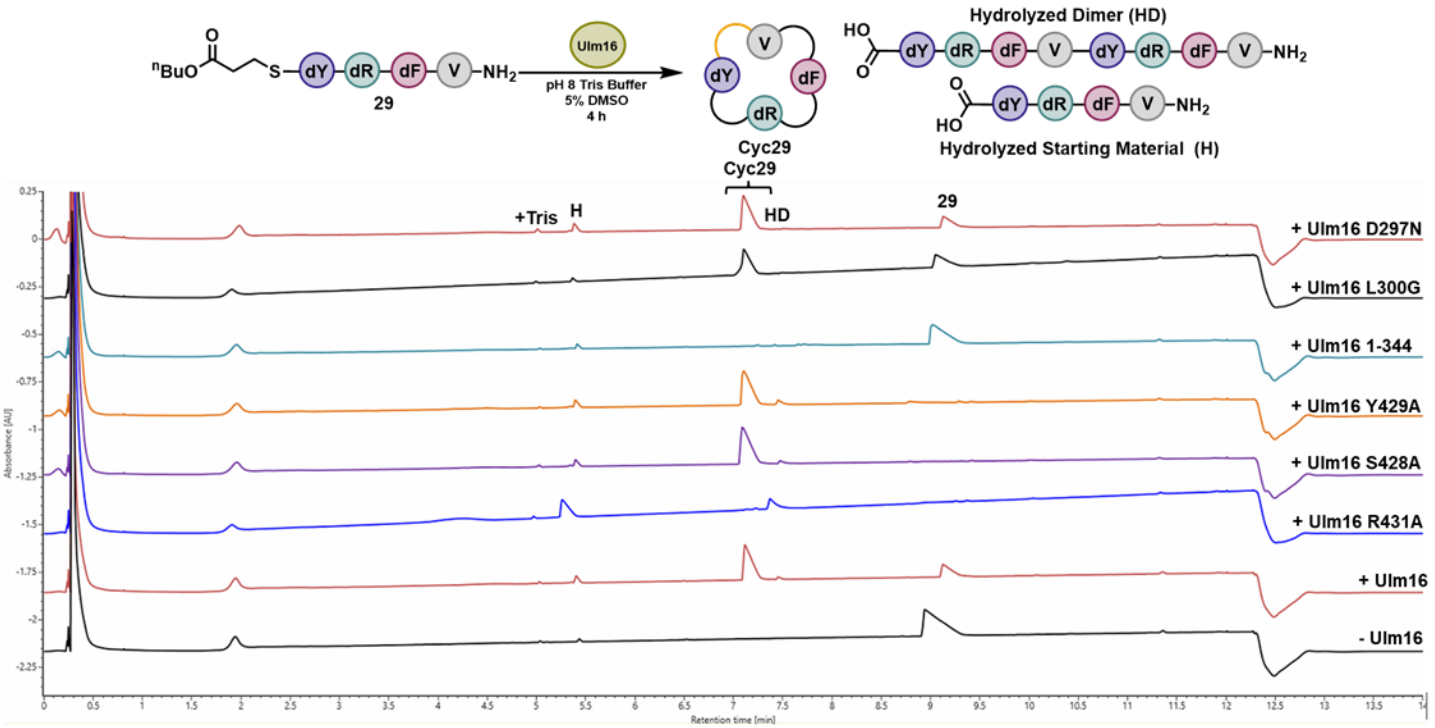


Supplementary Fig. 46. UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 mutant reactions with peptide 14. The enzyme and substrate concentrations can be found in

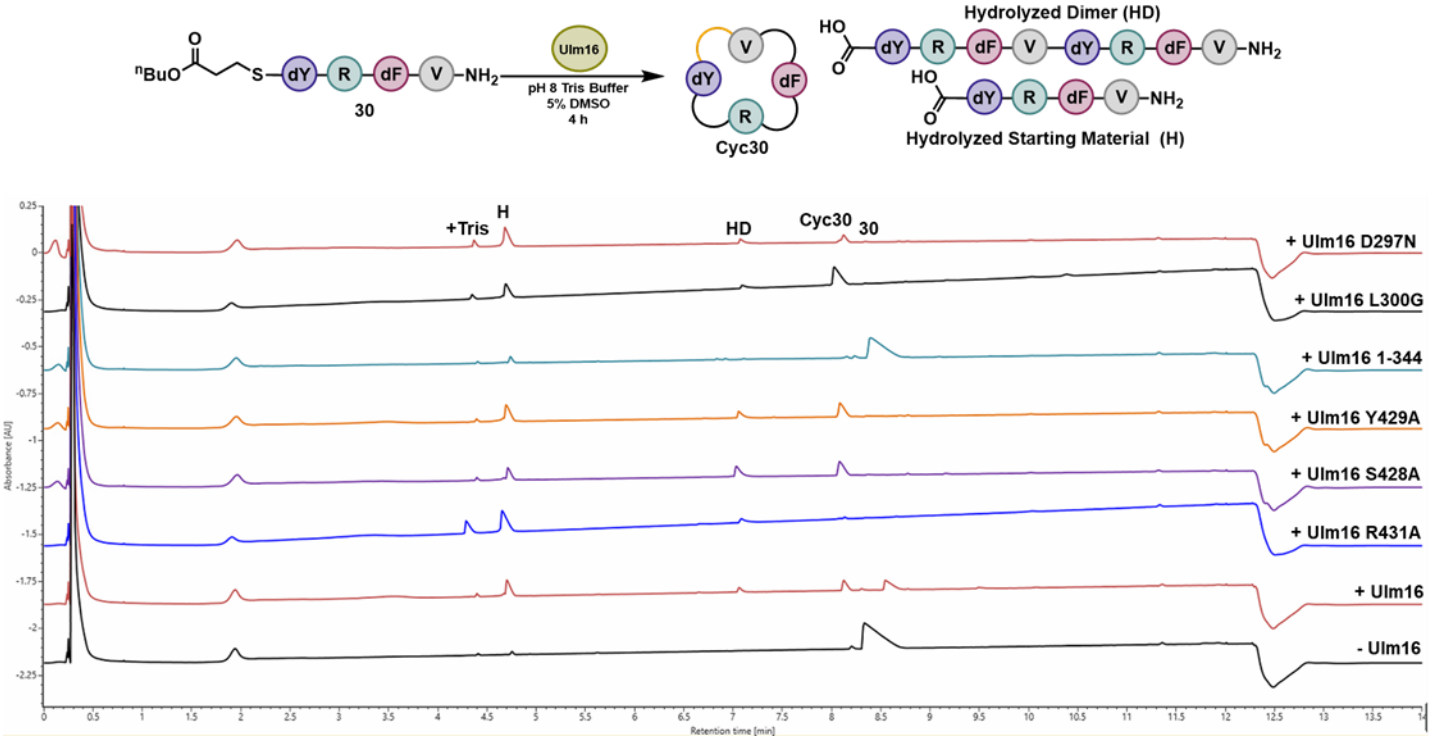
Supplementary Table 2. Each UPLC trace is labeled with the Ulm16 Mutant tested. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively.



Supplementary Fig. 47. UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 mutant reactions with peptide 16. The enzyme and substrate concentrations can be found in Supplementary Table 2. Each UPLC trace is labeled with the Ulm16 Mutant tested. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. Other side reactions included dimerization followed by hydrolysis (HD). The UPLC trace is representative of three individual replicates.

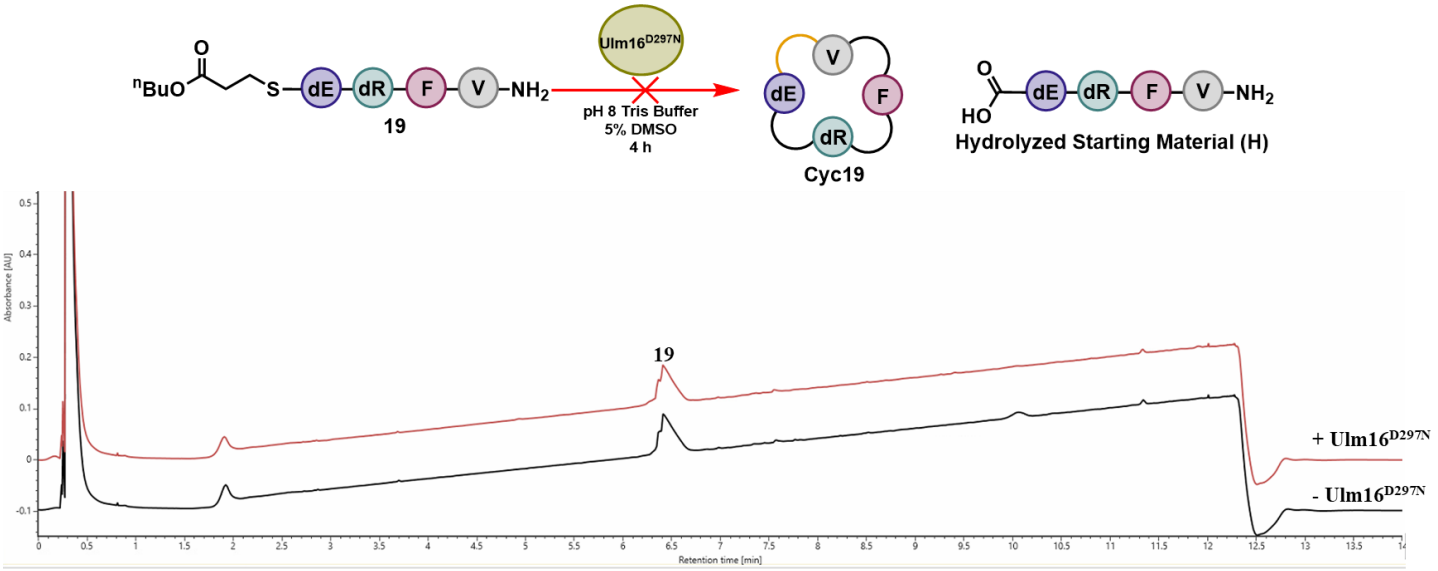


Supplementary Fig. 48. UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 mutant reactions with peptide 29. The enzyme and substrate concentrations can be found in Supplementary Table 2. Each UPLC trace is labeled with the Ulm16 Mutant tested. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. Other side reactions included dimerization followed by hydrolysis (HD) and addition of Tris to the C-terminus of the peptide (+Tris). The UPLC trace is representative of three individual replicates.



Supplementary Fig. 49. UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16 mutant reactions with peptide 30. The enzyme and substrate concentrations can be found in

Supplementary Table 2. Each UPLC trace is labeled with the Ulm16 Mutant tested. The substrates are labeled with their corresponding numbers from the paper, while 'Cyc' and 'H' represent head-to-tail cyclic and hydrolyzed linear compounds, respectively. Other side reactions included dimerization followed by hydrolysis (HD) and addition of Tris to the C-terminus of the peptide (+Tris) The UPLC trace is representative of three individual replicates.



Supplementary Fig. 50. UPLC UV trace (214 nm) is shown for the total turnover number (TTN) assay of Ulm16^{D297N} mutant reaction with peptide 19. A concentration of 70 nM Ulm16 and 280 µM of substrate was used however like with the wild type reaction no cyclic peptide or hydrolyzed peptide was observed. The UPLC trace is representative of three individual replicates.

Supplementary note 1: Gene sequence for Ulm16

ATGCACGGGGACTATGCGGATCCAGCGGATTGCGGCGCCGGTGACGGCGCCCCCGTCGGCCTCGACCT
GGACCGCCTCGCCCGTGACTGCGACGTCGTCGGCGGGCAGCTGGCCCTCCATCACCAAGGCACCCTGA
CCACCTGGGAGTTCGGCACCGAGGAGCACGCCGGCGGGCGTCCGGTACACGTCGGGTCGGCGTTCCC
GTACGGGTCGGTGACCAAGGCGTTCACCGCCACCGCCGTTCTGCAACTGGCCGGTGACGGGGACCTGG
ACCTCGACCGACCGGTACGGGAACTGCTCCCCGAGGCCGAGGCCGAGGCCGAGATCGAGGTCGAGGCA
GGGTCCGGGACCGGTGCGCGCGCGGACGGCGGCCACCCGGCCCTCGCCGCCACCCTCCGCCAACTCC
TCAGCCACACCGCCGGACTCCCGTCCGACCACGACGACGAGCGCGCCCCCTCGCTGCGCCGCTGGCTC
ACCGGCTTCCTGGCACTGCCGGTCGGCCCCTGGCCCGCACCCGGCTCCTTCTCCTACTCCAACGTCGGC
TACGGCATCGCGGGCCGCGTCGTGGAGGCCGTCACCGGACTGACCTGGTCCGAGGCGGTACGGGACTT
CCTGCTGCACCCGCTGGGCACCGCCATCACCGTGCTGCCCACCGACCCCGGCTCGCTGCCCGCGGGCG
GTCTCGCGGGCAGCGCCGCCGACCTGGTACGGCTGGGCCGGCTGCATCTGGACGAGCCCGGTGACCC
GGACCTGGCCCGCCTCGCCGACCCGGACGCCCTGCGGGAGATGGCCCGGCCCACCGCCGGCGCCGAC
CCCTTCGGGCTGGCGGACGGCTGGGGACCGGGCCTGGGCCGGTTCGGCCCCGCCGGCAACCGCTGGC
TCGGCCACGACGGCACCCTGGACGGCGCCACCTGCCATCTGCGCATCCACCCCGGGCGCGGCACCGTC
GTCGCGCTGACCACCAACTCCCCGACCGGGCAGGCCCTGTGGGACGCCGTGGTCGACGCACTGCGGGA
CGCGGACATCGACGTCGGCGTCCACCGCCCCGCGCCCCCGCCTGCCATCGCCGCGGCGGCCTTCGCG
GACTGTACGGGTACCTACCGCAACGGCGACCTGGCGGTGACGGTCGGCATCGACGGCCCGTACCTCGT
ACTTGAACTCCCCGGCGGCGCACGGGAGTTGGCGCAGCCACTGGCTCACCGGACGTTCTCCTCGCGGG
GGGCCGGCTTCCTCGGGCGCTTTGTCACCGACGCGCGCTCCGACGCCGTCCACGCCCTCCAGTACAGC
GGACGCACCCTCCTGCGGGAAGCGGGAGAGTCCCGCCGCGCACGGTCGCACTGA

Supplementary note 2: Gene sequence for SurE

ATGGGTGCCGAGGGGGCGGAACGGGACGCGGTCGGCGCGCTCTTCGAGGAGCTGGTGCGCGAGCACC
GGGTCACCGGGGCGCAGCTGGCCGTCTACCGCGACGGCGCCCTCAGCGAGTACGCCACCGGCCTCGC
CTCGGTCCGCACCGGTGAACCGGTCACCCCCCGGACGGGTTTCCCCTTCGGCTCGGTGACCAAGTTCCT
CACCGCCGAGCTGGTCATGCAGTTCGTCTGCGACGGCGACCTGGACCTCGACGACCCCCTCGCCGGGC
TCCTCCCCGACCTGGGGCGCGCCGCCGGCCCGGCCCTCGGCACCGCCACCGTCCGCCAGCTCCTCAG
CCACACCGCCGGCGTGGTGGACAGCATCGAGTACGACGAGATGCGCGGTCCCTCCTACCGGCGGTTCG
CCGCGACGTGCGCCCGGCAGCCCGCGCTCTTCCCGCCGGGCCTCGCCTTCTCCTACTCCAACACCGGC
TACTGCCTGCTGGGCGCGGTGATCGAGGCGGCGTCGGGGATGGACTGGTGGACGGCGATGGACAGTTG
CCTGCTGCGTCCGCTCGGCATCGAGCCGGCCTTCCTGCACGACCCGCGCCCCGGCCAGGGCGGCGCC
GCCCGGCCGGTGGCCGAAGGCCACGCGCTGCGCGCCGGCGGCGAGCGGGCCGAGCGCGTCGACCAC
ATGGCCTCGCTCTCGCTGGCCGCCGCCGGCGGGCTGGTCGGCAGCGCCACCGACCTGGTCACCGCGG
CCCGCCCGCACCTGGCCGACCGGAAGACCTTCGCCCAGCACGACCTGCTCCCCGAGGACGCCGTCCTC
GCCATGCGCACCTGCGTGCCGGACGCCGAGCCGTTCGGCCTGGCCGACGGCTGGGGGCTGGGCCTGA
TGCGCCACGGCACCGGCGACGGCGCCTGGTACGGCCACGACGGCGCGGTGGGCGGCGCCTCCTGCAA
CCTCCGTATCCACCCGGACCGTTCGCTGGCGCTGGCGCTGACCGCCAACTCCACCGCCGGGCCGAAGC
TGTGGGAGGCGCTGGTCGCGCGGCTGCCGGAGGCCGGGCTCGACGTGGGCCACTACGCGCTGCCCGT
CCCCGACTCCGCGCCGCTGGCCCCGGACGTCGGCCACCTCGGCACCTACGCCAACGGCGACCTGGAGC
TGATGGTGACCCACGATGCCGCCGGCGACCTCTTCCTGACCCGCGAGAGCTACTCCGACTACCGCCTCT
CCCTGCACGAGGACGACCTCTTCGTGGCCCGGAGCGGCGAGCCCGGCGCGCTCCCGATCACCGGCCG
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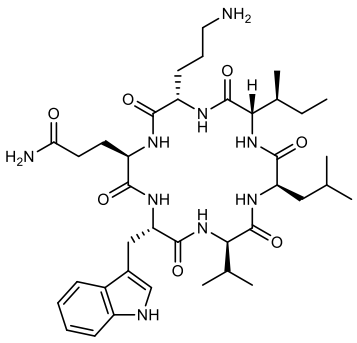
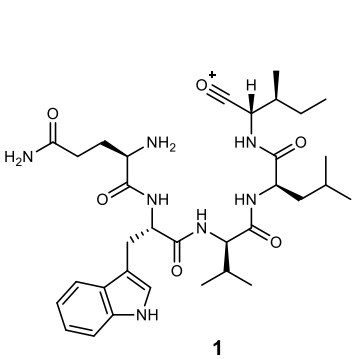
CTCTGA



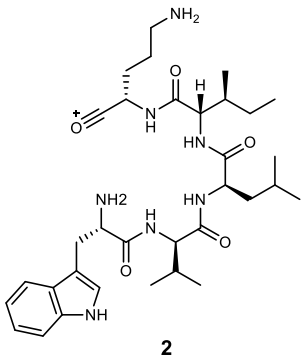
Cyc1

Chemical Formula: $C_{38}H_{59}N_9O_7$
Expected Neutral Mass: 753.4537
Observed Neutral Mass: 753.4538
Mass Error (ppm): < 0.1



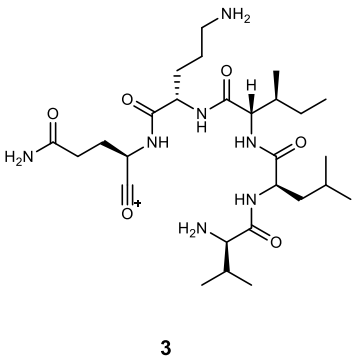
1

Chemical Formula: $C_{33}H_{50}N_7O_6^+$
Expected m/z: 640.382
Observed m/z: 640.383
Mass Error (mDa): 1.0



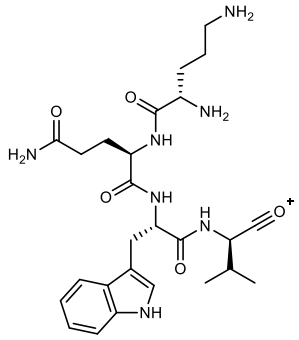
2

Chemical Formula: $C_{33}H_{52}N_7O_5^+$
Expected m/z: 626.402
Observed m/z: 626.400
Mass Error (mDa): 2.0



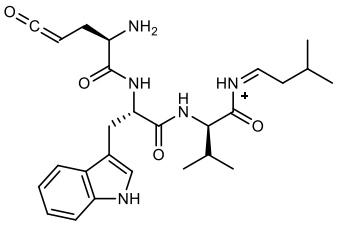
3

Chemical Formula: $C_{27}H_{50}N_7O_6^+$
Expected m/z: 568.382
Observed m/z: 568.381
Mass Error (mDa): 1.0



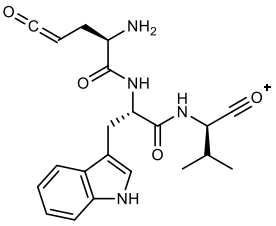
4

Chemical Formula: $C_{26}H_{38}N_7O_5^+$
Expected m/z: 528.293
Observed m/z: 528.297
Mass Error (mDa): 4.0



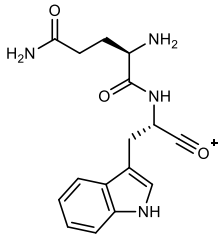
5

Chemical Formula: $C_{26}H_{36}N_5O_4^+$
Expected m/z: 482.276
Observed m/z: 482.279
Mass Error (mDa): 3.0



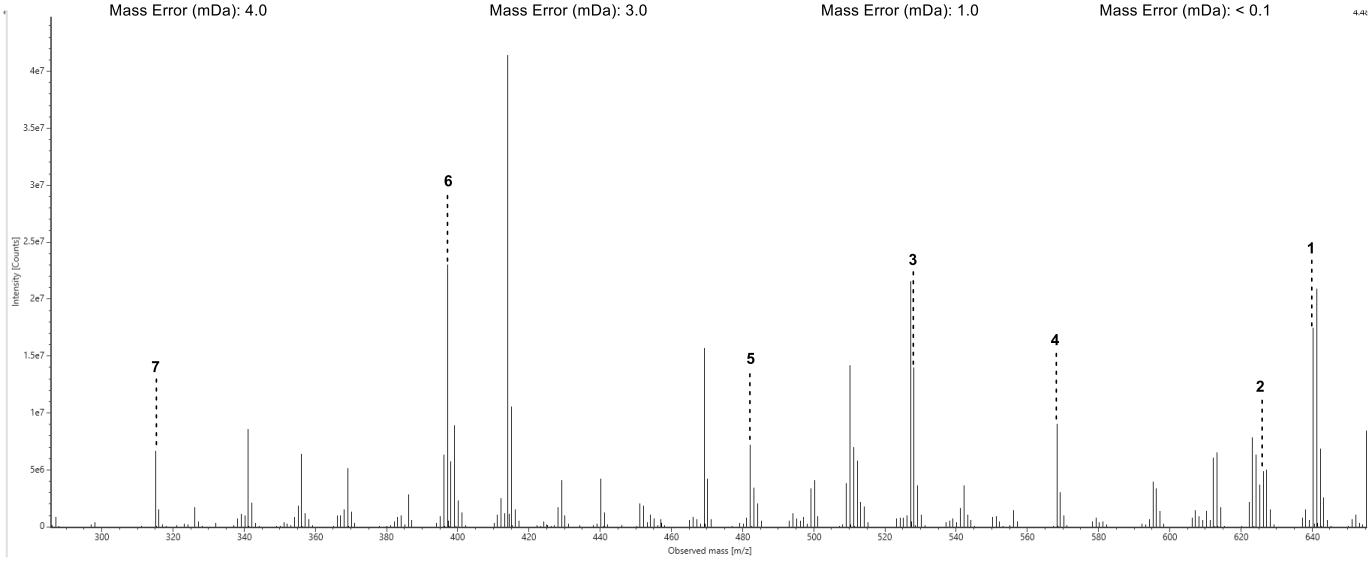
6

Chemical Formula: $C_{21}H_{25}N_4O_4^+$
Expected m/z: 397.187
Observed m/z: 397.188
Mass Error (mDa): 1.0



7

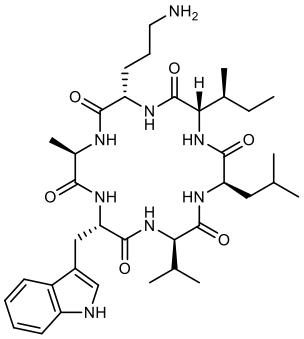
Chemical Formula: $C_{16}H_{19}N_4O_3^+$
Expected m/z: 315.145
Observed m/z: 315.145
Mass Error (mDa): < 0.1



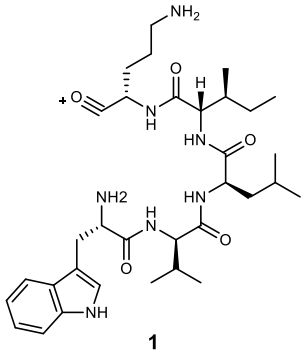
MS/MS Spectra of Cyclic Peptides Identified in this Study

Supplementary Fig. 51: MS2 spectrum for Cyclic peptide 1 (Cyc1)

Supplementary Fig. 52: MS2 spectrum for Cyclic peptide 5 (Cyc5)

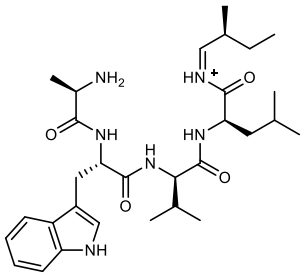


Chemical Formula: $C_{36}H_{56}N_8O_6$
Expected Neutral Mass: 696.4322
Observed Neutral Mass: 696.4322
Mass Error (ppm): < 0.1



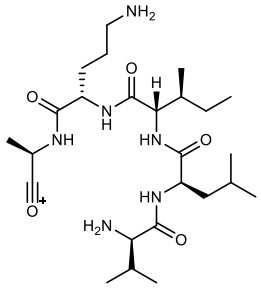
1

Chemical Formula: $C_{33}H_{52}N_7O_5^+$
Expected m/z: 626.402
Observed m/z: 626.403
Mass Error (mDa): 1.0



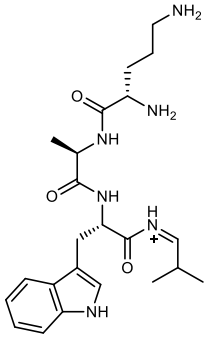
2

Chemical Formula: $C_{30}H_{47}N_6O_4^+$
Expected m/z: 555.365
Observed m/z: 555.367
Mass Error (mDa): 2.0



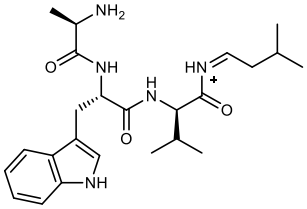
3

Chemical Formula: $C_{25}H_{47}N_6O_5^+$
Expected m/z: 511.360
Observed m/z: 511.359
Mass Error (mDa): 1.0



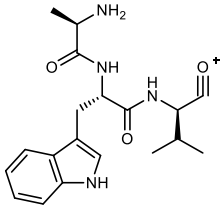
4

Chemical Formula: $C_{23}H_{35}N_6O_3^+$
Expected m/z: 443.277
Observed m/z: 443.278
Mass Error (mDa): 1.0



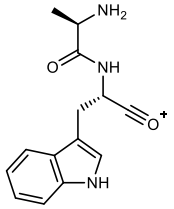
5

Chemical Formula: $C_{24}H_{36}N_5O_3^+$
Expected m/z: 442.281
Observed m/z: 442.283
Mass Error (mDa): 2.0



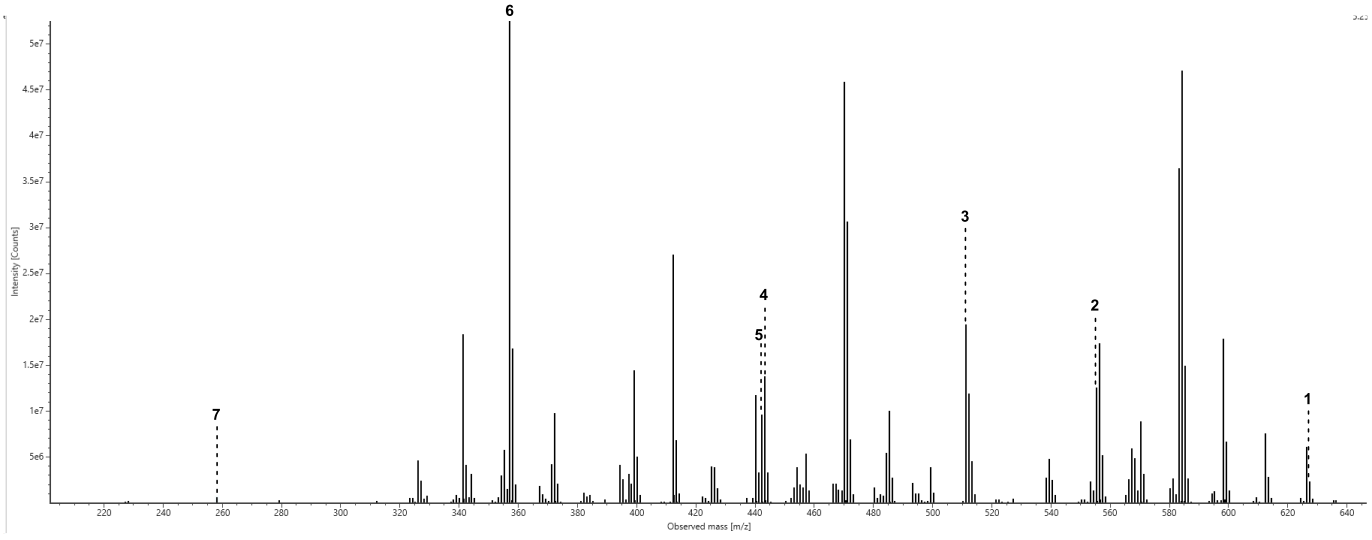
6

Chemical Formula: $C_{19}H_{25}N_4O_3^+$
Expected m/z: 357.192
Observed m/z: 357.192
Mass Error (mDa): < 1.0

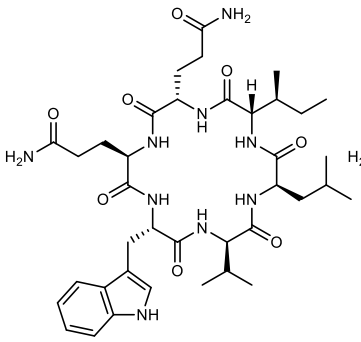


7

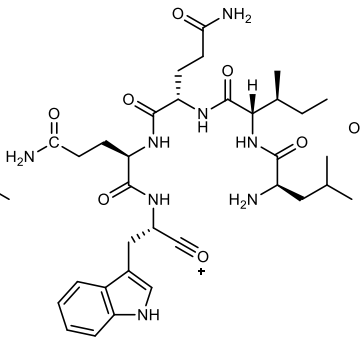
Chemical Formula: $C_{14}H_{16}N_3O_2^+$
Expected m/z: 258.124
Observed m/z: 258.126
Mass Error (mDa): 1.0



Supplementary Fig. 53: MS2 spectrum for Cyclic Peptide 6 (Cyc 6)

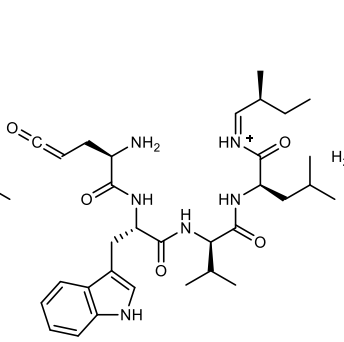


Chemical Formula: $C_{38}H_{57}N_9O_8$
Expected Neutral Mass: 767.4330
Observed Neutral Mass: 767.4315
Mass Error (ppm): 1.9



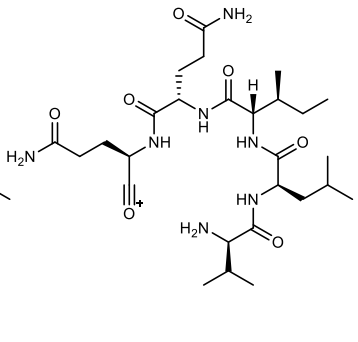
1

Chemical Formula: $C_{33}H_{49}N_8O_7^+$
Expected m/z: 669.372
Observed m/z: 669.370
Mass Error (mDa): 2.0



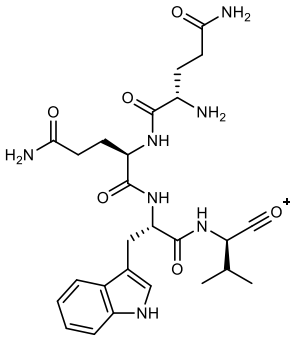
2

Chemical Formula: $C_{32}H_{47}N_6O_5^+$
Expected m/z: 595.360
Observed m/z: 595.358
Mass Error (mDa): 2.0



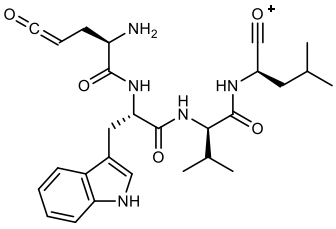
3

Chemical Formula: $C_{27}H_{48}N_7O_7^+$
Expected m/z: 582.361
Observed m/z: 582.359
Mass Error (mDa): 2.0



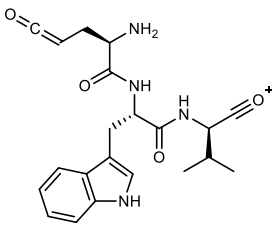
4

Chemical Formula:$C_{26}H_{36}N_7O_6^+$
Expected m/z: 542.272
Observed m/z: 542.271
Mass Error (mDa): 1.0



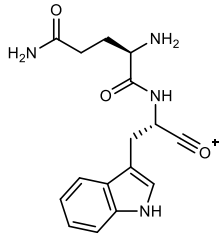
5

Chemical Formula: $C_{27}H_{36}N_5O_5^+$
Expected m/z: 510.271
Observed m/z: 510.270
Mass Error (mDa): 1.0



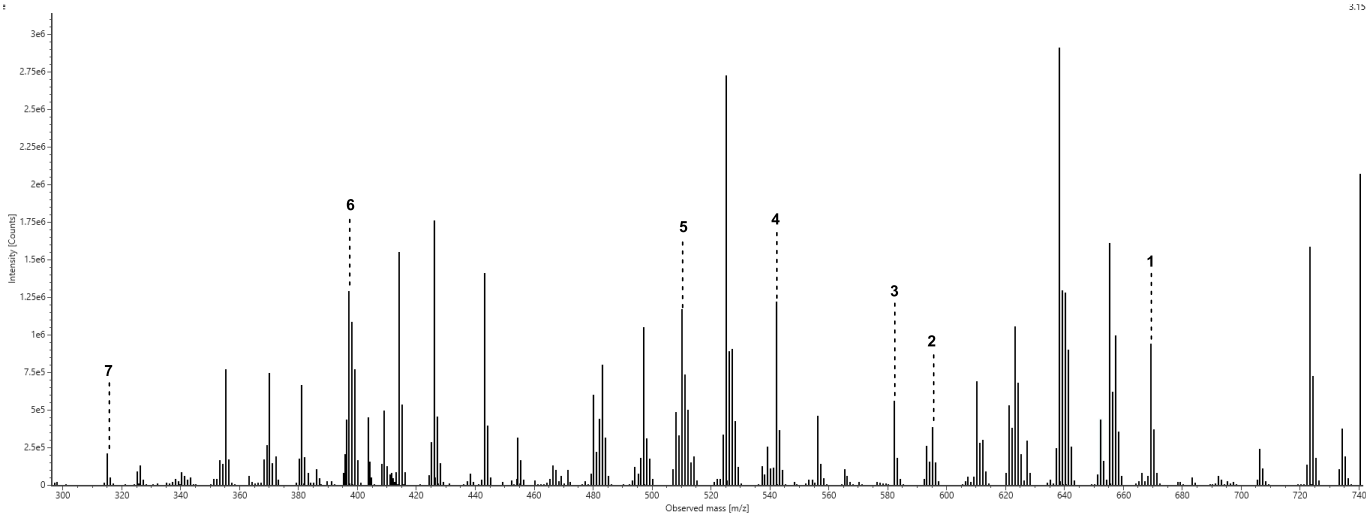
6

Chemical Formula:$C_{21}H_{25}N_4O_4^+$
Expected m/z: 397.187
Observed m/z: 397.188
Mass Error (mDa): 1.0

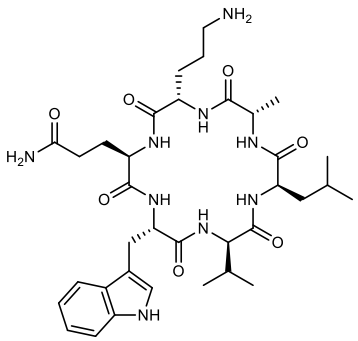


7

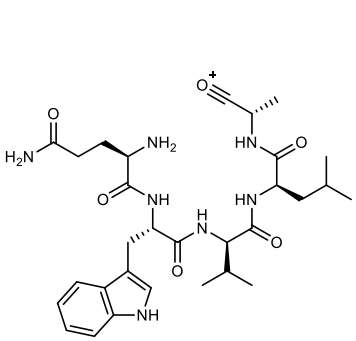
Chemical Formula: $C_{16}H_{19}N_4O_3^+$
Expected m/z: 315.145
Observed m/z: 315.144
Mass Error (mDa): 1.0



Supplementary Fig. 54: MS2 spectrum for Cyclic Peptide 7 (Cyc 7)

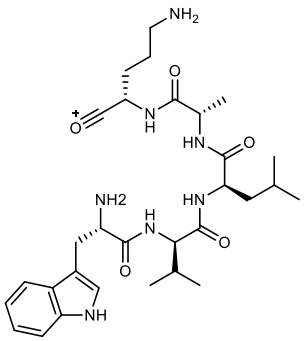


Chemical Formula: $C_{35}H_{53}N_9O_7$
Expected Neutral Mass: 711.4068
Observed Neutral Mass: 711.4061
Mass Error (ppm): 1.0



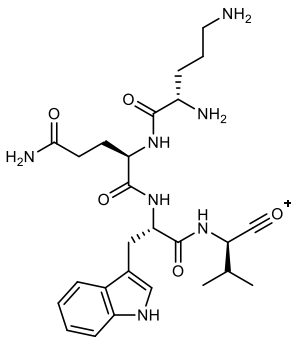
1

Chemical Formula: $C_{30}H_{44}N_7O_6^+$
Expected m/z: 598.335
Observed m/z: 598.335
Mass Error (mDa): > 1.0



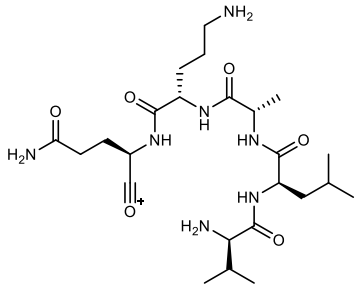
2

Chemical Formula: $C_{30}H_{46}N_7O_5^+$
Expected m/z: 584.355
Observed m/z: 584.355
Mass Error (mDa): > 1.0



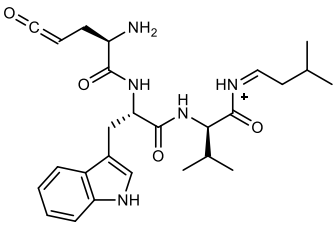
3

Chemical Formula: $C_{26}H_{38}N_7O_5^+$
Expected m/z: 528.293
Observed m/z: 528.297
Mass Error (mDa): 4.0



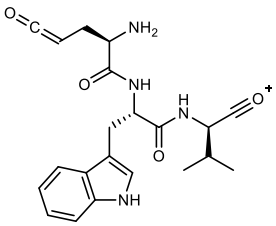
4

Chemical Formula: $C_{24}H_{44}N_7O_6^+$
Expected m/z: 526.335
Observed m/z: 526.333
Mass Error (mDa): 2.0



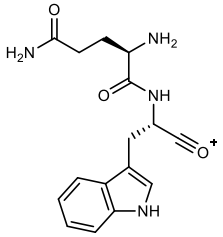
5

Chemical Formula: $C_{26}H_{36}N_5O_4^+$
Expected m/z: 482.276
Observed m/z: 482.274
Mass Error (mDa): 2.0



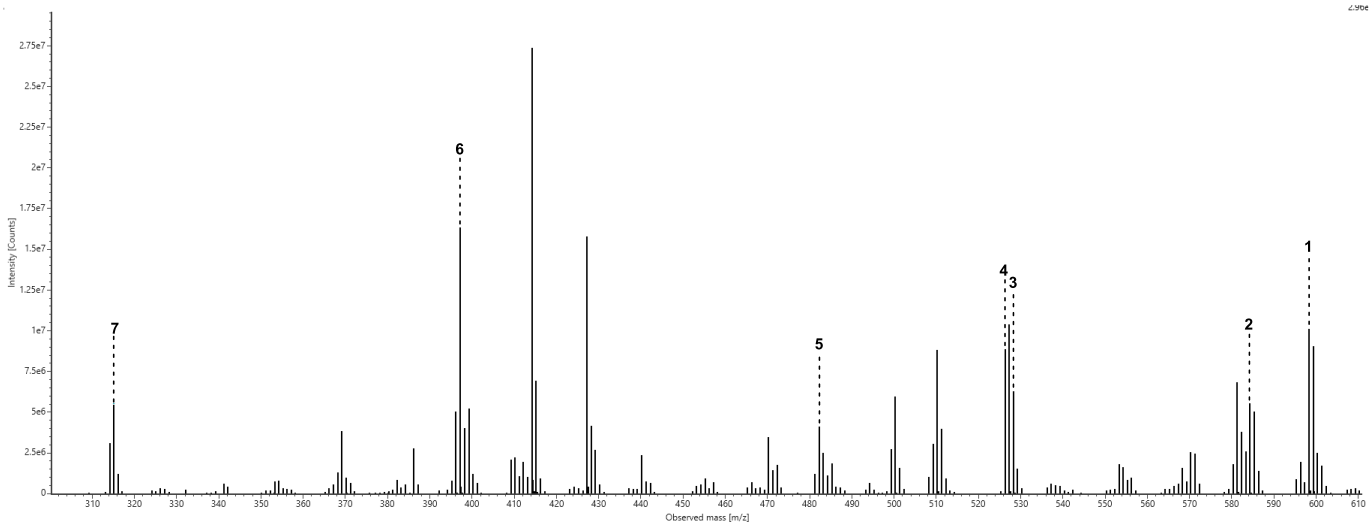
6

Chemical Formula: $C_{21}H_{25}N_4O_4^+$
Expected m/z: 397.187
Observed m/z: 397.188
Mass Error (mDa): 1.0

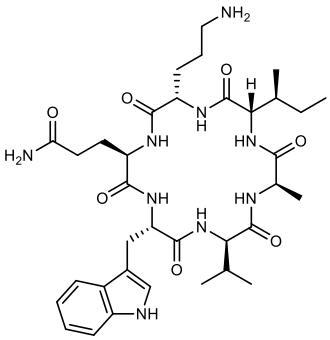


7

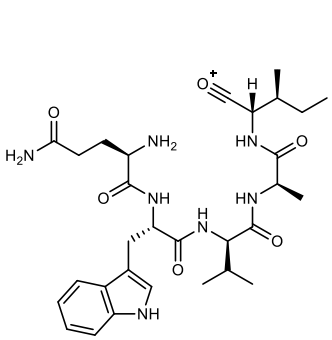
Chemical Formula: $C_{16}H_{19}N_4O_3^+$
Expected m/z: 315.145
Observed m/z: 315.148
Mass Error (mDa): < 3.0



Supplementary Fig. 55: MS2 spectrum for Cyclic Peptide 8 (Cyc 8)

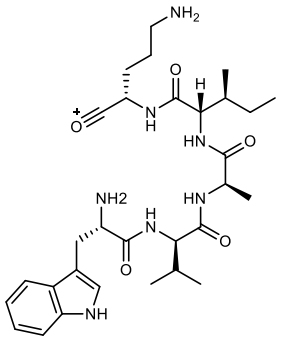


Chemical Formula: $C_{38}H_{59}N_9O_7$
Expected Neutral Mass: 711.4068
Observed Neutral Mass: 711.4078
Mass Error (ppm): 1.4



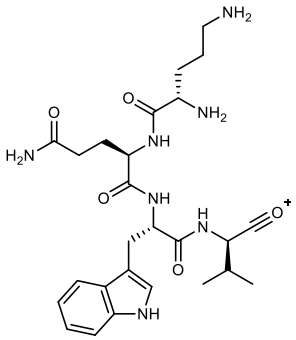
1

Chemical Formula: $C_{30}H_{44}N_7O_6^+$
Expected m/z: 598.335
Observed m/z: 598.336
Mass Error (mDa): 1.0



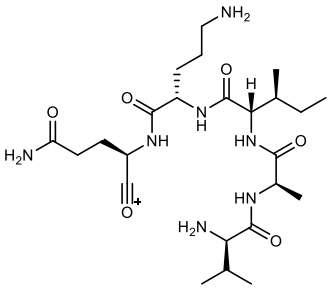
2

Chemical Formula: $C_{30}H_{46}N_7O_5^+$
Expected m/z: 584.355
Observed m/z: 584.356
Mass Error (mDa): 1.0



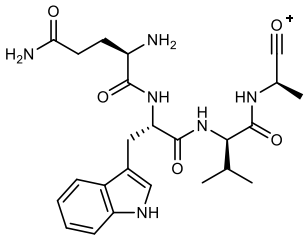
3

Chemical Formula: $C_{26}H_{38}N_7O_5^+$
Expected m/z: 528.293
Observed m/z: 528.296
Mass Error (mDa): 3.0



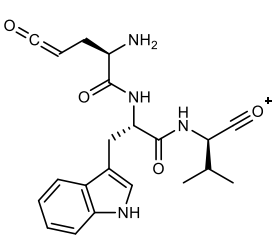
4

Chemical Formula: $C_{24}H_{44}N_7O_6^+$
Expected m/z: 526.335
Observed m/z: 526.335
Mass Error (mDa): > 1.0



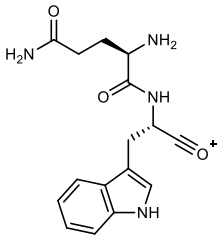
5

Chemical Formula: $C_{24}H_{33}N_6O_5^+$
Expected m/z: 485.251
Observed m/z: 485.252
Mass Error (mDa): 1.0



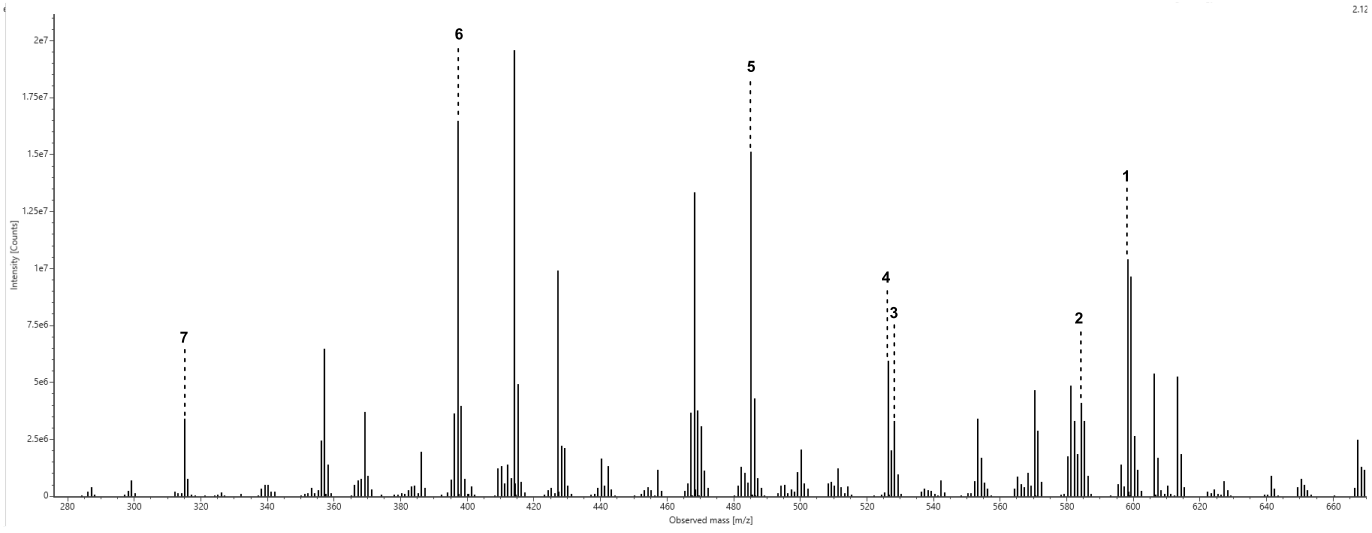
6

Chemical Formula: $C_{21}H_{25}N_4O_4^+$
Expected m/z: 397.187
Observed m/z: 397.188
Mass Error (mDa): 1.0

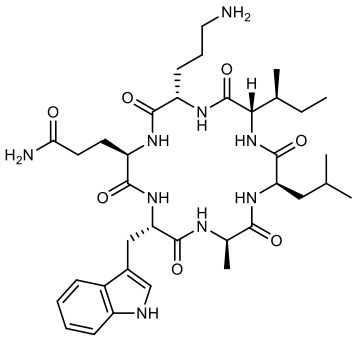


7

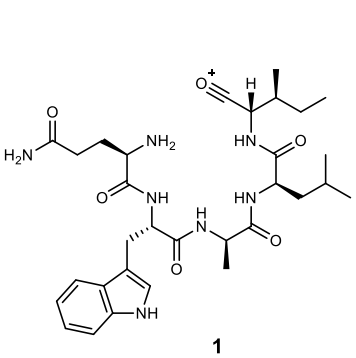
Chemical Formula: $C_{16}H_{19}N_4O_3^+$
Expected m/z: 315.145
Observed m/z: 315.146
Mass Error (mDa): 1.0



Supplementary Fig. 56: MS2 spectrum for Cyclic peptide 9 (Cyc 9)

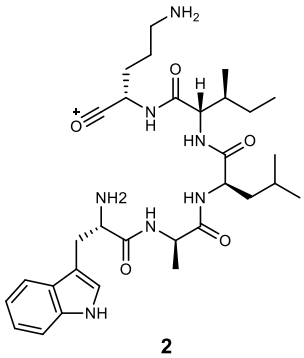


Chemical Formula: $C_{36}H_{55}N_9O_7$
Expected Neutral Mass: 725.4224
Observed Neutral Mass: 725.4227
Mass Error (ppm): 0.3



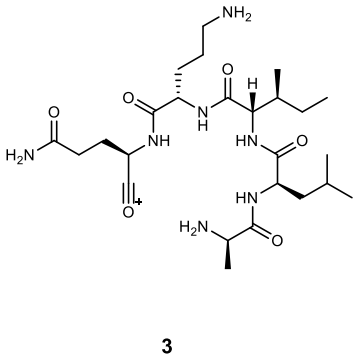
1

Chemical Formula: $C_{31}H_{46}N_7O_6^+$
Expected m/z: 612.350
Observed m/z: 612.352
Mass Error (mDa): 2.0



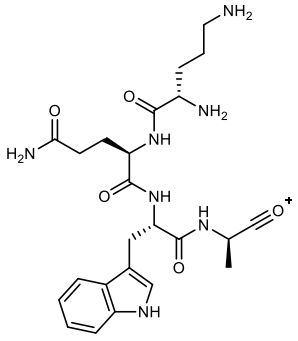
2

Chemical Formula: $C_{31}H_{48}N_7O_5^+$
Expected m/z: 598.371
Observed m/z: 598.371
Mass Error (mDa): < 1.0



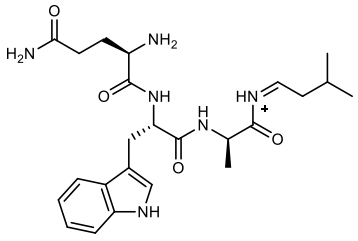
3

Chemical Formula: $C_{25}H_{46}N_7O_6^+$
Expected m/z: 540.350
Observed m/z: 540.350
Mass Error (mDa): < 1.0



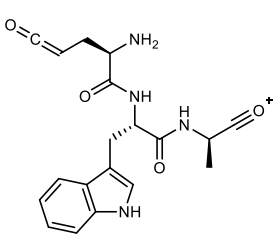
4

Chemical Formula: $C_{24}H_{34}N_7O_5^+$
Expected m/z: 500.262
Observed m/z: 500.266
Mass Error (mDa): 4.0



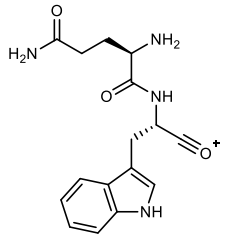
5

Chemical Formula: $C_{24}H_{35}N_6O_4^+$
Expected m/z: 471.271
Observed m/z: 471.274
Mass Error (mDa): 3.0



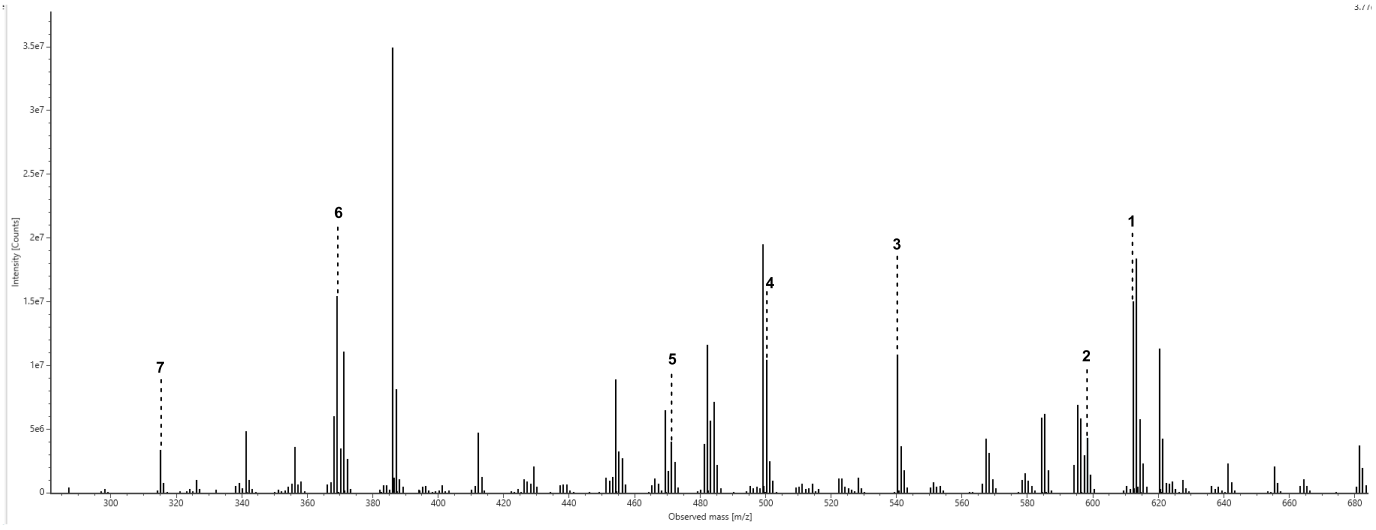
6

Chemical Formula: $C_{19}H_{21}N_4O_4^+$
Expected m/z: 369.156
Observed m/z: 369.157
Mass Error (mDa): 1.0

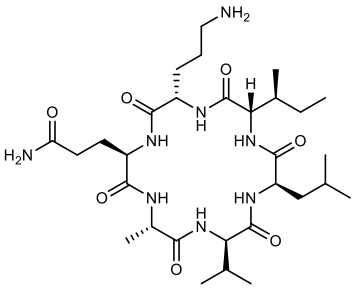


7

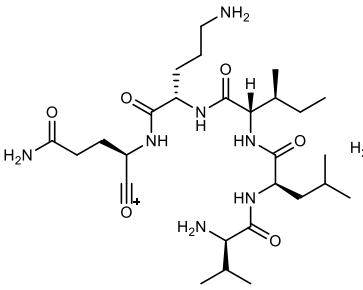
Chemical Formula: $C_{16}H_{19}N_4O_3^+$
Expected m/z: 315.145
Observed m/z: 315.145
Mass Error (mDa): < 0.1



Supplementary Fig. 57: MS2 spectrum for Cyclic Peptide 10 (Cyc 10)

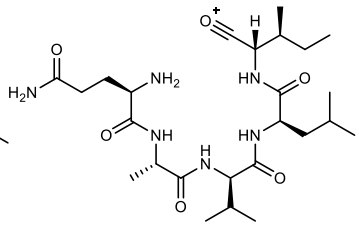


Chemical Formula: $C_{30}H_{54}N_8O_7$
Expected Neutral Mass: 638.4115
Observed Neutral Mass: 638.4120
Mass Error (ppm): 0.7



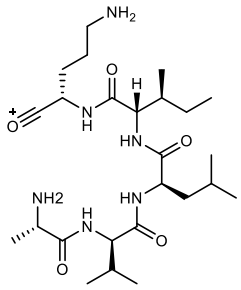
1

Chemical Formula: $C_{27}H_{50}N_7O_6^+$
Expected m/z: 568.382
Observed m/z: 568.381
Mass Error (mDa): 1.0



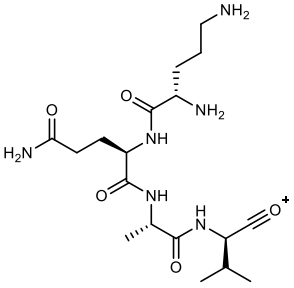
2

Chemical Formula: $C_{25}H_{45}N_6O_6^+$
Expected m/z: 525.340
Observed m/z: 525.341
Mass Error (mDa): 1.0



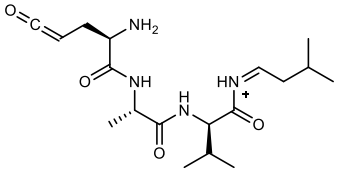
3

Chemical Formula: $C_{25}H_{47}N_6O_5^+$
Expected m/z: 511.360
Observed m/z: 511.358
Mass Error (mDa): 2.0



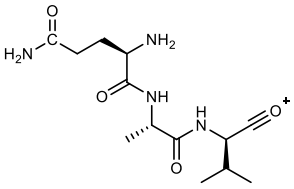
4

Chemical Formula: $C_{18}H_{33}N_6O_5^+$
Expected m/z: 413.251
Observed m/z: 413.255
Mass Error (mDa): 4.0



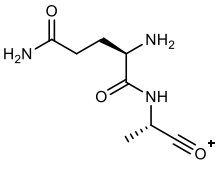
5

Chemical Formula: $C_{18}H_{31}N_4O_4^+$
Expected m/z: 367.234
Observed m/z: 367.236
Mass Error (mDa): 2.0



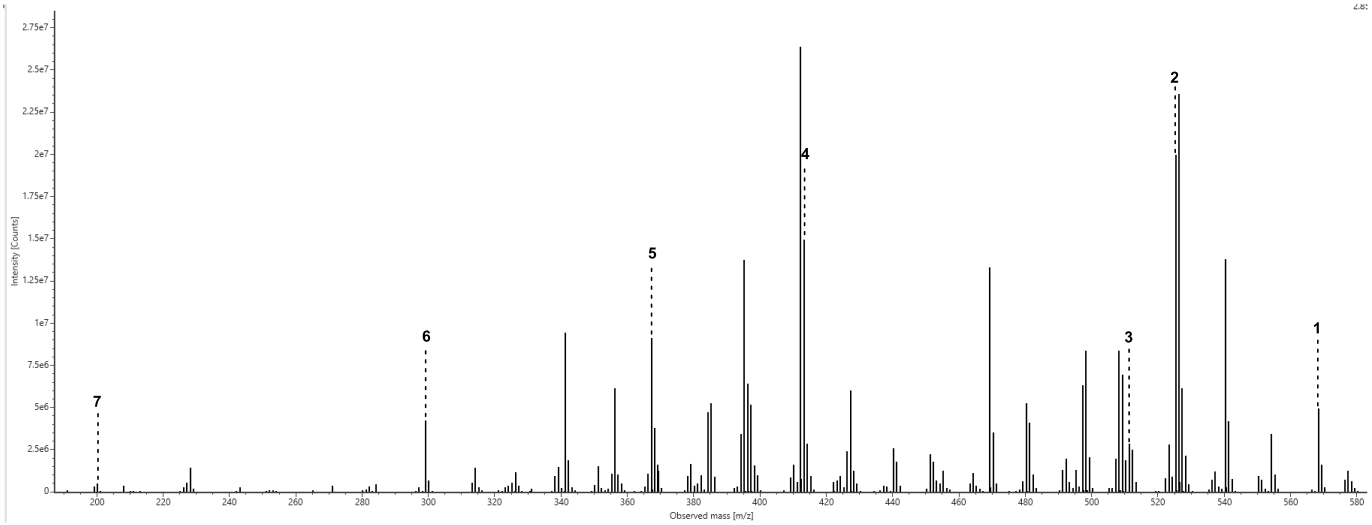
6

Chemical Formula: $C_{13}H_{23}N_4O_4^+$
Expected m/z: 299.171
Observed m/z: 299.173
Mass Error (mDa): 2.0

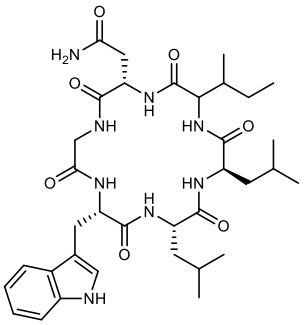


7

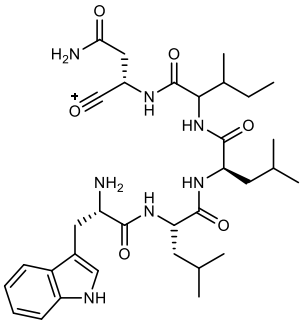
Chemical Formula: $C_8H_{14}N_3O_3^+$
Expected m/z: 200.103
Observed m/z: 200.103
Mass Error (mDa): < 0.1



Supplementary Fig. 58: MS2 spectrum for Cyclic Peptide 11 (Cyc11)

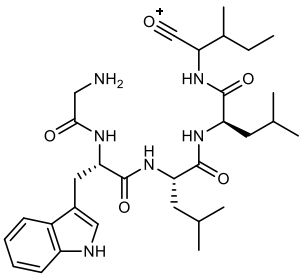


Chemical Formula: $C_{35}H_{52}N_8O_7$
Expected Neutral Mass: 696.3959
Observed Neutral Mass: 696.3978
Mass Error (ppm): 2.7



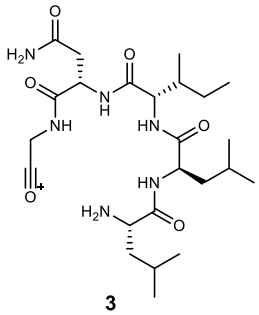
1

Chemical Formula: $C_{33}H_{50}N_7O_6^+$
Expected m/z: 640.382
Observed m/z: 640.379
Mass Error (mDa): 3.0



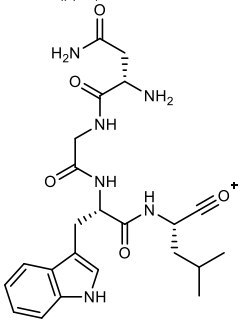
2

Chemical Formula: $C_{31}H_{47}N_6O_5^+$
Expected m/z: 583.360
Observed m/z: 583.360
Mass Error (mDa): < 1.0



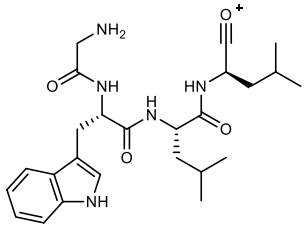
3

Chemical Formula: $C_{24}H_{43}N_6O_6^+$
Expected m/z: 511.324
Observed m/z: 511.318
Mass Error (mDa): 6.0



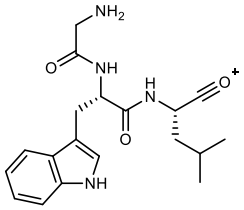
4

Chemical Formula: $C_{23}H_{31}N_6O_5^+$
Expected m/z: 471.235
Observed m/z: 471.236
Mass Error (mDa): 1.0



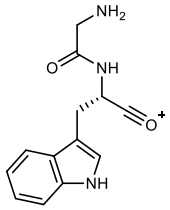
5

Chemical Formula: $C_{25}H_{36}N_5O_4^+$
Expected m/z: 470.276
Observed m/z: 470.276
Mass Error (mDa): < 1.0



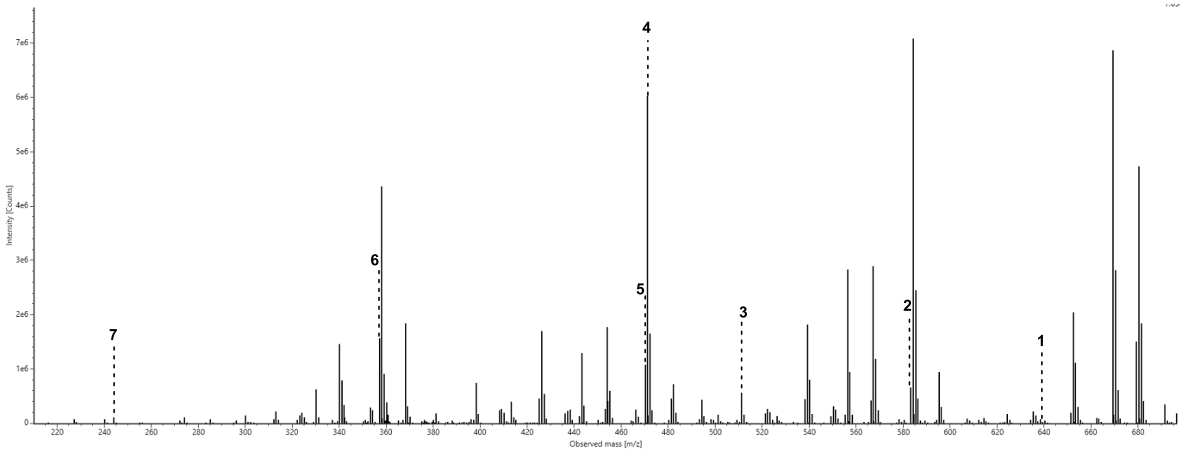
6

Chemical Formula: $C_{19}H_{25}N_4O_3^+$
Expected m/z: 357.192
Observed m/z: 357.190
Mass Error (mDa): 2.0

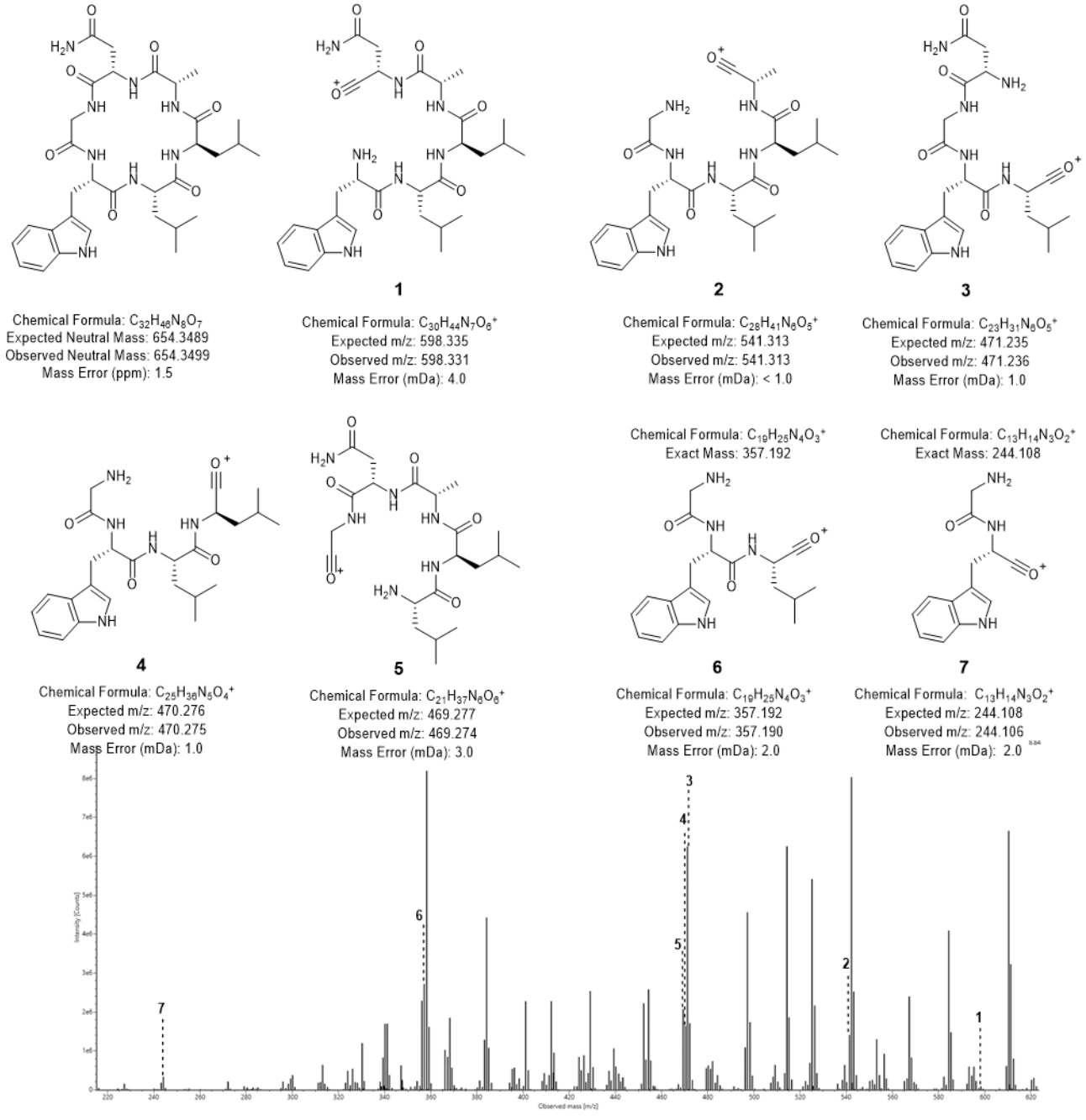


7

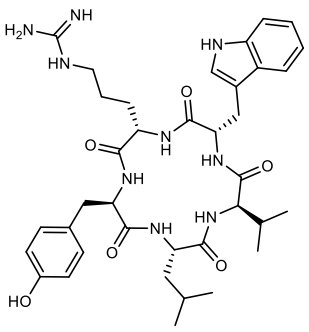
Chemical Formula: $C_{13}H_{14}N_3O_2^+$
Expected m/z: 244.108
Observed m/z: 244.105
Mass Error (mDa): 3.0



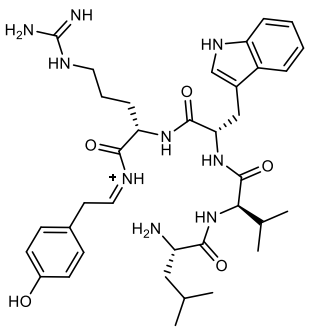
Supplementary Fig. 59: MS2 spectrum for Cyclic Peptide 12 (Cyc12)



Supplementary Fig. 60: MS2 spectrum for Cyclic Peptide 13 (Cyc13)

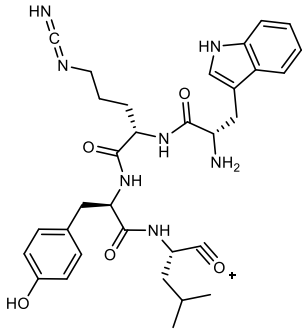


Chemical Formula: $C_{37}H_{51}N_9O_6$
Expected Neutral Mass: 717.3962
Observed Neutral Mass: 717.3969
Mass Error (ppm): 1.0



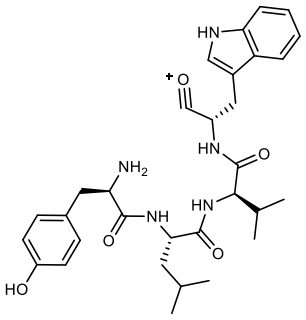
1

Chemical Formula: $C_{36}H_{52}N_9O_5^+$
Expected m/z: 690.409
Observed m/z: 690.409
Mass Error (mDa): < 1.0



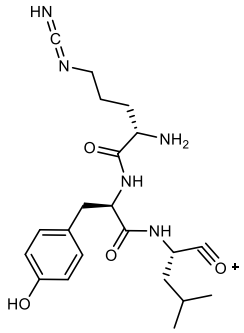
2

Chemical Formula: $C_{32}H_{40}N_7O_5^+$
Expected m/z: 602.309
Observed m/z: 602.309
Mass Error (mDa): 1.0



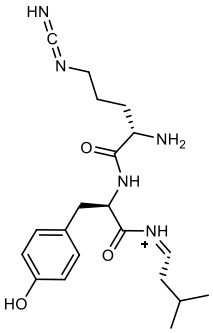
3

Chemical Formula: $C_{31}H_{40}N_5O_5^+$
Expected m/z: 562.302
Observed m/z: 562.304
Mass Error (mDa): 2.0



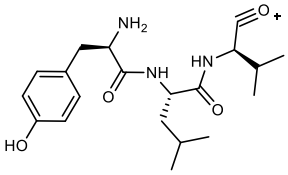
4

Chemical Formula: $C_{21}H_{30}N_5O_4^+$
Expected m/z: 416.229
Observed m/z: 416.230
Mass Error (mDa): 1.0



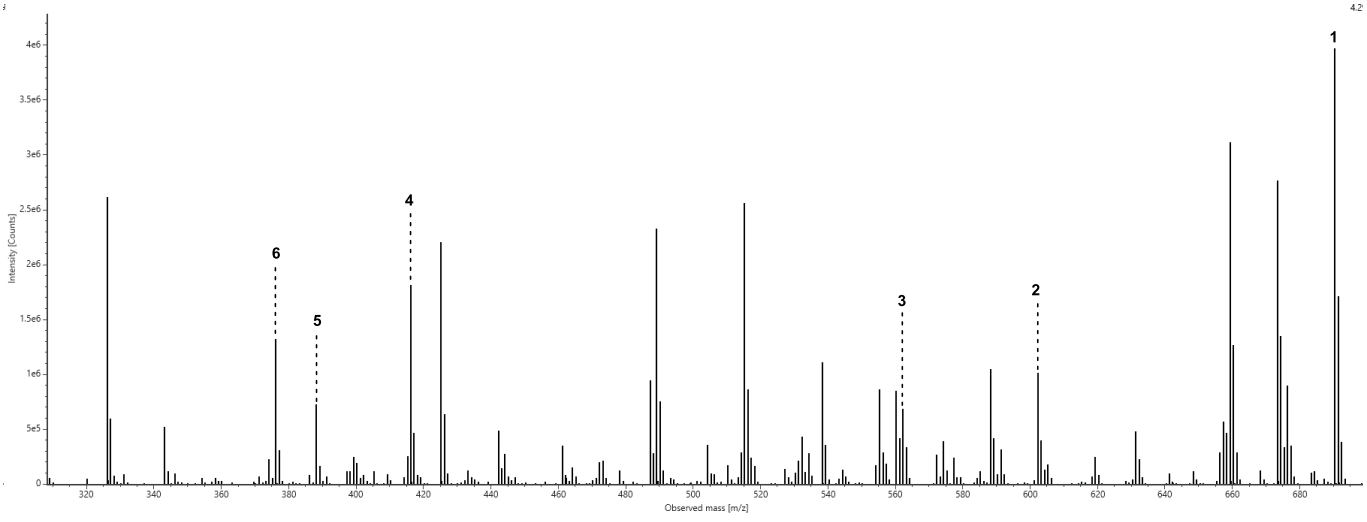
5

Chemical Formula: $C_{20}H_{30}N_5O_3^+$
Expected m/z: 388.234
Observed m/z: 388.234
Mass Error (mDa): < 1.0

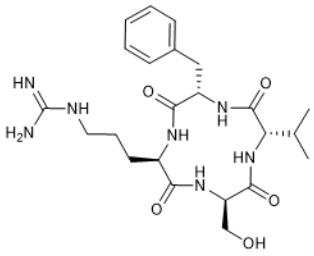


6

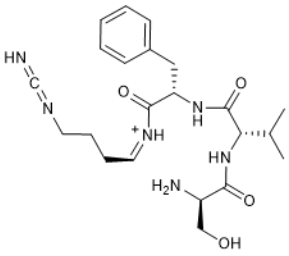
Chemical Formula: $C_{20}H_{30}N_3O_4^+$
Expected m/z: 376.223
Observed m/z: 376.223
Mass Error (mDa): < 1.0



Supplementary Fig. 61: MS2 spectrum for Cyclic Peptide 14 (Cyc14)

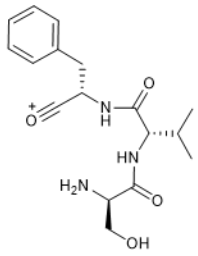


Chemical Formula: $C_{23}H_{35}N_7O_5$
Expected Neutral Mass: 489.2699
Observed Neutral Mass: 489.2697
Mass Error (ppm): 0.6



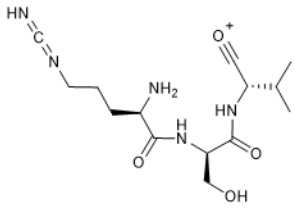
1

Chemical Formula: $C_{22}H_{33}N_6O_4^+$
Expected m/z: 445.256
Observed m/z: 445.256
Mass Error (mDa): < 1.0



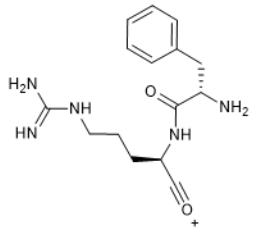
2

Chemical Formula: $C_{17}H_{24}N_3O_4^+$
Expected m/z: 334.176
Observed m/z: 334.176
Mass Error (mDa): < 1.0



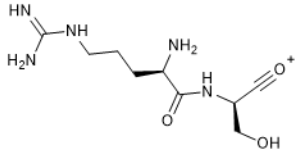
3

Chemical Formula: $C_{14}H_{24}N_5O_4^+$
Expected m/z: 326.182
Observed m/z: 326.183
Mass Error (mDa): 1.0



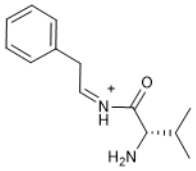
4

Chemical Formula: $C_{15}H_{22}N_5O_2^+$
Expected m/z: 304.177
Observed m/z: 304.176
Mass Error (mDa): 1.0



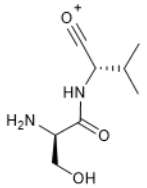
5

Chemical Formula: $C_9H_{18}N_5O_3^+$
Expected m/z: 244.140
Observed m/z: 244.140
Mass Error (mDa): < 1.0



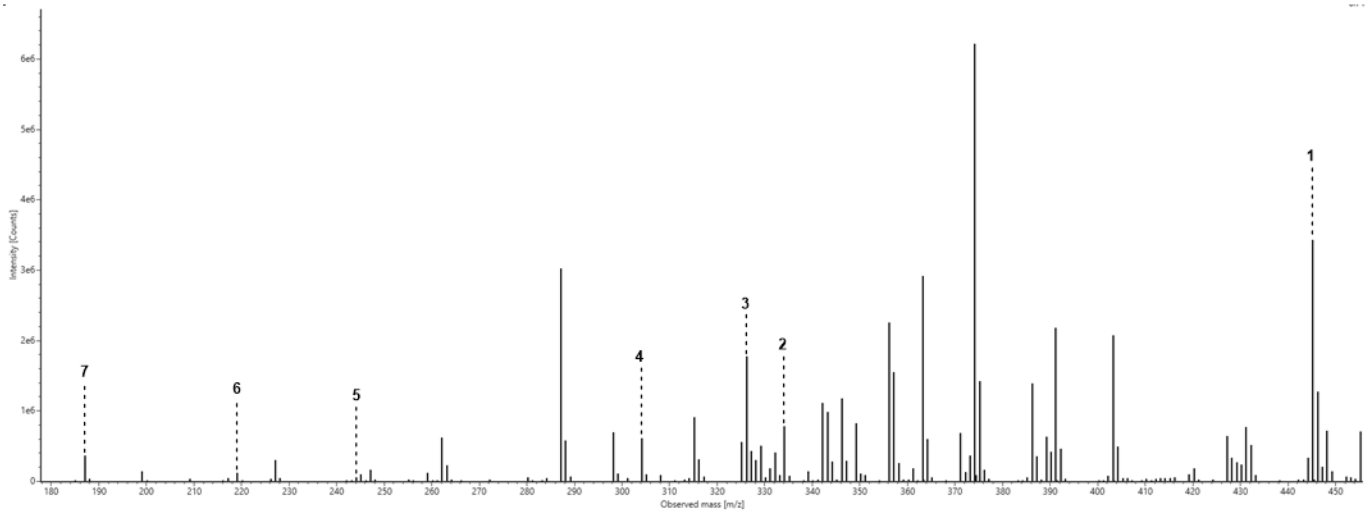
6

Chemical Formula :$C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.148
Mass Error (mDa): 1.0

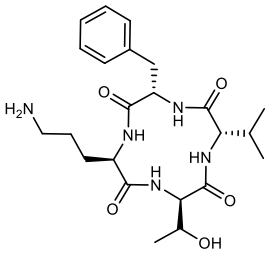


7

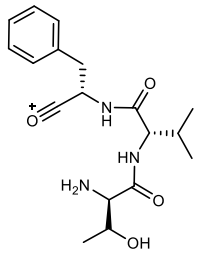
Chemical Formula: $C_8H_{15}N_2O_3^+$
Expected m/z: 187.108
Observed m/z: 187.107
Mass Error (mDa): 1.0



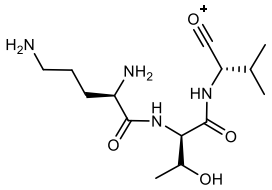
Supplementary Fig. 62: MS2 spectrum for Cyclic Peptide 15 (Cyc15)



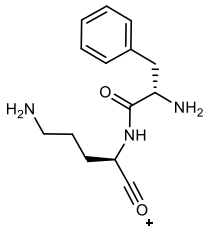
Chemical Formula:$C_{23}H_{35}N_5O_5$
Expected Neutral Mass: 461.2638
Observed Neutral Mass: 461.2635
Mass Error (ppm): 0.7



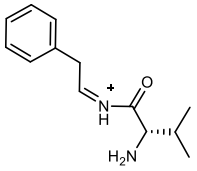
1
Chemical Formula: $C_{18}H_{26}N_3O_4^+$
Expected m/z: 348.192
Observed m/z: 348.192
Mass Error (mDa): < 1.0



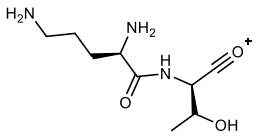
2
Chemical Formula: $C_{14}H_{27}N_4O_4^+$
Expected m/z: 315.203
Observed m/z: 315.202
Mass Error (mDa): 1.0



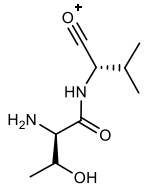
3
Chemical Formula: $C_{14}H_{20}N_3O_2^+$
Expected m/z: 262.155
Observed m/z: 262.155
Mass Error (mDa): < 1.0



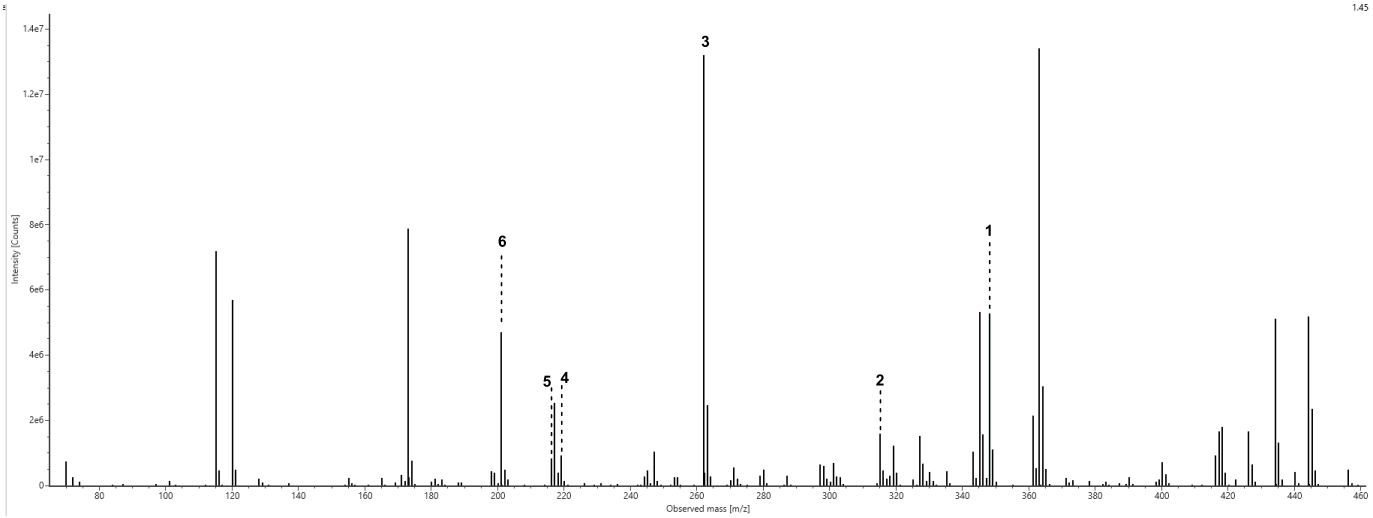
4
Chemical Formula: $C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.148
Mass Error (mDa): 1.0



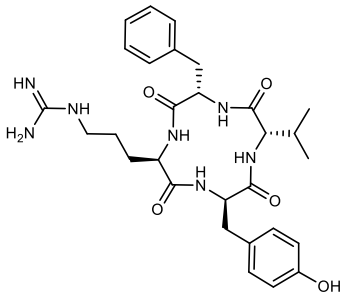
5
Chemical Formula: $C_9H_{18}N_3O_3^+$
Expected m/z: 216.134
Observed m/z: 216.134
Mass Error (mDa): < 1.0



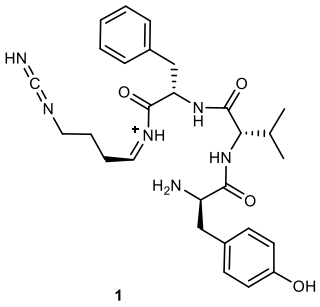
6
Chemical Formula: $C_9H_{17}N_2O_3^+$
Expected m/z: 201.123
Observed m/z: 201.123
Mass Error (mDa): < 1.0



Supplementary Fig. 63: MS2 spectrum for Cyclic Peptide 16 (Cyc16)

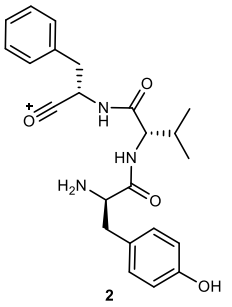


Chemical Formula: $C_{29}H_{39}N_7O_5$
Expected Neutral Mass: 565.3012
Observed Neutral Mass: 565.3019
Mass Error (ppm): 1.1



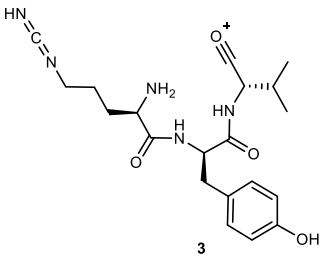
1

Chemical Formula: $C_{28}H_{37}N_6O_4^+$
Expected m/z: 521.287
Observed m/z: 521.287
Mass Error (mDa): < 1.0



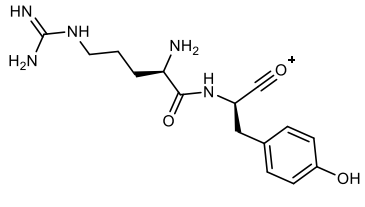
2

Chemical Formula: $C_{23}H_{28}N_3O_4^+$
Expected m/z: 410.207
Observed m/z: 410.208
Mass Error (mDa): 1.0



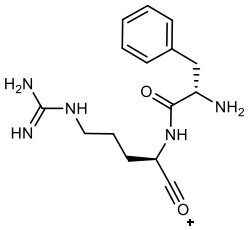
3

Chemical Formula: $C_{20}H_{28}N_5O_4^+$
Expected m/z: 402.214
Observed m/z: 402.216
Mass Error (mDa): 2.0



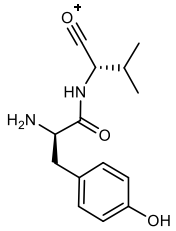
4

Chemical Formula: $C_{15}H_{22}N_5O_3^+$
Expected m/z: 320.172
Observed m/z: 320.172
Mass Error (mDa): < 1.0



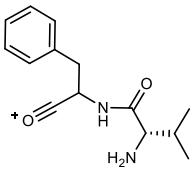
5

Chemical Formula: $C_{15}H_{22}N_5O_2^+$
Expected m/z: 304.177
Observed m/z: 304.175
Mass Error (mDa): 2.0



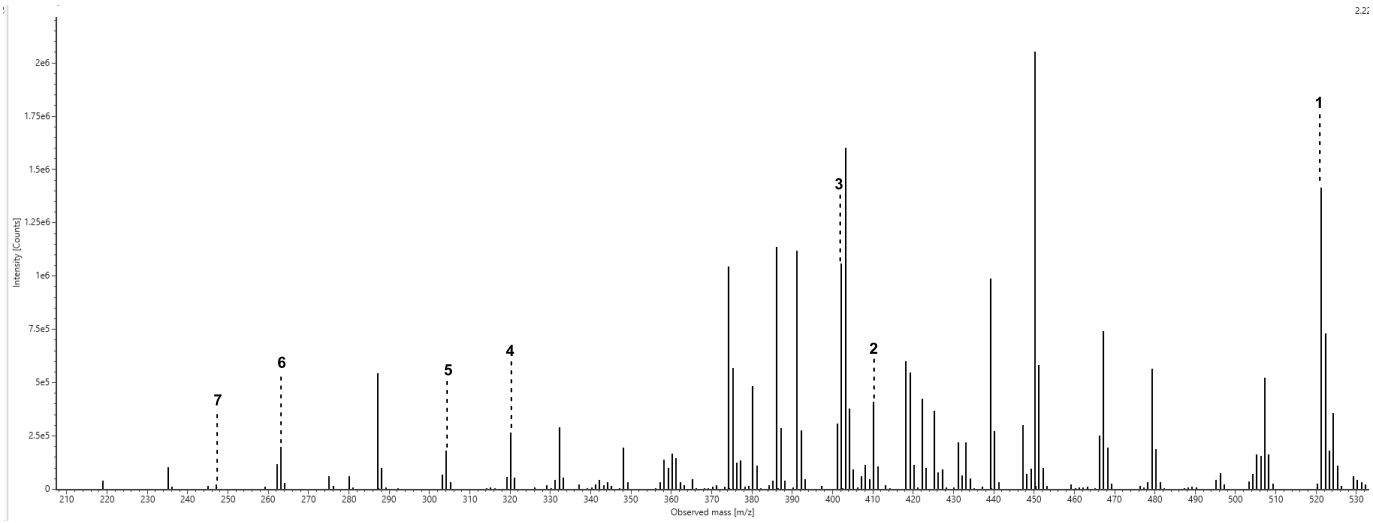
6

Chemical Formula: $C_{14}H_{19}N_2O_3^+$
Expected m/z: 263.139
Observed m/z: 263.141
Mass Error (mDa): 2.0

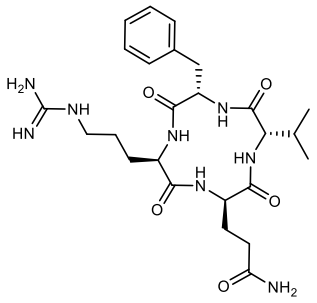


7

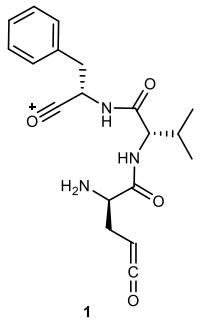
Chemical Formula :$C_{14}H_{19}N_2O_2^+$
Expected m/z: 247.144
Observed m/z: 247.146
Mass Error (mDa): 2.0



Supplementary Fig. 64: MS2 spectrum for Cyclic Peptide 17 (Cyc17)

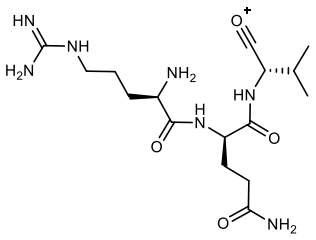


Chemical Formula: $C_{25}H_{38}N_8O_5$
Expected Neutral Mass: 530.2965
Observed Neutral Mass: 530.2965
Mass Error (ppm): 0.1



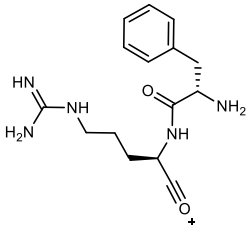
1

Chemical Formula: $C_{19}H_{27}N_4O_4^+$
Expected m/z: 358.176
Observed m/z: 358.181
Mass Error (mDa): 5.0



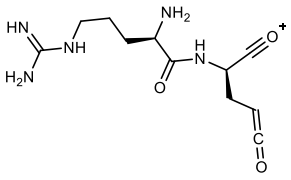
2

Chemical Formula: $C_{16}H_{30}N_7O_4^+$
Expected m/z: 384.235
Observed m/z: 384.237
Mass Error (mDa): 2.0



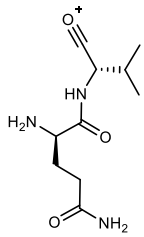
3

Chemical Formula: $C_{15}H_{22}N_5O_2^+$
Expected m/z: 304.176
Observed m/z: 304.175
Mass Error (mDa): 1.0



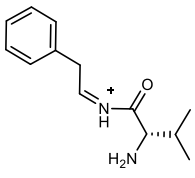
4

Chemical Formula: $C_{11}H_{18}N_5O_3^+$
Expected m/z: 268.140
Observed m/z: 268.139
Mass Error (mDa): 1.0



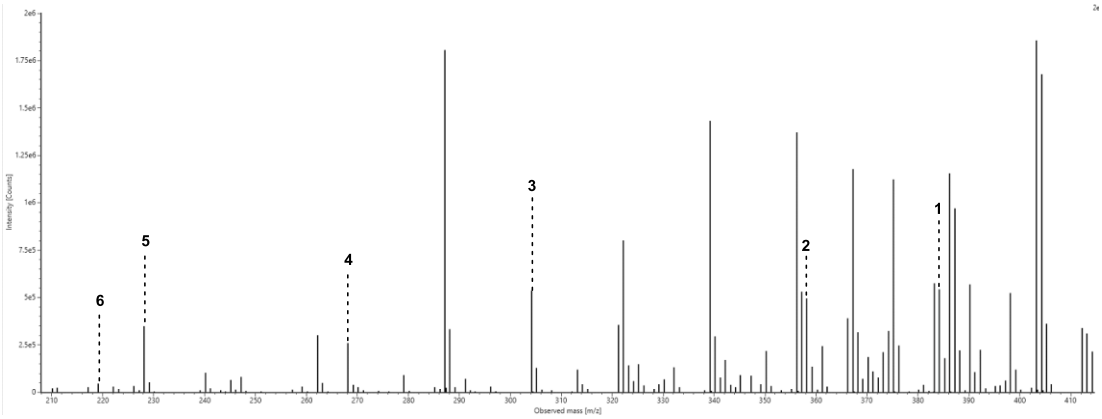
5

Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.139
Mass Error (mDa): 5.0

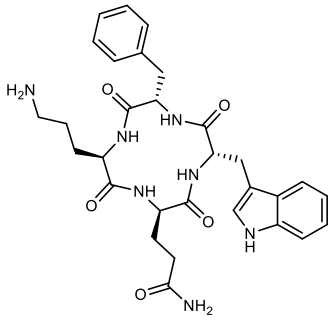


6

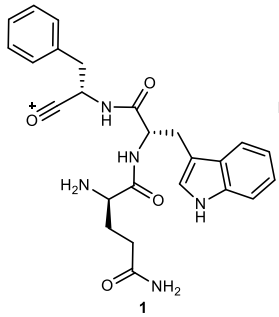
Chemical Formula: $C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.147
Mass Error (mDa): 2.0



Supplementary Fig. 65: MS2 spectrum for Cyclic Peptide 18 (Cyc18)

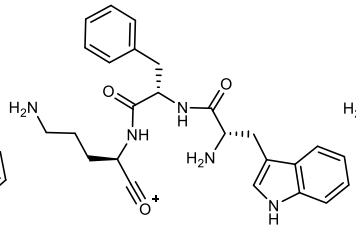


Chemical Formula: $C_{30}H_{37}N_7O_5$
Expected Neutral Mass: 575.2856
Observed Neutral Mass: 575.2852
Mass Error (ppm): 0.7



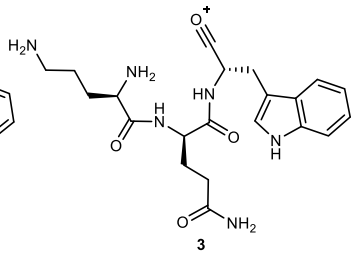
1

Chemical Formula: $C_{25}H_{28}N_5O_4^+$
Expected m/z: 462.214
Observed m/z: 462.214
Mass Error (mDa): < 1.0



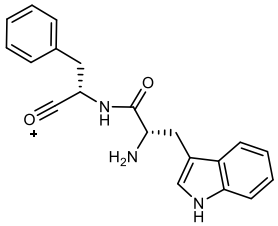
2

Chemical Formula: $C_{25}H_{30}N_5O_3^+$
Expected m/z: 448.234
Observed m/z: 448.234
Mass Error (mDa): < 1.0



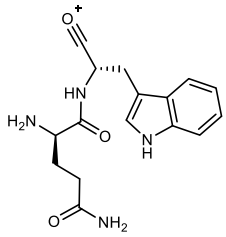
3

Chemical Formula: $C_{21}H_{29}N_6O_4^+$
Expected m/z: 429.224
Observed m/z: 429.223
Mass Error (mDa): 1.0



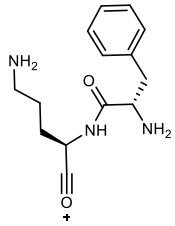
4

Chemical Formula: $C_{20}H_{20}N_3O_2^+$
Expected m/z: 334.155
Observed m/z: 334.154
Mass Error (mDa): 1.0



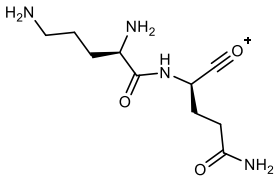
5

Chemical Formula: $C_{16}H_{19}N_4O_3^+$
Expected m/z: 315.145
Observed m/z: 315.145
Mass Error (mDa): < 1.0



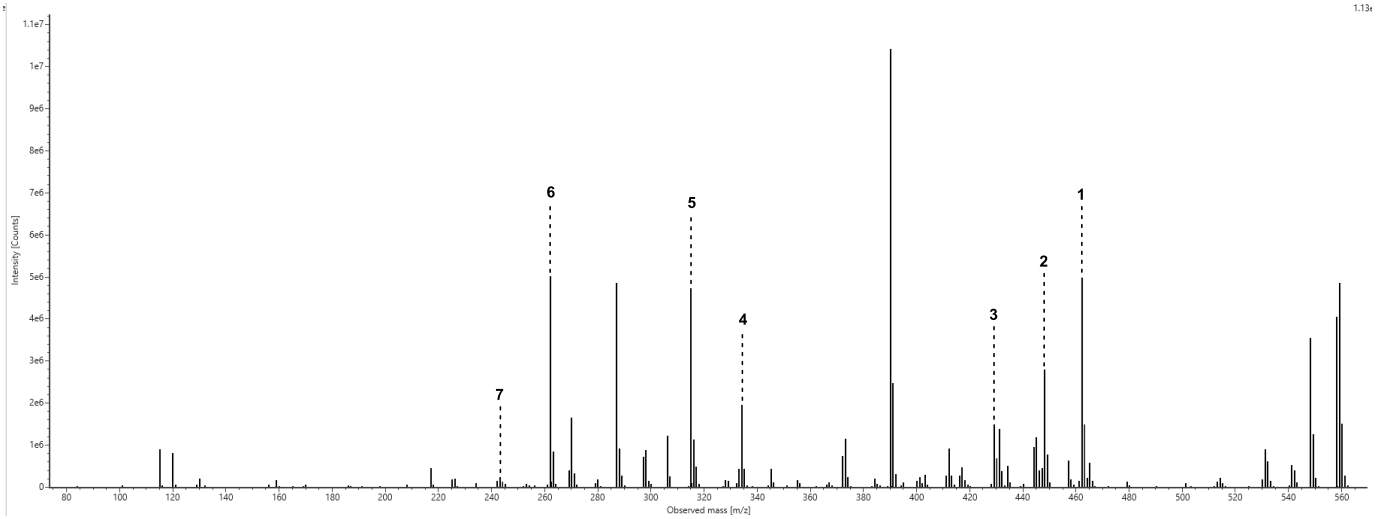
6

Chemical Formula: $C_{14}H_{20}N_3O_2^+$
Expected m/z: 262.155
Observed m/z: 262.155
Mass Error (mDa): < 1.0

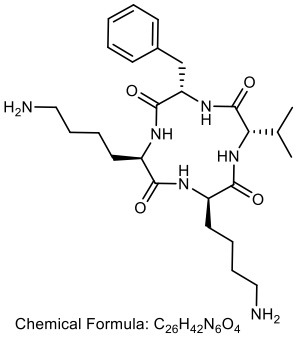


7

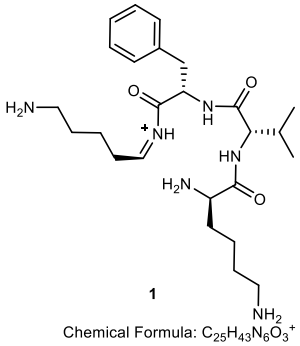
Chemical Formula: $C_{10}H_{19}N_4O_3^+$
Expected m/z: 243.145
Observed m/z: 243.144
Mass Error (mDa): 1.0



Supplementary Fig. 66: MS2 spectrum for Cyclic Peptide 20 (Cyc20)

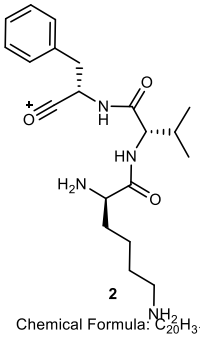


Chemical Formula: $C_{26}H_{42}N_6O_4$
Expected Neutral Mass: 502.3267
Observed Neutral Mass: 502.3260
Mass Error (ppm): 1.4



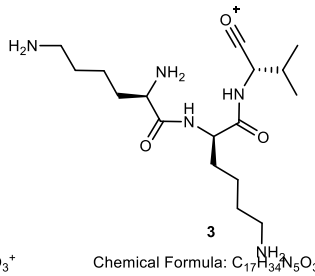
1

Chemical Formula: $C_{25}H_{43}N_6O_3^+$
Expected m/z: 475.339
Observed m/z: 475.337
Mass Error (mDa): 2.0



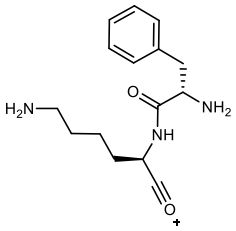
2

Chemical Formula: $C_{20}H_{31}N_4O_3^+$
Expected m/z: 375.239
Observed m/z: 375.238
Mass Error (mDa): 1.0



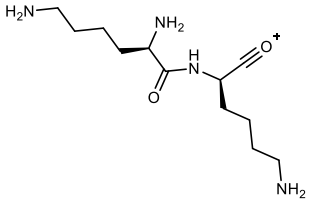
3

Chemical Formula: $C_{17}H_{34}N_5O_3^+$
Expected m/z: 356.266
Observed m/z: 356.264
Mass Error (mDa): 2.0



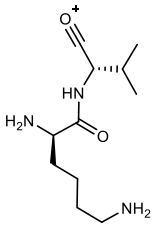
4

Chemical Formula: $C_{15}H_{22}N_3O_2^+$
Expected m/z: 276.171
Observed m/z: 276.170
Mass Error (mDa): 1.0



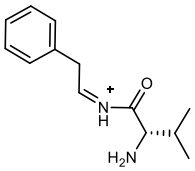
5

Chemical Formula: $C_{12}H_{25}N_4O_2^+$
Expected m/z: 257.197
Observed m/z: 257.195
Mass Error (mDa): 2.0



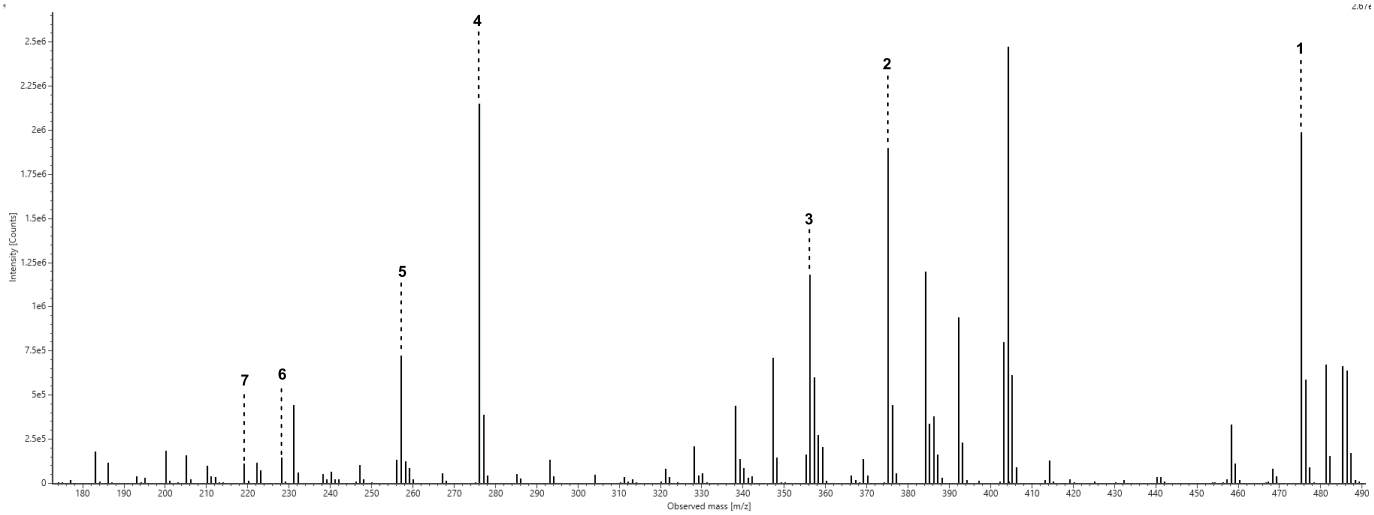
6

Chemical Formula: $C_{11}H_{22}N_3O_2^+$
Expected m/z: 228.171
Observed m/z: 228.170
Mass Error (mDa): 1.0

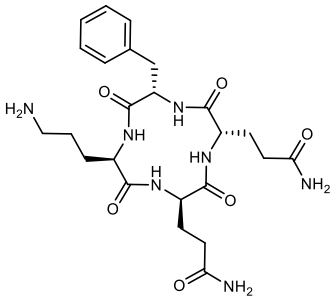


7

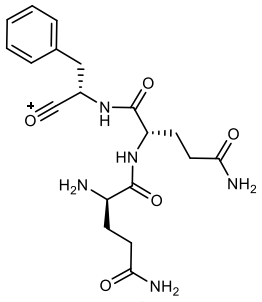
Chemical Formula :$C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.148
Mass Error (mDa): 1.0



Supplementary Fig. 67: MS2 spectrum for Cyclic Peptide 21 (Cyc21)

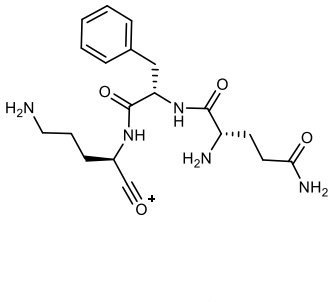


Chemical Formula: $C_{24}H_{35}N_7O_6$
Expected Neutral Mass: 517.2648
Observed Neutral Mass: 517.2640
Mass Error (ppm): 1.7



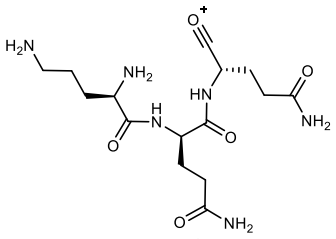
1

Chemical Formula: $C_{19}H_{26}N_5O_5^+$
Expected m/z: 404.193
Observed m/z: 404.192
Mass Error (mDa): 1.0



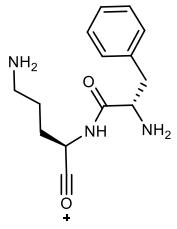
2

Chemical Formula: $C_{19}H_{28}N_5O_4^+$
Expected m/z: 390.214
Observed m/z: 390.213
Mass Error (mDa): 1.0



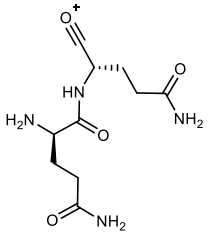
3

Chemical Formula: $C_{15}H_{27}N_6O_5^+$
Expected m/z: 371.204
Observed m/z: 371.202
Mass Error (mDa): 2,0



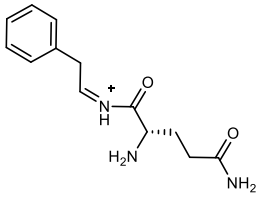
4

Chemical Formula: $C_{14}H_{20}N_3O_2^+$
Expected m/z: 262.155
Observed m/z: 262.154
Mass Error (mDa): 1.0



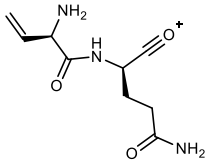
5

Chemical Formula: $C_{10}H_{17}N_4O_4^+$
Expected m/z: 257.124
Observed m/z: 257.124
Mass Error (mDa): < 1.0



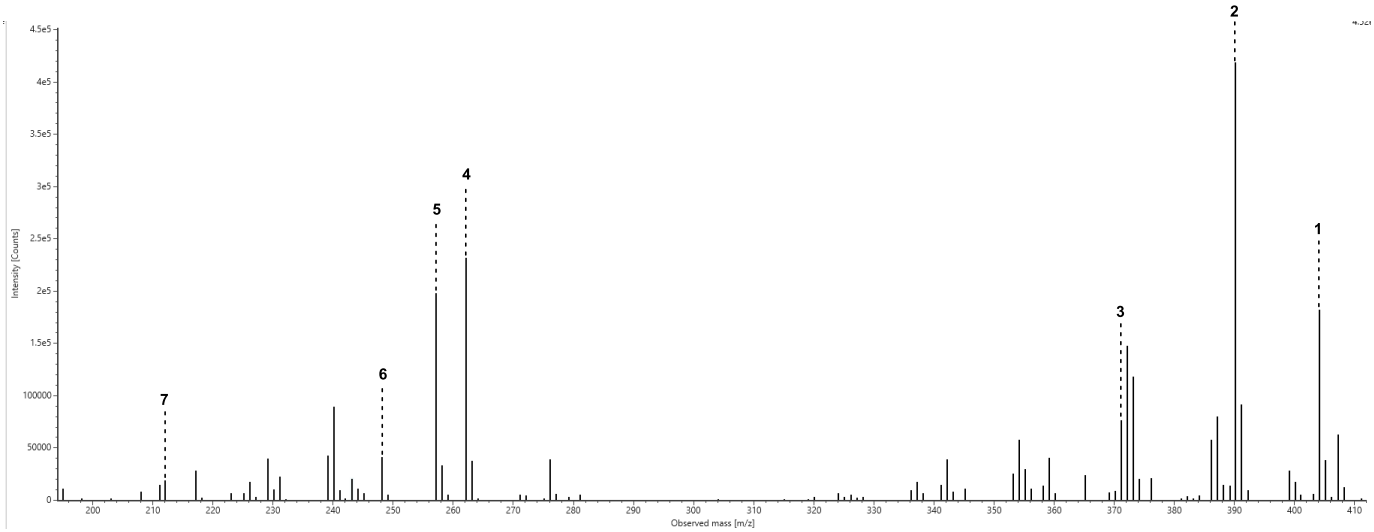
6

Chemical Formula: $C_{13}H_{18}N_3O_2^+$
Expected m/z: 248.139
Observed m/z: 248.138
Mass Error (mDa): 1.0

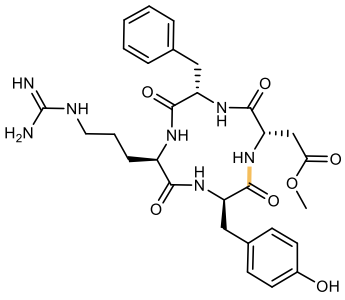


7

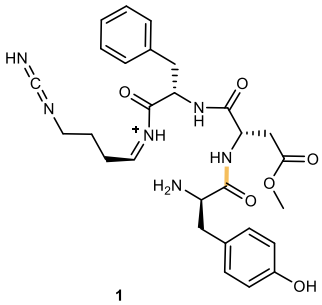
Chemical Formula: $C_9H_{14}N_3O_3^+$
Expected m/z: 212.103
Observed m/z: 212.103
Mass Error (mDa): < 1.0



Supplementary Fig. 68: MS2 spectrum for Cyclic Peptide 22 (Cyc22)

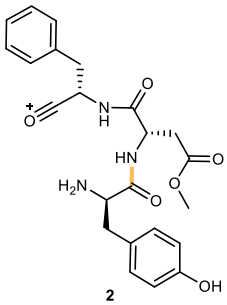


Chemical Formula: $C_{29}H_{37}N_7O_7$
Expected Neutral Mass: 595.2754
Observed Neutral Mass: 595.2767
Mass Error (ppm): 2.1



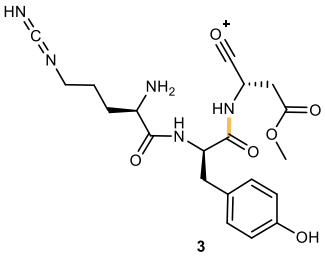
1

Chemical Formula: $C_{28}H_{35}N_6O_6^+$
Expected m/z: 551.261
Observed m/z: 551.261
Mass Error (mDa): < 1.0



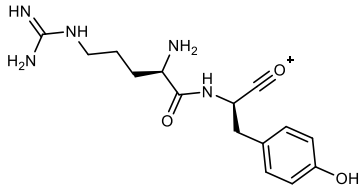
2

Chemical Formula: $C_{23}H_{26}N_3O_6^+$
Expected m/z: 440.182
Observed m/z: 440.182
Mass Error (mDa): < 1.0



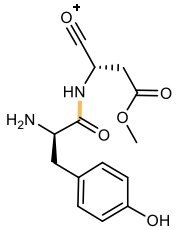
3

Chemical Formula: $C_{20}H_{26}N_5O_6^+$
Expected m/z: 432.188
Observed m/z: 432.188
Mass Error (mDa): > 1.0



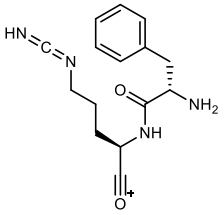
4

Chemical Formula: $C_{15}H_{22}N_5O_3^+$
Expected m/z: 320.172
Observed m/z: 320.171
Mass Error (mDa): 1.0



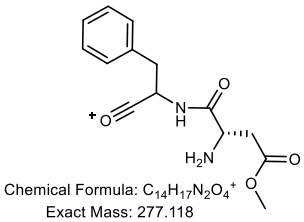
5

Chemical Formula: $C_{14}H_{17}N_2O_5^+$
Expected m/z: 293.113
Observed m/z: 293.112
Mass Error (mDa): < 1.0



6

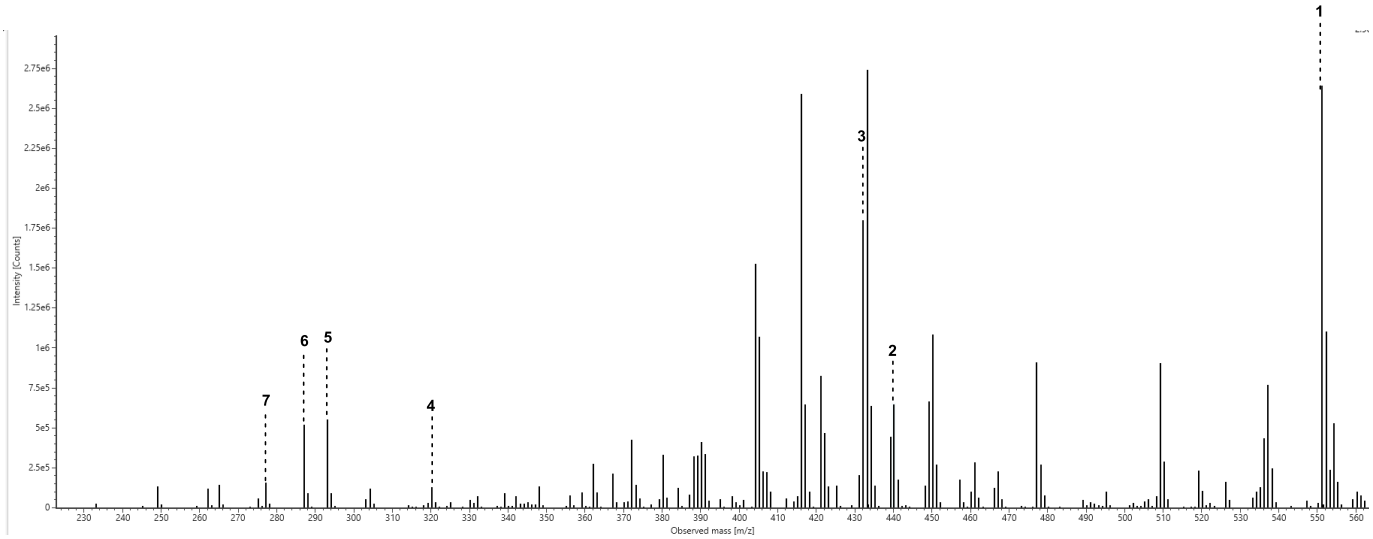
Chemical Formula: $C_{15}H_{19}N_4O_2^+$
Expected m/z: 287.150
Observed m/z: 287.149
Mass Error (mDa): 2.0



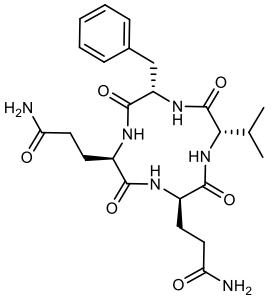
Chemical Formula: $C_{14}H_{17}N_2O_4^+$
Exact Mass: 277.118

7

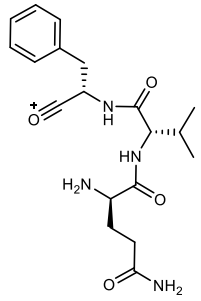
Chemical Formula :$C_{14}H_{17}N_2O_4^+$
Expected m/z: 277.118
Observed m/z: 277.117
Mass Error (mDa): 1.0



Supplementary Fig. 69: MS2 spectrum for Cyclic Peptide 23 (Cyc23)

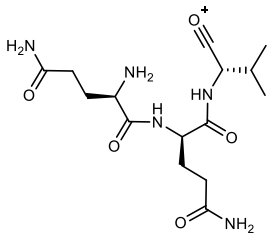


Chemical Formula: $C_{24}H_{34}N_6O_6$
Expected Neutral Mass: 502.2539
Observed Neutral Mass: 502.2538
Mass Error (ppm): 0.3



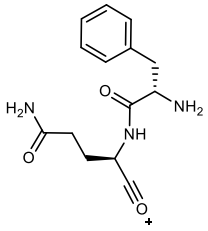
1

Chemical Formula: $C_{19}H_{27}N_4O_4^+$
Expected m/z: 375.203
Observed m/z: 375.203
Mass Error (mDa): < 1.0



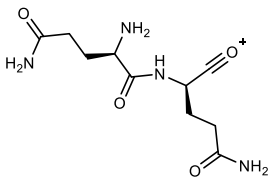
2

Chemical Formula: $C_{15}H_{26}N_5O_5^+$
Expected m/z: 356.193
Observed m/z: 356.192
Mass Error (mDa): 1.0



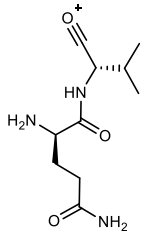
3

Chemical Formula: $C_{14}H_{18}N_3O_3^+$
Expected m/z: 276.134
Observed m/z: 276.134
Mass Error (mDa): < 1.0



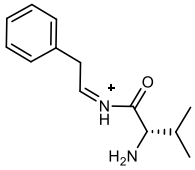
4

Chemical Formula: $C_{10}H_{17}N_4O_4^+$
Expected m/z: 257.124
Observed m/z: 257.124
Mass Error (mDa): < 1.0



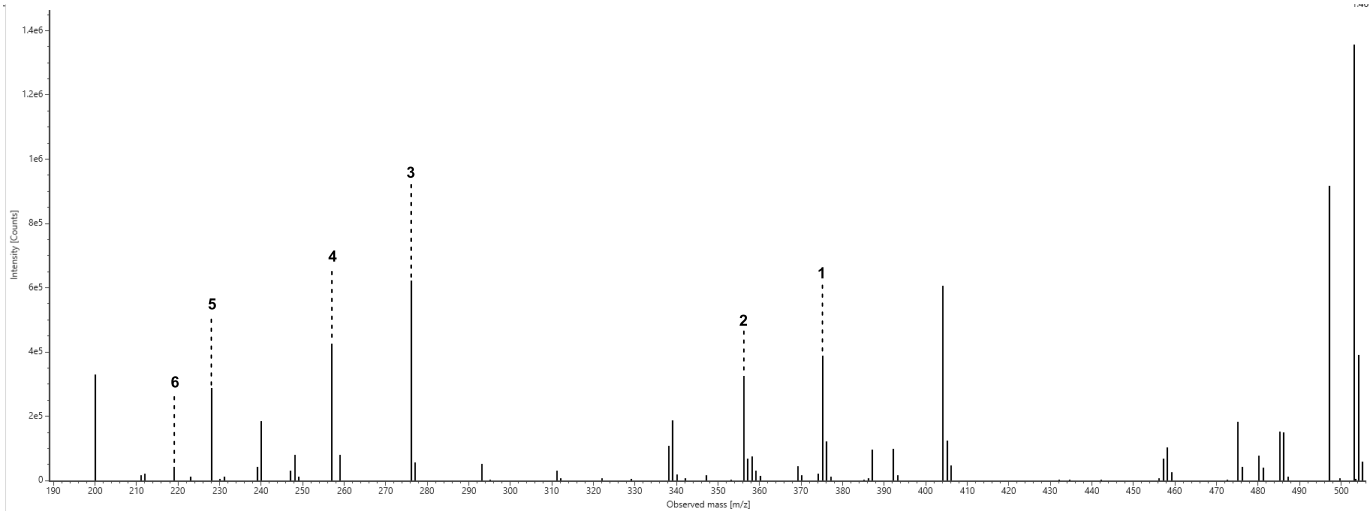
5

Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.133
Mass Error (mDa): 1.0

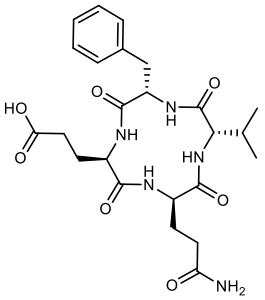


6

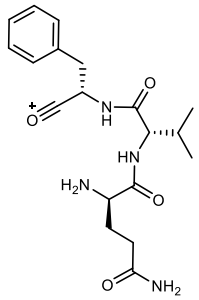
Chemical Formula: $C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.148
Mass Error (mDa): 1.0



Supplementary Fig. 70: MS2 spectrum for Cyclic Peptide 24 (Cyc24)

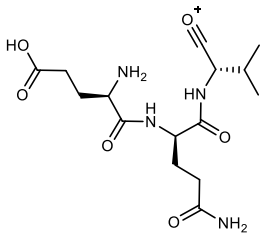


Chemical Formula:$C_{24}H_{33}N_5O_7$
Expected Neutral Mass: 503.2380
Observed Neutral Mass: 503.2372
Mass Error (ppm): 1.6



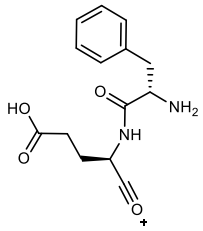
1

Chemical Formula: $C_{19}H_{27}N_4O_4^+$
Expected m/z: 375.203
Observed m/z: 375.202
Mass Error (mDa): 1.0



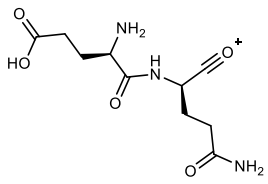
2

Chemical Formula: $C_{15}H_{25}N_4O_6^+$
Expected m/z: 357.177
Observed m/z: 357.177
Mass Error (mDa): < 1.0



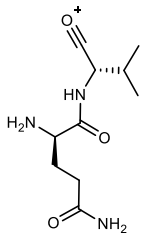
3

Chemical Formula: $C_{14}H_{17}N_2O_4^+$
Expected m/z: 277.118
Observed m/z: 277.117
Mass Error (mDa): 1.0



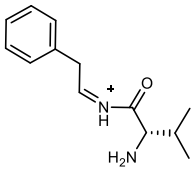
4

Chemical Formula: $C_{10}H_{16}N_3O_5^+$
Expected m/z: 258.108
Observed m/z: 258.108
Mass Error (mDa): < 1.0



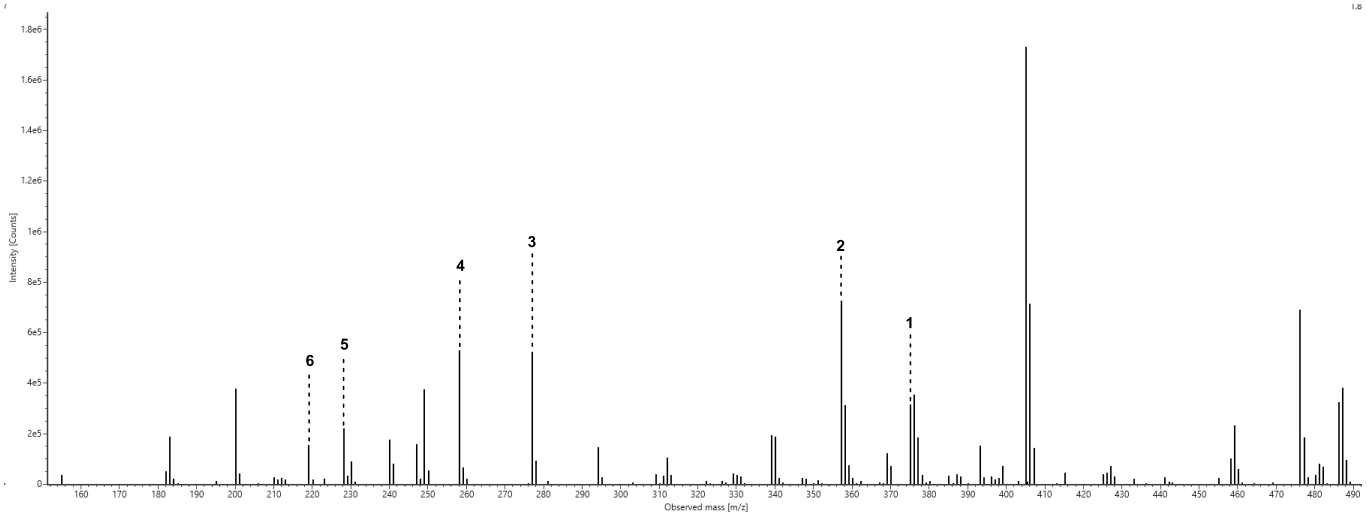
5

Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.133
Mass Error (mDa): 1.0

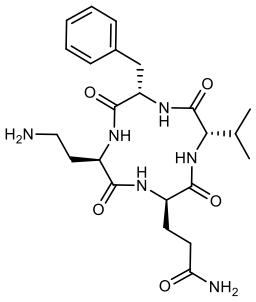


6

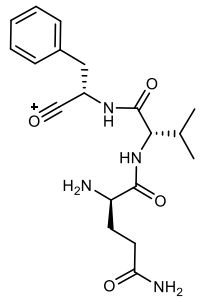
Chemical Formula: $C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.148
Mass Error (mDa): 1.0



Supplementary Fig. 71: MS2 spectrum for Cyclic Peptide 25 (Cyc25)

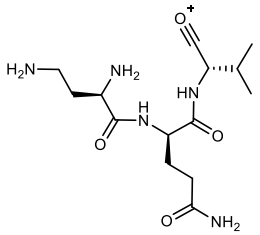


Chemical Formula:$C_{23}H_{34}N_6O_5$
Expected Neutral Mass: 474.2590
Observed Neutral Mass: 474.2577
Mass Error (ppm): 2.9



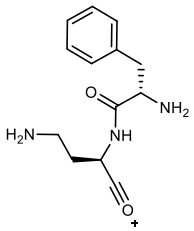
1

Chemical Formula: $C_{19}H_{27}N_4O_4^+$
Expected m/z: 375.203
Observed m/z: 375.202
Mass Error (mDa): 1.0



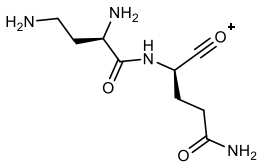
2

Chemical Formula: $C_{14}H_{26}N_5O_4^+$
Expected m/z: 328.198
Observed m/z: 328.197
Mass Error (mDa): 1.0



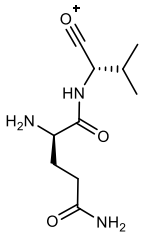
3

Chemical Formula: $C_{13}H_{18}N_3O_2^+$
Expected m/z: 248.139
Observed m/z: 248.138
Mass Error (mDa): 1.0



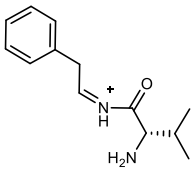
4

Chemical Formula: $C_8H_{17}N_4O_3^+$
Expected m/z: 229.130
Observed m/z: 229.130
Mass Error (mDa): < 1.0



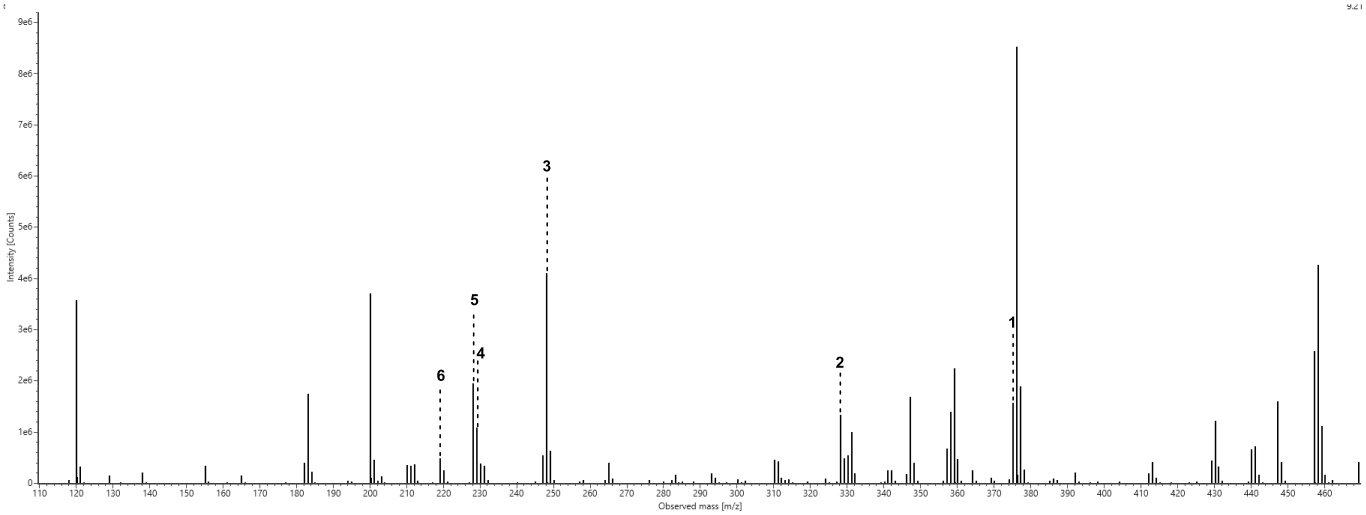
5

Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.133
Mass Error (mDa): 1.0

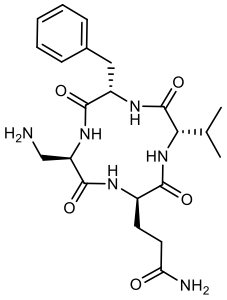


6

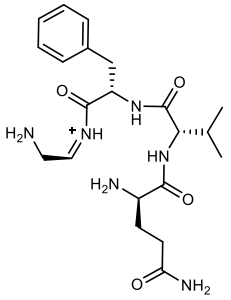
Chemical Formula: $C_{13}H_{19}N_2O^+$
Expected m/z: 219.149
Observed m/z: 219.148
Mass Error (mDa): 1.0



Supplementary Fig. 72: MS2 spectrum for Cyclic Peptide 26 (Cyc26))

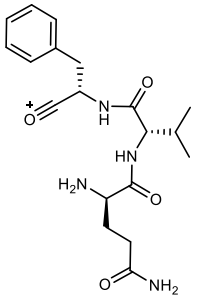


Chemical Formula:$C_{22}H_{32}N_6O_5$
Expected Neutral Mass: 460.2434
Observed Neutral Mass: 460.2423
Mass Error (ppm): 2.3



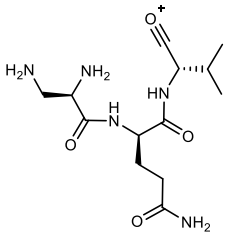
1

Chemical Formula: $C_{21}H_{33}N_6O_4^+$
Expected m/z: 433.256
Observed m/z: 433.255
Mass Error (mDa): 1.0



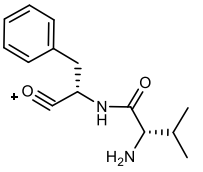
2

Chemical Formula: $C_{19}H_{27}N_4O_4^+$
Expected m/z: 375.203
Observed m/z: 375.202
Mass Error (mDa): 1.0



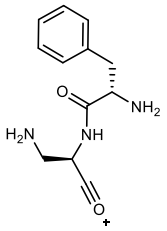
3

Chemical Formula: $C_{13}H_{24}N_5O_4^+$
Expected m/z: 314.182
Observed m/z: 314.183
Mass Error (mDa): 1.0



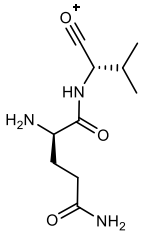
4

Chemical Formula: $C_{14}H_{19}N_2O_2^+$
Expected m/z: 247.144
Observed m/z: 247.144
Mass Error (mDa): > 1.0



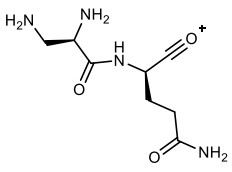
5

Chemical Formula: $C_{12}H_{16}N_3O_2^+$
Expected m/z :234.124
Observed m/z: 234.125
Mass Error (mDa): 1.0



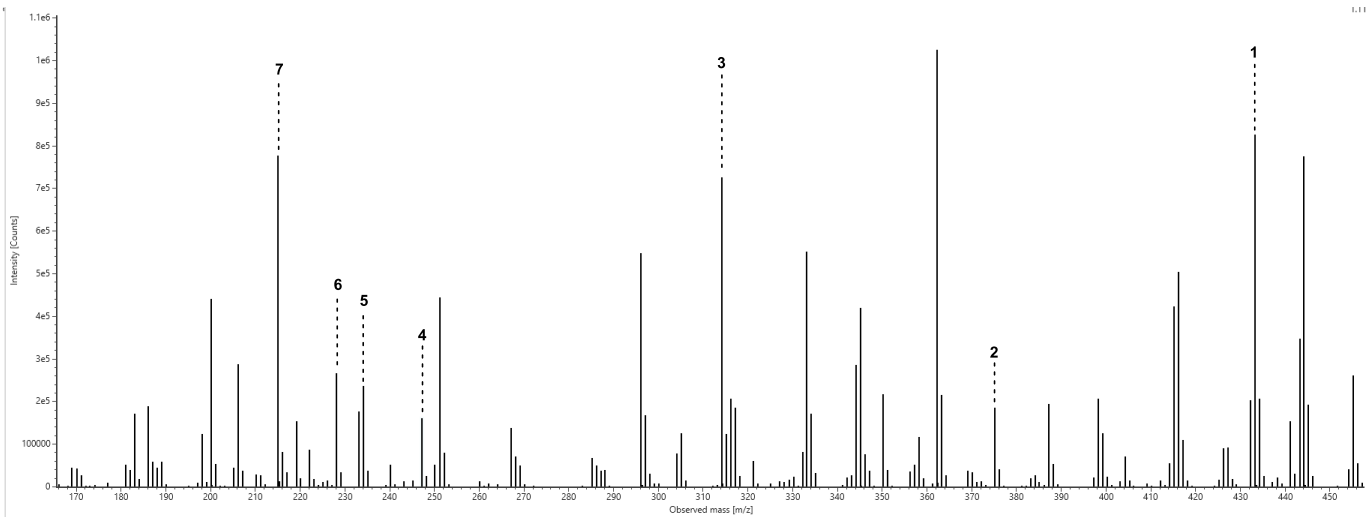
6

Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.134
Mass Error (mDa): > 1.0

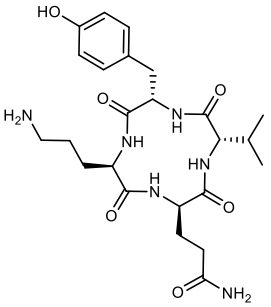


7

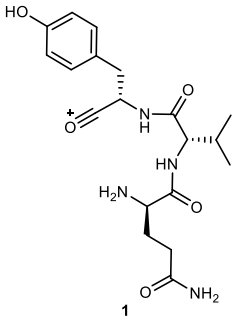
Chemical Formula: $C_8H_{15}N_4O_3^+$
Expected m/z: 215.114
Observed m/z: 215.114
Mass Error (mDa): < 1.0



Supplementary Fig. 73: MS2 spectrum for Cyclic Peptide 27 (Cyc27)

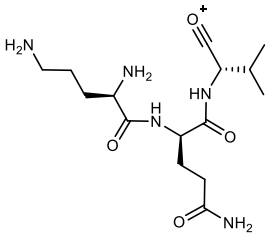


Chemical Formula: $C_{24}H_{36}N_6O_6$
Expected Neutral Mass: 504.2696
Observed Neutral Mass: 504.2694
Mass Error (ppm): 0.5



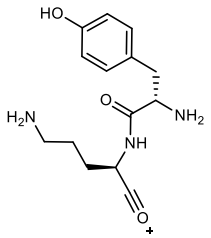
1

Chemical Formula: $C_{19}H_{27}N_4O_5^+$
Expected m/z: 391.197
Observed m/z: 391.195
Mass Error (mDa): 2.0



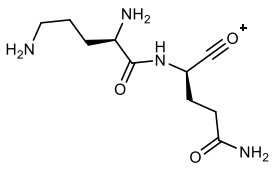
2

Chemical Formula: $C_{15}H_{28}N_5O_4^+$
Expected m/z: 342.213
Observed m/z: 342.210
Mass Error (mDa): 3.0



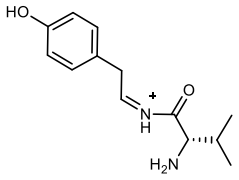
3

Chemical Formula: $C_{14}H_{20}N_3O_3^+$
Expected m/z: 278.150
Observed m/z: 278.147
Mass Error (mDa): 3.0



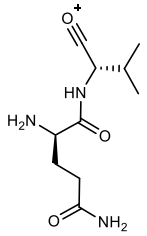
4

Chemical Formula: $C_{10}H_{19}N_4O_3^+$
Expected m/z: 243.145
Observed m/z: 243.141
Mass Error (mDa): 4.0



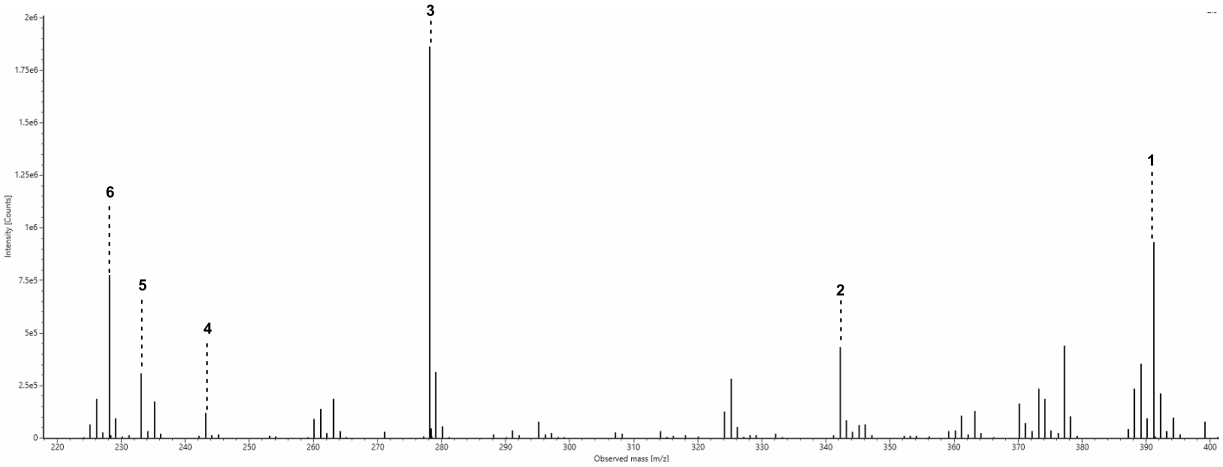
5

Chemical Formula: $C_{13}H_{19}N_2O_2^+$
Expected m/z: 235.144
Observed m/z: 235.140
Mass Error (mDa): 4.0

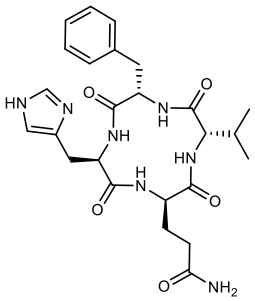


6

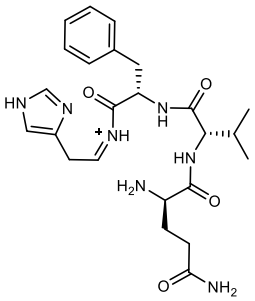
Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.130
Mass Error (mDa): 4.0



Supplementary Fig. 74: MS2 spectrum for Cyclic Peptide 28 (Cyc28)

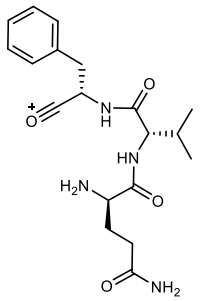


Chemical Formula: $C_{25}H_{33}N_7O_5$
Expected Neutral Mass: 511.2543
Observed Neutral Mass: 511.2544
Mass Error (ppm): 0.2



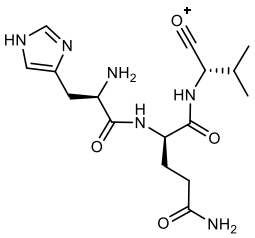
1

Chemical Formula: $C_{24}H_{34}N_7O_4^+$
Expected m/z: 484.267
Observed m/z: 484.266
Mass Error (mDa): 1.0



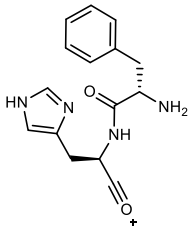
2

Chemical Formula: $C_{19}H_{27}N_4O_4^+$
Expected m/z: 375.203
Observed m/z: 375.202
Mass Error (mDa): 1.0



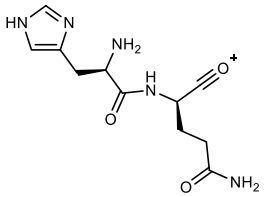
3

Chemical Formula: $C_{16}H_{25}N_6O_4^+$
Expected m/z: 365.193
Observed m/z: 365.192
Mass Error (mDa): 1.0



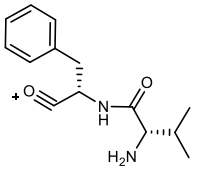
4

Chemical Formula: $C_{15}H_{17}N_4O_2^+$
Expected m/z : 285.135
Observed m/z: 285.133
Mass Error (mDa): 2.0



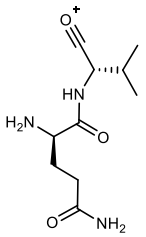
5

Chemical Formula: $C_{11}H_{16}N_5O_3^+$
Expected m/z: 266.125
Observed m/z: 266.123
Mass Error (mDa): 1.0



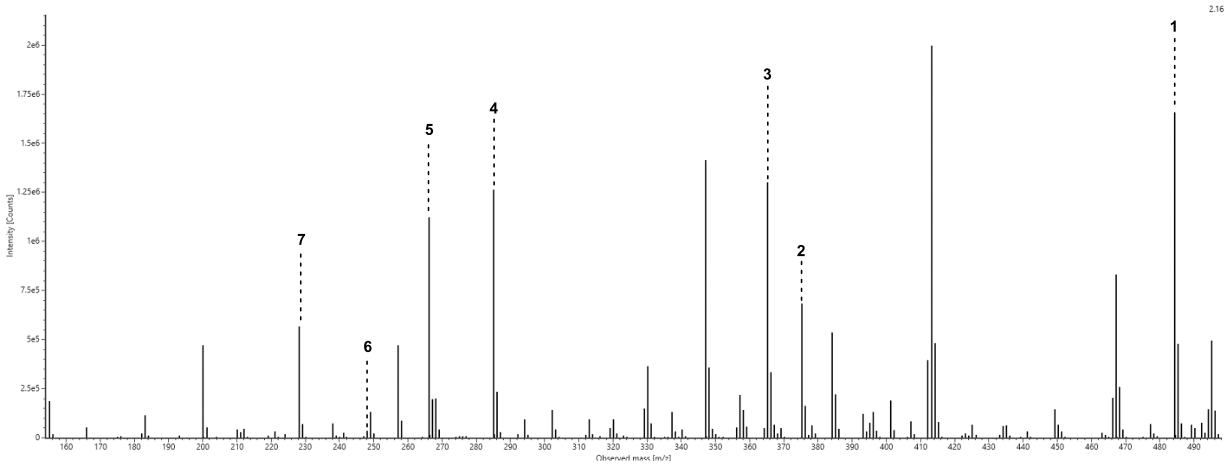
6

Chemical Formula: $C_{14}H_{19}N_2O_2^+$
Expected m/z: 247.144
Observed m/z: 247.144
Mass Error (mDa): < 1.0

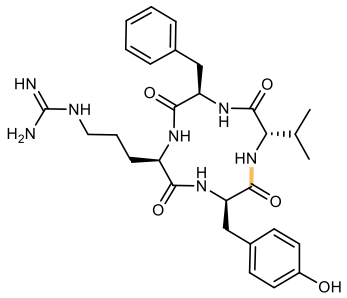


7

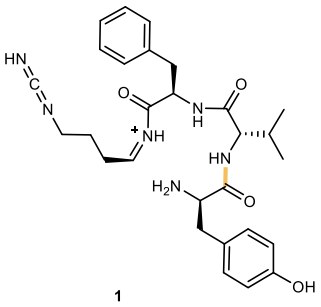
Chemical Formula: $C_{10}H_{18}N_3O_3^+$
Expected m/z: 228.134
Observed m/z: 228.135
Mass Error (mDa): 1.0



Supplementary Fig. 75: MS2 spectrum for Cyclic Peptide 29 (Cyc29)

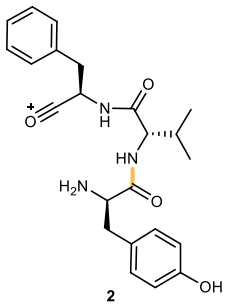


Chemical Formula: $C_{29}H_{39}N_7O_5$
Expected Neutral Mass: 565.3012
Observed Neutral Mass: 565.3012
Mass Error (ppm): < 0.1



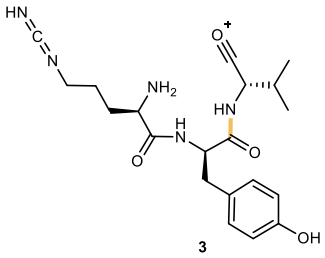
1

Chemical Formula: $C_{28}H_{37}N_6O_4^+$
Expected m/z: 521.287
Observed m/z: 521.286
Mass Error (mDa): 1.0



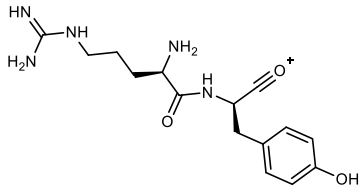
2

Chemical Formula: $C_{23}H_{28}N_3O_4^+$
Expected m/z: 410.207
Observed m/z: 410.206
Mass Error (mDa): 1.0



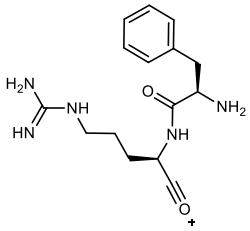
3

Chemical Formula: $C_{20}H_{28}N_5O_4^+$
Expected m/z: 402.214
Observed m/z: 402.214
Mass Error (mDa): < 1.0



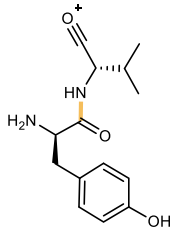
4

Chemical Formula: $C_{15}H_{22}N_5O_3^+$
Expected m/z: 320.172
Observed m/z: 320.171
Mass Error (mDa): 1.0



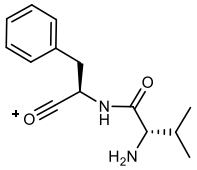
5

Chemical Formula: $C_{15}H_{22}N_5O_2^+$
Expected m/z: 304.177
Observed m/z: 304.174
Mass Error (mDa): 3.0



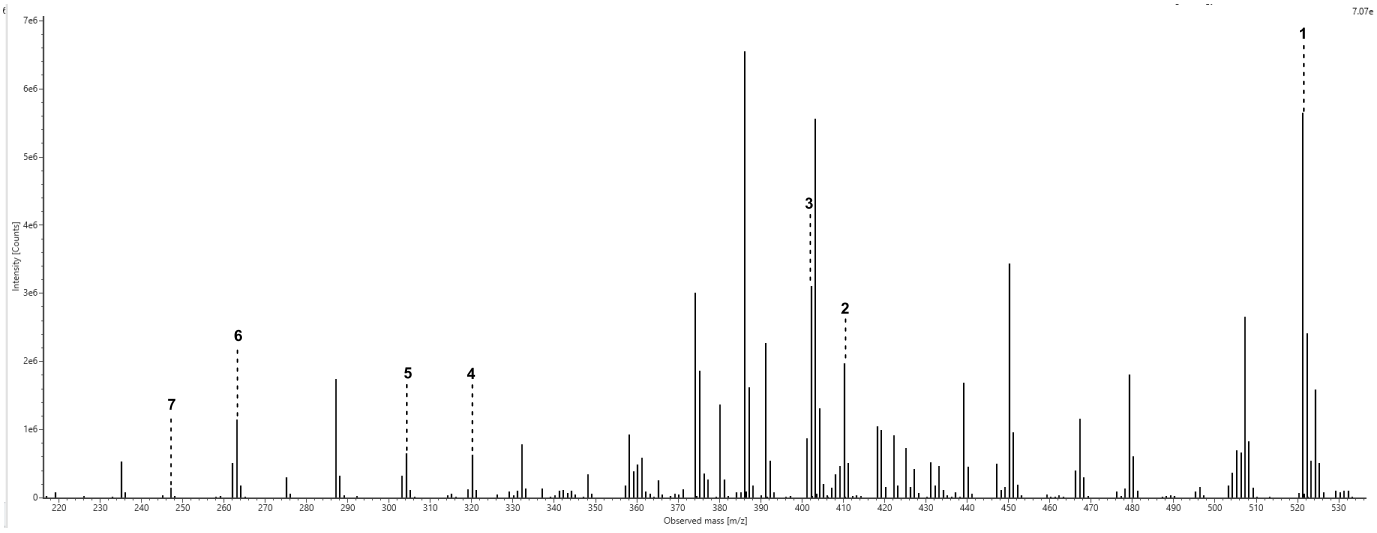
6

Chemical Formula: $C_{14}H_{19}N_2O_3^+$
Expected m/z: 263.139
Observed m/z: 263.139
Mass Error (mDa): < 1.0

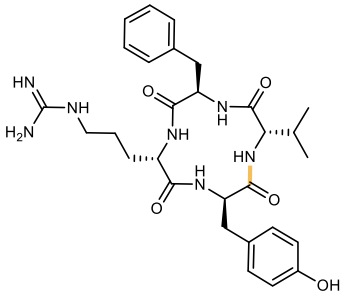


7

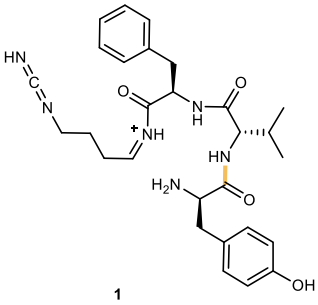
Chemical Formula :$C_{14}H_{19}N_2O_2^+$
Expected m/z: 247.144
Observed m/z: 247.143
Mass Error (mDa): 1.0



Supplementary Fig. 76: MS2 spectrum for Cyclic Peptide 30 (Cyc30)

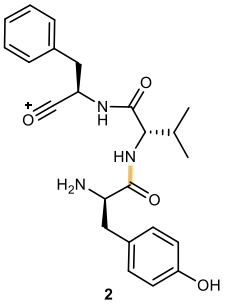


Chemical Formula: $C_{29}H_{39}N_7O_5$
Expected Neutral Mass: 565.3012
Observed Neutral Mass: 565.3016
Mass Error (ppm): 0.5



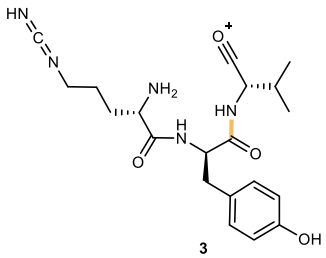
1

Chemical Formula: $C_{28}H_{37}N_6O_4^+$
Expected m/z: 521.287
Observed m/z: 521.286
Mass Error (mDa): 1.0



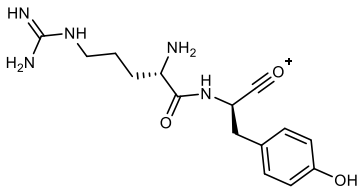
2

Chemical Formula: $C_{23}H_{28}N_3O_4^+$
Expected m/z: 410.207
Observed m/z: 410.205
Mass Error (mDa): 2.0



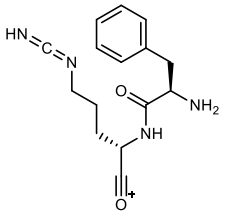
3

Chemical Formula: $C_{20}H_{28}N_5O_4^+$
Expected m/z: 402.214
Observed m/z: 402.214
Mass Error (mDa): < 1.0



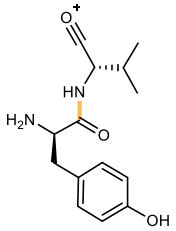
4

Chemical Formula: $C_{15}H_{22}N_5O_3^+$
Expected m/z: 320.172
Observed m/z: 320.170
Mass Error (mDa): 2.0



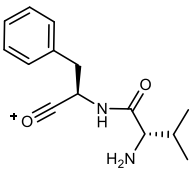
5

Chemical Formula: $C_{15}H_{19}N_4O_2^+$
Expected m/z: 287.150
Observed m/z: 287.149
Mass Error (mDa): 1.0



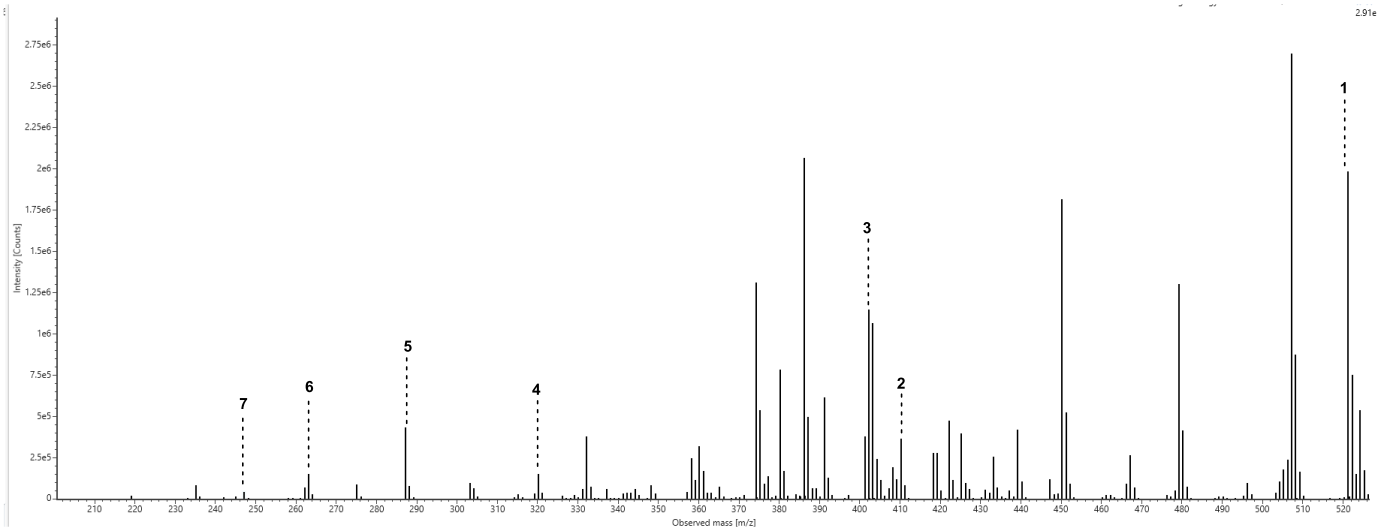
6

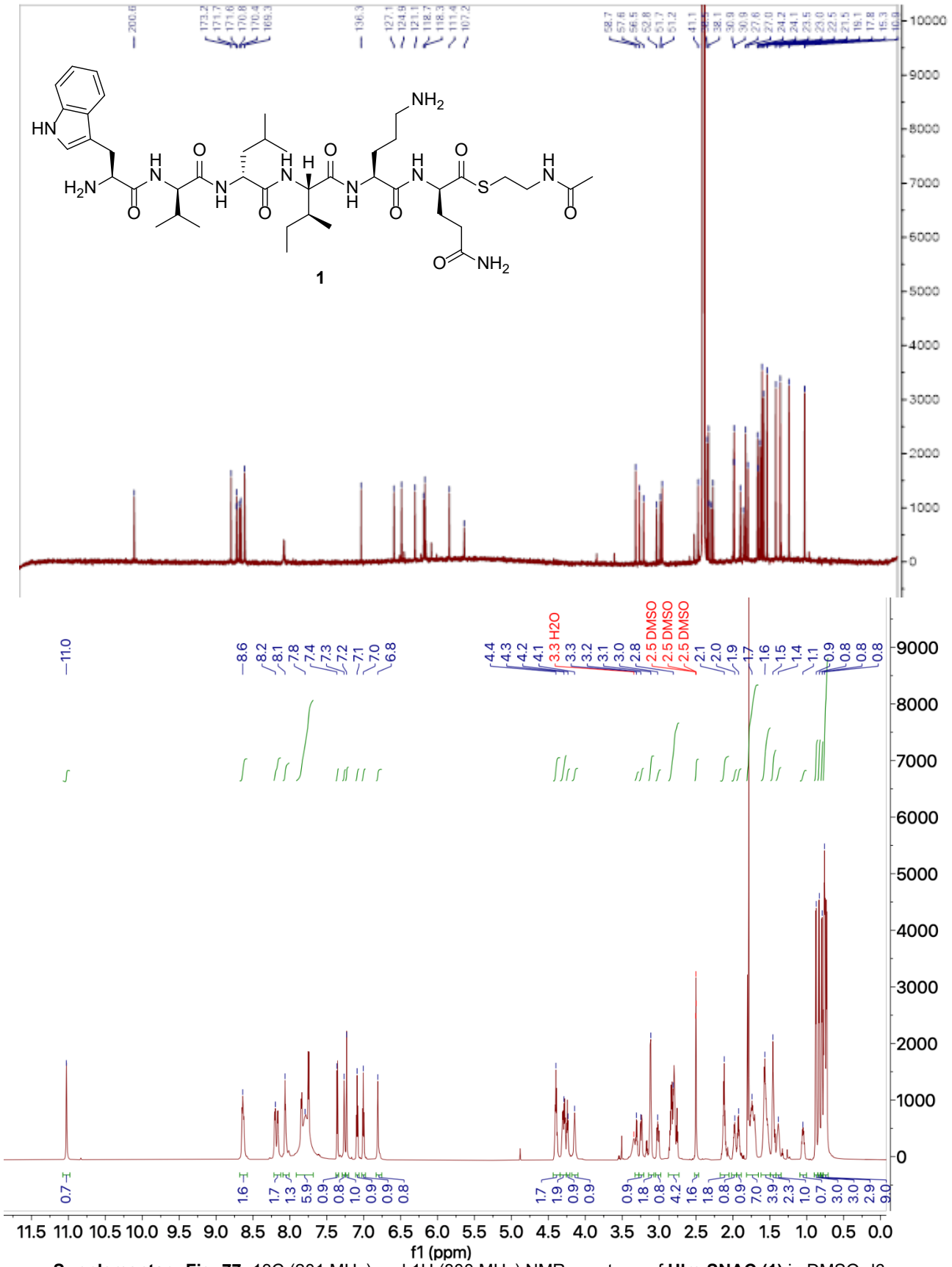
Chemical Formula: $C_{14}H_{19}N_2O_3^+$
Expected m/z: 263.139
Observed m/z: 263.138
Mass Error (mDa): 1.0



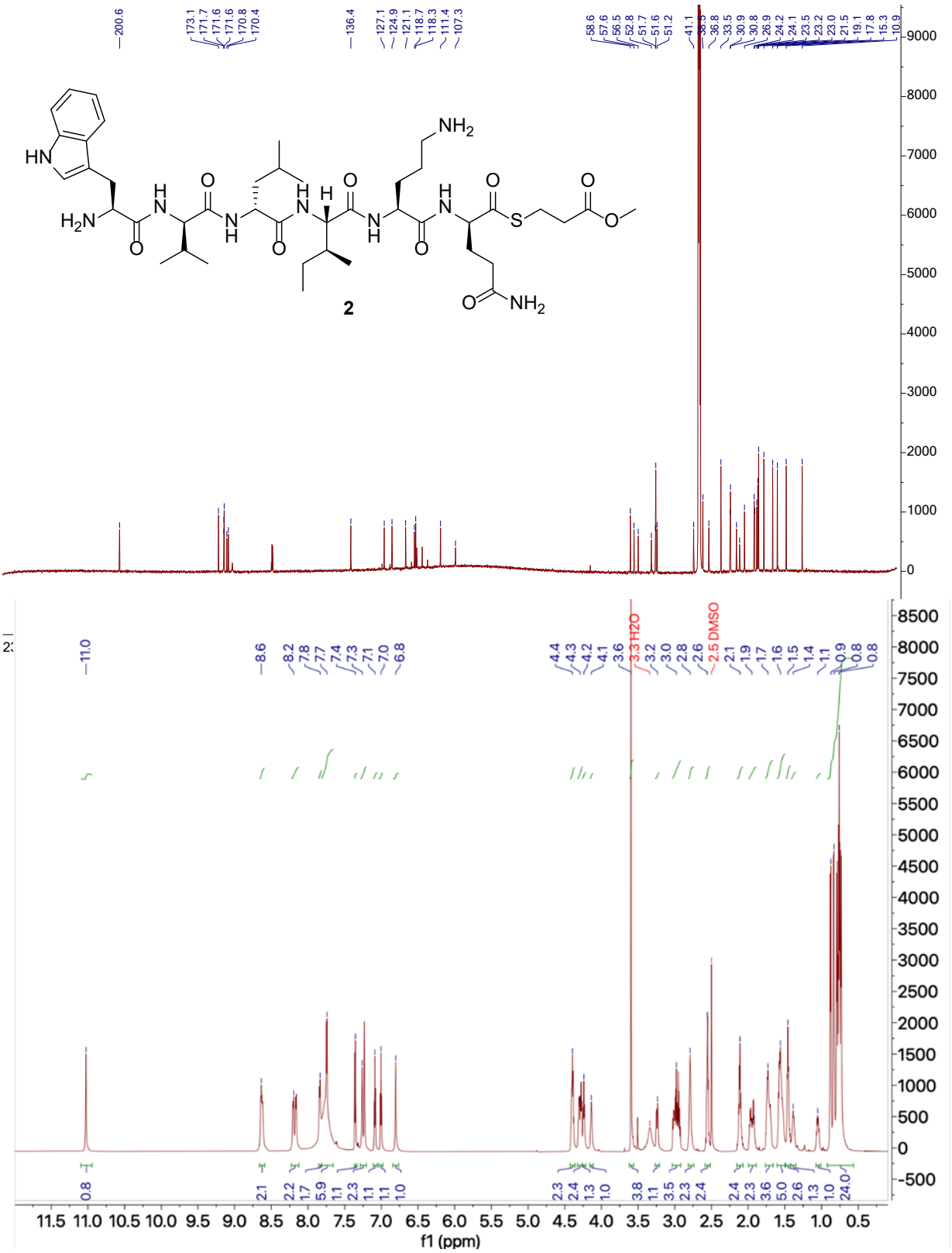
7

Chemical Formula :$C_{14}H_{19}N_2O_2^+$
Expected m/z: 247.144
Observed m/z: 247.142
Mass Error (mDa): 2.0

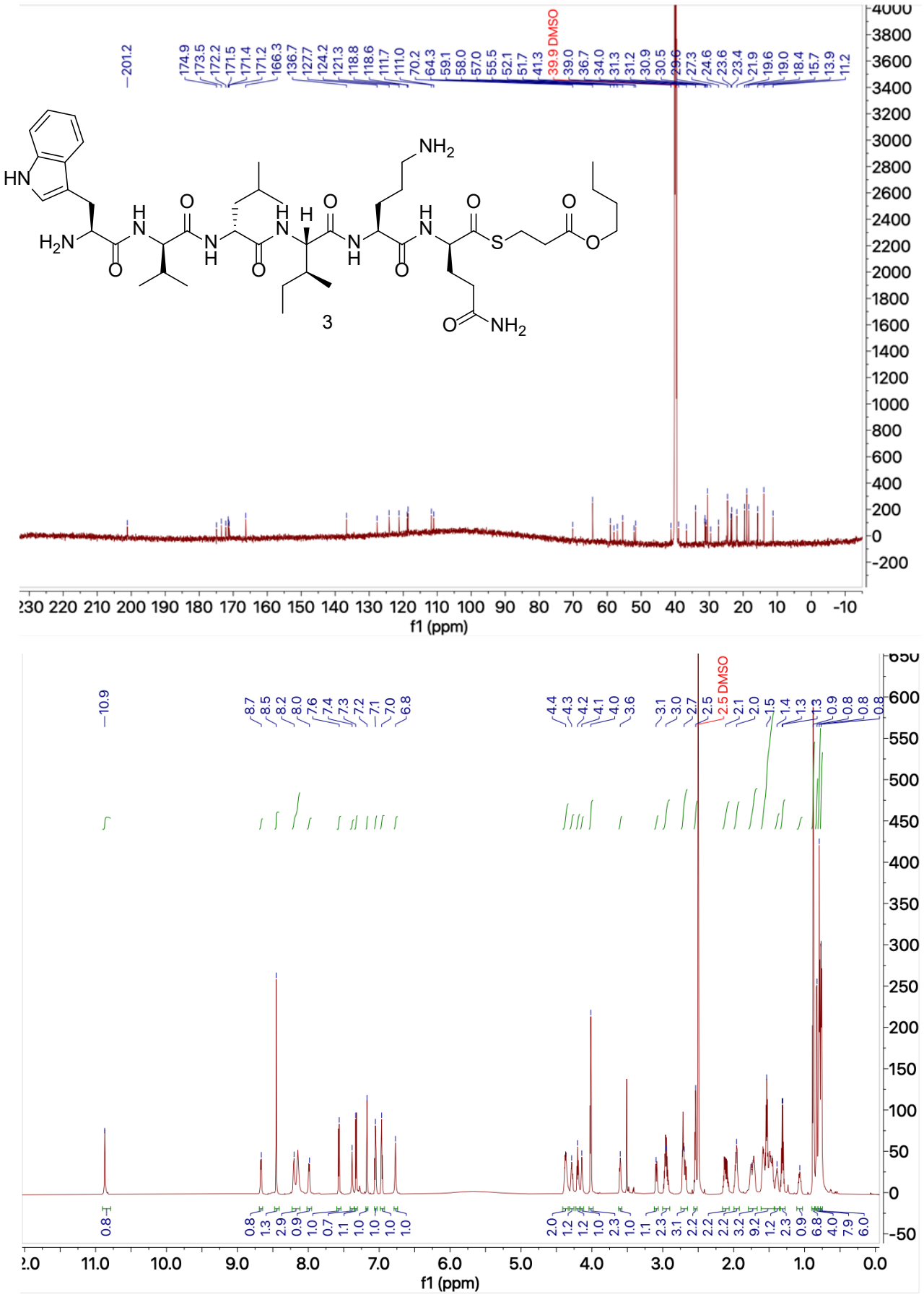




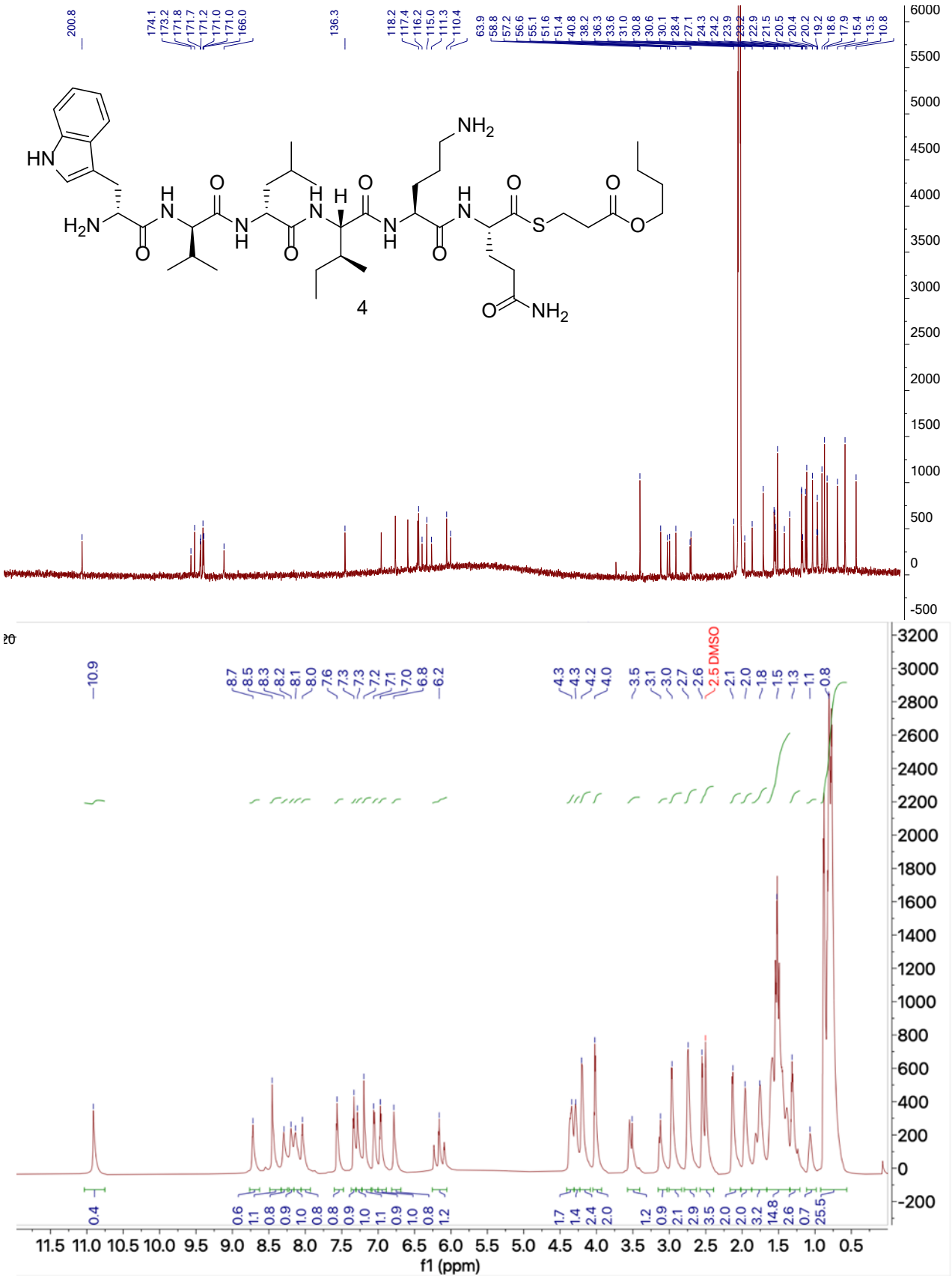
Supplementary Fig. 77: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **Ulm-SNAC (1)** in DMSO-d6



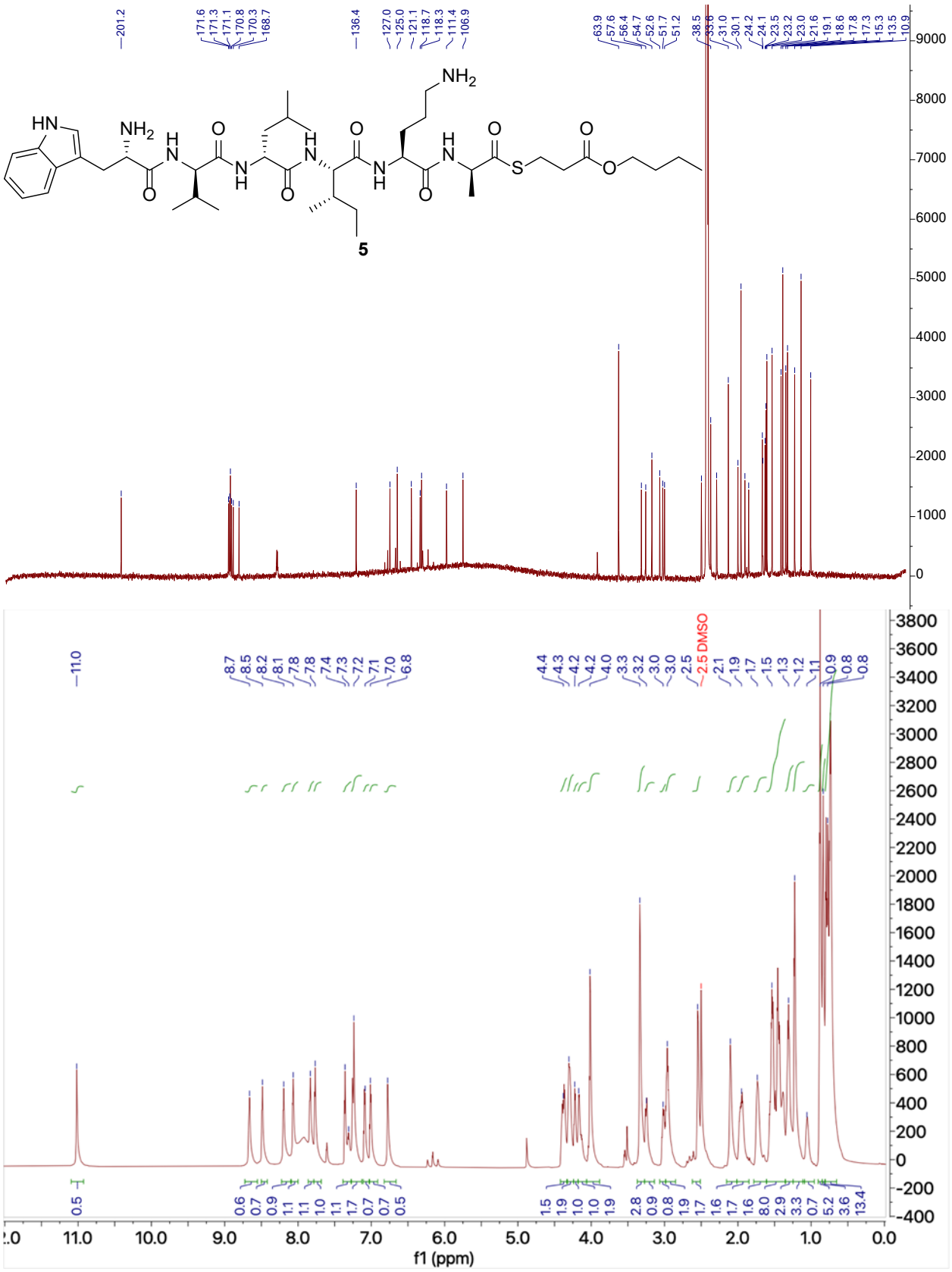
Supplementary Fig. 78: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **Ulm-SMMP (2)** in DMSO-d6



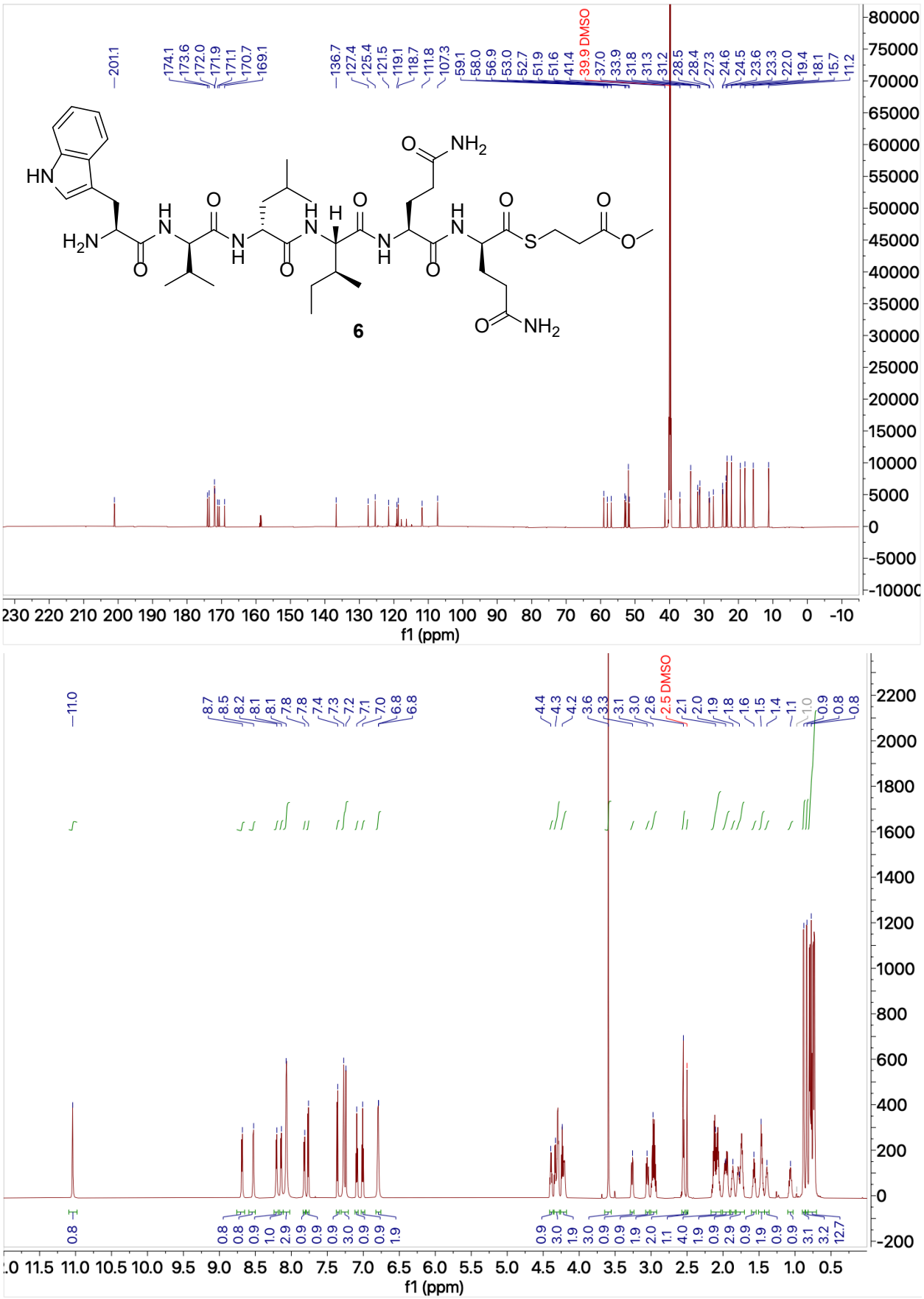
Supplementary Fig. 79: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **Ulm-SBMP (3)** in DMSO-d6



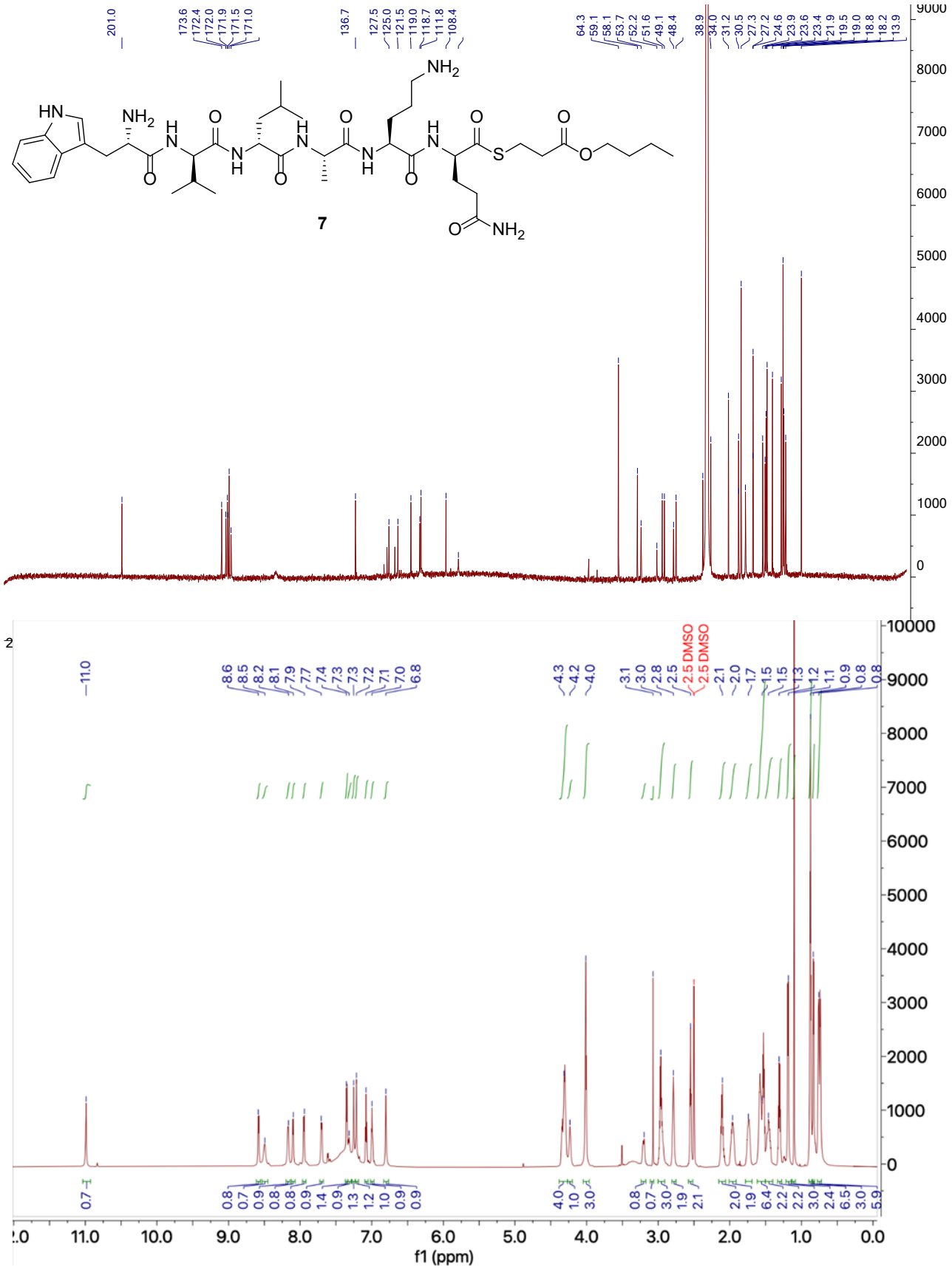
Supplementary Fig. 80: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **4** in DMSO-d6



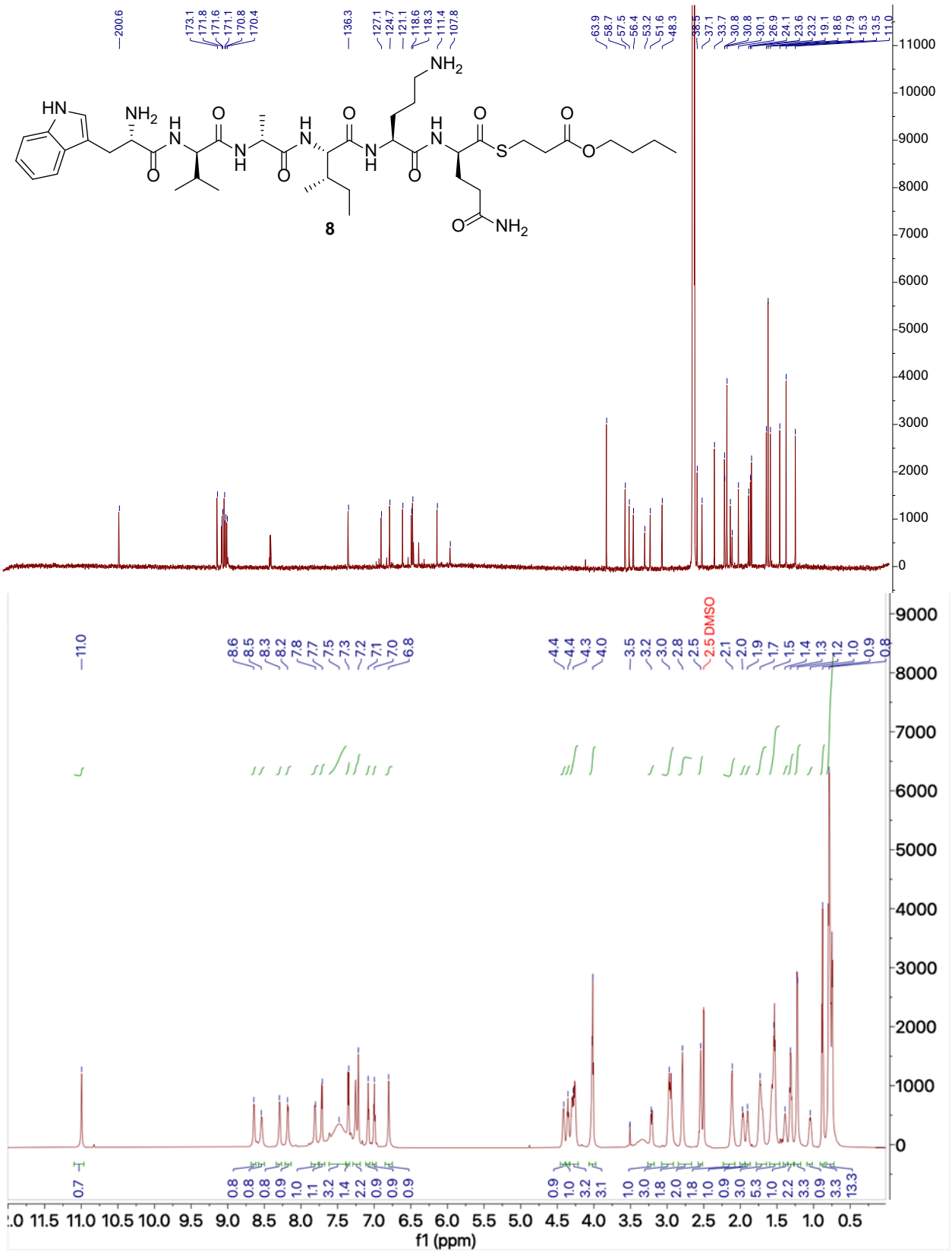
Supplementary Fig. 81:13C (201 MHz) and 1H (800 MHz) NMR spectrum of **5** in DMSO-d6



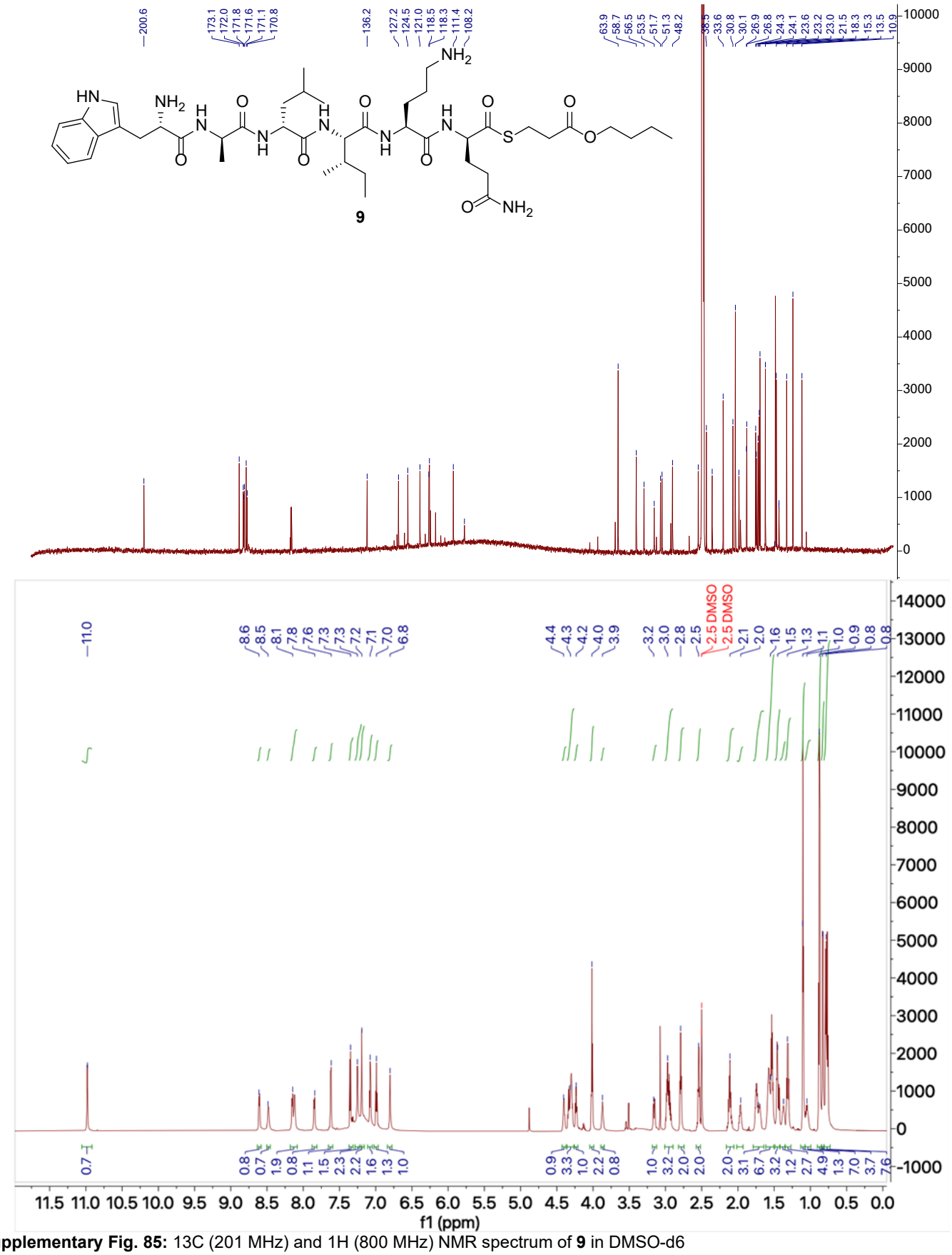
Supplementary Fig. 82:13C (201 MHz) and 1H (800 MHz) NMR spectrum of **6** in DMSO-d6



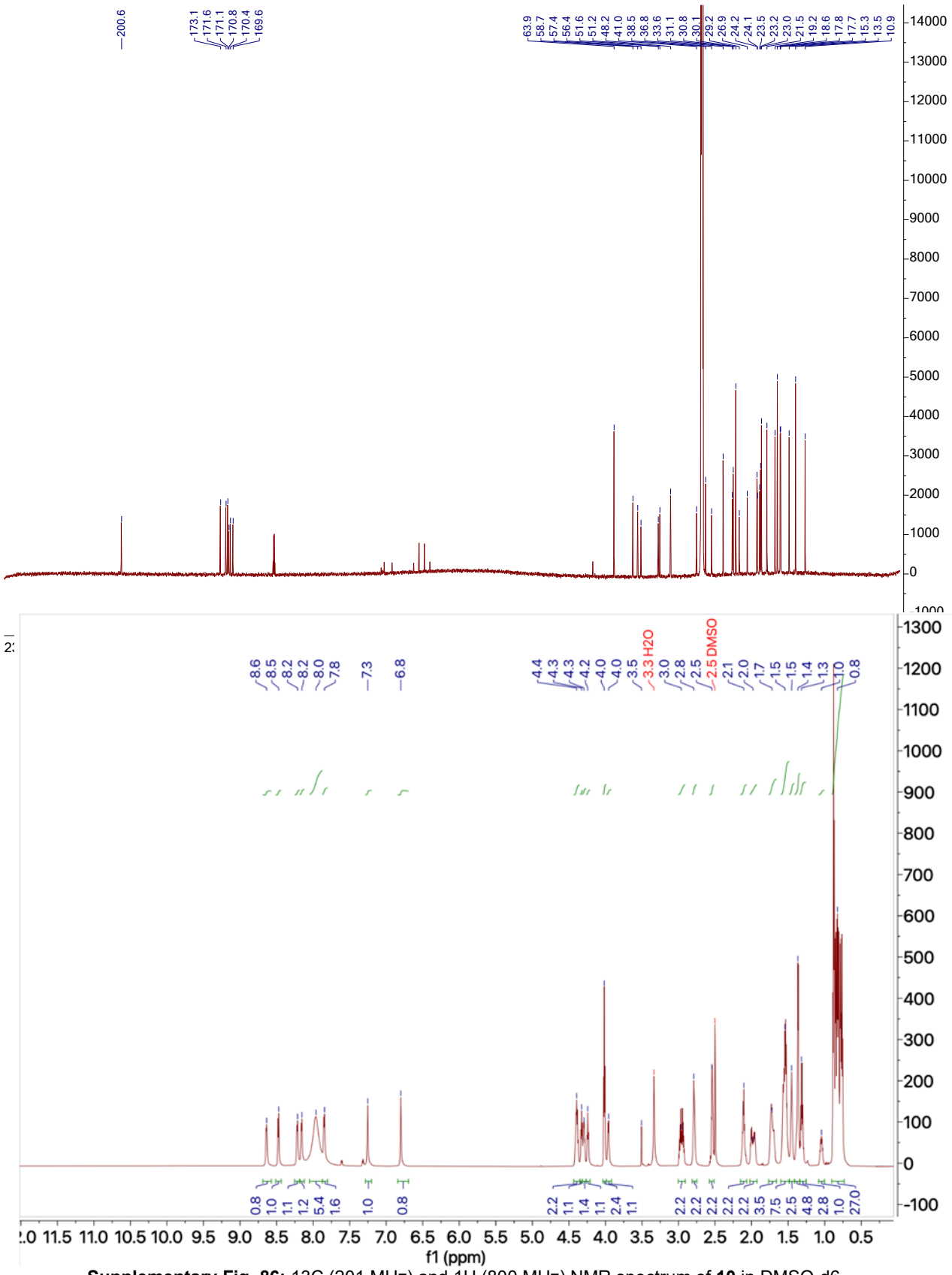
Supplementary Fig. 83: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **7** in DMSO-d6



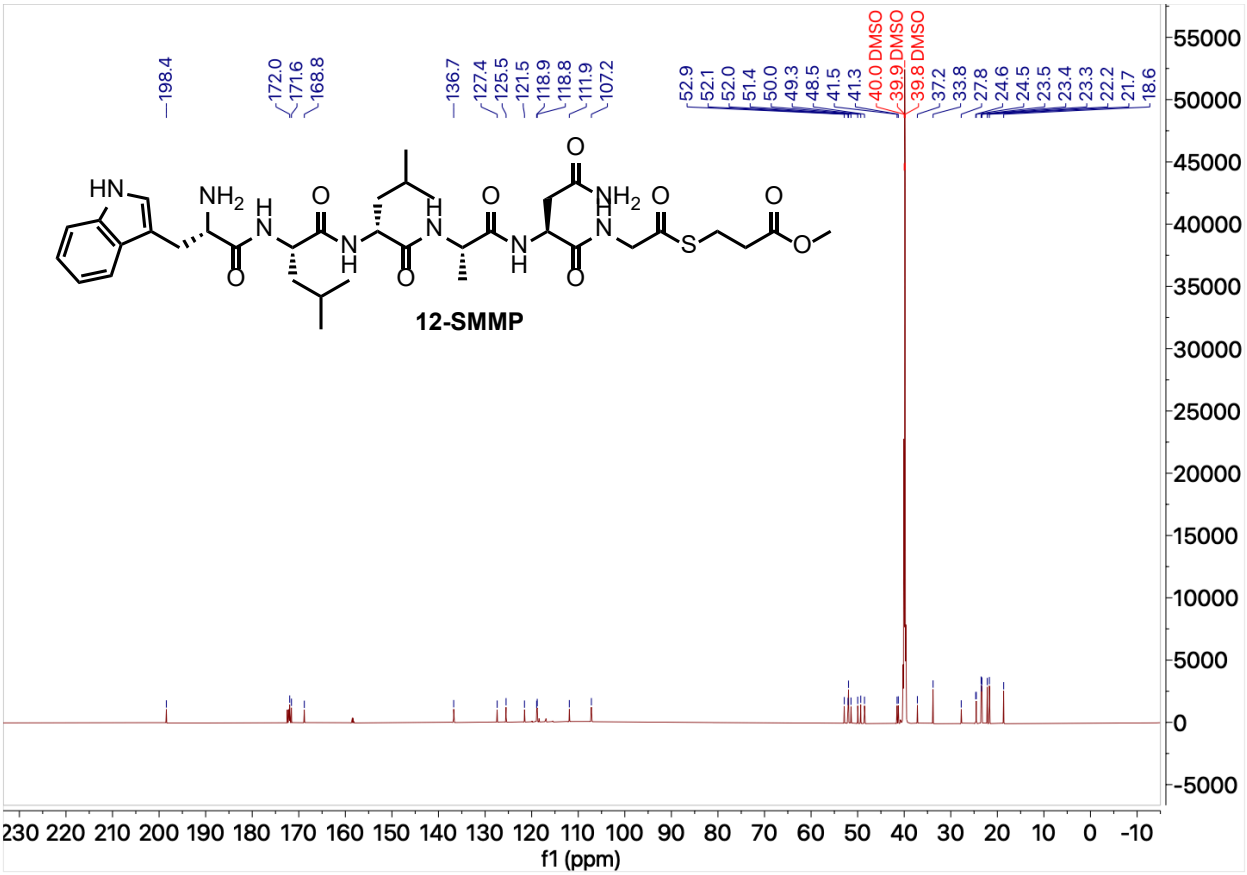
Supplementary Fig. 84: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **8** in DMSO-d6

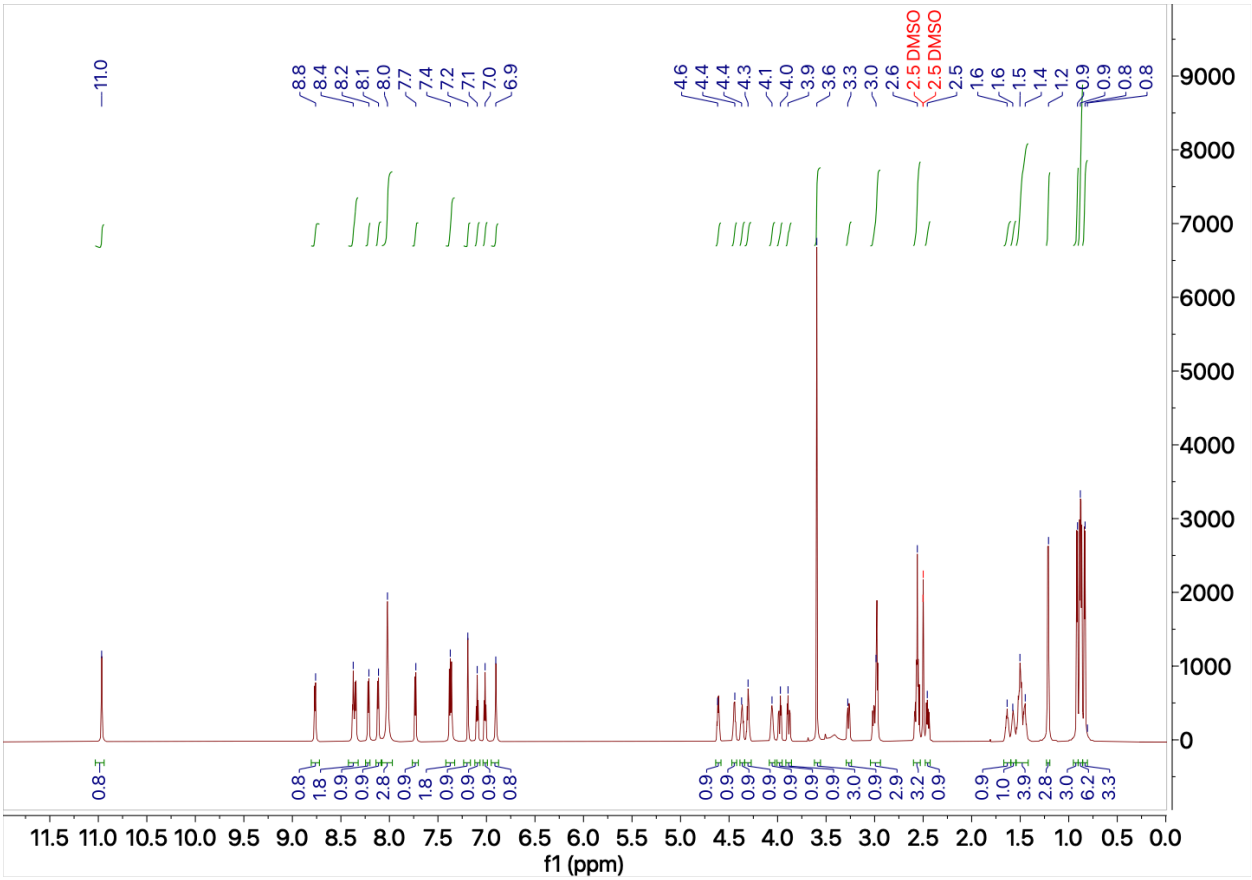


Supplementary Fig. 85: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **9** in DMSO-d6

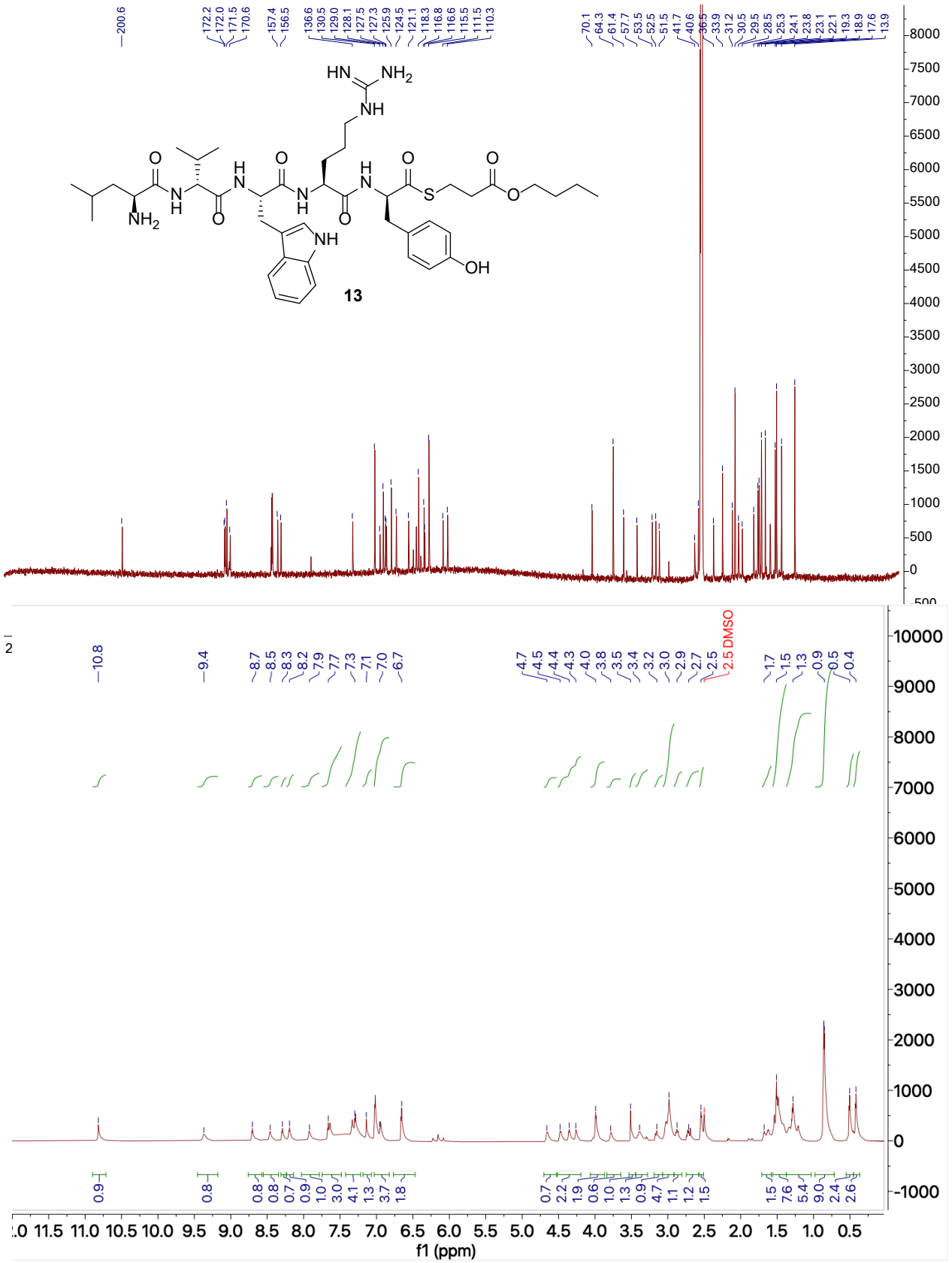


Supplementary Fig. 86: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **10** in DMSO-d6

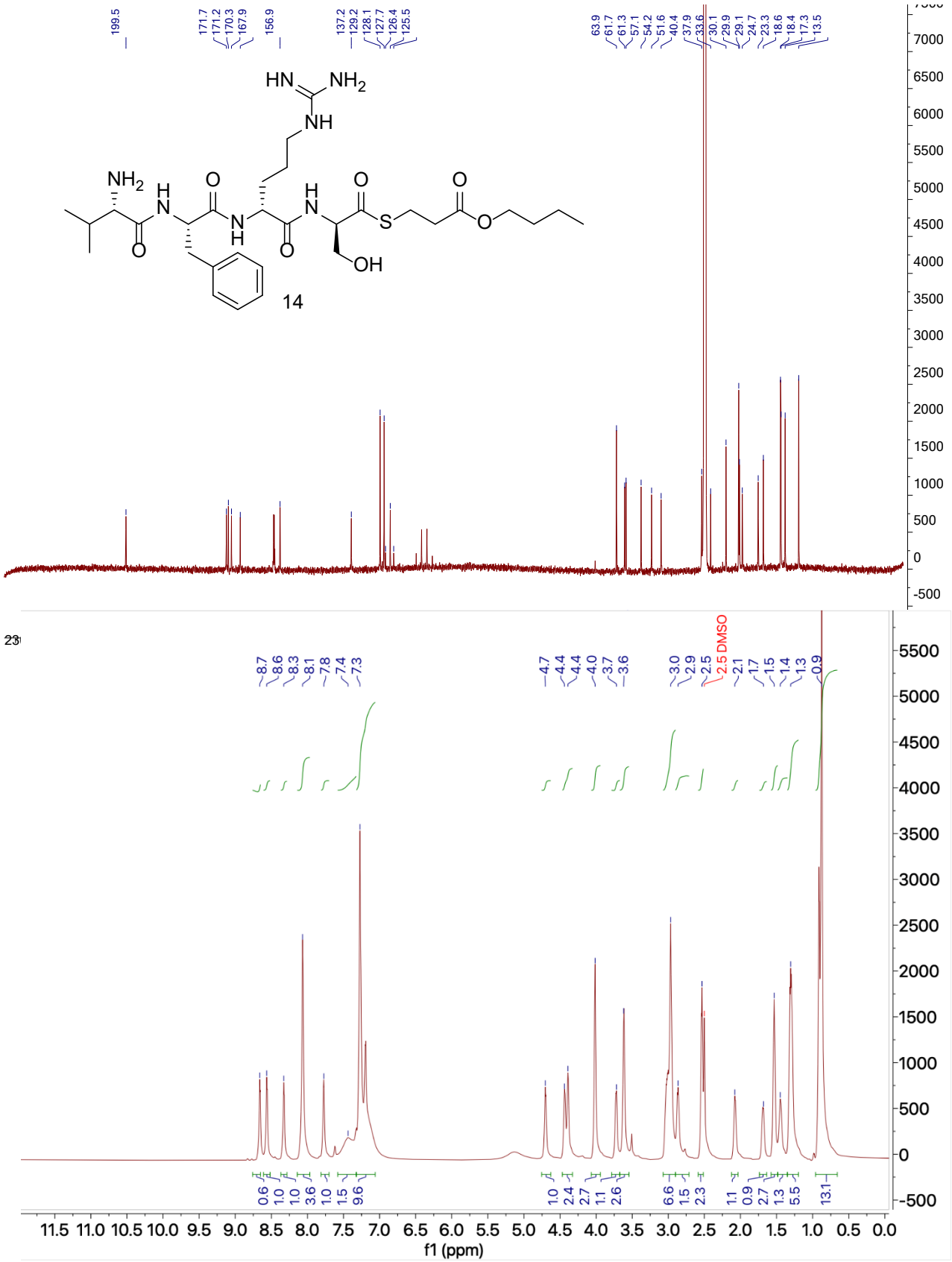




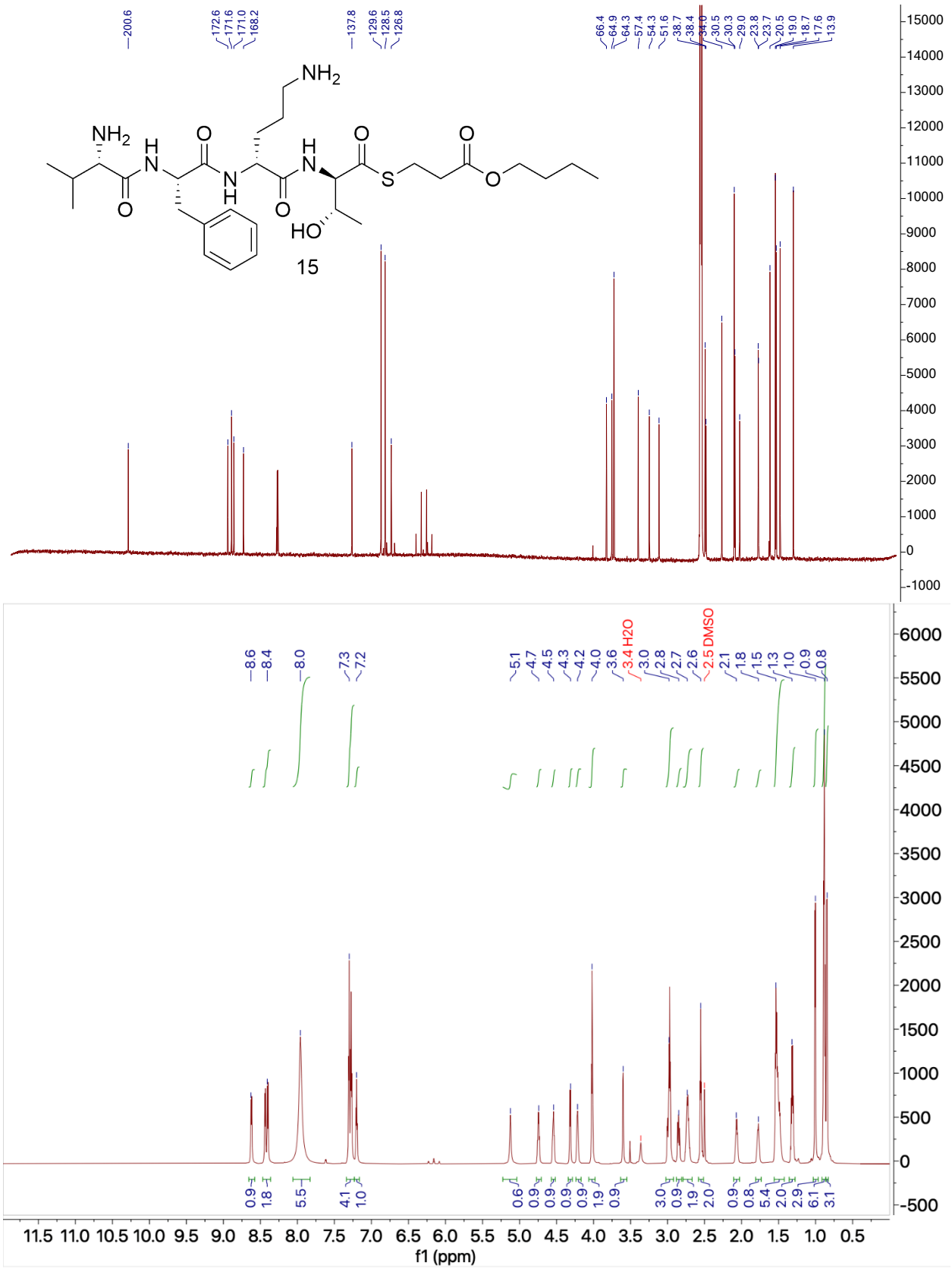
Supplementary Fig. 87: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **12-SMMP** in DMSO-d6



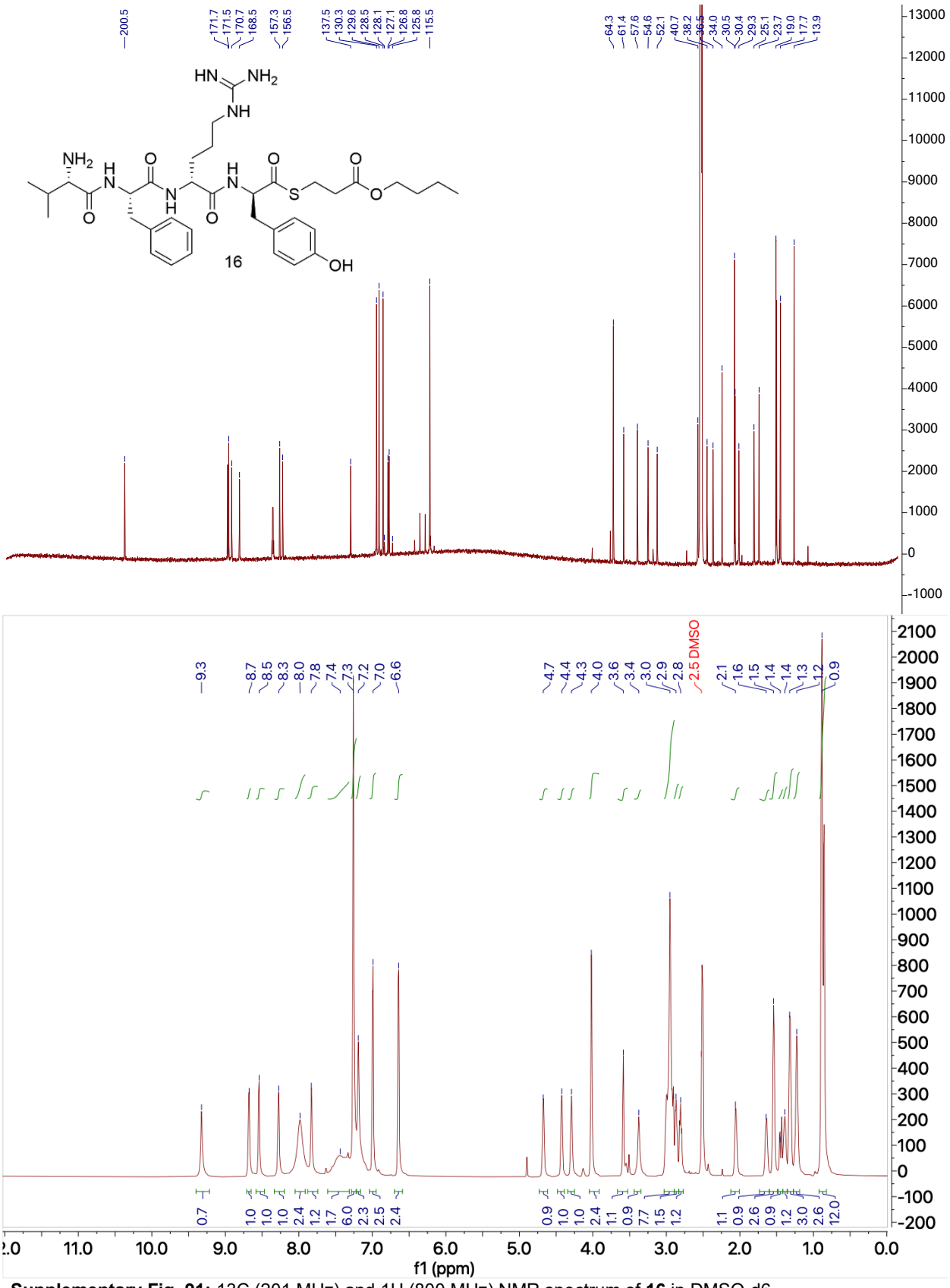
Supplementary Fig. 88: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **13** in DMSO-d6



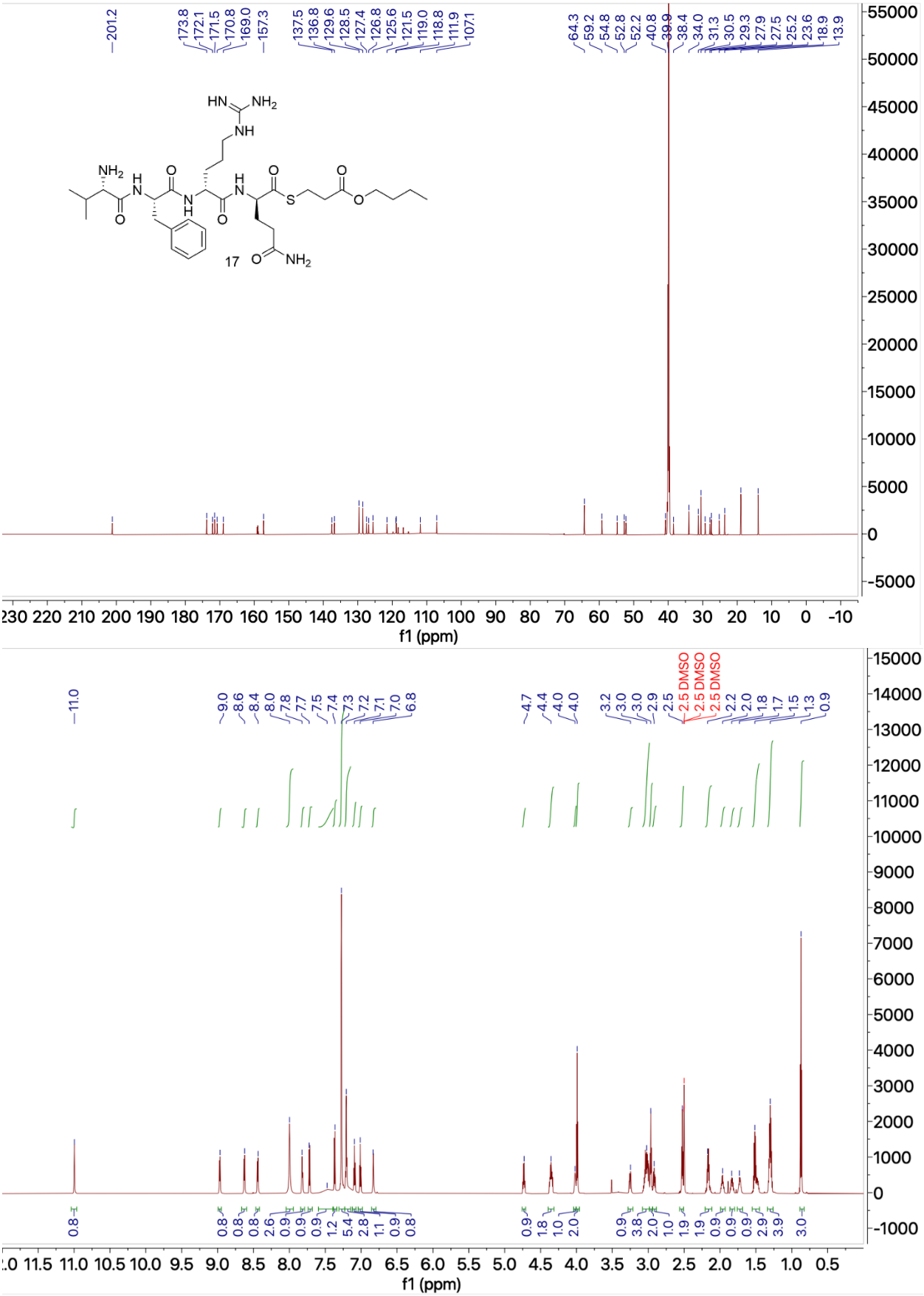
Supplementary Fig. 89: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **14** in DMSO-d6



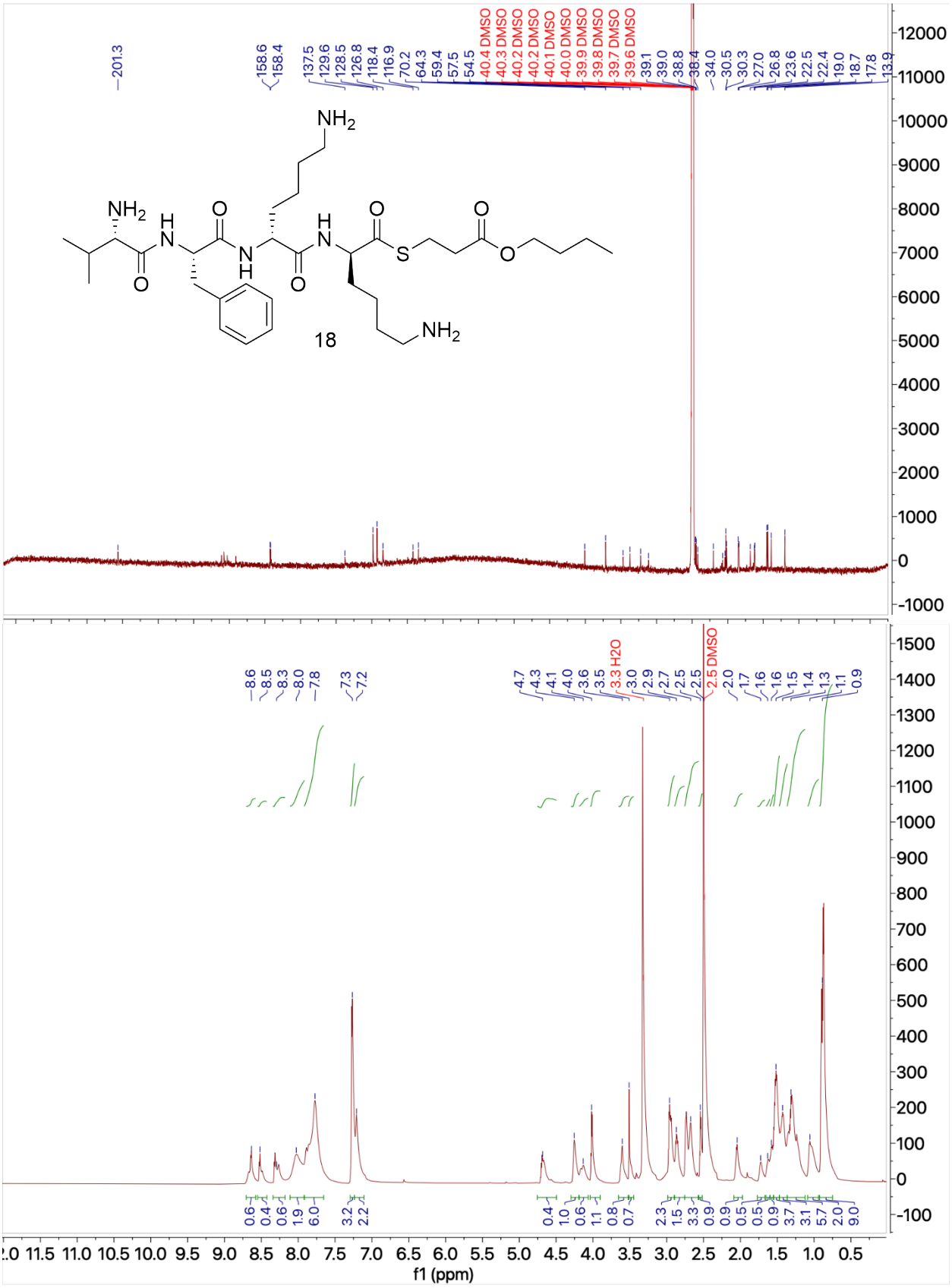
Supplementary Fig. 90: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **15** in DMSO-d6



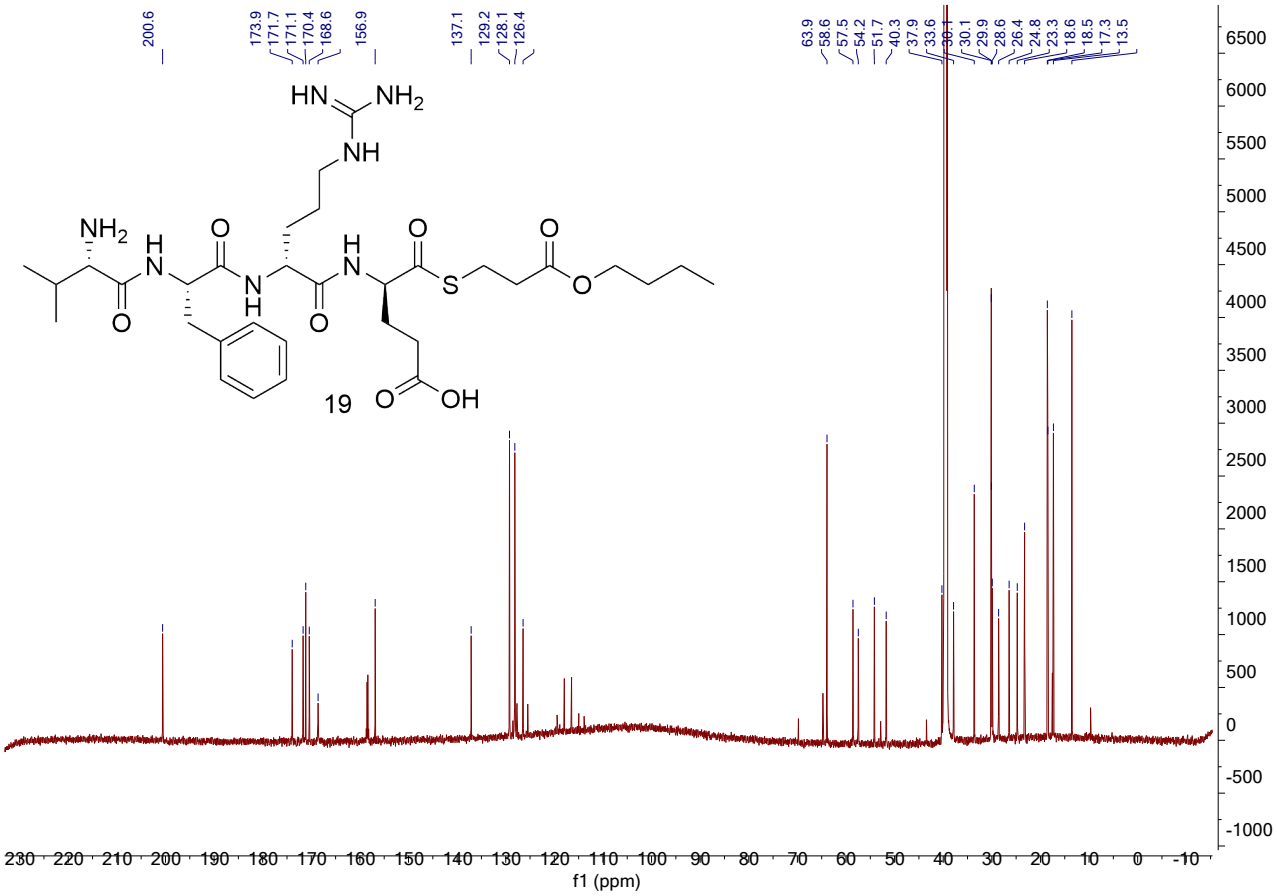
Supplementary Fig. 91: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **16** in DMSO-d6

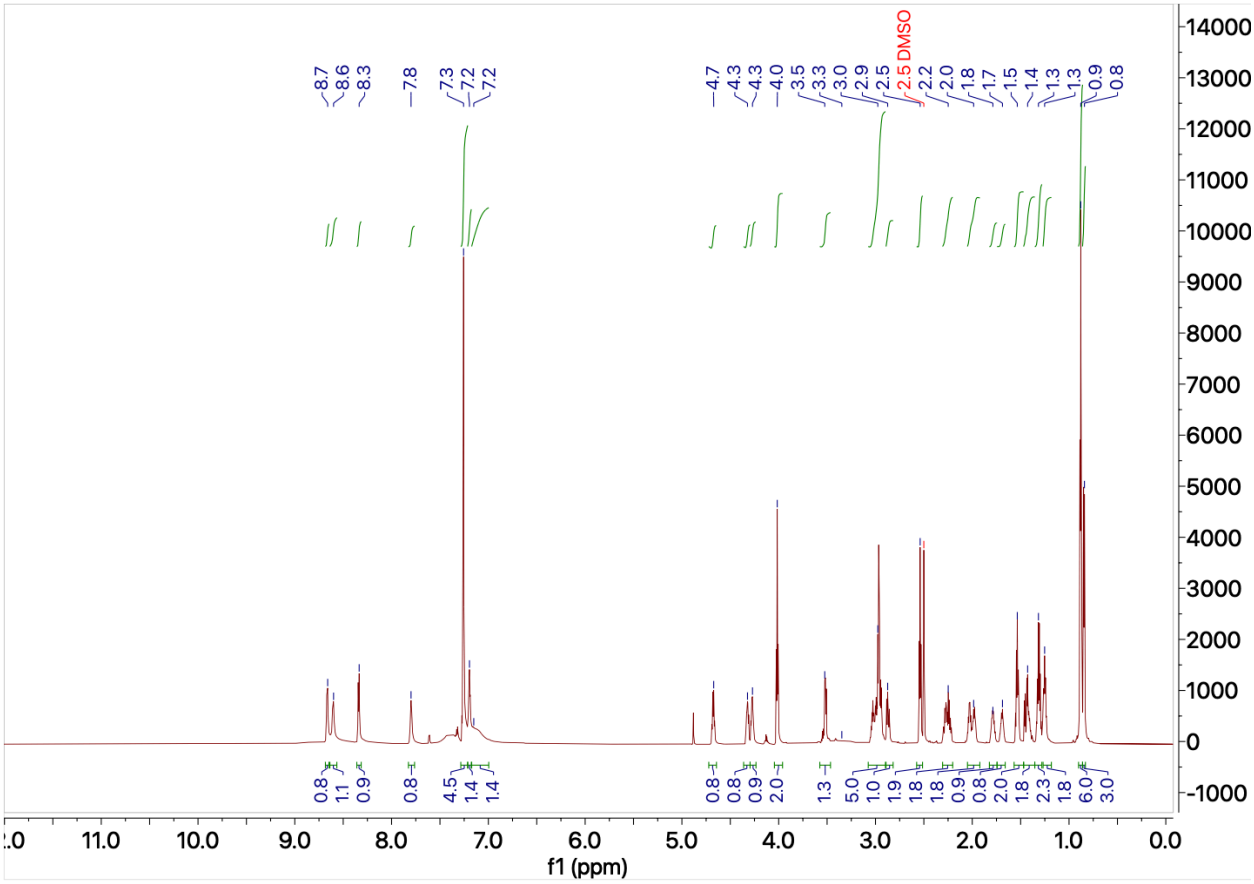


Supplementary Fig. 92: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **17** in DMSO-d6

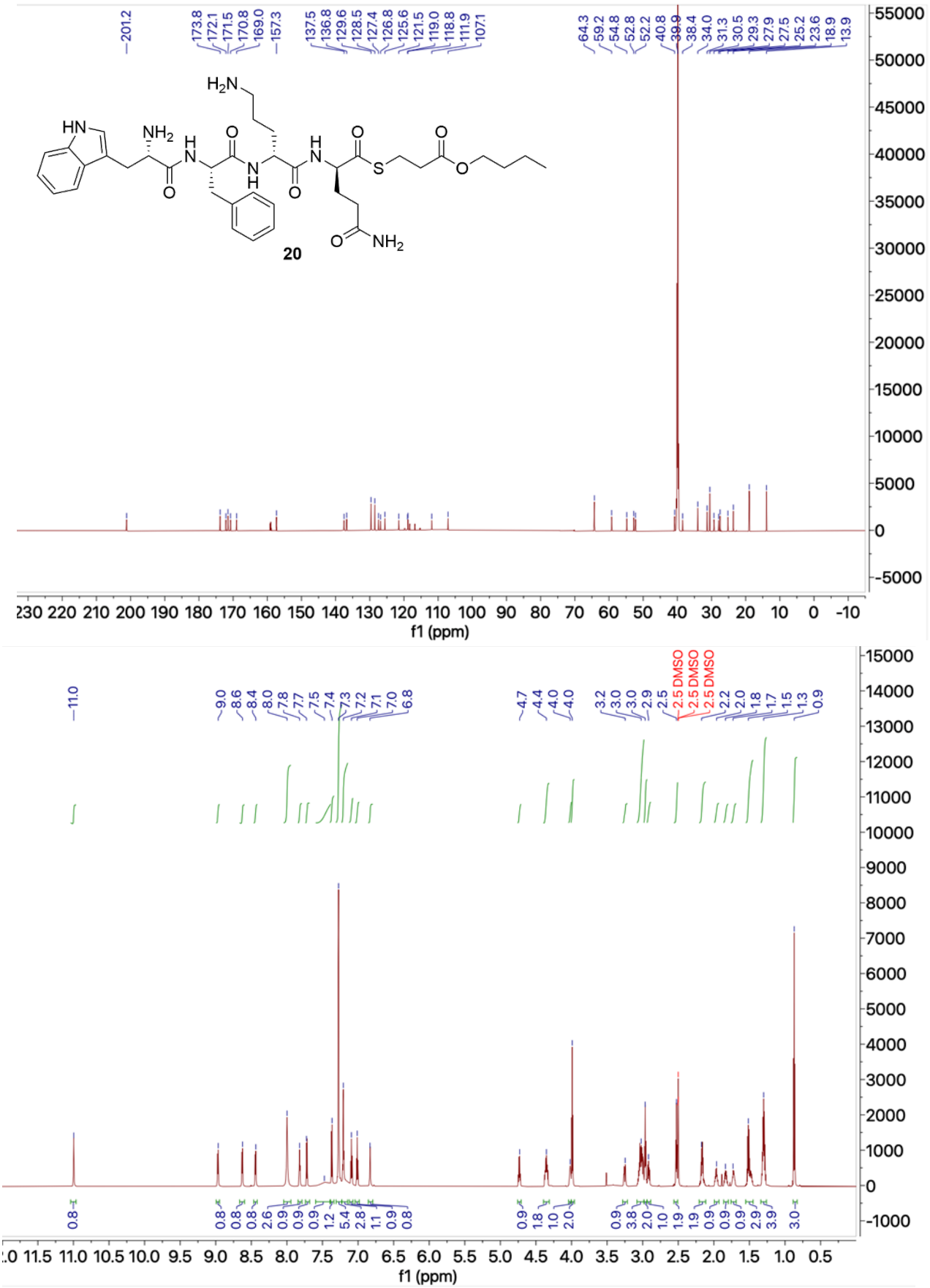


Supplementary Fig. 93: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **18** in DMSO-d6

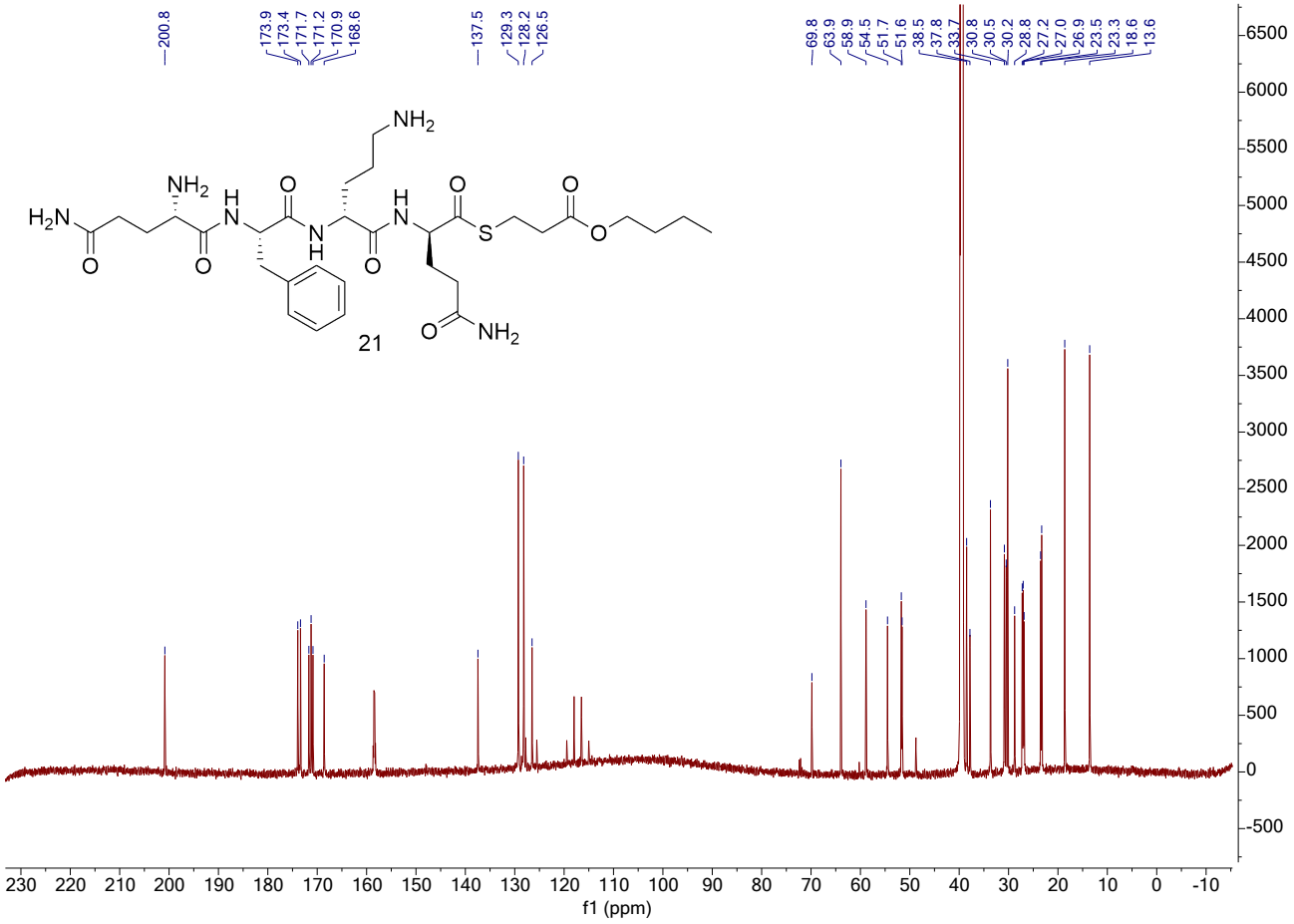

200.6
173.9
171.7
171.1
170.4
168.6
156.9
137.1
129.2
128.1
126.4
63.9
58.6
57.5
54.2
51.7
40.3
37.9
33.6
30.1
29.9
28.6
26.4
24.8
23.3
18.6
18.5
17.3
13.5
19
f1 (ppm)

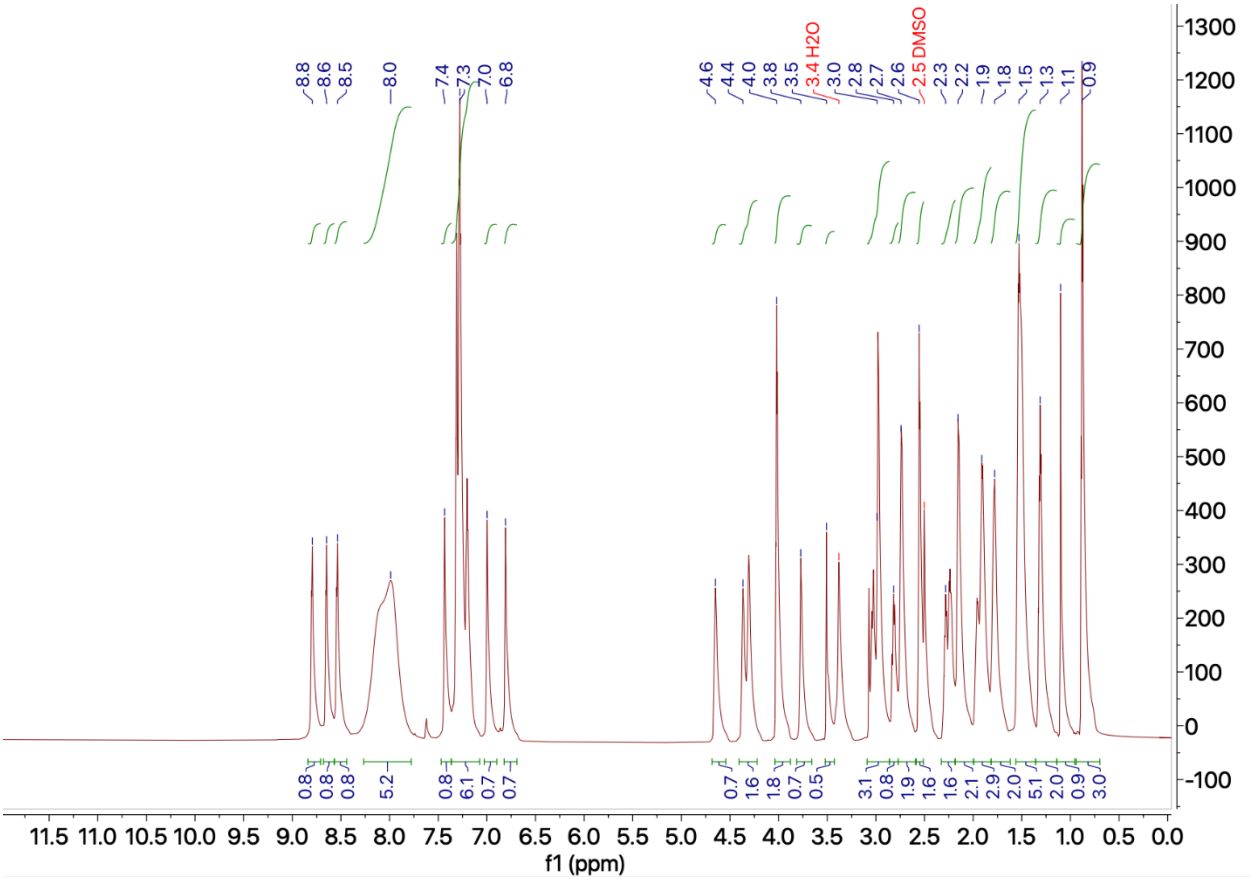


Supplementary Fig. 94: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **19** in DMSO-d6

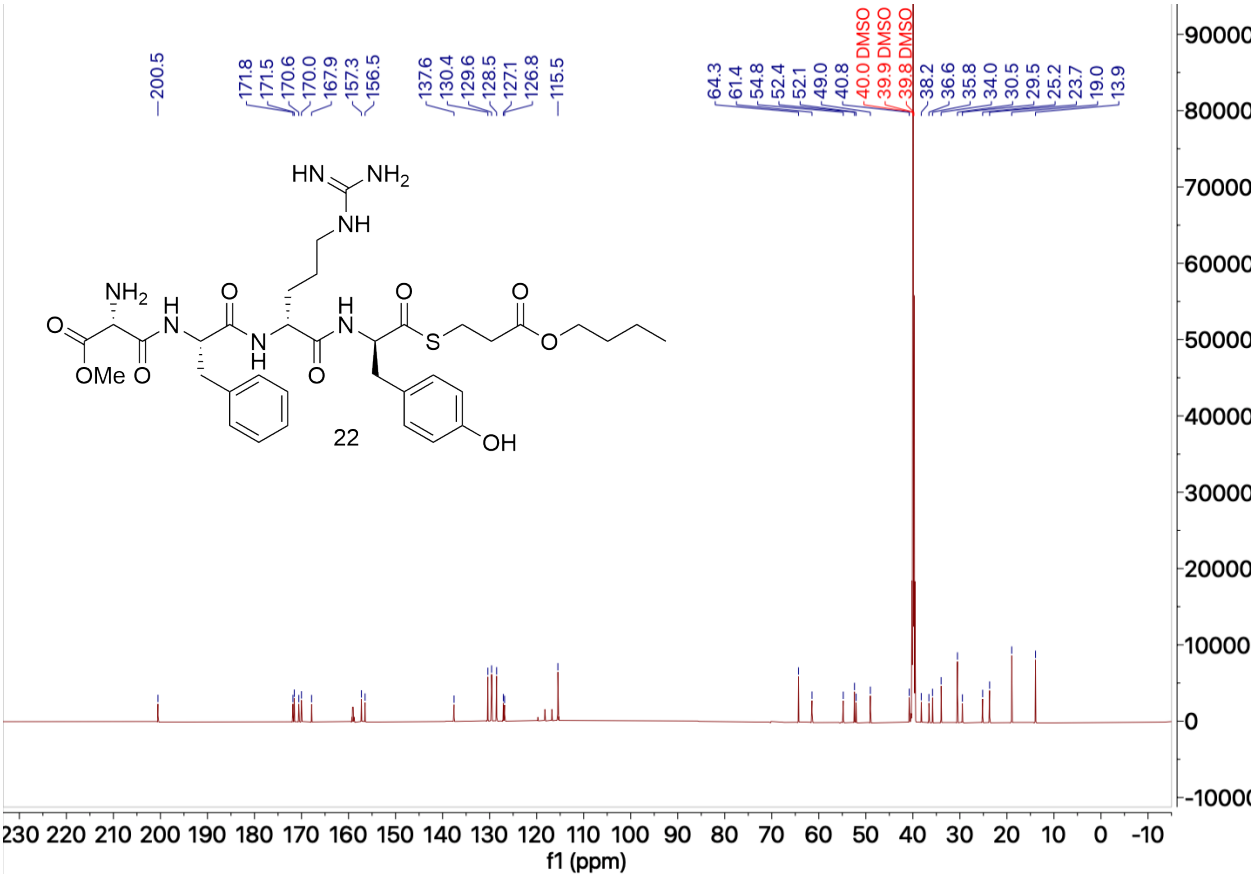


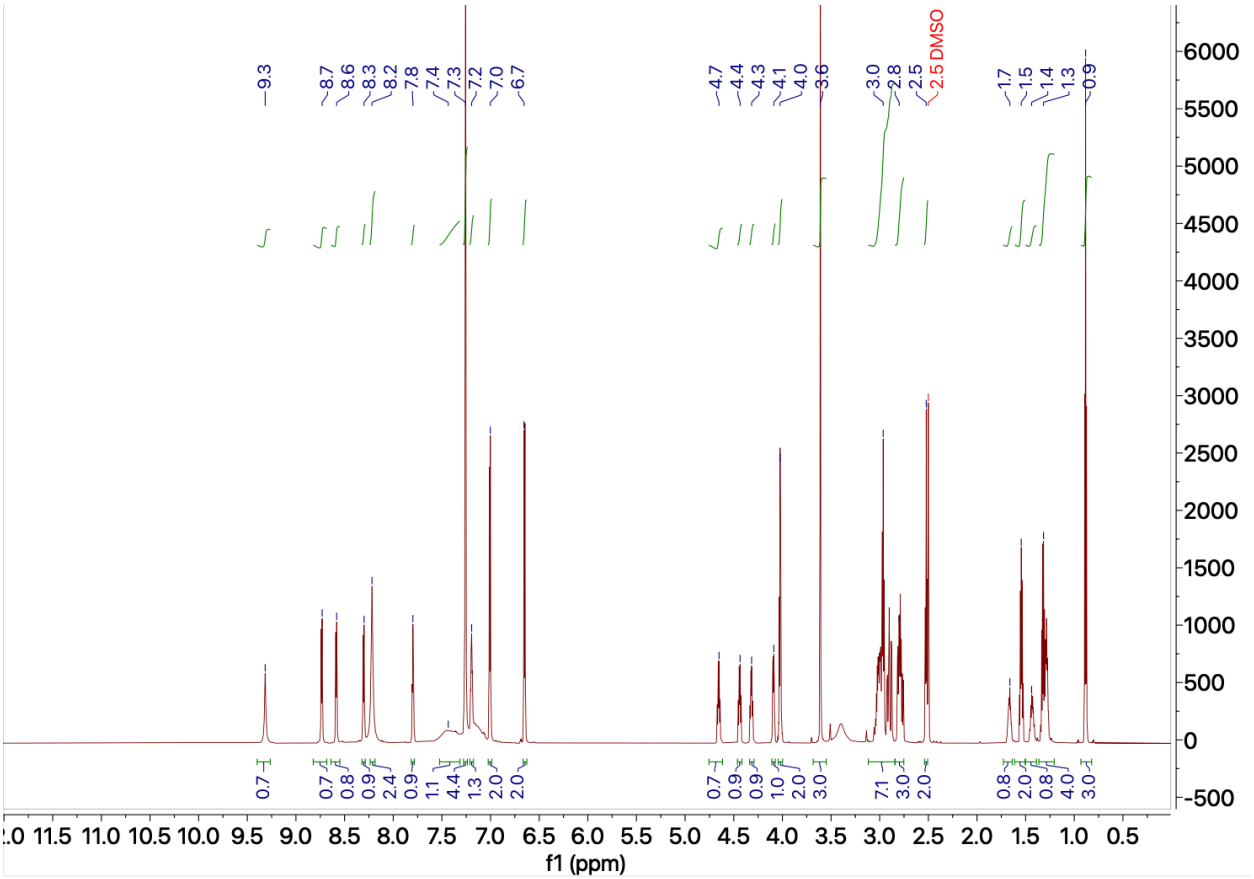
Supplementary Fig. 95: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **20** in DMSO-d6



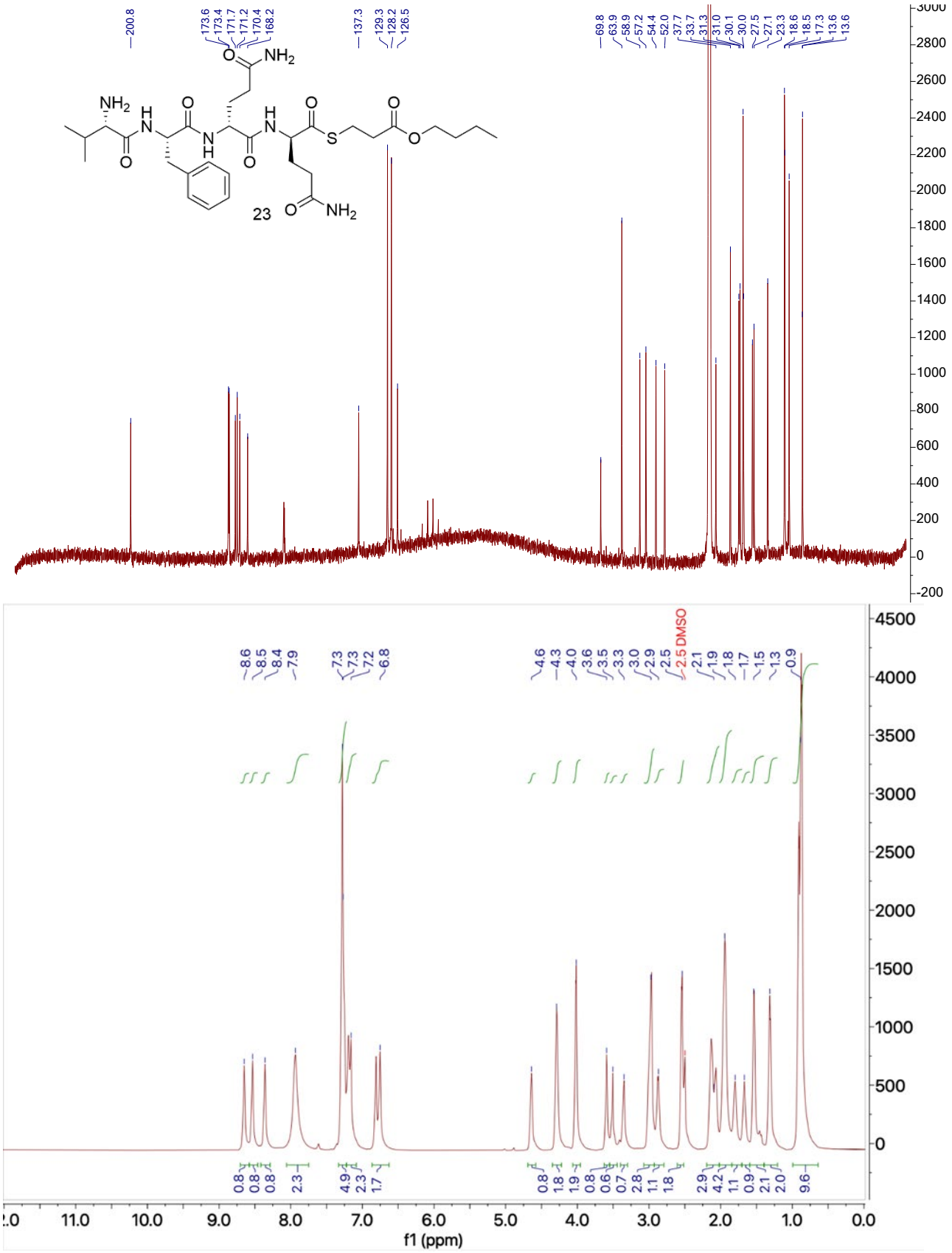


Supplementary Fig. 96: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **21** in DMSO-d6

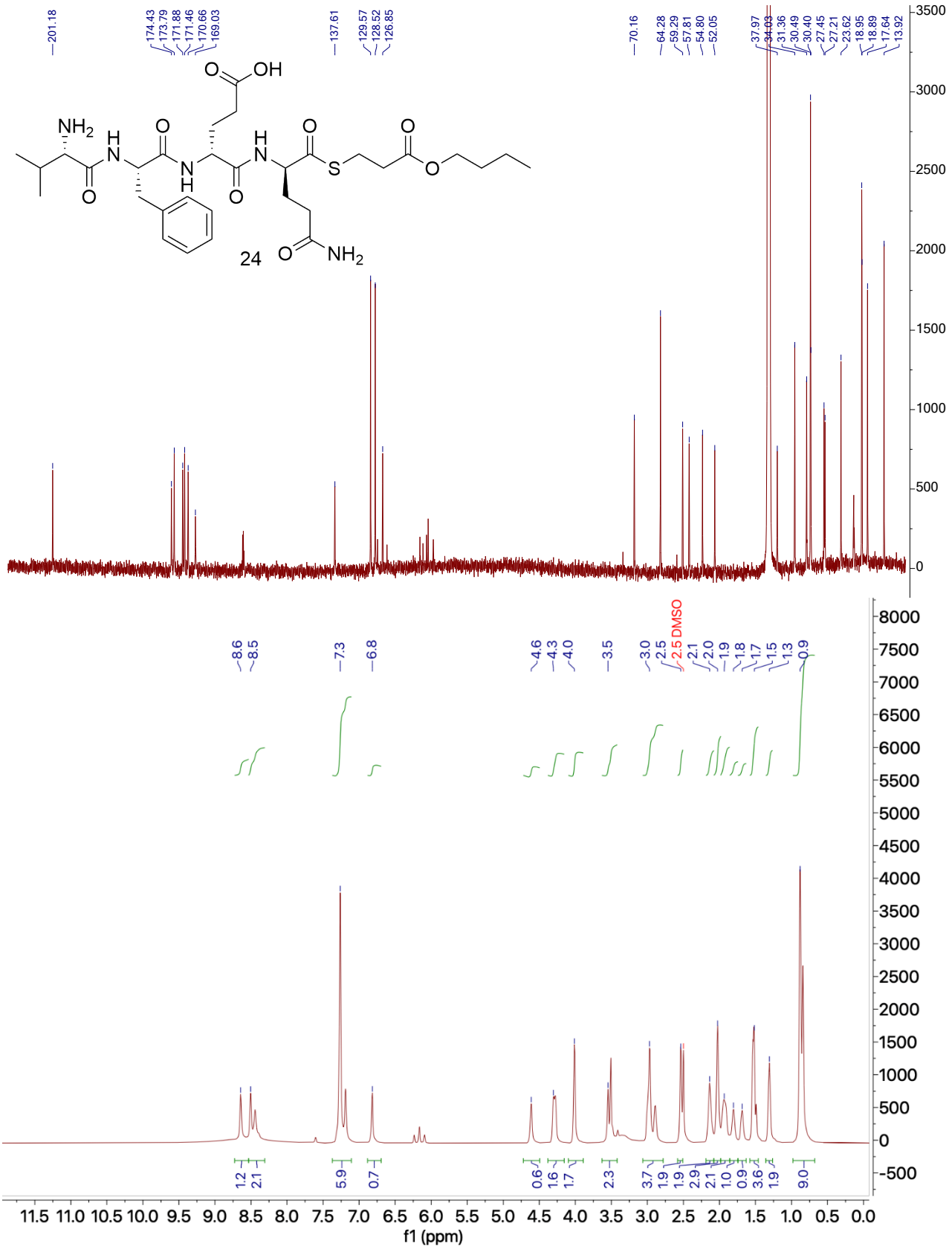

200.5
171.8
171.5
170.6
170.0
167.9
157.3
156.5
137.6
130.4
129.6
128.5
127.1
126.8
115.5
64.3
61.4
54.8
52.4
52.1
49.0
40.8
40.0 DMSO
39.9 DMSO
39.8 DMSO
38.2
36.6
35.8
34.0
30.5
29.5
25.2
23.7
19.0
13.9
HN
NH2
NH
NH2
O
H
N
O
N
H
O
H
N
O
S
O
O
OMe O
22
OH
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
-10000
230 220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)



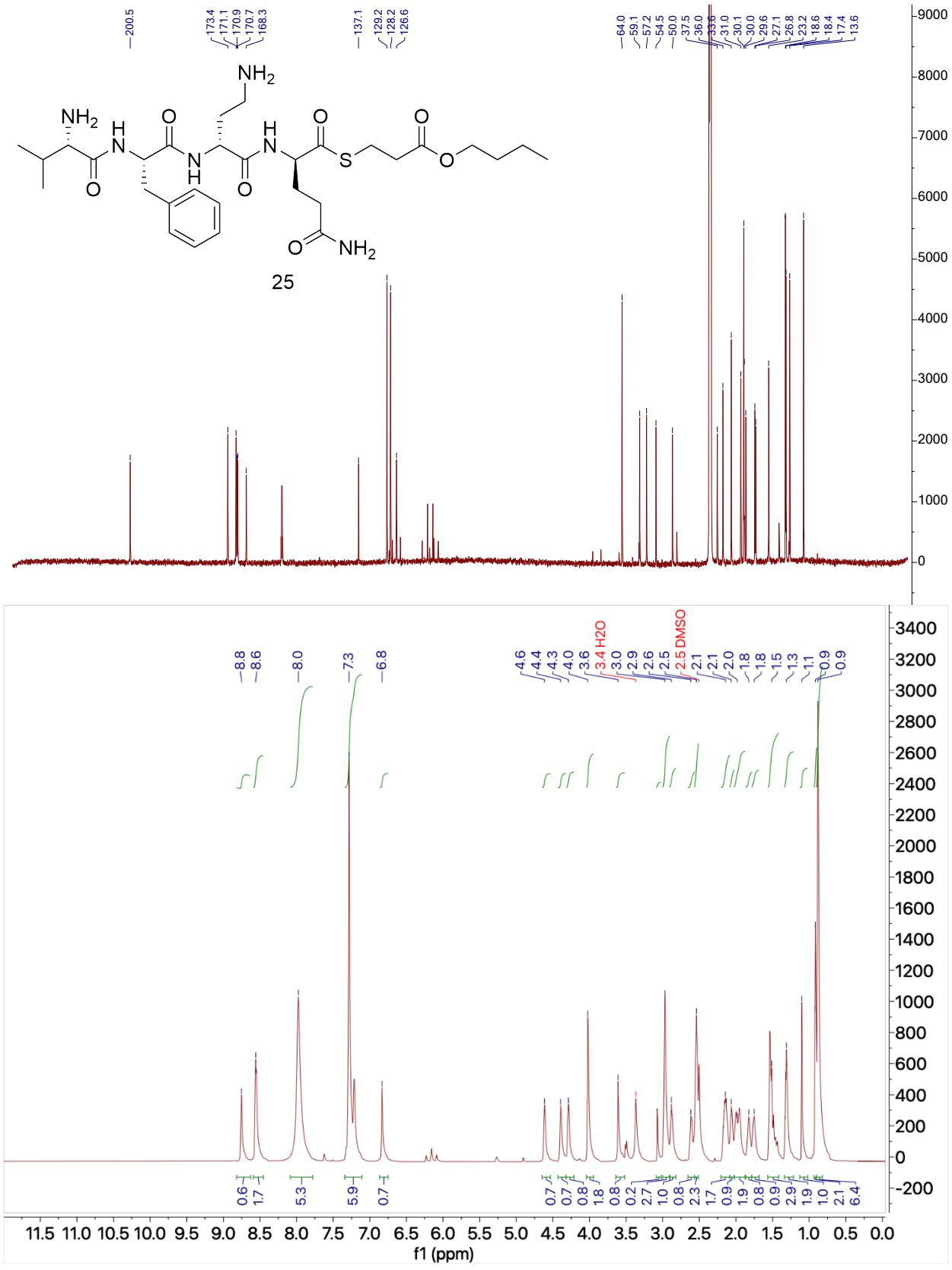
Supplementary Fig. 97: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **22** in DMSO-d6



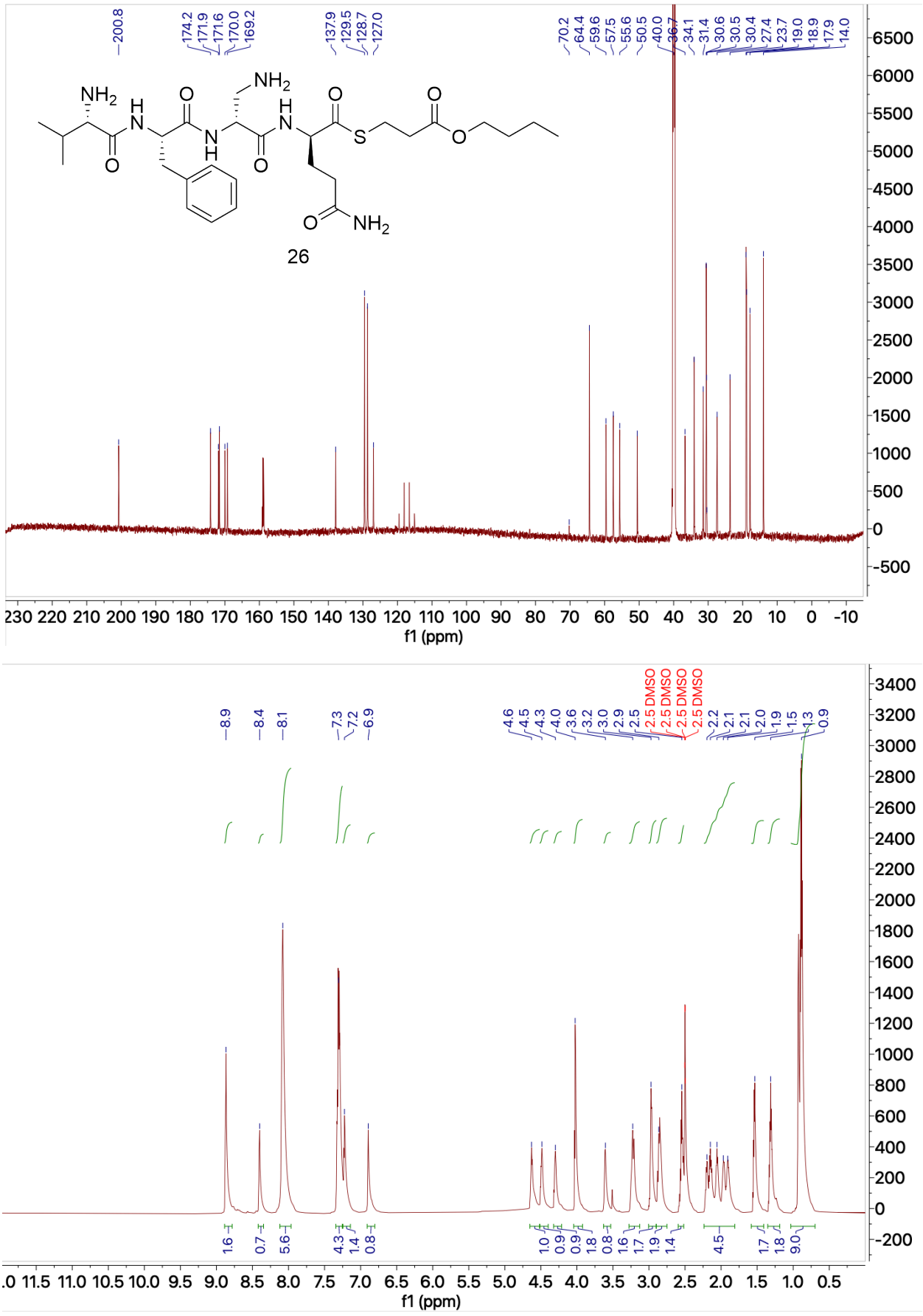
Supplementary Fig. 98: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **23** in DMSO-d6



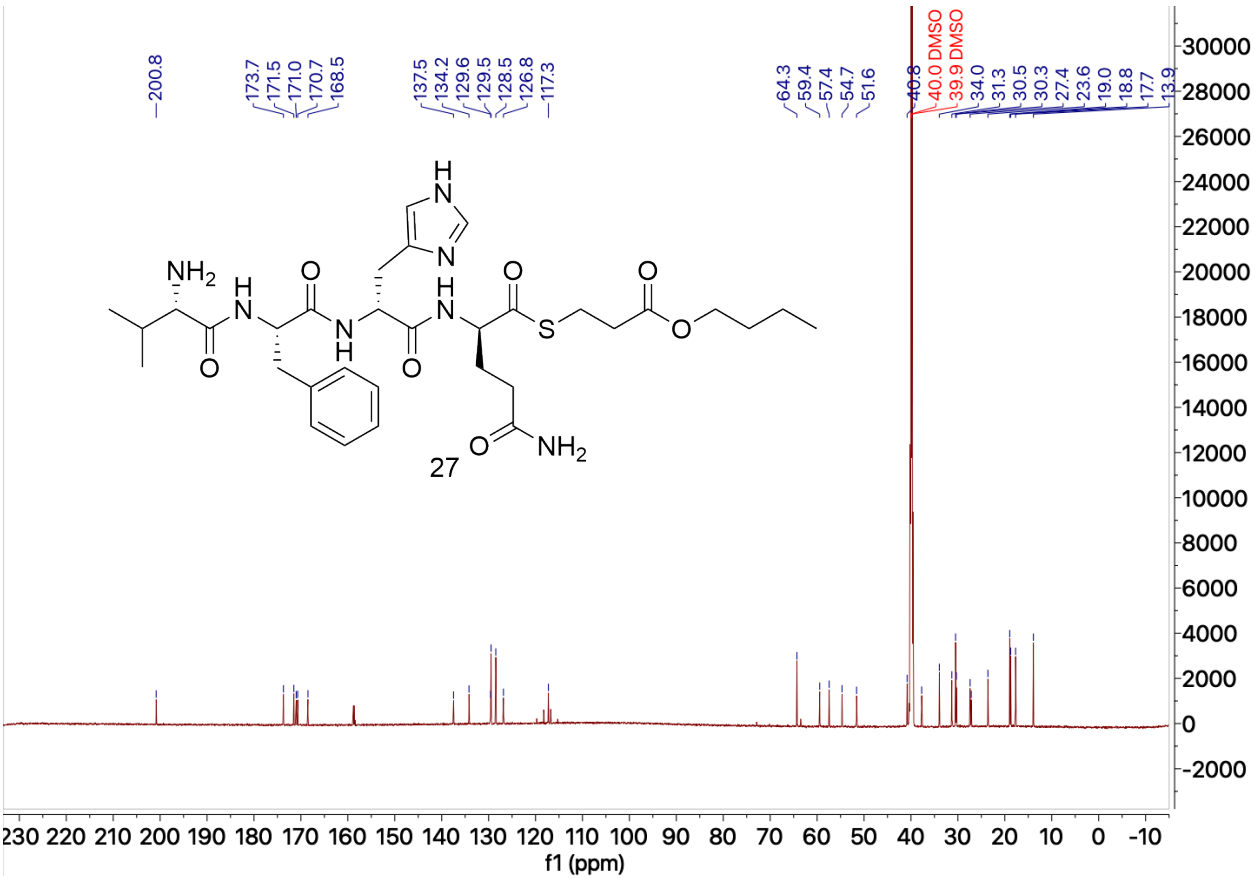
Supplementary Fig. 99: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **24** in DMSO-d6

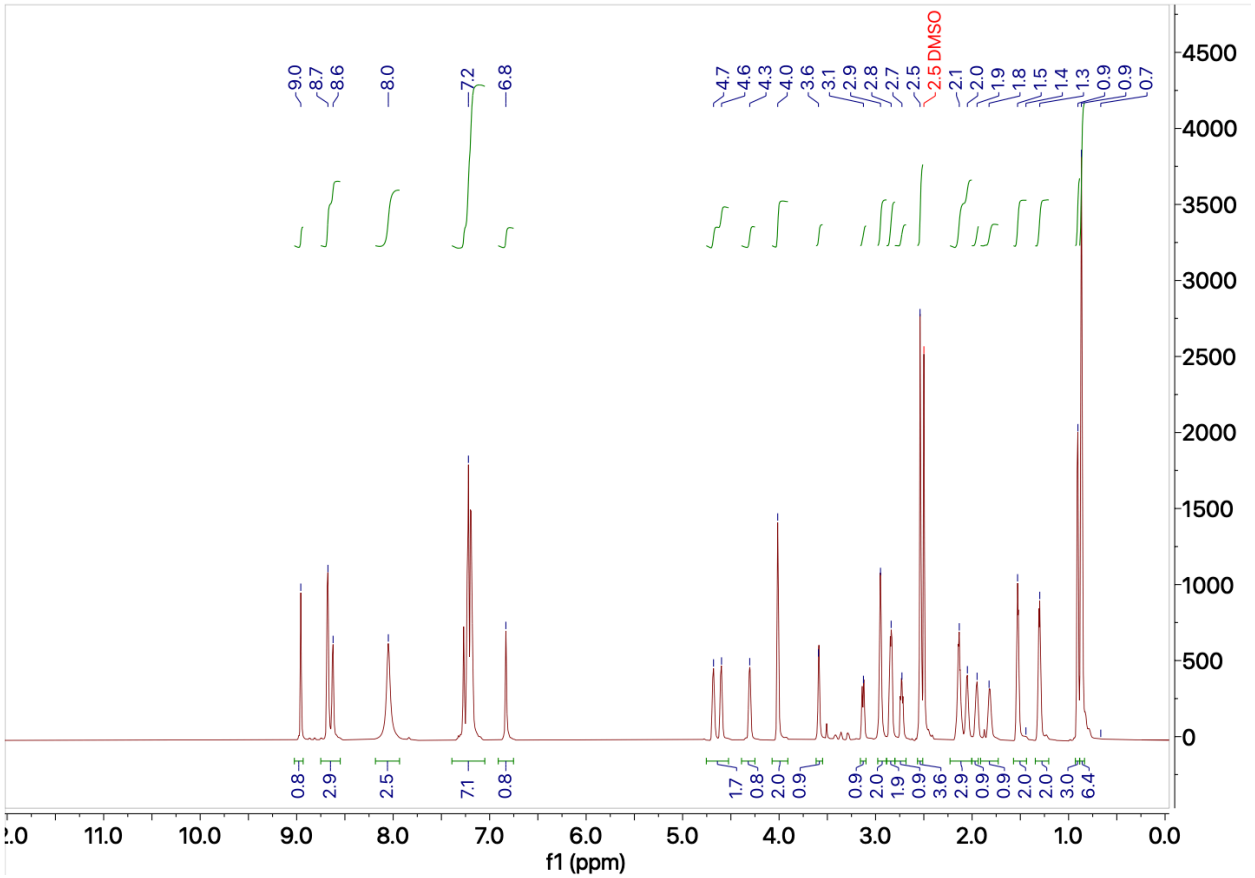


Supplementary Fig. 100: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **25** in DMSO-d6

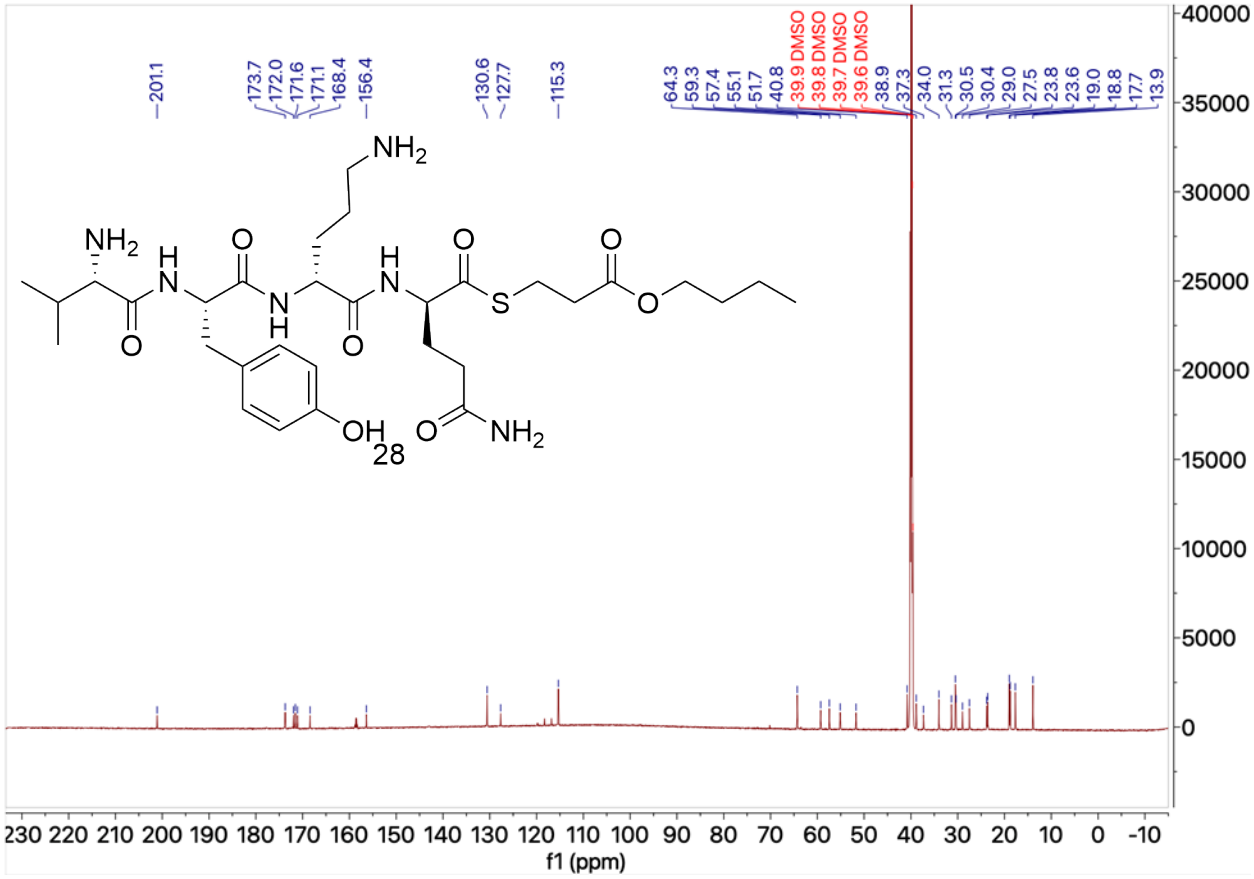


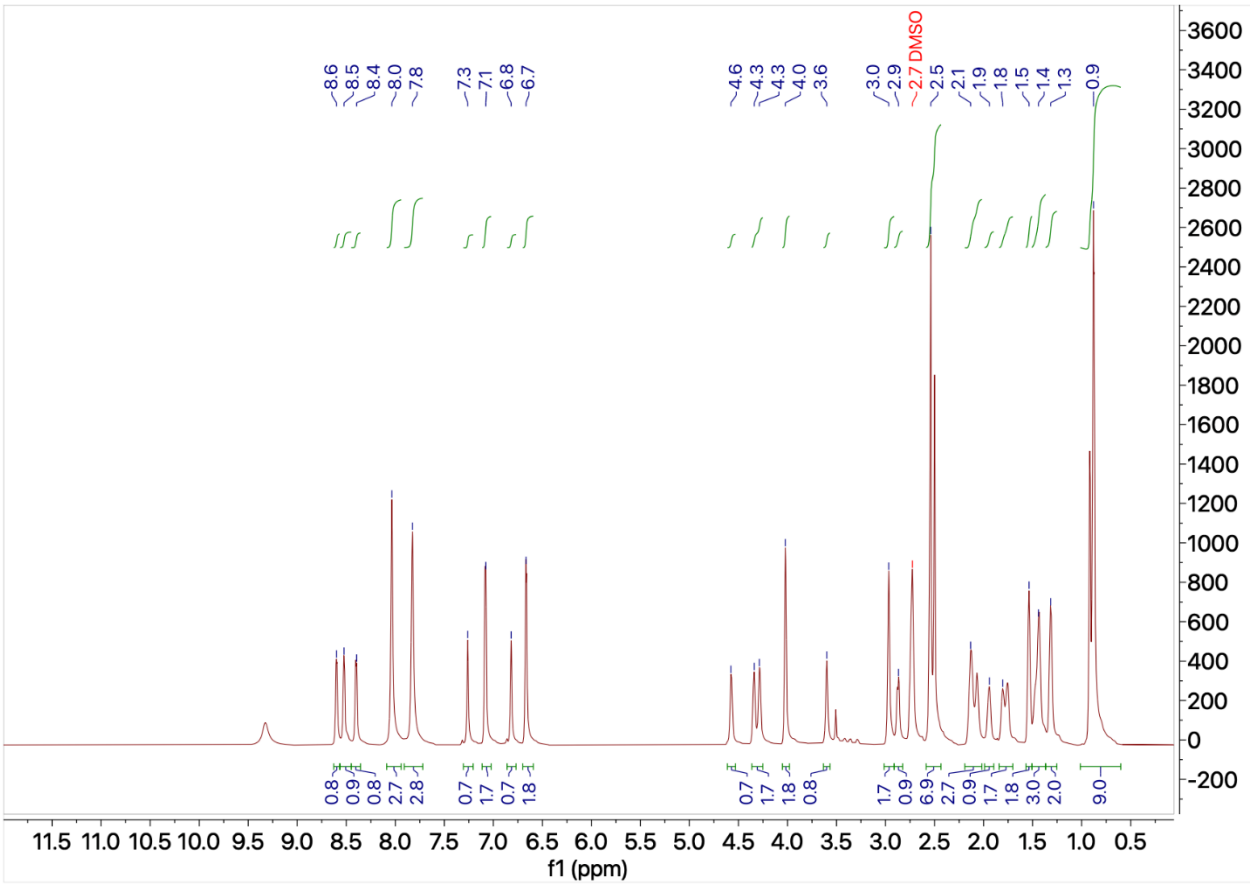
Supplementary Fig. 101: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **26** in DMSO-d6



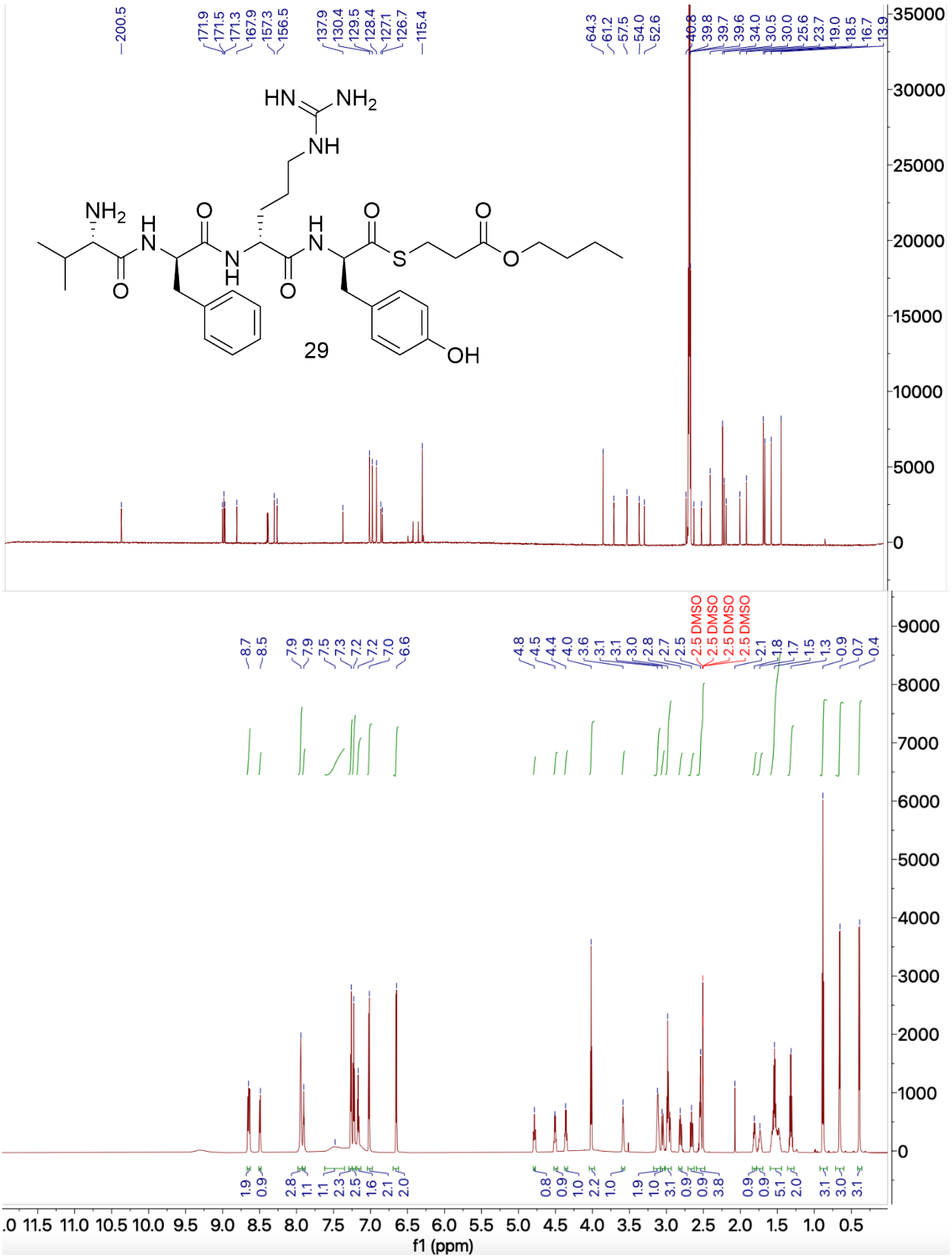


Supplementary Fig. 102: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **27** in DMSO-d6

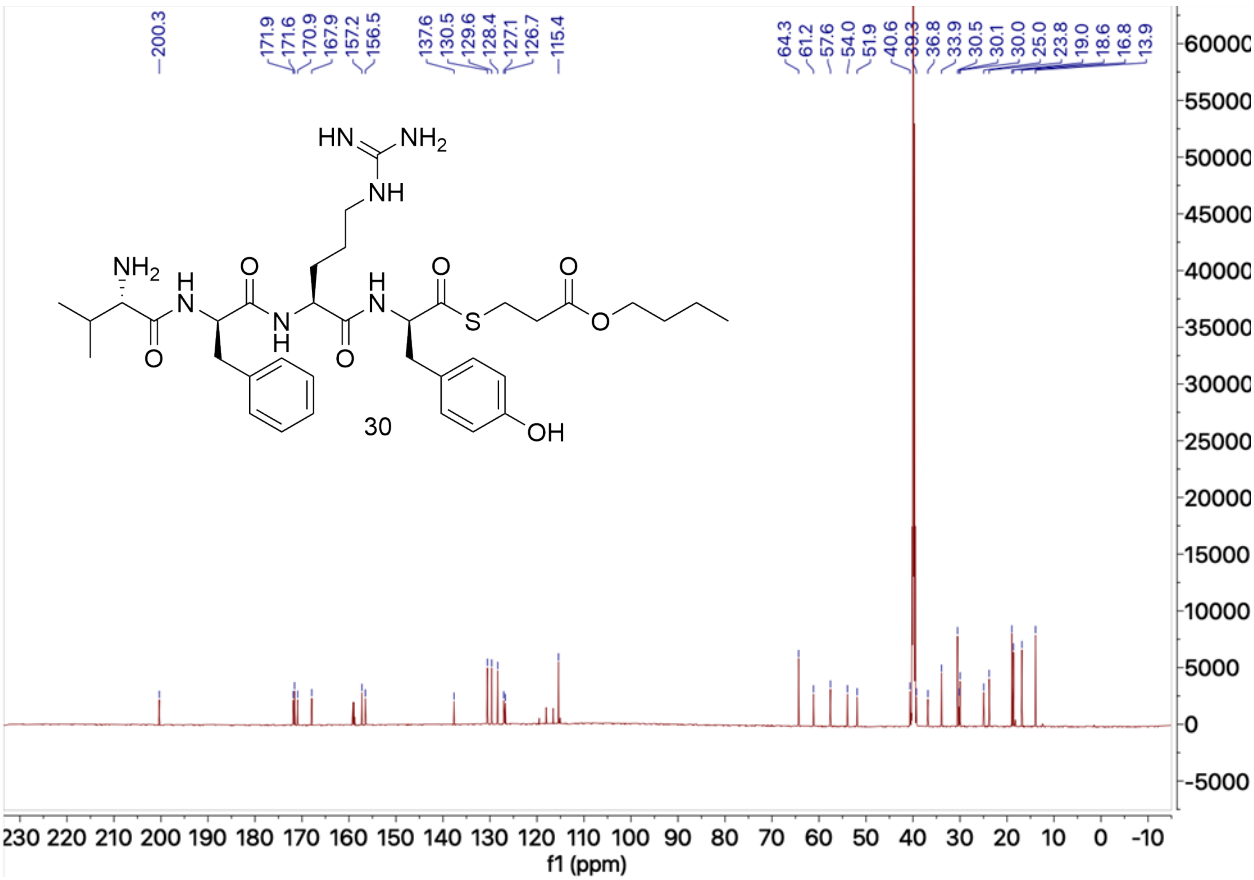


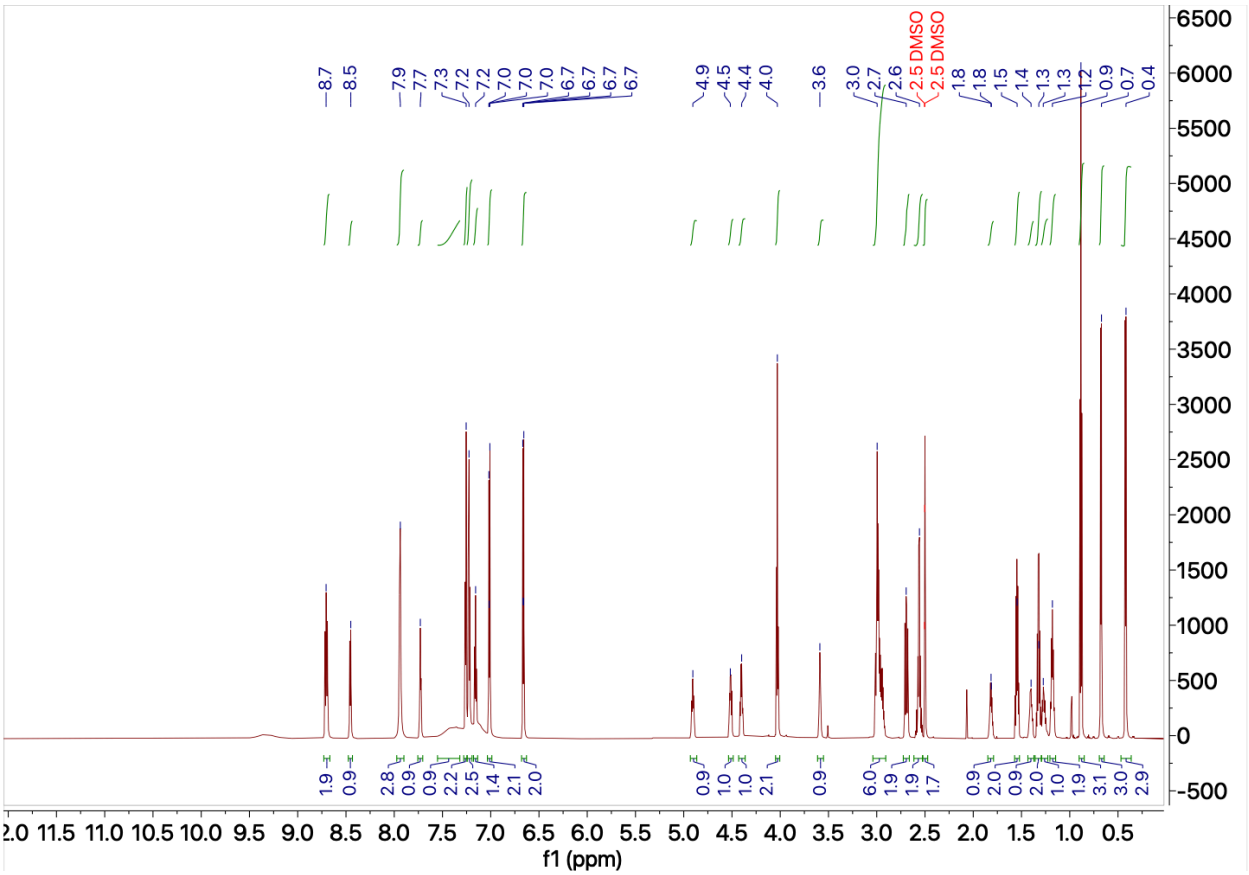


Supplementary Fig. 103: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **28** in DMSO-d6

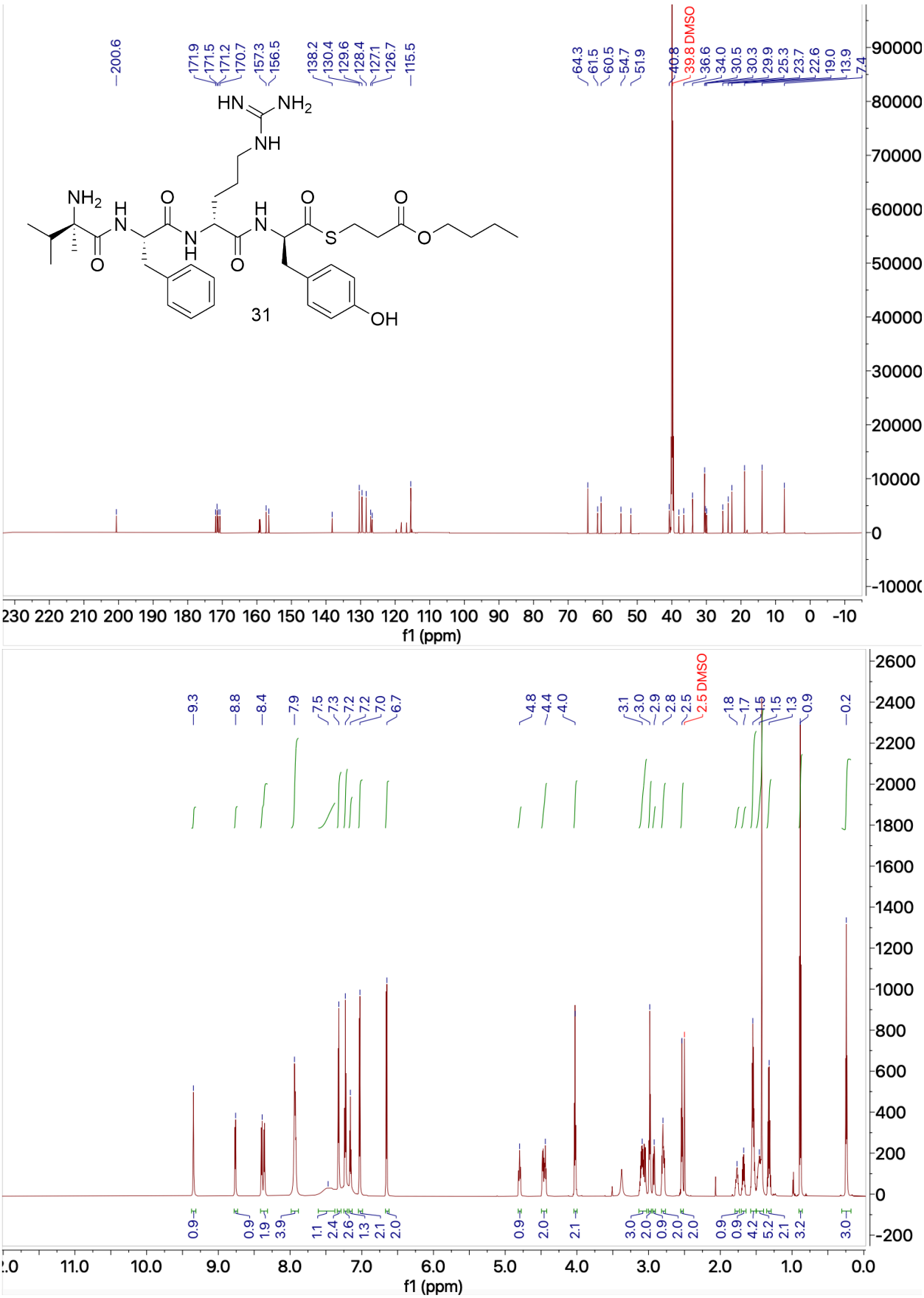


Supplementary Fig. 104: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **29** in DMSO-d6





Supplementary Fig. 105: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **30** in DMSO-d6



Supplementary Fig. 106: 13C (201 MHz) and 1H (800 MHz) NMR spectrum of **31** in DMSO-d6

References

1. Matsuda, K. *et al.* Heterochiral coupling in non-ribosomal peptide macrolactamization. *Nat. Catal.* **3**, 507–515 (2020).

2. Hostetler, M. A. *et al.* Synthetic Natural Product Inspired Cyclic Peptides. *ACS Chem. Biol.* **16**, 2604–2611 (2021).