

Supporting Information

Solid-Phase Photochemical Peptide Homologation Cyclization

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General Information

Definition of abbreviations used:

| | |
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| COMU | (1-Cyano-2-ethoxy-2-oxoethylideneaminoxy)dimethylamino-morpholino-carbenium hexafluorophosphate |
| DIC | <i>N, N'</i> -Diisopropylcarbodiimide |
| DIPEA | <i>N, N</i> -Diisopropylethylamine |
| DMF | <i>N, N</i> -Dimethylformamide |
| DMP | Dimethylphenyl |
| ESI | Electrospray Ionization |
| Fmoc | 9-Fluorenylmethoxycarbonyl |
| HATU | 1-[Bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxide hexafluorophosphate |
| HE | Diethyl 1,4-dihydro-2,6-dimethyl-3,5-pyridinedicarboxylate (Hantzsch ester) |
| NHPI | <i>N</i> -Hydroxyphthalimide |
| Orn | L-Ornithine |
| PyAOP | (7-Azabenzotriazol-1-yl)oxytrispyrrolidinophosphonium hexafluorophosphate |
| SPPS | Solid-phase peptide synthesis |
| TFA | Trifluoroacetic acid |
| TIPS | Triisopropylsilane |
| TMP | 2,2,6,6-Tetramethylpiperidine |
| TSP | 2,2,3,3-d(4)-3-(Trimethylsilyl)propionic acid sodium salt |

Reagents:

All commercially available reagents were used as received. Rink-amide polystyrene resin (0.36 mmol/g) was purchased from Novabiochem. All Fmoc-amino acids including Fmoc-Trp(Boc)-OH, Fmoc-Cys(tBu)-OH, Fmoc-His(Trt)-OH and COMU were purchased from Chem-Impex. TIPS was purchased from Oakwood Chemical. DIPEA, TFA, and pyridine was purchased from Fischer Scientific. DMF, CH₂Cl₂, Fenoprofen, methanol, and Et₂O were purchased from Sigma-Aldrich. D₂O was purchased from Cambridge Isotope Laboratories. TSP was purchased from Alfa-Aesar. Allyl acrylate was purchased from TCI.

Instrumentation:

Photochemistry was performed using two Kessil PR160L linear reflector 440 nm photoreactors with an average intensity in 2x4 cm area of 137 mW/cm². Reactions were conducted in polypropylene fritted syringes with stir bars placed on a stir plate centered 8 cm from the light source. HPLC was performed using either an Agilent Infinity II Analytical HPLC equipped with a Phenomenex Aeris C18 column 250 x 4.6 mm for analytical (1 mL/min) in an Agilent G7116A column heater; or Agilent 1260 II prep HPLC equipped with a Phenomenex Aeris C18 column (5 μM particle size, 250 x 21.2 mm (22 mL/min)). LCMS-ESI was performed on a Waters Acquity UPLC SQD equipped with an Acquity UPLC HSS C18 column (1.8 μm, 2.1 X 50 mm). High-resolution mass spectra (HRMS) were recorded on an Agilent LC/MSD TOF mass spectrometer by electrospray ionization time of flight experiments. All NMR samples were analyzed on a Bruker Ultrashield Plus 600 MHz equipped with a BBO Prodigy probe. Watergate was used for water suppression.

General Procedure for Peptide Synthesis

All equivalents are based on resin loading.

Fmoc Deprotections

Resin with peptide was treated with a solution of 1% HOBT (w/v), 2% DBU (v/v), in DMF at 50 mM concentration of peptide to solution (2 mL for 0.1 mmol resin) for 2 min. This was repeated two additional times, then the resin was washed with DMF (5x).

Resin Loading

Resin loading was calculated using the manufacturer's loading on label. The resin was swelled in DMF in a fritted plastic syringe with a stir bar for 20 min. The resin was subsequently Fmoc deprotected, then allowed to equilibrate in NMP and drained. Separately, Fmoc-amino acid (5 equiv) and COMU (5 equiv) were dissolved in NMP (0.2-0.3 M), then TMP (7.5 equiv) was added, and the solution was allowed to react for 5 min then added to the resin. The reaction was allowed to proceed for 30 min, then washed with DMF (5x).

Peptide Elongation

Fmoc-amino acids (5 equiv) and COMU (5 equiv) were dissolved in NMP (0.2-0.3 M), then TMP (7.5 equiv) was added, and the solution was allowed to react for 5 min. The solution was then added to resin equilibrated in NMP and allowed to react for 45 min. The resin was then washed with DMF (5x).

Fmoc-Asp(DMP)-OH (4 equiv) and COMU (4 equiv) were dissolved in NMP (0.2-0.3 M), then TMP (6 equiv) was added, and the solution was allowed to react for 5 min. The solution was then added to resin equilibrated in NMP and allowed to react for 45 min. The resin was then washed with DMF (5x).

Peptide Acylation

Peptide on-resin with a free N-terminus was equilibrated in DMF. To the drained resin was added 5% acetic anhydride in pyridine (50 mM based on resin loading) and allowed to react for 5 min. The procedure was repeated two additional times. The resin was then washed with DMF (5x).

N-Terminal Trityl Protection

Peptide on resin was washed with CH_2Cl_2 , then equilibrated with dry CH_2Cl_2 . Trityl chloride (10 equiv) was dissolved in dry CH_2Cl_2 (0.5 M trityl chloride), then DIPEA (5 equiv) was added. The solution was taken up into the fritted syringe with the plunger on to prevent evaporation, and the reaction was allowed to proceed for 2 h. The solution was ejected from the syringe, and a second portion of the trityl solution was taken up into the syringe and allowed to react overnight.

Upon completion of the reaction, a small amount of resin (~3 mg) was acylated, cleaved, and analyzed by LCMS to determine the presence of free amine versus acylated amine.

N-Terminal Trityl Deprotection

To resin equilibrated in CH_2Cl_2 was added a solution of 4% TFA, 5% TIPS, 91% CH_2Cl_2 (50 mM based on resin loading), and the mixture was allowed to react for 45 sec (solution turns yellow then clear). This was repeated three additional times. The resin was then washed with CH_2Cl_2 (5x). The resin was then equilibrated in DMF containing DIPEA (5 equiv) for 2 min to neutralize any acid, then washed four times with DMF.

Allyl Deprotection

Dry CH_2Cl_2 was taken up into a fritted syringe, with the back plunger on, containing Et_2O -dried resin. The resin was allowed to equilibrate in the dry CH_2Cl_2 for 10 min, then ejected. Argon was then taken up into the syringe and ejected (3x). Separately, $\text{Pd}(\text{PPh}_3)_4$ (0.1 equiv) in a dry vial was dissolved in dry CH_2Cl_2 (50 mM based on resin loading) then PhSiH_3 (20 equiv) was injected into the vial. The $\text{Pd}(\text{PPh}_3)_4$ solution was then taken up into the syringe under argon, and the reactions were allowed to proceed for 20 min. The reaction was ejected, and the resin was washed once with dry CH_2Cl_2 . Argon was then taken up into the syringe and ejected (3x). The reaction was then carried out a second time. Upon completion the resin was washed with CH_2Cl_2 (5x).

DMP Deprotection

To resin equilibrated in CH₂Cl₂ was added a solution of 4% TFA, 5% TIPS, 91% CH₂Cl₂ (50 mM based on resin loading) and allowed to react for 45 sec (solution turns yellow then clear). This was repeated three additional times. The resin was then washed with CH₂Cl₂ (5x). The resin was then equilibrated in DMF containing DIPEA (5 equiv) for 2 min to neutralize any acid, then washed four times with DMF.

Peptide Acrylation

Peptide on-resin with a free N-terminus was equilibrated in NMP. Acrylic acid (5 equiv) and COMU (5 equiv) were dissolved in NMP (0.2-0.3 M), then TMP (7.5 equiv) was added, and the solution was allowed to react for 5 min. The solution was then added to resin equilibrated in NMP and allowed to react for 45 min. The resin was then washed with DMF (5x).

Peptide Cleavage/Deprotection

Atosiban peptides:

To Et₂O-dried resin was added a solution of 5% H₂O, 5% TIPS, 90% TFA (20 mM based on resin loading), and the mixture was allowed to react for 60 min. The solution was ejected into a 15 mL conical tube. The resin was then washed with 1 mL of TFA and injected into the conical tube. The TFA was blown off with a stream of air to less than 2 mL. The tube was filled to 10 mL with cold Et₂O, vortexed, then centrifuged. The tube was decanted and washed a second time with cold Et₂O. The peptide pellet was dried with a stream of air, dissolved in 500 μL of 50/50 H₂O/MeCN, and filtered into a HPLC vial. The tube was washed with an additional 500 μL of 50/50 H₂O/MeCN and filtered into a HPLC vial.

Di-Peptides:

To Et₂O dried resin was added a solution of 5% H₂O, 5% TIPS, 90% TFA (20 mM based on resin loading) and allowed to react for 60 min. The solution was ejected into a 15 mL conical tube. The resin was then washed with 1 mL of TFA and injected into the conical tube. The TFA was blown off with a stream of air to less than 500 μL. The crude peptide solution was dissolved in 500 μL of 50/50 H₂O/MeCN and filtered into a HPLC vial. The tube was washed with an additional 500 μL of 50/50 H₂O/MeCN, and filtered into a HPLC vial.

Di-Peptide Quantitative HPLC Analysis:

To 5 mg of resin in a 2 mL Eppendorf tube was added 25 μL of H₂O, 25 μL of TIPS, and the tube was filled to 500 μL with TFA. The resin was allowed to react for 10 min, then was placed under a stream of air until dry. To the tube was added 500 μL of 50/50 H₂O/MeCN and filtered into an Eppendorf tube through a glass pipette with a cotton ball. To a low volume vial was added 45 μL of H₂O, 40 μL of peptide sample, and 15 μL of 0.5 mM Fenoprofen. Three separate portions of resin were weighed to perform the reaction in triplicate.

General Procedure for On-Resin Redox Active Ester Synthesis

Peptide on resin with a free carboxylic acid was equilibrated in dry DMF, then drained. In a separate vial, NHPI (10 equiv) was dissolved in dry DMF (100 mM based on resin loading), then DIPEA (10 equiv) was added (solution turns dark red). The solution was added to the resin and allowed to equilibrate for 5 min. In a separate vial, HATU (5 equiv) was dissolved in dry DMF (100 mM based on resin loading), then added dropwise to the resin (solution becomes clear with yellow hue) and allowed to react for 45 min. The resin was then washed with DMF (5x) then drained.

Procedure using DIC from literature.¹

General Procedure for Solid Phase Hydroalkylation

Hantzsch ester (10 equiv) was dissolved in dry DMF (30 mM based on resin loading). For the homologation, allyl acrylate (10 equiv) or styrene (10 equiv) was added to the solution. The mixture was suspended and added to the drained resin, in a fritted plastic syringe containing the NHPI-peptide. The reaction was stirred under a Kessil lamp at 440 nm for 2 h. The resin was then washed with DMF.

Crude LCMS Chromatographs

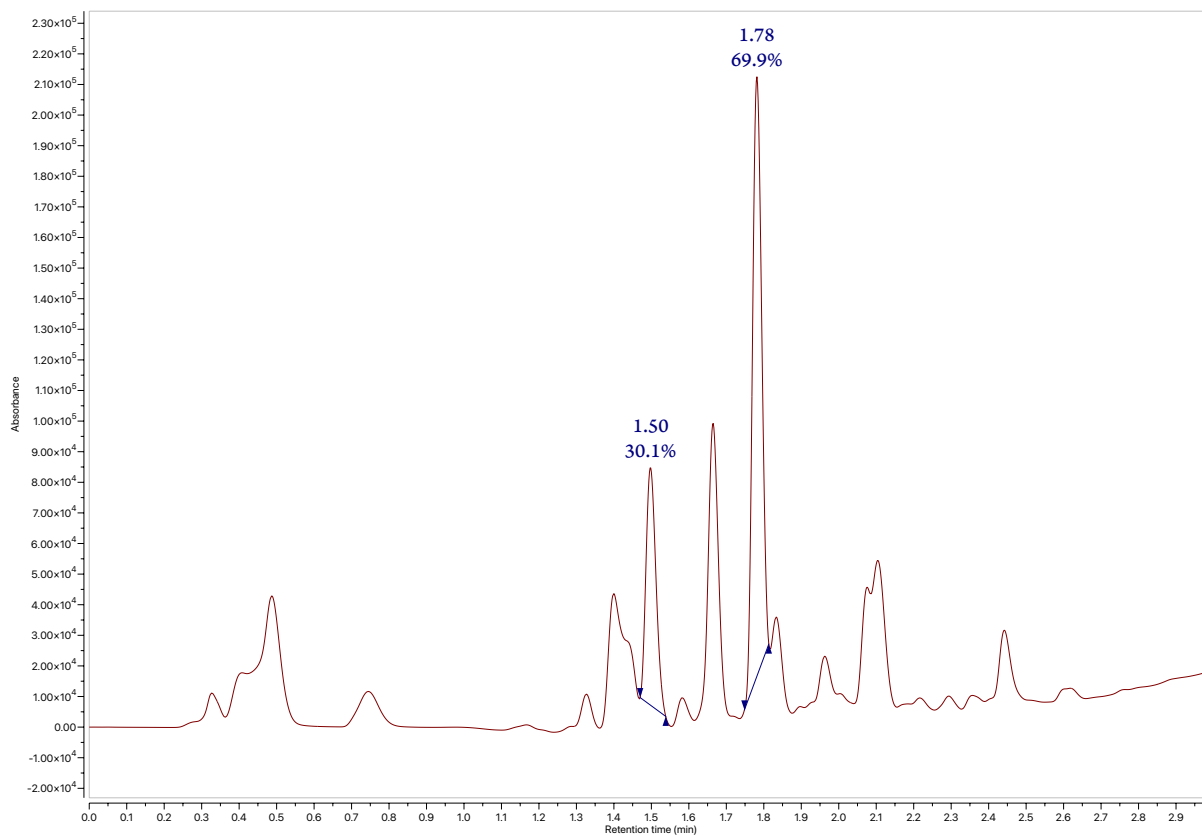
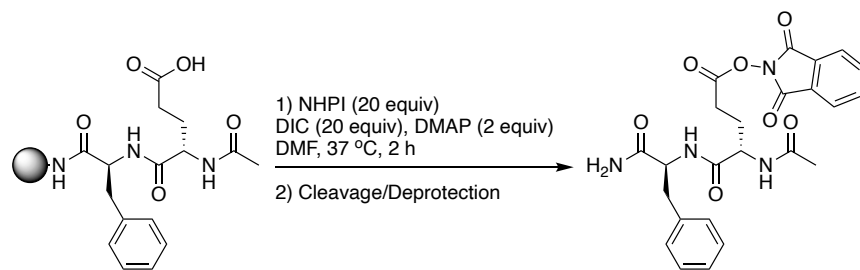


Figure 1. LCMS spectra of crude peptide using a linear gradient of 5-50% MeCN/H₂O over 2 min. The peak at 1.50 min has a mass consistent with the cyclized product (Calc: 318.138 m/z [M+H]⁺; Obs: 318.392 m/z [M+H]⁺). The peak at 1.78 min has a mass consistent with the redox active ester product (Calc: 481.164 m/z [M+H]⁺; Obs: 481.495 m/z [M+H]⁺).

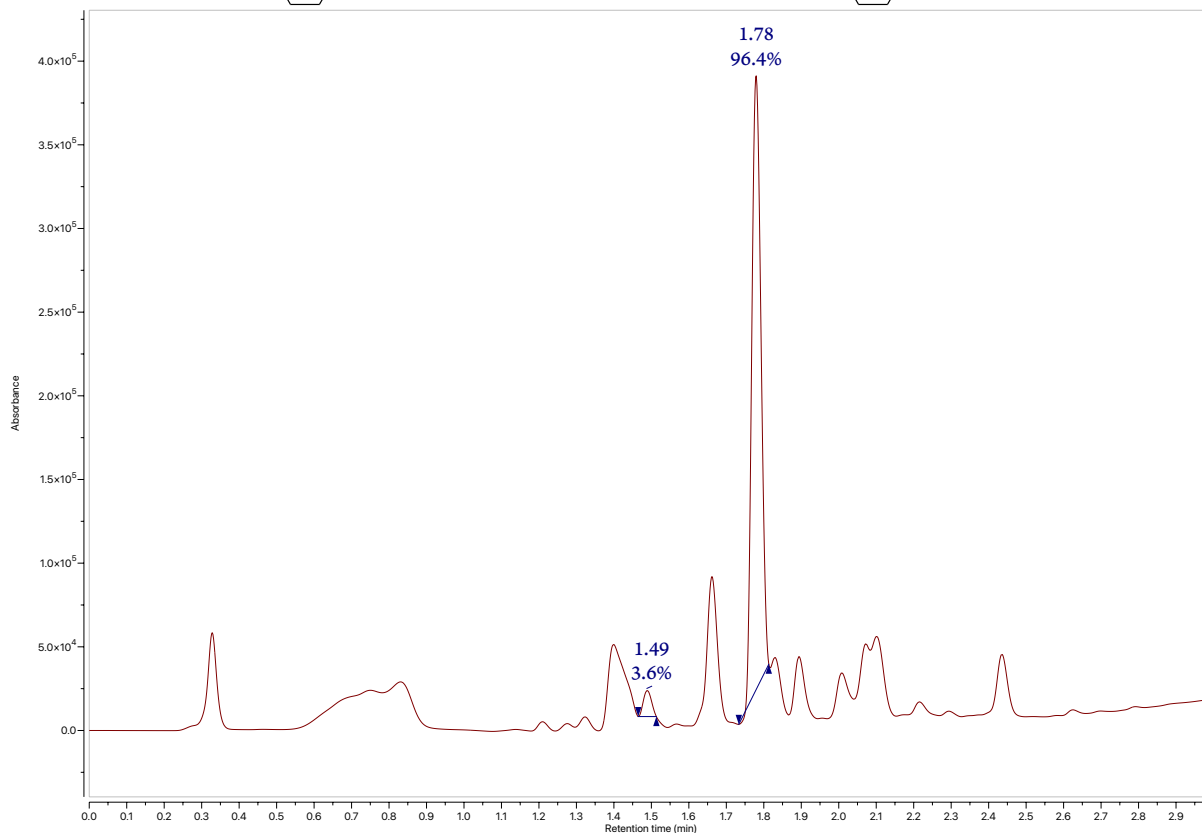
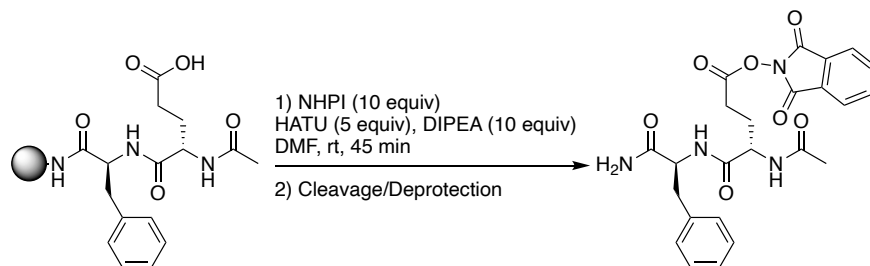


Figure 2. LCMS spectra of crude peptide using a linear gradient of 5-50% MeCN/ H₂O over 2 min. The peak at 1.50 min has a mass consistent with the cyclized product (Calc: 318.138 m/z [M+H]⁺; Obs: 318.392 m/z [M+H]⁺). The peak at 1.78 min has a mass consistent with the redox active ester product (Calc: 481.164 m/z [M+H]⁺; Obs: 481.495 m/z [M+H]⁺).

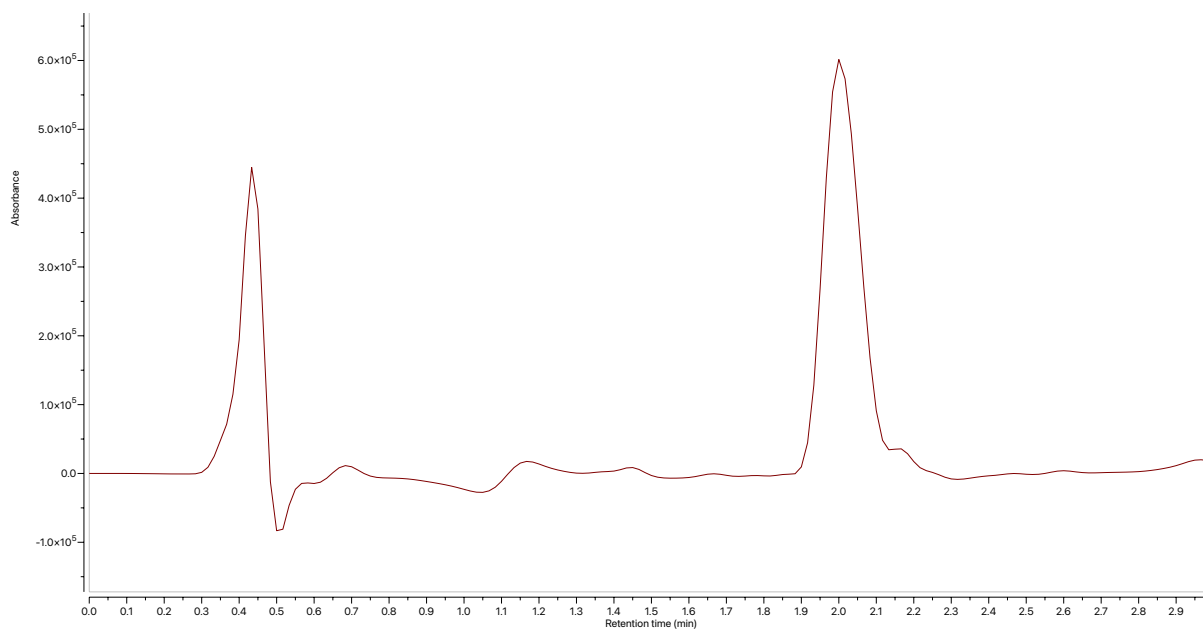
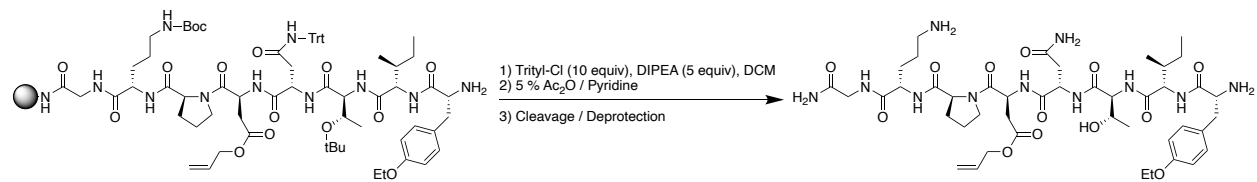


Figure 3. LCMS spectra of crude peptide using a linear gradient of 5-50% MeCN/H₂O over 2 min. The peak at 2.00 min has a mass of 960.802 m/z [M+H]⁺ (Calc 960.508 m/z [M+H]⁺).

Crude HPLC analysis of reactions

Procedure for dipeptide analysis: 5 mg of resin containing peptide were weighed in triplicate and cleaved with 500 μL of cleavage/deprotection solution in Eppendorf tubes. After 20 min the solutions were evaporated with a stream of air, and the dry crude peptides were then dissolved in 500 μL 50/50 MeCN/H₂O and filtered through cotton in a glass pipette. Then 40 μL of the stock solution was diluted with 45 μL of H₂O and 15 μL of Fenoprofen (0.5 mM), then analyzed by HPLC. The ratio of the unmodified peptide to Fenoprofen is used as theoretical 100% yield.

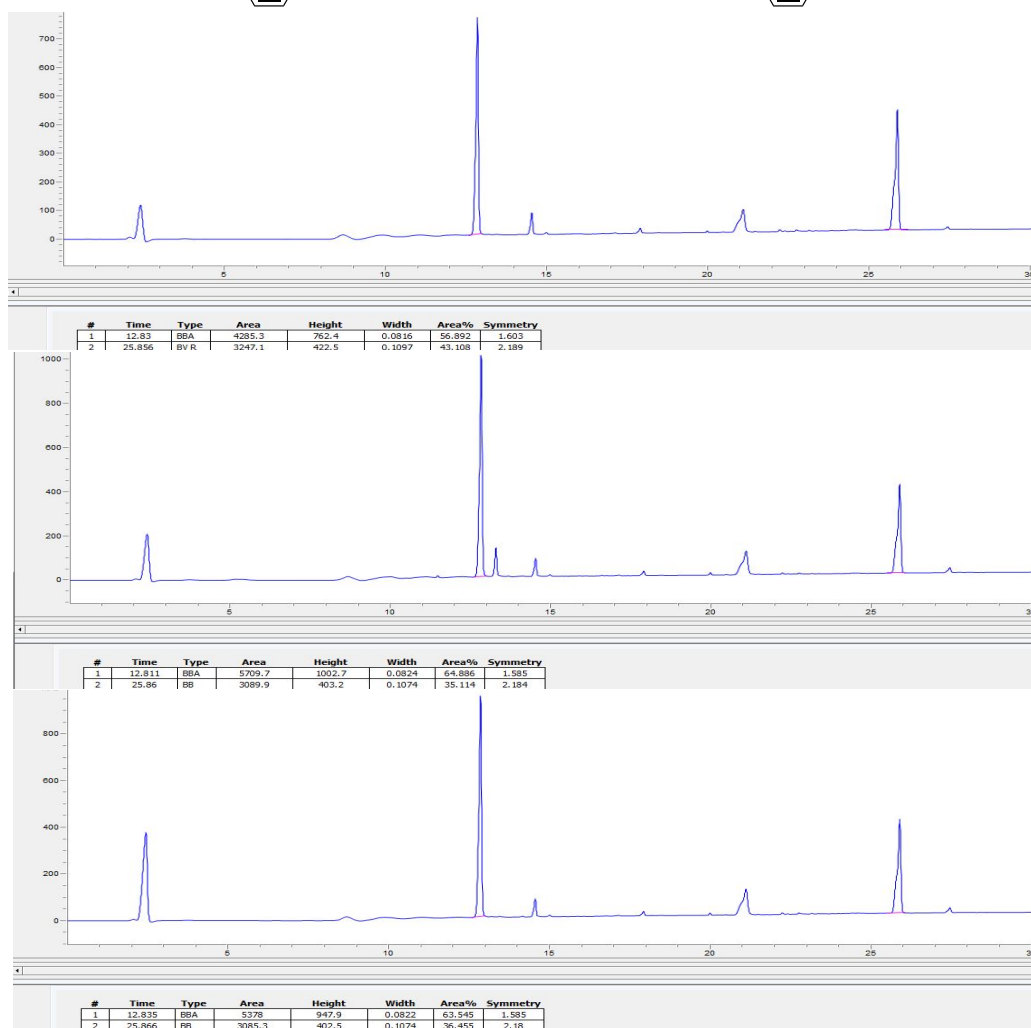
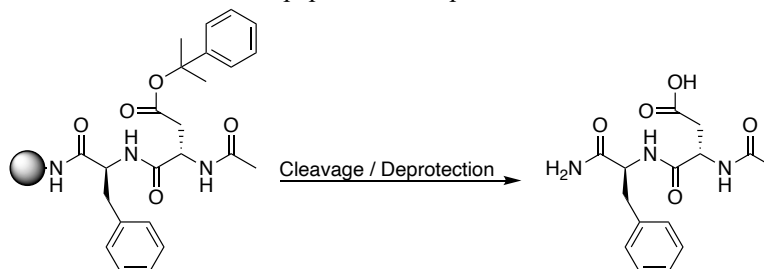


Figure 4. Analytical HPLC spectra for each trial of crude peptide containing 75 μM Fenoprofen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 $^{\circ}\text{C}$, data collected at 214 nm. Fenoprofen (25.8 min) has an average area of 3140.8. Product (12.8 min) has an average area of 5124.3. The average ratio of product area to Fenoprofen area is 1.631.

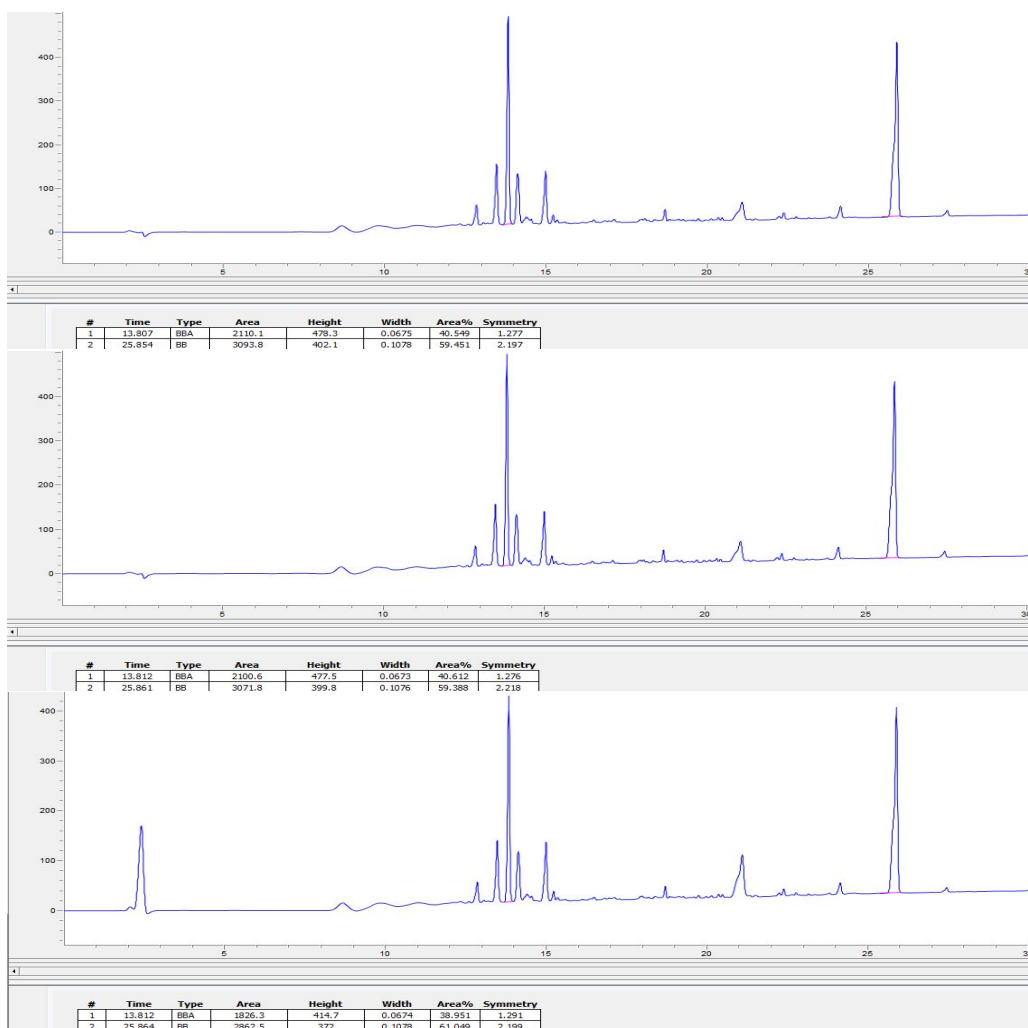
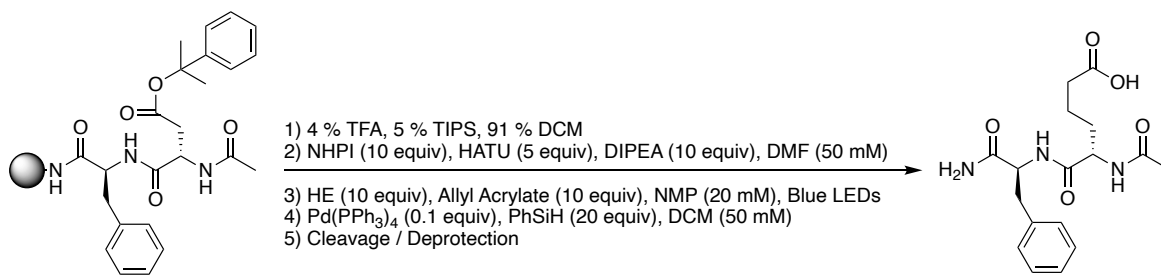


Figure 5. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenoprofen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 $^{\circ}$ C, data collected at 214 nm. Fenoprofen (25.8 min) has an average area of 3099.4. Product (13.8 min) has an average area of 2012.3. The average ratio of product area to Fenoprofen area is 0.67. The peak at 13.4 min is the decarboxylated/protonated product.

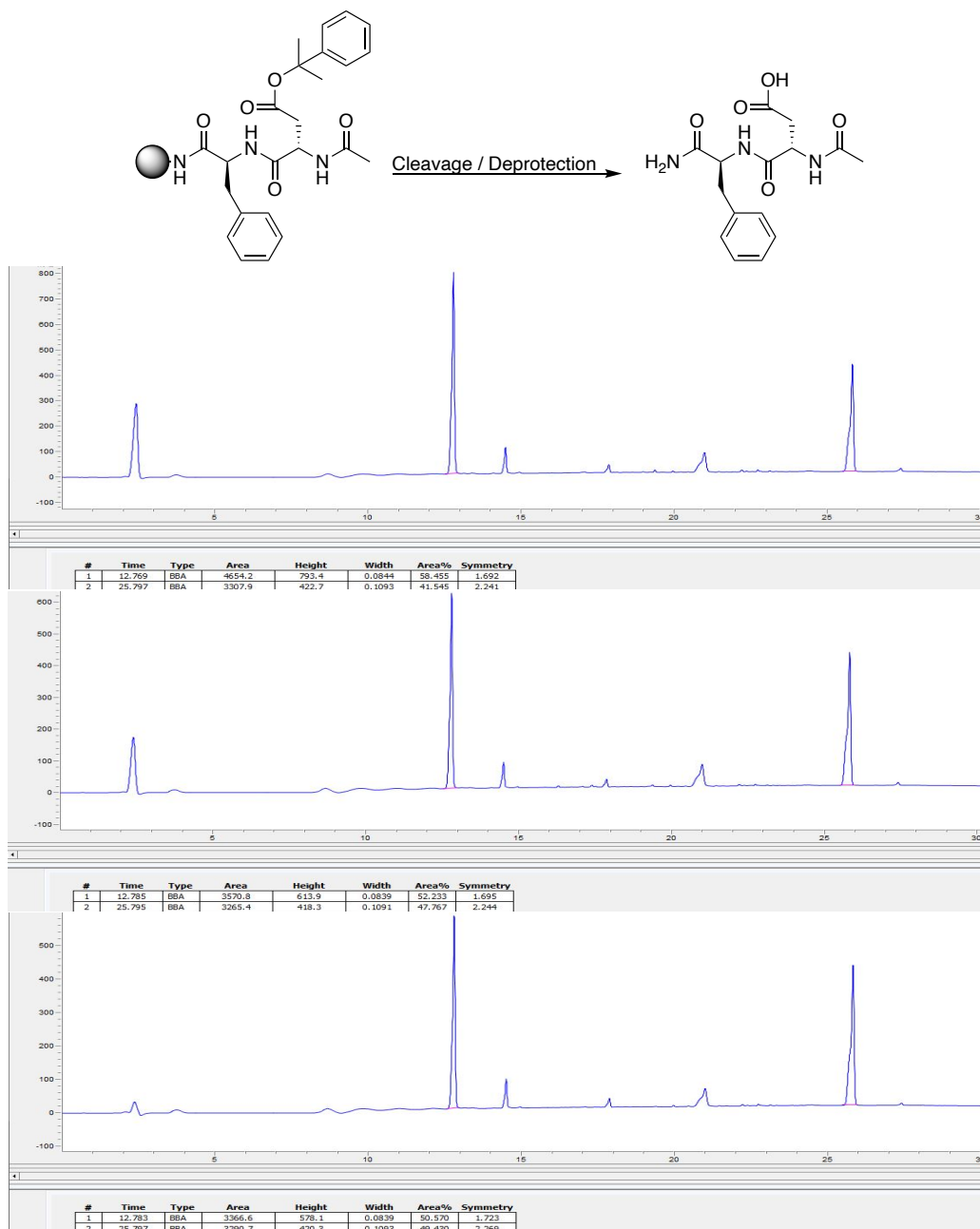


Figure 6. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenopropfen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 °C, data collected at 214 nm.. Fenopropfen (25.8 min) has an average area of 3288.0. Product (12.8 min) has an average area of 3863.9. The average ratio of product area to Fenopropfen area is 1.175.

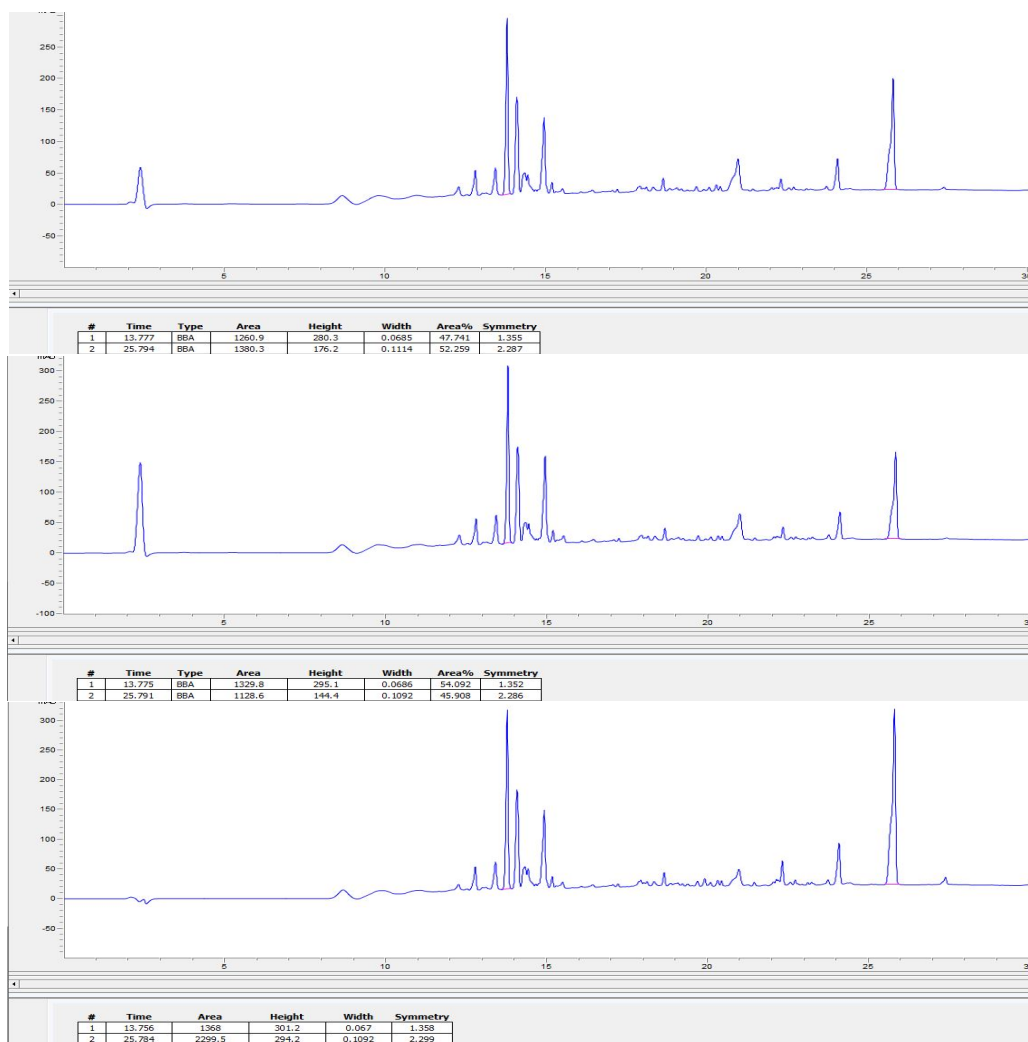
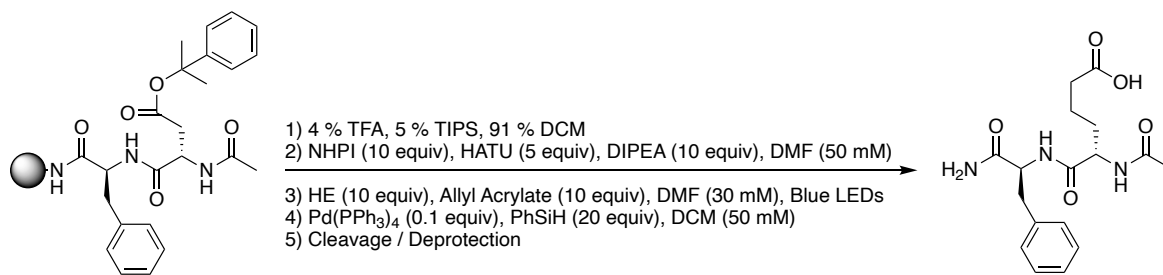


Figure 7. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenoprofen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 $^{\circ}$ C, data collected at 214 nm. Fenoprofen (25.8 min) has an average area of 1602.8. Product (13.8 min) has an average area of 1319.6. The average ratio of product area to Fenoprofen area is 0.823.

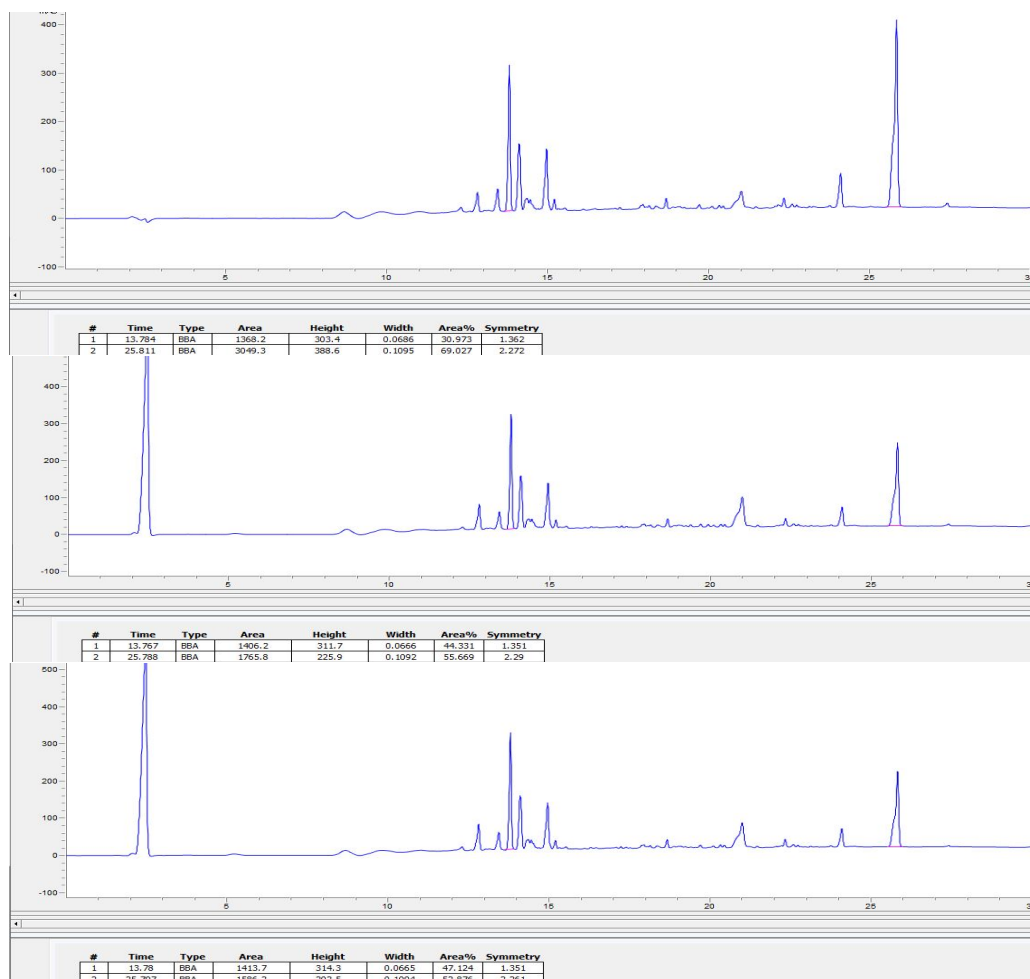
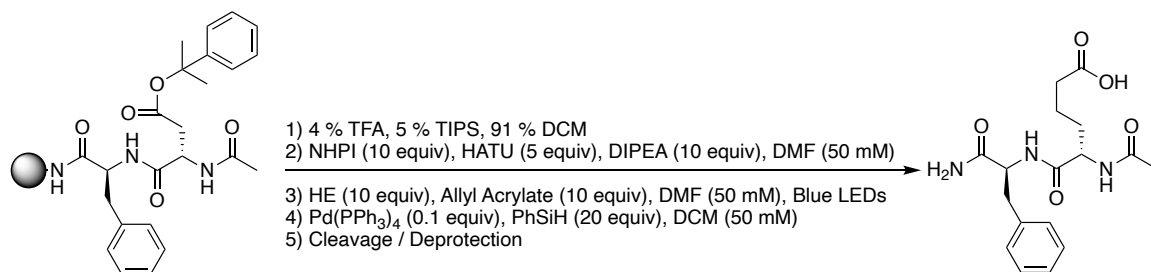


Figure 8. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenopropfen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 $^{\circ}$ C, data collected at 214 nm. Fenopropfen (25.8 min) has an average area of 2133.8. Product (13.8 min) has an average area of 1396.0. The average ratio of product area to Fenopropfen area is 0.654.

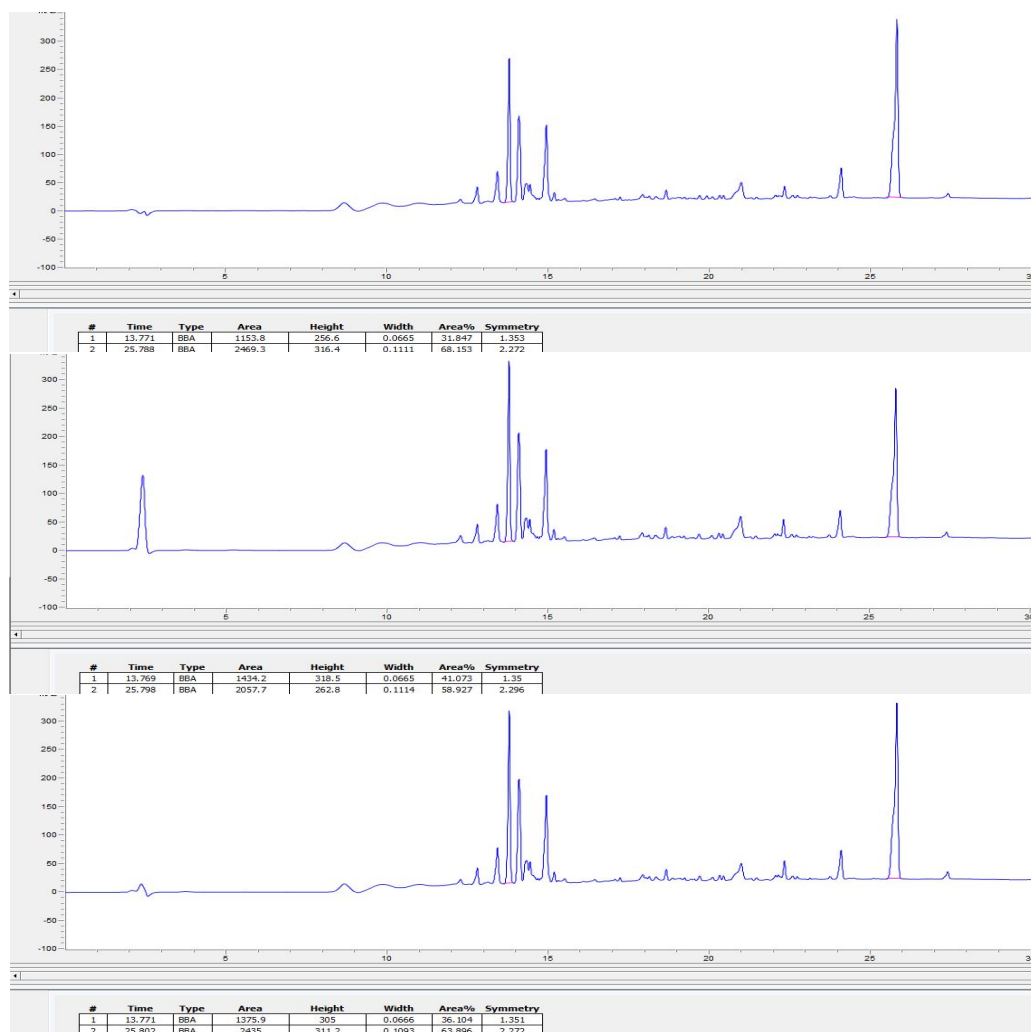
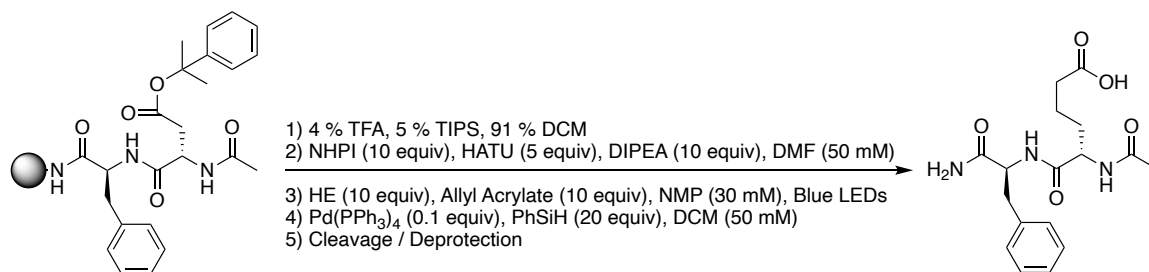


Figure 9. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenopropfen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 °C, data collected at 214 nm. Fenopropfen (25.8 min) has an average area of 2320.7. Product (13.8 min) has an average area of 1321.3. The average ratio of product area to Fenopropfen area is 0.569.

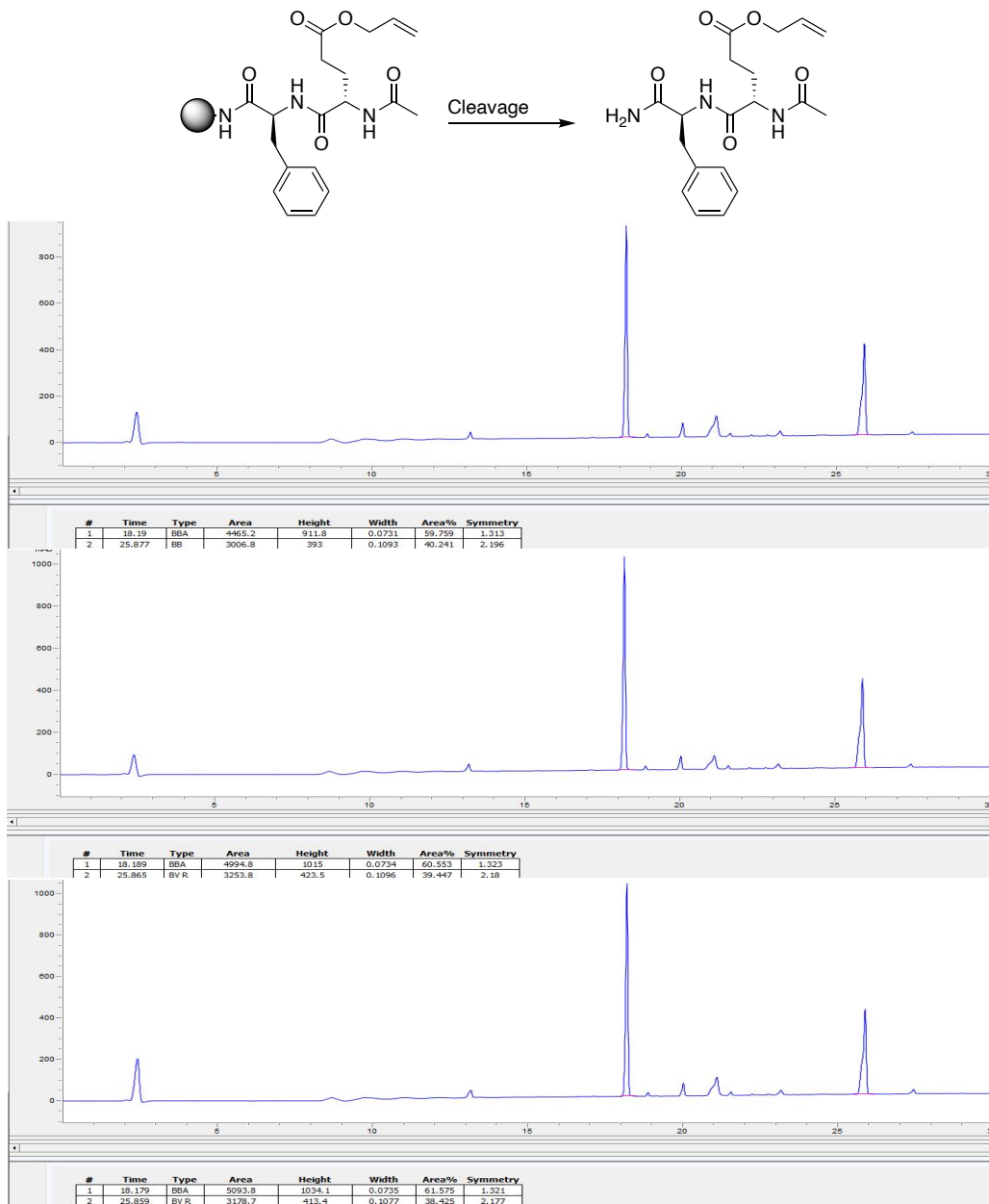


Figure 10. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenopropfen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 °C, data collected at 214 nm. Fenopropfen (25.8 min) has an average area of 3146.4. Product (18.2 min) has an average area of 4851.3. The average ratio of product area to Fenopropfen area is 1.54.

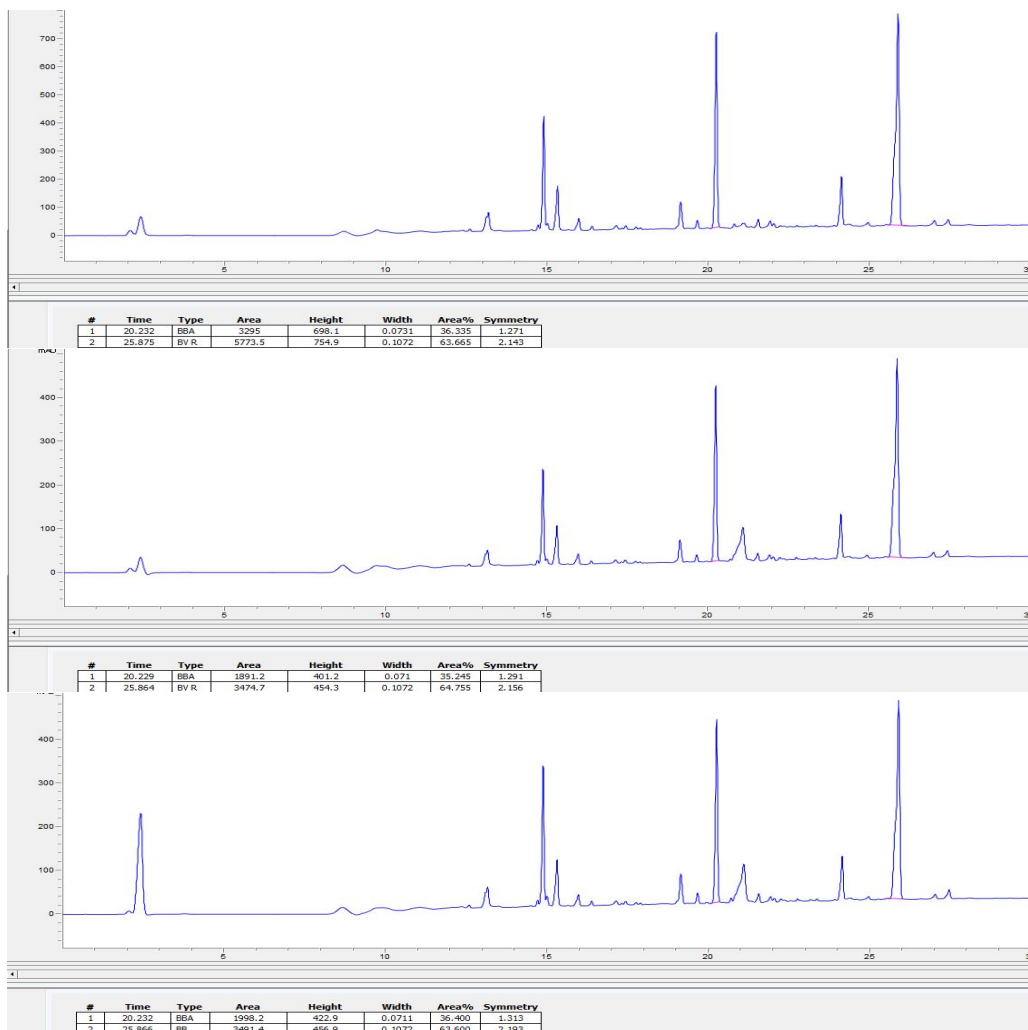
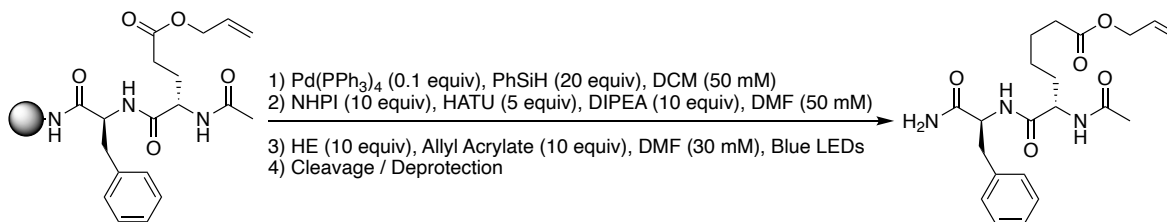


Figure 11. Analytical HPLC spectra for each trial of crude peptide containing 75 μ M Fenoprofen using a linear gradient of 1-60% MeCN/H₂O over 30 min at 60 °C, data collected at 214 nm. Fenoprofen (25.8 min) has an average area of 4246.5. Product (20.2 min) has an average area of 2394.8. The average ratio of product area to Fenoprofen area is 0.56. The peak at 14.8 min is the decarboxylated/protonated product.

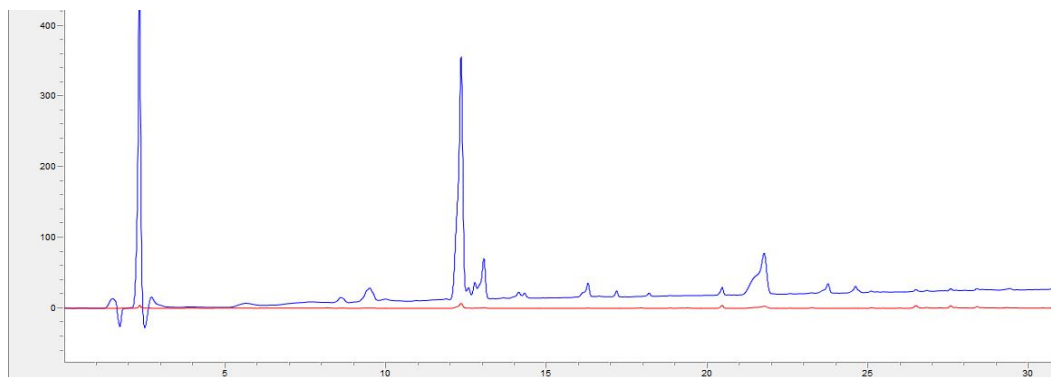
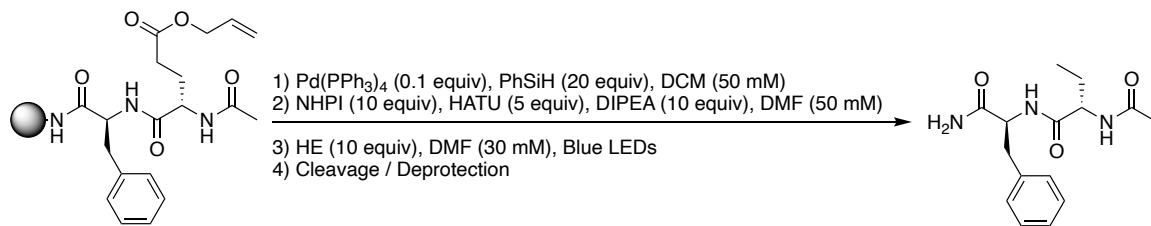


Figure 12. Analytical HPLC spectra of crude peptide using a linear gradient of 5-40% MeCN/H₂O over 30 min at room temperature. Product observed as major peak at 12.3 min. Obtained 2.9 mg (0.010 mmol, 22% isolated yield) of dipeptide.

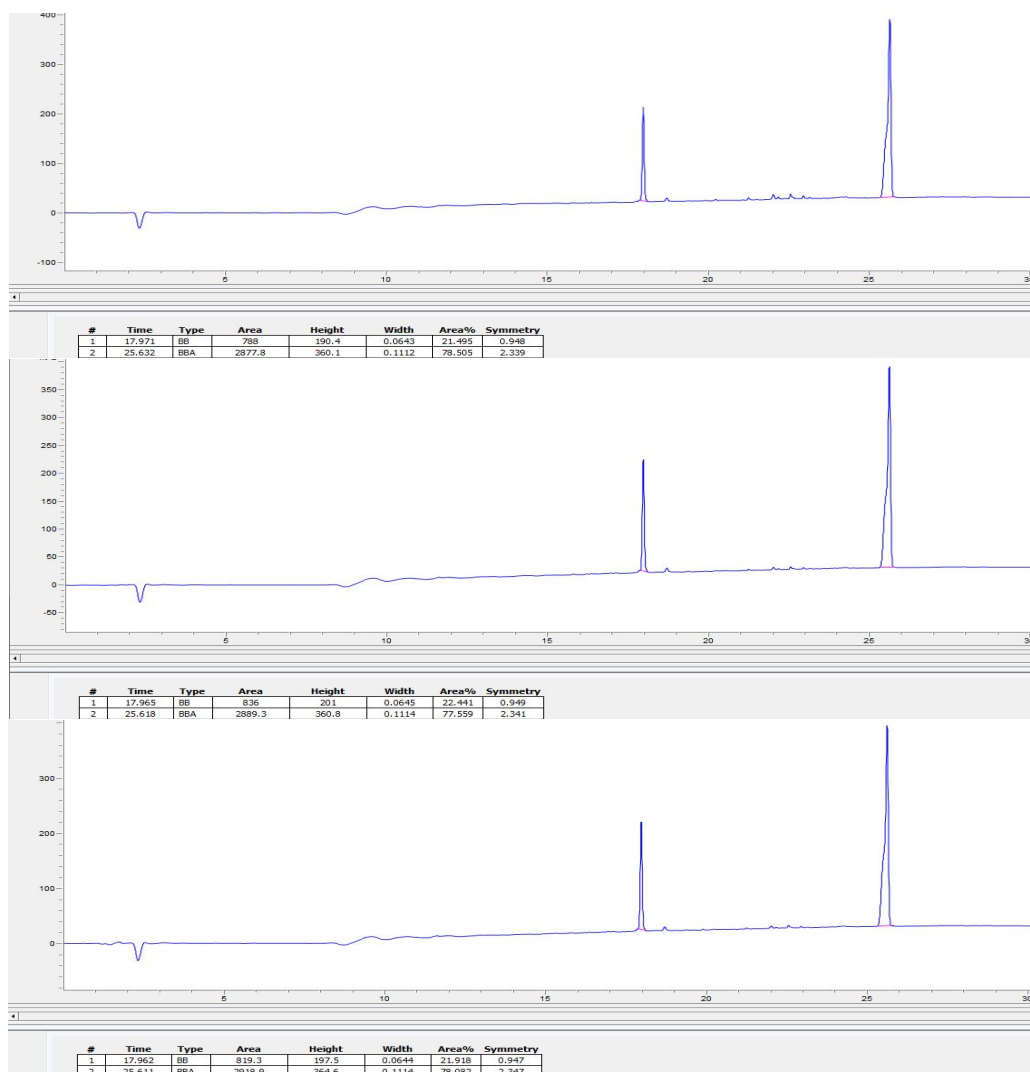
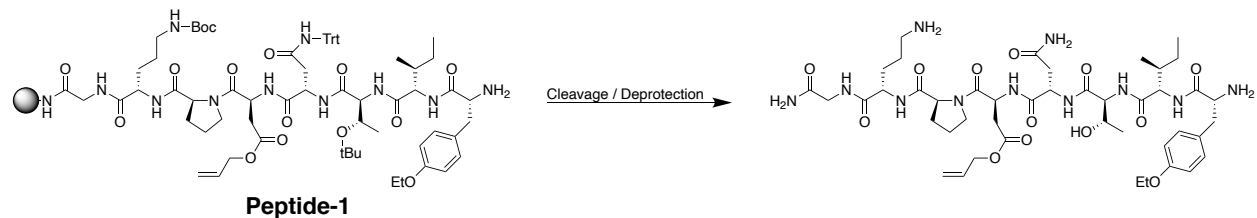


Figure 13. Analytical HPLC spectra of 5 μ L of crude diluted peptide (5 μ L of 1 mL stock was diluted to 20 μ L) to 50 μ L containing 75 μ M Fenopropfen using a linear gradient of 2-60% MeCN/H₂O over 30 min at 60 °C. Fenopropfen (25.6 min) has an average area of 2895.3. Product (17.9 min) has an average area of 814.4. The average ratio of product area to Fenopropfen area is 0.281 (P/IS) indicating the 1 mL stock is 4.490 mM (0.0045 mmol).

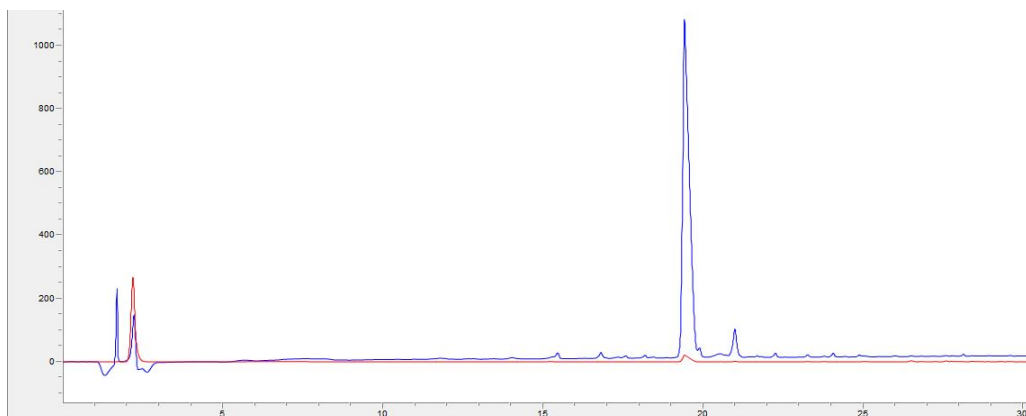
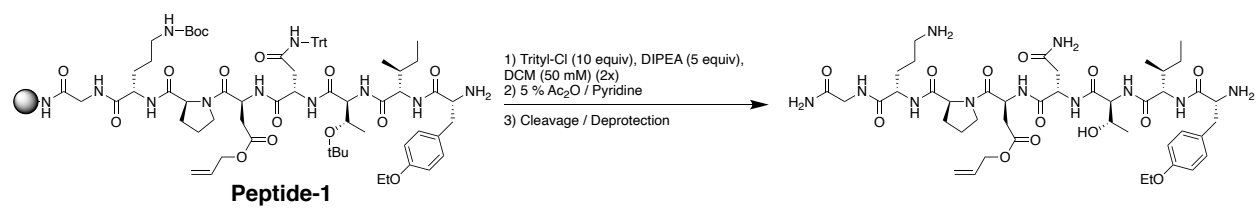


Figure 14. Analytical HPLC spectra of crude peptide using a linear gradient of 5-40% MeCN/H₂O over 30 min at 60 °C. The peak at 19.4 min was collected and analyzed by TOF-MALDI-MS and found to have a mass of 960.487 m/z [M+H]⁺ (Calculated 960.508 m/z [M+H]⁺).

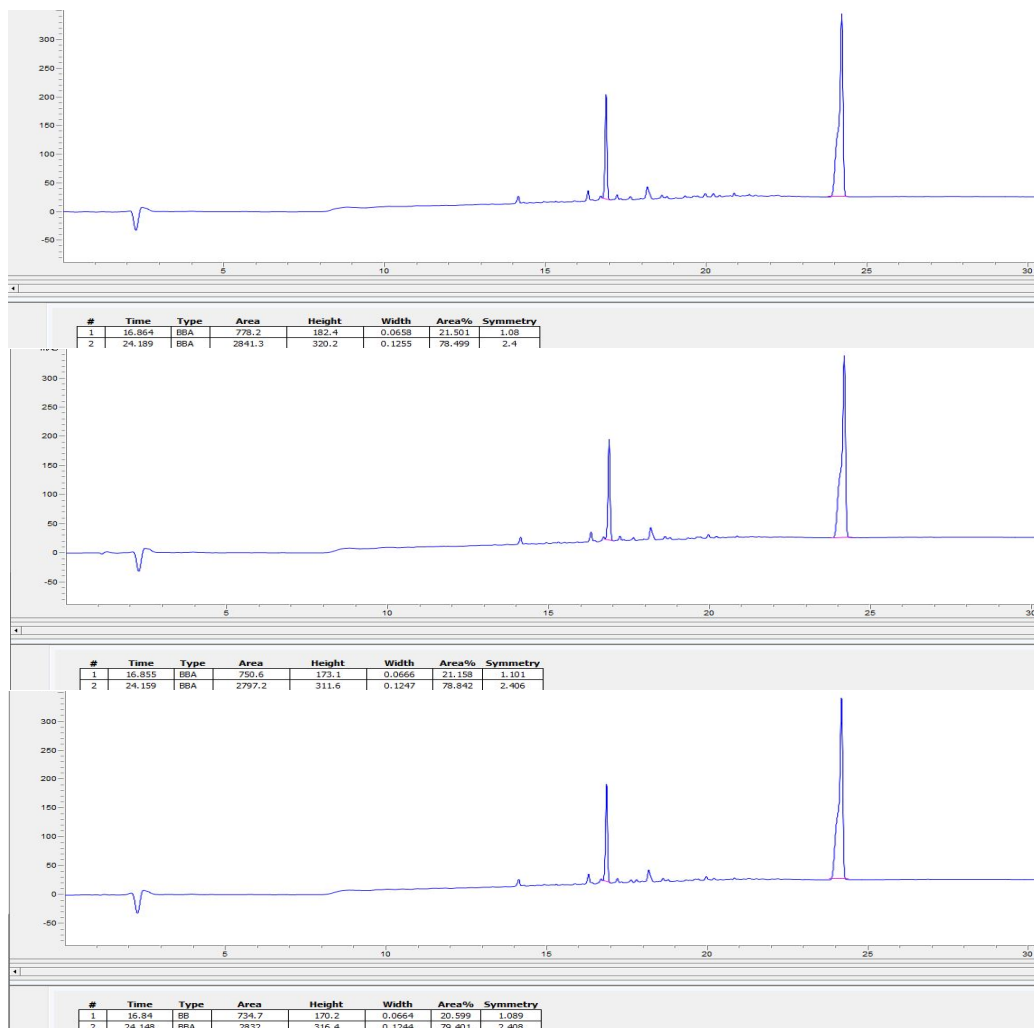
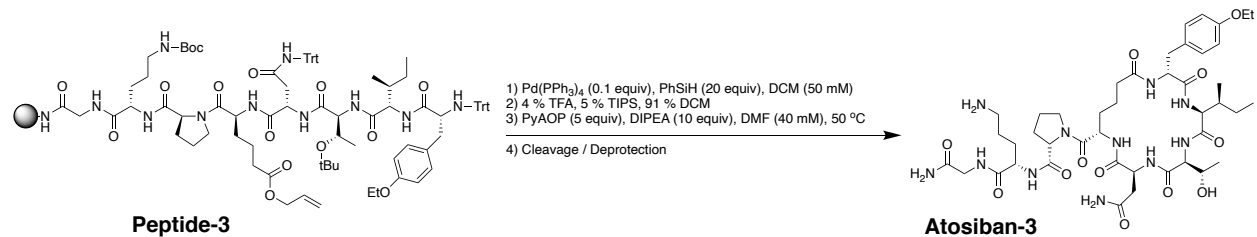
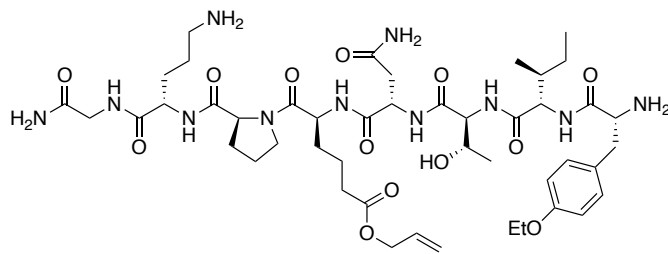


Figure 17. Analytical HPLC spectra of 5 μ L of crude peptide (1 mL total, 0.005 mmol) diluted to 50 μ L containing 75 μ M Fenoprofen using a linear gradient of 10-60% MeCN/H₂O over 30 min at 60 °C. Fenoprofen (24.1 min) has an average area of 2823.5. Product (16.8 min) has an average area of 754.5. The average ratio of product area to Fenoprofen area is 0.267 (P/IS) indicating the crude 1 mL stock solution is 0.915 mM (0.000915 mmol).



Peptide-3

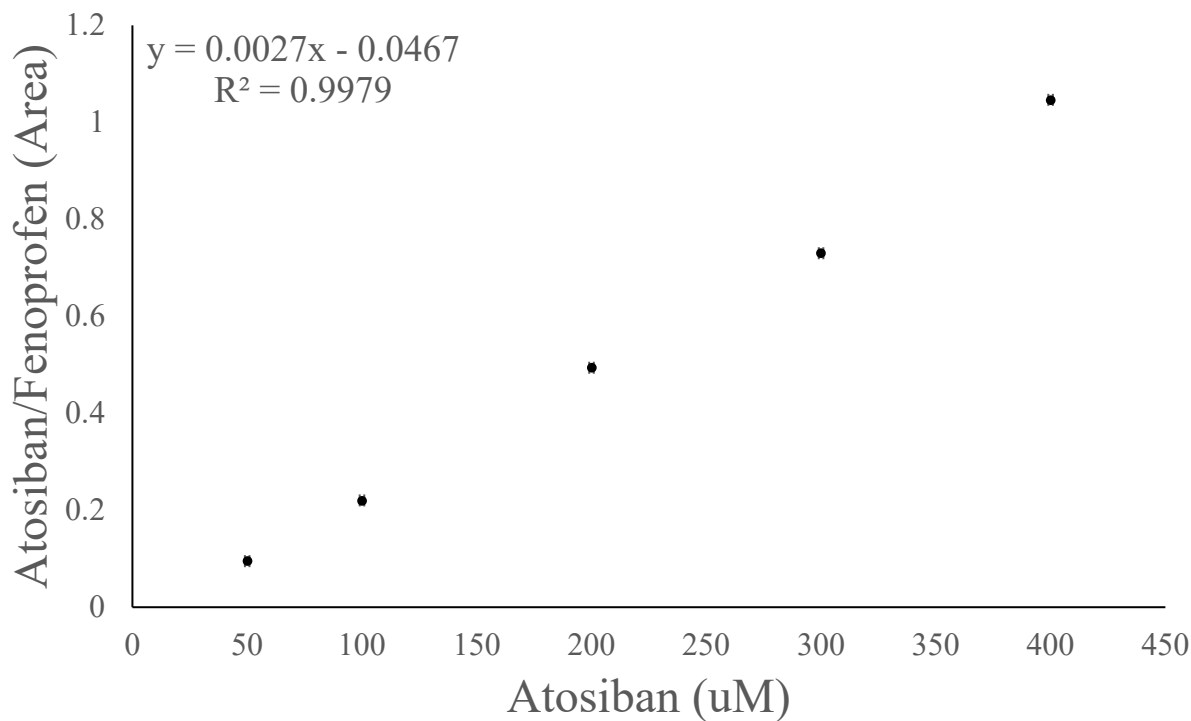
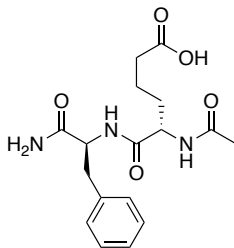


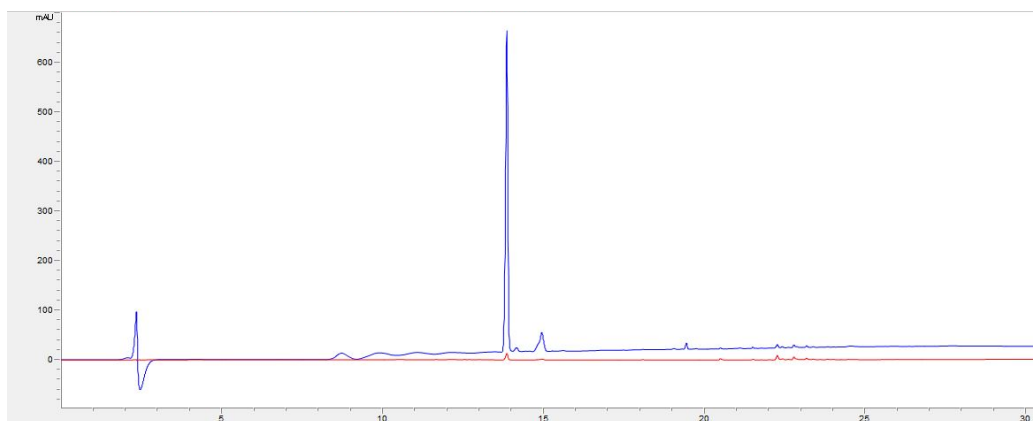
Figure 20. Standard curve of above peptide. NMR solution of Peptide-3 was diluted to 0.5 mM with water. The stock was then diluted to given concentrations with water in triplicate, then Fenopfen (0.5 mM) was added for a final concentration of 75 μ M. Samples were then injected (10 μ L) on analytical HPLC using a gradient of 2-60% MeCN/H₂O over 30 min at 60 °C.

Purification and Characterization Data for all Peptides



Purification: Peptide was purified on preparative HPLC using a focused gradient of 2-20% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 13.0 min.

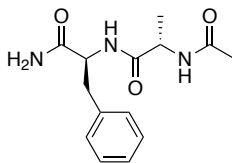
Analytical HPLC Gradient 1-60% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 13.8 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₁₇H₂₄N₃O₅ 350.1716; Found 350.1700

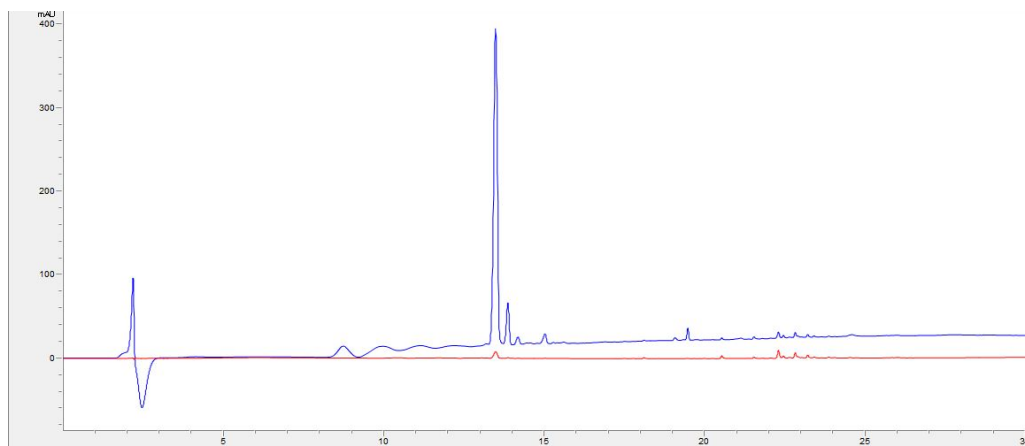
¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.33 – 7.04 (m, 5H), 4.39 (dd, J = 9.3, 4.8 Hz, 1H), 4.10 (dd, J = 7.9, 5.4 Hz, 1H), 3.02 (dd, J = 13.8, 4.8 Hz, 1H), 2.81 (dd, J = 13.8, 9.3 Hz, 1H), 2.21 – 2.09 (m, 2H), 1.83 (s, 3H), 1.56 – 1.33 (m, 4H).

¹³C NMR (151 MHz, 5% D₂O/DMSO-*d*₆) δ 174.7, 173.2, 171.8, 170.3, 138.2, 129.6, 128.5, 126.7, 54.0, 53.1, 37.6, 33.7, 31.4, 22.8, 21.3.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 2-20% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 11.8 min.

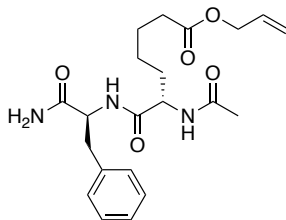
Analytical HPLC Gradient 1-60% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 13.4 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₁₄H₂₀N₃O₃ 278.1505; Found 278.1483

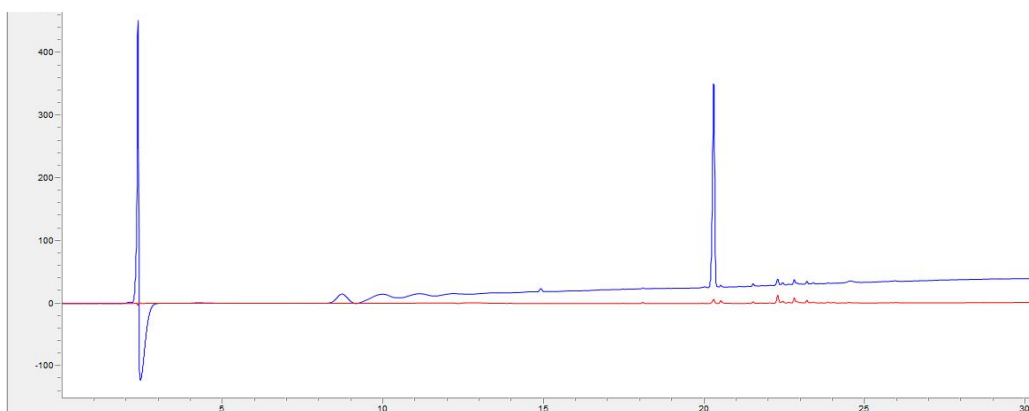
¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.26 – 7.18 (m, 5H), 4.36 (dd, $J = 9.3, 4.8$ Hz, 1H), 4.12 (q, $J = 7.1$ Hz, 1H), 3.03 (dd, $J = 13.8, 4.9$ Hz, 1H), 2.82 (dd, $J = 13.8, 9.3$ Hz, 1H), 1.82 (s, 3H), 1.09 (d, $J = 7.1$ Hz, 3H).

¹³C NMR (151 MHz, 5% D₂O/DMSO-*d*₆) δ 173.3, 172.6, 170.1, 138.3, 129.6, 128.5, 126.7, 53.9, 49.0, 37.5, 22.9, 18.0.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 5-40% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 20.7 min.

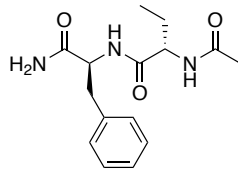
Analytical HPLC Gradient 1-60% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 20.3 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₁H₃₀N₃O₅ 404.2185; Found 404.2171

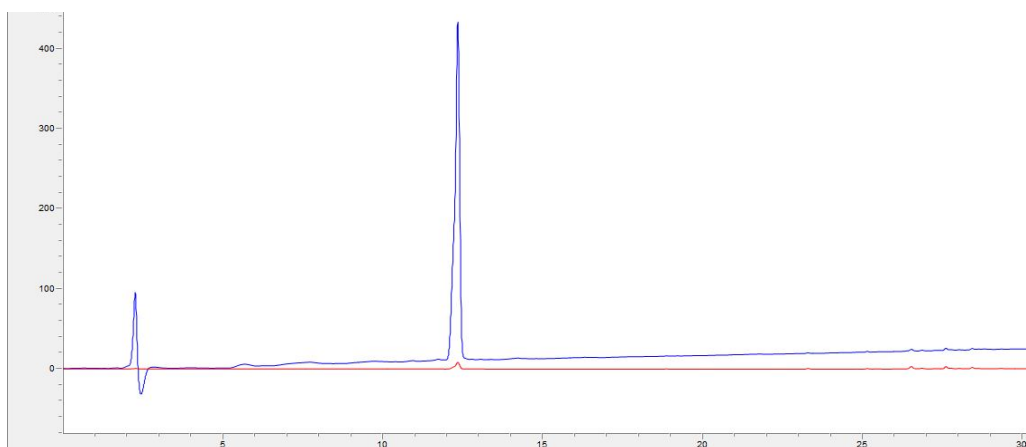
¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.26 – 7.16 (m, 5H), 5.94 – 5.87 (m, 1H), 5.28 (dq, $J = 17.1, 1.5$ Hz, 1H), 5.21 (dq, $J = 10.6, 1.3$ Hz, 1H), 4.55 – 4.51 (m, 2H), 4.39 (dd, $J = 9.4, 4.8$ Hz, 1H), 4.07 (dd, $J = 8.7, 5.4$ Hz, 1H), 3.02 (dd, $J = 13.9, 4.8$ Hz, 1H), 2.81 (dd, $J = 13.9, 9.4$ Hz, 1H), 2.26 (td, $J = 7.4, 3.4$ Hz, 2H), 1.82 (s, 3H), 1.50 – 1.40 (m, 4H), 1.18 – 1.12 (m, 2H).

¹³C NMR (151 MHz, 5% D₂O/DMSO-*d*₆) δ 173.3, 173.0, 171.9, 170.3, 138.3, 133.2, 129.6, 128.5, 126.7, 118.1, 64.7, 53.9, 53.2, 37.6, 33.7, 31.6, 25.1, 24.6, 22.9.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 2-25% MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 16.9 min.

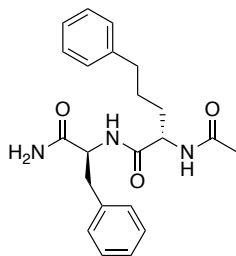
Analytical HPLC Gradient 5-40% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 12.3 min.



HRMS (ESI-TOF) m/z : $[M + Na]^+$ Calc'd for C₁₅H₂₁N₃O₃Na 314.1481; Found 314.1466

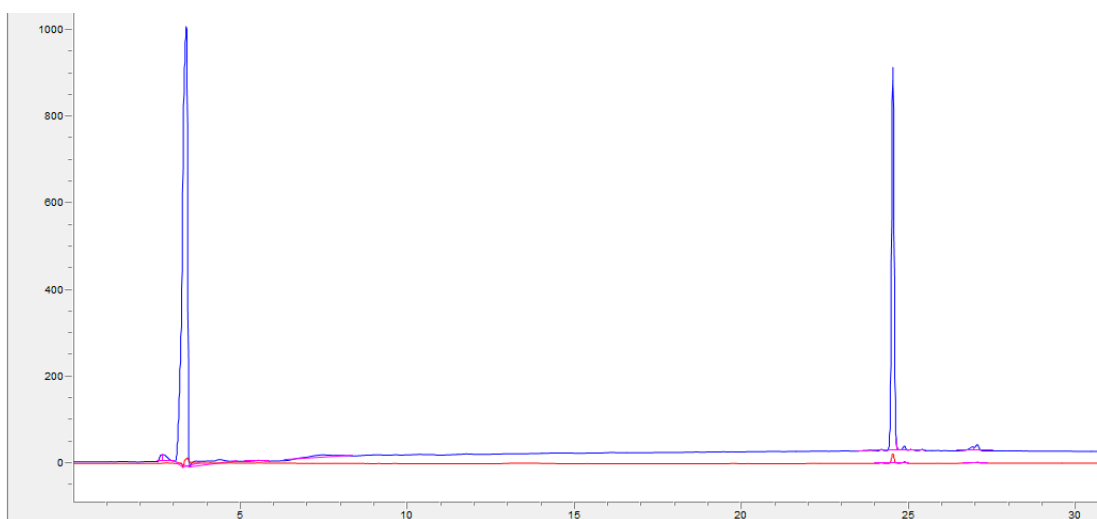
¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.27 – 7.15 (m, 5H), 4.40 (dd, J = 9.3, 4.8 Hz, 1H), 4.03 (dd, J = 8.1, 5.6 Hz, 1H), 3.02 (dd, J = 13.9, 4.8 Hz, 1H), 2.81 (dd, J = 13.9, 9.4 Hz, 1H), 1.83 (s, 3H), 1.55 (m, 1H), 1.43 (m, 1H), 0.74 (t, J = 7.4 Hz, 3H).

¹³C NMR (151 MHz, 5% D₂O/DMSO-*d*₆) δ 173.3, 171.8, 170.3, 138.3, 129.6, 128.5, 126.7, 54.7, 53.9, 37.6, 25.3, 22.9, 10.6.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 10-60 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 19.1 min. Obtained 1.1 mg (2.9 μ moles) of product for 12 % yield.

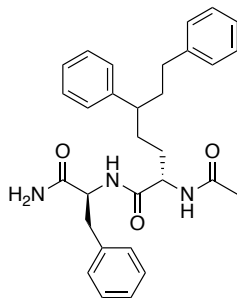
Analytical HPLC Gradient 5-46% MeCN/H₂O (0.1% TFA) at room temperature over 30 min (blue 214 nm, red 254 nm). Product eluted at 24.5 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₂H₂₈N₃O₃ 382.2131; Found 382.2145

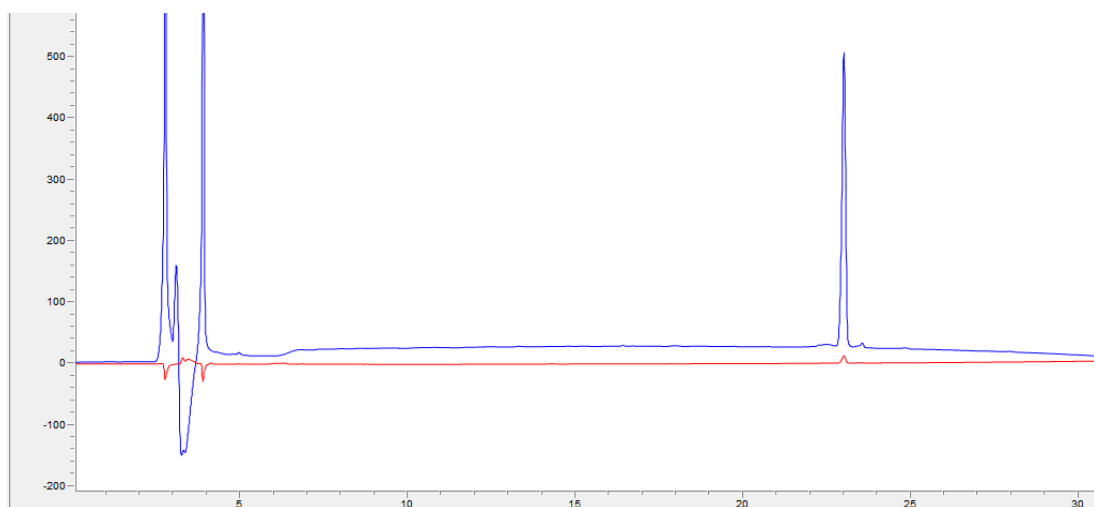
¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.27 (t, J = 7.6 Hz, 2H), 7.24 – 7.12 (m, 8H), 4.38 (dd, J = 9.4, 4.8 Hz, 1H), 4.12 (t, J = 6.6 Hz, 1H), 3.02 (dd, J = 13.9, 4.7 Hz, 1H), 2.81 (dd, J = 13.8, 9.4 Hz, 1H), 1.83 (s, 3H), 1.57 – 1.38 (m, 4 H), 1.23 (m, 2H).

¹³C NMR (151 MHz, 5% D₂O/DMSO-*d*₆) δ 173.3, 171.9, 170.3, 142.3, 138.3, 129.6, 128.73, 128.71, 128.5, 126.7, 126.15, 53.9, 53.3, 37.6, 35.3, 31.7, 27.5, 22.9.



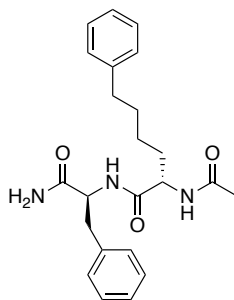
Purification: Peptide was purified on preparative HPLC using a focused gradient of 10-60 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 27.3 min. Obtained 1.1 mg (2.3 μmoles) of product for 9 % yield.

Analytical HPLC Gradient 20-80% MeCN/H₂O (0.1% TFA) at room temperature over 30 min (blue 214 nm, red 254 nm). Product eluted at 23.0 min.



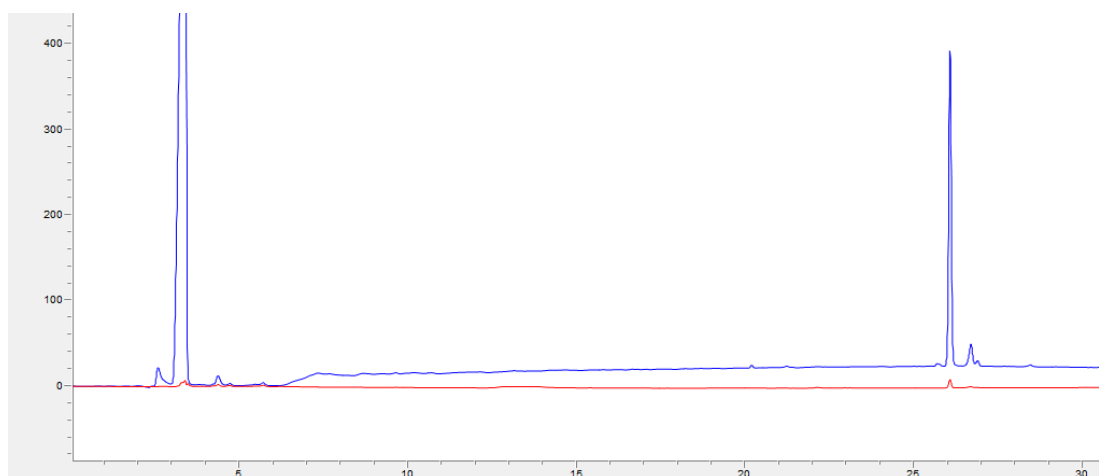
HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₃₀H₃₆N₃O₃ 486.2757; Found 486.2944

¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.32 (td, $J = 7.8, 3.6$ Hz, 2H), 7.26 (m, 2H), 7.23 – 7.11 (m, 8H), 7.09 (d, $J = 7.2$ Hz, 2H), 4.38 (m, 1H), 3.98 (m, 1H), 3.01 (ddd, $J = 13.8, 8.6, 4.7$ Hz, 1H), 2.78 (m, 1H), 2.43 (m, 1H), 2.34 (m, 2H), 1.80 (m, 5H), 1.48 (m, 2H), 1.37 – 1.32 (m, 1H), 1.17 (m, 1H).



Purification: Peptide was purified on preparative HPLC using a focused gradient of 10-60 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 21.1 min. Obtained 1.3 mg (3.3 μmoles) of product for 13 % yield.

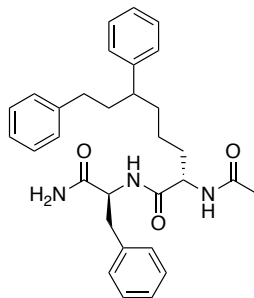
Analytical HPLC Gradient 5-60% MeCN/H₂O (0.1% TFA) at room temperature over 30 min (blue 214 nm, red 254 nm). Product eluted at 26.1 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₃H₃₀N₃O₃ 396.2287; Found 396.2283

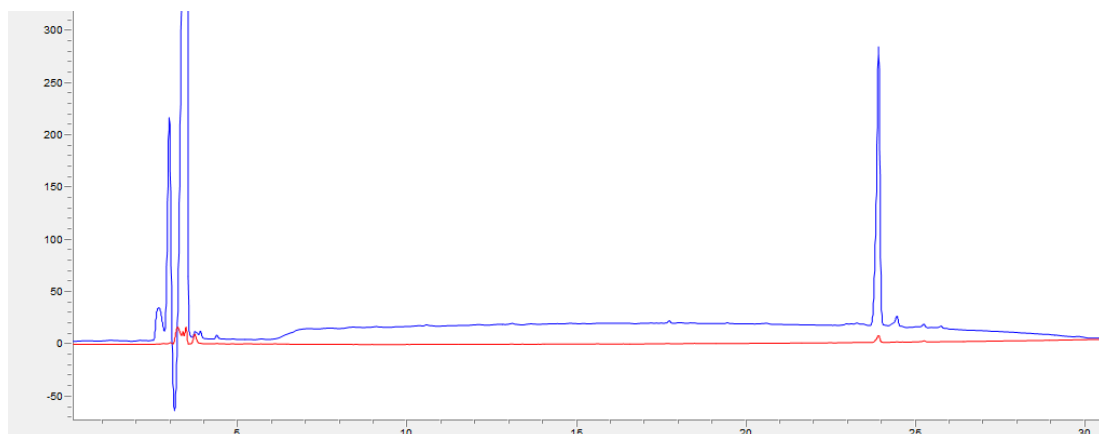
¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.25 (dt, $J = 13.8, 7.3$ Hz, 4H), 7.20 (d, $J = 8.1$ Hz, 2H), 7.16 (m, 4H), 4.40 (m, 1H), 4.07 (dd, $J = 8.6, 5.5$ Hz, 1H), 3.02 (dd, $J = 13.9, 4.8$ Hz, 1H), 2.81 (dd, $J = 13.8, 9.4$ Hz, 1H), 1.82 (s, 3H), 1.58 – 1.37 (m, 5H), 1.18 (m, 3H).

¹³C NMR (151 MHz, 5% D₂O/DMSO-*d*₆) δ 173.3, 172.0, 170.3, 142.6, 138.3, 129.6, 128.71, 128.69, 128.5, 126.7, 126.1, 53.9, 53.3, 37.6, 35.5, 31.8, 31.2, 25.4, 22.9.



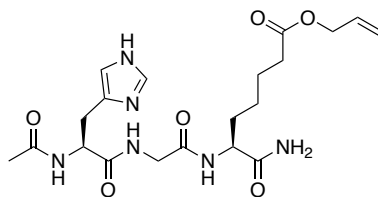
Purification: Peptide was purified on preparative HPLC using a focused gradient of 10-60 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 28.4 min. Obtained 0.8 mg (1.6 μmoles) of product for 6 % yield.

Analytical HPLC Gradient 20-80% MeCN/H₂O (0.1% TFA) at room temperature over 30 min (blue 214 nm, red 254 nm). Product eluted at 23.9 min.



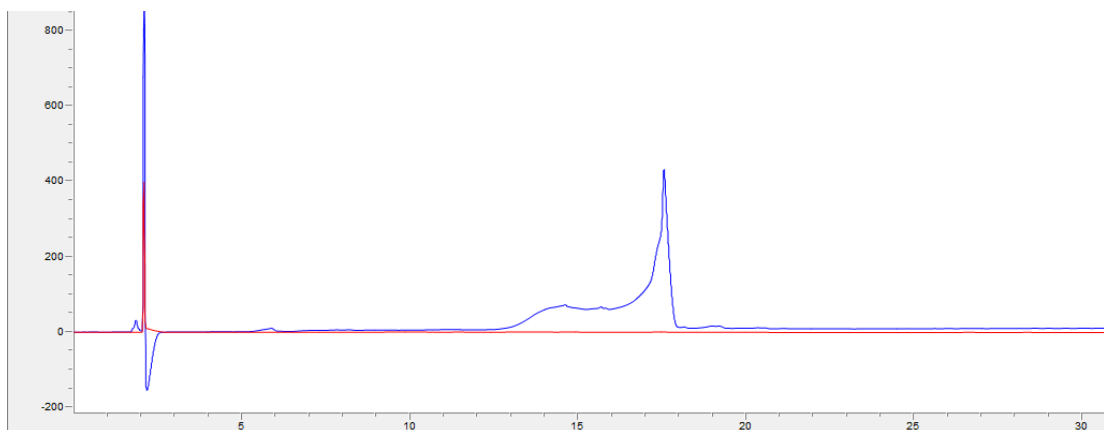
HRMS (ESI-TOF) m/z: [M + H]⁺ Calc'd for C₃₁H₃₈N₃O₃ 500.2913; Found 500.2938

¹H NMR (600 MHz, 5% D₂O/DMSO-*d*₆) δ 7.31 (t, *J* = 7.7 Hz, 2H), 7.25 (td, *J* = 7.6, 1.9 Hz, 2H), 7.23 – 7.13 (m, 7H), 7.09 (d, *J* = 7.8 Hz, 2H), 4.36 (dd, *J* = 9.3, 4.8 Hz, 1H), 4.00 (m, 1H), 3.00 (m, 1H), 2.78 (m, 1H), 2.45 (m, 1H), 2.34 (m, 2H), 1.86 (m, 1H), 1.81 – 1.74 (m, 4H), 1.58 – 1.40 (m, 4H), 1.33 (m, 1H), 1.04 (m, 1H).



Purification: Peptide was purified on preparative HPLC using a focused gradient of 2-20 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 18.1 min. Obtained 2.0 μ mole (6 % yield) as determined by NMR.

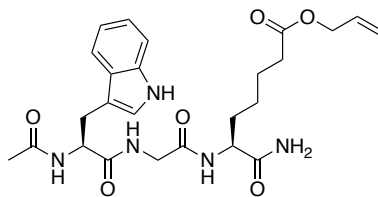
Analytical HPLC Gradient 2-25% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 17.5 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₀H₃₁N₆O₆ 451.2305; Found 451.2304

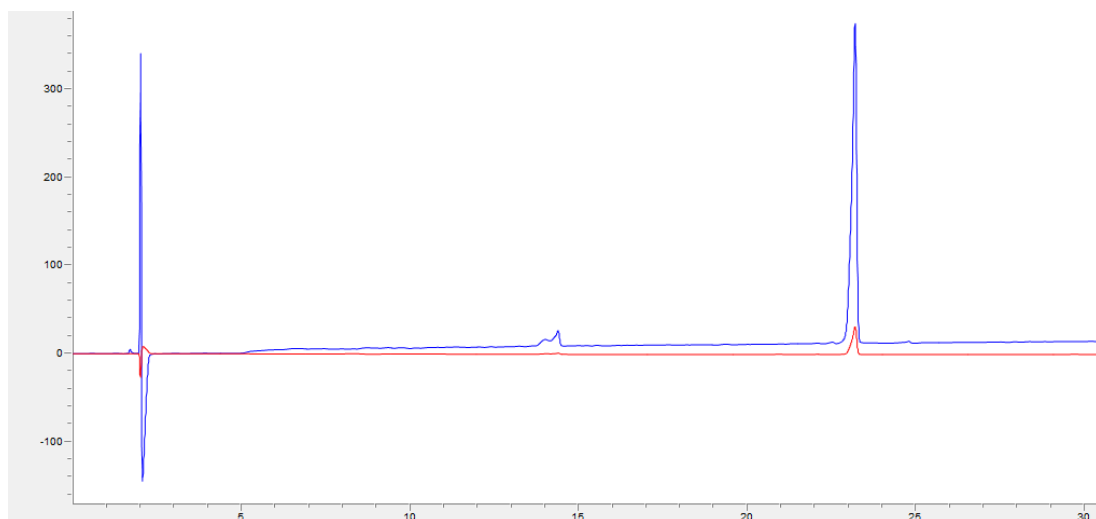
¹H NMR (600 MHz, D₂O) δ 8.62 (d, J = 1.4 Hz, 1H), 7.31 (s, 1H), 5.96 (m, 1H), 5.33 (dd, J = 17.3, 1.5 Hz, 1H), 5.27 (dd, J = 10.5, 1.4 Hz, 1H), 4.68 (dd, J = 8.5, 5.9 Hz, 1H), 4.61 (d, J = 5.6 Hz, 2H), 4.27 (dd, J = 9.3, 5.1 Hz, 1H), 3.95 (s, 2H), 3.30 (dd, J = 15.4, 5.8 Hz, 1H), 3.16 (dd, J = 15.4, 8.5 Hz, 1H), 2.43 (t, J = 7.5 Hz, 2H), 2.00 (s, 3H), 1.83 (m, 1H), 1.73 (m, 1H), 1.64 (m, 2H), 1.38 (m, 2H).

¹³C NMR (151 MHz, D₂O) δ 176.8, 176.3, 174.3, 172.5, 171.2, 133.5, 131.9, 128.5, 118.3, 117.2, 65.7, 53.4, 52.7, 42.3, 33.6, 30.5, 26.2, 24.4, 23.7, 21.7.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 5-40 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 19.1 min. Obtained 4.2 μ mole (8 % yield) as determined by NMR.

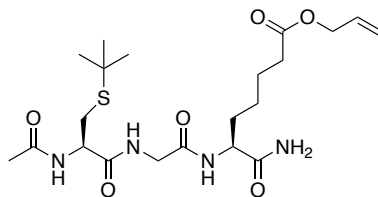
Analytical HPLC Gradient 5-40% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 23.2 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₅H₃₄N₅O₆ 500.2509; Found 500.2530

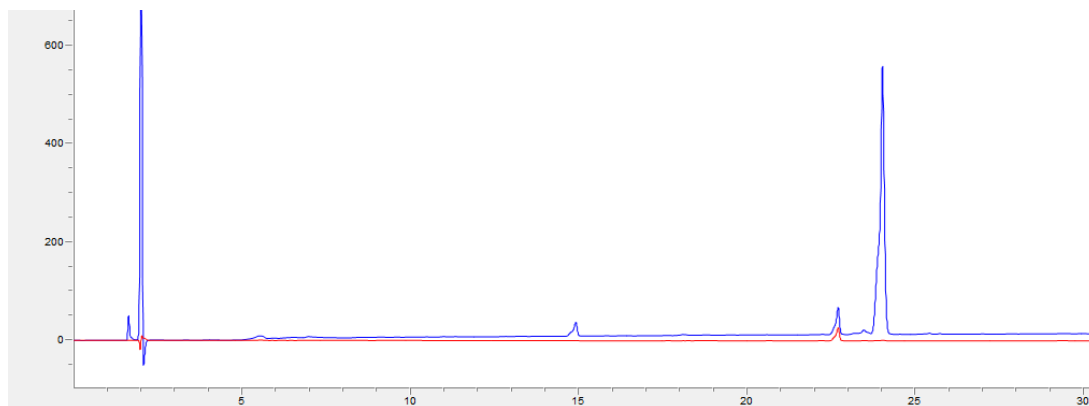
¹H NMR (600 MHz, 10% DMSO-*d*₆/D₂O) δ 7.63 (d, J = 7.9 Hz, 1H), 7.48 (d, J = 8.2 Hz, 1H), 7.25 (s, 1H), 7.23 (t, J = 8.2 Hz, 1H), 7.15 (t, J = 7.5 Hz, 1H), 5.92 (m, 1H), 5.31 (dd, J = 17.3, 1.5 Hz, 1H), 5.25 (dd, J = 10.5, 1.4 Hz, 1H), 4.58 (m, 3H), 4.20 (dd, J = 9.6, 4.9 Hz, 1H), 3.82 (d, J = 16.9 Hz, 1H), 3.72 (d, J = 16.9 Hz, 1H), 3.29 (dd, J = 14.6, 6.9 Hz, 1H), 3.19 (dd, J = 14.7, 7.8 Hz, 1H), 2.67 (p, J = 2.0 Hz, 1H), 2.40 (t, J = 7.4 Hz, 2H), 1.95 (s, 2H), 1.80 (m, 1H), 1.63 (m, 3H), 1.32 (m, 2H)

¹³C NMR (151 MHz, 10% DMSO-*d*₆/D₂O) δ 176.6, 176.1, 174.4, 174.1, 171.2, 136.1, 131.9, 126.9, 124.3, 121.9, 119.3, 118.3, 111.9, 109.1, 65.7, 55.0, 53.3, 42.6, 33.6, 30.5, 26.9, 24.5, 23.7, 21.8.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 5-40 % MeCN/H₂O 0.1% TFA over 30 min at room temperature. Product eluted at 22.8 min. Obtained 7.9 μ mole (16 % yield) as determined by NMR.

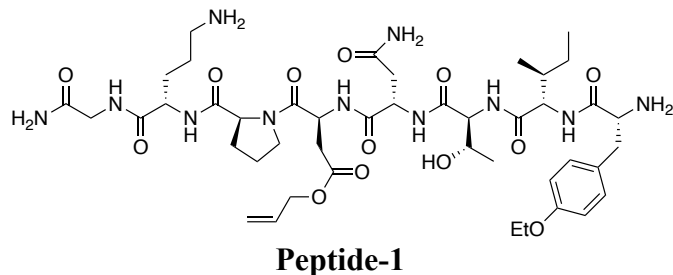
Analytical HPLC Gradient 5-40% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 24.0 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₁H₃₇N₃N₄O₆S 473.2356; Found 473.2430

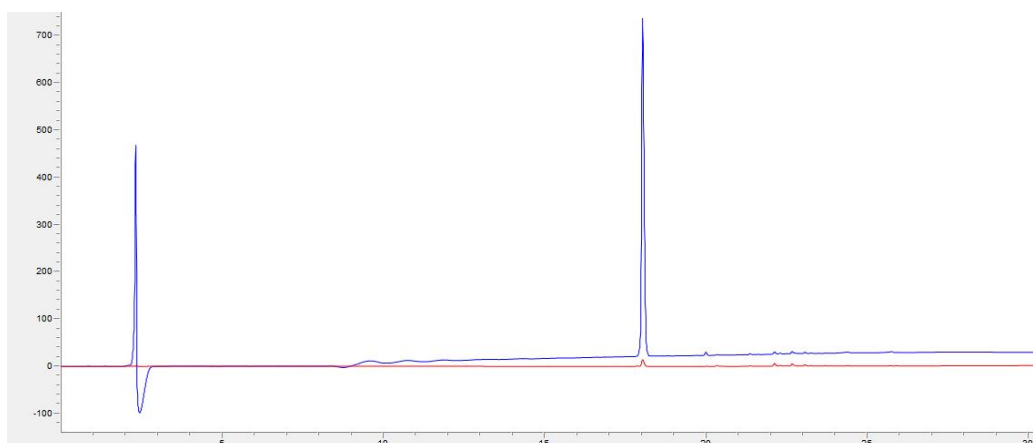
¹H NMR (600 MHz, 5% D₂O/CD₃OD) δ 5.91 (m, 1H), 5.28 (dd, J = 17.2, 1.6 Hz, 1H), 5.19 (dd, J = 10.4, 1.5 Hz, 1H), 4.55 (d, J = 5.6 Hz, 2H), 4.37 (dd, J = 8.1, 6.2 Hz, 1H), 4.31 (dd, J = 9.5, 4.8 Hz, 1H), 3.94 (d, J = 16.7 Hz, 1H), 3.79 (d, J = 16.7 Hz, 1H), 2.98 (dd, J = 12.9, 6.2 Hz, 1H), 2.84 (dd, J = 12.9, 8.1 Hz, 1H), 2.35 (t, J = 7.4 Hz, 2H), 1.99 (s, 3H), 1.84 (m, 1H), 1.69 (m, 1H), 1.62 (m, 2H), 1.38 (m, 2H), 1.32 (s, 9H).

¹³C NMR (151 MHz, 5% D₂O/CD₃OD) δ 175.5, 173.5, 172.34, 172.27, 170.1, 132.4, 116.9, 64.7, 54.5, 53.0, 42.4, 42.1, 33.3, 31.2, 29.9, 29.3, 25.0, 24.1, 21.1.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 5-40% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 20.6 min.

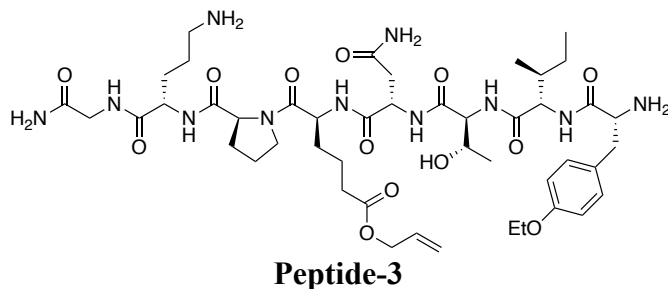
Analytical HPLC Gradient 2-60% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 18.0 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₄₄H₇₀N₁₁O₁₃ 960.5155; Found 960.5143

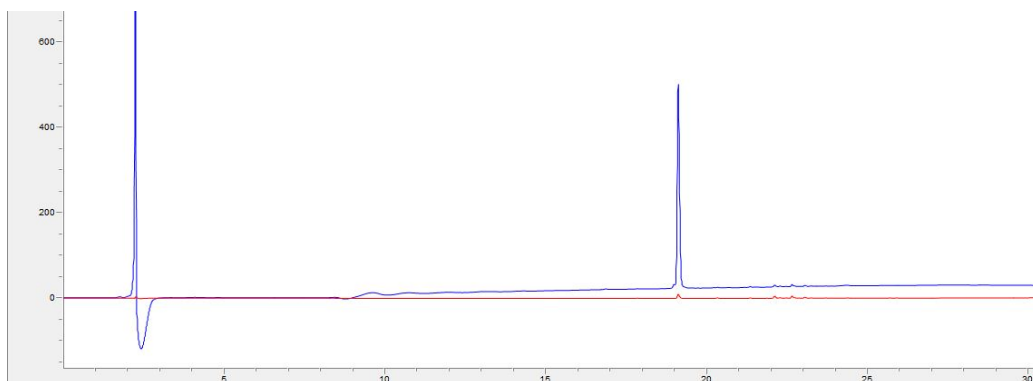
¹H NMR (600 MHz, 90% H₂O+D₂O) δ 8.46 (m, 2.7 H), 8.37 (m, 1.8 H), 8.28 (d, $J = 7.8$ Hz, 1.0 H), 7.58 (s, 0.9 H), 7.46 (s, 0.7 H), 7.21 (d, $J = 8.8$ Hz, 2.2 H), 7.07 (s, 0.6 H), 6.99 (d, $J = 8.7$ Hz, 2.1 H), 6.86 (s, 0.6 H), 5.94 (m, 0.8 H), 5.32 (m, 1.7 H), 5.02 (q, $J = 7.3$ Hz, 1.0 H), 4.62 (d, $J = 5.6$ Hz, 1.6 H), 4.41 (dd, $J = 8.7, 4.8$ Hz, 1.0 H), 4.32 (m, 2.1 H), 4.22 (dd, $J = 10.1, 5.8$ Hz, 1.2 H), 4.16 (m, 1.1 H), 4.11 (q, $J = 7.1$ Hz, 2.3 H), 3.96 (t, $J = 7.4$ Hz, 1.3 H), 3.91 (dd, $J = 5.9, 3.7$ Hz, 2.0 H), 3.72 (td, $J = 6.8, 2.6$ Hz, 2.0 H), 3.23 (dd, $J = 13.4, 5.8$ Hz, 1.1 H), 3.02 (m, 3.5 H), 2.95 (dd, $J = 16.6, 6.6$ Hz, 1.1 H), 2.78 (m, 1.7 H), 2.72 (m, 1.6 H), 2.27 (m, 1.1 H), 2.01 (m, 2.1 H), 1.94 (m, 2.2 H), 1.76 (m, 3.5 H), 1.56 (m, 1.1 H), 1.37 (t, $J = 7.0$ Hz, 3.2 H), 1.18 (d, $J = 6.5$ Hz, 3.09 H), 0.96 (m, 1.1 H), 0.70 (m, 7.5 H).

¹³C NMR (151 MHz, 90% H₂O+D₂O) δ 174.34, 174.26, 173.9, 173.7, 171.8, 171.5, 171.2, 169.9, 169.4, 157.6, 131.6, 130.6, 126.4, 118.6, 115.3, 67.1, 66.3, 64.4, 60.8, 59.2, 58.8, 54.7, 53.4, 50.4, 48.4, 48.0, 42.2, 39.1, 36.3, 36.1, 35.9, 35.4, 29.4, 27.6, 24.5, 24.4, 23.4, 18.8, 14.5, 13.9, 10.1.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 5-40% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 20.7 min.

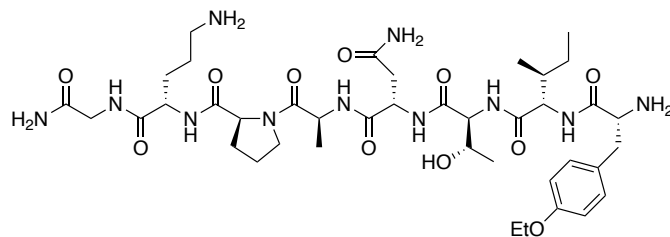
Analytical HPLC Gradient 2-60% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 19.1 min.



HRMS (ESI-TOF) m/z: [M + H]⁺ Calc'd for C₄₆H₇₄N₁₁O₁₃ 988.5468; Found 988.5446

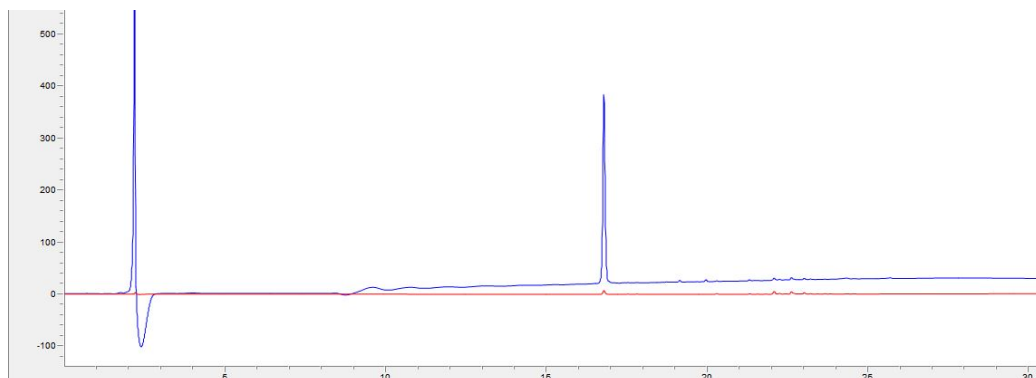
¹H NMR (600 MHz, 90% H₂O+D₂O) δ 8.58 (d, *J* = 6.7 Hz, 0.9 H), 8.45 (m, 1.8 H), 8.36 (d, *J* = 6.9 Hz, 1.0 H), 8.28 (d, *J* = 7.6 Hz, 1.0 H), 8.21 (d, *J* = 6.9 Hz, 0.9 H), 7.57 (s, 1.0 H), 7.47 (s, 0.9 H), 7.23 (d, *J* = 8.8 Hz, 2.2 H), 7.07 (s, 0.9 H), 6.99 (d, *J* = 8.8 Hz, 2.2H), 6.86 (s, 0.9H), 5.96 (m, 0.7 H), 5.31 (m, 2.3 H), 4.62 (d, *J* = 5.6 Hz, 1.6 H), 4.56 (m, 0.5 H), 4.41 (dd, *J* = 8.4, 5.9 Hz, 1.0 H), 4.32 (m, 2.1 H), 4.23 (dd, *J* = 10.2, 5.9 Hz, 1.1 H), 4.16 (m, 1.1 H), 4.11 (q, *J* = 7.0 Hz, 2.3 H), 3.95 (t, *J* = 7.4 Hz, 1.3 H), 3.92 (m, 2.1 H), 3.80 (dt, *J* = 10.4, 6.7 Hz, 1.1 H), 3.62 (dt, *J* = 10.6, 7.1 Hz, 1.1 H), 3.24 (dd, *J* = 13.6, 5.9 Hz, 1.2 H), 3.02 (m, 3.6 H), 2.79 (dd, *J* = 15.6, 5.6 Hz, 1.2 H), 2.70 (dd, *J* = 15.5, 8.0 Hz, 1.1 H), 2.46 (m, 2.1 H), 2.30 (m, 1.2 H), 2.02 (m, 2.6 H), 1.90 (m, 2.5 H), 1.85 – 1.62 (m, 8.1 H), 1.57 (m, 1.3 H), 1.37 (t, *J* = 7.0 Hz, 3.4 H), 1.18 (d, *J* = 6.5 Hz, 3.4 H), 0.95 (m, 1.2 H), 0.76 – 0.67 (m, 7.8 H).

¹³C NMR (151 MHz, 90% H₂O+D₂O) δ 175.6, 174.4, 174.3, 174.0, 173.7, 171.6, 171.8, 171.2, 169.5, 157.6, 131.9, 130.6, 126.4, 118.4, 115.3, 67.1, 65.8, 64.4, 60.5, 59.2, 58.9, 54.7, 53.4, 51.6, 50.3, 47.9, 42.2, 39.1, 36.1, 35.9, 33.1, 29.4, 27.7, 24.7, 24.4, 23.4, 20.4, 18.8, 14.6, 13.9, 10.1.



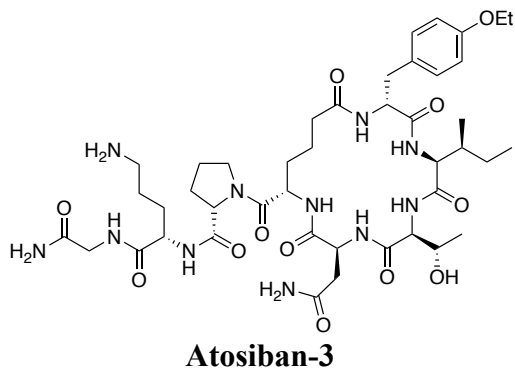
Purification: Peptide was purified on preparative HPLC using a focused gradient of 5-40% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 15.8 min.

Analytical HPLC Gradient 2-60% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 16.8 min.



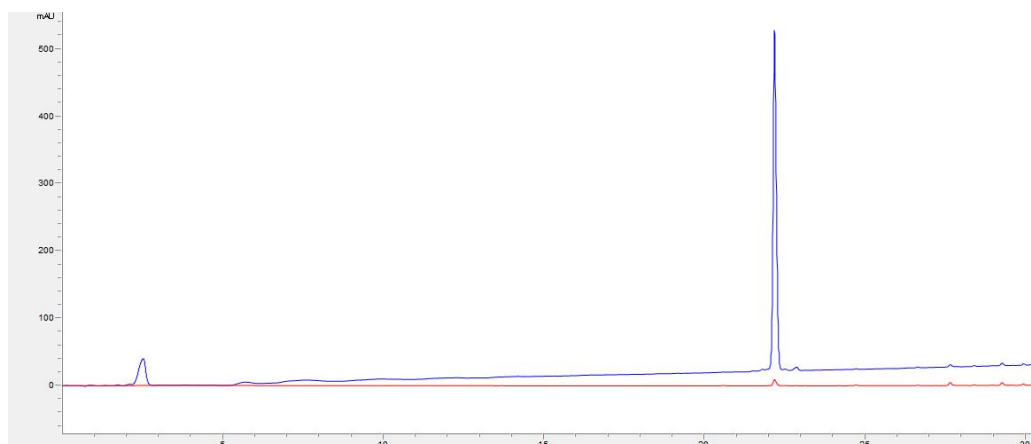
HRMS (ESI-TOF) m/z : $[M + Na]^+$ Calc'd for C₄₀H₆₅N₁₁O₁₁Na 898.4763; Found 898.4763

¹H NMR (600 MHz, 90% H₂O+D₂O) δ 8.60 (d, $J = 7.0$ Hz, 0.9 H), 8.48 (d, $J = 7.8$ Hz, 0.8 H), 8.45 (t, $J = 5.9$ Hz, 1.0 H), 8.37 (d, $J = 7.0$ Hz, 0.9 H), 8.31 (d, $J = 7.8$ Hz, 1.0 H), 8.21 (d, $J = 5.9$ Hz, 0.9 H), 7.58 (s, 1.0 H), 7.47 (s, 0.8 H), 7.22 (d, $J = 8.2$ Hz, 2.1 H), 7.08 (s, 0.9 H), 6.99 (d, $J = 8.4$ Hz, 2.1 H), 6.87 (s, 0.8H), 4.57 (m, 1.6 H), 4.41 (m, 1.4 H), 4.33 (m, 2.3 H), 4.22 (m, 1.2 H), 4.16 (m, 1.0 H), 4.12 (q, $J = 7.2$ Hz, 2.3 H), 3.97 (t, $J = 7.1$ Hz, 1.3 H), 3.92 (m, 2.0 H), 3.79 (m, 1.0 H), 3.64 (m, 1.0 H), 3.23 (dd, $J = 13.5, 6.5$ Hz, 1.1 H), 3.02 (m, 3.3 H), 2.78 (dd, $J = 15.9, 5.4$ Hz, 1.1 H), 2.70 (dd, $J = 15.8, 8.2$ Hz, 1.1 H), 2.39 (q, $J = 7.0$ Hz, 0.5 H), 2.30 (dq, $J = 15.0, 7.1$ Hz, 1.1 H), 2.02 (m, 2.6 H), 1.90 (m, 2.2 H), 1.76 (m, 3.7 H), 1.57 (m, 1.3 H), 1.37 (t, $J = 7.0$ Hz, 3.3 H), 1.33 (d, $J = 7.0$ Hz, 2.7 H), 1.19 (d, $J = 6.5$ Hz, 3.2 H), 0.95 (s, 1.2 H), 0.70 (m, 7.4 H).



Purification: Peptide was purified on preparative HPLC using a focused gradient of 10-30% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 22.4 min. Obtained 2.28 μmole of peptide as determined by NMR.

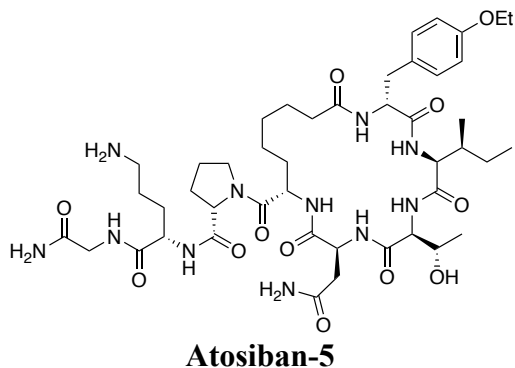
Analytical HPLC Gradient 5-40% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 20.3 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₄₃H₆₈N₁₁O₁₂ 930.5049; Found 930.5059

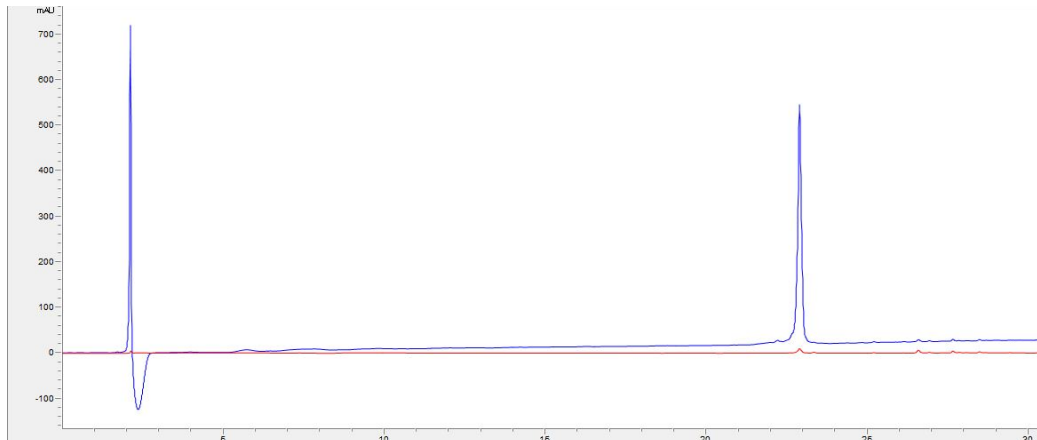
¹H NMR (600 MHz, 90% H₂O/D₂O) δ 8.63 (d, J = 6.9 Hz, 0.9 H), 8.48 (d, J = 6.6 Hz, 1 H), 8.45 (m, 1.8 H), 8.07 (dd, J = 10.7, 7.6 Hz, 1.7H), 7.79 (d, J = 8.1 Hz, 0.9H), 7.62 (s, 1.5 H), 7.49 (s, 0.6 H), 7.22 (d, J = 8.8 Hz, 2.1 H), 7.09 (s, 0.7 H), 6.96 (d, J = 8.8 Hz, 2.2 H), 6.90 (s, 0.5 H), 4.63 (m, 0.9H), 4.55 (m, 0.7 H), 4.47 (m, 1.0 H), 4.41 (m, 2.0 H), 4.35 (m, 1.0 H), 4.19 (m, 1.1 H), 4.10 (m, 3.2 H), 3.93 (dd, J = 5.9, 2.0 Hz, 2.1 H), 3.77 (m, 1.0 H), 3.64 (dt, J = 10.3, 7.0 Hz, 1.0 H), 3.05 (m, 3.4 H), 2.93 (dd, J = 13.4, 10.1 Hz, 1.1 H), 2.88 (dd, J = 15.5, 6.4 Hz, 1.2 H), 2.77 (dd, J = 15.6, 7.9 Hz, 1.0 H), 2.29 (m, 3.3 H), 2.03 (m, 2.5 H), 1.91 (m, 2.3 H), 1.86 – 1.71 (m, 6.8 H), 1.61 (t, J = 7.7 Hz, 2.0 H), 1.36 (t, J = 7.0 Hz, 3.2H), 1.16 (d, J = 6.3 Hz, 3.3H), 1.03 (m, 1.1 H), 0.81 (m, 1.1 H), 0.75 (t, J = 7.2 Hz, 3.3 H), 0.52 (d, J = 6.9 Hz, 3.1 H).

¹³C NMR (151 MHz, D₂O) δ 176.2, 174.8, 174.4, 173.9, 173.9, 173.8, 173.5, 171.8, 171.6, 170.8, 157.1, 130.4, 128.7, 115.1, 66.9, 64.5, 60.5, 58.8, 58.3, 55.7, 53.2, 51.7, 51.2, 48.0, 42.0, 38.8, 35.9, 35.5, 34.8, 34.6, 29.8, 29.3, 27.7, 24.7, 23.7, 23.2, 21.9, 18.6, 14.7, 13.9, 10.9.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 15-30% MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 20.0 min. Obtained 1.19 μmole of peptide as determined by NMR.

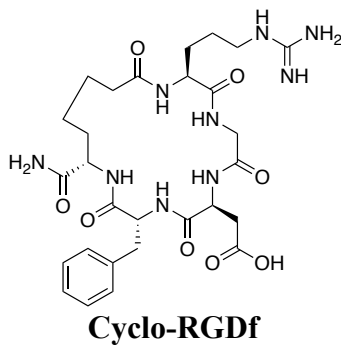
Analytical HPLC Gradient 5-40% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 20.3 min.



HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₄₅H₇₂N₁₁O₁₂ 958.5362; Found 958.5381

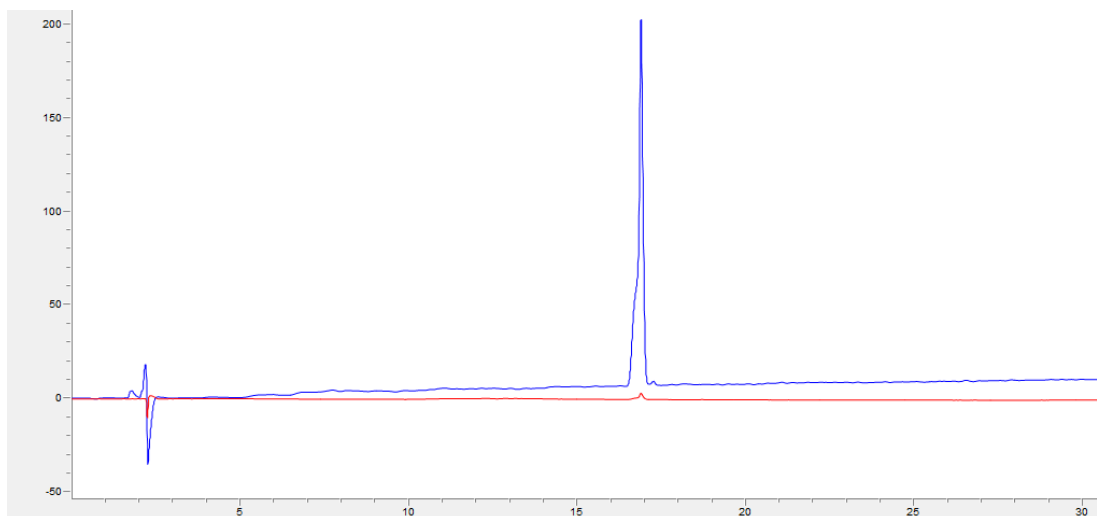
¹H NMR (600 MHz, 90% H₂O+D₂O) δ 8.59 (d, J = 6.9 Hz, 0.9 H), 8.49 (d, J = 6.6 Hz, 0.9 H), 8.45 (t, J = 5.9 Hz, 1.0 H), 8.39 (d, J = 6.2 Hz, 0.9 H), 8.01 (m, 1.7 H), 7.82 (d, J = 7.3 Hz, 0.8 H), 7.60 (s, 1.2 H), 7.48 (s, 0.8 H), 7.22 (d, J = 8.8 Hz, 2.2 H), 7.08 (s, 0.8 H), 6.95 (d, J = 8.7 Hz, 2.2 H), 6.90 (s, 0.6 H), 4.61 (m, 1.5 H), 4.57 (m, 0.9 H), 4.41 (m, 1.0 H), 4.34 (m, 1.2 H), 4.27 (m, 1.0 H), 4.20 (m, 2.1 H), 4.10 (q, J = 7.0 Hz, 2.3 H), 3.92 (dd, J = 6.1, 2.9 Hz, 2.1H), 3.77 (m, 1.0 H), 3.62 (dt, J = 10.3, 6.8 Hz, 1.1 H), 3.05 (m, 3.6 H), 2.95 (dd, J = 13.4, 9.8 Hz, 1.1 H), 2.83 (dd, J = 15.5, 6.7 Hz, 1.1 H), 2.73 (dd, J = 15.4, 7.6 Hz, 1.1 H), 2.36 (dd, J = 14.3, 7.6 Hz, 1.0 H), 2.30 (m, 1.3 H), 2.19 (dt, J = 14.1, 6.8 Hz, 1.2 H), 2.02 (m, 2.4 H), 1.90 (m, 2.4 H), 1.86 – 1.69 (m, 5.9 H), 1.63 (m, 1.2 H), 1.56 (t, J = 7.1 Hz, 2.2H), 1.36 (t, J = 7.0 Hz, 3.7 H), 1.32 (s, 3.0 H), 1.23 (d, J = 6.5 Hz, 3.5 H), 1.06 (m, 1.1 H), 0.86 (m, 1.2 H), 0.75 (t, J = 7.4 Hz, 3.4 H), 0.51 (d, J = 6.9 Hz, 3.0 H).

¹³C NMR (151 MHz, 90% H₂O+D₂O) δ 177.5, 174.7, 174.5, 174.0, 173.6, 171.8, 171.0, 157.0, 130.4, 128.7, 115.1, 67.3, 64.5, 60.5, 59.3, 58.5, 55.9, 53.3, 51.6, 50.9, 47.8, 42.2, 39.1, 36.1, 35.5, 35.2, 34.9, 30.1, 29.3, 27.8, 27.4, 25.0, 24.7, 23.9, 23.8, 23.3, 19.0, 14.8, 14.0, 11.0.



Purification: Peptide was purified on preparative HPLC using a focused gradient of 2-10 % MeCN/H₂O 0.1% TFA over 30 min at 60 °C. Product eluted at 16.1 min. Obtained 7.54 μmole (15 % yield) of isolated peptide from PyAOP cyclization route as determined by NMR. Obtained 5.9 μmole (24 %) of isolated peptide from photochemical cyclization route as determined by NMR.

Analytical HPLC Gradient 2-25% MeCN/H₂O (0.1% TFA) at 60 °C over 30 min (blue 214 nm, red 254 nm). Product eluted at 16.9 min.

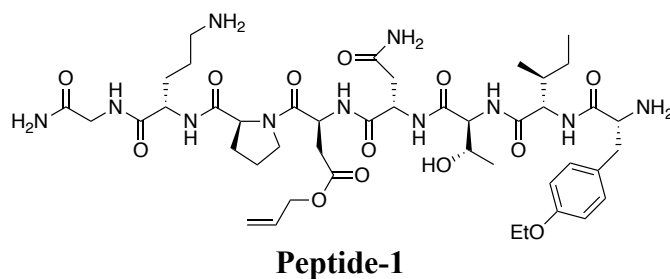


HRMS (ESI-TOF) m/z : $[M + H]^+$ Calc'd for C₂₈H₄₂N₉O₈ 632.3156; Found 632.3145

¹H NMR (600 MHz, 90% H₂O+D₂O) δ 8.41 (d, J = 7.8 Hz, 3H), 8.32 (t, J = 5.7 Hz, 1H), 7.83 (d, J = 6.2 Hz, 1H), 7.56 (s, 1H), 7.37 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.4 Hz, 1H), 7.26 (d, J = 8.4 Hz, 2H), 7.18 (t, J = 5.8 Hz, 1H), 7.08 (s, 1H), 4.64 (m, 1H), 4.51 (q, J = 6.8 Hz, 1H), 4.22 (m, 2H), 3.97 (dd, J = 17.0, 5.7 Hz, 1H), 3.88 (dd, J = 16.9, 5.7 Hz, 1H), 3.34 (dd, J = 14.1, 5.0 Hz, 1H), 3.20 (q, J = 6.7 Hz, 2H), 2.94 (dd, J = 14.1, 10.4 Hz, 1H), 2.56 (d, J = 6.7 Hz, 2H), 2.34 (m, 2H), 1.90 – 1.70 (m, 5H), 1.70 – 1.54 (m, 4H), 1.37 (m, 2H).

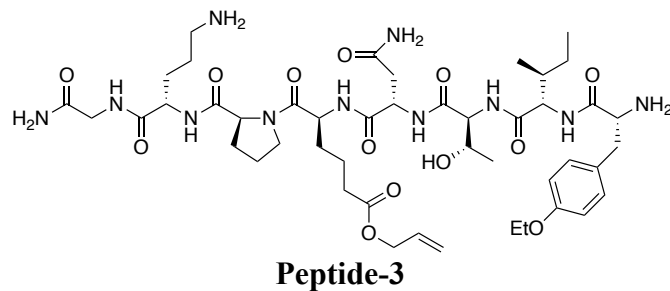
¹³C NMR (151 MHz, 90% H₂O+D₂O) δ 177.1, 176.9, 174.5, 173.6, 173.4, 172.7, 171.2, 156.9, 136.8, 129.2, 128.7, 127.1, 55.2, 54.7, 54.0, 50.5, 42.5, 40.6, 36.3, 35.2, 34.7, 30.0, 27.2, 24.4, 24.1, 24.0.

¹H NMR Assignment of Atosiban Peptides



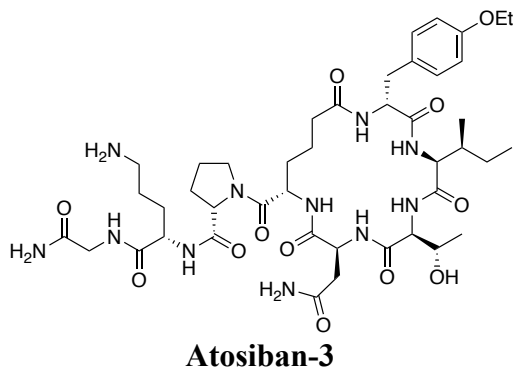
| Amino Acid | δ , H ^N | ³ J _{αN} | δ , H _{α} | δ , H _{β} | δ , H _{γ} | δ , H _{δ} | Other |
|------------|---------------------------|--|---|--|---|---|--|
| Gly | 8.37 | ^a - | 3.91 | N/A | N/A | N/A | N/A |
| Orn | 8.46 | ^a - | 4.36 | 1.93 / 1.81 | 1.93 / 1.81 | 3.03 | N/A |
| Pro | N/A | N/A | 4.42 | 2.31 | 2.02, 1.94 | 3.73 | N/A |
| Asp(Allyl) | 8.45 | ^a - | 4.69 | 2.75 | N/A | N/A | 5.94, 5.32, 4.63 |
| Asn | 8.46 | ^a - | 5.03 | 2.96, 2.77 | N/A | N/A | N/A |
| Thr | 8.28 | 7.8 | 4.33 | 4.16 | 1.18 (<i>J</i> = 6.5) | N/A | N/A |
| Ile | 8.37 | ^a - | 3.97 | 1.58 | 0.97, 0.72 0.72 | 0.72 | N/A |
| D-Tyr(Oet) | N/A | ^a - | 4.36 | 3.23, 3.02 | N/A | N/A | 1.37 (<i>J</i> = 7.0) 4.11 (<i>J</i> = 7.1) 6.99, (<i>J</i> = 8.7) 7.21, (<i>J</i> = 8.8) |

Table 1. Proton assignments for above peptide using TOCSY data for chemical shifts. Data was recorded in 50 mM sodium phosphate (pH 4.0), 100 μ M TSP, 90% H₂O/D₂O or 100% D₂O, at 298.1 K. ^aCoupling values were not obtained due to spectral overlap.



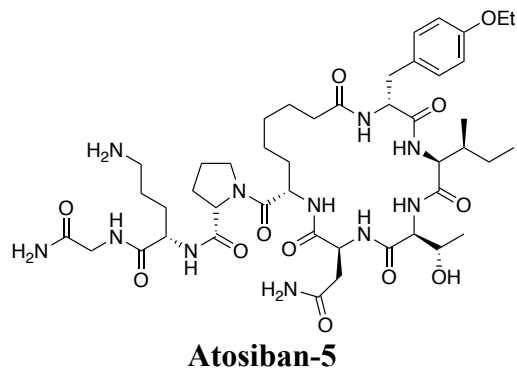
| Amino Acid | δ , H ^N | $^3J_{\alpha N}$ | δ , H $_{\alpha}$ | δ , H $_{\beta}$ | δ , H $_{\gamma}$ | δ , H $_{\delta}$ | Other |
|------------|---------------------------|------------------|--------------------------|-------------------------|--------------------------|--------------------------|--|
| Gly | 8.44 | ^a - | 3.91 | N/A | N/A | N/A | N/A |
| Orn | 8.57 | 6.7 | 4.33 | 1.91 / 1.81 | 1.91 / 1.81 | 3.02 | N/A |
| Pro | N/A | N/A | 4.41 | 2.30 | 2.03, 1.90 | 3.80, 3.65 | N/A |
| Asp(Allyl) | 8.21 | 6.9 | 4.58 | 2.43 | 1.68 | 1.81 | 5.97, 5.33, 4.62 |
| Asn | 8.44 | ^a - | 4.73 | 2.79, 2.71 | N/A | N/A | N/A |
| Thr | 8.28 | 7.6 | 4.33 | 4.16 | 1.19 ($J = 6.4$) | N/A | N/A |
| Ile | 8.36 | 6.9 | 3.96 | 1.56 | 0.96, 0.72 0.71 | 0.71 | N/A |
| D-Tyr(OEt) | N/A | N/A | 4.23 | 3.24, 3.02 | N/A | N/A | 1.38 ($J = 7.0$) 4.12 ($J = 7.0$) 7.00, ($J = 8.8$) 7.22, ($J = 8.7$) |

Table 2. Proton assignments for above peptide using TOCSY data for chemical shifts. Data was recorded in 50 mM sodium phosphate (pH 4.0), 100 μ M TSP, 90% H₂O/D₂O or 100% D₂O, at 298.1 K. ^aCoupling values were not obtained due to spectral overlap.



| Amino Acid | δ , H ^N | ³ J _{αN} | δ , H _{α} | δ , H _{β} | δ , H _{γ} | δ , H _{δ} | Other |
|------------|---------------------------|--|---|--|---|---|--|
| Gly | 8.45 | ^a - | 3.93 | N/A | N/A | N/A | N/A |
| Orn | 8.63 | 6.9 | 4.36 | 1.93 / 1.82 | 1.93 / 1.82 | 3.04 | N/A |
| Pro | N/A | N/A | 4.42 | 2.31 | 2.03, 1.91 | 3.77, 3.64 | N/A |
| Linker | 8.07 | 7.2 | 4.55 | 2.29 | 1.63 | 1.80, 1.77 | N/A |
| Asn | 8.48 | 6.6 | 4.47 | 2.87, 2.77 | N/A | N/A | N/A |
| Thr | 7.79 | 8.1 | 4.41 | 4.18 | 1.17 (<i>J</i> = 6.4) | N/A | N/A |
| Ile | 8.06 | 8.1 | 4.12 | 1.83 | 1.04, 0.52 (<i>J</i> = 6.9) | 0.75 (<i>J</i> = 6.9) | N/A |
| D-Tyr(OEt) | 8.43 | ^a - | 4.60 | 3.05, 2.93 | N/A | N/A | 1.36 (<i>J</i> = 7.0) 4.11 6.96, (<i>J</i> = 8.7) 7.22, (<i>J</i> = 8.6) |

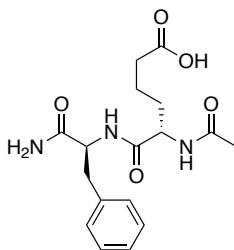
Table 3. Proton assignments for above peptide using TOCSY data for chemical shifts. Data was recorded in 50 mM sodium phosphate (pH 4.0), 100 μ M TSP, 90% H₂O/D₂O or 100% D₂O, at 298.1 K. ^aCoupling values were not obtained due to spectral overlap.



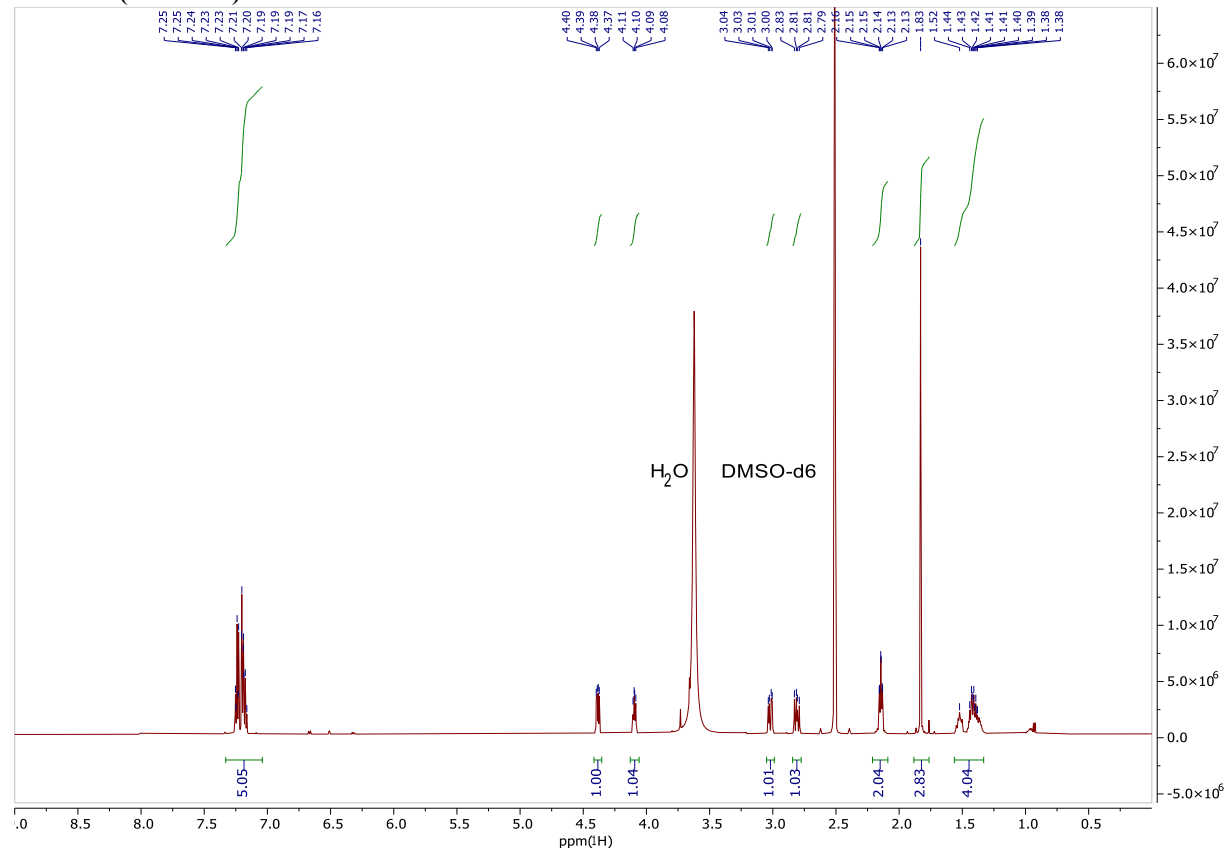
| Amino Acid | δ , H ^N | $^3J_{\alpha N}$ | δ , H α | δ , H β | δ , H γ | δ , H δ | Other |
|------------|---------------------------|------------------|-----------------------|----------------------|--------------------------------|-----------------------|---|
| Gly | 8.44 | 6.0 | 3.92 | N/A | N/A | N/A | N/A |
| Orn | 8.59 | 6.9 | 4.35 | 1.93 / 1.81 | 1.93 / 1.81 | 3.04 | N/A |
| Pro | N/A | N/A | 4.41 | 2.30 | 2.02, 1.91 | 3.77, 3.62 | N/A |
| Linker | 8.49 | 6.6 | 4.57 | 1.74, 1.64 | 1.56 | 1.31 | 2.35, 2.20 |
| Asn | 8.00 | 7.3 | 4.61 | 2.83, 2.73 | N/A | N/A | N/A |
| Thr | 7.82 | 7.5 | 4.28 | 4.20 | 1.24 ($J=6.5$) | N/A | N/A |
| Ile | 8.01 | 8.7 | 4.21 | 1.84 | 1.08, 0.86 0.52 ($J=6.7$) | 0.76 ($J=7.4$) | N/A |
| D-Tyr(Oet) | 8.39 | 6.2 | 4.63 | 3.04, 2.95 | N/A | N/A | 1.37 ($J=7.0$) 4.10, ($J=7.1$) 6.95, ($J=8.8$) 7.22, ($J=8.7$) |

Table 4. Proton assignments for above peptide using TOCSY data for chemical shifts. Data was recorded in 50 mM sodium phosphate (pH 4.0), 100 μ M TSP, 90% H₂O/D₂O or 100% D₂O, at 298.1 K.

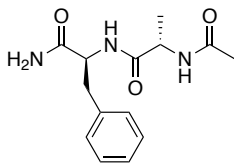
¹H NMR Spectra of Peptides



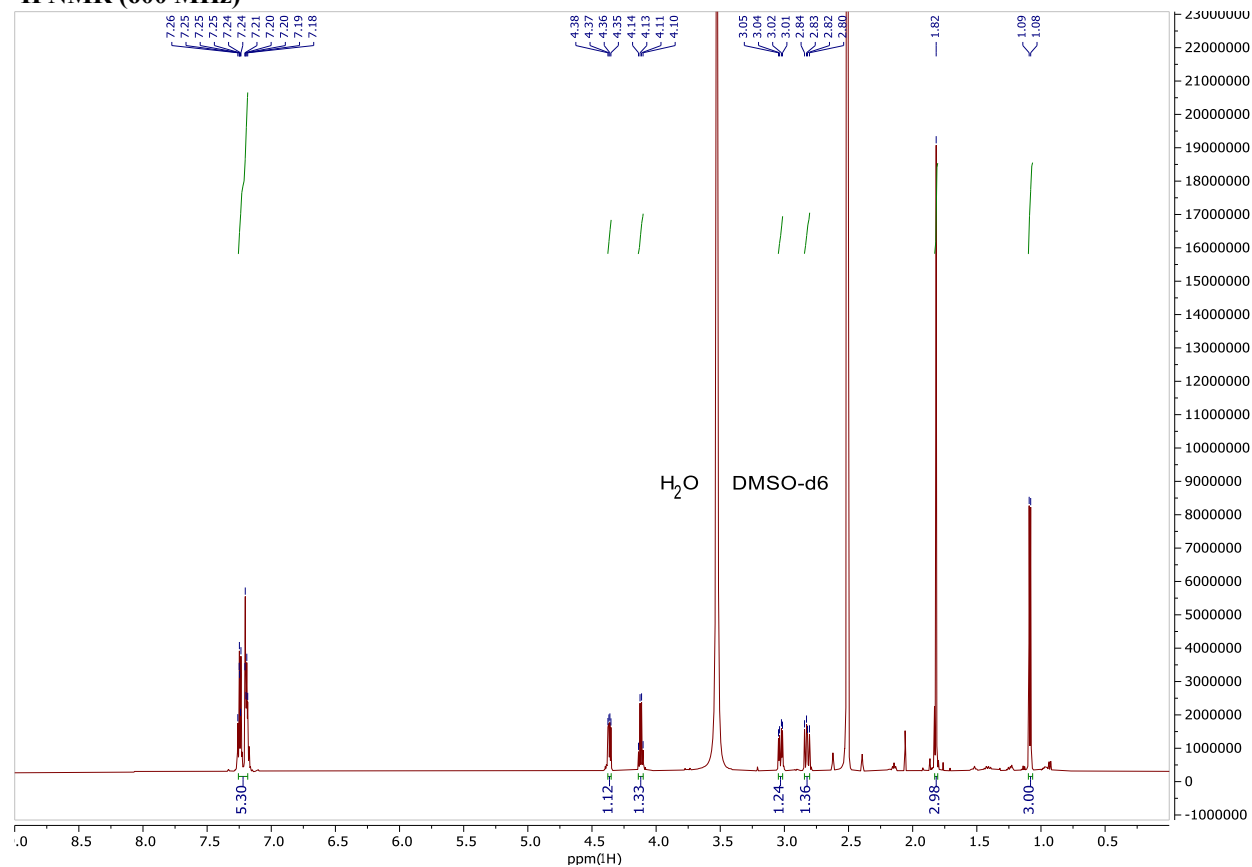
¹H NMR (600 MHz)



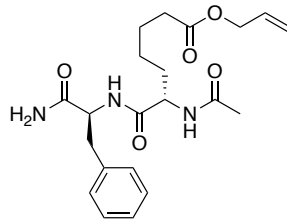
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



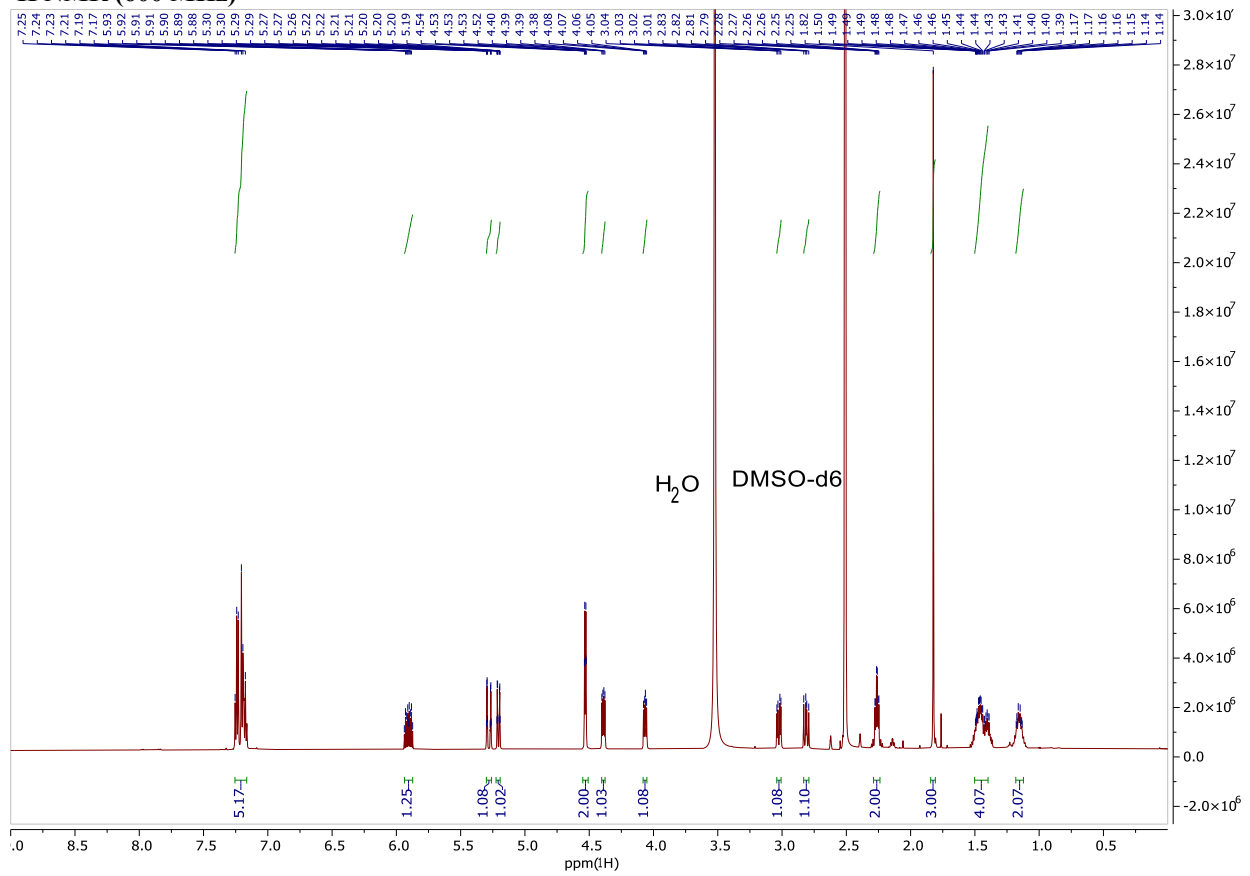
¹H NMR (600 MHz)



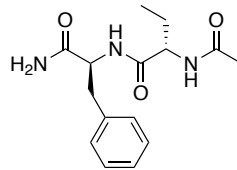
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



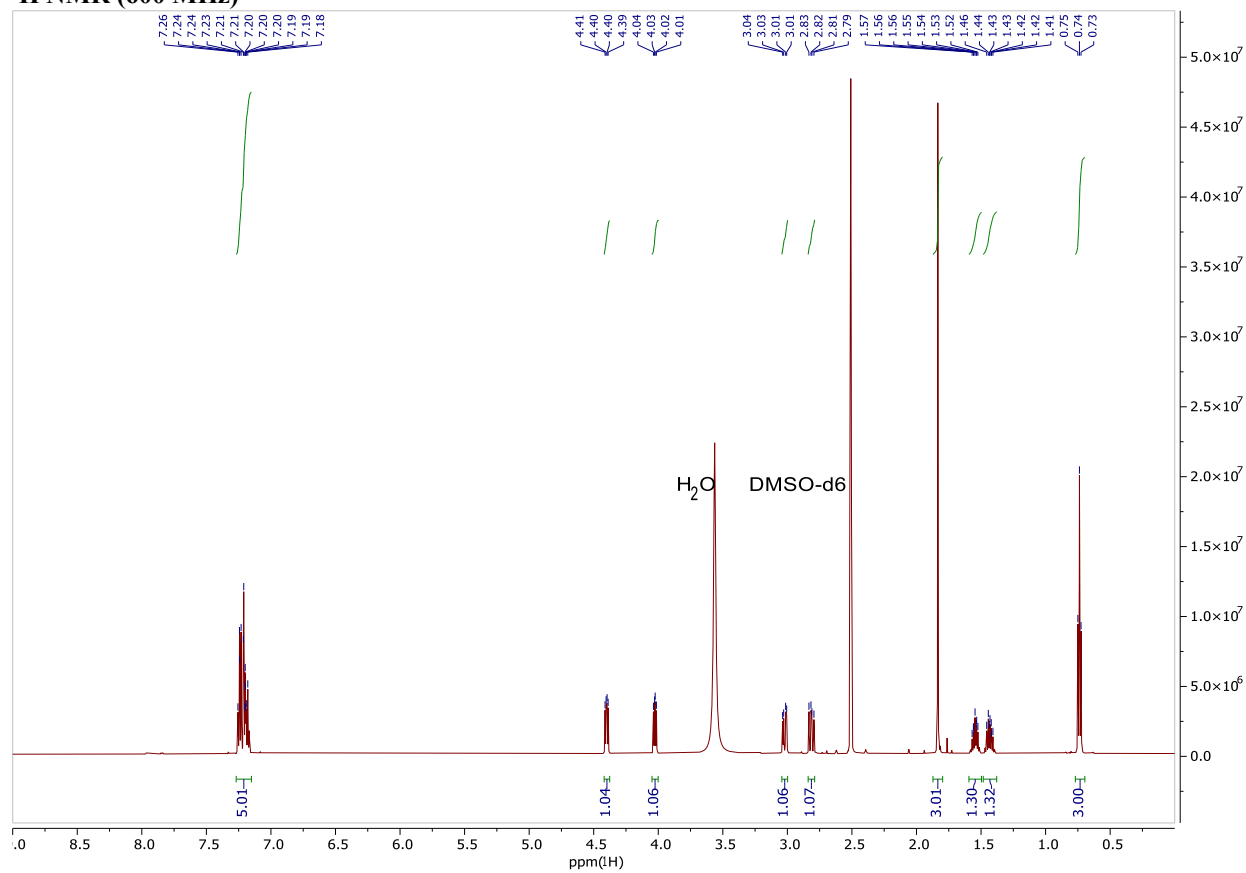
¹H NMR (600 MHz)



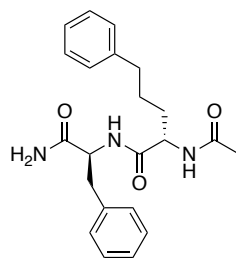
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



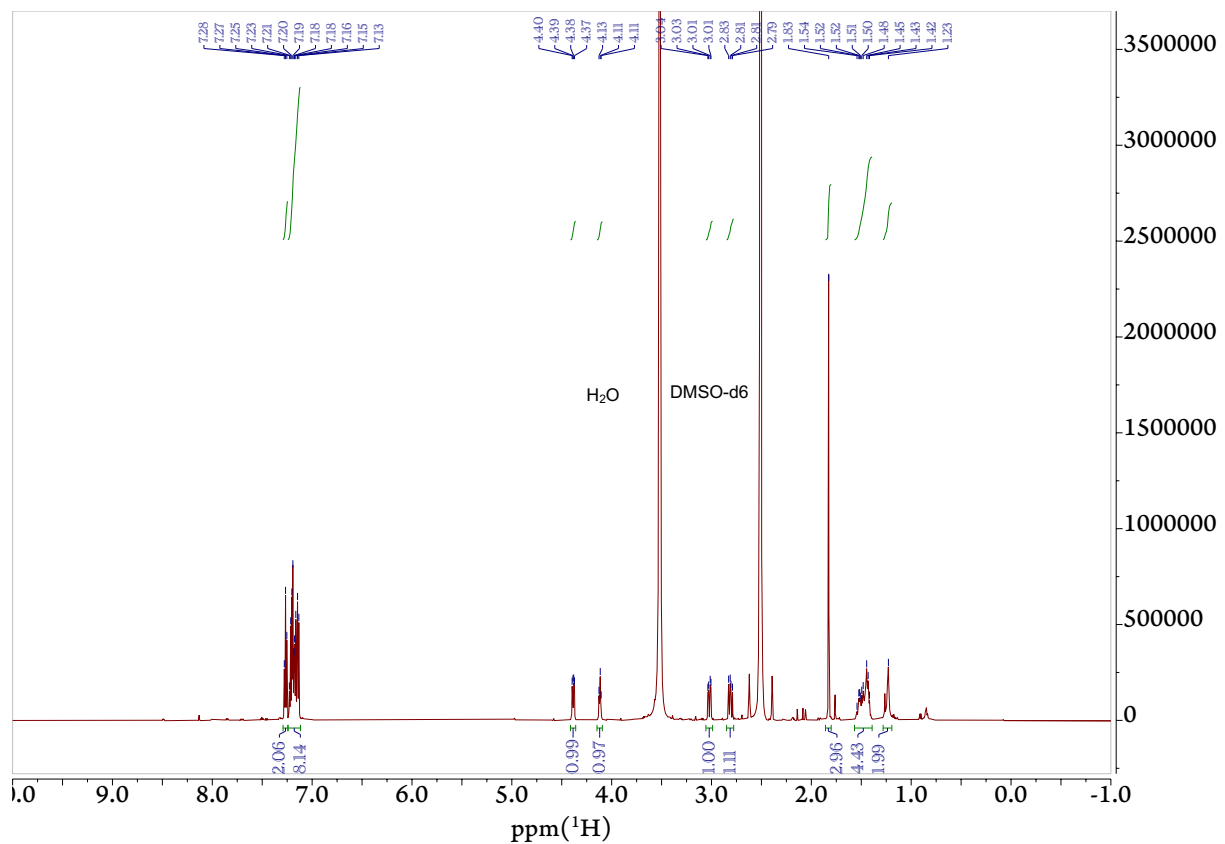
¹H NMR (600 MHz)



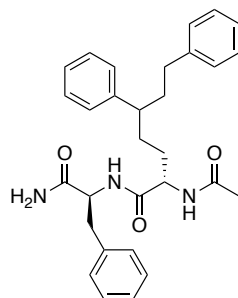
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



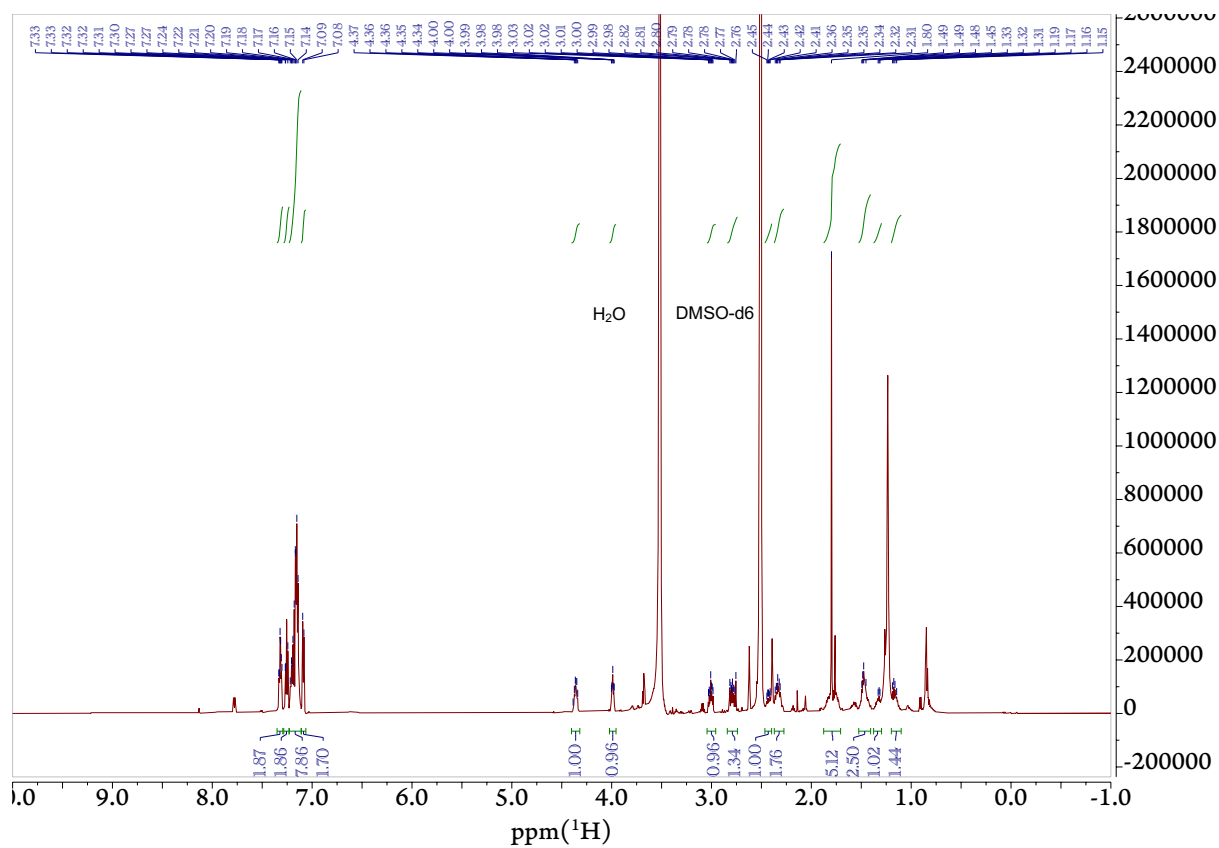
¹H NMR (600 MHz)



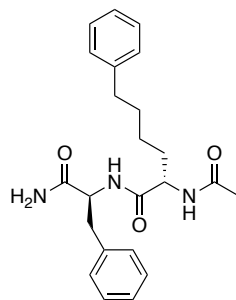
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



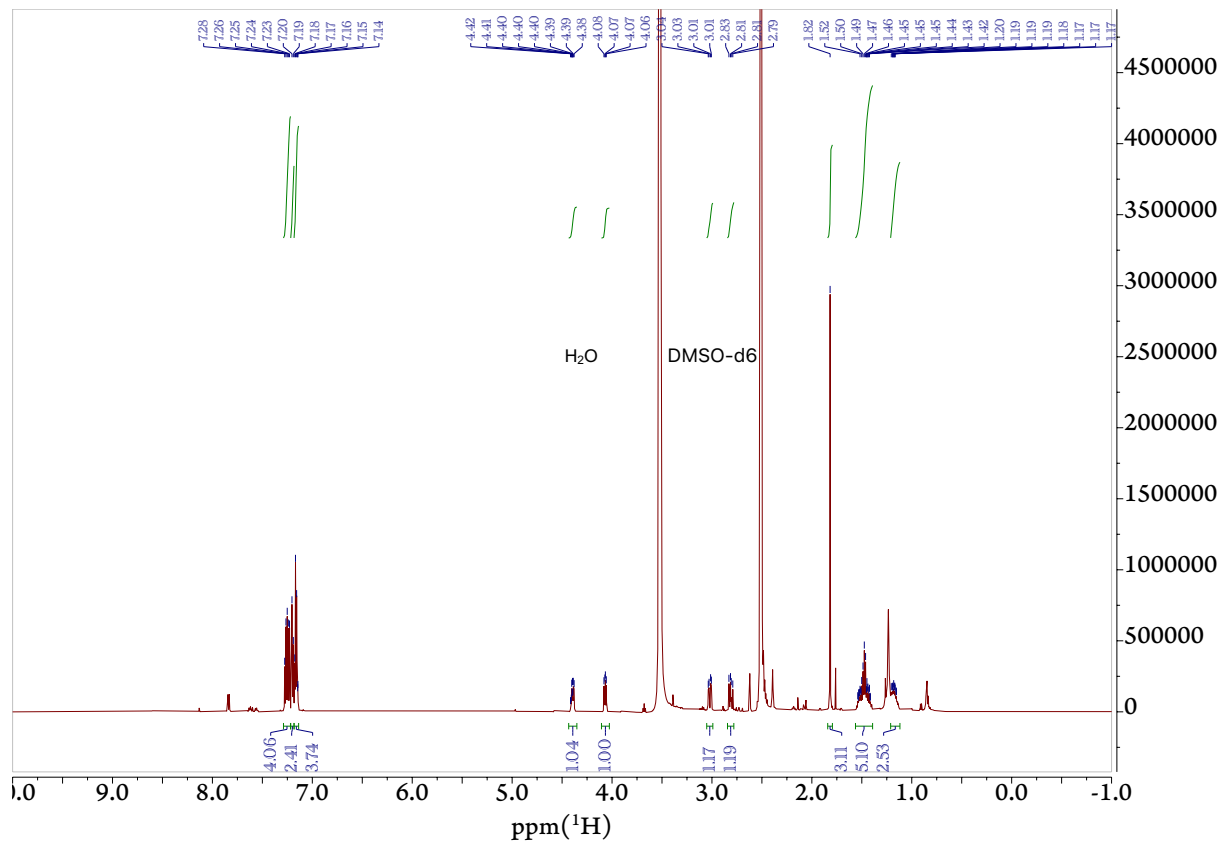
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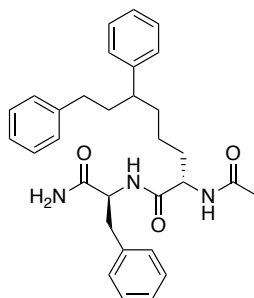
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



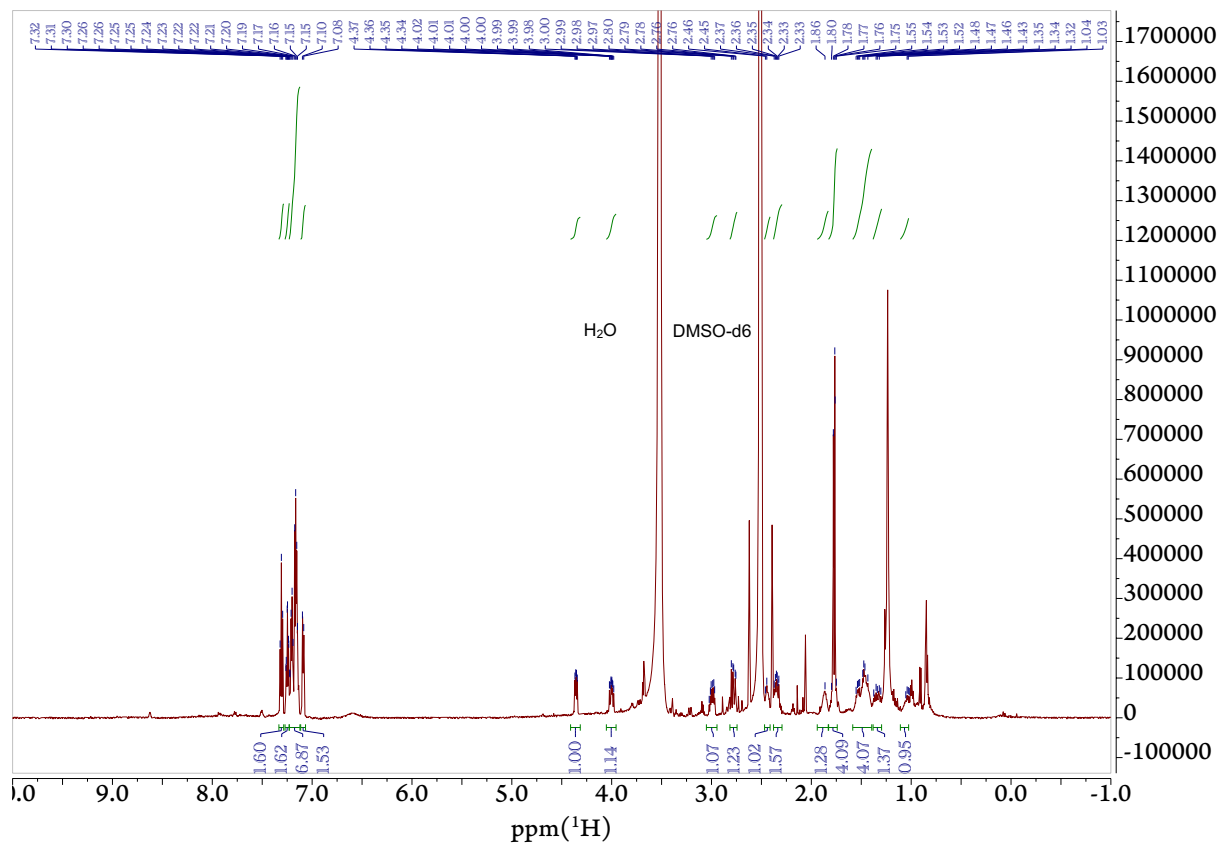
¹H NMR (600 MHz)



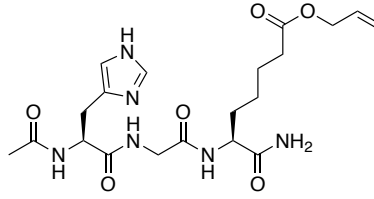
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



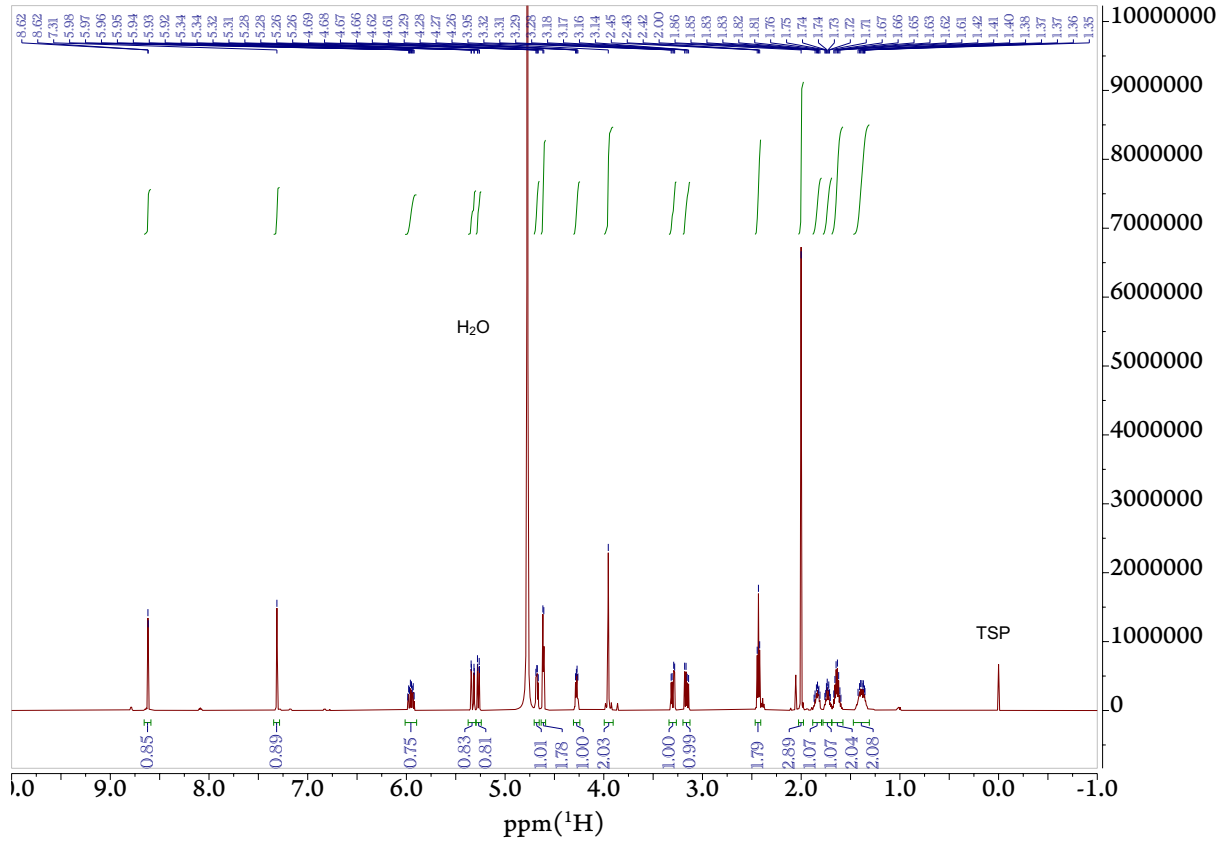
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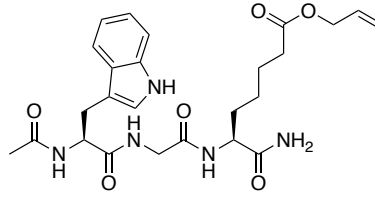
¹H NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



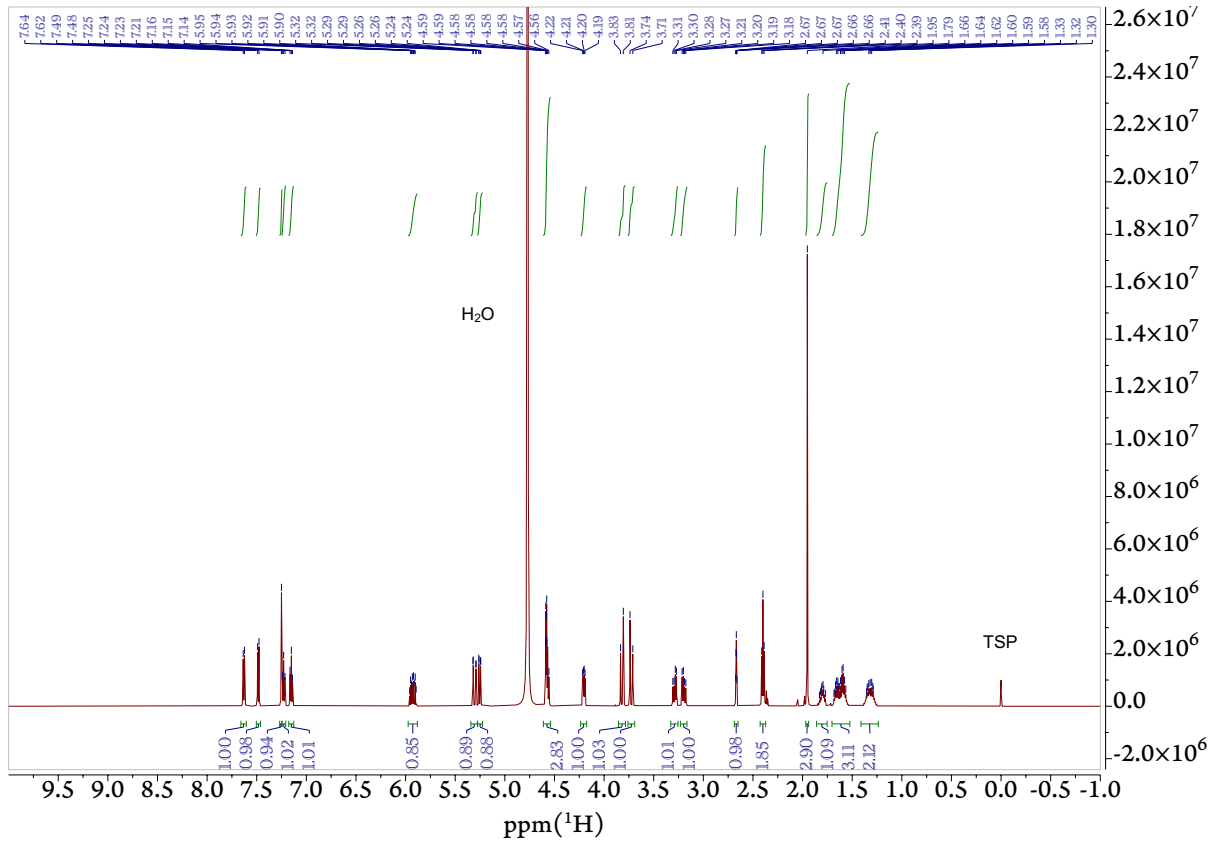
¹H NMR (600 MHz)



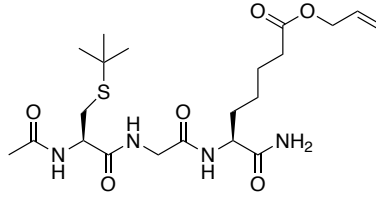
¹H NMR of above peptide in D₂O with 100 μM TSP at 298.1 K



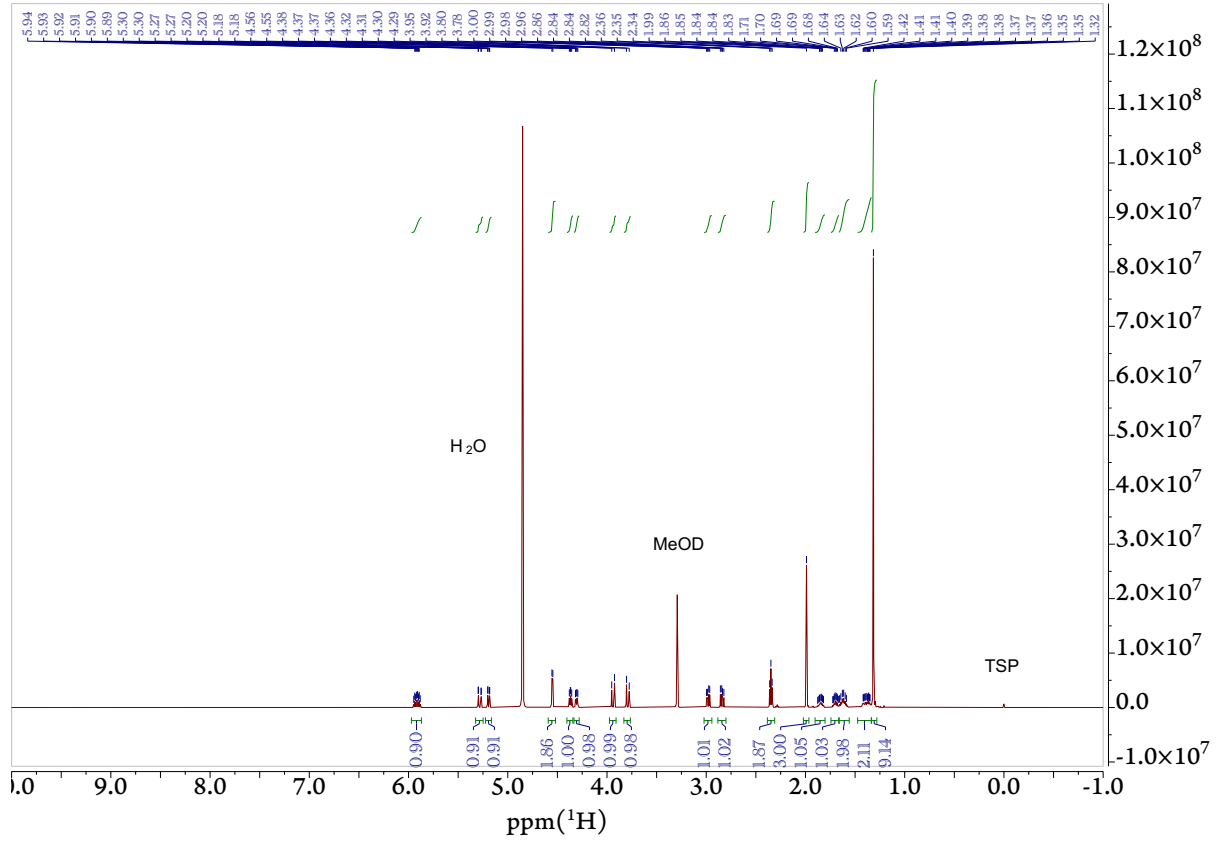
¹H NMR (600 MHz)



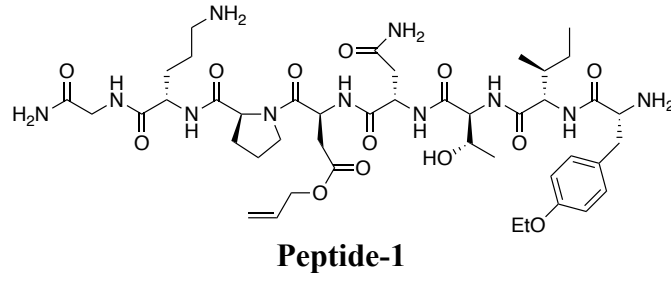
¹H NMR of above peptide in 10 % DMSO-*d*₆ /D₂O with 100 μM TSP at 298.1 K.



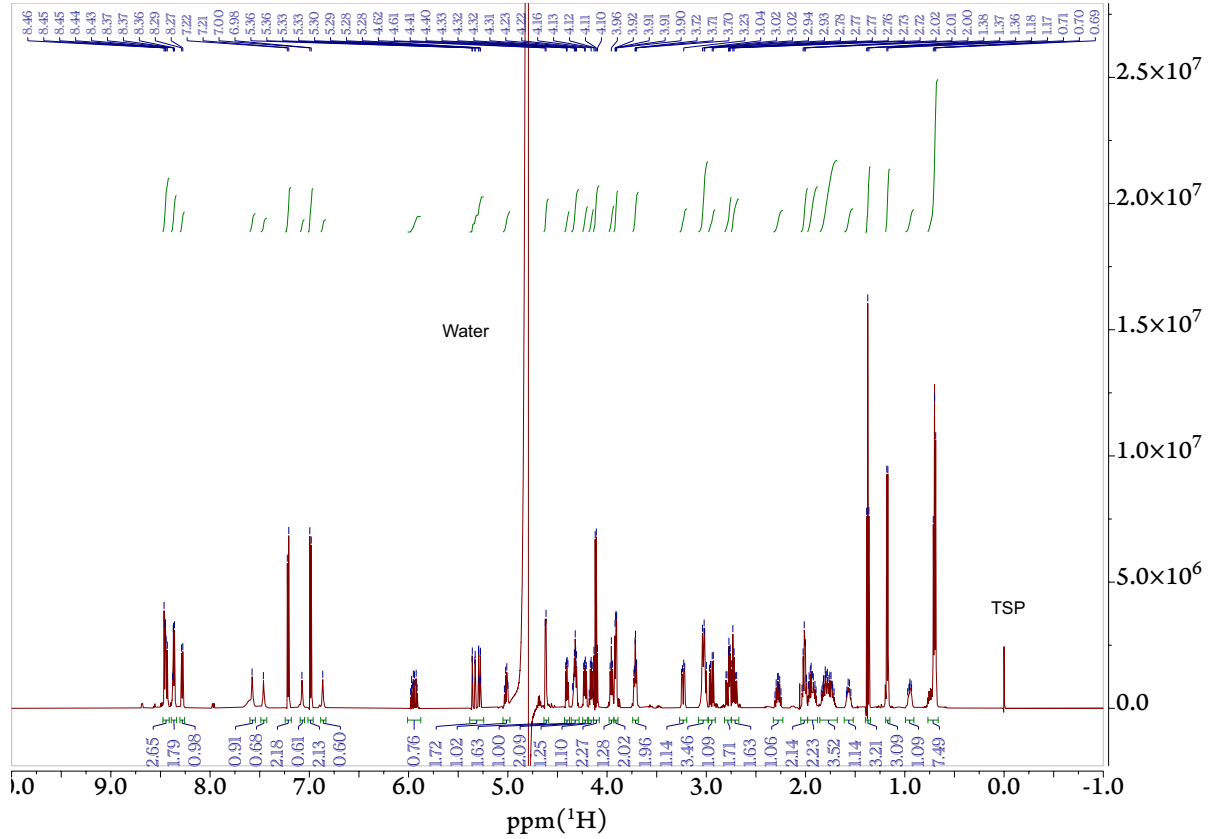
¹H NMR (600 MHz)



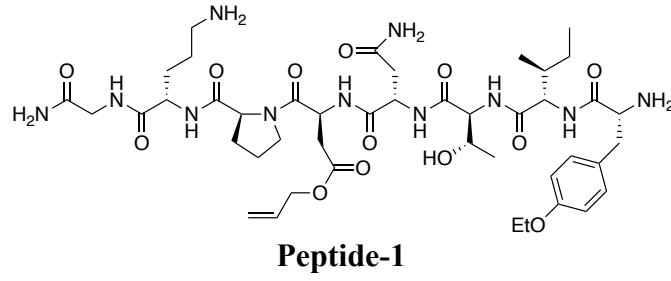
¹H NMR of above peptide in 5 % D₂O/CD₃OD with 100 μM TSP at 298.1 K.



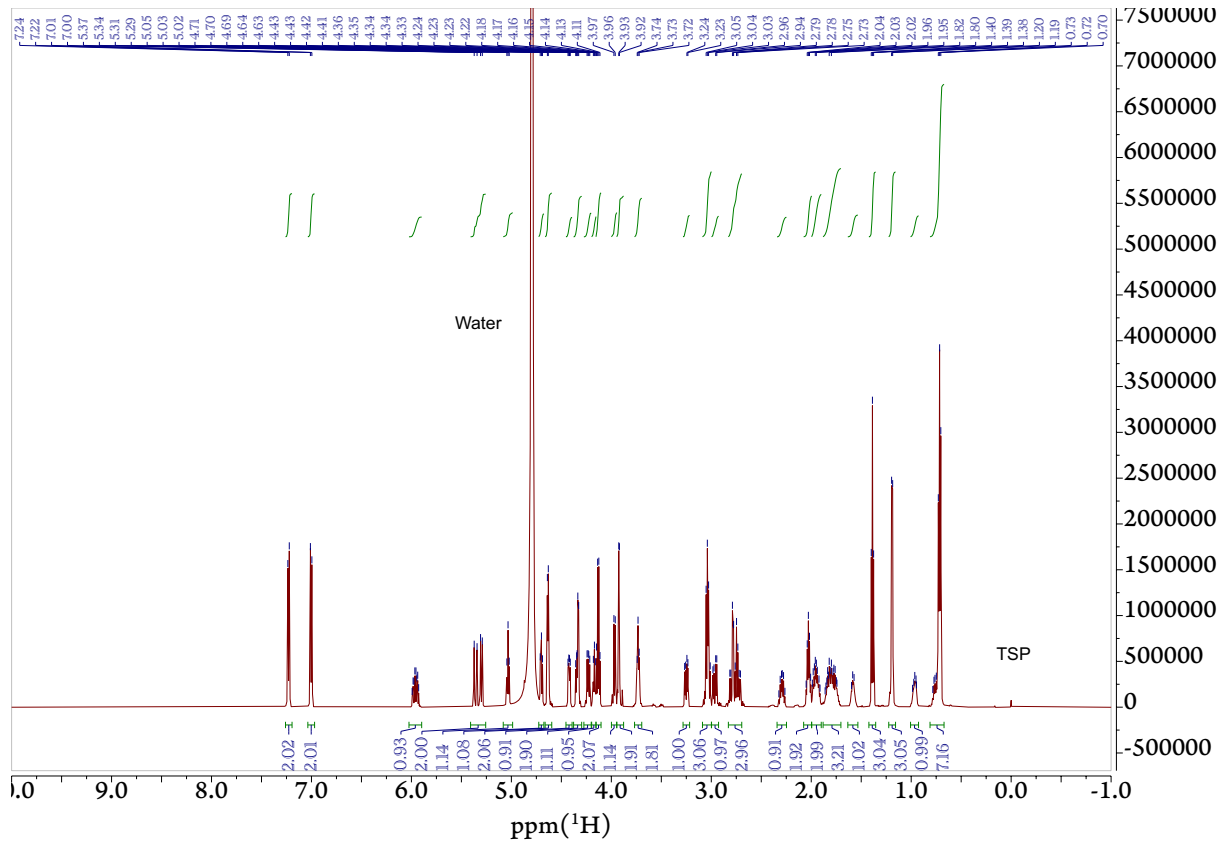
¹H NMR (600 MHz)



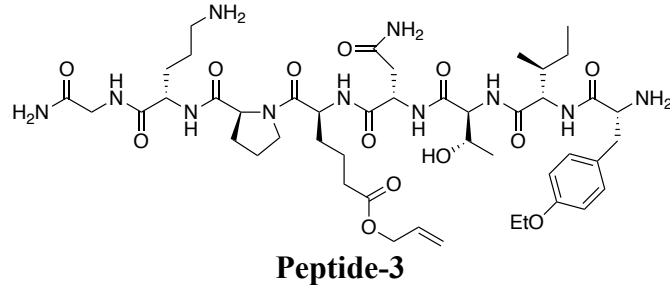
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



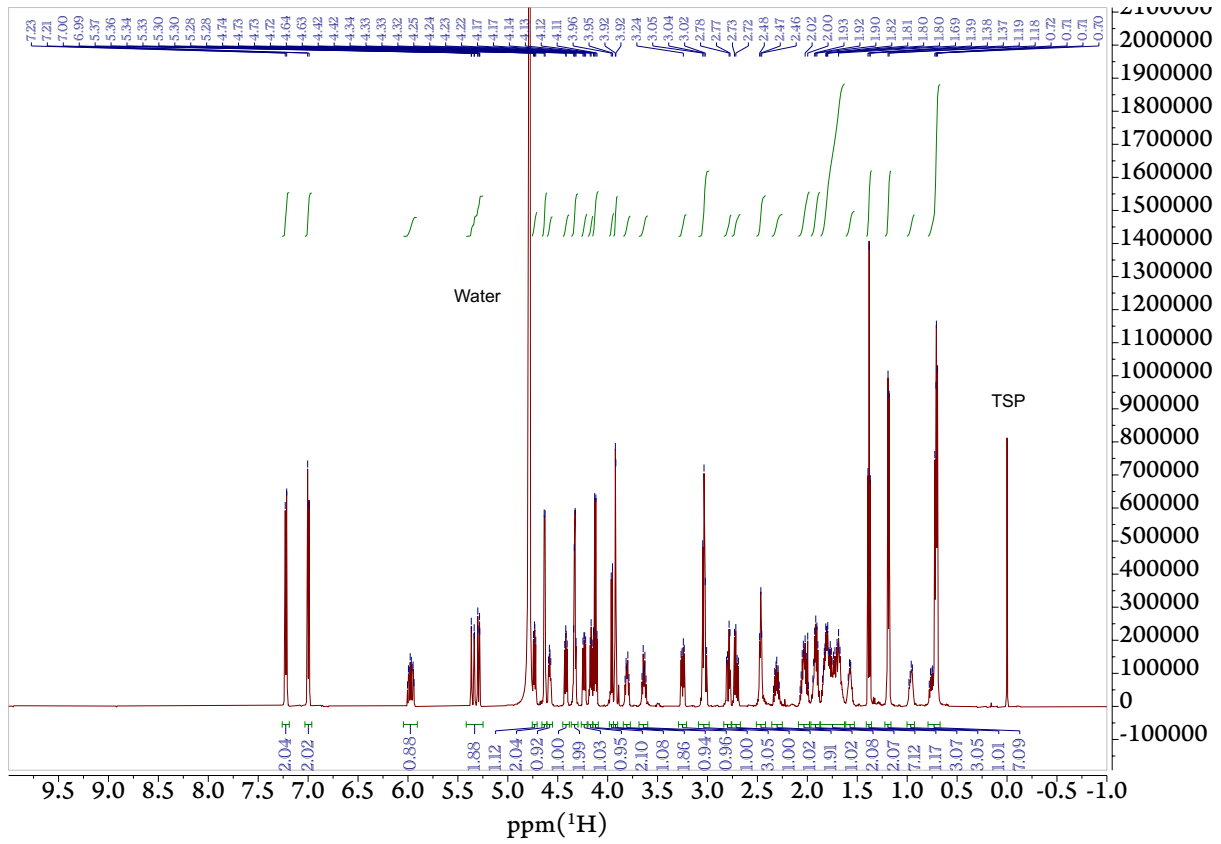
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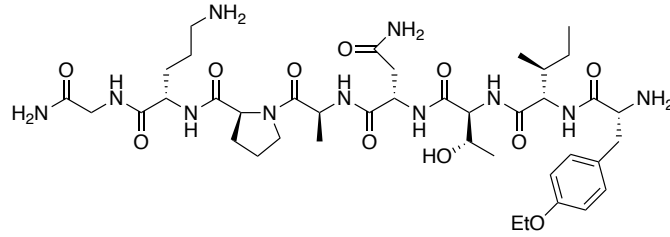
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



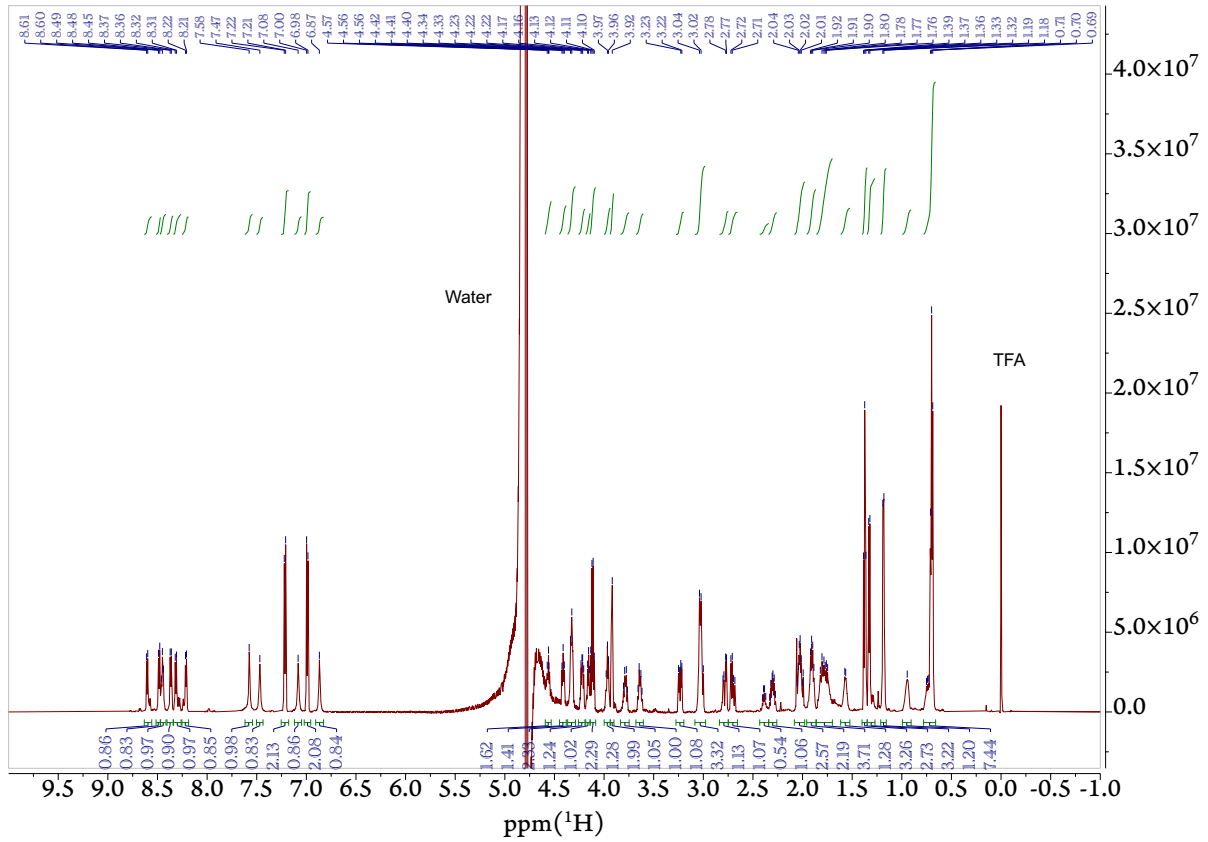
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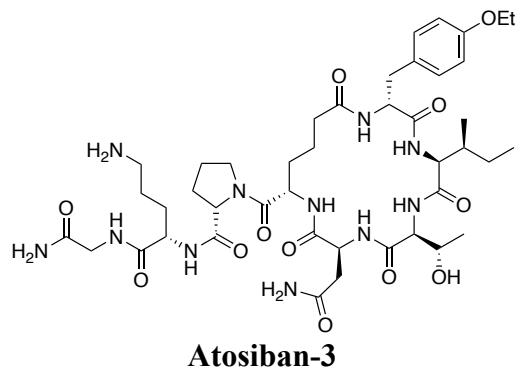
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



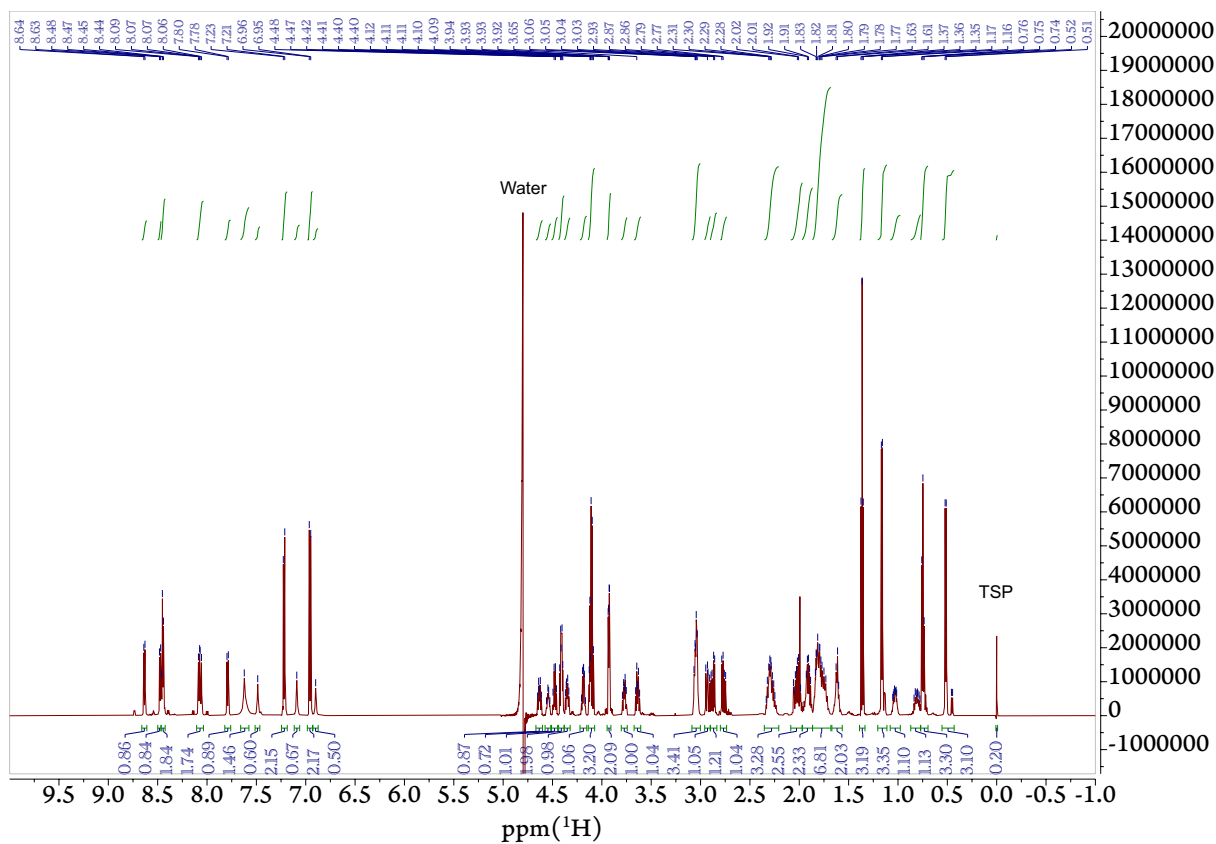
¹H NMR (600 MHz)



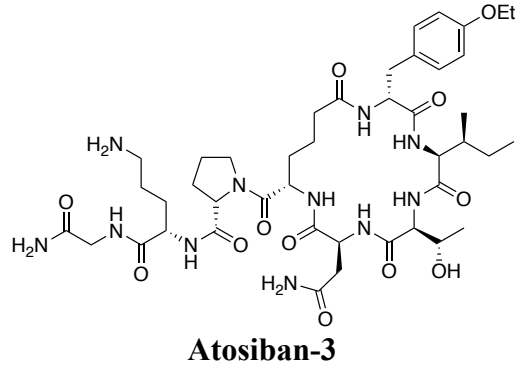
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



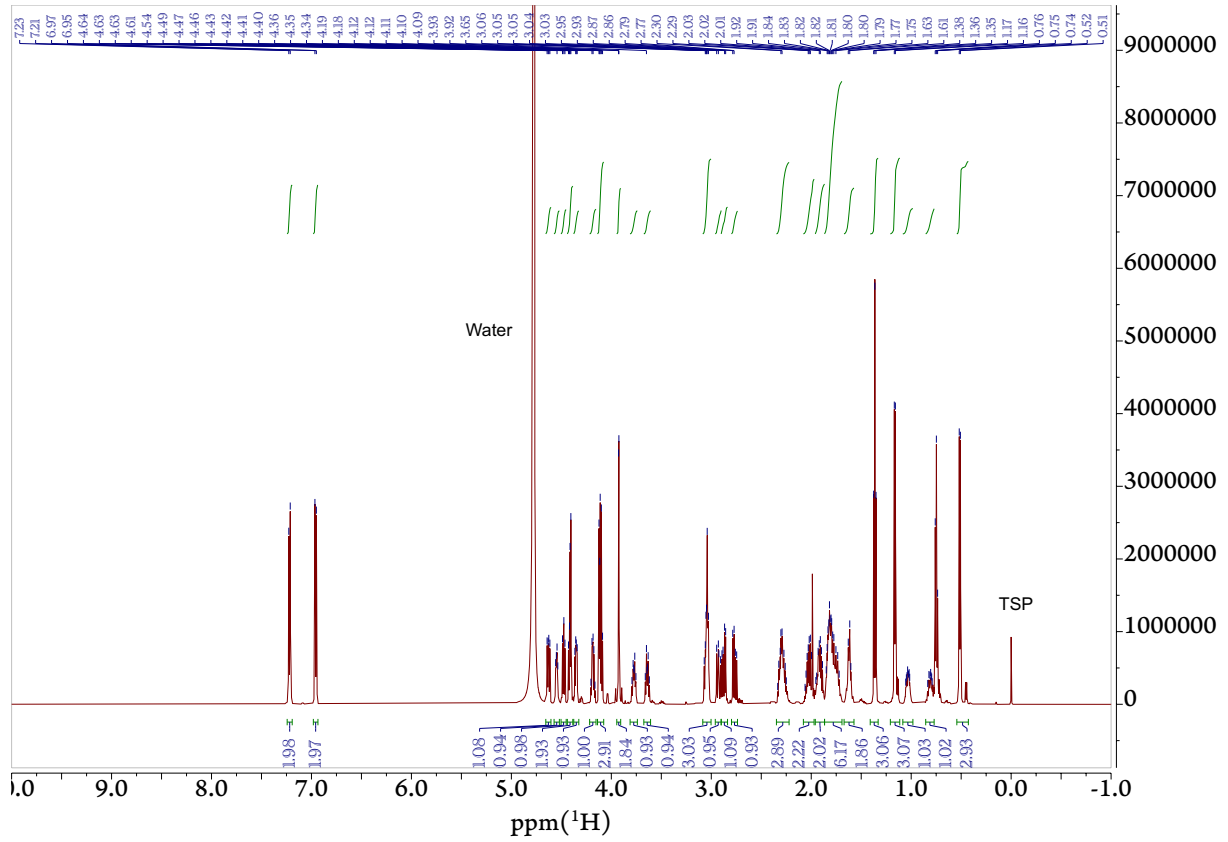
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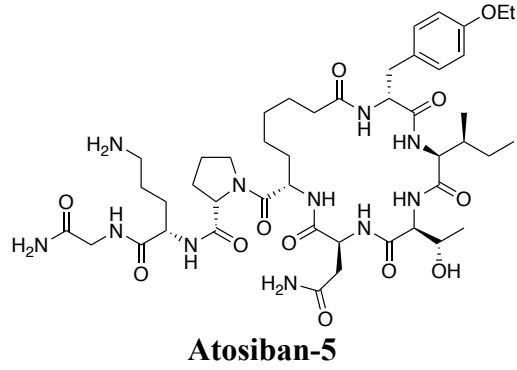
¹H NMR of above peptide in 50 mM Na₃PO₄ e (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



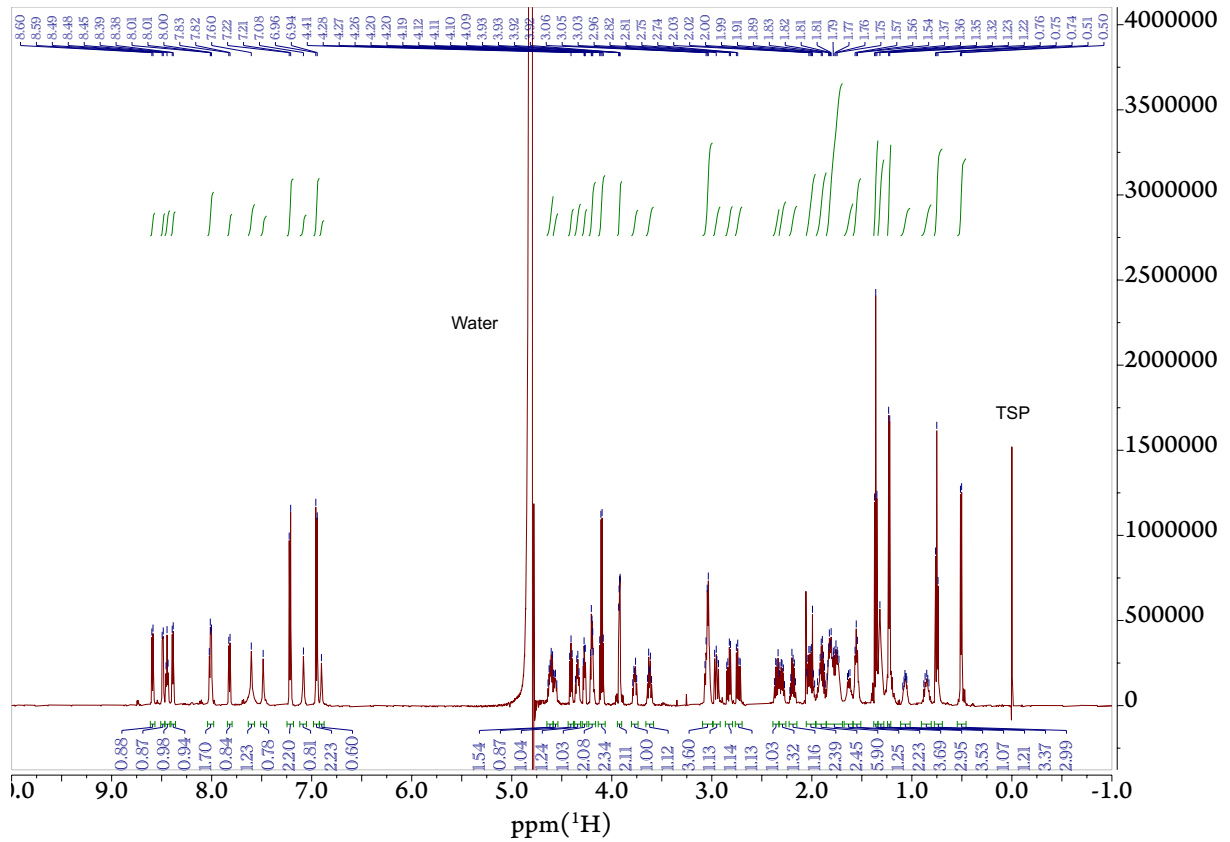
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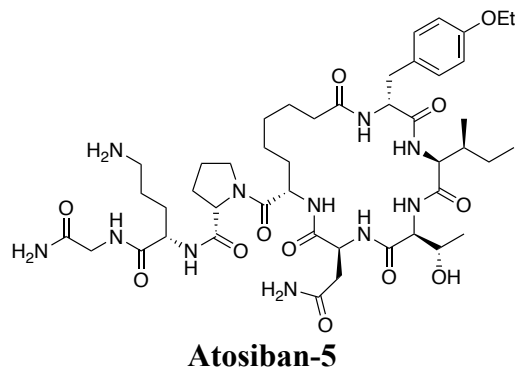
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



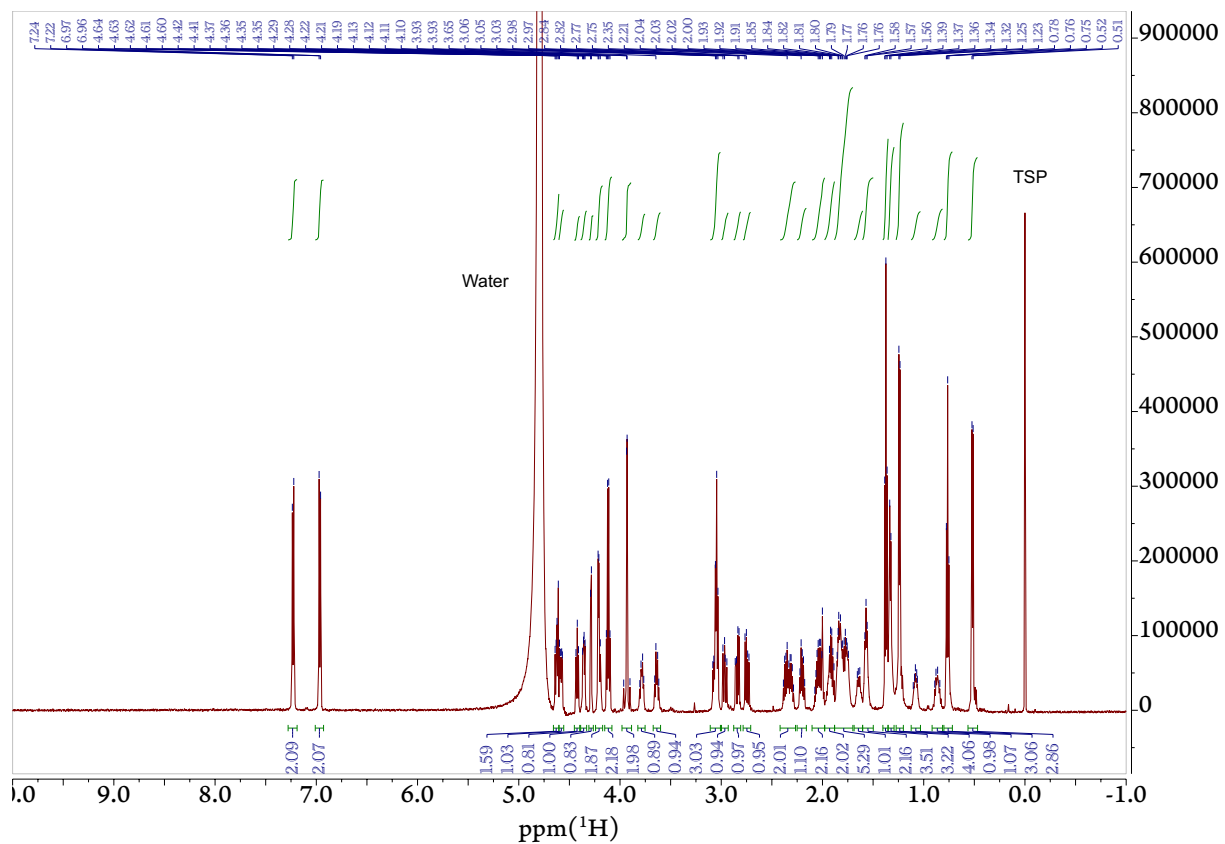
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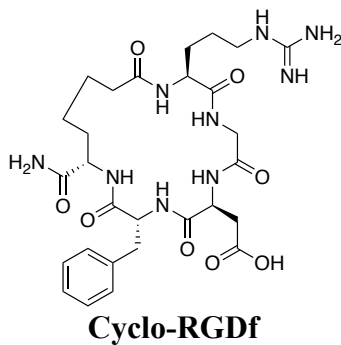
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



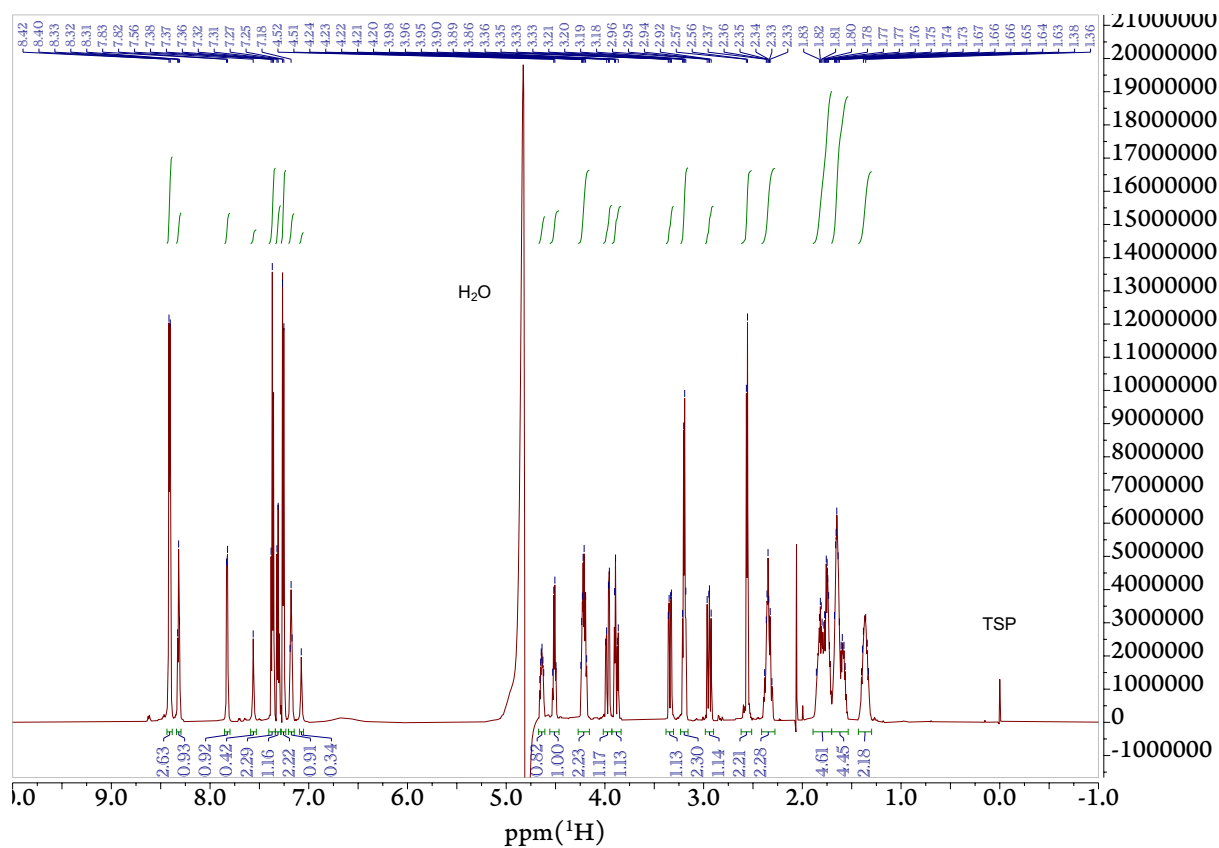
¹H NMR (600 MHz)



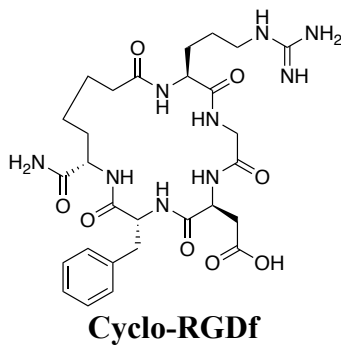
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



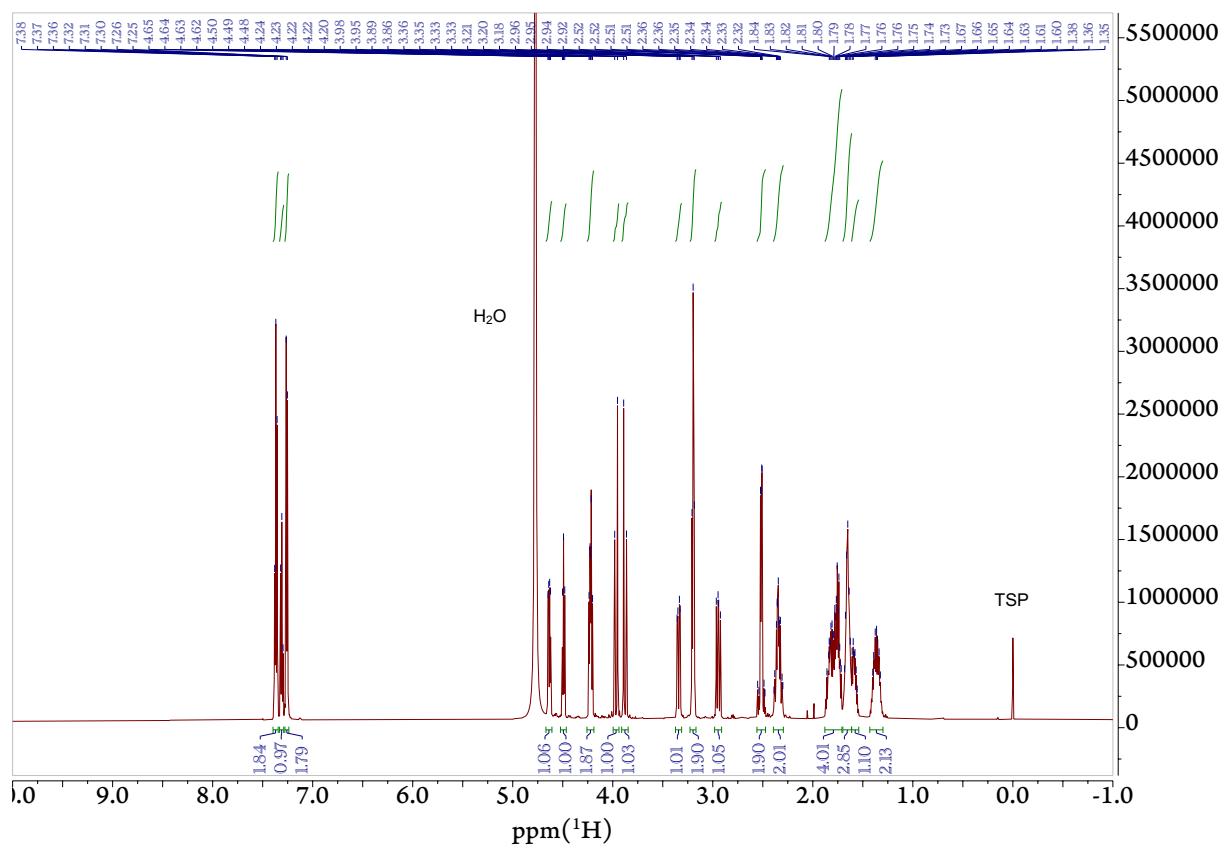
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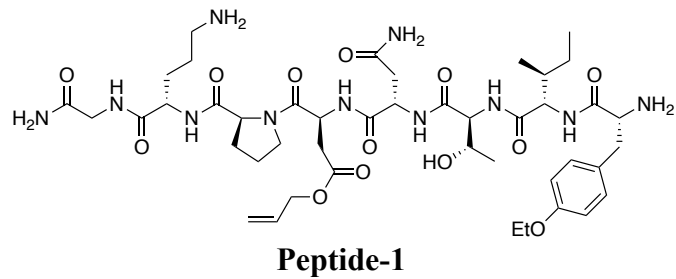
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



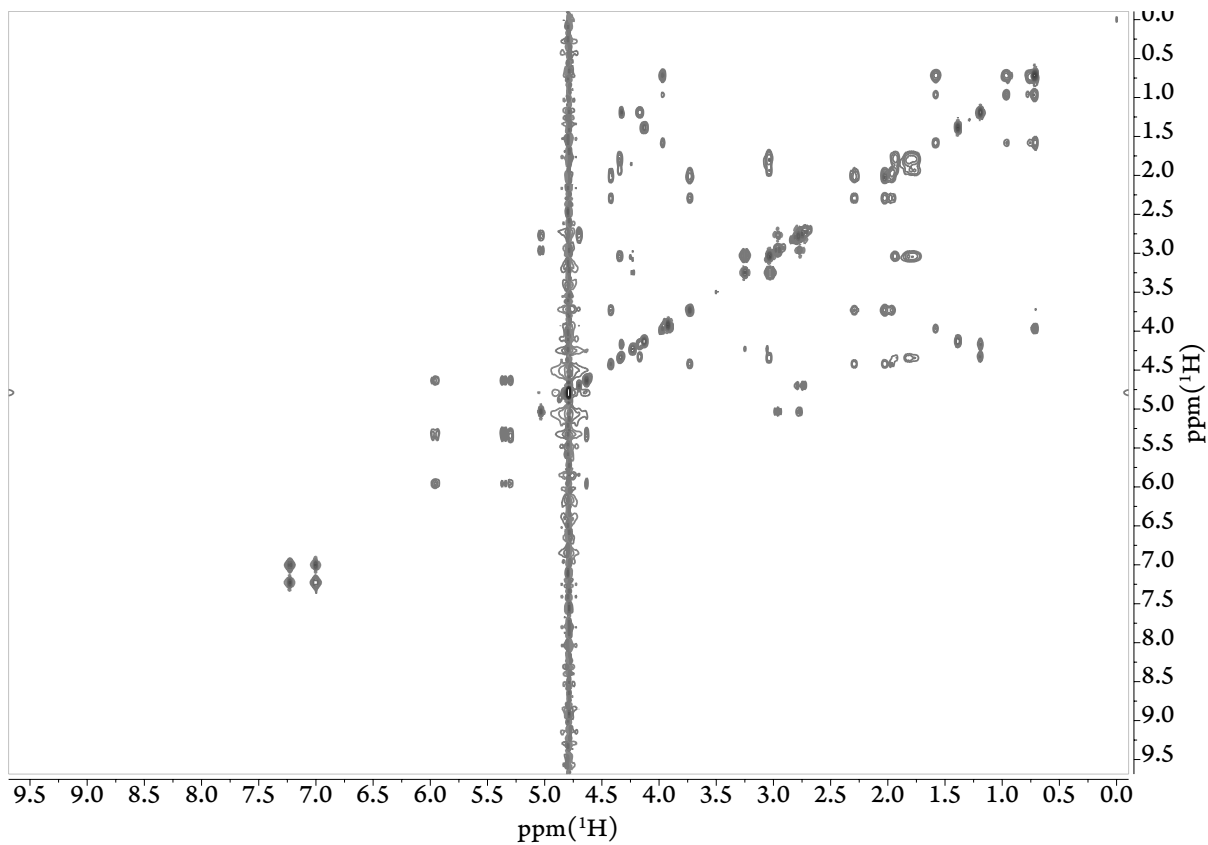
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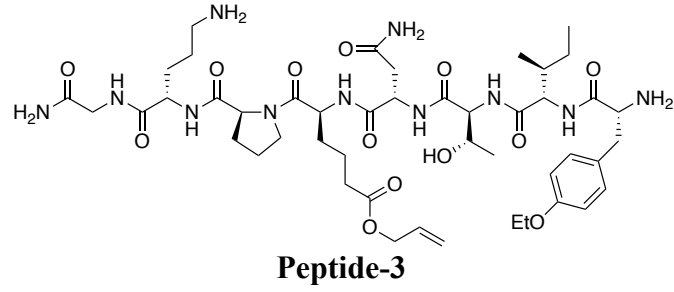
¹H NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, D₂O, at 298.1 K.



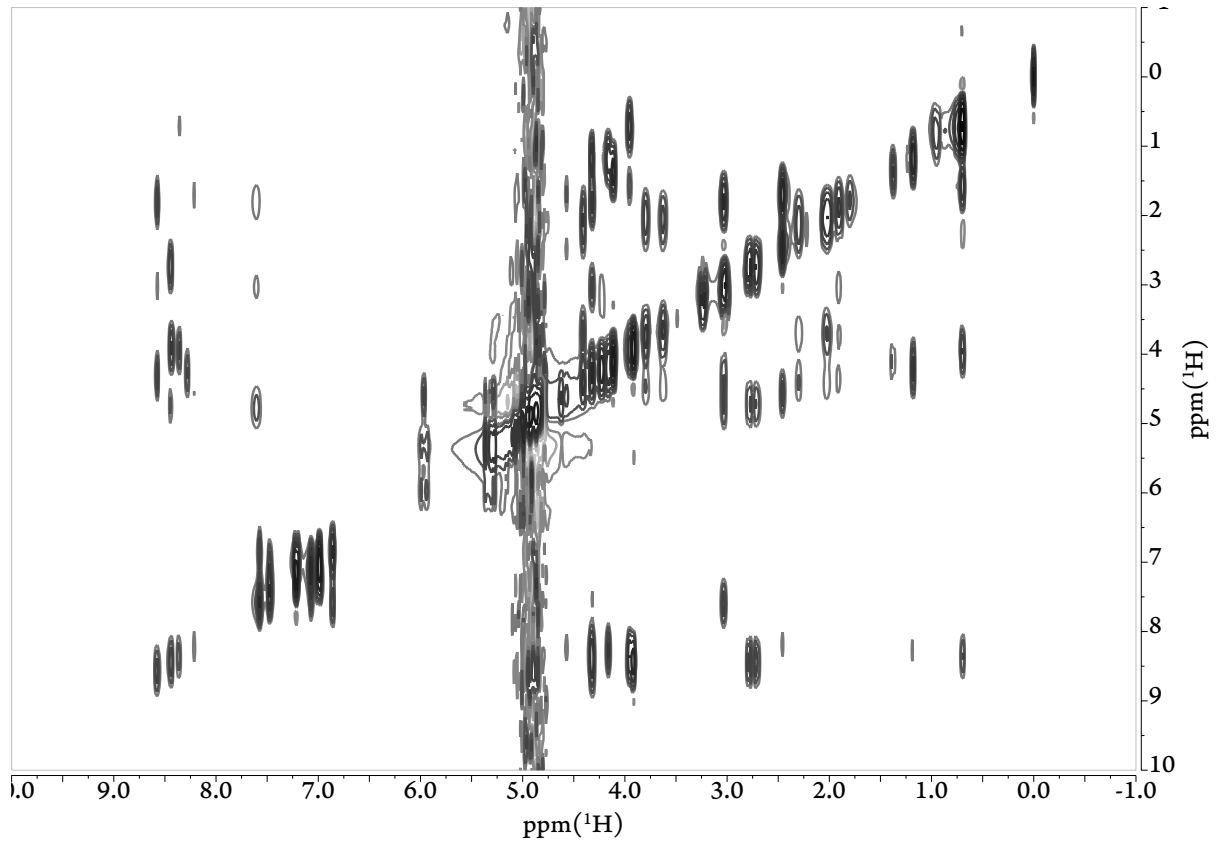
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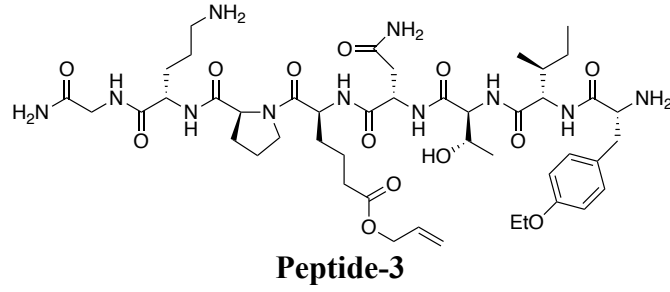
¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



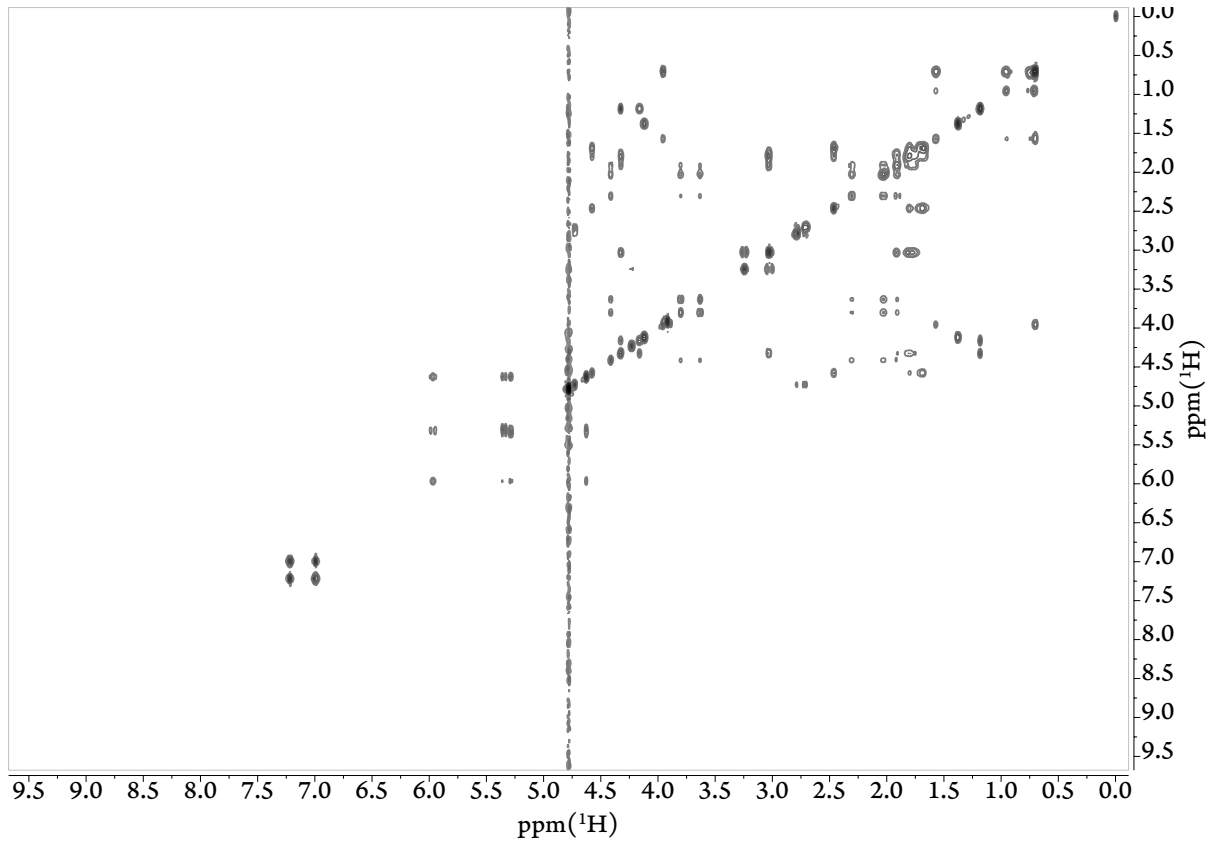
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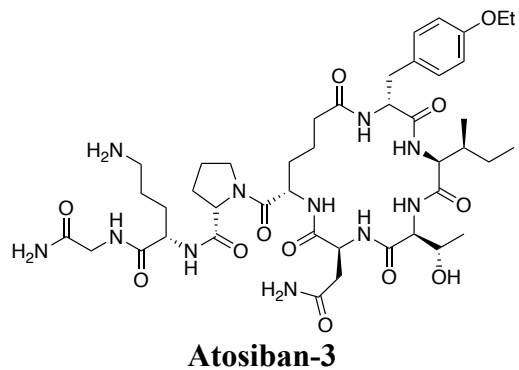
¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



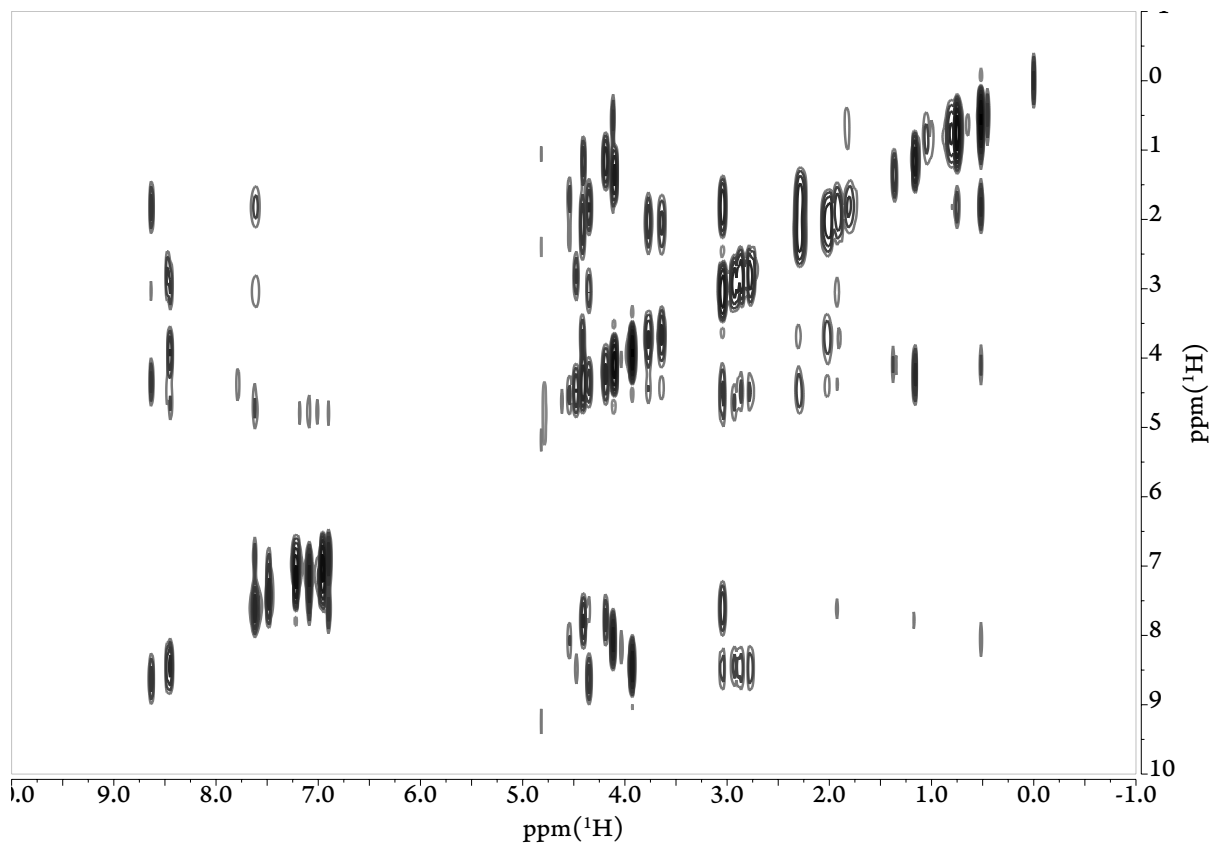
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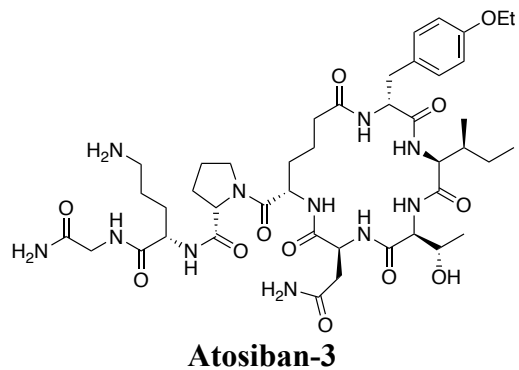
¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



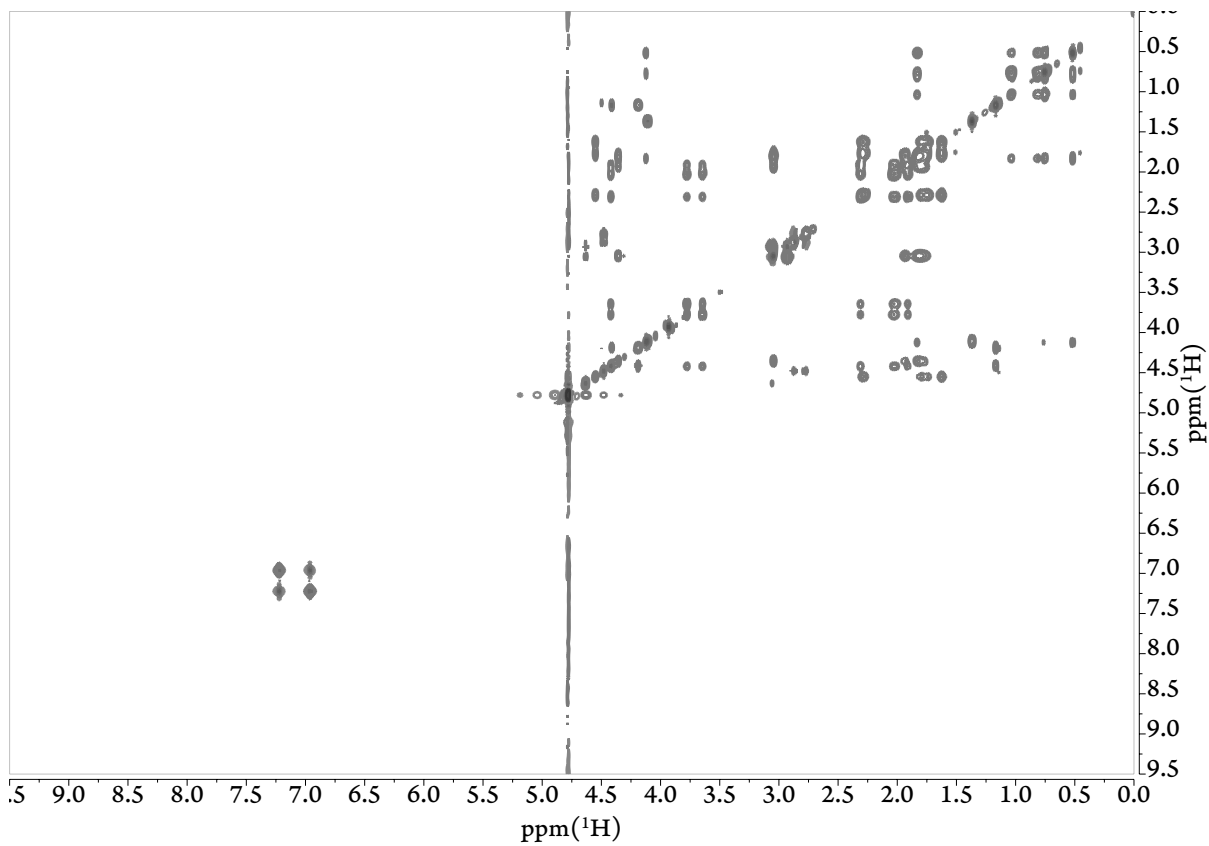
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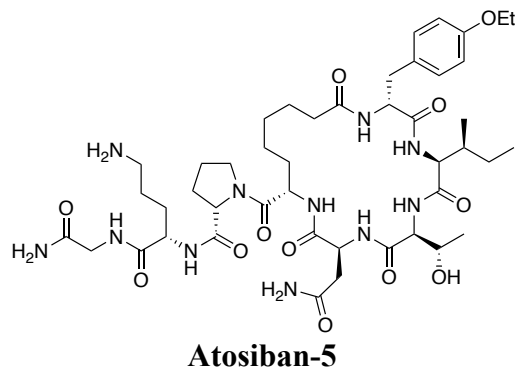
¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



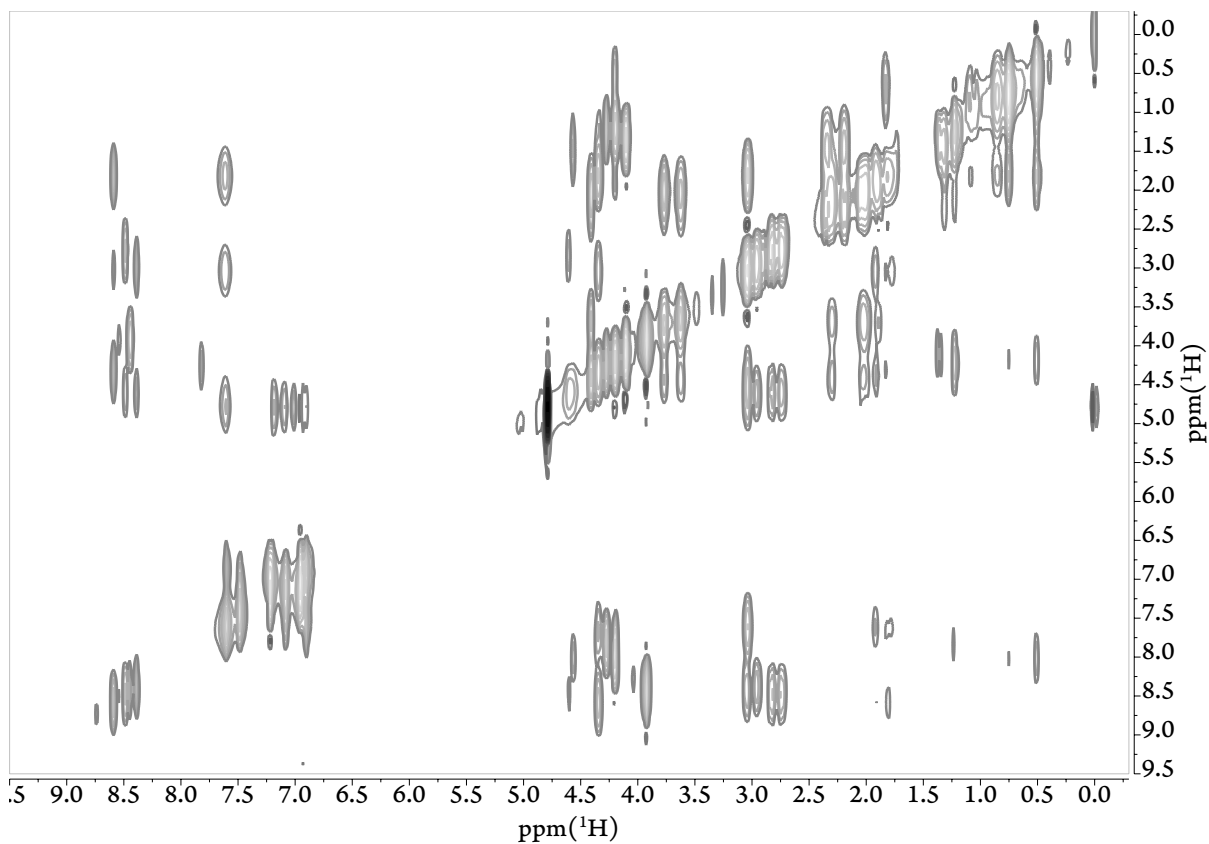
¹H NMR (600 MHz)



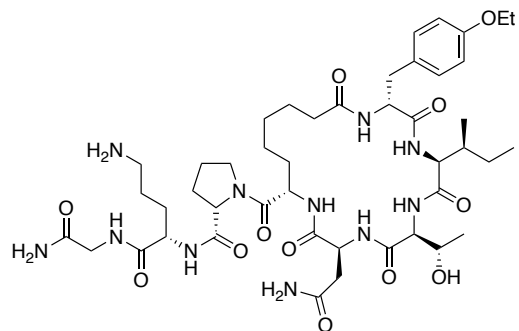
¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



¹H NMR (600 MHz)

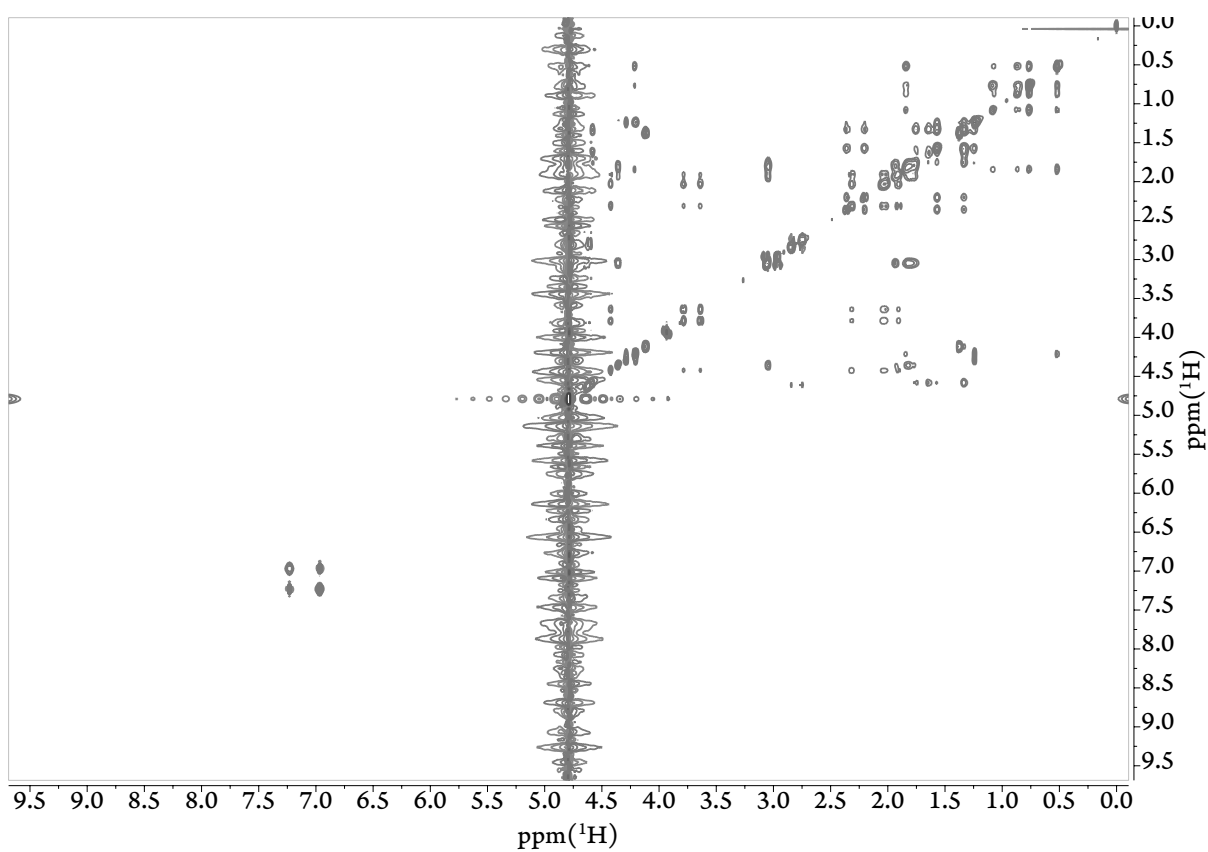


¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.

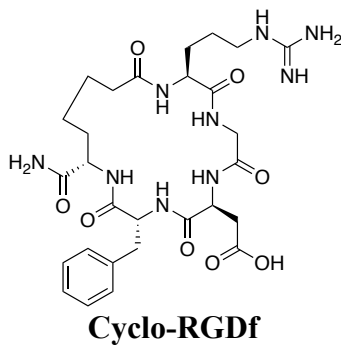


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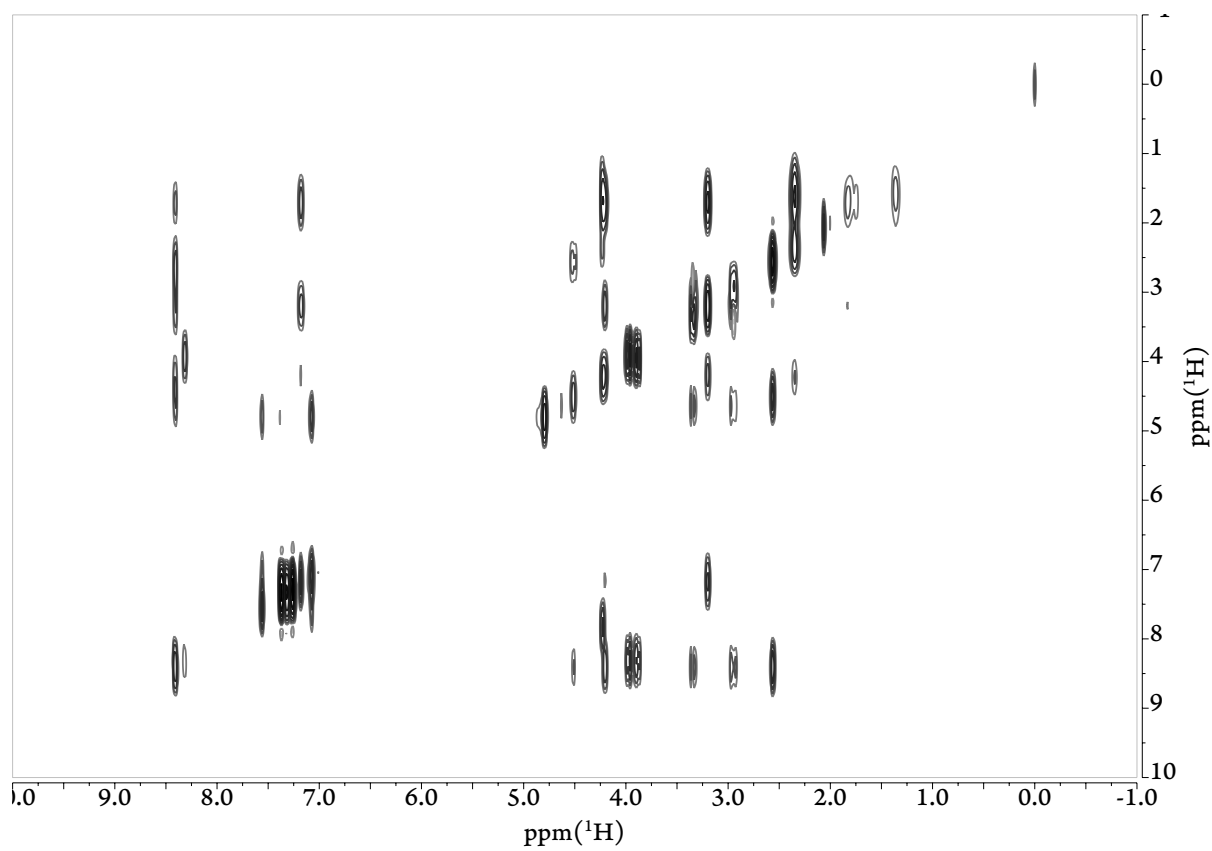
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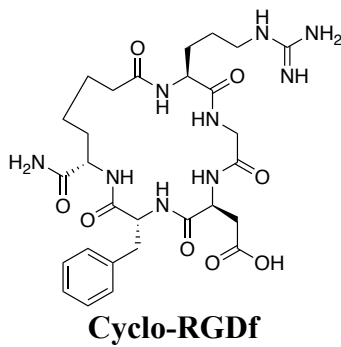
¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



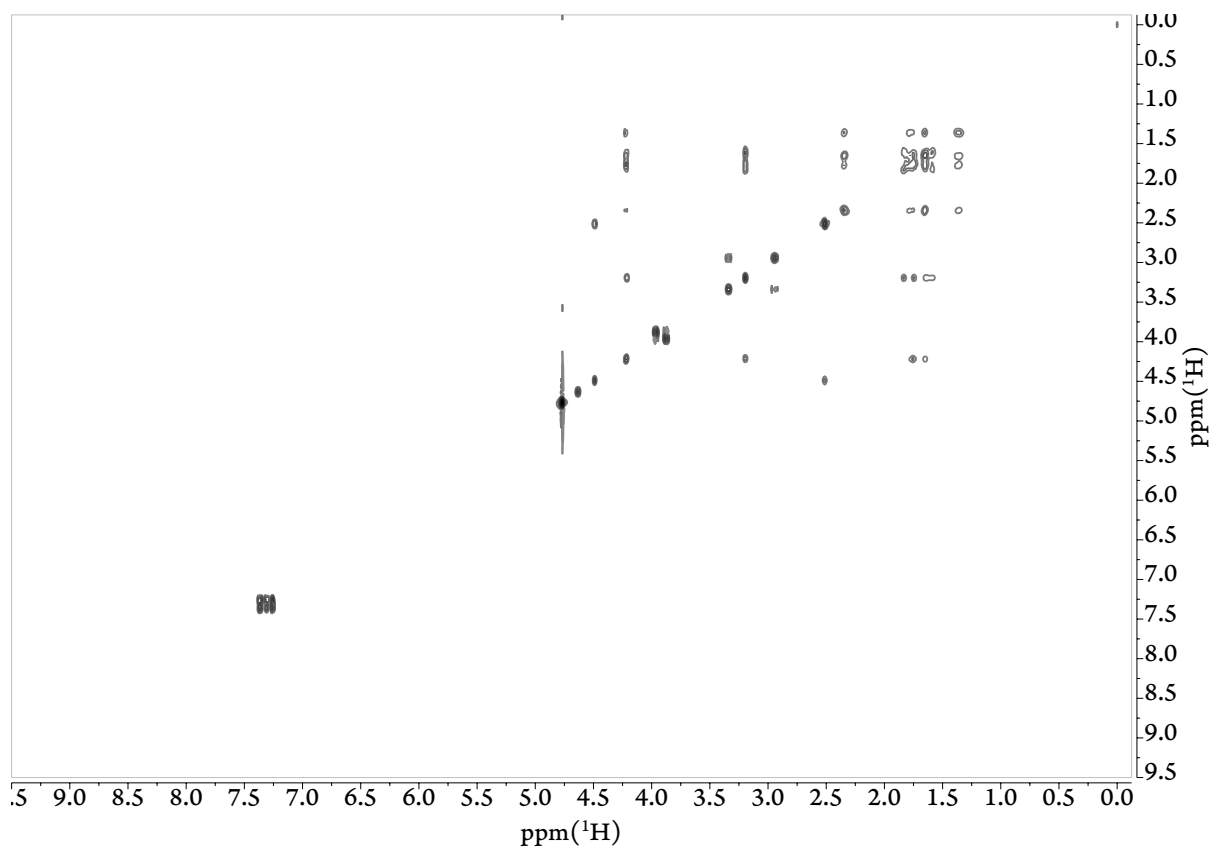
¹H NMR (600 MHz)



¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.

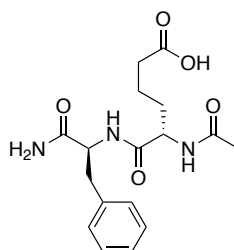


¹H NMR (600 MHz)

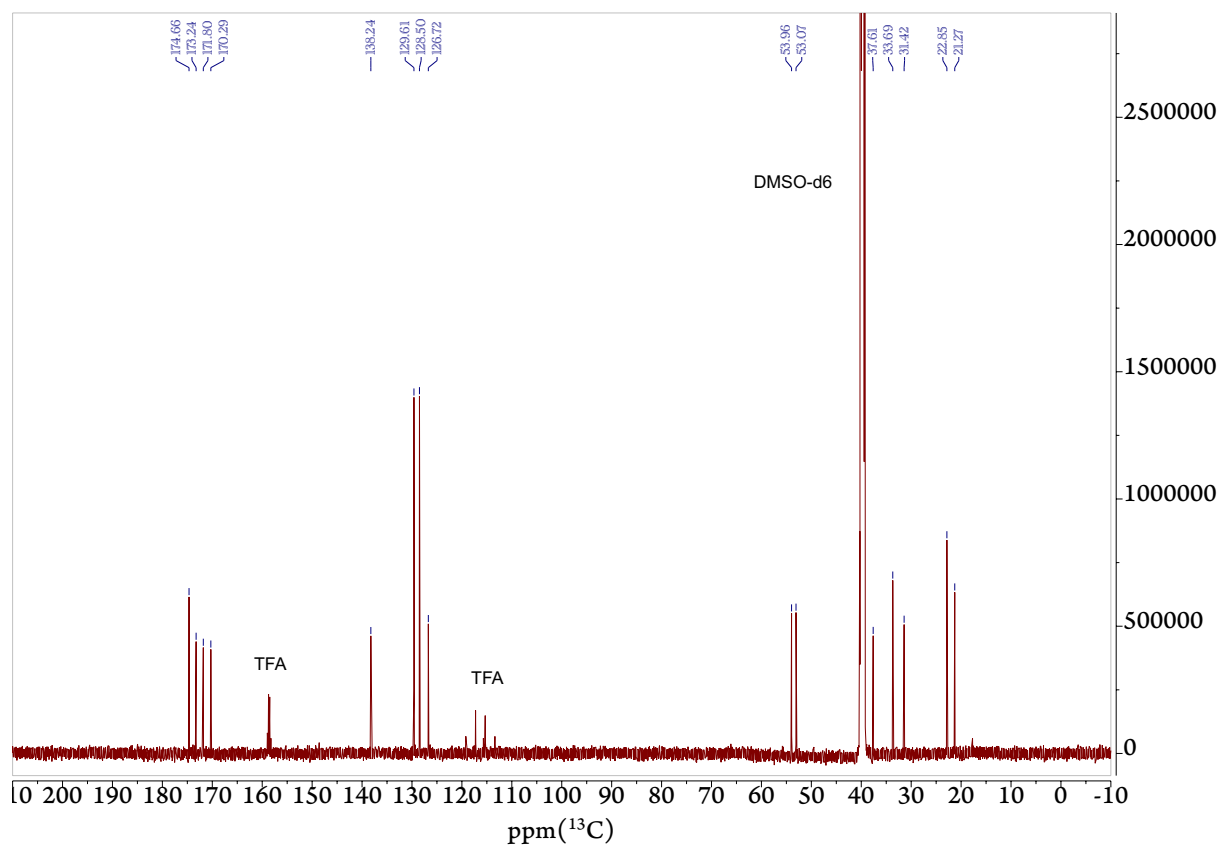


¹H TOCSY NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, D₂O, at 298.1 K.

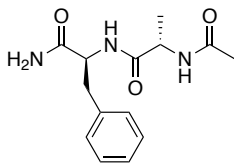
¹³C NMR Spectra of Peptides



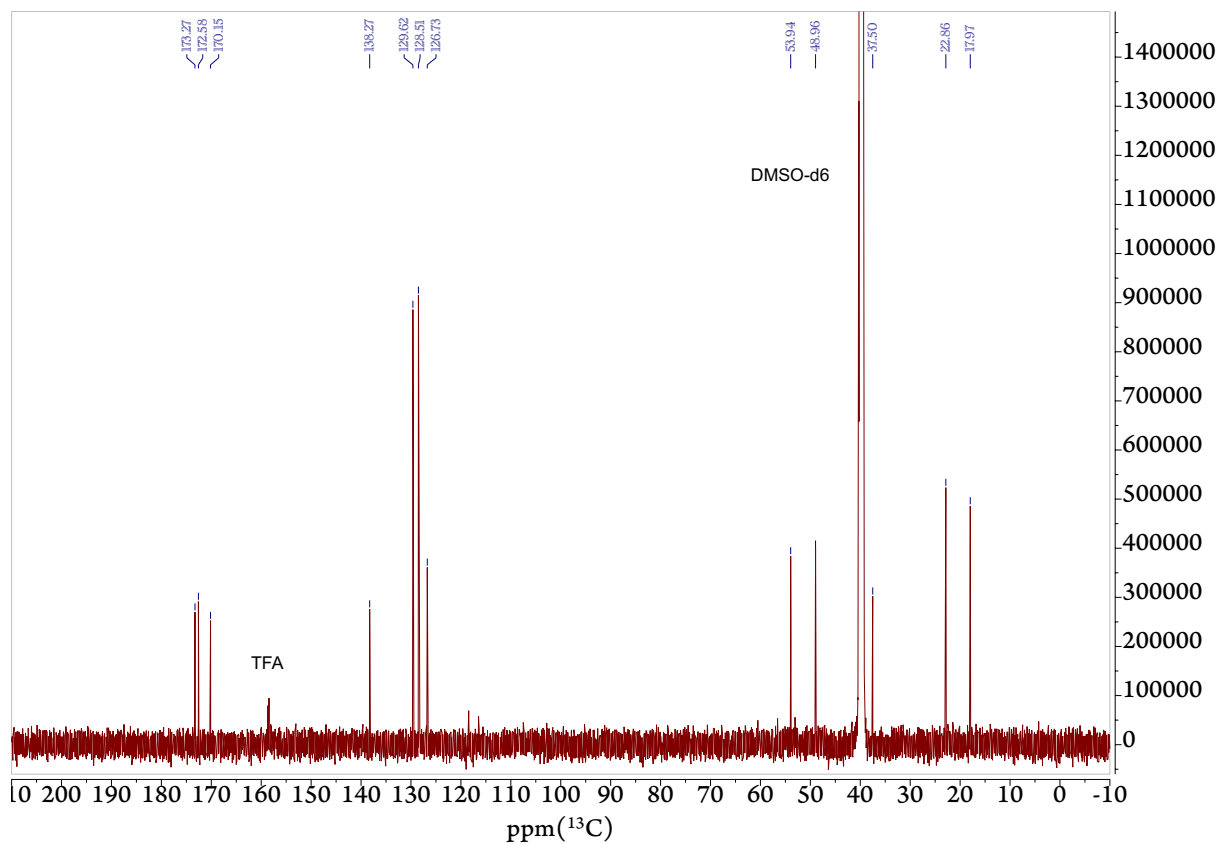
¹³C NMR (151 MHz)



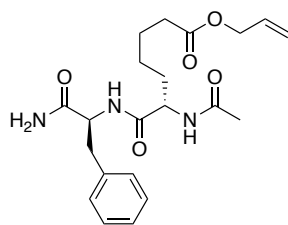
¹³C NMR of above peptide in 5% D₂O/DMSO-*d*₆ at 298.1 K.



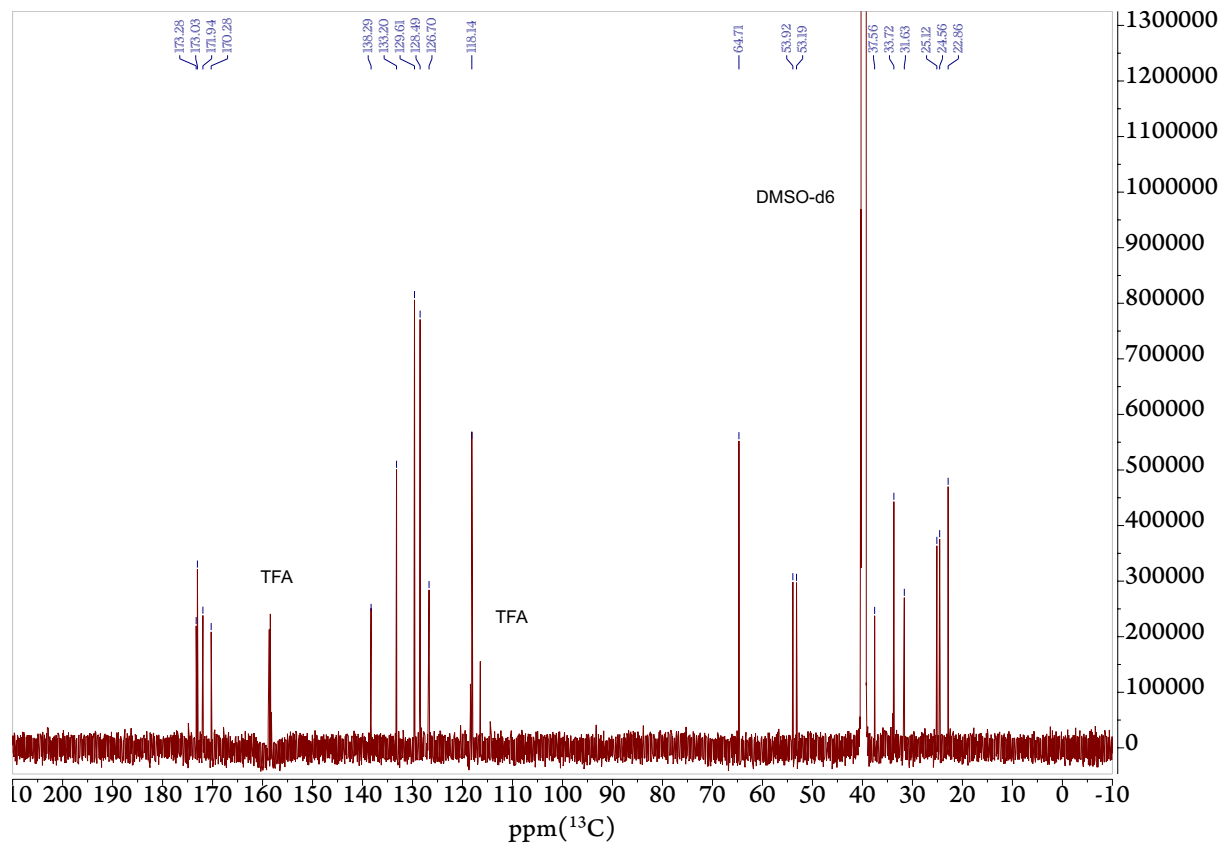
^{13}C NMR (151 MHz)



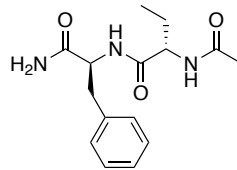
^{13}C NMR of above peptide in 5% $\text{D}_2\text{O}/\text{DMSO-}d_6$ at 298.1 K.



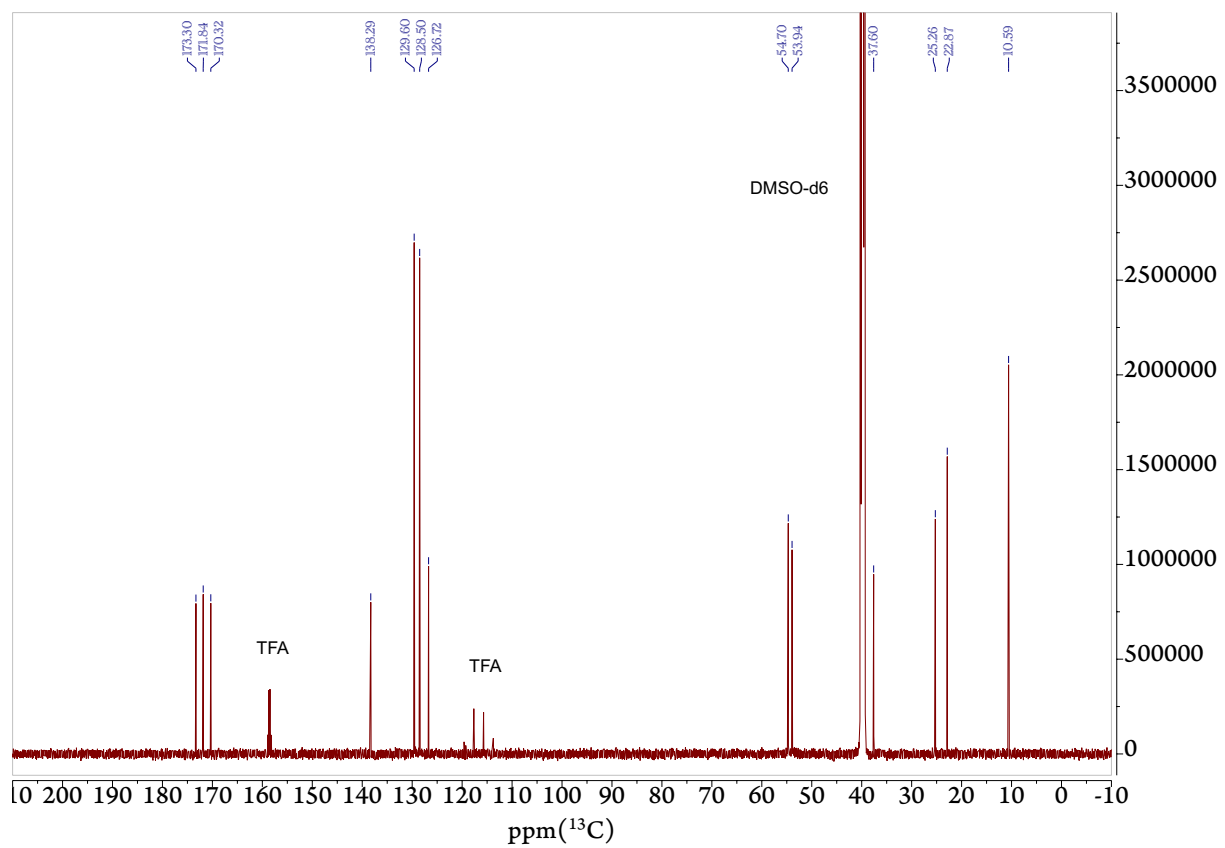
^{13}C NMR (151 MHz)



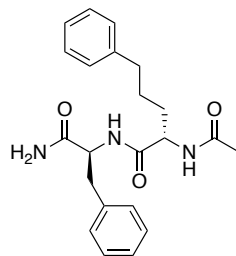
^{13}C NMR of above peptide in 5% $\text{D}_2\text{O}/\text{DMSO-}d_6$ at 298.1 K.



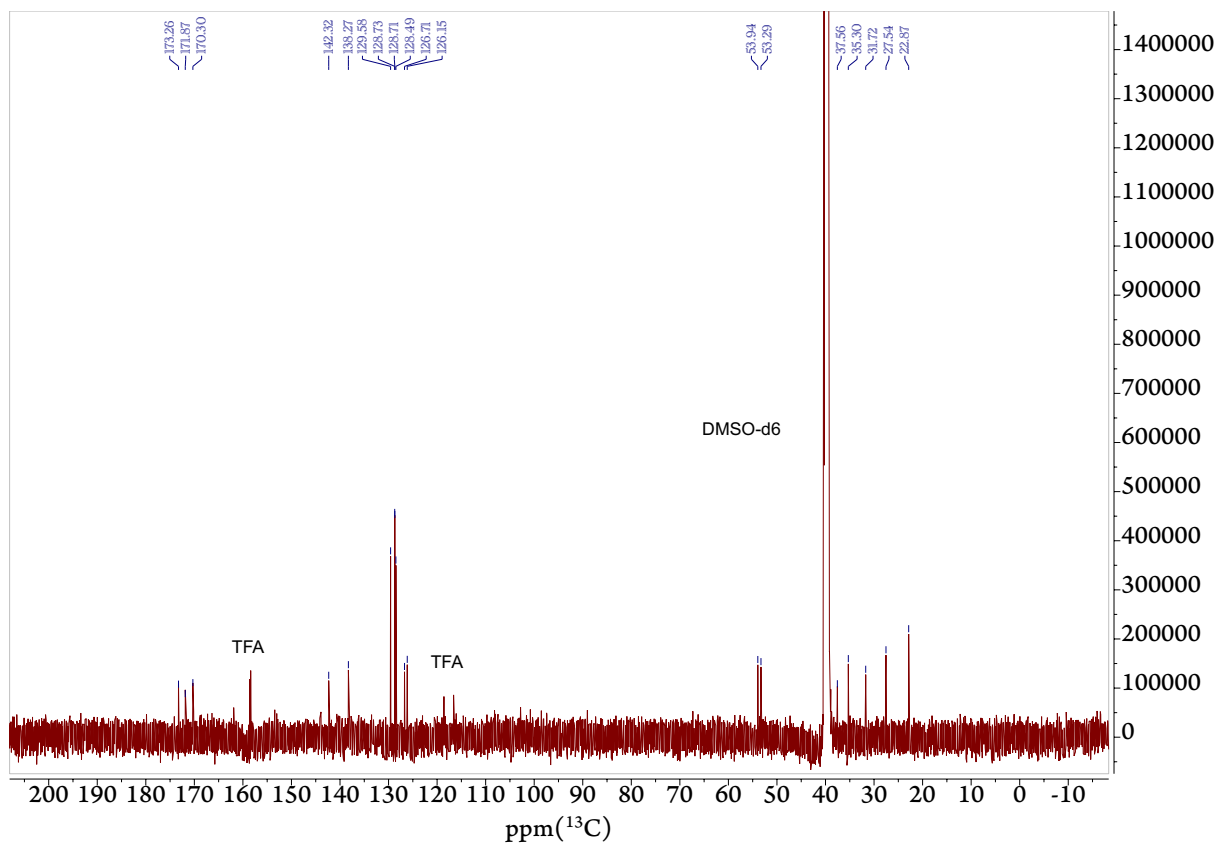
^{13}C NMR (151 MHz)



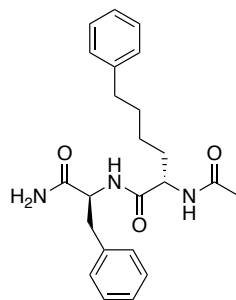
^{13}C NMR of above peptide in 5% $\text{D}_2\text{O}/\text{DMSO}-d_6$ at 298.1 K.



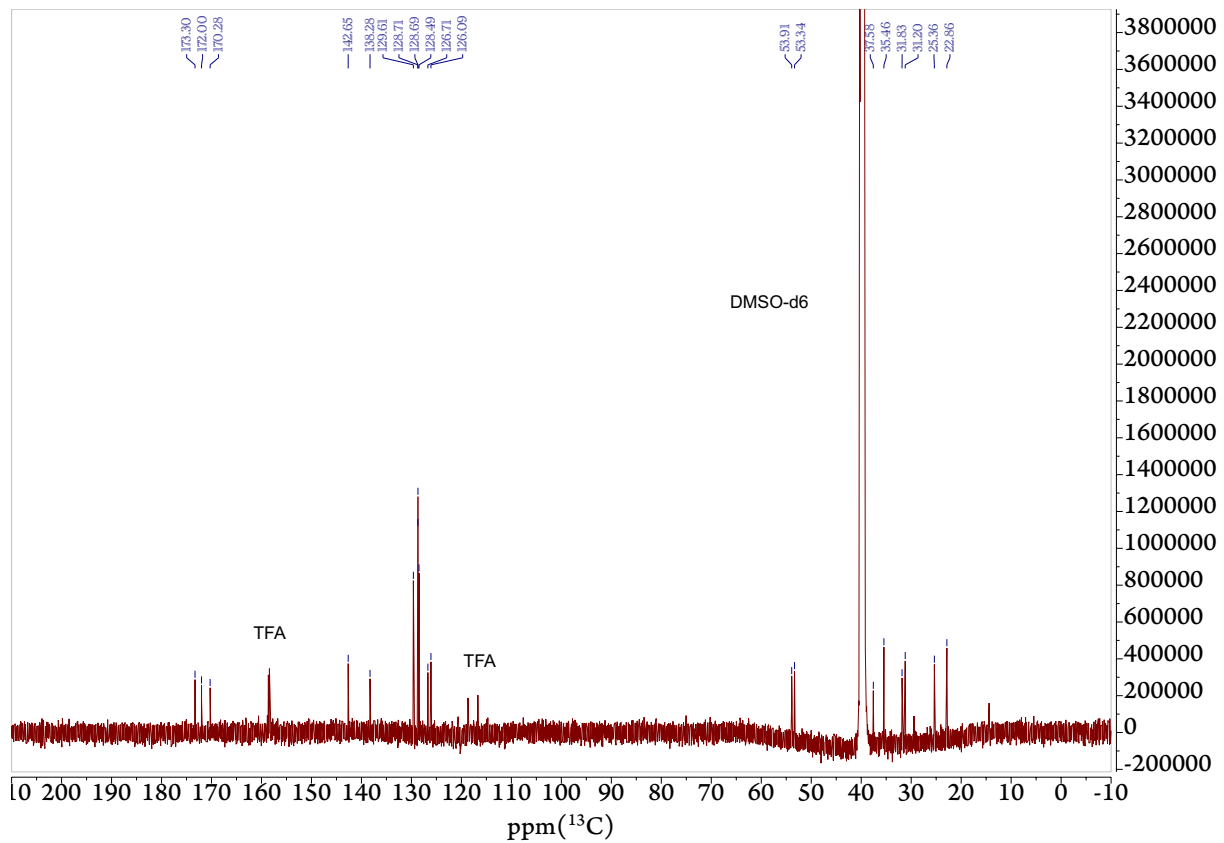
^{13}C NMR (151 MHz)



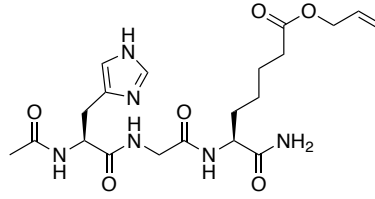
^{13}C NMR of above peptide in 5% $\text{D}_2\text{O}/\text{DMSO-}d_6$ at 298.1 K.



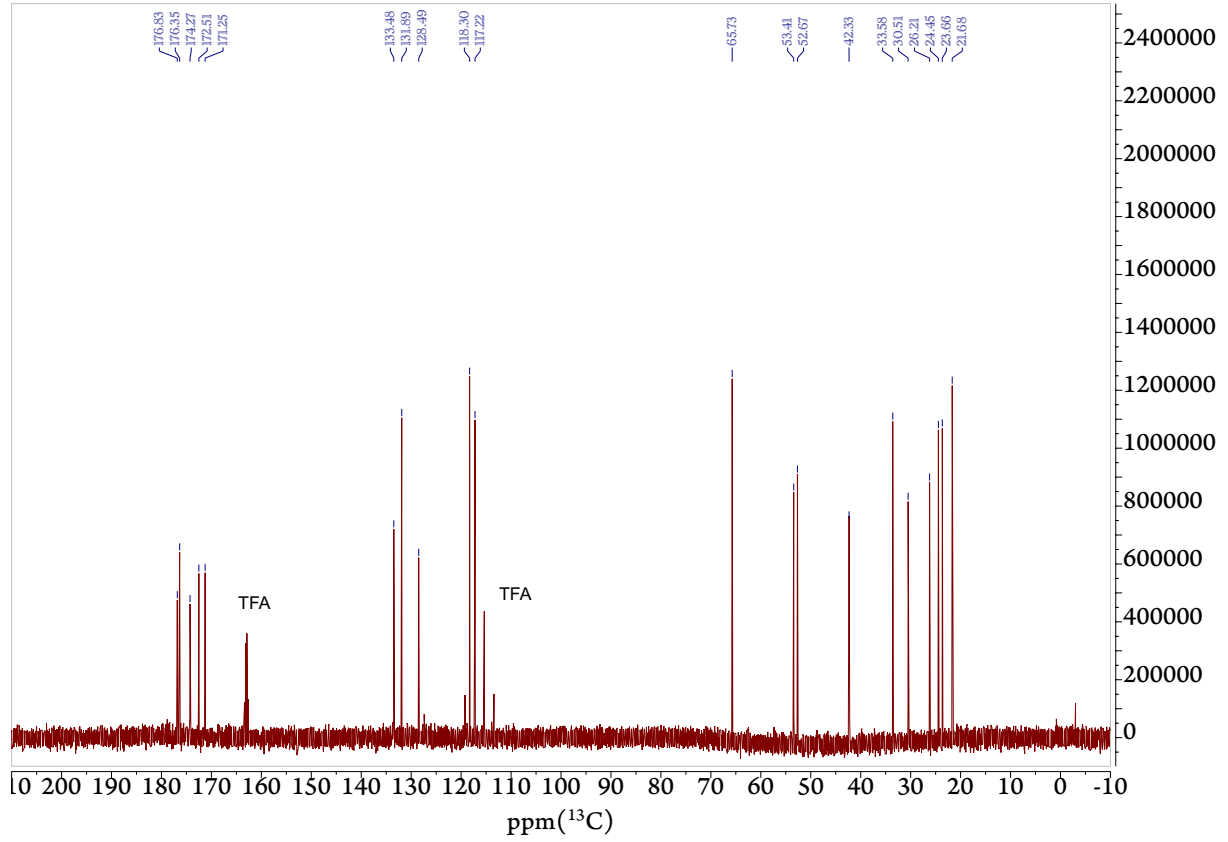
^{13}C NMR (151 MHz)



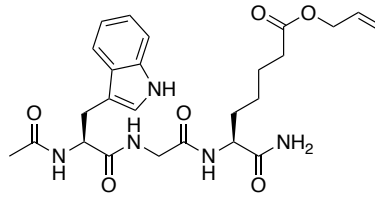
^{13}C NMR of above peptide in 5% $\text{D}_2\text{O}/\text{DMSO-}d_6$ at 298.1 K.



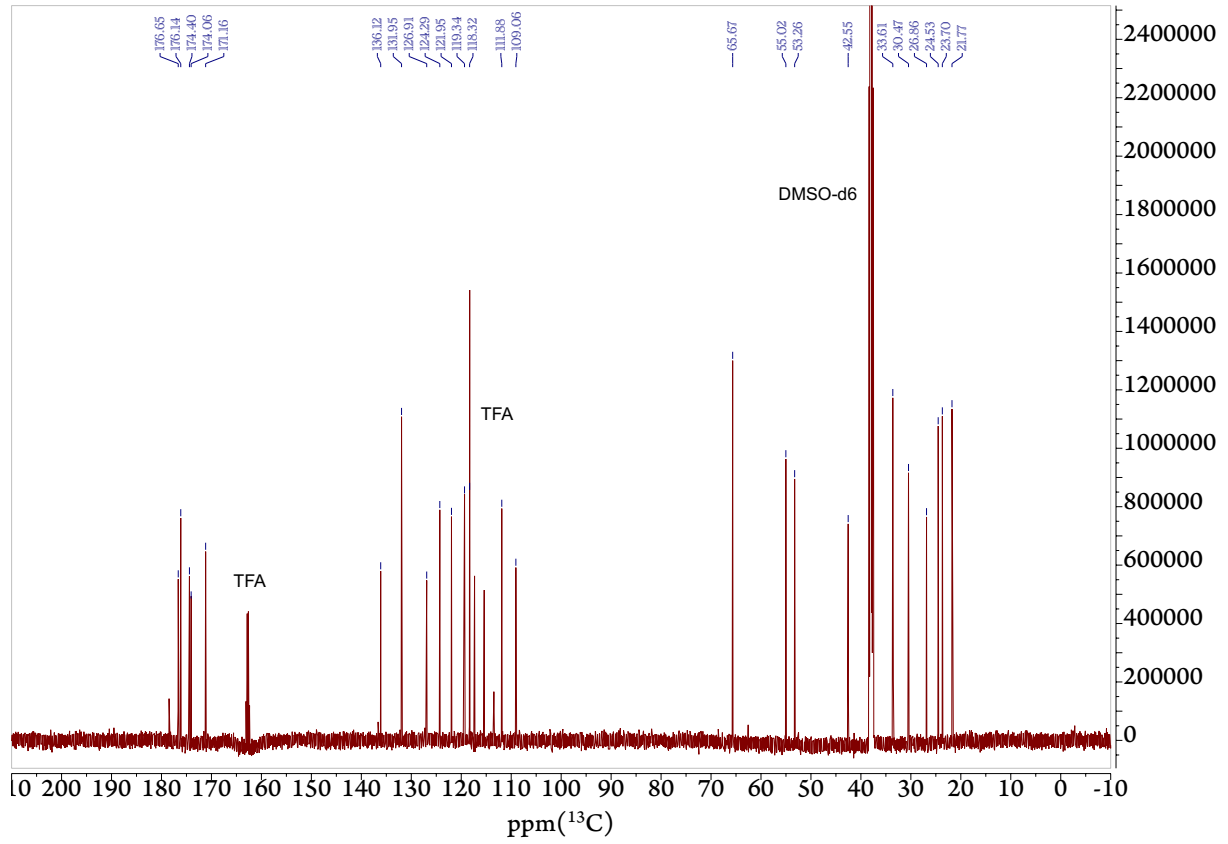
^{13}C NMR (151 MHz)



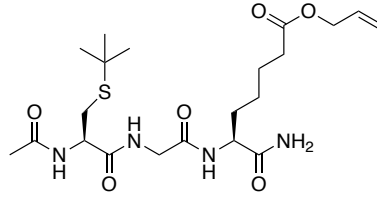
^{13}C NMR of above peptide in D_2O at 298.1 K.



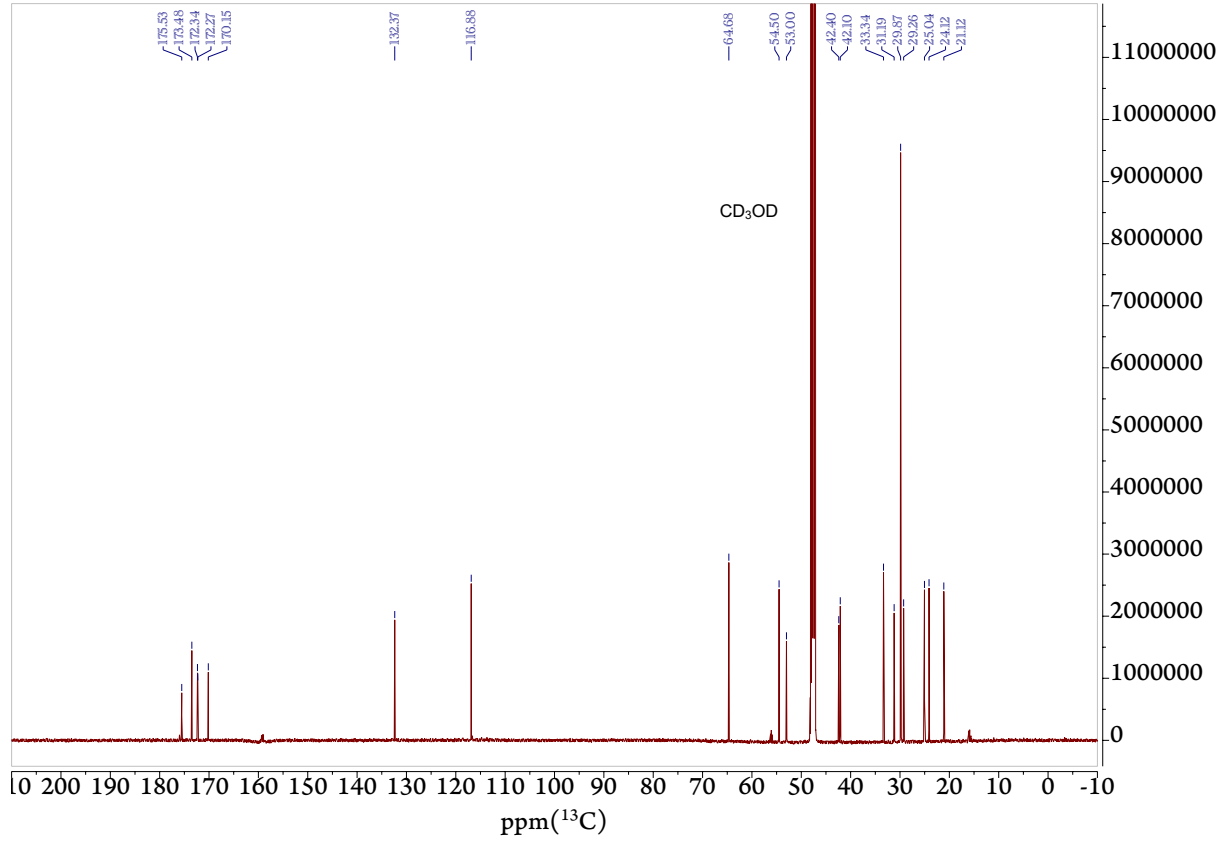
^{13}C NMR (151 MHz)



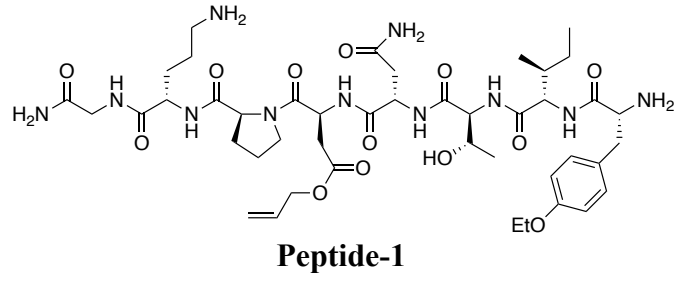
^{13}C NMR of above peptide in 10 % DMSO-*d*6/D₂O at 298.1 K.



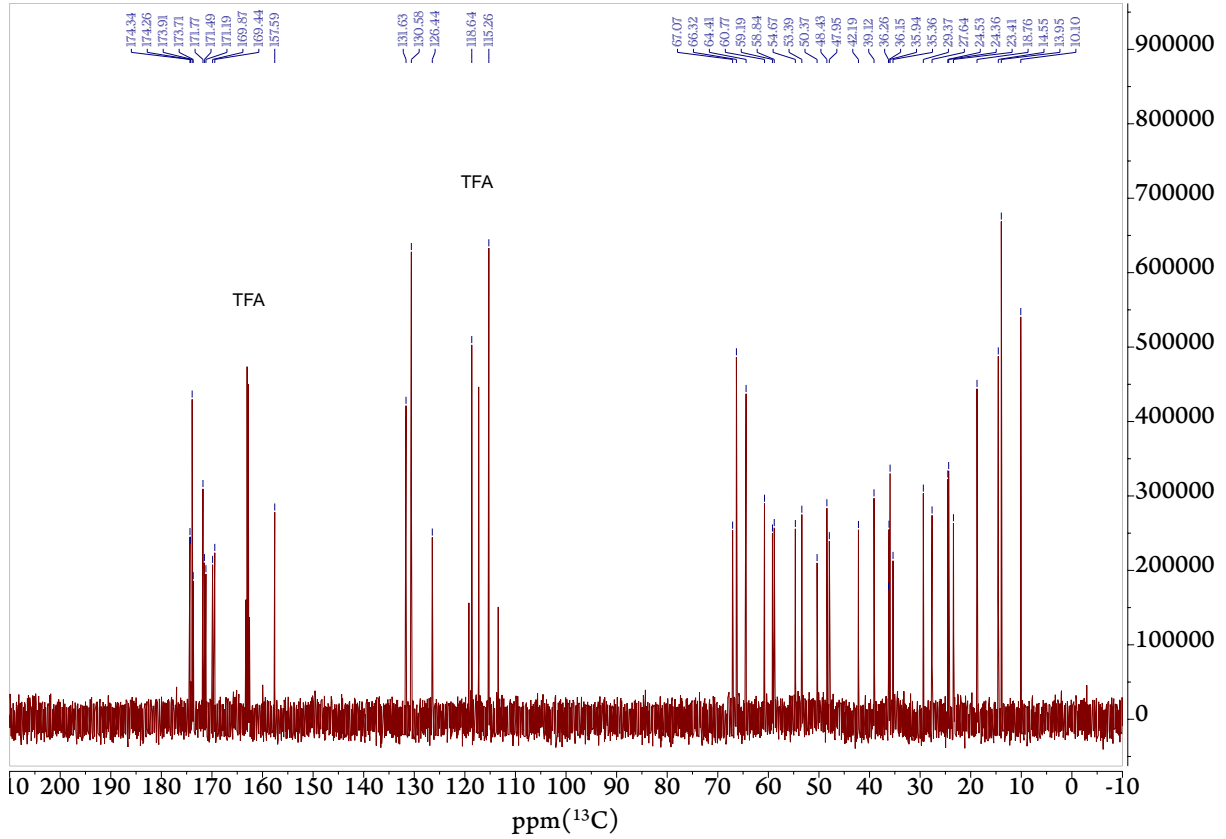
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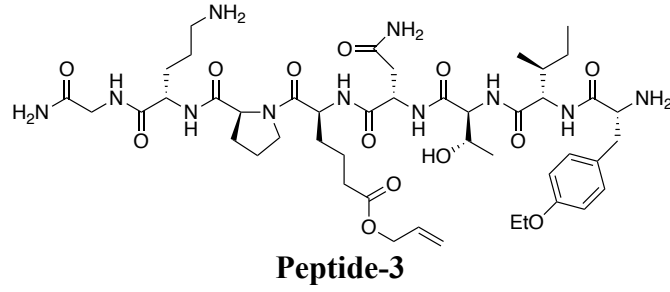
^{13}C NMR of above peptide in 5 % D₂O/CD₃OD at 298.1 K.



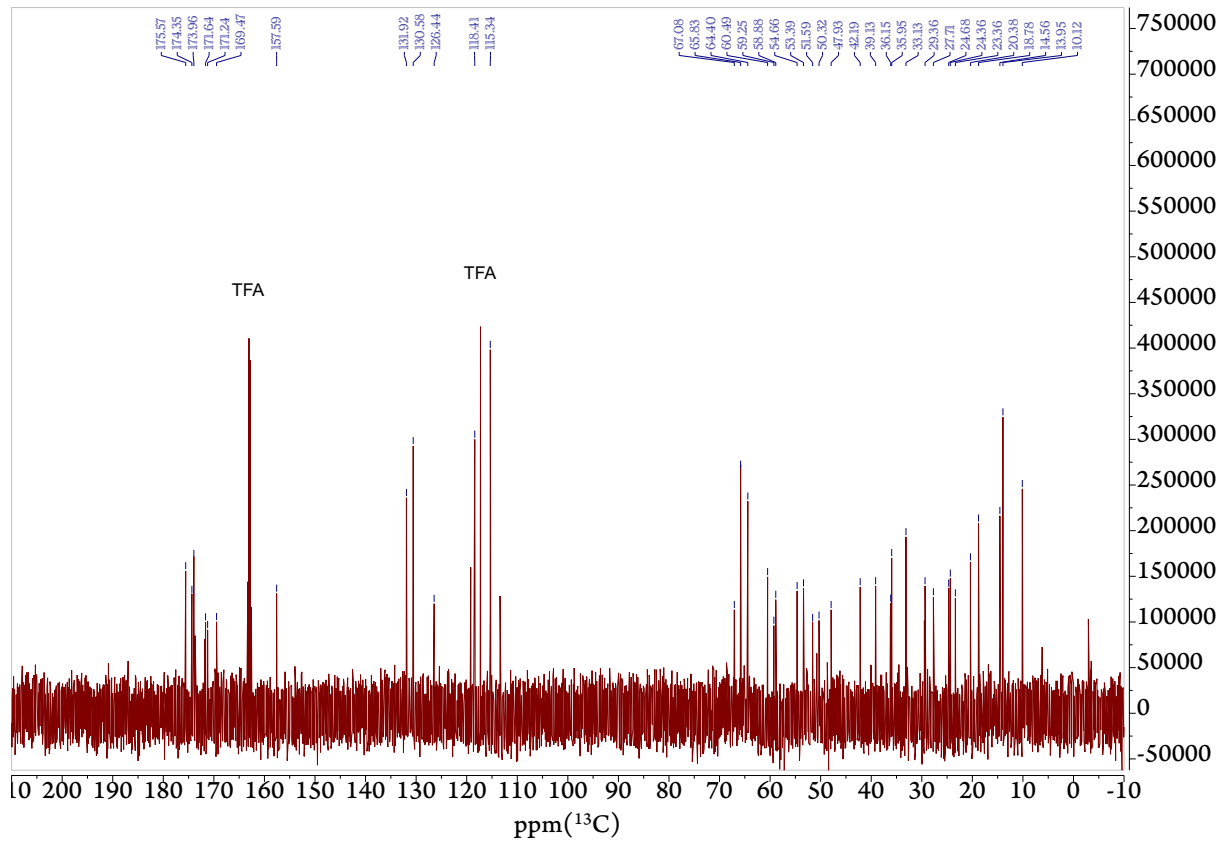
¹³C NMR (151 MHz)



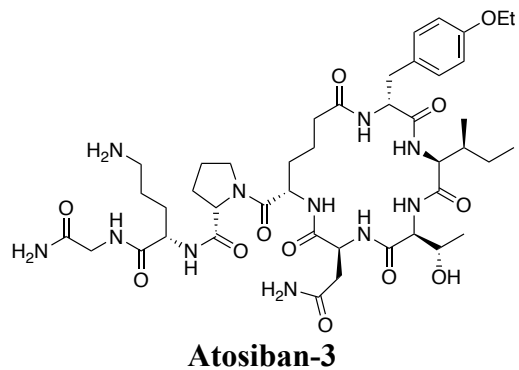
¹³C NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 90% H₂O/D₂O, at 298.1 K.



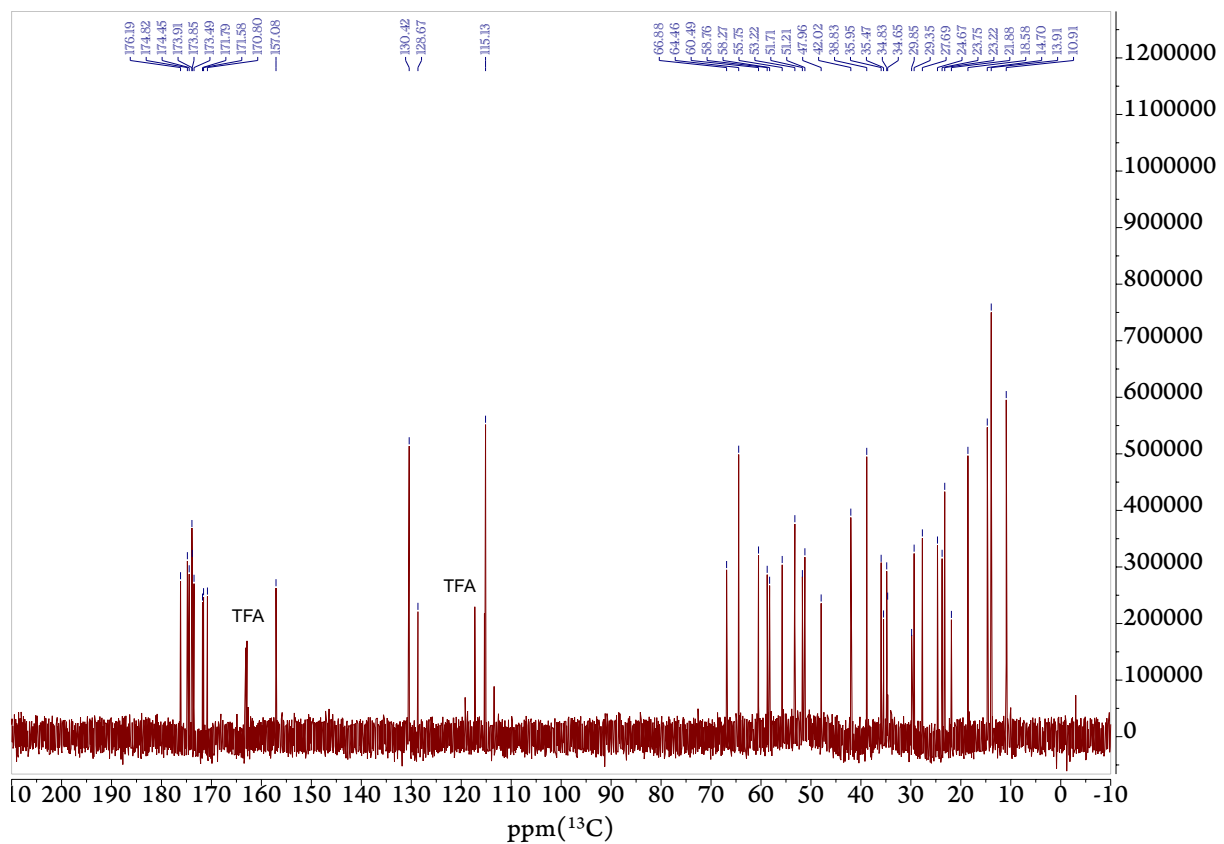
^{13}C NMR (151 MHz)



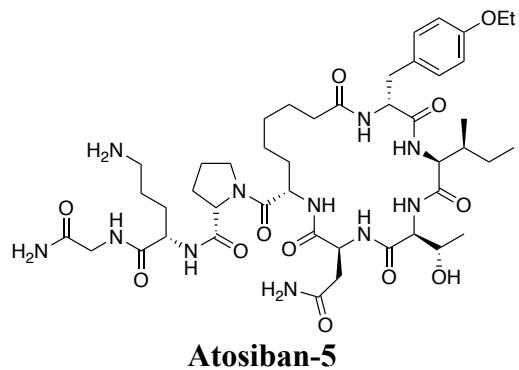
^{13}C NMR of above peptide in 50 mM Na_3PO_4 (pH 4.0), 100 μM TSP, 90% $\text{H}_2\text{O}/\text{D}_2\text{O}$, at 298.1 K.



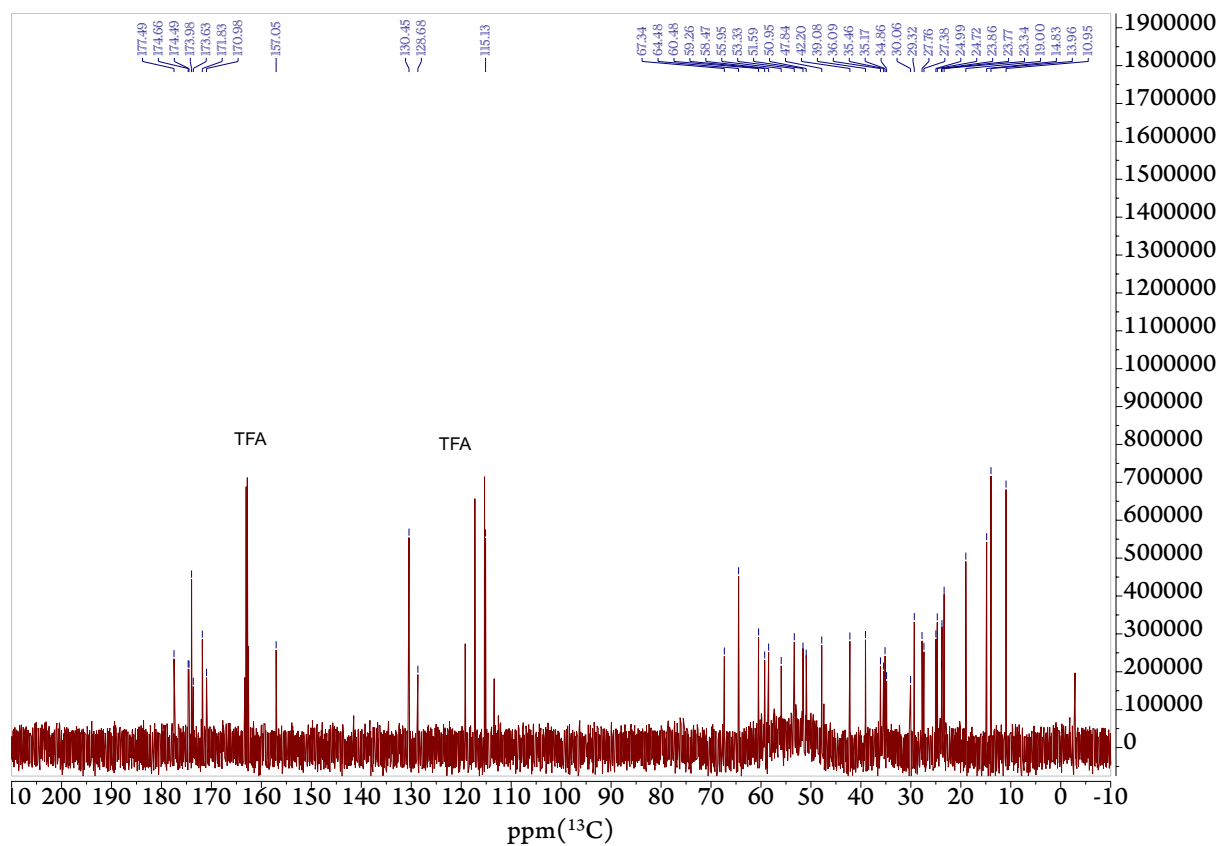
¹³C NMR (151 MHz)



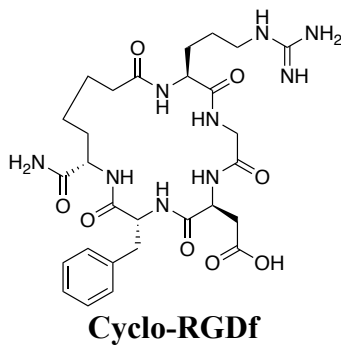
¹³C NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



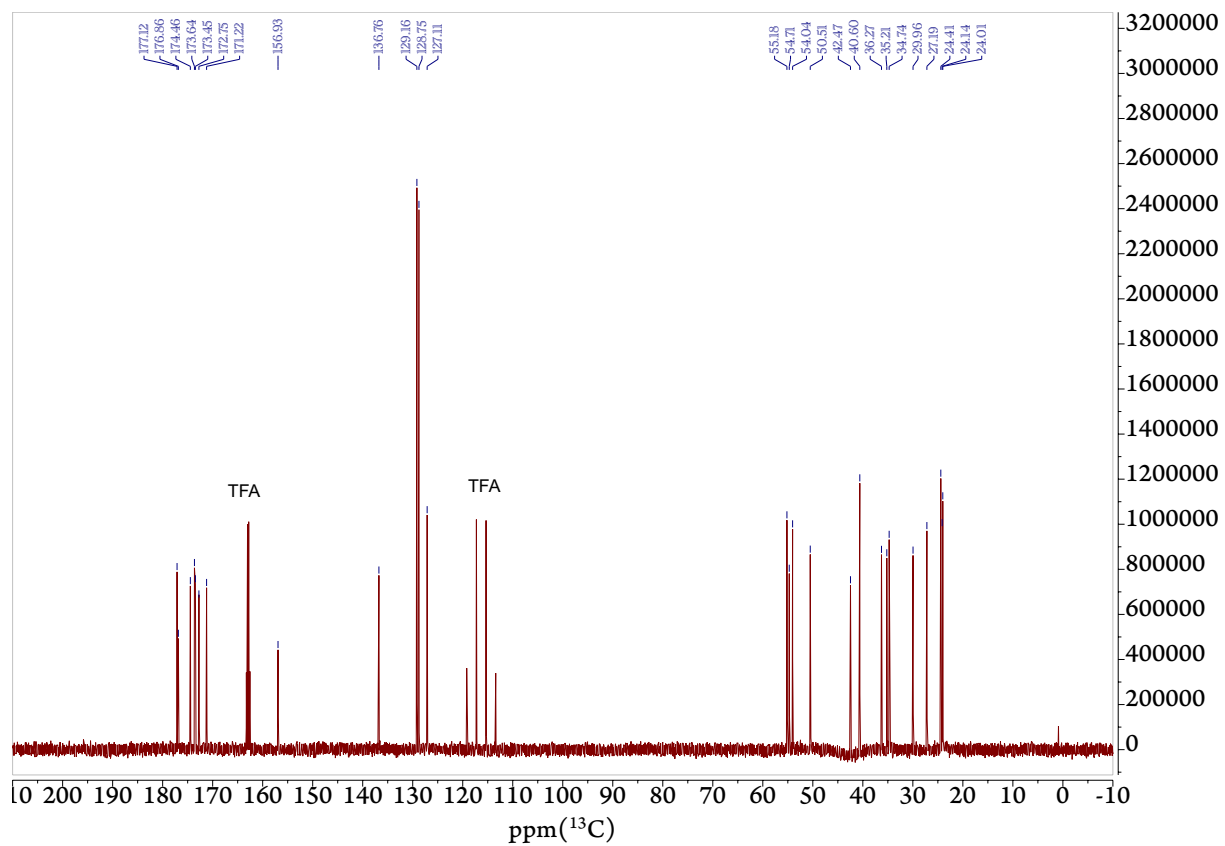
¹³C NMR (151 MHz)



¹³C NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 100% D₂O, at 298.1 K.



¹³C NMR (151 MHz)



¹³C NMR of above peptide in 50 mM Na₃PO₄ (pH 4.0), 100 μM TSP, 10% D₂O/H₂O, at 298.1 K.

References

- (1) Qin, T.; Malins, L. R.; Edwards, J. T.; Merchant, R. R.; Novak, A. J. E.; Zhong, J. Z.; Mills, R. B.; Yan, M.; Yuan, C.; Eastgate, M. D.; Baran, P. S. Nickel-Catalyzed Barton Decarboxylation and Giese Reactions: A Practical Take on Classic Transforms. *Angew. Chem. Int. Ed.* **2017**, *56* (1), 260–265. <https://doi.org/10.1002/anie.201609662>.