

Supporting information

Oxidative modification of tryptophan-containing peptides

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Contents

| | |
|---|-----------|
| Abbreviations | S2 |
| General Methods | S2 |
| Experimental Methods | S3 |
| Crude RP-UPLC, MS, ¹H and ¹³C NMR Data | S4 |

Abbreviations

Ac: acetate; BEH: ethylene bridged hybrid; DAD: diode-array detector; DMF: dimethyl formamide; DMSO: dimethyl sulfoxide; Et: ethyl; ESI: electrospray ionization; Fmoc: 9-fluorenylmethoxycarbonyl; Fmoc-Rink amide linker: 4-[(2,4-dimethoxyphenyl)(Fmoc-amino)methyl]phenoxyacetic acid; HATU: *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; DDQ: 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; HMBA: 4-hydroxymethylbenzoic acid; HPLC: high-performance liquid chromatography; HRMS: high resolution mass spectrometry; LED: light-emitting diode; LC: liquid chromatography; Me: methyl; MS: mass spectrometry; MSNT: 1-(2-mesitylenesulfonyl)-3-nitro-1*H*-1,2,4-triazole; NEM: *N*-ethyl morpholine; NMP: *N*-methyl-2-pyrrolidone; NMR: nuclear magnetic resonance; PBS: phosphate buffered saline; PDA: photodiode-array detector; PEGA: poly(ethylene glycol) dimethyl acrylamide; PE: polyethylene; PP: polypropylene; RP: reverse phase; SQD: single quadropole detector; TBTU: *N*-[(1*H*-Benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium tetrafluoroborate *N*-oxide; TFA: trifluoroacetic acid; UPLC: ultra-performance liquid chromatography; UV: ultra violet.

General Methods

All reagents utilised were commercially available and used without further purification, moreover solvents employed were of HPLC quality. Solid-phase synthesis was carried out using plastic syringes fitted with Teflon filters, which permit suction to be applied to the syringes for removal of reagents and solvents. Base mediated release of products was preceded in glass vials. Solid-phase peptide synthesis was performed on ChemMatrix beads (150-300 µm) supplied from PCAS Biomatrix Inc. and with a loading of 0.3 mmol/g.

Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC) was carried out on an analytical Waters Alliance 2695 HPLC system using Symmetry® C-18 column (3.5 µm, 4.6 x 75 mm; column temp.: 25 °C) with UV detection at 215 nm and 254 nm provided by a diode array detector. The elution was performed using a linear gradient from 100% eluent A (0.1% TFA in H₂O) to 100% eluent B (0.1% TFA in CH₃CN) with a run time of 12.70 min at a flow rate of 1 mL/min.

Analytical LC-MS (ESI) analysis was done using a Waters AQUITY UPLC equipped with a photo diode array and a single quadrupole electrospray mass spectrometer detector. The system utilizes an AQUITY UPLC BEH C-18 column (1.7 μm , 2.1 \times 50 mm; column temp.: 65 $^{\circ}\text{C}$). Eluent A (0.1% HCO_2H in H_2O) and B (0.1% HCO_2H in CH_3CN) were used in a linear gradient (5% B to 100% B) in a run time of 2.6 min at a flow rate of 0.6 mL/min.

A Varian Mercury-300 spectrometer operating at 300 MHz for proton and 75 MHz for carbon was employed for the recording of ^1H NMR and ^{13}C NMR. All spectra were recorded using $\text{DMSO-}d_6$ as solvent, and chemical shifts were measured relative to the solvent signal for $\text{DMSO-}d_6$ (singlet at 2.50 ppm for ^1H NMR, and the septet at 39.43 ppm for ^{13}C NMR). Chemical shifts (δ) are given in ppm and the coupling constants (J) in Hz. Multiplicities are described using the abbreviation s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet. Compounds synthesized in solid-phase were not analyzed through ^{13}C NMR.

Fluorescence intensity was detected by a Tecan Infinite M200pro microplate reader, which first records the absorbance properties to identify the required wavelength for excitation of the compound. The fluorescence was then measured in the arbitrary unit 'Relative Fluorescence Units' (RFU) and plotted against their respective wavelengths. The measurements were acquired setting the gain to 70. Methanol was used as a solvent.

Experimental Methods

Prior to solid-phase synthesis, PEGA800 (0.4 mmol/g) resin was sequentially washed with EtOH (x 6), H_2O (x 6), DMF (x 6), MeOH (x 6), CH_2Cl_2 (x 6) and finally dried overnight under vacuum. An amount of PEGA800 resin was then transferred to a plastic syringe fitted with a Teflon filter, and the resin was swelled with DMF (x 4) before each of the subsequent couplings. Attachment of the Fmoc-protected amino acid to the amino functionalized PEGA800 resin was carried out by premixing TBTU (2.88 equiv.), NEM (4 equiv.) and the amino acid (3 equiv.) for 5 min in DMF. The solution was then added to the preswelled resin and allowed to react for 2 hours. The volume of DMF was kept at a level that allowed the resin to be fully swelled and at the same time

maintained a high concentration of reagents. The reaction mixture was removed with suction from below the syringes, and the resin was subsequently washed with DMF (x 6) and CH₂Cl₂ (x 6). The Kaiser test after each coupling to check if the couplings had been successful. Three solutions were prepared for the Kaiser test: 1) 500 mg ninhydrin in 10 mL ethanol, 2) 80 g of liquefied phenol in 20 mL EtOH, 3) 2 mL 0.001 M KCN diluted to 100 mL with pyridine. A small amount of resin was placed in a glass tube and two drops of each solution were added, the glass tube was then shortly heated with a heat gun. The presence of free amino group was detected if the beads turned blue.

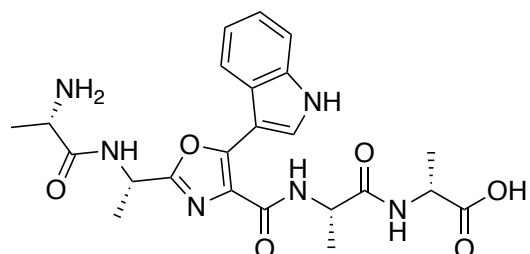
Fmoc-deprotection was accomplished by applying 20% piperidine in DMF to the preswelled resin in DMF for 2 min, followed by washing with DMF (x 2), and then further treatment with 20% piperidine in DMF for 18 min. The resin was afterwards washed with DMF (x 6). A HATU-mediated coupling was applied to couple an Fmoc-protected amino acid to the linker. The preswelled linker functionalised resin was treated with a solution of HATU (2.88 equiv.), DIPEA (4 equiv.) and amino acid (3 equiv.) in DMF after 5 min of preactivation and was left to react for 1 hour. Reagents were removed by DMF (x 6) and CH₂Cl₂ (x 6).

DDQ-oxidation was achieved by mixing 100-200 mg resin, 2 equiv. of DDQ and 4 mL MeCN in a 4.5 mL glass vial. The reaction was left overnight under vigorous shaking followed by removal of the reaction mixture and subsequently washing the resins with DMF (x6). The syringe containing 250-400 mg resin was added 10 mL of 20 % piperidine in DMF and placed on the shaker for 1 hour (repeated 2 times in a row), followed by wash with DMF (x 6) and CH₂Cl₂ (x 6).

Material adequate for ¹H NMR and fluorescent studies was obtained by cleaving from 150-250 mg resin. The resin-bound products were then released by treating the resin with a 0.1 N NaOH solution (aq) overnight, followed by neutralization with a 0.1 N HCl solution (aq). The resin was afterwards filtered and washed with CH₂Cl₂, and the filtrate and washings were concentrated *in vacuo* on either a rotary evaporator or on a speed vacuum concentrator.

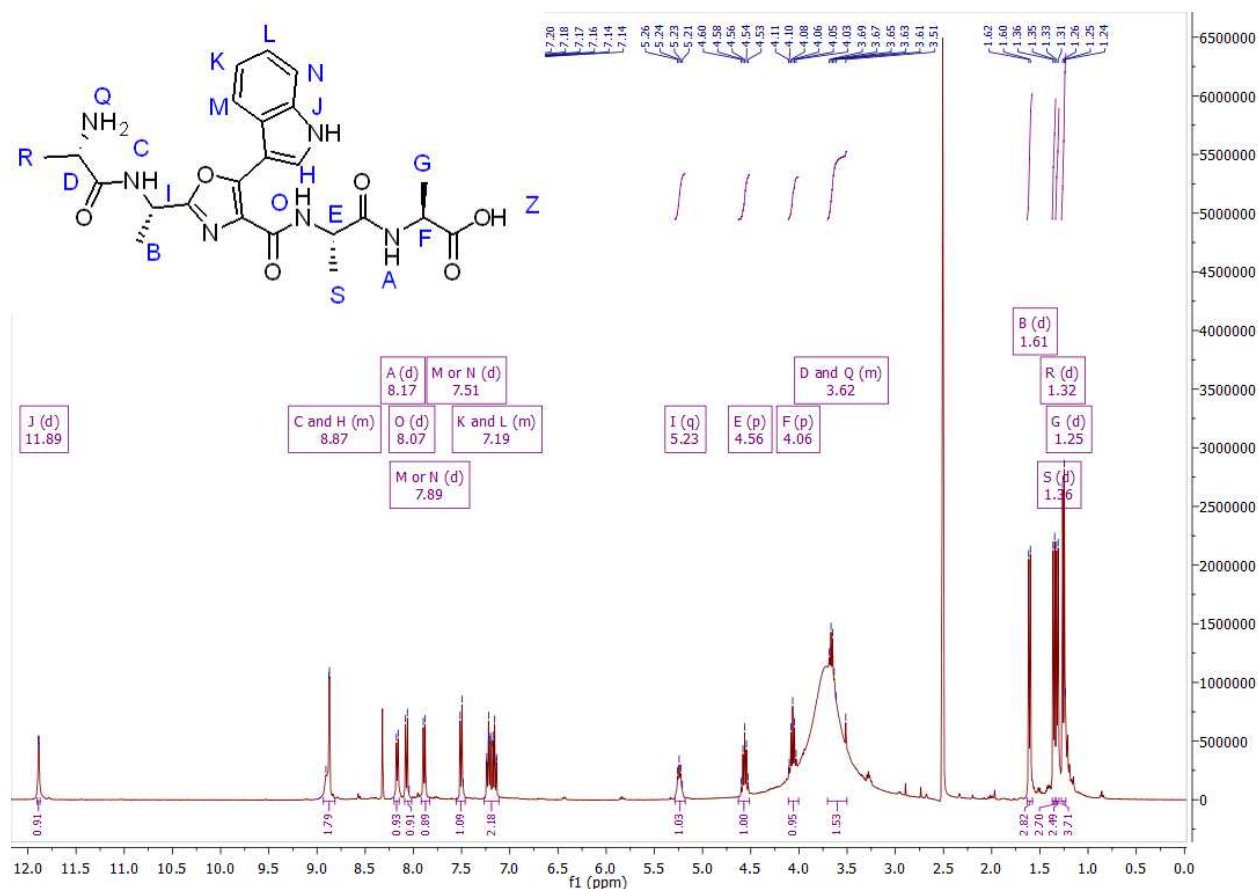
Crude RP-HPLC, RP-UPLC, MS, ¹H and ¹³C NMR Data

(2-((S)-1-((S)-2-aminopropanamido)ethyl)-5-(1*H*-indol-3-yl)oxazole-4-carbonyl)-*L*-alanine-*D*-alanine (10J)

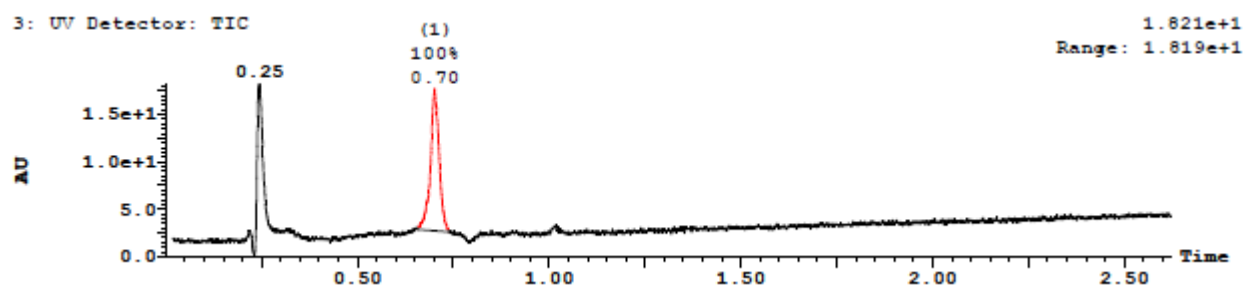


¹H NMR (400 MHz, DMSO-*d*₆): δ = 11.88 (d, *J* = 2.8 Hz, 1H), 8.93 – 8.84 (m, 2H) 8.16 (d, *J* = 7.1 Hz, 1H), 8.06 (d, *J* = 8.0 Hz, 1H), 7.88 (d, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.26 – 7.10 (m, 2H), 5.28 – 5.19 (m, 1H), 4.60 – 4.51 (m, 1H), 4.06

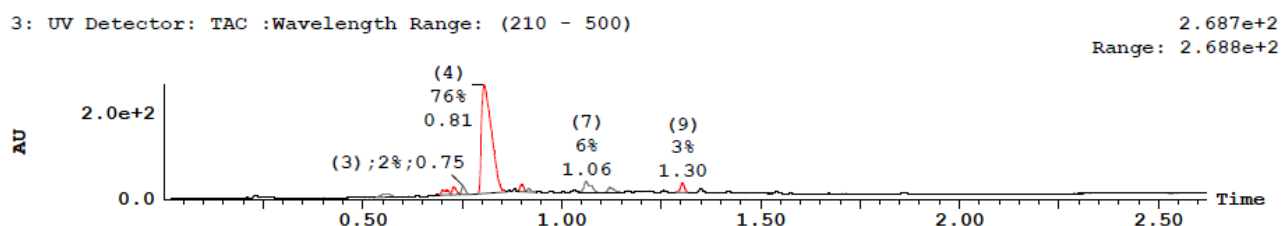
(t, *J* = 7.1 Hz, 1H), 3.90-3.50 (m, 3H), 1.60 (d, *J* = 7.0 Hz, 3H), 1.35 (d, *J* = 6.9 Hz, 3H), 1.31 (d, *J* = 6.9 Hz, 3H), 1.25 (d, *J* = 7.1 Hz, 3H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): *m/z* calcd. for C₂₃H₂₈N₆O₆ [M+H]⁺ = 485.1, found 485.1; HRMS (ESI): *m/z* calcd. for C₂₃H₂₈N₆O₆ [M+H]⁺ = 485.2143, found 485.2141. Purity = 88%, Rt = 0.81 min. Yield: 25 %.



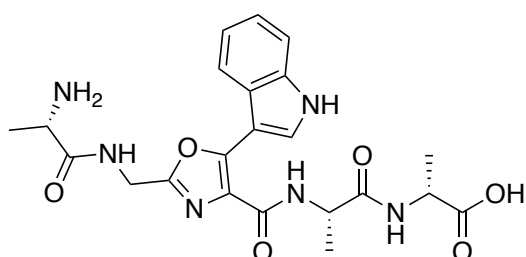
UPLC of crude peptide before oxidation (peak 1):



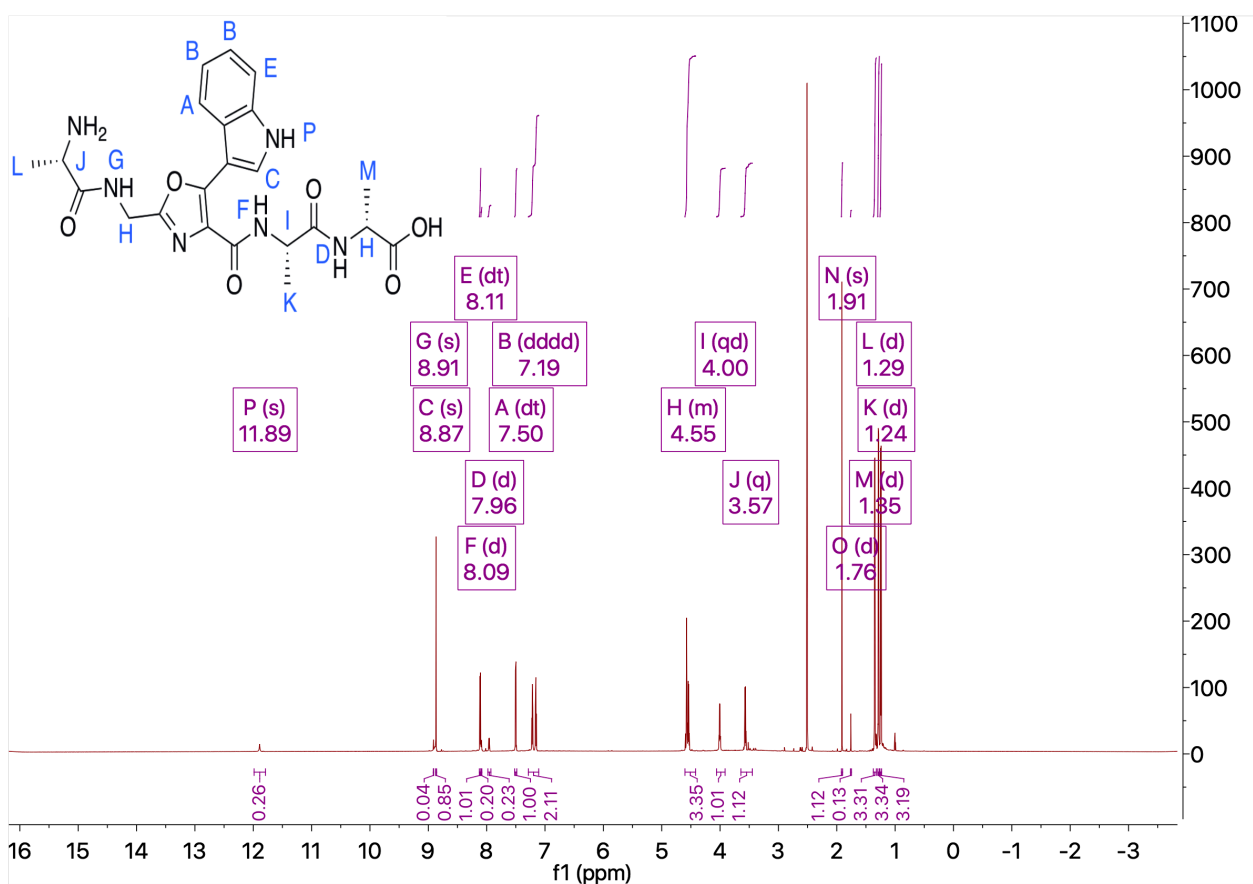
UPLC of crude peptide after oxidation (**10J**: peak 4, *rt* = 0.81):



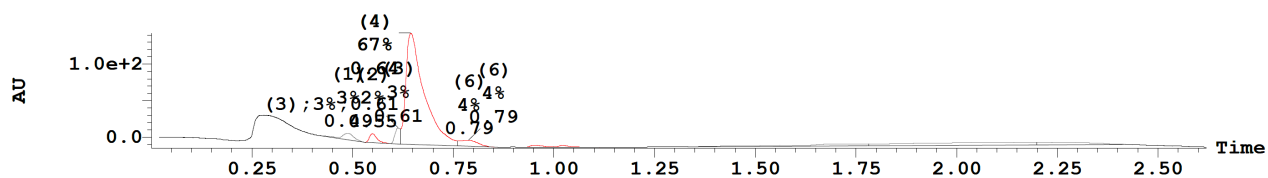
(2-(((*S*)-2-aminopropanamido)methyl)-5-(1*H*-indol-3-yl)oxazole-4-carbonyl)-*L*-alanyl-*D*-alanine (10L**)**



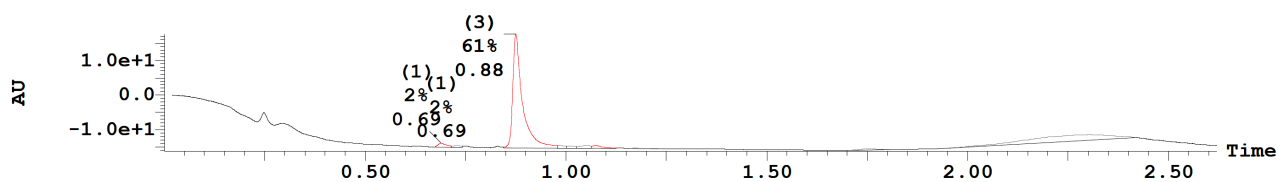
¹H NMR (800 MHz, DMSO-*d*₆) δ 11.89 (s, 1H), 8.91 (s, 1H), 8.87 (s, 1H), 8.11 (dt, *J* = 8.1, 1.0 Hz, 1H), 8.09 (d, *J* = 7.0 Hz, 1H), 7.96 (d, *J* = 8.0 Hz, 1H), 7.50 (dt, *J* = 8.1, 1.0 Hz, 1H), 7.19 (dddd, *J* = 49.6, 8.0, 6.9, 1.1 Hz, 2H), 4.60 – 4.42 (m, 3H), 4.00 (qd, *J* = 7.1, 3.5 Hz, 1H), 3.57 (q, *J* = 6.9 Hz, 1H), 1.91 (s, 1H, impurity), 1.35 (d, *J* = 7.0 Hz, 3H), 1.29 (d, *J* = 6.9 Hz, 3H), 1.24 (d, *J* = 7.1 Hz, 3H). UPLC/MS (ESI): *m/z* calcd. for C₂₂H₂₆N₆O₆ [*M*+*H*]⁺ = 471.2, found 471.2. HRMS (ESI): *m/z* calcd. for C₂₂H₂₇N₆O₆ [*M*+*H*]⁺ = 471.1987, found 471.1990. Purity: >95 %, *Rt* = 0.88 min. Yield: 31 %.



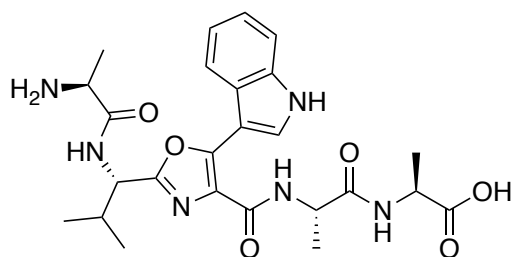
UPLC of crude peptide before oxidation (peak 4, *rt* = 0.68):



UPLC of crude peptide after oxidation (**10L**: peak 3, *rt* = 0.88):

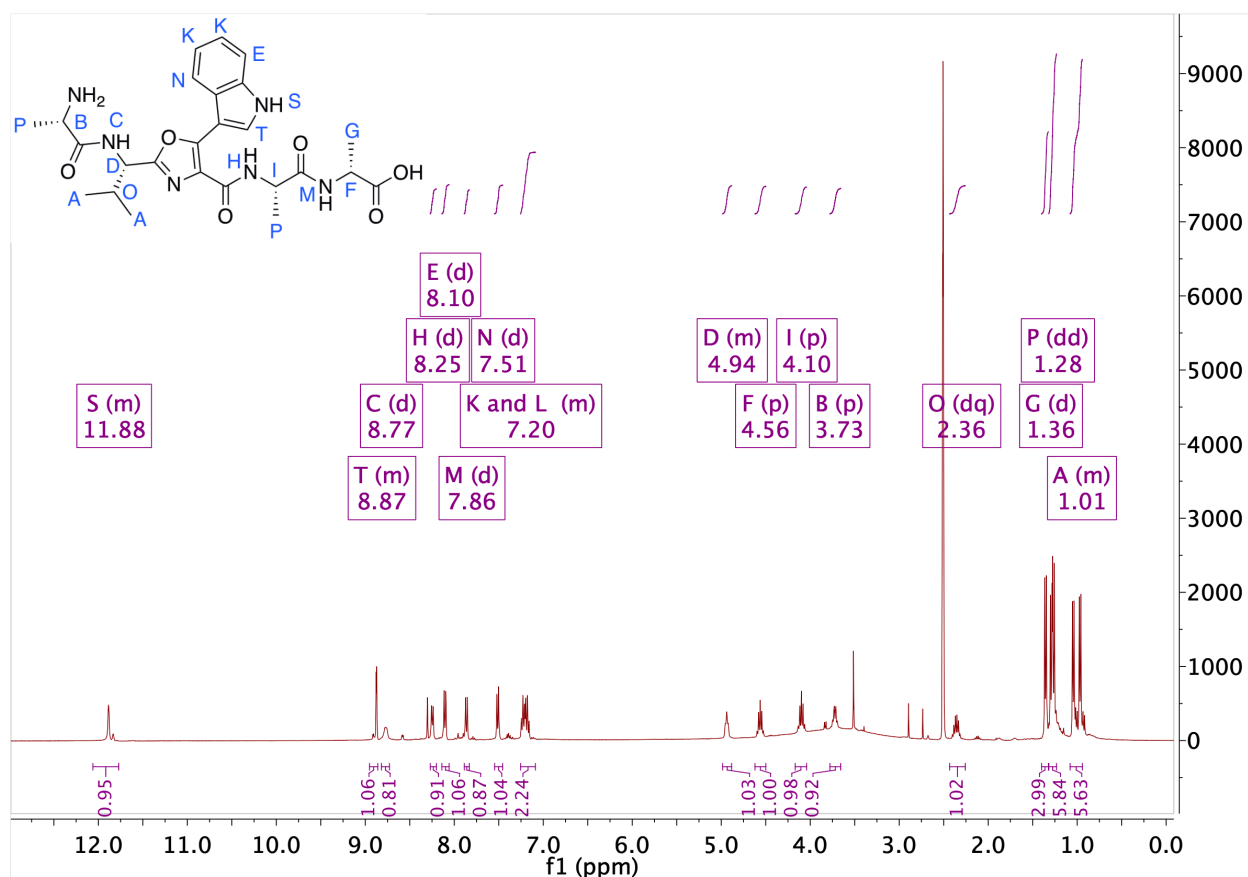


(2-((S)-1-((S)-2-aminopropanamido)-2-methylpropyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10M)

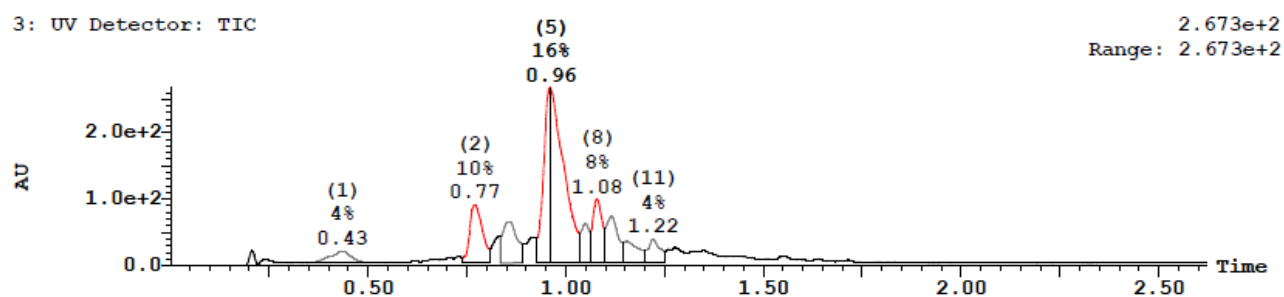


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 12.06 – 11.77 (m, 1H), 8.95 – 8.86 (m, 1H), 8.77 (d, J = 8.2 Hz, 1H), 8.25 (d, J = 7.1 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.51 (d, J = 7.9 Hz, 1H), 7.25 – 7.14 (m, 2H), 4.98 – 4.88 (m, 1H), 4.56

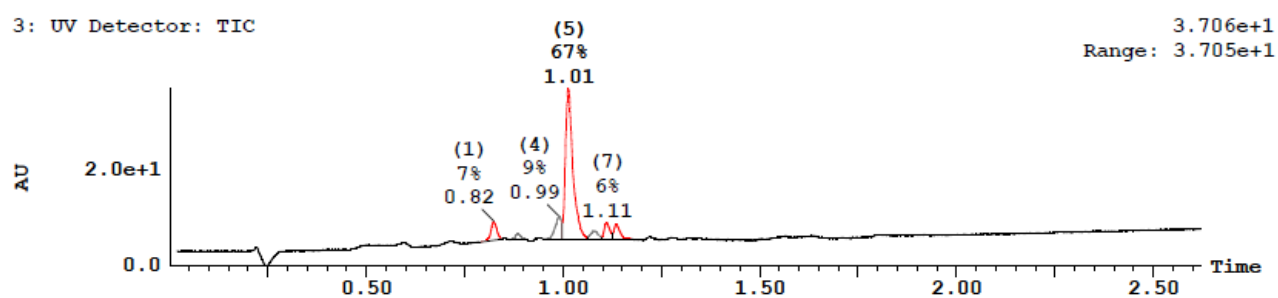
(p, J = 6.9 Hz, 1H), 4.10 (p, J = 7.1 Hz, 1H), 3.73 (m, 1H), 2.36 (dq, J = 13.9, 6.9 Hz, 1H), 1.36 (d, J = 6.9 Hz, 3H), 1.28 (dd, J = 10.5, 7.1 Hz, 6H), 1.03 (dd, J = 14.3, 6.7 Hz, 3H), 0.95 (dd, J = 17.0, 6.8 Hz, 3H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. for $\text{C}_{23}\text{H}_{28}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 513.2$, found 513.2. $[\text{M}+\text{H}]^+$. HRMS (ESI): m/z calcd. for $\text{C}_{25}\text{H}_{33}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 513.2456$, found 513.2457. Purity: 75 %, R_t = 1.01 min. Yield: 15 %.



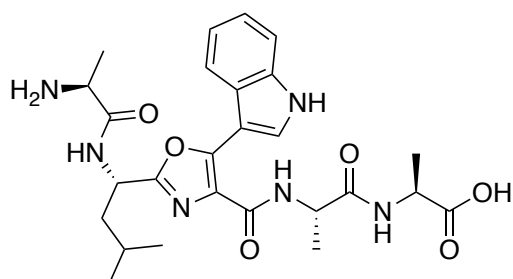
UPLC of crude peptide before oxidation (peak 5, $t_r = 0.96$ min):



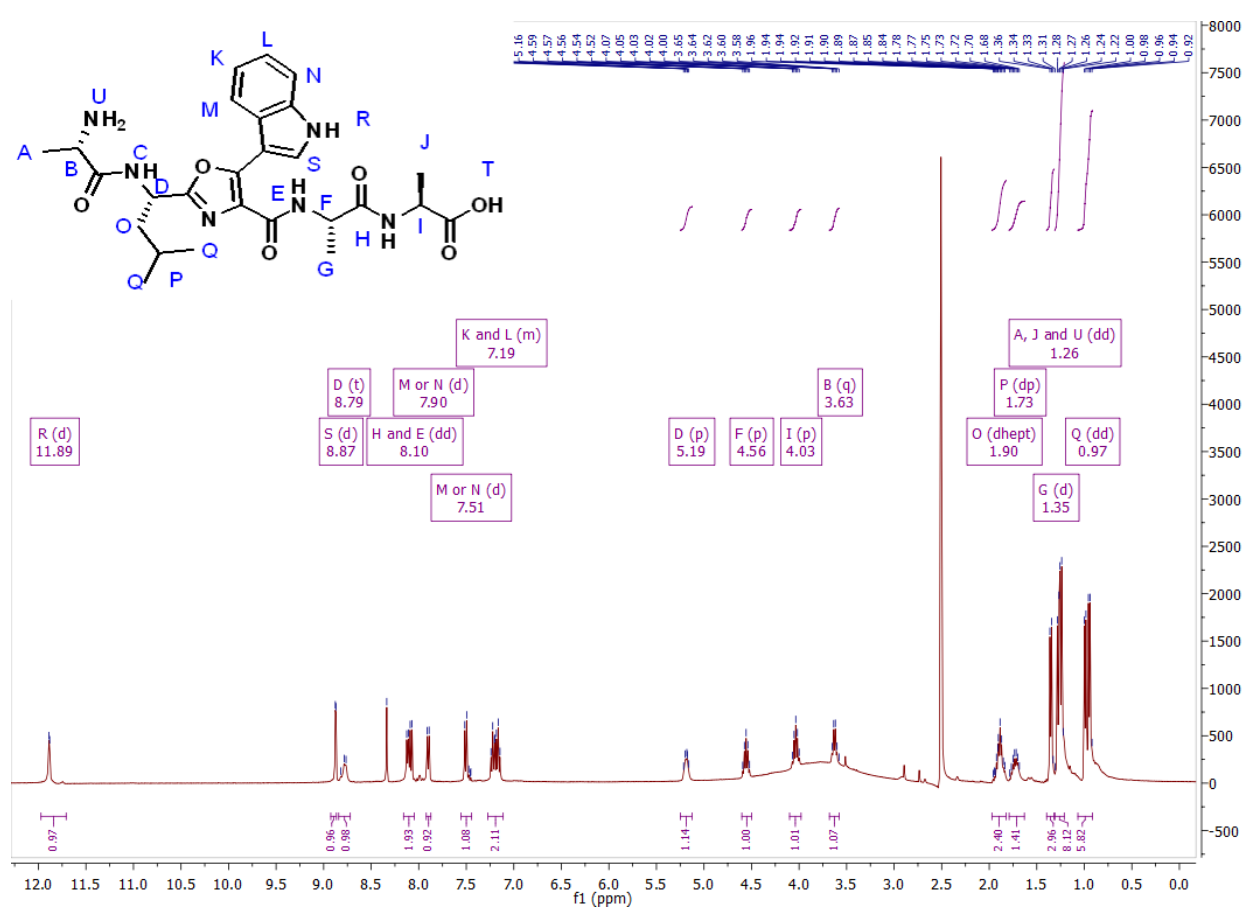
UPLC of crude peptide after oxidation (**10M**: $t_r = 1.01$ min):



(2-((S)-1-((S)-2-aminopropanamido)-3-methylbutyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10N)



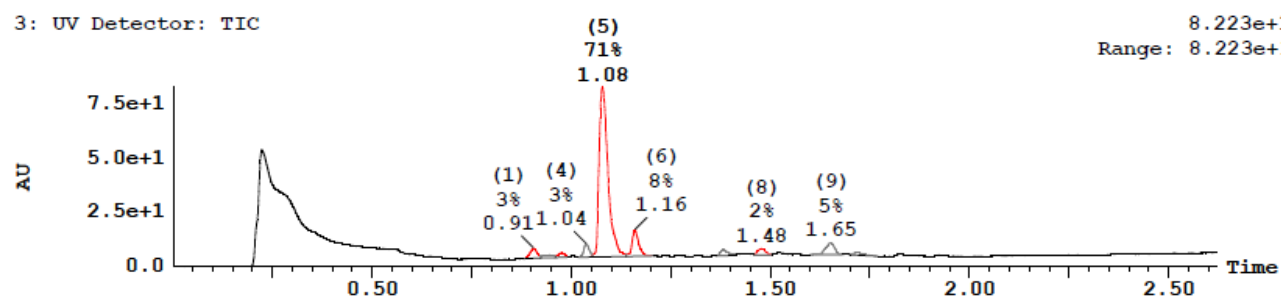
^1H NMR (300 MHz, $\text{DMSO-}d_6$): δ = 11.89 (d, J = 2.9 Hz, 1H), 8.87 (d, J = 2.6 Hz, 1H), 8.79 (t, J = 11.1 Hz, 1H), 8.10 (dd, J = 15.0, 7.5 Hz, 2H), 7.90 (d, J = 7.7 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.27 – 7.11 (m, 2H), 5.19 (p, J = 6.2, 5.7 Hz, 1H), 4.56 (p, J = 7.0 Hz, 1H), 4.03 (p, J = 7.1 Hz, 1H), 3.63 (q, J = 6.9 Hz, 1H),, 1.90 (m, 1H), 1.73 (m, 1H), 1.35 (d, J = 6.9 Hz, 3H), 1.26 (dd, J = 11.2, 7.0 Hz, 7H), 0.97 (dd, J = 17.1, 6.5 Hz, 6H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. for $\text{C}_{26}\text{H}_{35}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 527.3$, found 527.3. HRMS (ESI): m/z calcd. for $\text{C}_{26}\text{H}_{35}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 527.2613$, found 527.2617. Crude purity: 75 %, R_t = 1.08 min. Yield: 20 %.



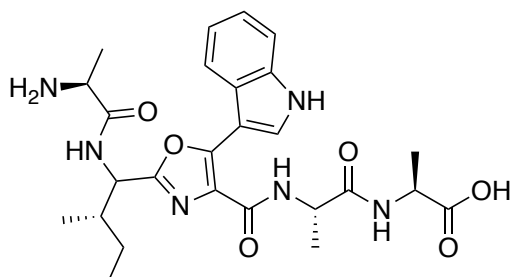
UPLC data of crude peptide after oxidation (**10N**: rt = 1.08 min):

3: UV Detector: TIC

8.223e+1
Range: 8.223e+1

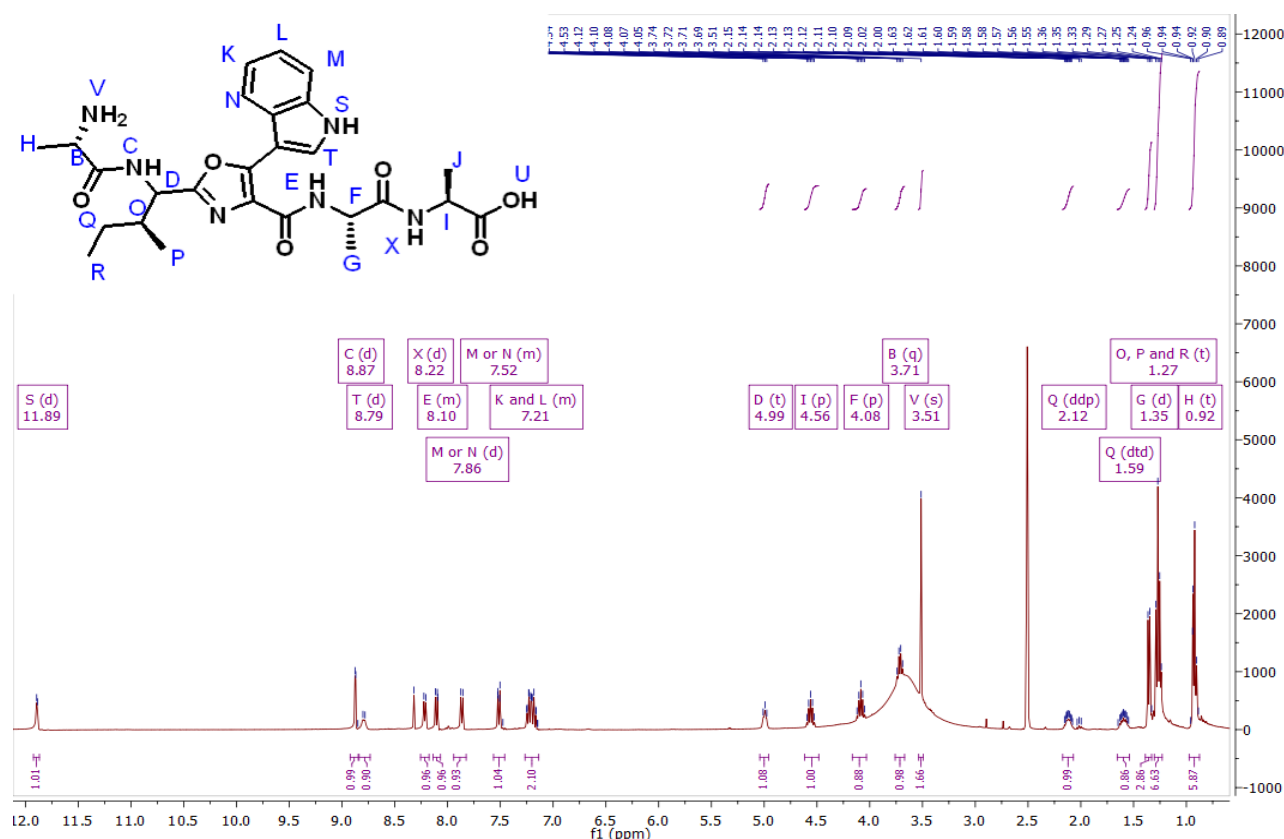


(2-((2S)-1-((S)-2-aminopropanamido)-2-methylbutyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (100)



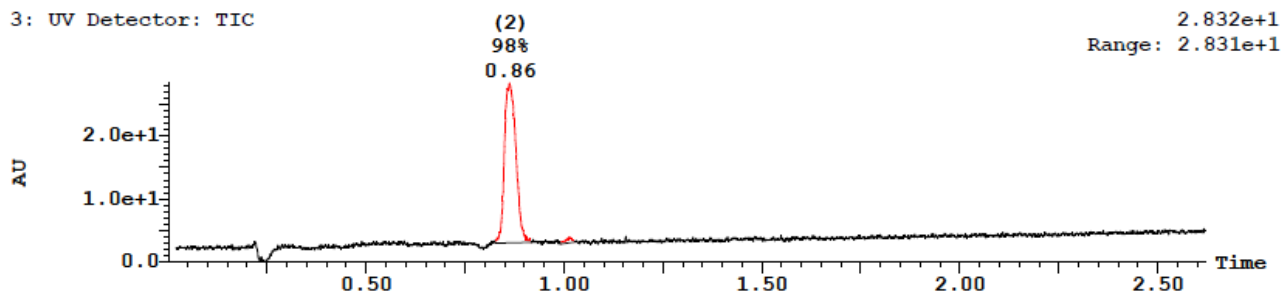
^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.89 (d, J = 2.9 Hz, 1H), 8.87 (d, J = m, 1H), 8.79 (d, J = 8.0 Hz, 1H), 8.22 (d, J = 7.2 Hz, 1H), 8.14 – 8.07 (m, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.56 – 7.46 (m, 1H), 7.27 – 7.14 (m, 2H), 4.99 (t, J = 6.9 Hz, 1H), 4.56 (p, J = 6.9 Hz, 1H), 4.08 (p, J = 7.1 Hz, 1H), 3.71 (q, J = 6.9

Hz, 1H), 3.51 (s, 2H), 2.12 (m, J = 10.9, 7.0, 3.6 Hz, 1H), 1.59 (m, J = 14.8, 7.4, 4.0 Hz, 1H), 1.35 (d, J = 6.9 Hz, 3H), 1.27 (t, J = 7.1 Hz, 7H), 0.92 (t, J = 6.8 Hz, 6H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{26}\text{H}_{35}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 527.3$, found 527.3. HRMS (ESI): m/z calcd. for $\text{C}_{26}\text{H}_{35}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 527.2613$, found 527.2617. Purity: 75 %, R_t = 1.05 min. Yield: 17 %.



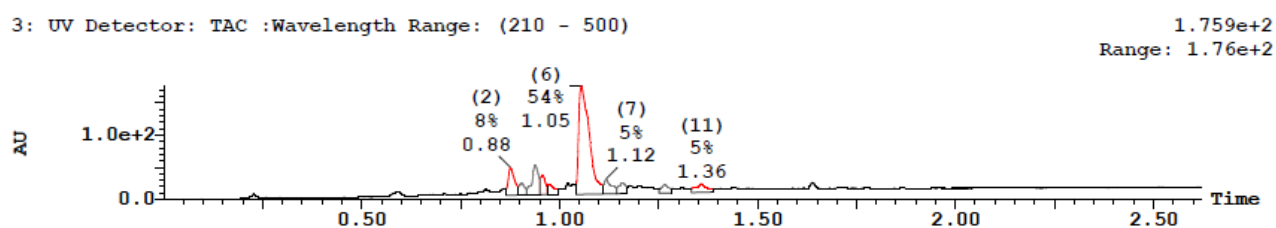
UPLC of crude peptide before oxidation (peak 2, r_t = 0.86 min):

3: UV Detector: TIC

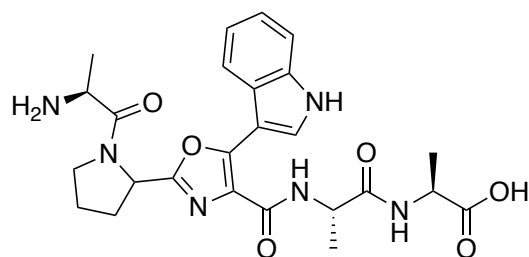


UPLC of crude peptide after oxidation (**100**: peak (6), rt = 1.05 min):

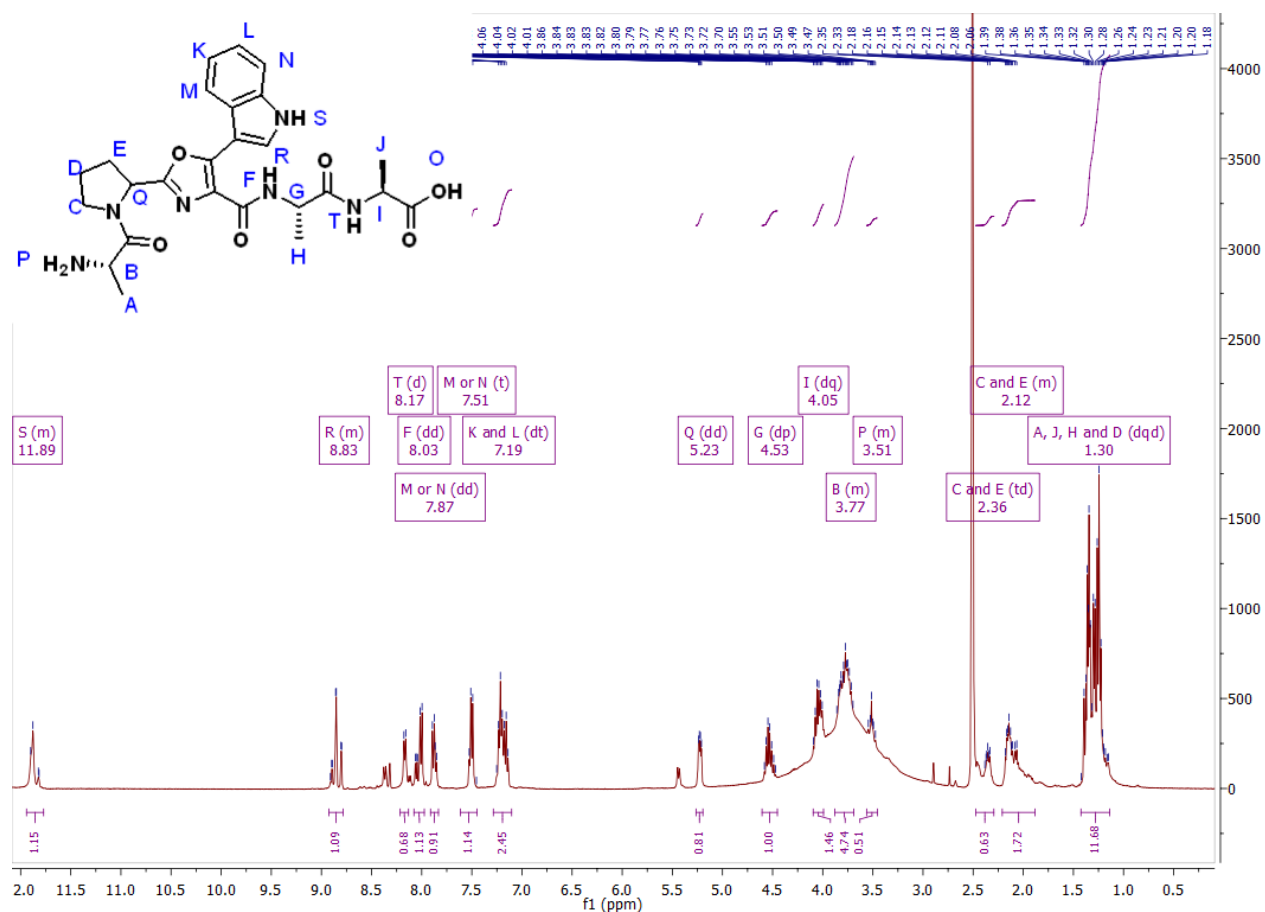
3: UV Detector: TAC :Wavelength Range: (210 - 500)



(2-(1-(L-alanyl)pyrrolidin-2-yl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10P)

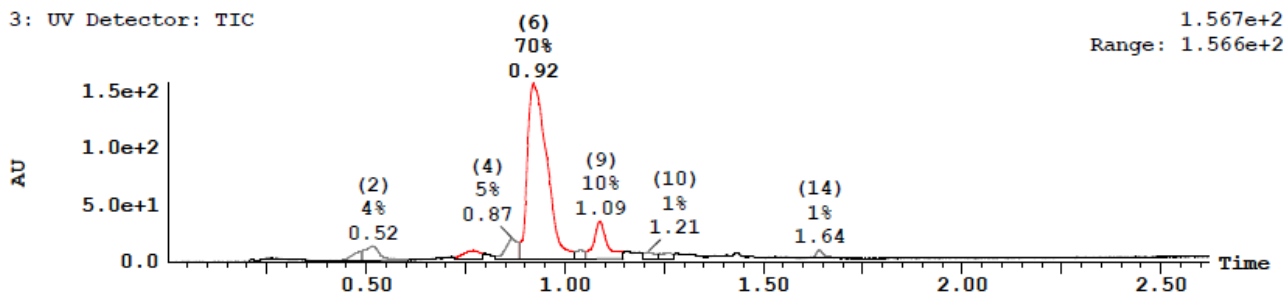


^1H NMR (400 MHz, $\text{DMSO-}d_6$): δ = 11.94 – 11.77 (m, 1H), 8.99 – 8.77 (m, 1H), 8.17 (d, J = 7.1 Hz, 1H), 8.03 (dd, J = 19.6, 7.3 Hz, 1H), 7.87 (dd, J = 9.8, 6.8 Hz, 1H), 7.51 (t, J = 6.9 Hz, 1H), 7.19 (m, 2H), 5.23 (dd, J = 8.0, 4.0 Hz, 1H), 4.53 (p, J = 7.2 Hz, 1H), 4.05 (dq, J = 13.7, 7.0 Hz, 1H), 3.88 – 3.69 (m, 1H) 3.56 – 3.46 (m, 2H), 2.36 (m, 2H), 2.21 – 1.88 (m, 2H), 1.30 (m, 11H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{25}\text{H}_{34}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 511.2$, found 511.2. HRMS (ESI): m/z calcd. for $\text{C}_{25}\text{H}_{31}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 511.2300$, found 511.2341. Purity calc.: 77 %, R_t = 0.92 min. Yield: 31 %.

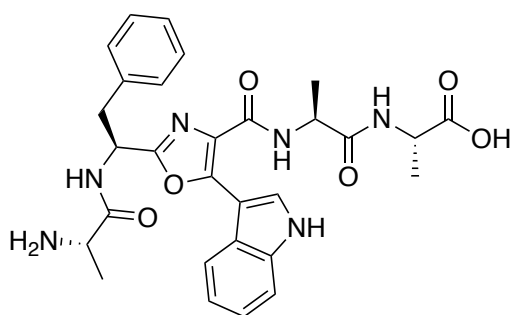


UPLC data for crude peptide after oxidation (10P: peak (6), r_t = 0.92 min):

3: UV Detector: TIC

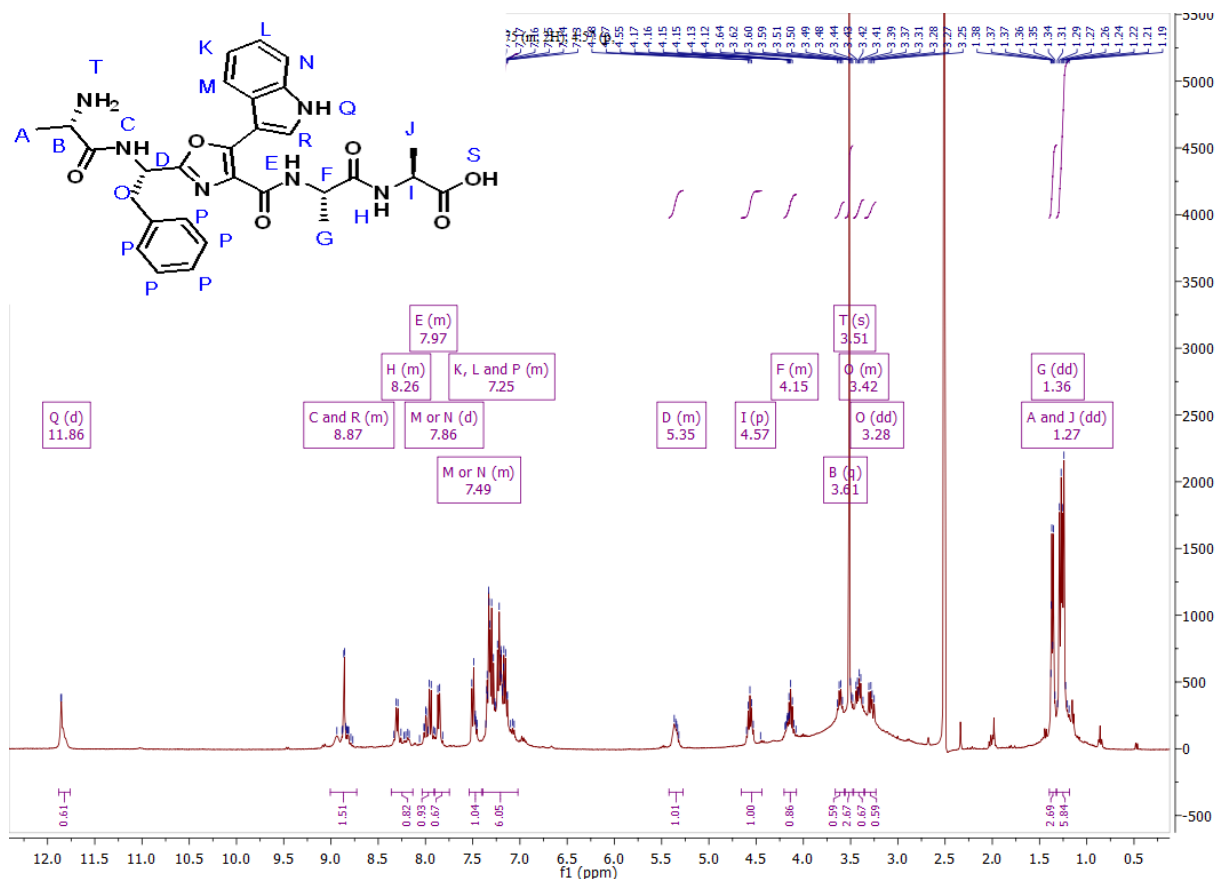


(2-((S)-1-((S)-2-aminopropanamido)-2-phenylethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10Q)



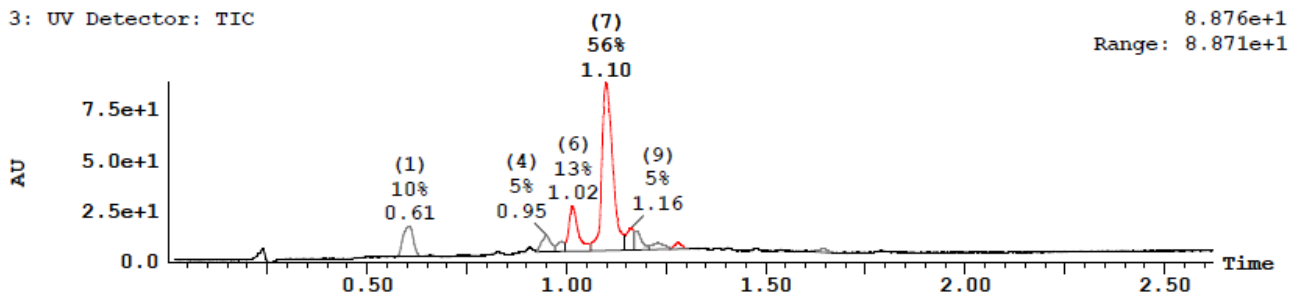
^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.88 (d, J = 3.0 Hz, 1H), 8.86 (m, 2H), 8.36 – 8.13 (m, 1H) 7.95 (d, J = 7.9 Hz, 1H), 8.04 - 7.89 (m, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.38 – 7.09 (m, 6H), 5.36 (t, J = 7.5 Hz, 1H), 4.56 (p, J = 7.0 Hz, 1H), 4.07 (p, J = 7.1 Hz, 1H), 3.61 (m, 1H), 3.51 (s, 2H), 3.41 (dd, J = 13.8,

6.3 Hz, 1H), 3.28 (dd, J = 13.8, 8.8 Hz, 1H), 1.36 (d, J = 6.9 Hz, 3H), 1.27 (m, 6H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{29}\text{H}_{36}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 561.2$, found 561.2. HRMS (ESI): m/z calcd. for $\text{C}_{29}\text{H}_{33}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+ = 561.2456$, found 561.2459. Purity: 63 %, R_t = 1.10 min. Yield: 24 %.

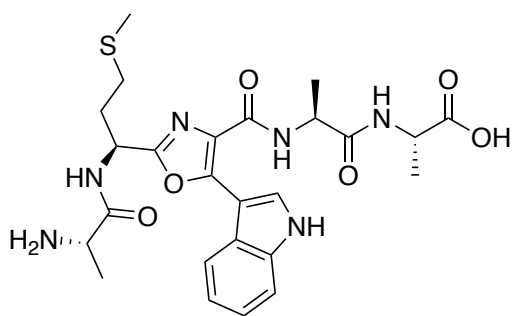


UPLC data of crude peptide after oxidation (**10Q**: peak (7), rt = 1.10):

3: UV Detector: TIC

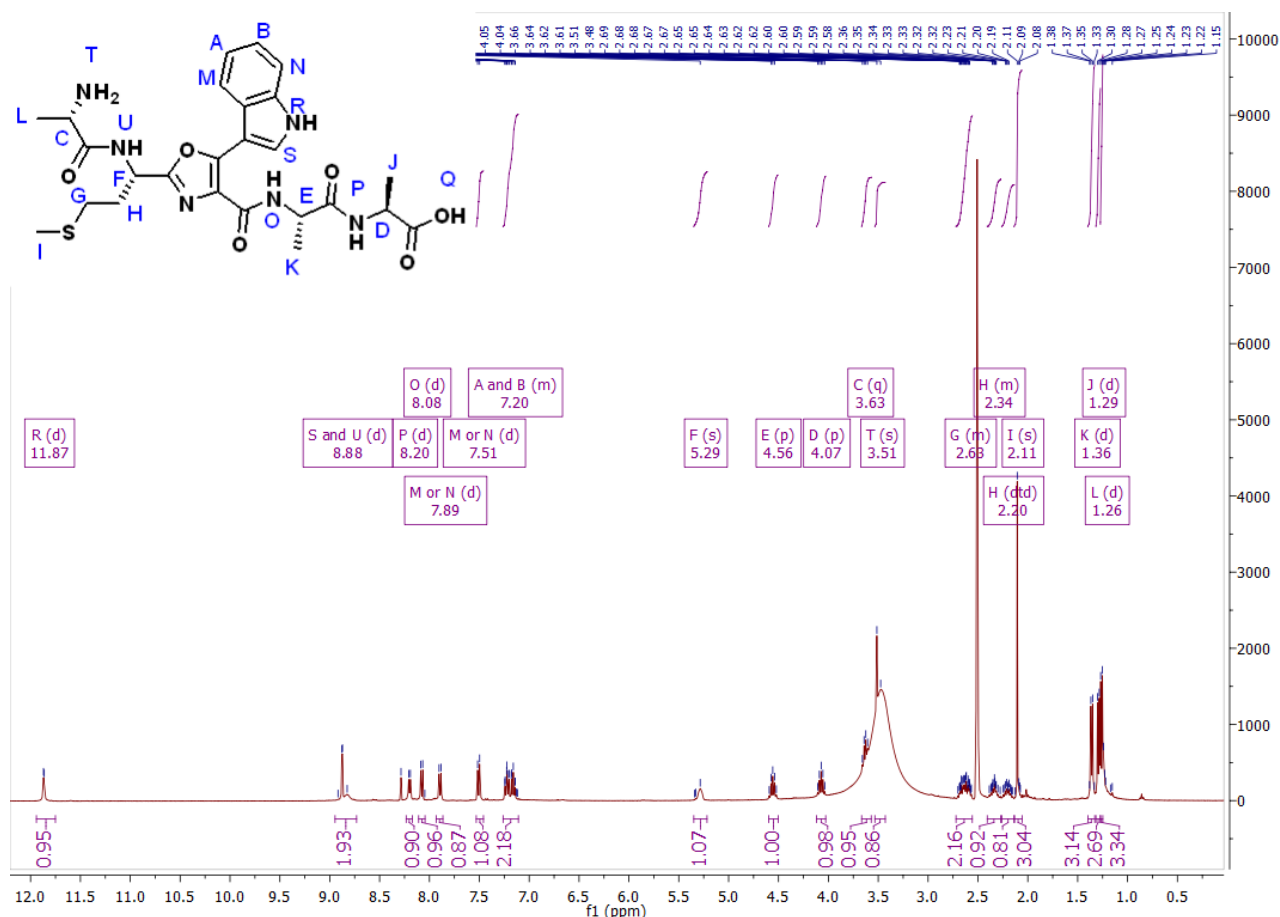


(2-((S)-1-((S)-2-aminopropanamido)-3-(methylthio)propyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10R)

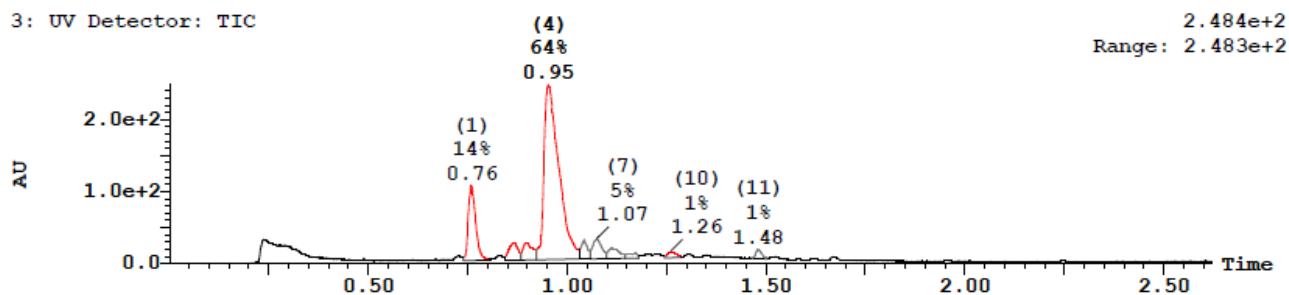


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.87 (d, J = 3.0 Hz, 1H), 8.88 (d, J = 2.8 Hz, 2H), 8.20 (d, J = 7.0 Hz, 1H), 8.08 (d, J = 7.9 Hz, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.26 – 7.11 (m, 2H), 5.29 (s, 1H), 4.56 (p, J = 7.0 Hz, 1H), 4.07 (p, J = 7.1 Hz, 1H), 3.63 (q, J = 6.9 Hz, 1H), 3.50 (s, 2H), 2.72 – 2.56 (m, 2H), 2.41 – 2.27 (m, 1H), 2.20 (dtd, J = 14.0, 8.5, 5.7 Hz, 1H), 2.11 (s, 3H),

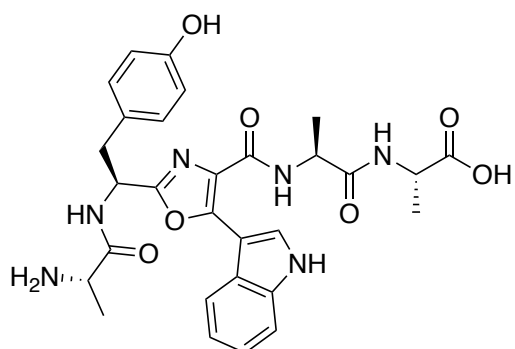
1.36 (d, $J = 6.9$ Hz, 3H), 1.29 (d, $J = 6.9$ Hz, 3H), 1.26 (d, $J = 7.1$ Hz, 3H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $C_{25}H_{36}N_6O_6S$ $[M+H]^+ = 545.2$, found 545.2. HRMS (ESI): m/z calcd. for $C_{25}H_{33}N_6O_6S$ $[M+H]^+ = 545.2177$, found 545.2173. Purity: 76 %, $R_t = 0.95$ min. Yield: 40 %.



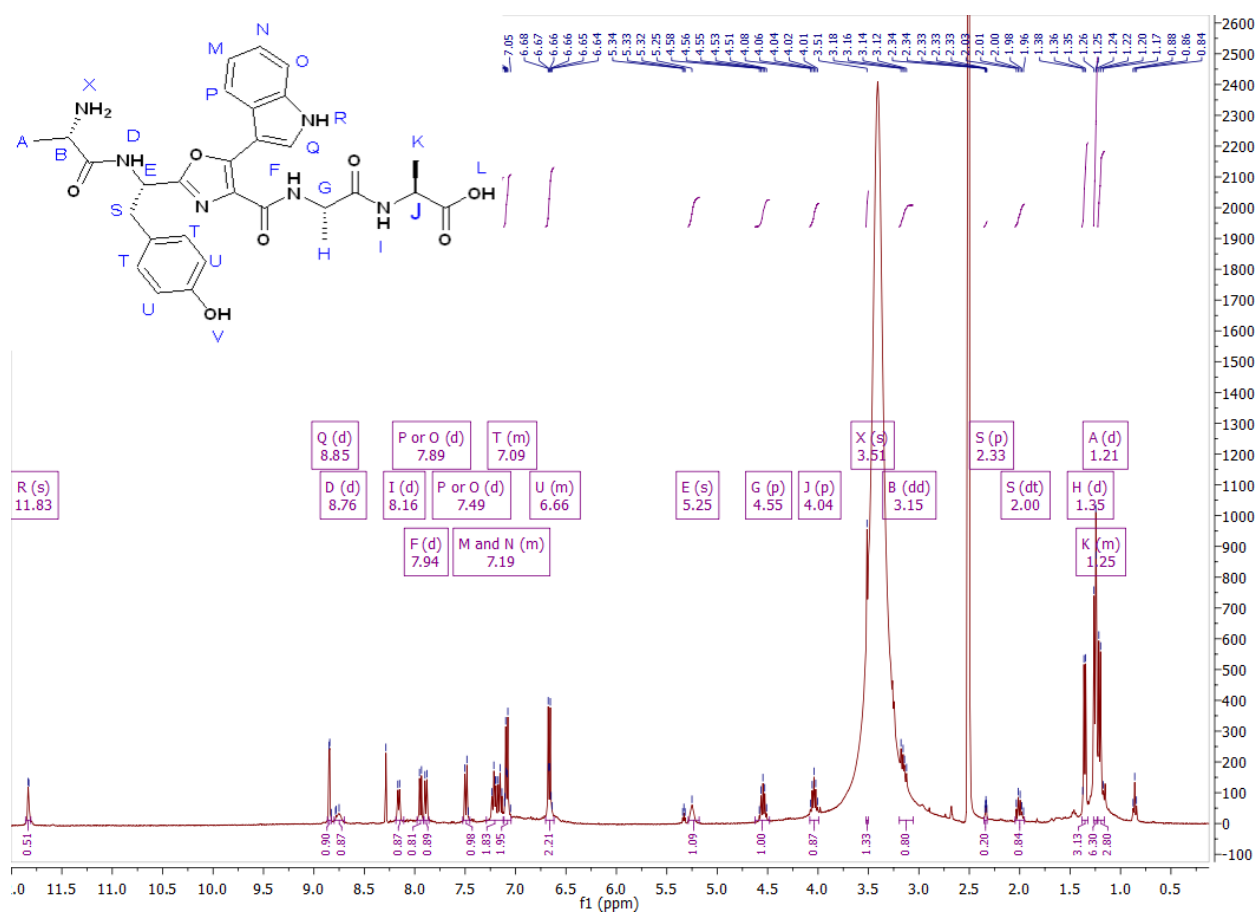
UPLC data of crude peptide after oxidation (**10R**: peak (4), $r_t = 0.95$):



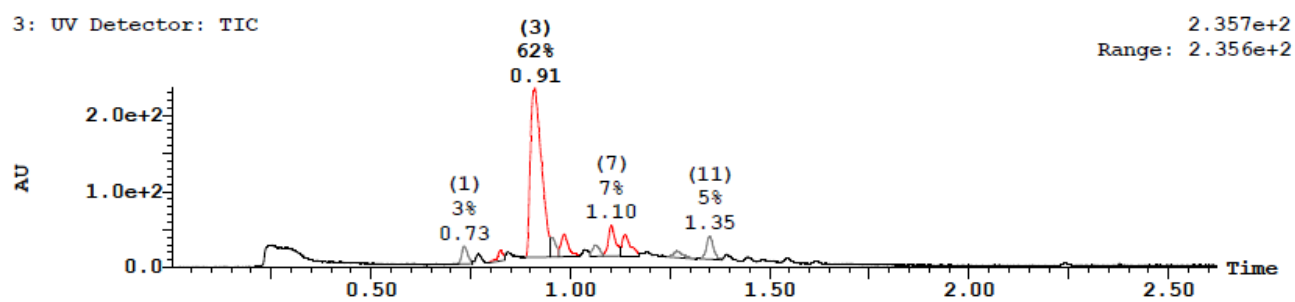
(2-((S)-1-((S)-2-aminopropanamido)-2-(4-hydroxyphenyl)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10S)



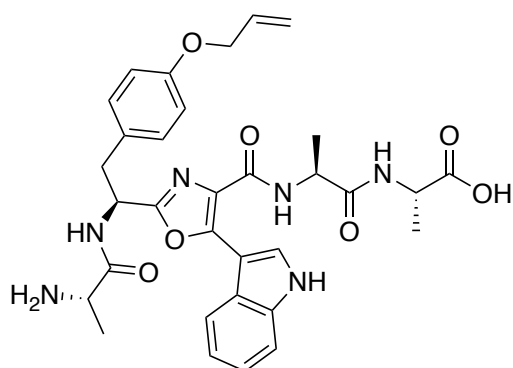
^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.83 (d, J = 3.0 Hz, 1H), 8.85 (d, J = 2.8 Hz, 1H), 8.76 (m, 1H), 8.16 (d, J = 7.0 Hz, 1H), 7.94 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.29 – 7.12 (m, 2H), 7.11 – 7.04 (m, 2H), 6.70 – 6.62 (m, 2H), 5.25 (s, 1H), 4.55 (p, J = 7.0 Hz, 1H), 4.04 (p, J = 7.0 Hz), 3.51 (m, 2H), 3.15 (dd, J = 13.8, 8.3 Hz, 1H), 2.33 (m, 1H), 2.00 (m, 1H), 1.35 (d, J = 6.9 Hz, 3H), 1.27 – 1.23 (m, 3H), 1.21 (d, J = 6.9 Hz, 3H). (Exchangeable COOH and Ar-OH protons not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{29}\text{H}_{32}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 577.3$, found 577.2. HRMS (ESI): m/z calcd. for $\text{C}_{29}\text{H}_{33}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 577.2405$, found 577.2410. Crude purity: 81 %, R_t = 0.91 min. Yield: 25 %.



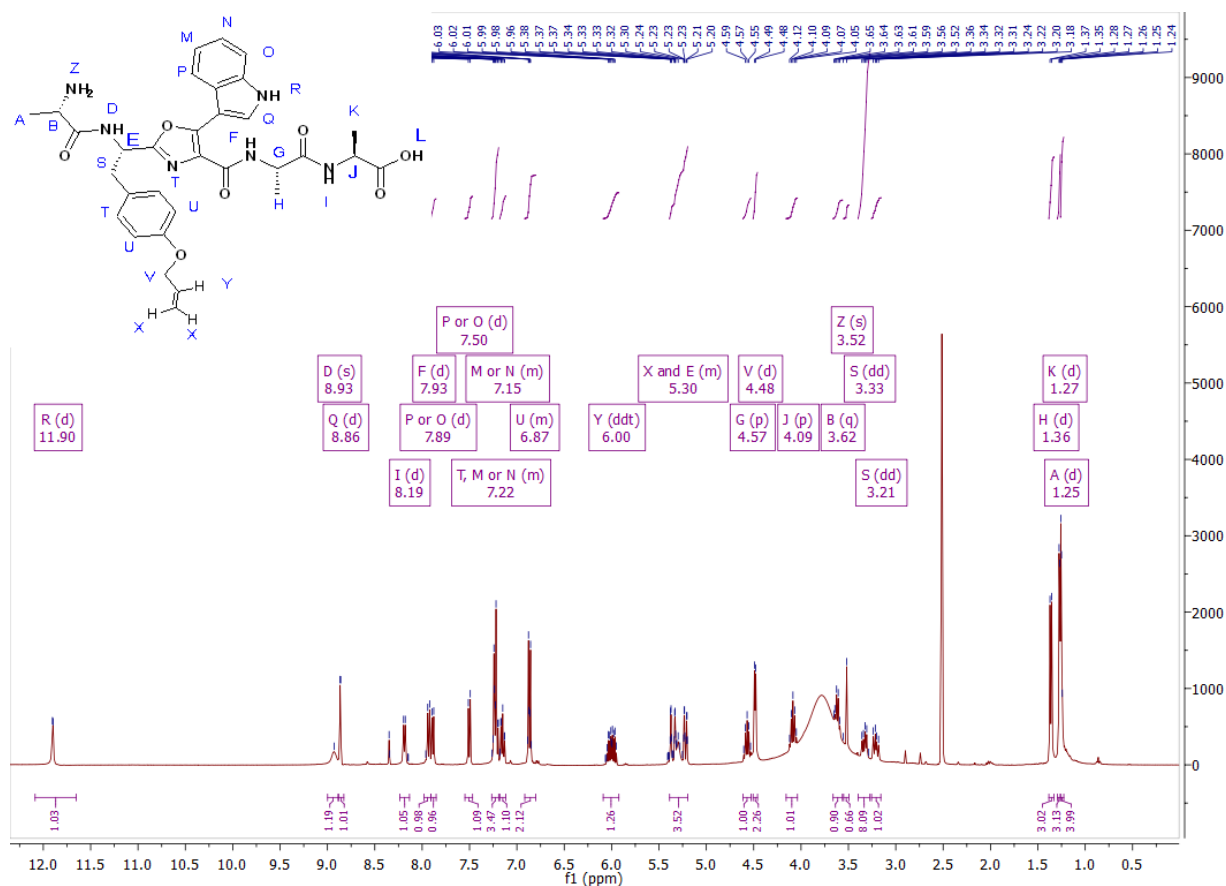
UPLC data of crude peptide after oxidation (**10S**: peak (3), $t_r = 0.91$):



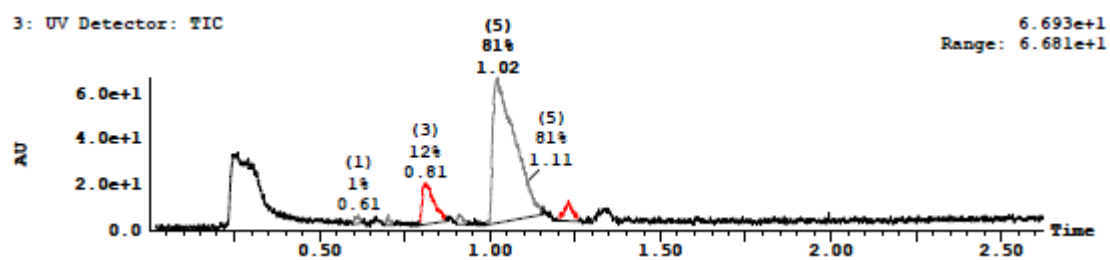
(2-((S)-2-(4-(allyloxy)phenyl)-1-((S)-2-aminopropanamido)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10T)



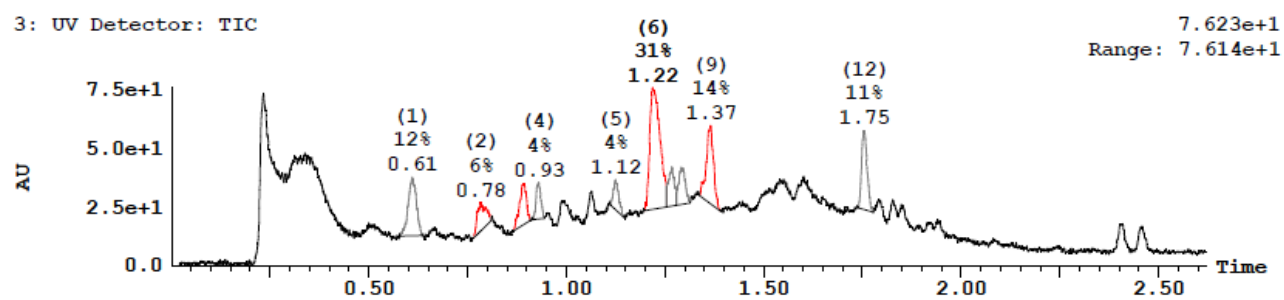
^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.90 (d, J = 2.9 Hz, 1H), 8.93 (m, 1H), 8.86 (d, J = 2.7 Hz, 1H), 8.19 (d, J = 7.1 Hz, 1H), 7.93 (d, J = 8.0 Hz, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.27 – 7.19 (m, 3H), 7.18 – 7.12 (m, 1H), 6.92 – 6.80 (m, 2H), 6.00 (ddt, J = 17.5, 10.5, 5.2 Hz, 1H), 5.46 – 5.18 (m, 3H), 4.57 (p, J = 7.0 Hz, 1H), 4.48 (d, J = 5.3 Hz, 1H), 4.09 (p, J = 7.1 Hz, 1H), 3.62 (q, J = 7.0 Hz, 1H), 3.52 (s, 1H), 3.33 (dd, J = 13.8, 6.6 Hz, 1H), 3.21 (dd, J = 13.8, 8.5 Hz, 1H), 1.36 (d, J = 6.9 Hz, 3H), 1.27 (d, J = 3.3 Hz, 3H), 1.25 (d, J = 3.1 Hz, 3H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{31}\text{H}_{34}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 616.3$, found 616.3. Crude purity: 38 %, R_t = 1.22 min. Yield: 16 %.



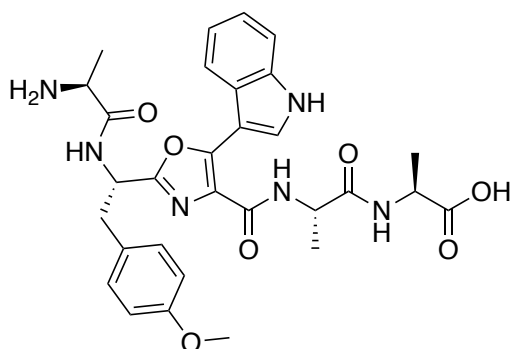
UPLC of crude peptide before oxidation (peak (5), r_t = 1.02 min):



UPLC data of crude peptide after oxidation (**10T**: peak (6), rt = 1.22):

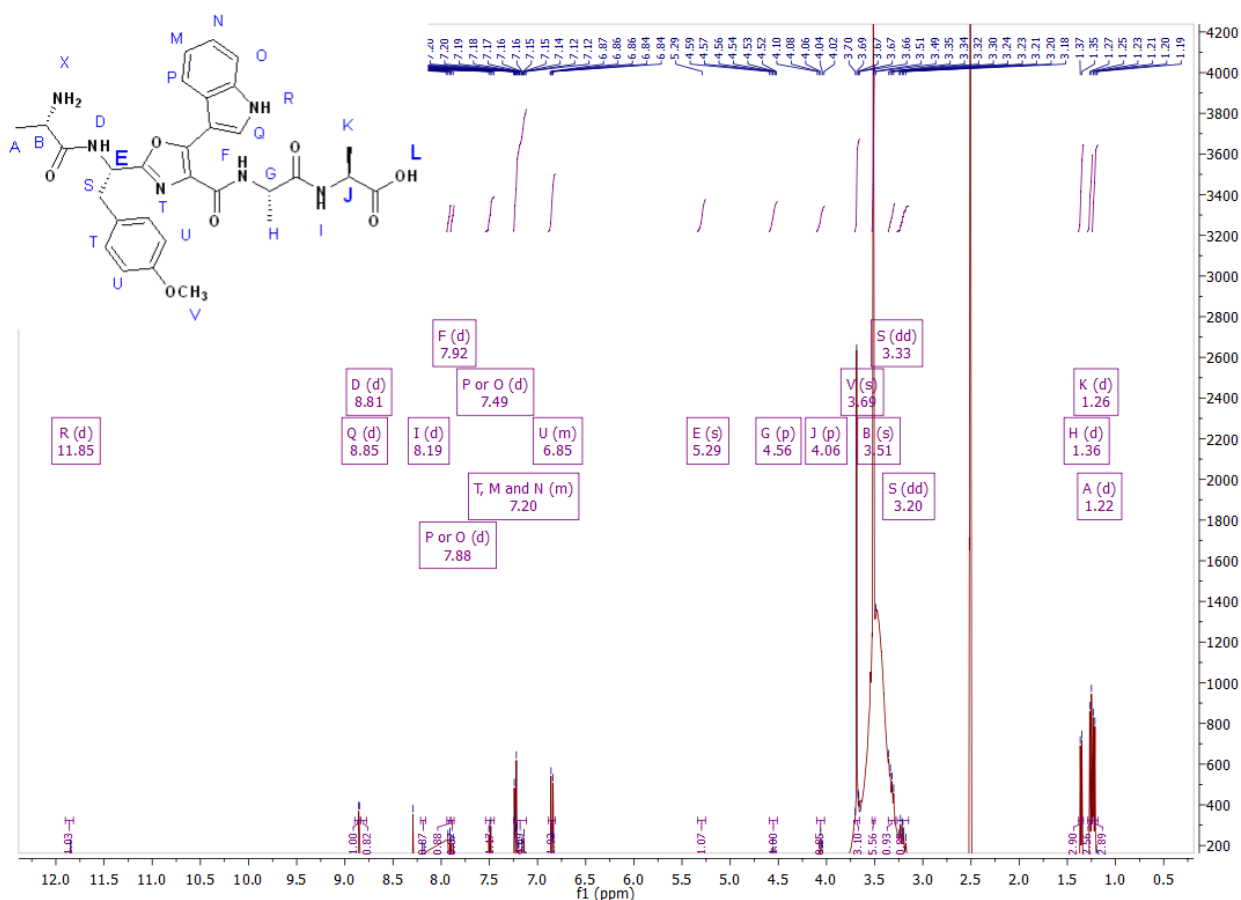


(2-((S)-1-((S)-2-aminopropanamido)-2-(4-methoxyphenyl)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10U)



^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.85 (d, J = 2.9 Hz, 1H), 8.85 (d, J = 2.8 Hz, 1H), 8.81 (d, J = 8.7 Hz, 1H), 8.19 (d, J = 7.1 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.88 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.27 – 7.12 (m, 4H), 6.89 – 6.82 (m, 2H), 5.29 (s, 1H), 4.56 (p, J = 7.0 Hz, 1H), 4.06 (p, J = 7.1 Hz, 1H), 3.69 (s, 3H), 3.51 (m, 1H), 3.33 (dd, J = 13.9,

6.7 Hz, 1H), 3.20 (dd, J = 13.8, 8.5 Hz, 1H), 1.36 (d, J = 6.9 Hz, 3H), 1.26 (d, J = 7.1 Hz, 3H), 1.22 (d, J = 7.0 Hz, 3H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. UPLC/MS (ESI): m/z calcd. $\text{C}_{30}\text{H}_{34}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 591.3$, found 591.3. Purity: 92 %, R_t = 1.02 min. Yield: 18 %.

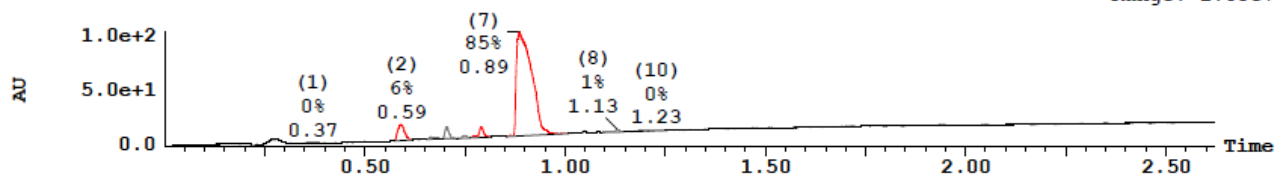


UPLC of crude peptide before oxidation (peak (7), $r_t = 0.89$ min.):

3: UV Detector: TAC :Wavelength Range: (210 - 500)

1.031e+2

Range: 1.05e+2

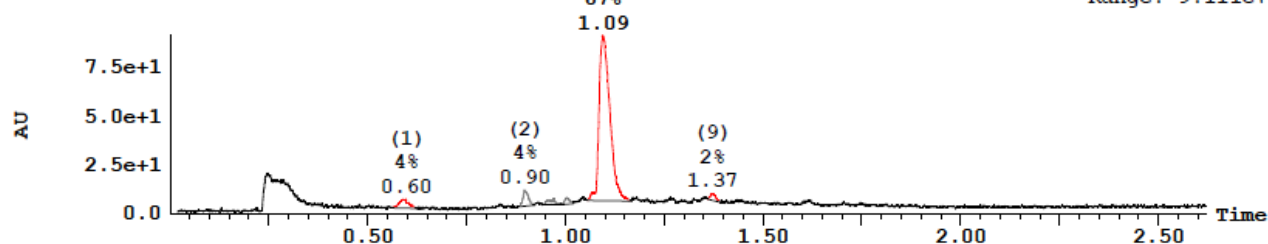


UPLC data of crude peptide after oxidation (**10U**: peak (6), $r_t = 1.09$):

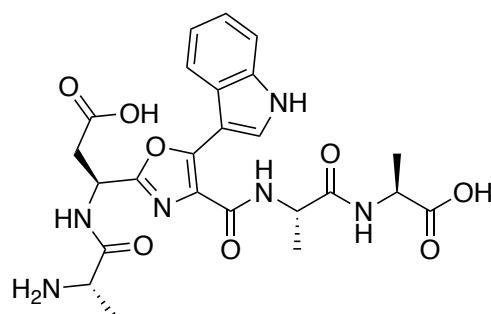
3: UV Detector: TIC

9.147e+1

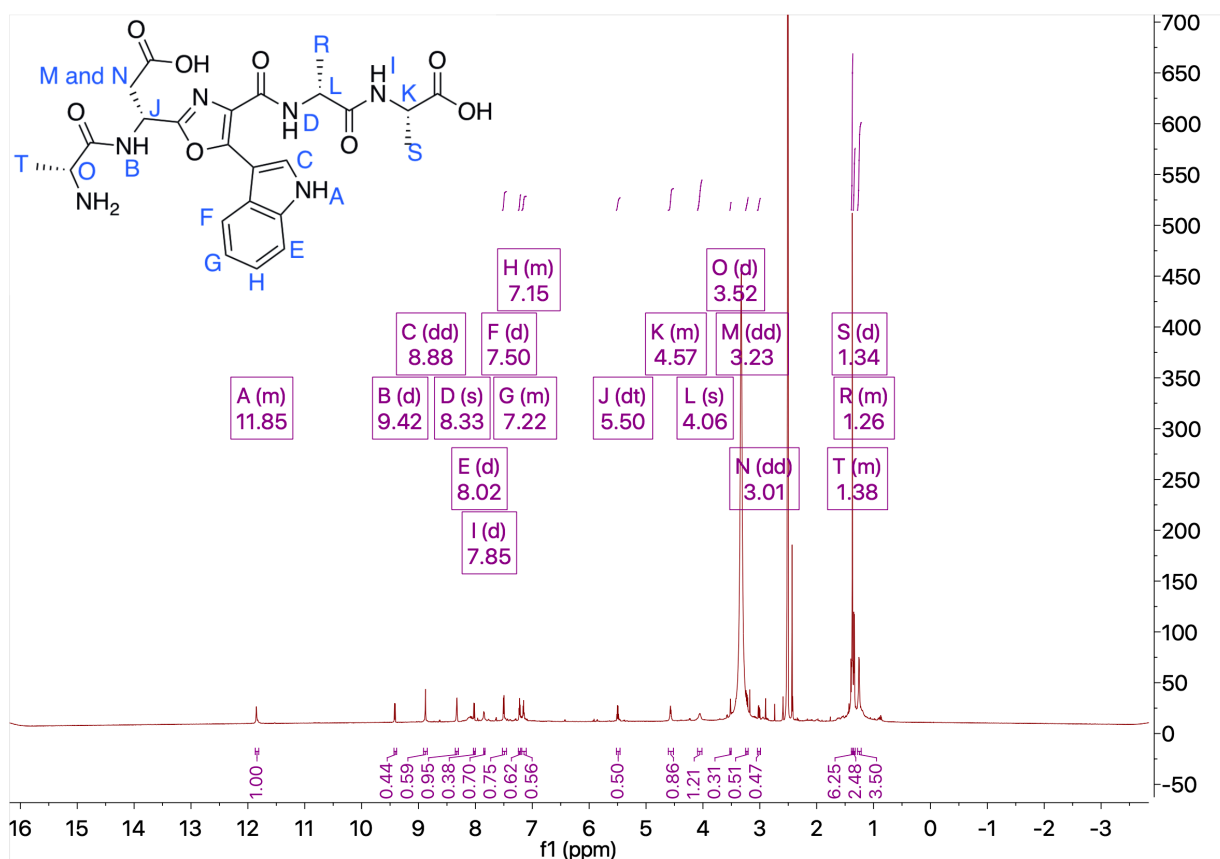
Range: 9.111e+1



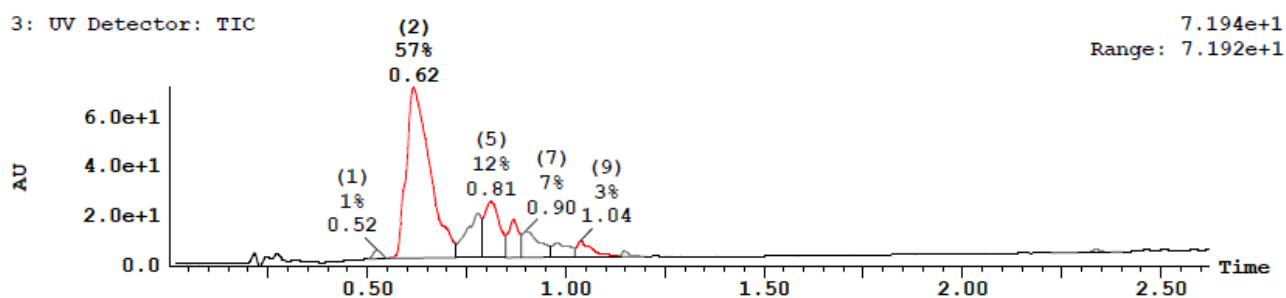
(S)-3-((S)-2-aminopropanamido)-3-(4-(((S)-1-(((S)-1-carboxyethyl)amino)-1-oxopropan-2-yl)carbamoyl)-5-(1H-indol-3-yl)oxazol-2-yl)propanoic acid (10V)



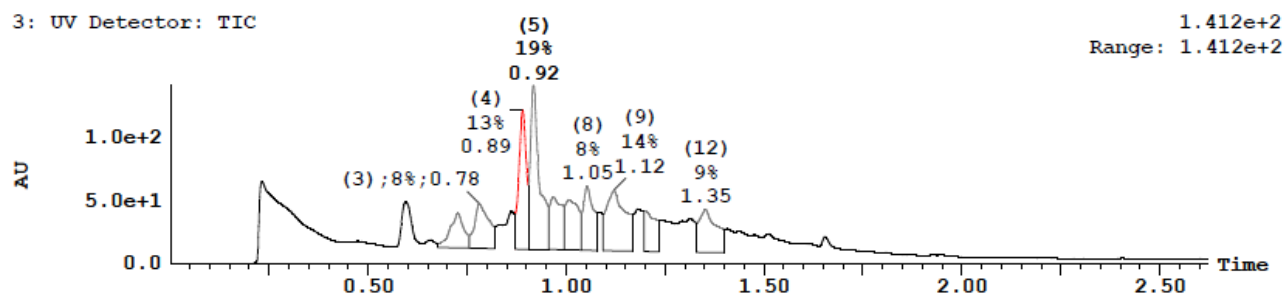
^1H NMR (800 MHz, $\text{DMSO}-d_6$) δ 11.87 – 11.81 (m, 1H, A), 9.42 (d, $J = 8.7$ Hz, 1H, B), 8.88 (m, 1H, C), 8.33 (m, 1H, D), 8.02 (d, $J = 8.0$ Hz, 1H, E), 7.85 (d, $J = 7.5$ Hz, 1H, I), 7.50 (d, $J = 8.1$ Hz, 1H, F), 7.25 – 7.20 (m, 1H, G), 7.18 – 7.11 (m, 1H, H), 5.50 (dt, $J = 8.7, 7.4$ Hz, 1H, J), 4.61 – 4.52 (m, 1H, K), 4.06 (s, 1H, L), 3.52 (m, 1H, O (Found in DQF-COSY)), 3.23 (dd, $J = 16.3, 7.4$ Hz, 1H, M), 3.01 (dd, $J = 16.3, 7.3$ Hz, 1H, N), 1.39 – 1.36 (m, 6H, T and impurity), 1.34 (d, $J = 7.0$ Hz, 3H, S), 1.28 – 1.22 (m, 3H, R). Exchangeable protons from carboxylic acids and amine not observed, UPLC/MS (ESI): m/z calcd. $\text{C}_{24}\text{H}_{28}\text{N}_6\text{O}_8$ $[\text{M}+\text{H}]^+ = 529.2$, found 529.2. HRMS (ESI): m/z calcd. for $\text{C}_{24}\text{H}_{29}\text{N}_6\text{O}_8$ $[\text{M}+\text{H}]^+ = 529.2041$, found 529.2035. Purity: 65 %, $R_t = 0.90$ min. Yield: 15 %.



UPLC of crude peptide before oxidation (peak (2), rt = 0.62 min):

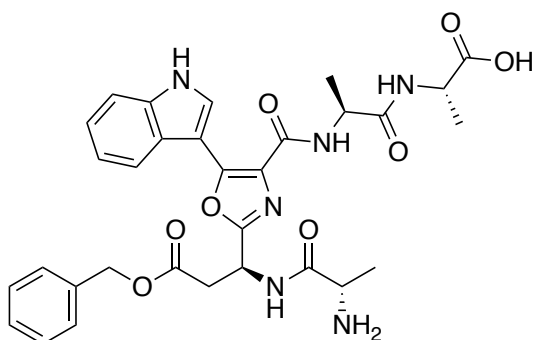


UPLC data of crude peptide after oxidation (**10V**: peak (4)-(5), rt = 0.90):

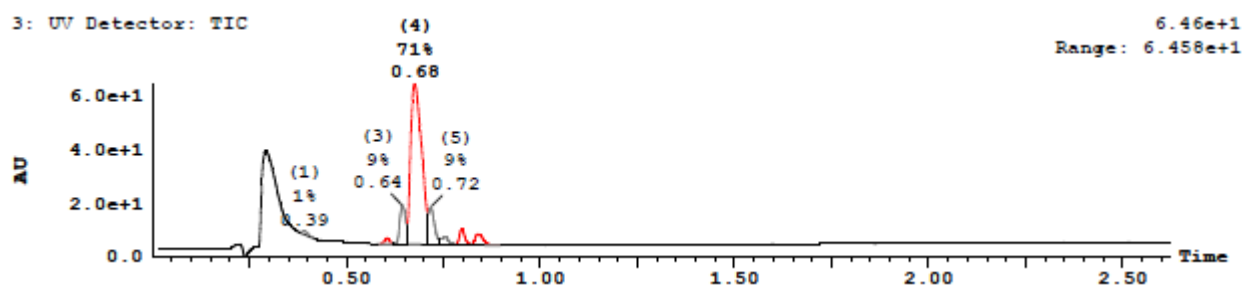


(2-((S)-1-((S)-2-aminopropanamido)-3-(benzyloxy)-3-oxopropyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10W)

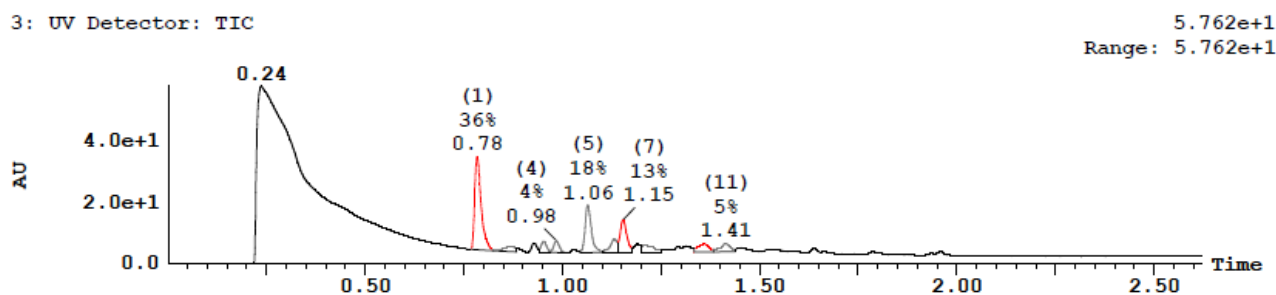
UPLC/MS (ESI): m/z calcd. $C_{31}H_{34}N_6O_8$ $[M+H]^+ = 619.3$, found 619.2. Purity: 48 %.



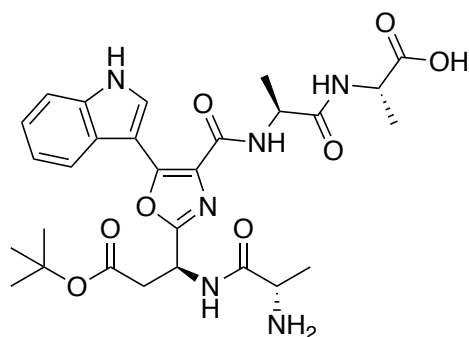
UPLC of crude peptide before oxidation (rt = 0.68 min):



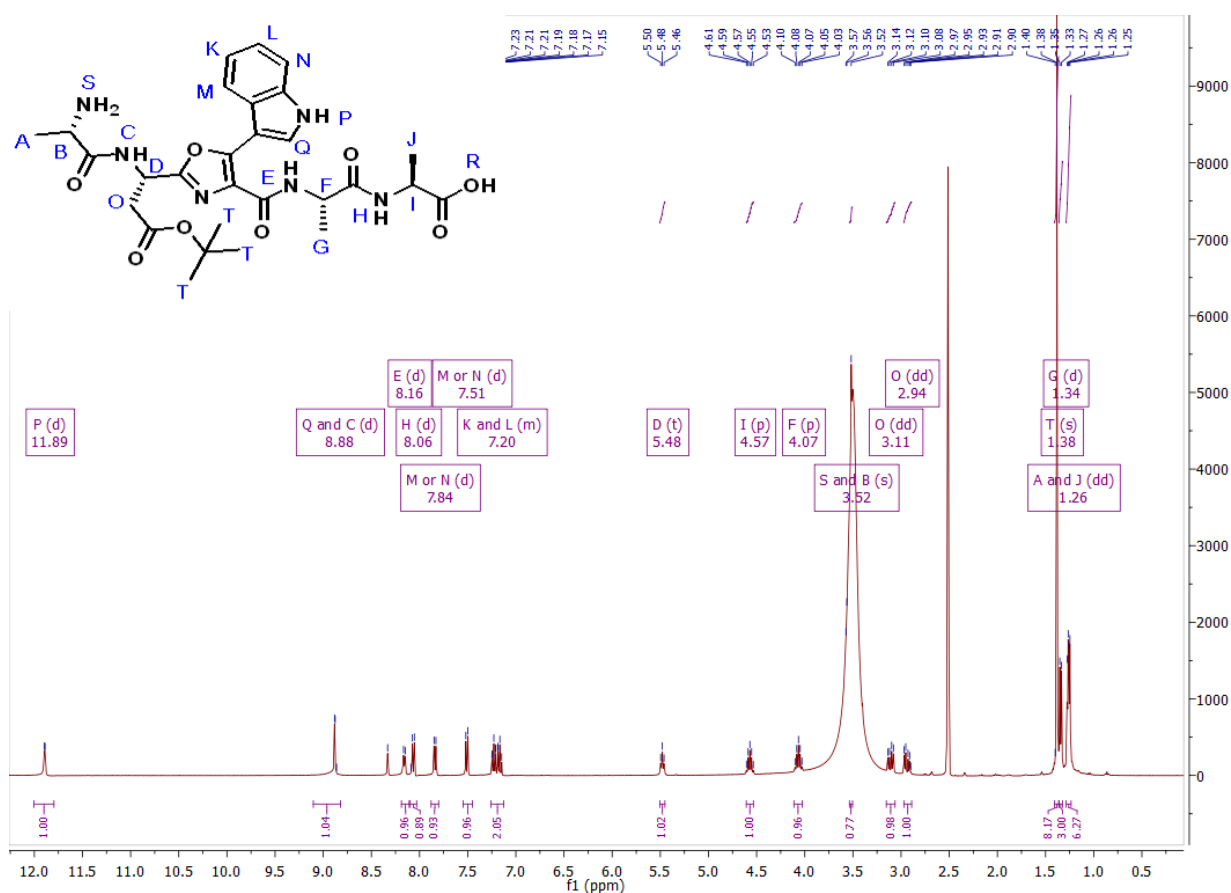
UPLC data of crude peptide after oxidation (**10W**). Partial OBzl deprotection was observed (rt = 0.78: deprotection of OBzl group, rt = 1.06: with OBzl group):



(2-((S)-1-((S)-2-aminopropanamido)-3-(tert-butoxy)-3-oxopropyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10X)



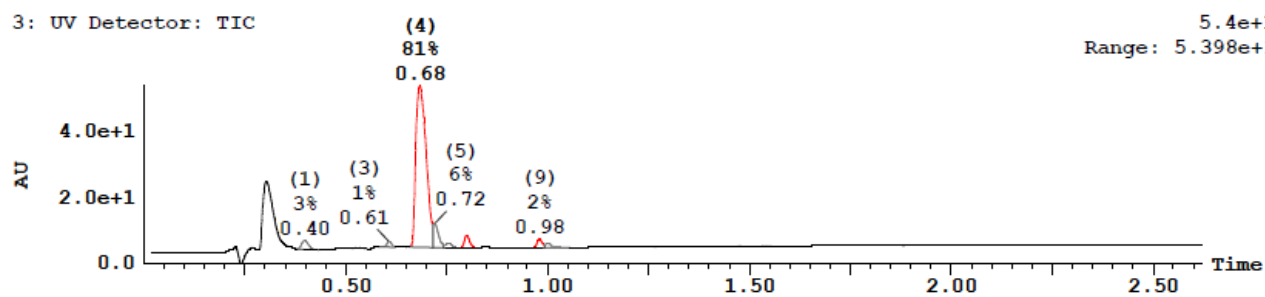
^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.89 (d, J = 2.9 Hz, 1H), 8.88 (m, 2H), 8.16 (d, J = 7.1 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.84 (d, J = 7.7 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.26 – 7.13 (m, 2H), 5.48 (t, J = 7.0 Hz, 1H), 4.57 (p, J = 7.0 Hz, 1H), 4.07 (p, J = 7.1 Hz, 1H), 3.52 (m, 3H), 3.11 (dd, J = 16.2, 7.4 Hz, 1H), 2.94 (dd, J = 16.2, 6.7 Hz, 1H), 1.38 (s, 9H), 1.34 (d, J = 6.9 Hz, 3H), 1.26 (dd, J = 7.0, 4.1 Hz, 6H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{28}\text{H}_{36}\text{N}_6\text{O}_8$ $[\text{M}+\text{H}]^+ = 585.3$, found 585.3. Purity: 81 %, R_t = 1.09 min. Yield = 30 %.



UPLC of crude peptide before oxidation (peak (4), r_t = 0.68 min):

3: UV Detector: TIC

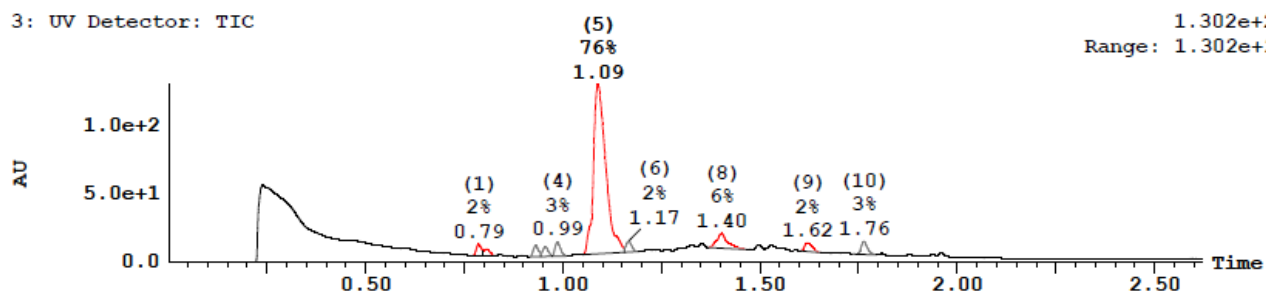
5.4e+1
Range: 5.398e+1



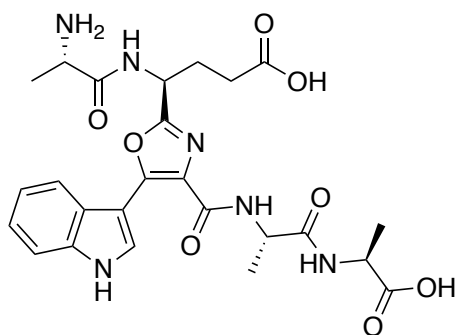
UPLC data of crude peptide after oxidation (**10X**: peak (5), rt = 1.09):

3: UV Detector: TIC

1.302e+2
Range: 1.302e+2

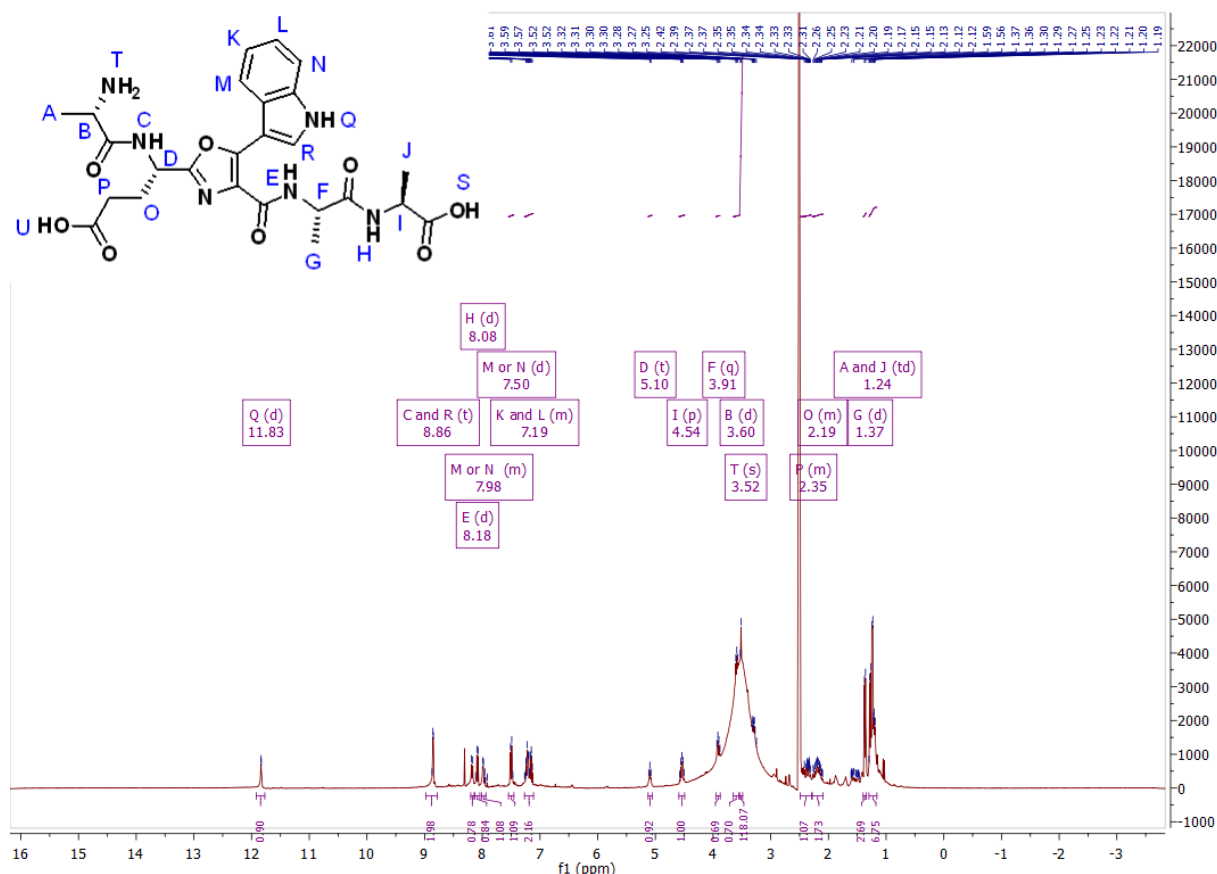


(S)-4-((S)-2-aminopropanamido)-4-(4-(((S)-1-(((S)-1-carboxyethyl)amino)-1-oxopropan-2-yl)carbamoyl)-5-(1H-indol-3-yl)oxazol-2-yl)butanoic acid (10Y)

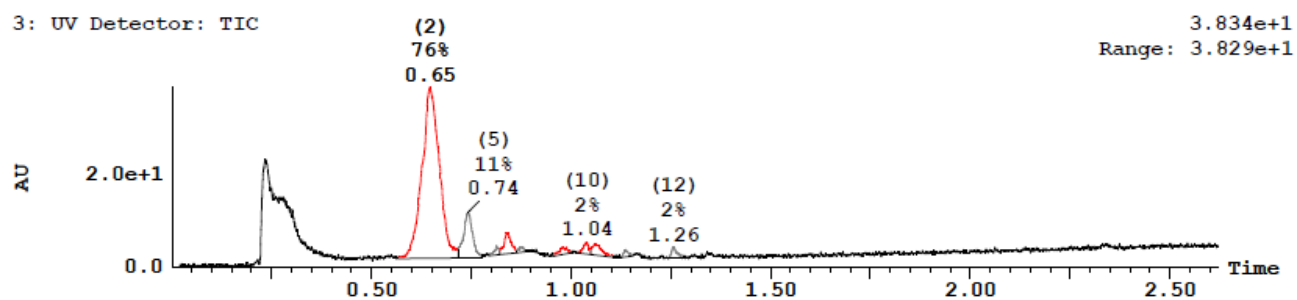


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.83 (d, J = 2.9 Hz, 1H, Q), 8.86 (m, J = 4.6 Hz, 2H, R and C), 8.18 (d, J = 8.2 Hz, 1H, E), 8.08 (d, J = 8.0 Hz, 1H), 8.01 – 7.94 (m, 1H), 7.50 (d, J = 8.0 Hz, 1H), 7.27 – 7.11 (m, 2H), 5.10 (t, J = 7.5 Hz, 1H), 4.54 (p, J = 7.1 Hz, 1H), 3.91 (q, J = 7.0 Hz, 1H), 3.60 (d, J = 6.9 Hz, 1H), 3.52 (s, 2H), 2.47 – 2.31 (m, 2H), 2.28 – 2.10 (m, 2H), 1.37 (d, J = 7.0 Hz, 3H), 1.24 (td, J = 15.2, 7.0 Hz, 6H). (Exchangeable COOH proton not observed);

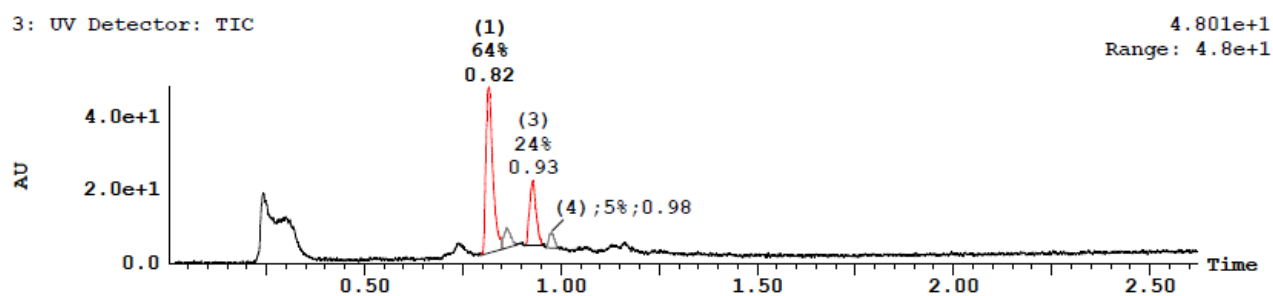
UPLC/MS (ESI): m/z calcd. $\text{C}_{25}\text{H}_{30}\text{N}_6\text{O}_8$ $[\text{M}+\text{H}]^+ = 543.2$, found 543.2. Purity: 64 %, R_t = 0.82 min. Yield = 23 %.



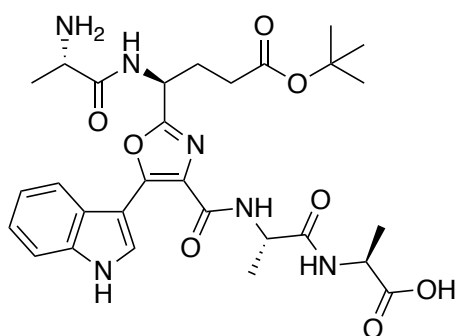
UPLC of crude peptide before oxidation (rt = 0.65 min):



UPLC data of crude peptide after oxidation (**10Y**: rt = 0.82):

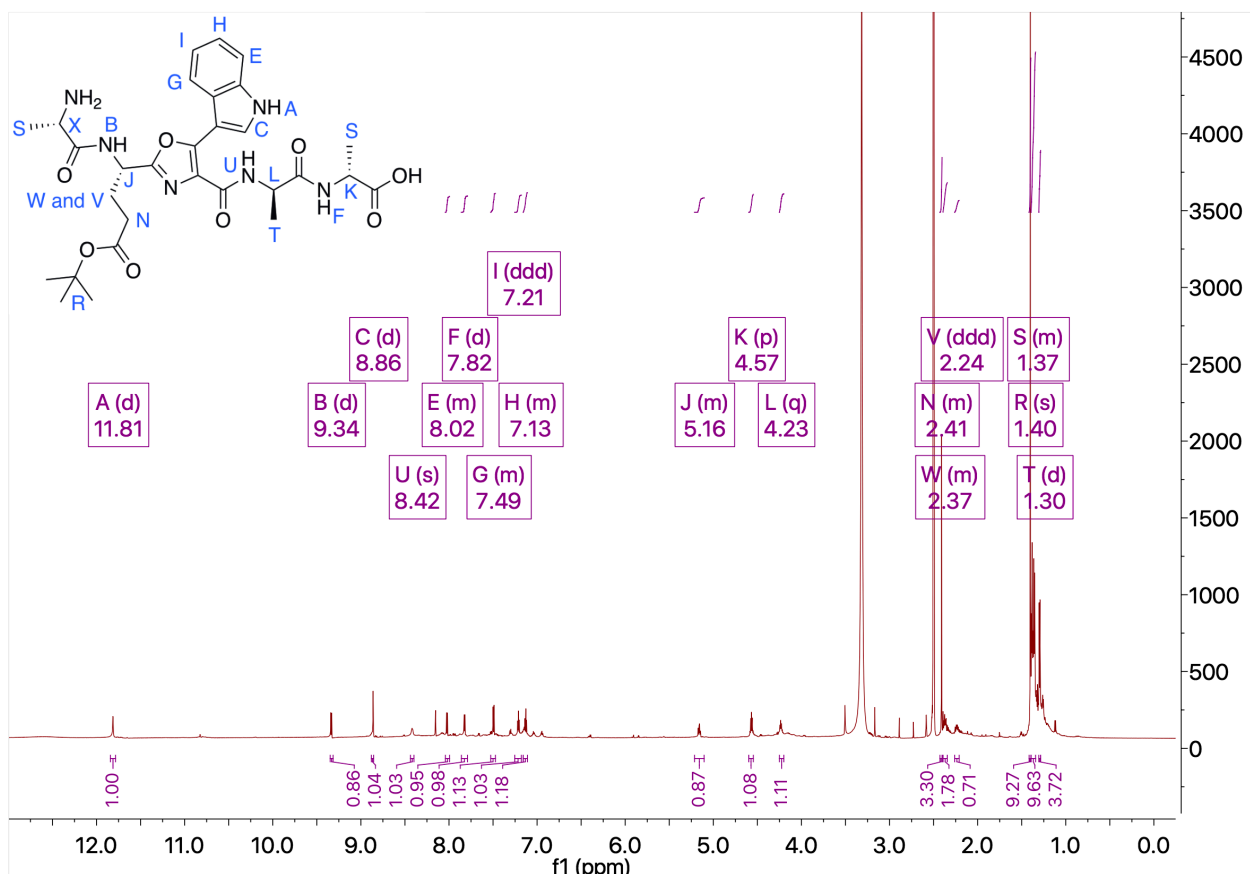


(2-((S)-1-((S)-2-aminopropanamido)-4-(tert-butoxy)-4-oxobutyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10Z)



^1H NMR (800 MHz, $\text{DMSO}-d_6$) δ 11.81 (d, $J = 2.9$ Hz, 1H, A), 9.34 (d, $J = 8.5$ Hz, 1H, B), 8.86 (d, $J = 2.9$ Hz, 1H, C), 8.42 (s, 1H, U), 8.04 – 7.99 (m, 1H, E), 7.82 (d, $J = 7.7$ Hz, 1H, F), 7.53 – 7.47 (m, 1H, G), 7.21 (m, 1H, I), 7.16 – 7.11 (m, 1H, H), 5.21 – 5.10 (m, 1H, J), 4.57 (p, $J = 7.1$ Hz, 1H, K), 4.23 (q, $J = 7.3$ Hz, 1H, L), 3.23 (m, 1H, X (Found by DQF-COSY)), 2.43 – 2.40 (m, 2H, N), 2.39 – 2.34 (m, 1H, W), 2.24 (m, 1H, V), 1.40 (s, 9H, R), 1.39 – 1.34 (m, 6H, S), 1.30 (d, $J = 7.3$ Hz, 3H, T). Exchangeable protons on carboxylic acids and amine not observed.

UPLC/MS (ESI): m/z calcd. $\text{C}_{29}\text{H}_{34}\text{N}_6\text{O}_8$ $[\text{M}+\text{H}]^+ = 598.3$, found 598.3. Purity: 89 %. $R_t = 1.16$. Yield = 14 %.

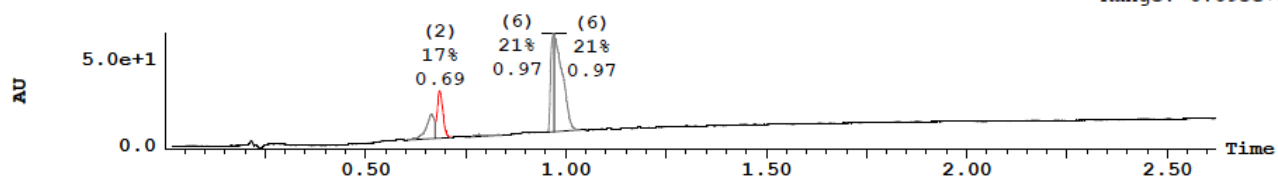


UPLC of crude peptide before oxidation ($r_t = 0.97$, $r_t = 0.69$: OtBu deprotection):

3: UV Detector: TAC :Wavelength Range: (210 - 500)

6.558e+1

Range: 6.693e+1

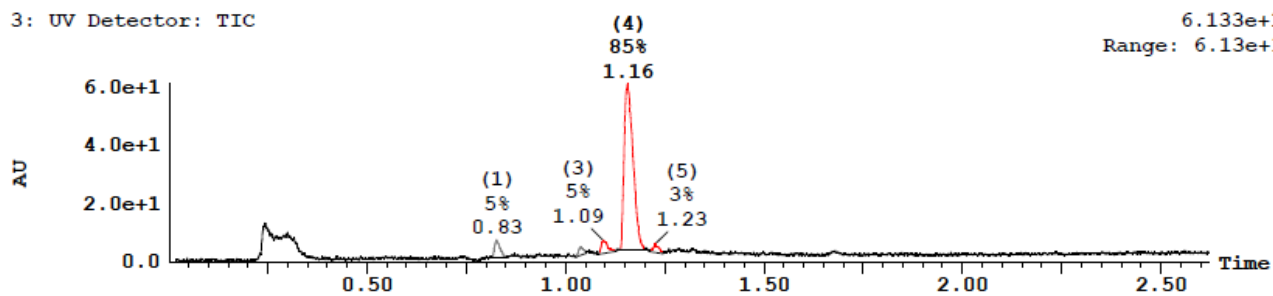


UPLC data of crude peptide after oxidation (**10Z**: rt = 1.16):

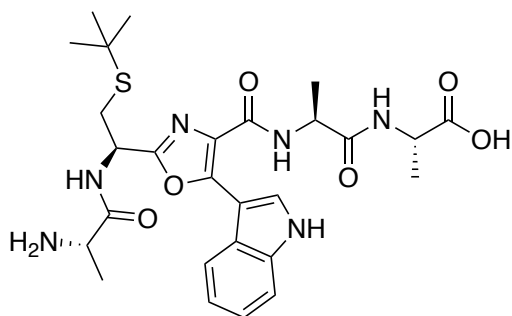
3: UV Detector: TIC

6.133e+1

Range: 6.13e+1

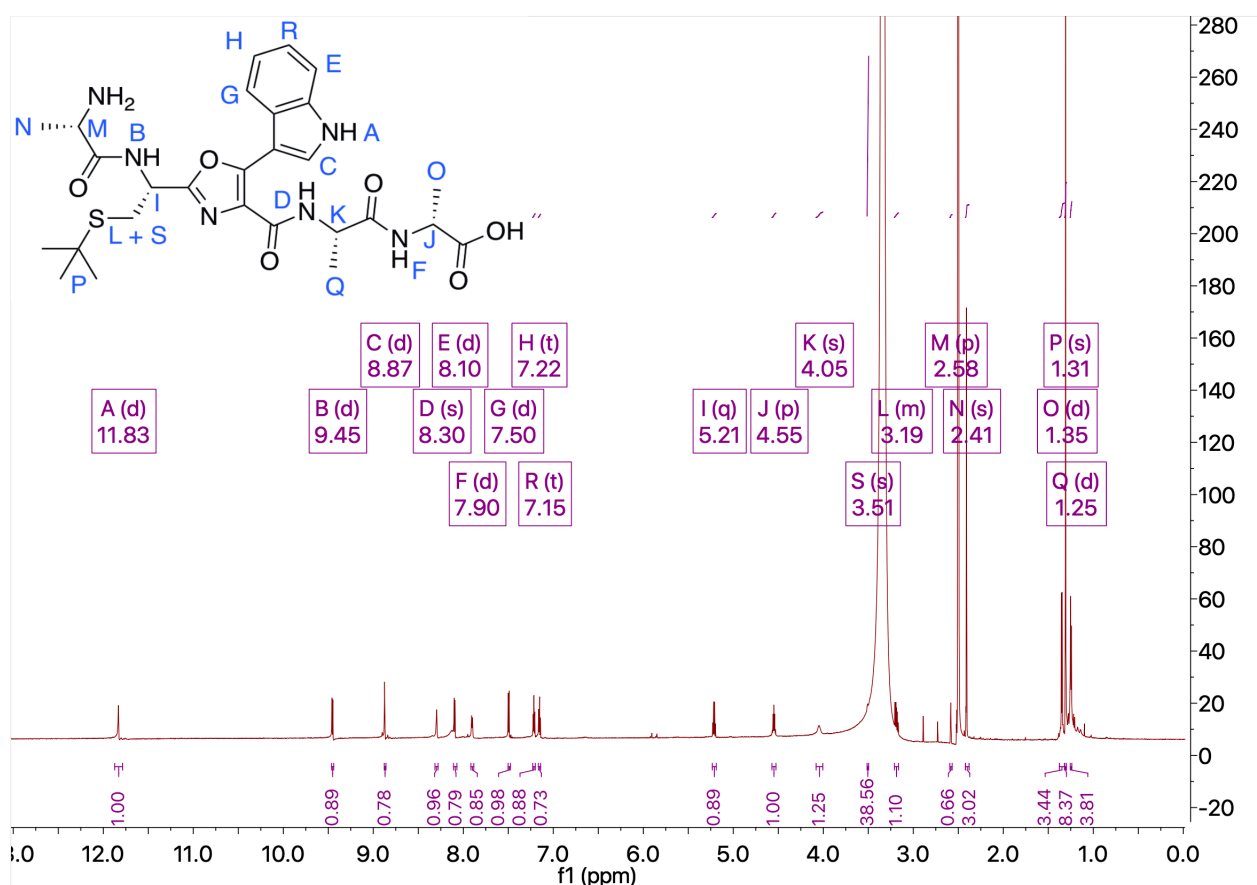


(2-((R)-1-((S)-2-aminopropanamido)-2-(tert-butylthio)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AA)



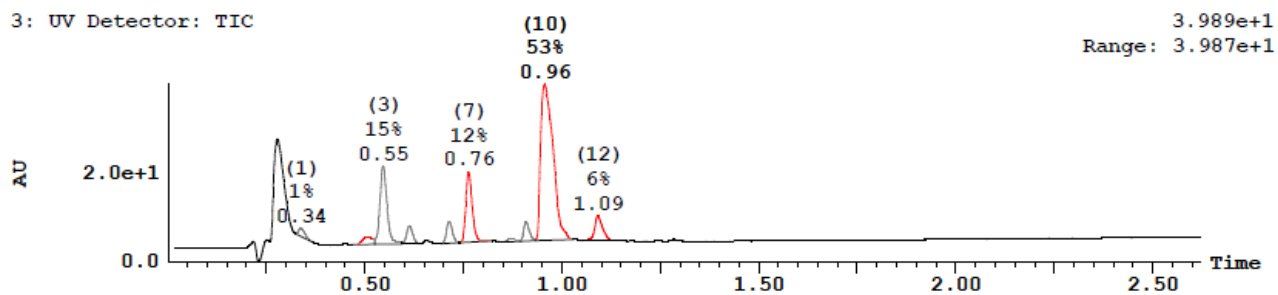
^1H NMR (800 MHz, $\text{DMSO}-d_6$) δ 11.83 (s, 1H, A), 9.45 (d, $J = 8.5$ Hz, 1H, C), 8.87 (d, $J = 2.9$ Hz, 1H, B), 8.30 (s, 1H, D), 8.10 (d, $J = 8.0$ Hz, 1H, E), 7.90 (d, $J = 7.7$ Hz, 1H, F), 7.50 (d, $J = 8.1$ Hz, 1H, G), 7.22 (t, $J = 7.5$ Hz, 1H, H), 7.15 (t, $J = 7.5$ Hz, 1H, R), 5.21 (q, $J = 7.8$ Hz, 1H, I), 4.55 (p, $J = 7.1$ Hz, 1H, J), 4.05 (s, 1H, K), 3.36 (m, 1H, S (From DQF-

COSY)), 3.21 – 3.16 (m, 1H, L), 2.58 (m, 1H, M), 2.41 (s, 3H, N), 1.35 (d, $J = 7.0$ Hz, 3H, O), 1.31 (s, 9H, P), 1.25 (d, $J = 7.0$ Hz, 3H, Q). UPLC/MS (ESI): m/z calcd. $\text{C}_{27}\text{H}_{36}\text{N}_6\text{O}_6\text{S}$ $[\text{M}]^+ = 573.3$, found 573.7. Purity: 66 %. $R_t = 1.09$. Yield = 17 %.



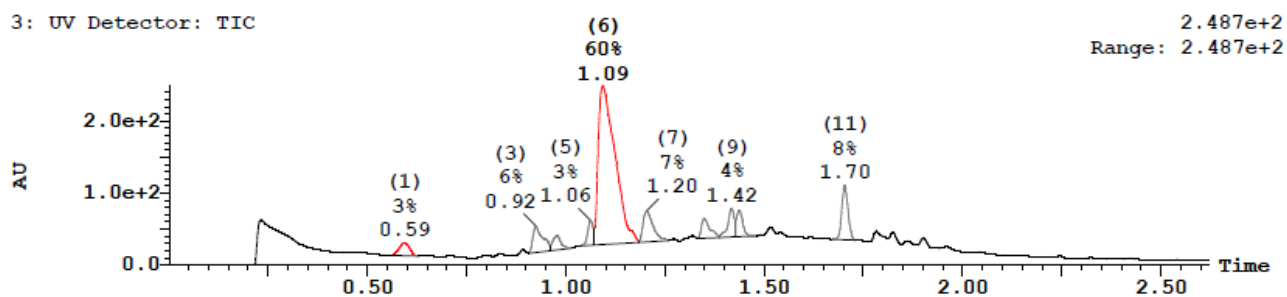
UPLC of crude peptide before oxidation ($r_t = 0.96$ min):

3: UV Detector: TIC

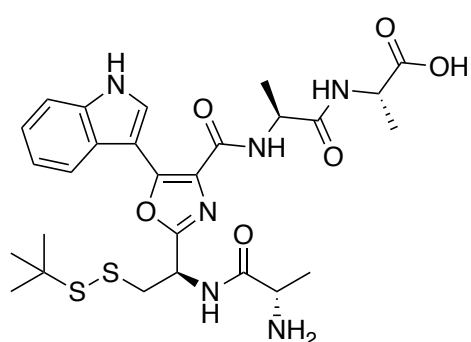


UPLC data of crude peptide after oxidation (**10AA**: rt = 1.09 min):

3: UV Detector: TIC

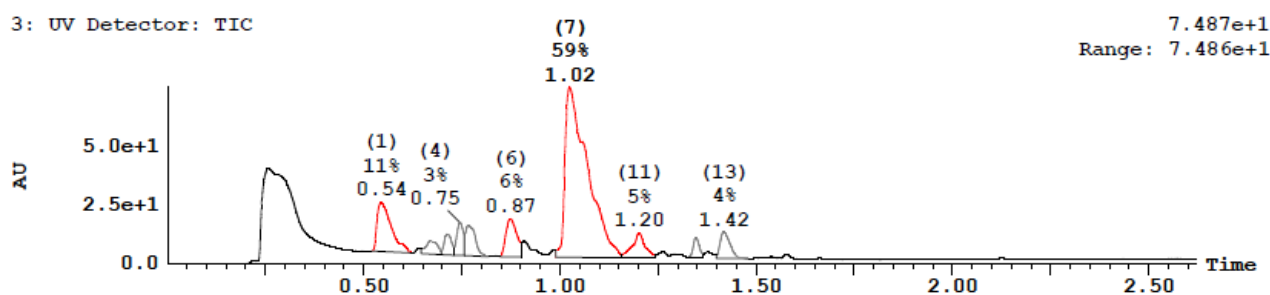


(2-((R)-1-((S)-2-aminopropanamido)-2-(tert-butylidysulfaneyl)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AB)

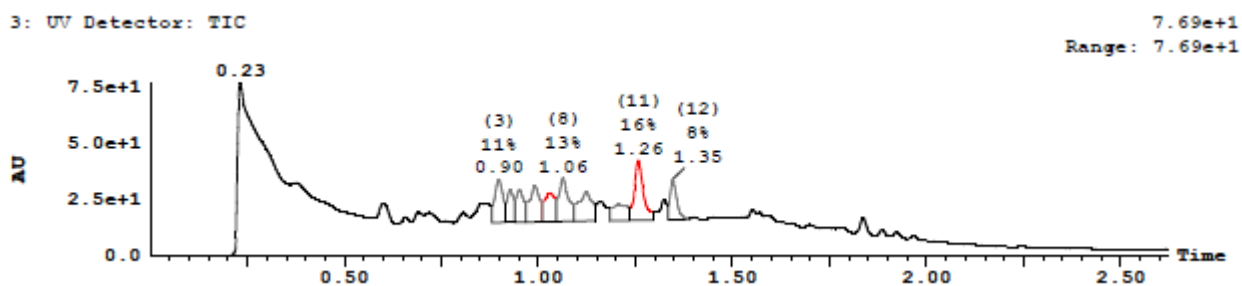


UPLC/MS (ESI): m/z calcd. $C_{27}H_{36}N_6O_6S_2$ $[M+H]^+ = 605.3$, found 605.3. Purity: 34 %. $R_t = 1.26$.

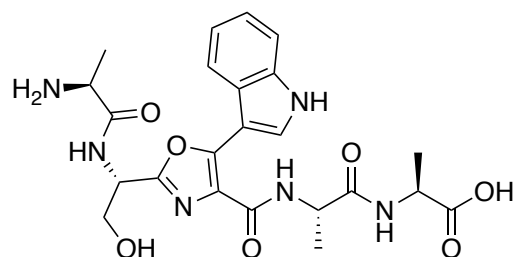
UPLC of crude peptide before oxidation ($r_t = 1.02$):



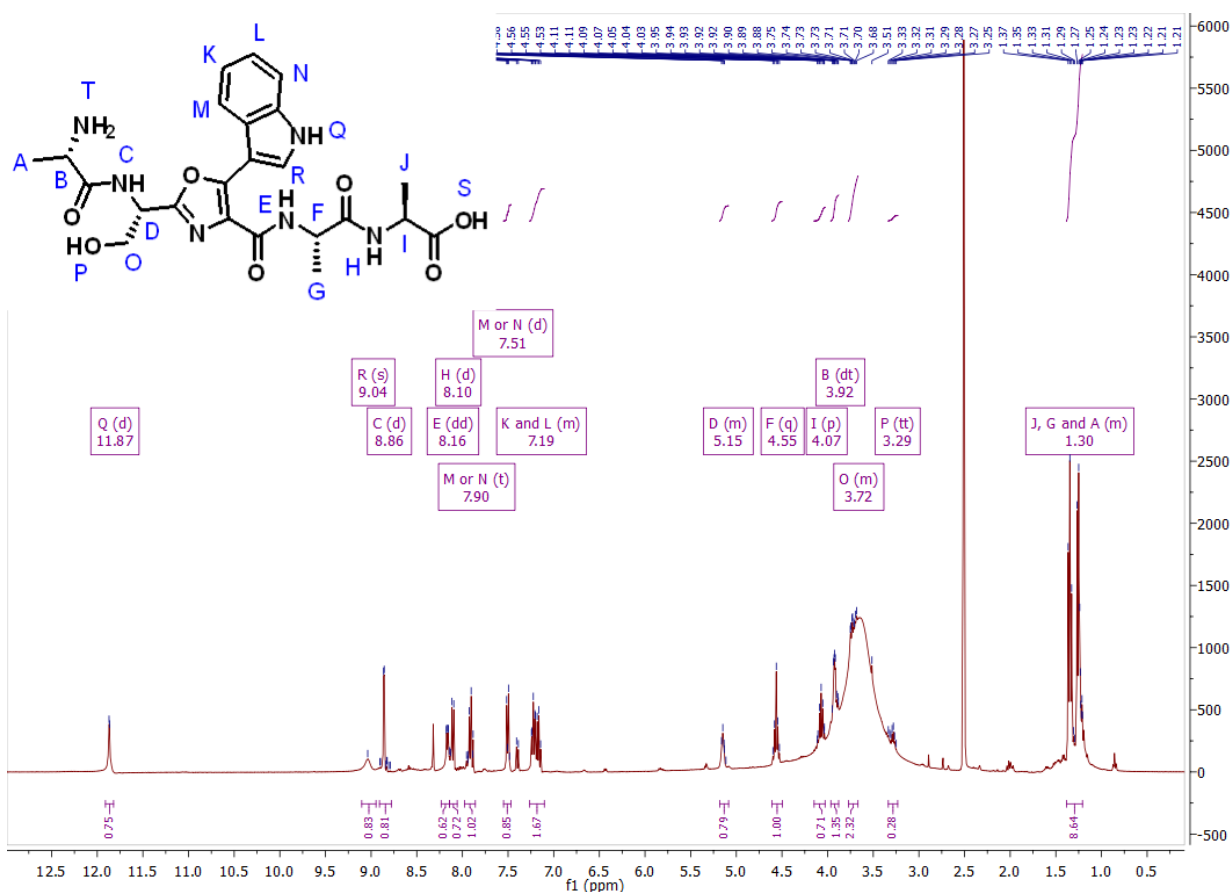
UPLC data of crude peptide after oxidation (**10AB**: $r_t = 1.26$):



(2-((S)-1-((S)-2-aminopropanamido)-2-hydroxyethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AC)



^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.87 (d, J = 2.9 Hz, 1H), 9.04 (s, 1H), 8.86 (d, J = 2.8 Hz, 1H), 8.16 (dd, J = 7.1, 2.0 Hz, 1H), 8.10 (d, J = 7.9 Hz, 1H), 7.90 (t, J = 8.3 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.26 – 7.10 (m, 2H), 5.18 – 5.08 (m, 1H), 4.55 (q, J = 7.4, 7.0 Hz, 1H), 4.07 (p, J = 7.1 Hz, 1H), 3.92 (dt, J = 8.5, 4.2 Hz, 1H), 3.72 (m, 2H), 3.29 (m, 1H), 1.38 – 1.21 (m, 9H). (Exchangeable COOH proton not observed, Amine NH_2 protons lies under water peak); UPLC/MS (ESI): m/z calcd. $\text{C}_{23}\text{H}_{28}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 501.2$, found 501.3. Purity: 81 %. R_t = 0.72. Yield = 15 %.

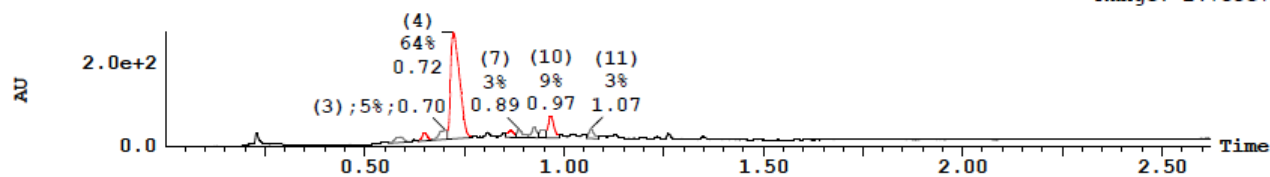


UPLC data of crude peptide after oxidation (**10AC**: peak 4, r_t = 0.72):

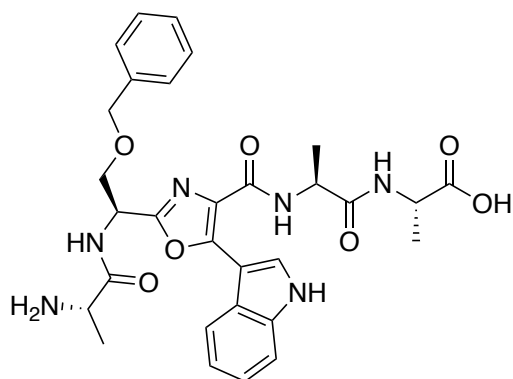
3: UV Detector: TAC :Wavelength Range: (210 - 500)

2.733e+2

Range: 2.733e+2

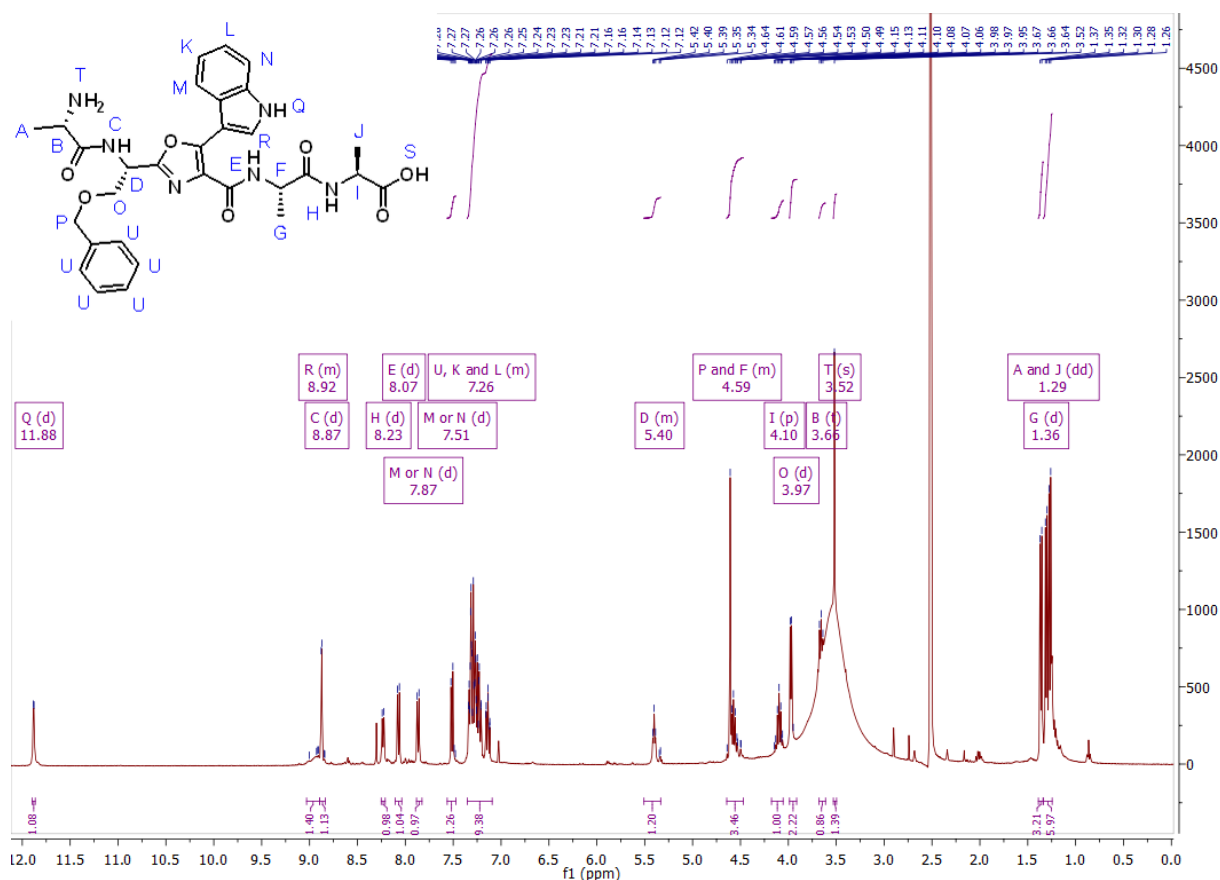


(2-((S)-1-((S)-2-aminopropanamido)-2-(benzyloxy)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AD)

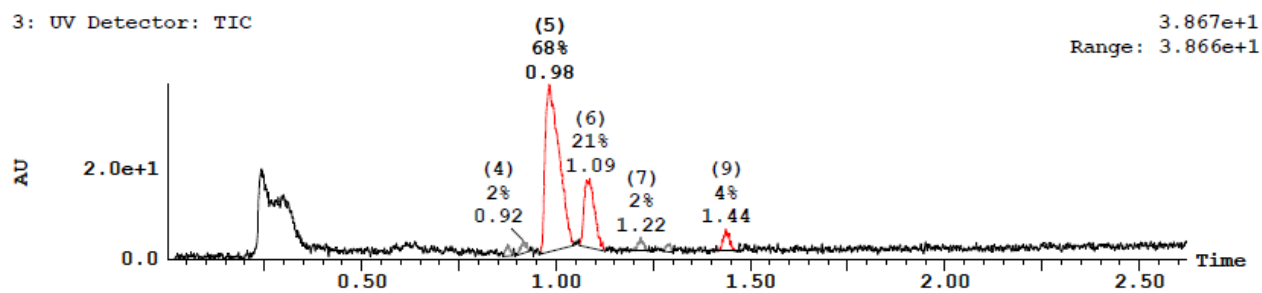


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.88 (d, J = 2.9 Hz, 1H), 9.03 – 8.90 (m, 1H), 8.87 (d, J = 2.8 Hz, 1H), 8.23 (d, J = 7.1 Hz, 1H), 8.07 (d, J = 8.0 Hz, 1H), 7.87 (d, J = 7.7 Hz, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.35 – 7.09 (m, 7H), 5.51 – 5.33 (m, 1H), 4.67 – 4.49 (m, 3H), 4.10 (p, J = 7.1 Hz, 1H), 3.97 (d, J = 5.8 Hz, 2H), 3.66 (t, J = 7.1 Hz, 1H), 3.52 (s, 2H),

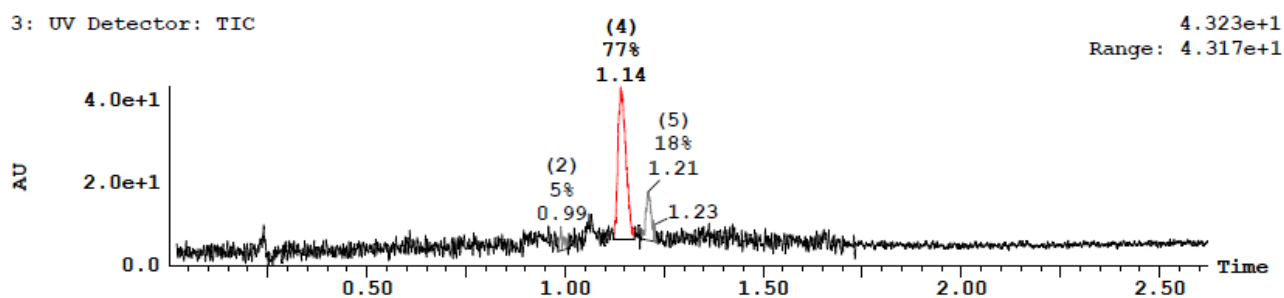
1.36 (d, J = 6.9 Hz, 3H), 1.29 (m, 6H) (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{30}\text{H}_{34}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 591.3$, found 591.7. Purity: 77 %. R_t = 1.14. Yield = 19 %.



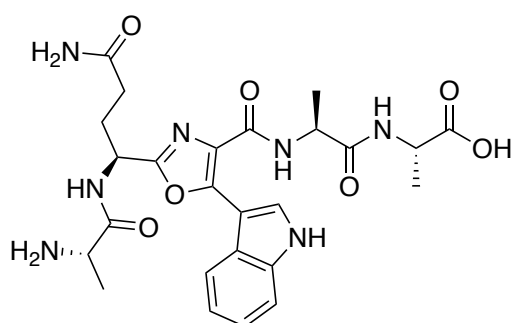
UPLC data of crude peptide before oxidation (peak 5, $r_t = 0.98$):



UPLC data of crude peptide after oxidation (**10AD**: peak 4, $r_t = 1.14$):

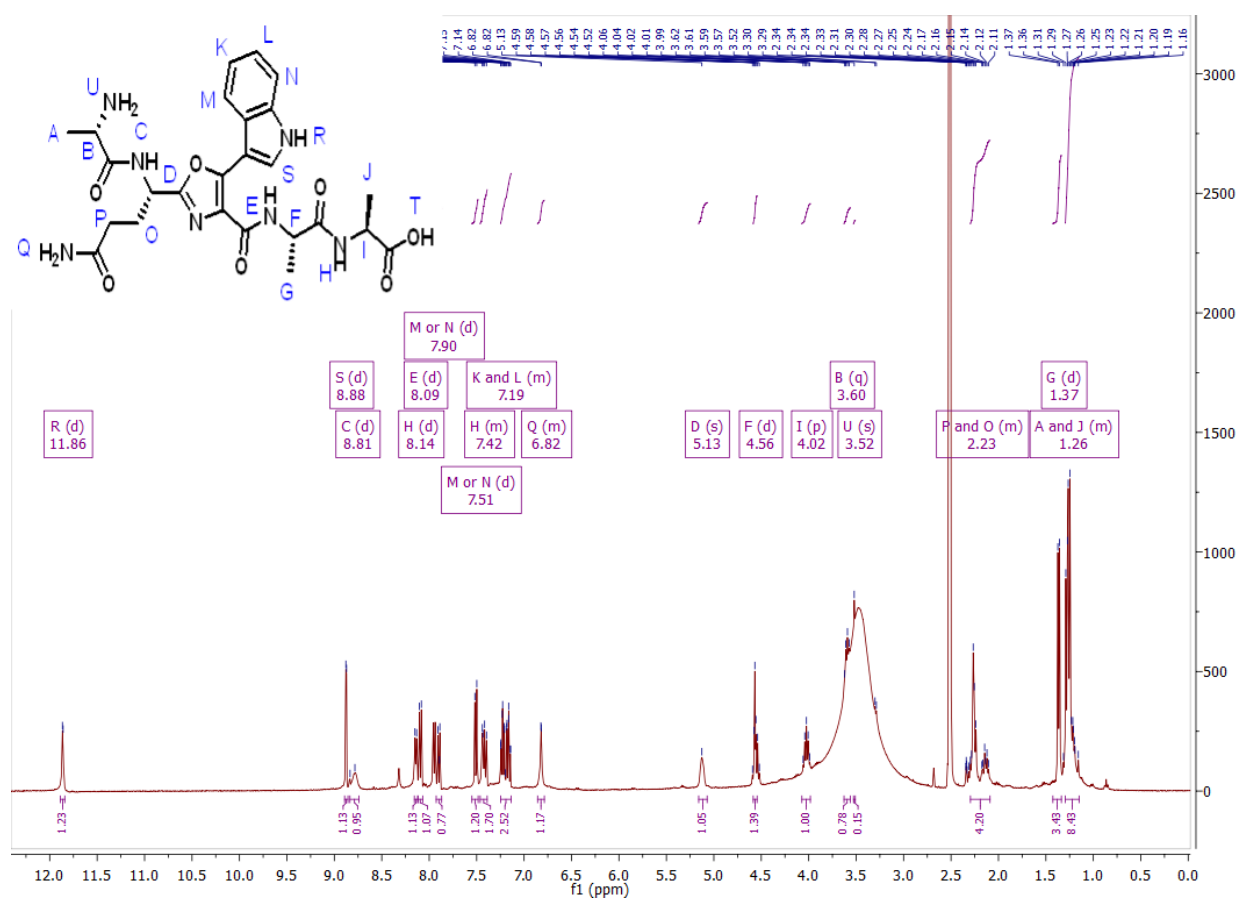


(2-((S)-4-amino-1-((S)-2-aminopropanamido)-4-oxobutyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AE)

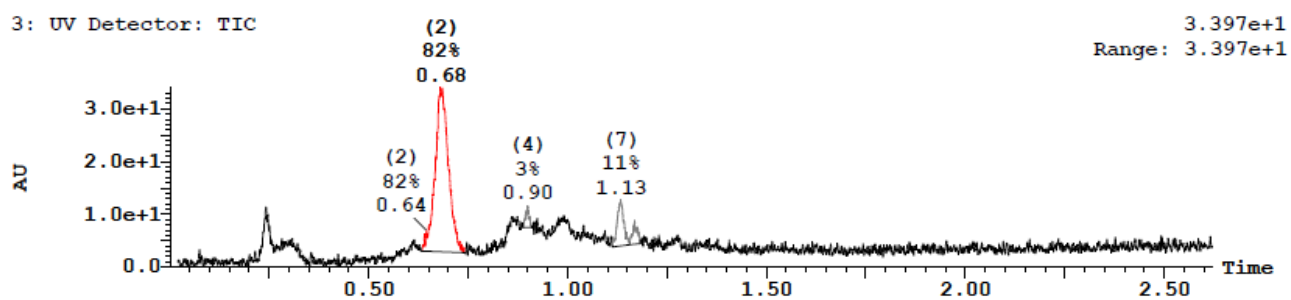


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.86 (d, J = 2.9 Hz, 1H), 8.88 (d, J = m, 1H), 8.81 (d, J = m, 1H), 8.14 (d, J = 7.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 8.1 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.26 – 7.13 (m, 2H), 6.86 – 6.79 (m, 2H), 5.13 (s, 1H), 4.60 – 4.51 (m, 2H), 4.02 (p, J = 7.0 Hz, 1H), 3.60 (q, J = 7.1, 5.8 Hz, 1H), 3.52 (s, 2H), 2.30 –

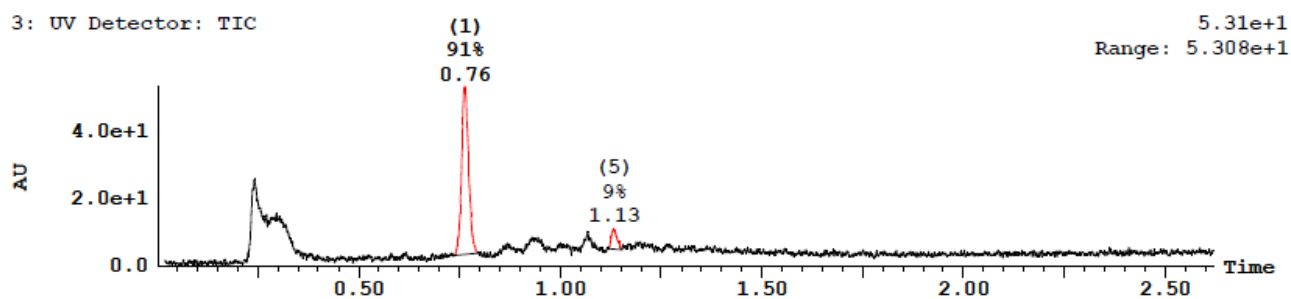
2.09 (m, 4H), 1.37 (d, J = 6.9 Hz, 3H), 1.31 – 1.15 (m, 6H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{25}\text{H}_{31}\text{N}_7\text{O}_7$ $[\text{M}+\text{H}]^+ = 542.2$, found 541.9. Purity: 91 %. R_t = 0.76. Yield = 12 %.



UPLC data of crude peptide before oxidation (peak 2, $t_r = 0.68$):



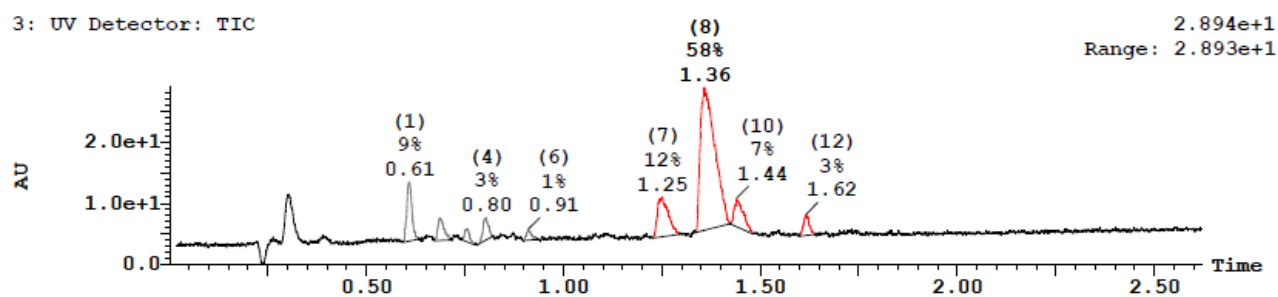
UPLC data of crude peptide after oxidation (**10AE**: peak 1, $t_r = 0.76$):



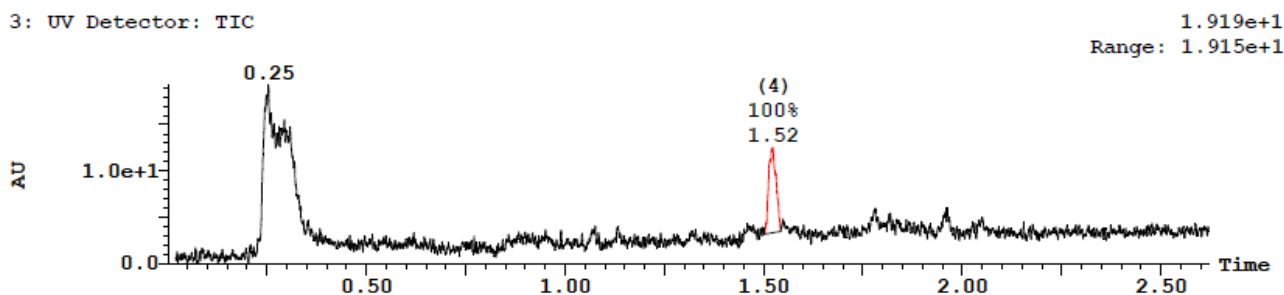
(2-((S)-1-((S)-2-aminopropanamido)-4-oxo-4-(tritylamino)butyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AF)

UPLC/MS (ESI): m/z calcd. $C_{44}H_{45}N_7O_7$ $[M+H]^+ = 784.4$, found 784.5. Purity: >95%. Rt = 1.52.

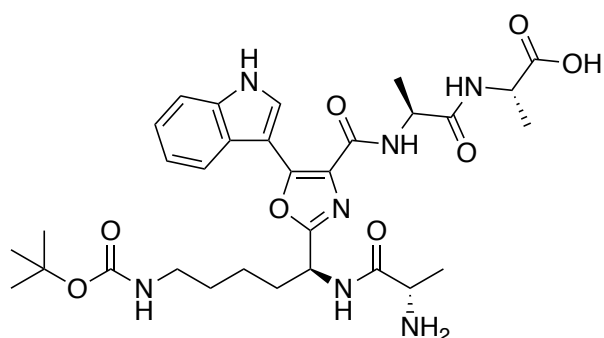
UPLC data of crude peptide before oxidation (peak (8), rt = 1.76; peak (7), rt = 1.25: trityl cleaved under the LCMS conditions).



UPLC data of crude peptide after oxidation (**10AF**: peak (4), rt = 1.52):

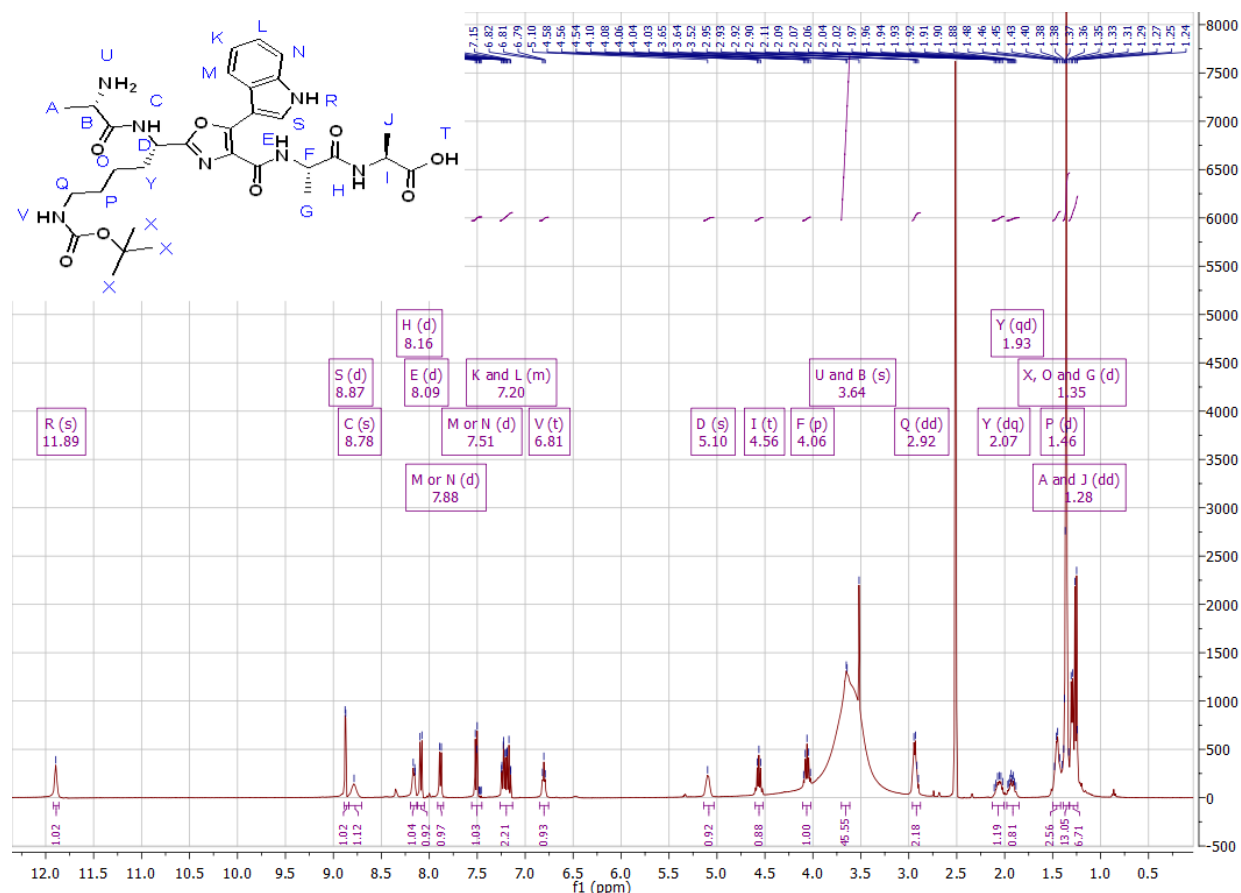


(2-((S)-1-((S)-2-aminopropanamido)-5-((tert-butoxycarbonyl)amino)-pentyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AH)

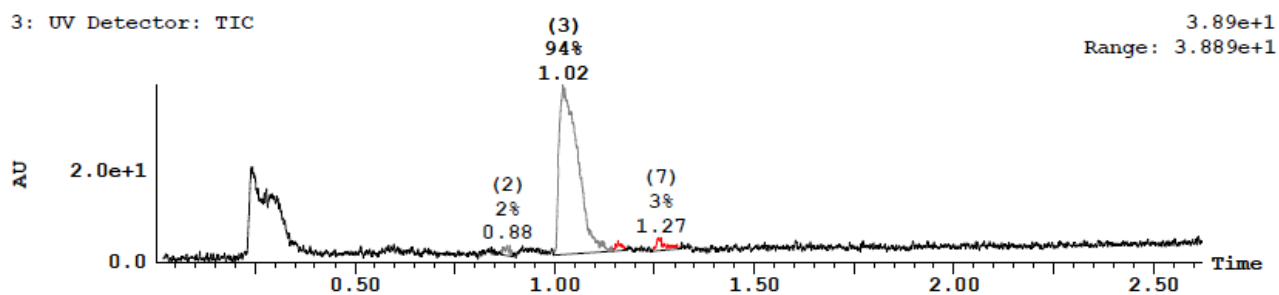


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.89 (d, 1H), 8.87 (m, 1H), 8.78 (s, 1H), 8.16 (d, J = 6.2 Hz, 1H), 8.09 (d, J = 7.9 Hz, 1H), 7.88 (d, J = 7.7 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.26 – 7.13 (m, 2H), 6.81 (t, J = 5.7 Hz, 1H), 5.10 (s, 1H), 4.56 (t, J = 7.2 Hz, 1H), 4.06 (p, J = 7.1 Hz, 1H), 3.64–3.52 (m, 3H), 2.92 (dd, J =

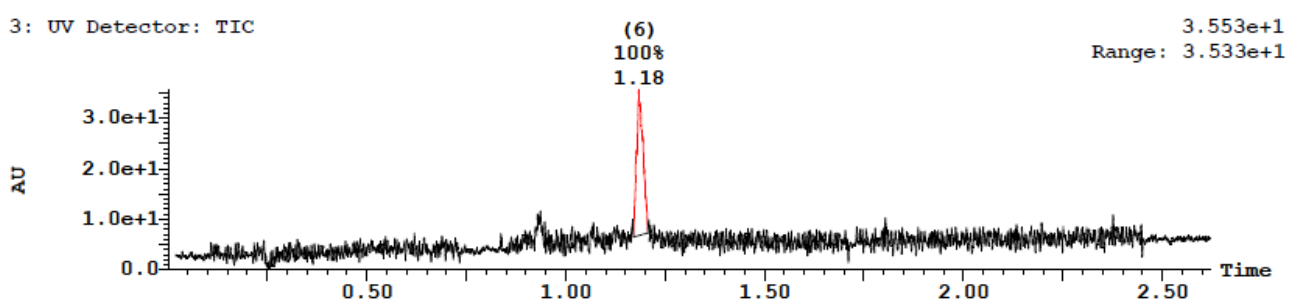
12.5, 6.2 Hz, 2H), 2.07 (dq, J = 13.6, 6.5 Hz, 1H), 1.93 (qd, J = 10.6, 10.0, 5.7 Hz, 1H), 1.46 (d, J = 6.3 Hz, 2H), 1.35 (m, 14H), 1.28 (m, 6H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{31}\text{H}_{43}\text{N}_7\text{O}_8$ $[\text{M}+\text{H}]^+ = 642.3$, found 642.1. Purity: >95 %. Rt = 1.18. Yield = 13 %.



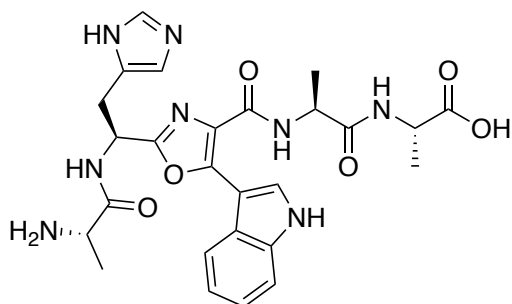
UPLC data of crude peptide bore oxidation (peak (3), rt = 1.02):



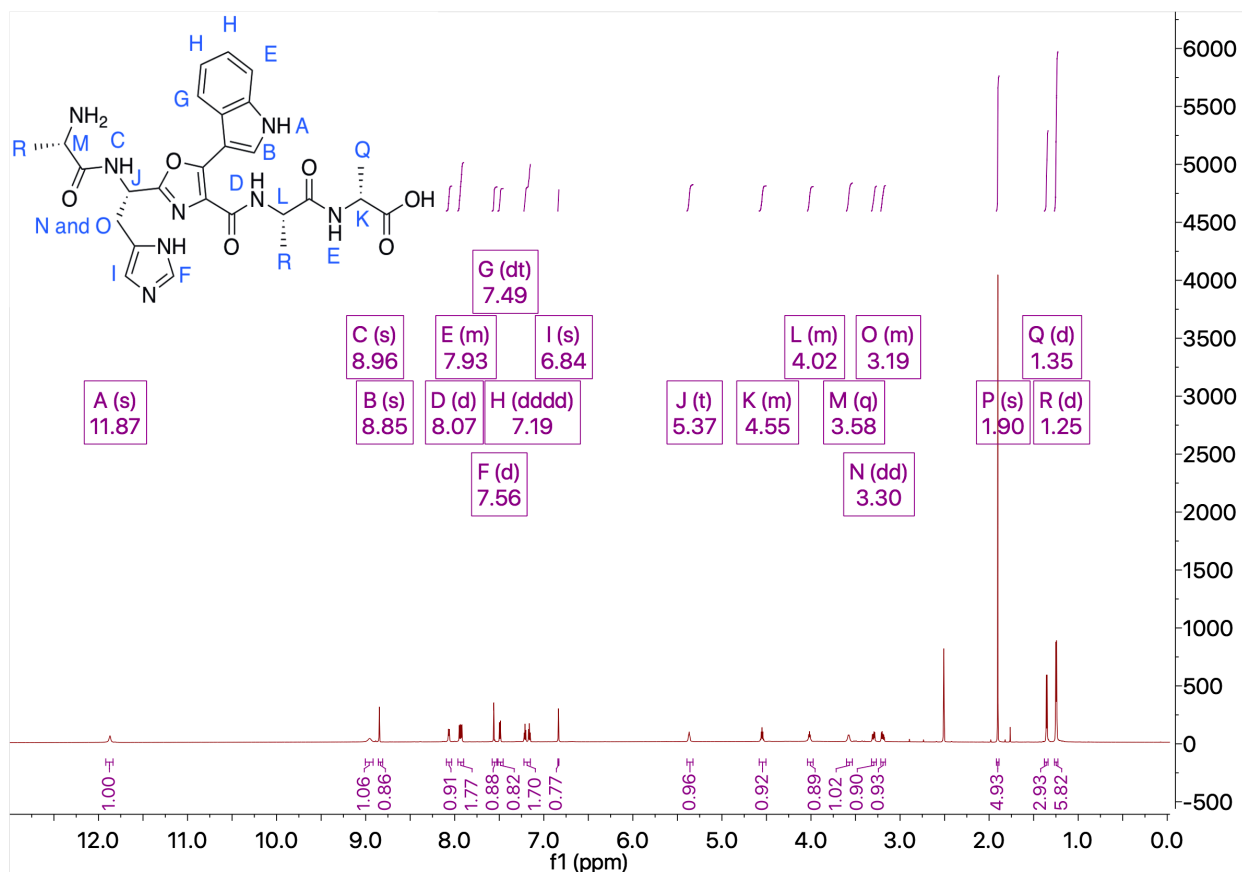
UPLC data of crude peptide after oxidation (**10AH**: peak (6), rt = 1.18):



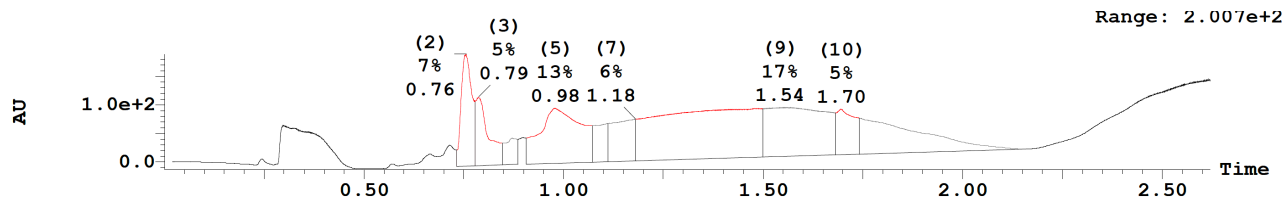
(2-((S)-1-((S)-2-aminopropanamido)-2-(1H-imidazol-5-yl)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AI)



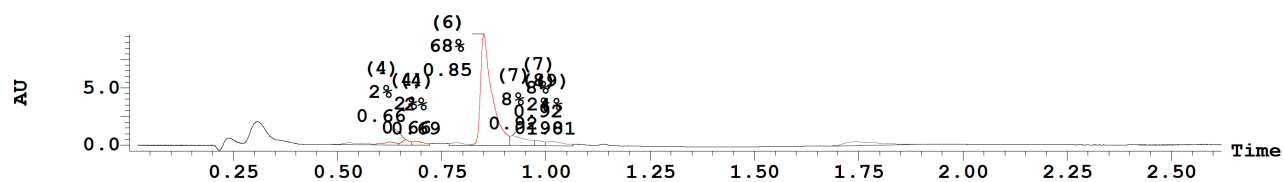
^1H NMR (800 MHz, $\text{DMSO}-d_6$) δ 11.87 (s, 1H), 8.96 (s, 1H), 8.85 (s, 1H), 8.07 (d, $J = 7.0$ Hz, 1H), 7.96 – 7.90 (m, 2H), 7.56 (d, $J = 1.1$ Hz, 1H), 7.49 (dt, $J = 8.1, 0.9$ Hz, 1H), 7.19 (m, 2H), 6.84 (s, 1H), 5.37 (t, $J = 7.1$ Hz, 1H), 4.58 – 4.51 (m, 1H), 4.04 – 3.98 (m, 1H), 3.58 (q, $J = 7.2, 5.6$ Hz, 1H), 3.30 (dd, $J = 14.7, 6.6$ Hz, 1H), 3.22 – 3.16 (m, 1H), 1.90 (s, 5H, impurity), 1.35 (d, $J = 7.0$ Hz, 3H), 1.25 (d, $J = 7.1$ Hz, 6H). UPLC/MS (ESI): m/z calcd. $\text{C}_{26}\text{H}_{30}\text{N}_8\text{O}_6$ $[\text{M}+\text{H}]^+ = 551.6$, found 551.6. Purity: > 95 %. $R_t = 0.85$. Yield = 10 %.



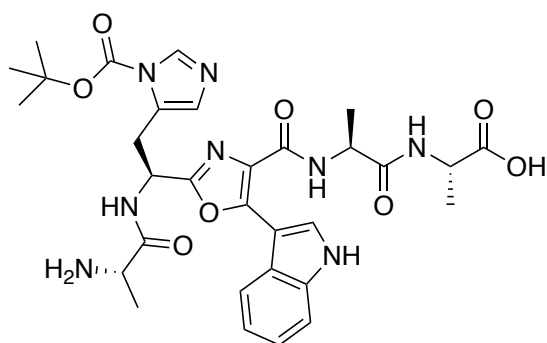
UPLC data of crude peptide before oxidation (peak (5), $r_t = 0.98$, peak (2), (3) $r_t = 1.76$, 0.79 corresponds to trityl deprotection under LCMS conditions):



UPLC data of crude peptide after oxidation (**10A**): peak (5), rt = 0.85:

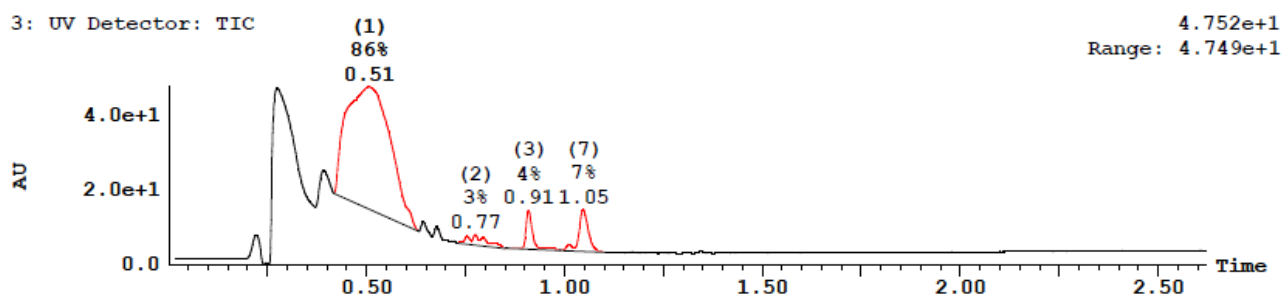


(2-((S)-1-((S)-2-aminopropanamido)-2-(1-(tert-butoxycarbonyl)-1H-imidazol-5-yl)ethyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AJ)

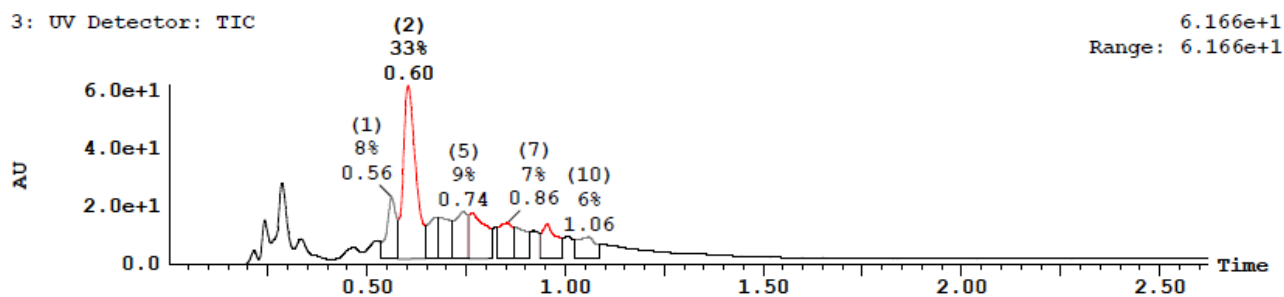


UPLC/MS (ESI): m/z calcd. $C_{31}H_{38}N_8O_8$ $[M+H]^+ = 651.3$, found 651.3. Purity: 53%. $R_t = 0.60$.

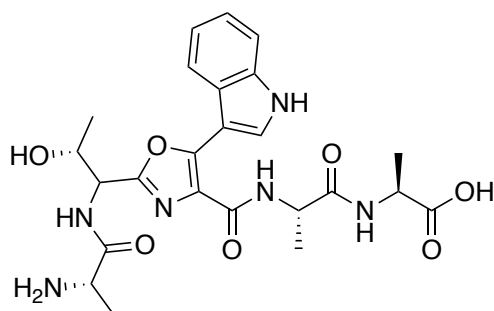
UPLC data of crude peptide before oxidation (peak (1), $r_t = 0.51$):



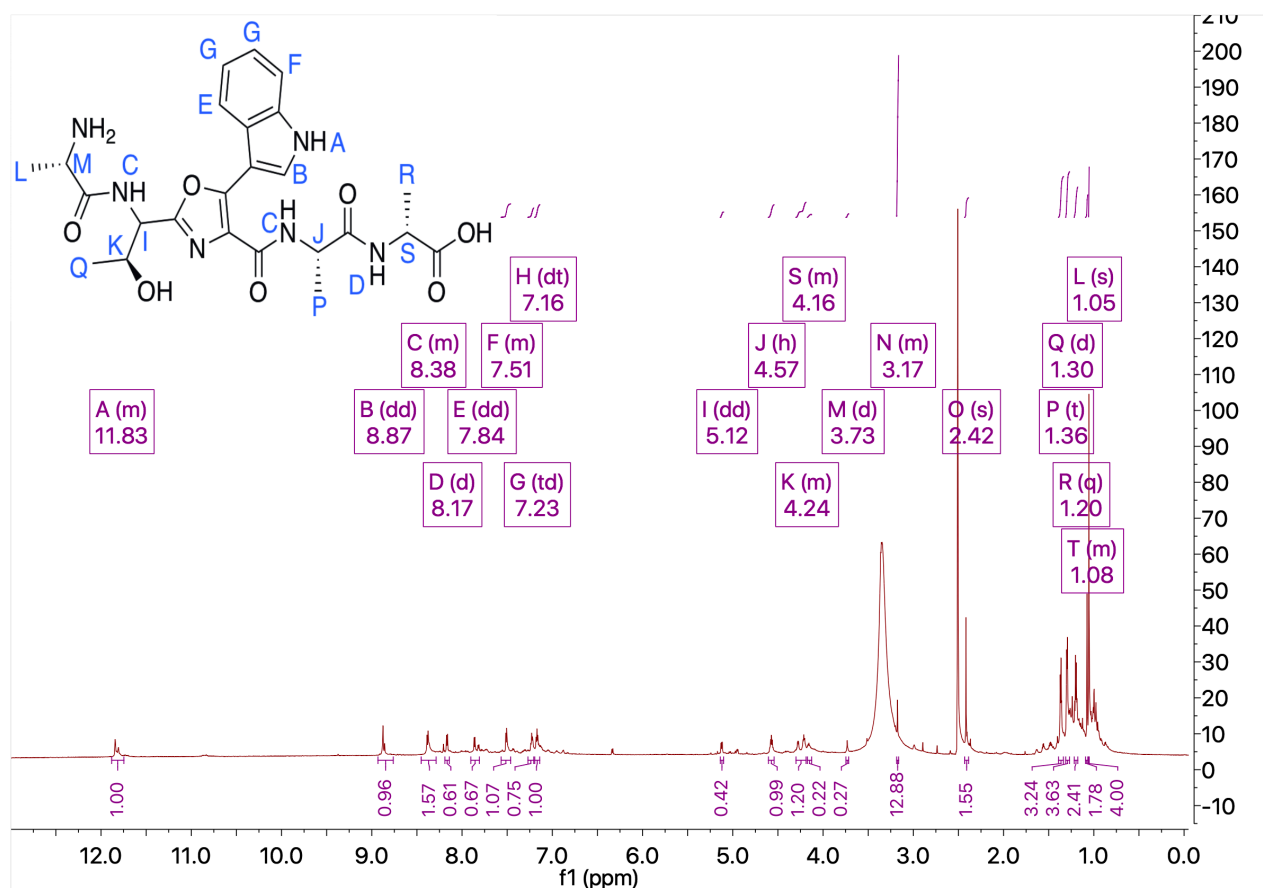
UPLC data of crude peptide after oxidation (**10AJ**: peak (2), $r_t = 0.60$):



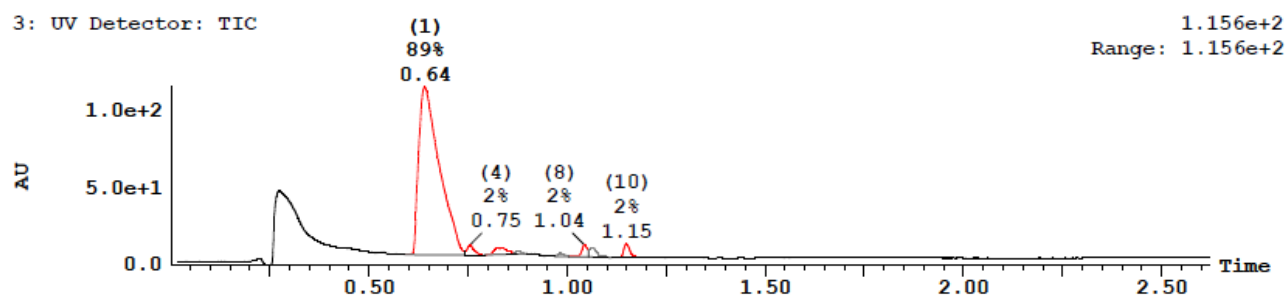
(2-((2R)-1-((S)-2-aminopropanamido)-2-hydroxypropyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AK)



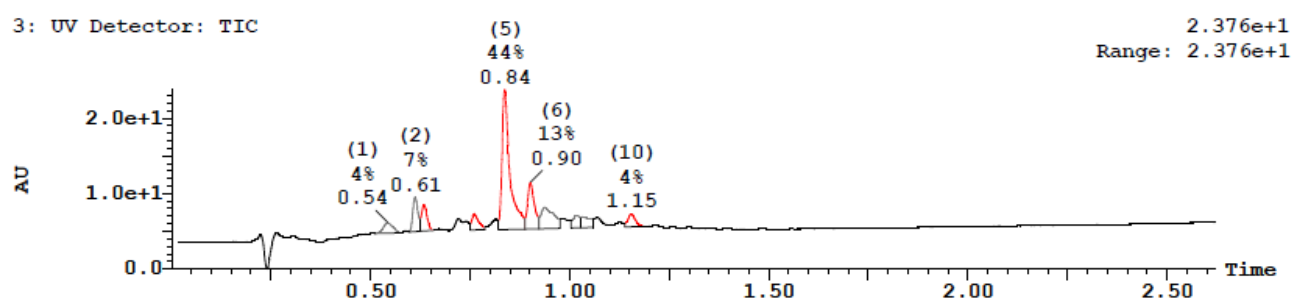
^1H NMR (800 MHz, $\text{DMSO}-d_6$) δ 11.88 – 11.75 (m, 1H), 8.87 (dd, J = 15.3, 2.9 Hz, 1H), 8.45 – 8.29 (m, 2H), 8.17 (d, J = 8.0 Hz, 1H), 7.84 (dd, J = 35.0, 7.7 Hz, 1H), 7.57 – 7.46 (m, 1H), 7.23 (td, J = 7.7, 3.4 Hz, 1H), 7.16 (dt, J = 13.5, 6.3 Hz, 1H), 5.12 (dd, J = 8.8, 3.7 Hz, 1H), 4.57 (h, J = 7.1 Hz, 1H), 4.30 – 4.19 (m, 1H), 4.17 – 4.13 (m, 1H), 3.73 (d, J = 6.6 Hz, 1H), 3.18 – 3.16 (m, 1H, Impurity), 2.42 (s, 2H), 1.36 (t, J = 6.1 Hz, 3H), 1.30 (d, J = 7.0 Hz, 3H), 1.20 (q, J = 6.9, 5.8 Hz, 3H), 1.09 – 1.07 (m, 3H), 1.05 (s, 4H, impurity). UPLC/MS (ESI): m/z calcd. $\text{C}_{24}\text{H}_{30}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 515.3$, found 515.2. Purity: 62%. R_t = 0.84. Yield = 12 %.



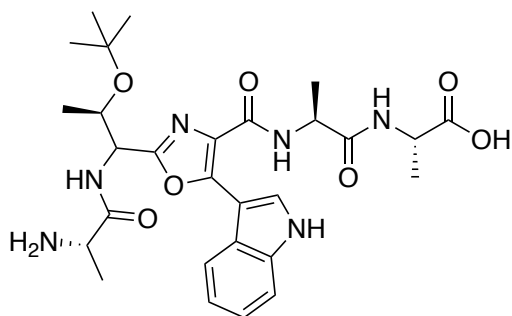
UPLC data of crude peptide before oxidation (peak (1), $r_t = 0.64$):



UPLC data of crude peptide after oxidation (**10AK**: peak (5), $r_t = 0.84$):

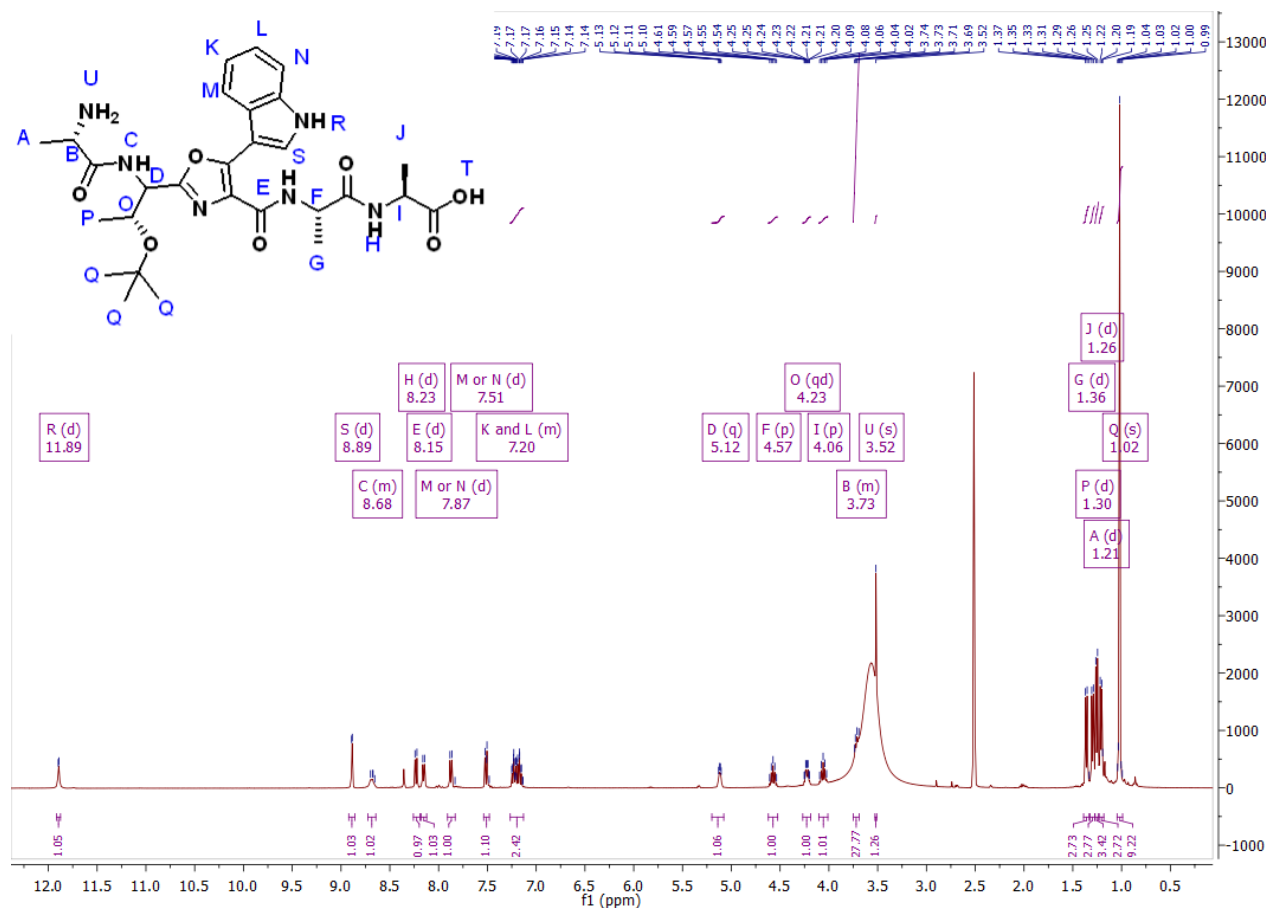


(2-((2R)-1-((S)-2-aminopropanamido)-2-(tert-butoxy)propyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AL)

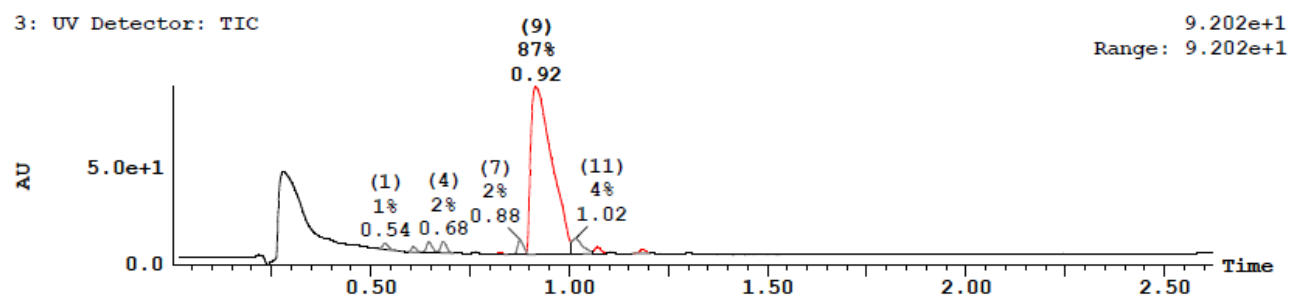


^1H NMR (400 MHz, $\text{DMSO}-d_6$): δ = 11.89 (d, J = 3.0 Hz, 1H), 8.89 (m, 1H), 8.73 – 8.64 (m, 1H), 8.23 (d, J = 7.9 Hz, 1H), 8.15 (d, J = 7.1 Hz, 1H), 7.87 (d, J = 7.6 Hz, 1H), 7.51 (d, J = 8.0 Hz, 1H), 7.27 – 7.13 (m, 2H), 5.12 (m, 1H), 4.57 (p, J = 7.0 Hz, 1H), 4.23 (qd, J = 6.2, 3.0 Hz, 1H), 4.06 (p, J = 7.1 Hz, 1H), 3.75 – 3.69 (m, 1H), 3.52 (s, 2H), 1.36 (d, J = 6.9 Hz, 3H),

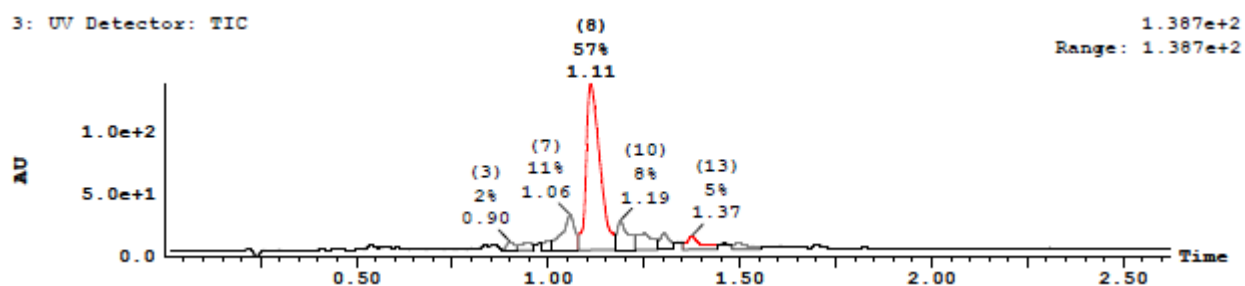
1.30 (d, J = 6.9 Hz, 3H), 1.26 (d, J = 7.2 Hz, 3H), 1.21 (d, J = 6.2 Hz, 3H), 1.02 (s, 9H). (Exchangeable COOH proton not observed); UPLC/MS (ESI): m/z calcd. $\text{C}_{28}\text{H}_{38}\text{N}_6\text{O}_7$ $[\text{M}+\text{H}]^+ = 574.3$, found 574.3. Purity: 69%. R_t = 1.11. Yield = 9%.



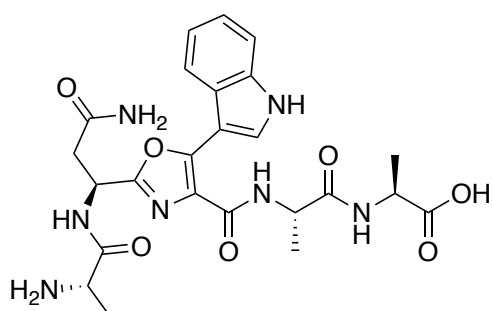
UPLC data of crude peptide before oxidation (peak (9), $t_r = 0.92$):



UPLC data of crude peptide after oxidation (**10AL**: peak (8), $t_r = 1.11$):

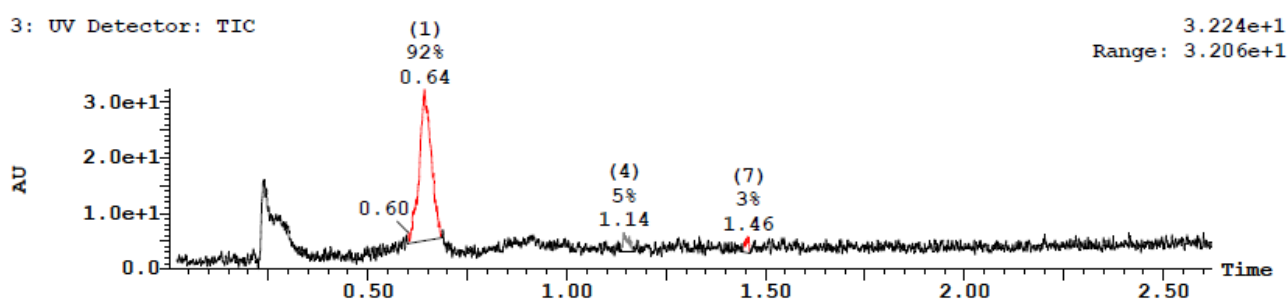


(2-((S)-3-amino-1-((S)-2-aminopropanamido)-3-oxopropyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AM)

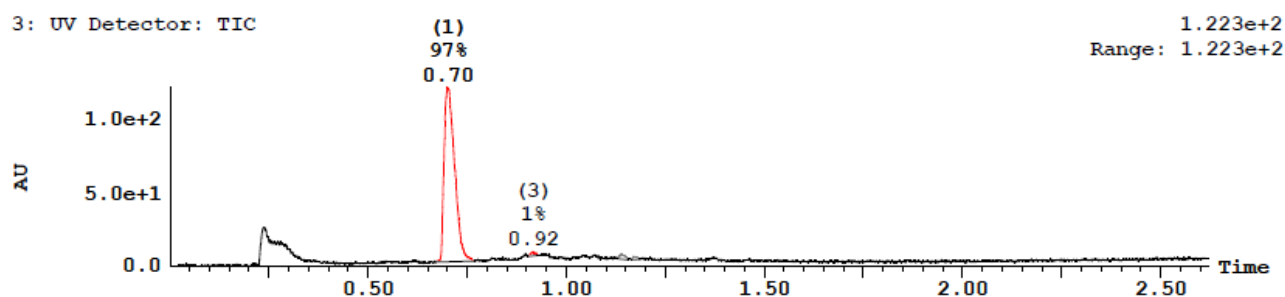


UPLC/MS (ESI): m/z calcd. $C_{24}H_{29}N_7O_7$ $[M+H]^+ = 532.3$, found 532.3. Purity: >95%. $R_t = 0.70$.

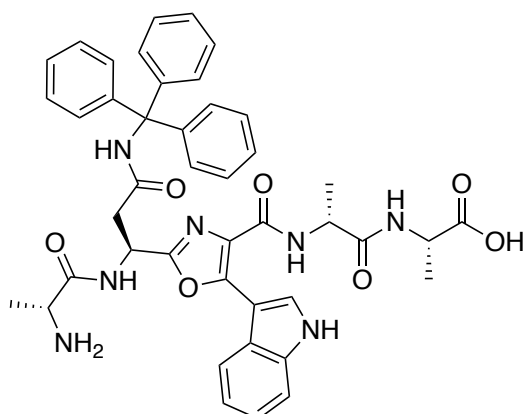
UPLC data of crude peptide before oxidation (peak (1), $r_t = 0.64$):



UPLC data of crude peptide after oxidation (**10AM**: peak (1), $r_t = 0.70$):

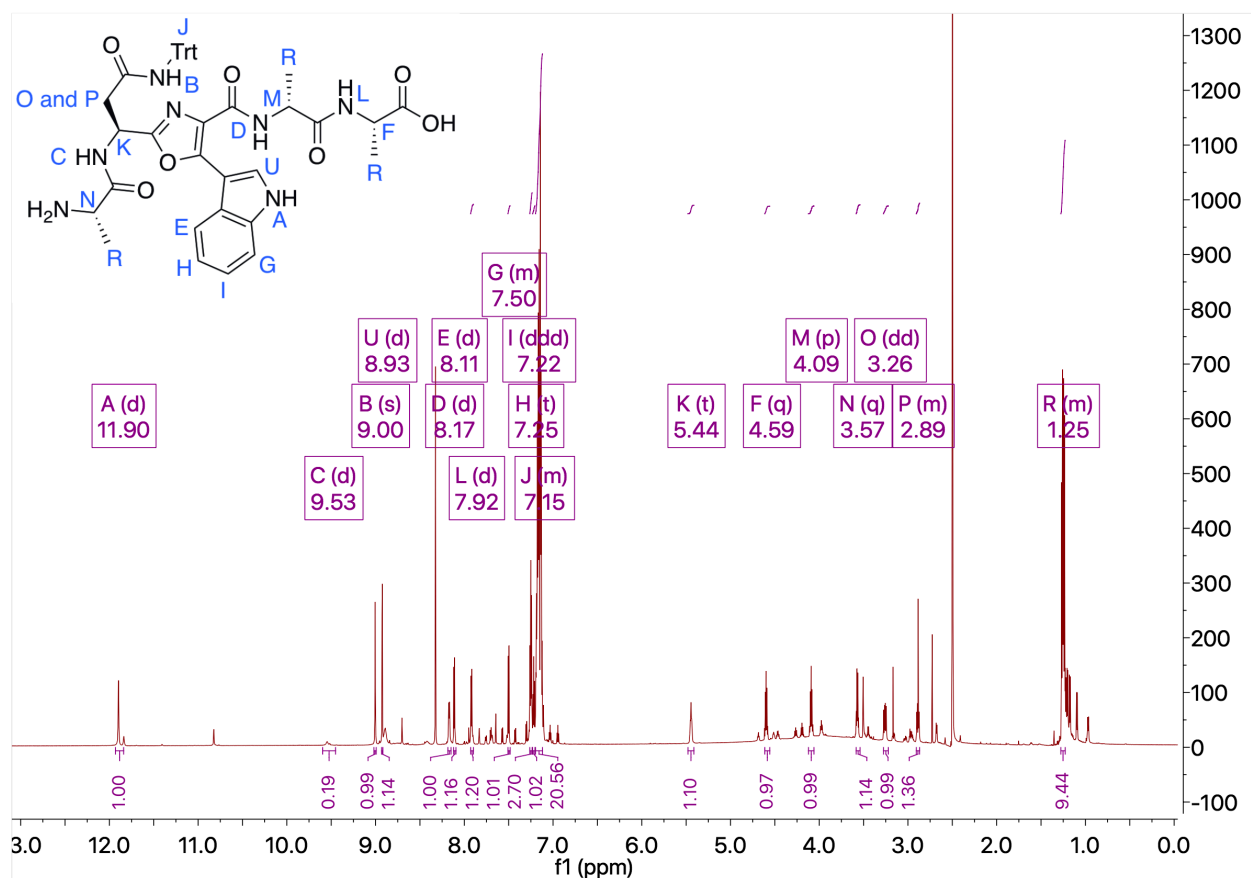


(2-((*R*)-1-aminoethyl)-5-(tritylcarbamoyl)oxazole-4-carbonyl)-*L*-tryptophyl-*D*-alanyl-*L*-alanine (10AN)



^1H NMR (800 MHz, $\text{DMSO}-d_6$) δ 11.90 (d, $J = 3.0$ Hz, 1H, A), 9.53 (m, 1H, C (Confirmed from DQF-COSY)) 9.00 (s, 1H, B), 8.93 (d, $J = 2.8$ Hz, 1H, U), 8.17 (d, $J = 7.2$ Hz, 1H, D), 8.11 (d, $J = 8.0$ Hz, 1H, E), 7.92 (d, $J = 7.8$ Hz, 1H, F), 7.51 – 7.48 (m, 1H, G), 7.25 (t, $J = 7.8$ Hz, 1H, H), 7.22 (t, $J = 8.1$ Hz, 1H, I), 7.20 – 7.12 (m, 15H, J), 5.44 (t, $J = 7.4$ Hz, 1H, K), 4.59 (q, $J = 7.2$ Hz, 1H, L), 4.09 (p, $J = 7.2$

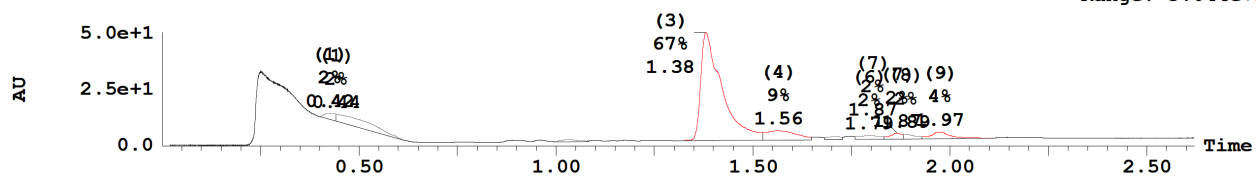
Hz, 1H, M), 3.57 (q, $J = 7.0$ Hz, 1H, N), 3.26 (dd, $J = 15.4, 8.1$ Hz, 1H, O), 2.91 – 2.87 (m, 1H, P), 1.28 – 1.23 (m, 9H, R). (Exchangeable amine and carboxyl protons not observed, peak at 8.35 might be amine(No HSQC), but shows no DQF-COSY correlation) UPLC/MS (ESI): m/z calcd. for $\text{C}_{43}\text{H}_{43}\text{N}_7\text{O}_7$ $[\text{M}+\text{H}]^+ = 769,3$ found 769.8. Purity: 88 %, $R_t = 1.38$. Yield = 25 %.



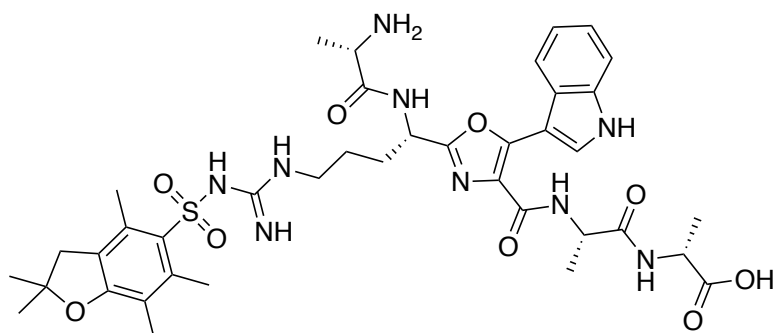
UPLC data of crude peptide after oxidation (**10AN**: peak (3), rt = 1.38):

3: UV Detector: TAC: Wavelength Range: (210 - 500) Smooth (Mn, 2x3)

5.023e+1
Range: 5.044e+1

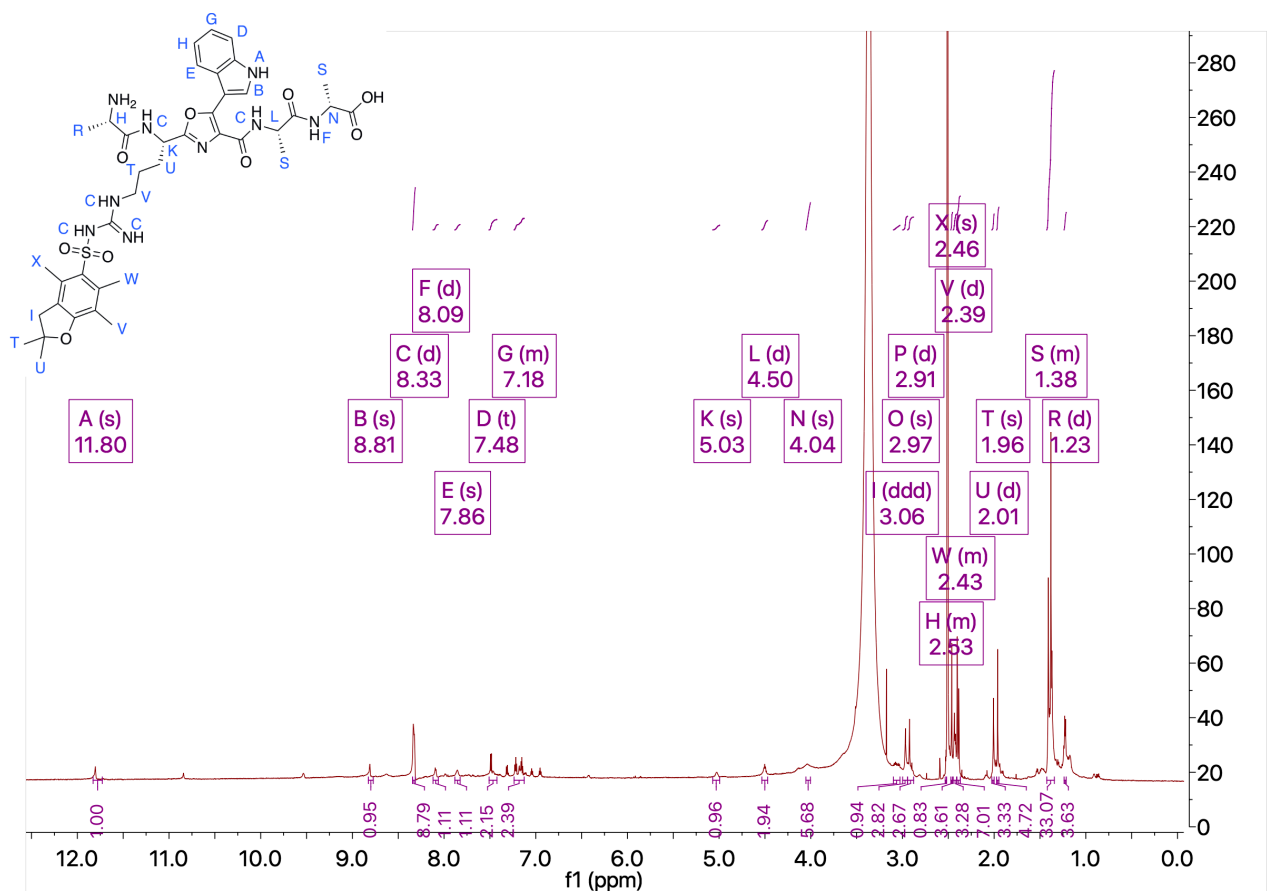


(2-((S)-1-((S)-2-aminopropanamido)-4-(3-((2,2,5,7,8-pentamethyl-chroman-6-yl)sulfonyl)guanidino)butyl)-5-(1H-indol-3-yl)oxazole-4-carbonyl)-L-alanyl-L-alanine (10AO)

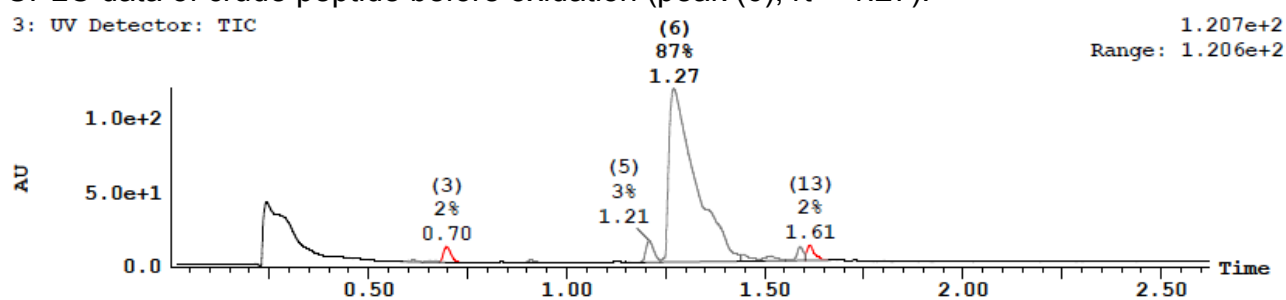


^1H NMR (800 MHz, $\text{DMSO-}d_6$) δ 11.80 (s, 1H), 8.81 (s, 1H), 8.33 (d, $J = 9.4$ Hz, 1H), 8.09 (d, $J = 7.9$ Hz, 1H), 7.86 (s, 1H), 7.48 (t, $J = 10.4$ Hz, 1H), 7.24 – 7.13 (m, 2H), 5.03 (s, 1H), 4.50 (d, $J = 10.3$ Hz, 1H), 4.04 (s, 1H), 3.06

(ddd, $J = 28.7, 13.5, 6.4$ Hz, 1H), 2.97 (s, 3H, impurity), 2.91 (d, $J = 21.2$ Hz, 3H, impurity), 2.46 (s, 3H), 2.45 – 2.41 (m, 3H), 2.39 (d, $J = 13.8$ Hz, 3H), 2.01 (d, $J = 2.6$ Hz, 3H), 1.96 (s, 3H), 1.43 – 1.34 (m, 6H), 1.23 (d, $J = 6.9$ Hz, 3H). UPLC/MS (ESI): m/z calcd. $\text{C}_{40}\text{H}_{54}\text{N}_9\text{O}_9\text{S}$ $[\text{M}+\text{H}]^+ = 836.4$, found 836.4. Purity: 62 %. $R_t = 1.38$. Yield = 18 %.

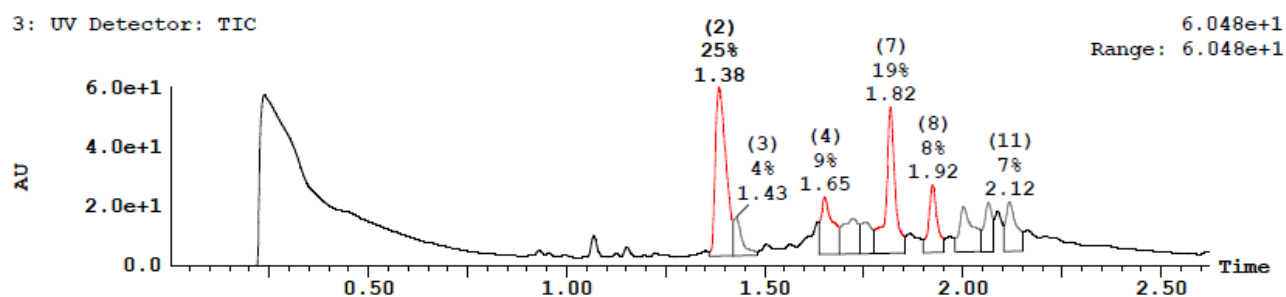


UPLC data of crude peptide before oxidation (peak (6), $rt = 1.27$):

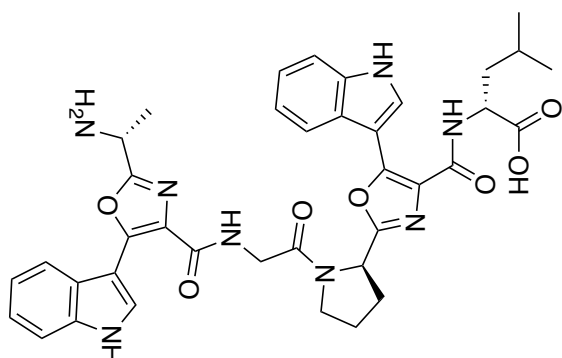


UPLC data of crude peptide after oxidation (**10AN**: peak (2) and (7), $rt = 1.38$ and $rt = 1.82$):

Peptide before oxidation (2) is $[M+1]^+$ + (7) is $[M-1]^+$

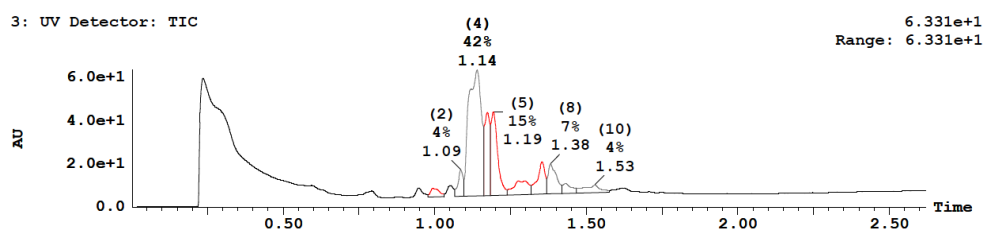


(2-((*R*)-1-((2-((*R*)-1-aminoethyl)-5-(1*H*-indol-3-yl)oxazole-4-carbonyl)glycyl)pyrrolidin-2-yl)-5-(1*H*-indol-3-yl)oxazole-4-carbonyl)-*D*-leucine (10AO)

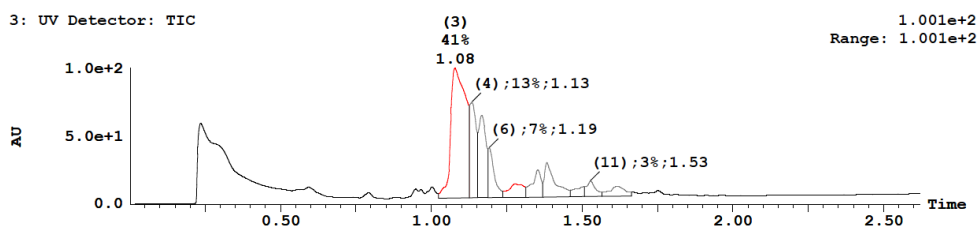


UPLC/MS (ESI): m/z calcd. $C_{40}H_{54}N_9O_9S$
 $[M+H]^+ = 721.3$, found 721.3. Purity: 83%. $R_t = 1.35$

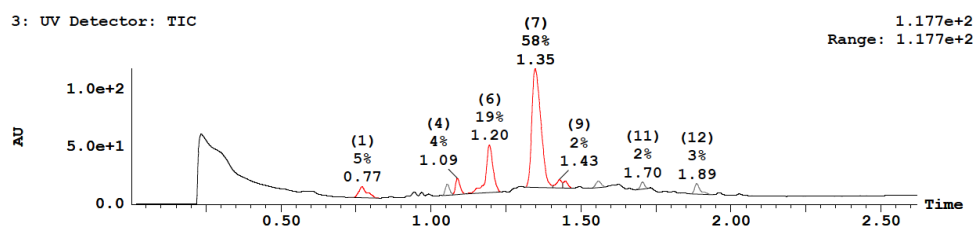
0.5 equiv. of DDQ with respect to tryptophan. Product is (8)



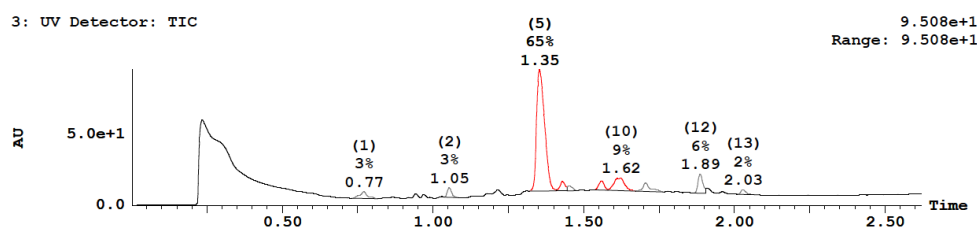
1.0 equiv. of DDQ with respect to tryptophan. Product is not reported



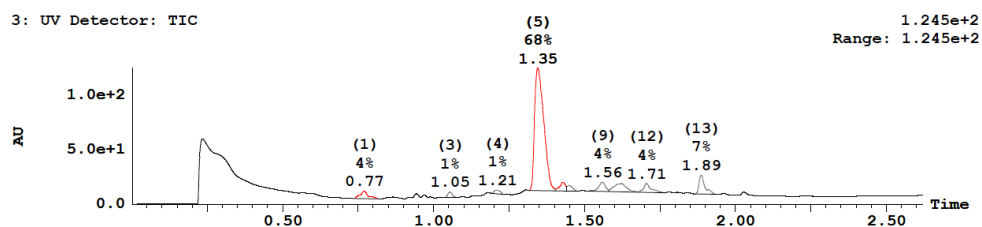
1.5 equiv. of DDQ with respect to tryptophan. Product is (7)



2.0 equiv. of DDQ with respect to tryptophan. Product is (5)



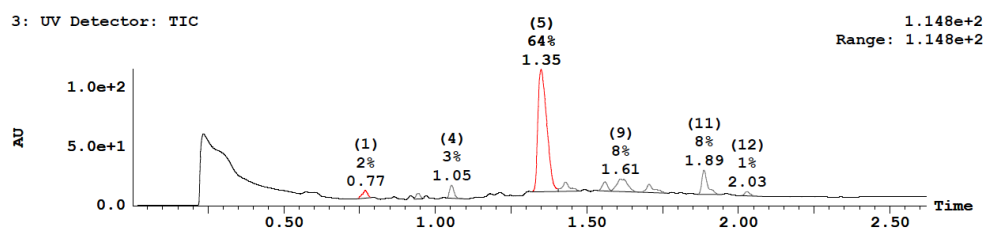
2.5 equiv. of DDQ with respect to tryptophan. Product is (5)



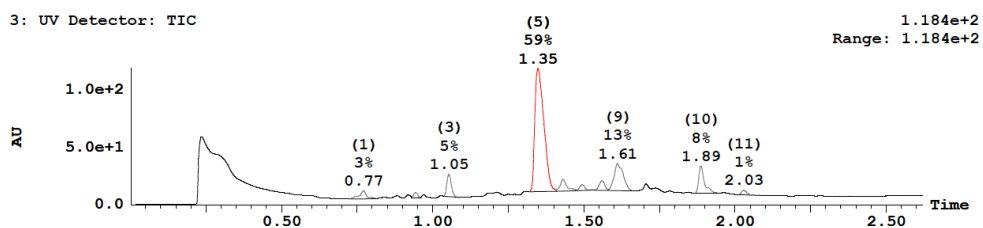
3.0 equiv.

of

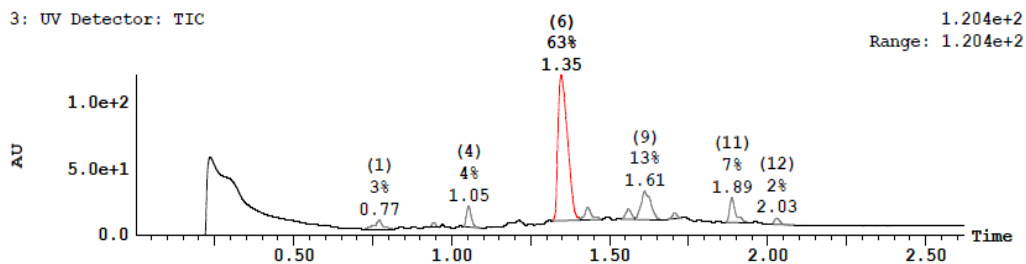
DDQ with respect to tryptophan. Product is (5)



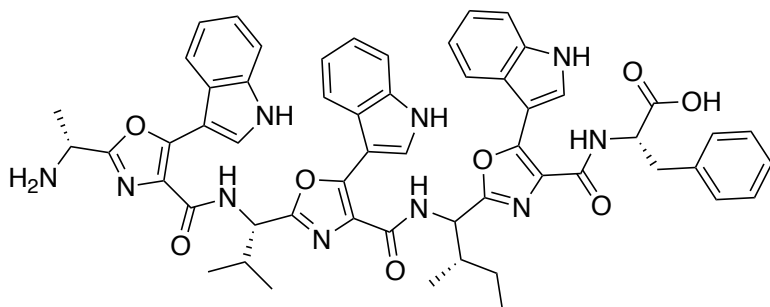
3.5 equiv. of DDQ with respect to tryptophan. Product is (5)



4.0 equiv of DDQ with respect to tryptophan. Product is (6)

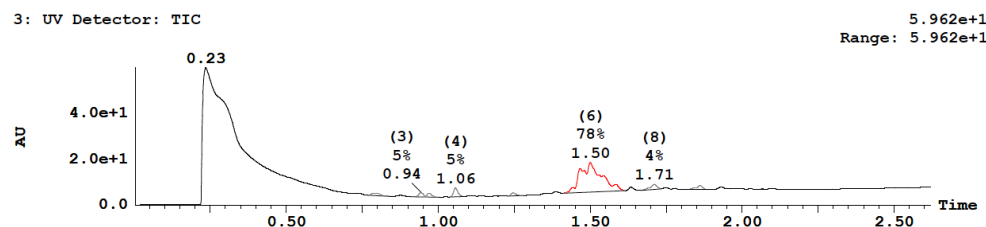


((3S)-2-((S)-2-((S)-2-((S)-2-((S)-2-aminopropanamido)-3-(1H-indol-3-yl)-propanamido)-3-methylbutanamido)-3-(1H-indol-3-yl)propanamido)-3-methylpentanoyl)-L-tryptophyl-L-phenylalanine (10AP)

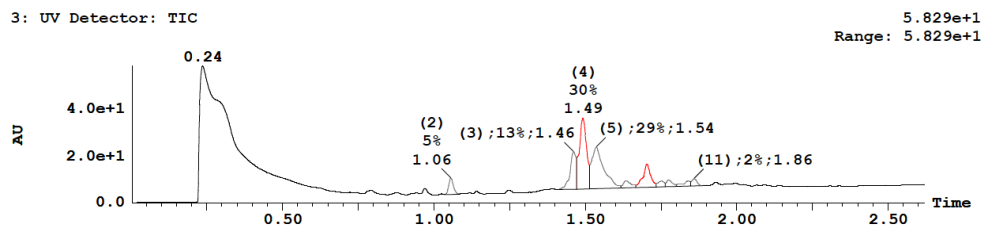


UPLC/MS (ESI): m/z calcd.
 $C_{40}H_{54}N_9O_9S$ $[M+H]^+ = 995.4$,
 found 995.3 Purity: 75 %. Rt =
 1.82.

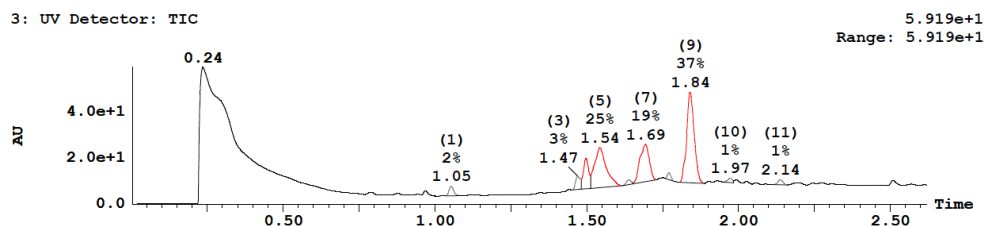
0.5 equiv. of DDQ with respect to tryptophan. Product is not traced.



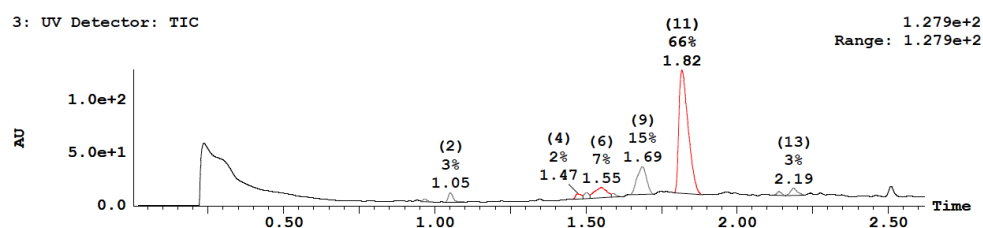
1.0 equiv. of DDQ with respect to tryptophan. Product is (11)



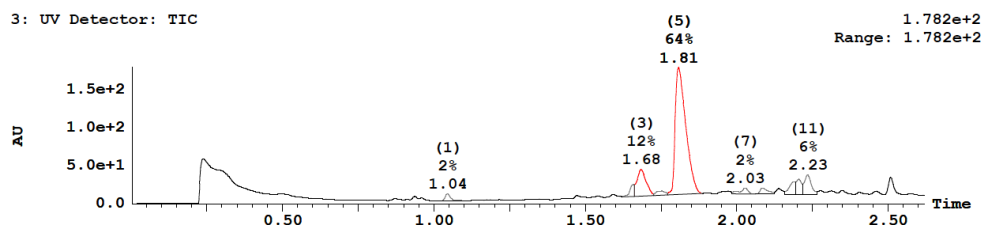
1.5 equiv. of DDQ with respect to tryptophan. Product is (9)



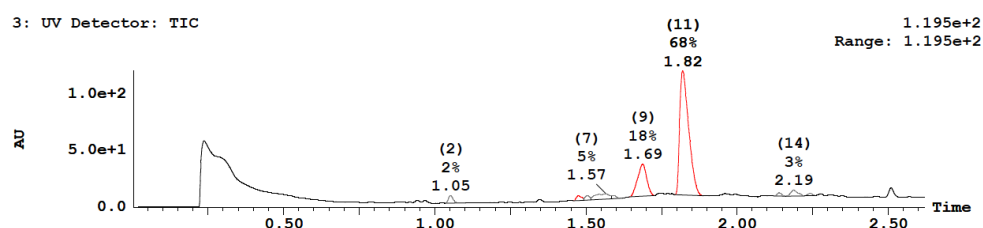
2.0 equiv. of DDQ with respect to tryptophan. Product is (11)



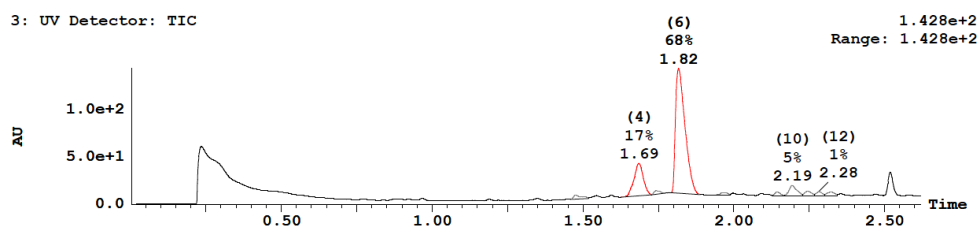
2.5 equiv. of DDQ with respect to tryptophan. Product is (5)



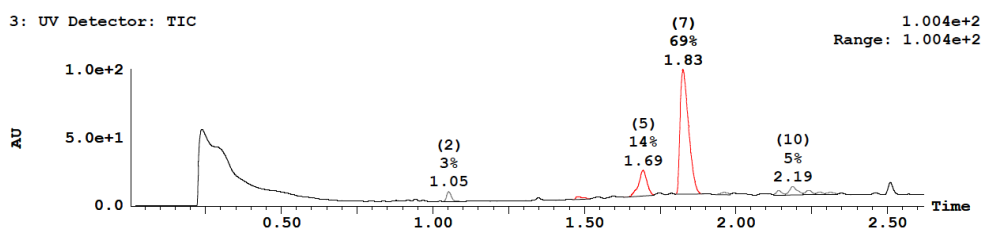
3.0 equiv. of DDQ with respect to tryptophan. Product is (11)



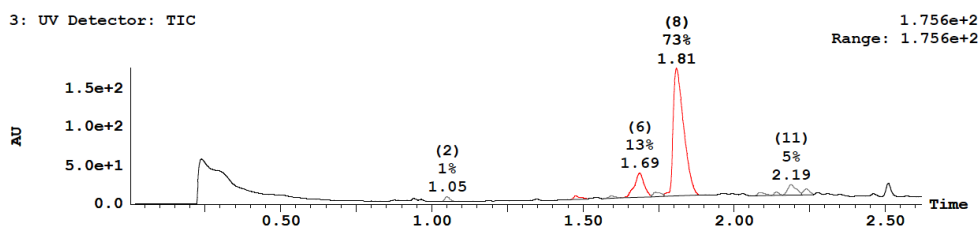
3.5 equiv. of DDQ with respect to tryptophan. Product is (6)



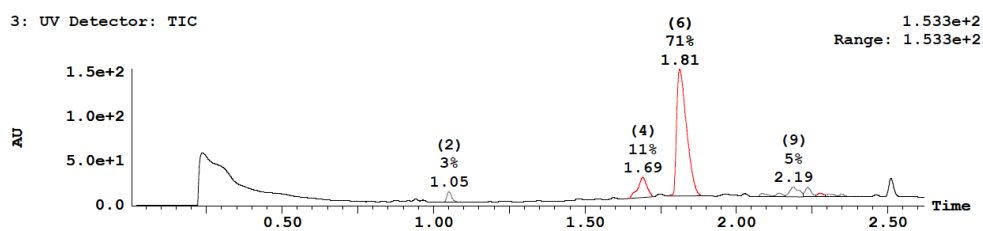
4.0 equiv. of DDQ with respect to tryptophan. Product is (7)



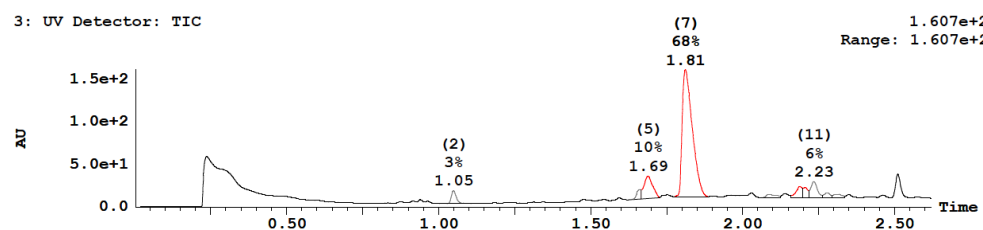
4.5 equiv. of DDQ with respect to tryptophan. Product is (8)



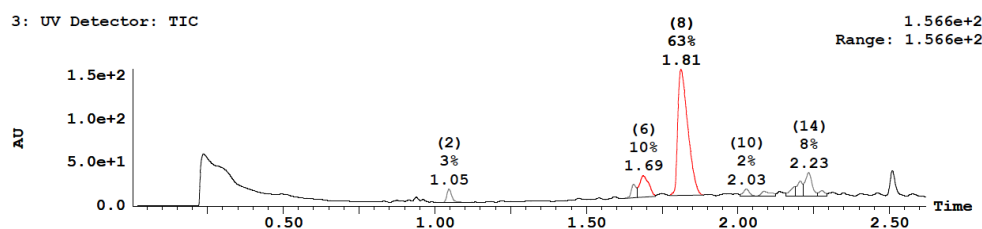
5.0 equiv. of DDQ with respect to tryptophan. Product is (6)



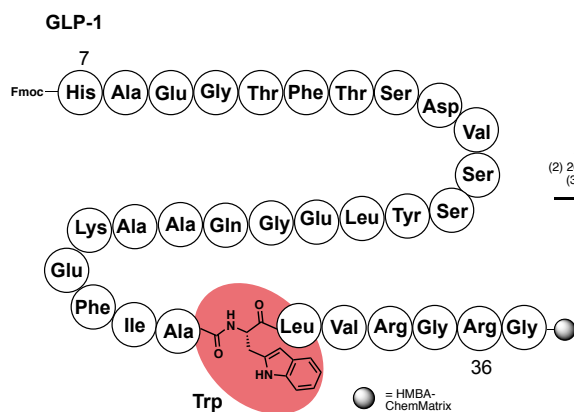
5.5 equiv. of DDQ with respect to tryptophan. Product is (7)



6.0 equiv. of DDQ with respect to tryptophan. Product is (8)



Fluorescent labeling of GLP-1:



(1) DDQ, CH₃CN
(2) 20% Piperidine in DMF
(3) 0.1M NaOH (aq)

Fluorescent labeled GLP-1

