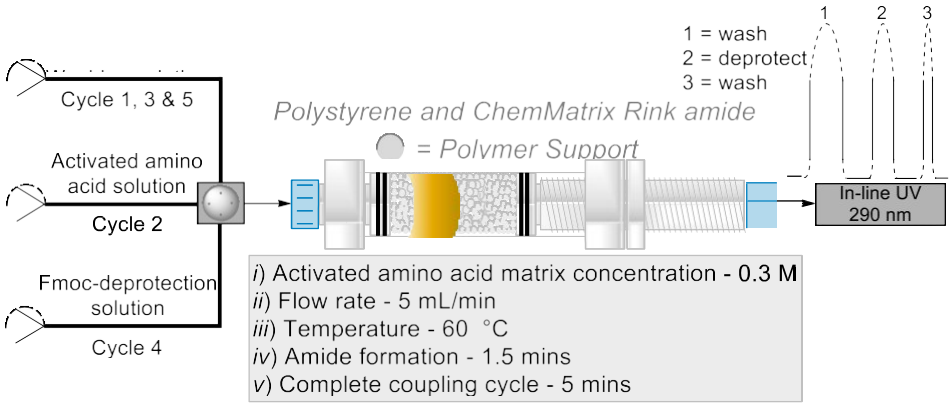
An optimised approach for continuous-flow solid-phase peptide synthesis utilising a rudimentary flow reactor

Electronic Supporting Information

Lawson K. Spare,[a] Victoire laude,[b] David G. Harman,[c,d] Janice R. Aldrich-Wright,[a,e] and Christopher P. Gordon*[a,d]



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Contents

1.1 General information

Fmoc-protected amino acids were purchased from Auspep and used as received as was Rink Amide functionalised polystyrene resin (0.7 mmol/g). Rink Amide functionalised Chem Matrix Resin (0.48 mmol/g) was purchased from Biotage. All solvents were bulk, and distilled in glass prior to use. Materials were purchased from either Aldrich Chemical Co or ChemSupply. Peptide sequences were monitored using analytical RP-HPLC, performed using an Agilent instrument comprised of six modules; 1260 Bin Pump, 1260 HIP Degasser, 1260 ALS, 1260 TCC, 1260 DAD and 1260 FC-AS. Analytical RP-HPLC was performed using Phenomenex Onyx Monolithic reversed-phase C18 column (4.6 x 150 mm). Solvent A: 0.06% TFA in H_2O and solvent B: 0.06% TFA in CH_3CN:H_2O (9:1), flow rate of 1.0 mL/min, gradient 10-100 (%B), curve = 6, over 15.0 mins, and detection at 214 and 254 nm. Mass spectrometry data was obtained in the Western Sydney Mass Spectrometry Facility. Low resolution electrospray ionisation mass spectrometry (ESIMS) experiments were performed using a Waters TQ-MS triple quadrupole mass spectrometer fitted with an ESI source. Spectra were recorded in positive ion mode from analyte solutions injected (10 µL) into 0.1% formic acid in 50% aqueous methanol flowing at 0.1 mL min−1. A capillary voltage of 3.0 kV, cone voltage of 30 V, desolvation temperature of 300 °C and desolvation flow rate (nitrogen) of 500 L h−1 were employed. Spectra were collected over 1min with an m/z range of 100–1500. High resolution accurate mass spectra were obtained using a Waters Xevo QToF MS mass spectrometer. The instrument was fitted with an ESI probe and mass-corrected sample spectra were recorded in positive ion mode, incorporating leucine encephalin (200 pg/µL in 50% aqueous acetonitrile + 0.1% formic acid) as a lockmass compound. Prior to obtaining spectra, the m/z range of 50-2000 was calibrated against sodium iodide solution. Samples were prepared in 0.1% aqueous formic acid at concentrations of approximately 10 ng/mL and infused via syringe pump at 3 µL/min.

1.2 Apparatus setup

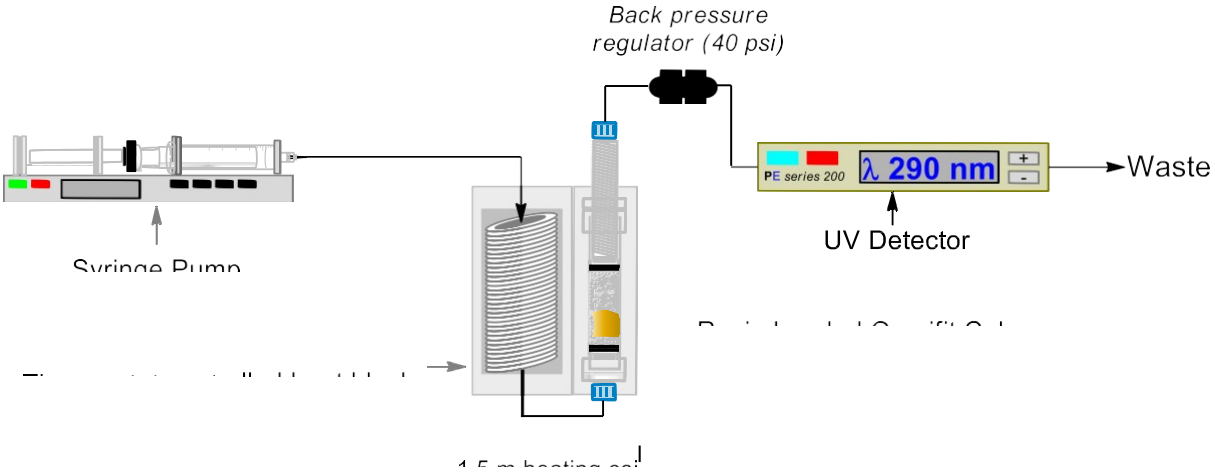


Figure 1.2.1: Schematic overview of continuous infusion method with Syringe pump, thermostat controlled heating apparatus (Volcano), 1.5 m heating coil, omnifit column, back pressure regulator and in-line HPLC UV/Vis detector set to 290 nm.

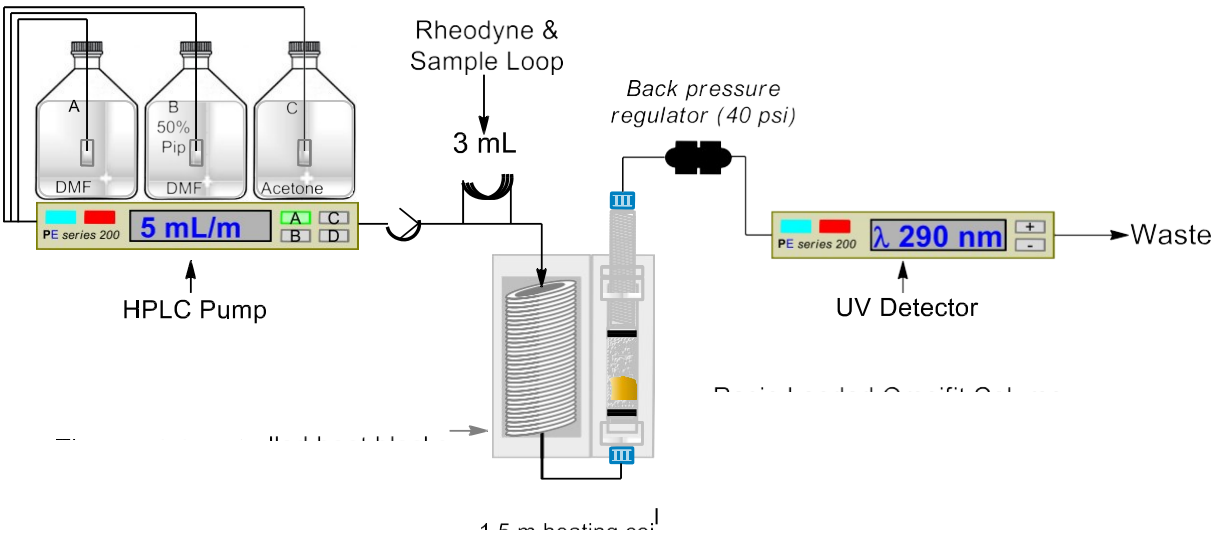


Figure 1.2.2: Schematic overview of continuous flow injection method with LC Pump, Rheodyne injection module with 3 mL injection loop, thermostat controlled heating apparatus (Volcano), 1.5 m heating coil, omnifit column, back pressure regulator and in-line HPLC UV/Vis detector set to 290 nm.

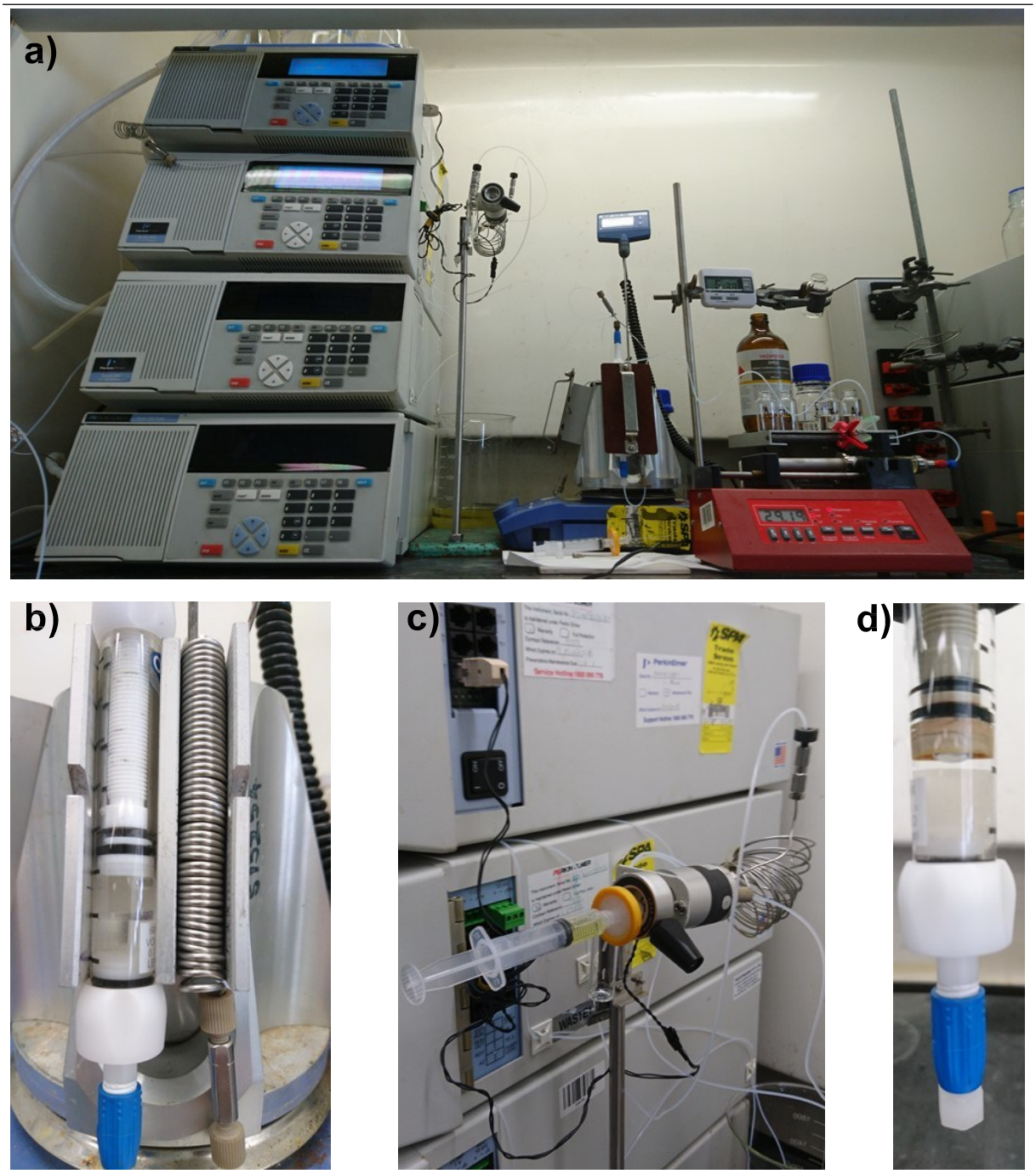
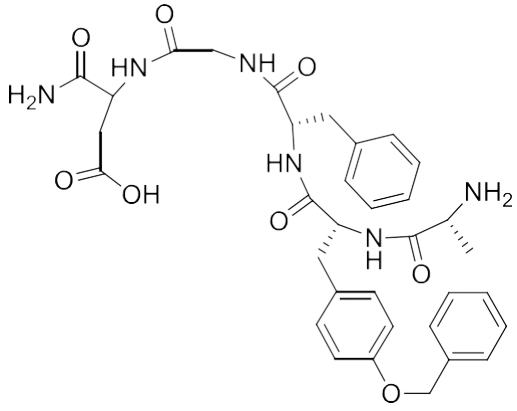


Figure 1.2.3: **a**) Overall system layout comprised of a PerkinElmer Series 200 LC pump, PerkinElmer *Series 200 UV*/VIS *Detector, Rheodyne 7725(i) injection module, FRX volcano heat block and IKA* RCT basic heater stirrer fitted with temperature sensor; **b**) Omnifit column and 1.5 m heating coil (stainless steel tubing 1/16" OD x .005" ID) fitted within FRX volcano heat block adaptor; **c**) *Rheodyne 7725(i) injection module fitted with a 3 mL injection loop;* ***d****) Resin packed within an omnifit with reaction void adjusted to with 5 mm of swollen resin surface.*

1.3 General procedure

(9S,12R,15R)-15-amino-9-benzyl-12-(4-(benzyloxy)benzyl)-3-carbamoyl-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (2)



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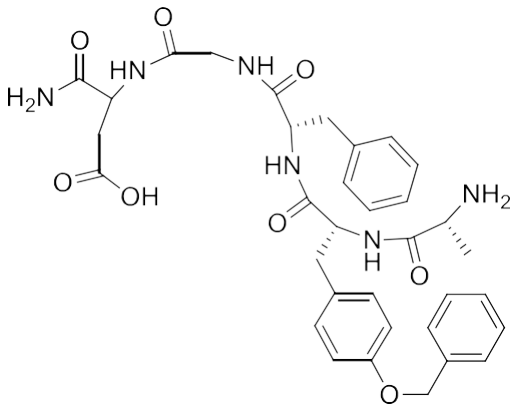
Optimized procedure for the construction of 2 using PS-RAM. Rink amide polystyrene resin (0.1 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (89 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(Bz)-OH (148 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. The dried resin was cleaved using the cleavage cocktail (10 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) for 12 hours. The resin was filtered off and solution evaporated to dryness on rotary evaporator. The solution was resuspended in H_2O:MeOH mixture (1 mL, H_2O:MeOH 1:1) and purified using preparative HPLC. The combined fractions were lyophilized and subjected to analysis. MS (ESI^+) *m/z* 661 (M + 1, 100 %), 683 (M + Na, 30%). HRMS (ESI^+) for $C_{34}H_{41}N_6O_8$; calculated 661.2986, found, 661.2986. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15

min, t_R 7.6 min. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.51 (d, *J* = 7.1 Hz, 1H), 8.41 (t, *J* = 7.5 Hz, 1H), 8.34 (m, 2H), 8.25 (d, *J* = 7.6 Hz, 1H), 7.47 – 7.26 (m, 8H), 7.26 – 7.19 (m, *J* = 4.0 Hz, 5H), 7.20 – 7.13 (m, 2H), 7.10 (d, *J* = 8.3 Hz, 2H), 7.05 (d, *J* = 6.8 Hz, 1H), 6.84 (d, *J* = 8.4 Hz, 3H), 5.00 (s, 2H), 4.56 – 4.33 (m, 3H), 3.60 – 3.46 (m, 1H), 3.12 – 2.98 (m, 1H), 2.97 – 2.77 (m, 2H), 2.75 – 2.59 (m, 1H), 2.57 – 2.37 (m, 3H), 1.13 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 174.1, 174.0, 172.1, 171.9, 171.6, 168.9, 168.2, 157.3, 138.1, 137.6, 130.8, 130.2, 129.6, 128.9, 128.6, 128.2, 128.1, 126.8, 114.7, 69.5, 55.1, 54.8, 50.7, 49.3, 42.8, 38.5, 37.5, 18.9.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS-RAM this was 1 cm^3).

Optimized procedure for the construction of 2 using CM-RAM. Rink amide ChemMatrix resin (0.146 g, loading = 0.48 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (247 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (178 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (232 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(Bz)-OH (296 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol),



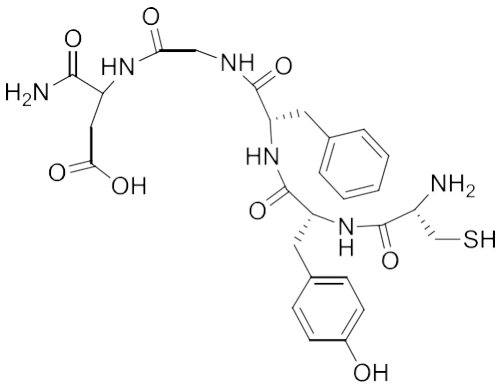
2

step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 661 (M + 1, 100 %), 683 (M + Na, 30%). HRMS (ESI^+) for $C_{34}H_{41}N_6O_8$; calculated 661.2986, found, 661.2986. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 7.6 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.146g of CM-RAM this was 2 cm^3).

1.4 Procedures for other peptide analogues

(9S,12R,15S)-15-amino-9-benzyl-3-carbamoyl-12-(4-hydroxybenzyl)-16-mercapto-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (4)



4

Optimized procedure for the construction of 4 using PS-RAM. Rink amide polystyrene resin (0.1 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (89 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step

1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(OtBu)-OH (138 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Cys(trt)-OH (176 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. The dried resin was cleaved using the cleavage cocktail (10 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) for 12 hours. The resin was filtered off and solution evaporated to dryness on rotary evaporator. The solution was resuspended in H_2O:MeOH mixture (1 mL, H_2O:MeOH 1:1) and purified using preparative HPLC. The combined fractions were lyophilized and subjected to analysis. MS (ESI^+) *m/z* 604 (M + 1, 100%), 626 (M + Na, 35%). HRMS (ESI^+) for $C_{27}H_{35}N_6O_8S$; calculated 603.2237, found, 603.2242. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.6 min. ^1H NMR (300 MHz, DMSO-d_6) δ 9.21 (s, 1H), 8.47 (d, *J* = 8.0 Hz, 1H), 8.39 (d, *J* = 7.9 Hz, 1H), 8.22 (t, *J* = 5.6 Hz, 1H), 8.11 (d, *J* = 8.2 Hz, 2H), 7.25 (m, 5H), 7.18 (dd, *J* = 9.4, 4.5 Hz, 2H), 7.04 (d, *J* = 8.5 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 2H), 4.64 – 4.42 (m, 3H), 3.90 (m, 1H), 3.83 – 3.65 (m, 2H), 3.13 – 2.52 (m, 10H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.99, 172.90, 172.32, 171.75, 171.38, 168.91, 167.11, 156.30, 138.09, 130.51, 129.57, 128.52, 127.95, 126.68, 115.38, 55.06, 54.39, 49.73, 42.56, 40.79, 37.95, 36.62, 26.02.

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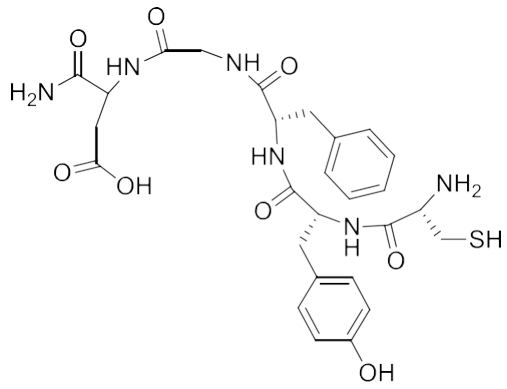

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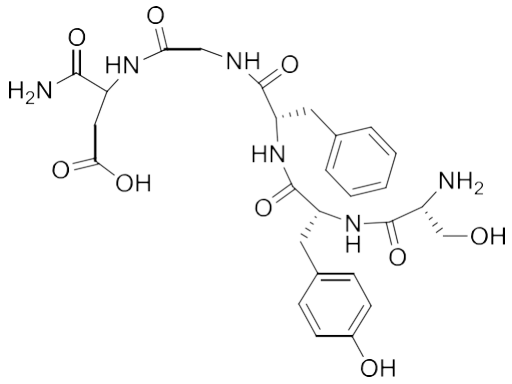


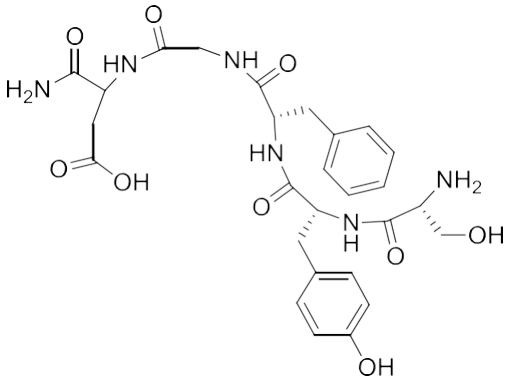
5

Optimized procedure for the construction of 5 using PS-RAM. Rink amide polystyrene resin (0.1 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (89 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(OtBu)-OH (138 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Ser(OtBu)-OH, 115 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. The dried resin was cleaved using the cleavage cocktail (10 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) for 12 hours. The resin was filtered off and solution evaporated to dryness on rotary evaporator. The solution was resuspended in H_2O:MeOH mixture (1 mL, H_2O:MeOH 1:1) and purified using preparative HPLC. The combined fractions were lyophilized and subjected to analysis. MS (ESI^+) *m/z* 588 (M + 1, 100 %), 610 (M + Na, 45%). HRMS (ESI^+) for $C_{27}H_{35}N_6O_9$; calculated 587.2466, found, 587.2461. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.4 min. ^1H NMR (300 MHz, DMSO-d_6) δ 9.20 (bs, 1H), 8.47 (d, *J* = 8.1 Hz, 1H), 8.24 (d, *J* = 7.9

Hz, 1H), 8.19 (t, *J* = 5.6 Hz, 1H), 8.09 (d, *J* = 8.1 Hz, 1H), 8.03 (s, 3H), 7.24 (m, 5H), 7.18 (dd, *J* = 6.6, 2.4 Hz, 1H), 7.13 (d, *J* = 2.2 Hz, 1H), 6.99 (d, *J* = 8.5 Hz, 2H), 6.61 (d, *J* = 8.4 Hz, 2H), 5.48 (bs, 1H), 4.58 – 4.37 (m, 3H), 3.71 (m, 4H), 3.57 (m, 2H), 3.03 (dd, *J* = 14.0, 4.6 Hz, 1H), 2.94 – 2.73 (m, 2H), 2.73 – 2.51 (m, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.92, 172.31, 171.79, 171.15, 168.92, 167.05, 156.28, 138.08, 130.55, 129.60, 128.53, 127.92, 126.72, 115.37, 60.91, 55.10, 54.56, 54.45, 49.74, 42.57, 37.89, 36.93, 36.61.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS-RAM this was 1 cm^3).

(9S,12R,15R)-15-amino-9-benzyl-3-carbamoyl-16-hydroxy-12-(4-hydroxybenzyl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (5)



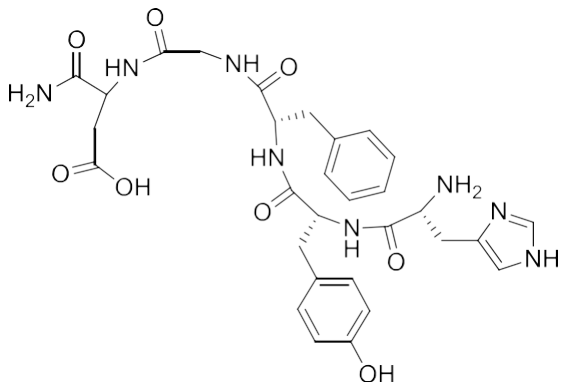
5

Optimized procedure for the construction of 5 using CM-RAM. Rink amide ChemMatrix resin (0.146 g, loading = 0.48 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (247 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (178 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (232 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(OtBu)-OH (275 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Ser(OtBu)-OH, 230 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the

heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 588 (M + 1, 100 %), 610 (M + Na, 45%). HRMS (ESI^+) for $C_{27}H_{35}N_6O_9$; calculated 587.2466, found, 587.2461. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.4 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.146g of CM-RAM this was 2 cm^3).

(9S,12R,15R)-15-amino-9-benzyl-3-carbamoyl-12-(4-hydroxybenzyl)-16-(1H-imidazol-4-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (6)



6

Optimized procedure for the construction of 6 using PS-RAM. Rink amide polystyrene resin (0.1 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (89 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(OtBu)-OH (138

mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1 (Fmoc-His(trt)-OH, 185 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. The dried resin was cleaved using the cleavage cocktail (10 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) for 12 hours. The resin was filtered off and solution evaporated to dryness on rotary evaporator. The solution was resuspended in H_2O:MeOH mixture (1 mL, H_2O:MeOH 1:1) and purified using preparative HPLC. The combined fractions were lyophilized and subjected to analysis. MS (ESI^+) *m/z* 638 (M + 1, 100 %) 660 (M + Na, 50%). HRMS (ESI^+) for $C_{30}H_{37}N_8O_8$; calculated 637.2734, found, 637.2728. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.0 min. ^1H NMR (300 MHz, DMSO-d_6) δ 9.14 (s, 1H), 8.75 (s, 1H), 8.28 (d, *J* = 7.7 Hz, 1H), 8.19 (t, *J* = 5.5 Hz, 1H), 8.07 (dd, *J* = 14.3, 8.1 Hz, 2H), 7.85 (d, *J* = 7.9 Hz, 1H), 7.23 (d, *J* = 3.5 Hz, 5H), 7.16 (dd, *J* = 7.4, 3.5 Hz, 3H), 6.94 (d, *J* = 8.5 Hz, 2H), 6.59 (d, *J* = 8.5 Hz, 2H), 4.50 (q, *J* = 7.8 Hz, 3H), 4.37 (q, *J* = 4.4 Hz, 1H), 3.72 (qd, *J* = 16.6, 5.5 Hz, 3H), 3.03 (dd, *J* = 13.9, 4.8 Hz, 2H), 2.84 (dt, *J* = 14.1, 7.3 Hz, 4H), 2.75 – 2.60 (m, 2H), 2.53 (d, *J* = 7.8 Hz, 1H).

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS-RAM this was 1 cm^3).

(9S,12R,15R)-15-amino-9-benzyl-3-carbamoyl-12-(4-hydroxybenzyl)-16-(1H-imidazol-4-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (6)

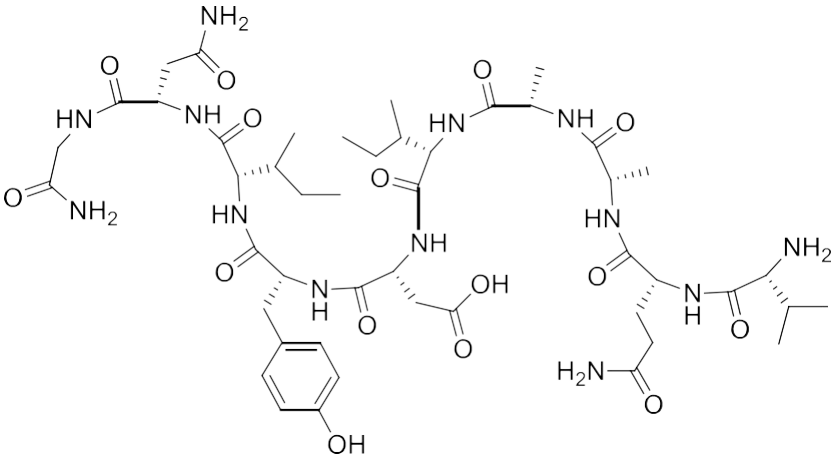
6

Optimized procedure for the construction of 6 using CM-RAM. Rink amide ChemMatrix resin (0.146 g, loading = 0.48 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Asp(OtBu)-OH (247 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™

column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1 (Fmoc-Gly-OH (178 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Phe-OH (232 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-Tyr(OtBu)-OH (275 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1 (Fmoc-His(trt)-OH, 371 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 638 (M + 1, 100 %) 660 (M + Na, 50%). HRMS (ESI^+) for $C_{30}H_{37}N_8O_8$; calculated 637.2734, found, 637.2728. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.0 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.146g of CM-RAM this was 2 cm^3).

VQAAIDYING-CONH$_2$ (ACP$_{65-74}$); (3R,6S,9S,12S,15R,18R)-18-amino-3-(((R)-1-(((2S,3S)-1-(((S)-4-amino-1-((2-amino-2-oxoethyl)amino)-1,4-dioxobutan-2-yl)amino)-3-methyl-1-oxopentan-2-yl)amino)-3-(4-hydroxyphenyl)-1-oxopropan-2-yl)carbamoyl)-15-(3-amino-3-oxopropyl)-6-((R)-sec-butyl)-9,12,19-trimethyl-5,8,11,14,17-pentaoxo-4,7,10,13,16-pentaazaicosan-1-oic acid

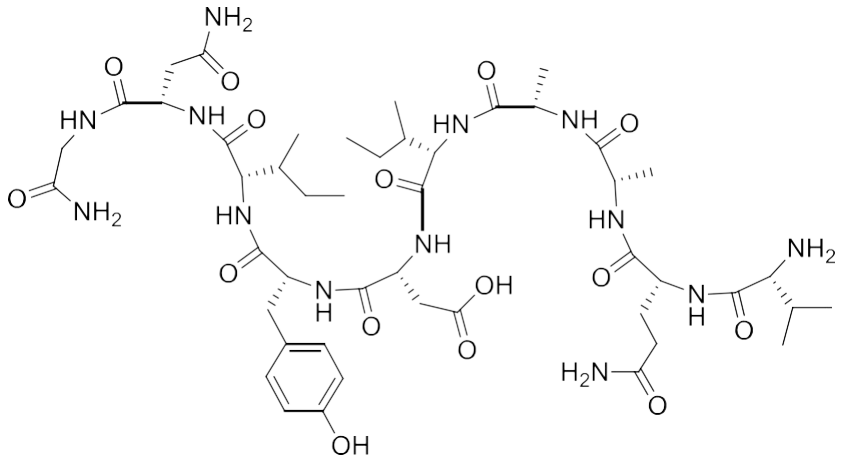


VQAAIDYING-CONH2 (**ACP$_{65-74}$**)

Optimized procedure for the construction of (ACP$_{65-74}$) using PS-RAM. Rink amide polystyrene resin (0.1 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† Fmoc-Gly-OH (89 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1, Fmoc-Asn(trt)-OH (179 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (138 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114

mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Gln(trt)-OH (183 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Val-OH (102 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (104 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2× 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 543 ($[M + H + Na]^{2+}$, 100%), 532 ($[M + 2H]^{2+}$, 25%), 554 ($[M + 2Na]^{2+}$, 55%), 1063 ([M + H], 10%), 1085 ([M + Na], 15%). HRMS (ESI^+) for $C_{47}H_{76}N_{13}O_{15}$; calculated 1062.5584, found, 1062.5565. RP-HPLC Onyx Monolithic C18 150 × 4.6 mm, 10-60% B in 15 min, t_R 6.7 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.1g of PS-RAM this was 1 cm^3).



VQAAIDYING-CONH2 (**ACP_{65-74}**)

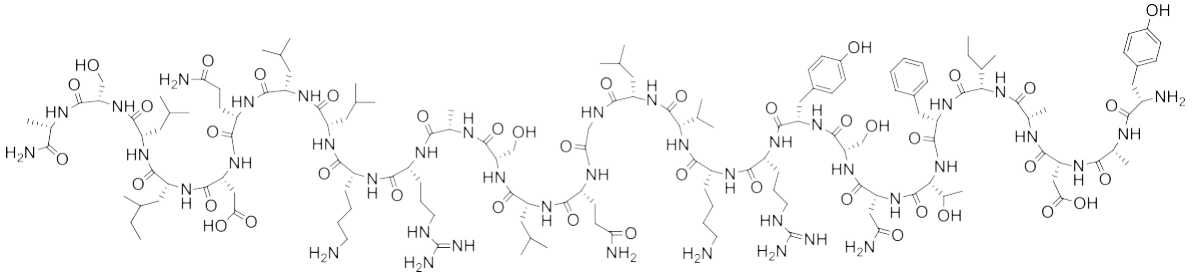
Optimized procedure for the construction of (ACP_{65-74}) using CM-RAM. Rink amide ChemMatrix resin (0.146 g, loading = 0.48 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† Fmoc-Gly-OH (178 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed

utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1, Fmoc-Asn(trt)-OH (357 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (212 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (275 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Asp(OtBu)-OH (246 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (212 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (186 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Gln(trt)-OH (366 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. Step 1, Fmoc-Val-OH (203 mg, 0.6 mmol), HATU (228 mg, 0.6 mmol), DMF (1 mL) and DIPEA (209 µL, 1.2 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 543 ($[M + H + Na]^{2+}$, 100%), 532 ($[M + 2H]^{2+}$, 25%), 554 ($[M + 2Na]^{2+}$, 55%), 1063 ([M + H], 10%), 1085 ([M + Na], 15%). HRMS (ESI^+) for $C_{47}H_{76}N_{13}O_{15}$; calculated 1062.5584, found, 1062.5565. RP-HPLC Onyx Monolithic C18 150 × 4.6 mm, 10-60% B in 15 min, t_R 6.7 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.146g of CM-RAM this was 2 cm^3).

YADAIFTNSYRKVLGQLSARKLLQDILSA-CONH2 (GHRH analogue)

Optimized procedure for the construction of GHRH Analogue using PS-RAM. Rink amide polystyrene



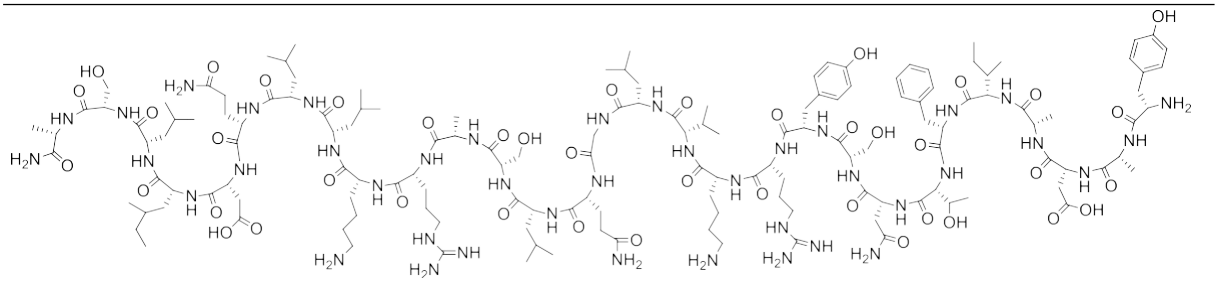
YADAIFTNSYRKVLGQLSARKLLQDILSA-CONH2 (**GHRH analogue**)

resin (0.05 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution†, Fmoc-Ala-OH (47 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1, Fmoc-Ser(OtBu)-OH (58 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Asp(OtBu)-OH (62 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Gln(trt)-OH (82 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Lys(Boc)-OH (70 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Arg(Pbf)-OH (97 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (47 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-

Ser(OtBu)-OH (58 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Gln(trt)-OH (92 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Gly-OH (45 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Val-OH (51 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Lys(Boc)-OH (70 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Arg(Pbf)-OH (97 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (69 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Ser(OtBu)-OH (58 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Asn(trt)-OH (90 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Thr(OtBu)-OH (60 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Phe-OH (58 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (54 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (47 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Asp(OtBu)-OH (62 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (47 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (69 mg, 0.15 mmol), HATU (57 mg, 0.15 mmol), DMF (1 mL) and DIPEA (53 µL, 0.3 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 814 ($[M + 4H]^{4+}$, 100 %), 1086 ($[M + 3H]^{3+}$, 50%). HRMS (ESI^+) for $C_{147}H_{242}N_{41}O_{42}$; calculated $[M + 3H]^{3+}$ 1085.2739, found 1085.2698. RP-HPLC Onyx Monolithic C18 150 × 4.6 mm, 10-100% B in 15 min, t_R 11.99 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.05 g of PS-RAM this was 0.5 cm^3).

YADAIFTNSYRKVLGQLSARKLLQDILSA-CONH2 (GHRH analogue)



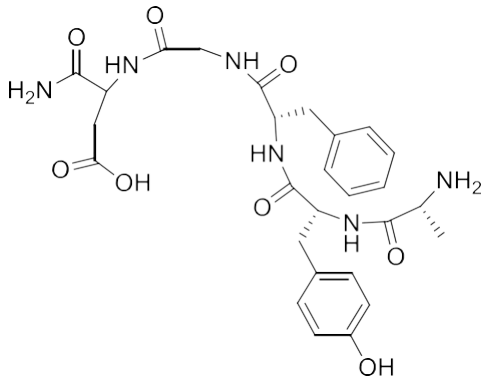
YADAIFTNSYRKVLGQLSARKLLQDILSA-CONH2 (**GHRH analogue**)

Optimized procedure for the construction of GHRH Analogue using CM-RAM. Rink amide ChemMatrix resin (0.073 g, loading = 0.48 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (3 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 1.5 mins using solution A. The amino acid (AA) solution† (Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 1.5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1, Fmoc-Ser(OtBu)-OH (115 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Gln(trt)-OH (183 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Lys(Boc)-OH (140 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Arg(Pbf)-OH (194 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1,

Fmoc-Ser(OtBu)-OH (115 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Gln(trt)-OH (183 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Gly-OH (89 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Leu-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Val-OH (102 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Lys(Boc)-OH (140 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Arg(Pbf)-OH (194 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (138 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ser(OtBu)-OH (115 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Asn(trt)-OH (179 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Thr(OtBu)-OH (120 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Phe-OH (116 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ile-OH (107 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Asp(OtBu)-OH (123 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (93 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (138 mg, 0.3 mmol), HATU (114 mg, 0.3 mmol), DMF (1 mL) and DIPEA (105 µL, 0.6 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 10 mL), EtOAc (2 × 10 mL), hexanes (2 × 5.0 mL), DCM (2 × 5.0 mL) and Et_2O (2 × 10 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 814 ($[M + 4H]^{4+}$, 100 %), 1086 ($[M + 3H]^{3+}$, 50%). HRMS (ESI^+) for $C_{147}H_{242}N_{41}O_{42}$; calculated $[M + 3H]^{3+}$ 1085.2739, found 1085.2698. RP-HPLC Onyx Monolithic C18 150 × 4.6 mm, 10-100% B in 15 min, t_R 11.99 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.073g of CM-RAM this was 1 cm^3).

(9S,12R,15R)-15-amino-9-benzyl-3-carbamoyl-12-(4-hydroxybenzyl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (7)



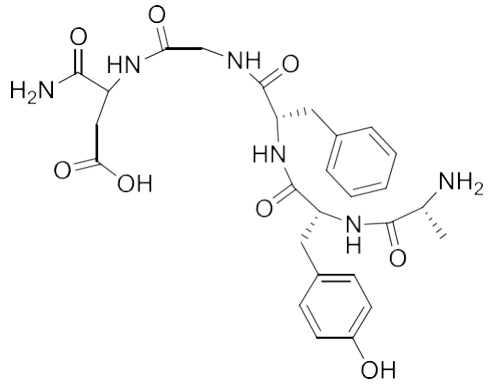
7

Optimized procedure for the construction of 7 using PS-RAM. Rink amide polystyrene resin (0.4 g, loading = 0.7 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (8 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 5 mins using solution A. The amino acid (AA) solution† Fmoc-Asp(OtBu)-OH (430 mg, 1.05 mmol), HATU (399 mg, 1.05 mmol), DMF (2.5 mL) and DIPEA (364 µL, 2.1 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1, Fmoc-Gly-OH (312 mg, 1.05 mmol), HATU (399 mg, 1.05 mmol), DMF (2.5 mL) and DIPEA (365 µL, 2.1 mmol), step 2, step 3. Step 1, Fmoc-Phe-OH (406 mg, 1.05 mmol), HATU (399 mg, 1.05 mmol), DMF (2.5 mL) and DIPEA (364 µL, 2.1 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (483 mg, 1.05 mmol), HATU (399 mg, 1.05 mmol), DMF (2.5 mL) and DIPEA (364 µL, 2.1 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (326 mg, 1.05 mmol), HATU (399 mg, 1.05 mmol), DMF (2.5 mL) and DIPEA (364 µL, 2.1 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH (2 × 30 mL), EtOAc (2 × 30 mL), hexanes (2 × 15 mL), DCM (2 × 15 mL) and Et_2O (2 × 30 mL) and dried *in vacuo*. The dried resin was cleaved using the cleavage cocktail (10 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) for 12 hours. The resin was filtered off and solution evaporated to dryness on rotary evaporator. The solution was resuspended in H_2O:MeOH mixture (1 mL, H_2O:MeOH 1:1) and purified using preparative HPLC. The combined fractions were lyophilized and subjected to analysis. MS (ESI^+) *m/z* 572 (M + 1, 100 %), 594 (M + Na, 40%). HRMS (ESI^+) for $C_{27}H_{35}N_6O_8$; calculated 571.2516, found 571.2515. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.3 min. ^1H NMR (300 MHz, DMSO-d_6) δ 9.12 (bs, 1H), 8.14 (t, *J* = 5.5 Hz, 1H), 8.03 (dd, *J* = 8.0, 3.5 Hz, 2H), 7.96 (d, *J* = 7.3 Hz,

1H), 7.74 (d, *J* = 8.1 Hz, 1H), 7.30 – 7.07 (m, 7H), 6.91 (d, *J* = 8.1 Hz, 2H), 6.57 (d, *J* = 8.1 Hz, 2H), 4.57–4.39 (m, 2H), 4.30 (td, *J* = 8.7, 4.5 Hz, 1H), 4.15 (p, *J* = 7.1 Hz, 1H), 3.69 (m, 2H), 3.01 (dd, *J* = 14.0, 4.8 Hz, 2H), 2.90 – 2.74 (m, 2H), 2.74 – 2.49 (m, 3H), 1.77 (s, 3H), 1.05 (d, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.91, 172.54, 172.32, 171.80, 171.50, 169.64, 168.94, 156.11, 138.04, 130.52, 129.57, 128.51, 128.07, 126.71, 115.21, 54.51, 49.75, 48.58, 36.54, 22.88, 18.25.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.4 g of PS-RAM this was 3.5 cm^3).

(9S,12R,15R)-15-amino-9-benzyl-3-carbamoyl-12-(4-hydroxybenzyl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazahexadecan-1-oic acid (7)



7

Optimized procedure for the construction of 7 using CM-RAM. Rink amide ChemMatrix resin (0.4 g, loading = 0.48 mmol/g) was placed in an Omnifit™ BenchMark™ column assembly with one fixed end and one adjustable end, the resin was swelled with DMF (8 mL) for 0.5 h. After this time, the adjustable end was carefully wound down to allow no movement of the resin, but not to compress it. The Omnifit™ column was placed in line and in the thermostat controlled heating block (60 °C) Utilising the HPLC driven flow synthesizer with inline UV detector (baseline to DMF), the resin was washed with solution A (100% DMF, 5 mL min^{-1}) until the UV detector read < 0.1 AU. The resin was then Fmoc-deprotected using solution B (1:1 DMF:piperidine, 5 mL min^{-1}) until the UV detector reached maximum detection and subsequently returned to <0.1 AU, then washed for 5 mins using solution A. The amino acid (AA) solution† Fmoc-Asp(OtBu)-OH (553 mg, 1.35 mmol), HATU (513 mg, 1.35 mmol), DMF (2.5 mL) and DIPEA (350 µL, 2.7 mmol) was injected into the sample loop of the inline Rheodyne™, which was subsequently released inline into the heating coil followed by the Omnifit™ column. Once the UV detector returned to <0.3 AU the Fmoc protecting group was removed utilizing solvent B until the UV detector reached maximum detection and returned to <0.1 AU, this was subsequently washed for 5 mins using solution A. The above steps of; injection of AA solution (step 1), Fmoc-Deprotect (step 2) and wash (step 3) was followed for all additional AA's in the sequence. Step 1, Fmoc-Gly-OH (400 mg, 1.35 mmol), HATU (513 mg, 1.35 mmol), DMF (2.5 mL) and DIPEA (350 µL, 2.7 mmol), step 2, step 3. Step 1, Fmoc-Phe-OH (522 mg, 1.35 mmol), HATU (513 mg, 1.35 mmol), DMF (2.5 mL) and DIPEA (350 µL, 2.7 mmol), step 2, step 3. Step 1, Fmoc-Tyr(OtBu)-OH (621 mg, 1.35 mmol), HATU (513 mg, 1.35 mmol), DMF (2.5 mL) and DIPEA (350 µL, 2.7 mmol), step 2, step 3. Step 1, Fmoc-Ala-OH (420 mg, 1.35 mmol), HATU (513 mg, 1.35 mmol), DMF (2.5 mL) and DIPEA (350 µL, 2.7 mmol), step 2, step 3. After the final Fmoc-deprotection, the resin was removed from the heating block and washed with MeOH

(2 × 30 mL), EtOAc (2 × 30 mL), hexanes (2 × 15 mL), DCM (2 × 15 mL) and Et_2O (2 × 30 mL) and dried *in vacuo*. An aliquot (10 mg) of the resin was added to a scintillation vial to which a cleavage cocktail (1 mL, TFA:AcOH:H_2O:TIPS 7:2.5:0.25:0.25) was added and allowed to cleave for 12 hours. The cleaved peptide solution was filtered and the filtrate treated with ACN (0.5 mL) and K_2CO_3 (0.5 mL, 1 M). The peptide solution was then subjected to HPLC and LCMS. MS (ESI^+) *m/z* 572 (M + 1, 100 %), 594 (M + Na, 40%). HRMS (ESI^+) for $C_{27}H_{35}N_6O_8$; calculated 571.2516, found 571.2515. RP-HPLC Onyx Monolithic C18 100 × 4.6 mm, 10-100% B in 15 min, t_R 4.3 min.

†The AA solution was calculated based upon the final swollen resin volume; reagents were calculated accordingly for a 0.3 M solution based on the swollen volume (for 0.4 g of CM-RAM this was 4.5 cm^3).

1.5 Flow peptide construction optimisation data and HPLC traces

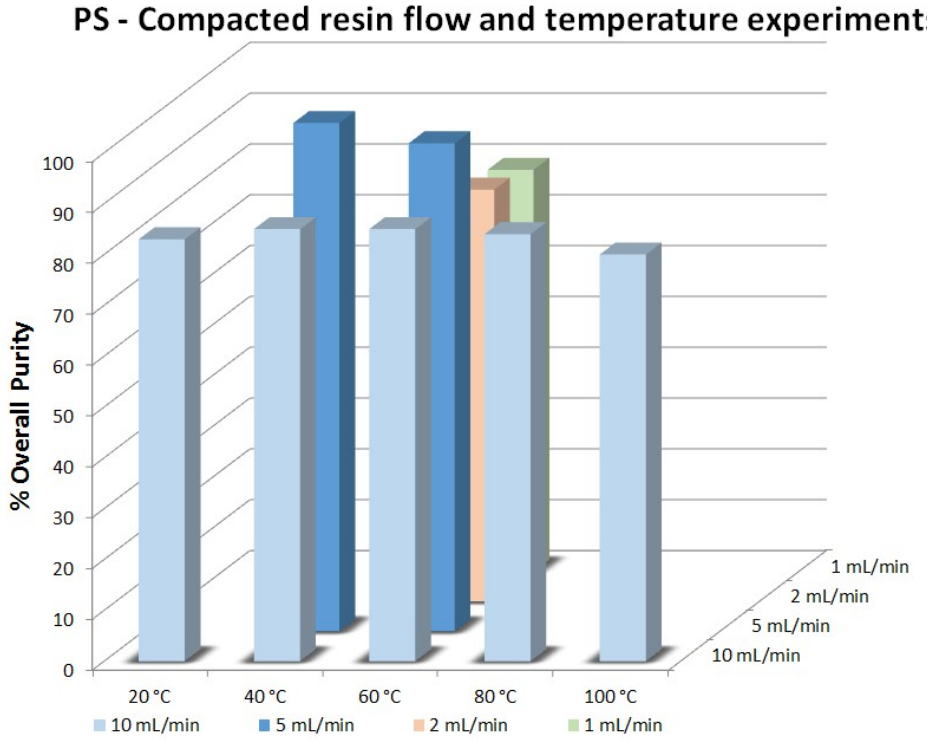


Figure 1.5.1: Comparison of crude purities for initial optimisation of flow rate experiments using polystyrene resin and injection method.

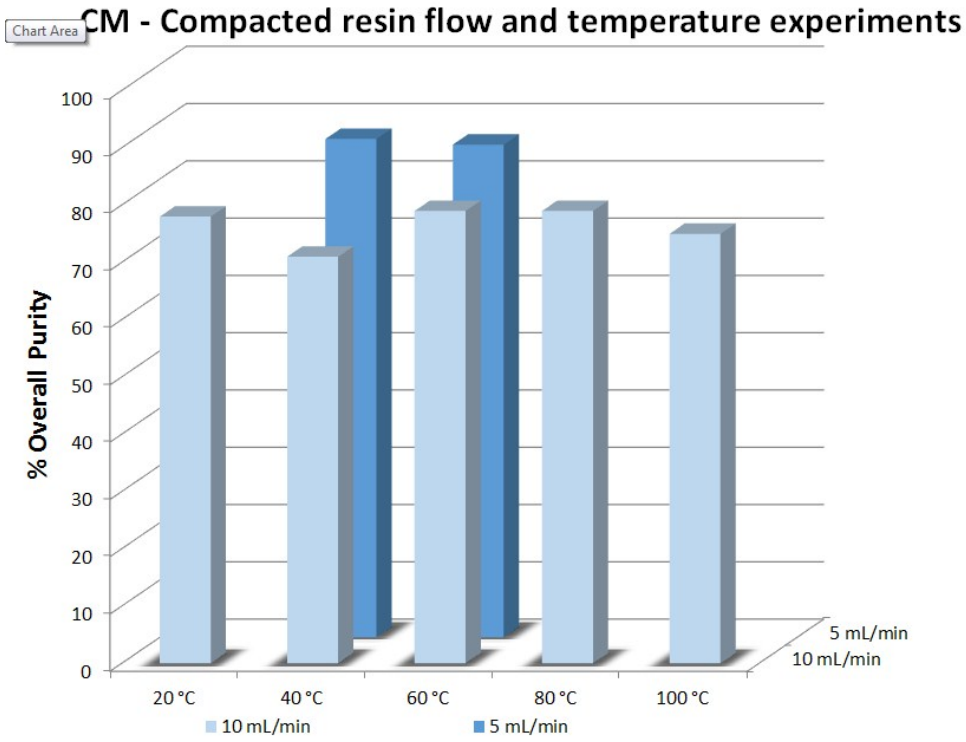


Figure 1.5.2: Comparison of crude purities for initial optimisation of flow rate experiments using Chemmatrix resin and injection method.

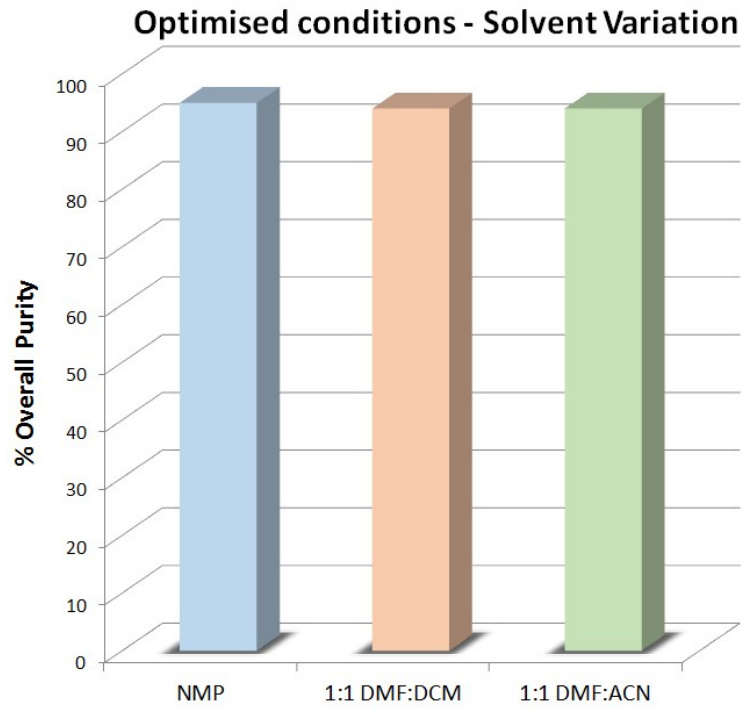


Figure 1.5.3: Comparison of crude purities using the injection method optimised conditions varying the solvent.

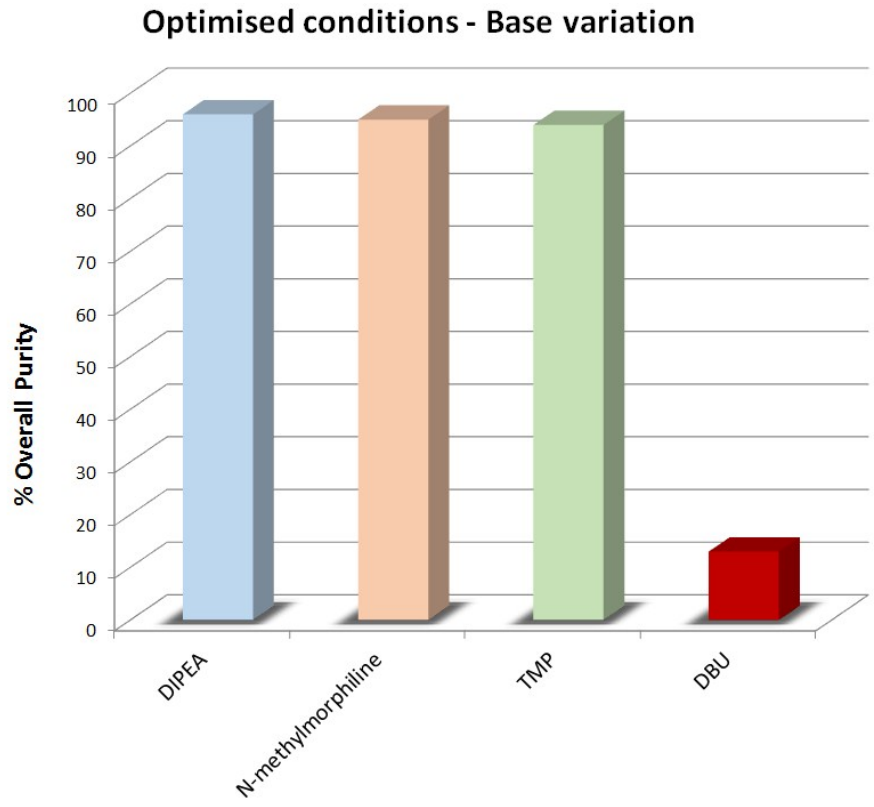


Figure 1.5.4: Comparison of crude purities using the injection method optimised conditions varying the base used for amino acid coupling.

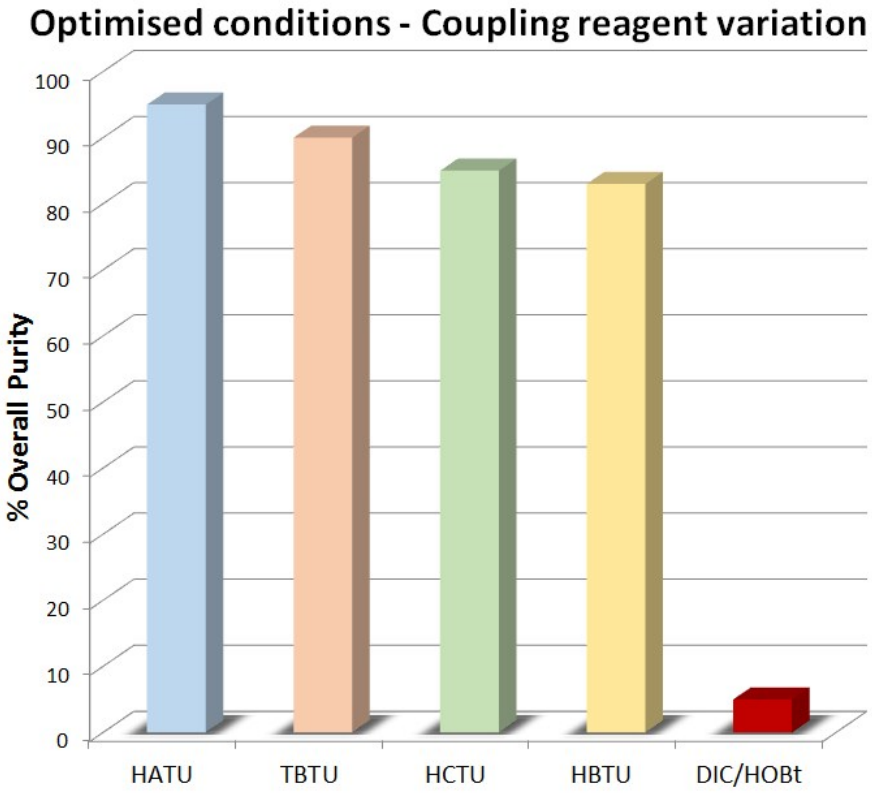


Figure 1.5.5: Comparison of crude purities using the injection method optimised conditions varying the coupling reagent used for amino acid coupling.

HPLC traces of batch construction 10 and 30 minute couplings for benchmark/standard

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\052-0201.D
Sample Name: Batch 10 mins

=====================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   2
Acq. Instrument : LC1260                      Location : Vial 52
Injection Date  : 1/18/2018 5:02:40 PM             Inj :   1
                                             Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:00:40 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Batch 10 mins
```

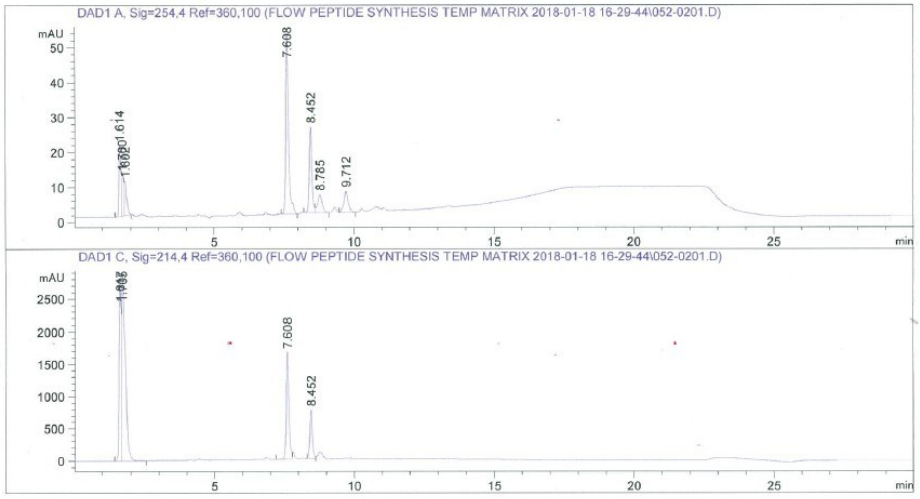


Figure 1.5.6: HPLC trace of **2** under batch conditions, 10 minute couplings

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\053-0301.D
Sample Name: Batch 2nd 10 mins

=====================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   3
Acq. Instrument : LC1260                      Location : Vial 53
Injection Date  : 1/18/2018 5:34:07 PM             Inj :   1
                                             Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:01:12 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Batch 2nd 10 mins
```

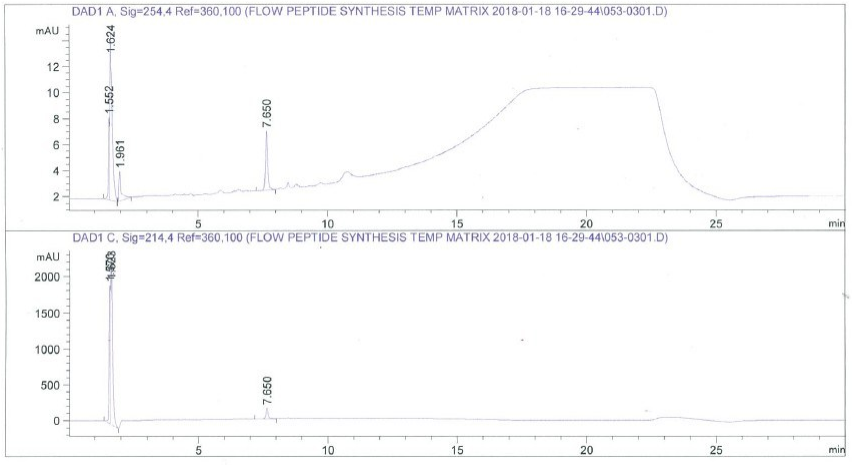


Figure 1.5.7: HPLC trace of **2** under batch conditions, 10 minute couplings

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\051-0101.D
Sample Name: Batch 30 mis

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   1
Acq. Instrument : LC1260                        Location : Vial 51
Injection Date  : 1/18/2018 4:31:14 PM             Inj :   1
                                               Inj Volume : 10.000 µl
Method          : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Batch 30 mis
```

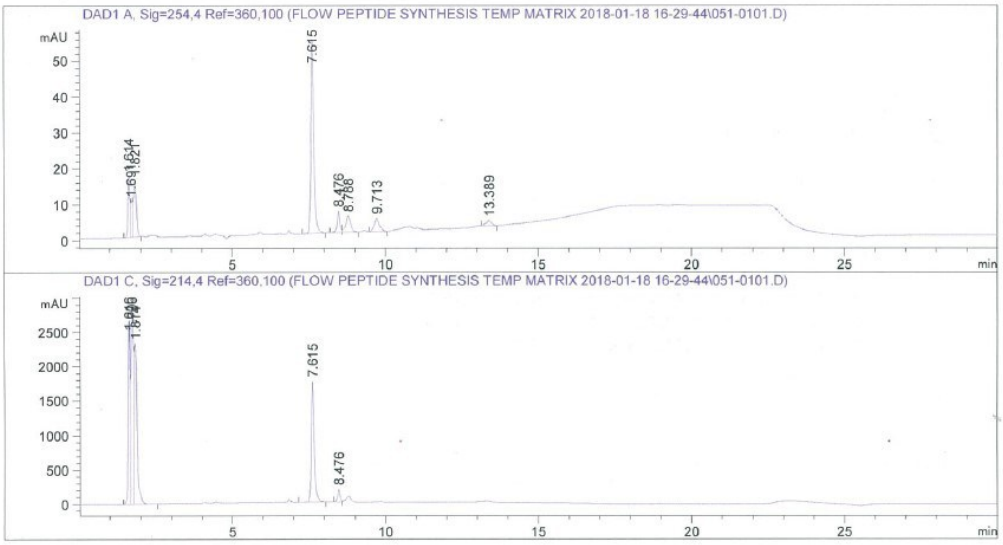


Figure 1.5.8: HPLC trace of **2** under batch conditions, 30 minute couplings


```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\051-0101.D
Sample Name: Batch 30 mis

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   1
Acq. Instrument : LC1260                        Location : Vial 51
Injection Date  : 1/18/2018 4:31:14 PM             Inj :   1
                                               Inj Volume : 10.000 µl
Method          : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Batch 30 mis
```

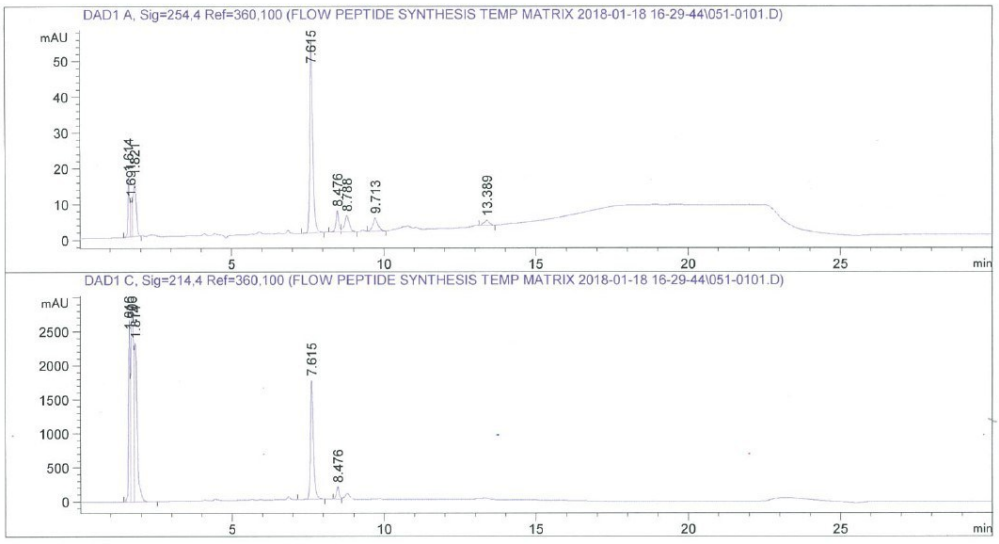



Figure 1.5.9: HPLC trace of **2** under batch conditions, 30 minute couplings

Data File C:\CHEM32\...A\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\081-0101.D
Sample Name: PS Batch0.5hcouplig 24h depro

```
======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   1
Acq. Instrument : LC1260                       Location : Vial 81
Injection Date  : 1/22/2018 9:12:35 AM              Inj :   1
                                              Inj Volume : 10.000 µl
Method          : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:11:09 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : PS Batch0.5hcouplig 24h depro
```

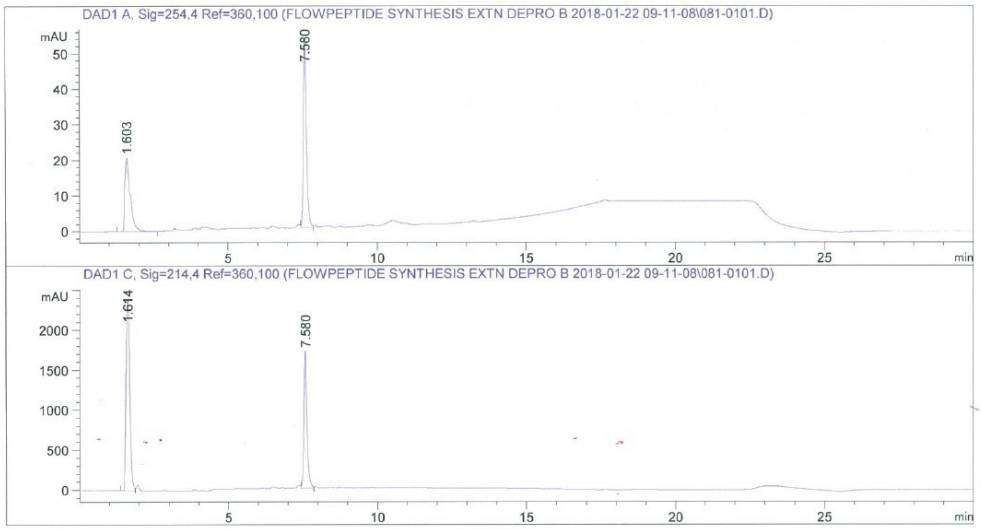


Figure 1.5.10: HPLC trace of **2** under batch conditions, 30 minute couplings with 24 hour cleavage/deprotection solution

Construction using continuous Infusion method at 10 mL/min and 5mL/min

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\054-0401.D
Sample Name: Contin 5 mLmin

======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   4
Acq. Instrument : LC1260                      Location : Vial 54
Injection Date  : 1/18/2018 6:05:32 PM            Inj :   1
                                             Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:02:13 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Contin 5 mLmin
```

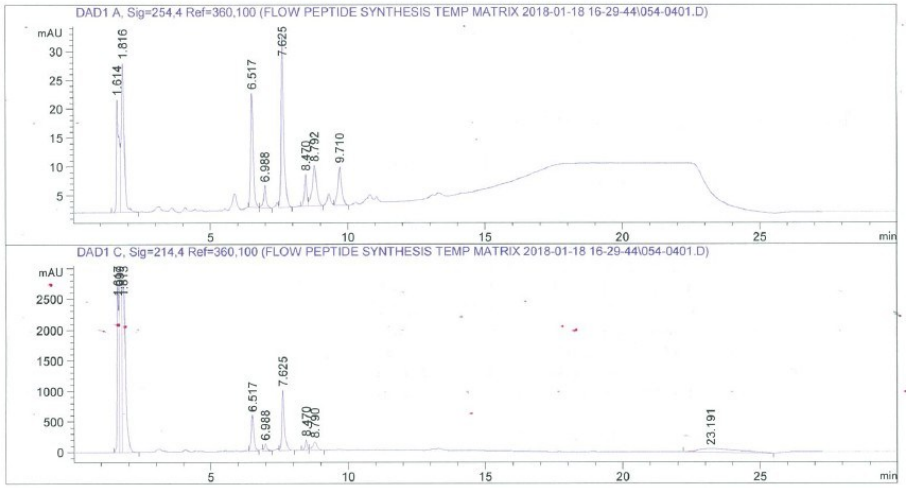


Figure 1.5.11: HPLC trace of **2** using continuous infusion method at 5 mL min^{-1}

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\055-0501.D
Sample Name: Contin 10 mLmin

======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   5
Acq. Instrument : LC1260                      Location : Vial 55
Injection Date  : 1/18/2018 6:36:58 PM            Inj :   1
                                             Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:02:48 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Contin 10 mLmin
```

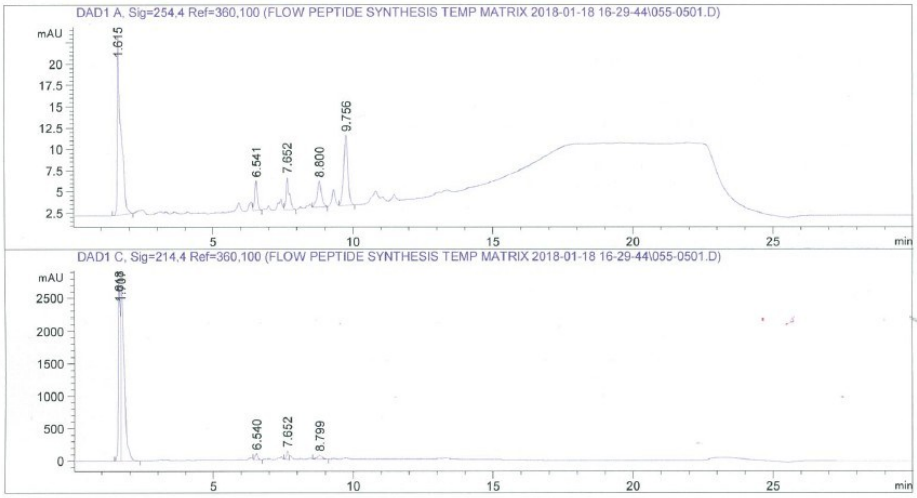


Figure 1.5.12: HPLC trace of **2** using continuous infusion method at 10 mL min^{-1}

Continuous flow using Injection method, 2 fixed ends free flowing resin

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\056-0601.D
Sample Name: large voild inject 1 mLmin

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   6
Acq. Instrument : LC1260                        Location : Vial 56
Injection Date  : 1/18/2018 7:08:23 PM             Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:03:27 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : large voild inject 1 mLmin
```

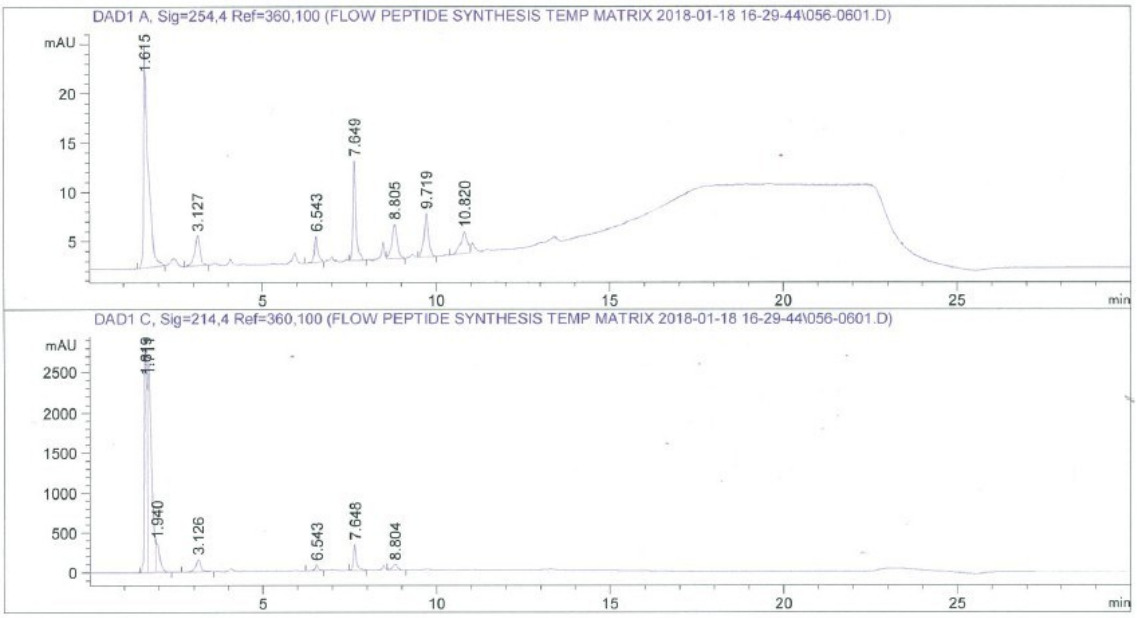


Figure 1.5.13: HPLC trace of **2** using Injection method at 1 mL min^{-1} with resin allowed free movement within column.

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin –Flow rate optimisation

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\057-0701.D
Sample Name: inject 1 mlmin 60 deg c

==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   7
Acq. Instrument : LC1260                        Location : Vial 57
Injection Date  : 1/18/2018 7:39:47 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:04:00 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 1 mlmin 60 deg c
```

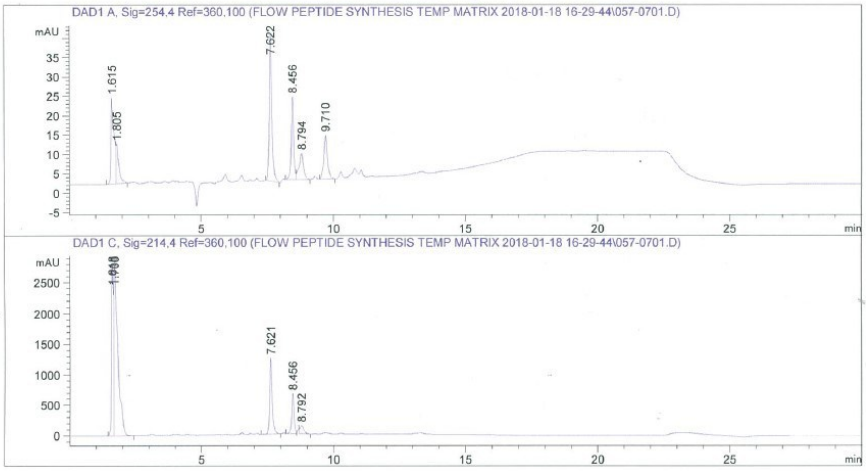


Figure 1.5.14: HPLC trace of **PS-2** using Injection method at 1 mL min^{-1}

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\058-0801.D
Sample Name: inject 2 mlmin 60 deg c

==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   8
Acq. Instrument : LC1260                        Location : Vial 58
Injection Date  : 1/18/2018 8:11:10 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:04:16 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 2 mlmin 60 deg c
```

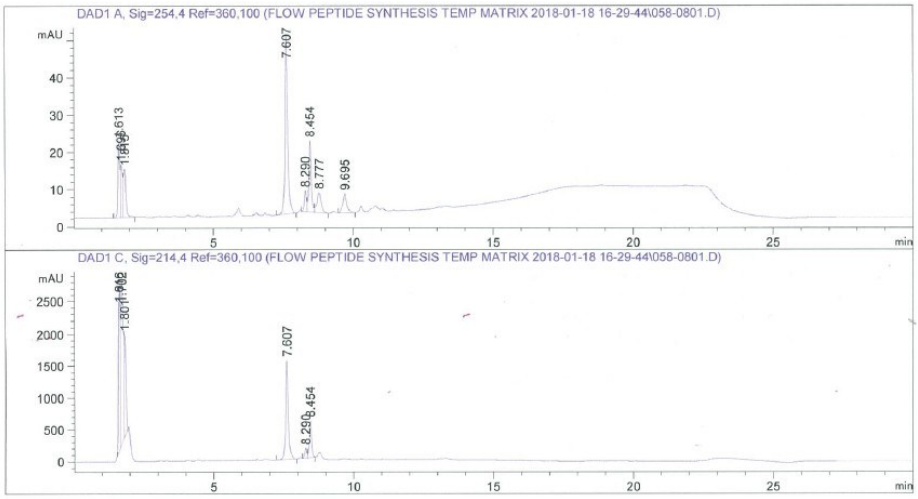


Figure 1.5.15: HPLC trace of **PS-2** using Injection method at 2 mL min^{-1}

Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\060-1001.D
Sample Name: inject 5 mlmin 60 deg c B

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :  10
Acq. Instrument : LC1260                        Location : Vial 60
Injection Date  : 1/18/2018 9:13:59 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:05:36 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 5 mlmin 60 deg c B
```

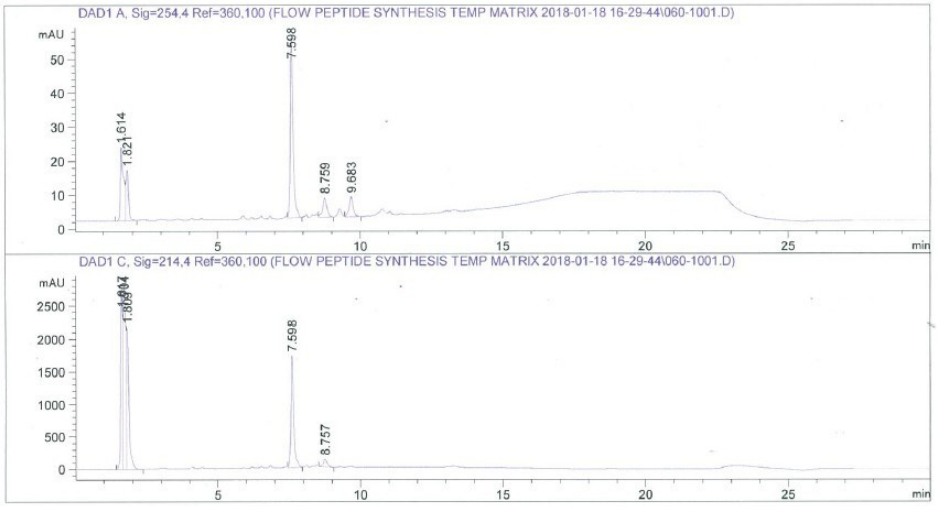


Figure 1.5.16: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}

Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\060-1001.D
Sample Name: inject 5 mlmin 60 deg c B

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :  10
Acq. Instrument : LC1260                        Location : Vial 60
Injection Date  : 1/18/2018 9:13:59 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 2:11:59 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 5 mlmin 60 deg c B
```

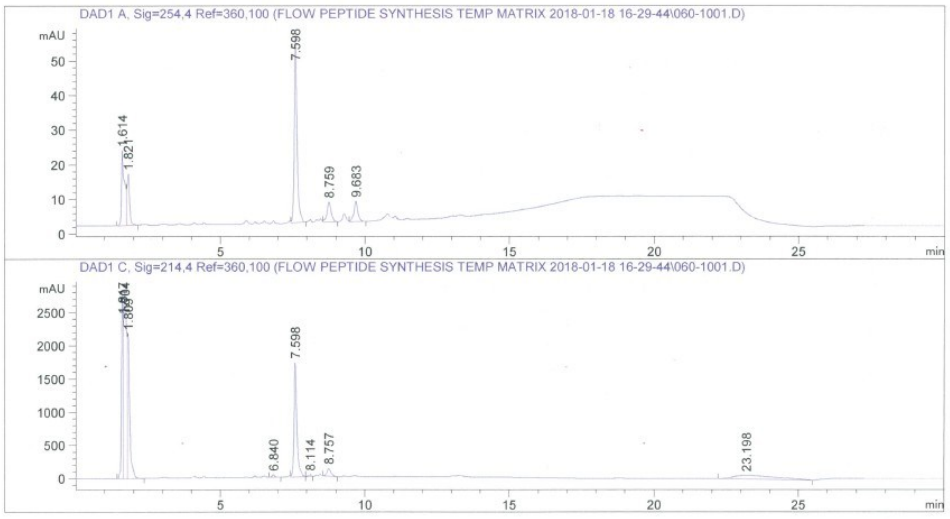


Figure 1.5.17: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}

```
Data File C:\CHEM32\...A\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\083-0301.D
Sample Name: PS 5mLmin60degC 24h depro

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   3
Acq. Instrument : LC1260                        Location : Vial 83
Injection Date  : 1/22/2018 10:15:23 AM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/22/2018 9:11:09 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 2:13:43 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : PS 5mLmin60degC 24h depro
```

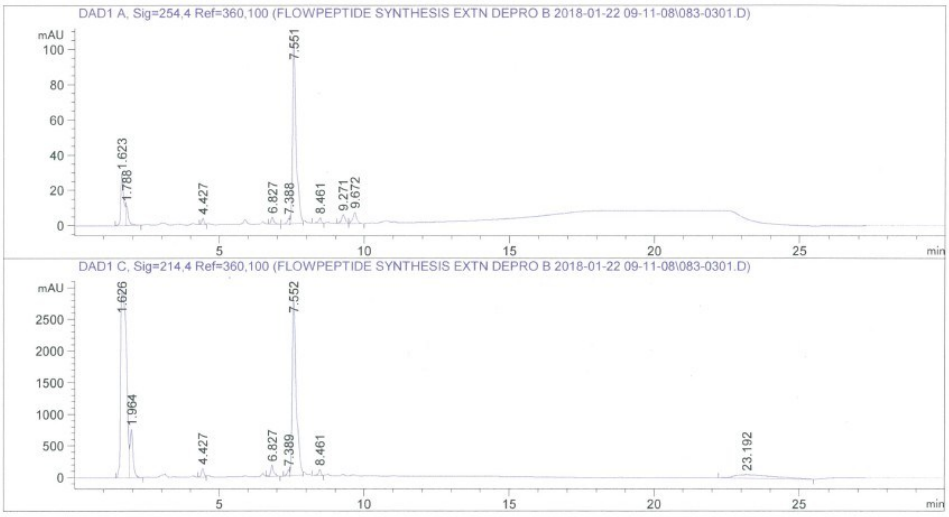


Figure 1.5.18: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}

```
Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\061-1101.D
Sample Name: inject 10 mlmin 60 deg c A

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  11
Acq. Instrument : LC1260                        Location : Vial 61
Injection Date  : 1/18/2018 9:45:25 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:06:08 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 60 deg c A
```

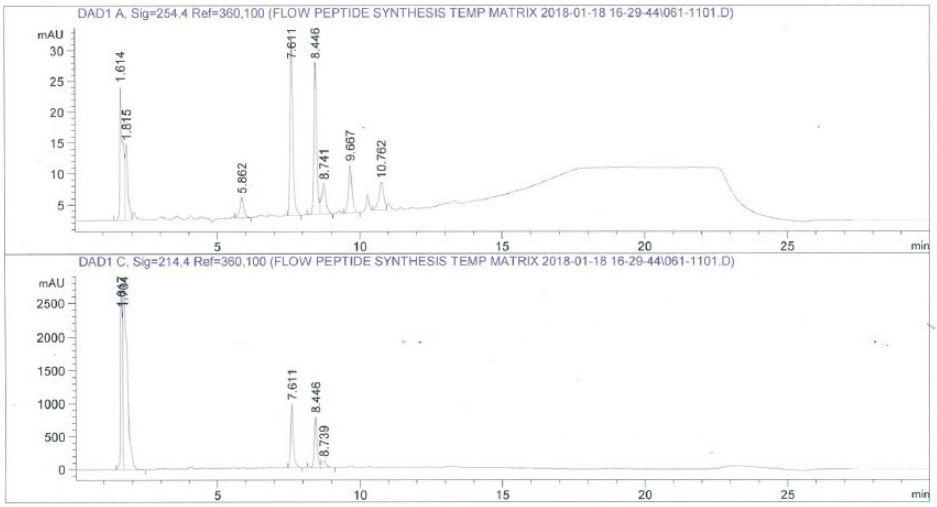


Figure 1.5.19: HPLC trace of **2** using Injection method at 10 mL min^{-1}

Data File C:\CHEM32\...A\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\062-1201.D
Sample Name: inject 10 mlmin 60 deg c B

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  12
Acq. Instrument : LC1260                        Location : Vial 62
Injection Date  : 1/18/2018 10:16:51 PM              Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/18/2018 4:29:45 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS TEMP MATRIX 2018-01-18 16-29-44\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 10:06:41 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 60 deg c B
```

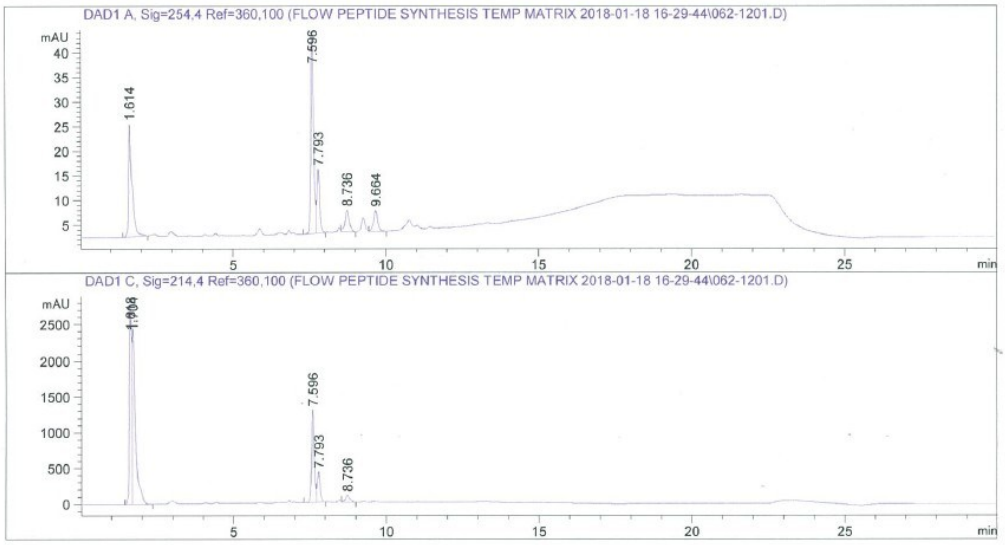


Figure 1.5.20: HPLC trace of **2** using Injection method at 10 mL min^{-1}

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\064-0101.D
Sample Name: inject 10 mlmin 60 deg c

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   1
Acq. Instrument : LC1260                        Location : Vial 64
Injection Date  : 1/19/2018 8:36:01 AM               Inj :   1
                                                Inj Volume : 10.000 µl
Method          : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 60 deg c
```

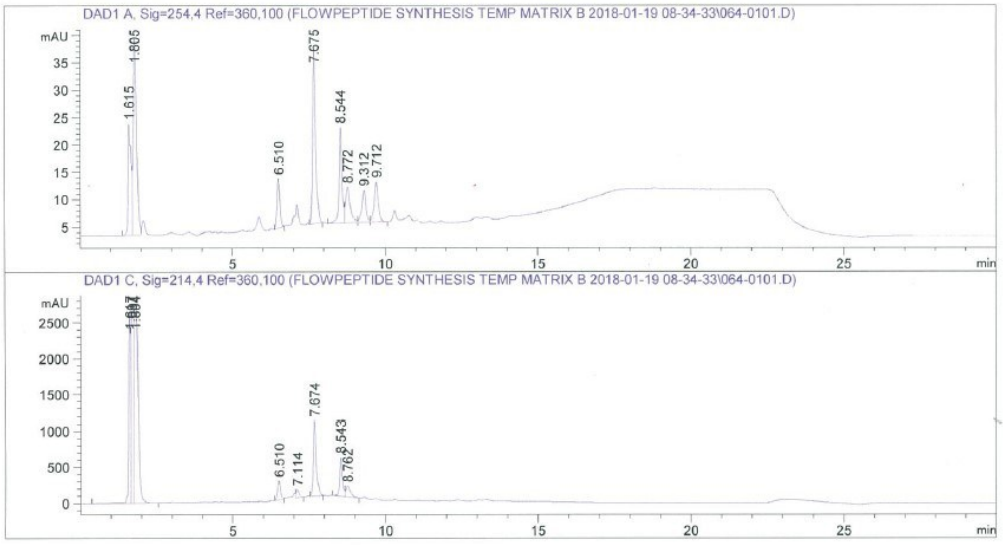


Figure 1.5.21: HPLC trace of **2** using Injection method at 10 mL min^{-1}

```
Data File C:\CHEM32\...A\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\085-0501.D
Sample Name: PS 10mLmin60degC 24h depro

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : LC1260                        Location : Vial 85
Injection Date  : 1/22/2018 11:18:09 AM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/22/2018 9:11:09 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 11:49:17 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : PS 10mLmin60degC 24h depro
```

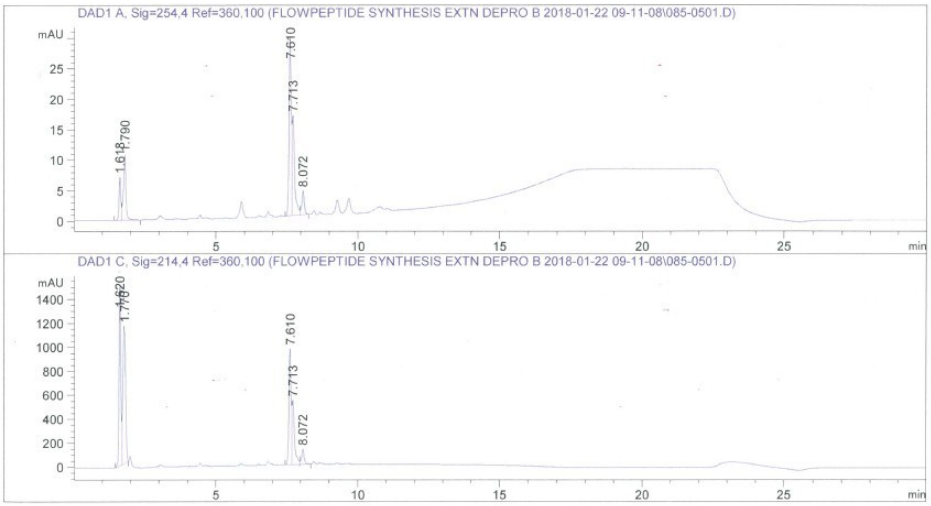


Figure 1.5.22: HPLC trace of **PS-2** using Injection method at 10 mL min^{-1}

```
Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\067-0401.D
Sample Name: inject 10 mlmin 60 deg c

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   4
Acq. Instrument : LC1260                        Location : Vial 67
Injection Date  : 1/19/2018 10:10:19 AM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:51:49 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 60 deg c
```

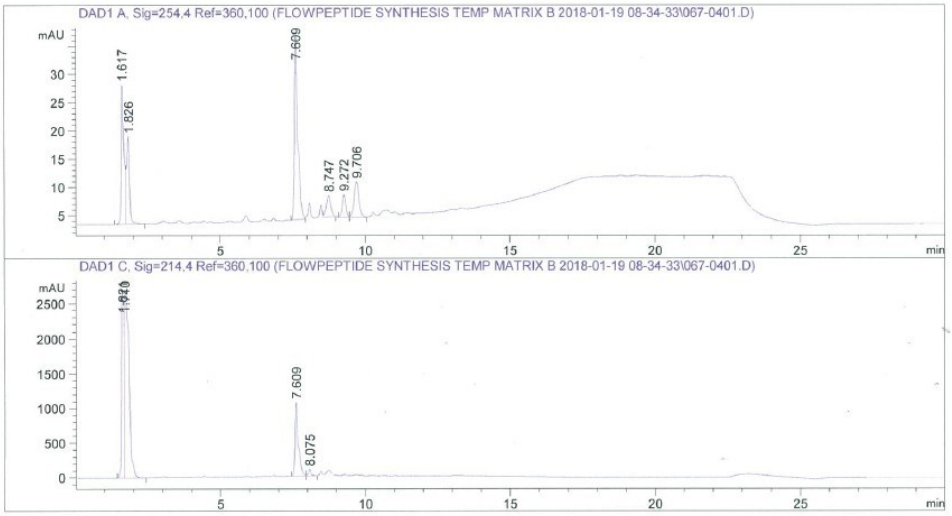


Figure 1.5.23: HPLC trace of **PS-2** using Injection method at 10 mL min^{-1}

Data File C:\CHEM32\1\DATA\20180124_FLOWLOADING AND BASES 2018-01-24 11-00-30\021-1001.D
Sample Name: CM-5mL-min_60d

```
======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :  10
Acq. Instrument : LC1260                      Location : Vial 21
Injection Date  : 1/24/2018 3:47:11 PM             Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180124_FLOWLOADING AND BASES 2018-01-24 11-00-30\10 TO
                  100 OVER 15 MINS 10UL.M
Last changed    : 1/24/2018 11:00:31 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180124_FLOWLOADING AND BASES 2018-01-24 11-00-30\10 TO
                  100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 12:20:16 PM by SYSTEM
                  (modified after loading)
```

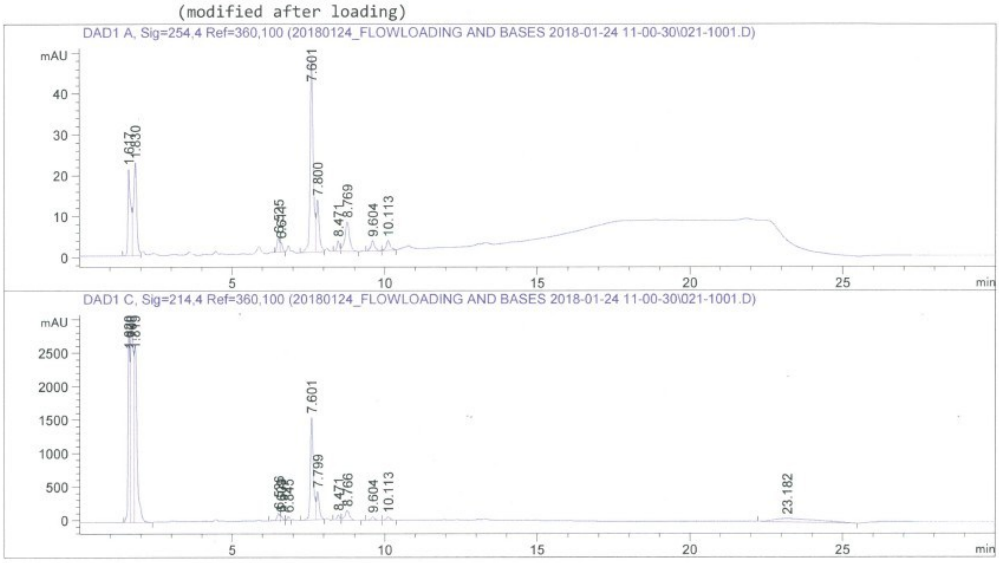


Figure 1.5.24: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin –Temperature optimisation

```
Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\065-0201.D
Sample Name: inject 10 mlmin 20 deg c

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   2
Acq. Instrument : LC1260                        Location : Vial 65
Injection Date  : 1/19/2018 9:07:28 AM             Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:50:40 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 20 deg c
```

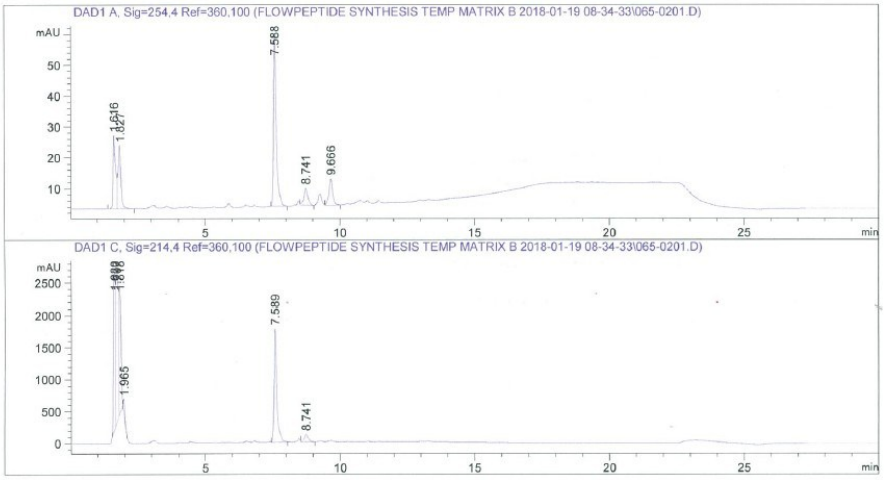


Figure 1.5.25: HPLC trace of **PS-2** using Injection method at 10 mL min^{-1}, 20 °C.

```
Data File C:\CHEM32\...A\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\082-0201.D
Sample Name: PS 5mLmin40degC 24h depro

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   2
Acq. Instrument : LC1260                        Location : Vial 82
Injection Date  : 1/22/2018 9:43:59 AM             Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/22/2018 9:11:09 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS EXTN DEPRO B 2018-01-22 09-11-08\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 11:38:53 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : PS 5mLmin40degC 24h depro
```

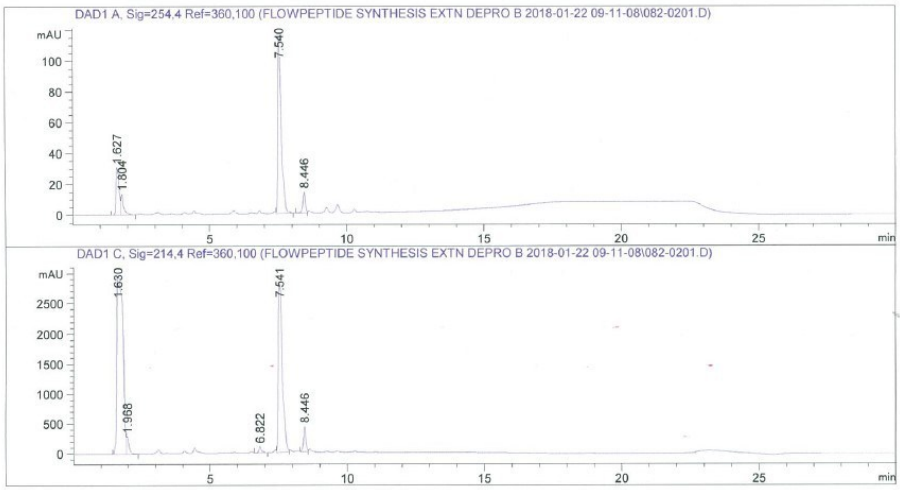


Figure 1.5.26: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 40 °C.

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\066-0301.D
Sample Name: inject 10 mlmin 40 deg c

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   3
Acq. Instrument : LC1260                        Location : Vial 66
Injection Date  : 1/19/2018 9:38:53 AM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:51:27 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 40 deg c
```

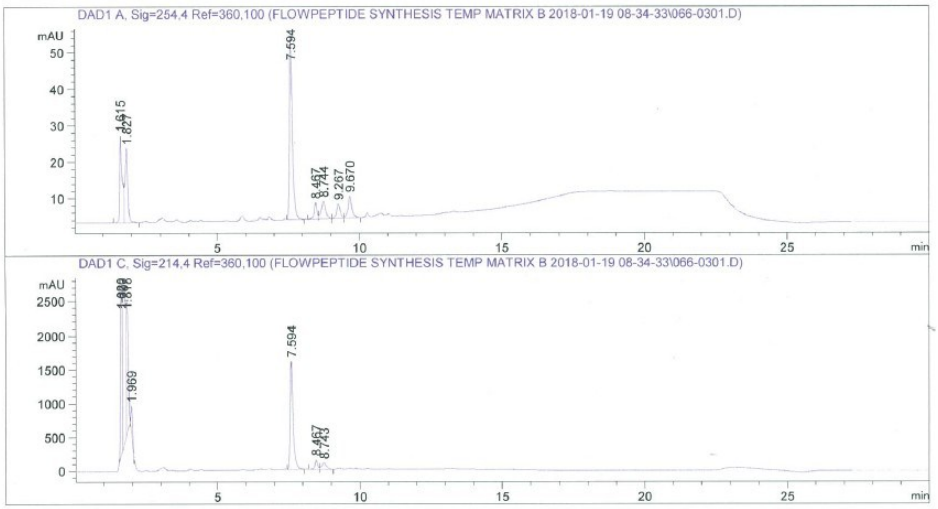


Figure 1.5.27: HPLC trace of **PS-2** using Injection method at 10 mL min^{-1}, 40 °C.

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\068-0501.D
Sample Name: inject 10 mlmin 80 deg c

```
==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   5
Acq. Instrument : LC1260                        Location : Vial 68
Injection Date  : 1/19/2018 10:41:44 AM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:52:52 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 80 deg c
```

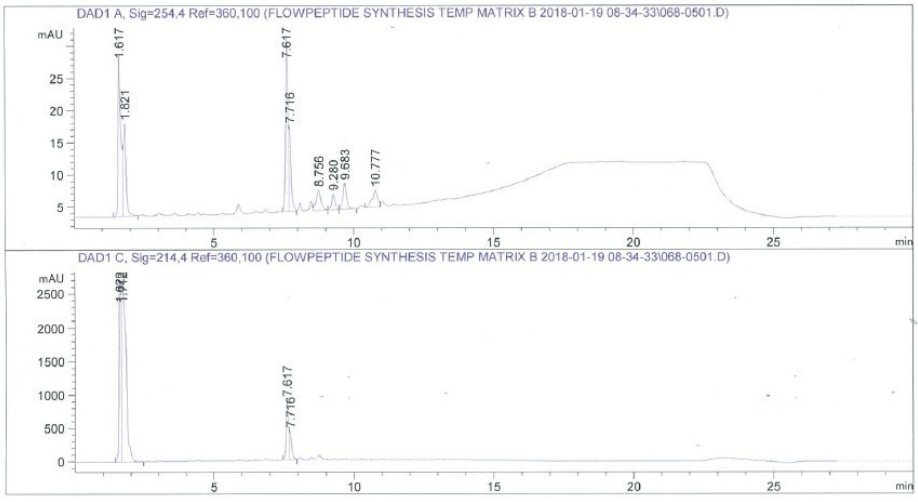


Figure 1.5.28: HPLC trace of **PS-2** using Injection method at 10 mL min^{-1}, 80 °C.

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\069-0601.D
Sample Name: inject 10 mlmin 100 deg c

```
==========================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   6
Acq. Instrument : LC1260                      Location : Vial 69
Injection Date  : 1/19/2018 11:13:07 AM            Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:54:24 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : inject 10 mlmin 100 deg c
```

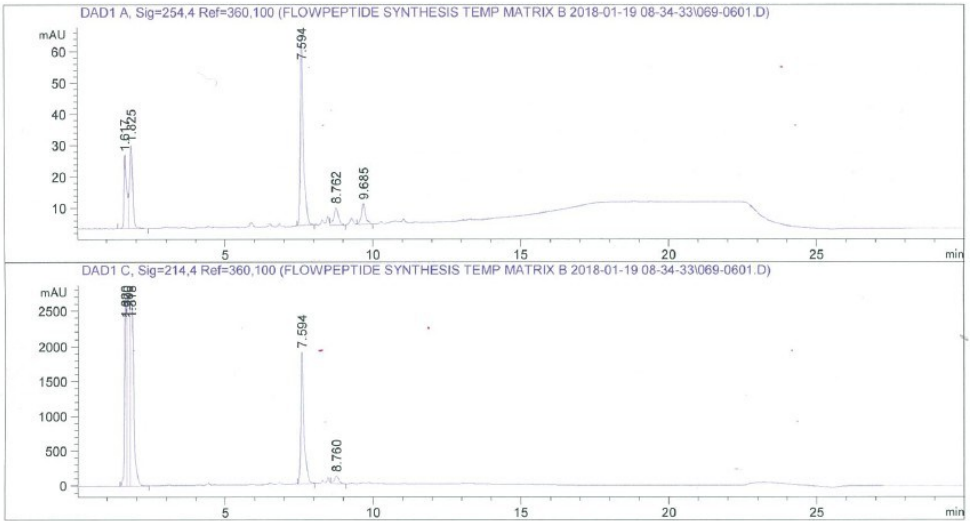


Figure 1.5.29: HPLC trace of **PS-2** using Injection method at 10 mL min^{-1}, 100 °C.

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\070-0701.D
Sample Name: CM inject 10 mlmin 20 deg c

```
==========================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   7
Acq. Instrument : LC1260                      Location : Vial 70
Injection Date  : 1/19/2018 11:44:33 AM            Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:54:29 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : CM inject 10 mlmin 20 deg c
```

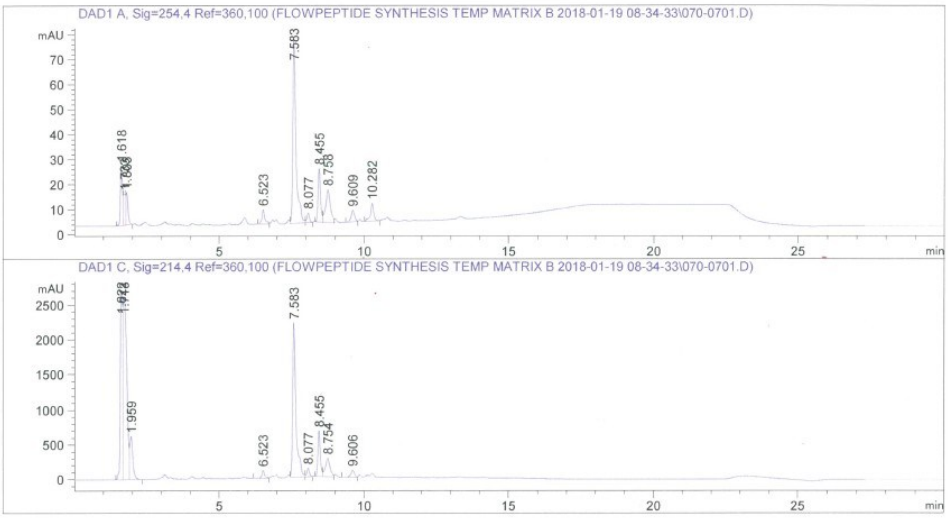


Figure 1.5.30: HPLC trace of **CM-2** using Injection method at 10 mL min^{-1}, 20 °C.

```
Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\071-0801.D
Sample Name: CM inject 10 mlmin 40 deg c

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   8
Acq. Instrument : LC1260                        Location : Vial 71
Injection Date  : 1/19/2018 12:15:54 PM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:55:20 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : CM inject 10 mlmin 40 deg c
```

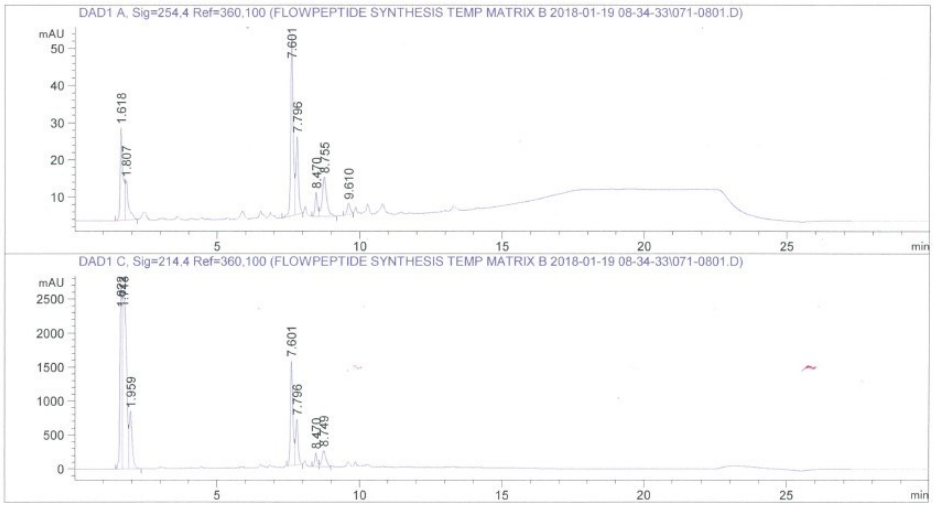


Figure 1.5.31: HPLC trace of **CM-2** using Injection method at 10 mL min^{-1}, 40 °C.

```
Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\076-1301.D
Sample Name: CM inject 10 mlmin 40 deg c

======================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  13
Acq. Instrument : LC1260                        Location : Vial 76
Injection Date  : 1/19/2018 2:52:59 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:58:20 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : CM inject 10 mlmin 40 deg c
```

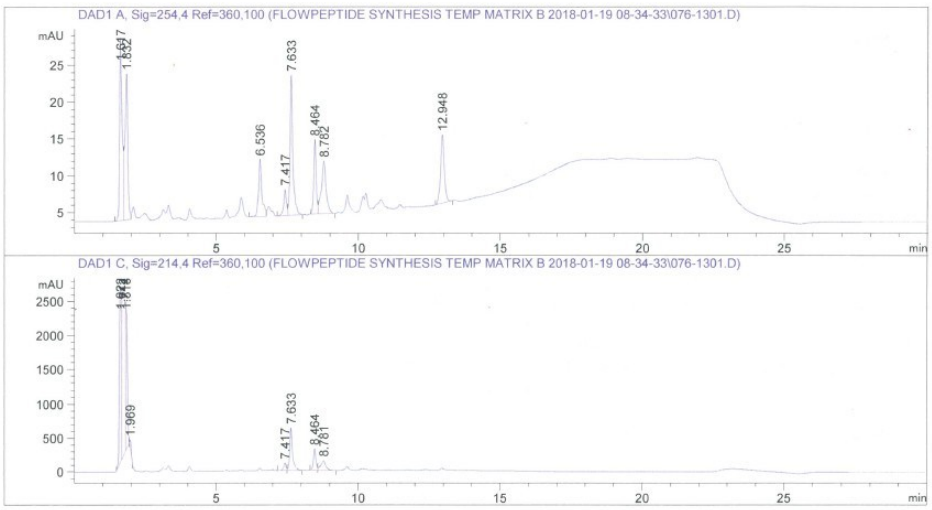


Figure 1.5.32: HPLC trace of **CM-2** using Injection method at 10 mL min^{-1}, 40 °C.

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\073-1001.D
Sample Name: CM inject 10 mlmin 80 deg c

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :  10
Acq. Instrument : LC1260                       Location : Vial 73
Injection Date  : 1/19/2018 1:18:39 PM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:56:31 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : CM inject 10 mlmin 80 deg c
```

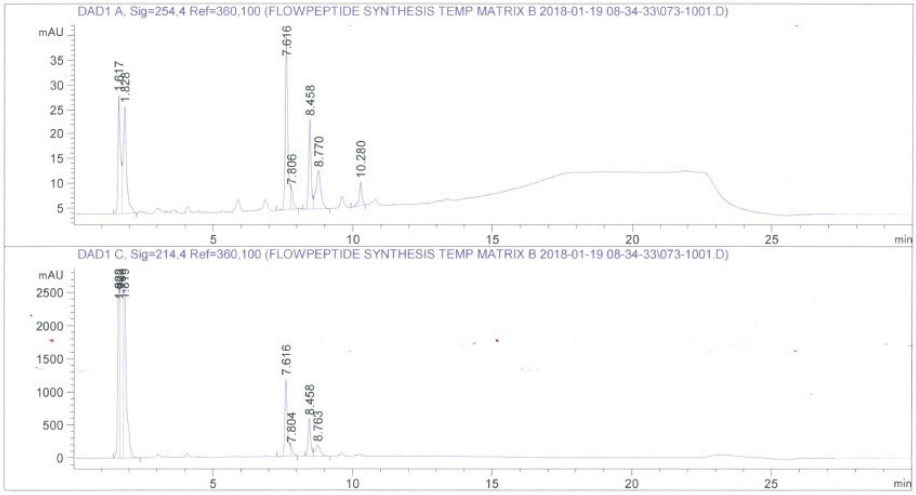


Figure 1.5.33: HPLC trace of **CM-2** using Injection method at 10 mL min^{-1}, 80 °C.

Data File C:\CHEM32\...\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\074-1101.D
Sample Name: CM inject 10 mlmin 100 deg c

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :  11
Acq. Instrument : LC1260                       Location : Vial 74
Injection Date  : 1/19/2018 1:50:06 PM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/19/2018 8:34:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOWPEPTIDE SYNTHESIS TEMP MATRIX B 2018-01-19 08-34-33\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/22/2018 9:57:08 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : CM inject 10 mlmin 100 deg c
```

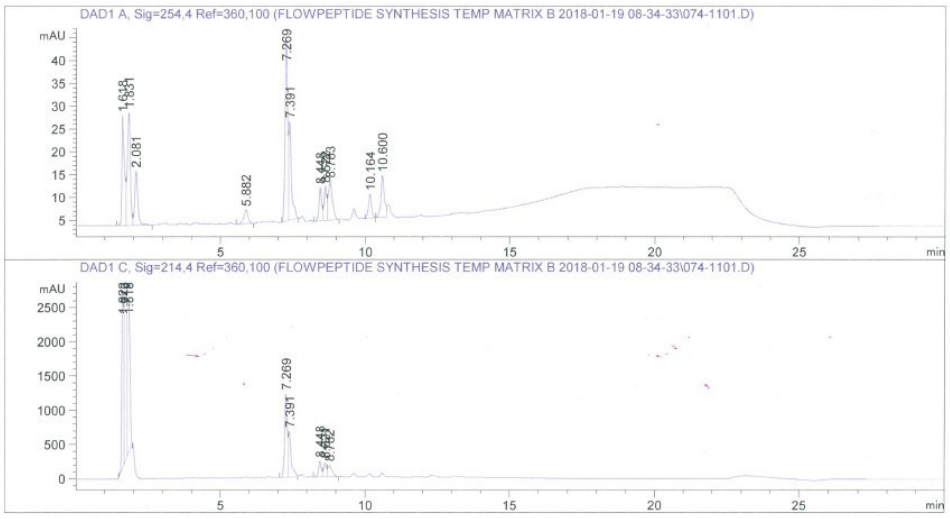


Figure 1.5.34: HPLC trace of **CM-2** using Injection method at 10 mL min^{-1}, 100 °C.

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin –Equivalents and Concentration

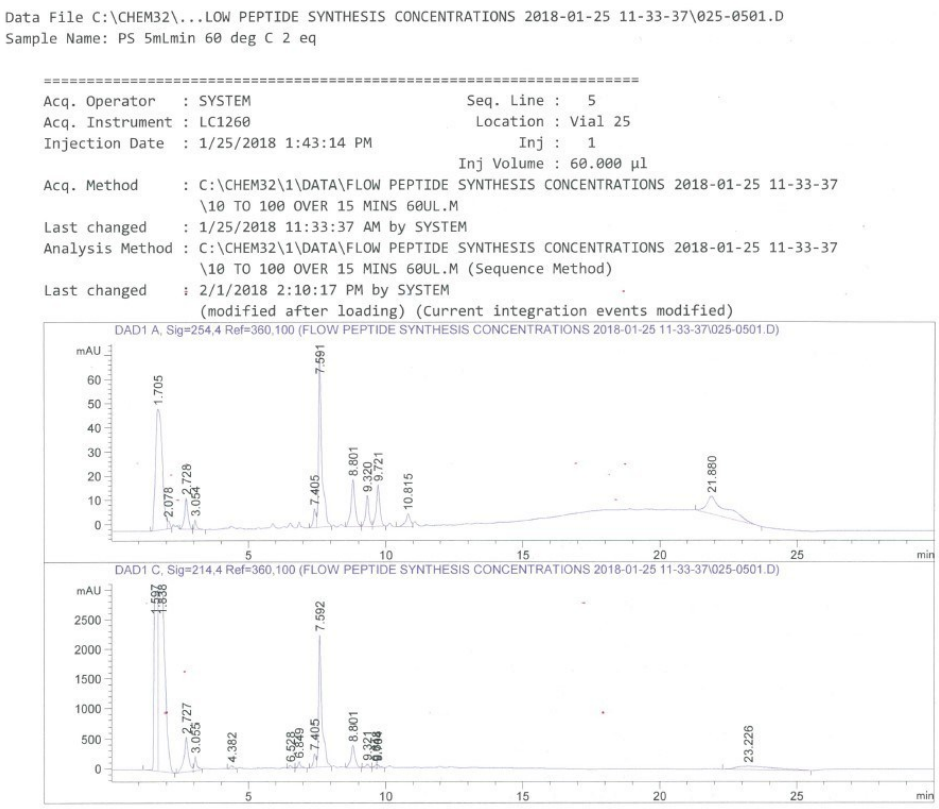


Figure 1.5.35: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 2 equivalents of AA in 1 mL Injection

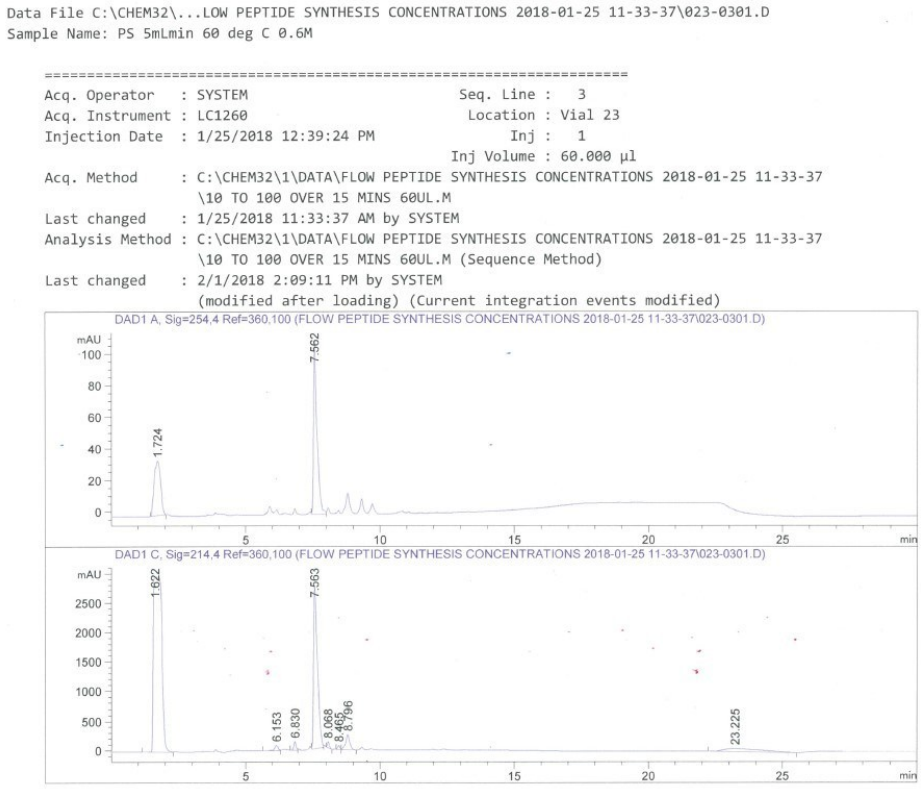


Figure 1.5.36: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.6 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\073-0701.D
Sample Name: CM 5mLmin 60degC 2 eq

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   7
Acq. Instrument : LC1260                        Location : Vial 73
Injection Date  : 1/30/2018 11:56:20 AM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M
Last changed    : 1/30/2018 11:48:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M (Sequence Method)
Last changed    : 2/1/2018 12:50:44 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

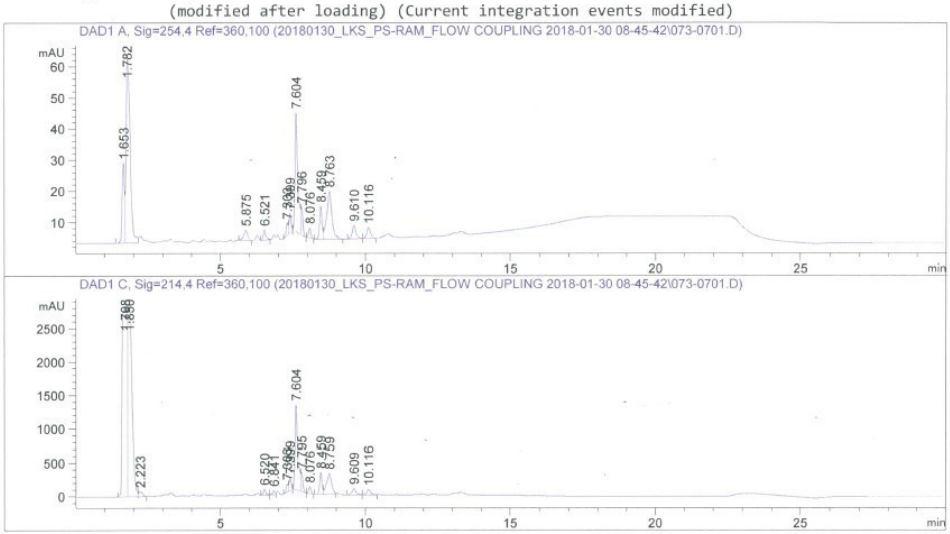


Figure 1.5.37: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, 2 equivalents of AA in 1 mL Injection

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\071-0501.D
Sample Name: CM 5mLmin 60degC 0.15M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   5
Acq. Instrument : LC1260                        Location : Vial 71
Injection Date  : 1/30/2018 10:53:18 AM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M
Last changed    : 1/30/2018 10:32:19 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M (Sequence Method)
Last changed    : 2/1/2018 12:49:37 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

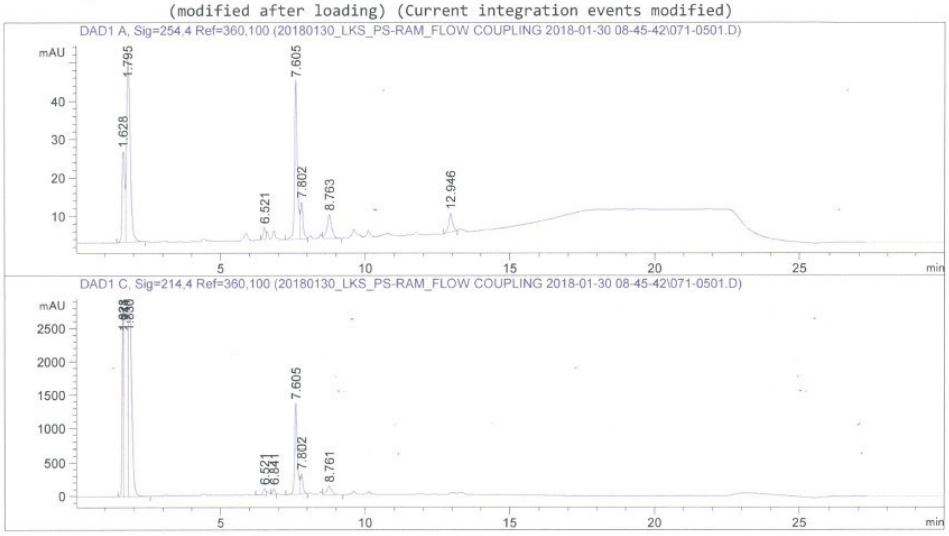


Figure 1.5.38: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.15 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\074-0801.D
Sample Name: CM 5mLmin 60degC 0.2M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   8
Acq. Instrument : LC1260                        Location : Vial 74
Injection Date  : 1/30/2018 12:27:53 PM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M
Last changed    : 1/30/2018 11:48:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M (Sequence Method)
Last changed    : 2/1/2018 12:51:07 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

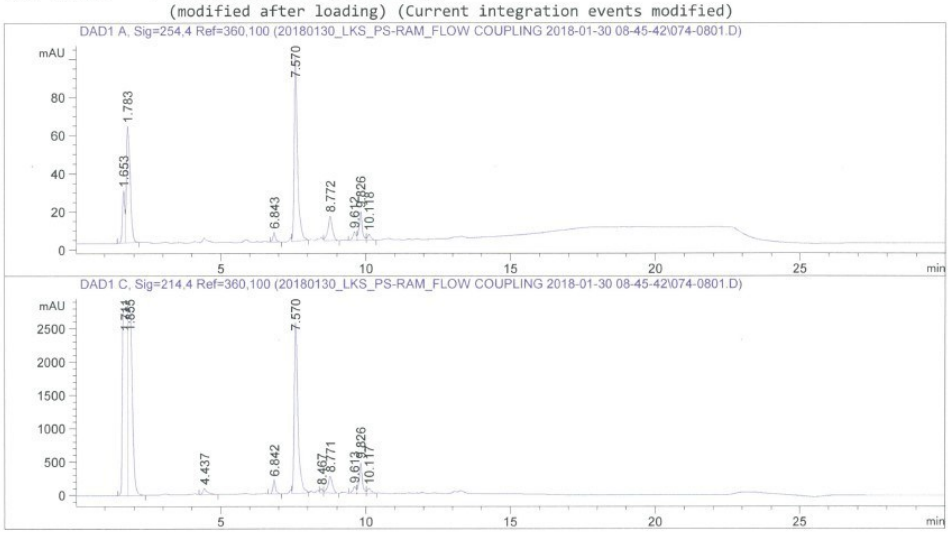


Figure 1.5.39: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.2 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\075-0901.D
Sample Name: CM 5mLmin 60degC 0.28M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   9
Acq. Instrument : LC1260                        Location : Vial 75
Injection Date  : 1/30/2018 12:59:25 PM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M
Last changed    : 1/30/2018 11:48:34 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M (Sequence Method)
Last changed    : 2/1/2018 12:51:25 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

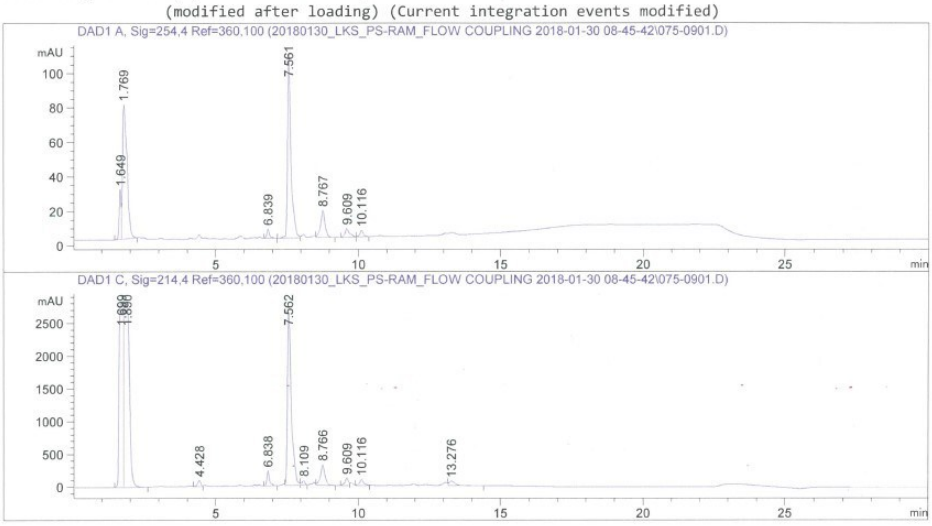


Figure 1.5.40: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.28 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\071-0501.D
Sample Name: CM 5mLmin 60degC 0_3M 1mL

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   5
Acq. Instrument : LC1260                       Location : Vial 71
Injection Date  : 1/31/2018 11:22:57 AM            Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/31/2018 9:14:13 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 9:42:54 AM by SYSTEM
                  (modified after loading)
```

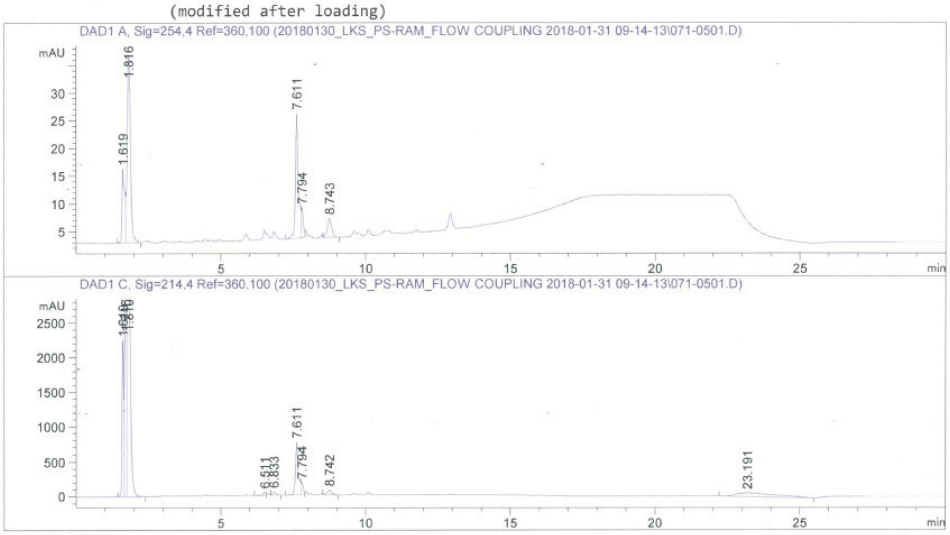


Figure 1.5.41: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.3 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\076-1001.D
Sample Name: CM 5mLmin 60degC 0_4M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :  10
Acq. Instrument : LC1260                       Location : Vial 76
Injection Date  : 1/31/2018 2:00:04 PM             Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/31/2018 11:43:41 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 9:42:54 AM by SYSTEM
                  (modified after loading)
```

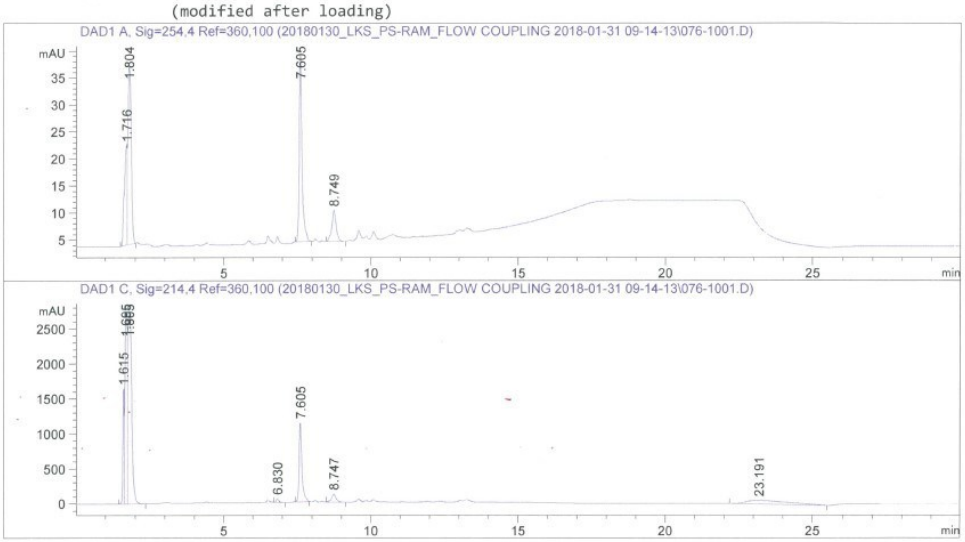


Figure 1.5.42: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.4 M

Data File C:\CHEM32\...OW PEPTIDE SYNTHESIS CM 0.4 AND 0.6M 2018-02-01 13-00-54\076-0101.D
Sample Name: CM 5mLmin 60 deg C 0.4M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   1
Acq. Instrument : LC1260                        Location : Vial 76
Injection Date  : 2/1/2018 1:02:20 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS CM 0.4 AND 0.6M 2018-02-01 13-00-54
                  \10 TO 100 OVER 15 MINS 10UL.M
Last changed    : 2/1/2018 1:00:55 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS CM 0.4 AND 0.6M 2018-02-01 13-00-54
                  \10 TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 2:06:36 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Batch 30 mis
```

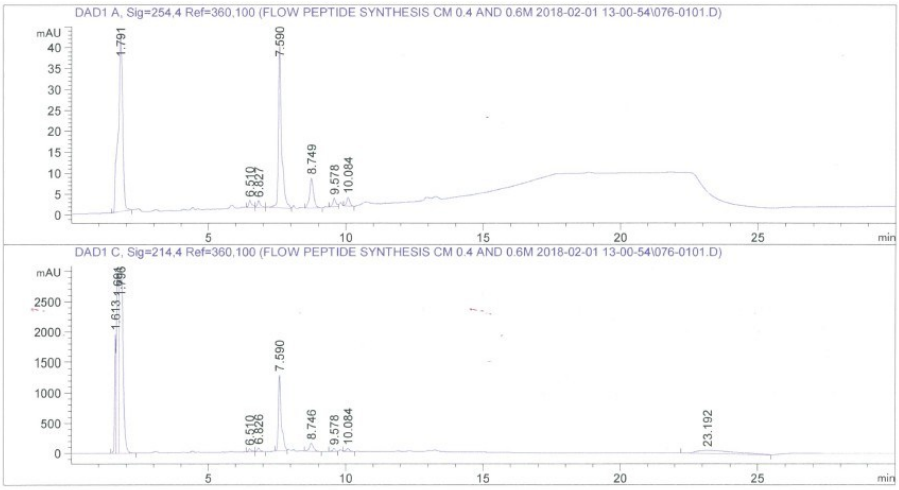


Figure 1.5.43: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.4 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\077-1101.D
Sample Name: CM 5mLmin 60degC 0_6M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :  11
Acq. Instrument : LC1260                        Location : Vial 77
Injection Date  : 1/31/2018 2:31:31 PM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/31/2018 11:43:41 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 9:42:54 AM by SYSTEM
                  (modified after loading)
```

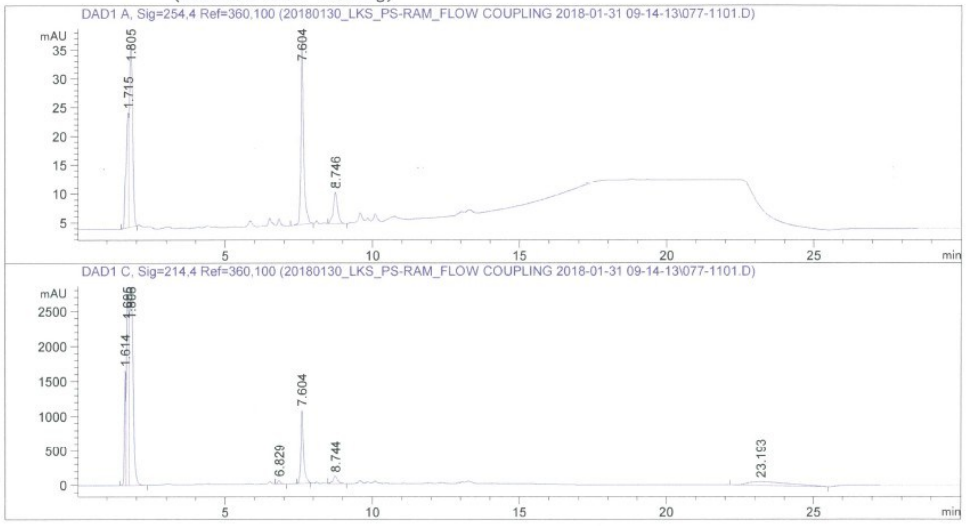


Figure 1.5.44: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.6 M

Data File C:\CHEM32\...OW PEPTIDE SYNTHESIS CM 0.4 AND 0.6M 2018-02-01 13-00-54\076-0201.D
Sample Name: CM 5mLmin 60 deg C 0.6M

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   2
Acq. Instrument : LC1260                        Location : Vial 76
Injection Date  : 2/1/2018 1:33:47 PM                Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS CM 0.4 AND 0.6M 2018-02-01 13-00-54
                  \10 TO 100 OVER 15 MINS 10UL.M
Last changed    : 2/1/2018 1:00:55 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS CM 0.4 AND 0.6M 2018-02-01 13-00-54
                  \10 TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/1/2018 2:06:32 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

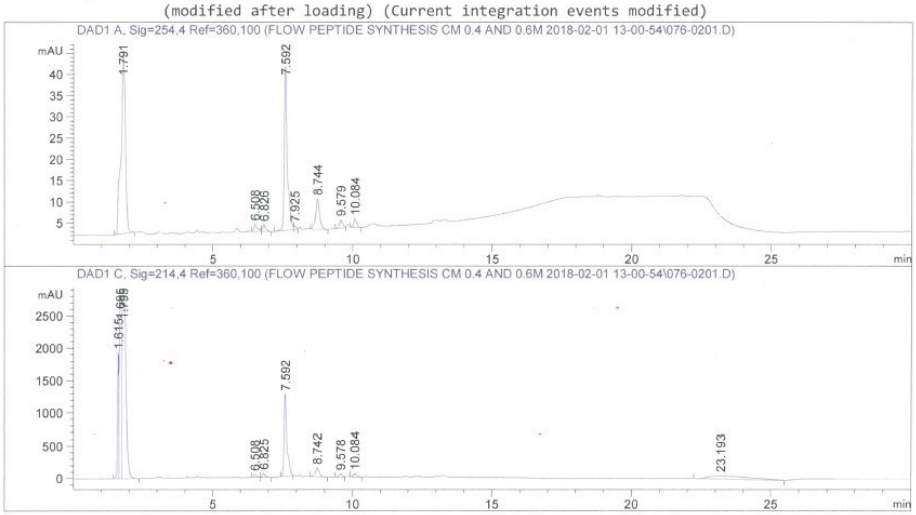


Figure 1.5.45: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.6 M

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\072-0601.D
Sample Name: CM 5mLmin 60degC 0.6M 0.5mL solution

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   6
Acq. Instrument : LC1260                        Location : Vial 72
Injection Date  : 1/30/2018 11:24:48 AM              Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M
Last changed    : 1/30/2018 10:32:19 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M (Sequence Method)
Last changed    : 2/1/2018 12:49:37 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

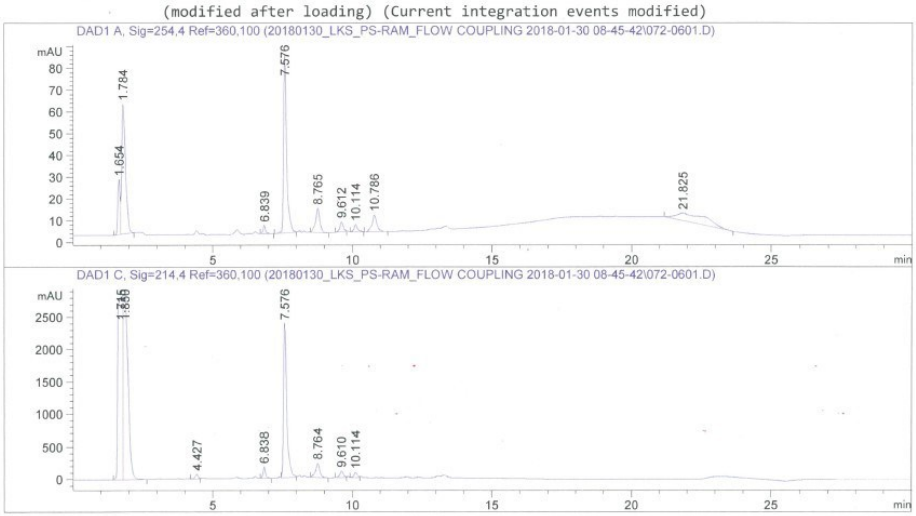


Figure 1.5.46: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, AA Injection of 0.6 M, 0.5 mL injection.

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin – Overall Optimised Conditions

```
Data File C:\CHEM32\...20180202_LKS_FLOW PEPTIDE 0.3M SWELL 2018-02-02 09-33-35\041-0101.D
Sample Name: PS  5mL 60d 0.3M based on 1ml swell

======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   1
Acq. Instrument : LC1260                     Location : Vial 41
Injection Date  : 2/2/2018 9:34:58 AM              Inj :   1
                                           Inj Volume : 10.000 µl
Method          : C:\CHEM32\1\DATA\20180202_LKS_FLOW PEPTIDE 0.3M SWELL 2018-02-02 09-33-35
                  \10 TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/2/2018 9:33:36 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
Sample Info     : Batch 30 mis
```

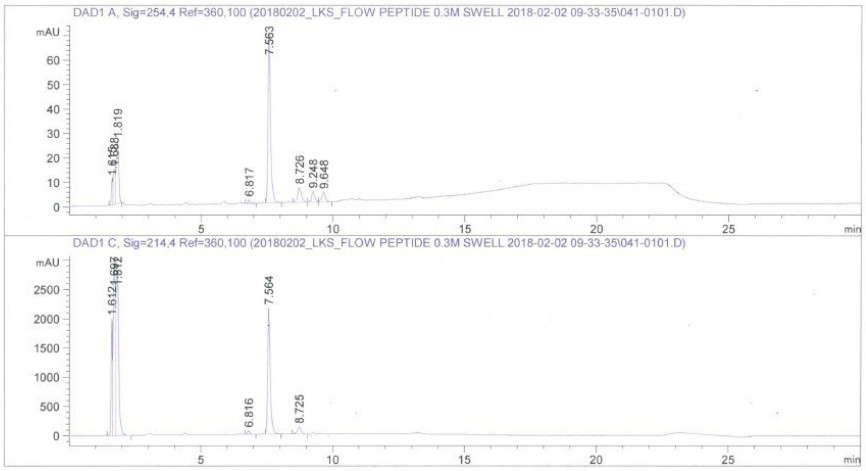


Figure 1.5.47: HPLC trace of **PS-2** using optimised Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection (Based on 1 mL resin swell) , HATU, DIPEA

```
Data File C:\CHEM32\...20180202_LKS_FLOW PEPTIDE 0.3M SWELL 2018-02-02 09-33-35\042-0201.D
Sample Name: CM  5mL 60d 0.3M based on 2ml swell

======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   2
Acq. Instrument : LC1260                     Location : Vial 42
Injection Date  : 2/2/2018 10:06:24 AM             Inj :   1
                                           Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180202_LKS_FLOW PEPTIDE 0.3M SWELL 2018-02-02 09-33-35
                  \10 TO 100 OVER 15 MINS 10UL.M
Last changed    : 2/2/2018 9:33:36 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180202_LKS_FLOW PEPTIDE 0.3M SWELL 2018-02-02 09-33-35
                  \10 TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/2/2018 10:36:36 AM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

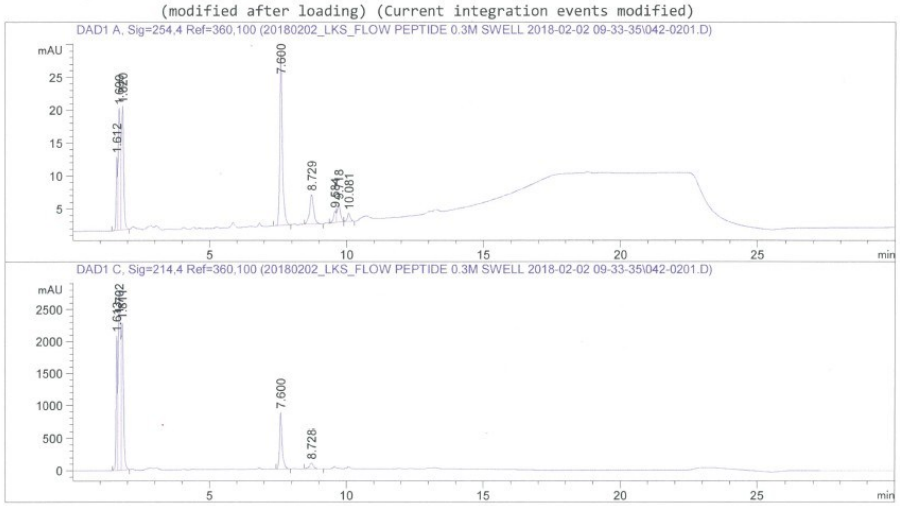


Figure 1.5.48: HPLC trace of **CM-2** using optimised Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection (Based on 2 mL resin swell) , HATU, DIPEA

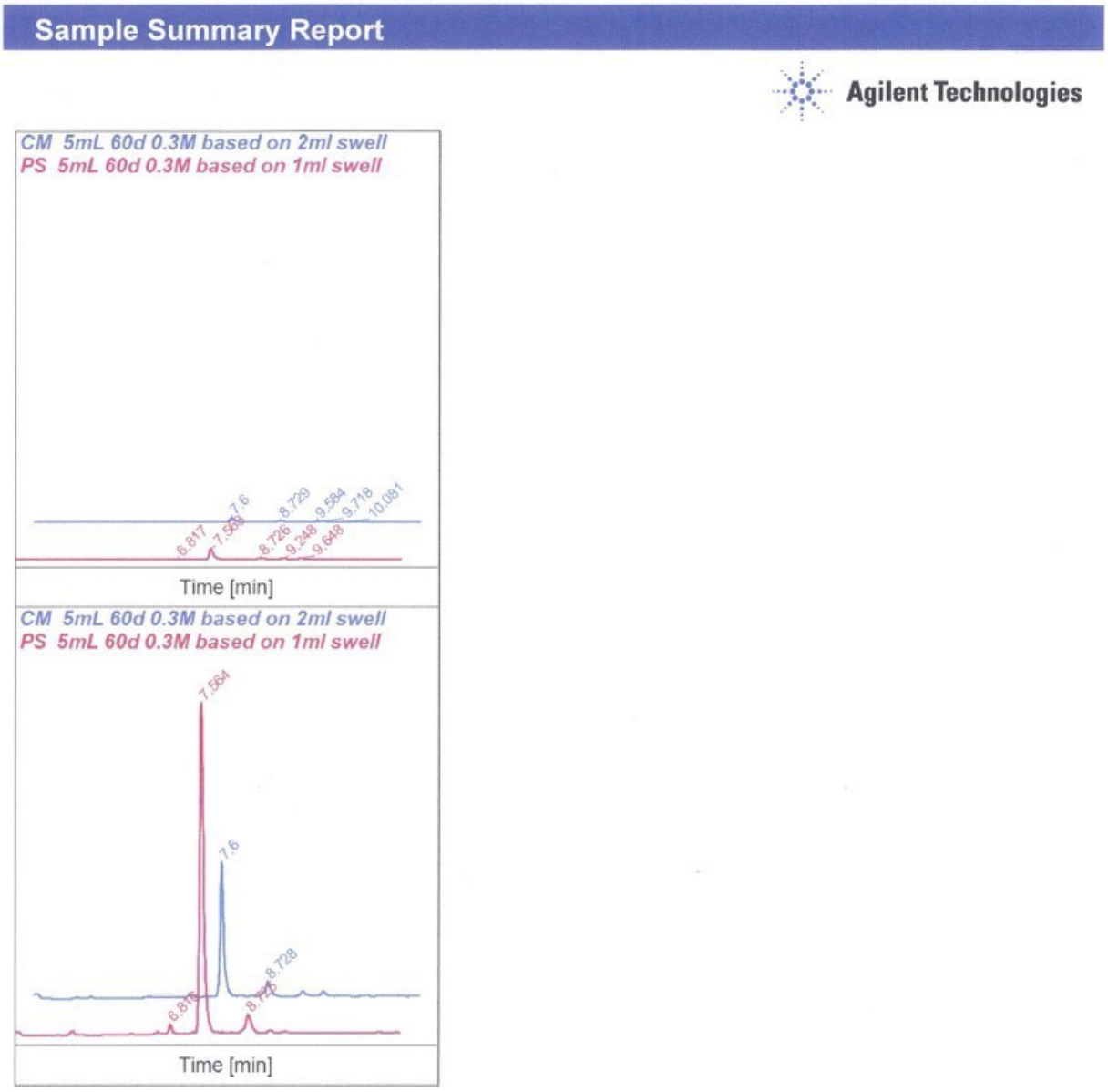


Figure 1.5.49: Stacked HPLC Traces of the CM and PS resin optimised conditions for the construction of **2**. (Top trace) 254 nm. (Bottom trace) 214 nm.

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin, optimised conditions – Solvent Variation

```
Data File C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS2 2018-04-10 10-32-21\020-0101.D
Sample Name: AspGlyTyr(Bzl)PheAla solvent NMP

    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   1
    Acq. Instrument : LC1260                        Location : Vial 20
    Injection Date  : 4/10/2018 10:33:45 AM             Inj :   1
                                                  Inj Volume : 5.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS2 2018-04-10 10-32-21\10 TO
                      100 OVER 15 MINS 5UL.M
    Last changed    : 4/10/2018 10:32:23 AM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS2 2018-04-10 10-32-21\10 TO
                      100 OVER 15 MINS 5UL.M (Sequence Method)
    Last changed    : 4/10/2018 4:19:18 PM by SYSTEM
                      (modified after loading) (Current integration events modified)
    Sample Info     : Batch 30 mis
```

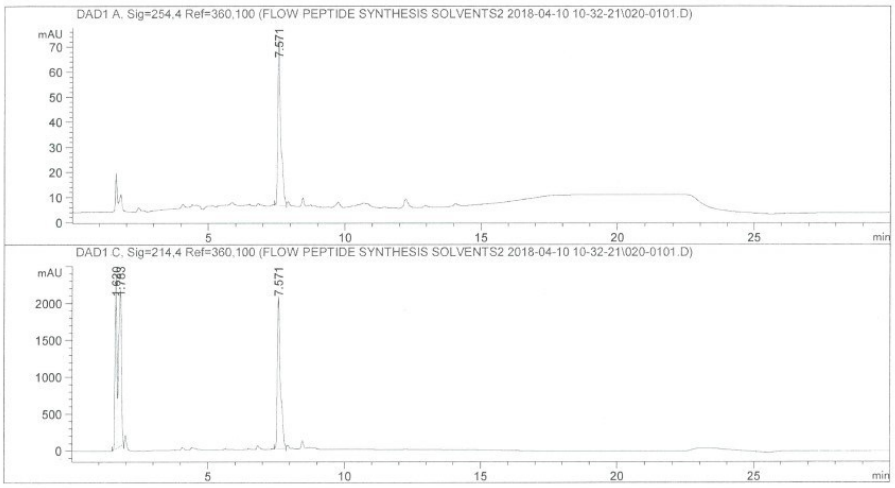


Figure 1.5.50: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, DIPEA. Substituting NMP for DMF.

Data File C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS4 2018-04-11 13-16-37\012-0201.D
Sample Name: AspGlyPheTyr(Bzl)Ala solvent DMF DCM

```
======================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   2
Acq. Instrument : LC1260                     Location : Vial 12
Injection Date  : 4/11/2018 1:49:20 PM            Inj :   1
                                             Inj Volume : 1.500 µl
Method          : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS4 2018-04-11 13-16-37\10 TO
                  100 OVER 15 MINS 1.5UL.M (Sequence Method)
Last changed    : 4/11/2018 1:16:38 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

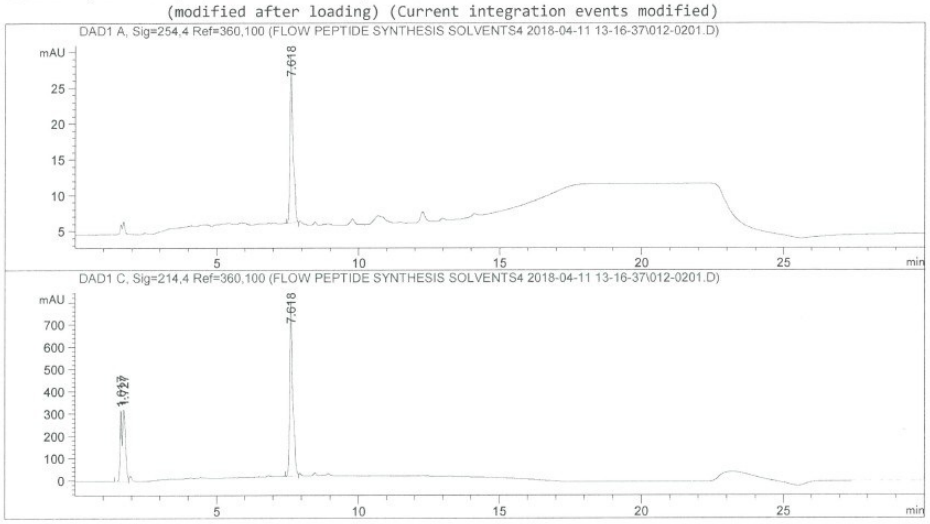


Figure 1.5.51: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, DIPEA. Substituting 1:1 DMF:DCM for DMF.

```
Data File C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS3 2018-04-11 08-58-45\013-0301.D
Sample Name: AspGlyPheTyr(Bzl)Ala solvent DMF AcN

======================================================================
Acq. Operator   : SYSTEM                          Seq. Line :   3
Acq. Instrument : LC1260                          Location : Vial 13
Injection Date  : 4/11/2018 10:02:57 AM                Inj :   1
                                                  Inj Volume : 5.000 µl
Acq. Method     : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS3 2018-04-11 08-58-45\10 TO
                  100 OVER 15 MINS 5UL.M
Last changed    : 4/11/2018 8:58:46 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\FLOW PEPTIDE SYNTHESIS SOLVENTS3 2018-04-11 08-58-45\10 TO
                  100 OVER 15 MINS 5UL.M (Sequence Method)
Last changed    : 4/11/2018 3:34:46 PM by SYSTEM
                  (modified after loading) (Current integration events modified)
```

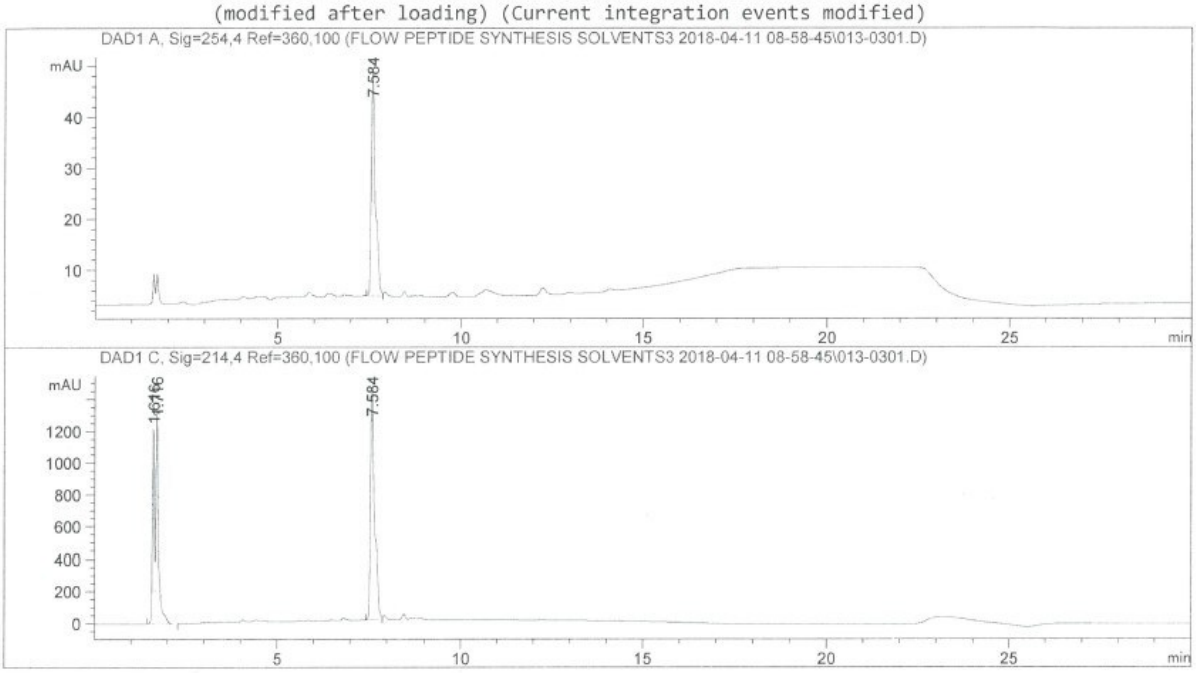


Figure 1.5.52: HPLC trace of **CM-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, DIPEA. Substituting 1:1 DMF:ACN for DMF.

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin, optimised conditions – Base Variation

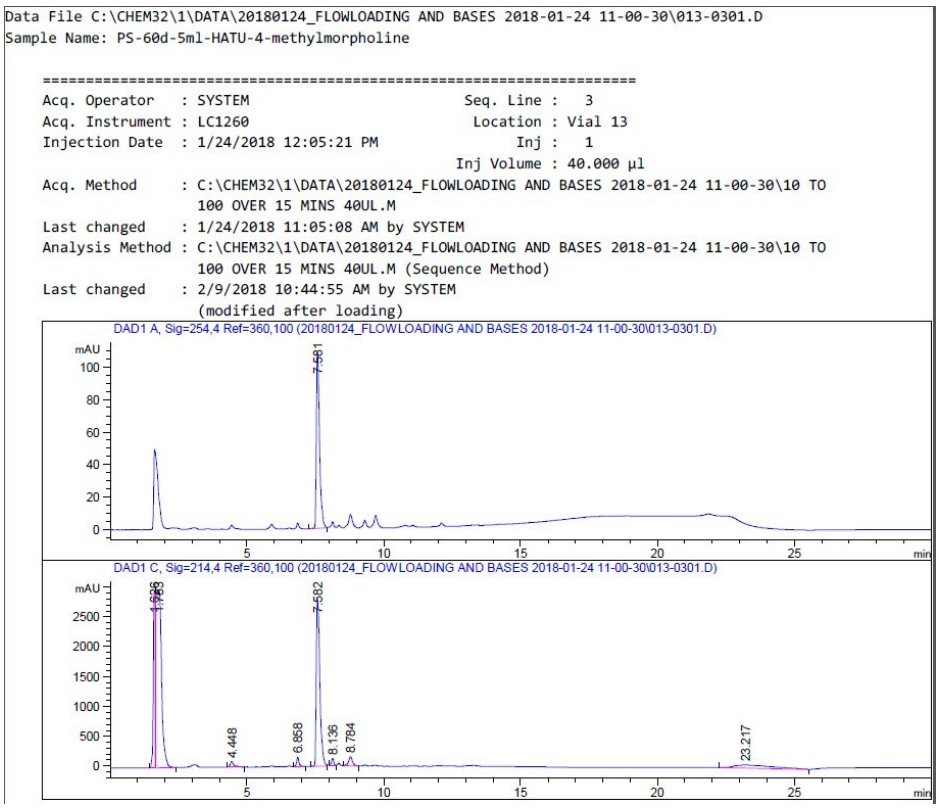


Figure 1.5.53: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, 4-methylmorpholine

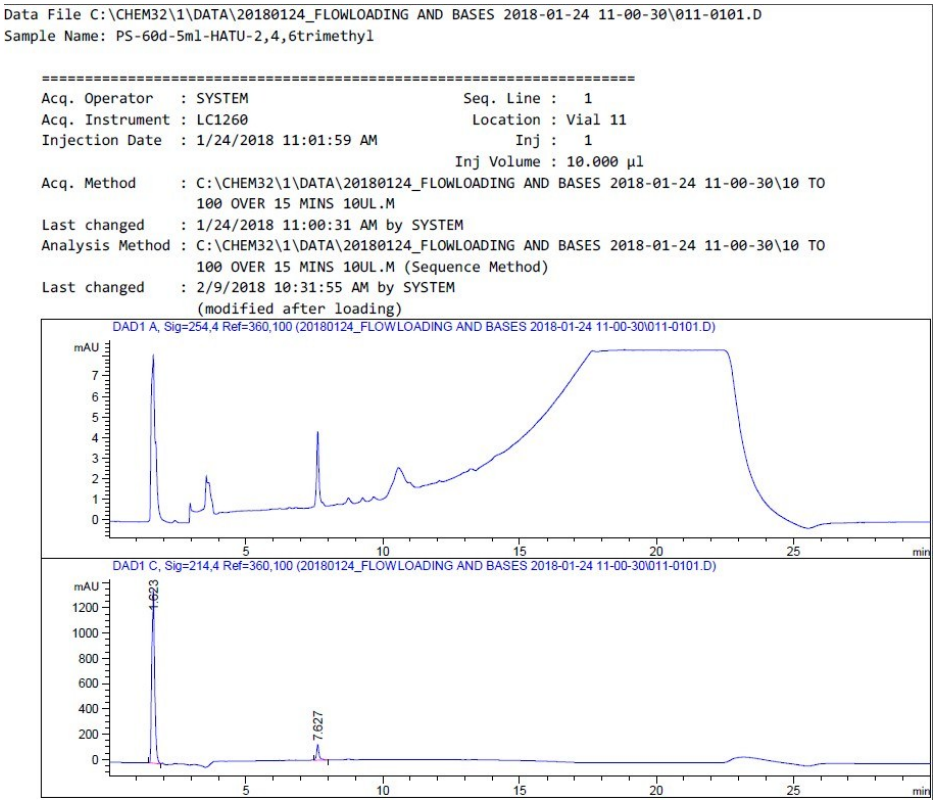


Figure 1.5.54: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, 2,4,6-trimethylpyridine

Data File C:\CHEM32\1\DATA\20180124_FLOWLOADING AND BASES 2018-01-24 11-00-30\012-0201.D
Sample Name: PS-60d-5ml-HATU-DBU

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   2
Acq. Instrument : LC1260                       Location : Vial 12
Injection Date  : 1/24/2018 11:33:37 AM             Inj :   1
                                              Inj Volume : 40.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180124_FLOWLOADING AND BASES 2018-01-24 11-00-30\10 TO
                  100 OVER 15 MINS 40UL.M
Last changed    : 1/24/2018 11:05:08 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180124_FLOWLOADING AND BASES 2018-01-24 11-00-30\10 TO
                  100 OVER 15 MINS 40UL.M (Sequence Method)
Last changed    : 2/9/2018 10:44:10 AM by SYSTEM
                  (modified after loading)
```

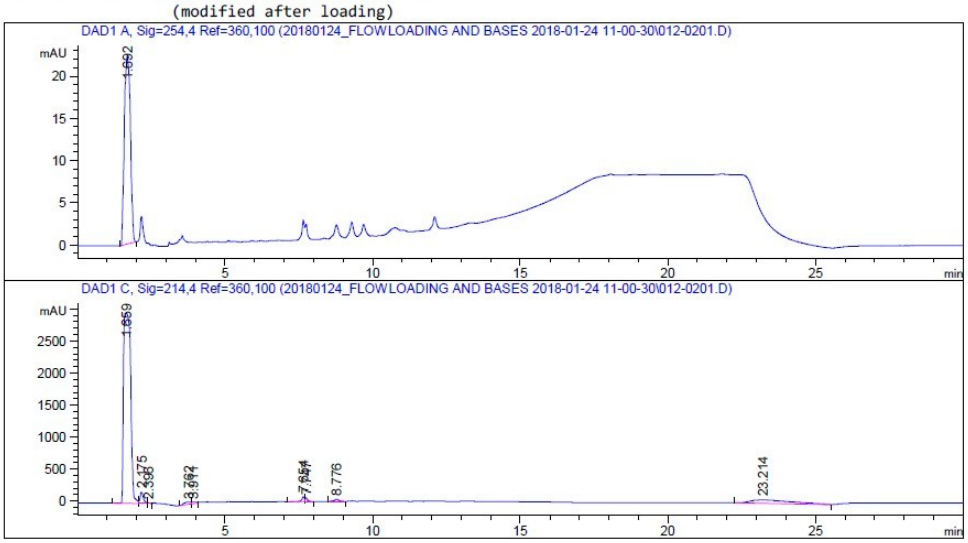


Figure 1.5.55: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, DBU

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\032-0201.D
Sample Name: PS-60d_5ml_HATU_load-dip_couple-246

```
======================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   2
Acq. Instrument : LC1260                       Location : Vial 32
Injection Date  : 1/31/2018 9:48:44 AM              Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/31/2018 9:14:13 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/31/2018 11:43:41 AM by SYSTEM
```

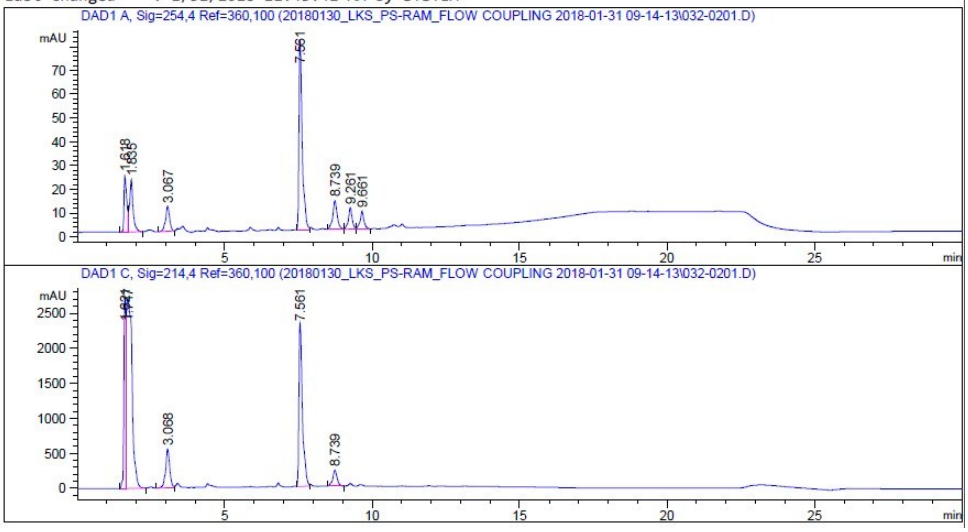


Figure 1.5.56: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, First AA loaded with DIPEA, subsequent couplings with 2,4,6-trimethylpyridine

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\031-0101.D
Sample Name: PS-60d_5ml_HATU_load-dip_couple-DBU

```
=====================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   1
Acq. Instrument : LC1260                        Location : Vial 31
Injection Date  : 1/30/2018 8:47:12 AM             Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M
Last changed    : 1/30/2018 8:45:43 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-30 08-45-42\10
                  TO 100 OVER 15 MINS 20UL.M (Sequence Method)
Last changed    : 2/1/2018 12:53:25 PM by SYSTEM
```

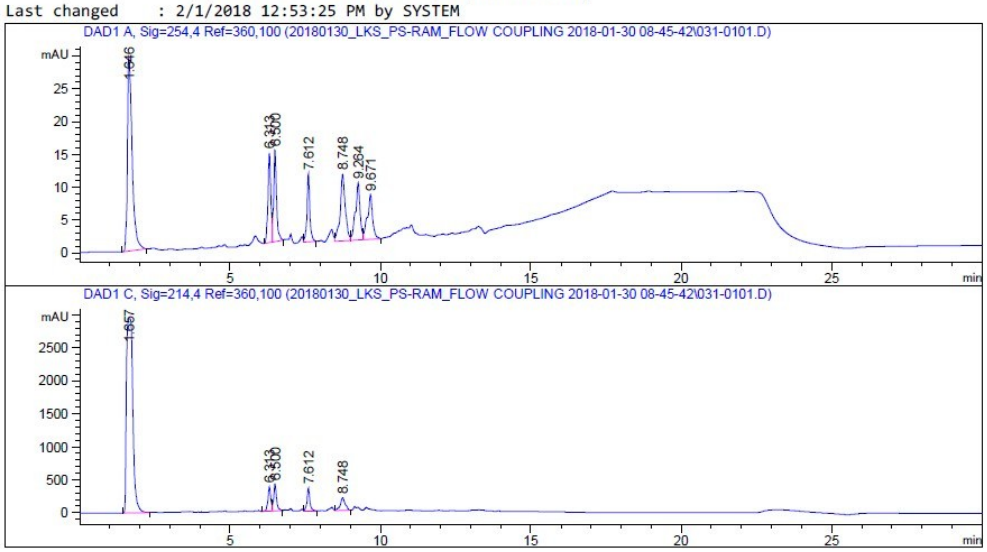


Figure 1.5.57: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HATU, First AA loaded with DIPEA, subsequent couplings with DBU

Continuous flow using Injection method, 1 fixed end, 1 adjustable end, immobilised resin, optimised conditions – Coupling Reagent Variation

```
Data File C:\CHEM32\...180204_LKS_FLOW PEPTIDE BASE- COUPLE 2018-02-04 11-19-41\053-0301.D
Sample Name: PS  5mL 60d 0.3M TBTU Dip

=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   3
Acq. Instrument : LC1260                         Location : Vial 53
Injection Date  : 2/4/2018 12:24:00 PM                Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180204_LKS_FLOW PEPTIDE BASE- COUPLE 2018-02-04 11-19-41
                  \10 TO 100 OVER 15 MINS 10UL.M
Last changed    : 2/4/2018 11:19:41 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180204_LKS_FLOW PEPTIDE BASE- COUPLE 2018-02-04 11-19-41
                  \10 TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/9/2018 11:20:11 AM by SYSTEM
                  (modified after loading)
```

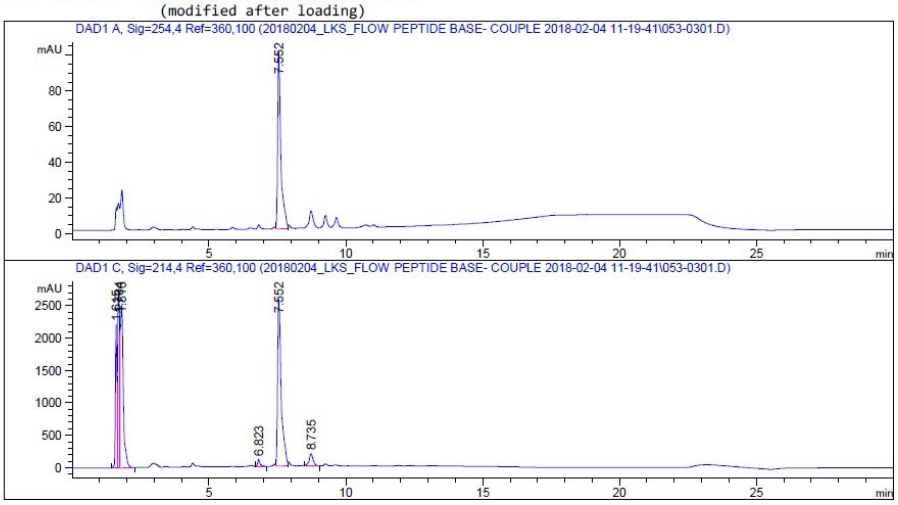


Figure 1.5.58: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, TBTU, DIPEA

```
Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\033-0301.D
Sample Name: PS-60d_5ml_Dip_HBTU

=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   3
Acq. Instrument : LC1260                         Location : Vial 33
Injection Date  : 1/31/2018 10:20:09 AM               Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/31/2018 9:14:13 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/31/2018 11:43:41 AM by SYSTEM
```

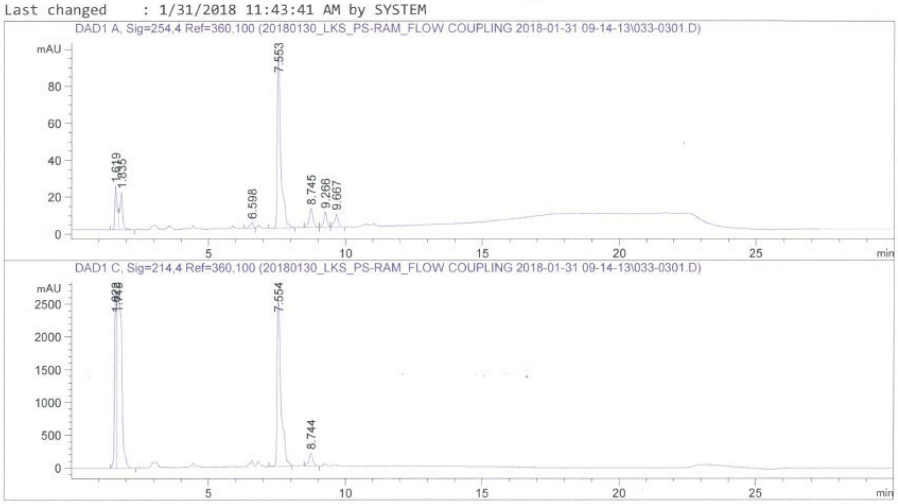


Figure 1.5.59: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HBTU, DIPEA

Data File C:\CHEM32\...TA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\034-0401.D
Sample Name: PS-60d_5ml_Dip_HCTU

```
==================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   4
Acq. Instrument : LC1260                       Location : Vial 34
Injection Date  : 1/31/2018 10:51:34 AM             Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M
Last changed    : 1/31/2018 9:14:13 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180130_LKS_PS-RAM_FLOW COUPLING 2018-01-31 09-14-13\10
                  TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 1/31/2018 11:43:41 AM by SYSTEM
```

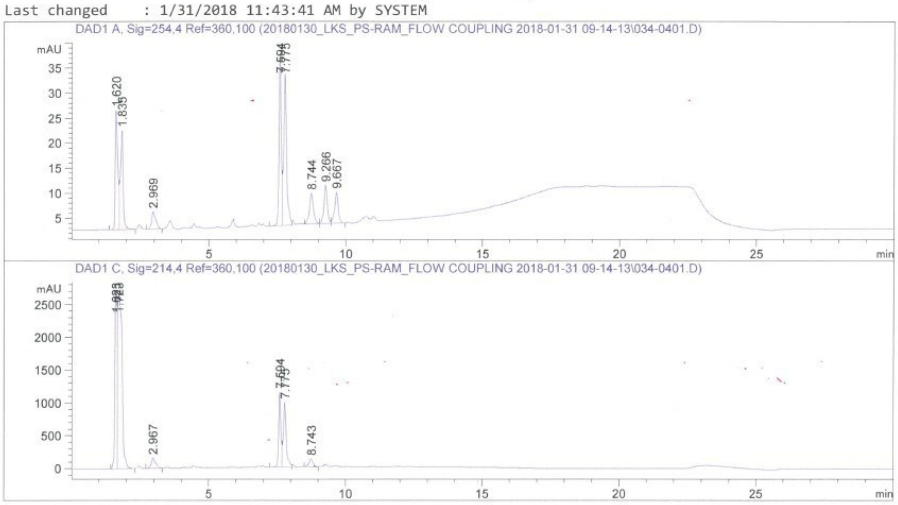


Figure 1.5.60: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HCTU, DIPEA

Data File C:\CHEM32\...180204_LKS_FLOW PEPTIDE BASE- COUPLE 2018-02-04 11-19-41\054-0401.D
Sample Name: PS 5mL 60d 0.3M DiC-HOBt Dip

```
==================================================================
Acq. Operator   : SYSTEM                      Seq. Line :   4
Acq. Instrument : LC1260                       Location : Vial 54
Injection Date  : 2/4/2018 12:55:27 PM              Inj :   1
                                              Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180204_LKS_FLOW PEPTIDE BASE- COUPLE 2018-02-04 11-19-41
                  \10 TO 100 OVER 15 MINS 10UL.M
Last changed    : 2/4/2018 11:19:41 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180204_LKS_FLOW PEPTIDE BASE- COUPLE 2018-02-04 11-19-41
                  \10 TO 100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 2/9/2018 11:20:11 AM by SYSTEM
                  (modified after loading)
```

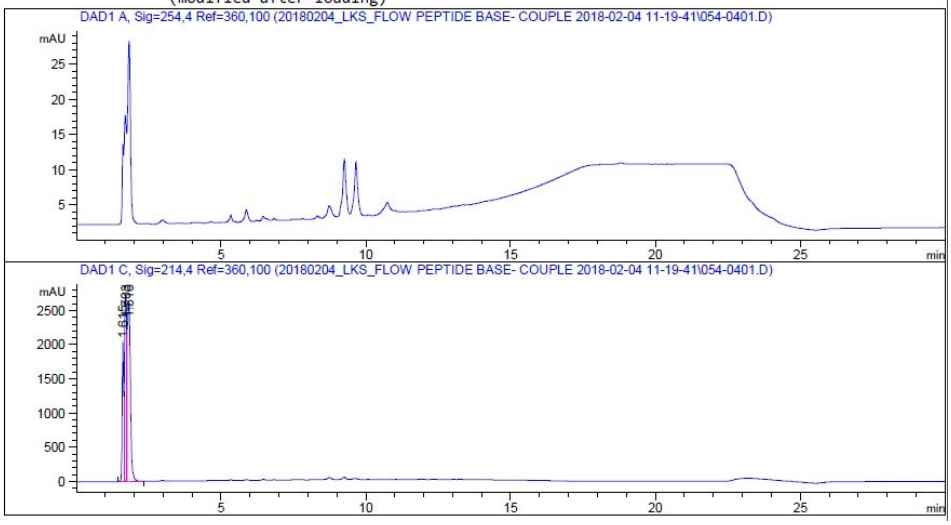


Figure 1.5.61: HPLC trace of **PS-2** using Injection method at 5 mL min^{-1}, 60 °C, 0.3 M Injection, HOBt/DIC, DIPEA

1.6 Copies of HPLC traces of final products

Figure 1.6.1: HPLC trace of **2** using PS-RAM

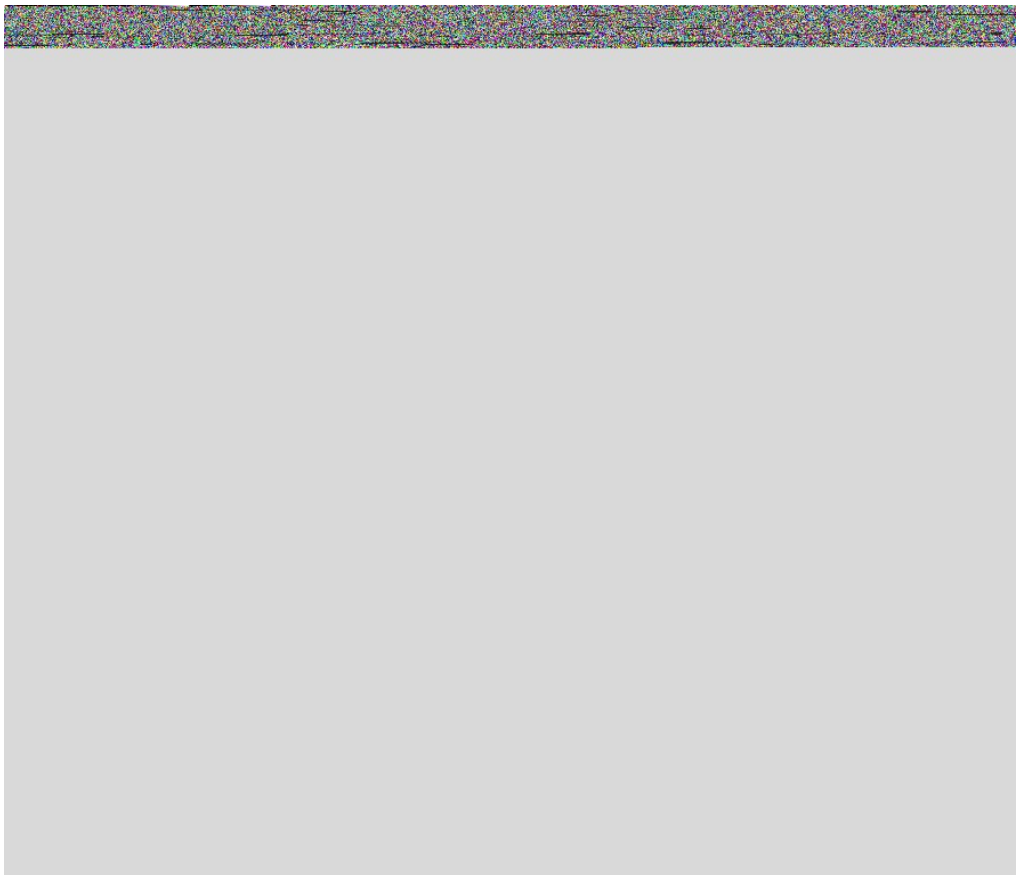


Figure 1.6.2: HPLC trace of **2** using CM-RAM

```
Data File C:\CHEM32\1\DATA\20180221_DEPROTECTTRITYL CHECKS 2018-02-21 17-24-57\033-0301.D
Sample Name: PS_Cys-end_Hex

    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   3
    Acq. Instrument : LC1260                        Location : Vial 33
    Injection Date  : 2/21/2018 6:29:16 PM               Inj :   1
                                                  Inj Volume : 10.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180221_DEPROTECTTRITYL CHECKS 2018-02-21 17-24-57\10 TO
                      100 OVER 15 MINS 10UL.M
    Last changed    : 2/21/2018 5:24:59 PM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\20180221_DEPROTECTTRITYL CHECKS 2018-02-21 17-24-57\10 TO
                      100 OVER 15 MINS 10UL.M (Sequence Method)
    Last changed    : 3/19/2018 2:55:30 PM by SYSTEM
                      (modified after loading)
```

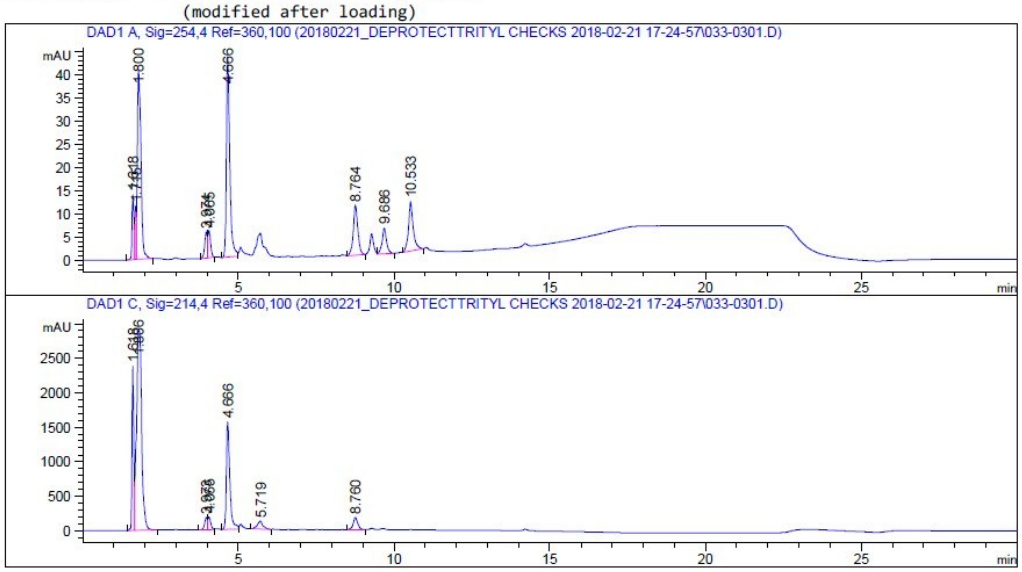


Figure 1.6.3: HPLC trace of **4** using PS-RAM

```
Data File C:\CHEM32\1\DATA\20180221_DEPROTECTTRITYL CHECKS 2018-02-21 17-24-57\034-0401.D
Sample Name: CM_Cys-end_Hex

    ======================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :   4
    Acq. Instrument : LC1260                        Location : Vial 34
    Injection Date  : 2/21/2018 7:00:40 PM               Inj :   1
                                                  Inj Volume : 10.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180221_DEPROTECTTRITYL CHECKS 2018-02-21 17-24-57\10 TO
                      100 OVER 15 MINS 10UL.M
    Last changed    : 2/21/2018 5:24:59 PM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\20180221_DEPROTECTTRITYL CHECKS 2018-02-21 17-24-57\10 TO
                      100 OVER 15 MINS 10UL.M (Sequence Method)
    Last changed    : 3/19/2018 2:55:30 PM by SYSTEM
                      (modified after loading)
```

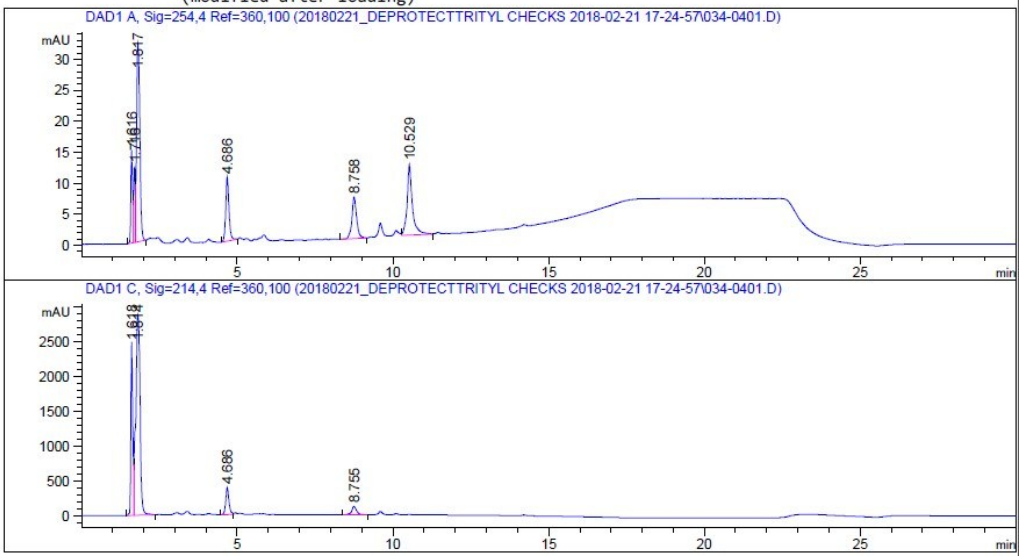


Figure 1.6.4: HPLC trace of **4** using CM-RAM

```
Data File C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\009-0901.D
Sample Name: PS_AspGlyPheTyr(Tbu)Ser(tbu)_Hexextract

==================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   9
Acq. Instrument : LC1260                        Location : Vial 9
Injection Date  : 2/22/2018 8:39:17 PM               Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                  OVER 15 MINS 10UL.M
Last changed    : 2/22/2018 4:26:43 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                  OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 3/19/2018 2:50:39 PM by SYSTEM
                  (modified after loading)
```

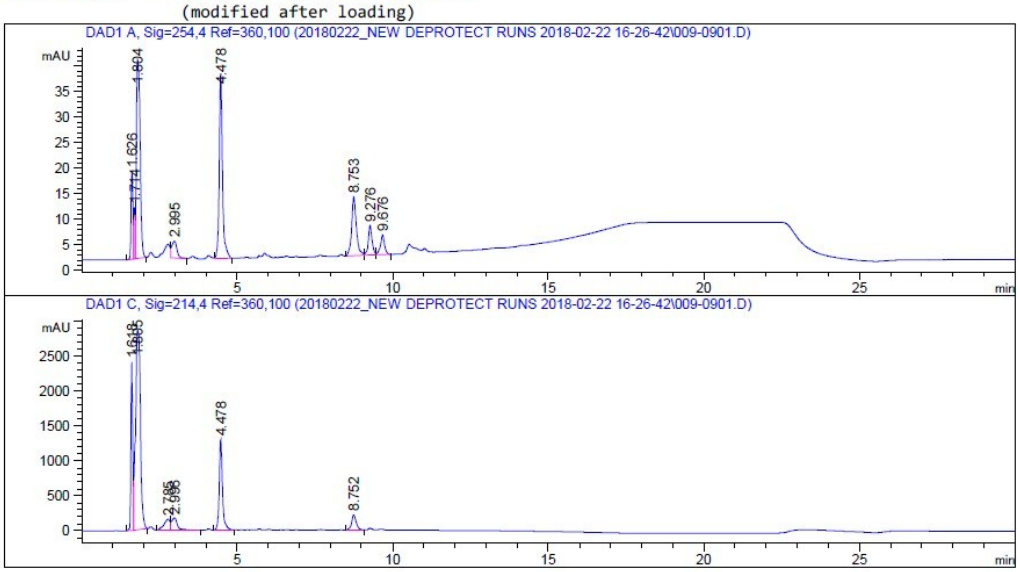


Figure 1.6.5: HPLC trace of **5** using PS-RAM

```
Data File C:\CHEM32\1\DATA\20180215_PEPTIDE COLLECTION_TFA 2018-02-15 10-55-10\009-0401.D
Sample Name: CM_AspGlyPheTyr(tbu)Ser(tbu)_TFA

==================================================================
Acq. Operator   : SYSTEM                       Seq. Line :   4
Acq. Instrument : LC1260                        Location : Vial 9
Injection Date  : 2/15/2018 12:30:59 PM              Inj :   1
                                               Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180215_PEPTIDE COLLECTION_TFA 2018-02-15 10-55-10\10 TO
                  100 OVER 15 MINS 10UL.M
Last changed    : 2/15/2018 10:55:11 AM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180215_PEPTIDE COLLECTION_TFA 2018-02-15 10-55-10\10 TO
                  100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 3/19/2018 2:58:20 PM by SYSTEM
                  (modified after loading)
```

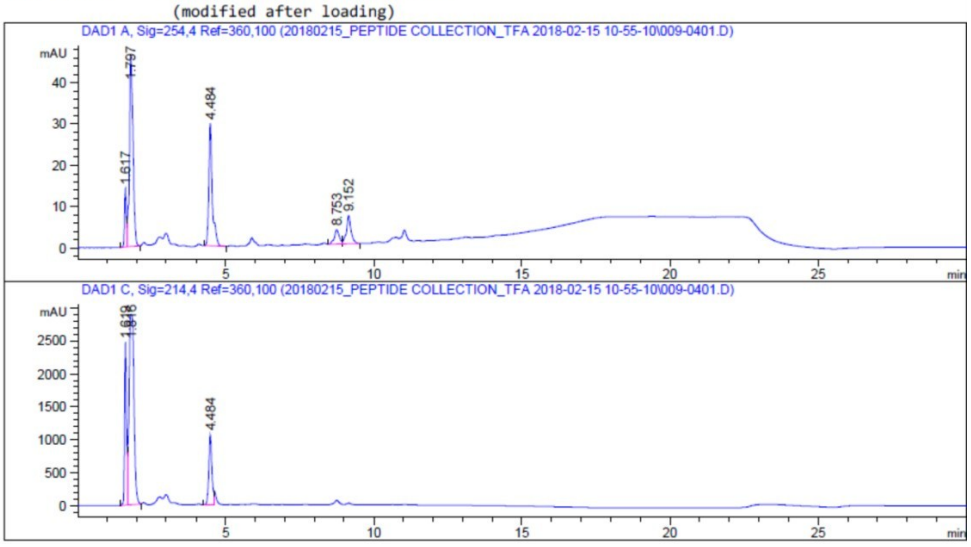


Figure 1.6.6: HPLC trace of **5** using CM-RAM

Data File C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\007-0701.D
Sample Name: PS_AspGlyPheTyr(Tbu)His(Trt)_Hexextract

```
Acq. Operator   : SYSTEM                        Seq. Line :   7
Acq. Instrument : LC1260                         Location : Vial 7
Injection Date  : 2/22/2018 7:36:34 PM                Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                  OVER 15 MINS 10UL.M
Last changed    : 2/22/2018 4:26:43 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                  OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 3/19/2018 2:50:39 PM by SYSTEM
                  (modified after loading)
```

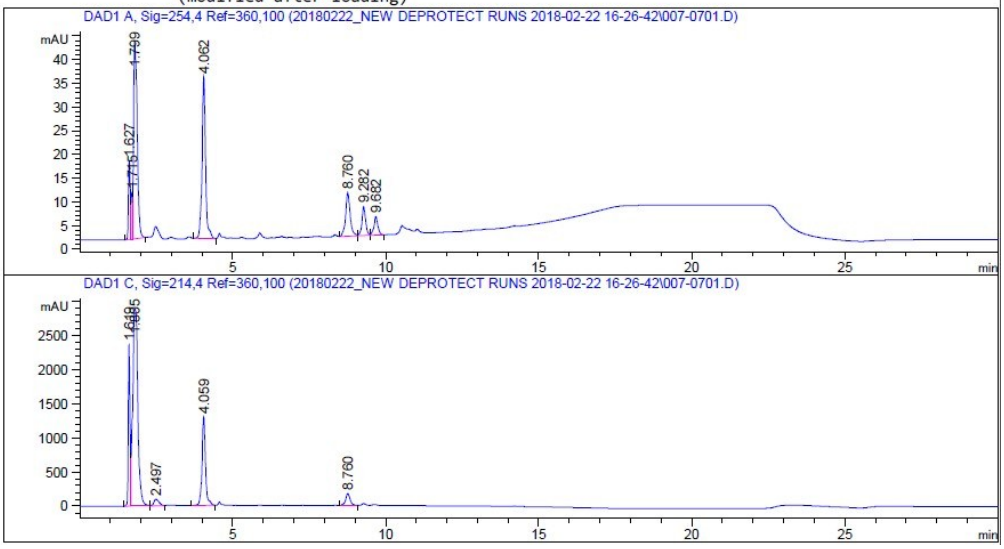


Figure 1.6.7: HPLC trace of **6** using PS-RAM

Data File C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\008-0801.D
Sample Name: CM_AspGlyPheTyr(Tbu)His(Trt)_Hexextract

```
Acq. Operator   : SYSTEM                        Seq. Line :   8
Acq. Instrument : LC1260                         Location : Vial 8
Injection Date  : 2/22/2018 8:07:56 PM                Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                  OVER 15 MINS 10UL.M
Last changed    : 2/22/2018 4:26:43 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                  OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 3/19/2018 2:50:39 PM by SYSTEM
                  (modified after loading)
```

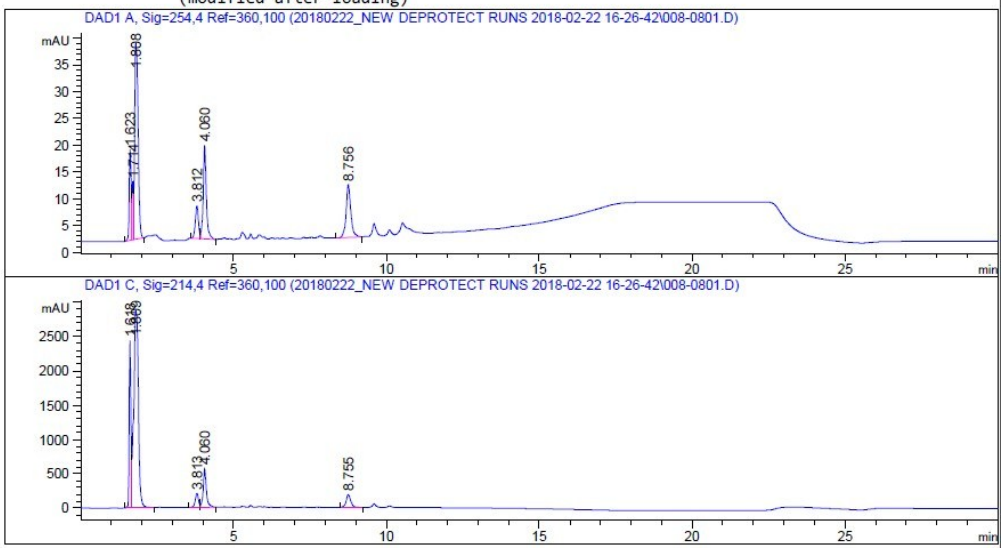


Figure 1.6.8: HPLC trace of **6** using CM-RAM

```
Data File C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\012-1101.D
Sample Name: PS_Lge-Scale_AspGlyPheTyr(Bz)Ala

    ====================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :  11
    Acq. Instrument : LC1260                        Location : Vial 12
    Injection Date  : 2/22/2018 9:42:06 PM               Inj :   1
                                                  Inj Volume : 10.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                      OVER 15 MINS 10UL.M
    Last changed    : 2/22/2018 4:26:43 PM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                      OVER 15 MINS 10UL.M (Sequence Method)
    Last changed    : 3/19/2018 2:50:39 PM by SYSTEM
                      (modified after loading)
```

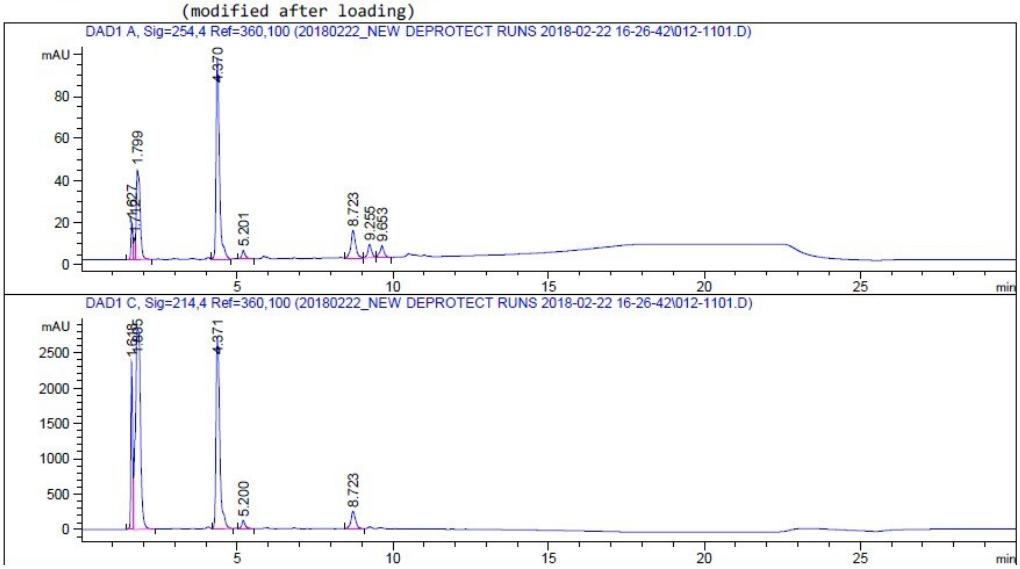


Figure 1.6.9: HPLC trace of **7** using PS-RAM

```
Data File C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\013-1201.D
Sample Name: CM_Lge-Scale_AspGlyPheTyr(Bz)Ala

    ====================================================================
    Acq. Operator   : SYSTEM                       Seq. Line :  12
    Acq. Instrument : LC1260                        Location : Vial 13
    Injection Date  : 2/22/2018 10:13:31 PM              Inj :   1
                                                  Inj Volume : 10.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                      OVER 15 MINS 10UL.M
    Last changed    : 2/22/2018 4:26:43 PM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\20180222_NEW DEPROTECT RUNS 2018-02-22 16-26-42\10 TO 100
                      OVER 15 MINS 10UL.M (Sequence Method)
    Last changed    : 3/19/2018 2:50:39 PM by SYSTEM
                      (modified after loading)
```

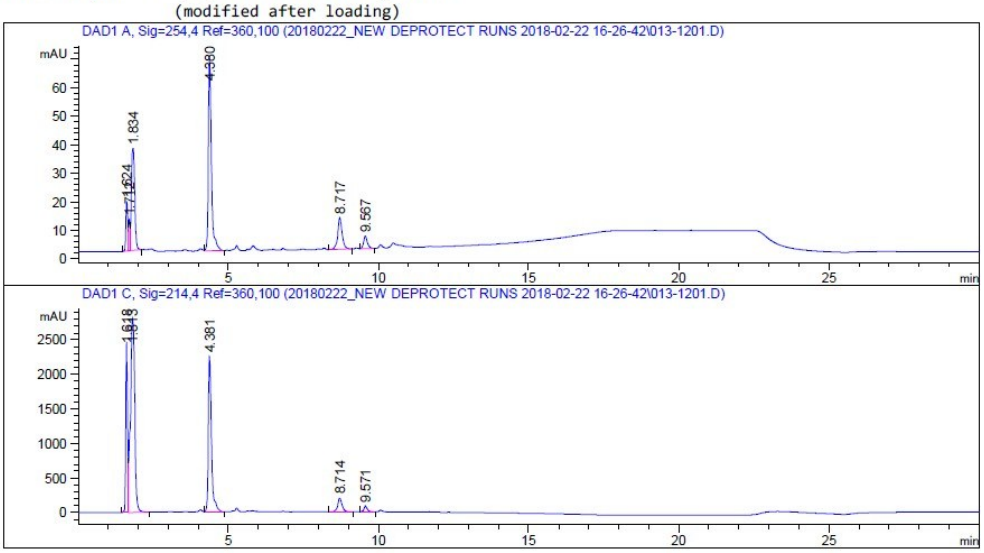


Figure 1.6.10: HPLC trace of **7** using CM-RAM

```
Data File C:\CHEM32\1\DATA\20180327_ACP PEPTIDE 2018-03-27 11-31-40\063-0301.D
Sample Name: PS_ACP

    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   3
    Acq. Instrument : LC1260                        Location : Vial 63
    Injection Date  : 3/27/2018 12:35:06 PM              Inj :   1
                                                   Inj Volume : 60.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180327_ACP PEPTIDE 2018-03-27 11-31-40\10 TO 60 OVER 15
                      MINS 150 X 4.6MM 60UL.M
    Last changed    : 3/27/2018 11:44:17 AM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\20180327_ACP PEPTIDE 2018-03-27 11-31-40\10 TO 60 OVER 15
                      MINS 150 X 4.6MM 60UL.M (Sequence Method)
    Last changed    : 4/3/2018 9:08:17 AM by SYSTEM
                      (modified after loading)
```

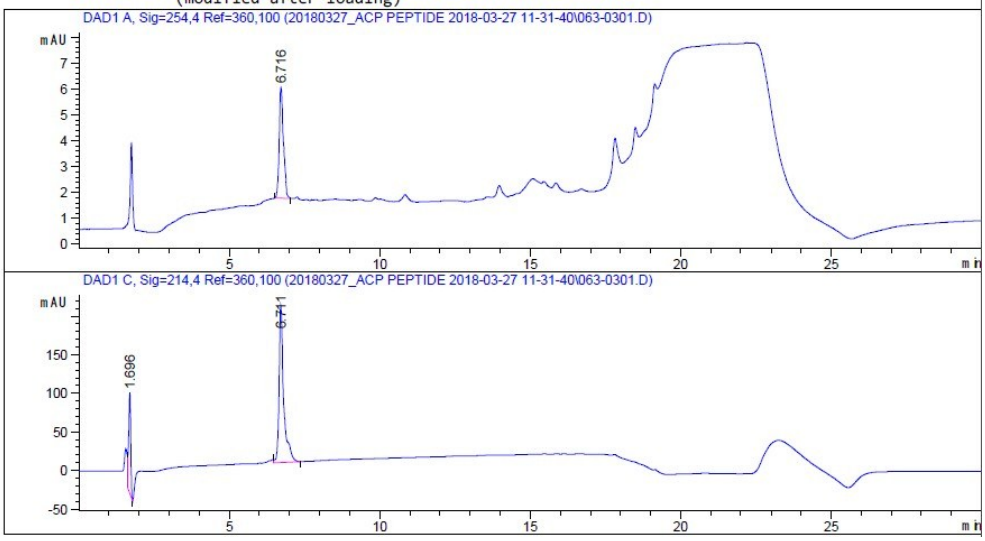


Figure 1.6.11: HPLC trace of **ACP$_{65-74}$** using PS-RAM

```
Data File C:\CHEM32\1\DATA\20180327_ACP PEPTIDE 2018-03-27 11-31-40\064-0401.D
Sample Name: CM_ACP

    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :   4
    Acq. Instrument : LC1260                        Location : Vial 64
    Injection Date  : 3/27/2018 1:06:27 PM               Inj :   1
                                                   Inj Volume : 60.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180327_ACP PEPTIDE 2018-03-27 11-31-40\10 TO 60 OVER 15
                      MINS 150 X 4.6MM 60UL.M
    Last changed    : 3/27/2018 11:44:17 AM by SYSTEM
    Analysis Method : C:\CHEM32\1\DATA\20180327_ACP PEPTIDE 2018-03-27 11-31-40\10 TO 60 OVER 15
                      MINS 150 X 4.6MM 60UL.M (Sequence Method)
    Last changed    : 4/3/2018 9:10:07 AM by SYSTEM
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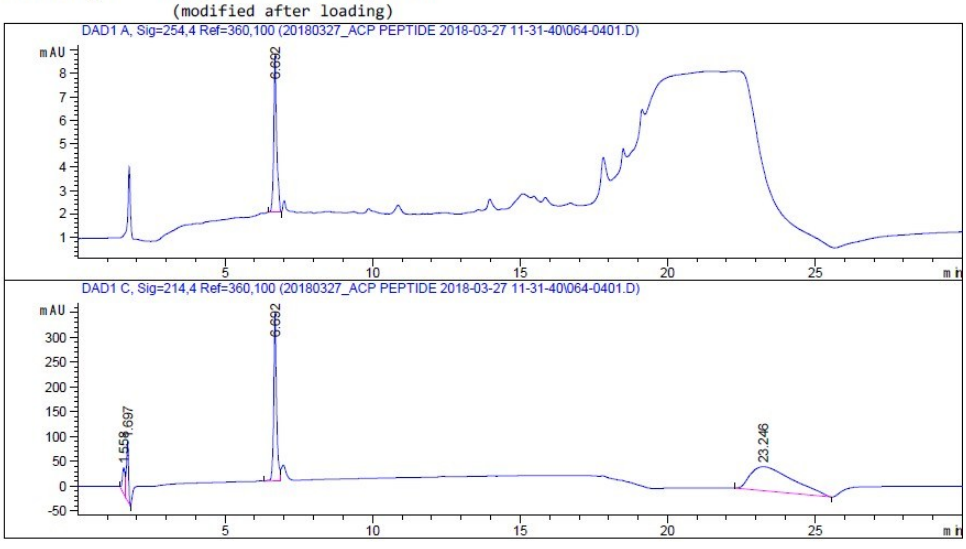

Figure 1.6.12: HPLC trace of **ACP$_{65-74}$** using CM-RAM

Data File C:\CHEM32\1\DATA\20180214_PEPTIDE SEQUENCES_TFA 2018-02-14 16-45-07\014-1301.D
Sample Name: PS_GHRH

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==========================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  13
Acq. Instrument : LC1260                        Location : Vial 14
Injection Date  : 2/14/2018 11:03:13 PM              Inj :   1
                                                Inj Volume : 10.000 µl
Acq. Method     : C:\CHEM32\1\DATA\20180214_PEPTIDE SEQUENCES_TFA 2018-02-14 16-45-07\10 TO
                  100 OVER 15 MINS 10UL.M
Last changed    : 2/14/2018 4:45:08 PM by SYSTEM
Analysis Method : C:\CHEM32\1\DATA\20180214_PEPTIDE SEQUENCES_TFA 2018-02-14 16-45-07\10 TO
                  100 OVER 15 MINS 10UL.M (Sequence Method)
Last changed    : 4/3/2018 9:14:25 AM by SYSTEM
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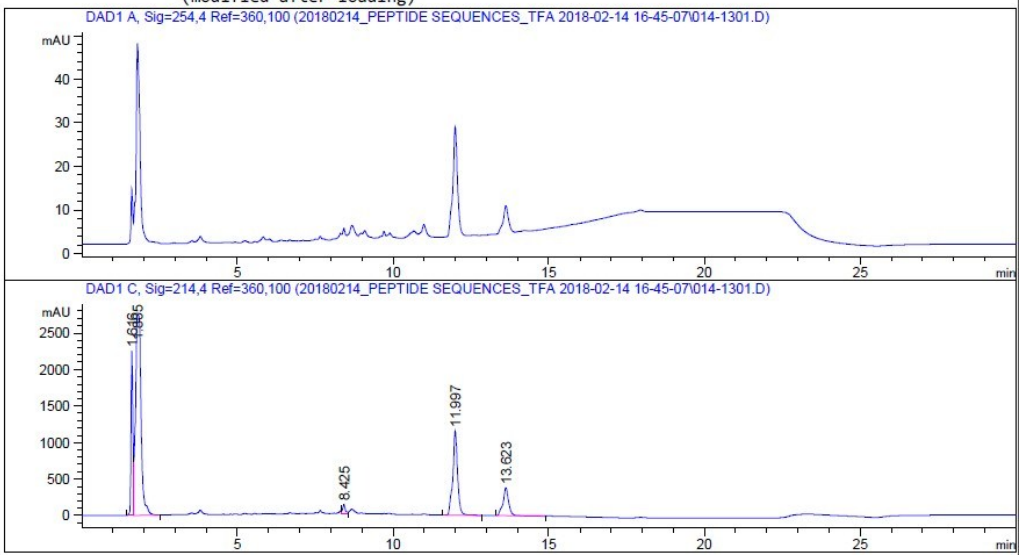

Figure 1.6.13: HPLC trace of **GHRH Analogue** using PS-RAM

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Data File C:\CHEM32\1\DATA\20180214_PEPTIDE SEQUENCES_TFA 2018-02-14 16-45-07\015-1401.D
Sample Name: CM_GHRH

    ======================================================================
    Acq. Operator   : SYSTEM                        Seq. Line :  14
    Acq. Instrument : LC1260                        Location : Vial 15
    Injection Date  : 2/14/2018 11:34:38 PM              Inj :   1
                                                   Inj Volume : 10.000 µl
    Acq. Method     : C:\CHEM32\1\DATA\20180214_PEPTIDE SEQUENCES_TFA 2018-02-14 16-45-07\10 TO
                      100 OVER 15 MINS 10UL.M
    Last changed    : 2/14/2018 4:45:08 PM by SYSTEM
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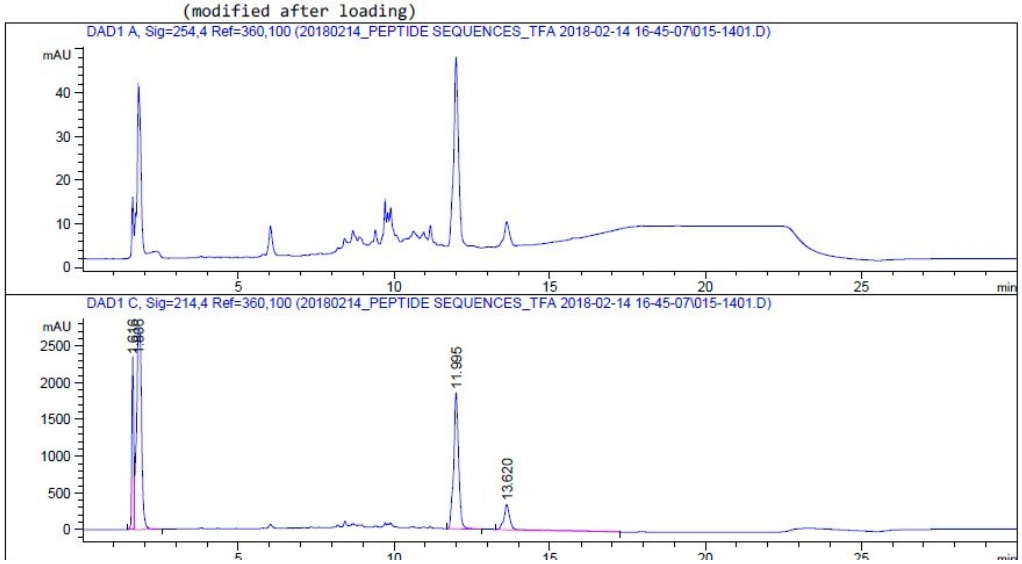


Figure 1.6.14: HPLC trace of **GHRH Analogue** using CM-RAM

1.7 Copies of mass spectra of final products

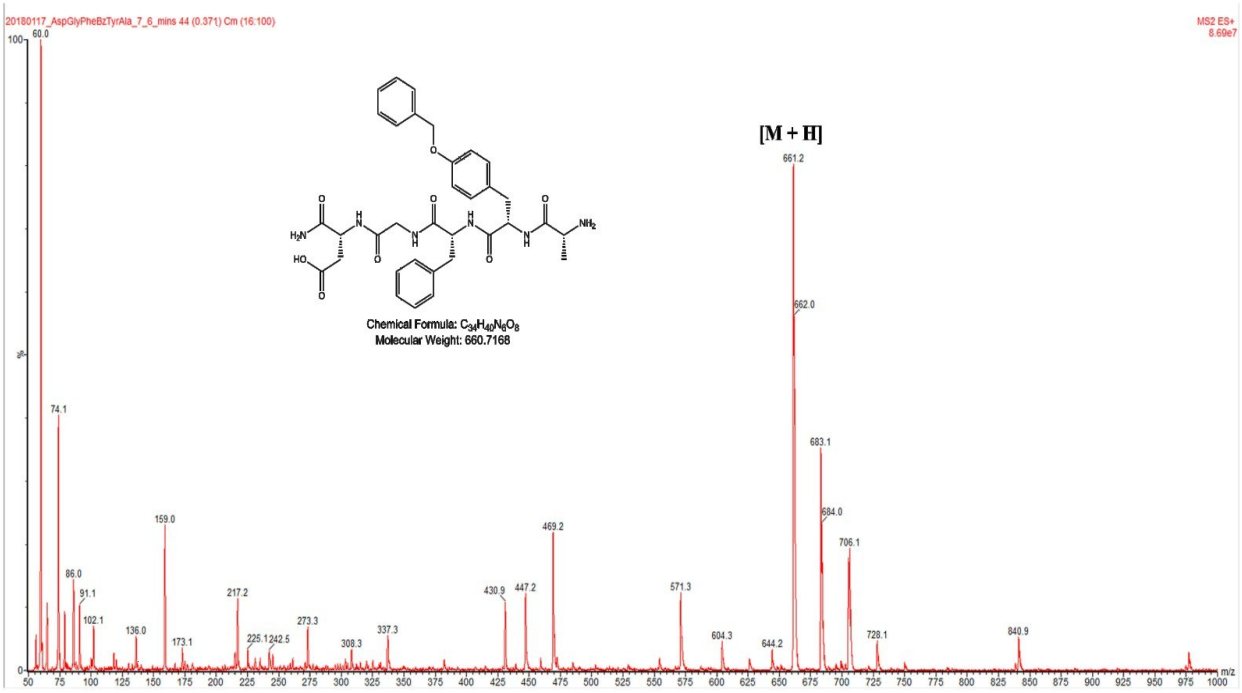


Figure 1.7.1: LRMS of **2**

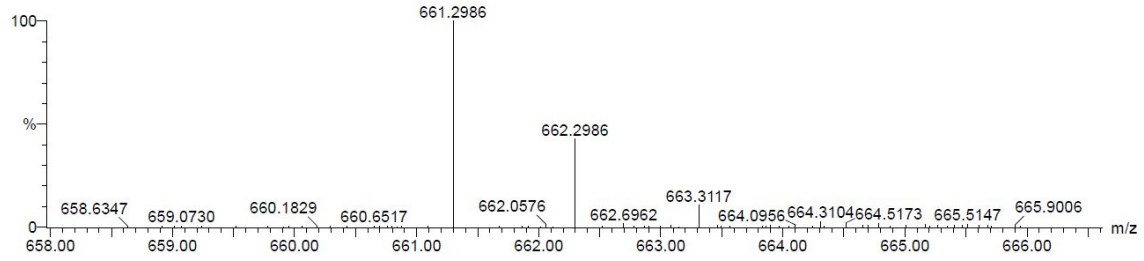
Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
8 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 30-40 H: 40-45 N: 5-10 O: 7-10

20180320_Lawson_1 25 (0.455)

1: TOF MS ES+
6.62e+002



Minimum:				0.0			
Maximum:		5.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
661.2986	661.2986	0.0	0.0	17.5	54.5	0.5	C34 H41 N6 O8
	661.3098	-11.2	-16.9	17.5	55.0	1.1	C33 H41 N8 O7
	661.3197	-21.1	-31.9	12.5	56.6	2.7	C31 H45 N6 O10
	661.3310	-32.4	-49.0	12.5	57.9	4.0	C30 H45 N8 O9

Figure 1.7.2: HRMS of **2**

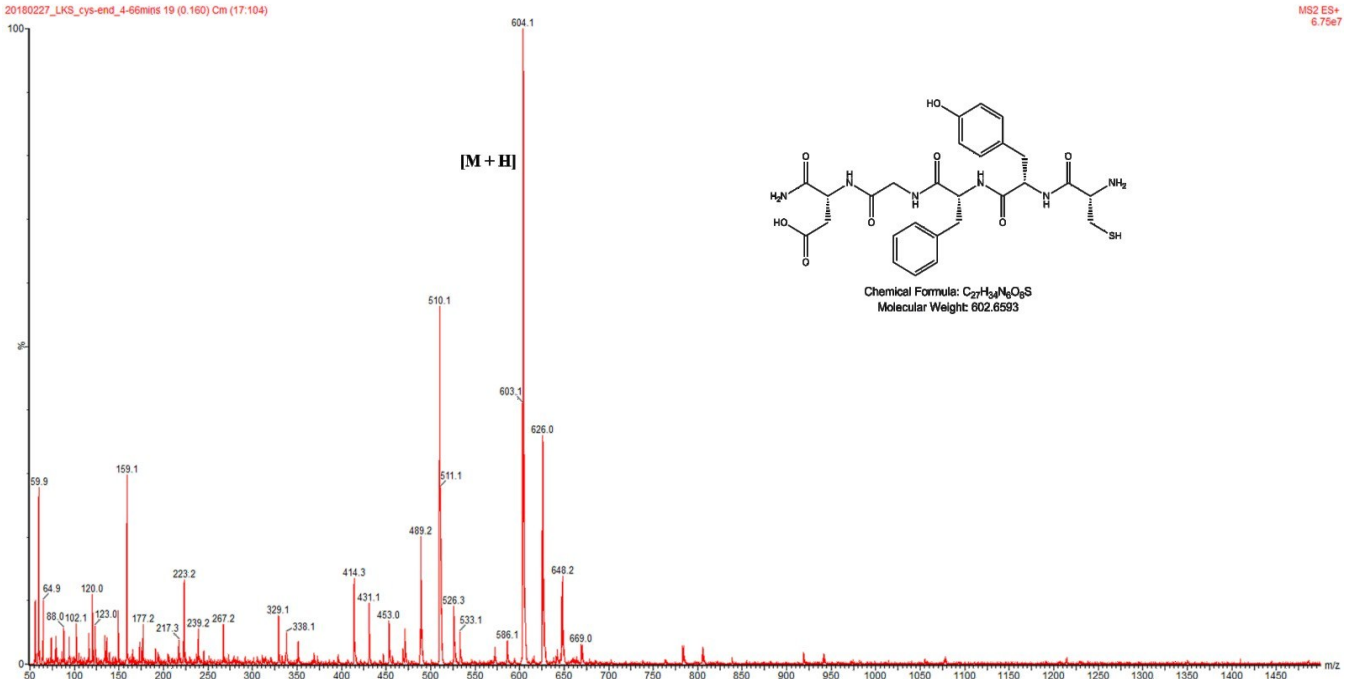


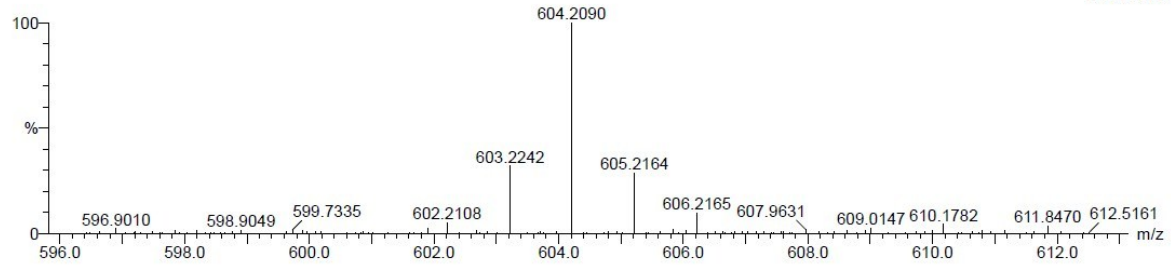
Figure 1.7.3: LRMS of **4**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 4 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 35-37 N: 5-10 O: 7-10 S: 0-2
20180320_Lawson_7 38 (0.689)

1: TOF MS ES+
4.56e+002



Minimum:				0.0			
Maximum:		5.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
603.2242	603.2237	0.5	0.8	13.5	52.5	1.1	C27 H35 N6 O8 S
	603.2349	-10.7	-17.7	13.5	52.7	1.3	C26 H35 N8 O7 S
	603.2415	-17.3	-28.7	13.5	53.0	1.5	C27 H35 N6 O10
	603.2527	-28.5	-47.2	13.5	53.4	1.9	C26 H35 N8 O9

Figure 1.7.4: HRMS of **4**

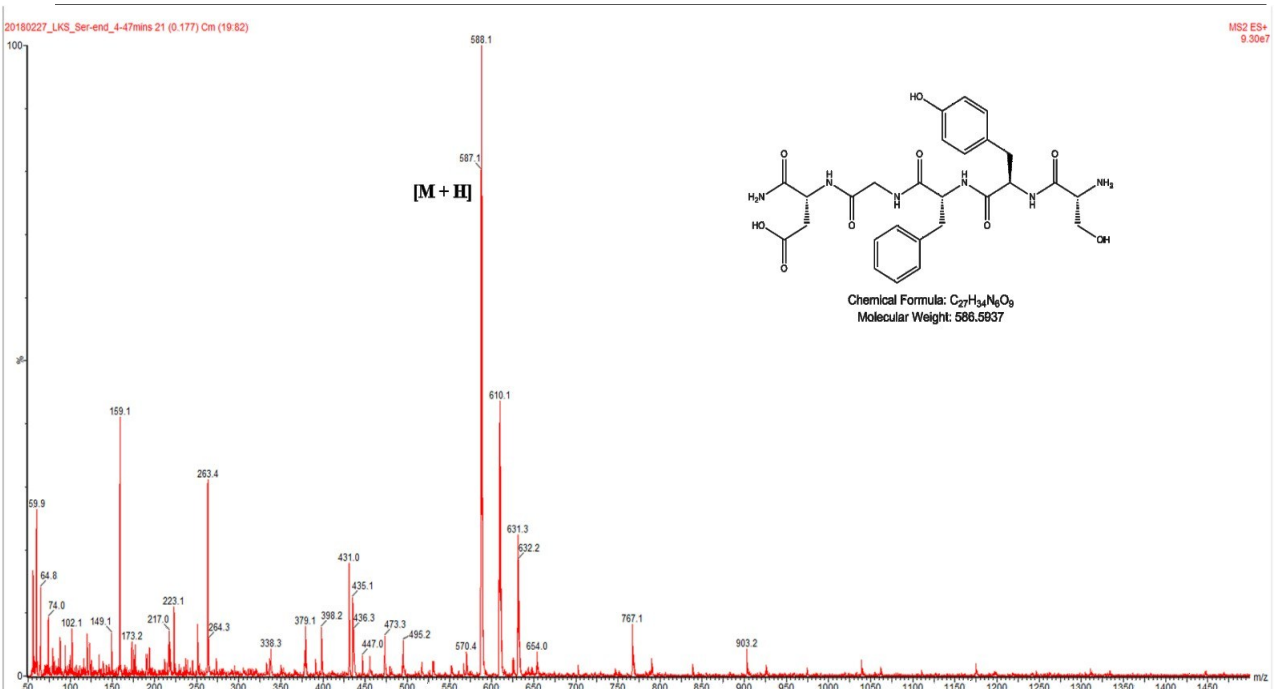


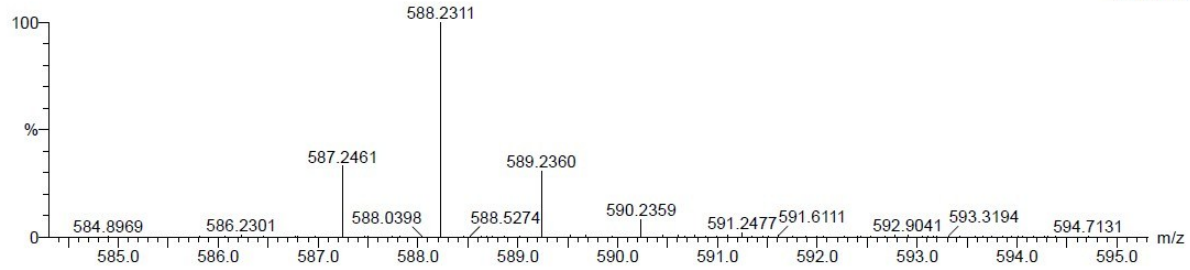
Figure 1.7.5: LRMS of **5**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
21 formula(e) evaluated with 8 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 30-40 N: 5-10 O: 5-10
20180320_Lawson_5 74 (1.314)

1: TOF MS ES+
3.14e+003



Minimum:				0.0			
Maximum:		5.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
587.2461	587.2479	-1.8	-3.1	18.5	86.2	2.0	C28 H31 N10 O5
	587.2367	9.4	16.0	18.5	86.2	2.0	C29 H31 N8 O6
	587.2254	20.7	35.2	18.5	86.2	2.0	C30 H31 N6 O7
	587.2466	-0.5	-0.9	13.5	86.3	2.1	C27 H35 N6 O9
	587.2214	24.7	42.1	14.5	86.3	2.1	C25 H31 N8 O9
	587.2578	-11.7	-19.9	13.5	86.3	2.1	C26 H35 N8 O8
	587.2730	-26.9	-45.8	17.5	86.3	2.1	C30 H35 N8 O5
	587.2690	-22.9	-39.0	13.5	86.4	2.2	C25 H35 N10 O7

Figure 1.7.6: HRMS of **5**

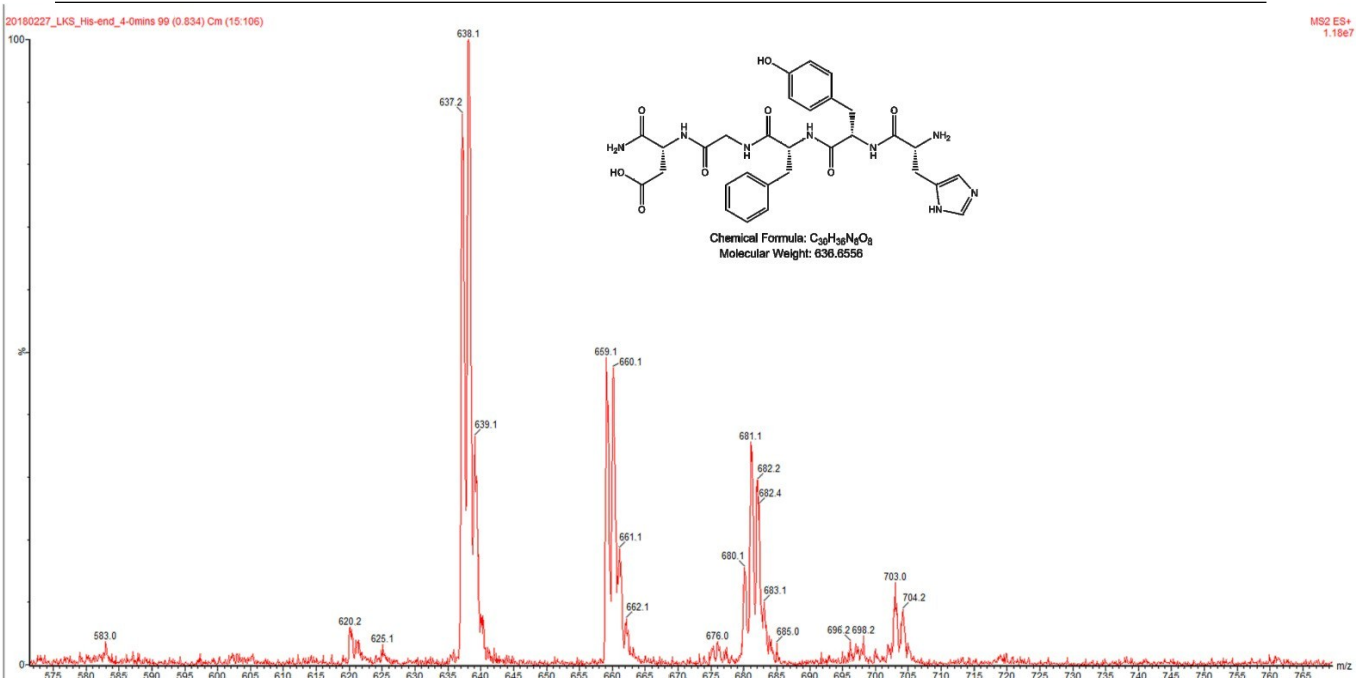


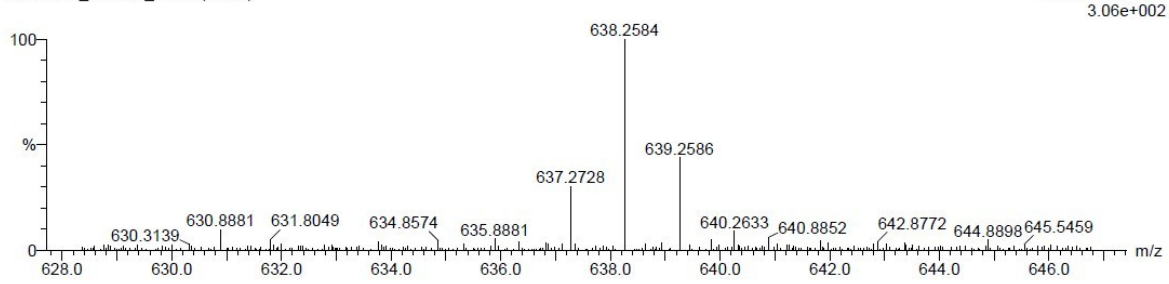
Figure 1.7.7: LRMS of **6**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
23 formula(e) evaluated with 7 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 30-35 H: 30-40 N: 5-10 O: 5-10
20180320_Lawson_4 230 (4.044)

1: TOF MS ES+
3.06e+002



Minimum:				0.0			
Maximum:		5.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
637.2728	637.2635	9.3	14.6	21.5	95.1	1.7	C32 H33 N10 O5
	637.2523	20.5	32.2	21.5	95.1	1.8	C33 H33 N8 O6
	637.2622	10.6	16.6	16.5	95.2	1.8	C31 H37 N6 O9
	637.2411	31.7	49.7	21.5	95.4	2.0	C34 H33 N6 O7
	637.2734	-0.6	-0.9	16.5	95.4	2.0	C30 H37 N8 O8
	637.2775	-4.7	-7.4	20.5	95.4	2.0	C35 H37 N6 O6
	637.2887	-15.9	-25.0	20.5	95.8	2.4	C34 H37 N8 O5

Figure 1.7.8: HRMS of **6**

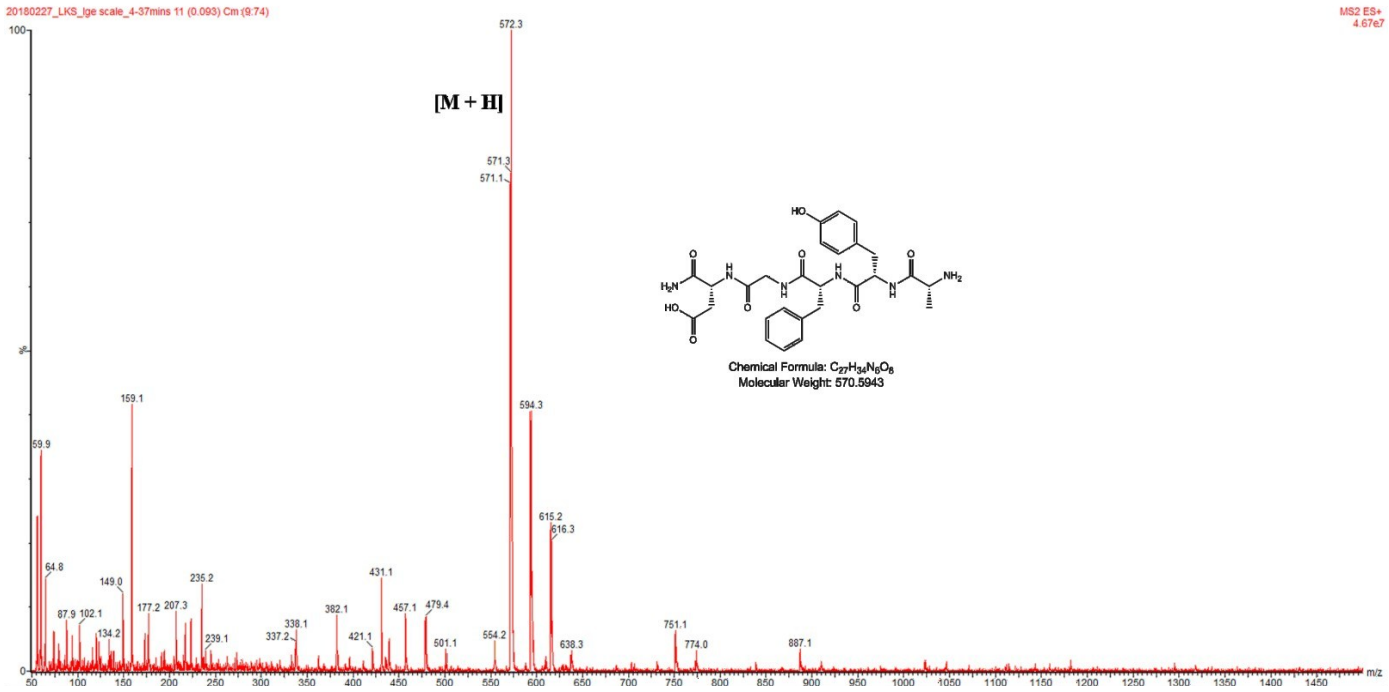


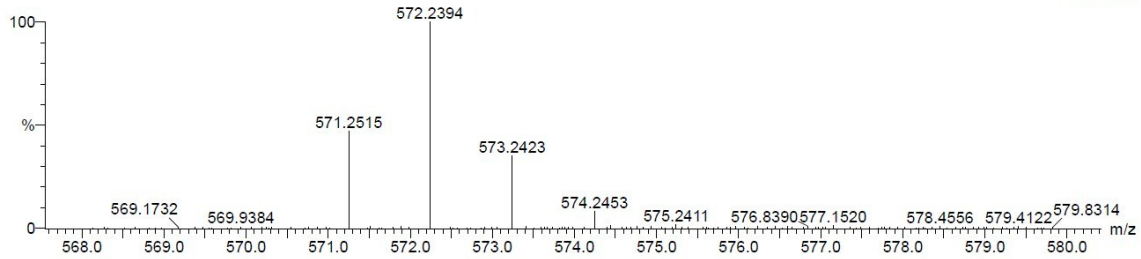
Figure 1.7.9: LRMS of **7**

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 50.0 PPM / DBE: min = 0.0, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
20 formula(e) evaluated with 6 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 25-30 H: 30-40 N: 5-10 O: 5-10

20180320_Lawson_6 120 (2.122) 1: TOF MS ES+ 1.36e+003



Minimum:				0.0			
Maximum:		5.0	50.0	50.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
571.2515	571.2417	9.8	17.2	18.5	89.8	1.7	C29 H31 N8 O5
	571.2305	21.0	36.8	18.5	89.9	1.7	C30 H31 N6 O6
	571.2516	-0.1	-0.2	13.5	90.0	1.8	C27 H35 N6 O8
	571.2265	25.0	43.8	14.5	90.0	1.8	C25 H31 N8 O8
	571.2629	-11.4	-20.0	13.5	90.0	1.8	C26 H35 N8 O7
	571.2741	-22.6	-39.6	13.5	90.2	2.0	C25 H35 N10 O6

Figure 1.7.10: HRMS of **7**

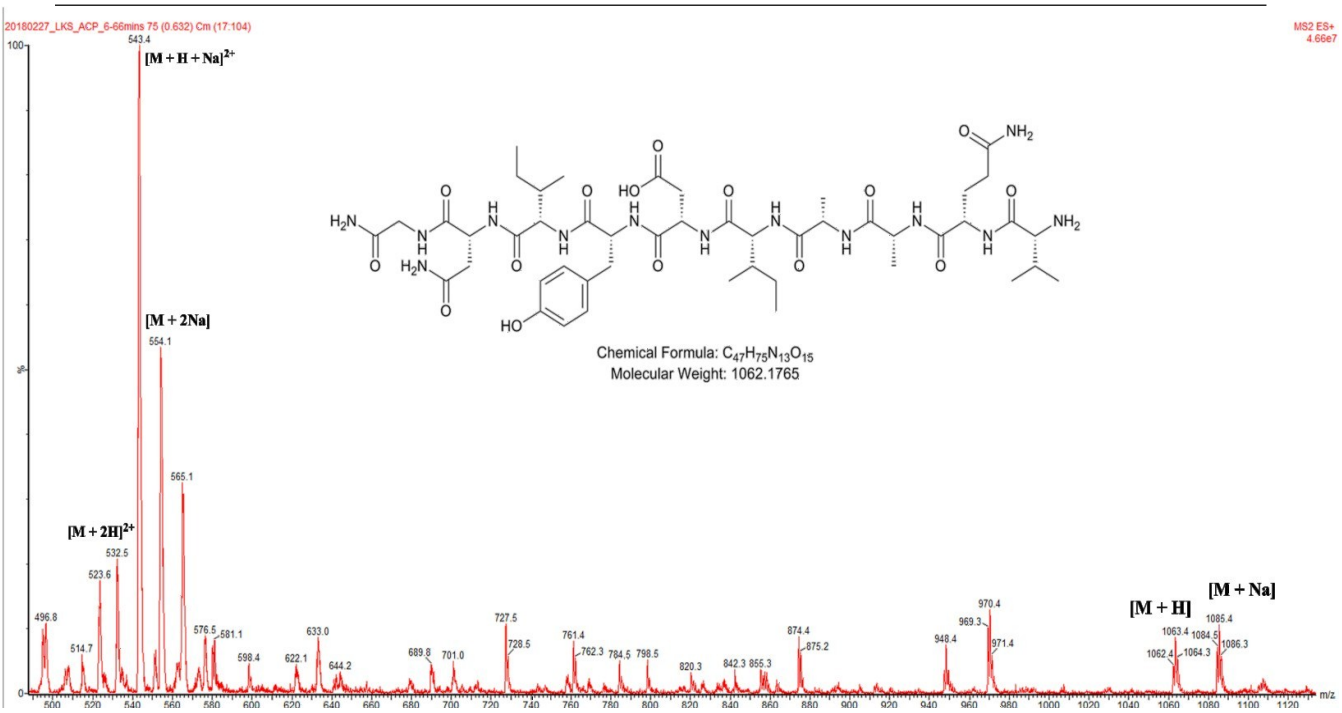
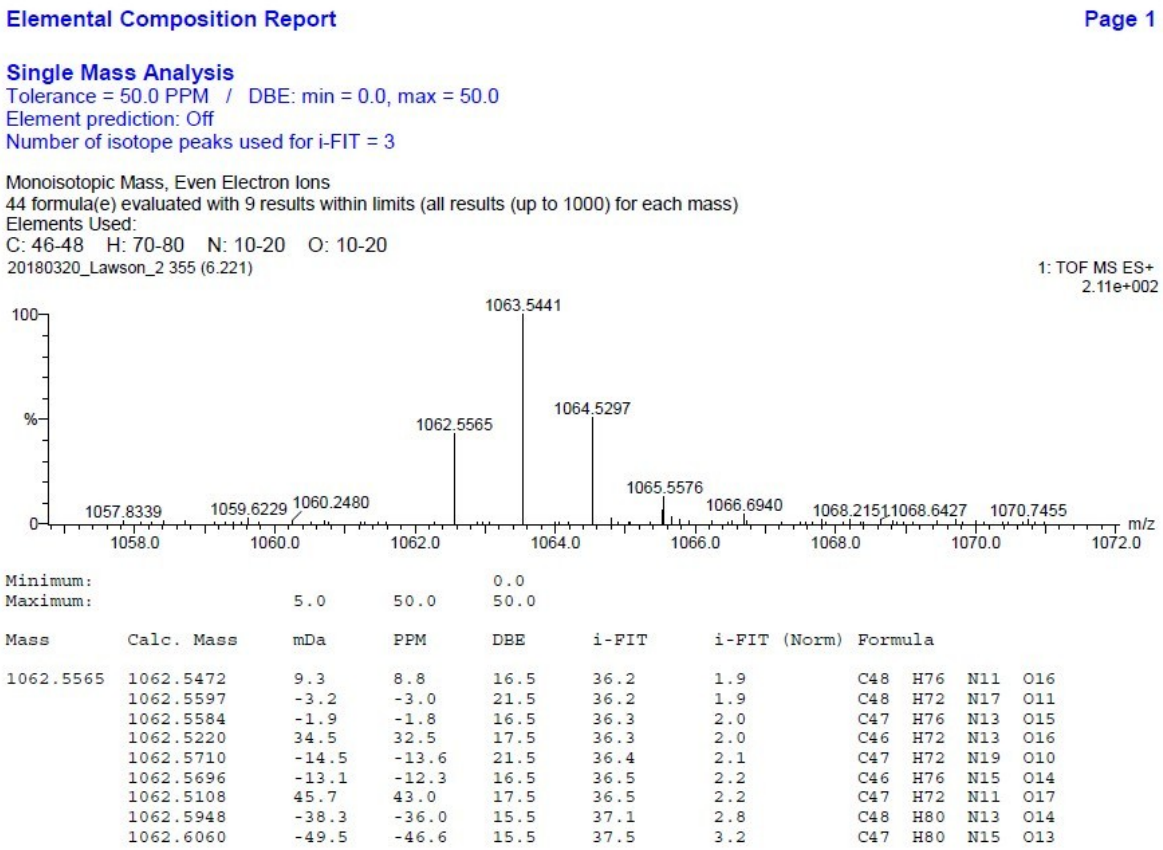


Figure 1.7.11: LRMS of **ACP$_{65-74}$**



Minimum:				0.0						
Maximum:		5.0	50.0	50.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
1062.5565	1062.5472	9.3	8.8	16.5	36.2	1.9	C48	H76	N11	O16
	1062.5597	-3.2	-3.0	21.5	36.2	1.9	C48	H72	N17	O11
	1062.5584	-1.9	-1.8	16.5	36.3	2.0	C47	H76	N13	O15
	1062.5220	34.5	32.5	17.5	36.3	2.0	C46	H72	N13	O16
	1062.5710	-14.5	-13.6	21.5	36.4	2.1	C47	H72	N19	O10
	1062.5696	-13.1	-12.3	16.5	36.5	2.2	C46	H76	N15	O14
	1062.5108	45.7	43.0	17.5	36.5	2.2	C47	H72	N11	O17
	1062.5948	-38.3	-36.0	15.5	37.1	2.8	C48	H80	N13	O14
	1062.6060	-49.5	-46.6	15.5	37.5	3.2	C47	H80	N15	O13

Figure 1.7.12: HRMS of **ACP$_{65-74}$**

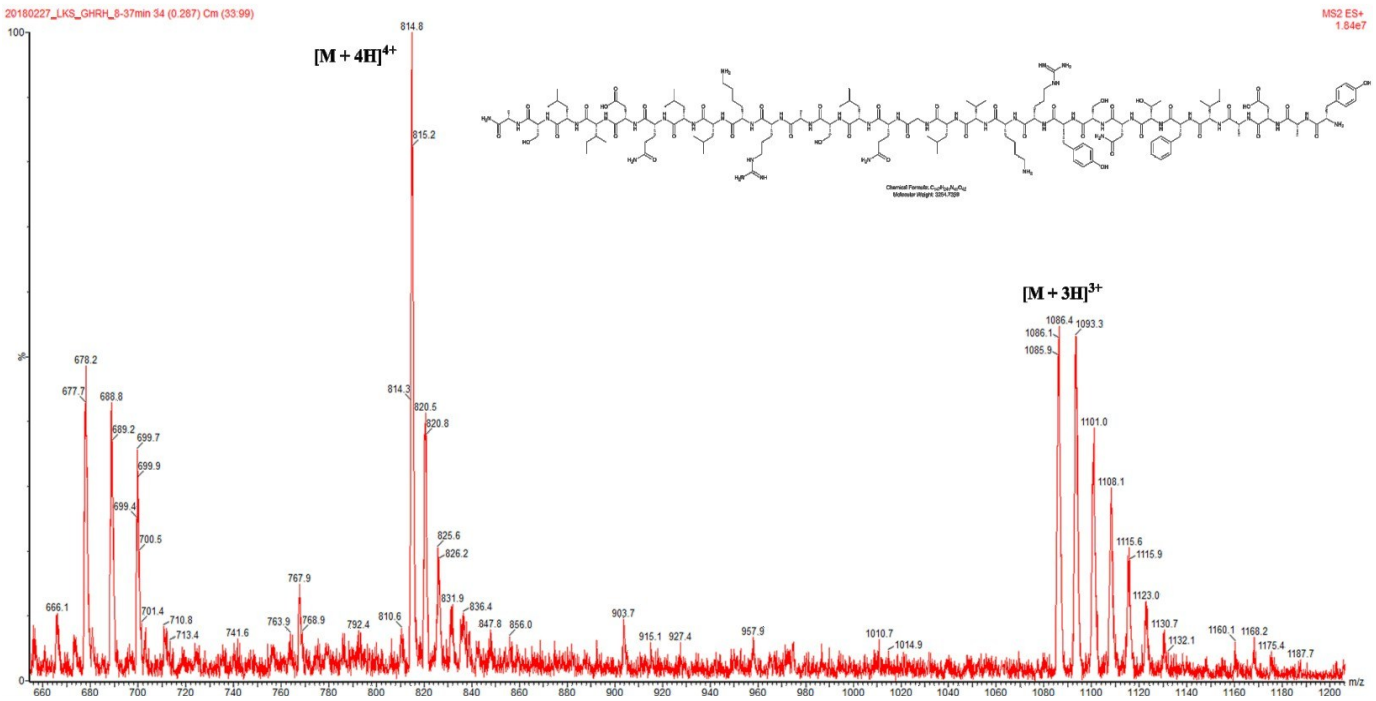


Figure 1.7.13: LRMS of **GHRH Analogue**

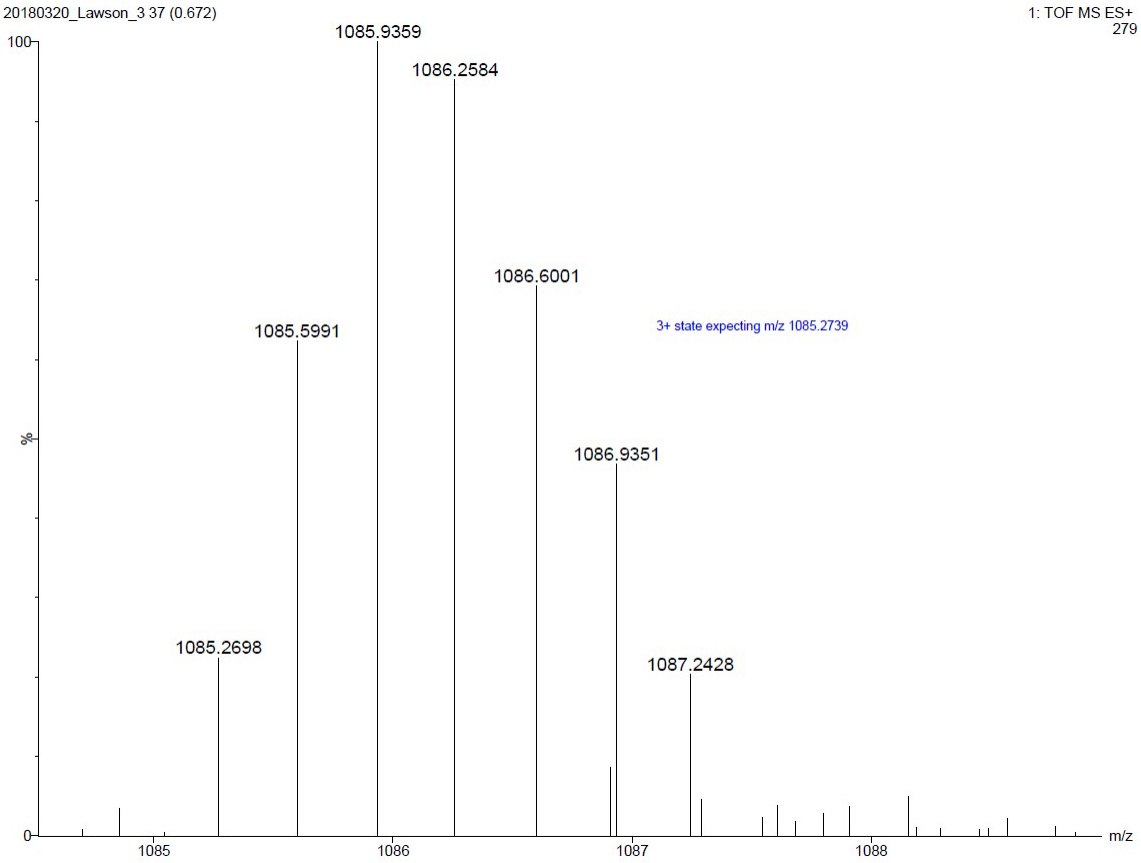


Figure 1.7.14: HRMS of **GHRH Analogue**

1.8 NMR Spectra of final products

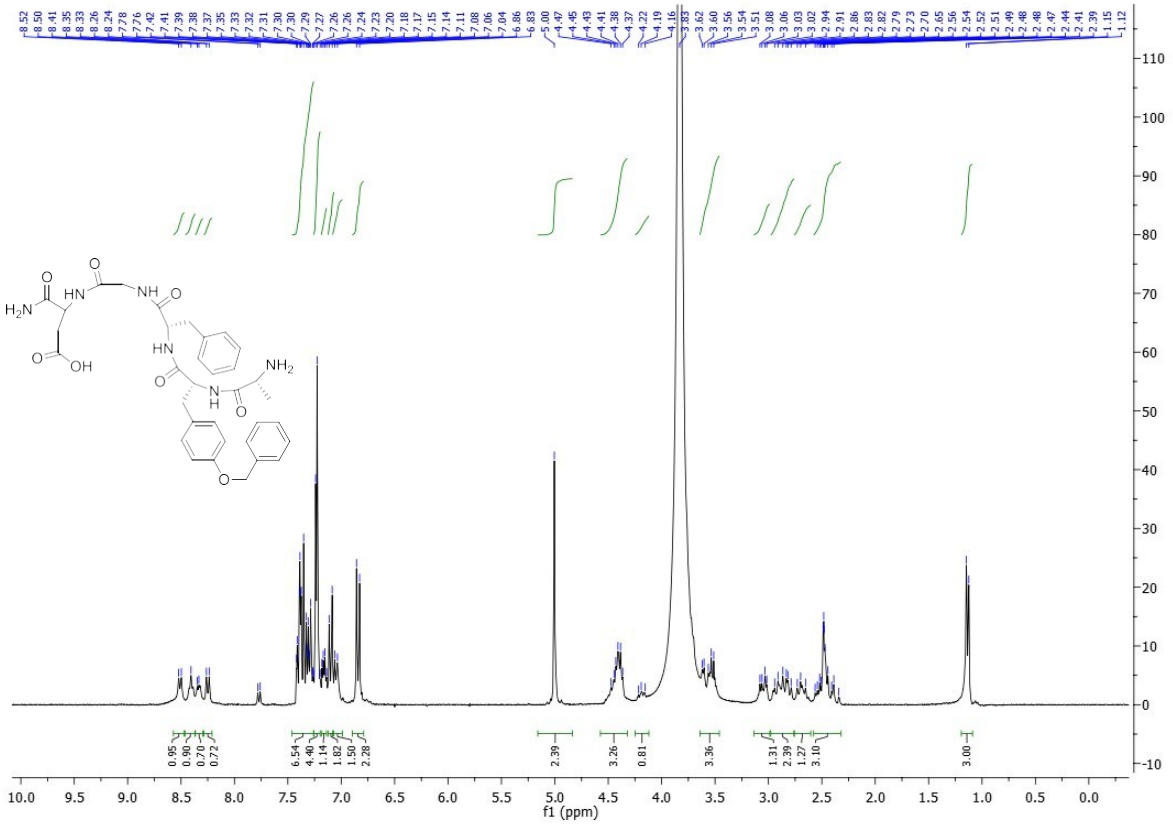


Figure 1.8.1: ^{1}H NMR spectra of **2**

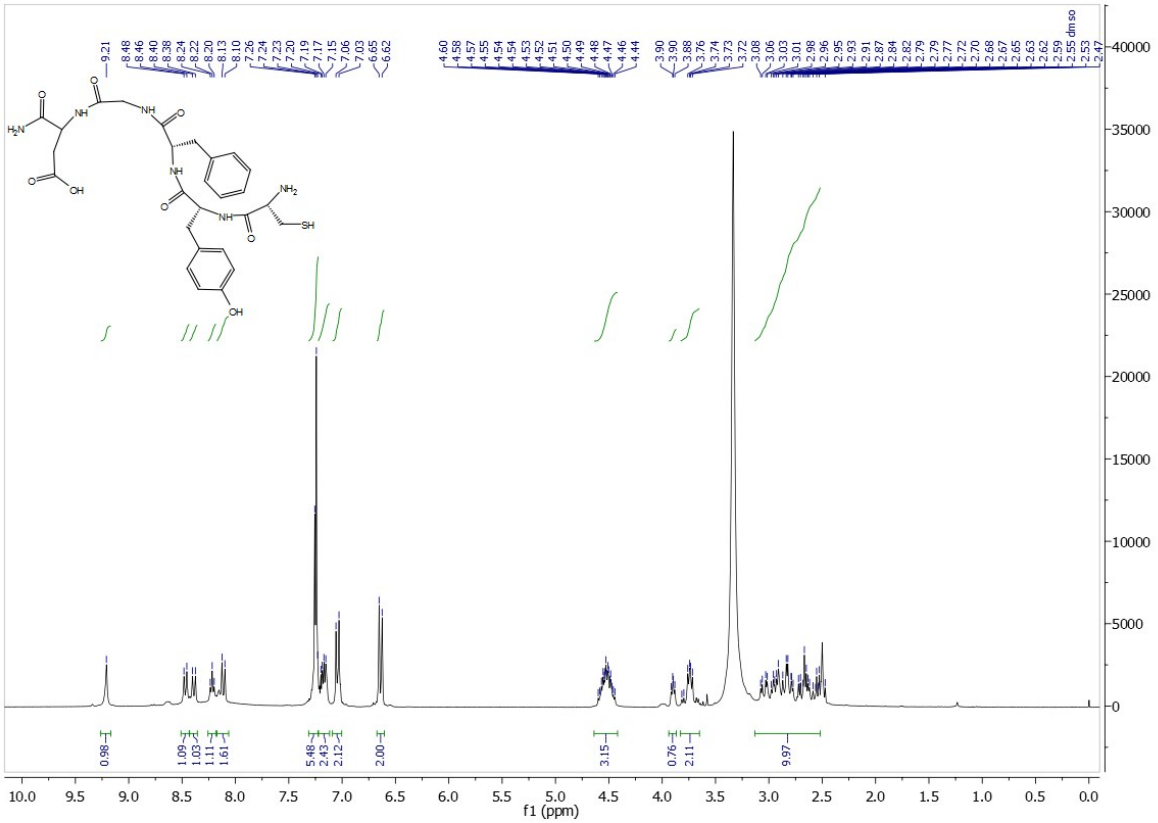


Figure 1.8.2: ^{1}H NMR spectra of **4**

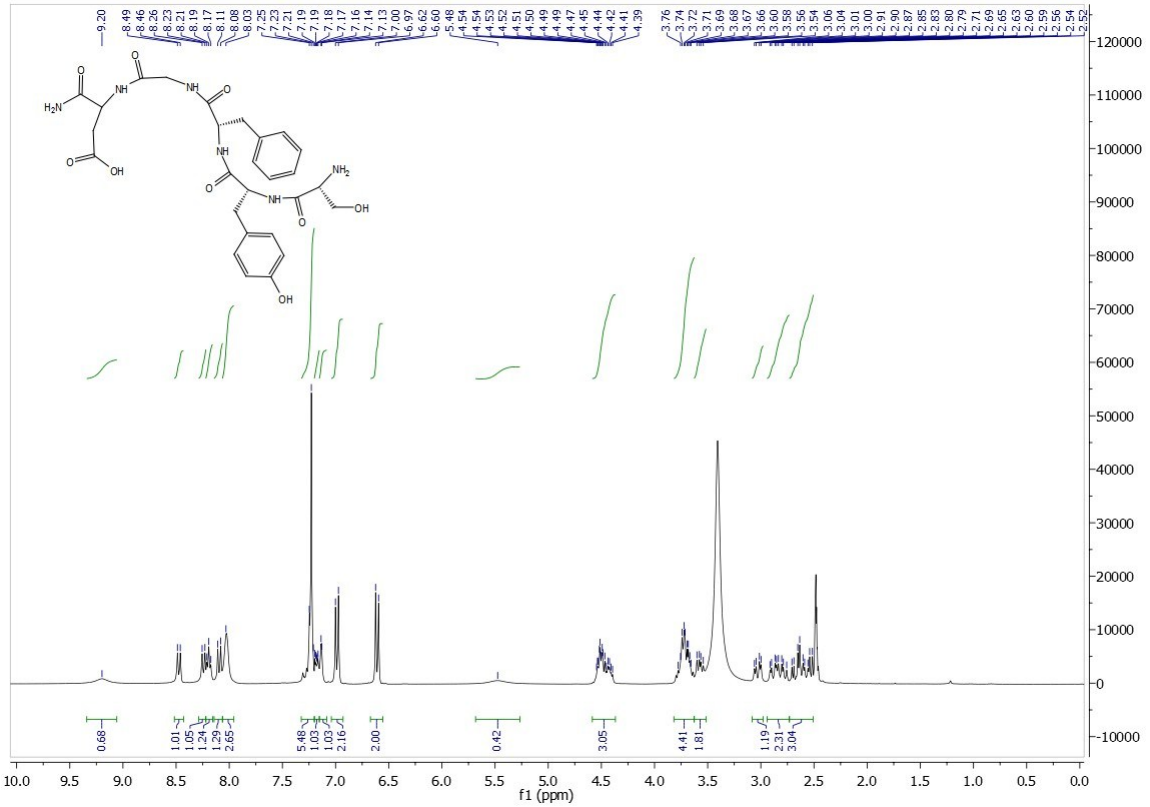


Figure 1.8.3: ^{1}H NMR spectra of **5**

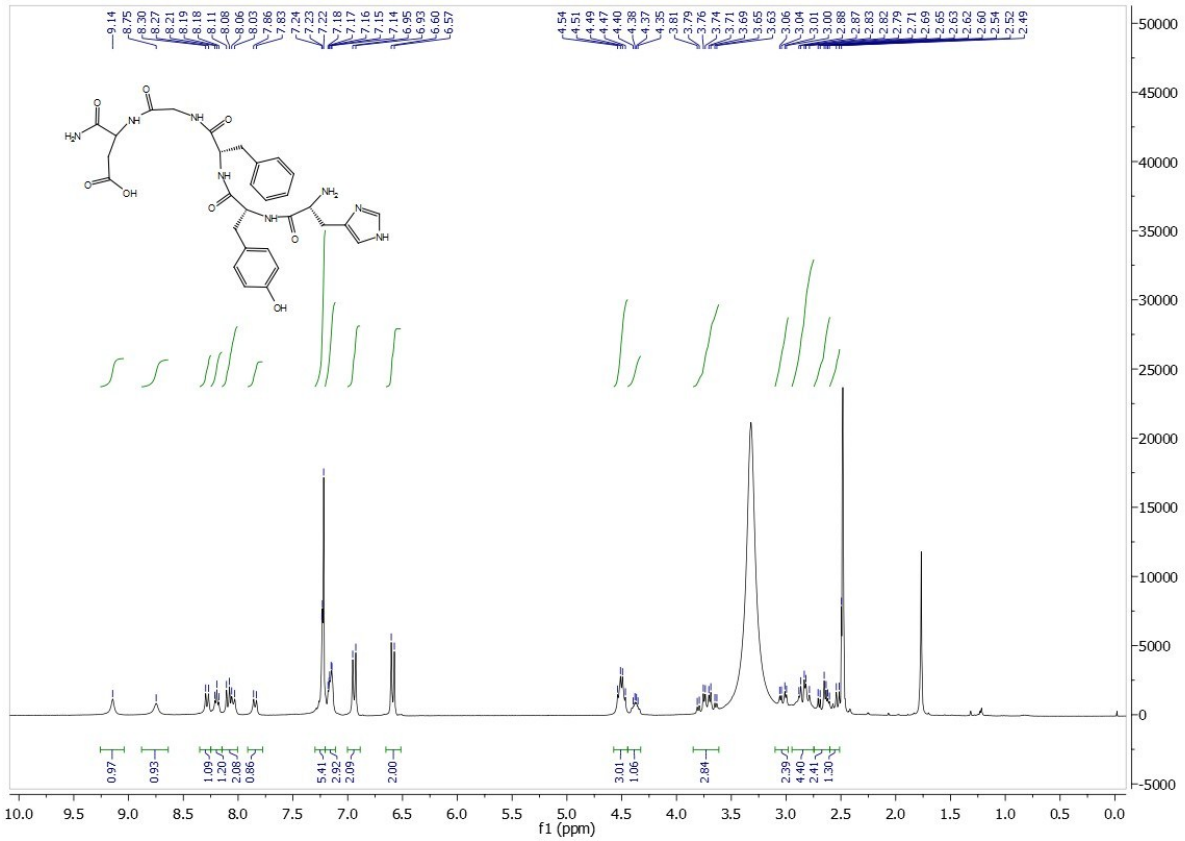


Figure 1.8.4: ^{1}H NMR spectra of **6**

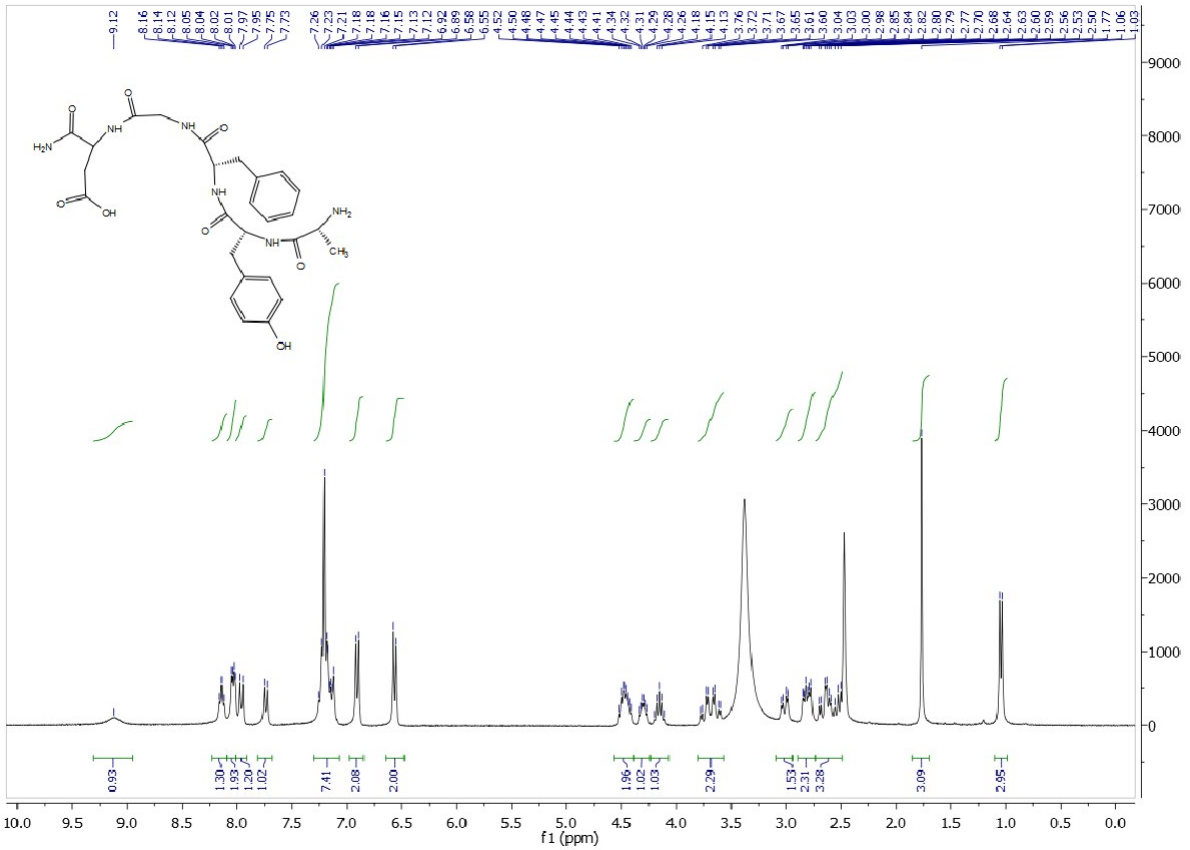


Figure 1.8.5: ^{1}H NMR spectra of **7**

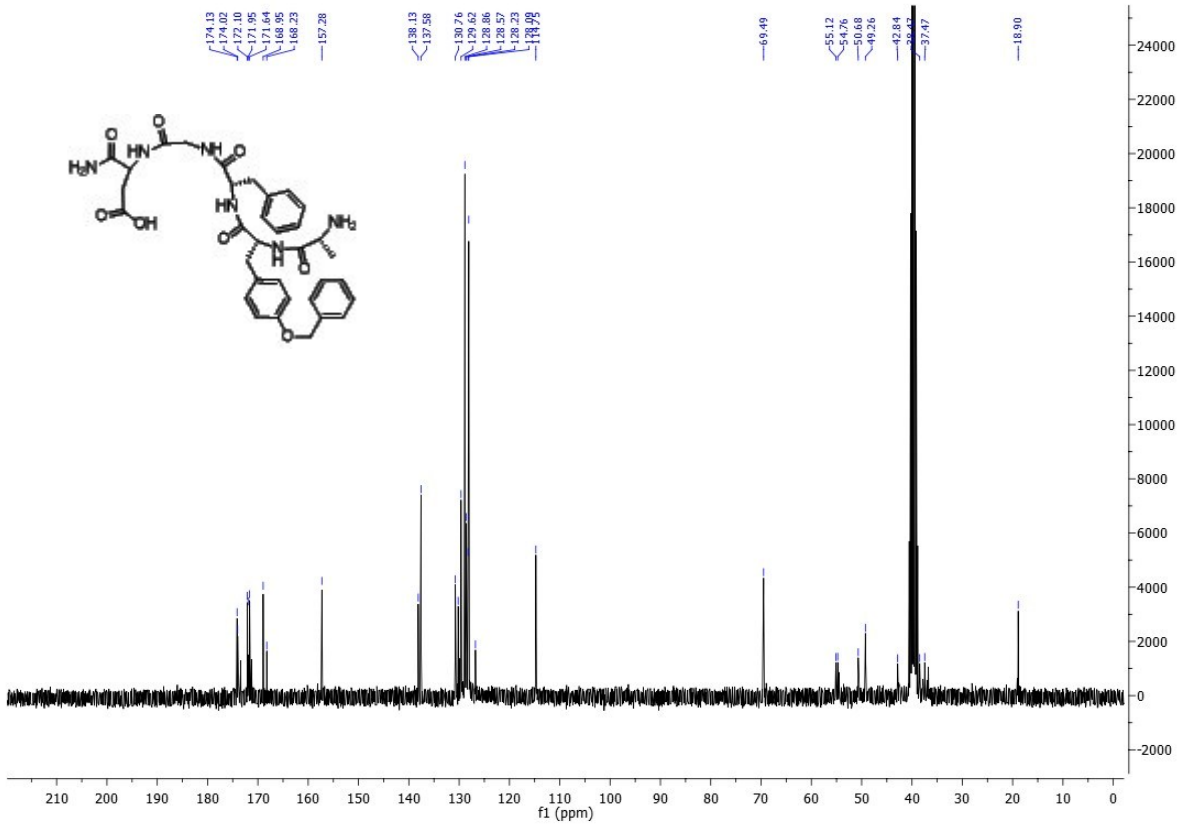


Figure 1.8.6: ^{13}C NMR spectra of **2**

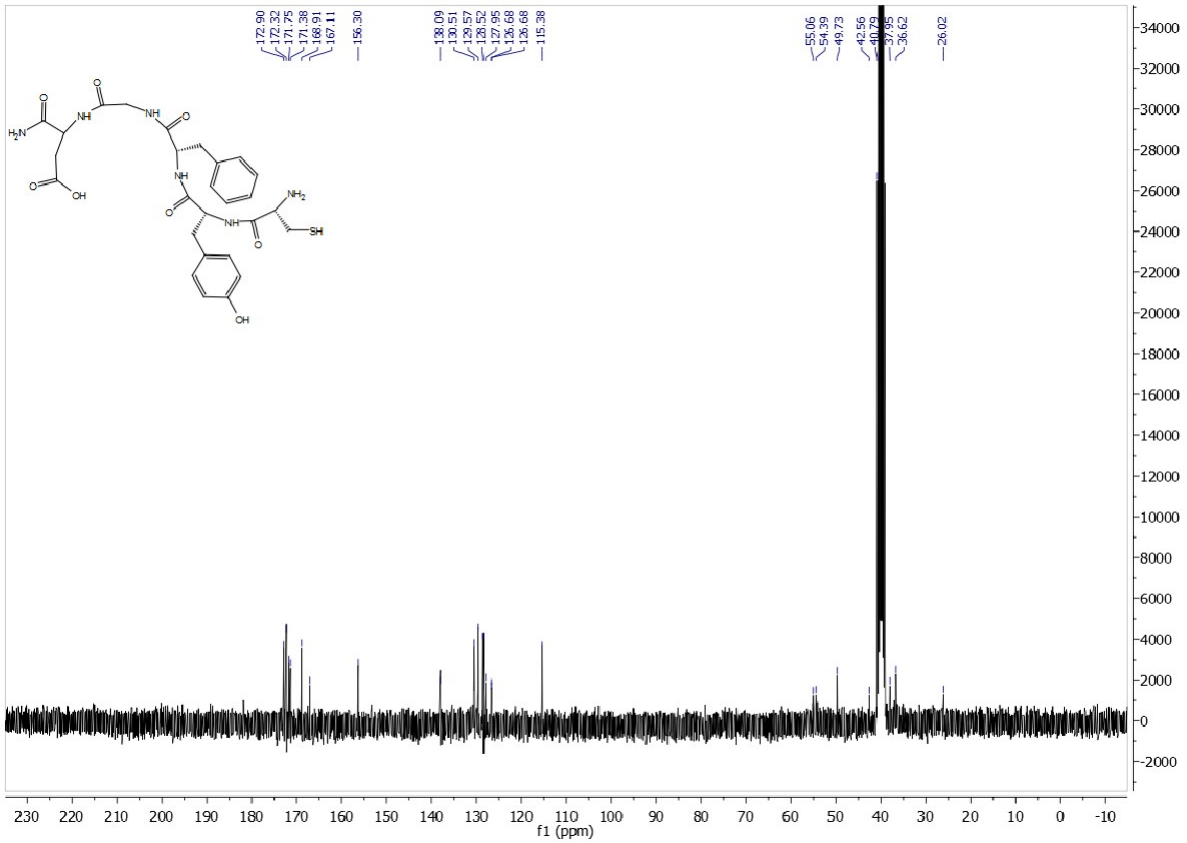


Figure 1.8.7: ^{13}C NMR spectra of **4**

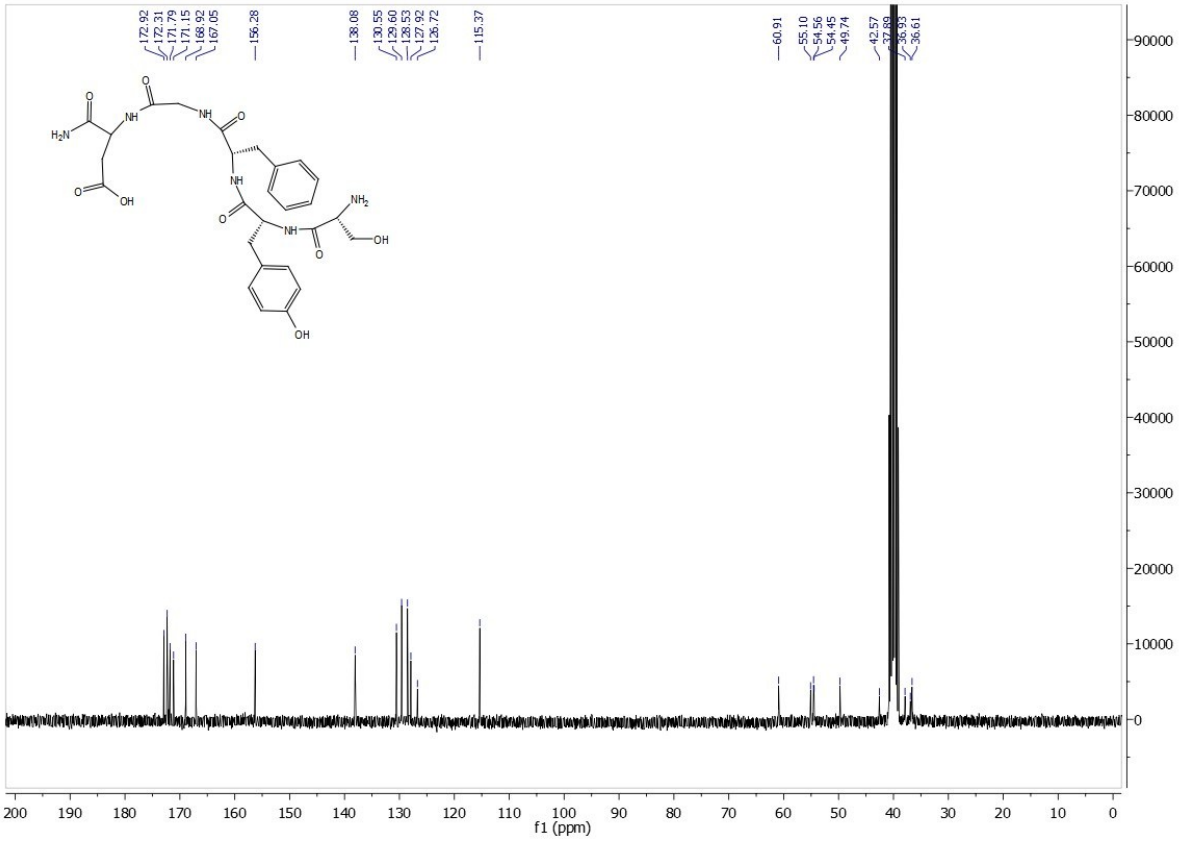


Figure 1.8.8: ^{13}C NMR spectra of **5**

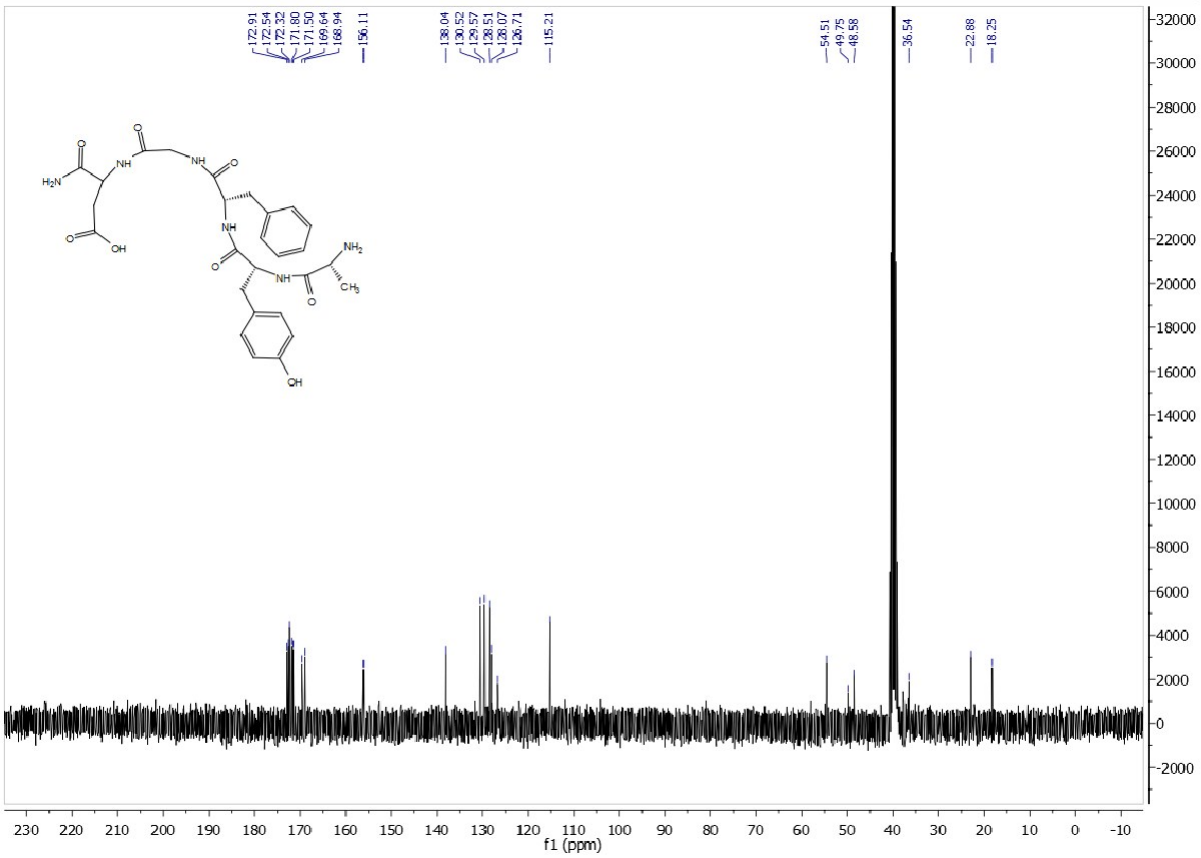


Figure 1.8.9: ^{13}C NMR spectra of **7**

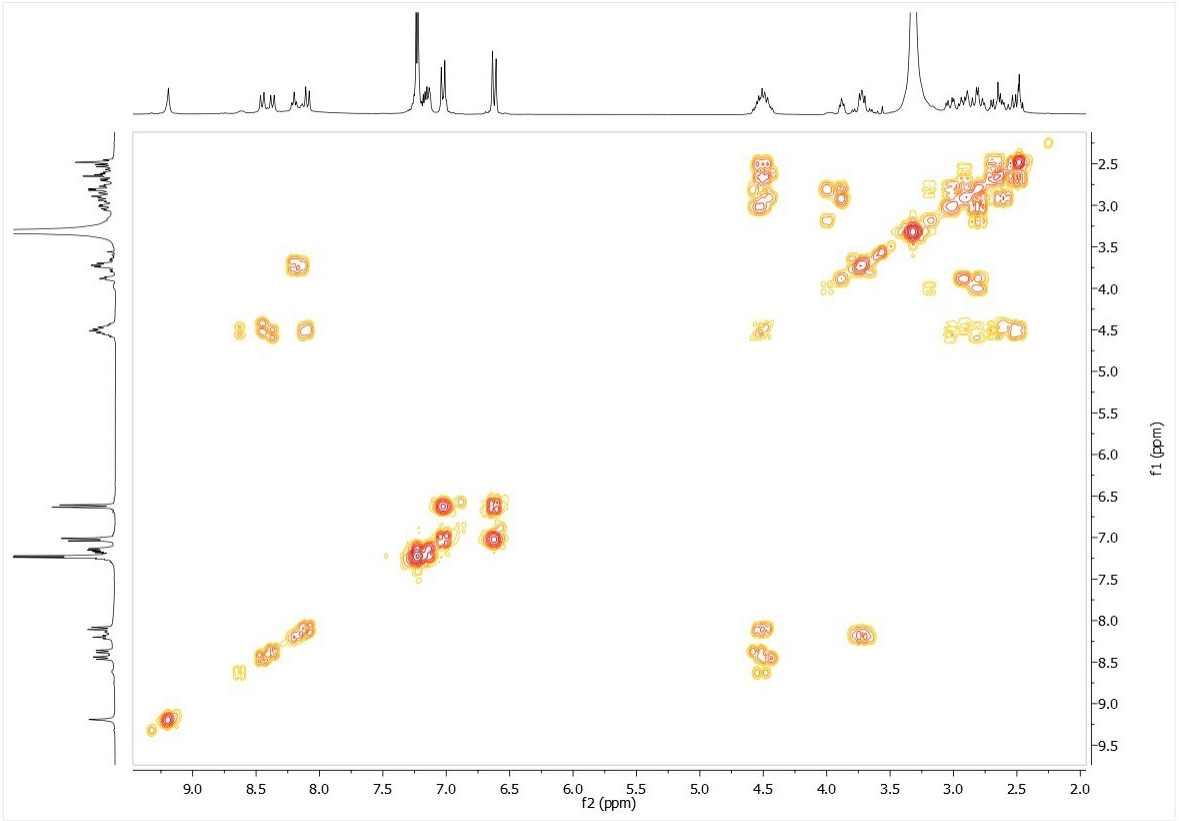


Figure 1.8.11: Cosy NMR spectra of **4**

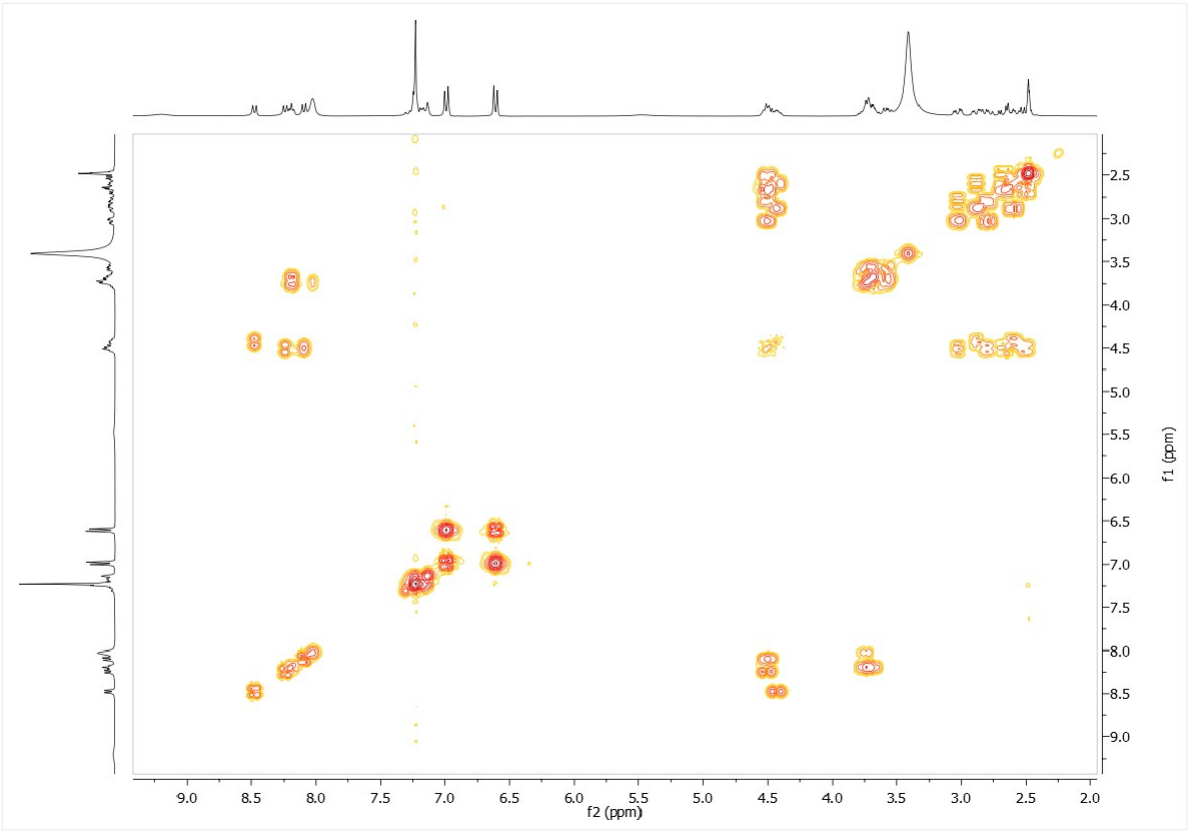


Figure 1.8.12: Cosy NMR spectra of **5**

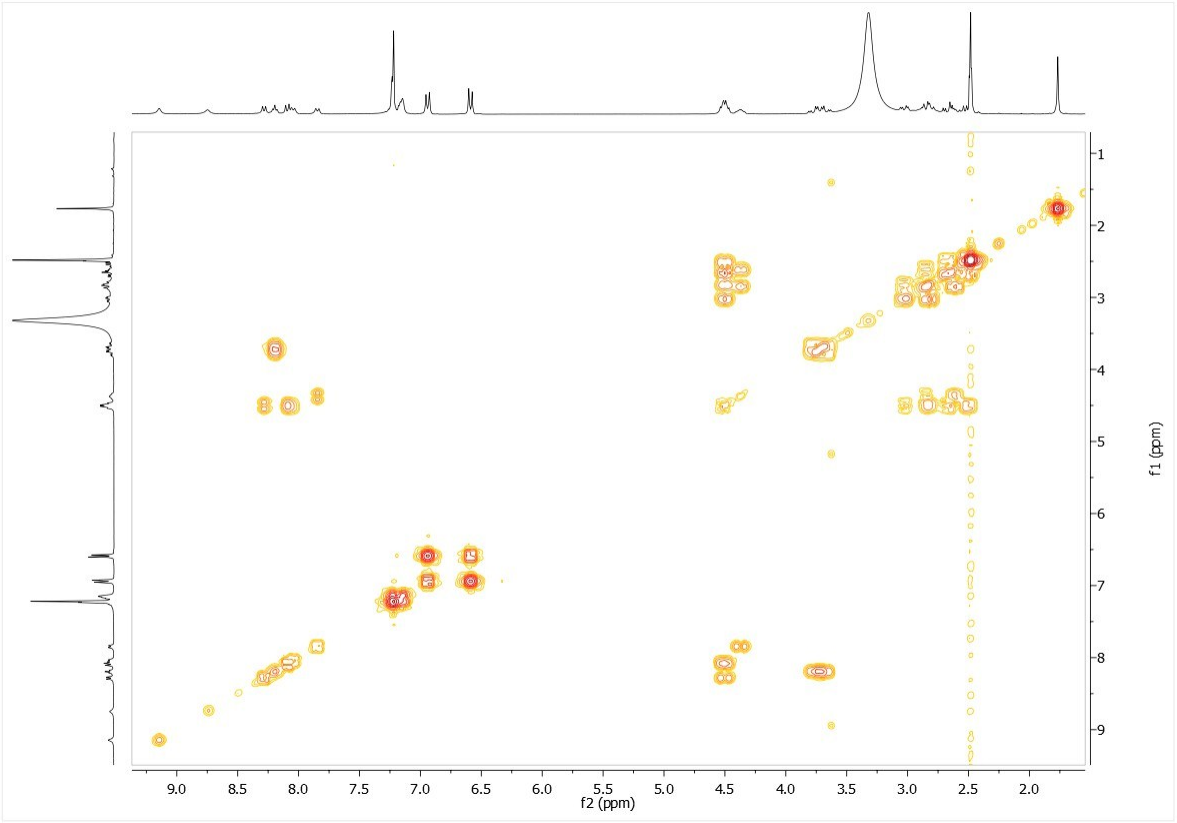


Figure 1.8.13: Cosy NMR spectra of **6**

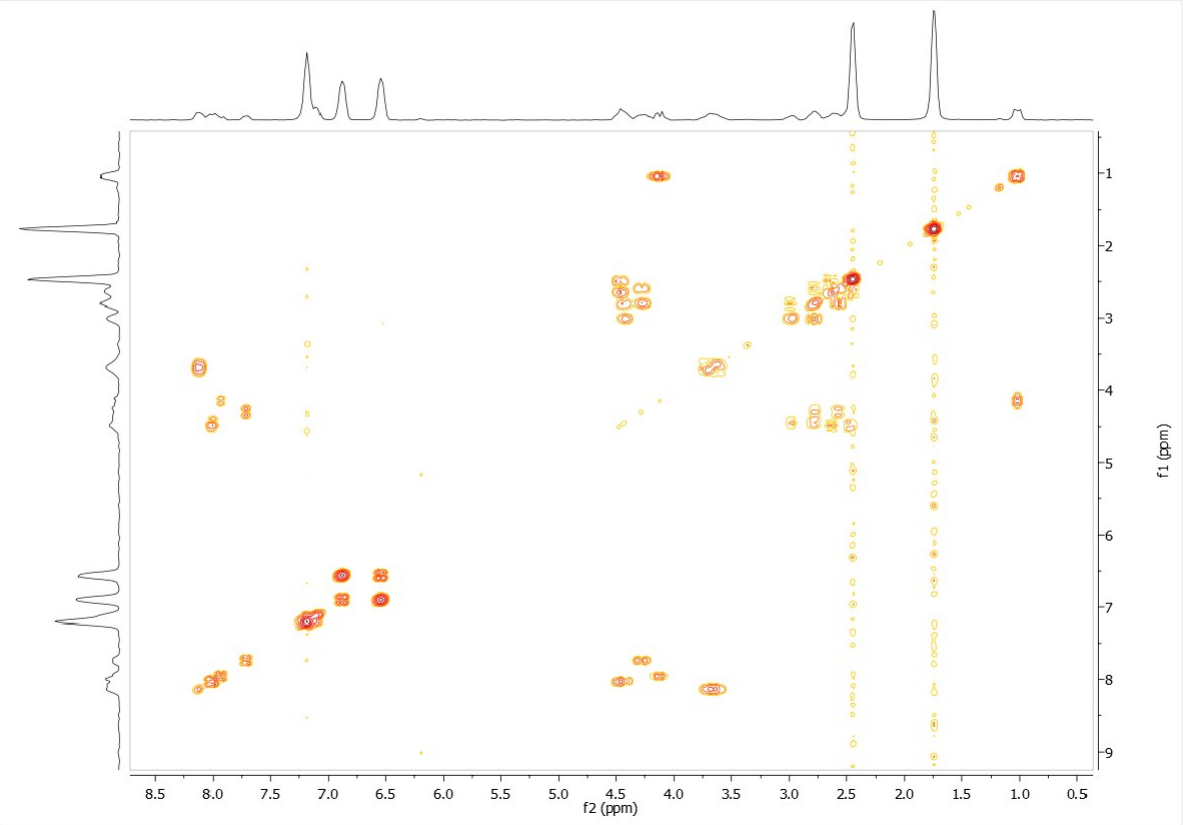


Figure 1.8.14: Cosy NMR spectra of **7**