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Supporting Information

De novo design of heat shock protein 90 inhibitors: direct inhibition of the C-terminus

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Supplementary Figure 1 (Figure S1)

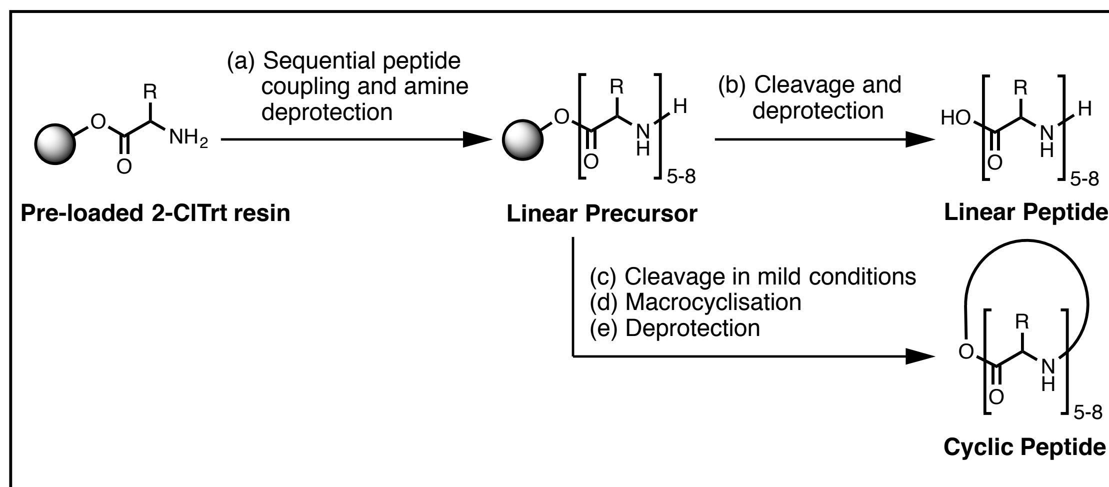


Figure S1. Overview of synthetic approach. Reaction conditions for each step are as follows: **(a)** Fmoc-protected amino acid (3 eq.), HOAt or HOBt (3 eq.), DIC (6 eq.) in DMF (0.3 M) followed by washing with 20% piperidine in DMF. **(b)** TFA (4 mL/g of resin with anisole (2 eq./ side chain protecting group). **(c)** TFE:CH_2Cl_2 (1:1, 10 mL/g resin). **(d)** HATU (1 eq.), TBTU (0.8 eq.), DMTMM (0.8 eq.), DIPEA (8 eq.) in CH_2Cl_2 (0.001 M). **(e)** TFA (4 mL/g of peptide with anisole (2 eq./side chain protecting group).

Supplementary Figure 2 (Figure S2)

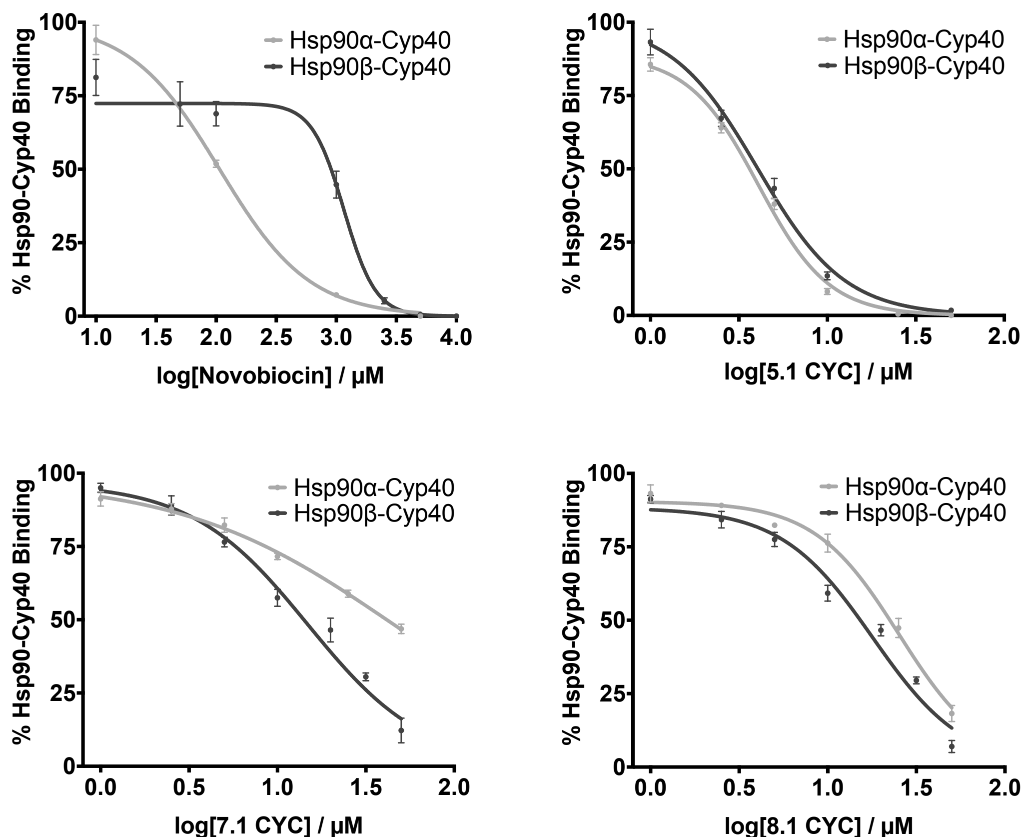


Figure S2. Impact of novobiocin, 5.1 CYC, 7.1 CYC and 8.1 CYC on binding of both α and β isoforms of Hsp90 with Cyp40. Graphs represent mean ± SEM, *n* = 3.

General Remarks

All chemicals were purchased from commercial suppliers (Chem-Impex International, Peptide International, GL-Biochem and Sigma Aldrich) and used without further purification. All moisture sensitive reactions were performed using anhydrous solvents under nitrogen gas. Removal of solvent was carried out under reduced pressure using a Buchi R-210 rotary evaporator.

Thin Layer Chromatography (TLC) was performed on aluminium silica gel sheets (Merck TLC silica gel 60 F254). Spots were visualised under ultraviolet light (λ = 254 nm) and developed by heating with ninhydrin solution.

LC/MS analyses were performed using a Waters Symmetry® C18 column (3.5 µm, 4.65 x 75 mm) on a Shimadzu Prominence High Performance LCMS 2010EV system connected to a Shimadzu LCMS 2010EV mass spectrometer. The mobile phase consisted of milli-Q water with 0.1% (v/v) formic acid (Mobile Phase A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (Mobile Phase B) at a flow rate of 0.5 mL/min, starting at 95% Mobile Phase A and 5% Mobile Phase B.

Semi-preparative HPLC for purification was performed using a GRACE VisionHT C18 column (5 µm, 22 x 150 mm) on a Shimadzu Prominence High Performance LCMS 2010EV system. The mobile phase consisted of milli-Q water with 0.1% (v/v) formic acid (Mobile Phase A), and HPLC grade acetonitrile with 0.1% (v/v) formic acid (Mobile Phase B) at a flow rate of 5 mL/min, starting at 95% Mobile Phase A and 5% Mobile Phase B.

^{1}H and ^{13}C NMR spectra were obtained on Bruker Avance III 600 MHz. All samples were dissolved in deuterium oxide (D_2O). Linear peptide spectra were obtained at 318 K (45 °C) while cyclic peptide spectra were obtained at 308 K (35 °C). Multiplicity of NMR signals were represented by the following abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad, dd = doublet of doublet.

High-resolution mass spectrometry (HRMS) analyses were recorded on a Thermo LTQ Orbitrap XL ESI/APCI with UPLC system at the Bioanalytical Mass Spectrometry Facility in Mark Wainwright Analytical Centre at the University of New South Wales.

General Procedures for Synthesis of Linear Peptides

Solid-Phase Peptide Synthesis
Stepwise SPPS was performed in a polypropylene solid-phase extraction cartridge fitted with a 20 µm polyethylene frit purchased from Applied Separations (Allentown, PA) using pre-loaded 2-ClTrt resins with loading scales between 0.2-0.9 mmol/g. The resin was weighed, transferred to the cartridge and swelled in DMF for 30 minutes prior to the first coupling reaction.

Coupling Reaction
Couplings were performed in DMF at a concentration of 0.3 M. Fmoc-protected amino acid (3 eq.) and HOBt (3 eq.) were mixed with the resin. DIC (6 eq.) was then added to activate the reaction. Coupling was allowed to proceed for a minimum of 4 hours while shaking (Labquake tube shaker, Thermo Fisher Scientific) at room temperature. Reaction was monitored using thin layer chromatography (TLC) with a ninhydrin test. Upon completion, the reaction solution was drained and the resin was subjected to *Fmoc Removal*. (Note: For particularly hindered coupling reactions, HOBt was replaced with HOAt and the reaction was allowed to proceed overnight.)

Fmoc Removal
After the peptide coupling reaction was complete, the Fmoc protecting group was removed using the following washes: DMF (3 x 1 min), 20% piperidine in DMF (1 x 5 min), 20% piperidine in DMF (1 x 10 min), DMF (2 x 1 min), IPA (1 x 1 min), DMF (1 x 1 min), IPA (1 x 1 min) and DMF (3 x 1 min). The resin was then ready for the next coupling reaction.

Cleavage
Once the desired peptide was generated, the final Fmoc protecting group was removed following *Fmoc Removal* procedure with the following additional washes: DMF (3 x 1 min), IPA (3 x 1 min) and MeOH (3 x 1 min). The resin-bound peptide was then dried *in vacuo* overnight. The resin was then cleaved from the linear peptide using TFE and CH_2Cl_2 (1:1 v/v) at a concentration of 10 mL/g resin. The reaction was allowed to stir at room temperature for 48 hours. The suspension was then filtered through a Büchner funnel and the resin was washed with additional CH_2Cl_2 to fully extract the linear peptide. The filtrate was then evaporated and the dried solid was redissolved in CH_2Cl_2 and evaporated multiple times to remove residual entrapped TFE. The linear peptide was then dried *in vacuo* overnight.

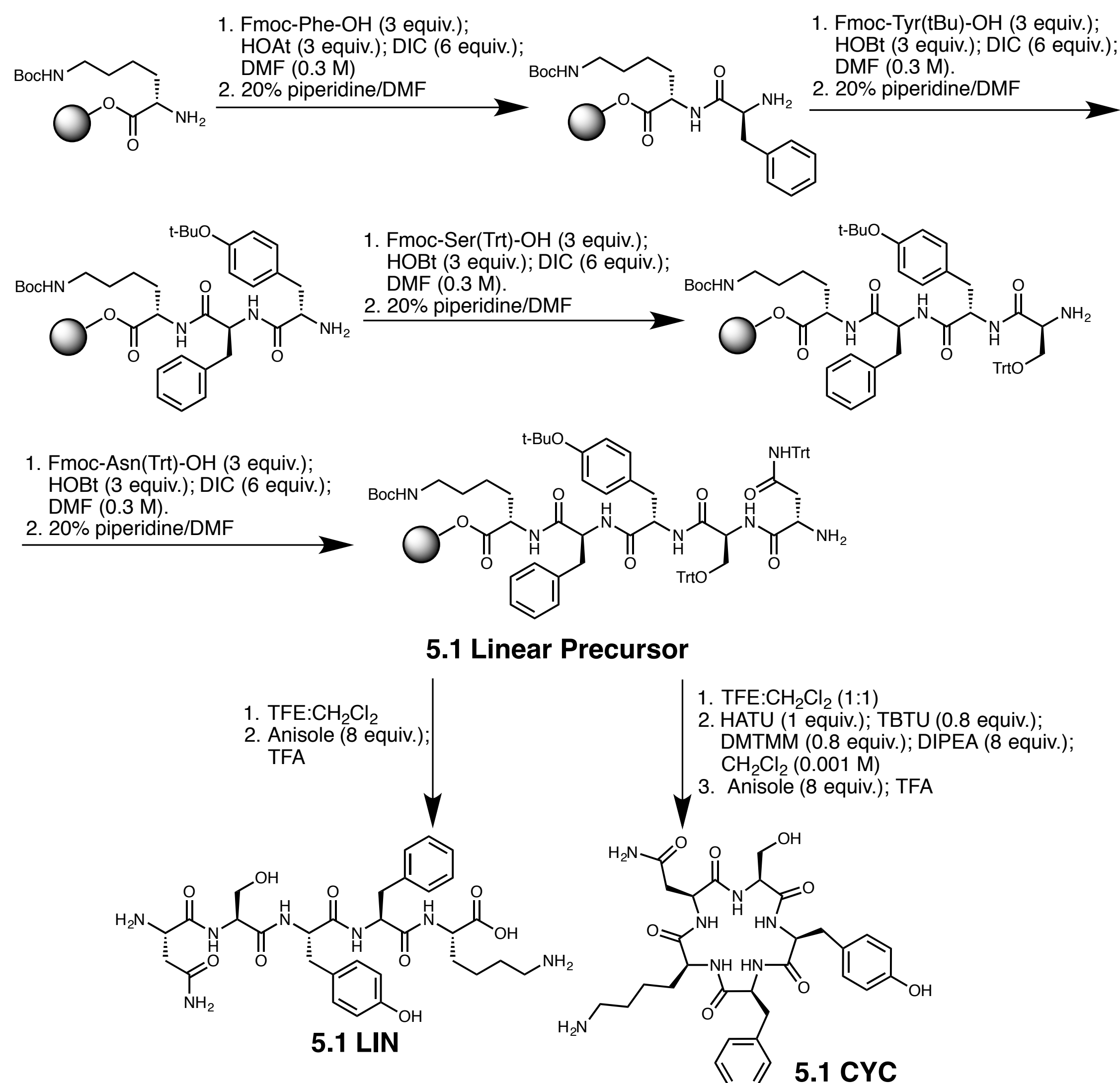
General Procedure for Synthesis of Cyclic Peptides

Macrocyclisation of the linear peptide was achieved using a cocktail of 3 coupling reagents: HATU (1 eq.), TBTU (0.5 eq.) and DMTMM (0.5 eq.). The reaction was performed in dilute conditions using anhydrous solvents at concentration of 0.001 M. The linear peptide and coupling reagents were dissolved separately in CH_2Cl_2, where 20% of the final volume was used to dissolve the linear peptide and the other 80% dissolved the coupling reagents. DIPEA (4 eq.) was added to each solution. The linear peptide solution was then added drop-wise to the coupling reagents solution *via* a syringe pump over approximately 2 hours. The reaction was stirred overnight and monitored using LC/MS. (Note: if the reaction failed to reach completion after stirring overnight, additional HATU (1 eq.) was added and the reaction was monitored using LC/MS.) Upon completion, the reaction mixture was evaporated and the dry solid was subjected to acid-base work-up with saturated sodium bicarbonate ($NaHCO_3$) and milli-Q water acidified to pH 3 using ammonium chloride (NH_4Cl) and hydrochloric acid (HCl) to remove excess DIPEA. The organic layer was then dried over Na_2SO_4, filtered and evaporated under reduced pressure before the compound was dried *in vacuo* overnight.

General Procedure for Removal of Side Chain Protecting Groups

Amino acid side chain protecting groups were removed using neat TFA and anisole. 2 equivalents of anisole was added per protecting group to be removed. Anisole was added to the linear or cyclic peptide, whilst stirring, followed by neat TFA at a concentration of 1 mL/250 mg compound. The reaction was left stirring at room temperature for 4 hours. The reaction was monitored using LC/MS and once complete the solvent was evaporated before redissolving in CH_2Cl_2 and evaporating multiple times to remove residual entrapped TFA. The peptide was then dried *in vacuo* overnight.

Synthesis of 5.1 LIN and 5.1 CYC



Experimental Procedures for 5.1 LIN

Resin-O-Lys(Boc)-Phe-NH_2
The resin-bound dipeptide Resin-O-Lys(Boc)-Phe-NH_2 was synthesised following the *Coupling Reaction* procedure using 1.0 g H-Lys(Boc)-2-ClTrt resin (0.50 mmol, 1 eq.), 0.58 g Fmoc-Phe-OH (1.5 mmol, 3 eq.), 0.21 g HOAt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run overnight and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-NH_2.

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-NH_2
The resin-bound tripeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Lys(Boc)-Phe-NH_2 synthesised from previous coupling reaction, together with 0.69 g Fmoc-Tyr(t-Bu)-OH (1.5 mmol, 3 eq.), 0.21 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-NH_2.

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2
The resin-bound tetrapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-NH_2 synthesised from previous coupling reaction, together with 0.86 g Fmoc-Ser(Trt)-OH (1.5 mmol, 3 eq.), 0.21 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2.

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2
The resin-bound pentapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2 synthesised from previous coupling reaction, together with 0.92 g Fmoc-Asn(Trt)-OH (1.5 mmol, 3 eq.), 0.21 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2.
The resin-bound pentapeptide was then divided into 2 equal portions of 1.3 g, where one part was reserved for the synthesis of the 6.1 compound series and the other was taken forward to complete the synthesis of the 5.1 compound series.

HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2
The linear pentapeptide HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 3.6 mL of TFE and 3.6 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 as a white solid (286 mg, overall 87%).

HO-Lys-Phe-Tyr-Ser-Asn-NH_2
The free linear peptide HO-Lys-Phe-Tyr-Ser-Asn-NH_2 was generated by removing the side chain protecting groups on HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2. 30 mg HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 (0.02 mmol, 1 eq.) was deprotected using a mixture of 120 µL of TFA and anisole (8 eq.). The free linear peptide then underwent HPLC purification to generate pure final compound **5.1 LIN** in a 29% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+2H]^{2+}$ calcd for $C_{31}H_{43}N_7O_9$, 329.66; found, 329.50.
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{31}H_{43}N_7O_9$, 658.3100; found, 658.3196.
^{1}H NMR (600 MHz, D_2O) δ 7.53-7.33 (m, 5H, Phe), 7.21-7.15 (d, *J* = 8.58 Hz, 2H, δH Tyr), 6.96-6.91 (d, *J* = 8.58 Hz, 2H, εH Tyr), 4.59-4.55 (t, *J* = 5.76 Hz, 1H, αH Ser), 4.36-4.29 (m, 1H, αH Asn), 4.27-4.22 (t, *J* = 5.58 Hz, 1H, αH Lys), 3.92-3.82 (d, *J* = 5.76 Hz, 2H, βCH_2 Ser), 3.32-3.28 & 3.15-3.06 (m, 2H, βCH_2 Phe), 3.15-3.06 (m, 2H, εCH_2 Lys), 3.15-3.06 & 3.05-2.89 (m, 2H, βCH_2 Tyr), 3.05-2.89 (m, 2H, βCH_2 Asn), 1.97-1.88 (m, 2H, βCH_2 Lys), 1.86-1.77 (m, 2H, δCH_2 Lys), 1.52-1.45 (m, 2H, γCH_2 Lys).

Experimental Procedures for 5.1 CYC

Resin-O-Phe-Tyr(t-Bu)-NH_2
The resin-bound dipeptide Resin-O-Phe-NH_2 was synthesised following the *Coupling Reaction* procedure using 0.50 g H-Phe-2-ClTrt resin (0.29 mmol, 1 eq.), 0.39 g Fmoc-Tyr(t-Bu)-OH (0.86 mmol, 3 eq.), 0.12 g HOBt (0.86 mmol, 3 eq.), 0.26 mL DIC (1.71 mmol, 6 eq.) and 2.9 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O- Phe-Tyr(t-Bu)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Phe-Tyr(t-Bu)-NH_2.

Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2
The resin-bound tripeptide Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Phe-Tyr(t-Bu)-NH_2 synthesised from previous coupling reaction, together with 0.49 g Fmoc-Ser(Trt)-OH (0.89 mmol, 3 eq.), 0.12 g HOAt (0.89 mmol, 3 eq.), 0.26 mL DIC (3.0 mmol, 6 eq.) and 2.9 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run overnight and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2.

Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2
The resin-bound tetrapeptide Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-NH_2 synthesised from previous coupling reaction, together with 0.53 g Fmoc-Asn(Trt)-OH (0.89 mmol, 3 eq.), 0.12 g HOAt (0.89 mmol, 3 eq.), 0.26 mL DIC (3.0 mmol, 6 eq.) and 2.9 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run overnight and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2.

Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2
The resin-bound pentapeptide Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 synthesised from previous coupling reaction, together with 0.40 g Fmoc-Lys(Boc)-OH (0.89 mmol, 3 eq.), 0.12 g HOAt (0.89 mmol, 3 eq.), 0.26 mL DIC (3.0 mmol, 6 eq.) and 2.9 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run overnight and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2.

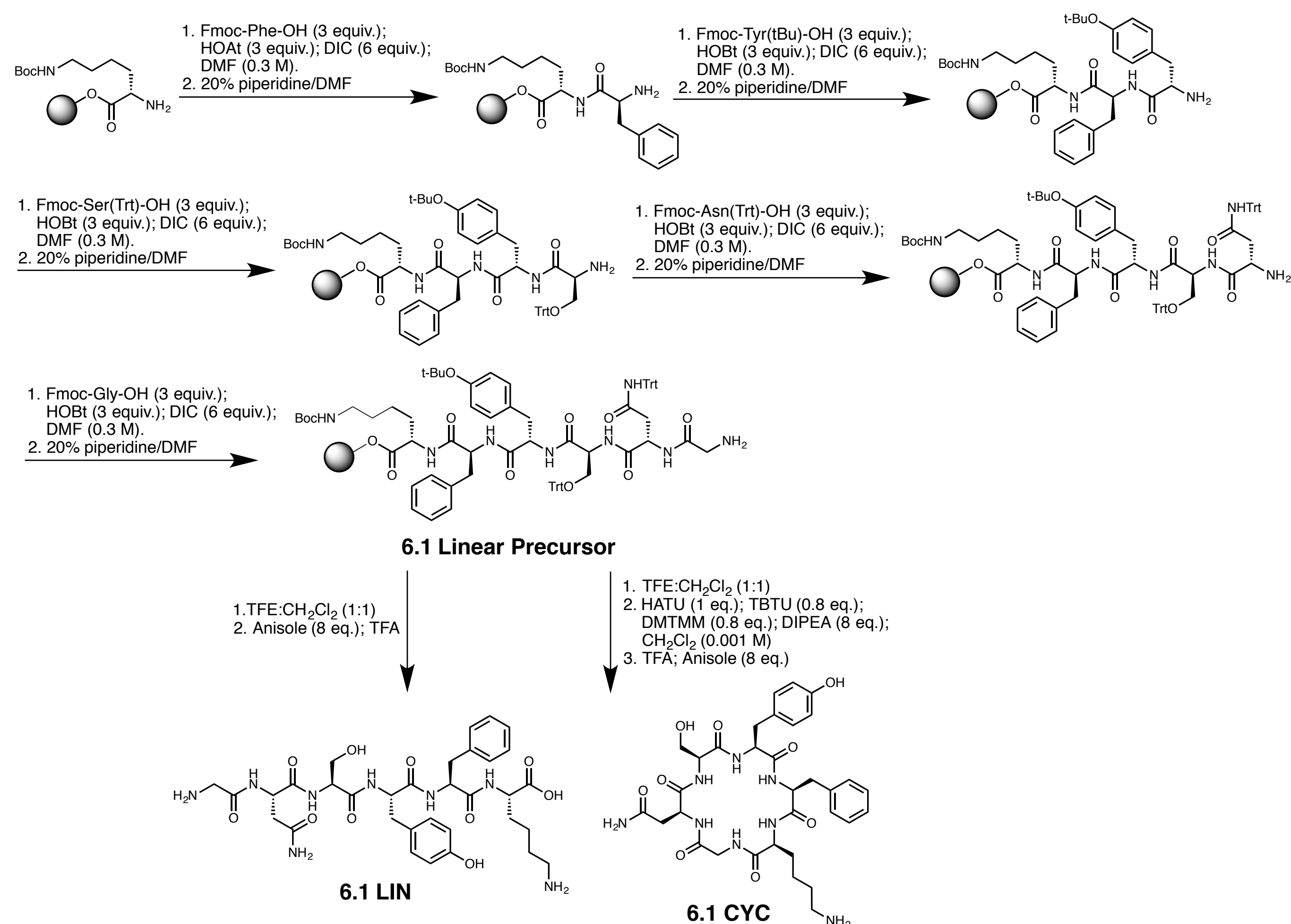
HO-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2
The linear pentapeptide HO-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 3.5 mL of TFE and 3.5 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2 as a white solid (267 mg, overall 72%).

***cyclo*-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)**
cyclo-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc) was synthesised using 0.16 g HO-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc)-NH_2 (0.12 mmol, 1 eq.), 0.046 g HATU (0.12 mmol, 1 eq.), 0.021 g TBTU (0.060 mmol, 0.5 eq.), 0.018 g DMTMM (0.060 mmol, 0.5 eq.), 0.09 mL DIPEA (0.96 mmol, 8 eq.) in anhydrous CH_2Cl_2 (122 mL, 0.001 M) following the *General Procedure for Synthesis of Cyclic Peptides.* The reaction was allowed to stir overnight and the reaction was monitored *via* LC/MS. Once complete, the reaction mixture was subjected to an acid-base work-up before drying over Na_2SO_4, filtering, evaporating under reduced pressure and drying *in vacuo* to yield crude *cyclo*-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc).

***cyclo*-Phe-Tyr-Ser-Asn-Lys**
The free cyclic peptide *cyclo*-Phe-Tyr-Ser-Asn-Lys was generated by removing the side chain protecting groups on *cyclo*-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc). 100 mg *cyclo*-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Lys(Boc) (0.08 mmol, 1 eq.) was deprotected using a mixture of 400 μL of TFA and anisole (8 eq.). The free cyclic peptide then underwent HPLC purification to generate pure final compound **5.1 CYC** in a 42% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{31}H_{41}N_7O_8$, 640.30; found, 640.20.
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{31}H_{41}N_7O_8$, 640.3000; found, 640.3090.

^{1}H NMR (600 MHz, D_2O) δ 7.57-7.03 (m, 5H, Phe), 7.57-7.03 (m, 2H, δH Tyr), 7.03-6.86 (m, 2H, εH Tyr), 4.55-4.39 (m, 1H, αH Tyr), 4.39-4.16 (m, 1H, αH Ser), 4.09-3.96 (m, 2H, βCH_2 Ser), 3.96-3.79 (m, 1H, αH Lys), 3.42-3.33 & 3.33-3.19 (m, 2H, βCH_2 Phe), 3.19-3.07 (m, 2H, εCH_2 Lys), 3.19-2.80 (m, 2H, βCH_2 Tyr), 3.19-2.80 (m, 2H, βCH_2 Asn), 2.22-1.71 (m, 2H, βCH_2 Lys), 1.89-1.77 (m, 2H, δCH_2 Lys), 1.59-1.43 (m, 2H, γCH_2 Lys).

<u>Synthesis of 6.1 LIN and 6.1 CYC</u>



<u>Experimental Procedures for 6.1 LIN</u>

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2

The resin-bound hexapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2 was synthesised following the *Coupling Reaction* procedure using 1.3 g Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 (0.25 mmol, 1 eq.) reserved from the synthesis of **5.1 LIN**, together with 0.24 g Fmoc-Gly-OH (0.75 mmol, 3 eq.), 0.10 g HOBt (0.75 mmol, 3 eq.), 0.23 mL DIC (1.5 mmol, 6 eq.) and 2.5 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2.

HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2

The linear pentapeptide HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 3.6 mL of TFE and 3.6 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 as a white solid (286 mg, overall 87%).

HO-Lys-Phe-Tyr-Ser-Asn-Gly-NH_2

The free linear peptide HO-Lys-Phe-Tyr-Ser-Asn-Gly-NH_2 was generated by removing the side chain protecting groups on HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2. 70 mg HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-

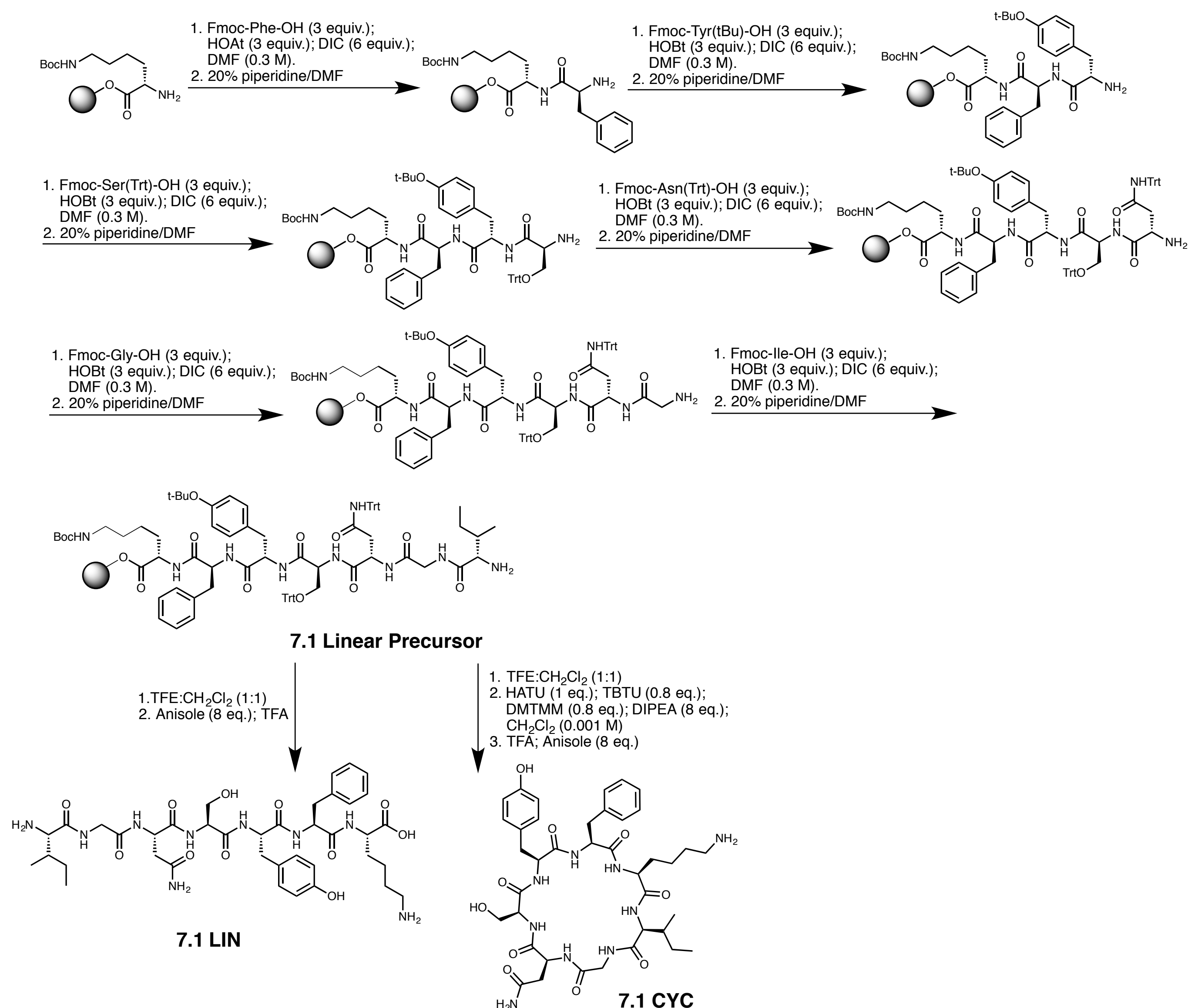
Asn(Trt)-NH_2 (0.05 mmol, 1 eq.) was deprotected using a mixture of 280 µL of TFA and anisole (8 eq.). The free linear peptide then underwent HPLC purification to generate pure final compound **6.1 LIN** in a 29% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+2H]^{2+}$ calcd for $C_{33}H_{46}N_8O_{10}$, 358.17; found, 357.95.
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{33}H_{46}N_8O_{10}$, 715.3300; found, 715.3412.
^{1}H NMR (600 MHz, D_2O) δ 7.56-7.38 (m, 5H, Phe), 7.25-7.17 (d, *J* = 8.40 Hz, 2H, δH Tyr), 7.01-6.93 (d, *J* = 8.40 Hz, 2H, εH Tyr), 4.97-4.92 (t, *J* = 7.20 Hz, 1H, αH Asn), 4.59-4.53 (t, *J* = 5.58 Hz, 1H, αH Ser), 4.31-4.25 (t, *J* = 6.66 Hz, 1H, αH Lys), 4.02-3.94 (m, 2H, αCH_2 Gly), 3.94-3.86 (m, 2H, βCH_2 Ser), 3.37-3.29 & 3.21-3.09 (m, 2H, βCH_2 Phe), 3.21-3.09 (m, 2H, εCH_2 Lys), 3.21-3.09 & 2.97-2.86 (m, 2H, βCH_2 Tyr), 2.97-2.86 (qd, *J* = 5.70, 15.75 Hz, 2H, βCH_2 Asn), 2.00-1.91 & 1.91-1.78 (m, 2H, βCH_2 Lys), 1.91-1.78 (m, 2H, δCH_2 Lys), 1.63-1.47 (m, 2H, γCH_2 Lys).

Experimental Procedures for 6.1 CYC

***cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly**
cyclo-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly was synthesised using 0.10 g HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2 (0.074 mmol, 1 eq.), 0.028 g HATU (0.074 mmol, 1 eq.), 0.012 g TBTU (0.037 mmol, 0.5 eq.), 0.011 g DMTMM (0.037 mmol, 0.5 eq.), 0.10 mL DIPEA (0.59 mmol, 8 eq.) in anhydrous CH_2Cl_2 (74 mL, 0.001 M) following the *General Procedure for Synthesis of Cyclic Peptides.* The reaction was allowed to stir overnight and the reaction was monitored *via* LC/MS. Once complete, the reaction mixture was subjected to an acid-base work-up before drying over Na_2SO_4, filtering, evaporating under reduced pressure and drying *in vacuo* to yield crude *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly.

***cyclo*-Lys-Phe-Tyr-Ser-Asn-Gly**
The free cyclic peptide *cyclo*-Lys-Phe-Tyr-Ser-Asn-Gly was generated by removing the side chain protecting groups on *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly. 56 mg *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly (0.042 mmol, 1 eq.) was deprotected using a mixture of 224 µL of TFA and anisole (8 eq.). The free cyclic peptide then underwent HPLC purification to generate pure final compound **6.1 CYC** in a 40% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{33}H_{44}N_8O_9$, 697.32; found, 696.85.
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{33}H_{44}N_8O_9$, 697.3200; found, 697.3301.
^{1}H NMR (600 MHz, D_2O) δ 7.49-7.16 (m, 5H, Phe), 7.16-6.91 (m, 2H, δH Tyr), 6.91-6.78 (m, 2H, εH Tyr), 4.85-4.79 (t, *J* = 5.58 Hz, 1H, αH Asn), 4.49-4.43 (m, 1H, αH Phe), 4.43-4.38 (t, *J* = 4.98 Hz, 1H, αH Tyr), 4.21-4.16 (t, *J* = 7.03 Hz, 1H, αH Ser), 4.14-4.05 (m, 1H, αH Lys), 3.85-3.72 (m, 2H, αCH_2 Gly), 3.85-3.72 (m, 2H, βCH_2 Ser), 3.30-3.22 & 3.13-3.03 (m, 2H, βCH_2 Phe), 2.97-2.70 (m, 2H, εCH_2 Lys), 2.97-2.70 (m, 2H, βCH_2 Tyr), 2.97-2.70 (m, 2H, βCH_2 Asn), 1.91-1.77 (m, 2H, βCH_2 Lys), 1.77-1.63 (m, 2H, δCH_2 Lys), 1.46-1.27 (m, 2H, γCH_2 Lys).

Synthesis of 7.1 LIN and 7.1 CYC



Experimental Procedures for 7.1 LIN

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2
Resin-bound pentapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 was synthesised as described for compound **5.1 LIN**.

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2
The resin-bound hexapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2 synthesised from the previous coupling reaction, together with 0.45 g Fmoc-Gly-OH (1.5 mmol, 3 eq.), 0.20 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2.

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2
The resin-bound heptapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2 synthesised from the previous coupling reaction, together with 0.53 g Fmoc-Ile-OH (1.5 mmol, 3 eq.), 0.20 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The

coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2.
The resin-bound pentapeptide was then divided into 2 equal portions of 1.4 g, where one part was reserved for the synthesis of the 8.1 compound series and the other was taken forward to complete the synthesis of the 7.1 compound series.

HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2
The linear heptapeptide HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 3.1 mL of TFE and 3.1 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2 as a white solid (255 mg, overall 68%).

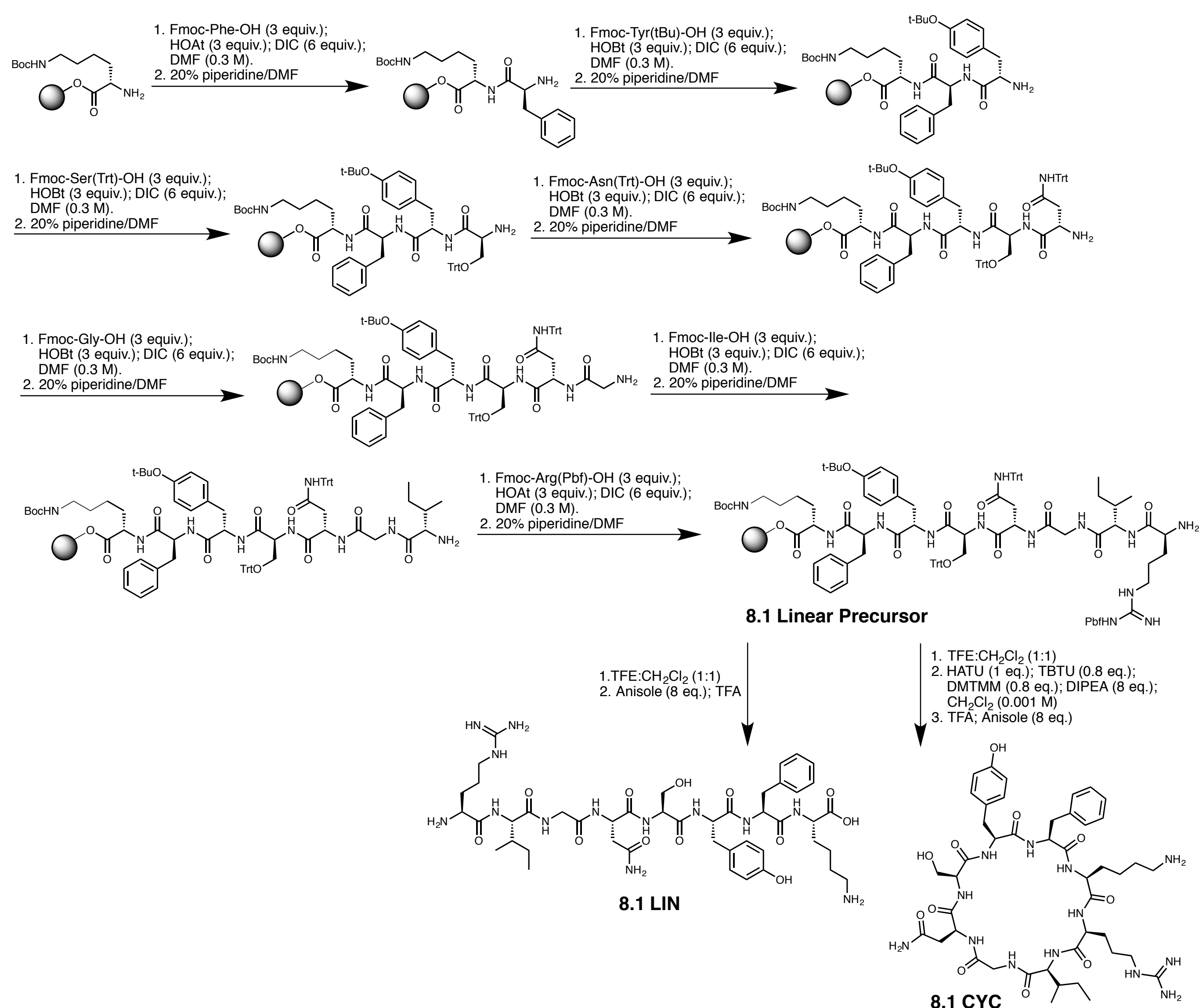
HO-Lys-Phe-Tyr-Ser-Asn-Gly-Ile-NH_2
The free linear peptide HO-Lys-Phe-Tyr-Ser-Asn-Gly-Ile-NH_2 was generated by removing the side chain protecting groups on HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2. 62 mg HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2 (0.04 mmol, 1 eq.) was deprotected using a mixture of 250 µL of TFA and anisole (8 eq.). The free linear peptide then underwent HPLC purification to generate pure final compound **7.1 LIN** in a 48% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+2H]^{2+}$ calcd for $C_{39}H_{57}N_9O_{11}$, 414.71; found, 414.55.
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{57}N_9O_{11}$, 828.42; found, 828.4255.
^{1}H NMR (600 MHz, D_2O) δ 7.56-7.28 (m, 5H, Phe), 7.21-7.15 (d, *J* = 8.04 Hz, 2H, δH Tyr), 7.02-6.93 (m, 2H, εH Tyr), 4.91-4.86 (t, *J* = 6.49 Hz, 1H, αH Asn), 4.57-4.51 (t, *J* = 5.52 Hz, 1H, αH Ser), 4.31-4.24 (m, 1H, αH Lys), 4.22-4.15 (m, 2H, αCH_2 Gly), 4.06-4.00 (m, 1H, αH Ile), 3.96-3.85 (m, 2H, βCH_2 Ser), 3.39-3.33 & 3.18-3.05 (m, 2H, βCH_2 Phe), 3.18-3.05 (m, 2H, εCH_2 Lys), 3.18-2.97 (m, 2H, βCH_2 Tyr), 2.97-2.85 (m, 2H, βCH_2 Asn), 2.16-2.07 (m, 1H, βH Ile), 2.00-1.90 & 1.90-1.79 (m, 2H, βCH_2 Lys), 1.90-1.79 (m, 2H, δCH_2 Lys), 1.73-1.65 & 1.46-1.36 (m, 2H, δCH_3 Ile), 1.59-1.36 (m, 2H, γCH_2 Lys), 1.19-1.12 (d, *J* = 6.90 Hz, 3H, γCH_2 Ile), 1.11-1.00 (m, 3H, γCH_3 Ile).

<u>**Experimental Procedures for 7.1 CYC**</u>

***cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile**
cyclo-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile was synthesised using 0.10 g HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2 (0.068 mmol, 1 eq.), 0.029 g HATU (0.068 mmol, 1 eq.), 0.014 g TBTU (0.034 mmol, 0.5 eq.), 0.012 g DMTMM (0.034 mmol, 0.5 eq.), 0.10 mL DIPEA (0.55 mmol, 8 eq.) in anhydrous CH_2Cl_2 (68 mL, 0.001 M) following the *General Procedure for Synthesis of Cyclic Peptides.* The reaction was allowed to stir overnight and the reaction was monitored *via* LC/MS. Once complete, the reaction mixture was subjected to an acid-base work-up before drying over Na_2SO_4, filtering, evaporating under reduced pressure and drying *in vacuo* to yield crude *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile.

***cyclo*-Lys-Phe-Tyr-Ser-Asn-Gly-Ile**
The free cyclic peptide *cyclo*-Lys-Phe-Tyr-Ser-Asn-Gly-Ile was generated by removing the side chain protecting groups on *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile. 62 mg *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile (0.043 mmol, 1 eq.) was deprotected using a mixture of 250 µL of TFA and anisole (8 eq.). The free cyclic peptide then underwent HPLC purification to generate pure final compound **7.1 CYC** in a 30% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{55}N_9O_{10}$, 810.41; found, 810.05.
HRMS (ESI-TOF) *m/z*: $[M+H]^+$ calcd for $C_{39}H_{55}N_9O_{10}$, 810.0500; found, 810.4141.
^{1}H NMR (600 MHz, D_2O) δ 7.52-7.13 (m, 5H, Phe), 7.13-6.90 (m, 2H, δH Tyr), 6.90-6.74 (m, 2H, εH Tyr), 4.88-4.82 (t, *J* = 6.66 Hz, 1H, αH Asn), 4.57-4.52 (dd, *J* = 5.52, 9.90 Hz, 1H, αH Phe), 4.49-4.44 & 4.41-4.35 (t, *J* = 4.80 Hz, 1H, αH Tyr), 4.32-4.21 (m, 2H, αCH_2 Gly), 4.32-4.21 (m, 2H, αH Lys), 4.21-4.05 (m, 1H, αH Ile), 4.05-3.65 (m, 2H, βCH_2 Ser), 3.33-3.22 & 3.12-2.67 (m, 2H, βCH_2 Phe), 3.12-2.67 (m, 2H, εCH_2 Lys), 3.12-2.67 (m, 2H, βCH_2 Tyr), 3.12-2.67 (m, 2H, βCH_2 Asn), 1.98-1.77 (m, 1H, βH Ile), 1.98-1.77 (m, 2H, βCH_2 Lys), 1.77-1.63 (m, 2H, δCH_2 Lys), 1.63-1.51 & 1.45-1.21 (m, 2H, γCH_2 Ile), 1.45-1.21 (m, 2H, γCH_2 Lys), 1.10-0.83 (m, 3H, δCH_3 Ile), 1.10-0.83 (m, 3H, γCH_3 Ile).

Synthesis of 8.1 LIN and 8.1 CYC



Experimental Procedures for 8.1 LIN

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2

The resin-bound octapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2 was synthesised following the *Coupling Reaction* procedure using 1.4 g Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-NH_2 (0.25 mmol, 1 eq.) reserved from the synthesis of 7.1 LIN, together with 0.49 g Fmoc-Arg(Pbf)-OH (0.75 mmol, 3 eq.), 0.10 g HOAt (0.75 mmol, 3 eq.), 0.23 mL DIC (1.5 mmol, 6 eq.) and 2.5 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run overnight and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2.

HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-NH_2

The linear octapeptide HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 3.7 mL of TFE and 3.7 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2 as a white solid (364 mg, overall 77%).

HO-Lys-Phe-Tyr-Ser-Asn-Gly-NH_2

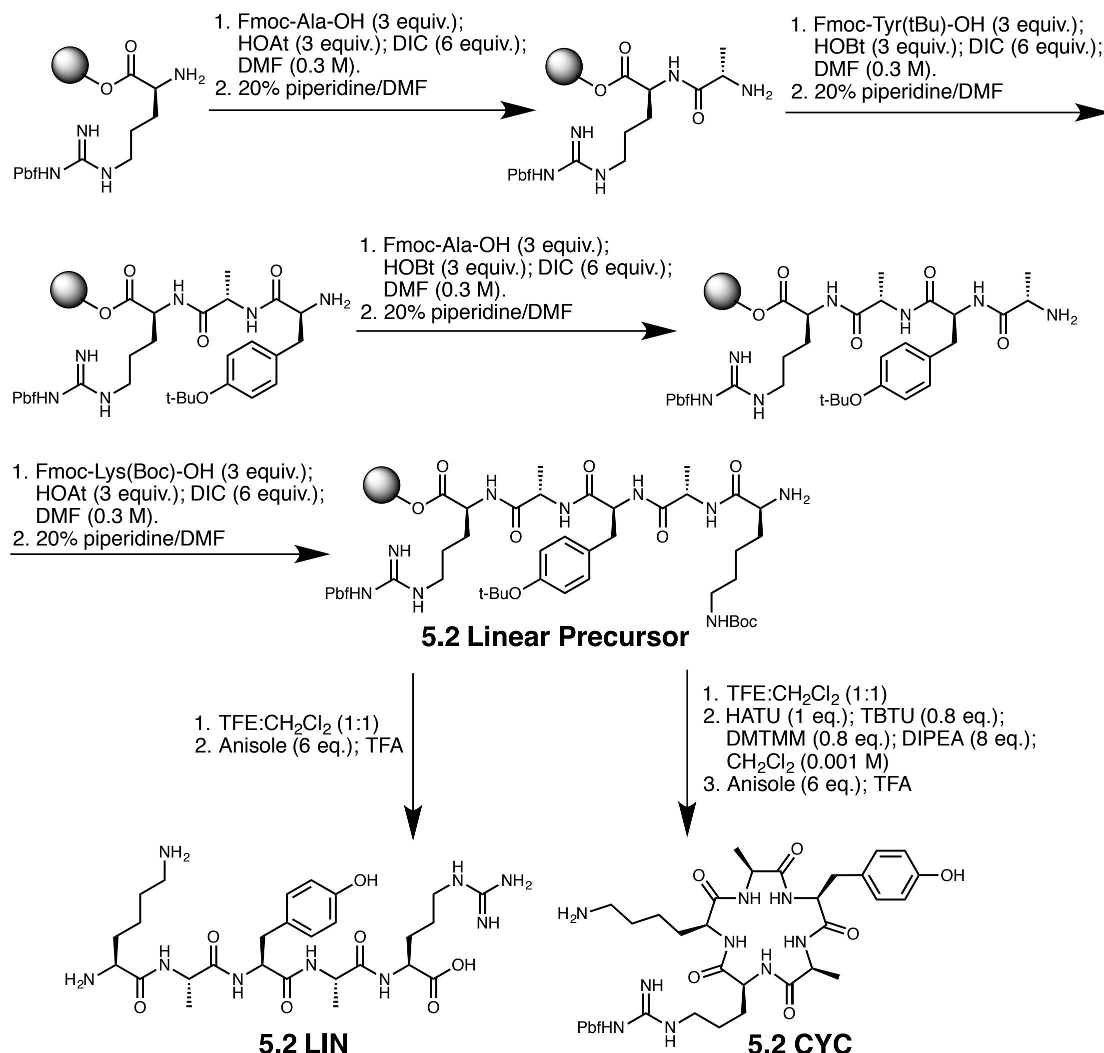
The free linear peptide HO-Lys-Phe-Tyr-Ser-Asn-Gly-Ile-Arg-NH_2 was generated by removing the side chain protecting groups on HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2. 62 mg HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2 (0.033 mmol, 1 eq.) was deprotected using a mixture of 250 μL of TFA and anisole (8 eq.). The free linear peptide then underwent HPLC purification to generate pure final compound **8.1 LIN** in a 32% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+2H]^{2+}$ calcd for $C_{45}H_{69}N_{13}O_{12}$, 492.76; found, 492.70.
HRMS (ESI-TOF) *m/z*: $[M+2H]^{2+}$ calcd for $C_{45}H_{69}N_{13}O_{12}$, 492.7600; found, 492.7666.
^{1}H NMR (600 MHz, D_2O) δ 7.55-7.35 (m, 5H, Phe), 7.22-7.14 (d, *J* = 8.53 Hz, 2H, δH Tyr), 6.99-6.93 (d, *J* = 8.41 Hz, 2H, εH Tyr), 4.90-4.86 (t, *J* = 6.58 Hz, 1H, αH Asn), 4.56-4.51 (t, *J* = 5.46 Hz, 1H, αH Ser), 4.42-4.38 (d, *J* = 7.74 Hz, 1H, αH Ile), 4.31-4.23 (t, *J* = 7.27 Hz, 1H, αH Lys), 4.21-4.09 (m, 1H, αH Arg), 4.21-4.09 (m, 2H, αCH_2 Gly), 3.95-3.85 (m, 2H, βCH_2 Ser), 3.37-3.29 (m, 2H, δCH_2 Arg), 3.37-3.29 & 3.20-2.85 (m, 2H, βCH_2 Phe), 3.20-2.85 (m, 2H, εCH_2 Lys), 3.20-2.85 (m, 2H, βCH_2 Tyr), 3.20-2.85 (m, 2H, βCH_2 Asn), 2.08-1.99 (m, 1H, βH Ile), 2.08-1.99 (m, 2H, βCH_2 Arg), 1.99-1.90& 1.88-1.73 (m, 2H, βCH_2 Lys), 1.88-1.73 (m, 2H, δCH_2 Lys), 1.88-1.73 (m, 2H, γCH_2 Arg), 1.71-1.63 & 1.42-1.33 (m, 2H, γCH_2 Ile), 1.58-1.47 (m, 2H, γCH_2 Lys), 1.12-1.07 (d, *J* = 6.78 Hz, 3H, δCH_3 Ile), 1.07-1.00 (t, *J* = 7.38 Hz, 3H, γCH_3 Ile).

Experimental Procedures for 8.1 CYC

***cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)**
cyclo-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf) was synthesised using 0.15 g HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-NH_2 (0.080 mmol, 1 eq.), 0.033 g HATU (0.080 mmol, 1 eq.), 0.014 g TBTU (0.040 mmol, 0.5 eq.), 0.012 g DMTMM (0.040 mmol, 0.5 eq.), 0.11 mL DIPEA (0.64 mmol, 8 eq.) in anhydrous CH_2Cl_2 (80 mL, 0.001 M) following the *General Procedure for Synthesis of Cyclic Peptides.* The reaction was allowed to stir overnight and the reaction was monitored *via* LC/MS. Once complete, the reaction mixture was subjected to an acid-base work-up before drying over Na_2SO_4, filtering, evaporating under reduced pressure and drying *in vacuo* to yield crude *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf).

***cyclo*-Lys-Phe-Tyr-Ser-Asn-Gly-Ile**
The free cyclic peptide *cyclo*-Lys-Phe-Tyr-Ser-Asn-Gly-Ile-Arg was generated by removing the side chain protecting groups on *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf). 45 mg *cyclo*-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile (0.024 mmol, 1 eq.) was deprotected using a mixture of 180 μL of TFA and anisole (10 eq.). The free cyclic peptide then underwent HPLC purification to generate pure final compound **8.1 CYC** in a 67% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+2H]^{2+}$ calcd for $C_{45}H_{67}N_{13}O_{11}$, 483.76; found, 483.65.
HRMS (ESI-TOF) *m/z*: $[M+2H]^{2+}$ calcd for $C_{45}H_{67}N_{13}O_{11}$, 483.7600; found, 483.7610.
^{1}H NMR (600 MHz, D_2O) δ 7.57-7.20 (m, 5H, Phe), 7.20-6.73 (m, 2H, δH Tyr), 7.20-6.73 (m, 2H, εH Tyr), 4.90-4.84 (m, 1H, αH Asn), 4.55-4.67 (s, br, 1H, αH Tyr), 4.39-4.35 (t, *J* = 5.16 Hz, 1H, αH Ser), 4.33-4.26 (t, *J* = 8.04 Hz, 1H, αH Lys), 4.21-4.09 (m, 2H, αCH_2 Gly), 4.05-4.00 (m, 1H, αH Ile), 4.05-4.00 (m, 1H, αH Arg), 4.05-3.72 (m, 2H, βCH_2 Ser), 3.45-3.36 & 3.09-2.96 (m, 2H, βCH_2 Phe), 3.31-3.17 (m, 2H, δCH_2 Arg), 3.09-2.96 (m, 2H, εCH_2 Lys), 2.96-2.87 (m, 2H, βCH_2 Tyr), 2.87-2.68 (m, 2H, βCH_2 Asn), 2.15-1.88 (m, 1H, βH Ile), 2.15-1.88 (m, 1H, βCH_2 Arg), 1.88-1.59 (m, 2H, βCH_2 Lys), 1.79-1.59 (m, 2H, δCH_2 Lys), 1.59-1.53 & 1.30-1.10 (m, 2H, γCH_2 Ile), 1.47-1.30 (m, 2H, γCH_2 Lys), 1.00-0.82 (m, 3H, δCH_3 Ile), 1.00-0.82 (m, 3H, γCH_3 Ile).

Synthesis of 5.2 LIN and 5.2 CYC



Experimental Procedures for 5.2 LIN

Resin-O-Arg(Pbf)-Ala-NHFmoc
The dipeptide Resin-O-Arg(Pbf)-Ala-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 1.003 g (0.60 mmol, 1.0 equivalent) of Resin-O-Arg(Pbf)-NH_2, 0.560 g (1.80 mmol, 3.0 equivalents) of HO-Ala-NHFmoc, 245 mg of HOAt (1.80 mmol, 3.0 equivalents), 0.56 mL of DIC (3.60 mmol, 6.0 equivalents) and 3.0 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound dipeptide.

Resin-O-Arg(Pbf)-Ala-NH_2
The dipeptide Resin-O-Arg(Pbf)-Ala-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-NHFmoc
The tripeptide Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 1.003 g (0.60 mmol, 1.0 equivalent) of Resin-O-Arg(Pbf)-Ala-NH_2, 0.827 g (1.80 mmol, 3.0 equivalents) of HO-Tyr(tBu)-NHFmoc, 243 mg of HOBt (1.80 mmol, 3.0 equivalents), 0.56 mL of DIC (3.60 mmol, 6.0 equivalents) and 3.0 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound tripeptide.

Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-NH_2
The tripeptide Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NHFmoc
The tetrapeptide Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.003 g (0.60 mmol, 1.0 equivalent) of Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-NH_2, 0.560 g (1.80 mmol, 3.0 equivalents) of HO-Ala-NHFmoc, 243 mg of HOBt (1.80 mmol, 3.0 equivalents), 0.56 mL of DIC (3.60 mmol, 6.0 equivalents) and 3.0 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound tetrapeptide.

Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2
The tetrapeptide Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NHFmoc
The pentapeptide Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.003 g (0.60 mmol, 1.0 equivalent) of Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2, 0.843 g (1.80 mmol, 3.0 equivalents) of HO-Lys(Boc)-NHFmoc, 245 mg of HOAt (1.80 mmol, 3.0 equivalents), 0.56 mL of DIC (3.60 mmol, 6.0 equivalents) and 3.0 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound pentapeptide.

Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2
The pentapeptide Resin-O-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.
HO-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2
The double deprotected linear precursor (DDLP) HO-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 was synthesized using the resin-bound peptide prepared from previous step following "Linear peptide cleavage from resin" procedure utilizing 3.9 mL of TFE and 3.9 mL of CH_2Cl_2. The resulting slurry was filtered and dried *in vacuo* to yield HO-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 as a pale yellow solid (270 mg, overall 44%).
LC/MS (ESI): *m/z* calculated $C_{49}H_{78}N_9O_{12}S$ [M + H^+] = 1016.55, found 1016.20

HO-Arg-Ala-Tyr-Ala-Lys-NH_2
The HO-Arg-Ala-Tyr-Ala-Lys-NH_2 was synthesized utilizing 25.0 mg (0.025 mmol, 1.0 equivalent) of the DDLP HO-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2, 5.00 mL (0.050 mmol, 2.0 equivalents) of Anisole, 0.125 mL of TFA and 0.125 mL of CH_2Cl_2. The resulting slurry was dried *in vacuo*. The residue was redissolved in MeOH, and the solution was centrifuged. The supernatant was injected into the HPLC to yield HO-Arg-Ala-Tyr-Ala-Lys-NH_2 (13.2 mg, overall 87%).
LC/MS (ESI): *m/z* calculated $C_{27}H_{46}N_9O_7$ [M + H^+] = 608.35, found 608.10.
HRMS (ESI-TOF): M+H+, found 608.3508 $C_{27}H_{47}N_9O_7$ requires 608.3520
^{1}H NMR (600 MHz, $CDCl_3$, 298K): δ = 1.22-1.32 (m, 6H, CH_3β Ala); 1.22-1.35 (m, 2H, CH_2γ Lys); 1.51-1.62 (m, 2H, CH_2δ Lys); 1.56-1.65 (m, 2H, CH_2γ Arg); 1.65-1.73 & 1.81-1.89 (m, 2H, CH_2β Lys); 1.71-1.81 (m, 2H, CH_2β Arg); 2.83-2.98 & 3.03-3.08 (m, 2H, CH_2β Tyr); 2.85-2.93 (m, 2H, CH_2δ Arg); 3.09-3.18 (m, 2H, CH_2ε Lys); 3.61-3.66 & 3.84-3.91 (m, 1H, CHα Arg); 4.19-4.33 (m, 2H, CHα Ala); 4.20-4.28 (m, 1H, CHα Lys); 4.41-4.46 & 4.49-4.54 (m, 1H, CHα Tyr); 6.73-6.76 & 7.13-7.17 (d, J = 8.55 Hz, 2H, Ph Tyr); 7.04-7.08 & 7.19-7.23 (d, J = 8.55 Hz, 2H, Ph Tyr).
^{13}C NMR (150 MHz, $CDCl_3$, 298K): δ = 16.61, 21.06, 24.40, 26.25, 27.90, 30.31, 36.27, 36.66, 38.96, 40.49, 49.28, 52.78, 52.85, 54.35, 54.63, 55.04, 115.37, 121.43, 128.00, 130.52, 154.39, 156.71, 169.10, 172.19, 172.45, 174.08, 175.83

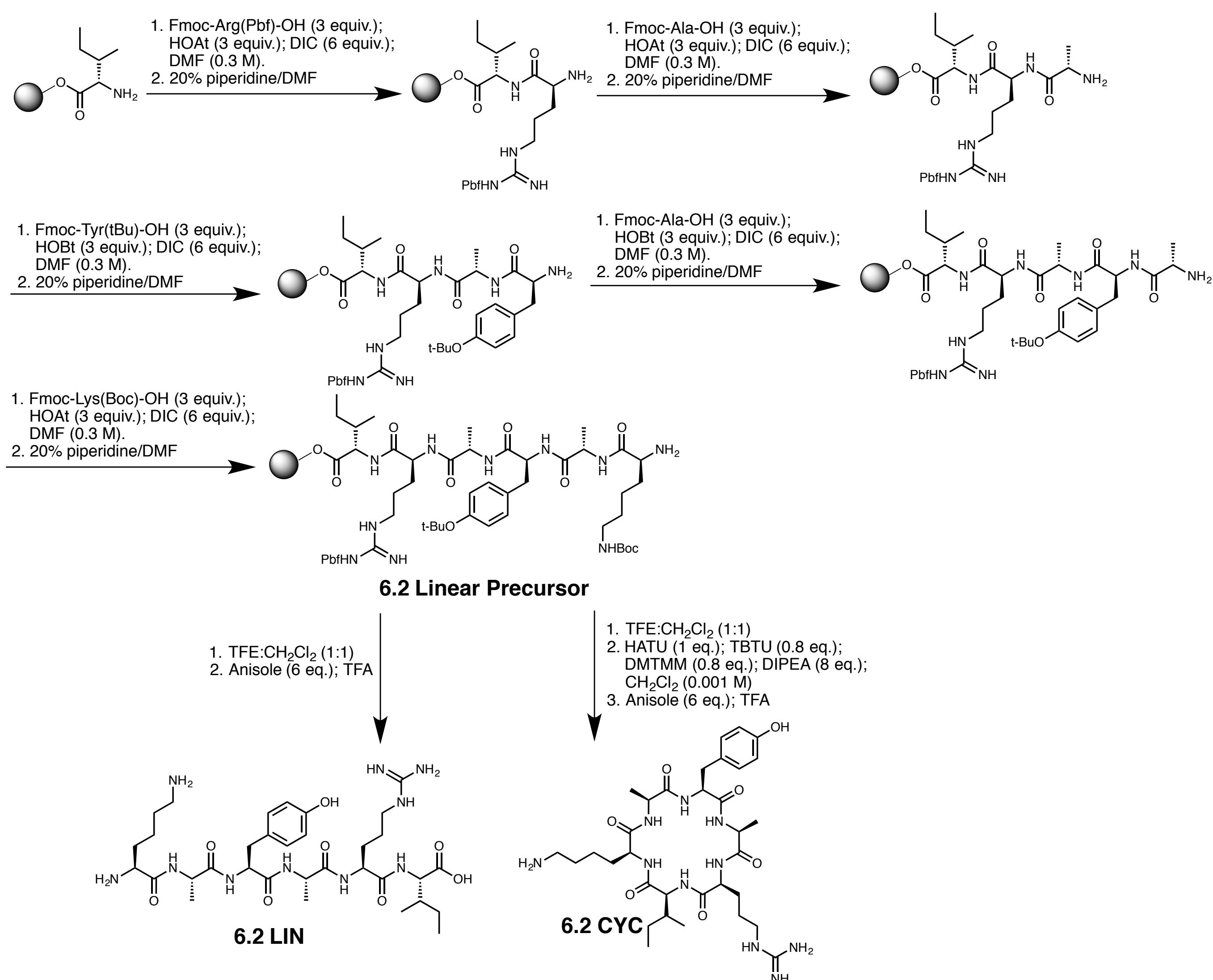
Experimental Procedures for 5.2 CYC

***cyclo*-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)**
The macrocycle Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc) was synthesized following the "Syringe pump macrocyclization" procedure utilizing 107 mg (0.105 mmol, 1.0 equivalent) of DDLP HO-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2, 0.11 mL (0.632 mmol, 6.0 equivalents) of DIPEA, 17 mg (0.053 mmol, 0.5 equivalents) of TBTU, 40 mg (0.105 mmol, 1.0 equivalents) HATU, and 15 mg (0.053 mmol, 0.5 equivalents) of DMTMM in 105 mL CH_2Cl_2. The crude reaction was dried in vacuo to yield 126 mg of macrocycle. The macrocycle was taken onto the next reaction without further purification.

***cyclo*-Arg-Ala-Tyr-Ala-Lys**
Macrocycle Arg-Ala-Tyr-Ala-Lys was synthesized utilizing 126 mg (0.127 mmol, 1.0 equivalent) of the Macrocycle Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc), 27.0 mL (0.254 mmol, 2.0 equivalents) of Anisole, 0.65 mL of TFA and 0.65 mL of CH_2Cl_2. The resulting slurry was dried *in vacuo*. The residue was redissolved in MeOH, and the solution was

centrifuged. The supernatant was injected into the HPLC to yield Macrocycle Arg-Ala-Tyr-Ala-Lys (21.7 mg, overall 17%). LCMS: m/z calcd for $C_{27}H_{44}N_9O_6$ (M + H$^+$) = 590.34, found 590.05.
HRMS (ESI-TOF): M+H+, found 590.3408 $C_{27}H_{45}N_9O_6$ requires 590.3414
^{1}H NMR (600 MHz, $CDCl_3$, 318K): δ = 1.42-1.53 (d, *J*= 6.60 Hz, 3H, CH_3β Ala); 1.45-1.51 & 1.59-1.64 (d, *J*= 7.23 Hz, 3H, CH_3β Ala); 1.52-1.63 (m, 2H, CH_2γ Lys); 1.73-1.87 (m, 2H, CH_2γ Arg); 1.79-1.90 (m, 2H, CH_2δ Lys); 1.97-2.06 (m, 2H, CH_2β Lys); 2.05-2.11 (m, 2H, CH_2β Arg); 3.11-3.19 (m, 2H, CH_2δ Arg); 3.19-3.25 & 3.28-3.32 (m, 2H, CH_2β Tyr); 3.30-3.43 & 3.33-3.39 (m, 2H, CH_2ε Lys); 4.24-4.29 (m, 1H, CHα Ala); 4.40-4.44 (m, 1H, CHα Ala); 4.38-4.43 (m, 1H, CHα Lys); 4.38-4.44 (m, 1H, CHα Arg); 4.58-4.64 (m, 1H, CHα Tyr); 7.01-7.06 & 7.24-7.27 (d, J = 8.77 Hz, 2H, Ph Tyr); 7.32-7.36 & 7.91-7.94 (d, J = 8.77 Hz, 2H, Ph Tyr).
^{13}C NMR (150 MHz, $CDCl_3$, 318K): δ = 15.64, 16.63, 22.36, 24.82, 26.27, 27.56, 29.51, 35.43, 39.29, 40.65, 42.64, 50.82, 51.21, 54.17, 54.82, 55.53, 114.37, 115.68, 127.58, 130.78, 154.39, 156.71, 169.10, 172.19, 172.45, 174.08, 175.83

Synthesis of 6.2 LIN and 6.2 CYC



Experimental Procedures for 6.2 LIN

Resin-O-Ile-Arg(Pbf)-NH$_2$

The resin-bound dipeptide Resin-O-Ile-Arg(Pbf)-NH$_2$ was synthesised following the *Coupling Reaction* procedure using 1.00 g H-Ile-2-ClTrt resin (0.9 mmol, 1 eq.), 1.75 g Fmoc-Phe-OH (2.7 mmol, 3 eq.), 0.38 g HOBt (2.7 mmol, 3 eq.), 0.83 mL DIC (5.4 mmol, 6 eq.) and 9.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Ile-Arg(Pbf)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Ile-Arg(Pbf)-NH$_2$.

Resin-O-Ile-Arg(Pbf)-Ala-NH_2

The resin-bound tripeptide Resin-O-Ile-Arg(Pbf)-Ala-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Ile-Arg(Pbf)-NH_2 synthesised from previous coupling reaction, together with 0.84 g Fmoc-Ala-OH (2.7 mmol, 3 eq.), 0.37 g HOAt (2.7 mmol, 3 eq.), 0.83 mL DIC (5.4 mmol, 6 eq.) and 9.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run overnight and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Ile-Arg(Pbf)-Ala-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Ile-Arg(Pbf)-Ala-NH_2 .

Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-NH_2

The resin-bound tetrapeptide Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Ile-Arg(Pbf)-Ala-NH_2 synthesised from previous coupling reaction, together with 1.24 g Fmoc-Tyr(t-Bu)-OH (2.7 mmol, 3 eq.), 0.38 g HOBt (2.7 mmol, 3 eq.), 0.83 mL DIC (5.4 mmol, 6 eq.) and 9.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-NH_2.

Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-NH_2

The resin-bound pentapeptide Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-NH_2 synthesised from previous coupling reaction, together with 0.84 g Fmoc-Ala-OH (2.7 mmol, 3 eq.), 0.37 g HOBt (2.7 mmol, 3 eq.), 0.83 mL DIC (5.4 mmol, 6 eq.) and 9.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-NH_2.

Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2

The resin-bound pentapeptide Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-NH_2 synthesised from previous coupling reaction, together with 1.27 g Fmoc-Lys(Boc)-OH (2.7 mmol, 3 eq.), 0.37 g HOBt (2.7 mmol, 3 eq.), 0.83 mL DIC (5.4 mmol, 6 eq.) and 9.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2.

HO-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2

The linear hexapeptide HO-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 6.4 mL of TFE and 6.4 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 as a white solid (450 mg, overall 45%).

HO-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2

The free linear peptide HO-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2 was generated by removing the side chain protecting groups on HO-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2. 71 mg HO-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 (0.063 mmol, 1 eq.) was deprotected using a mixture of 284 µL of TFA and anisole (6 eq.). The free linear peptide then underwent HPLC purification to generate pure final compound **6.2 LIN** in a 69% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+2H]^{2+}$ calcd for $C_{33}H_{56}N_{10}O_8$, 361.22; found, 361.10.
HRMS (ESI-TOF) *m/z*: $[M+2H]^{2+}$ calcd for $C_{33}H_{56}N_{10}O_8$, 361.2200; found, 361.2216.
^{1}H NMR (600 MHz, D_2O) δ 7.47-7.25 (m, 2H, δH Tyr), 7.02-6.96 (d, *J* = 8.40 Hz, 2H, εH Tyr), 4.56-4.46 (m, 1H, αH Arg), 4.56-4.46 (m, 1H, αH Ala), 4.56-4.46 (m, 1H, αH Ala), 4.29-4.25 (d, *J* = 6.19 Hz, 1H, βH Ile), 4.16-4.11 (m, 1H, αH Lys), 3.41-3.34 (t, *J* = 5.52 Hz, 2H, δCH_2 Arg), 3.23-3.06 (m, 2H, εCH_2 Lys), 3.23-3.06 (m, 2H, βCH_2 Tyr), 2.07-1.95 (m, 1H, βH Ile), 2.07-1.95 (m, 1H, βCH_2 Lys), 2.07-1.95 & 1.97-1.75 (m, 1H, βCH_2 Arg), 1.95-1.75 (m, 2H, γCH_2 Arg), 1.95-1.75 (m, 2H, δCH_2 Lys), 1.63-1.45 (m, 2H, γCH_2 Lys), 1.63-1.45 (m, 3H, βCH_3 Ala), 1.63-1.45 (m, 3H, βCH_3 Ala), 1.63-1.45 & 1.35-1.25 (m, 2H, γCH_2 Ile), 1.06-1.00 (m, 3H, δCH3 Ile), 1.06-1.00 (m, 3H, γCH3 Ile).

Experimental Procedures for 6.2 CYC

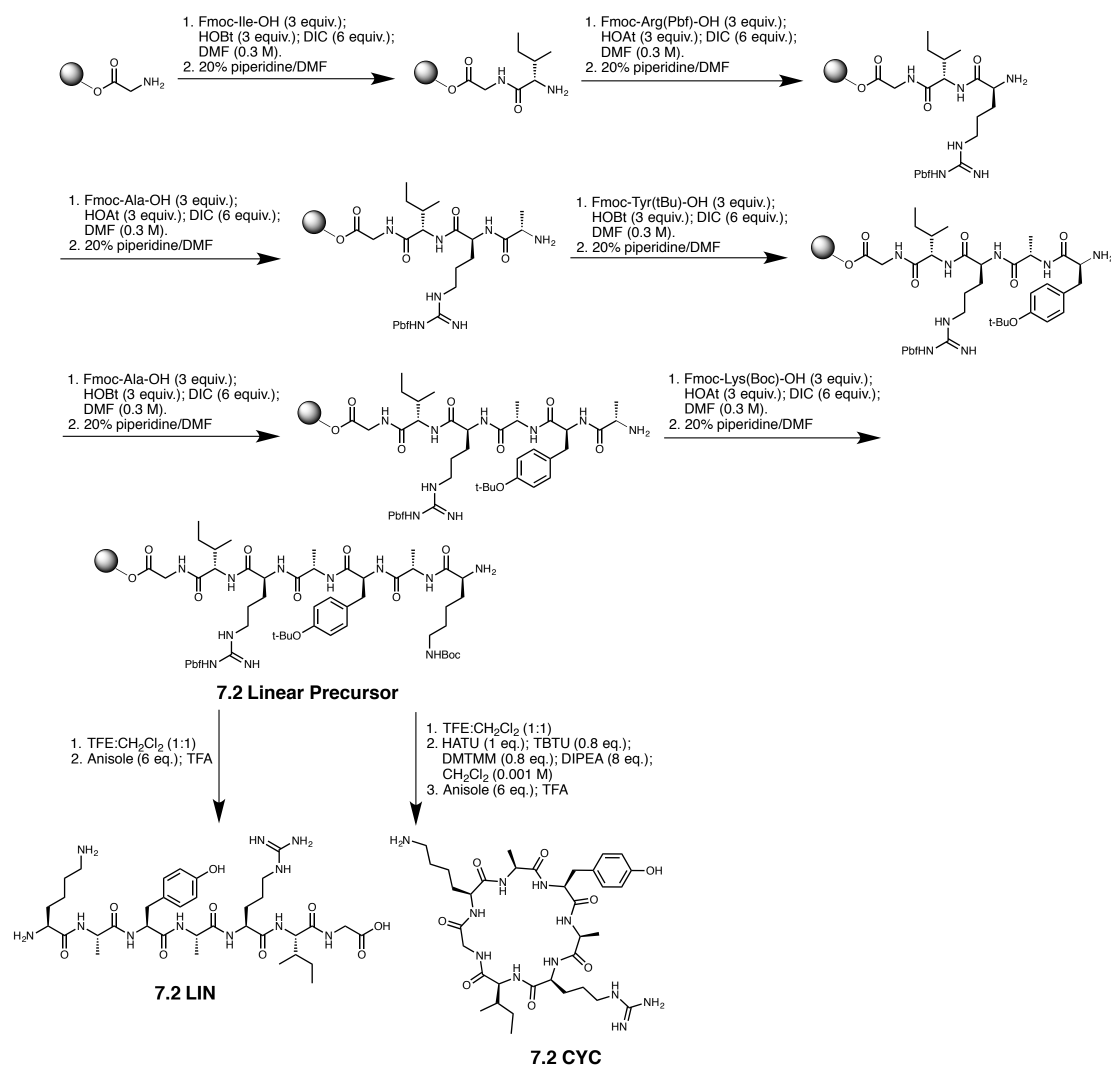
cyclo-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)

cyclo-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc) was synthesised using 0.12 g HO-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 (0.11 mmol, 1 eq.), 0.042 g HATU (0.11 mmol, 1 eq.), 0.020 g TBTU (0.050 mmol, 0.5 eq.), 0.012 g DMTMM (0.050 mmol, 0.5 eq.), 0.15 mL DIPEA (0.88 mmol, 8 eq.) in anhydrous CH_2Cl_2 (106 mL, 0.001 M) following the *General Procedure for Synthesis of Cyclic Peptides.* The reaction was allowed to stir overnight and the reaction was monitored *via* LC/MS. Once complete, the reaction mixture was subjected to an acid-base work-up before drying over Na_2SO_4, filtering, evaporating under reduced pressure and drying *in vacuo* to yield crude *cyclo*-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc).

cyclo-Ile-Arg-Ala-Tyr-Ala-Lys

The free cyclic peptide *cyclo*-Ile-Arg-Ala-Tyr-Ala-Lys was generated by removing the side chain protecting groups on *cyclo*-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc). 45 mg *cyclo*-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc) (0.040 mmol, 1 eq.) was deprotected using a mixture of 180 µL of TFA and anisole (6 eq.). The free cyclic peptide then underwent HPLC purification to generate pure final compound **6.2 CYC** in a 26% yield as a white solid.
LC/MS (ESI) *m/z*: $[M-H]^-$ calcd for $C_{33}H_{54}N_{10}O_7$, 701.42; found, 700.90.
HRMS (ESI-TOF) *m/z*: $[M+2H]^{2+}$ calcd for $C_{33}H_{54}N_{10}O_7$, 352.2100; found 352.2162.
^1H NMR (600 MHz, D_2O) δ 7.35-7.12 (m, 2H, δH Tyr), 6.95-6.83 (dd, *J* = 7.68, 24.31 Hz, 2H, εH Tyr), 4.62-4.49 (m, 1H, αH Tyr), 4.49-4.16 (m, 1H, αH Arg), 4.49-4.16 (m, 1H, αH Ala), 4.49-4.16 (m, 1H, αH Ala), 4.16-4.13 (d, *J* = 6.18 Hz, 1H, βH Ile), 4.13-4.06 (m, 1H, αH Lys), 3.34-3.22 (m, 2H, $δCH_2$ Arg), 3.22-2.83 (m, 2H, $εCH_2$ Lys), 3.22-2.83 (m, 2H, $βCH_2$ Tyr), 1.97-1.95 (m, 1H, βH Ile), 1.97-1.78 (m, 1H, $βCH_2$ Lys), 1.78-1.58 (m, 1H, $βCH_2$ Arg), 1.78-1.58 (m, 2H, $γCH_2$ Arg), 1.78-1.58 (m, 2H, $δCH_2$ Lys), 1.58-1.47 & 1.27-1.12 (m, 2H, $γCH_2$ Ile), 1.47-1.27 (m, 2H, $γCH_2$ Lys), 1.47-1.27 (m, 3H, $βCH_3$ Ala), 1.47-1.27 (m, 3H, $βCH_3$ Ala), 1.01-0.84 (m, 3H, δCH3 Ile), 1.01-0.84 (m, 3H, γCH3 Ile).

Synthesis of 7.2 LIN and 7.2 CYC



Experimental Procedures for 7.2 LIN

Resin-O-Gly-Ile-NHFmoc
The dipeptide Resin-O-Gly-Ile-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.012 g (0.90 mmol, 1.0 equivalent) of Resin-O-Gly-NH_2, 0.954 g (2.70 mmol, 3.0 equivalents) of HO-Ile-NHFmoc, 365 mg of HOBt (2.70 mmol, 3.0 equivalents), 0.85 mL of DIC (5.40 mmol, 6.0 equivalents) and 4.5 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound dipeptide.

Resin-O-Gly-Ile-NH_2
The dipeptide Resin-O-Gly-Ile-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Gly-Ile-Arg(Pbf)-NHFmoc
The tripeptide Resin-O-Gly-Ile-Arg(Pbf)-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.012 g (0.90 mmol, 1.0 equivalent) of Resin-O-Gly-Ile-NH_2, 1.150 g (2.70 mmol, 3.0 equivalents) of HO-Arg(Pbf)-NHFmoc, 367 mg of HOAt (2.70 mmol, 3.0 equivalents), 0.85 mL of DIC (5.40 mmol, 6.0 equivalents) and 4.5 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound tripeptide.

Resin-O-Gly-Ile-Arg(Pbf)-NH_2
The tripeptide Resin-O-Gly-Ile-Arg(Pbf)-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-NHFmoc
The tetrapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.012 g (0.90 mmol, 1.0 equivalent) of Resin-O-Gly-Ile-Arg(Pbf)-NH_2, 0.841 g (2.70 mmol, 3.0 equivalents) of HO-Ala-NHFmoc, 367 mg of HOAt (2.70 mmol, 3.0 equivalents), 0.85 mL of DIC (5.40 mmol, 6.0 equivalents) and 4.5 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound tetrapeptide.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-NH_2
The tetrapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NHFmoc
The pentapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.012 g (0.90 mmol, 1.0 equivalent) of Resin-O-Gly-Ile-Arg(Pbf)-Ala-NH_2, 1.240 g (2.70 mmol, 3.0 equivalents) of HO-Tyr(tBu)-NHFmoc, 365 mg of HOBt (2.70 mmol, 3.0 equivalents), 0.85 mL of DIC (5.40 mmol, 6.0 equivalents) and 4.5 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound pentapeptide.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NH_2
The pentapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NHFmoc
The hexapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.012 g (0.90 mmol, 1.0 equivalent) of Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NH_2, 0.841 g (2.70 mmol, 3.0 equivalents) of HO-Ala-NHFmoc, 365 mg of HOBt (2.70 mmol, 3.0 equivalents), 0.85 mL of DIC (5.40 mmol, 6.0 equivalents) and 4.5 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound hexapeptide.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2
The hexapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NHFmoc

The heptapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 1.012 g (0.90 mmol, 1.0 equivalent) of Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2, 1.265 g (2.70 mmol, 3.0 equivalents) of HO-Lys(Boc)-NHFmoc, 367 mg of HOAt (2.70 mmol, 3.0 equivalents), 0.85 mL of DIC (5.40 mmol, 6.0 equivalents) and 4.5 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound heptapeptide.

Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2
The heptapeptide Resin-O-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.
HO-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2
The double deprotected linear precursor (DDLP) HO-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 was synthesized using the resin-bound peptide prepared from previous step following "Linear peptide cleavage from resin" procedure utilizing 5.0 mL of TFE and 5.0 mL of CH_2Cl_2. The resulting slurry was filtered and dried *in vacuo* to yield HO-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 as a pale yellow solid (172 mg, overall 16%).
LC/MS (ESI): *m/z* calculated $C_{57}H_{92}N_{11}O_{14}S$ [M + H^+] = 1186.65, found 1186.15

HO-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2
The HO-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2 was synthesized utilizing 12.0 mg (0.010 mmol, 1.0 equivalent) of the DDLP HO-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2, 2.00 mL (0.020 mmol, 2.0 equivalents) of Anisole, 0.050 mL of TFA and 0.050 mL of CH_2Cl_2. The resulting slurry was dried *in vacuo*. The residue was redissolved in MeOH, and the solution was centrifuged. The supernatant was injected into the HPLC to yield HO-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2 (4.1 mg, overall 52%).
LC/MS (ESI): *m/z* calculated $C_{35}H_{60}N_{11}O_9$ [M + H^+] = 778.46, found 778.05.
HRMS (ESI-TOF): M+H+, found 778.4564 $C_{35}H_{61}N_{11}O_9$ requires 778.4575
^{1}H NMR (600 MHz, $CDCl_3$, 298K): δ = 0.76-0.81 (t, *J* = 7.37 Hz, 3H, CH_3δ Ile); 0.84-0.87 (d, *J* = 6.72 Hz, 3H, CH_3γ Ile); 1.10-1.16 & 1.40-1.46 (m, 2H, CH_2γ Ile); 1.23-1.25 (d, *J* = 7.11 Hz, 3H, CH_3β Ala); 1.25-1.29 (d, *J* = 7.24 Hz, 3H, CH_3β Ala); 1.26-1.34 (m, 2H, CH_2γ Lys); 1.47-1.60 (m, 2H, CH_2δ Lys); 1.57-1.65 (m, 2H, CH_2γ Arg); 1.65-1.77 (m, 2H, CH_2β Lys); 1.74-1.80 (m, 2H, CH_2β Arg); 1.77-1.83 (m, 2H, CHβ Ile); 2.84-2.90 & 2.92-2.96 (m, 2H, CH_2β Tyr); 2.86-2.92 (m, 2H, CH_2δ Arg); 3.10-3.16 (m, 2H, CH_2ε Lys); 3.81-3.85 & 3.90-3.94 & 3.92-3.96 & 3.97-4.00 (m, 2H, CH_2 Gly); 3.86-3.91 (m, 2H, CHα Arg); 4.12-4.17 (m, 1H, CHα Ile); 4.19-4.25 (m, 1H, CHα Ala); 4.19-4.25 (m, 1H, CHα Lys); 4.27-4.33 (m, 1H, CHα Ala); 4.41-4.46 (m, 1H, CHα Tyr); 6.72-6.76 & 7.13-7.16 (d, J = 8.42 Hz, 2H, Ph Tyr); 7.04-7.09 & 7.19-7.22 (d, J = 8.42 Hz, 2H, Ph Tyr).
^{13}C NMR (150 MHz, $CDCl_3$, 298K): δ = 9.96, 14.58, 16.63, 17.69, 21.06, 24.35, 24.41, 26.25, 28.05, 30.31, 36.11, 36.28, 38.96, 40.37, 40.52, 41.03, 44.53, 49.19, 49.29, 52.66, 53.23, 55.04, 58.08, 115.36, 117.30, 128.01, 130.55, 154.37, 156.69, 162.87, 169.05, 172.44, 173.02, 173.21, 173.69, 173.99, 174.08.

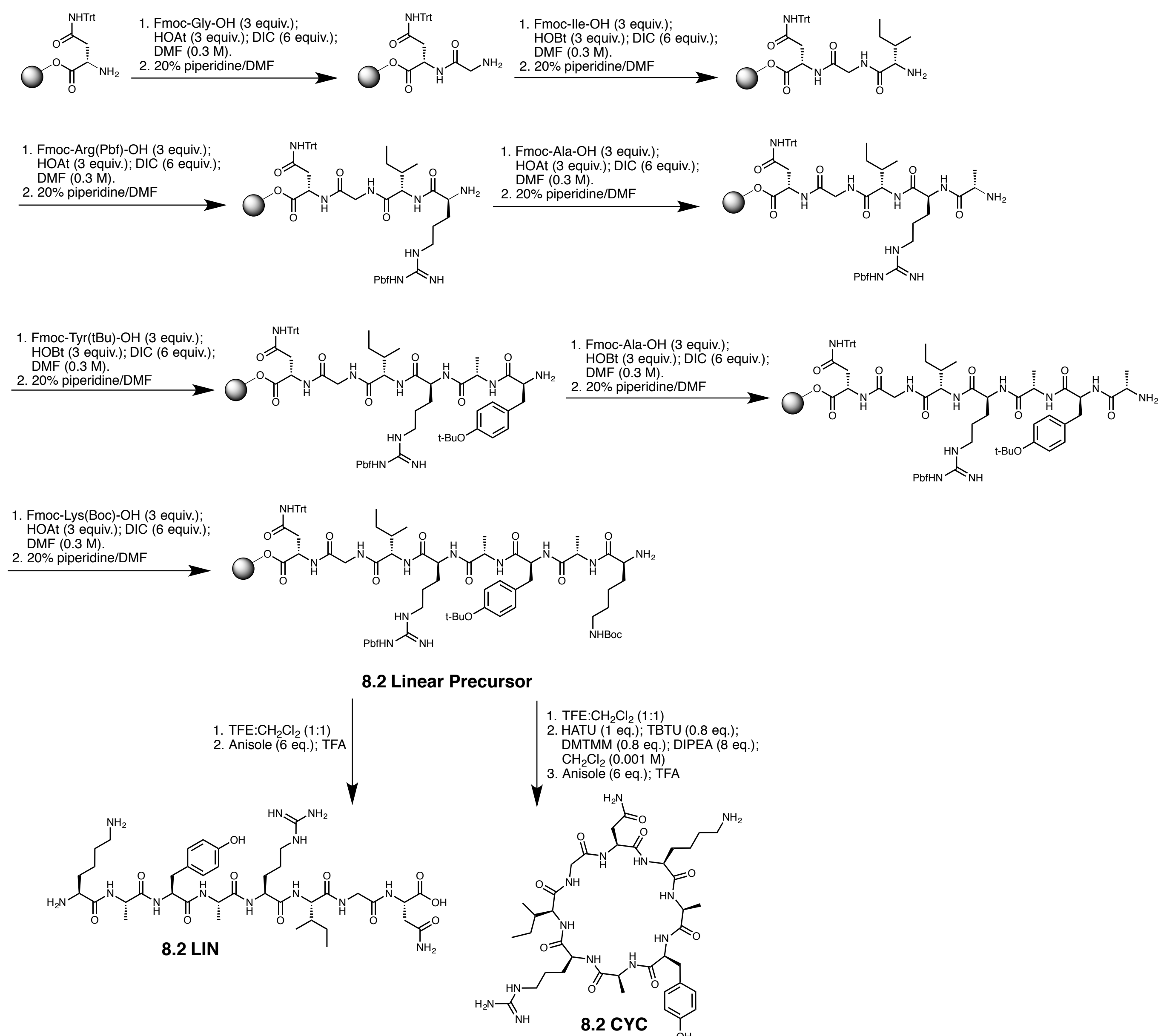
Experimental Procedures for 7.2 CYC

***cyclo*-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)**
The macrocycle Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc) was synthesized following the "Syringe pump macrocyclization" procedure utilizing 67 mg (0.056 mmol, 1.0 equivalent) of DDLP HO-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2, 0.058 mL (0.336 mmol, 6.0 equivalents) of DIPEA, 9.00 mg (0.028 mmol, 0.5 equivalents) of TBTU, 21.3 mg (0.056 mmol, 1.0 equivalents) HATU, and 7.75 mg (0.028 mmol, 0.5 equivalents) of DMTMM in 56 mL CH_2Cl_2. The crude reaction was dried in vacuo to yield 25.4 mg of macrocycle. The macrocycle was taken onto the next reaction without further purification.

***cyclo*-Gly-Ile-Arg-Ala-Tyr-Ala-Lys**
Macrocycle Gly-Ile-Arg-Ala-Tyr-Ala-Lys was synthesized utilizing 25.4 mg (0.022 mmol, 1.0 equivalent) of the Macrocycle Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc), 4.70 mL (0.044 mmol, 2.0 equivalents) of Anisole, 0.11 mL of TFA and 0.11 mL of CH_2Cl_2. The resulting slurry was dried *in vacuo*. The residue was redissolved in MeOH, and the solution was centrifuged. The supernatant was injected into the HPLC to yield Macrocycle Gly-Ile-Arg-Ala-Tyr-Ala-Lys (3.2 mg, overall 19%). LCMS: m/z calcd for $C_{35}H_{58}N_{11}O_8$ (M + H^+) = 760.45, found 760.30.
HRMS (ESI-TOF): M+H+, found 760.4462 $C_{35}H_{59}N_{11}O_8$ requires 760.4470
^{1}H NMR (600 MHz, $CDCl_3$, 308K): δ = 0.90-0.96 (m, 3H, CH_3δ Ile); 0.94-1.01 (m, 3H, CH_3γ Ile); 1.24-1.29 & 1.52-1.56 (m, 2H, CH_2γ Ile); 1.32-1.48 (m, 6H, CH_3β Ala); 1.41-1.51 (m, 2H, CH_2γ Lys); 1.57-1.72 (m, 2H, CH_2δ Lys); 1.69-1.79 (m, 2H, CH_2γ Arg); 1.79-1.84 & 1.85-1.98 (m, 2H, CH_2β Lys); 1.71-1.76 & 1.85-1.90 (m, 2H, CH_2β Arg); 1.90-1.97 (m, 2H, CHβ Ile); 3.01-3.08 (m, 2H, CH_2β Tyr); 3.22-3.30 (m, 2H, CH_2δ Arg); 3.46-3.50 & 3.52-3.58 (m, 2H, CH_2ε Lys); 3.99-4.09 & 4.12-4.23 (m, 2H, CH_2 Gly); 3.93-3.95 & 4.00-4.03 (m, 2H, CHα Arg); 4.12-4.18 & 4.25-4.28 (m, 1H, CHα Ala); 4.20-4.28 (m, 1H, CHα Ile); 4.31-4.39 (m, 1H, CHα Ala); 4.31-4.34 & 4.35-4.44 (m, 1H, CHα Lys); 4.43-4.45 & 4.50-4.60 (m, 1H, CHα Tyr); 6.83-6.93 (m, 2H, Ph Tyr); 7.10-7.20 (m, 2H, Ph Tyr).

^{13}C NMR (150 MHz, $CDCl_3$, 308K): δ = 10.11, 14.68, 16.24, 16.67, 21.94, 24.71, 24.51, 25.02, 26.27, 28.03, 30.72, 36.14, 39.10, 40.50, 41.00, 42.40, 43.80, 46.10, 49.50, 50.80, 52.10, 53.20, 54.10, 55.00, 55.30, 55.40, 58.40, 115.43, 130.66, 154.37, 156.69, 162.87, 169.05, 172.44, 173.02, 173.21, 173.69, 173.99, 174.08.

<u>Synthesis of 8.2 LIN and 8.2 CYC</u>



<u>Experimental Procedures for 8.2 LIN</u>

Resin-O-Asn(Trt)-Gly-NHFmoc

The dipeptide Resin-O-Asn(Trt)-Gly-NHFmoc was synthesized following the "Solid phase peptide synthesis" procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-NH_2, 0.589 g (1.98 mmol, 3.0 equivalents) of HO-Gly-NHFmoc, 268 mg of HOBt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound dipeptide.

Resin-O-Asn(Trt)-Gly-NH_2

The dipeptide Resin-O-Asn(Trt)-Gly-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Asn(Trt)-Gly-Ile-NHFmoc

The tripeptide Resin-O-Asn(Trt)-Gly-Ile-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-Gly-NH_2, 0.70 g (1.98 mmol, 3.0 equivalents) of HO-Ile-NHFmoc, 268 mg of HOBt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound tripeptide.

Resin-O-Asn(Trt)-Gly-Ile-NH_2
The tripeptide Resin-O-Asn(Trt)-Gly-Ile-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-NHFmoc
The tetrapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-Gly-Ile-NH_2, 0.845 g (1.98 mmol, 3.0 equivalents) of HO-Arg(Pbf)-NHFmoc, 269 mg of HOAt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound tetrapeptide.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2
The tetrapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-NHFmoc
The pentapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-NH_2, 0.616 g (1.98 mmol, 3.0 equivalents) of HO-Ala-NHFmoc, 269 mg of HOAt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound pentapeptide.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-NH_2
The pentapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NHFmoc
The hexapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-NH_2, 0.910 g (1.98 mmol, 3.0 equivalents) of HO-Tyr(tBu)-NHFmoc, 268 mg of HOBt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound hexapeptide.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NH_2
The hexapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NHFmoc
The heptapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-NH_2, 0.616 g (1.98 mmol, 3.0 equivalents) of HO-Ala-NHFmoc, 268 mg of HOBt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound heptapeptide.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2
The heptapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2 was synthesized following the “Fmoc removal” procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NHFmoc
The octapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NHFmoc was synthesized following the “Solid phase peptide synthesis” procedure utilizing 0.984 g (0.66 mmol, 1.0 equivalent) of Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-NH_2, 0.928 g (1.98 mmol, 3.0 equivalents) of HO-Lys(Boc)-NHFmoc, 269 mg of

HOAt (1.98 mmol, 3.0 equivalents), 0.62 mL of DIC (3.96 mmol, 6.0 equivalents) and 3.3 mL of DMF (0.2 M). The reaction was run for 2 hr and a negative ninhydrin test was performed to verify the reaction completion. The reaction mixture was drained to give the Fmoc-protected resin-bound octapeptide.

Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2

The octapeptide Resin-O-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 was synthesized following the "Fmoc removal" procedure. A positive ninhydrin test was performed to verify the completion of Fmoc removal.

HO-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2

The double deprotected linear precursor (DDLP) HO-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 was synthesized using the resin-bound peptide prepared from previous step following "Linear peptide cleavage from resin" procedure utilizing 6.5 mL of TFE and 6.5 mL of CH_2Cl_2. The resulting slurry was filtered and dried *in vacuo* to yield HO-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2 as a pale yellow solid (691 mg, overall 68%).
LC/MS (ESI): *m/z* calculated $C_{80}H_{112}N_{13}O_{16}S$ [M + H^+] = 1542.81, found 1542.25

HO-Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2

The HO-Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2 was synthesized utilizing 43.7 mg (0.028 mmol, 1.0 equivalent) of the DDLP HO-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2, 6.00 mL (0.056 mmol, 2.0 equivalents) of Anisole, 0.140 mL of TFA and 0.140 mL of CH_2Cl_2. The resulting slurry was dried *in vacuo*. The residue was redissolved in MeOH, and the solution was centrifuged. The supernatant was injected into the HPLC to yield HO-Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2 (15.9 mg, overall 63%).
LC/MS (ESI): *m/z* calculated $C_{39}H_{66}N_{13}O_{11}$ [M + H^+] = 892.50, found 893.30.
HRMS (ESI-TOF): M+H+, found 892.4990 $C_{39}H_{67}N_{13}O_{11}$ requires 892.5004
^{1}H NMR (600 MHz, $CDCl_3$, 298K): δ = 0.75-0.80 (t, *J* = 7.41 Hz, 3H, CH_3δ Ile); 0.82-0.85 (d, *J* = 6.83 Hz, 3H, CH_3γ Ile); 1.07-1.15 & 1.37-1.44 (m, 2H, CH_2γ Ile); 1.22-1.27 (dd, *J* = 10.88, 7.29 Hz, 3H, CH_3β Ala); 1.22-1.34 (m, 2H, CH_2γ Lys); 1.47-1.60 (m, 2H, CH_2δ Lys); 1.55-1.63 (m, 2H, CH_2γ Arg); 1.62-1.79 (m, 2H, CH_2β Lys); 1.71-1.81 (m, 2H, CH_2β Arg); 1.73-1.83 (m, 2H, CHβ Ile); 2.71-2.81 (m, 2H, CH_2β Asn); 2.82-2.98 (m, 2H, CH_2β Tyr); 2.85-2.92 (m, 2H, CH_2δ Arg); 3.08-3.16 (m, 2H, CH_2ε Lys); 3.84-3.94 (m, 2H, CH_2 Gly); 3.84-3.92 (m, 2H, CHα Arg); 4.08-4.12 (d, J = 8.65 Hz, 1H, CHα Ile); 4.17-4.24 (m, 1H, CHα Ala); 4.19-4.27 (m, 1H, CHα Lys); 4.26-4.33 (m, 1H, CHα Ala); 4.39-4.47 & 4.48-4.52 (m, 1H, CHα Tyr); 4.65-4.73 (m, 1H, CHα Asn); 6.72-6.76 & 7.12-7.15 (d, J = 8.29 Hz, 2H, Ph Tyr); 7.03-7.06 & 7.17-7.20 (d, J = 8.29 Hz, 2H, Ph Tyr).
^{13}C NMR (150 MHz, $CDCl_3$, 298K): δ = 9.99, 14.65, 16.63, 21.09, 24.34, 24.50, 26.26, 28.05, 30.32, 36.03, 36.25, 38.99, 40.53, 42.18, 49.24, 49.28, 52.67, 53.19, 54.65, 55.03, 58.29, 113.40, 115.33, 117.26, 119.23, 121.41, 128.01, 130.49, 154.38, 156.69, 162.78, 169.04, 170.73, 172.46, 173.30, 173.77, 173.80, 173.95, 174.03, 174.06, 174.65.

Experimental Procedures for 8.2 CYC

***cyclo*-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)**

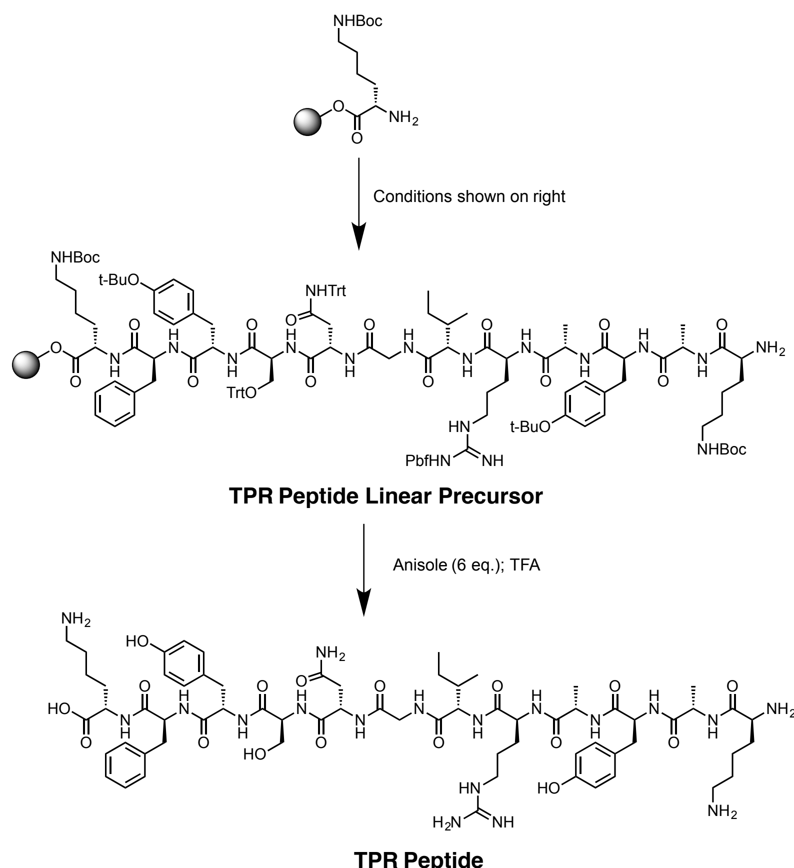
The macrocycle Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc) was synthesized following the "**Syringe pump macrocyclization**" procedure utilizing 321 mg (0.208 mmol, 1.0 equivalent) of DDLP HO-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc)-NH_2, 0.220 mL (1.25 mmol, 6.0 equivalents) of DIPEA, 33.5 mg (0.104 mmol, 0.5 equivalents) of TBTU, 79.2 mg (0.208 mmol, 1.0 equivalents) HATU, and 29.0 mg (0.104 mmol, 0.5 equivalents) of DMTMM in 208 mL CH_2Cl_2. The crude reaction was dried in vacuo to yield 361 mg of macrocycle. The macrocycle was taken onto the next reaction without further purification.

***cyclo*-Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys**

Macrocycle Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys was synthesized utilizing 107 mg (0.070 mmol, 1.0 equivalent) of the Macrocycle Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(tBu)-Ala-Lys(Boc), 15.0 mL (0.140 mmol, 2.0 equivalents) of Anisole, 0.35 mL of TFA and 0.35 mL of CH_2Cl_2. The resulting slurry was dried *in vacuo*. The residue was redissolved in MeOH, and the solution was centrifuged. The supernatant was injected into the HPLC to yield Macrocycle Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys (11.4 mg, overall 19%). LCMS: m/z calcd for $C_{39}H_{64}N_{13}O_{10}$ (M + H^+) = 874.49, found 874.15.
HRMS (ESI-TOF): M+H+, found 874.4880 $C_{39}H_{65}N_{13}O_{10}$ requires 874.4899
^{1}H NMR (600 MHz, $CDCl_3$, 308K): δ = 0.89-0.95 (m, 3H, CH_3δ Ile); 0.92-0.98 (m, 3H, CH_3γ Ile); 1.27-1.30 & 1.44-1.46 (d, *J* = 7.27 Hz, 3H, CH_3β Ala); 1.36-1.42 (m, 3H, CH_3β Ala); 1.21-1.26 & 1.53-1.61 (m, 2H, CH_2γ Ile); 1.38-1.55 (m, 2H, CH_2γ Lys); 1.62-1.72 (m, 2H, CH_2δ Lys); 1.65-1.77 (m, 2H, CH_2γ Arg); 1.77-1.81 & 1.90-1.94 (m, 2H, CH_2β Lys); 1.78-1.88 (m, 2H, CH_2β Arg); 1.87-1.94 (m, 2H, CHβ Ile); 2.80-2.91 (m, 2H, CH_2β Asn); 2.98-3.09 (m, 2H, CH_2β Tyr); 3.17-3.30 (m, 2H, CH_2δ Arg); 3.20-3.28 (m, 2H, CH_2ε Lys); 3.62-3.66 & 3.72-3.81 (m, 1H, CHα Arg); 3.81-3.84 & 3.85-3.89 & 3.99-4.03 & 4.12-4.15 & 4.17-4.19 (m, 2H, CH_2 Gly); 4.10-4.16 & 4.23-4.30 & 4.32-4.40 (m, 1H, CHα Ala); 4.15-4.19 & 4.21-4.29 (m, 1H, CHα Ile); 4.22-4.27 & 4.34-4.41 (m, 1H, CHα Lys); 4.49-4.53 & 4.54-4.59 & 4.59-4.64 (m, 1H, CHα Tyr); 4.71-4.75 & 4.77-4.83 & 4.86-4.92 (m, 1H, CHα Asn); 6.84-6.96 (m, 2H, Ph Tyr); 7.12-7.23 (m, 2H, Ph Tyr).
^{13}C NMR (150 MHz, $CDCl_3$, 308K): δ = 10.13, 14.66, 15.47, 15.61, 16.37, 17.70, 22.15, 24.37, 24.87, 24.99, 26.12, 29.04, 29.90, 35.70, 36.03, 39.22, 40.54, 42.55, 42.75, 42.42, 42.80, 49.42, 50.08, 50.22, 50.55, 51.26, 51.91,

52.71, 53.04, 53.28, 54.36, 54.40, 54.97, 55.16, 58.35, 59.10, 113.40, 115.40, 117.26, 119.23, 121.41, 128.01, 130.31, 154.38, 156.69, 162.78, 169.04, 170.73, 172.46, 173.30, 173.77, 173.80, 173.95, 174.03, 174.06, 174.65.

Synthesis of TPR Peptide



Coupling Reaction Conditions

1. (a) Fmoc-Phe-OH (3 equiv.); HOAt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
2. (a) Fmoc-Tyr(t-Bu)-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
3. (a) Fmoc-Ser(Trt)-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
4. (a) Fmoc-Asn(Trt)-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
5. (a) Fmoc-Gly-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
6. (a) Fmoc-Ile-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
7. (a) Fmoc-Arg(Pbf)-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
8. (a) Fmoc-Ala-OH (3 equiv.); HOAt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
9. (a) Fmoc-Tyr(t-Bu)-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
   (b) 20% Piperidine in DMF
10. (a) Fmoc-Ala-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
    (b) 20% Piperidine in DMF
11. (a) Fmoc-Lys(Boc)-OH (3 equiv.); HOBt (3 equiv.); DIC (6 equiv.) in DMF (0.2 M)
    (b) 20% Piperidine in DMF

Experimental Procedures for TPR Peptide

Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2

Resin-bound dodecapeptide Resin-O-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 was synthesised using a Biotage Initiator + Alstra Automated Microwave Peptide Synthesiser. 0.50 g H-Lys(Boc)-2-ClTrt resin was weighed and added to a 10 mL-capacity dedicated reactor vial. All amino acids were pre-dissolved in DMF to produce 3 mL solutions with concentrations of 0.25 M. Coupling reagents HOBt and HOAt were dissolved separately in a mix of DIC and DMF (1:1 v/v) also with concentrations of 0.25 M. The system was set up according to the manufacturer's protocol.
The resin underwent sequential coupling reactions with each amino acid as described in the *Coupling Reaction* procedure, with the following modifications:

- Reaction time was shortened to 35 minutes
- Reaction temperature was increased to 70°C

Fmoc removal washes were performed as described in the *Fmoc Removal* procedure. Once the final amino acid coupling reaction was complete, the system automatically performed the pre-cleavage washes as described in the *Cleavage* procedure. Once the synthesis was complete, the resin-bound peptide was removed from the system and dried *in vacuo* overnight.

HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2

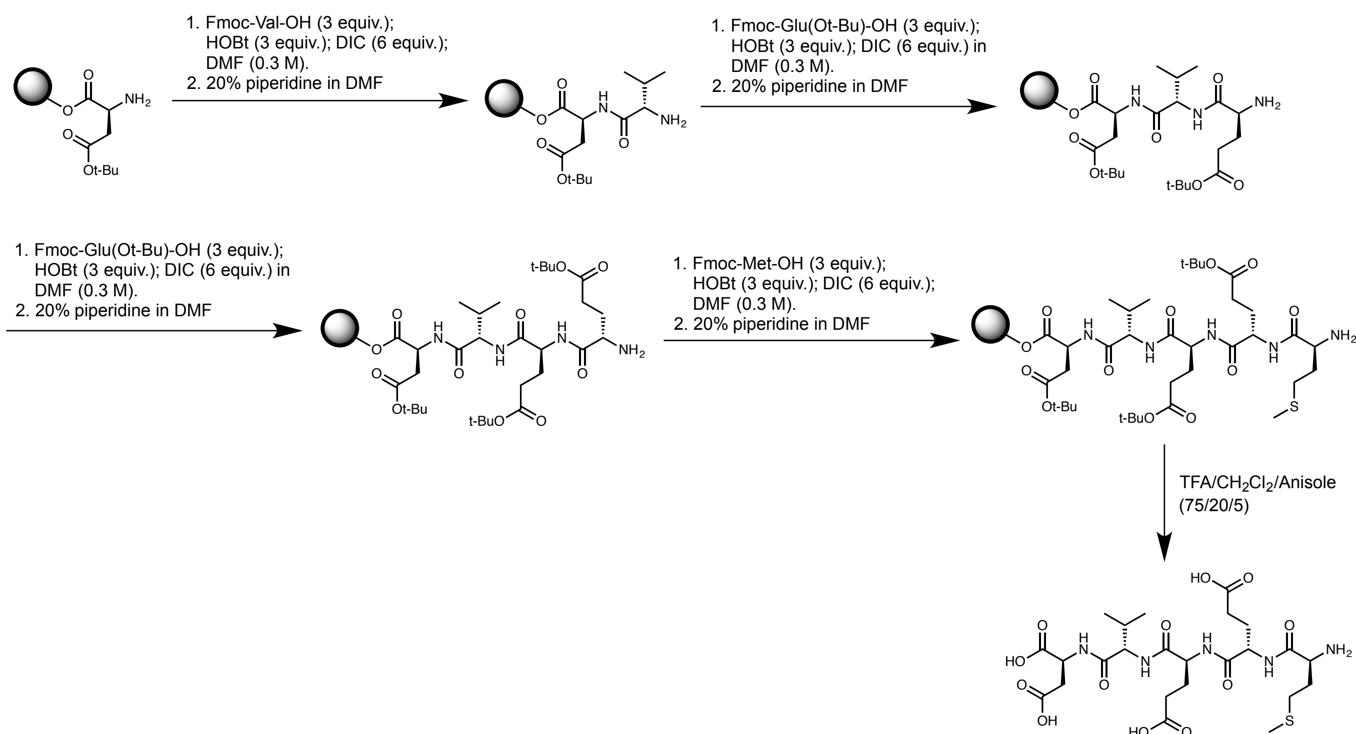
The linear dodecapeptide HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 was generated following the *Cleavage* procedure. The linear peptide was cleaved from the resin using a mixed solution of 3.3 mL of TFE and 3.3 mL of CH_2Cl_2. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 (156 mg, overall 25%).

HO-Lys-Phe-Tyr-Ser-Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2

The free linear peptide HO-Lys-Phe-Tyr-Ser-Asn-Gly-Ile-Arg-Ala-Tyr-Ala-Lys-NH_2 was generated by removing the side chain protecting groups on HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-

Lys(Boc)-NH_2. 156 mg HO-Lys(Boc)-Phe-Tyr(t-Bu)-Ser(Trt)-Asn(Trt)-Gly-Ile-Arg(Pbf)-Ala-Tyr(t-Bu)-Ala-Lys(Boc)-NH_2 (0.063 mmol, 1 eq.) was deprotected using a mixture of 624 µL of TFA and anisole (14 eq.).
The free linear peptide was then purified by washing with MeOH. MeOH was added to the crude, dry linear peptide and the suspension was vortexed before undergoing centrifugation. The supernatant was removed and this process was repeated until the supernatant was observed to be clear and colourless for 3 successive washes. This generated pure final compound **12 LIN** in a 16% yield as a white solid.
LC/MS (ESI) *m/z*: $[M+3H]^{3+}$ calcd for $C_{66}H_{100}N_{18}O_{17}$, 473.25; found, 473.15.
HRMS (ESI-TOF) *m/z*: $[M+2H]^{2+}$ calcd for $C_{66}H_{100}N_{18}O_{17}$, 709.3800; found, 709.3831.
^1H NMR (600 MHz, D_2O) δ 7.56-7.21 (m, 5H, Phe), 7.21-7.01 (dd, *J* = 8.43, 16.29 Hz, 4H, δH Tyr), 7.01-6.92 (dd, *J* = 8.35, 21.19 Hz, 4H, εH Tyr), 4.91-4.87 (t, *J* = 6.12 Hz, 1H, αH Asn), 4.57-4.52 (m, 1H, αH Ser), 4.57-4.52 (m, 1H, αH Ala), 4.51-4.46 (m, 1H, αH Arg), 4.51-4.46 (m, 1H, αH Ala), 4.42-4.32 (m, 1H, αH Ile), 4.42-4.32 (t, 1H, αH Lys), 4.23-4.04 (m, 1H, αH Lys), 4.23-4.04 (m, 2H, $αCH_2$ Gly), 3.96-3.87 (m, 2H, $βCH_2$ Ser), 3.38-3.24 (m, 2H, $δCH_2$ Arg), 3.38-3.24 & 3.24-2.84 (m, 2H, $βCH_2$ Phe), 3.24-2.84 (m, 4H, $εCH_2$ Lys), 3.24-2.84 (m, 4H, $βCH_2$ Tyr), 3.24-2.84 (m, 2H, $βCH_2$ Asn), 2.08-1.93 (m, 1H, βH Ile), 2.08-1.93 & 1.93-1.80 (m, 2H, $βCH_2$ Arg), 2.08-1.93 & 1.93-1.80 (m, 4H, $βCH_2$ Lys), 1.93-1.80 (m, 4H, $δCH_2$ Lys), 1.80-1.68 (m, 2H, $γCH_2$ Arg), 1.67-1.59 & 1.39-1.29 (m, 2H, $γCH_2$ Ile), 1.59-1.43 (m, 4H, $γCH_2$ Lys), 1.59-1.43 (m, 6H, CH_3 Ala), 1.11-1.05 (d, *J* = 6.72 Hz, 3H, $δCH_3$ Ile), 1.05-0.98 (t, *J* = 7.38 Hz, 3H, $γCH_3$ Ile).

Synthesis of MEEVD Peptide



Experimental Procedures for MEEVD Peptide

Resin-O-Asp(Ot-Bu)-Val-NH₂

The resin-bound dipeptide Resin-O-Asp(O-tBu)-Val-NH_2 was synthesised following the *Coupling Reaction* procedure using 1.0 g H-Asp(Ot-Bu)-2-ClTrt resin (0.50 mmol, 1 eq.), 0.51 g Fmoc-Val-OH (1.5 mmol, 3 eq.), 0.20 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run over 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O- Asp(O-tBu)-Val-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Asp(Ot-Bu)-Val-NH_2.

Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-NH_2

The resin-bound tripeptide Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Asp(Ot-Bu)-Val-NH_2 synthesised from previous coupling reaction, together with 0.64 g Fmoc-Glu(Ot-Bu)-OH (1.5 mmol, 3 eq.), 0.20 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative

ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-NH_2.

Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-NH_2

The resin-bound tetrapeptide Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-NH_2 synthesised from previous coupling reaction, together with 0.64 g Fmoc-Glu(Ot-Bu)-OH (1.5 mmol, 3 eq.), 0.20 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-NH_2.

Resin-O-Asp(O-tBu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-Met-NH_2

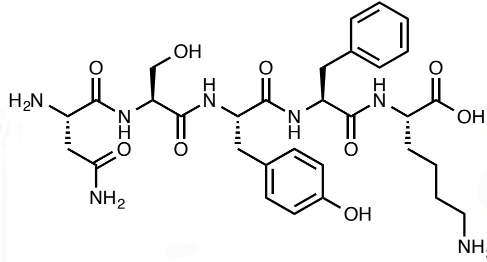
The resin-bound pentapeptide Resin-O-Asp(O-tBu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-Met-NH_2 was synthesised following the *Coupling Reaction* procedure using Resin-O-Asp(Ot-Bu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-NH_2 synthesised from previous coupling reaction, together with 0.56 g Fmoc-Met-OH (1.5 mmol, 3 eq.), 0.20 g HOBt (1.5 mmol, 3 eq.), 0.46 mL DIC (3.0 mmol, 6 eq.) and 5.0 mL DMF to generate a concentration of 0.30 M. The coupling reaction was run for 4 hours and a negative ninhydrin test was used to confirm the reaction was complete. The reaction mixture was then drained to produce Resin-O-Asp(O-tBu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-Met-NHFmoc. The Fmoc protecting group was removed following the *Fmoc Removal* procedure and a positive ninhydrin test was used to confirm complete removal, producing Resin-O-Asp(O-tBu)-Val-Glu(Ot-Bu)-Glu(Ot-Bu)-Met-NH_2.

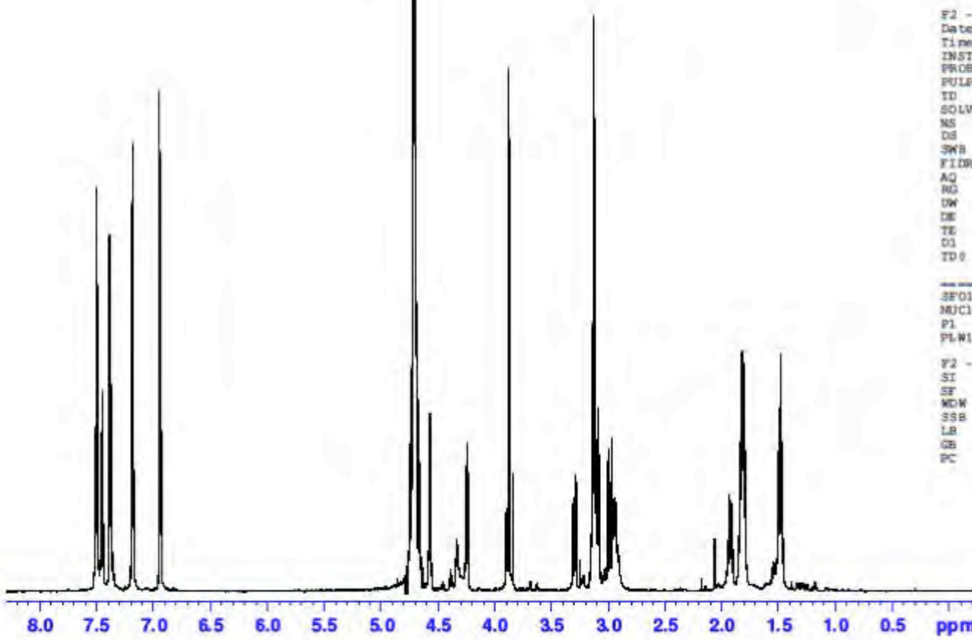
HO-Asp-Val-Glu-Glu-Met-NH_2

The linear pentapeptide HO-Asp-Val-Glu-Glu-Met-NH_2 was generated by simultaneously cleaving the resin and deprotecting the side chain protecting groups using a mixture of TFA/CH_2Cl_2/Anisole (75/24/1) at a concentration of 10 mL/g of resin. The resin-containing solution was filtered and dried *in vacuo* to yield HO-Asp-Val-Glu-Glu-Met-NH_2 as a white solid (560 mg, overall 81%). The free linear peptide was then precipitated in methanol and pelleted by centrifugation then dried *in vacuo* to generate pure final compound **MEEVD Peptide** in a 60% yield as a white solid.

^{1}H NMR (600 MHz, D_2O) δ 4.49-4.44 (dd, *J* = 7.6, 5.1 Hz, 1H, αH Asp), 4.38-4.29 (m, 2H, αH Glu), 4.13-4.05 (dd, *J* = 7.7, 6.0 Hz, 1H, αH Val), 3.68-3.60 (m, 1H, αH Met), 2.81-2.66 (m, 2H, βCH_2 Asp), 2.60-2.49 (m, 2H, γCH_2 Met), 2.44-2.27 (m, 4H, γCH_2 Glu), 2.16-2.08 & 2.08-1.97 (m, 3H, δCH_3 Met), 2.08-1.97 (m, 5H, βCH_2 Glu & αH Val), 1.96-1.86 (m, 2H, βCH_2 Met), 0.90-0.84 (dd, *J* = 7.7, 6.8 Hz, 6H, γCH_3 Val).

5.1 LIN: ^{1}H NMR and ^{13}C NMR





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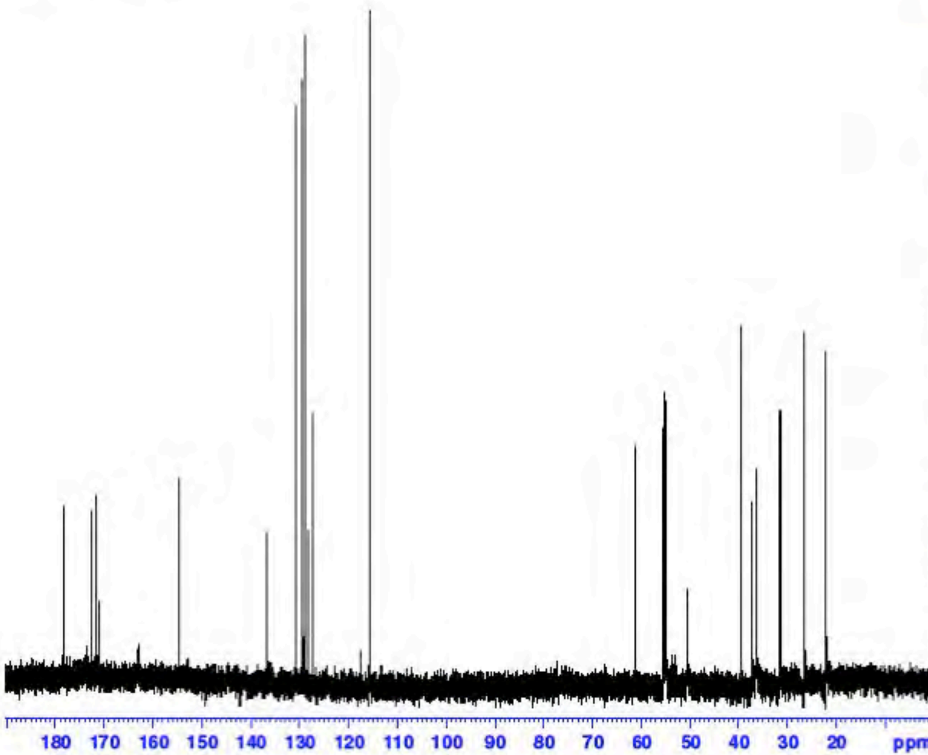

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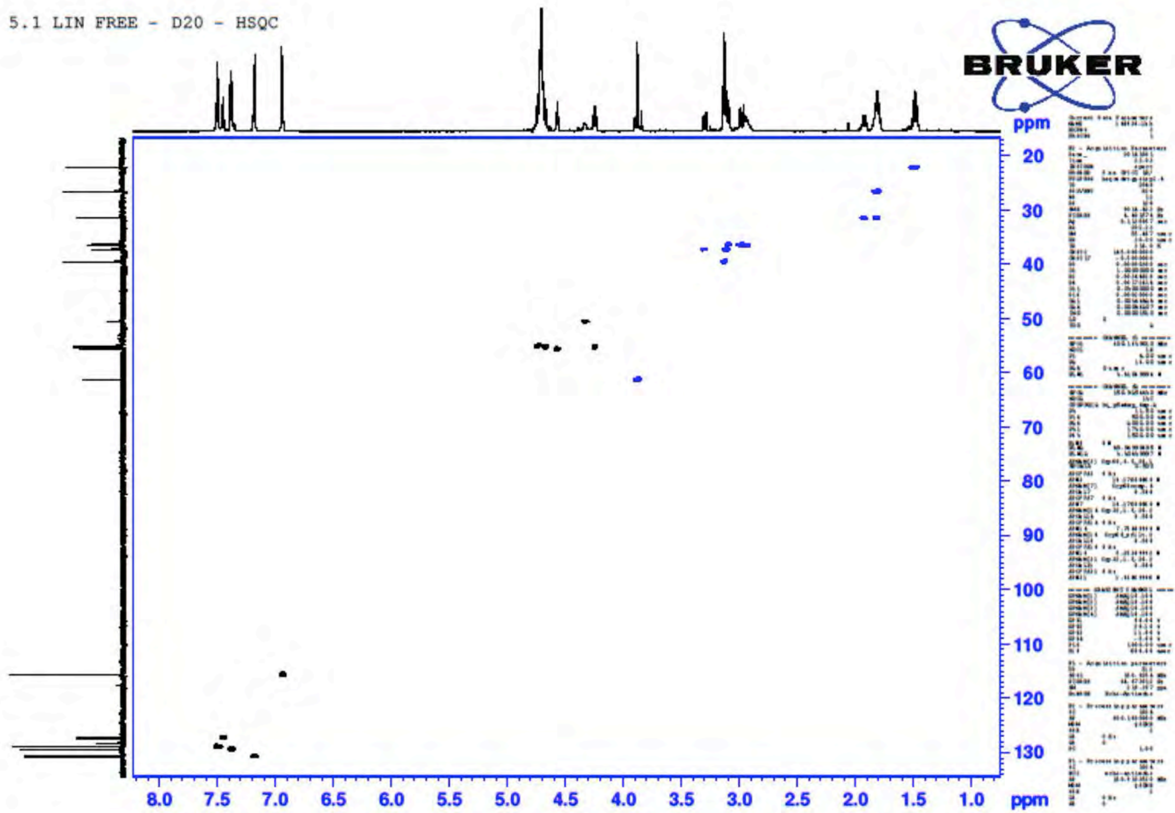
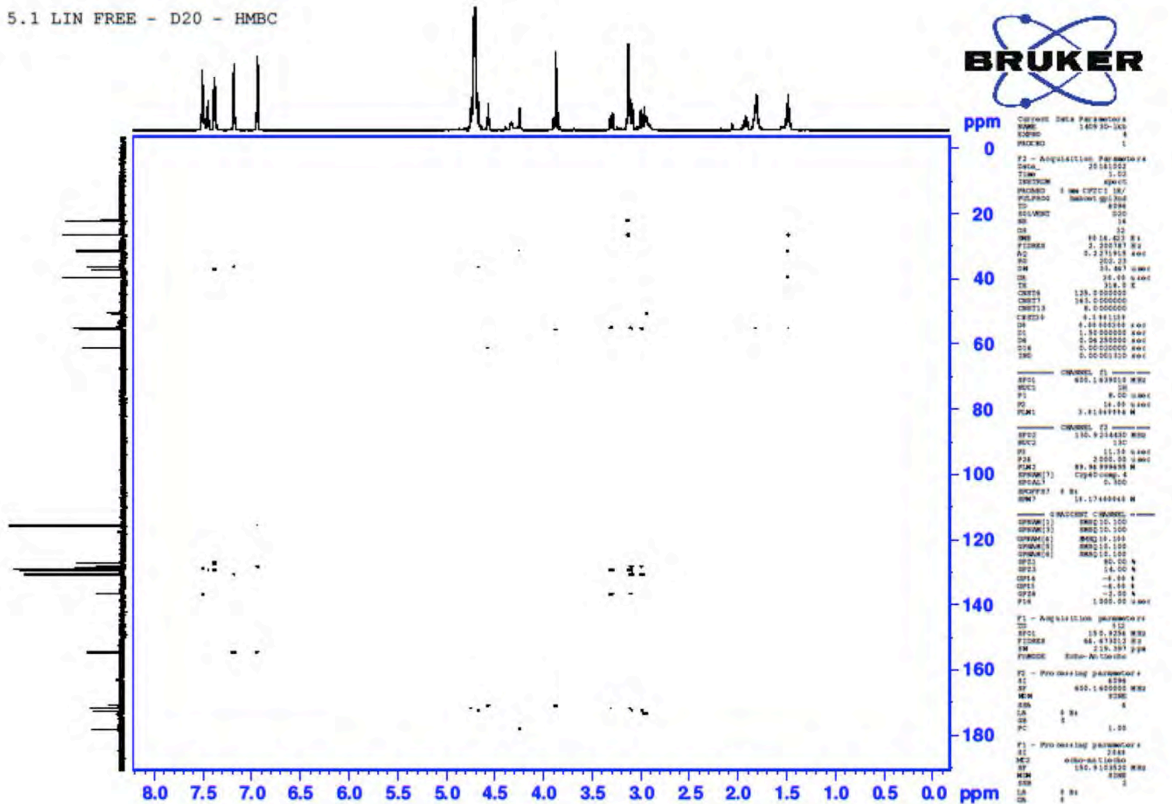
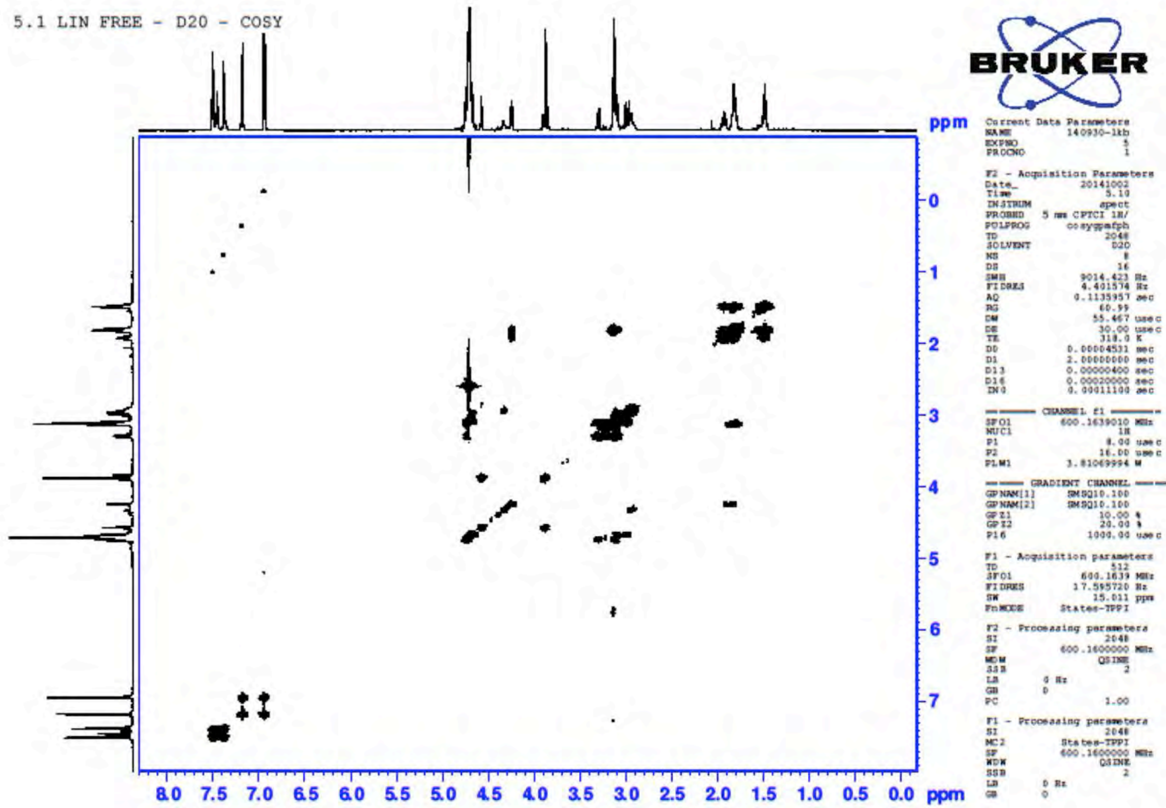
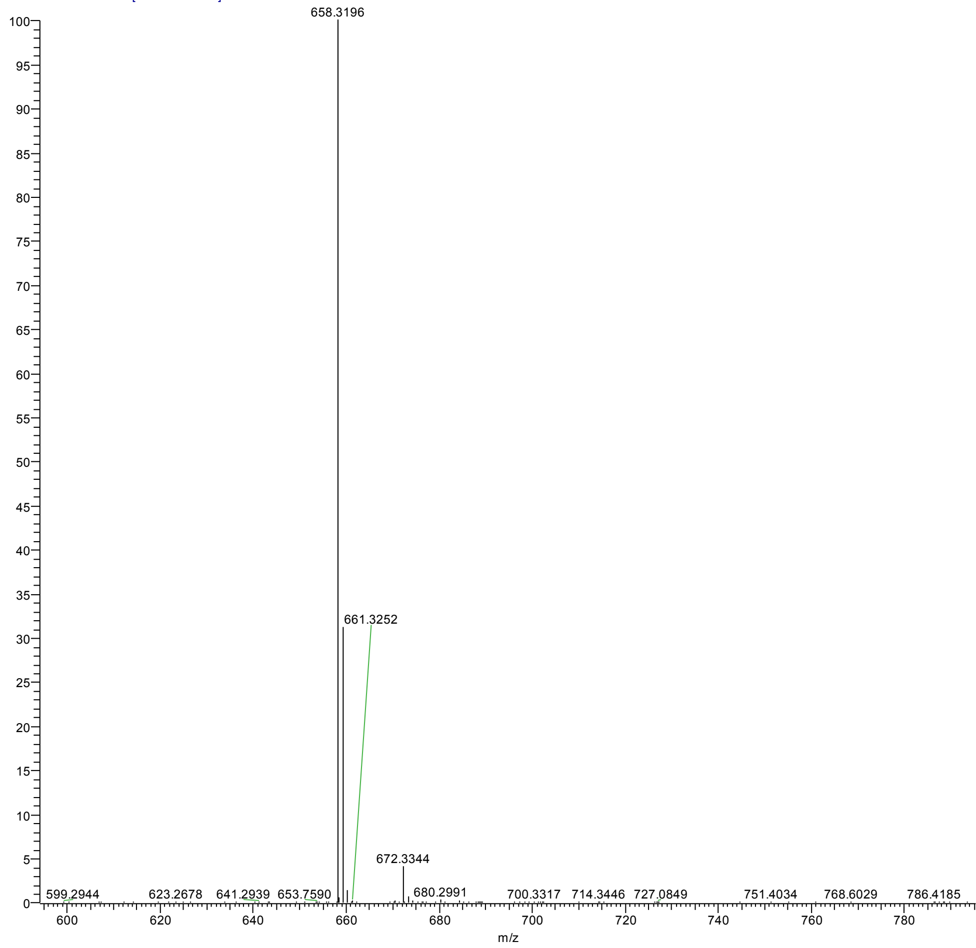
5.1 LIN: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





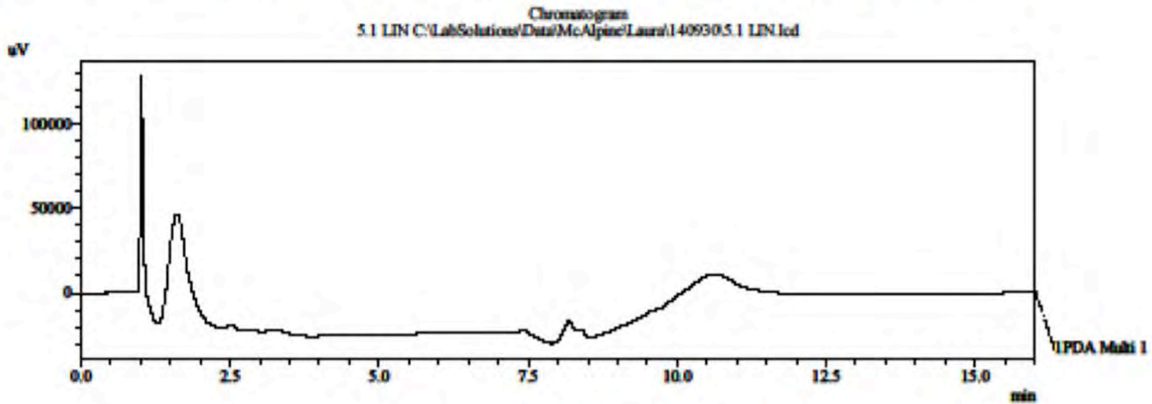
5.1 LIN: ^{1}H-^{1}H COSY NMR and HRMS

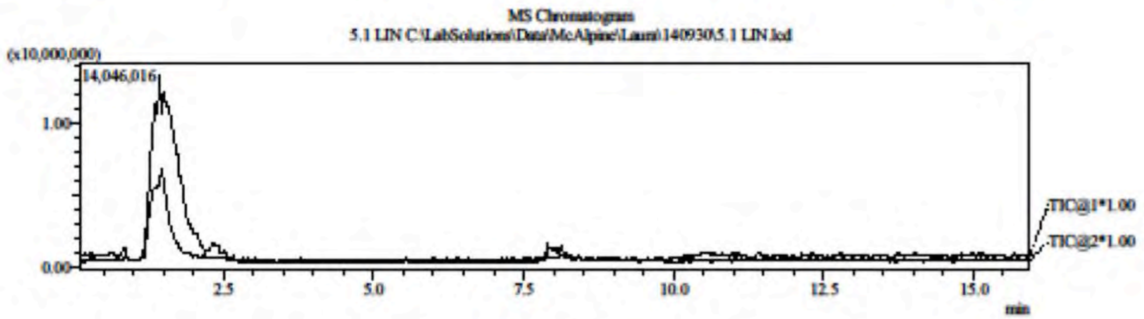


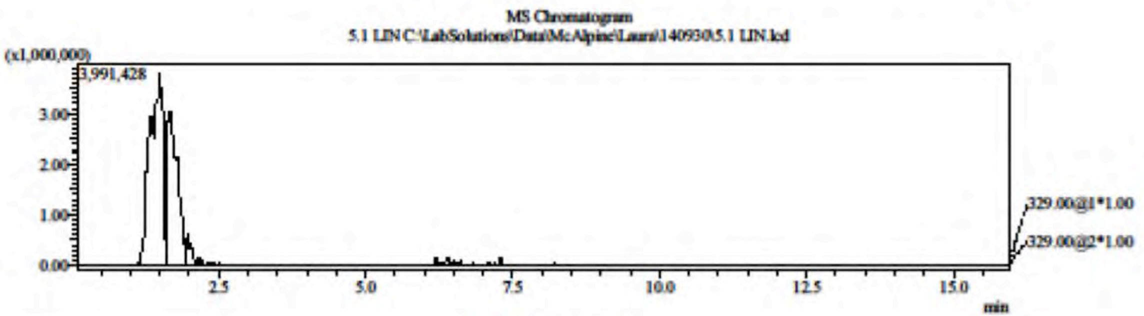


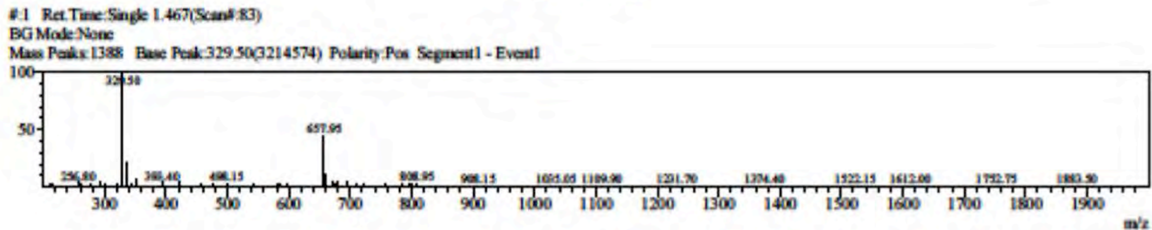
5.1 LIN: LC/MS

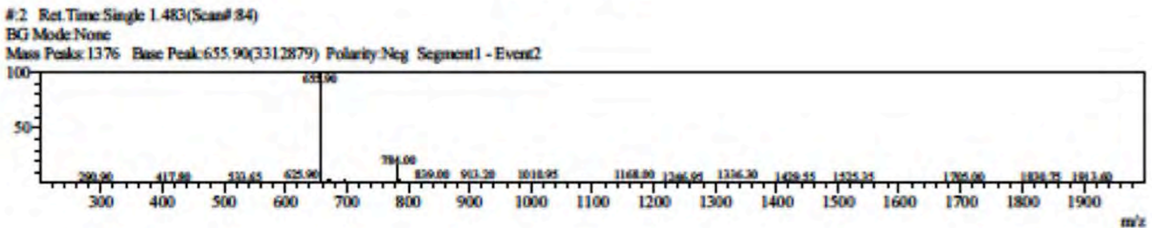
==== Shimadzu LCMSsolution Analysis Report ====











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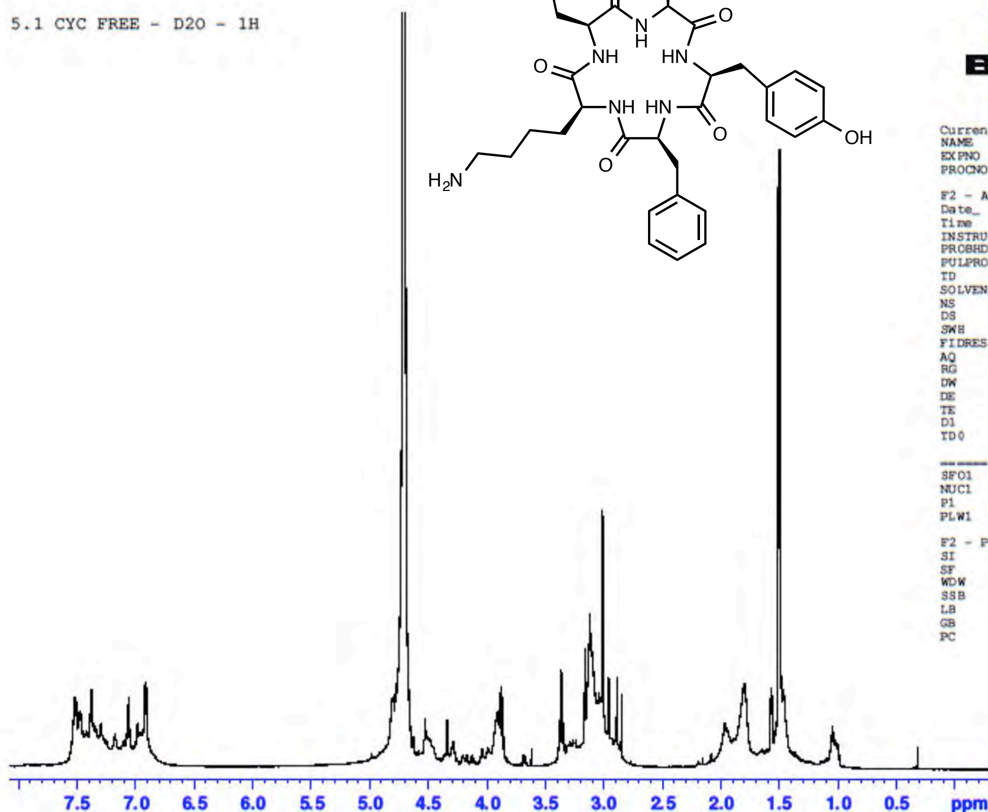

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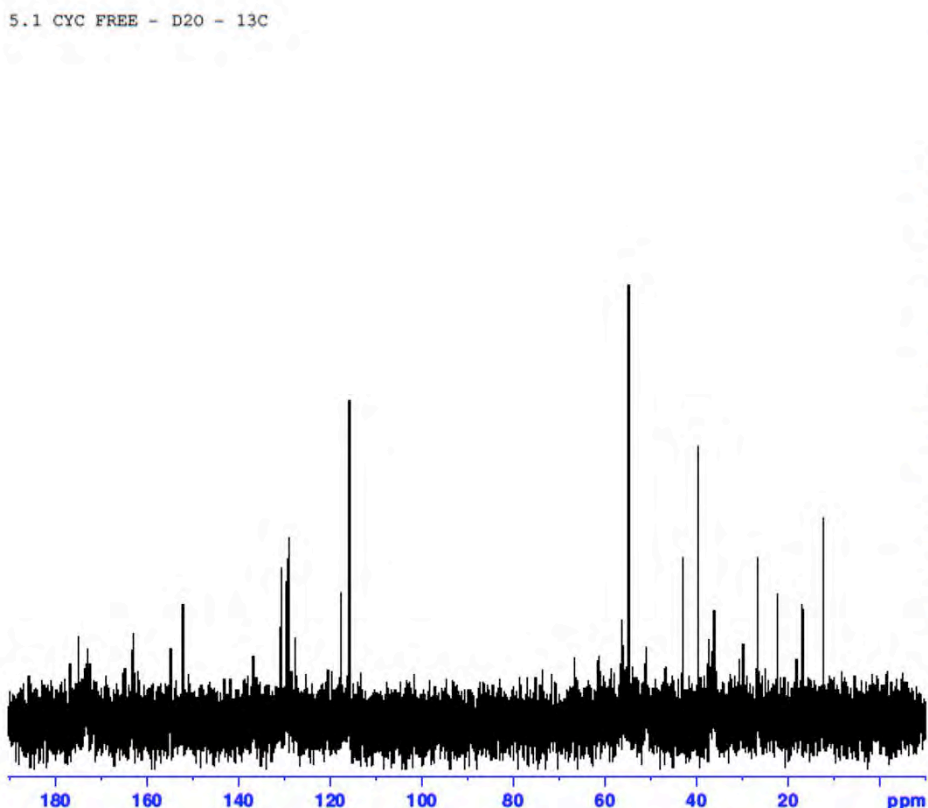

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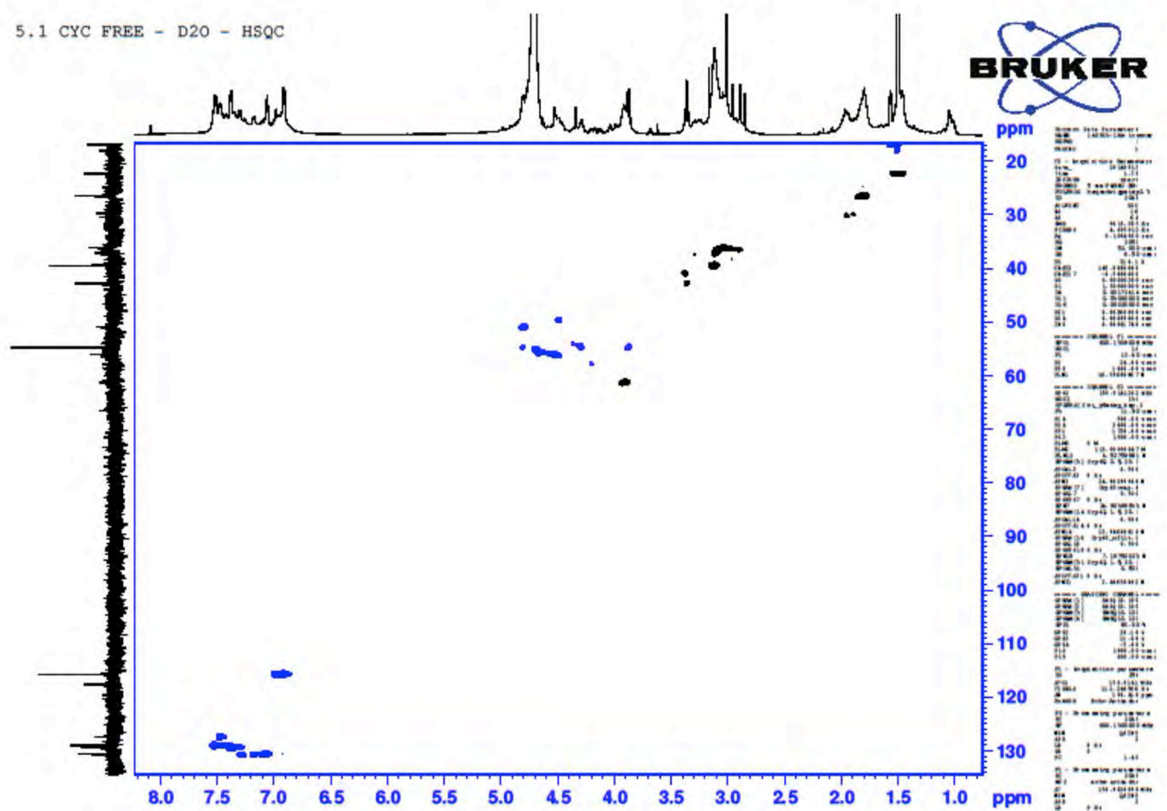
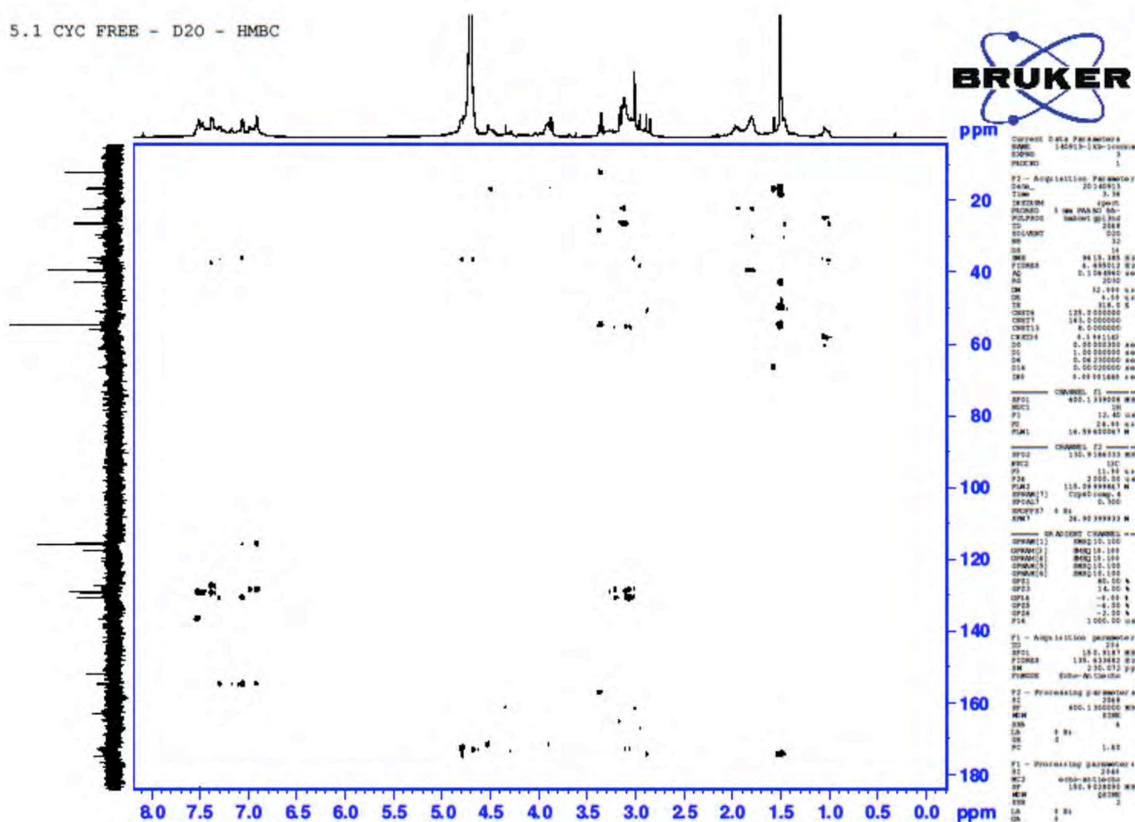
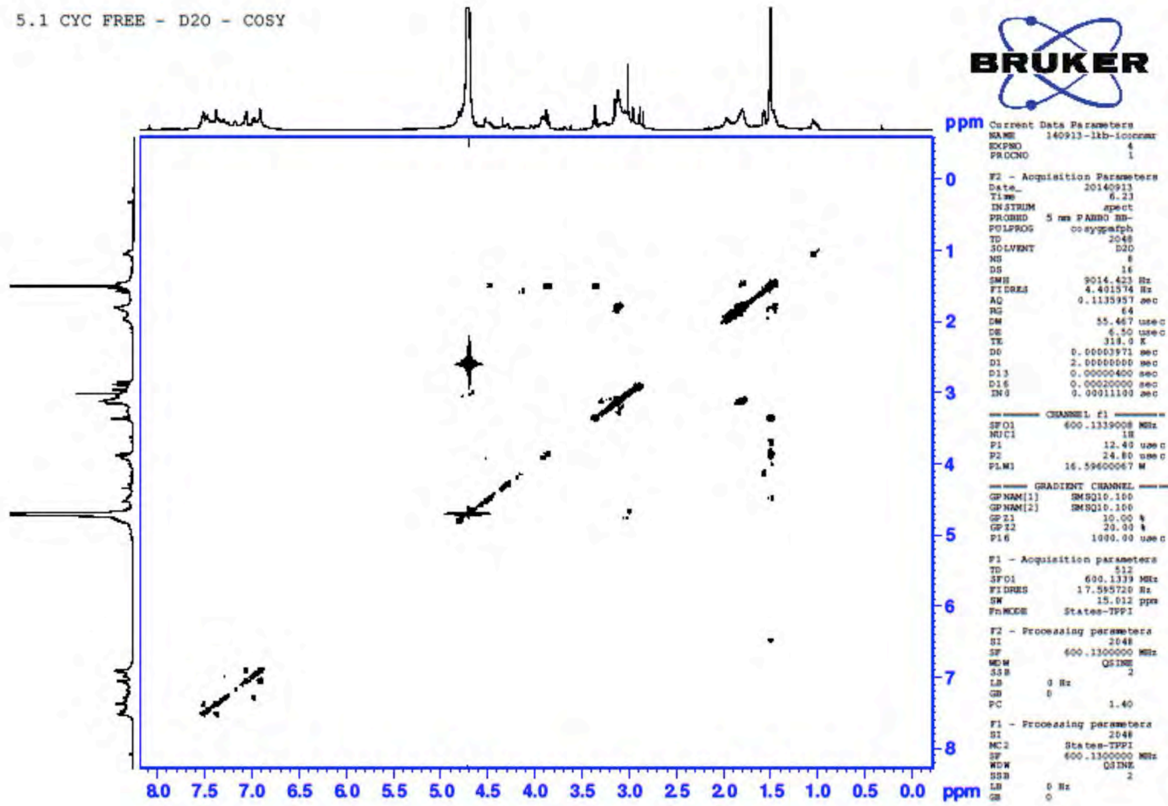
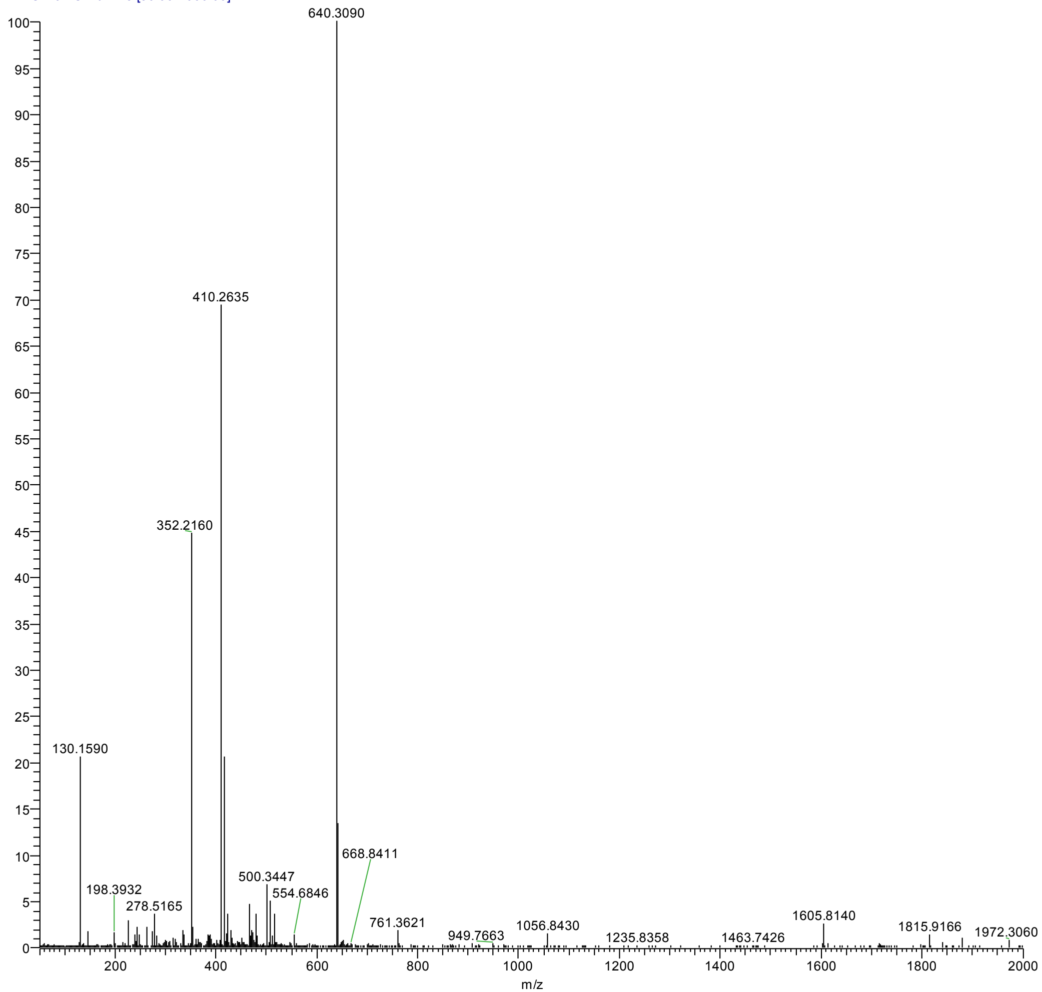
5.1 CYC: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





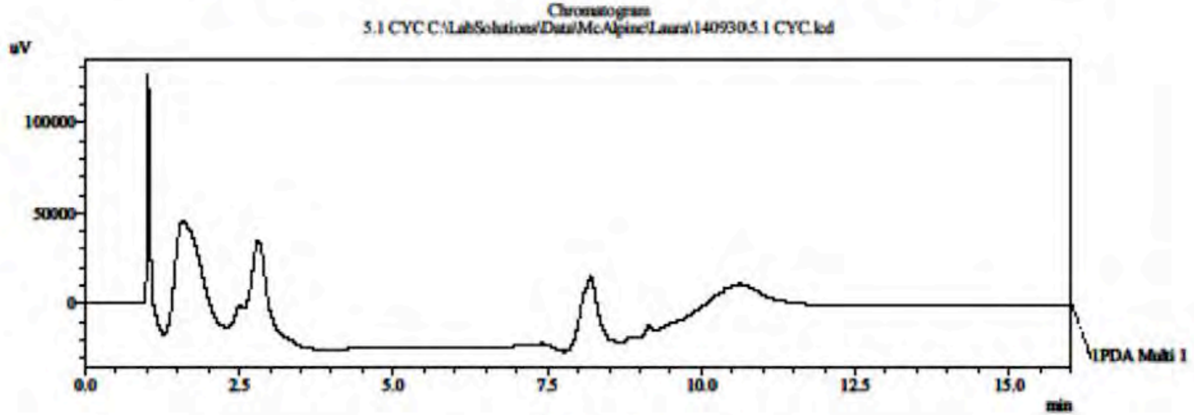
5.1 CYC: ^{1}H-^{1}H COSY NMR and HRMS

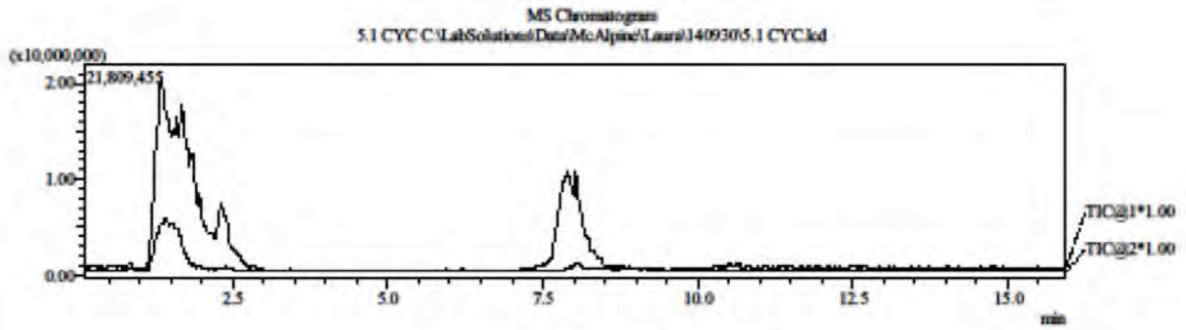


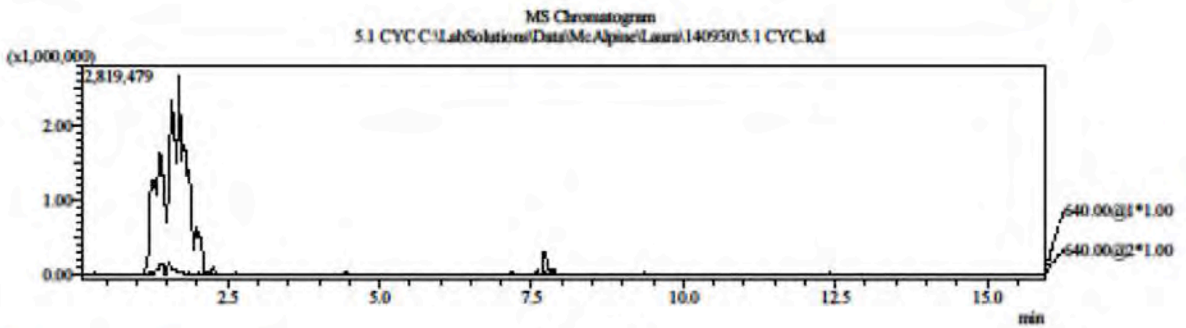


5.1 CYC: LC/MS

==== Shimadzu LCMSsolution Analysis Report ====







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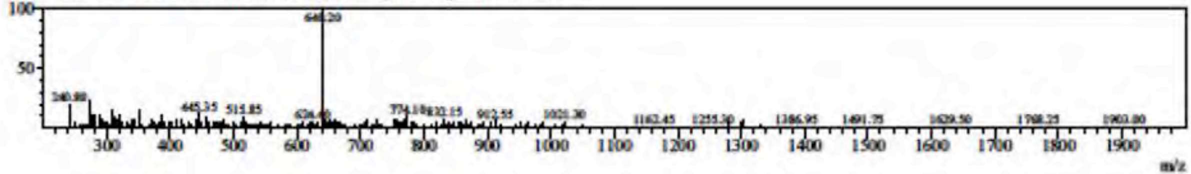

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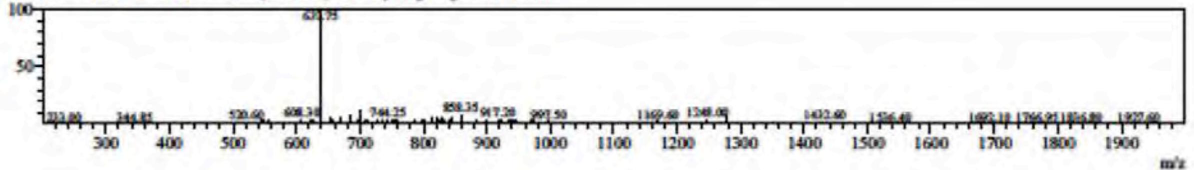
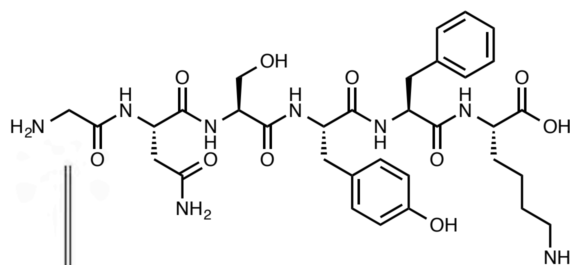
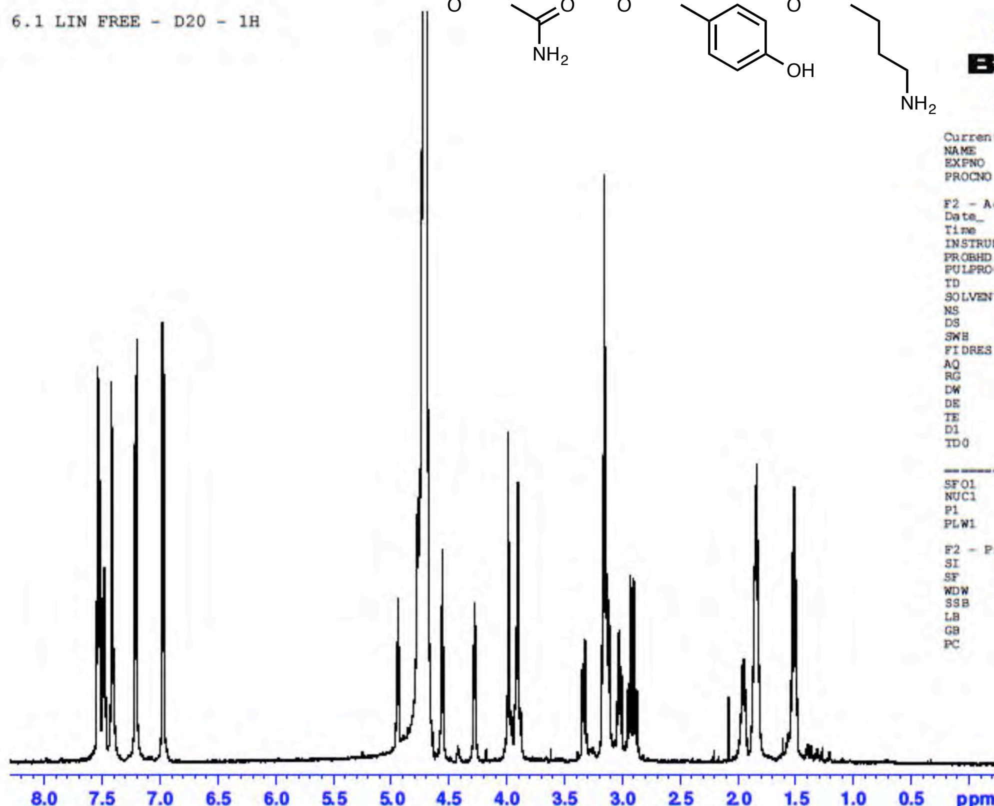


6.1 LIN: ^{1}H NMR and ^{13}C NMR





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TE                  318.1 K
D1            5.00000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          600.1339008 MHz
NUC1                   1H
P1                  12.40 usec
PLW1          16.59600067 W

F2 - Processing parameters
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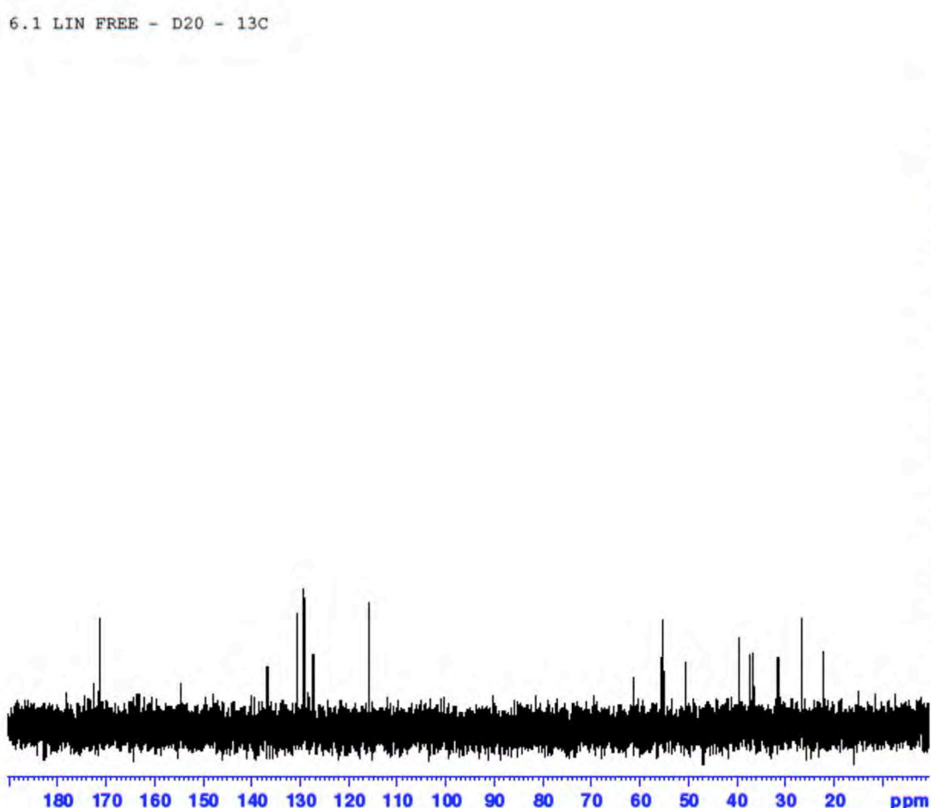

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FIDRES           0.461455 Hz
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D11           0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          150.9163903 MHz
NUC1                  13C
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PLW1         115.09999847 W

======== CHANNEL f2 ========
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NUC2                   1H
CPDPRG[2   bi_waltz65_256
PCPD2               70.00 usec
PLW2          16.59600067 W
PLW12          0.52078003 W
PLW13          0.25518000 W

F2 - Processing parameters
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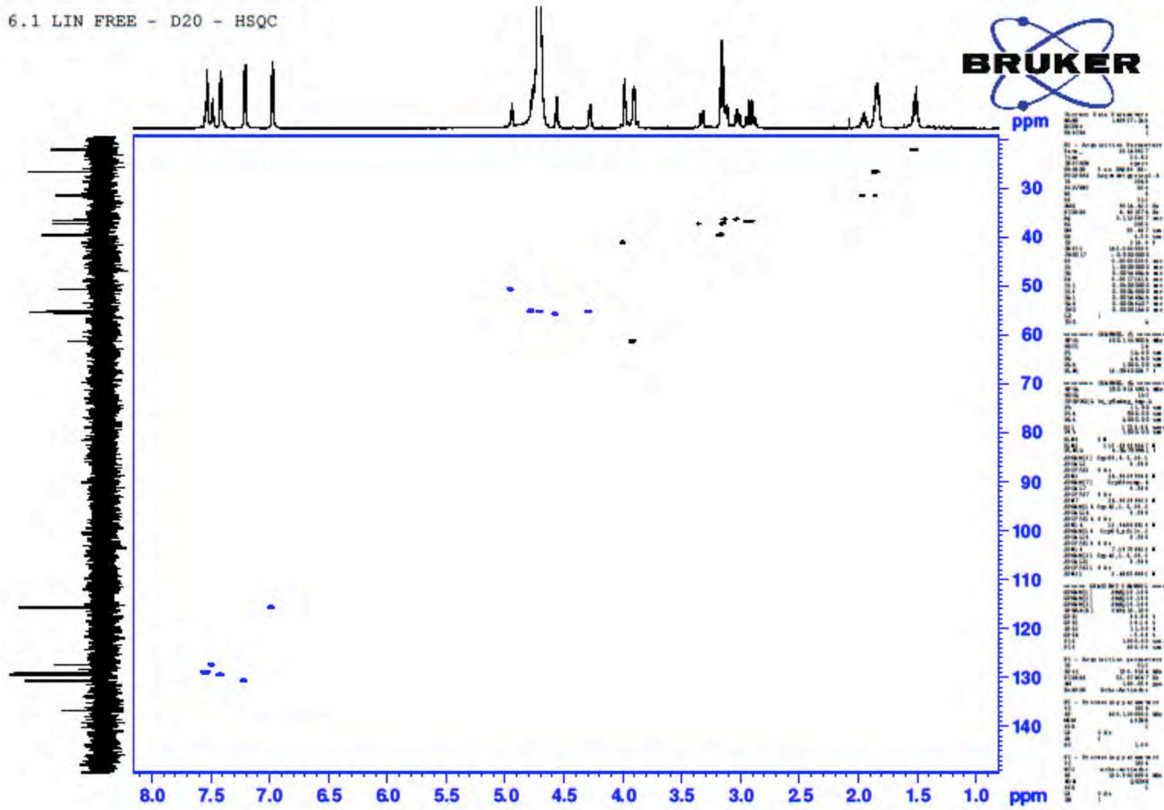
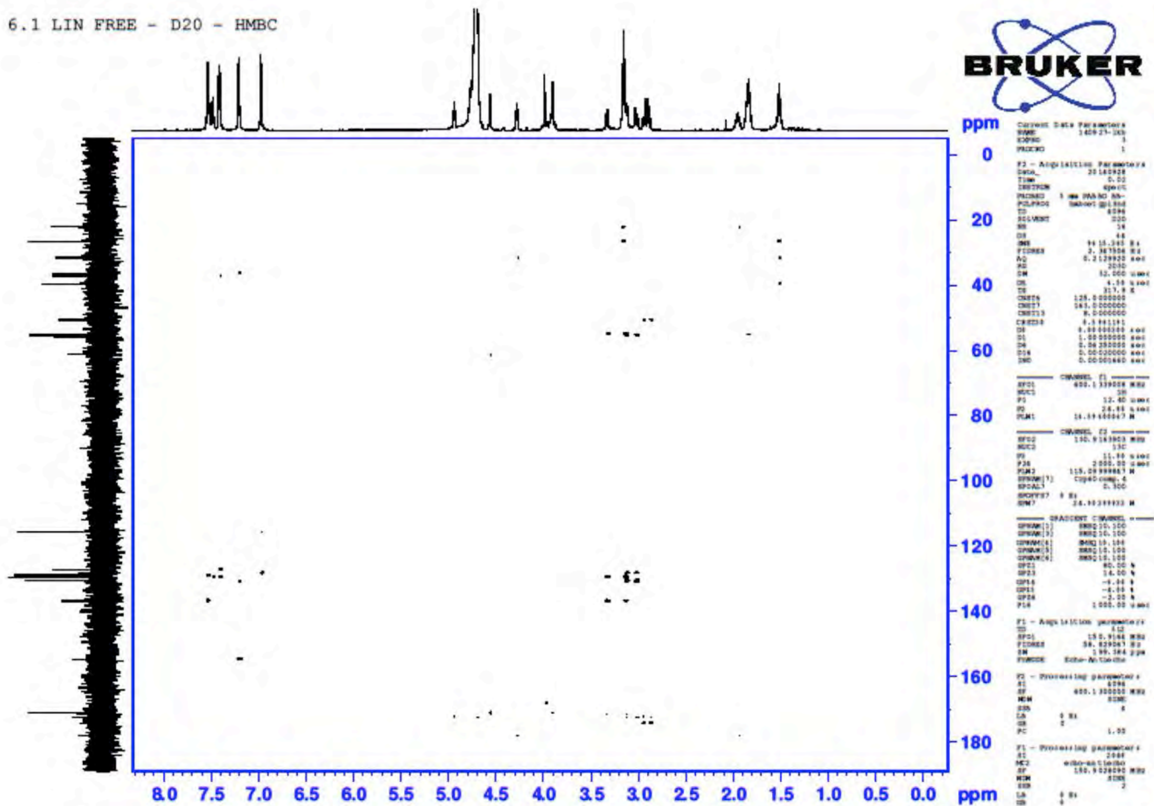
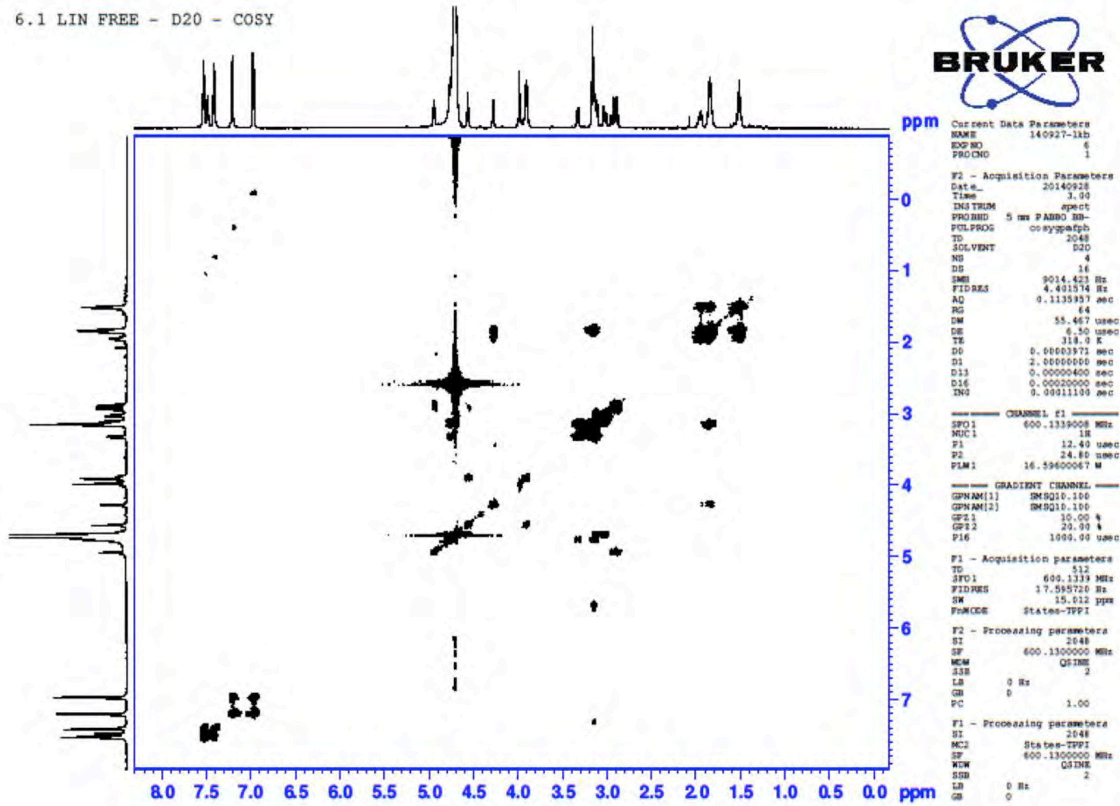
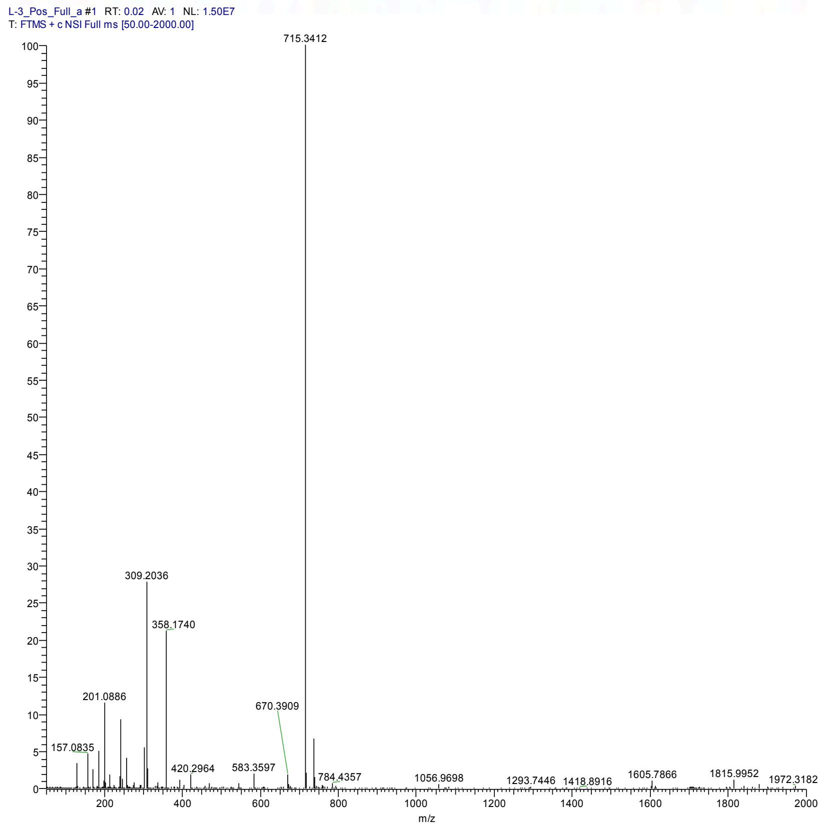
6.1 LIN: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





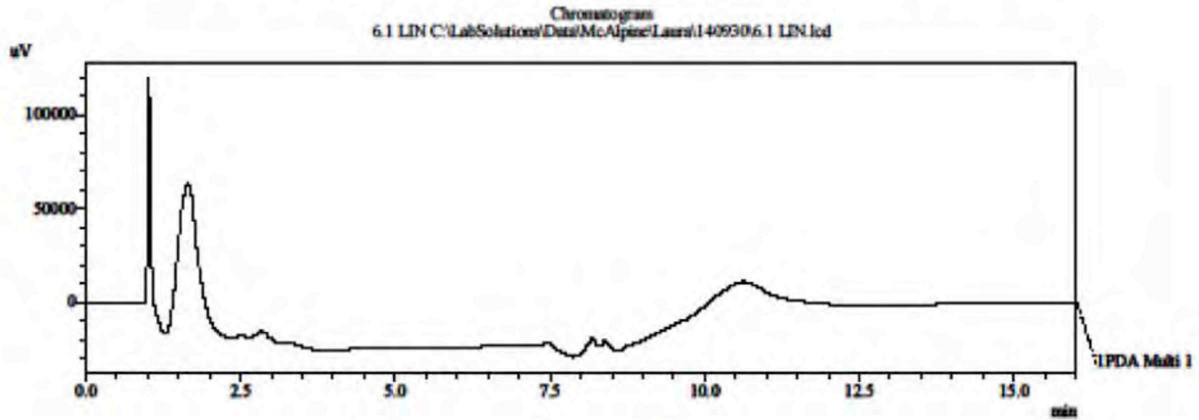
6.1 LIN: ^{1}H-^{1}H COSY NMR and HRMS

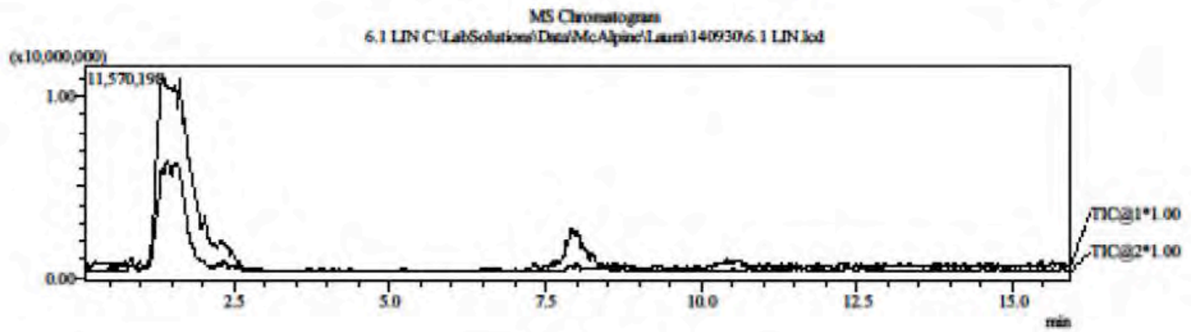


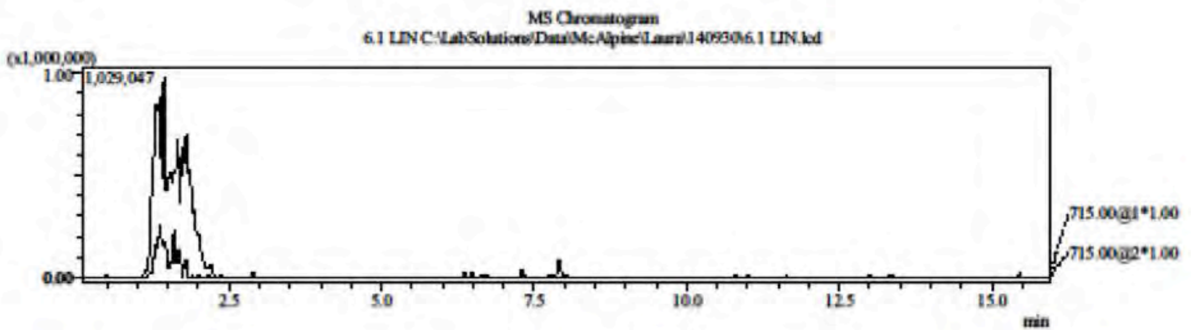


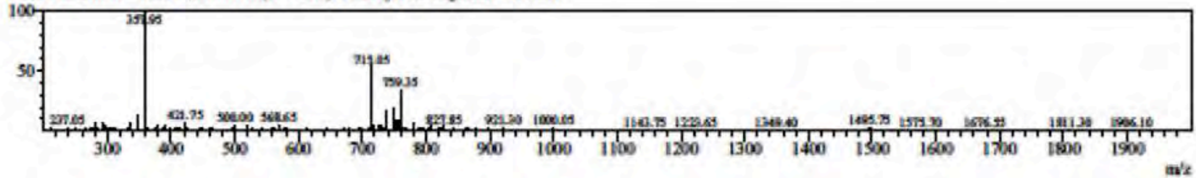
6.1 LIN: LC/MS

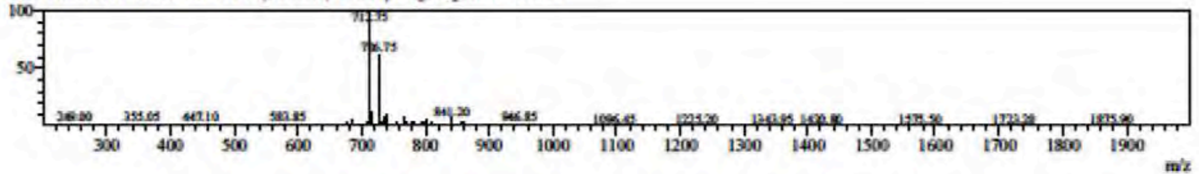
==== Shimadzu LCMSsolution Analysis Report ====



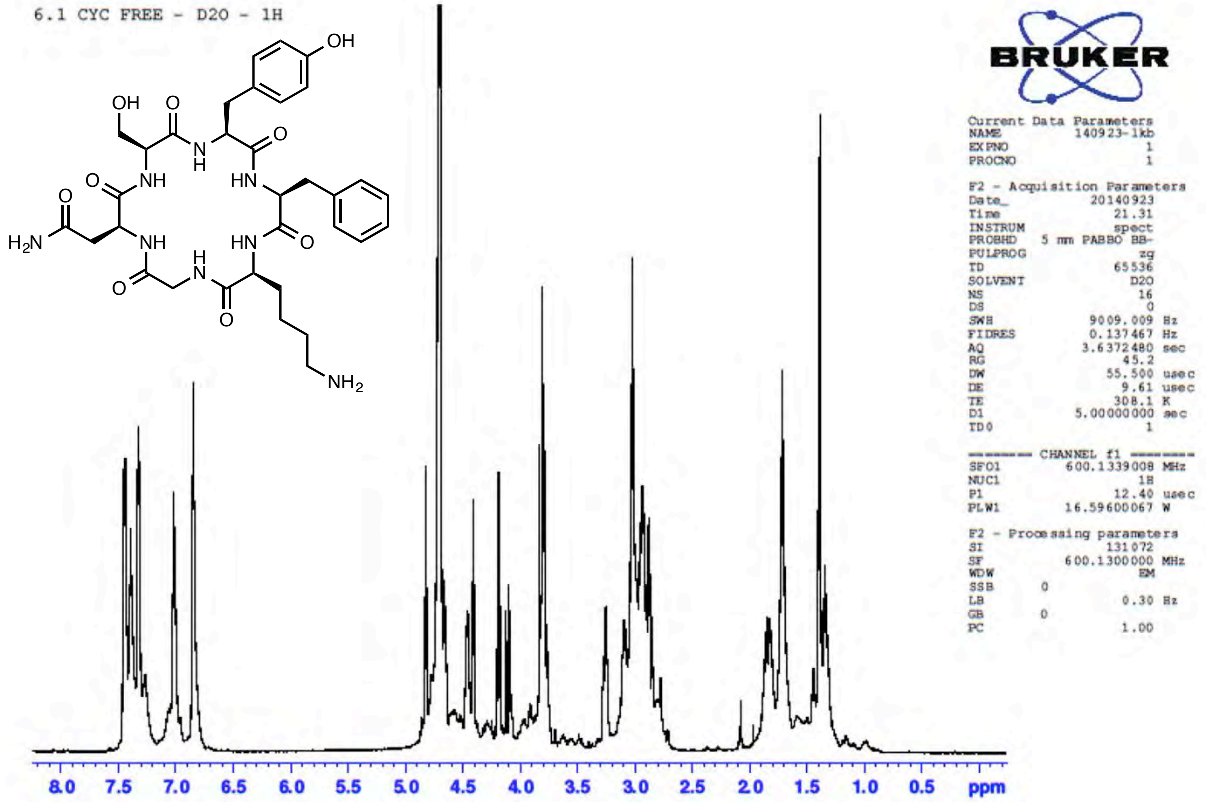


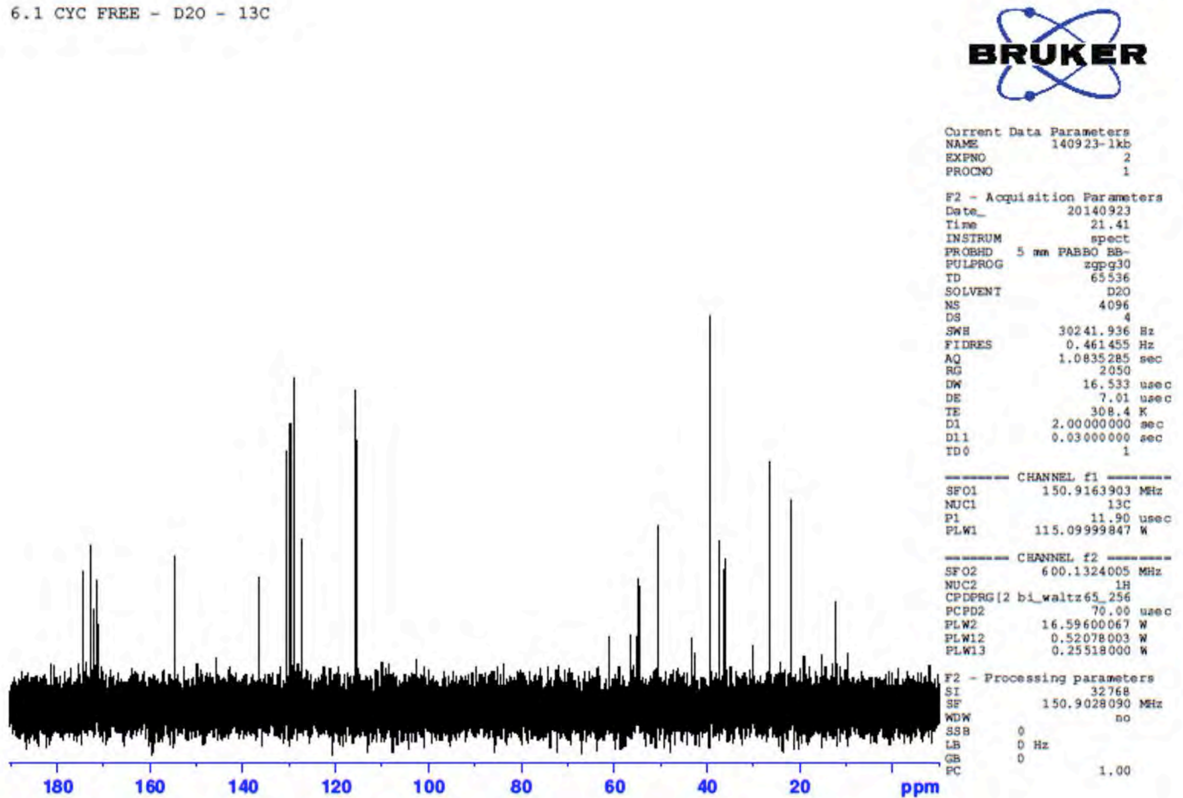




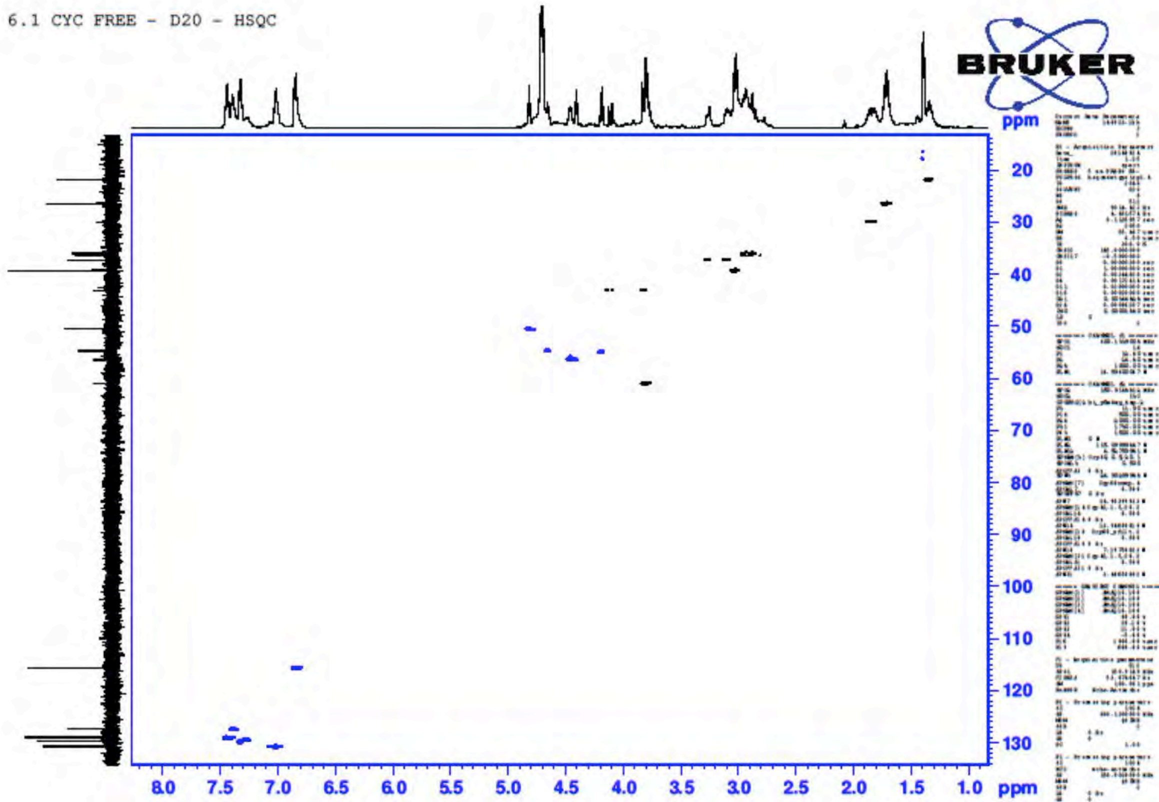


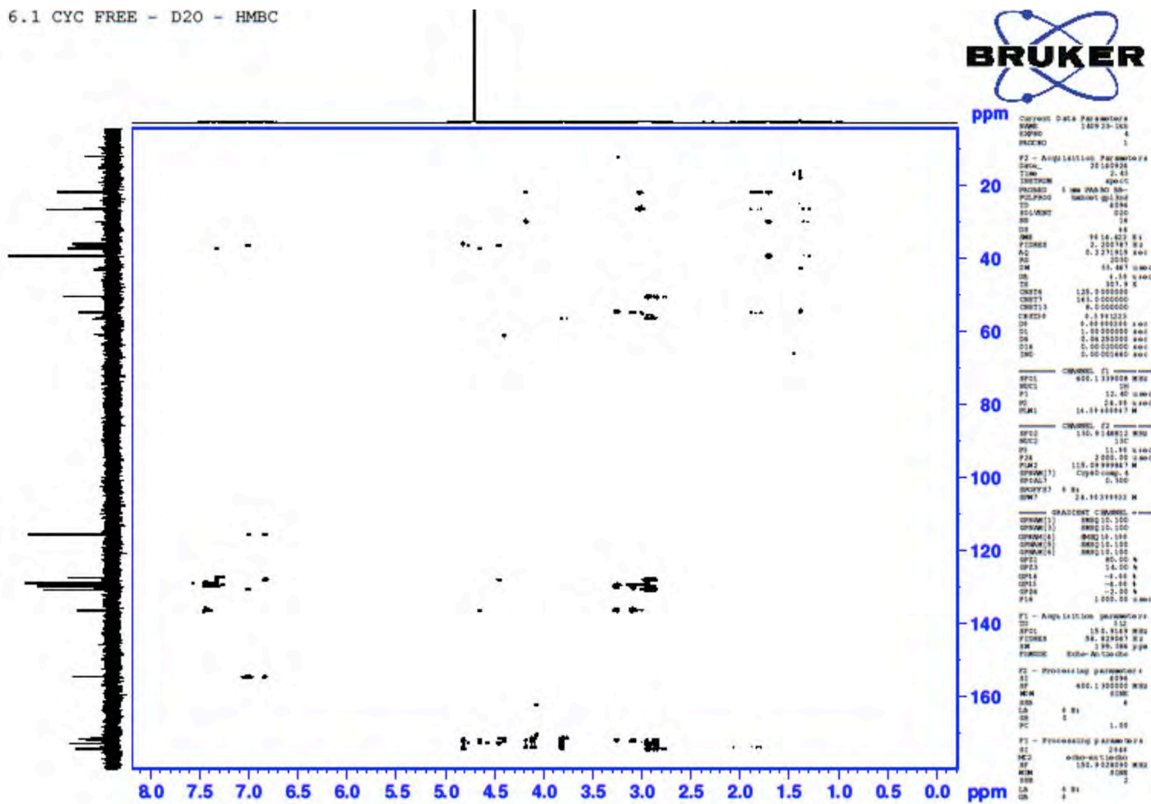
6.1 CYC: ^{1}H NMR and ^{13}C N MR



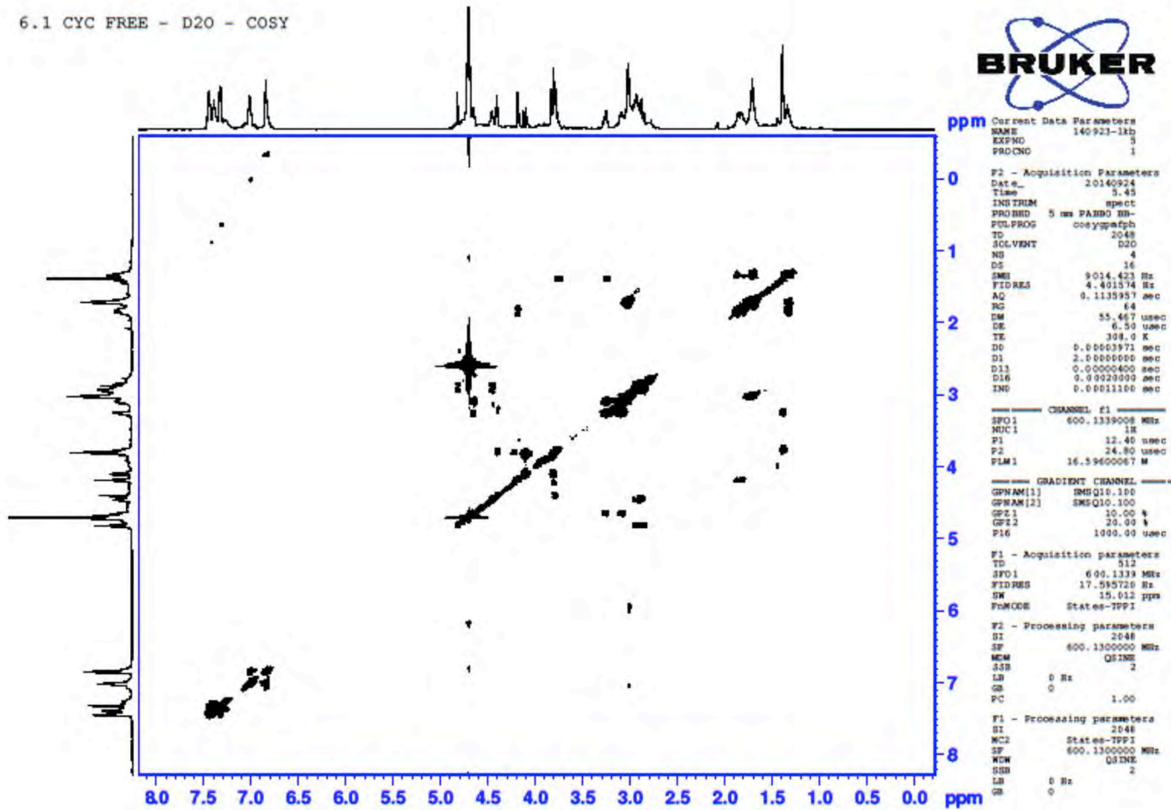


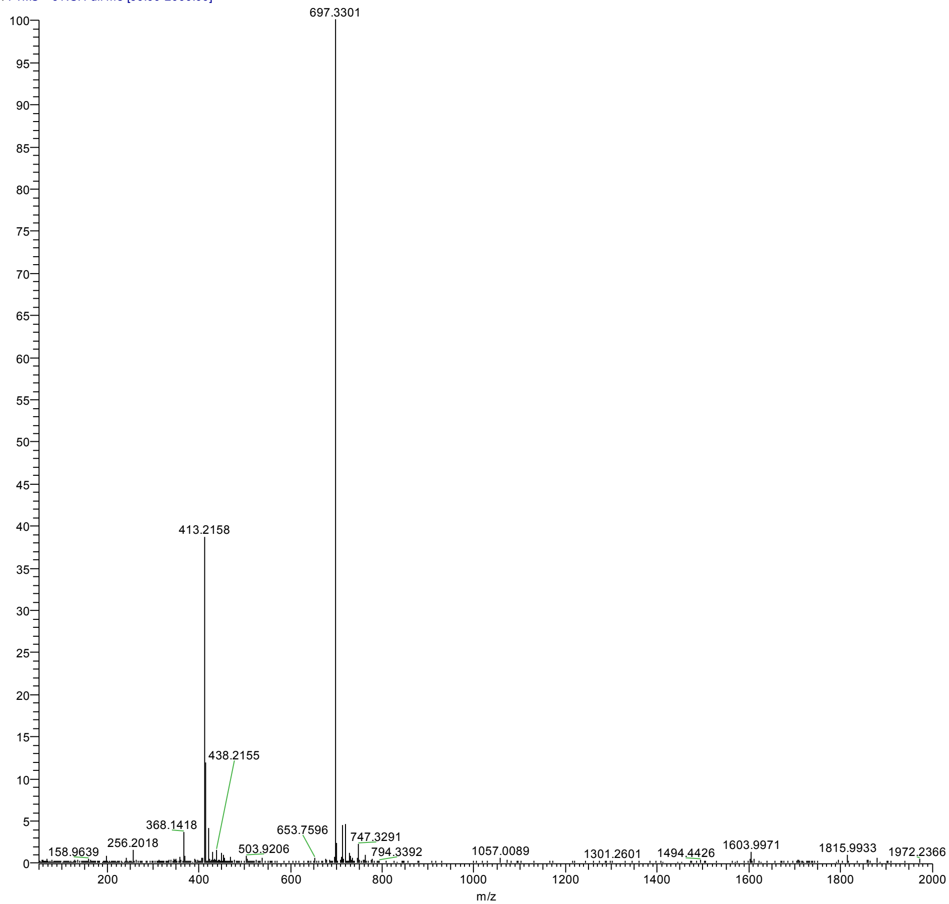
6.1 CYC: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





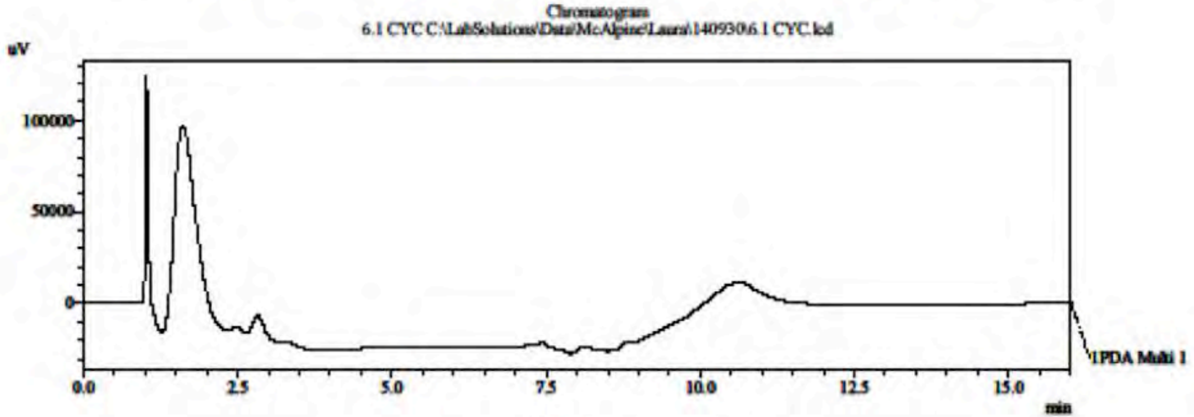
6.1 CYC: ^{1}H-^{1}H COSY NMR and HRMS

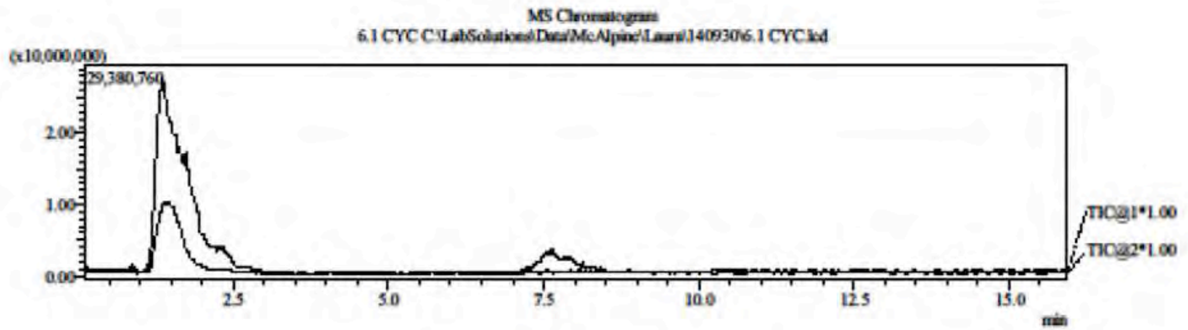


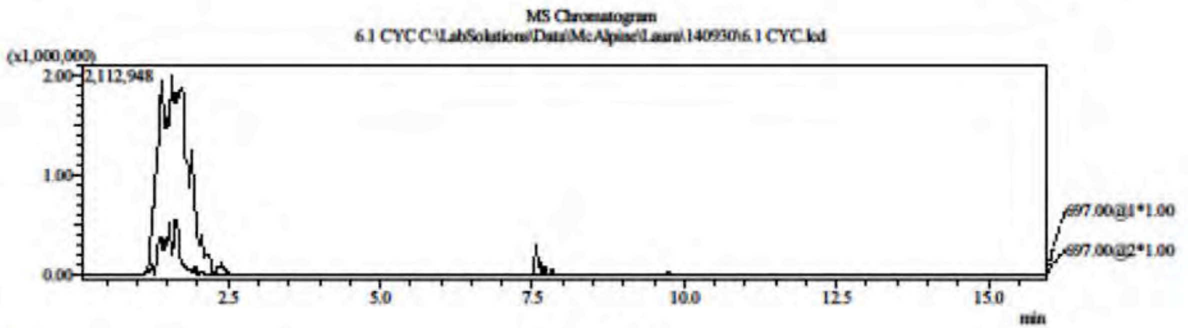


6.1 CYC: LC/MS

==== Shimadzu LCMSsolution Analysis Report ====







MS Spectrum Graph

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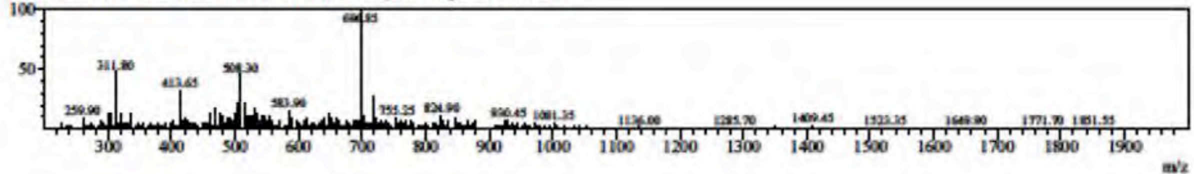

MS Spectrum Graph

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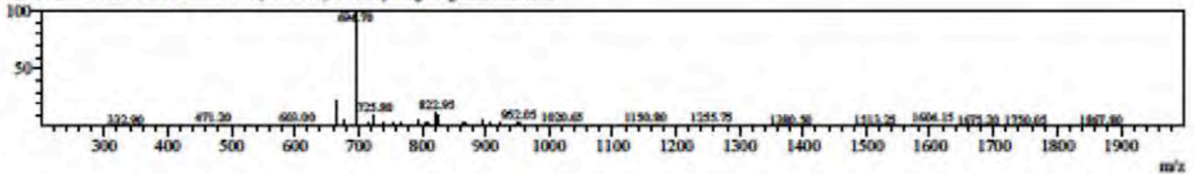
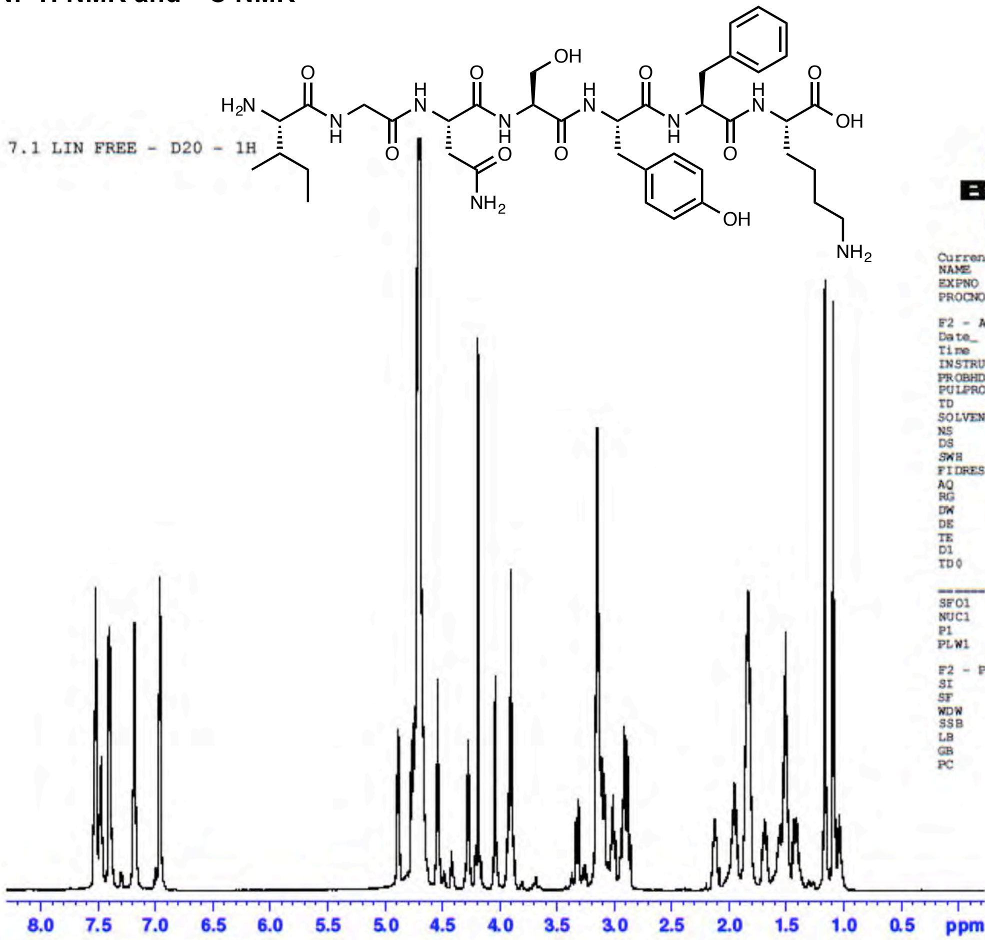
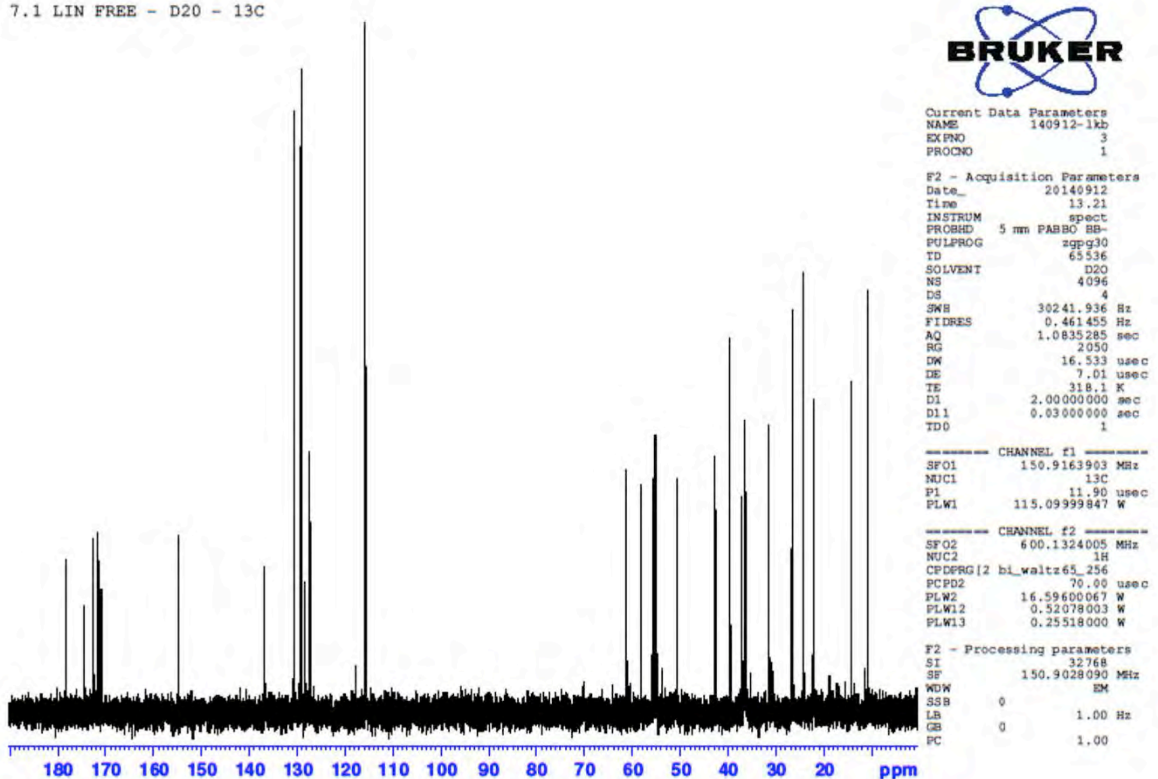
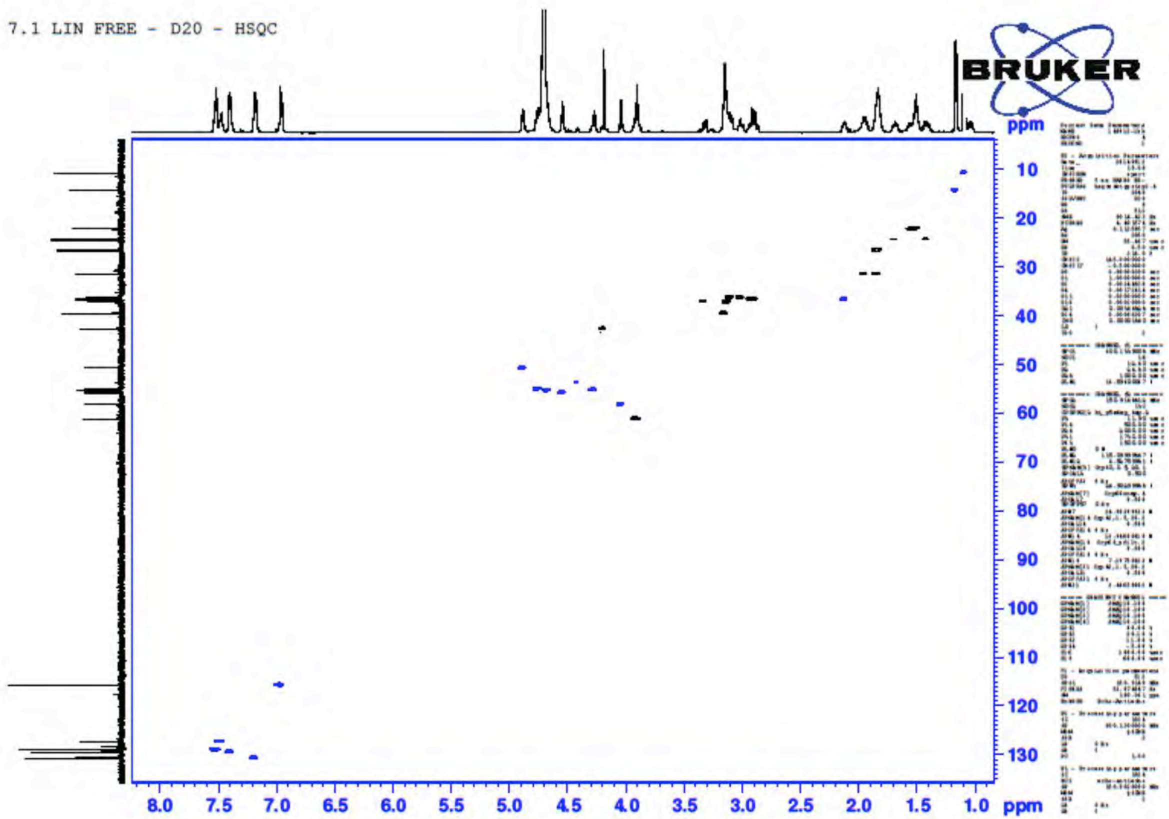


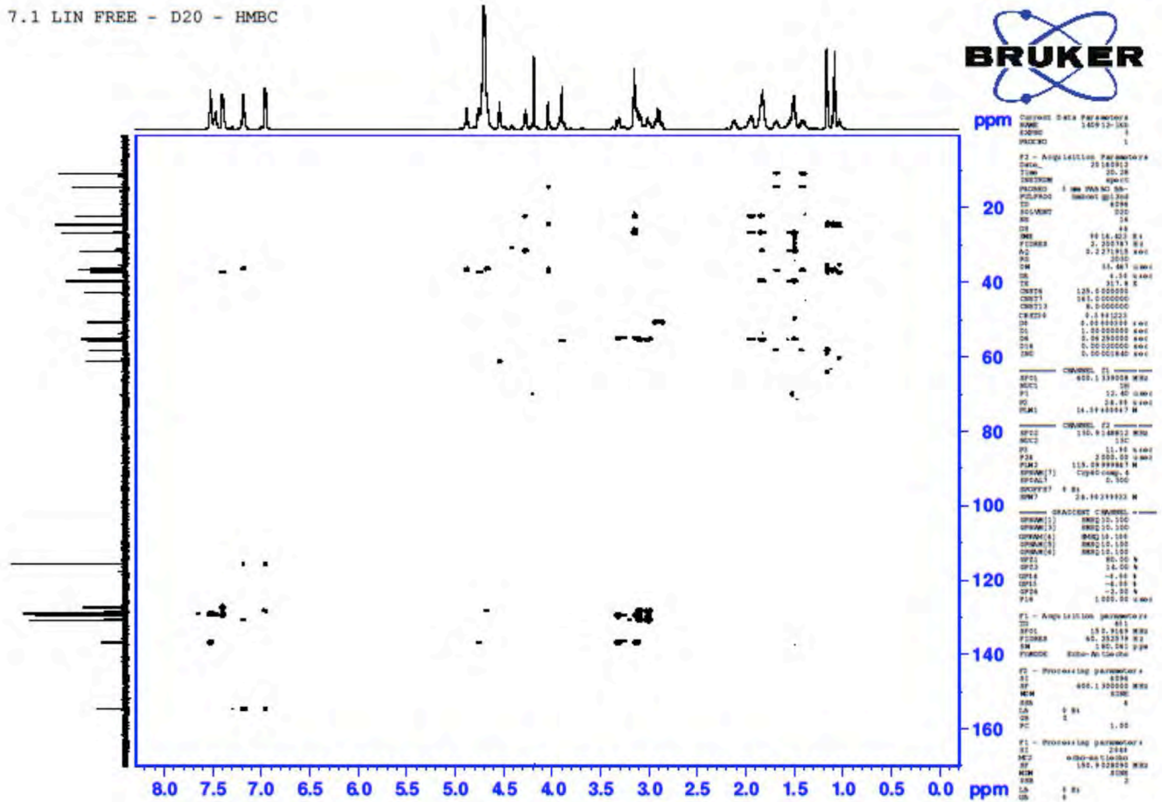
7.1 LIN: ^{1}H NMR and ^{13}C NMR



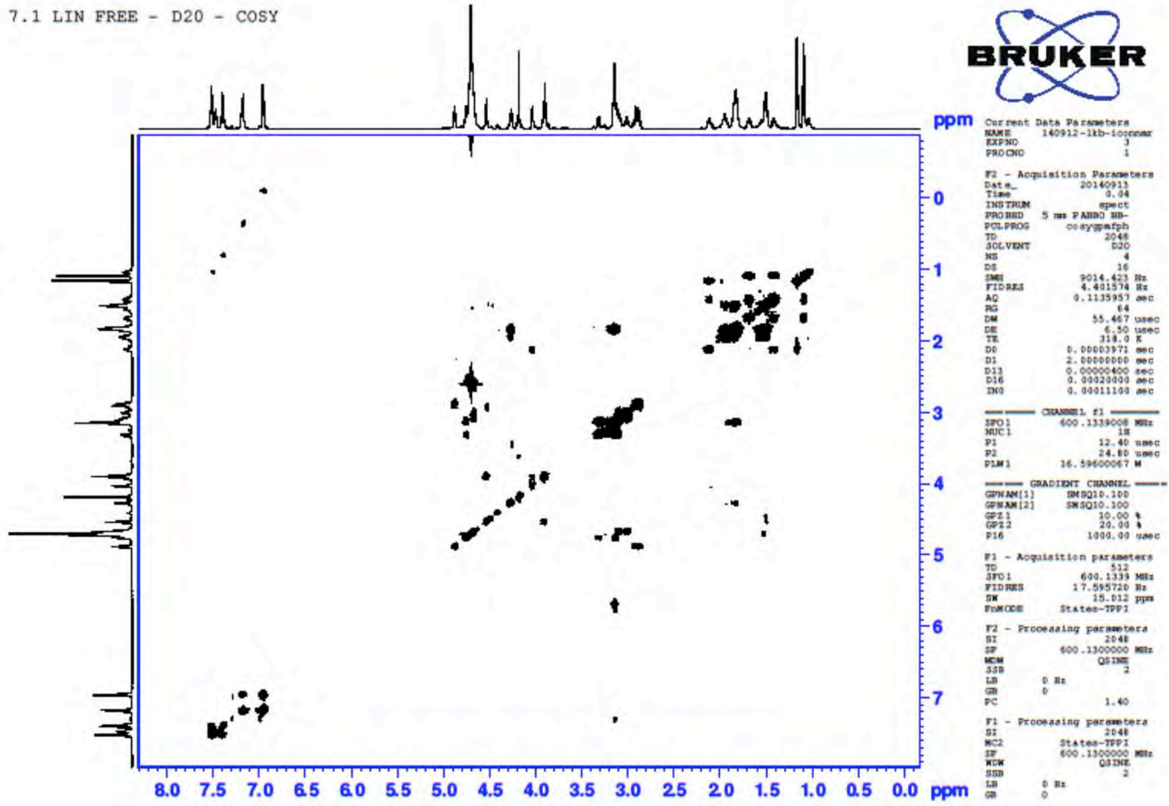


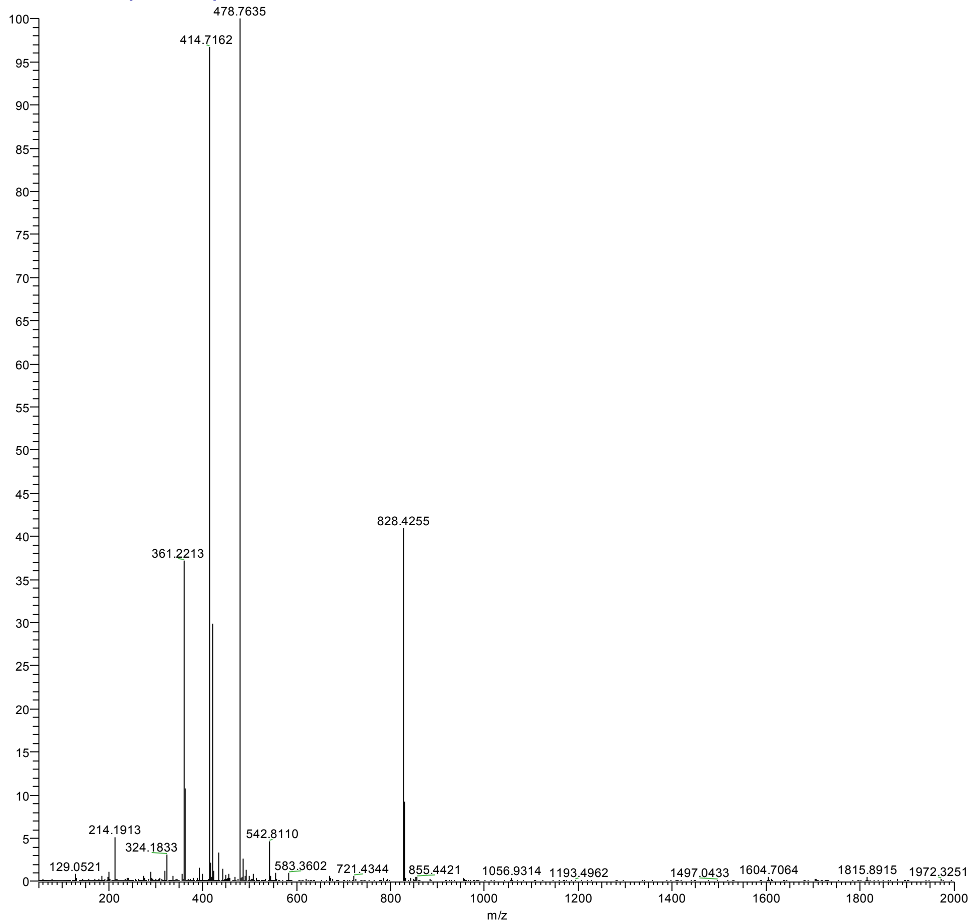
7.1 LIN: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





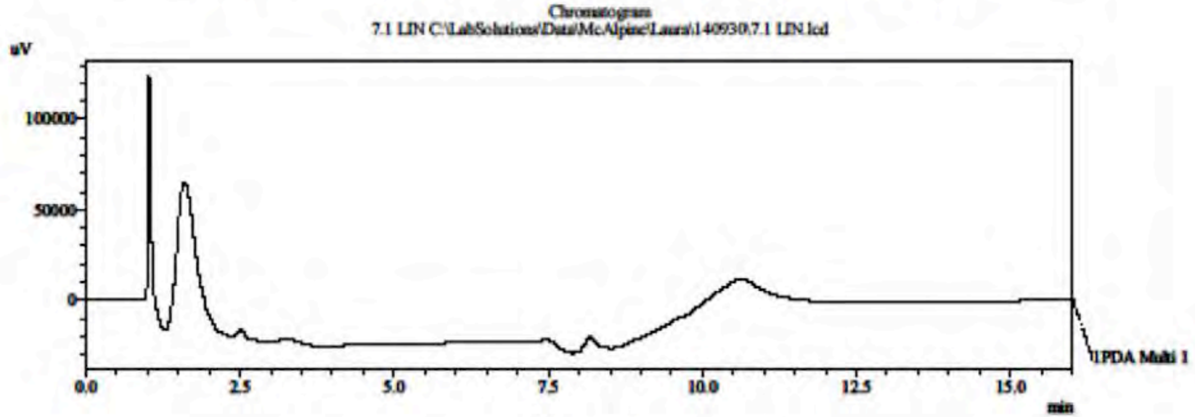
7.1 LIN: ^{1}H-^{1}H COSY NMR and HRMS

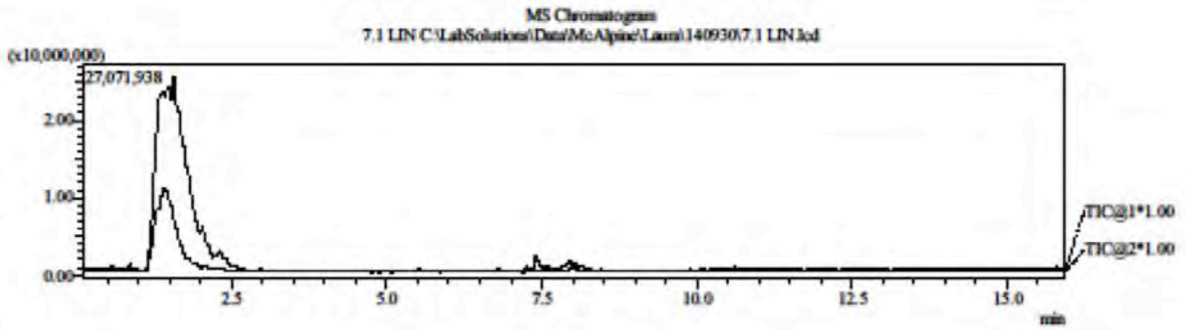


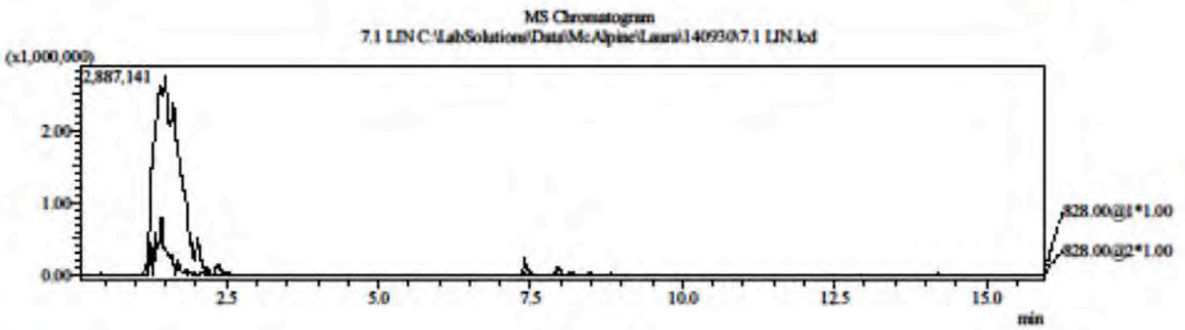


7.1 LIN: LC/MS

==== Shimadzu LCMSsolution Analysis Report ====







MS Spectrum Graph

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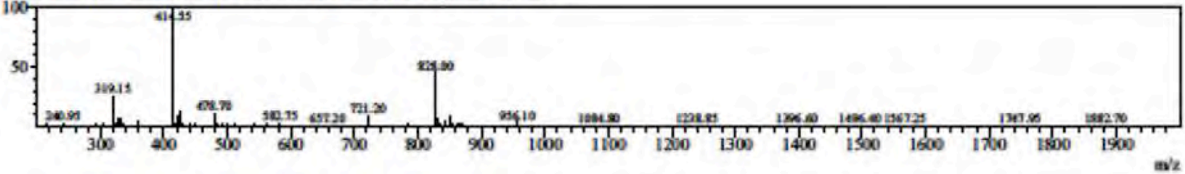

MS Spectrum Graph

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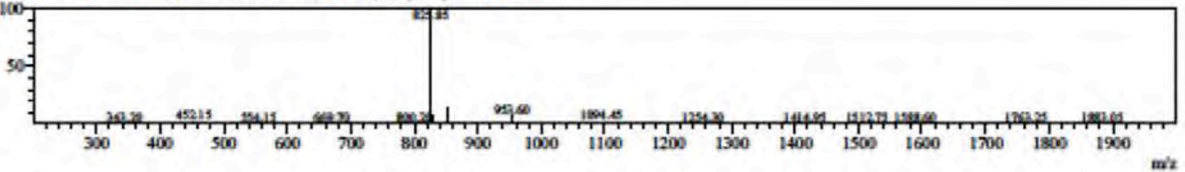
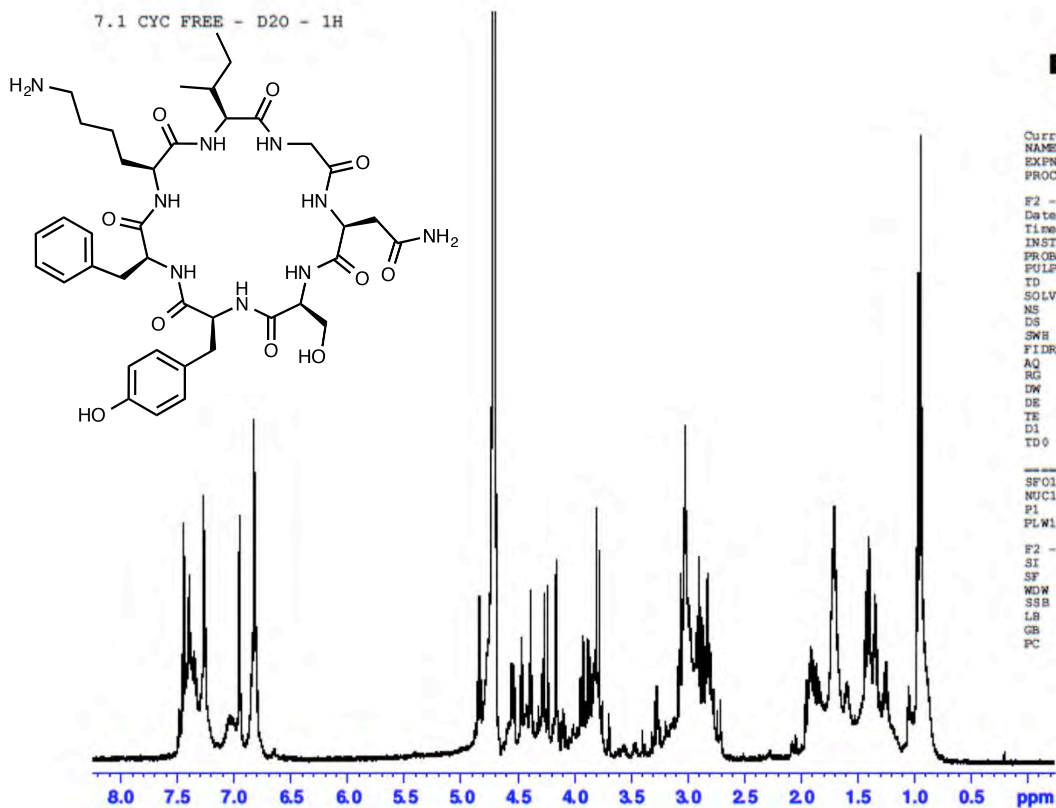


7.1 CYC: ^{1}H NMR and ^{13}C NMR



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PROCNO 1

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SOLVENT D2O
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DS 0
SWH 9009.009 Hz
FIDRES 0.137467 Hz
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RG 45.2
DW 55.500 usec
DE 9.39 usec
TE 308.1 K
D1 5.00000000 sec
TD0 1

======== CHANNEL f1 ========
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NUC1 1H
P1 12.74 usec
PLW1 16.59600067 W

F2 - Processing parameters
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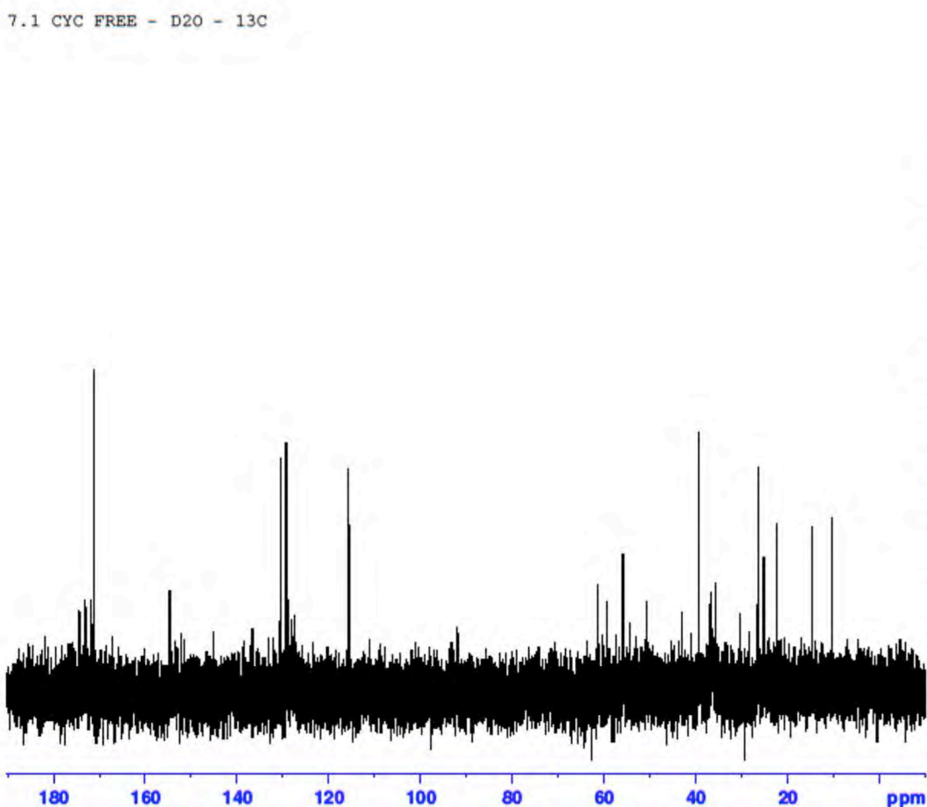
SF 600.1300000 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00



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EXPNO 6
PROCNO 1

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PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 4096
DS 4
SWH 30241.936 Hz
FIDRES 0.461455 Hz
AQ 1.0835285 sec
RG 2050
DW 16.533 usec
DE 7.01 usec
TE 308.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 150.9163903 MHz
NUC1 13C
P1 11.90 usec
PLW1 115.09999847 W

======== CHANNEL f2 ========
SFO2 600.1324005 MHz
NUC2 1H
CPDPRG[2 bi_waltz65_256
PCPD2 70.00 usec
PLW2 16.59600067 W
PLW12 0.52078003 W
PLW13 0.25518000 W

F2 - Processing parameters
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SF 150.9028090 MHz
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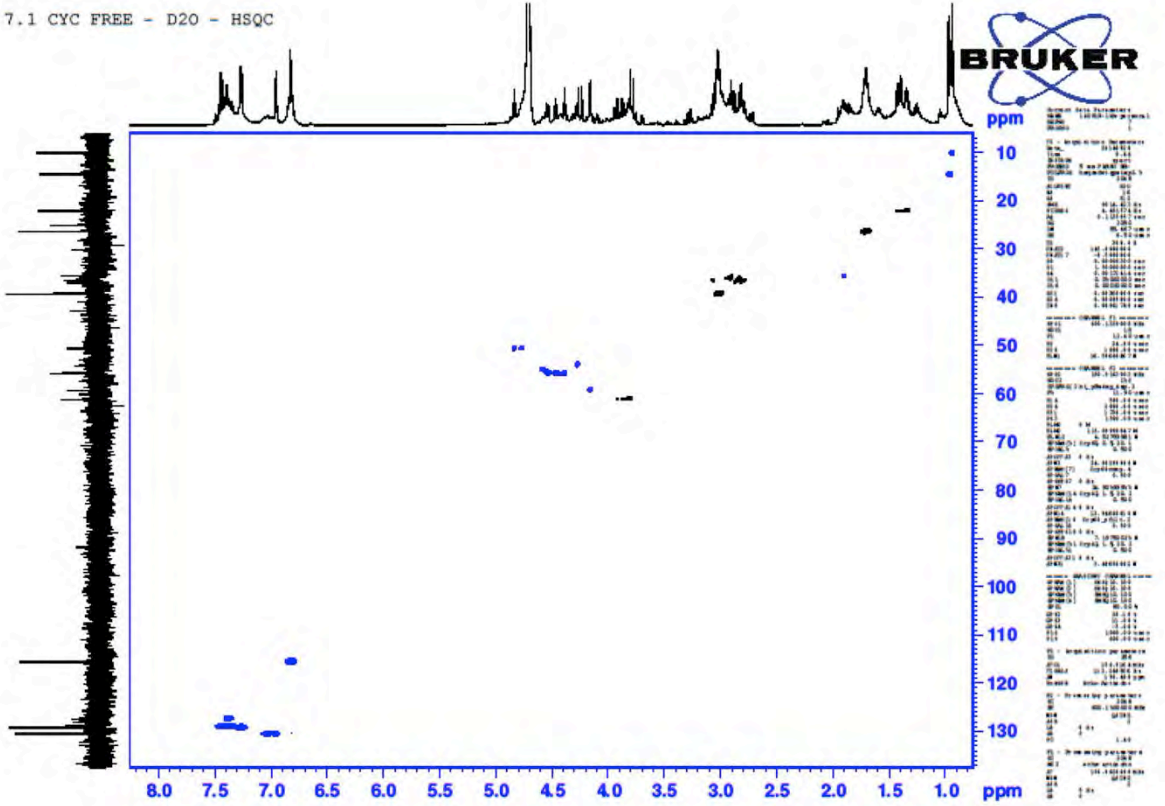
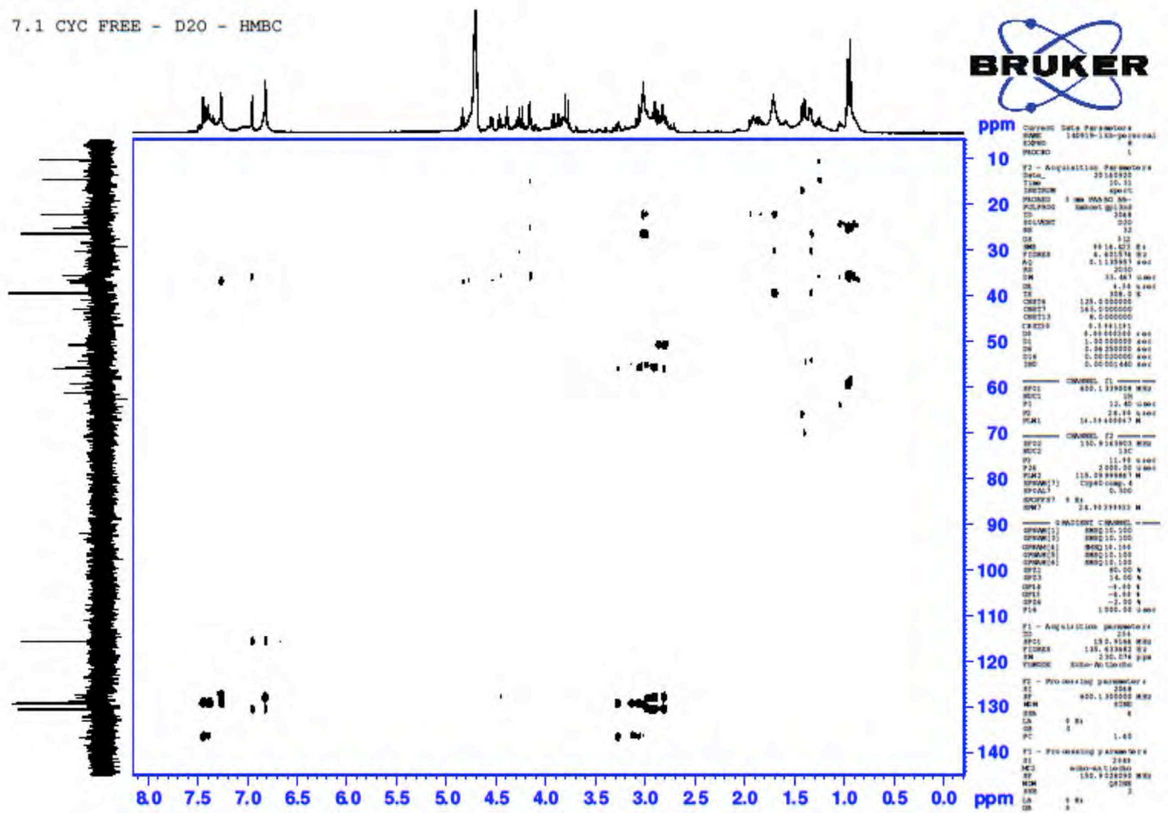
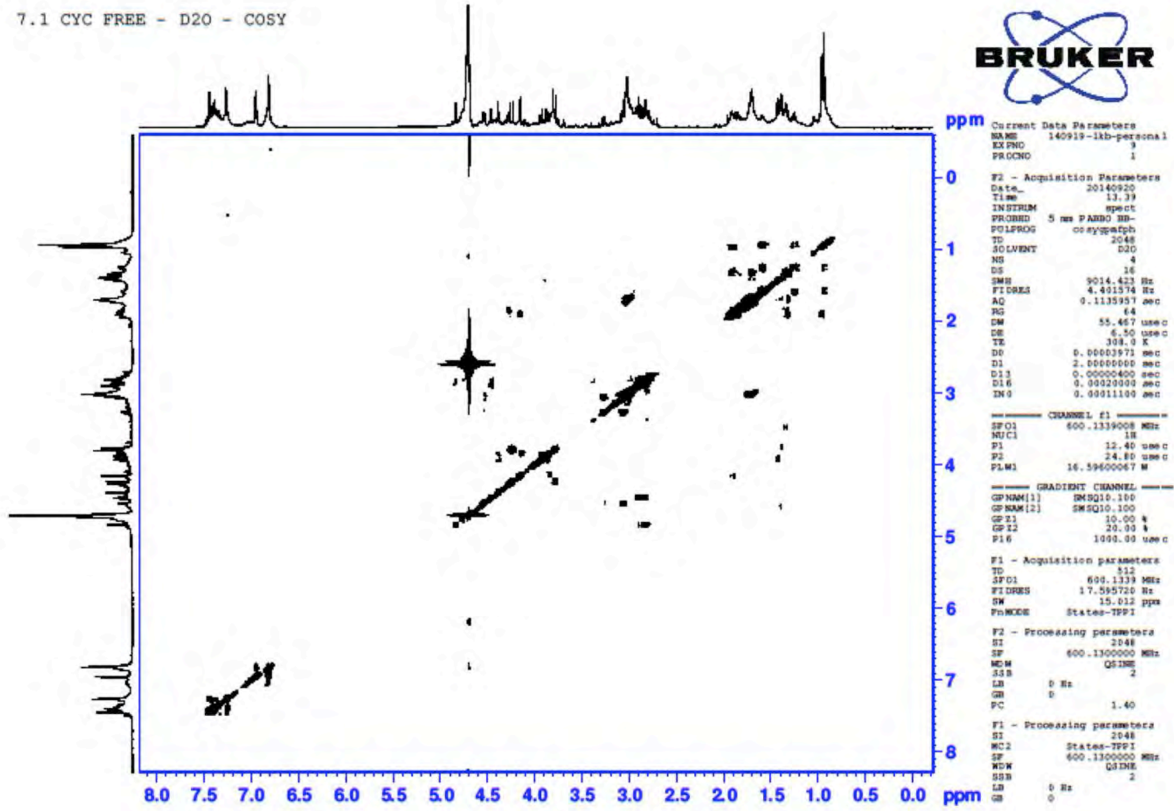
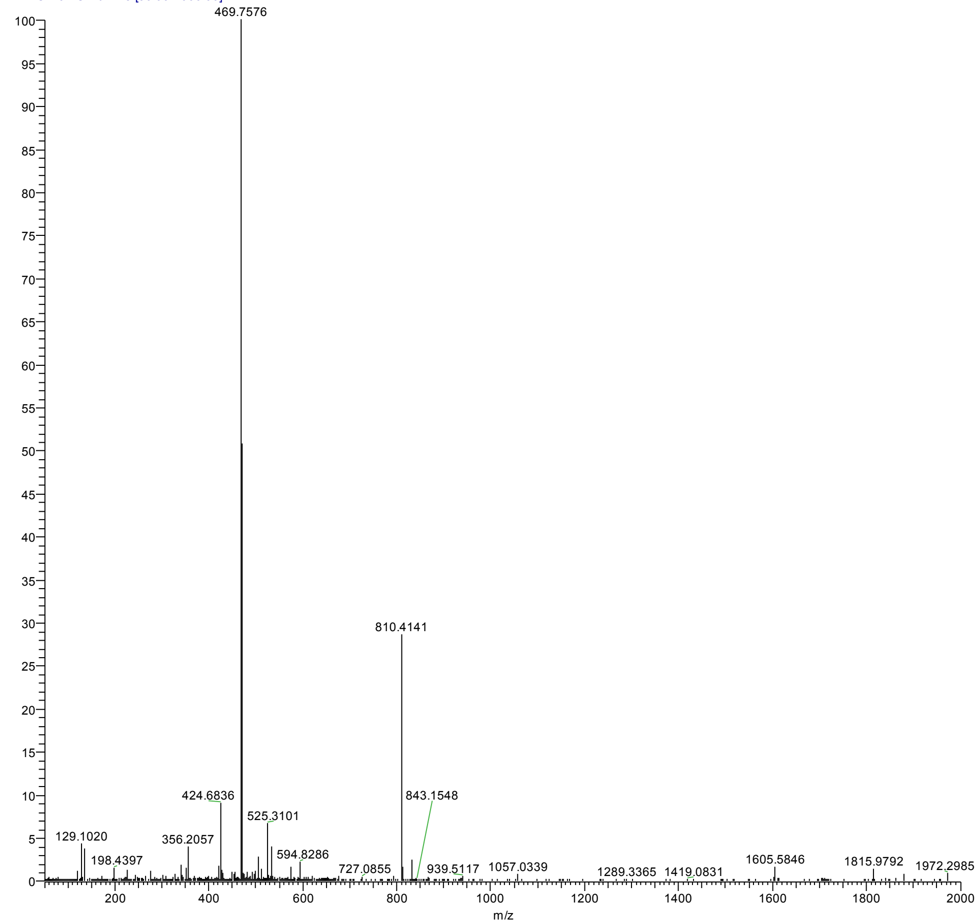
7.1 CYC: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





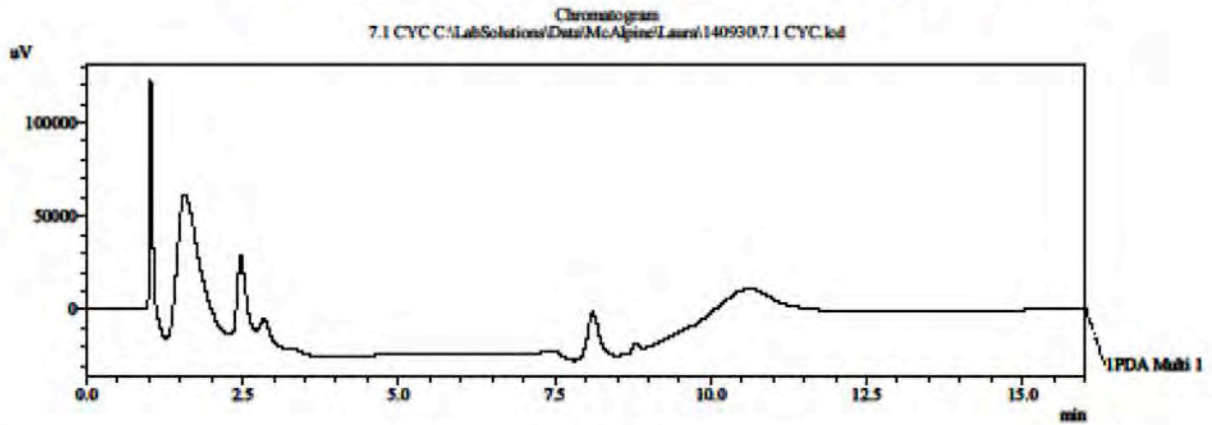
7.1 CYC: ^{1}H-^{1}H COSY NMR and HRMS

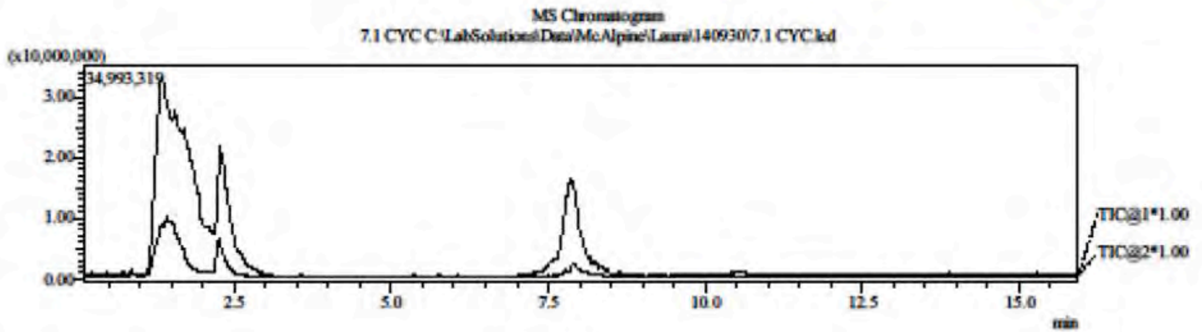


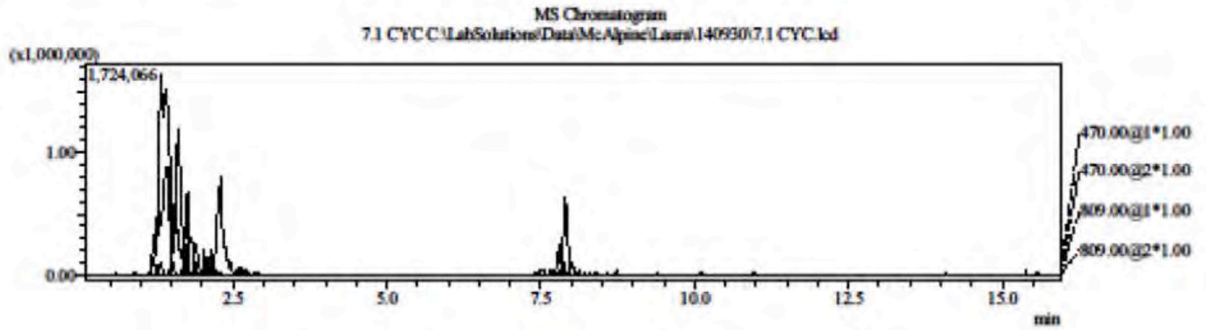


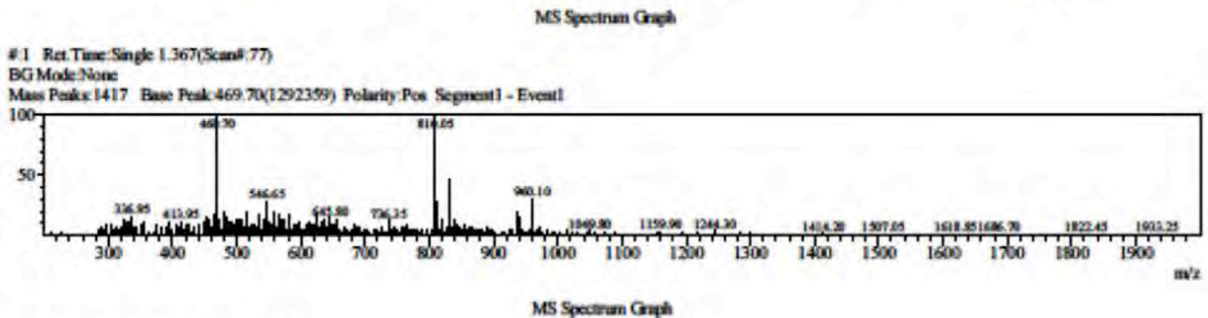
7.1 CYC: LC/MS

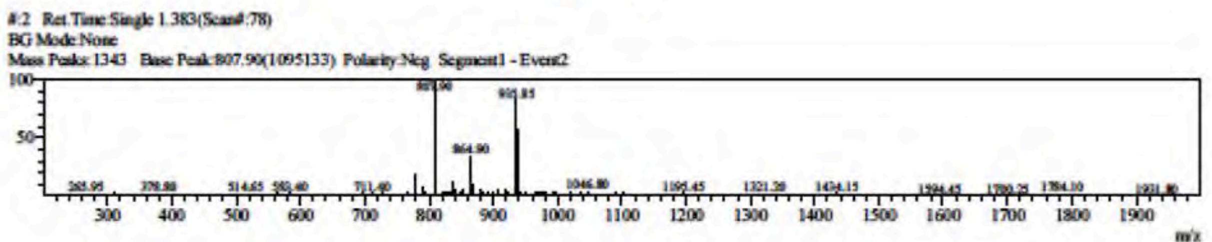
==== Shimadzu LCMSsolution Analysis Report ====



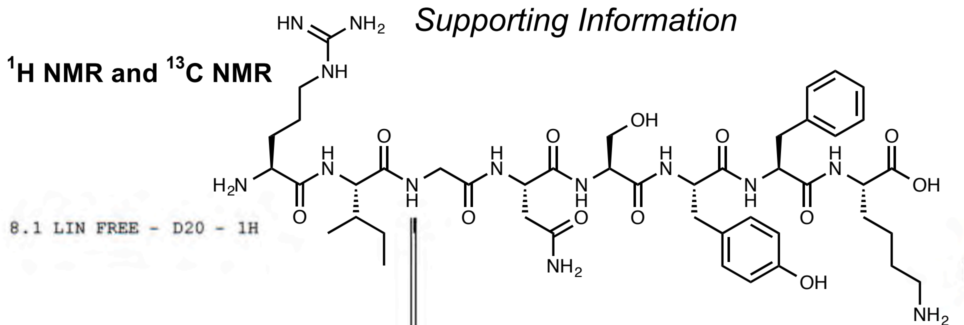


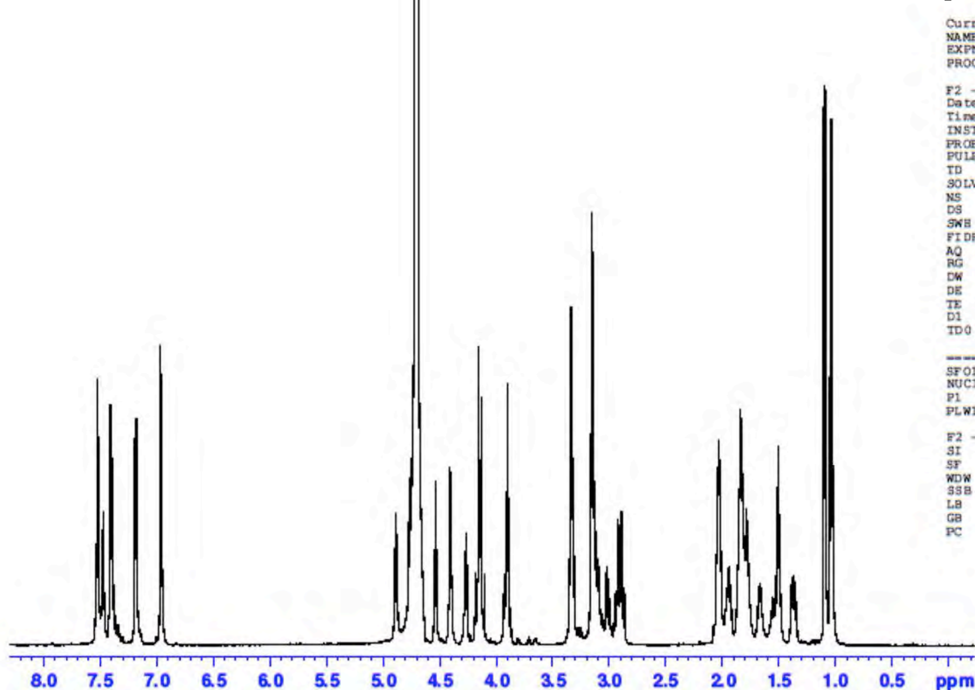


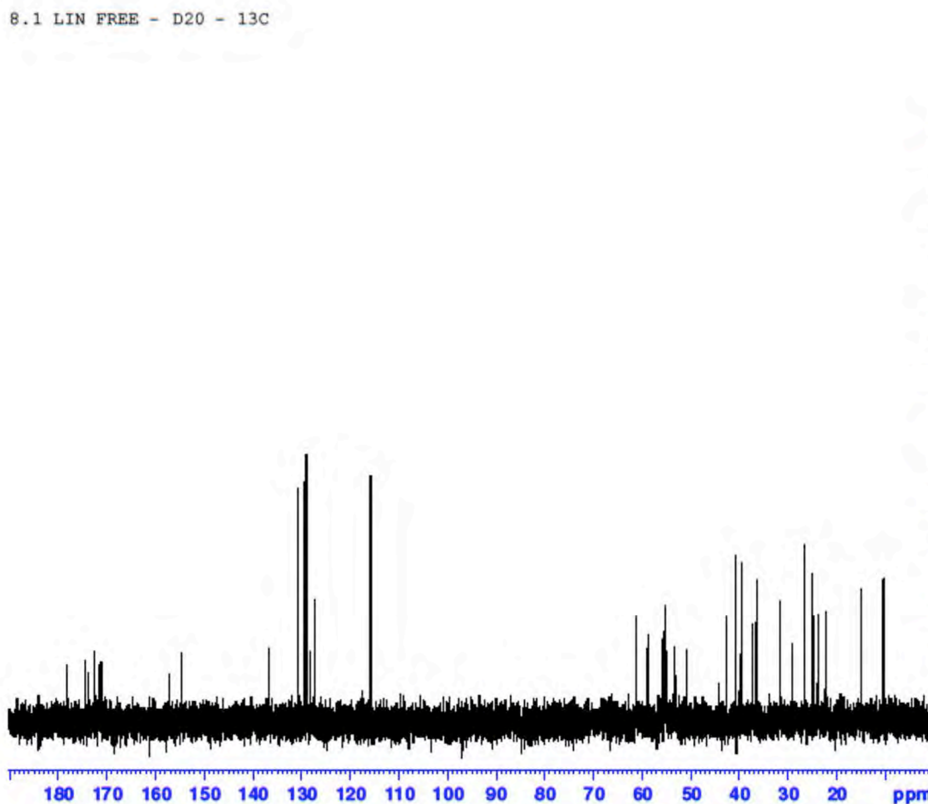




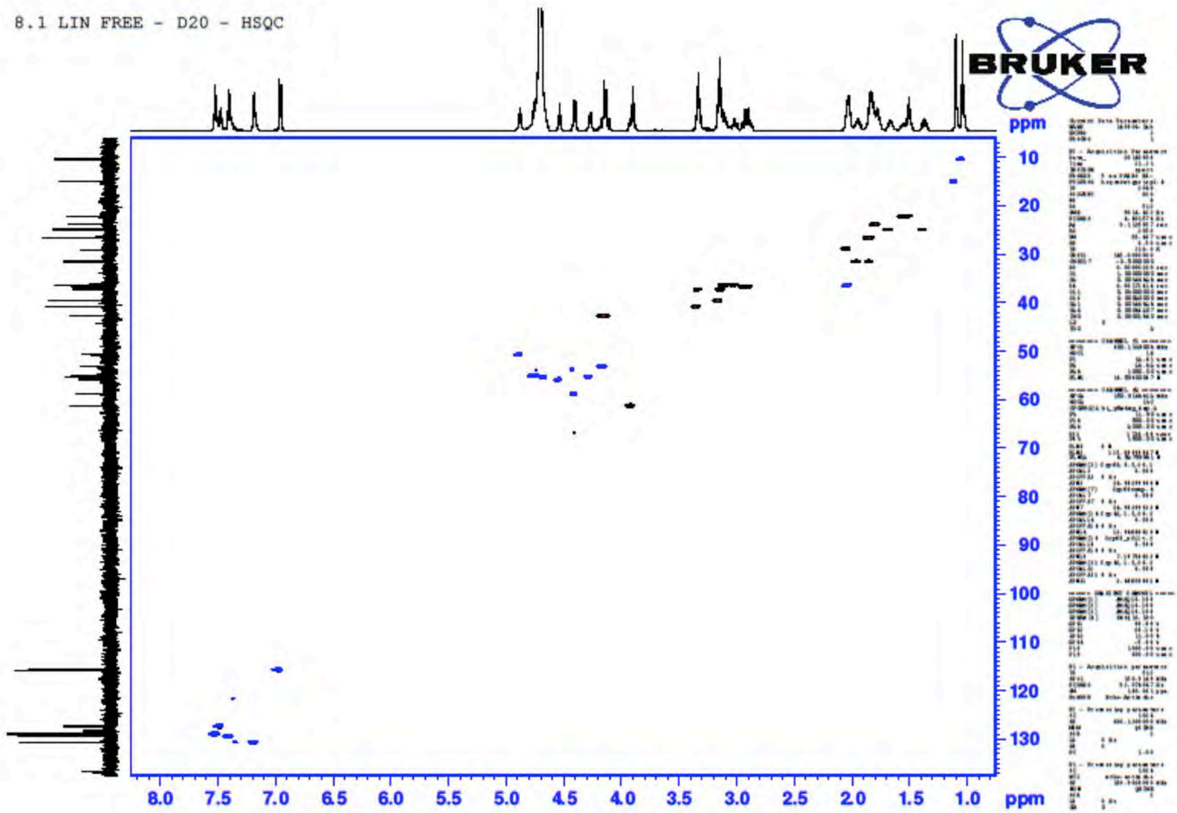
8.1 LIN: ^{1}H NMR and ^{13}C NMR

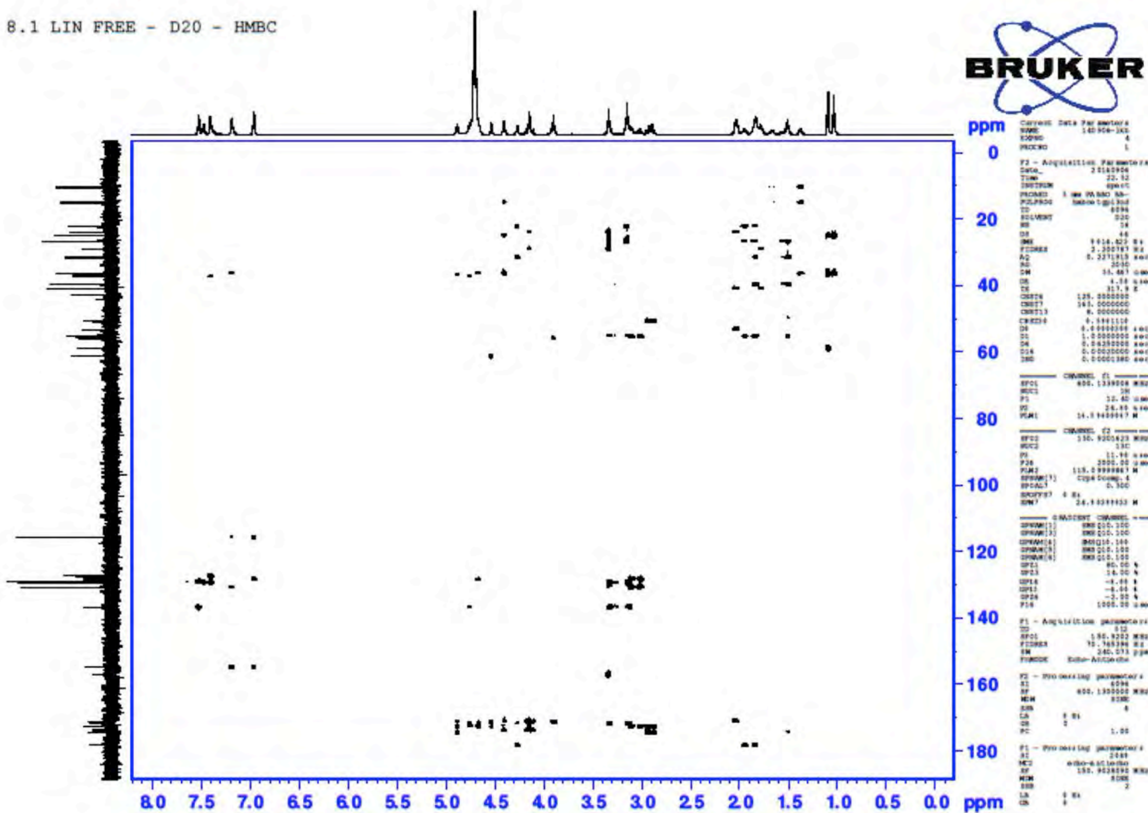




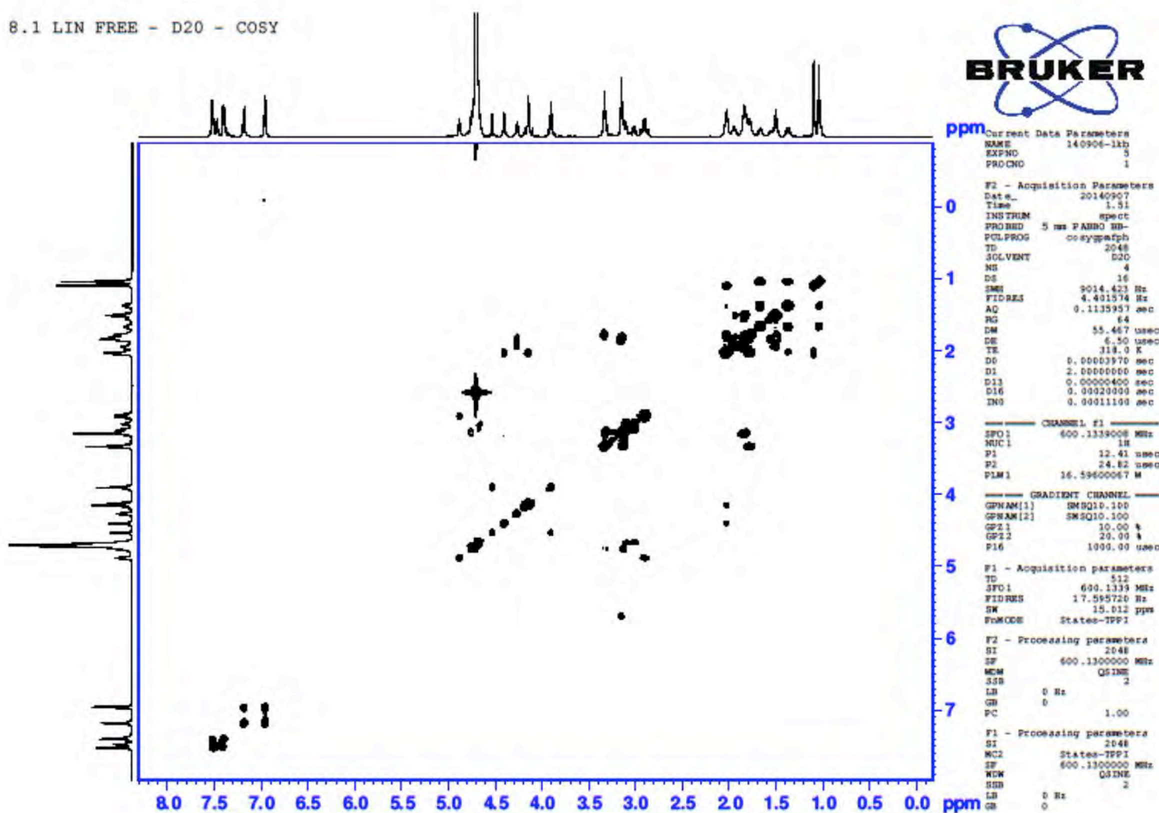


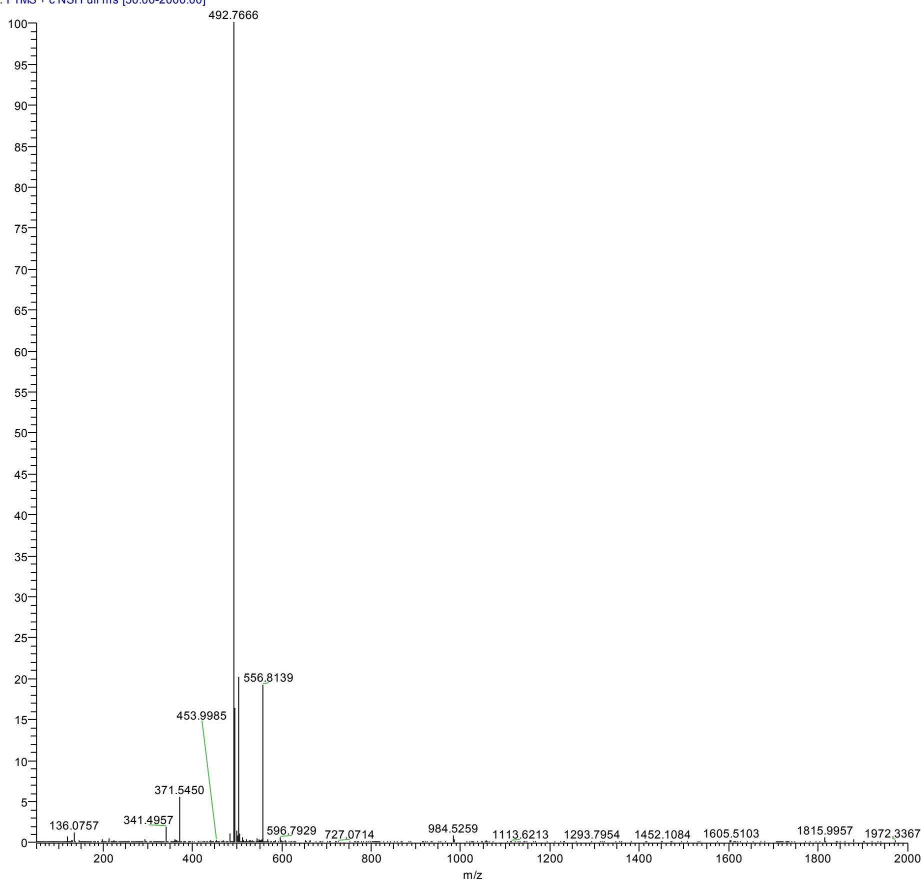
8.1 LIN: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





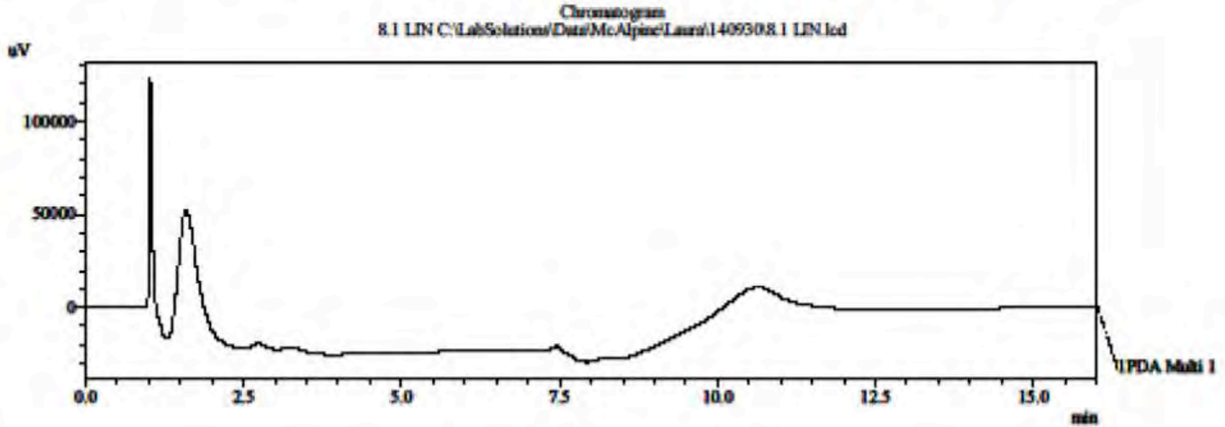
8.1 LIN: ^{1}H-^{1}H COSY NMR and HRMS

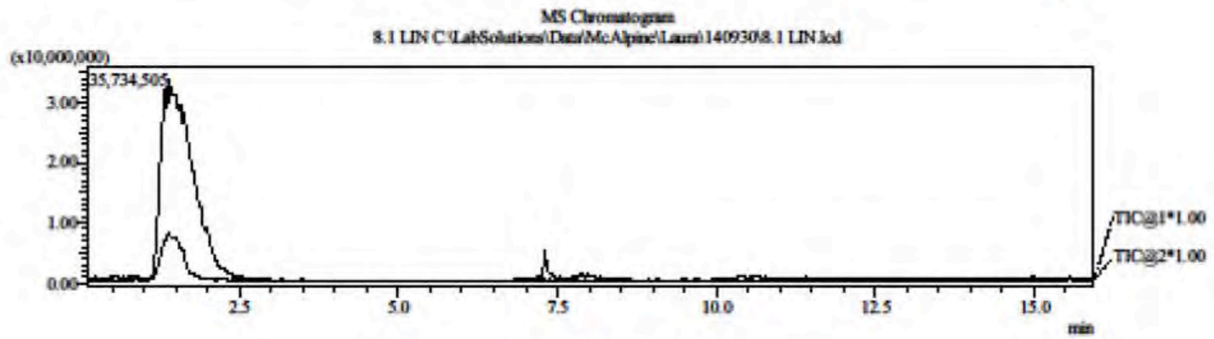


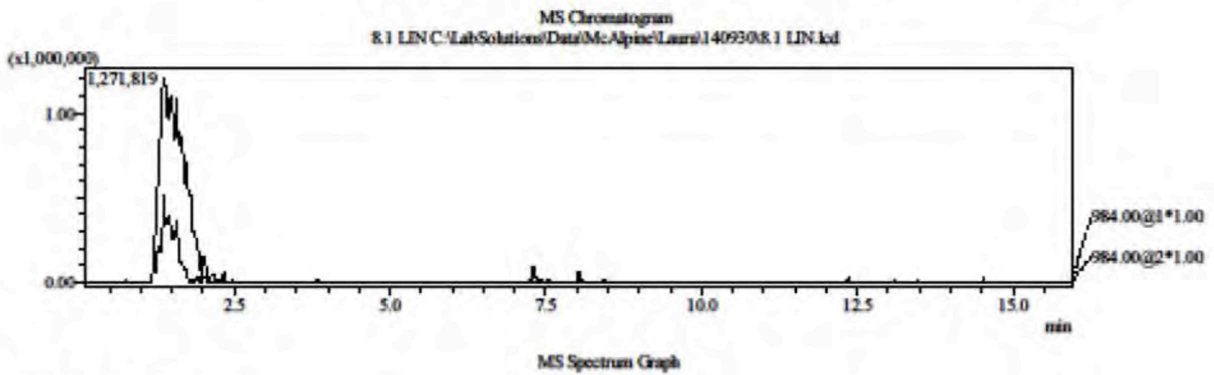


8.1 LIN: LC/MS

==== Shimadzu LCMSsolution Analysis Report ====







MS Spectrum Graph

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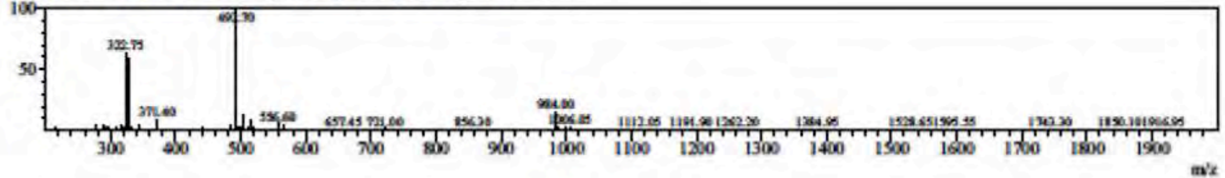

MS Spectrum Graph

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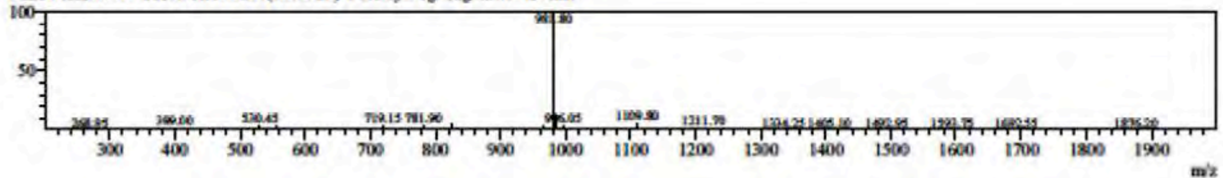
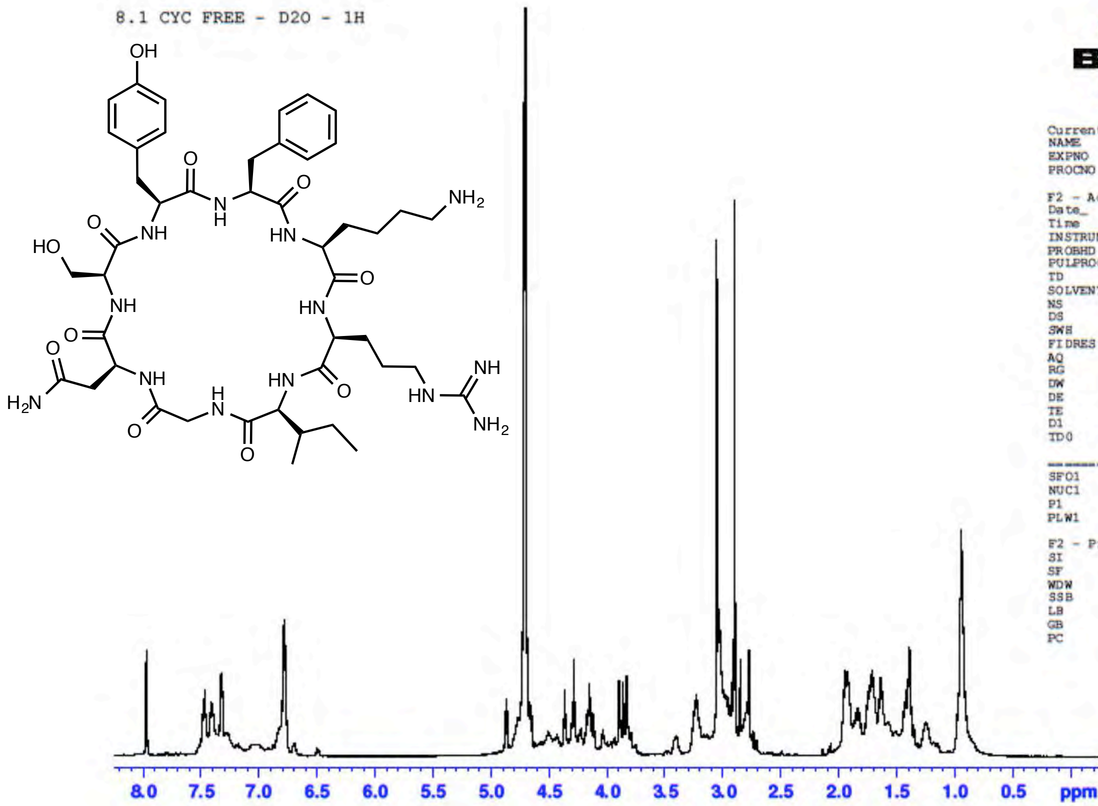
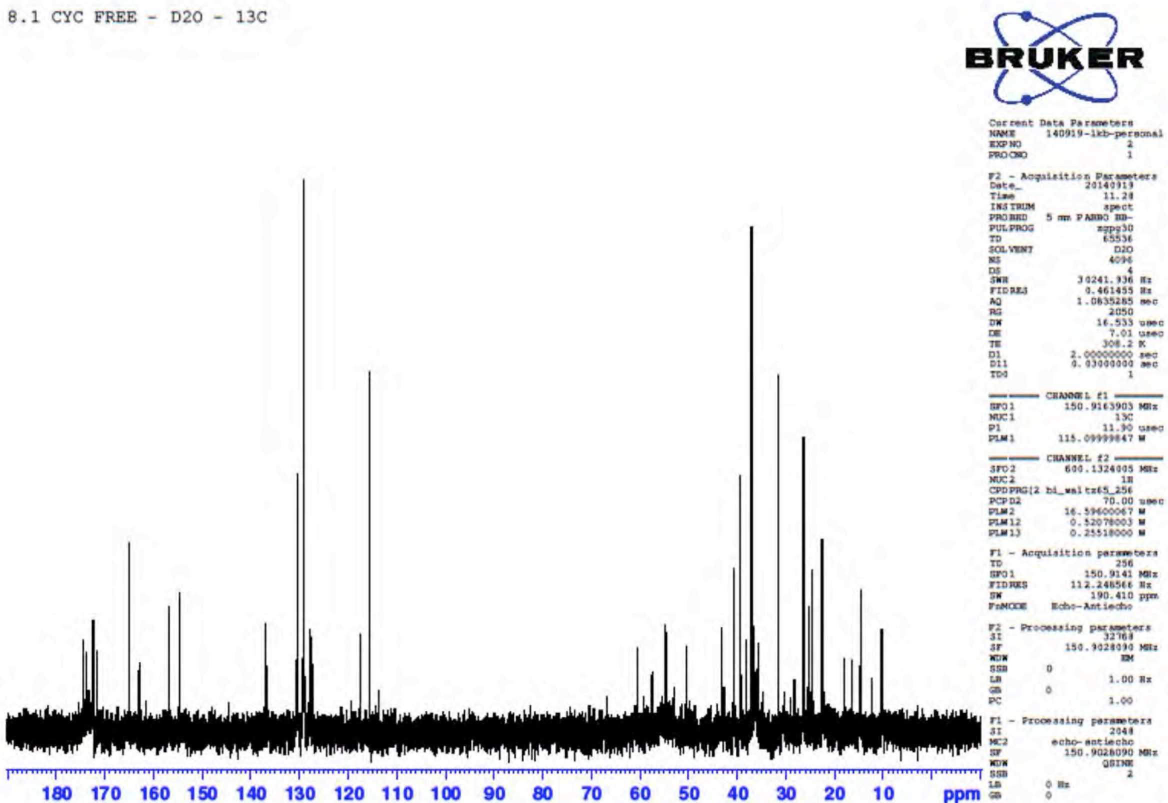
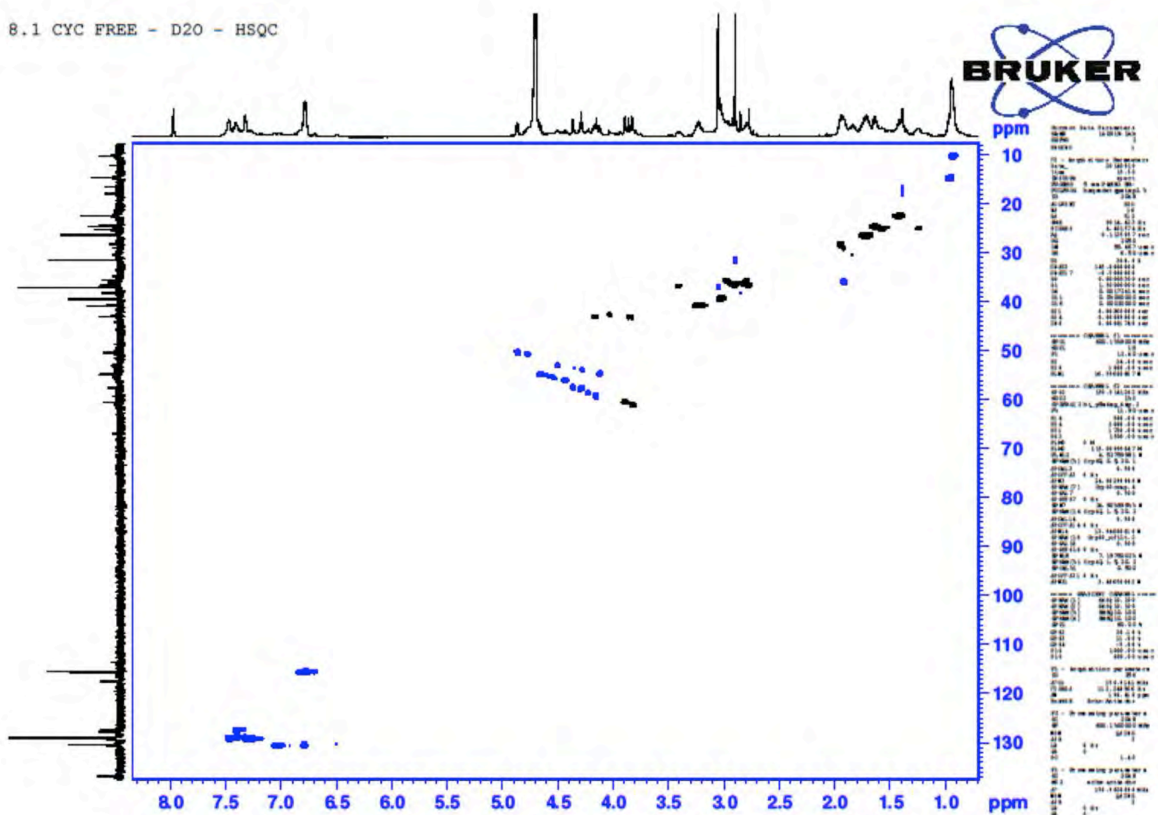


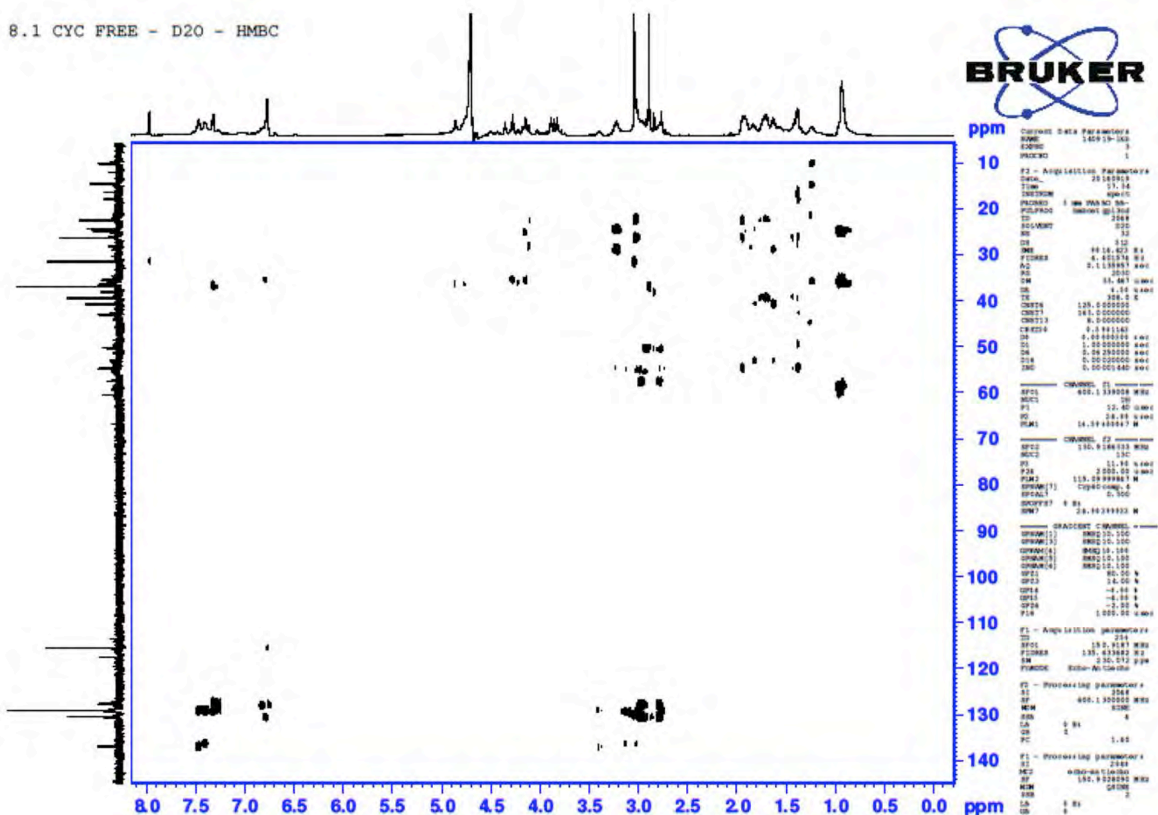
8.1 CYC: ^{1}H NMR and ^{13}C NMR



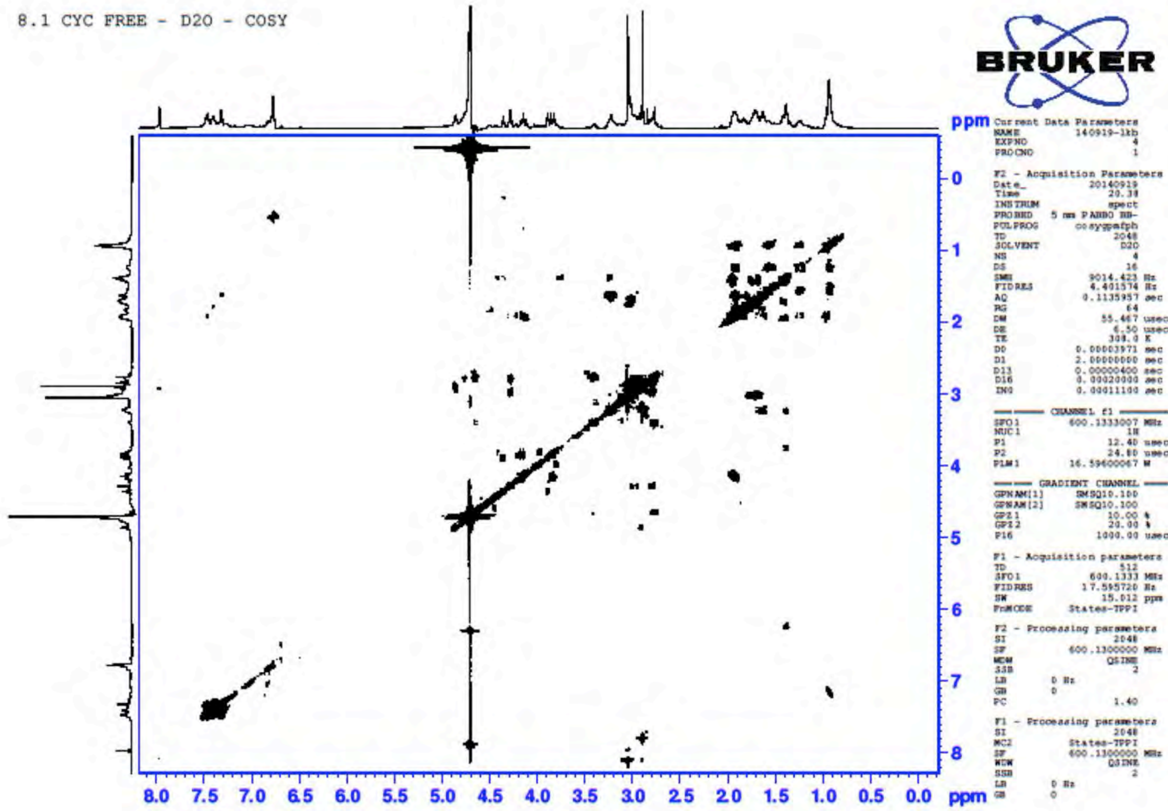


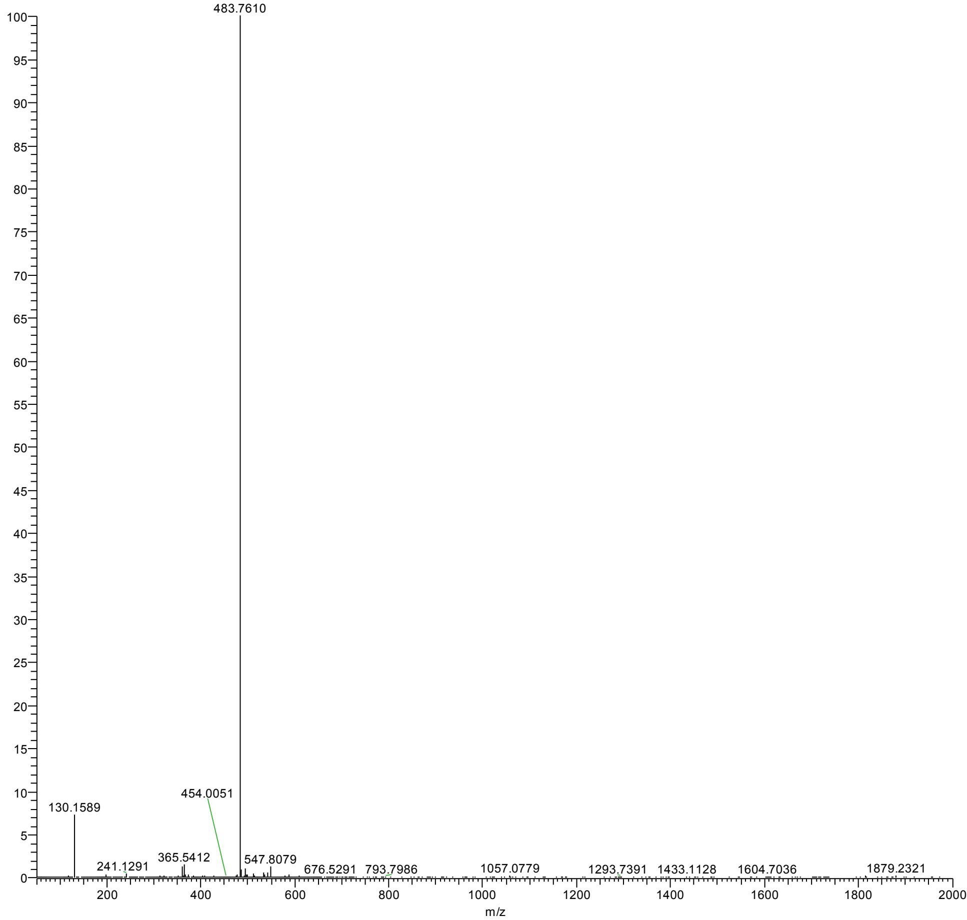
8.1 CYC: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





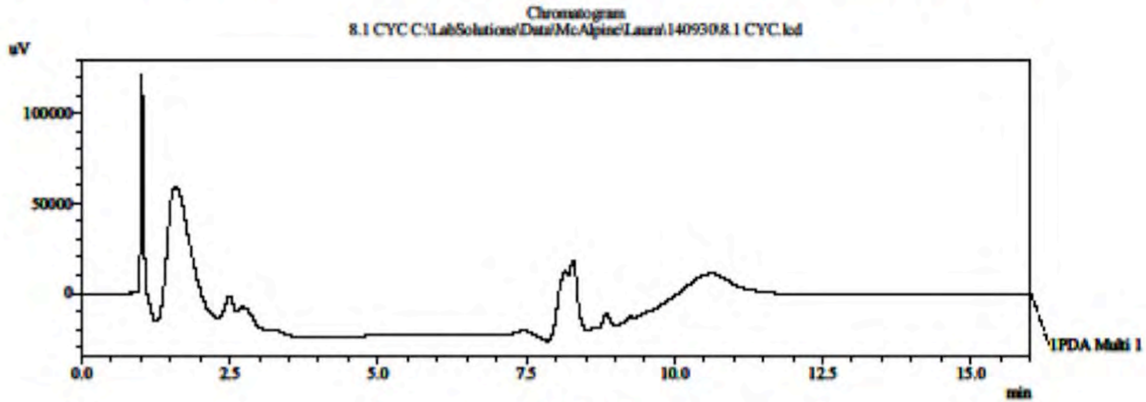
8.1 CYC: ^1H-^1H COSY NMR and HRMS

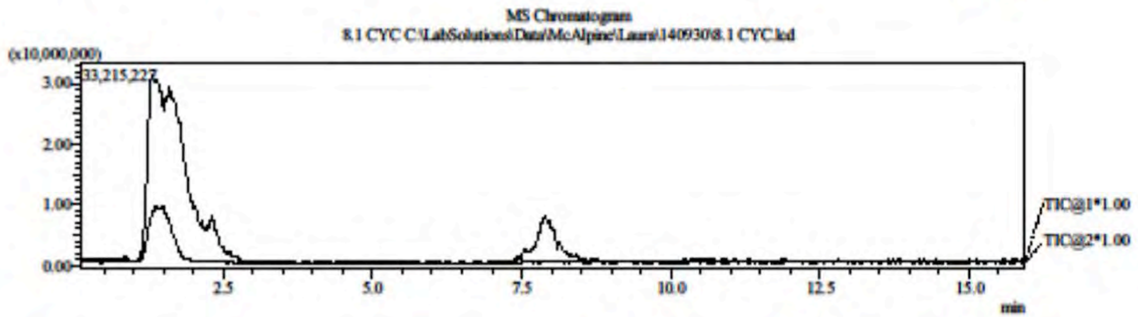


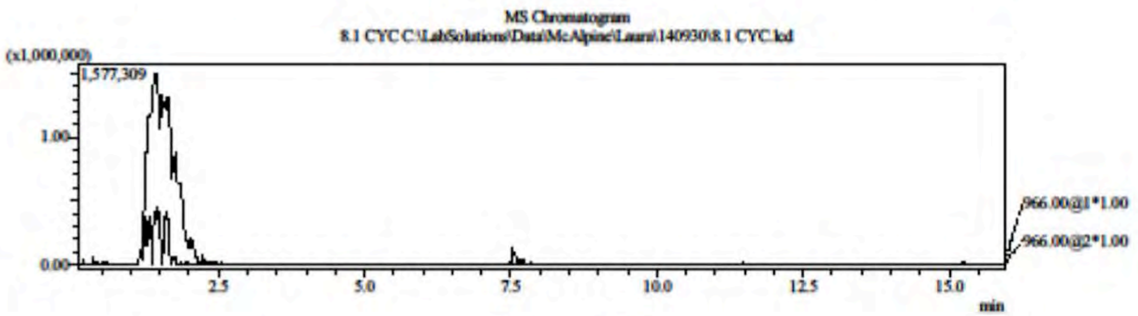


8.1 CYC: LC/MS

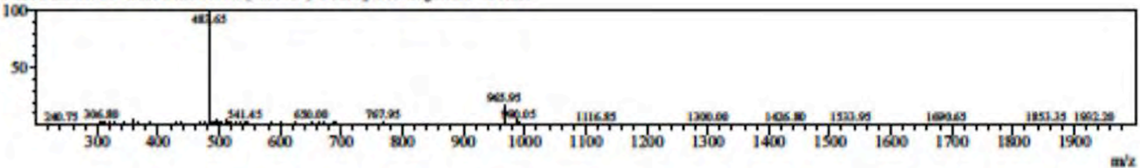
==== Shimadzu LCMSsolution Analysis Report ====



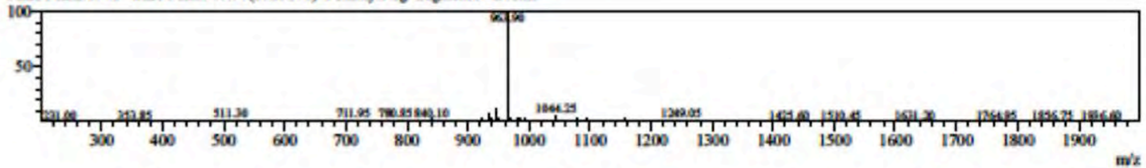




MS Spectrum Graph

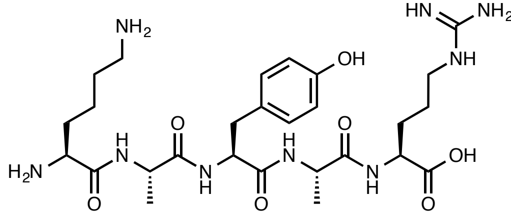


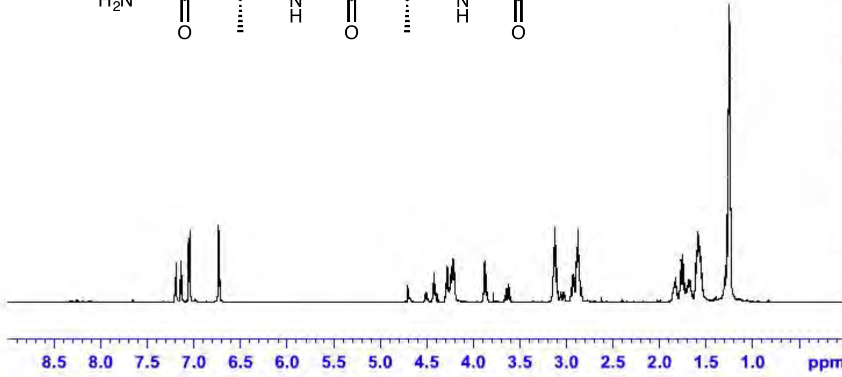
MS Spectrum Graph



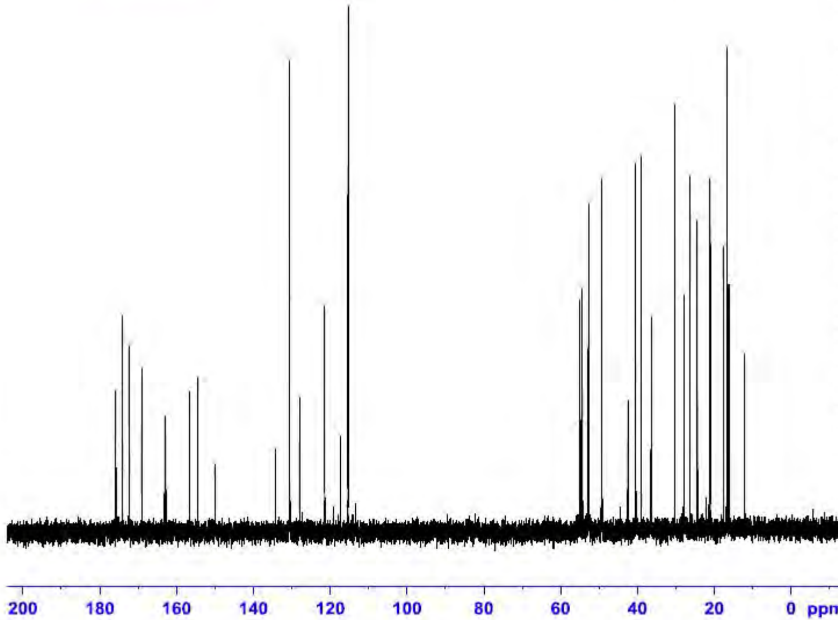
5.2 LIN: ^{1}H NMR and ^{13}C NMR

Lin 5.2 free

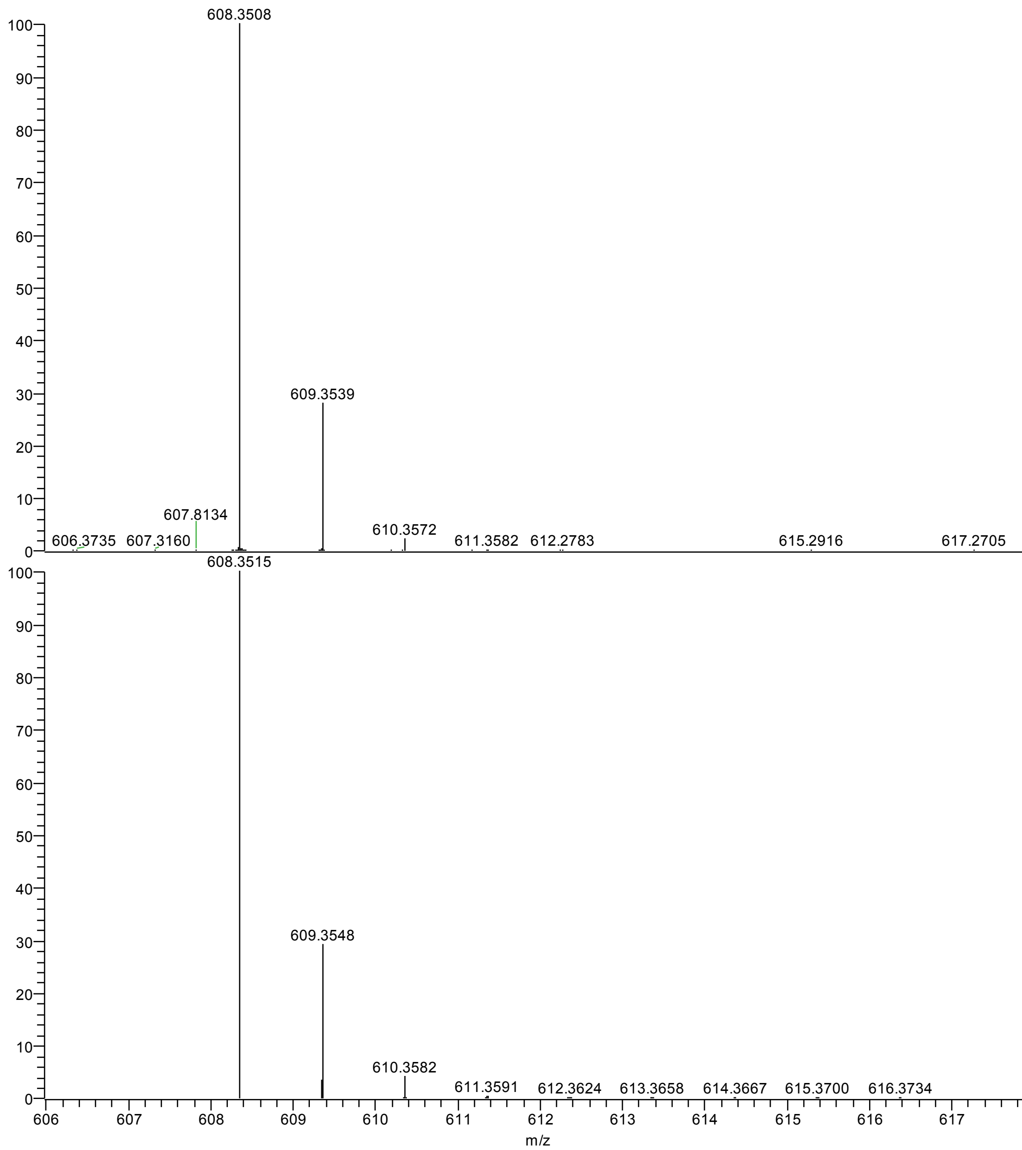




Lin 5.2 free C-NMR

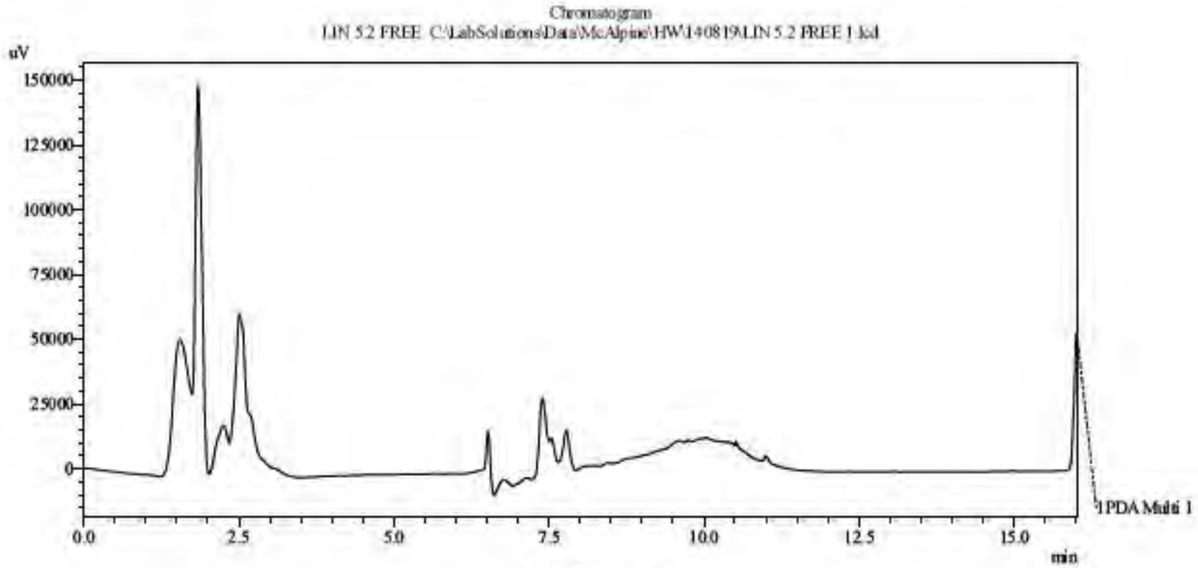


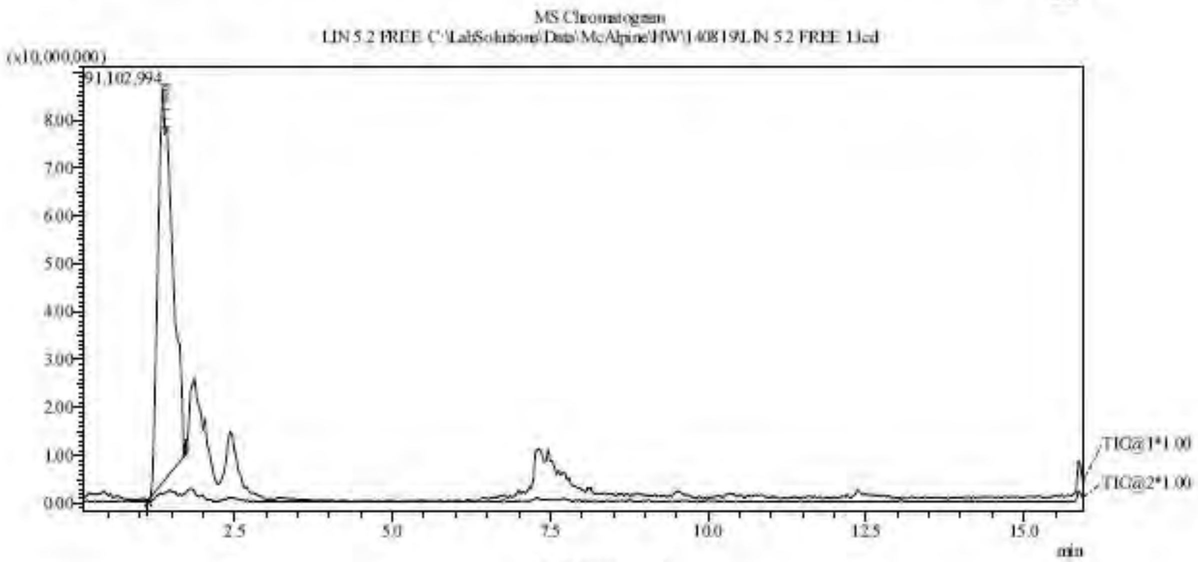
5.2 LIN: HRMS

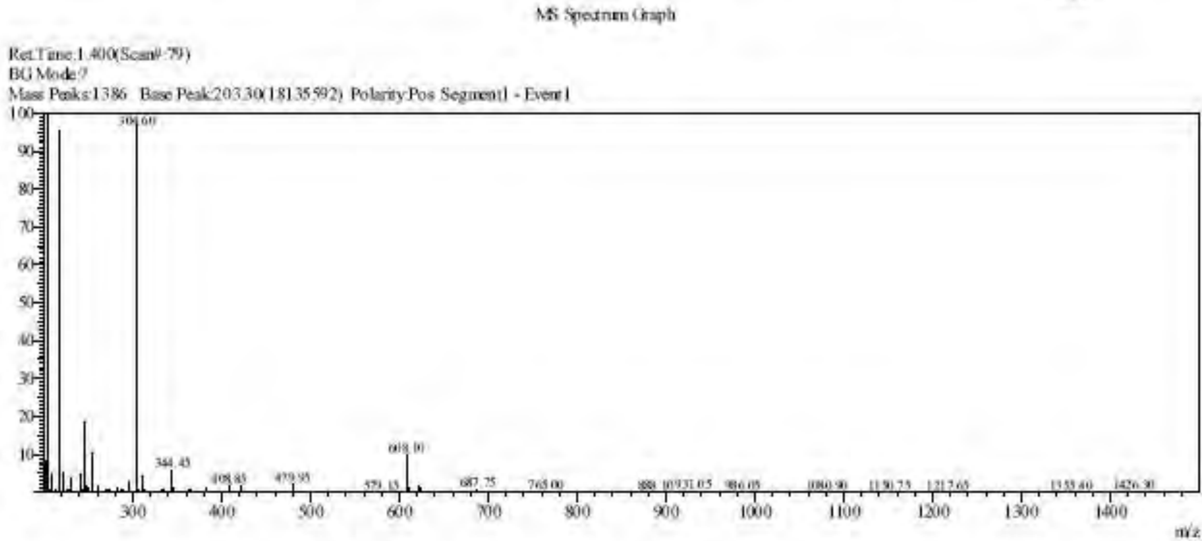


5.2 LIN: LC/MS

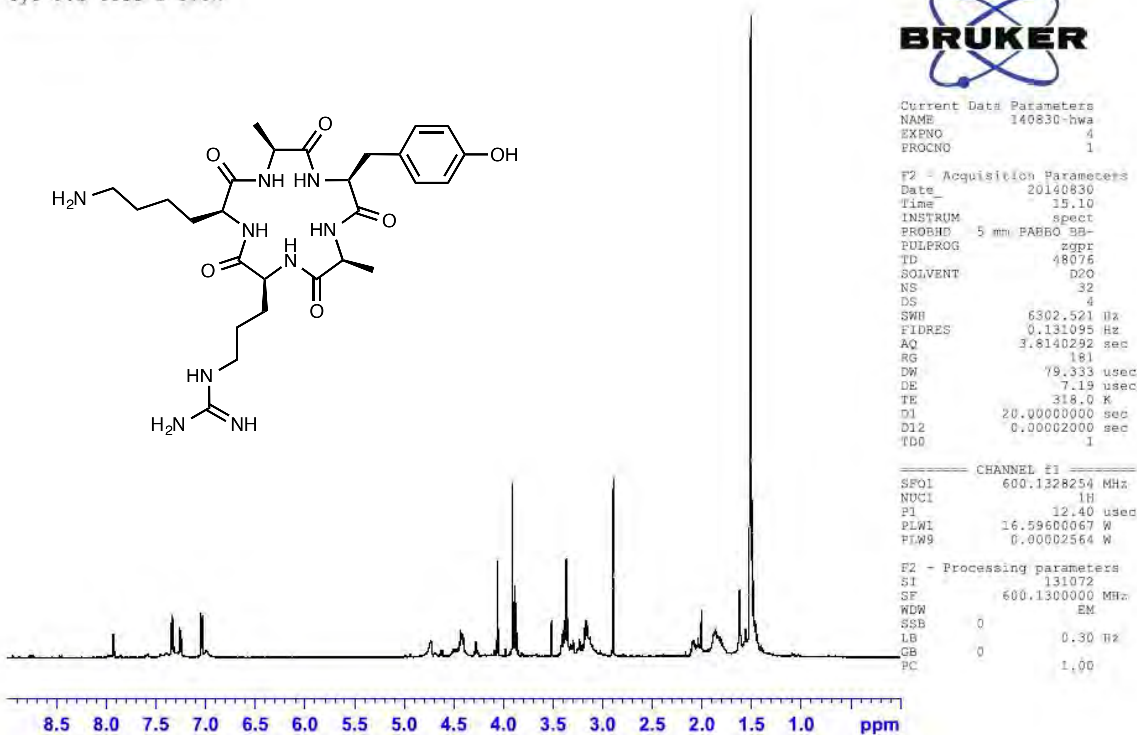
==== Shimadzu LCMSsolution Analysis Report ====

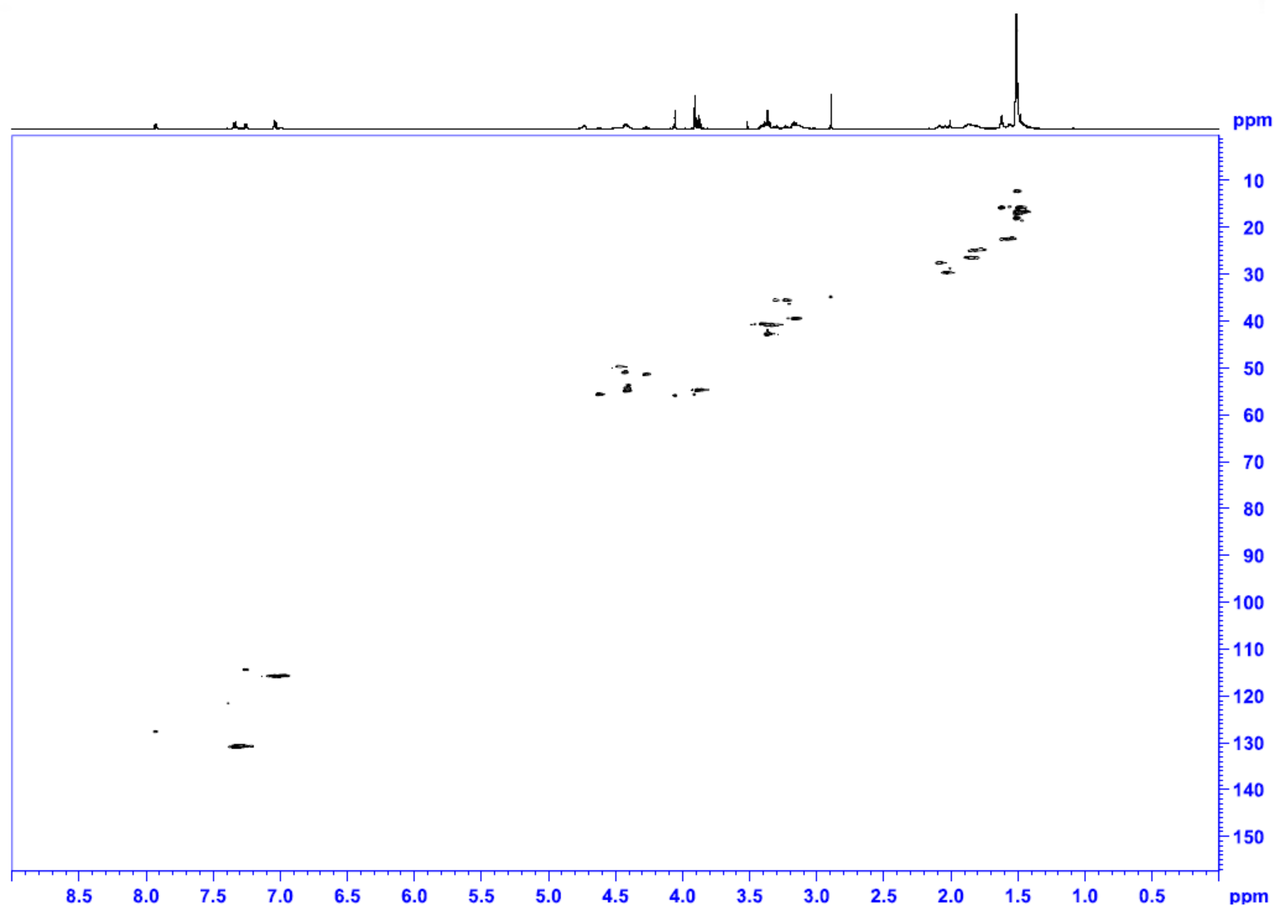




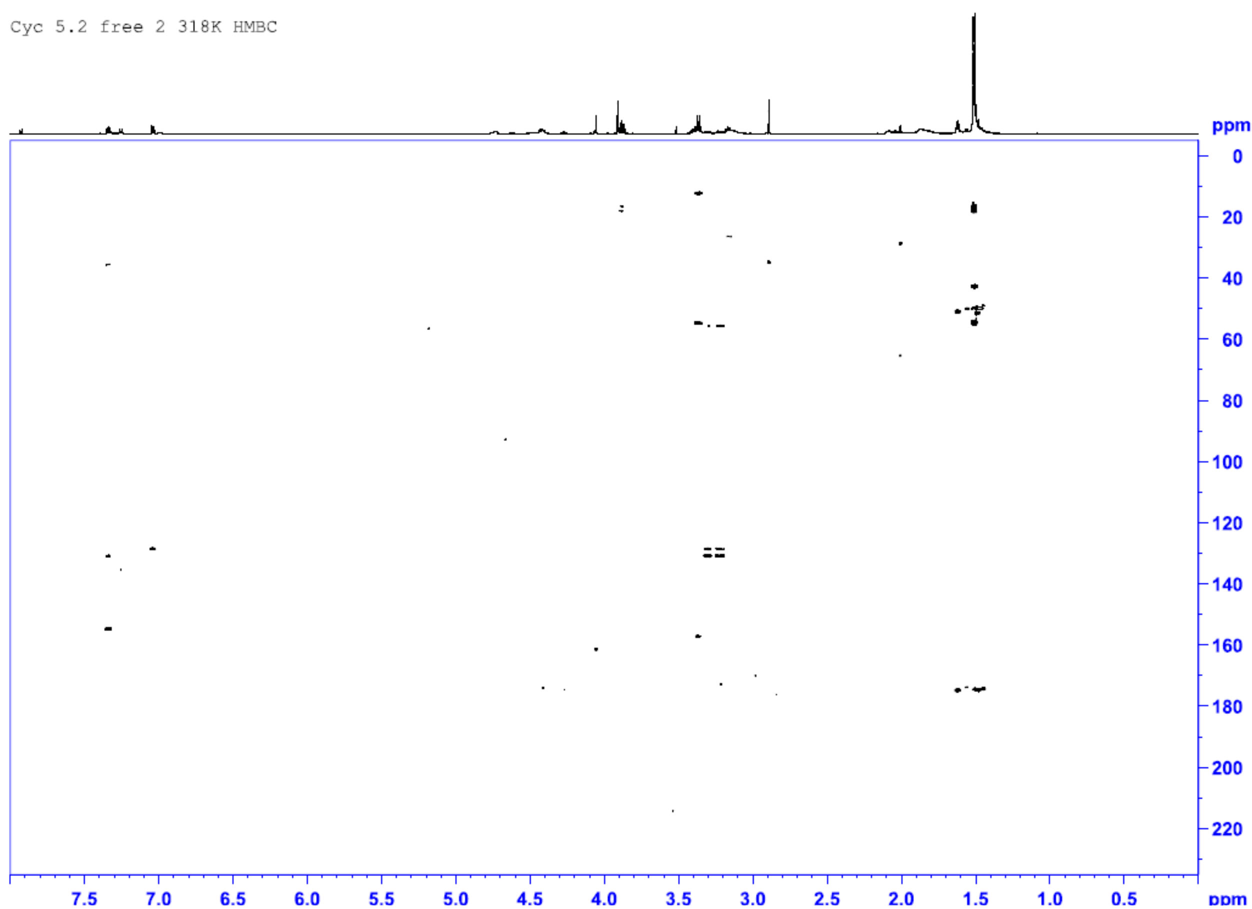


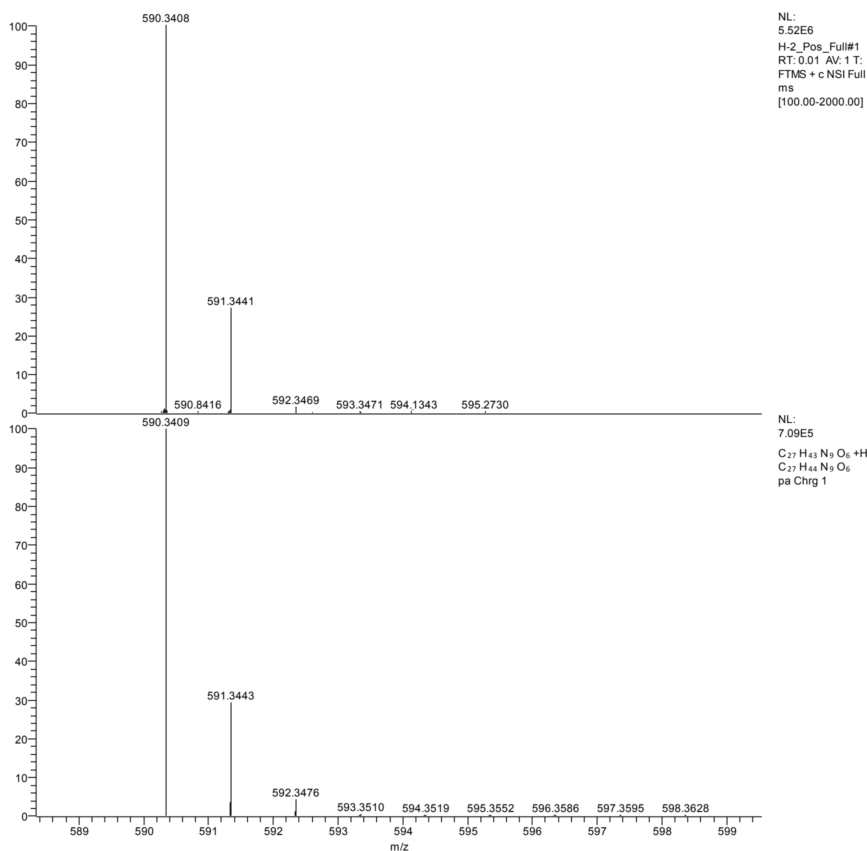
5.2 CYC: ^{1}H NMR and ^{1}H-^{13}C HSQC NMR





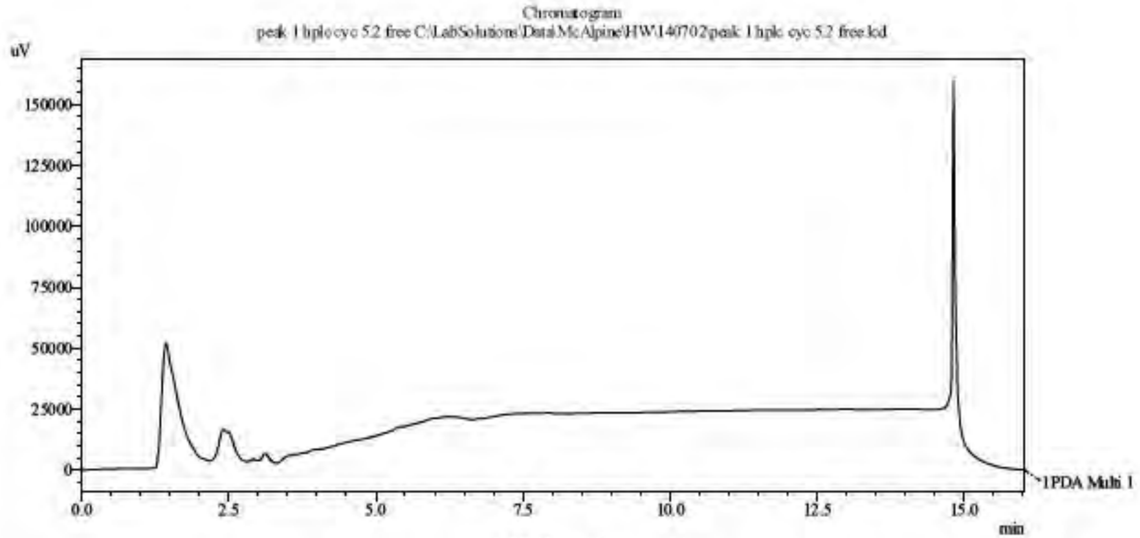
5.2 CYC: ^{1}H-^{13}C HMBC NMR and HRMS

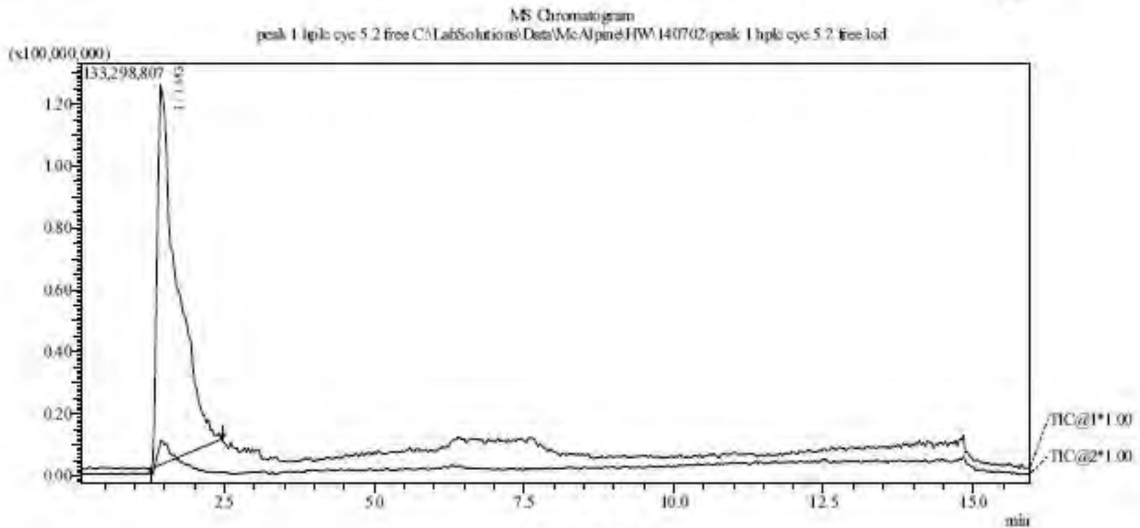


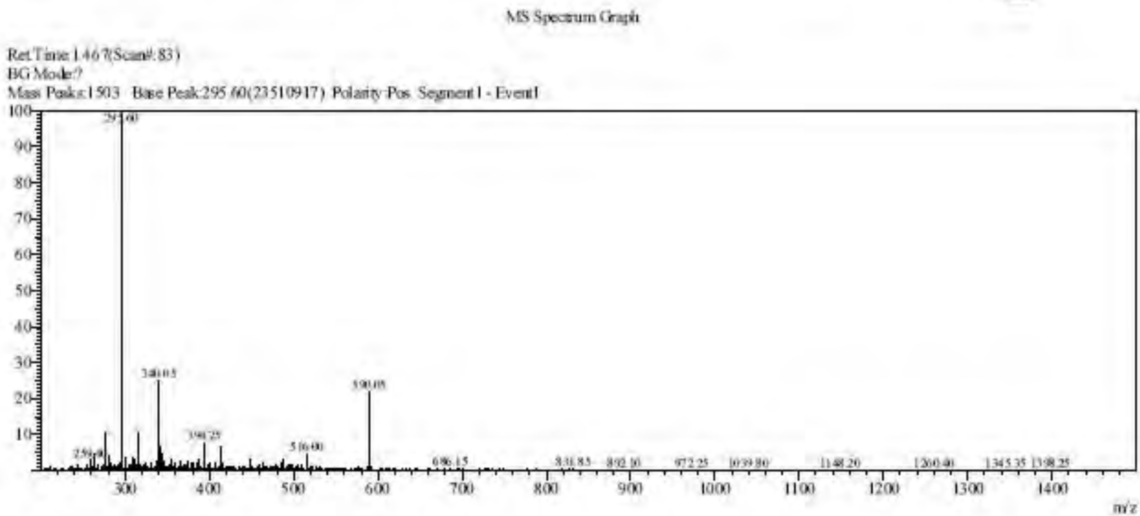


5.2 CYC: LC/MS

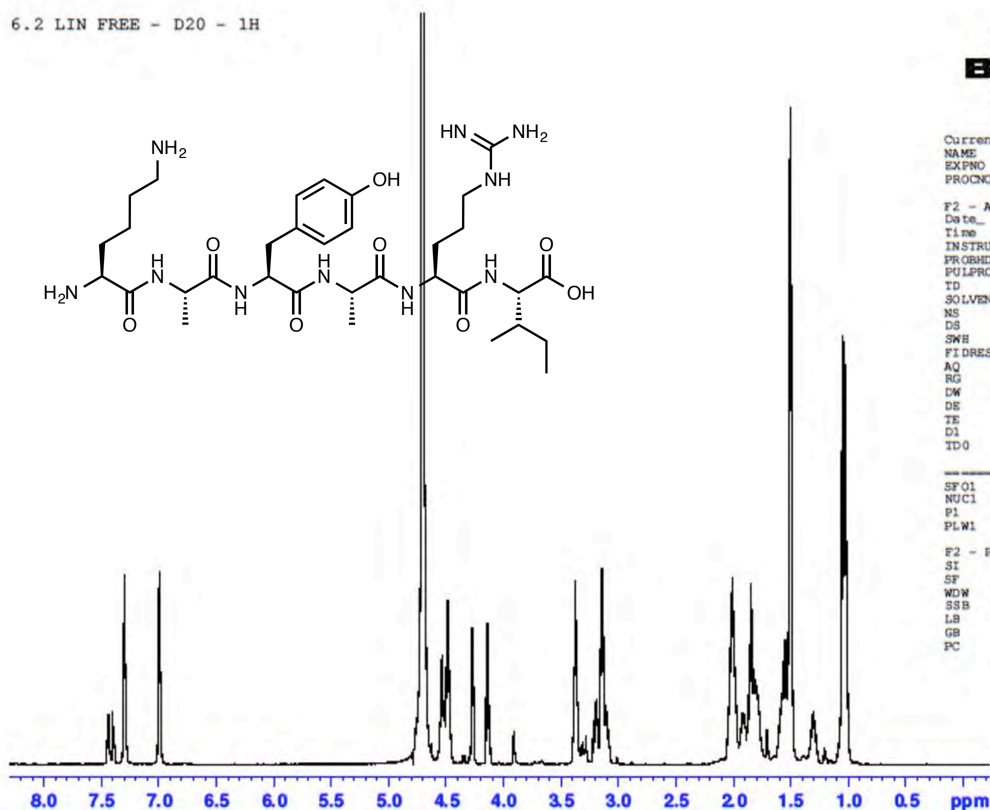
==== Shimadzu LCMSsolution Analysis Report ====







6.2 LIN: ^{1}H NMR and ^{13}C NMR



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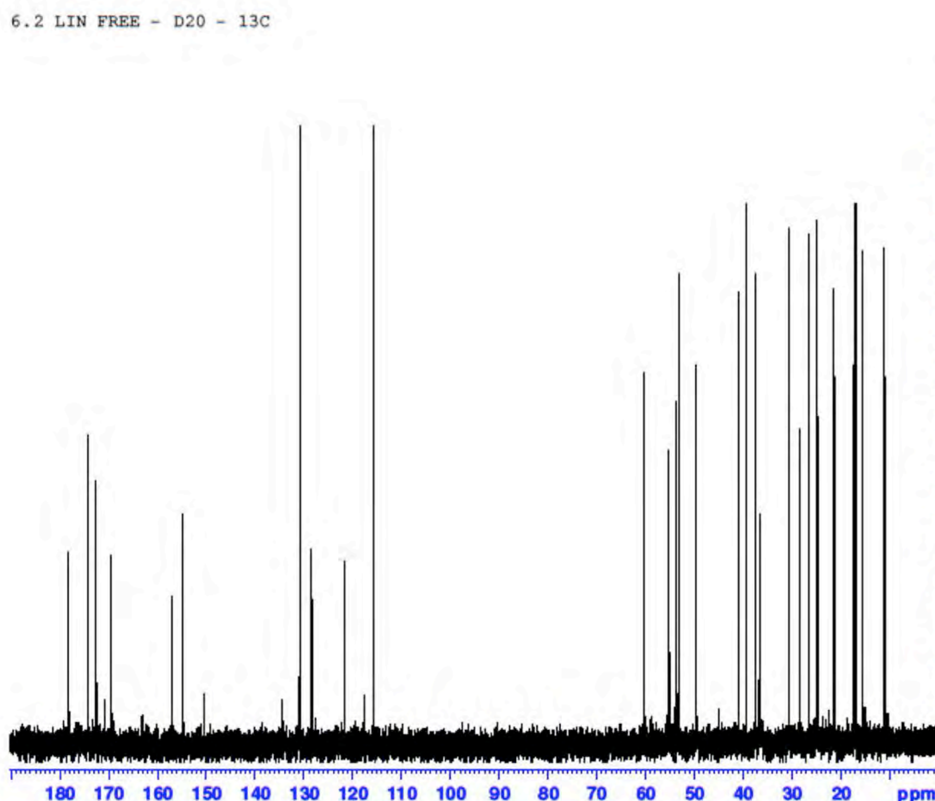

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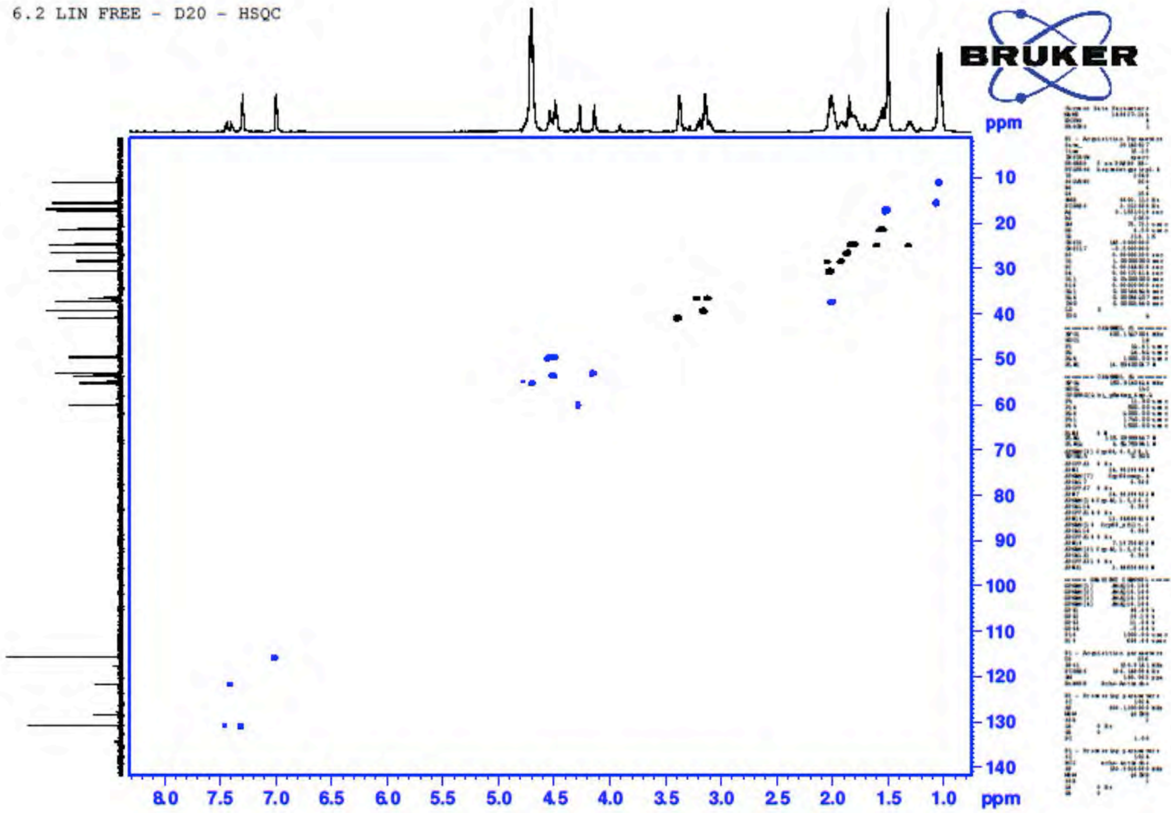
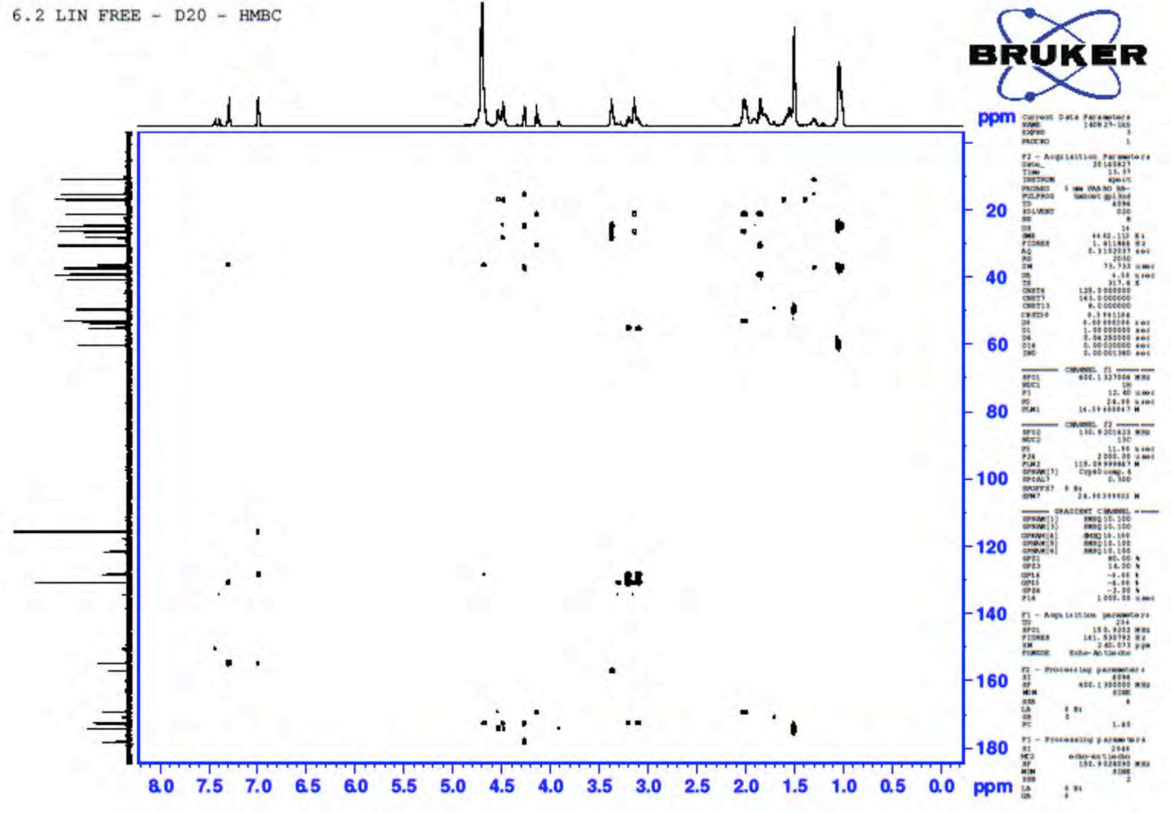
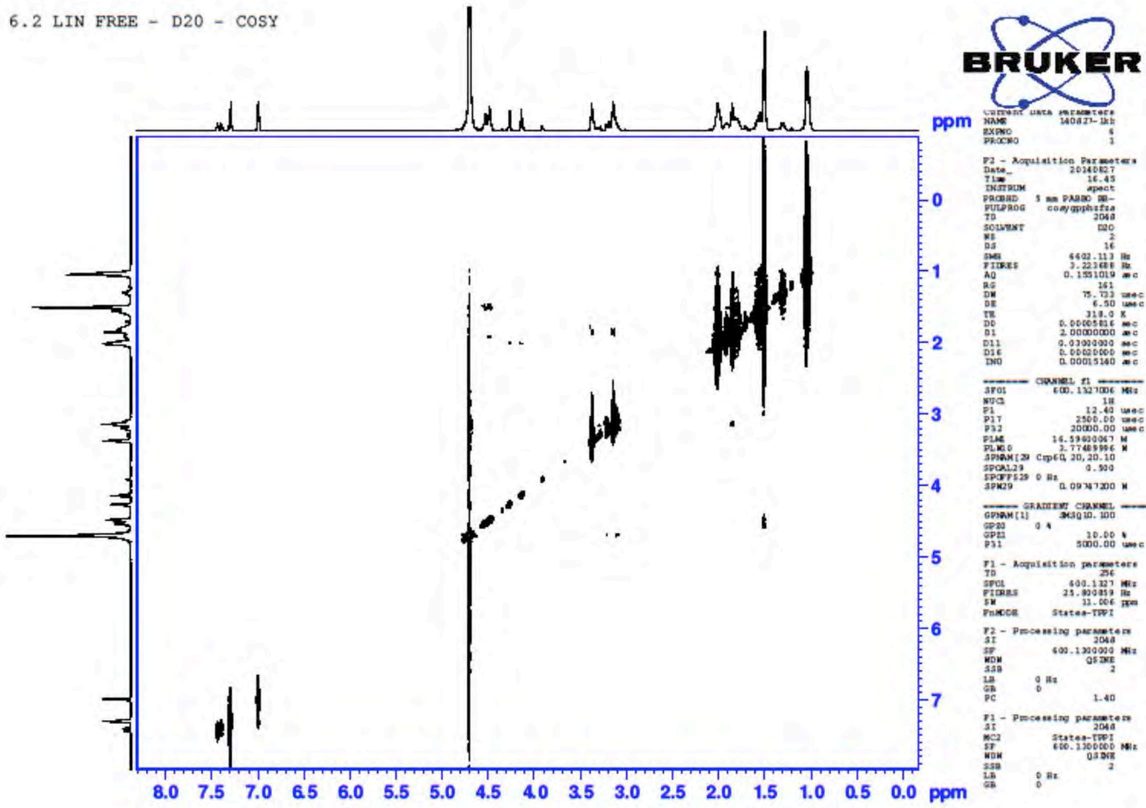
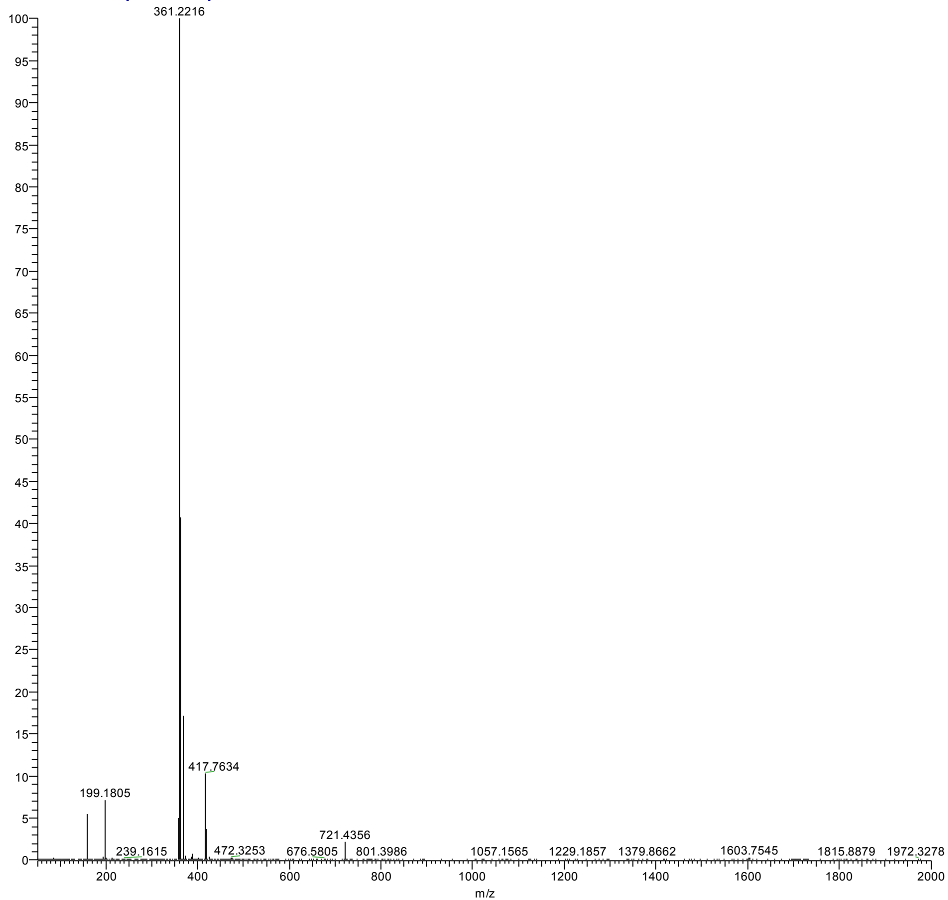
6.2 LIN: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





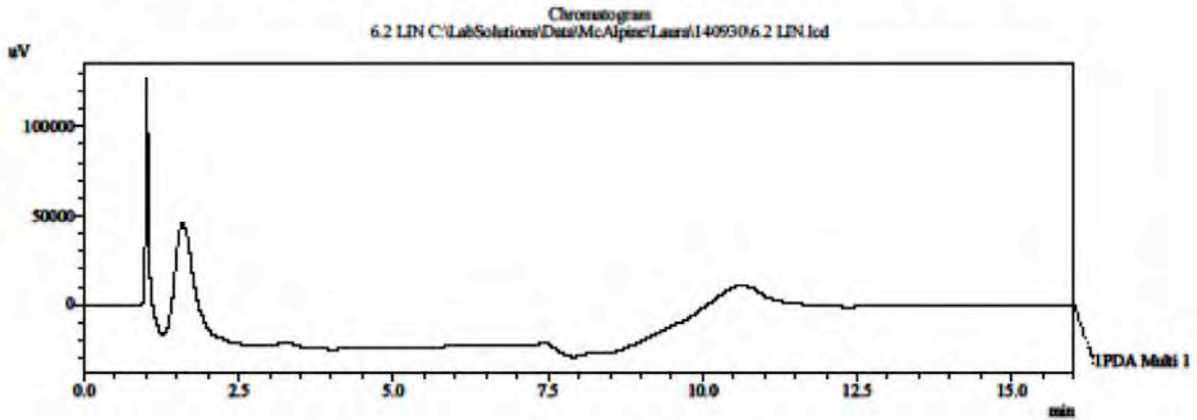
6.2 LIN: ^{1}H-^{1}H COSY NMR and HRMS

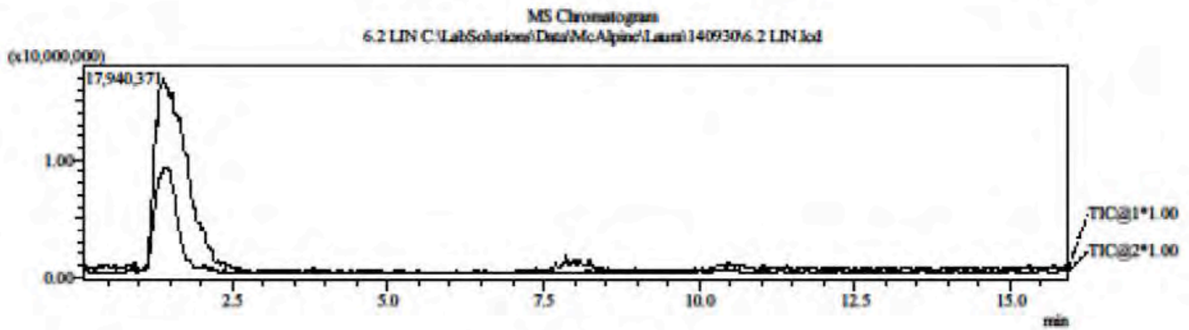


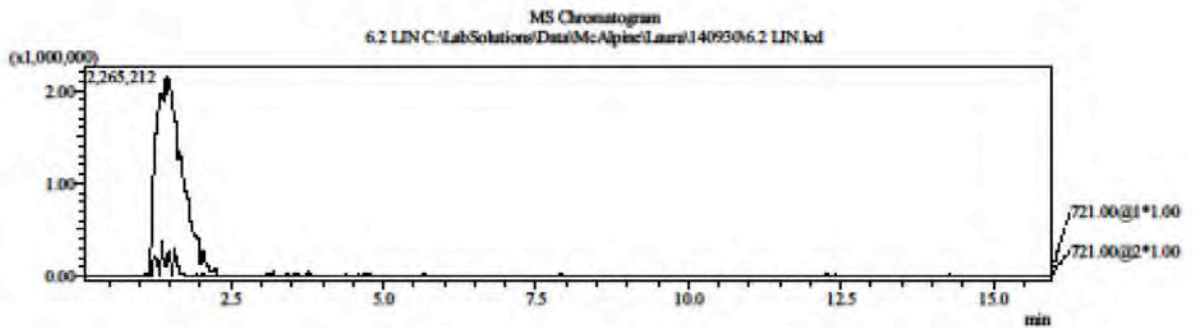


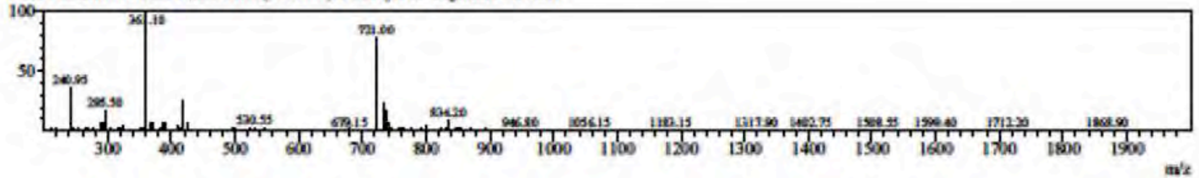
6.2 LIN: LC/MS

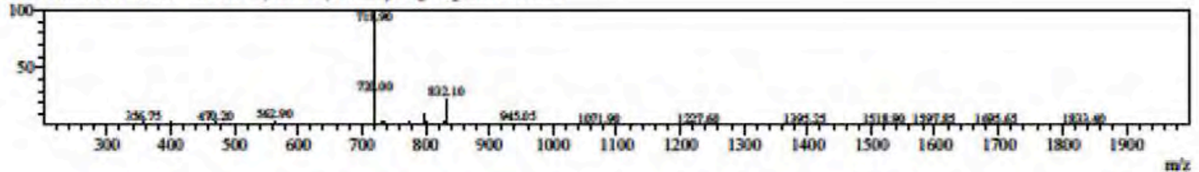
==== Shimadzu LCMSsolution Analysis Report ====



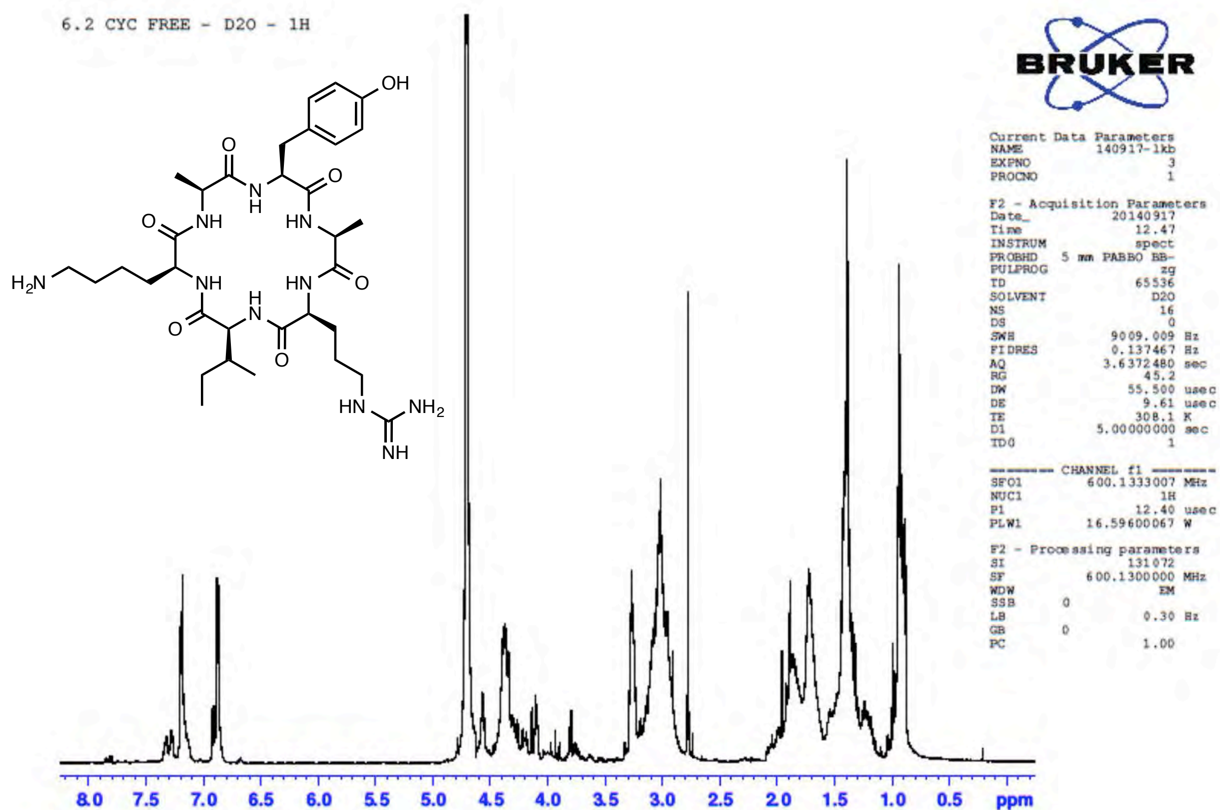


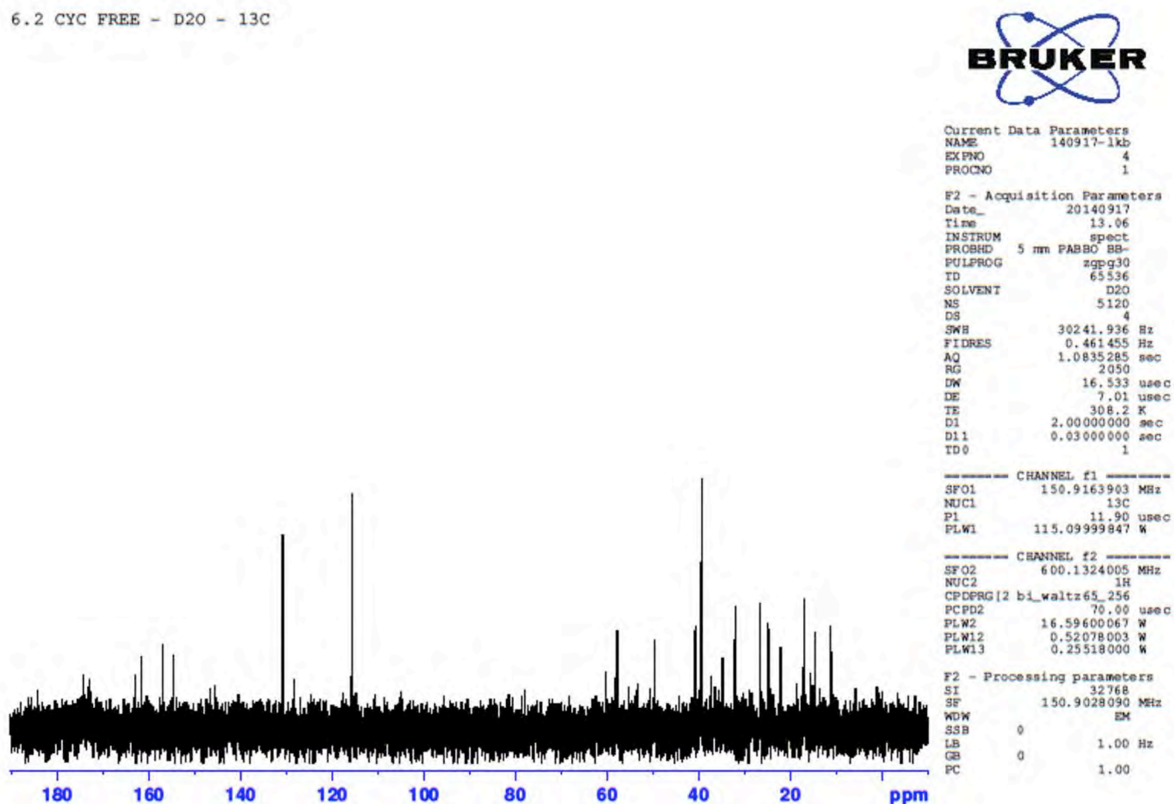




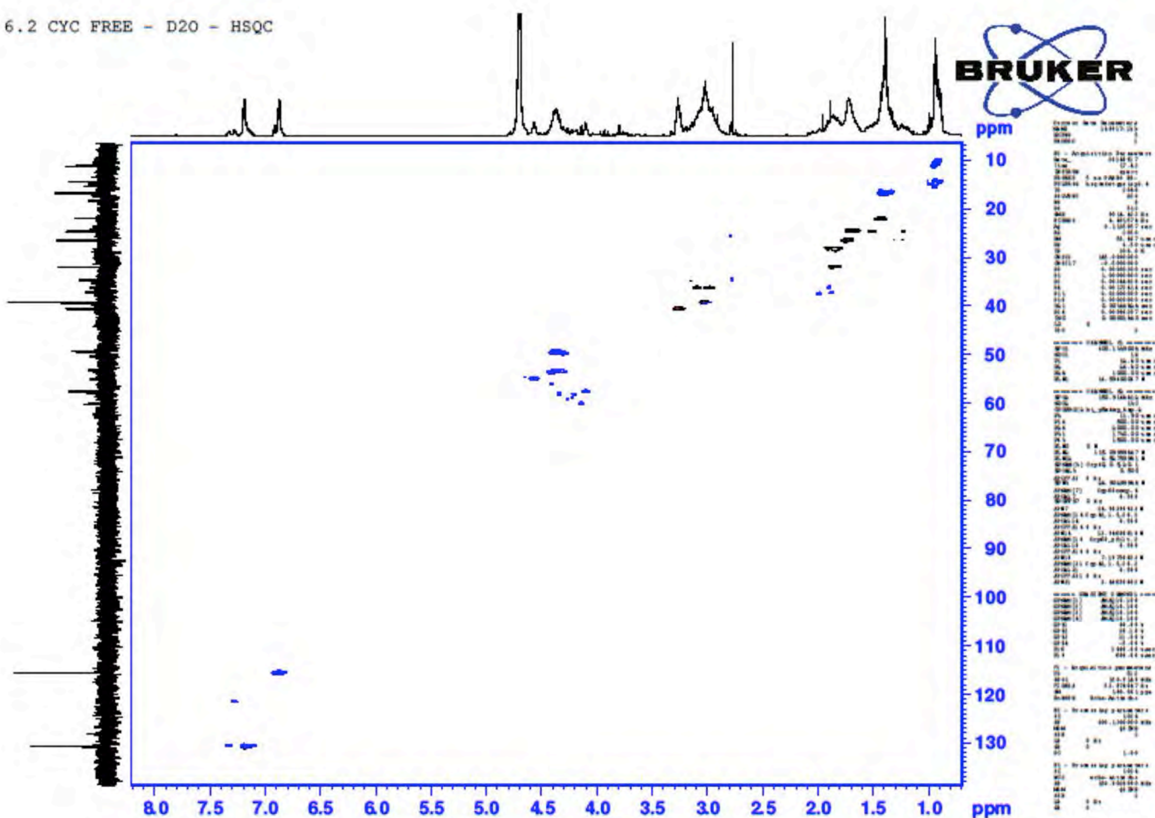


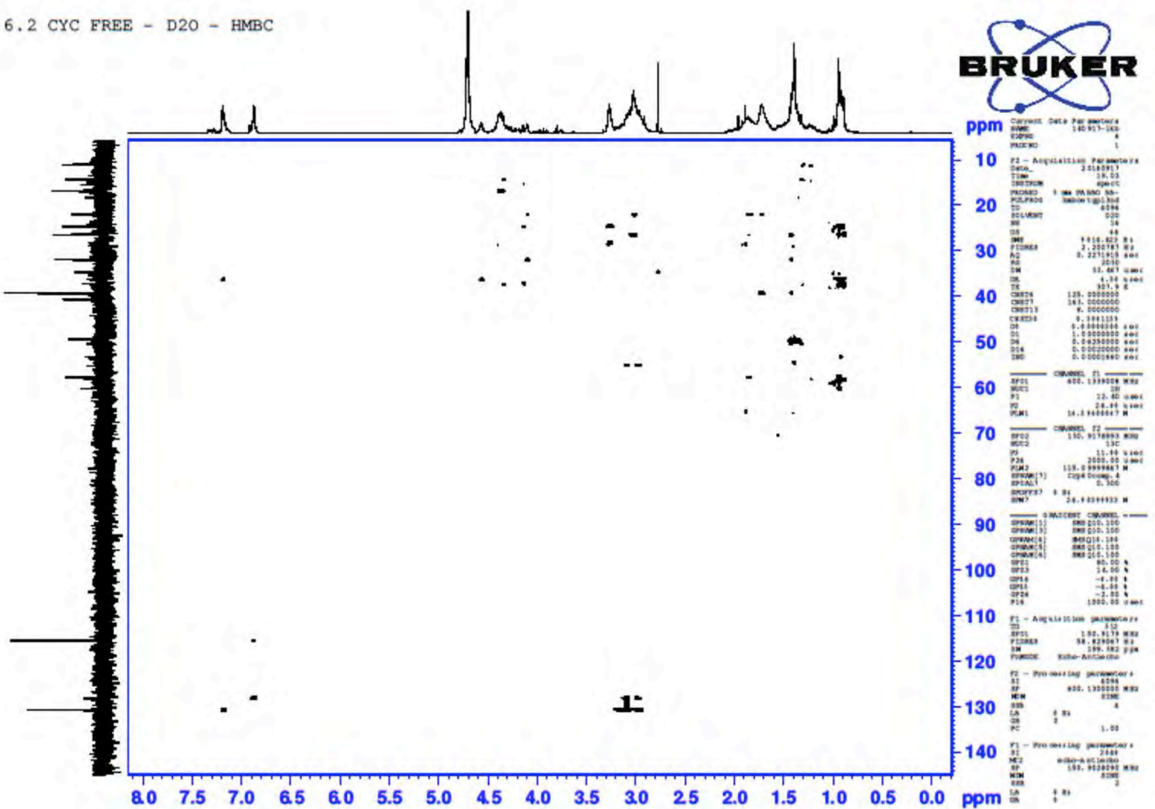
6.2 CYC: ^{1}H NMR and ^{13}C NMR



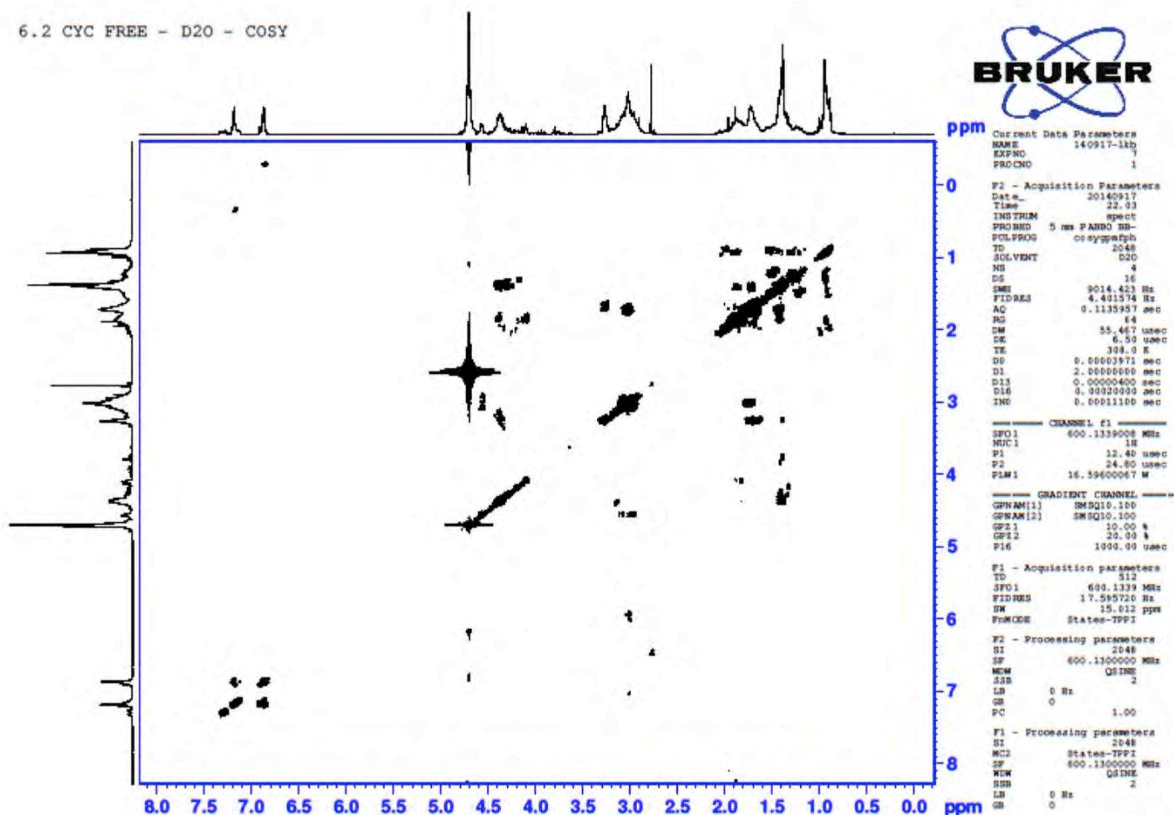


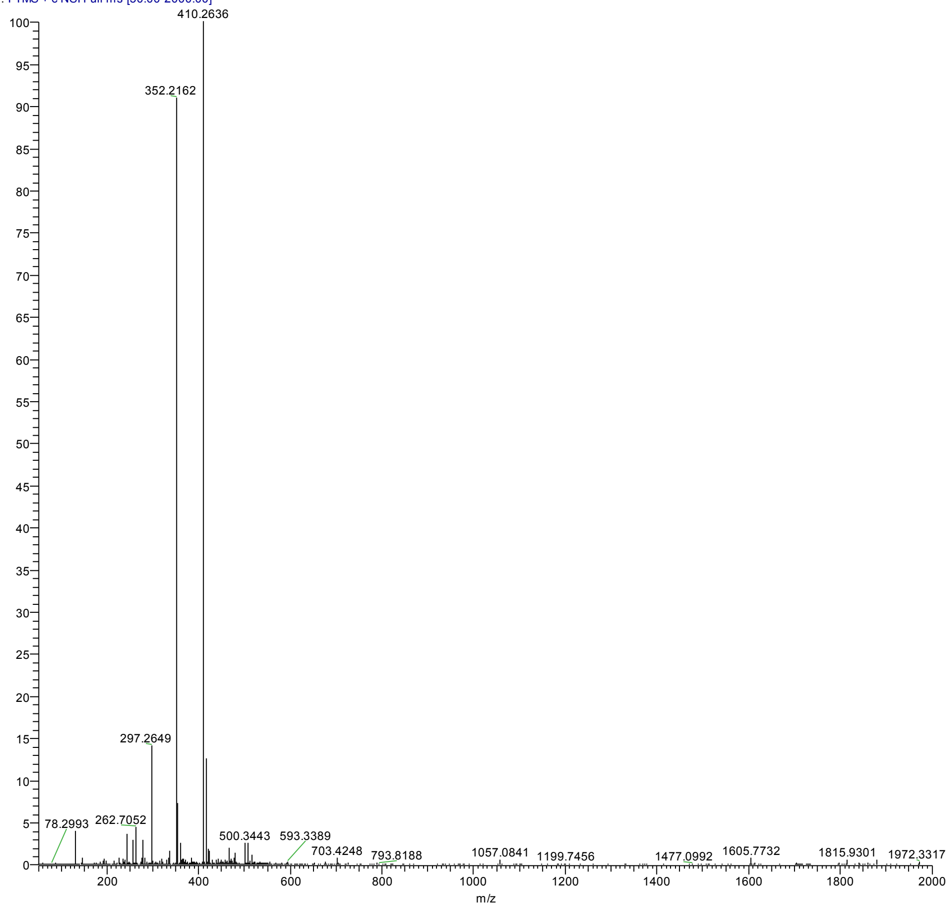
6.2 CYC: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





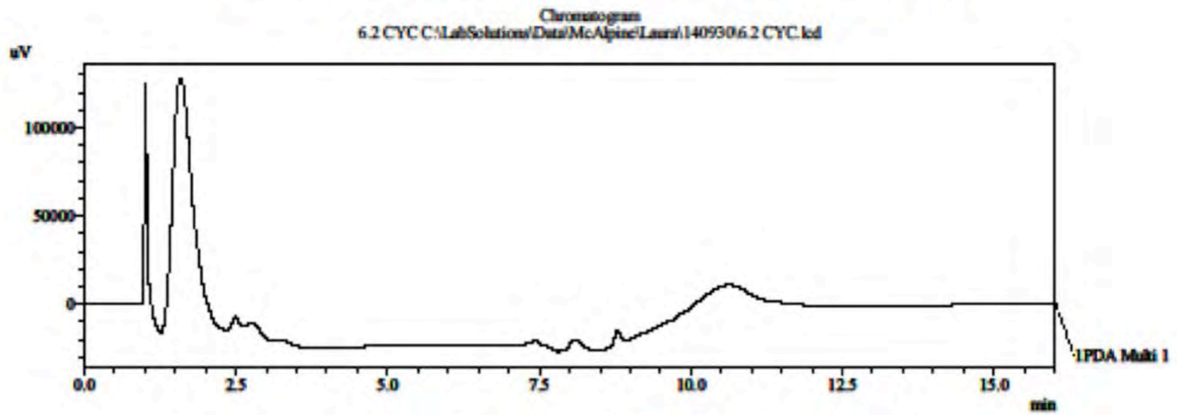
6.2 CYC: ^{1}H-^{1}H COSY NMR and HRMS

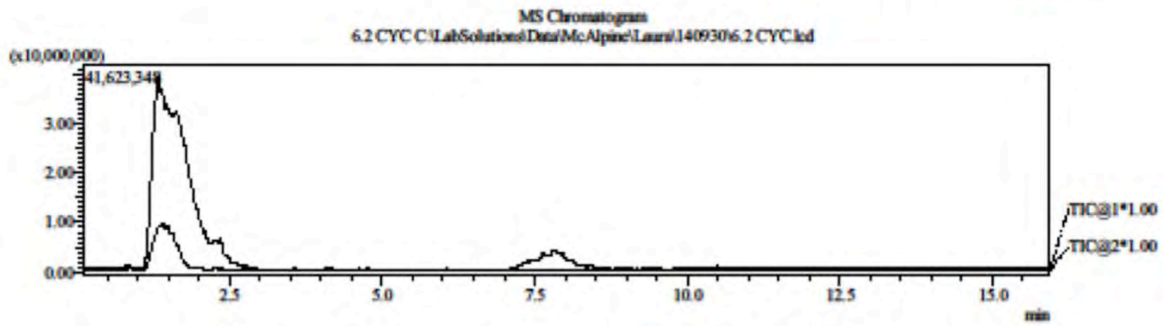


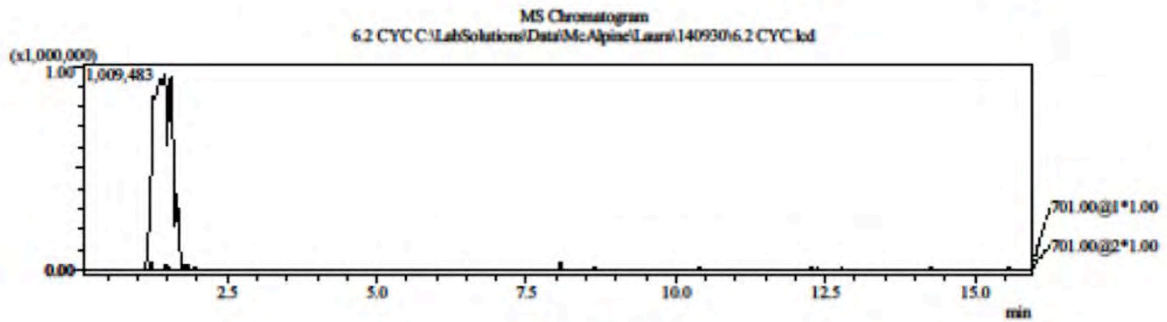


6.2 CYC: LC/MS

==== Shimadzu LCMSsolution Analysis Report ====







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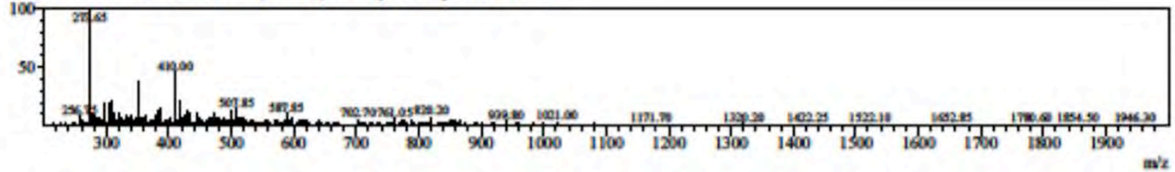

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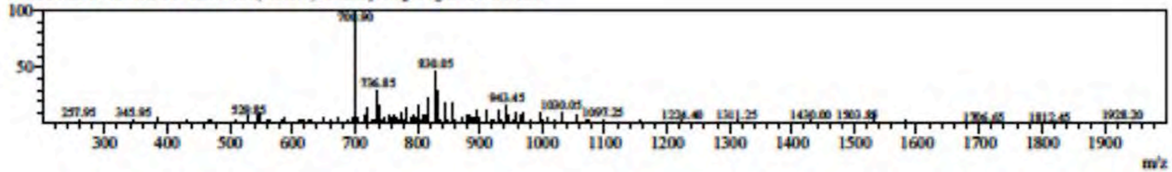
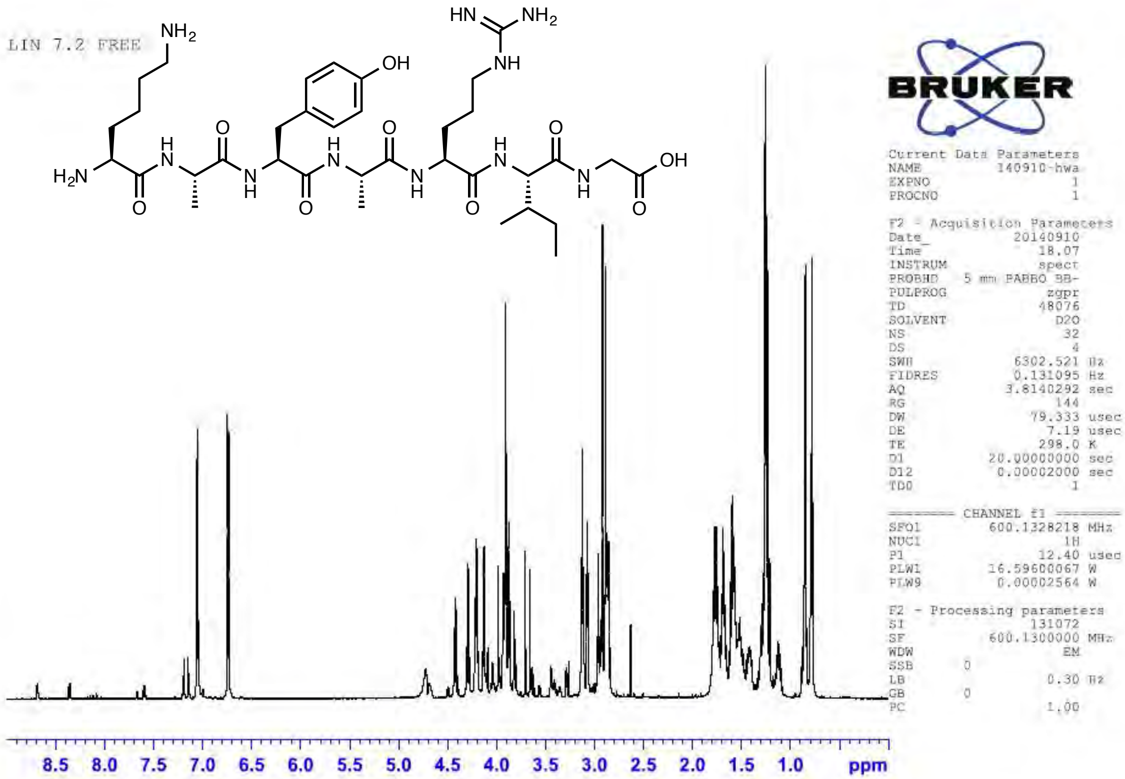
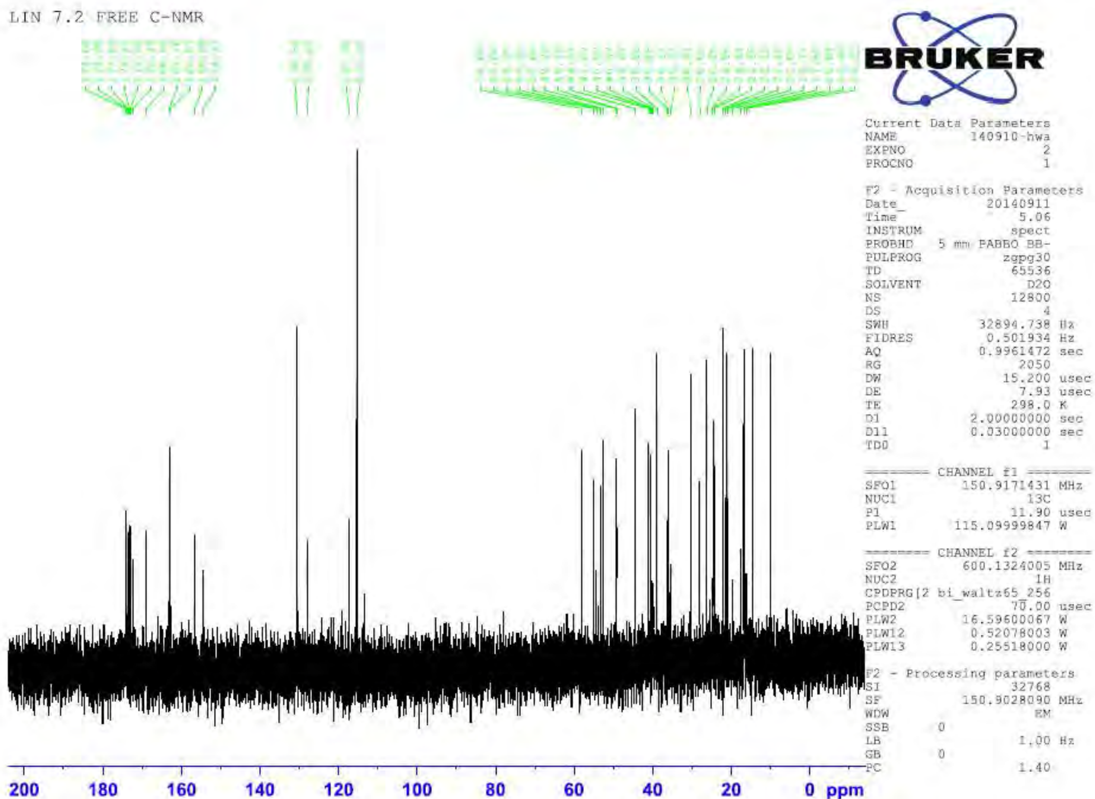
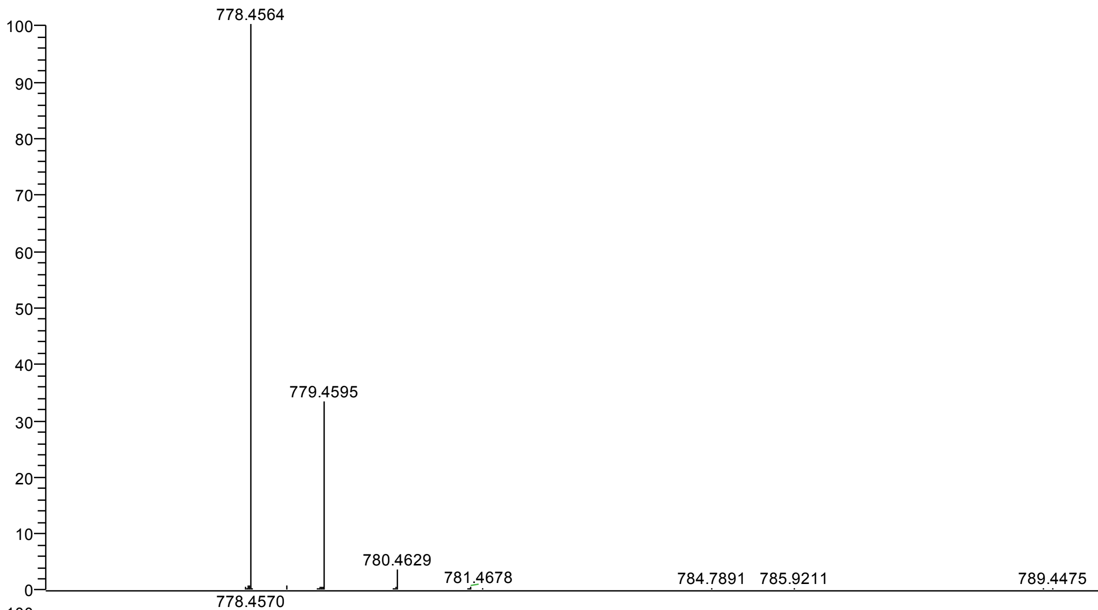


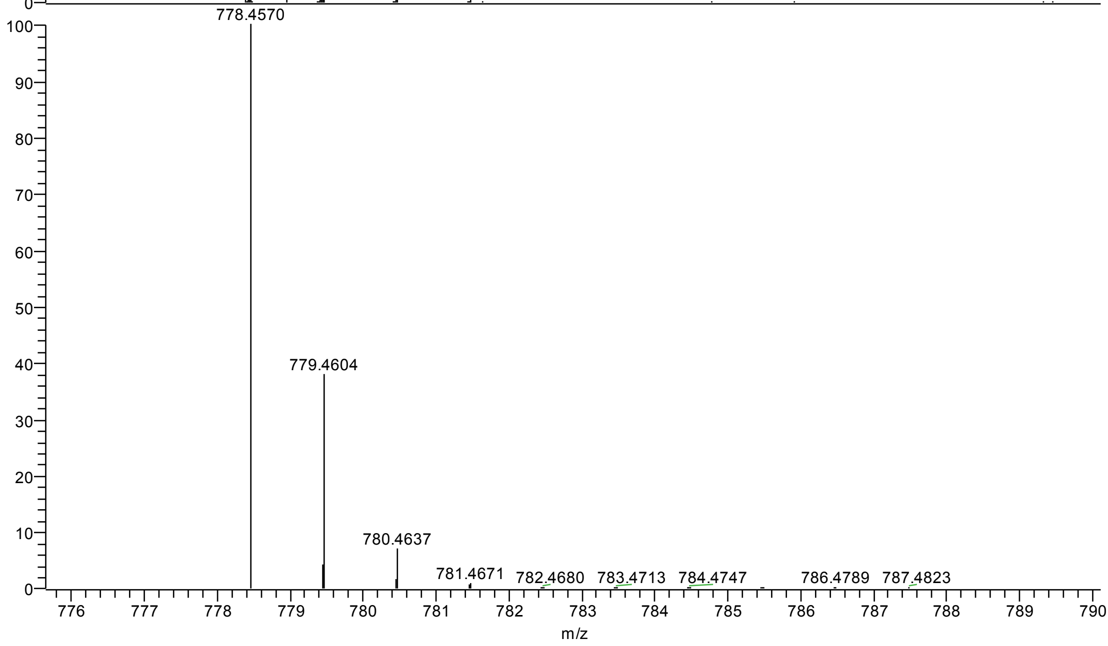
7.2 LIN: ^{1}H NMR and ^{13}C NMR





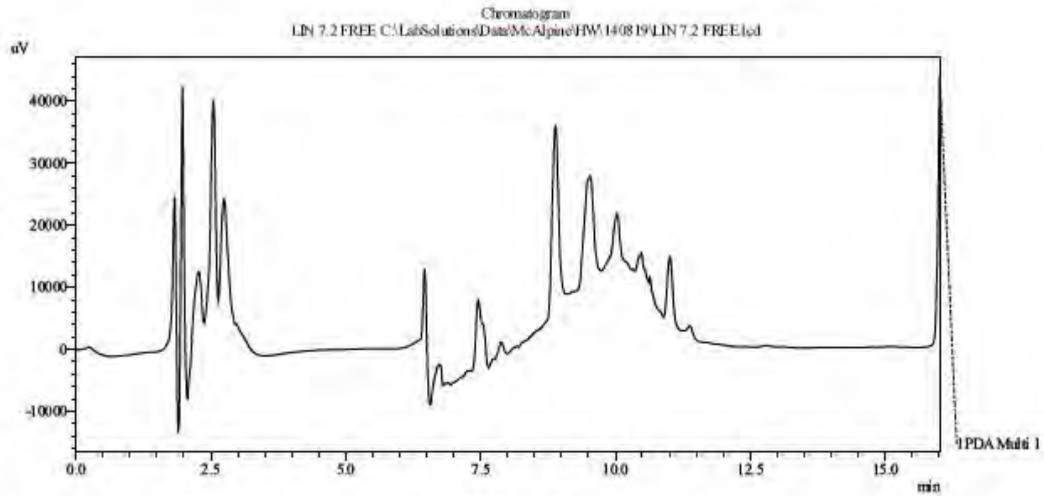
7.2 LIN: HRMS

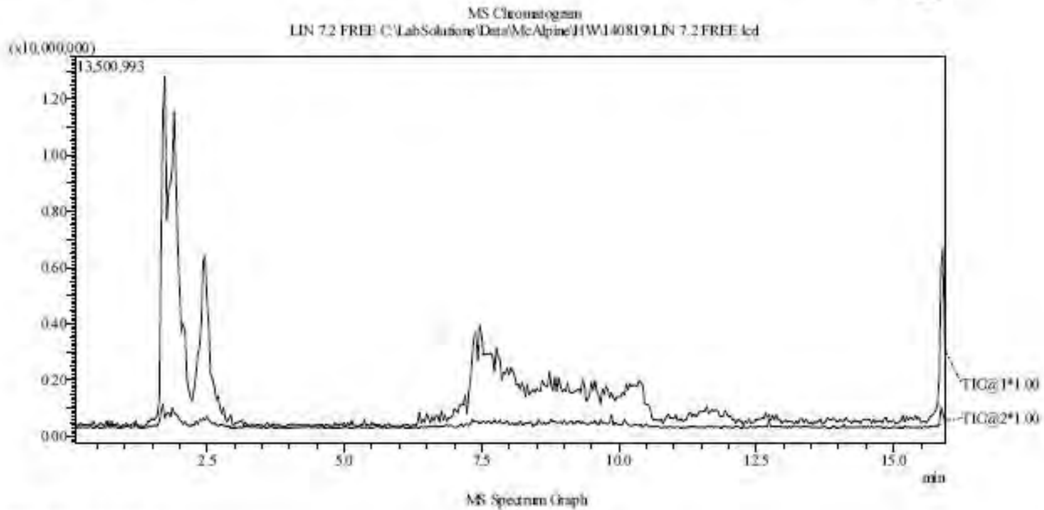


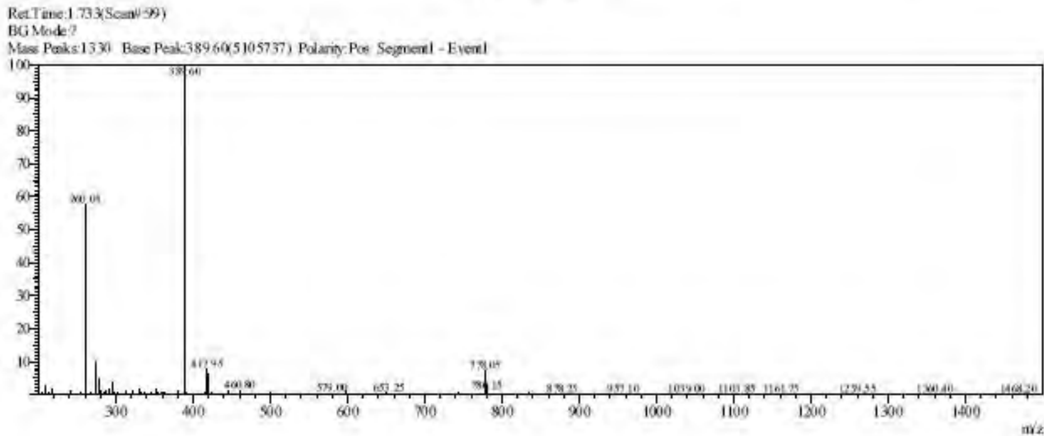


7.2 LIN: LC/MS

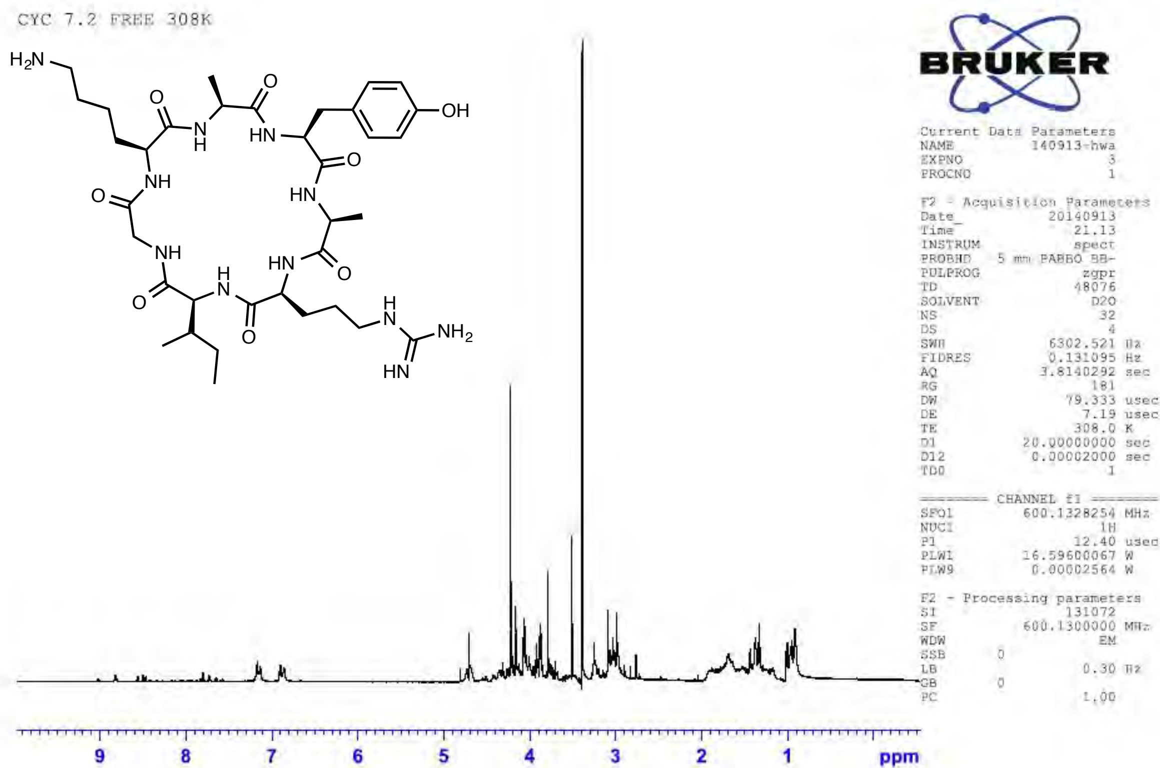
==== Shimadzu LCMSsolution Analysis Report ====

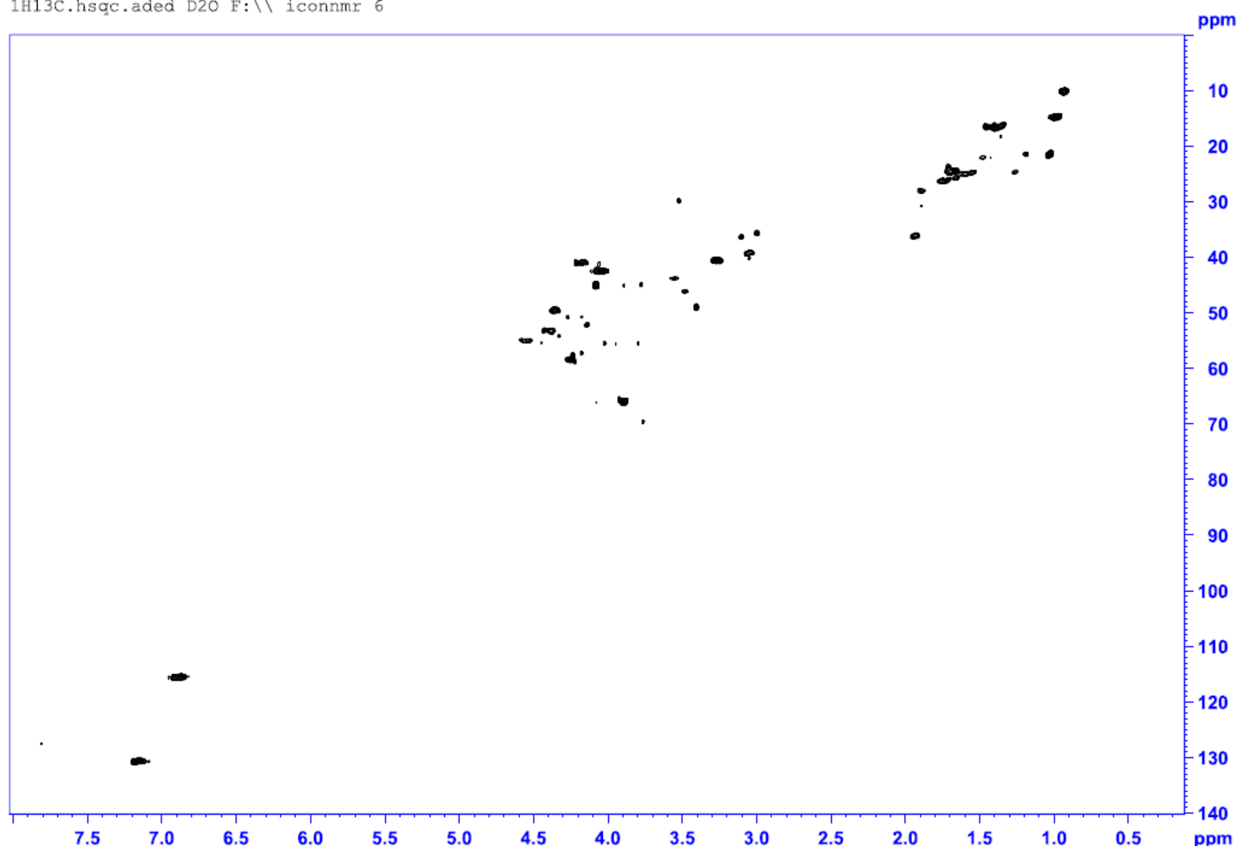




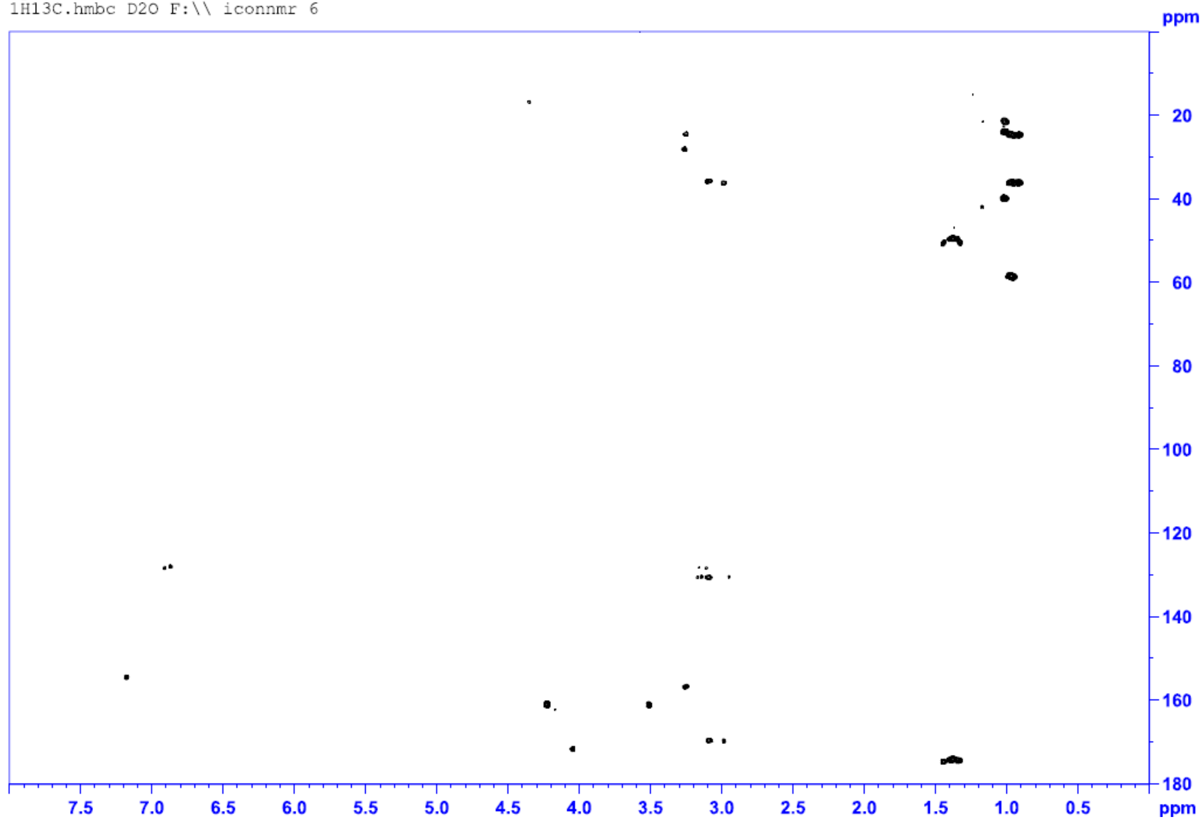


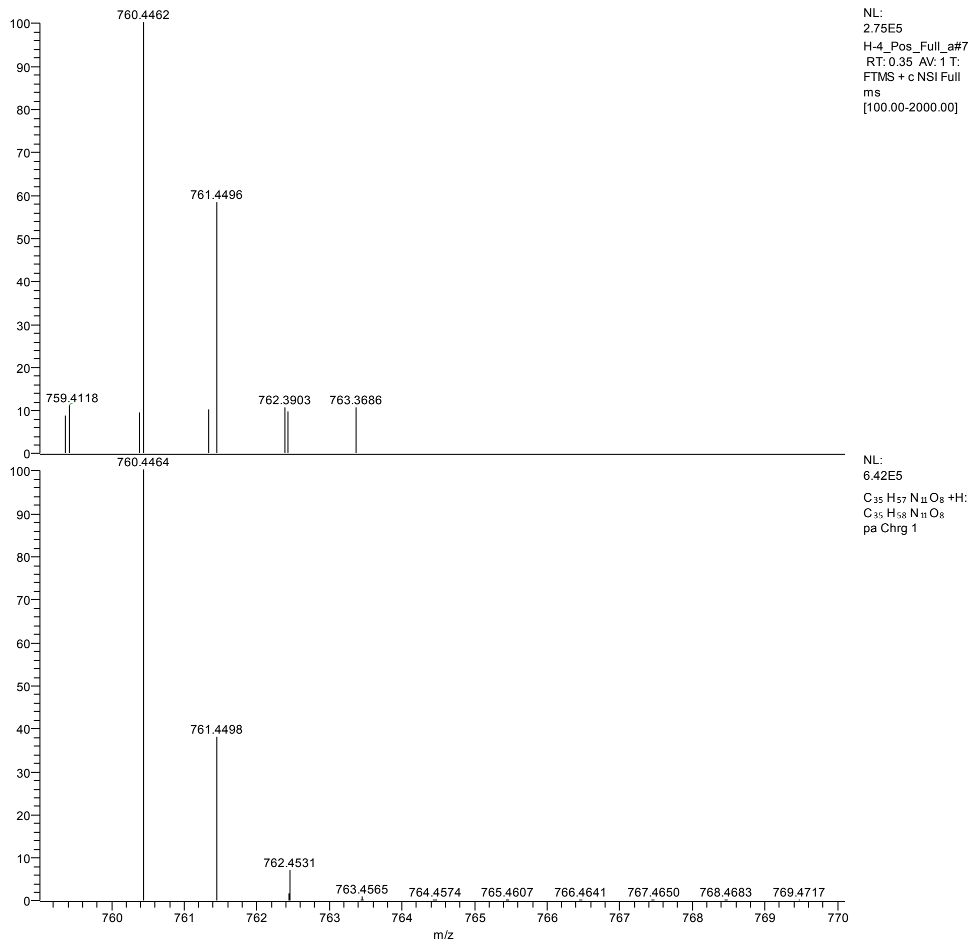
7.2 CYC: ^{1}H NMR and ^{1}H-^{13}C HSQC NMR





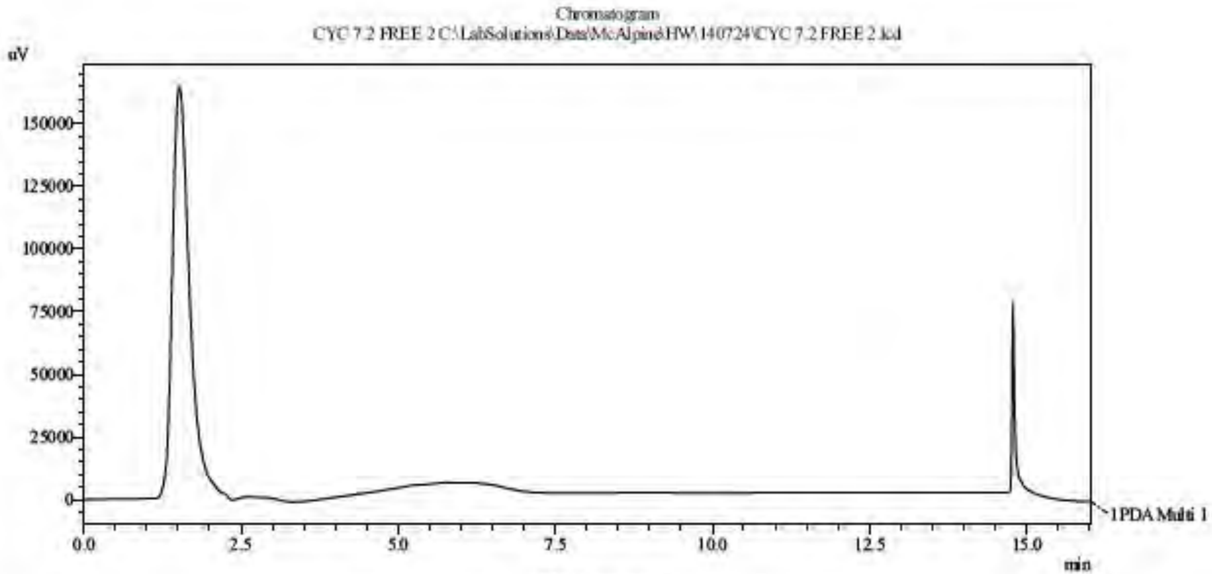
7.2 CYC: ^{1}H-^{13}C HMBC NMR and HRMS

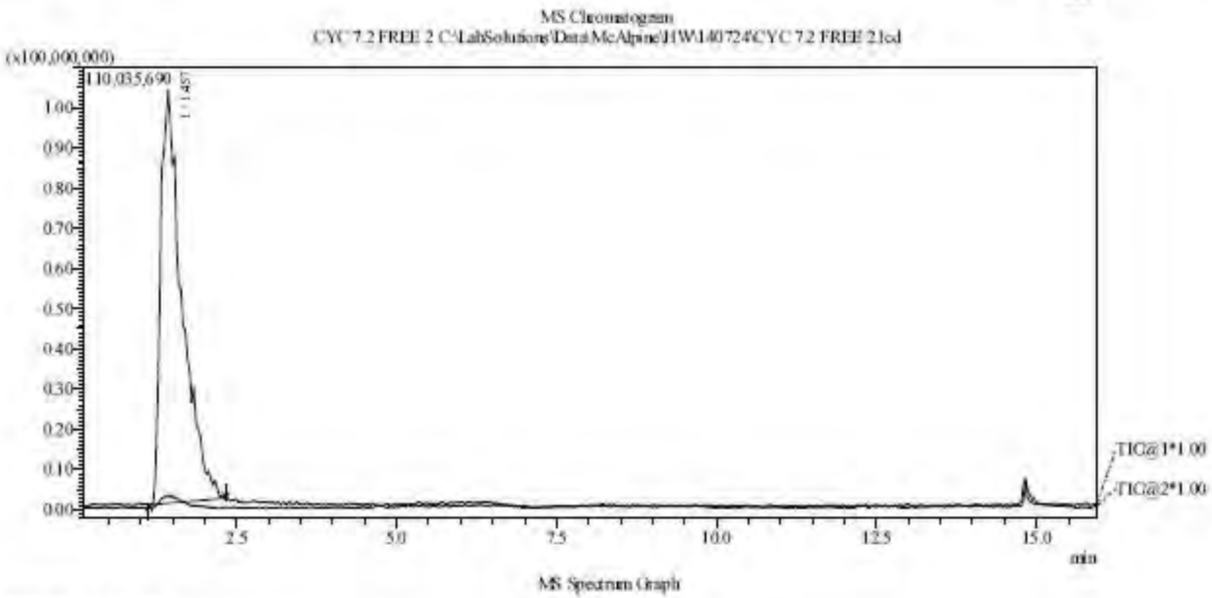


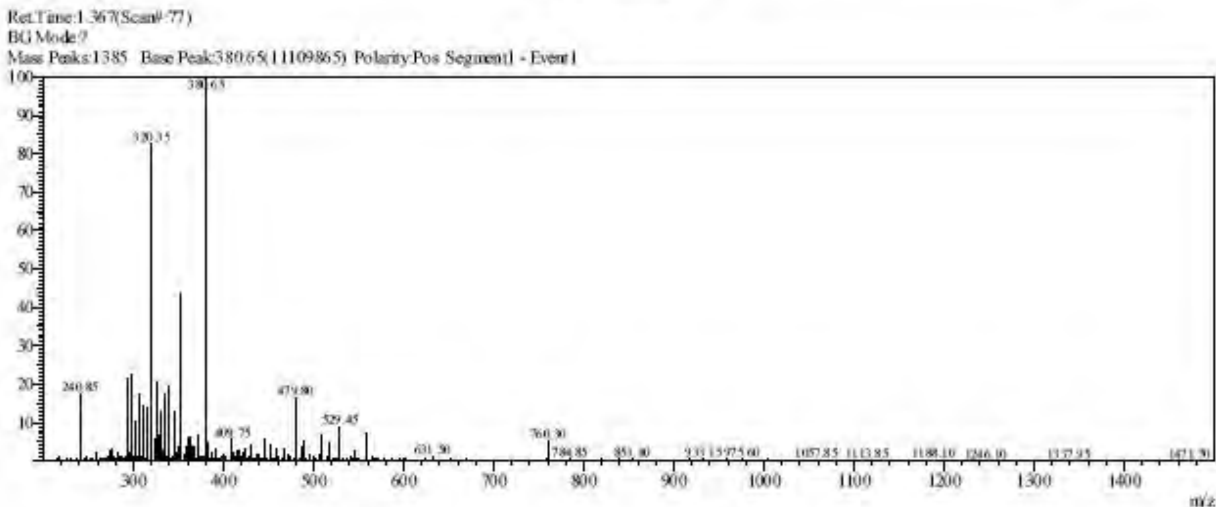


7.2 CYC: LC/MS

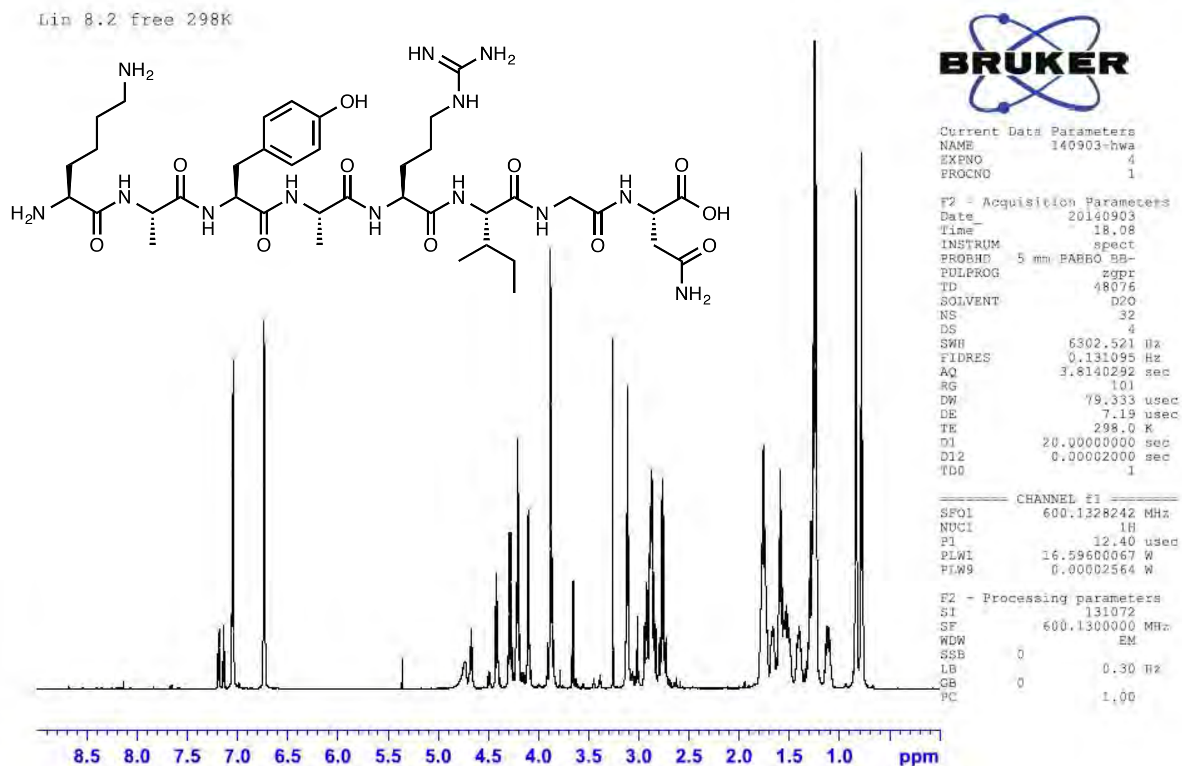
==== Shimadzu LCMSsolution Analysis Report ====

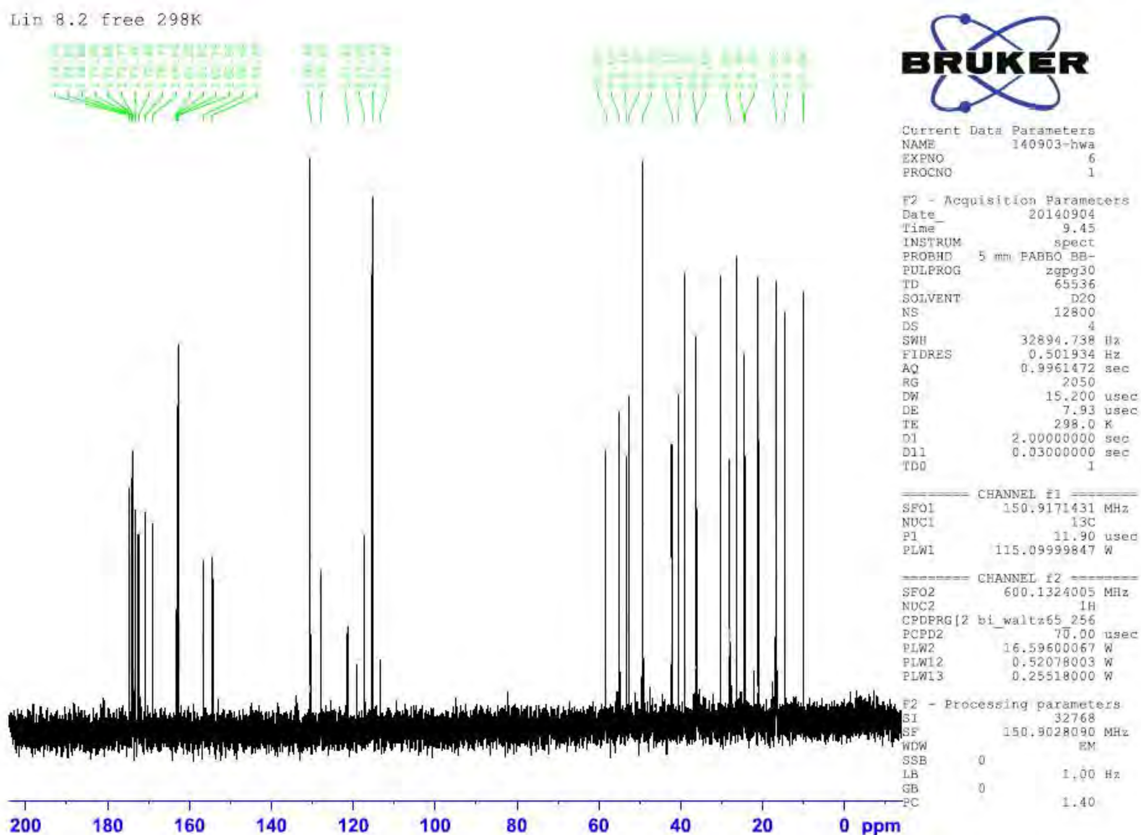




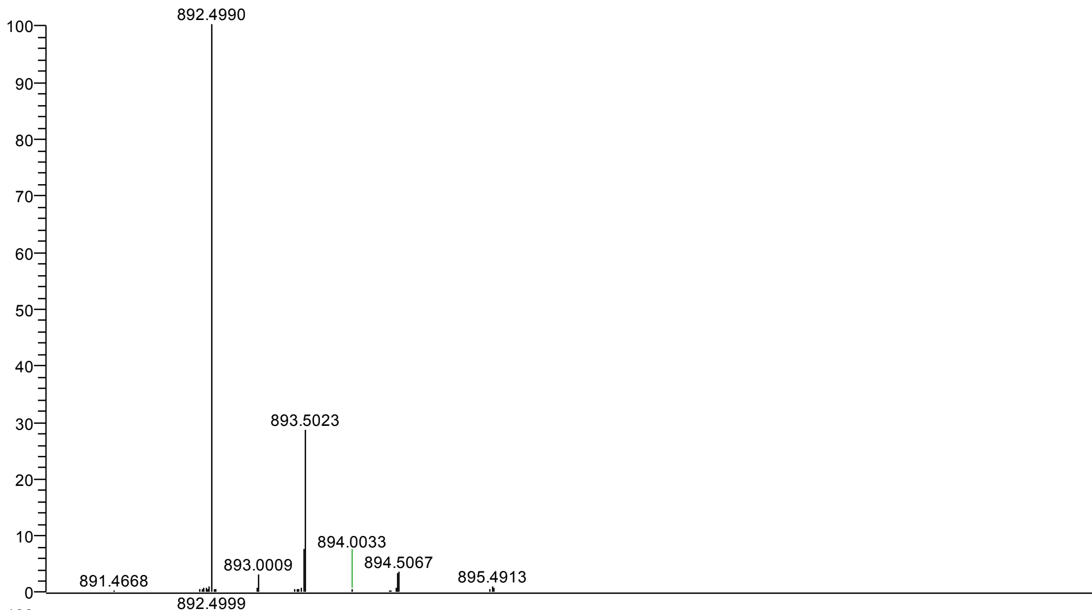


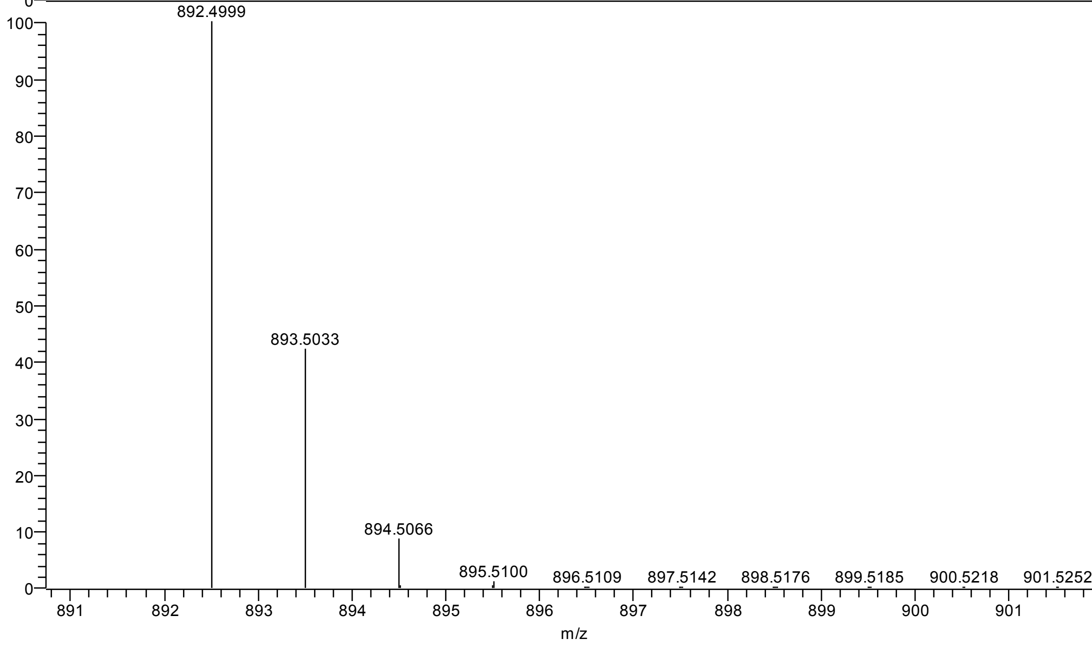
8.2 LIN: ^{1}H NMR and ^{13}C NMR





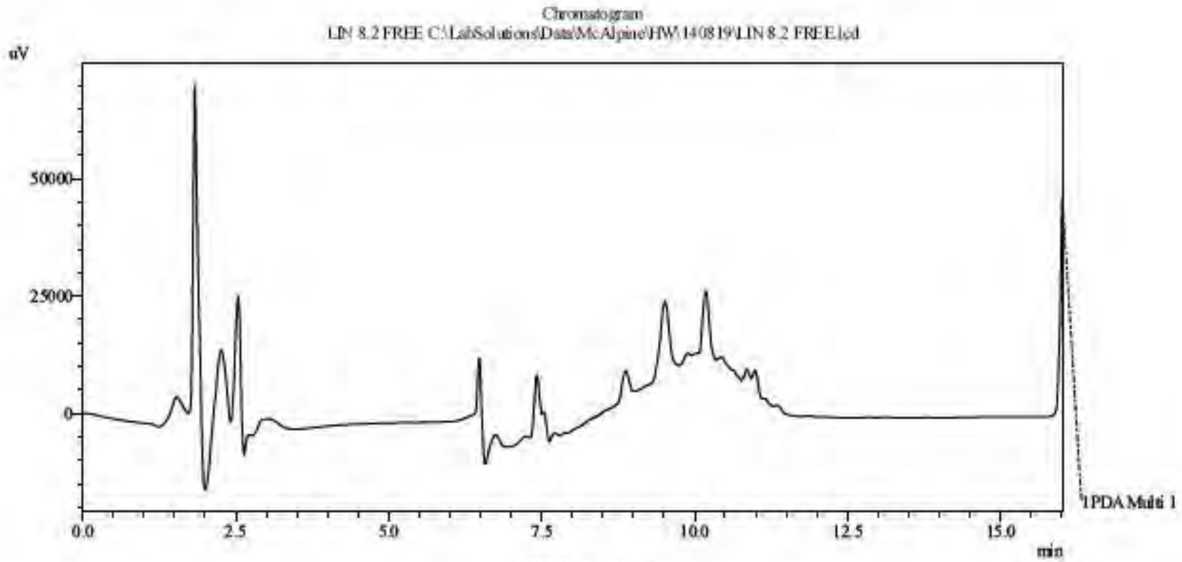
8.2 LIN: HRMS

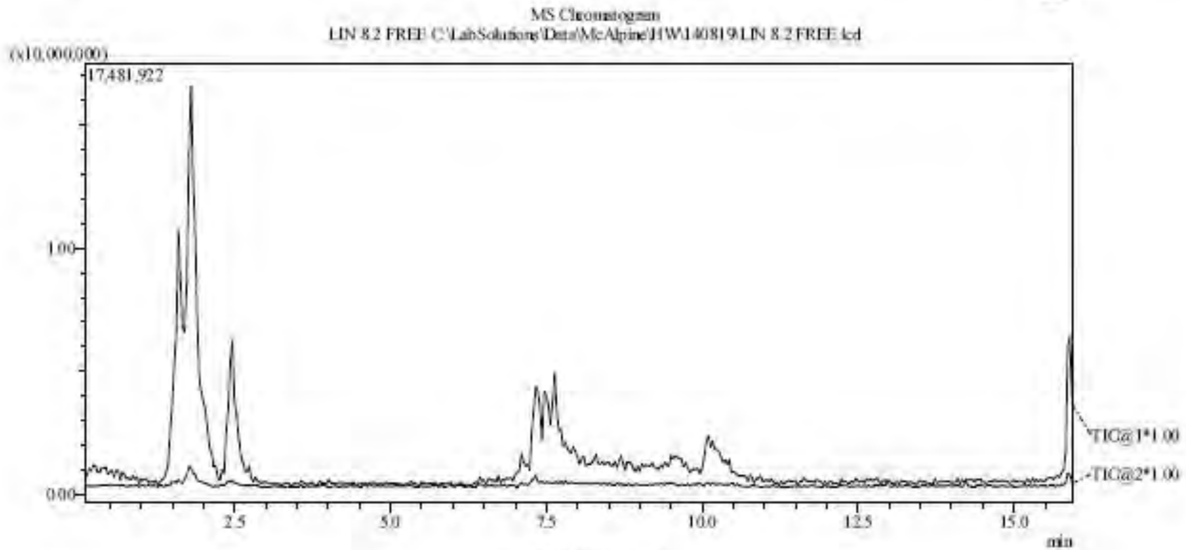


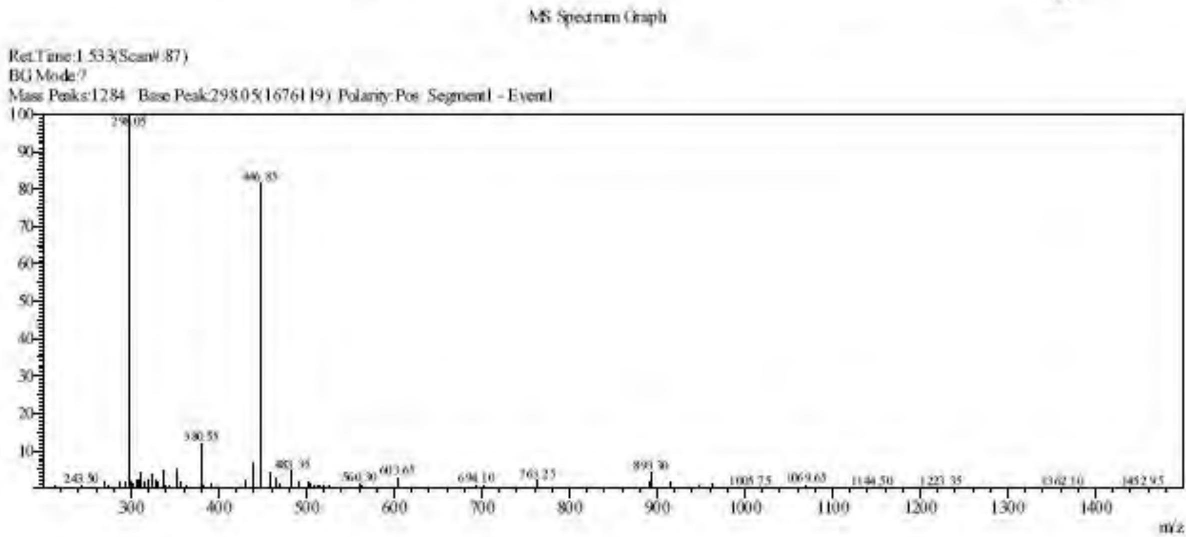


8.2 LIN: LC/MS

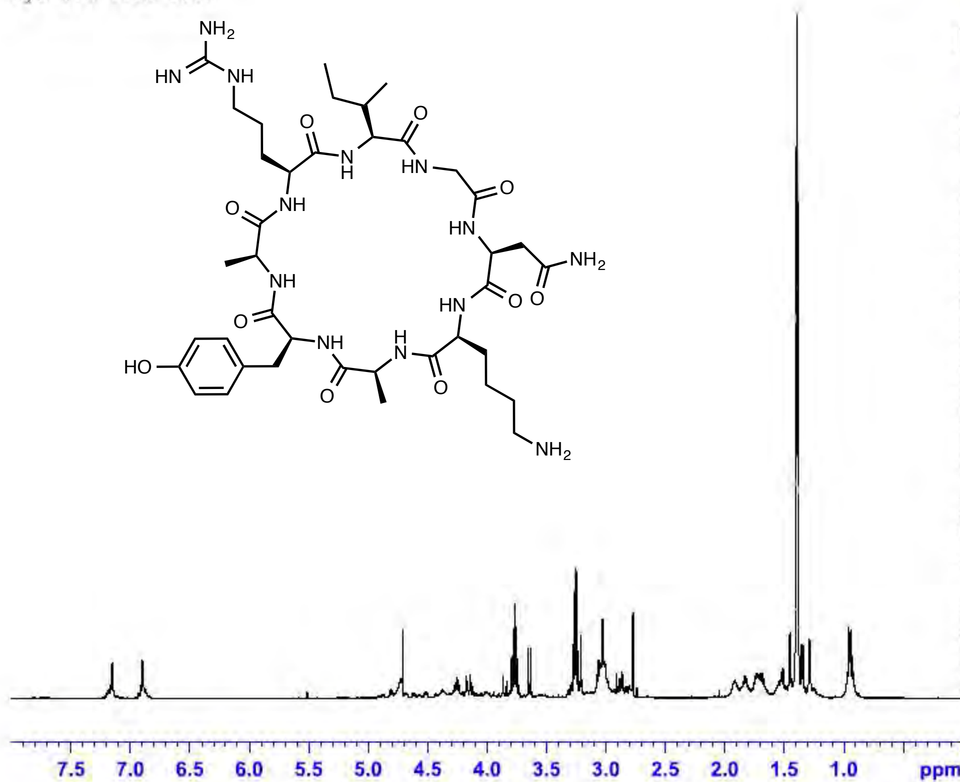
==== Shimadzu LCMSsolution Analysis Report ====

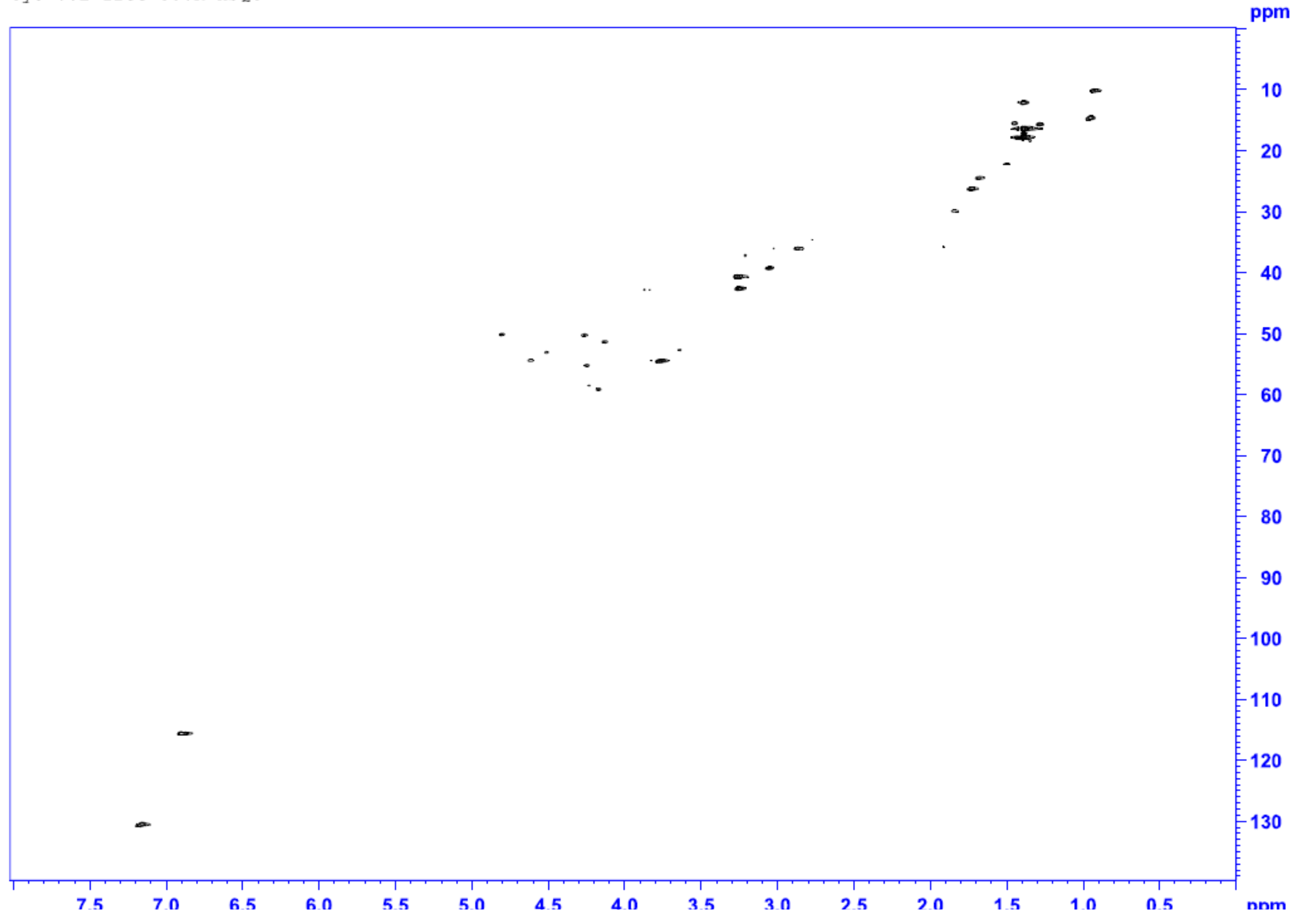




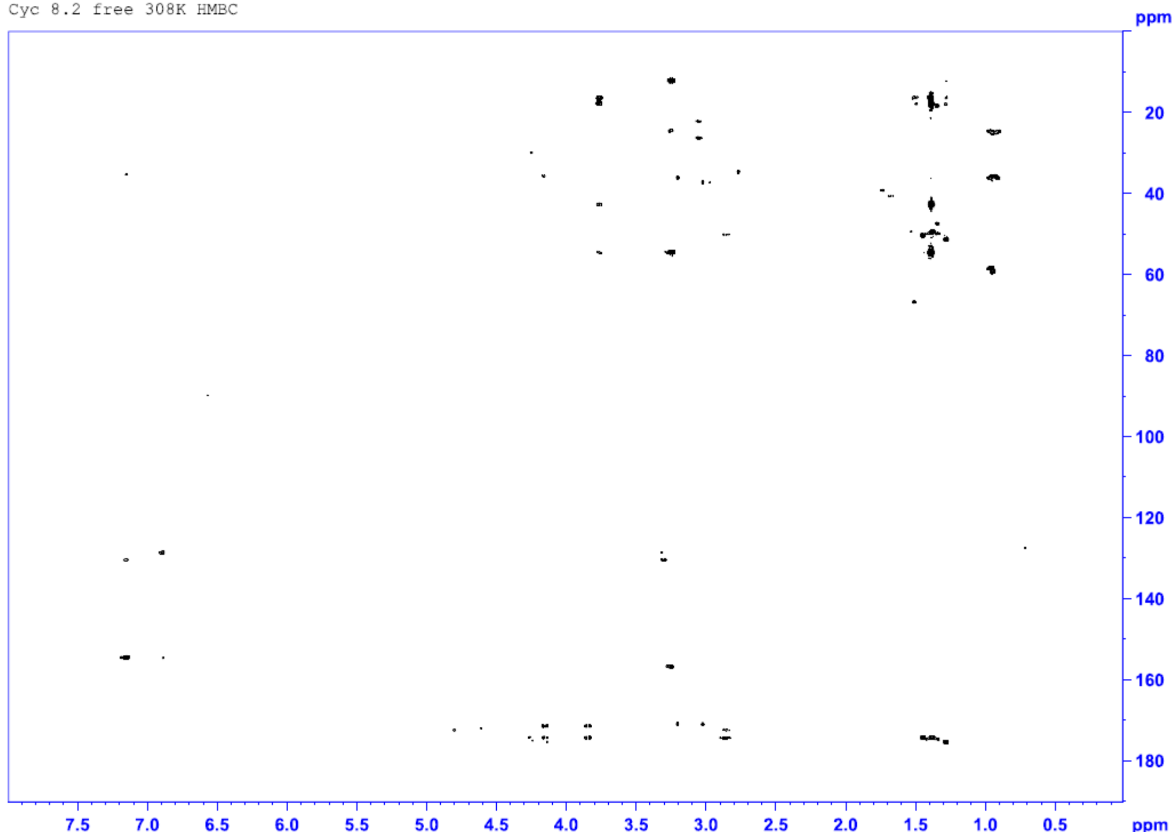


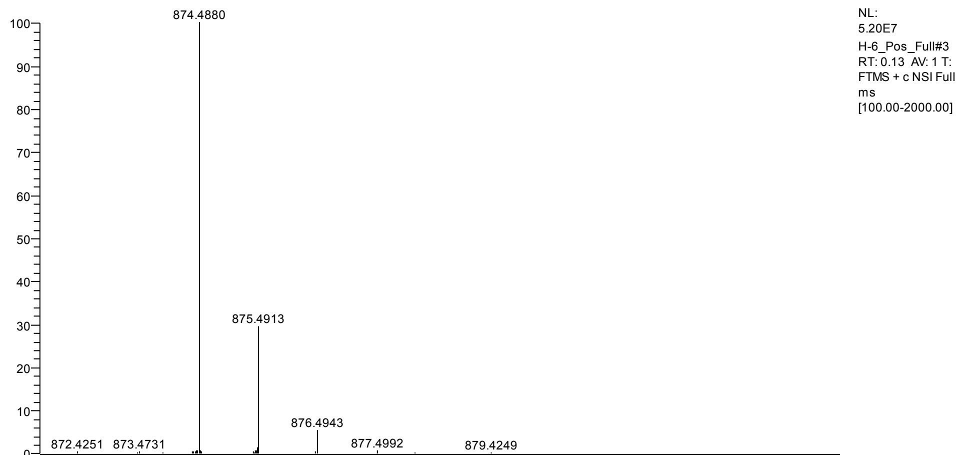
8.2 CYC: ^{1}H NMR and ^{1}H-^{13}C HSQC NMR

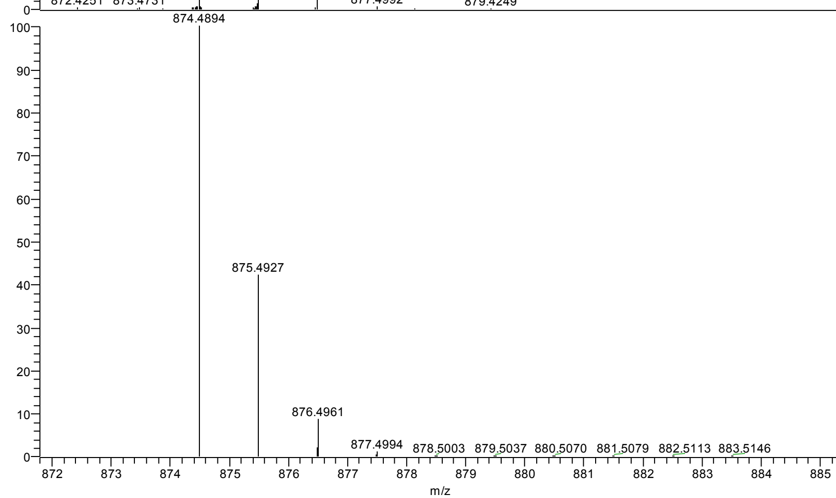




8.2 CYC: ^{1}H-^{13}C HMBC NMR and HRMS







8.2 CYC: LC/MS

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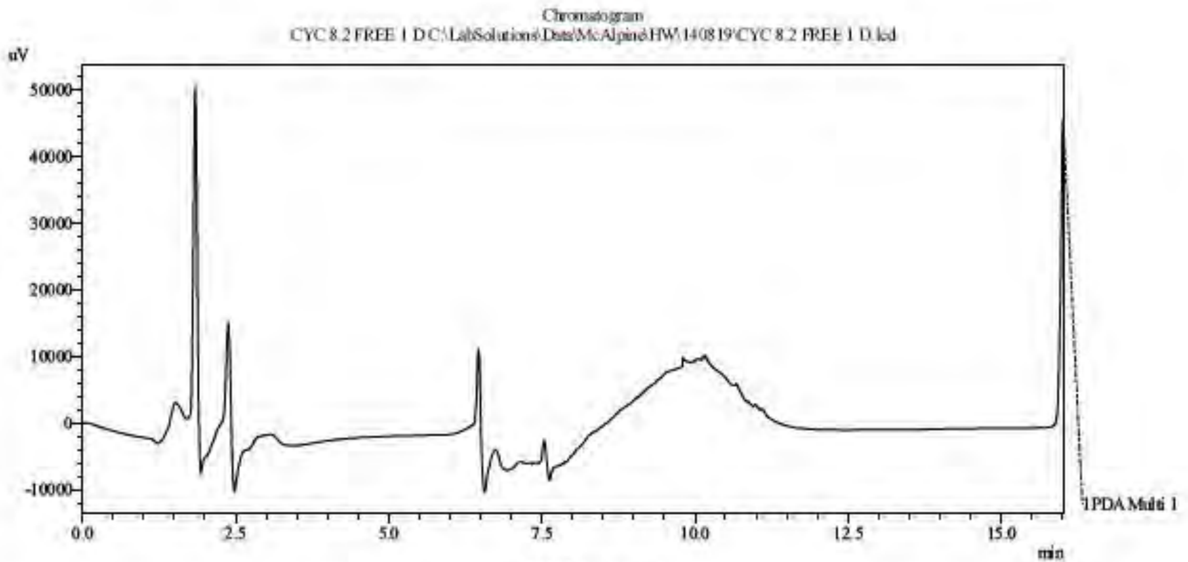
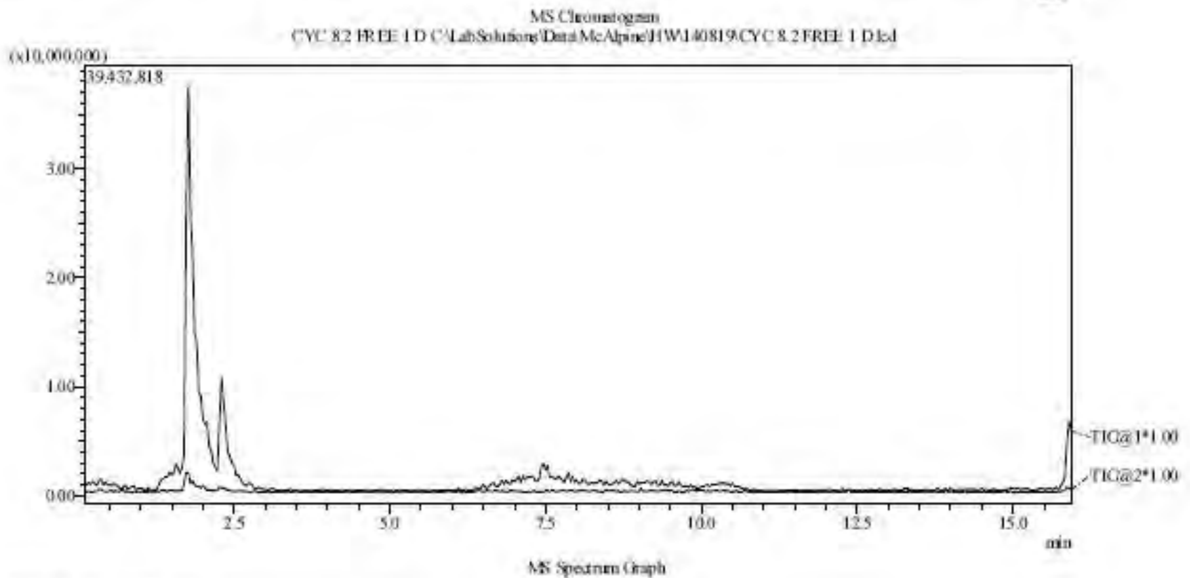
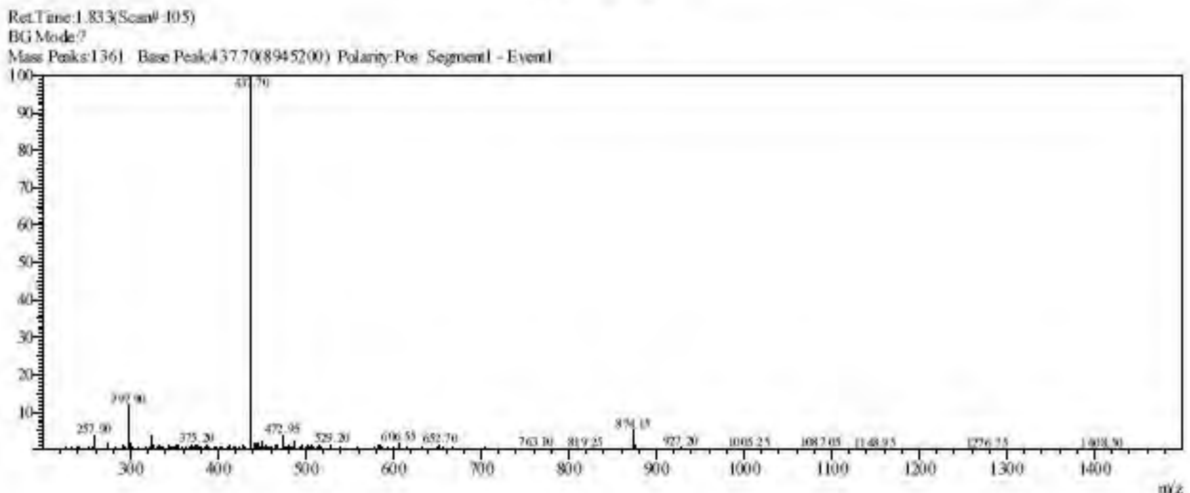
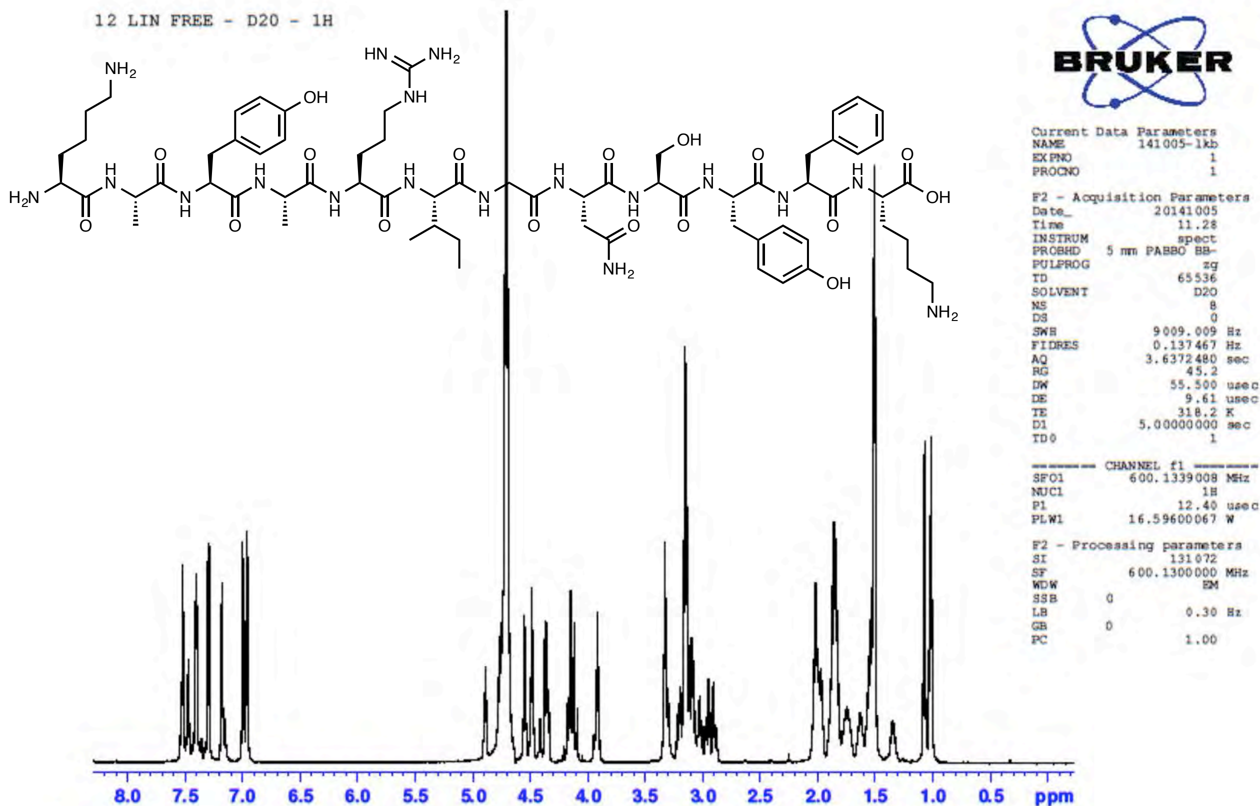
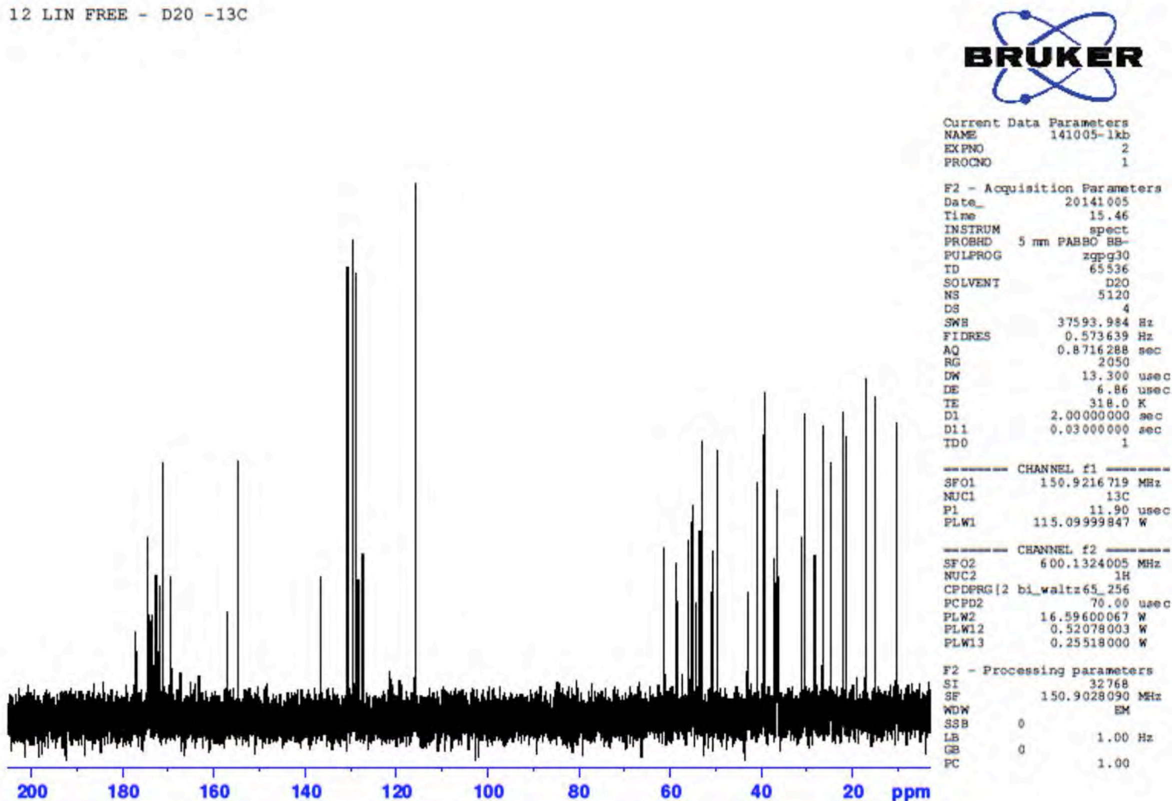




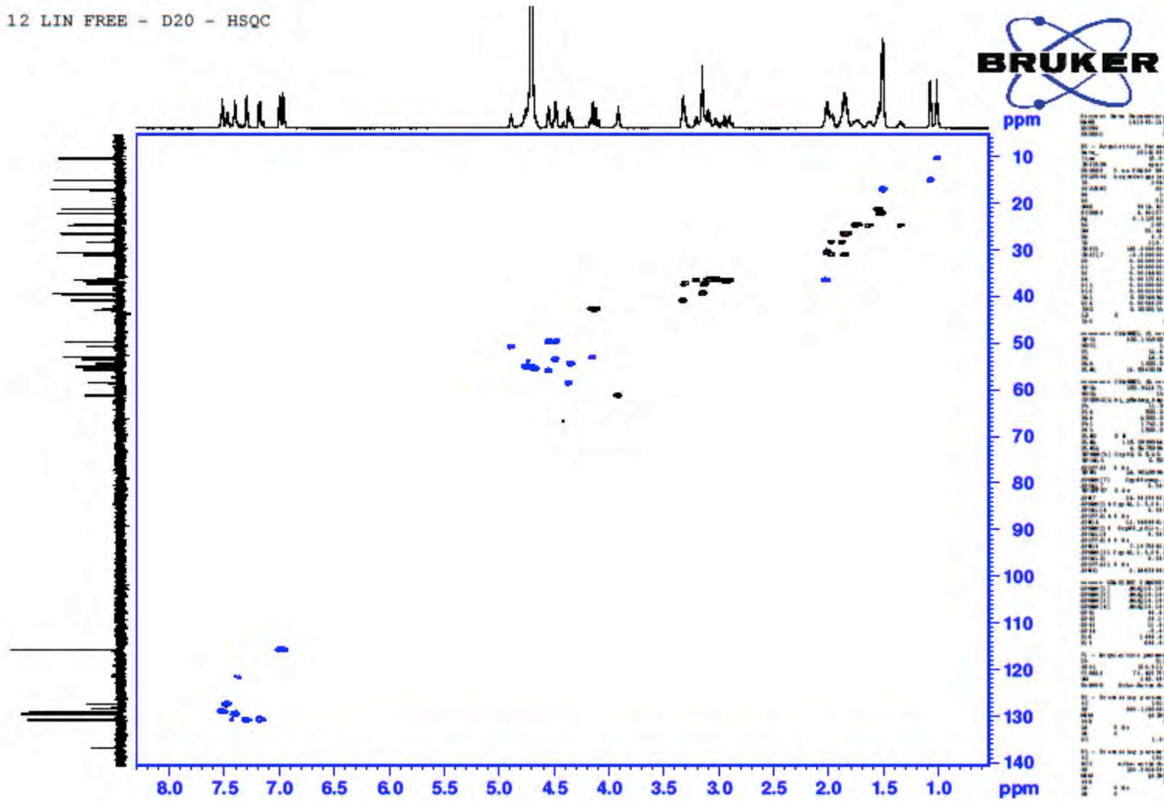


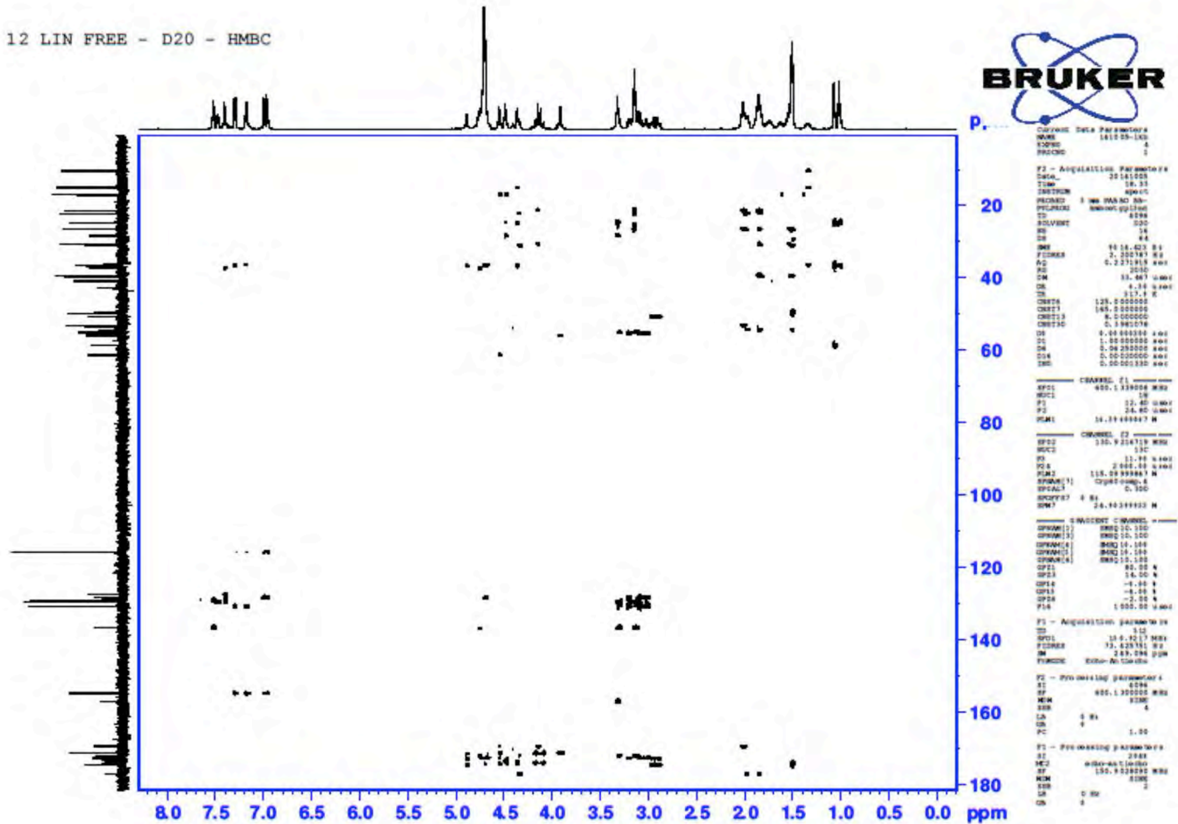
TPR Peptide: ^{1}H NMR and ^{13}C NMR



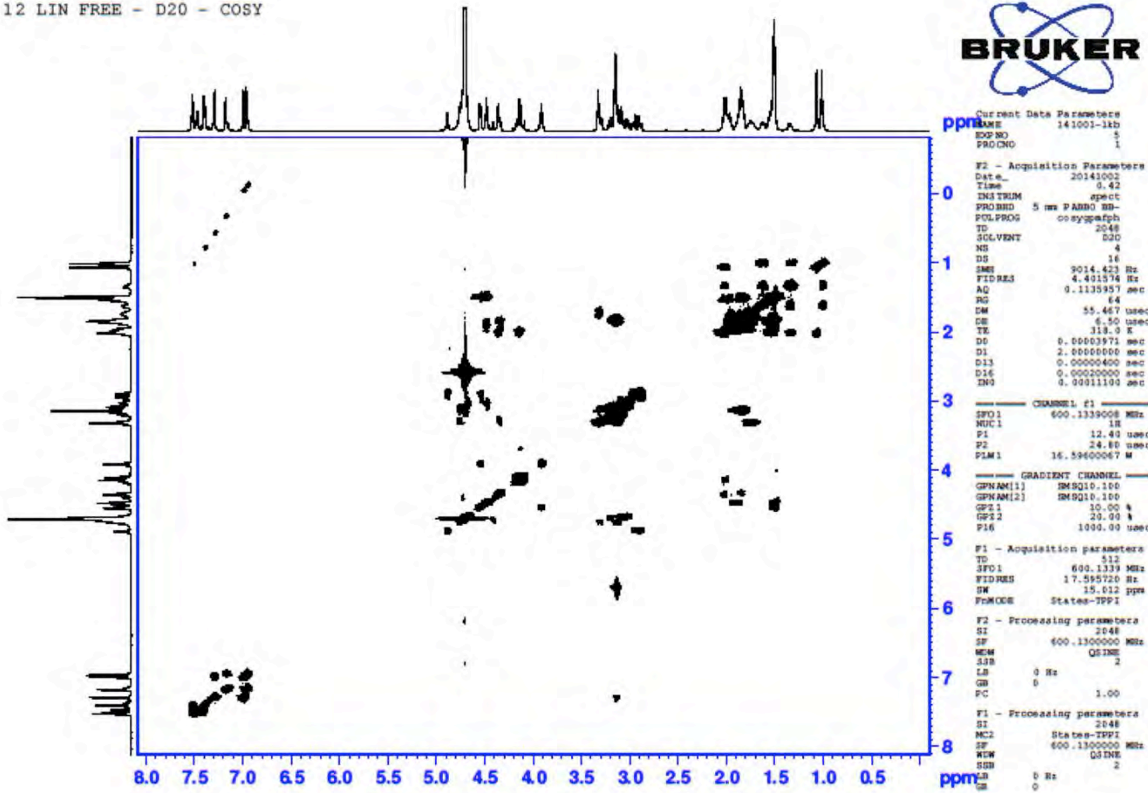


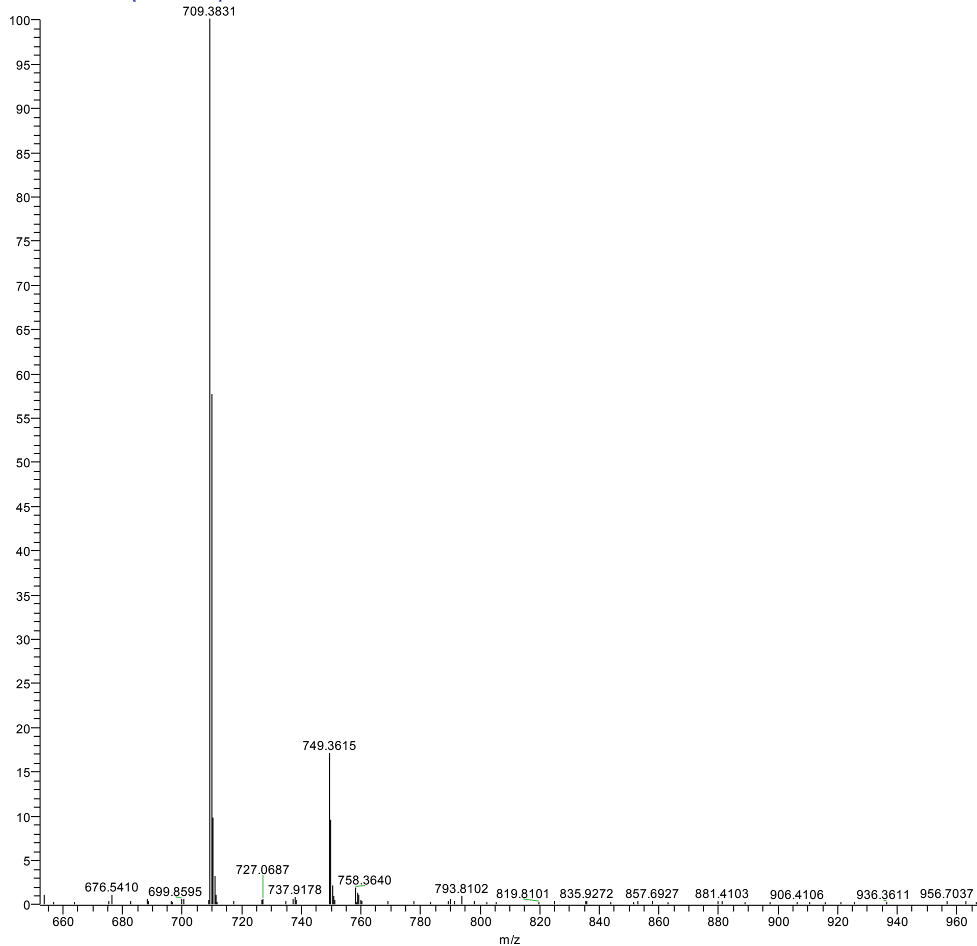
TPR Peptide: ^{1}H-^{13}C HSQC NMR and ^{1}H-^{13}C HMBC NMR





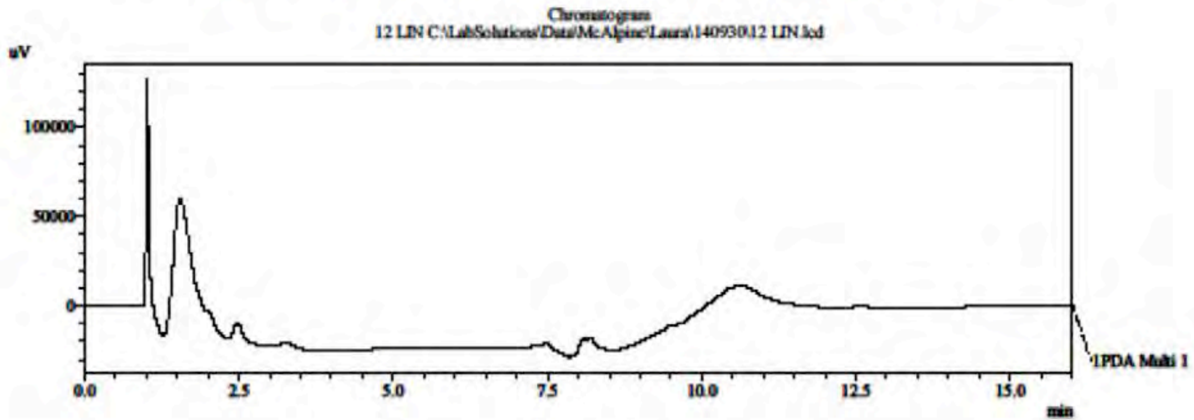
TPR Peptide: ^{1}H-^{1}H COSY NMR and HRMS

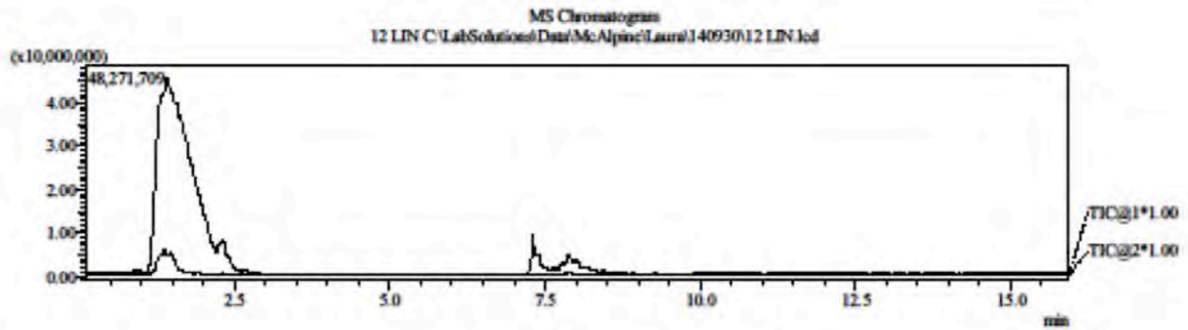


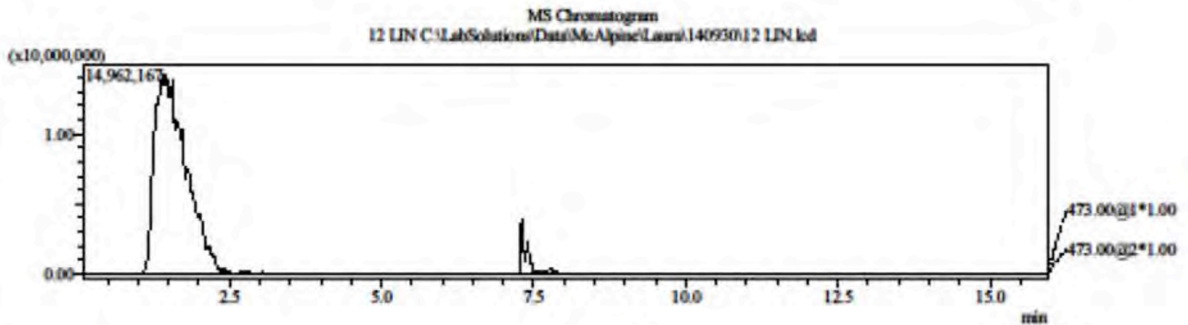


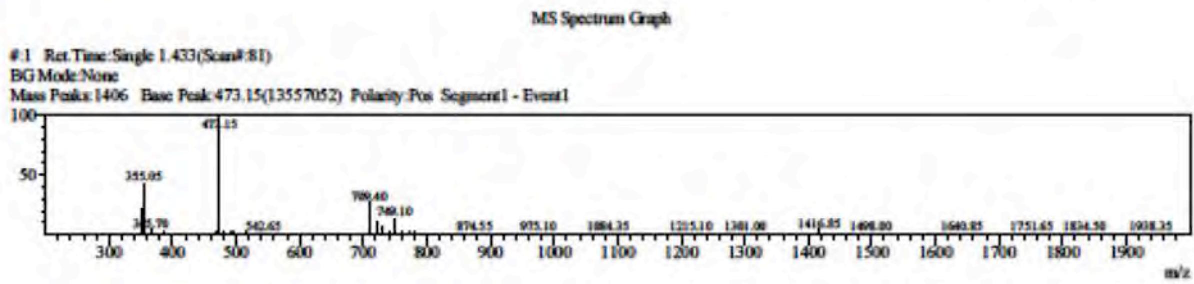
TPR Peptide: LC/MS

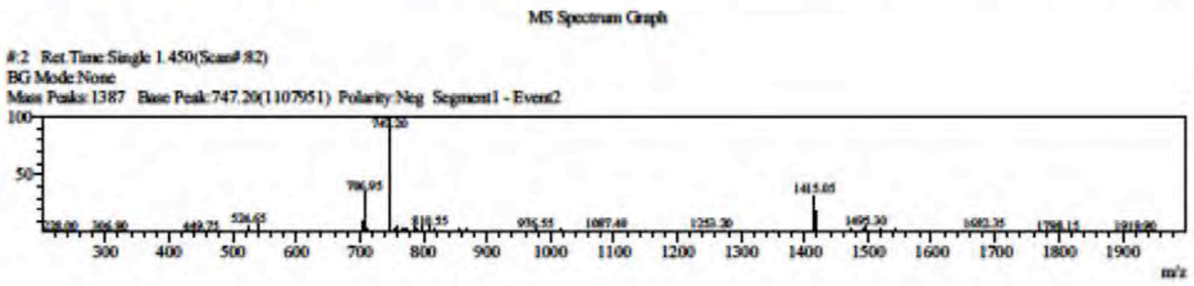
==== Shimadzu LCMSsolution Analysis Report ====











Protein Binding Assay

The binding assays were performed using either a HSP90α (C-terminal) Inhibitor Screening Kit (cat. 50317) or HSP90β (C-terminal) Inhibitor Screening Kit (cat. 50314) purchased from BPS Bioscience. The assay was performed according to the manufacturors protocol and utilised AlphaScreen technology (PerkinElmer). The test compounds were dissolved in 100% DMSO and diluted with water to the desired concentration so that the final dilution was dissolved in 5% DMSO with water. 2 μL of the dilution was added to a 10 μL reaction so that the final concentration of DMSO was 1% in all reactions. The reactions were conducted at room temperature for 30 min in a 10 μL mixture containing assay buffer, 6 ng (24 nM) of a C-terminal fragment of either HSP90α (Uniprot P07900, a.a. 535-732) or HSP90β (Uniprot P08238, a.a. 527-724), 40 ng (100 nM) cyp40, and the test compound. After the 30 min incubation, 10 μl of detection buffer containing 20 μg/ml glutathione acceptor beads (Perkin Elmer) were added to the reaction mix and incubated for 30 min in the dark. 10 μL of 40 μg/ml streptavidin donor beads (Perkin Elmer) were then added and the final 30 μl mixture was incubated for one hour the dark. The AlphaScreen signal was measured using EnSpire multimode plate reader (Perkin Elmer).

Luciferase Protein Renaturation Assay

Protocol was adapted from the following paper:
L. Galam, M. K. Hadden, Z. Ma, Q. Z. Ye, B. G. Yun, B. S. Blagg and R. L. Matts, *Bioorganic & medicinal chemistry*, 2007, **15**, 1939.

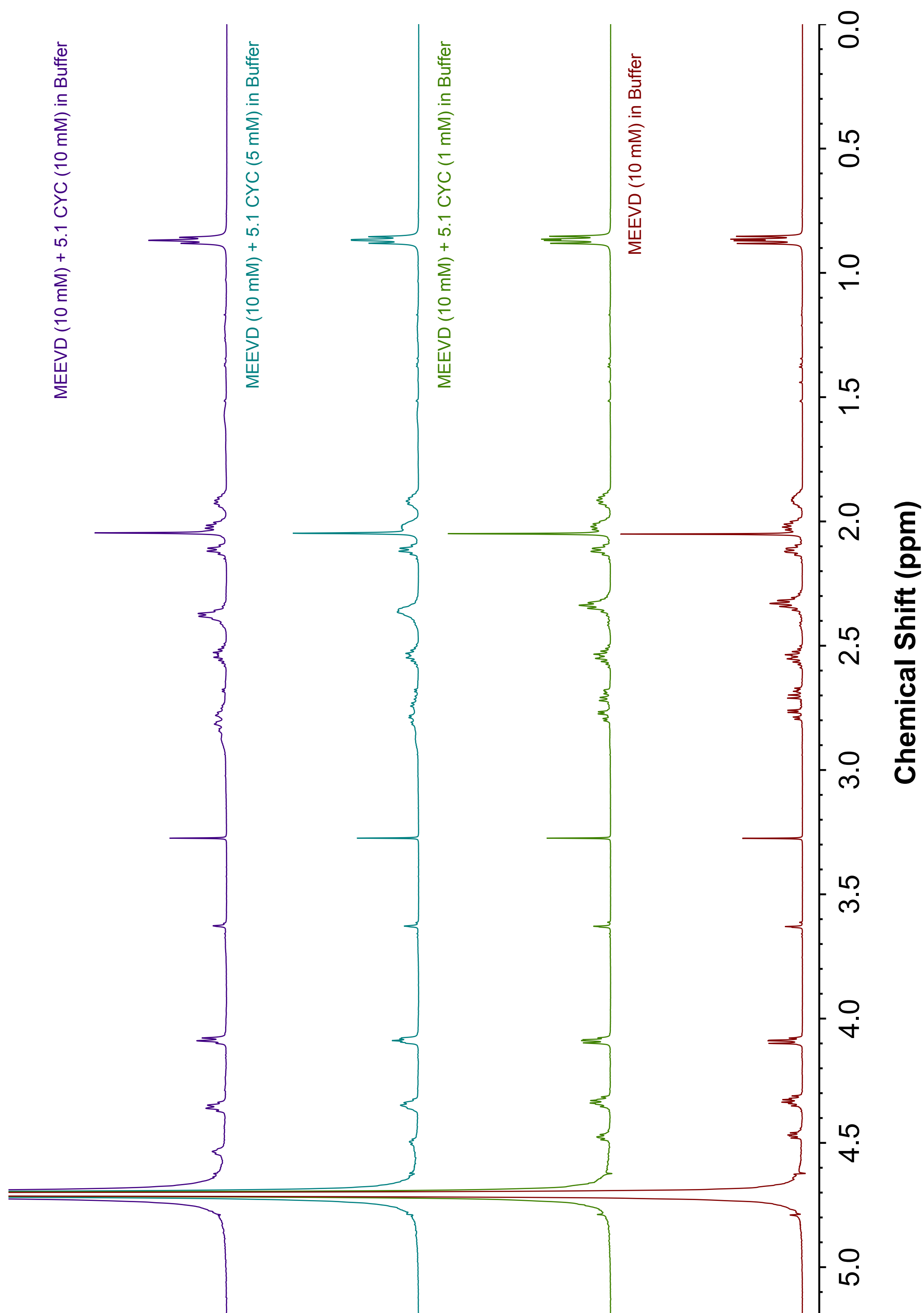
Luciferase (L9506, Sigma-Aldrich) was dissolved in stability buffer (25 mM Tricine HCl (pH 7.8), 8 mM $MgSO_4$, 0.1 mM EDTA and 10 mg/mL acetylated BSA) before adding 1% Triton X-100 and 10% glycerol. Luciferase solution was then aliquoted and stored at -80˚C. 1 μL of protein was thawed on ice and denatured at 40˚C for 1.5 minutes. Denatured luciferase was immediately stored on ice before being diluted with 64 μL cold mix (100 mM TrisHCl pH 7.7, 10 mM $Mg(OAc)_2$, 375 mM KCl, 15 mM ATP, and 25 mM creatine phosphate), 6.4 μL creatine phosphokinase (CPK) in 50% glycerol and 8.4 μL milli-Q water to produce a final volume of 80 μL. The denatured luciferase solution was then diluted 1:10 with the same ratio of cold mix, CPK and milli-Q water. 10 μL of diluted luciferase solution was added to rabbit reticulocyte lysate (RRL, Promega) that had been diluted with RNase-free water (1:4 v/v) and pre-incubated at room temperature with DMSO (1%), test compound or control compound for 5 hours. Denatured luciferase was incubated for 3 hours with the lysate before 30 μL of the reaction mixture was removed and combined with 40 μL of Bright-Glo Luciferase Assay System (Promega) and read on an illuminometer (Berthold Orion Microplate Luminometer).

^{1}H NMR Titration Experiments

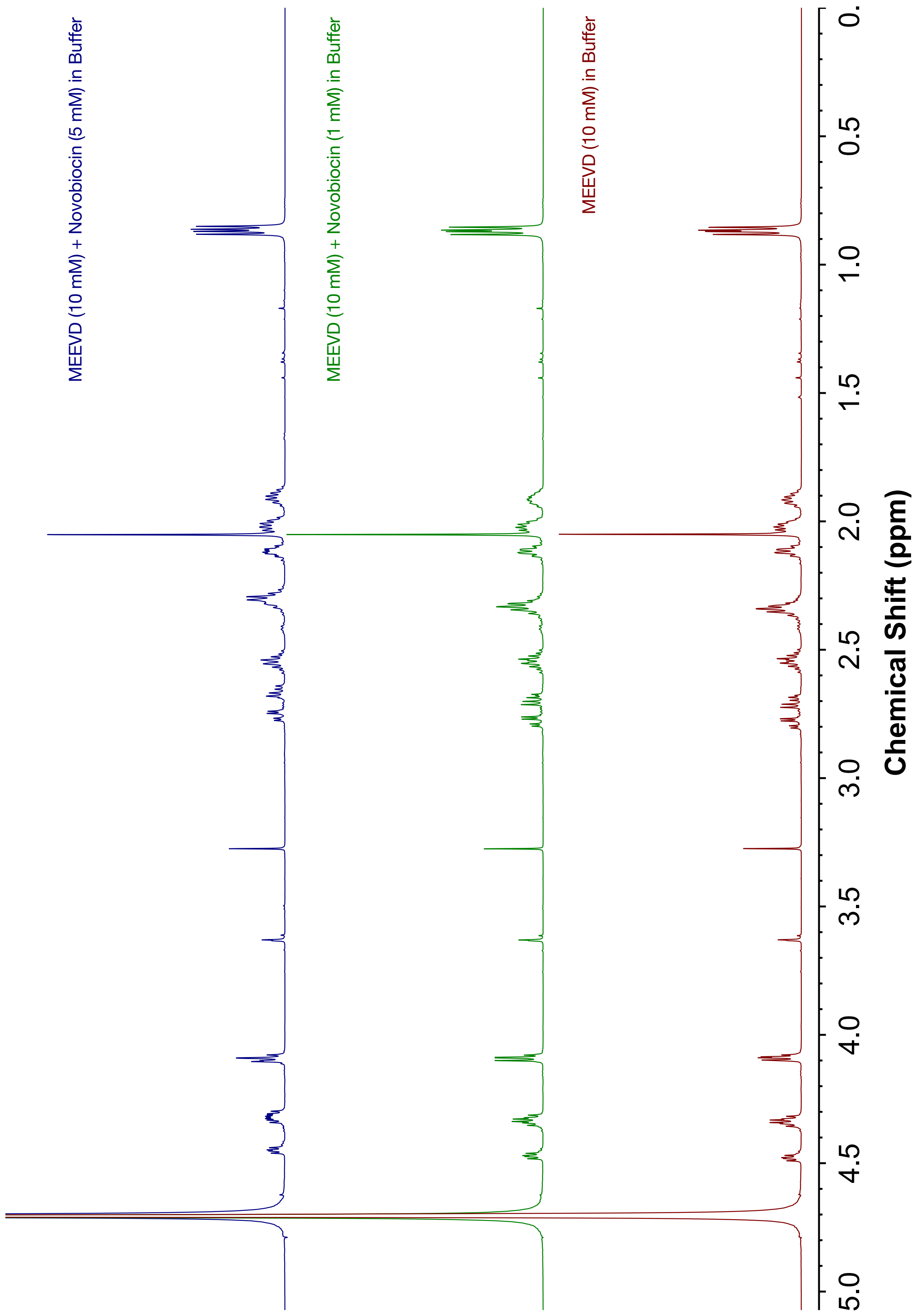
^{1}H NMR spectra were obtained on Bruker Avance III 600 MHz. All samples were dissolved in a buffer consisting of 25 mM dibasic sodium phosphate and 25 mM sodium chloride in deuterium oxide (D_2O) with a pH of 7.2. Spectra were obtained at 298K (25˚C).

Full Spectra of ^{1}H NMR Titration Experiment

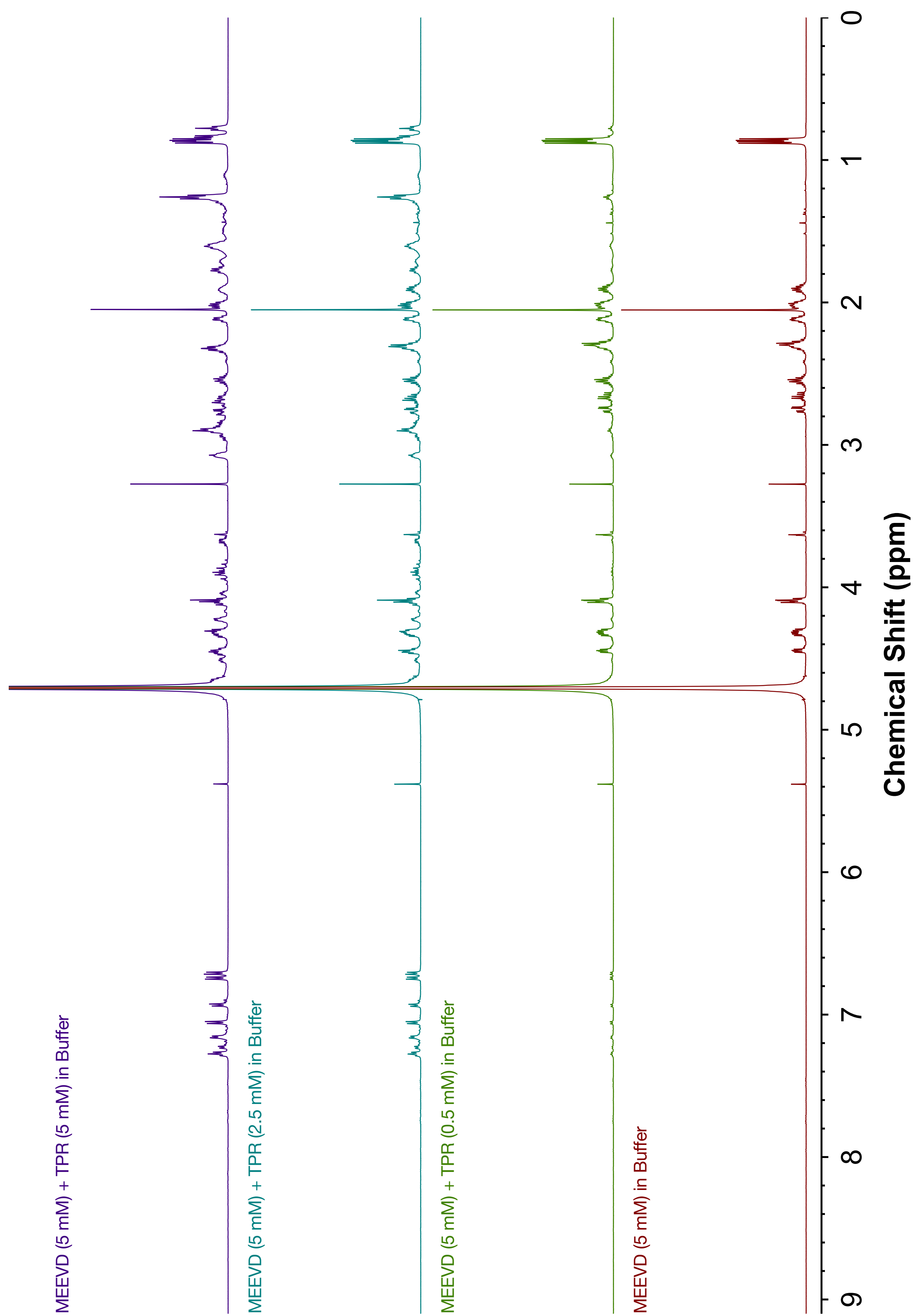
MEEVD + 5.1 CYC



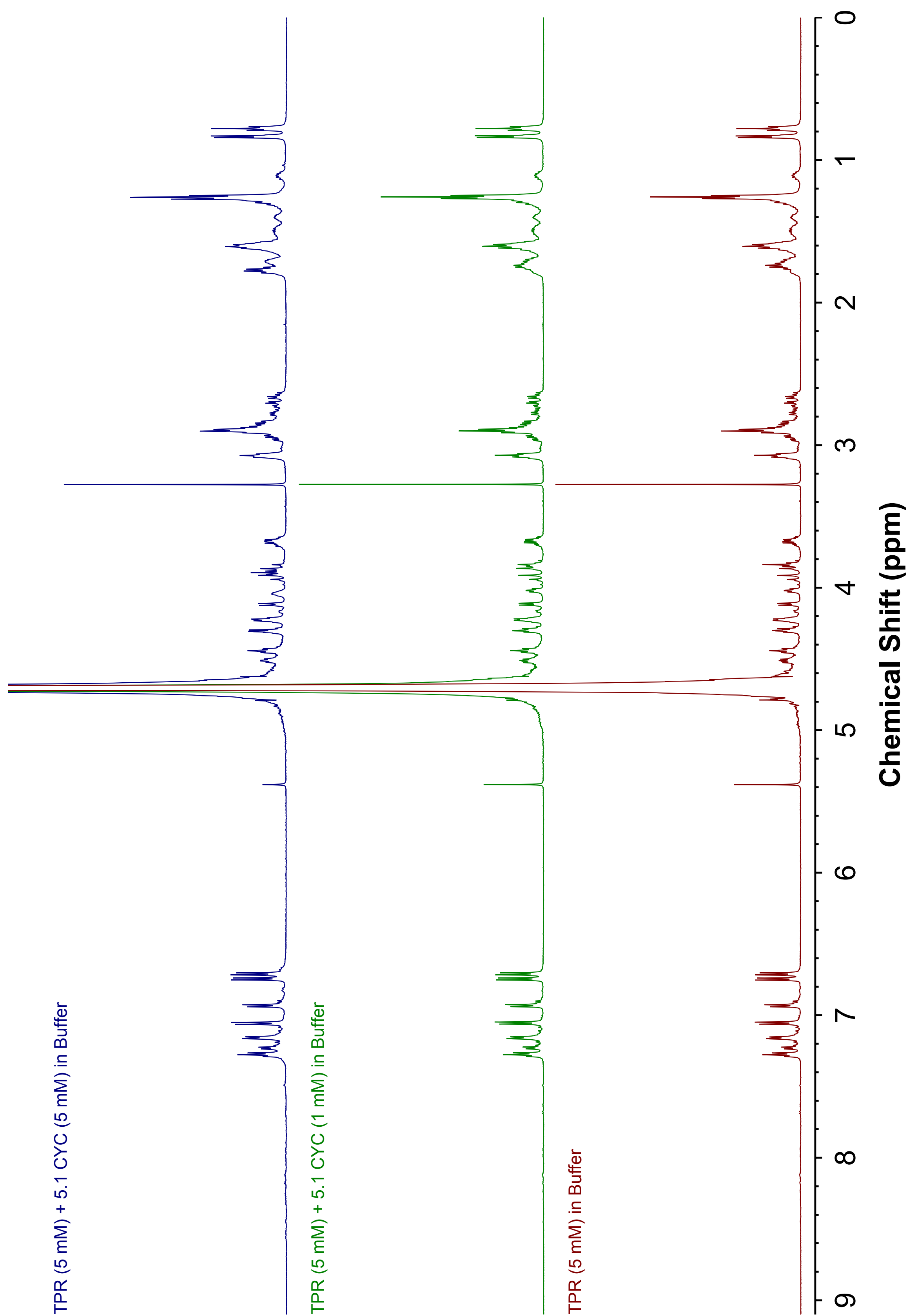
MEEVD + Novobiocin



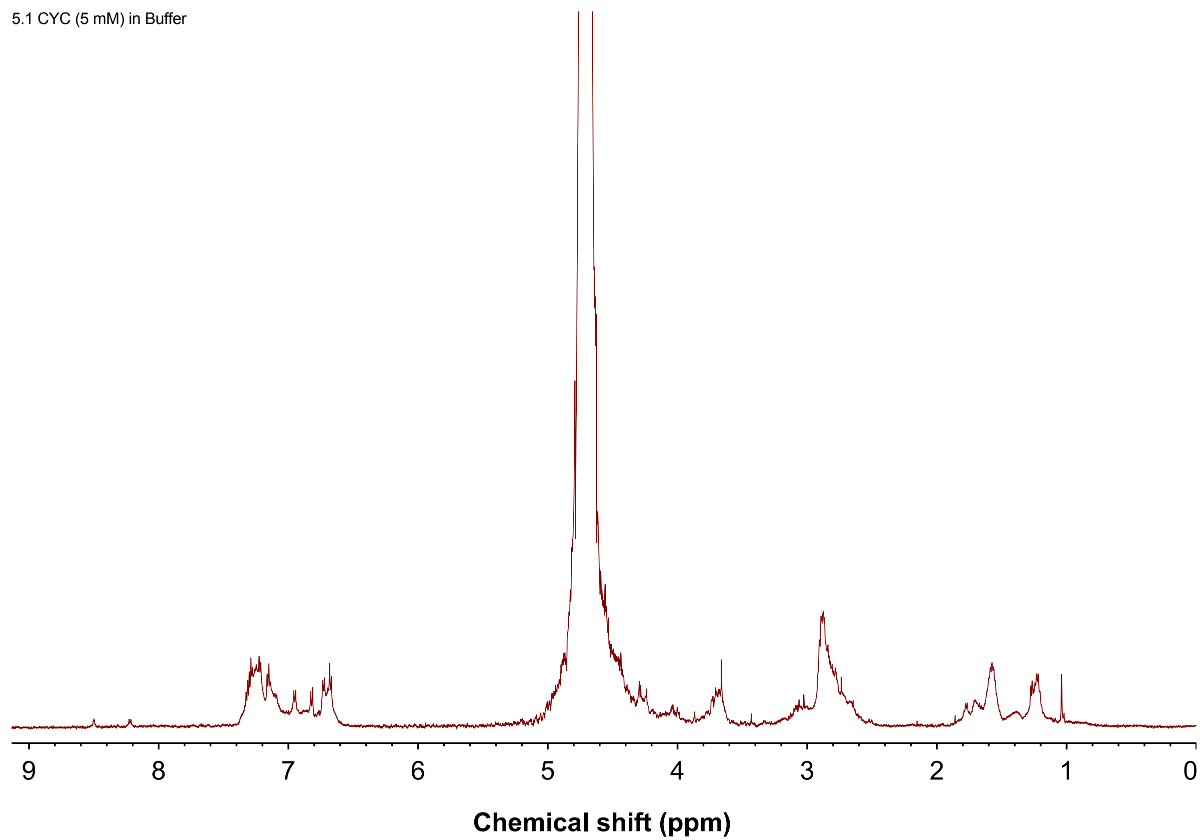
MEEVD + TPR



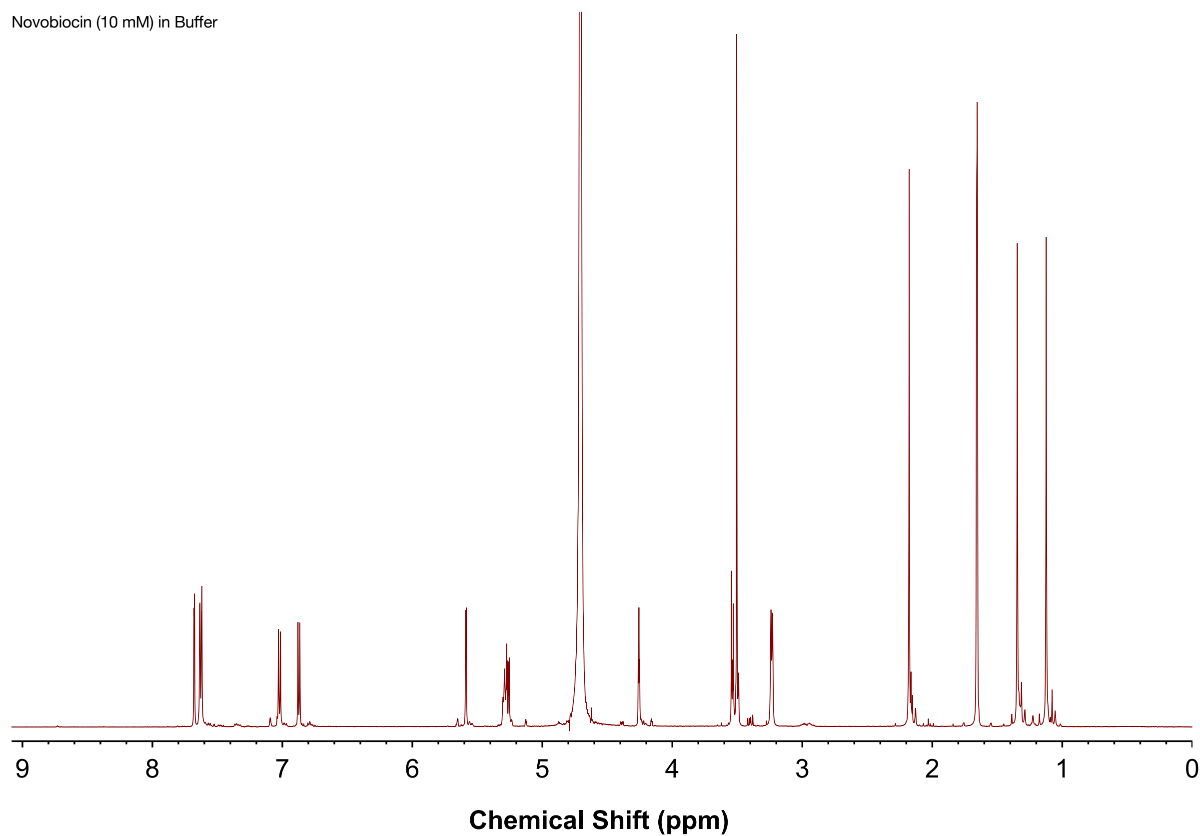
TPR + 5.1 CYC



5.1 CYC

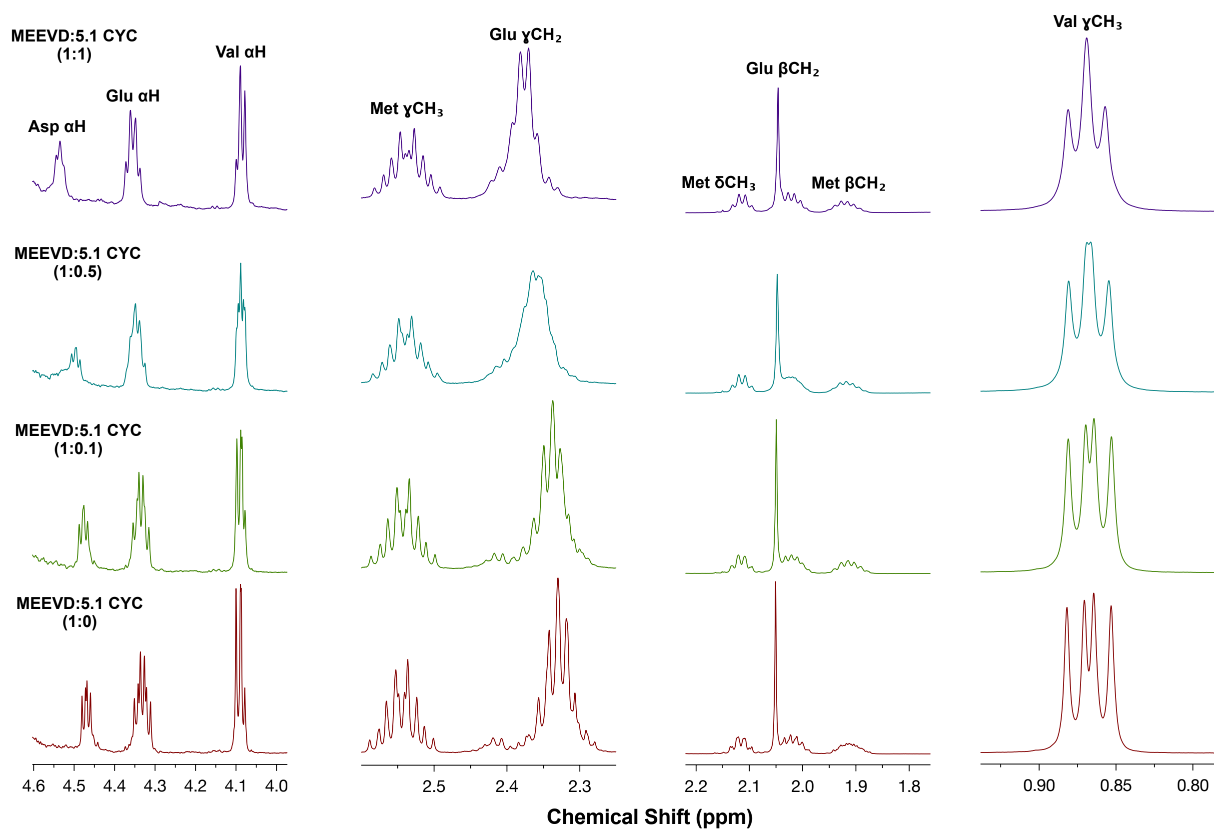


Novobiocin

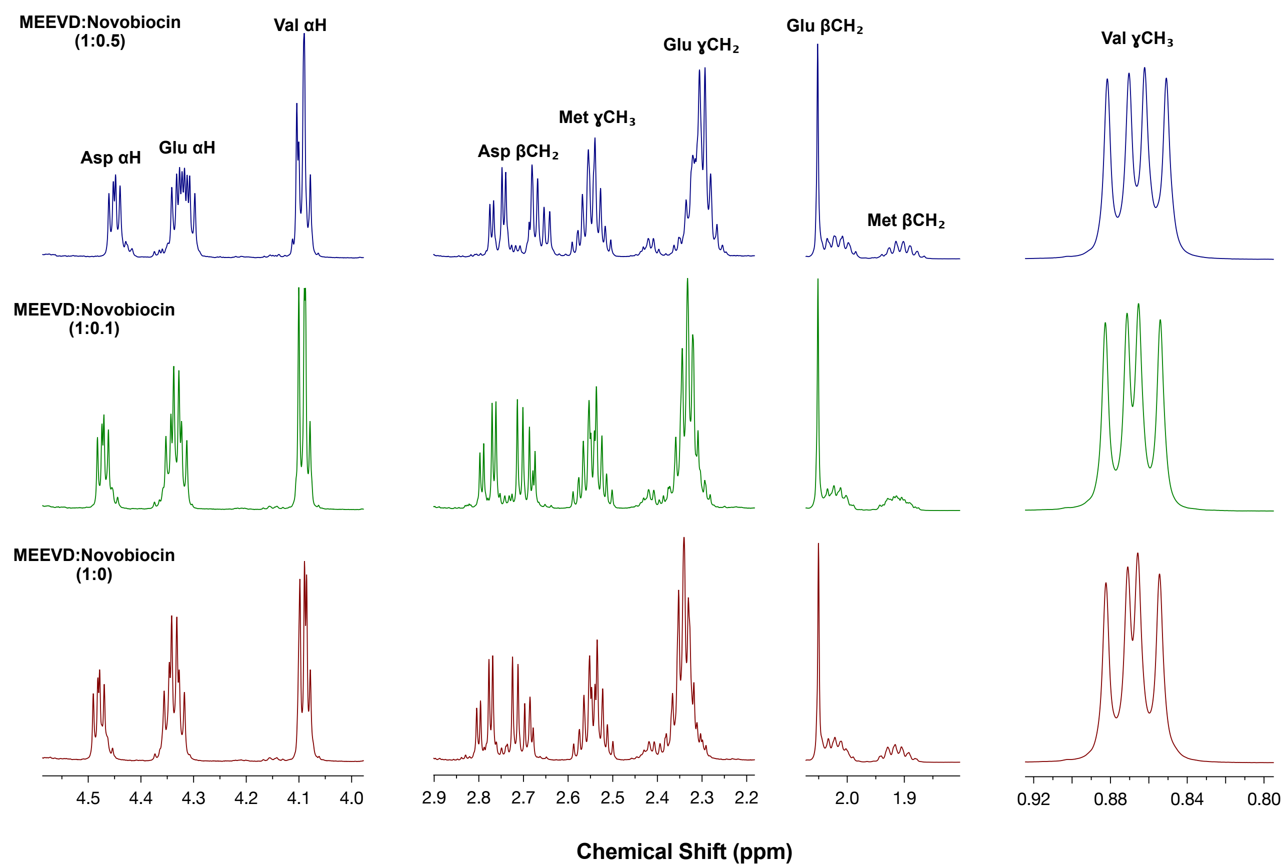


Enlarged Spectra of ^{1}H NMR Titration Experiment

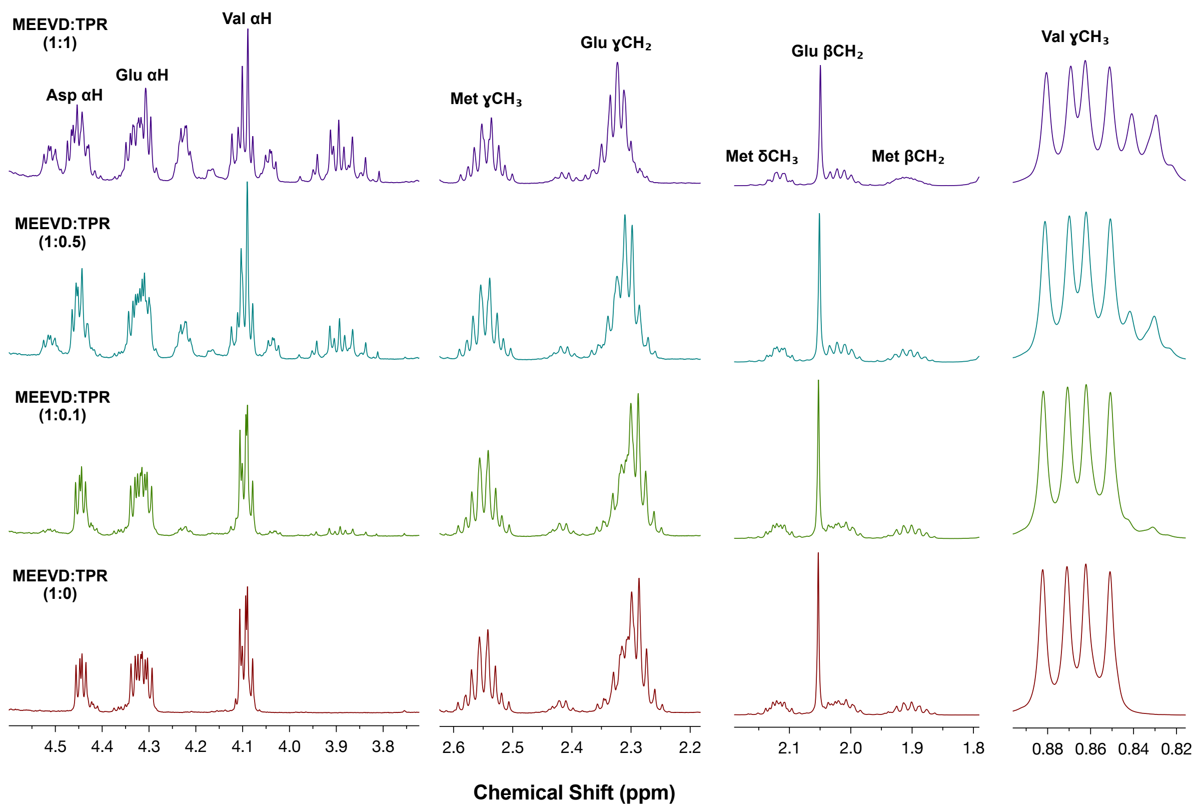
MEEVD + 5.1 CYC



MEEVD + Novobiocin



MEEVD + TPR



TPR + 5.1 CYC