Electronic Supplementary Information

A novel route towards cycle-tail peptides using oxime resin: Teaching an old dog a new trick

Christopher Bérubé[1], Alexandre Borgia[1] and Normand Voyer[1]

[1]Département de Chimie and PROTEO, Université Laval, Pavillon Alexandre-Vachon, Faculté des sciences et de génie, 1045 avenue de la Médecine, Québec, QC, Canada, G1V 0A6

E-mail: normand.voyer@chm.ulaval.ca

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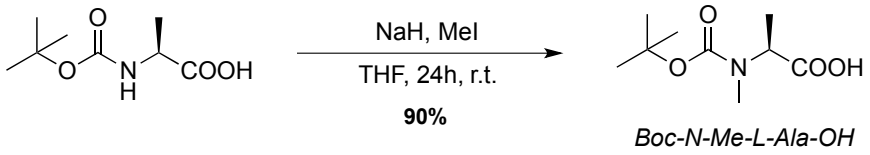
Experimental

General information

Oxime resin (0.30 mmol/g), coupling reagents and *N*-Boc-protected amino acids were purchased from Matrix Innovation, Bachem, Advanced Chemtech and GL Shangai. Unless otherwise indicated, other starting materials were purchased from commercial sources (Sigma-Aldrich and VWR) and used without further purification. ^{1}H and $^{13}C\{1H\}$-NMR spectra were recorded on an Agilent DD2 500 MHz spectrometer. The coupling constants are reported in hertz (Hz). Splitting patterns are designated as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), br (broad singlet), ddd (doublet of doublet of doublet), ddt (doublet of doublet of triplets), and m (multiplet). Mass spectra were obtained on an Agilent 6210 LC Time of Flight Mass Spectrometer in direct injection mode. HPLC purities data were recorded on Agilent 1260 Infinity instrument equipped with an auto sampler, a quaternary high-pressure mixing pump and a photodiode array detector. Chromatography analysis was performed with a Grace Vydac C18 column (5 µm, 250 x 4.6 mm i.d.). The gradient was performed at a flow rate of 1mL/min with a mobile phase composed of H_2O containing 0.1% TFA (A) and CH_3CH (B). All solvents were degassed and a gradient of (100% H_2O) to (46.4% H_2O /54.4% CH_3CN) in 35 min followed with 100% H_2O between 35 to 42 min was used for compounds **1**-**2** and **5**-**8**. The column temperature was kept at 22.5°C. The UV absorbance was recorded at 220 and 280 nm. Retention time is in minute followed by the percentage integration of the total chromatogram. Semi-preparative HPLC was carried out using a HP 1260 system with a photodiode array detector using an ACE C18 column (5µm, 250 x 10.0 mm i.d) with a gradient of (60% H_2O) to (30% H_2O /70% CH_3CN) in 45 min followed with 30% H_2O between 45 to 50 min was used. Optical rotations were measured at ambient temperature on a Jasco DIP-360 digital polarimeter using a sodium lamp.

Preparation of Boc-N-Me-Ala-OH

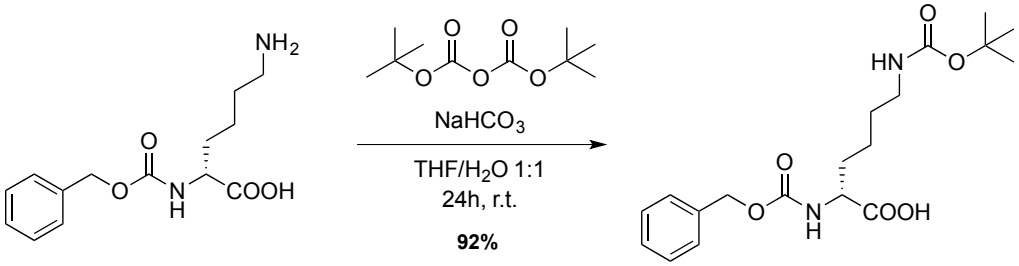


Boc-N-Me-L-Ala-OH

Sodium hydride (60% dispersion in mineral oil, (21.3 g, 528.5 mmol, 10.0 equiv.) was added in small portions to a stirred solution of *N*-Boc-L-Ala-OH (10 g, 52.9 mmol, 1.0 equiv.) and methyl iodide (32.9 mL, 528.5 mmol, 10.0 equiv.) in anhydrous THF at 0 °C. the mixture was allowed to stir at room temperature for 24 h under nitrogen atmosphere. The reaction was then quenched with

water (60 mL). Ethyl acetate (100 mL) was added and the mixture was evaporated *in vacuo*. The concentrate was diluted in water (200 mL) and washed with ethyl acetate (200 mL). The aqueous solution was acidified to pH 3 with a solution of HCl 1N and extracted with ethyl acetate (600 mL). The extract was washed with brine, dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give the Boc-*N*-Me-Ala-OH in 90% yield as a yellow powder. **[a]$_D^{22}$** -32° (c 0.5 in EtOH). **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 10.98 (s, 1H), 4.85 (brq, 1H), 4.43 (s, 1H), 2.80 (s, 3H), 1.42 (s, 12H). **^{13}C{1H}-NMR** (126 MHz, DMSO-d_6): δ 177.8, 155.9, 80.5, 55.0, 28.3, 14.6. **HRMS** (ESI) m/z calcd for $C_9H_{18}NNaO_4$ $(M+Na)^+$: 226.1050, found 226.1043.

Preparation of Z-D-Lys(Boc)-OH



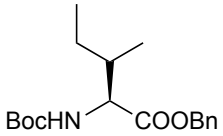
Z-D-Lys(Boc)-OH

To a solution of Z-D-Lys-OH (10.0g, 35.70 mmol, 1.0 equiv.) in a mixture of 100 mL of water and 100 mL of THF was added $NaHCO_3$ (70 mmol, 6.0g). Boc_2O (8.61g, 39.27 mmol, 1.1 equiv.) was added at room temperature and the mixture was allowed to stir overnight. The solution was then concentrated *in vacuo* and acidified with HCl 1N to pH 3. The mixture was extracted three times with EtOAc (3 X 150 mL), washed with brine and dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give the Z-D-Lys(Boc)-OH in 92% yield as a white solid. **[a]$_D^{22}$** +6.8° (c 1.0 in MeOH). **^{1}H-NMR** (500 MHz, DMSO-d_6): 7.49 (d, J = 7.9 Hz, 1H), 7.36 (m, 4H), 7.30 (m, 1H), 6.75 (t, J = 5.7 Hz, 1H), 5.02 (s, 2H), 3.89 (ddd, J = 9.4, 7.8, 4.6 Hz, 1H), 2.87 (dt, J = 9.7, 6.5 Hz, 2H), 1.66 (m, 1H), 1.56 (m, 1H), 1.35 (s, 9H), 1.28 (m, 4H). **^{13}C{1H}-NMR** (125 MHz, DMSO-d_6): δ 174.4, 156.6, 156.0, 137.5, 128.8, 128.2, 128.2, 127.4, 77.8, 65.8, 54.3, 40.0, 31.0, 30.9, 29.5, 29.4, 28.7, 28.6, 23.3.**HRMS** (ESI) m/z calcd for $C_{19}H_{29}N_2O_6$ $(M+H)^+$: 381.2020, found 381.2028.

General procedure for the preparation of *N*-Boc amino benzyl esters

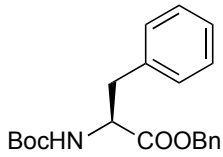
To a stirred solution of *N*-Boc amino acid (24.0 mmol, 1.0 equiv.) in 80 mL and CH_3CN was added K_2CO_3 (6.64 g, 48.0 mmol, 2.0 equiv.) and benzyl bromide (4.2 mL, 36.0 mmol, 1.5 equiv.). The reaction mixture was stirred for 16 h at room temperature and extracted two times with EtOAc. The organic layers were combined, washed with brine, dried with anhydrous $MgSO_4$ and concentrated *in vacuo* and further purified by flash chromatography (5:95 EtOAc/hexanes) yielding to compounds **9** and **10**.

*Boc-L-Ile-OBzl (**9**)*



(9). White powder, 82% yield. **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 7.35 (m, 5H), 5.16 (d, *J* = 12.3 Hz, 1H), 5.12 (d, *J* = 12.3 Hz, 1H), 4.31 (dd, *J* = 9.1, 4.7 Hz, 1H), 1.87 (m, 1H), 1.43 (s, 9H), 1.36 (m, 1H), 1.13 (m, 1H), 0.90 (d, *J* = 6.9 Hz, 3H), 0.86 (t, *J* = 7.4 Hz, 3H). **^{13}C{1H}-NMR** (100 MHz, $CDCl_3$): δ 172.3, 155.6, 128.5, 128.4, 128.3, 79.7, 66.8, 57.9, 38.0, 28.3, 24.9, 15.5, 11.6. **HRMS** (ESI) m/z calcd for $C_{18}H_{28}NO_4$ $(M+H)^+$: 322.12013, found 322.2046.

*Boc-L-Phe-OBzl (**10**)*

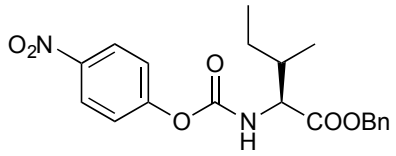


(10). White powder, 90% yield. **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 7.36 (m, 3H), 7.31-7.28 (m, 2H), 7.23 (m, 3H), 7.05 (m, 2H), 5.17 (d, *J* = 12.3 Hz, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 4.64 (q, *J* = 6.3 Hz, 1H), 3.10 (dq, *J* = 14.6, 7.9, 6.9 Hz, 1H), 1.42 (s, 9H). **^{13}C{1H}-NMR** (100 MHz, $CDCl_3$): δ 171.7, 155.1, 135.9, 135.2, 129.3, 128.6, 128.5, 128.5, 128.4, 127.0, 79.9, 67.1, 54.4, 38.3, 28.3. **HRMS** (ESI) m/z calcd for $C_{21}H_{26}NO_4$ $(M+H)^+$: 356.1856, found 356.1866.

General procedure for the preparation of *ONp amino benzyl esters*

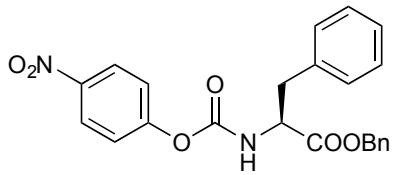
A solution of benzylester *N*-Boc amino acid (12.4 mmol, 1.0 equiv.) and *p*-toluenesulfonic acid (2.14 g, 12.4 mmol, 1.0 equiv.) in DCM/EtOH 1:1 was stirred for 30 min and evaporated under reduce pressure at 40°C until no starting material remained. A solution of the *N*-unprotected benzylester amino acids in DCM was added to a solution of 4-nitrophenylchloroformate in DCM and pyridine (8.0 mL, 0.099 mmol, 8.0 equiv.) with cooling in an ice/salt bath. After stirring and allowing the temperature to increase to 5°C over 3 h, the reaction mixture was acidified with by addition of HCl 1N. The organic layer was separated and the aqueous phase was extracted with dichloromethane. Organic layers were combined, dried over anhydrous $MgSO_4$, and filtered. The solvent was remove *in vacuo* and the crude product was purified three times by trituration using cold Et_2O leading to compounds **11** and **12**.

*ONp-L-Ile-OBzl (**11**)*



*(**11**).* White powder, 75% yield. **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.34 (d, *J* = 9.1 Hz, 2H), 7.70 (d, *J* = 9.1 Hz, 1H), 7.44 (d, *J* = 8. 1Hz, 2H), 7.34 (m, 3H), 7.31 (d, *J* = 3.3 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 2H), 5.23 (d, *J* = 12.2 Hz, 1H), 5.18 (d, *J* = 12.2 Hz, 1H), 4.00 (brs, 1H), 1.83 (m, 1H), 1.21 (m, 2H), 0.81 (m, 6H). **^{13}C{1H}-NMR** (100 MHz, $CDCl_3$): δ 169.1, 155.3, 146.0, 138.1, 135.4, 129.0, 128.9, 128.5, 126.0, 125.9, 123.1, 67.6, 56.3, 36.5, 25h.3, 21.2, 14.6, 11.9. **HRMS** (ESI) m/z calcd for $C_{20}H_{23}N_2O_6$ $(M+H)^+$: 387.1551, found 387.1562.

*Preparation of ONp-L-Phe-OBzl (**12**)*



*(**12**).* White powder, 80% yield. **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.88 (d, *J* = 5.3 Hz, 1H), 8.00 (m, 1H), 7.46 (d, *J* = 8.0 Hz, 2H), 7.30 (m, 3H), 7.22 (m, 4H), 7.14 (m, 2H), 7.08 (d, *J* = 7.9 Hz, 2H), 5.10 (d, *J* = 12.2 Hz, 1H), 5.06 (d, *J* = 12.2 Hz, 1H), 4.30 (t, *J* = 6.8 Hz, 1H), 3.15 (dd, *J* = 13.9, 5.7 Hz, 1H), 3.03 (dd, *J* = 14.0, 7.8 Hz, 1H). **^{13}C{1H}-NMR** (100 MHz, $CDCl_3$): δ 169.3,

145.8, 142.9, 138.3, 135.2, 134.9, 129.8, 129.0, 128.8, 128.5, 127.7, 127.5, 125.9, 67.5, 53.6, 36.4, 21.2. **HRMS** (ESI) m/z calcd for $C_{23}H_{21}N_2O_6$ $(M+H)^+$: 421.1394, found 421.1388.

* Compound **12** was difficult to purifiy from traces of *p*-nitrophenol. Since it is used in excess in the tail ligation reaction, we decided to use it as is.

Preparation of cyclic peptides 5-6

Coupling of the first N-Boc amino acid on oxime resin

10.0 g of oxime resin (0.30 mmol/g) was added to a peptide synthesis vessel. The resin was treated three times with CH_2Cl_2. Amino acid (3.0 equiv, 9.0 mmol) and 6-Cl-HOBt (3.0 equiv, 9.0 mmol) were dissolved in DMF (60 mL) in a 100 mL flask and the mixture was stirred for 10 minutes at 0 °C. DIC (3.0 equiv, 9.0 mmol), DIEA (6.0 equiv, 18.0 mmol) and DMAP (0.1 equiv, 0.3 mmol) were added and the mixture was introduced into the peptide synthesis vessel and stirred mechanically for 3h. The mixture was filtered under vacuum and the resin was washed [DMF (3 x 40 mL), MeOH (3 x 40 mL), DMF (3 x 40 mL), MeOH (3 x 40 mL)] and dried under reduced pressure.

Acetylation of unreacted sites on oxime resin

The resin was treated three times with DMF (3 x 40 mL). A solution of 50% v/v DMF/acetic anhydride (60 mL) and DIEA (3.0 mL) were added to the peptide synthesis vessel and shaken for 1 hour. Then, the mixture was filtered under vacuum and the resin was washed [DMF (3 x 40 mL), MeOH (3 x 40 mL), DMF (3 x 40 mL), MeOH (3 x 40 mL)] and dried under reduced pressure.

Removal of the N-Boc protecting group

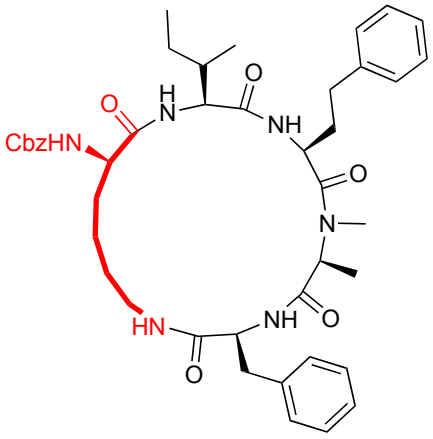
The resin was treated three times with CH_2Cl_2 (25 mL). A 50% v/v solution (25 mL) of trifluoroacetic acid (TFA) in CH_2Cl_2 was added to the peptide synthesis vessel and shaken for 30 minutes. Then, the mixture was filtered under vacuum and the resin was washed with DMF (3 x 40 mL), MeOH (3 x 40 mL), DMF (3 x 40 mL), MeOH (3 x 40 mL) and with a solution of 10% v/v DIEA in CH_2Cl_2 (40 mL).

Coupling of the subsequent N-Boc protected α-amino acids

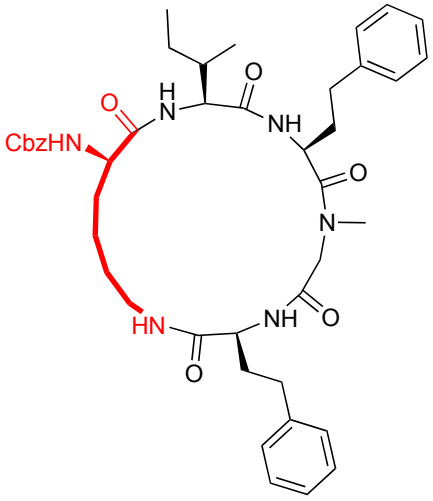
The amino acid (3.0 equiv, 9.0 mmol) was dissolved in DMF (60 mL) in a 100 mL flask. The solution was cooled to 0 °C, then HCTU (3.0 equiv, 9.0 mmol) and 6-Cl-HOBt (3.0 equiv, 9.0 mmol) were added. The mixture was poured into the peptide synthesis vessel, in which the resin had been previously treated with CH_2Cl_2 (3 X 40 mL). DIEA (6.0 equiv, 18.0 mmol) was also added to the vessel and the mixture was shaken for 3 h. After filtration under vacuum, the resin was washed [DMF (3 x 40 mL), MeOH (3 x 40 mL), DMF (3 x 40 mL) and MeOH (3 x 40 mL)] and dried under reduce pressure. The Kaiser ninhydrin test was performed to monitor the efficiency of the coupling, and the coupling procedure was repeated if needed.

Cyclization/cleavage from the resin

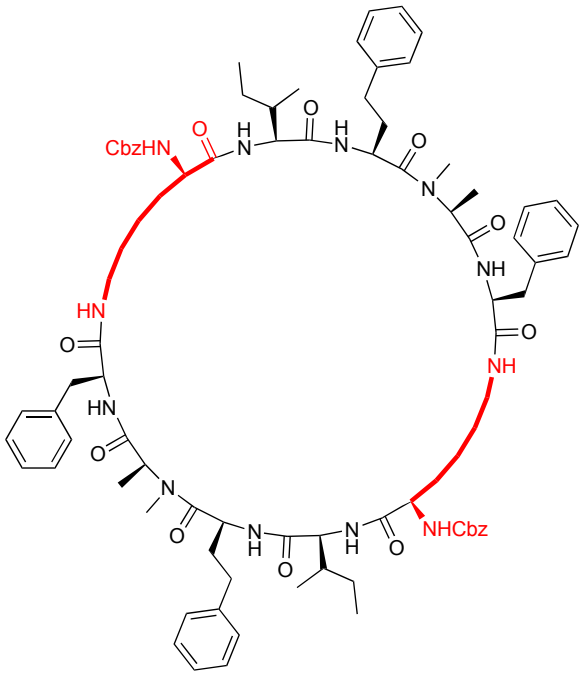
First, the *N*-Boc group was removed using the procedure described above, but without the 10% v/v DIEA/CH_2Cl_2 washing step. After drying, CH_2Cl_2 and DIEA (2.5 equiv.) were added to the peptide synthesis vessel and the mixture was shaken for 2 min. Acetic acid (5.0 equiv.) was then added and the contents were shaken for 24 h. The filtrate was collected and the resin was rinsed several times with CH_2Cl_2 and MeOH. All the filtrates were combined and evaporated. Trituration in a minimum of cold ether was performed and led to the desired compounds **3** and **4** by simple filtration.



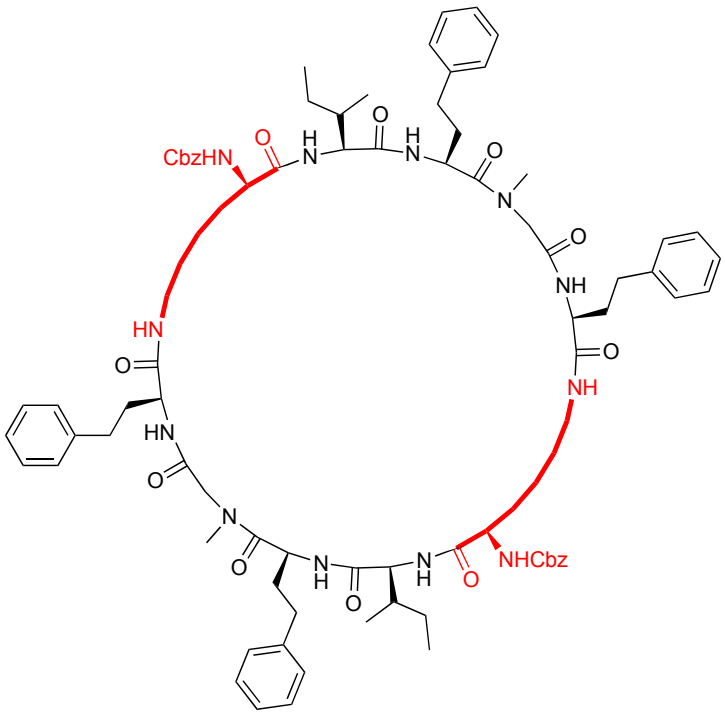
(3). White powder, 74% yield. **HRMS** (ESI) m/z calcd for $C_{43}H_{57}N_6O_7$ $(M+H)^+$: 769.4283, found 769.5238. **HPLC** (Retention time): 11.82 min.



(4). White powder, 72% yield. **HRMS** (ESI) m/z calcd for $C_{43}H_{57}N_6O_7$ $(M+H)^+$: 769.4283, found 769.5490. **HPLC** (Retention time): 12.18 min.



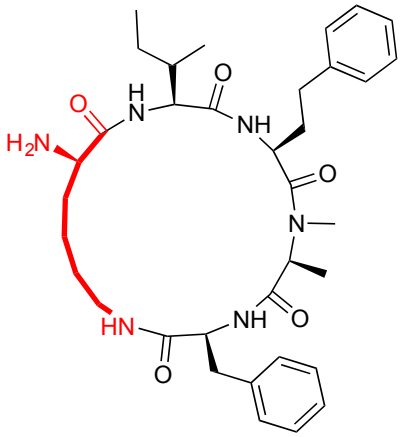
(3-dimer). White powder, 12% yield. **HRMS** (ESI) m/z calcd for $C_{86}H_{113}N_{12}O_{14}$ $(M+H)^+$: 1537.8494, found 1538.8451. **LC-MS** (Retention time): 17.09 min.



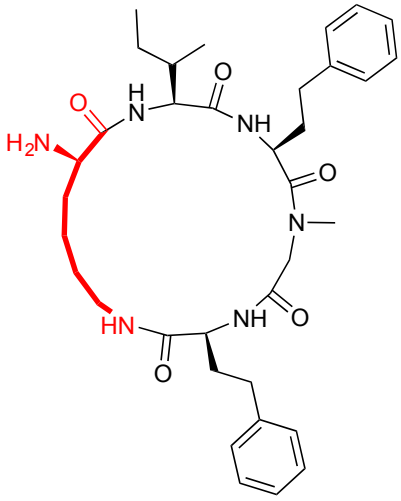
(4-dimer). White powder, 11% yield. **HRMS** (ESI) m/z calcd for $C_{86}H_{113}N_{12}O_{14}$ $(M+H)^+$: 1537.8494, found 1538.8480. **LC-MS** (Retention time): 16.59 min.

Hydrogenolysis

The protected cyclic peptide was poured into a microwave vial and was suspended in EtOH. Pd/C 10% was then added. Then, a balloon full of H_2 was introduce to the reaction mixture and the reaction was stirred for 48 h. The reaction mixture was filtered on a Celite® pad. The filtrate was concentrated *in vacuo* and the crude product was isolated as pure white solid and further purified by normal-phase chromatography using a gradient composed of DCM (100% to 80%) and MeOH (0% to 20%) in 30 min to afford pure cyclic monomer **6** and **7** as well as cyclic dimers as a white powder.

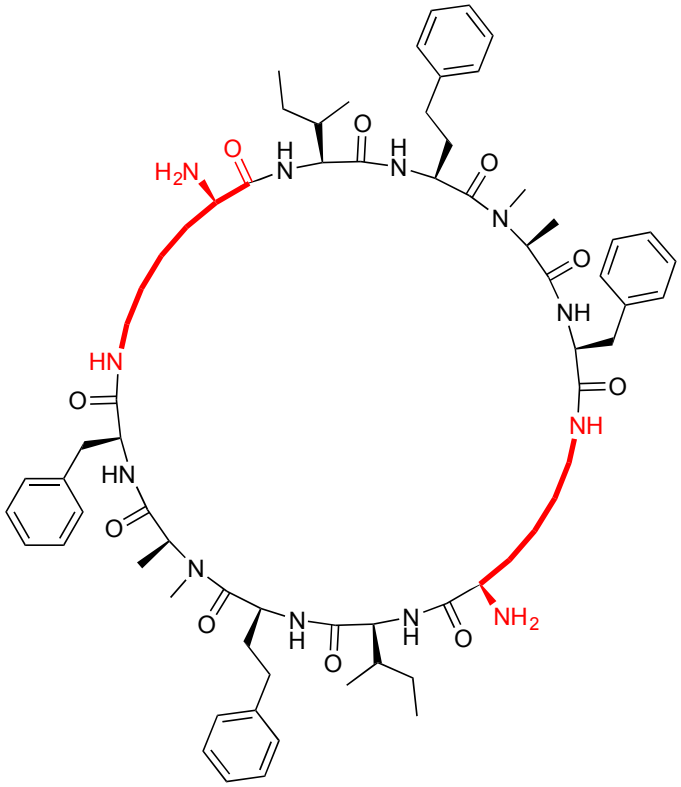


(5). White powder, 60% yield. **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 8.93 (d, *J* = 4.7 Hz, 1H), 8.68 (d, *J* = 8.8 Hz, 1H), 7.69 (d, *J* = 8.1 Hz, 1H), 7.28 (m, 2H), 7.20 (m, 4H), 7.14 (m, 2H), 7.11 (d, *J* = 3.4 Hz, 1H), 7.08 (m, 2H), 4.79 (m, 2H), 4.35 (ddd, *J* = 12.5, 8.8, 3.4 Hz, 1H), 4.14 (t, *J* = 8.1 Hz, 1H), 3.55 (m, 1H), 2.80 (m, 2H), 2.74 (dd, *J* = 11.9, 4.4 Hz, 1H), 2.56 (ddd, *J* = 13.5, 11.2, 6.1 Hz, 1H), 1.81 (s, 3H), 1.76 (m, 2H), 1.62 (m, 2H), 1.51 (m, 2H), 1.39 (m, 2H), 1.19 (m, 4H), 1.07 (d, *J* = 6.7 Hz, 3H), 1.02 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 7.4 Hz, 3H). **^{13}C{1H}-NMR** (126 MHz, DMSO-d_6): δ δ 175.4, 173.3, 171.3, 171.2, 170.4, 141.5, 138.8, 129.36, 128.9, 128.7, 128.7, 128.6, 126.5, 56.3, 55.8, 55.5, 54.8, 49.4, 40.5, 39.4, 38.8, 37.9, 36.7, 34.5, 33.3, 31.8, 28.8, 27.6, 25.1, 20.0, 15.5, 14.3, 11.1. **HRMS** (ESI) m/z calcd for $C_{35}H_{51}N_6O_5$ $(M+H)^+$: 635.3915, found 635.3880. **HPLC** (Retention time, purity): 25.25 min, 98 %.

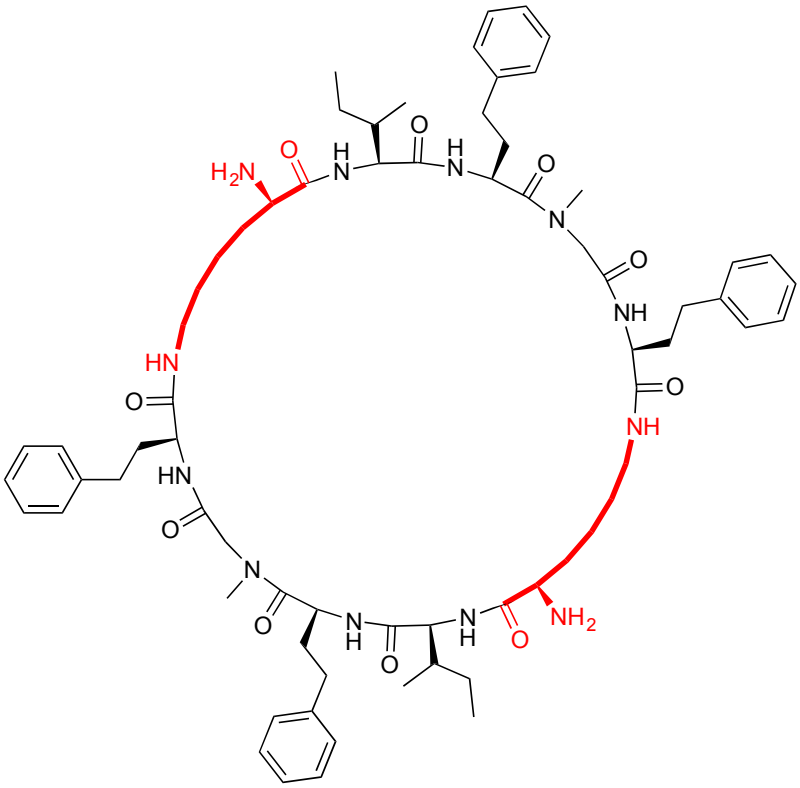


(6). White powder, 58% yield. **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 8.90 (d, *J* = 5.1, 1H), 8.68 (d, *J* = 8.6 Hz, 1H), 7.92 (d, *J* = 8.9 Hz, 1H), 7.60 (d, *J* = 8.2 Hz, 1H), 7.29 (m, 2H), 7.24 (m, 4H), 7.20-

7.11 (m, 6H), 4.68 (dt, J = 9.0, 5.4 Hz, 1H), 4.60 (d, J = 16.7 Hz, 1H), 4.47 (J = 16.2, 1H), 4.15 (dt, J = 9.5, 4.5 Hz, 1H), 4.08 (m, 1H), 3.63 (d, J = 16.7 Hz, 1H), 2.81 (s, 2H), 2.09 (m, 2H), 1.90 (s, 3H), 1.70 (m, 2H), 1.56 (m, 2H), 1.45 (m, 2H), 1.32 (m, 4H), 1.09 (m, 3H), 0.95 (d, J = 6.7 Hz, 3H), 0.79 (dt, J = 27.6, 7.3 Hz, 3H). **^{13}C{1H}-NMR** (126 MHz, DMSO-d_6): δ 173.2, 171.5, 171.4, 169.4, 168.8, 141.8, 141.7., 141.6, 128.8, 128.7, 128.6, 126.2, 126.1, 57.9, 56.5, 55.5, 53.5, 52.8, 49.9, 38.7, 37.3, 36.5, 34.4, 32.8, 32.4, 32.1, 31.5, 31.3, 28.7, 25.1, 21.5, 20.0, 15.7, 15.6, 11.2. **HRMS** (ESI) m/z calcd for $C_{35}H_{51}N_6O_5$ $(M+H)^+$: 635.3915, found 635.3880. **HPLC** (Retention time, purity): 26.06 min, 97 %.



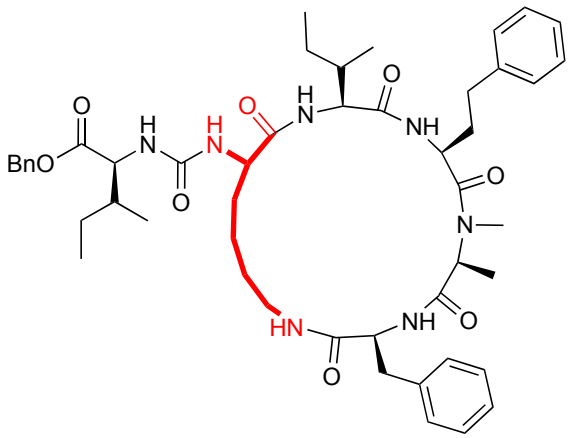
(7). White powder, 11% yield. **HRMS** (ESI) m/z calcd for $C_{70}H_{101}N_{12}O_{10}$ $(M+H)^+$: 1269.7758, found 1269.7790. **HPLC** (Retention time, purity): 37.10 min, 92 %.



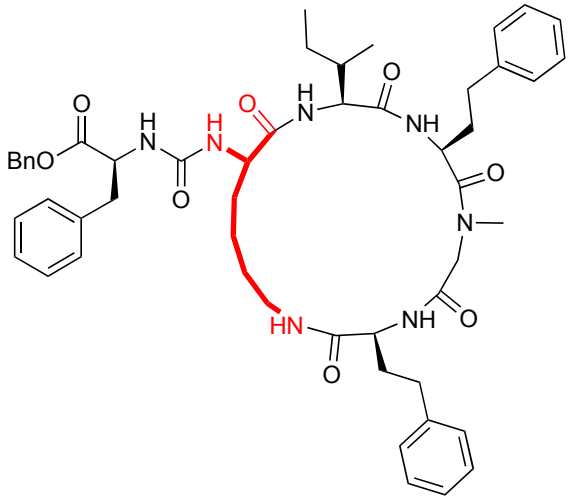
*(**8**)*. White powder, 13% yield. **HRMS** (ESI) m/z calcd for $C_{70}H_{101}N_{12}O_{10}$ $(M+H)^+$: 1269.7758, found 1269.7649. **HPLC** (Retention time, purity): 35.16 min, 94%.

General procedure for the preparation of protected anabaenopeptins

In a 5 mL round bottom flask, to a solution of cyclic peptide **5** or **6** (1.0 equiv,) in DMF was added the corresponding *p*-nitrophenylcarbamate amino benzyl esters (5.0 equiv)) followed by Et_3N (10.0 equiv.) and DMAP (0.1 equiv.). The reaction mixture was stirred for 48 h and the crude product was subjected to semi-preparative HPLC to afford compounds **13** and **14**.



(13). White powder, 55% yield. **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 8.98 (d, *J* = 4.8 Hz, 1H), 8.69 (d, *J* = 8.9 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 1H), 7.33 (m, 7H), 7.19 (m, 3H), 7.06 (d, *J* = 7.0 Hz, 1H), 6.93 (d, *J* = 7.2 Hz, 1H), 6.49 (d, *J* = 8.8 Hz, 1H), 5.10 (m, 3H), 4.83 (q, *J* = 7.0 Hz, 1H), 4.76 (dd, *J* = 13.6, 5.1 Hz, 1H), 4.38 (ddd, *J* = 12.6, 8.9, 3.4 Hz, 1H), 4.15 (dt, *J* = 8.8, 5.9 Hz, 1H), 3.98 (qd, *J* = 6.8, 1.8 Hz, 1H), 3.91 (dd, *J* = 11.3, 5.2 Hz, 1H), 2.76 (m, 2H), 2.54 (m, 2H), 1.93 (m, 2H), 1.77 (s, 3H), 1.72 (m, 2H), 1.47 (m, 3H), 1.31 (m, 4H), 1.22 (m, 2H), 1.15 (t, *J* = 7.1 Hz, 3H), 1.07 (d, *J* = 6.8 Hz, 3H), 1.02 (m, 2H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.86 (m, 2H), 0.80 (m, 9H). **HRMS** (ESI) m/z calcd for $C_{49}H_{68}N_7O_8$ $(M+XX_X)^+$: 882.5124, found 882.5077 **HPLC** (Retention time, purity): 38.10 min, 98 %.

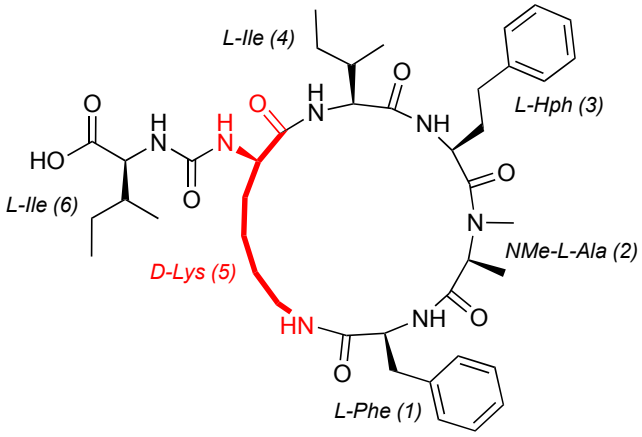


(14). White powder, 51% yield. **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 8.93 (d, *J* = 5.2 Hz, 1H), 8.64 (d, *J* = 8.6, 1H), 8.32 (s, 1H), 7.91 (d, *J* = 8.9 Hz, 1H), 7.32 (m, 4H), 7.23 (m, 7H), 7.12 (m, 4H), 6.84 (d, *J* = 7.3 Hz, 1H), 6.49 (d, *J* = 8.1 Hz, 1H), 5.05 (d, *J* = 9.6 Hz, 2H), 4.65 (m, 1H), 4.42 (m,

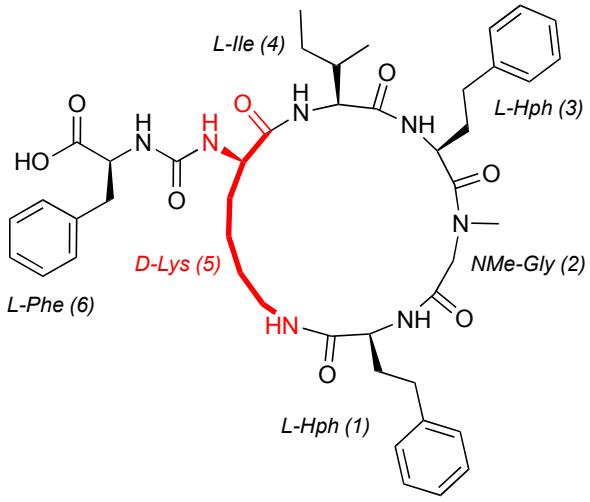
1H), 4.17 (td, *J* = 9.7, 4.2 Hz, 1H), 4.08 (ddd, *J* = 11.7, 8.5, 3.3 Hz, 1H), 3.96 (dd, *J* = 9.0, 7.3 Hz, 1H), 3.87 (m, 1H), 3.58 (d, *J* = 16.5 Hz, 1H), 2.96 (m, 1H), 2.89 (m, 1H), 2.80 (s, 3H), 2.13 (m, 2H), 1.92 (m, 2H), 1.62 (m, 1H), 1.49 (m, 2H), 1.33 (m, 2H), 1.18 (m, 2H), 0.91 (d, *J* = 6.7 Hz, 3H), 0.72 (dt, *J* = 23.2, 7.4 Hz, 3H). **HRMS** (ESI) m/z calcd for $C_{52}H_{66}N_7O_8$ $(M+H)^+$: 916.4367, found 916.4303. **HPLC** (Retention time, purity): 38.89 min, 99 %.

General procedure for hydrogenolysis of protected anabaenopeptins

The protected anabaenopeptin was poured into a microwave vial and was suspended in a EtOH/THF 1:1 mixture. Pd/C 10% was added. Then, a balloon full of H_2 was introduce to the reaction mixture and the reaction was stirred for 48 h. The reaction mixture was filtered on a Celite® pad. The filtrate was concentrated *in vacuo* and the crude product was isolated as pure white solid and further purified by semi-preparative HPLC.



(1). White powder, 91% yield. **[a]$_D^{22}$** -44.5° (c 3.0 in MeOH). **^{1}H-NMR** (500 MHz, DMSO-*d*$_6$): δ 8.86 (d, *J* = 8.9 Hz, 1H), 8.76 (d, *J* = 4.5 Hz, 1H), 7.74 (m, 1H), 7.68 (m, 1H), 7.57 (m, 1H), 7.35 (dd, *J* = 8.5, 2.7 Hz, 2H), 7.30 (m, 1H), 7.18 (t, *J* = 7.2 Hz, 2H), 7.13 (t, *J* = 7.2 Hz, 1H), 7.05 (d, *J* = 7.0 Hz, 2H), 6.84 (d, *J* = 7.2 Hz, 1H), 6.44 (d, *J* = 6.9 Hz, 1H), 6.31 (d, *J* = 8.8 Hz, 1H), 5.05 (q, *J* = 6.8 Hz, 1H), 4.69 (td, *J* = 7.2, 4.5 Hz, 1H), 4.39 (ddd, *J* = 12.5, 8.9, 3.3 Hz, 1H), 4.05 (dd, *J* = 8.8, 4.9 Hz, 1H), 3.95 (dd, *J* = 9.0, 7.2 Hz, 1H), 3.89 (q, *J* = 5.5 Hz, 1H), 3.60 (m, 2H), 2.76 (m, 3H), 1.75 (s, 3H), 1.71 (m, 1H), 1.65 (m, 2H), 1.59 (m, 2H), 1.48 (m, 2H), 1.37 (m, 2H), 1.26 (m, 2H), 1.20 (m, 2H), 1.11 (m, 2H), 1.07 (d, *J* = 6.8 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.83 (m, 6H), 0.78 (d, *J* = 7.5 Hz, 3H). **HRMS** (ESI) m/z calcd for $C_{42}H_{61}N_7O_7Na$ $(M\text{-}OH + Na)^+$: 798.4525, found 798.5186. **HPLC** (Retention time, purity): 31.52 min, 95 %.



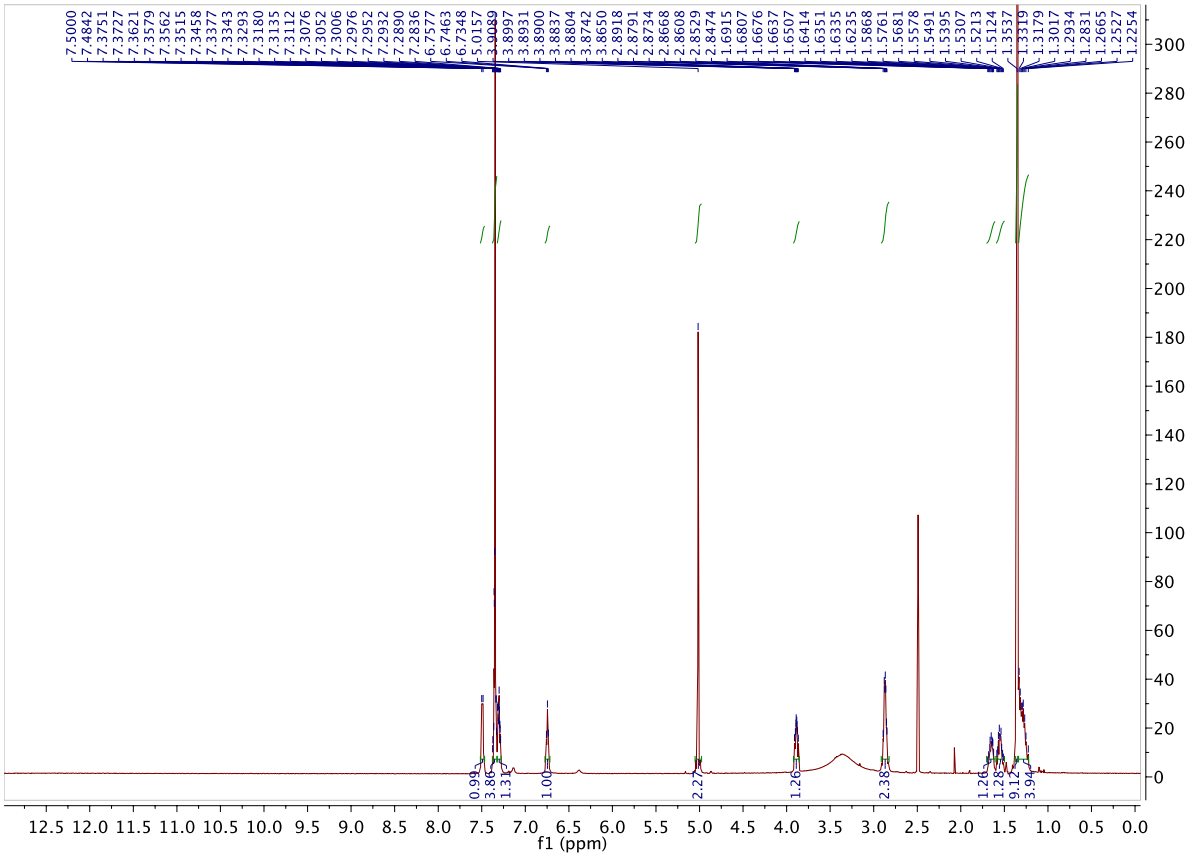
(2). White powder, 90% yield. **[a]$_D^{22}$** -6.7° (c 0.004 in MeOH). **^{1}H-NMR** (500 MHz, DMSO-d_6): δ ^{1}H NMR (500 MHz, DMSO-d_6) δ **^{1}H-NMR** (500 MHz, DMSO-d_6): δ 12.69 (s, 1H), 8.93 (d, J = 5.2 Hz, 1H), 8.64 (d, J = 8.6 Hz, 1H), 7.24 (m, 13H), 7.15 (m, 1H), 7.10 (m, 2H), 6.83 (d, J = 5.7 Hz, 1H), 6.45 (m, 1H), 6.25 (m, 1H), 4.67 (m, 1H), 4.61 (m, 1H), 4.42 (m, 1H), 4.07 (m, 1H), 3.95 (m, 1H), 3.87 (m, 1H), 3.59 (d, J = 16.5 Hz, 1H), 3.49 (m, 1H), 2.98 (m, 1H), 2.87 (m, 1H), 2.80 (s, 3H), 2.73 (m, 1H), 2.62 (m, 1H), 2.57 (m, 1H), 2.42 (m, 2H), 2.11 (m, 1H), 1.90 (m, 3H), 1.59 (m, 1H), 1.51 (m, 2H), 1.46 (m, 1H), 1.35 (m, 1H), 1.17 (m, 3H), 1.02 (m, 1H), 0.92 (d, J = 6.7 Hz, 3H), 0.76 (t, J = 7.4 Hz, 3H). **HRMS** (ESI) m/z calcd for $C_{45}H_{60}N_7O_8$ $(M+H)^+$: 826.4498, found 826.4584. **HPLC** (Retention time, purity): 31.51 min, 96 %.

		Isolated peptide	*Synthesized peptide* (**1**)
	Position	δ_H, mult., *J* (Hz)	δ_H, mult., *J* (Hz)
Phe	1	-	-
	2	4.38 ddd 12.3, 8.9, 3.3	4.39 ddd 12.5, 8.9, 3.3
	3	2.78 m	2.76 m
		3.35 m	3.59 m
	4	-	-
	5, 5’	7.05 d 7.3	7.05 d 7.0
	6, 6’	7.18 t 7.3	7.18 t 7.2
	7	7.14 t 7.3	7.13 t 7.2
	NH	8.64 d 8.9	8.86 d 8.9
NMeAla	1	-	-
	2	4.80 q 6.7	5.05 q 6.8
	3	1.06 d 6.7	1.07 d 6.8
	NMe	1.77 s	1.75 s
Hph	1	-	-
	2	4.74 ddd 8.0, 5.2, 4.8	4.69 td 7.2, 4.5
	3	1.76 m	1.72 m
		1.92 m	1.64 m
	4	2.55 dd 6.2, 11.3	2.56 dd 6.2, 11.3
		2.77 m	2.76 m
	5	-	-
	6, 6’	7.22 d 7.3	7.18 d 7.1
	7, 7’	7.28 t 7.3	7.31 t 7.1
	8	7.19 t 7.3	7.22 t 7.0
	NH	8.95 d 4.8	8.76 d 4.5
Ile(1)	1	-	-
	2	3.98 dd 8.6, 6.9	3.95 dd 9.0, 7.2
	3	1.74 m	1.64 m
	4	1.16 m	1.19 m
		1.37 m	1.37 m
	5	0.81 t 7.4	0.83 m
	6	1.00 d 6.8	0.96 d 6.8
	NH	6.91 d 6.9	6.84 d 7.2
Lys	1	-	-
	2	3.92 dt 7.1, 5.7	3.89 q 5.5
	3	1.62 q	1.59 m
	4	1.12 m	1.11 m
		1.35 m	1.37 m
	5	1.44 m	1.48 m
	6	2.80 m	2.76 m
		3.57 m	3.59 m
	α-NH	6.49 d 7.1	6.44 d 6.9
	ε-NH	7.13 t 7.3	7.13 t 7.2
Urea	1	-	-
Ile(2)	1	-	-
	2	4.07 dd 8.9, 5.1	4.05 dd 8.8, 4.9
	3	1.70 m	1.59 m
	4	1.12 m	1.11 m
		1.32 m	1.19 m
	5	0.84 t 7.3	0.83 m
	6	0.83 d 6.9	0.78 d 7.5
	NH	6.32 d 8.9	6.31 d 8.8

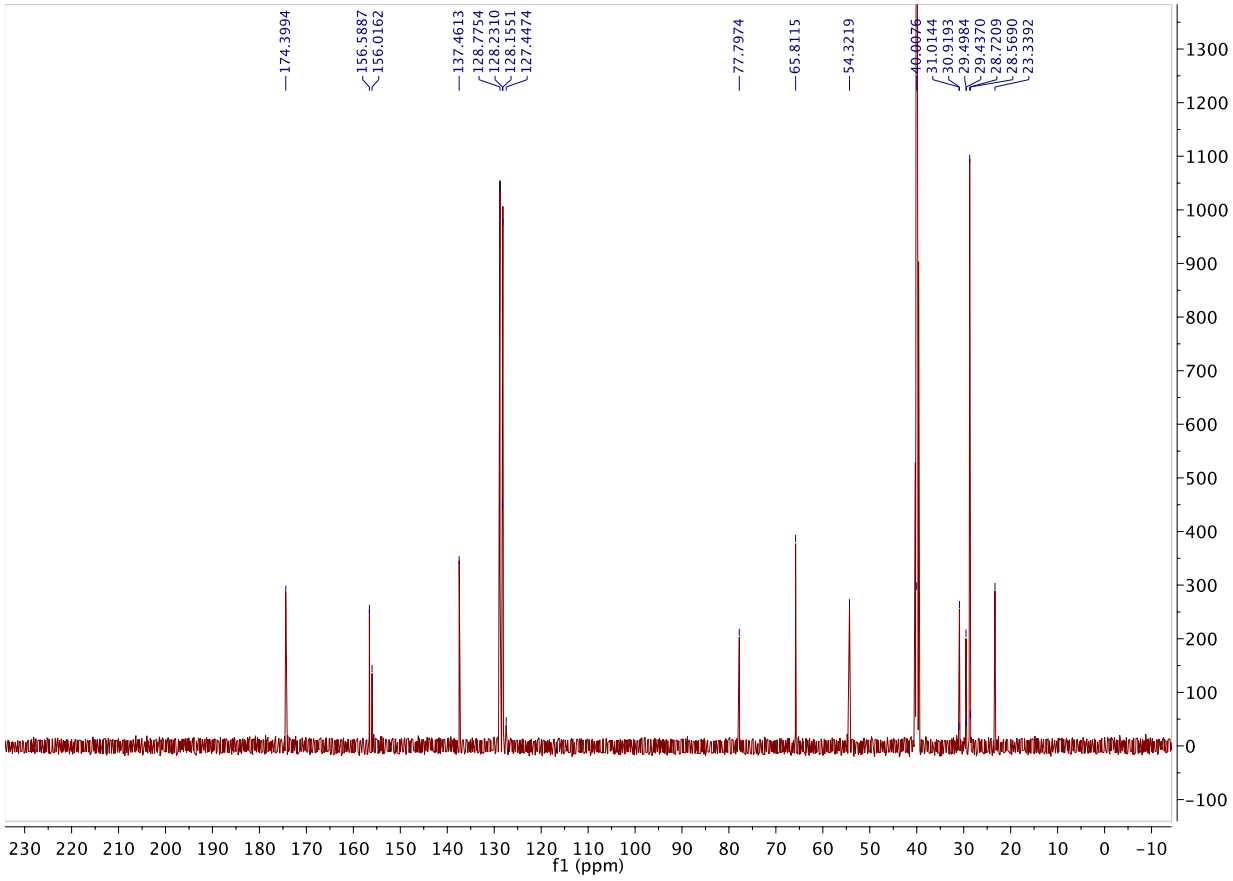
		Isolated peptide	*Synthesized peptide* (**2**)
	Position	δ_H, mult., *J* (Hz)	δ_H, mult., *J* (Hz)
Hph(1)	1	-	-
	2	4.10 ddd, 11.5, 9.0, 2.7	4.07 m
	3	2.15 m	2.11 m
		1.90 m	1.90 m
	4	2.48 m	2.42 m
	5	-	-
	6, 6’	7.17 m	7.24 m
	7, 7’	7.23 m	7.24 m
	8	7.20 m	7.24 m
	NH	8.55 d 8.0	8.64 d 8.6
NMeGly	1	-	-
	2	3.60 d 16.0	3.59 d 16.5
		4.55 d 16.0	4.61 m
	NMe	2.80 s	2.80 s
Hph(2)	1	-	-
	2	4.65 ddt	4.67 m
	3	1.88 m	1.90 m
	4	2.74 m	2.62 m
		2.56 m	2.57 m
	5	-	-
	6, 6’	7.17 m	7.24 m
	7, 7’	7.23 m	7.24 m
	8	7.20 m	7.24 m
	NH	8.87 d 8.0	8.93 d 5.2
Ile	1	-	-
	2	3.97 t 8.0	3.95 m
	3	1.60 m	1.59 m
	4	1.05 m	1.02 m
		1.45 m	1.45 m
	5	0.72 t 7.2	0.76 t 7.4
	6	0.91 d 6.8	0.92 d 6.7
	NH	6.78 d 7.1	6.83 d 5.7
Lys	1	-	-
	2	3.88 m	3.87 m
	3	1.54 m	1.51 m
		1.50 m	1.51 m
	4	1.08 m	1.19 m
		1.18 m	1.19 m
	5	1.35 m	1.34 m
		1.18 m	1.19 m
	6	2.75 m	2.74 m
		3.46 m	3.48 m
	α-NH	7.14 m	7.14 m
	ε-NH	6.47 d 7.2	6.45 m
Urea	1	-	-
Phe	1	-	-
	2	4.35 m	4.42 m
	3	2.88 m	2.87 m
		2.98 m	2.98 m
	4	-	-
	5,5’	7.15 d 8.0	7.10 m
	6, 6’	7.20 m	7.24 m
	7	7.17 m	7.24 m
	NH	6.25 d 8.2	6.25 m

*Differences in the chemical shifts (compounds **1** and **2)** are due to concentration effect and/or presence of water traces.

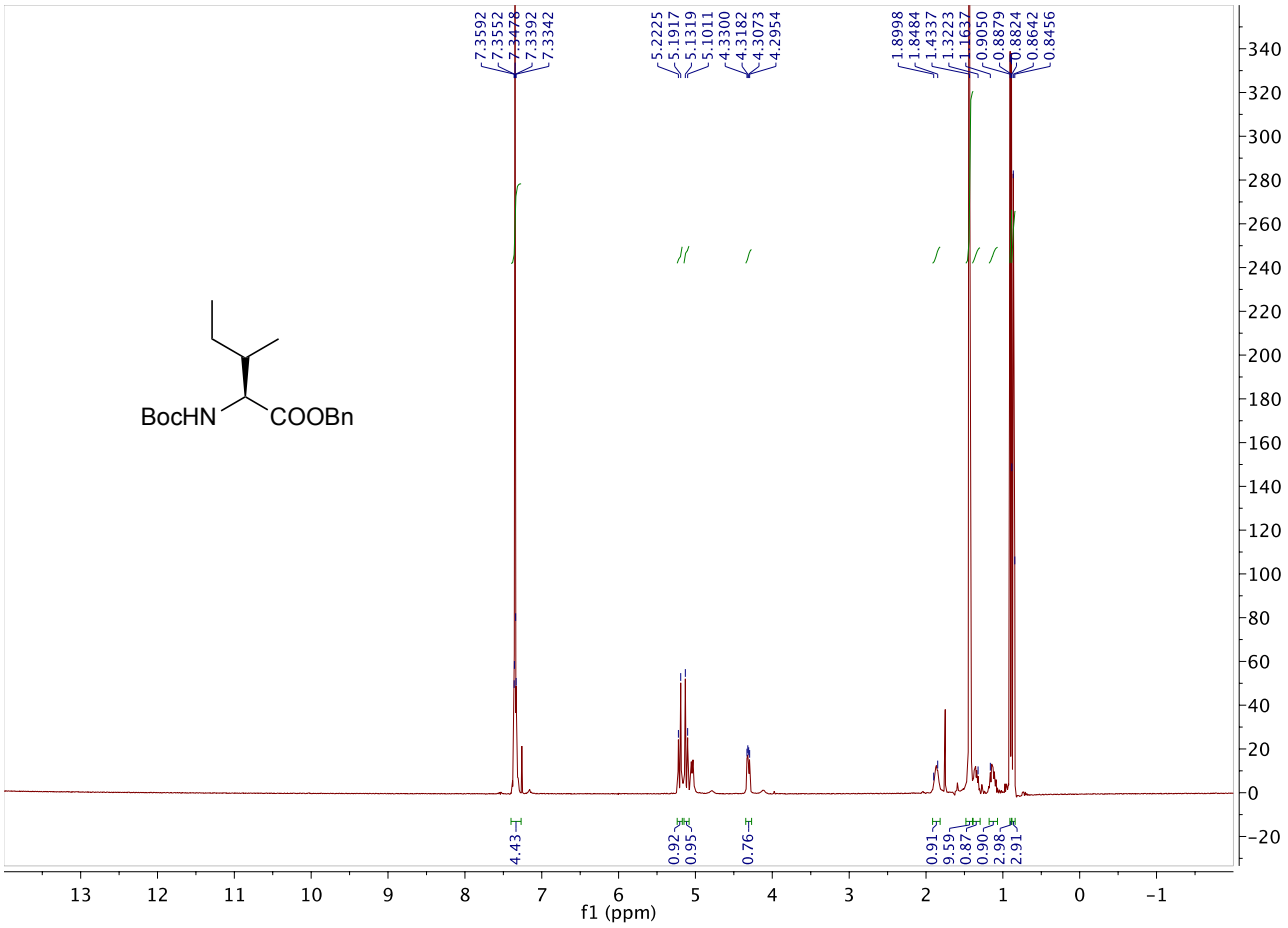
[1]H-NMR spectrum of Z-D-Lys(Boc)-OH



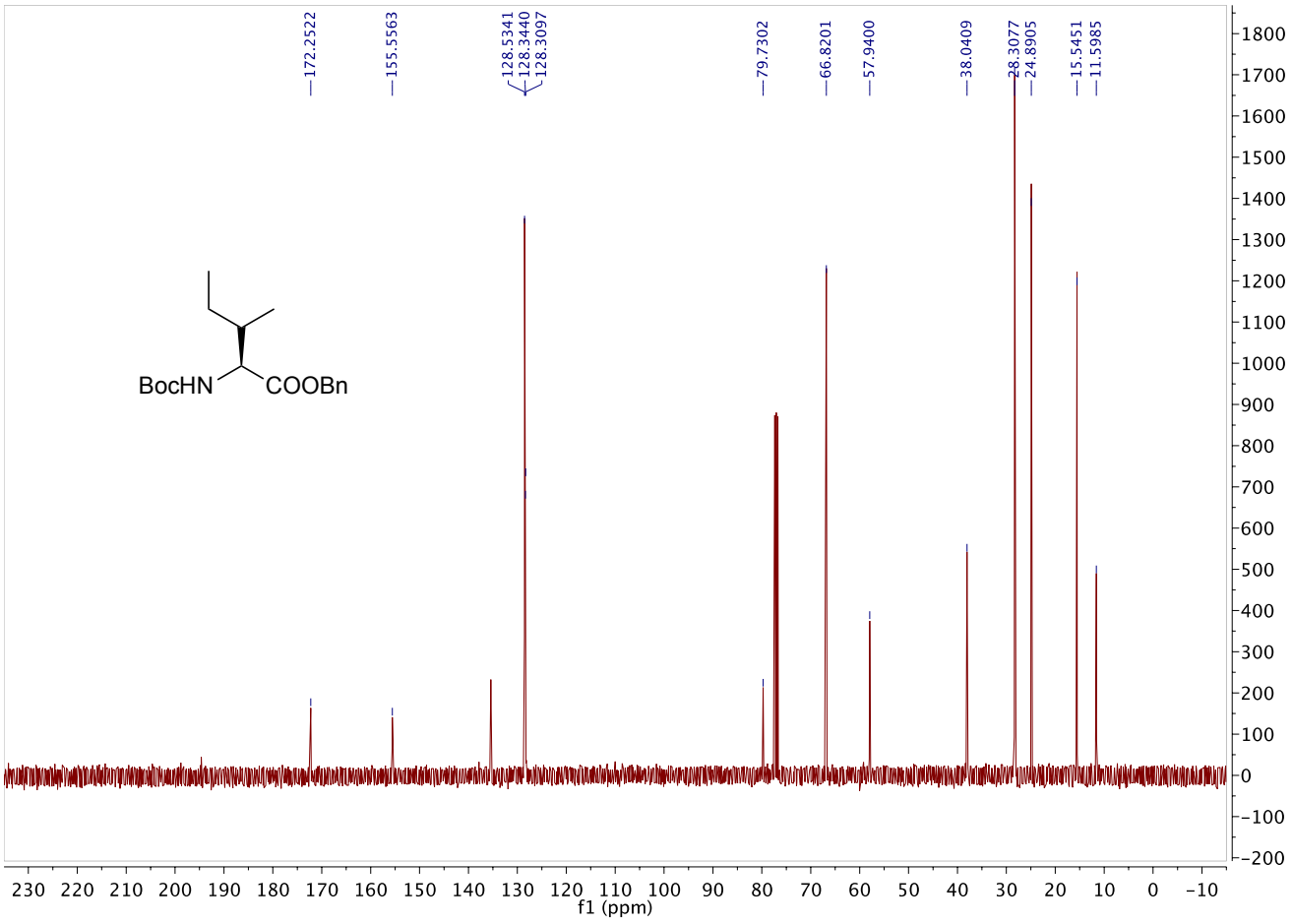
$^{13}C\{1H\}$-NMR spectrum of Z-D-Lys(Boc)-OH



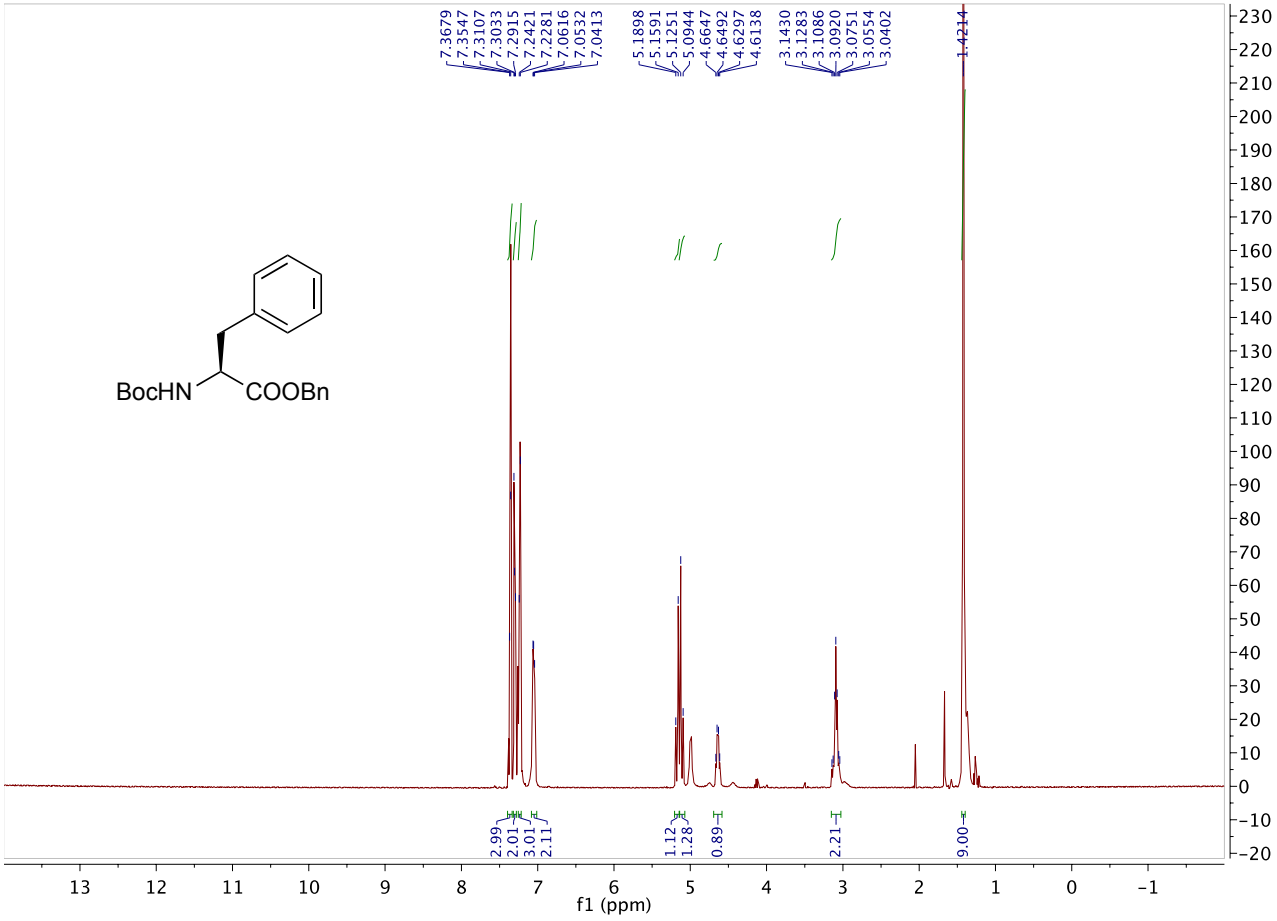
[1]H-NMR spectrum of ***9***



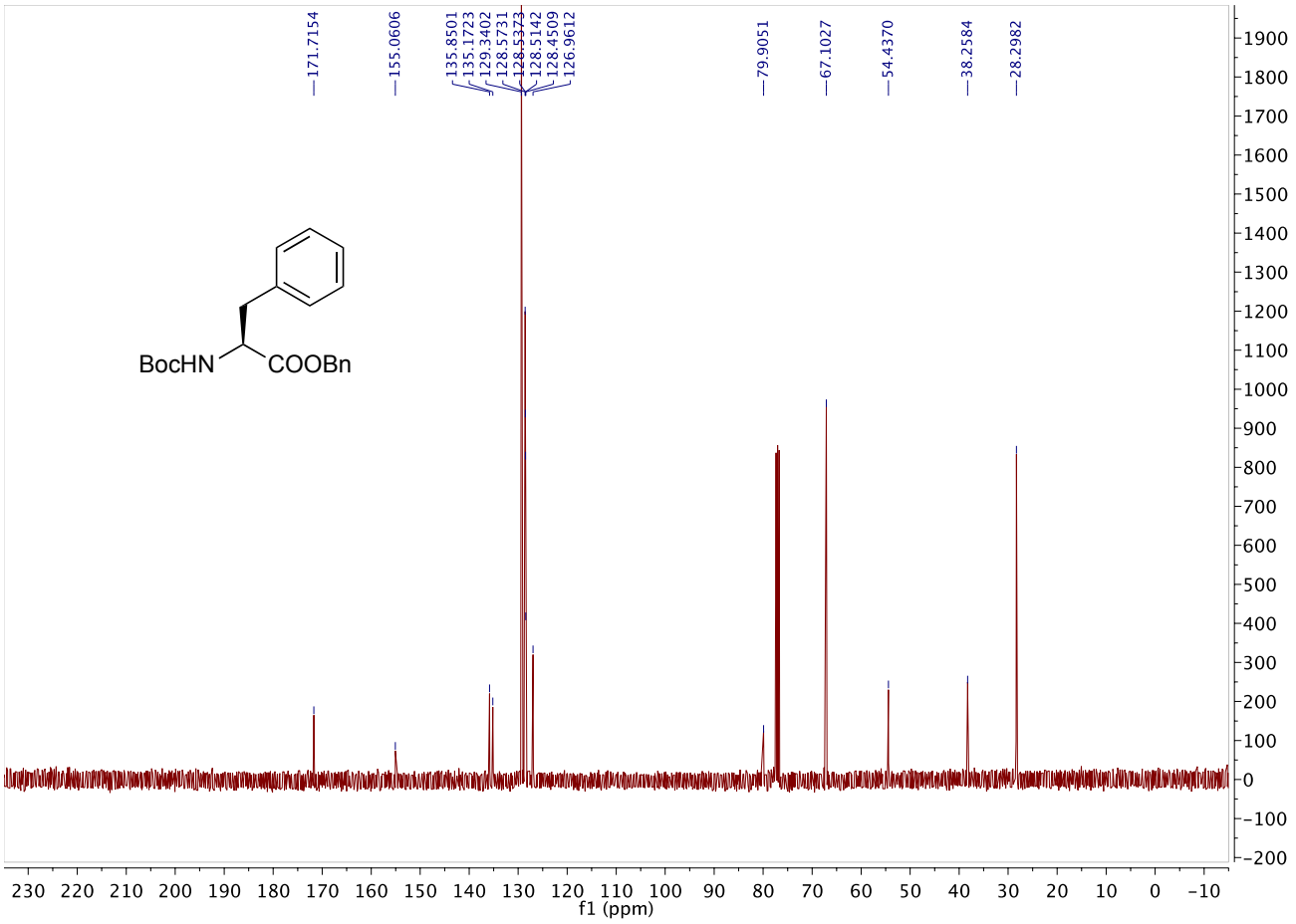
*$^{13}C\{1H\}$-NMR spectrum of **9***



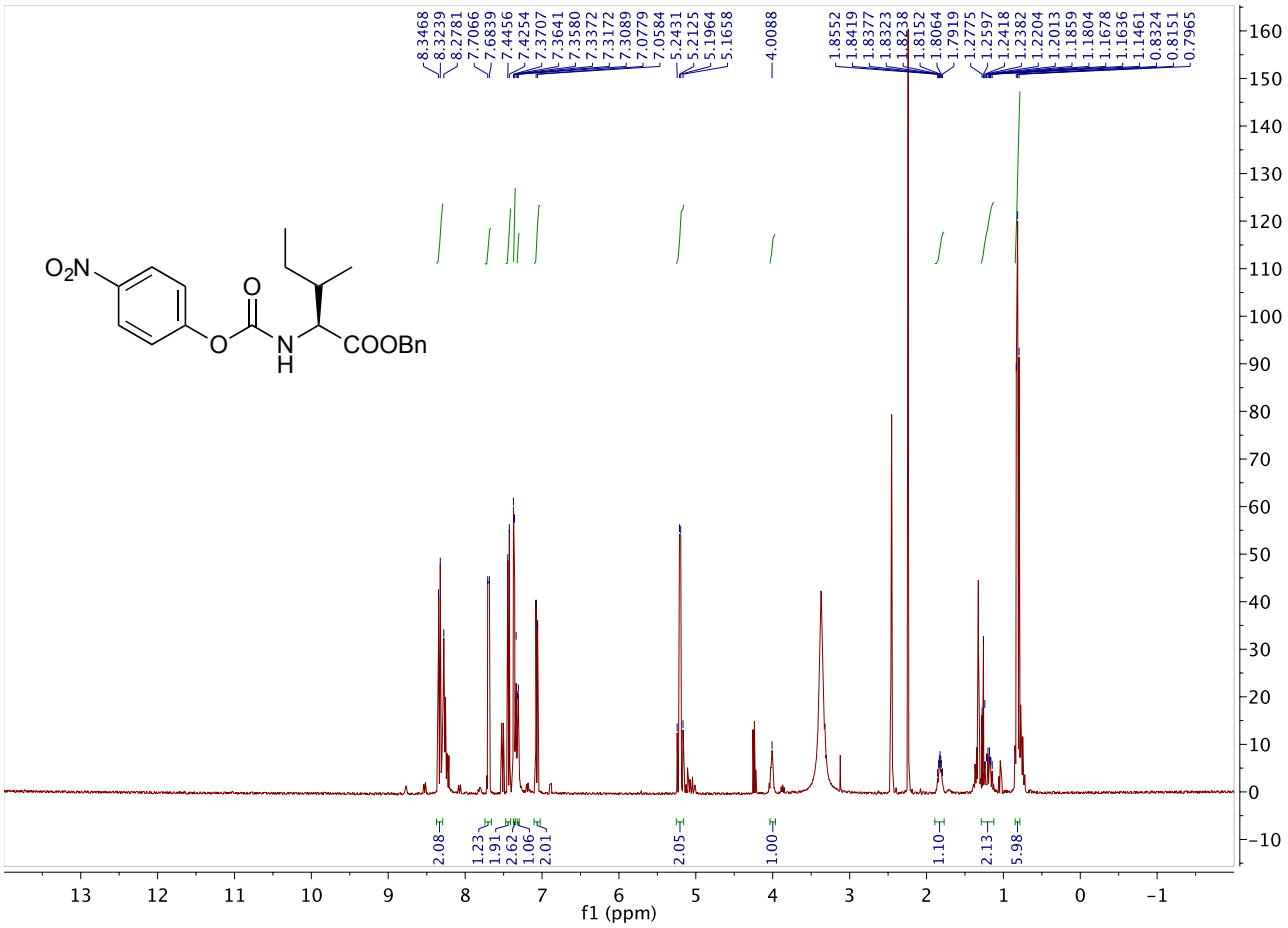
*[1]H-NMR spectrum of **10***



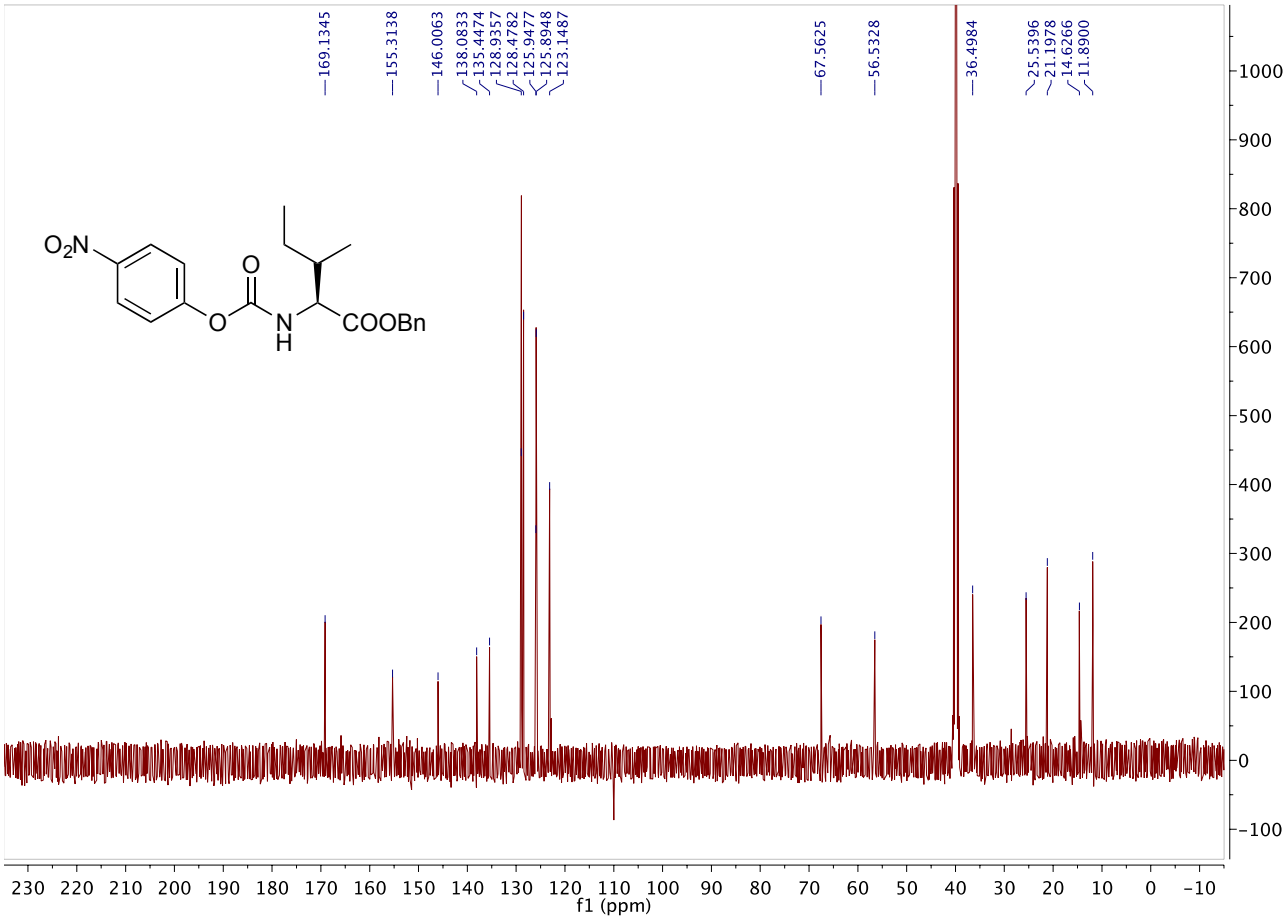
*$^{13}C\{1H\}$-NMR spectrum of **10***



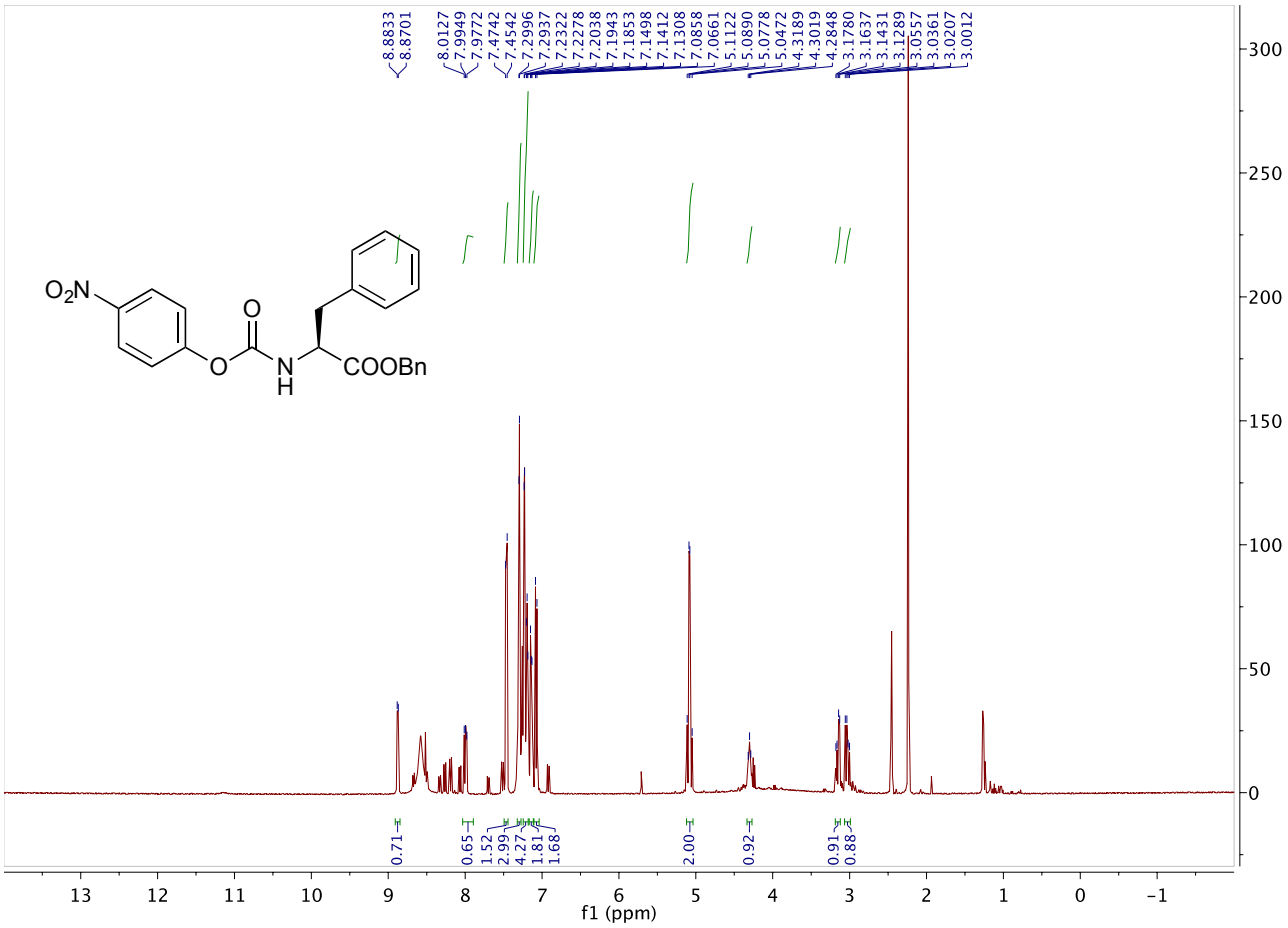
[1]H-NMR spectrum of ***11***



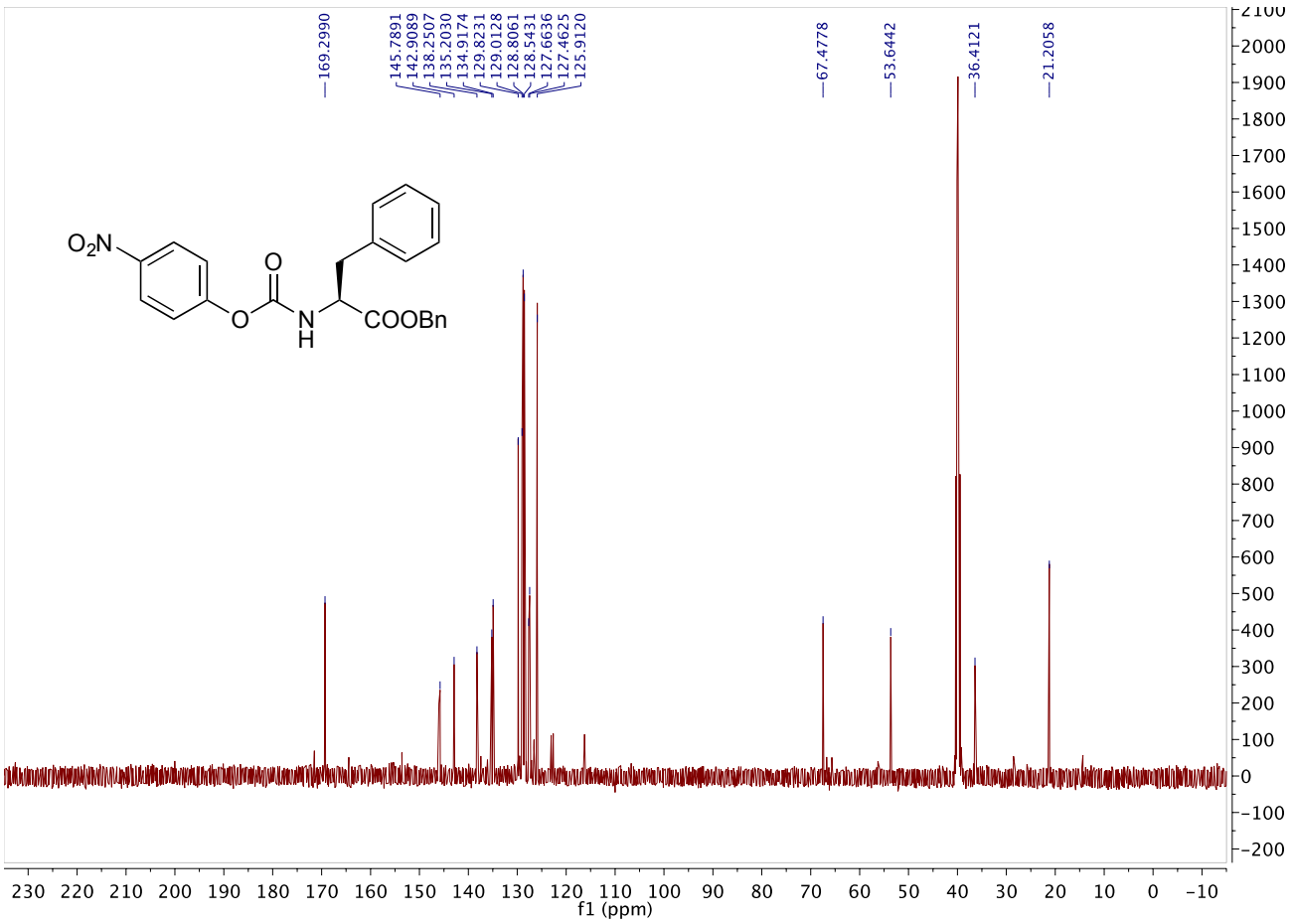
*$^{13}C\{1H\}$-NMR spectrum of **11***



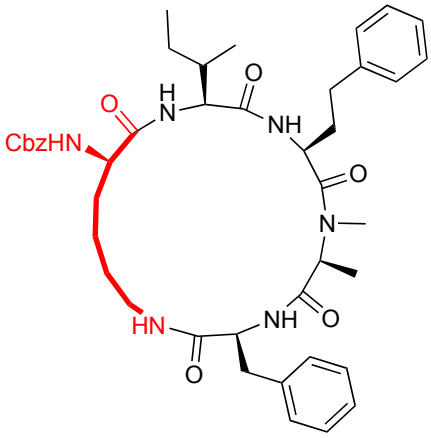
[1]H-NMR spectrum of ***12***

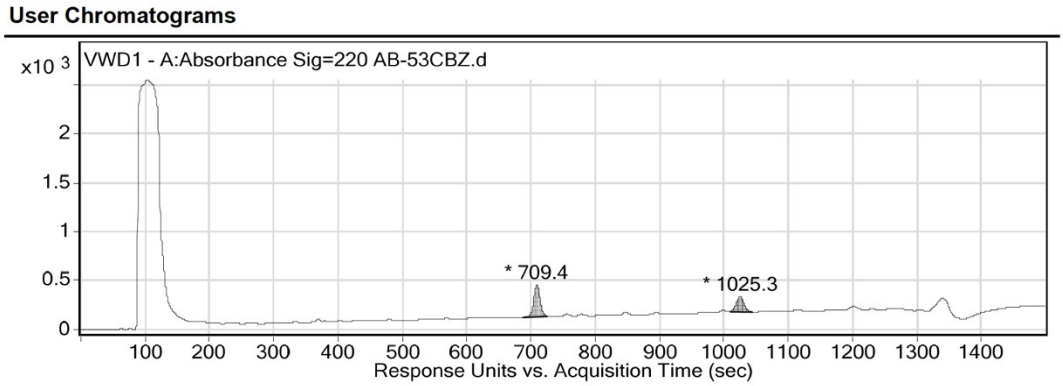


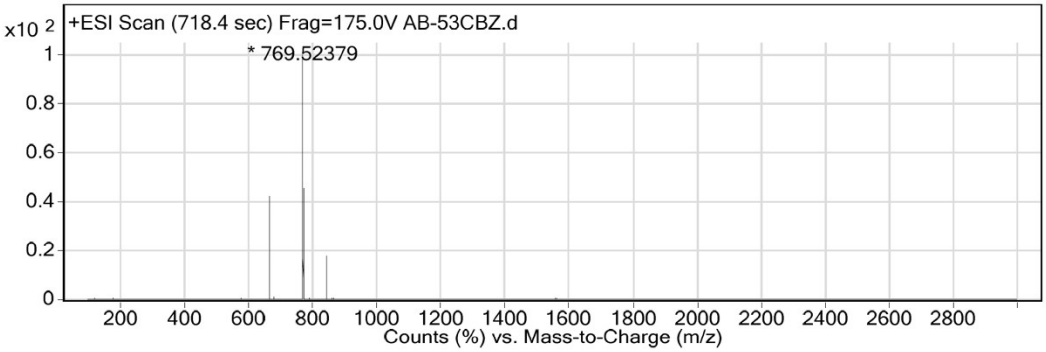
*$^{13}C\{1H\}$-NMR spectrum of **12***



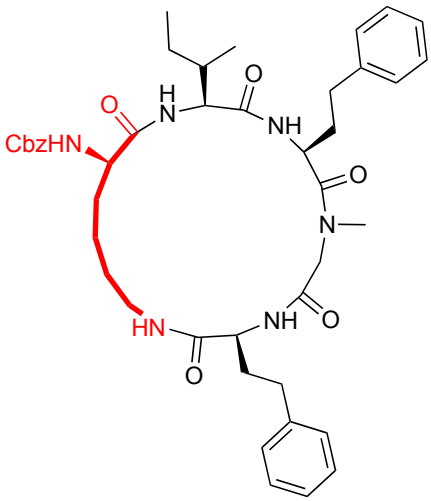
*LC-MS spectrum of **3** (retention time : 709.4 sec)*

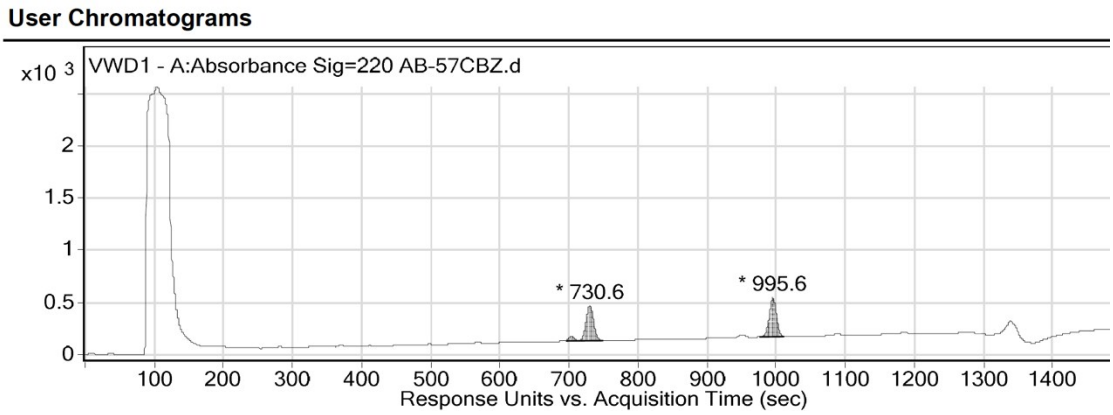


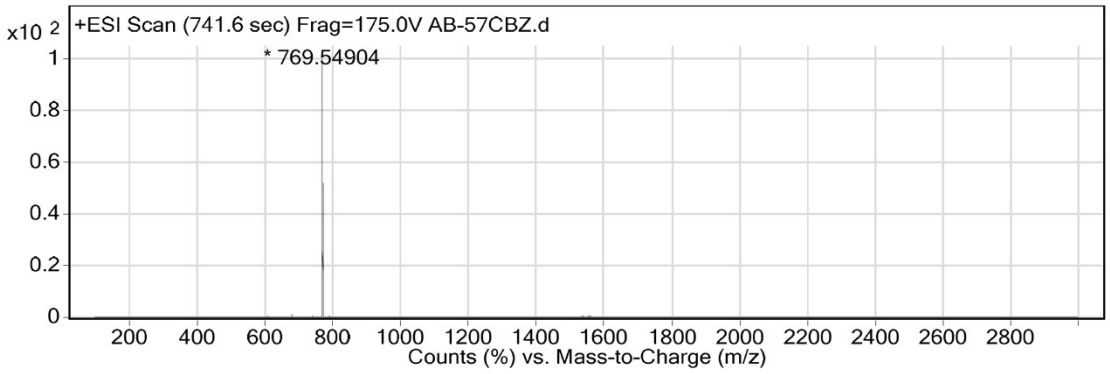




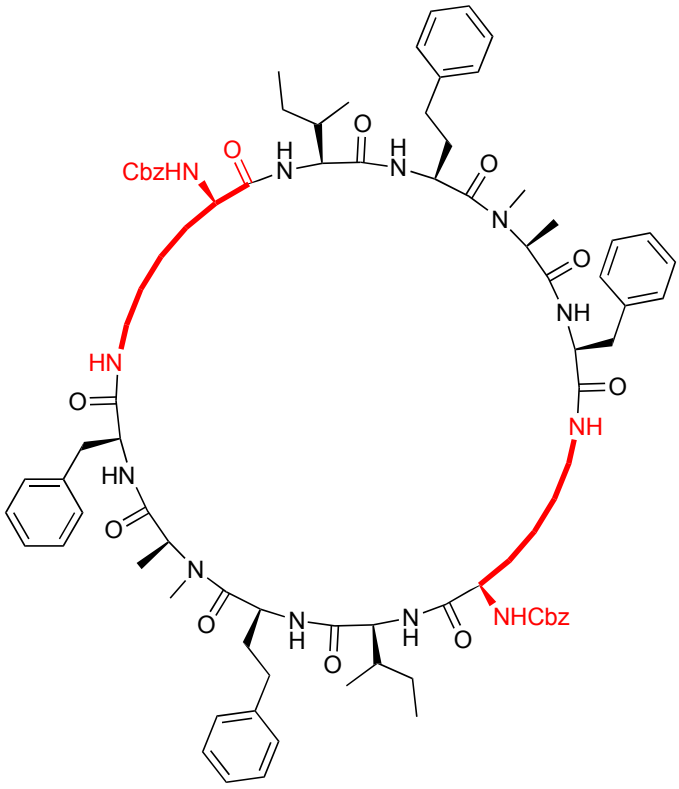
*LC-MS spectrum of **4** (retention time : 730.6 sec)*

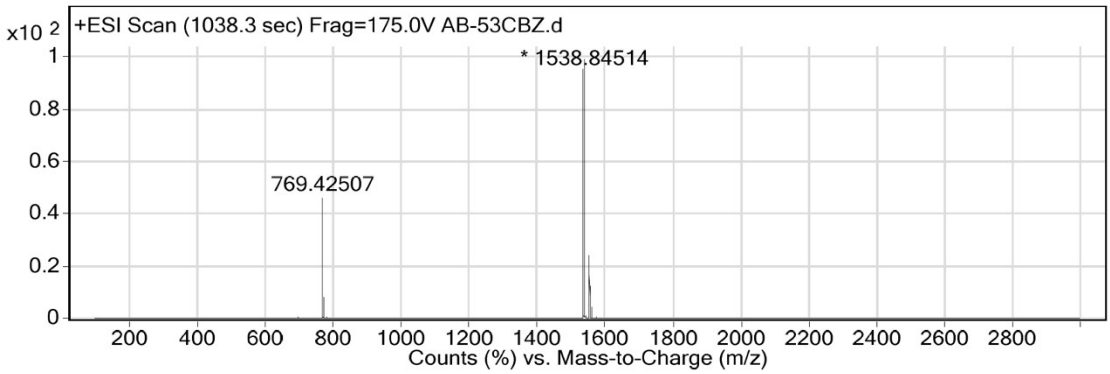




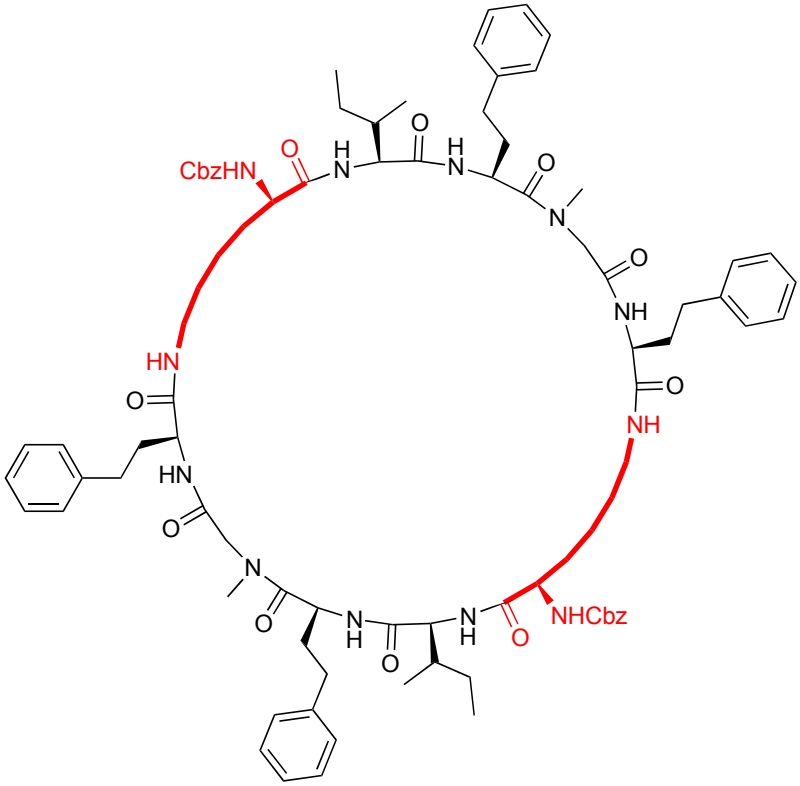


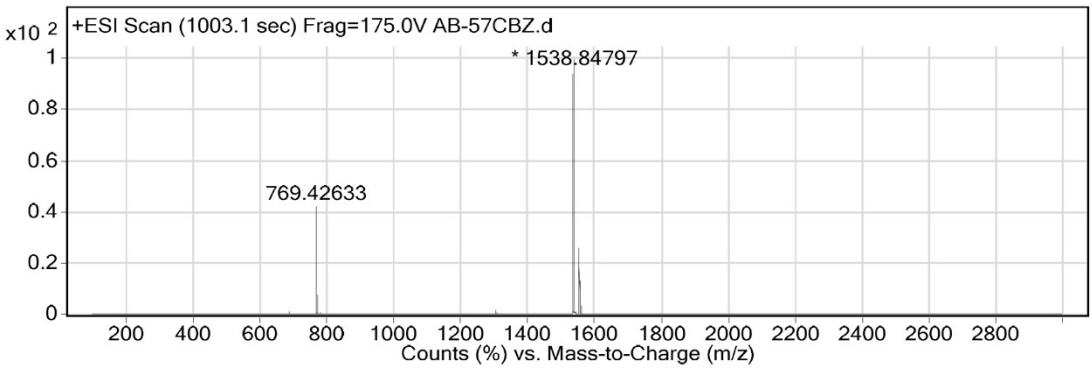
*LC-MS spectrum of **3-dimer** (retention time : 1025.3 sec)*



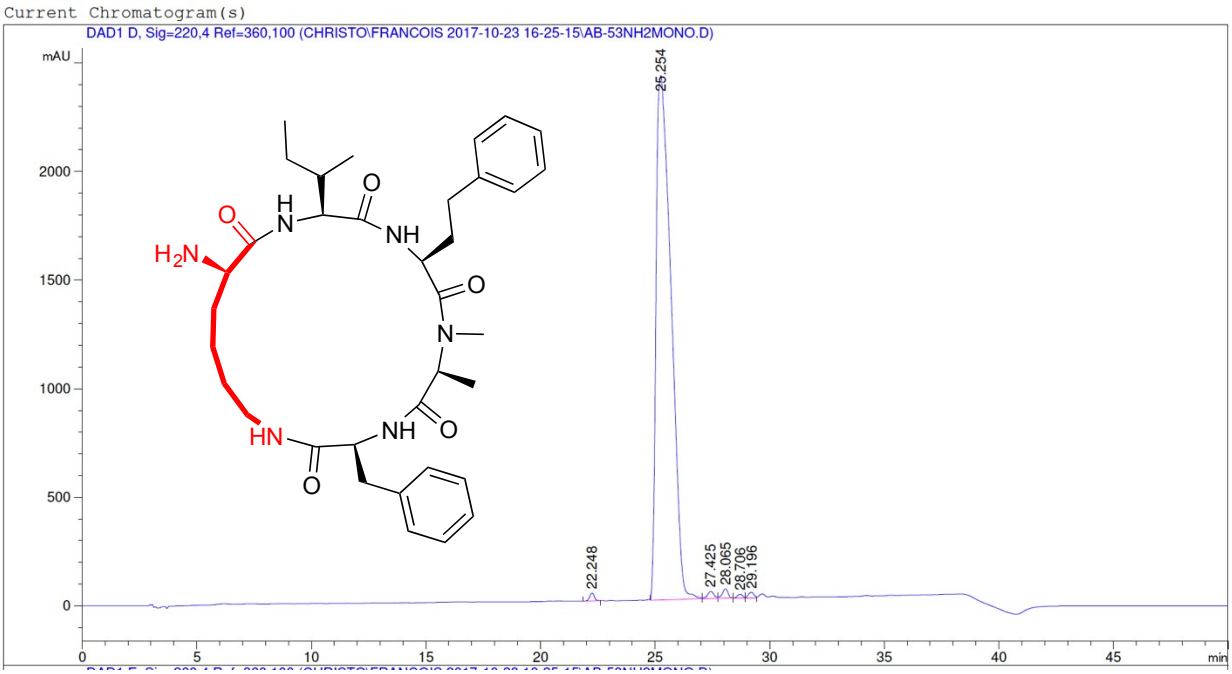


*LC-MS spectrum of **4-dimer** (retention time : 995.6 sec)*



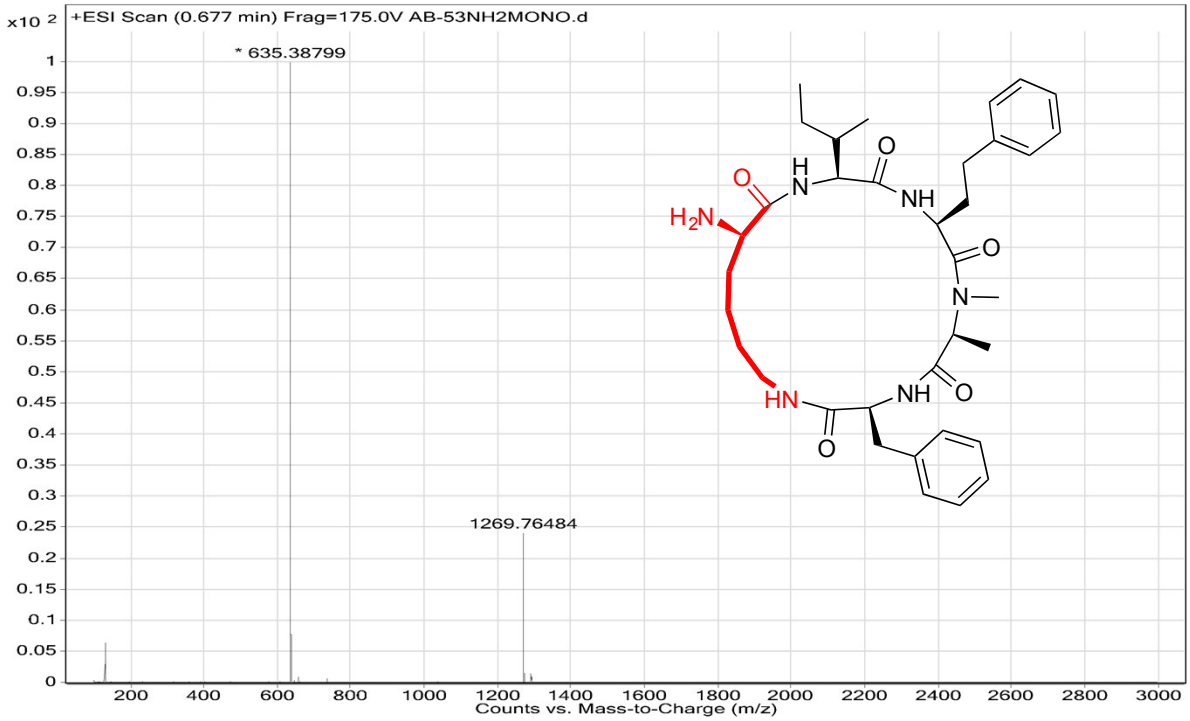


*HPLC spectrum of **5***



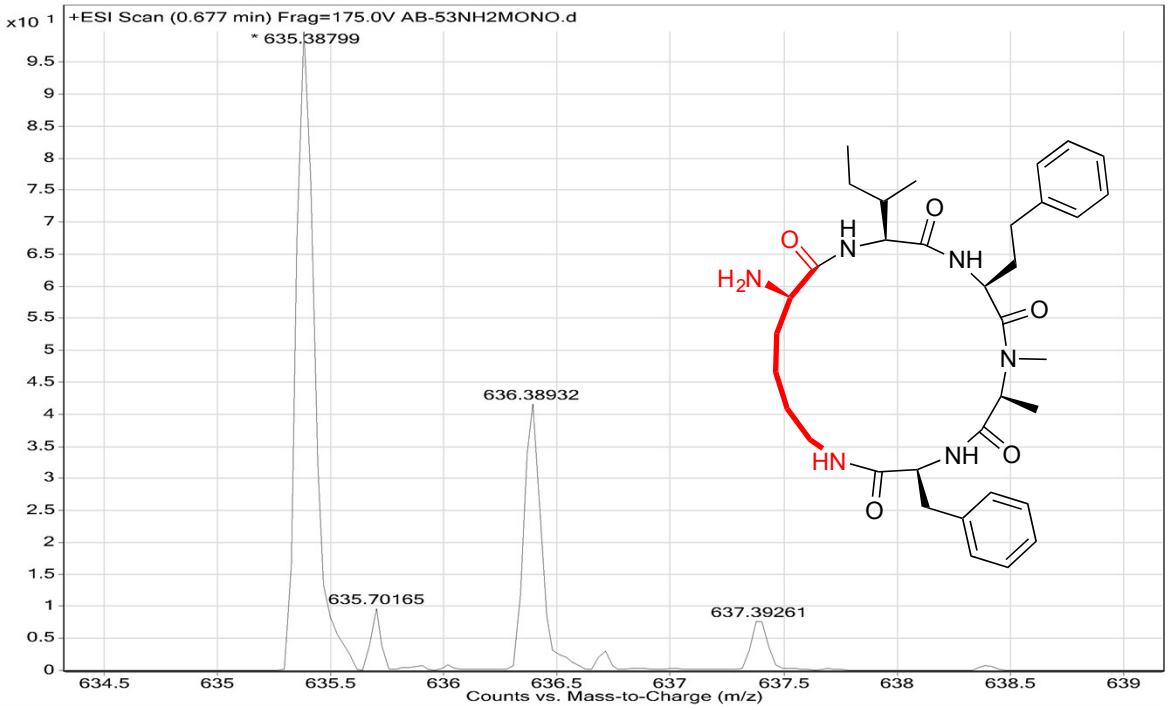
HRMS-TOF spectrum of ***5***

Sample Name	AB-53NH2MONO	Position	Vial 3	Instrument Name	Instrument 1	User Name	Luis MISAL
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-53NH2MONO.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	10/23/2017 3:27:48 PM

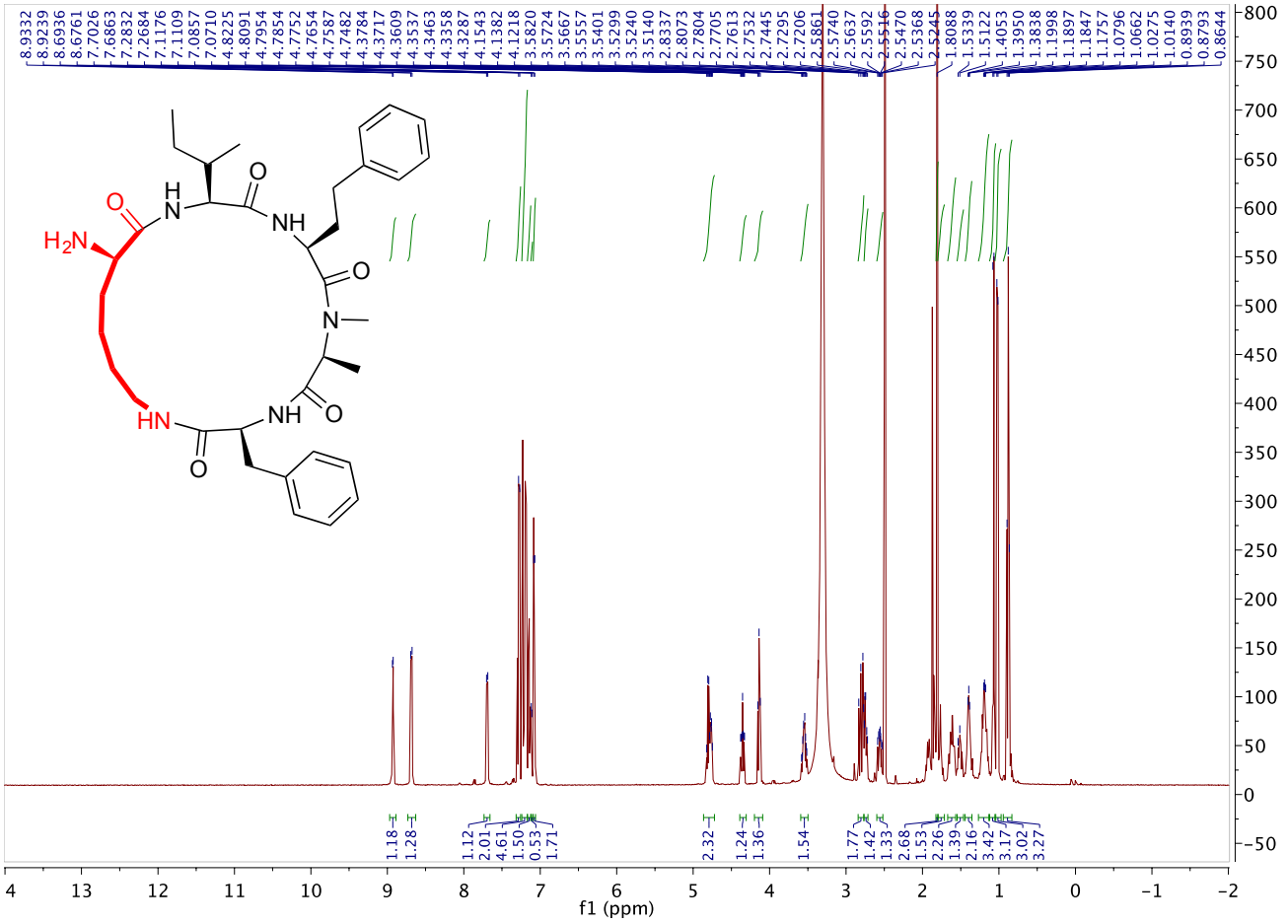


HRMS-TOF zoom spectrum of ***5***

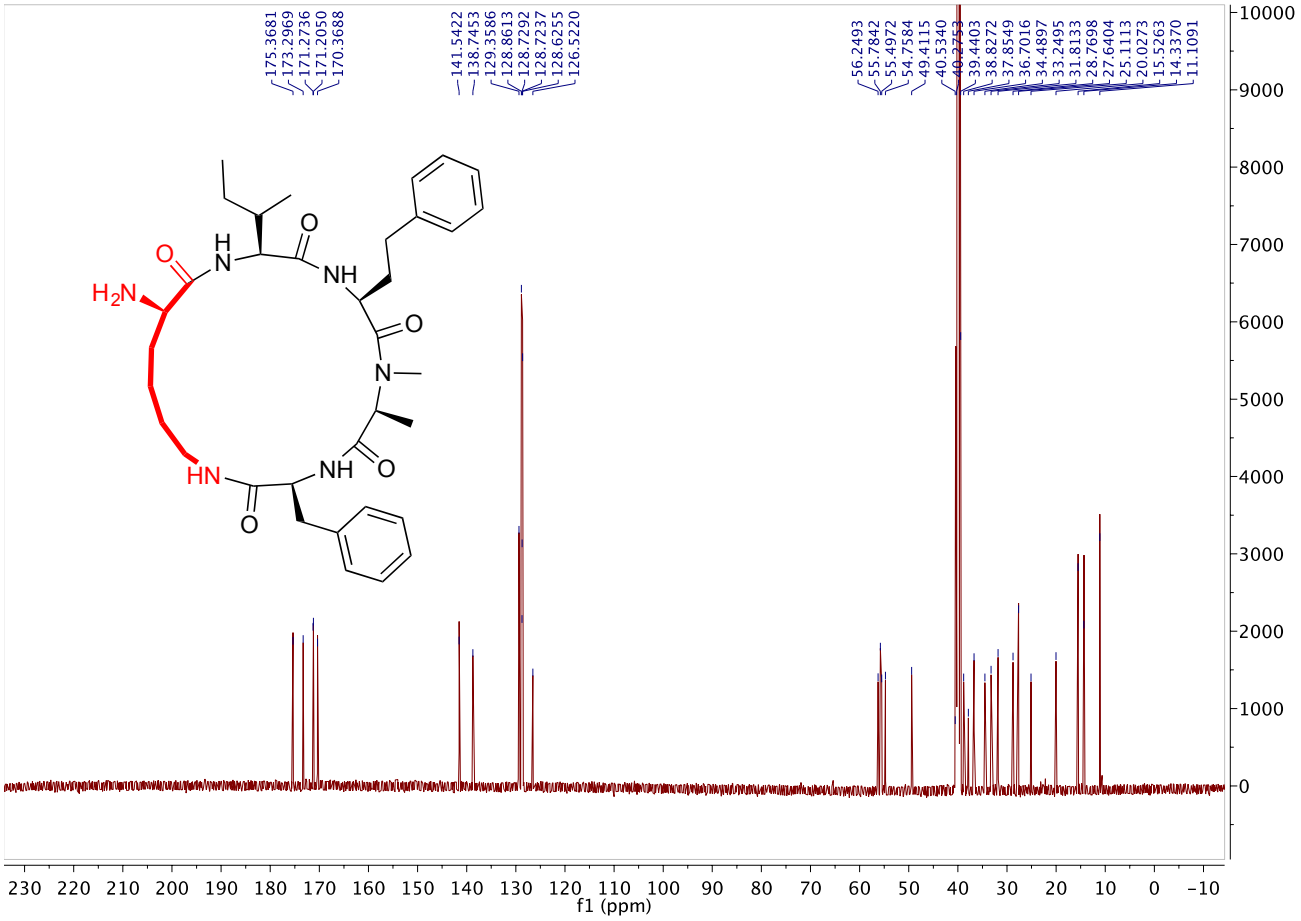
Sample Name	AB-53NH2MONO	Position	Vial 3	Instrument Name	Instrument 1	User Name	Luis MISAL
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-53NH2MONO.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	10/23/2017 3:27:48 PM



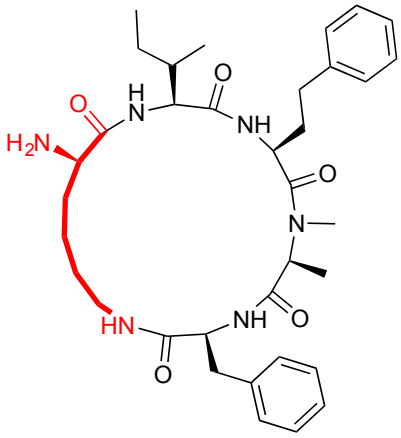
*[1]H-NMR spectrum of **5** in DMSO-d_6*

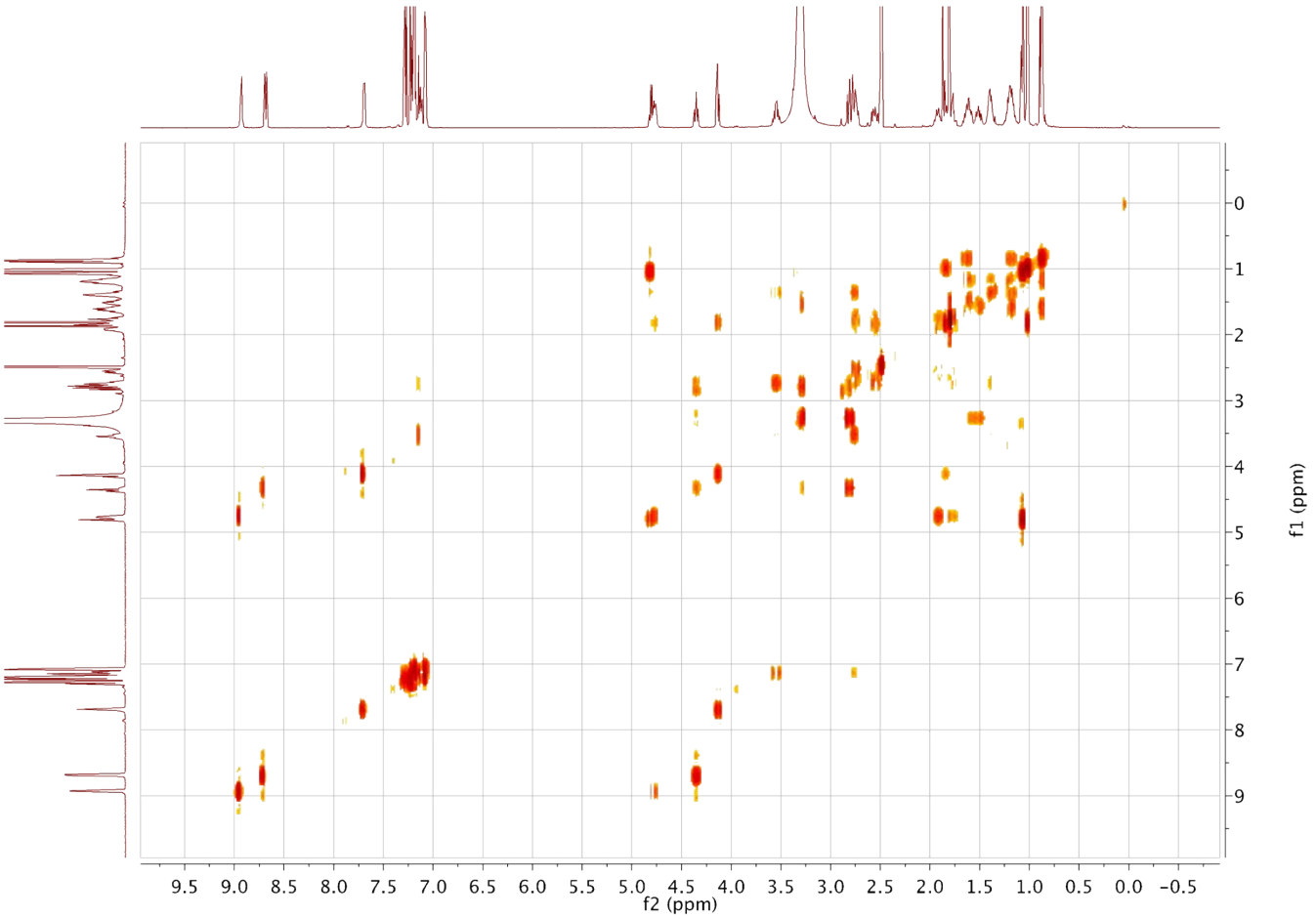


*$^{13}C\{1H\}$-NMR spectrum of **5** in DMSO-d_6*

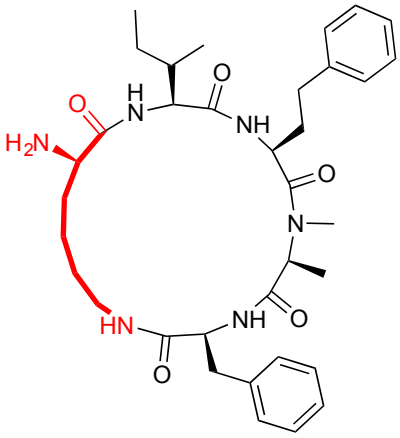


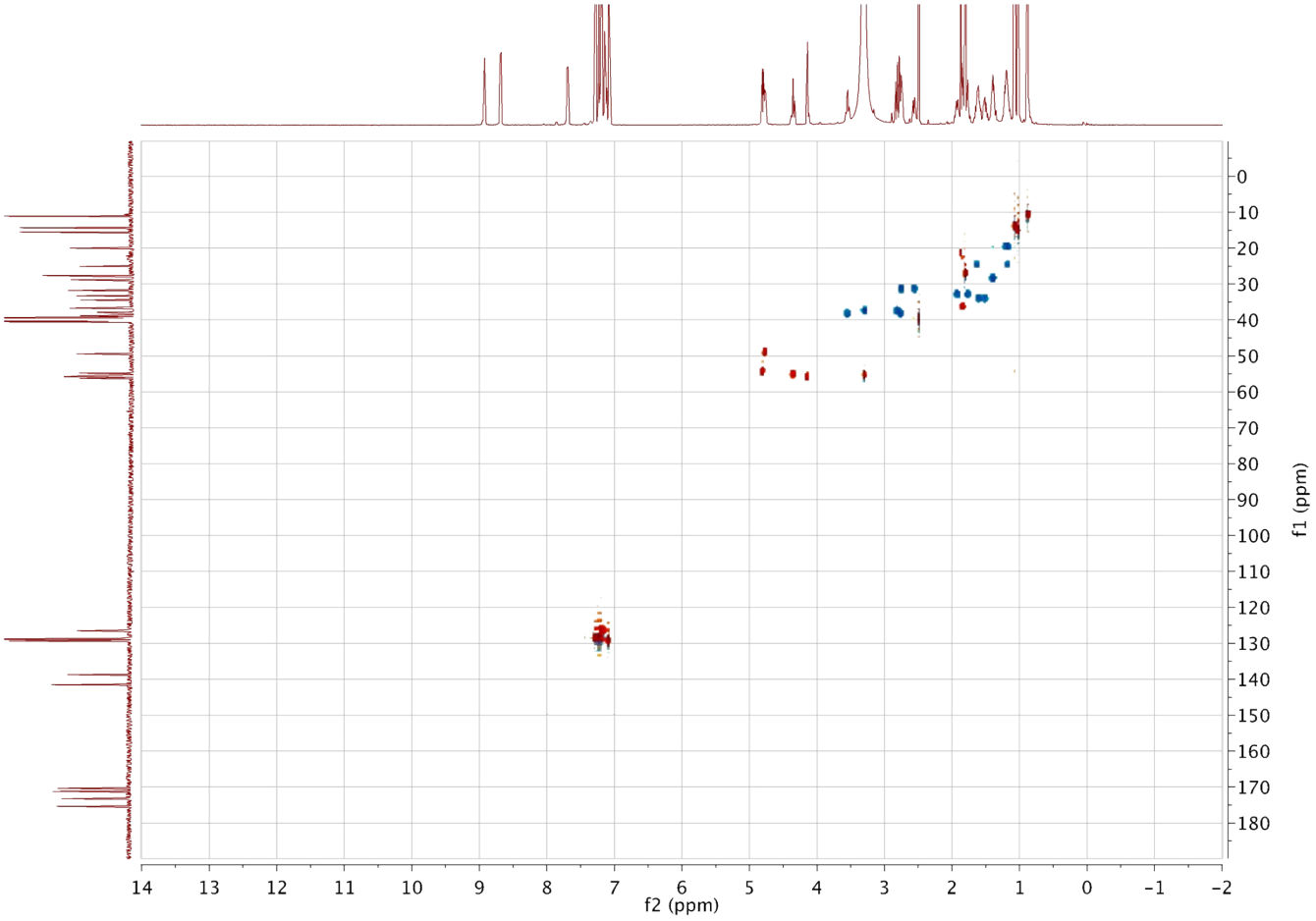
*^{1}H-^{1}H COSY spectrum of A **5** in DMSO-d_6*



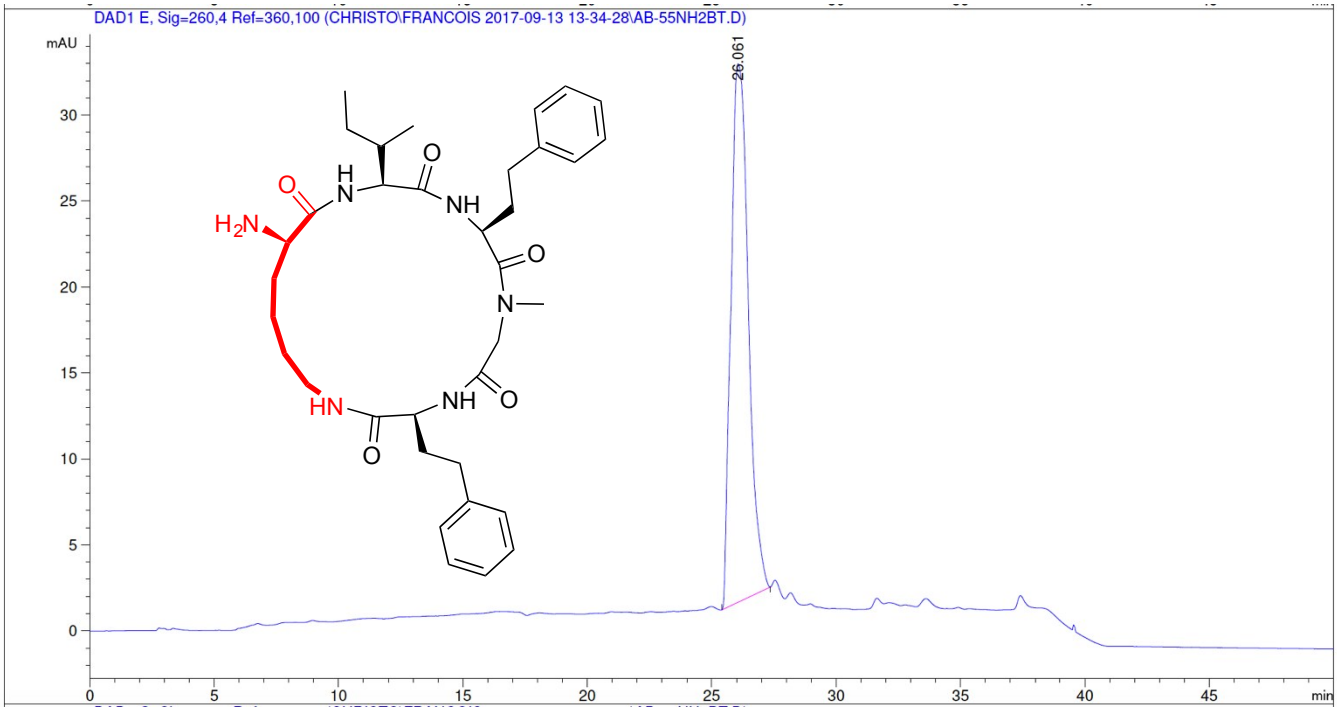


HSQC spectrum of A ***5*** *in DMSO-d_6*



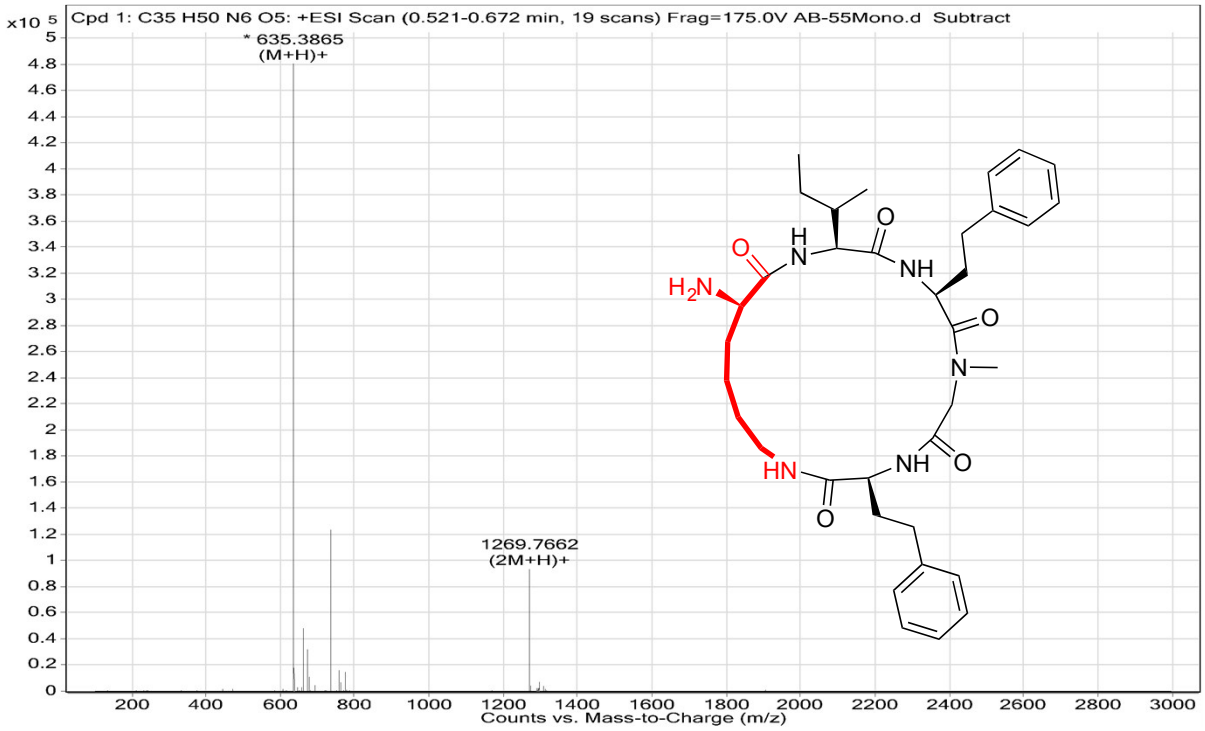


HPLC spectrum of ***6***



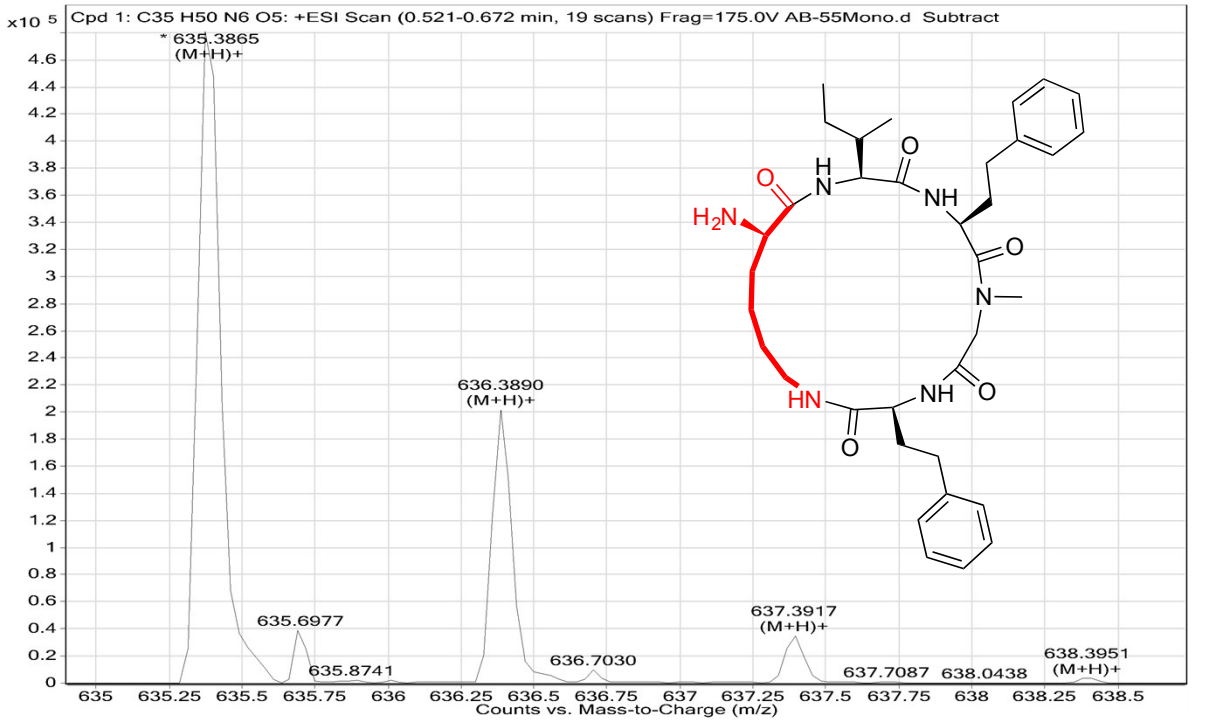
HRMS-TOF spectrum of ***6***

Sample Name	AB-55Mono	Position	Vial 3	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	2	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-55Mono.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	4/23/2018 8:09:19 PM

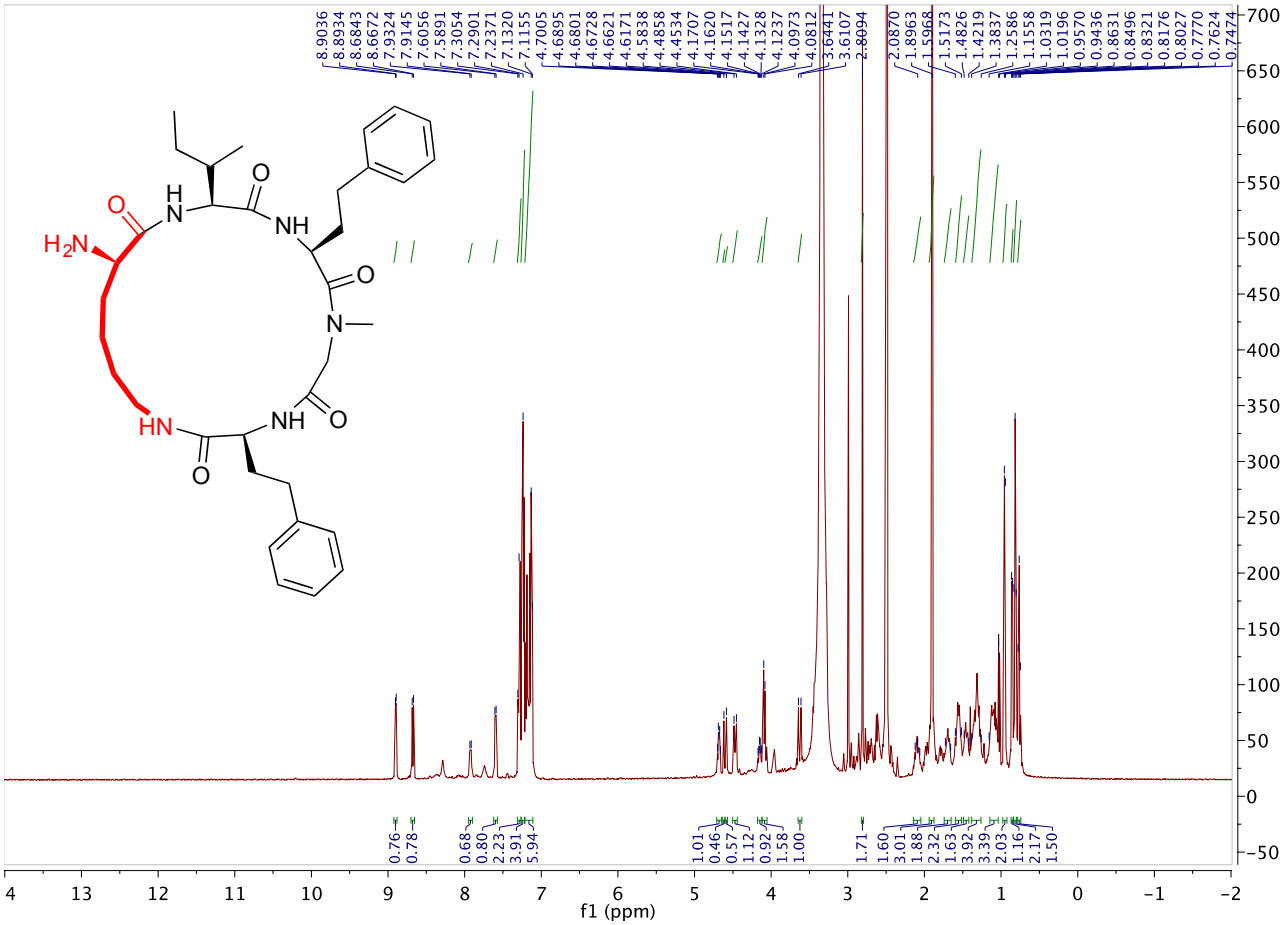


HRMS-TOF zoom spectrum of ***6***

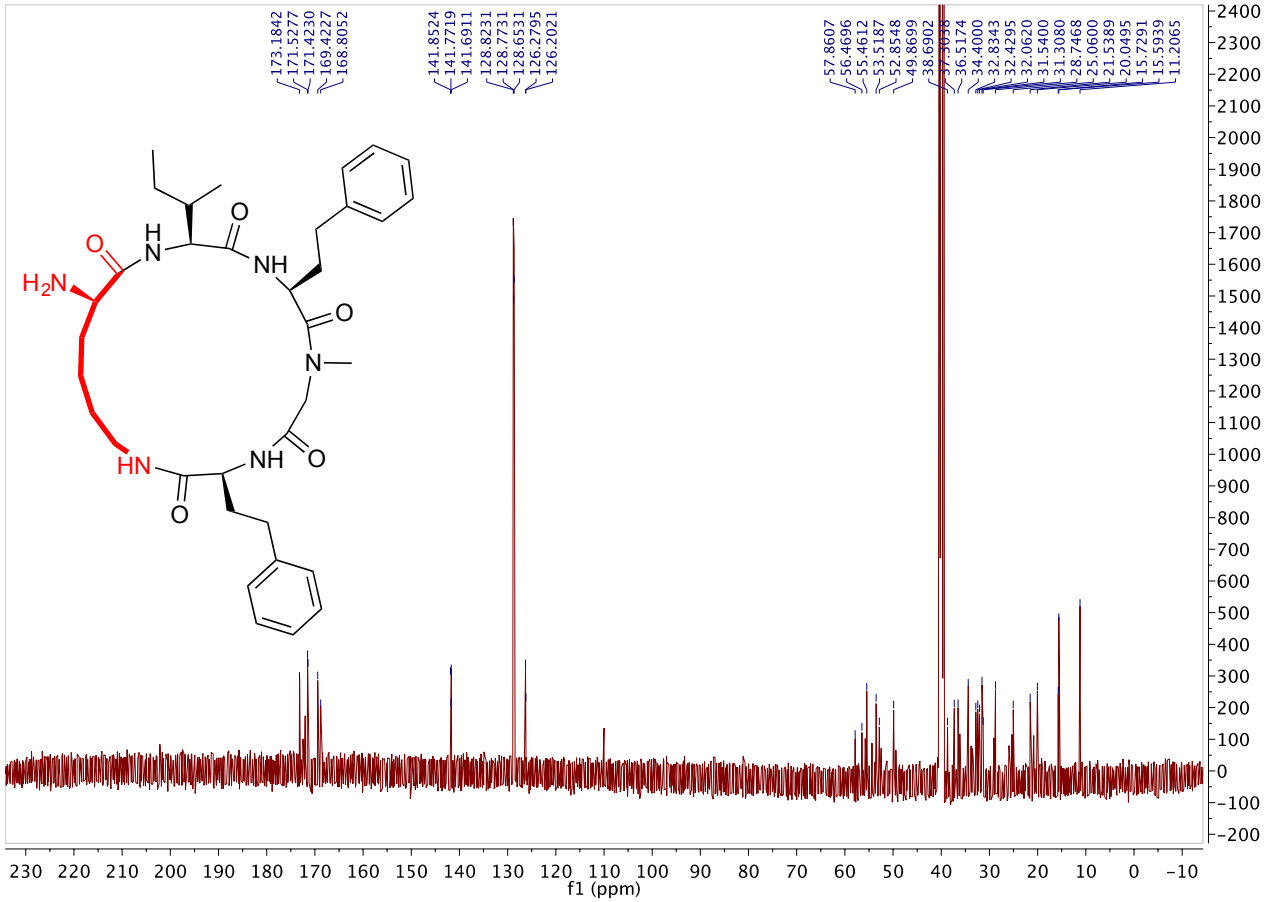
Sample Name	AB-55Mono	Position	Vial 3	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	2	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-55Mono.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	4/23/2018 8:09:19 PM



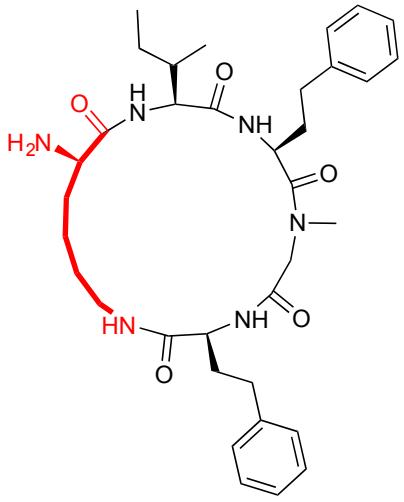
*[1]H-NMR spectrum of **6** in DMSO-d_6*

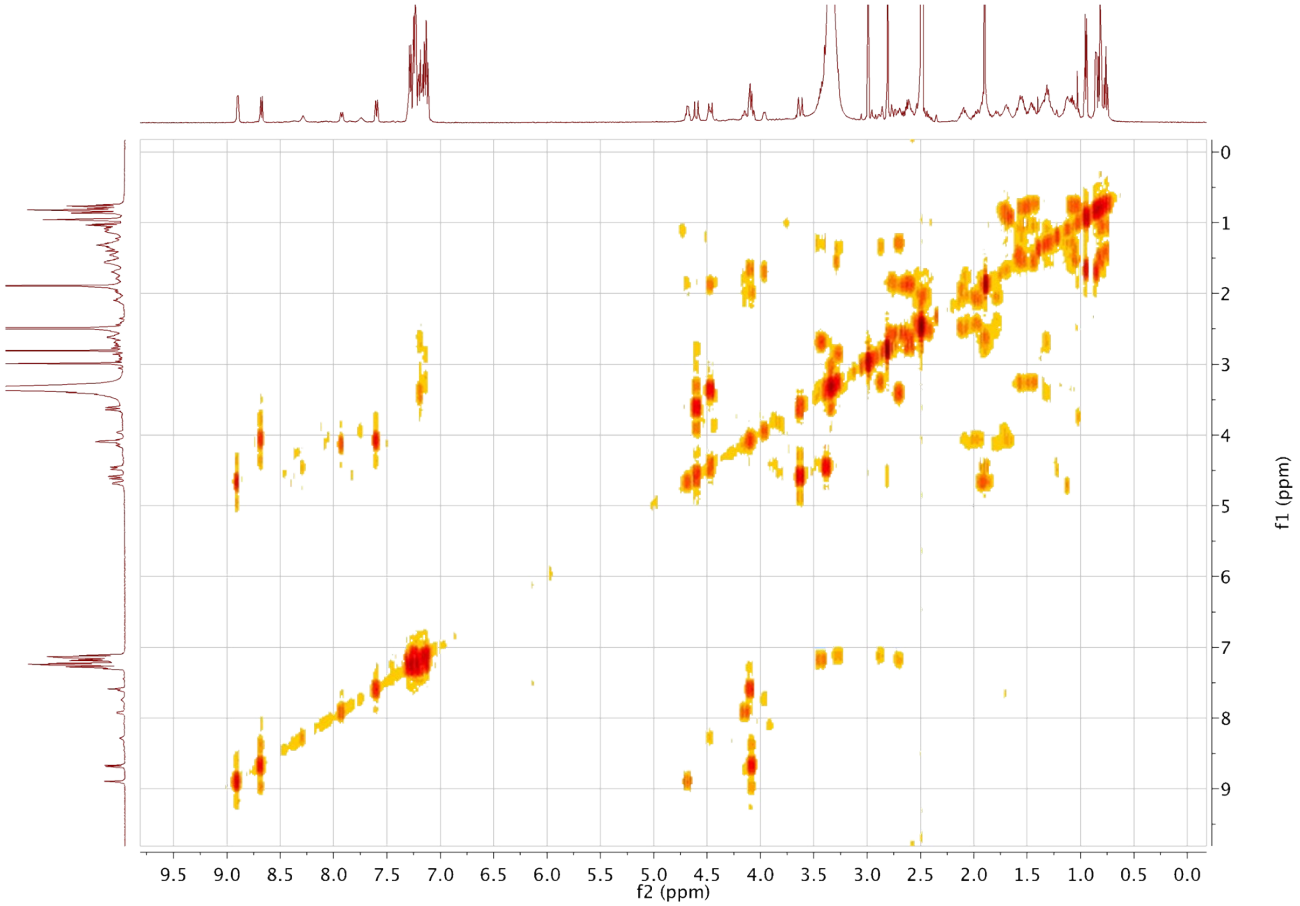


*$^{13}C\{1H\}$-NMR spectrum of **6** in DMSO-d_6*

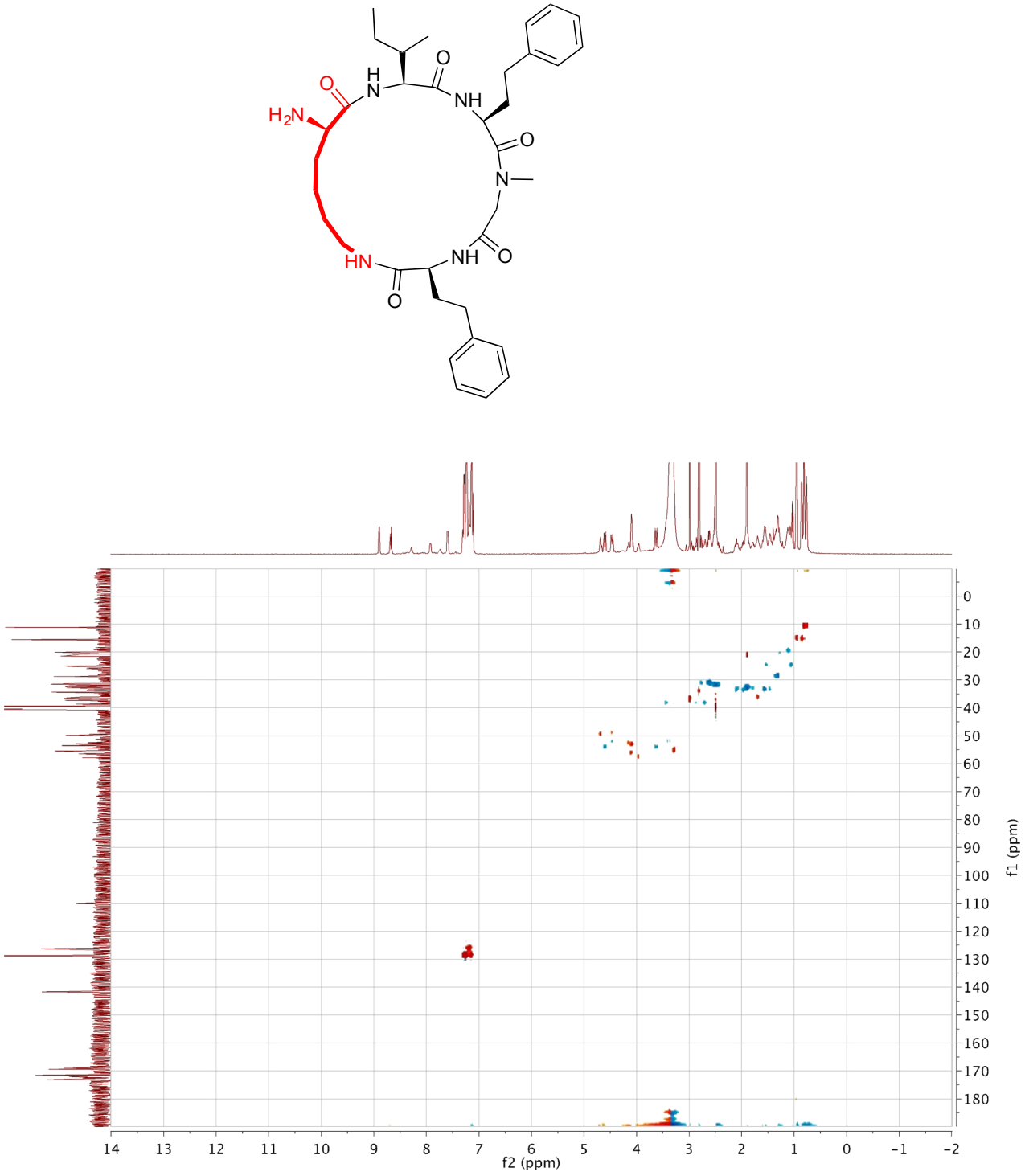


*^{1}H-^{1}H COSY spectrum of **6** in DMSO-d_6*



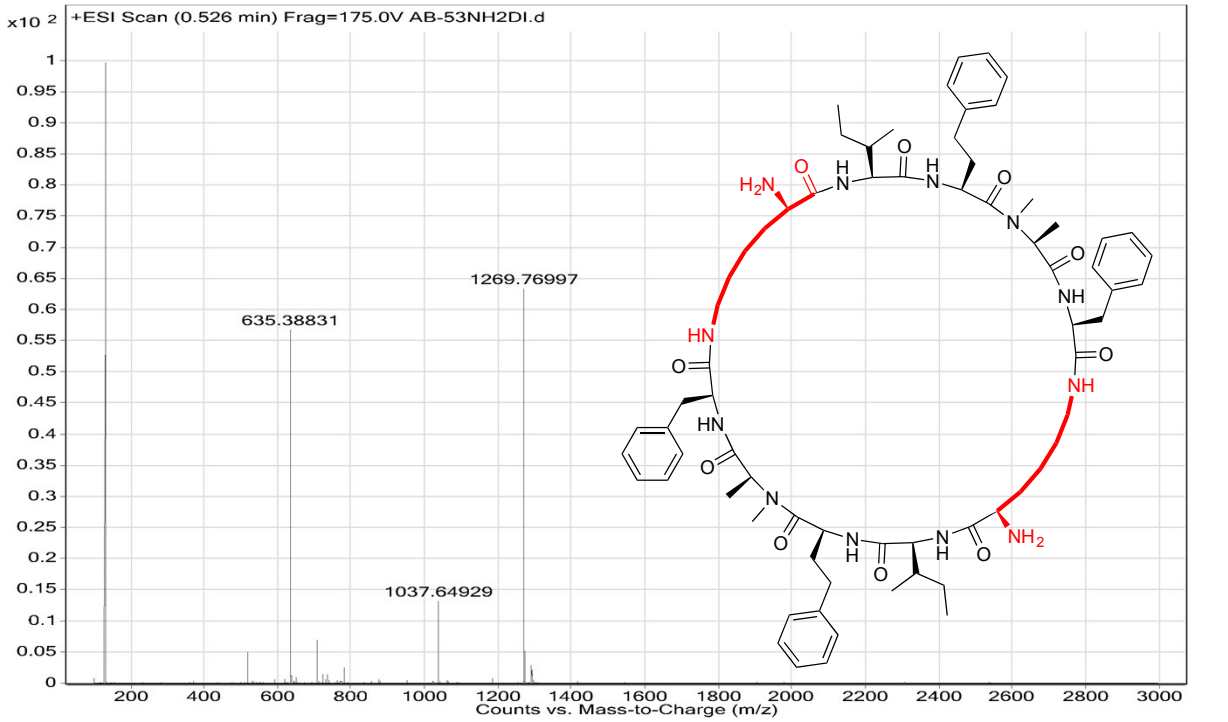


HSQC spectrum of A ***6*** *in DMSO-d_6*



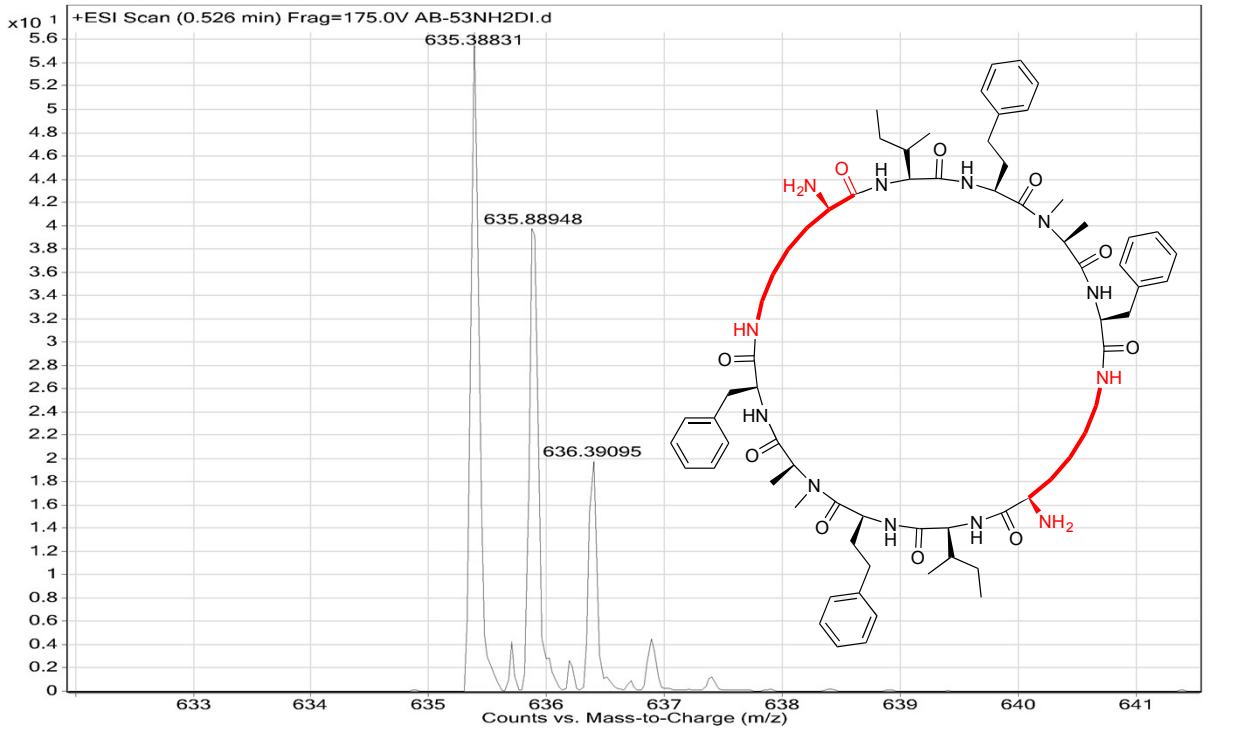
HRMS-TOF spectrum of 7

Sample Name	AB-53NH2DI	Position	Vial 4	Instrument Name	Instrument 1	User Name	Luis MISAL
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-53NH2DI.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	10/23/2017 3:16:05 PM



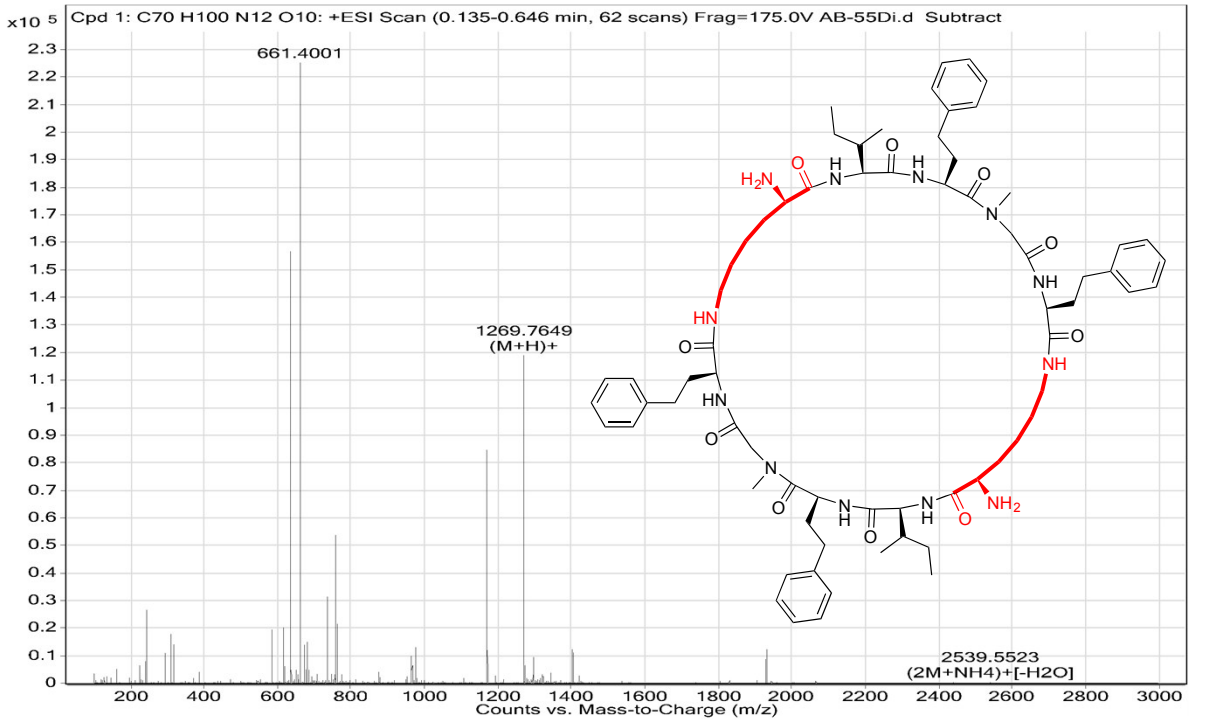
HRMS-TOF spectrum of 7 zoom

Sample Name	AB-53NH2DI	Position	Vial 4	Instrument Name	Instrument 1	User Name	Luis MISAL
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-53NH2DI.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	10/23/2017 3:16:05 PM



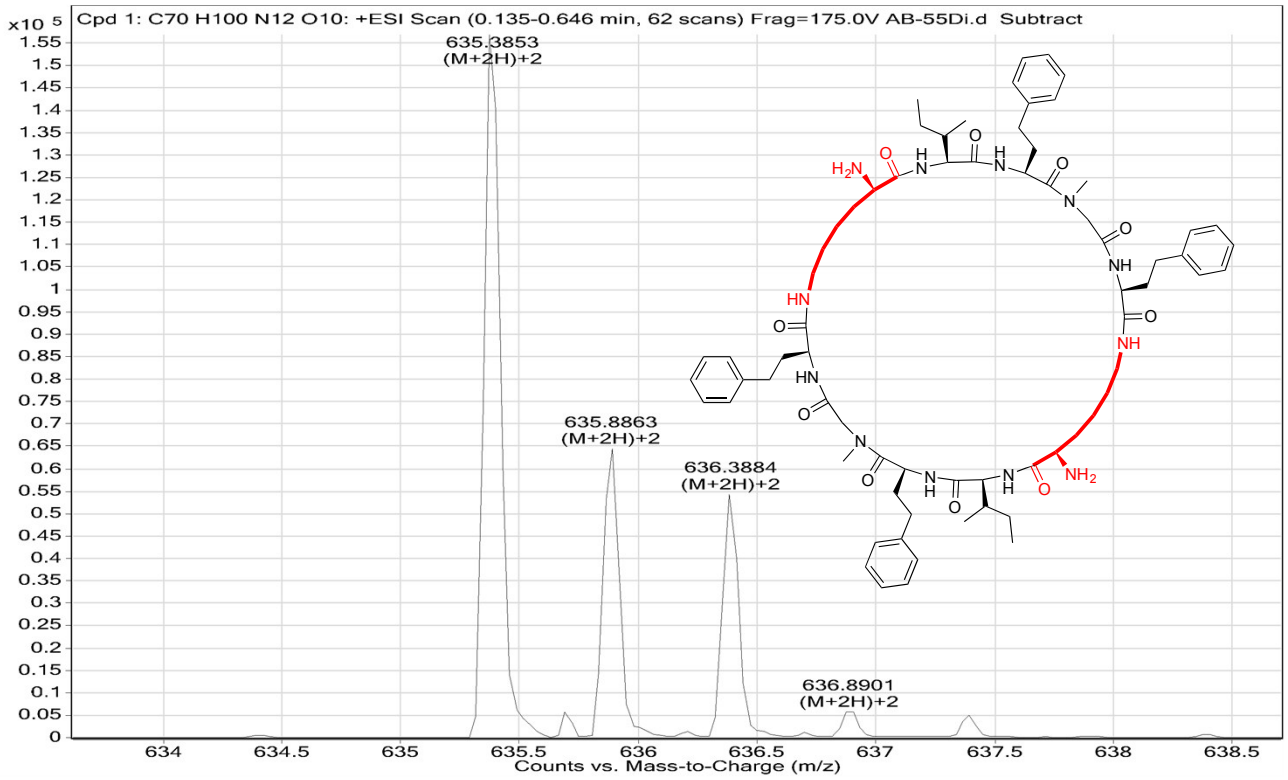
HRMS-TOF spectrum of ***8***

Sample Name	AB-55Di	Position	Vial 4	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	2	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-55Di.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	4/23/2018 8:12:21 PM



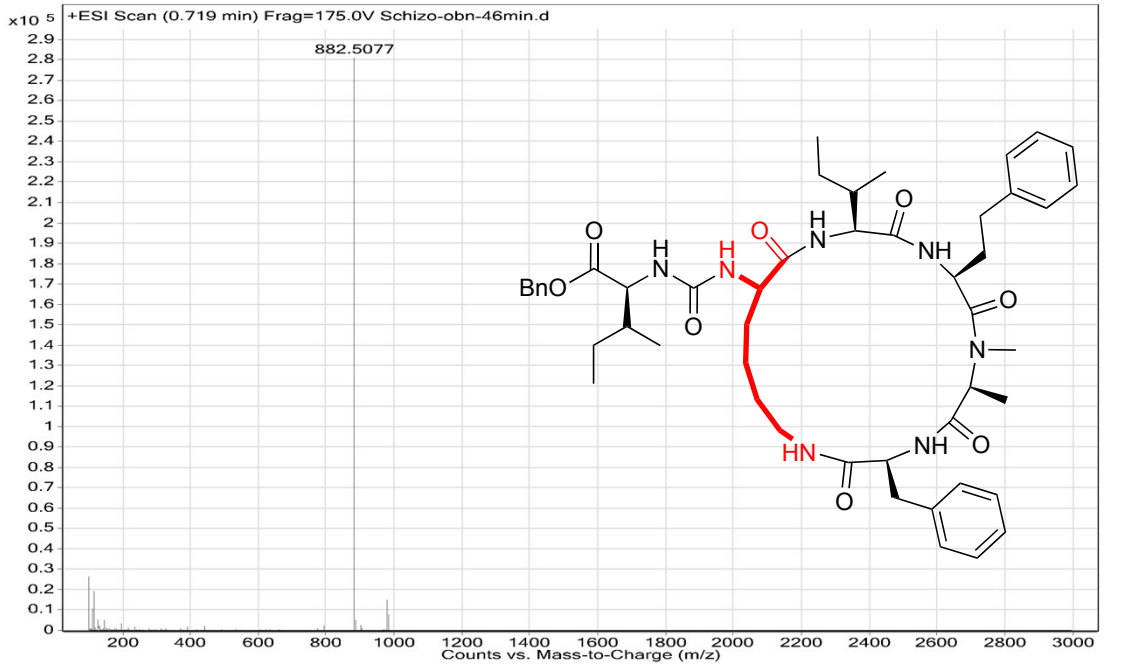
*HRMS-TOF spectrum of **8** zoom*

Sample Name	AB-55Di	Position	Vial 4	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	2	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AB-55Di.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	4/23/2018 8:12:21 PM

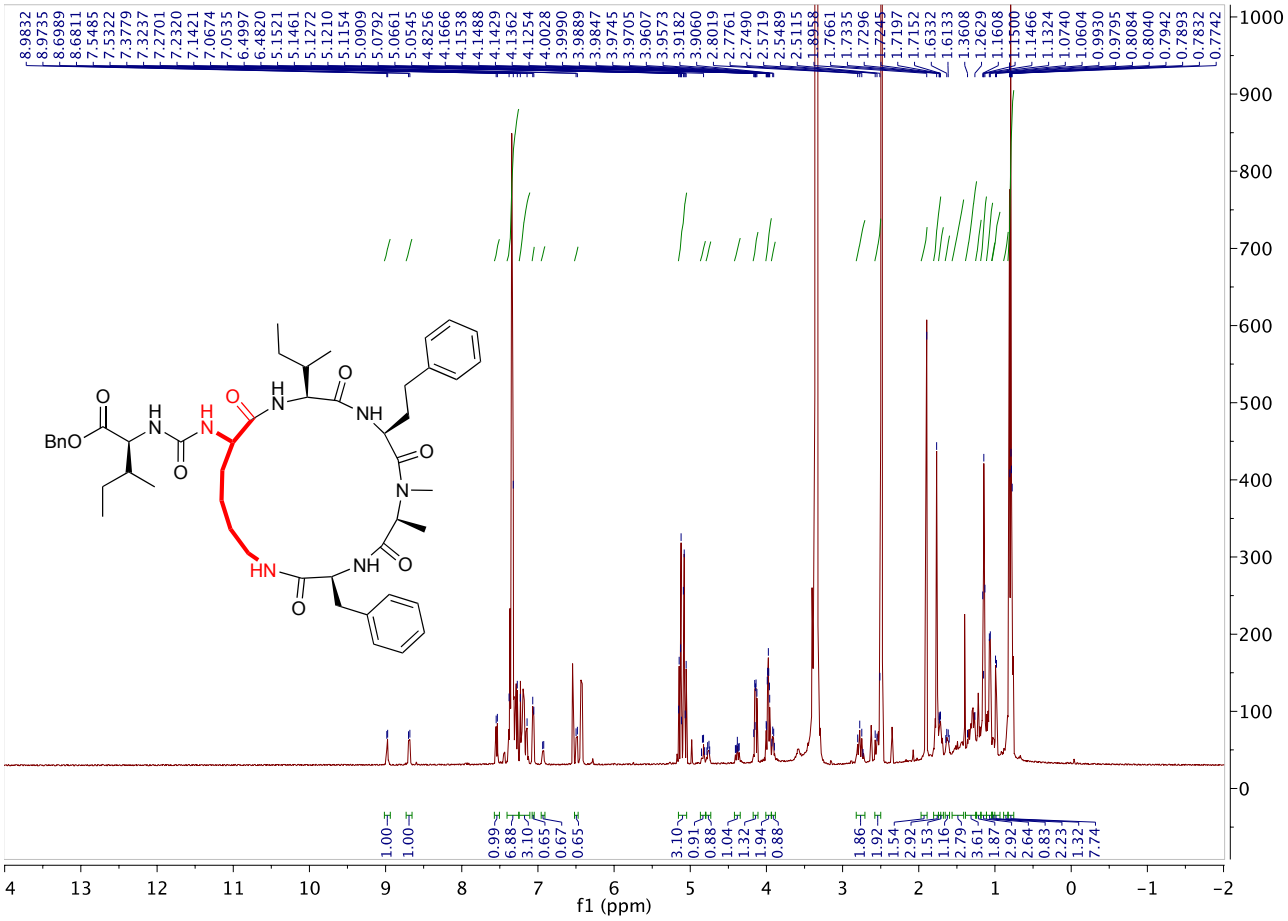


HRMS-TOF spectrum of ***13***

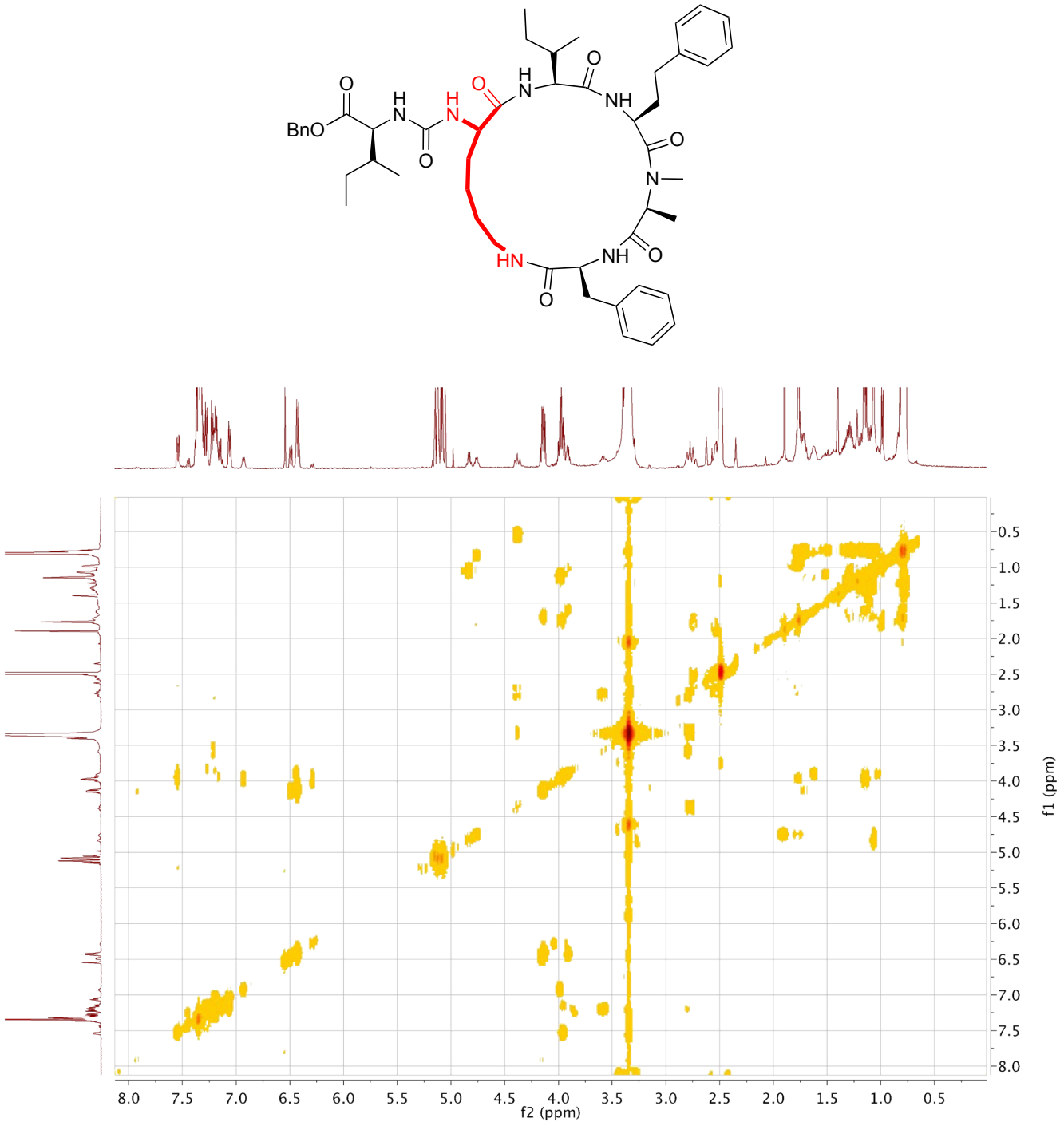
Sample Name	Schizo-obn-46min	Position	Vial 11	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	Schizo-obn-46min.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	3/14/2018 5:15:01 PM



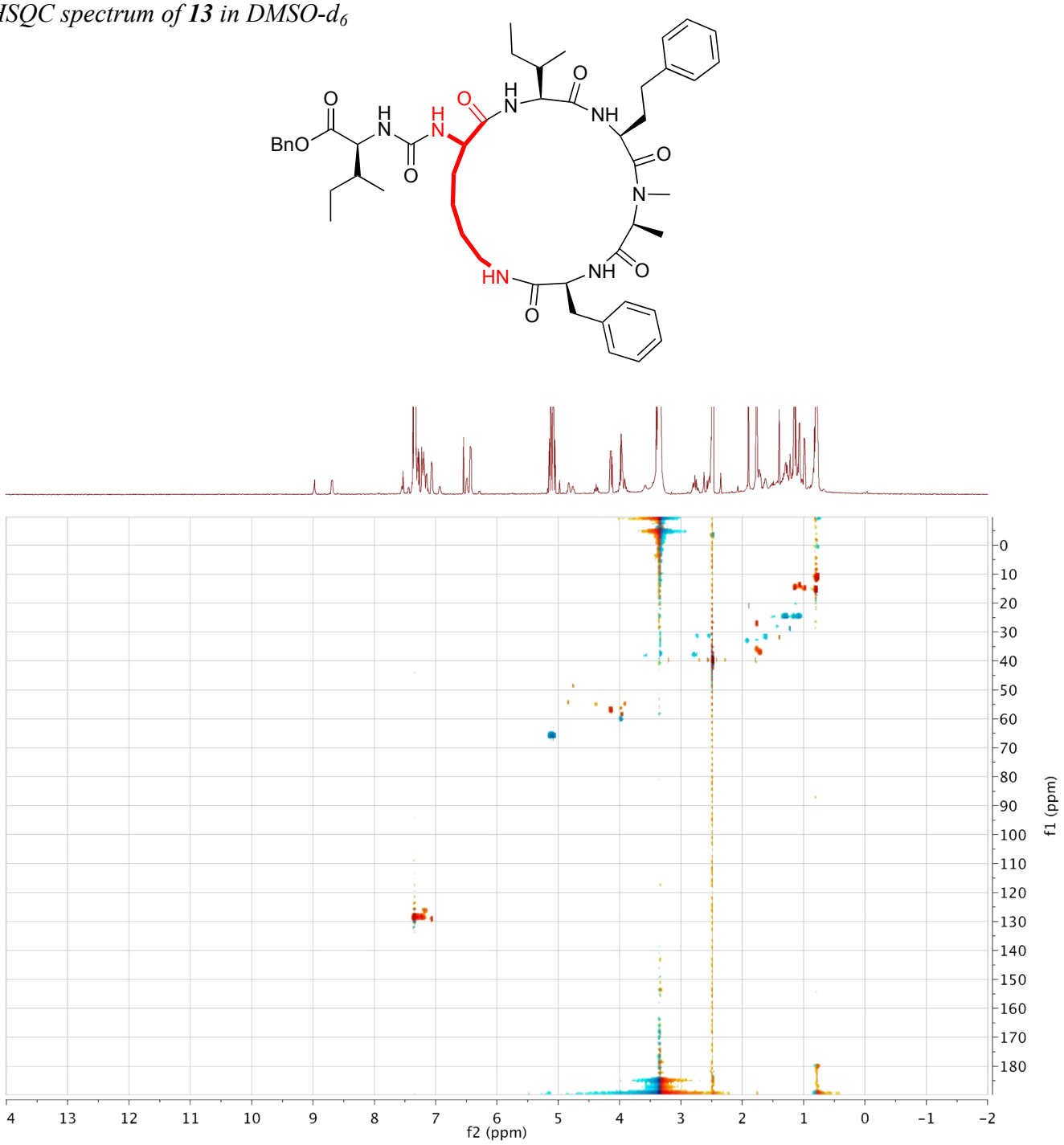
*[1]H-NMR spectrum of **13** in DMSO-d_6*



*^{1}H-^{1}H COSY spectrum of **13** in DMSO-d_6*

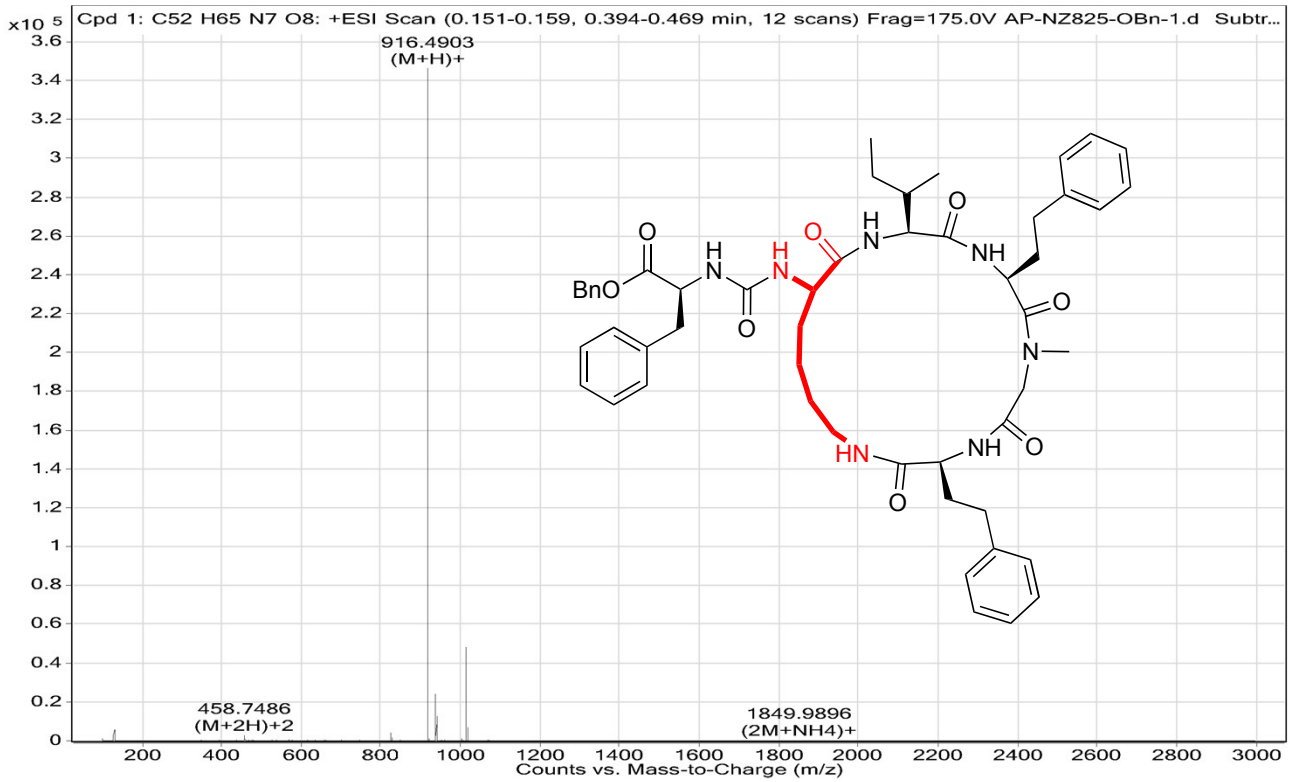


*HSQC spectrum of **13** in DMSO-d_6*

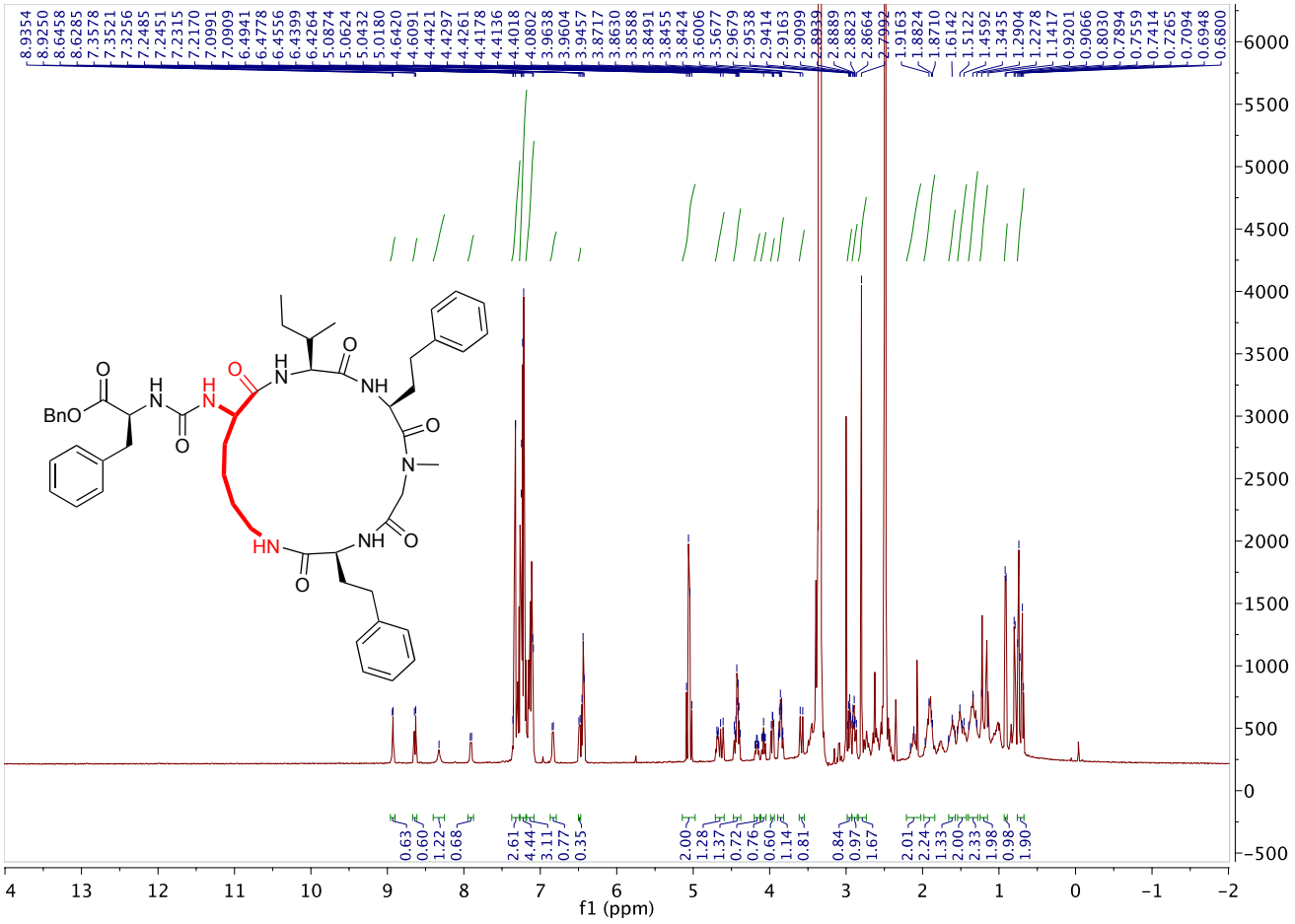


HRMS-TOF spectrum of ***14***

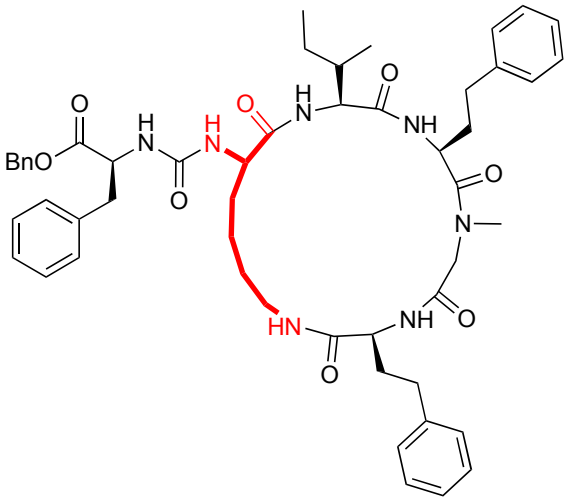
Sample Name	AP-NZ825-OBn-1	Position	Vial 11	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	5	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AP-NZ825-OBn-1.d	ACQ Method	DIESIchrist-3000.m	Comment		Acquired Time	5/2/2018 12:02:26 PM



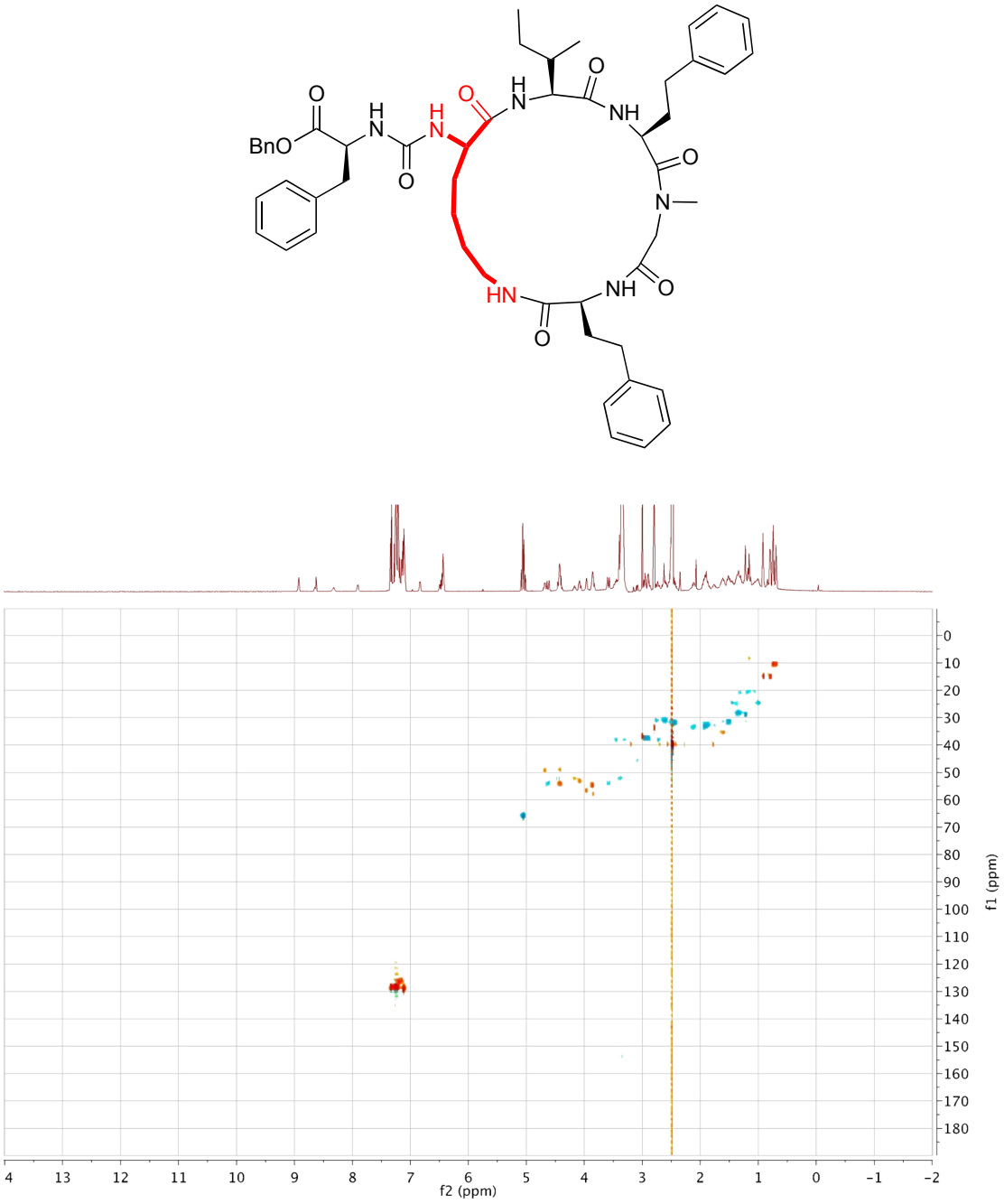
*[1]H-NMR spectrum of **14** in DMSO-d_6*



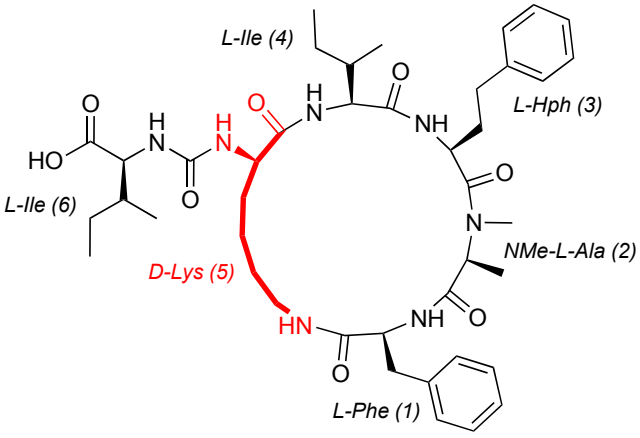
*^{1}H-^{1}H COSY spectrum of **14** in DMSO-d_6*

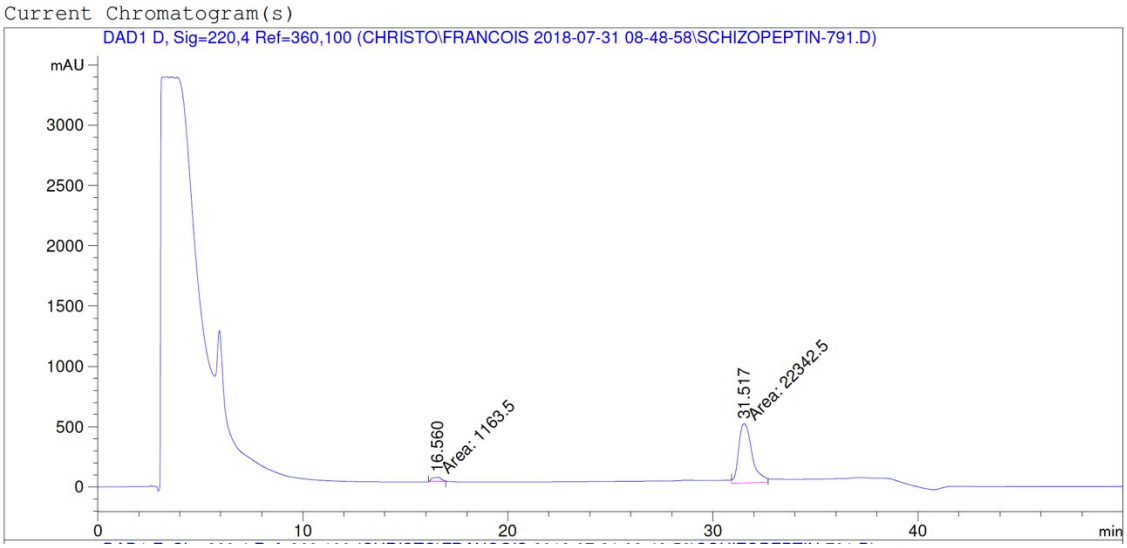


*HSQC spectrum of **14** in DMSO-d_6*



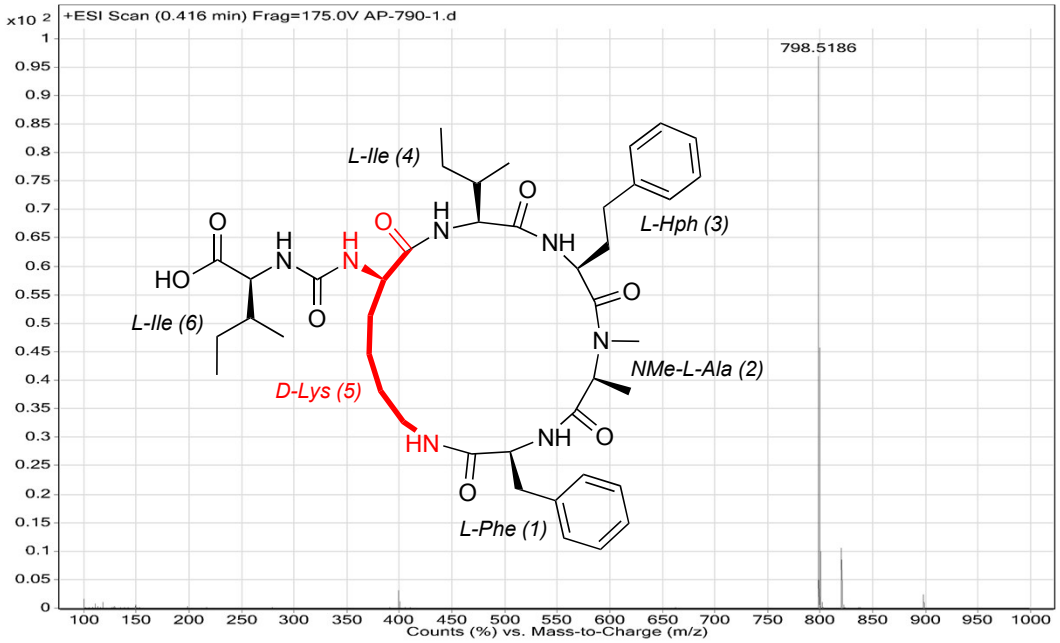
HPLC spectrum of ***1***



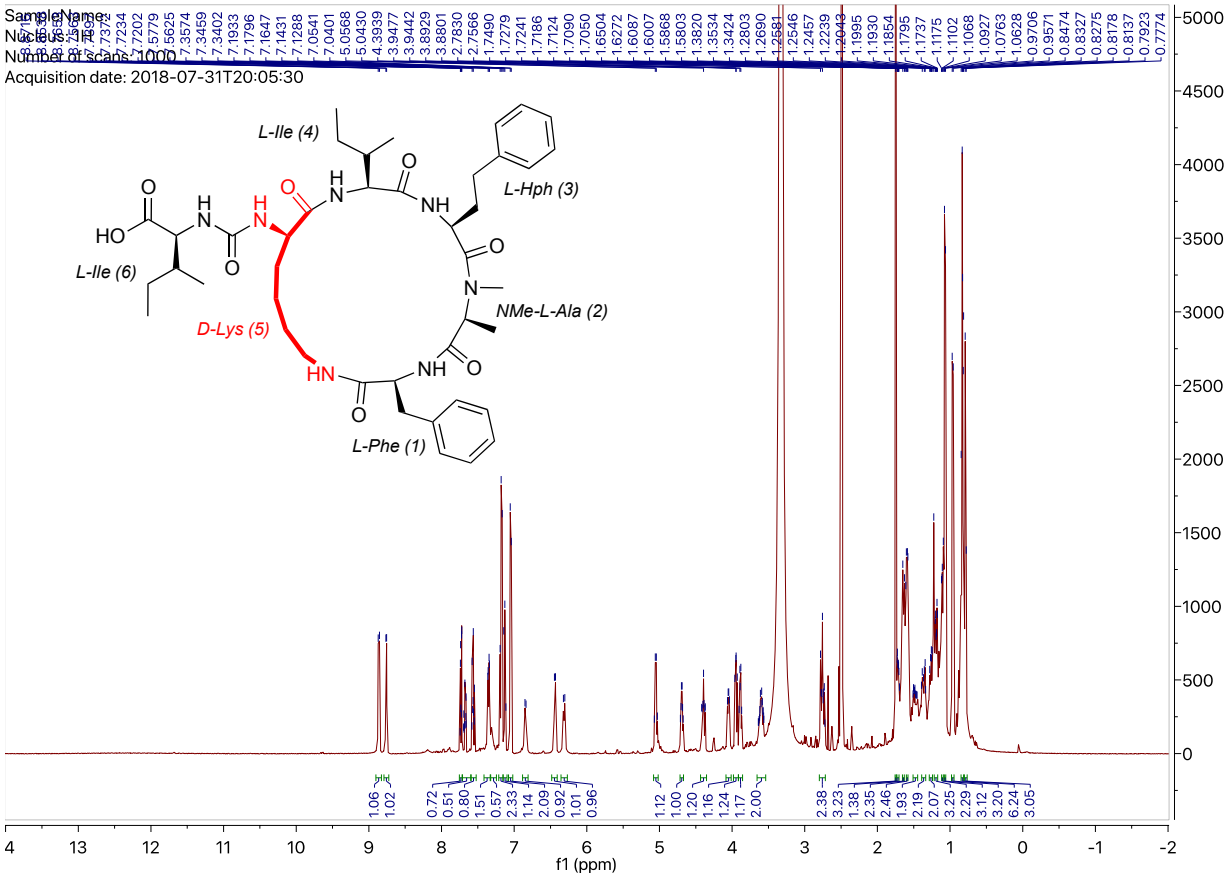


*HRMS-TOF spectrum of **1***

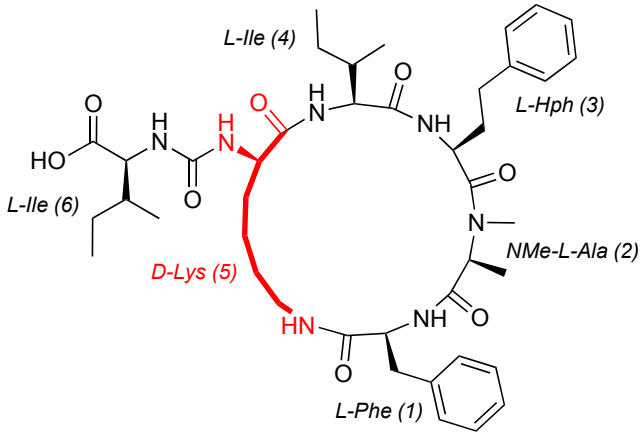
Sample Name	AP-790-1	Position	Vial 11	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	AP-790-1.d	ACQ Method	Direct_Injection_ESI	Comment		Acquired Time	7/30/2018 10:43:19 AM

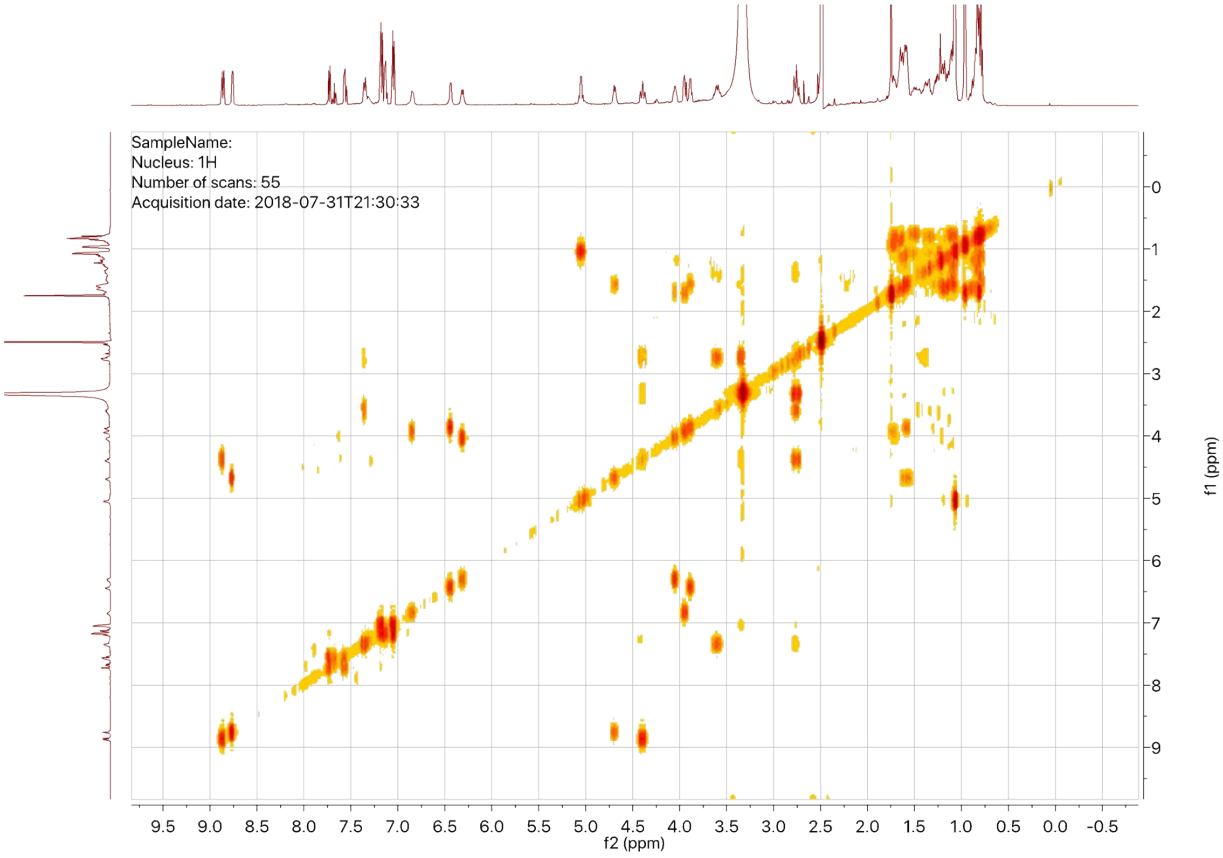


*[1]H-NMR spectrum of **1** in DMSO-d_6*

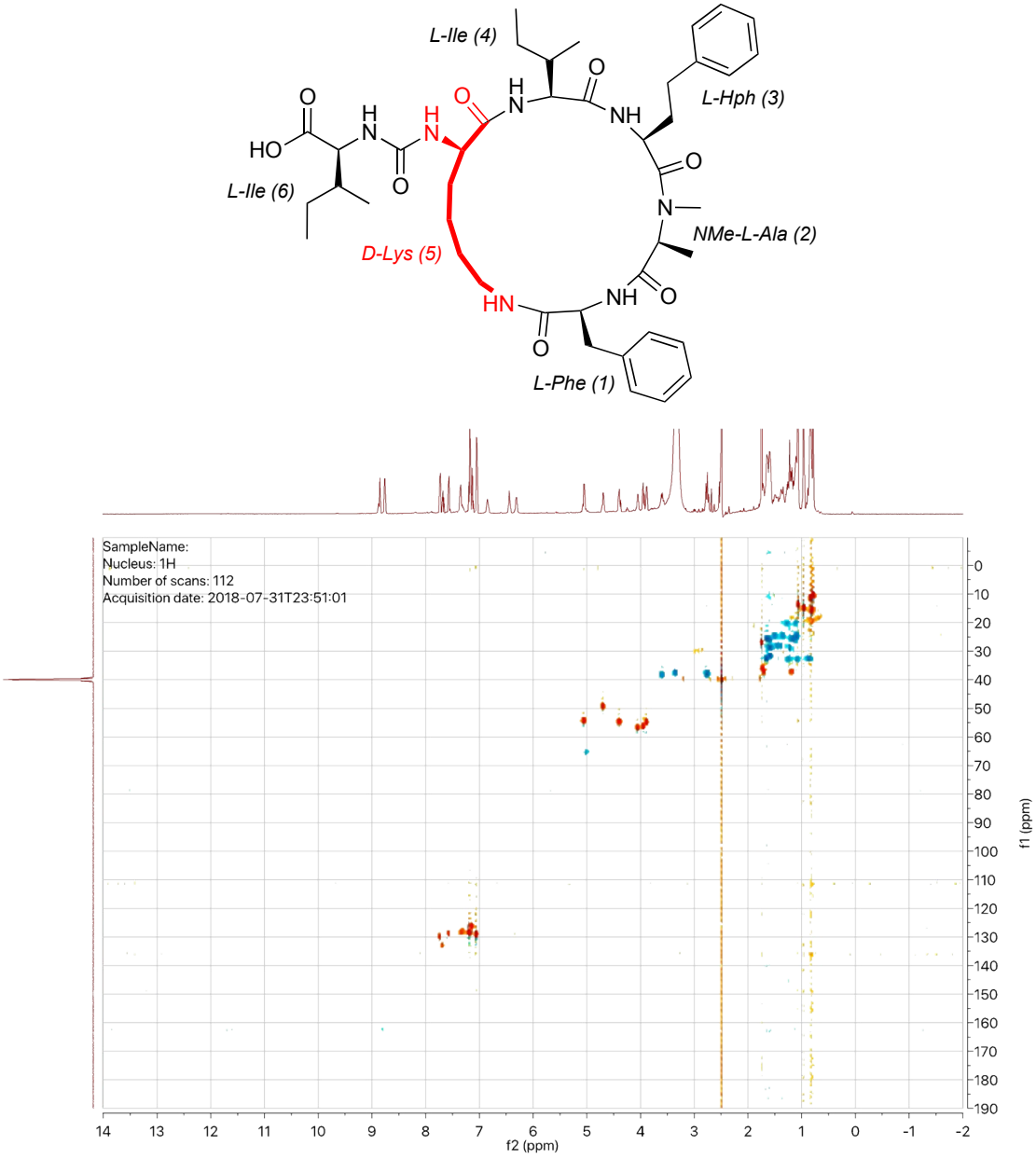


*^{1}H-^{1}H COSY spectrum of **1** in DMSO-d_6*





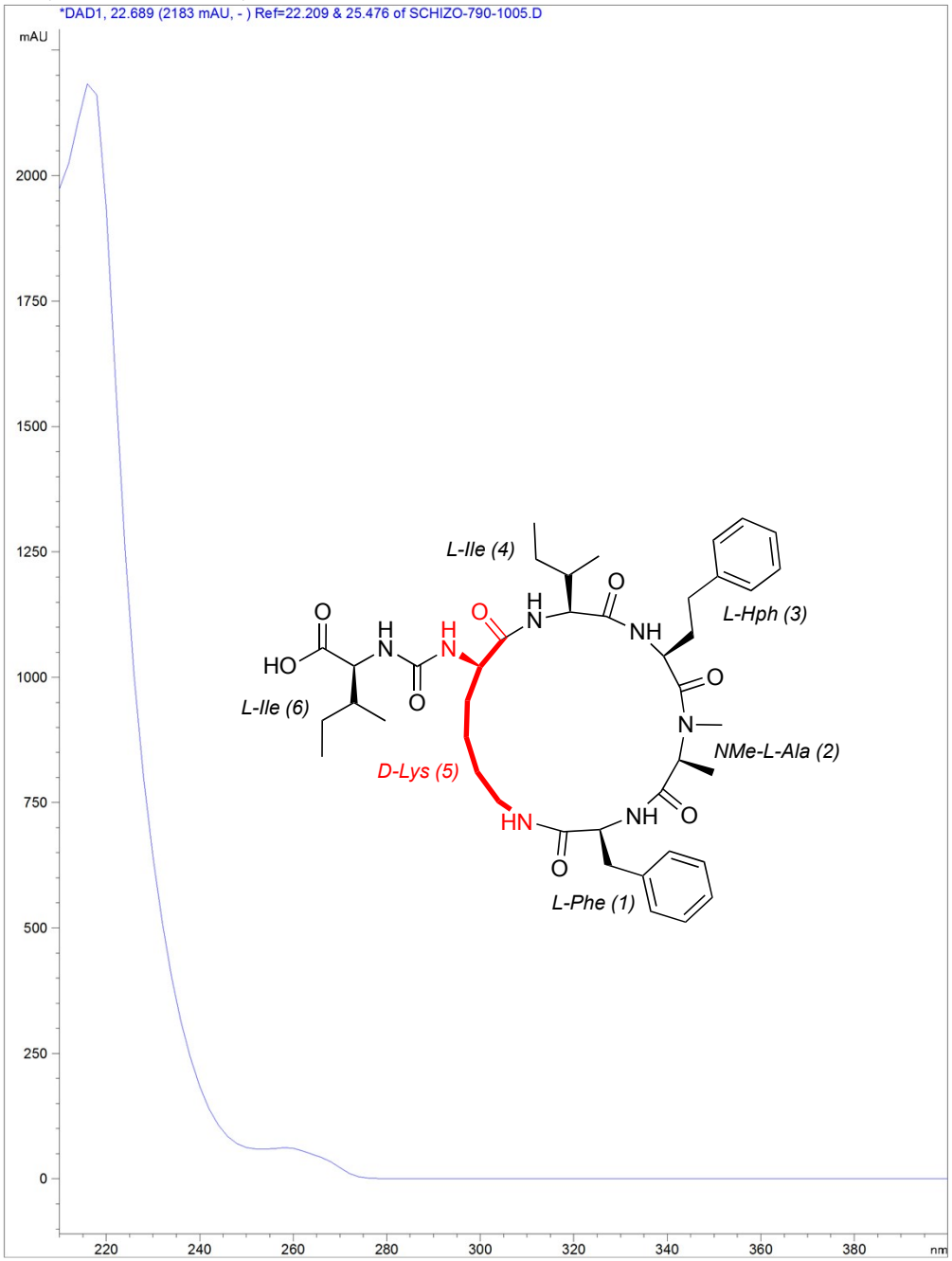
*HSQC spectrum of A **1** in DMSO-d_6*



*UV spectrum of **1***

Print of window 39: DAD1, 22.689 (2183 mAU, -) Ref=22.209 & 25.476 of SCHIZO-790-1005.D

DAD1, 22.689 (2183 mAU, -) Ref=22.209 & 25.476 of SCHIZO-790-1005.D



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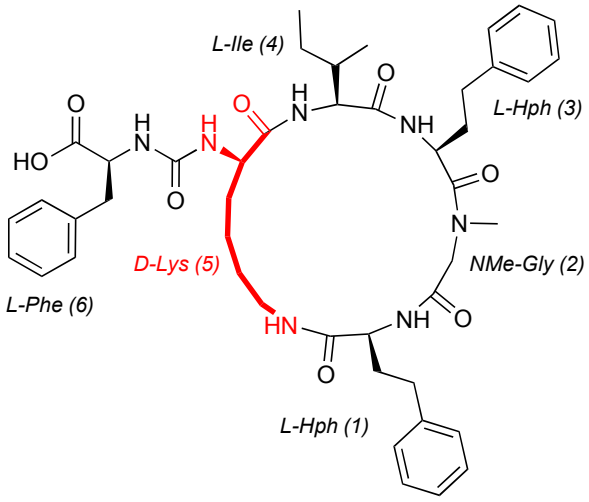
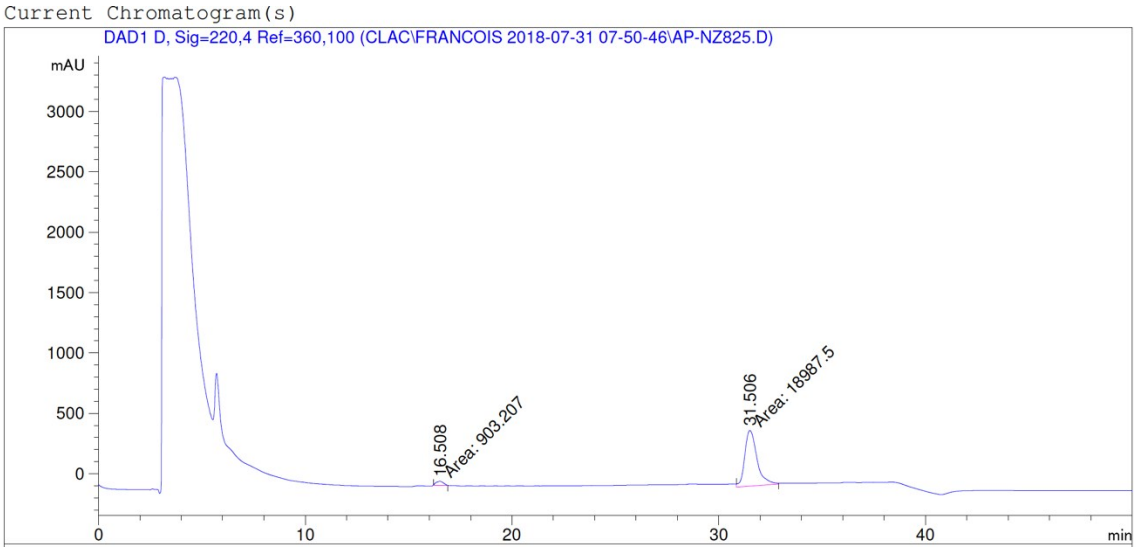
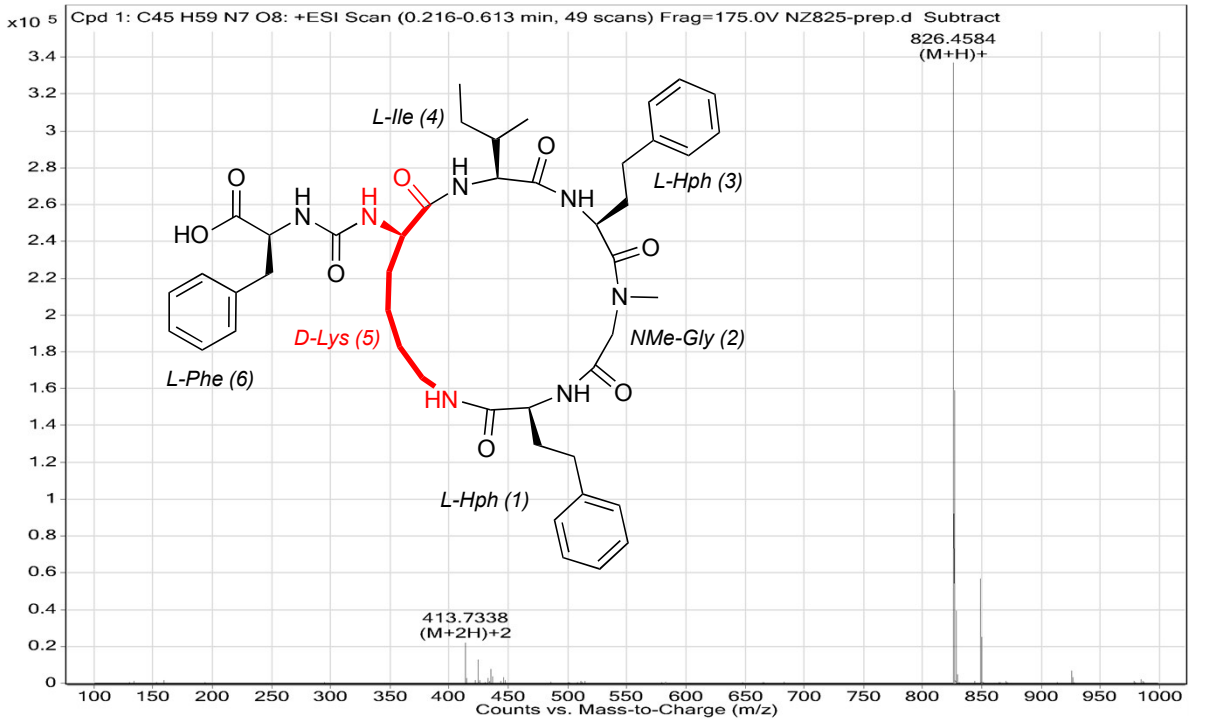
HPLC spectrum of ***2***



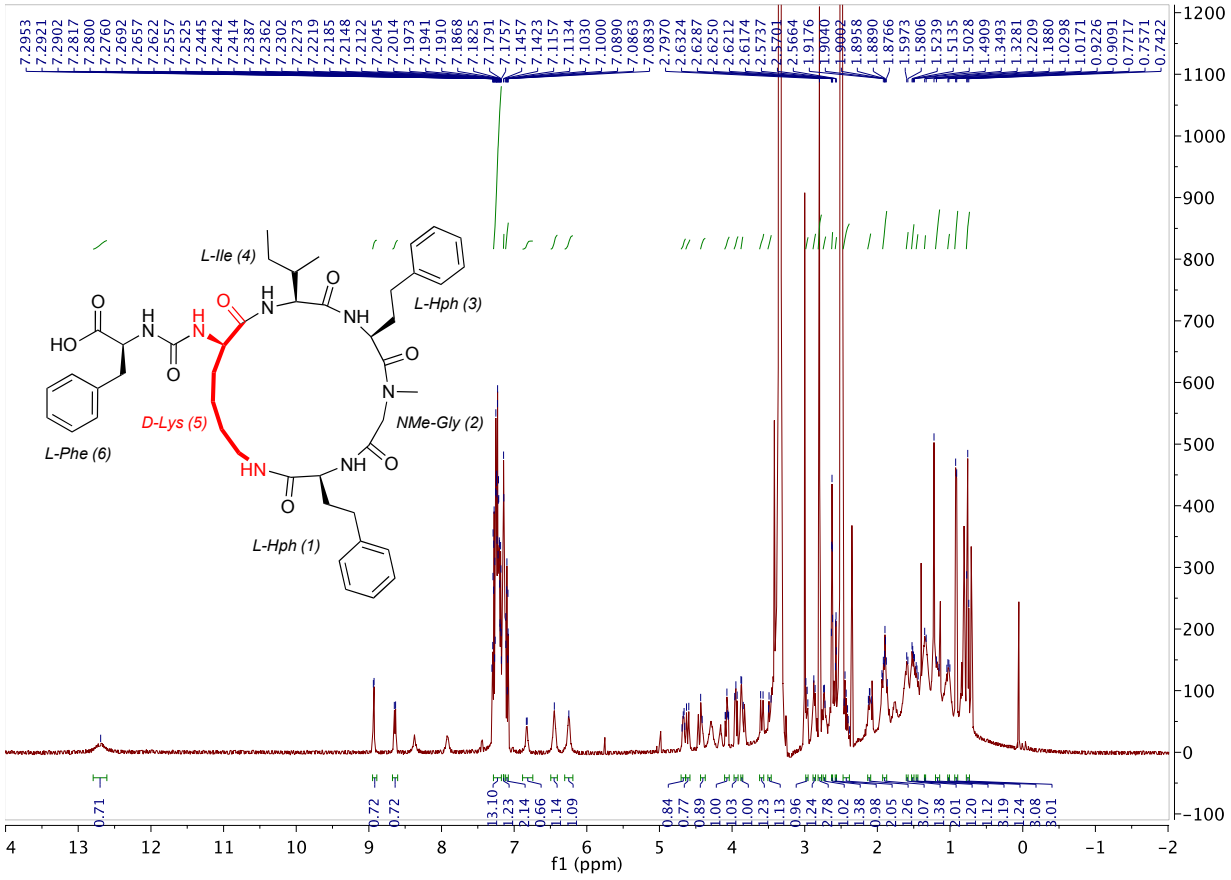


HRMS-TOF spectrum of ***2***

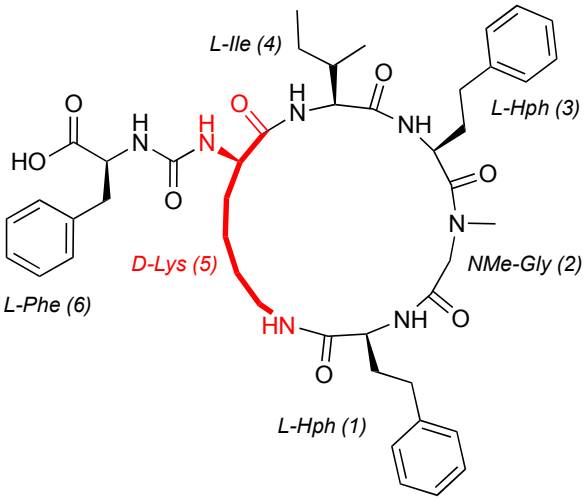
Sample Name	NZ825-prep	Position	Vial 1	Instrument Name	Instrument 1	User Name	Hugo Boutin
Inj Vol	10	InjPosition		SampleType	Sample	IRM Calibration Status	Success
Data Filename	NZ825-prep.d	ACQ Method	Direct_Injection_ESI	Comment		Acquired Time	6/5/2018 2:00:32 PM

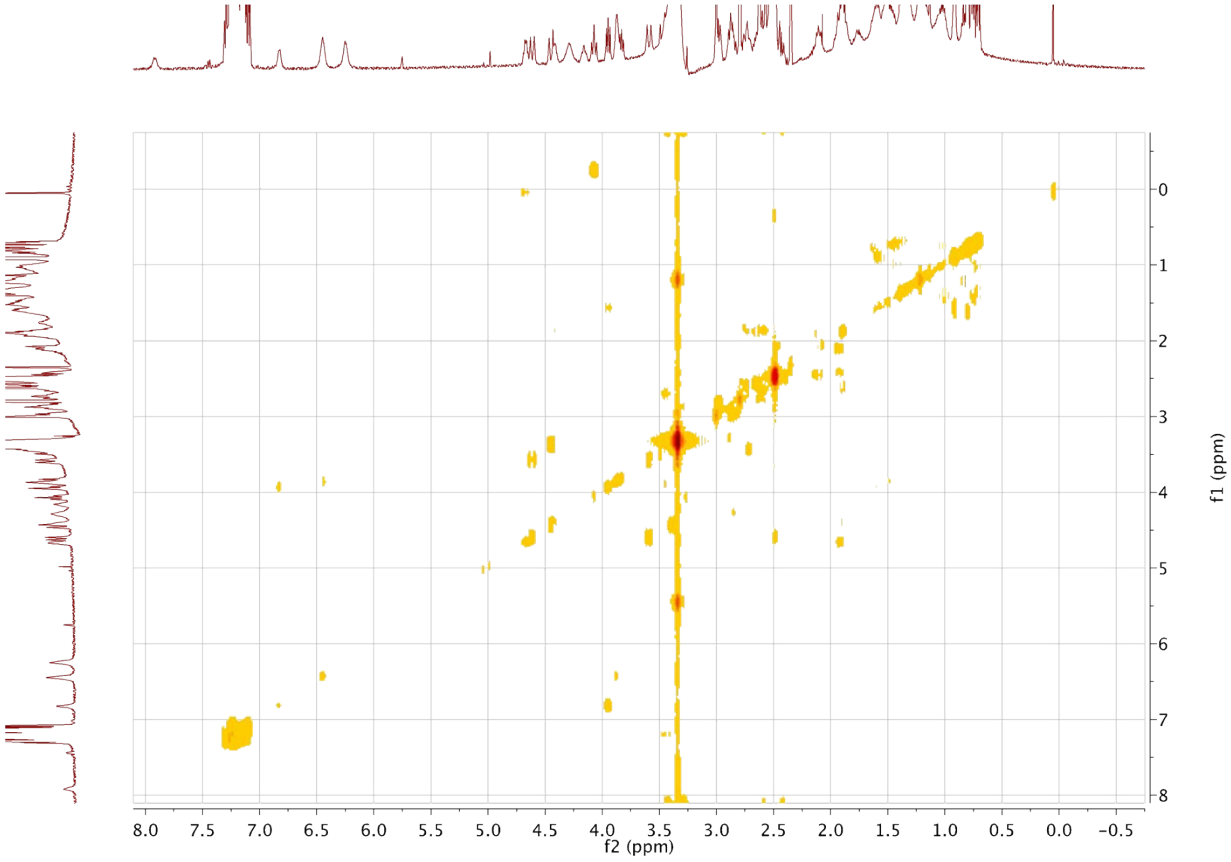


*[1]H-NMR spectrum of **2** in DMSO-d_6*

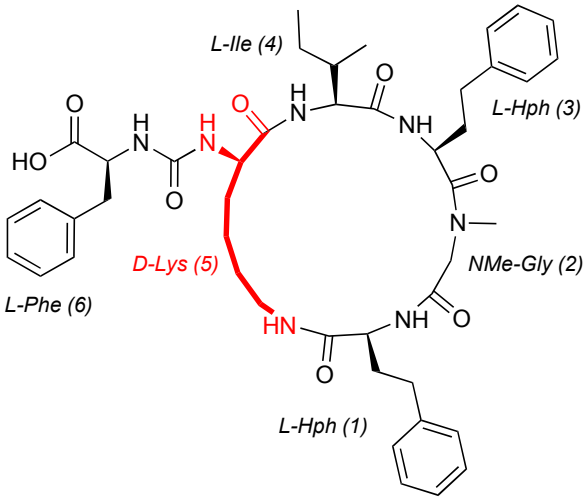


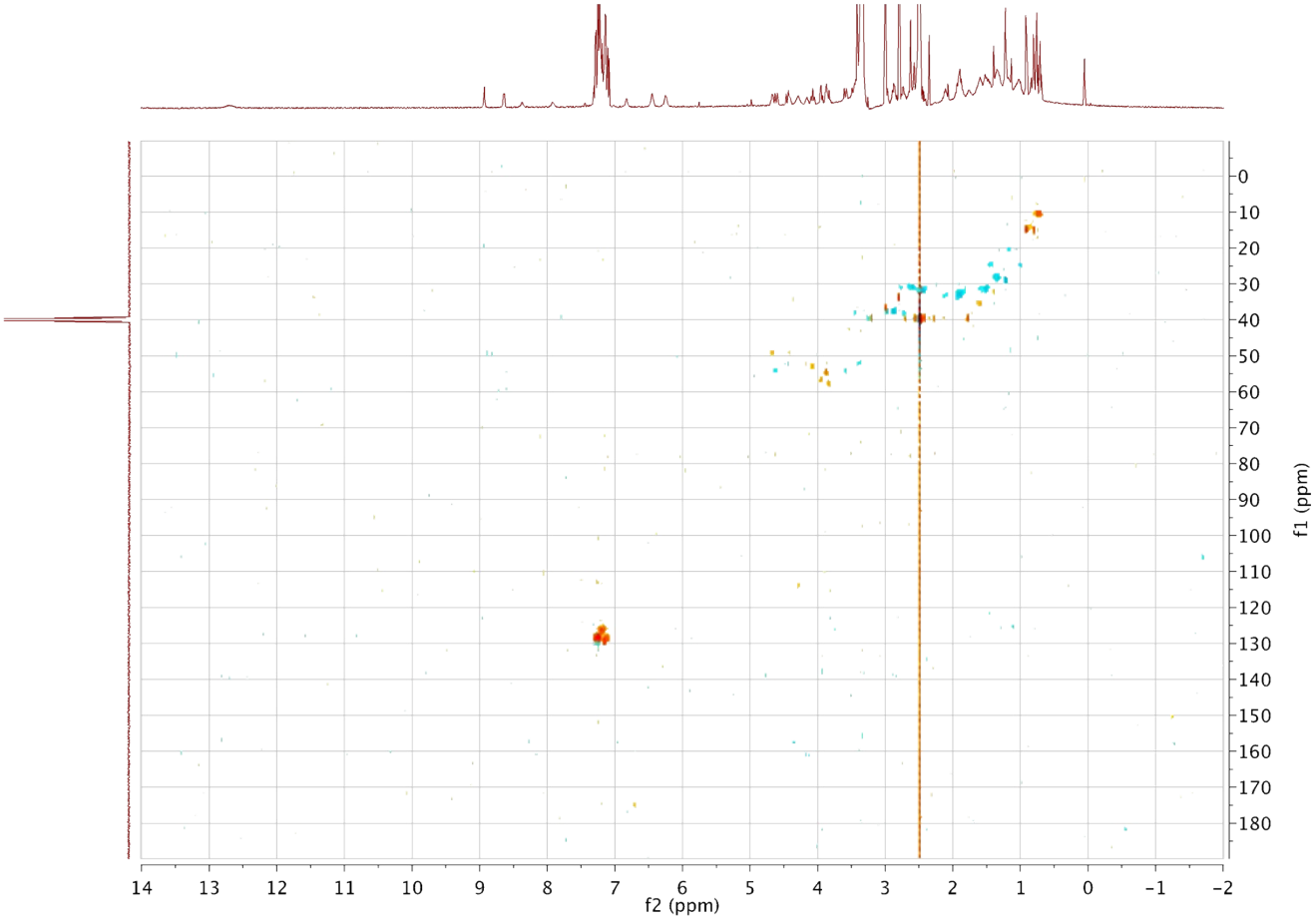
*^{1}H-^{1}H COSY spectrum of **2** in DMSO-d_6*





*HSQC spectrum of A **2** in DMSO-d_6*

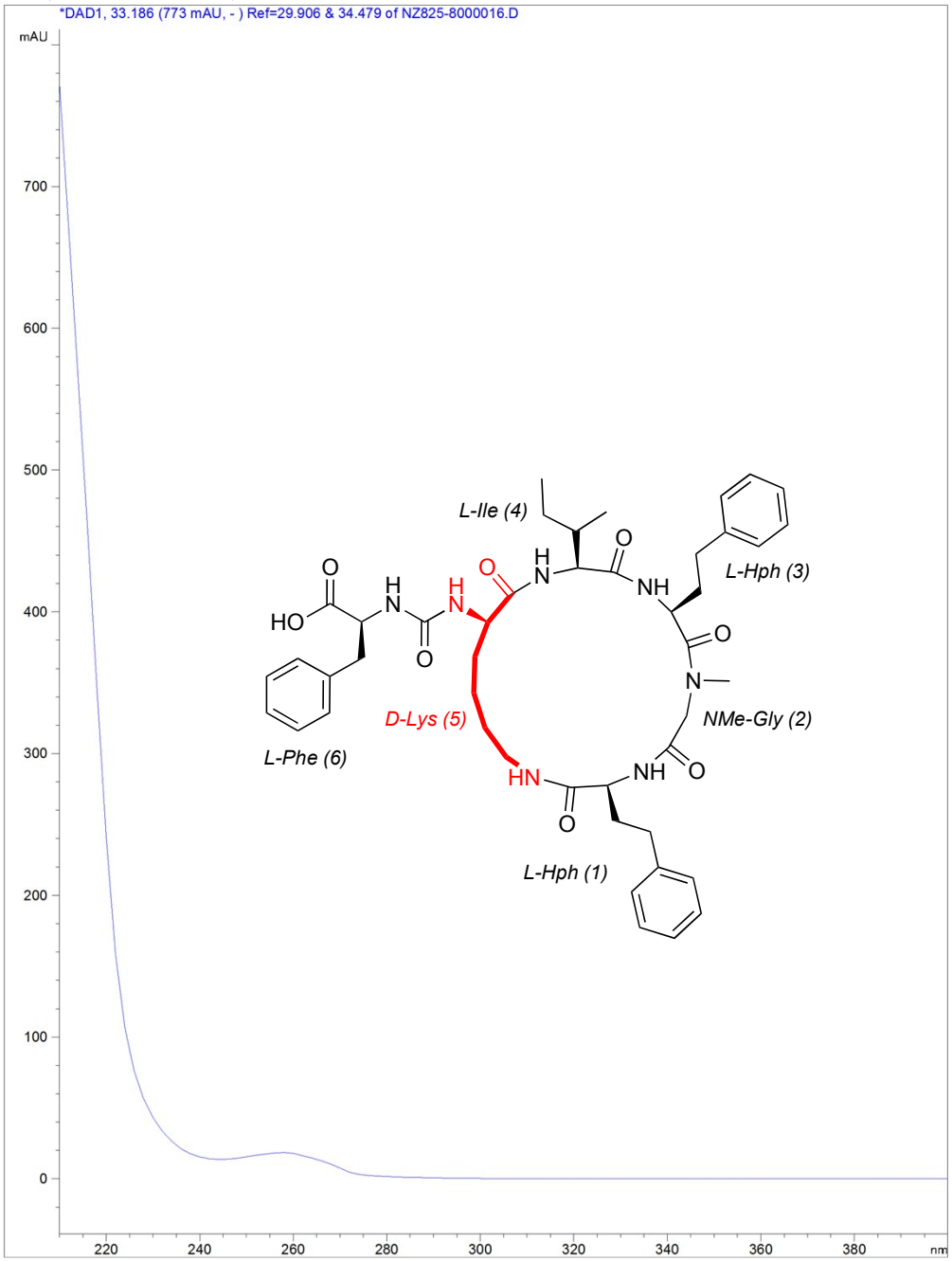




UV spectrum of ***2***

Print of window 39: DAD1, 33.186 (773 mAU, -) Ref=29.906 & 34.479 of NZ825-8000016.D

DAD1, 33.186 (773 mAU, -) Ref=29.906 & 34.479 of NZ825-8000016.D



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