Three-Component Synthesis of Neoglycopeptides using a Cu(II)-triggered aminolysis of peptide hydrazide resin and azide-alkyne cycloaddition sequence

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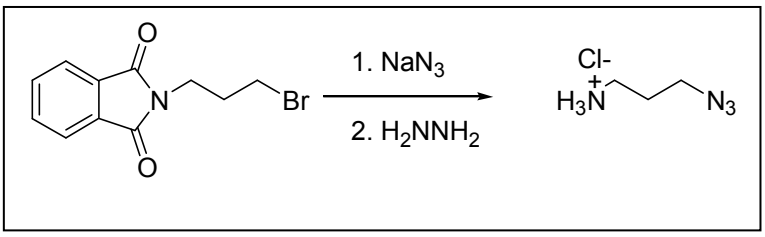
Synthesis of 3-azidopropylamine hydrochloride 4



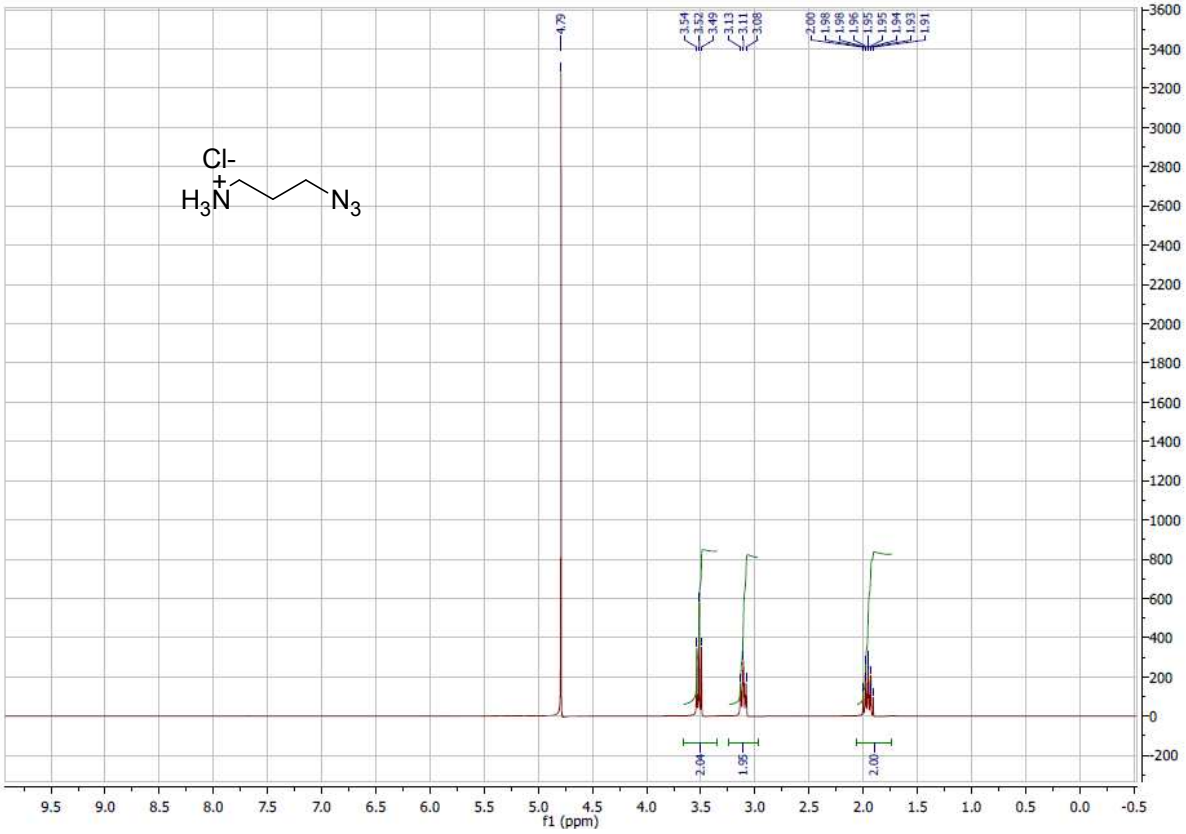
To a solution of *N*-(3-bromopropyl)phtalimide (15.0 g, 56.0 mmol) in DMF (200 mL) was added sodium azide (7.25 g, 112.0 mmol, 2.0 equiv). After stirring at 70°C for 2 hours, the reaction mixture was cooled to room temperature, then concentrated *in vacuo* to remove most of the DMF and co-evaporated 3 times with toluene (3 × 100 mL) to dryness. The resulting yellow oil dissolved in Et_2O (150 mL) was washed with water (2 × 150 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated *in vacuo* to afford 2-(3-azidopropyl)-isoindole-1,3-dione as a powdery white solid (12.4 g, 54.1 mmol, 97% yield). This compound was directly used in the nextr step without further purification.
NMR-^{1}H ($CDCl_3$, 300 MHz): δ ppm 7.78 (m, 2H), 7.65 (m, 2H), 3.72 (t, 2H, J = 6.9 Hz), 3.31 (t, 2H, J = 6.9 Hz), 1.90 (tt, 2H, J = 6.9 Hz).
NMR-^{13}C (75 MHz $CDCl_3$): δ ppm 168.28, 134.04, 131.96, 123.31, 49.00, 35.35, 28.0.
MALDI-TOF (α-cyano-4-hydroxycinnaminic acid, monoisotopic): calculated for $C_{11}H_{10}N_4O_2$ $[M + H]^+$ 231.09, found 231.10.

To a round-bottom flask equipped with a condenser, 2-(3-azidopropyl)-isoindole-1,3-dione (3.00 g, 12.0 mmol) was dissolved in CH_2Cl_2 (10 mL). A hydrazine hydrate solution 24-26% (RT) in water (9.6 mL, 32.0 mmol, 2.6 equiv) was then added and the reaction mixture was stirred for overnight at 60°C. The mixture was cooled to room temperature and quenched with a 1 N hydrochloric aqueous solution (100 mL). The aqueous layer was washed with CH_2Cl_2 (3 × 50 mL). The aqueous layer was basified to pH 14 with a 5 N sodium hydroxide aqueous solution and was extracted with CH_2Cl_2 (3 × 40 mL). The combined organic layers were extracted with a 1 N hydrochloric aqueous solution (3 × 40 mL). The combined aqueous extracts were finally frozen and lyophilized to afford 3-azidopropylamine hydrochloride as a white solid (1.49 g, 10.97 mmol, 84 % yield).
NMR-^{1}H (D_2O, 300 MHz): δ ppm 3.52 (t, 2H, J = 6.6 Hz), 3.11 (t, 2H, J = 7.2 Hz), 1.96 (tt, 2H, J = 6.6 Hz).
NMR-^{13}C (75 MHz D_2O, TMS): δ ppm 39.02, 26.48, 15.67.
MALDI-TOF (α-cyano-4-hydroxycinnaminic acid, monoisotopic): calculated for $C_3H_8N_4$ $[M + H]^+$ 101.08, found 101.09.

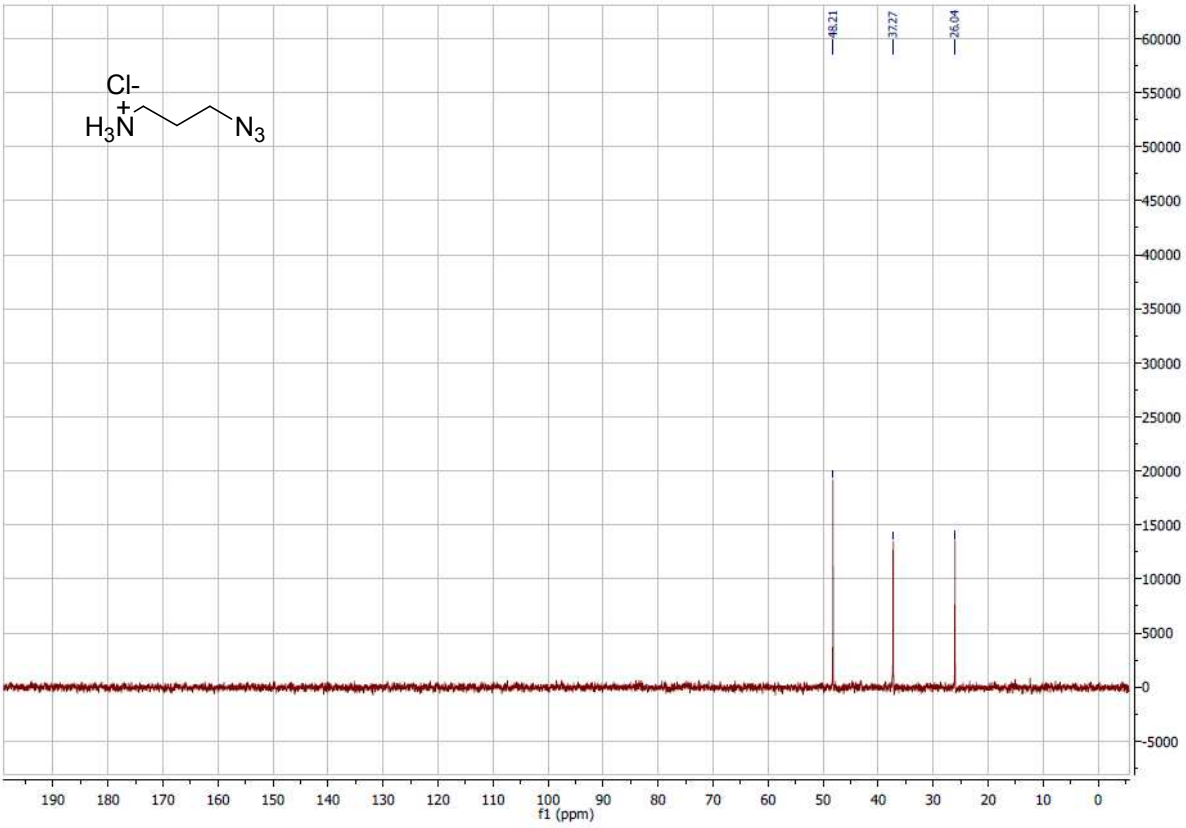
3-azidopropylamine hydrochloride is hygroscopic, thus elemental analysis was carried out on the tosylate salt instead (see later).

Characterization of 3-azidopropylamine hydrochloride 4

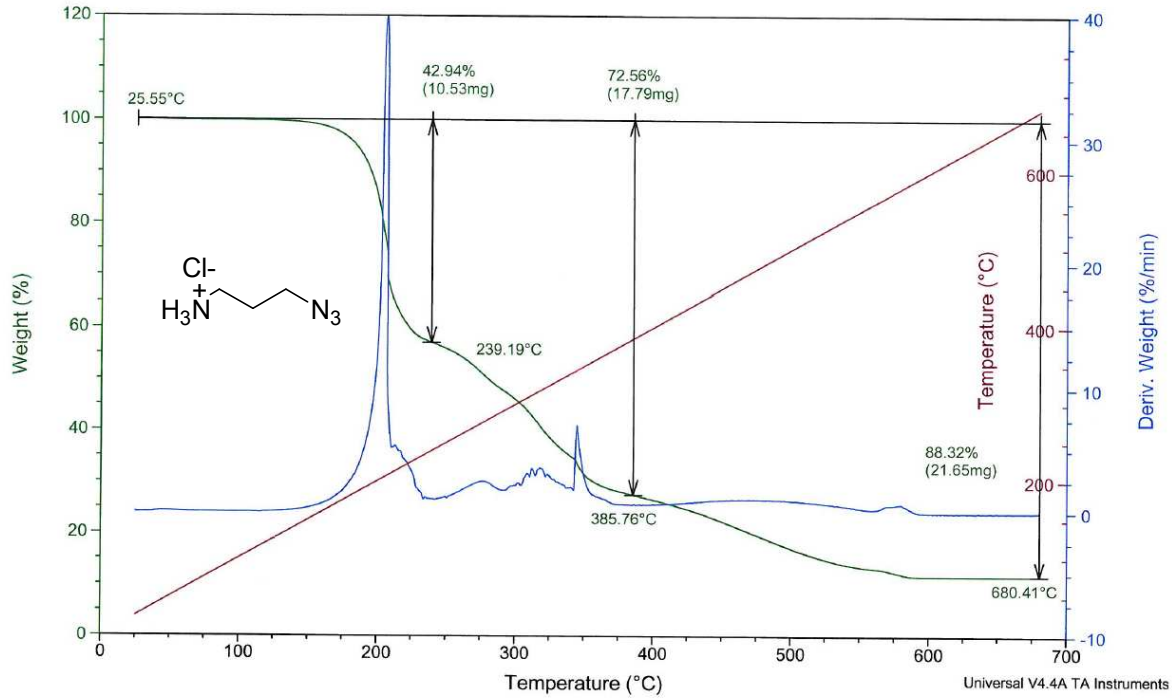
^{1}H NMR spectrum of 3-azidopropylamine hydrochloride 4 (H_2O/D_2O : 1/9 by vol)

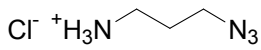


^{13}C NMR spectrum of 3-azidopropylamine hydrochloride 4 (H_2O/D_2O : 1/9 by vol)

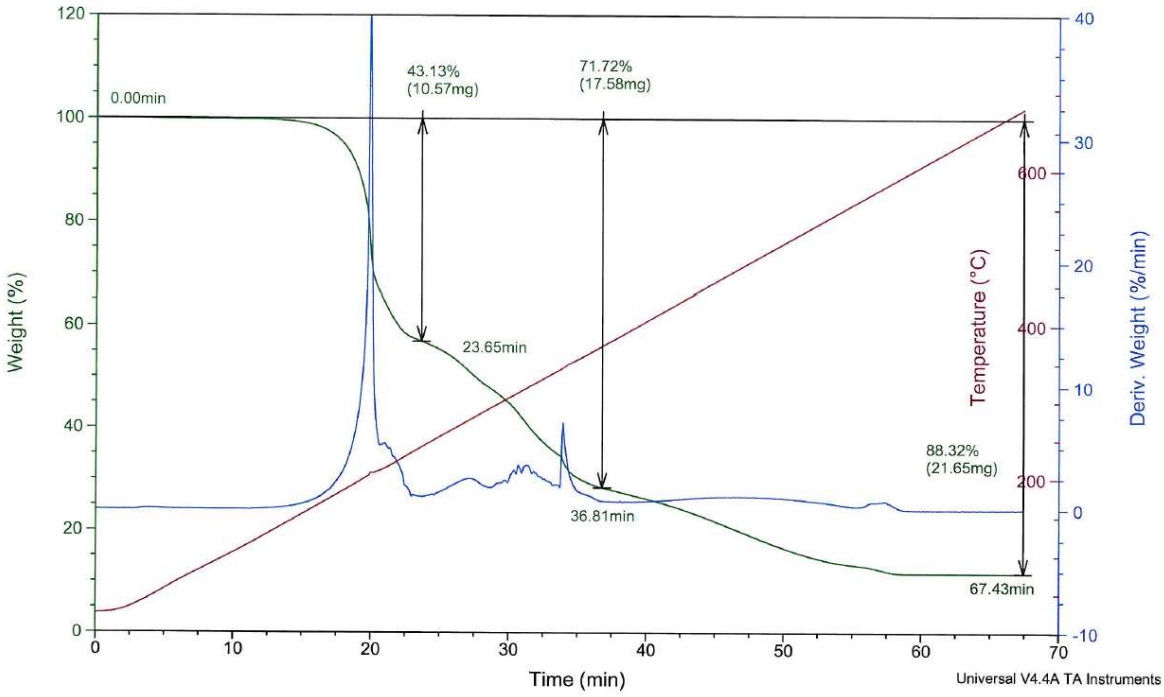


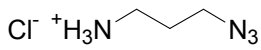
Thermogravimetric analysis of 3-azidopropylamine hydrochloride 4





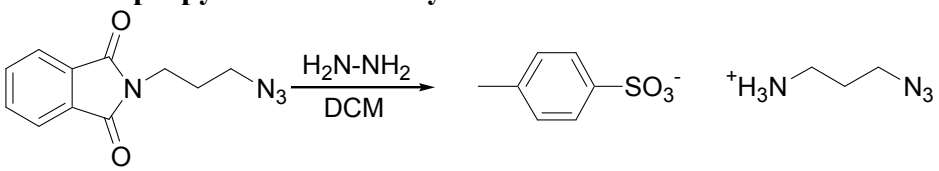
3-azidopropylamine hydrochloride





3-azidopropylamine hydrochloride

Synthesis of 3-azidopropylammonium tosylate



To a round-bottom flask equipped with a condenser, 2-(3-azidopropyl)-isoindole-1,3-dione (3.00 g, 12.0 mmol) was dissolved in CH_2Cl_2 (10 mL). A hydrazine hydrate solution 24-26% (RT) in water (9.6 mL, 32.0 mmol, 2.6 equiv) was then added and the reaction mixture was stirred for overnight at 60°C. The mixture was cooled to room temperature and quenched with a 1 N hydrochloric aqueous solution (100 mL). The aqueous layer was washed with CH_2Cl_2 (3 × 50 mL). The aqueous layer was basified to pH 14 with a 5 N sodium hydroxide aqueous solution and was extracted with CH_2Cl_2 (3 × 40 mL). To the combined organic layer, was added paratoluene sulfonic acid-monohydrate (2.51 g, 13.2 mmol) and the solution was concentrated in vacuo. The solid was then recrystallised in AcOEt to afford after filtration a white powder (2.65 g, 9.72 mmol, 81 % yield)

NMR-^{1}H (D_2O, 300 MHz): δ δ 7.70 (dd, J = 8.6, 0.3 Hz, 2H), 7.37 (dd, J = 8.6, 0.7 Hz, 2H), 3.48 (t, J = 6.5 Hz, 2H), 3.08 (t, J = 7.4 Hz, 2H), 2.39 (s, 3H), 2.01 – 1.83 (m, 2H).

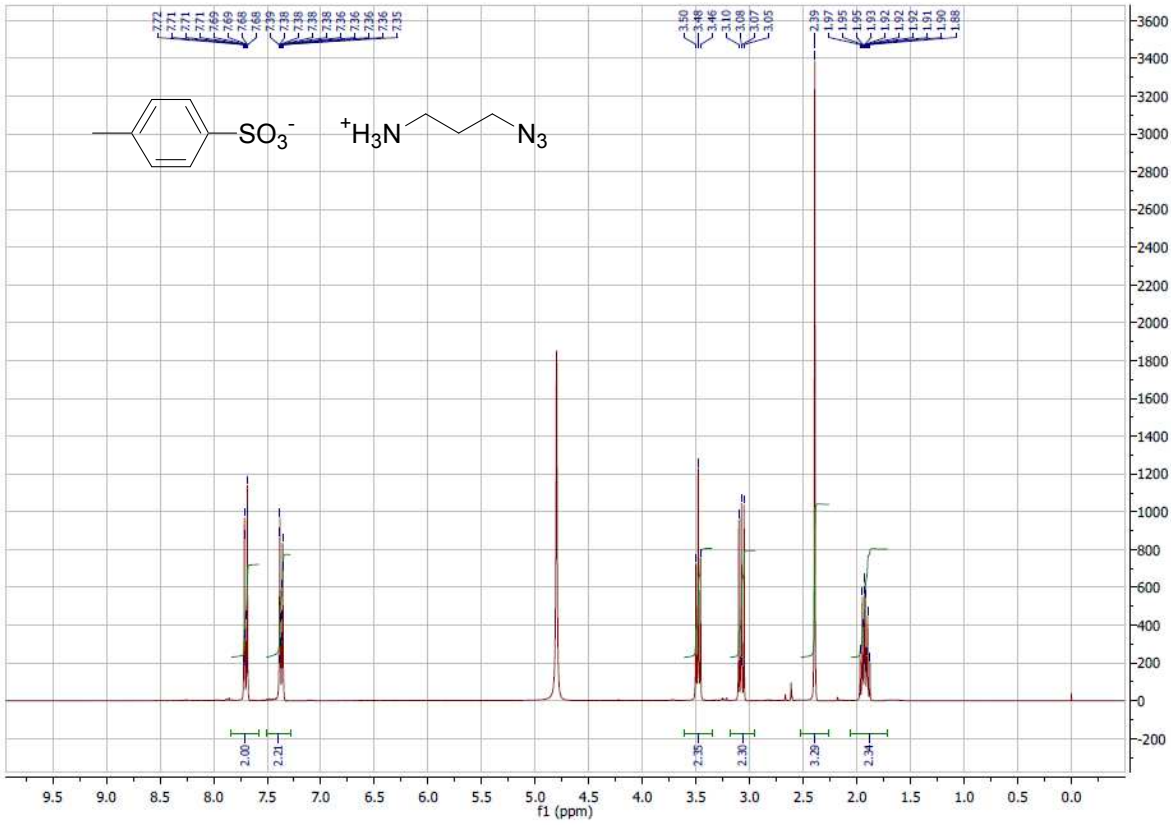
NMR-^{13}C (75 MHz D_2O, TMS): δ 143.2, 140.2, 130.9, 126.8, 49.1, 38.0, 26.8, 21.9.

MALDI-TOF (α-cyano-4-hydroxycinnaminic acid, monoisotopic): calculated for $C_3H_8N_4$ $[M + H]^+$ 101.08, found 101.07.

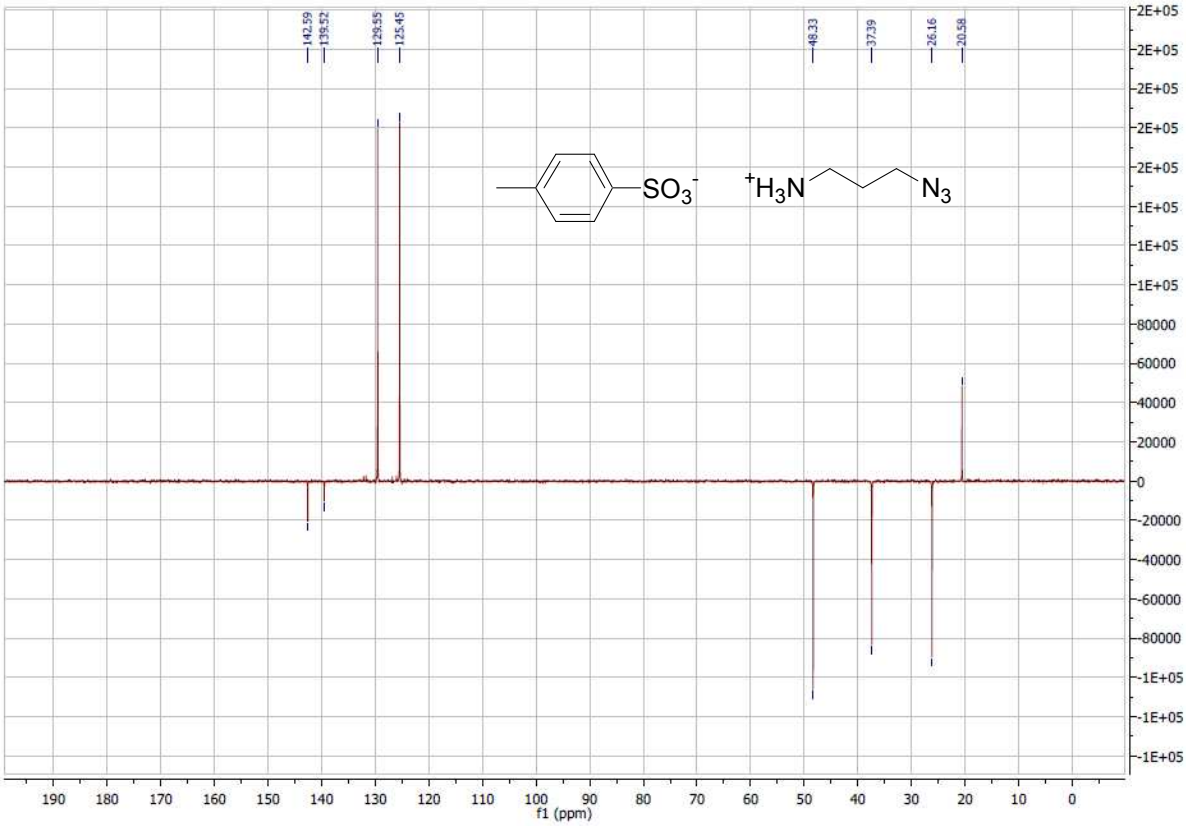
Elementary analysis :

% calculated:	C 44.27	H 5.57	N 20.65	O 17.69	S 11.82
% found:	C 43.86	H 5.83	N 20.49	O 17.29	S 11.80

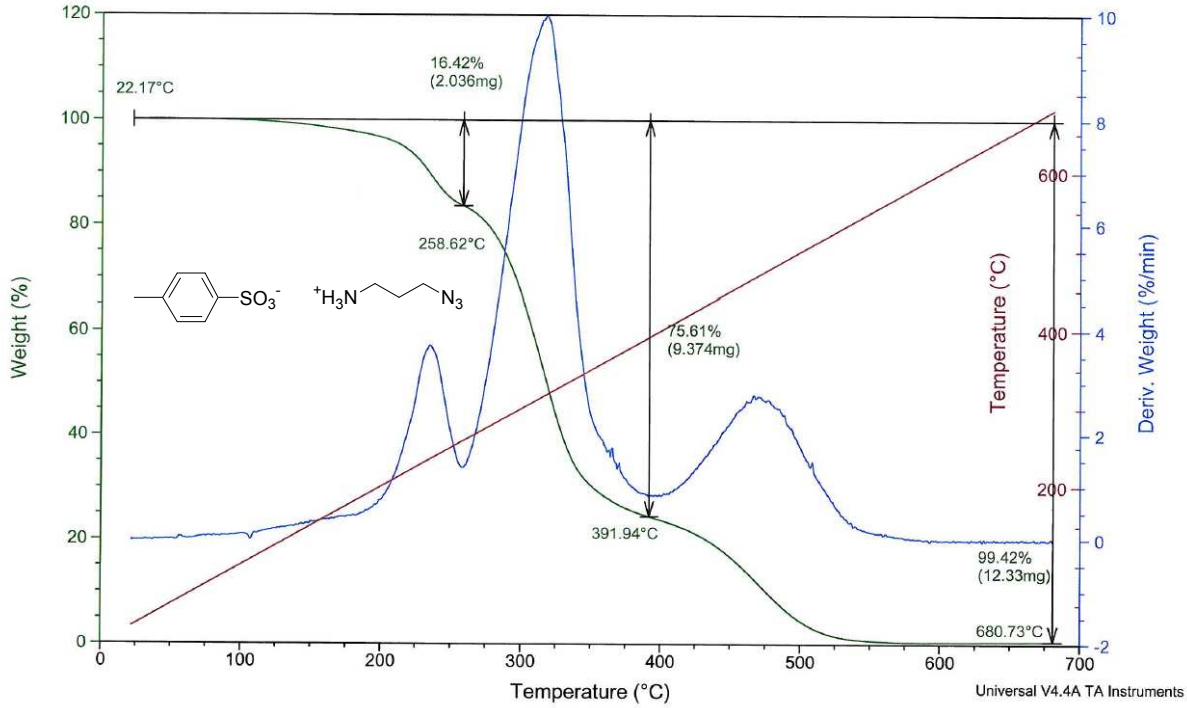
^{1}H NMR spectrum of 3-azidopropylammonium tosylate (H_2O/D_2O : 1/9 by vol)

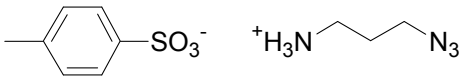


^{13}C NMR spectrum of 3-azidopropylammonium tosylate (H_2O/D_2O : 1/9 by vol)

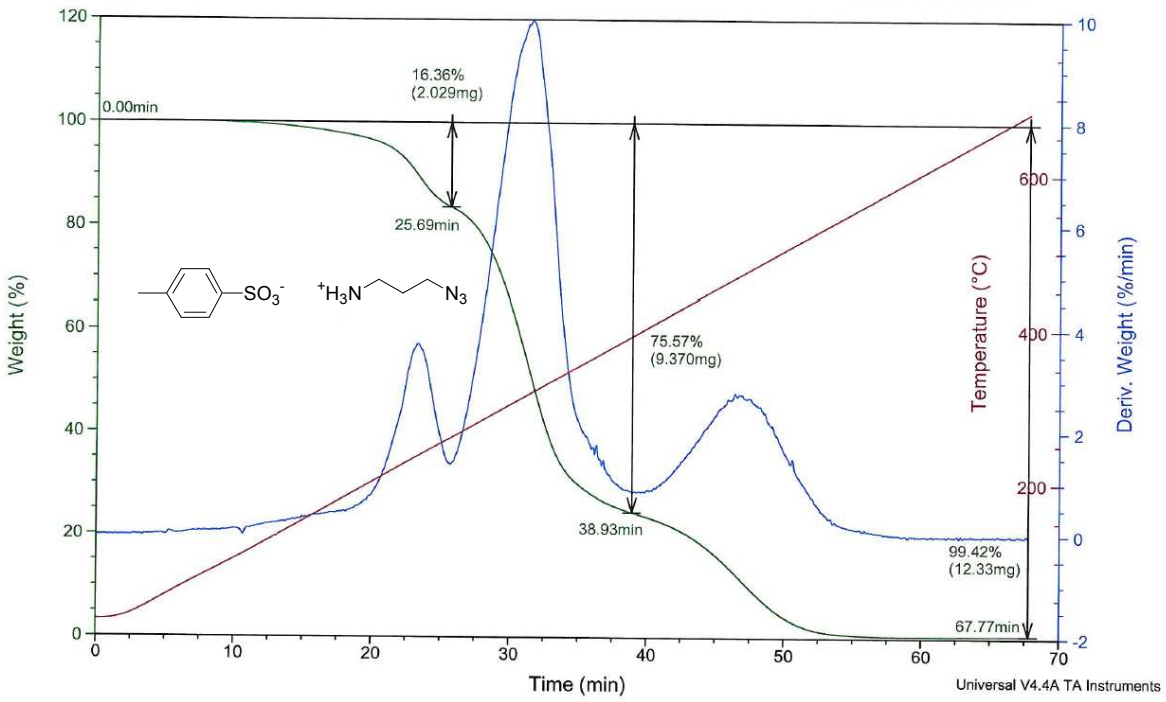


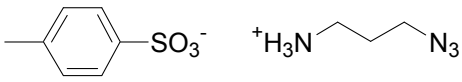
Thermogravimetric analysis of 3-azidopropylammonium tosylate





3-azidopropylammonium tosylate





3-azidopropylammonium tosylate

Peptide SPPS. Synthesis of peptidyl hydrazide resin 2

Peptide elongation was performed on 4-Fmoc-hydrazinobenzoyl AM NovaGel™ (0.76 mmol/g) using standard Fmoc/*tert*-butyl chemistry on a microwaves peptides synthesizer (CEM µ WAVES, Saclay, France) on a 0.1 mmol scale. Couplings were performed using 3-fold molar excess of each Fmoc L-amino acid, 4.5-fold molar excess of HBTU, and 10-fold molar excess of DIEA. A capping step was performed after each coupling with Ac_2O/DIEA. At the end of the synthesis, the resin was washed with CH_2Cl_2, diethyl ether (2×2 min) and dried *in vacuo*.

Oxidation and aminolysis of the hydrazide resin 2.

General procedure

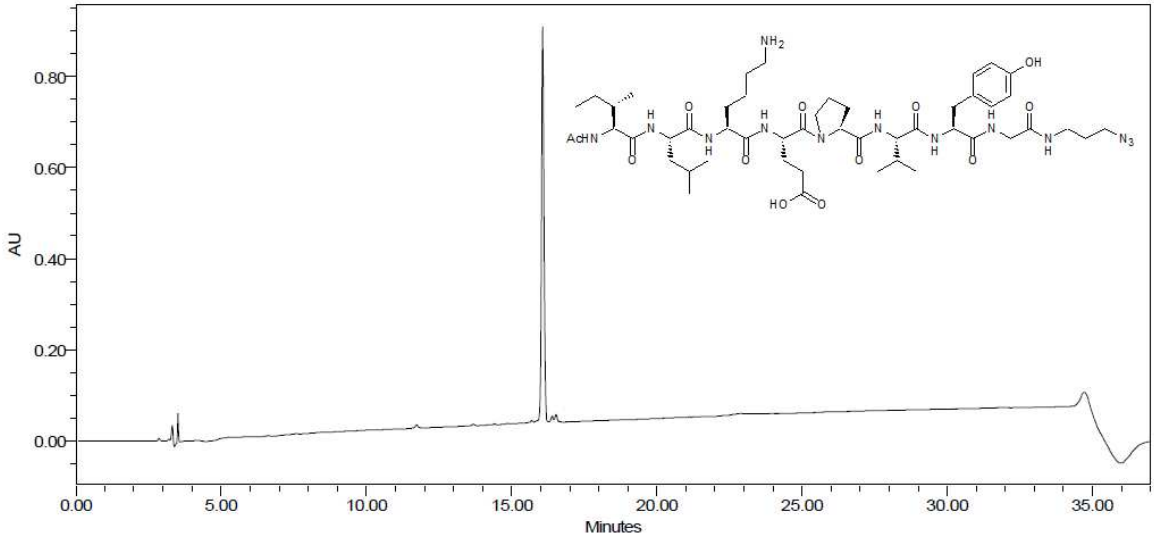
The corresponding peptide-hydrazide resin **2** (0.05 mmol) was swollen in anhydrous DCM for 10 min and drained. Copper (II) acetate (4.6 mg, 0.5 equiv), 3-azidopropylamine hydrochloride **4a** (34.2 mg, 0.25 mmol) and diisopropylethylamine (87.0 µL, 0.5 mmol) were dissolved in anhydrous pyridine (41 µL, 0.5 mmol) and anhydrous DCM (5 mL). This mixture was added to the peptide resin and the aminolysis was kept for 6 hours at room temperature while oxygen was bubbled through. After filtration, the resin was washed five times with DCM and the combined filtrates were concentrated in vacuo. To the resulting oil was added a solution of TFA/H_2O/TriIsopropylSilane : 95/2.5/2.5 by vol. (10 mL) at room temperature. After 2 hours, the peptide was precipitated in a mixture of diethylether/heptane : 1/1 by vol. (100 mL). The peptide was separated by centrifugation. The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 9a:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 12.0 mg of pure peptide **9a** (23 % yield).

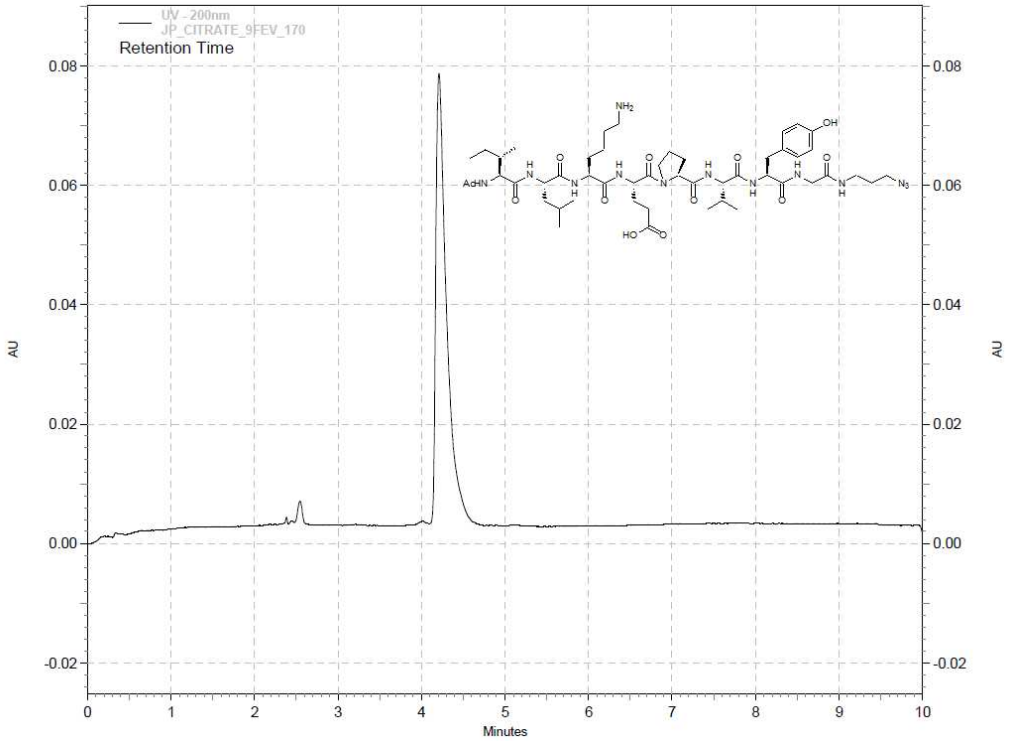
RP-HPLC analysis of 9a

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol. RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 9a

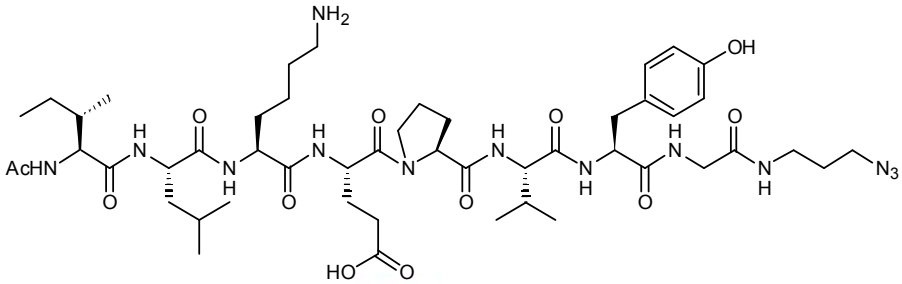
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV

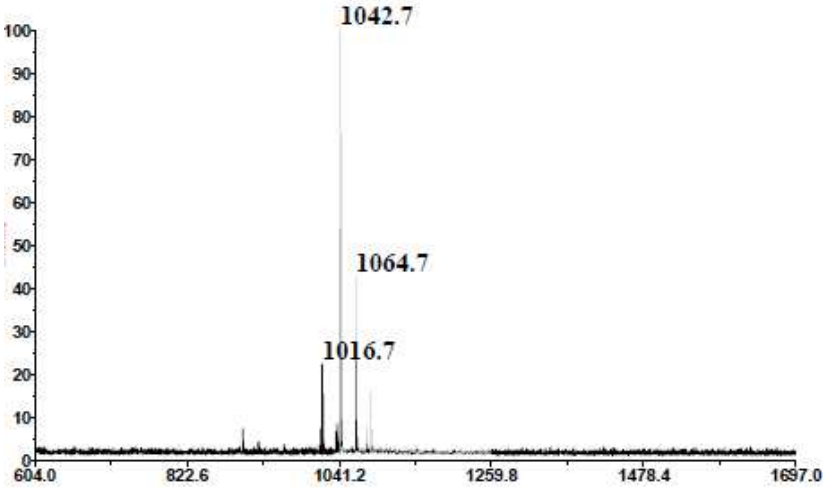


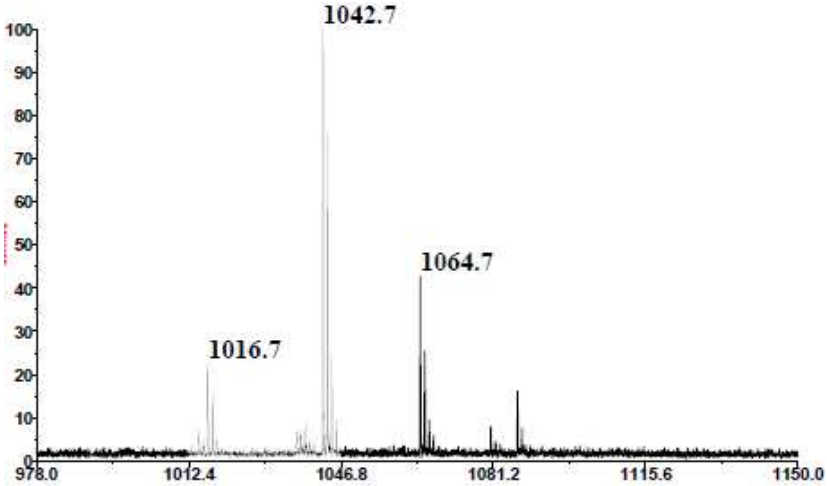
MALDI-TOF spectrum of 9a

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{49}H_{79}N_{13}O_{12}$ $[M+H]^+$ 1042.6, found 1042.7 ; $[M+Na]^+$ calcd. 1064.6, found 1064.7.





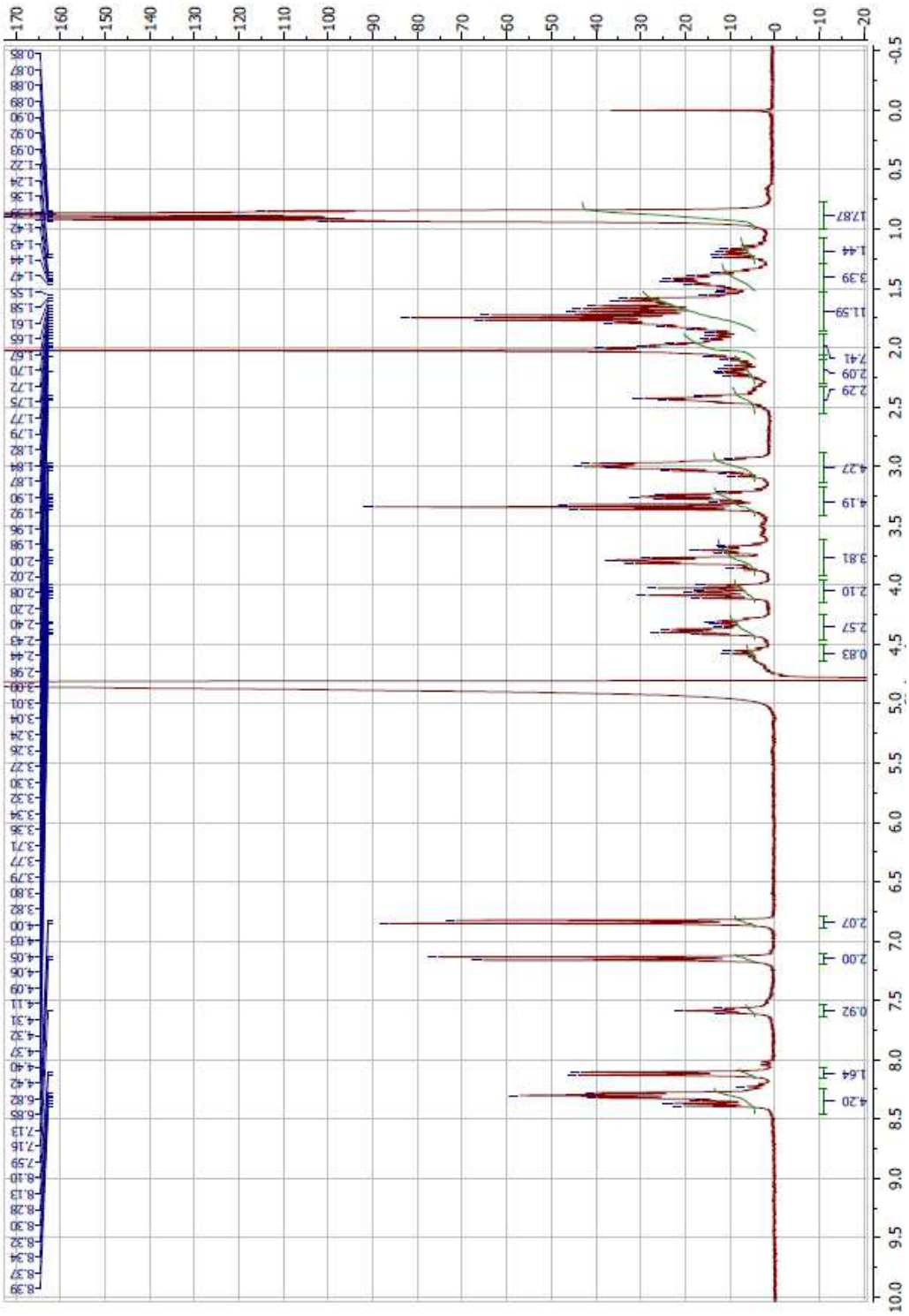


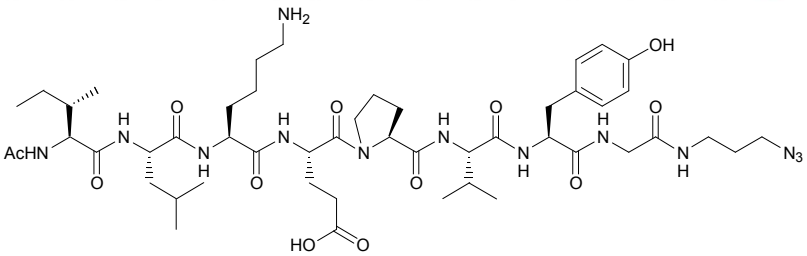
^{1}H and ^{13}C NMR for peptide 9a

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.39-8.28 (m, 5H), 8.12 (m, 2H), 7.59 (t, *J* = 5.9 Hz, 1H), 7.15 (d, *J* = 8.5 Hz, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 4.57 (m, 2 H), 4.42 – 4.31 (m, 3H), 4.09 (t, *J* = 7.4 Hz, 1H), 4.03 (t, *J* = 7.9 Hz, 1H), 3.76 (m, 4H), 3.34 (t, *J* = 6.3 Hz, 1H), 3.26 (m, 3H), 3.04 (m, 4 H), 2.43 (m, 2H), 2.31 – 2.00 (m, 2H), 2.09 – 1.88 (m, 8H), 1.86 – 1.53 (m, 13H), 1.53 – 1.29 (m, 4H), 1.20 (m, 2H), 1.01 – 0.77 (m, 18H).

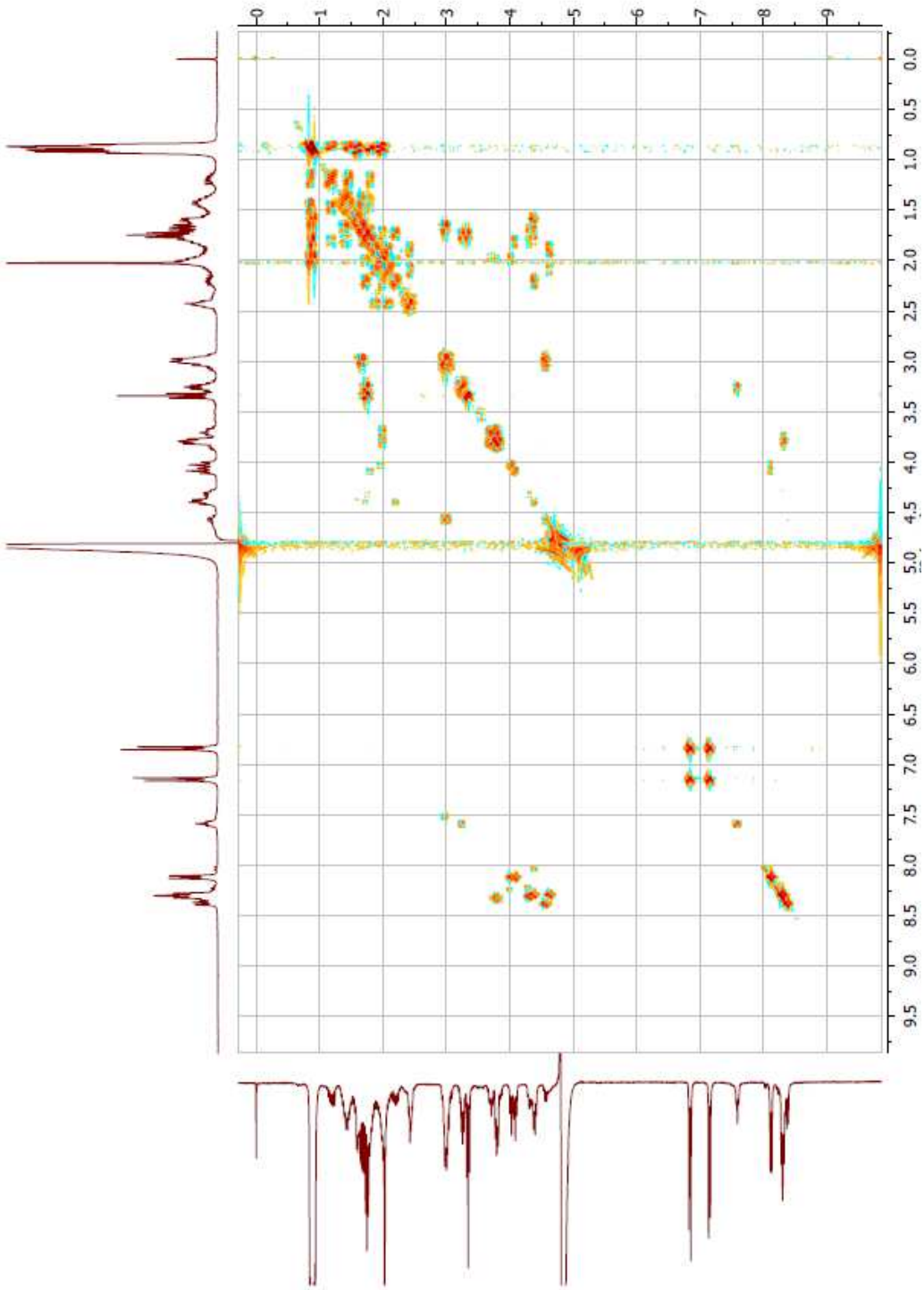
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 181.3, 177.1, 177.0, 176.6, 176.5, 176.3, 176.1, 174.2, 173.8, 157.3, 133.2, 130.6, 118.3, 103.8, 63.0, 62.7, 61.9, 61.5, 58.1, 56.0, 55.0, 53.9, 51.3, 50.7, 45.5, 42.4, 42.2, 39.6, 38.7, 33.7, 33.1, 32.9, 32.1, 30.4, 29.1, 28.9, 27.4, 27.4, 27.0, 24.8, 24.7, 24.4, 23.6, 21.1, 20.6, 17.5, 13.0.

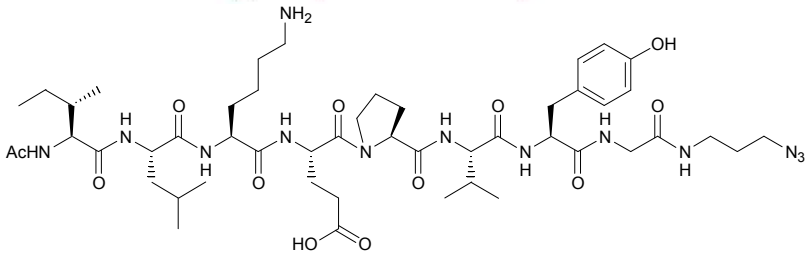
NMR ^{1}H experiment for compound **9a**



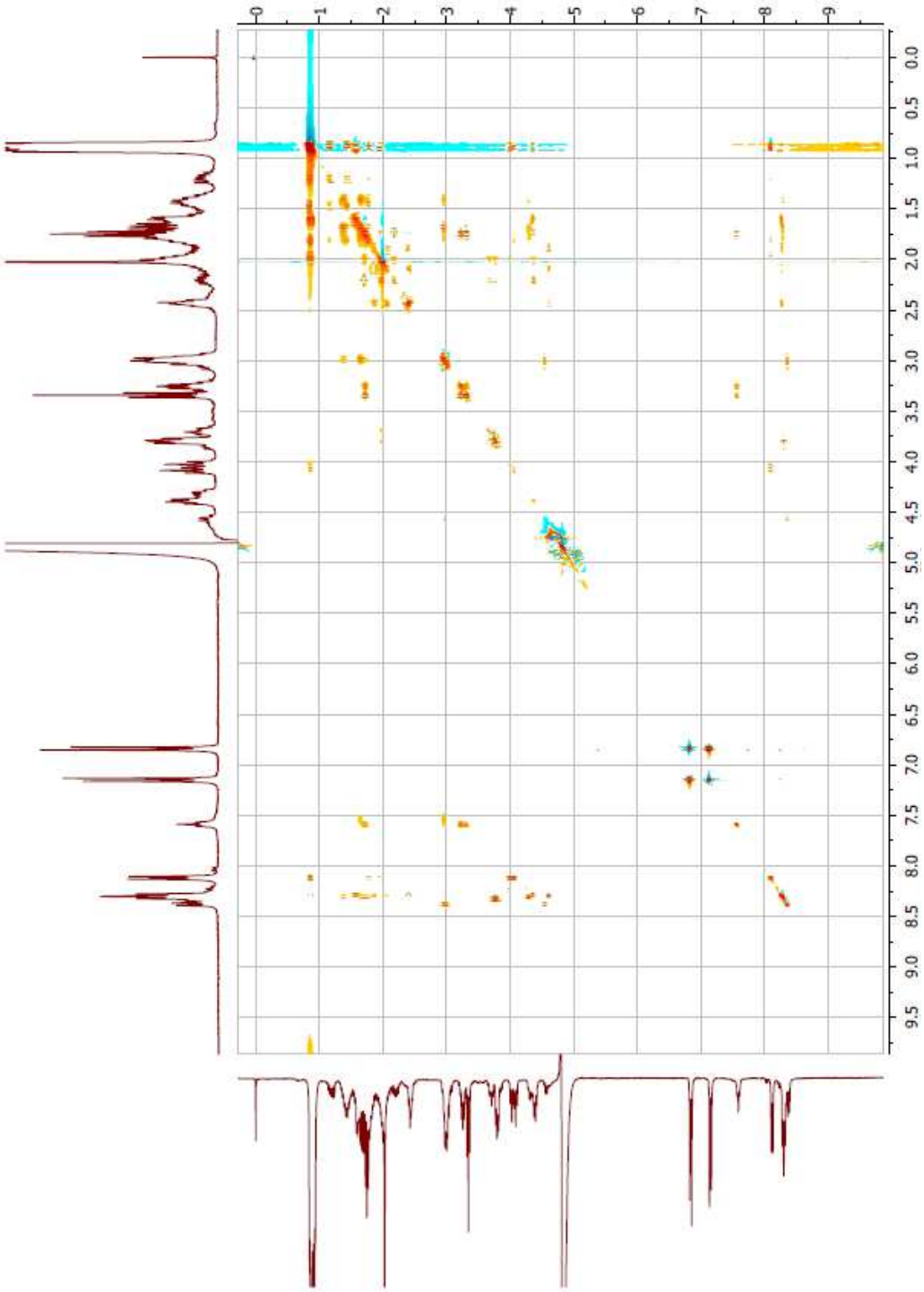


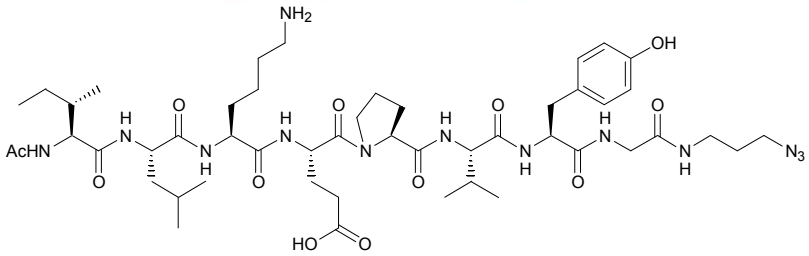
COSY experiment for compound **9a**



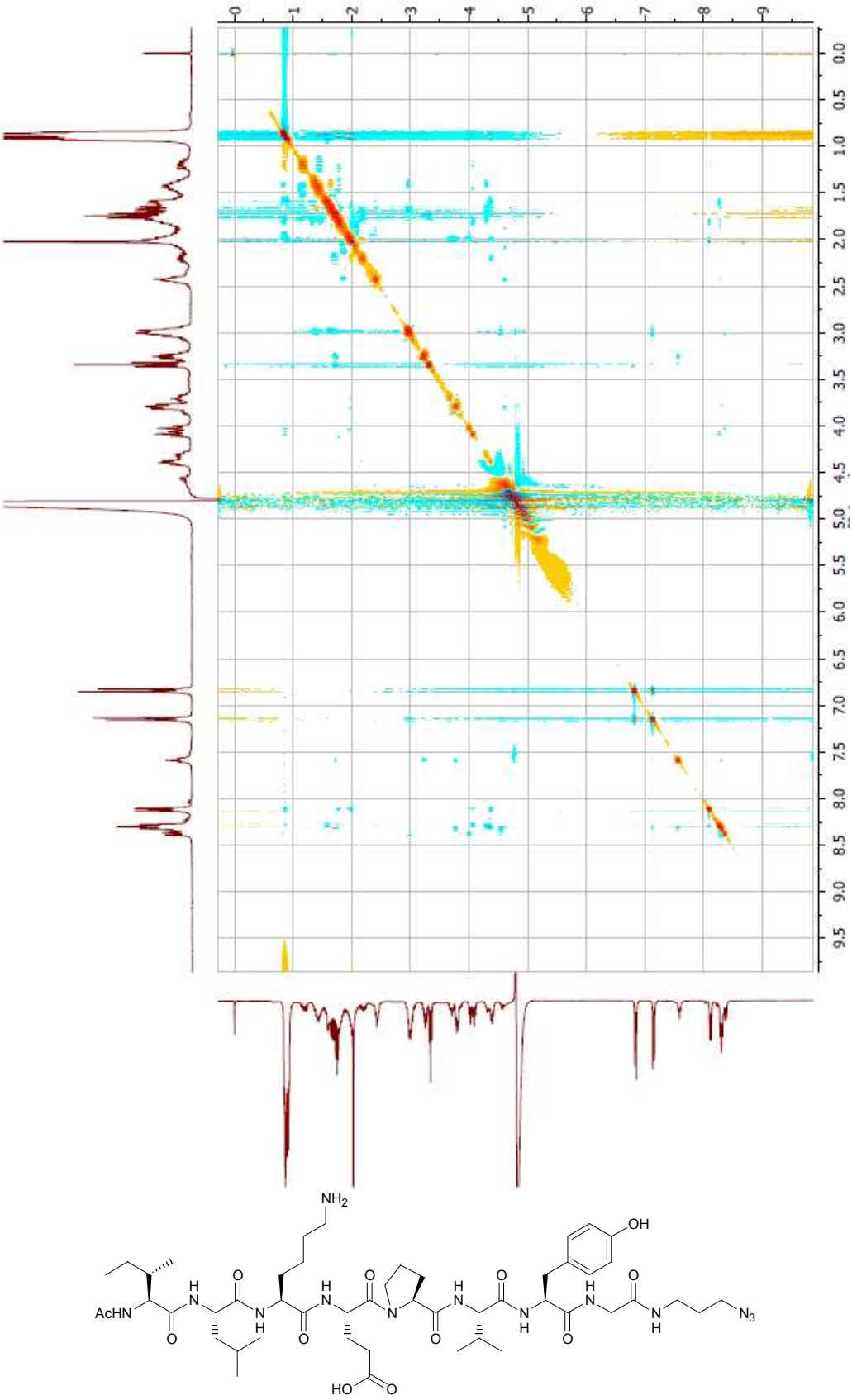


TOCSY experiment for compound **9a**

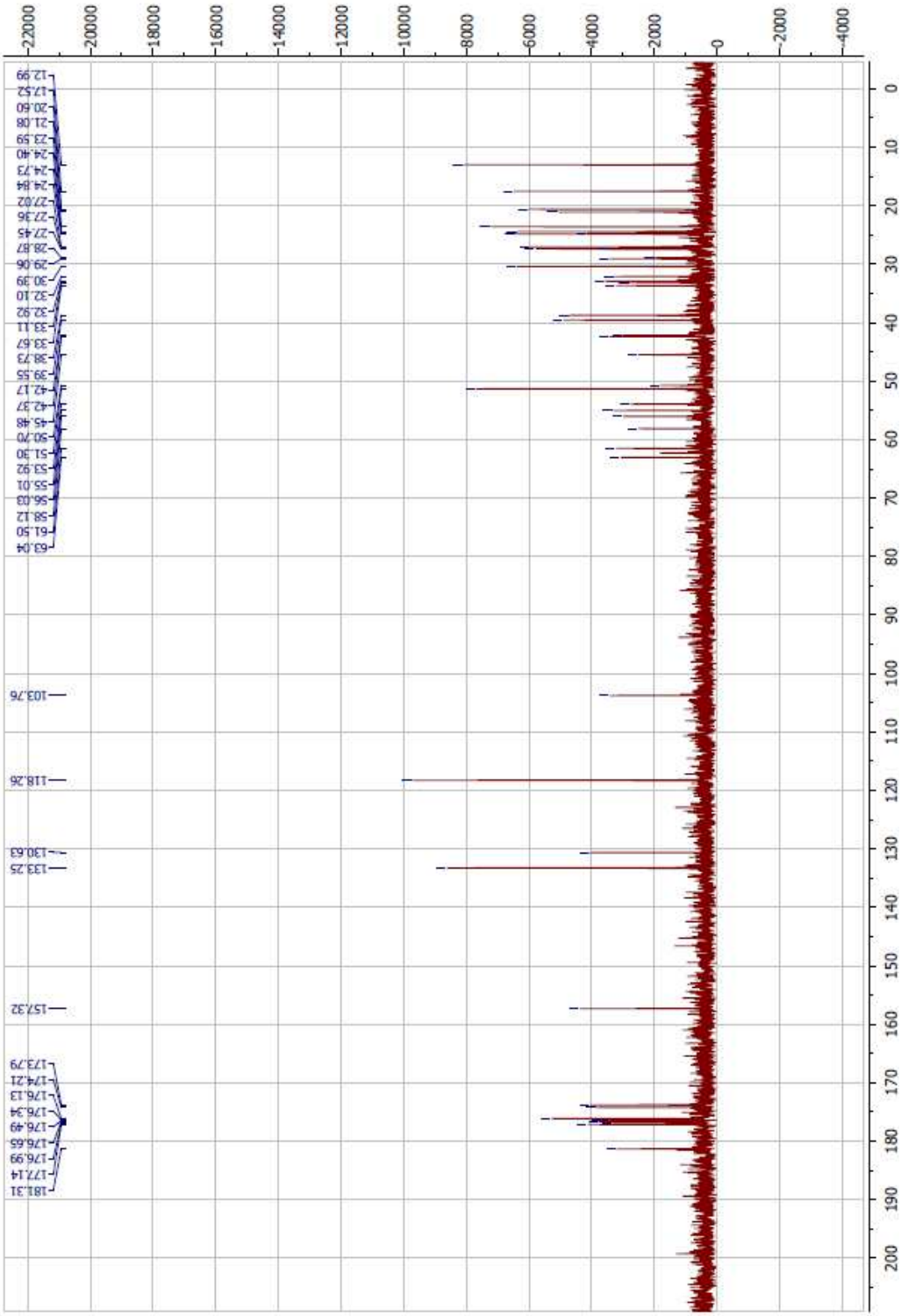


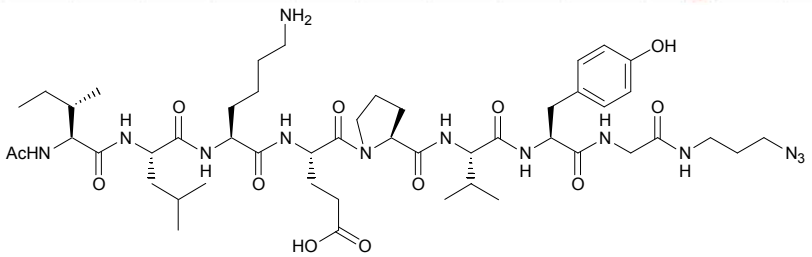


ROESY experiment for compound **9a**

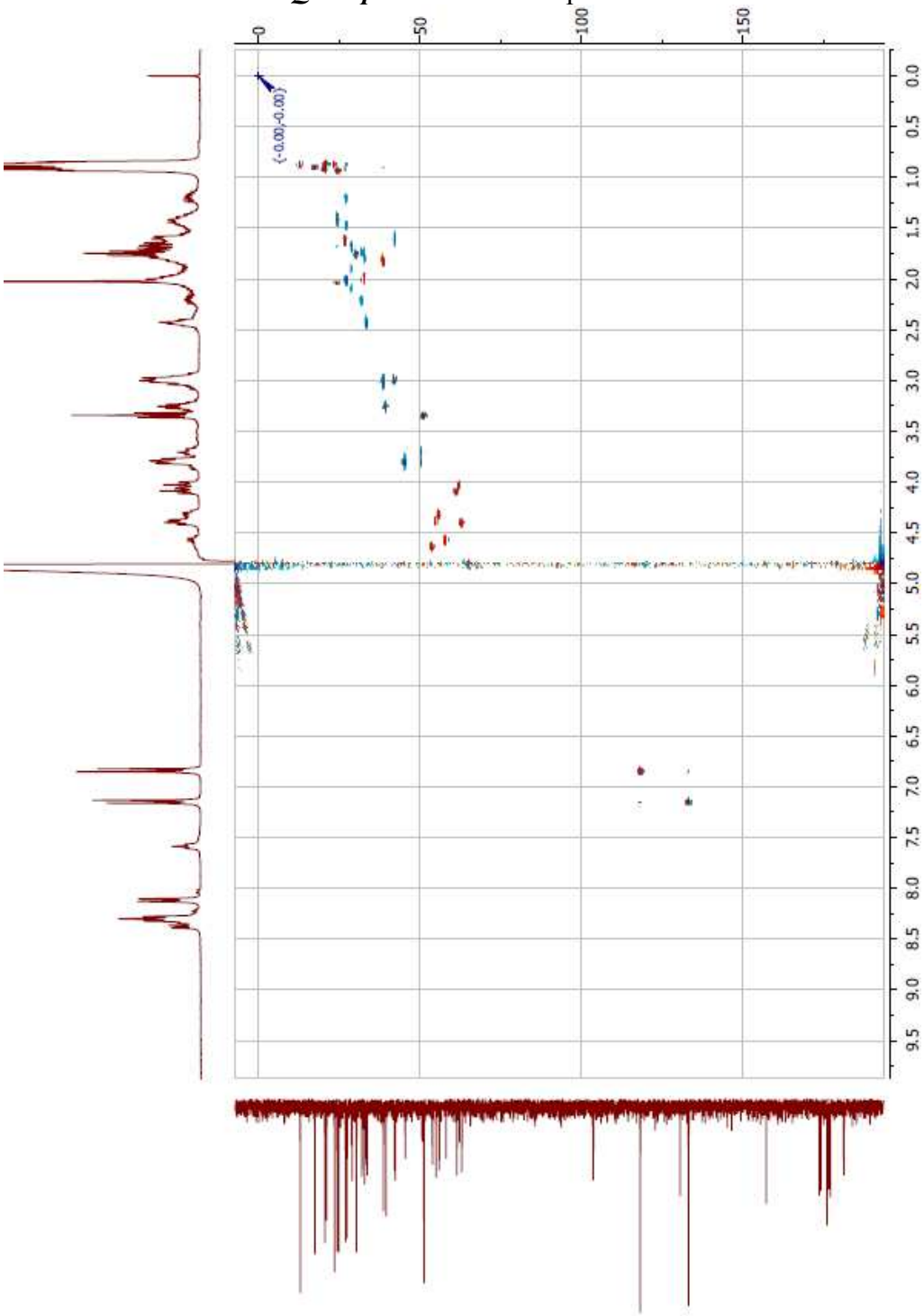


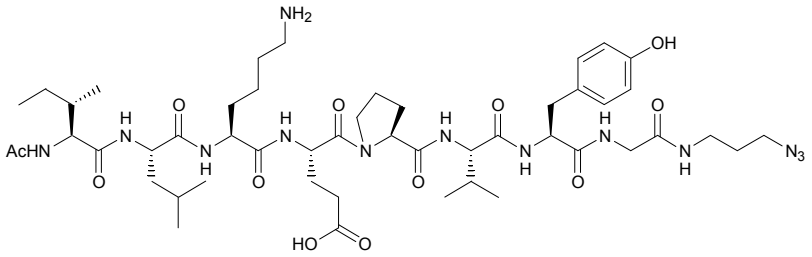
NMR ^{13}C experiment for compound **9a**





HSQC experiment for compound **9a**



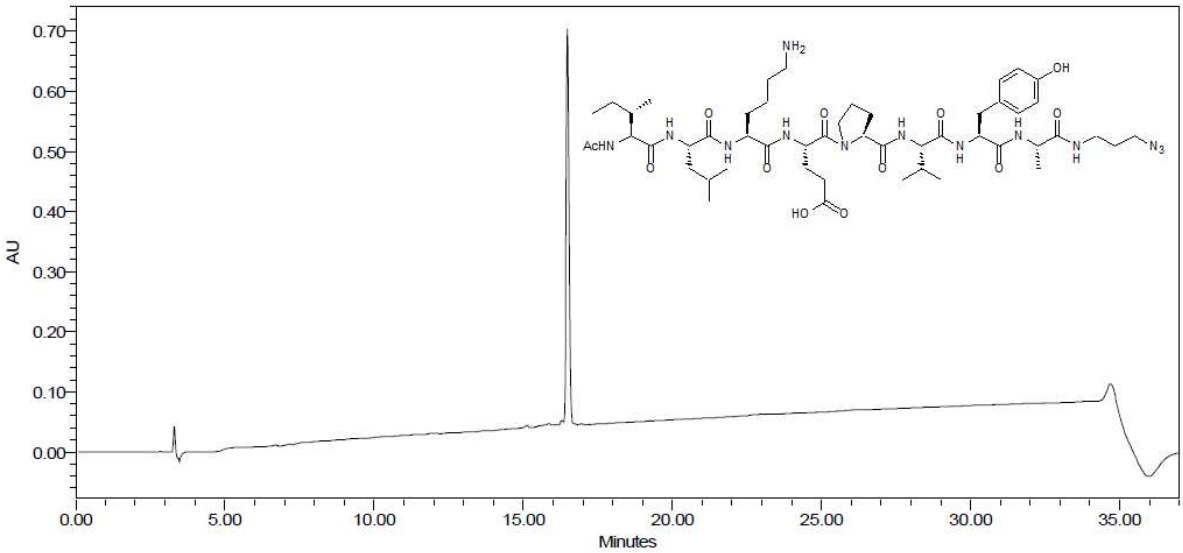


Characterization of peptide 9b:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 15.3 mg of pure peptide **9b** (29 % yield).

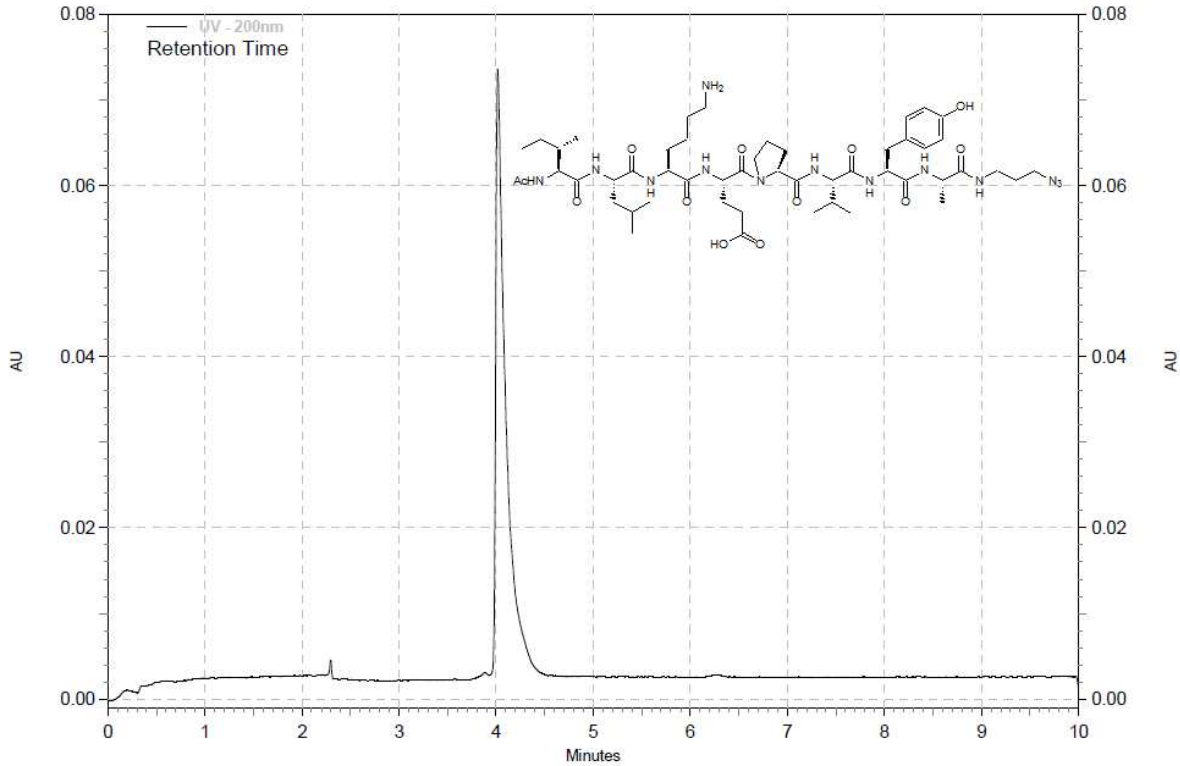
RP-HPLC analysis of 9b

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH3CN/water: 4/1 by vol. RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 9b

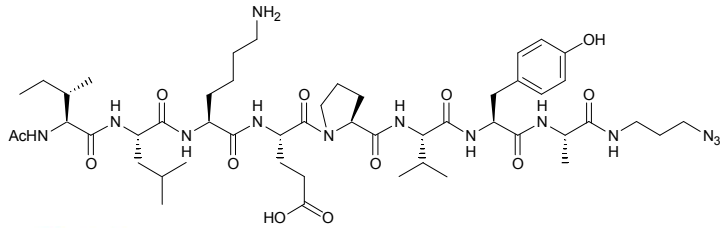
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV

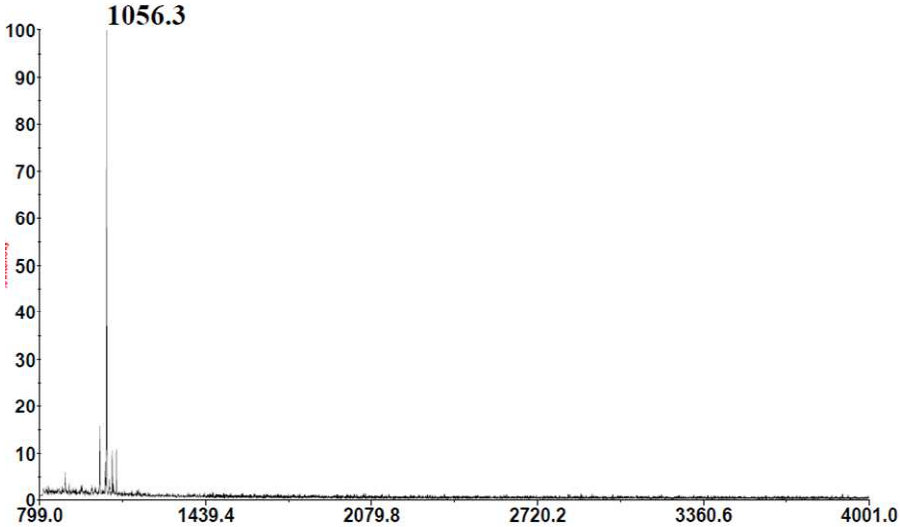


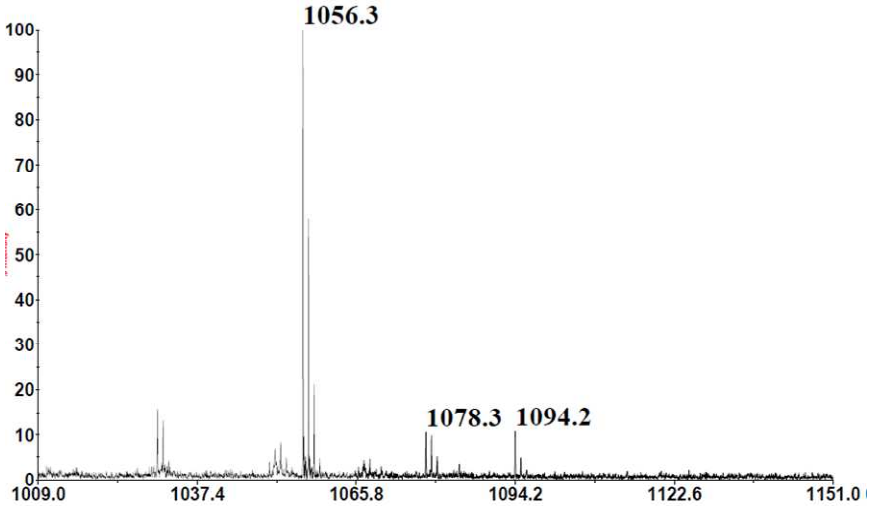
MALDI-TOF spectrum of 9b

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{50}H_{81}N_{13}O_{12}$ $[M+H]^+$ 1056.6, found 1056.3 ; $[M+Na]^+$ calcd. 1078.6, found 1078.3.





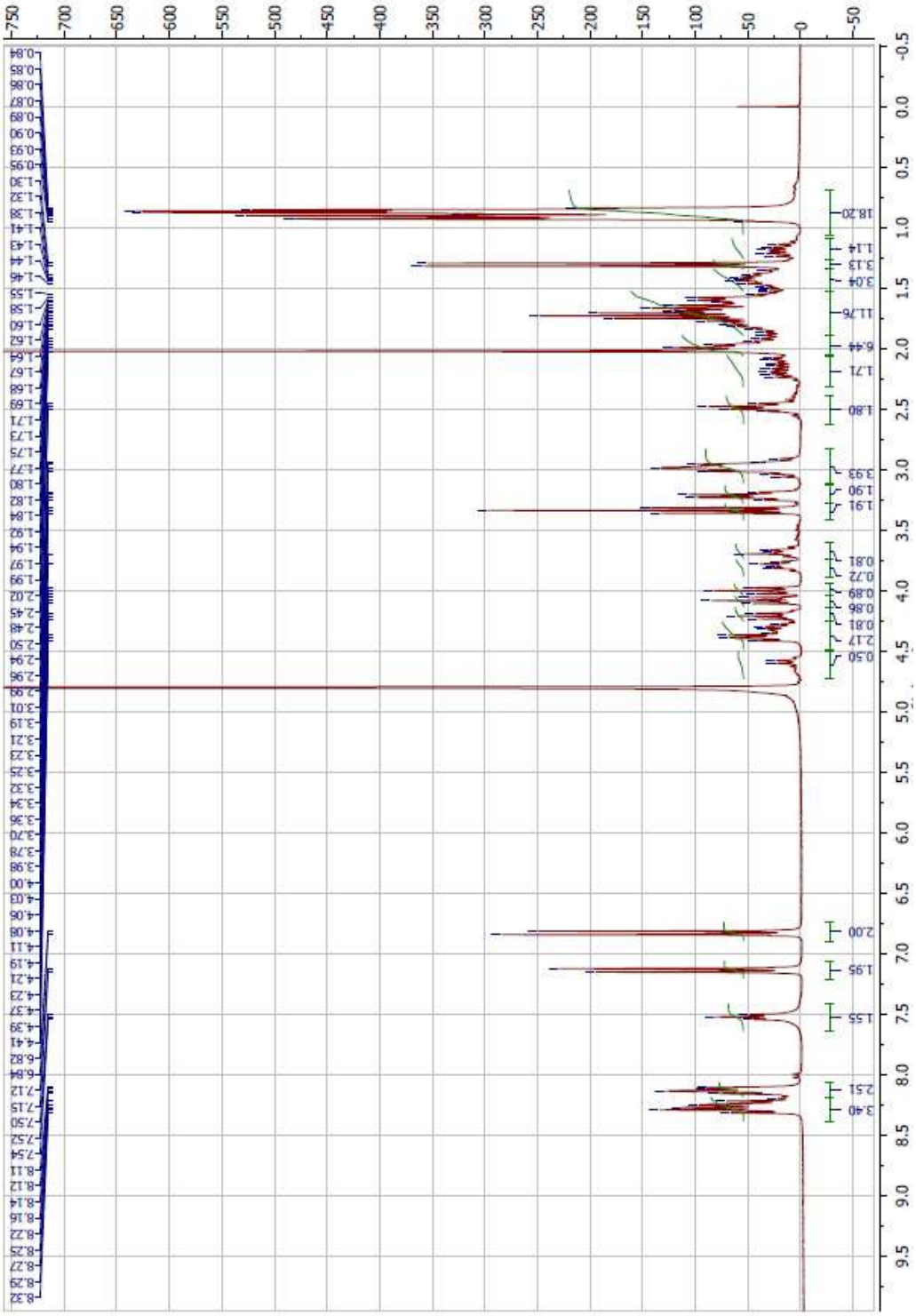


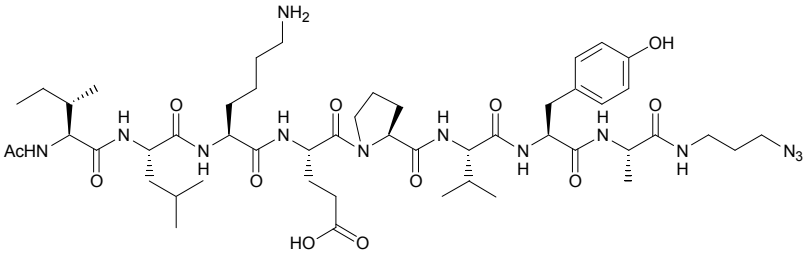
^{1}H and ^{13}C NMR for peptide 9b

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.38 – 8.19 (m, 4H), 8.19 – 8.07 (m, 3H), 7.52 (t, *J* = 5.8 Hz, 1H), 7.14 (d, *J* = 8.6 Hz, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.69 – 4.52 (m, 2H), 4.48 – 4.26 (m, 3H), 4.21 (t, *J* = 6.9 Hz, 1H), 4.08 (t, *J* = 7.4 Hz, 1H), 4.00 (t, *J* = 7.6 Hz, 1H), 3.86 – 3.74 (m, 1H), 3.74 – 3.63 (m, 1H), 3.34 (t, *J* = 6.7 Hz, 2H), 3.22 (d, *J* = 6.6 Hz, 2H), 3.10 – 2.84 (m, 4H), 2.49 (dd, *J* = 13.2, 6.7 Hz, 1H), 2.30 – 2.07 (m, 2H), 2.06 – 1.92 (m, 4H), 1.89 – 1.35 (m, 12H), 1.31 (d, *J* = 7.2 Hz, 3H), 1.25 – 1.07 (m, 1H), 1.04 – 0.74 (m, 18H).

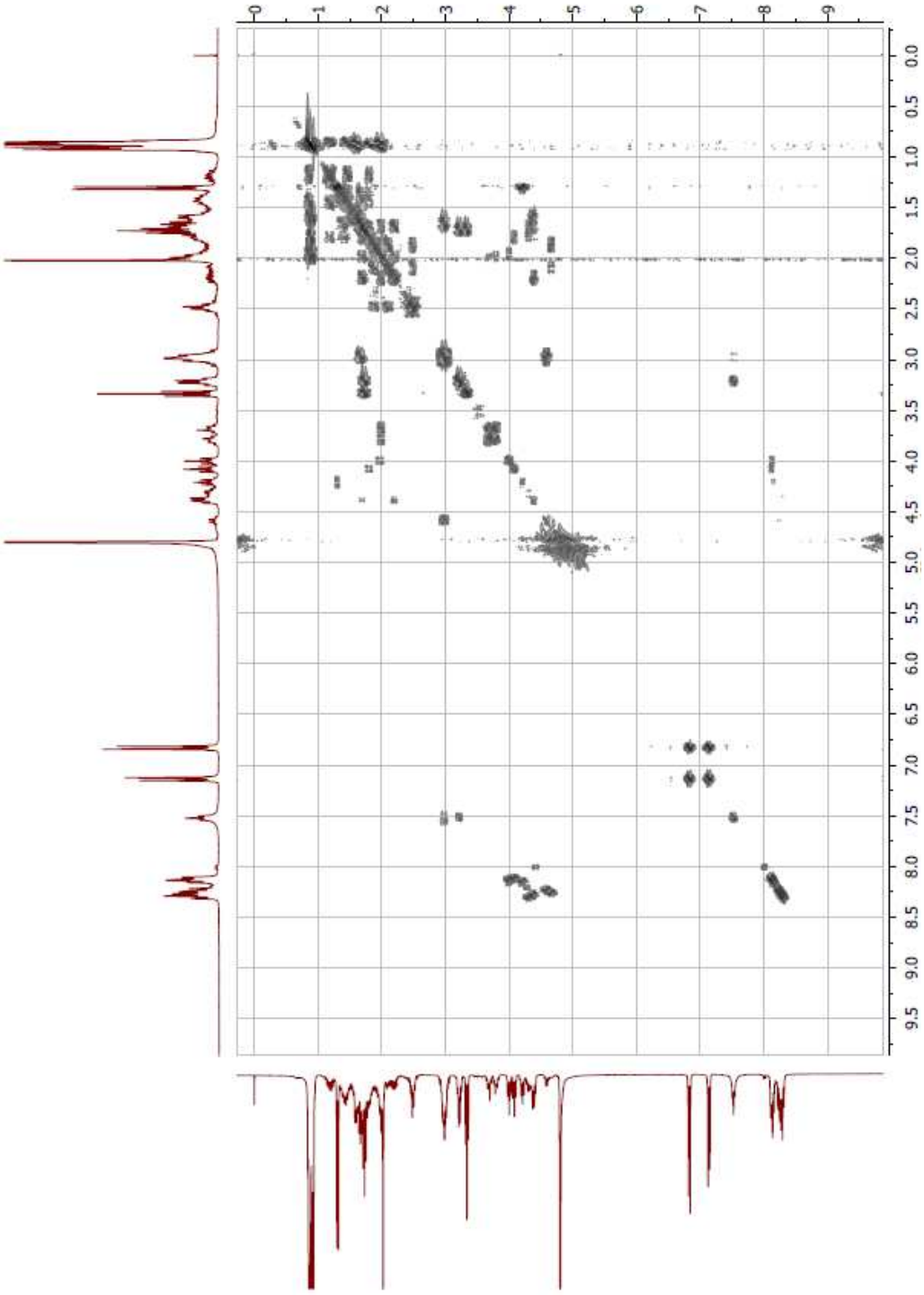
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 180.0, 177.1, 177.0, 177.0, 176.6, 176.5, 176.2, 176.0, 175.3, 174.0, 157.3, 133.2, 130.5, 118.3, 63.0, 62.4, 61.5, 57.6, 56.0, 55.0, 53.6, 52.6, 51.3, 50.7, 42.4, 42.2, 39.5, 38.8, 38.7, 33.1, 32.9, 32.5, 32.1, 30.4, 29.1, 28.4, 27.4, 27.4, 27.0, 24.8, 24.8, 24.4, 23.6, 21.0, 20.7, 19.6, 17.5, 13.0.

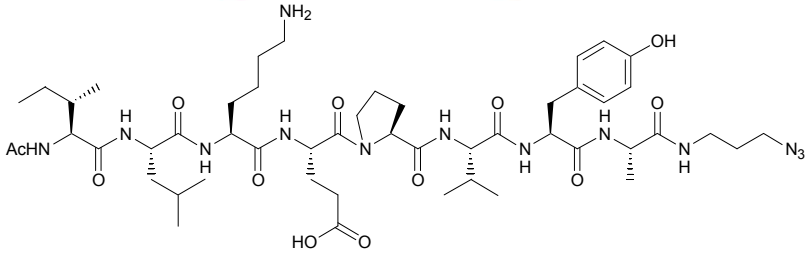
NMR ^{1}H experiment for compound **9b**



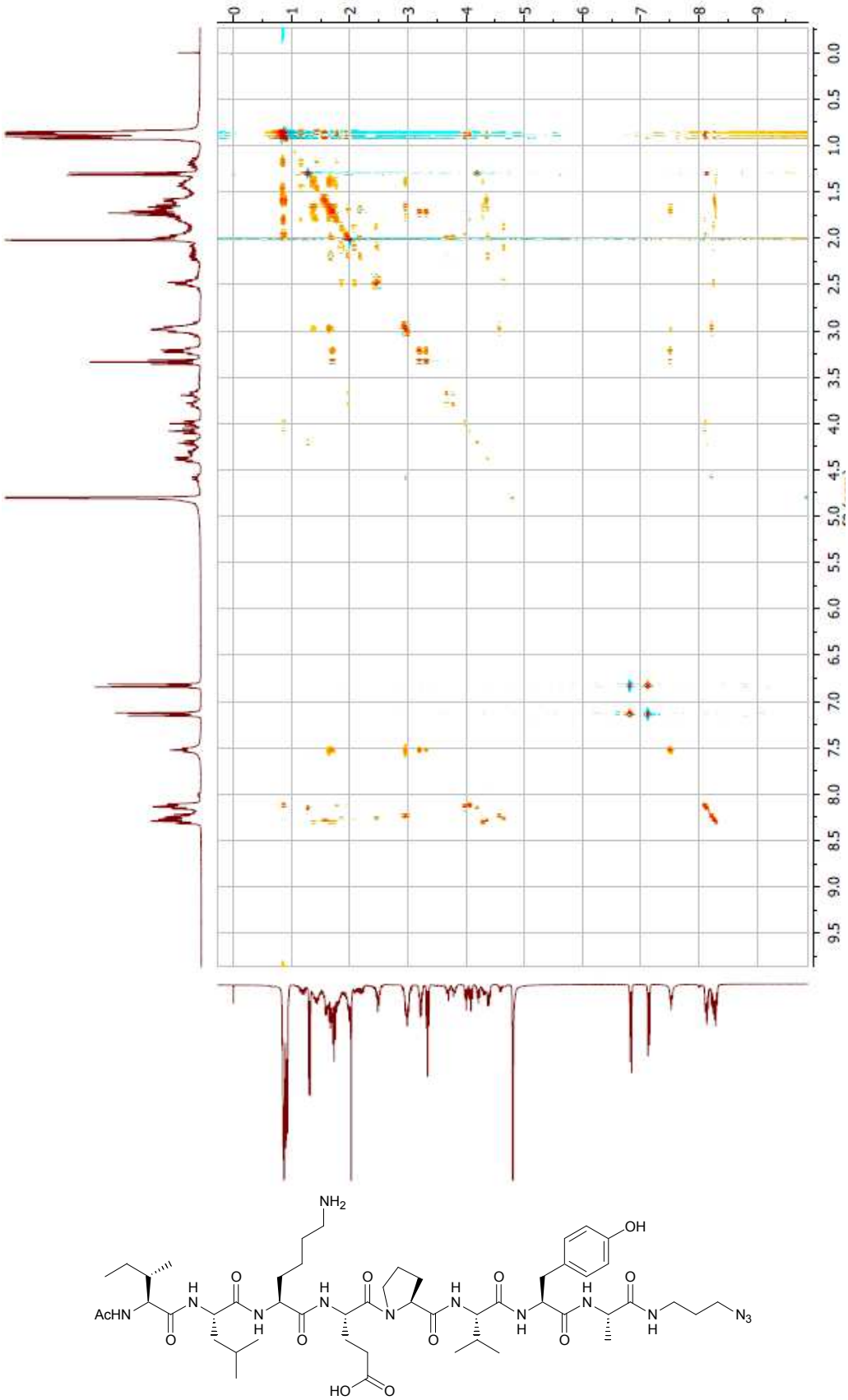


COSY experiment for compound **9b**

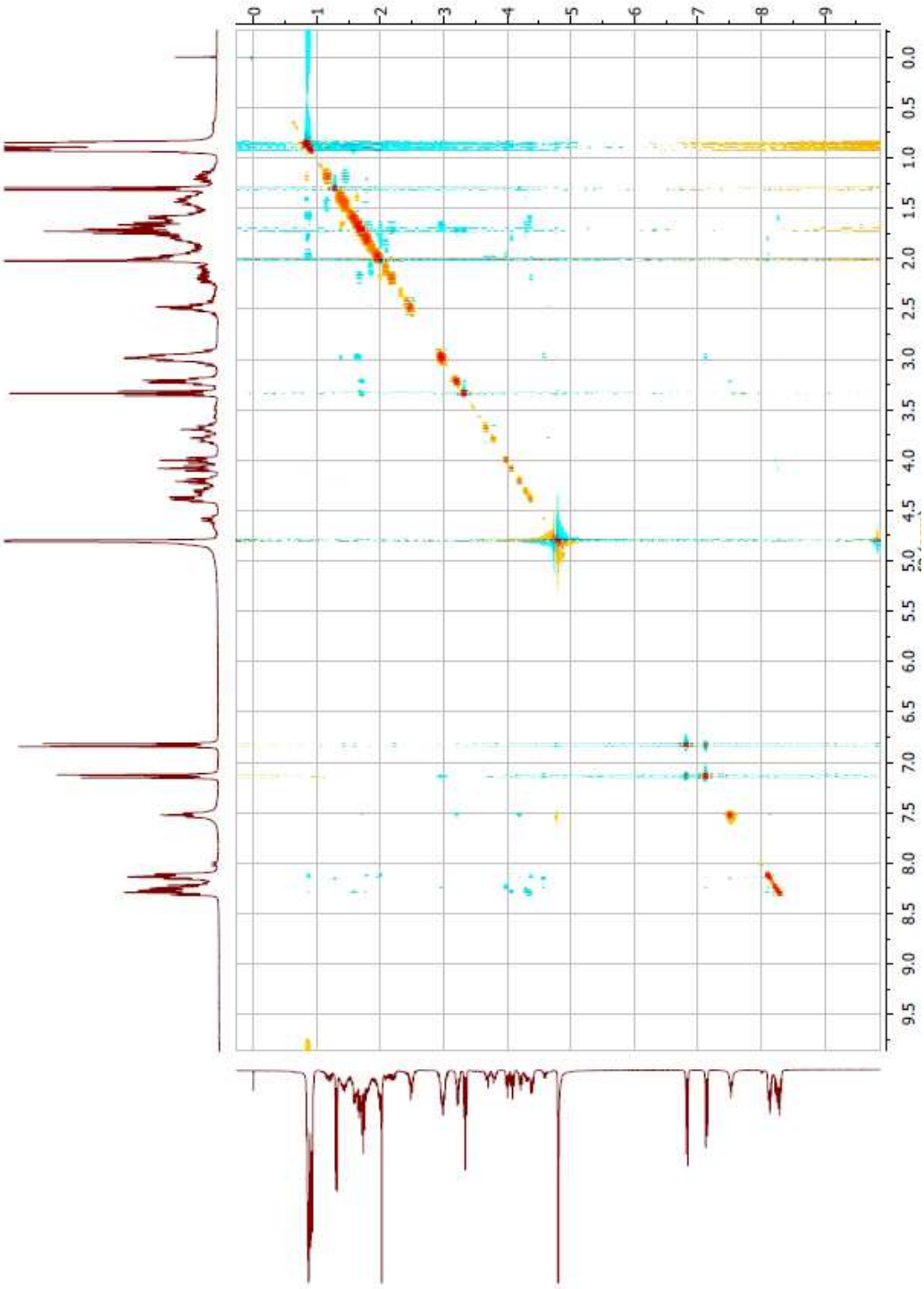


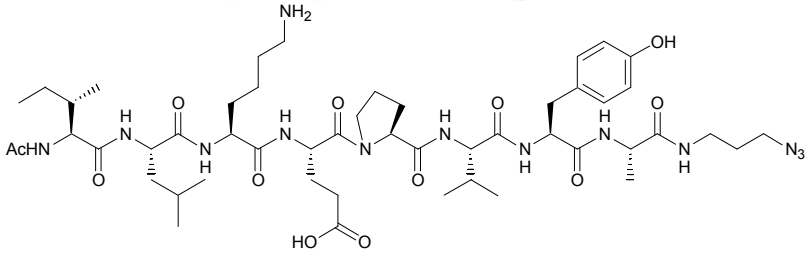


TOCSY experiment for compound **9b**

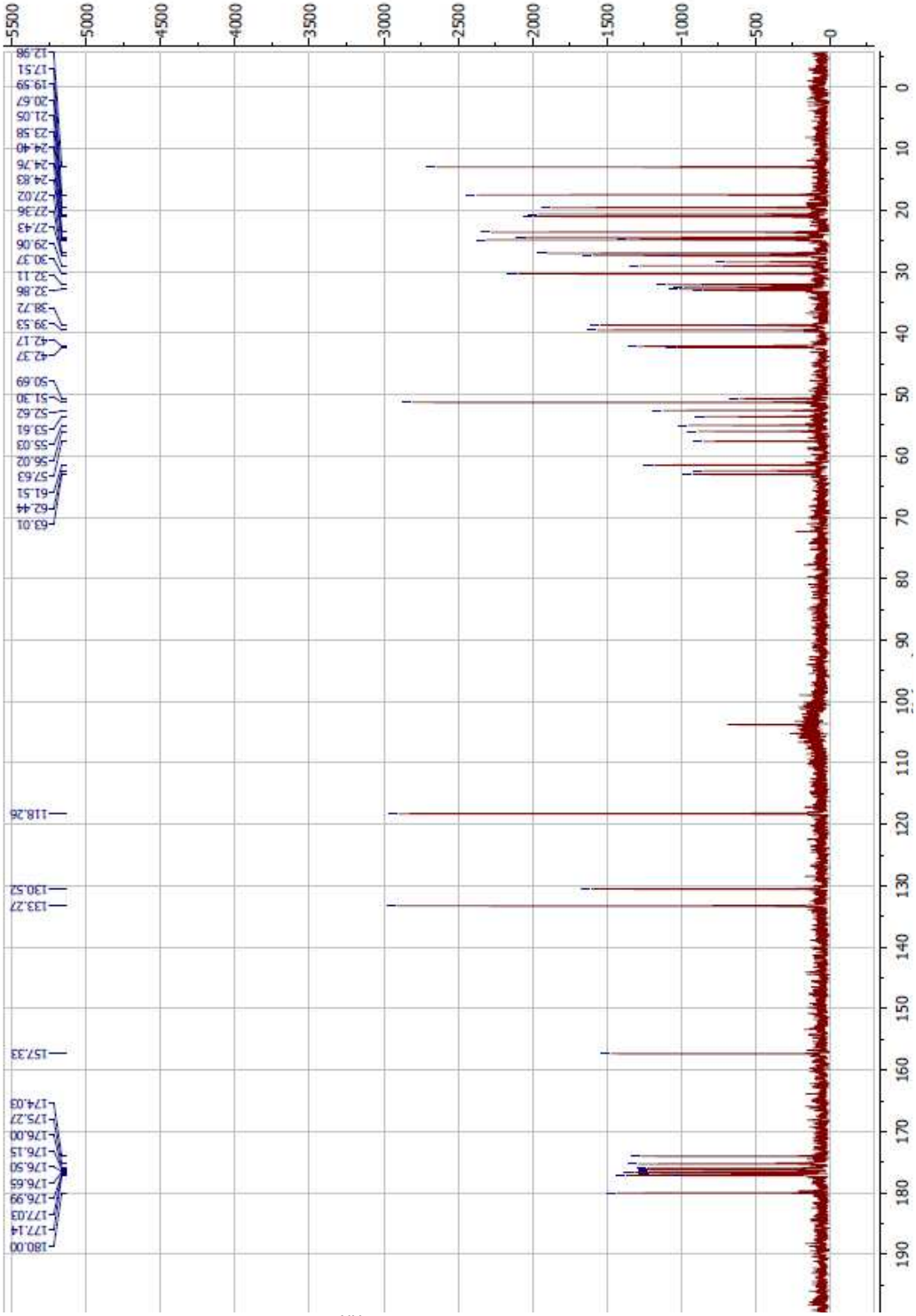


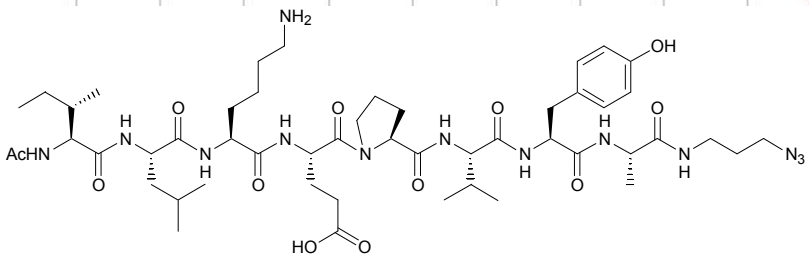
ROESY experiment for compound **9b**



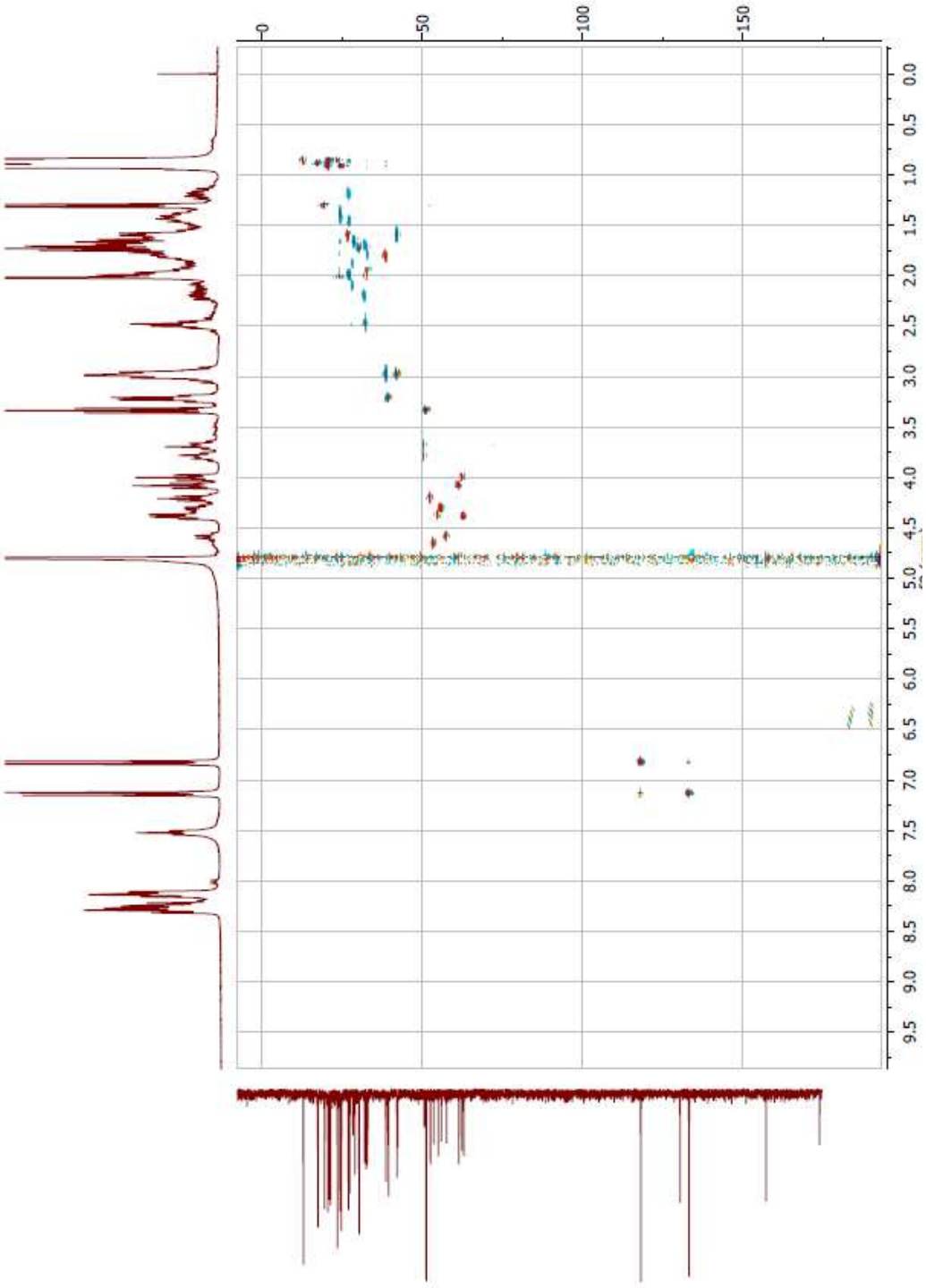


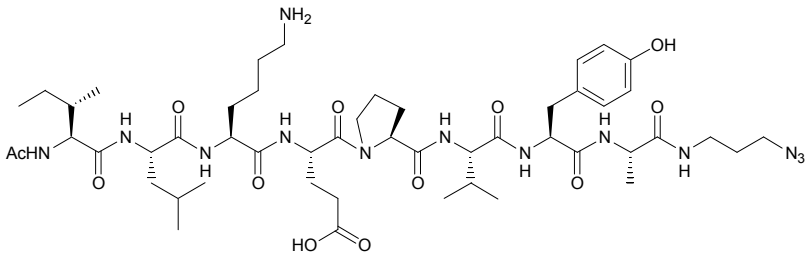
NMR ^{13}C experiment for compound **9b**





HSQC experiment for compound **9b**



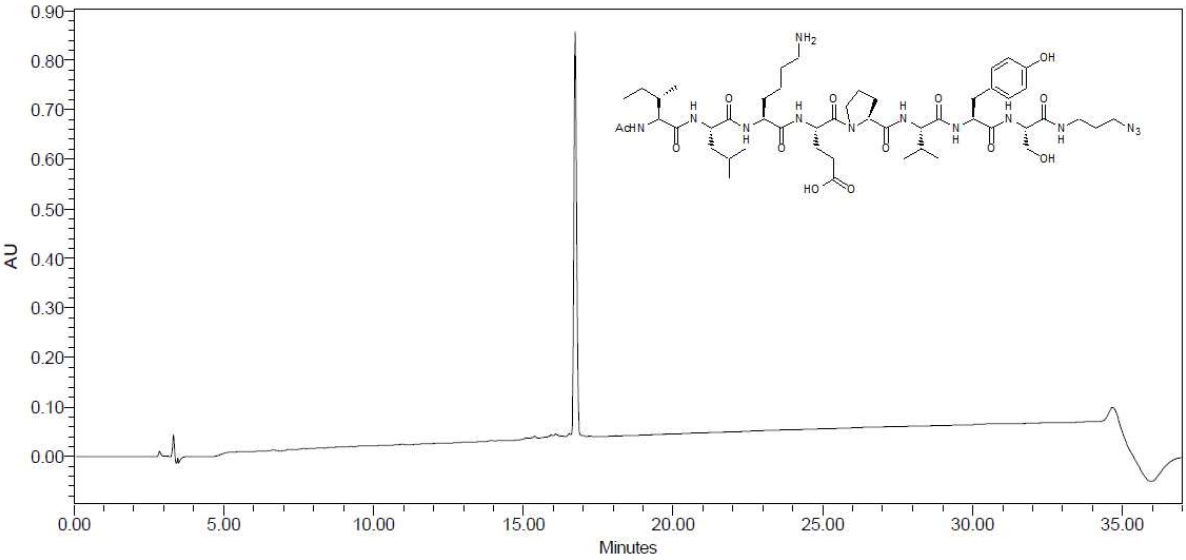


Characterization of peptide 9c:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 6.4 mg of pure peptide **9c** (12 % yield).

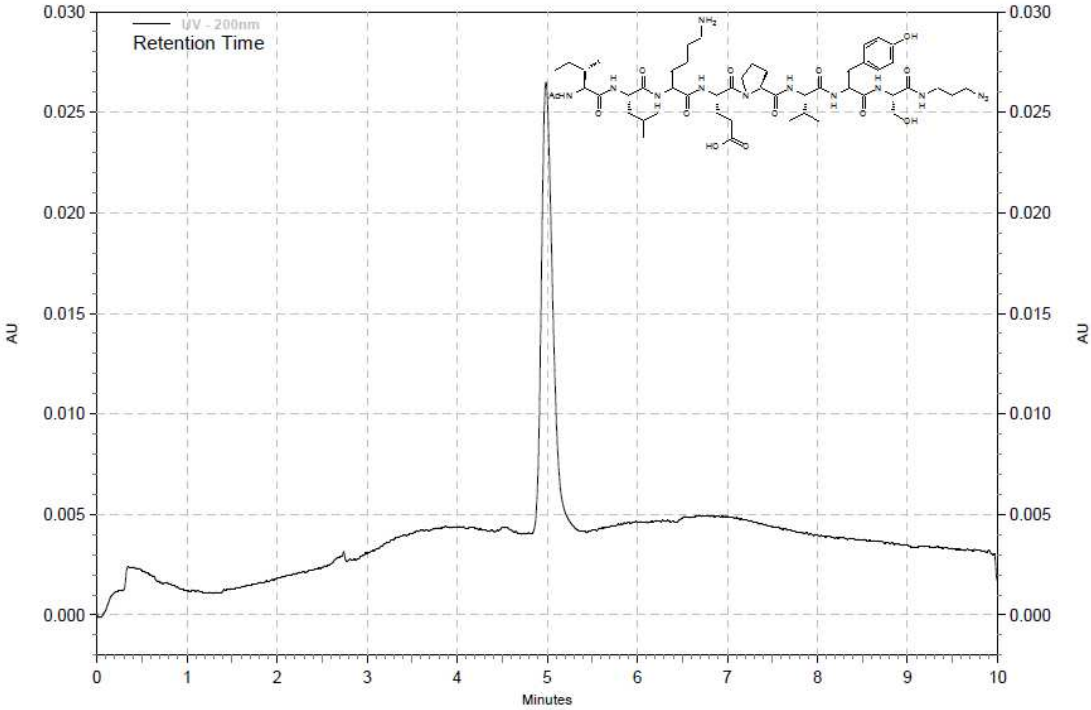
RP-HPLC analysis of 9c

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol. RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 9c

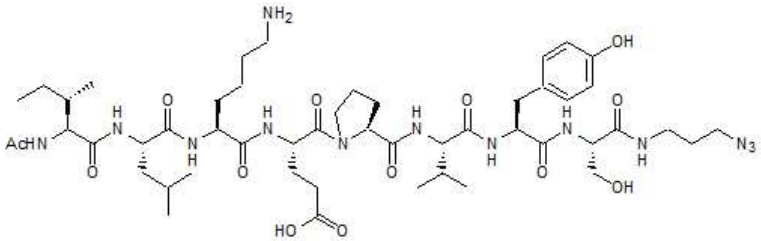
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV

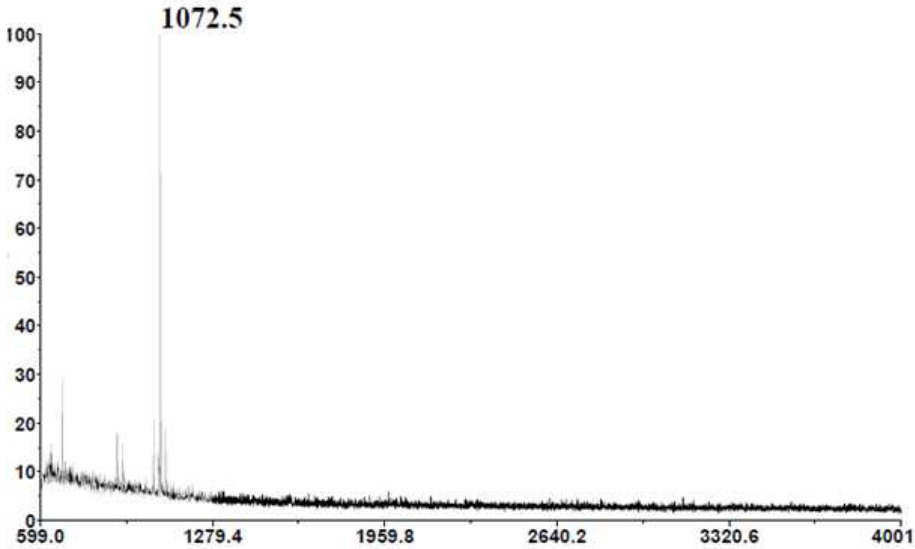


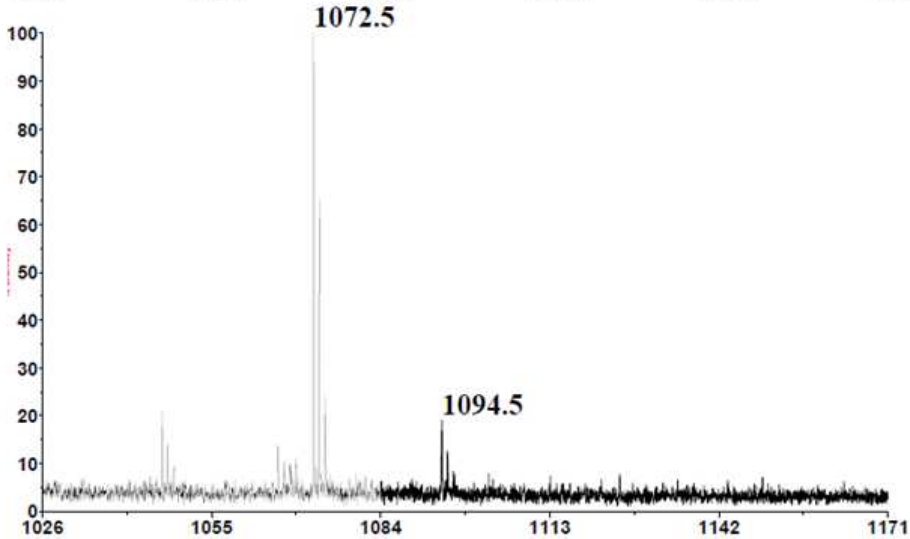
MALDI-TOF spectrum of 9c

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{50}H_{81}N_{13}O_{13}$ $[M+H]^+$ 1072.6, found 1072.5 ; $[M+Na]^+$ calcd. 1094.6, found 1094.5.





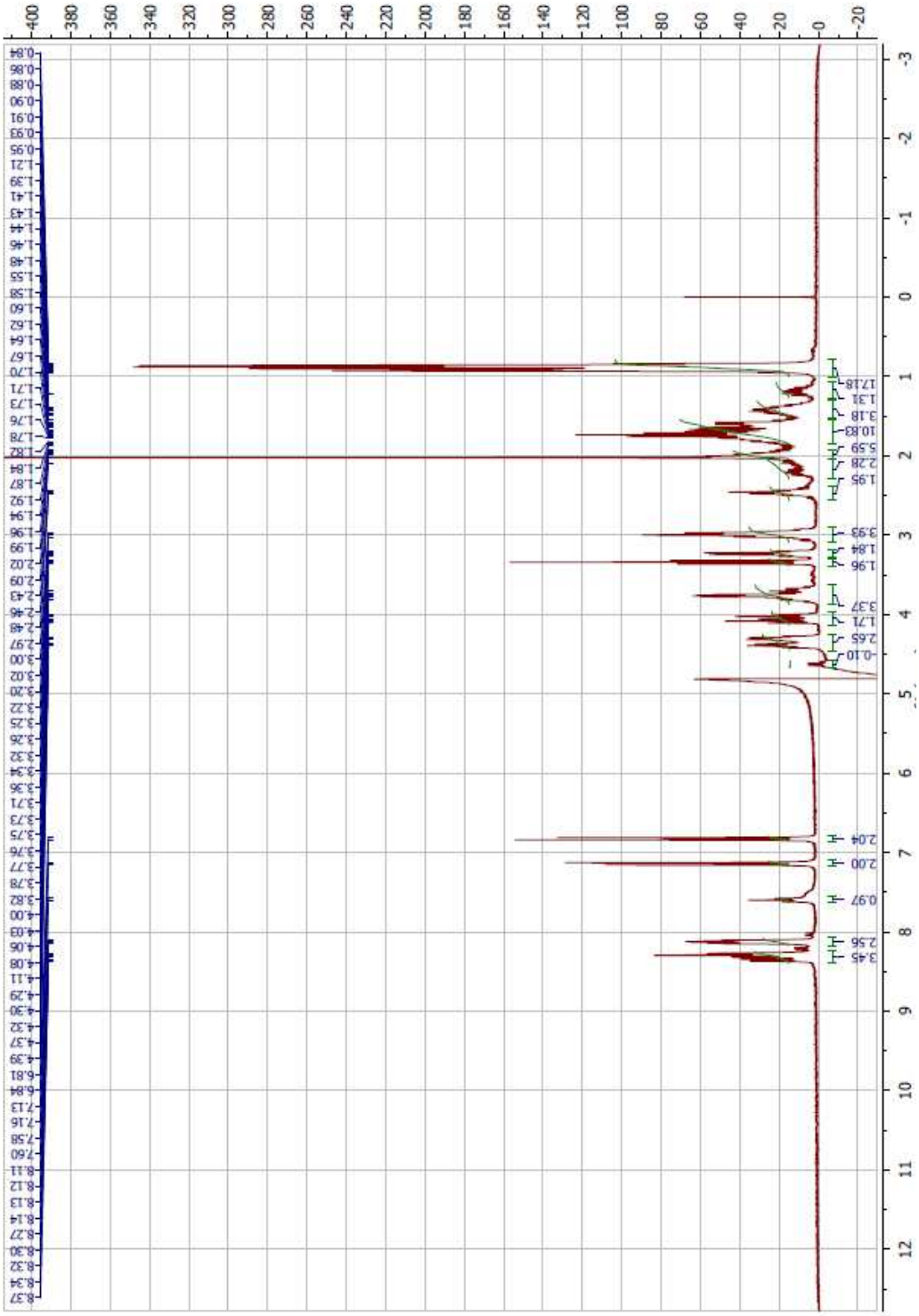


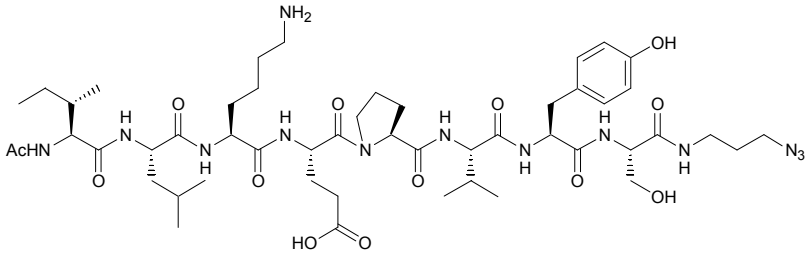
^{1}H and ^{13}C NMR for peptide 9c

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.37 – 8.37 (m, 4H), 8.14 – 8.11 (m, 3H), 7.60 (t, J = 5.9 Hz, 1H), 7.14 (d, J = 8.6 Hz, 2H), 6.83 (d, J = 8.6 Hz, 2H), 4.64 (m, 2H), 4.42 – 4.27 (m, 4H), 4.08 (t, J = 7.3 Hz, 1H), 4.03 (t, J = 7.7 Hz, 1H), 3.82 – 3.67 (m, 4H), 3.34 (t, J = 6.6 Hz, 2H), 3.23 (m, 2H), 3.00 (m, 4H), 2.48 (m, 2H), 2.20 (m, 2H), 2.04 – 1.93 (m, 6H), 1.85 – 1.54 (m, 11H), 1.52 – 1.30 (m, 3H), 1.27 – 1.07 (m, 1H), 1.01 – 0.78 (m, 18H).

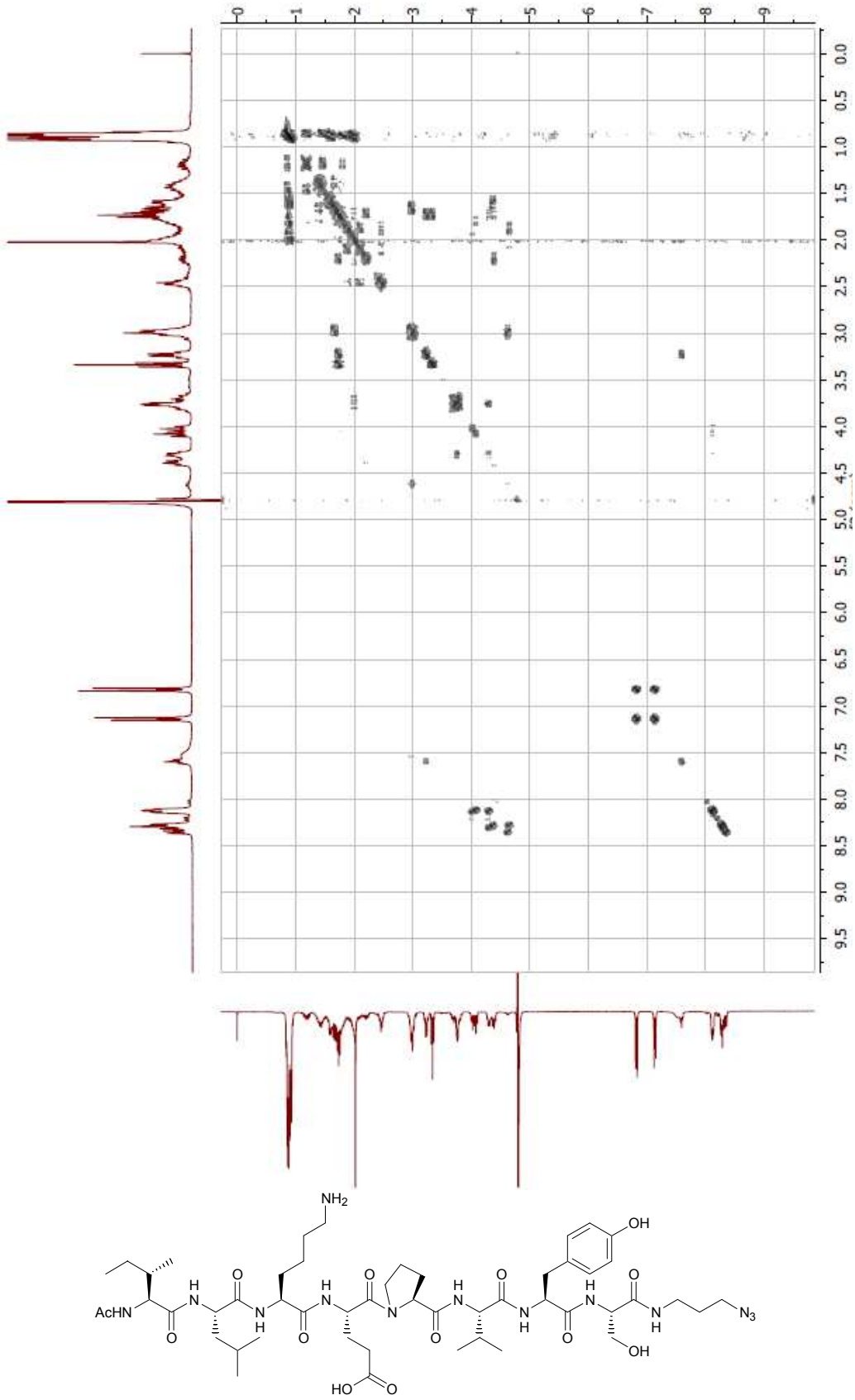
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 216.6, 180.4, 177.0, 176.8, 176.5, 176.3, 176.0, 175.8, 175.5, 173.9, 173.6, 157.2, 133.1, 130.3, 118.1, 103.6, 77.8, 63.8, 62.1, 61.3, 58.2, 57.7, 55.8, 54.8, 53.6, 51.1, 50.5, 42.2, 42.0, 39.5, 38.6, 32.8, 32.8, 31.9, 30.2, 28.9, 27.4, 27.3, 27.2, 26.8, 24.6, 24.6, 24.2, 23.4, 20.9, 20.5, 17.3, 12.8.

NMR ^{1}H experiment for compound **9c**

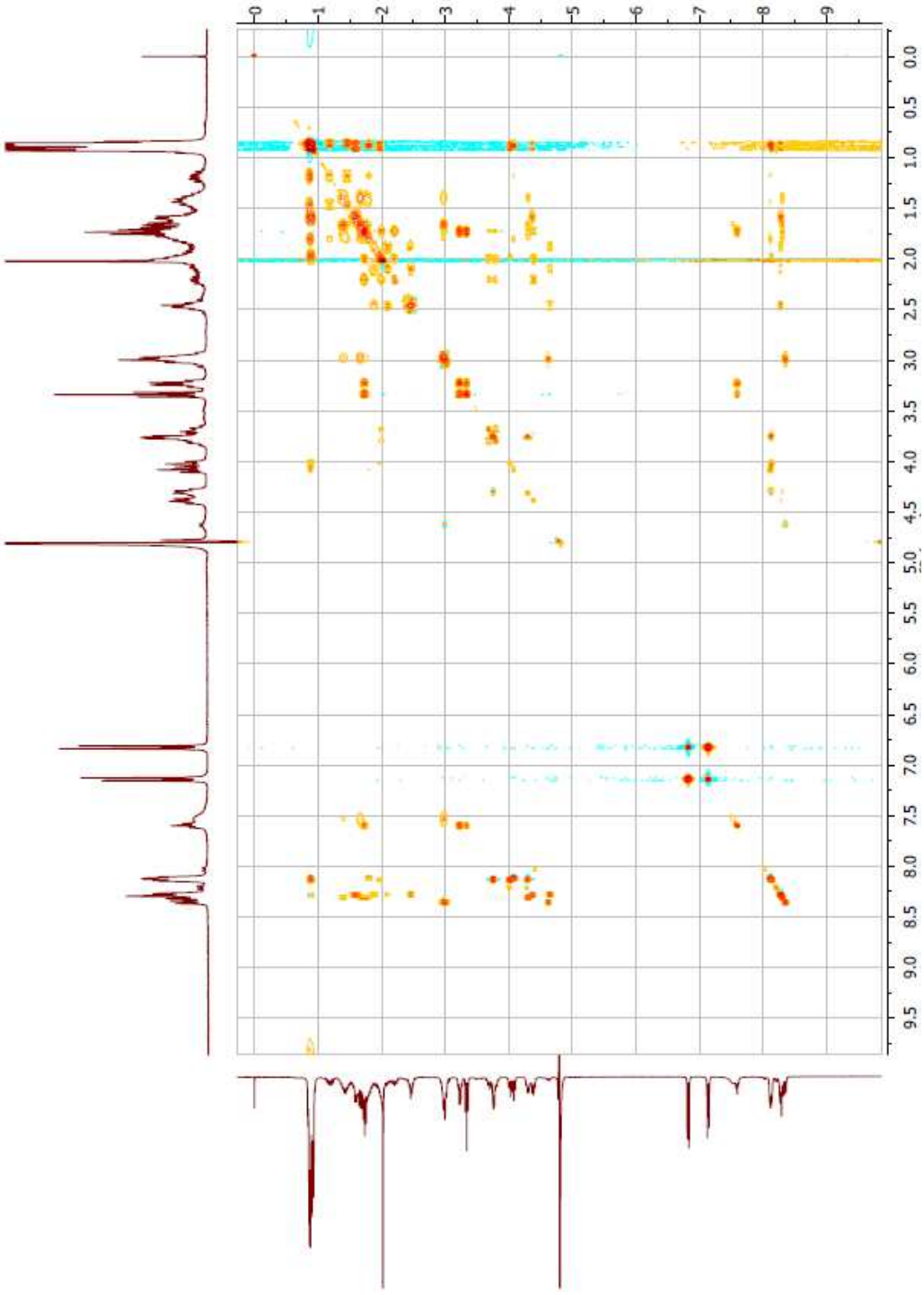


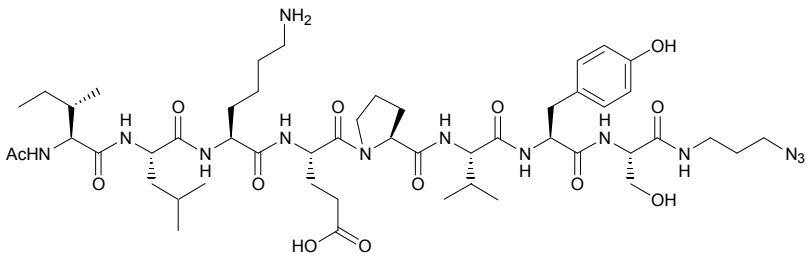


COSY experiment for compound **9c**

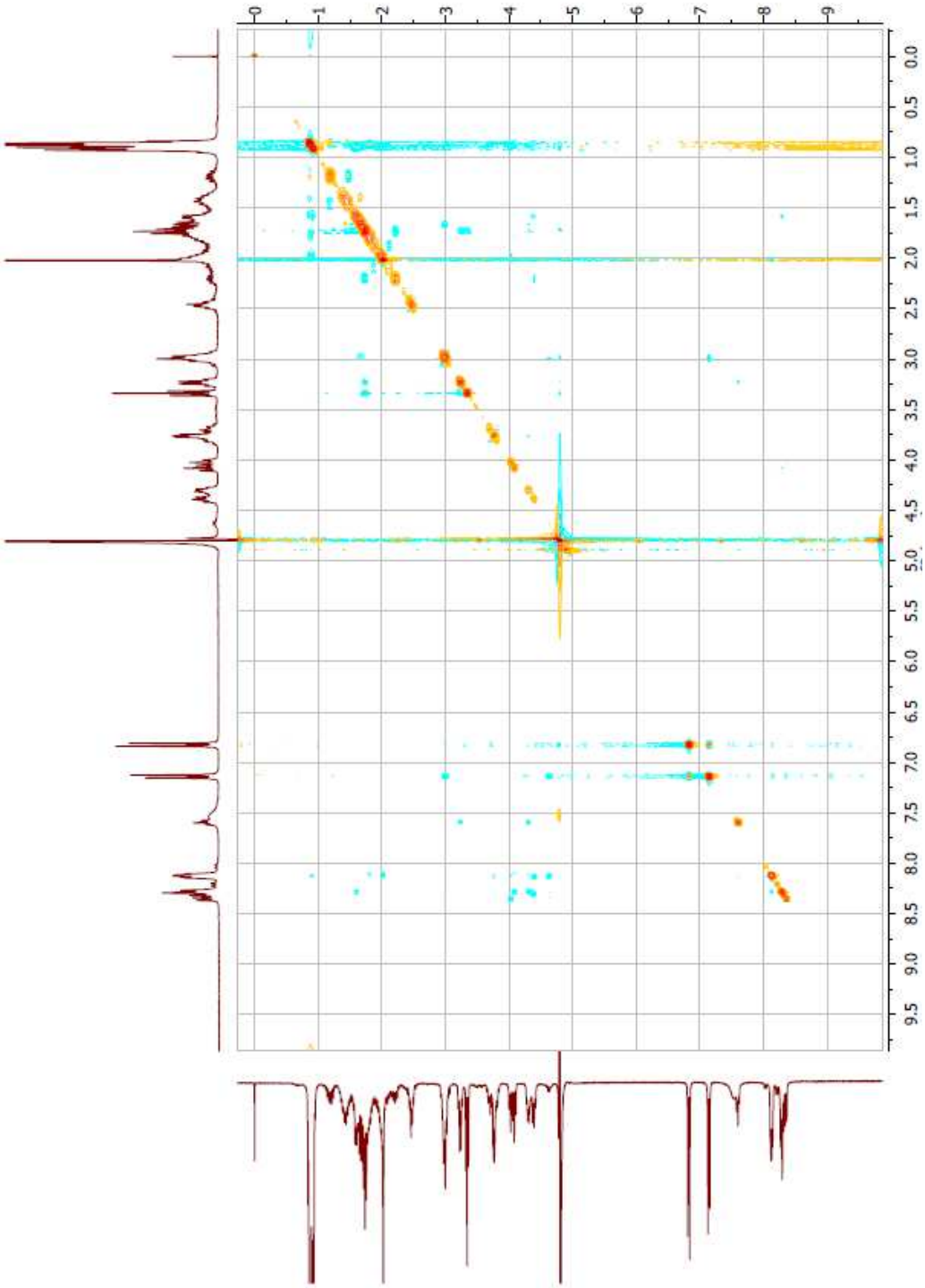


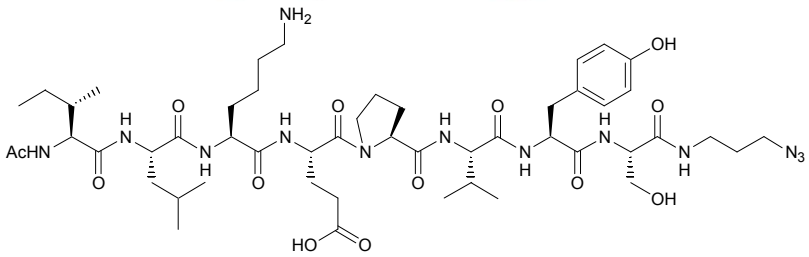
TOCSY experiment for compound **9c**



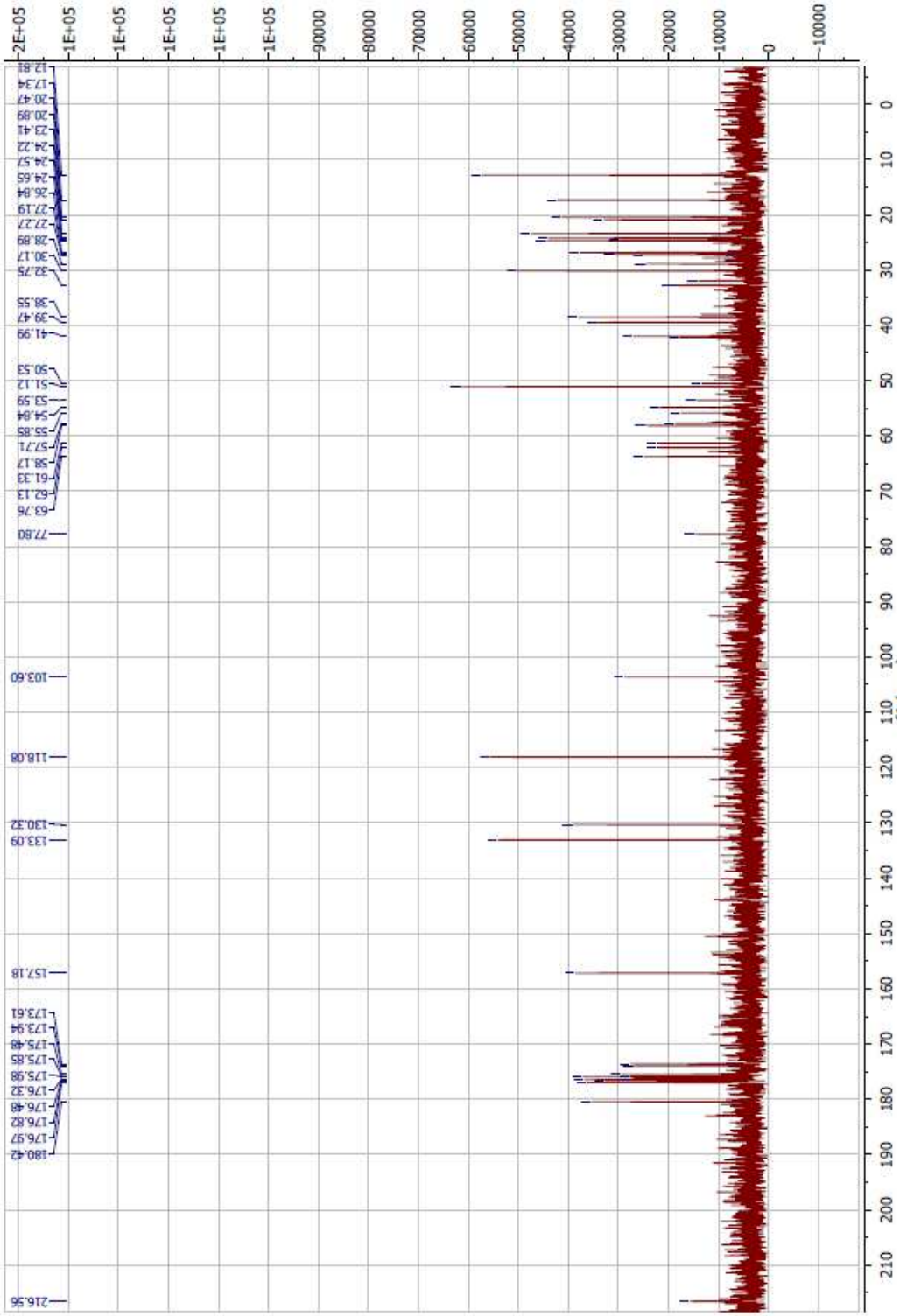


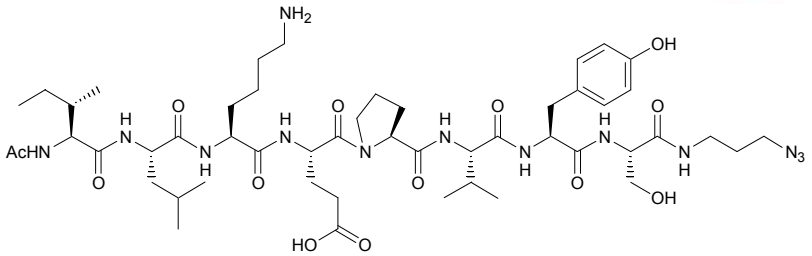
ROESY experiment for compound **9c**



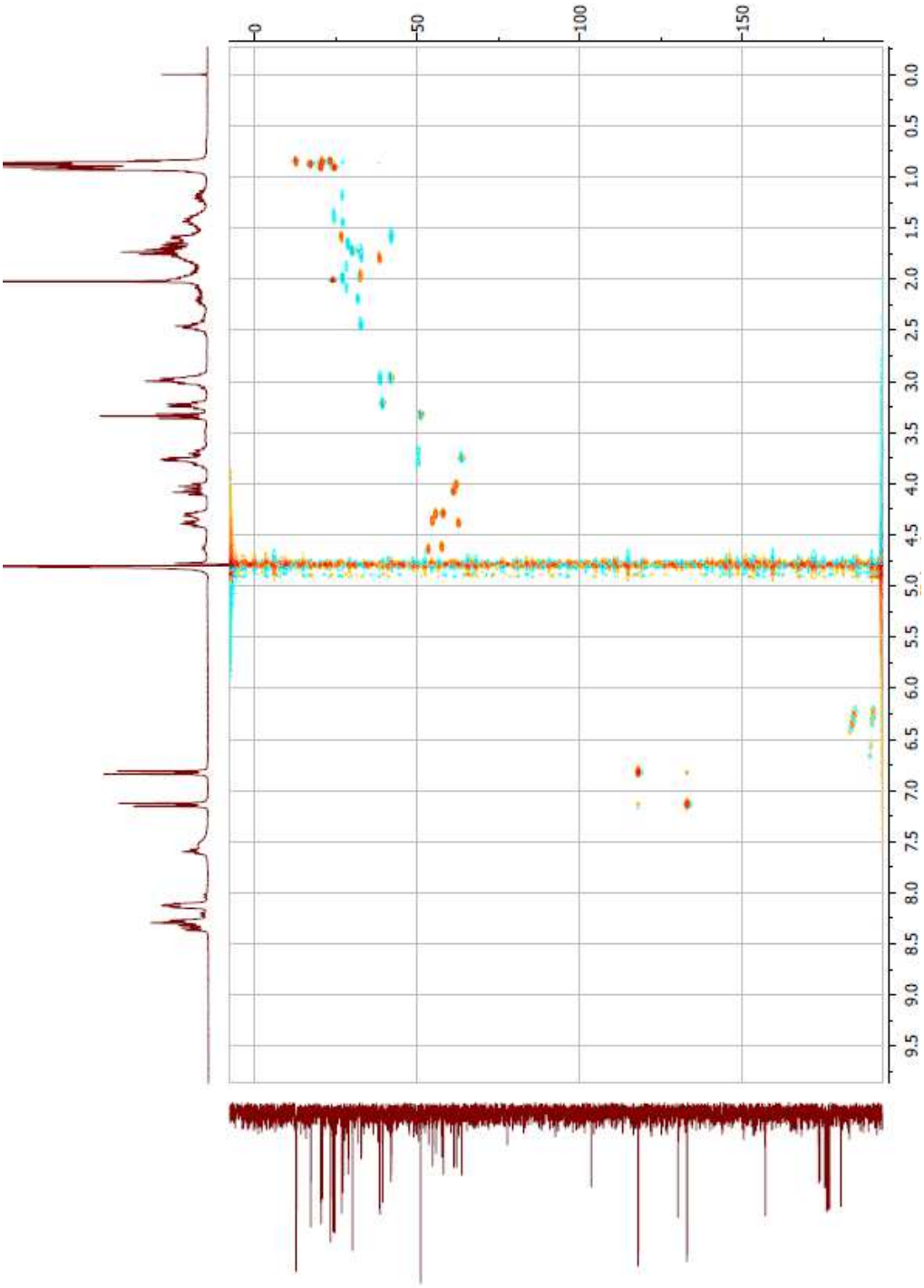


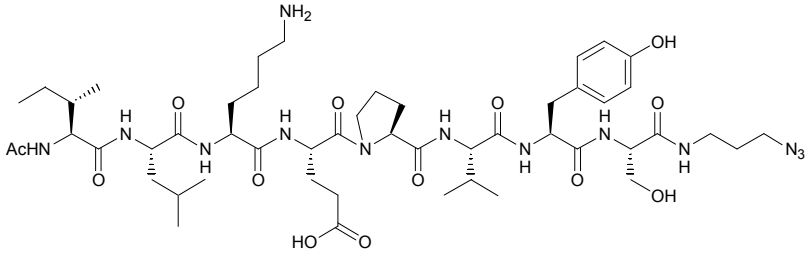
NMR ^{13}C experiment for compound **9c**





HSQC experiment for compound **9c**



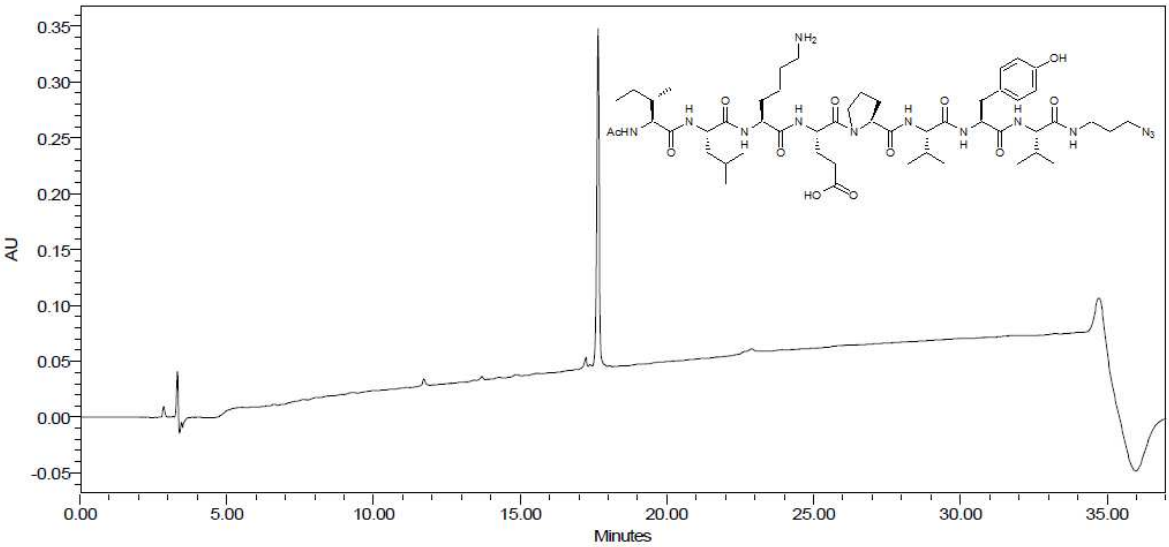


Characterization of peptide 9d:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 7.6 mg of pure peptide **9d** (14 % yield).

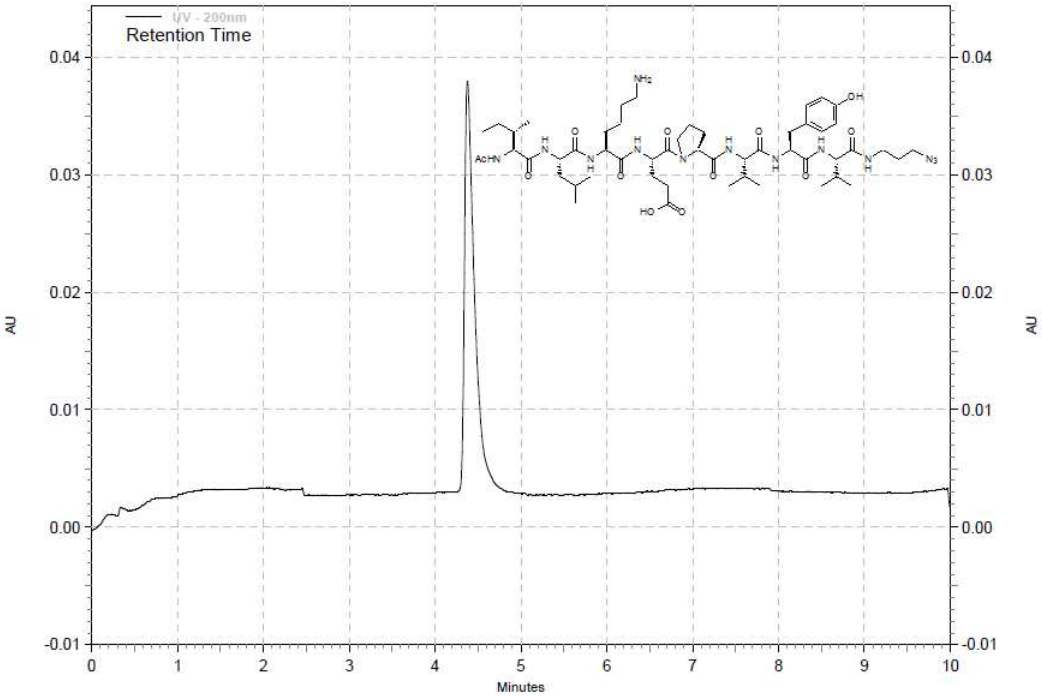
RP-HPLC analysis of 9d

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 9d

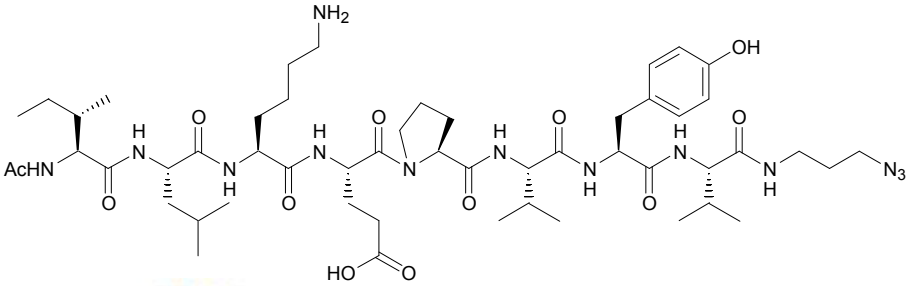
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.

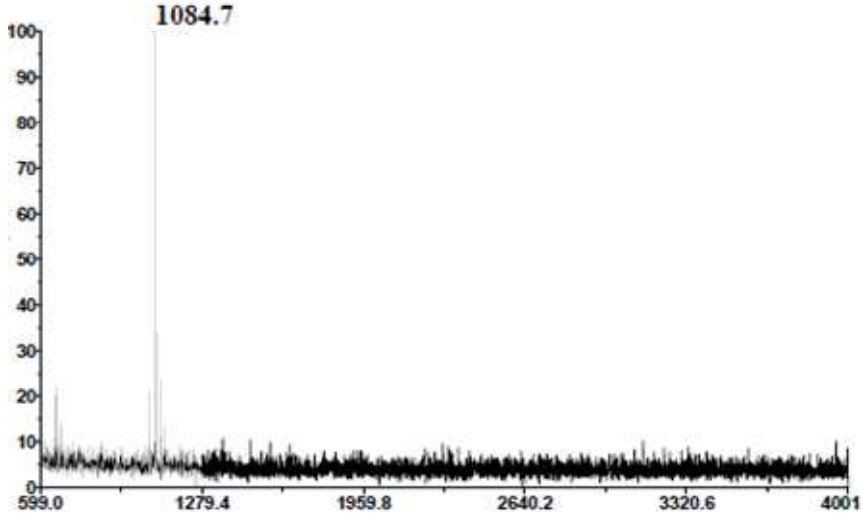


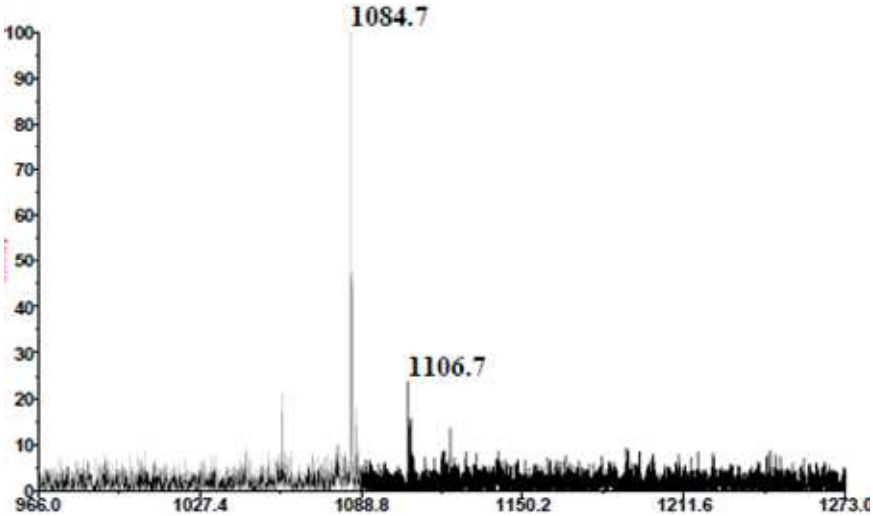
MALDI-TOF spectrum of 9d

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{52}H_{85}N_{13}O_{12}$ $[M+H]^+$ 1084.6, found 1084.7 ; $[M+Na]^+$ calcd. 1106.6, found 1106.7.





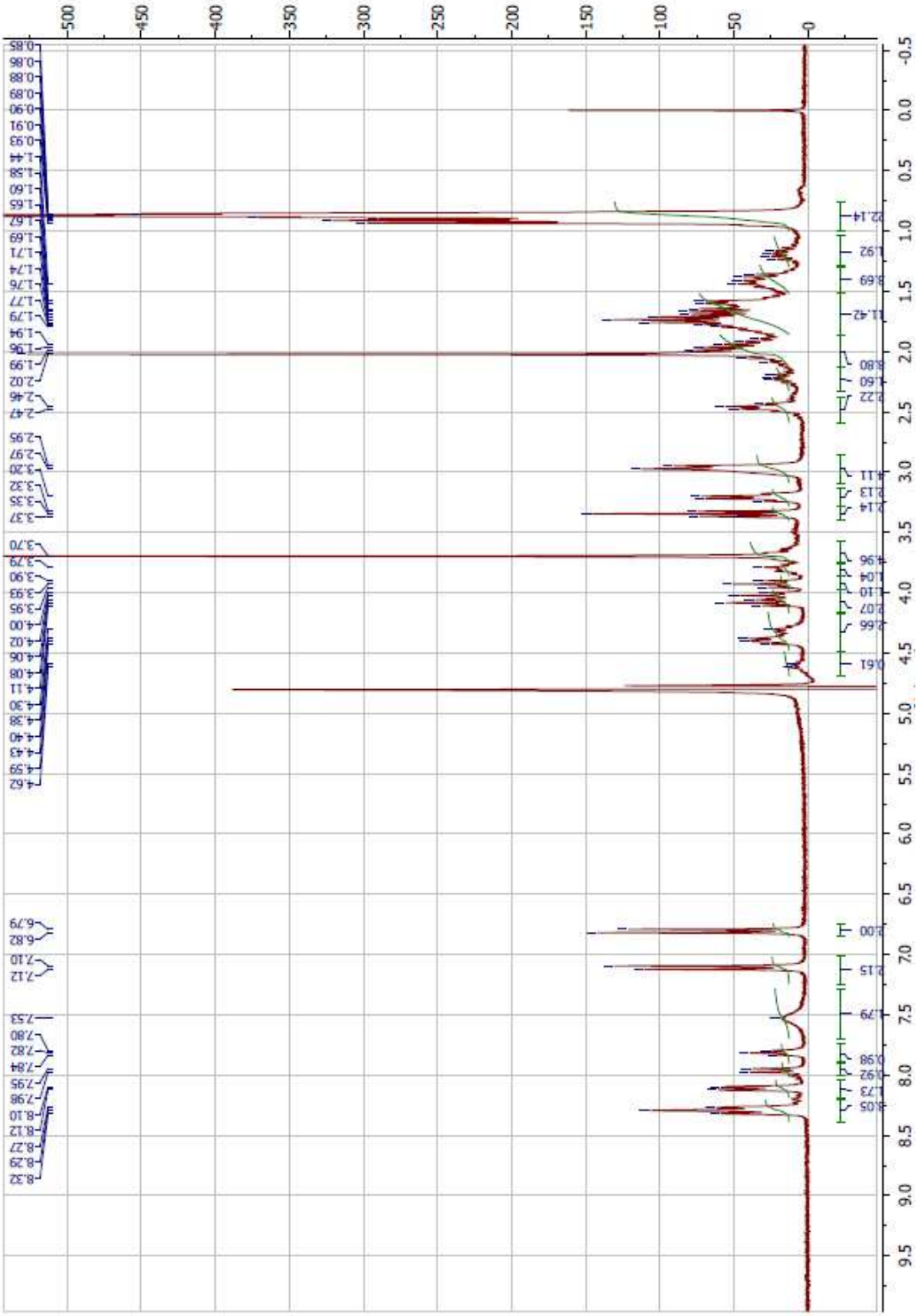


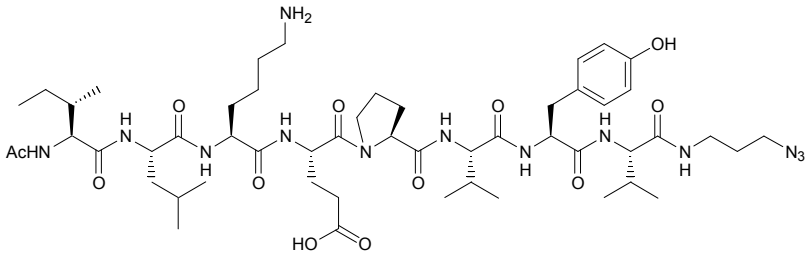
^{1}H and ^{13}C NMR for peptide 9d

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.38 – 8.23 (m, 3H), 8.18 – 8.05 (m, 2H), 8.02 – 7.89 (m, 1H), 7.82 (t, *J* = 5.9 Hz, 1H), 7.53 (s, 2H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 2H), 4.67 – 4.51 (m, 1H), 4.46 – 4.24 (m, 3H), 4.16 – 3.97 (m, 2H), 3.93 (t, *J* = 8.3 Hz, 1H), 3.85 – 3.75 (m, 1H), 3.77 – 3.60 (m, 1H), 3.35 (t, *J* = 6.7 Hz, 2H), 3.21 (d, *J* = 6.4 Hz, 2H), 3.08 – 2.84 (m, 4H), 2.56 – 2.39 (m, 2H), 2.30 – 2.15 (m, 1H), 2.14

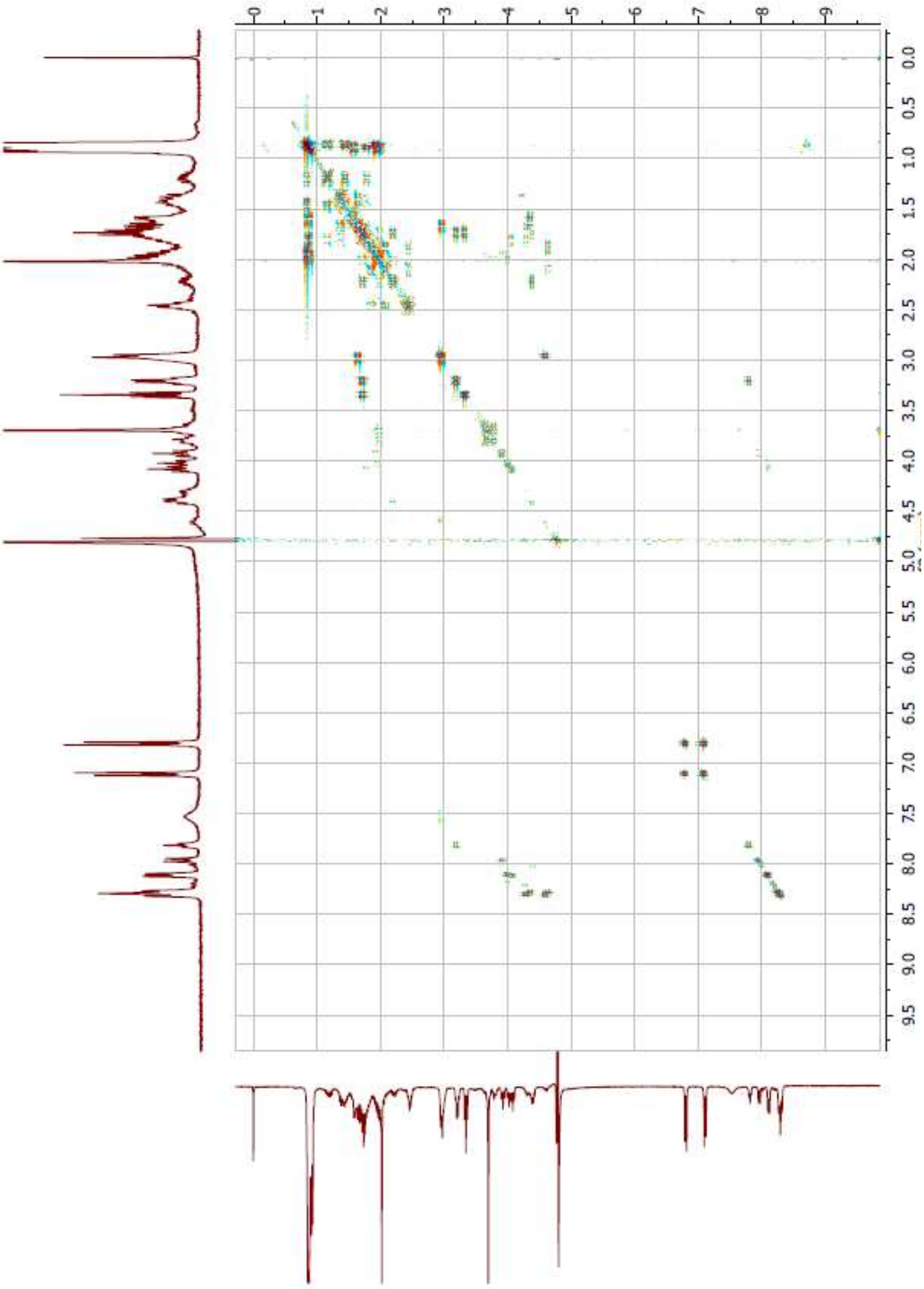
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 181.4, 180.6, 177.2, 177.0, 176.6, 176.5, 176.1, 175.8, 175.4, 175.3, 175.3, 174.1, 157.3, 132.9, 130.5, 118.2, 103.7, 63.1, 62.6, 61.5, 56.0, 55.1, 53.8, 51.4, 50.7, 42.4, 42.2, 39.5, 39.0, 38.8, 32.9, 32.1, 30.3, 29.1, 27.5, 27.4, 27.1, 26.3, 24.8, 24.4, 23.6, 21.1, 20.7, 20.6, 17.5, 13.0.

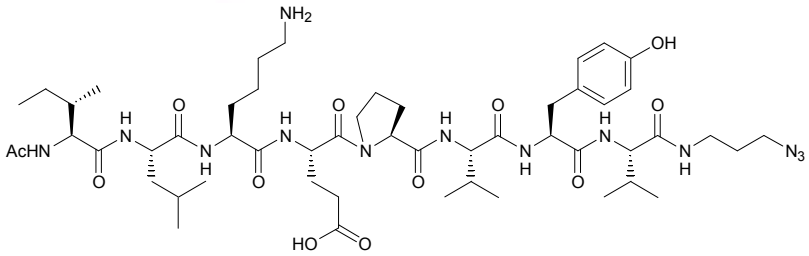
NMR ^{1}H experiment for compound **9d**



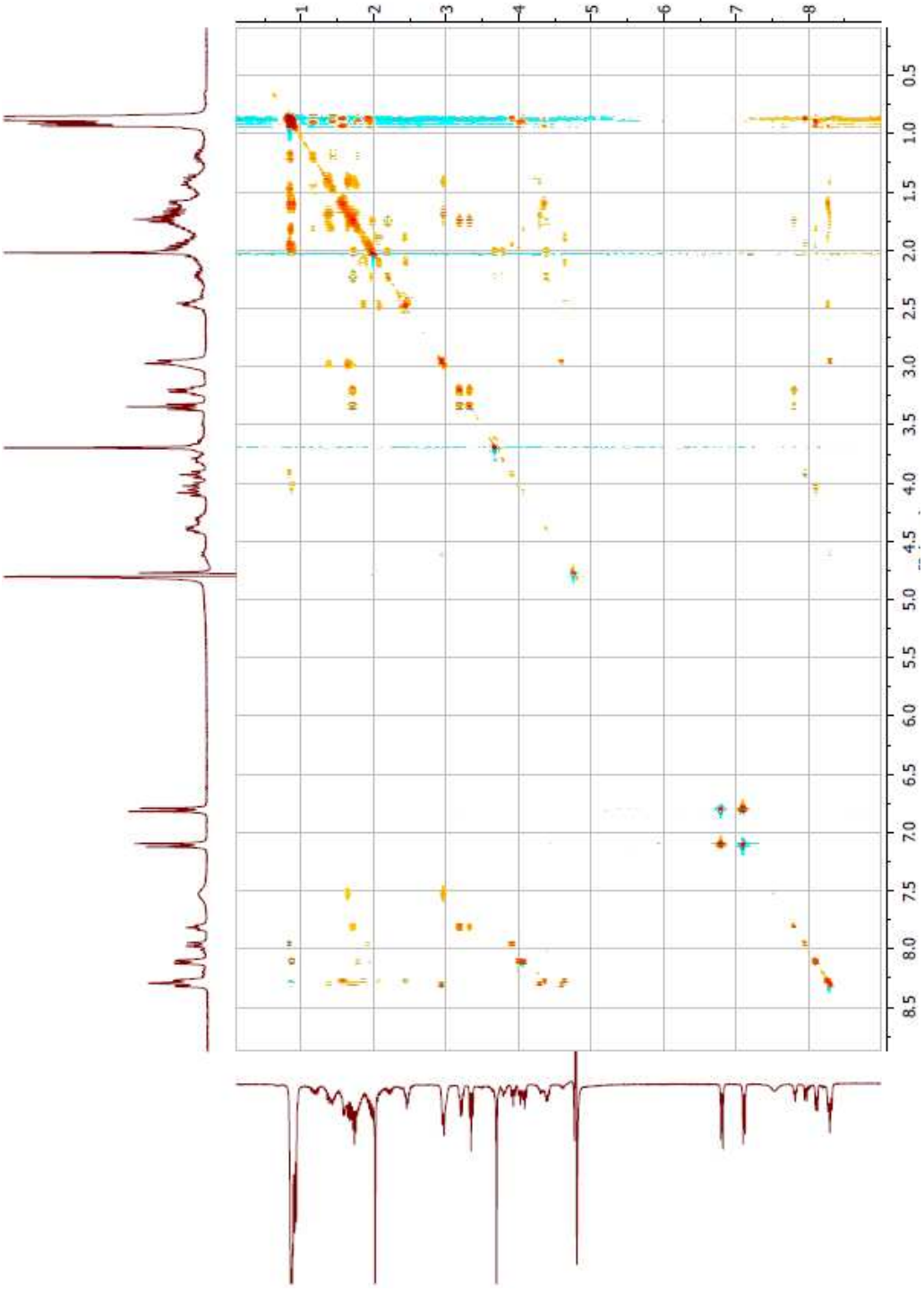


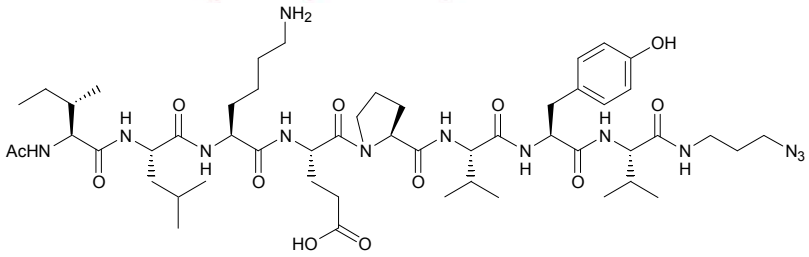
COSY experiment for compound **9d**



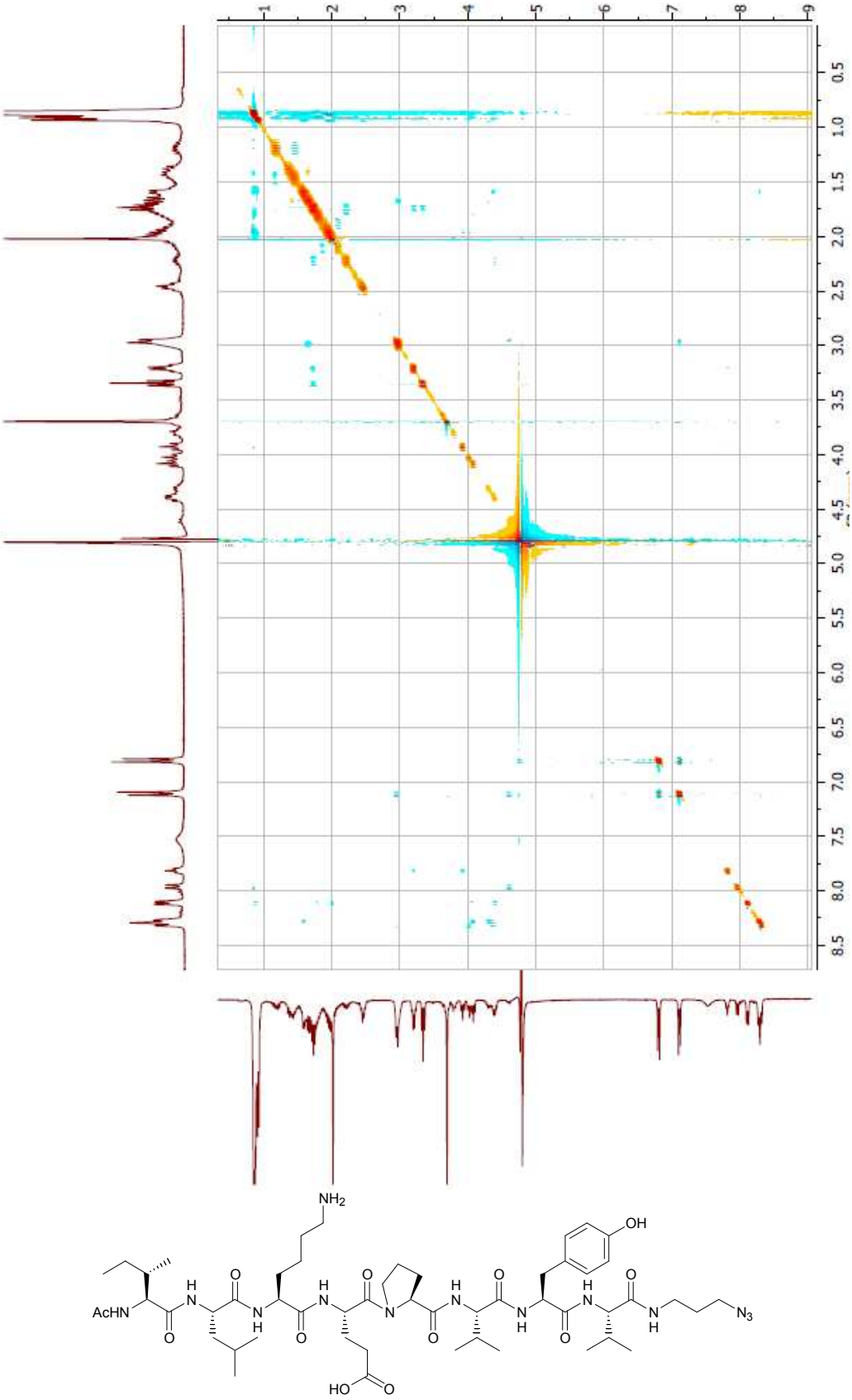


TOCSY experiment for compound **9d**

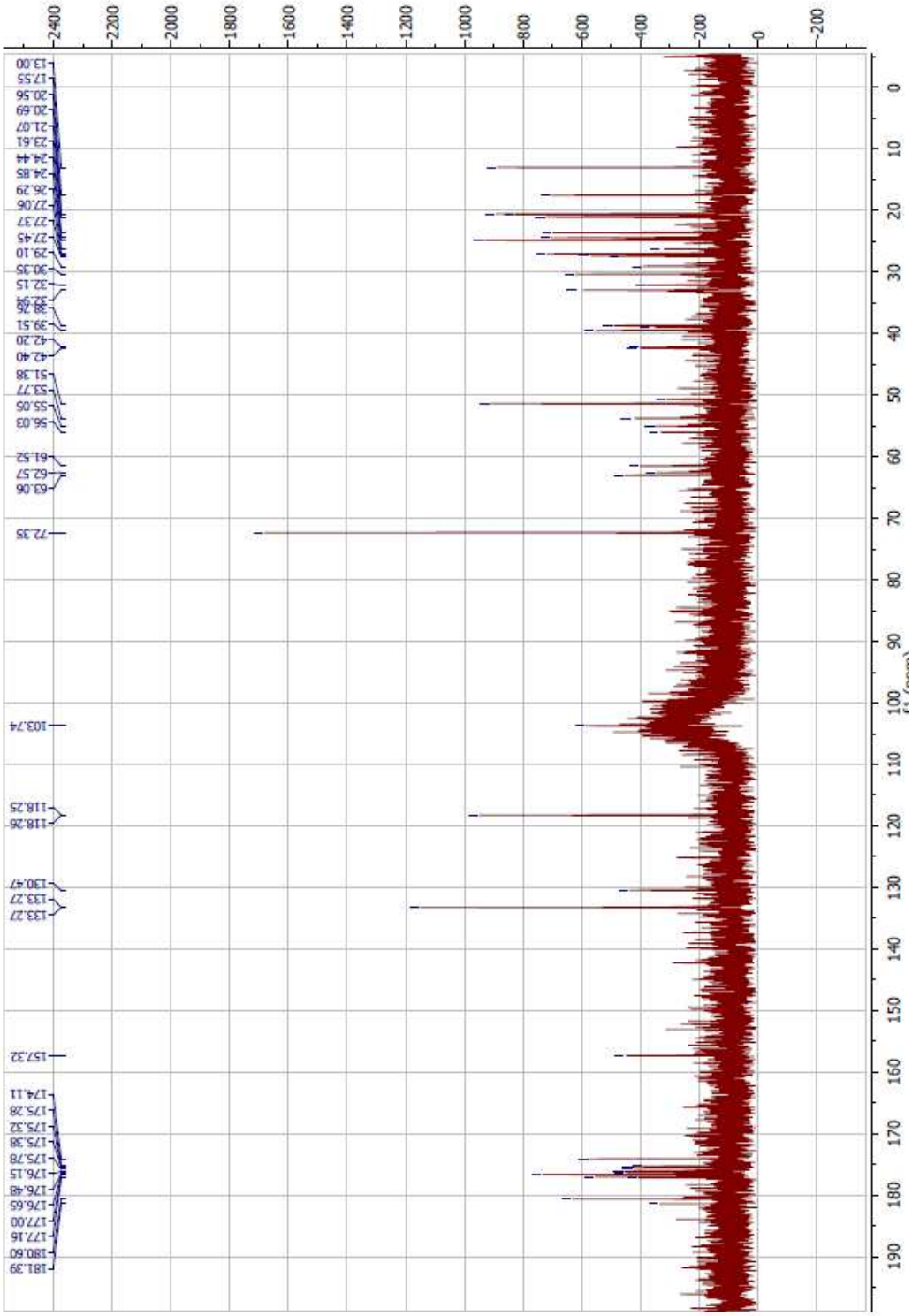


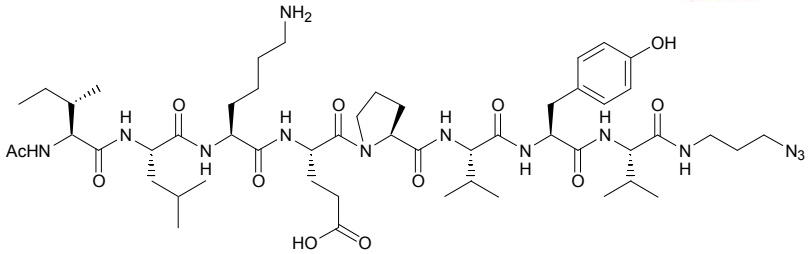


ROESY experiment for compound **9d**

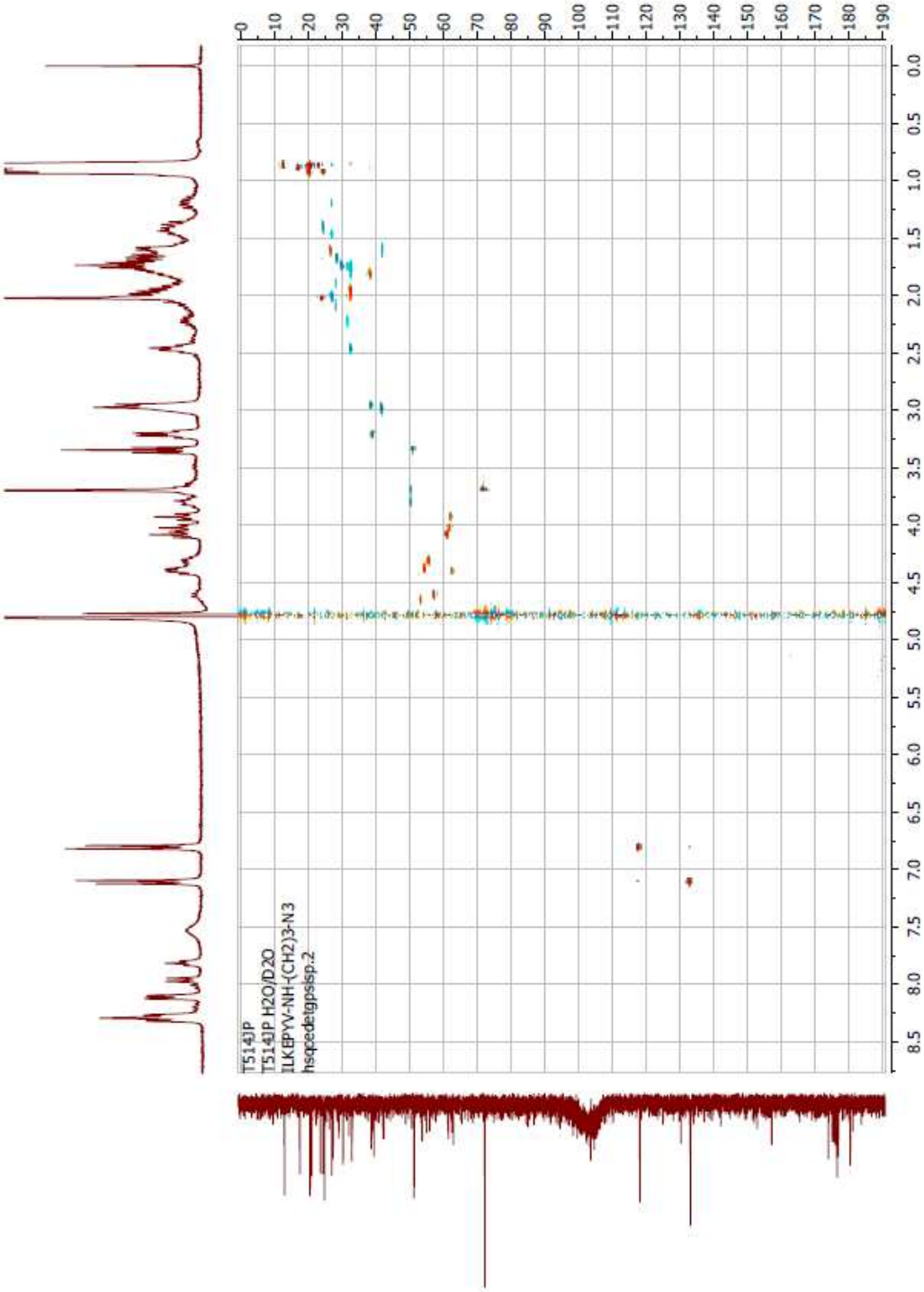


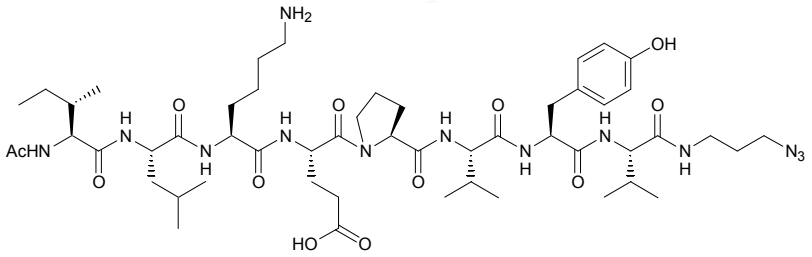
NMR ^{13}C experiment for compound **9d**





HSQC experiment for compound **9d**



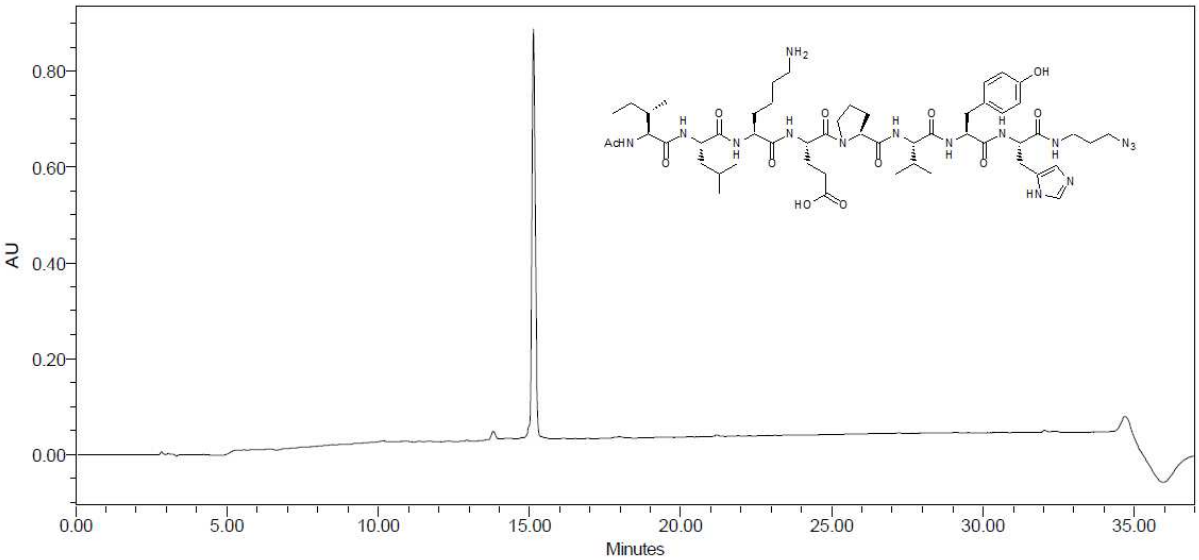


Characterization of peptide 9e:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 2.2 mg of pure peptide **9e** (4 % yield).

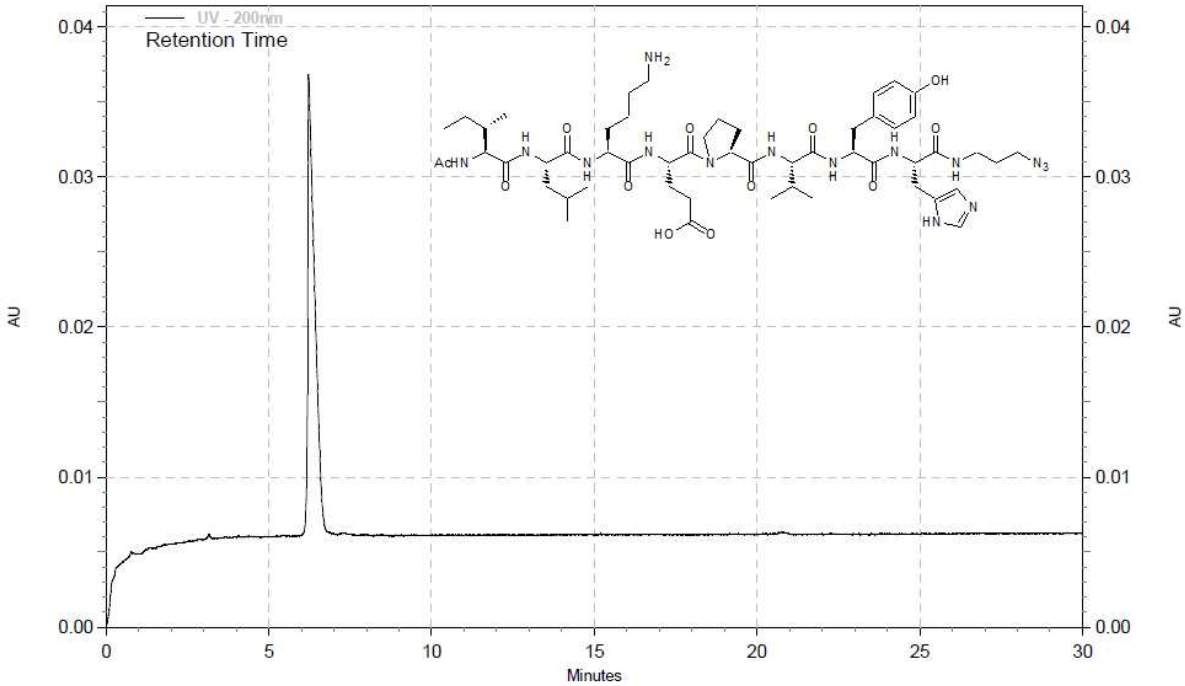
RP-HPLC analysis of 9e

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 9e

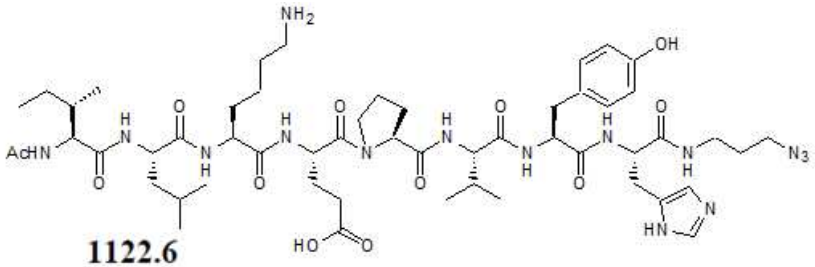
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 50 mM phosphate buffer pH = 2.5 ; Run time = 30 min ; Separate voltage = 30 kV

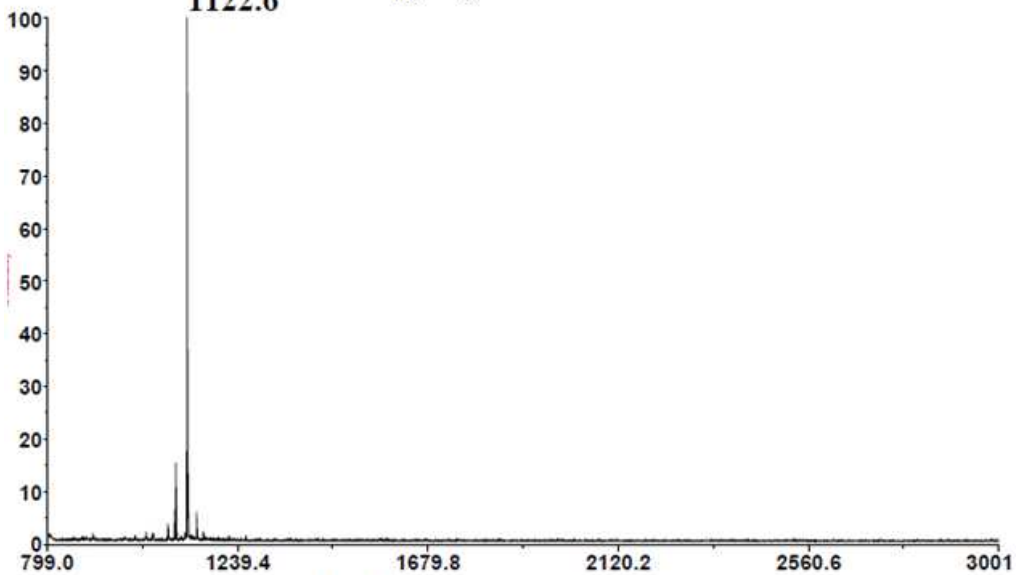


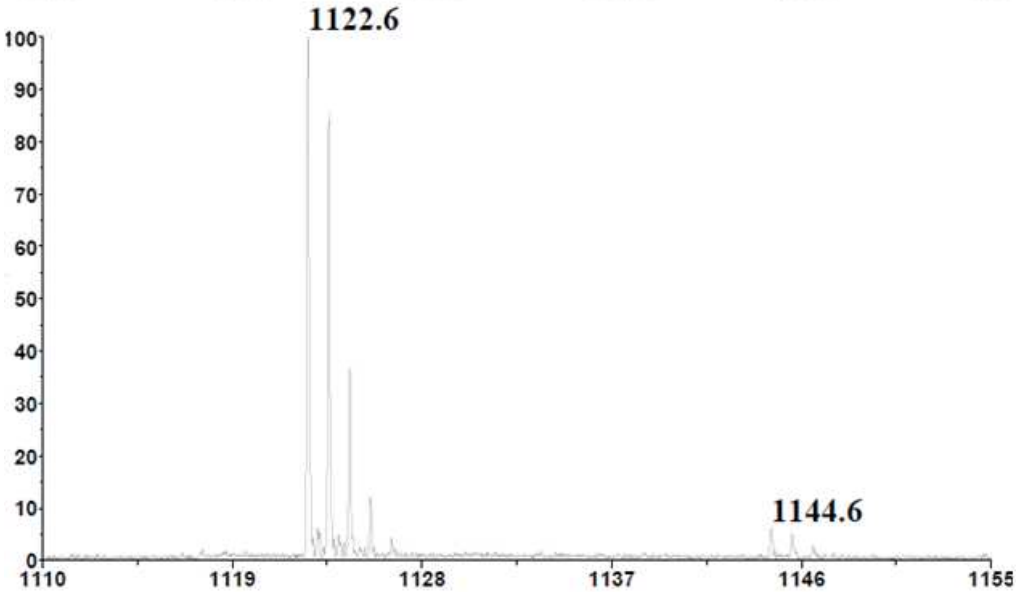
MALDI-TOF spectrum of 9e

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{53}H_{83}N_{15}O_{12}$ $[M+H]^+$ 1122.6, found 1122.6 ; $[M+Na]^+$ calcd. 1144.6, found 1144.6.





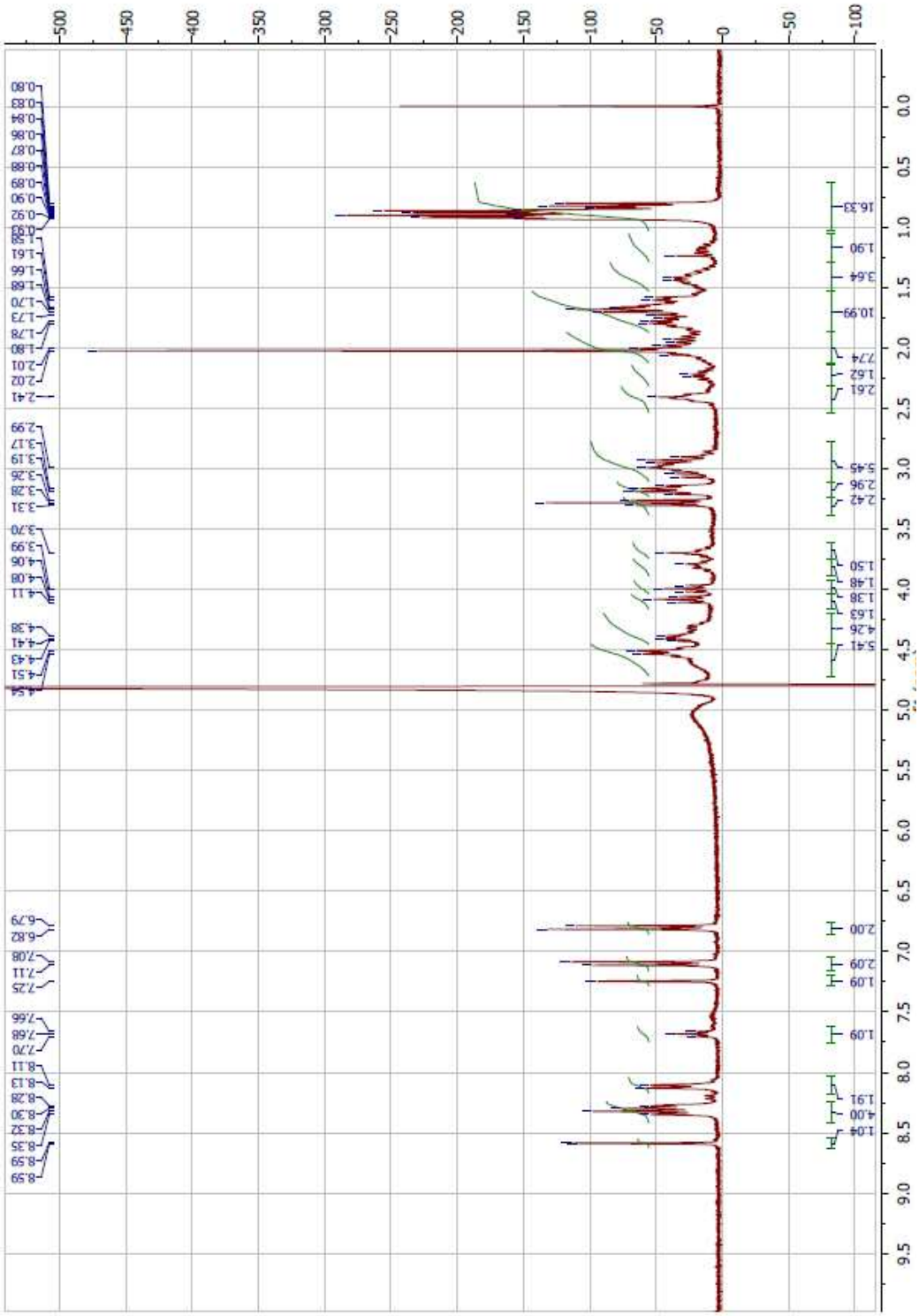


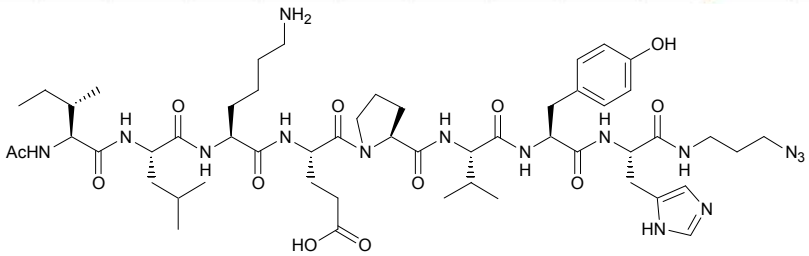
^{1}H and ^{13}C NMR for peptide 9e

^{1}H NMR (300 MHz, H_2O+D_2O) δ 8.59 (d, *J* = 1.3 Hz, 1H), 8.41 – 8.24 (m, 5H), 8.17 – 8.05 (m, 2H), 7.68 (t, *J* = 6.0 Hz, 1H), 7.25 (s, 1H), 7.10 (d, *J* = 8.6 Hz, 2H), 6.80 (d, *J* = 8.6 Hz, 2H), 4.72 – 4.46 (m, 5H), 4.46 – 4.24 (m, 4H), 4.08 (t, *J* = 7.4 Hz, 1H), 3.99 (t, *J* = 7.7 Hz, 1H), 3.87 – 3.74 (m, 1H), 3.74 – 3.61 (m, 1H), 3.28 (t, *J* = 6.7 Hz, 2H), 3.23 – 3.12 (m, 3H), 3.12 – 2.78 (m, 5H), 2.54 – 2.33 (m, 2H), 2.33 – 2.12 (m, 2H), 2.12 – 1.87 (m, 7H), 1.87 – 1.53 (m, 10H), 1.53 – 1.29 (m, 3H), 1.29 – 1.05 (m, 2H), 1.02 – 0.63 (m, 15H).

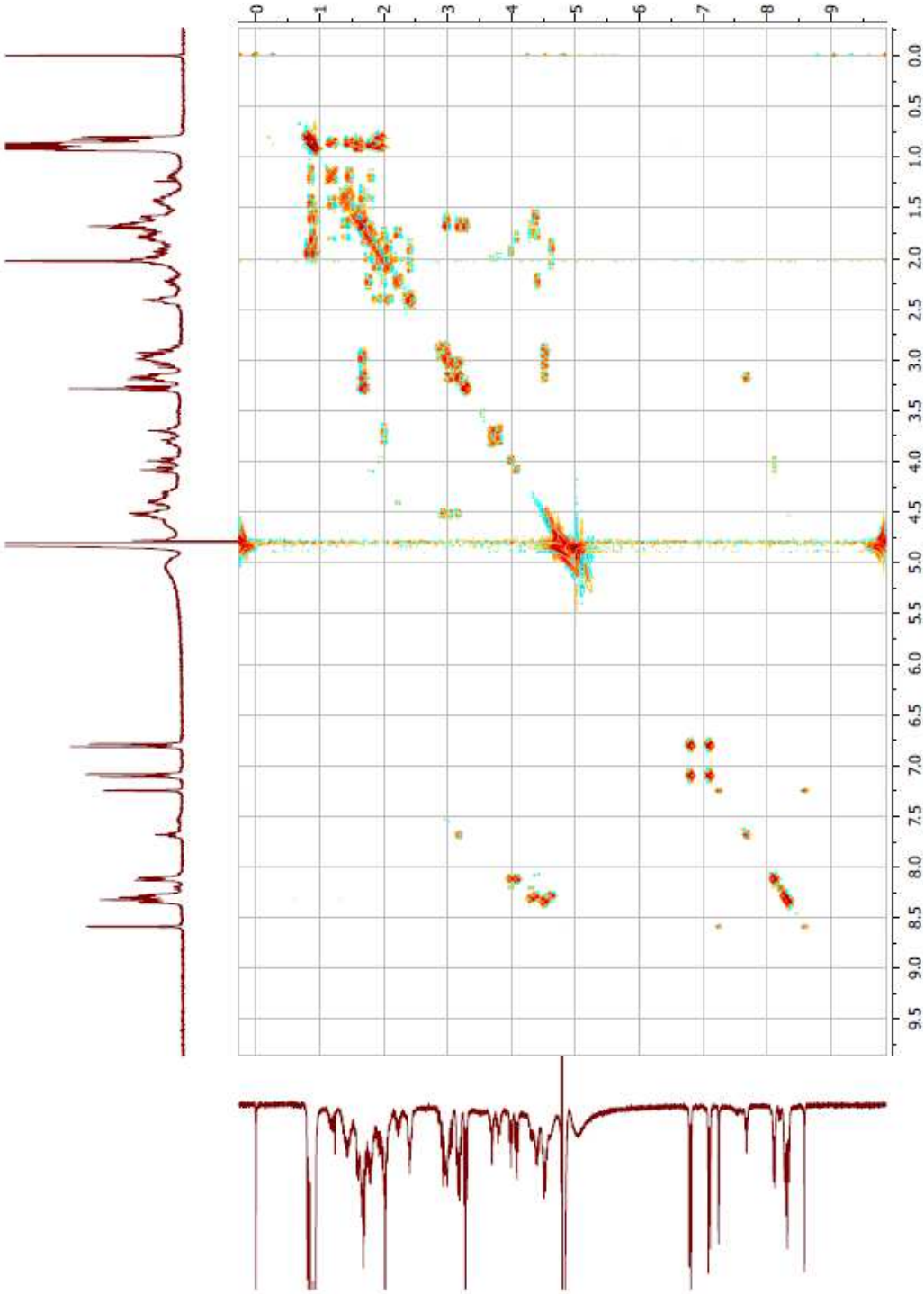
^{13}C NMR (75 MHz, H_2O+D_2O) δ 181.6, 177.2, 177.0, 176.7, 176.5, 176.2, 175.7, 175.3, 174.2, 173.4, 157.3, 136.4, 133.2, 131.3, 130.3, 120.0, 118.2, 103.7, 63.04, 62.3, 61.5, 57.9, 56.0, 55.3, 55.0, 54.0, 51.3, 50.7, 42.3, 42.2, 39.7, 38.7, 33.9, 32.9, 32.1, 30.3, 29.0, 27.4, 27.0, 24.84, 24.7, 24.4, 23.6, 21.0, 20.7, 17.5, 13.0.

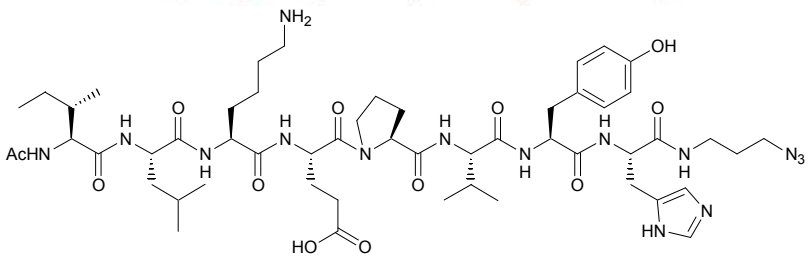
NMR ^{1}H experiment for compound **9e**



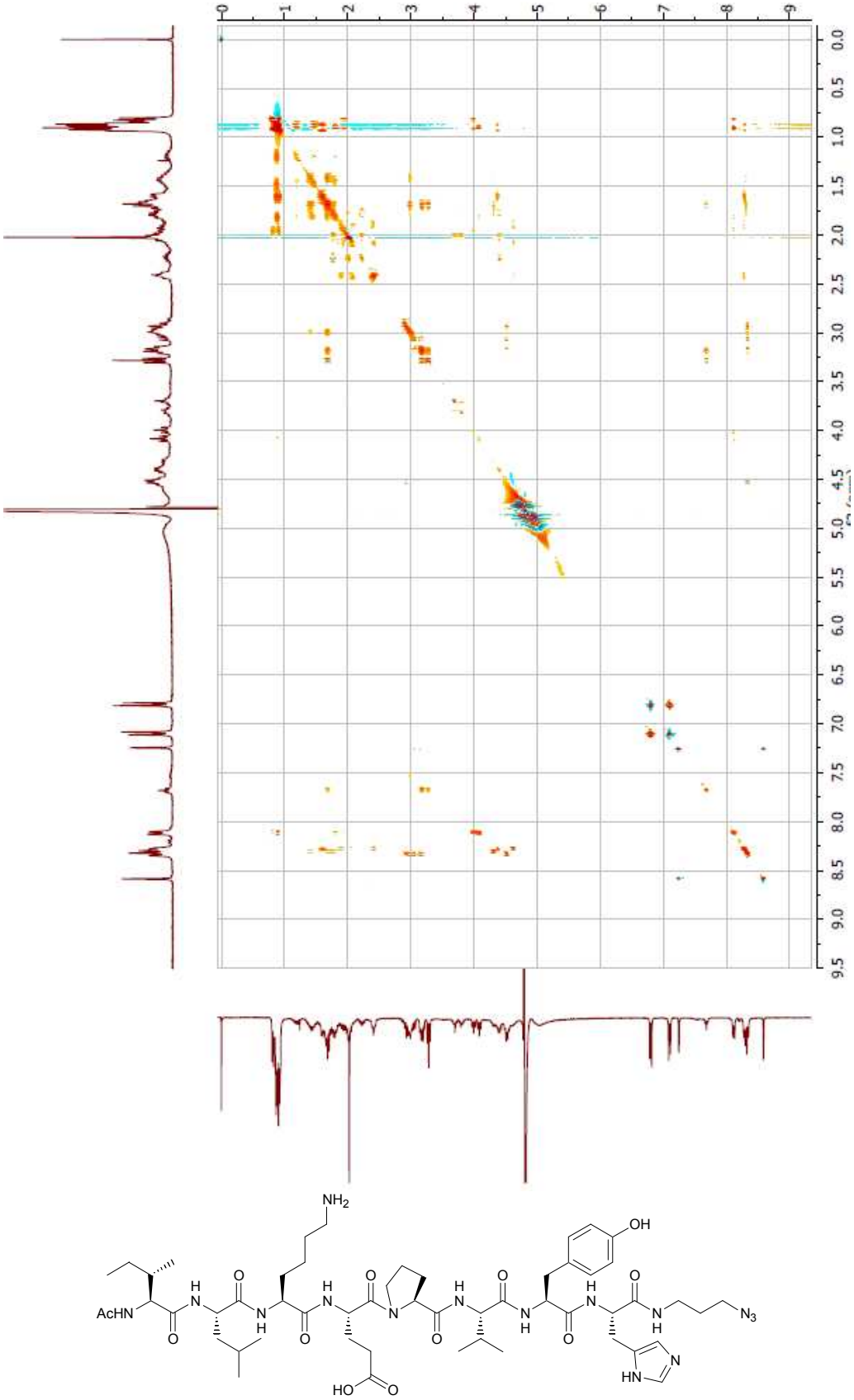


COSY experiment for compound **9e**

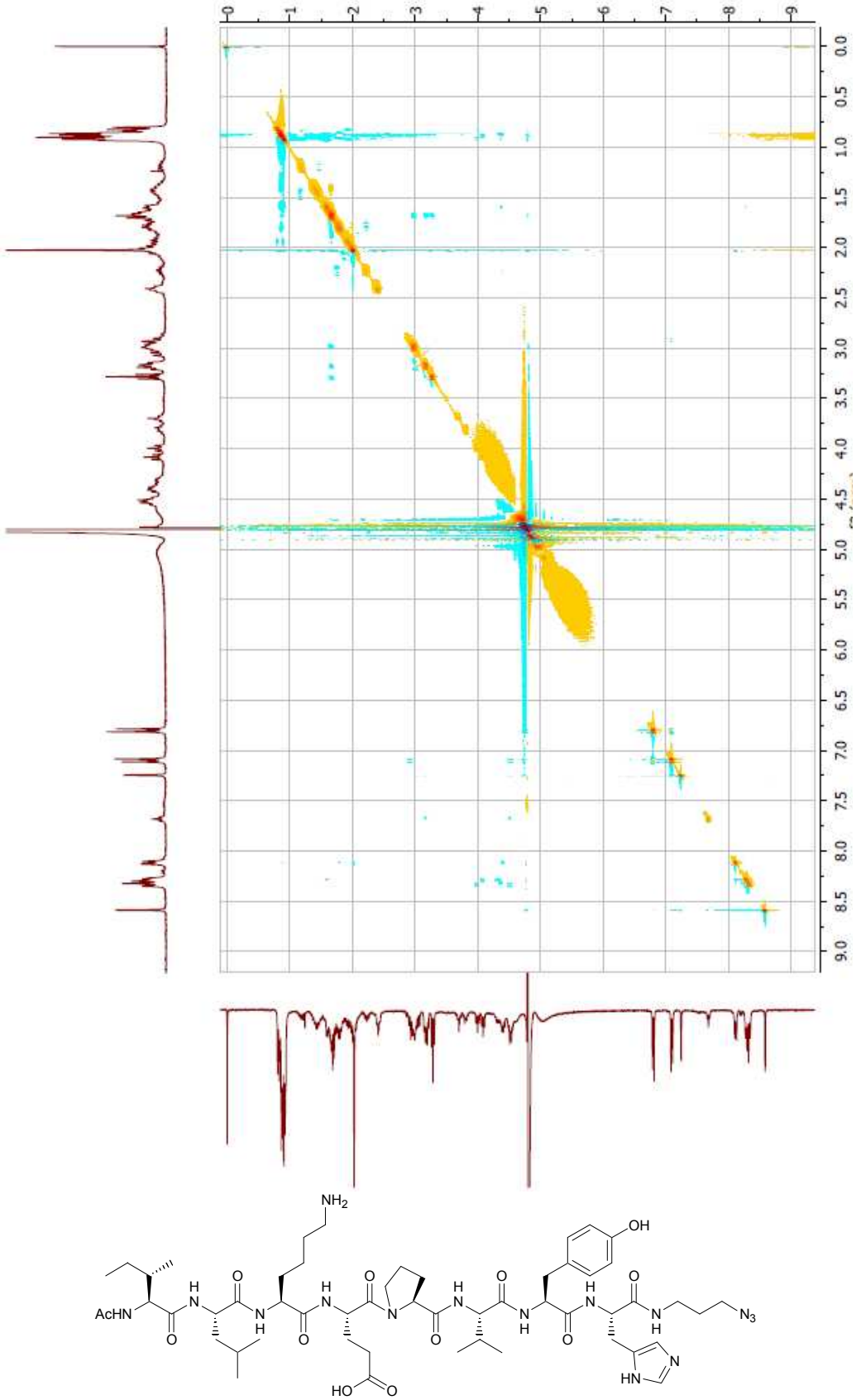




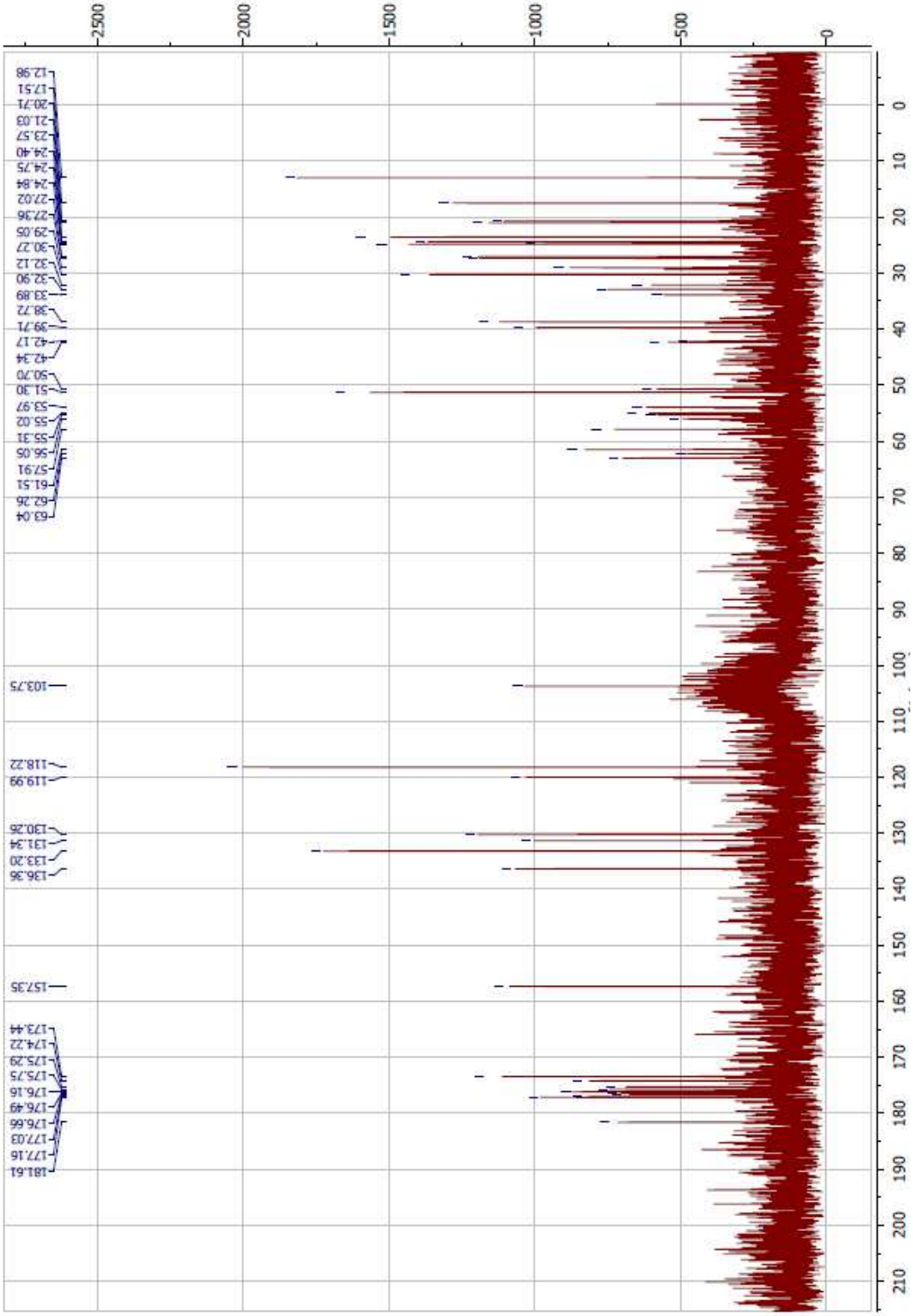
TOCSY experiment for compound **9e**

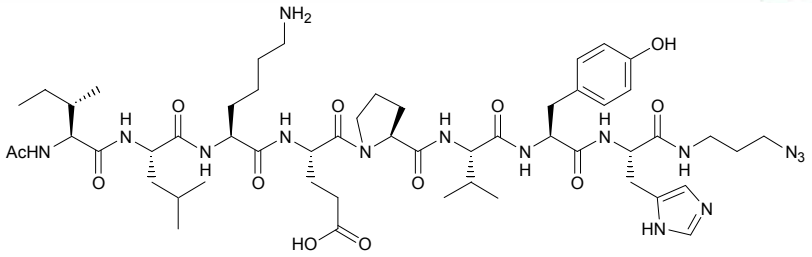


ROESY experiment for compound **9e**

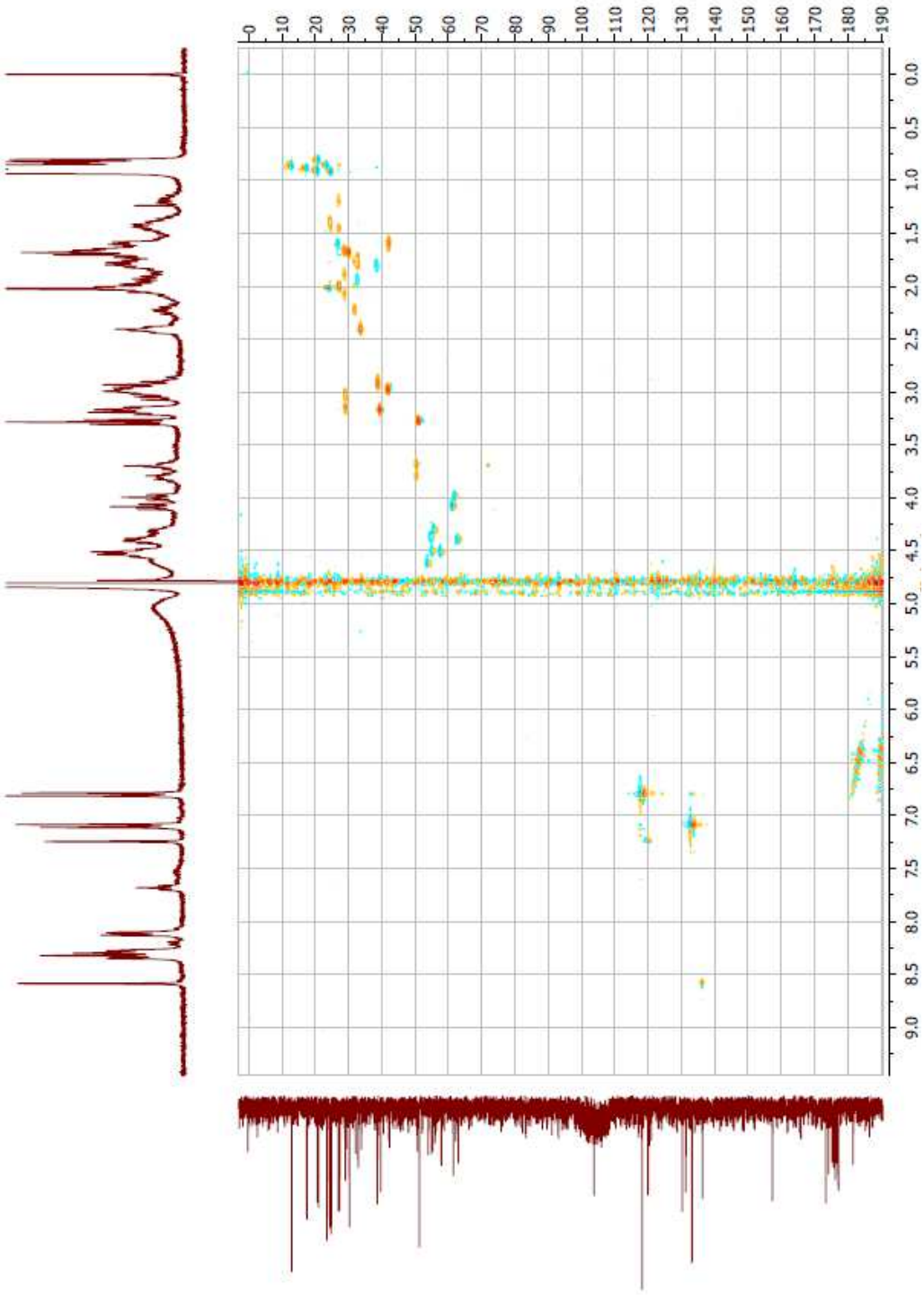


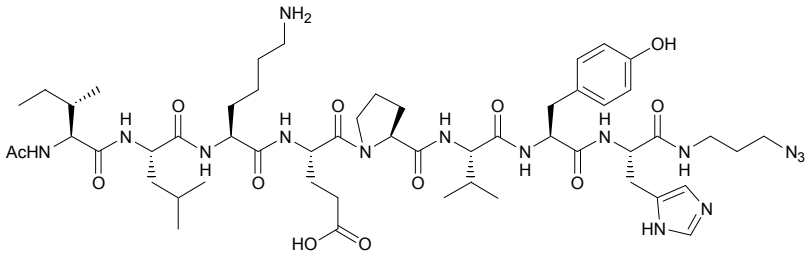
NMR ^{13}C experiment for compound **9e**





HSQC experiment for compound **9e**





Copper Catalyzed Peptide-Azides Carbohydrate-Alkynes Cycloadditions

Synthesis of peptide 10

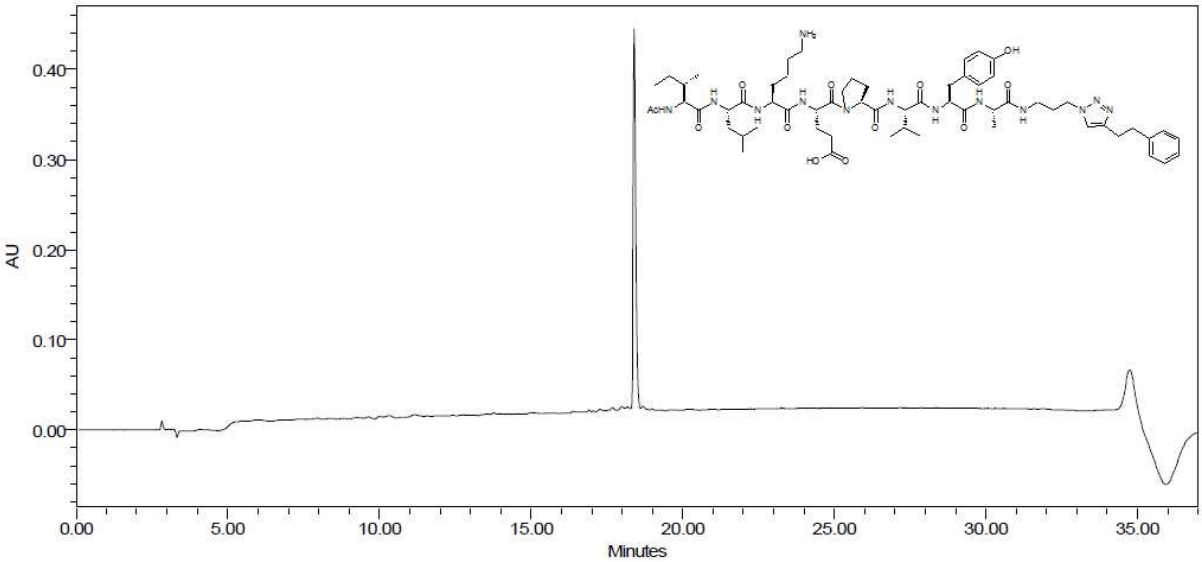
To a stirred solution of **9b** (5.3 mg, 5 µmol) and 4-Phenyl-1-bytyne **6a** (1.3 mg, 10 µmol) in a 2.5:1 mixture of *tert*-butyl alcohol and water (1.15 mL) were successively added at rt copper (II) sulfate pentahydrate (250 µL of 2 mM in water, 0.5 µmol) followed by sodium ascorbate (250 µL of freshly prepared 20 mM in water, 5µmol). After 20 h, HPLC analysis indicated complete consumption of starting material (Rt 16.7 min, linear H_2O/CH_3CN gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm) and formation of a major product (Rt 18.6 min). The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 10:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 5.3 mg of pure peptide (89 % yield).

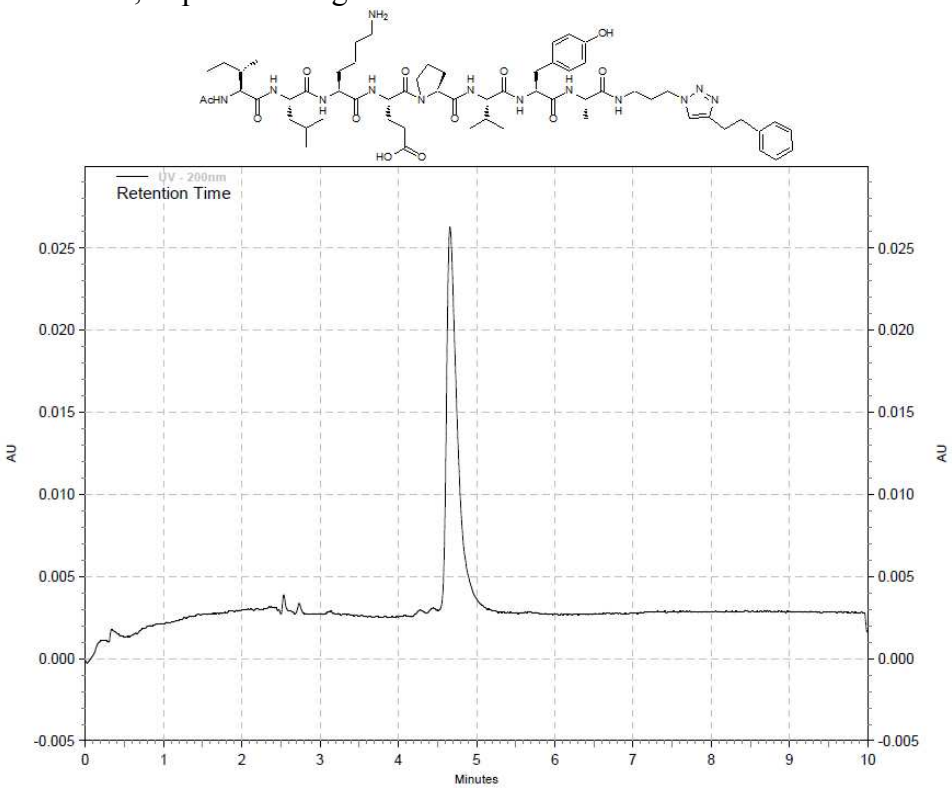
RP-HPLC analysis of 10

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 10

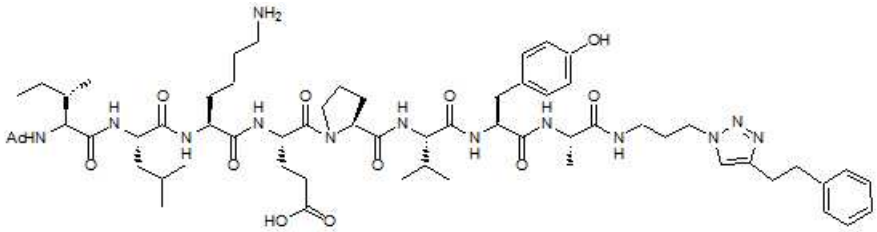
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.

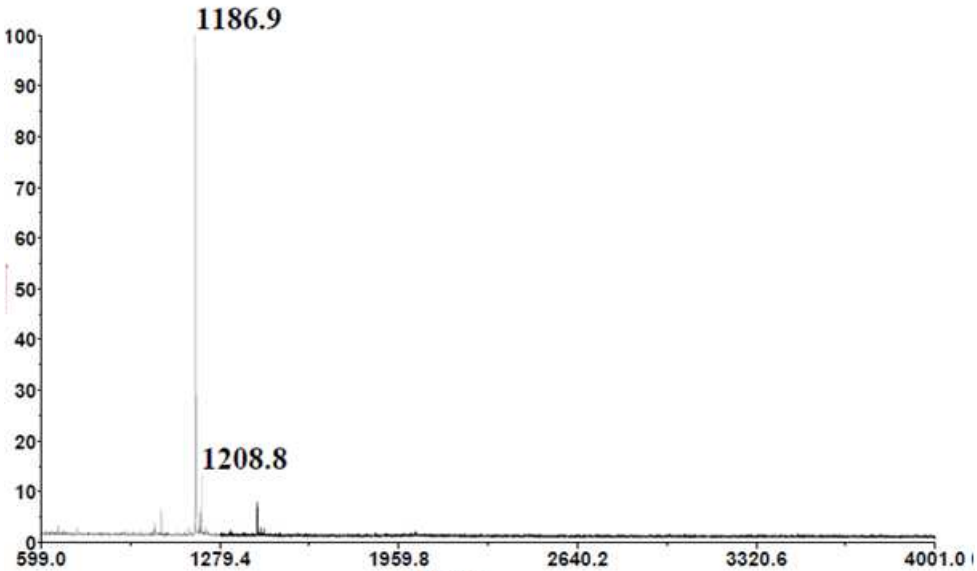


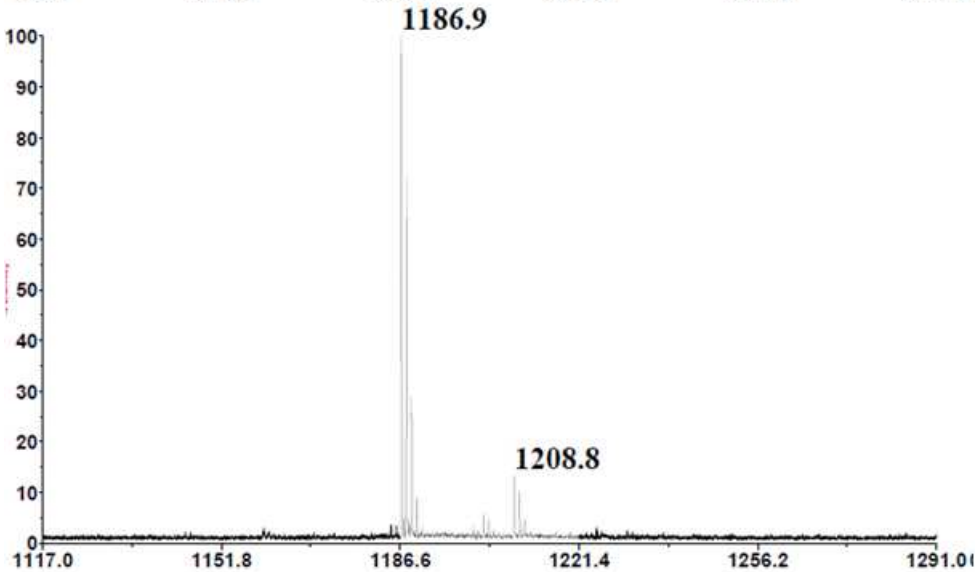
MALDI-TOF spectrum of 10

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{60}H_{91}N_{13}O_{12}$ $[M+H]^+$ 1186.7 found 1186.9 ; $[M+Na]^+$ calcd. 1208.7, found 1208.8.





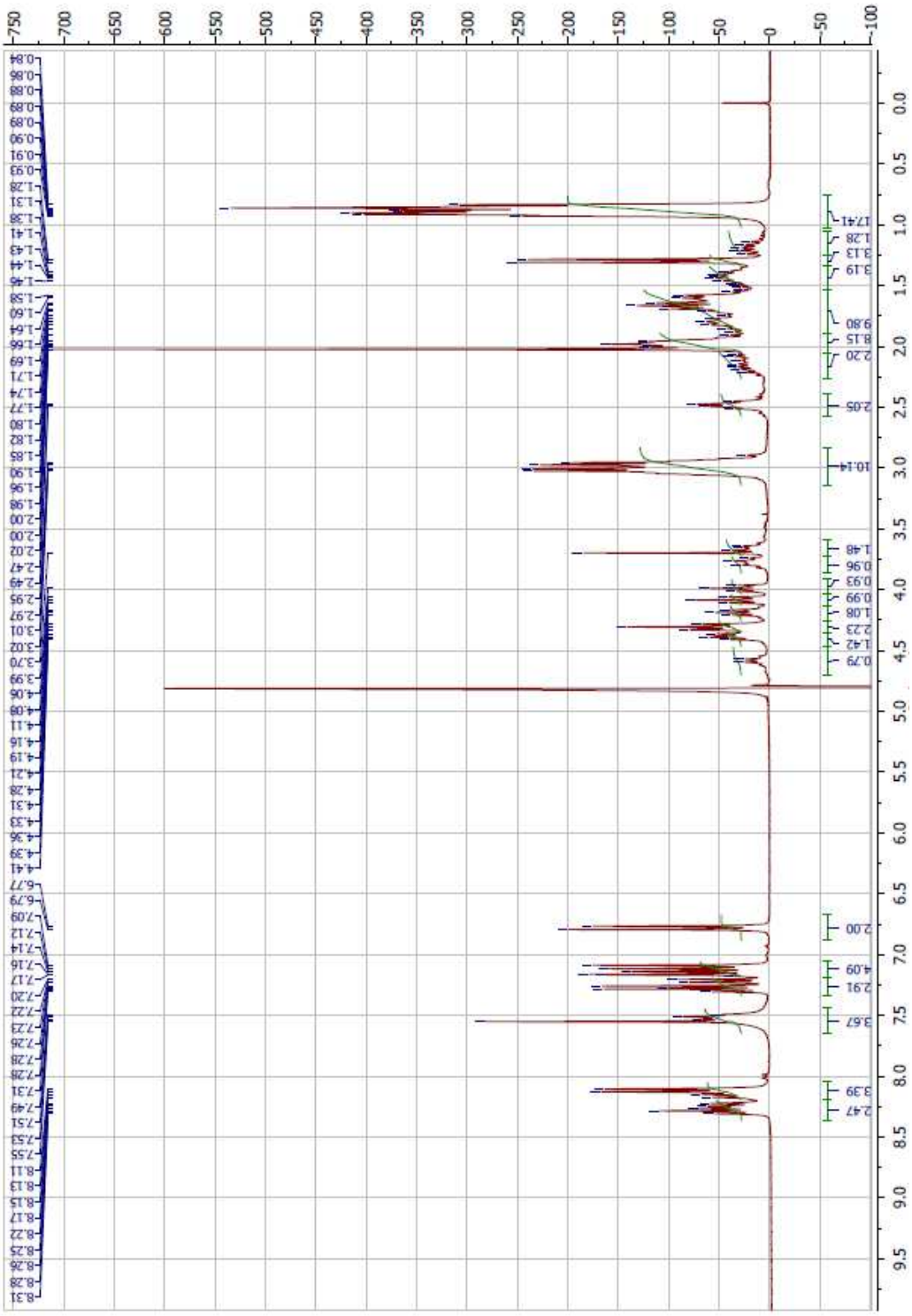


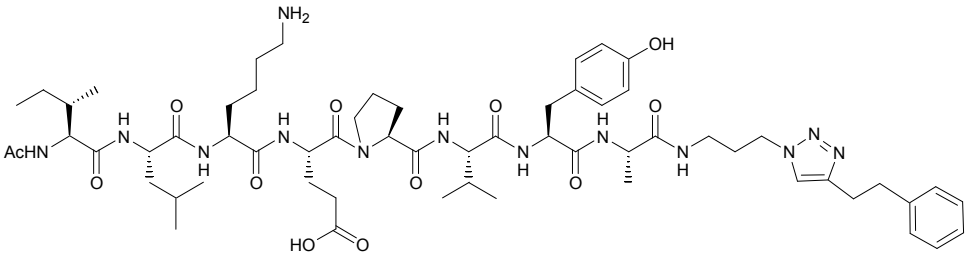
^{1}H and ^{13}C NMR for peptide 10

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.37 – 8.20 (m, 3H), 8.20 – 8.05 (m, 4H), 7.55 (s, 2H), 7.51 (t, *J* = 5.7 Hz, 2H), 7.34 – 7.19 (m, 3H), 7.19 – 7.05 (m, 4H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.71 – 4.47 (m, 1H), 4.47 – 4.35 (m, 1H), 4.31 (t, *J* = 6.8 Hz, 2H), 4.19 (d, *J* = 6.8 Hz, 1H), 4.08 (t, *J* = 7.4 Hz, 1H), 3.99 (t, *J* = 7.5 Hz, 1H), 3.87 – 3.73 (m, 1H), 3.73 – 3.59 (m, 2H), 3.14 – 2.83 (m, 10H), 2.57 – 2.39 (m, 2H), 2.27 – 2.05 (m, 2H), 2.05 – 1.89 (m, 8H), 1.89 – 1.53 (m, 10H), 1.53 – 1.34 (m, 3H), 1.30 (d, *J* = 7.2 Hz, 3H), 1.25 – 1.05 (m, 1H), 1.03 – 0.76 (m, 18H).

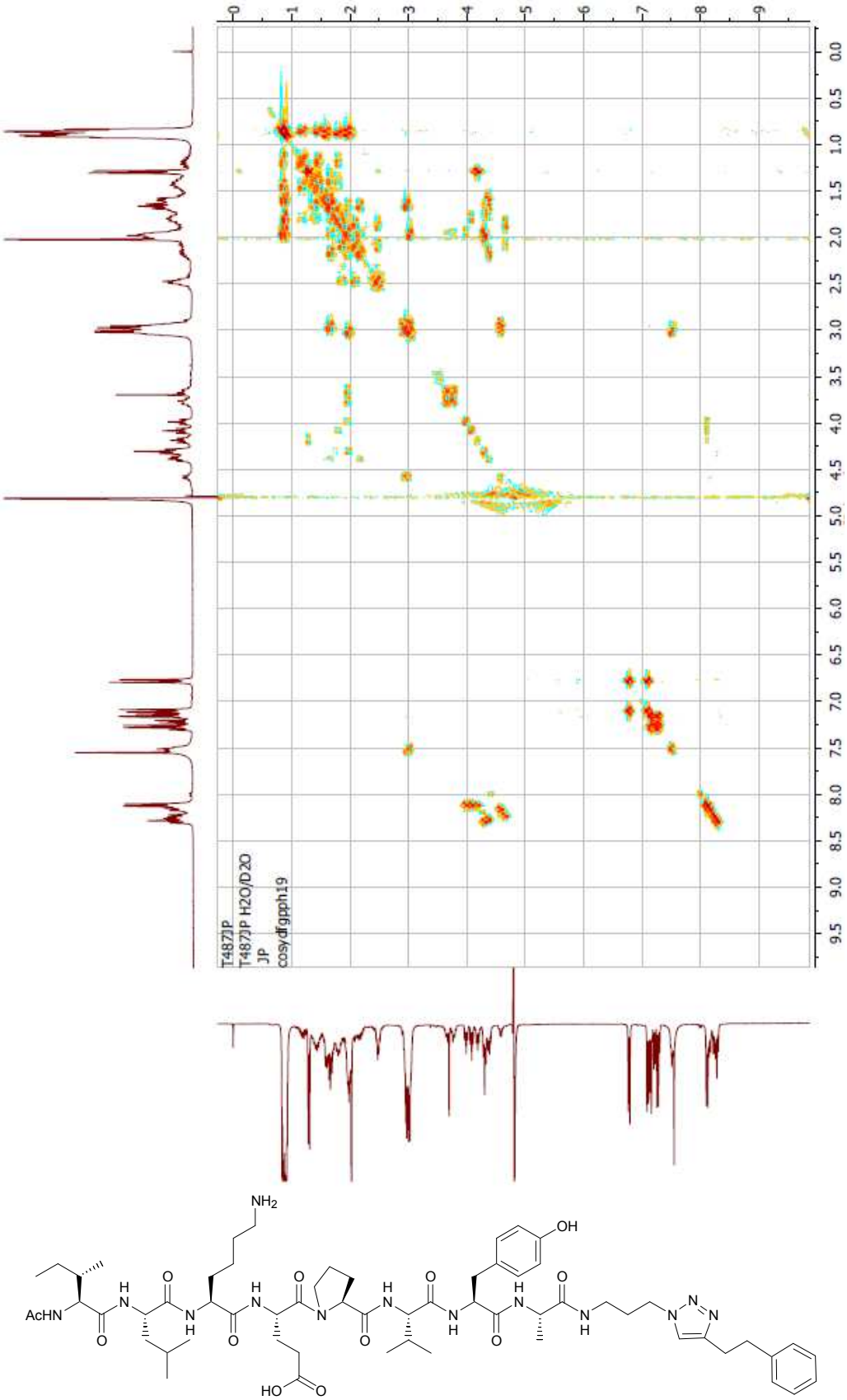
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 179.7, 177.1, 177.0, 177.0, 176.6, 176.5, 176.1, 176.0, 175.2, 174.0, 157.3, 143.7, 133.3, 131.4, 131.3, 131.3, 130.4, 129.0, 126.6, 118.2, 72.3, 63.0, 62.5, 61.5, 57.6, 56.0, 55.0, 53.5, 52.6, 50.4, 42.4, 42.2, 39.0, 38.7, 37.3, 33.0, 32.8, 32.3, 32.1, 31.5, 29.0, 28.9, 28.4, 27.4, 27.0, 24.8, 24.4, 23.6, 21.0, 20.7, 19.5, 17.5, 13.0.

NMR ^{1}H experiment for compound **10**

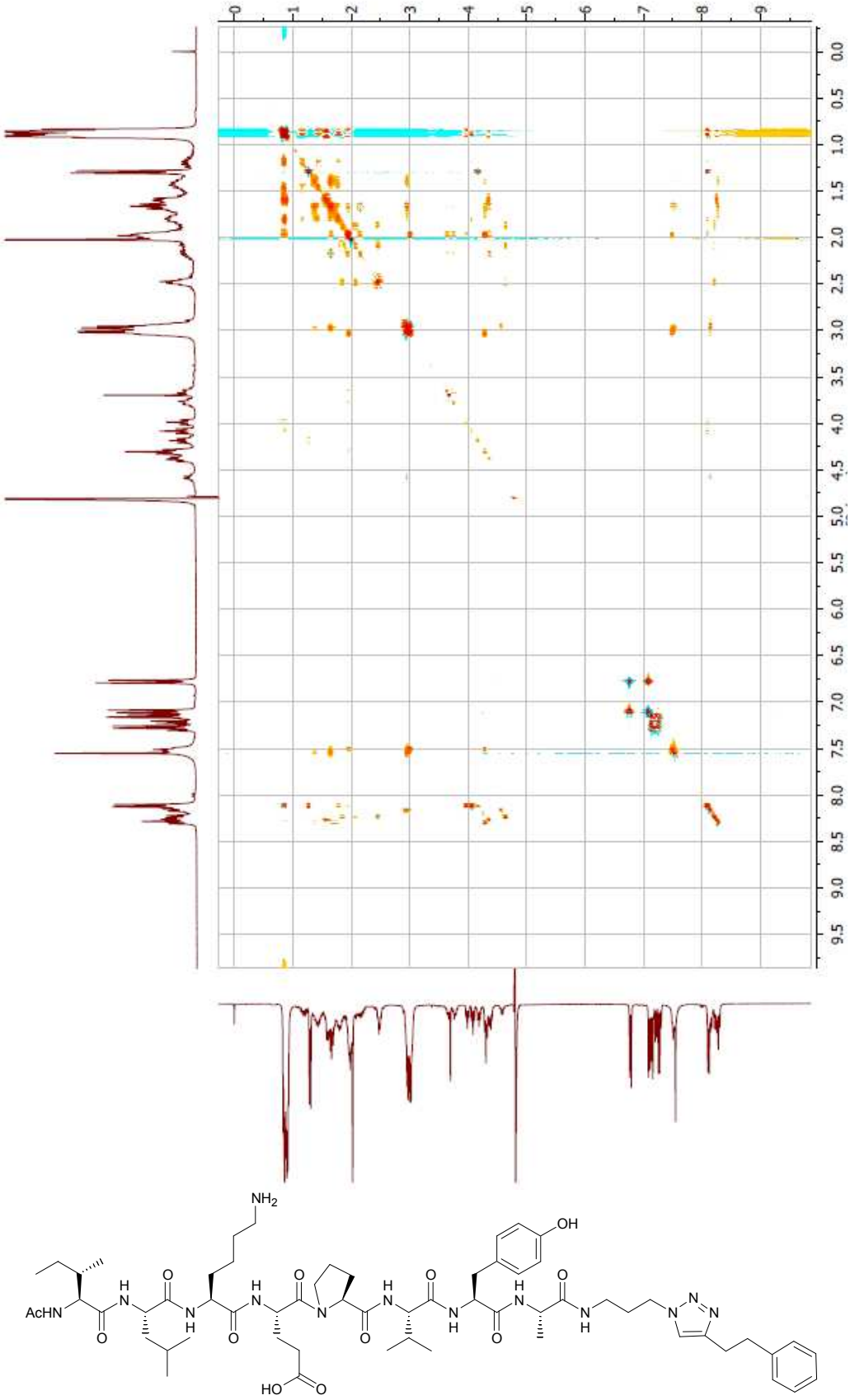




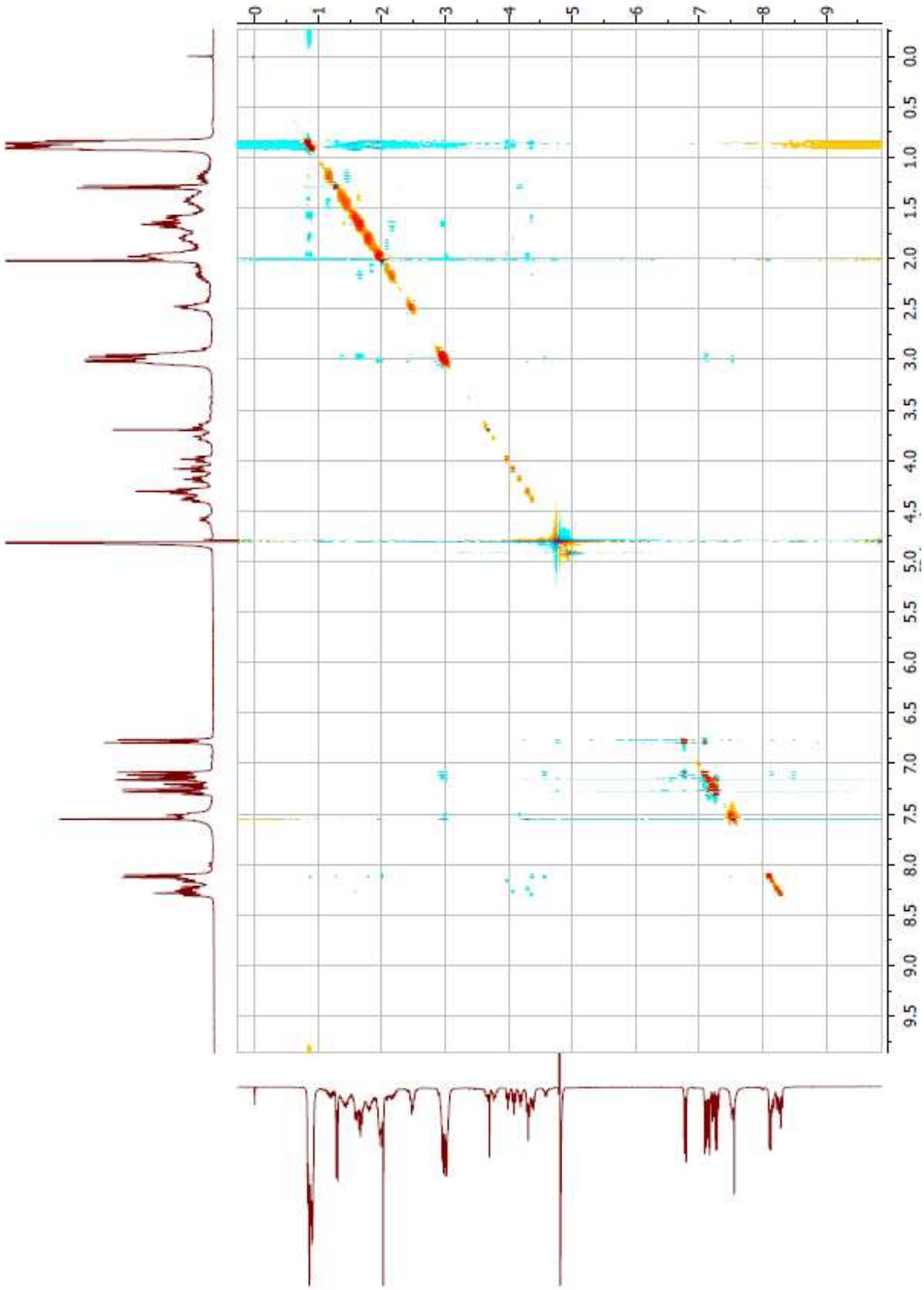
COSY experiment for compound **10**

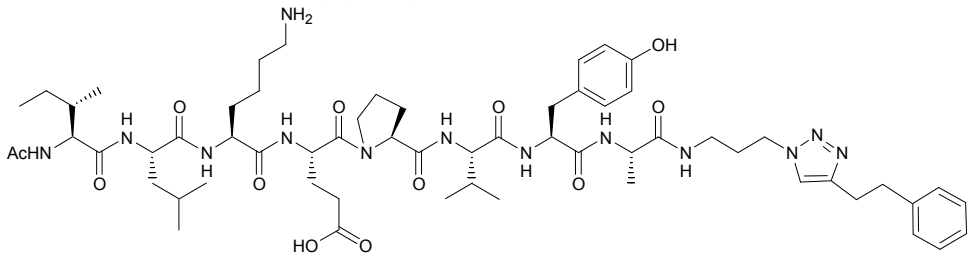


TOCSY experiment for compound **10**

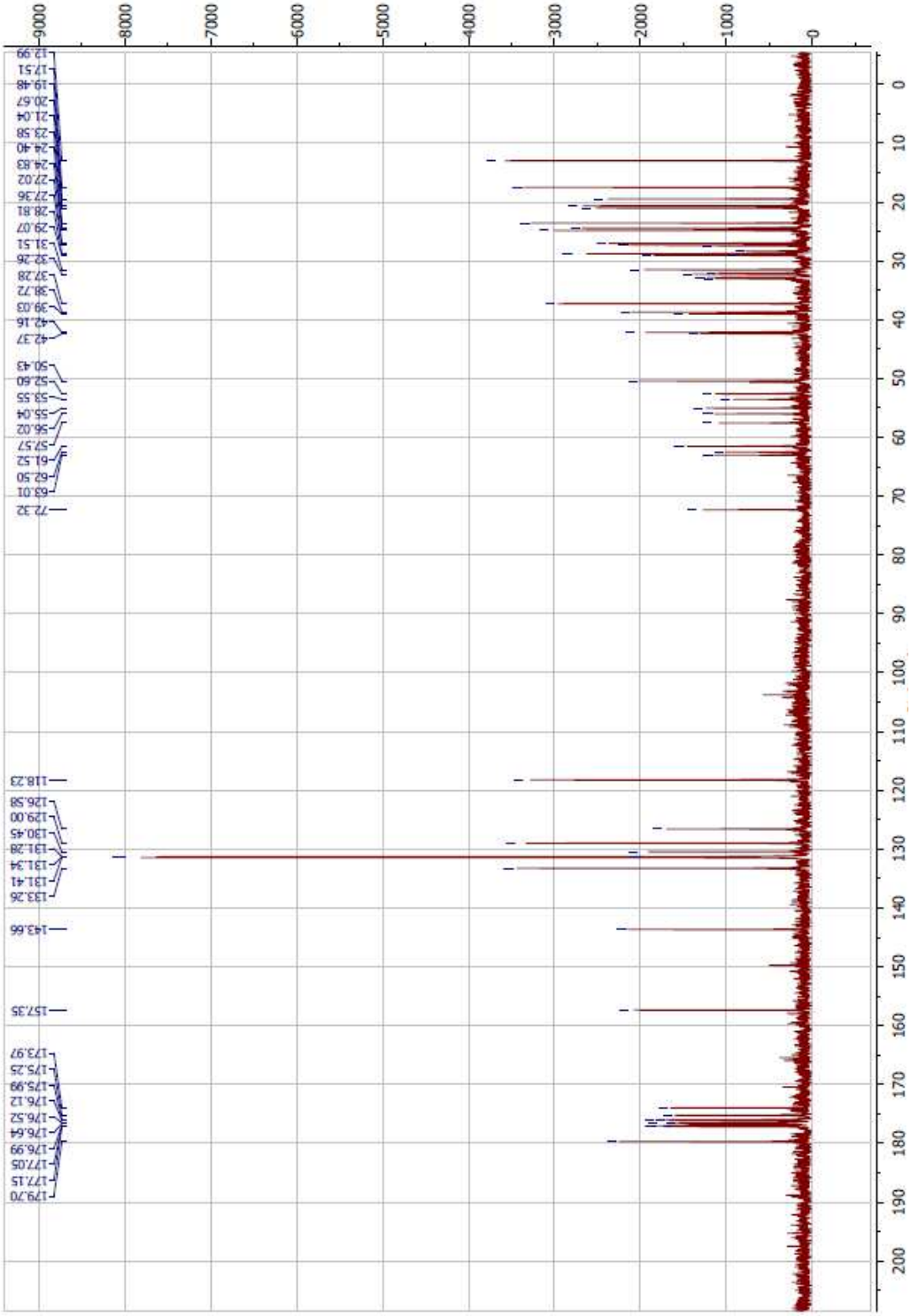


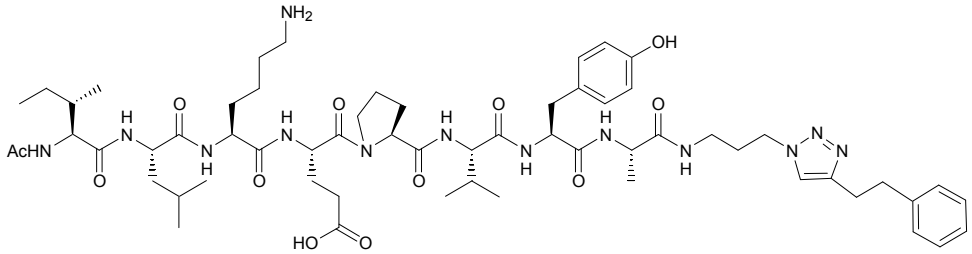
ROESY experiment for compound **10**



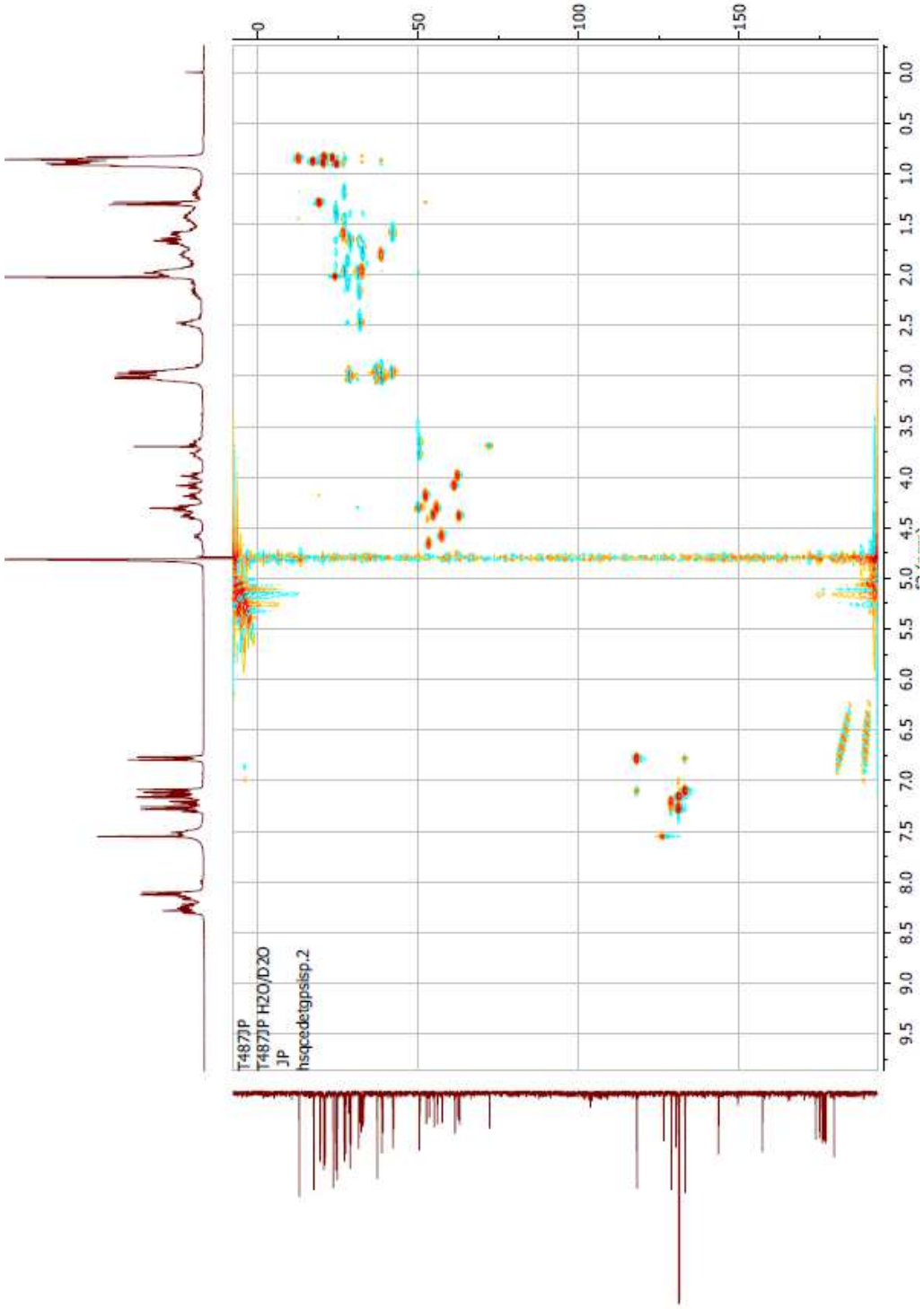


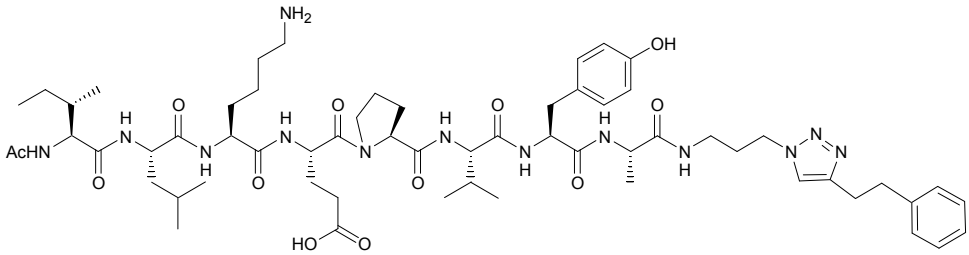
NMR ^{13}C experiment for compound **10**





HSQC experiment for compound **10**





Synthesis of peptide 11:

To a stirred solution of **9b** (10.6 mg, 10 µmol) and Glucose derivative **6b** (4.9 mg, 20 µmol) in *tert*-butyl alcohol (1.0 mL) were successively added at rt copper (II) sulfate pentahydrate (500 µL of 2 mM in water, 1.0 µmol) followed by sodium ascorbate (500 µL of freshly prepared 20 mM in water, 10 µmol). After 4 h, HPLC analysis indicated complete consumption of starting material (Rt 16.7 min, linear H_2O/CH_3CN gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm) and formation of a major product (Rt 14.1 min). The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

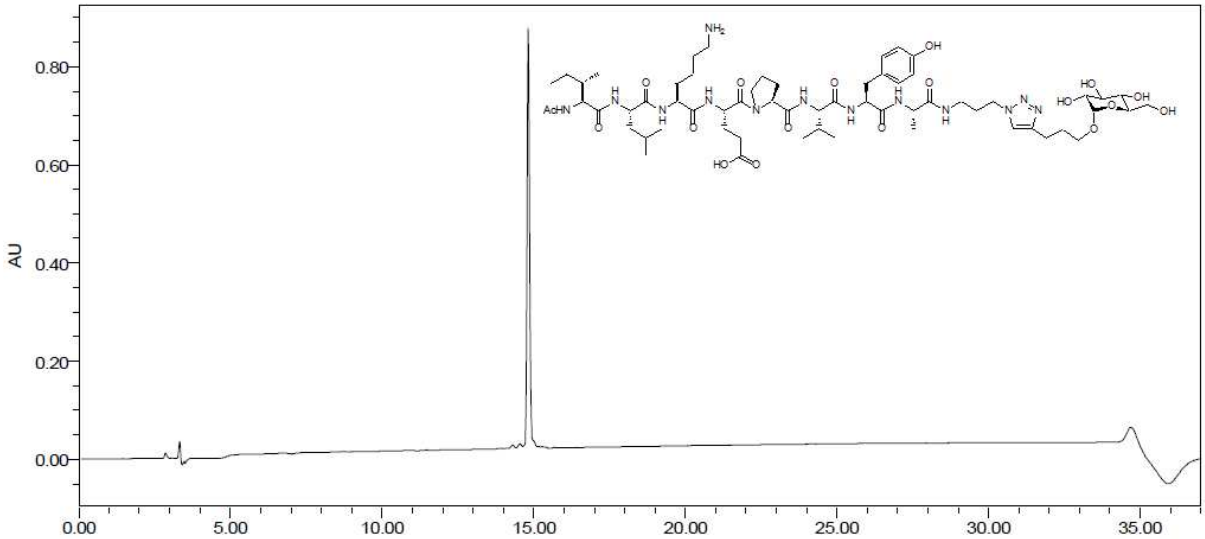
Characterization of peptide 11:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 10.7 mg of pure peptide **11** (82 % yield).

RP-HPLC analysis of 11

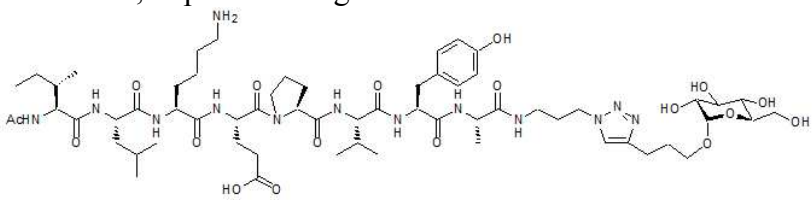
Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.

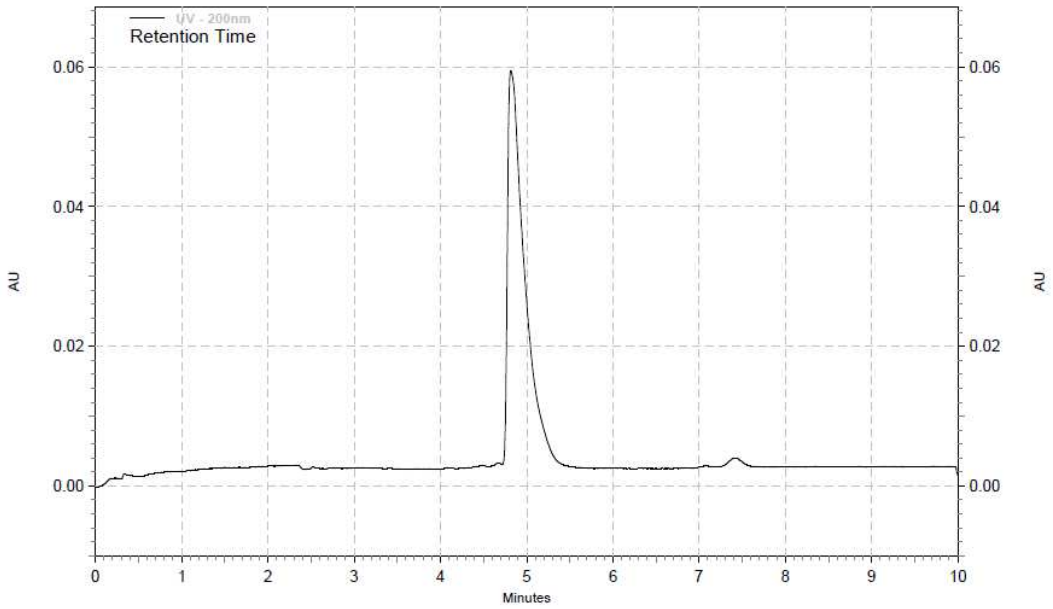
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 11

T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV

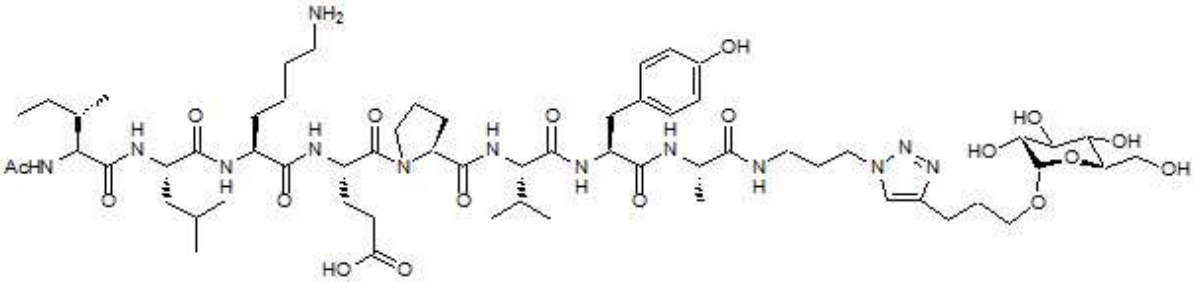


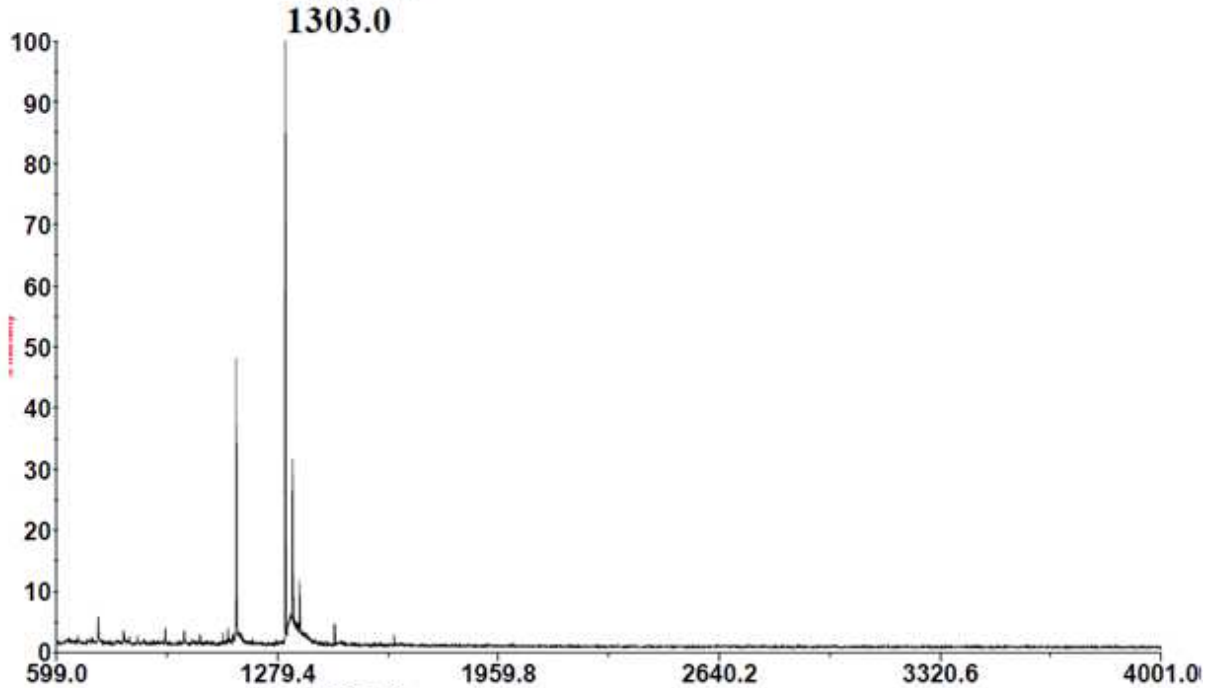


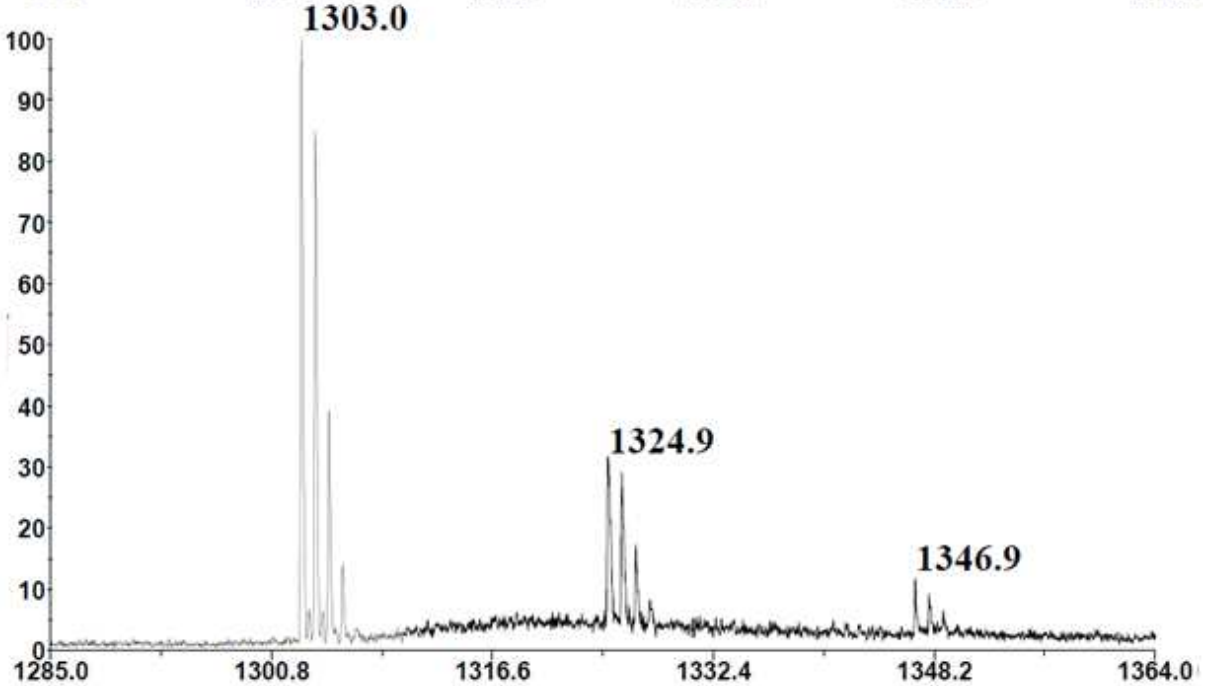
MALDI-TOF spectrum of 11

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{61}H_{99}N_{13}O_{18}$ $[M+H]^+$ 1302.7 found 1303.0 ; $[M+Na]^+$ calcd. 1324.7, found 1324.9.





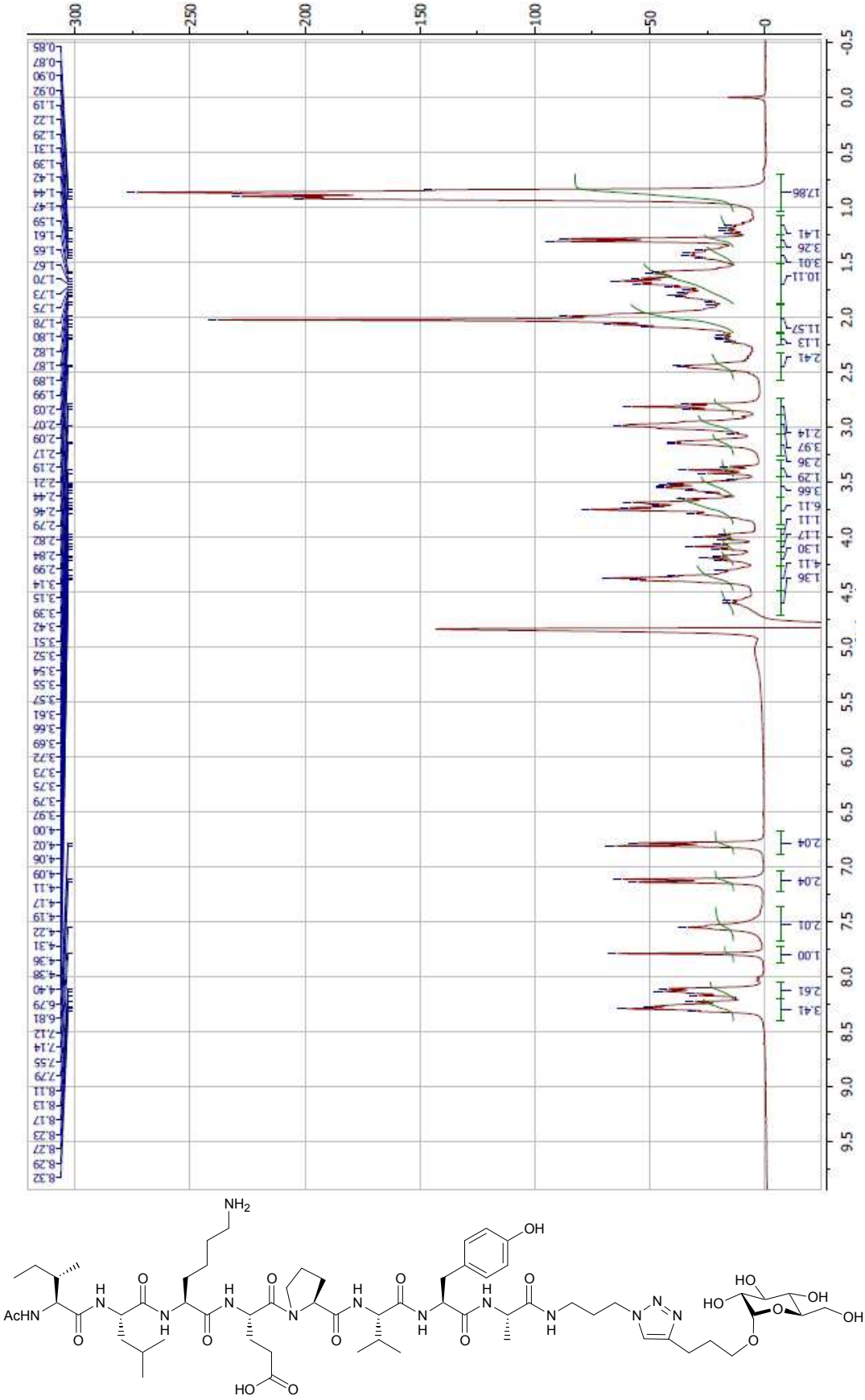


^{1}H and ^{13}C NMR for peptide 11

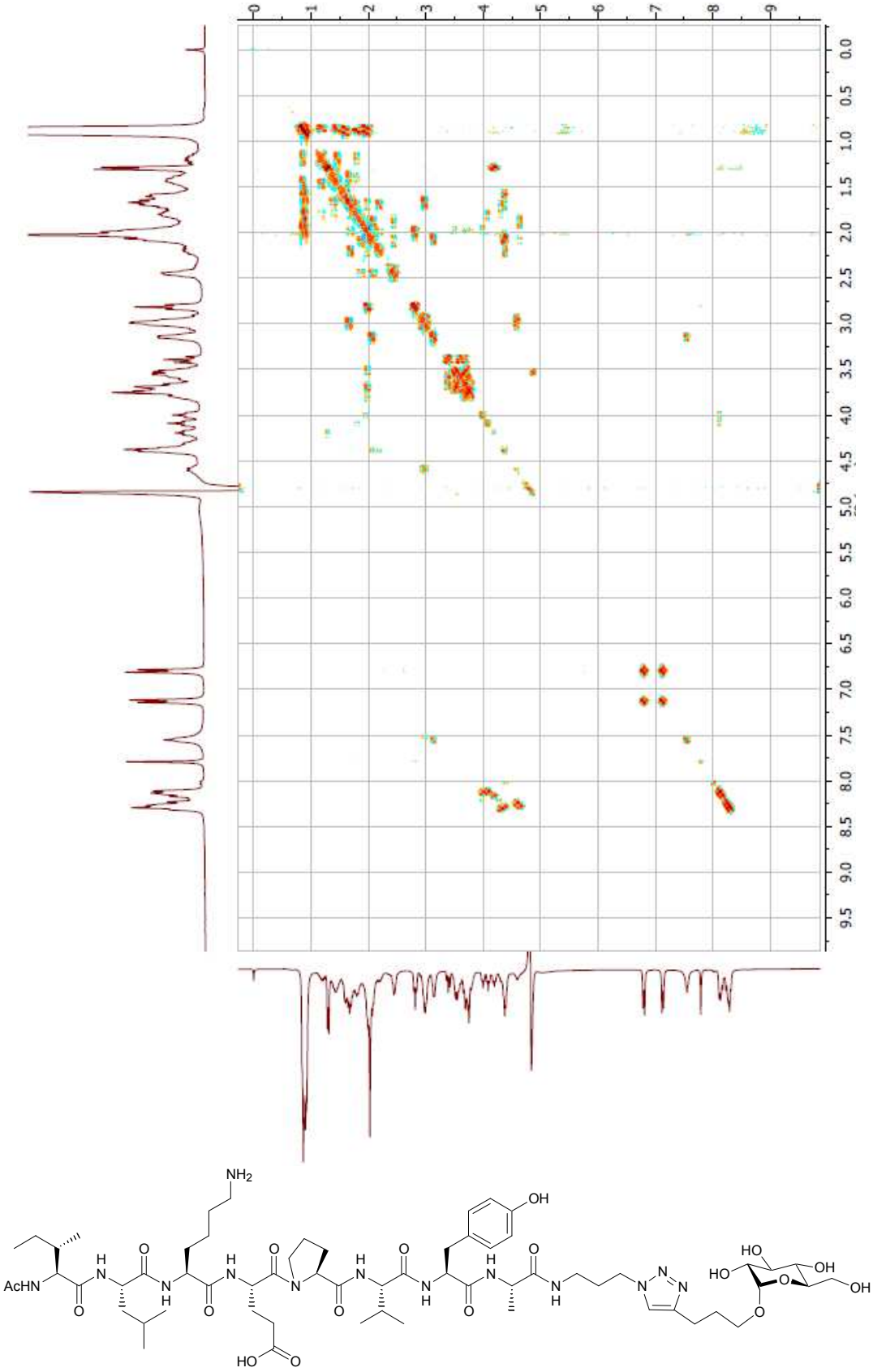
^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.42 – 8.21 (m, 4H), 8.21 – 8.05 (m, 3H), 7.79 (s, 1H), 7.55 (s, 1H), 7.13 (d, *J* = 8.0 Hz, 2H), 6.80 (d, *J* = 8.0 Hz, 2H), 4.73 – 4.48 (m, 2H), 4.49 – 4.25 (m, 5H), 4.19 (d, *J* = 6.6 Hz, 1H), 4.09 (t, *J* = 7.2 Hz, 1H), 4.00 (t, *J* = 7.2 Hz, 1H), 3.90 – 3.64 (m, 6H), 3.64 – 3.46 (m, 3H), 3.39 (t, *J* = 9.4 Hz, 1H), 3.24 – 3.07 (m, 2H), 3.07 – 2.89 (m, 4H), 2.82 (t, *J* = 7.2 Hz, 2H), 2.60 – 2.31 (m, 2H), 2.29 – 1.53 (m, 24H), 1.53 – 1.36 (m, 4H), 1.30 (d, *J* = 7.0 Hz, 3H), 1.27 – 1.10 (m, 2H), 1.06 – 0.70 (m, 18H).

^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 180.8, 177.1, 177.1, 177.0, 176.6, 176.5, 176.1, 176.0, 175.3, 174.1, 157.3, 150.4, 133.3, 130.5, 126.1, 118.2, 103.8, 100.9, 76.0, 74.5, 74.2, 72.4, 69.6, 65.4, 63.3, 63.0, 62.4, 61.5, 57.6, 56.0, 55.0, 53.8, 52.6, 50.7, 50.4, 42.4, 42.2, 39.2, 38.7, 33.2, 33.1, 32.8, 32.1, 31.6, 30.9, 29.1, 28.7, 27.4, 27.4, 27.0, 24.8, 24.7, 24.4, 24.0, 23.6, 21.0, 20.7, 19.5, 17.5, 13.0.

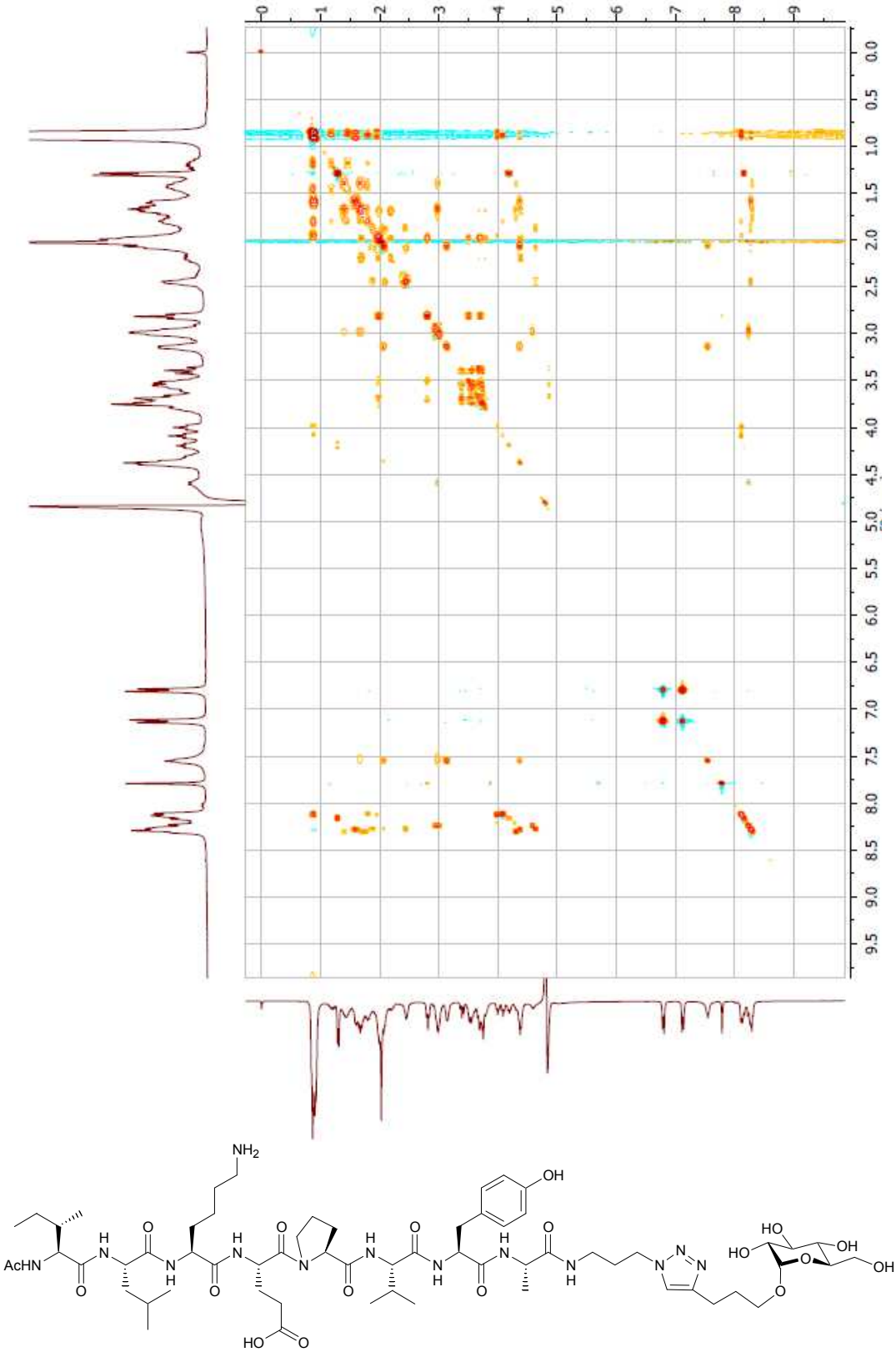
NMR ^{1}H experiment for compound **11**



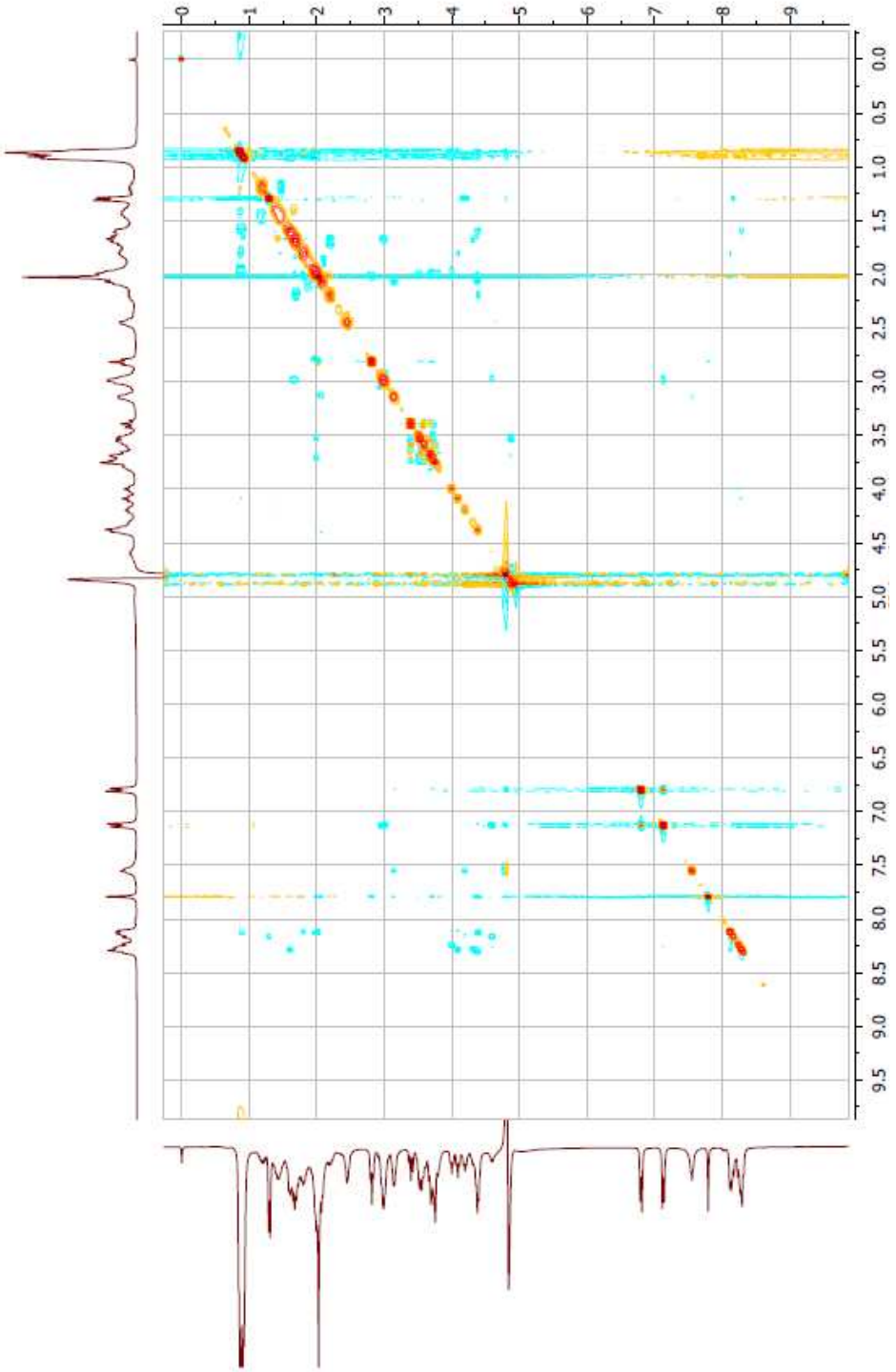
COSY experiment for compound **11**

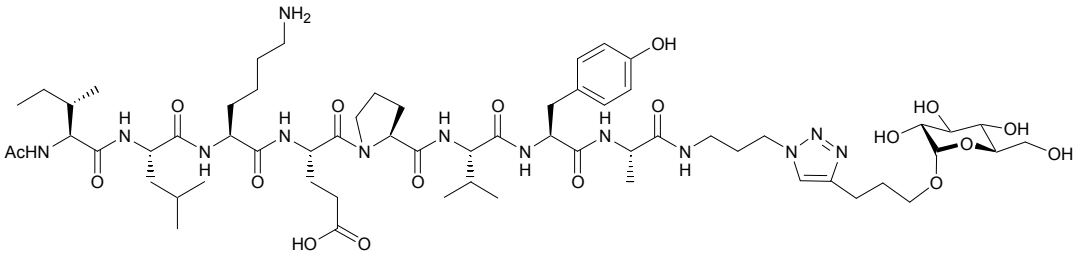


TOCSY experiment for compound **11**

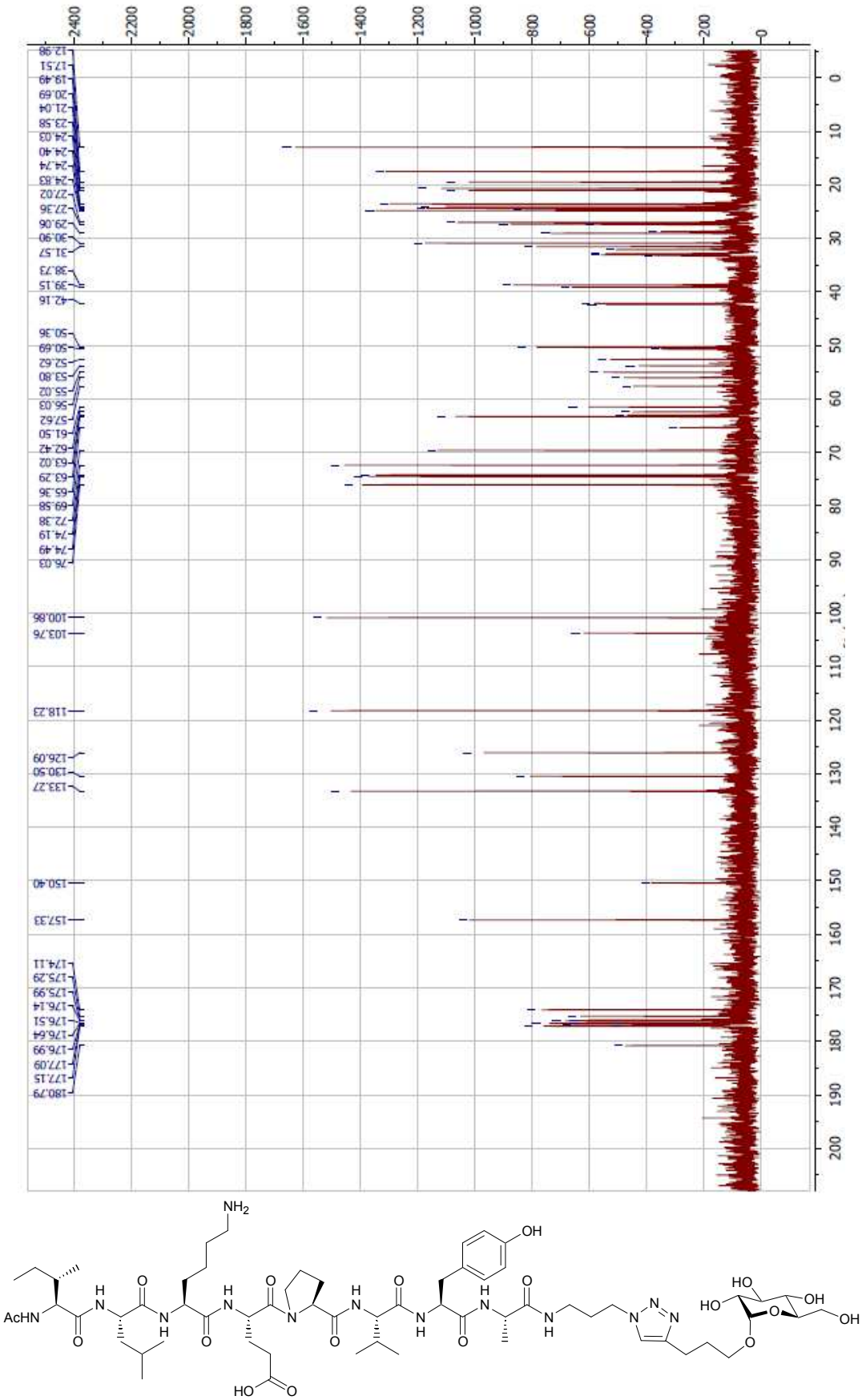


ROESY experiment for compound **11**

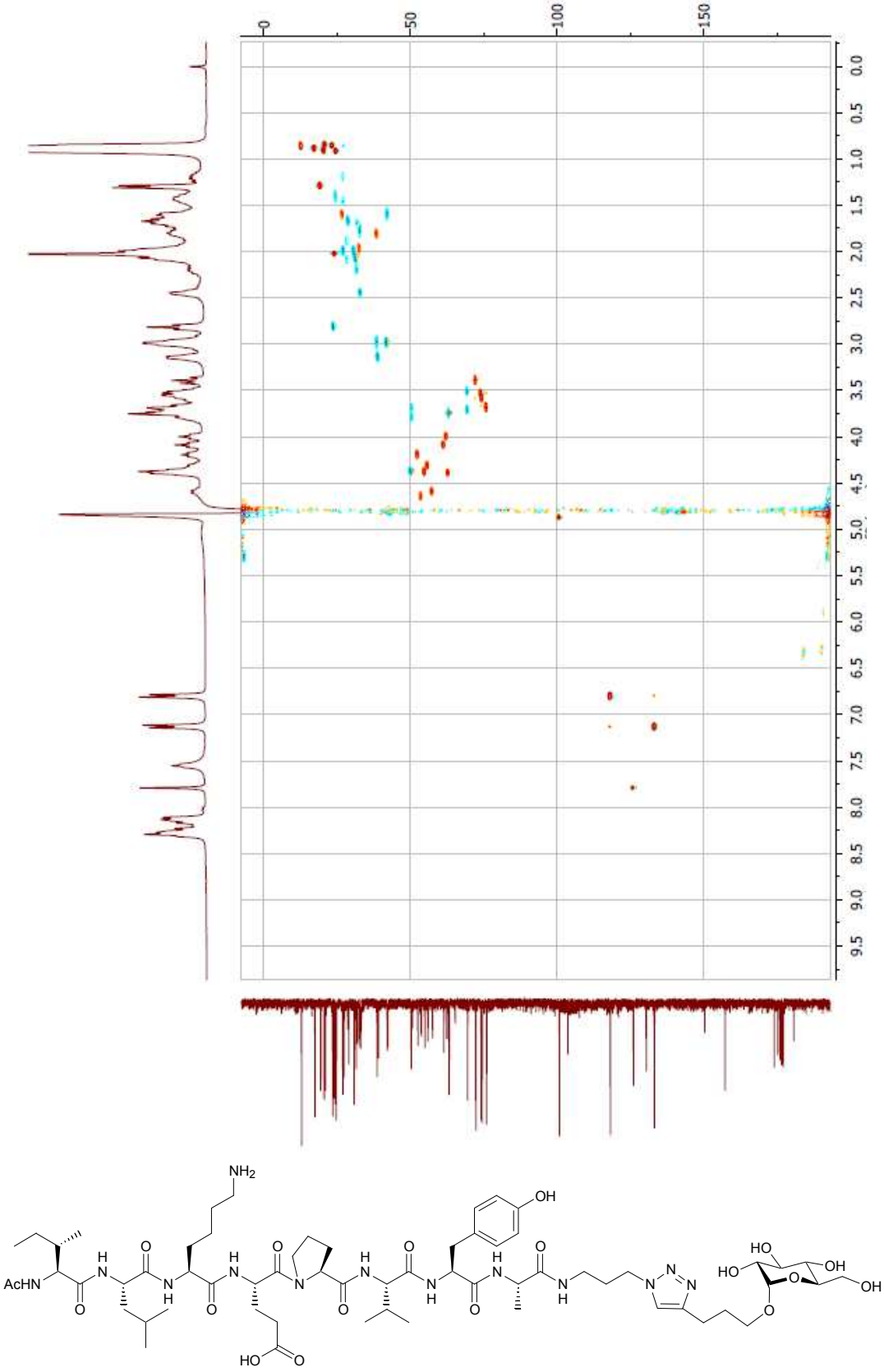




NMR ^{13}C experiment for compound **11**



HSQC experiment for compound **11**



Synthesis of peptide 12:

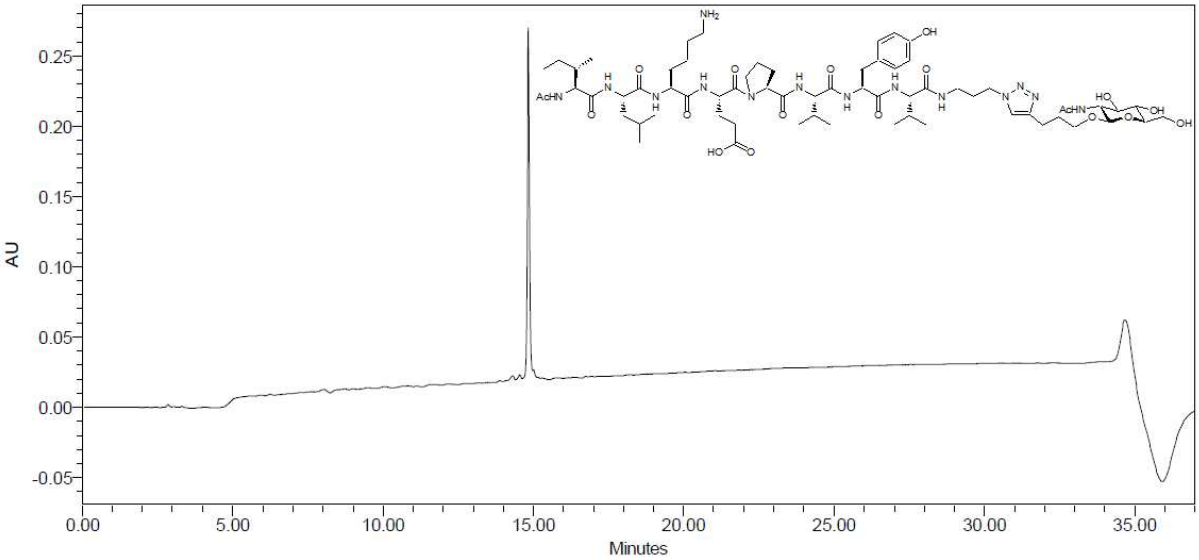
To a stirred solution of **9d** (8.8 mg, 8.1 µmol) and *N*-Acetyl Glucosamine derivative **6c** (4.7 mg, 16.3 µmol) in *tert*-butyl alcohol (910 µL) were successively added at rt copper (II) sulfate pentahydrate (405 µL of 2 mM in water, 0.81 µmol) followed by sodium ascorbate (405 µL of freshly prepared 20 mM in water, 8.1 µmol). After 4 h, HPLC analysis indicated complete consumption of starting material (Rt 17.8 min, linear H_2O/CH_3CN gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm) and formation of a major product (Rt 14.8 min). The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 12:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-40% B in 60 min) to give 8.7 mg of pure peptide **12** (78 % yield).

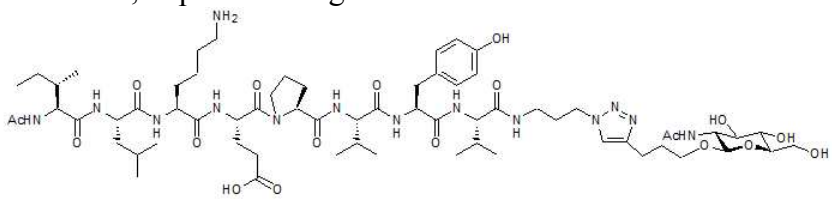
RP-HPLC analysis of 12

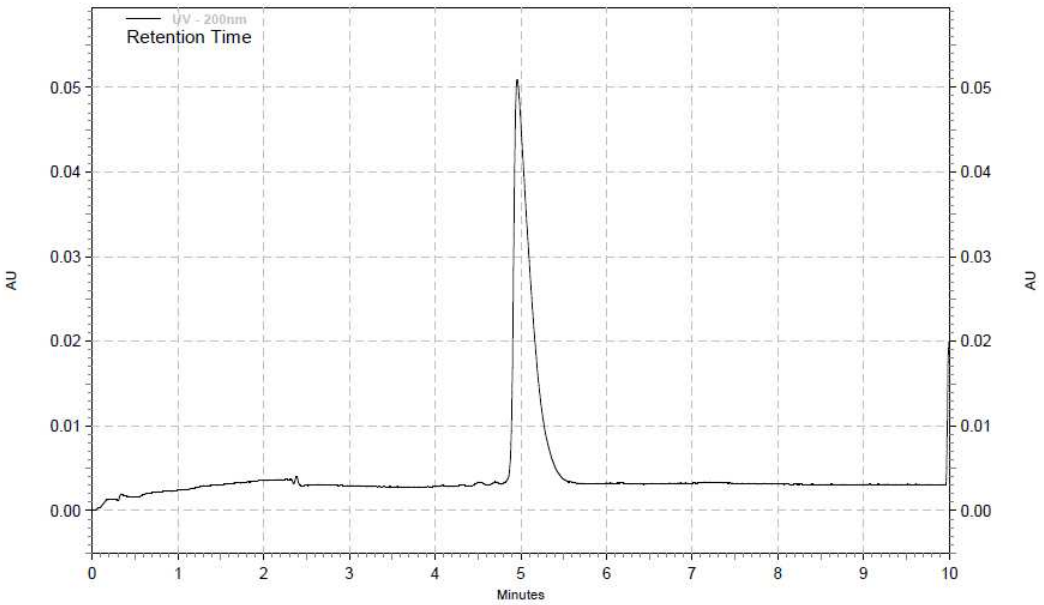
Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 12

T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.

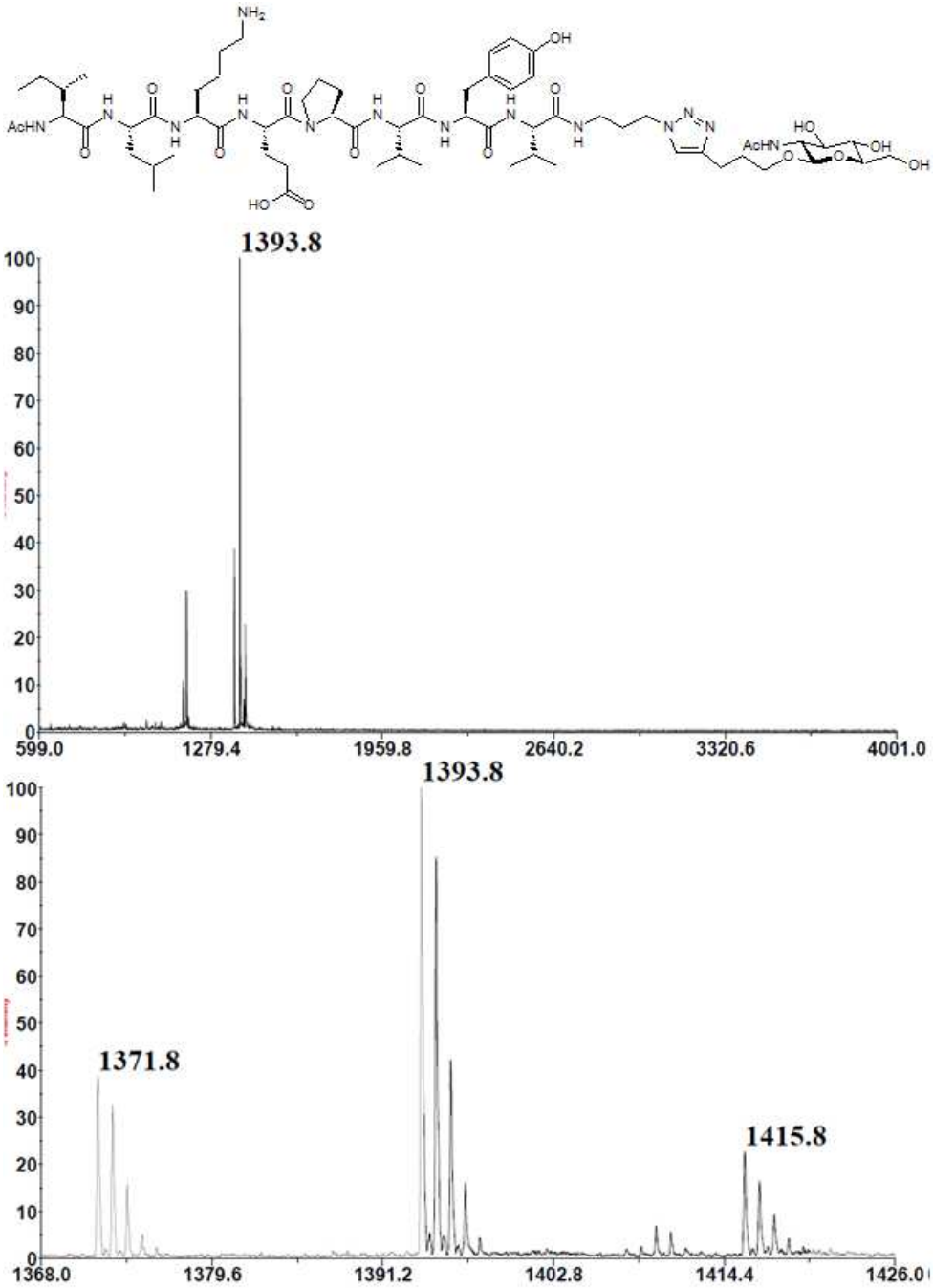




MALDI-TOF spectrum of 12

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{65}H_{106}N_{14}O_{18}$ $[M+H]^+$ 1371.8 found 1371.8 ; $[M+Na]^+$ calcd. 1393.8, found 1393.8.

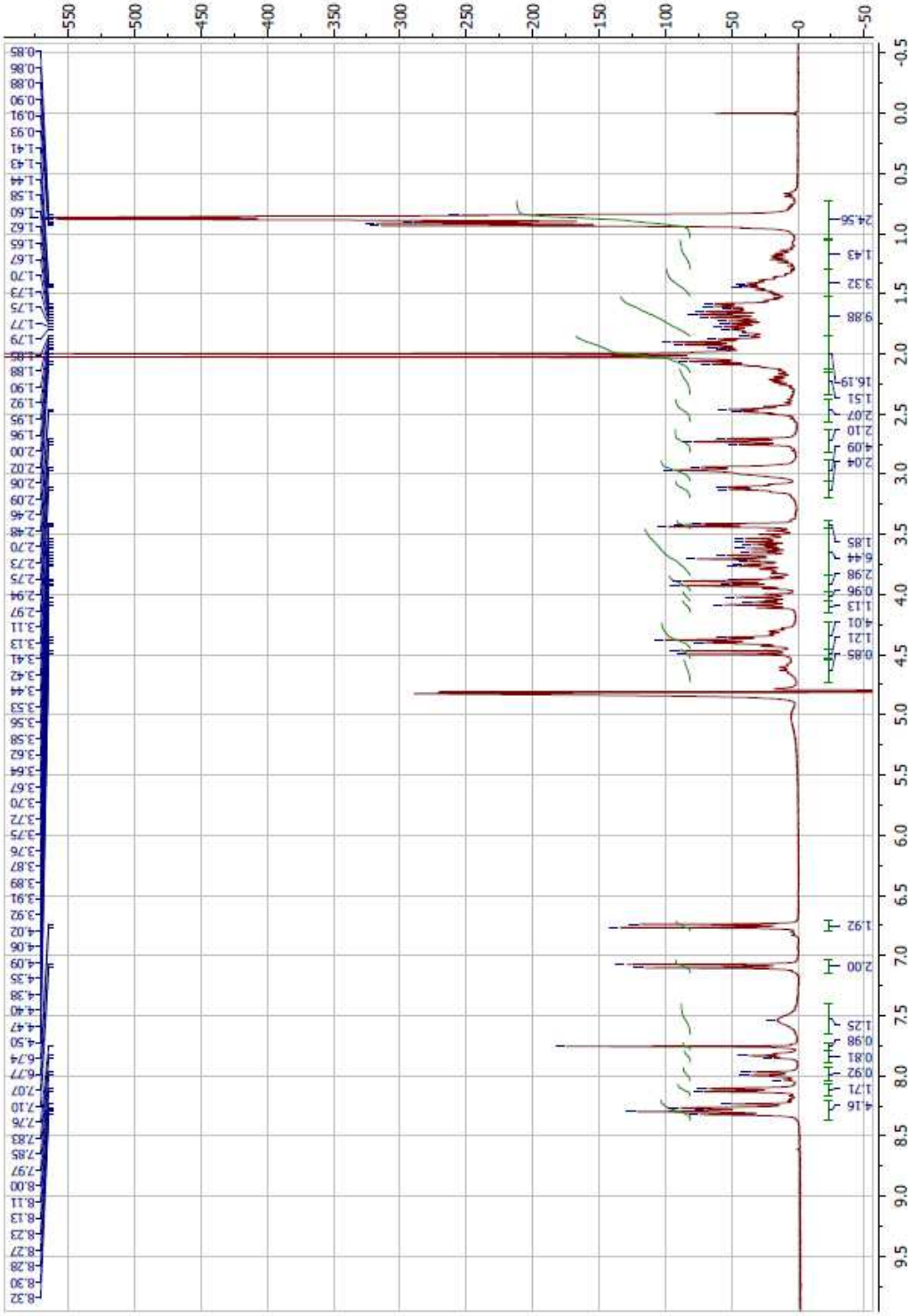


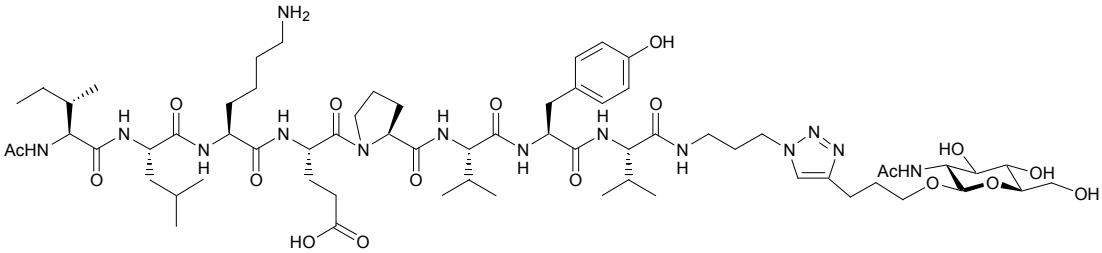
^{1}H and ^{13}C NMR for peptide 12

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.38 – 8.20 (m, 4H), 8.17 – 8.08 (m, 2H), 7.98 (d, *J* = 8.3 Hz, 1H), 7.84 (t, *J* = 5.6 Hz, 1H), 7.76 (s, 1H), 7.54 (s, 1H), 7.09 (d, *J* = 8.6 Hz, 2H), 6.75 (d, *J* = 8.6 Hz, 2H), 4.74 – 4.54 (m, 2H), 4.48 (d, *J* = 8.4 Hz, 1H), 4.45 – 4.22 (m, 5H), 4.09 (t, *J* = 7.4 Hz, 1H), 4.02 (t, *J* = 7.6 Hz, 1H), 3.98 – 3.84 (m, 3H), 3.82 – 3.47 (m, 6H), 3.46 – 3.38 (m, 2H), 3.20 – 3.06 (m, 2H), 3.06 – 2.89 (m, 4H), 2.73 (t, *J* = 7.5 Hz, 2H), 2.55 – 2.40 (m, 2H), 2.32 – 2.16 (m, 2H), 2.14 – 1.53 (m, 23H), 1.52 – 1.32 (m, 4H), 1.29 – 1.06 (m, 2H), 1.03 – 0.76 (m, 18H).

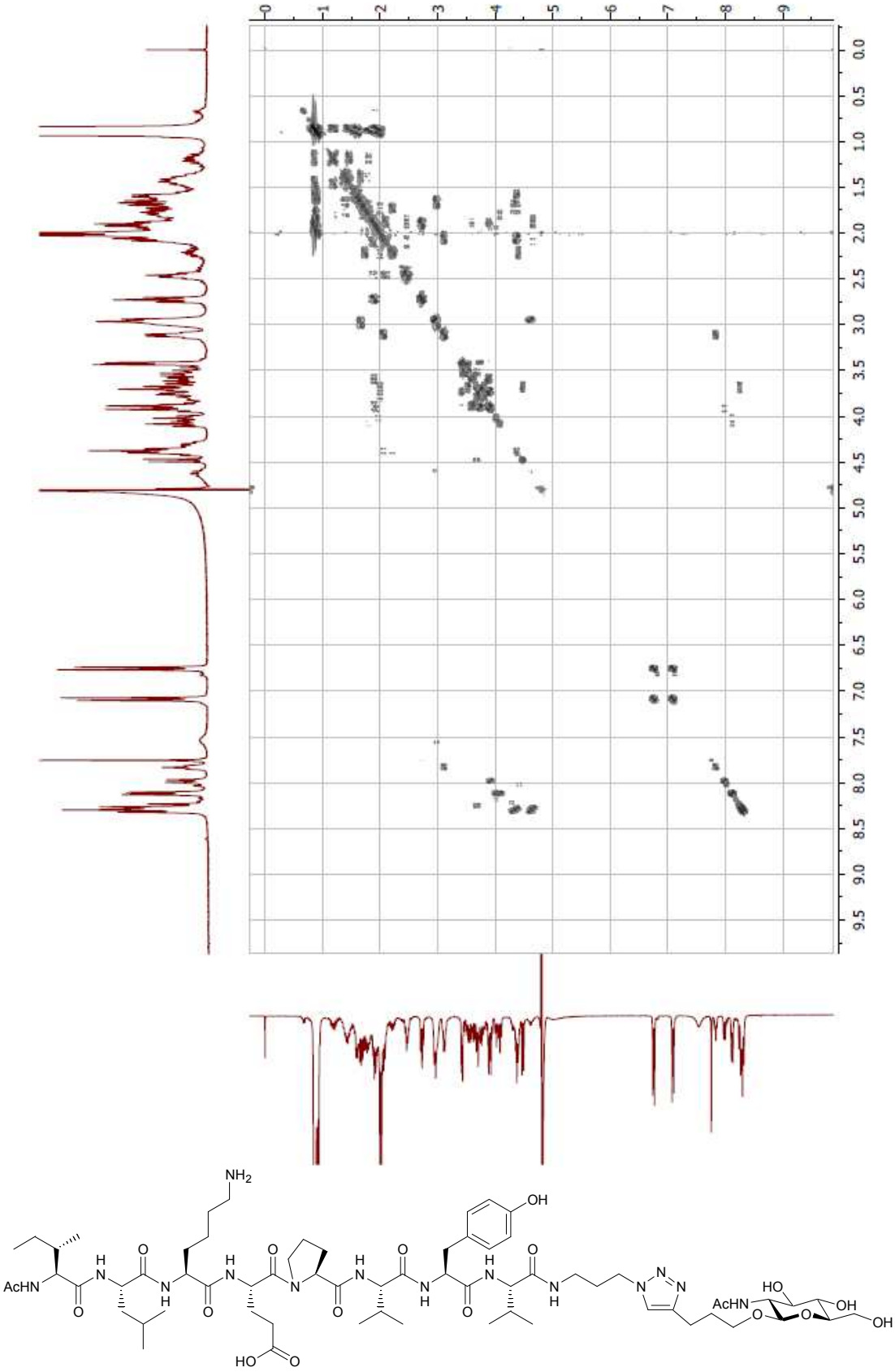
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 180.5, 177.3, 177.1, 177.0, 176.6, 176.4, 176.1, 175.7, 175.4, 175.3, 174.1, 157.3, 150.3, 133.2, 130.4, 125.9, 118.2, 103.9, 103.8, 78.6, 76.7, 72.7, 71.9, 63.6, 63.0, 62.6, 62.3, 61.5, 58.4, 57.7, 56.0, 55.0, 53.7, 50.7, 50.4, 42.4, 42.2, 39.1, 38.7, 33.1, 33.0, 32.9, 32.1, 31.6, 31.0, 29.1, 28.6, 27.4, 27.3, 27.0, 24.9, 24.8, 24.7, 24.4, 23.7, 23.6, 21.1, 20.7, 20.6, 17.5, 13.0.

NMR ^{1}H experiment for compound **12**

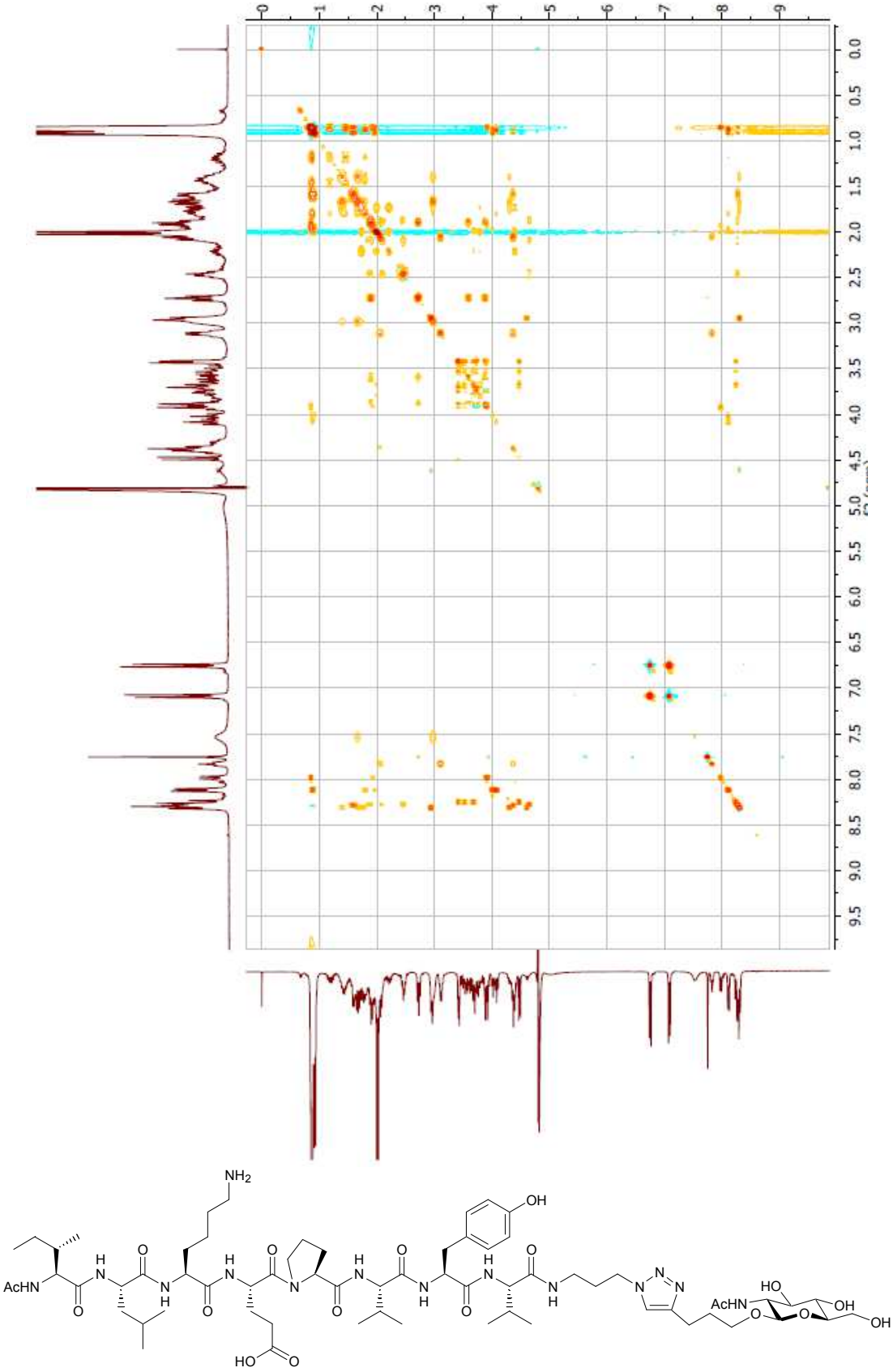




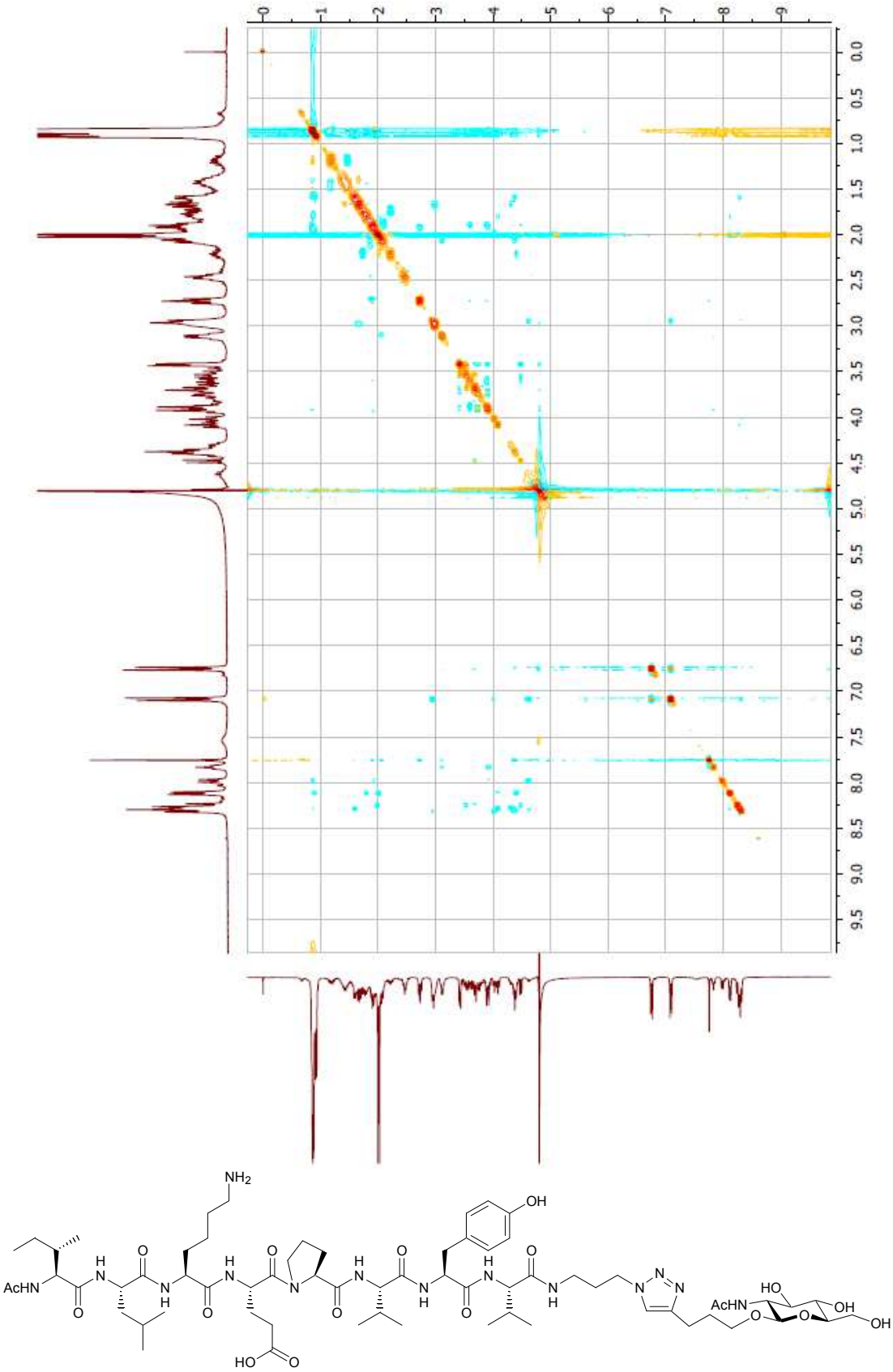
COSY experiment for compound **12**



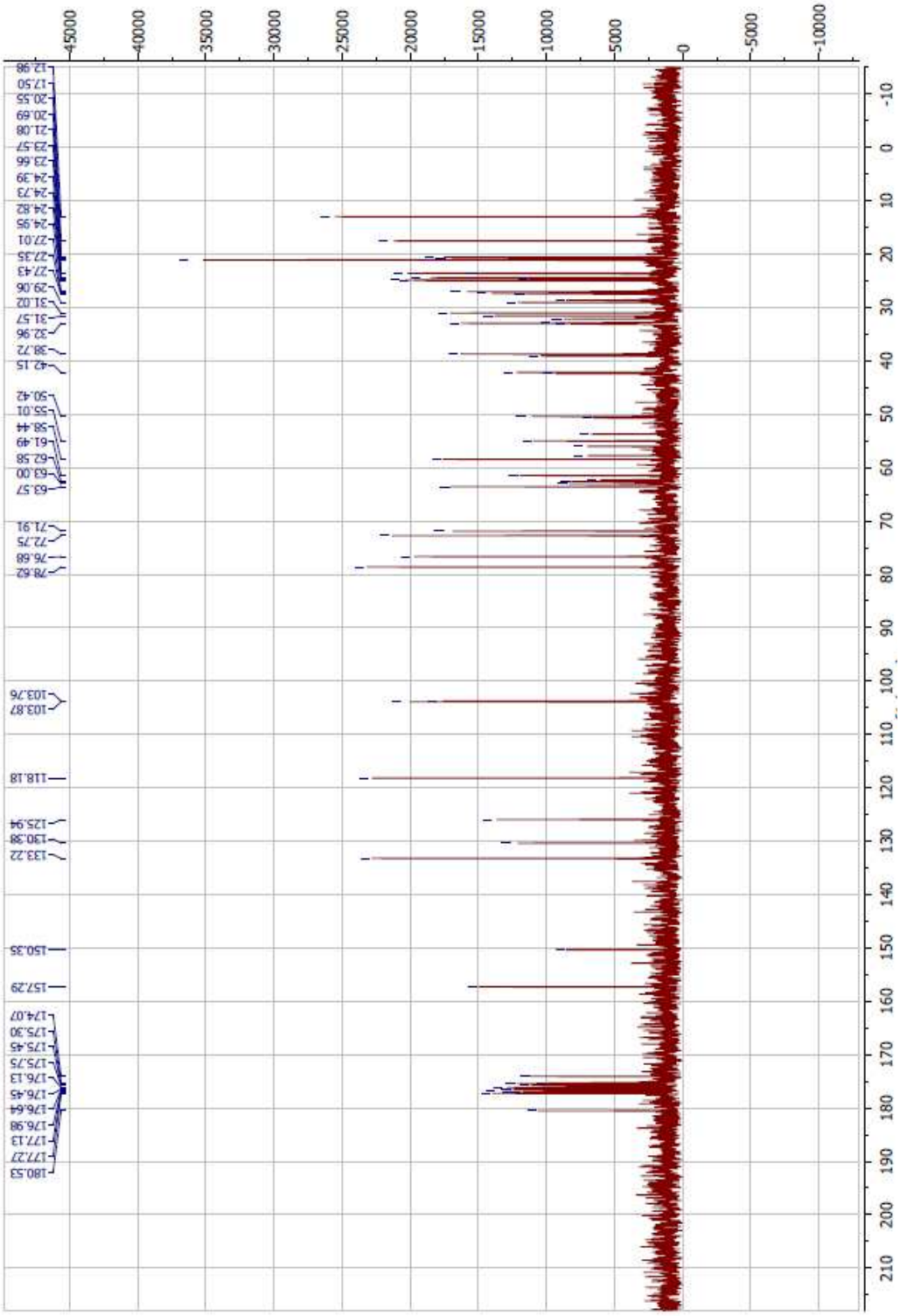
TOCSY experiment for compound **12**

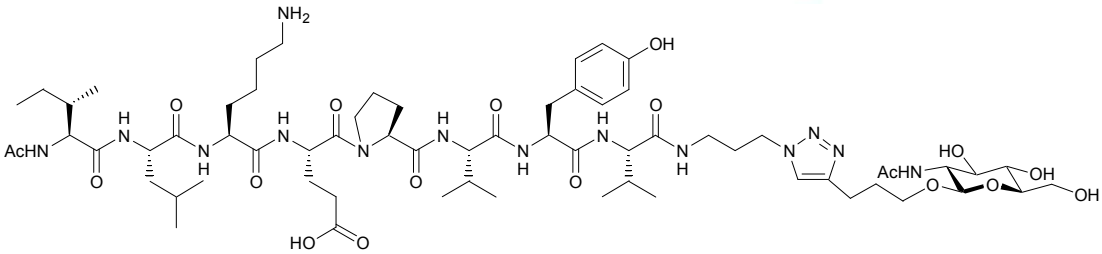


ROESY experiment for compound **12**

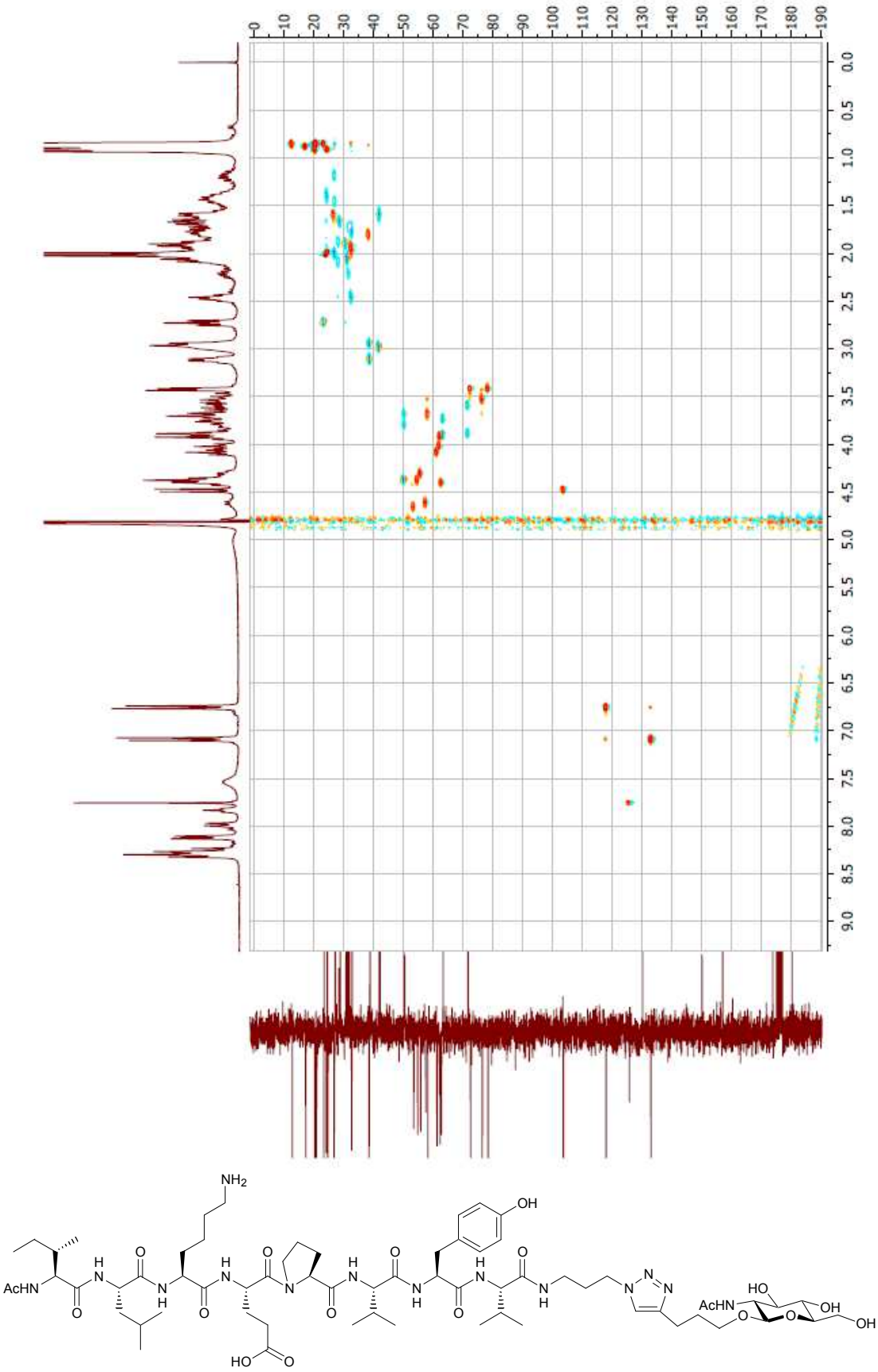


NMR ^{13}C experiment for compound **12**





HSQC experiment for compound **12**



Synthesis of peptide 13:

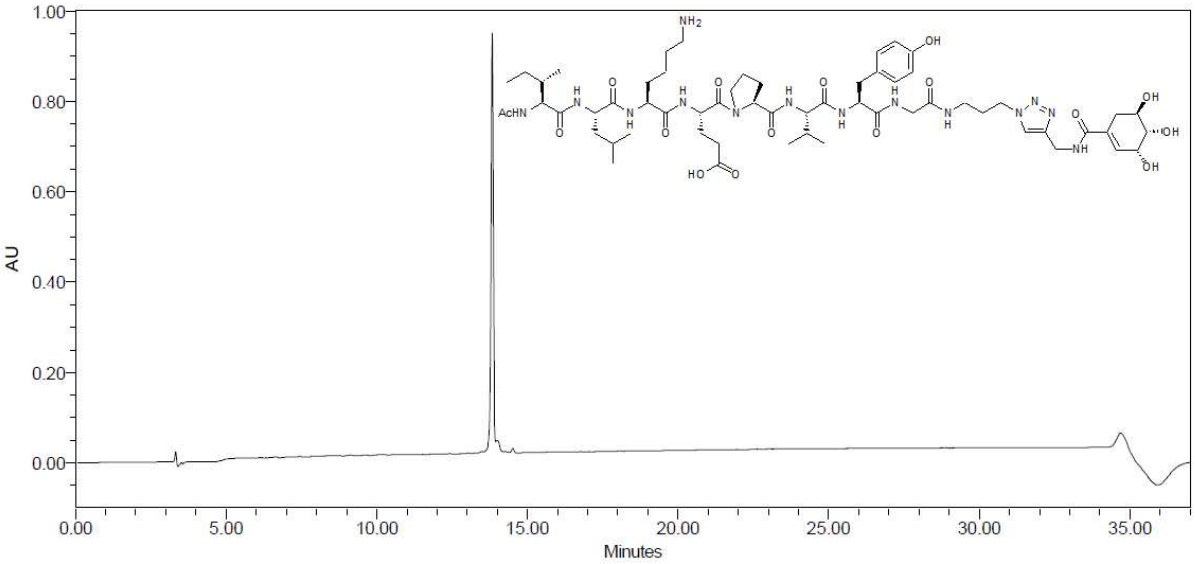
To a stirred solution of **9a** (10.4 mg, 10 µmol) and Shikimic derivative **6d** (4.2 mg, 20 µmol) in *tert*-butyl alcohol (1.0 mL) were successively added at rt copper (II) sulfate pentahydrate (500 µL of 2 mM in water, 1.0 µmol) followed by sodium ascorbate (500 µL of freshly prepared 20 mM in water, 10 µmol). After 4 h, HPLC analysis indicated complete consumption of starting material (Rt 16.2 min, linear H_2O/CH_3CN gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm) and formation of a major product (Rt 13.9 min). The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 13:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-40% B in 60 min) to give 10.1 mg of pure peptide **13** (79 % yield).

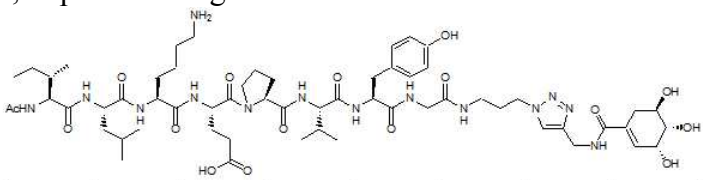
RP-HPLC analysis of 13

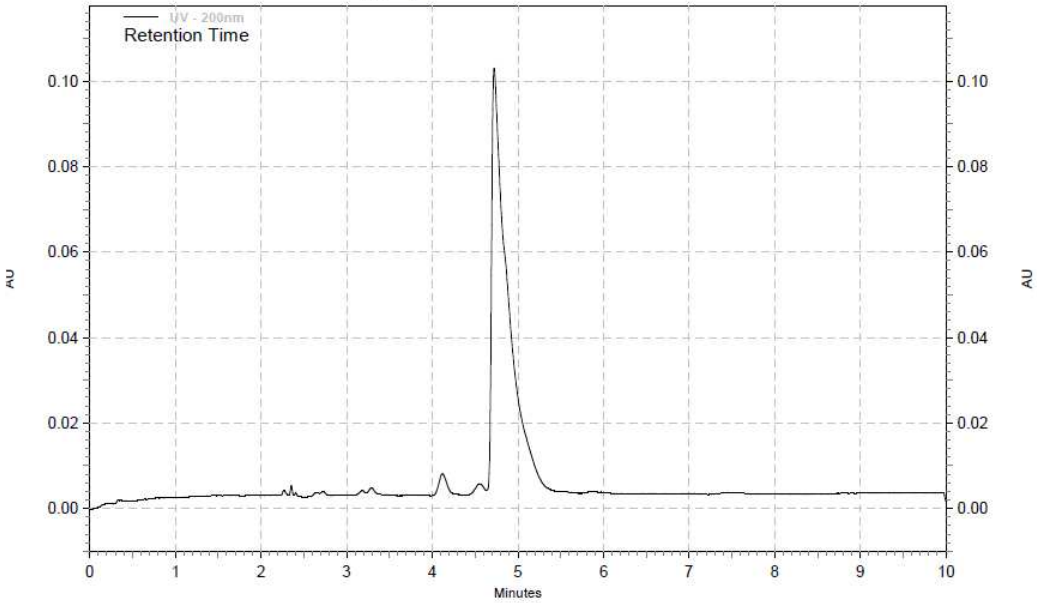
Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 13

T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.

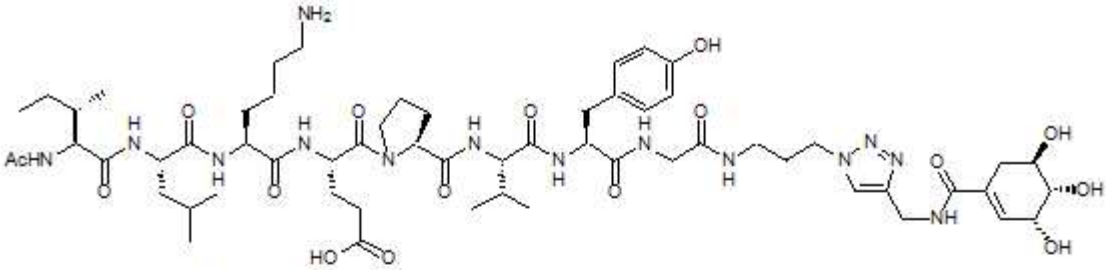


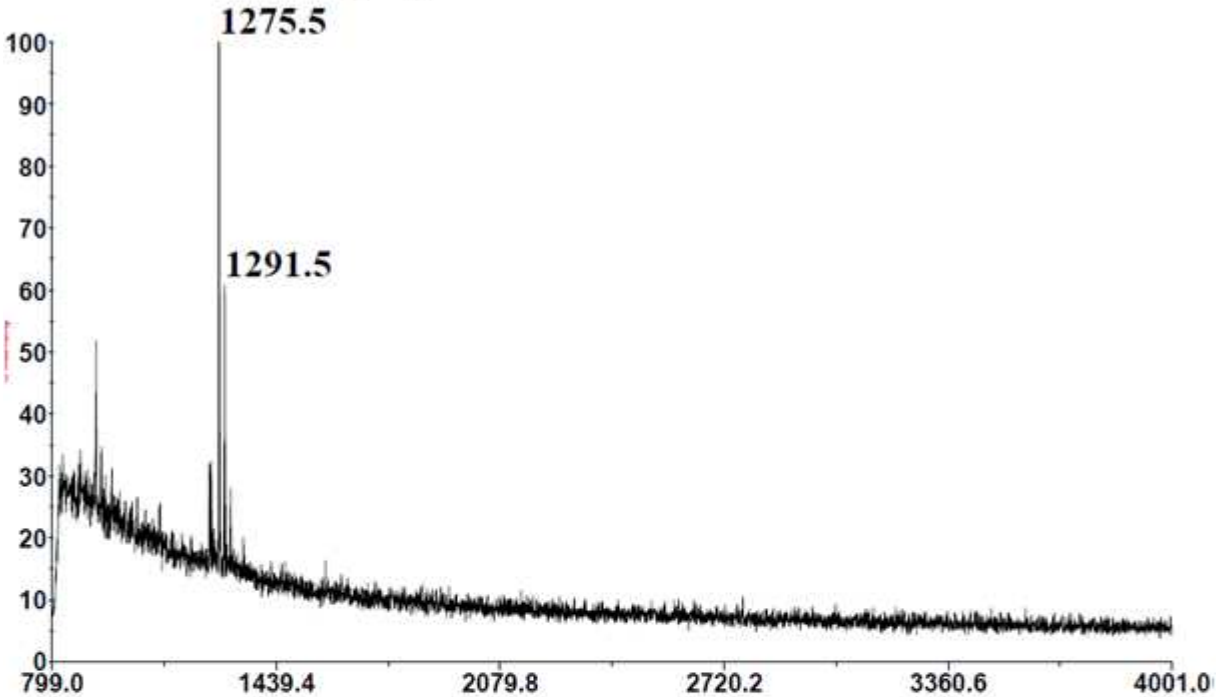


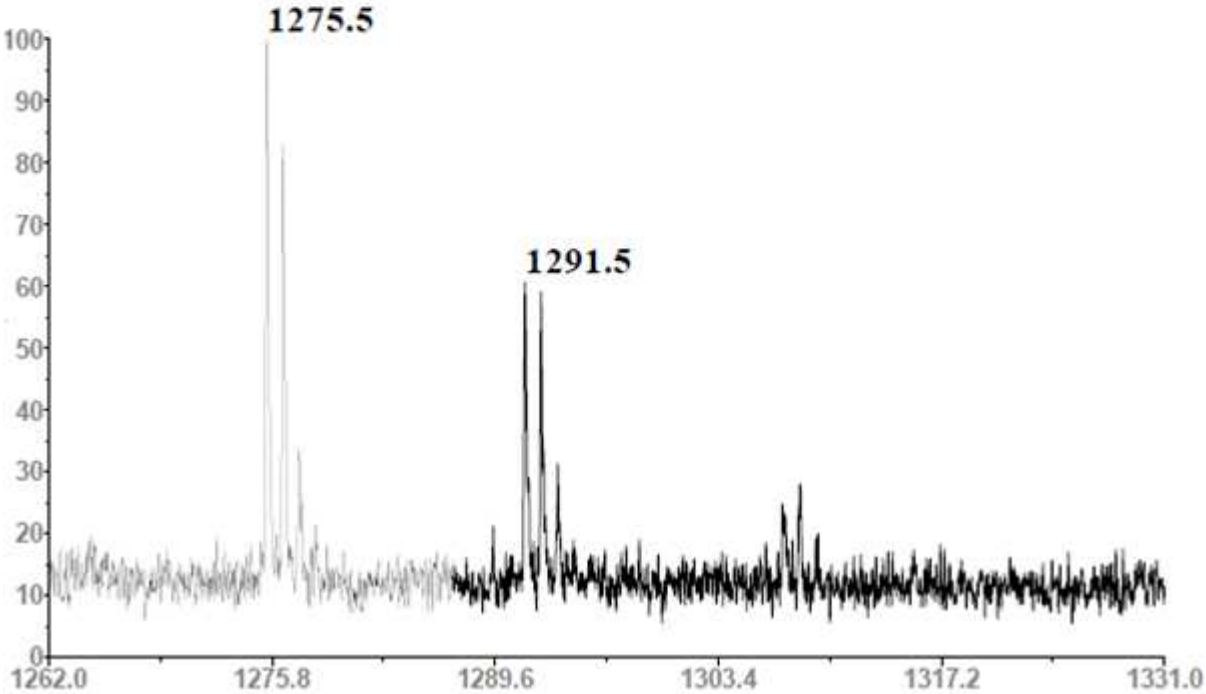
MALDI-TOF spectrum of 13

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{59}H_{92}N_{14}O_{16}$ $[M+Na]^+$ 1275.7 found 1275.5 ; $[M+K]^+$ calcd. 1291.6, found 1291.5.





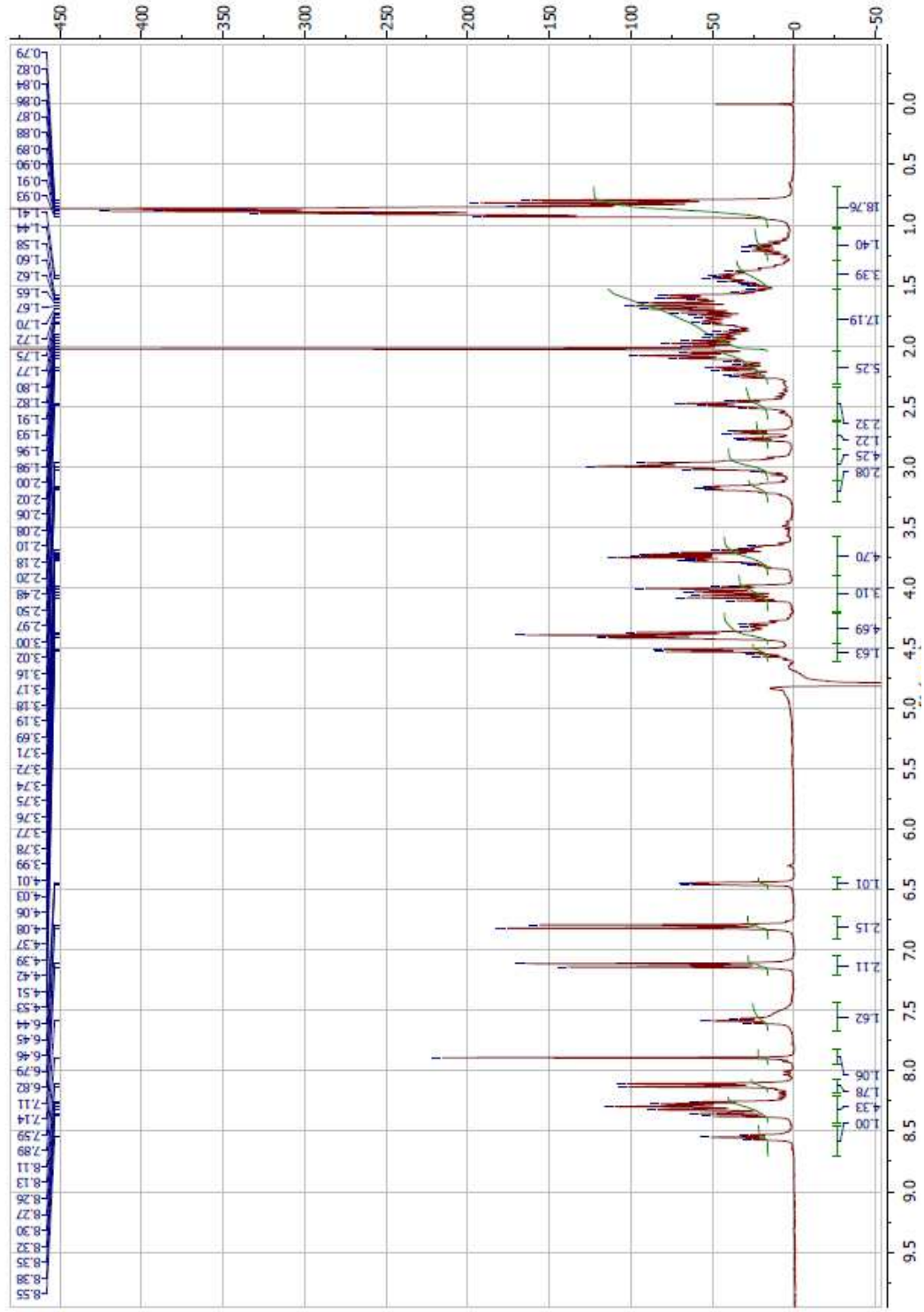


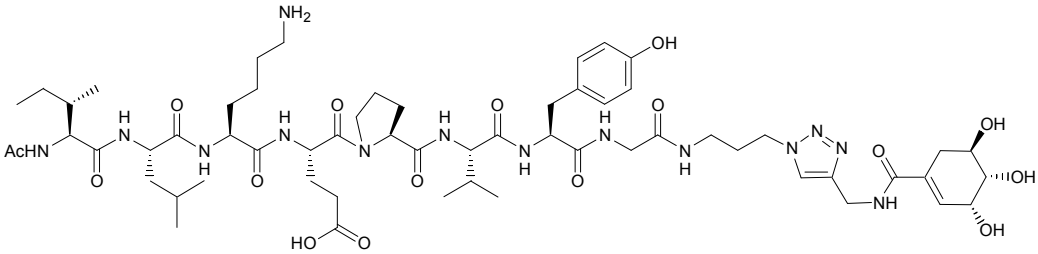
^{1}H and ^{13}C NMR for peptide 13

^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.55 (t, *J* = 5.8 Hz, 1H), 8.42 – 8.24 (m, 5H), 8.12 (d, *J* = 7.3 Hz, 2H), 7.89 (s, 1H), 7.59 (t, *J* = 5.8 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 2H), 6.81 (d, *J* = 8.5 Hz, 2H), 6.49 – 6.43 (m, 1H), 4.70 – 4.55 (m, 2H), 4.53 (d, *J* = 5.2 Hz, 2H), 4.45 – 4.25 (m, 6H), 4.14 – 3.96 (m, 3H), 3.87 – 3.63 (m, 5H), 3.26 – 3.13 (m, 2H), 3.08 – 2.90 (m, 4H), 2.74 (dd, *J* = 17.7, 5.2 Hz, 2H), 2.57 – 2.39 (m, 2H), 2.28 – 2.06 (m, 6H), 2.02 – 1.56 (m, 17H), 1.50 – 1.34 (m, 3H), 1.25 – 1.10 (m, 2H), 1.00 – 0.75 (m, 18H).

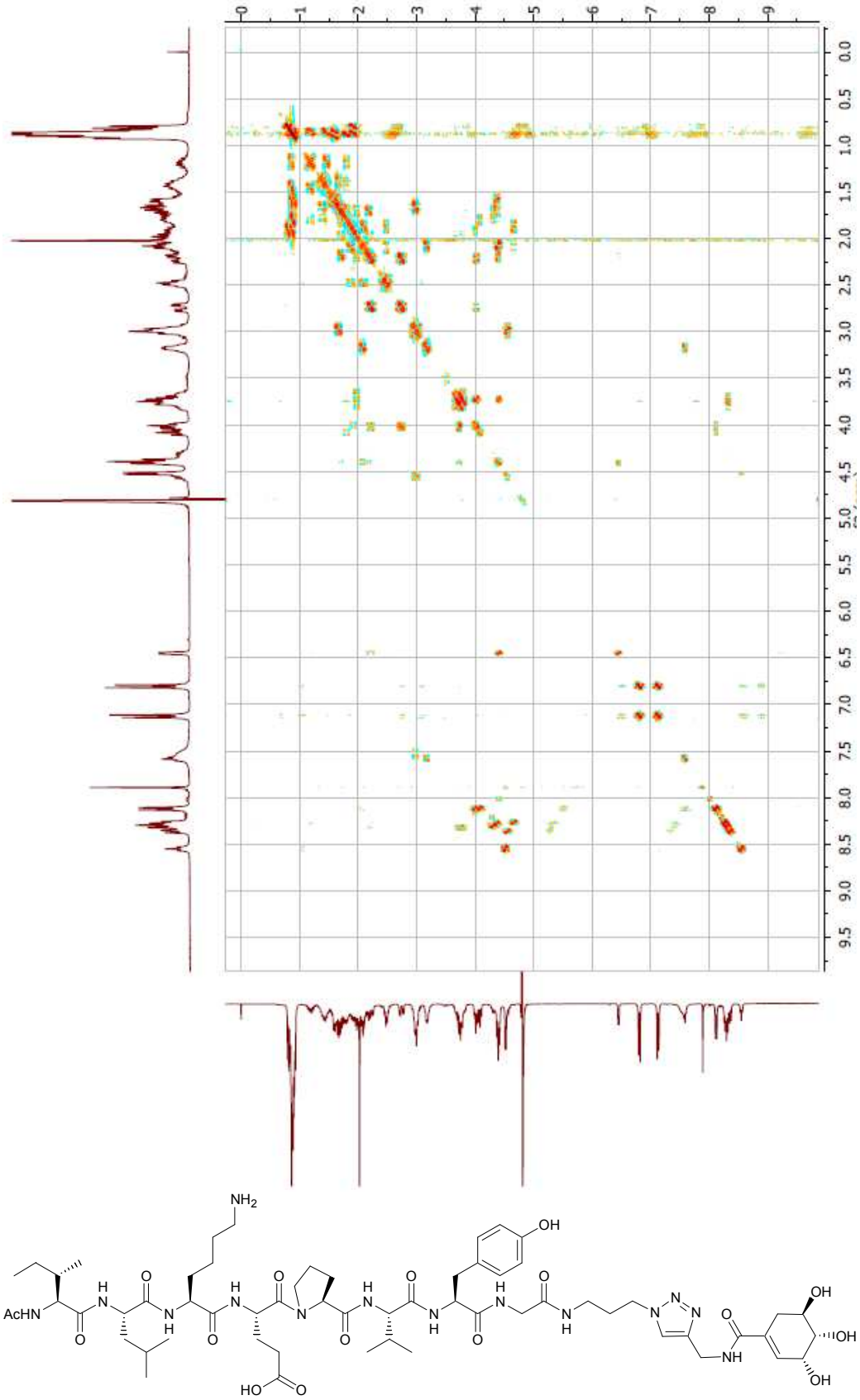
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 180.0, 177.1, 177.0, 176.6, 176.5, 176.3, 176.1, 176.1, 174.0, 173.8, 172.7, 165.9, 165.4, 157.3, 147.3, 135.5, 134.4, 133.2, 130.6, 126.7, 121.0, 118.2, 117.1, 103.7, 74.2, 69.2, 68.6, 63.0, 62.2, 61.5, 58.1, 56.0, 55.0, 53.6, 50.7, 50.5, 45.5, 42.4, 42.2, 39.1, 38.7, 37.5, 33.7, 33.1, 32.9, 32.5, 32.1, 31.6, 29.1, 28.4, 27.4, 27.4, 27.0, 24.8, 24.7, 24.4, 23.6, 21.0, 20.6, 17.5, 13.0.

NMR ^{1}H experiment for compound **13**

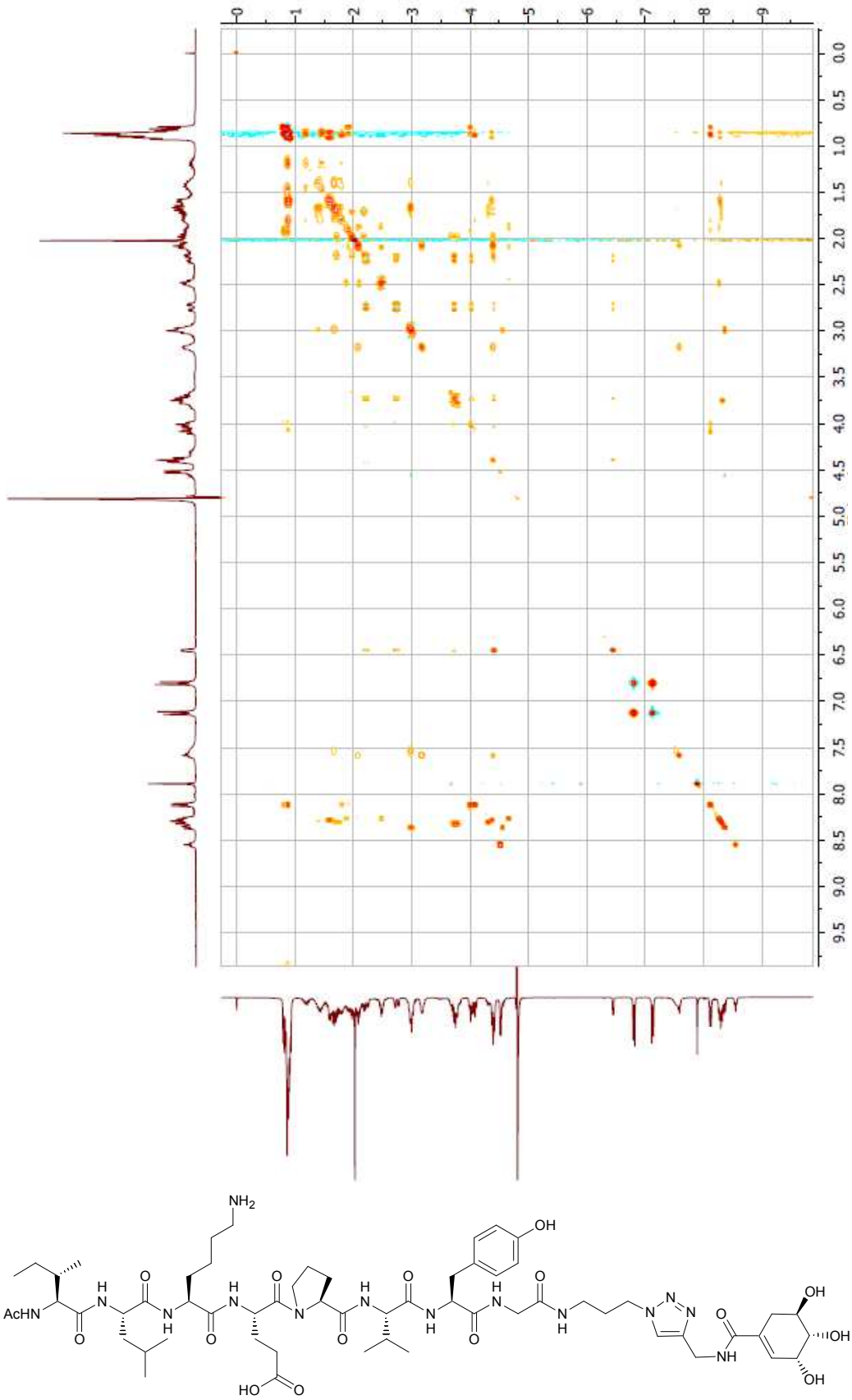




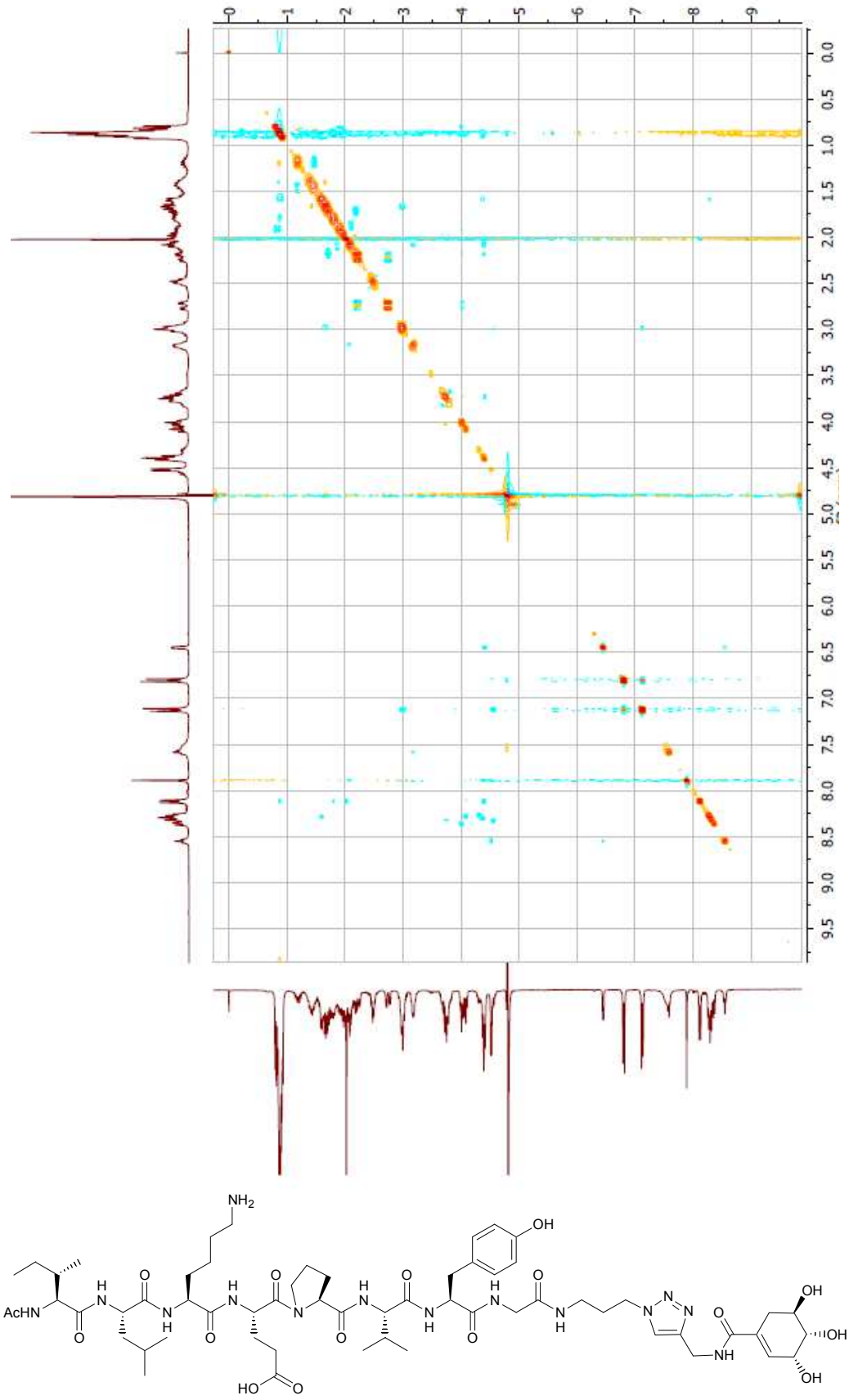
COSY experiment for compound **13**



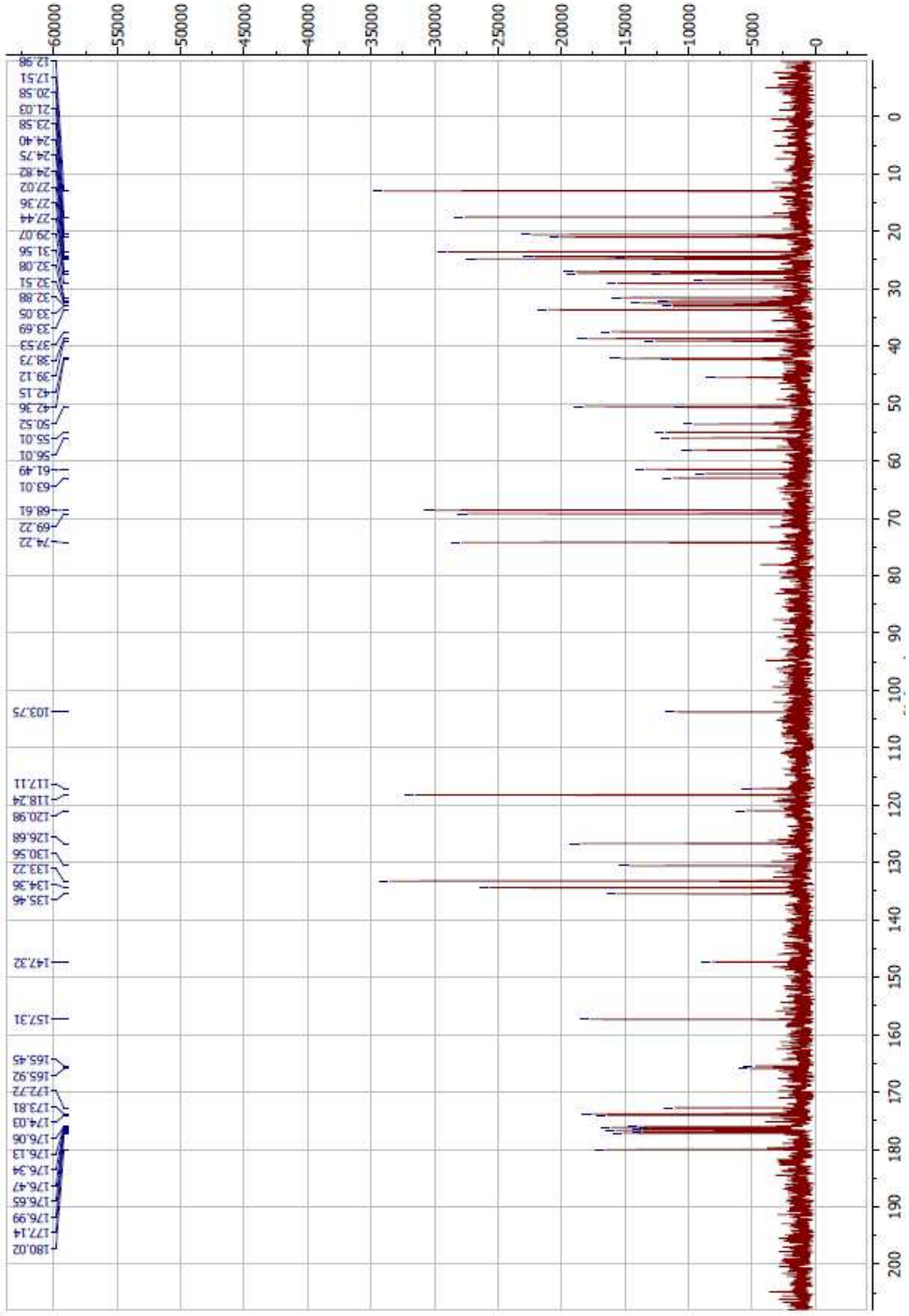
TOCSY experiment for compound **13**

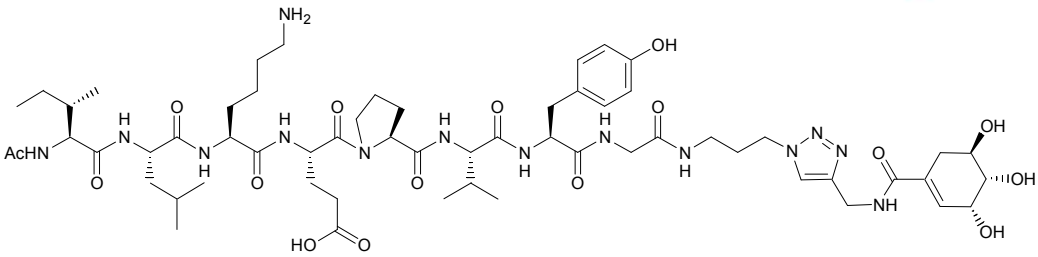


ROESY experiment for compound **13**

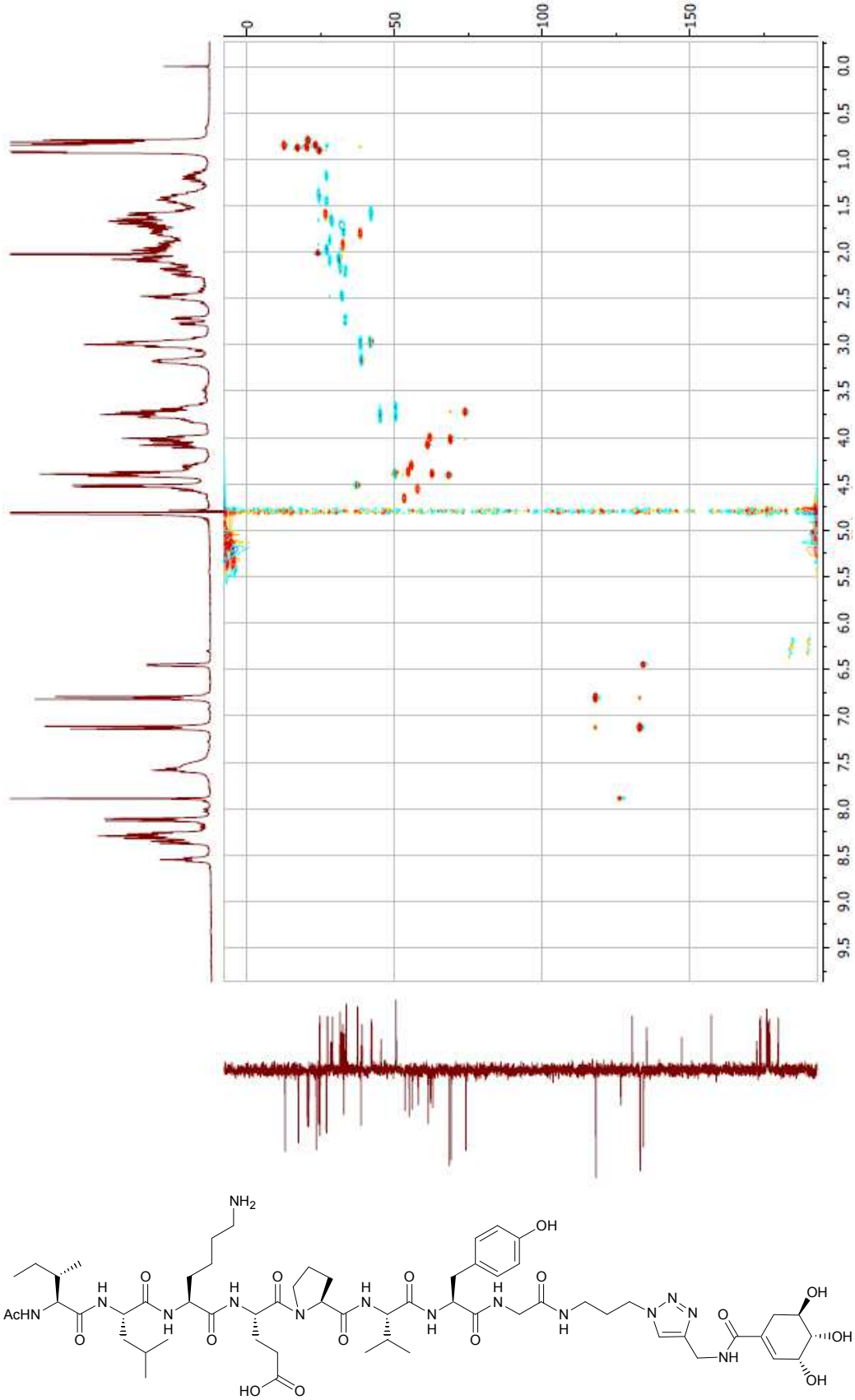


NMR ^{13}C experiment for compound **13**





HSQC experiment for compound **13**



Synthesis of peptide 14:

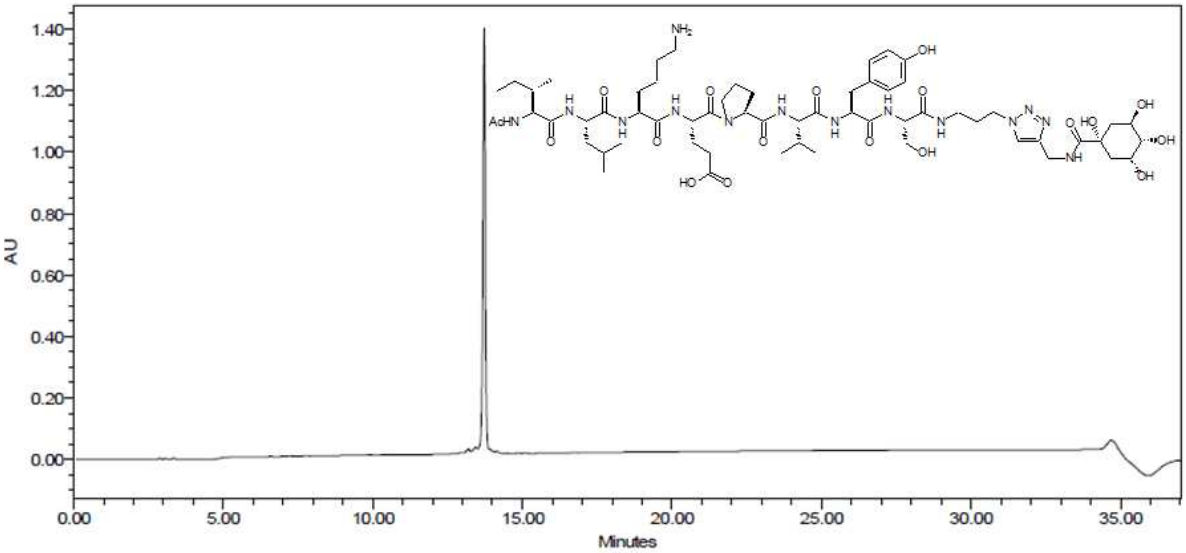
To a stirred solution of **9c** (3.6 mg, 3.3 µmol) and Quinic derivative **6e** (1.56 mg, 6.7 µmol) in *tert*-butyl alcohol (335 µL) were successively added at rt copper (II) sulfate pentahydrate (165 µL of 2 mM in water, 0.33 µmol) followed by sodium ascorbate (500 µL of freshly prepared 20 mM in water, 3.3 µmol). After 4 h, HPLC analysis indicated complete consumption of starting material (Rt 16.1 min, linear H_2O/CH_3CN gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm) and formation of a major product (Rt 13.7 min). The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 14:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-65% B in 40 min) to give 3.1 mg of pure peptide **14** (72 % yield).

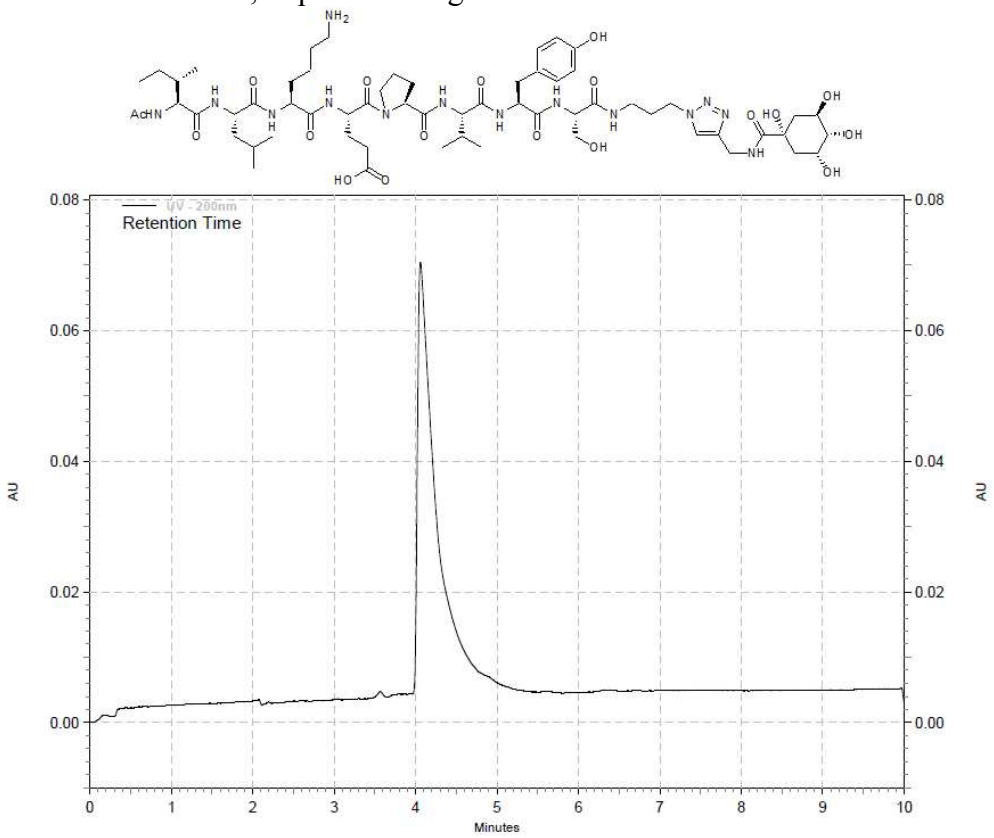
RP-HPLC analysis of 14

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 14

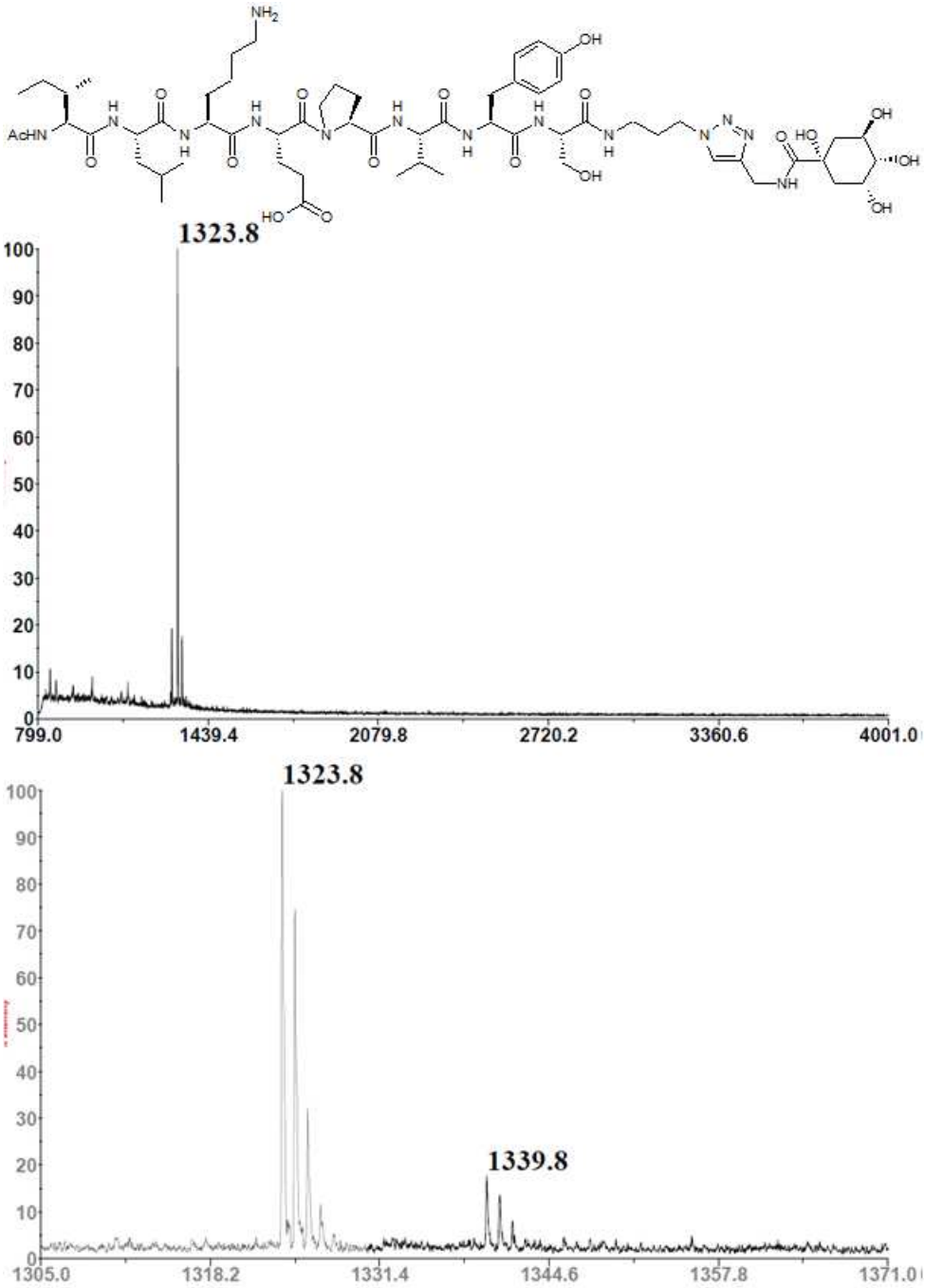
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.



MALDI-TOF spectrum of 14

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{60}H_{96}N_{14}O_{18}$ $[M+Na]^+$ 1323.7 found 1323.8 ; $[M+K]^+$ calcd. 1339.7, found 1339.8.

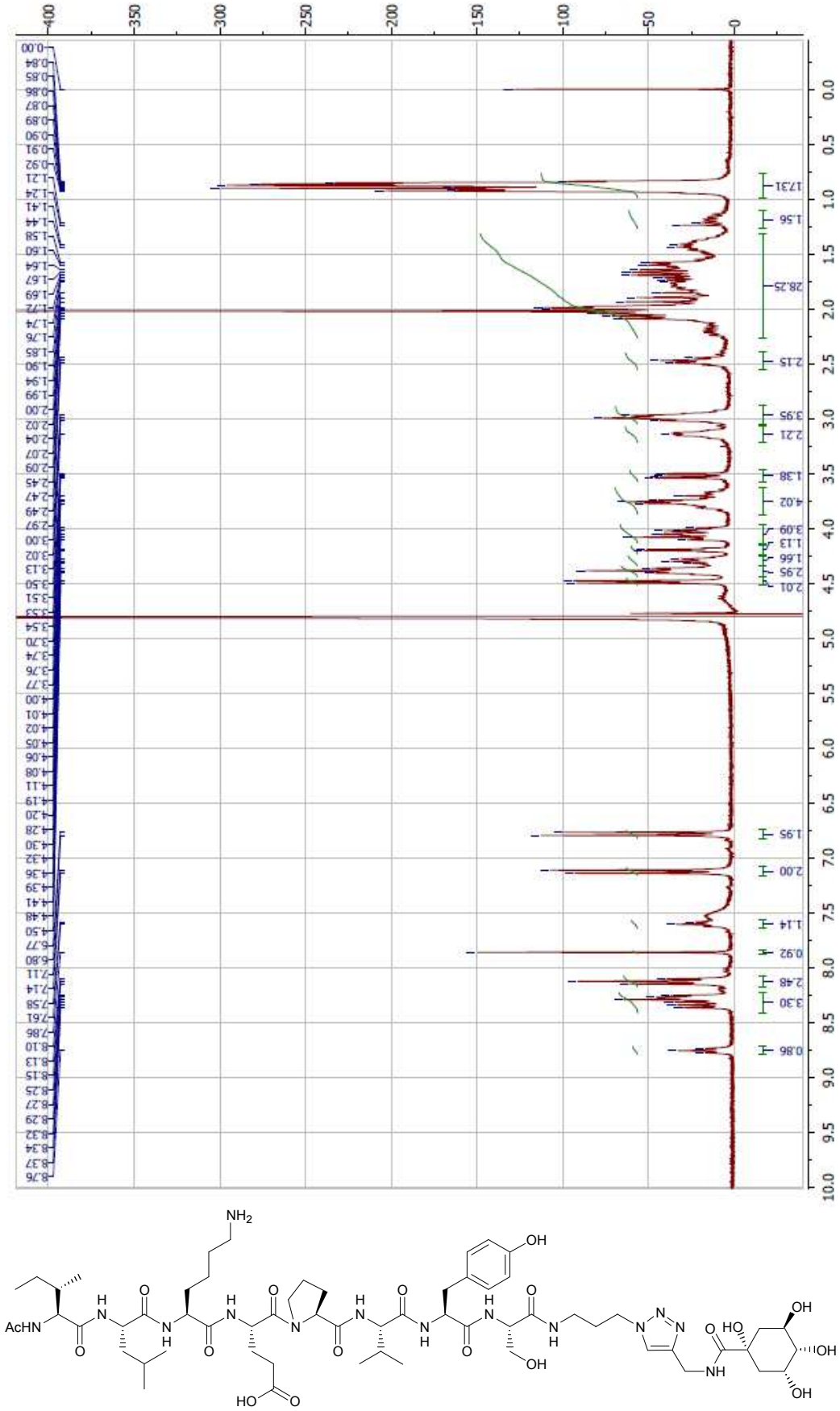


^{1}H and ^{13}C NMR for peptide 14

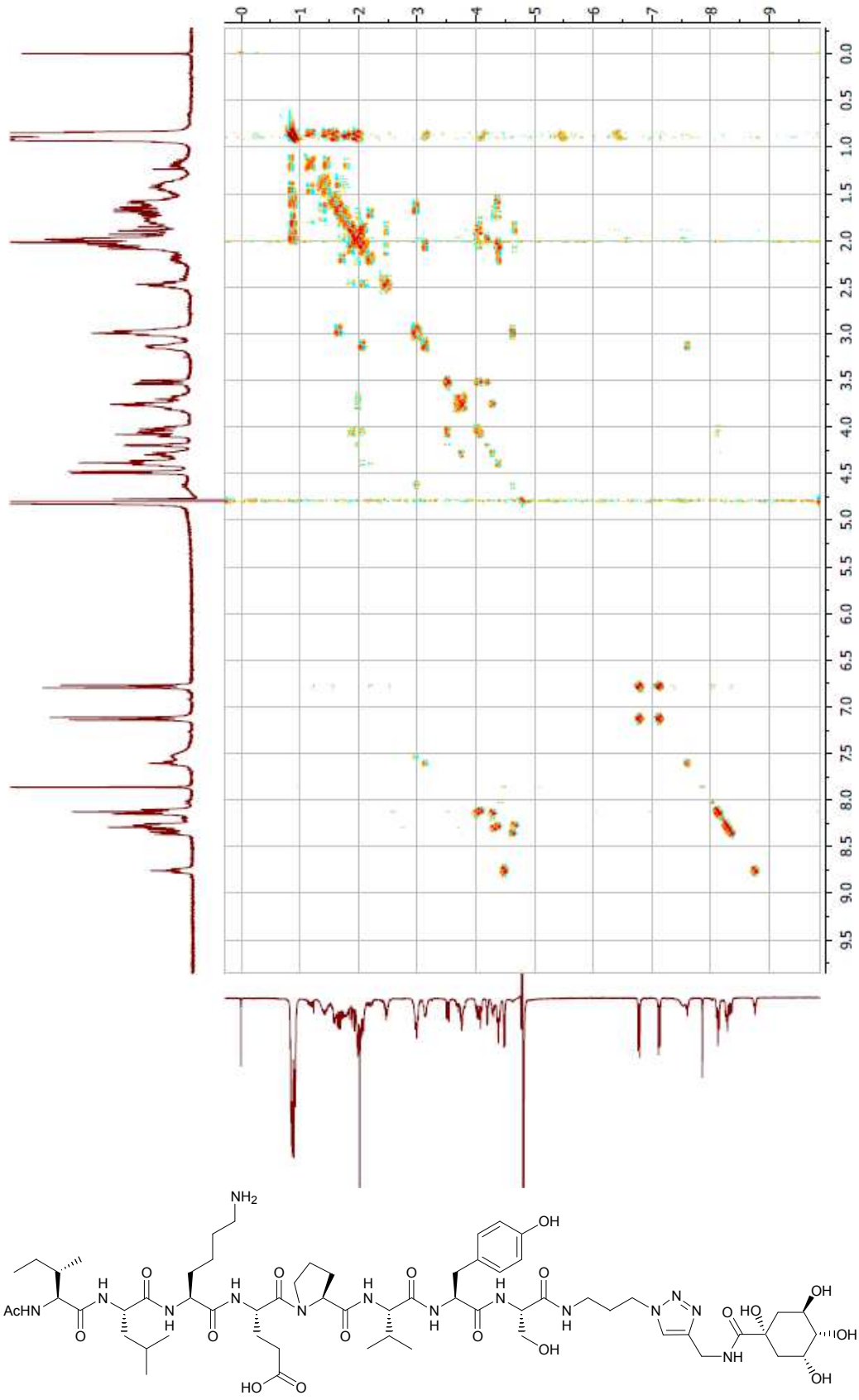
^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.76 (t, *J* = 6.0 Hz, 1H), 8.41 – 8.23 (m, 4H), 8.18 – 8.07 (m, 3H), 7.86 (s, 1H), 7.60 (t, *J* = 6.7 Hz, 1H), 7.13 (d, *J* = 8.5 Hz, 2H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.73 – 4.54 (m, 2H), 4.49 (d, *J* = 6.1 Hz, 2H), 4.45 – 4.35 (m, 4H), 4.34 – 4.23 (m, 2H), 4.20 (dd, *J* = 6.3, 3.1 Hz, 1H), 4.13 – 3.97 (m, 3H), 3.85 – 3.64 (m, 4H), 3.52 (dd, *J* = 9.6, 3.2 Hz, 1H), 3.22 – 3.09 (m, 2H), 3.06 – 2.90 (m, 4H), 2.54 – 2.41 (m, 2H), 2.27 – 1.31 (m, 28H), 1.27 – 1.10 (m, 1H), 0.99 – 0.77 (m, 18H).

^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 180.2, 179.8, 177.1, 177.0, 176.7, 176.5, 176.1, 176.0, 175.6, 174.1, 173.9, 164.8, 157.3, 147.3, 133.3, 130.5, 126.6, 118.2, 79.5, 77.8, 73.2, 69.1, 63.9, 63.0, 62.3, 61.5, 58.9, 58.3, 57.3, 56.0, 55.0, 53.7, 50.4, 43.0, 42.3, 42.2, 39.8, 39.1, 38.7, 37.3, 33.1, 32.9, 32.6, 32.1, 31.5, 29.1, 27.3, 27.0, 24.8, 24.7, 24.4, 23.6, 21.1, 20.6, 17.5, 13.0.

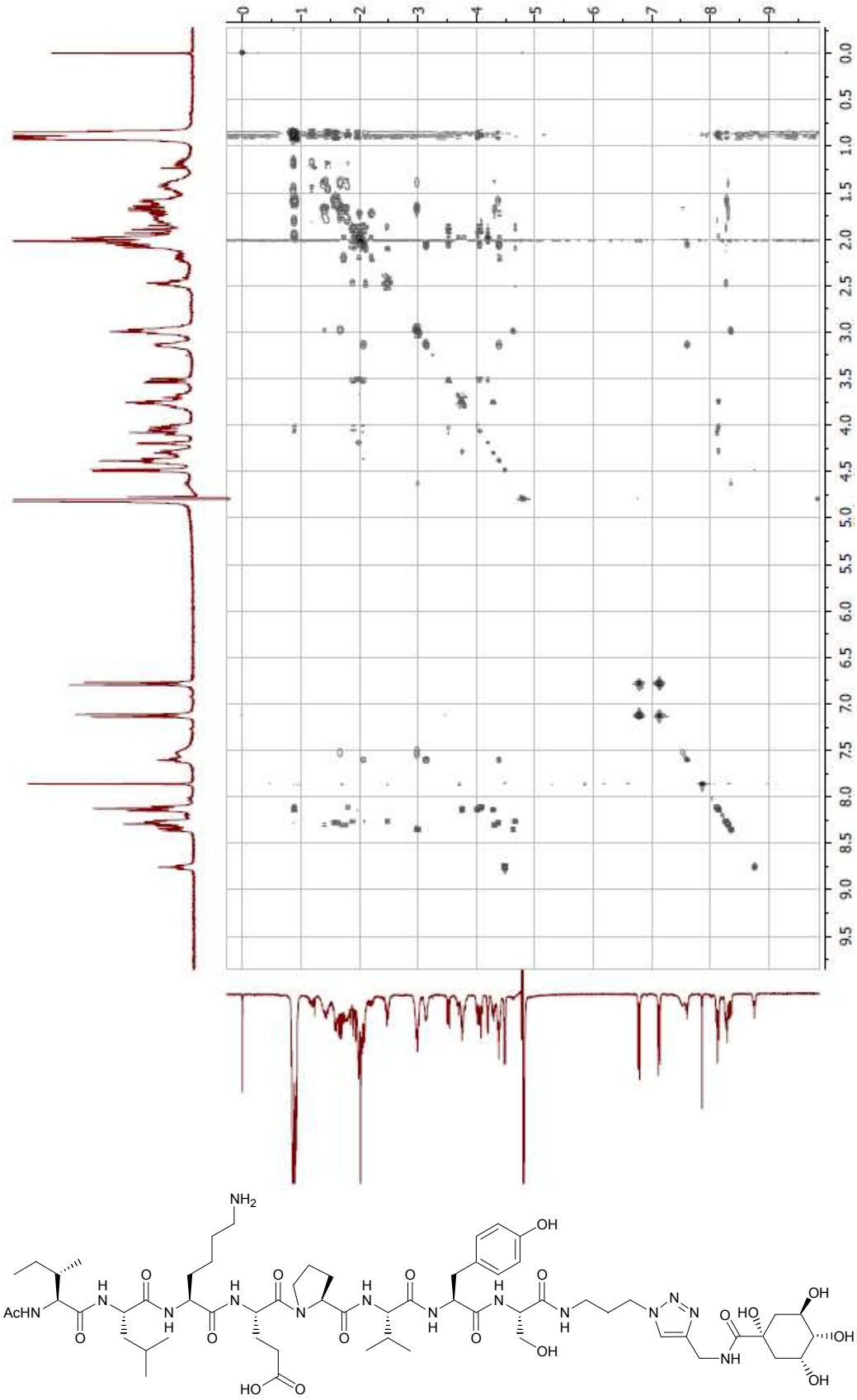
NMR ^{1}H experiment for compound **14**



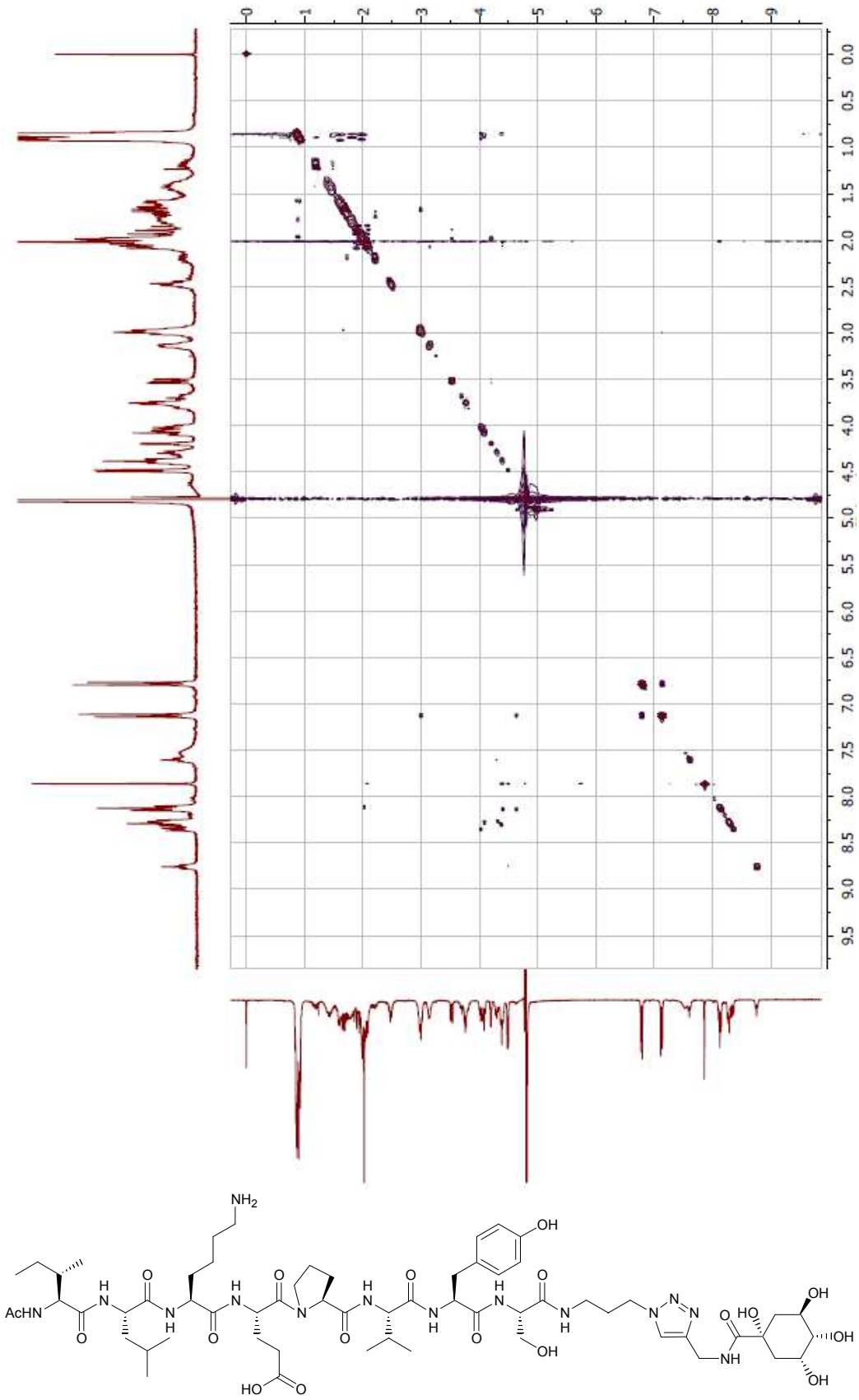
COSY experiment for compound **14**



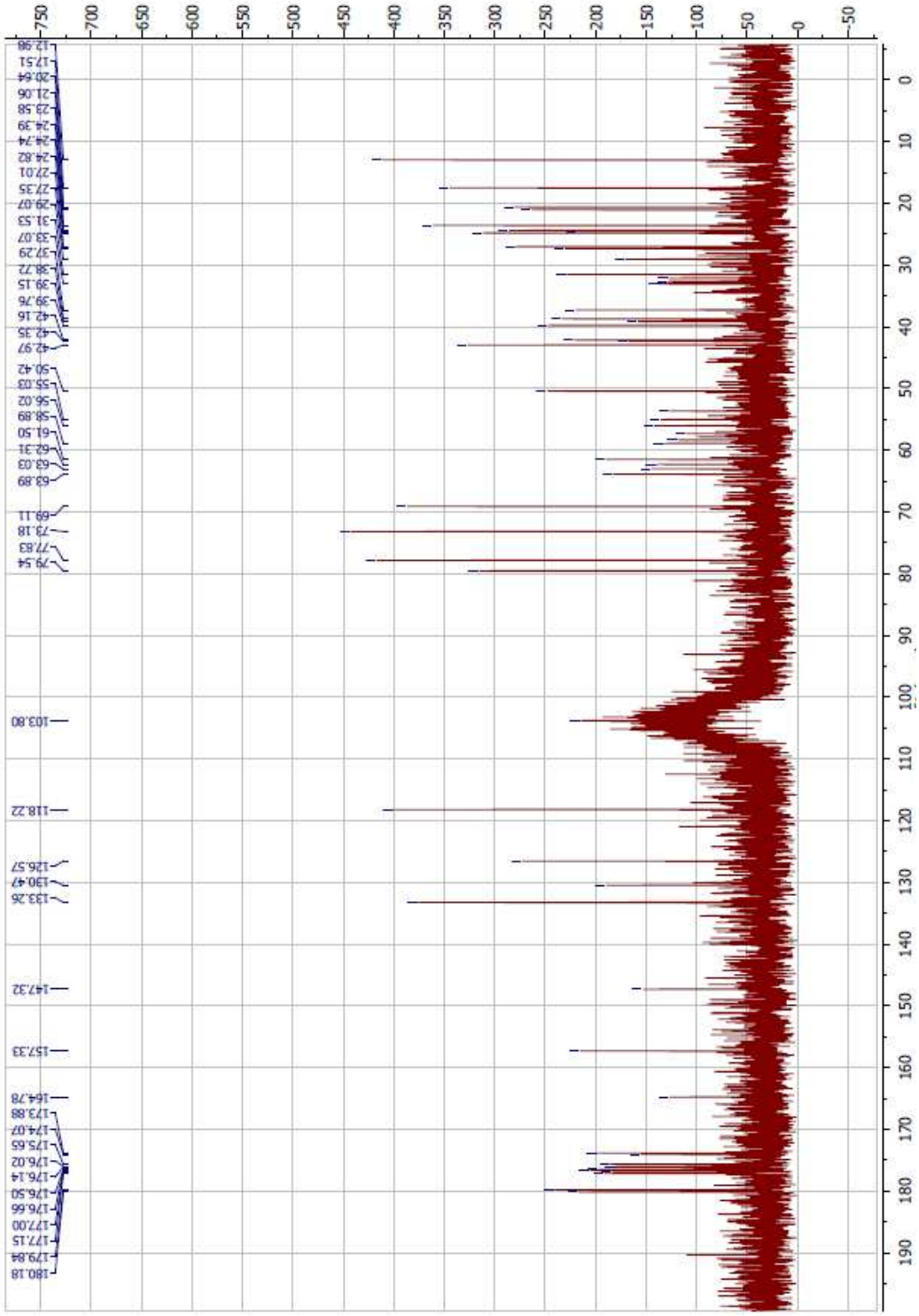
TOCSY experiment for compound **14**

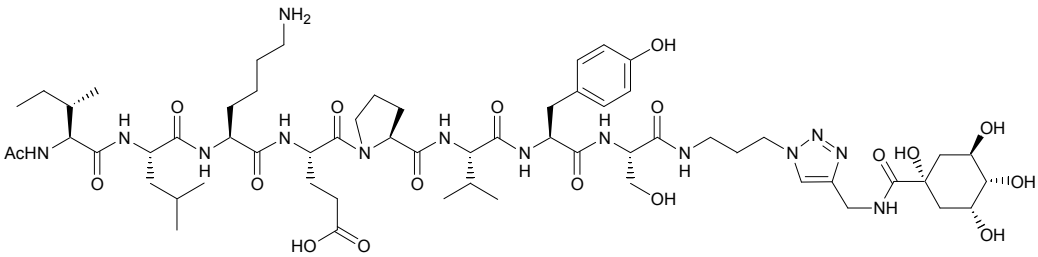


ROESY experiment for compound **14**

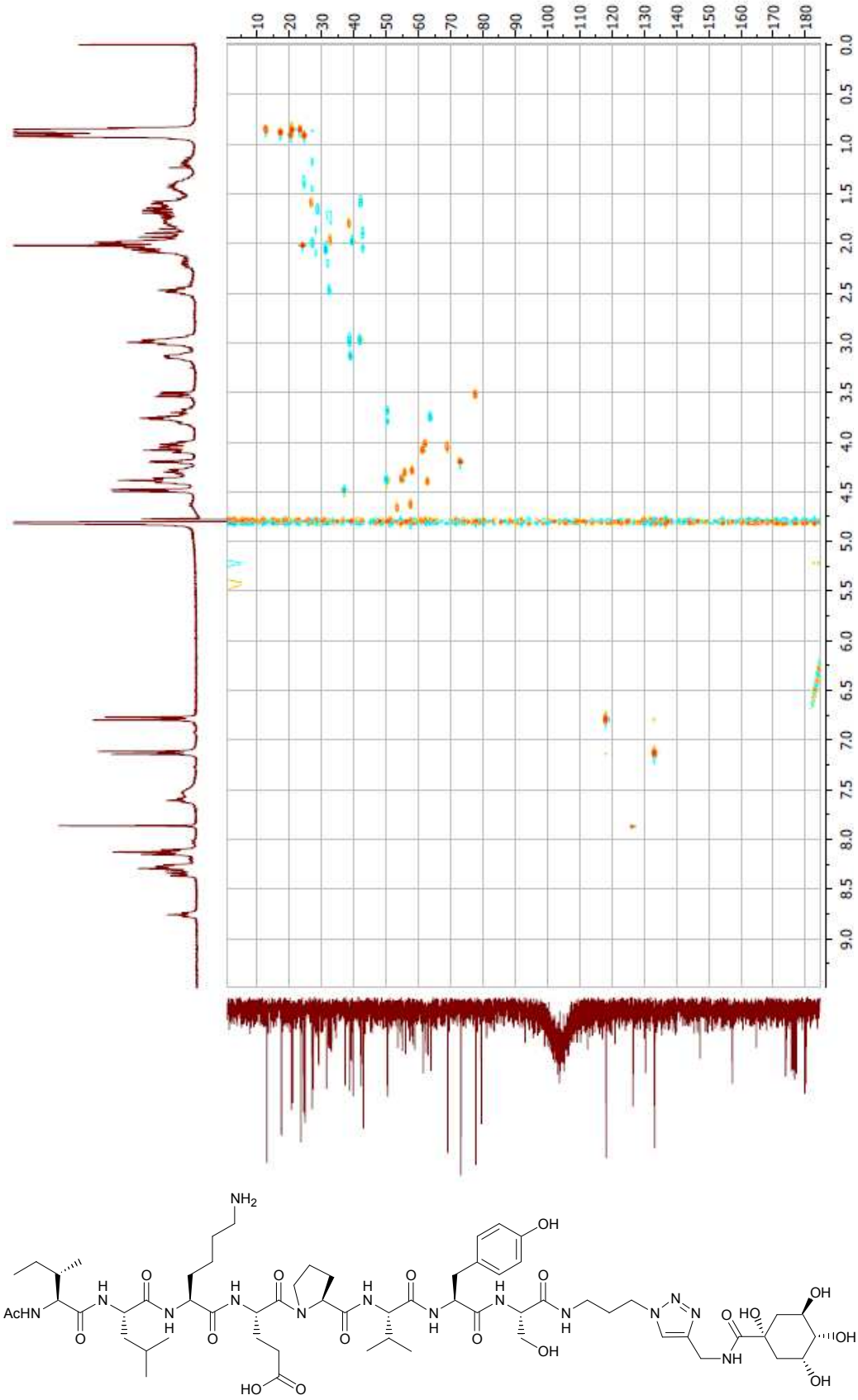


NMR ^{13}C experiment for compound **14**





HSQC experiment for compound **14**



Synthesis of peptide 15:

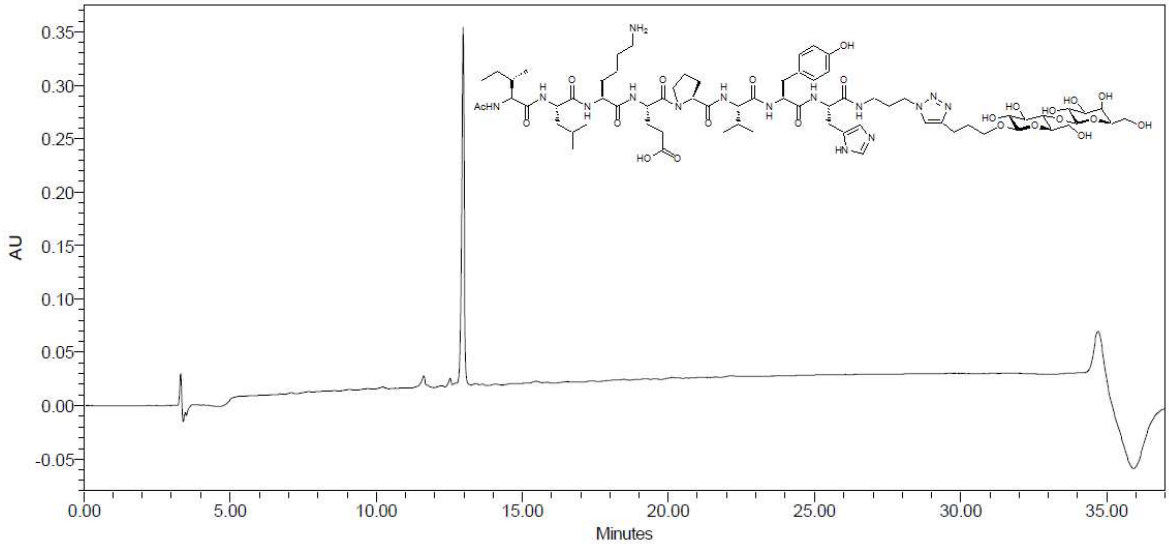
To a stirred solution of **9e** (3.9 mg, 3.5 µmol) and Lactose derivative **6f** (2.84 mg, 7 µmol) in *tert*-butyl alcohol (350 µL) were successively added at rt copper (II) sulfate pentahydrate (175 µL of 2 mM in water, 0.35 µmol) followed by sodium ascorbate (175 µL of freshly prepared 20 mM in water, 3.5 µmol). After 4 h, HPLC analysis indicated complete consumption of starting material (Rt 15.0 min, linear H_2O/CH_3CN gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm) and formation of a major product (Rt 13.2 min). The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 15:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-65% B in 50 min) to give 1.9 mg of pure peptide **15** (36 % yield).

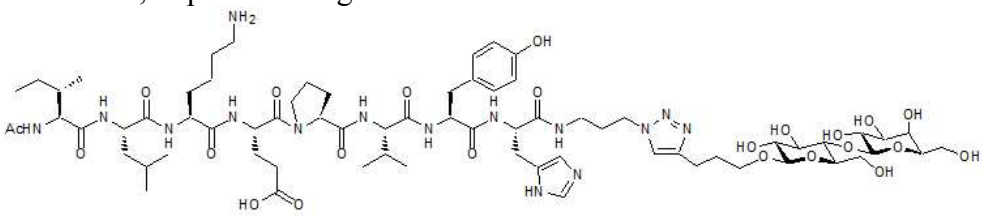
RP-HPLC analysis of 15

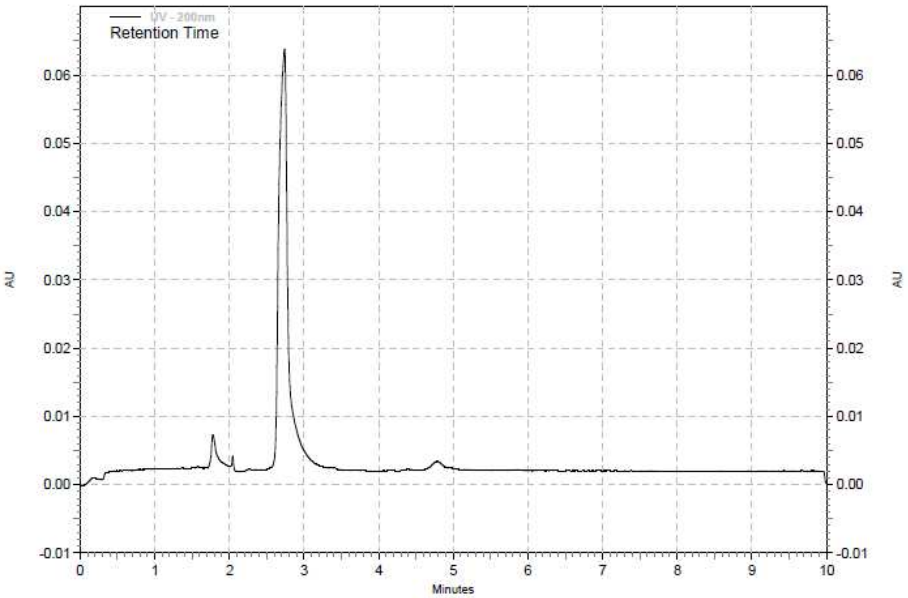
Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 15

T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.

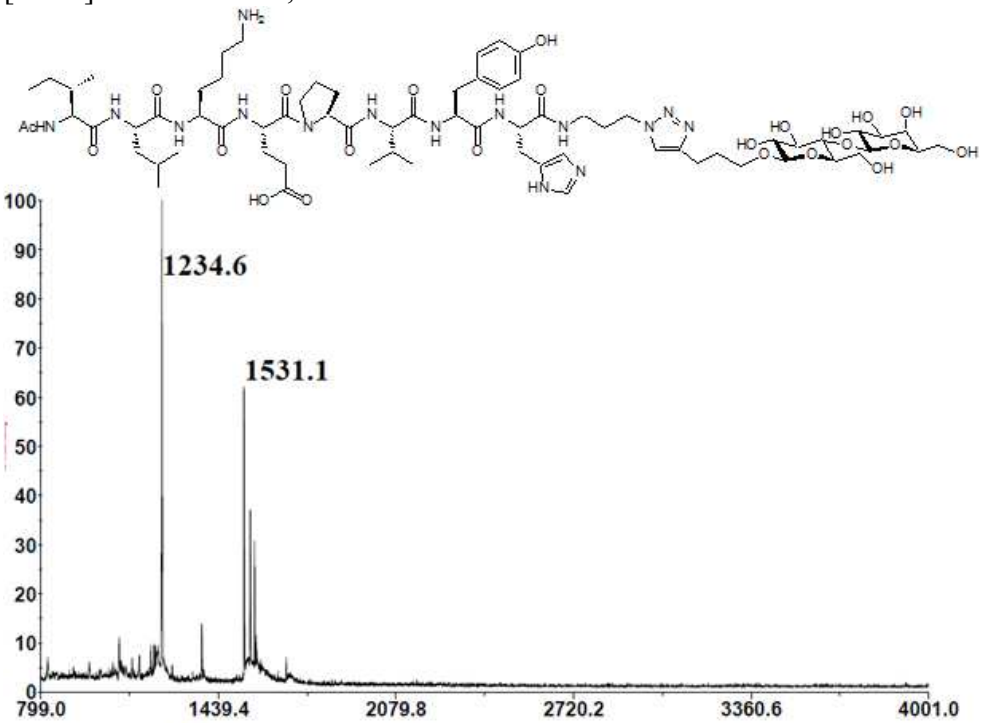


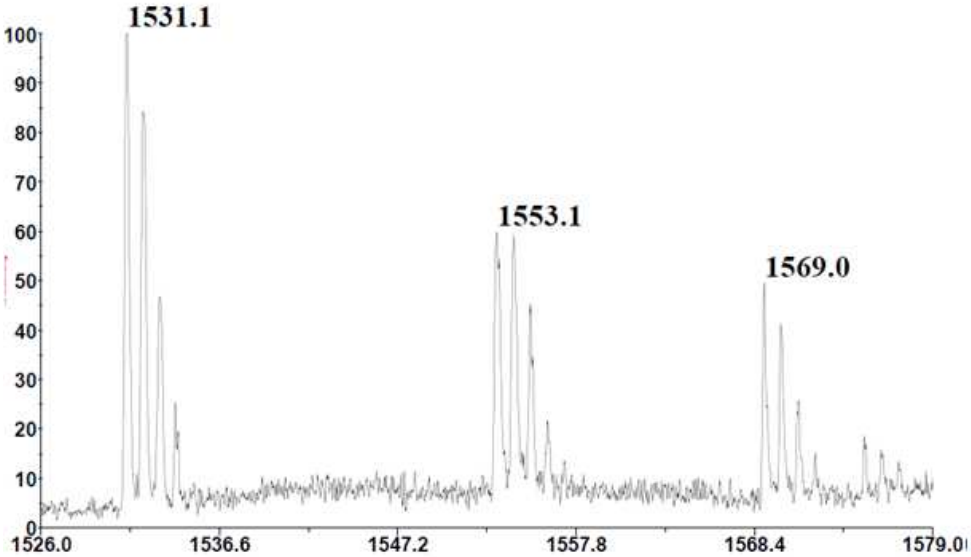


MALDI-TOF spectrum of 15

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{70}H_{111}N_{15}O_{23}$ $[M+H]^+$ 1530.8 found 1531.1 ; $[M+Na]^+$ 1552.8 found 1553.1 ; $[M+K]^+$ calcd. 1568.8, found 1569.0.





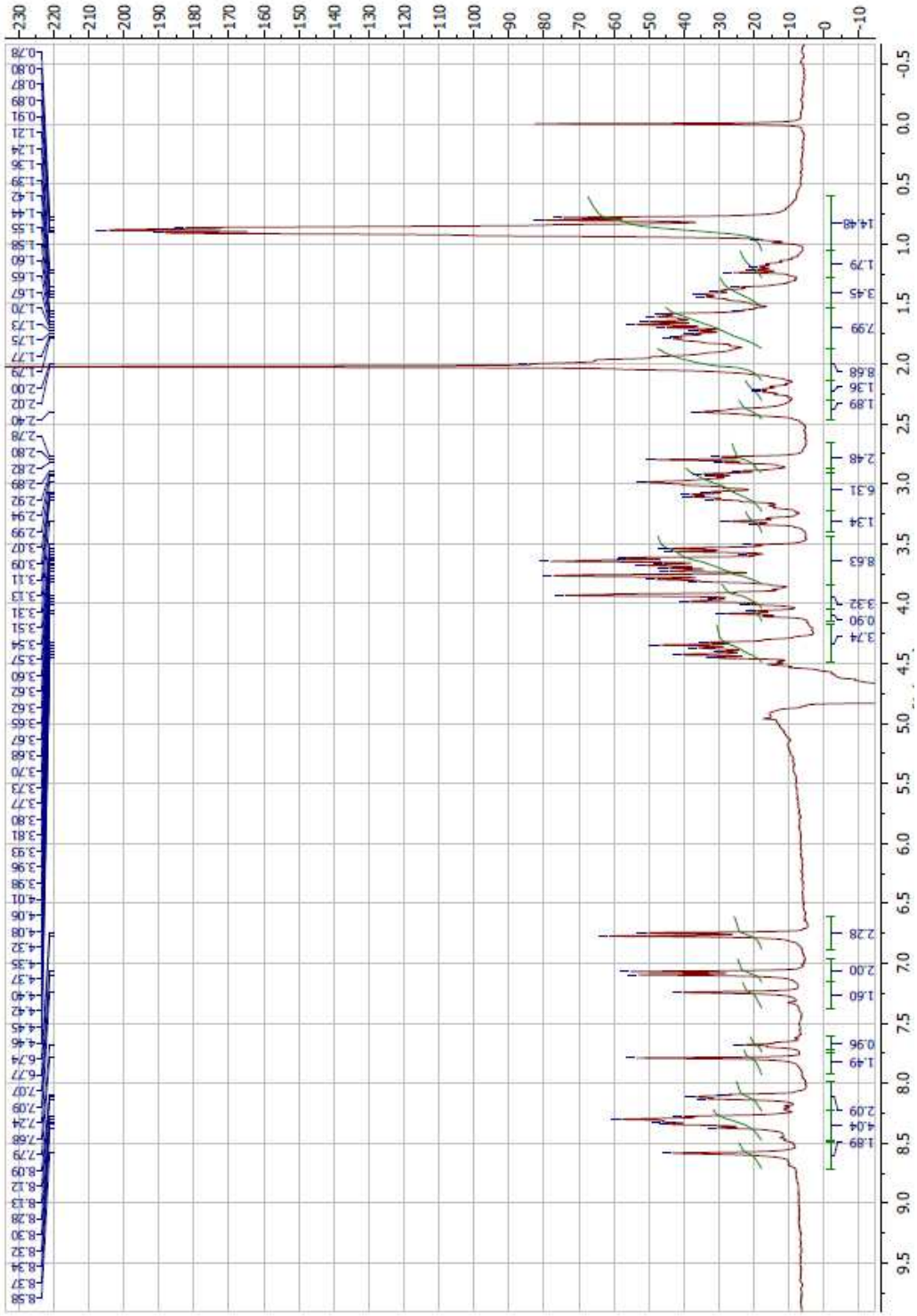
^{1}H and ^{13}C NMR for peptide 15

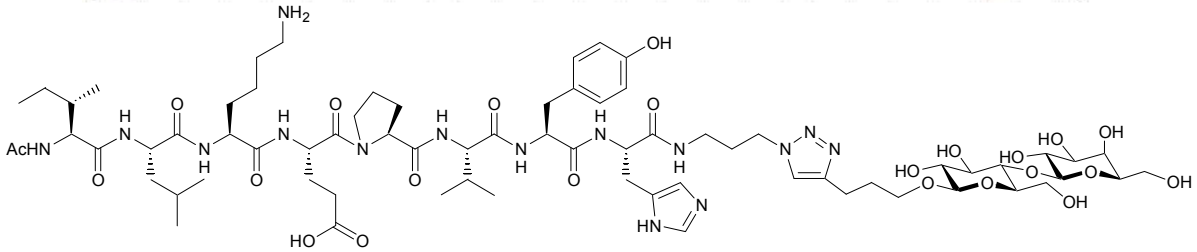
^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.58 (s, 1H), 8.42 – 8.23 (m, 5H), 8.19 – 8.03 (m, 2H), 7.79 (s, 1H), 7.68 (s, 1H), 7.24 (s, 1H), 7.08 (d, *J* = 8.2 Hz, 2H), 6.76 (d, *J* = 8.2 Hz, 2H), 4.60 – 4.20 (m, 10H), 4.07 (t, *J* = 7.2 Hz, 1H), 4.03 – 3.85 (m, 4H), 3.87 – 3.48 (m, 13H), 3.33 (t, *J* = 7.8 Hz, 1H), 3.22 – 2.87 (m, 8H), 2.80 (t, *J* = 7.2 Hz, 2H), 2.48 – 2.32 (m, 2H), 2.29 – 2.15 (m, 2H), 2.15 – 1.08 (m, 20H), 1.06 – 0.62 (m, 18H).

Selected data:

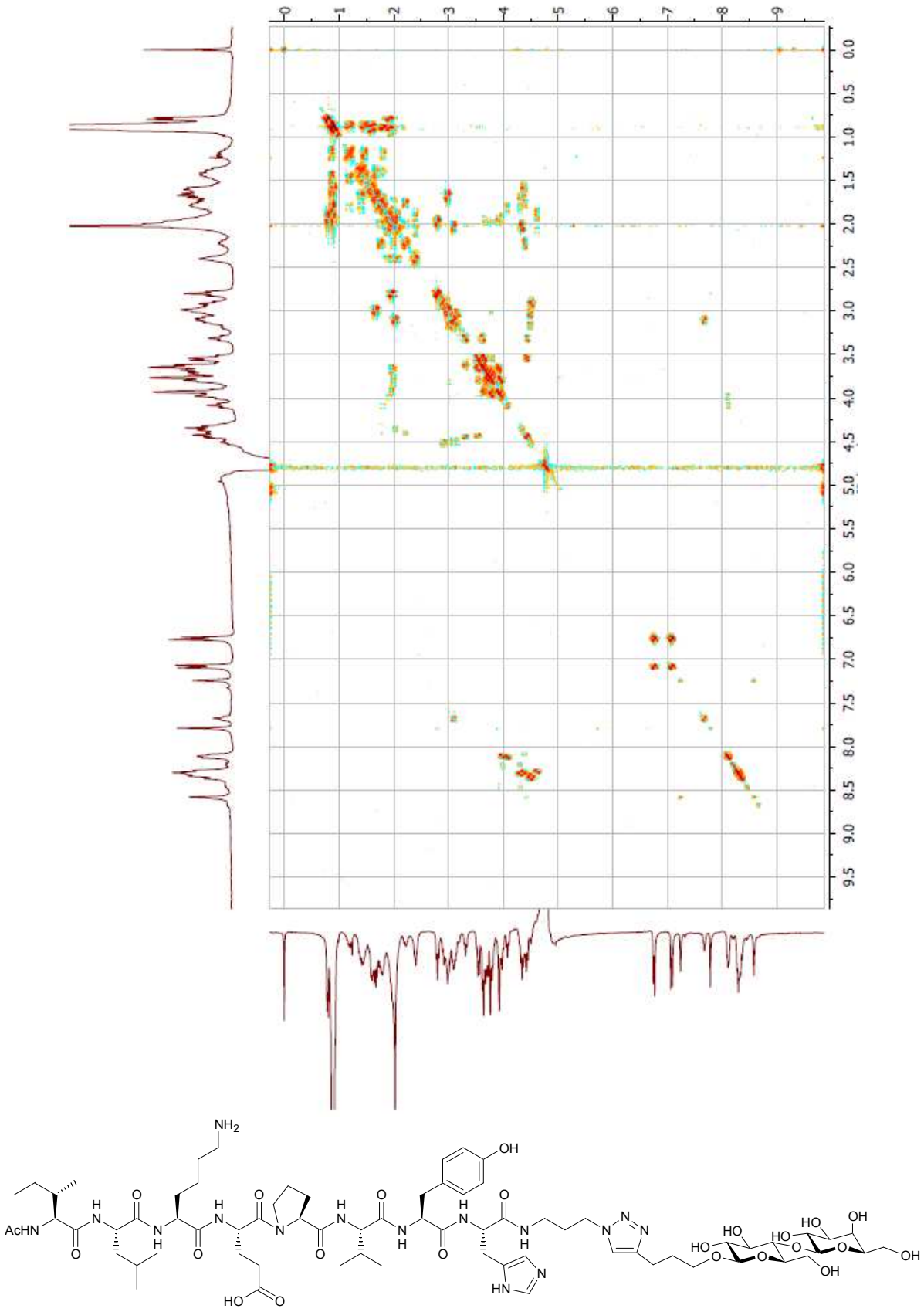
^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 136.6, 125.6, 119.6, 133.1, 118.0, 105.6, 104.8, 81.5, 78.6, 77.7, 77.6, 76.1, 75.7, 74.3, 72.5, 71.3, 64.1, 63.4, 63.4, 62.6, 61.8, 58.3, 56.3, 55.5, 55.3, 54.3, 51.1, 50.6, 42.8, 42.3, 39.6, 39.5, 38.8, 34.3, 33.4, 33.0, 32.4, 31.4, 29.5, 29.5, 27.8,27.8, 27.3, 25.2, 25.2, 24.6, 23.8, 23.8, 21.2, 21.1, 17.6, 13.3.

NMR ^{1}H experiment for compound **15**

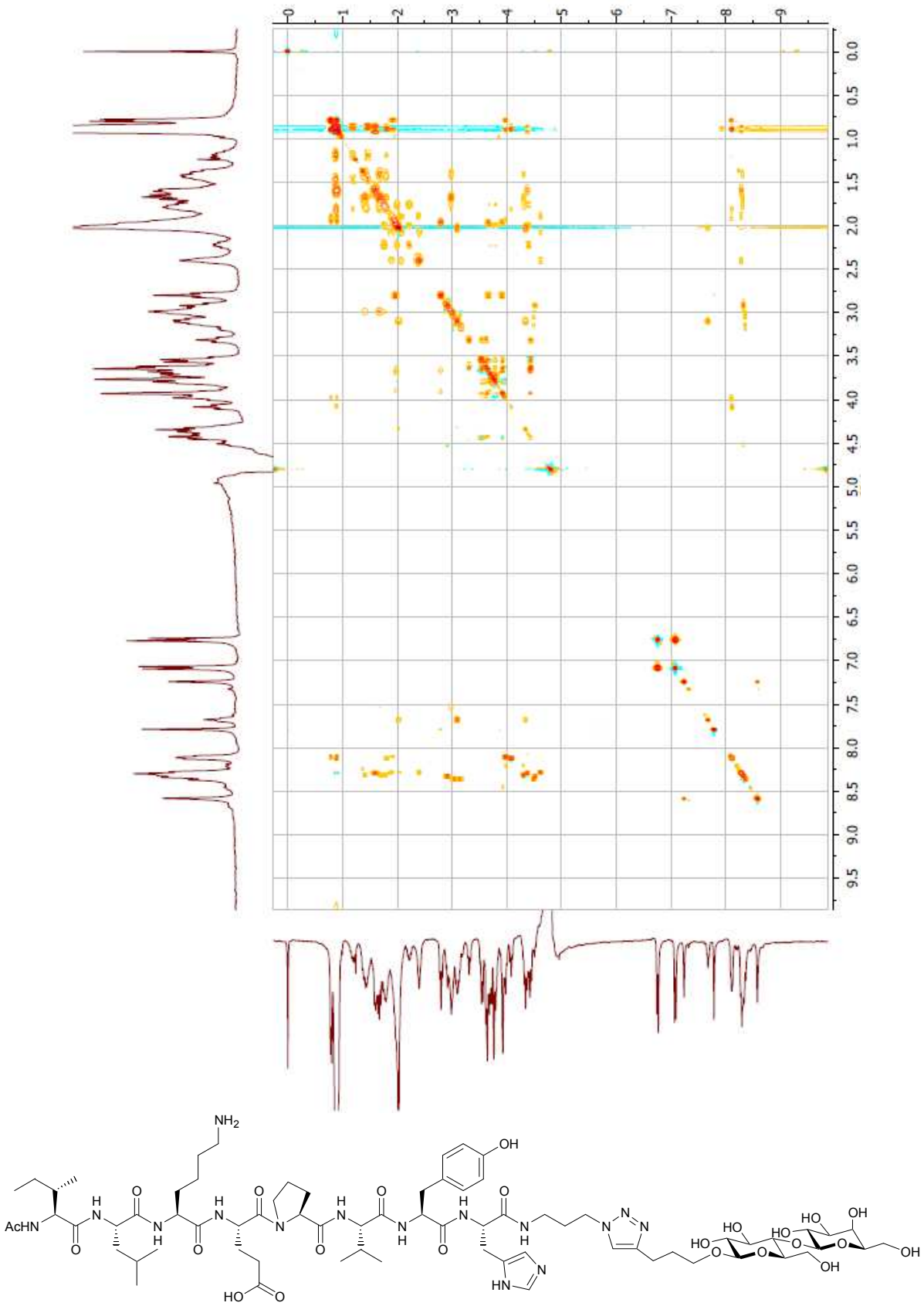




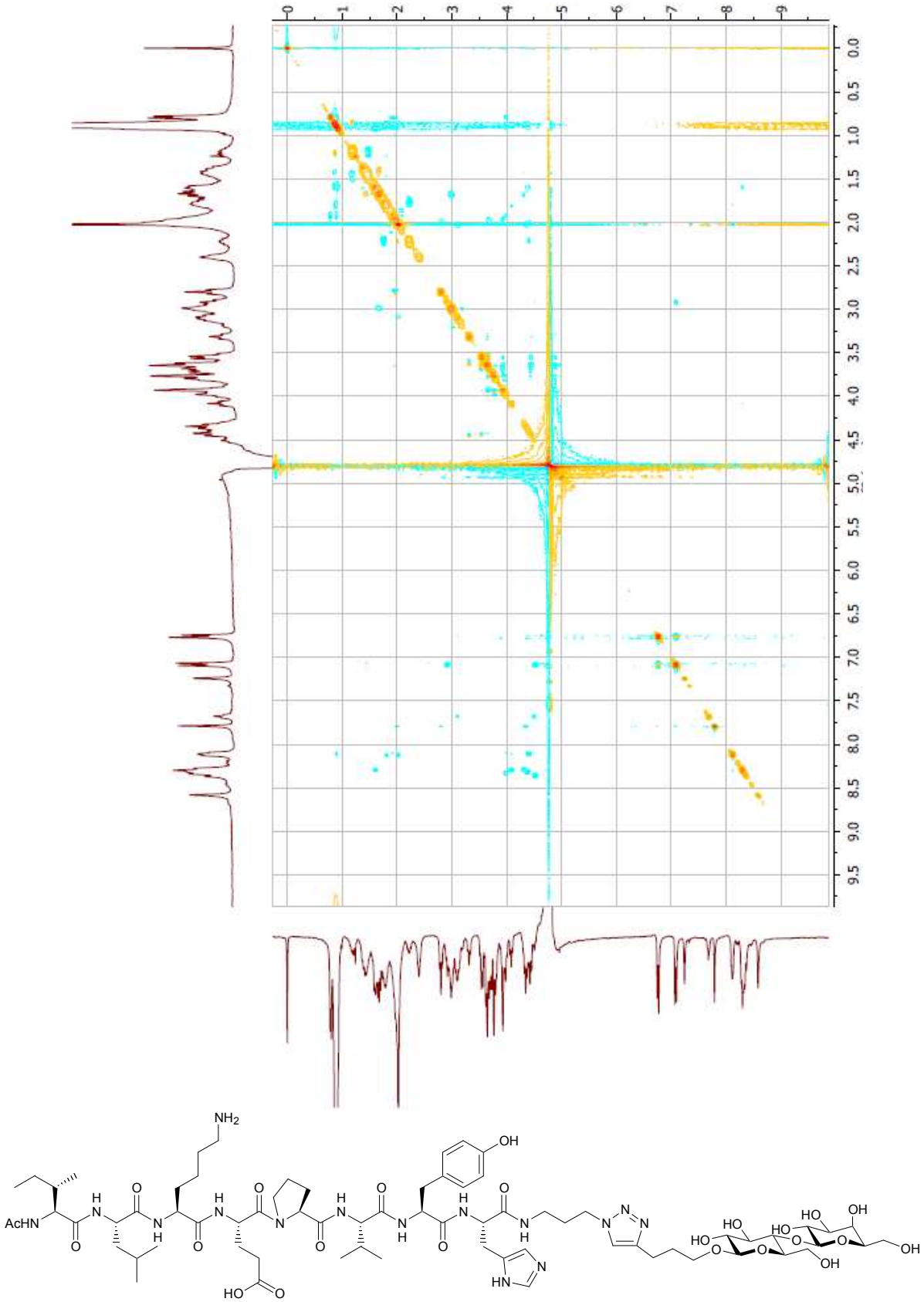
COSY experiment for compound **15**



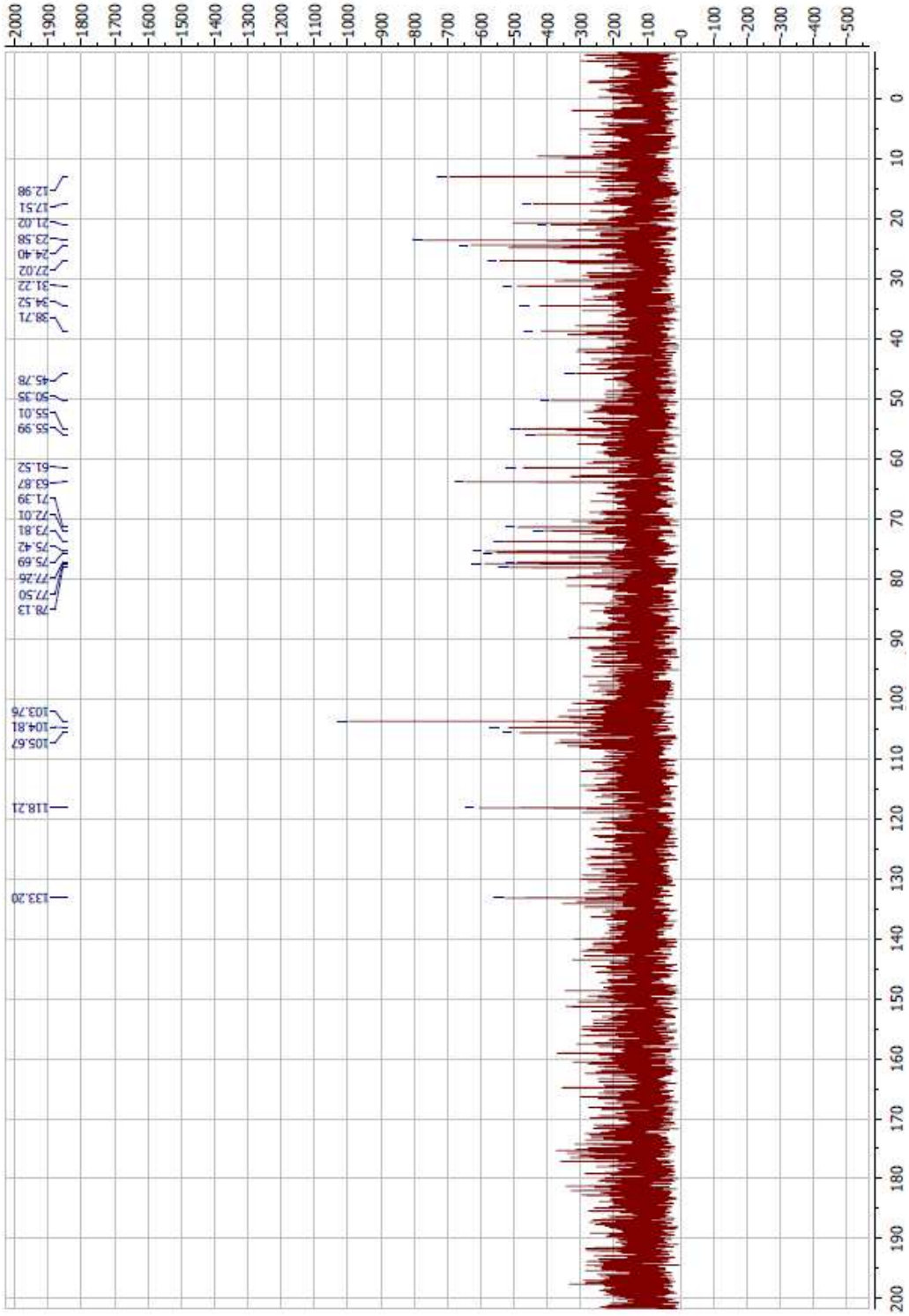
TOCSY experiment for compound **15**

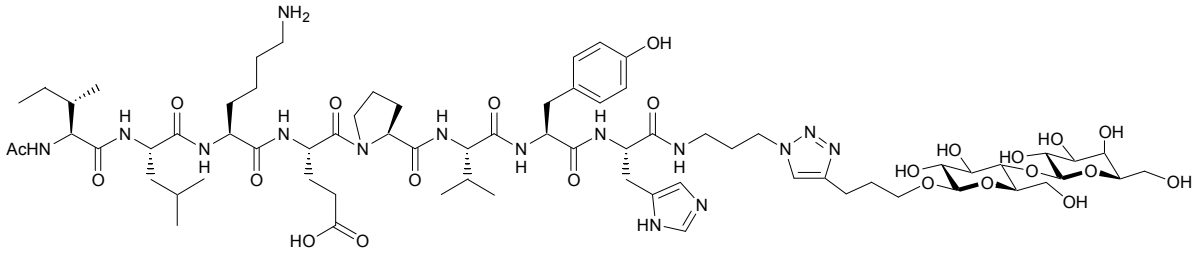


ROESY experiment for compound **15**

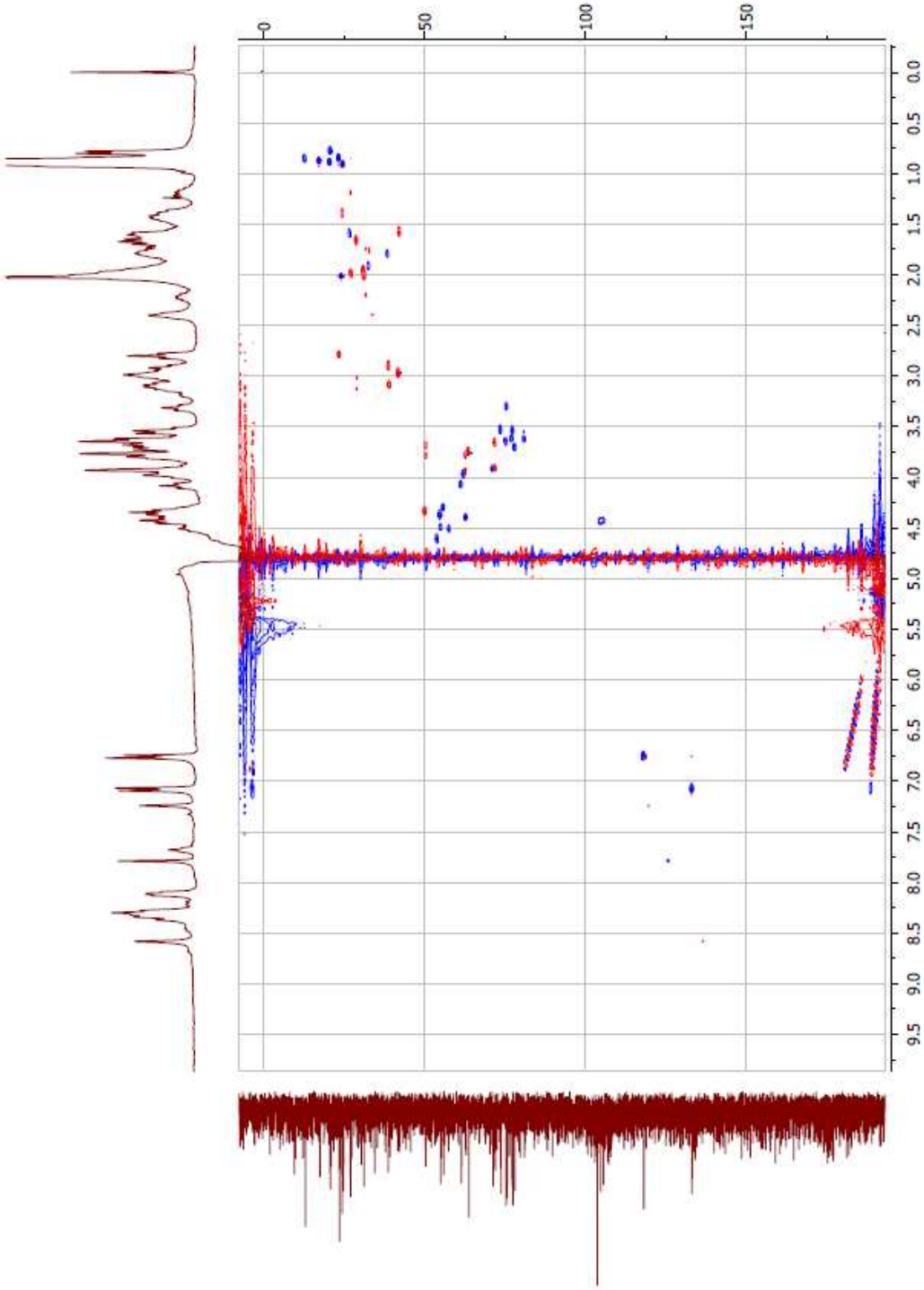


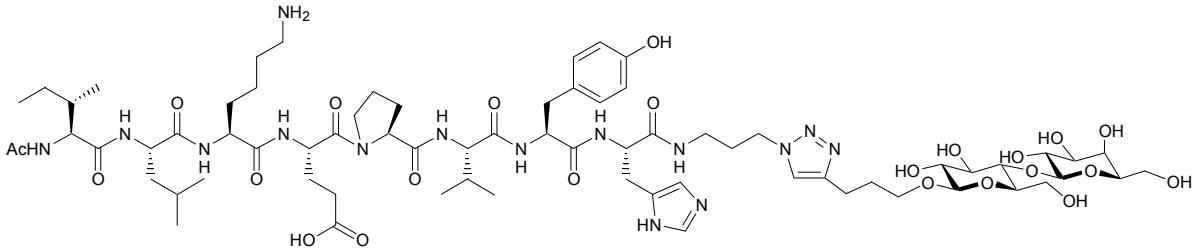
NMR ^{13}C experiment for compound **15**





HSQC experiment for compound **15**





Three-component CuAAC. Synthesis of 10

To a well stirred suspension of the corresponding hydrazide resin **2** (50 µmol) swollen in anhydrous DCM (250 µL) was added a solution of 3-azidopropylamine hydrochloride **4a** (13.7 mg, 0.1 mmol), 4-Phenyl-1-butyne **6a** (19.5 mg, 0.15 mmol) and diisopropylethylamine (87.0 µL, 0.5 mmol) in dichloromethane (250 µL). Copper (II) acetate (22.7 mg, 0.125 mmol) dissolved in anhydrous pyridine (20.2 µL, 0.25 mmol) and anhydrous DCM (500 µL) was added and the reaction was kept for 24 hours at room temperature in a glove-box. After filtration, the resin was washed five times with DCM and the combined filtrates were concentrated in vacuo. To the resulting oil was added a solution of TFA/H_2O/triisopropylsilane: 95/2.5/2.5 by vol. (5 mL) at room temperature. After 2 hours, the peptide was precipitated in a mixture of diethylether/heptane: 1/1 by vol. (100 mL). The peptide was separated by centrifugation. The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 10:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-50% B in 60 min) to give 7.5 mg of pure peptide (14.2 % yield).

Three-component CuAAC. Synthesis of 16 (control experiment with Cu(I))

Synthesis of 3-(4-Phenethyl-1H-1,2,3-triazolyl)propanamine 16

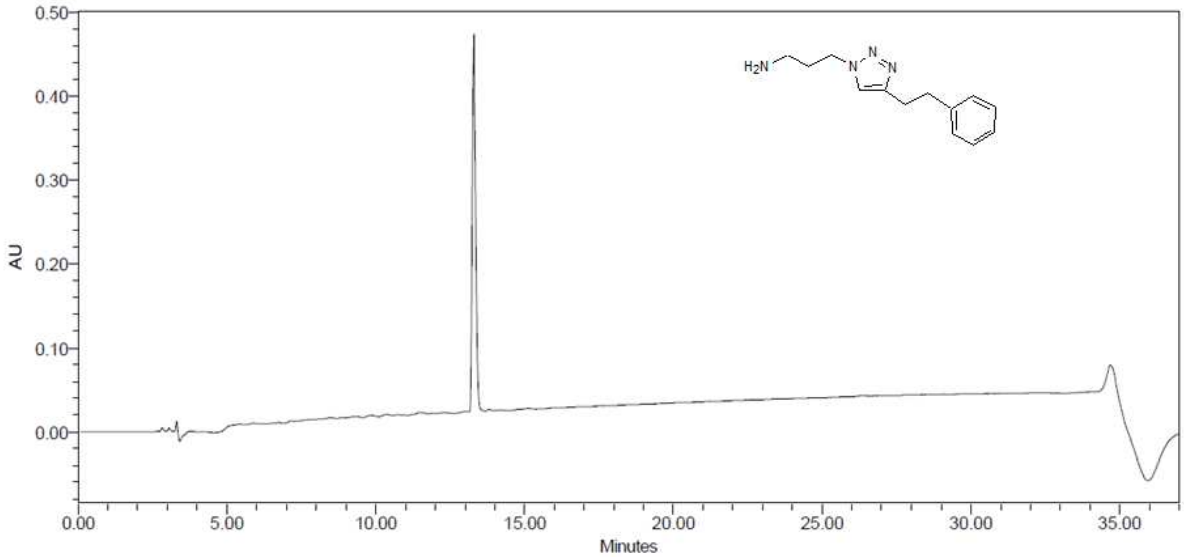
To a well stirred suspension of the corresponding hydrazide resin **2b** (50 µmol) swollen in anhydrous DCM (250 µL) was added a solution of 3-azidopropylamine hydrochloride **4a** (13.7 mg, 0.1 mmol), 4-Phenyl-1-butyne **6a** (19.5 mg, 0.15 mmol) and diisopropylethylamine (87.0 µL, 0.5 mmol) in dichloromethane (250 µL). Copper (I) acetate (15.3 mg, 0.125 mmol) dissolved in anhydrous pyridine (20.2 µL, 0.25 mmol) and anhydrous DCM (500 µL) was added and the reaction was kept for 24 hours at room temperature in a glove-box. After filtration, the resin was washed five times with DCM and the combined filtrates were concentrated in vacuo. The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude amine-triazole.

Characterization of 16:

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-40% B in 40 min) to give 19.6 mg of pure amine-azide **16** (84 % yield).

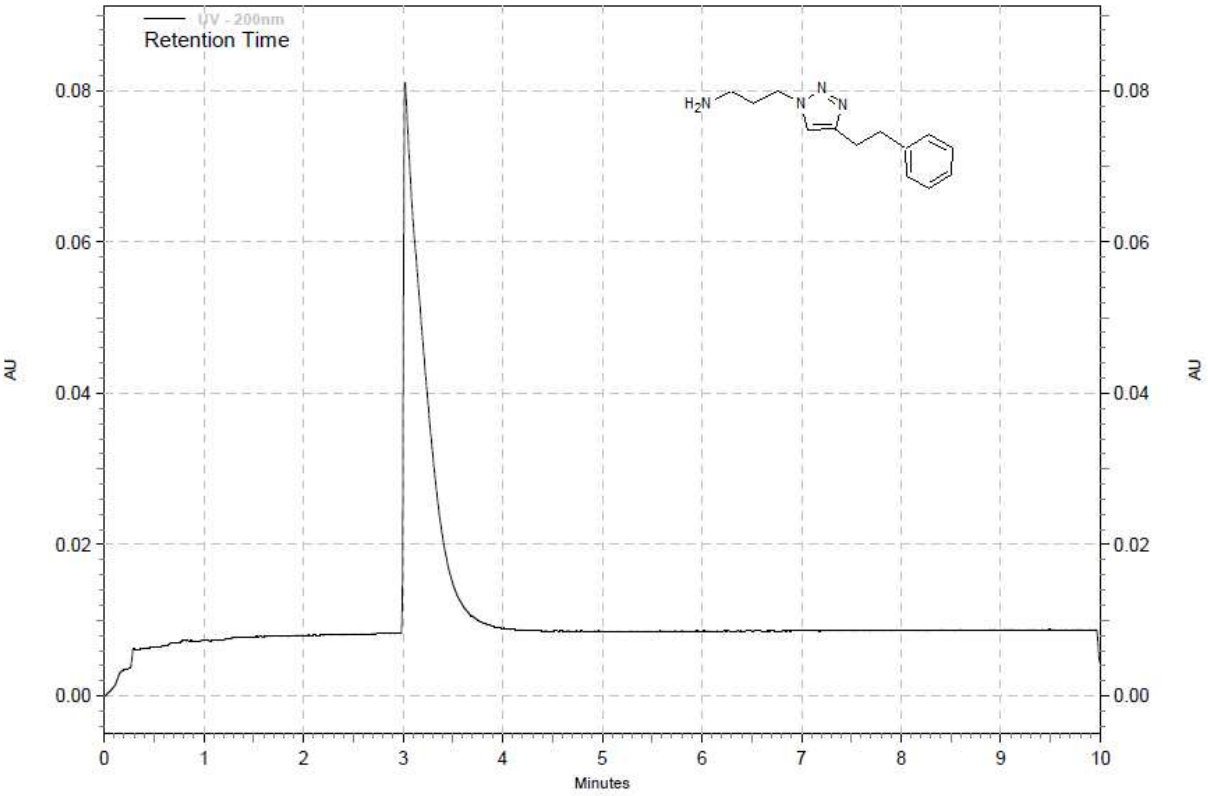
RP-HPLC analysis of 16

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 16

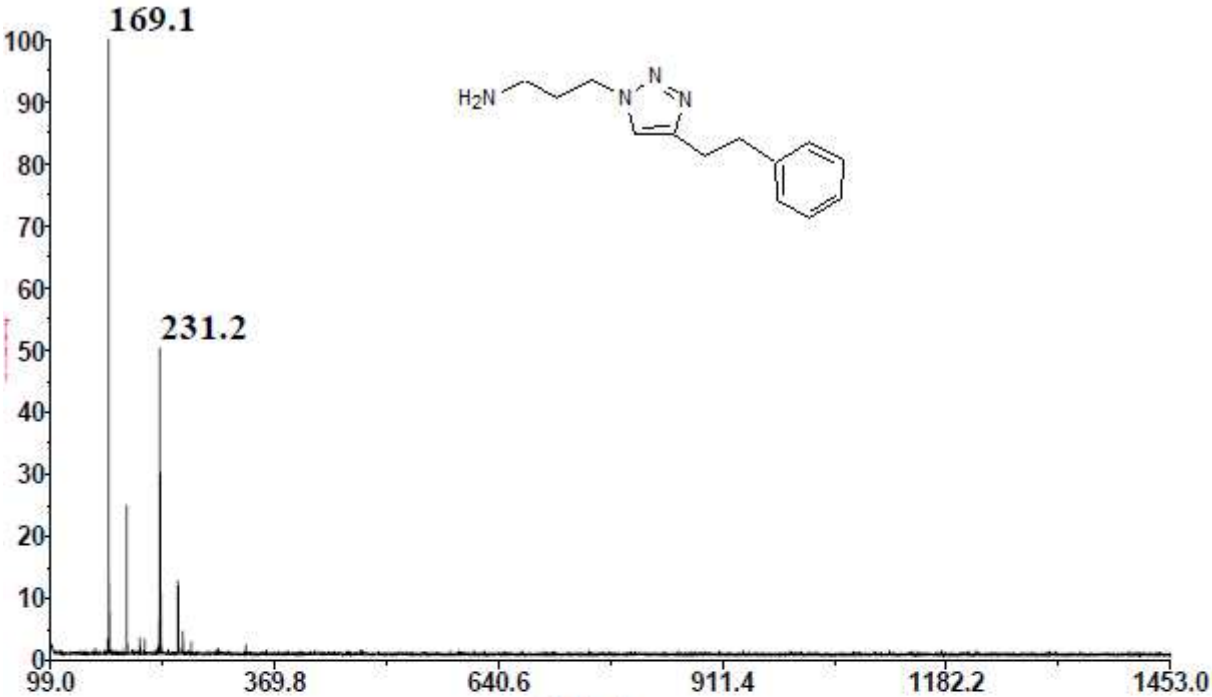
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 50 mM phosphate buffer pH = 2.5 ; Run time = 10 min ; Separate voltage = 30 kV.

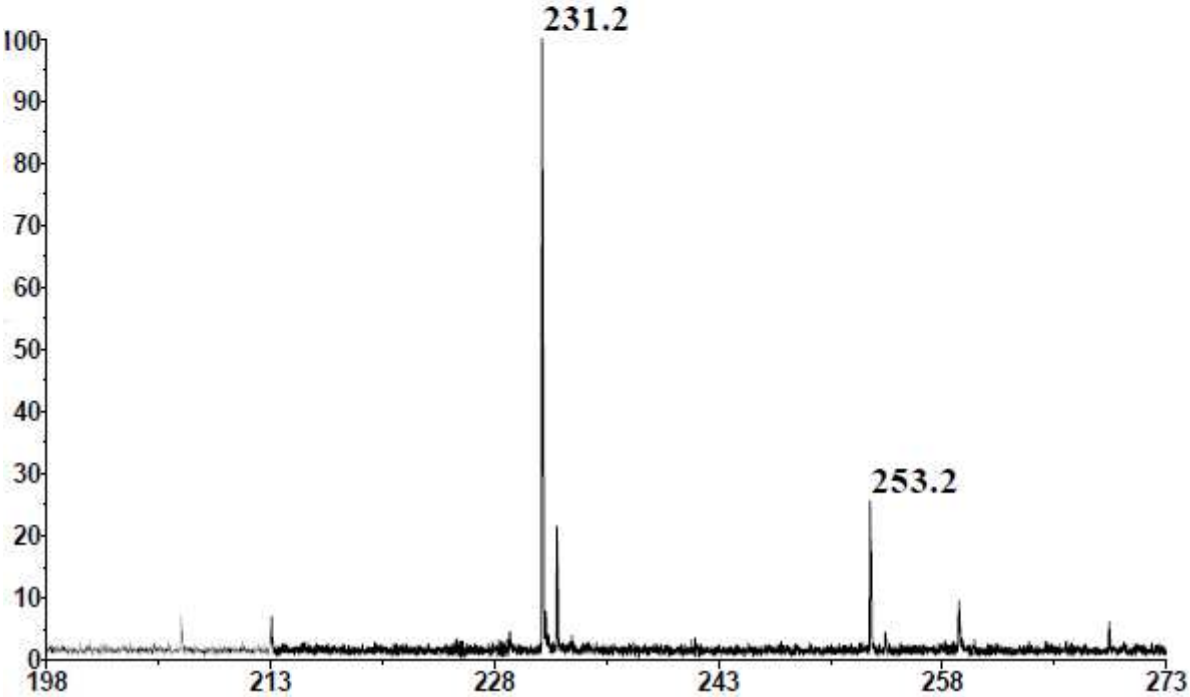


MALDI-TOF spectrum of 16

MALDO-TOF matrix: 2,4,6-Trihydroxyacetophenone

Calcd. for $C_{13}H_{18}N_4$ $[M+H]^+$ 231.2, found 231.2 ; $[M+Na]^+$ calcd. 253.1, found 253.2.



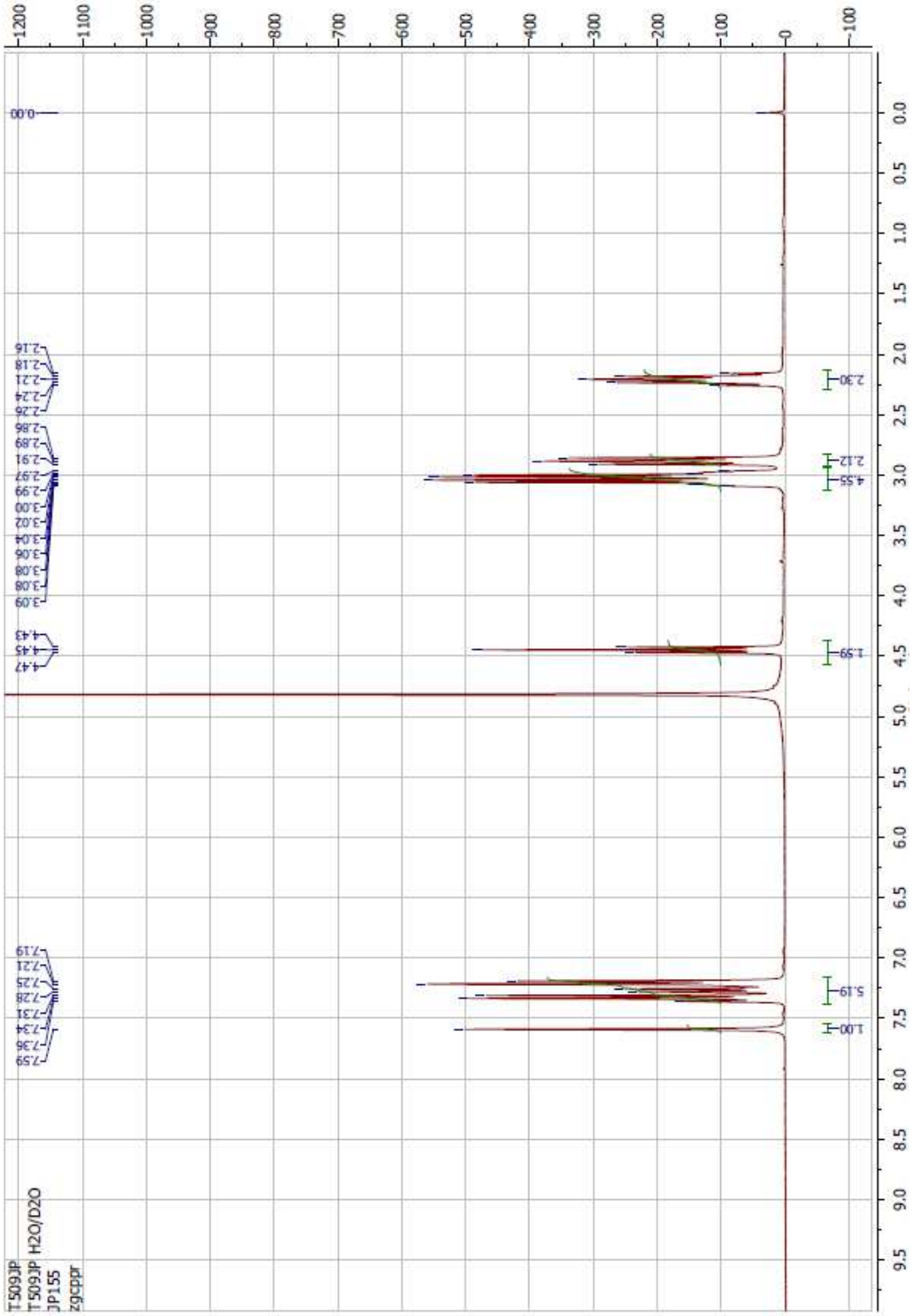


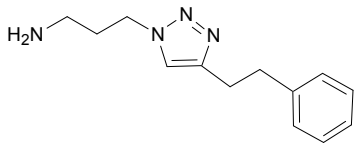
^{1}H and ^{13}C NMR for peptide 16

^{1}H NMR (300 MHz, H_2O+D_2O) δ 7.59 (s, 1H), 7.36 – 7.19 (m, 5H), 4.45 (t, J = 6.7 Hz, 2H), 3.09 – 2.97 (m, 4H), 2.89 (t, J = 7.6 Hz, 2H), 2.21 (q, J = 6.9 Hz, 2H).

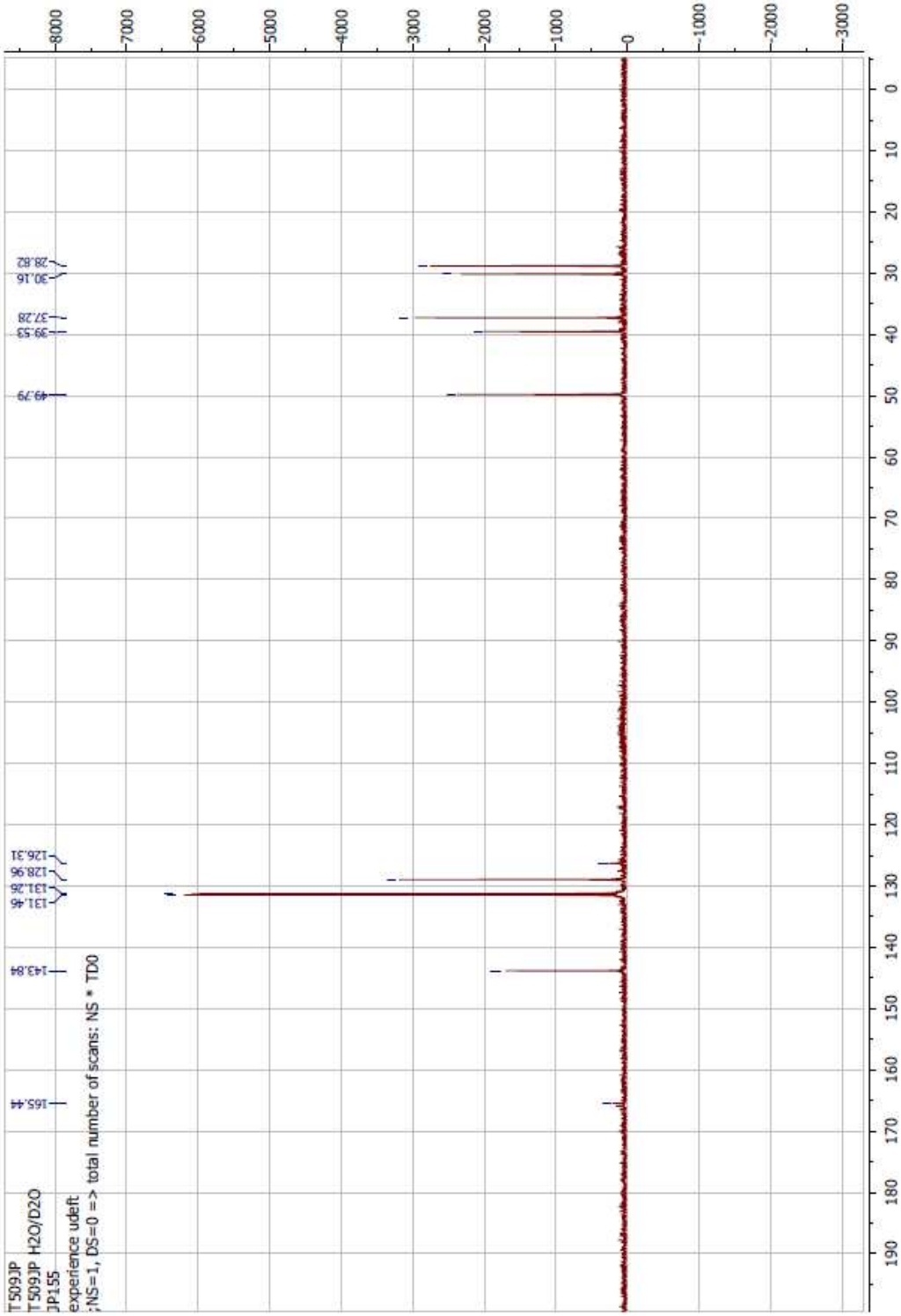
^{13}C NMR (75 MHz, H_2O+D_2O) δ 165.4, 143.8, 131.46, 131.3, 129.0, 126.3, 49.8, 39.5, 37.3, 30.2, 28.8.

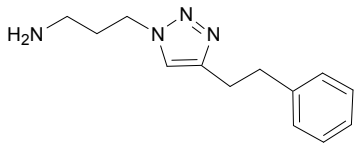
NMR ^{1}H experiment for compound **16**





NMR ^{13}C experiment for compound **16**





Three-component CuAAC. Synthesis of 17

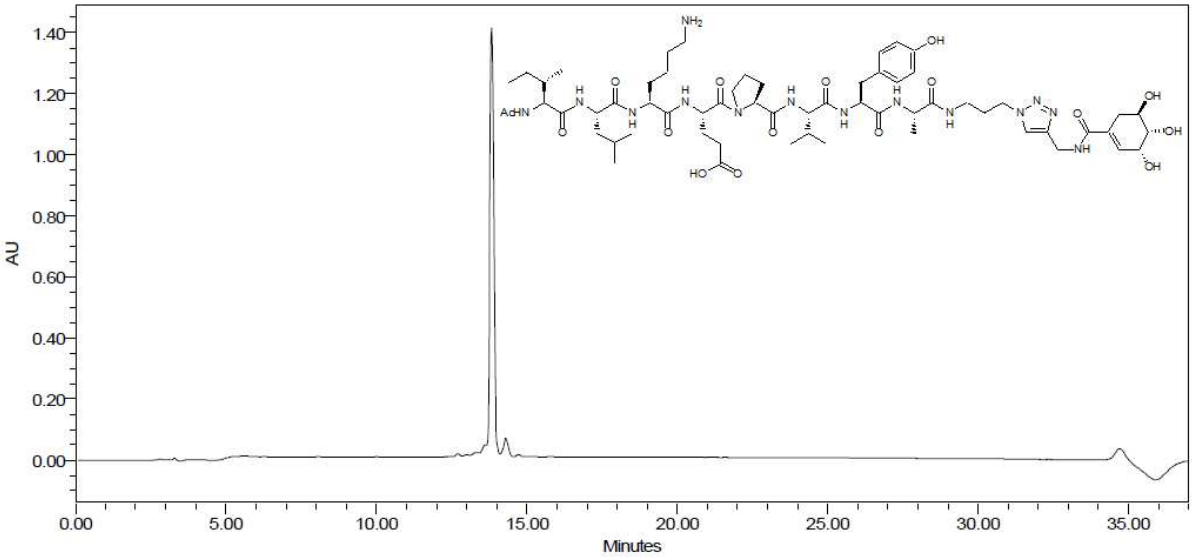
To a well stirred suspension of the corresponding hydrazide resin **2b** (50 µmol) swollen in anhydrous DCM (250 µL) was added a solution of 3-azidopropylamine hydrochloride **4a** (10.2 mg, 0.075 mmol), Shikimic derivative **6d** (21.1 mg, 0.1 mmol) and diisopropylethylamine (87.0 µL, 0.5 mmol) in dichloromethane (250 µL). Copper (II) acetate (22.7 mg, 0.125 mmol) dissolved in anhydrous pyridine (20.2 µL, 0.25 mmol) and anhydrous DCM (500 µL) was added and the reaction was kept for 24 hours at room temperature in a glove-box. After filtration, the resin was washed five times with DCM and the combined filtrates were concentrated in vacuo. To the resulting oil was added a solution of TFA/H_2O/triisopropylsilane: 95/2.5/2.5 by vol. (5 mL) at room temperature. After 2 hours, the peptide was precipitated in a mixture of diethylether/heptane: 1/1 by vol. (100 mL). The peptide was separated by centrifugation. The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 17

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-45% B in 60 min) to give 8.9 mg of pure peptide **17** (14.1 % yield).

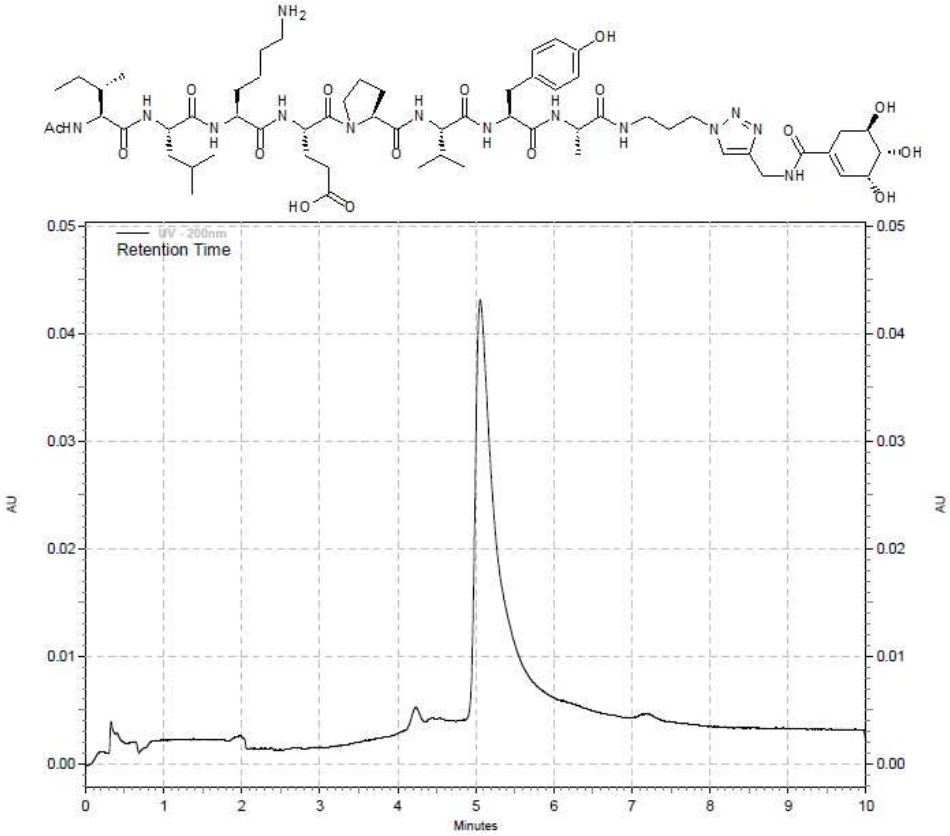
RP-HPLC analysis of 17

Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 17

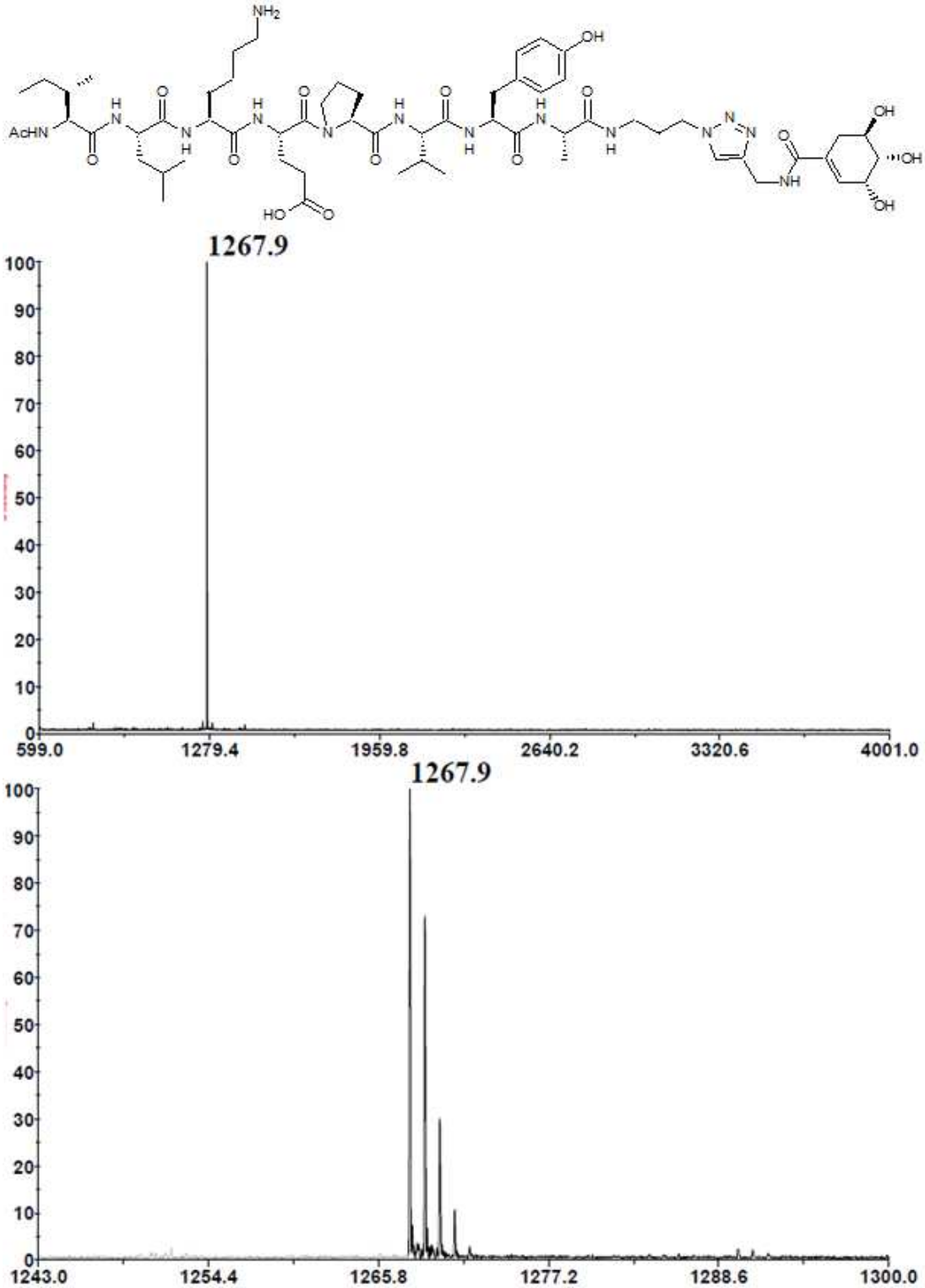
T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.



MALDI-TOF spectrum of 17

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{60}H_{94}N_{14}O_{16}$ $[M+H]^+$ 1267.7 found 1267.9.

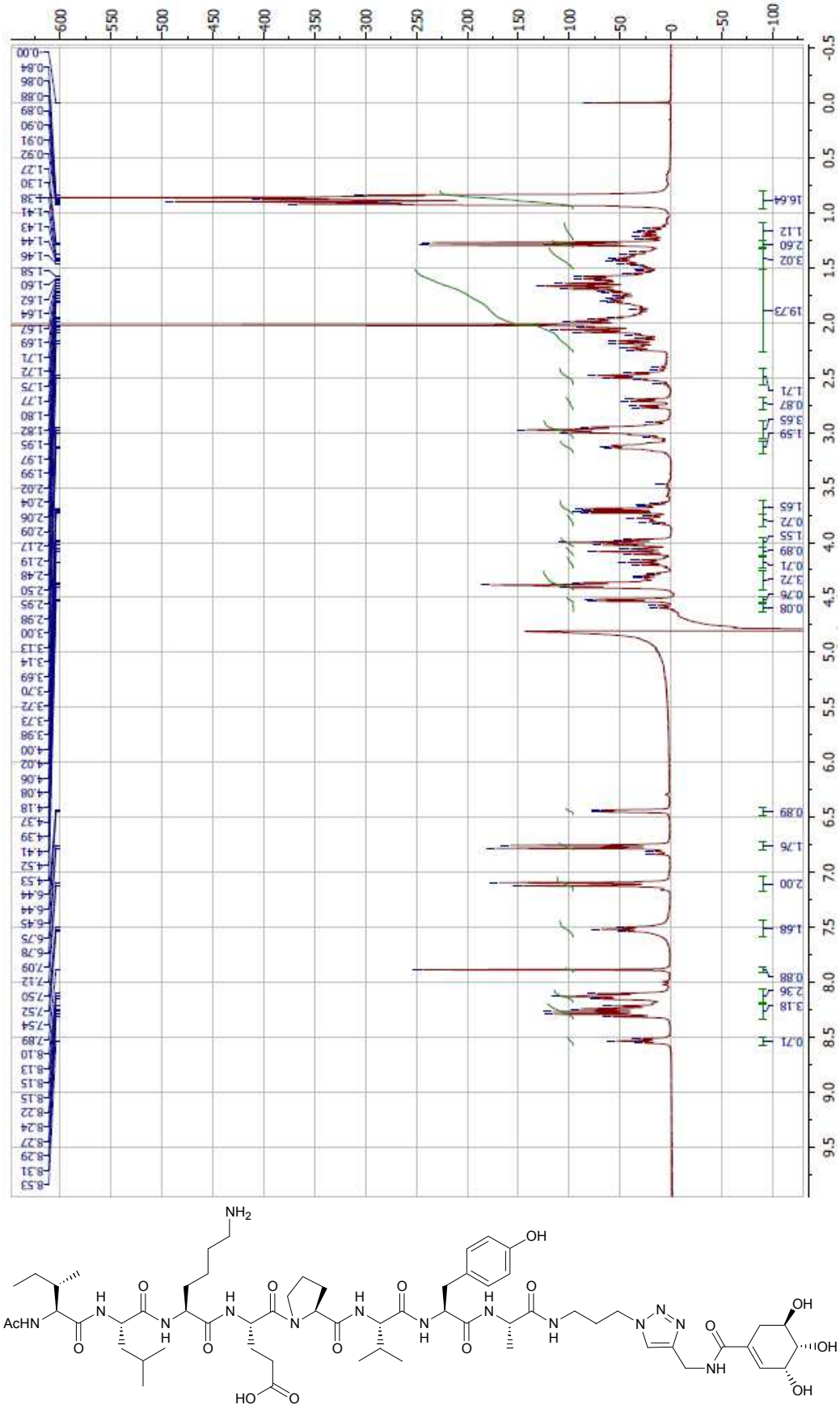


^{1}H and ^{13}C NMR for peptide 17

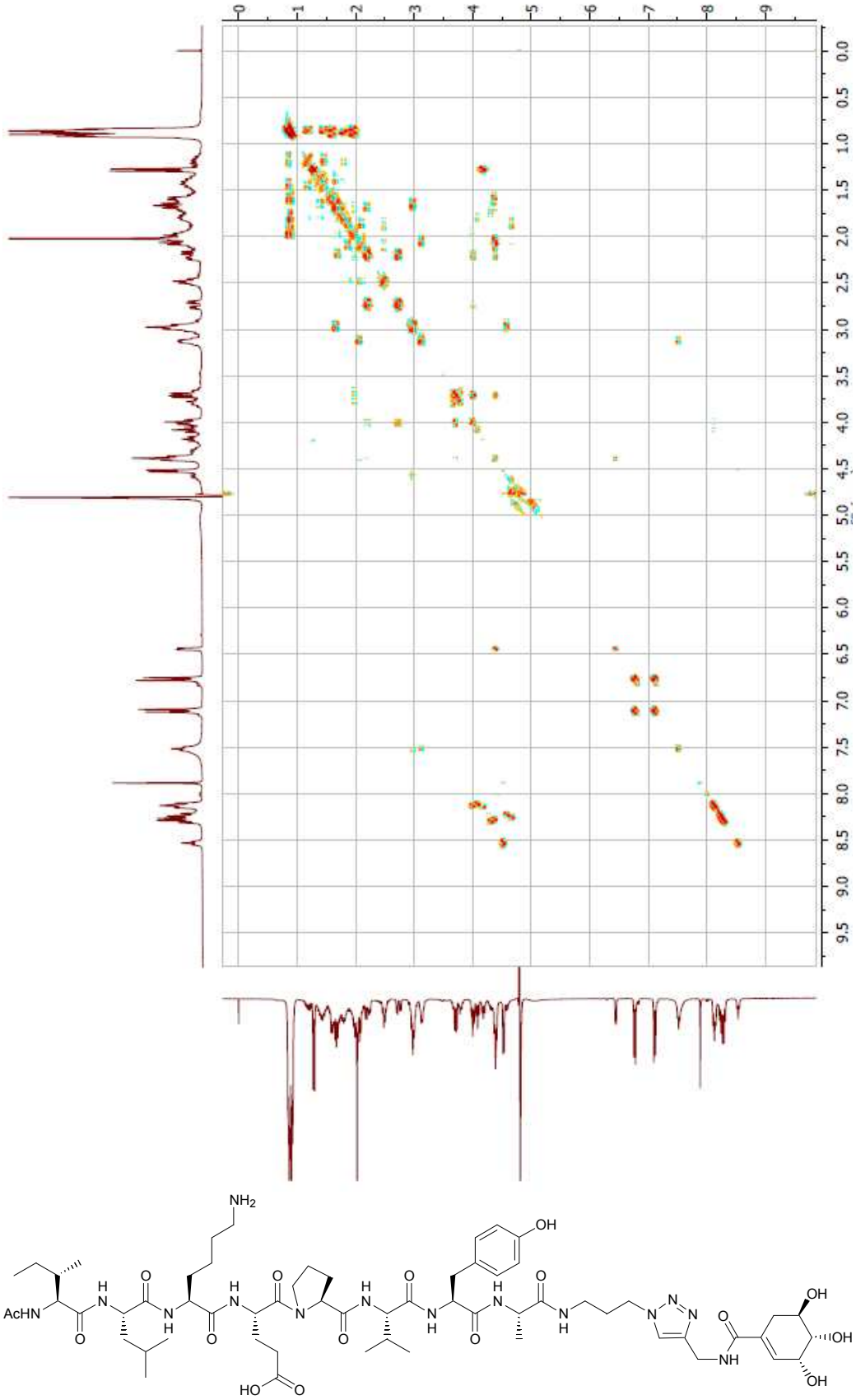
^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.53 (t, *J* = 5.7 Hz, 1H), 8.35 – 8.19 (m, 4H), 8.19 – 8.06 (m, 3H), 7.89 (s, 1H), 7.52 (t, *J* = 5.7 Hz, 1H), 7.11 (d, *J* = 8.5 Hz, 2H), 6.78 (t, *J* = 8.1 Hz, 2H), 6.49 – 6.41 (m, 1H), 4.65 – 4.55 (m, 2H), 4.53 (d, *J* = 5.7 Hz, 2H), 4.46 – 4.23 (m, 6H), 4.23 – 4.13 (m, 1H), 4.13 – 4.04 (m, 1H), 4.04 – 3.93 (m, 2H), 3.86 – 3.75 (m, 1H), 3.71 (dd, *J* = 8.6, 4.2 Hz, 1H), 3.68 – 3.61 (m, 1H), 3.21 – 3.07 (m, 2H), 3.07 – 2.86 (m, 4H), 2.74 (dd, *J* = 17.7, 5.2 Hz, 1H), 2.59 – 2.39 (m, 2H), 2.30 – 1.52 (m, 19H), 1.28 (d, *J* = 7.1 Hz, 3H), 1.25 – 1.04 (m, 1H), 1.03 – 0.76 (m, 18H).

^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 180.0, 177.2, 177.1, 177.0, 176.6, 176.5, 176.1, 176.0, 175.2, 174.0, 172.7, 157.3, 147.3, 135.5, 134.4, 133.3, 130.5, 126.7, 118.2, 103.7, 74.2, 69.2, 68.6, 63.0, 62.4, 61.5, 57.6, 56.0, 55.0, 53.6, 52.6, 50.7, 50.5, 42.4, 42.2, 39.1, 38.7, 37.5, 33.7, 33.0, 32.9, 32.4, 32.1, 31.5, 29.1, 27.4, 27.4, 27.0, 24.8, 24.7, 24.4, 23.6, 21.0, 20.7, 19.5, 17.5, 13.0.

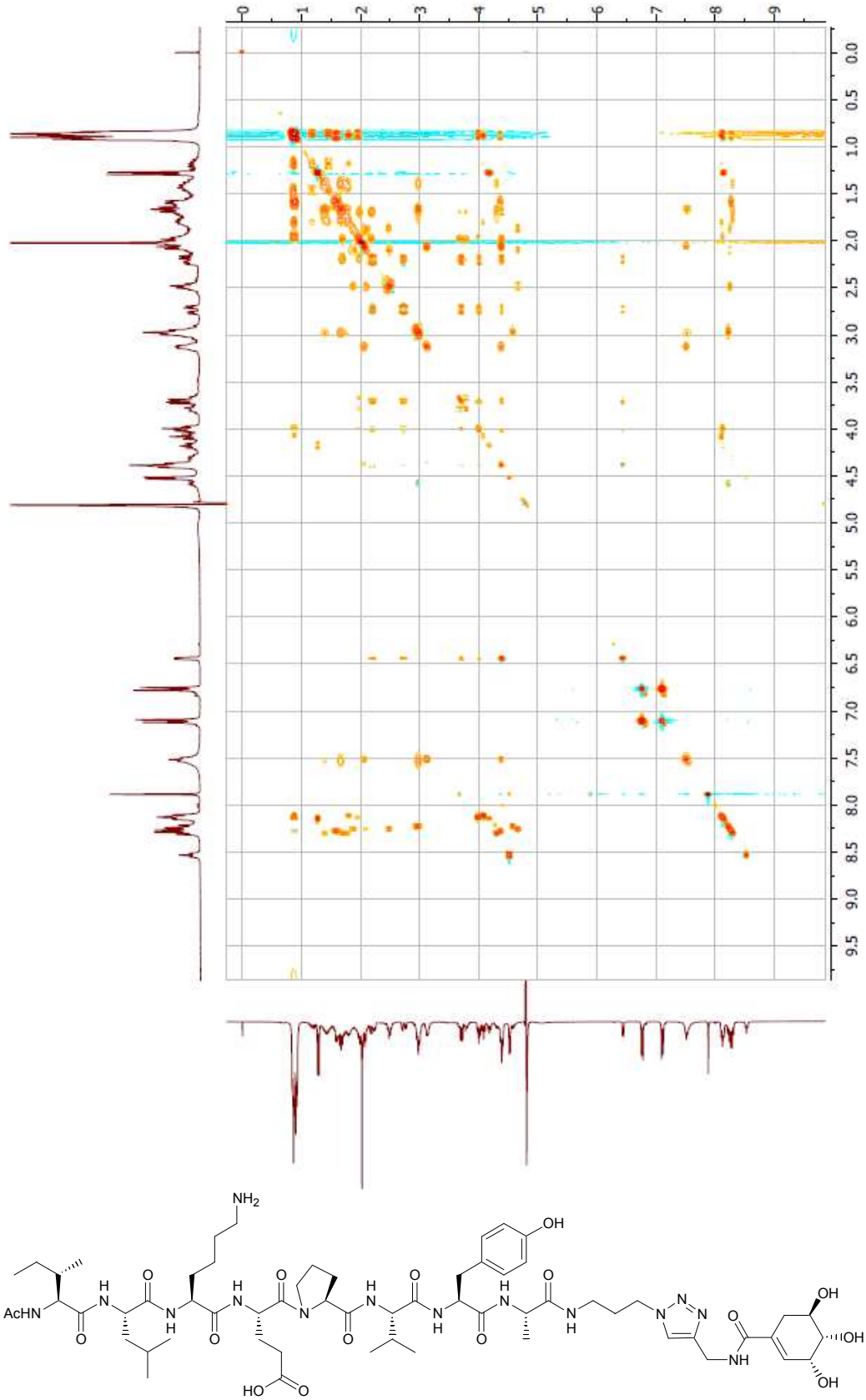
NMR ^{1}H experiment for compound **17**



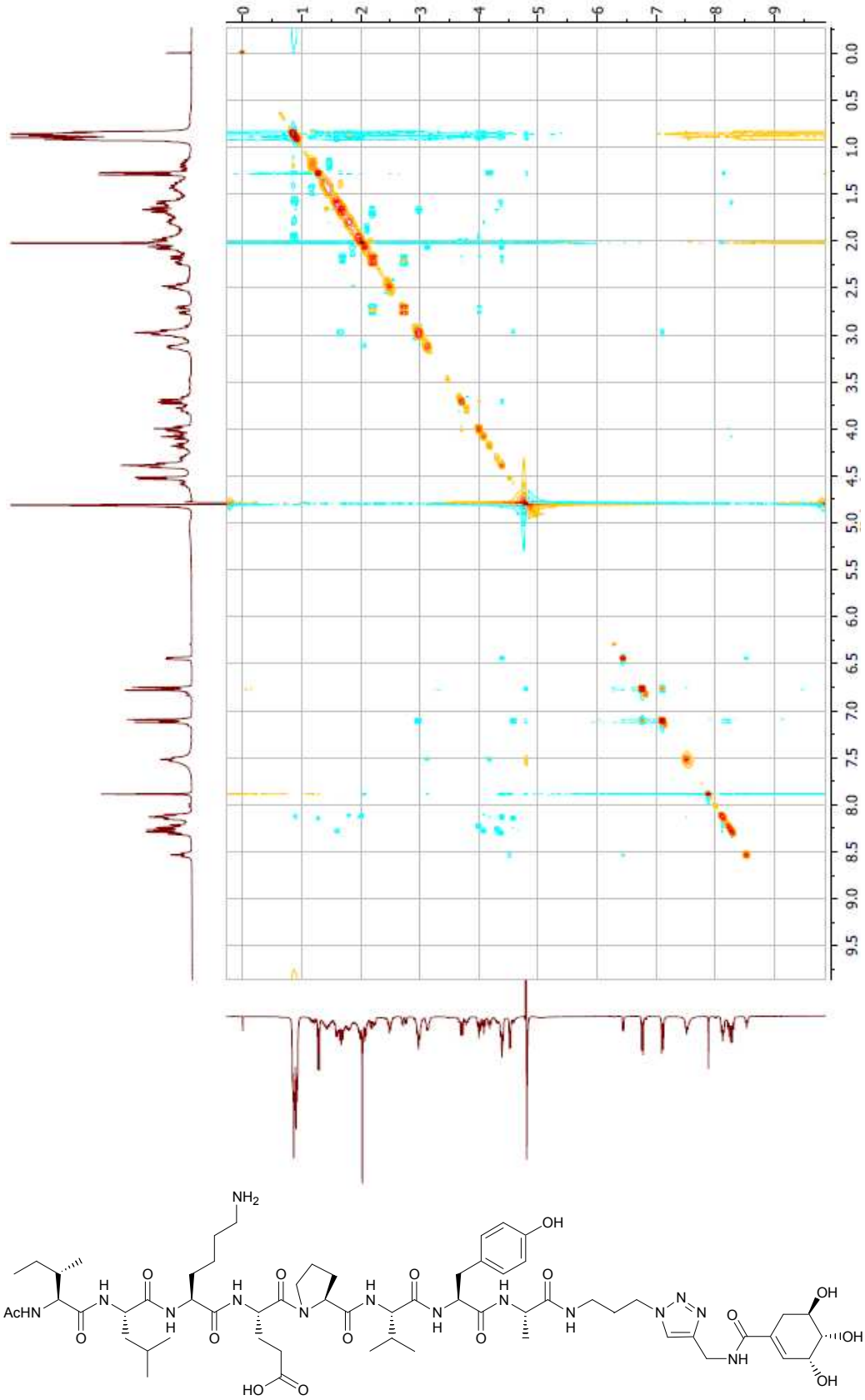
COSY experiment for compound **17**



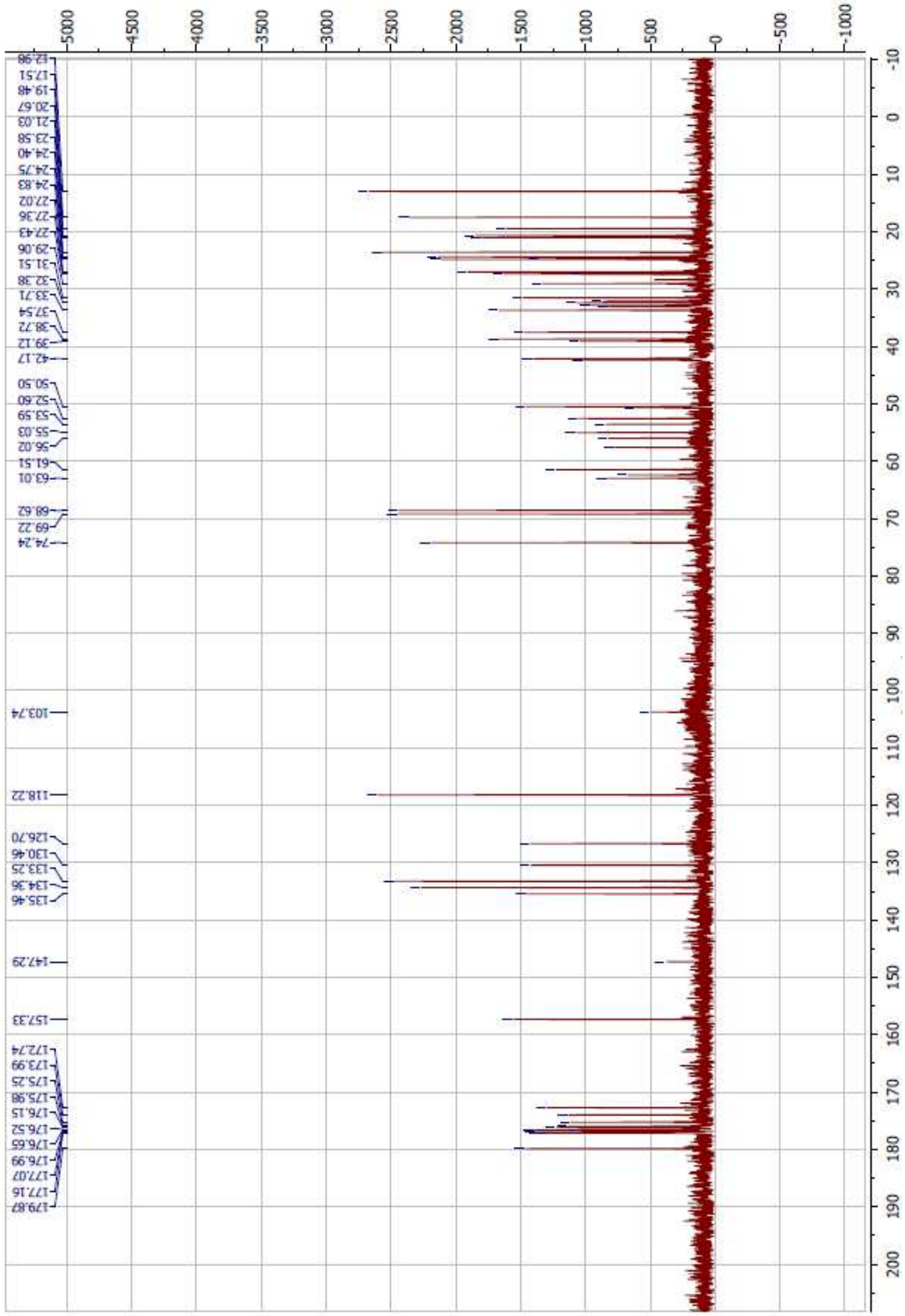
TOCSY experiment for compound **17**

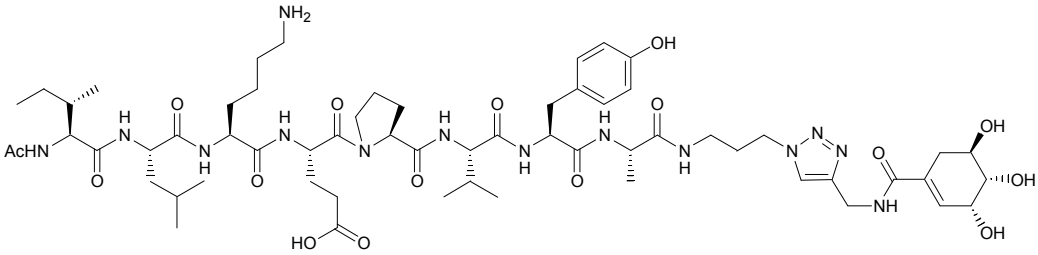


ROESY experiment for compound **17**

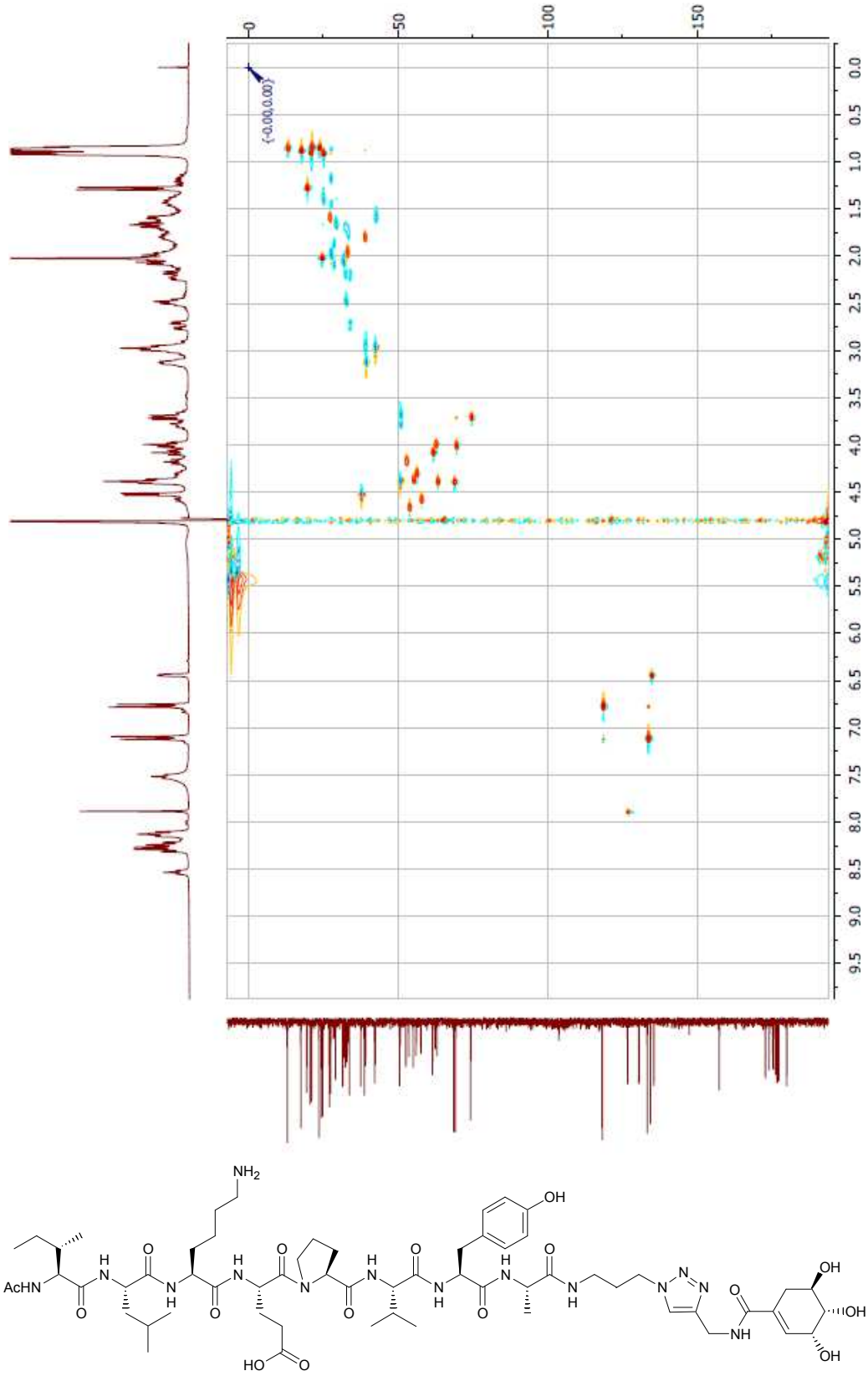


NMR ^{13}C experiment for compound **17**





HSQC experiment for compound **17**



Three-component CuAAC. Synthesis of 18

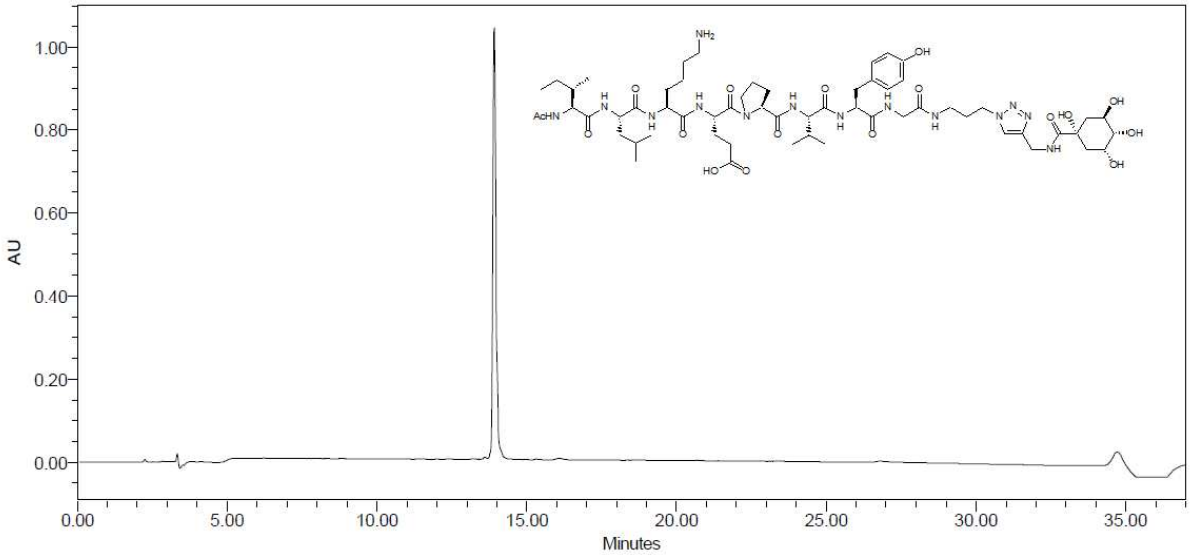
To a well stirred suspension of the corresponding hydrazide resin **2a** (50 µmol) swollen in anhydrous DCM (250 µL) was added a solution of 3-azidopropylamine hydrochloride **4a** (10.2 mg, 0.075 mmol), quinic derivative **6e** (22.9 mg, 0.1 mmol) and diisopropylethylamine (87.0 µL, 0.5 mmol) in dichloromethane (250 µL). Copper (II) acetate (22.7 mg, 0.125 mmol) dissolved in anhydrous pyridine (20.2 µL, 0.25 mmol) and anhydrous DCM (500 µL) was added and the reaction was kept for 24 hours at room temperature in a glove-box. After filtration, the resin was washed five times with DCM and the combined filtrates were concentrated in vacuo. To the resulting oil was added a solution of TFA/H_2O/triisopropylsilane: 95/2.5/2.5 by vol. (5 mL) at room temperature. After 2 hours, the peptide was precipitated in a mixture of diethylether/heptane: 1/1 by vol. (100 mL). The peptide was separated by centrifugation. The copper salts were removed by using a Chromabond C18-cartridge in buffer A (H_2O, TFA 0.05 %). The C18-cartridge was then washed with buffer B (CH_3CN/H_2O 80:20 TFA 0.05%), affording after lyophilization the crude peptide.

Characterization of peptide 18

Purification by RP-HPLC on a C18 nucleosil 120 Å, 5 µm column (215 nm, 6 mL/min, rt, buffer A water containing 0.05% TFA, buffer B CH_3CN/water 4/1 containing 0.05% TFA, linear gradient of 0-45% B in 60 min) to give 6.9 mg of pure peptide **18** (11.0 % yield).

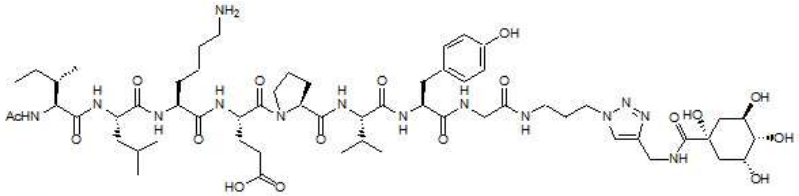
RP-HPLC analysis of 18

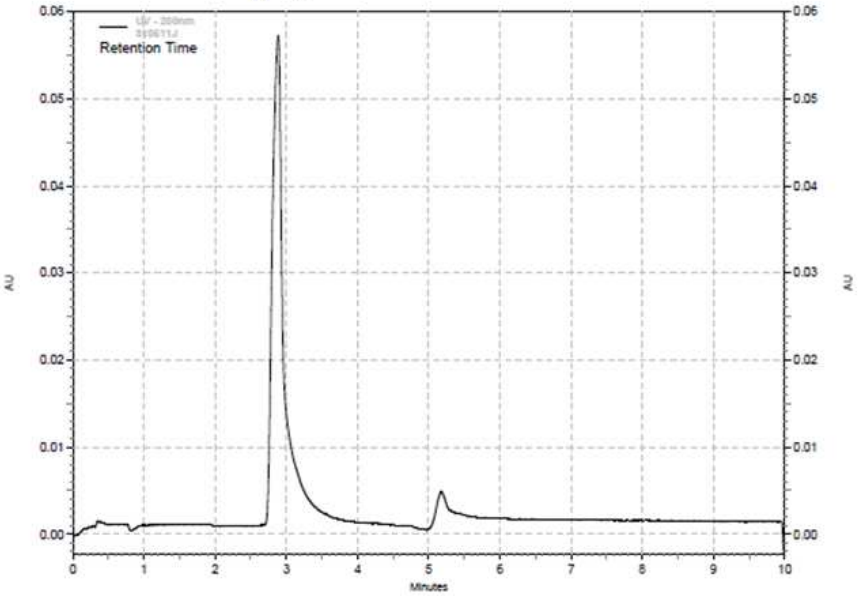
Eluent A is 0.05% TFA in water, eluent B is 0.05% TFA in CH_3CN/water: 4/1 by vol.
RP-HPLC on a 100 Å 5 µm C18 Nucleosil column (2.1x250 mm) using a linear water/acetonitrile gradient 0-100%B in 30 min (1.0 mL/min, detection 215 nm).



Capillary electrophoresis chromatogram of peptide 18

T = 30°C ; λ = 200 nm ; Capillary length = 20 cm ; Buffer = 20 mM Citrate buffer pH = 3.0 ; Run time = 10 min ; Separate voltage = 30 kV.

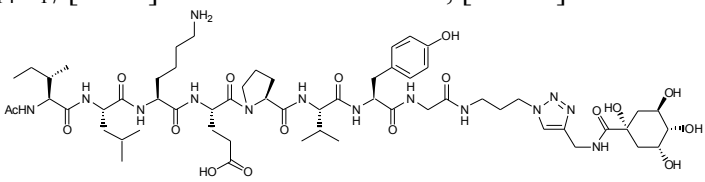


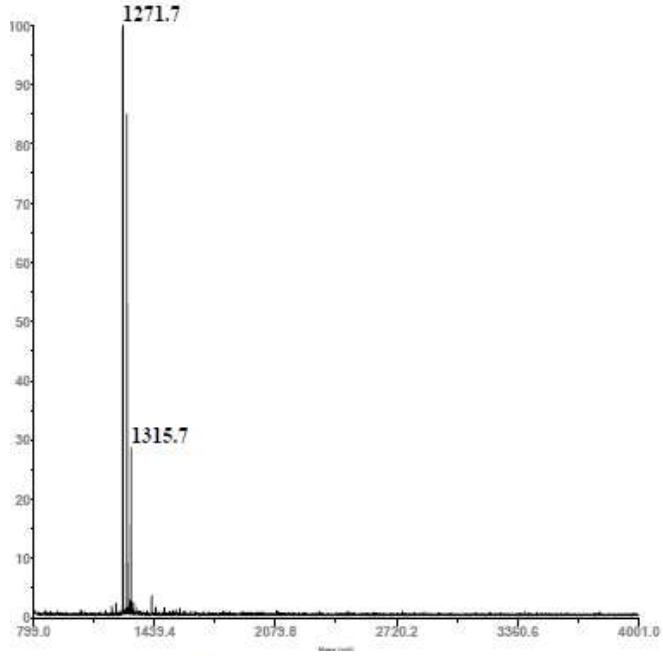


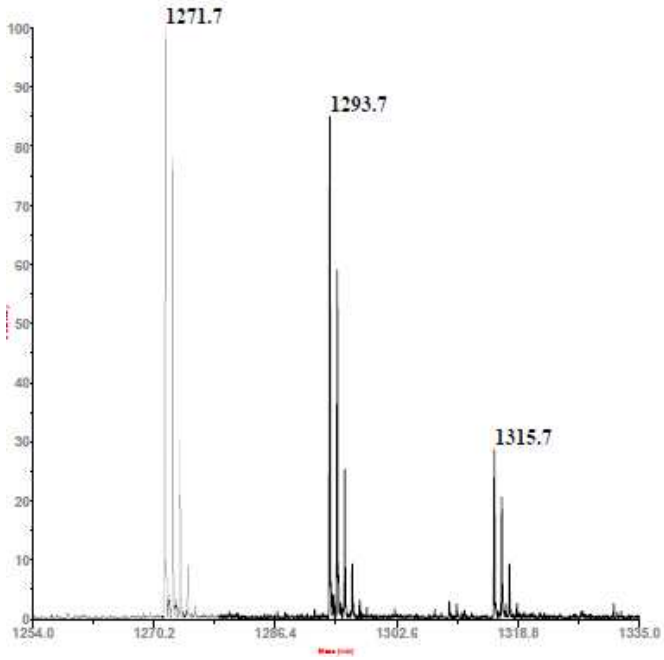
MALDI-TOF spectrum of 18

MALDO-TOF matrix: 2,5-Dihydroxybenzoic acid

Calcd. for $C_{59}H_{94}N_{14}O_{17}$ $[M+H]^+$ 1271.7 found 1271.7, $[M+Na]^+$ 1293.7 found 1293.7.





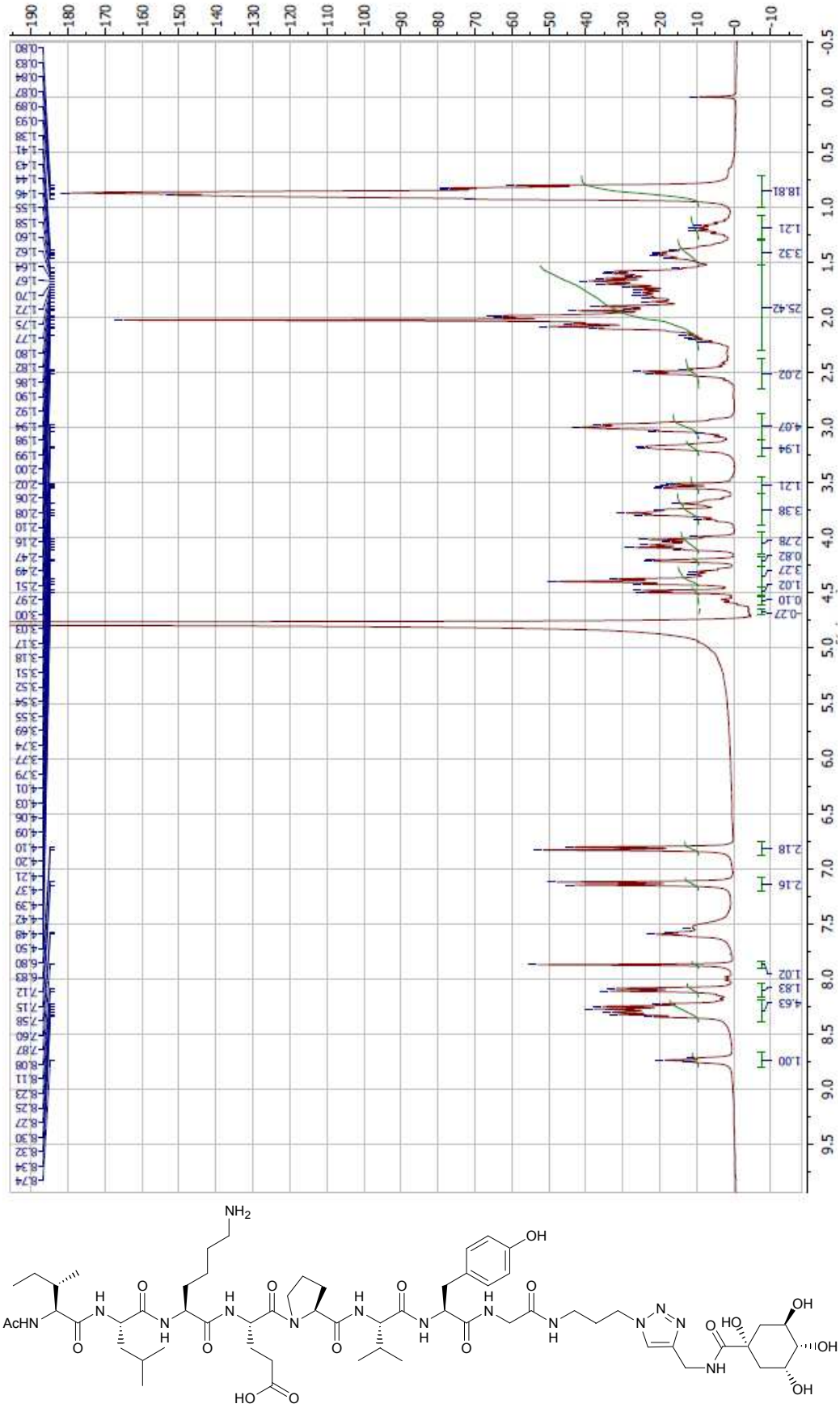


^{1}H and ^{13}C NMR for peptide 18

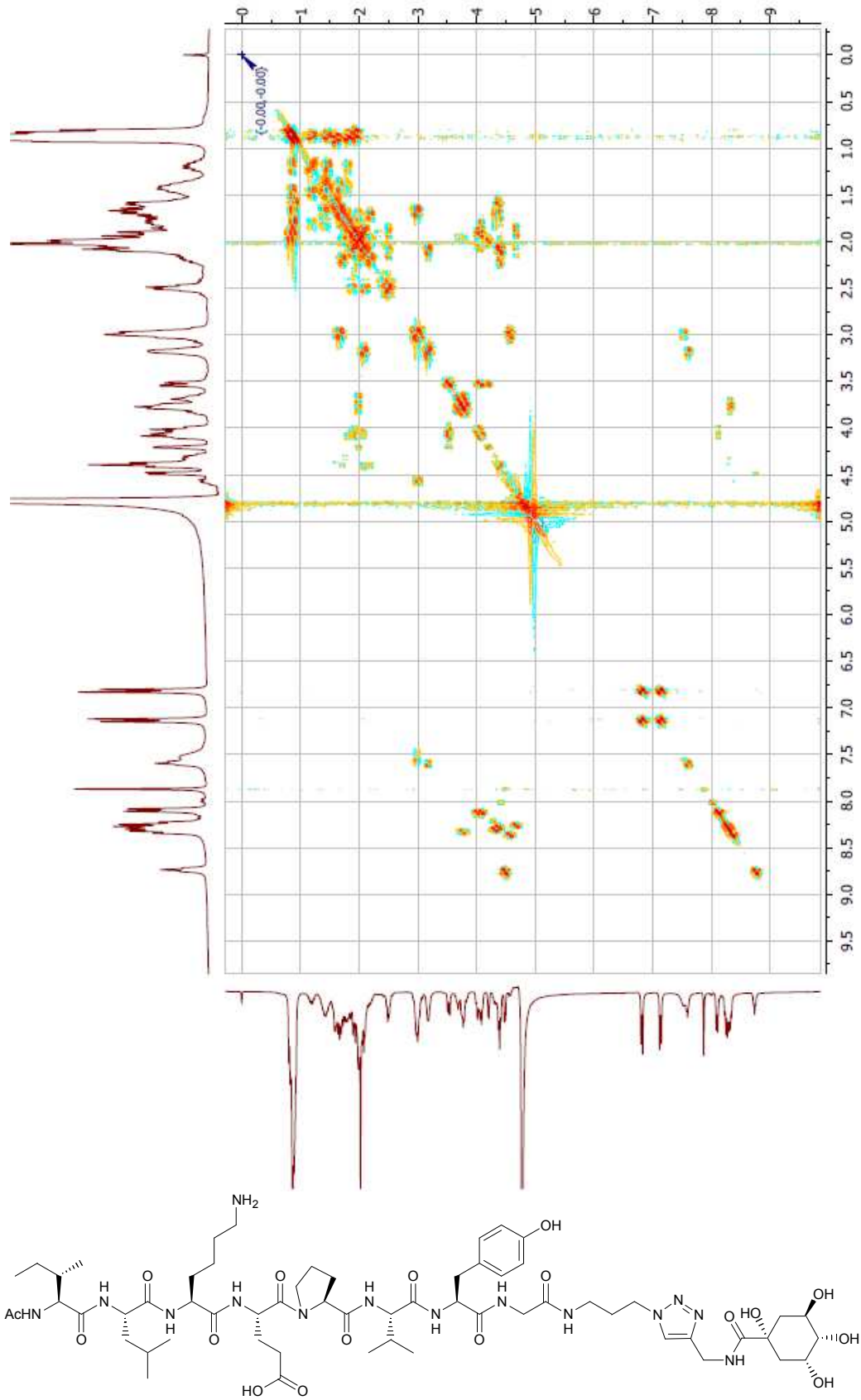
^{1}H NMR (300 MHz, H_2O+D_2O) δ (ppm): 8.74 (t, *J* = 5.6 Hz, 1H), 8.39 – 8.19 (m, 5H), 8.10 (d, *J* = 7.3 Hz, 2H), 7.87 (s, 1H), 7.66 – 7.42 (m, 2H), 7.13 (d, *J* = 8.2 Hz, 2H), 6.82 (d, *J* = 8.2 Hz, 2H), 4.70 – 4.64 (m, 1H), 4.61 – 4.53 (m, 1H), 4.49 (d, *J* = 5.7 Hz, 1H), 4.44 – 4.26 (m, 3H), 4.21 (d, *J* = 2.8 Hz, 1H), 4.14 – 3.94 (m, 3H), 3.88 – 3.60 (m, 4H), 3.53 (dd, *J* = 9.6, 2.9 Hz, 1H), 3.26 – 3.11 (m, 2H), 3.10 – 2.87 (m, 4H), 2.64 – 2.38 (m, 2H), 2.30 – 1.53 (m, 25H), 1.53 – 1.29 (m, 3H), 1.30 – 1.08 (m, 1H), 1.00 – 0.71 (m, 19H).

^{13}C NMR (75 MHz, H_2O+D_2O) δ (ppm): 179.8, 179.7, 177.2, 177.0, 176.6, 176.4, 176.3, 176.1, 176.0, 174.0, 173.8, 157.3, 147.3, 133.2, 130.6, 126.6, 118.2, 79.5, 77.8, 73.2, 69.1, 63.0, 62.3, 61.5, 58.1, 56.3, 55.4, 55.0, 53.9, 53.2, 50.5, 45.5, 43.0, 42.4, 42.2, 39.8, 39.2, 38.7, 37.3, 37.1, 33.0, 32.9, 32.3, 32.0, 31.6, 29.1, 27.4, 27.0, 24.8, 24.4, 23.6, 21.0, 20.6, 17.5, 13.0.

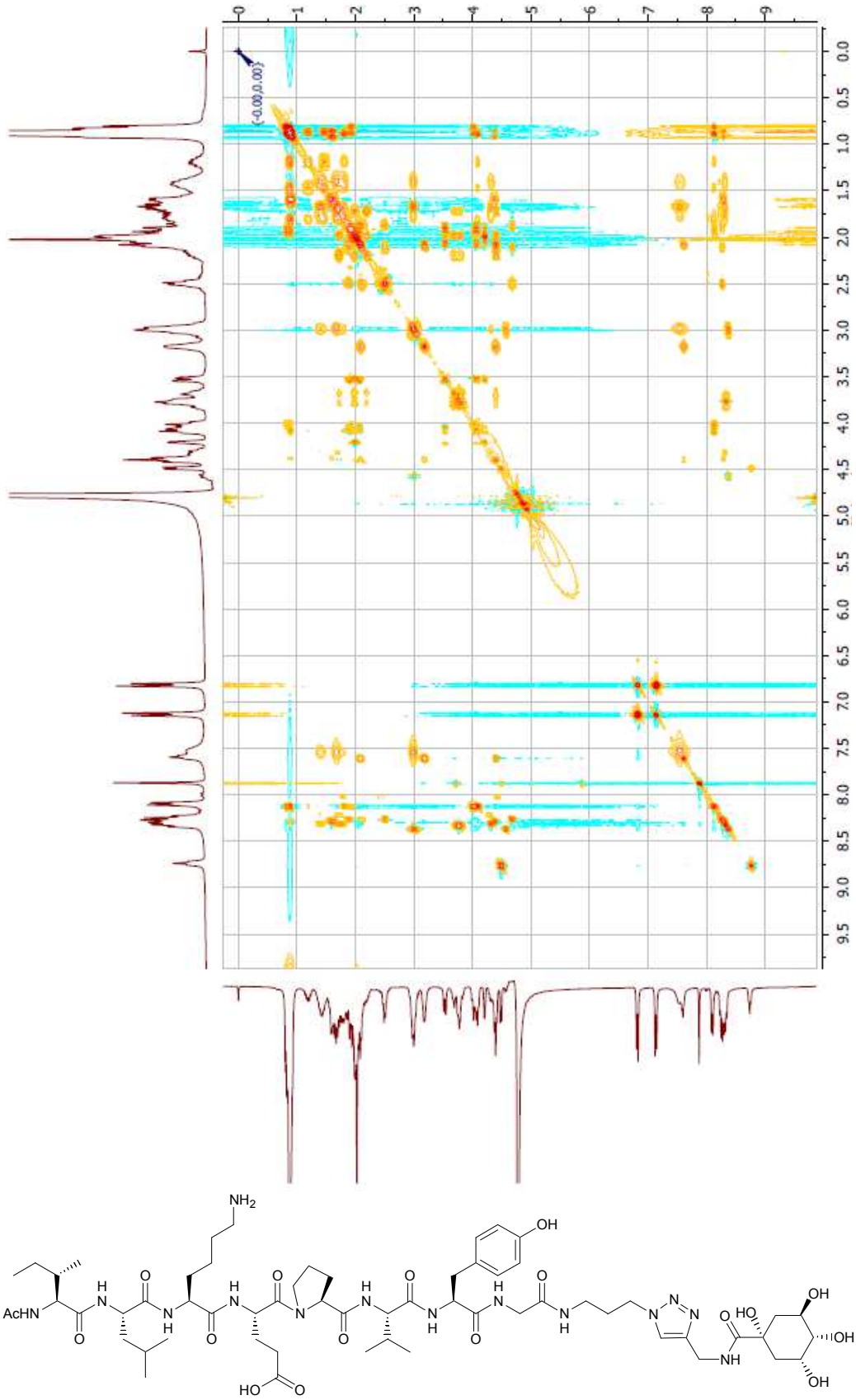
NMR ^{1}H experiment for compound **18**



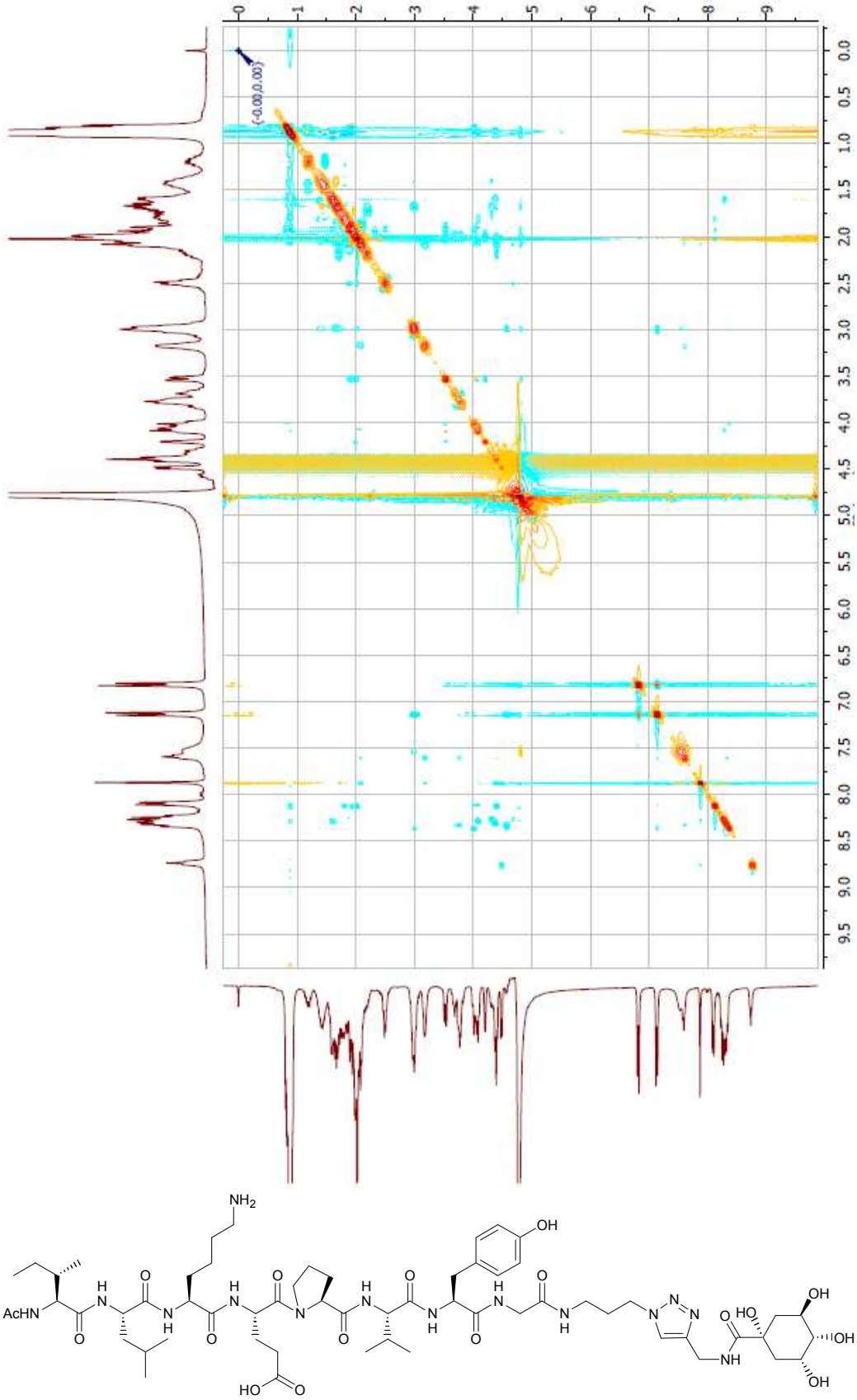
COSY experiment for compound **18**



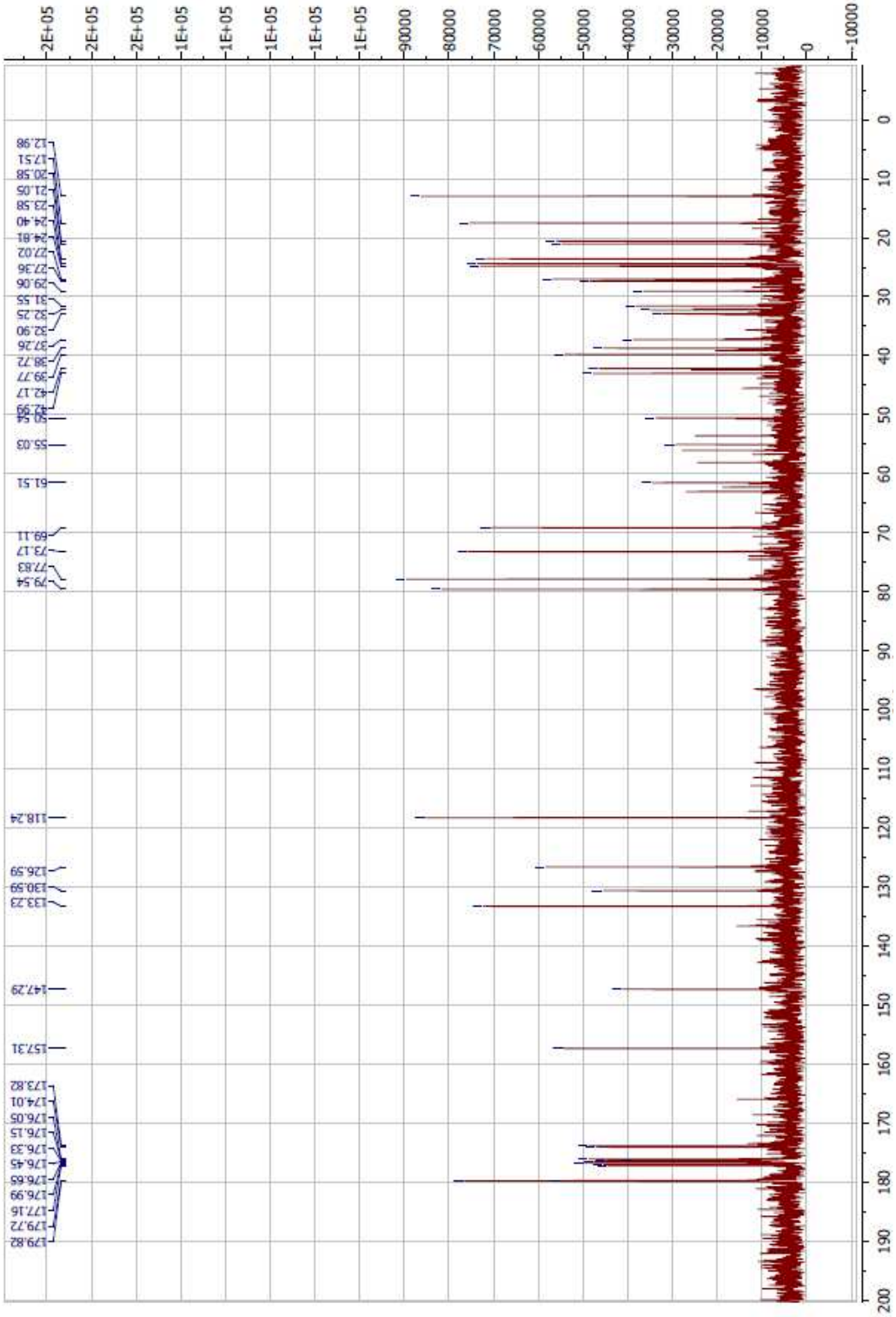
TOCSY experiment for compound **18**

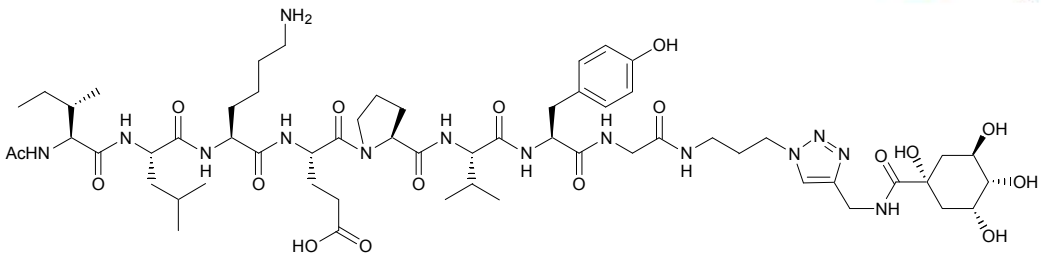


ROESY experiment for compound **18**



NMR ^{13}C experiment for compound **18**





HSQC experiment for compound **18**