Synthesis aided structural determination of amyloid-β (1-15) glycopeptide, a new biomarker for Alzheimer's disease

Supporting Information

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Materials and Instruments

Unless otherwise indicated, all starting materials, reagents and solvents were obtained from commercial suppliers and used as supplied without further purification. Amino acids were purchased from ChemImpex. H-Gln(Trt)-2-ClTrt resin was purchased from Advanced ChemTech. All other reagents were purchased from Sigma-Aldrich. ^{1}H and ^{13}C NMR spectra were recorded on an Agilent-500M spectrometer. HRMS spectra were recorded on a Water Xevo G2-S Q-TOF LC-MS instrument. Peptide purification was performed on Shimadzu (LC-8A Liquid Chromatograph Pump, DGU-14A Degasser and SPD-10A UV-Vis Detector) with a Phenomenex C-18 HPLC column. UV-Vis absorbance spectra were recorded on a UNICAM UV/Vis spectrometer.

Experimental Procedures

Synthesis of α-GalNAc Tyr

1, 3, 4, 6-Tetra-O-acetyl-2-azido-2-deoxy-D-galactopyranoside **(S1)**

Sodium azide (15.1 g, 232 mmol) was dissolved in water (37.5 mL) and cooled to 0 ℃. Then toluene (37.5 mL) was added followed by drop wise addition of triflic anhydride (7.7 mL, 46.4 mmol). The reaction mixture was stirred at 0℃ for 2 h. A saturated aqueous solution of sodium bicarbonate was added to the reaction till no more bubbles were formed. The organic phase was separated and the aqueous phase was extracted with toluene twice. The combined organic phase was dried over anhydrous sodium sulfate, (Note: this solution should never be concentrated.) and used directly for the next step without further purification.

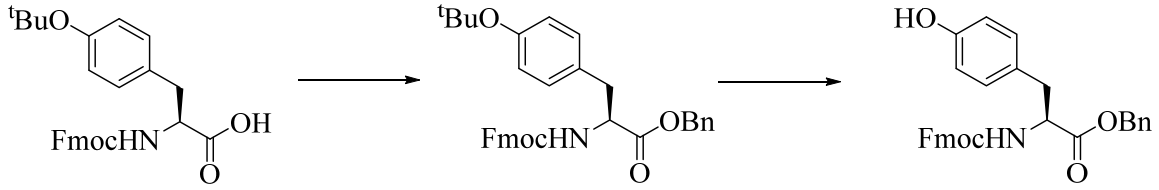
Galactosamine hydrochloride (**1**) (5 g, 23.2 mmol) was dissolved in water (30 mL). Potassium carbonate (4.8 g, 3.8 mmol), copper sulfate pentahydrate (57.9 mg, 0.232 mmol) and MeOH (50 mL) were added. The triflic azide solution from last step was added followed by addition of more MeOH until the solution was homogeneous. The mixture was allowed to stir at room temperature (r.t.) overnight, and the color changed from blue to green. The solvent was coevaporated with toluene several times until near dryness. Then pyridine (25 mL, 302 mmol) was added and the reaction was cooled to 0℃ followed by addition of acetic anhydride (50 mL, 464 mmol) slowly and 4-dimethylaminopyridine (DMAP) (0.718 g, 5.8 mmol). The reaction was allowed to warm to r.t. slowly and continued to stir at r.t. for 1 day. Upon completion, excess acetic anhydride was quenched by slow addition of MeOH. The mixture was concentrated under vacuum, diluted with dichloromethane (DCM) and washed with 1 M HCl, Na_2CO_3 and water. The organic phase was then dried over anhydrous Na_2SO_4, filtered and concentrated. After purification by column chromatography (Hex:EtOAc = 1:1), compound **S1** was obtained as a white foam in an overall yield of 71%. Comparison with literature data[1] confirms its identity.

***3, 4, 6-Tri-O-acetyl-2-azido-2-deoxy-D-galactopyranose* (S2)**

Compound **S1** (5.98 g, 16 mmol) was dissolved in THF (200 mL), and hydrazine acetate (2.15 g, 24 mmol) was added. After stirred at r.t. overnight, the solution was concentrated, diluted with DCM and washed with water twice. The organic phase was dried. Purification by column chromatography (Hex:EtOAc = 3:2) produced compound **S2** as colorless oil in 82% yield. Comparison with literature data[2] confirms its identity.

***O-(3, 4, 6-Tri-O-acetyl-2-azido-2-deoxy-D-galactopyranoside) trichloroacetimidate* (2)**

Compound **S2** (0.199 g, 0.56 mmol) was dissolved in anhydrous DCM (2 mL) under nitrogen. Potassium carbonate (0.142 g, 0.76 mmol) was added to the reaction mixture, which was followed by a solution of trichloroacetonitrile (0.14mL, 1.32mmol) in DCM (2 mL). The reaction was vigorously stirred overnight. Upon completion, the mixture was filtered through Celite. Purification by column chromatography produced compound **2** as clear oil in 75% yield. Comparison with literature data[2] confirms its identity.



S3 **S4** **3**

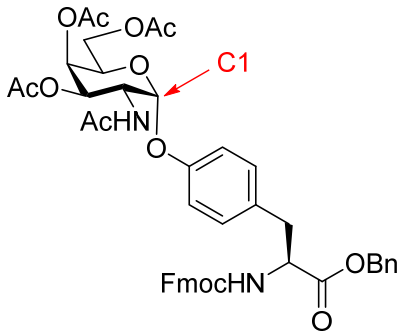
***N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-O-tert-butoxy-L-tyrosine benzyl ester* (S4)**

Compound **S3** (2.29 g, 5.0 mmol), potassium bicarbonate (1.52 g, 15.2 mmol) and tetrabutyl ammonium iodide (TBAI) (390 mg, 1.05 mmol) were dissolved in DMSO (12 mL). Benzyl bromide (5.22 g, 30 mmol, 6 eq) was added into the mixture, which was stirred for 20 h. The reaction was quenched with water, diluted with EtOAc and washed with sat. $NaHCO_3$, $Na_2S_2O_3$ and brine. The organic phase was dried and concentrated. Recrystallization in EtOAc/hexanes gave compound **S4** as white powder in an 82% yield. Comparison with literature data[3] confirms its identity.

***N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-L-tyrosine benzyl ester* (3)**

Compound **S4** (549 mg, 1 mmol) was dissolved in TFA/DCM (6 ml/6 mL) and stirred for 3 h. The solvent was evaporated and the solid was recrystallized to produce compound **3** as a white powder in 71% yield. Comparison with literature data[4] confirms its identity.

***N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-tyrosine benzyl ester* (4)**



Compound **2** (714 mg, 1.5 mmol), compound **3** (494 mg, 1 mmol) were mixed with 1 g pre-activated 4 A MS and dissolved in DCM/Et_2O (20 mL/ 20 mL) under nitrogen. The solution was stirred for 1 h and then cooled to -30℃. TMSOTf (90 μL, 0.3 mmol) was added dropwise and the reaction was stirred for another 2 h. Upon completion, the reaction was washed with sat. $NaHCO_3$ and then dried and concentrated. The product was separated by column chromatography (Hex:EtOAc = 3:1) and then dissolved in THF (30 mL). Zinc dust (866 mg, 13.3 mmol), acetic anhydride (679 mg, 6.7 mmol) and AcOH (400 mg, 6.7 mmol) were added and the reaction was stirred overnight. Upon completion, the reaction mixture was filtered and concentrated. Then it was diluted with DCM and washed with sat. $NaHCO_3$. The solution was dried and concentrated. Then the crude product was purified through column (Toluene/Acetone = 2:1) to obtain compound **4** as white powder in a 56% yield. ^{1}HNMR (500 MHz, $CDCl_3$): δ = 1.90 (s, 3H), 1.97 (s, 3H), 2.05 (s, 3H), 2.19 (s, 3H), 3.00-3.12 (m, 2H), 4.01 (dd, 1H, J = 7.0, 11.0 Hz), 4.09 (dd, 1H, J = 6.5, 11.5 Hz), 4.20 (t, 2H, J = 6.5 Hz), 4.35-4.38 (m, 1H), 4.46 (dd, 1H, J = 7.0, 11.0 Hz), 4.67-4.70 (m, 1H), 4.73-4.78 (m, 1H), 5.13 (d, 1H, J = 12.0 Hz), 5.20 (d, 1H, J = 12.5 Hz), 5.24 (d, 1H, J = 8.5 Hz), 5.38 (dd, 1H, J = 3.0, 11.0 Hz), 5.42 (d, 1H, J = 2.5 Hz), 5.54 (d, 1H, J = 3.0 Hz, C1-H), 5.70 (d, 1H, J = 9.5 Hz), 6.89 (s, 4H), 7.14-7.18 (m, 1H), 7.24-7.42 (m, 8H), 7.53-7.57 (m, 2H), 7.76-7.79 (m, 2H). ^{13}CNMR (125 MHz, $CDCl_3$): δ = 20.51, 20.71, 20.78, 23.31, 37.27, 47.17, 47.87, 54.74, 61.48, 66.87, 67.12, 67.36, 67.51, 68.14, 96.27, 116.47, 119.99, 120.02, 125.03, 127.03, 127.75, 128.20, 128.65, 128.68, 129.01, 130.64, 141.32, 155.05, 170.08, 170.22, 170.25, 171.05, 171.14 ppm. HRMS: m/z calc. for $C_{45}H_{47}N_2O_{13}$: 823.3078; found: 823.3058 $[M+H]^+$.

***N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-α-D-galactopyranosyl)-L-tyrosine* (5)**

Compound **4** (411 mg, 0.5 mmol) was dissolved in DCM (25 mL) along with Pd/C (60 mg). AcOH (5 mL) and MeOH (5 mL) were added into the reaction. It was stirred for 1 h under a hydrogen atmosphere. Upon completion, the reaction was filtered and concentrated. Compound **5** was obtained through column chromatography (DCM:MeOH = 12:1 with 1% of AcOH) as white powder in a 94% yield. ^{1}HNMR (500 MHz, d^6-DMSO): δ = 1.83 (s, 3H, NH-CO-CH_3), 1.85 (s, 3H, O-CO-CH_3), 1.94 (s, 3H, O-CO-CH_3), 2.13 (s, 3H, O-CO-CH_3), 2.86 (dd, 1H, J = 8.0, 13.5 Hz, Tyr-CH_2^{β}), 3.06 (dd, 1H, J = 4.0, 13.5 Hz, Tyr-CH_2^{β}), 3.93-4.01 (m, 3H, Fmoc-CH_2, Tyr-CH^{α}), 4.10-4.18

(m, 2H, GalNAc-C6-H_2, Fmoc-CH), 4.25-4.30 (m, 2H, GalNAc-C5-H, GalNAc-C6-H_2), 4.34-4.39 (m, 1H, GalNAc-C2-H), 5.21 (dd, 1H, J = 3.5, 12.0 Hz, GalNAc-C3-H), 5.38 (d, 1H, J = 2.5 Hz, GalNAc-C4-H), 5.45 (d, 1H, J = 3.5 Hz, GalNAc-C1-H), 6.93 (d, 2H, J = 8.5 Hz, Tyr-C_6H_4), 7.12 (d, 2H, J = 8.5 Hz, Tyr-C_6H_4), 7.28-7.32 (m, 2H, Fmoc), 7.39-7.41 (m, 2H, Fmoc), 7.61-7.63 (m, 2H, Fmoc), 7.88 (d, 2H, J = 7.5 Hz, Fmoc), 8.24 (d, 1H, J = 8.0 Hz, NH-Ac). ^{13}CNMR (125 MHz, d^6-DMSO): δ = 20.80, 20.82, 21.02, 22.82, 47.14, 47.63, 62.00, 65.68, 67.29, 67.40, 67.82, 97.08, 117.36, 120.48, 125.70, 127.46, 127.99, 130.84, 141.12, 144.30, 155.13, 155.79, 170.18, 170.23, 170.33, 170.46 ppm. HRMS: m/z calc. for $C_{38}H_{41}N_2O_{13}$: 733.2609; found: 733.2613 $[M+H]^+$.

Synthesis of β-GalNAc Tyr

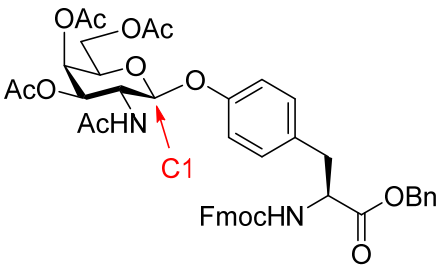
1, 3, 4, 6-Tetra-O-acetyl-2-(2, 2, 2-trichloroethoxycarbonylamino)-2-deoxy-D-galactopyranoside **(S5)**

Compound **S1** (3.74 g, 10 mmol) was dissolved in THF (60 mL) with *p*-TsOH·H_2O (1.90 g, 10 mmol) and Pd/C (600 mg) under a hydrogen atmosphere. The reaction was stirred at rt. for 20 h. Then the reaction was filtered, washed with sat. $NaHCO_3$ and concentrated. It was then diluted with THF (80 mL). The solution was cooled to 0 ℃ followed by addition of trichloroethoxycarbonyl chloride (4 mL, 30 mmol) and triethylamine (8 mL). The reaction was stirred for 30 min and quenched by sat. $NaHCO_3$. The product was purified by column chromatography (Hex:EtOAc = 2:1) as colorless oil in an 80% yield. Comparison with literature data[5] confirms its identity.

p-Tolyl 3, 4, 6-Tri-O-acetyl-2-(2, 2, 2-trichloroethoxycarbonylamino)-2-deoxy-1-thio-β-D-galactopyranoside **(6)**

Compound **S5** (4.21 g, 8.0 mmol) and *p*-toluenethiol (3.40 g, 27 mmol) was dissolved in anhydrous DCM (80 mL). Then borontrifluoride etherate (3.14 mL, 25 mmol) was added and the reaction was stirred for 1 h. The reaction was washed with sat. $NaHCO_3$, dried and concentrated. Compound **6** was obtained through column chromatography (Hex:EtOAc =2:1) as colorless oil in an 85% yield. Comparison with literature data[5] confirms its identity.

N^{α}-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-L-tyrosine benzyl ester **(7)**



Compound **6** (2.06 g, 3.5 mmol), compound **3** (1.50 g, 3.0 mmol) were mixed with 2 g pre-activated 4 A MS and *N*-iodosuccinimide (1.1g, 4.9 mmol). They were dissolved in DCM (80 mL) under nitrogen. The solution was stirred for 1 h and then cooled to -20℃. Triflic acid (50 μL, 0.33 mmol) was added dropwise and the reaction was stirred for 1 h. Upon completion, the reaction was washed with sat. $NaHCO_3$ and then dried and concentrated. The crude product was separated by column chromatography (Hex:EtOAc = 3:2) and then dissolved in THF (60 mL). Zinc dust (2.5 g, 38.5 mmol), acetic anhydride (2.0 g, 19.6 mmol) and AcOH (1.2 g, 20 mmol) were added and the reaction was stirred overnight. Upon completion, the reaction was filtered and concentrated. Then it was diluted with DCM and washed with sat. $NaHCO_3$. The solution was dried and concentrated. Then the crude product was purified through column (Toluene/Acetone = 2:1) to obtain compound **7** (β isomer) as white powder in a 26% yield. ^{1}HNMR (500 MHz, $CDCl_3$): δ = 1.93 (s, 3H), 2.02 (s, 3H), 2.03 (s, 3H), 2.15 (s, 3H), 3.01 (dd, 1H, J = 6.5, 13.0 Hz), 3.14 (dd, 1H, J = 5.5, 13.5 Hz), 3.78 (t, 1H, J = 6.0 Hz), 4.09-4.16 (m, 3H), 4.21-4.26 (m, 2H), 4.67 (dd, 1H, J = 6.0, 10.5 Hz), 4.70-4.75 (m, 1H), 4.94 (d, 1H, J = 8.5 Hz, C1-H), 5.13-5.21 (m, 2H), 5.23-5.28 (m, 2H), 5.33 (d, 1H, J = 2.0 Hz), 5.37 (d, 1H, J = 8.5 Hz), 6.84-6.95 (m, 4H), 7.29-7.43 (m, 9 H), 7.50-7.57 (m, 2H), 7.79 (d, 2H, J = 7.5 Hz). ^{13}CNMR (125 MHz, $CDCl_3$): δ = 20.64, 20.67, 23.40, 37.55, 47.21, 51.34, 54.68, 61.43, 66.53, 67.01, 67.36, 69.70, 70.86, 99.66, 117.40, 120.07, 120.11, 125.07, 125.30, 127.09, 127.18, 127.72, 127.83, 128.54, 128.63, 128.67, 130.48, 135.00, 141.20, 155.50, 156.25, 170.22, 170.30, 170.44, 171.32 ppm. HRMS: m/z calc. for $C_{45}H_{47}N_2O_{13}$: 823.3078; found: 823.3083 $[M+H]^+$.

N$^{\alpha}$-(Fluoren-9-ylmethoxycarbonyl)-O-(2-acetamido-3, 4, 6-tri-O-acetyl-2-deoxy-β-D-galactopyranosyl)-L-tyrosine **(8)**

Compound **7** (411 mg, 0.5 mmol) was dissolved in DCM (15 mL) along with Pd/C (60 mg). AcOH (3 mL) and MeOH (3 mL) were added into the reaction. It was stirred for 1 h under hydrogen atmosphere. Upon completion, the reaction was filtered and concentrated. Compound **8** was obtained through column chromatography (DCM:MeOH = 12:1 with 1% AcOH) as white powder in a 90% yield. ^{1}HNMR (500 MHz, d^6-DMSO): δ = 1.77 (s, 3H, NH-CO-CH_3), 1.92 (s, 3H, O-CO-CH_3), 1.98 (s, 3H, O-CO-CH_3), 2.13 (s, 3H, O-CO-CH_3), 2.82 (dd, 1H, J = 10.5, 14.0 Hz, Tyr-CH_2^{β}), 3.02 (dd, 1H, J = 4.0, 14.0 Hz, Tyr-CH_2^{β}), 4.05 (d, 2H, J = 6.5 Hz, Fmoc-CH_2), 4.09-4.23 (m, 5H, Tyr-CH^{α}, GalNAc-C6-H_2, Fmoc-CH, GalNAc-C2-H), 4.25 (t, 1H, J = 6.5 Hz, GalNAc-C5-H), 5.08 (dd, 1H, J = 4.0, 11.0 Hz, GalNAc-C3-H), 5.16 (d, 1H, J = 8.5 Hz, GalNAc-C1-H), 5.29 (d, 1H, J = 3.5 Hz, GalNAc-C4-H), 6.90 (d, 2H, J = 8.5 Hz, Tyr-C_6H_4), 7.21 (d, 2H, J = 8.5 Hz, Tyr-C_6H_4), 7.28-7.33 (m, 2H, Fmoc), 7.41 (t, 2H, J = 7.5 Hz, Fmoc), 7.66 (dd, 2H, J = 4.0, 7.5 Hz, Fmoc), 7.69 (d, 1H, J = 8.5 Hz, Tyr-NH), 7.88 (d, 2H, J = 8.0 Hz, Fmoc), 7.92 (d, 1H, J = 9.0 Hz, NH-Ac). ^{13}CNMR (125 MHz, d^6-DMSO): δ = 20.44, 20.48, 21.03, 22.76, 35.60, 46.55, 49.29, 55.71, 61.35, 65.58, 66.58, 70.18, 70.27, 98.84, 116.16, 120.06, 125.21, 127.02, 127.60, 130.14, 132.05, 140.65, 143.73, 155.60, 155.92, 169.47, 169.61, 169.80, 169.98, 171.96, 173.29 ppm. HRMS: m/z calc. for $C_{38}H_{41}N_2O_{13}$: 733.2609; found: 733.2594 $[M+H]^+$.

Synthesis of Glycosylated Aβ(1-15)

The Aβ1-15 peptide was prepared by the Fmoc-chemistry based solid phase peptide synthesis. Resin with pre-loaded Gln (1.0 mmol/g, 200 mg) was loaded into a plastic syringe fitted with a filter. The next amino acid (10 eq) was activated by O-(Benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate (HBTU, 9.9 eq) in DIPEA (20 eq) and anhydrous DMF (5 mL). The solution was extracted into the syringe and mixed with the resin on a nutator for 12 h. The resin was then washed with DMF and DCM. The unreacted N-terminus was capped with acetic anhydride and N-terminal protecting group Fmoc was removed by 20% piperidine in DMF. To synthesize the glycopeptide, the glycosylated Tyr (2 eq) was activated by *O*-(7-azabenzotriazol-1-yl)-N, N, N', N'-tetramethyluronium hexafluorophosphate (HATU, 1.98 eq). After the peptide chain was completed, Fmoc was removed and the peptide was cleaved from the resin by TFA/TIPS/H_2O (95%: 2.5%: 2.5%). Protecting groups on galactosamine was removed by 5% aqueous hydrazine. The peptide was then purified through HPLC with a C-18 column. Glycopeptide **9**/**10**/**11** were obtained in 3%, 5% and 9% yield respectively.

9: HRMS: m/z calc. for $C_{38}H_{41}N_2O_{13}$: 677.2932; found: 677.2919 $[M+3H]^{3+}$.

10: HRMS: m/z calc. for $C_{38}H_{41}N_2O_{13}$: 677.2932; found: 677.2935 $[M+3H]^{3+}$.

11: HRMS: m/z calc. for $C_{38}H_{41}N_2O_{13}$: 609.6001; found: 609.5994 $[M+3H]^{3+}$.

LC-MS/MS analyses

Nanoflow LC coupled to electrospray ionization (ESI) LQIT/FTICR-MS/MS was performed with an Ettan MDLC (GE Healthcare, Uppsala, Sweden) and an LTQ FT Classic/Ultra (Thermo Fisher Scientific, Bremen, Germany). A nanoscale C4 column (150x0.075 mm, G&T SepTech) and a linear gradient of 0-60% mobile phase B at 400 nL/min for 50 min was used. Mobile phase A was HPLC grade water with 0.1% formic acid and mobile phase B was 84% HPLC grade aqueous ACN with 0.1% formic acid. The CID normalized collision energy was set to 30 and the ECD energy was 1.9 eV with 70 ms irradiation time. For more detailed description see Ref. [6].

Cu-Aβ(1-15) Binding

Stock solutions of $Cu(NO_3)_2$ (25 μM), BC (6.0 mM), ascorbate (30.0 mM), NaOH (2.5 M), glycopeptide **9** (2.40 mM) and peptide **11** (2.40 mM) were prepared and stored at 4℃. Baseline absorbance was obtained with 1 mL H_2O, 10 μL BC and 80 μL ascorbate in both reference and sample cuvettes. 1 mL $Cu(NO_3)_2$ was added into the sample cuvette and argon was bubbled into the sample for 10 min. 10 μL BC, 40 μL ascorbate and 4μL NaOH were then added and the solution was checked every 10 min until the absorbance at 483 nm reached a maximum. An additional 40 μL ascorbate was added prior to titration to avoid oxidation of Cu^+. Solution **9, 10** or **11** (20 μL) was added every time. The solution was bubbled with argon and Abs_{483} was monitored until minimum.

According to the equation:

$$Cu^{+} + 2BC^{2-} \rightleftharpoons CuBC_2^{3-}$$

The total concentration of $CuBC^{3-}$ is determined by Abs_{483}. The concentration of the other species involved in the equilibrium is as follows:

$$[Cu^{+}]_{free} = \frac{[CuBC_2{}^{3-}]}{([BC]_{total} - 2[CuBC_2{}^{3-}])^2 \beta}$$

β is the formation constant of $CuBC_2^{3-}$. Upon the addition of Aβ (glycopeptide **9** or **11**), a new equilibrium is established:

$$CuBC_2^{3-} + A\beta \rightleftharpoons Cu^{+}A\beta + 2BC^{2-}$$

The new concentration of $CuBC_2^{3-}$ is also calculated by Abs_{483}. To achieve mass balance, $[Cu^{+}A\beta] = [Cu]_{total} - [CuBC_2{}^{3-}] - [Cu^{+}]_{free}$

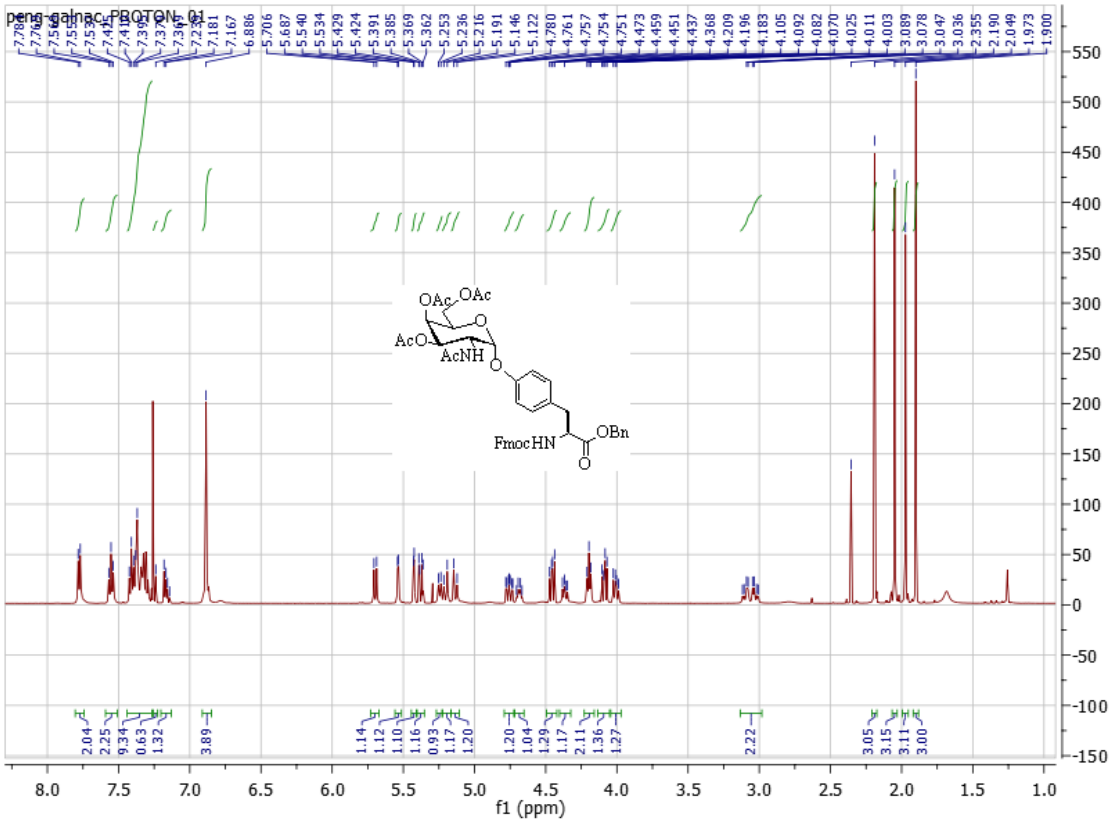
$$[A\beta]_{free} = [A\beta]_{total} - [Cu^{+}A\beta]$$

Finally, the dissociation constant of $Cu^{+}A\beta$ complex can be calculated from:

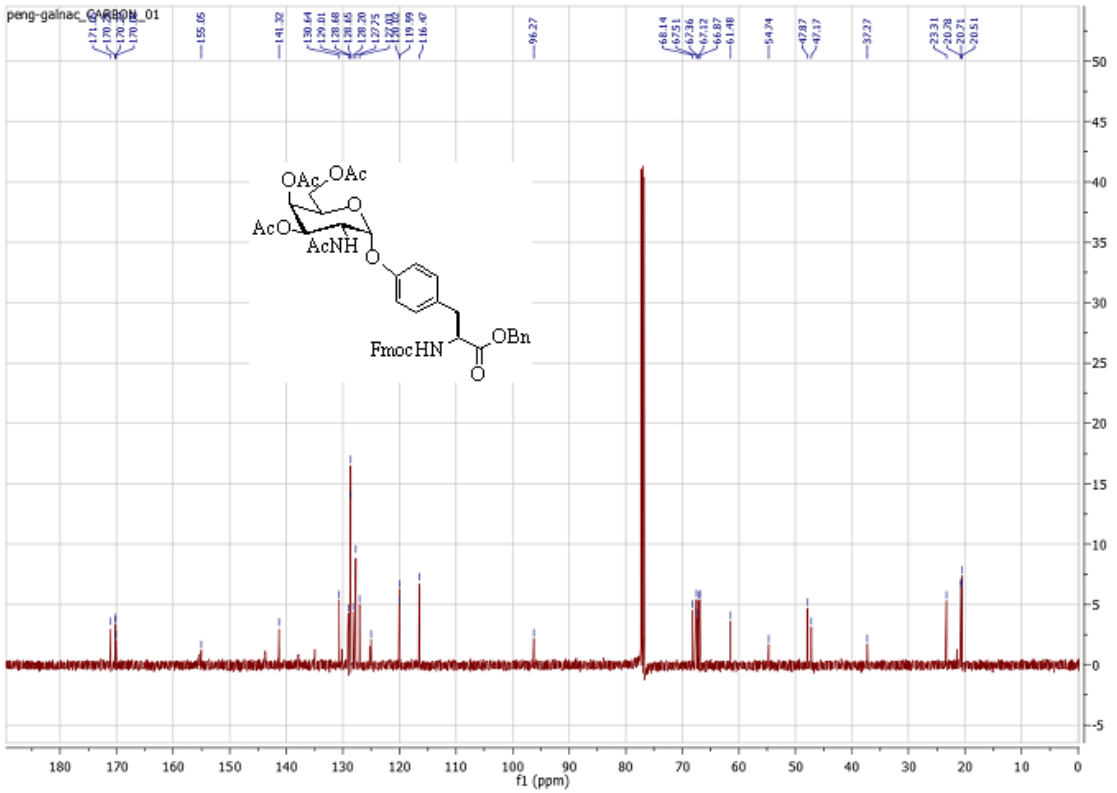
$$K_d = \frac{[Cu^{+}]_{free}[A\beta]_{free}}{[Cu^{+}A\beta]}$$

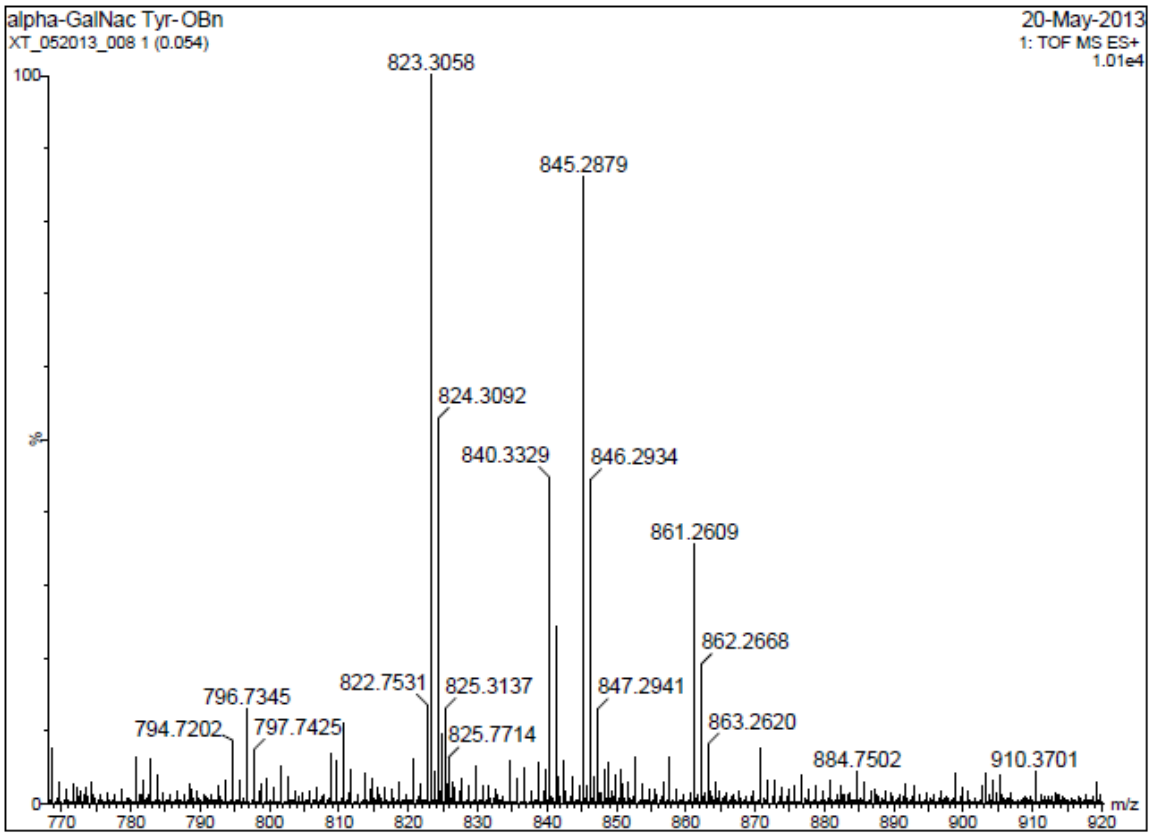
Product Characterization Spectra

Compound **4** ^{1}HNMR (500 MHz, $CDCl_3$)

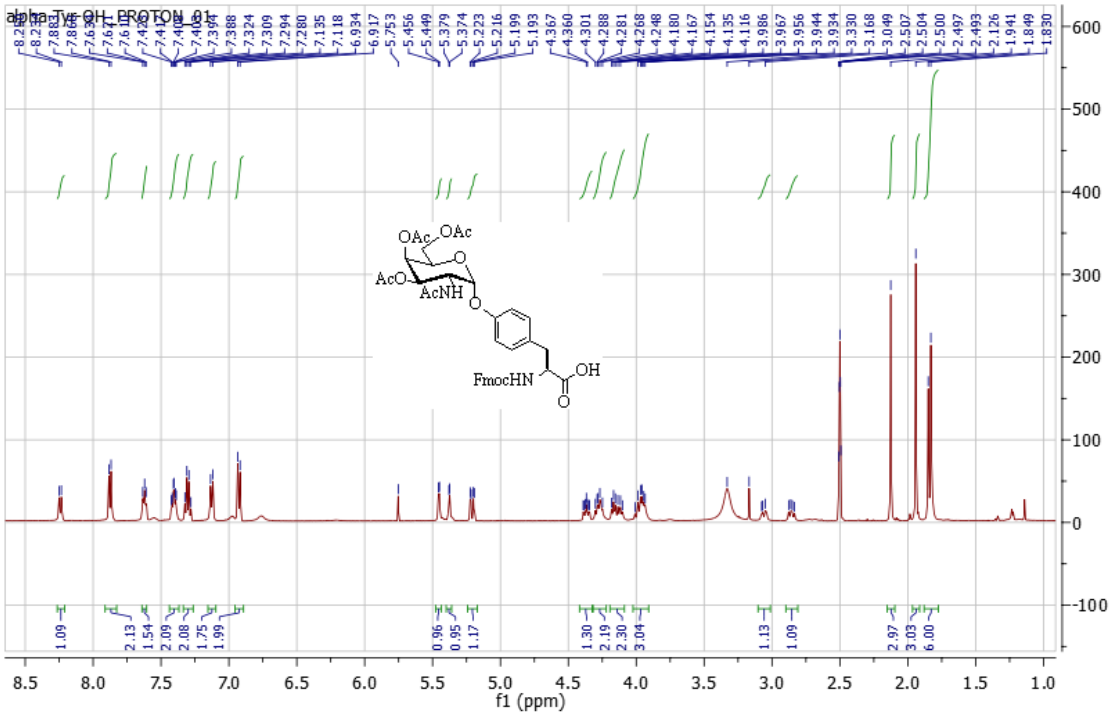


Compound **4** ^{13}CNMR (125 MHz, $CDCl_3$)

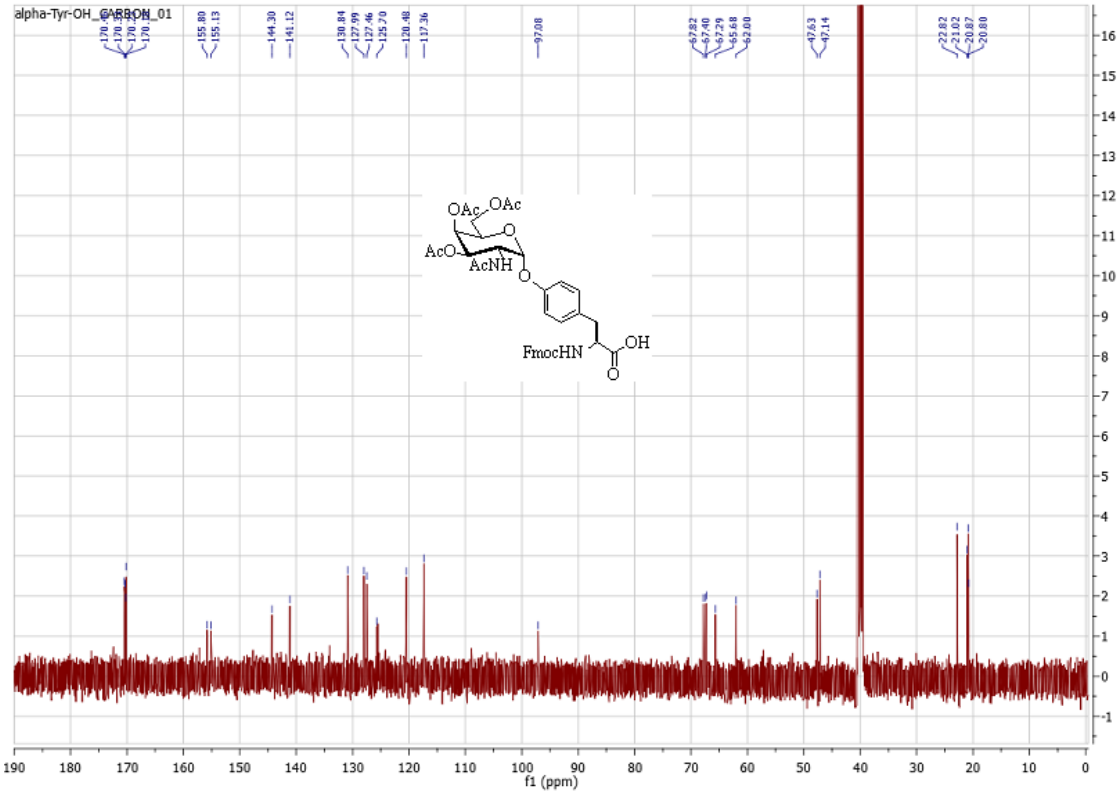


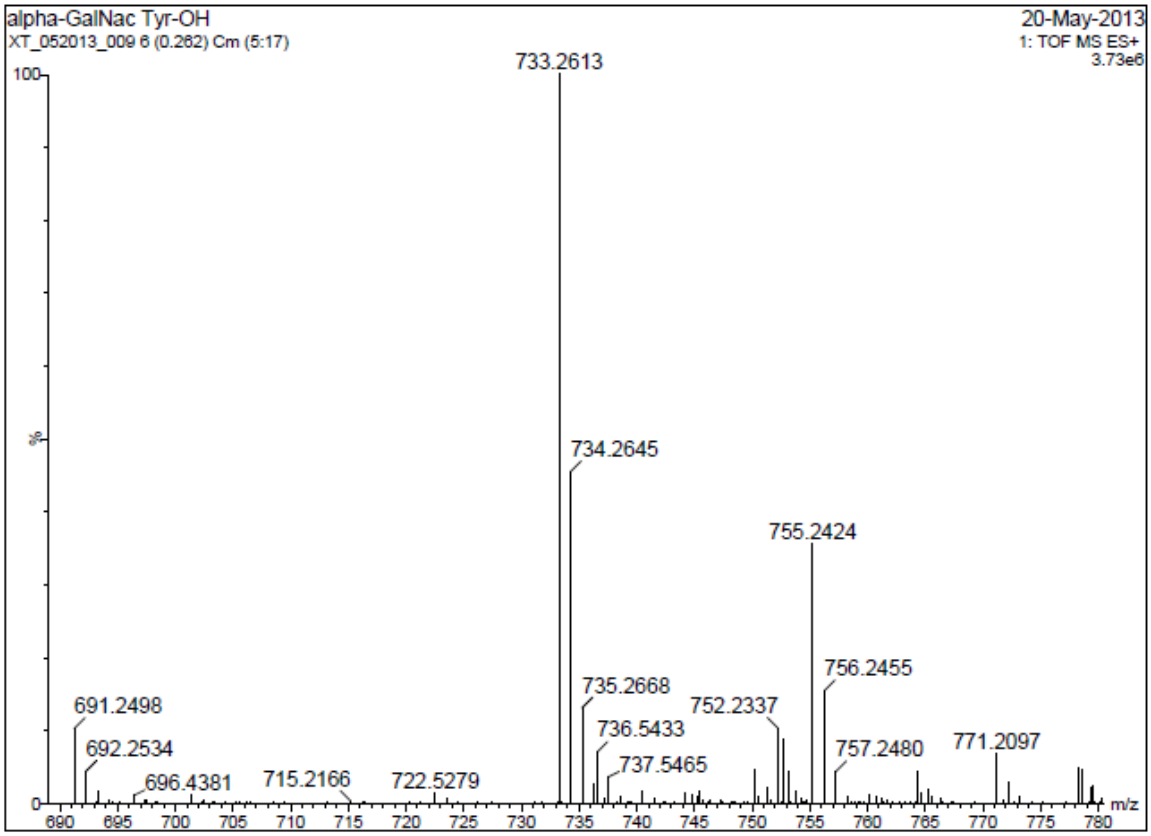


Compound **5** ^{1}HNMR (500 MHz, d^{6}-DMSO)

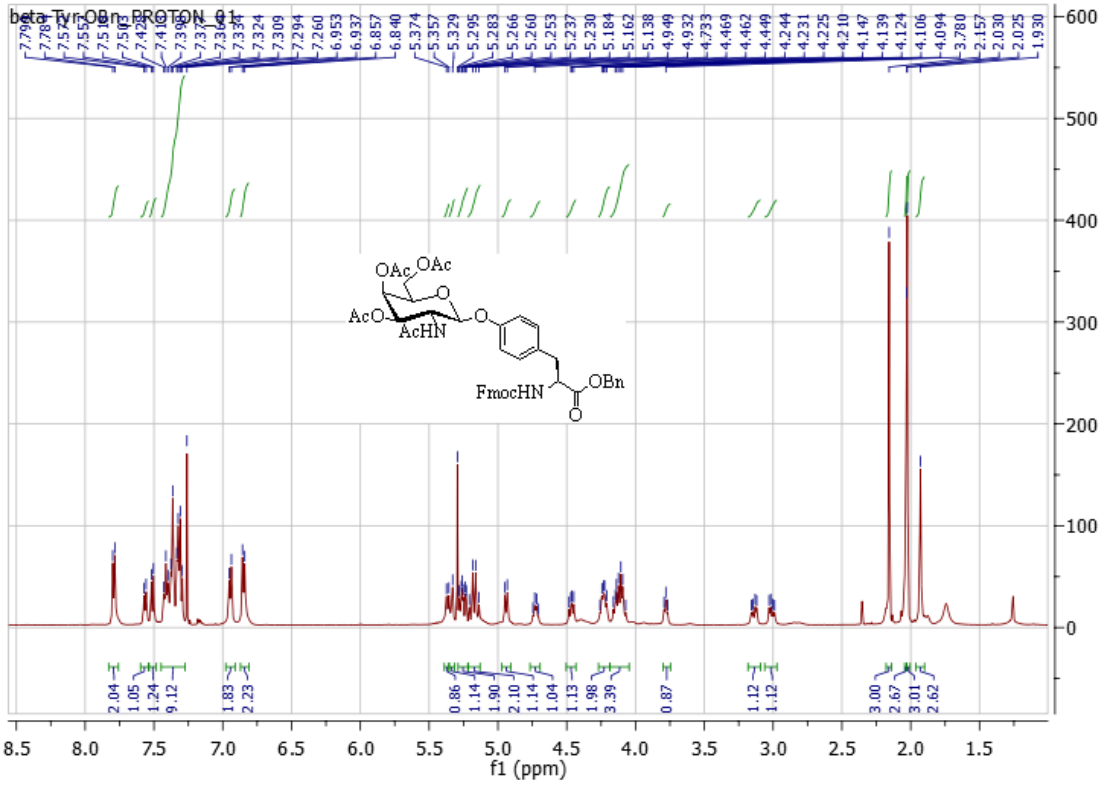


Compound **5** ^{13}CNMR (125 MHz, d^{6}-DMSO)

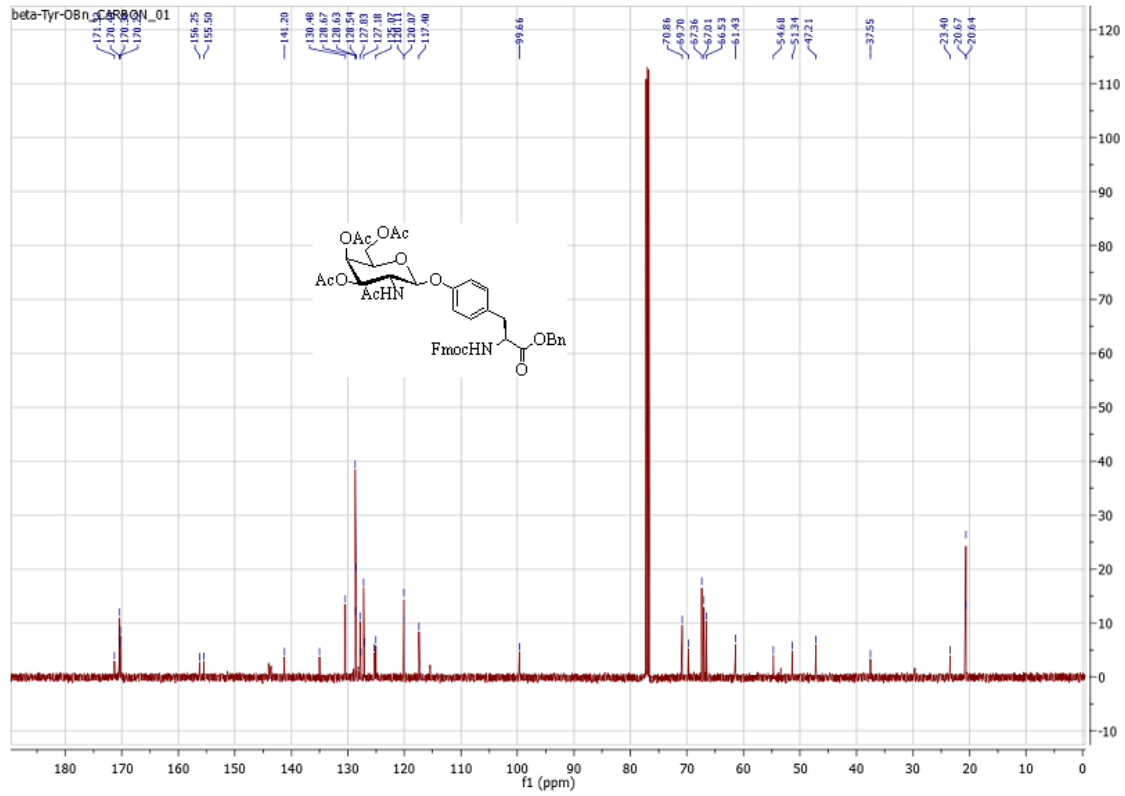


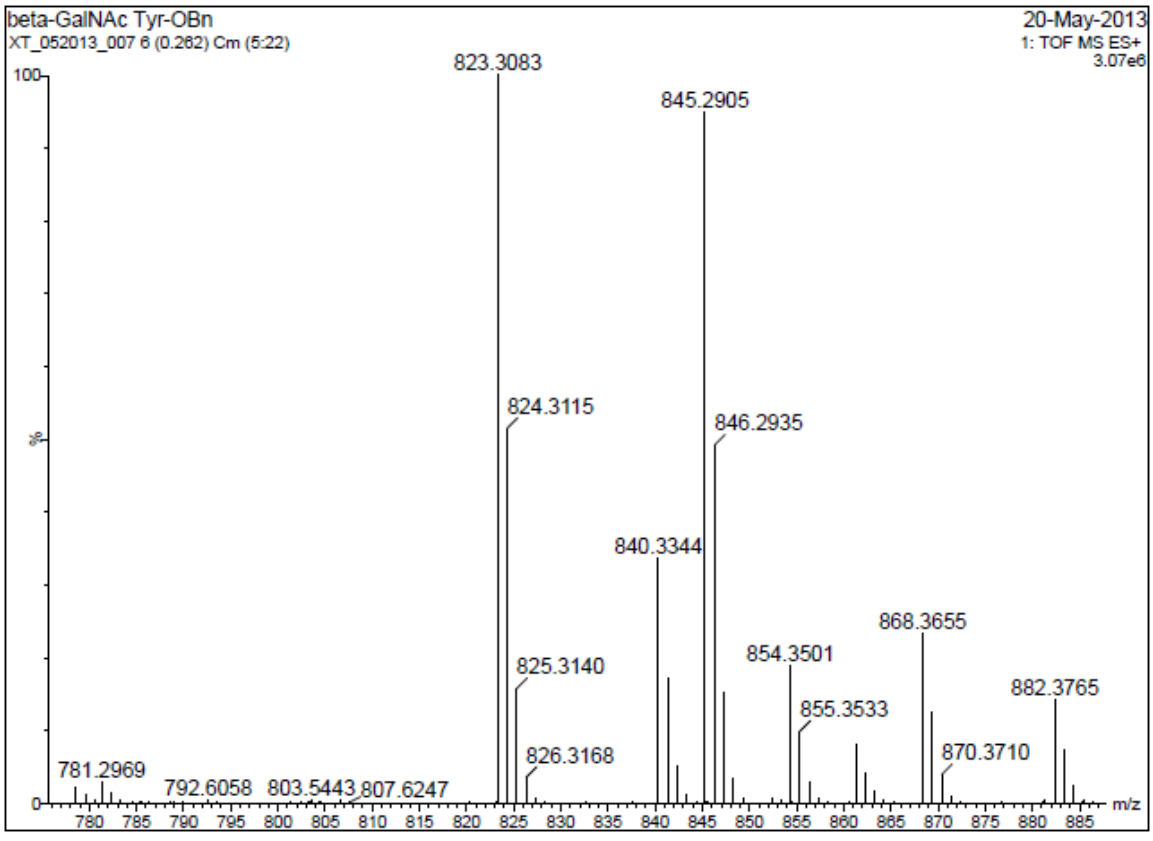


Compound **7** ^{1}HNMR (500 MHz, $CDCl_3$)

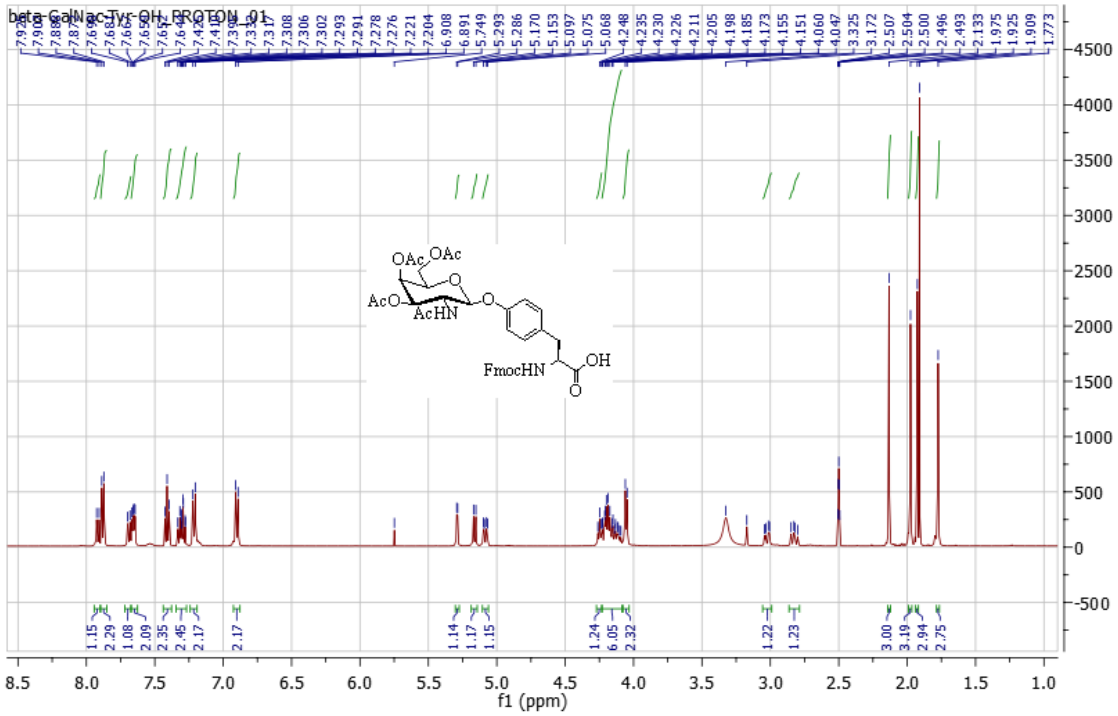


Compound **7** ^{13}CNMR (125 MHz, $CDCl_3$)

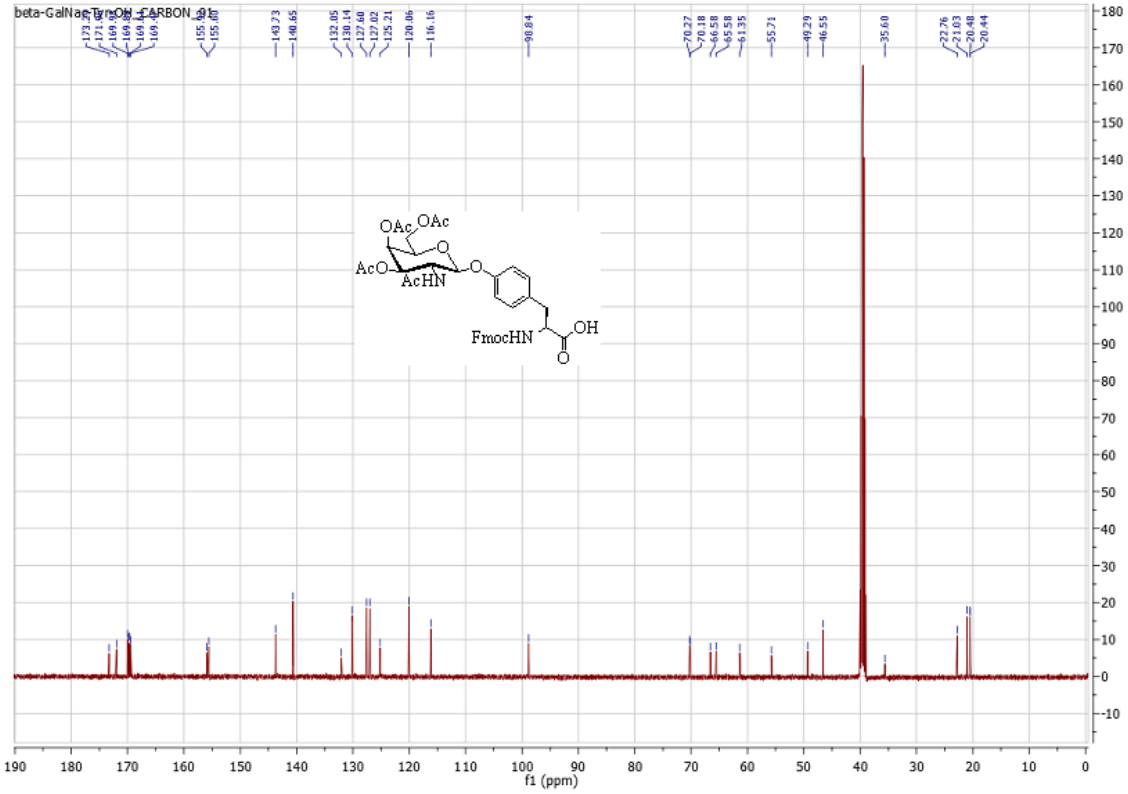


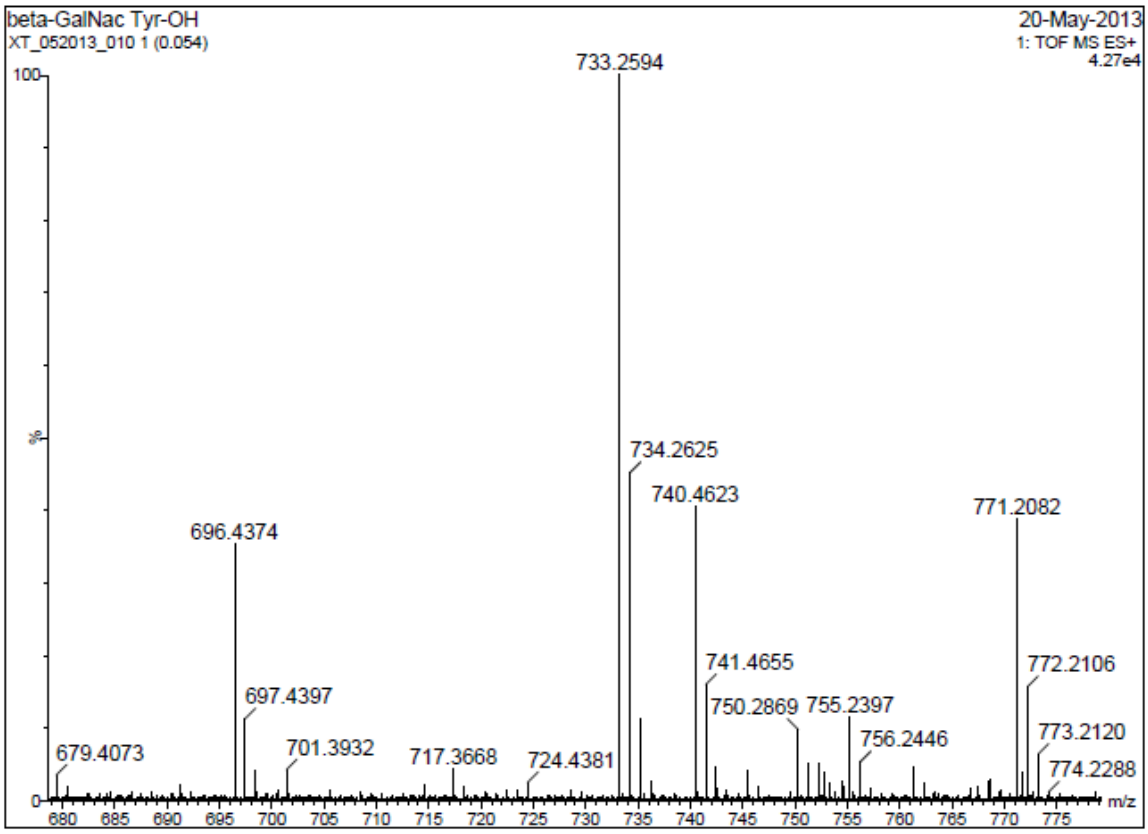


Compound **8** ^{1}HNMR (500 MHz, d^{6}-DMSO)

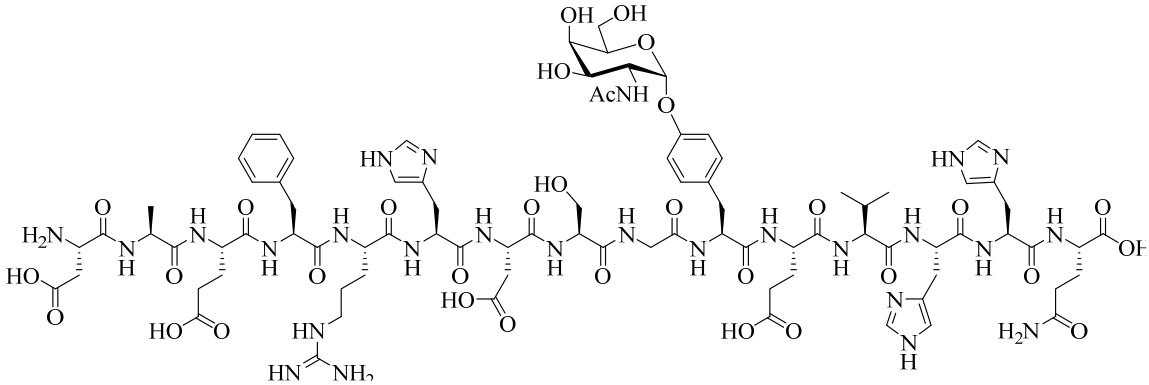


Compound **8** ^{13}CNMR (125 MHz, d^{6}-DMSO)

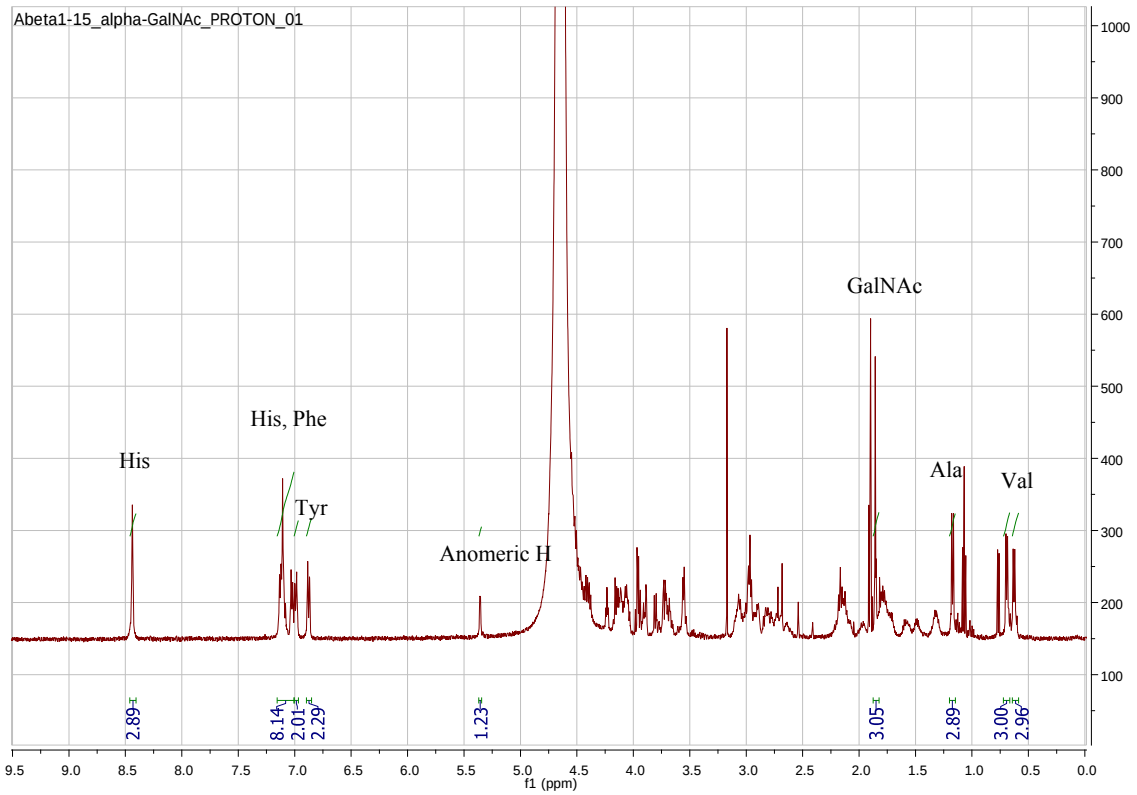



beta-GalNac Tyr-OH
XT_052013_010 1 (0.054)
20-May-2013
1: TOF MS ES+
4.27e4
100
%
0
733.2594
734.2625
740.4623
771.2082
696.4374
741.4655
772.2106
697.4397
750.2869
755.2397
773.2120
679.4073
701.3932
717.3668
724.4381
756.2446
774.2288
m/z
680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775

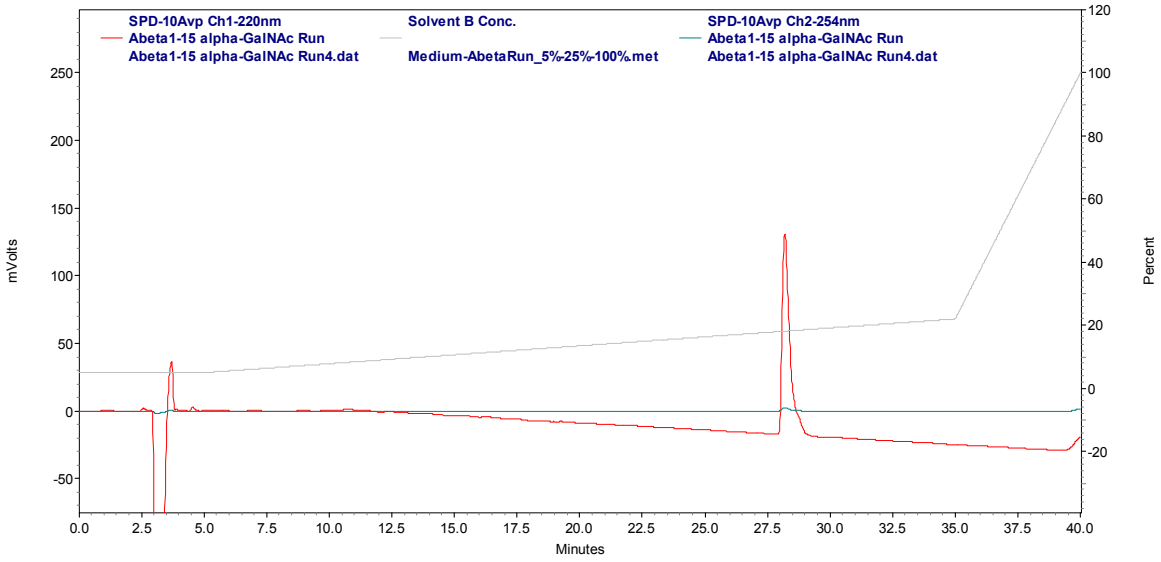
Glycopeptide **9**



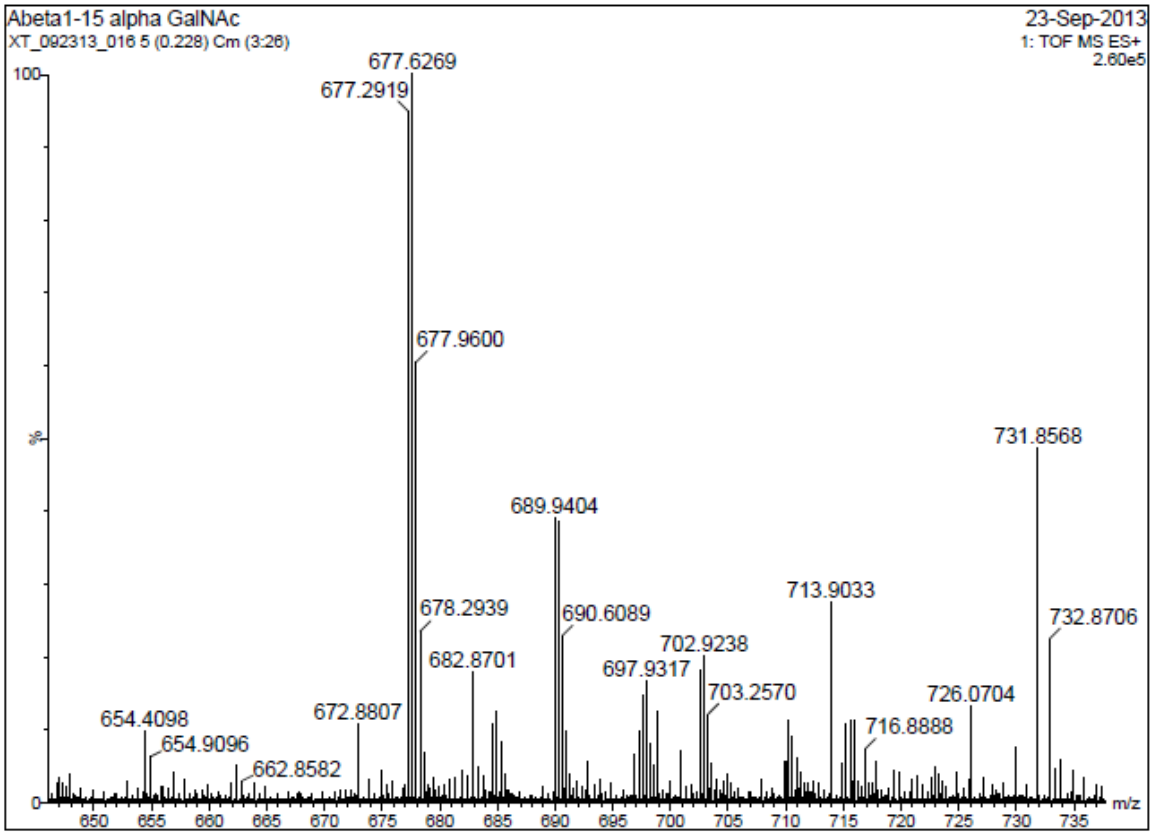
^{1}HNMR (500 MHz, D_2O)



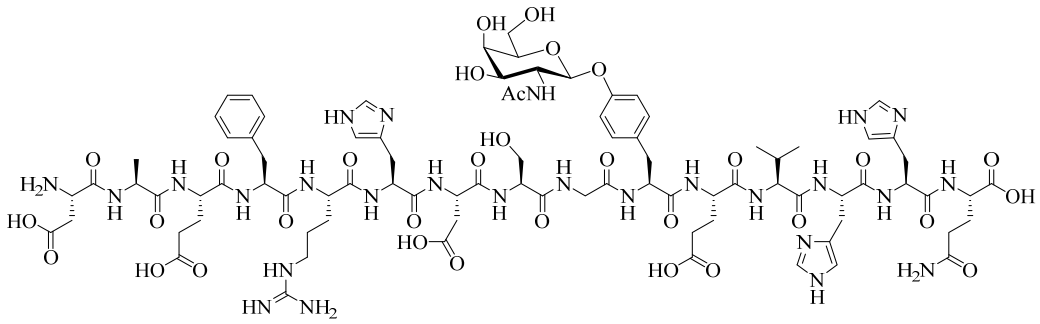
HPLC chromatogram of purified glycopeptide **9**



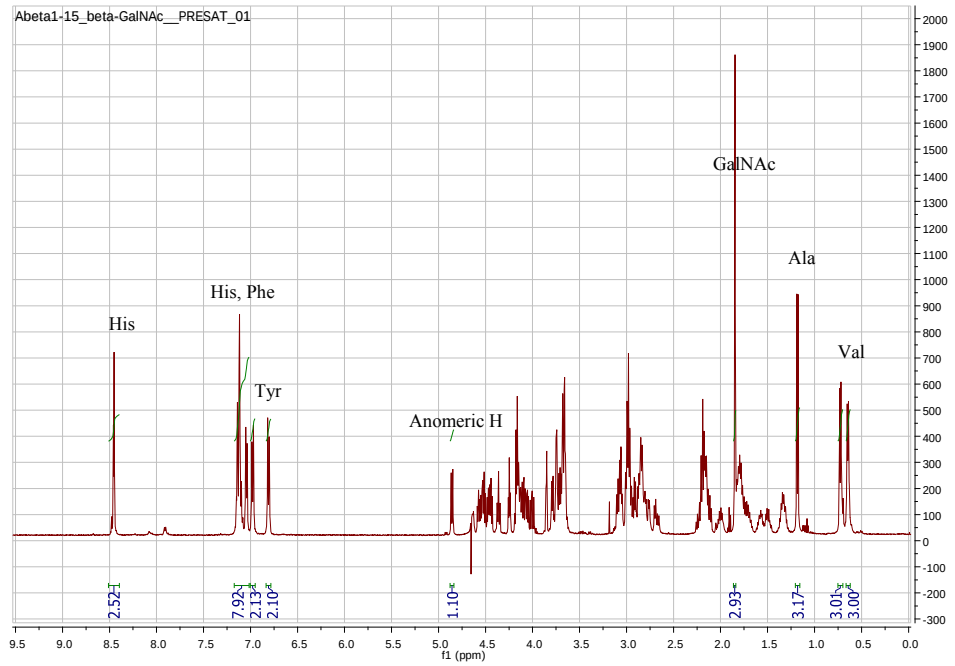
ESI-MS of glycopeptide **9**



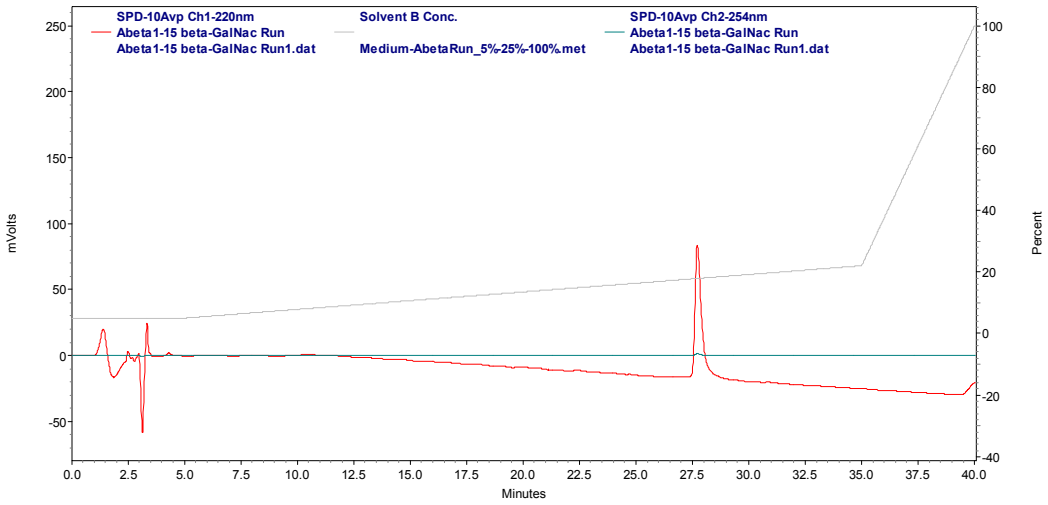
Glycopeptide **10**



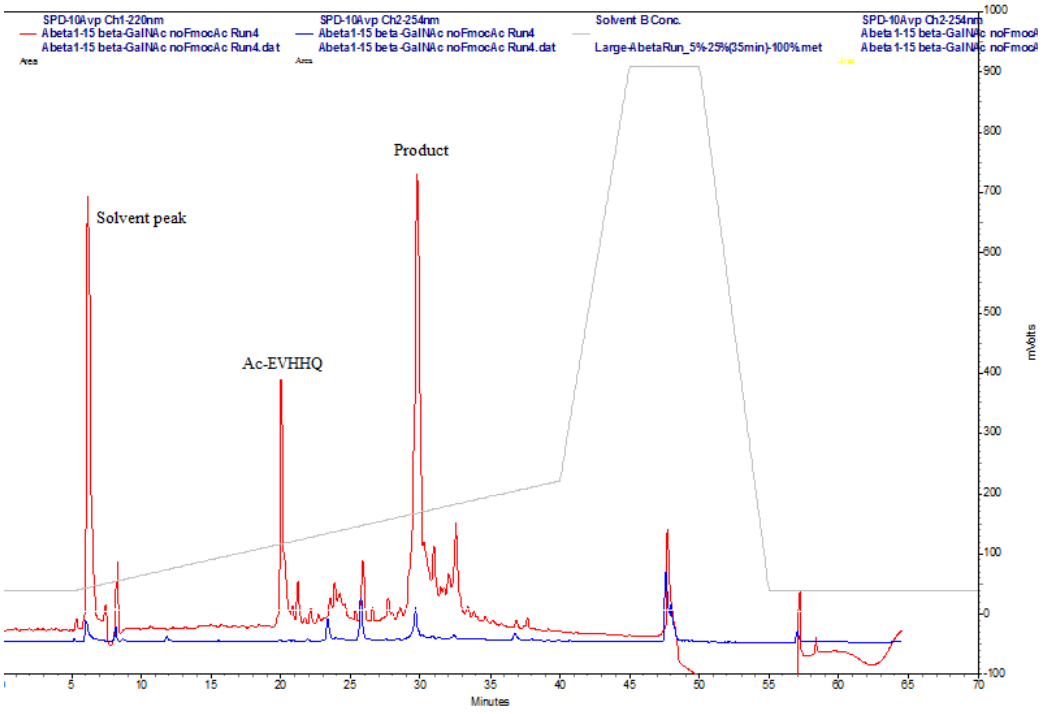
^{1}HNMR (500 MHz, D_2O)



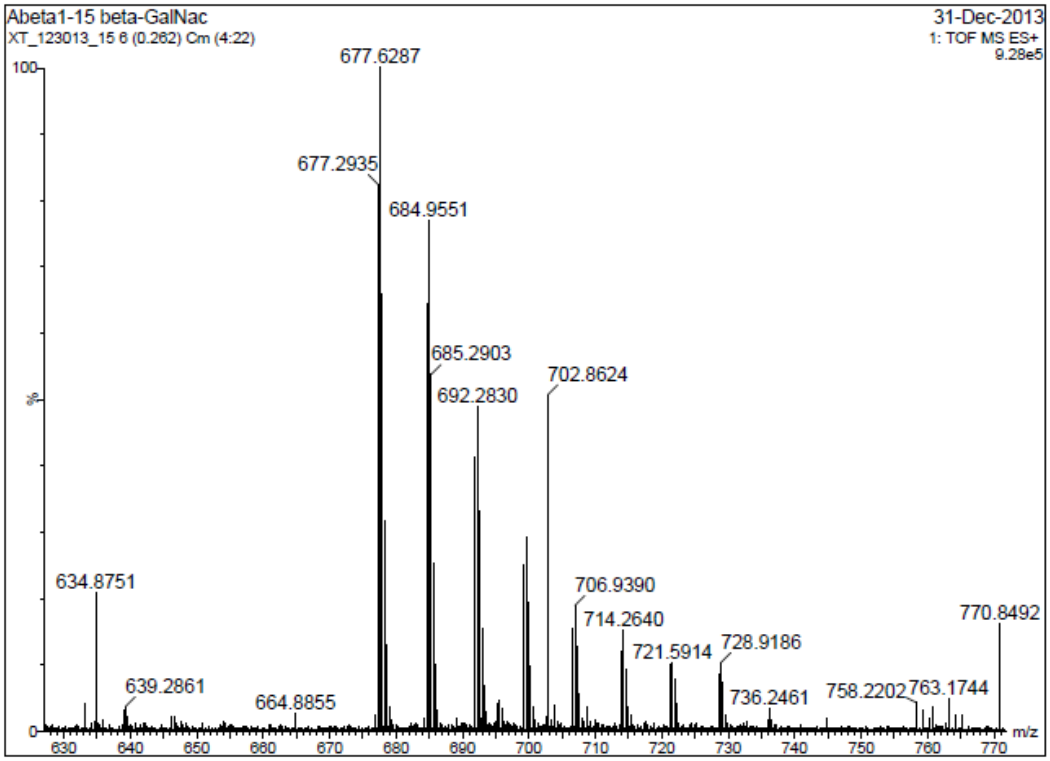
HPLC chromatogram of purified glycopeptide **10**



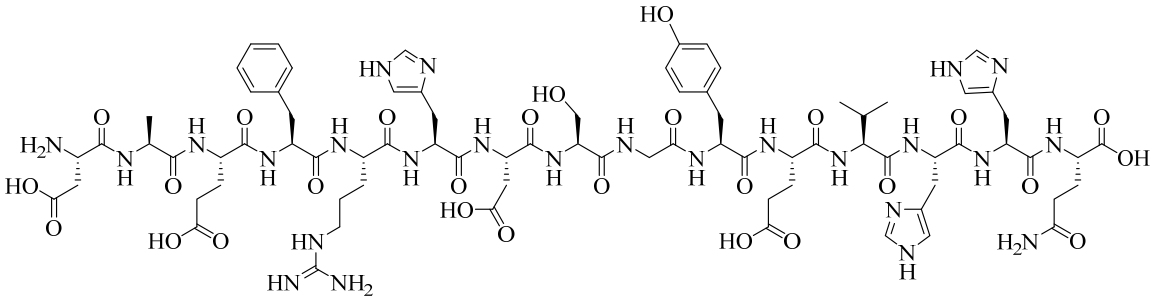
HPLC chromatogram of crude glycopeptide **10**



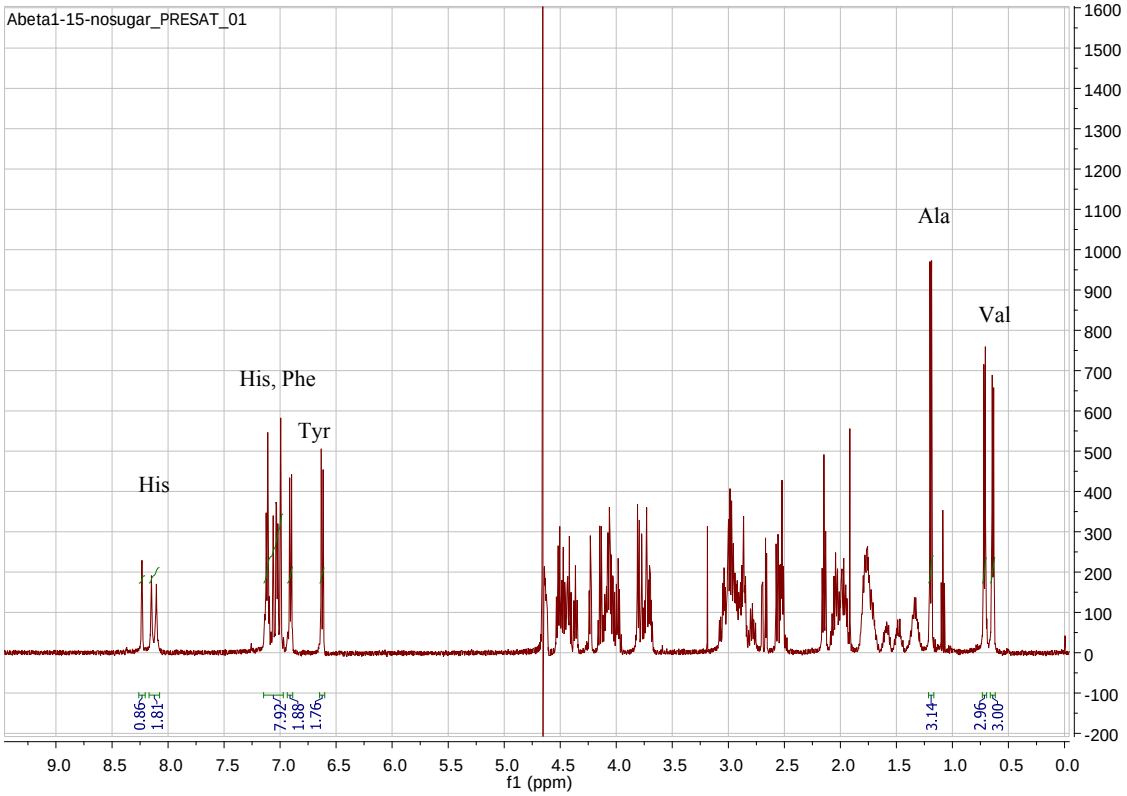
ESI-MS of glycopeptide **10**



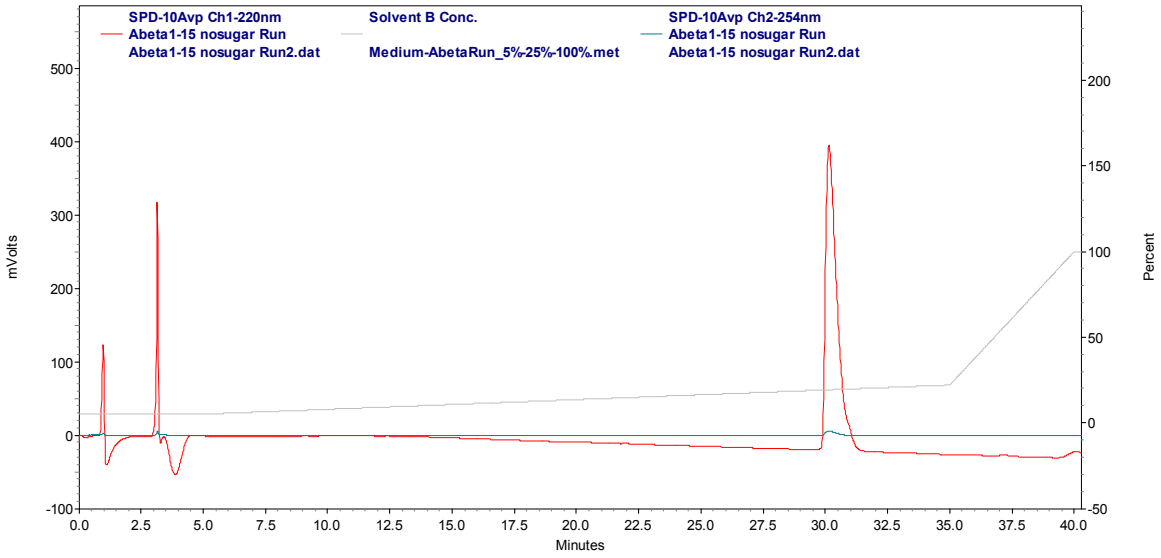
Peptide **11**



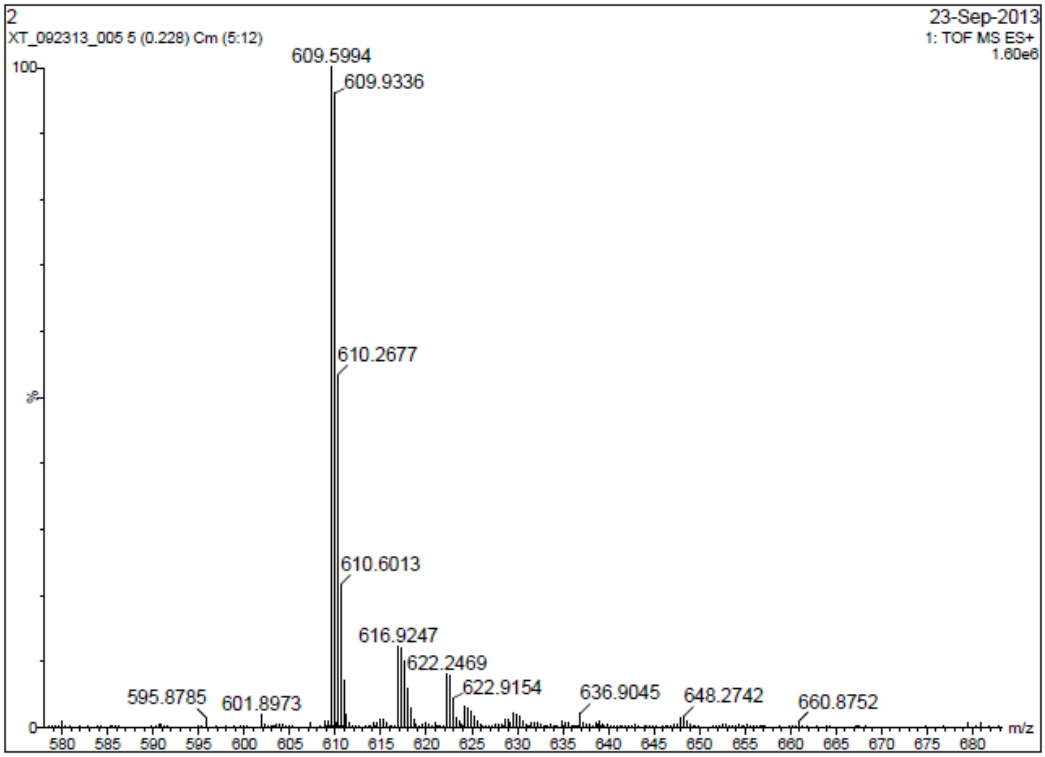
^{1}HNMR (500 MHz, D_2O)



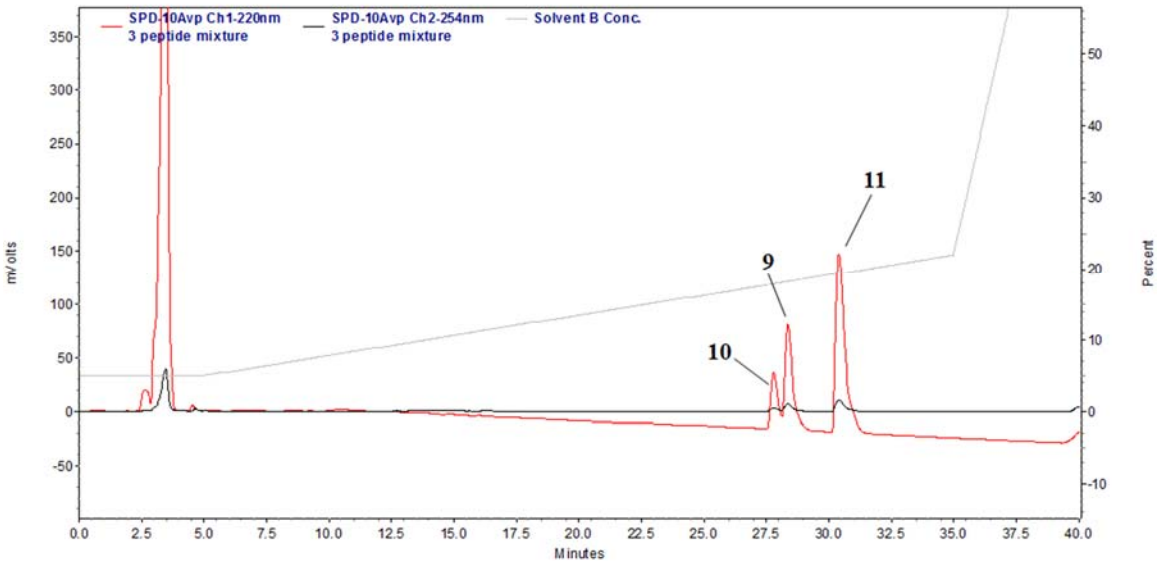
HPLC chromatogram of purified peptide **11**



ESI-MS of peptide **11**



HPLC of Peptide Mixture



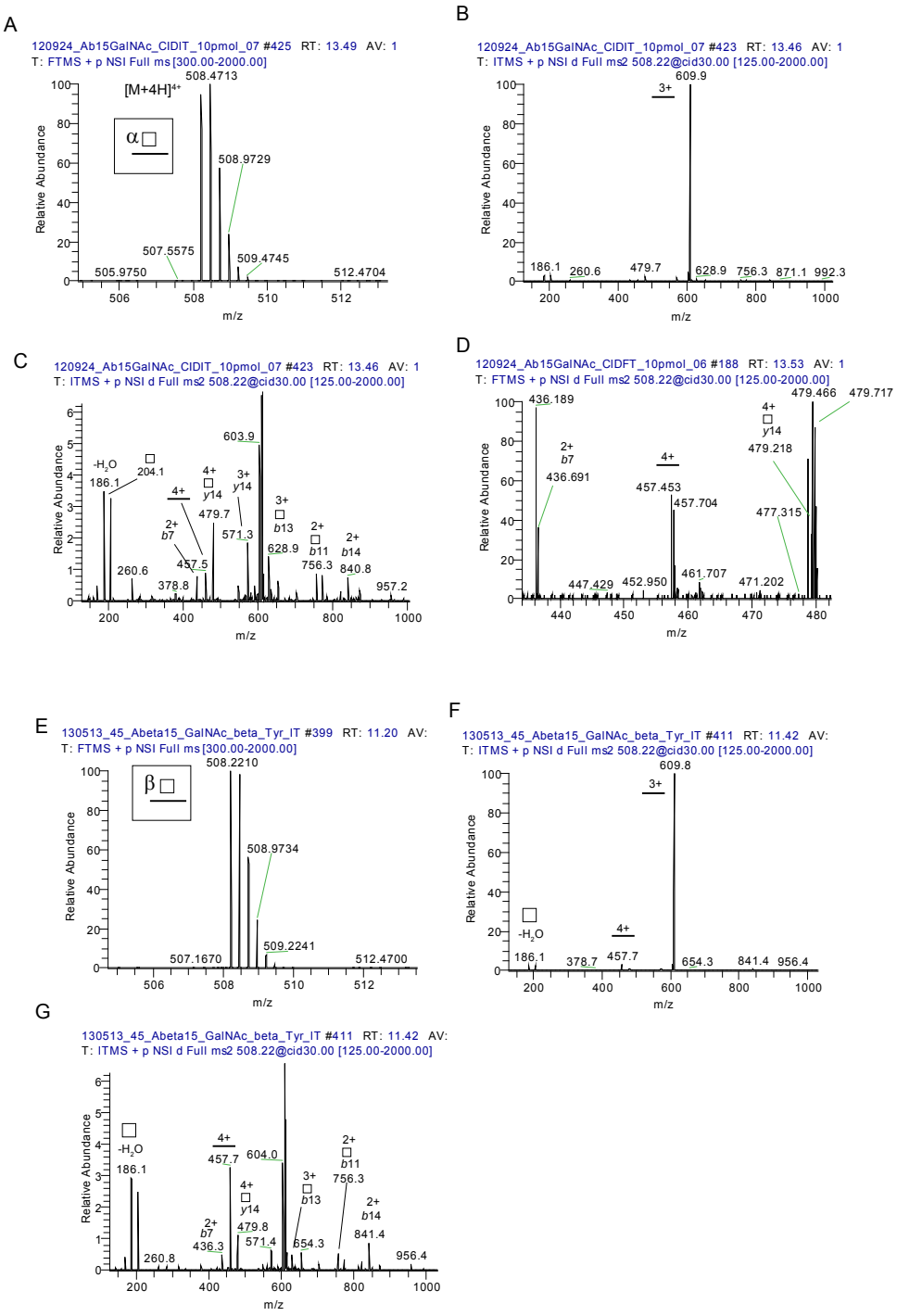


Fig. S1. CID-MS2 spectra of glycopeptides **9** and **10** (A) Full MS of the $[M+4H]^{4+}$ ion of **9**; (B) the resulting CID-MS2 spectrum; and (C) an intensity expansion of the CID-MS2 spectrum. (D) A *m/z* expansion of the CID-MS2 spectrum of **9** collected in the ion cyclotron resonance (ICR) detector demonstrating the exact masses and charge state of the peptide backbone fragments. (E) Full MS of the $[M+4H]^{4+}$ ion of **10**; (F) the resulting CID-MS2 spectrum; and (G) an intensity expansion of the CID-MS2 spectrum.

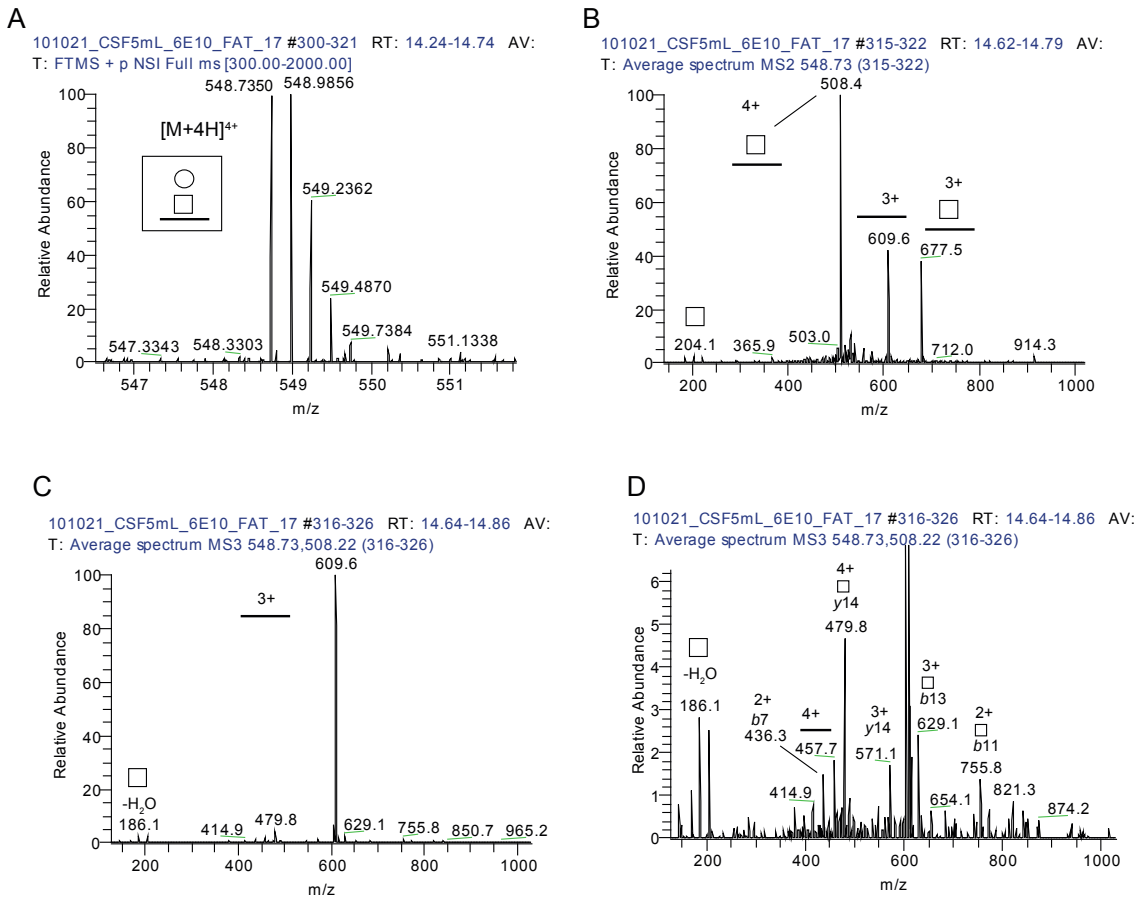
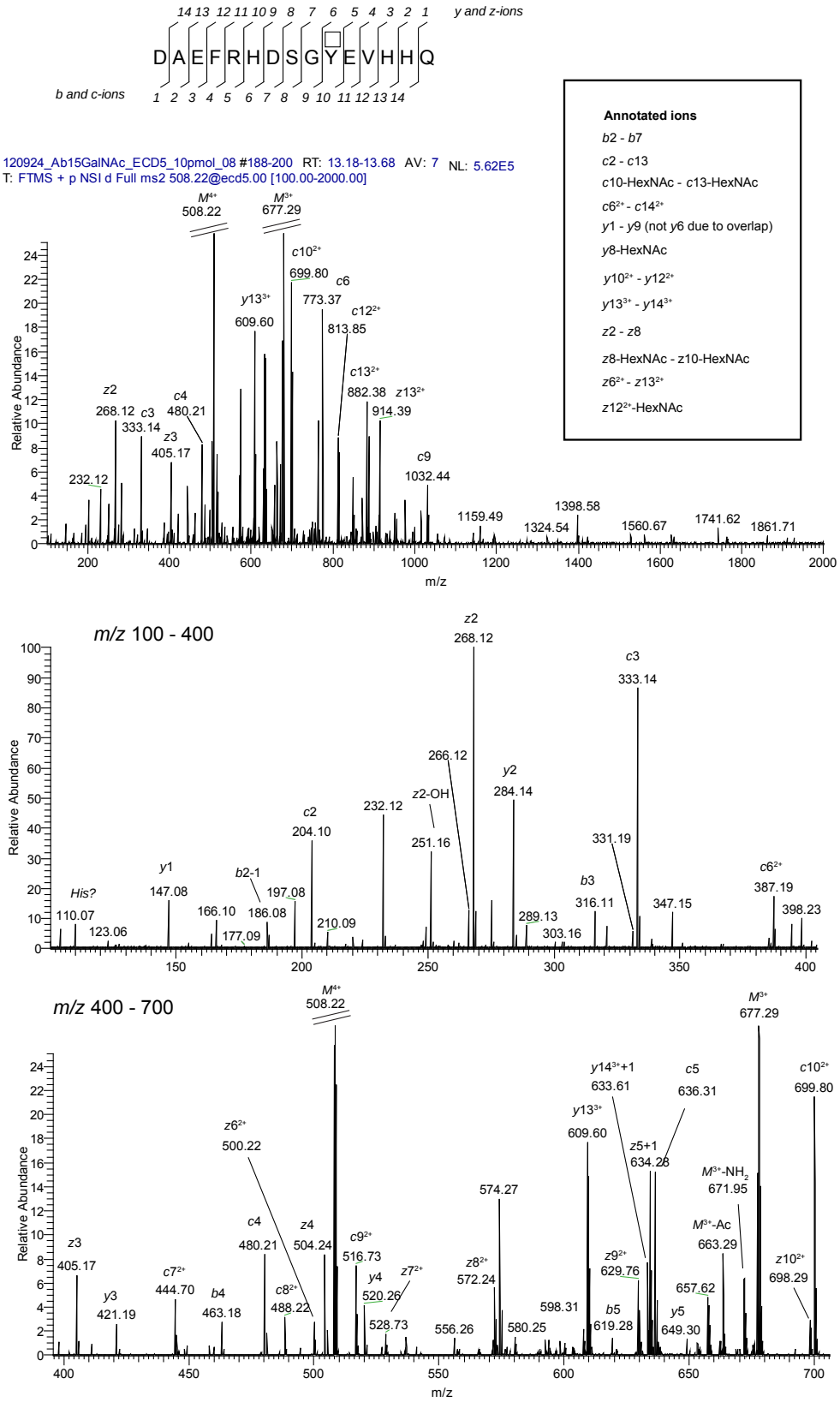


Fig. S2. CID-MS2 and MS3 of native HexHexNAc substituted Aβ(1-15) peptide isolated from human cerebrospinal fluid. (A) Full MS of the $[M+4H]^{4+}$ ion and (B) the resulting CID-MS2 spectrum. (C) CID-MS3 spectrum of the Aβ(1-15)+HexNAc fragment (at *m/z* 508.4); and (D) an intensity expansion of the same spectrum.


14 13 12 11 10 9 8 7 6 5 4 3 2 1
y and z-ions
D A E F R H D S G Y E V H H Q
b and c-ions
1 2 3 4 5 6 7 8 9 10 11 12 13 14
Annotated ions
b2 - b7
c2 - c13
c10-HexNAc - c13-HexNAc
c6²⁺ - c14²⁺
y1 - y9 (not y6 due to overlap)
y8-HexNAc
y10²⁺ - y12²⁺
y13³⁺ - y14³⁺
z2 - z8
z8-HexNAc - z10-HexNAc
z6²⁺ - z13²⁺
z12²⁺-HexNAc
120924_Ab15GalNAc_ECD5_10pmol_08 #188-200 RT: 13.18-13.68 AV: 7 NL: 5.62E5
T: FTMS + p NSI d Full ms2 508.22@ecd5.00 [100.00-2000.00]
M⁴⁺ 508.22
M³⁺ 677.29
c10²⁺ 699.80
c6
773.37
y13³⁺ 609.60
c12²⁺ 813.85
c13²⁺ 882.38
z13²⁺ 914.39
z2 268.12
c3 333.14
c4 480.21
z3 405.17
232.12
c9 1032.44
1159.49
1324.54
1398.58
1560.67
1741.62
1861.71
Relative Abundance
m/z
m/z 100 - 400
z2 268.12
c3 333.14
266.12
y2 284.14
z2-OH 232.12
c2 204.10
251.16
331.19
y1 147.08
b2-1 197.08
b3 316.11
c6²⁺ 387.19
347.15
398.23
His? 110.07
123.06
166.10
186.08
177.09
210.09
289.13
303.16
m/z 400 - 700
M⁴⁺ 508.22
M³⁺ 677.29
y14³⁺+1 633.61
c5 636.31
c10²⁺ 699.80
y13³⁺ 609.60
z6²⁺ 500.22
z5+1 634.28
M³⁺-NH₂ 671.95
574.27
M³⁺-Ac 663.29
c4 480.21
z4 504.24
c9²⁺ 516.73
z3 405.17
z9²⁺ 629.76
z10²⁺ 698.29
z8²⁺ 572.24
c7²⁺ 444.70
y4 520.26
z7²⁺
657.62
y3 421.19
b4 463.18
c8²⁺ 488.22
528.73
598.31
b5 619.28
y5 649.30
556.26
580.25

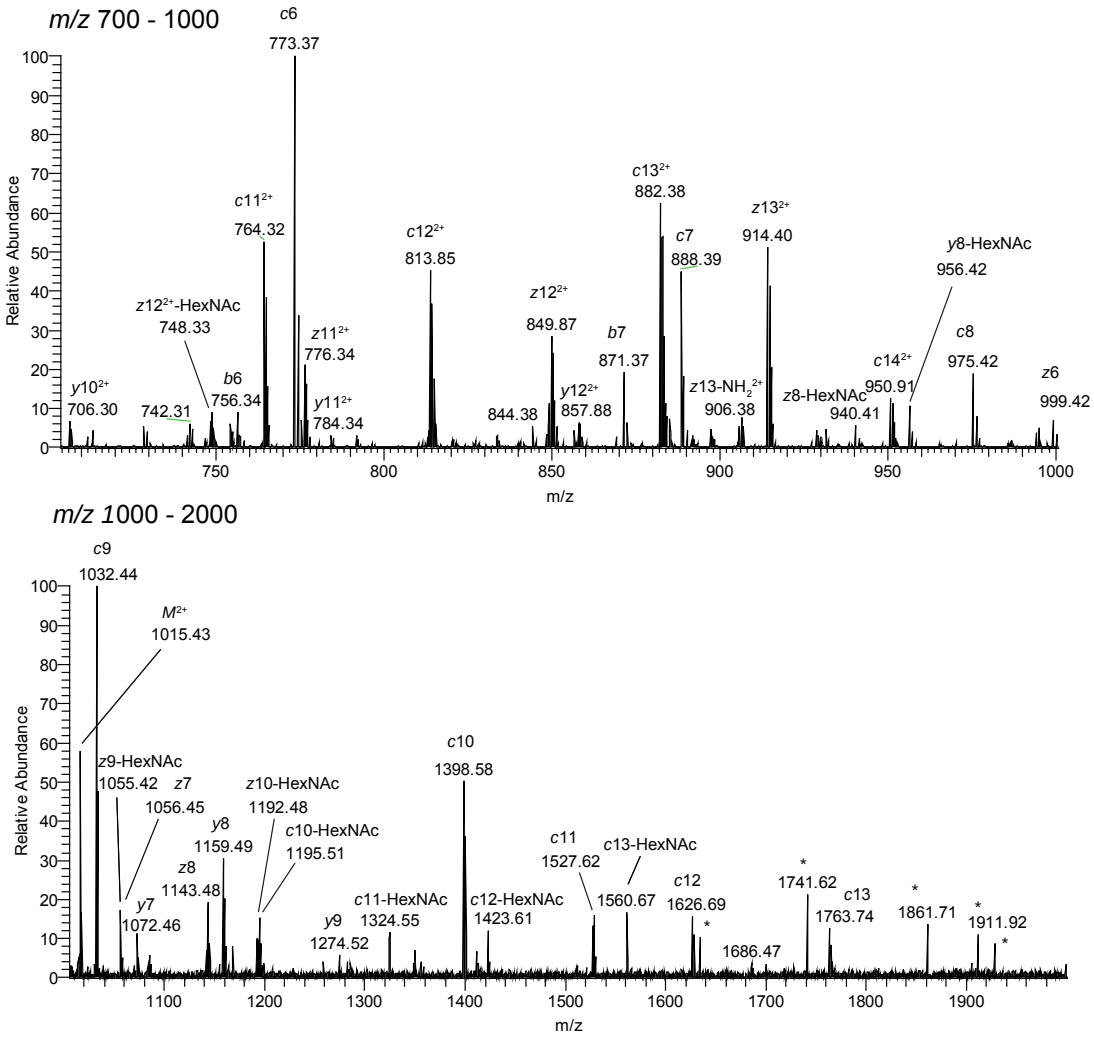


Fig. S3. ECD spectrum of glycopeptide **9**. All the fragment ions identified are summarized in the box. * are background peaks originating from external frequencies picked up by the detector.

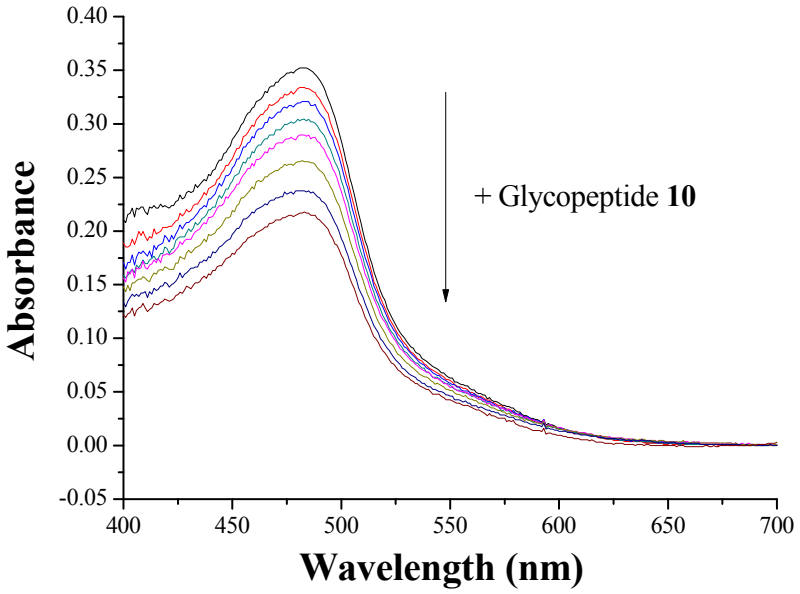


Fig. S4. Competitive chelation of BC and glycopeptide **10** to Cu^{+}. Addition of glycopeptide **10** competed with the formation of $[CuBC_2]^{3-}$ complex resulting in reduction of the absorbance at 483 nm.

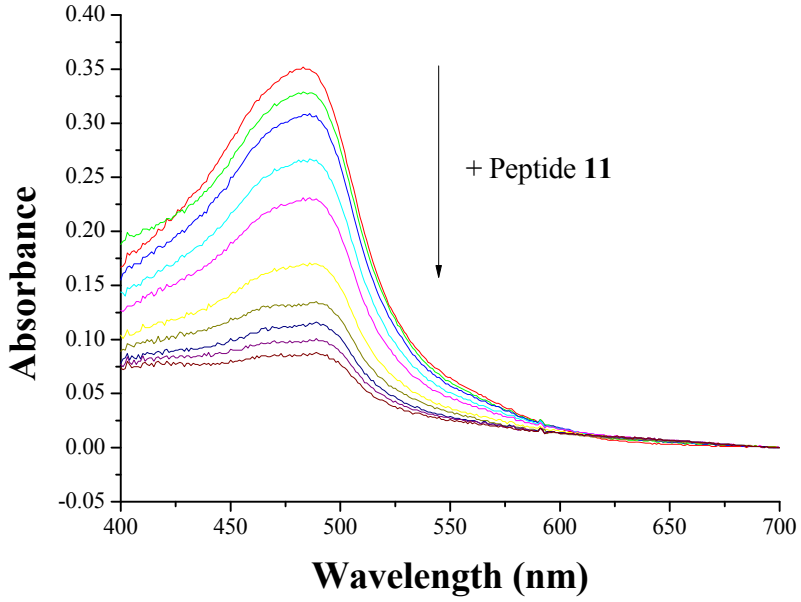


Fig. S5. Competitive chelation of BC and peptide **11** to Cu^{+}. Addition of peptide **11** competed with the formation of $[CuBC_2]^{3-}$ complex resulting in reduction of the absorbance at 483 nm.

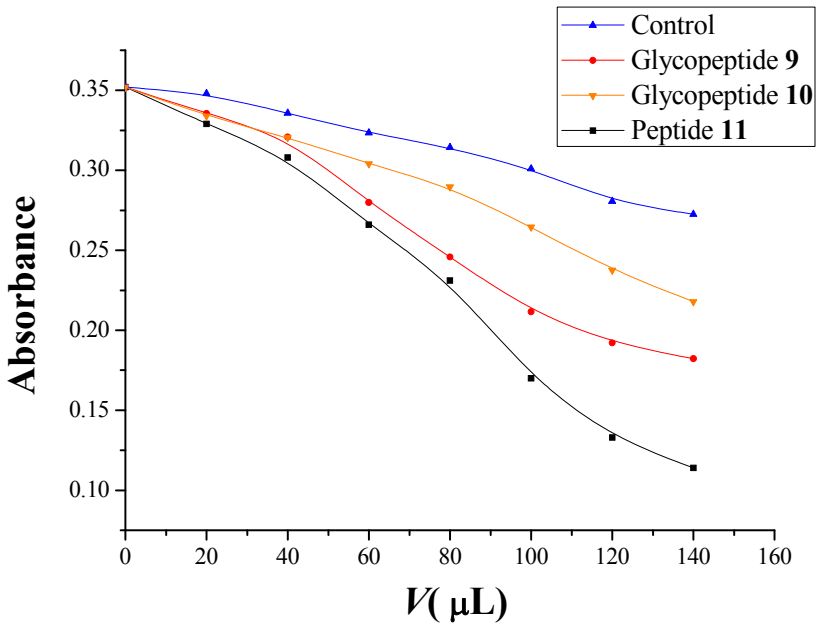


Fig. S6. Absorbance changes at 483 nm of solutions of BC and Cu^{+} upon addition of increasing amounts of glycopeptide **9**, **10** or **11** respectively based on data from Fig. 2, S4-S5. Water was added instead of peptide solutions in the control. From the absorbance changes, the dissociation constants of [Cu^{+} - **9**], [Cu^{+} - **10**] and [Cu^{+} -**11**] complexes were calculated to be $1.69 \pm 0.84 \times 10^{-14}$ M, $5.25 \pm 2.87 \times 10^{-14}$ M, and $2.72 \pm 1.26 \times 10^{-15}$ M respectively.

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[5] Z. Wang, L. Zhou, K. El-Boubbou, X.-s. Ye, X. Huang, J. Org. Chem. 2007, 72, 6409-6420.

[6] G. Brinkmalm, E. Portelius, A. Öhrfelt, N. Mattsson, R. Persson, M. K. Gustavsson, C. H. Vite, J. Gobom, J.-E. Månsson, J. Nilsson, A. Halim, G. Larson,

U. Rüetschi, H. Zetterberg, K. Blennow and A. Brinkmalm, J. Mass Spectrometry, 47 (2012) 591-603