Glycopeptide Biomarkers in Serum Haptoglobin for Hepatocellular Carcinoma Detection in Patients with Non-Alcoholic Steatohepatitis

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Supporting Information

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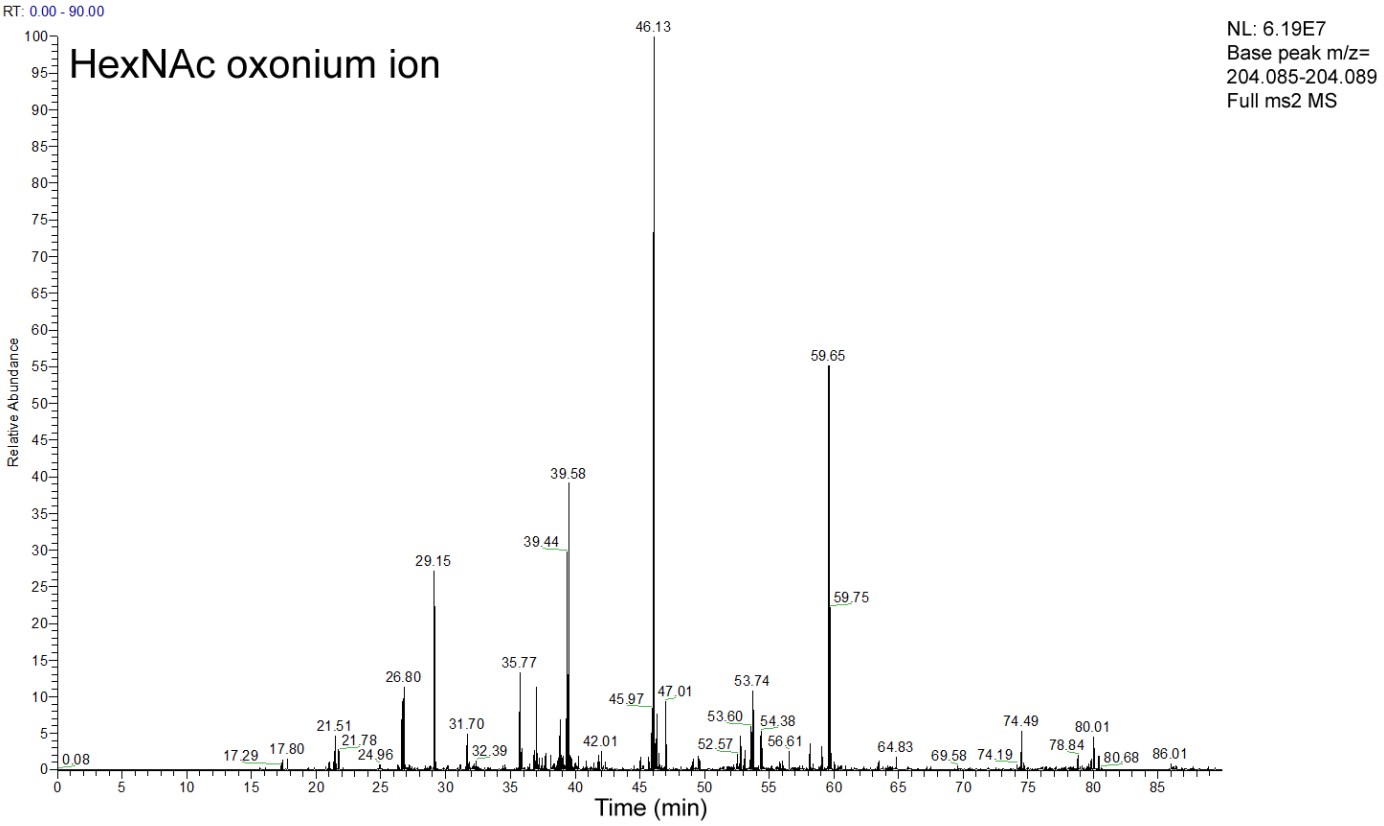

Figure S1. Representative extracted ion chromatograms (XICs) of MS/MS spectra of the $HexNAc^+$ ion within the m/z range 204.085-204.089. The major peaks correspond to the $HexNAc^+$ ion derived from glycopeptides of MVSHHN^{184}LTTGATLINE (~29 and ~39 min), NLFLN^{207}HSE (~36 min), VVLHPN^{241}YSQVD (~46 min) and VVLHPN^{241}YSQVDIGLIK (~60 min), respectively. The short peptide N^{211}ATAK was difficult to detect by RP C18 chromatography due to its high hydrophilicity.

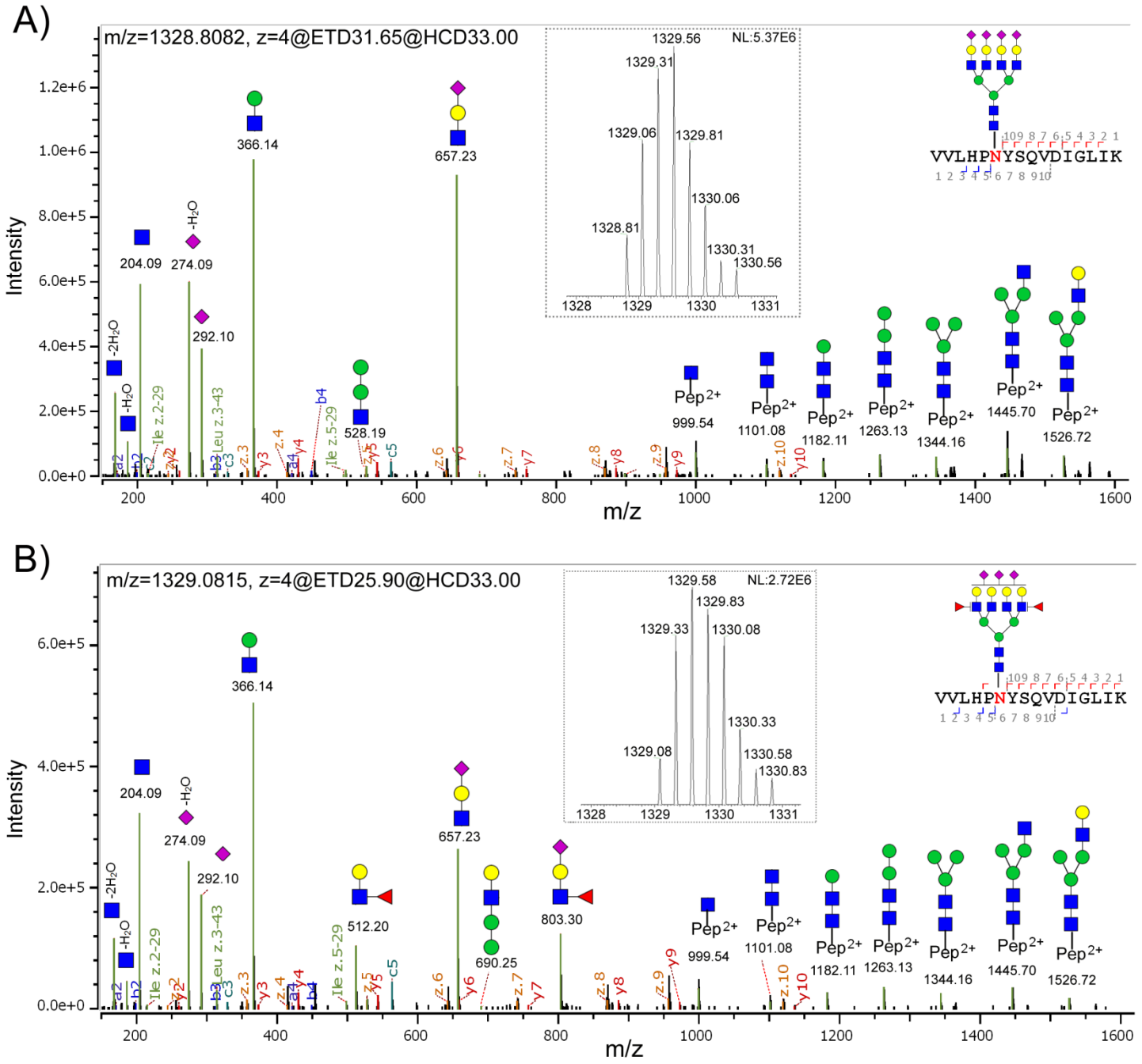

Figure S2. Representative MS/MS spectra of *N*-glycopeptides of VVLHPN^{241}YSQVDIGLIK with the glycan A4G4S4 (A) and A4G4F2S3 (B) to elucidate the difference in MS2 spectrum caused by the change in glycan moiety between 1 sialic acid and 2 fucoses. In the case of 2Fuc-1.02 = 1NeuAc, the monoisotopic evidence of the precursor ions at *m/z* 1328.81 and *m/z* 1329.08 (inserts in A and B) was used to confirm the assignment. Specific diagnostic fragment ions at *m/z* 512.20 and *m/z* 803.30 (B) further confirmed the outer arm fucosylation. (The symbols used in the structural formulas: blue square = GlcNAc; green circle = Man; yellow circle = Gal; red triangle = Fuc; purple diamond = NeuAc)

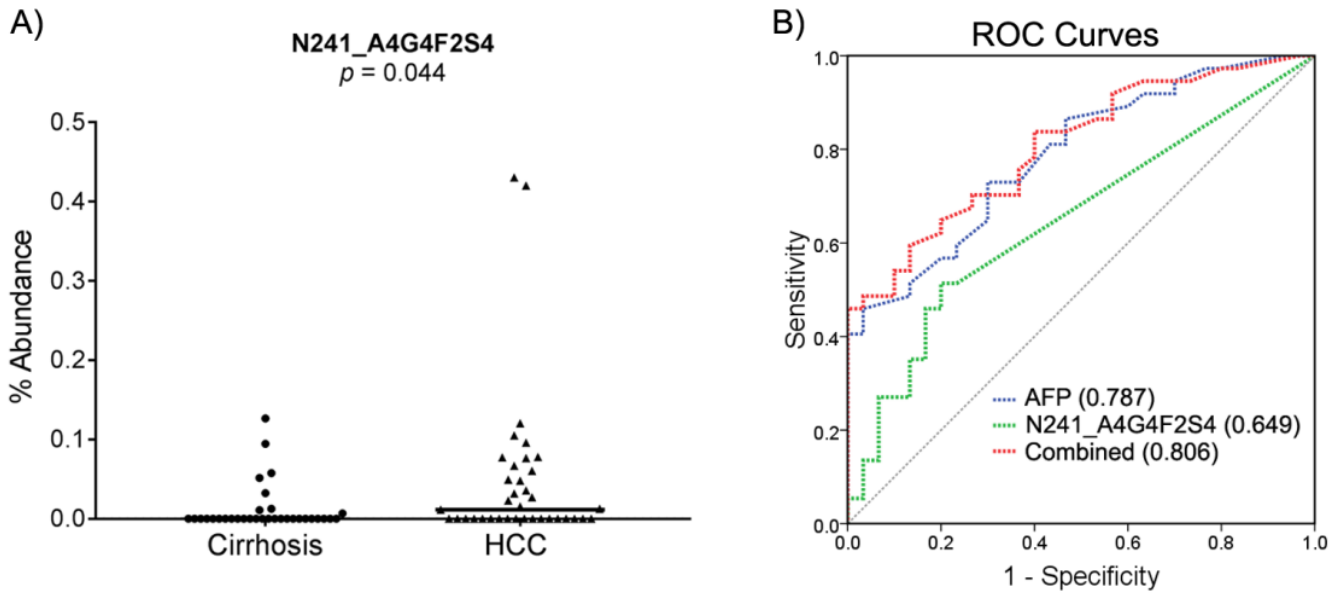

Figure S3. (A) Scatter plot of the relative abundance of the *N*-glycopeptide N241_A4G4F2S4 in HCC and cirrhosis, respectively. (B) ROC curves of the *N*-glycopeptide N241_A4G4F2S4 and its combination with AFP to differentiate HCCs from cirrhosis patients.

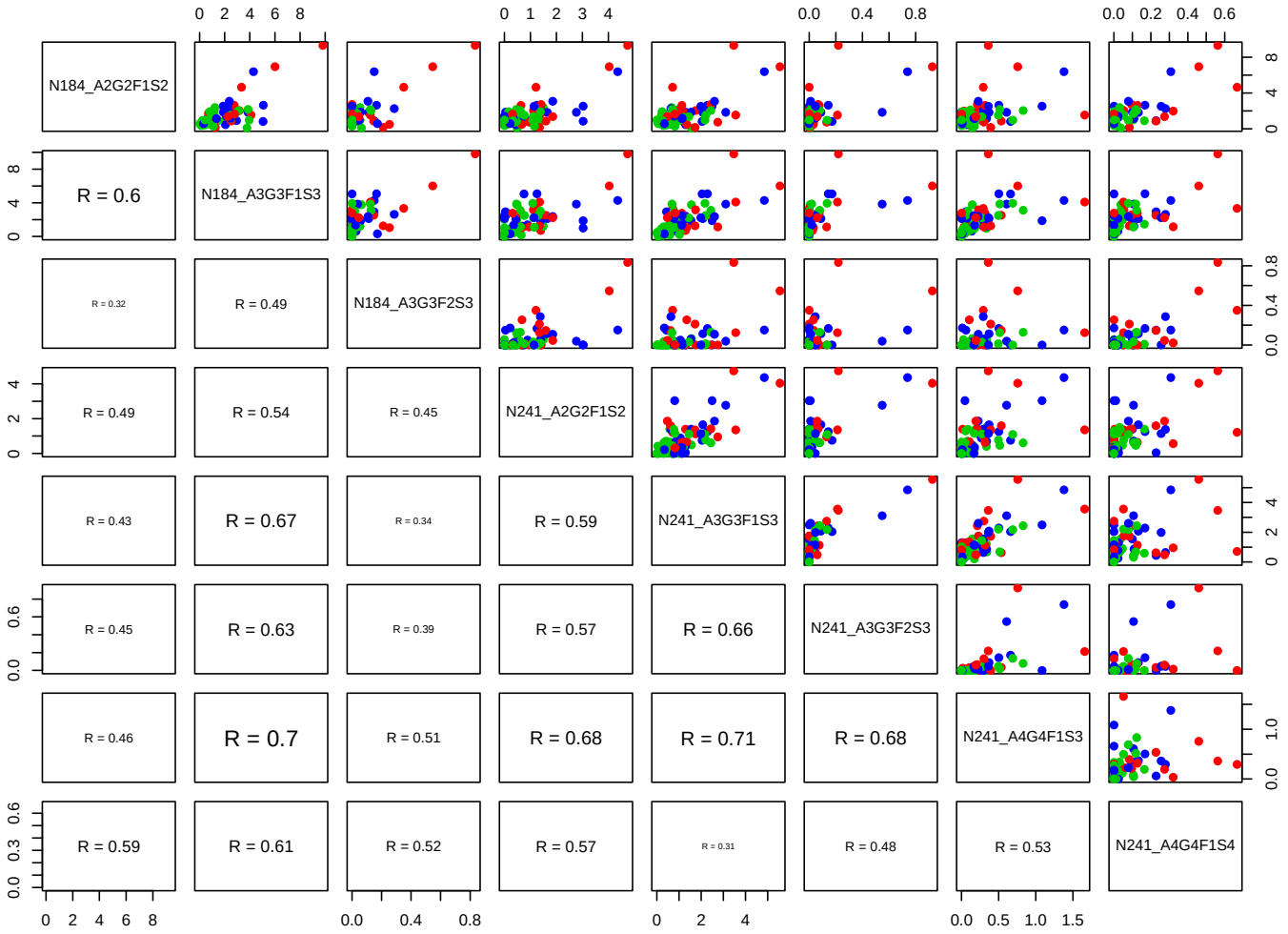

Figure S4. The correlation plot of the differentially expressed *N*-glycopeptides at sites N184 and N241. The Spearman correlation coefficients are labeled on the lower panels, and the size of the text is proportional to the correlations. The color of the dots represents the diagnosis (green=cirrhosis control, blue=early HCC, red=late HCC). N241_A4G4F1S3 was highly correlated with N241_A3G3F1S3 and N184_A3G3F1S3, with the correlation coefficients greater than or equal to 0.7.

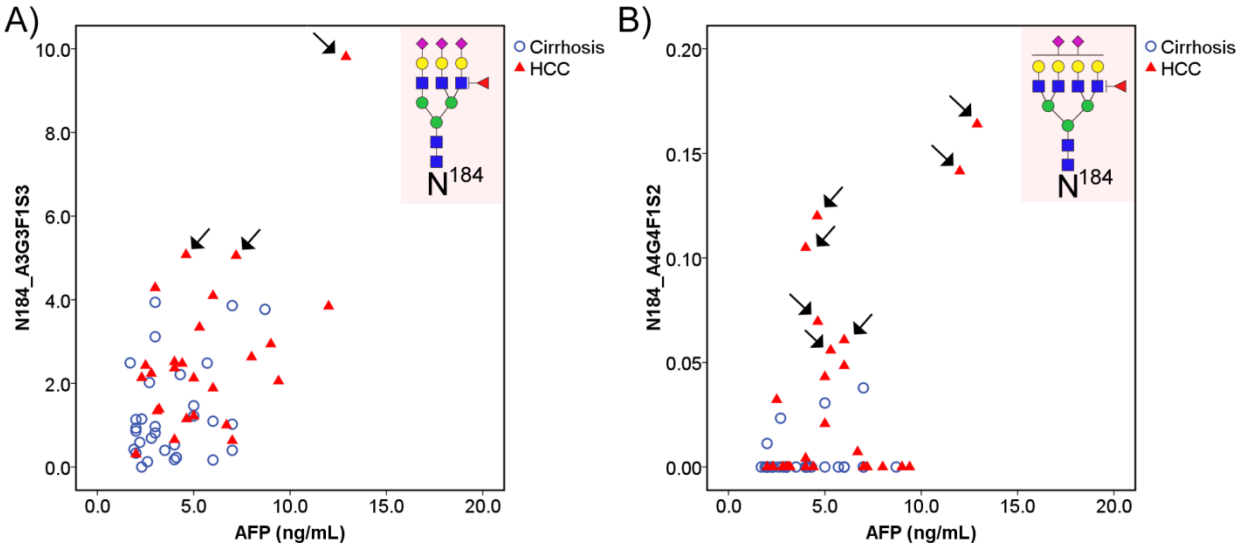

Figure S5. 2D scatter plot of AFP value against Hp *N*-glycopeptides N184_A3G3F1S3 and N184_A4G4F1S2, respectively, in cirrhosis (*blue*) and HCC (*red*) patients with AFP<20 ng/mL. HCC patients with negative AFP but elevated level of Hp *N*-glycopeptide are marked with arrows.

Table S1. An *N*-glycan database containing 53 human *N*-glycans employed in this study.

N-glycans	M-H_2O
HexNAc(3)Hex(3)	1095.3966
HexNAc(3)Hex(4)	1257.4494
HexNAc(3)Hex(4)Fuc(1)NeuAc(1)	1694.6027
HexNAc(3)Hex(4)NeuAc(1)	1548.5448
HexNAc(4)Hex(3)Fuc(1)	1444.5339
HexNAc(4)Hex(4)	1460.5288
HexNAc(4)Hex(4)Fuc(1)	1606.5867
HexNAc(4)Hex(4)Fuc(2)NeuAc(1)	2043.7400
HexNAc(4)Hex(4)NeuAc(1)	1751.6242
HexNAc(4)Hex(5)	1622.5816
HexNAc(4)Hex(5)Fuc(1)NeuAc(1)	2059.7349
HexNAc(4)Hex(5)Fuc(1)NeuAc(2)	2350.8304
HexNAc(4)Hex(5)Fuc(2)NeuAc(1)	2205.7928
HexNAc(4)Hex(5)NeuAc(1)	1913.6770
HexNAc(4)Hex(5)NeuAc(2)	2204.7724
HexNAc(4)Hex(6)Fuc(1)NeuAc(1)	2221.7878
HexNAc(4)Hex(6)NeuAc(1)	2075.7299
HexNAc(5)Hex(4)NeuAc(1)	1954.7036
HexNAc(5)Hex(4)NeuAc(2)	2245.7990
HexNAc(5)Hex(5)Fuc(1)NeuAc(1)	2262.8143
HexNAc(5)Hex(5)Fuc(1)NeuAc(2)	2553.9097
HexNAc(5)Hex(5)NeuAc(2)	2407.8518
HexNAc(5)Hex(6)Fuc(1)	2133.7717
HexNAc(5)Hex(6)Fuc(1)NeuAc(1)	2424.8671
HexNAc(5)Hex(6)Fuc(1)NeuAc(2)	2715.9626
HexNAc(5)Hex(6)Fuc(1)NeuAc(3)	3007.0580
HexNAc(5)Hex(6)Fuc(2)NeuAc(1)	2570.9250
HexNAc(5)Hex(6)Fuc(2)NeuAc(2)	2862.0204
HexNAc(5)Hex(6)Fuc(2)NeuAc(3)	3153.1158
HexNAc(5)Hex(6)Fuc(3)NeuAc(1)	2716.9829
HexNAc(5)Hex(6)Fuc(4)NeuAc(1)	2863.0408
HexNAc(5)Hex(6)NeuAc(1)	2278.8092
HexNAc(5)Hex(6)NeuAc(2)	2569.9046
HexNAc(5)Hex(6)NeuAc(3)	2861.0001
HexNAc(6)Hex(6)Fuc(1)NeuAc(2)	2919.0419
HexNAc(6)Hex(6)NeuAc(1)	2481.8886
HexNAc(6)Hex(7)Fuc(1)NeuAc(1)	2789.9993
HexNAc(6)Hex(7)Fuc(1)NeuAc(2)	3081.0947
HexNAc(6)Hex(7)Fuc(1)NeuAc(3)	3372.1902

HexNAc(6)Hex(7)Fuc(1)NeuAc(4)	3663.2856
HexNAc(6)Hex(7)Fuc(2)NeuAc(1)	2936.0572
HexNAc(6)Hex(7)Fuc(2)NeuAc(2)	3227.1526
HexNAc(6)Hex(7)Fuc(2)NeuAc(3)	3518.2480
HexNAc(6)Hex(7)Fuc(2)NeuAc(4)	3809.3434
HexNAc(6)Hex(7)Fuc(3)NeuAc(1)	3082.1151
HexNAc(6)Hex(7)Fuc(3)NeuAc(3)	3664.3059
HexNAc(6)Hex(7)Fuc(4)NeuAc(1)	3228.1730
HexNAc(6)Hex(7)Fuc(5)NeuAc(1)	3374.2309
HexNAc(6)Hex(7)NeuAc(1)	2643.9414
HexNAc(6)Hex(7)NeuAc(2)	2935.0368
HexNAc(6)Hex(7)NeuAc(3)	3226.1323
HexNAc(6)Hex(7)NeuAc(4)	3517.2277
HexNAc(7)Hex(8)NeuAc(1)	3009.0736