SUPPORTING INFORMATION for the article "**Novel O-linked Sialoglycan Structures in Human Urinary Glycoproteins**."

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Figure S-16 and S-17 illustrate certain cases when in-source fragments were selected for MS/MS experiments.
Each example was selected from file 180417_08 , in which only doubly charged ions were permitted in the precursor ion selection.

Figure S-16 shows with XIC chromatograms, MS spectra, and some MS/MS data that the sialyl-Tn antigen-bearing glycoforms of AVAVTLQSH, indicated in scans 8961 and 12464, from precursors 710.435(2+) (Supplement 2) are in-source fragments of two different glycoforms featuring larger disialo-glycans.

Figure S-17 present similar data for in-source fragment featuring AVAVTLQSH bearing a glycan of $HexNAc_2Hex_2NeuAc$ composition. This 'glycoform' was indicated in scans 8384 and 9774 (Supplement 2); and this also could derive from two different larger structures.

In both cases the larger structures bear two sialic acids, while the products display only one. Glycopeptides with a different degree of sialylation do not coelute [Trinidad, J. C.; Schoepfer, R., Burlingame, A. L.; Medzihradszky, K. F. *Mol Cell Proteomics.* **2013**, *12,* 3474-3488.; Medzihradszky, K.F.; Kaasik, K.; Chalkley, R.J. *Anal Chem.* **2015,** *87,* 3064-3071.].

Figure S-18 indicates that the larger monosialo structures identified on peptide DFTAAFPR are not fragments but rather intact glycans. The monosialo glycoforms eluted slightly before the disialo glycoforms.

Figure S-19 presents the HCD and EThcD spectra of the VGPVRPT($HexNAcHexNeuAc_2$)GQDWN($HexNAc_2$)HTPQK peptide. This sequence was identified in the primary search bearing mucin-type O-linked sugars only.

Figure S-20 presents the HCD and EThcD spectra of two coeluting glycopeptides, DFT($HexNAc_4Hex_4NeuAc_1$)AAFPR (m/z 892.699 (3+)) and AQDGGPVGT($HexNAc_2Hex_3Fuc_1NeuAc_1$)ELFR (m/z 892.718 (3+))

Figure S-21 shows the XICs of m/z 892.699 and 892.7182 representing the triply charged precursors of the glycopeptides depicted on Figure S-20

Figure S-16

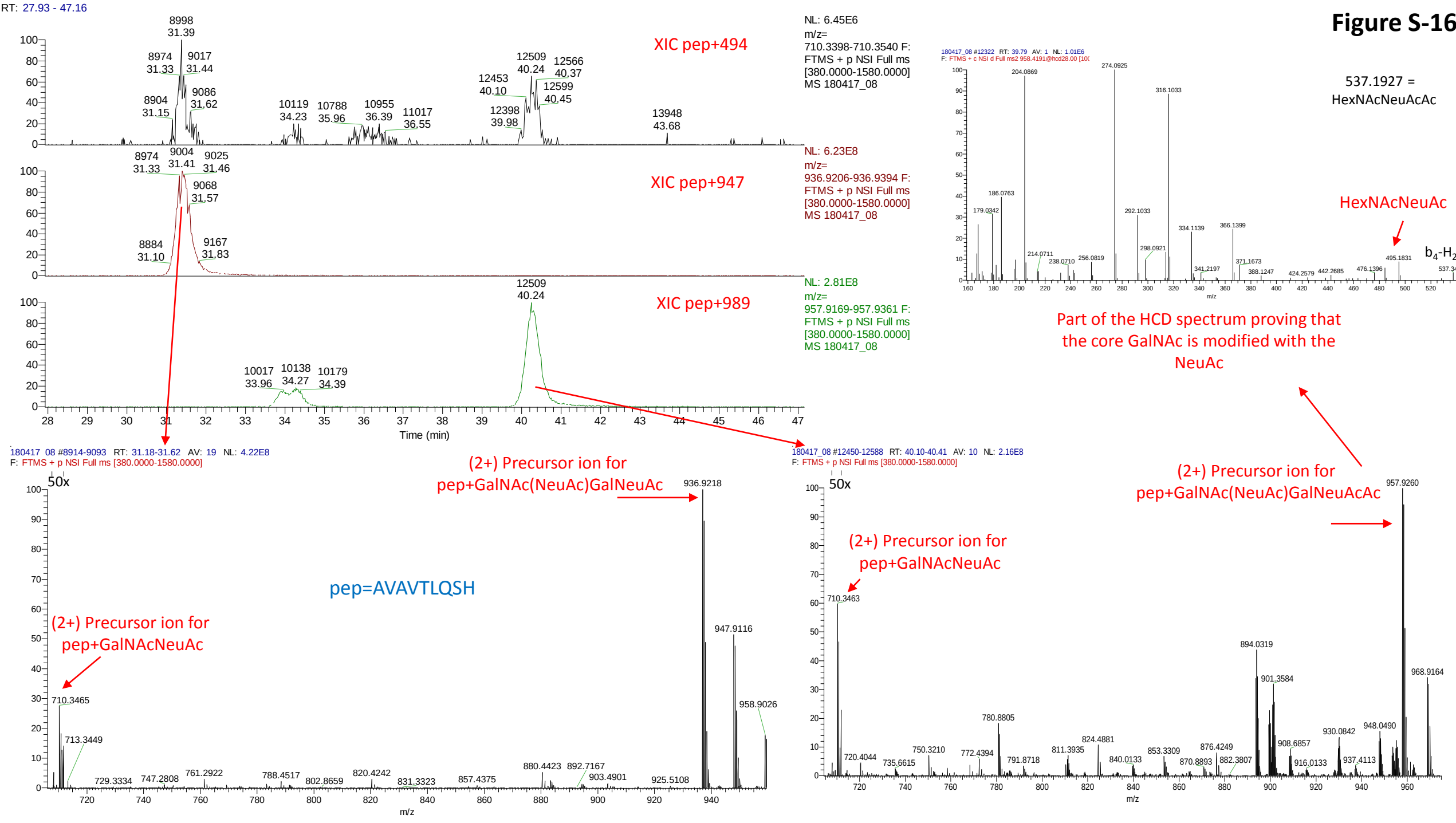
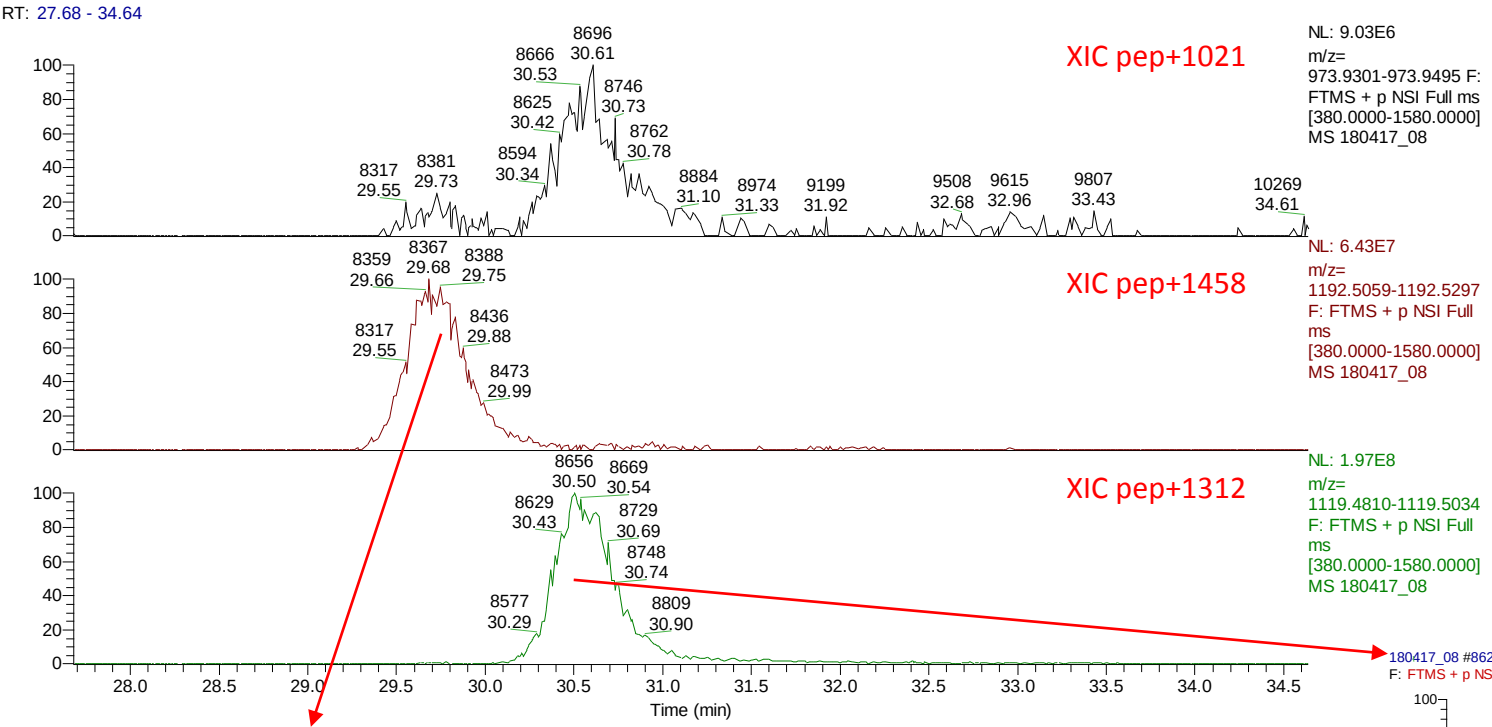
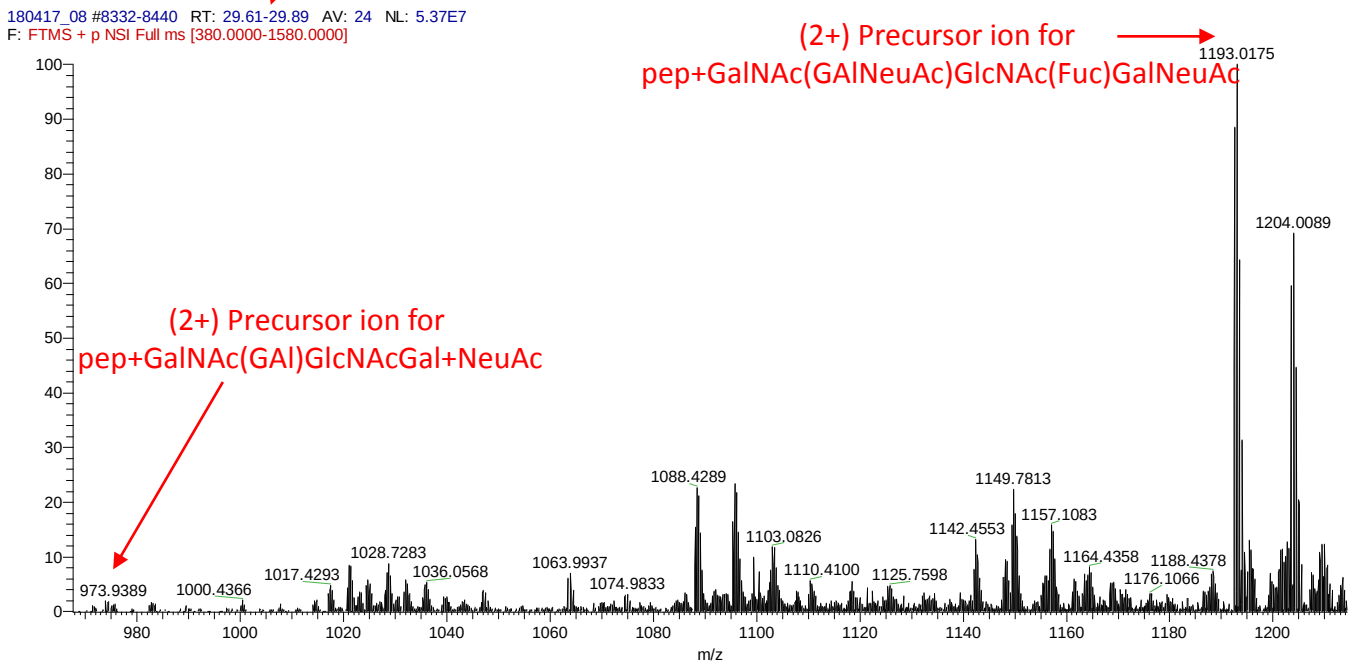

RT: 27.93 - 47.16
XIC pep+494
NL: 6.45E6
m/z= 710.3398-710.3540 F: FTMS + p NSI Full ms [380.0000-1580.0000] MS 180417_08
XIC pep+947
NL: 6.23E8
m/z= 936.9206-936.9394 F: FTMS + p NSI Full ms [380.0000-1580.0000] MS 180417_08
XIC pep+989
NL: 2.81E8
m/z= 957.9169-957.9361 F: FTMS + p NSI Full ms [380.0000-1580.0000] MS 180417_08
Time (min)
537.1927 = HexNAcNeuAcAc
HexNAcNeuAc
b_4-H_2O
Part of the HCD spectrum proving that the core GalNAc is modified with the NeuAc
180417_08 #8914-9093 RT: 31.18-31.62 AV: 19 NL: 4.22E8
F: FTMS + p NSI Full ms [380.0000-1580.0000]
(2+) Precursor ion for pep+GalNAc(NeuAc)GalNeuAc
pep=AVAVTLQSH
(2+) Precursor ion for pep+GalNAcNeuAc
180417_08 #12450-12588 RT: 40.10-40.41 AV: 10 NL: 2.16E8
F: FTMS + p NSI Full ms [380.0000-1580.0000]
(2+) Precursor ion for pep+GalNAc(NeuAc)GalNeuAcAc
(2+) Precursor ion for pep+GalNAcNeuAc
m/z

Figure S-17



pep=AVAVTLQSH



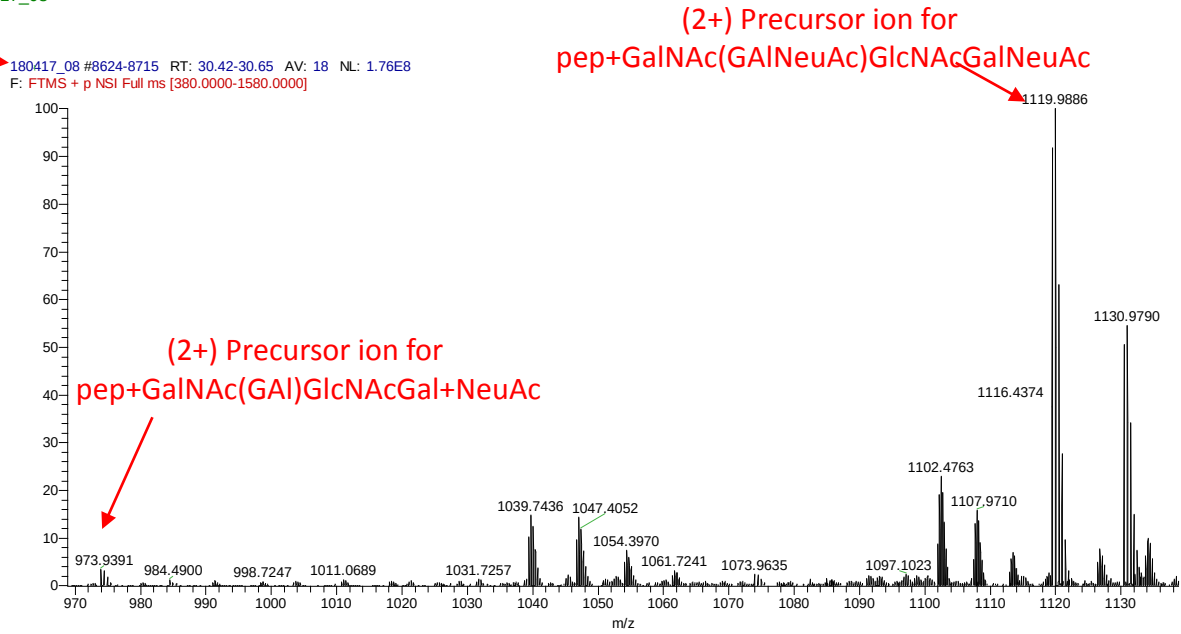
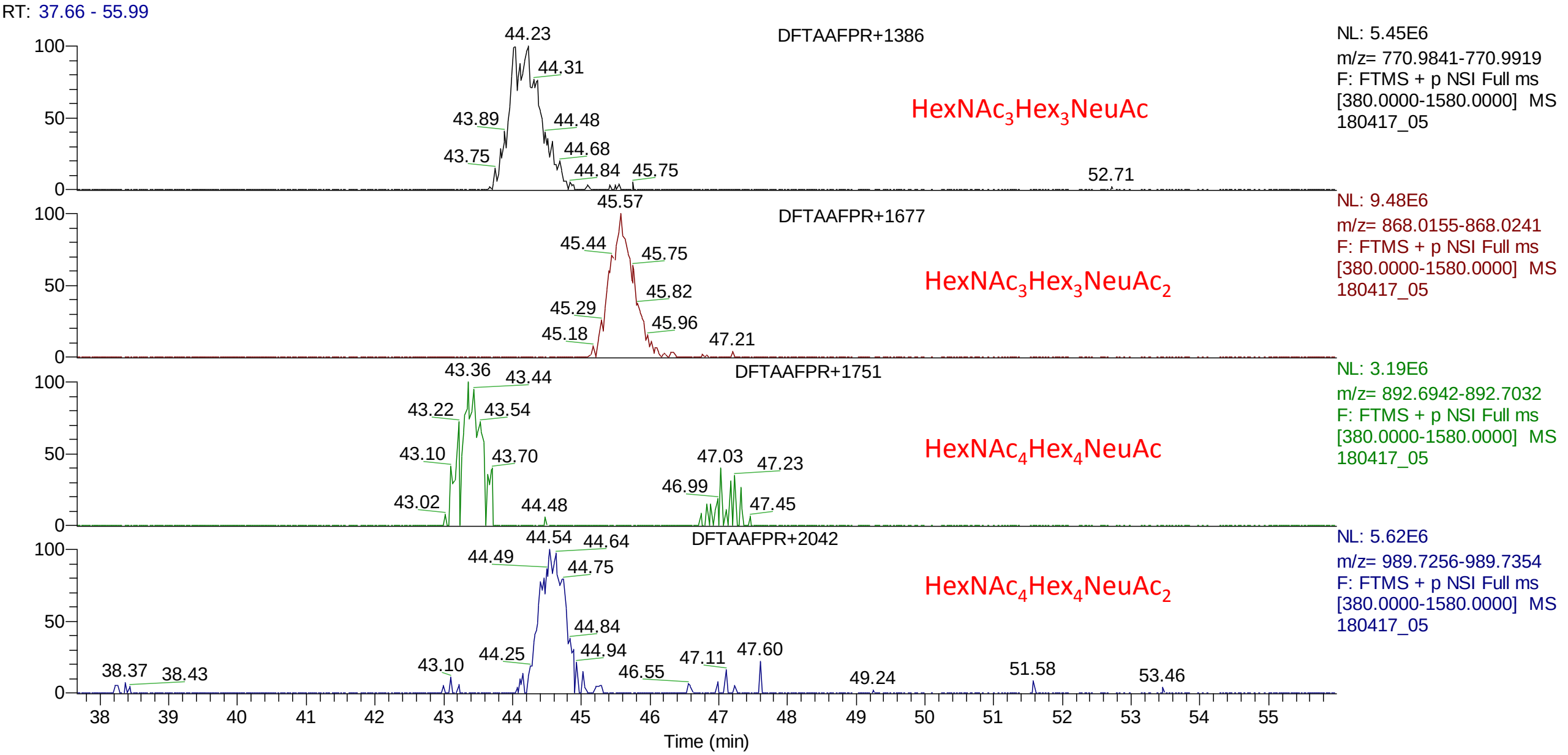


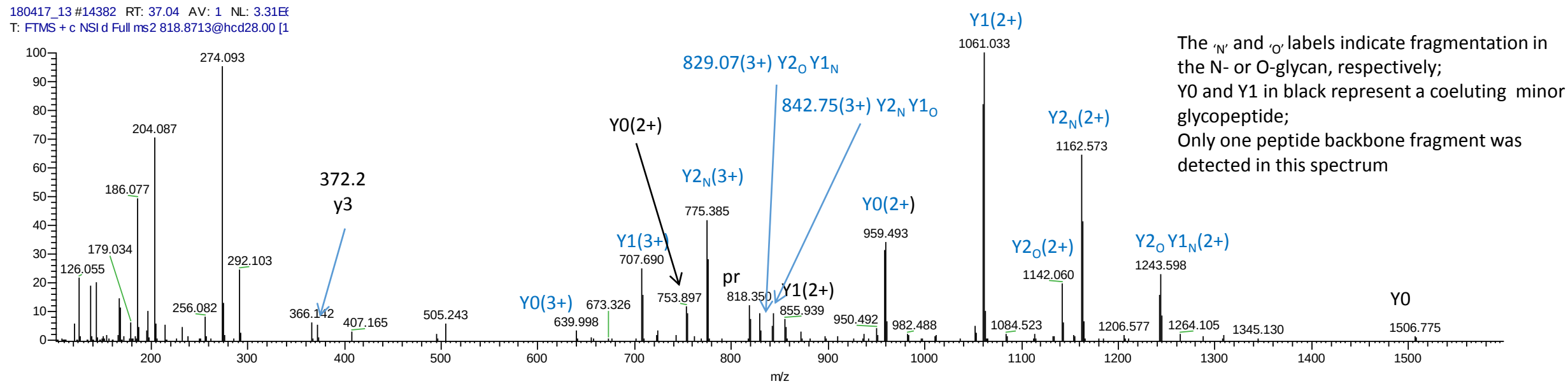
Figure S-18

XIC data of triply charged ions representing DFTAAFPR glycoforms

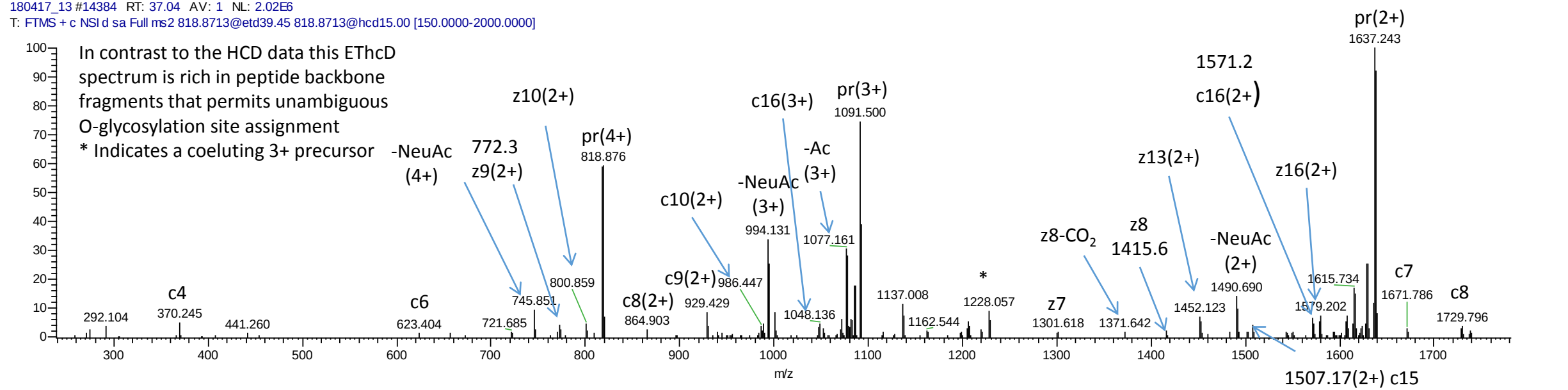


D:\Data\Urine2\180417_13
50mL, 5/12.5ul

VGPVRPT(HexNAcHexNeuAc2)GQDWN(HexNAc2)HTPQK, 818.3707(4+)



The 'N' and 'O' labels indicate fragmentation in the N- or O-glycan, respectively;
Y0 and Y1 in black represent a coeluting minor glycopeptide;
Only one peptide backbone fragment was detected in this spectrum



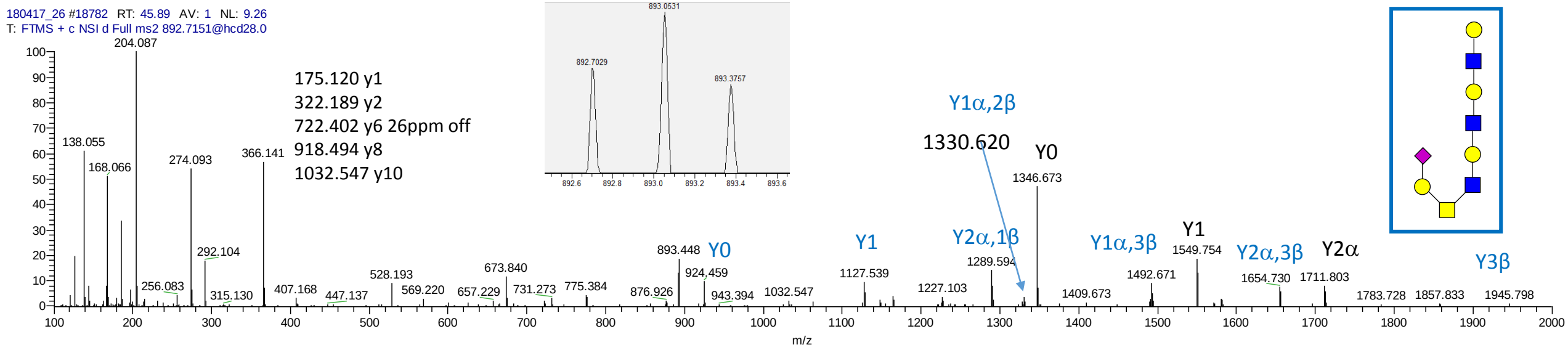
DFT(1751.6242)AAFPR & AQDGGPVGT(1329.4705)ELFR, 892.7182(3+)

Figure S-20

D:\Data\Urine2\180417_26
50mL, 5/12.5ul

4/18/2018 8:24:29 PM

Calc. 3+ ions = 892.6987 vs 892.7183



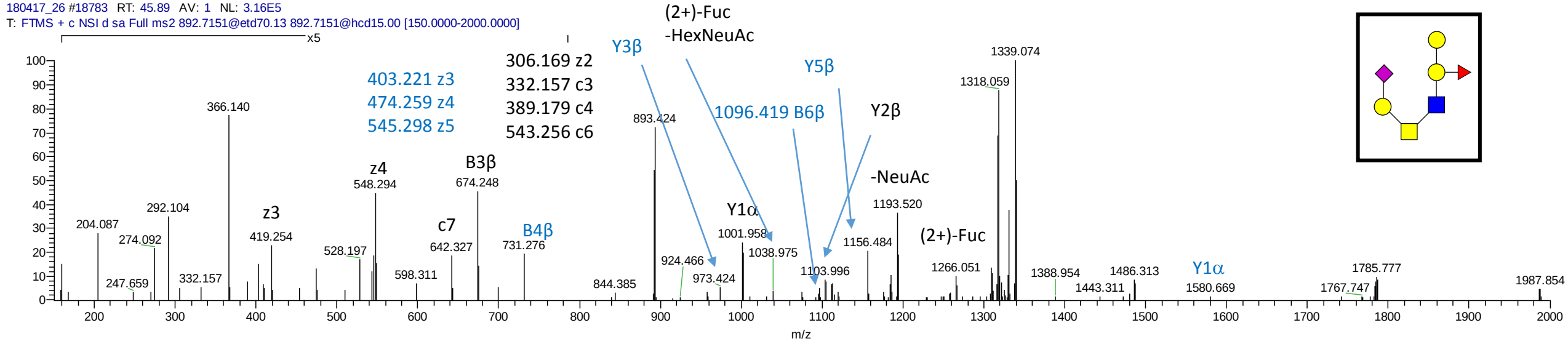
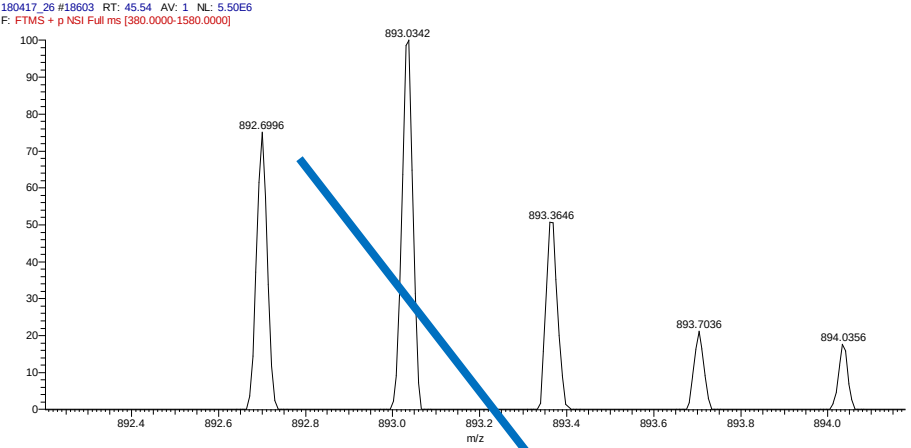
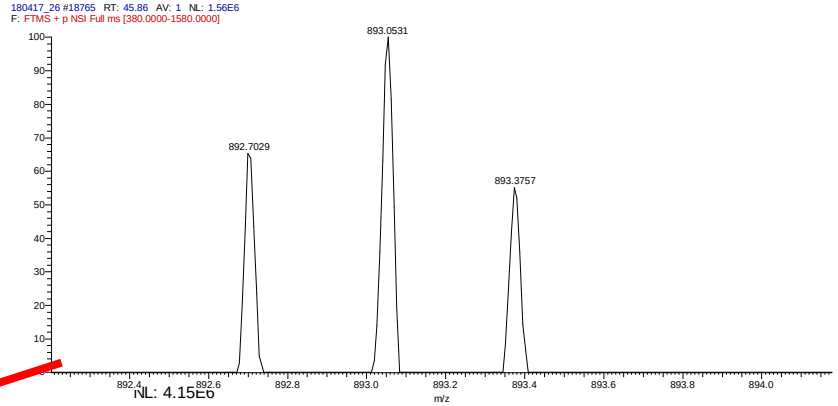


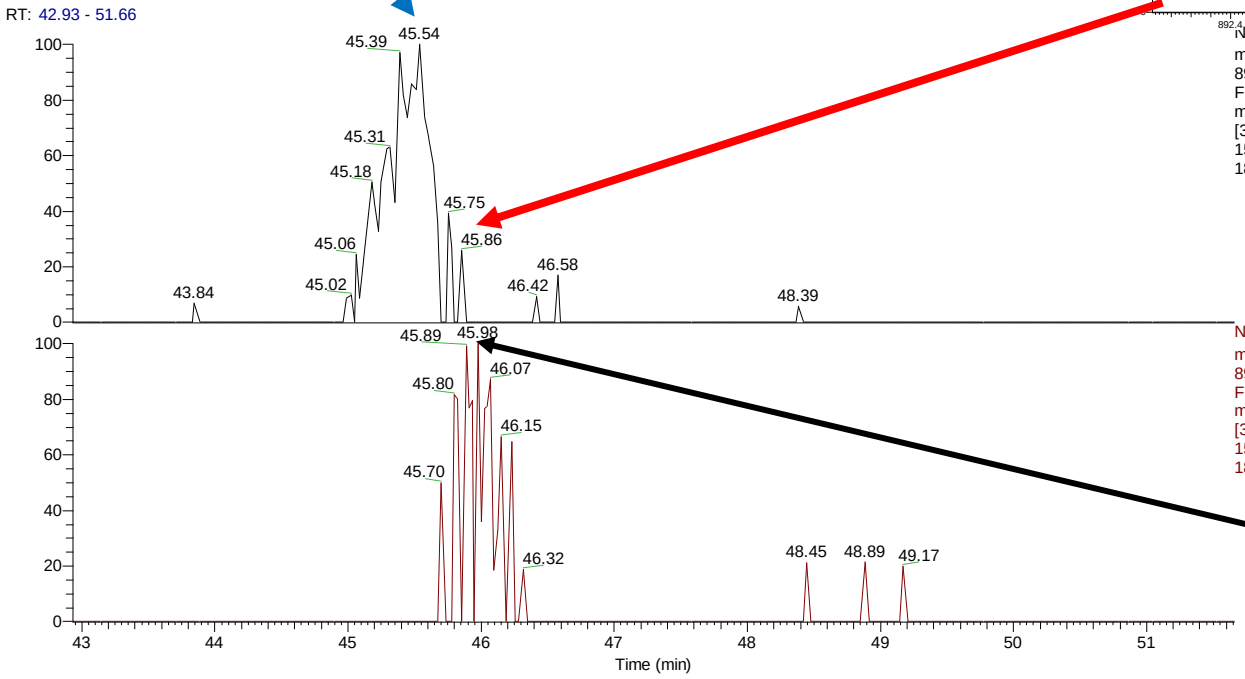
Figure S-21

precursor ion selection scan





NL: 4.15E6
m/z=
892.6942-892.7032
F: FTMS + p NSI Full
ms
[380.0000-
1580.0000] MS
180417_26



NL: 1.34E6
m/z=
892.7138-892.7228
F: FTMS + p NSI Full
ms
[380.0000-
1580.0000] MS
180417_26