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SUPPORTING INFORMATION for the article

"Novel O-linked Sialoglycan Structures in Human Urinary Glycoproteins"

A. Pap, E. Tasnadi, K.F. Medzihradszky and Z. Darula

The most likely structure of new glycoforms was deciphered manually, using both HCD and EThcD data. Obviously, the identity of the sugar units and their linkage positions cannot be assigned from these data. However, this supplement demonstrates that **EThcD spectra acquired with 15% NCE may deliver structural information on the glycan part of a glycopeptide.** All AVAVTLQSH spectra presented here have proof for the site assignment (modified c_7 and/or unmodified z_4 peptide fragments).

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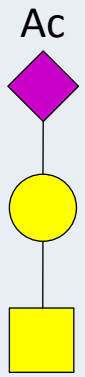
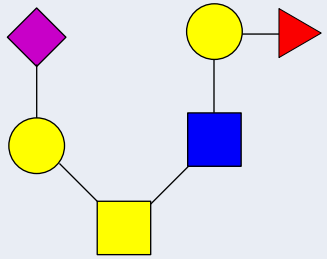
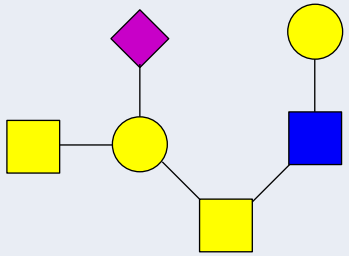
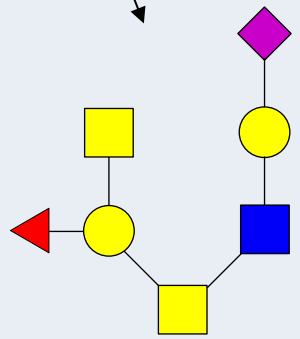
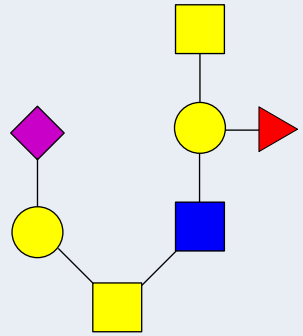
Table S-1 Novel glycans

This isomer has been reported in [Darula, Z.; Pap A.; Medzihradszky, K. F. *J Proteome Res.* **2019,** *18,* 280-291.]

N: HexNAc, H: Hex, F: Fuc, A: NeuAc, AAc: NeuAcAc

Monoisotopic additive mass	**698.2382**	**1167.4177**	**1224.4392**	**1370.4971**	
Composition	*NHAAc*	N_2H_2FA	N_3H_2A	N_3H_2FA	
Structure					

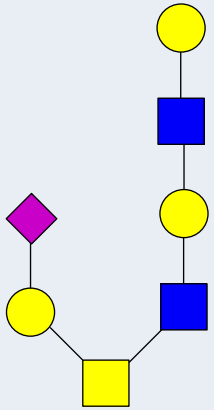
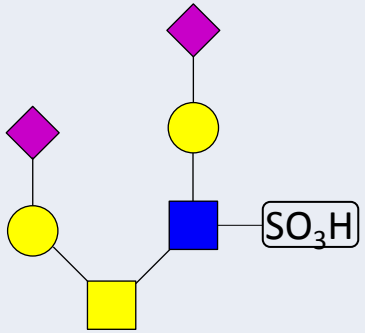
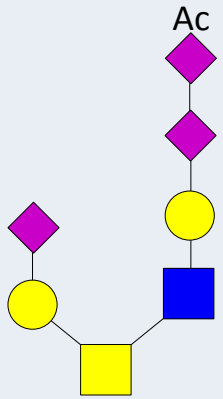
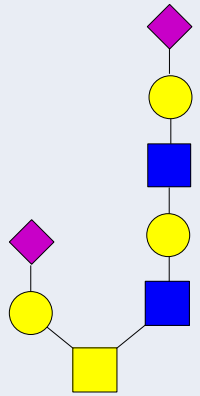
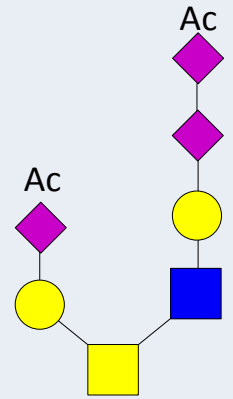
Monoisotopic additive mass	**1386.4920**	**1392.4120**	**1645.5612**	**1677.5874**	**1687.5718**
Composition	N_3H_3A	$N_2H_2A_2Sulfo$	$N_2H_2A_2AAc$	$N_3H_3A_2$	$N_2H_2AAAc_2$
Structure					

Table S-1 Novel glycans (contd.)

N: HexNAc, H: Hex, F: Fuc, A: NeuAc

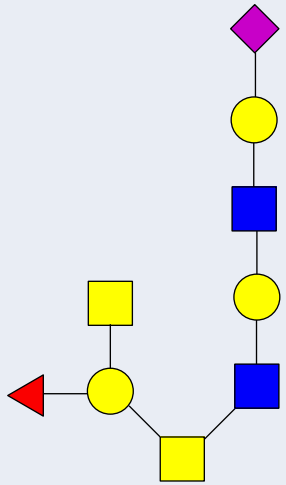
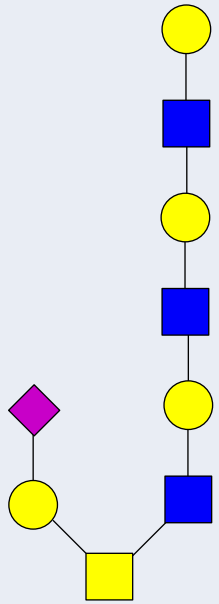
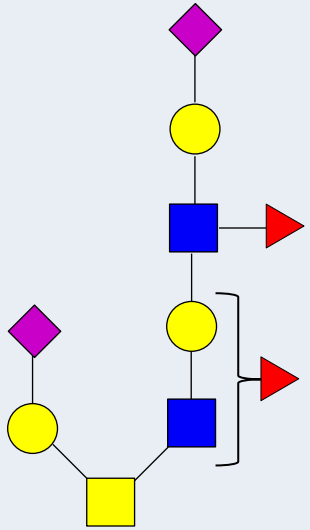
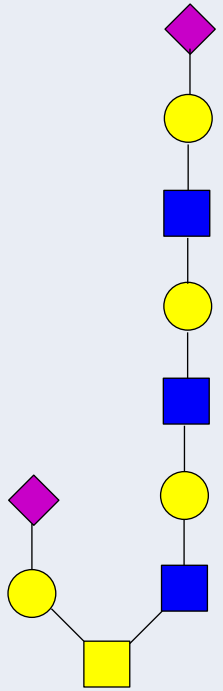
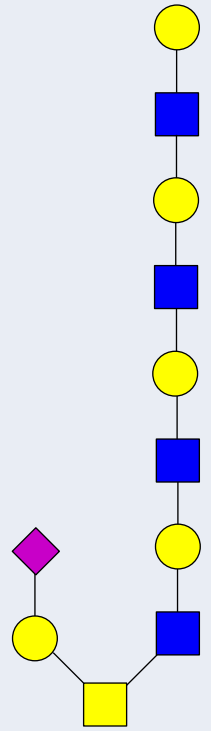
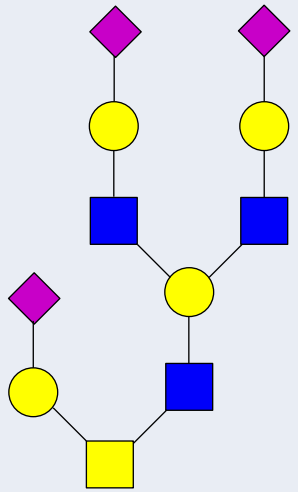
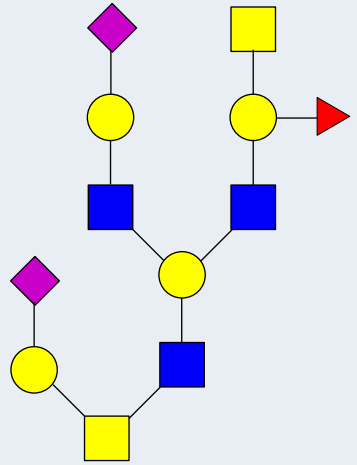
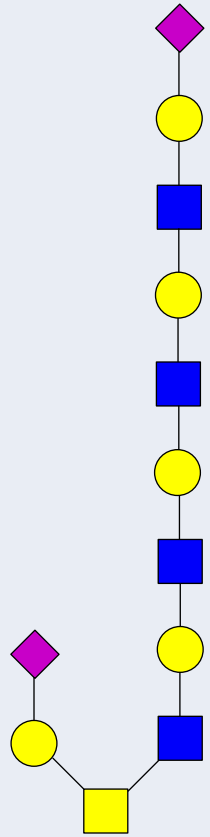
Monoisotopic additive mass	**1735.6293**	**1751.6242**	**1969.7032**	**2042.7196**	**2116.7564**
Composition	N_4H_3FA	N_4H_4A	$N_3H_3F_2A_2$	$N_4H_4A_2$	N_5H_5A
Structure					

Table S-1 Novel glycans (contd.)

N: HexNAc, H: Hex, F: Fuc, A: NeuAc

Monoisotopic additive mass	**2333.8150**	**2391.8569**	**2407.8518**
Composition	$N_4H_4A_3$	$N_5H_4FA_2$	$N_5H_5A_2$
Structure			

Internal & non-reducing end glycan fragments (calculated masses)

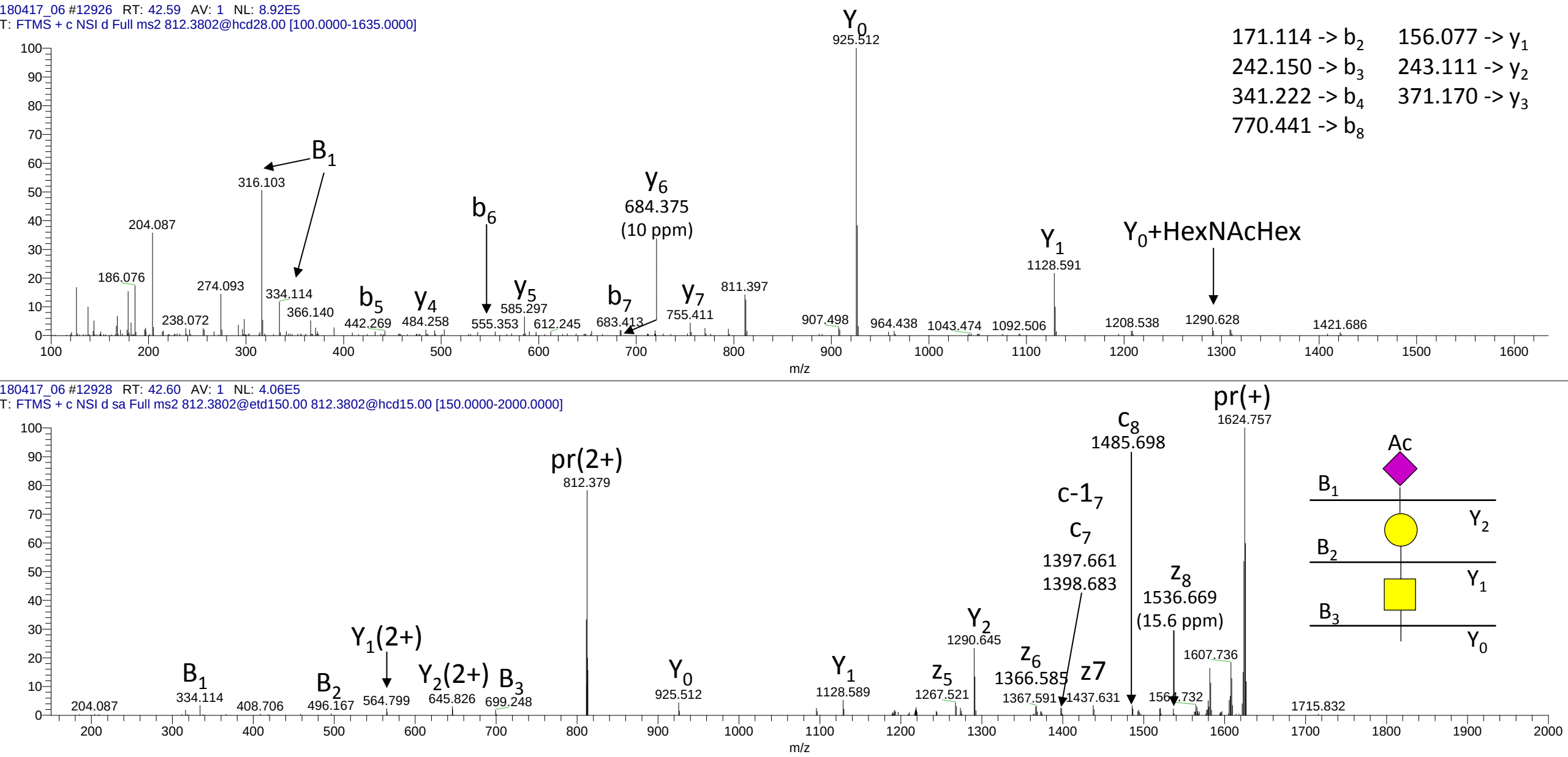
m/z	Glycan composition
186.0761	HexNAc-H_2O
204.0867	HexNAc
274.0921	NeuAc-H_2O
292.1027	NeuAc
314.0846	NeuAc+Na
316.1027	NeuAcAc-H_2O
334.1133	NeuAcAc
366.1395	HexNAcHex
388.1214	HexNAcHex+Na
407.1661	$HexNAc_2$
454.1555	HexNeuAc
476.1374	HexNeuAc+Na
495.1821	HexNAcNeuAc
512.1974	HexNAcHexFuc
528.1923	$HexNAcHex_2$

m/z	Glycan composition
569.2189	$HexNAc_2Hex$
657.2349	HexNAcHexNeuAc
698.2615	$HexNAc_2NeuAc$
699.2455	HexNAcHexNeuAcAc
715.2768	$HexNAc_2HexFuc$
731.2717	$HexNAc_2Hex_2$
803.2928	HexNAcHexFucNeuAc
1022.3671	$HexNAc_2Hex_2NeuAc$
1096.4039	$HexNAc_3Hex_3$
1168.4250	$HexNAc_2Hex_2FucNeuAc$
1387.4993	$HexNAc_3Hex_3NeuAc$
1371.5044	$HexNAc_3Hex_2FucNeuAc$
1678.5947	$HexNAc_3Hex_3NeuAc_2$

In **Figures S-1-14** HCD spectra are presented in the upper panels, EThcD data are shown in the lower panels. Peptide fragments are labeled according to the Biemann nomenclature [Biemann K. Nomenclature for peptide fragment ions (positive ions). *Methods Enzymol*. **1990,** *193*, 886-887.]. In most cases the peptide fragments are rather weak and the HCD data may reveal the presence of coeluting/cofragmenting components. Glycan fragments are labeled according to the Domon-Costello nomenclature [Domon, B.; Costello, C. E. *Glycoconjugate J*. **1988,** *5,* 397-409.], and the cleavages are indicated in the schemes included. Internal glycan fragments are labeled with cartoons, according to the CFG recommendations. In the EThcD spectra the precursor and its charge reduced forms are indicated as „pr(charge)". The charge reduced form of coeluting molecules are labeled with „Coel.(charge)". When a fragment is formed via multiple glycosidic bond cleavages than the following label format is used: $Y_{2\alpha,3\beta.}$ When a particular mass may represent different structures, the following format is used: $Y_{2\alpha}|Y_{2\beta}$.

Figure S-15 – displays the EThcD data of isomeric blood-type A antigens, Type 3 (upper panel), Type 1 or 2 (lower panel).

As we reported earlier [Darula, Z.; Pap, Á.; Medzihradszky, K. F. *J Proteome Res*. **2019**, *18*, 280–291.], in some of the spectra we observed fragments that indicated sialic acid or fucose migration, a known phenomenon in glycan analysis [Wuhrer, M.; Deelder, A. M.; van der Burgt, Y. E. *Mass Spectrom Rev.* **2011**, *30*, 664-680.]. Such fragments are clearly annotated.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242.150 -> b3
341.222 -> b4
770.441 -> b8
156.077 -> y1
243.111 -> y2
371.170 -> y3
Y0 925.512
B1 316.103
y6 684.375 (10 ppm)
b6
Y1 1128.591
Y0+HexNAcHex
204.087
186.076
274.093
334.114
366.140
238.072
b5 442.269
y4 484.258
y5 585.297
555.353
612.245
b7 683.413
y7 755.411
811.397
907.498
964.438
1043.474
1092.506
1208.538
1290.628
1421.686
m/z
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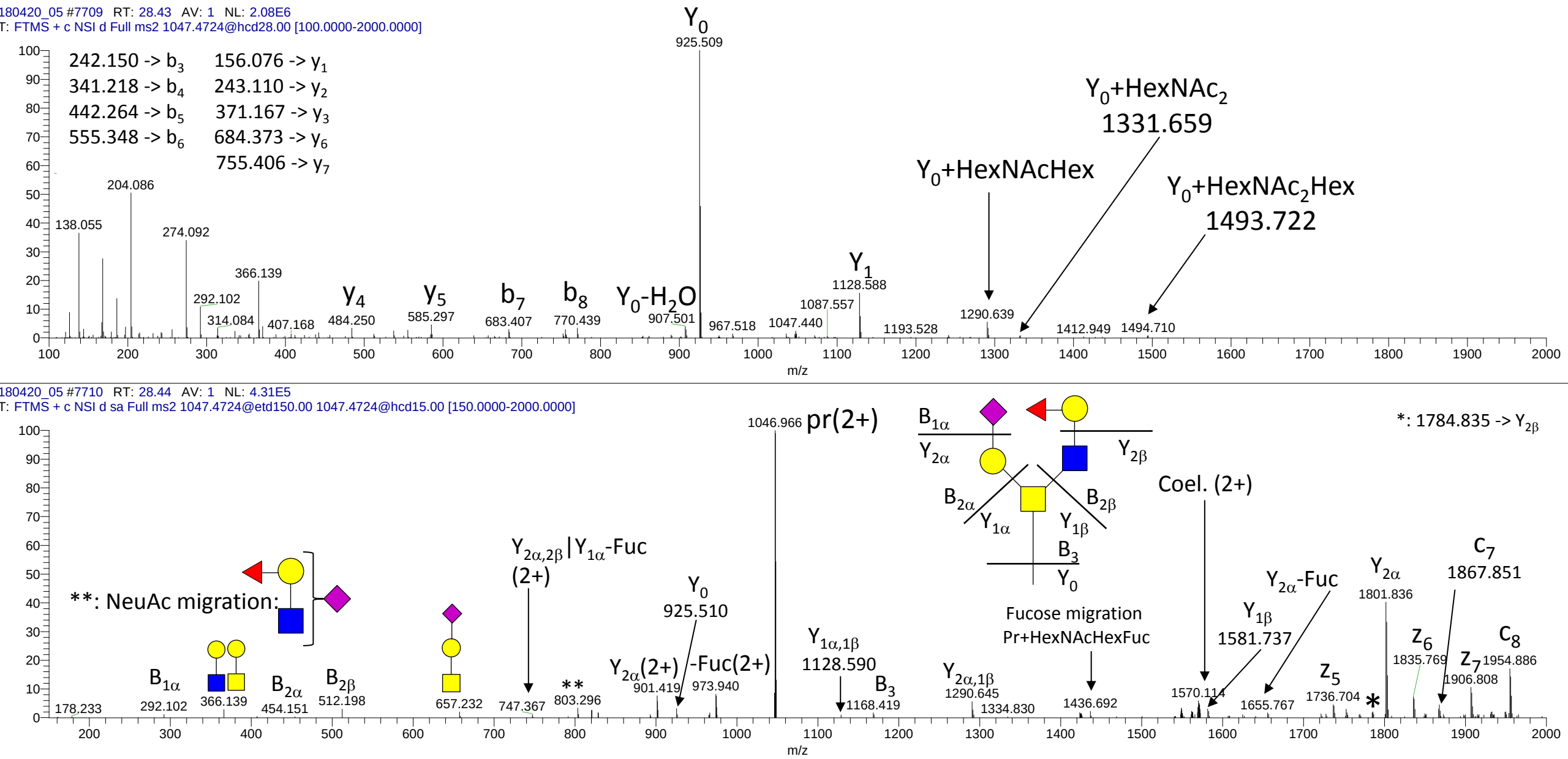
pr(2+) 812.379
pr(+) 1624.757
c8 1485.698
c-17
c7 1397.661
1398.683
z8 1536.669 (15.6 ppm)
Y1(2+)
B1 334.114
B2 496.167
Y2(2+) 645.826
B3 699.248
564.799
Y0 925.512
Y1 1128.589
Y2 1290.645
z5 1267.521
z6 1366.585
z7 1437.631
1367.591
1607.736
1564.732
1715.832
204.087
408.706
Ac
B1
Y2
B2
Y1
B3
Y0
m/z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341.218 -> b4
442.264 -> b5
555.348 -> b6
156.076 -> y1
243.110 -> y2
371.167 -> y3
684.373 -> y6
755.406 -> y7
Y0
925.509
Y0+HexNAc2
1331.659
Y0+HexNAcHex
Y0+HexNAc2Hex
1493.722
Y1
1128.588
y4
y5
b7
b8
Y0-H2O
204.086
138.055
274.092
366.139
292.102
314.084
407.168
484.250
585.297
683.407
770.439
907.501
967.518
1047.440
1087.557
1193.528
1290.639
1412.949
1494.710
m/z
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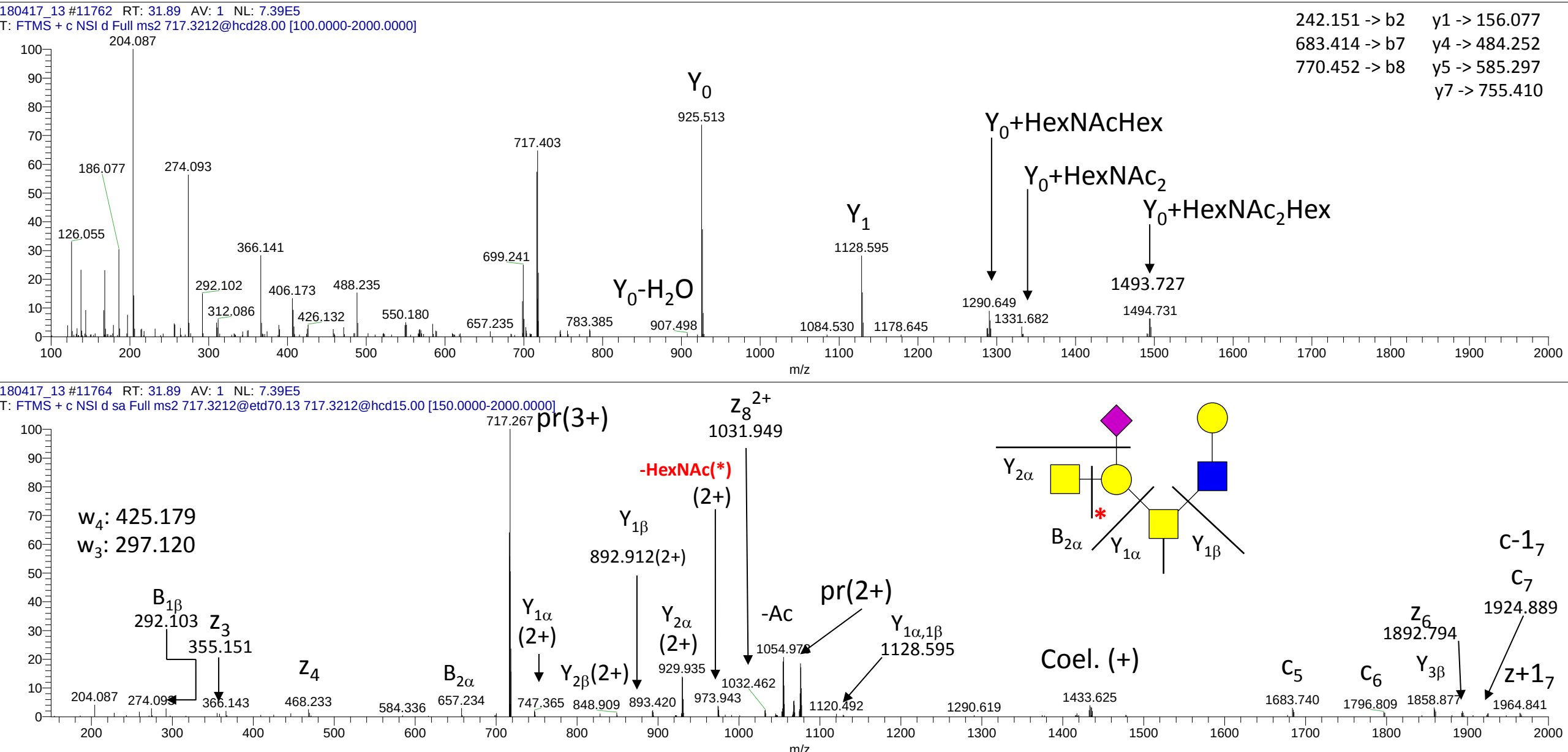
1046.966 pr(2+)
B1α
Y2α
Y2β
B2α
B2β
Y1α
Y1β
B3
Y0
*: 1784.835 -> Y2β
Coel. (2+)
**: NeuAc migration:
Y2α,2β|Y1α-Fuc
(2+)
Y0
925.510
Y2α(2+)
-Fuc(2+)
Y1α,1β
1128.590
B3
1168.419
Y2α,1β
1290.645
1334.830
Fucose migration
Pr+HexNAcHexFuc
1436.692
Y2α-Fuc
Y1β
1581.737
Y2α
1801.836
C7
1867.851
Z6
1835.769
C8
Z7
1906.808
1954.886
Z5
1736.704
1570.114
1655.767
B1α
292.102
366.139
B2α
454.151
B2β
512.198
178.233
657.232
747.367
803.296
901.419
973.940
m/z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242.151 -> b2
683.414 -> b7
770.452 -> b8
y1 -> 156.077
y4 -> 484.252
y5 -> 585.297
y7 -> 755.410
204.087
186.077
274.093
126.055
366.141
292.102
312.086
406.173
426.132
488.235
550.180
657.235
699.241
717.403
783.385
907.498
925.513
Y0
Y0-H2O
1084.530
1128.595
Y1
1178.645
1290.649
1331.682
Y0+HexNAcHex
Y0+HexNAc2
1493.727
1494.731
Y0+HexNAc2Hex
m/z
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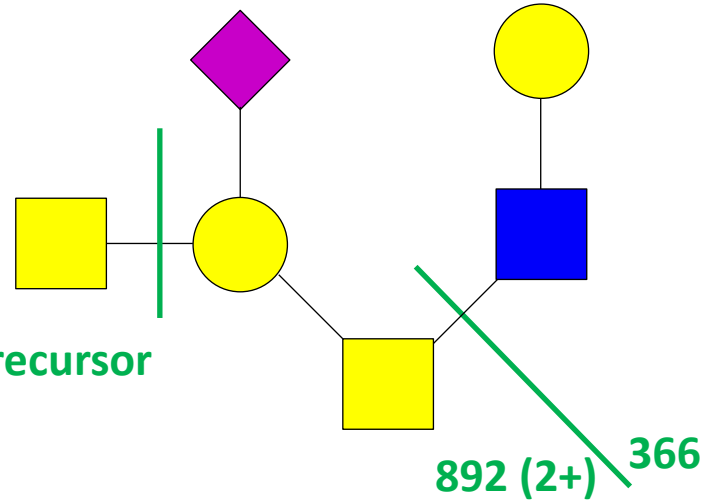
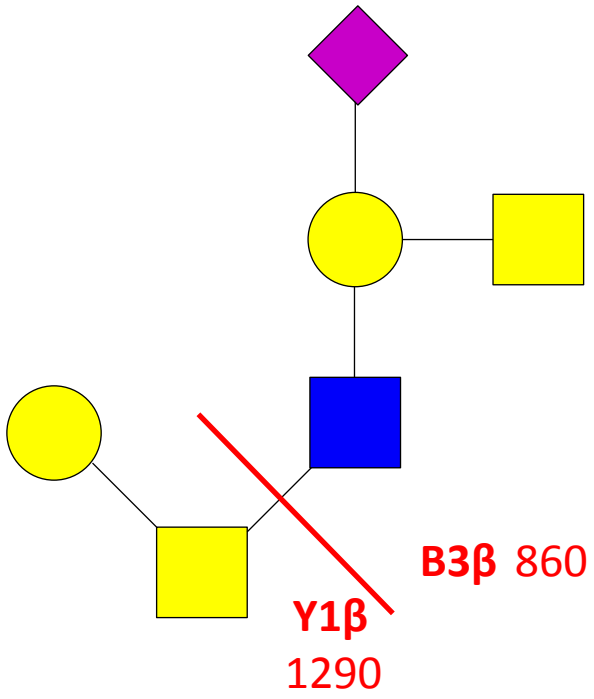
T: FTMS + c NSI d sa Full ms2 717.3212@etd70.13 717.3212@hcd15.00 [150.0000-2000.0000]
w4: 425.179
w3: 297.120
717.267
pr(3+)
z8 2+
1031.949
-HexNAc(*)
(2+)
Y1β
892.912(2+)
B1β
292.103
z3
355.151
Y1α
(2+)
Y2α
(2+)
-Ac
pr(2+)
Y1α,1β
1128.595
1054.978
z4
B2α
Y2β(2+)
929.935
1032.462
973.943
204.087
274.093
366.143
468.233
584.336
657.234
747.365
848.909
893.420
1120.492
1290.619
Coel. (+)
1433.625
c5
1683.740
c6
1796.809
Y3β
1858.877
z6
1892.794
c7
1924.889
c-17
z+17
1964.841
Y2α
B2α
Y1α
Y1β
m/z

Potential structures for the $HexNAc_3Hex_2NeuAc$ (1224.4392) composition:

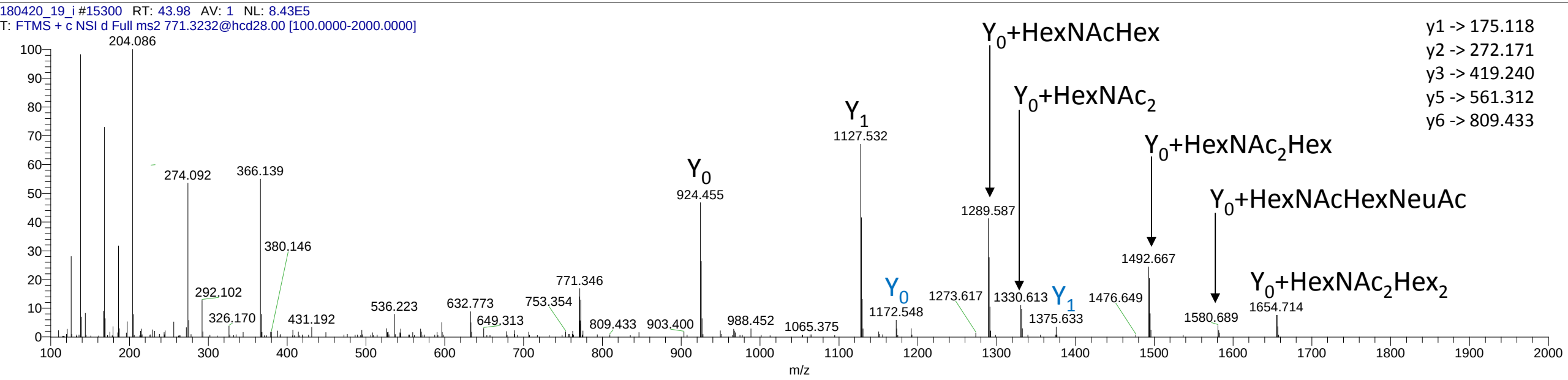


The most likely structure

The other option



These fragment ions were not detected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y2 -> 272.171
y3 -> 419.240
y5 -> 561.312
y6 -> 809.433
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y1
1127.532
Y0
924.455
Y0
1172.548
Y1
1375.633
1289.587
1330.613
1492.667
1580.689
1654.714
1273.617
1476.649
204.086
274.092
366.139
380.146
292.102
326.170
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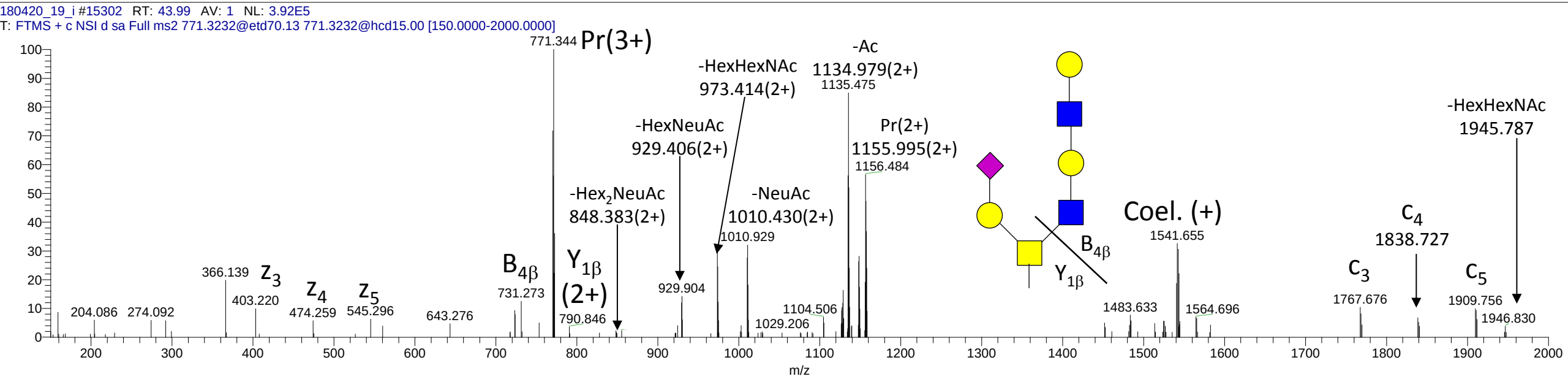
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-HexHexNAc
973.414(2+)
-Ac
1134.979(2+)
1135.475
-HexNeuAc
929.406(2+)
Pr(2+)
1155.995(2+)
1156.484
-Hex2NeuAc
848.383(2+)
-NeuAc
1010.430(2+)
1010.929
-HexHexNAc
1945.787
Coel. (+)
1541.655
B4β
Y1β
Y1β
(2+)
B4β
731.273
z3
z4
z5
c3
c4
c5
1767.676
1838.727
1909.756
1946.830
366.139
403.220
474.259
545.296
204.086
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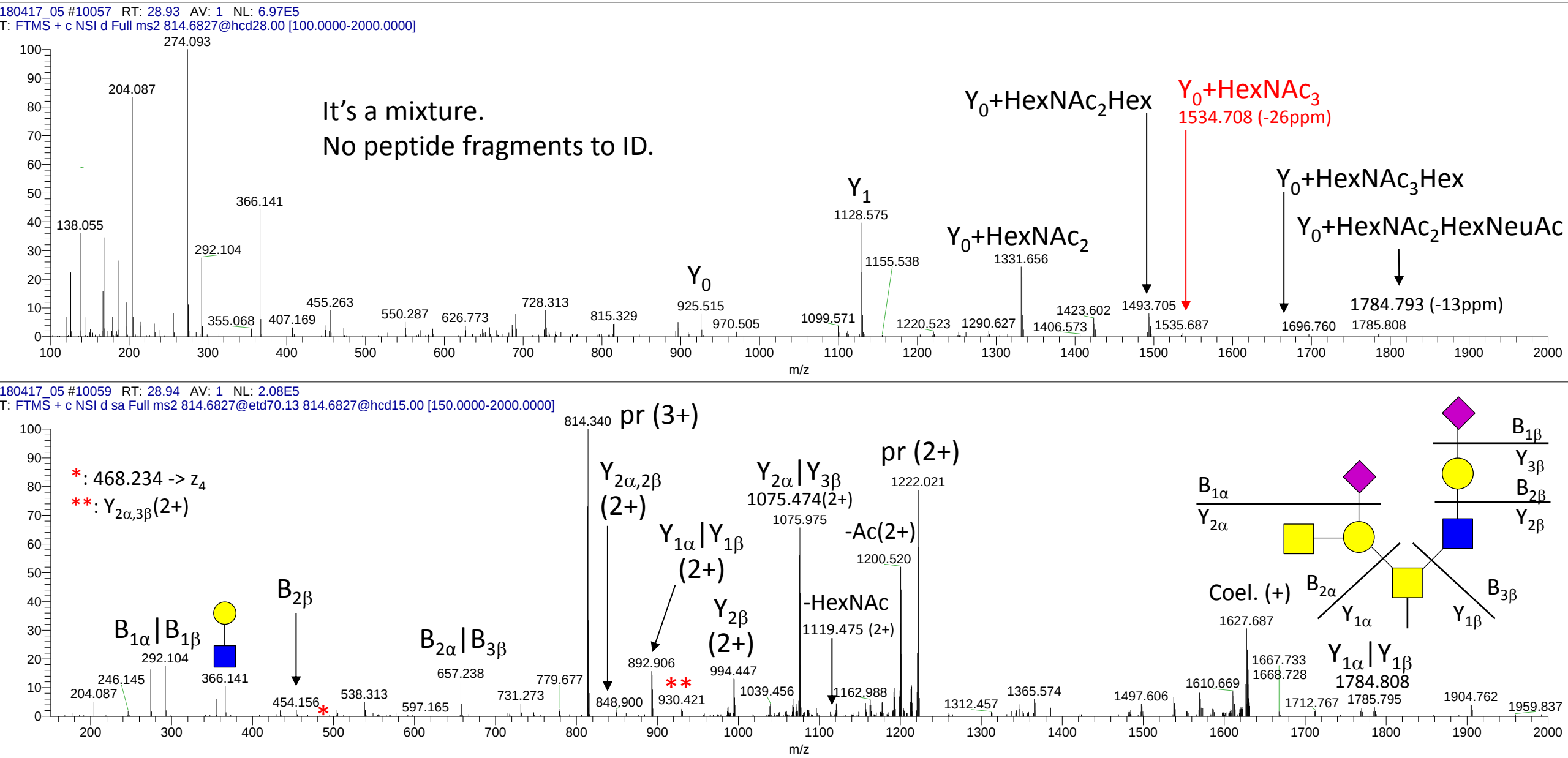
1029.206
1104.506
1483.633
1564.696
m/z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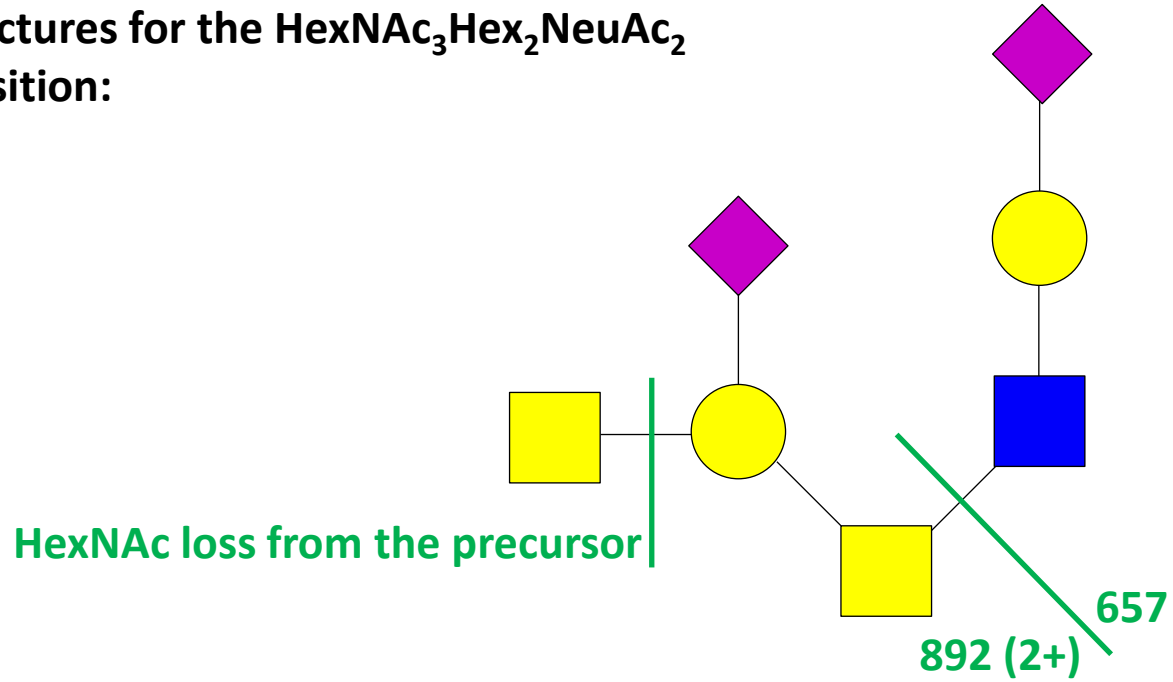
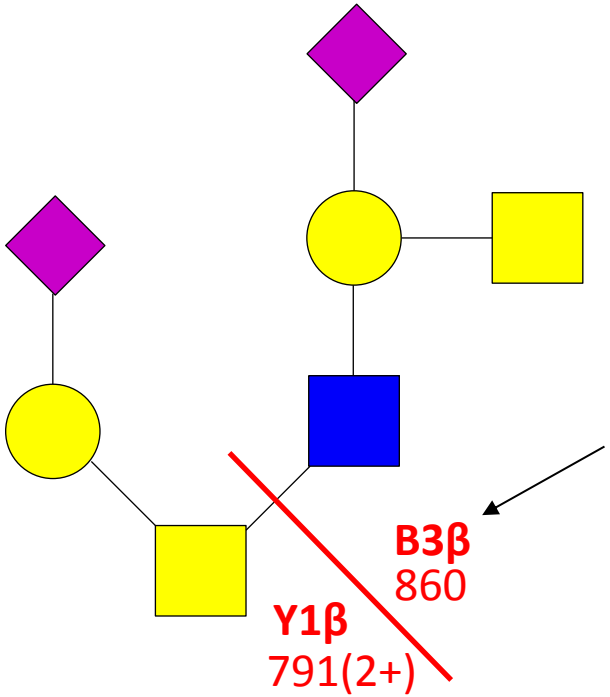
No peptide fragments to ID.
Y0+HexNAc2Hex
Y0+HexNAc3
1534.708 (-26ppm)
Y0+HexNAc3Hex
Y0+HexNAc2HexNeuAc
Y1
1128.575
Y0+HexNAc2
1331.656
Y0
925.515
1784.793 (-13ppm)
m/z
180417_05 #10059 RT: 28.94 AV: 1 NL: 2.08E5
T: FTMS + c NSI d sa Full ms2 814.6827@etd70.13 814.6827@hcd15.00 [150.0000-2000.0000]
*: 468.234 -> z4
**: Y2α,3β(2+)
814.340 pr (3+)
Y2α,2β (2+)
Y1α|Y1β (2+)
Y2α|Y3β
1075.474(2+)
1075.975
pr (2+)
1222.021
-Ac(2+)
1200.520
-HexNAc
1119.475 (2+)
Y2β (2+)
B2β
B1α|B1β
B2α|B3β
B1β
Y3β
B1α
Y2α
B2β
Y2β
Coel. (+)
B2α
B3β
Y1α
Y1β
Y1α|Y1β
1784.808
m/z

Potential other structures for the $HexNAc_3Hex_2NeuAc_2$ (1515.5346) composition:

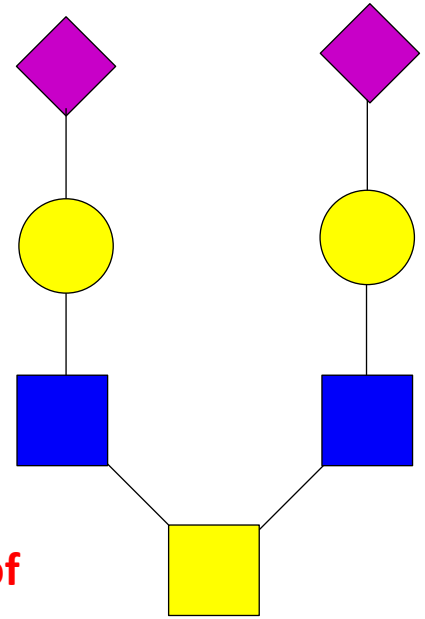


The most likely structure

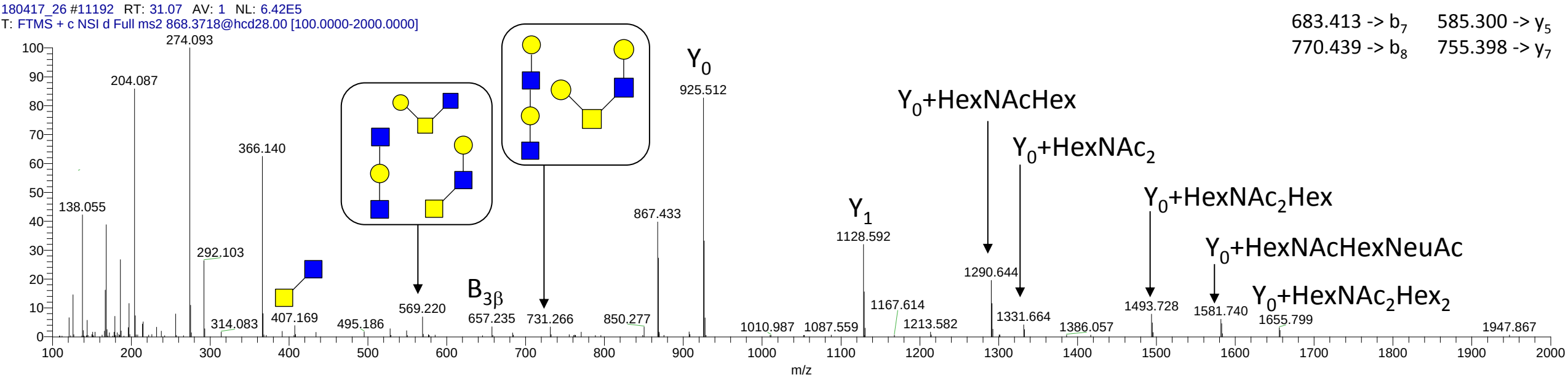


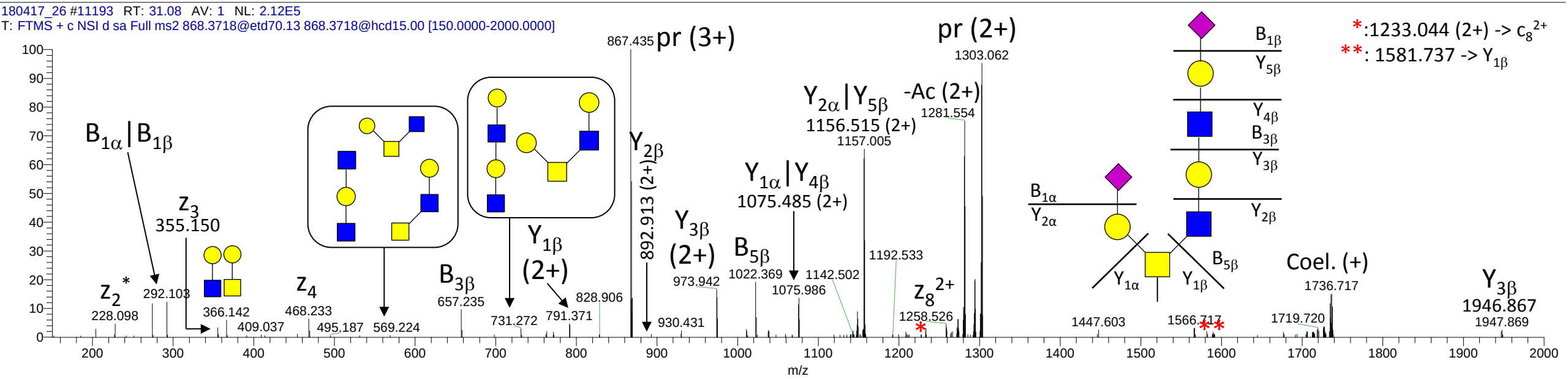
Was not detected

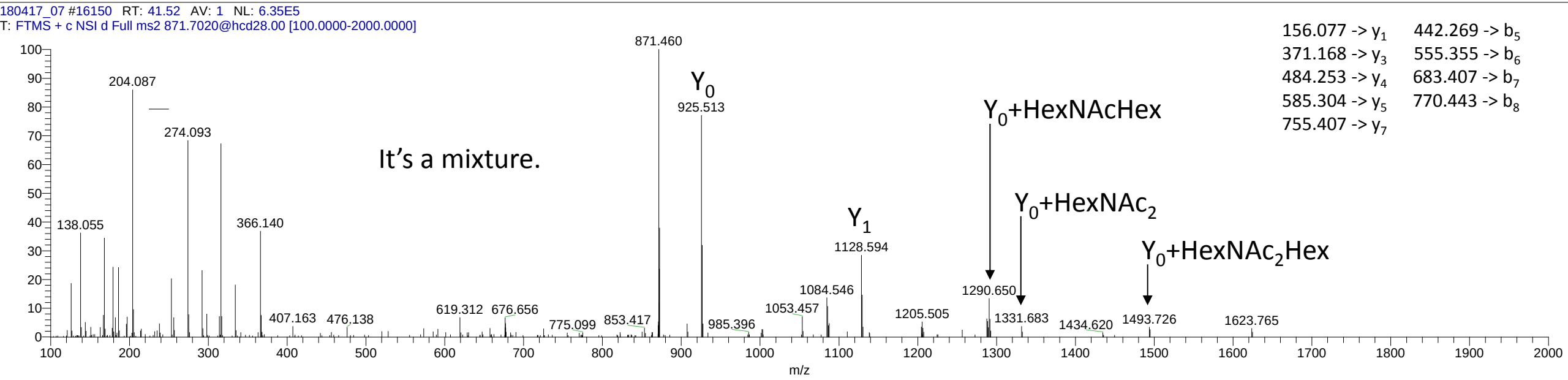
Other options

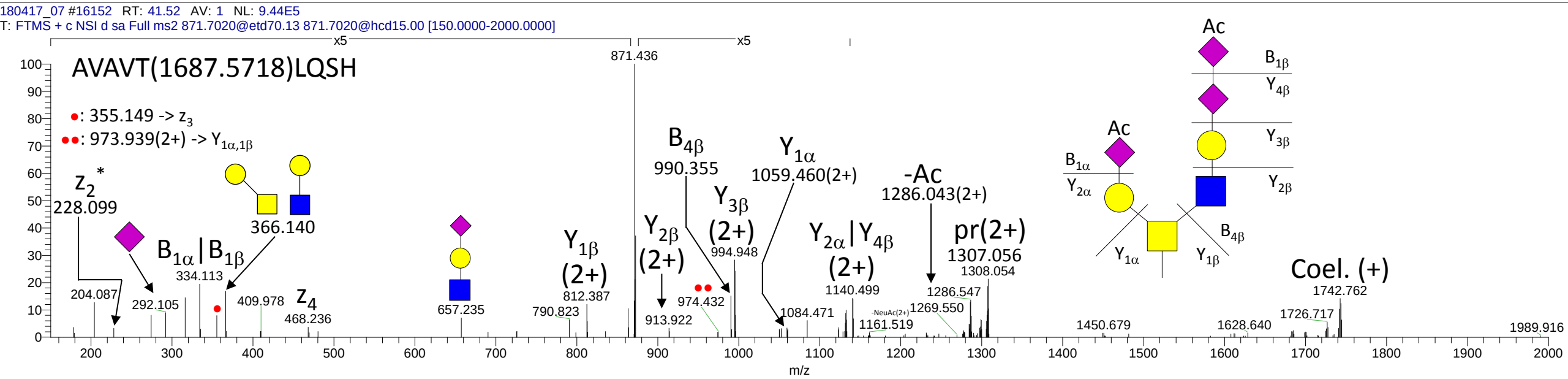


The HCD fragment that could prove this structure (peptide+$HexNAc_3$) is outside of the acceptable mass accuracy range.

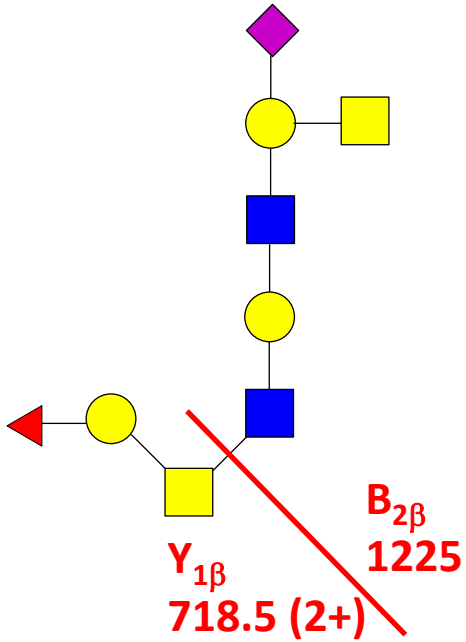

180417_26 #11192 RT: 31.07 AV: 1 NL: 6.42E5
T: FTMS + c NSI d Full ms2 868.3718@hcd28.00 [100.0000-2000.0000]
683.413 -> b7
770.439 -> b8
585.300 -> y5
755.398 -> y7
Y0
925.512
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y1
1128.592
B3β
657.235
274.093
204.087
366.140
138.055
292.103
407.169
314.083
495.186
569.220
731.266
850.277
867.433
1010.987
1087.559
1167.614
1213.582
1290.644
1331.664
1386.057
1493.728
1581.740
1655.799
1947.867
m/z

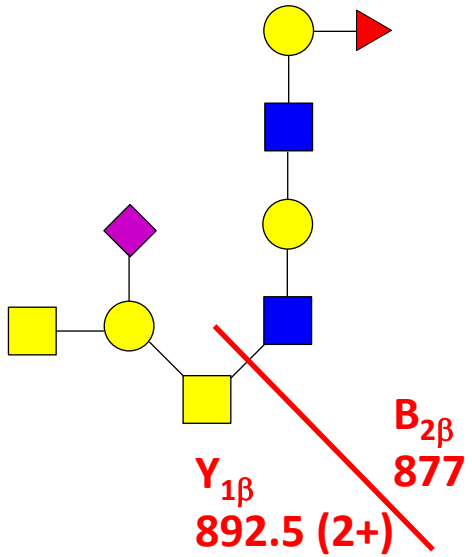

180417_26 #11193 RT: 31.08 AV: 1 NL: 2.12E5
T: FTMS + c NSI d sa Full ms2 868.3718@etd70.13 868.3718@hcd15.00 [150.0000-2000.0000]
*:1233.044 (2+) -> c8 2+
**: 1581.737 -> Y1β
B1α | B1β
z3
355.150
z2 *
228.098
292.103
366.142
409.037
z4
468.233
495.187
569.224
B3β
657.235
Y1β
(2+)
731.272
791.371
828.906
867.435 pr (3+)
892.913 (2+)
Y2β
930.431
Y3β
(2+)
973.942
Y1α | Y4β
1075.485 (2+)
B5β
1022.369
1075.986
1142.502
Y2α | Y5β
1156.515 (2+)
1157.005
-Ac (2+)
1281.554
pr (2+)
1303.062
1192.533
z8 2+
1258.526
B1β
Y5β
Y4β
B3β
Y3β
Y2β
B1α
Y2α
B5β
Y1α
Y1β
Coel. (+)
1736.717
1447.603
1566.717
1719.720
Y3β
1946.867
1947.869
m/z

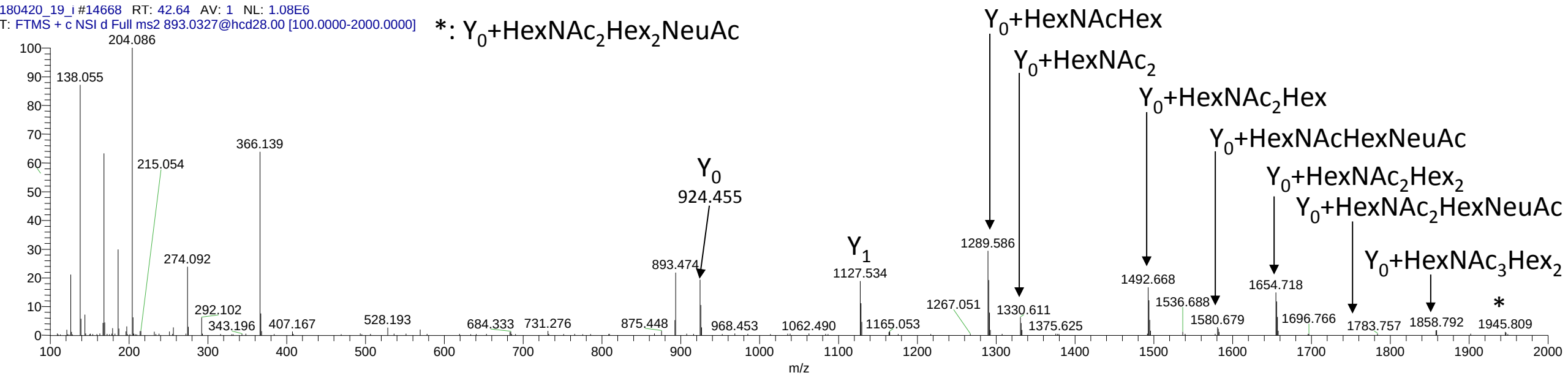

180417_07 #16150 RT: 41.52 AV: 1 NL: 6.35E5
T: FTMS + c NSI d Full ms2 871.7020@hcd28.00 [100.0000-2000.0000]
156.077 -> y1
371.168 -> y3
484.253 -> y4
585.304 -> y5
755.407 -> y7
442.269 -> b5
555.355 -> b6
683.407 -> b7
770.443 -> b8
It's a mixture.
Y0
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y1
m/z

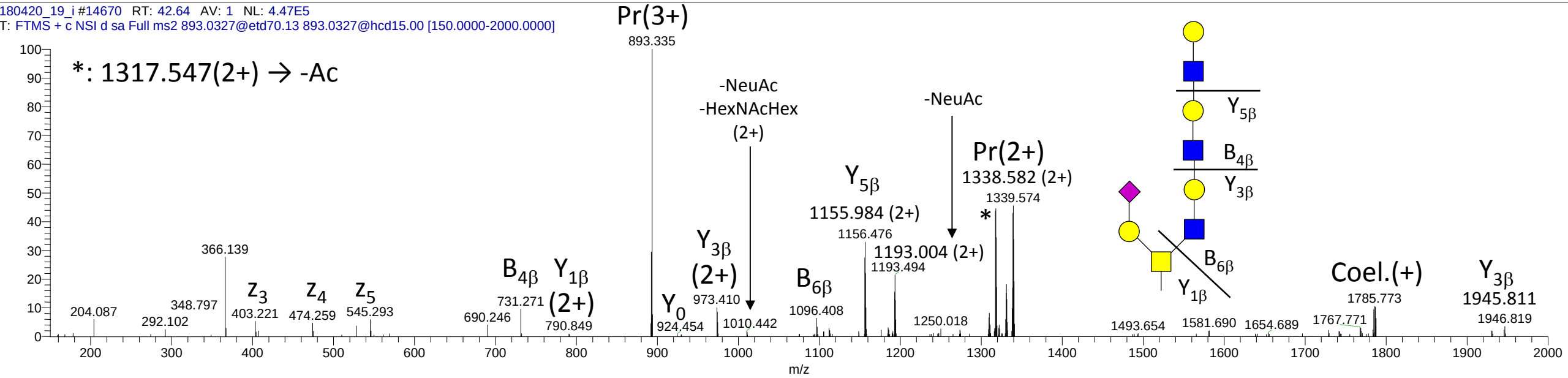

180417_07 #16152 RT: 41.52 AV: 1 NL: 9.44E5
T: FTMS + c NSI d sa Full ms2 871.7020@etd70.13 871.7020@hcd15.00 [150.0000-2000.0000]
AVAVT(1687.5718)LQSH
●: 355.149 -> z3
●●: 973.939(2+) -> Y1α,1β
z2*
228.099
B1α|B1β
334.113
366.140
Y1β (2+)
812.387
B4β
990.355
Y2β (2+)
913.922
Y3β (2+)
994.948
Y1α
1059.460(2+)
Y2α|Y4β (2+)
1140.499
-Ac
1286.043(2+)
pr(2+)
1307.056
1308.054
Coel. (+)
1742.762
Ac
B1β
Y4β
Y3β
Y2β
B4β
Y1β
B1α
Y2α
Y1α
m/z

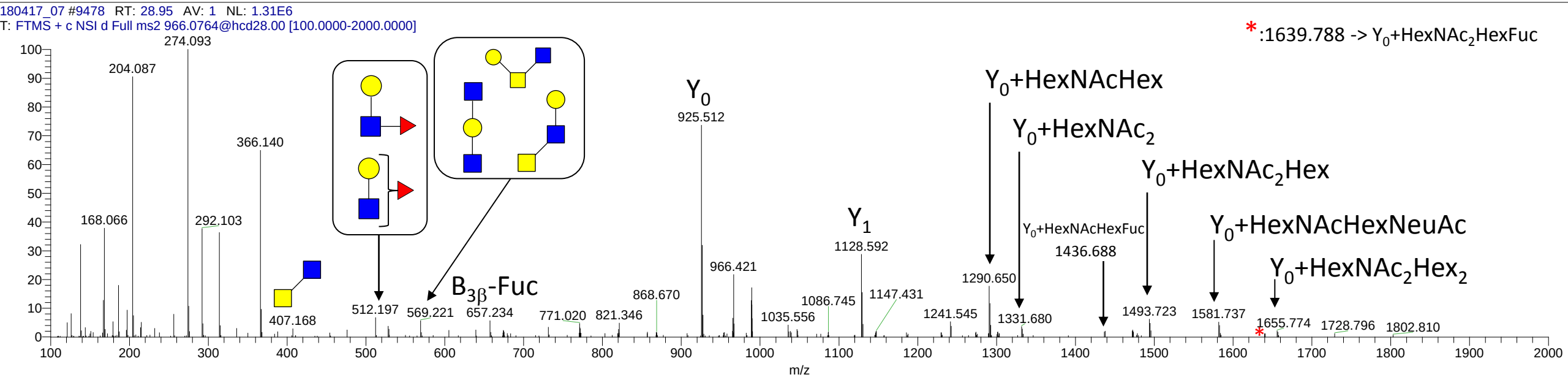
Another potential structure for the $HexNAc_4Hex_3FucNeuAc$ (1735.6293) composition:
(The most likely structure and the supporting EThcD spectrum can be found in the article as Figure 2.)

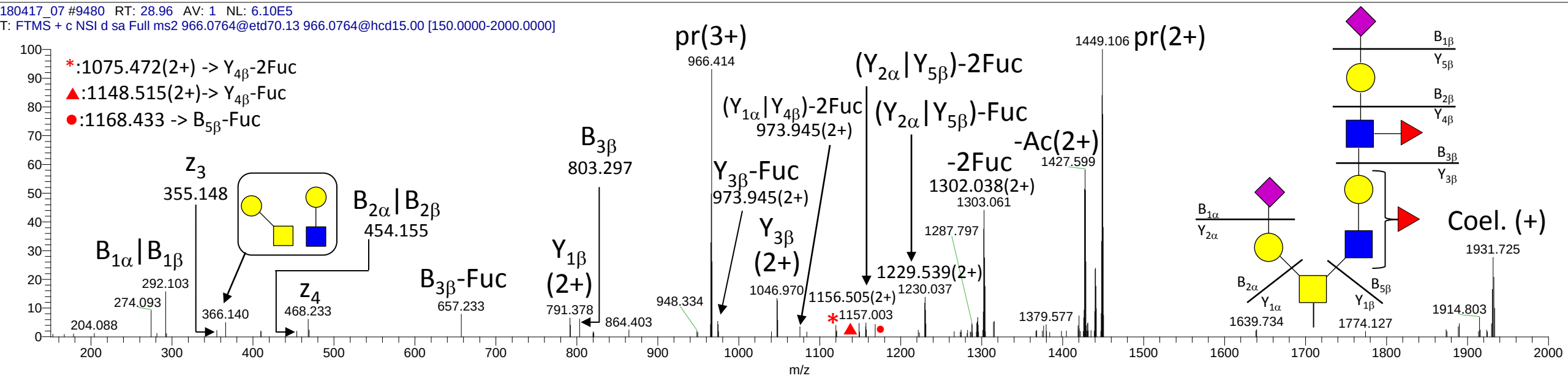


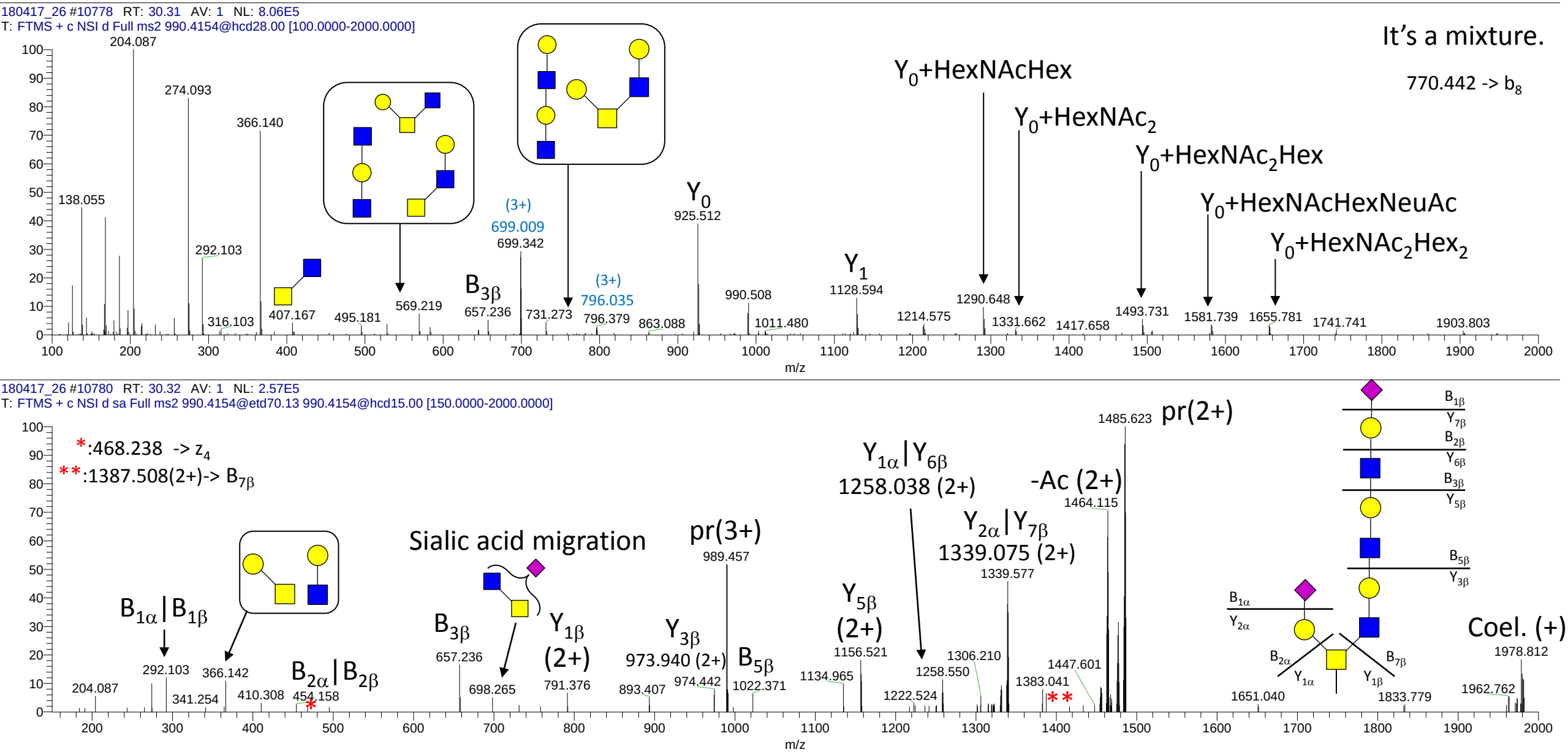


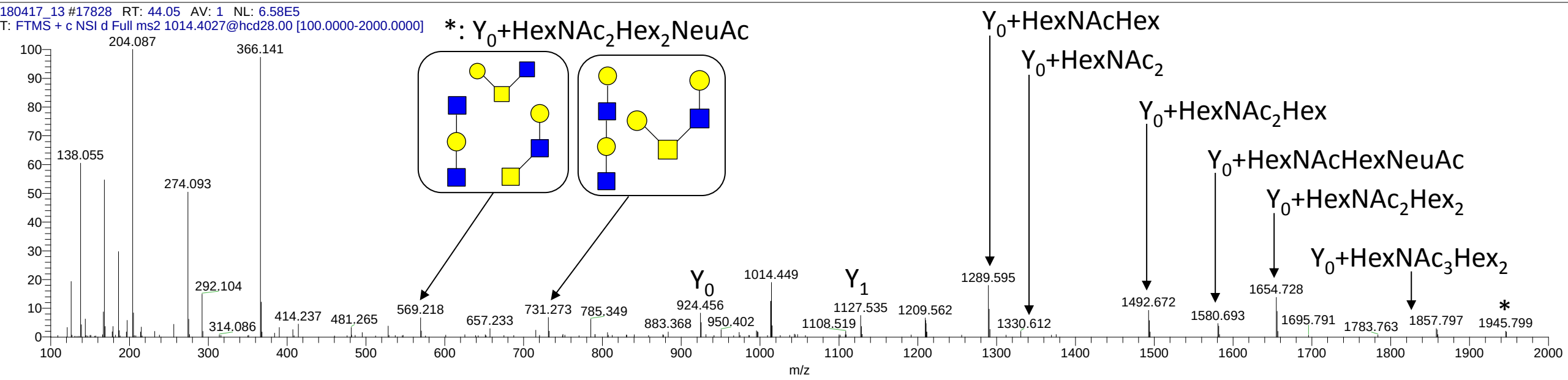

180420_19_i #14668 RT: 42.64 AV: 1 NL: 1.08E6
T: FTMS + c NSI d Full ms2 893.0327@hcd28.00 [100.0000-2000.0000]
*: Y0+HexNAc2Hex2NeuAc
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y0+HexNAc2HexNeuAc
Y0+HexNAc3Hex2
Y0 924.455
Y1 1127.534
204.086
138.055
366.139
215.054
274.092
292.102
343.196
407.167
528.193
684.333
731.276
875.448
893.474
968.453
1062.490
1165.053
1267.051
1289.586
1330.611
1375.625
1492.668
1536.688
1580.679
1654.718
1696.766
1783.757
1858.792
1945.809
m/z

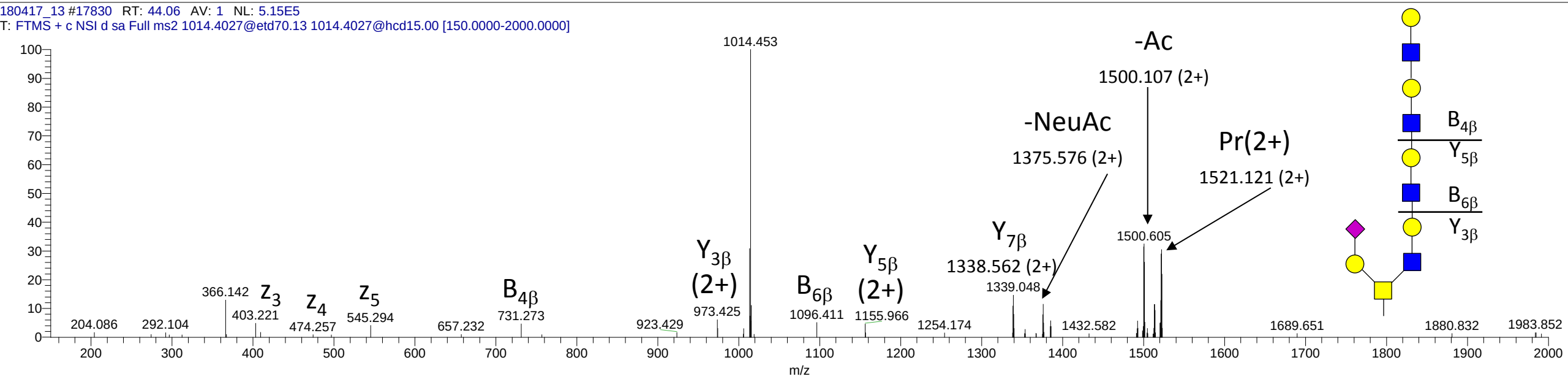

180420_19_i #14670 RT: 42.64 AV: 1 NL: 4.47E5
T: FTMS + c NSI d sa Full ms2 893.0327@etd70.13 893.0327@hcd15.00 [150.0000-2000.0000]
*: 1317.547(2+) → -Ac
Pr(3+) 893.335
-NeuAc -HexNAcHex (2+)
-NeuAc
Pr(2+) 1338.582 (2+)
Y5β 1155.984 (2+)
Y3β (2+)
B4β Y1β (2+)
B6β
Y0 924.454
973.410
1010.442
1096.408
1156.476
1193.004 (2+)
1193.494
1250.018
1339.574
366.139
204.087
292.102
348.797
403.221
474.259
545.293
690.246
731.271
790.849
Y5β
B4β
Y3β
B6β
Y1β
Coel.(+) 1785.773
Y3β 1945.811
1946.819
1493.654
1581.690
1654.689
1767.771
m/z

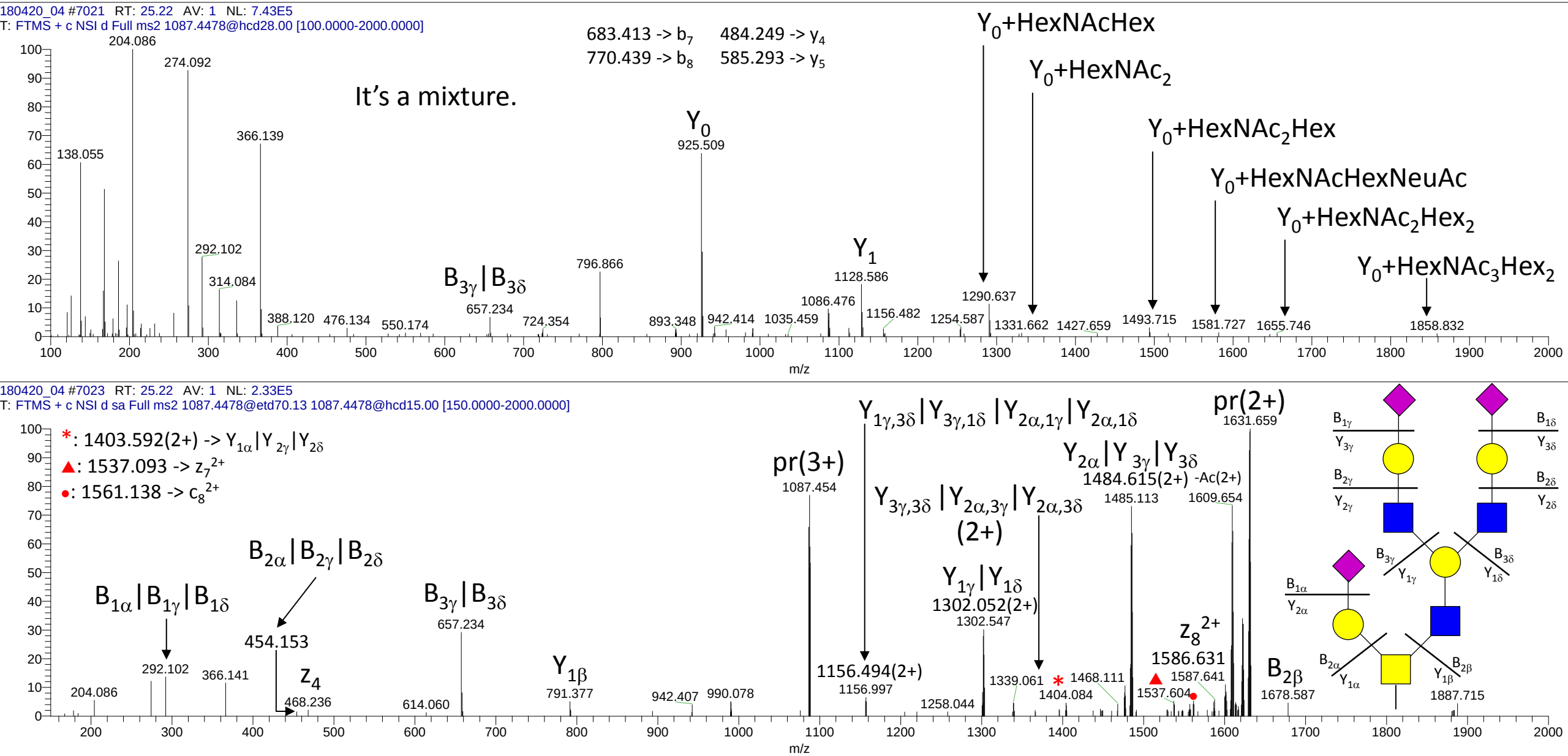

180417_07 #9478 RT: 28.95 AV: 1 NL: 1.31E6
T: FTMS + c NSI d Full ms2 966.0764@hcd28.00 [100.0000-2000.0000]
*:1639.788 -> Y0+HexNAc2HexFuc
Y0
925.512
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y0+HexNAcHexFuc
Y1
1128.592
B3β-Fuc
274.093
204.087
366.140
168.066
292.103
407.168
512.197
569.221
657.234
771.020
821.346
868.670
966.421
1035.556
1086.745
1147.431
1241.545
1290.650
1331.680
1436.688
1493.723
1581.737
1655.774
1728.796
1802.810
m/z

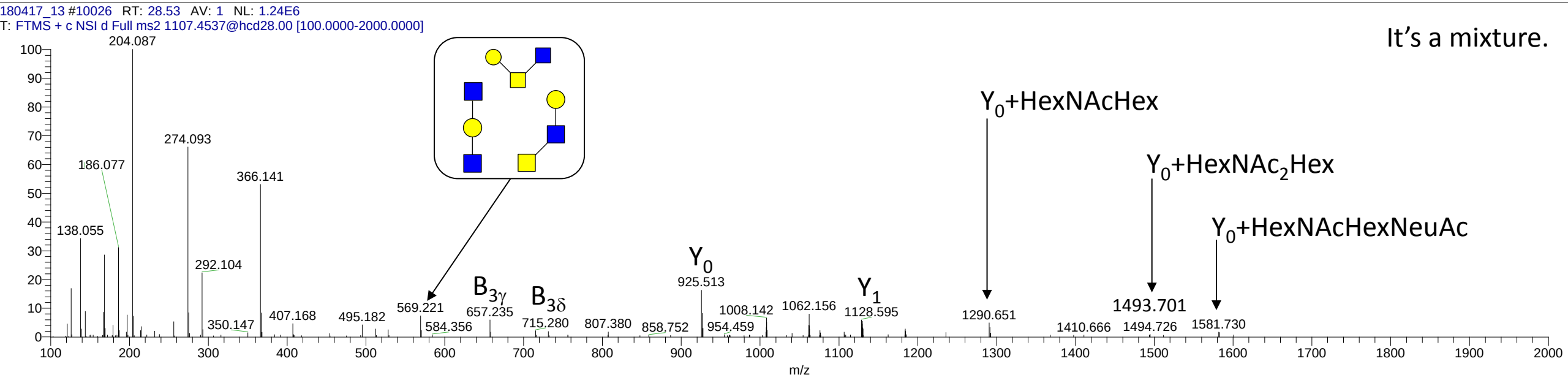

180417_07 #9480 RT: 28.96 AV: 1 NL: 6.10E5
T: FTMS + c NSI d sa Full ms2 966.0764@etd70.13 966.0764@hcd15.00 [150.0000-2000.0000]
*:1075.472(2+) -> Y4β-2Fuc
▲:1148.515(2+)-> Y4β-Fuc
●:1168.433 -> B5β-Fuc
pr(3+)
966.414
pr(2+)
1449.106
(Y2α|Y5β)-2Fuc
(Y1α|Y4β)-2Fuc
973.945(2+)
(Y2α|Y5β)-Fuc
-Ac(2+)
1427.599
-2Fuc
1302.038(2+)
1303.061
Y3β-Fuc
973.945(2+)
Y3β
(2+)
1229.539(2+)
1230.037
1287.797
B3β
803.297
Y1β
(2+)
791.378
z3
355.148
B2α|B2β
454.155
B1α|B1β
292.103
274.093
204.088
366.140
z4
468.233
B3β-Fuc
657.233
948.334
864.403
1046.970
1156.505(2+)
*1157.003
1379.577
Coel. (+)
1931.725
1914.803
1639.734
1774.127
B1β
Y5β
B2β
Y4β
B3β
Y3β
B1α
Y2α
B2α
Y1α
B5β
Y1β
m/z

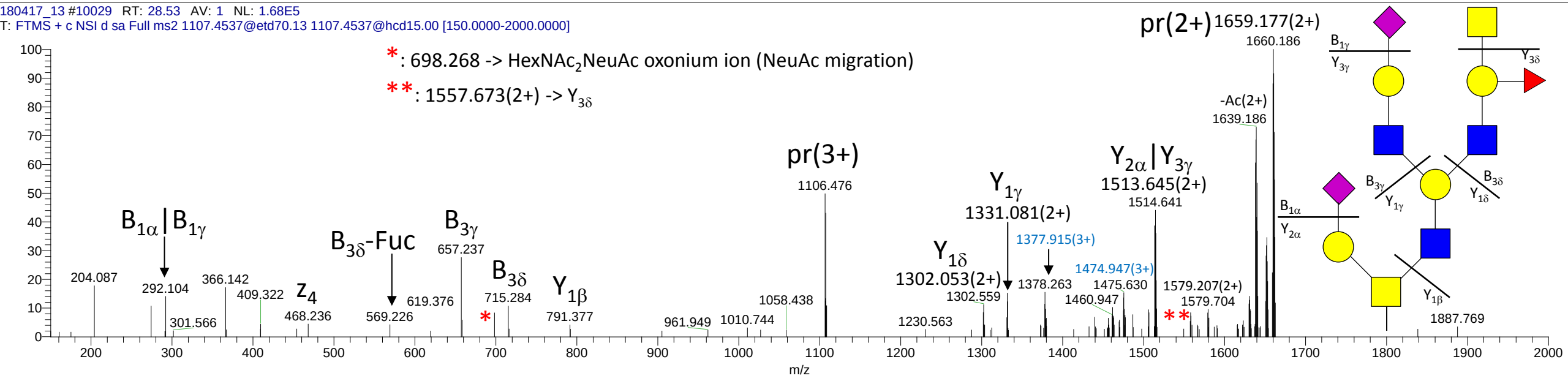

180417_26 #10778 RT: 30.31 AV: 1 NL: 8.06E5
T: FTMS + c NSI d Full ms2 990.4154@hcd28.00 [100.0000-2000.0000]
It's a mixture.
770.442 -> b8
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
204.087
274.093
366.140
138.055
292.103
316.103
407.167
495.181
569.219
(3+)
699.009
699.342
B3β
657.236
731.273
(3+)
796.035
796.379
863.088
Y0
925.512
990.508
1011.480
Y1
1128.594
1214.575
1290.648
1331.662
1417.658
1493.731
1581.739
1655.781
1741.741
1903.803
m/z
180417_26 #10780 RT: 30.32 AV: 1 NL: 2.57E5
T: FTMS + c NSI d sa Full ms2 990.4154@etd70.13 990.4154@hcd15.00 [150.0000-2000.0000]
*:468.238 -> z4
**:1387.508(2+)-> B7β
B1α|B1β
292.103
366.142
204.087
341.254
410.308
B2α|B2β
454.158
Sialic acid migration
B3β
657.236
698.265
Y1β
(2+)
791.376
Y3β
973.940 (2+)
893.407
974.442
pr(3+)
989.457
B5β
1022.371
Y5β
(2+)
1156.521
1134.965
Y1α|Y6β
1258.038 (2+)
1258.550
1222.524
Y2α|Y7β
1339.075 (2+)
1339.577
1306.210
-Ac (2+)
1464.115
1485.623
pr(2+)
1447.601
1383.041
**
B1β
Y7β
B2β
Y6β
B3β
Y5β
B5β
Y3β
B1α
Y2α
B2α
Y1α
Y1β
B7β
1651.040
1833.779
1978.812
1962.762
Coel. (+)
m/z


180417_13 #17828 RT: 44.05 AV: 1 NL: 6.58E5
T: FTMS + c NSI d Full ms2 1014.4027@hcd28.00 [100.0000-2000.0000]
*: Y0+HexNAc2Hex2NeuAc
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y0+HexNAc3Hex2
204.087
366.141
138.055
274.093
292.104
314.086
414.237
481.265
569.218
657.233
731.273
785.349
883.368
924.456
950.402
1014.449
1108.519
1127.535
1209.562
1289.595
1330.612
1492.672
1580.693
1654.728
1695.791
1783.763
1857.797
1945.799
Y0
Y1
m/z


180417_13 #17830 RT: 44.06 AV: 1 NL: 5.15E5
T: FTMS + c NSI d sa Full ms2 1014.4027@etd70.13 1014.4027@hcd15.00 [150.0000-2000.0000]
1014.453
-Ac
1500.107 (2+)
-NeuAc
1375.576 (2+)
Pr(2+)
1521.121 (2+)
Y7β
1338.562 (2+)
1339.048
1500.605
Y3β
(2+)
973.425
B6β
1096.411
Y5β
(2+)
1155.966
B4β
731.273
366.142
z3
403.221
z4
474.257
z5
545.294
204.086
292.104
657.232
923.429
1254.174
1432.582
1689.651
1880.832
1983.852
B4β
Y5β
B6β
Y3β
m/z

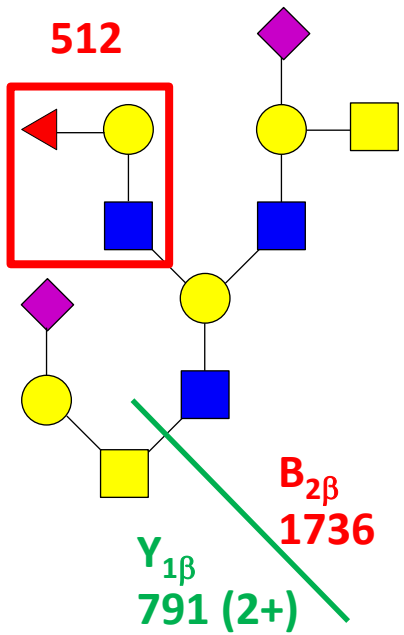

180420_04 #7021 RT: 25.22 AV: 1 NL: 7.43E5
T: FTMS + c NSI d Full ms2 1087.4478@hcd28.00 [100.0000-2000.0000]
683.413 -> b7
770.439 -> b8
484.249 -> y4
585.293 -> y5
It's a mixture.
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y0+HexNAc3Hex2
Y0
925.509
Y1
1128.586
B3γ|B3δ
657.234
204.086
274.092
366.139
138.055
292.102
314.084
388.120
476.134
550.174
724.354
796.866
893.348
942.414
1035.459
1086.476
1156.482
1254.587
1290.637
1331.662
1427.659
1493.715
1581.727
1655.746
1858.832
m/z
180420_04 #7023 RT: 25.22 AV: 1 NL: 2.33E5
T: FTMS + c NSI d sa Full ms2 1087.4478@etd70.13 1087.4478@hcd15.00 [150.0000-2000.0000]
*: 1403.592(2+) -> Y1α|Y2γ|Y2δ
▲: 1537.093 -> z7 2+
●: 1561.138 -> c8 2+
Y1γ,3δ|Y3γ,1δ|Y2α,1γ|Y2α,1δ
pr(2+)
1631.659
Y2α|Y3γ|Y3δ
1484.615(2+) -Ac(2+)
1485.113
1609.654
pr(3+)
1087.454
Y3γ,3δ|Y2α,3γ|Y2α,3δ
(2+)
B2α|B2γ|B2δ
B1α|B1γ|B1δ
B3γ|B3δ
657.234
Y1γ|Y1δ
1302.052(2+)
1302.547
454.153
z4
468.236
292.102
366.141
204.086
614.060
Y1β
791.377
942.407
990.078
1156.494(2+)
1156.997
1258.044
1339.061
1404.084
1468.111
z8 2+
1586.631
1587.641
1537.604
B2β
1678.587
1887.715
m/z

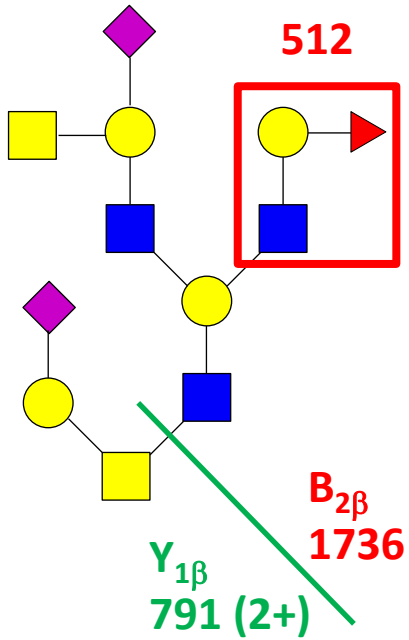

180417_13 #10026 RT: 28.53 AV: 1 NL: 1.24E6
T: FTMS + c NSI d Full ms2 1107.4537@hcd28.00 [100.0000-2000.0000]
It's a mixture.
Y0+HexNAcHex
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
204.087
274.093
186.077
366.141
138.055
292.104
350.147
407.168
495.182
569.221
584.356
B3γ
657.235
B3δ
715.280
807.380
858.752
Y0
925.513
954.459
1008.142
1062.156
Y1
1128.595
1290.651
1410.666
1493.701
1494.726
1581.730
m/z

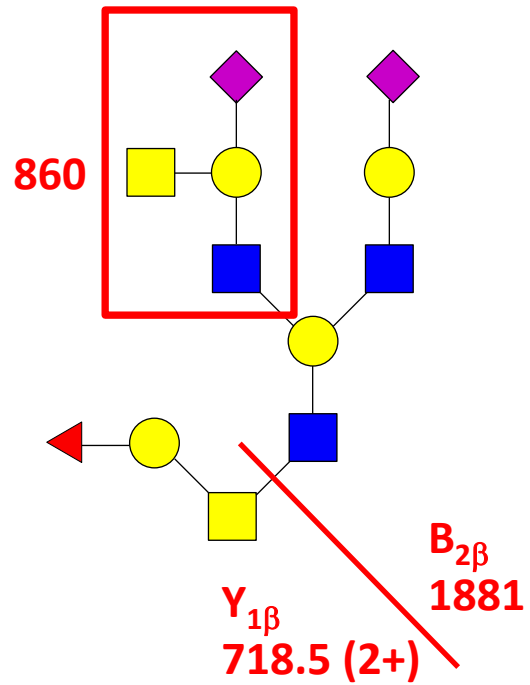

180417_13 #10029 RT: 28.53 AV: 1 NL: 1.68E5
T: FTMS + c NSI d sa Full ms2 1107.4537@etd70.13 1107.4537@hcd15.00 [150.0000-2000.0000]
*: 698.268 -> HexNAc2NeuAc oxonium ion (NeuAc migration)
**: 1557.673(2+) -> Y3δ
pr(2+) 1659.177(2+)
1660.186
-Ac(2+)
1639.186
B1γ
Y3γ
Y3δ
B3γ
Y1γ
B3δ
Y1δ
B1α
Y2α
Y1β
1887.769
pr(3+)
1106.476
B1α | B1γ
204.087
292.104
301.566
366.142
409.322
z4
468.236
B3δ-Fuc
569.226
619.376
B3γ
657.237
B3δ
715.284
*
Y1β
791.377
961.949
1010.744
1058.438
Y1γ
1331.081(2+)
1377.915(3+)
Y2α | Y3γ
1513.645(2+)
1514.641
Y1δ
1302.053(2+)
1302.559
1230.563
1378.263
1460.947
1474.947(3+)
1475.630
1579.207(2+)
1579.704
**
m/z

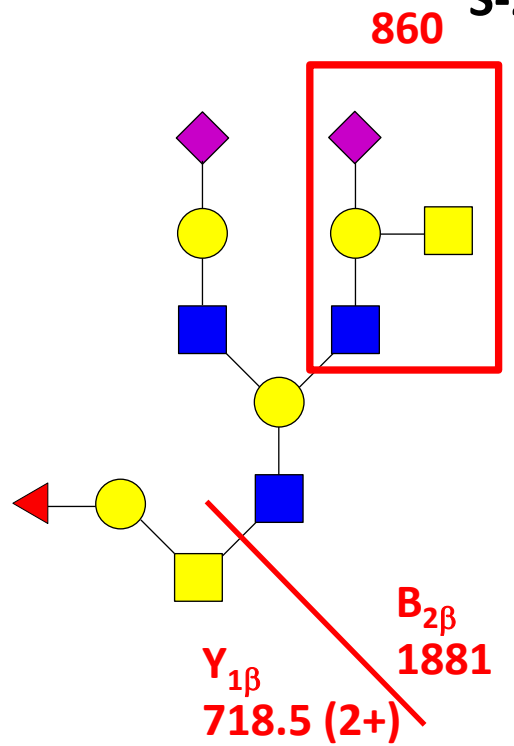
Additional potential structures for the $HexNAc_5Hex_4FucNeuAc_2$ (2391.8569) composition:

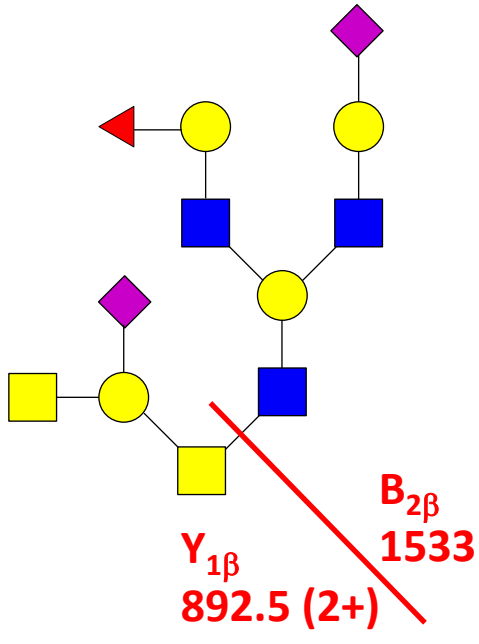
Fragments in red are missing from the EThcD spectrum

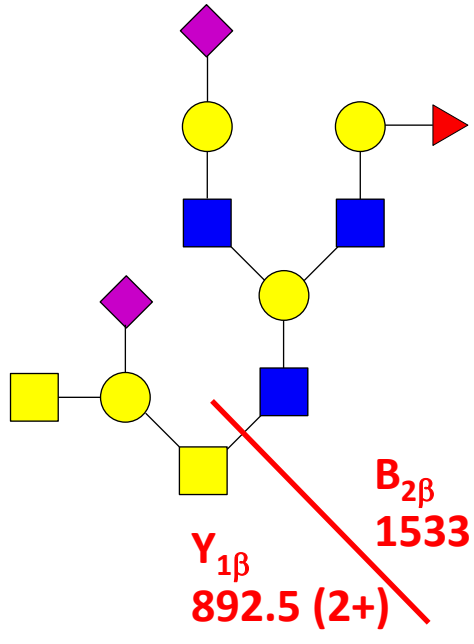


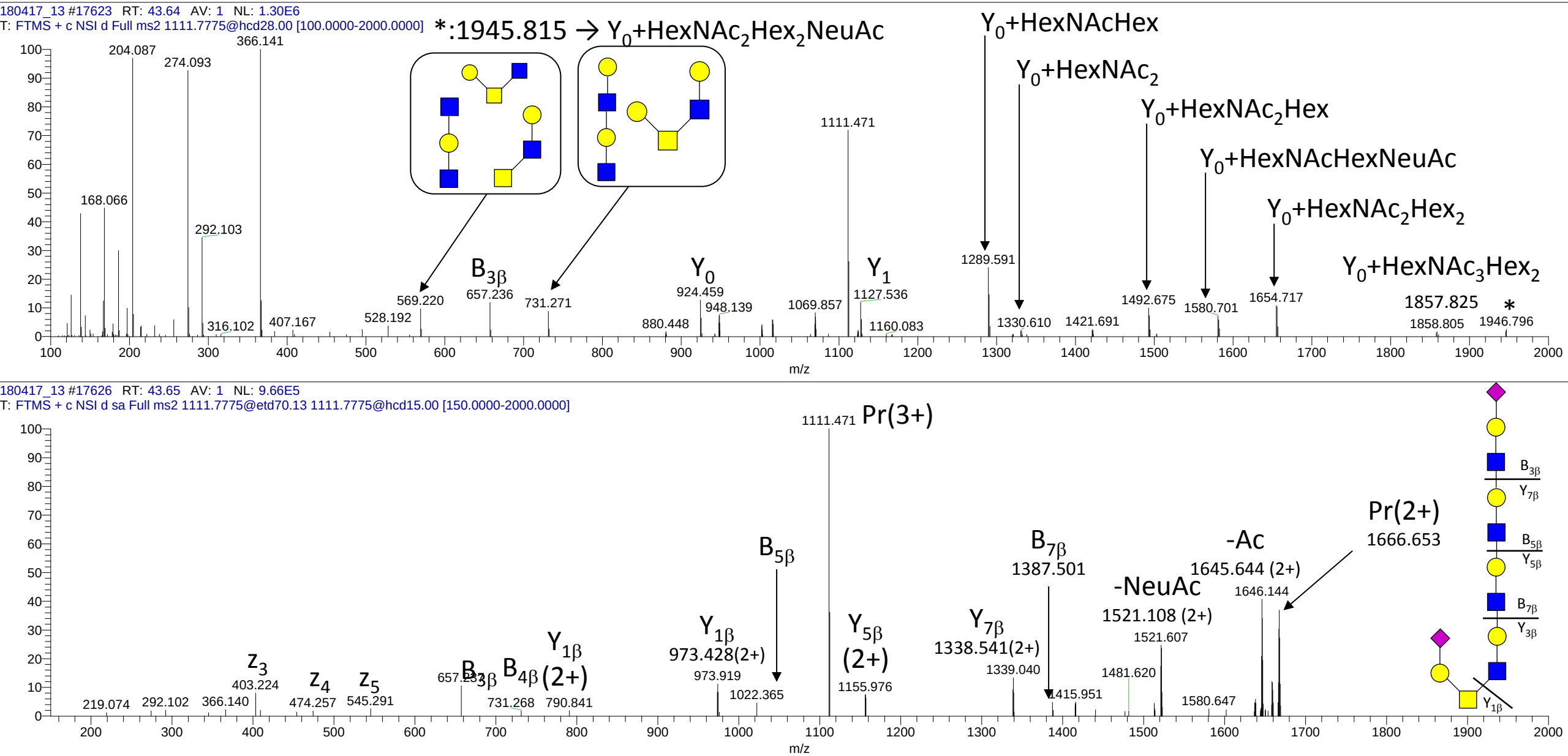


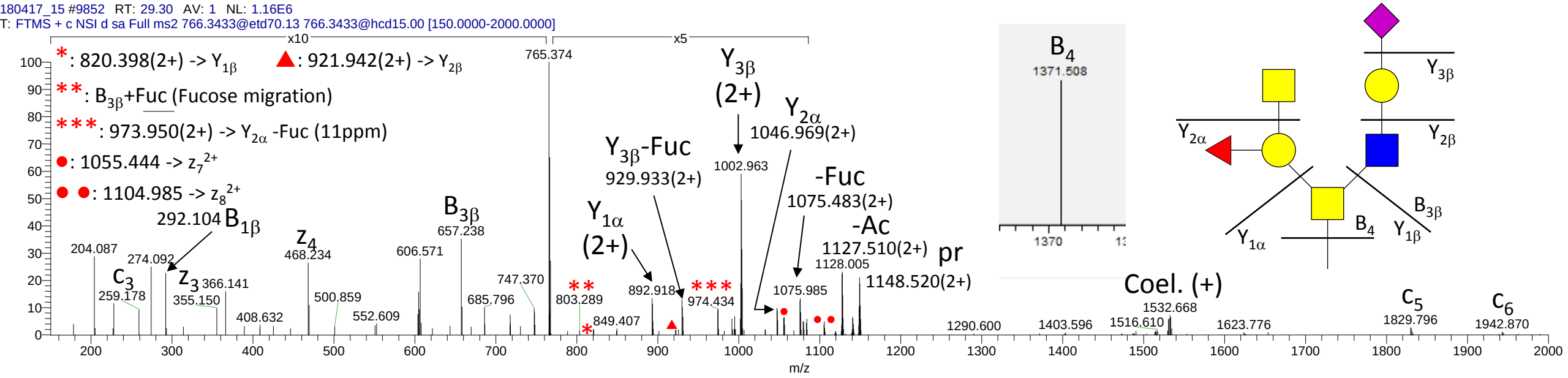


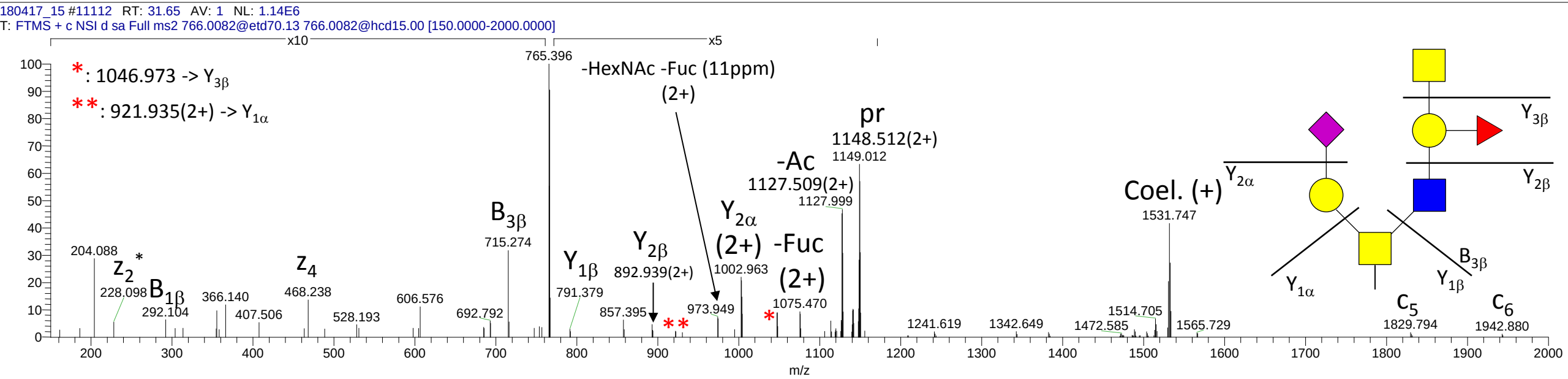







180417_13 #17623 RT: 43.64 AV: 1 NL: 1.30E6
T: FTMS + c NSI d Full ms2 1111.7775@hcd28.00 [100.0000-2000.0000]
*:1945.815 → Y0+HexNAc2Hex2NeuAc
Y0+HexNAcHex
Y0+HexNAc2
Y0+HexNAc2Hex
Y0+HexNAcHexNeuAc
Y0+HexNAc2Hex2
Y0+HexNAc3Hex2
204.087
274.093
366.141
168.066
292.103
316.102
407.167
528.192
569.220
B3β
657.236
731.271
Y0
924.459
948.139
880.448
1069.857
1111.471
Y1
1127.536
1160.083
1289.591
1330.610
1421.691
1492.675
1580.701
1654.717
1857.825
1858.805
1946.796
m/z
180417_13 #17626 RT: 43.65 AV: 1 NL: 9.66E5
T: FTMS + c NSI d sa Full ms2 1111.7775@etd70.13 1111.7775@hcd15.00 [150.0000-2000.0000]
1111.471 Pr(3+)
B5β
Y1β
973.428(2+)
973.919
1022.365
Y5β
(2+)
1155.976
B7β
1387.501
Y7β
1338.541(2+)
1339.040
1415.951
-NeuAc
1521.108 (2+)
1521.607
1481.620
-Ac
1645.644 (2+)
1646.144
1580.647
Pr(2+)
1666.653
z3
403.224
z4
474.257
z5
545.291
219.074
292.102
366.140
B3β
B4β (2+)
657.233
731.268
790.841
B3β
Y7β
B5β
Y5β
B7β
Y3β
Y1β
m/z


180417_15 #9852 RT: 29.30 AV: 1 NL: 1.16E6
T: FTMS + c NSI d sa Full ms2 766.3433@etd70.13 766.3433@hcd15.00 [150.0000-2000.0000]
x10
x5
*: 820.398(2+) -> Y1β
▲: 921.942(2+) -> Y2β
**: B3β+Fuc (Fucose migration)
***: 973.950(2+) -> Y2α -Fuc (11ppm)
●: 1055.444 -> z7 2+
● ●: 1104.985 -> z8 2+
292.104 B1β
204.087
274.092
c3
259.178
z3
355.150
366.141
408.632
z4
468.234
500.859
552.609
606.571
B3β
657.238
685.796
747.370
765.374
803.289
849.407
Y1α
(2+)
892.918
Y3β-Fuc
929.933(2+)
974.434
Y3β
(2+)
1002.963
Y2α
1046.969(2+)
1075.985
-Fuc
1075.483(2+)
-Ac
1127.510(2+)
1128.005
pr
1148.520(2+)
1290.600
1403.596
1516.610
1532.668
1623.776
Coel. (+)
c5
1829.796
c6
1942.870
m/z
B4
1371.508
1370
Y3β
Y2α
Y2β
B3β
B4
Y1α
Y1β


180417_15 #11112 RT: 31.65 AV: 1 NL: 1.14E6
T: FTMS + c NSI d sa Full ms2 766.0082@etd70.13 766.0082@hcd15.00 [150.0000-2000.0000]
x10
x5
*: 1046.973 -> Y3β
**: 921.935(2+) -> Y1α
765.396
-HexNAc -Fuc (11ppm)
(2+)
204.088
z2 *
228.098
B1β
292.104
366.140
407.506
z4
468.238
528.193
606.576
692.792
B3β
715.274
Y1β
791.379
Y2β
892.939(2+)
857.395
973.949
Y2α
(2+)
1002.963
-Fuc
(2+)
1075.470
-Ac
1127.509(2+)
1127.999
pr
1148.512(2+)
1149.012
1241.619
1342.649
1472.585
1514.705
Coel. (+)
1531.747
1565.729
c5
1829.794
c6
1942.880
m/z
Y3β
Y2α
Y2β
B3β
Y1α
Y1β

Additional potential structures for the $HexNAc_3Hex_2FucNeuAc$ (1370.4971) composition:

Fragments in red are missing from the EThcD spectrum

Neutral loss of HexNeuAc (921 (2+)) from the precursor is present which cannot be explained with this structure.