Legends to supplemental Figures

Supplemental Figure 1: Annotation of Srr1 sequence. The following colour code was used. Blue: signal peptide, yellow: peptide detected in MS experiments, gray: identified glycopeptide, green: LPXTG motif.

Supplemental Figures 2a to 2d: Automated identification of peptides after neutral loss event using X! Tandem . Detection parameters were set to detect fragmentation ions at low signal to noise ratio.

Supplemental Fig 2a: neutral loss of HexNAc on [234-247] peptide.

Supplemental Fig 2b: neutral Loss of HexNAc on [275-285] peptide.

Supplemental Fig 2c: neutral Loss of HexNAc on [214-233] peptide.

Supplemental Fig 2d: neutral Loss of *O*-AcHexNAc on [214-233] peptide.

The figures were obtained with the Global Proteome Machine XML data viewer. (http://bacteria.thegpm.org/tandem/thegpm_upview.html).

Supplemental Figures 3a-3t: **CID MS/MS spectra of glycopeptides described in Table 1.** Glycan symbols: HexNAc (Square), Hex (Circle), *O*-AcHexNAc (Crossed square).

Supplemental Figure 4: **Successive neutral loss events of [192-211] peptide modified with +3(HexNAc) detected by CID MS/MS cycles on LTQ-Orbitrap.** Precursor ion m/z 911.4414 z=3. Sample Srr1 MSn4. Scan 2236-2240. Peak noted * corresponds to the most abundant ion selected for next CID event.

Supplemental Figures 5a-5ag: HCD MS/MS spectra of glycopeptides described in Table 2. Glycan symbols: HexNAc (Square), Hex (Circle), *O*-AcHexNAc (Crossed square).

Supplemental Figure 6: **Extracted ion chromatograms (XIC) of MS spectra of non-glycosylated and glycosylated forms of peptide [192-211] peptide described in Table 2.** The reported data were extracted in the time window 29-39 min. The glycan modification is reported in each panel.

Supplemental figure 7: SDS PAGE of medium after overnight cultures of *S. agalactiae* (A) strain H36B *srtA** and (B) strain H36B *srtA** Δ*gtfCH.* Gel was stained with Coomassie Blue. Arrowheads points to Srr1 bands which were analyzed by MS. Note the apparent molecular weight difference between the two proteins which likely reflects the glycosylation default in H36B *srtA**Δ*gtfCH.*

Supplemental Figures 8a to 8j: ETD MS/MS spectra of glycopeptides described in Table 3. The deduced glycosylation sites are underlined. Z' = Z + H·.

MSQKTFGKQLTVVDTKSRVKMHKSGKNWVRTVMSHFNLFKAIKGRATVEADVCIQDVEKEDRLSSGNLTYLKGI
LAAGALVGGASLTSRVYADETPVVQEQSSSVPTLAEQTEVTVKTTTVQNHQDGTVSKNIIDSNSVSMSESASTS
TSESVSMSMSGSTLTSVSESVSTSALTSASESISTSASESVSKSTSISEVSNILETQASLTDKGRESFSANQIV
TESSLVTDAGKNASVSSLIEITKPKSELQTSKMSNESLITPEKSQVMIASDKTGNESLTPTIRLKSVIQPRSMN
LMTLSSEMDLIPLEEVSDTEMLGKDVSSELQKVNIALKDNTLSEPGTVKLDSSENLVLNFAFSIASVNEGDVFT
VKLSDNLDTQGIGTILKVQDIMDETGQLLATGSYSPLTHNITYTWTRYASTLNNIKARVNMPVWPDQRIISKTT
SDKQCFTATLNNQVASIEERVQYNSPSVTEHTNVKTNVRSRIMKLDDERQTETYITQINPEGKEMYFASGLGNL
YTIIGSDGTSGSPVNLLNAEVKILKTNSKNLTDSMDQNYDSPEFEDVTSQYSYTNDGSKITIDWKTNSISSTTS
YVVLVKIPKQSGVLYSTVSDINQTYGSKYSYGHTNISGDSDANAEIKLLSESASTSASTSASTSASMSASTSAS
TSASMSASTSASTSASMSASMSASTSASMSASTSASTSASMSASMSASTSASMSASTSASTSASTSASMSASTS
ASTSASTSASTSASTSTSTSASTSASTSASTSASMSASTSASTSASTSASMSASTSASISASTSASMSASTSAS
TSASTSASTSASMSASTSASTSASTSASTSASMSASTSASTSASTSASTSASTSASTSASTSASTSSSTSASTS
ASTSASTSASMSASTSASTSASMSASTSASTSASMSASTSASTSASMSASTSASTSASTSASMSASTSSSTSAS
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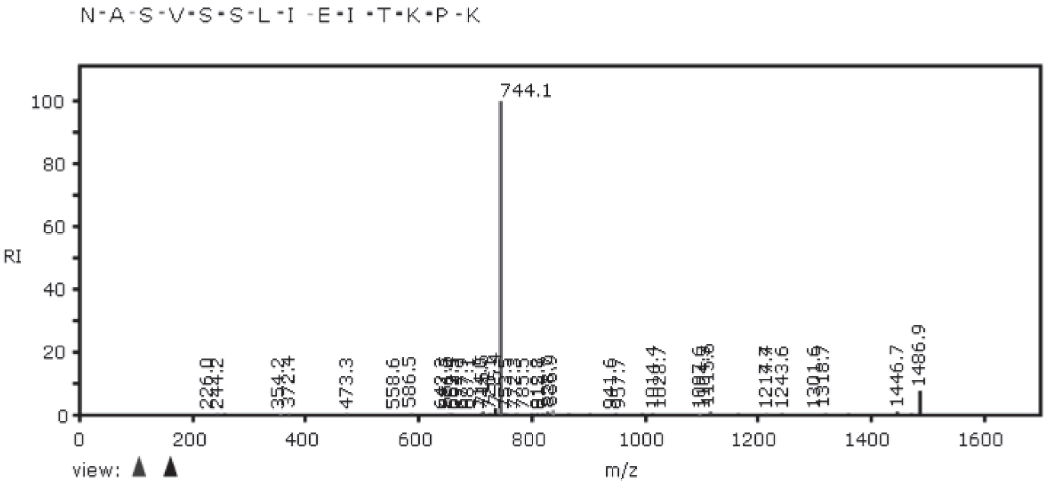
ASTSASMSVSTSASMSASTSASTSASMSASTSASTSASMSASTSASTSASTSASMSASTSASMSASTSASTSAS
MSASMSASTSASTSSSSSVTSNSSKEKVYSALPSTGDQDYSVTATALGLGLMTGATLLGRKKSKKDKN

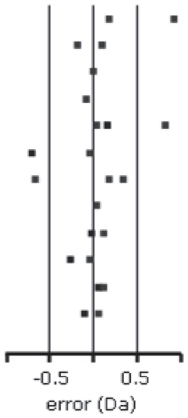
SKKDKN covered sequence
SKKDKN LPXTG site
SKKDKN Signal peptide
MSNESLITPEK Glycopeptide identified in the present study

Supplemental Figure 1

NASVSSLIEITKPK+ (HexNAc)1,
m/z [2+] = 743.92

#	log (e)	log (I)	m+h	delta
1633	-5	4.25	1689.9273	0.0001





matched/total: # ions: 48% intensity: 91% σ: 0.16 Da

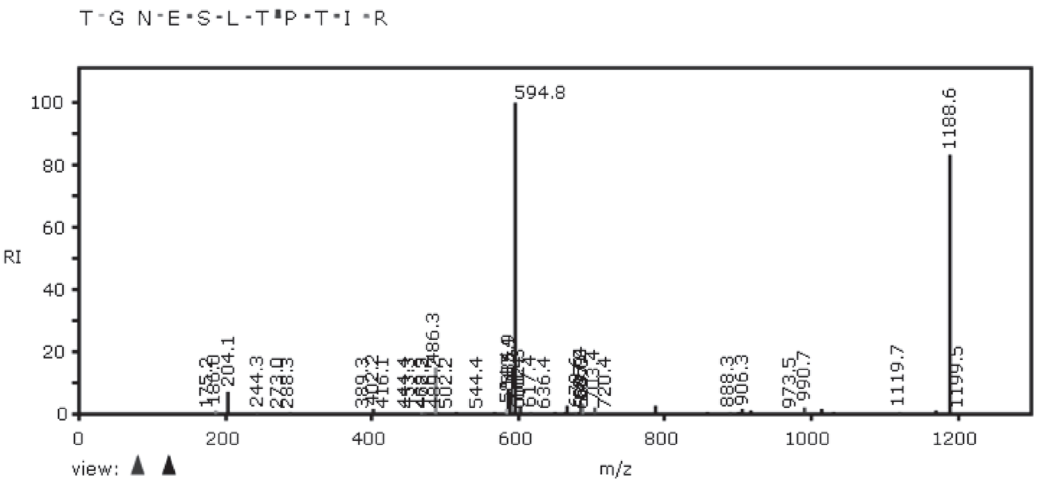
bond	^{+1}y	$^{+1}y^{-17}$	$^{+1}y^{-18}$	^{+1}b	$^{+1}b^{-17}$	$^{+1}b^{-18}$
N_1	1575.884	1558.858	1557.874	115.05	98.024	97.04
A_2	1504.847	1487.821	1486.837	186.087	169.061	168.077
S3	1417.815 $^{2+}$	1400.789	1399.805	273.119	256.093	255.109
V_4	1318.747 $^{+1,2}$	1301.72	1300.736	372.188	355.161	354.177
S_5	1231.715	1214.688	1213.704	459.22	442.193	441.209
S6	941.603	924.577	923.593	749.331	732.305	731.321
L_7	828.519	811.493	810.509	862.415	845.389	844.405
I8	715.435	698.409	697.425	975.499	958.473	957.489
E_9	586.393	569.366	568.382	1104.542	1087.515	1086.531
I10	473.309	456.282	455.298	1217.626	1200.6	1199.616
T_{11}	372.261	355.234	354.25	1318.674 $^{+1,2}$	1301.647	1300.663
K_{12}	244.166	227.139	226.155	1446.769	1429.742	1428.758
P_{13}	147.113	130.087	129.103	1543.821 $^{+2}$	1526.795	1525.811

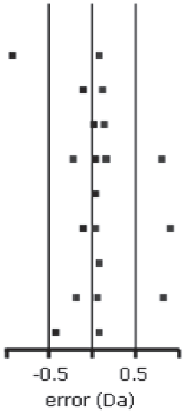
Other observed ions:

Ion type	**m/z**
by[12-14]	354.25
ay[5-10]	818.451
ay[2-8]	833.461
by[3-13]	1358.741
ay[4-13]	1243.714
by[3-14]	1486.836
$(\text{parent-NH}_3)^{+2}$	836.954
$(\text{parent-K})^{+2}$	772.415
$(\text{parent-2NH}_3)^{+2}$	828.441
$(\text{parent-H}_2\text{O})^{+2}$	836.462

Supplemental Figure 2a

TGNESLTPTIR+ (HexNAc)1
m/z [2+] = 594.81





#	log (e)	log (I)	m+h	delta
1219	-2.1	4.69	1391.7017	0.0006

matched/total: # ions: 38% intensity: 14% σ: 0.40 Da

bond	^{+1}y	$^{+1}y^{-17}$	$^{+1}y^{-18}$	^{+1}b	$^{+1}b^{-17}$	$^{+1}b^{-18}$
T$_1$	1290.654	1273.627	1272.643	102.055	85.029	84.045
G$_2$	1233.632 $^{+2}$	1216.606	1215.621	159.077	142.05	141.066
N$_3$	1119.589	1102.563	1101.579	273.119	256.093	255.109
E$_4$	990.547	973.52	972.536	402.162	385.136	384.151
S$_5$	903.515	886.488	885.504	489.194	472.168	471.184
L$_6$	790.43	773.404	772.42	602.278	585.252	584.268
T$_7$	486.304	469.277	468.293	906.405 $^{+1,2}$	889.378	888.394
P$_8$	389.251	372.224	371.24	1003.458 $^{+2}$	986.431	985.447
T$_9$	288.203	271.177	270.193	1104.505	1087.479	1086.495
I$_{10}$	175.119	158.093	157.109	1217.589	1200.563	1199.579

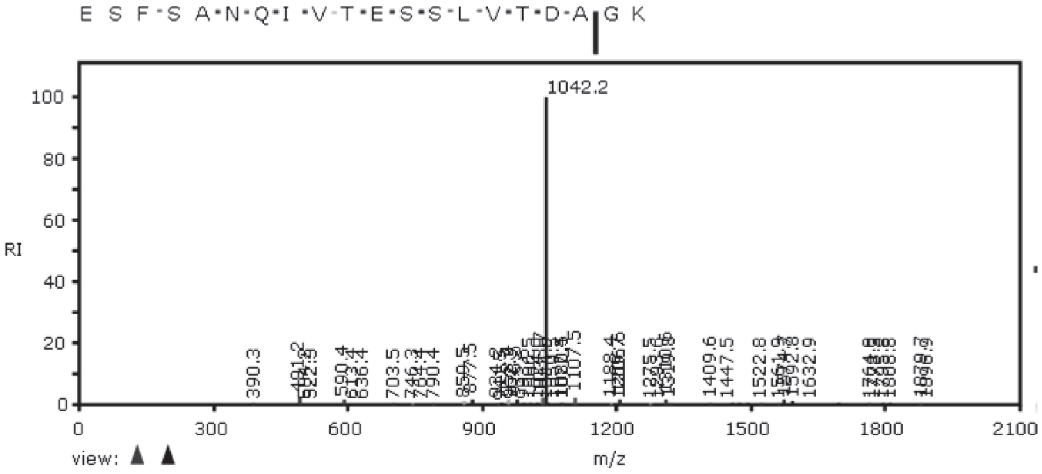
Other observed ions:

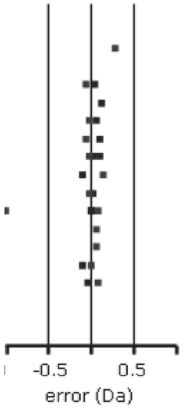
Ion type	**m/z**
by[3-4]	244.093
ay[2-4]	273.119
by[7-8]	402.187
by[3-6]	444.209
ay[3-6]	416.214
by[5-8]	602.303
by[8-11]	468.293
ay[4-8]	703.35
by[5-9]	703.35
ay[3-7]	720.341
(parent-H$_2$O)$^{+2}$	687.349
(parent-2H$_2$O)$^{+2}$	678.841
(parent-NH$_3$)$^{+2}$	687.8411

Supplemental Figure 2b

ESFSANQIVTESSLVTDAGK + (HexNAc)1
m/z [2+] = 1042.01

#	log (e)	log (I)	m+h	delta
2102	-7.2	4.69	2286.0988	-0.003





matched/total: # ions: 72% intensity: 95% σ: 0.07 Da

bond	$^{+1}$y	$^{+1}$y^{-17}	$^{+1}$y^{-18}	$^{+1}$b	$^{+1}$b^{-17}	$^{+1}$b^{-18}
E$_1$	2157.056	2140.03	2139.045	130.05	113.023	112.039
S$_2$	2070.024	2052.997	2052.013	217.082	200.055	199.071
F$_3$	1922.956	1905.929	1904.945	364.15	347.124	346.14
S$_4$	1835.924	1818.897	1817.913	451.182	434.156	433.172
A$_5$	1764.886	1747.86	1746.876	522.22	505.193	504.209
N$_6$	1650.844	1633.817	1632.833	636.262	619.236	618.252
Q$_7$	1522.785	1505.758	1504.774	764.321	747.295	746.311
I$_8$	1409.701	1392.674	1391.69	877.405	860.379	859.395
V$_9$	1310.632	1293.606	1292.622	976.474	959.447	958.463
T$_{10}$	1209.585	1192.558	1191.574	1077.521	1060.495	1059.511
E$_{11}$	1080.542	1063.516	1062.532	1206.564	1189.537	1188.553
S$_{12}$	993.51	976.484	975.5	1293.596	1276.569	1275.585
S$_{13}$	703.399	686.372	685.388	1583.707	1566.681	1565.697
L$_{14}$	590.315	573.288	572.304	1696.791	1679.765	1678.781
V$_{15}$	491.246	474.22	473.236	1795.86	1778.833	1777.849
T$_{16}$	390.199	373.172	372.188	1896.907	1879.881	1878.897
D$_{17}$	275.172	258.145	257.161	2011.934 $^{+2}$	1994.908	1993.924
A18	204.135	187.108	186.124	2082.971 $^{+2}$	2065.945	2064.961
G$_{19}$	147.113	130.087	129.103	2139.993	2122.966	2121.982

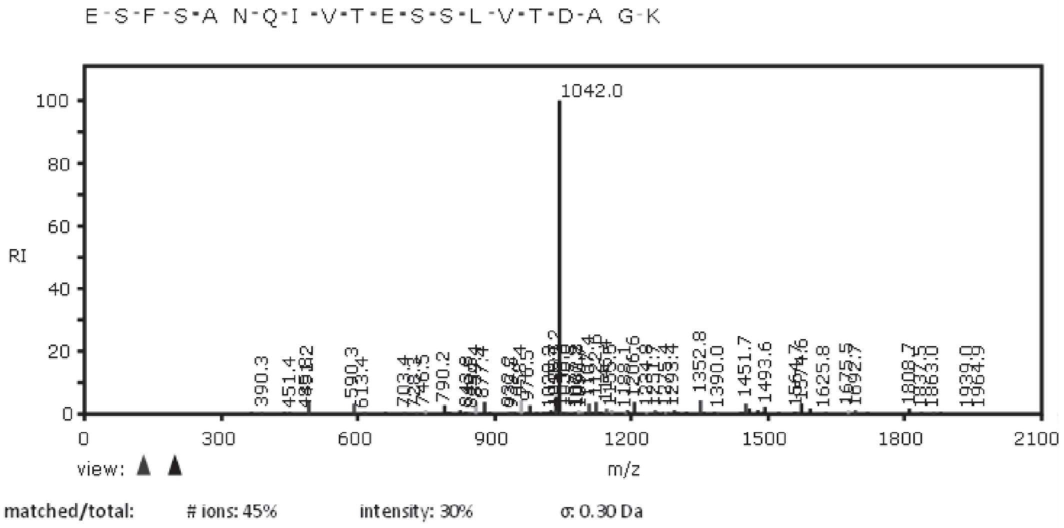
Other observed ions:

Ion type	**m/z**
by[12-14]	491.235
by[13-18]	790.383
by[12-19]	934.436
ay[7-14]	1033.541
by[3-12]	1077.521
by[10-18]	1107.505
by[8-18]	1319.658
by[5-17]	1561.759
by[3-17]	1795.86
by[12-15]	590.303
by[12-18]	877.415
by[11-18]	1006.458
by[2-11]	1077.521
ay[9-17]	1107.542
by[9-18]	1206.574
by[7-18]	1447.716
by[6-18]	1561.759

Supplemental Figure 2c

ESFSANQIVTESSLVTDAGK + (O-AcHexNAc)1
m/z [2+] = 1042.01

#	log (e)	log (I)	m+h	delta
2194	-8.8	3.71	2328.1107	-0.0047

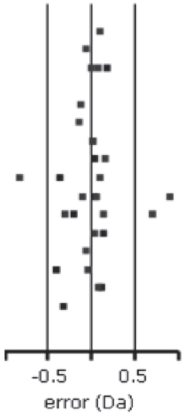


matched/total: # ions: 45% intensity: 30% σ: 0.30 Da

matched/total:	# ions: 52%	intensity: 28%		σ: 0.10 Da		
bond	^{+1}y	$^{+1}y^{-17}$	$^{+1}y^{-18}$	^{+1}b	$^{+1}b^{-17}$	$^{+1}b^{-18}$
E_1	2199.067	2182.04	2181.056	130.05	113.023	112.039
S_2	2112.035	2095.008	2094.024	217.082	200.055	199.071
F_3	1964.966	1947.94	1946.956	364.15	347.124	346.14
S_4	1877.934	1860.908	1859.924	451.182	434.156	433.172
A_5	1806.897	1789.87	1788.886	522.22	505.193	504.209
N_6	1692.854	1675.828	1674.844	636.262	619.236	618.252
Q_7	1564.796	1547.769	1546.785	764.321	747.295	746.311
I_8	1451.711	1434.685	1433.701	877.405	860.379	859.395
V_9	1352.643	1335.617	1334.632	976.474	959.447	958.463
T_{10}	1251.595	1234.569	1233.585	1077.521	1060.495	1059.511
E_{11}	1122.553	1105.526	1104.542	1206.564	1189.537	1188.553
S_{12}	1035.521	1018.494	1017.51	1293.596	1276.569	1275.585
S_{13}	703.399	686.372	685.388	1625.718	1608.691	1607.707
L_{14}	590.315	573.288	572.304	1738.802	1721.775	1720.791
V_{15}	491.246	474.22	473.236	1837.87	1820.844	1819.86
T_{16}	390.199	373.172	372.188	1938.918	1921.891	1920.907
D_{17}	275.172	258.145	257.161	2053.945	2036.918	2035.934
A18	204.135	187.108	186.124	2124.982	2107.955	2106.971
G_{19}	147.113	130.087	129.103	2182.003	2164.977	2163.993

Other observed ions:

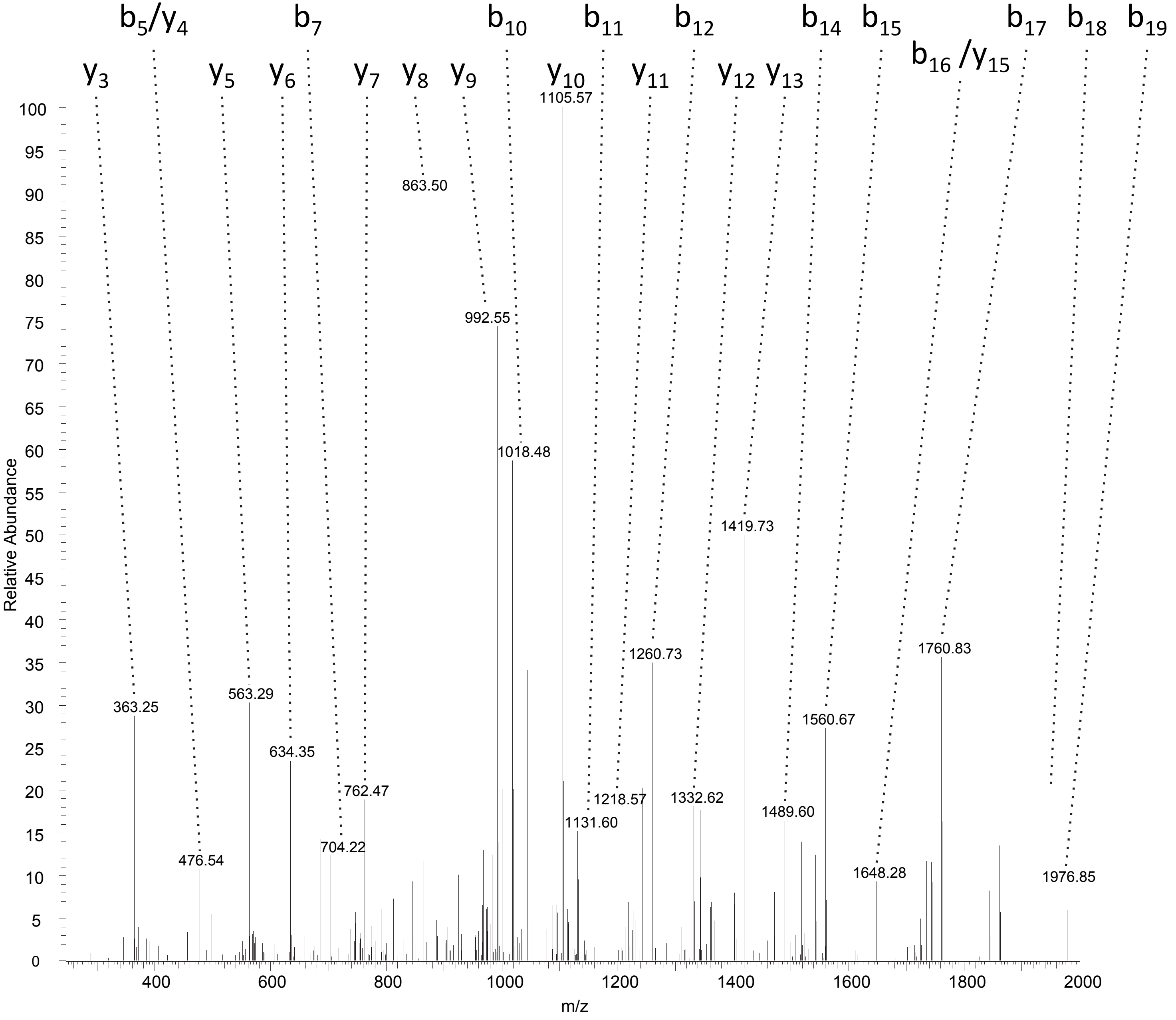
Ion type	**m/z**
by[4-11]	843.421
by[12-19]	976.447
$(parent-2H_2O)^{+2}$	1146.548
by[10-19]	1206.537
ay[7-16]	1275.668
by[5-12]	843.421
ay[8-15]	1046.562
$(parent-H_2O)^{+2}$	1155.553
ay[4-13]	1234.58



Supplemental Figure 2d

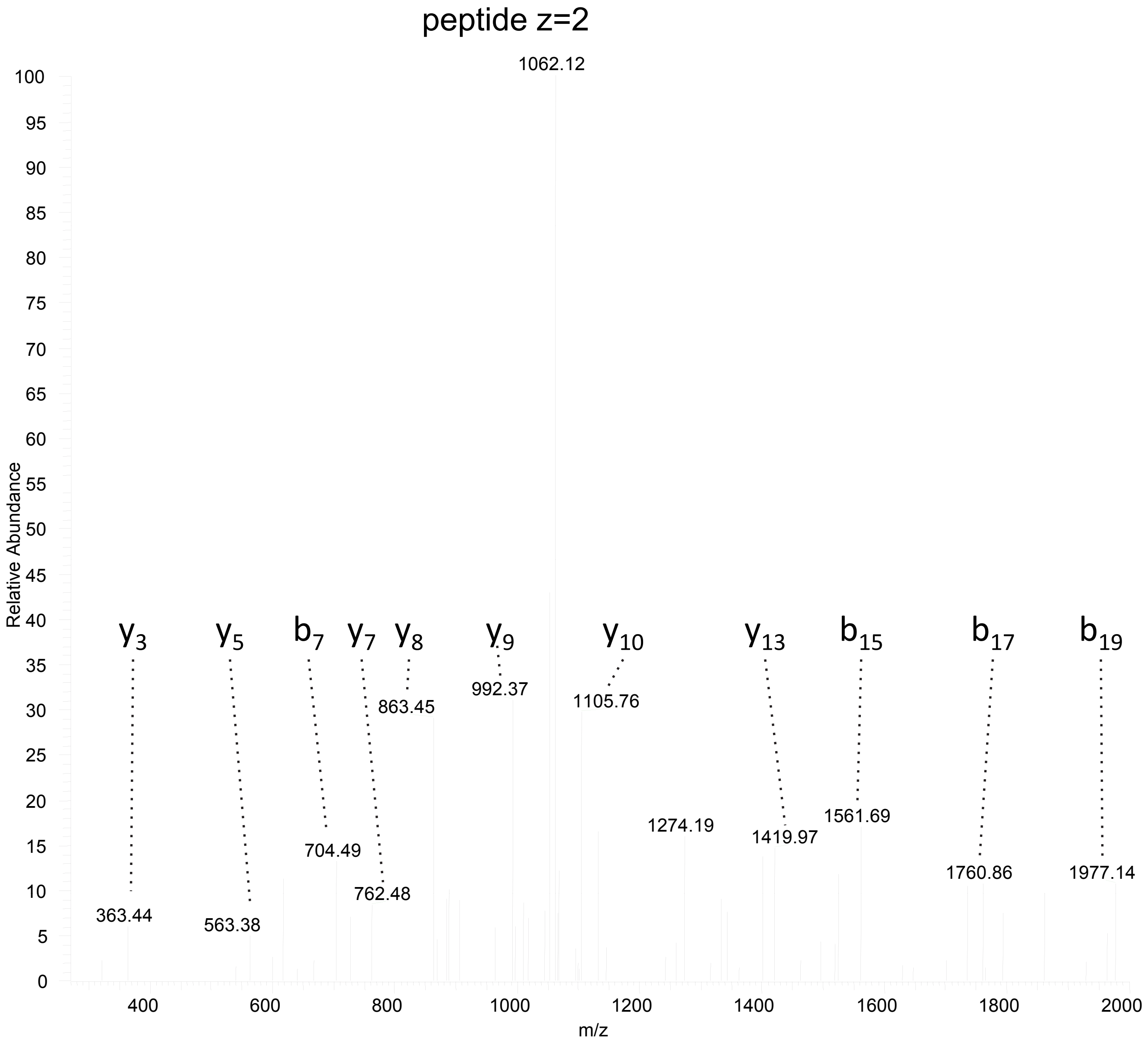
Supplemental Figure 3a

STSISEVSNILETQASLTDK nude
m/z =1062.54 (z=2) scan 2385 RT 28.81



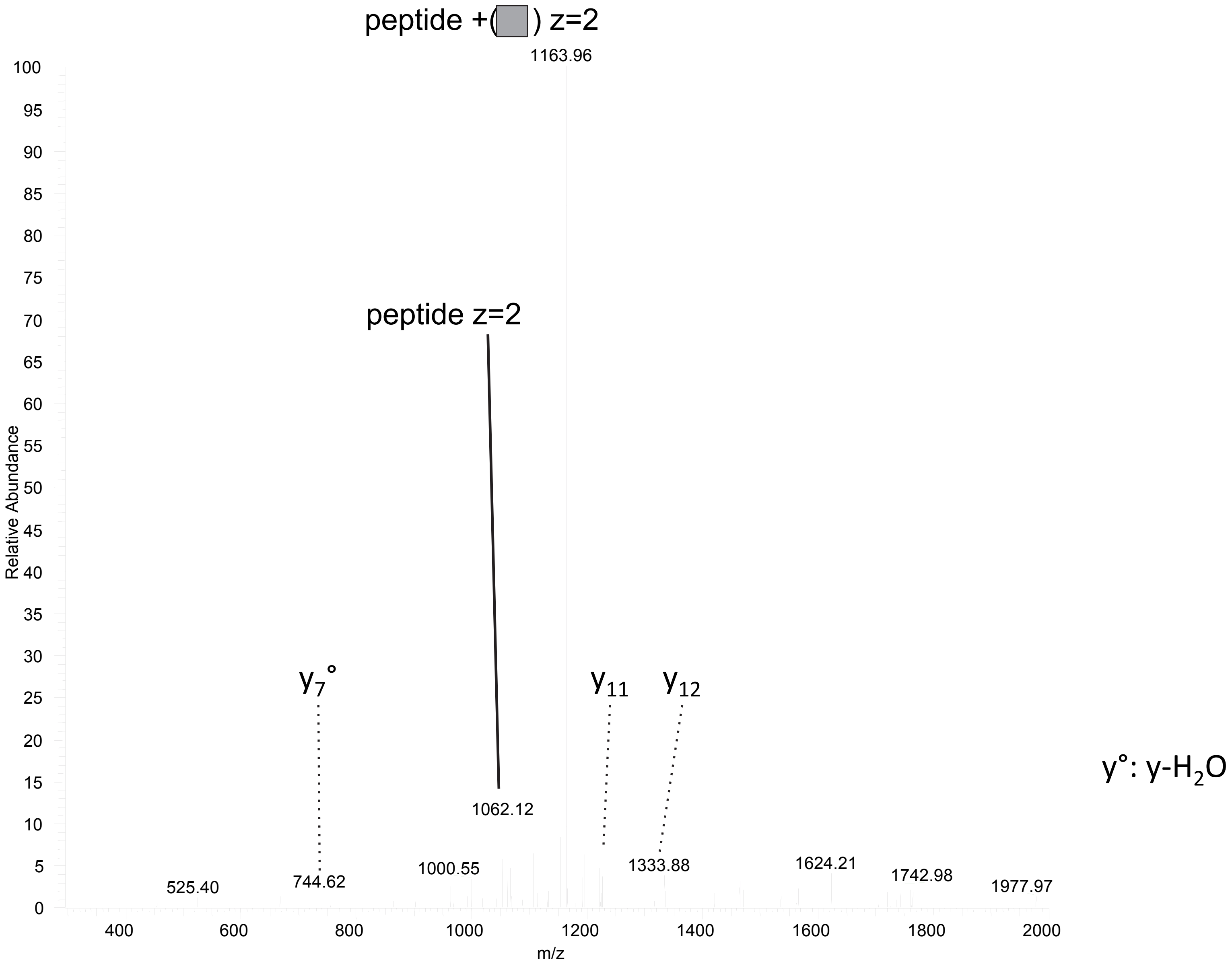
Supplemental Figure 3b

STSISEVSNILETQASLTDK +(HexNAc)
m/z =1163.57 (z=2), scan 2383 RT 27.22



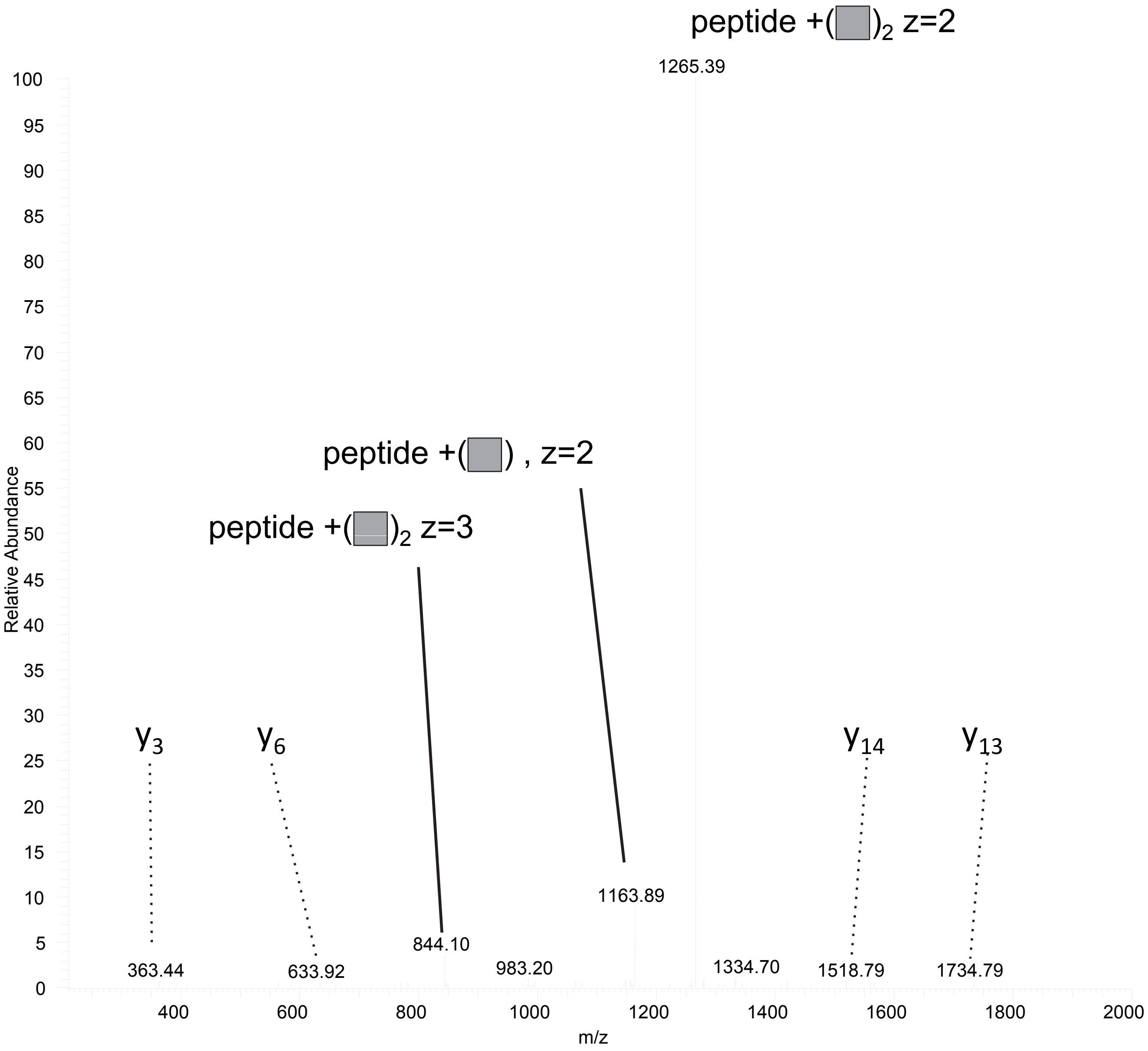
Supplemental Figure 3c

STSISEVSNILETQASLTDK +$(HexNAc)_2$
m/z=1265.61 z=2, scan 2299 RT 26.53



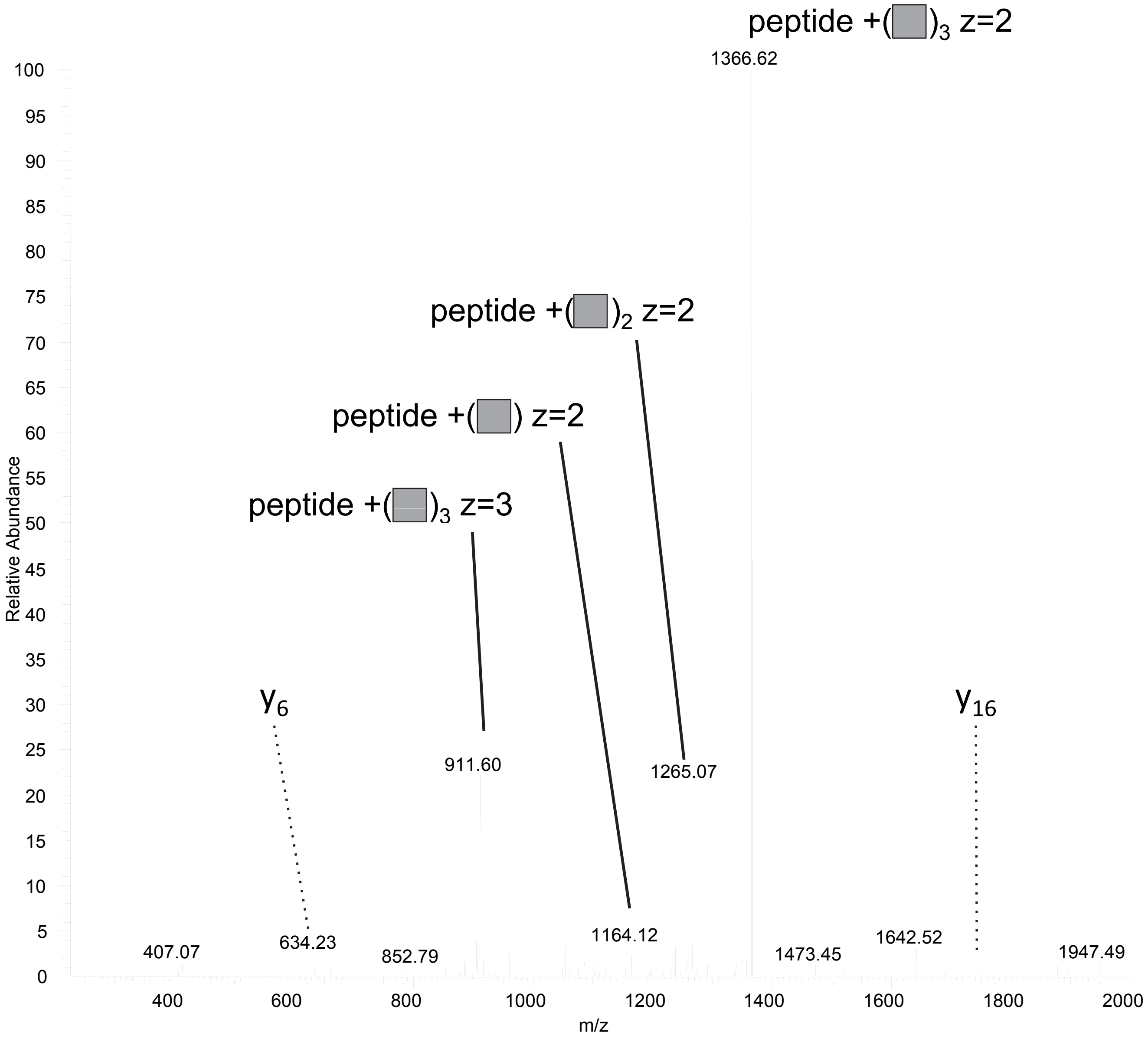
Supplemental Figure 3d

STSISEVSNILETQASLTDK +(HexNAc)$_3$
m/z= 912.11, z=3, scan 2245 RT 26.13



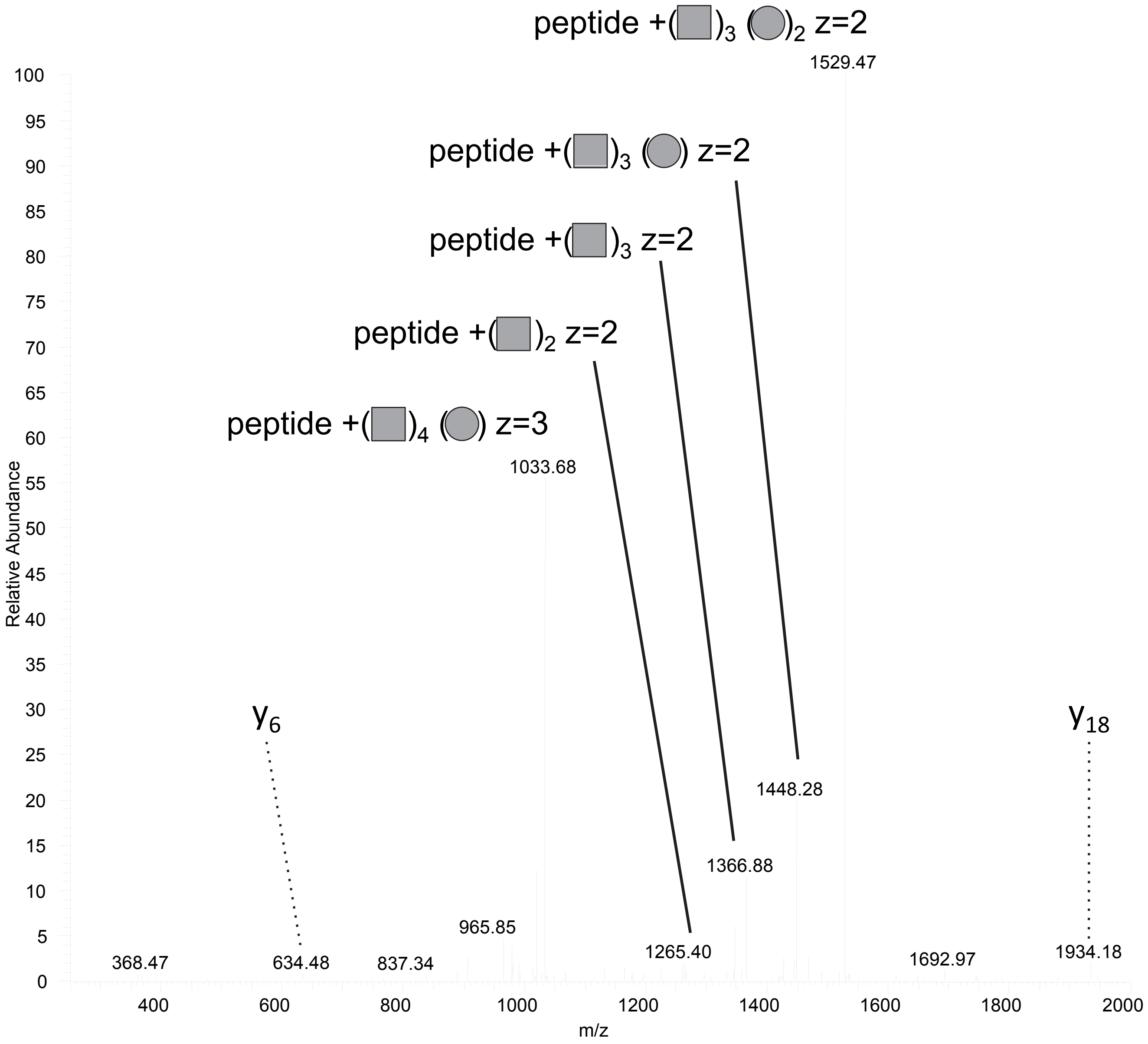
Supplemental Figure 3e

STSISEVSNILETQASLTDK +(HexNAc)$_4$
m/z= 979.46 z=3, scan 1946 RT 23.83



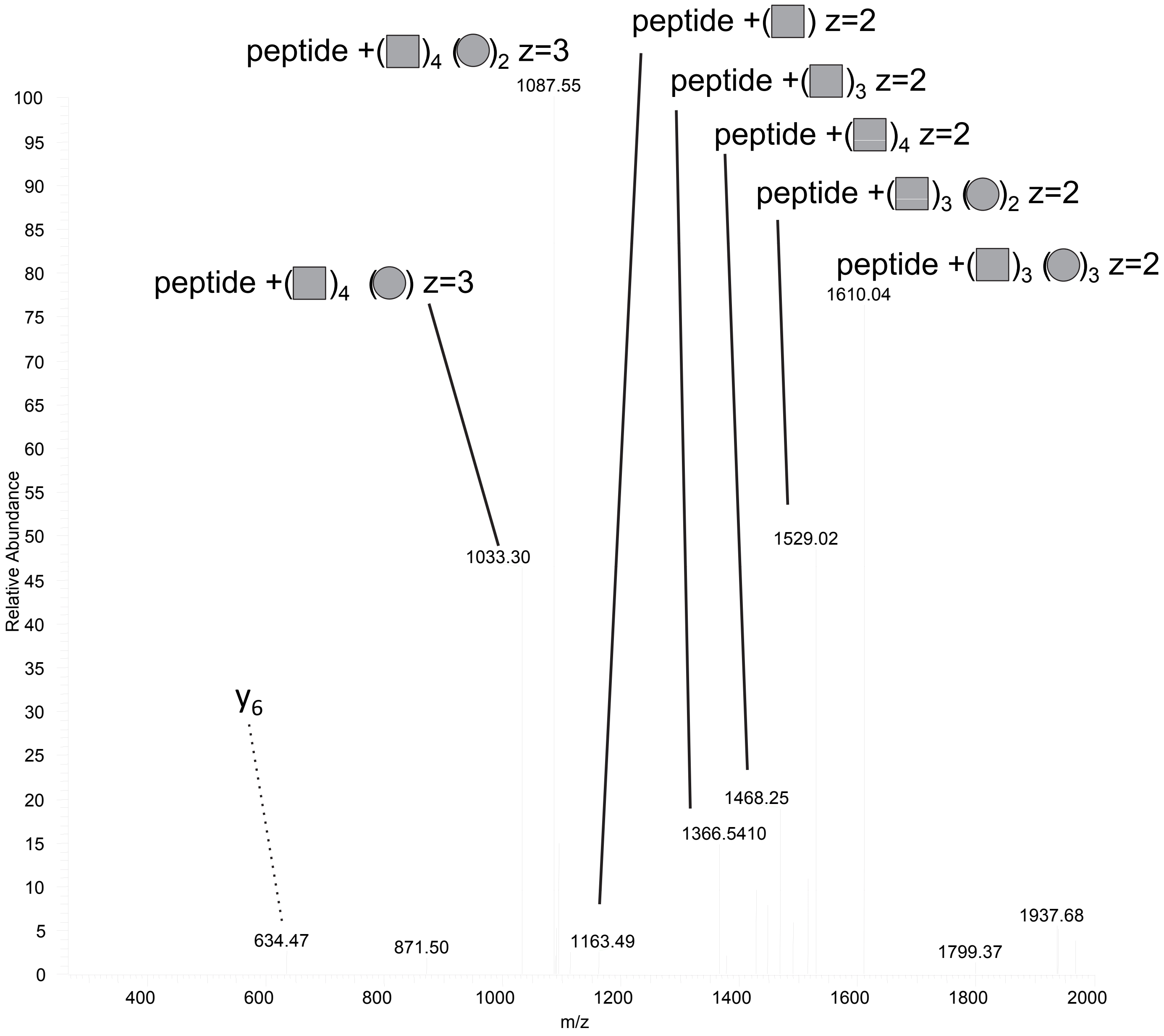
Supplemental Figure 3f

STSISEVSNILETQASLTDK +(HexNAc)$_4$ (Hex)$_2$
m/z= 1088.17 z=3, scan 1883 RT 23.36



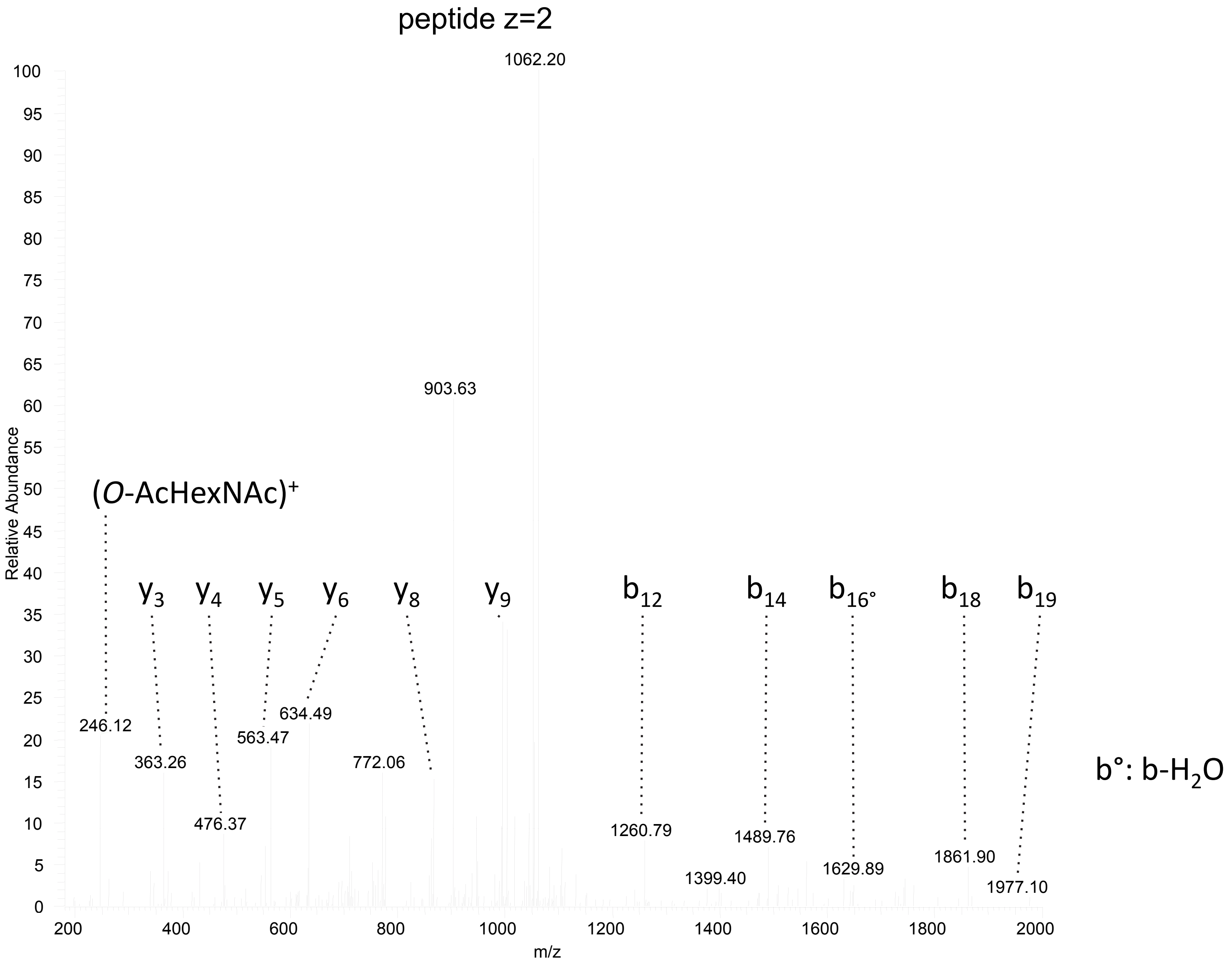
Supplemental Figure 3g

STSISEVSNILETQASLTDK +(HexNAc)$_4$ (Hex)$_3$
m/z= 1141.85 z=3, scan 1877 RT 23.30



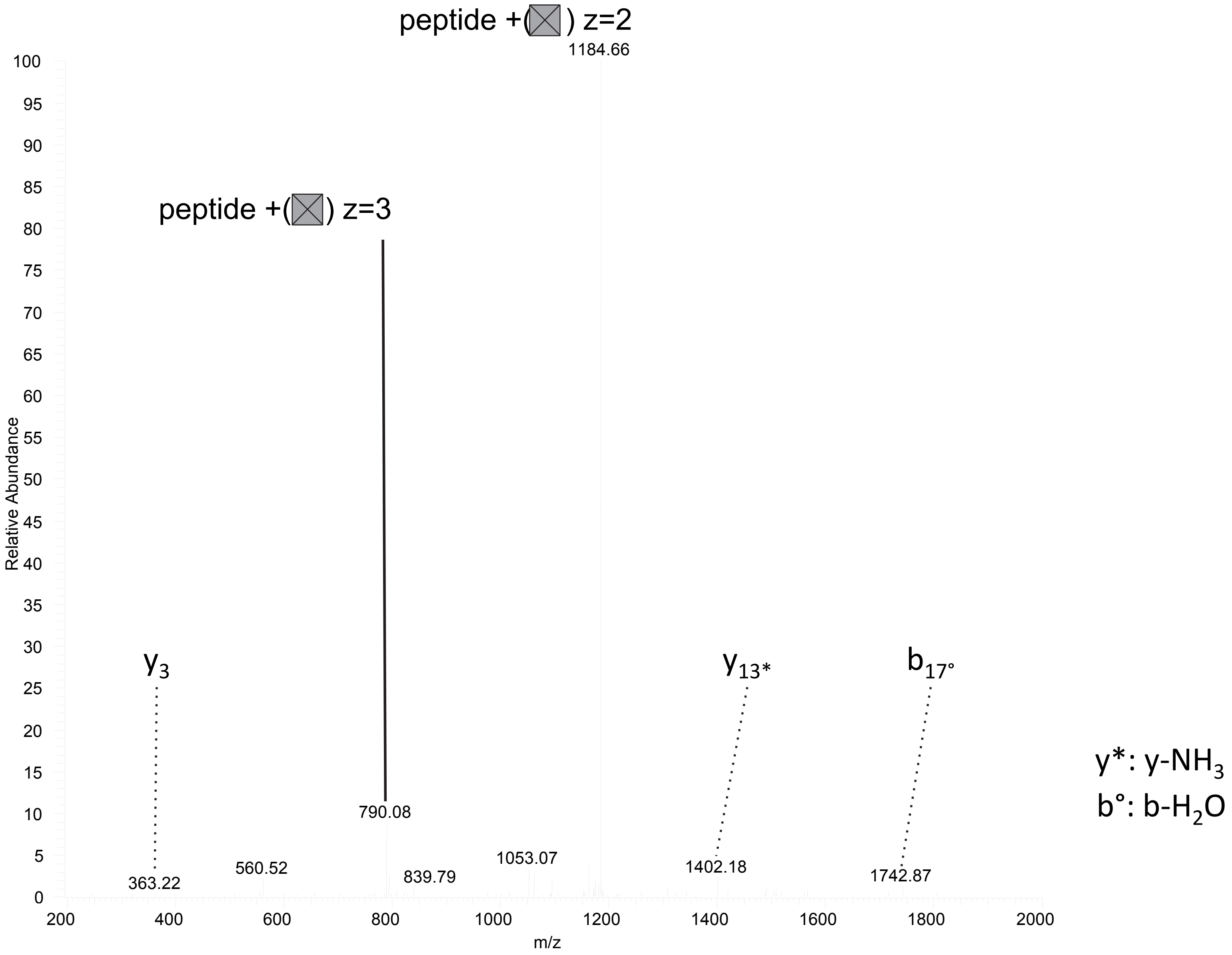
Supplemental Figure 3h

STSISEVSNILETQASLTDK +(AcHexNAc)
m/z= 790.39 z=3, scan 2345 RT 26.89



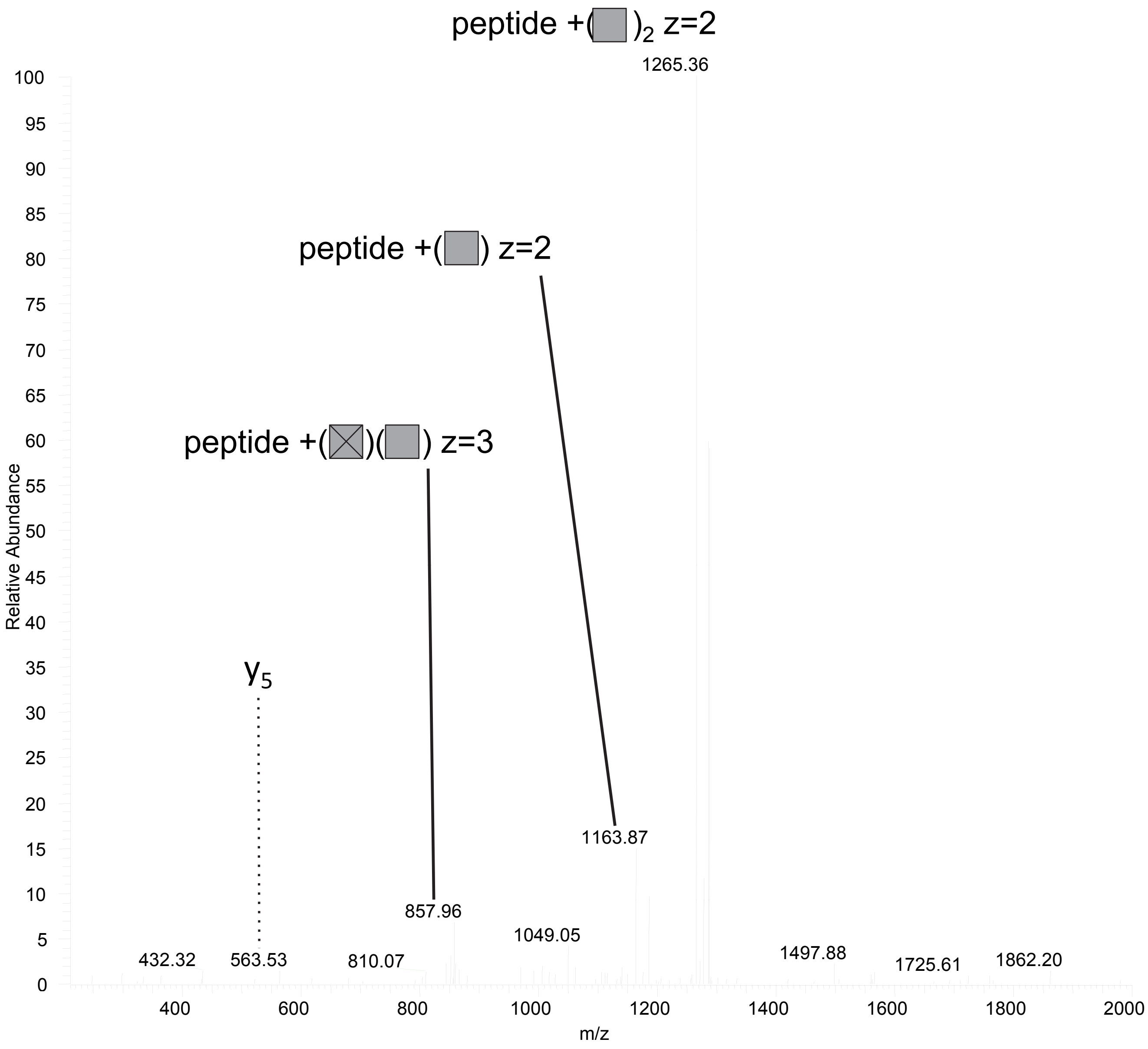
Supplemental Figure 3i

STSISEVSNILETQASLTDK +(AcHexNAc) +(HexNAc)
m/z= 857.75 z=3, scan 2338 RT 26.84



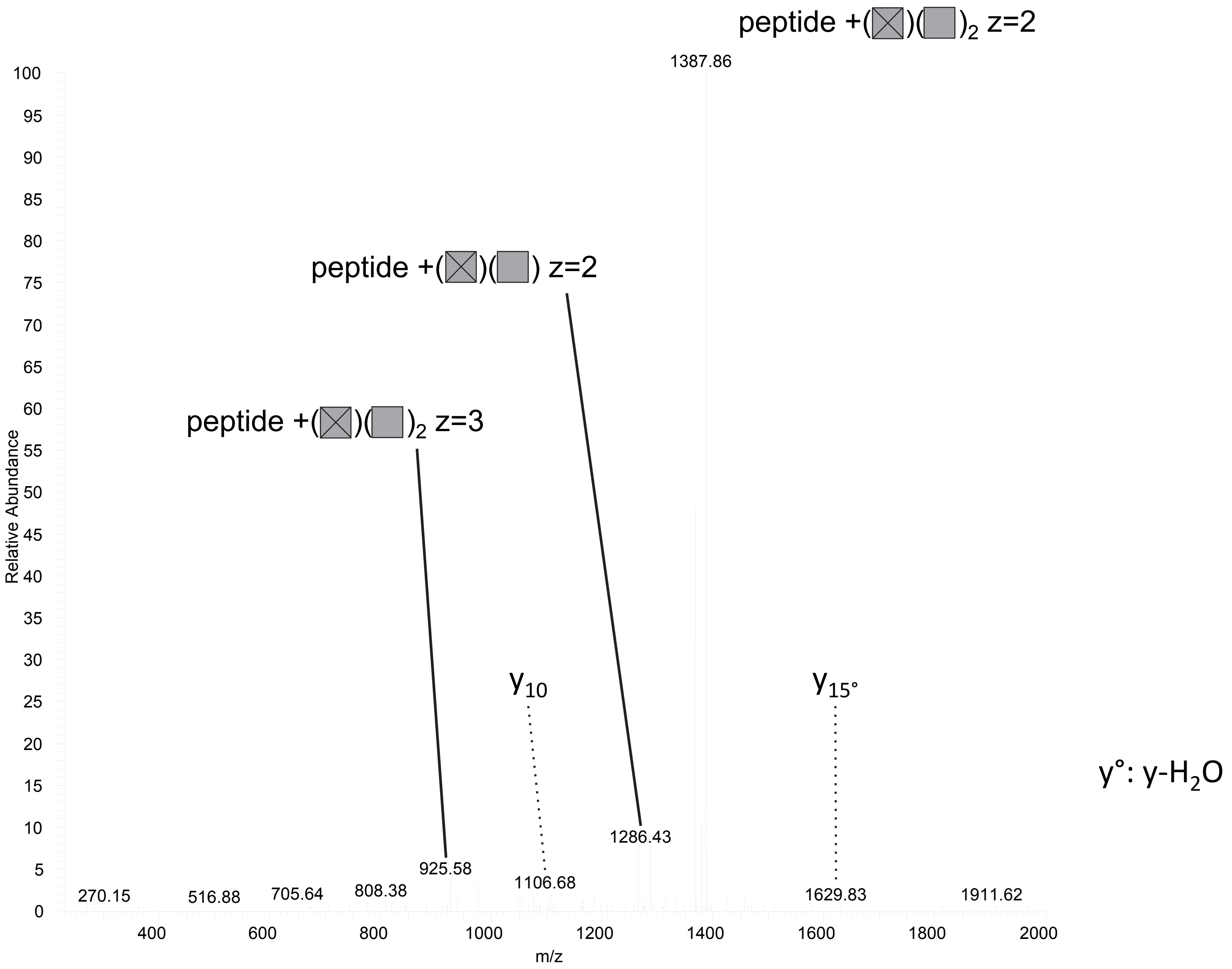
Supplemental Figure 3j

STSISEVSNILETQASLTDK +(AcHexNAc) +(HexNAc)$_2$
m/z= 926.44 z=3, scan 2330 RT 26.77



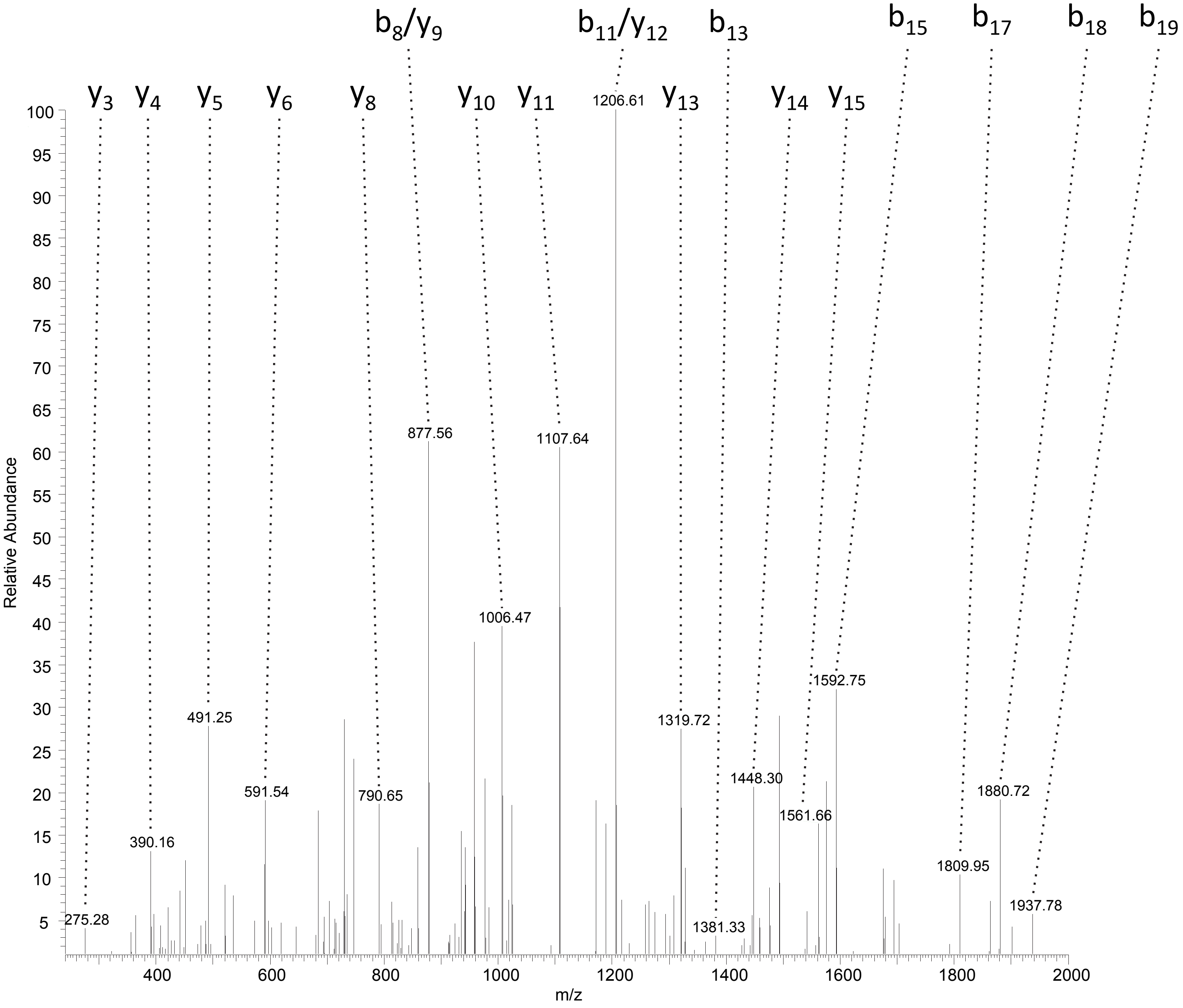
Supplemental Figure 3k

STSISEVSNILETQASLTDK +(AcHexNAc) +(HexNAc)$_3$
m/z= 993.80 z=3, scan 2048 RT 24.62



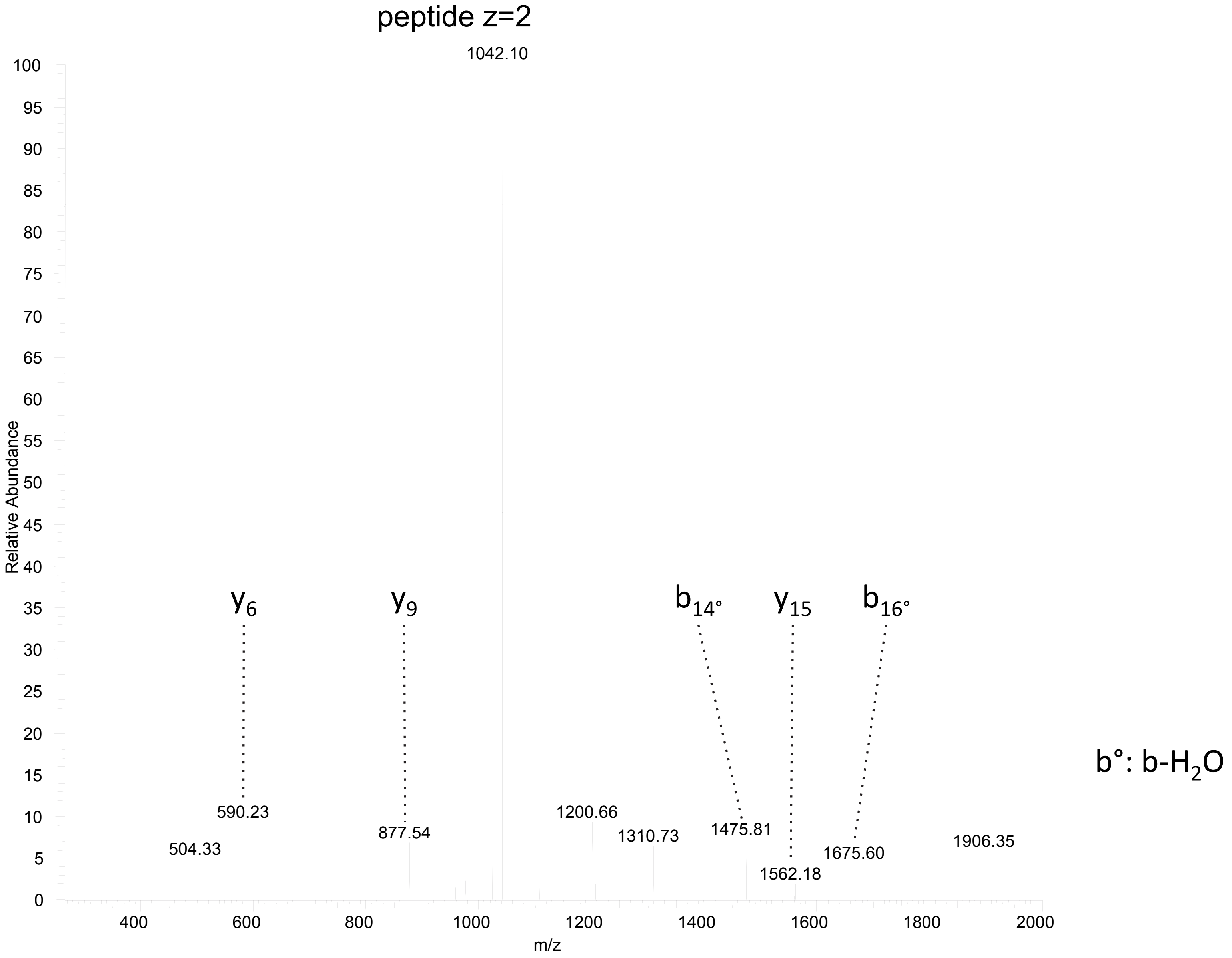
Supplemental Figure 3l

ESFSANQIVTESSLVTDAGK nude
m/z= 1042.51 z=2, scan 2178 RT 25.60



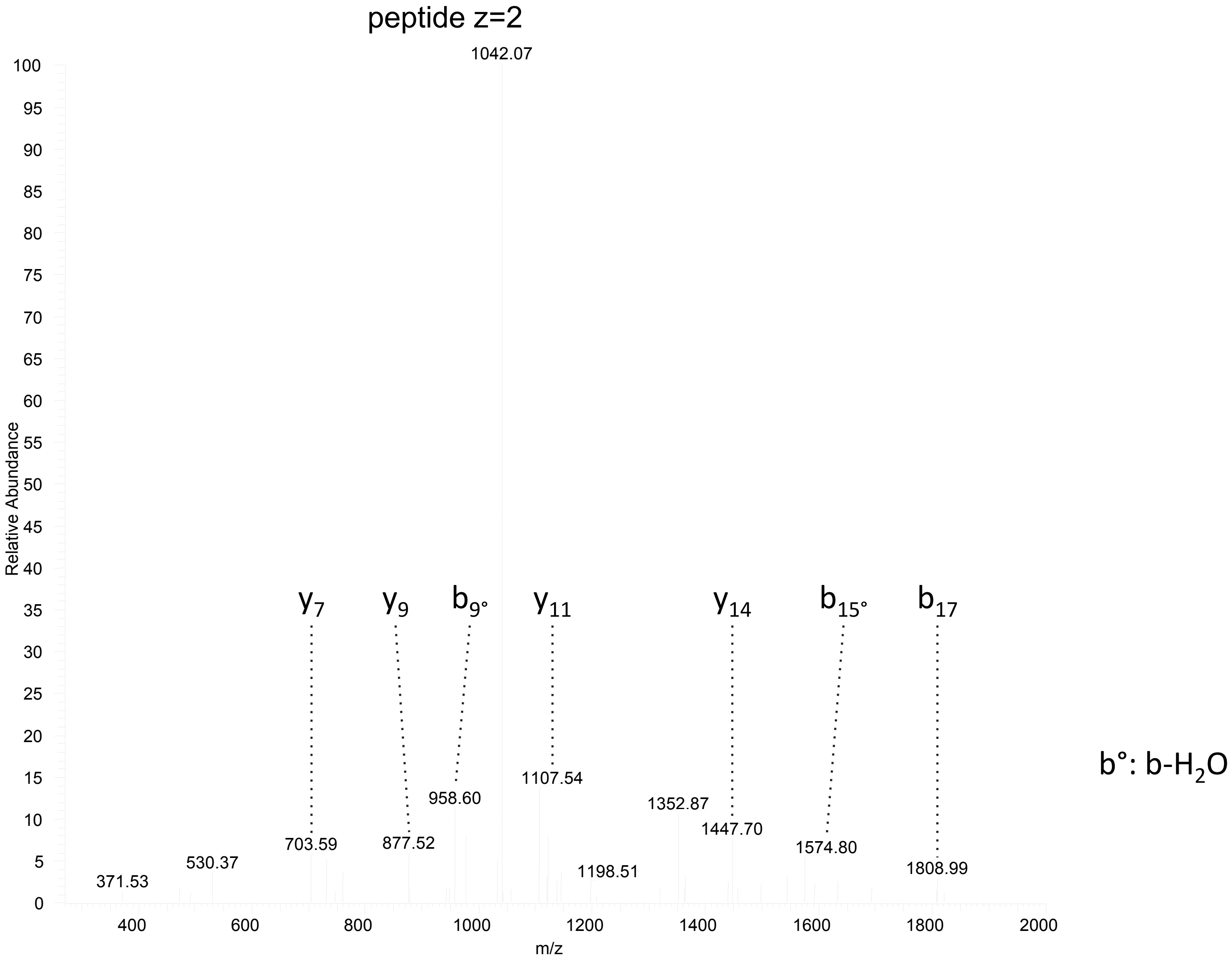
Supplemental Figure 3m

ESFSANQIVTESSLVTDAGK +(HexNAc)
m/z= 1143.55 z=2, scan 2113 RT 25.12



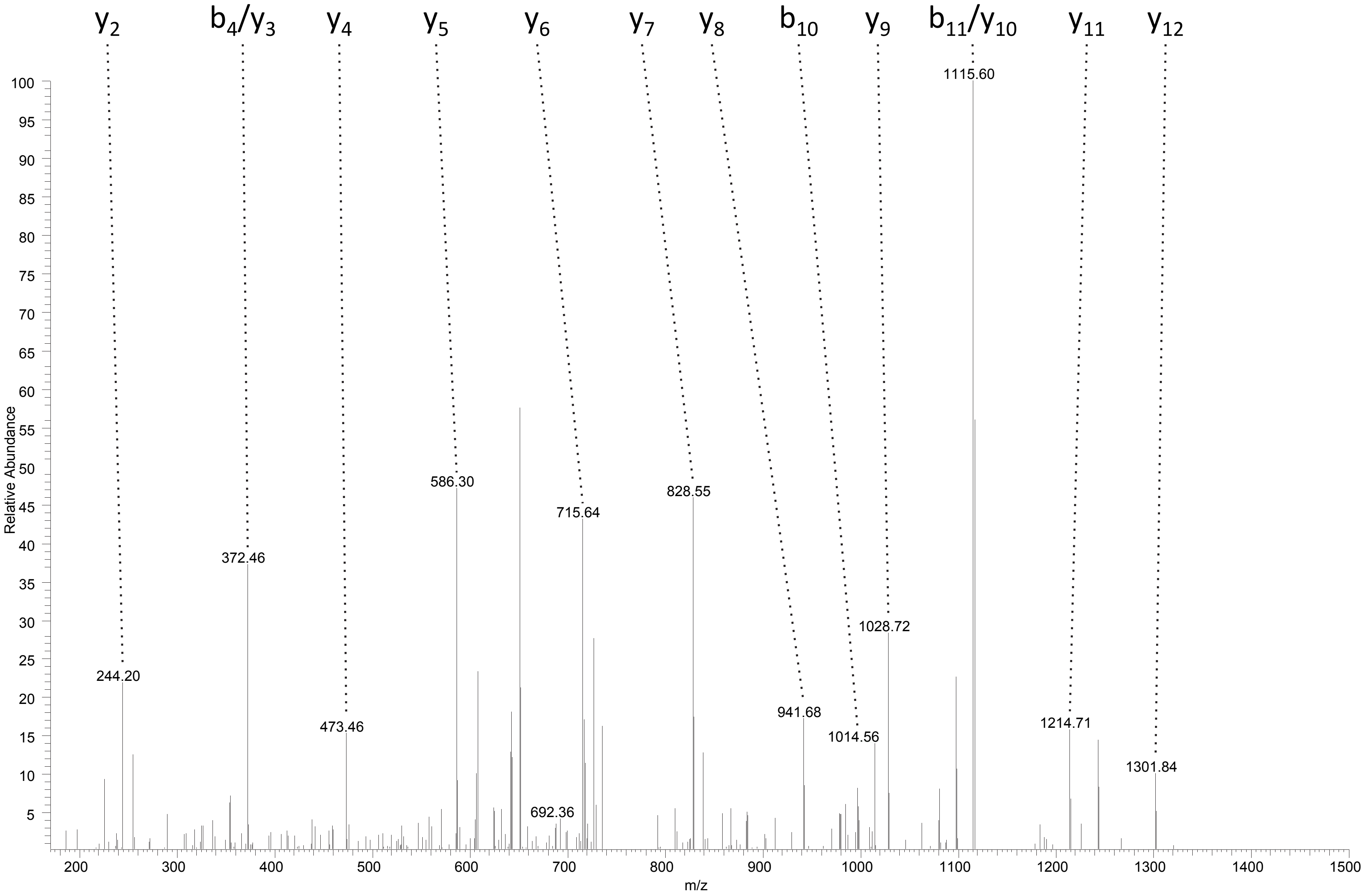
Supplemental Figure 3n

ESFSANQIVTESSLVTDAGK +(AcHexNAc)
m/z=1165.05 z=2, scan 2197 RT 25.75



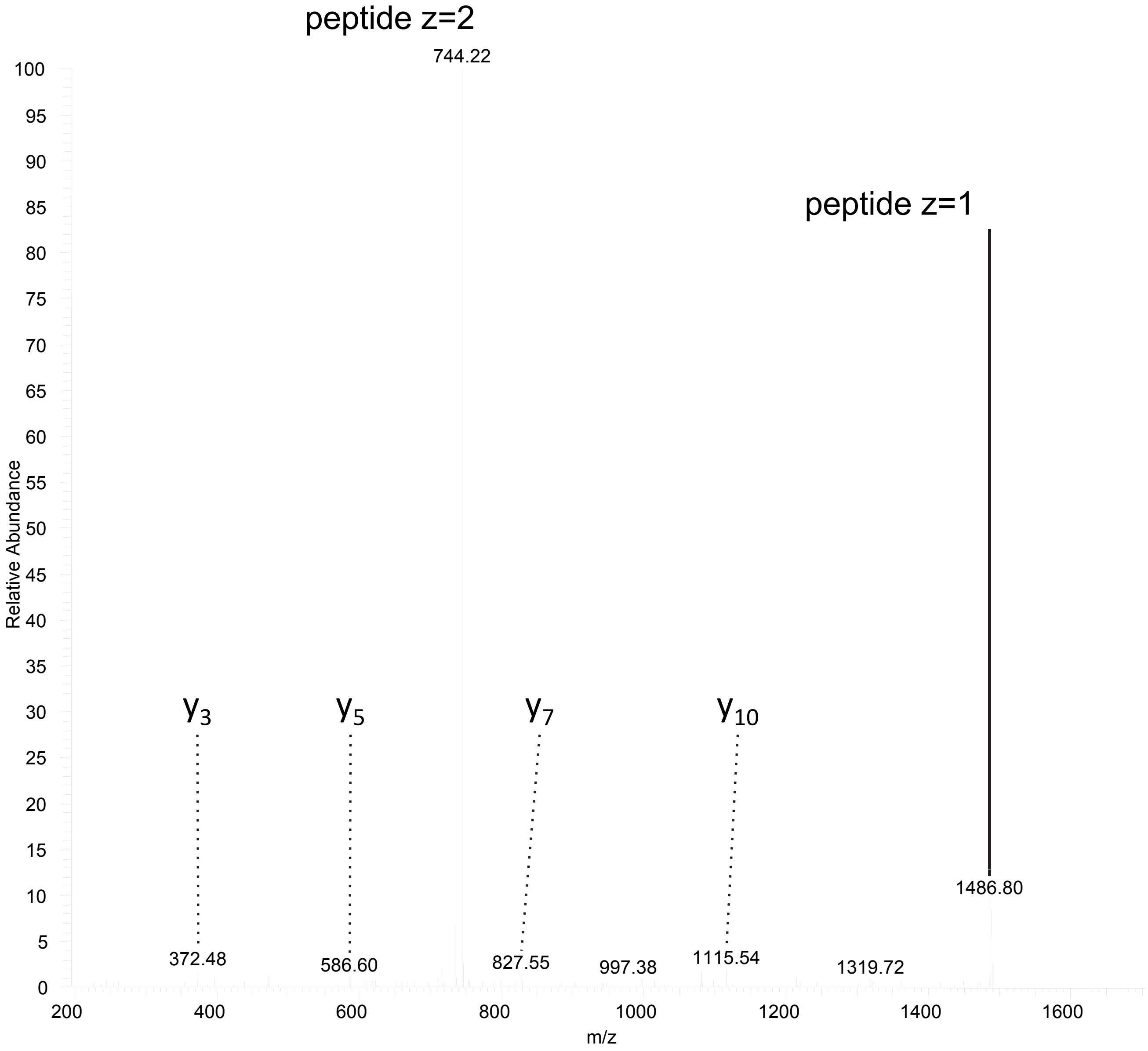
Supplemental Figure 3o

NASVSSLIEITKPK nude
m/z= 743.93 z=2, scan 1601 RT 21.04



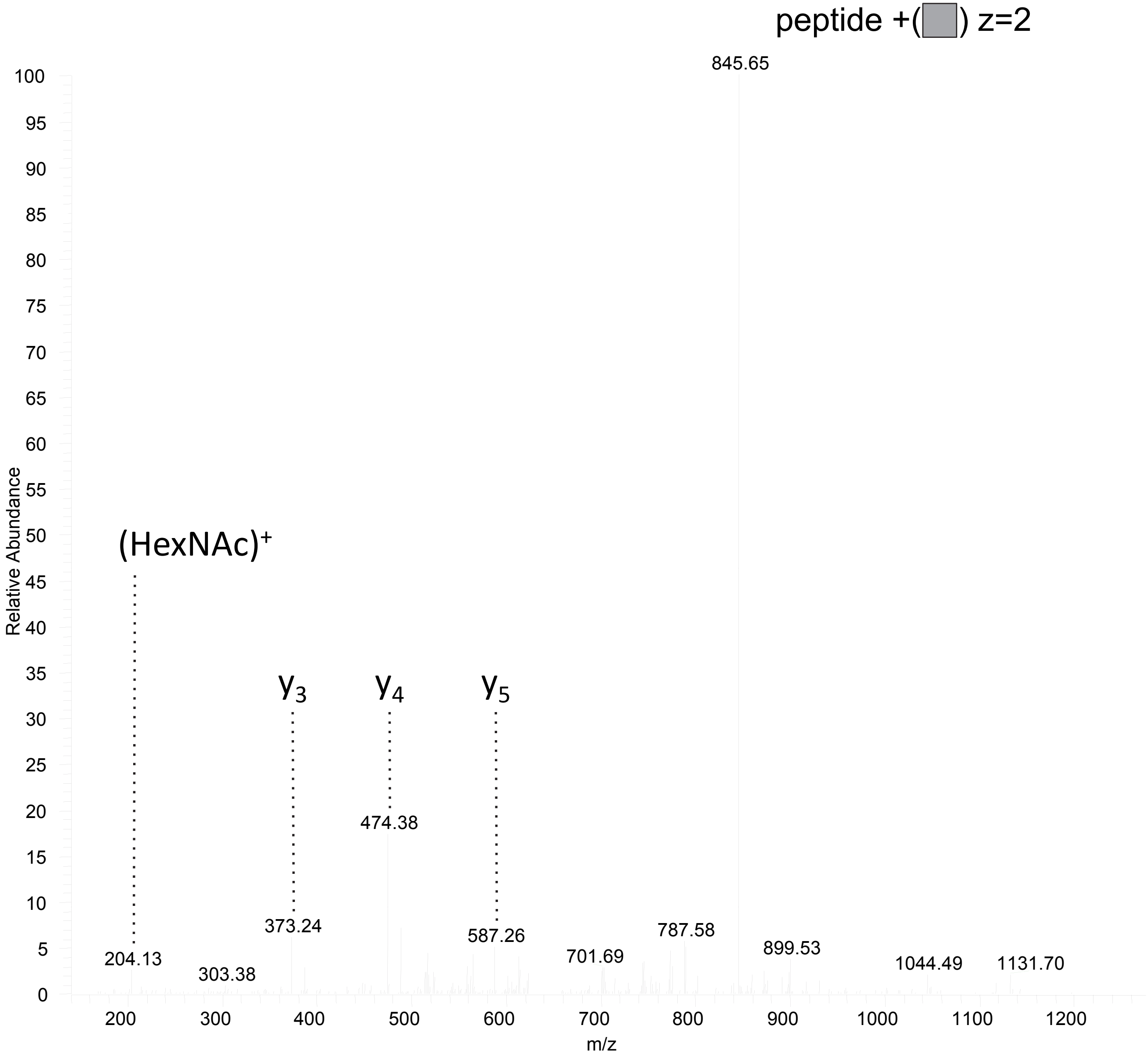
Supplemental Figure 3p

NASVSSLIEITKPK +(HexNAc)
m/z= 845.46 z=2, scan 1498 RT 20.21



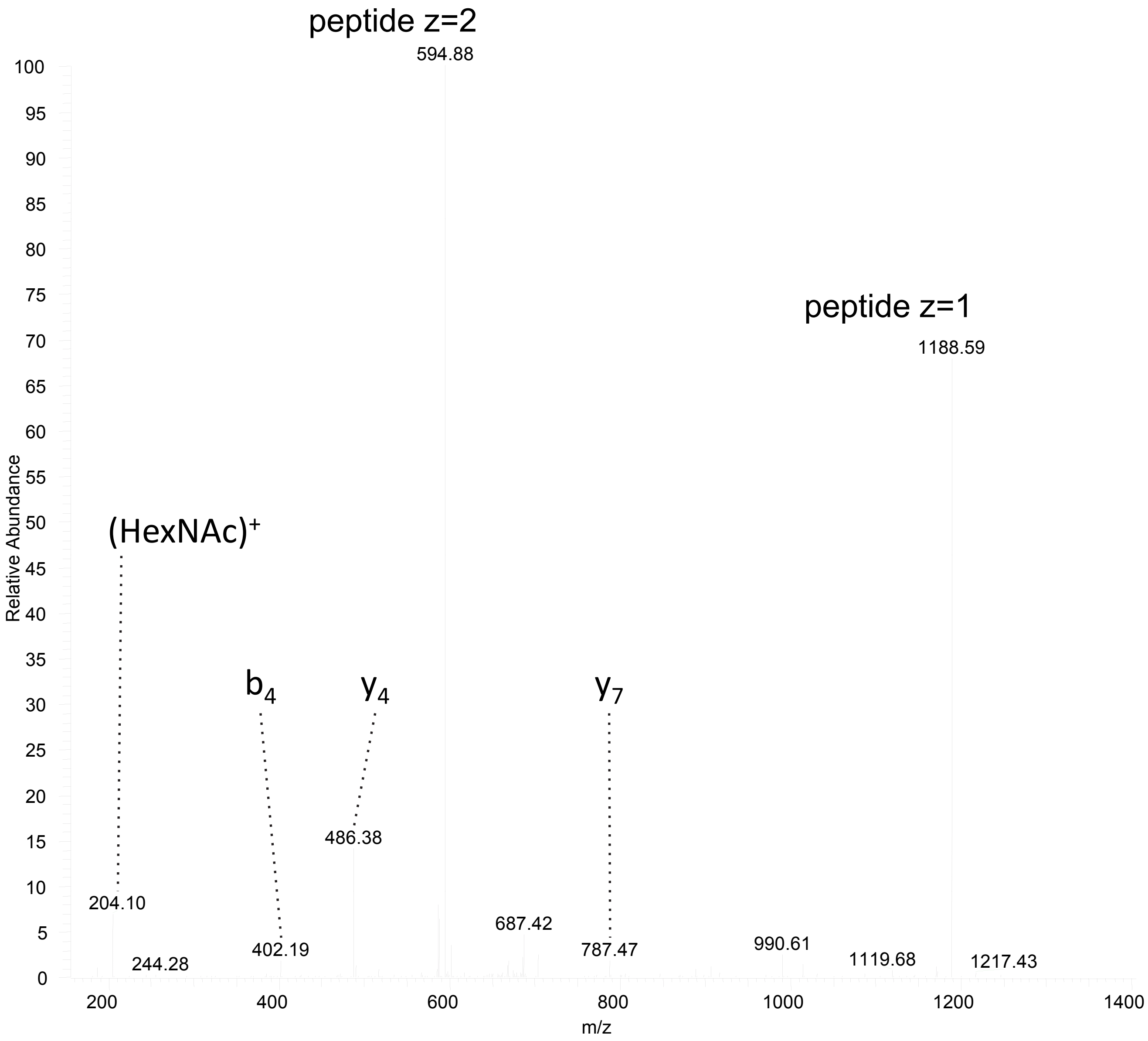
Supplemental Figure 3q

NASVSSLIEITKPK +(HexNAc)$_2$
m/z 631.67 z=3, scan 1340 RT 18.91



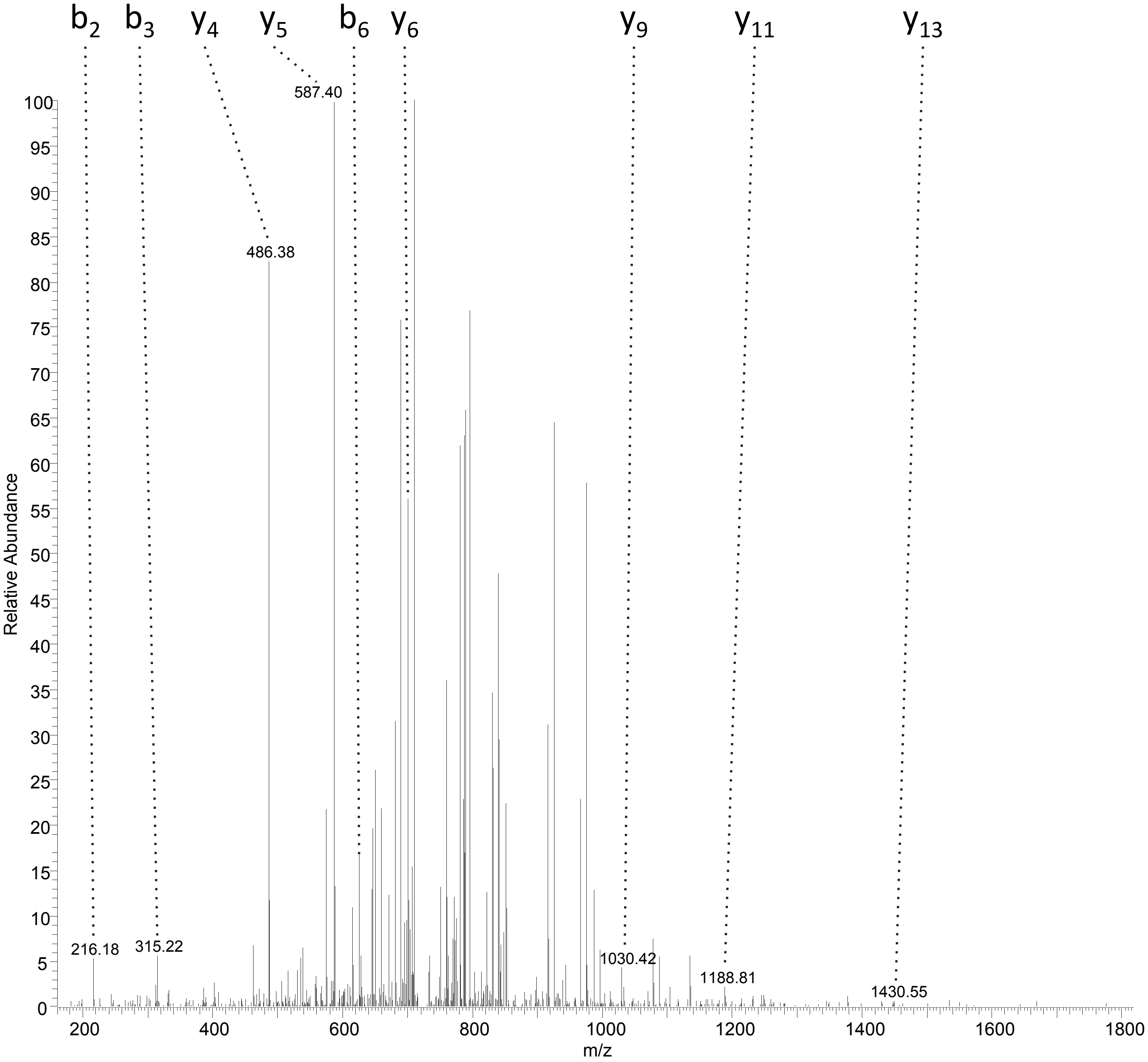
Supplemental Figure 3r

TGNESLTPTIR +(HexNAc) z=2
m/z= 696.85 z=2, scan 1045 RT 16.51



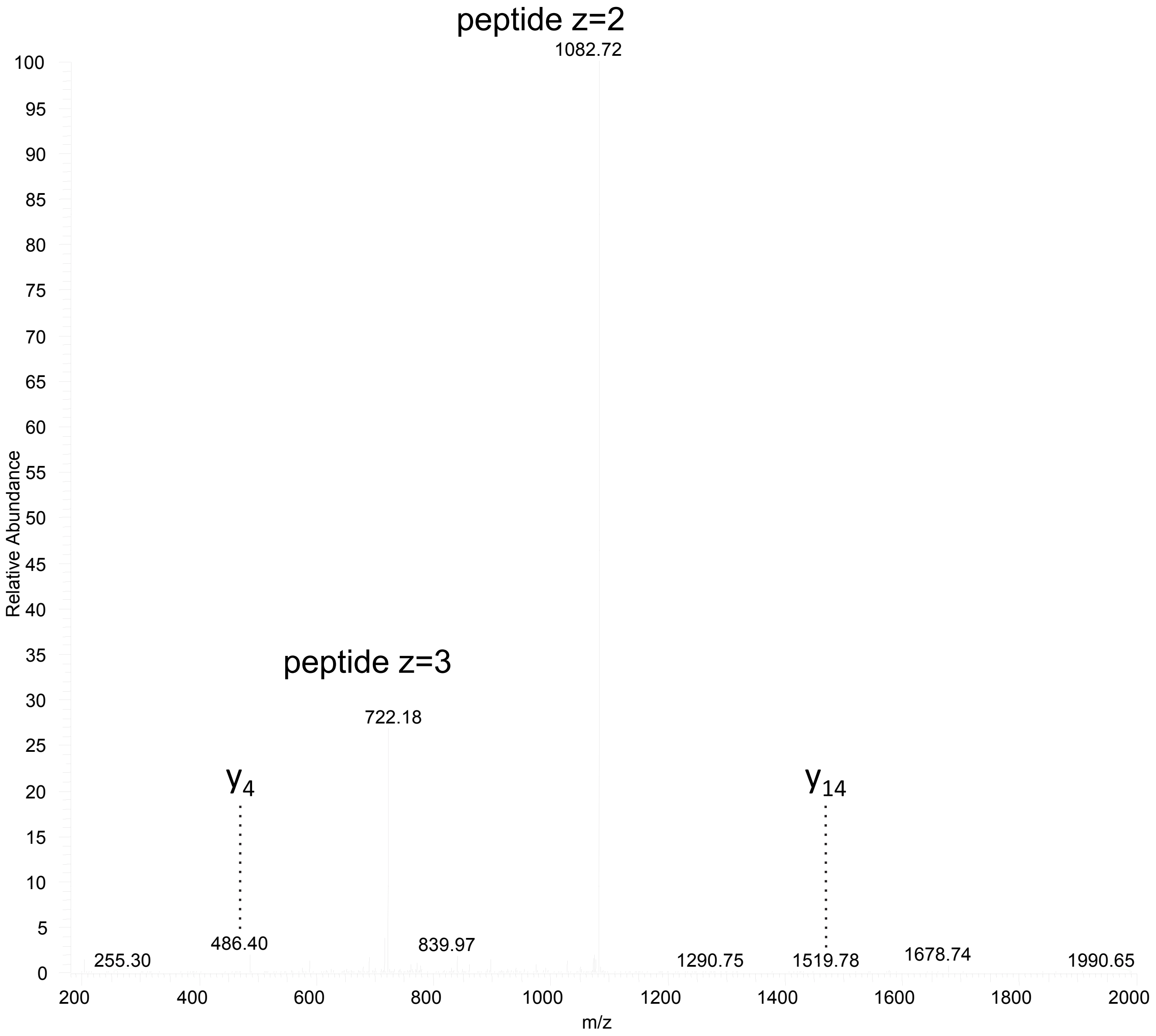
Supplemental Figure 3s

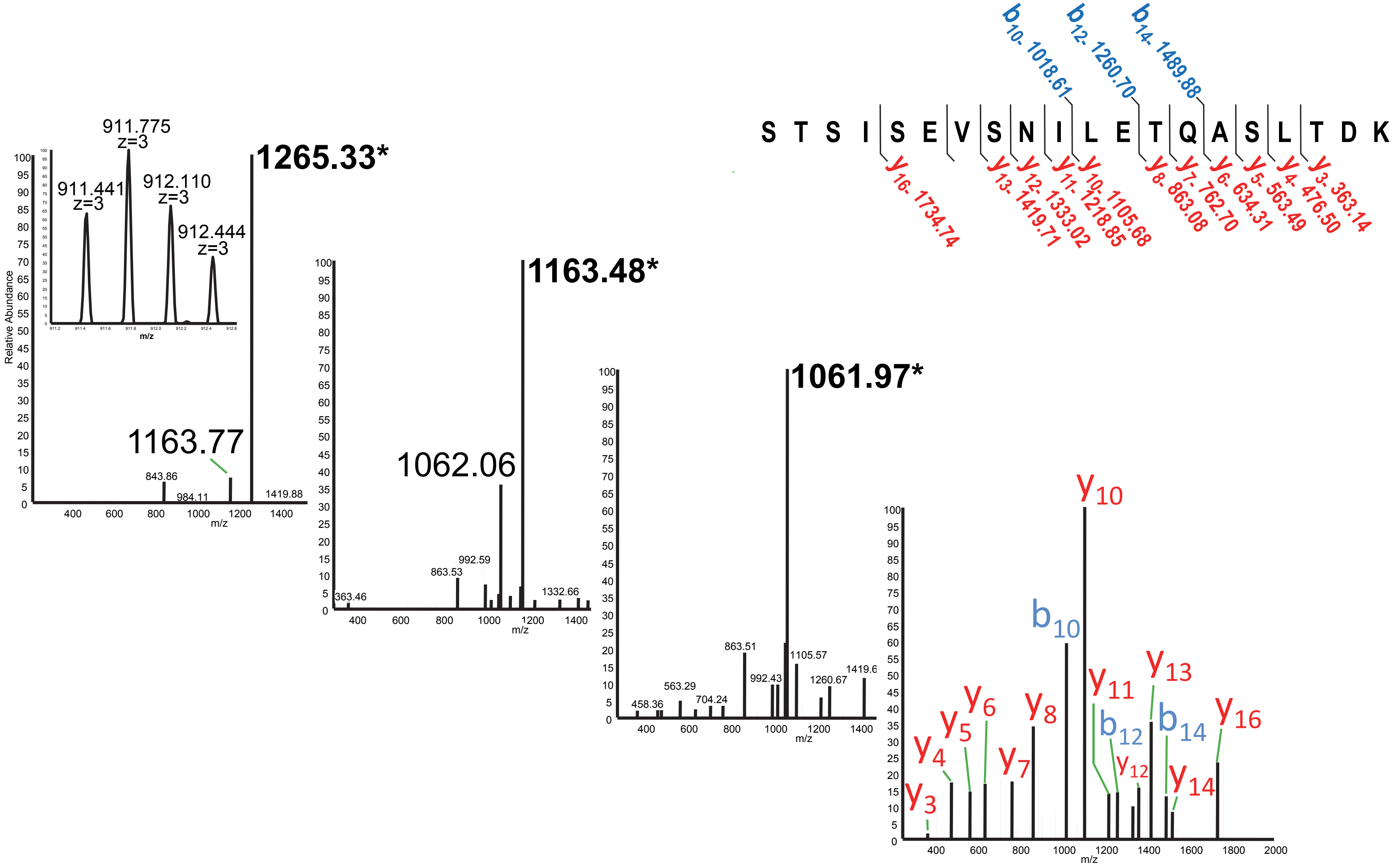
SQVMIASDKTGNESLTPTIR nude
m/z= 722.04 z=3, scan 1348 RT 19.75



Supplemental Figure 3t

SQVMIASDKTGNESLTPTIR +(HexNAc)
m/z= 789.73 z=3, scan 1316 RT 19.47





Supplemental Figure 4

Supplemental Figure 5a

S$_{192}$TSISEVSNILETQASLTDK$_{211}$

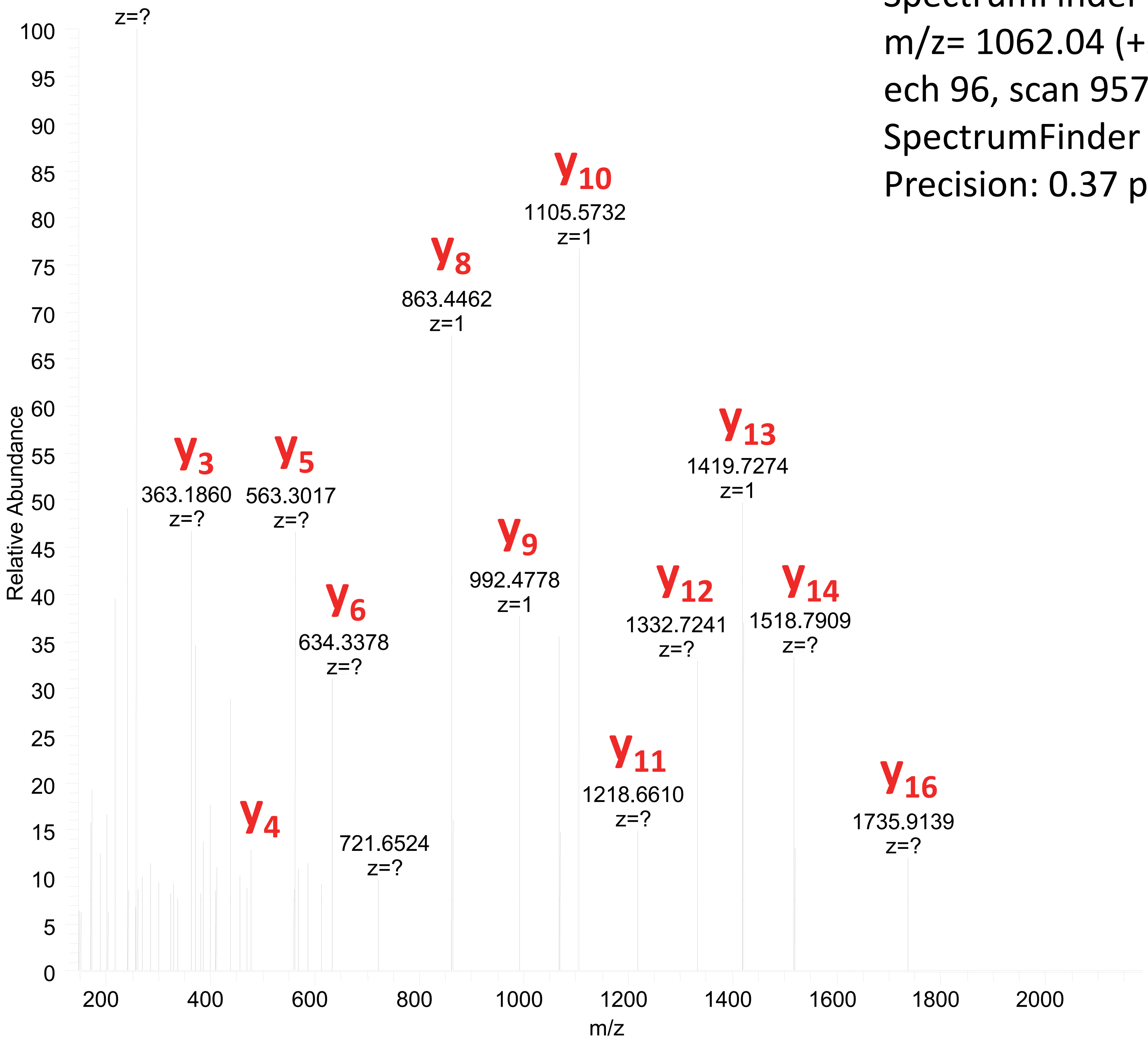
SpectrumFinder Reference

m/z= 1062.04 (+2)

ech 96, scan 9577

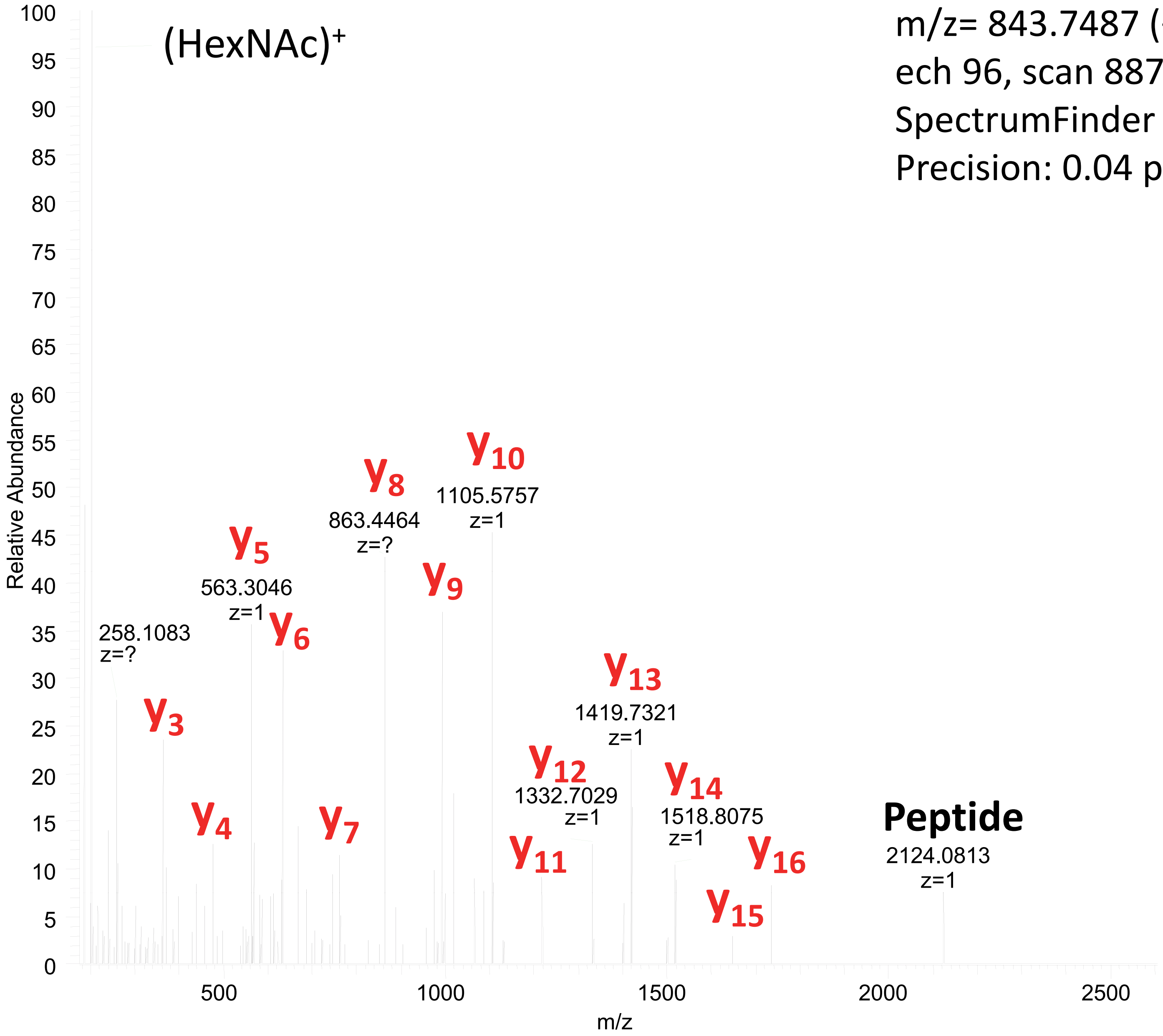
SpectrumFinder score: 0.00E+00

Precision: 0.37 ppm



Supplemental Figure 5b

S$_{192}$TSISEVSNILETQASLTDK$_{211}$
+ 2 HexNAc
m/z= 843.7487 (+3)
ech 96, scan 8871
SpectrumFinder score: 3.25E-09
Precision: 0.04 ppm



Supplemental Figure 5c

S$_{192}$TSISEVSNILETQASLTDK$_{211}$

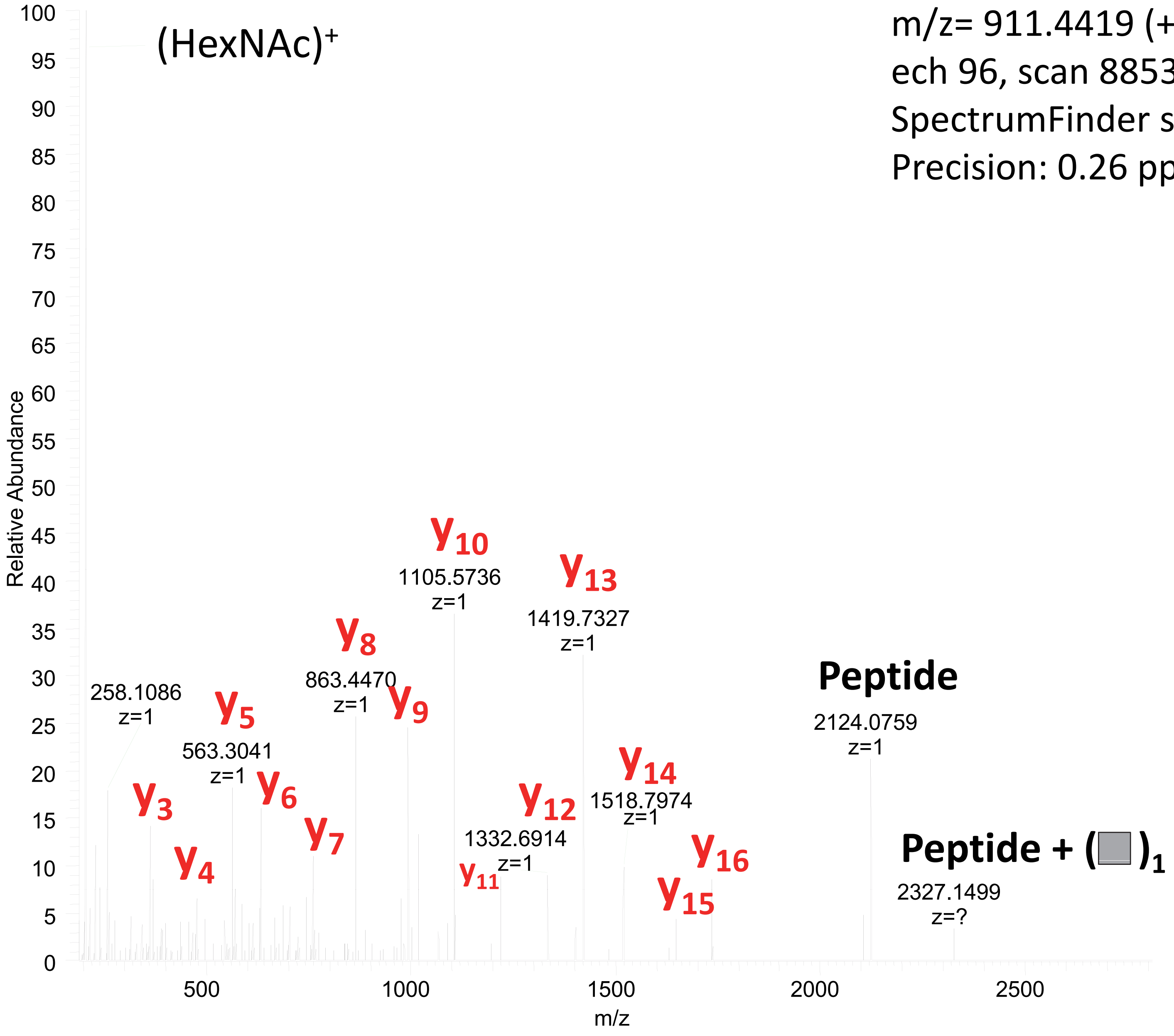
+ 3 HexNAc

m/z= 911.4419 (+3)

ech 96, scan 8853

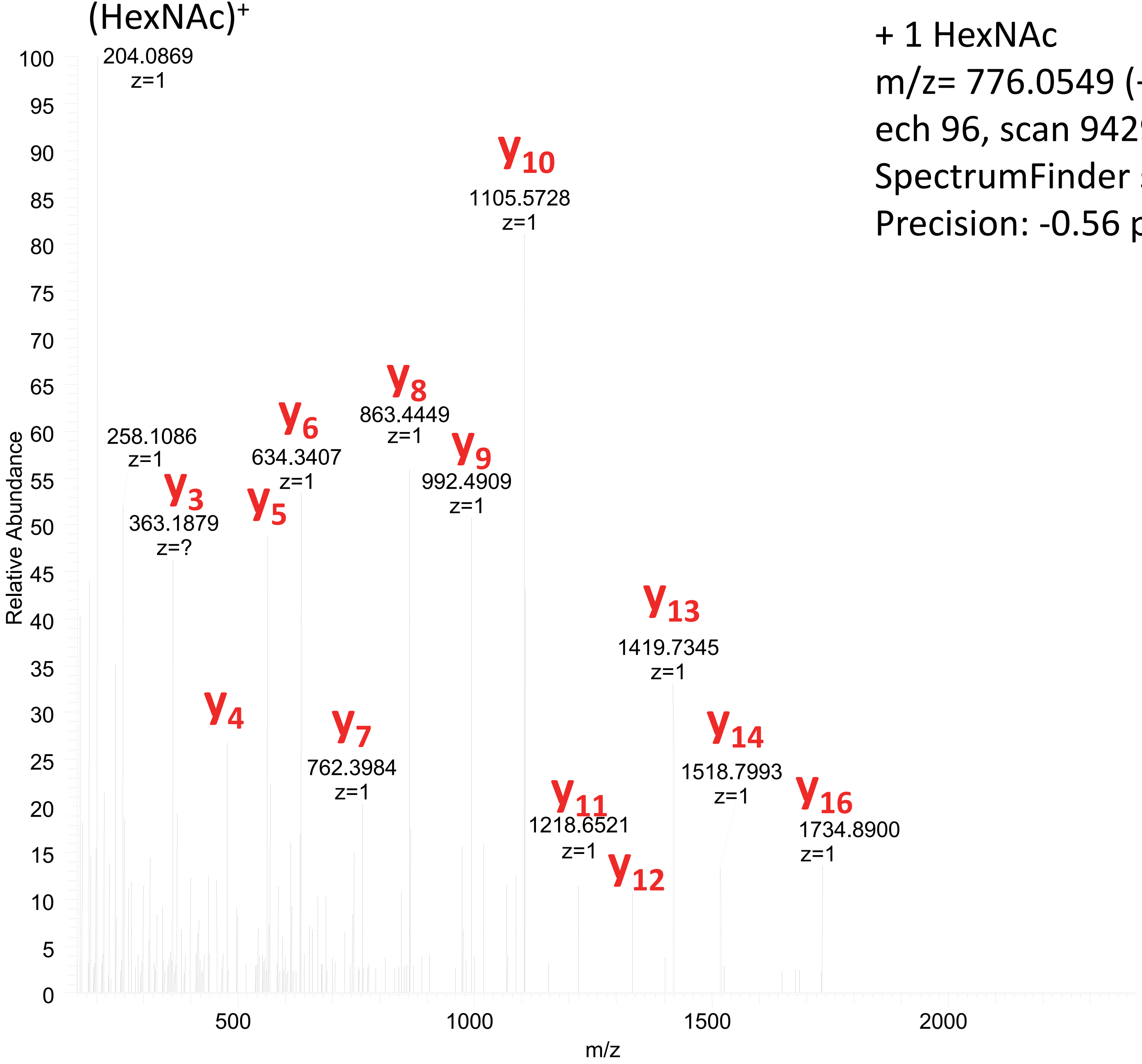
SpectrumFinder score: 1.61E-04

Precision: 0.26 ppm



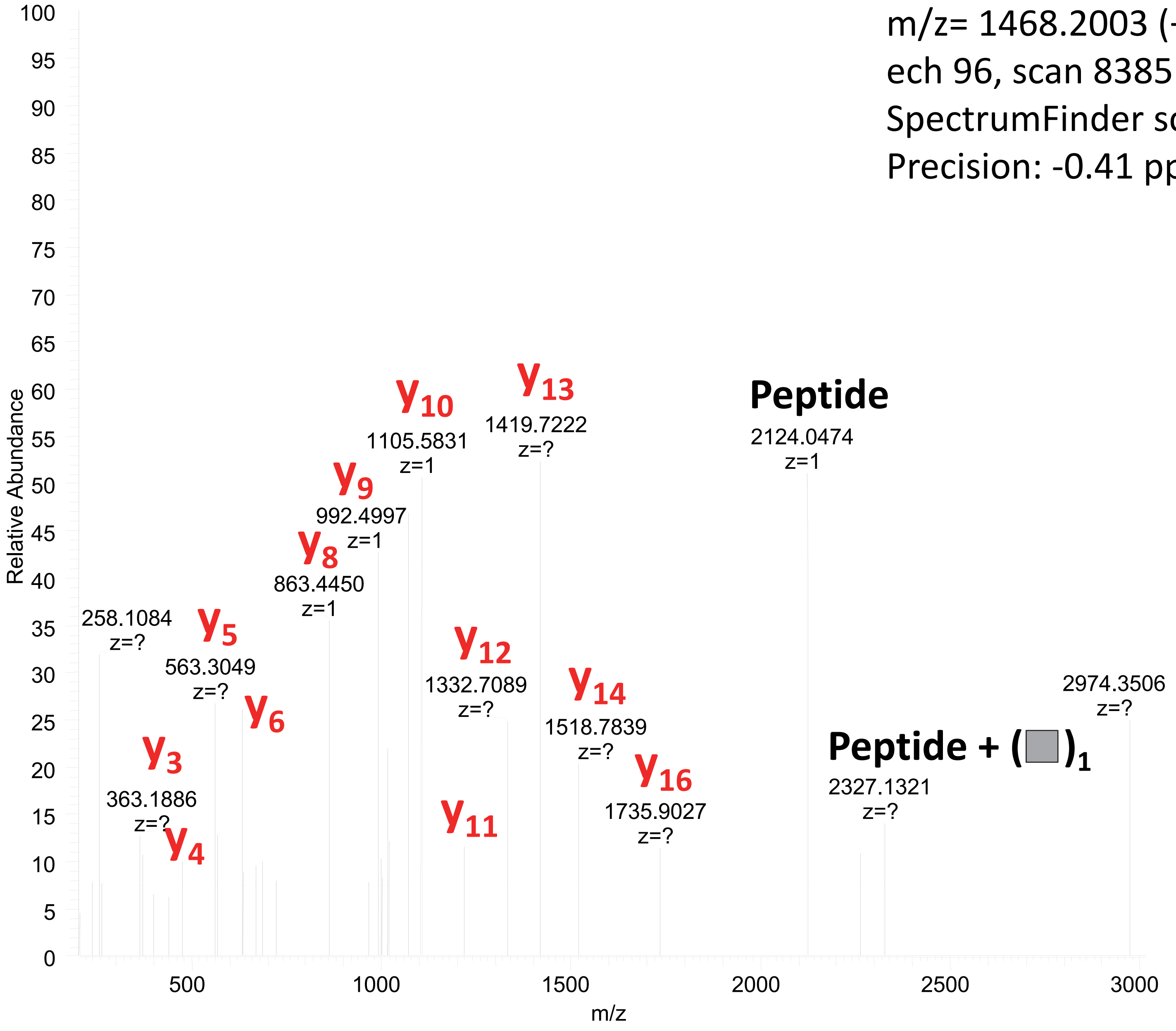
Supplemental Figure 5d

S$_{192}$TSISEVSNILETQASLTDK$_{211}$
+ 1 HexNAc
m/z= 776.0549 (+3)
ech 96, scan 9429
SpectrumFinder score: 1.08E-09
Precision: -0.56 ppm



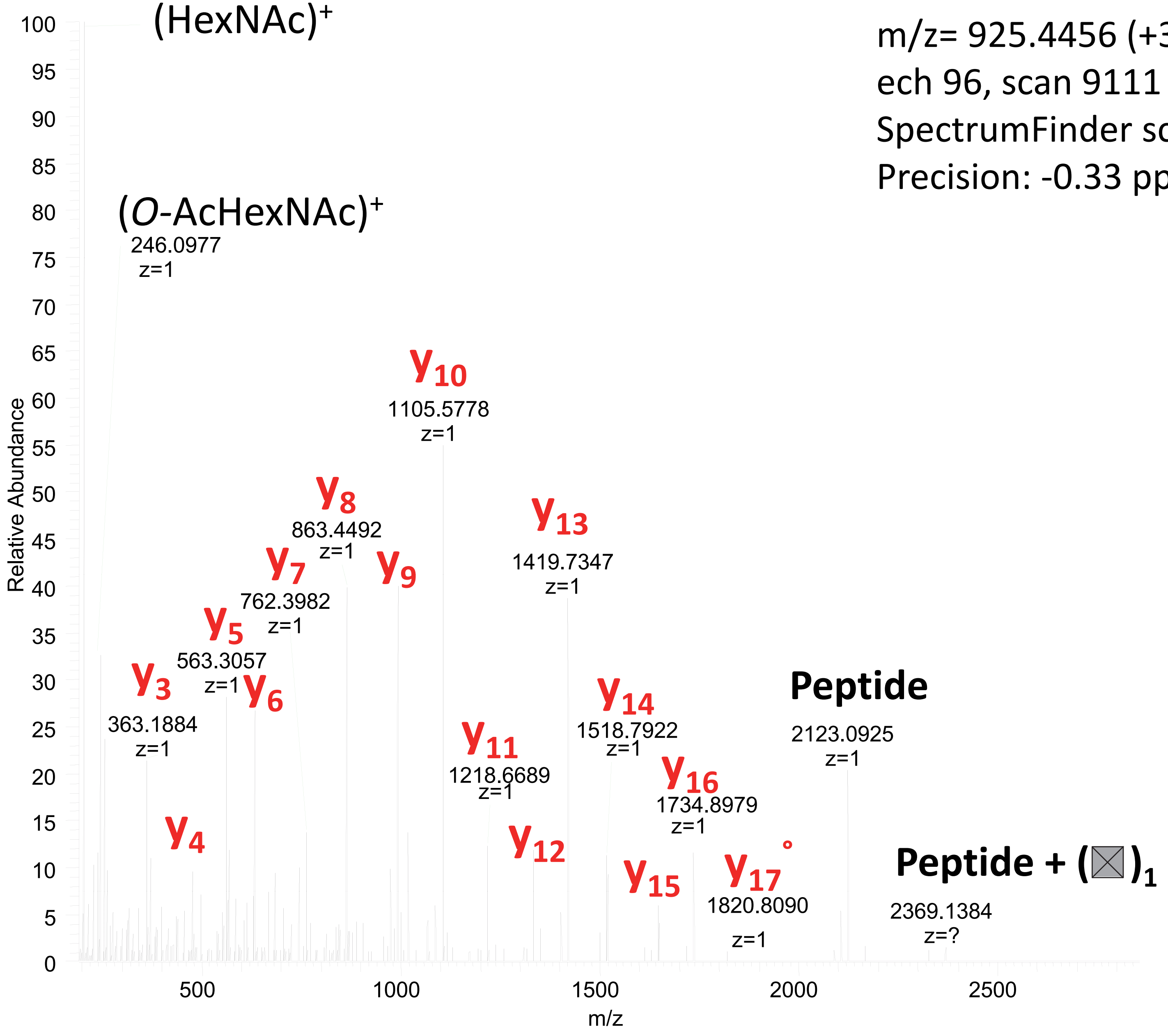
Supplemental Figure 5e

S$_{192}$TSISEVSNILETQASLTDK$_{211}$
+ 4 HexNAc
m/z= 1468.2003 (+2)
ech 96, scan 8385
SpectrumFinder score: 1.31E-03
Precision: -0.41 ppm



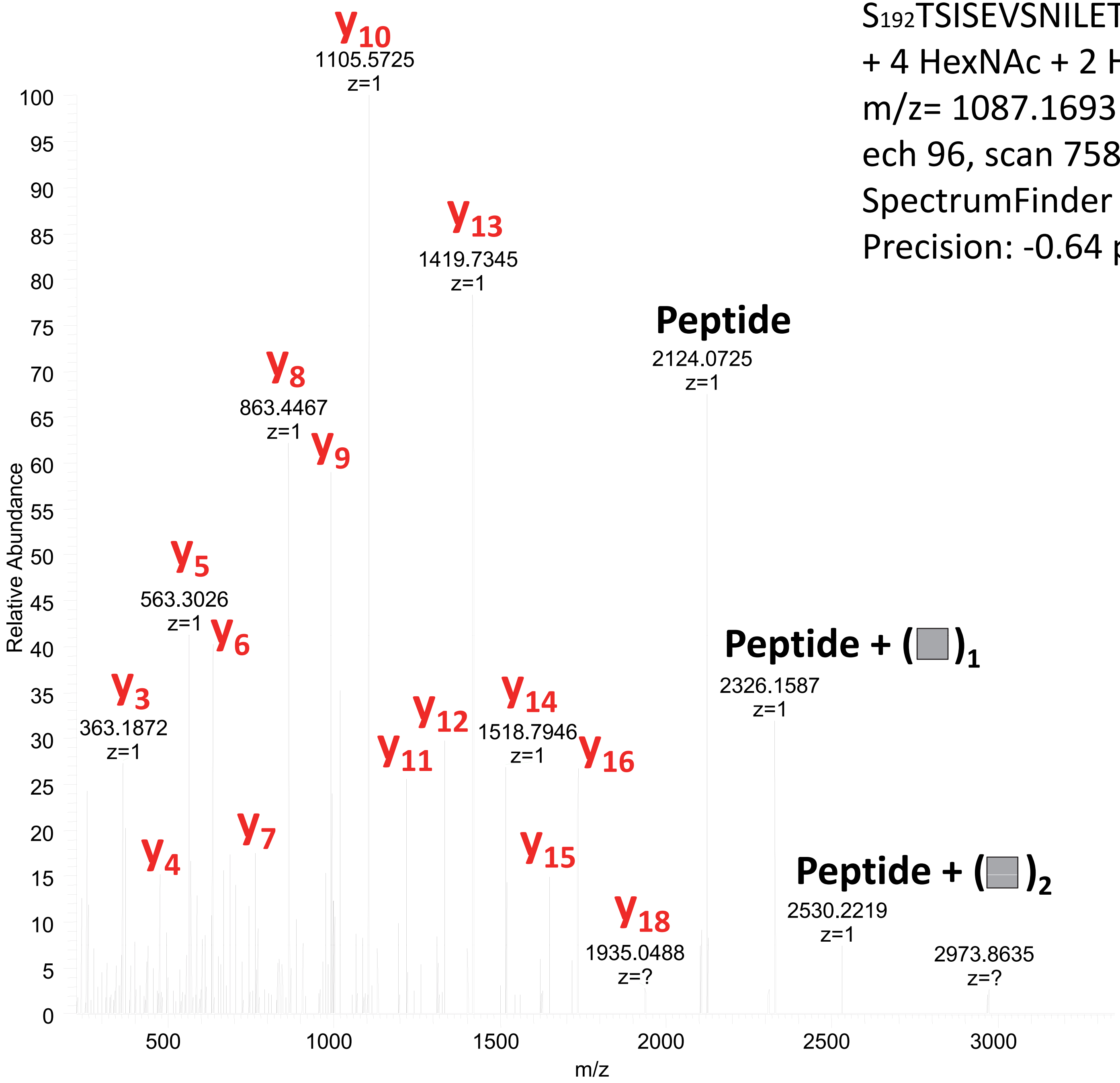
Supplemental Figure 5f

S_{192}TSISEVSNILETQASLTDK$_{211}$
+ 1 *O*-AcHexNAc + 2 HexNAc
m/z= 925.4456 (+3)
ech 96, scan 9111
SpectrumFinder score: 5.51E-06
Precision: -0.33 ppm



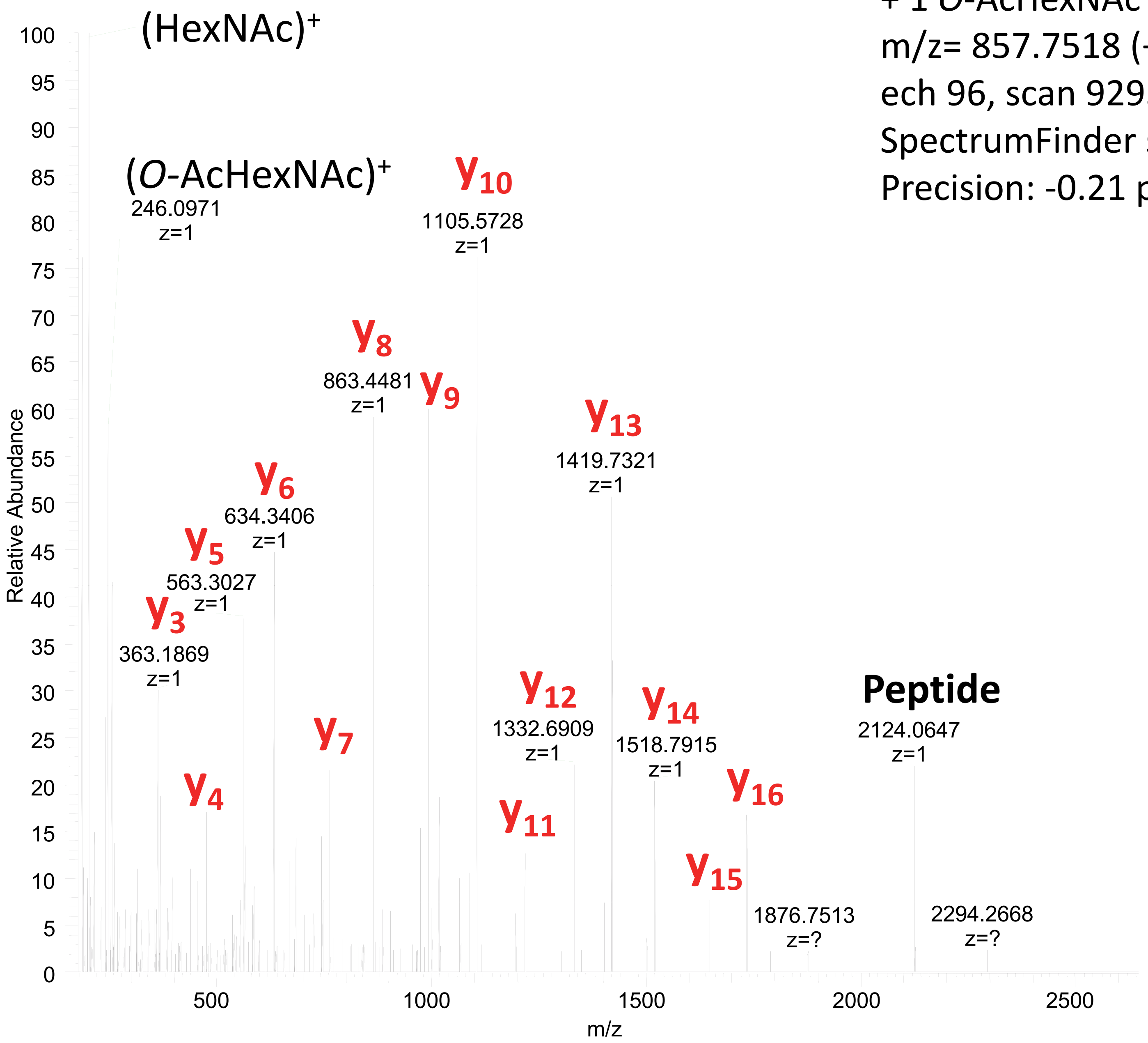
Supplemental Figure 5g

S$_{192}$TSISEVSNILETQASLTDK$_{211}$
+ 4 HexNAc + 2 Hex
m/z= 1087.1693 (+3)
ech 96, scan 7581
SpectrumFinder score: 4.61E-03
Precision: -0.64 ppm



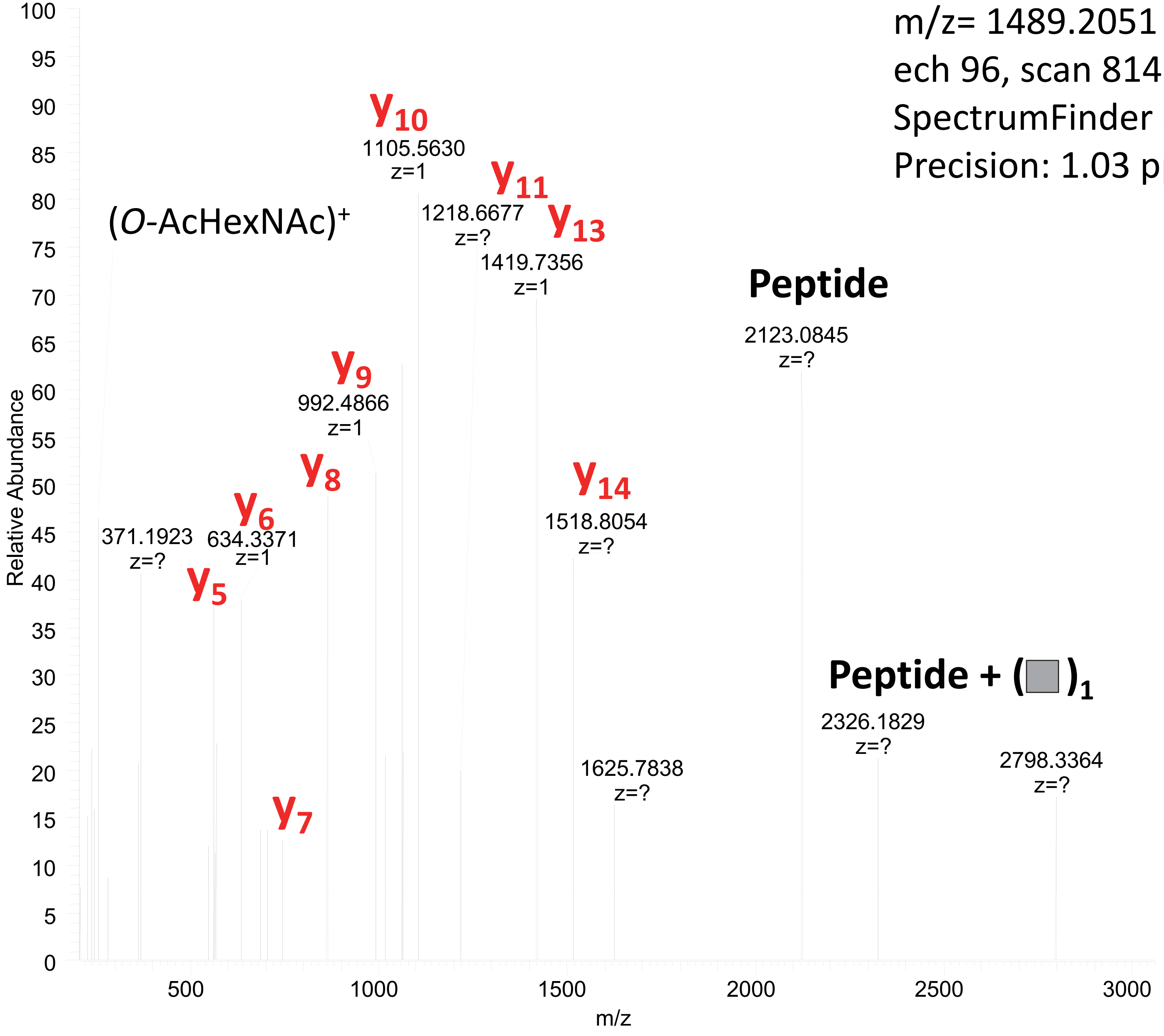
Supplemental Figure 5h

S_{192}TSISEVSNILETQASLTDK$_{211}$
+ 1 *O*-AcHexNAc + 1 HexNAc
m/z= 857.7518 (+3)
ech 96, scan 9293
SpectrumFinder score: 6.73E-08
Precision: -0.21 ppm



Supplemental Figure 5i

S_{192}TSISEVSNILETQASLTDK$_{211}$
+ 1 *O*-AcHexNAc + 3 HexNAc
m/z= 1489.2051 (+2)
ech 96, scan 8145
SpectrumFinder score: 4.62E-02
Precision: 1.03 ppm



Supplemental Figure 5j

$S_{192}TSISEVSNILETQASLTDK_{211}$

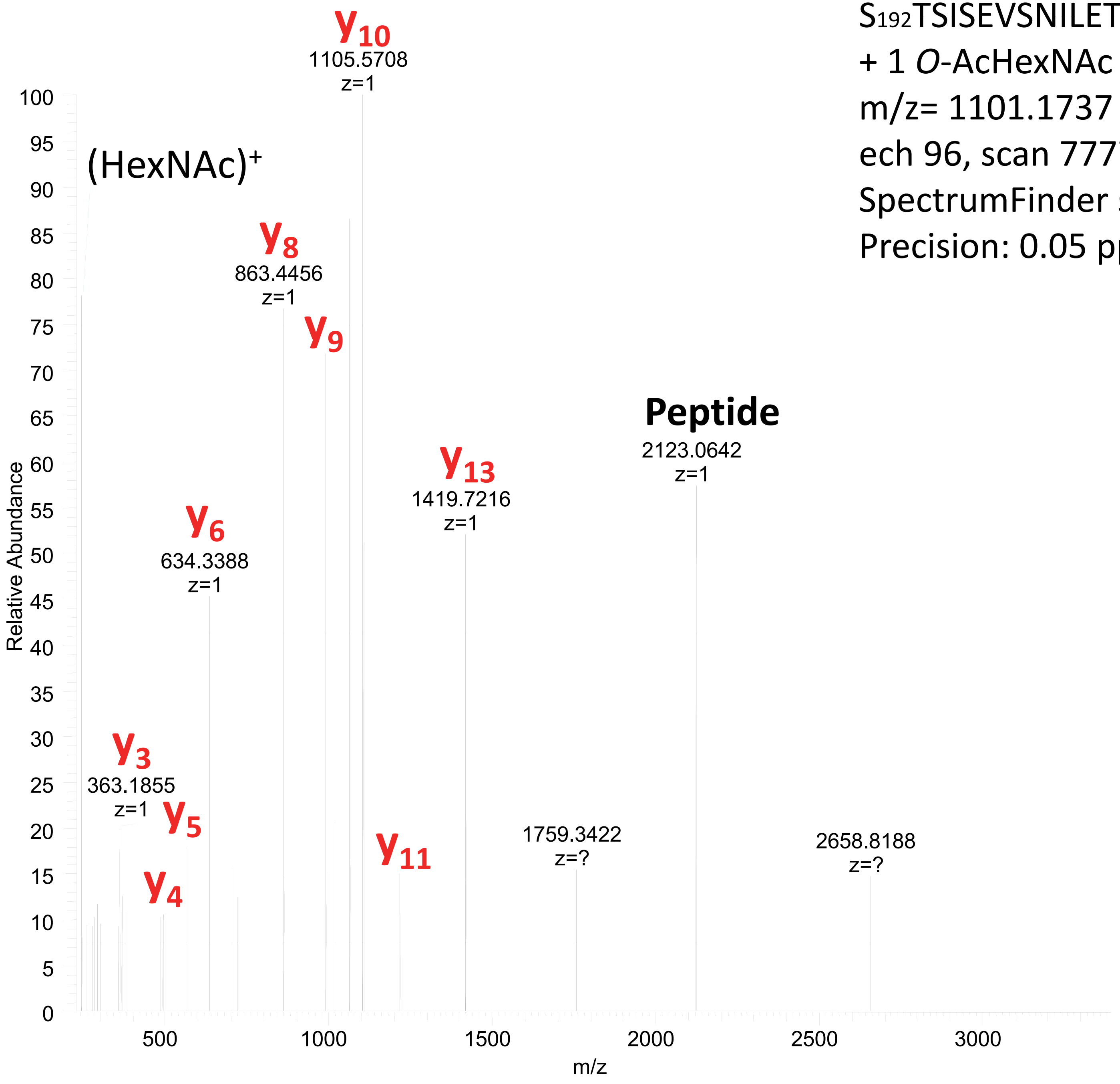
+ 1 *O*-AcHexNAc + 3 HexNAc + 2 Hex

m/z= 1101.1737 (+3)

ech 96, scan 7777

SpectrumFinder score: 1.38E-02

Precision: 0.05 ppm



Supplemental Figure 5k

$S_{192}TSISEVSNILETQASLTDK_{211}$

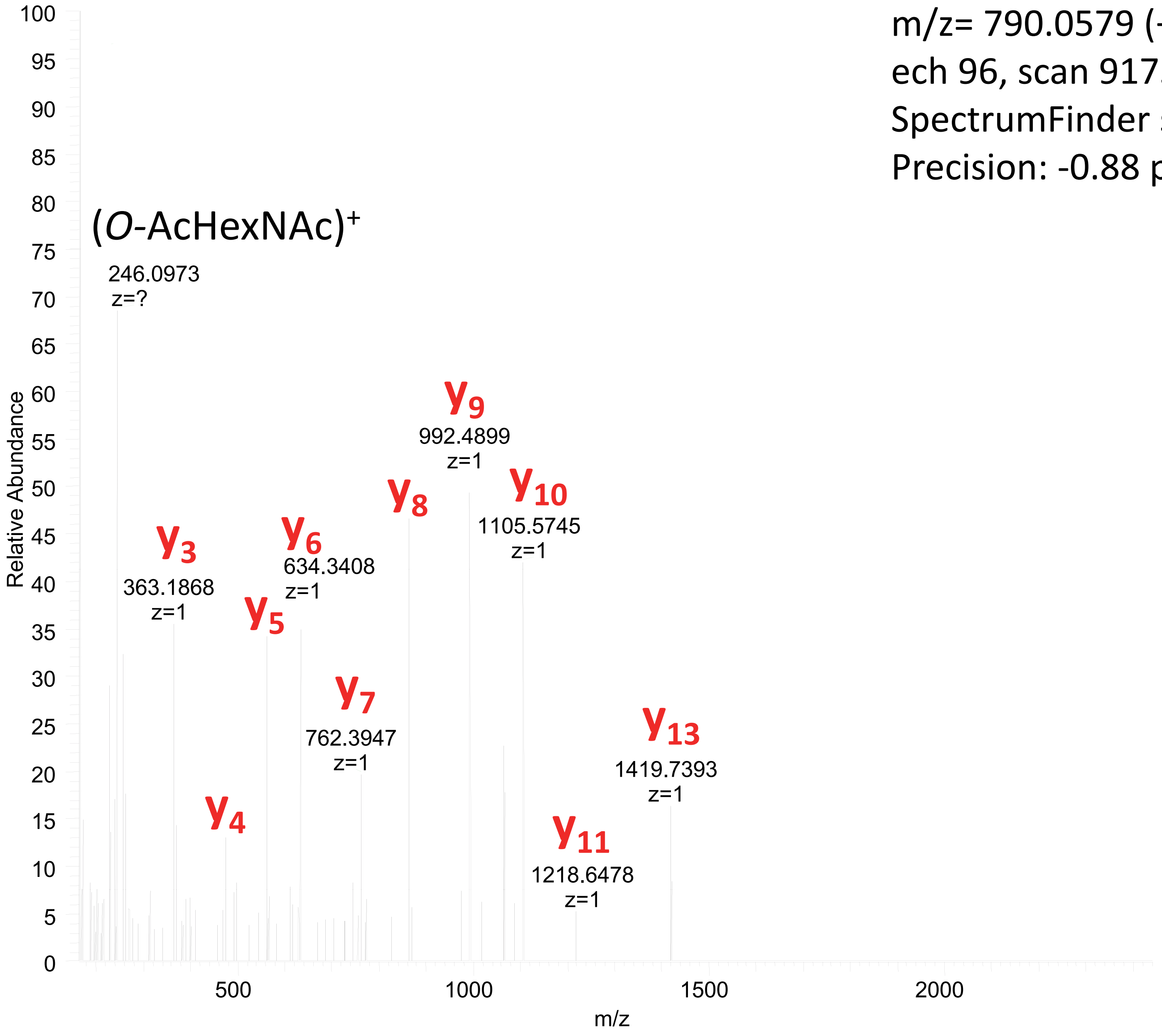
+ 1 *O*-AcHexNAc

m/z= 790.0579 (+3)

ech 96, scan 9175

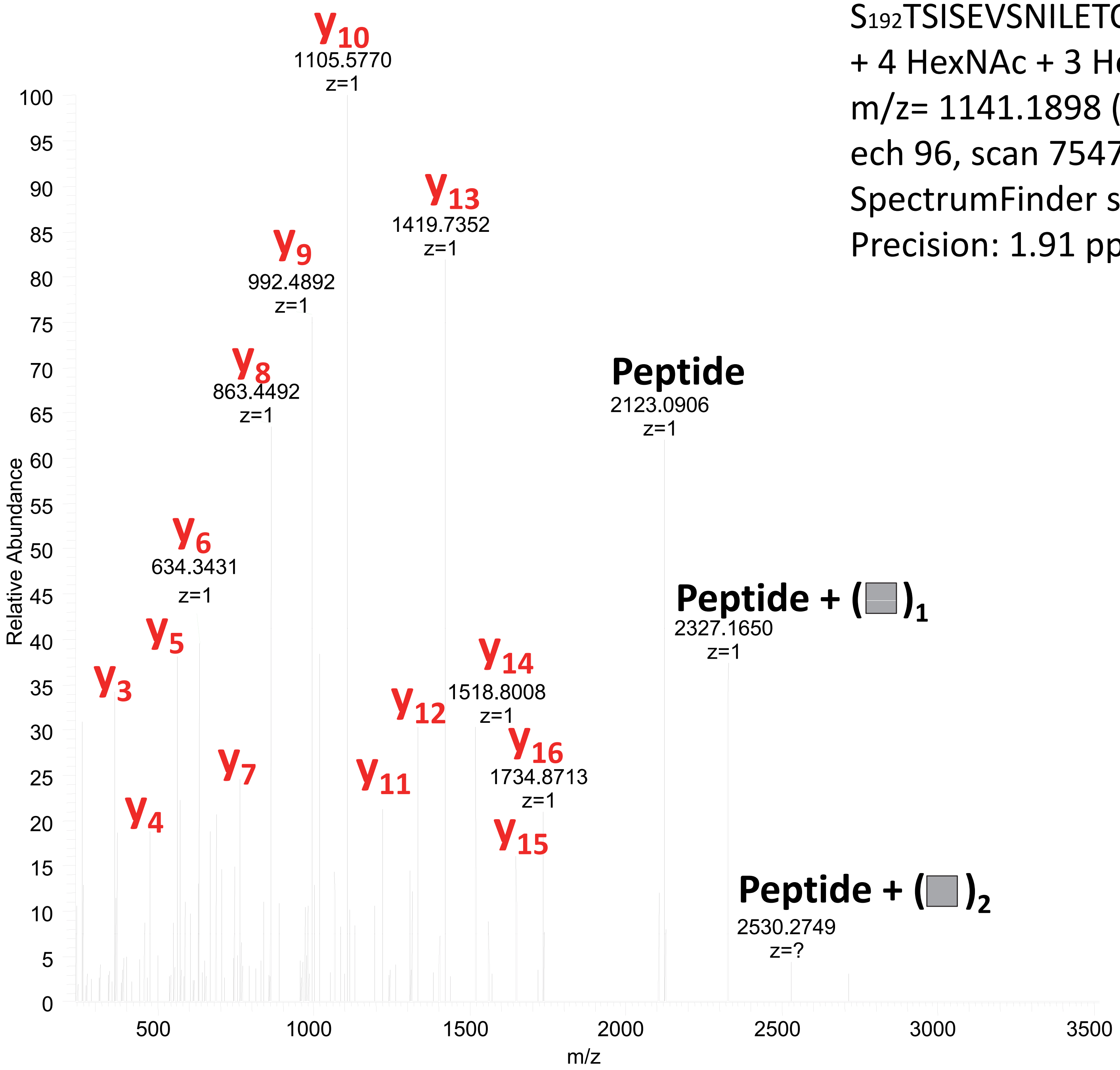
SpectrumFinder score: 7.42E-10

Precision: -0.88 ppm



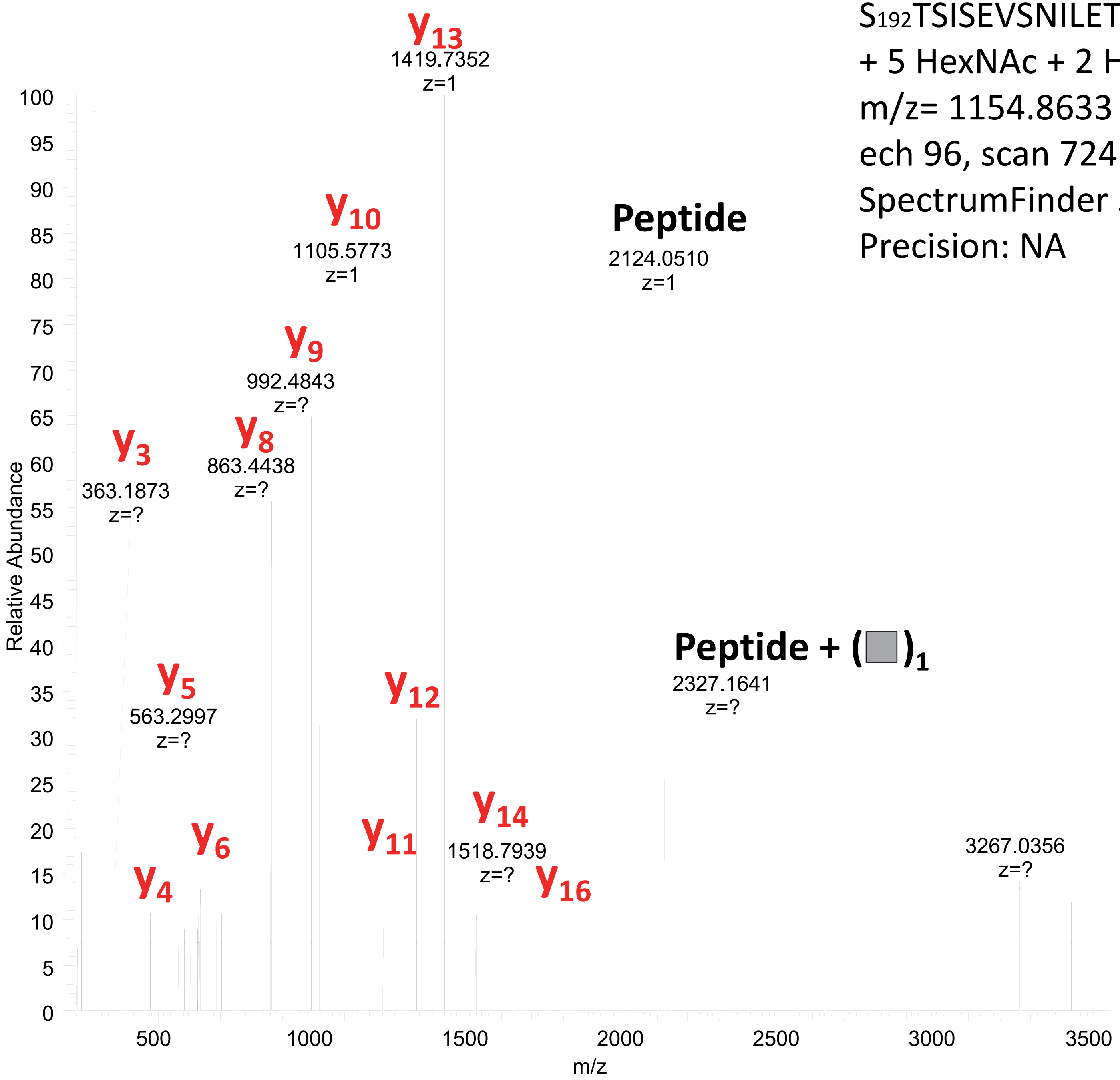
Supplemental Figure 5l

S_{192}TSISEVSNILETQASLTDK_{211}
+ 4 HexNAc + 3 Hex
m/z= 1141.1898 (+3)
ech 96, scan 7547
SpectrumFinder score: 1.13E-03
Precision: 1.91 ppm



Supplemental Figure 5m

S$_{192}$TSISEVSNILETQASLTDK$_{211}$
+ 5 HexNAc + 2 Hex
m/z= 1154.8633 (+3)
ech 96, scan 7241
SpectrumFinder score: Manual detection
Precision: NA



Supplemental Figure 5n

S_{192}TSISEVSNILETQASLTDK$_{211}$

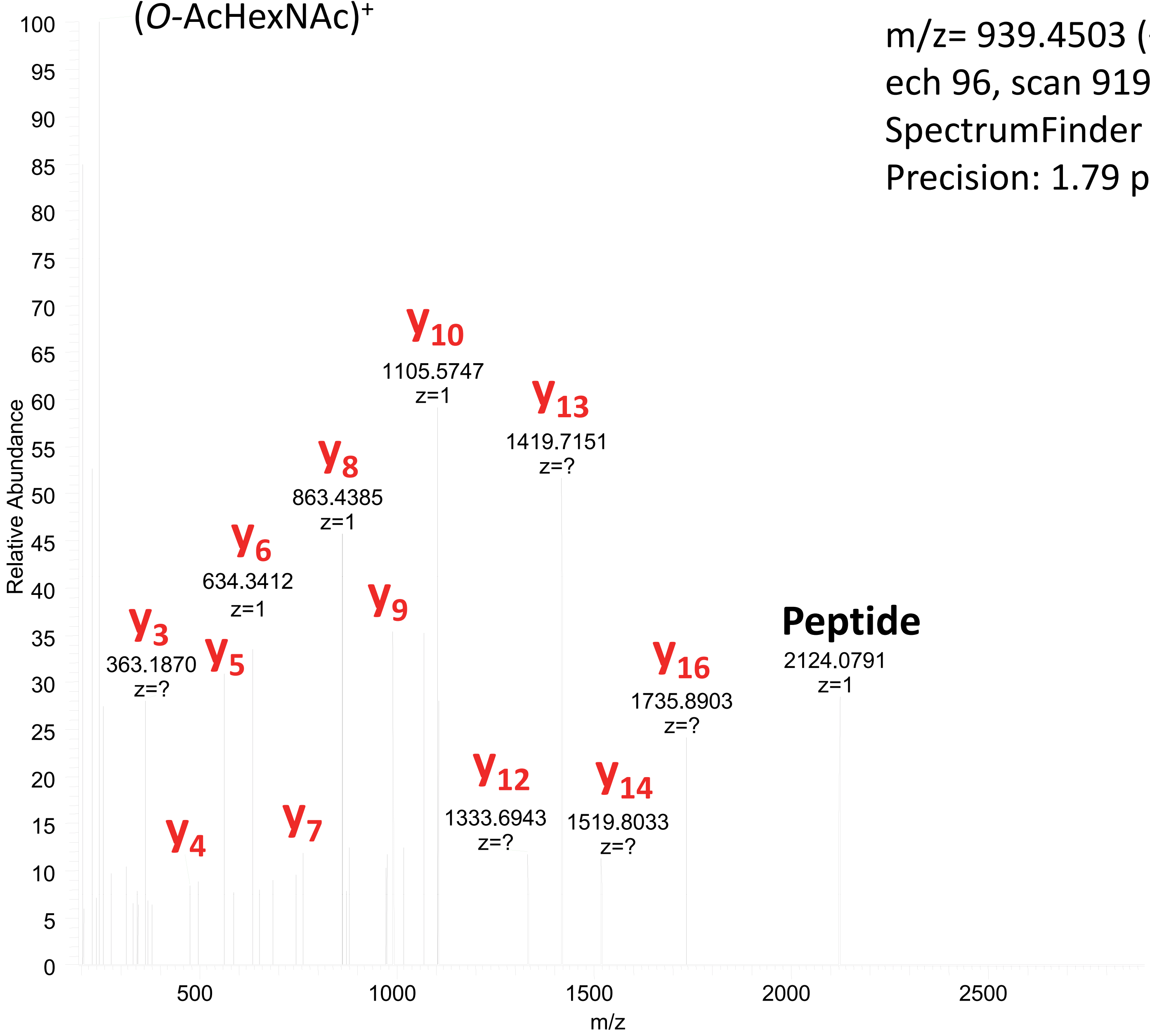
+ 2 *O*-AcHexNAc + 1 HexNAc

m/z= 939.4503 (+3)

ech 96, scan 9195

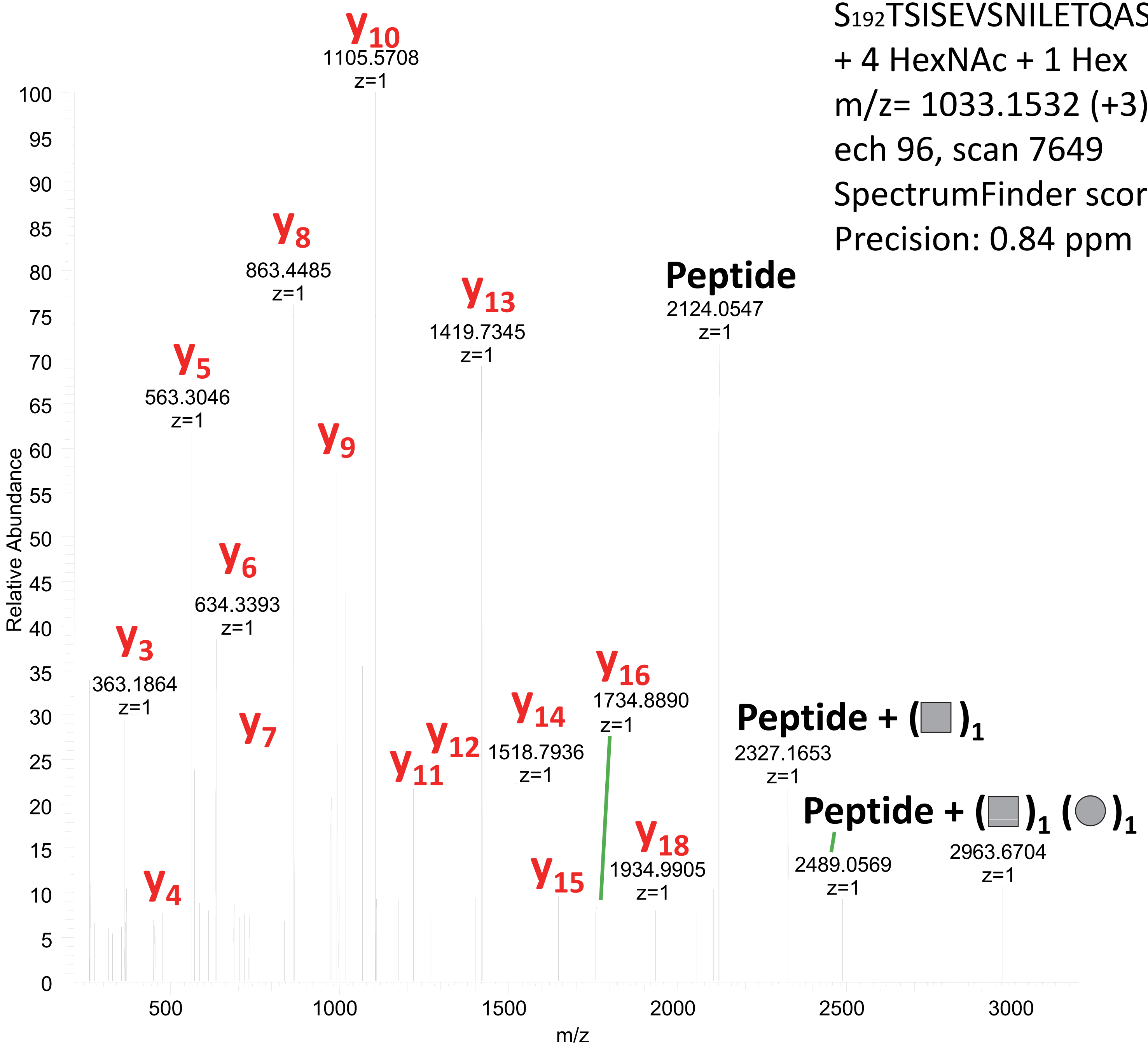
SpectrumFinder score: 1.31E-03

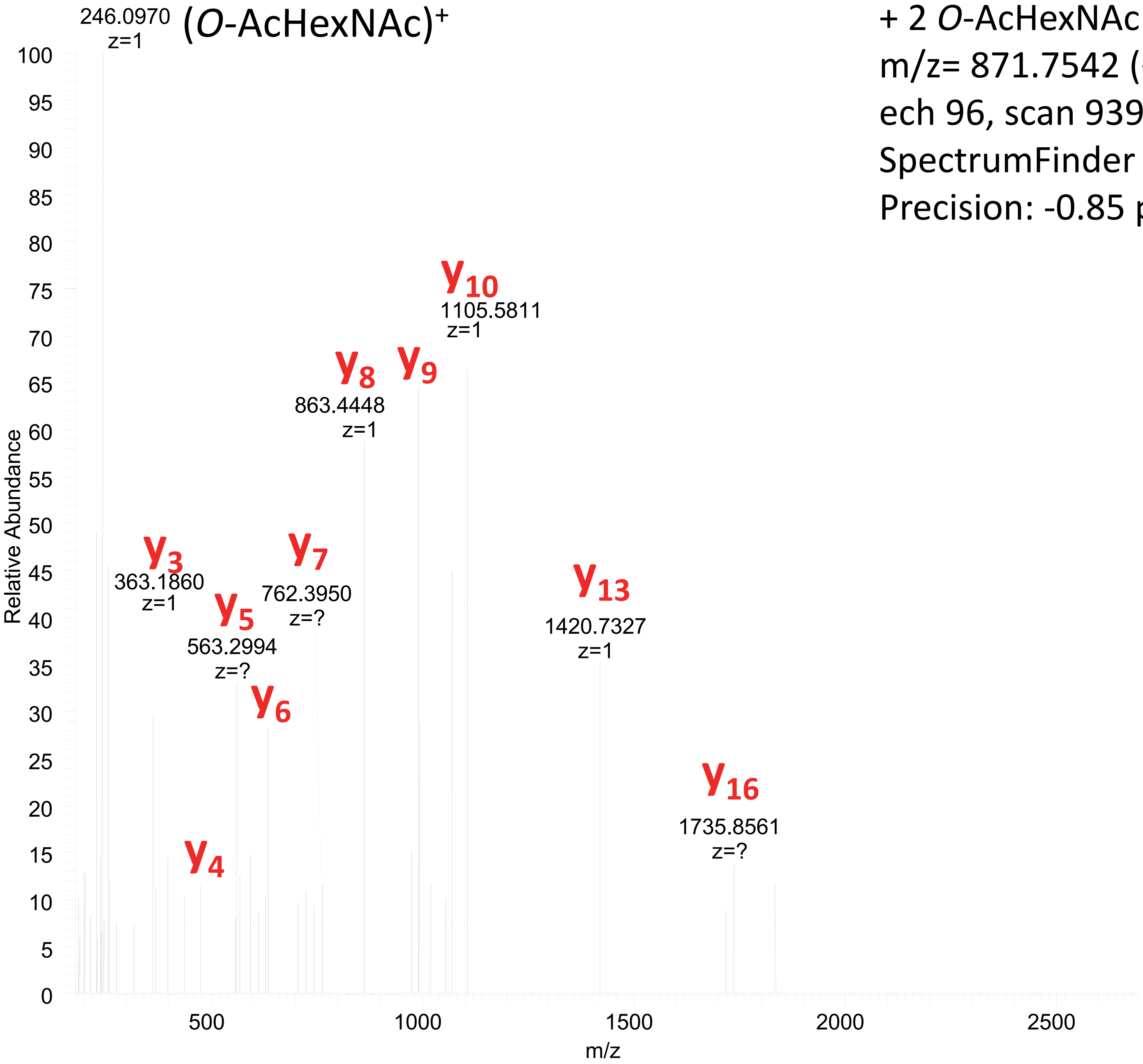
Precision: 1.79 ppm



Supplemental Figure 5o

S$_{192}$TSISEVSNILETQASLTDK$_{211}$
+ 4 HexNAc + 1 Hex
m/z= 1033.1532 (+3)
ech 96, scan 7649
SpectrumFinder score: 1.92E-02
Precision: 0.84 ppm





Supplemental Figure 5p

S_{192}TSISEVSNILETQASLTDK_{211}
+ 2 *O*-AcHexNAc
m/z= 871.7542 (+3)
ech 96, scan 9390
SpectrumFinder score: 1.13E-05
Precision: -0.85 ppm

Supplemental Figure 5q

S_{192}TSISEVSNILETQASLTDK_{211}

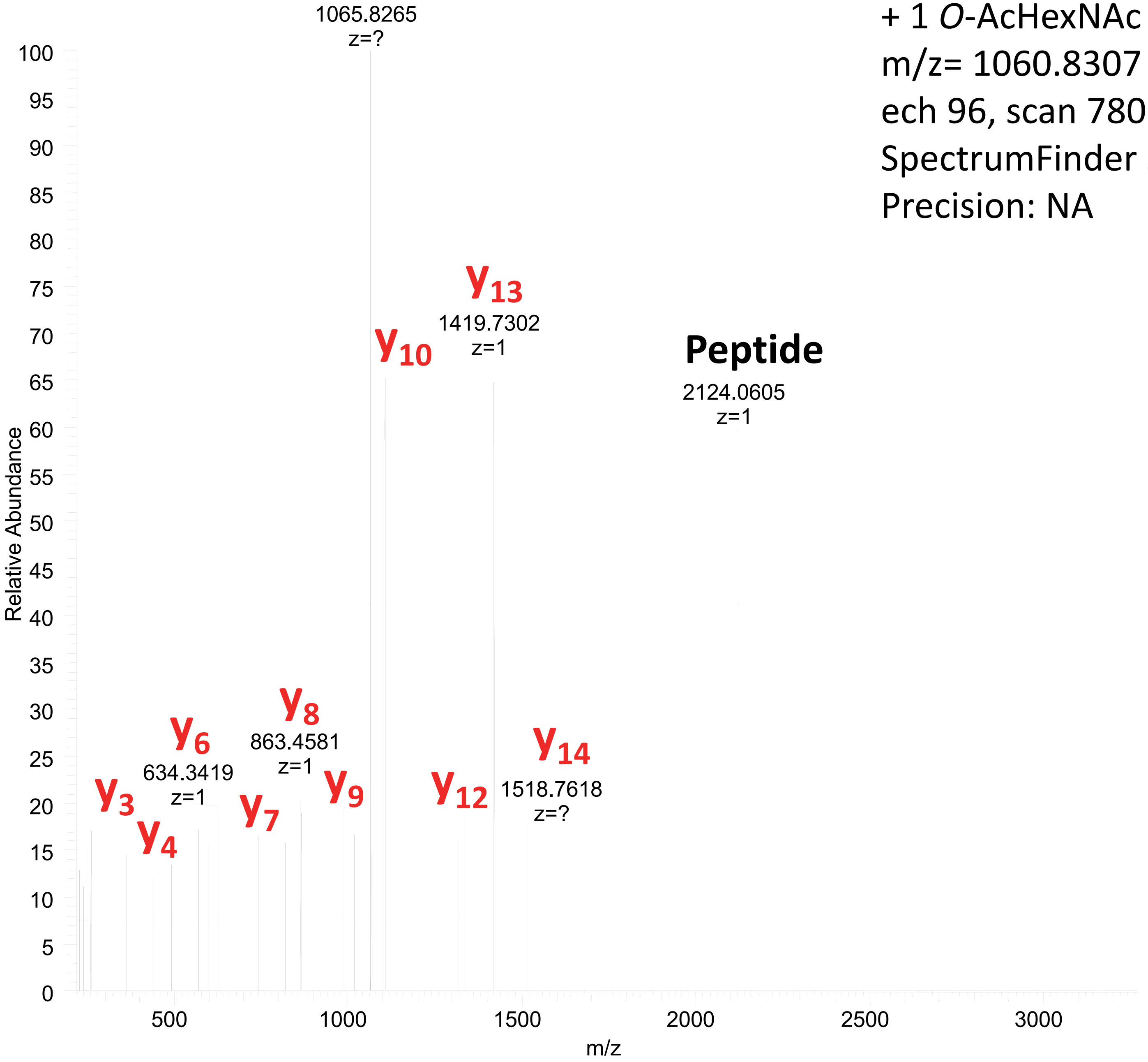
+ 1 *O*-AcHexNAc + 4 HexNAc

m/z= 1060.8307 (+3)

ech 96, scan 7801

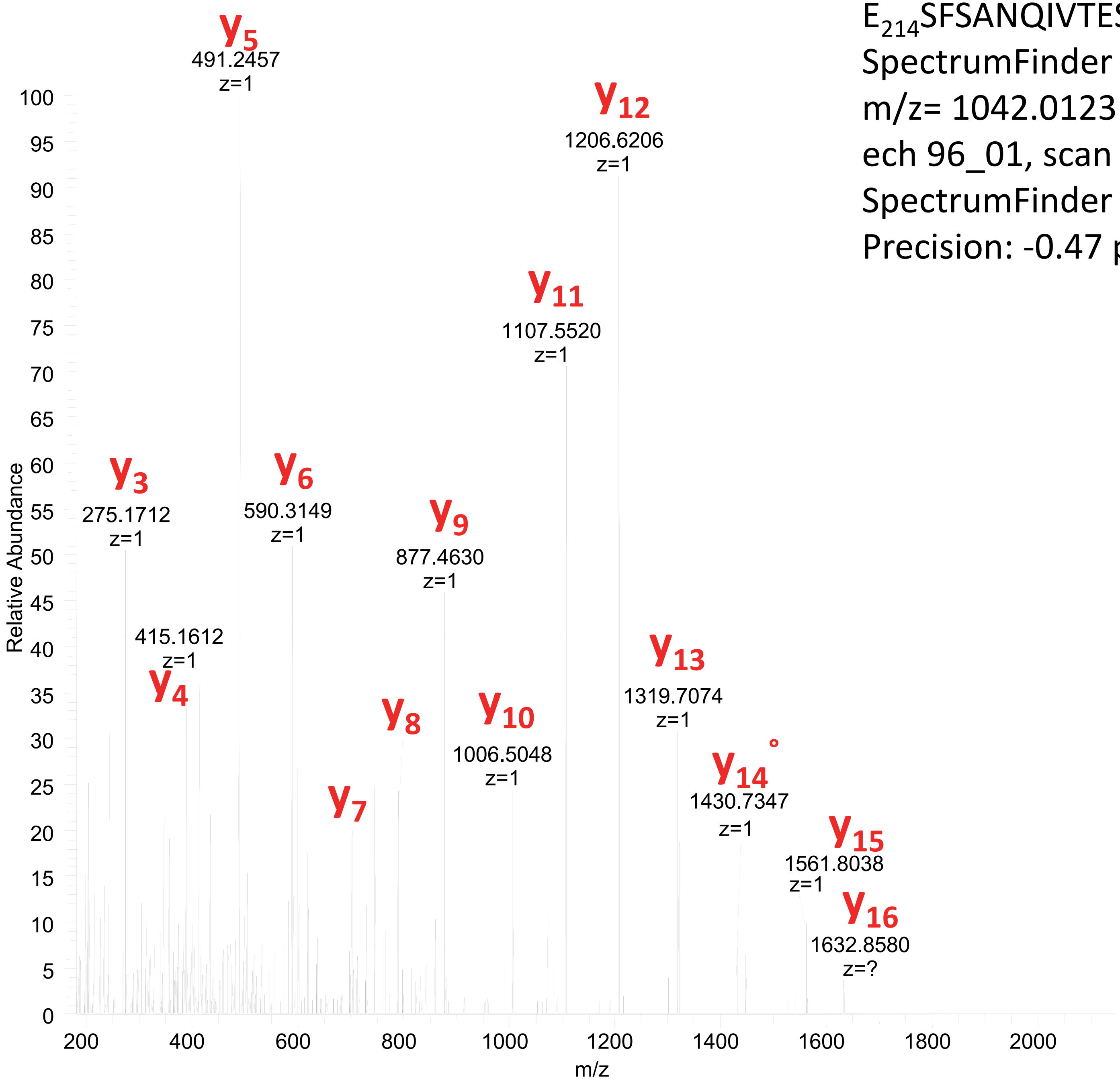
SpectrumFinder score: Manual detection

Precision: NA



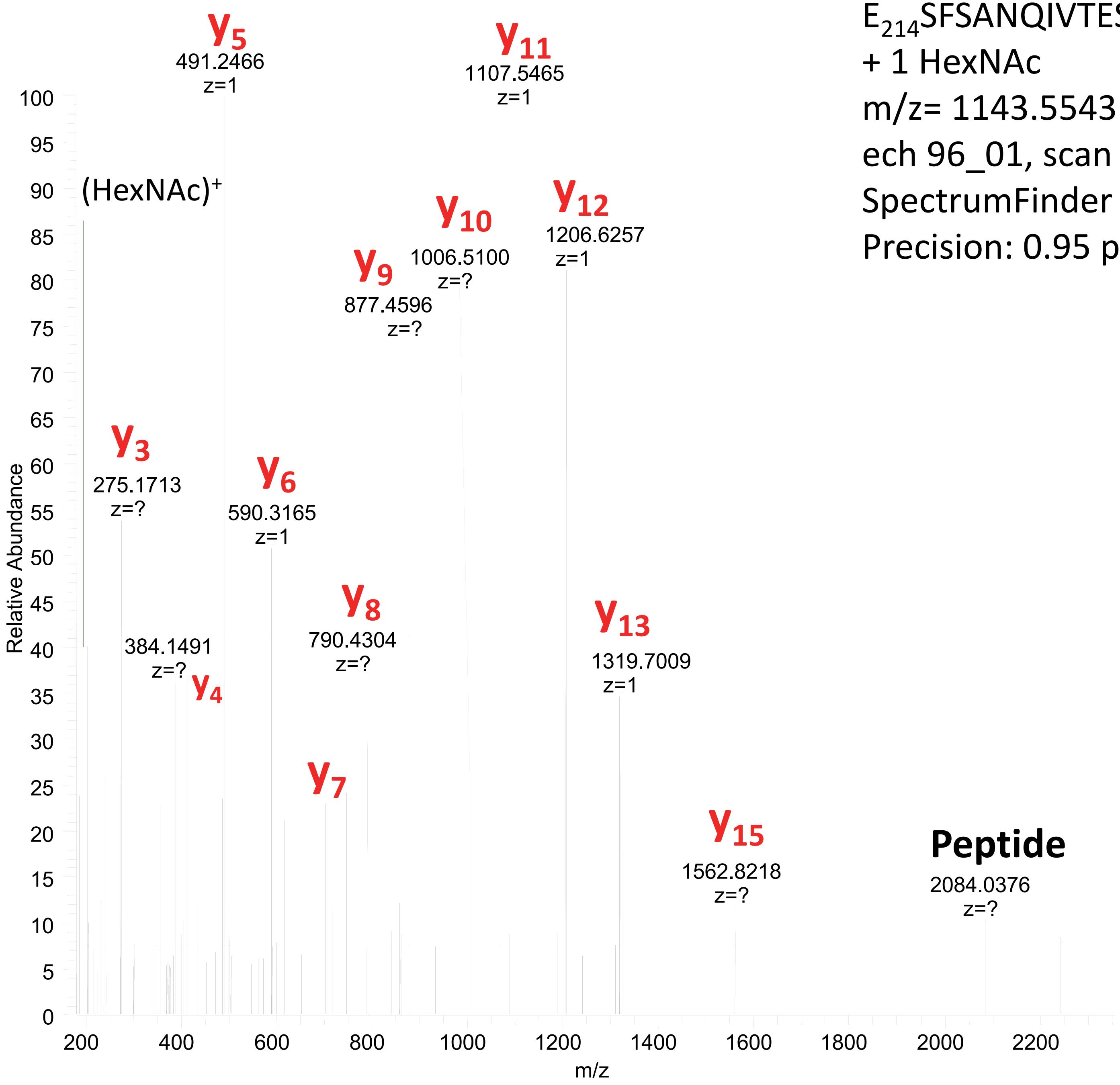
Supplemental Figure 5r

E_{214}SFSANQIVTESSLVTDAGK$_{233}$
SpectrumFinder reference
m/z= 1042.0123 (+2)
ech 96_01, scan 8526
SpectrumFinder score: 1.11E-16
Precision: -0.47 ppm



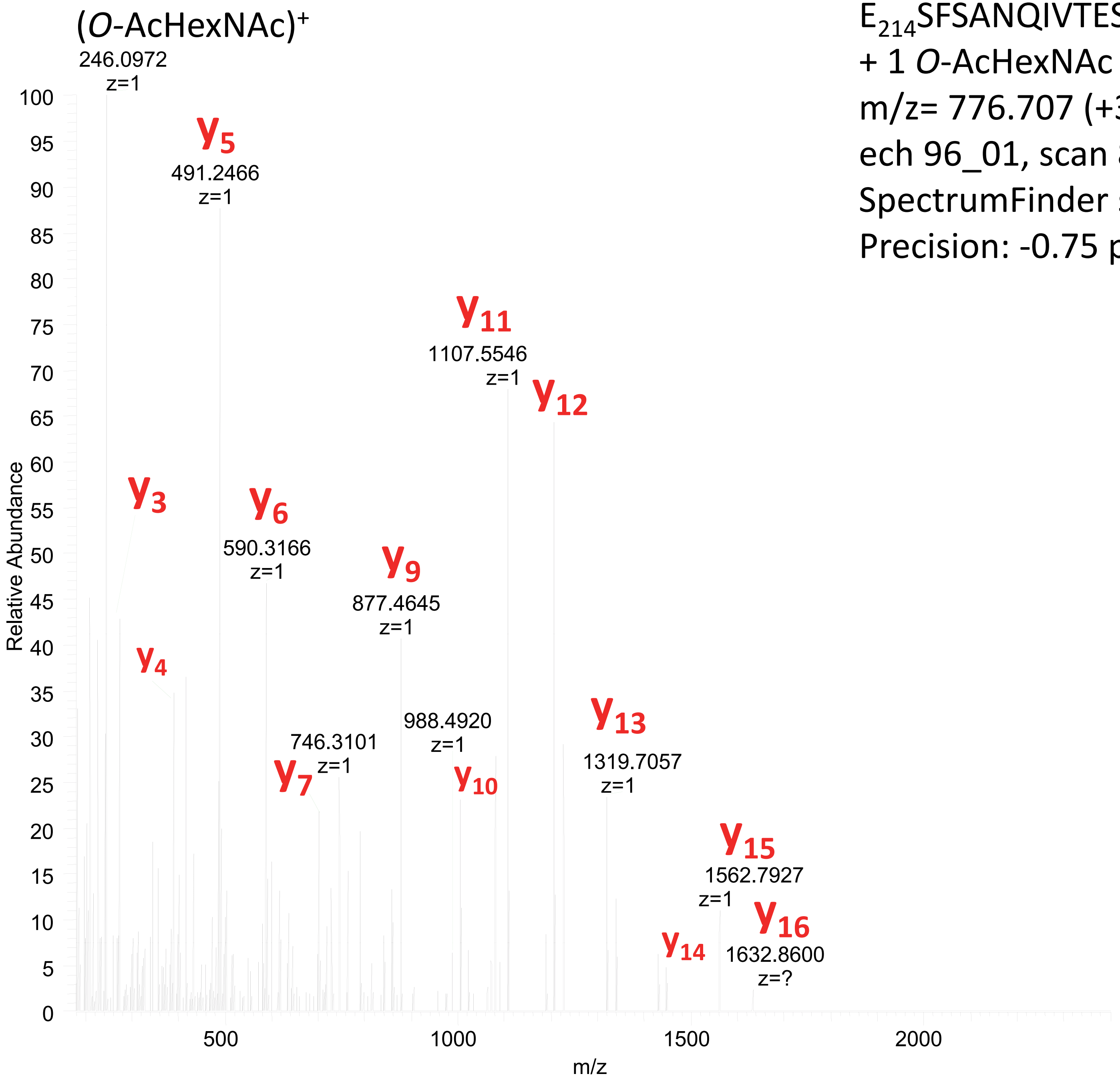
Supplemental Figure 5s

$E_{214}SFSANQIVTESSLVTDAGK_{233}$
+ 1 HexNAc
m/z= 1143.5543 (+2)
ech 96_01, scan 8238
SpectrumFinder score: 0.00E+00
Precision: 0.95 ppm



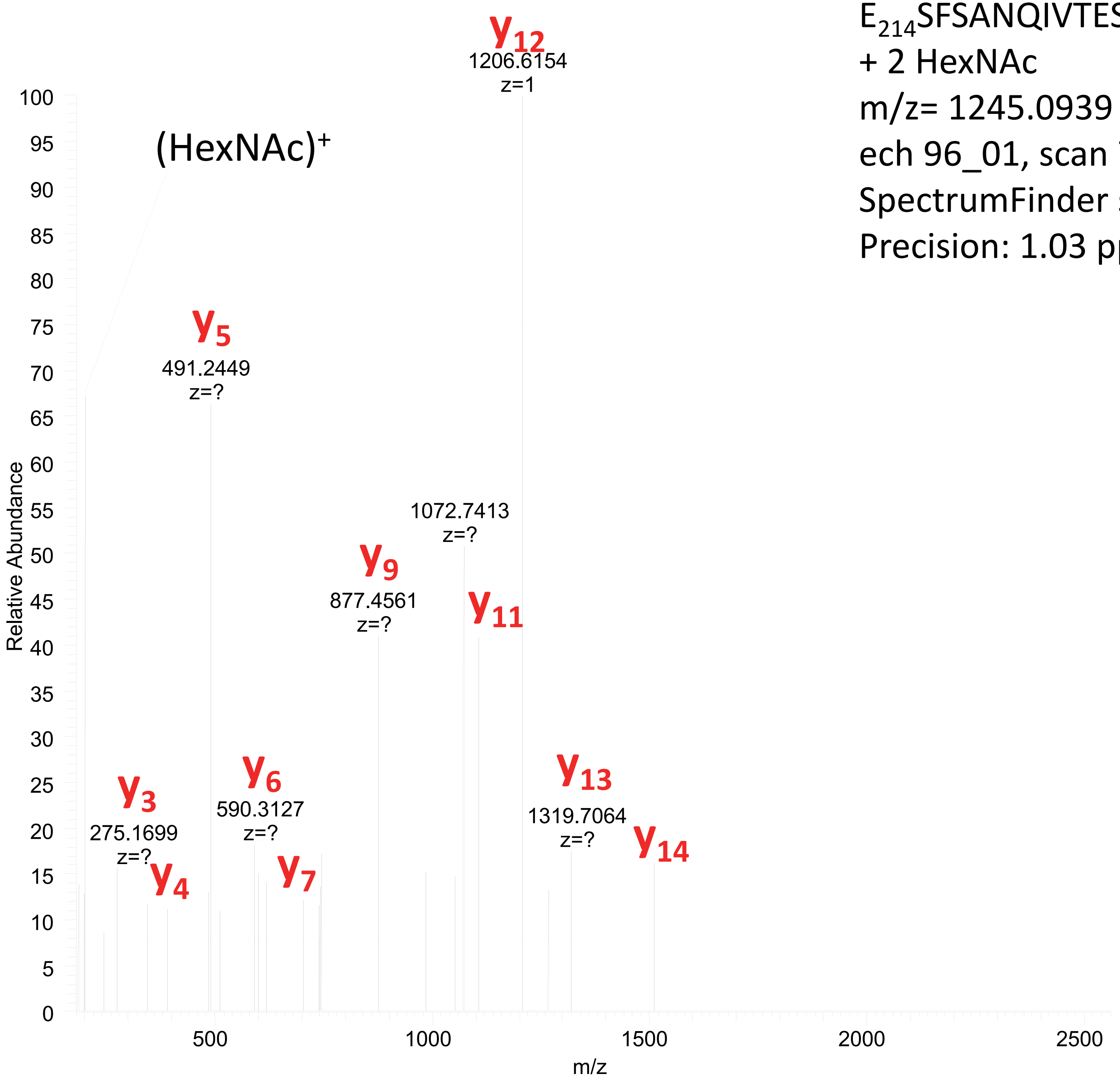
Supplemental Figure 5t

$E_{214}SFSANQIVTESSLVTDAGK_{233}$
+ 1 *O*-AcHexNAc
m/z= 776.707 (+3)
ech 96_01, scan 8433
SpectrumFinder score: 0.00E+00
Precision: -0.75 ppm



Supplemental Figure 5u

E_{214}SFSANQIVTESSLVTDAGK$_{233}$
+ 2 HexNAc
m/z= 1245.0939 (+2)
ech 96_01, scan 7636
SpectrumFinder score: 9.07E-05
Precision: 1.03 ppm



Supplemental Figure 5v

E_{214}SFSANQIVTESSLVTDAGK$_{233}$

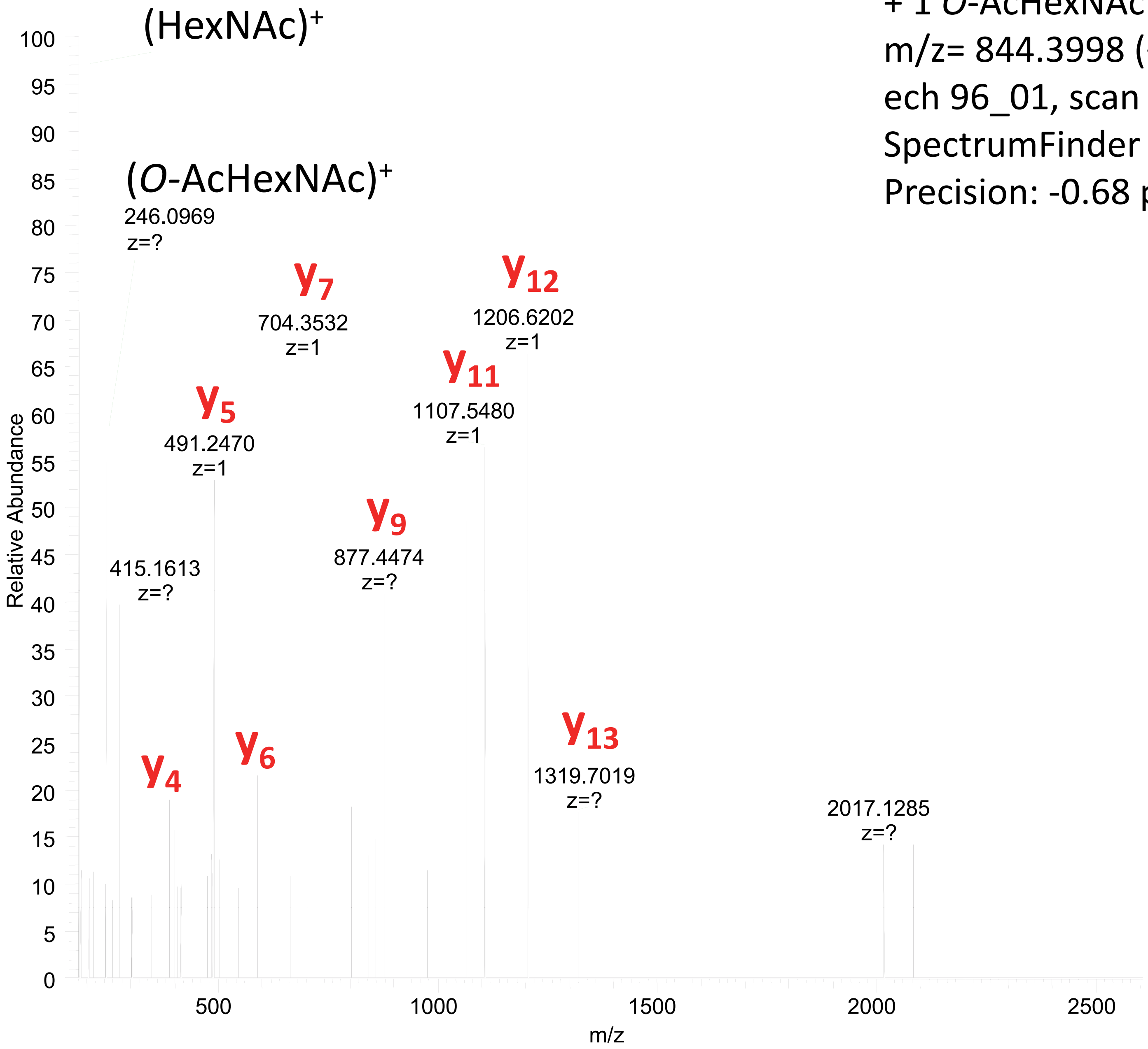
+ 1 *O*-AcHexNAc + 1 HexNAc

m/z= 844.3998 (+3)

ech 96_01, scan 7926

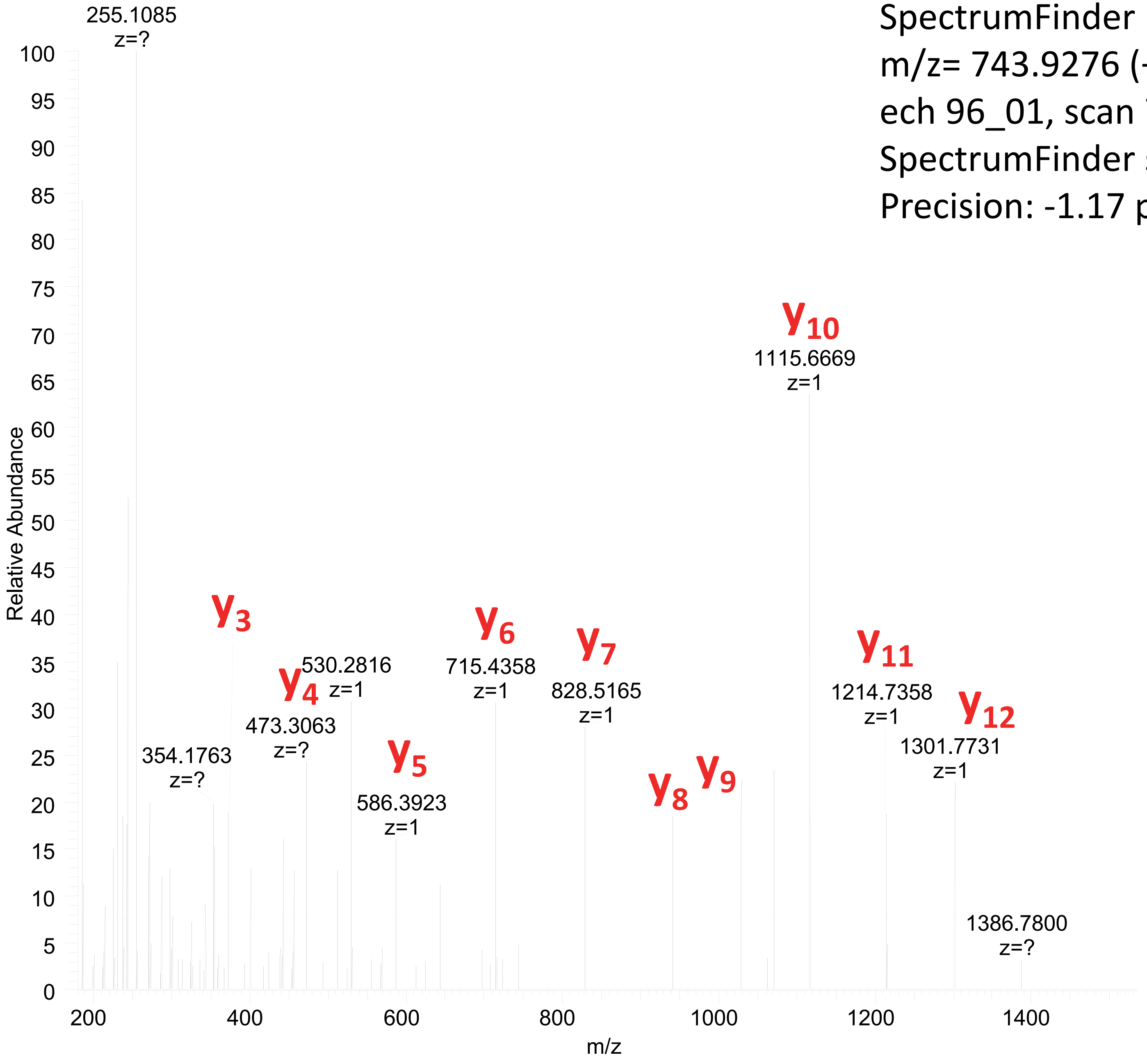
SpectrumFinder score: 1.21E-07

Precision: -0.68 ppm



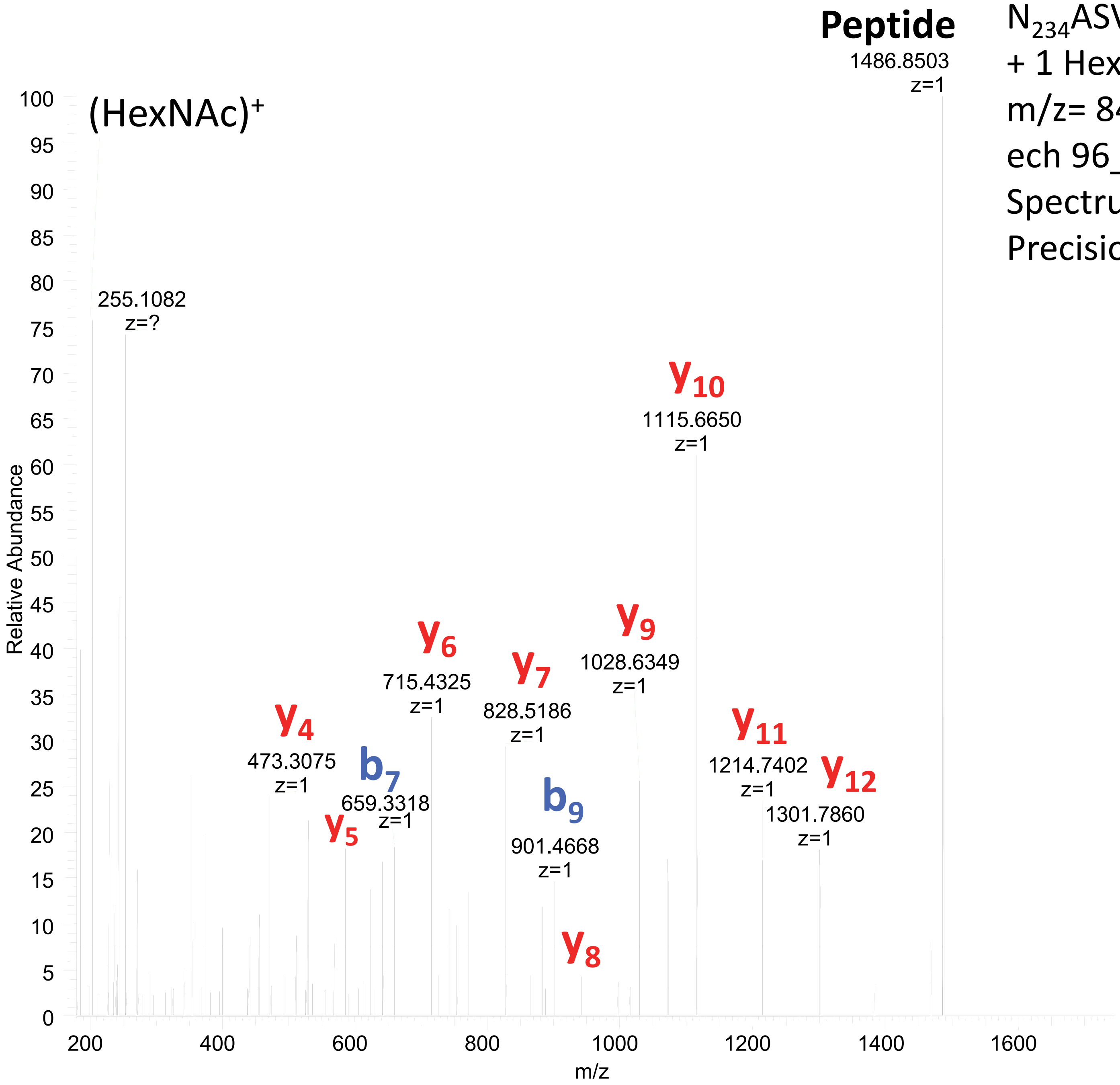
Supplemental Figure 5w

$N_{234}ASVSSLIEITKPK_{247}$
SpectrumFinder reference
m/z= 743.9276 (+2)
ech 96_01, scan 7059
SpectrumFinder score: 6.66E-16
Precision: -1.17 ppm



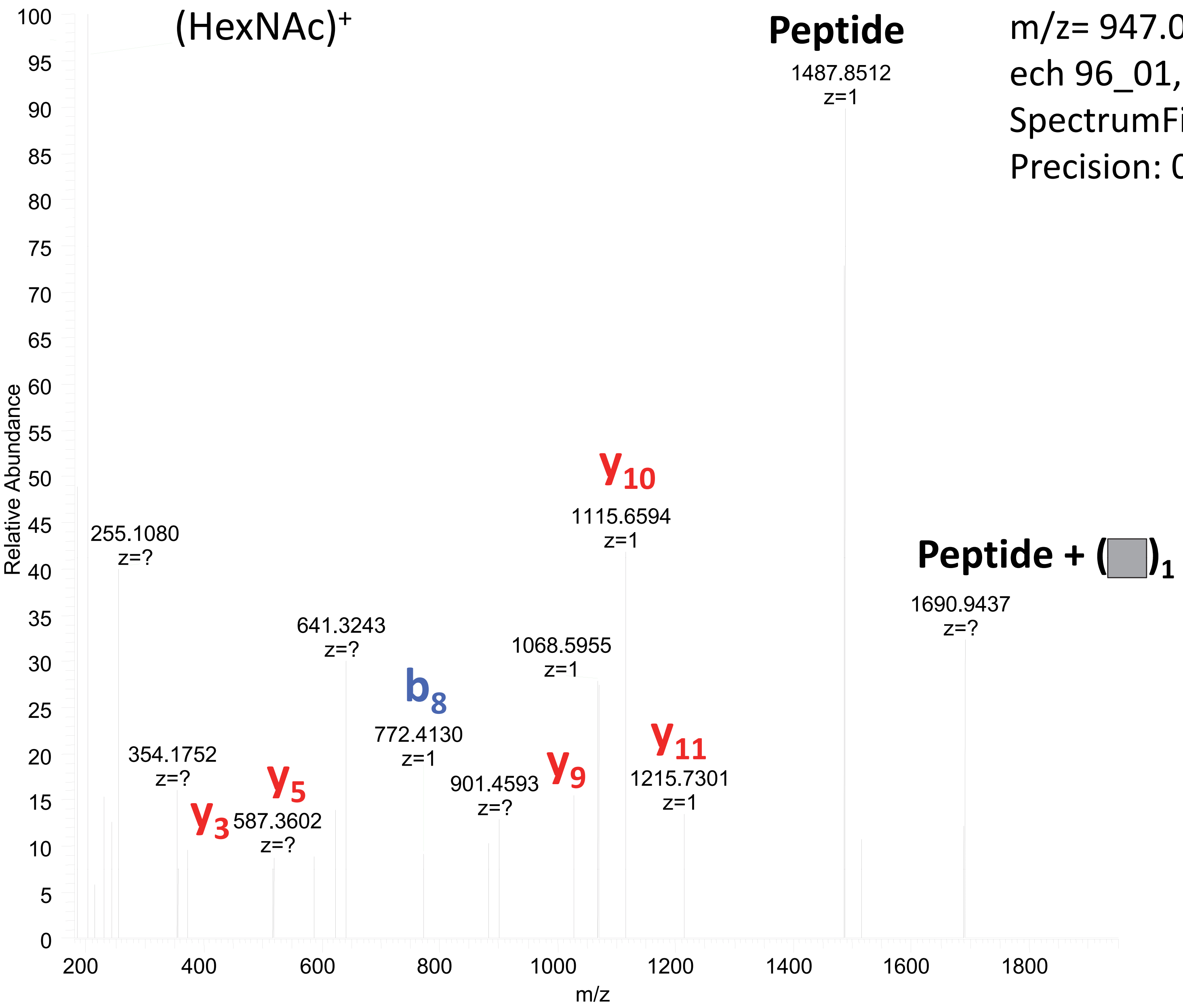
Supplemental Figure 5x

$N_{234}ASVSSLIEITKPK_{247}$
+ 1 HexNAc
m/z= 845.4673 (+2))
ech 96_01, scan 6994
SpectrumFinder score: 1.11E-16
Precision: 0.12 ppm



Supplemental Figure 5y

N_{234}ASVSSLIEITKPK$_{247}$
+ 2 HexNAc
m/z= 947.0071 (+2)
ech 96_01, scan 6074
SpectrumFinder score: 6.71E-04
Precision: 0.11 ppm



Supplemental Figure 5z

M_{255}SNESLITPEK $_{265}$

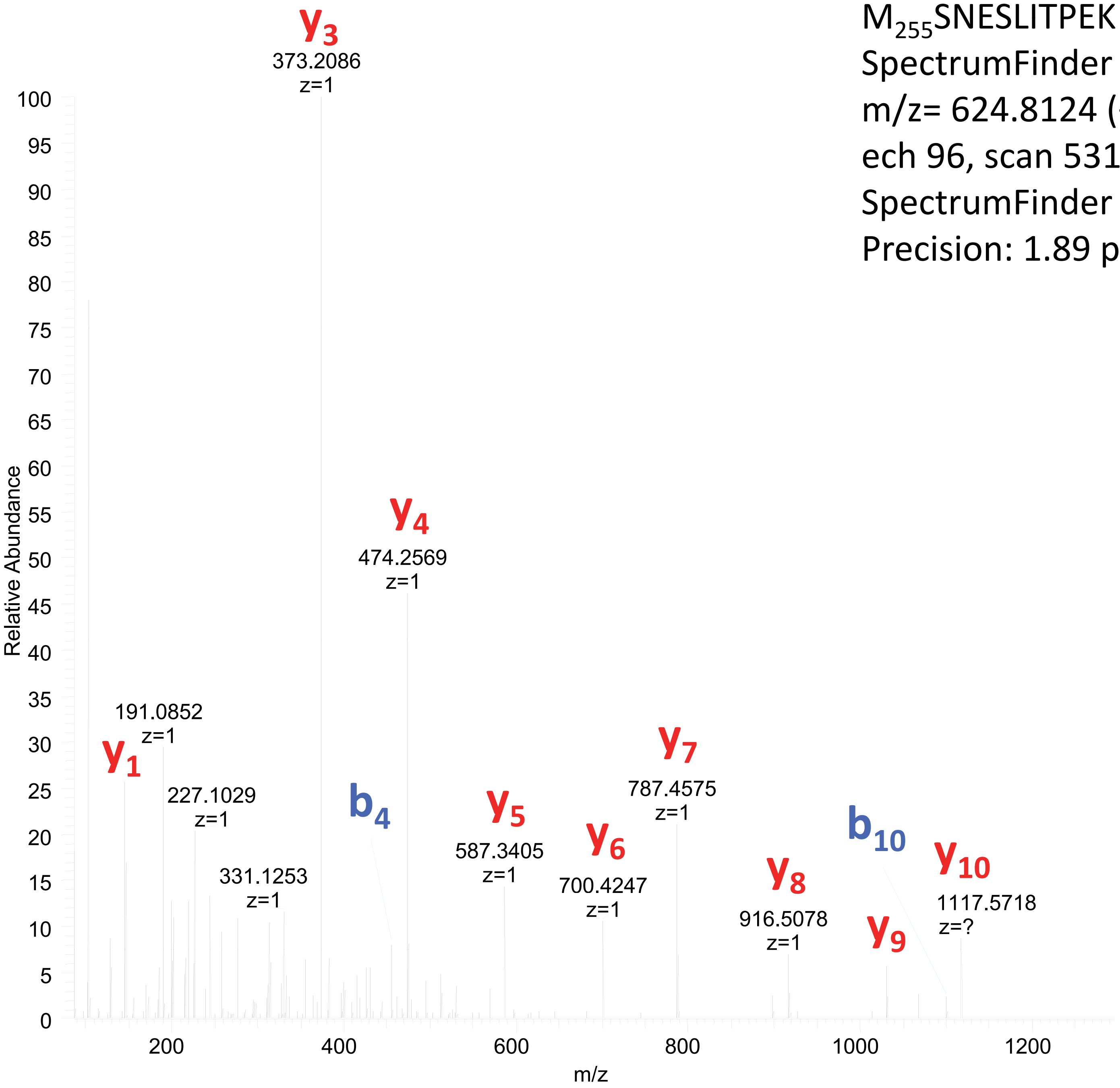
SpectrumFinder reference

m/z= 624.8124 (+2)

ech 96, scan 5316

SpectrumFinder score: 0.00E+00

Precision: 1.89 ppm



Supplemental Figure 5aa

$M_{255}SNESLITPEK_{265}$

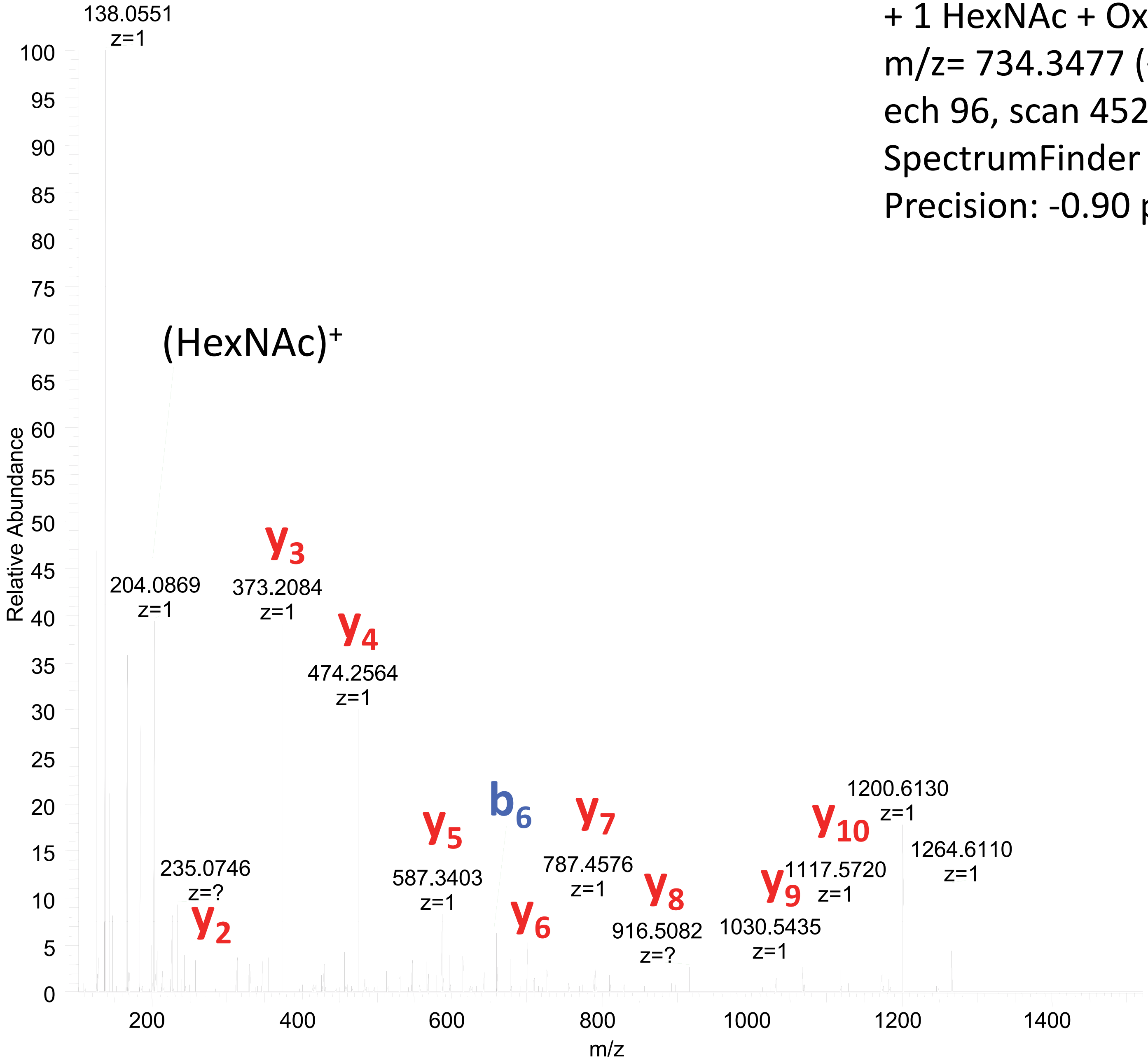
+ 1 HexNAc + Oxid. Methionine

m/z= 734.3477 (+2)

ech 96, scan 4524

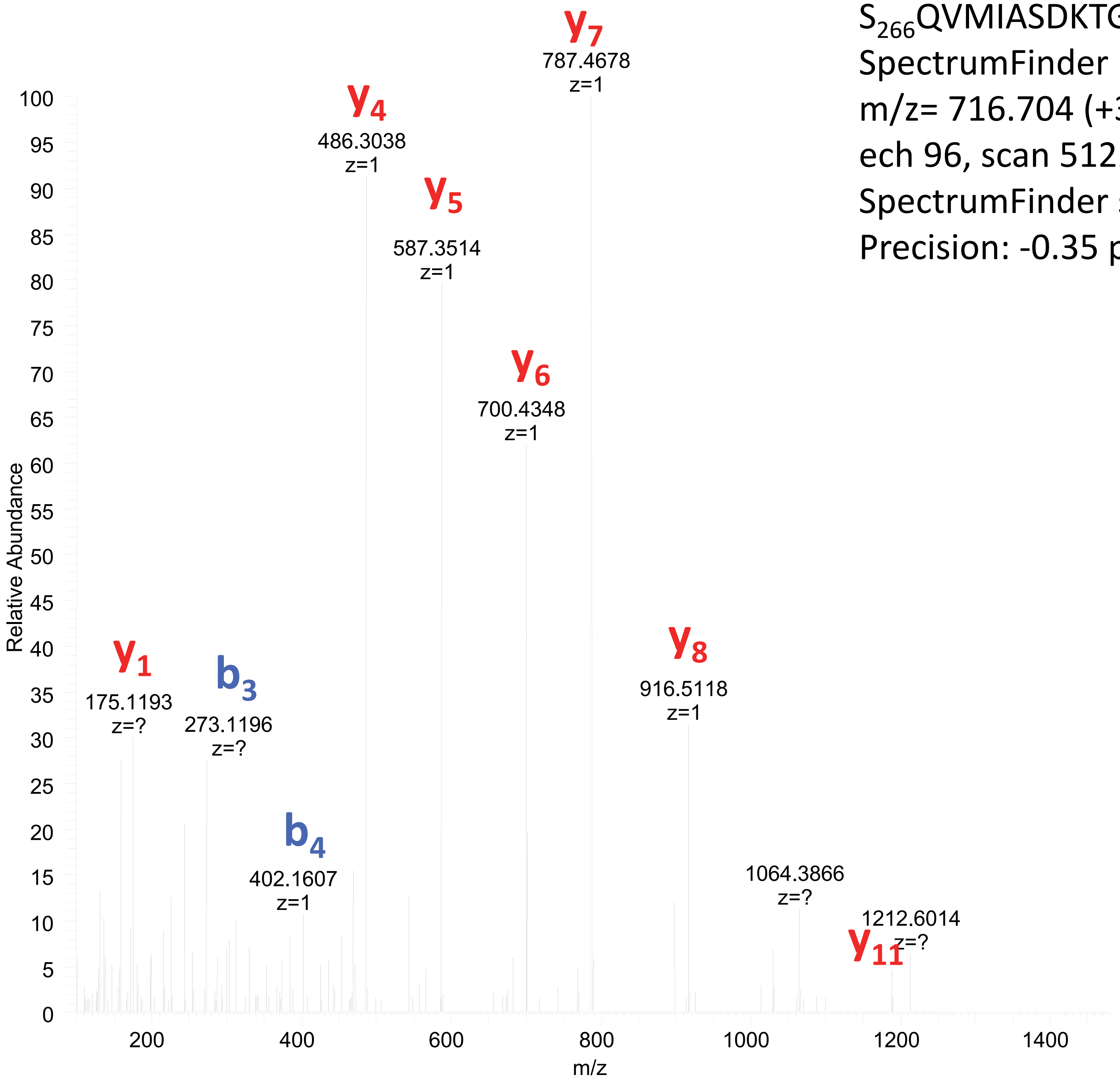
SpectrumFinder score: 5.71E-09

Precision: -0.90 ppm



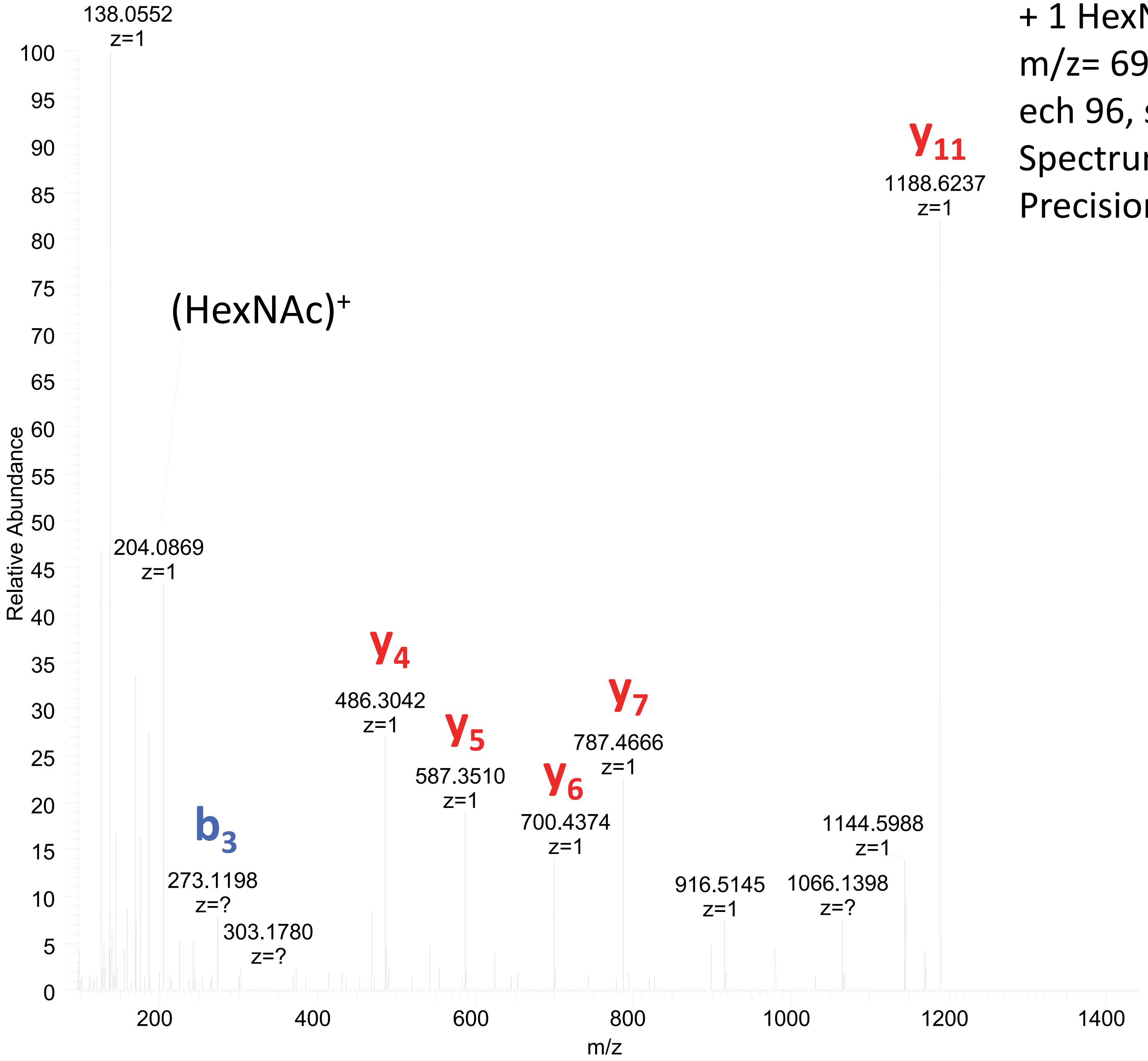
Supplemental Figure 5ab

S_{266}QVMIASDKTGNESLTPTIR$_{285}$
SpectrumFinder reference
m/z= 716.704 (+3)
ech 96, scan 5122
SpectrumFinder score: 3.33E-16
Precision: -0.35 ppm



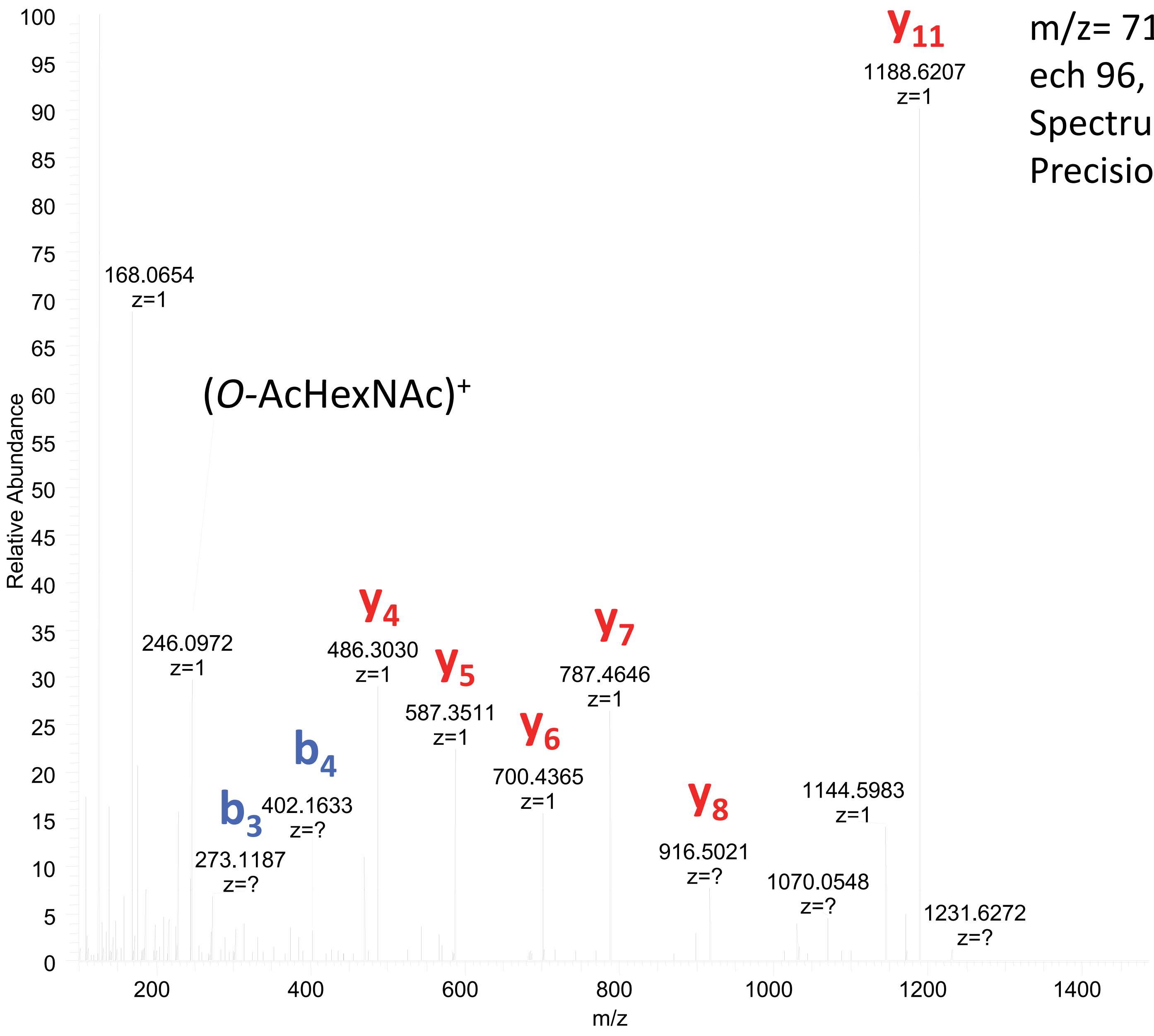
Supplemental Figure 5ac

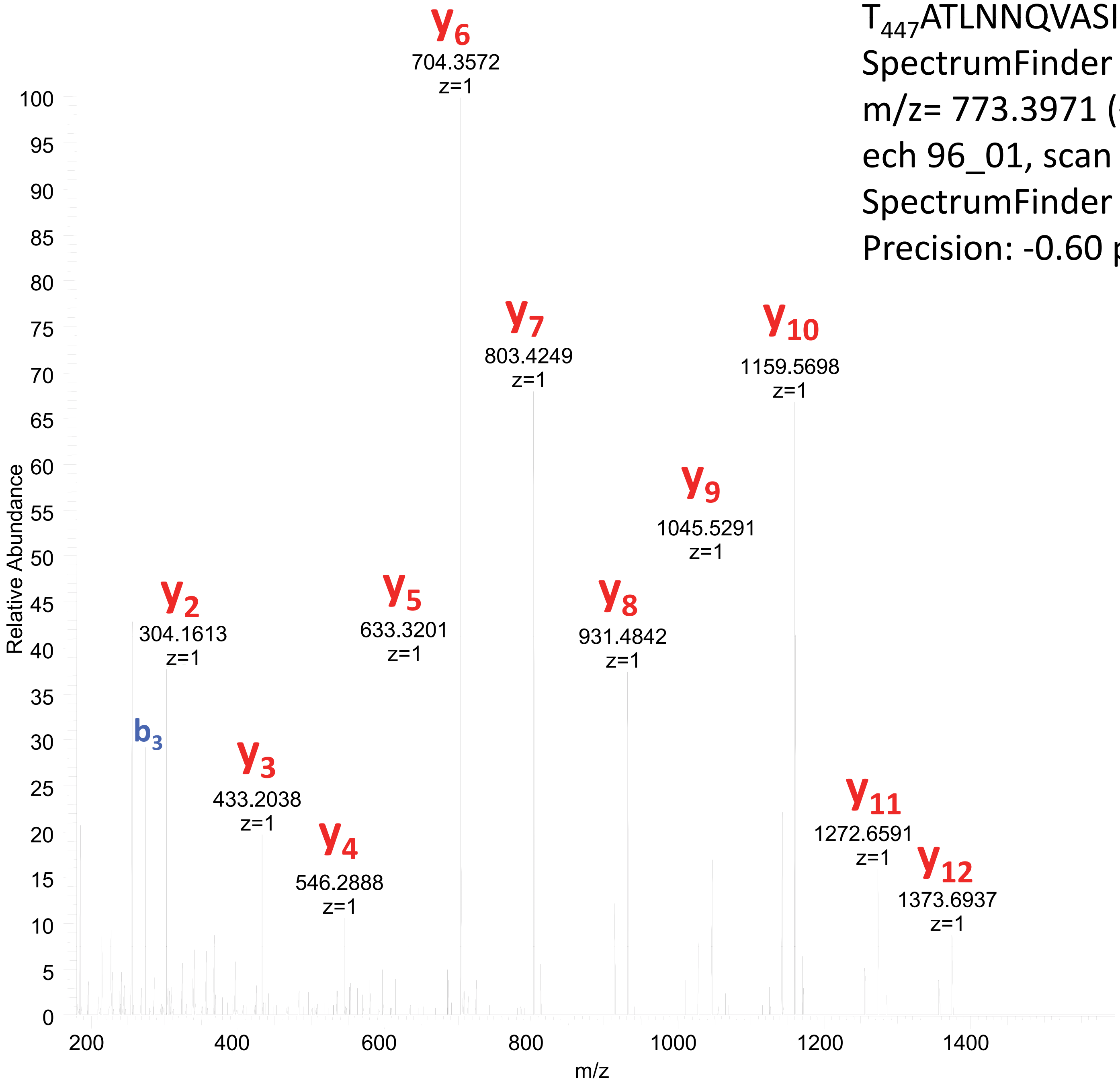
$T_{275}GNESLTPTIR_{285}$
+ 1 HexNAc
m/z= 696.3559 (+2)
ech 96, scan 4797
SpectrumFinder score: 2.01E-07
Precision: 1.53 ppm



Supplemental Figure 5ad

T_{275}GNESLTPTIR$_{285}$
+ 1 *O*-AcHexNAc
m/z= 717.359 (+2)
ech 96, scan 5481
SpectrumFinder score: 0.00E+00
Precision: 0.00 ppm



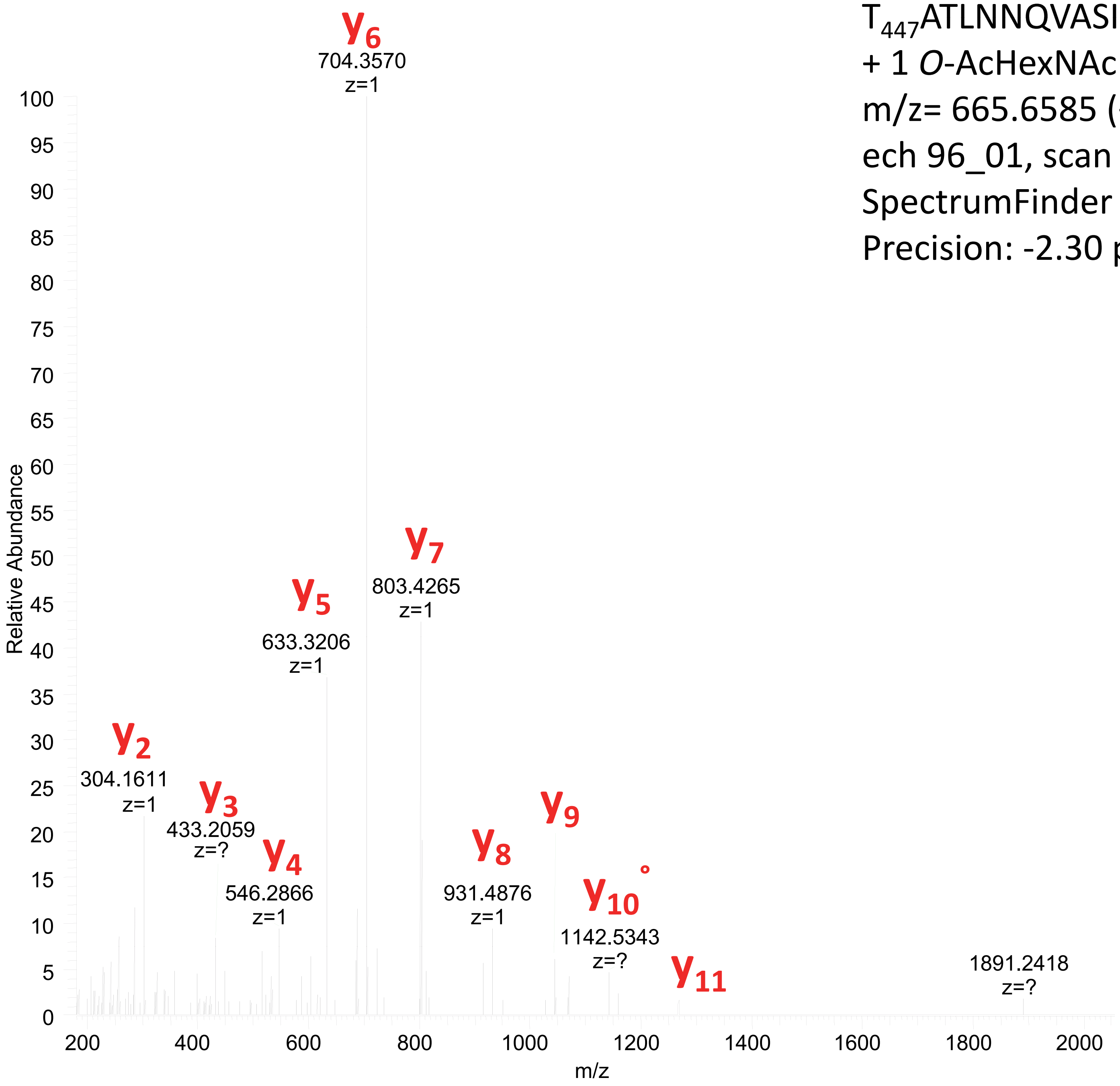


Supplemental Figure 5ae

T$_{447}$ATLNNQVASIEER$_{464}$
SpectrumFinder reference
m/z= 773.3971 (+2)
ech 96_01, scan 5875
SpectrumFinder score: 4.20E-05
Precision: -0.60 ppm

Supplemental Figure 5af

$T_{447}ATLNNQVASIEER_{464}$
+ 1 *O*-AcHexNAc + 1 HexNAc
m/z= 665.6585 (+3)
ech 96_01, scan 8034
SpectrumFinder score: 7.77E-03
Precision: -2.30 ppm



<u>Supplemental Figure 5ag</u>

$T_{447}ATLNNQVASIEER_{464}$

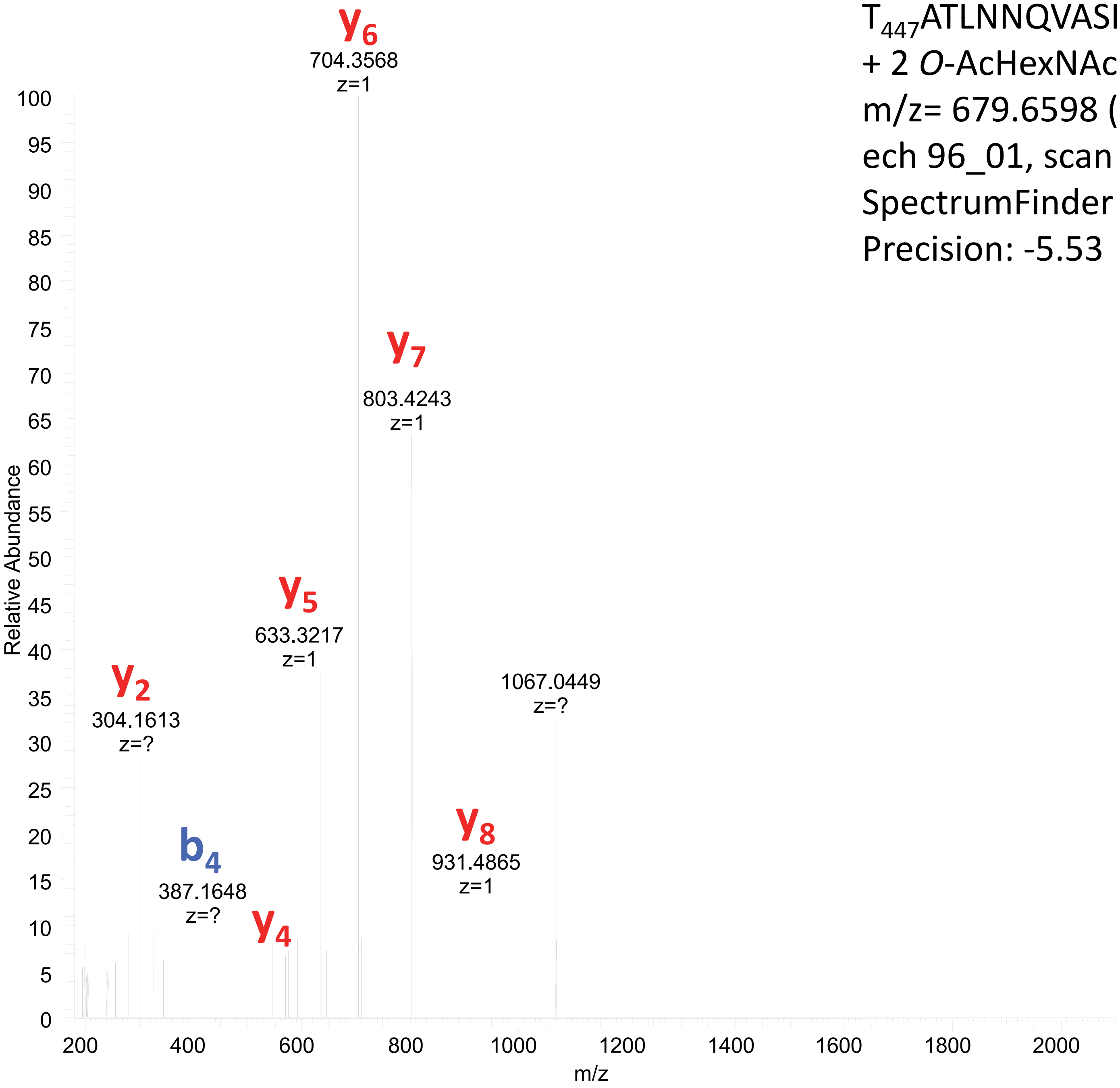
+ 2 *O*-AcHexNAc

m/z= 679.6598 (+3)

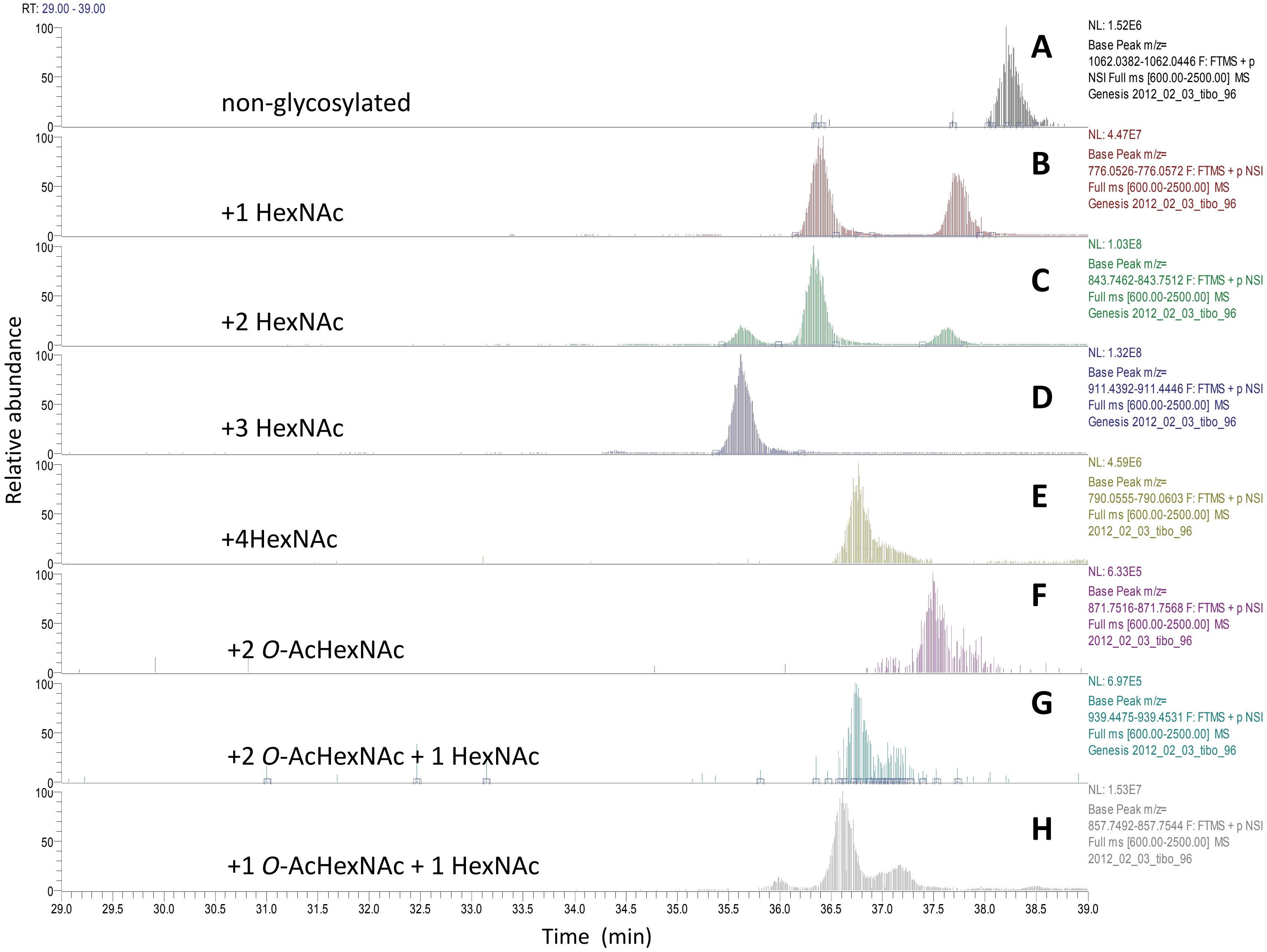
ech 96_01, scan 8518

SpectrumFinder score: 2.77E-03

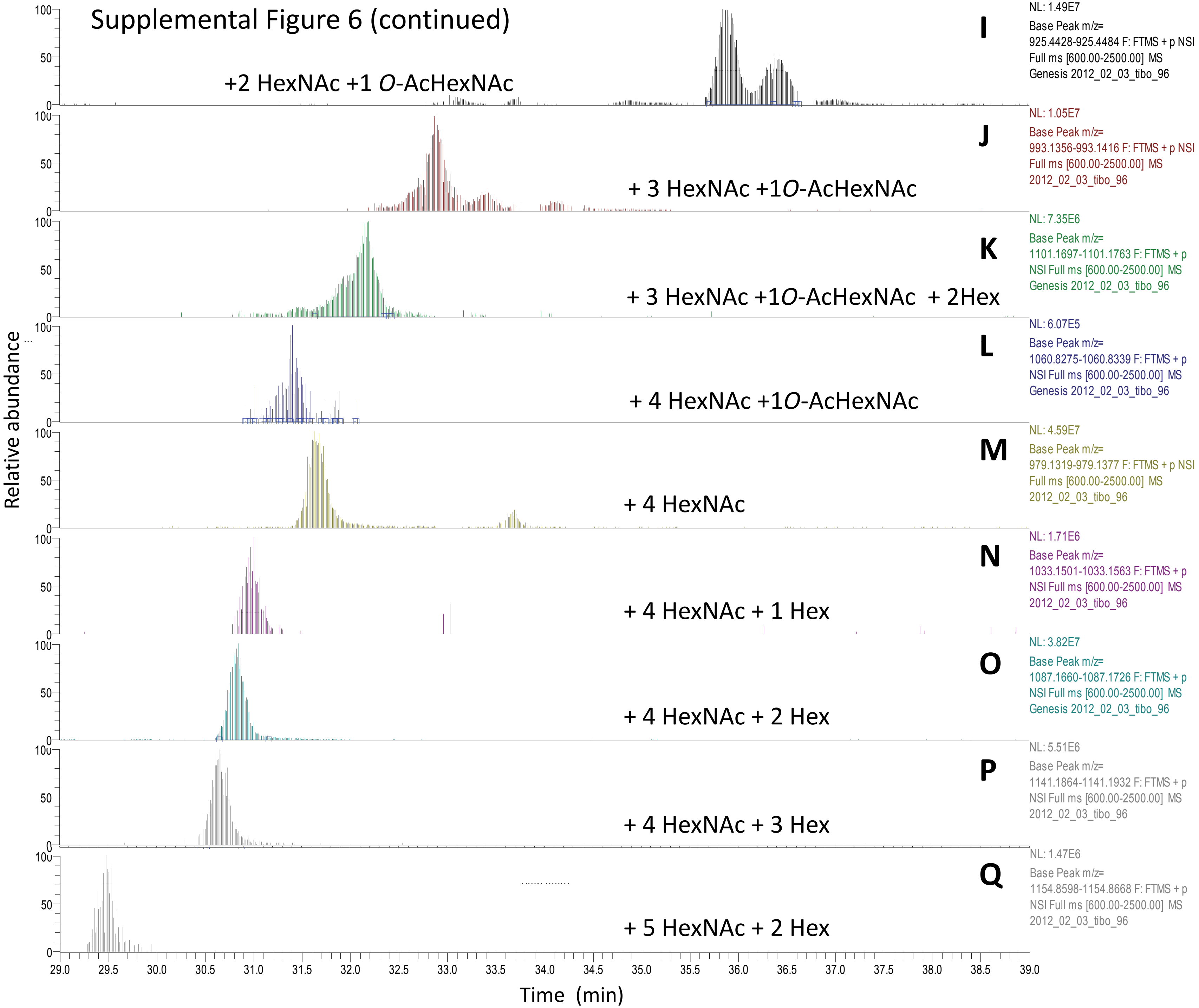
Precision: -5.53 ppm

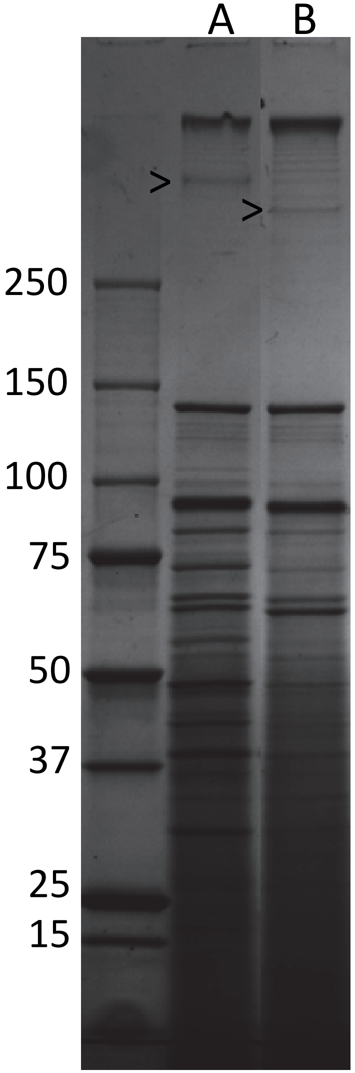


Supplemental Figure 6

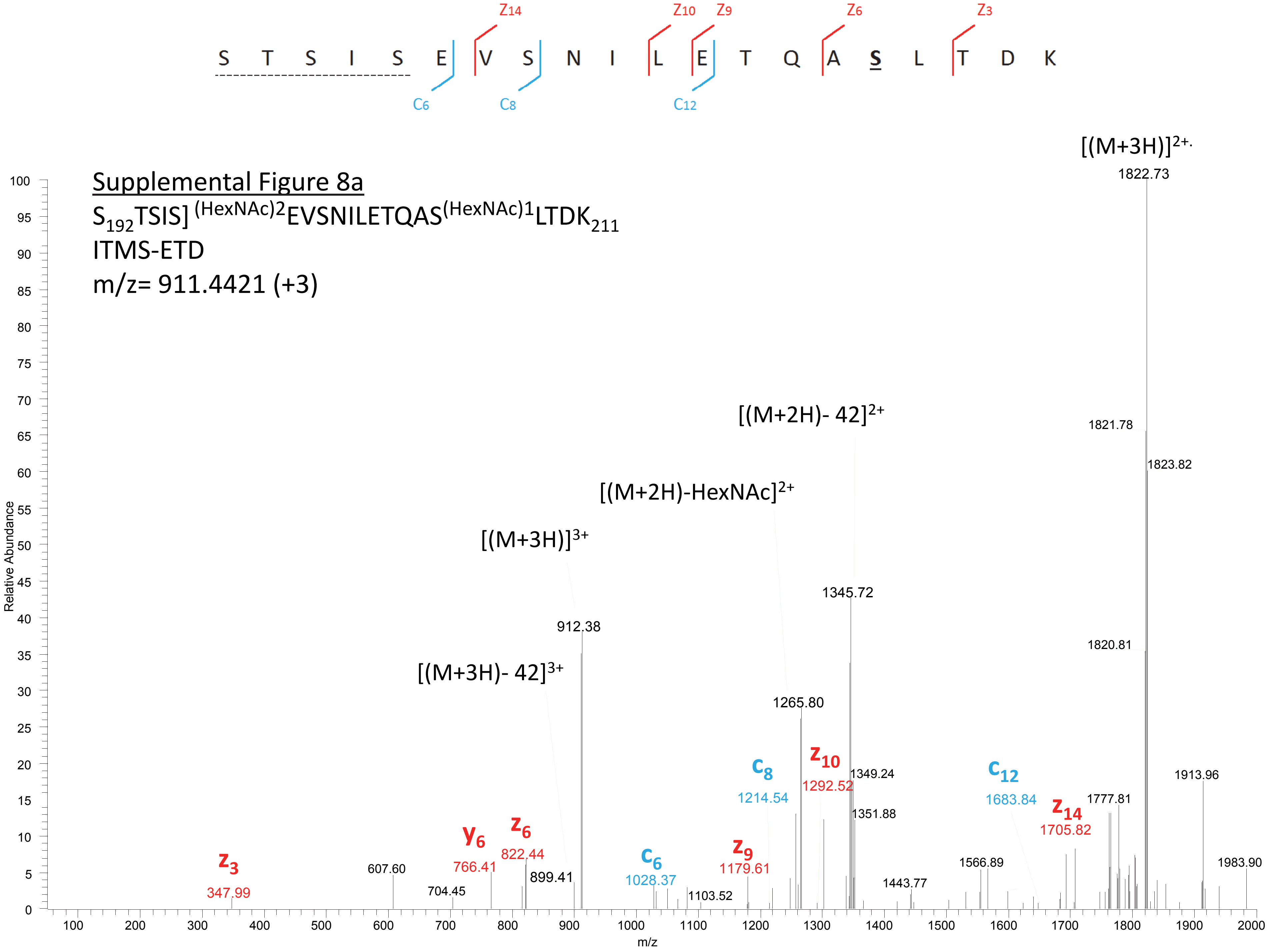


Supplemental Figure 6 (continued)





Supplemental Figure 7

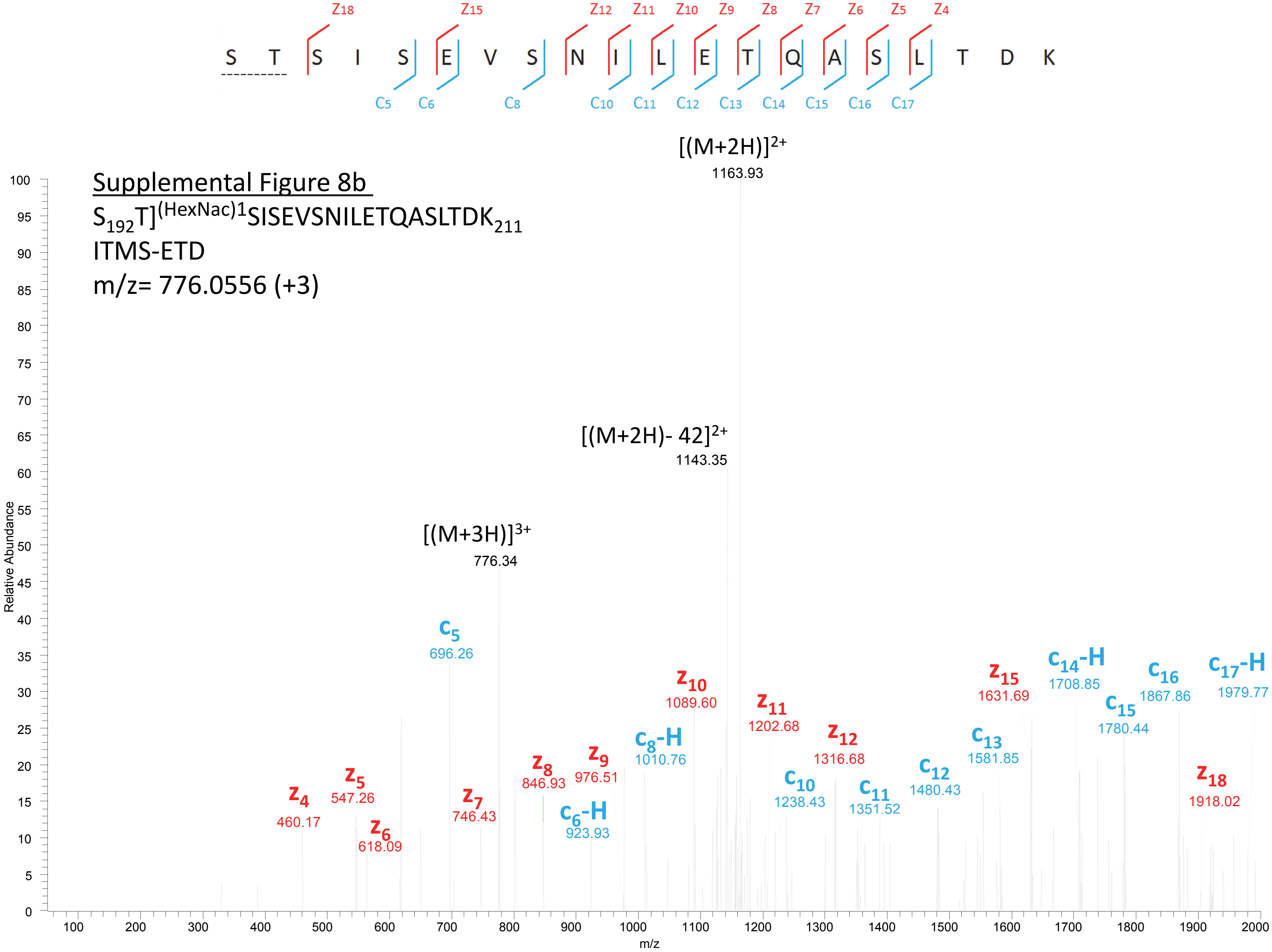


Supplemental Figure 8a

$S_{192}TSIS]^{(HexNAc)2}EVSNILETQAS^{(HexNAc)1}LTDK_{211}$

ITMS-ETD

m/z= 911.4421 (+3)

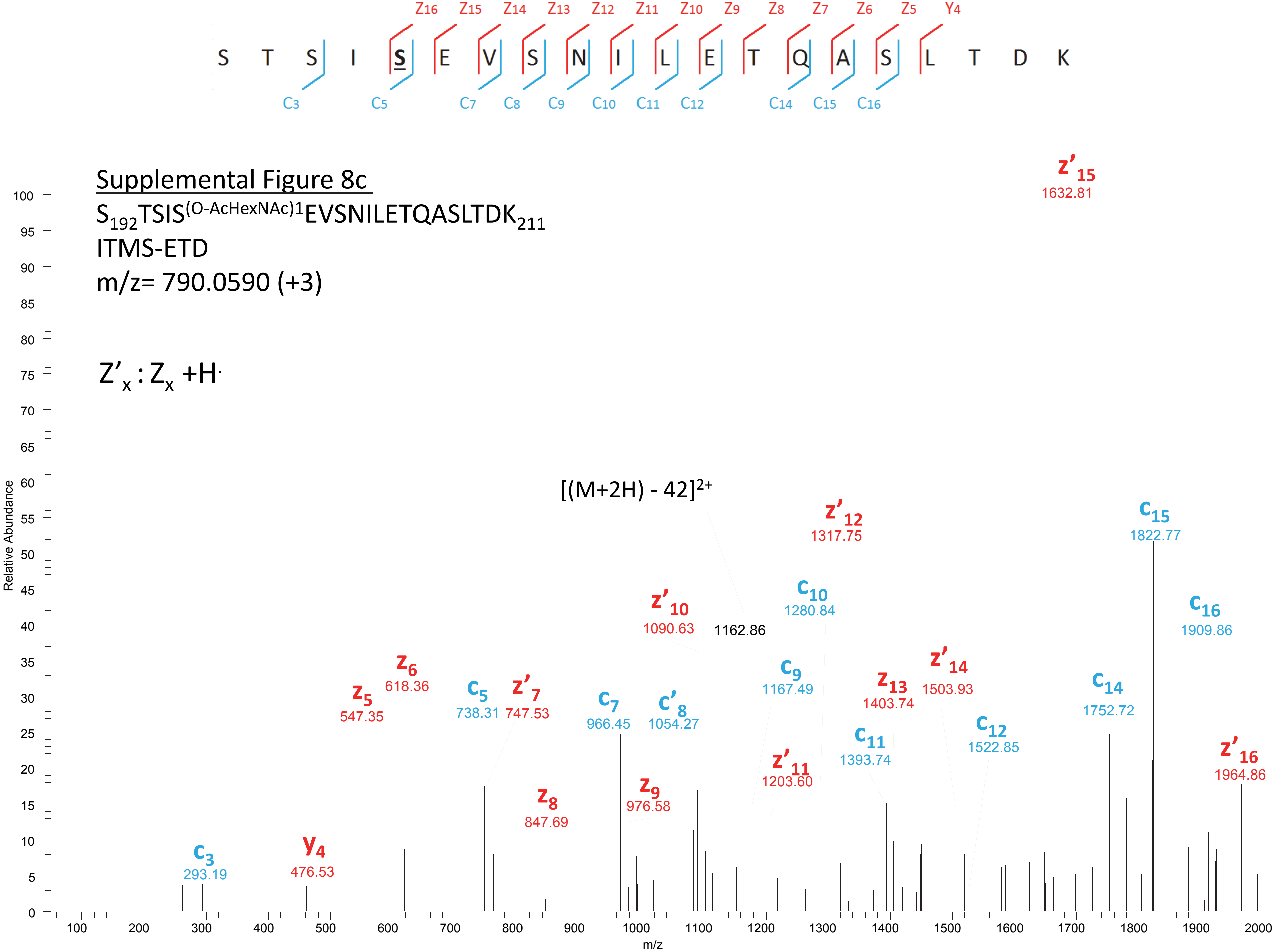


Supplemental Figure 8b

$S_{192}T]^{(HexNac)1}SISEVSNILETQASLTDK_{211}$

ITMS-ETD

m/z= 776.0556 (+3)



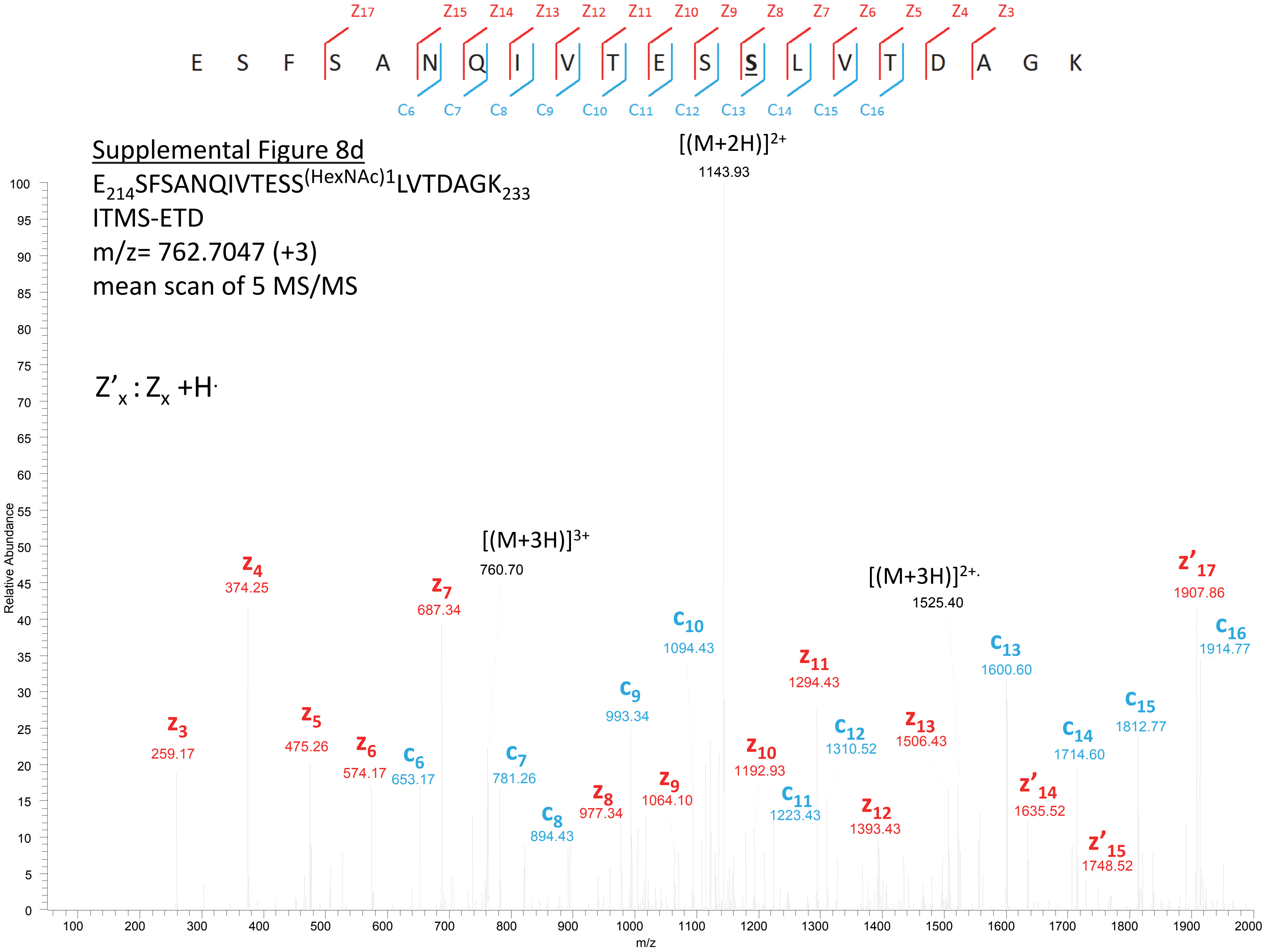
Supplemental Figure 8c

$S_{192}TSIS^{(O\text{-}AcHexNAc)1}EVSNILETQASLTDK_{211}$

ITMS-ETD

m/z= 790.0590 (+3)

$Z'_x : Z_x + H^{\cdot}$



Supplemental Figure 8d

$E_{214}SFSANQIVTESS^{(HexNAc)1}LVTDAGK_{233}$

ITMS-ETD

m/z= 762.7047 (+3)

mean scan of 5 MS/MS

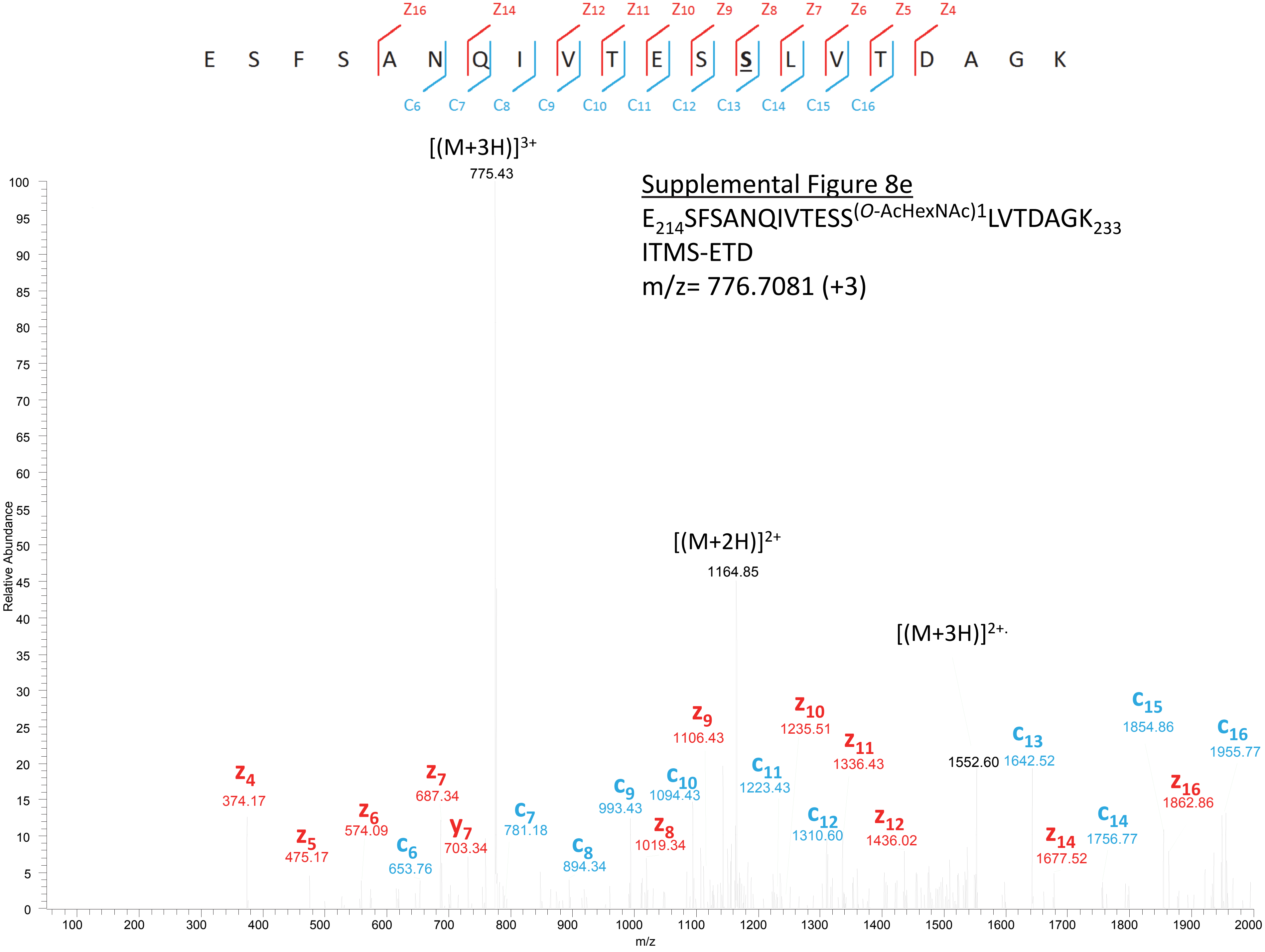
$Z'_x : Z_x + H^{\cdot}$

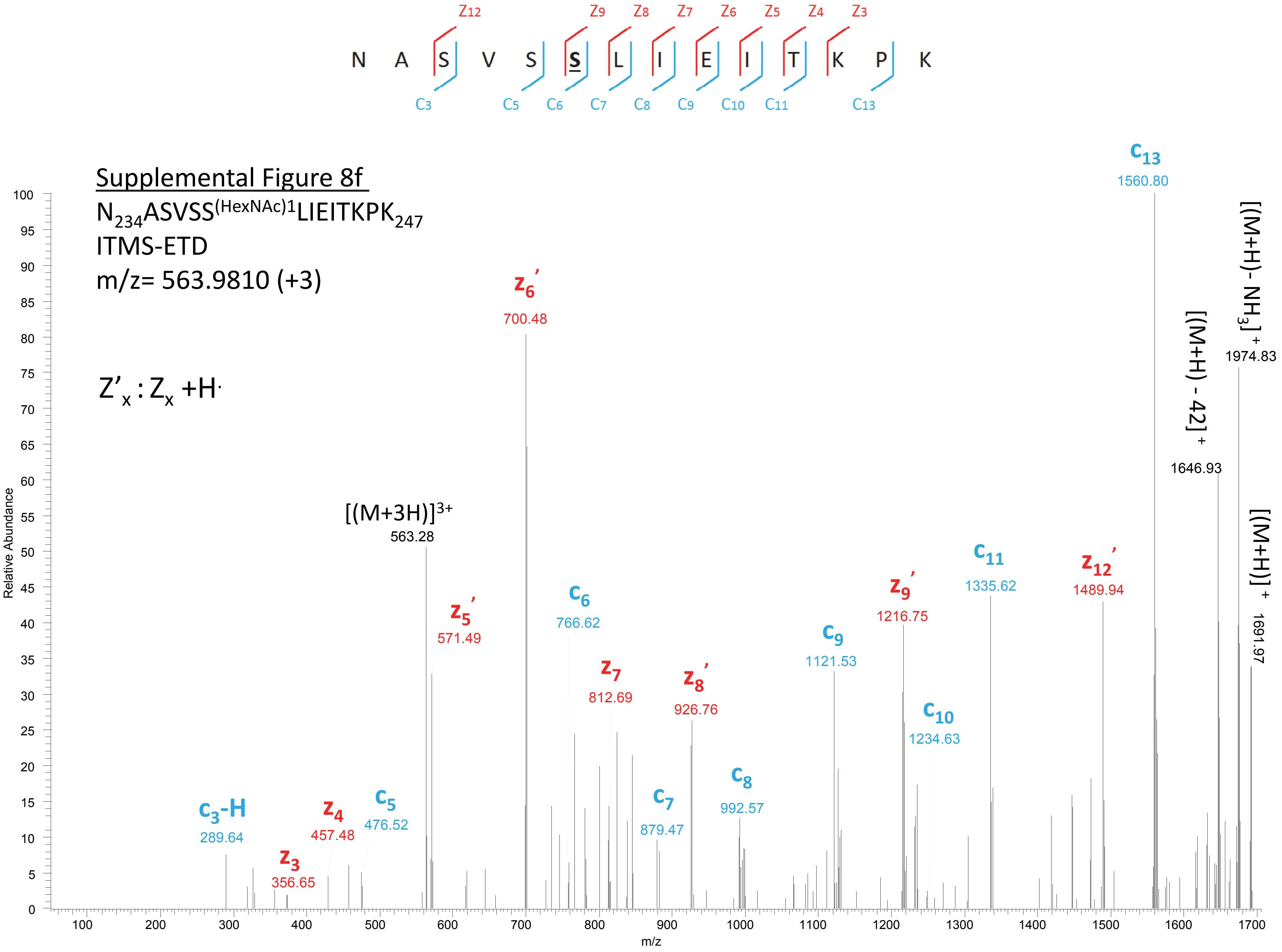
Supplemental Figure 8e

$E_{214}SFSANQIVTESS^{(O\text{-AcHexNAc})1}LVTDAGK_{233}$

ITMS-ETD

m/z= 776.7081 (+3)





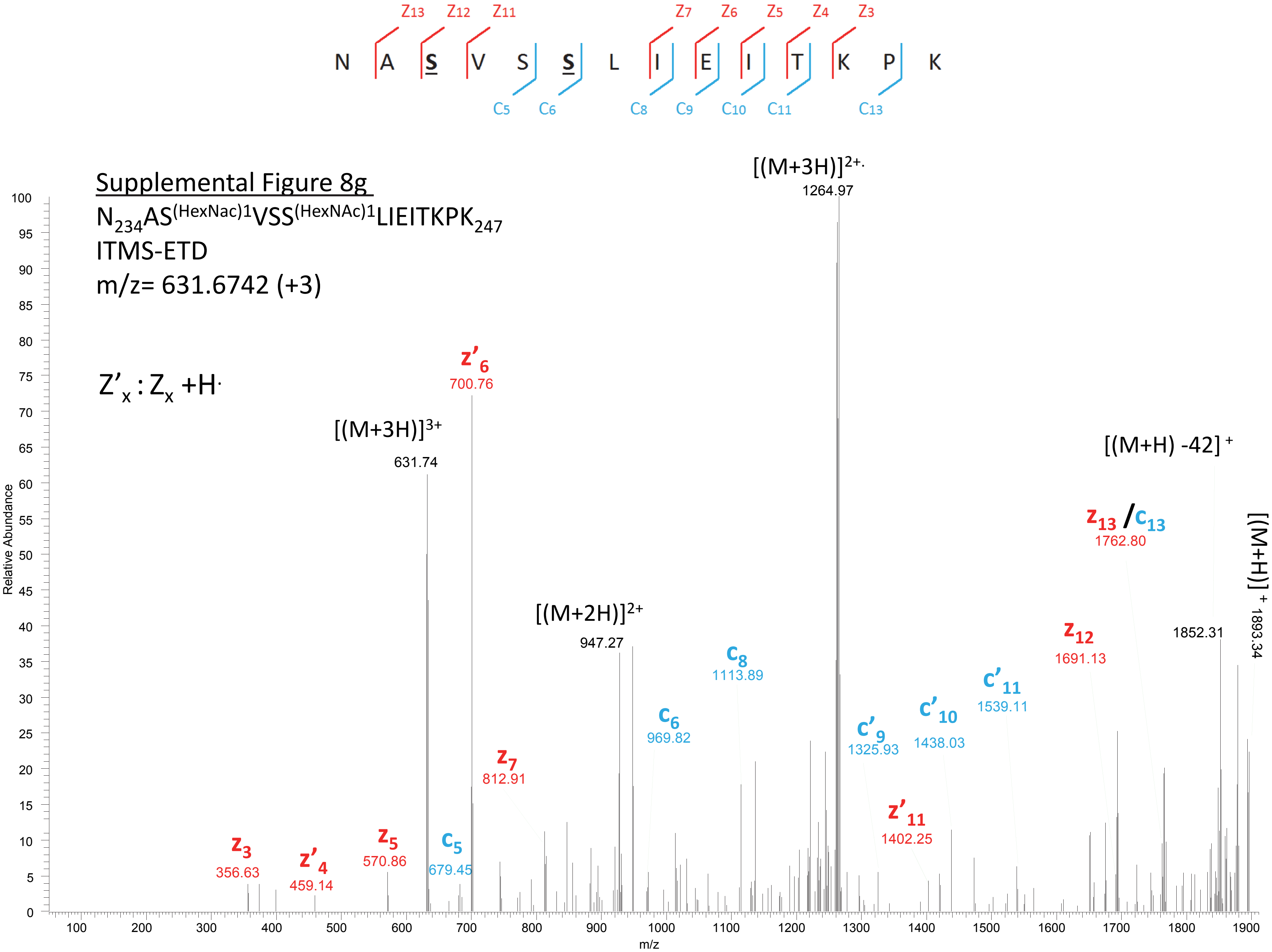
Supplemental Figure 8f

$N_{234}ASVSS^{(HexNAc)1}LIEITKPK_{247}$

ITMS-ETD

m/z= 563.9810 (+3)

$Z'_x : Z_x + H\cdot$



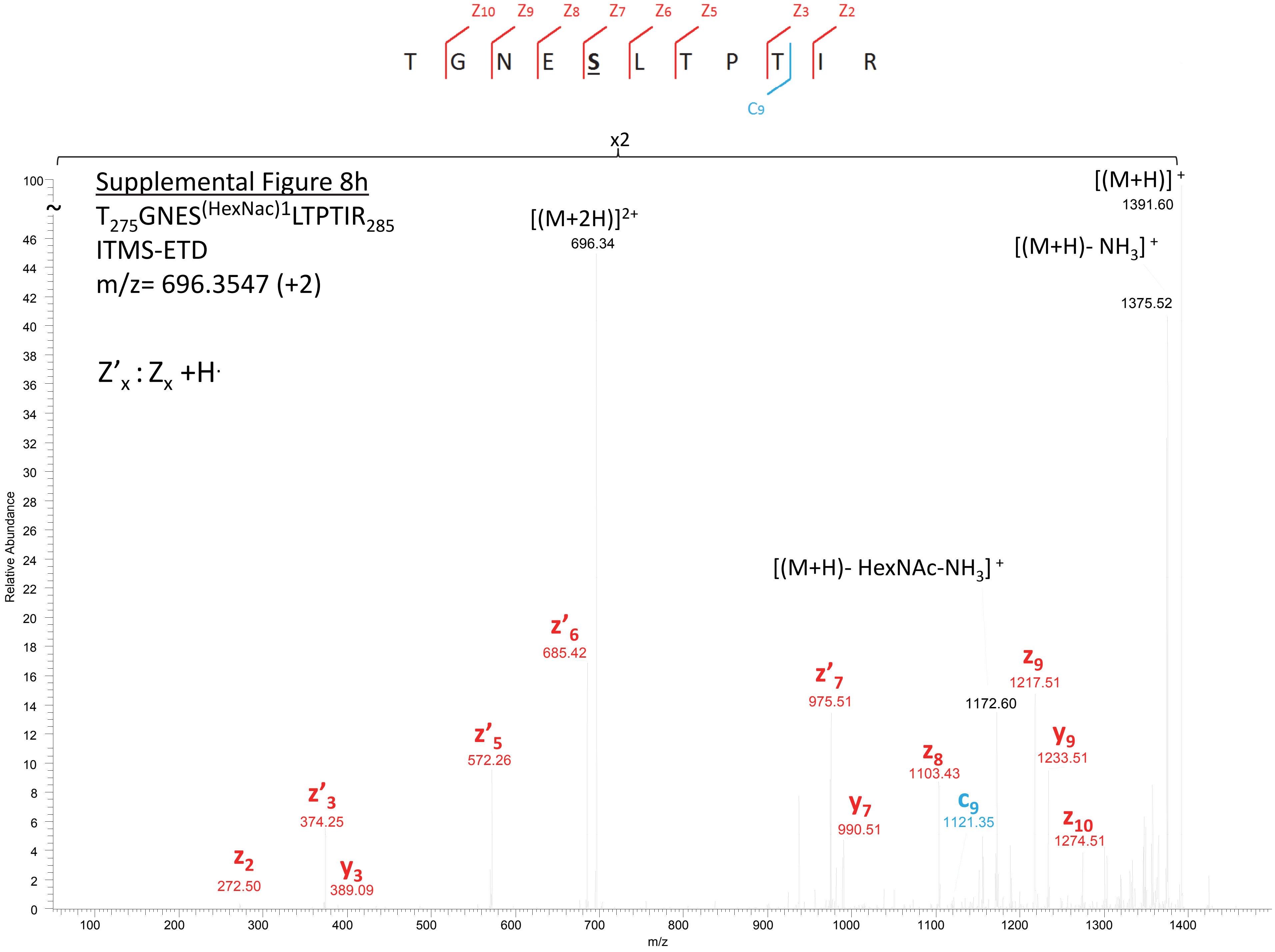
Supplemental Figure 8g

$N_{234}AS^{(HexNac)1}VSS^{(HexNAc)1}LIEITKPK_{247}$

ITMS-ETD

m/z= 631.6742 (+3)

$Z'_x : Z_x + H^{\cdot}$



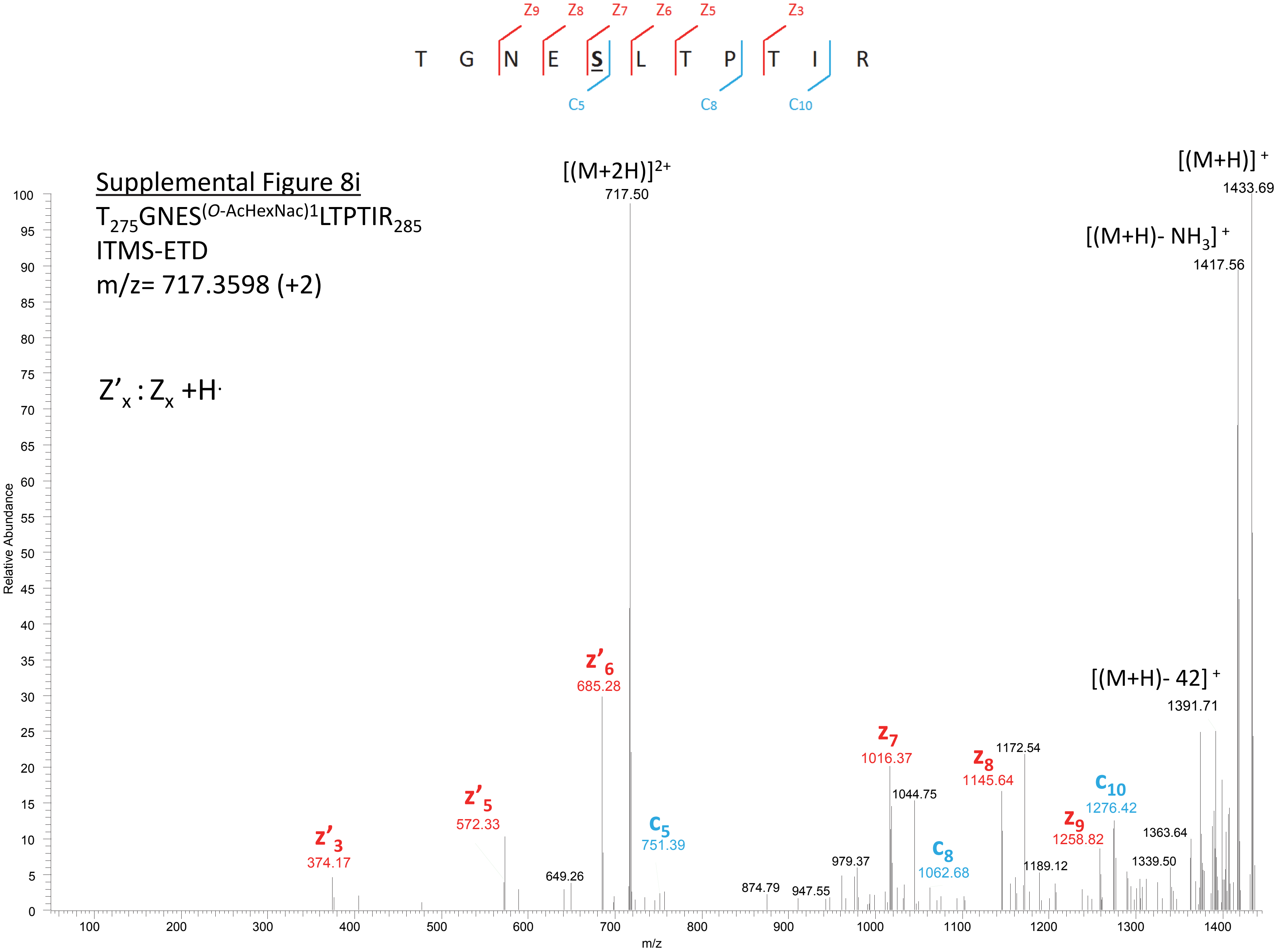
Supplemental Figure 8h

$T_{275}GNES^{(HexNac)1}LTPTIR_{285}$

ITMS-ETD

m/z= 696.3547 (+2)

$Z'_x : Z_x + H^{\cdot}$



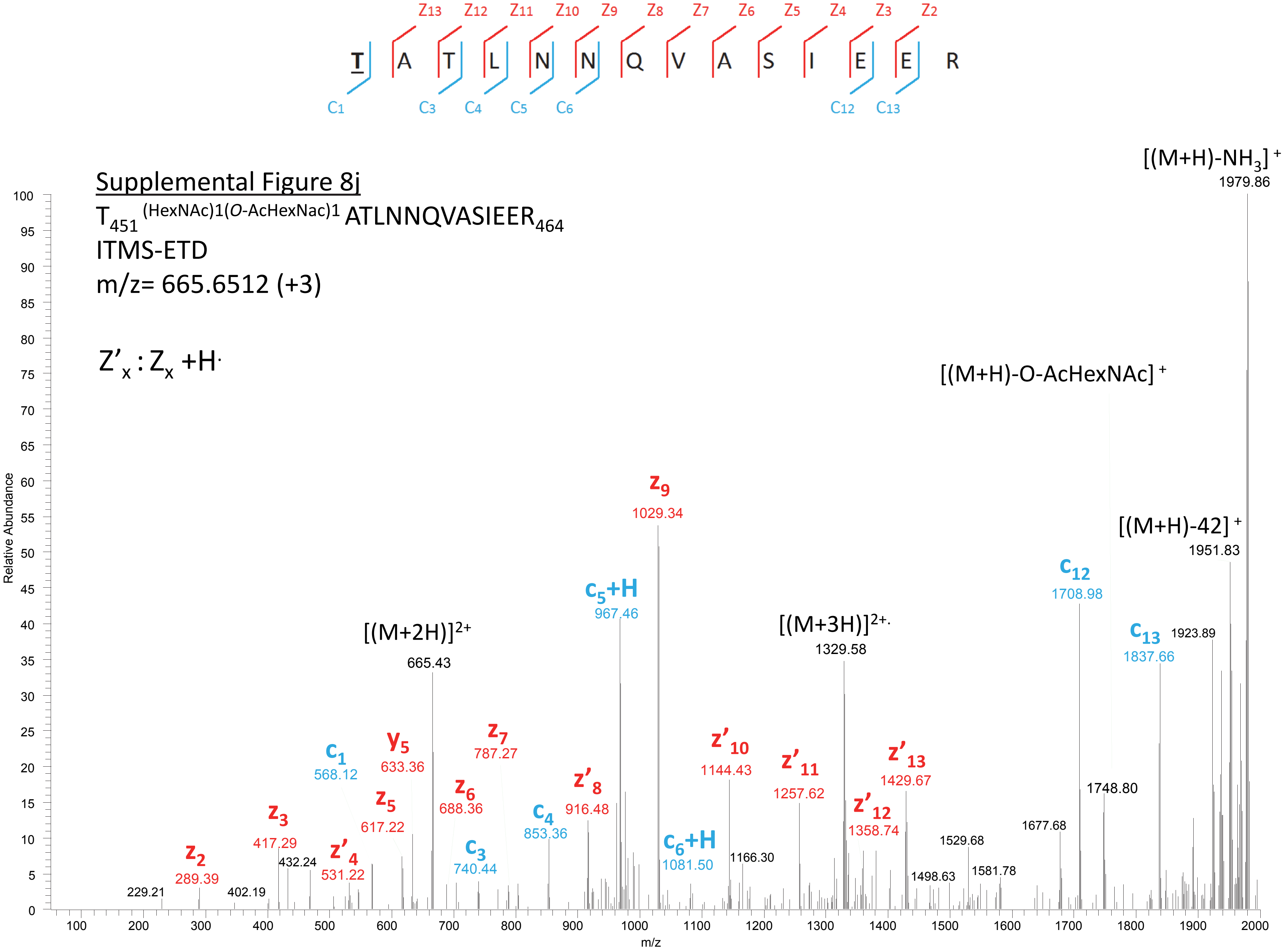
Supplemental Figure 8i

$T_{275}GNES^{(O\text{-AcHexNac})1}LTPTIR_{285}$

ITMS-ETD

m/z= 717.3598 (+2)

$Z'_x : Z_x + H^{\cdot}$



Supplemental Figure 8j

$T_{451}{}^{(\text{HexNAc})1(\textit{O}\text{-AcHexNac})1}\,\text{ATLNNQVASIEER}_{464}$

ITMS-ETD

m/z= 665.6512 (+3)

$Z'_x : Z_x + H^{\cdot}$