Supporting Information for:

The weaker-binding Fc γ receptor IIIa F158 allotype retains sensitivity to N-glycan composition and exhibits a destabilized antibody-binding interface

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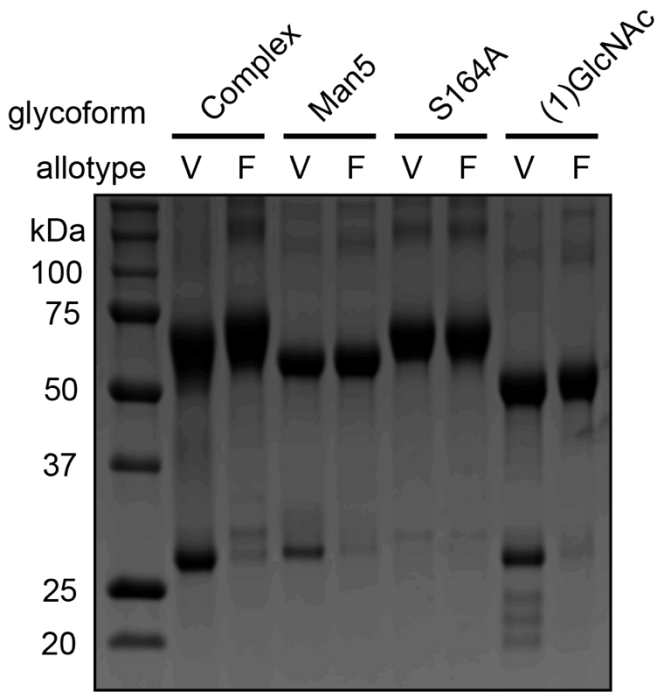
Supplemental Table 1. N162 glycan composition of CD16a expressed in HEK293F cells

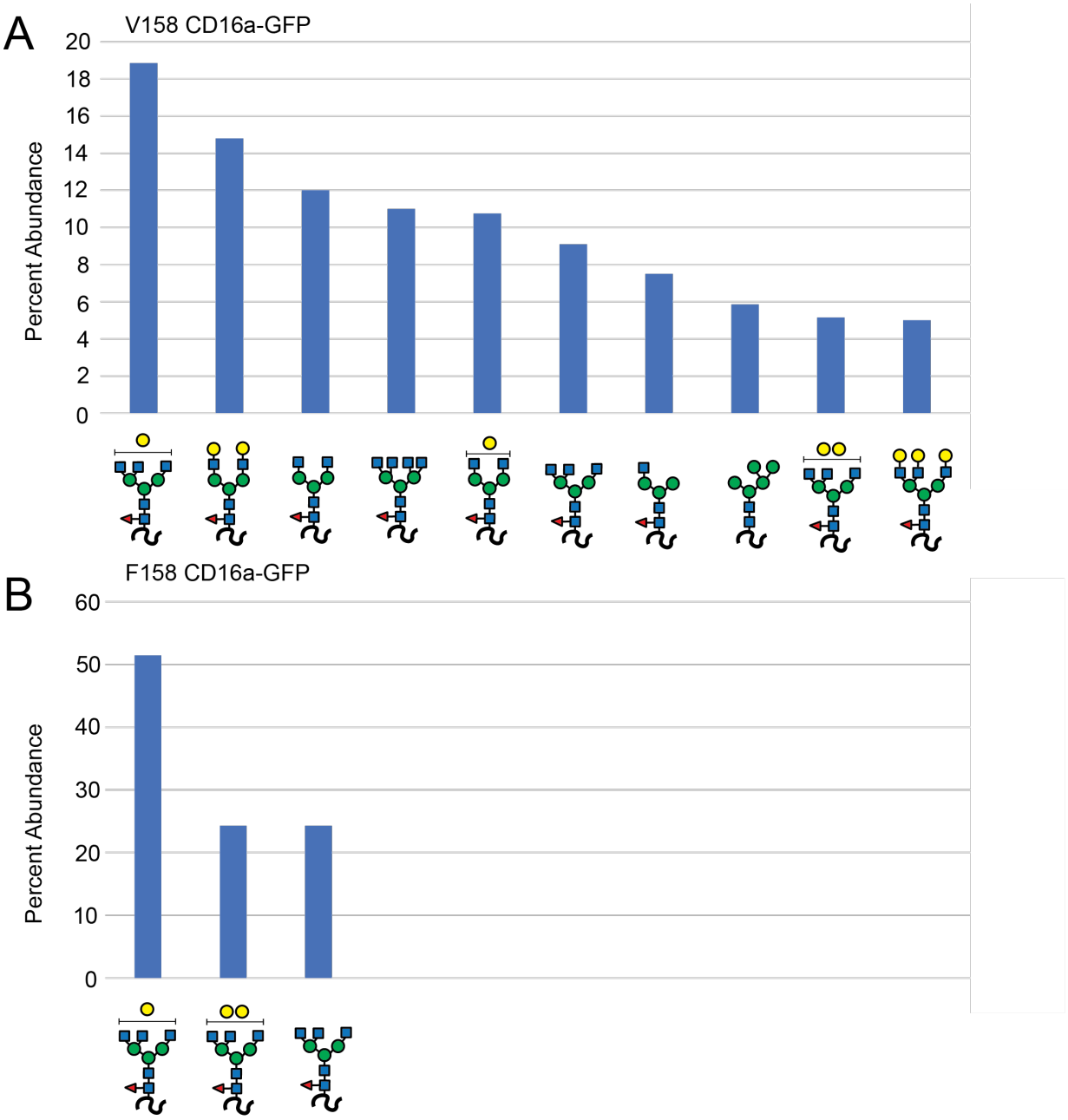
Allotype	Glycan	MS2	z	Calc $(M+zH)^{z+}$	Obs $(M+zH)^{z+}$	Intensity (arb)	RT	PPM error	Mass error (Da)	Peptide
F	HexNAc(5)Hex(5)Fuc(1)	X	3	927.0392	927.0381	2500	9	1.230	0.00114	GSKNVSSE
F	HexNAc(5)Hex(4)Fuc(1)	X	3	873.0216	873.0211	5300	9	0.607	0.00053	GSKNVSSE
F	HexNAc(5)Hex(3)Fuc(1)	X	3	819.0040	819.0026	2500	9	1.734	0.00142	GSKNVSSE
V	HexNAc(5)Hex(5)Fuc(1)	X	3	960.0620	960.0625	103000	11	-0.479	0.00046	VGSKNVSSE
V	HexNAc(5)Hex(4)Fuc(1)	X	3	906.0444	906.0444	377000	11	0.033	3E-05	VGSKNVSSE
V	HexNAc(5)Hex(3)Fuc(1)	X	3	852.0268	852.0268	182000	11	0.023	2E-05	VGSKNVSSE
V	HexNAc(4)Hex(3)Fuc(1)	X	3	784.3337	784.3337	240000	10	0.000	0	VGSKNVSSE
V	HexNAc(2)Hex(5)	X	3	870.3758	870.3763	117000	15	-0.574	0.0005	CRGLVGSKNVSSE
V	HexNAc(6)Hex(3)Fuc(1)	X	3	919.7200	919.7196	220000	10	0.381	0.00035	VGSKNVSSE
V	HexNAc(3)Hex(3)Fuc(1)	X	2	1074.4572	1074.4572	150000	10	0.019	2E-05	VGSKNVSSE
V	HexNAc(4)Hex(5)Fuc(1)	X	3	892.3689	892.3685	296000	10	0.471	0.00042	VGSKNVSSE
V	HexNAc(4)Hex(4)Fuc(1)	X	3	838.3513	838.3509	215000	10	0.489	0.00041	VGSKNVSSE
V	HexNAc(5)Hex(6)Fuc(1)	X	3	1014.0797	1014.0799	100000	9	-0.247	0.00025	VGSKNVSSE

Supplemental Table 2. CD16a affinities relative to the complex-type glycoform

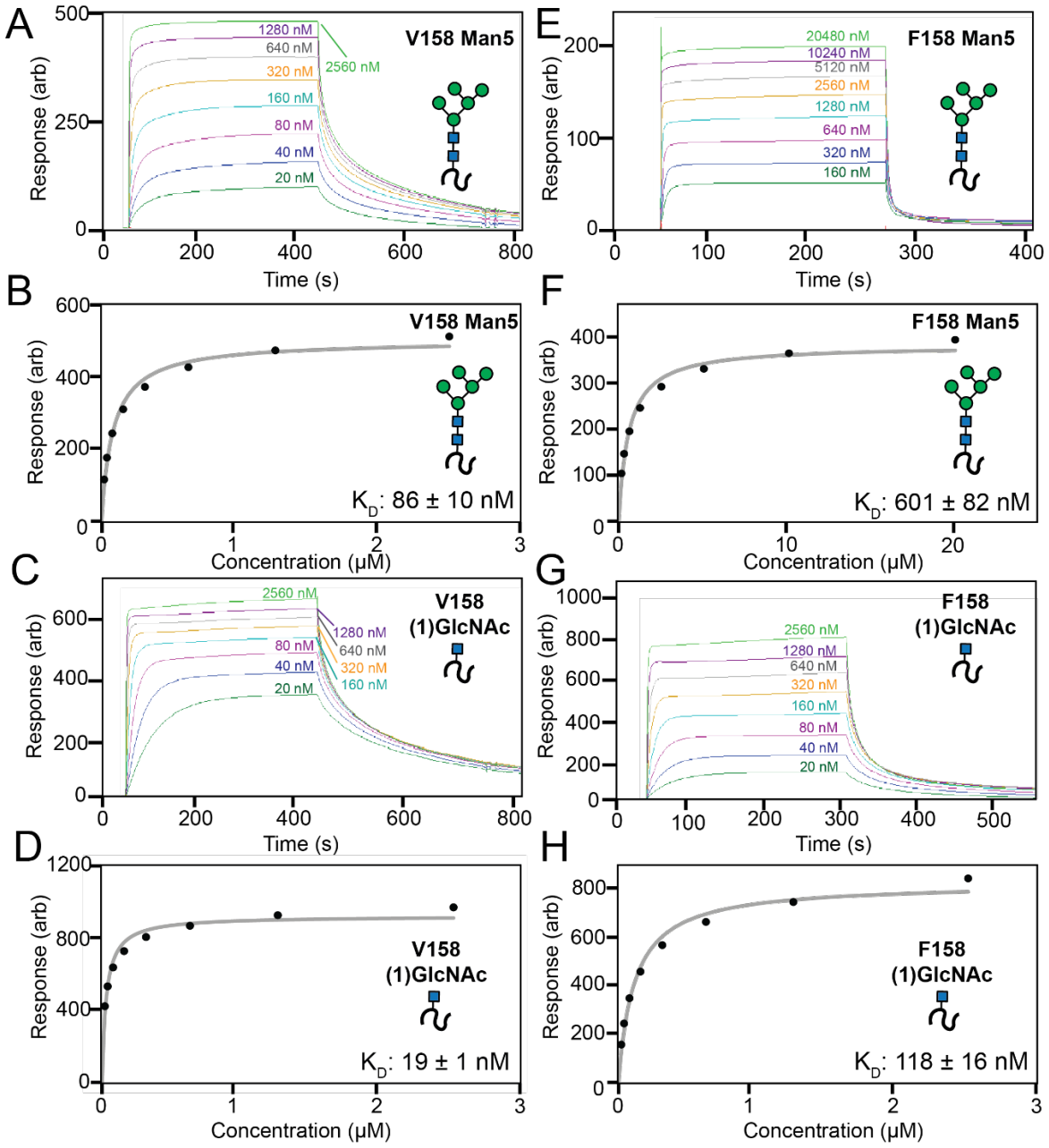
	Complex-type	Man5	(1)GlcNAc	S164A
V158	1.0	3.4	13	1.1
F158	1.0	1.6	13	1.1



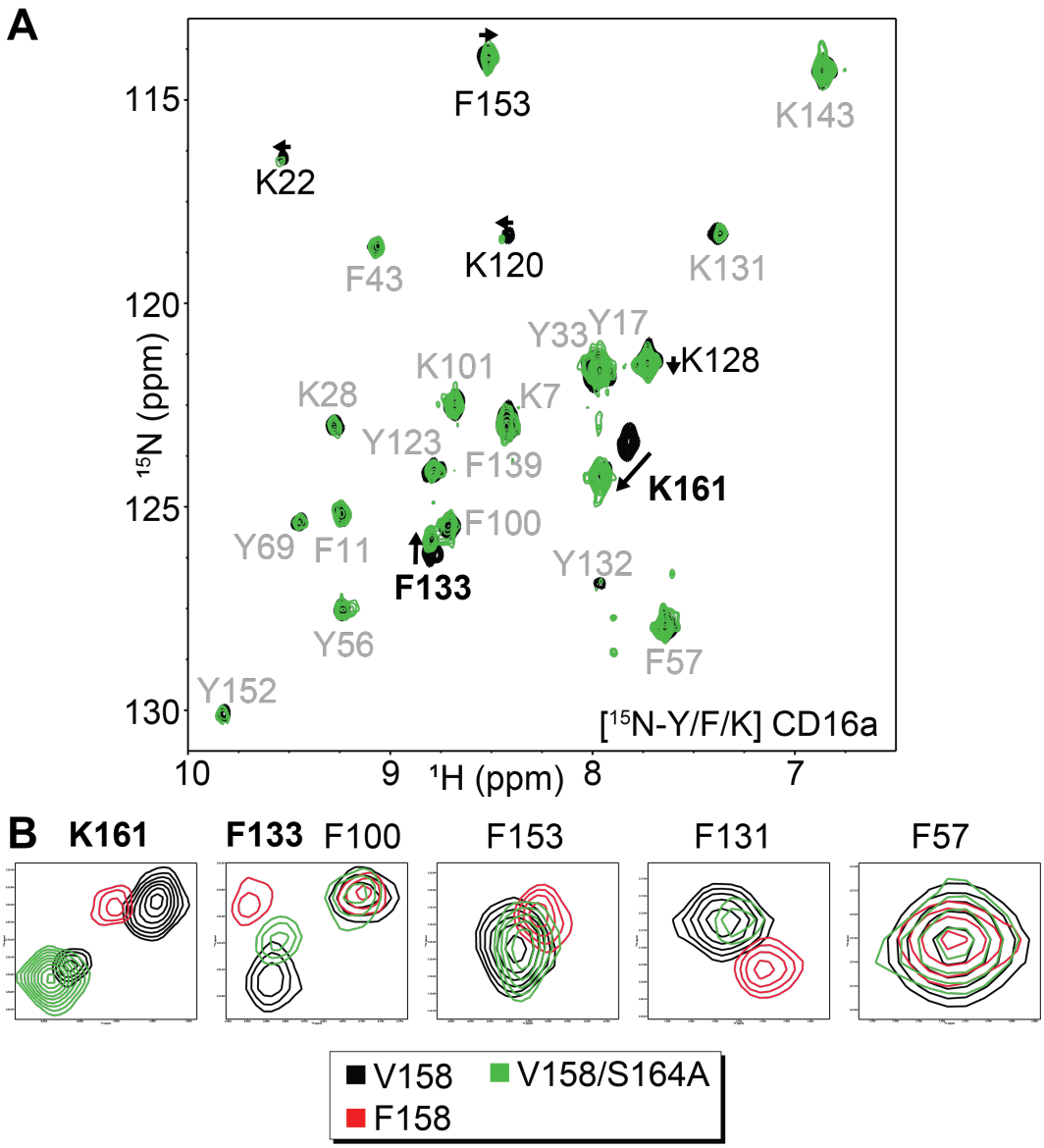
Supplemental Figure 1. SDS-PAGE of GFP-srCD16a fusions. The two different allotypes are expressed with four different glycosylation patterns. Distinct shifts in migration are observed between each type of glycosylated protein.



Supplemental Figure 2. Relative abundance of N162 glycans on the V158 and F158 allotypes as determined by glycoproteomics MS. The N162-containing glycopeptide for F158 provides less intense peaks by ESI-MS/MS as our lab previously noted (Mol Cell Proteomics. 2019 Nov;18(11):2178-2190; Mol Cell Proteomics. 2020 Feb;19(2):362-374). Possible N-glycan configurations are shown as cartoons below the chart.

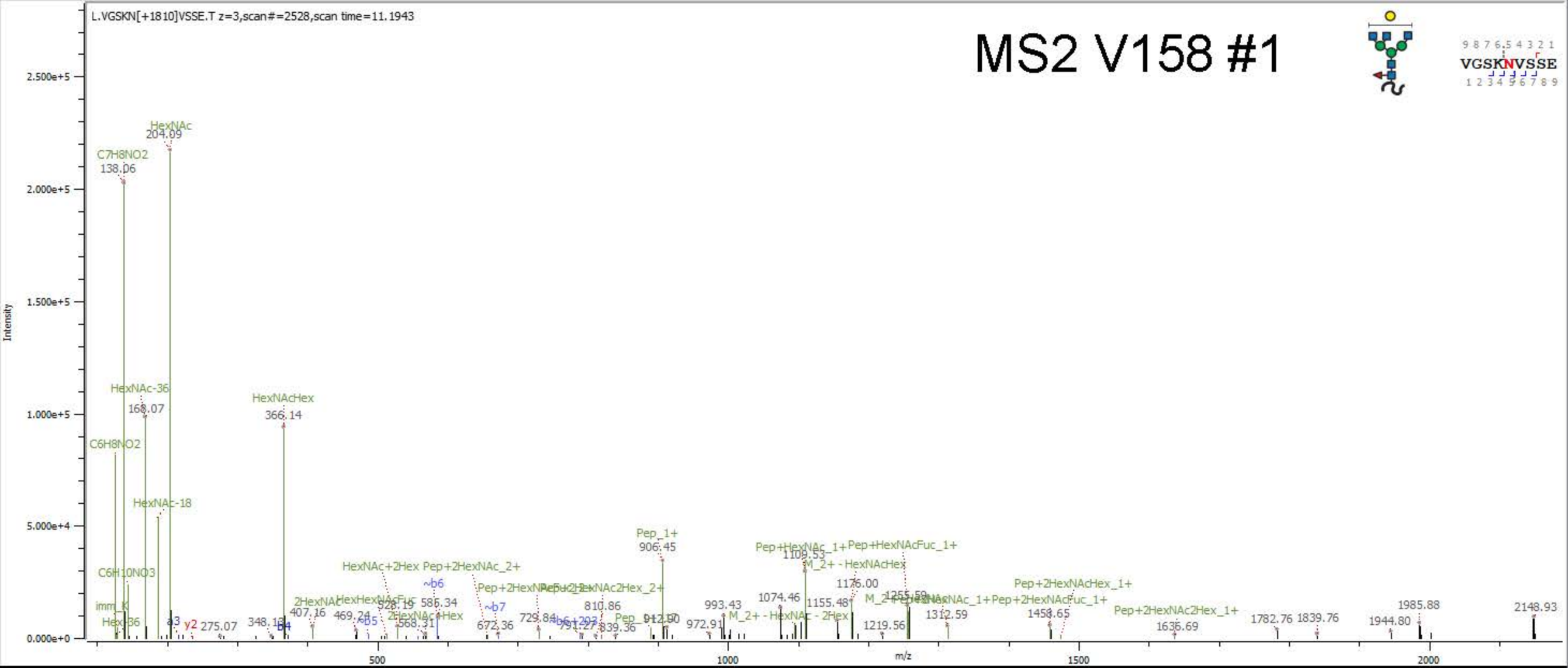


Supplemental Figure 3. Representative surface plasmon resonance (SPR) sensorgrams of CD16a allotypes with either Man5 oligomannose N-glycans (A,E) or (1)GlcNAc N-glycans (C,G). Fits of the dissociation constants and associated errors from equilibrium binding measurements are also shown (B,D,F,H). Cartoons show the expected N-glycan at the N162 site.



Supplemental Figure 4. (**A**) Overlay of HSQC-TROSY spectra of CD16a V158 with (black) and without (green) the N162 glycan. Residues with large chemical shift perturbations are bolded. (**B**) Highlighted peaks showing differences between the V158, V158/S16A and F158 spectra.

Supplemental Figure 5. Assigned N162 glycopeptide MS2 spectra (following pages)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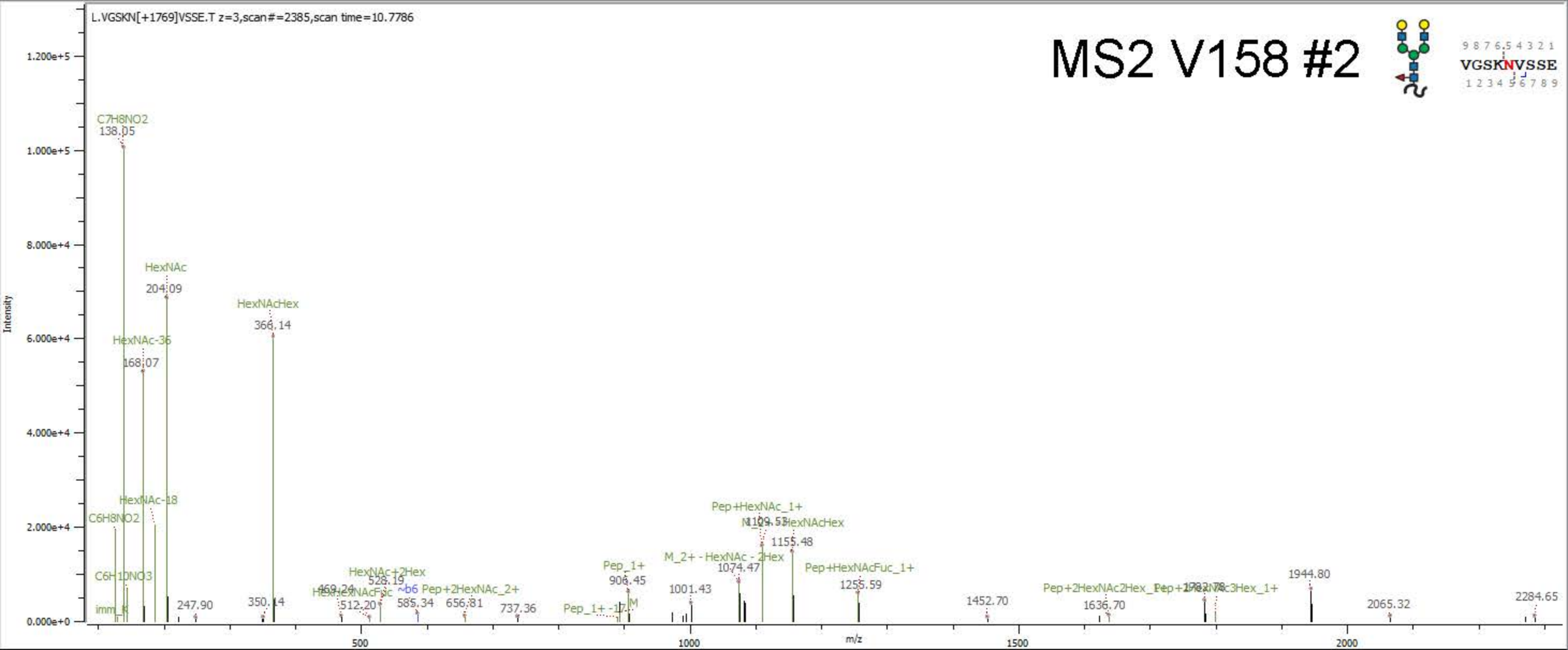
VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
2.500e+5
2.000e+5
1.500e+5
1.000e+5
5.000e+4
0.000e+0
m/z
500
1000
1500
2000
HexNAc
204.09
C7H8NO2
138.06
HexNAc-36
168.07
HexNAcHex
366.14
C6H8NO2
HexNAc-18
C6H10NO3
imm_K
Hex-36
b3
y2
275.07
348.13
b4
407.16
2HexNAc
469.24
~b5
528.19
2HexNAc+Hex
568.31
HexNAc+2Hex
585.34
~b6
672.36
Pep+2HexNAc_2+
~b7
729.84
~b6+203
791.27
839.36
Pep_2+
912.90
Pep_1+
906.45
810.86
Pep+2HexNAc2Hex_2+
972.91
993.43
M_2+ - HexNAc - 2Hex
1074.46
Pep+HexNAc_1+
1109.53
Pep+HexNAcFuc_1+
M_2+ - HexNAcHex
1155.48
1176.00
1219.56
1255.59
1312.59
Pep+2HexNAcHex_1+
1458.65
Pep+2HexNAc2Hex_1+
1636.69
1782.76
1839.76
1944.80
1985.88
2148.93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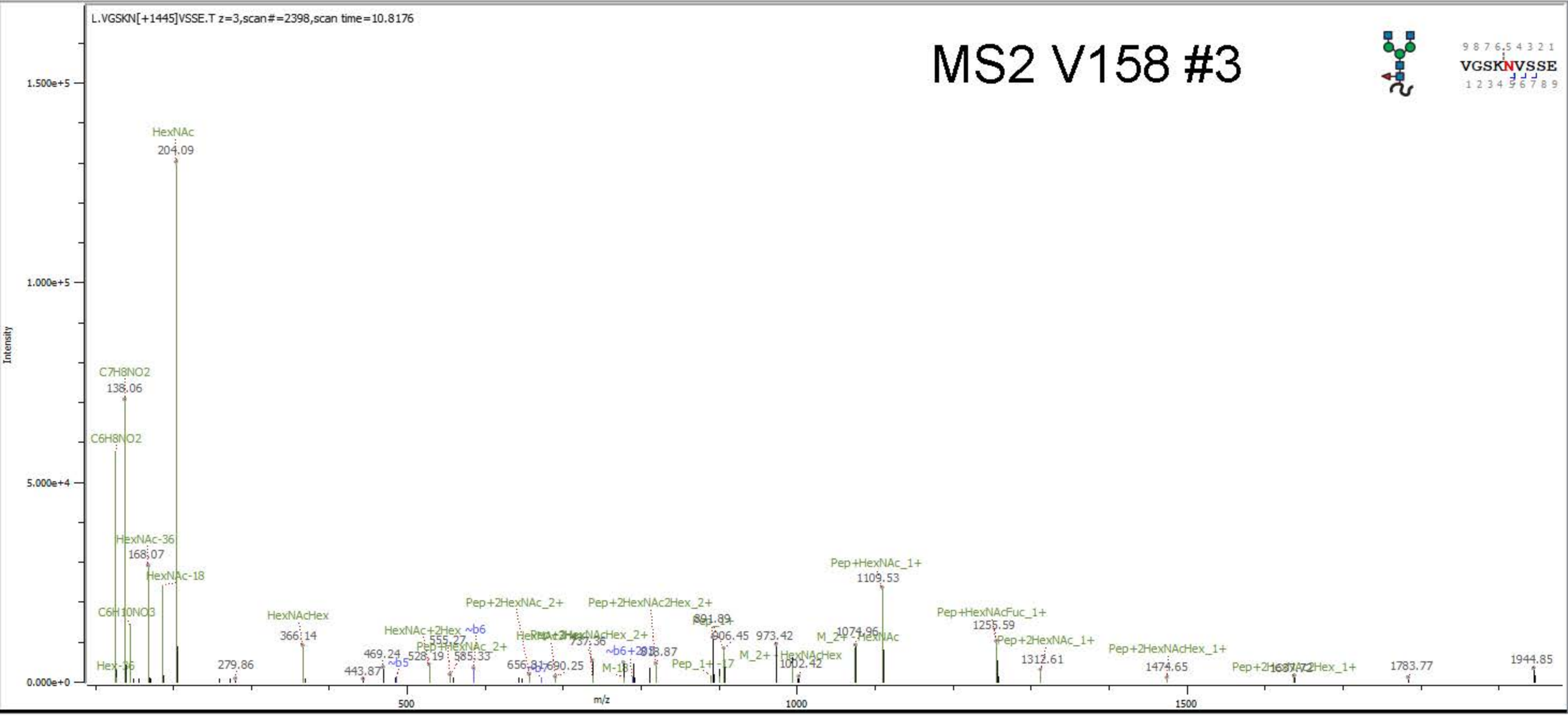
VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
m/z
C7H8NO2
138.05
HexNAc
204.09
HexNAcHex
366.14
HexNAc-36
168.07
HexNAc-18
C6H8NO2
C6H10NO3
imm_N
247.90
350.14
HexNAc+2Hex
469.24
528.19
HexNAcFuc
512.20
~b6
585.34
Pep+2HexNAc_2+
656.81
737.36
Pep_1+
906.45
Pep_1+ -17.M
1001.43
Pep+HexNAc_1+
M_2+ - HexNAc - 2Hex
1074.47
HexNAcHex
1155.48
Pep+HexNAcFuc_1+
1255.59
1452.70
Pep+2HexNAc2Hex_1+
1636.70
1944.80
2065.32
2284.65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VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
1.500e+5
1.000e+5
5.000e+4
0.000e+0
m/z
500
1000
1500
HexNAc
204.09
C7H8NO2
138.06
C6H8NO2
HexNAc-36
168.07
HexNAc-18
C6H10NO3
Hex-36
279.86
HexNAcHex
366.14
HexNAc+2Hex ~b6
555.27
Pep+HexNAc_2+
469.24
528.19
585.33
~b5
443.87
Pep+2HexNAc_2+
656.81
690.25
Pep+2HexNAc2Hex_2+
737.36
M-18
Pep_1+-17
891.89
906.45
973.42
M_2+-HexNAc
M_2+-HexNAcHex
1002.42
1074.96
Pep+HexNAc_1+
1109.53
Pep+HexNAcFuc_1+
1255.59
Pep+2HexNAc_1+
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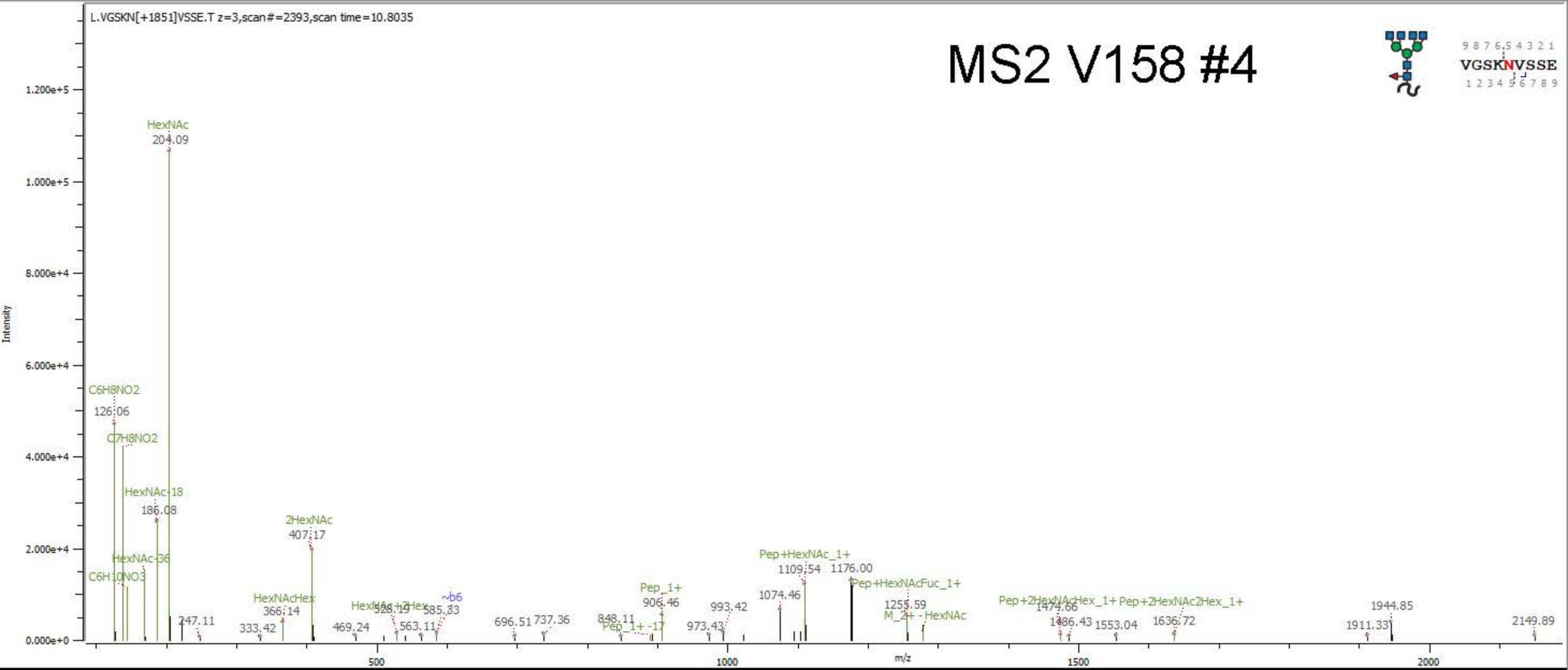
Pep+2HexNAcHex_1+
1474.65
1783.77
1944.85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VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
1.200e+5
1.000e+5
8.000e+4
6.000e+4
4.000e+4
2.000e+4
0.000e+0
HexNAc
204.09
C6H8NO2
126.06
C7H8NO2
HexNAc-18
186.08
2HexNAc
407.17
HexNAc-36
C6H10NO3
HexNAcHex
366.14
HexNAc+2Hex
~b6
247.11
333.42
469.24
528.19
563.11
585.33
696.51
737.36
848.11
Pep_1+ -17
Pep_1+
906.46
973.43
993.42
Pep+HexNAc_1+
1109.54
1074.46
1176.00
Pep+HexNAcFuc_1+
1255.59
M_2+ - HexNAc
Pep+2HexNAcHex_1+
1474.66
1486.43
1553.04
Pep+2HexNAc2Hex_1+
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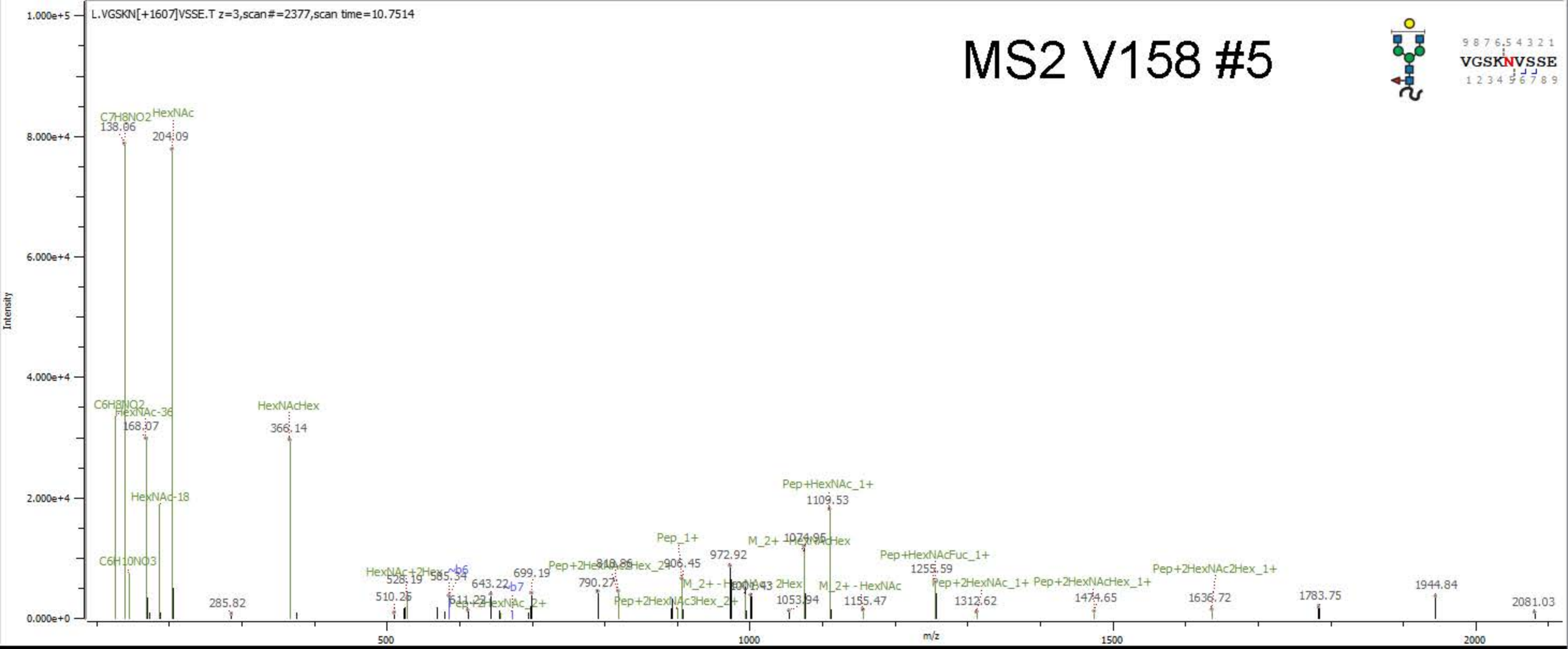
1911.33
1944.85
2149.89
500
1000
m/z
1500
2000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MS2 V158 #5
VGSKNVSSE
Intensity
m/z
C7H8NO2
138.06
HexNAc
204.09
C6H8NO2
HexNAc-36
168.07
HexNAcHex
366.14
HexNAc-18
C6H10NO3
285.82
HexNAc+2Hex
528.19
585.34
b6
643.22
b7
699.19
510.25
Pep+2HexNAc_2+
Pep+2HexNAc3Hex_2+
790.27
906.45
Pep_1+
M_2+ - HexNAc
M_2+ - 2Hex
M_2+ - HexNAcHex
972.92
1053.94
1074.95
Pep+HexNAc_1+
1109.53
1155.47
Pep+HexNAcFuc_1+
1255.59
Pep+2HexNAc_1+
1312.62
Pep+2HexNAcHex_1+
1474.65
Pep+2HexNAc2Hex_1+
1636.72
1783.75
1944.84
2081.03
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6.000e+4
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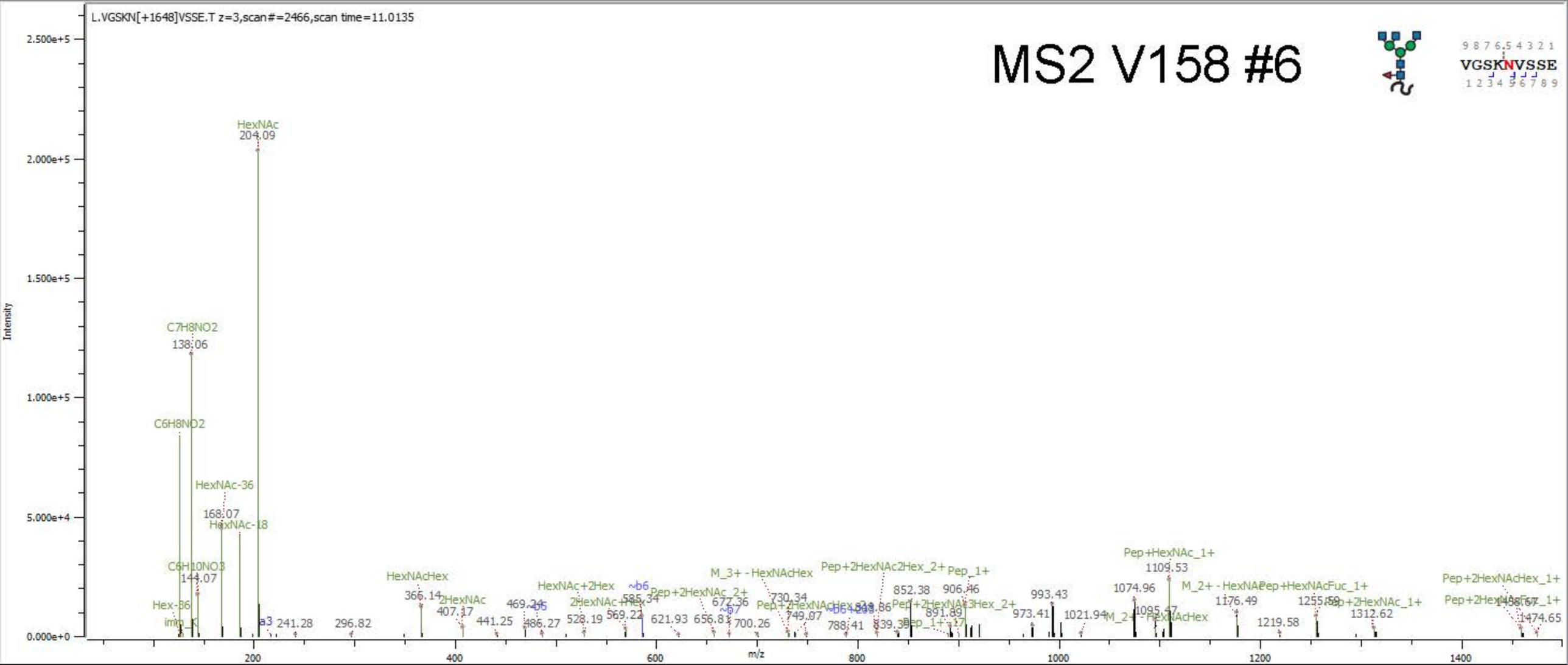
500
1000
1500
2000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VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
2.500e+5
2.000e+5
1.500e+5
1.000e+5
5.000e+4
0.000e+0
m/z
200
400
600
800
1000
1200
1400
HexNAc
204.09
C7H8NO2
138.06
C6H8NO2
HexNAc-36
168.07
HexNAc-18
C6H10NO3
144.07
Hex-36
imm_K
a3
241.28
296.82
HexNAcHex
366.14
2HexNAc
407.17
441.25
469.24
b6
486.27
HexNAc+2Hex
2HexNAc+Hex
528.19
585.34
569.22
b6
Pep+2HexNAc_2+
621.93
656.81
672.36
b7
700.26
M_3+ - HexNAcHex
730.34
749.07
Pep+2HexNAc2Hex_2+
Pep+2HexNAc3Hex_2+
788.41
839.38
Pep_1+
852.38
891.89
906.46
973.41
993.43
1021.94
Pep+HexNAc_1+
1109.53
1074.96
1095.47
M_2+ - HexNAcHex
Pep+HexNAcFuc_1+
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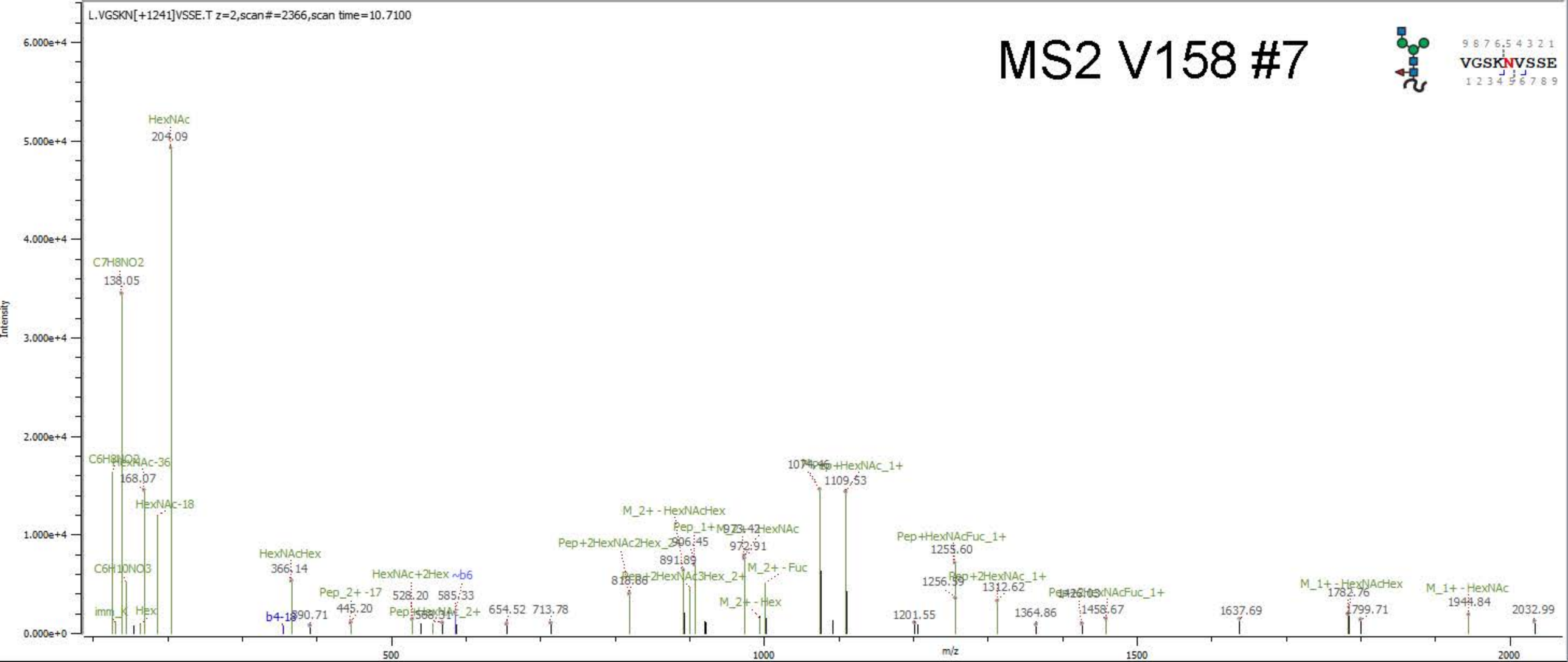
1219.58
1255.59
Pep+2HexNAc_1+
1312.62
Pep+2HexNAcHex_1+
1474.65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Intensity
m/z
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C7H8NO2
138.05
C6H8NO3
C6H10NO3
HexNAc-36
168.07
HexNAc-18
imm_K
Hex
HexNAcHex
366.14
b4-18
890.71
Pep_2+ -17
445.20
HexNAc+2Hex
~b6
528.20
585.33
654.52
713.78
M_2+ - HexNAcHex
Pep+2HexNAc2Hex_2+
818.86
Pep_1+
906.45
891.89
M_2+ - HexNAc
972.91
M_2+ - Fuc
M_2+ - Hex
Pep+HexNAc_1+
1109.53
Pep+HexNAcFuc_1+
1255.60
1256.59
Pep+2HexNAc_1+
1312.62
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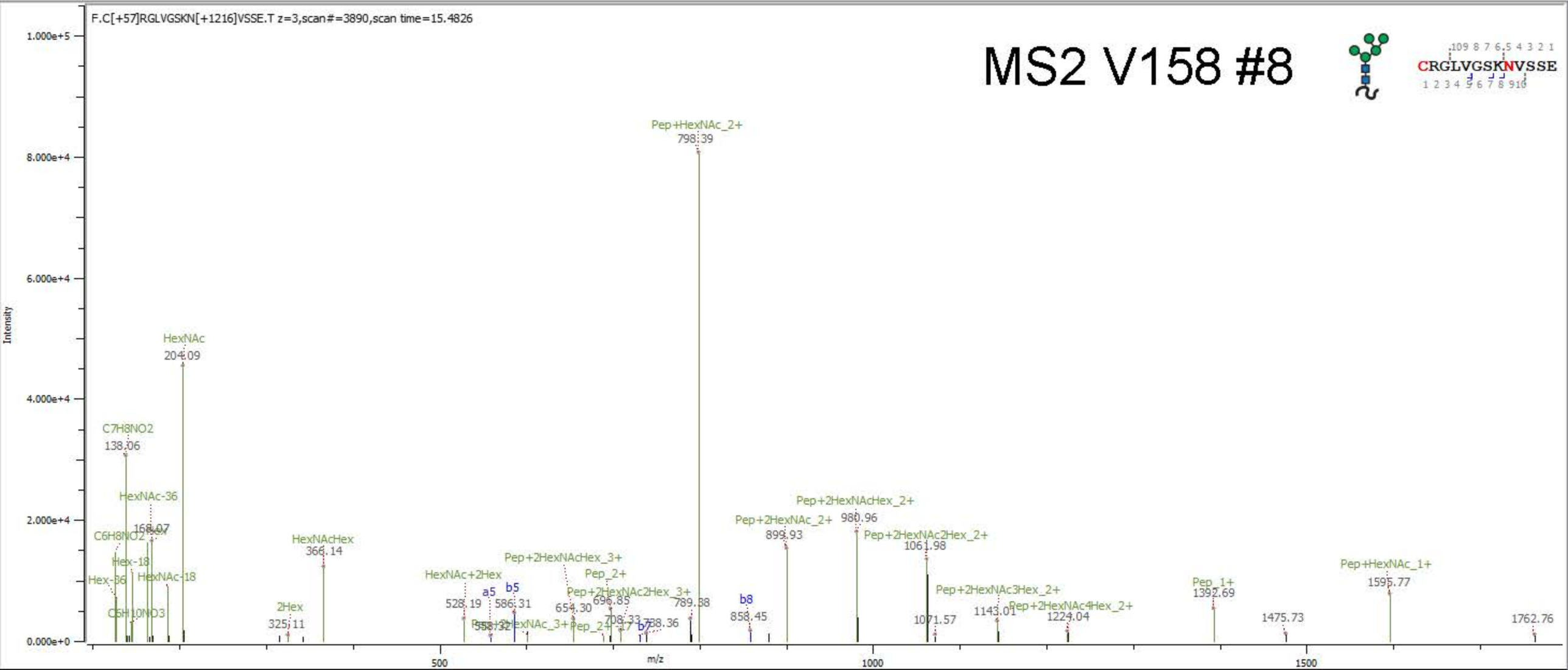
1944.84
2032.99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CRGLVGSKNVSSE
Intensity
m/z
Pep+HexNAc_2+
798.39
HexNAc
204.09
C7H8NO2
138.06
HexNAc-36
168.07
C6H8NO2
Hex-18
Hex-36
HexNAc-18
C6H10NO3
HexNAcHex
366.14
2Hex
325.11
HexNAc+2Hex
528.19
a5
b5
586.31
Pep+2HexNAcHex_3+
Pep_2+
654.30
696.85
Pep+2HexNAc2Hex_3+
789.88
708.33
728.36
b7
b8
858.45
Pep+2HexNAcHex_2+
Pep+2HexNAc_2+
899.93
980.96
Pep+2HexNAc2Hex_2+
1061.98
1071.57
Pep+2HexNAc3Hex_2+
1143.01
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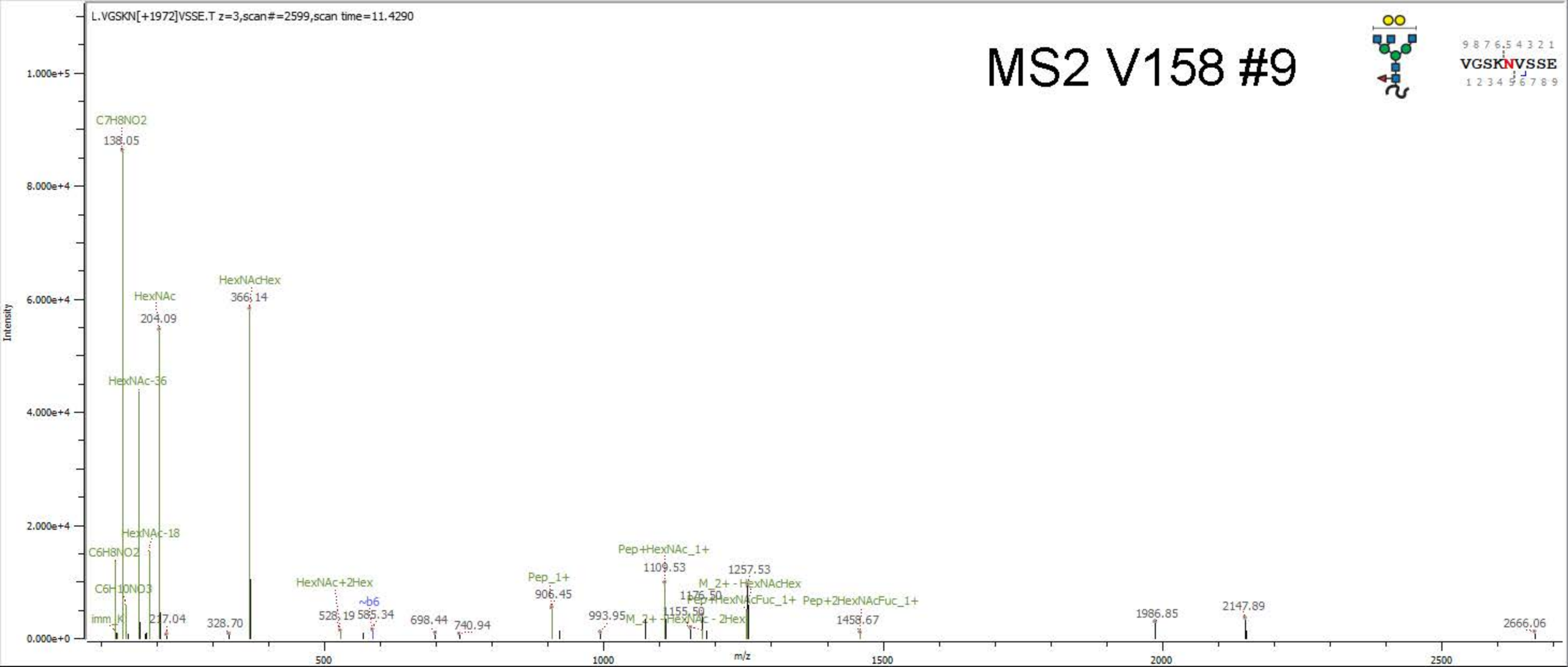
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VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
1.000e+5
8.000e+4
6.000e+4
4.000e+4
2.000e+4
0.000e+0
C7H8NO2
138.05
HexNAc
204.09
HexNAcHex
366.14
HexNAc-36
HexNAc-18
C6H8NO2
C6H10NO3
imm_K
217.04
328.70
HexNAc+2Hex
528.19
~b6
585.34
698.44
740.94
Pep_1+
906.45
993.95
Pep+HexNAc_1+
1109.53
M_2+ - HexNAc - 2Hex
1155.50
1176.50
1257.53
M_2+ - HexNAcHex
Pep+HexNAcFuc_1+
Pep+2HexNAcFuc_1+
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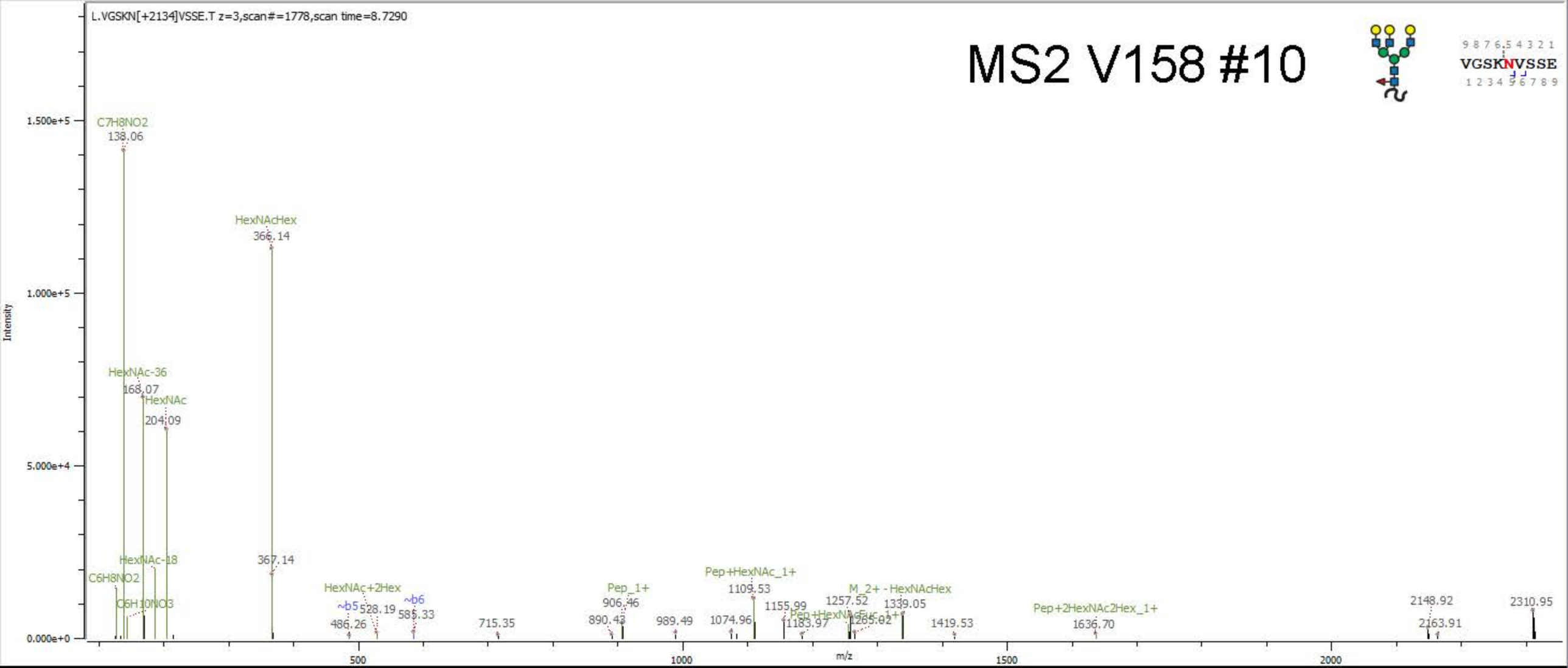
1986.85
2147.89
2666.06
500
1000
1500
2000
2500
m/z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VGSKNVSSE
1 2 3 4 5 6 7 8 9
Intensity
1.500e+5
1.000e+5
5.000e+4
0.000e+0
C7H8NO2
138.06
HexNAcHex
366.14
HexNAc-36
168.07
HexNAc
204.09
HexNAc-18
C6H8NO2
C6H10NO3
367.14
HexNAc+2Hex
~b5
528.19
~b6
585.33
486.26
715.35
Pep_1+
906.46
890.43
989.49
Pep+HexNAc_1+
1109.53
1074.96
1155.99
1183.97
M_2+ - HexNAcHex
1257.52
1339.05
Pep+HexNAcFuc_1+?
1265.02
1419.53
Pep+2HexNAc2Hex_1+
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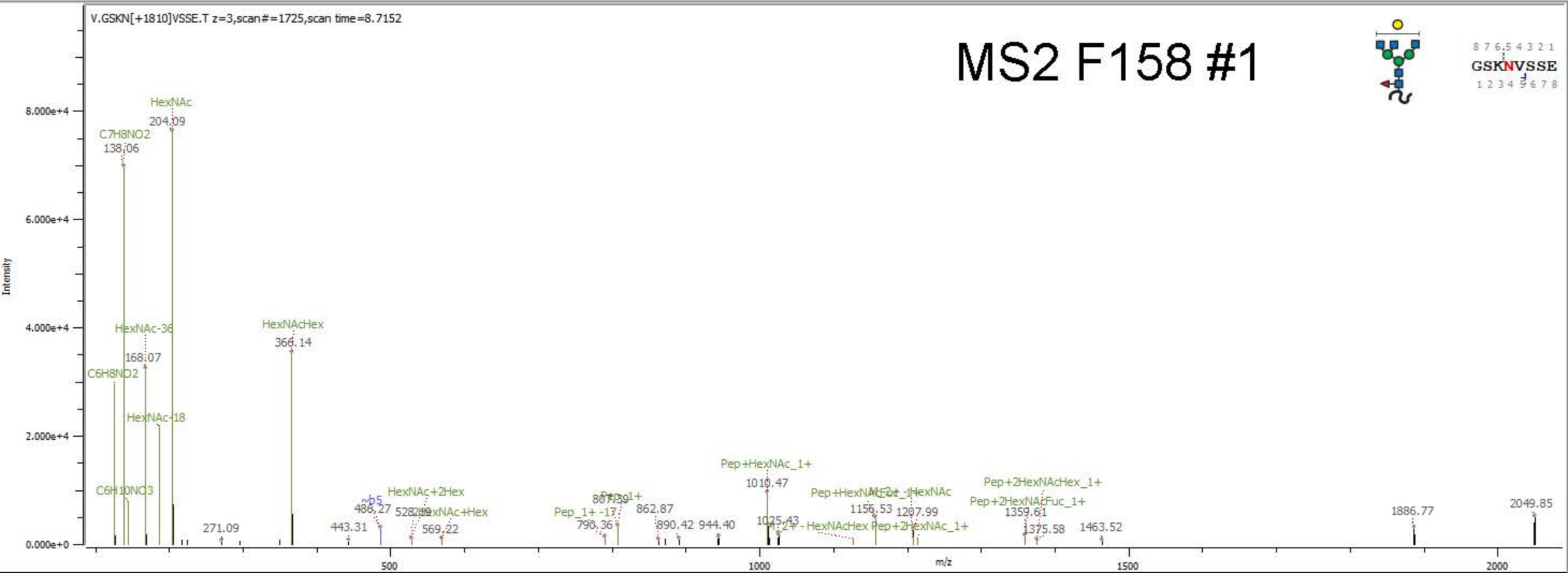
2148.92
2163.91
2310.95
500
1000
m/z
1500
2000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GSKNVSSE
1 2 3 4 5 6 7 8
Intensity
8.000e+4
6.000e+4
4.000e+4
2.000e+4
0.000e+0
m/z
500
1000
1500
2000
HexNAc
204.09
C7H8NO2
138.06
HexNAc-36
168.07
C6H8NO2
HexNAc-18
C6H10NO3
HexNAcHex
366.14
271.09
443.31
b5
486.27
HexNAc+2Hex
569.22
Pep_1+ -17
790.36
Pep_1+
862.87
890.42
944.40
Pep+HexNAc_1+
1010.47
1025.43
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1375.58
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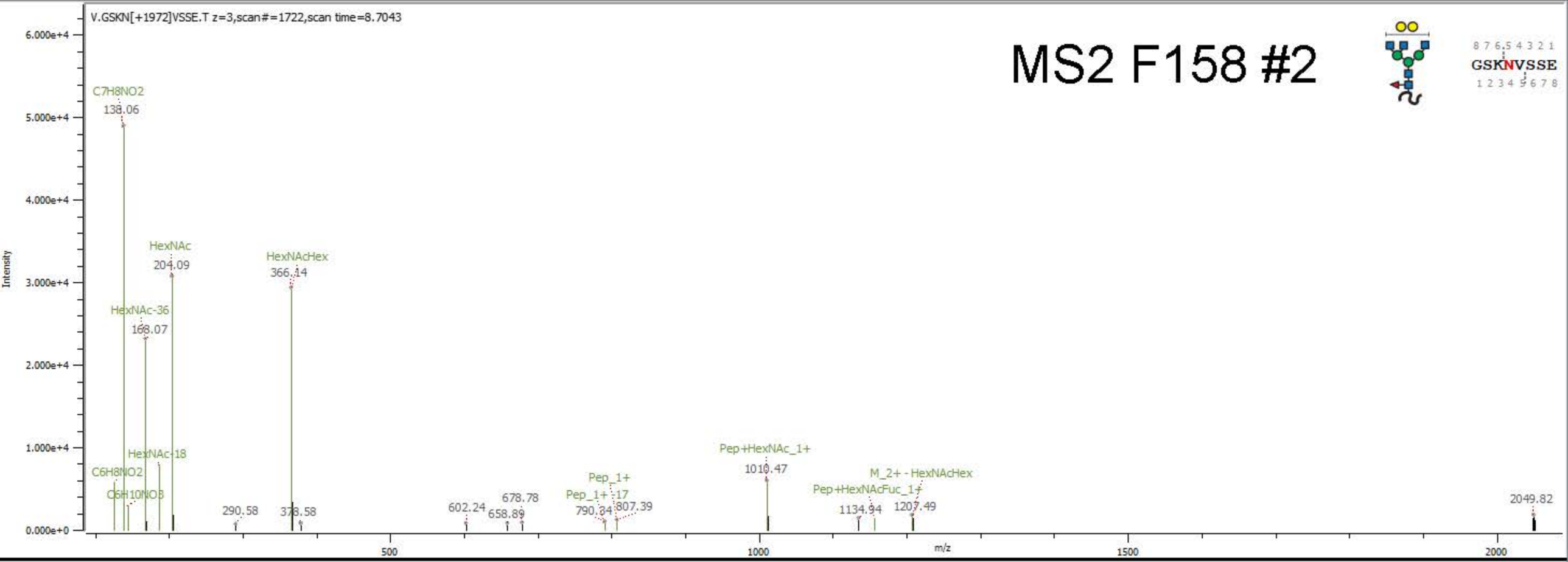
1886.77
2049.85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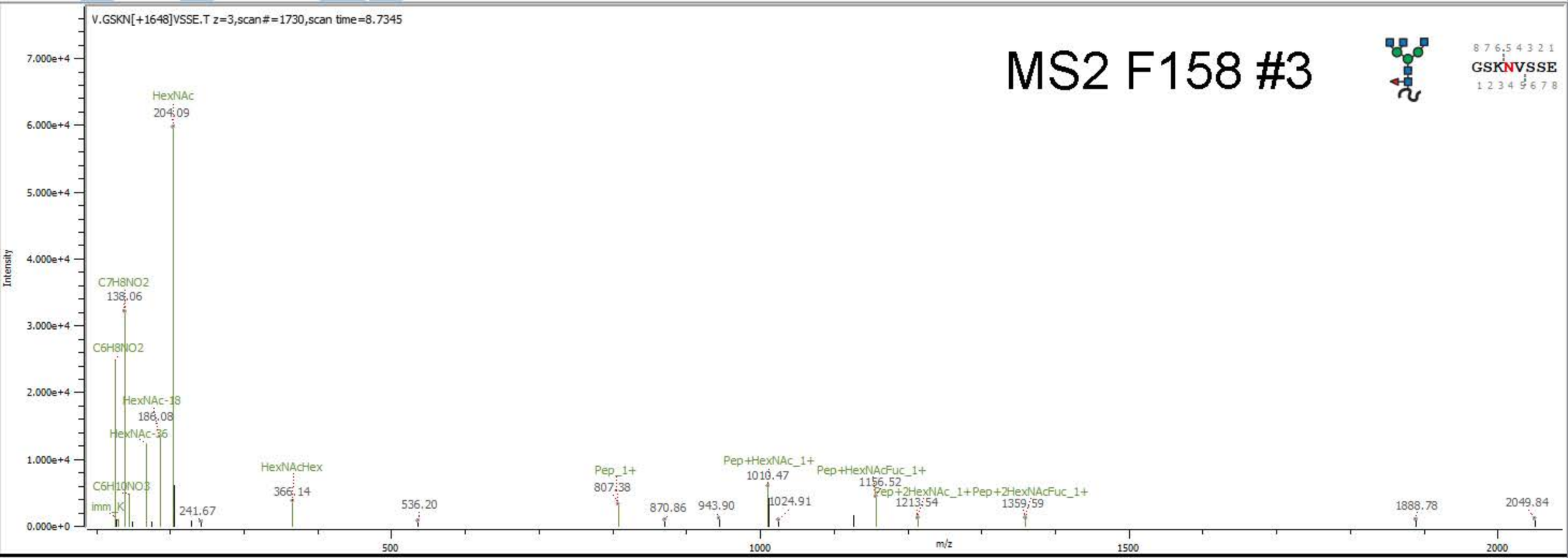
GSKNVSSE
1 2 3 4 5 6 7 8
Intensity
6.000e+4
5.000e+4
4.000e+4
3.000e+4
2.000e+4
1.000e+4
0.000e+0
C7H8NO2
138.06
HexNAc
204.09
HexNAcHex
366.14
HexNAc-36
168.07
HexNAc-18
C6H8NO2
C6H10NO3
290.58
378.58
602.24
658.89
678.78
Pep_1+
Pep_1+-17
790.34
807.39
Pep+HexNAc_1+
1010.47
M_2+ - HexNAcHex
Pep+HexNAcFuc_1+
1134.94
1207.49
2049.82
500
1000
m/z
1500
2000


V.GSKN[+1648]VSSE.T z=3,scan#=1730,scan time=8.7345
MS2 F158 #3
8 7 6 5 4 3 2 1
GSKNVSSE
1 2 3 4 5 6 7 8
Intensity
7.000e+4
6.000e+4
5.000e+4
4.000e+4
3.000e+4
2.000e+4
1.000e+4
0.000e+0
HexNAc
204.09
C7H8NO2
138.06
C6H8NO2
HexNAc-18
186.08
HexNAc-36
C6H10NO3
imm_K
241.67
HexNAcHex
366.14
536.20
Pep_1+
807.38
870.86
943.90
Pep+HexNAc_1+
1010.47
1024.91
Pep+HexNAcFuc_1+
1156.52
Pep+2HexNAc_1+
1213.54
Pep+2HexNAcFuc_1+
1359.59
1888.78
2049.84
500
1000
m/z
1500
2000