

EXPERIMENTAL PROCEDURES

Construction of Conditional Targeting Vector for GnT-Vb (Cre-lox) and generation of Mutant mice: A cre/loxP systems was used to generate GnT-Vb-deficient mouse. The targeting vector has been generated at Ingenious Targeting Labs, Inc. (Stony Brook, New York, USA). A ~10.7kb region used to construct the targeting vector was first sub cloned from a positively identified B6 BAC clone using a homologous recombination-based technique. The region was designed such that the short homology arm (SA) extends 2.0 kb 3' to the exon 6. The long homology arm (LA) starts at the 5' side of exon 6 and is ~8.7kb long. The single loxP site is inserted between exons 6 and 7, and the loxP-flanked Neo cassette is inserted 3' to exons 6. The target region is 0.8kb and includes exon 6. The BAC was sub cloned into a ~2.4kb backbone vector containing an ampicillin selection cassette for retransformation of the construct prior to electroporation. A pGKNeo cassette flanked by loxP sites was inserted into the gene (Supplementary Fig 1). The targeting construct was linearized using NotI and electroporated into C57BL/6 Embryonic Stem cell lines. (ES cell culture and generation of mouse strains was performed in the Medical College of Georgia Embryonic Stem Cell and Transgenic Core Facility.) Embryonic Stem Cells were lysed into 100 µl of lysis buffer (100mM NaCl, 20mM Tris.Cl, pH7.6, 10mM EDTA, 0.5% SDS). The resulting solutions were subject to phenol/chloroform extraction and ethanol precipitation. The DNA precipitates were dissolved in 100ul of distilled water for over night. Two microliters of genomic DNA solution were used as template for PCR genotyping. 8/125 isolated ES cell clones that survived G418 selection were homologous recombinant on the basis of PCR genotyping. For ES cell diagnostic PCR genotyping, genomic DNA were evaluated using one primer specific to the intron region between exon 6 and exon 7 (JKL91) (5'-GGAATCCAGTACAGGGAACTTGCTTGG-3'), one primer specific to the neo sequence (JKL92), (5'-ATGTGTGCGAGGCCAGAGGCCACTTGT-3') (expected size kb) and one primer specific to the 3' genomic sequence, outside the region used in the targeting vector (JKL93) (5'-CTGGGGACCCAATCAGCCCTGAATCAC-3') (Supplementary Fig. 1). PCR amplification was performed on ES DNA in a final volume of 20 µl with the HotStar Taq DNA polymerase (Qiagen Inc.). Samples were denatured at 94 °C, 15 min and then subjected to 35 cycles of amplification (94 °C, 30 s; 60 °C, 30 s; 72 °C, 2 min 30 s). As depicted in Figure 1, primers JKL91 and JKL93 result in a 261-bp product from wild type allele and a 2.3 kb from the targeted allele. But primers, JKL92 and JKL93, results in no PCR products from the wild type allele and a 460-bp product from the targeted allele. The 8 clones were further checked on the right side of the recombination event by Southern blot analysis. For Southern analysis, genomic DNA were digested with EcoRV or HindIII and probed with a PCR fragment containing intron sequence after exon 8 (Supplementary Fig 1). The wild type allele generates a 3.5kb fragment and targeted allele generates a 5.5kb fragment in EcoRV digestion and 9.8kb and 6.7kb fragment in HindIII digestion.

Eight ES cell clones were retained of which one (clone #116) was injected into C57BL/6 X Balb/C blastocysts, which were then reimplanted into recipient female mice. Eight highly chimeric B6 male mice (90% and 99% of coat color chimerism) were identified and were fully normal. The chimera mice were then crossed to C57BL/6 mice. Genotyping of GnT-Vb^{flxed/+} mice was carried out by PCR of genomic DNA isolated from tails using REDExtract-N-Amp Tissue PCR Kit (Sigma Inc.). Same primers and methods were used as in genotyping for ES cells screening in above. GnT-Vb^{flxed/+} mice were crossed to transgenic mice of ubiquitously expressing Cre recombinase (C57BL/6 Cre mouse from Dr. Nancy Manley in Department of Genetics, University of Georgia). Primers JKL91, JKL93 and JKL97 were used for screening of conventional knock out mouse of GnT-Vb. Primer JKL97 and JKL93 result in 370-bp product from conventional K/O allele and JKL91 and JKL93 result in 261-bp product from wild type allele.

Crossing GnT-Vb null mice into the GnT-V null background (from Dr. Jim Dennis, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, Canada) yielded a mouse that was null for both GnT-V and GnT-Vb.

FIGURE LEGENDS

Supplementary Figure 1. Generation of GnT-Vb Mutant mice: **A**, Map of the targeting vectors (GnT-Vb^{fl}) and the wild-type GnT-Vb (GnT-Vb⁺) locus. The single loxP site is inserted between exons 6 and 7 bearing GnT-Vb , and the loxP-flanked Neo cassette is inserted 3' to exons 6 as described in Methods and Materials. Homologous recombination produces the GnT-Vb^{dfl} alleles. **B**, PCR primers and Southern blots probe used in screening ES cells and mice. **C**, Southern blot analysis of ES clones and chimera mice. E⁺, the wild type of ES clone, E^{+/fl},

heterozygote with wild type and floxed ES clone, K⁺, wild type of chimera mouse, K^{+/fl}, heterozygote with wild type and floxed chimera mouse genomic DNA were digested with EcoRV or HindIII and probed with a PCR fragment containing intron sequence after exon 8 as shown in B. The wild type allele generates a 3.5kb fragment and targeted allele generates a 5.5kb fragment in EcoRV digestion and 9.8kb and 6.7kb fragment in HindIII digestion. **D**, PCR analysis of the genomic DNA from GnT-Vb^{dfn/dfl} (GnT-Vb(-/-)). GnT-Vb^{flxed/+} mice were crossed to transgenic mice of ubiquitously expressing Cre recombinase (C57BL/6 Cre mouse). Genotyping of GnT-Vb^{dfn/dfl} (conventional K/O, GnT-Vb(-/-)) mouse was carried out by PCR of genomic DNA isolated from tails using REDExtract-N-Amp Tissue PCR Kit (Sigma Inc.). Primers JKL91, JKL93 and JKL97 were used for screening of the mice. Primer JKL97 and JKL93 result in 370-bp product from GnT-Vb^{dfn/dfl} allele and JKL91 and JKL93 result in 261-bp product from wild type allele.

Supplementary Figure 2. MS/MS spectra of O-linked glycans in mouse brains from the five genotypes. Blue letter with Ø, <10% to <60% amounts compared to wild type; Blue letter with X, <5% amounts compared to wild type;

▼: Fuc, ● : Man, ○ : Gal, ■ :GlcNAc, □ :GalNAc, and ♦ :NeuAc

Supplementary Figure 3. MS/MS and MS³ spectra of N-linked glycans in mouse brains from the five genotypes. Blue with X, <5% amounts compared to wild type;

▼: Fuc, ● : Man, ○ : Gal, ■ :GlcNAc, □ :GalNAc, and ♦ :NeuAc

Supplemental Table 1. O-linked glycans observed in mouse brains from all genotypes by tandem mass spectrometric analysis.

No.	O-linked oligosaccharide composition	<i>m/z</i> (mono)					
		[M+Na] ⁺	[M+2Na] ²⁺				
1	(Hex)1(HexNAc)1[(HexNAc)1(Hex)1]	534.289	278.639				
2	(HexNAc)2	575.316	299.153				
3	(NeuAc)1(Hex)1	650.337	336.663				
4	(NeuAc)1(HexNAc)1	691.363	357.176				
5	(Deoxyhexose)1(HexNAc)1(Hex)1	708.378	365.684				
6	(Hex)1(HexNAc)1(Hex)1	738.389	380.689				
7	(HexNAc)2(Hex)1	779.415	401.202				
8	(NeuAc)1(Hex)1(HexNAc)1	895.463	459.226				
9	(Hex)1(Deoxyhexose)1(HexNAc)1(Hex)1	912.478	467.734				
10	(NeuGc)1(Hex)1(HexNAc)1	925.473	474.231				
11	(NeuAc)1(HexNAc)2	936.489	479.739				
12	(Hex)1(Deoxyhexose)1(HexNAc)2	953.505	488.247				
13	(Hex)1(HexNAc)2(Hex)1	983.515	503.252				
14	(NeuAc)2(Hex)1	1011.510	517.250				
15	(NeuAc)2(HexNAc)1	1052.537	537.763				
16	(NeuAc)1(Hex)1(Deoxyhexose)1(HexNAc)1	1069.552	546.271				
17	(NeuAc)1(Hex)1(HexNAc)1(Hex)1	1099.563	561.276				
18	(NeuAc)1(Hex)1(HexNAc)2	1140.589	581.789				
19	(Hex)1(Deoxyhexose)1(HexNAc)2(Hex)1	1157.604	590.297				
20	(Hex)2(HexNAc)2(Hex)1	1187.615	605.302				
21	(NeuAc)2(Hex)1(HexNAc)1	1256.637	639.813				
22	(NeuAc)1(NeuGc)1(Hex)1(HexNAc)1	1286.647	654.818				
23	(NeuAc)2(HexNAc)2	1297.663	660.326				
24	(NeuAc)1(Hex)1(Deoxyhexose)1(HexNAc)2	1314.678	668.834				
25	(NeuGc)2(Hex)1(HexNAc)1	1316.658	669.824				
26	(NeuAc)1(Hex)1(HexNAc)2(Hex)1	1344.689	683.839				
27	(Hex)2(Deoxyhexose)1(HexNAc)2(Hex)1	1361.704	692.347				
28	(NeuAc)2(Hex)1(HexNAc)1(Hex)1	1460.736	741.863				
29	(NeuAc)1(Hex)2(Deoxyhexose)1(HexNAc)2	1518.778	770.884				
30	(NeuAc)1(Hex)2(HexNAc)2(Hex)1	1548.789	785.889				
31	(NeuAc)3(Hex)1(HexNAc)1	1617.810	820.400				
32	(NeuAc)2(Hex)2(HexNAc)2	1705.863	864.426				
33	(NeuAc)1(Hex)3(Deoxyhexose)1(HexNAc)2	1722.878	872.934				
34	(NeuAc)2(Hex)2(HexNAc)2(Hex)1	1909.962	966.476				

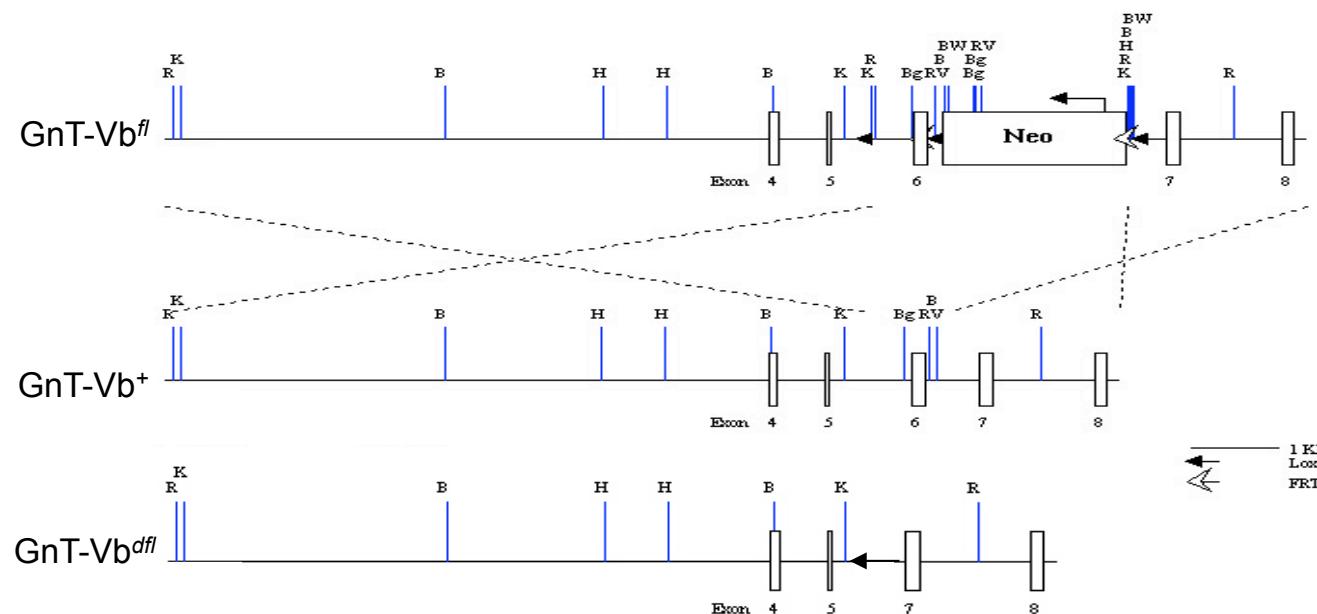
The detected glycans by TIM MS/MS are shown in Red.

Supplemental Table 2. N-linked glycans observed in mouse brains from all genotypes using tandem mass spectrometric analysis. The detected glycans by TIM MS/MS are shown in Red.

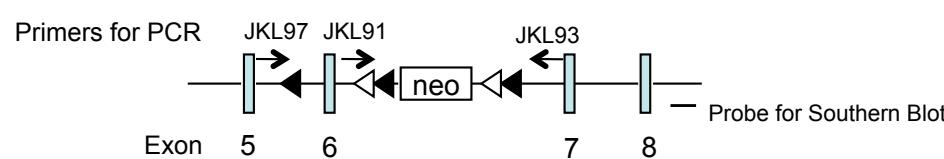
No.	N-linked oligosaccharide composition	m/z (mono)			
		[M+Na] ⁺	[M+2Na] ⁺⁺	[M+3Na] ⁺⁺⁺	[M+4Na] ⁺⁺⁺⁺
1	(Man)1(GlcNAc)2	763.384	393.187	267.788	209.088
2	(Man)1(GlcNAc)2(Fuc)1	902.433	456.251	307.108	251.111
3	(Man)2(GlcNAc)2	967.484	495.237	337.821	259.113
4	(Man)2(GlcNAc)2(Fuc)	1141.573	582.281	395.851	302.636
5	(Man)3(GlcNAc)2	1171.583	597.207	405.824	310.138
6	(Man)3(GlcNAc)2(Fuc)1	1348.633	681.351	439.944	355.069
7	(Man)4(GlcNAc)2	1376.683	699.336	473.888	361.163
8	(GlcNAc)1(Man)3(GlcNAc)2	1416.710	719.850	487.563	371.420
9	(Man)4(GlcNAc)2(Fuc)	1579.783	801.386	541.921	412.188
10	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	1589.783	801.386	541.920	412.188
11	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2	1620.810	821.900	555.593	422.445
12	(GlcNAc)1(Man)4(GlcNAc)2	1620.810	821.900	555.593	422.445
13	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	1661.836	842.413	566.272	432.701
14	(Man)5(GlcNAc)2	1735.873	880.453	609.044	460.113
15	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	1794.899	908.944	613.626	465.967
16	(GlcNAc)1(Man)5(GlcNAc)2	1824.909	923.950	623.630	473.470
17	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	1835.925	929.495	627.302	476.224
18	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2 or (GlcNAc)2(Man)4(GlcNAc)2	1900.935	949.483	631.010	480.225
19	(GlcNAc)3(Man)3(GlcNAc)2	1906.963	964.976	650.991	493.983
20	(Gal)1(GlcNAc)1(Fuc)1(Man)3(GlcNAc)2(Fuc)1	1968.998	995.989	671.656	509.489
21	(Man)4(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	1981.983	1002.467	675.988	512.736
22	(Man)7(GlcNAc)2	1987.983	1005.486	677.007	514.236
23	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	2008.998	1016.001	689.051	518.028
24	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1 or (GlcNAc)3(Man)2(GlcNAc)2(Fuc)1	2020.010	1023.000	699.663	522.085
25	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2	2029.009	1025.998	691.663	524.495
26	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2040.025	1031.507	695.333	527.249
27	(Gal)2(GlcNAc)2(Man)3(GlcNAc)2 or (GlcNAc)2(Man)5(GlcNAc)2	2070.038	1046.813	705.338	534.751
28	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2092.042	1052.000	709.010	535.005
29	(Gal)1(GlcNAc)3(Man)3(GlcNAc)2 or (GlcNAc)3(Man)4(GlcNAc)2	2111.062	1067.026	719.014	545.008
30	(GlcNAc)4(Man)3(GlcNAc)2	2152.089	1087.539	732.694	555.265
31	(NeuAc)1(Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	2156.073	1089.531	734.017	556.260
32	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2171.082	1100.001	746.017	565.005
33	(Gal)1(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2203.098	1113.044	749.693	568.017
34	(Gal)1(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2214.115	1119.562	753.365	570.771
35	(NeuAc)1(Gal)1(GlcNAc)2(Man)3(GlcNAc)2	2227.110	1125.050	757.694	574.020
36	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1 or (Gal)1(GlcNAc)2(Man)4(GlcNAc)2(Fuc)1	2233.109	1128.049	759.696	575.520
37	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2244.125	1130.567	763.368	578.274
38	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2	2274.136	1144.001	770.012	580.776
39	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2292.142	1154.071	777.044	588.259
40	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2 or (Gal)1(GlcNAc)3(Man)4(GlcNAc)2	2315.162	1169.076	787.047	596.033
41	(GlcNAc)3(Man)5(GlcNAc)2	2315.162	1169.076	787.047	596.033
42	(GlcNAc)4(Man)3(GlcNAc)2(Fuc)1	2326.178	1174.584	790.719	598.787
43	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	2356.189	1189.589	800.723	606.290
44	(NeuAc)1(Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	2360.172	1191.581	802.051	607.285
45	(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2373.182	1204.001	802.052	611.030
46	(NeuAc)1(Gal)1(GlcNAc)1(Man)3(GlcNAc)2	2390.183	1206.586	812.054	614.788
47	(Man)9(GlcNAc)2	2396.182	1209.586	814.054	616.288
48	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2397.215	1210.103	814.398	616.546
49	(Gal)1(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2401.219	1214.001	815.006	617.252
50	(Gal)2(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2418.214	1220.602	821.398	621.796
51	(NeuAc)1(Gal)2(GlcNAc)2(Man)3(GlcNAc)2	2431.210	1227.100	825.730	625.045
52	(Gal)1(GlcNAc)1(Man)4(GlcNAc)2	2437.209	1230.099	827.729	626.545
53	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2449.214	1240.001	831.071	632.053
54	(Gal)1(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	2459.241	1241.116	835.073	632.053
55	(NeuAc)1(Gal)1(GlcNAc)3(Man)3(GlcNAc)2	2472.233	1247.613	839.405	635.301
56	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2478.235	1250.613	841.405	636.801
57	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2491.247	1252.007	844.057	639.255
58	(GlcNAc)4(Man)3(GlcNAc)2(Fuc)1	2500.268	1261.629	848.749	642.309
59	(Gal)2(GlcNAc)3(Man)3(GlcNAc)2	2519.262	1271.126	855.091	647.058
60	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2530.278	1276.634	858.753	649.812
61	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2539.286	1286.001	860.056	651.174
62	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2571.305	1297.147	872.428	660.069
63	(NeuAc)1(Gal)1(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2575.288	1299.139	873.756	661.064
64	(Gal)2(GlcNAc)2(Fuc)2(Man)3(GlcNAc)2(Fuc)1	2592.303	1307.647	879.428	665.318
65	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2600.312	1311.001	885.057	667.013
66	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2	2601.315	1312.152	882.431	667.371
67	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2622.314	1322.652	889.431	672.821
68	(NeuAc)1(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1 or (GlcNAc)3(Man)2(GlcNAc)2(Fuc)1	2635.309	1329.150	893.763	676.070
69	(NeuAc)1(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1 or (GlcNAc)3(Man)2(GlcNAc)2(Fuc)1	2648.325	1334.688	897.435	678.824
70	(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2652.325	1337.657	899.435	680.324
71	(Gal)2(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2663.341	1343.165	903.107	683.078
72	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2676.336	1348.001	907.439	686.326
73	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2689.341	1358.171	911.10	689.050
74	(GlcNAc)3(Man)2(GlcNAc)2(Fuc)1	2700.347	1363.679	916.782	693.334
75	(Gal)1(GlcNAc)1(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2707.367	1370.176	921.114	696.583
76	(NeuAc)1(Gal)1(GlcNAc)1(Man)3(GlcNAc)2	2717.363	1373.176	923.114	698.083
77	(Gal)1(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	2723.362	1379.362	926.006	700.037
78	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2737.369	1386.001	926.006	701.036
79	(Gal)1(GlcNAc)5(Man)3(GlcNAc)2(Fuc)1	2775.405	1399.197	940.461	711.093
80	(NeuAc)1(Gal)2(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2779.398	1401.169	941.789	712.089
81	(NeuAc)1(Gal)2(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2792.383	1407.693	946.121	715.336
82	(NeuAc)1(Gal)1(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2800.389	1414.194	951.123	715.325
83	(NeuAc)1(Gal)1(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	2820.415	1421.702	955.465	722.346
84	(Gal)2(GlcNAc)3(Fuc)2(Man)3(GlcNAc)2(Fuc)1	2837.430	1430.210	961.136	726.600
85	(NeuGe)1(Gal)1(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1 or (GlcNAc)3(Man)2(GlcNAc)2(Fuc)1	2850.425	1436.707	965.468	729.849
86	(Gal)3(GlcNAc)3(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2867.441	1445.215	971.140	734.102
87	(NeuAc)1(Gal)3(GlcNAc)3(Man)3(GlcNAc)2	2880.430	1451.713	975.472	737.351
88	(Gal)1(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1 or (GlcNAc)4(Man)3(GlcNAc)2(Fuc)1	2891.452	1457.221	979.144	740.105
89	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	2897.451	1460.220	981.144	741.605
90	(Gal)2(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2909.457	1469.001	984.056	744.035
91	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2921.462	1472.236	989.147	747.608
92	(Gal)2(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	2928.478	1480.734	994.879	751.862
93	(GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2966.473	1494.731	1004.151	758.860
94	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	2983.488	1500.001	1009.822	763.114
95	(NeuAc)1(Gal)1(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	2993.493	1509.736	1014.144	765.053
96	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	3008.492	1515.736	1018.154	769.363
97	(NeuAc)1(Gal)2(GlcNAc)1(Fuc)1(Man)3(GlcNAc)2(Fuc)1	3024.514	1523.752	1023.498	773.371
98	(NeuAc)1(Gal)2(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	3037.510	1529.397	1027.830	776.620
99	(NeuAc)1(Gal)2(GlcNAc)3(Fuc)1(Man)3(GlcNAc)2(Fuc)1	3039.516	1530.759	1029.774	778.032
100	(NeuAc)1(Gal)2(GlcNAc)4(Man)3(GlcNAc)2(Fuc)1	3095.552	1559.271	1047.177	791.130
101	(NeuAc)1(Gal)3(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3125.562	1574.276	1057.181	798.633
102	(NeuAc)2(Man)2(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3211.592	1617.294	1085.860	820.142
103	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	3231.607	1620.001	1085.903	820.545
104	(NeuAc)2(Man)2(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3269.641	1648.815	1105.207	834.653
105	(NeuAc)2(Man)2(GlcNAc)4(Man)3(GlcNAc)2(Fuc)1	3282.636	1652.813	1109.539	837.901
106	(NeuAc)1(Gal)3(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3288.635	1655.813	1111.538	839.301
107	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	3311.643	1669.001	1118.024	840.009
108	(NeuAc)1(Gal)4(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	3329.662	1676.326	1125.214	849.658
109	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1 or (GlcNAc)2(Man)3(GlcNAc)2(Fuc)1	3385.689	1704.339	1143.889	863.664
110	(NeuAc)1(Gal)4(GlcNAc)1(Fuc)1(Man)3(GlcNAc)2(Fuc)1	3402.704	1712.847	1149.561	867.916
111	(NeuAc)1(Gal)4(GlcNAc)2(Fuc)1(Man)3(GlcNAc)2(Fuc)1	3411.710	1713.001	1153.303	871.067
112	(NeuAc)1(Gal)2(GlcNAc)2(Fuc)2(Man)3(GlcNAc)2(Fuc)1	3443.730	1733.360	1163.237	878.175
113	(NeuAc)2(Man)2(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3456.725	1739.686	1167.568	881.424
114	(NeuAc)1(Gal)4(GlcNAc)1(Man)3(GlcNAc)2(Fuc)1	3503.751	1763.339	1183.244	893.180
115	(GlcNAc)1(Man)5(GlcNAc)2(Fuc)1	3535.762	1777.001	1193.347	900.003
116	(NeuAc)1(Gal)4(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3574.788	1798.889	1206.923	910.939
117	(NeuAc)3(Gal)1(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3602.793	1812.887	1216.254	917.938
118	(NeuAc)2(Man)2(GlcNAc)3(Man)3(GlcNAc)2(Fuc)1	3619.799	182		

Supplemental Figure 1.

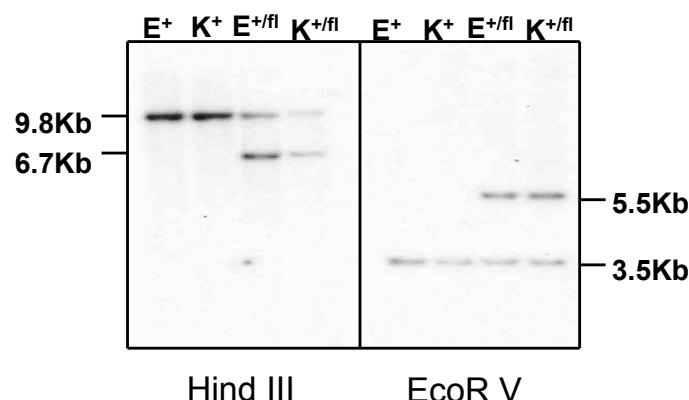
(A)



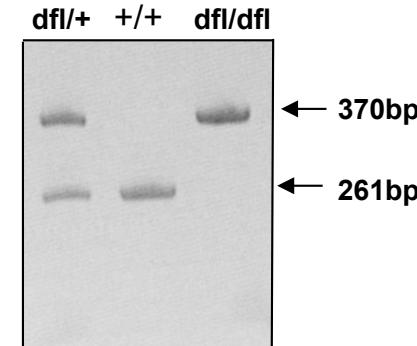
(B)



(C)

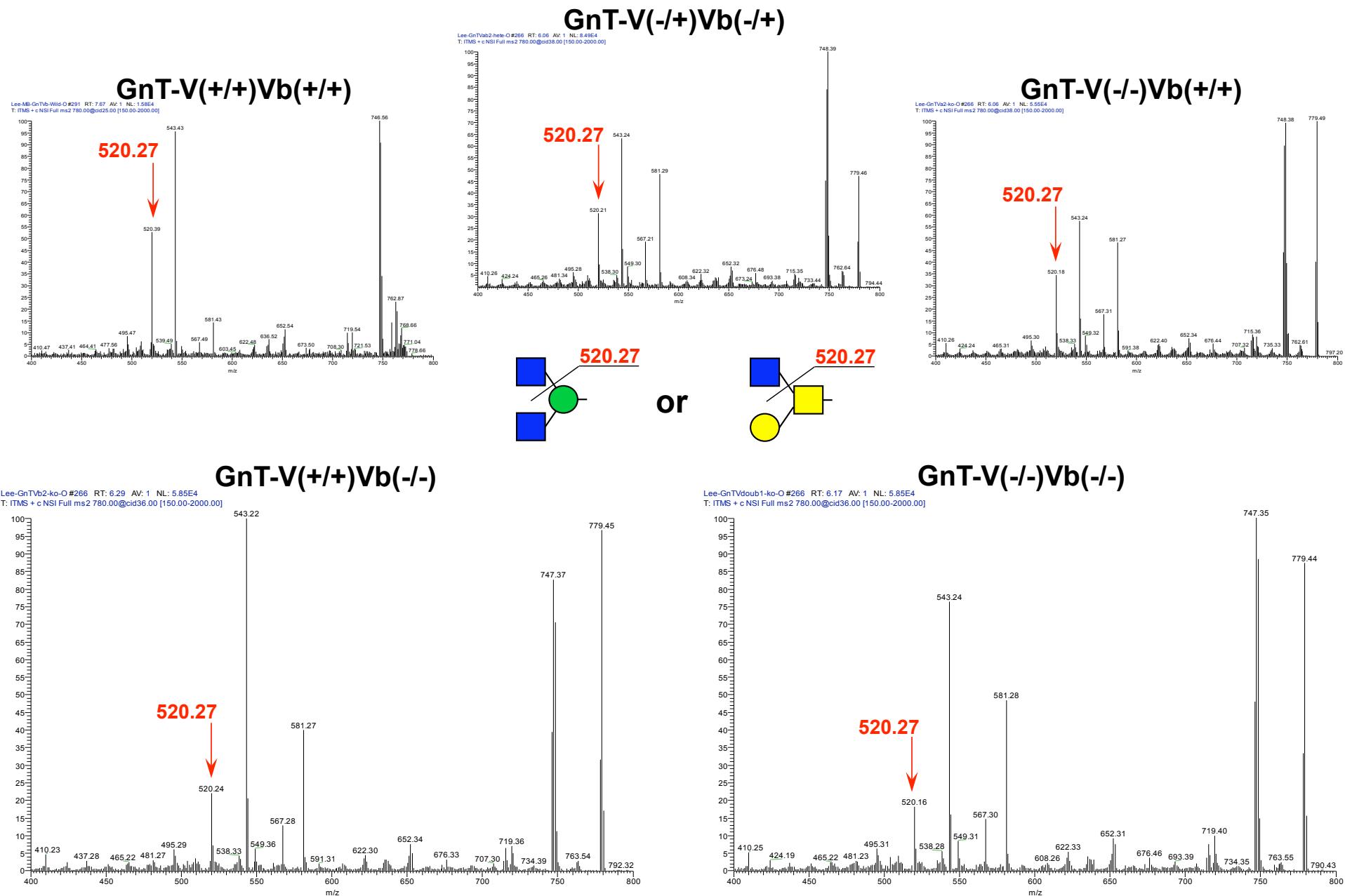


(D)



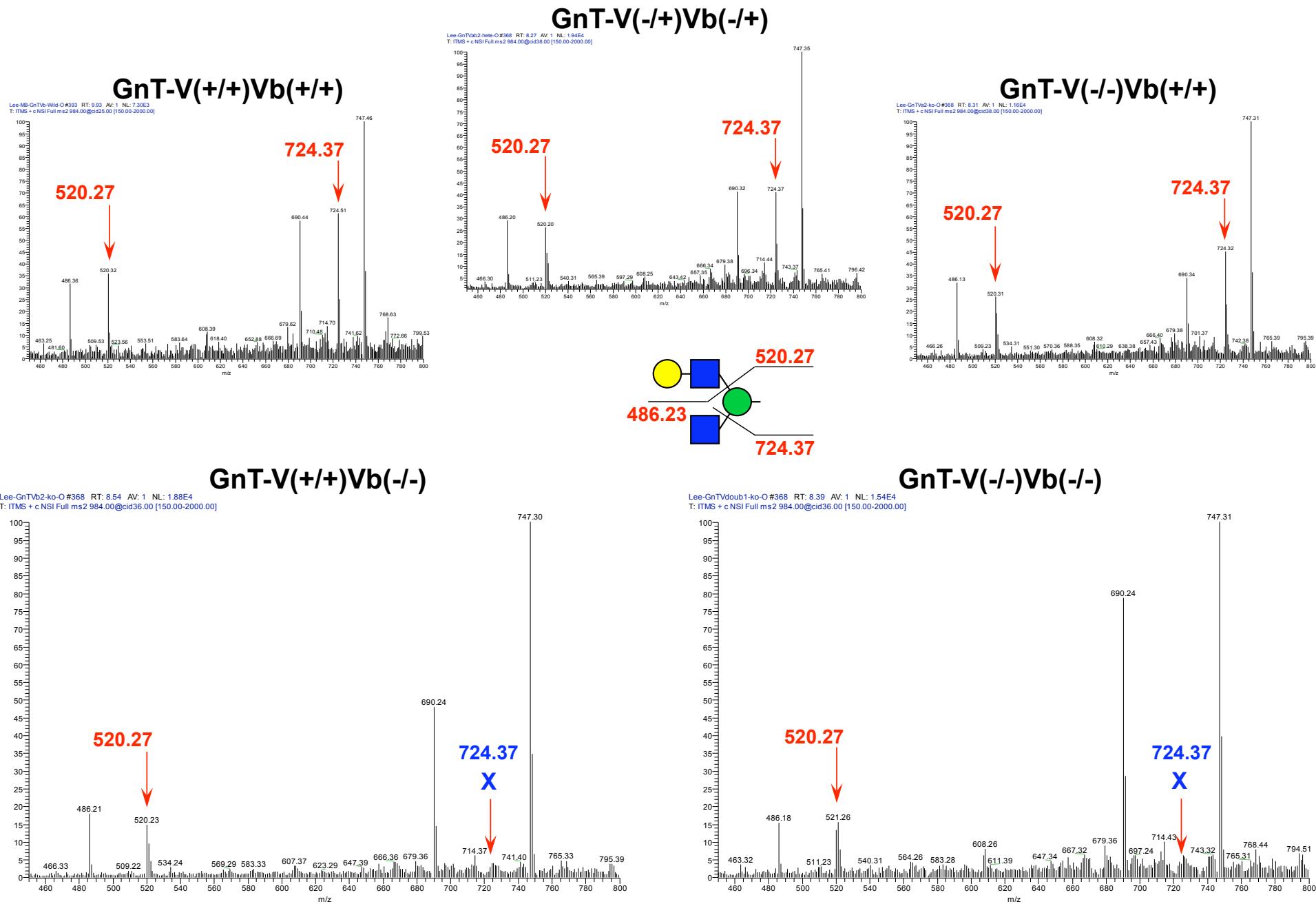
Supplemental Figure 2.

A. $[M+Na]^+$: 779.415 m/z (mono)



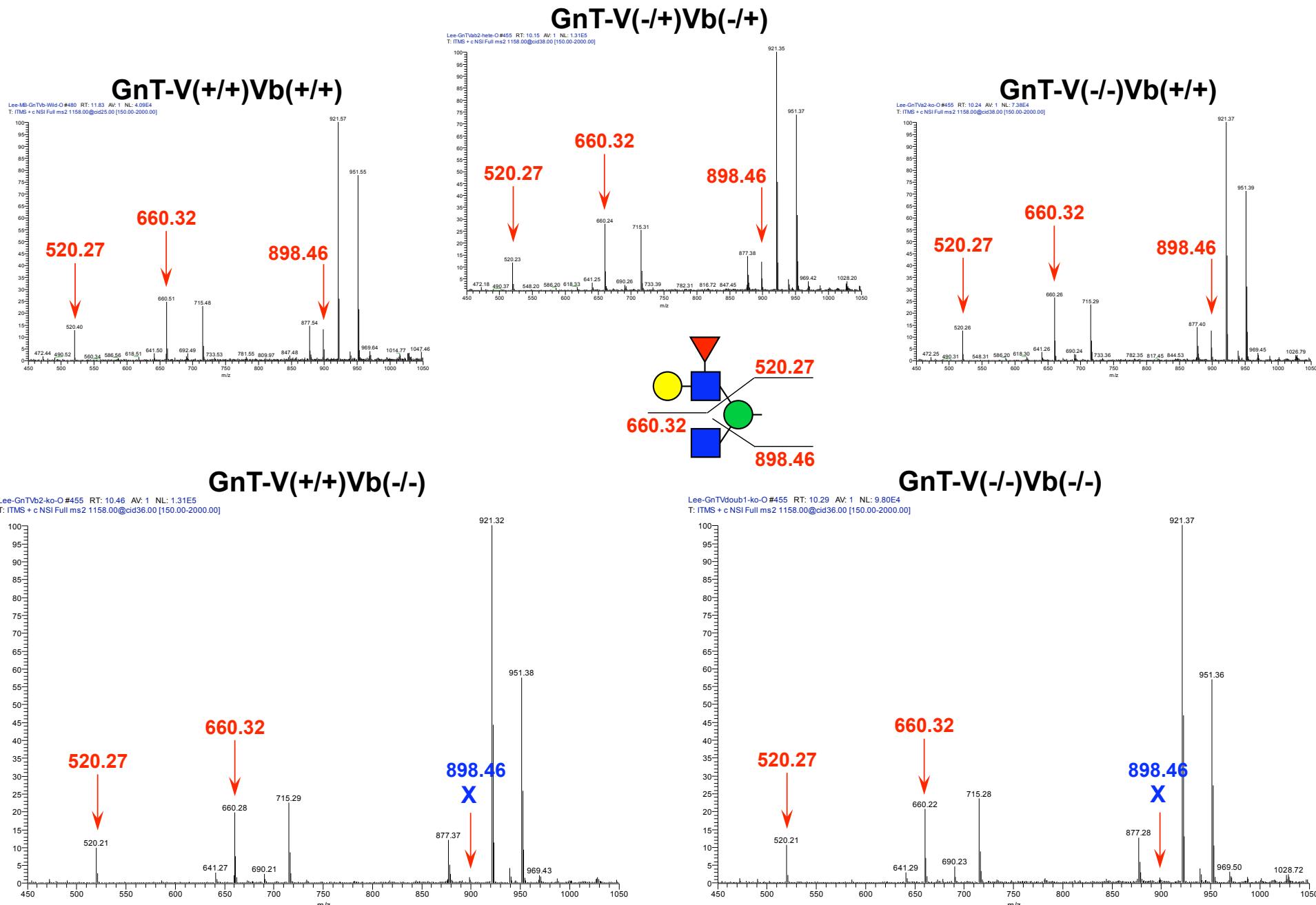
Supplemental Figure 2.

B. $[M+Na]^+$: 983.515 m/z (mono)



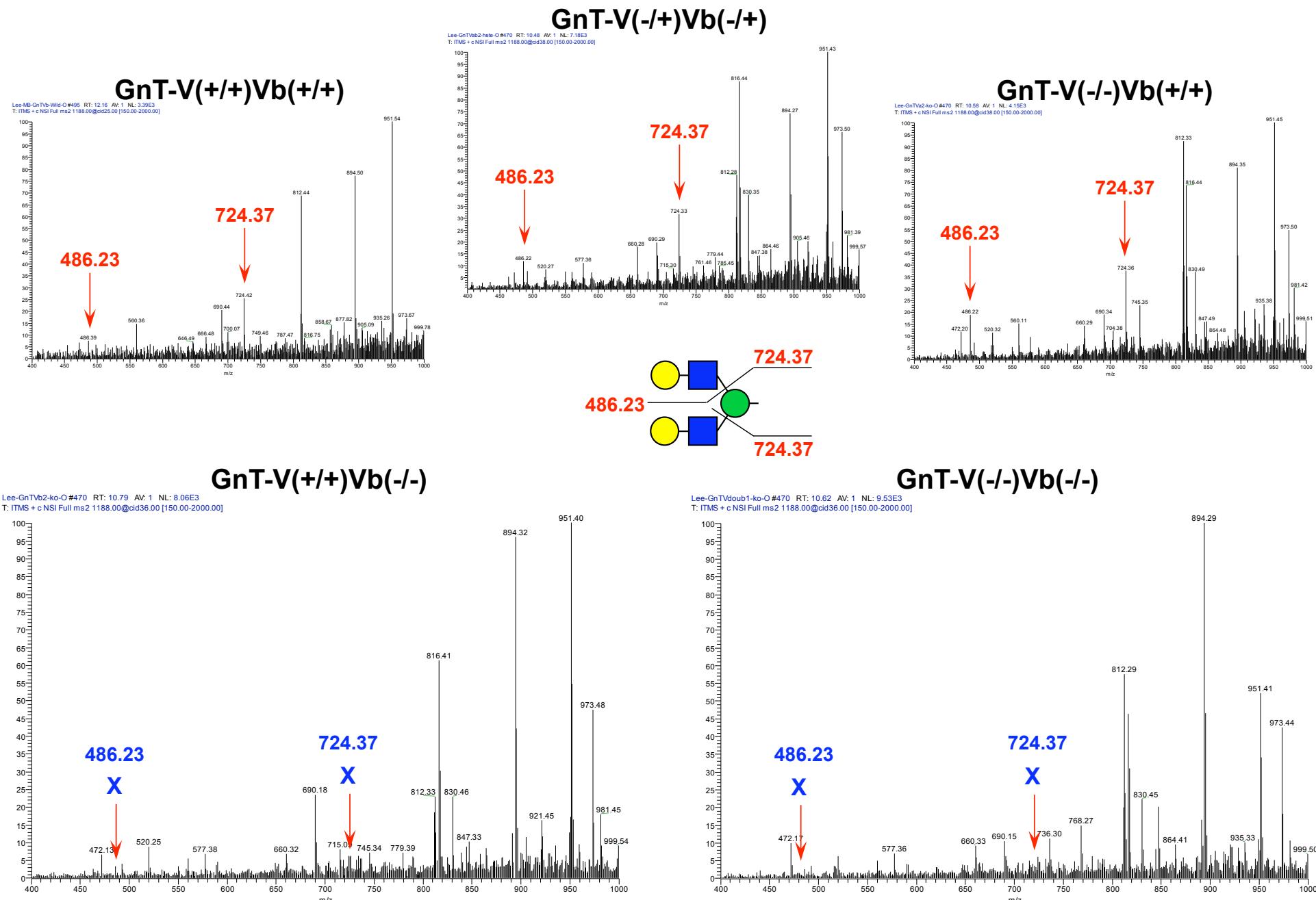
Supplemental Figure 2.

C. $[M+Na]^+$: 1157.604 m/z (mono)



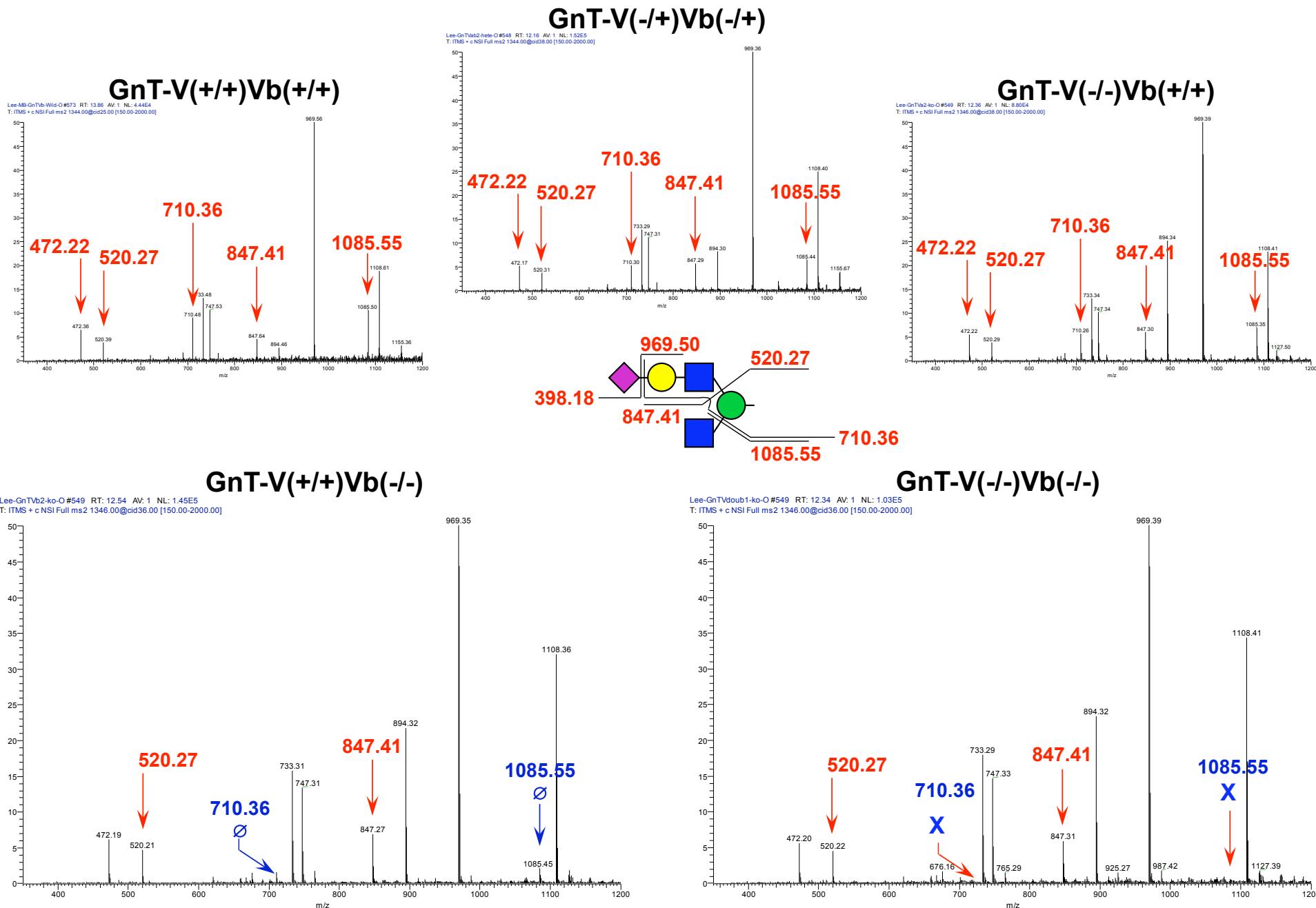
Supplemental Figure 2.

D. $[M+Na]^+$: 1187.615 m/z (mono)



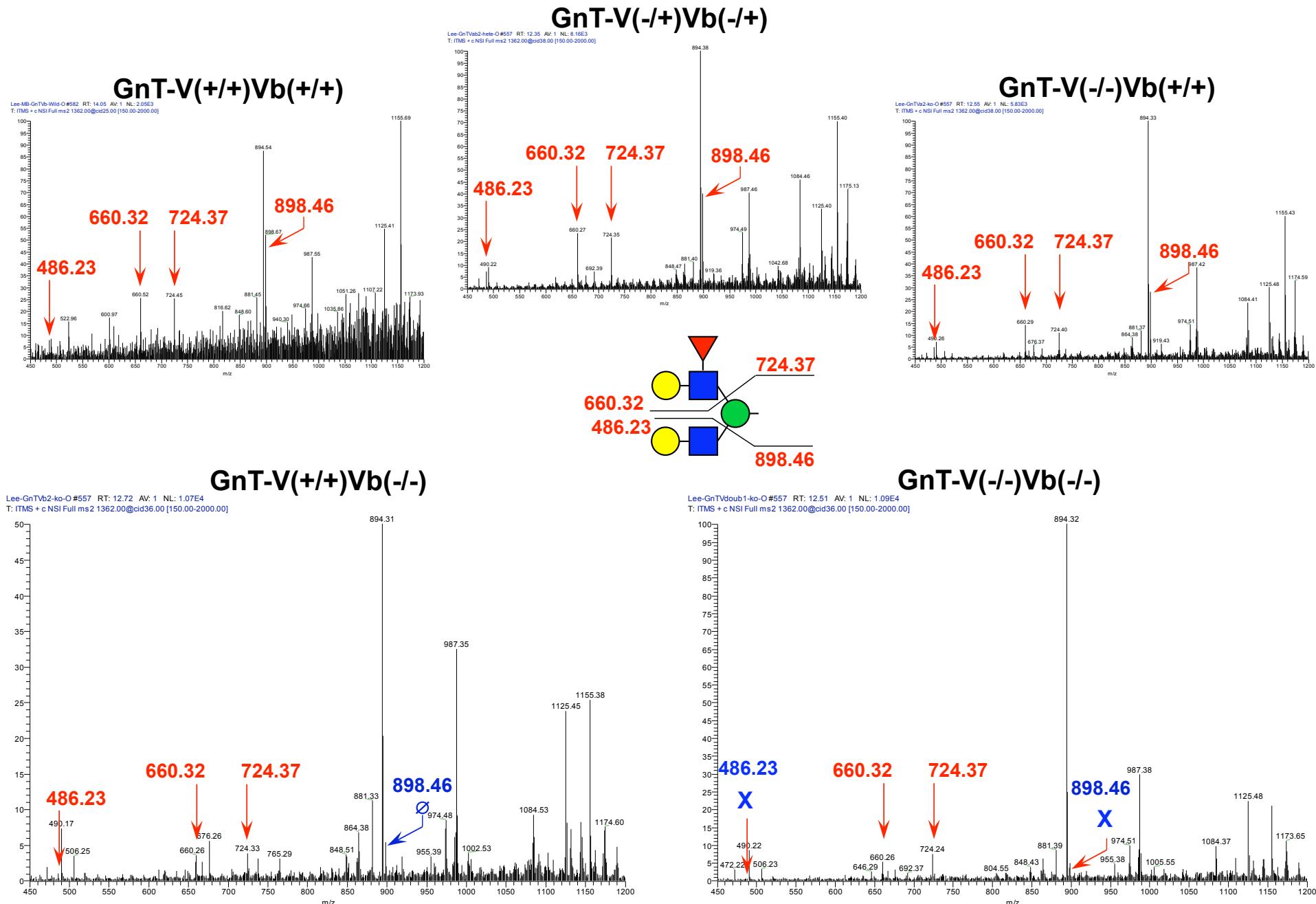
Supplemental Figure 2.

E. $[M+Na]^+$: 1344.689 m/z (mono)



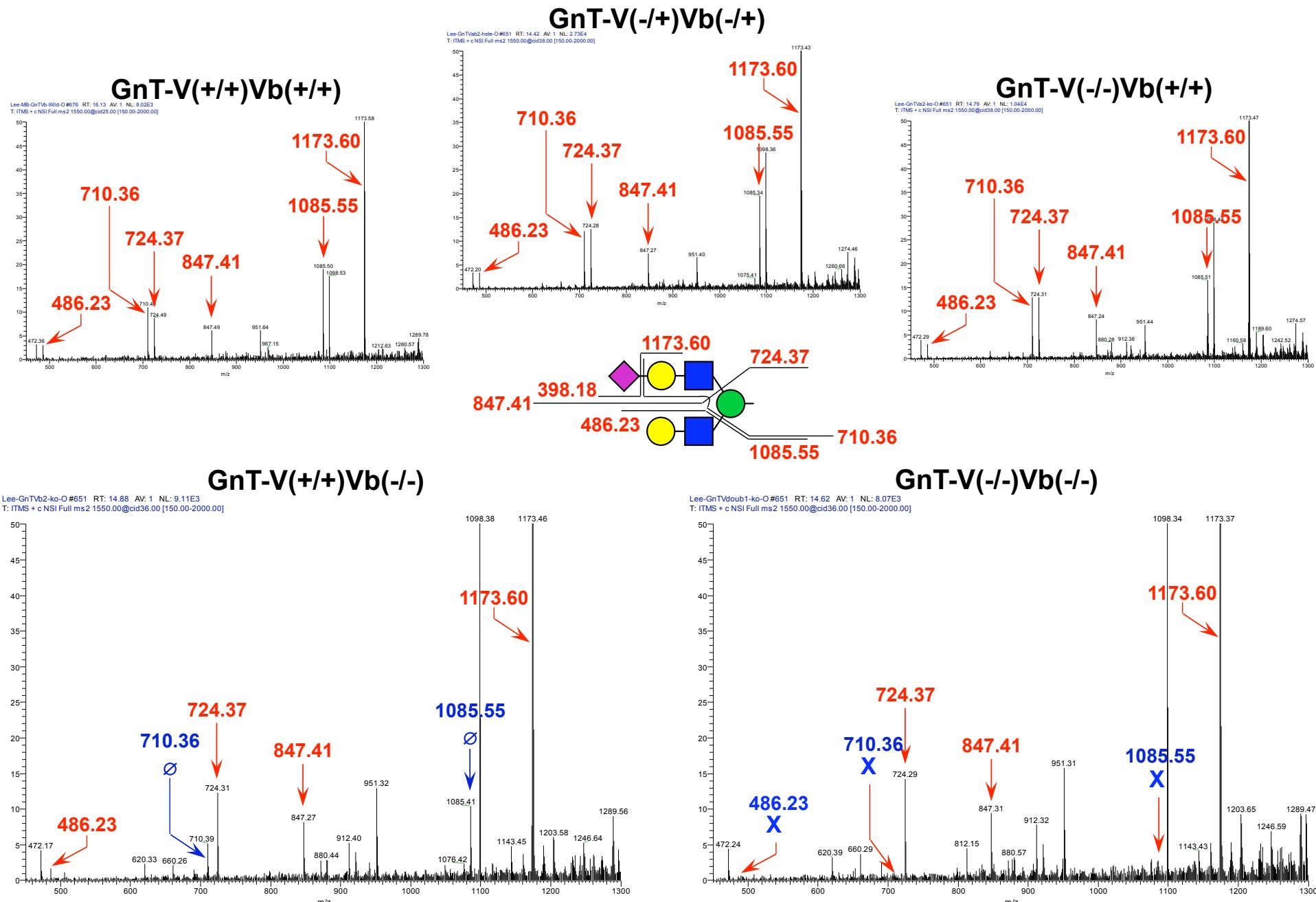
Supplemental Figure 2.

F. $[M+Na]^+$: 1361.704 m/z (mono)



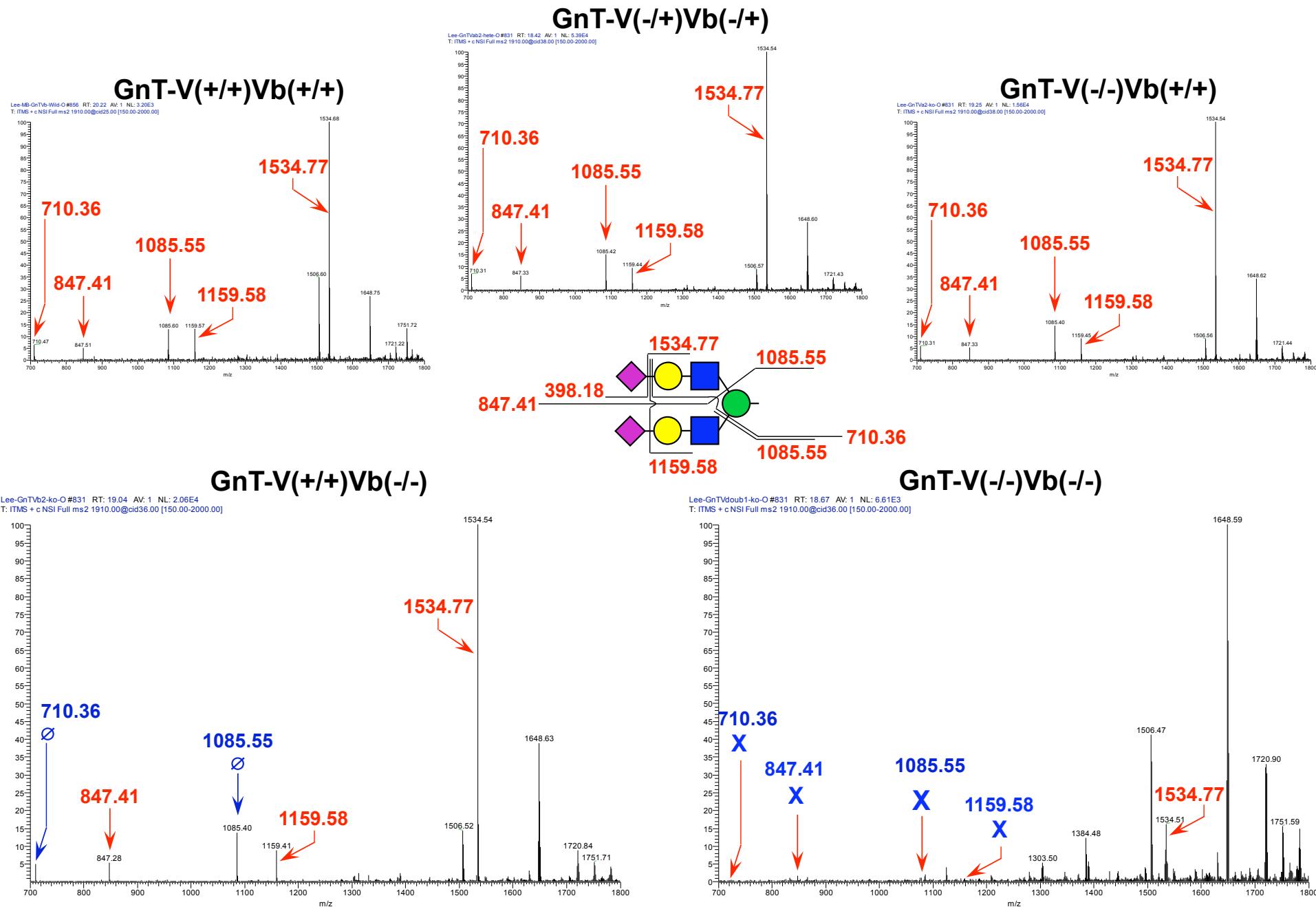
Supplemental Figure 2.

G. $[M+Na]^+$: 1548.789 m/z (mono)



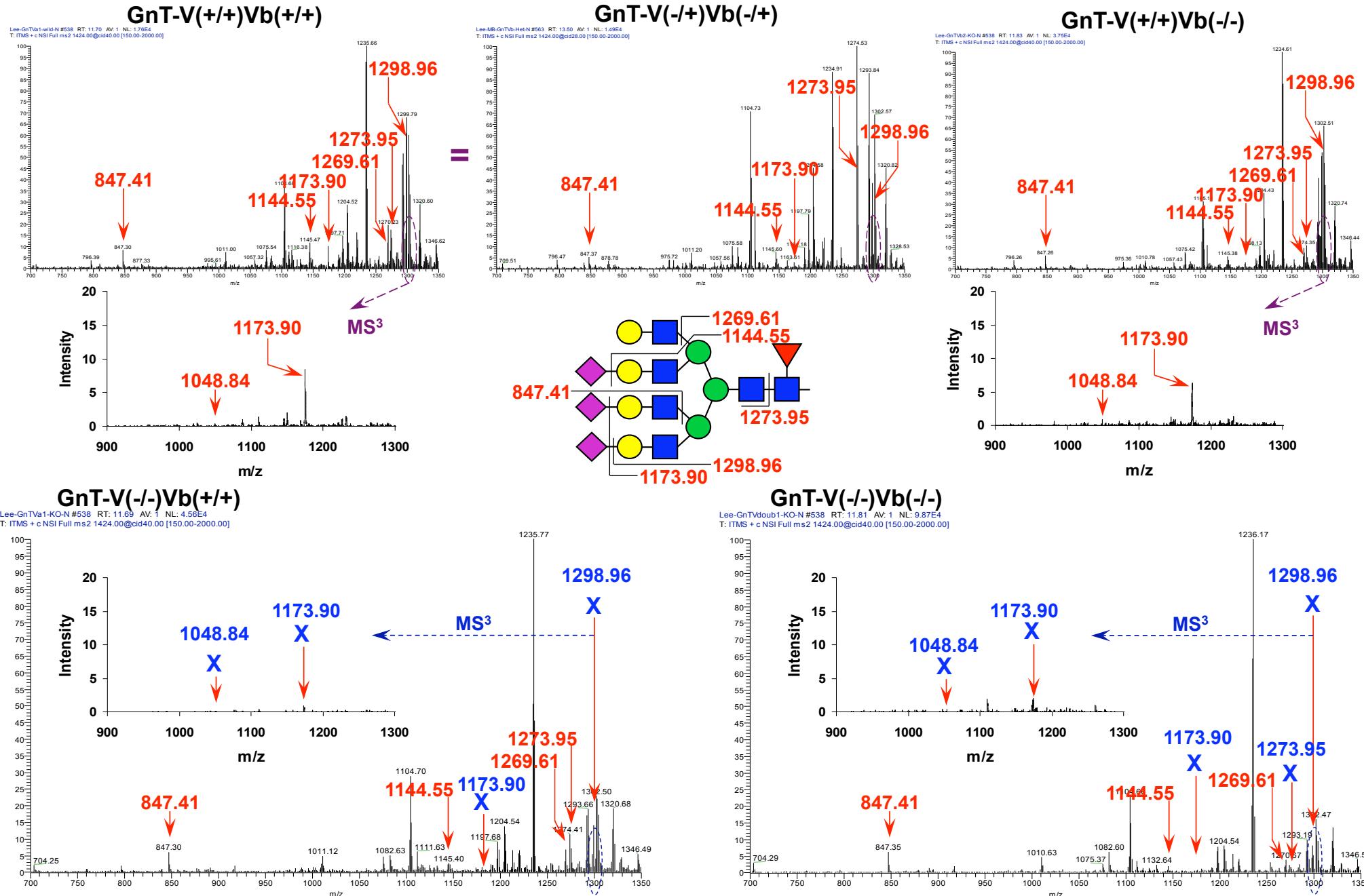
Supplemental Figure 2.

H. $[M+Na]^+$: 1909.962 m/z (mono)



Supplemental Figure 3.

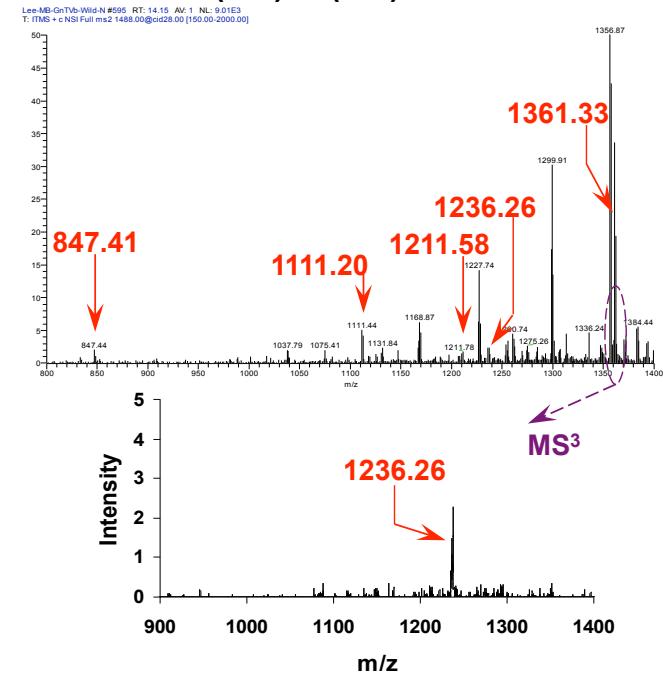
A. $[M+3Na]^{3+}$: 1424.026 m/z (mono)



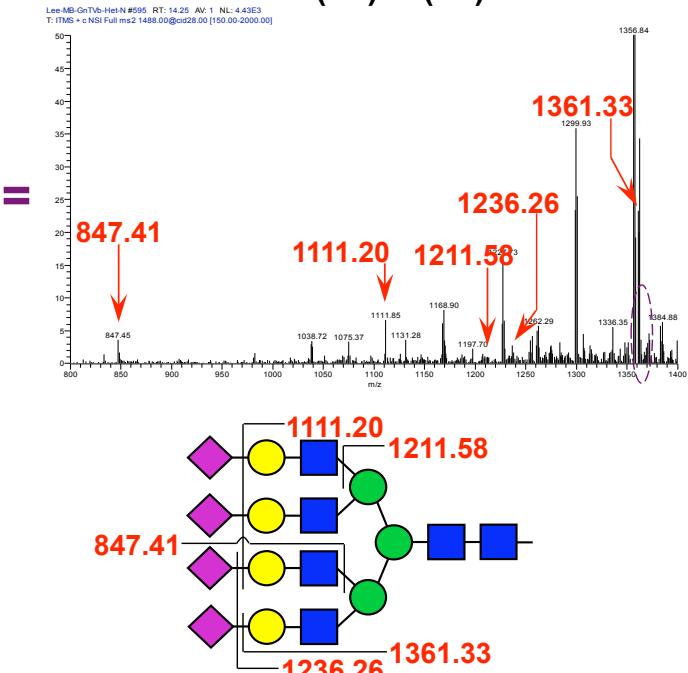
Supplemental Figure 3.

B. $[M+3Na]^{3+}$: 1486.388 m/z (mono)

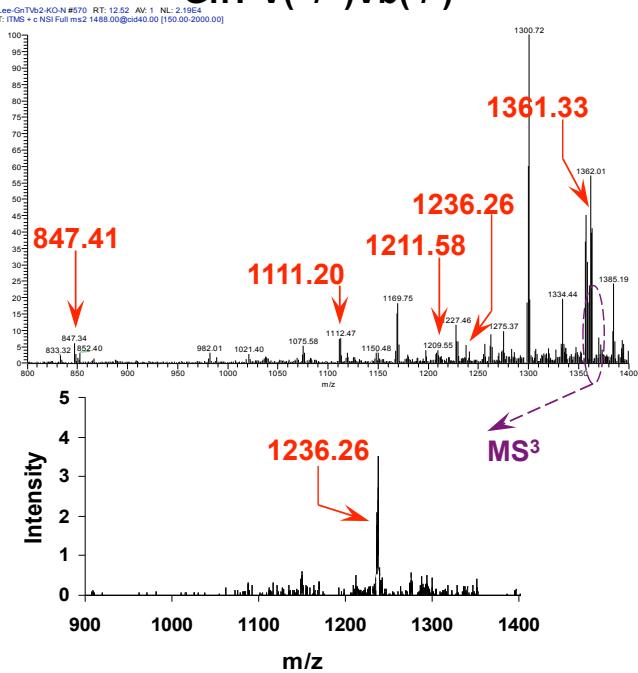
GnT-V(+/+)Vb(++)



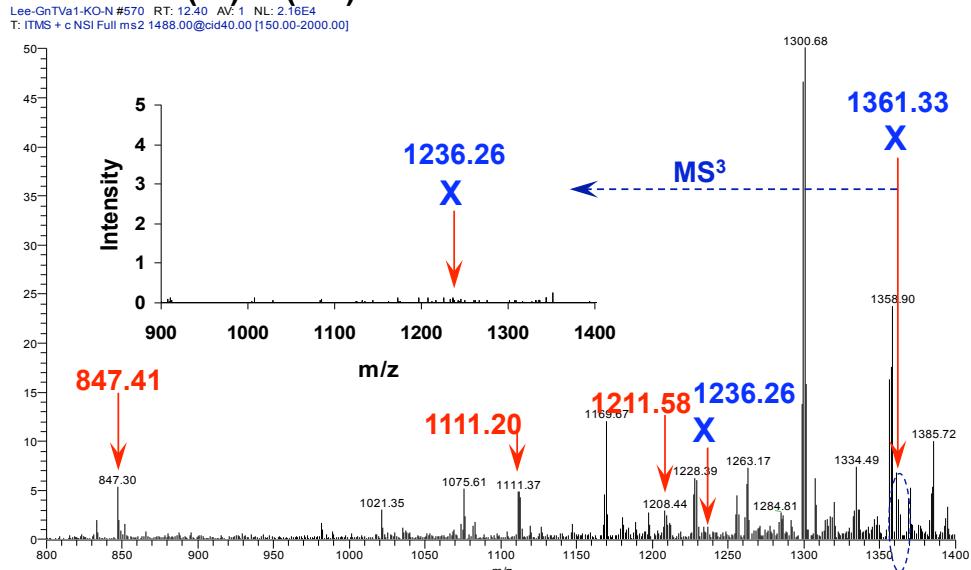
GnT-V(-/+)Vb(-+)



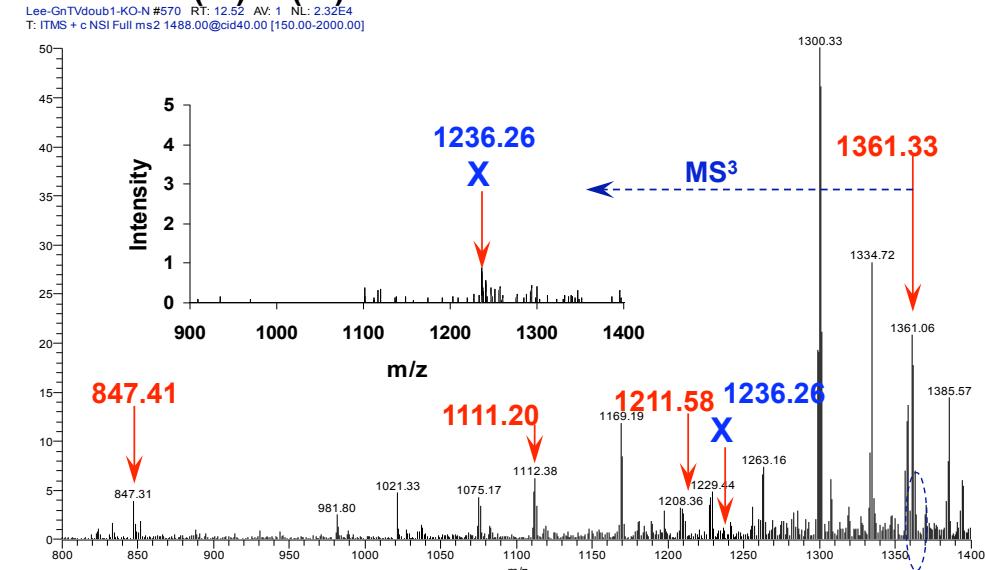
GnT-V(+/-)Vb(-/-)



GnT-V(-/-)Vb(++)



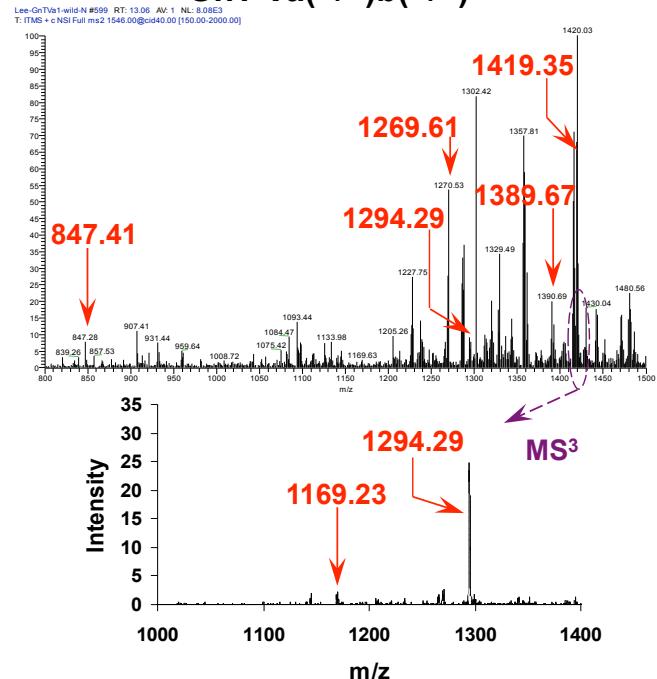
GnT-V(-/-)Vb(-/-)



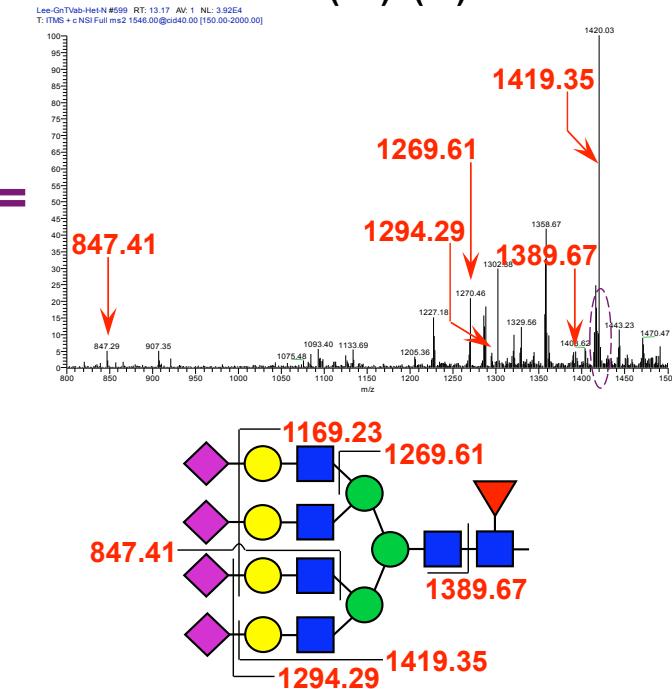
Supplemental Figure 3.

C. $[M+3Na]^{3+}$: 1544.417 m/z (mono)

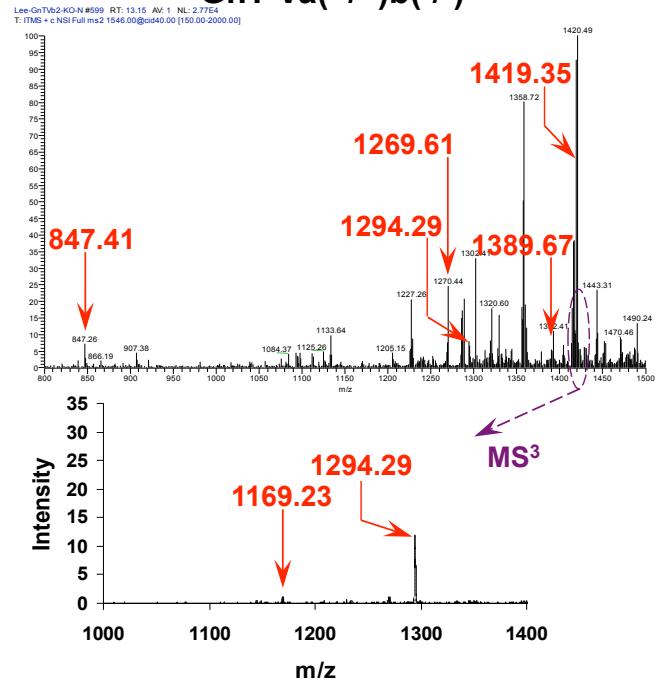
GnT-Va(+/+)b(+/+)



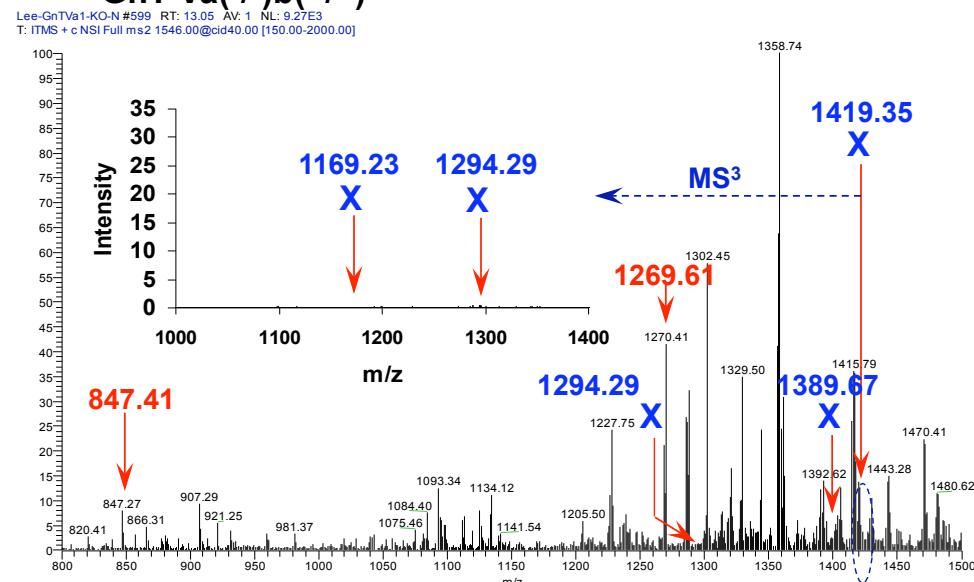
GnT-Va(-+)/b(-/-)



GnT-Va(+/-)b(-/-)



GnT-Va(-/-)b(+/+)



GnT-Va(-/-)b(-/-)

