

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'-GGAATCCAGTACAGGGAACCTTGCTTGG-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^{fl} 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, $K^{+/\Delta}$, 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^{dn/dn} (GnT-Vb(-/-)). GnT-Vb^{flxed/+} mice were crossed to transgenic mice of ubiquitously expressing Cre recombinase (C57BL/6 Cre mouse). Genotyping of GnT-Vb^{dn/dn} (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^{dn/dn} 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 \emptyset , <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

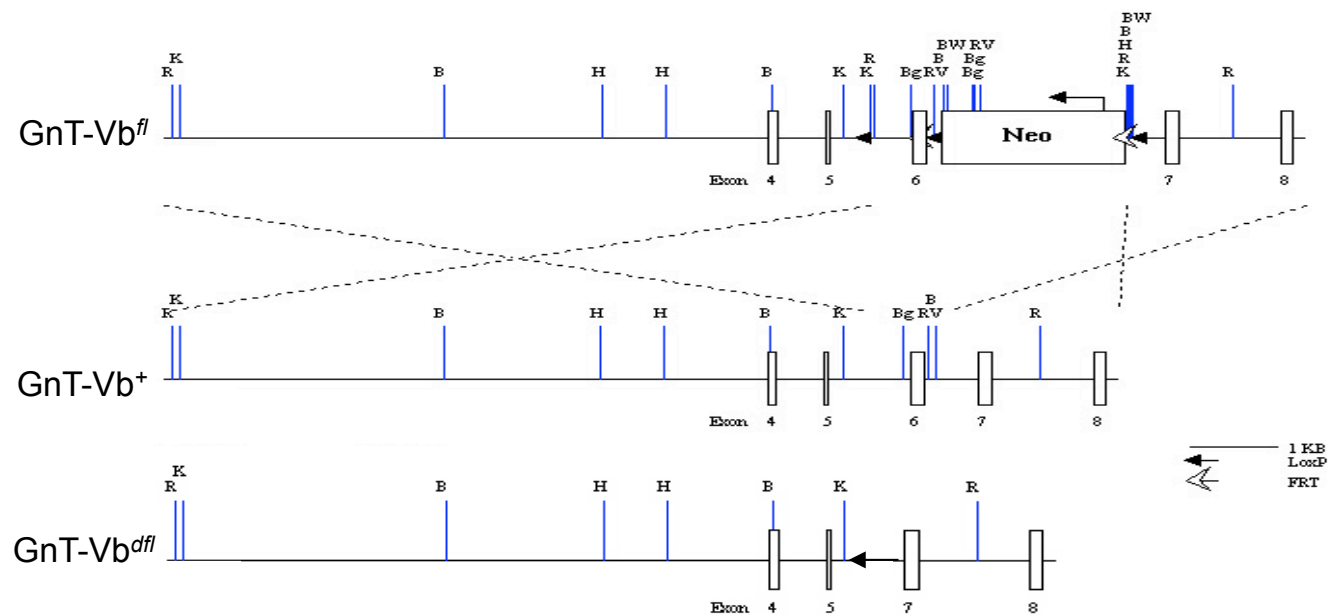
Supplemental Table 1. O-linked glycans observed in mouse brains from all genotypes by tandem mass spectrometric analysis.

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						

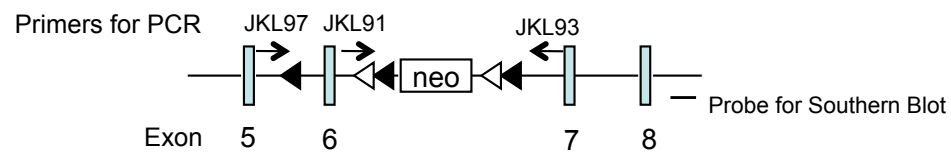
The detected glycans by TIM MS/MS are shown in Red.

Supplemental Figure 1.

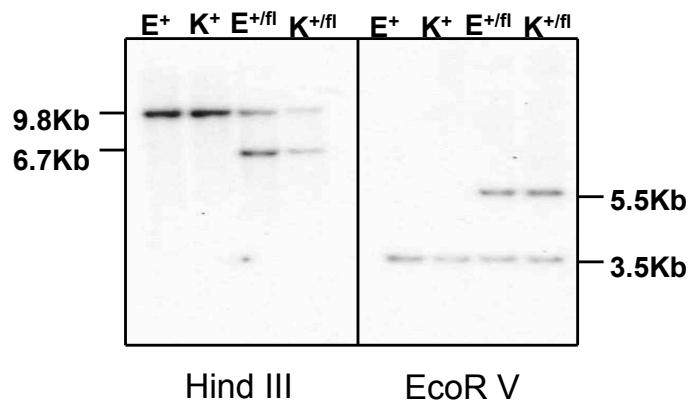
(A)



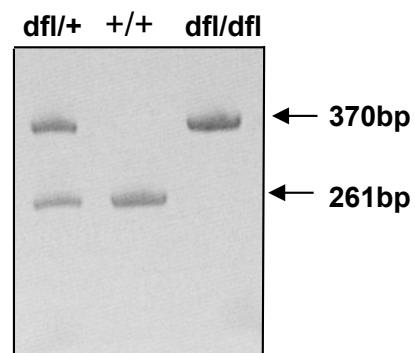
(B)



(C)



(D)



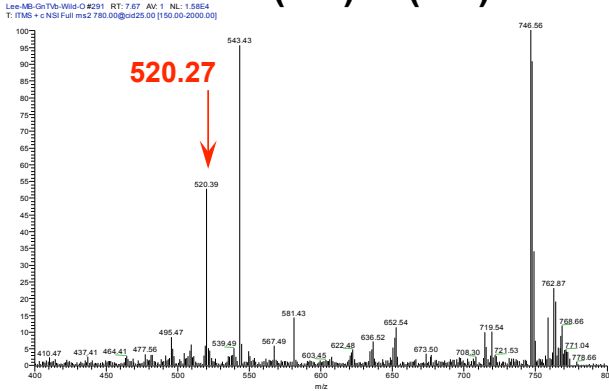
Supplemental Figure 2.

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

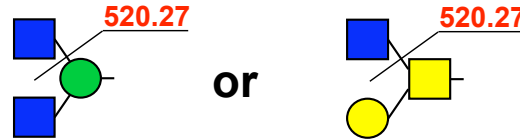
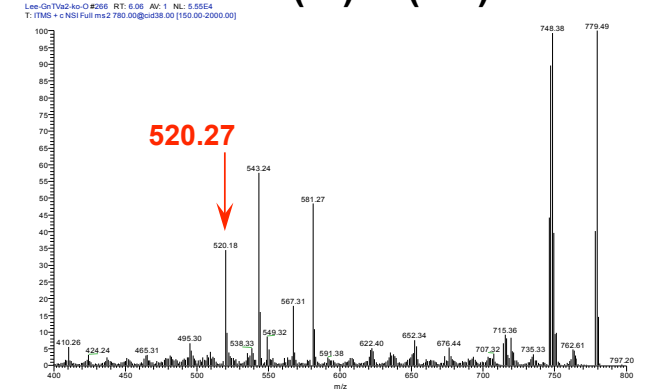
GnT-V(-/+)/Vb(-/+)



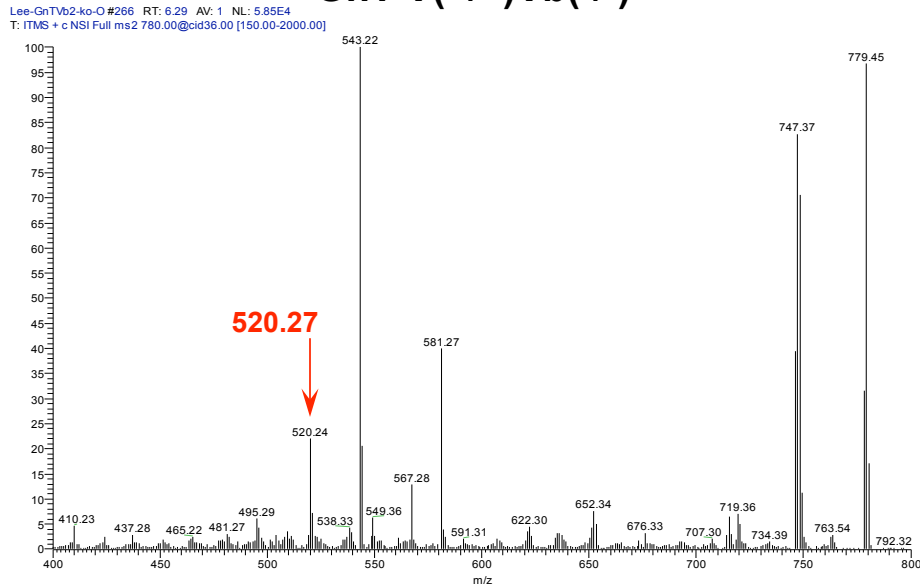
GnT-V(++)/Vb(++)



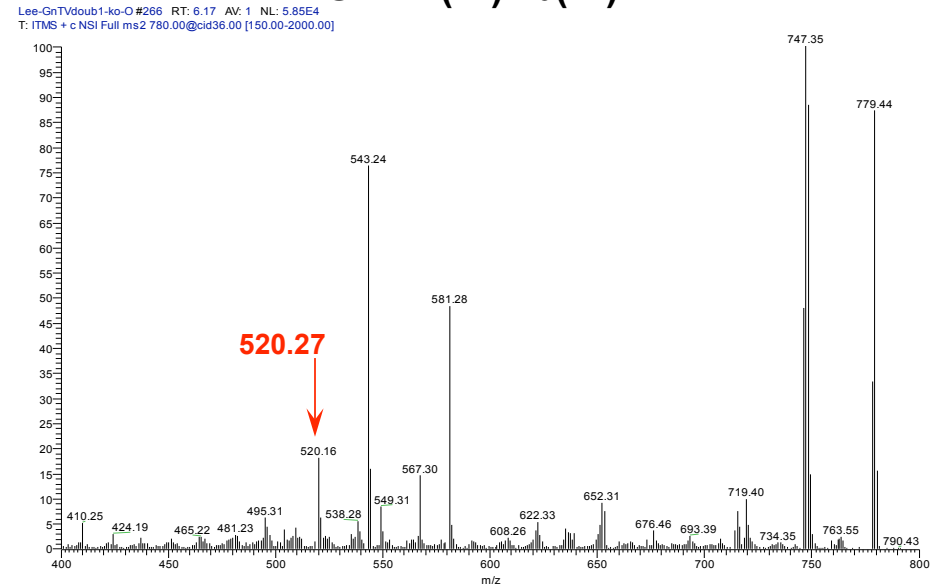
GnT-V(-/-)/Vb(++)



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



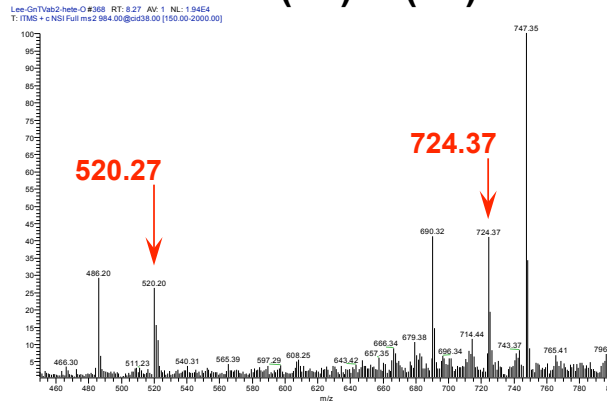
GnT-V(-/-)/Vb(-/-)



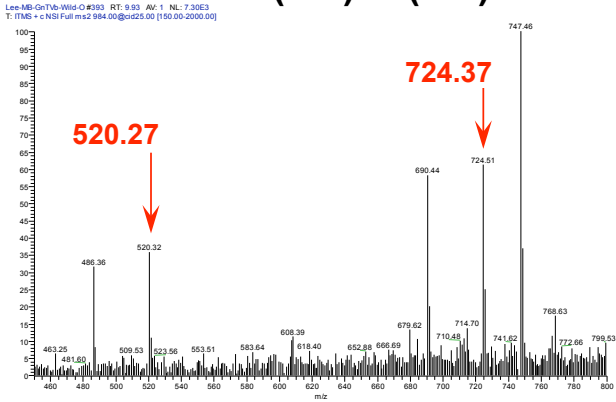
Supplemental Figure 2.

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

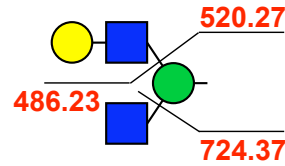
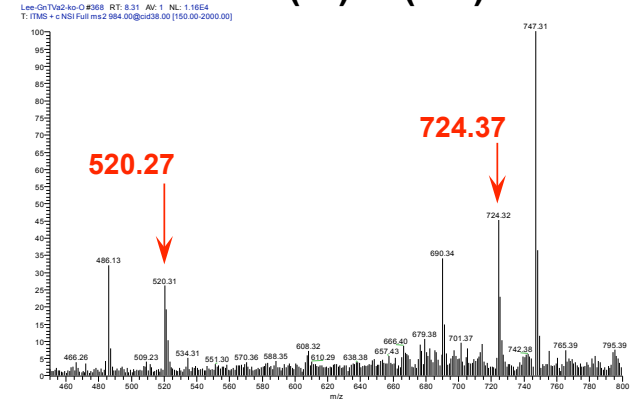
GnT-V(-/+)Vb(-/+)



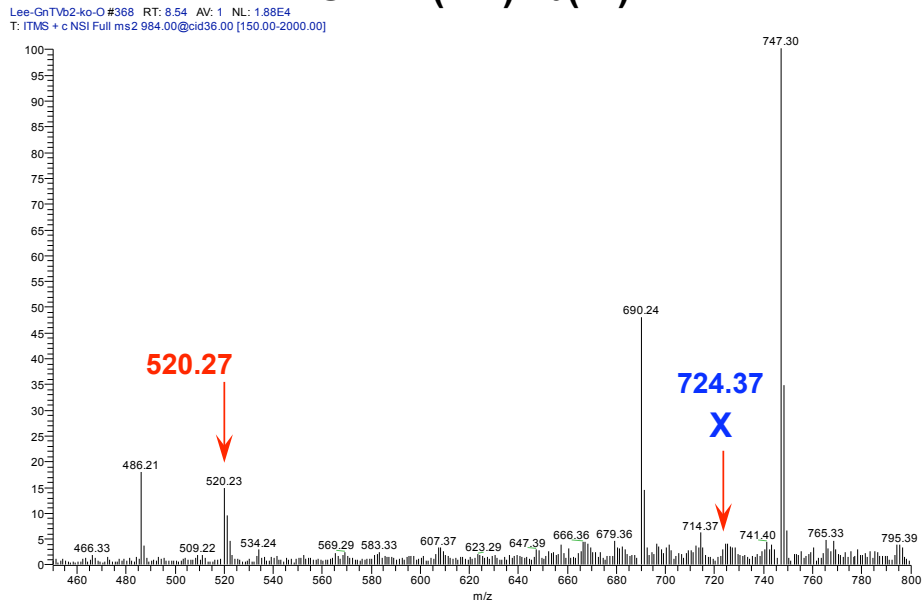
GnT-V(+/+)Vb(+/+)



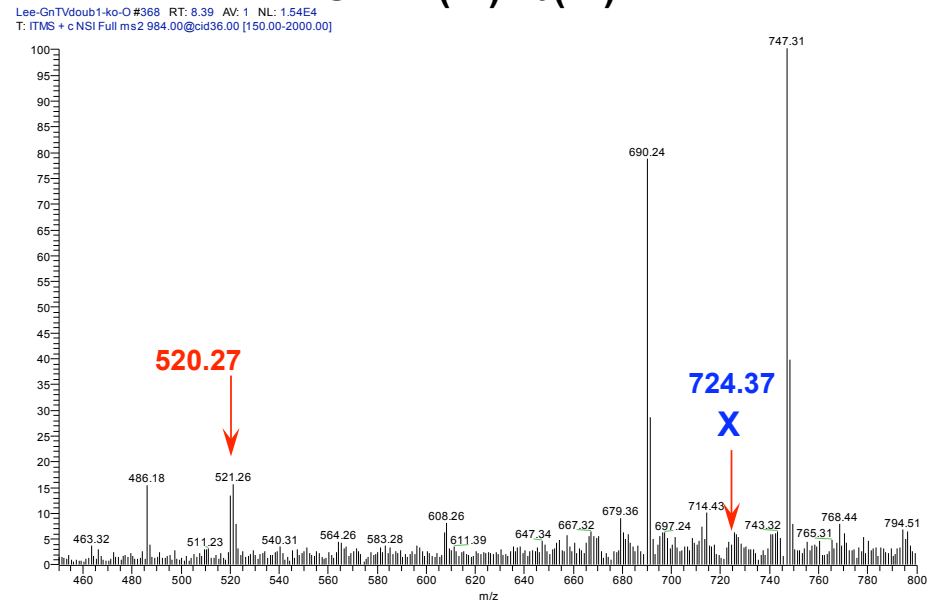
GnT-V(-/-)Vb(+/+)



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

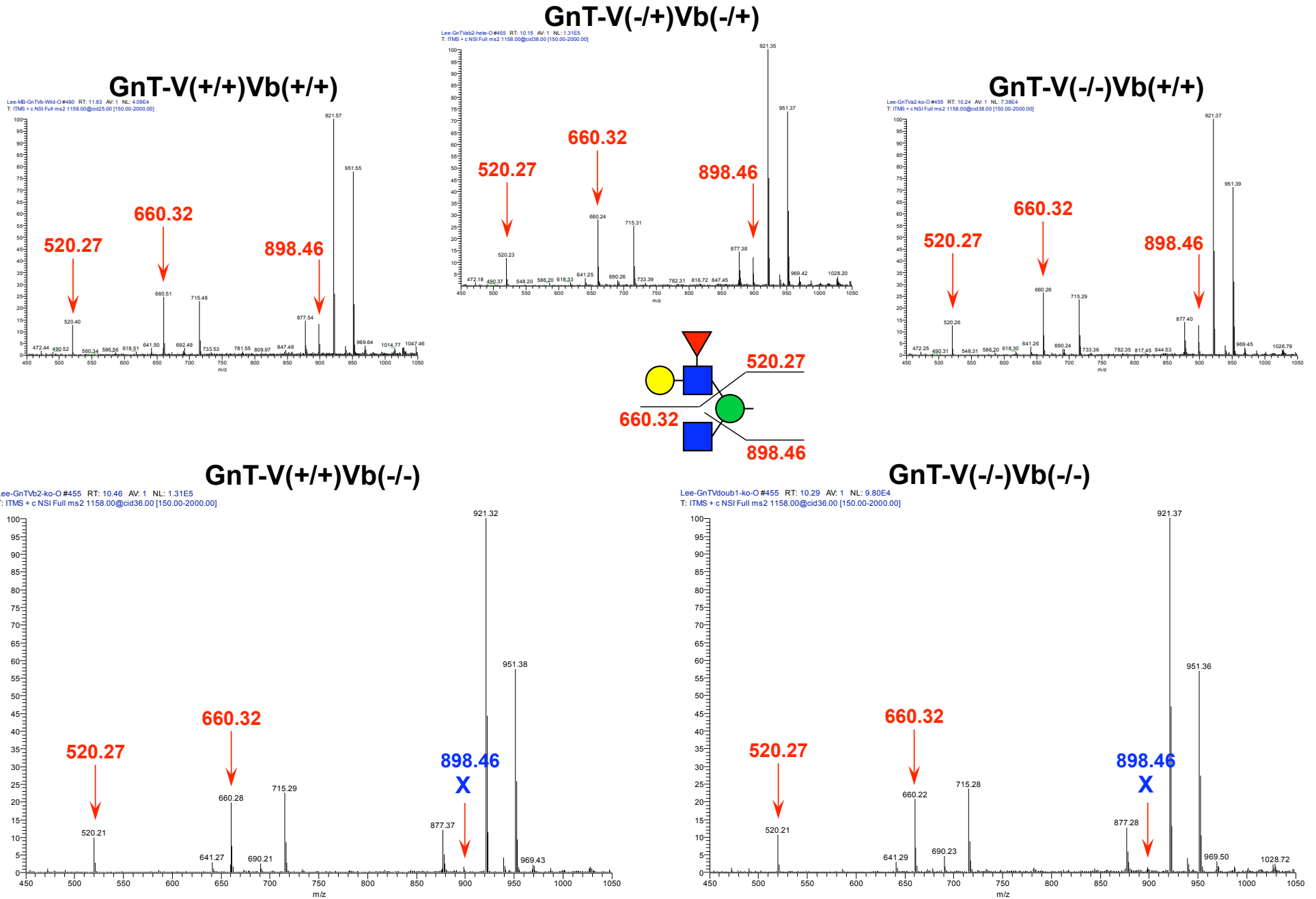


GnT-V(-/-)Vb(-/-)



Supplemental Figure 2.

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

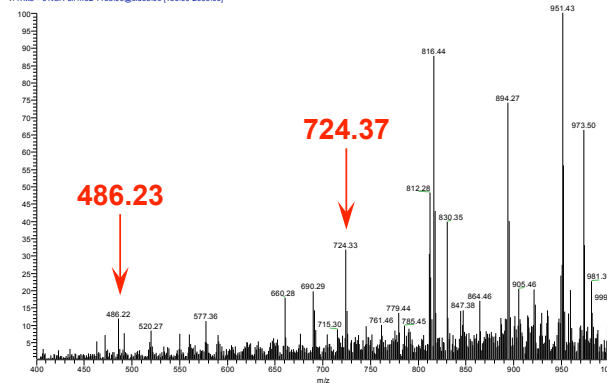


Supplemental Figure 2.

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

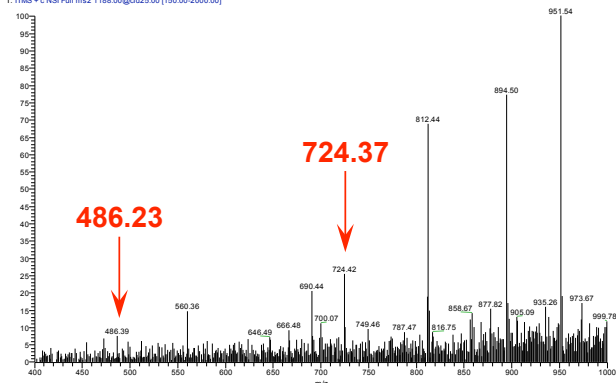
GnT-V(-/+)Vb(-/+)

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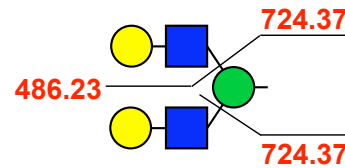
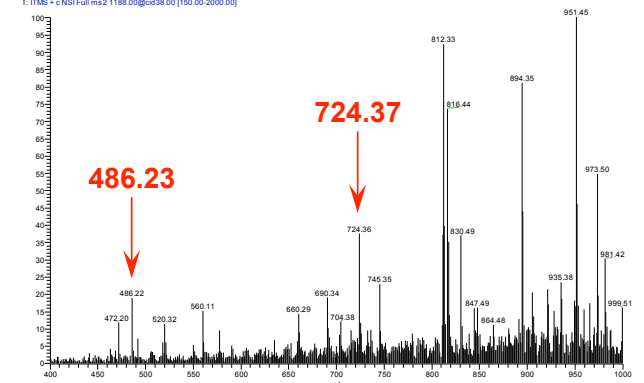
GnT-V(+/+)Vb(+/+)

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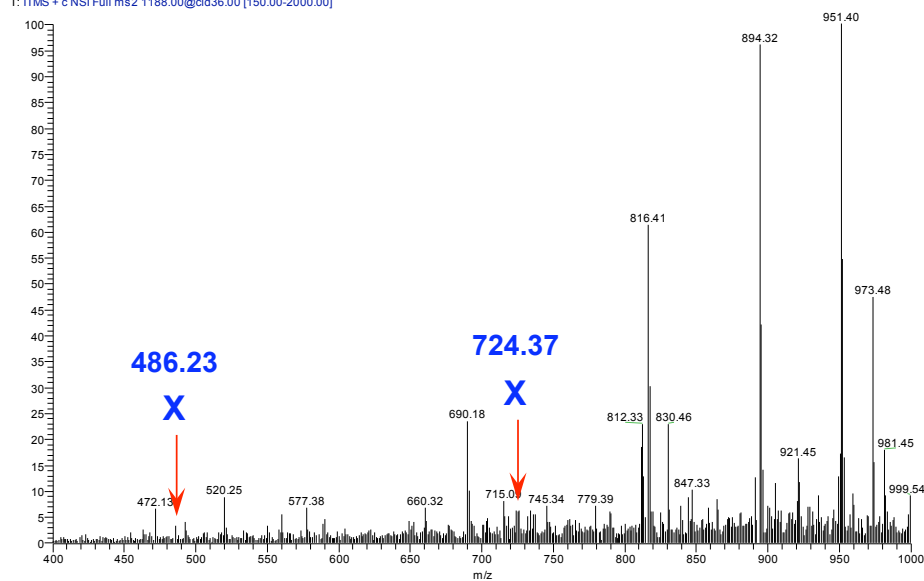
GnT-V(-/-)Vb(+/+)

Lee-GnTVb2-ko-O#470 RT: 10.58 Av: 1 NL: 4.19E3
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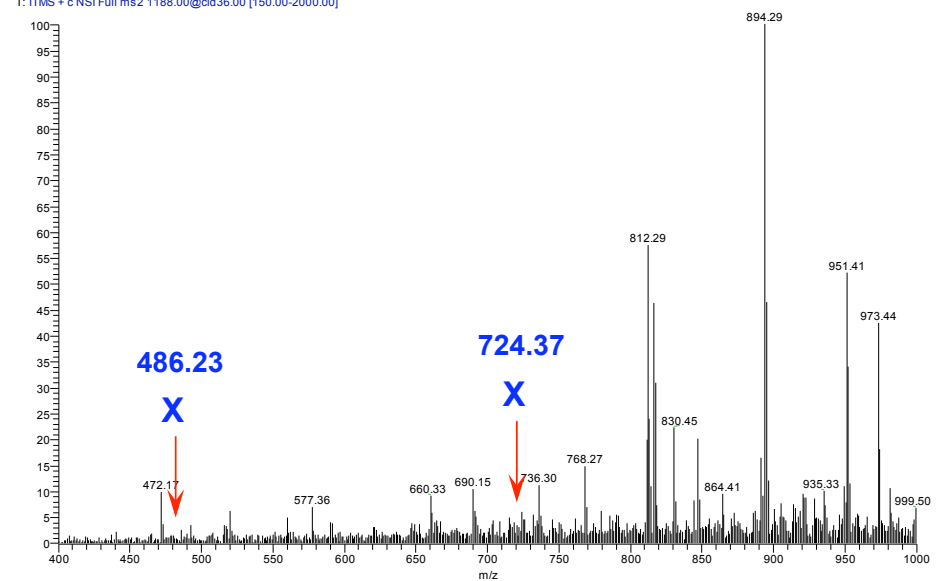
GnT-V(+/+)Vb(-/-)

Lee-GnTVb2-ko-O#470 RT: 10.79 Av: 1 NL: 8.06E3
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GnT-V(-/-)Vb(-/-)

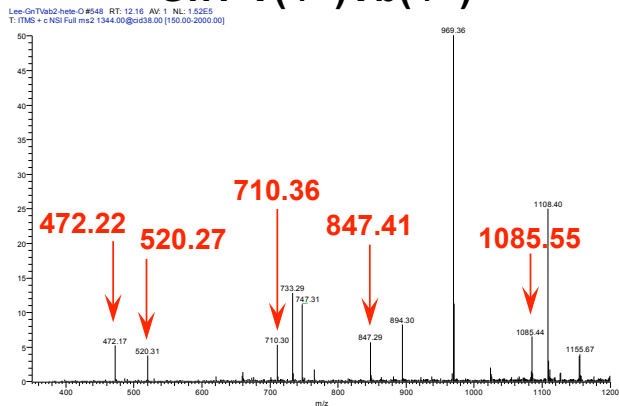
Lee-GnTVtoub1-ko-O#470 RT: 10.62 Av: 1 NL: 9.53E3
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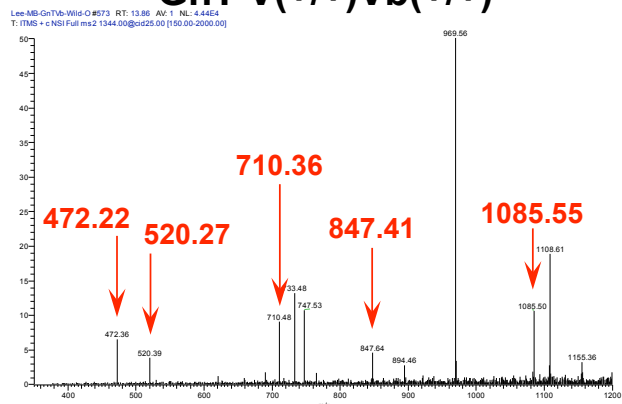
Supplemental Figure 2.

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

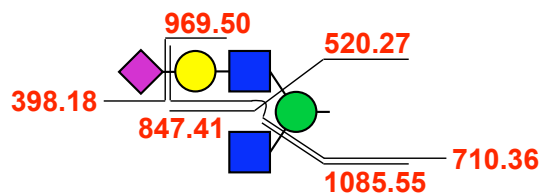
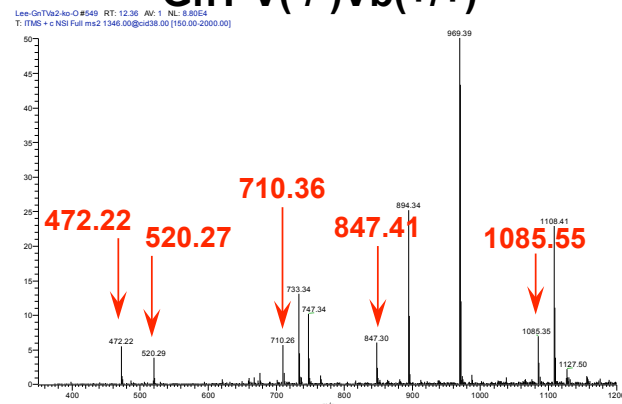
GnT-V(-/+)Vb(-/+)



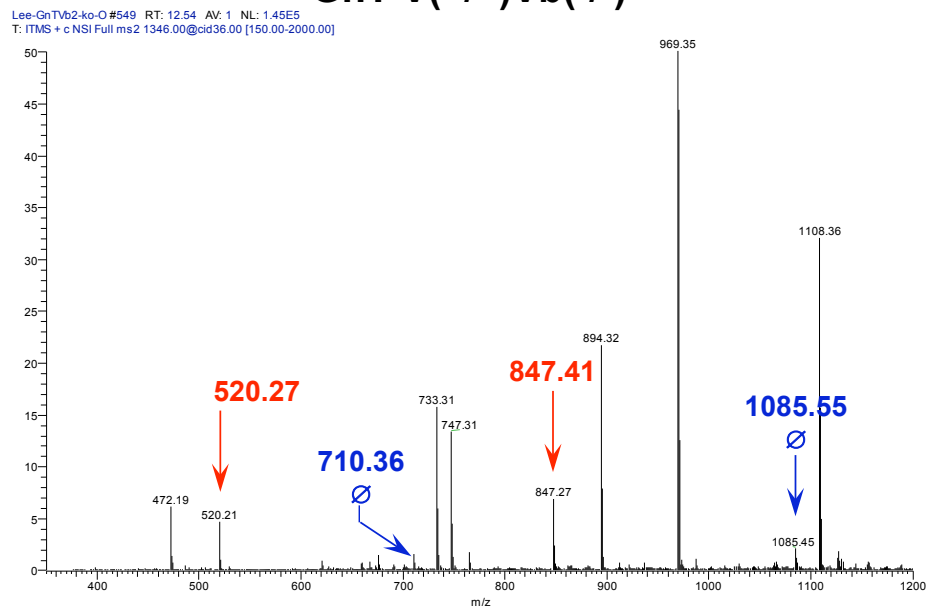
GnT-V(+/+)Vb(+/+)



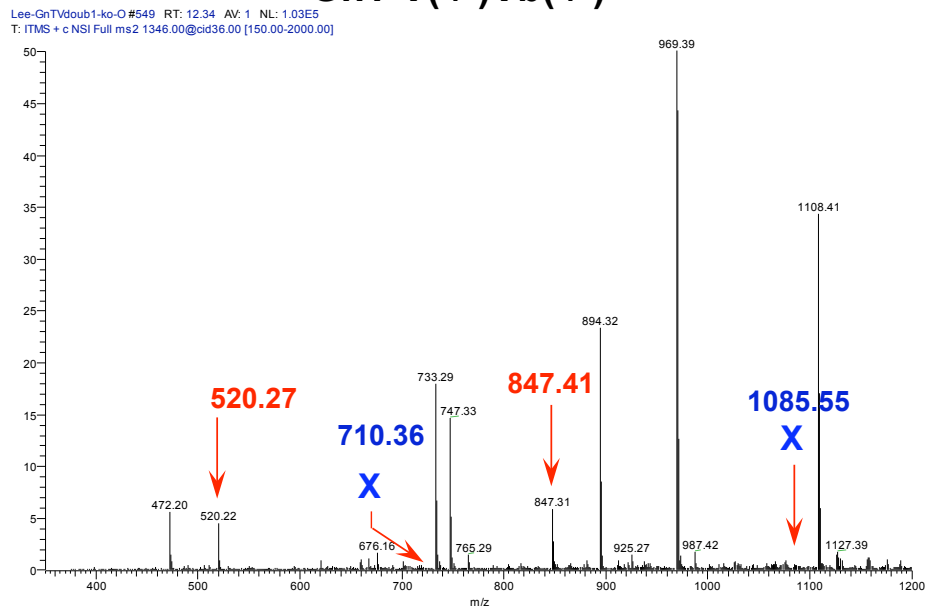
GnT-V(-/-)Vb(+/+)



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



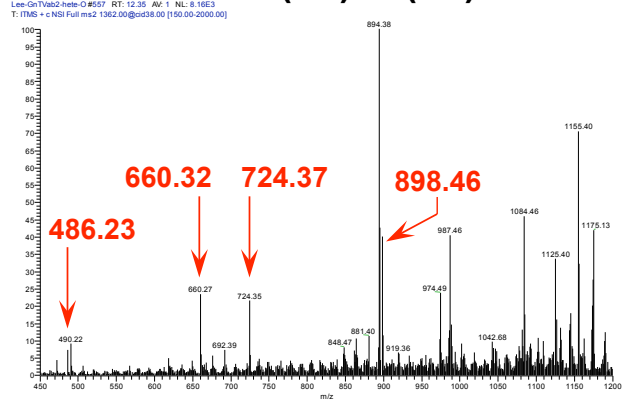
GnT-V(-/-)Vb(-/-)



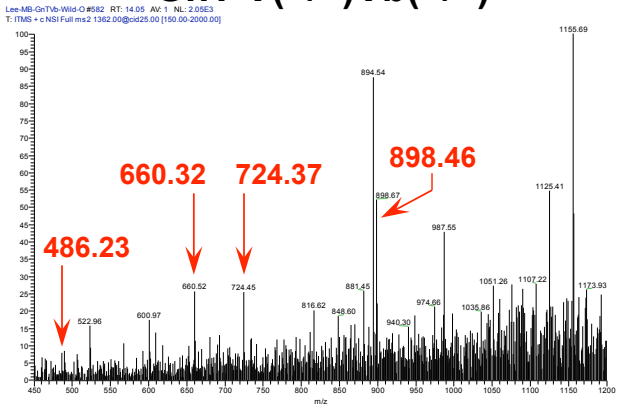
Supplemental Figure 2.

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

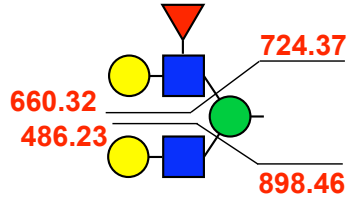
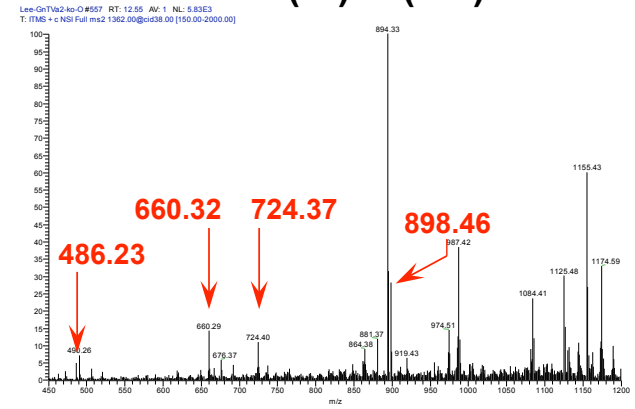
GnT-V(-/+)Vb(-/+)



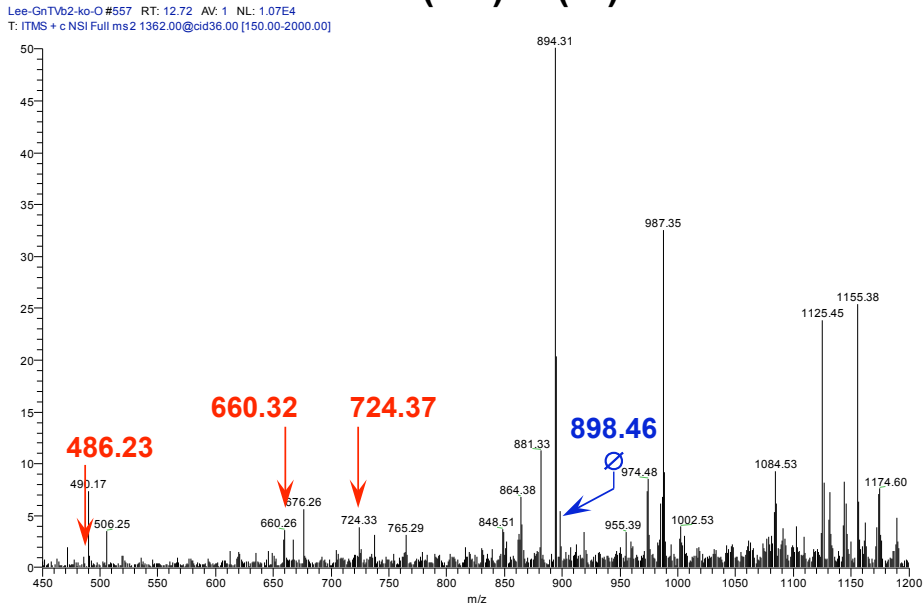
GnT-V(+/+)Vb(+/+)



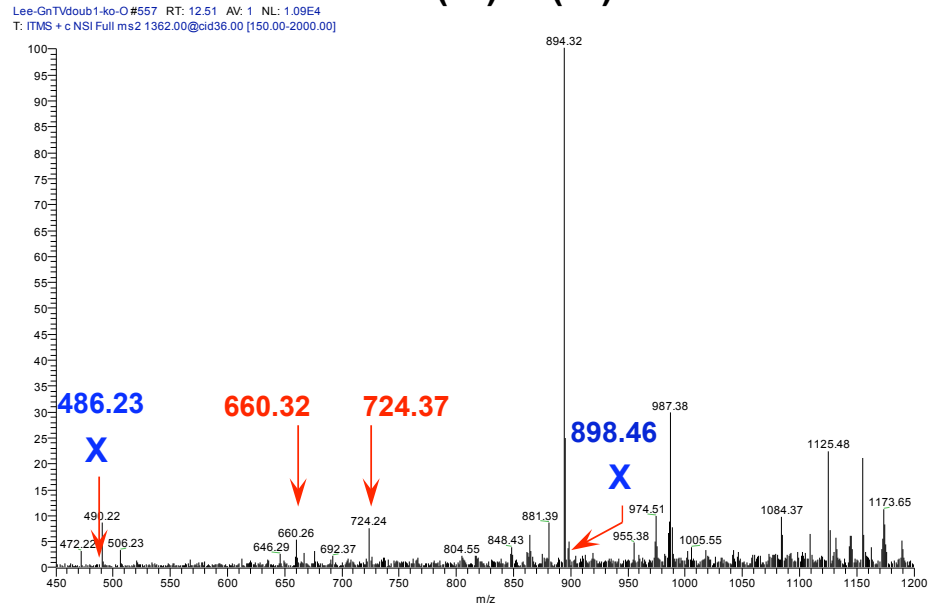
GnT-V(-/-)Vb(+/+)



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

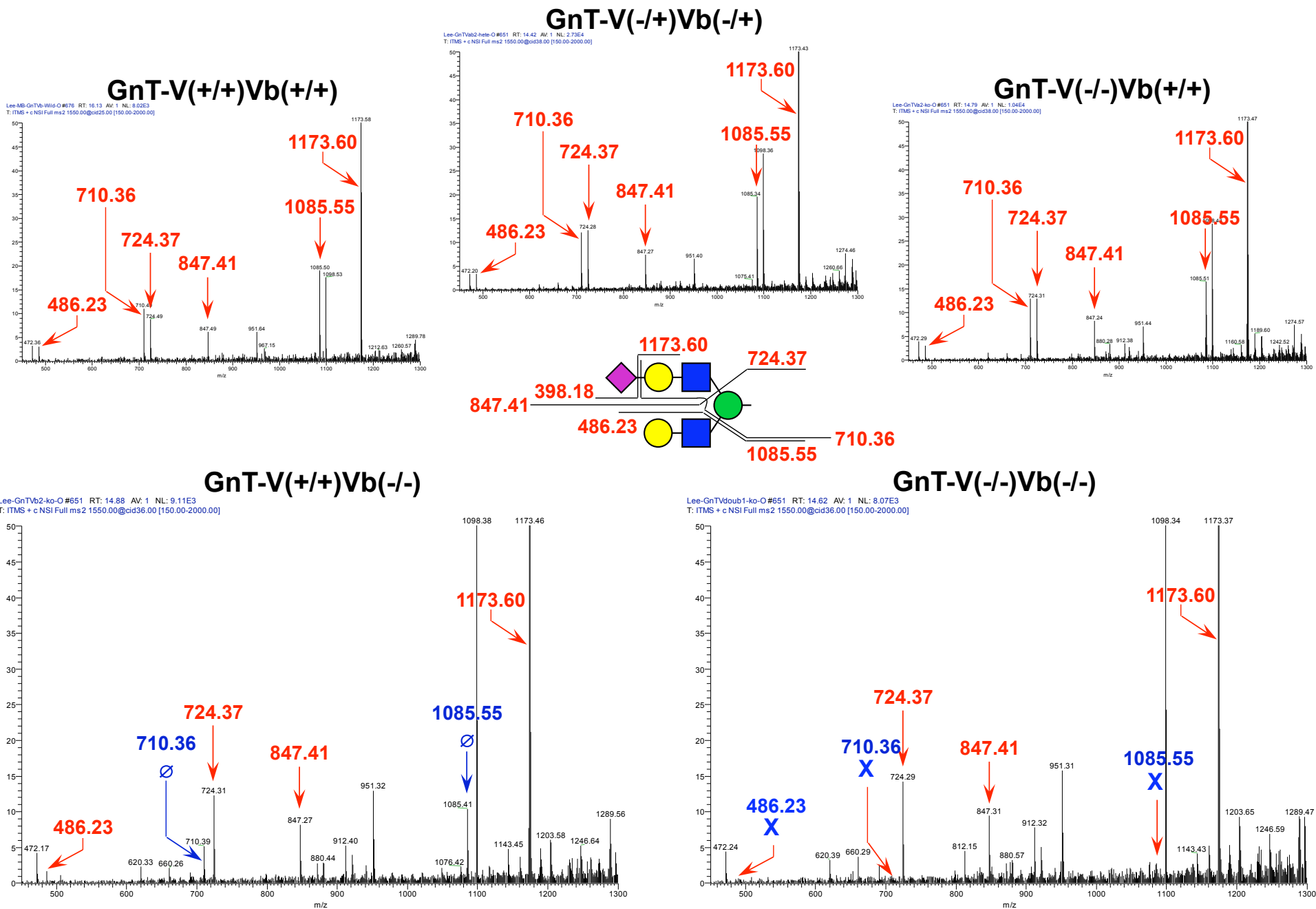


GnT-V(-/-)Vb(-/-)



Supplemental Figure 2.

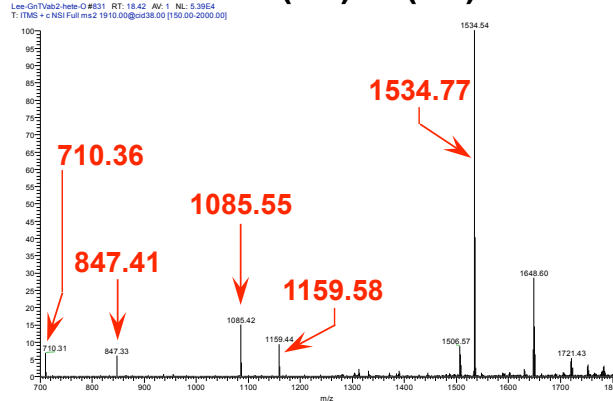
G. $[M+Na]^+$: 1548.789 m/z (mono)



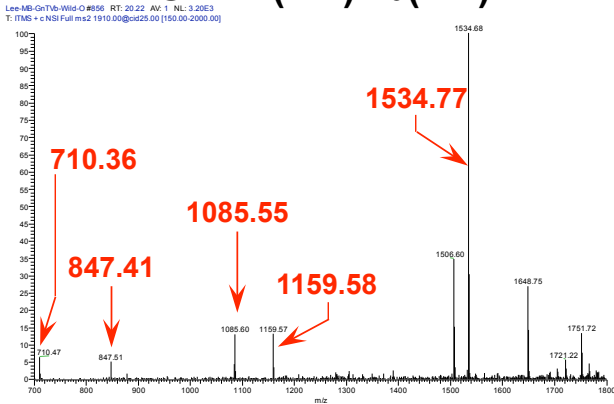
Supplemental Figure 2.

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

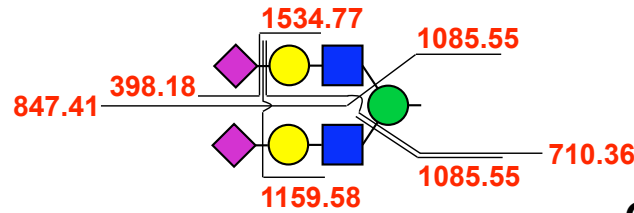
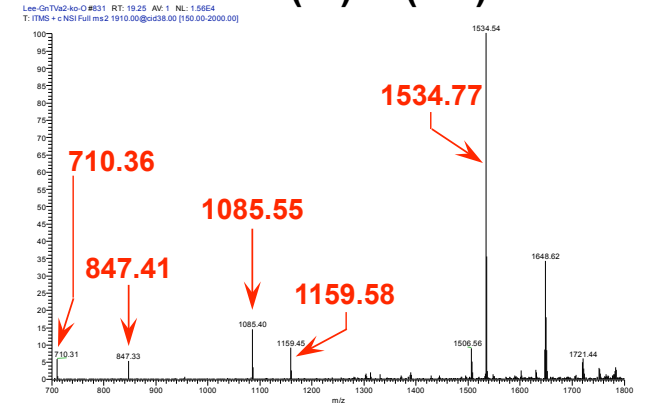
GnT-V(-/+)Vb(-/+)



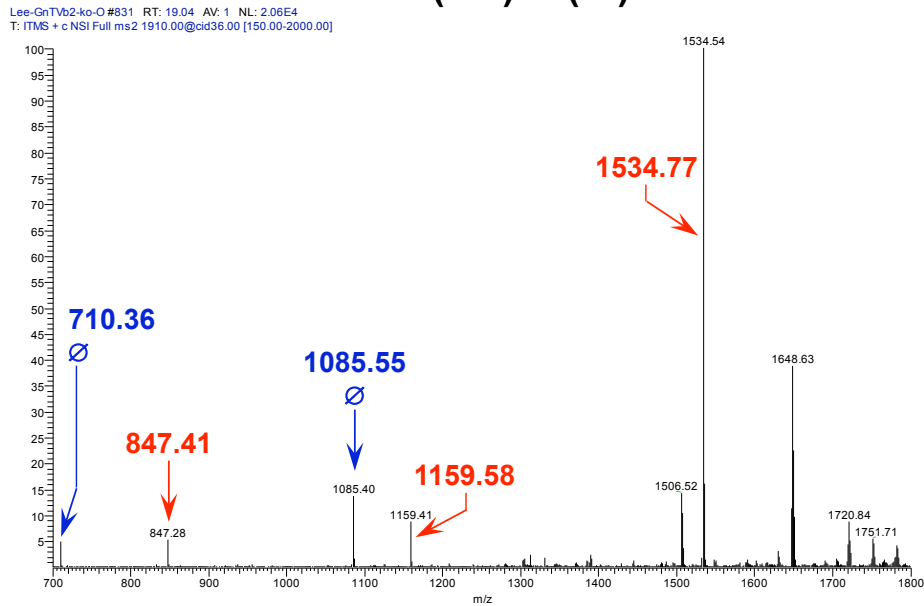
GnT-V(+/+)Vb(+/+)



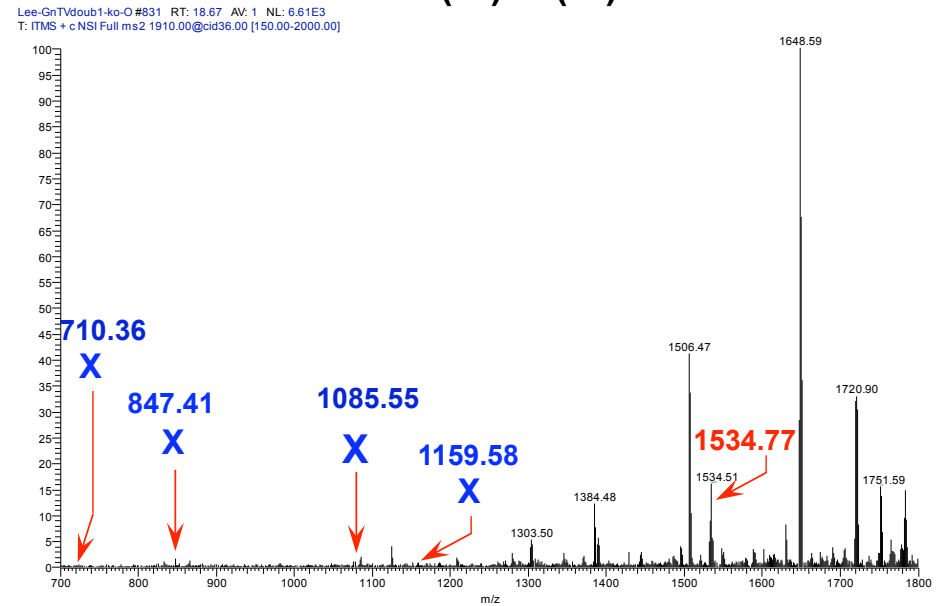
GnT-V(-/-)Vb(+/+)



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



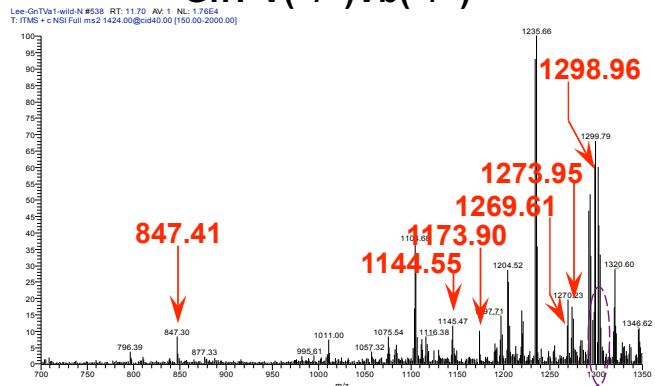
GnT-V(-/-)Vb(-/-)



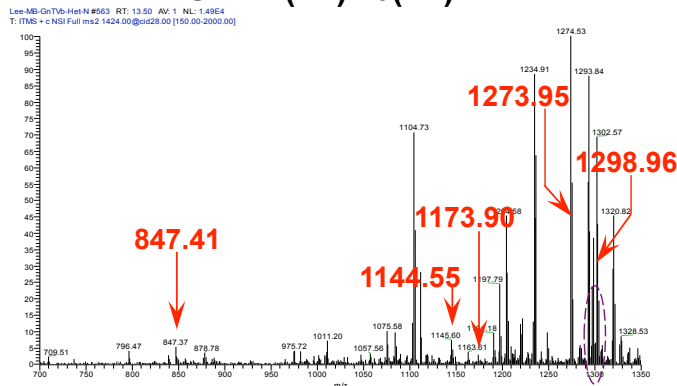
Supplemental Figure 3.

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

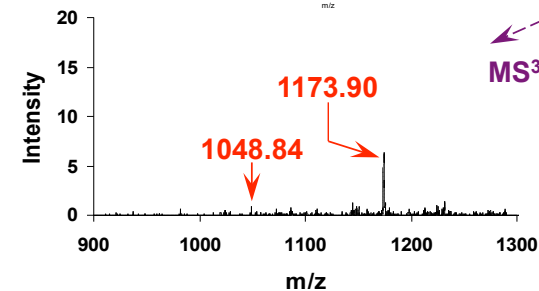
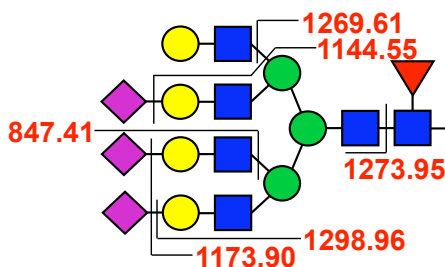
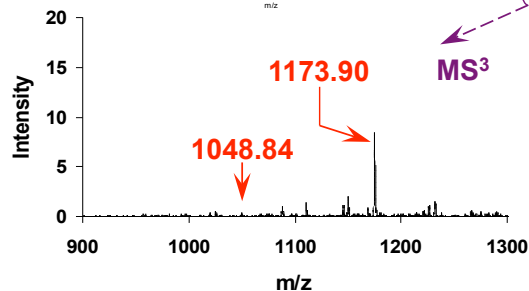
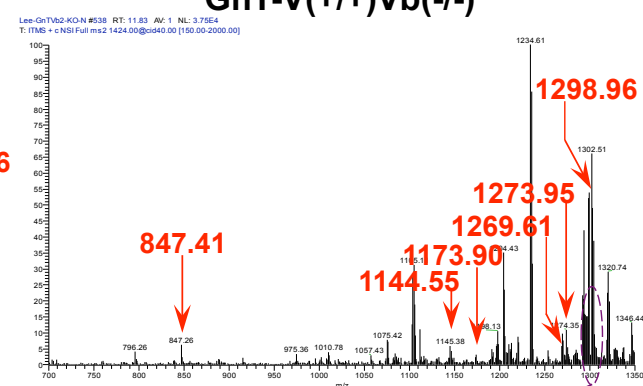
GnT-V(+/+)/Vb(+/+)



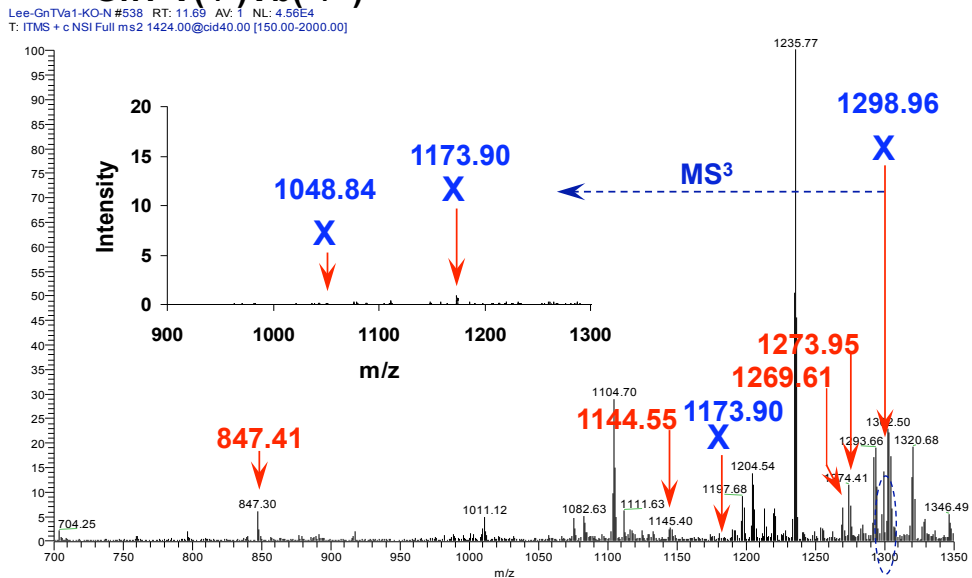
GnT-V(-/+)/Vb(-/+)



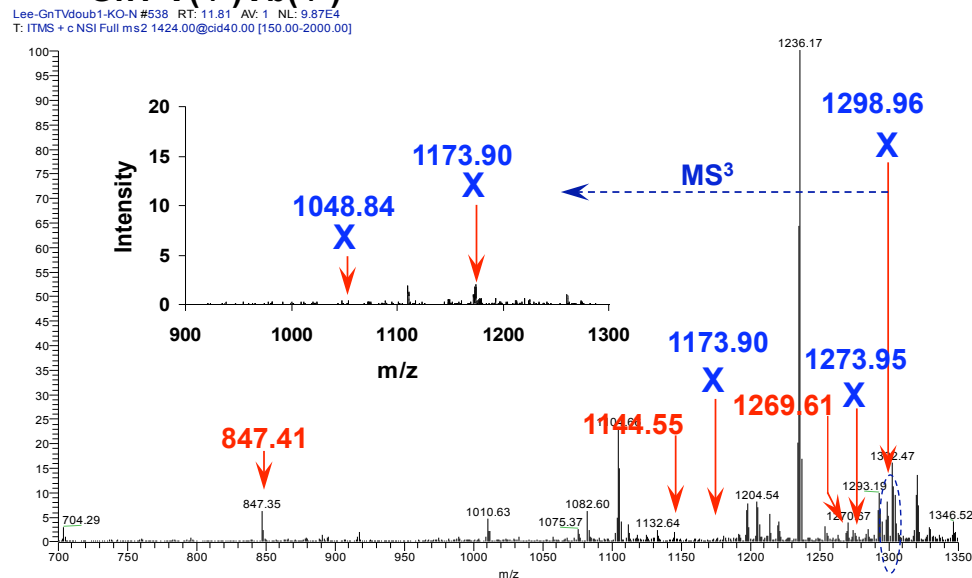
GnT-V(+/+)/Vb(-/-)



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



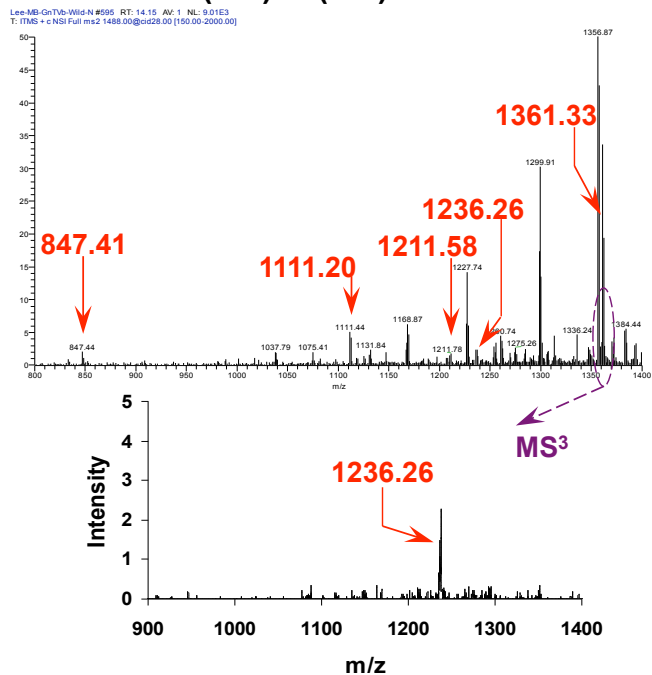
GnT-V(-/-)/Vb(-/-)



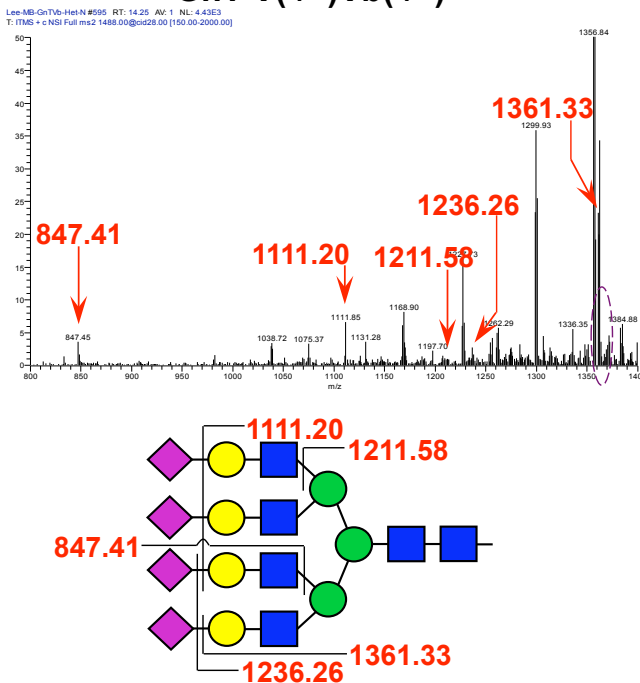
Supplemental Figure 3.

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

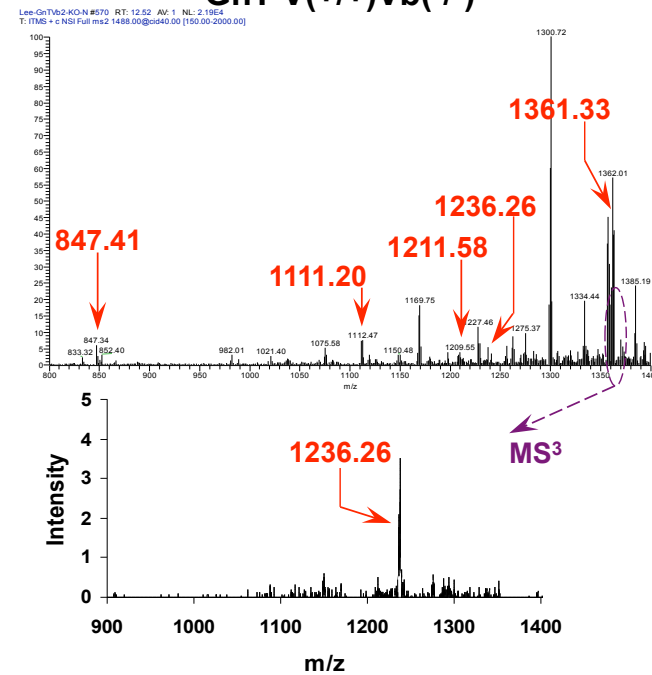
GnT-V(+/+)Vb(+/+)



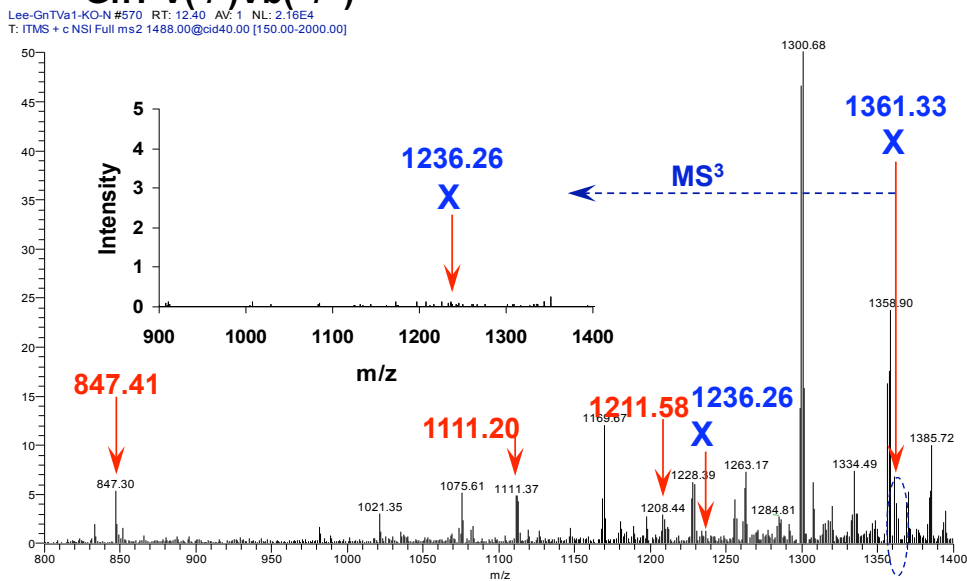
GnT-V(-/)Vb(-/)



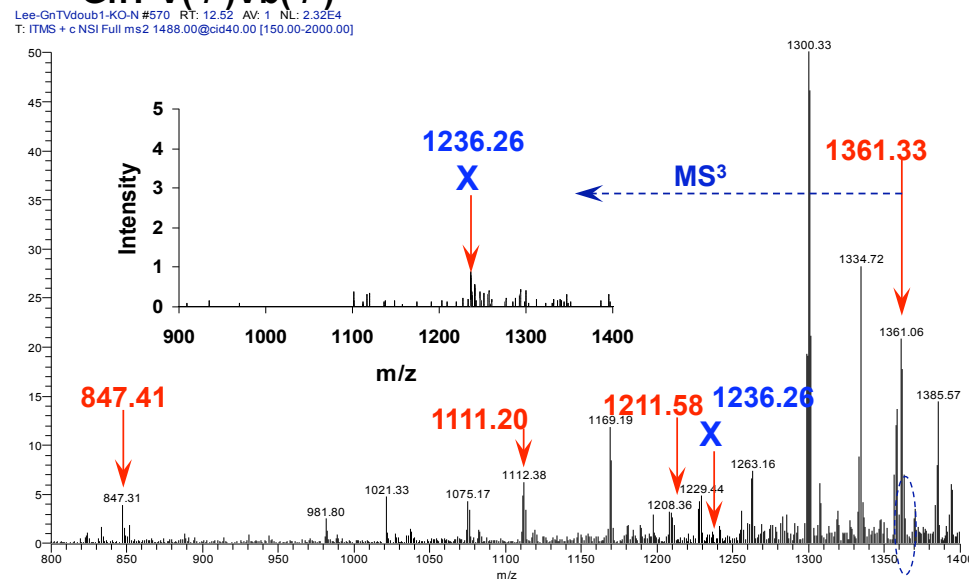
GnT-V(+/+)Vb(-/-)



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



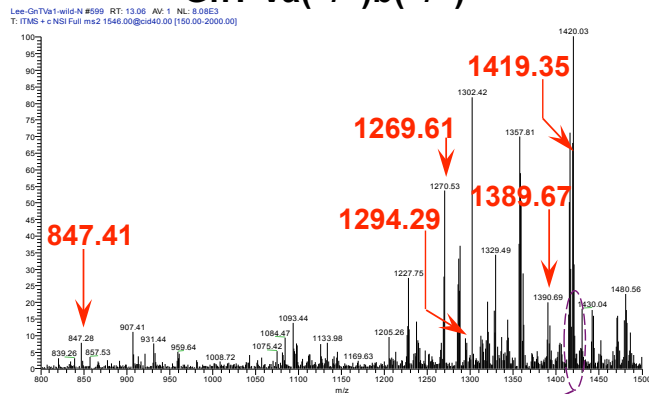
GnT-V(-/-)Vb(-/-)



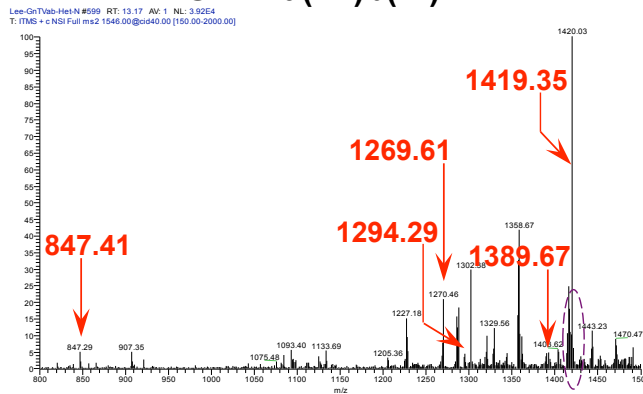
Supplemental Figure 3.

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

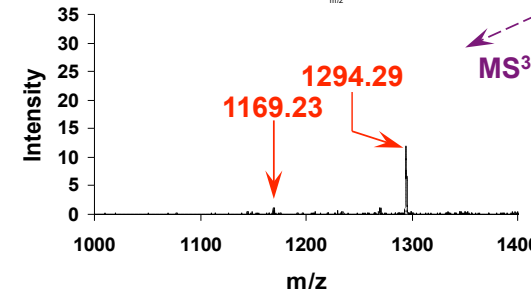
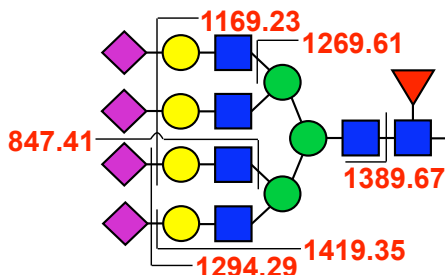
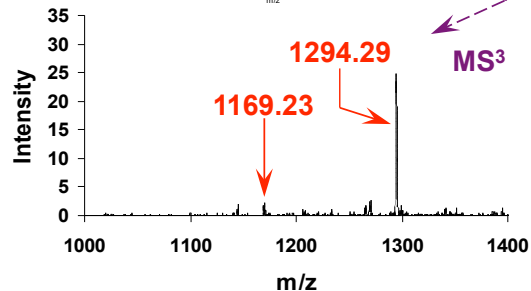
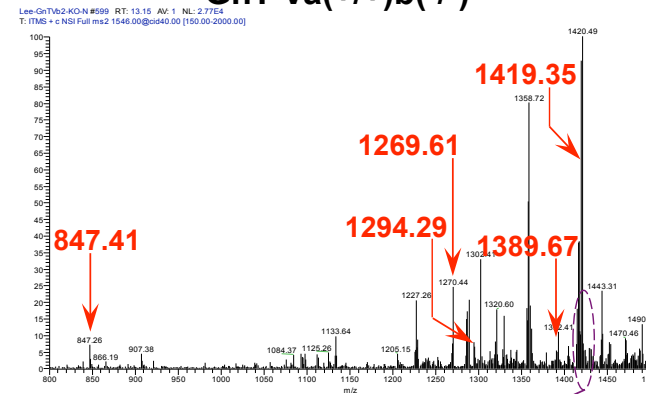
GnT-Va(+/+)**b**(+/+)



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

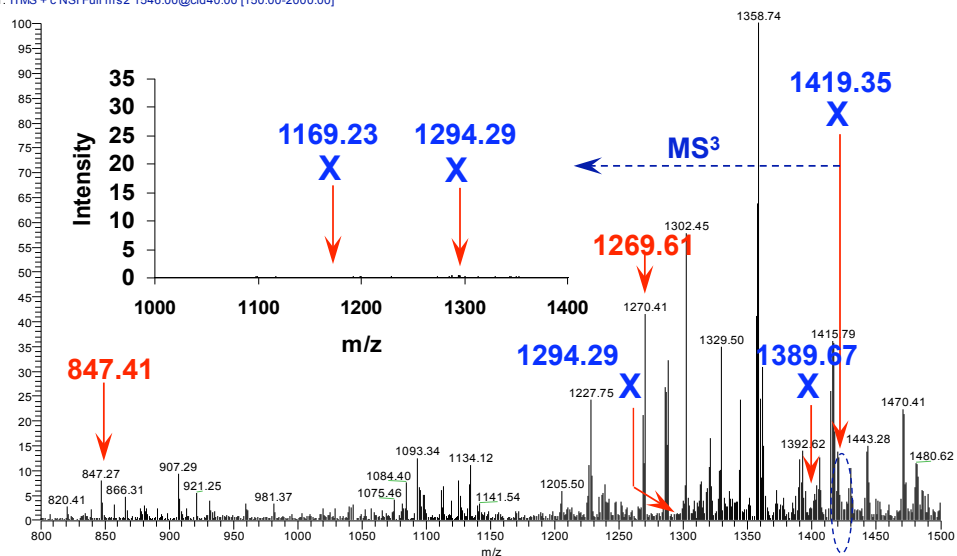


GnT-Va(+/+)**b**(-/-)



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

Lee-GnTv1-KO-N #599 RT: 13.05 AV: 1 NL: 9.27E3
T: ITMS + c NSI Full ms2 1546.00@cid40.00 [150.00-2000.00]



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

Lee-GnTv1-KO-N #599 RT: 13.17 AV: 1 NL: 1.77E4
T: ITMS + c NSI Full ms2 1546.00@cid40.00 [150.00-2000.00]

