I. Supplemental Figures S1-S7

Figure S1. Characteristic MS^3 spectra and ions generated from permethylated glycan standards carrying the target glycotopes.

Figure S2. RP C18 nanoLC separation of permethylated $LacNAc_{1-2}$-extended cores 1 and 2 O-glycans with 0-2 Fuc.

Figure S3. Trap CID-MS^2 and HCD-MS^3 spectra of select permethylated, sulfated O-glycans to demonstrate how individual sulfated O-glycans can be identified.

Figure S4. Graphic User Interface for GlyPick.

Figure S5. Summed ion intensities for individual MS^2 and MS^3 diagnostic ions across 10 fold differences in the applied sample amount of permethylayted AGS O-glycans.

Figure S6. An overlapping isotopic cluster for co-eluting N-glycans.

Figure S7. Collated set of 71 annotated MS^2 spectra from the mouse brain striatum N-glycans containing the pd-MS^3-validated diagnostic ion of disialyl LacNAc glycotope.

II. Supplemental Table S1-S4

Table S1. **Data and statistics for glycotope identification and relative quantification by MS^2/MS^3 ion intensities**.

Table S1.1 Statistics of total MS^1/MS^2/MS^3 scans acquired on permethylated AGS O-glycans across 10 fold variations in the injected sample amount

Table S1.2 Total number of MS^2 spectra in which a particular MS^2 ion could be detected when different amount of permethylated AGS O-glycan samples were applied.

Table S1.3 The summed intensity for each of the selected MS^2 ions afforded by the permethylated AGS O-glycan samples across 10x variations in the applied sample amount.

Table S1.4 The summed intensity for the diagnostic MS^3 ions afforded by the target MS^2 oxonium ions of permethylated AGS O-glycan samples across 10x variations in the applied sample amount.

Table S2. **Triplicate reproducibility of glycotope identification and relative quantification by MS^2/MS^3 ion intensities.**

Table S2.1 Relative abundance of selected glycotopes on AGS O-glycans based on spectral counting or sum total of its MS^2 ion intensity.

Table S2.2 Relative abundance of the isomeric constituents of mono- and difucosylated Gal-GlcNAc on AGS O-glycans based on sum of its diagnostic MS3 ion intensity.

Table S2.3 Relative abundance of selected glycotopes on AGS N-glycans based on spectral counting or sum total of its MS^2 ion intensity.

Table S2.4 Relative abundance of the isomeric constituents of mono- and difucosylated Gal-GlcNAc on AGS N-glycans based on sum of its diagnostic MS3 ion intensity.

Table S3. **NanoLC-MS^2-pd-MS^3 dataset on permethylated sulfated AGS O-Glycans.**

Table S4. **NanoLC-MS^2-pd-MS^3 dataset for the permethylated N-glycans of mouse brain striatum.**

Table S5. **List of MS^2 scans containing the diagnostic ion at m/z 1186 for the target disialyl LacNAc glycotope, which yielded the diagnostic pd-MS^3 ion at m/z 737.**

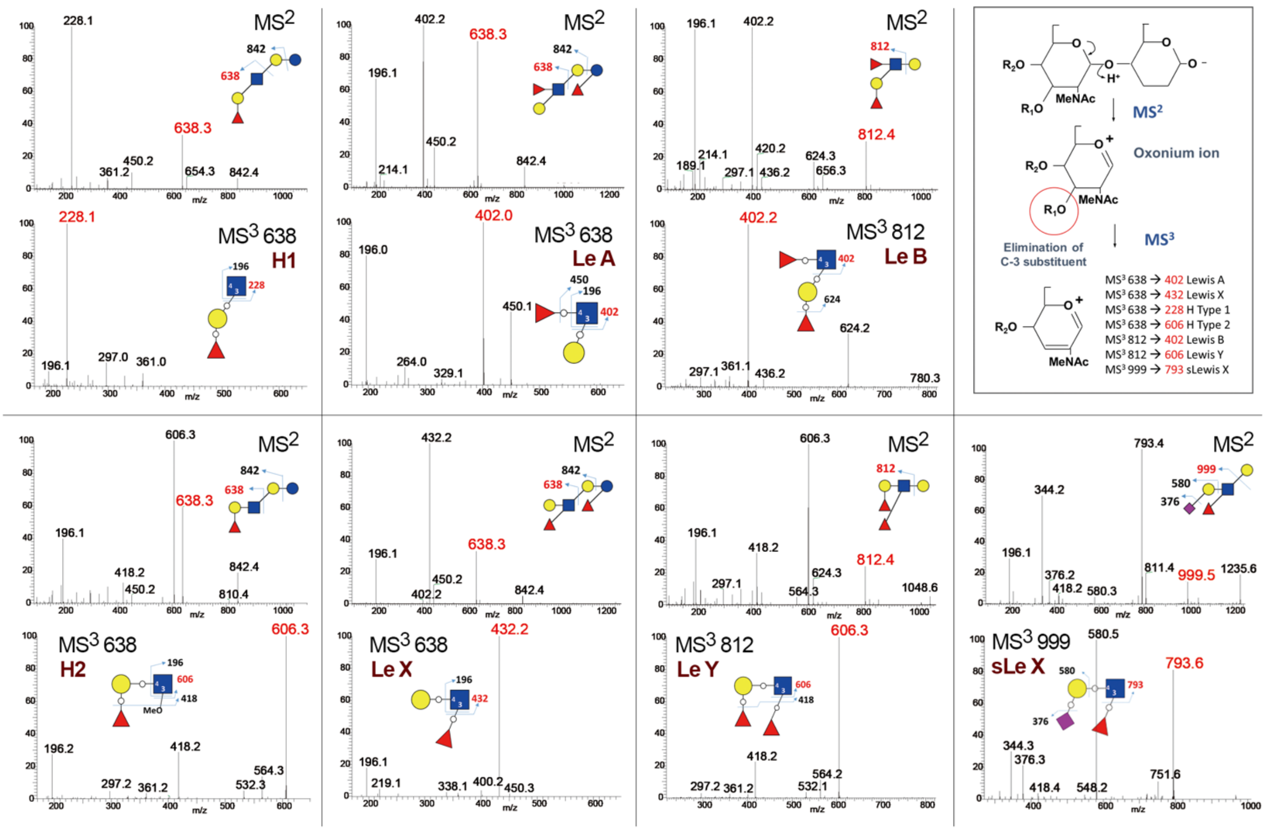

Figure S1. Characteristic MS^3 spectra and ions generated from protonated permethylated glycan standards carrying the target glycotopes. Identification of each of the 7 glycotopes *i.e.* H type 1, H type 2, LeA, LeB, LeX, LeY and sialyl LeX (sLeX) is based on detecting the diagnostic MS^3 ion corresponding to the targeted precursor MS^2 oxonium ion having eliminated its substituent on C3-position, as shown schematically in the boxed drawing. This diagnostic MS^3 ion can often also be detected as intense MS^2 ion but in the case when more than a single glycotope is carried on the glycan subjected to MS^2, its origin cannot be ascertained and hence the need for MS^3. For each glycotope, the MS^2 spectrum of its precursor and the MS^3 spectrum of its defining oxonium ion are shown in the upper and lower panel, respectively, with the diagnostic MS^2 and MS^3 ions labeled in red color. Apart from these diagnostic $MS^2 \rightarrow MS^3$ ion pairs, other more prominent MS^2 and/or MS^3 ions detected include those derived from further loss of an MeOH moiety (-32 u) from $HexNAc^+$ and $NeuAc^+$. Sialylated glycans always yielded the $NeuAc^+$ oxonium ion at m/z 376, along with m/z 344 after elimination of an MeOH. When the HexNAc is singly substituted at 3-position as in the case of H type 1 glycotope here, only a prominent m/z 228 was produced. When it is doubly substituted, further loss of an MeOH yielded the ion at m/z 196. Loss of Fuc to create a free OH group (-188 u) instead of elimination is commonly observed for Fuc moiety not on C3 position and thus produced the ions at m/z 418, 450 and 624 from the primary ions at m/z 606, 638 and 812, respectively. For the glycan standards that carries a fucosylated HexHexNAc glycotope on a reducing end Gal-GlcNAc, additional cleavage at the Gal produced the B ion at m/z 842, which is much less intense than the dominating oxonium ion at m/z 638. Similar B ion was also detected for $NeuAc\text{-}Gal^+$ at m/z 580. Other minor ions detected were not sequence- nor linkage-informative and not further assigned.

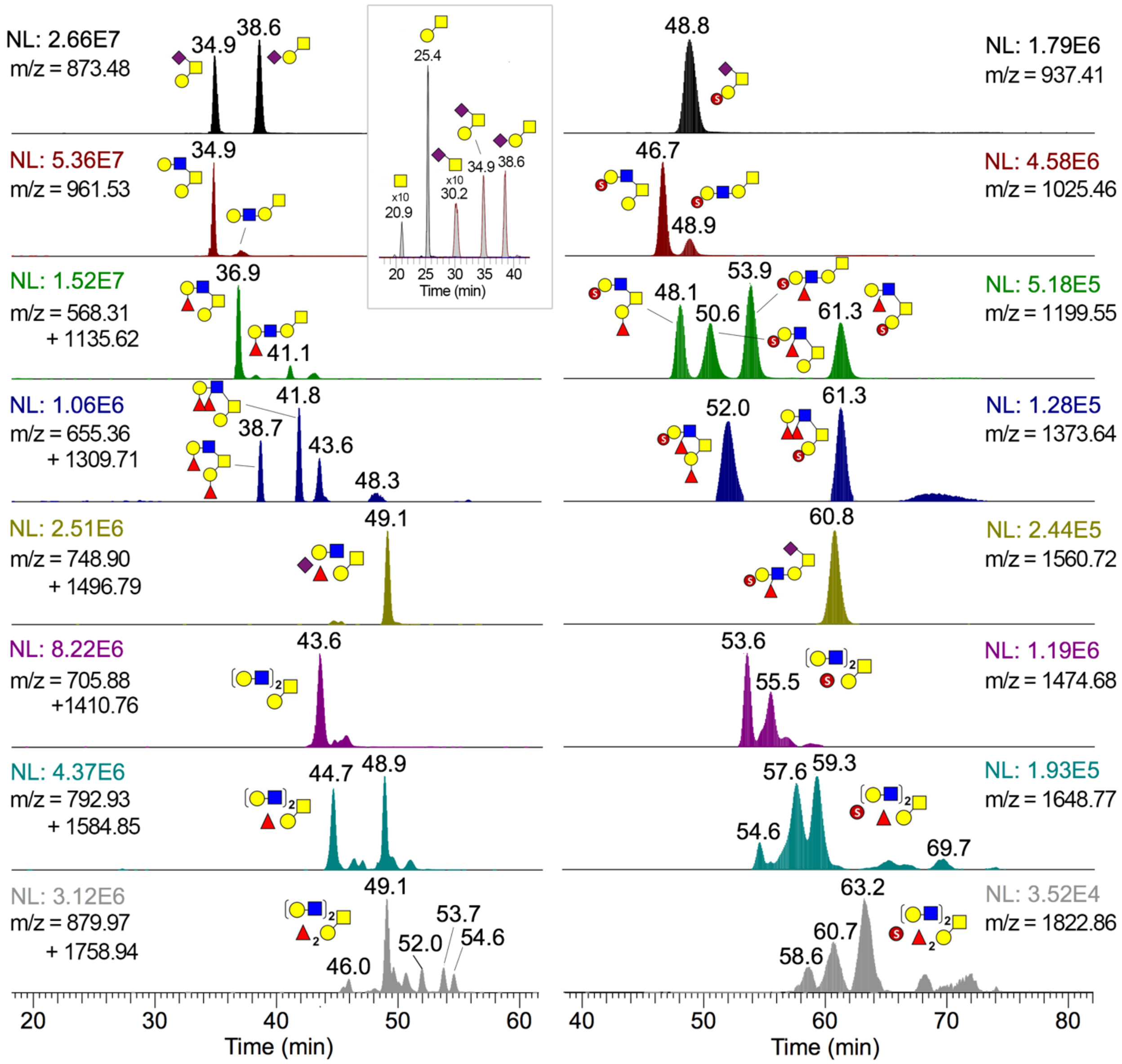

Figure S2. RP C18 nanoLC separation of permethylated LacNAc$_{1-2}$-extended cores 1 and 2 O-glycans with 0-2 Fuc. The permethylated non-sulfated (left panel) and mono-sulfated (right panel) AGS O-glycan sets were resolved using the same nanoLC column but with slightly different LC gradients (as described in the Methods section), and detected by MS in positive and negative ion modes, respectively. Larger permethylated, non-sulfated O-glycans were ionized predominantly as singly and doubly protonated in positive ion mode and their XIC intensity is a sum of both species. Permethylated, mono-sulfated O-glycans were detected as singly charged [M-H]$^-$ species in negative ion mode. The individual peaks for the smaller O-glycans can be resolved into their isomeric constituents based on MS^2-pd-MS^3 and are individually annotated, whereas larger ones cannot be unambiguously resolved. The Tn (HexNAcitol) , sialyl Tn and T (Hex-HexNAcitol) structures can be further resolved from the sialylated core 1 structures, as shown in the inset.

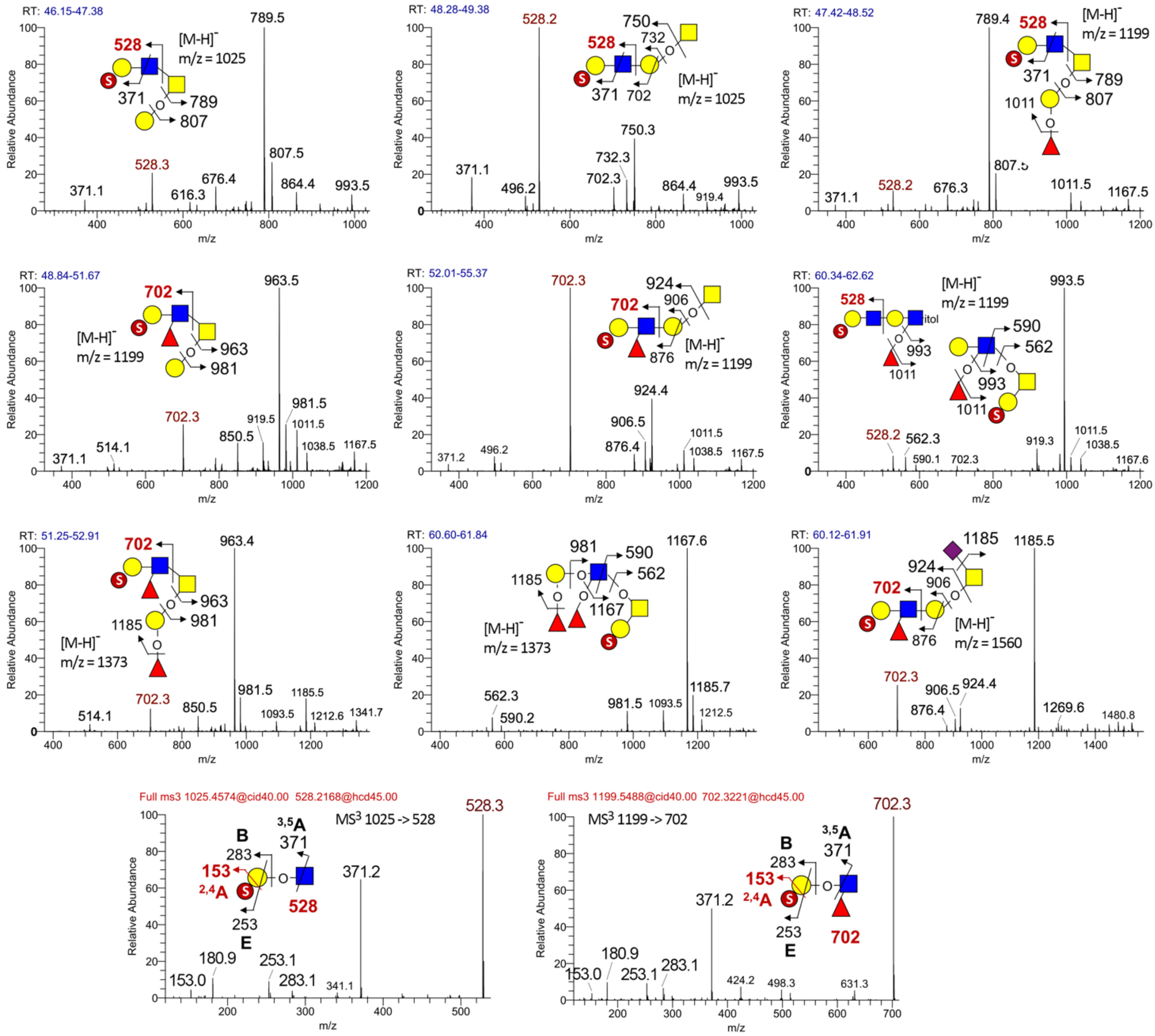

Figure S3. Trap CID-MS^2 and HCD-MS^3 spectra of select permethylated, sulfated O-glycans to demonstrate how individual sulfated O-glycans can be identified. The fragmentation pattern was established and reported previously (9) but assignment for the permethylated sulfated AGS O-glycans here is simpler due to 3-O-sulfation at terminal Gal being the predominant form of sulfation. Note that the critical low mass ions informative of the location of sulfate were missing in the trap CID-MS^2 spectra due to the cut-off.

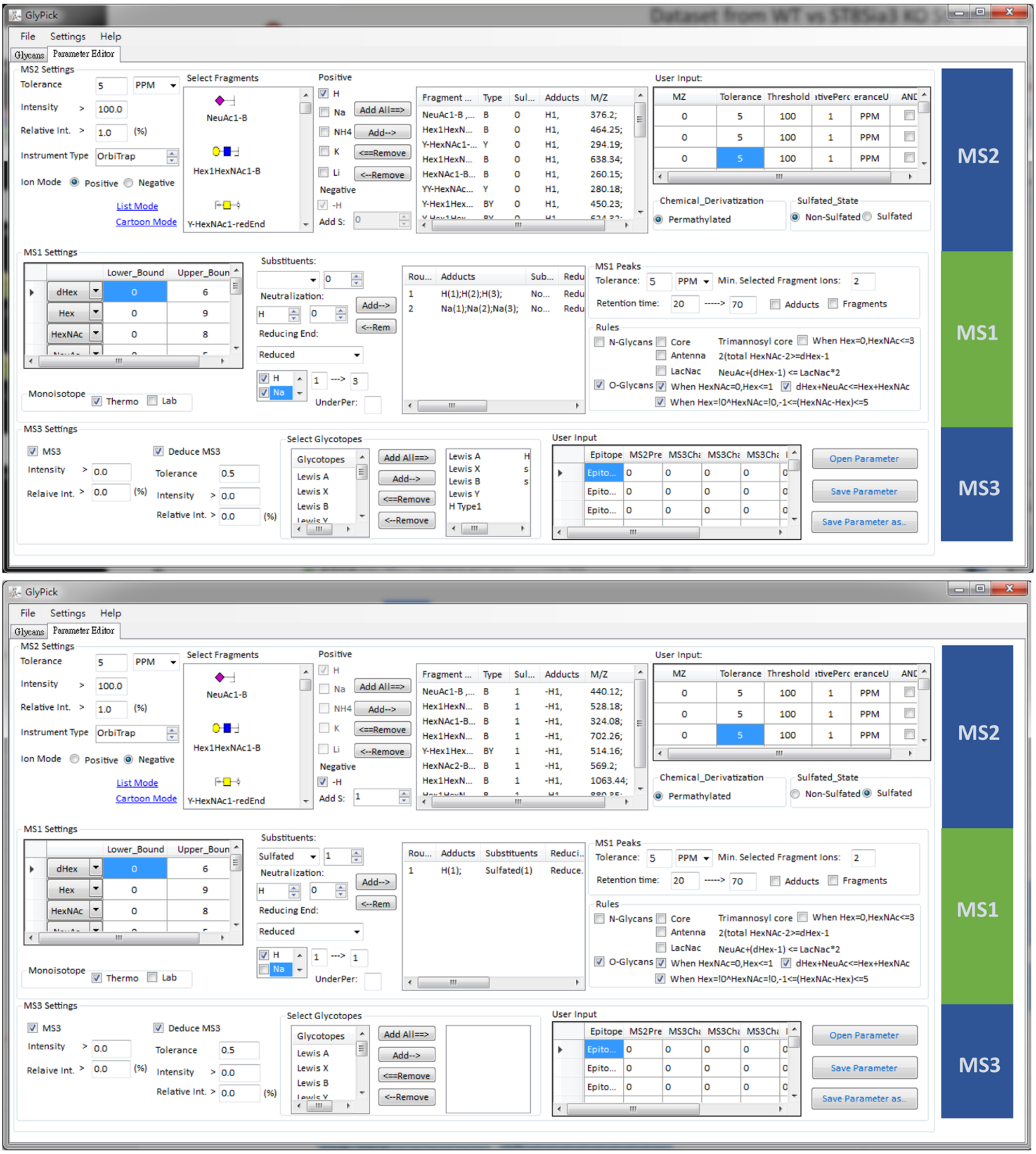

Figure S4. Graphic User Interface for GlyPick. The main user input is divided into 3 sections corresponding to MS2, MS1 and MS3 settings, respectively. A minimum user input would require specifying MS2 settings to select for glycan-related MS^2 spectra, with additional MS3 settings if product dependent MS^3 is acquired. Other than built-in MS^2/MS^3 ions for known glycotopes, additional ions for user defined glycotopes or glycosylation features can be input and included. Input for MS1 settings is required only if fitting glycosyl composition is to be performed. For non-sulfated permethylated glycans in positive ion mode (upper panel), no additional substituent is selected for MS1 setting, whereas considerations for Na^+ adducts (1-3 for mono- to triply charged) or degree of under-permethylation (to be specified), are optional. For sulfated permethylated glycans in negative ion mode (lower panel), the number of sulfate can be specified along with any additional H^+/Na^+ neutralization of the charge (usually no).

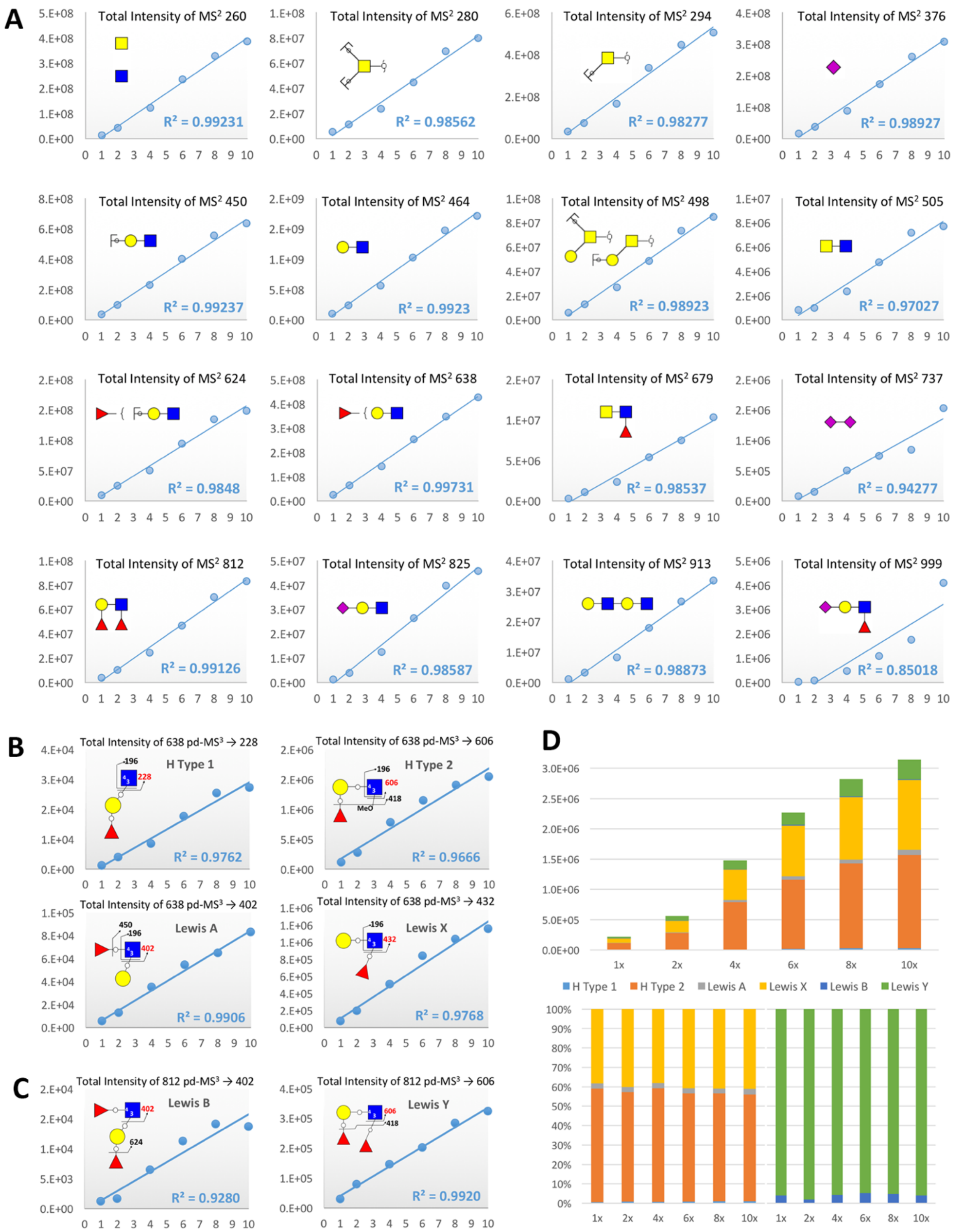

Figure S5. Summed ion intensities for individual MS^2 and MS^3 diagnostic ions across 10 fold differences in applied sample amount of permethylayted AGS O-glycans. (**A**) A panel of 16 glycotopes or glycosylation features defined by diagnostic MS^2 ions and sum of their respective total ion intensity in each of the 5 analyses (Table S1.3) representing a 10x concentration range. The 2 panels for *m/z* 638 and 812 are also shown in Fig. 3. Better linearity or R^2 values were obtained when the relative amount of the glycotope in question was higher. In this sample, the diagnostic ions for the disialyl (m/z 737) and sialyl Lewis (m/z 999) glycotopes were only detected in very few spectra at very low abundance compared to others to allow convincing identification and relative quantification. Summed pd-MS^3 ion intensities for (**B**) mono- and (**C**) di-fucosylated Gal-GlcNAc glycotopes (Table S1.4) show equally good linearity over 10x differences in sample amount. (**D**) The relative abundances of the MS^3- resolved isomeric constituents for the 2 target glycotopes can be totaled or calculated as % total based on the summed intensities of their respective diagnostic MS^3 ions.

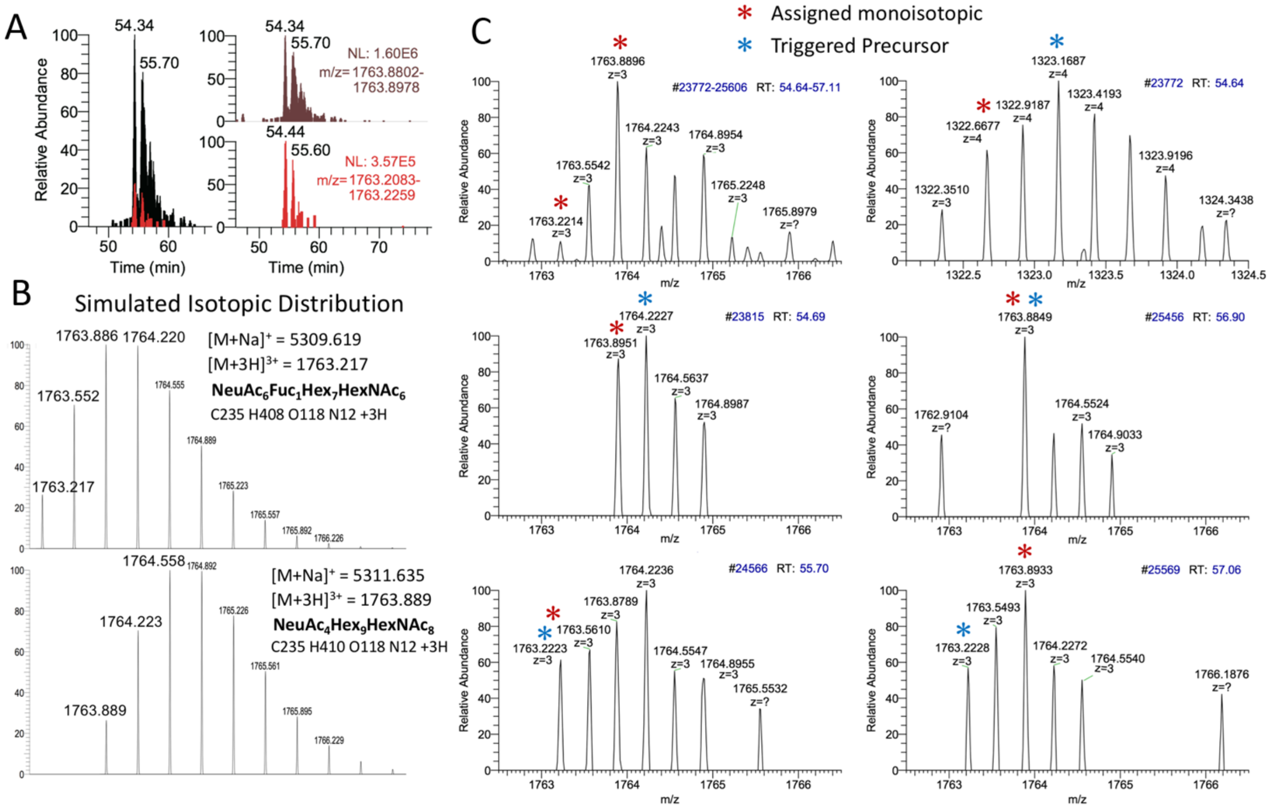

Figure S6. An overlapping isotopic cluster for co-eluting N-glycans. In the example shown here, rapid sampling of the MS^1 scans across the elution time period of 54.6 – 57.1 min resulted in MS^2 triggering of different precursors (marked with blue asterisk) based on signal intensity and the dynamic exclusion applied. The raw data processing software would attempt to assign the monoisotopic precursor (marked with red asterisk) for a particular MS^2 scan based on the detected isotopic cluster pattern in the immediate preceding MS^1 scan. Due to poor ion statistics (weak signals), complicated by co-elution of 2 or more different isobaric structures, or even those differed by as much as 2 Da shown in this example, correct assignment of monoisotopic mass is often problematic if not outright impossible. (**A**) Overlapping and non-overlapping XIC plots of the 2 precursors, using the accurate m/z values for the monoisotopic masses at 5 ppm window. (**B**) The simulated isotopic distribution pattern for the 2 structures in question when there is no overlapping interference. (**C**) The detected isotopic clusters for the precursors for each of the individual or averaged MS1 scans. In practice, irrespective of triggered and assigned precursors, as much as 2 Th for the quadrupole isolation width centered on the triggered precursor was typically applied. In samples of high complexity, multiplexed MS^2 spectra contributed by several precursors is inevitable.

Figure S7. Collated set of 71 annotated MS^2 spectra from the mouse brain striatum N-glycans containing the pd-MS^3-validated diagnostic ion of disialyl LacNAc glycotope. The details for the MS^2 scans including all the identified MS^2 ions, their respective MS^1 scans, the m/z values and intensities of monoisotopic precursors, fitted glycosyl compositions, and their associated pd-MS^3 scans are listed in Table S5.

[Note: This MS^2 spectral set is provided as a separate Supplemental data file in pdf format.]

Table S1. Data and Statistics for Glycotope Identification and Relative Quantification by MS2/MS3 ion intensities

Table S1.1 Statistics of total MS1/MS2/MS3 scans acquired on permethylated AGS O-glycans across 10 fold variations in the injected sample amount. The actual amount of glycans in each of the 6 runs was not determined but defined by different dilution factors applied on aliquots of C18 Zip-Tip eluates, with the undiluted one designated as 10x. For all subsequent data mining, only MS1 and MS3 scans associated with MS2 spectra acquired within 20-70 min of the nanoLC run and which contain at least 2 predefined diagnostic MS2 ions detected at an accuracy of 5 ppm were considered[1]. The total intensity of all selected MS2 ions[2] is a better indicator of the relative abundance of total glycans than the total MS2 spectral count[3], and can be used as a basis for normalization (**Fig. 3A**).

Sample amount injected on column:		1x	2x	4x	6x	8x	10x
Total Scans	MS1	3368	3213	3209	3136	2916	3107
	MS2	42524	42222	39948	38492	39062	37757
	MS3	1023	2372	5057	6959	7808	8271
Select Only within RT: 20-70 min	MS1	1327	1243	1210	1160	1127	1110
	MS2	26047	25485	23591	22373	21725	21383
	MS3	929	2160	4502	6107	7000	7455
With ≥ 2 diagnostic MS2 ions	MS1	1103	1143	1175	1143	1121	1108
	MS2	6313	9766	11676	12923	13621	13276
	MS3	691	1888	4209	5743	6590	7002
Total MS2 Spectral Count[1]		6313	9766	11676	12923	13621	13276
Total selected MS2 Spectral Count[3]		11620	26641	51166	71343	82874	88428
Total selected MS2 Ion intensity[2]		253821504	628733878	1466423291	2724173528	3846120070	4476947894

Table S1.2 Total number of MS2 spectra in which a particular MS2 ion could be detected when different amount of permethylated AGS O-glycan samples were applied. Any MS2 spectrum can contain more than one of the selected ions and hence counted as many times as needed.

MS2 ion	Total Spectral Count					
	1x	2x	4x	6x	8x	10x
260	1800	5227	10083	13191	14919	15171
280	278	515	1037	1799	2176	2554
294	759	1456	2852	4406	5209	5699
376	758	1710	4097	6465	7987	9256
450	1532	4202	8474	11501	13021	13232
464	4398	8001	11997	14785	16363	16758
498	227	472	813	1321	1552	1803
505	73	57	130	242	384	436
624	213	576	1319	2389	3153	3750
638	1125	3231	7433	9886	11211	11736
679	37	130	204	488	417	600
737	6	12	15	22	30	42
812	188	488	1117	1874	2586	3052
825	123	330	1058	1994	2534	2736
913	94	213	466	854	1135	1345
999	9	21	71	126	197	258
Total:	11620	26641	51166	71343	82874	88428

Table S1.3 The summed intensity for each of the selected MS2 ions afforded by the permethylated AGS O-glycan samples across 10x variations in the applied sample amount. The total intensity of all selected MS2 ions[1] is a good indication of the relative abundance of total glycans, which can be used as a basis for normalization. Alternatively, the summed intensity of each of the MS2 ions can be expressed as % of total intensity[2] for all selected MS2 ions (**Fig. S5A, 3C & 3D**)

MS2 ion	Summed Ion Intensity					
	1x	2x	4x	6x	8x	10x
260	13458205	42855934	122116582	235656085	328974184	386633703
280	5315276	11368502	23797315	44783863	69382278	79805074
294	34042513	74909009	166949294	338278432	446960392	504569091
376	15760184	37175664	88807671	173321485	260929425	308018023
450	35268060	97872503	230920548	401731591	554797842	635465840
464	102063264	241588488	562431014	1029793135	1475004624	1715871867
498	5822824	13006723	26900037	48522062	73460642	84876809
505	803025	967282	2352575	4735727	7168249	7711839
624	9696741	25318926	50266317	94693581	134508694	148729281
638	25022203	65194248	143246913	254411932	348318467	426730323
679	287068	1116973	2362511	5400149	7508810	10346322
737	79530	154160	505860	744542	845351	1527353
812	3872599	10206323	24530117	46756232	70120271	83282468
825	1210136	3765762	12541246	26401086	39839942	45765000
913	1096080	3151609	8232875	17858040	26548020	33522929
999	23796	81772	462416	1085586	1752879	4091972
Total[1]:	253821504	628733878	1466423291	2724173528	3846120070	4476947894

MS2 ion	% of Total Ion Intensity[2]							
	1x	2x	4x	6x	8x	10x	Avg.	SD.
260	5.30%	6.82%	8.33%	8.65%	8.55%	8.64%	7.71%	1.25%
280	2.09%	1.81%	1.62%	1.64%	1.80%	1.78%	1.79%	0.15%
294	13.41%	11.91%	11.38%	12.42%	11.62%	11.27%	12.00%	0.73%
376	6.21%	5.91%	6.06%	6.36%	6.78%	6.88%	6.37%	0.36%
450	13.89%	15.57%	15.75%	14.75%	14.42%	14.19%	14.76%	0.68%
464	40.21%	38.42%	38.35%	37.80%	38.35%	38.33%	38.58%	0.76%
498	2.29%	2.07%	1.83%	1.78%	1.91%	1.90%	1.96%	0.17%
505	0.32%	0.15%	0.16%	0.17%	0.19%	0.17%	0.19%	0.06%
624	3.82%	4.03%	3.43%	3.48%	3.50%	3.32%	3.60%	0.25%
638	9.86%	10.37%	9.77%	9.34%	9.06%	9.53%	9.65%	0.42%
679	0.11%	0.18%	0.16%	0.20%	0.20%	0.23%	0.18%	0.04%
737	0.03%	0.02%	0.03%	0.03%	0.02%	0.03%	0.03%	0.00%
812	1.53%	1.62%	1.67%	1.72%	1.82%	1.86%	1.70%	0.11%
825	0.48%	0.60%	0.86%	0.97%	1.04%	1.02%	0.83%	0.22%
913	0.43%	0.50%	0.56%	0.66%	0.69%	0.75%	0.60%	0.11%
999	0.01%	0.01%	0.03%	0.04%	0.05%	0.09%	0.04%	0.03%

MS2[2]	MS3[3]	Glycotope[1]	Summed Ion Intensity[4]					
			1x	2x	4x	6x	8x	10x
638	228	H Type 1	1142	3882	8447	17583	25343	27169
638	606	H Type 2	110997	272340	777605	1143989	1401769	1543694
638	402	Lewis A	5231	12476	35012	54329	64792	82904
638	432	Lewis X	72658	193758	506451	837734	1031916	1152137
812	402	Lewis B	1208	1610	6440	11268	14086	13682
812	606	Lewis Y	29119	78254	144982	201400	283288	323321
		Total:	220354	562320	1478937	2266302	2821194	3142906

Table S1.4 The summed intensity for the diagnostic MS3 ions afforded by the target MS2 oxonium ions of permethylated AGS O-glycan samples across 10x variations in the applied sample amount. The isomeric mono- and difucosylated glycotopes[1] sharing the same MS2 oxonium ion[2] were identified by their diagnostic MS3 ion[3] and quantified by sum of their respective ion intensity[4] in each of the product dependent-MS3 spectra where it was detected. See **Fig. S5B-D** for the corresponding charts.

Table S2. Triplicate Reproducibility of Glycotope Identification and Relative Quantification by MS2/MS3 ion intensities

Table S2.1 Relative abundance of selected glycotopes on AGS O-glycans based on spectral counting or sum total of its MS2 ion intensity. Relative quantification can be based on counting the number of MS2 spectra in which the target ion[1] was detected (spectral counting[2]), sum of its ion intensity[3] in each of these MS2 spectra, and/or expressed as % of total intensity[4] of all selected ions, or normalised against an arbitrarily chosen reference glycotope[5] (here LacNAc, represented by m/z 464). Reproducibility of quantified relative abundance of individual and all glycotopes was evaluated by triplicate analyses.

MS2 Ion[1]	Total Spectral Count[2]			Summed Ion Intensity[3]			% of Total Ion Intensity[4]					Total Ion Intensity /464[5]		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Avg.	SD	Run 1	Run 2	Run 3
260	10083	10241	10390	122116582	124578563	135497368	8.3%	8.0%	8.5%	8%	0.21%	21.7%	21.4%	22.9%
280	1037	1080	1106	23797315	25900976	26838516	1.6%	1.7%	1.7%	2%	0.03%	4.2%	4.4%	4.5%
294	2852	2875	3098	166949294	177401591	196612456	11.4%	11.5%	12.3%	12%	0.50%	29.7%	30.5%	33.2%
376	4097	4183	4337	88807671	96122218	104236343	6.1%	6.2%	6.5%	6%	0.23%	15.8%	16.5%	17.6%
450	8474	8477	8781	230920548	240386261	241043411	15.8%	15.5%	15.1%	15%	0.35%	41.1%	41.3%	40.7%
464	11997	12287	12382	562431014	582212238	592971901	38.4%	37.6%	37.1%	38%	0.66%	100.0%	100.0%	100.0%
498	813	831	875	26900037	29368870	29862395	1.8%	1.9%	1.9%	2%	0.03%	4.8%	5.0%	5.0%
505	130	150	134	2352575	2784667	2563217	0.2%	0.2%	0.2%	0%	0.01%	0.4%	0.5%	0.4%
624	1319	1285	1413	50266317	59670006	57545820	3.4%	3.9%	3.6%	4%	0.21%	8.9%	10.2%	9.7%
638	7433	7337	7577	143246913	156660396	157931384	9.8%	10.1%	9.9%	10%	0.18%	25.5%	26.9%	26.6%
679	204	221	258	2362511	2823710	3142534	0.2%	0.2%	0.2%	0%	0.02%	0.4%	0.5%	0.5%
812	1117	1078	1148	24530117	27181769	28848342	1.7%	1.8%	1.8%	2%	0.07%	4.4%	4.7%	4.9%
825	1058	1039	1058	12541246	13001088	13032857	0.9%	0.8%	0.8%	1%	0.02%	2.2%	2.2%	2.2%
913	466	427	491	8232875	9377115	9674183	0.6%	0.6%	0.6%	1%	0.03%	1.5%	1.6%	1.6%
999	71	70	82	462416	410790	574997	0.0%	0.0%	0.0%	0%	0.00%	0.1%	0.1%	0.1%
Total:	32893	33425	34102	1465917431	1547880258	1600375724	100%	100%	100%	100%				

Table S2.2 Relative abundance of the isomeric constituents of mono- and difucosylated Gal-GlcNAc on AGS O-glycans based on sum of its diagnostic MS3 ion intensity. The isomeric glycotope[1] sharing the same MS2 oxonium ion[2] is identified by its diagnostic MS3 ion[3] and quantified by sum of its ion intensity[4] in each of the product dependent-MS3 spectra where it was detected. Reproducibility of quantified relative abundance was evaluated by triplicate analyses.

			Summed Ion Intensity[4]				
MS2[2]	MS3[3]	Glycotope[1]	Run 1	Run 2	Run 3	Avg.	SD.
638	228	H Type 1	8447	8676	11874	9665	1916
638	606	H Type 2	777605	727267	766689	757187	26480
638	402	Lewis A	35012	34610	32197	33939	1523
638	432	Lewis X	506451	509044	535423	516973	16031
812	402	Lewis B	6440	4024	8452	6305	2217
812	606	Lewis Y	144982	147406	143771	145386	1851
		Total:	1478937	1431027	1498405	1469456	

Table S2.3 Relative abundance of selected glycotopes on AGS N-glycans based on spectral counting or sum total of its MS2 ion intensity. Relative quantification can be based on counting the number of MS2 spectra in which the target ion[1] was detected (spectral counting[2]), sum of its ion intensity[3] in each of these MS2 spectra, and/or expressed as % of total intensity[4] of all selected ions, or normalised against an arbitrarily chosen reference glycotope[5] (here LacNAc, represented by m/z 464). Reproducibility of quantified relative abundance of individual and all glycotopes was evaluated by triplicate analyses.

MS2 Ion[1]	Total Spectral Count[2]			Summed Ion Intensity[3]			% of Total Ion Intensity[4]					Total Ion Intensity /464[5]		
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	Avg.	SD	Run 1	Run 2	Run 3
260	15623	15390	15002	3373143903	3333405021	3362923822	20.2%	20.4%	20.4%	20%	0.11%	76.7%	77.3%	76.9%
280	9055	8817	8553	665232946	636408864	641687763	4.0%	3.9%	3.9%	4%	0.05%	15.1%	14.8%	14.7%
294	9645	9486	9171	4523786318	4502356955	4463534745	27.1%	27.5%	27.1%	27%	0.22%	102.8%	104.4%	102.1%
376	7424	7294	7135	572286482	551733979	574328076	3.4%	3.4%	3.5%	3%	0.06%	13.0%	12.8%	13.1%
450	17774	17698	17581	1703895791	1657354031	1664968674	10.2%	10.1%	10.1%	10%	0.05%	38.7%	38.4%	38.1%
464	18259	18233	18162	4399691875	4312472784	4371611290	26.4%	26.3%	26.6%	26%	0.12%	100.0%	100.0%	100.0%
505	613	592	556	16499887	16212208	14885554	0.1%	0.1%	0.1%	0%	0.00%	0.4%	0.4%	0.3%
624	14	14	7	45377	46147	26905	0.0%	0.0%	0.0%	0%	0.00%	0.0%	0.0%	0.0%
638	16652	16668	16597	1113034896	1065713088	1072172198	6.7%	6.5%	6.5%	7%	0.09%	25.3%	24.7%	24.5%
679	1208	1180	1154	41971650	40172677	39774548	0.3%	0.2%	0.2%	0%	0.00%	1.0%	0.9%	0.9%
812	6737	6481	6561	138161694	128843594	127249330	0.8%	0.8%	0.8%	1%	0.03%	3.1%	3.0%	2.9%
825	1090	1053	865	20373727	11896181	6568405	0.1%	0.1%	0.0%	0%	0.04%	0.5%	0.3%	0.2%
913	4625	4668	4720	115579543	114793209	115564059	0.7%	0.7%	0.7%	1%	0.01%	2.6%	2.7%	2.6%
999	292	258	290	2899975	2651411	2736851	0.0%	0.0%	0.0%	0%	0.00%	0.1%	0.1%	0.1%
Total:	109011	107832	106354	16686604064	16374060149	16458032220	100%	100%	100%	100%				

Table S2.4 Relative abundance of the isomeric constituents of mono- and difucosylated Gal-GlcNAc on AGS N-glycans based on sum of its diagnostic MS3 ion intensity. The isomeric glycotope[1] sharing the same MS2 oxonium ion[2] is identified by its diagnostic MS3 ion[3] and quantified by sum of its ion intensity[4] in each of the product dependent-MS3 spectra where it was detected. Reproducibility of quantified relative abundance was evaluated by triplicate analyses.

			Summed Ion Intensity[4]				
MS2[2]	MS3[3]	Glycotope[1]	Run 1	Run 2	Run 3	Avg.	Std.
638	228	H Type 1	15514	19392	11874	15593	3760
638	606	H Type 2	3434013	3481288	3450960	3455420	23951
638	402	Lewis A	211596	191883	203953	202477	9939
638	432	Lewis X	2307996	2309714	2291537	2303082	10035
812	402	Lewis B	38619	25744	34192	32852	6541
812	606	Lewis Y	1036361	990269	982985	1003205	28944
		Total:	7044099	7018290	6975500	7012630	

Table S3. NanoLC-MS2/MS3 Dataset on permethylated sulfated AGS O-Glycans. The data were acquired in negative ion mode by nanoLC-HCD MS2-pd-HCD MS3 targeting the MS2 product ions at m/z 528 and 702. The filtered and edited entries were sorted according to the *m/z* of singly charged [M-H]- and then by elution time (MS1 scan number).

MS1 Scan	RT	m/z (Mono)	MS1 Intensity	Fitted Glycosyl Composition	MS2 Scan	MS2 Selected Ions	MS3 Scan	pd-MS3	MS3 ions
3367	21.16	821.4	75387.3	H1N2S1	3369	153;181;283;371.1;528.2			
3390	21.34	821.4	199621.2	H1N2S1	3392	153;181;253;283;371.1;528.2			
3416	21.51	821.4	232585.5	H1N2S1	3418	153;181;253;283;301.1;371.1;528.2	3419	528.2	283;341.6;528.1
3443	21.68	821.4	116807.4	H1N2S1	3445	153;181;253;269;283;371.1;528.2			
3628	22.79	821.4	17746.6	H1N2S1	3630	253			
3653	22.96	821.4	18684.2	H1N2S1	3655	253			
3493	22.02	891.4	53536.0		3495	181;253			
3522	22.19	891.4	74873.4		3524	153;181;253			
3557	22.38	891.4	94778.0		3560	153;181;253			
3587	22.55	891.4	76494.3		3590	153;181;253			
3618	22.73	891.4	46680.1		3620	153;181			
3586	22.54	893.3	73367.4		3588	153;181			
3623	22.75	893.3	10993.6		3625	153;181			
4095	25.89	921.4	25569.3		4097	181			
4357	27.58	921.4	47623.1		4359	153;181			
4385	27.75	921.4	80815.1		4387	153;181			
3427	21.57	923.4	18356.6	H1N1Neu1S1(OH)1	3430	181			
3893	24.58	923.4	78279.9	H1N1Neu1S1(OH)1	3895	181;253			
3920	24.75	923.4	158908.7	H1N1Neu1S1(OH)1	3922	153;181;253;283			
3948	24.92	923.4	168804.9	H1N1Neu1S1(OH)1	3950	153;181;253;283.1			
3976	25.10	923.4	100747.8	H1N1Neu1S1(OH)1	3978	153;181;253			
4438	28.09	937.4	53254.1	H1N1Neu1S1	4441	153;253			
4467	28.26	937.4	196193.6	H1N1Neu1S1	4470	153;181;253;283			
4496	28.44	937.4	541440.1	H1N1Neu1S1	4499	153;181;253;283;371.1			
4524	28.61	937.4	1026299.3	H1N1Neu1S1	4527	153;181;253;269;283;301.1;371.1			
4554	28.78	937.4	1532180.1	H1N1Neu1S1	4556	153;181;253;269;283;301.1;371.1			
4584	28.95	937.4	1534412.2	H1N1Neu1S1	4586	153;181;253;269;283.1;301.1;371.1			
4615	29.13	937.4	1205643.9	H1N1Neu1S1	4618	153;181;253;269;283;301.1;371.1			
4646	29.30	937.4	699177.6	H1N1Neu1S1	4649	153;181;253;269;283;301.1;371.1			
4676	29.48	937.4	313053.2	H1N1Neu1S1	4679	153;181;253			
4708	29.65	937.4	119272.4	H1N1Neu1S1	4710	153;181;253			
4437	28.08	967.4	25250.8		4439	253			
4466	28.25	967.4	40569.8		4468	253;283			
3850	24.30	995.4	11175.3	F1H1N2S1	3852	153			
3876	24.47	995.4	21126.0	F1H1N2S1	3878	153			
3930	24.81	995.4	39233.8	F1H1N2S1	3932	153;181			
3959	24.98	995.4	26320.4	F1H1N2S1	3961	181			
3304	20.71	997.4	30064.9	H2N2S1(OH)2	3306	528.2			
3328	20.88	997.4	53589.3	H2N2S1(OH)2	3330	153;371.1;528.2			
3352	21.05	997.4	76942.1	H2N2S1(OH)2	3354	181;371.1;528.2	3355	528.2	253.4;371.4
3375	21.22	997.4	60441.2	H2N2S1(OH)2	3377	153;181;253;371.1;528.2			
3399	21.39	997.4	28120.5	H2N2S1(OH)2	3401	371.1;528.2			
4491	28.41	1005.4	27783.1		4494	253			
4519	28.58	1005.4	62091.0		4522	253			
4549	28.76	1005.4	88581.4		4552	253			
4578	28.93	1005.4	95573.9		4581	181;253			
4611	29.11	1005.4	92553.1		4614	253;283			
3581	22.51	1009.4	10094.7		3583	371.1			
3611	22.69	1009.4	10684.9		3614	181			
3805	23.99	1009.4	25198.5		3808	153;181;528.2			
3832	24.17	1009.4	26682.1		3834	153			
4578	28.93	1009.4	10177.8		4583	153;181			
3481	21.93	1011.4	18904.7	H2N2S1(OH)1	3483	528.2			
3857	24.34	1011.4	51261.3	H2N2S1(OH)1	3860	153;181;283;371.1			
3883	24.52	1011.4	123892.6	H2N2S1(OH)1	3886	153;181;371.1;528.2			
3910	24.69	1011.4	330086.9	H2N2S1(OH)1	3913	153;181;253;283;371.1;528.2	3914	528.2	371;371.8;528
3938	24.86	1011.4	467732.0	H2N2S1(OH)1	3941	153;181;253;283;371.1;528.2	3942	528.2	253.2;255.4;371.1
3966	25.03	1011.4	353755.3	H2N2S1(OH)1	3969	153;181;253;283;371.1;528.2	3970	528.2	204.9;253.4;371.1;528.2
3994	25.21	1011.4	171680.9	H2N2S1(OH)1	3996	153;181;253;283;371.1;528.2	3997	528.2	528.3
4020	25.38	1011.4	60128.6	H2N2S1(OH)1	4022	153;181;371.1;528.2			
4047	25.55	1011.4	29952.4	H2N2S1(OH)1	4049	153;181;371.1;528.2			
4229	26.78	1011.4	19937.0	H2N2S1(OH)1	4231	181			
4257	26.95	1011.4	25305.4	H2N2S1(OH)1	4259	371.1			
4196	26.58	1025.5	87490.7	H2N2S1	4199	153;181;253;283.1;301.1;371.1;528.2			
4225	26.76	1025.5	372880.8	H2N2S1	4227	153;181;253;283;301.1;371.1;528.2	4228	528.2	180.9;283.2;498.3
4253	26.93	1025.5	849763.8	H2N2S1	4255	153;181;253;283;301.1;371.1;528.2;614.2	4256	528.2	181.1;205.3;255.4;342;371.3;528.2
4281	27.10	1025.5	1462667.0	H2N2S1	4283	153;181;253;283;301.1;371.1;528.2;614.2	4284	528.2	153.3;255.2;283.2;285.1;371.3;528.4
4308	27.27	1025.5	1298067.5	H2N2S1	4310	153;181;253;283;301.1;371.1;528.2;614.2;702.3	4311	528.2	269.1;371;371.8;496.3;528.4
4308	27.27	1025.5	1298067.5	H2N2S1	4310	153;181;253;283;301.1;371.1;528.2;614.2;702.3	4312	702.3	283.1;501.7;528.4;672.2
4336	27.45	1025.5	756051.0	H2N2S1	4338	153;181;253;283;301.1;371.1;528.2;614.2;702.3	4339	528.2	181.3;205.1;341.1;371.3;528.2
4363	27.62	1025.5	395386.7	H2N2S1	4365	153;181;195;253;283;301.1;371.1;528.2;702.3	4366	528.2	342.4
4391	27.79	1025.5	134183.5	H2N2S1	4393	153;181;195;253;283.1;371.1;528.2			
4419	27.96	1025.5	54428.2	H2N2S1	4421	181;371.1			
4504	28.49	1025.5	28591.2	H2N2S1	4507	153;253;371.1;528.2			
4532	28.66	1025.5	107311.1	H2N2S1	4535	153;181;253;283;371.1;528.2;702.3	4536	528.2	282.9;528.5
4561	28.83	1025.5	246426.8	H2N2S1	4563	153;181;253;283;301.1;371.1;528.2;702.3	4565	528.2	165.8;225;339.4;371.6;528.2
4561	28.83	1025.5	246426.8	H2N2S1	4563	153;181;253;283;301.1;371.1;528.2;702.3	4566	702.3	371.1
4592	29.01	1025.5	396048.1	H2N2S1	4594	153;167;181;253;283;301.1;371.1;528.2;702.3	4596	528.2	153;181.8;225.2;253.2;341.1;372.5;528.1
4592	29.01	1025.5	396048.1	H2N2S1	4594	153;167;181;253;283;301.1;371.1;528.2;702.3	4597	702.3	370.9;427.2;457.1;528.4

4625	29.18	1025.5	340552.5	H2N2S1	4627	153;181;253;283;301.1;371.1;528.2;702.3	4628	528.2	153.1;253.2;371.1;528.3
4625	29.18	1025.5	340552.5	H2N2S1	4627	153;181;253;283;301.1;371.1;528.2;702.3	4629	702.3	301;529
4655	29.35	1025.5	223556.6	H2N2S1	4657	153;181;253;269;283;301.1;371.1;528.2;702.3	4658	528.2	138.1;151.1;181;182.6;225.4;371.1;528.3
4684	29.53	1025.5	99499.0	H2N2S1	4686	153;181;253;283.1;371.1;528.2;702.3	4687	528.2	180.9
4717	29.70	1025.5	33900.9	H2N2S1	4719	371.1;528.2			
4750	29.87	1025.5	15998.3	H2N2S1	4753	528.2			
4237	26.83	1093.4	28066.9		4239	181;371.1;528.2			
4266	27.00	1093.4	63721.6		4268	181;371.1;528.2			
4293	27.17	1093.4	88770.1		4295	153;181;253;371.1;528.2			
4320	27.34	1093.4	71779.2		4322	181;253;371.1;528.2			
4347	27.52	1093.4	42162.7		4349	181;371.1			
4573	28.90	1093.4	20561.1		4575	181			
4605	29.08	1093.4	21605.0		4607	181;253;528.2			
4638	29.26	1093.4	16863.6		4640	528.2			
3267	20.43	1171.5	30913.9	F1H2N2S1(OH)2	3269	371.1;702.3			
3290	20.60	1171.5	28119.6	F1H2N2S1(OH)2	3292	181;702.3			
3313	20.77	1171.5	28036.2	F1H2N2S1(OH)2	3315	253;371.1;702.3			
4298	27.21	1185.5	15478.8	F1H2N2S1(OH)1	4300	253			
4326	27.38	1185.5	66598.1	F1H2N2S1(OH)1	4328	153;181;371.1;528.2			
4353	27.55	1185.5	132047.3	F1H2N2S1(OH)1	4355	153;181;253;371.1;528.2			
4381	27.72	1185.5	191147.7	F1H2N2S1(OH)1	4383	153;181;253;283;371.1;528.2			
4407	27.89	1185.5	153856.4	F1H2N2S1(OH)1	4409	153;181;253;283;371.1;528.2			
4434	28.06	1185.5	80553.2	F1H2N2S1(OH)1	4436	153;181			
4462	28.23	1185.5	52202.6	F1H2N2S1(OH)1	4464	153;181;371.1			
4491	28.41	1185.5	48915.6	F1H2N2S1(OH)1	4493	153;181			
4519	28.58	1185.5	64329.0	F1H2N2S1(OH)1	4521	153;181			
4548	28.75	1185.5	55003.7	F1H2N2S1(OH)1	4550	283.1			
4608	29.10	1185.5	28458.6	F1H2N2S1(OH)1	4612	181;702.3			
4641	29.27	1185.5	33327.4	F1H2N2S1(OH)1	4643	253			
4670	29.45	1185.5	36387.6	F1H2N2S1(OH)1	4672	153;371.1;702.3			
4702	29.63	1185.5	34233.5	F1H2N2S1(OH)1	4704	153;702.3			
6008	37.95	1185.5	33391.8	F1H2N2S1(OH)1	6011	181			
6035	38.12	1185.5	68736.0	F1H2N2S1(OH)1	6038	153;253			
6065	38.29	1185.5	116514.5	F1H2N2S1(OH)1	6067	181;253			
6093	38.46	1185.5	133197.5	F1H2N2S1(OH)1	6096	153;181;253			
6121	38.64	1185.5	123602.0	F1H2N2S1(OH)1	6124	153;181;253			
6150	38.81	1185.5	69860.2	F1H2N2S1(OH)1	6152	253			
4420	27.97	1199.5	32243.6	F1H2N2S1	4423	528.2			
4447	28.14	1199.5	76859.5	F1H2N2S1	4450	153;181;253;371.1;528.2			
4476	28.31	1199.5	160593.8	F1H2N2S1	4478	153;181;253;371.1;528.2			
4504	28.49	1199.5	176004.4	F1H2N2S1	4506	153;181;253;283;371.1;528.2			
4532	28.66	1199.5	124896.2	F1H2N2S1	4534	153;181;253;283;371.1;528.2			
4561	28.83	1199.5	74295.0	F1H2N2S1	4564	181;371.1;528.2			
4592	29.01	1199.5	22446.8	F1H2N2S1	4595	181;371.1			
4763	29.95	1199.5	53739.1	F1H2N2S1	4766	153;181;371.1			
4790	30.12	1199.5	89307.9	F1H2N2S1	4792	181;371.1			
4816	30.30	1199.5	125704.9	F1H2N2S1	4819	153;181;253;283;371.1;702.3			
4844	30.47	1199.5	120757.7	F1H2N2S1	4846	153;181;253;371.1;702.3			
4870	30.64	1199.5	162708.1	F1H2N2S1	4873	153;181;253;371.1;702.3			
4899	30.81	1199.5	139836.3	F1H2N2S1	4901	153;181;253;283.1;371.1;702.3			
4926	30.98	1199.5	122204.1	F1H2N2S1	4928	153;181;253;371.1			
4952	31.15	1199.5	83776.1	F1H2N2S1	4954	153;181;253;283			
4978	31.33	1199.5	35957.0	F1H2N2S1	4980	153			
5211	32.77	1199.5	31405.8	F1H2N2S1	5213	702.3			
5240	32.94	1199.5	63888.1	F1H2N2S1	5242	181;253;702.3	5243	702.3	151.2;181.2;542;702.2
5267	33.11	1199.5	121494.5	F1H2N2S1	5269	153;181;253;283;371.1;702.3;876.4	5270	702.3	
5295	33.28	1199.5	159309.6	F1H2N2S1	5297	153;181;253;371.1;702.3;876.4	5298	702.3	153;181.3;283.5
5321	33.45	1199.5	195330.9	F1H2N2S1	5323	153;181;253;283;371.1;702.3;876.4	5324	702.3	206.9;702.4
5350	33.63	1199.5	149656.7	F1H2N2S1	5352	153;181;253;371.1;702.3;876.4	5353	702.3	152.2;181.1;224.2;514.2;660.5;702.3
5377	33.80	1199.5	112008.5	F1H2N2S1	5379	153;181;253;371.1;702.3;876.4	5381	702.3	151.1;253;283;702.7
5406	33.98	1199.5	58922.6	F1H2N2S1	5409	181;253;371.1;702.3	5410	702.3	128.3;514.1;702.2
5434	34.15	1199.5	29358.1	F1H2N2S1	5437	181;253;371.1;702.3			
6166	38.91	1199.5	39985.3	F1H2N2S1	6169	253			
6194	39.08	1199.5	116305.1	F1H2N2S1	6196	153;181;253;283			
6223	39.26	1199.5	229855.7	F1H2N2S1	6225	153;181;253;269;301.1;371.1			
6253	39.43	1199.5	394200.3	F1H2N2S1	6255	153;181;253;283;301.1;371.1			
6286	39.61	1199.5	504680.6	F1H2N2S1	6289	153;181;253;269;283;301.1;371.1;528.2			
6318	39.78	1199.5	511772.2	F1H2N2S1	6320	153;181;253;269;283;301.1;371.1;528.2			
6350	39.95	1199.5	403481.0	F1H2N2S1	6352	153;181;253;283;301.1;371.1;528.2			
6381	40.13	1199.5	269789.9	F1H2N2S1	6383	153;181;253;283;371.1			
6410	40.30	1199.5	128162.8	F1H2N2S1	6413	153;181;253;283;371.1;528.2			
6442	40.47	1199.5	58480.0	F1H2N2S1	6445	253;528.2			
6471	40.64	1199.5	24233.1	F1H2N2S1	6474	528.2			
4403	27.87	1226.6	36583.4	F1H1N3S1(OH)1	4405	153			
4431	28.04	1226.6	59574.8	F1H1N3S1(OH)1	4433	181;253			
4458	28.22	1226.6	48419.7	F1H1N3S1(OH)1	4461	153;253			
5459	34.31	1229.6	20967.7	H3N2S1	5461	167;253			
5540	34.82	1229.6	11833.9	H3N2S1	5542	167			
4592	29.01	1240.6	10734.9	F1H1N3S1	4598	253			
4626	29.19	1240.6	36635.0	F1H1N3S1	4631	253			
4659	29.37	1240.6	119817.7	F1H1N3S1	4661	181;253;283			
4688	29.54	1240.6	186606.2	F1H1N3S1	4690	153;181;253;283;301.1			
4720	29.72	1240.6	206692.2	F1H1N3S1	4723	153;181;253;269;283			
4754	29.89	1240.6	153613.0	F1H1N3S1	4756	153;181;253;283;301.1			

4780	30.06	1240.6	80431.9	F1H1N3S1	4782	153;181;253			
4806	30.23	1240.6	32660.6	F1H1N3S1	4809	253			
5343	33.59	1267.5	14245.8		5345	702.3			
6258	39.46	1267.5	32453.4		6260	253			
6291	39.63	1267.5	34591.2		6293	253			
6322	39.81	1267.5	30626.4		6324	253			
6355	39.98	1267.5	32135.3		6357	253			
6386	40.15	1267.5	16258.7		6388	253			
4644	29.29	1270.6	10961.1	H2N3S1	4647	371.1;528.2			
5010	31.52	1270.6	15055.0	H2N3S1	5013	181			
4662	29.39	1308.6	11957.4		4664	253			
5173	32.53	1345.6	27320.9		5176	153;253			
4886	30.73	1357.6	12957.8		4891	181			
6489	40.74	1357.6	21545.9		6491	283.1			
4916	30.92	1359.6	20459.7	F2H2N2S1(OH)1	4918	181			
4969	31.26	1359.6	50544.6	F2H2N2S1(OH)1	4971	153;181;371.1;702.3			
4996	31.43	1359.6	69474.4	F2H2N2S1(OH)1	4998	153;181;702.3			
5025	31.61	1359.6	66122.9	F2H2N2S1(OH)1	5028	181;702.3			
5054	31.78	1359.6	62796.8	F2H2N2S1(OH)1	5056	181;371.1			
5929	37.43	1359.6	26817.4	F2H2N2S1(OH)1	5932	153;181			
5956	37.61	1359.6	52618.2	F2H2N2S1(OH)1	5958	153;181;253			
5982	37.78	1359.6	94280.6	F2H2N2S1(OH)1	5985	153;181;253			
6010	37.96	1359.6	73979.7	F2H2N2S1(OH)1	6013	153;181;253			
6066	38.30	1359.6	21057.8	F2H2N2S1(OH)1	6069	181			
4982	31.35	1373.6	40485.4	F2H2N2S1	4984	181			
5010	31.52	1373.6	54533.1	F2H2N2S1	5012	181;283.1;702.3			
5040	31.70	1373.6	67632.4	F2H2N2S1	5042	153;181;253;371.1;702.3			
5068	31.87	1373.6	73986.7	F2H2N2S1	5070	181;253			
5096	32.04	1373.6	63257.6	F2H2N2S1	5098	153;181;253;371.1			
5125	32.21	1373.6	57367.3	F2H2N2S1	5127	153;181			
5151	32.38	1373.6	41585.5	F2H2N2S1	5153	371.1;702.3			
5178	32.55	1373.6	29170.2	F2H2N2S1	5180	153			
5233	32.90	1373.6	12655.5	F2H2N2S1	5236	702.3			
6178	38.98	1373.6	11844.8	F2H2N2S1	6181	181			
6209	39.16	1373.6	39827.3	F2H2N2S1	6211	181;253			
6236	39.33	1373.6	91340.1	F2H2N2S1	6239	153;181;253;269;283			
6266	39.50	1373.6	184709.1	F2H2N2S1	6269	153;181;253;283;301.1;371.1			
6300	39.68	1373.6	291115.2	F2H2N2S1	6303	153;181;253;269;283			
6332	39.85	1373.6	314516.2	F2H2N2S1	6335	153;181;253;283;301.1;371.1			
6364	40.02	1373.6	256770.0	F2H2N2S1	6367	153;181;253;269;283;301.1;371.1			
6392	40.20	1373.6	167452.9	F2H2N2S1	6396	153;181;253;283.1;301.1			
6424	40.38	1373.6	87265.0	F2H2N2S1	6427	153;253			
6455	40.55	1373.6	34227.6	F2H2N2S1	6458	253			
5775	36.44	1386.6	11196.2	H2N2Neu2S1	5777	528.2			
5806	36.65	1386.6	13257.6	H2N2Neu2S1	5808	181;371.1;528.2			
5834	36.83	1386.6	16276.7	H2N2Neu2S1	5838	528.2			
6264	39.49	1441.6	11352.1		6267	253			
6361	40.01	1441.6	15901.0		6365	253			
5115	32.16	1460.7	18600.5	H3N3S1(OH)1	5118	371.1;528.2			
5142	32.33	1460.7	21815.8	H3N3S1(OH)1	5144	153;371.1;528.2			
5167	32.50	1460.7	19086.2	H3N3S1(OH)1	5169	253;371.1;528.2			
5252	33.02	1460.7	13917.7	H3N3S1(OH)1	5254	371.1;528.2			
5279	33.19	1460.7	10649.1	H3N3S1(OH)1	5281	371.1			
5443	34.20	1474.7	10541.2	H3N3S1	5445	528.2			
5470	34.37	1474.7	32720.7	H3N3S1	5472	371.1;528.2			
5496	34.54	1474.7	52606.2	H3N3S1	5498	181;371.1;528.2	5500	528.2	153.1
5524	34.72	1474.7	68425.1	H3N3S1	5526	153;181;253;371.1;528.2	5527	528.2	180.9;371.2
5550	34.89	1474.7	44621.9	H3N3S1	5552	153;181;371.1;528.2	5553	528.2	
5577	35.06	1474.7	39792.1	H3N3S1	5579	181;528.2			
5601	35.23	1474.7	26952.7	H3N3S1	5603	181;371.1;528.2			
5624	35.41	1474.7	26016.7	H3N3S1	5627	528.2			
5648	35.58	1474.7	23091.1	H3N3S1	5653	528.2			
5761	36.36	1474.7	23346.5	H3N3S1	5763	181;528.2			
5788	36.54	1474.7	39393.5	H3N3S1	5790	153;181;371.1;528.2			
5816	36.71	1474.7	45744.3	H3N3S1	5818	181;371.1;528.2			
5843	36.89	1474.7	31760.2	H3N3S1	5845	181;371.1;528.2			
5870	37.06	1474.7	22536.5	H3N3S1	5872	181;371.1			
5895	37.22	1474.7	20966.0	H3N3S1	5898	528.2			
6418	40.35	1560.7	11704.6	F1H2N2Neu1S1	6422	702.3			
6479	40.69	1560.7	23842.1	F1H2N2Neu1S1	6482	702.3			
6508	40.86	1560.7	32574.8	F1H2N2Neu1S1	6510	181;702.3			
6537	41.03	1560.7	28296.8	F1H2N2Neu1S1	6539	253;702.3			
6565	41.20	1560.7	19631.5	F1H2N2Neu1S1	6567	702.3			
6451	40.52	1560.7	15745.3	F1H2N2Neu1S1	6453	371.1;702.3			
6591	41.38	1560.7	13221.3	F1H2N2Neu1S1	6594	702.3			
6440	40.46	1648.8	10146.3	F1H3N3S1	6443	528.2;702.3			

The MS2 scan entries were first filtered from the raw dataset based on its containing at least a sulfated glycotope-specific ion detected at 10 ppm. This created a full list of 297 MS2 scans, each tracked back to its preceding MS1 scans, including 4 pairs of duplicate entries due to the same MS2 scan was used to trigger 2 events of pd-MS3, namely m/z 528 and 702. Both the MS1 and MS2 scan columns were shaded in light blue with the aforementioned duplicate pairs in darker blue shades. Subsequently, additional filterings were applied in the following order: 1) retention time within 20-42 min, 2) eliminate those few entries with elution time inconsistent with its m/z, and 3) those MS2 scans containing only a single ion at m/z 195, 296 and 371. This led to an "edited" list of 258 scans in total. The cells in the column for monoisotopic m/z was colored if it can be fitted to glycosyl composition (H, Hex; N, HexNAc; F, Fuc; Neu, NeuAc; S, sulfate; OH, free hydroxyl group due to under methylation) and differentiated in color shades by whether its MS2 contained m/z 528 (gold), m/z 702 (light orange), both (darker orange), or neither (pale yellow). Cells in the MS2 selected Ions column were similarly color coded according to whether it contained m/z 528 (gold), 702 (orange) or both (darker orange). Cells in the column for MS1 precursor intensity with a value > 50,000 were colored in different shades of green to reflect abundance, except for the 3 larger glycan isomers at m/z 1460.7, 1474.7 and 1560.7, which peaked at 32.33 min, 36.71 min, and 40.86 min, respectively, at lower intensity values.

Table S4. **NanoLC-MS^2-pd-MS^3 dataset for the permethylated N-glycans of mouse brain striatum**, in multiple Excel sheets (available online as standalone Supplemental Data file):

1) **Info & Glycotope Sheet** (this page) tabulates the input parameters for data mining using GlyPick, and the summed intensity and spectral counts data for the selected MS^2 and MS^3 diagnostic ions. Under-methylation and the additional ammonium and sodium adducts were not considered in the initial round of glycosyl composition fitting to generate the **A-list**. Fitted MS^2 scans will be removed for a second iteration to generate the **B-list**. The remaining MS^2 scans, which could not be fitted were compiled into **C-list.**

2) **A-list Sheets** present the compiled A-list in 3 different views. The **first** is the most comprehensive list containing a total of 3840 MS^2 scans (after editing out unreasonable ones) grouped into 454 unique precursor N-glycans. MS^1 intensity and MS^2 entries containing the target MS^2 and MS^3 ions are color-shaded for ease of reference. The **second** is a simplified list after removing the individual entries, keeping only the headers for a total of **352** unique N-glycans with summed MS^1 and MS^2 ion intensities. Entries corresponding to the most abundant N-glycans (by summed MS1 intensity) are highlighted, whereas those seemingly fitting to unreasonable glycosyl composition were hidden (but not deleted). The **third** is a glycotope-centric list containing only MS^2 entries associated with successful pd-MS^3. In this case, there were **291** successful pd-MS^3 scans on either *m/z* 1186 or 737, but only **223** of which fitted to glycosyl composition having at least 2 sialic acids to make DiSia possible. 91 were contributed by m/z 737, 132 by m/z 1186.

3) **B-list Sheets** present the compiled B-list in 3 different views similar to the A-list. No further manual editing was made. A total of **345** successful pd-MS^3 scans were triggered on either *m/z* 1186 or 737, **221** of which fitted to glycosyl composition having at least 2 sialic acids, with 86 contributed by *m/z* 737, and 135 by *m/z* 1186.

4) **C-list Sheet** compiled the MS^2 entries that could not be fitted to glycosyl composition even after considering under-methylation and extra adducts. No grouping nor editing was attempted. Using the charge state determined for the *m/z* of precursor, the assumed $[M+H]^+$ *m/z* values were computed. This list contains an additional **118** productive MS^3 scans triggered on either *m/z* 1186 (66) or 737 (52) but no further filtering by the criteria of containing at least 2 Sia in deduced glycosyl composition could be applied.

Glycotope	Count	Intensity	%
260	22475	1509531830	32%
376	20120	865049643	19%
450	19897	1021687854	22%
464	17488	411365113	9%
505	2	13225	0%
624	326	1650824	0%
638	19291	569428526	12%
679	91	588653	0%
737	3805	16205788	0%
812	58	558097	0%
825	5889	192221267	4%
913	47	279377	0%
999	163	1251940	0%
1186	7683	71170101	2%
		4661002238	100%

MS2	MS3	Glycotope	Count	Intensity	%	%
638.3	228	H1	131	29897	1%	1%
638.3	606	H2	1931	1170971	28%	26%
638.3	402	LeA	158	63563	2%	1%
638.3	432	LeX	6796	2938237	70%	64%
				4202668	100%	92%
737.4	376	DiSia	273	102735		2%
1186.6	737	DiSia1	358	264043	93%	6%
1186.6	589	DiSia2	33	19448	7%	0%
				283491	100%	6%
		All Total:	9680	4588893		100%

Number of Spectra	MS1	MS2	MS3
Total	4141	26210	11608
Within RT	2018	14035	11429
Selected by MS2 ions	1705	11953	11380
Fitted Composition	704	2540	3629

Spectral Settings	
MS1 Tolerance	5 ppm
Starting Time	20 min
Ending Time	70 min
Min selected ms2 ion	2
MS2 Tolerance	5 ppm
MS2 Threshold	100
MS2 Relative %	1
MS3 Tolerance	0.5 Da
MS3 Threshold	0
MS3 Relative %	0

Glycan Settings		
Number of Residues	Max	Min
dHex	5	0
Hex	9	0
HexNAc	7	0
NeuAc	7	0
Reducing end	Free	
Form	Permethylated	
Ion Mode	Positive	
Under PerMe	2	
N-glycan Rules:		
Trimannosyl core		
Antenna (2(total HexNAc-2>=dHex-1)		
LacNac(NeuAc+(dHex-1) <= LacNac*2)		
Adducts & Charges		
Adduct Settings:	H, NH3, Na	
Charge State	2, 3, 4	
MS3 Glycotopes		
MS2 Precursors	Name	MS3 Ions
1186.596	DiSLN1	737.4
1186.596	DiSLN2	589.3
737.370	DiS	376.2

Table S5. List of MS2 scans containing the diagnostic ion at m/z 1186 for the target disialyl LacNAc glycotope, which yielded the diagnostic pd-MS3 ion at m/z 737. The entries compiled in this Table were filtered from the same nanoLC-MS/MS dataset reported in Table S4 but arranged in a slightly different format and sorted according to the MS2 scan numbers. The corresponding annotated MS2 spectra of these 71 entries are collated in a Supplemental Figure S7. MS2 scans containing m/z 1186 but not further triggered for MS3 or did not give m/z 737 in the resulting MS3 spectra were excluded. Those that could not be fitted with glycosyl compositions containing ≥2 NeuAc were also discarded.

MS2 Scan	MS2 Selected Ions	MS1 Scan	m/z (Trig)	m/z (Mono)	Z	Intensity	[M+Na]+ m/z	dHex	Hex	Hex NAc	Neu Ac	MS3 Scan	pd MS2 Ion	Matched m/z	Deduced Glycotopes
18331	260.1;376.2;464.2;638.3;1186.6	18273	1370.697	1370.697	2	663060	2762.373	1	4	4	2	18335	1186.6	737.7	DiSLN1
19290	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	19253	1493.769	1493.265	2	1551854	3007.499	1	4	5	2	19293	1186.6	737.5	DiSLN1
19502	260.1;376.2;450.2;464.3;638.3;825.4;1186.6	19461	1473.757	1472.752	2	1401877	2966.472	1	5	4	2	19506	1186.6	737.5	DiSLN1
19932	260.1;376.2;450.2;464.2;638.3;825.4;999.5;1186.6	19888	1077.891	1077.555	3	2940393	3252.625	1	4	6	2	19936	1186.6	737.8	DiSLN1
19989	260.1;376.2;450.2;464.2;638.3;1186.6	19922	1653.843	1653.343	2	842607	3327.646	1	5	4	3	19992	1186.6	737.5	DiSLN1
20100	260.1;376.2;450.2;464.2;1186.6	20032	1662.351	1661.851	2	757075	3344.661	2	6	4	2	20103	1186.6	737.7	DiSLN1
20432	260.1;376.2;450.2;464.2;638.3;1186.6	20396	1145.924	1145.590	3	5909167	3456.725	1	5	6	2	20443	1186.6	737.5	DiSLN1
20507	260.1;376.2;450.2;464.2;638.3;1186.6	20469	1112.900	1112.566	3	1844131	3357.657	0	6	4	3	20511	1186.6	737.6	DiSLN1
20803	260.1;376.2;450.2;464.3;638.3;1186.6	20766	1040.535	1040.201	3	4078534	3140.562	2	5	4	2	20807	1186.6	737.6	DiSLN1
20834	260.1;376.2;450.2;638.3;825.4;1186.6	20766	1682.360	1682.360	2	787536	3385.688	2	5	5	2	20838	1186.6	737.6	DiSLN1
20954	260.1;376.2;450.2;464.2;638.3;1186.6	20914	1311.004	1310.335	3	4265628	3950.961	2	6	5	3	20961	1186.6	737.6	DiSLN1
21482	260.1;376.2;450.2;464.2;638.3;1186.6	21433	1455.075	1454.407	3	1976968	4383.172	1	6	6	4	21485	1186.6	737.6	DiSLN1
21687	260.1;376.2;450.2;464.2;638.3;1186.6	21618	1405.712	1405.712	3	751699	4237.114	2	5	7	3	21691	1186.6	737.6	DiSLN1
22000	260.1;376.2;450.2;464.2;638.3;1186.6	21958	1252.974	1252.306	3	1811328	3776.872	1	6	5	3	22004	1186.6	737.6	DiSLN1
22259	260.1;376.2;450.2;464.2;638.3;999.5;1186.6	22220	1204.287	1203.617	3	6267915	3630.814	2	5	6	2	22262	1186.6	737.5	DiSLN1
22386	260.1;376.2;450.2;464.2;638.3;1186.6	22334	1266.981	1265.979	3	1825801	3817.899	1	5	6	3	22390	1186.6	737.5	DiSLN1
22642	260.1;376.2;450.2;464.2;638.3;1186.6	22598	1001.264	1000.764	4	1834442	4021.998	1	6	6	3	22648	1186.6	737.5	DiSLN1
22652	260.1;376.2;450.2;464.3;638.3;1186.6	22598	1305.997	1304.661	3	1118642	3933.946	1	5	5	4	22656	1186.6	737.4	DiSLN1
22661	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	22598	1575.463	1575.463	3	970759	4746.361	0	8	8	3	22665	1186.6	737.5	DiSLN1
22679	260.1;376.2;450.2;464.2;638.3;1186.6	22638	1120.821	1120.068	4	2558044	4499.219	1	6	5	5	22682	1186.6	737.5	DiSLN1
22709	260.1;376.2;450.2;464.2;638.3;1186.6	22638	1320.339	1320.339	3	1039343	3980.972	1	7	5	3	22714	1186.6	737.4	DiSLN1
22803	260.1;376.2;450.2;464.2;638.3;1186.6	22754	1455.741	1455.741	3	2619889	4387.192	4	7	6	2	22807	1186.6	737.4	DiSLN1
22896	260.1;376.2;450.2;464.2;638.3;1186.6	22836	1528.453	1528.112	3	905456	4604.287	2	8	6	3	22899	1186.6	737.6	DiSLN1
22914	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	22874	1431.395	1430.727	3	2260900	4312.135	2	6	5	4	22920	1186.6	737.7	DiSLN1
23066	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	23022	1493.755	1493.755	3	1497918	4501.235	0	8	7	3	23069	1186.6	737.6	DiSLN1
23080	260.1;376.2;450.2;464.2;638.3;1186.6	23022	1524.112	1523.776	3	859800	4591.292	4	8	6	2	23086	1186.6	737.6	DiSLN1
23103	260.1;376.2;450.2;464.3;638.3;1186.6	23063	1373.697	1372.696	3	2554051	4138.046	1	6	5	4	23108	1186.6	737.8	DiSLN1
23128	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	23063	1614.150	1614.150	3	823397	4862.409	0	8	7	4	23133	1186.6	737.1	DiSLN1
23230	260.1;376.2;450.2;464.2;638.3;1186.6	23170	1460.747	1460.079	3	2000960	4400.187	2	7	6	3	23235	1186.6	737.6	DiSLN1
23286	260.2;376.2;450.2;464.2;1186.6	23249	1523.107	1522.440	3	5414422	4587.272	1	7	6	4	23290	1186.6	737.5	DiSLN1
23559	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	23509	1473.752	1473.752	3	1265933	4441.214	2	6	7	3	23567	1186.6	737.6	DiSLN1
23649	260.1;376.2;450.2;464.2;638.3;1186.6	23583	1537.792	1537.452	3	913390	4632.318	4	7	7	2	23655	1186.6	737.3	DiSLN1
23661	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	23622	1186.105	1185.604	4	8732457	4761.361	2	7	6	4	23670	1186.6	737.7	DiSLN1
23807	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	23733	1378.370	1378.370	3	1422772	4155.061	2	7	5	3	23811	1186.6	737.5	DiSLN1
23997	260.1;376.2;450.2;464.2;638.3;1186.6	23960	1233.127	1232.376	4	10064271	4948.446	1	7	6	5	24001	1186.6	737.6	DiSLN1
24097	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	24035	1386.698	1386.369	3	975443	4179.072	1	5	6	4	24105	1186.6	737.5	DiSLN1
24435	260.1;376.2;450.2;464.2;638.3;1186.6	24383	1387.706	1387.706	3	1072652	4183.092	4	6	6	2	24444	1186.6	737.5	DiSLN1
24437	260.1;376.2;450.2;464.3;638.3;737.4;1186.6	24383	1513.438	1512.433	3	905481	4557.261	2	6	6	4	24442	1186.6	737.6	DiSLN1
24491	260.1;376.2;450.2;464.2;638.3;1186.6	24456	1139.334	1138.833	4	5078384	4574.277	3	7	6	3	24496	1186.6	737.5	DiSLN1
24627	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	24566	1764.224	1763.222	3	1068922	5309.619	1	7	6	6	24632	1186.6	737.6	DiSLN1
24681	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	24641	1503.750	1503.750	4	1560541	6033.982	0	9	8	6	24684	1186.6	737.6	DiSLN1
24717	260.1;376.2;450.2;464.2;638.3;1186.6	24680	1258.646	1257.978	3	8814630	3793.887	2	7	5	2	24723	1186.6	737.8	DiSLN1
24797	260.1;376.2;450.2;464.2;638.3;1186.6	24757	1127.580	1127.580	4	1953409	4529.266	2	7	8	2	24801	1186.6	737.7	DiSLN1
24853	260.1;376.2;450.2;464.3;638.3;737.4;1186.6	24794	1441.397	1440.726	3	827553	4342.146	1	7	5	4	24861	1186.6	737.4	DiSLN1
24875	260.1;376.2;450.2;464.3;638.3;825.4;1186.6	24832	1049.041	1048.540	4	3254083	4213.103	3	7	6	2	24881	1186.6	737.5	DiSLN1
25014	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	24952	1512.431	1508.754	3	953800	4546.245	1	8	5	4	25024	1186.6	737.6	DiSLN1
25054	260.1;376.2;450.2;464.2;638.3;1186.6	24994	1656.836	1656.500	3	882947	4989.472	1	6	7	5	25058	1186.6	737.6	DiSLN1
25073	376.2;464.3;638.3;737.4;1186.6	24994	1884.946	1884.946	3	713349	5674.813	4	8	6	5	25074	1186.6	737.5	DiSLN1
25154	260.1;376.2;450.2;464.2;638.3;737.4;1186.6	25113	1366.692	1366.692	4	2370569	5485.724	1	9	8	4	25162	1186.6	737.5	DiSLN1
25508	260.2;376.2;450.2;464.3;638.3;1186.6	25456	1763.885	1763.885	3	995552	5311.635	0	9	8	4	25512	1186.6	737.6	DiSLN1
25572	260.1;376.2;450.2;464.2;638.3;1186.6	25530	1271.651	1271.651	3	1854444	3834.914	2	6	6	2	25577	1186.6	737.5	DiSLN1
25581	260.1;376.2;450.2;464.2;638.3;1186.6	25530	1701.529	1701.529	3	1021070	5124.550	1	9	8	3	25585	1186.6	737.5	DiSLN1
25823	260.2;376.2;450.2;464.3;638.3;825.4;1186.6	25756	1498.762	1498.762	3	681820	4516.235	2	7	5	4	25828	1186.6	737.7	DiSLN1
25889	260.1;376.2;450.2;464.3;638.3;825.4;1186.6	25833	1513.106	1513.106	3	786230	4559.277	1	8	8	2	25893	1186.6	737.3	DiSLN1
26311	260.1;376.2;450.2;464.3;638.3;825.4;1186.6	26268	1431.726	1431.726	4	998725	5745.850	3	8	7	5	26315	1186.6	737.4	DiSLN1
26358	260.1;376.2;450.2;464.2;638.3;1186.6	26300	1600.475	1599.812	3	563429	4819.403	3	7	7	3	26365	1186.6	737.4	DiSLN1
26548	260.1;376.2;450.2;464.2;638.3;737.4;825.4;1186.6	26513	1330.350	1329.682	3	3688103	4009.003	3	6	6	2	26560	1186.6	737.4	DiSLN1
26563	260.1;376.2;450.2;464.2;638.3;1186.6	26513	1258.642	1258.642	4	1012299	5053.513	2	9	7	3	26567	1186.6	737.6	DiSLN1
26654	260.1;376.2;450.2;464.2;638.3;1186.6	26584	1658.834	1658.506	3	597124	4995.471	1	9	6	4	26659	1186.6	737.5	DiSLN1
26854	260.1;376.2;450.2;464.3;638.3;737.4;1186.6	26807	1450.729	1450.729	3	619511	4372.156	0	8	5	4	26863	1186.6	737.3	DiSLN1
26926	260.1;376.2;450.2;464.2;638.3;1186.6	26878	1668.515	1667.844	3	920447	5023.503	3	8	7	3	26935	1186.6	737.4	DiSLN1
26995	260.1;376.2;450.2;464.2;638.3;1186.6	26947	1382.701	1382.701	3	738692	4168.056	0	7	5	4	26999	1186.6	737.7	DiSLN1
27110	260.1;376.2;450.2;464.2;638.3;1186.6	27051	1600.803	1600.478	3	651839	4821.382	0	9	6	4	27114	1186.6	737.6	DiSLN1
27235	260.1;376.2;450.2;464.2;638.3;1186.6	27165	1595.815	1595.476	3	543484	4806.408	5	7	7	2	27238	1186.6	737.6	DiSLN1
27324	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	27277	1264.643	1264.643	4	1127432	5077.525	1	7	8	4	27333	1186.6	737.6	DiSLN1
27577	260.2;376.2;450.2;464.3;638.3;737.4;1186.6	27534	1418.070	1417.721	3	1119796	4273.124	1	9	6	2	27582	1186.6	737.6	DiSLN1
27875	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	27822	1518.771	1518.771	4	684789	6094.028	5	8	7	5	27879	1186.6	737.6	DiSLN1
27972	260.1;376.2;450.2;464.2;638.3;1186.6	27934	1606.153	1605.485	3	691610	4836.418	4	8	7	2	27980	1186.6	737.6	DiSLN1
27984	260.1;376.2;450.2;638.3;1186.6	27934	1551.786	1551.786	3	485410	4675.324	1	8	7	3	27988	1186.6	737.5	DiSLN1
28251	260.1;376.2;450.2;464.2;624.3;638.3;1186.6	28217	1360.445	1360.190	4	965910	5459.734	5	9	8	2	28259	1186.6	737.3	DiSLN1
28443	260.1;376.2;450.2;464.2;638.3;825.4;1186.6	28388	1192.613	1192.613	4	338337	4789.392	4	6	7	3	28447	1186.6	737.7	DiSLN1