Supporting Information

for

Converting *Pasteurella multocida* α2–3-sialyltransferase 1 (PmST1) to a regioselective α2–6-sialyltransferase by saturation mutagenesis and regioselective screening

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Table S1. ^{13}C NMR chemical shift assignment for LNnTβProN_3 and Neu5Acα2–6LNnTβProN_3.

Residue	Carbon atom	Chemical shift (ppm)	
	C	**Galβ1–4GlcNAcβ1–3Galβ1–4GlcβProN_3 (LNnTβProN_3)**	**Neu5Acα2–6LNnTβProN_3**
4Glcβ1–	1	101.98	101.98
	2	72.66	73.56
	3	74.22	74.23
	4	78.19	78.22
	5	74.64	74.65
	6	59.91	59.90
3Galβ 1–	1	102.73	102.82
	2	69.83	69.84
	3	81.92	81.89
	4	68.20	68.19
	5	74.75	74.76
	6	60.83	60.84
4GlcNAcβ 1–	1	102.81	102.47
	2	55.06	54.80
	3	72.05	72.11
	4	**77.99**	**80.30**
	5	74.42	74.13
	6	59.71	59.99
	C=O	174.83	174.78
	CH_3	22.03	22.16
6Galβ1–	1	102.63	103.33
	2	70.83	70.60
	3	72.37	72.41
	4	68.42	68.27
	5	75.22	72.66
	6	**60.91**	**63.22**
Neu5Acα2–	1		173.43
	2		100.00
	3		39.94
	4		68.08
	5		51.76
	6		72.29
	7		68.23
	8		71.59
	9		62.52
	C=O		174.81
	CH_3		21.91
ProN_3	$O\underline{C}H_2CH_2CH_2N_3$	67.24	67.24
	$OCH_2\underline{C}H_2CH_2N_3$	28.10	28.10
	$OCH_2CH_2\underline{C}H_2N_3$	47.73	47.73

Figure S1. pH profiles of sialylation yields and sialyl product linkage specificities of PmST1-catalyzed reactions. A, α2–3-sialyl product percentages; B, α2–6-sialyl product percentages; and C, sialylation yields.

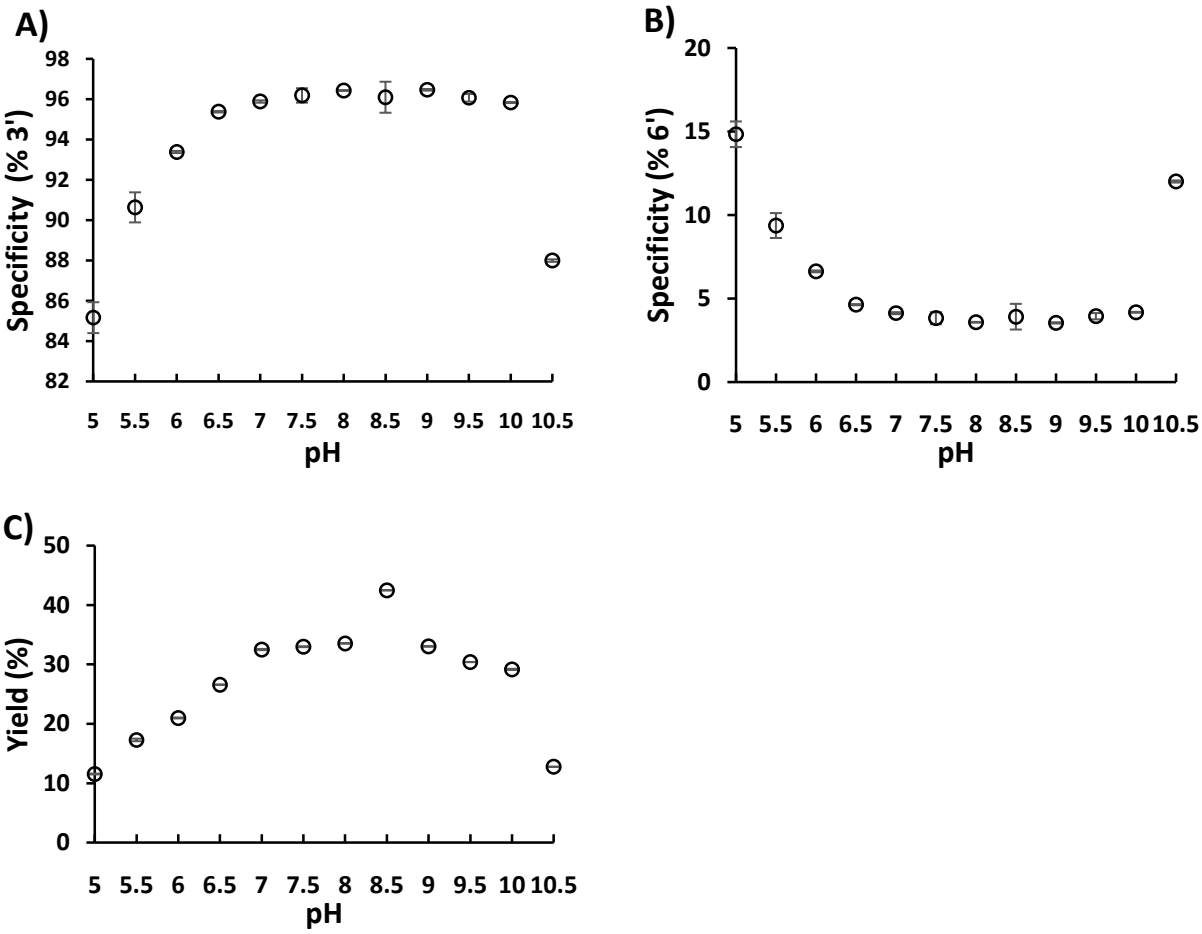


Figure S2. pH profiles of α2–6-sialyl product percentages (A) and sialylation yields (B) of PmST1 P34H/M144L-catalyzed reactions.

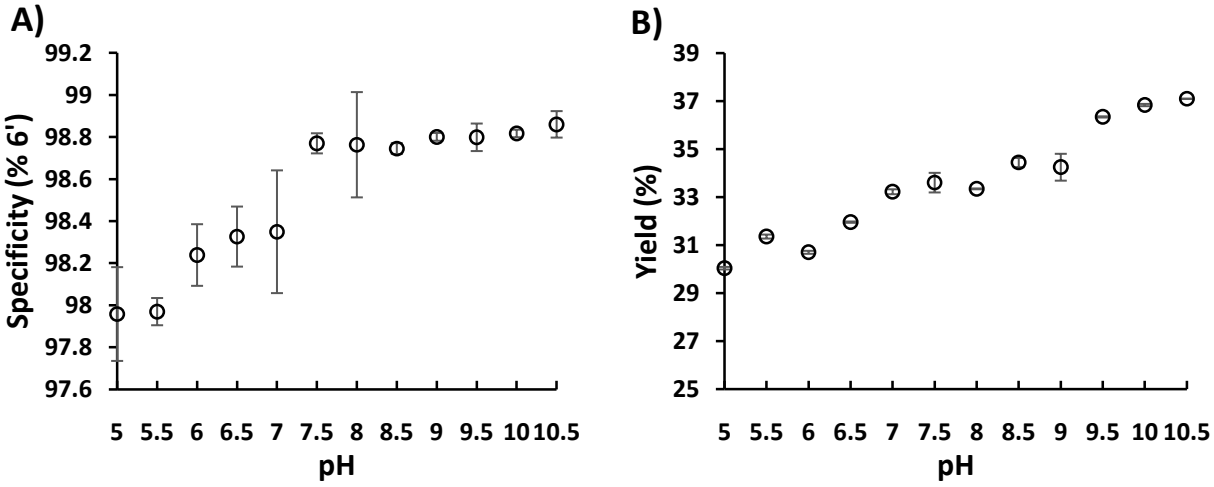
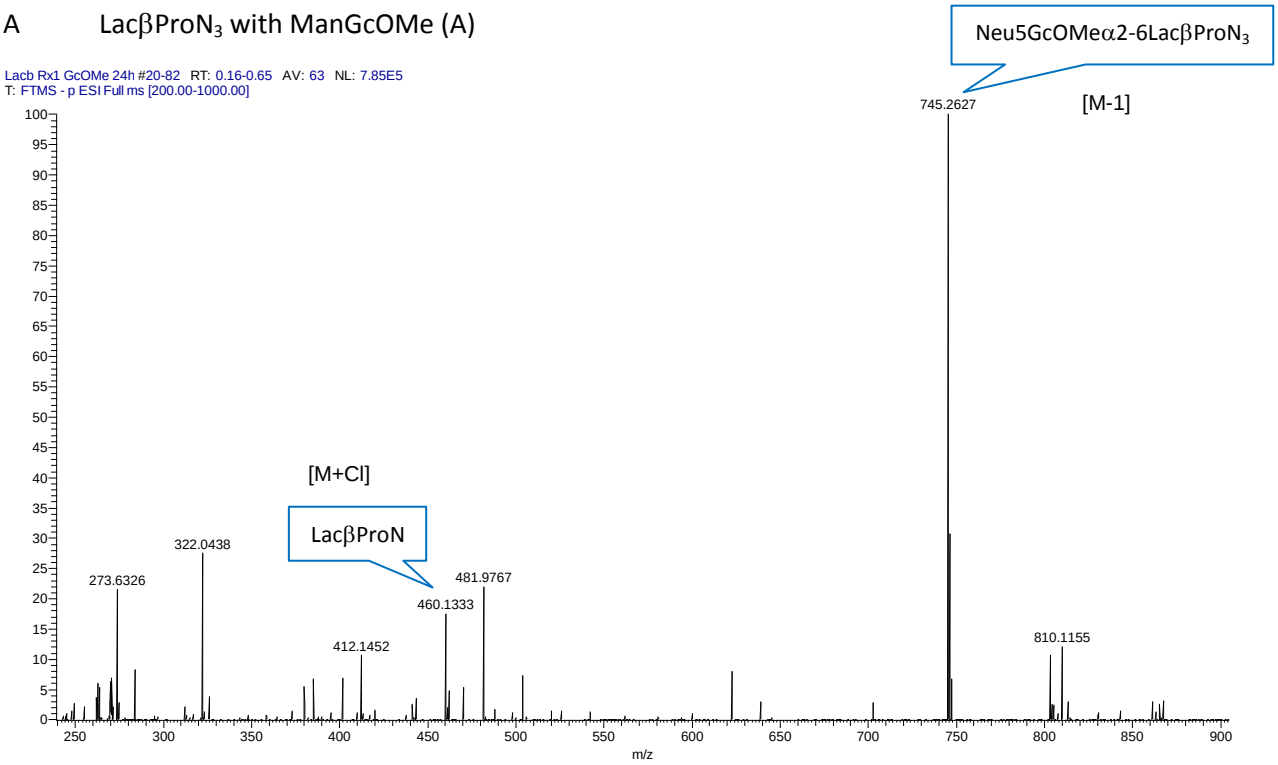
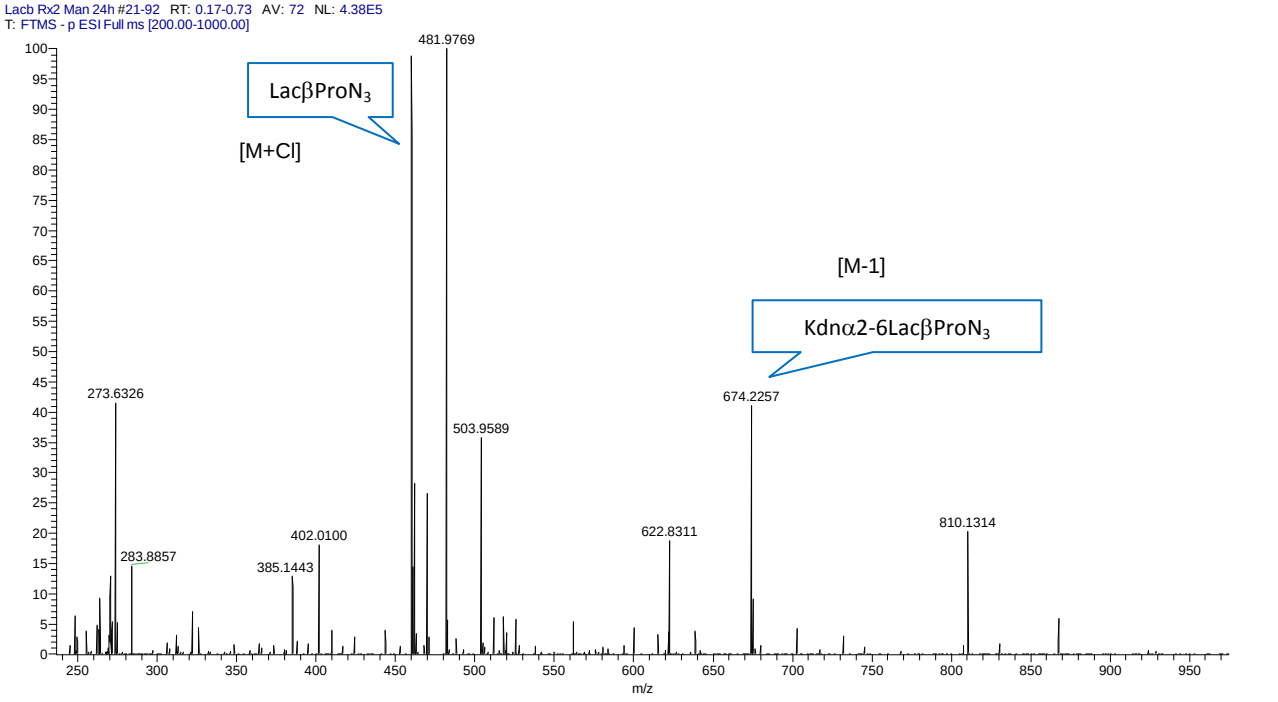


Figure S3. Donor substrate promiscuity of PmST1 P34H/M144L confirmed by HRMS results.

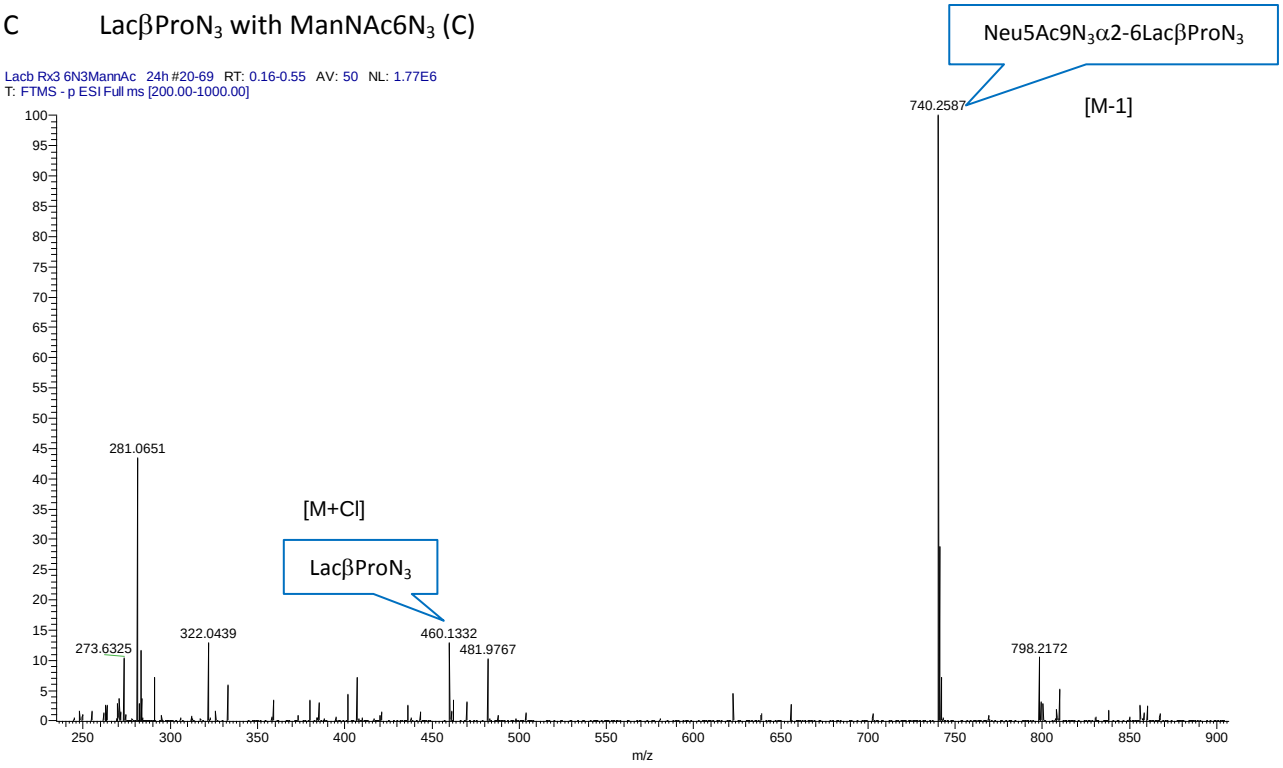
A Lacβ$ProN_3$ with ManGcOMe (A)



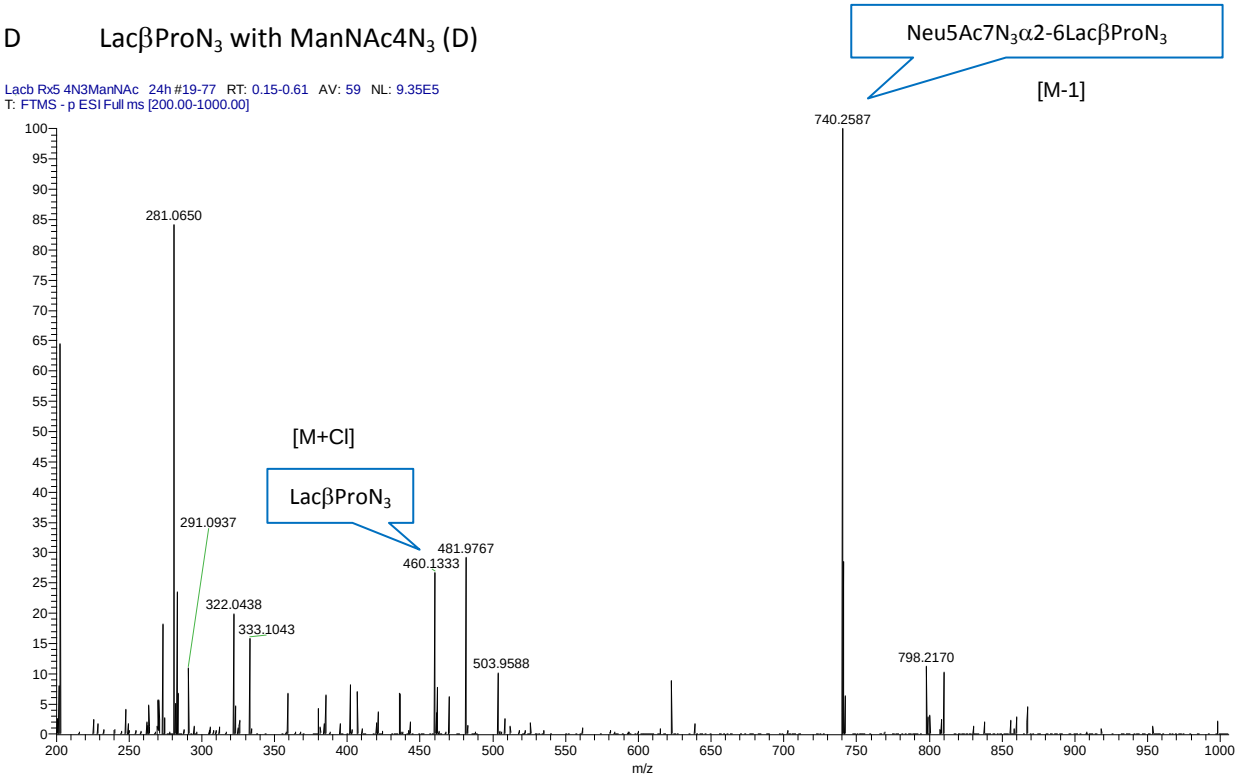
B Lacβ$ProN_3$ with mannose (B)



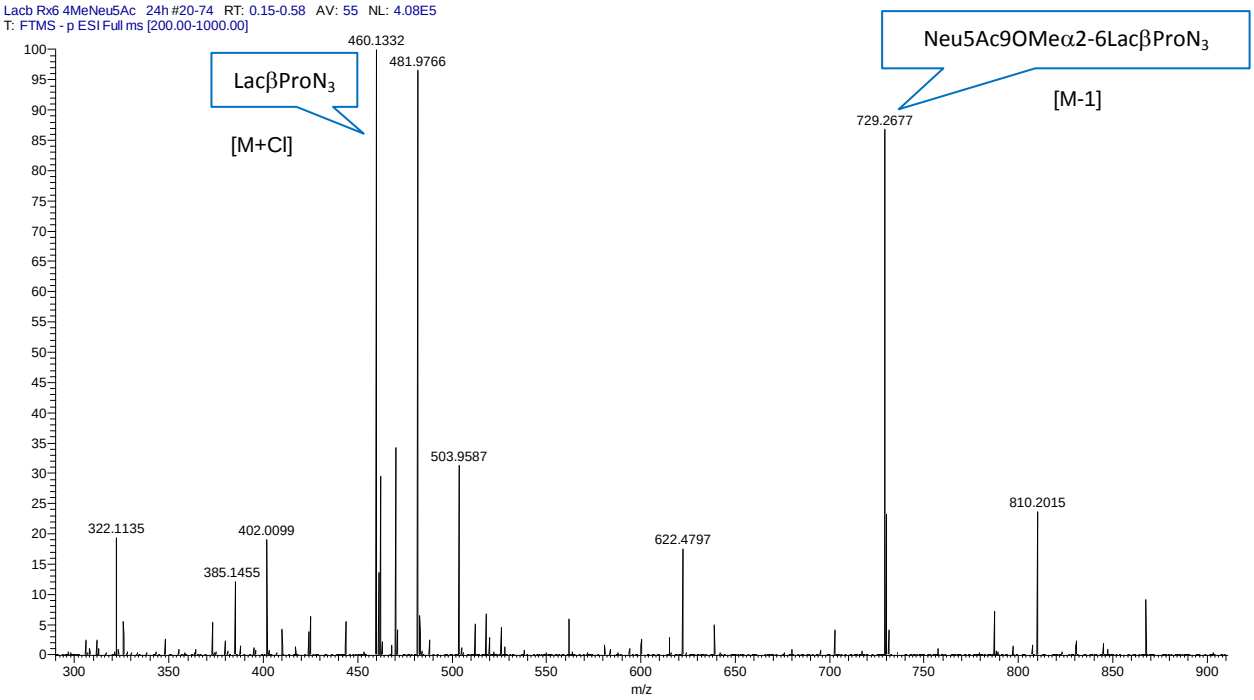
C LacβProN$_3$ with ManNAc6N$_3$ (C)



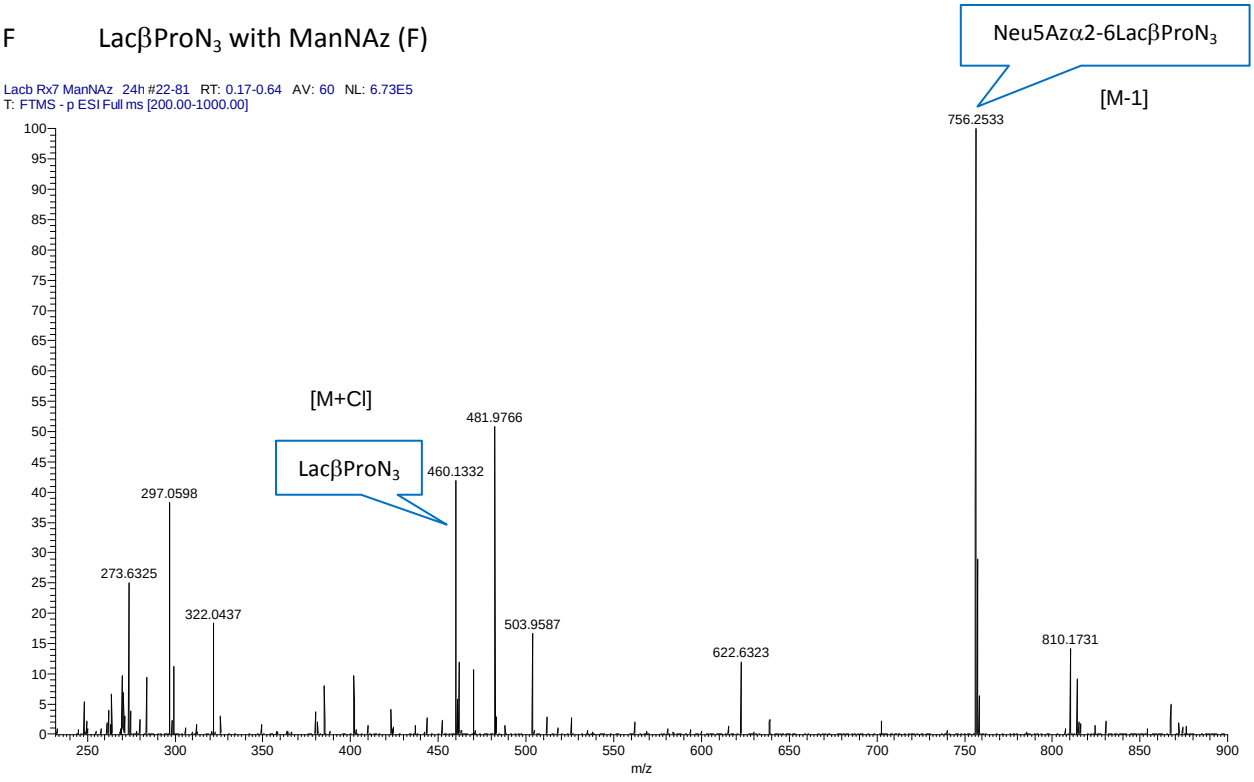
D LacβProN$_3$ with ManNAc4N$_3$ (D)



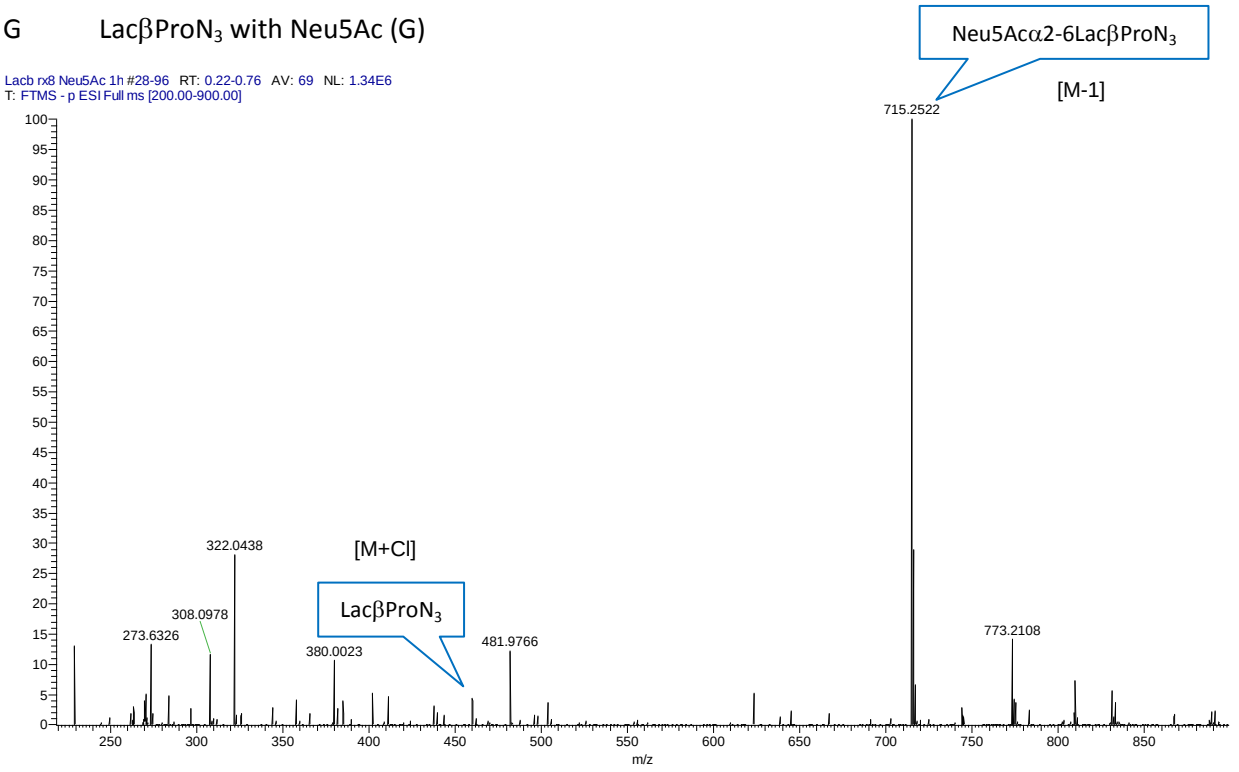
E Lacβ$ProN_3$ with ManNAc6OMe (E)



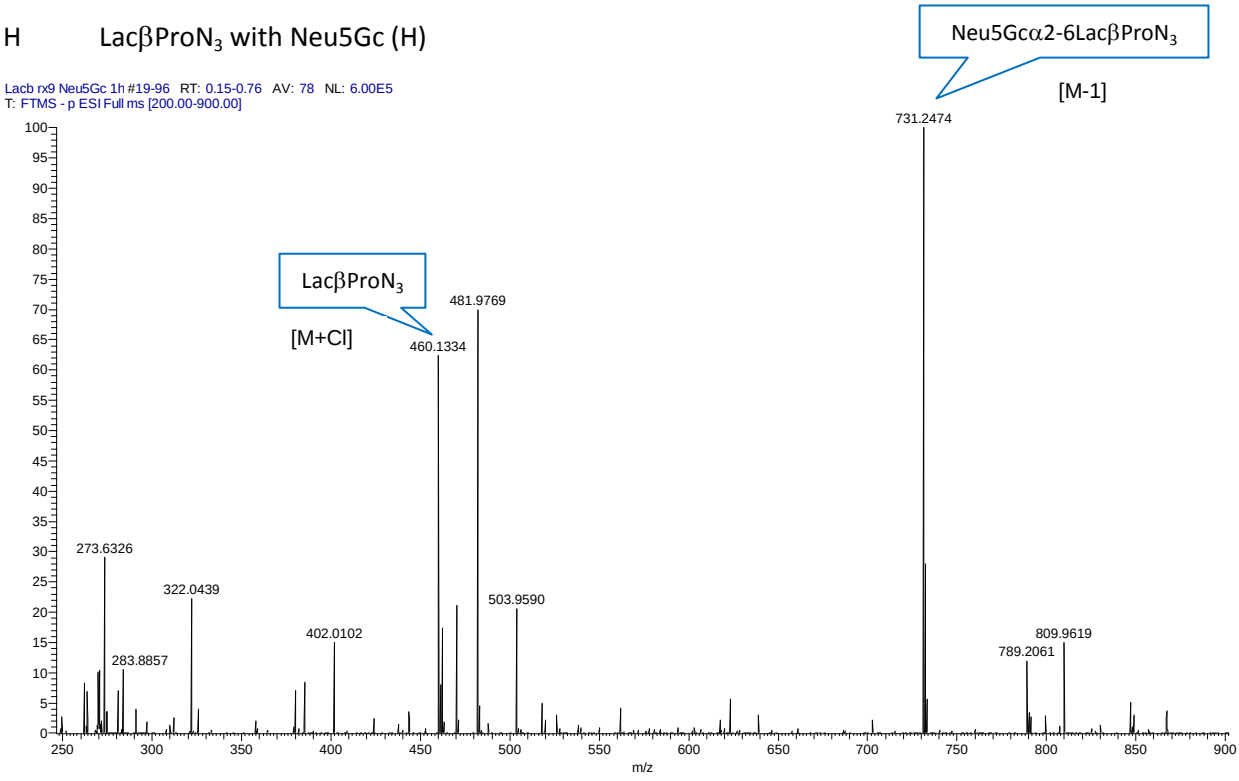
F Lacβ$ProN_3$ with ManNAz (F)



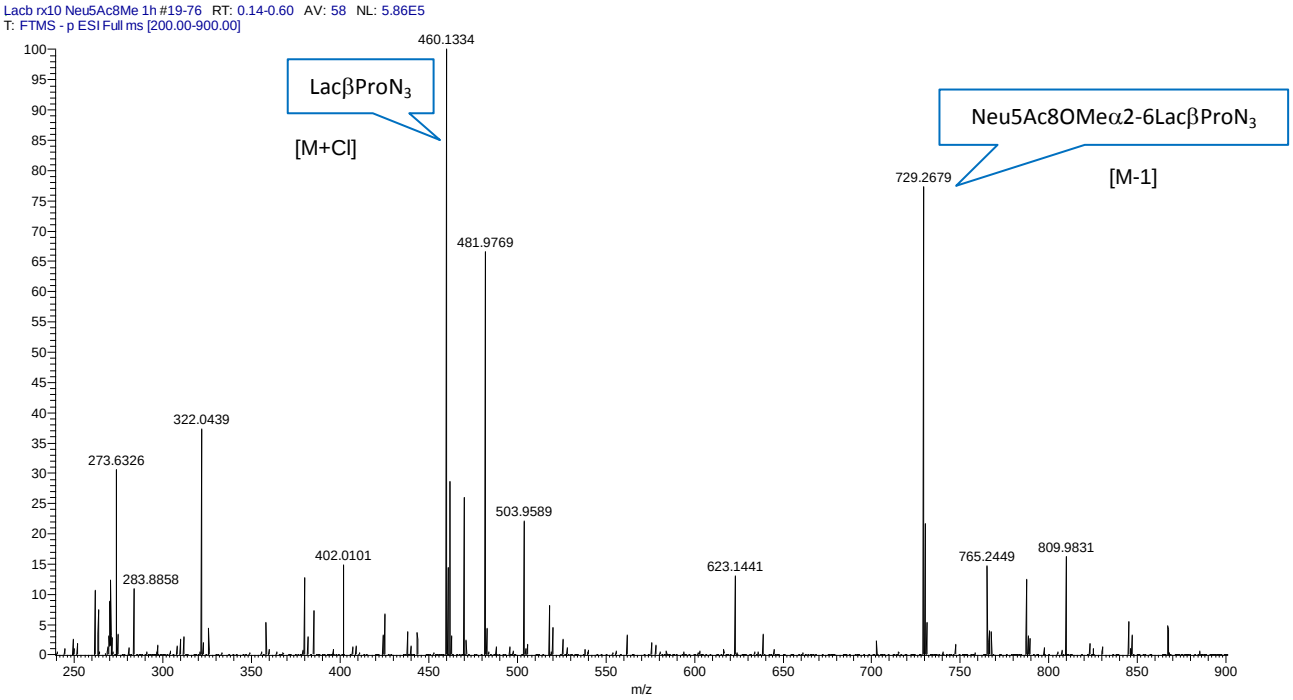
G LacβProN$_3$ with Neu5Ac (G)



H LacβProN$_3$ with Neu5Gc (H)



I LacβProN$_3$ with Neu5Ac8OMe (I)



J LacβProN$_3$ with Neu5,9Ac$_2$ (J)

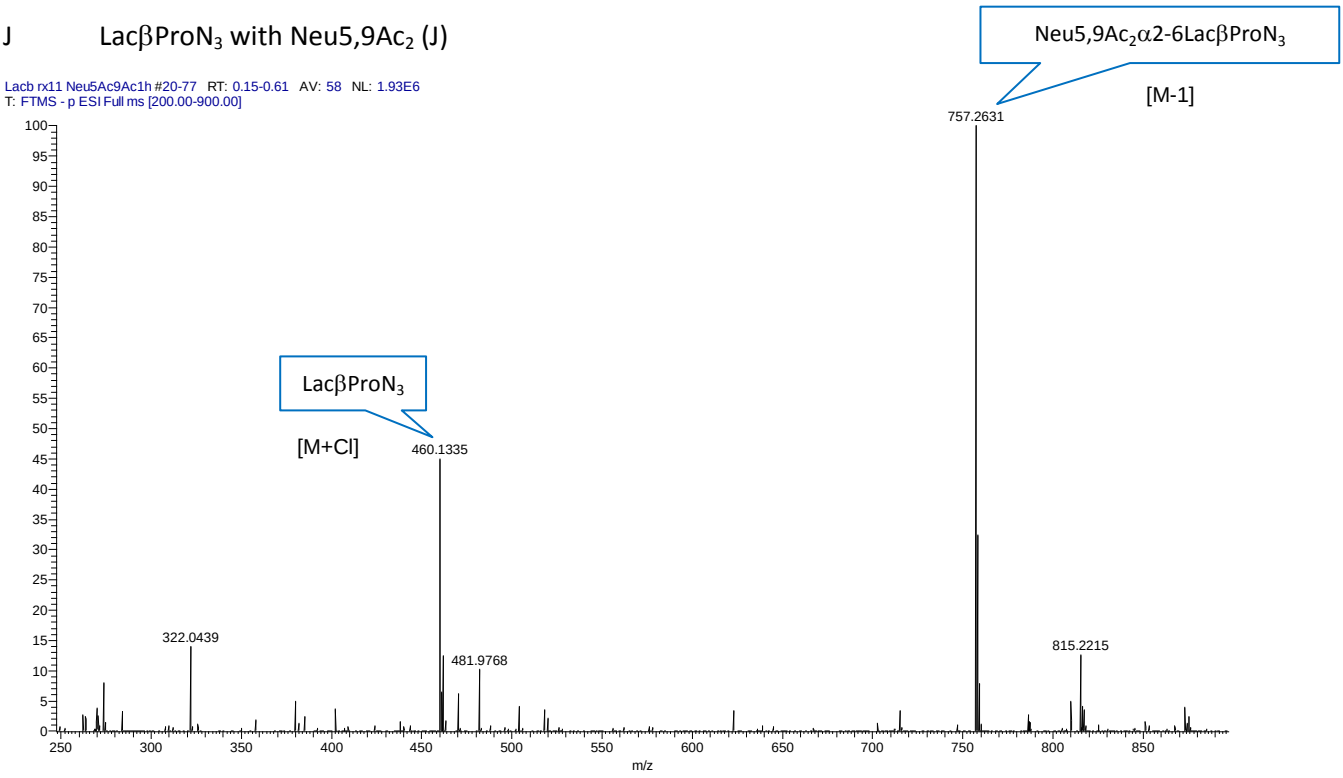


Figure S4. ^{1}H and ^{13}C NMR spectra of Neu5Acα2–6LNnTβProN$_3$

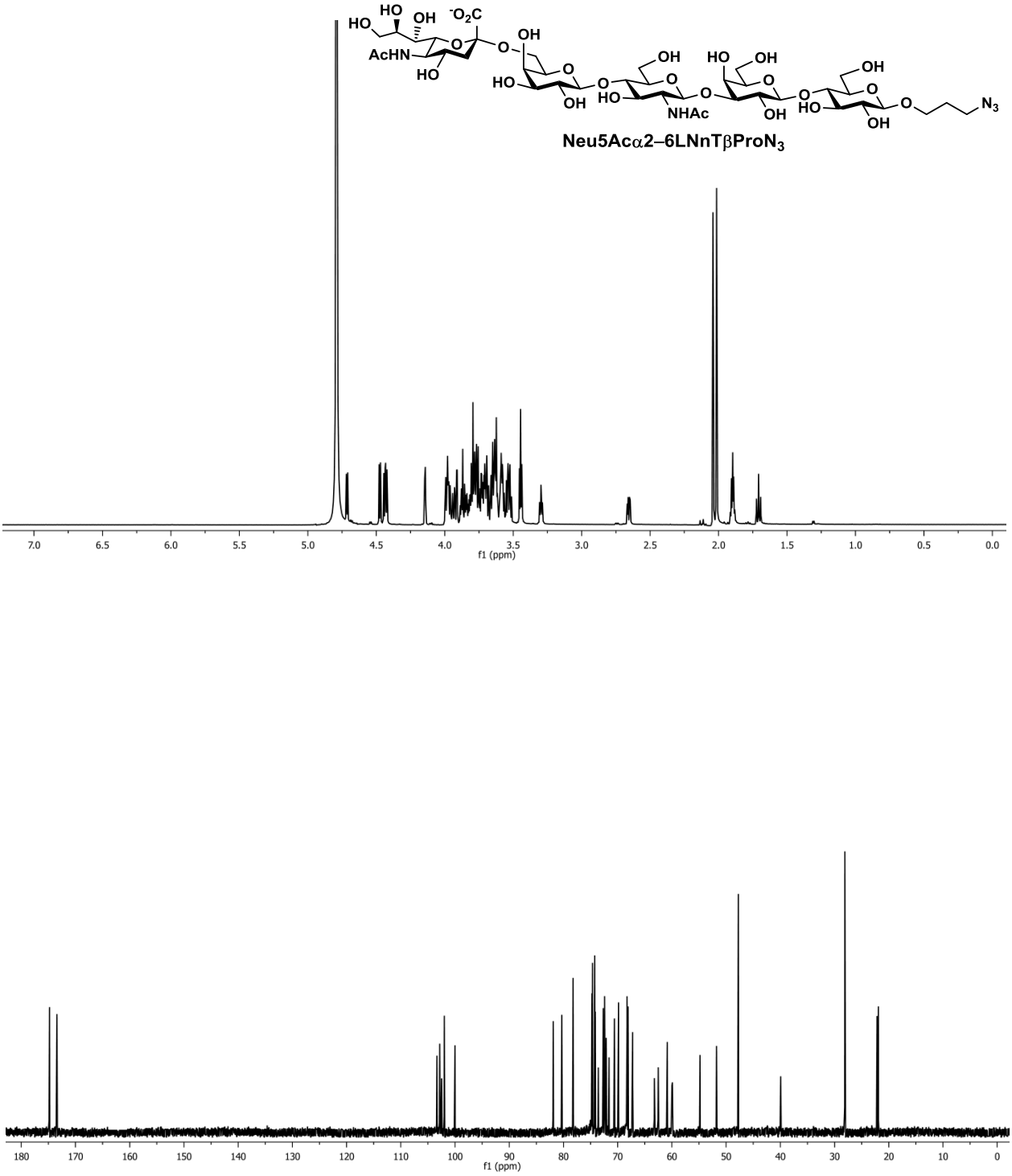


Figure S5. Comparison of ^{13}C spectra of LNnTβProN_3 and Neu5Acα2−6LNnTβProN_3 in the range of 56–65 ppm.

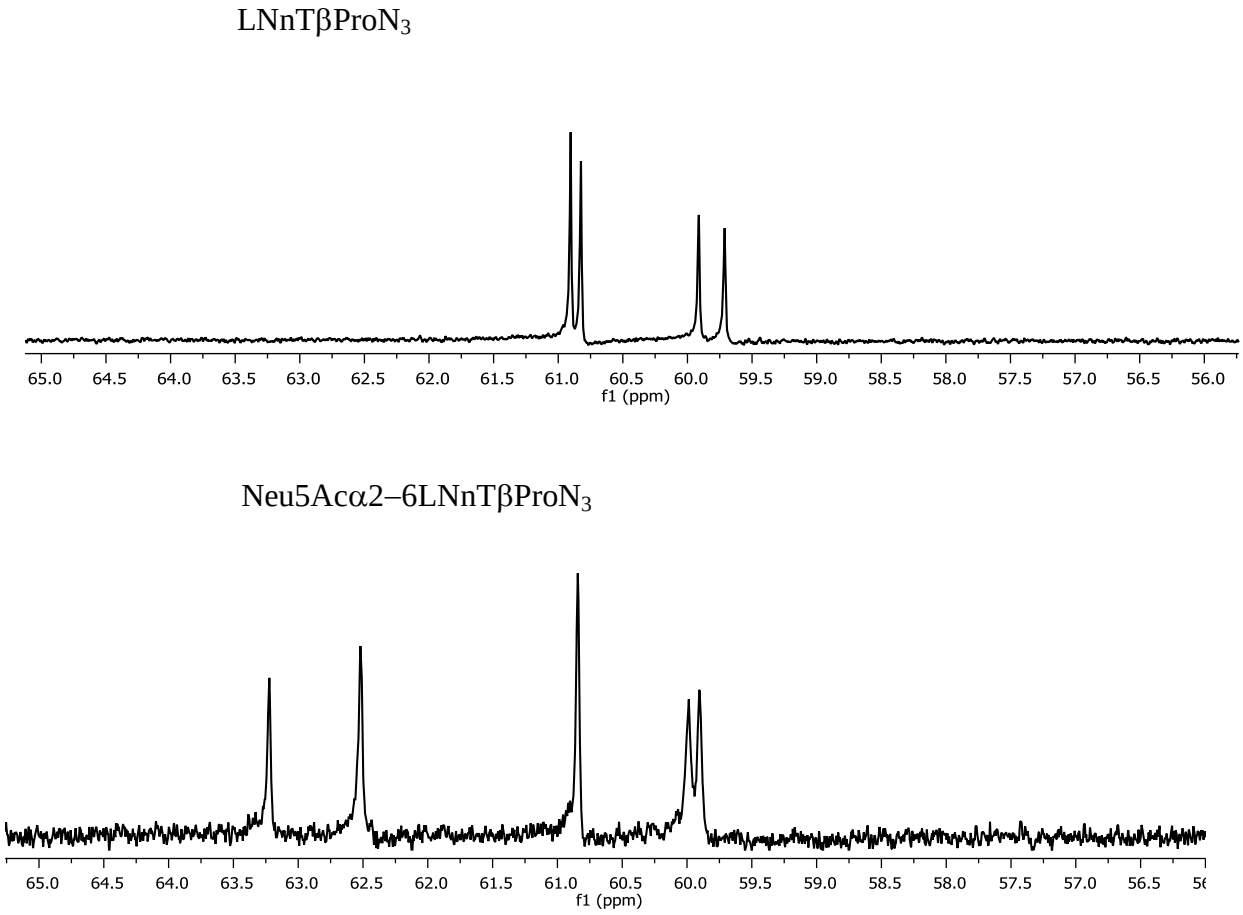


Figure S6. ^{1}H and ^{13}C NMR spectra of Neu5Acα2–6Galβ1–4GlcNAcβ1–3(Neu5Acα2–6)Galβ1–4GlcβProN_3