

## Supporting Information

for

### Converting *Pasteurella multocida* $\alpha$ 2–3-sialyltransferase 1 (PmST1) to a regioselective $\alpha$ 2–6-sialyltransferase by saturation mutagenesis and regioselective screening

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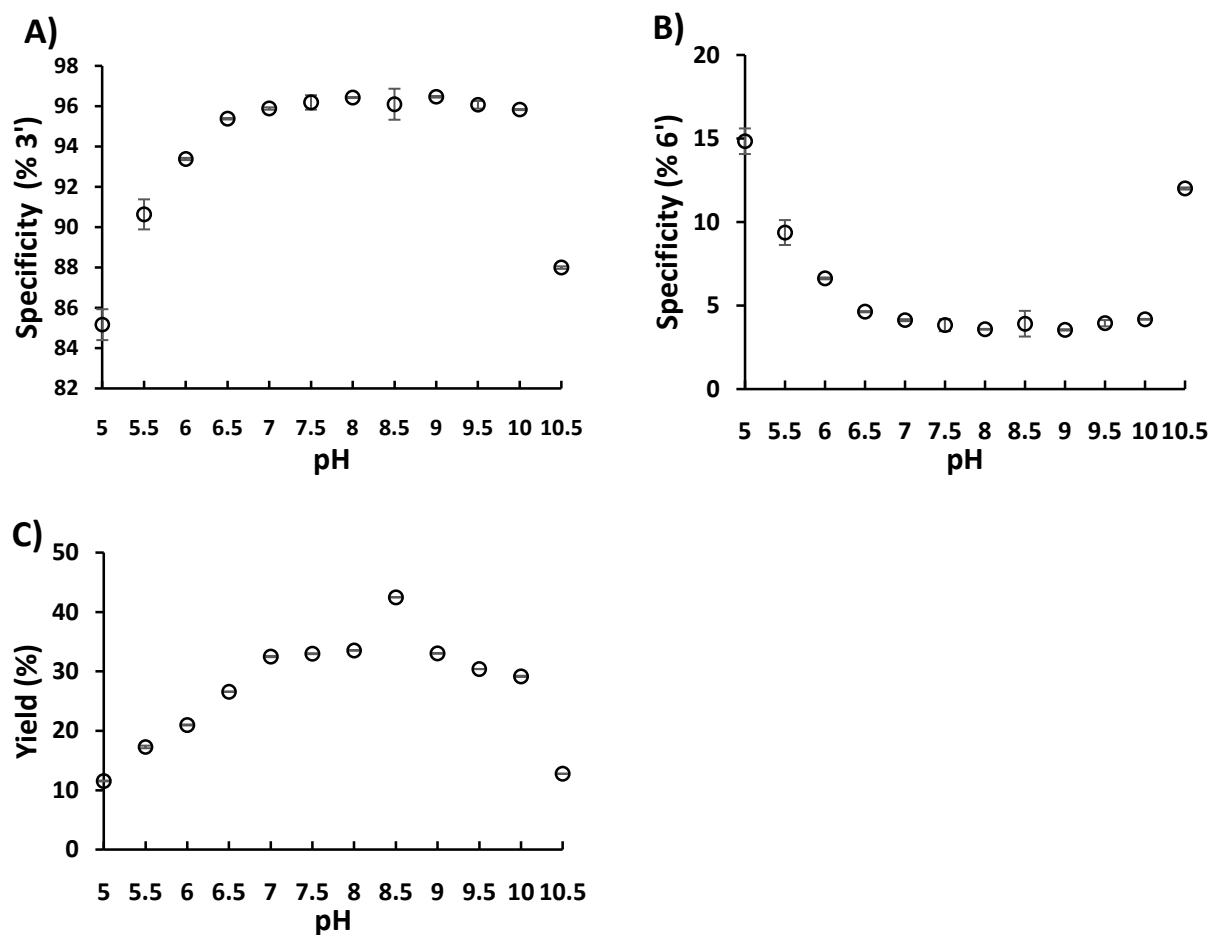
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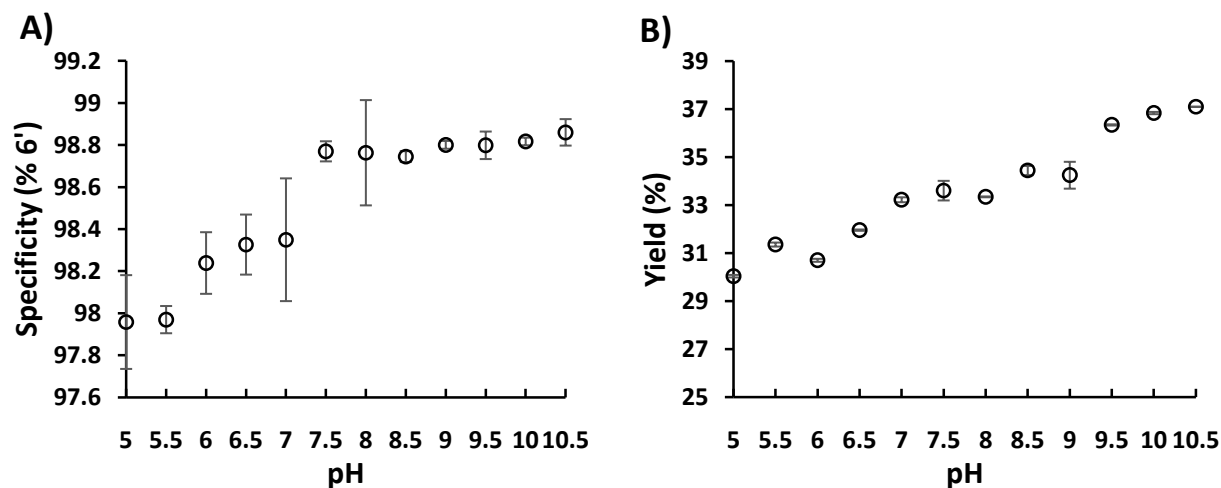
**Table S1.**  $^{13}\text{C}$  NMR chemical shift assignment for LNnT $\beta$ ProN $_3$  and Neu5Ac $\alpha$ 2–6LNnT $\beta$ ProN $_3$ .

Residue	Carbon atom	Chemical shift (ppm)	
		Gal $\beta$ 1–4GlcNAc $\beta$ 1–3Gal $\beta$ 1–4Glc $\beta$ ProN $_3$ (LNnT $\beta$ ProN $_3$ )	Neu5Ac $\alpha$ 2–6LNnT $\beta$ ProN $_3$
4Glc $\beta$ 1–	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
3Gal $\beta$ 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
4GlcNAc $\beta$ 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
6Gal $\beta$ 1–	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
Neu5Ac $\alpha$ 2–	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 $_3$	OCH $_2$ CH $_2$ CH $_2$ N $_3$	67.24	67.24
	OCH $_2$ CH $_2$ CH $_2$ N $_3$	28.10	28.10
	OCH $_2$ CH $_2$ CH $_2$ N $_3$	47.73	47.73

**Figure S1.** pH profiles of sialylation yields and sialyl product linkage specificities of PmST1-catalyzed reactions. A,  $\alpha$ 2-3-sialyl product percentages; B,  $\alpha$ 2-6-sialyl product percentages; and C, sialylation yields.



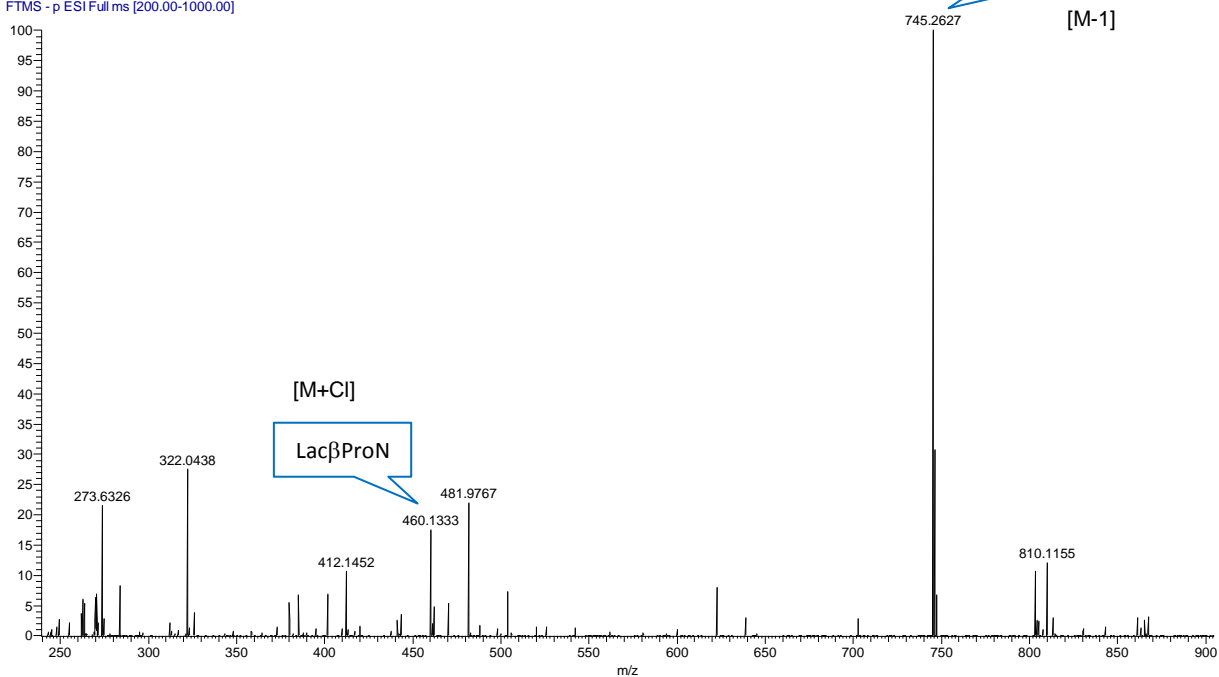
**Figure S2.** pH profiles of  $\alpha$ 2-6-sialyl product percentages (A) and sialylation yields (B) of PmST1 P34H/M144L-catalyzed reactions.



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

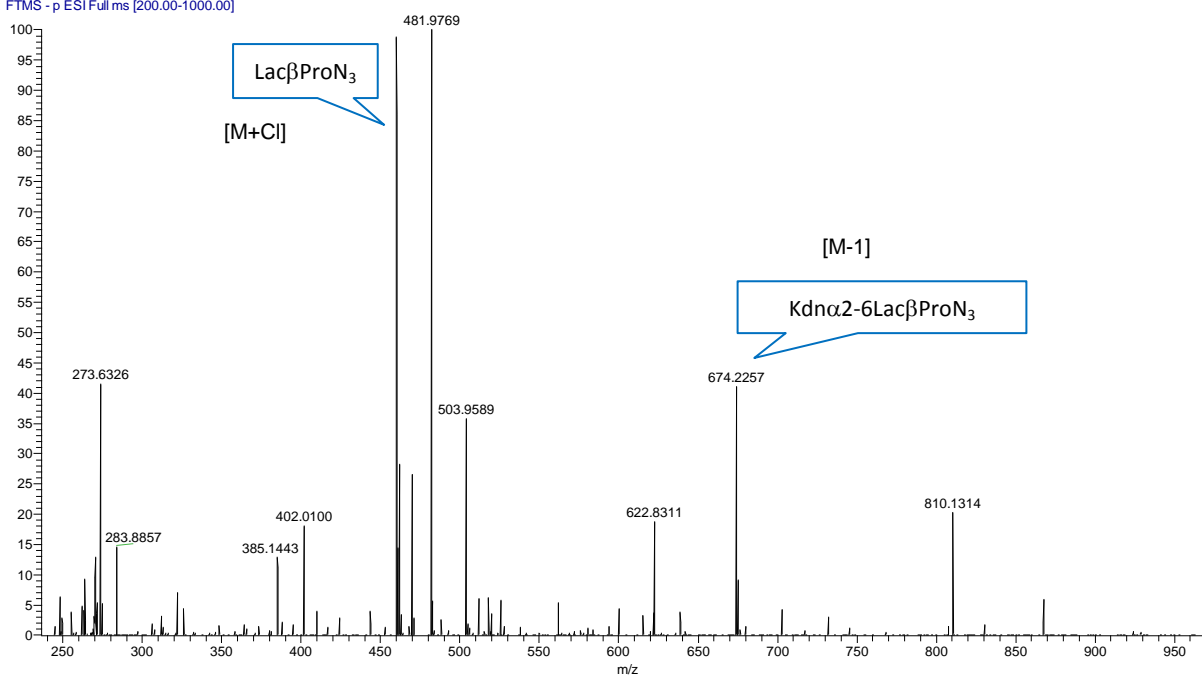
**A** Lac $\beta$ ProN<sub>3</sub> with ManGcOMe (A)

Lacβ Rx1 GcOMe 24h #20-82 RT: 0.16-0.65 AV: 63 NL: 7.85E5  
T: FTMS - p ESI Full ms [200.00-1000.00]



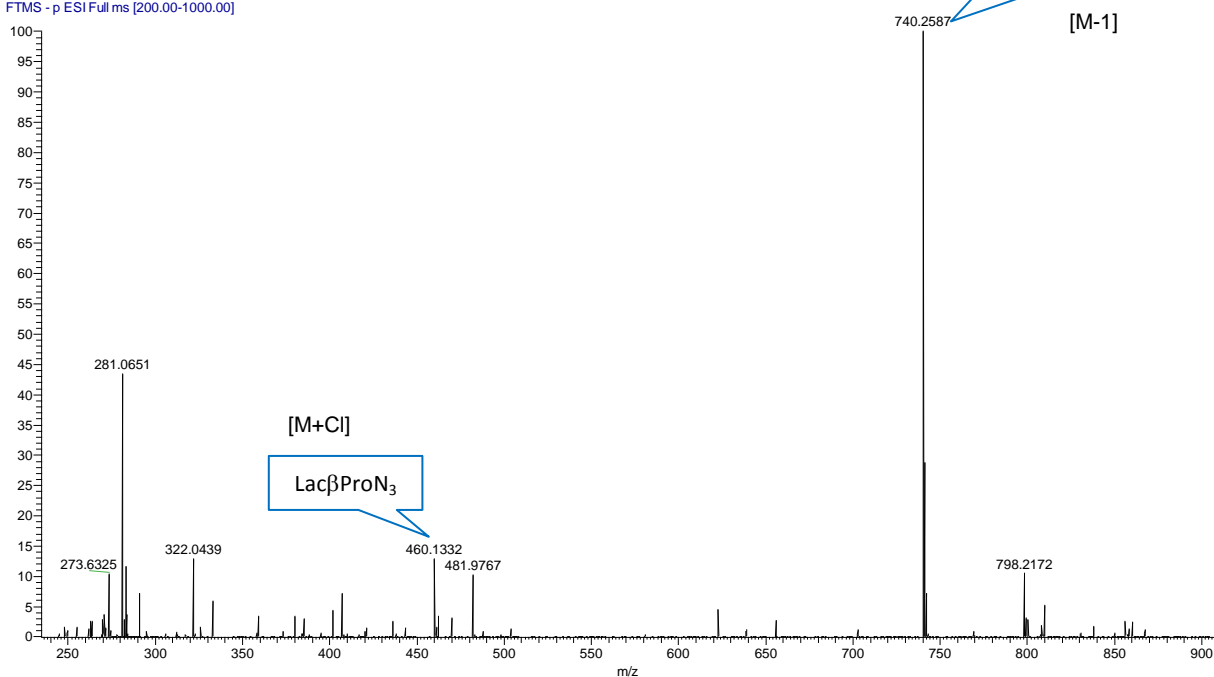
**B** Lac $\beta$ ProN<sub>3</sub> with mannose (B)

Lacβ Rx2 Man 24h #21-92 RT: 0.17-0.73 AV: 72 NL: 4.38E5  
T: FTMS - p ESI Full ms [200.00-1000.00]



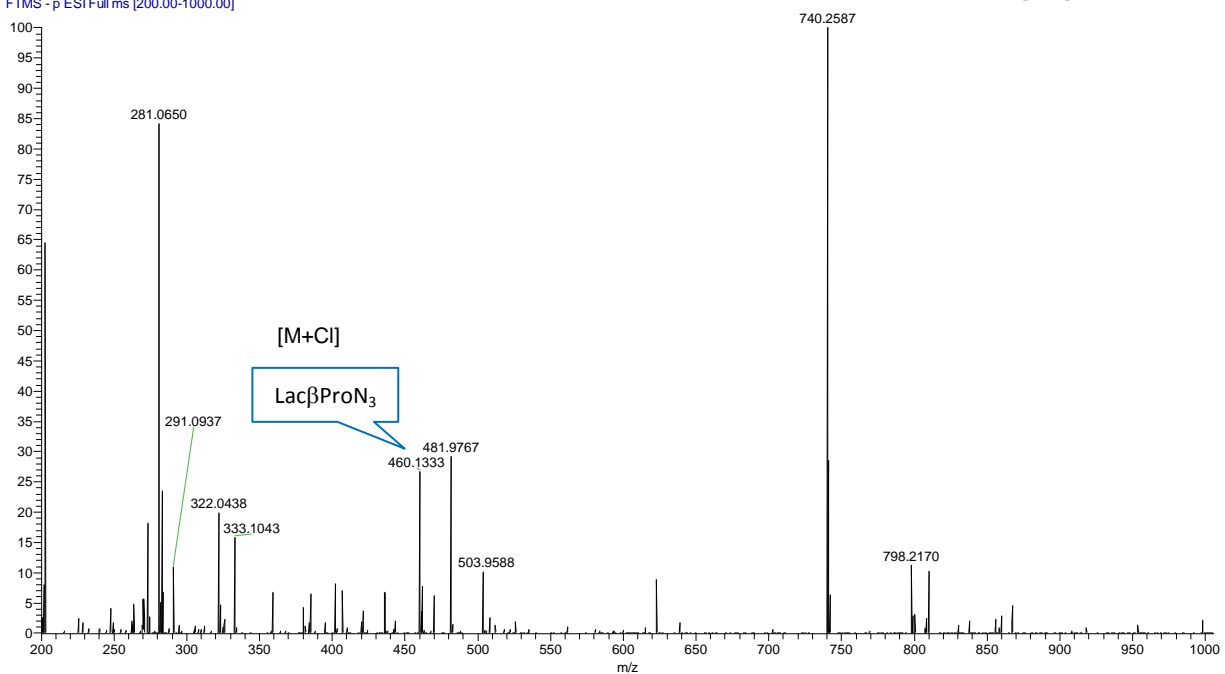
### C Lac $\beta$ ProN<sub>3</sub> with ManNAc6N<sub>3</sub> (C)

LacB Rx3 6N3ManNAc 24h #20-69 RT: 0.16-0.55 AV: 50 NL: 1.77E6  
T: FTMS - p ESI Full ms [200.00-1000.00]



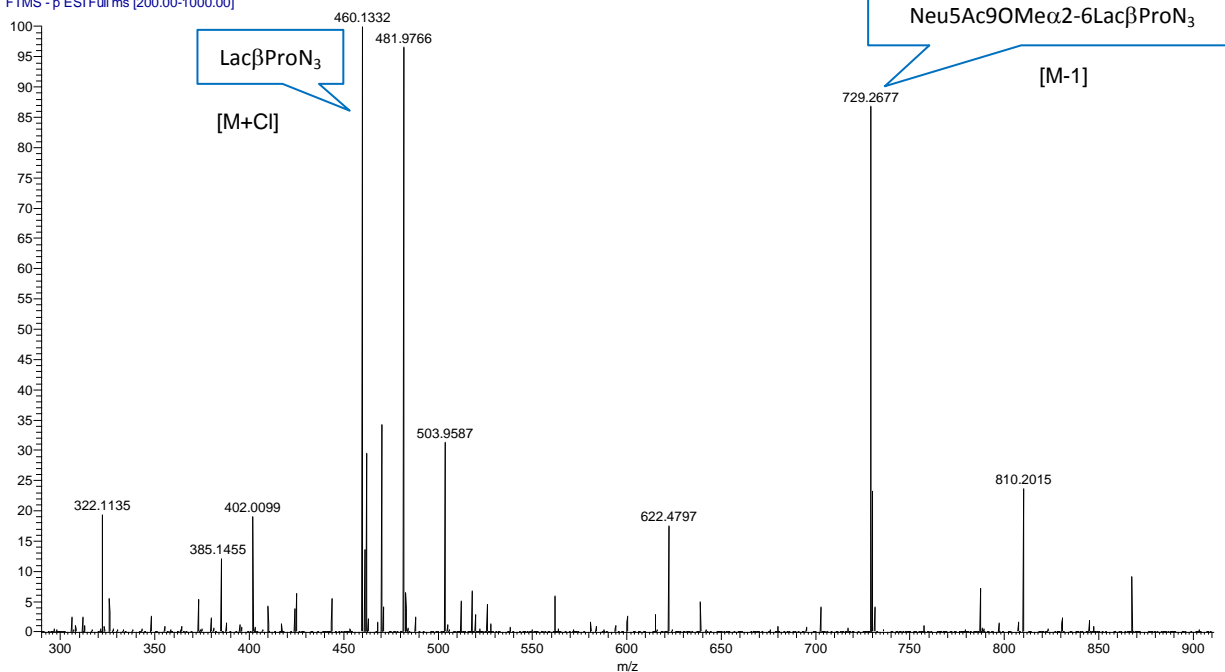
### D Lac $\beta$ ProN<sub>3</sub> with ManNAc4N<sub>3</sub> (D)

LacB Rx5 4N3ManNAc 24h #19-77 RT: 0.15-0.61 AV: 59 NL: 9.35E5  
T: FTMS - p ESI Full ms [200.00-1000.00]



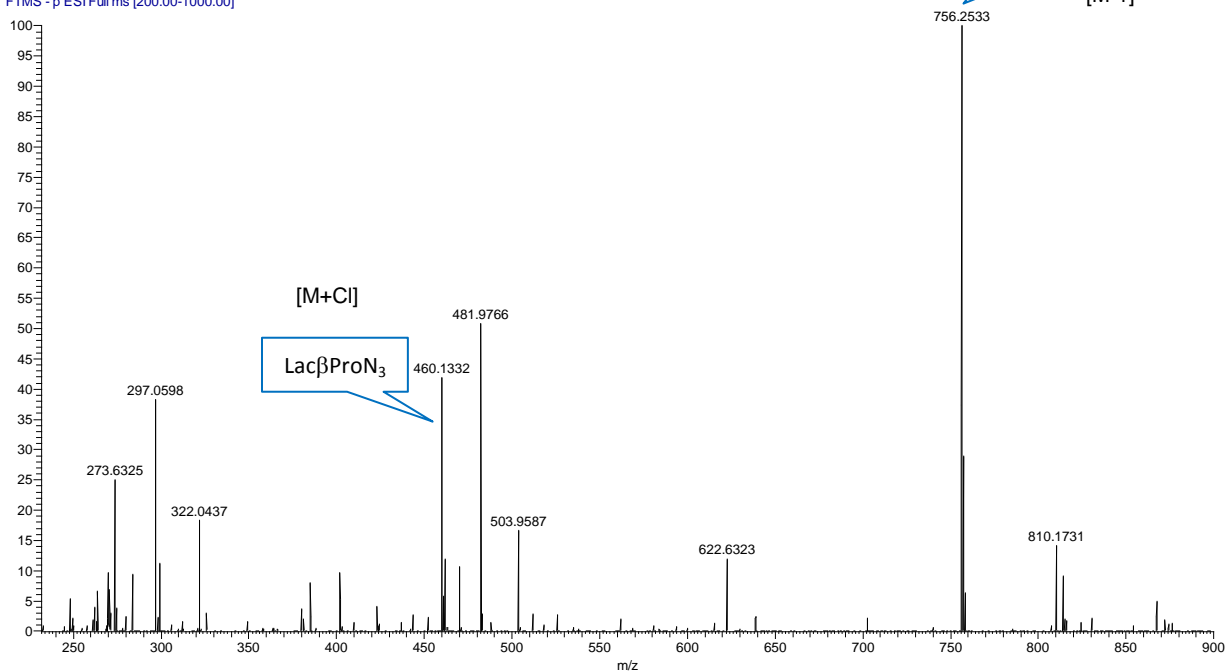
## E LacβProN<sub>3</sub> with ManNAc6OMe (E)

Lacβ Rx6 4MeNeu5Ac 24h #20-74 RT: 0.15-0.58 AV: 55 NL: 4.08E5  
T: FTMS - p ESI Full ms [200.00-1000.00]



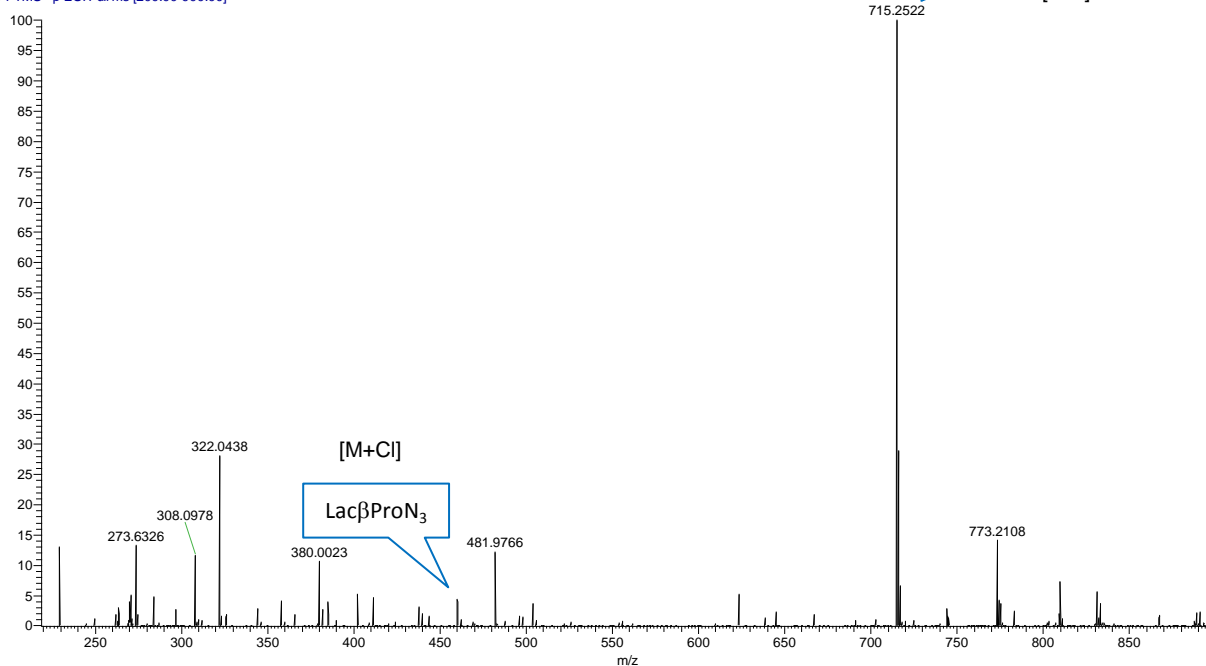
## F LacβProN<sub>3</sub> with ManNAz (F)

Lacβ Rx7 ManNAz 24h #22-81 RT: 0.17-0.64 AV: 60 NL: 6.73E5  
T: FTMS - p ESI Full ms [200.00-1000.00]



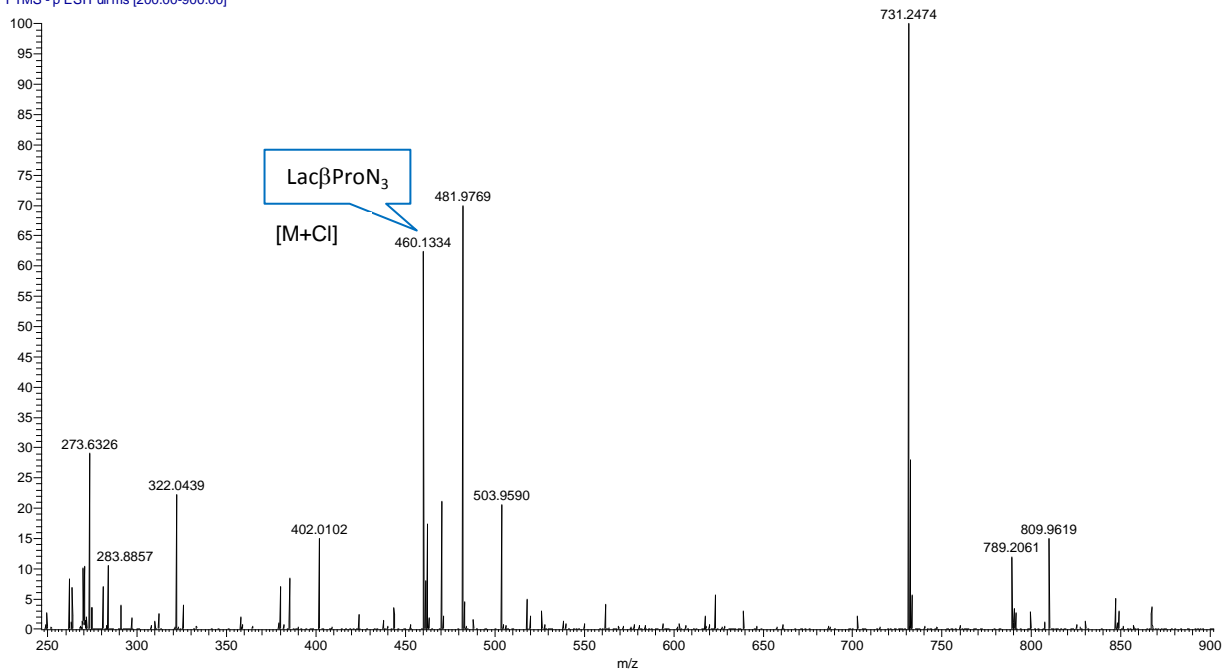
### G Lac $\beta$ ProN<sub>3</sub> with Neu5Ac (G)

Lac $\beta$  rx8 Neu5Ac 1h #28-96 RT: 0.22-0.76 AV: 69 NL: 1.34E6  
T: FTMS - p ESI Full ms [200.00-900.00]



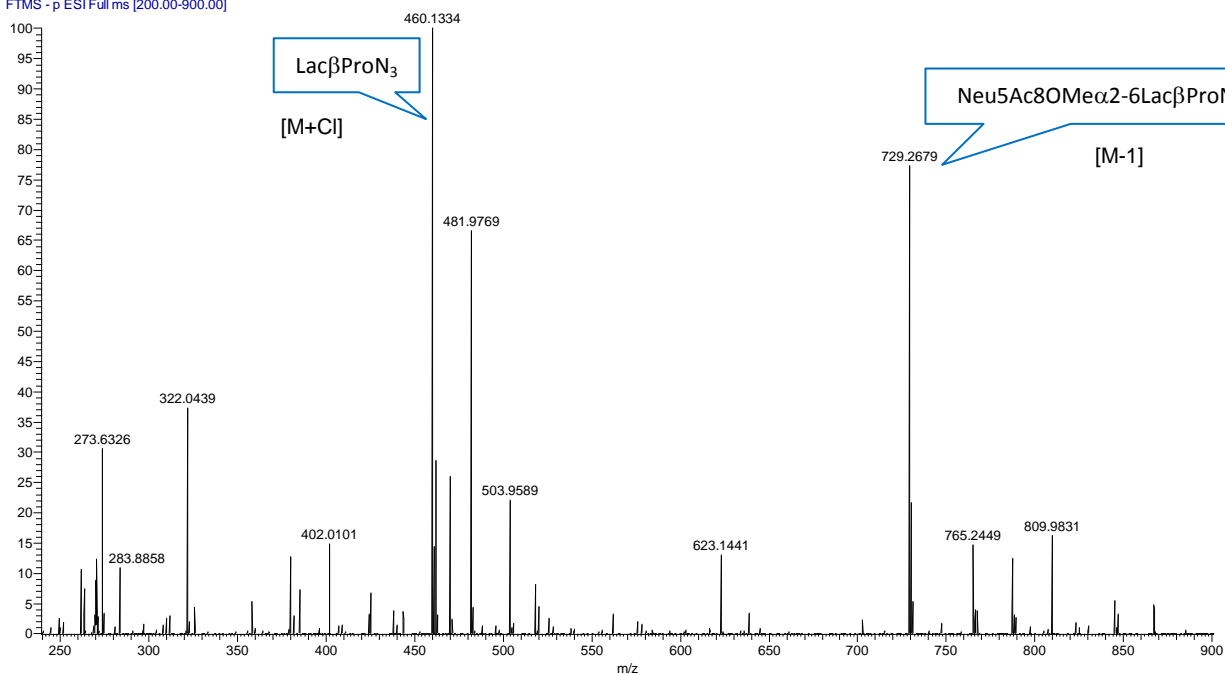
### H Lac $\beta$ ProN<sub>3</sub> with Neu5Gc (H)

Lac $\beta$  rx9 Neu5Gc 1h #19-96 RT: 0.15-0.76 AV: 78 NL: 6.00E5  
T: FTMS - p ESI Full ms [200.00-900.00]



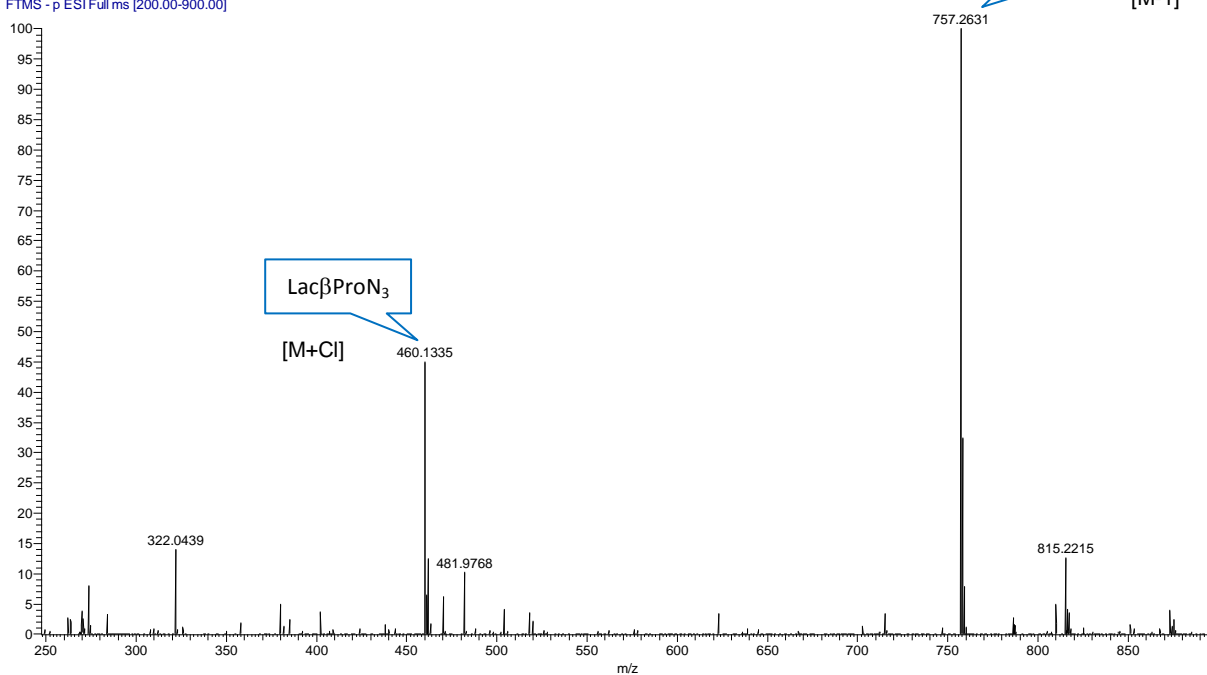
# I Lac $\beta$ ProN<sub>3</sub> with Neu5Ac8OMe (I)

Lacb rx10 Neu5Ac8Me 1h #19-76 RT: 0.14-0.60 AV: 58 NL: 5.86E5  
T: FTMS - p ESI Full ms [200.00-900.00]



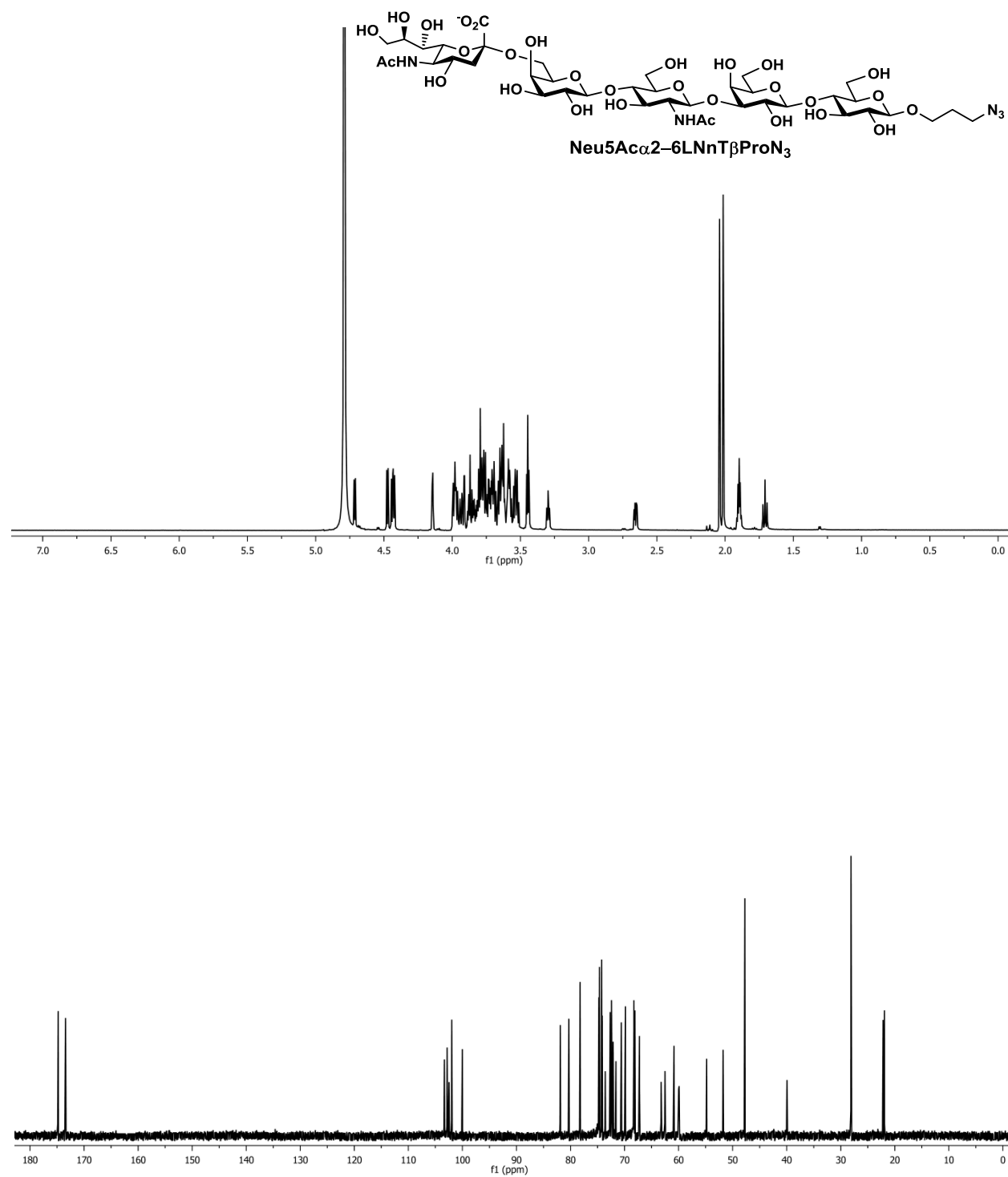
# J Lac $\beta$ ProN<sub>3</sub> with Neu5,9Ac<sub>2</sub> (J)

Lacb rx11 Neu5Ac9Ac1h #20-77 RT: 0.15-0.61 AV: 58 NL: 1.93E6  
T: FTMS - p ESI Full ms [200.00-900.00]

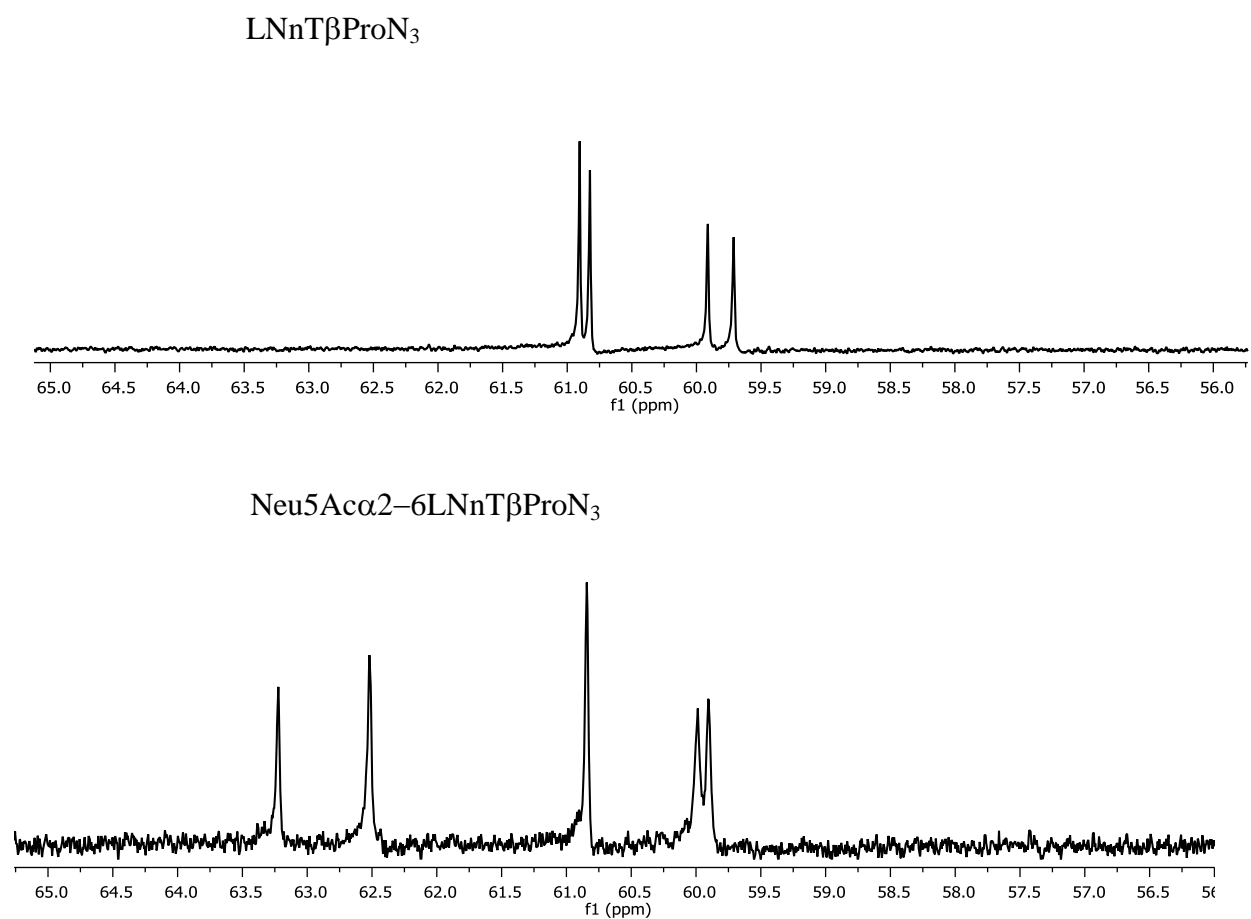




**Figure S4.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of Neu5Ac $\alpha$ 2-6LNnT $\beta$ ProN $_3$



**Figure S5.** Comparison of  $^{13}\text{C}$  spectra of  $\text{LNnT}\beta\text{ProN}_3$  and  $\text{Neu5Ac}\alpha 2\text{--}6\text{LNnT}\beta\text{ProN}_3$  in the range of 56–65 ppm.



**Figure S6.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of Neu5Ac $\alpha$ 2-6Gal $\beta$ 1-4GlcNAc $\beta$ 1-3(Neu5Ac $\alpha$ 2-6)Gal $\beta$ 1-4Glc $\beta$ ProN $_3$

