Supporting Information

for

Donor substrate promiscuity of bacterial β1–3-*N*-acetylglucosaminyltransferases and acceptor substrate flexibility of β1–4-galactosyltransferases

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Contents

Figure S1. SDS-PAGE (4–20% Tris-Glycine gel) analysis of Bβ4GalT. Lanes: BI, whole cell extract before induction; AI, whole cell extract after induction; L, lysate after induction; PP, Ni^{2+}-NTA column purified protein; M, protein markers (Bio-Rad precision Plus Protein Standards, 10–250 kDa).

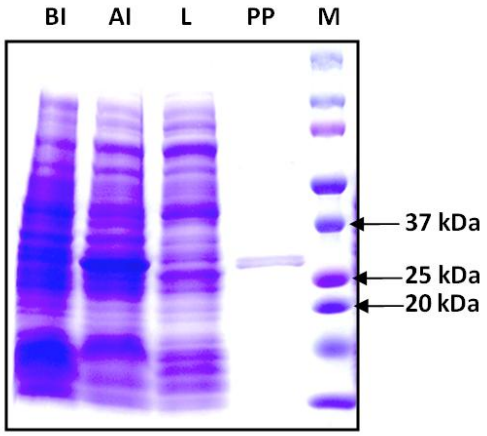
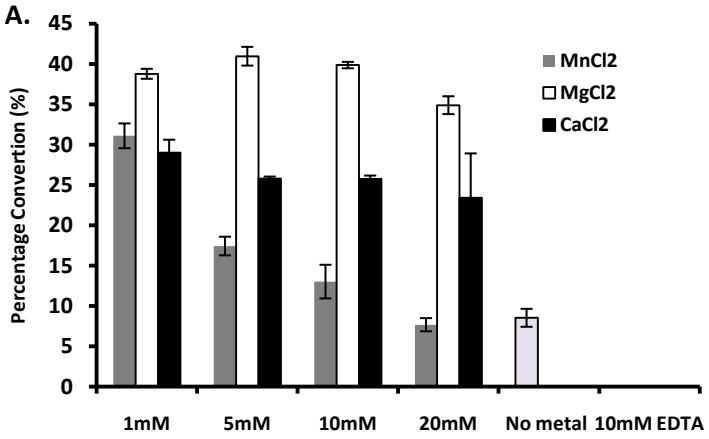


Figure S2. Metal effect of Hpβ3GlcNAcT (A) and NmLgtA (B). G*ray columns*, $MnCl_2$; *white columns*, $MgCl_2$; *black columns*, $CaCl_2$. The reactions were carried out with the addition of different concentrations (1, 5, 10, and 20 mM) of different metal ions ($MgCl_2$, $MnCl_2$, $CaCl_2$) or EDTA (10 mM) in Tris-HCl buffer (100 mM, pH 8.0) containing UDP-GlcNAc (1 mM), LacβMU (1 mM), and Hpβ3GlcNAcT (3 μg) or NmLgtA(2 μg). Reactions were performed at 30 °C for 10 min. The reaction without metal ion nor EDTA was used as a control.



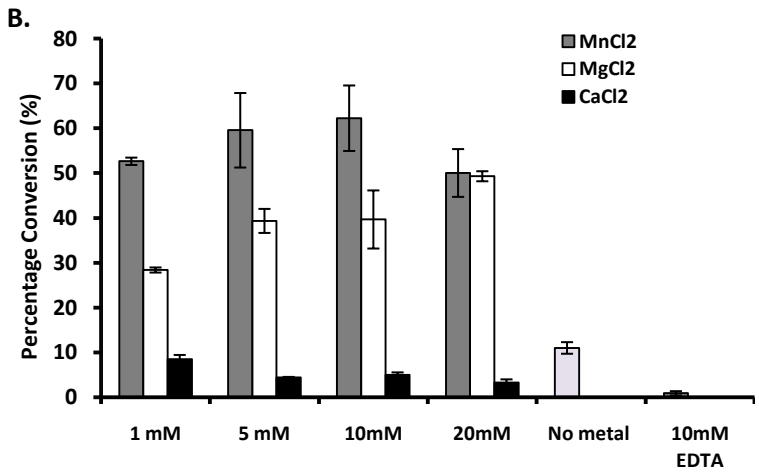


Figure S3. Sugar nucleotides and derivatives[1-6] used for donor substrate specificity studies of Hpβ3GlcNAcT and NmLgtA.

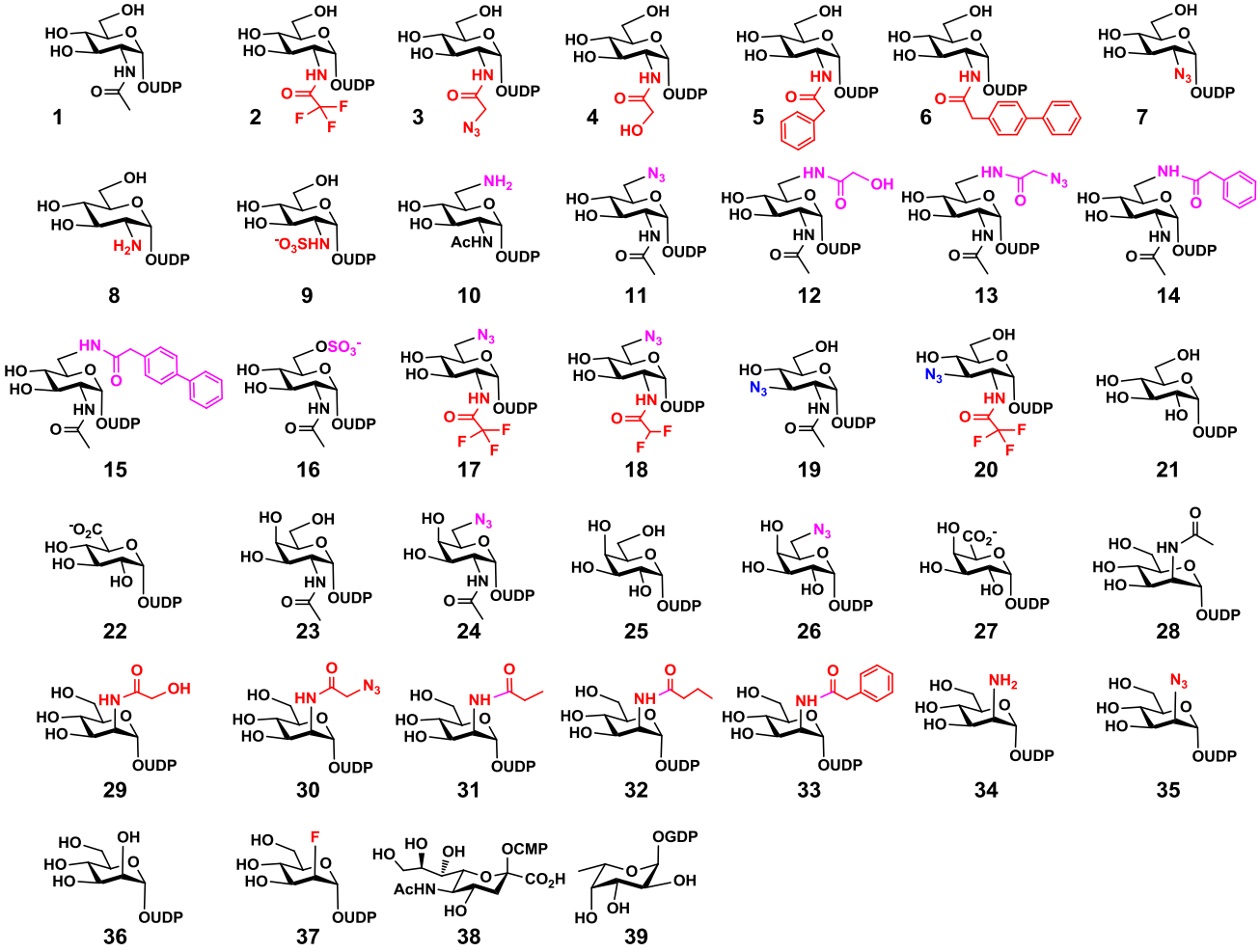


Figure S4. Protein sequences alignment of Hpβ4GalT, NmLgtB, and Bβ4GalT analyzed by software of ClustalW2 and GeneDoc.

```
Hpβ1-4GalT :  MRVFAISLN------QKVCDTFG---LVFRDTTTLLNSINATHHQAQIFLAIYSKTFEGGLHP :  54
NmLgtB     :  MQNHVISIASAAERRAHIADTFGRHGIPFQFFDAIMPSERIEQAMAELVPGLSAHPYLSGVEK :  63
Bβ1-4GalT  :  MRSLTACPEESPLLVGPMLIEFN-IPVDLKLVEQQNPKVKIG-GRYTPMLCISPHKVAIIIPF :  61

Hpβ1-4GalT :  LVKKHLHPYFITQNIKDMGITINLISEVSKFYYALKYHAKFMSIGFLGCYASHYSLWFKCIEL : 117
NmLgtB     :  ACFMSHAVLWKQAIDEGLPYITVFEDDVLLGEGAEKFLAEDAWLQER--FDPDTAFIVRIETM : 124
Bβ1-4GalT  :  RNRQEHLKYWLYYLHPILQRQQLDYGIYVINQAGFSMFNRAKLINVG--------FKEAIKDY : 116

Hpβ1-4GalT :  NEAICILEIDITLKEDFKEGLDFLE-KHIQELGYIRLMHILYDASVKSEPLSHKNHEIQERVG : 179
NmLgtB     :  --FMHVLTSPSGVADYCGRAFPLLESEHWGTAGYIISRKAMRFFLDRFAALPPEGLHPVD-IM : 184
Bβ1-4GalT  :  DYNCFVFSIVDLIPMNDHNTYRCFS-QPRHISVAMDKFGISLPYVQYFGGVSALSKQQFLSIN : 178

Hpβ1-4GalT :  IIKAYSEGVGTQGYVITPKIAKVFLKCSRKWVVPVDTIMDATFIHG-VKNLVLQPFVIADDEQ : 241
NmLgtB     :  MFSDFFDREGMPVCQLNPALCAQELHYAKFHDQ--NSALGSLIEHDRLLNRKQQRRDSFANTF : 245
Bβ1-4GalT  :  GFPNNYWGWGGEDDDIYNRLAFRGMSVSRPNAVIGKCRMIR-HSRDKKNEPNPQRFDRIAHIK : 240

Hpβ1-4GalT :  ISTIARKEEPYSPKIALMRELHFKYLKYWQFV--- : 273
NmLgtB     :  KHRLIRALTKISREREKRRQRREQFIVPFQ----- : 275
Bβ1-4GalT  :  ETMLSDGLNSLTYMVLEVQRYPLYTKITVDIGTPS : 275
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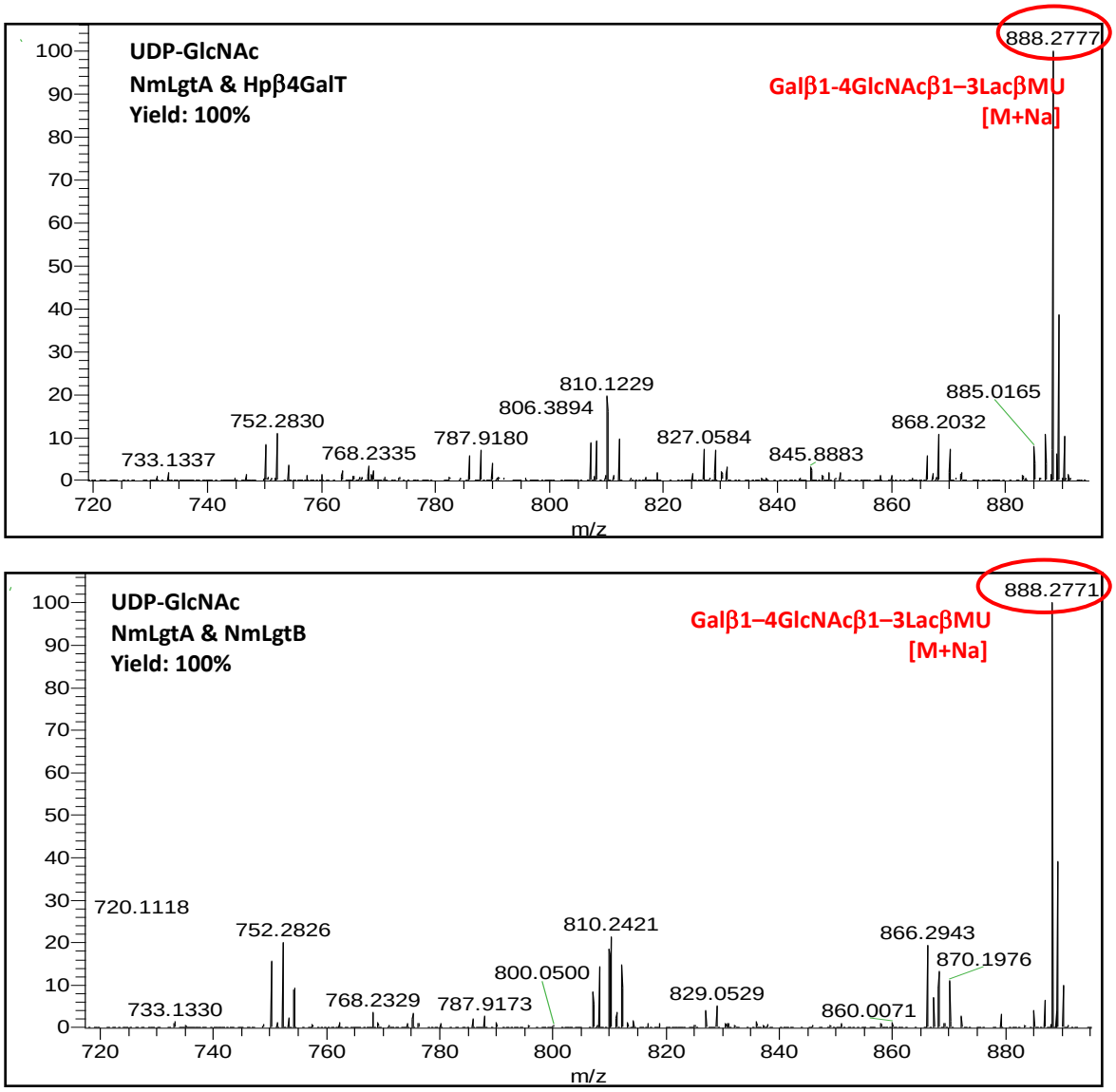
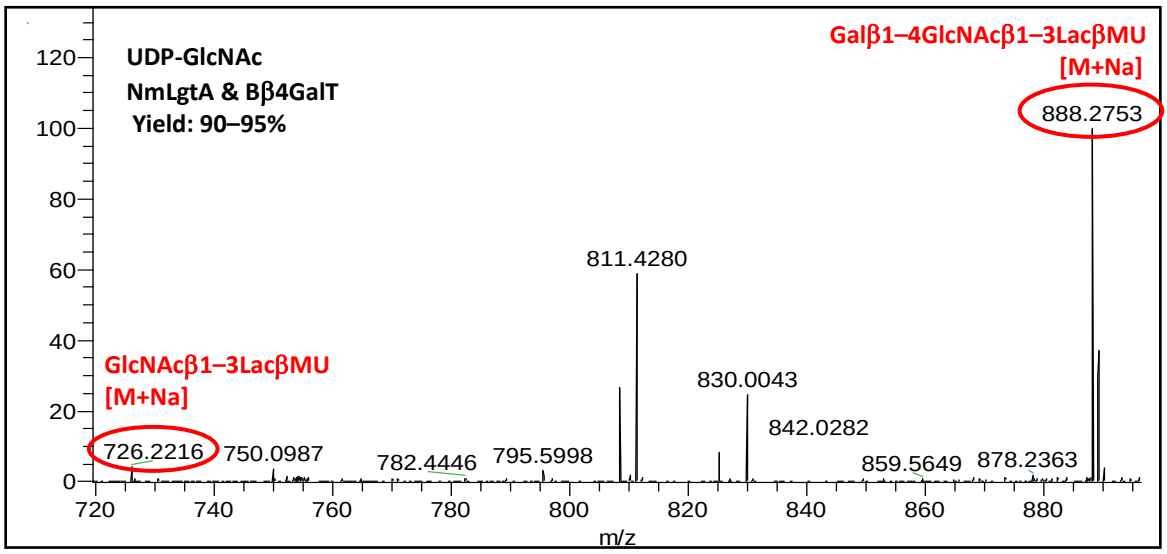
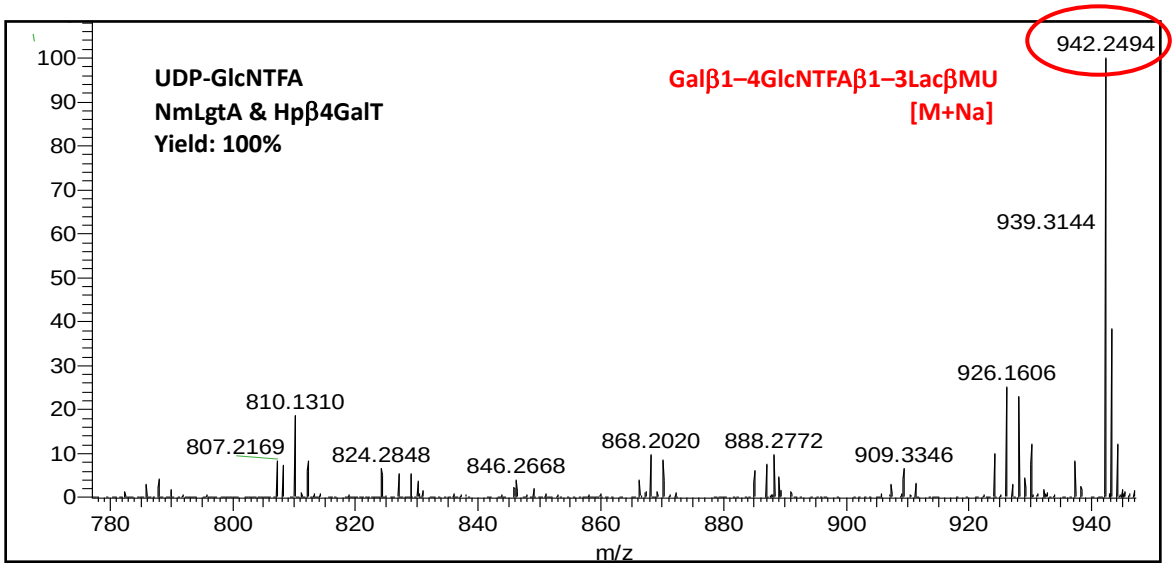
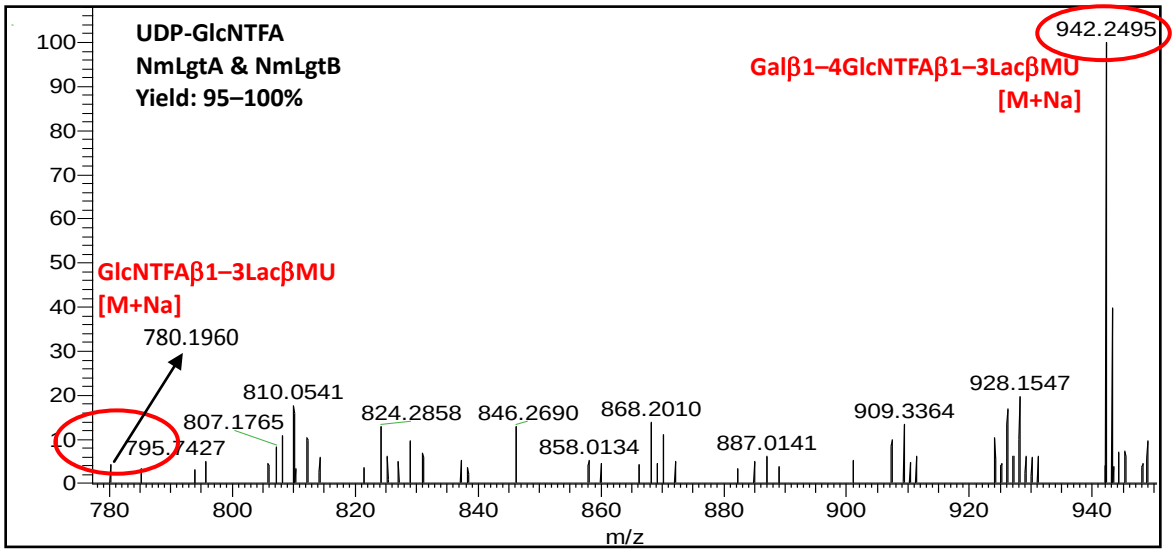
Figure S5. HRMS analysis of the acceptor substrate specificities of Hp4GalT, NmLgtB, and Bβ4GalT using products obtained from NmLgtA or Hpβ3GlcNAcT-catalyzed reactions. Starting materials and products (m/z values) are shown in red circles. Reference m/z values are shown in green circles. Estimated yields are shown.




UDP-GlcNAc
NmLgtA & Bβ4GalT
Yield: 90–95%
Galβ1–4GlcNAcβ1–3LacβMU
[M+Na]
888.2753
811.4280
830.0043
842.0282
GlcNAcβ1–3LacβMU
[M+Na]
726.2216
750.0987
782.4446
795.5998
859.5649
878.2363
m/z


UDP-GlcNTFA
NmLgtA & Hpβ4GalT
Yield: 100%
Galβ1–4GlcNTFAβ1–3LacβMU
[M+Na]
942.2494
939.3144
926.1606
810.1310
807.2169
824.2848
846.2668
868.2020
888.2772
909.3346
m/z


UDP-GlcNTFA
NmLgtA & NmLgtB
Yield: 95–100%
Galβ1–4GlcNTFAβ1–3LacβMU
[M+Na]
942.2495
GlcNTFAβ1–3LacβMU
[M+Na]
780.1960
810.0541
807.1765
795.7427
824.2858
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858.0134
868.2010
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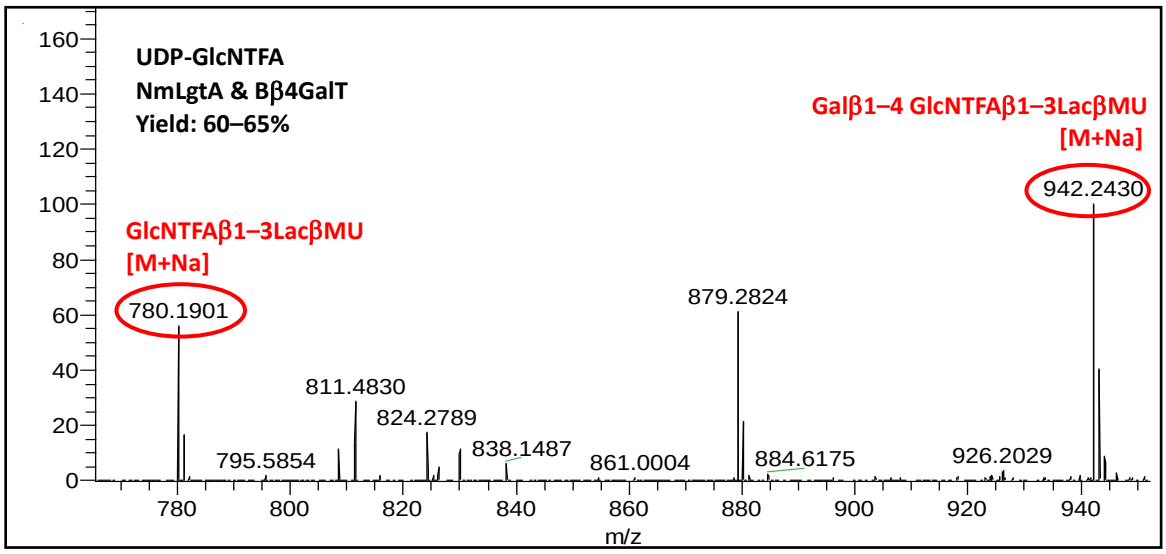
928.1547
m/z


UDP-GlcNTFA
NmLgtA & Bβ4GalT
Yield: 60–65%
Galβ1–4 GlcNTFAβ1–3LacβMU
[M+Na]
942.2430
GlcNTFAβ1–3LacβMU
[M+Na]
780.1901
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811.4830
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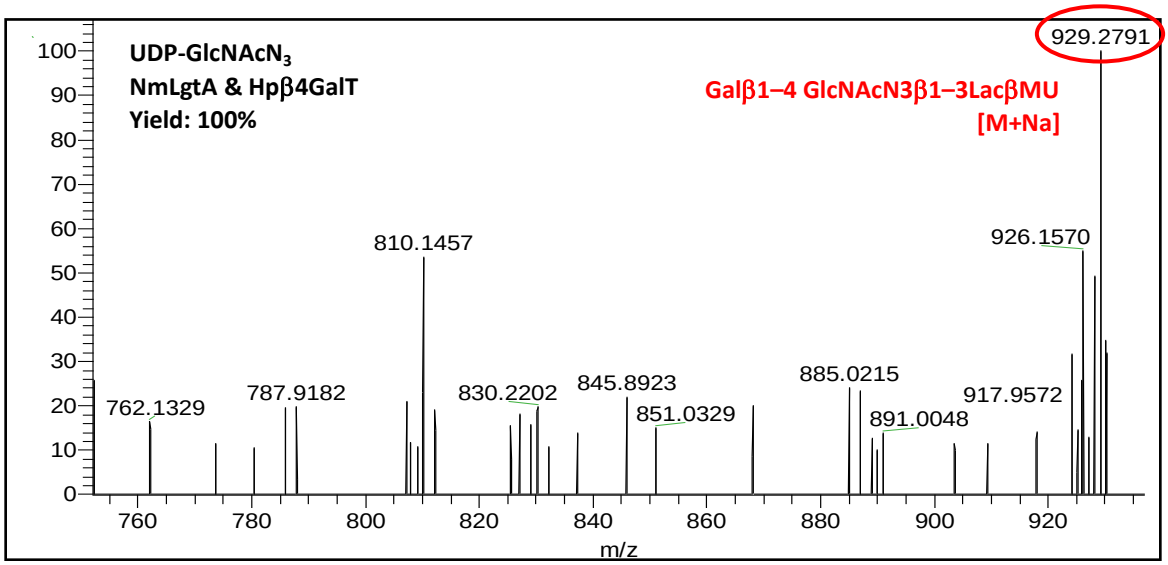
926.2029
m/z


UDP-GlcNAcN3
NmLgtA & Hpβ4GalT
Yield: 100%
Galβ1–4 GlcNAcN3β1–3LacβMU
[M+Na]
929.2791
810.1457
926.1570
762.1329
787.9182
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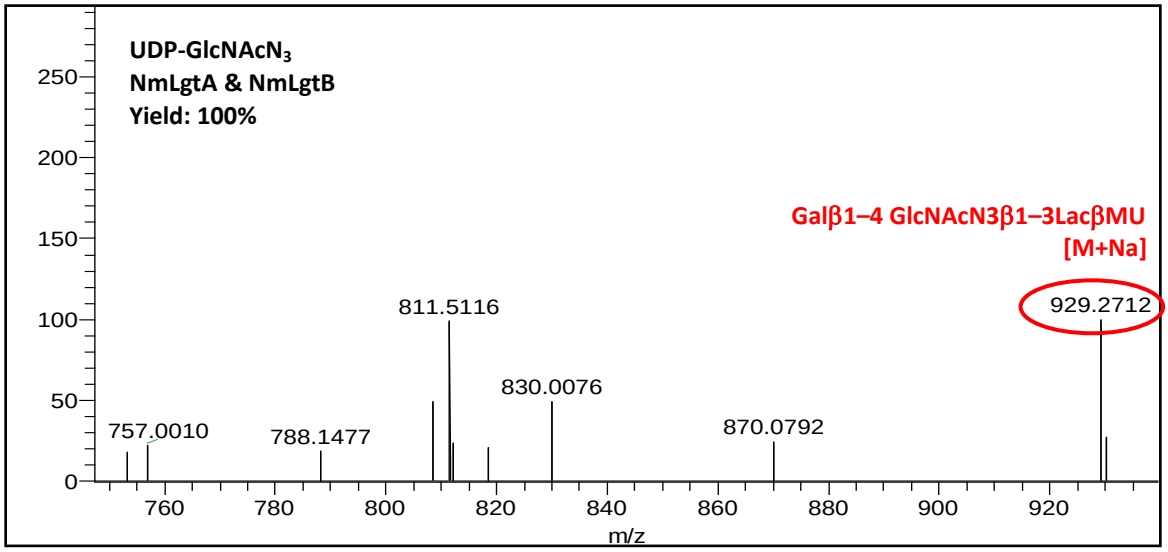
m/z


UDP-GlcNAcN3
NmLgtA & NmLgtB
Yield: 100%
Galβ1–4 GlcNAcN3β1–3LacβMU
[M+Na]
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757.0010
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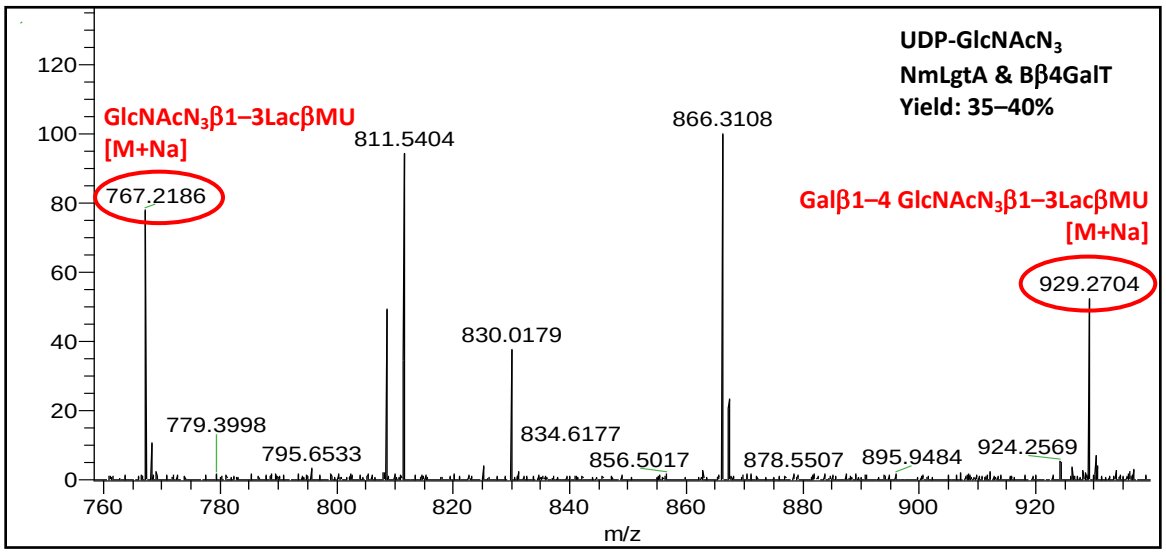
870.0792
m/z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Yield: 35–40%
GlcNAcN3β1–3LacβMU
[M+Na]
767.2186
811.5404
866.3108
Galβ1–4 GlcNAcN3β1–3LacβMU
[M+Na]
929.2704
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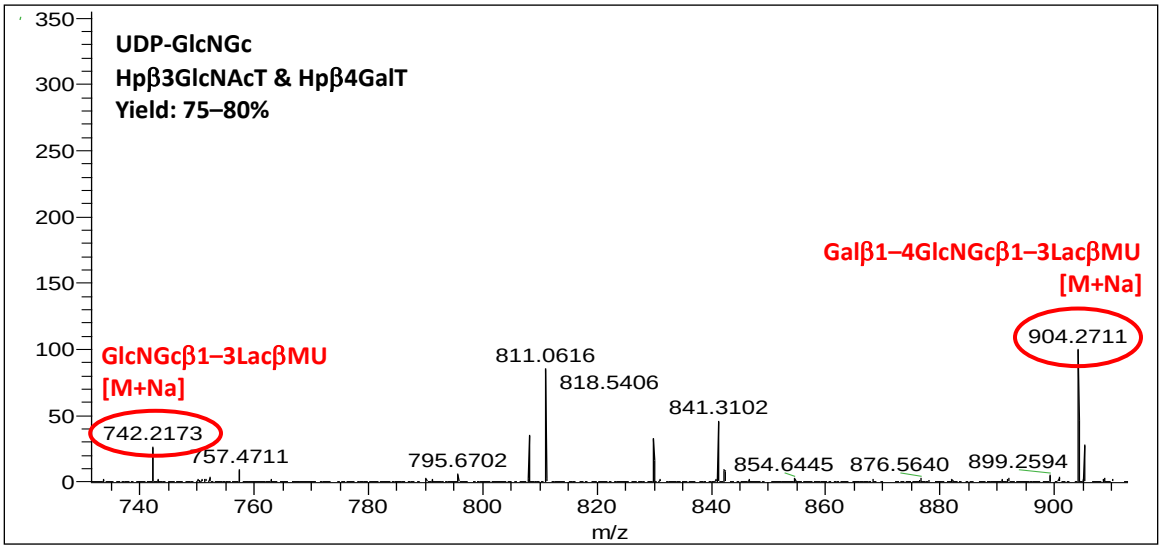
878.5507
895.9484
924.2569
m/z


UDP-GlcNGc
Hpβ3GlcNAcT & Hpβ4GalT
Yield: 75–80%
Galβ1–4GlcNGcβ1–3LacβMU
[M+Na]
904.2711
GlcNGcβ1–3LacβMU
[M+Na]
742.2173
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854.6445
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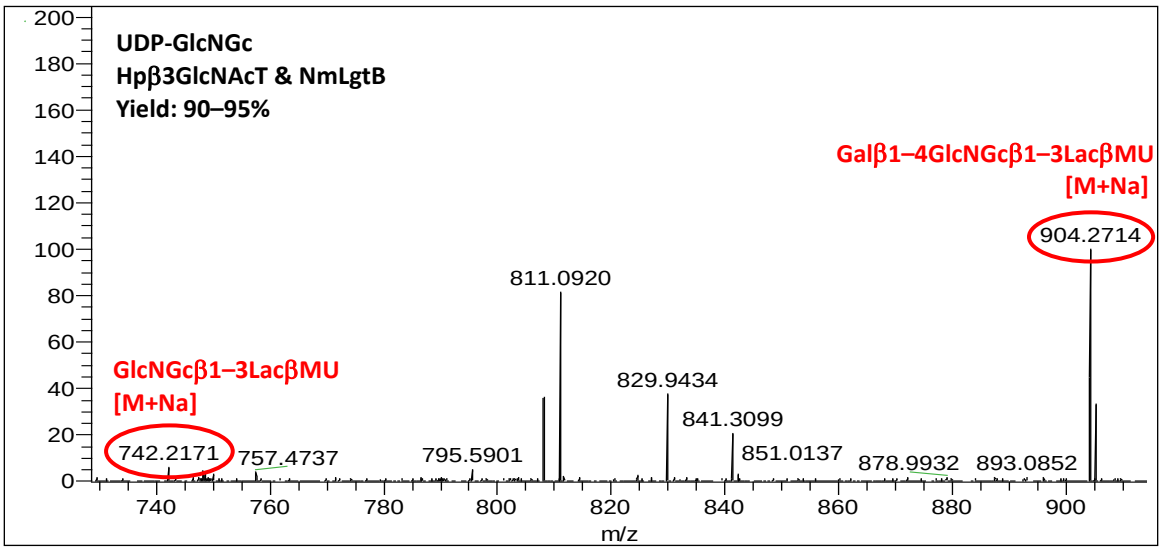
m/z


UDP-GlcNGc
Hpβ3GlcNAcT & NmLgtB
Yield: 90–95%
Galβ1–4GlcNGcβ1–3LacβMU
[M+Na]
904.2714
811.0920
GlcNGcβ1–3LacβMU
[M+Na]
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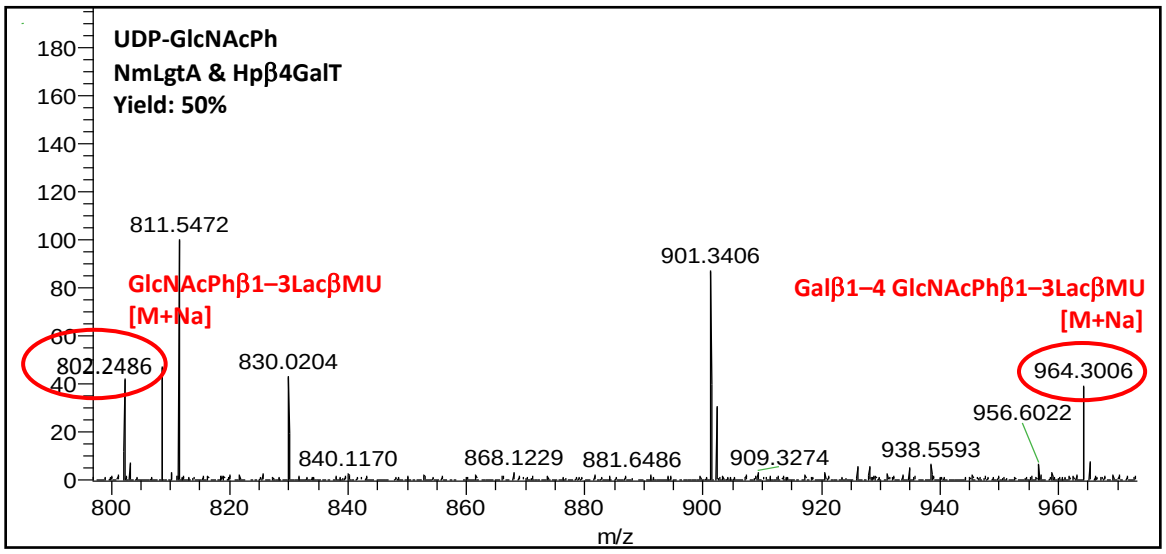
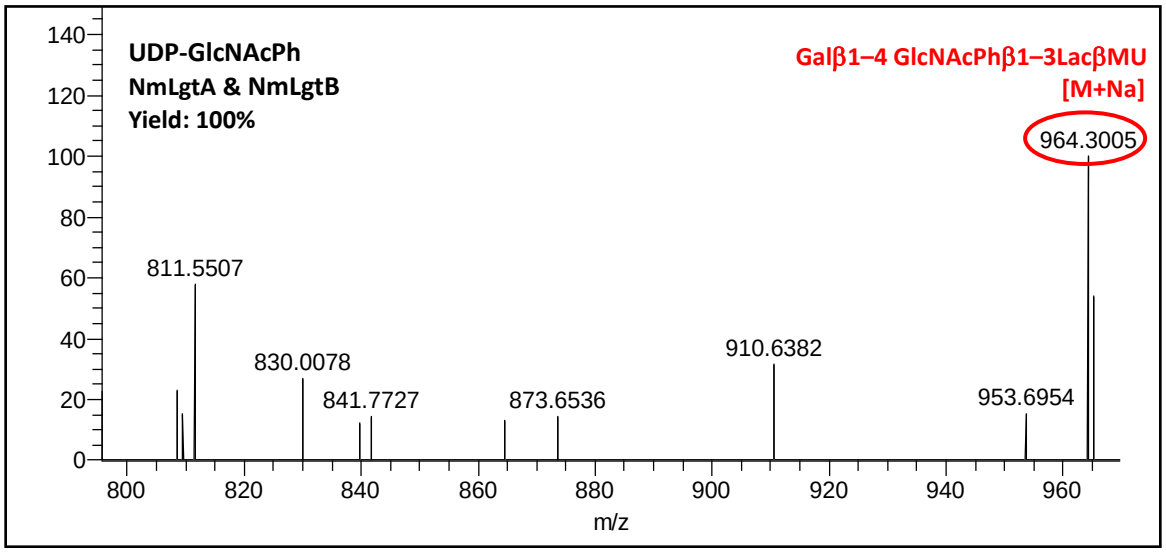

UDP-GlcNAcPh
NmLgtA & Hpβ4GalT
Yield: 50%
811.5472
GlcNAcPhβ1–3LacβMU
[M+Na]
802.2486
830.0204
840.1170
868.1229
881.6486
901.3406
909.3274
938.5593
956.6022
964.3006
Galβ1–4 GlcNAcPhβ1–3LacβMU
[M+Na]
m/z


UDP-GlcNAcPh
NmLgtA & NmLgtB
Yield: 100%
811.5507
830.0078
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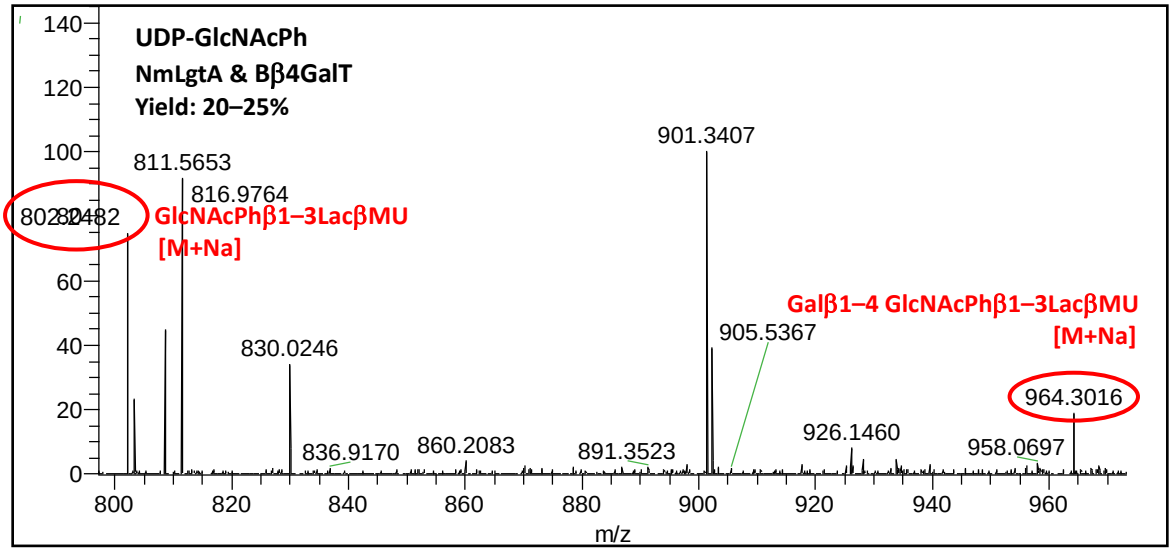
953.6954
964.3005
Galβ1–4 GlcNAcPhβ1–3LacβMU
[M+Na]
m/z


UDP-GlcNAcPh
NmLgtA & Bβ4GalT
Yield: 20–25%
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GlcNAcPhβ1–3LacβMU
[M+Na]
830.0246
836.9170
860.2083
891.3523
901.3407
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[M+Na]
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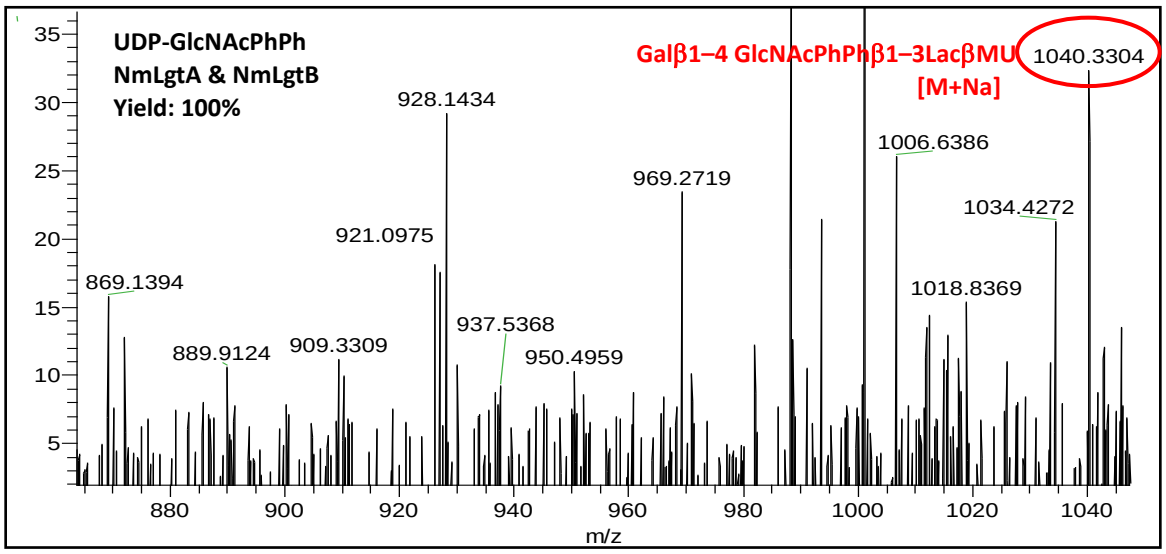
UDP-GlcNAcPhPh
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Yield: 100%
Galβ1–4 GlcNAcPhPhβ1–3LacβMU
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1006.6386
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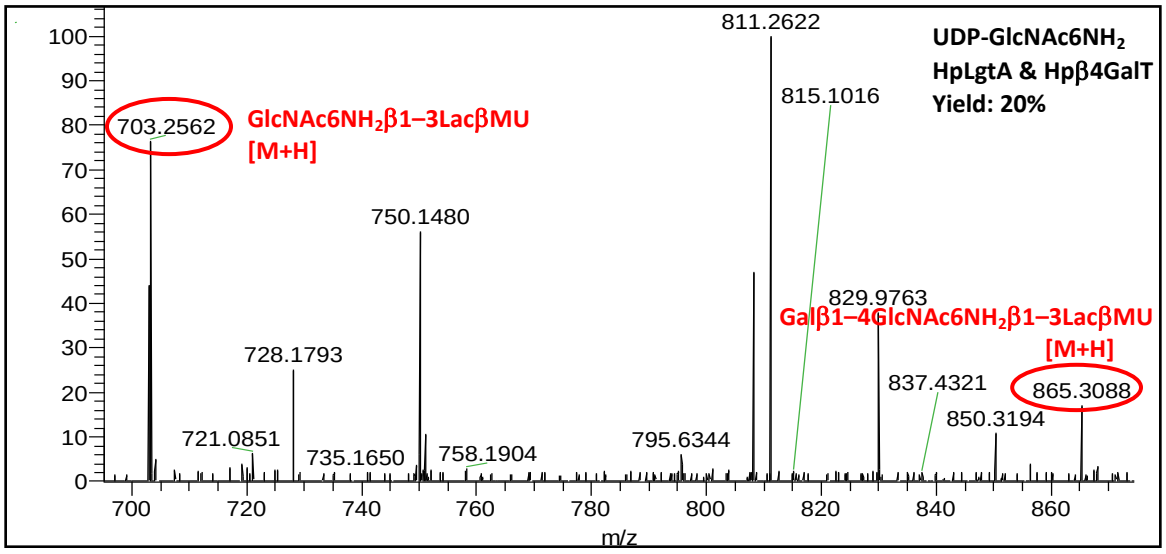
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HpLgtA & Hpβ4GalT
Yield: 20%
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815.1016
703.2562
GlcNAc6NH2β1–3LacβMU
[M+H]
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Galβ1–4GlcNAc6NH2β1–3LacβMU
[M+H]
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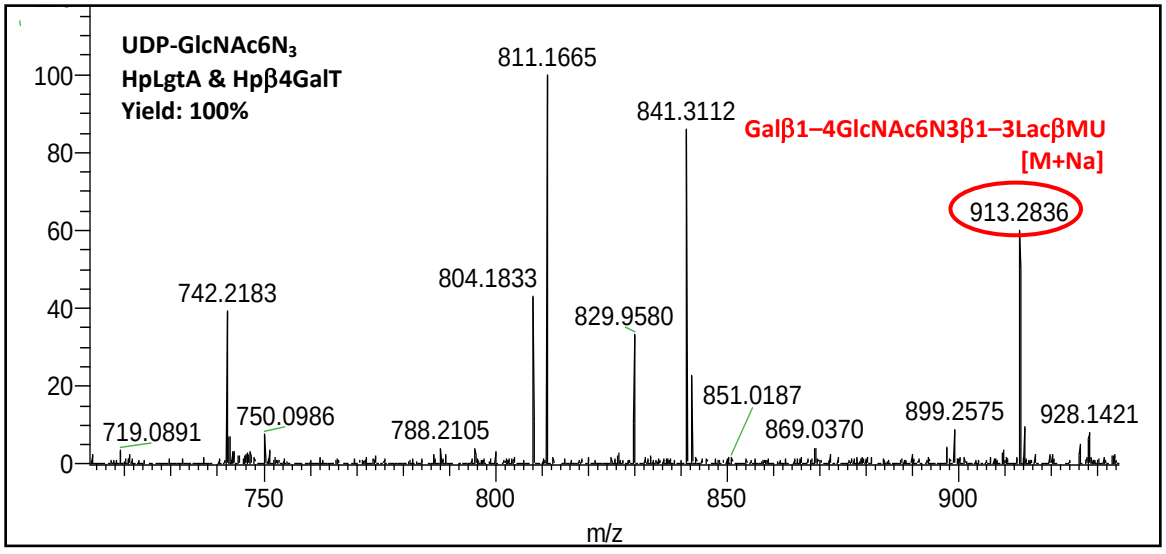
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HpLgtA & Hpβ4GalT
Yield: 100%
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Galβ1–4GlcNAc6N3β1–3LacβMU
[M+Na]
913.2836
804.1833
742.2183
829.9580
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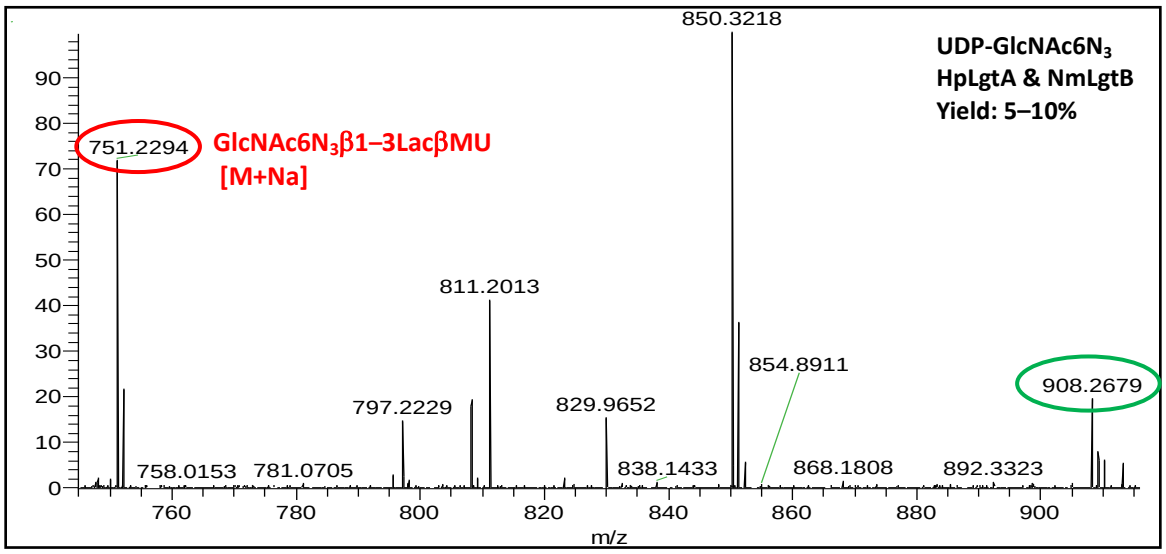
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UDP-GlcNAc6N3
HpLgtA & NmLgtB
Yield: 5–10%
751.2294
GlcNAc6N3β1–3LacβMU
[M+Na]
811.2013
854.8911
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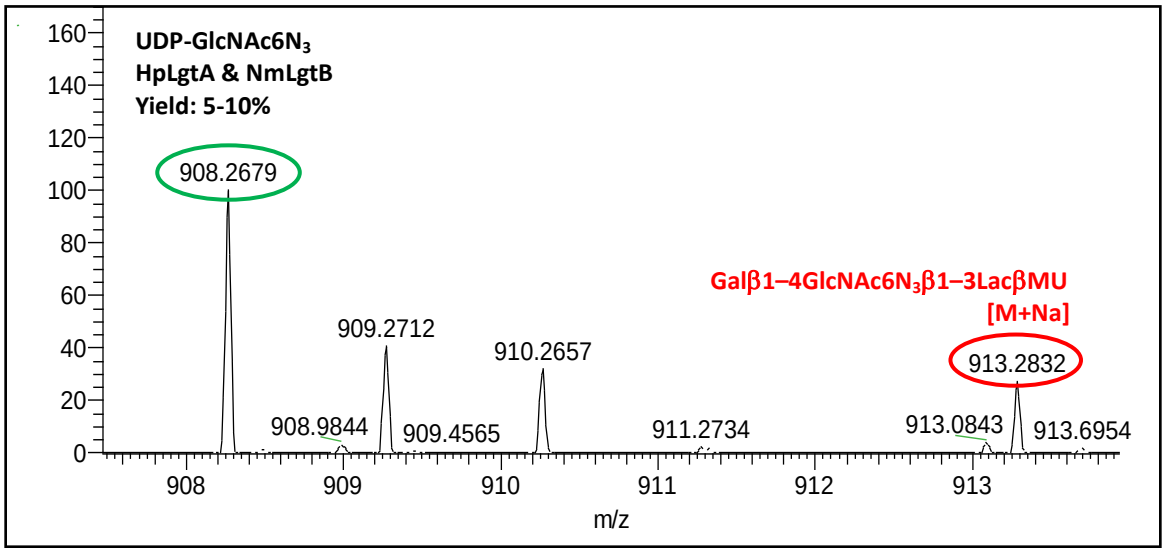
UDP-GlcNAc6N3
HpLgtA & NmLgtB
Yield: 5-10%
908.2679
Galβ1–4GlcNAc6N3β1–3LacβMU
[M+Na]
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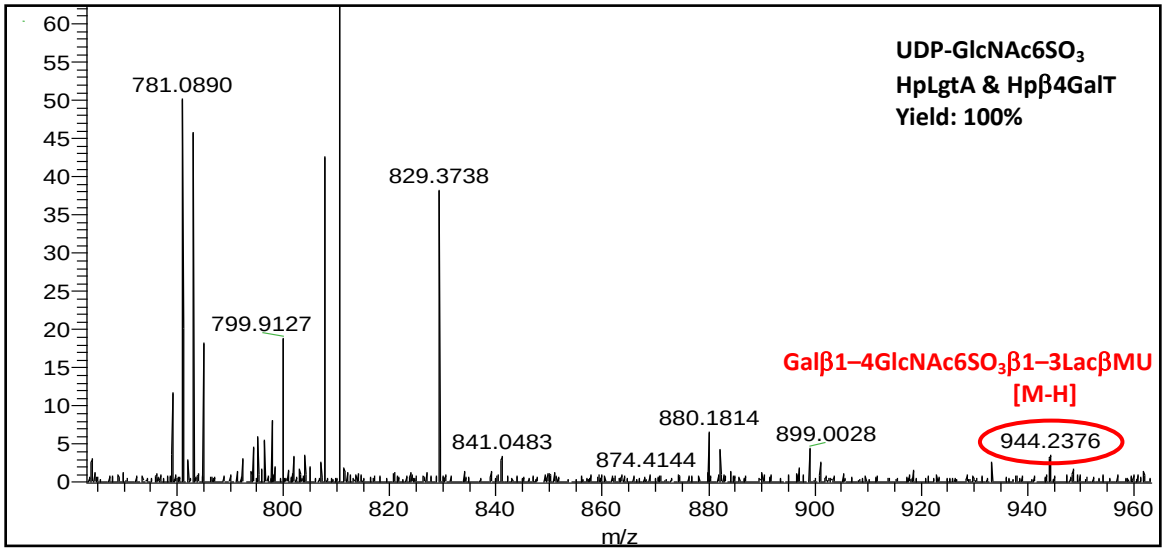
UDP-GlcNAc6SO3
HpLgtA & Hpβ4GalT
Yield: 100%
781.0890
829.3738
799.9127
Galβ1–4GlcNAc6SO3β1–3LacβMU
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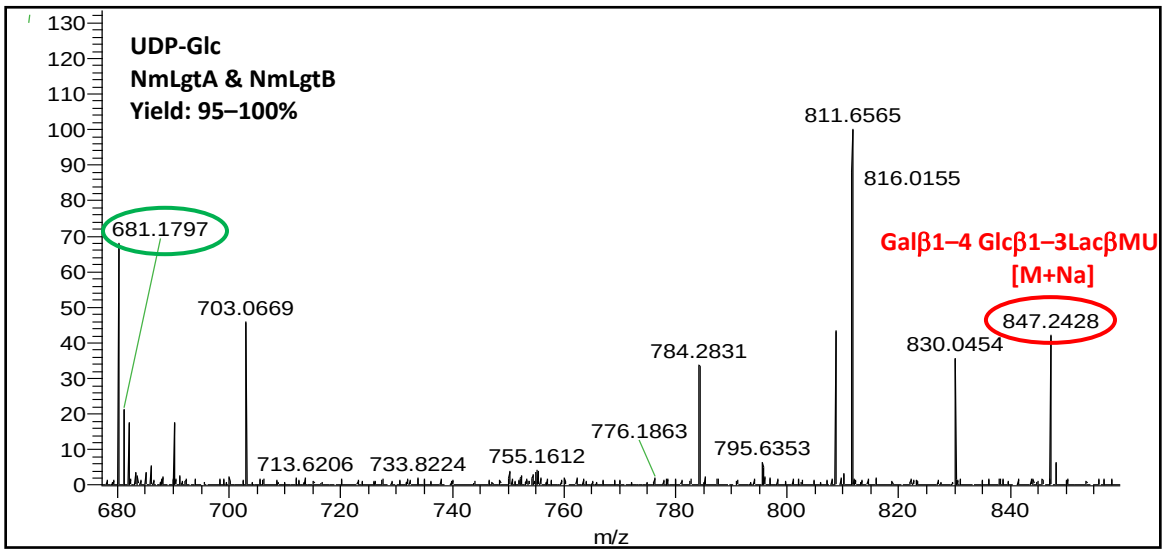
880.1814
899.0028
944.2376
841.0483
874.4144
m/z


UDP-Glc
NmLgtA & NmLgtB
Yield: 95–100%
811.6565
816.0155
681.1797
Galβ1–4 Glcβ1–3LacβMU
[M+Na]
703.0669
847.2428
784.2831
830.0454
776.1863
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m/z

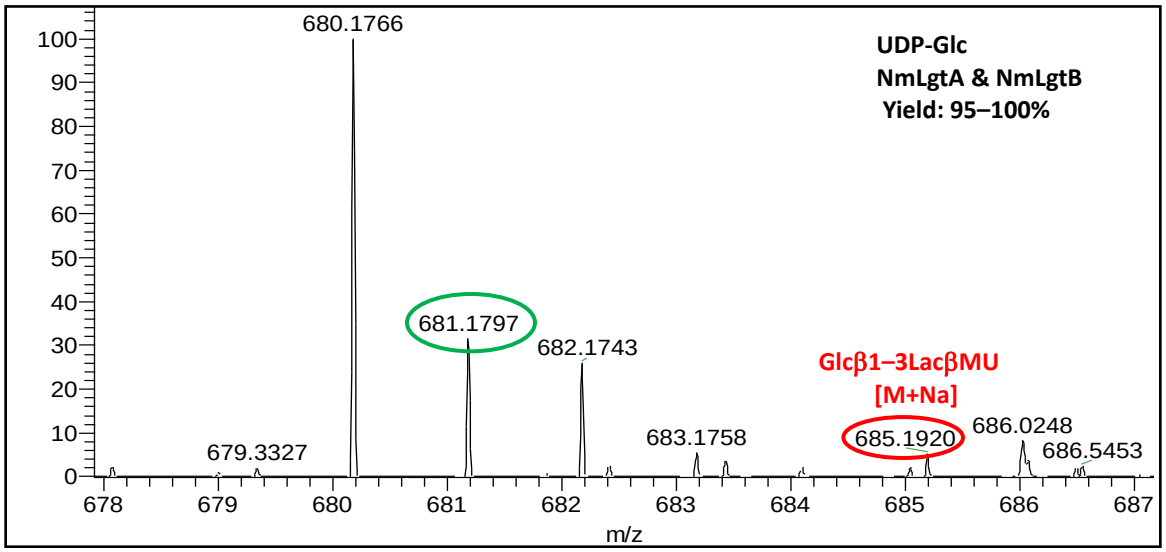

680.1766
UDP-Glc
NmLgtA & NmLgtB
Yield: 95–100%
681.1797
682.1743
Glcβ1–3LacβMU
[M+Na]
686.0248
685.1920
683.1758
679.3327
686.5453
m/z

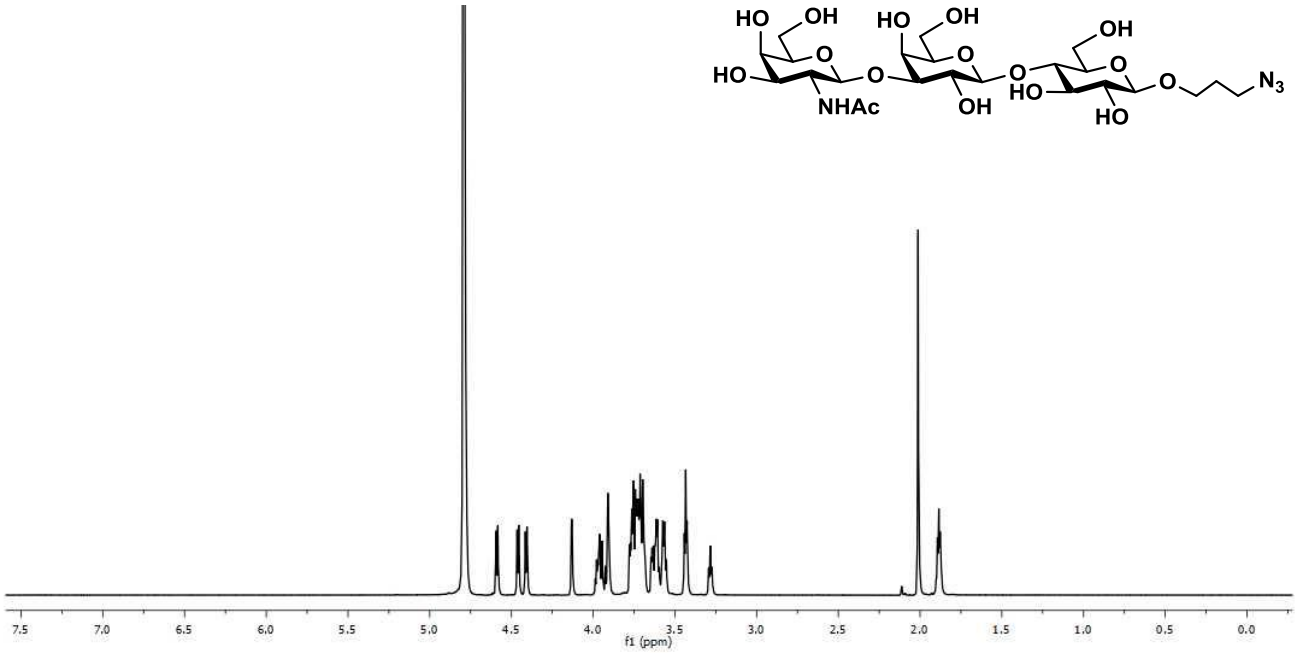
Table S1. ^{13}C NMR chemical shifts assignment of LacβProN_3 (Galβ1–4GlcβProN_3) and GalNAcβ1–3Galβ1–4GlcβProN_3 (**1**).

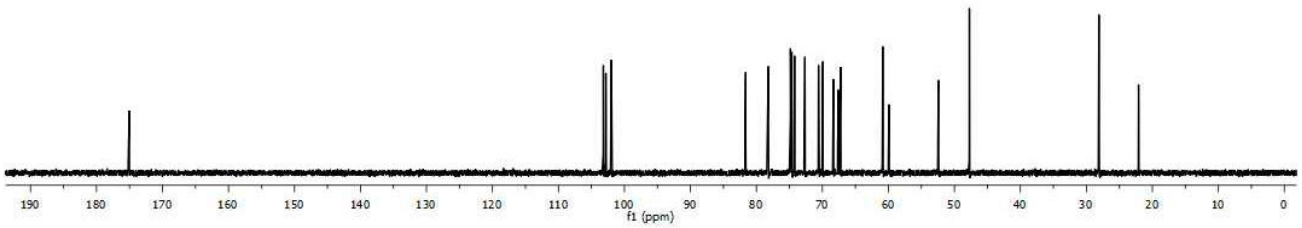
Residue	Carbon atom	Chemical shift (ppm)	
βGlc	C	LacβProN_3	GalNAcβ1–3Galβ1–4GlcβProN_3 (**1**)
	1	101.99	101.97
	2	72.67	72.65
	3	74.23	74.22
	4	78.22	78.20
	5	74.64	74.63
	6	59.92	59.92
βGal(1-4)	1	102.79	102.81
	2	70.82	69.93
	3	**72.37**	**81.63**
	4	68.41	67.61
	5	75.22	74.84
	6	60.89	60.82
βGalNAc(1-3)	1		103.20
	2		69.93
	3		70.59
	4		68.34
	5		74.74
	6		60.85
	C=O	174.61	175.03
	CH_3	22.37	22.09
ProN_3	$O\underline{C}H_2CH_2CH_2N_3$	67.24	67.23
	$OCH_2\underline{C}H_2CH_2N_3$	28.10	28.10
	$OCH_2CH_2\underline{C}H_2N_3$	47.73	47.73

Table S2. ^{13}C NMR chemical shifts assignment of Lac (Galβ1–4Glc) and Galβ1–3Galβ1–4Glc (**2**).

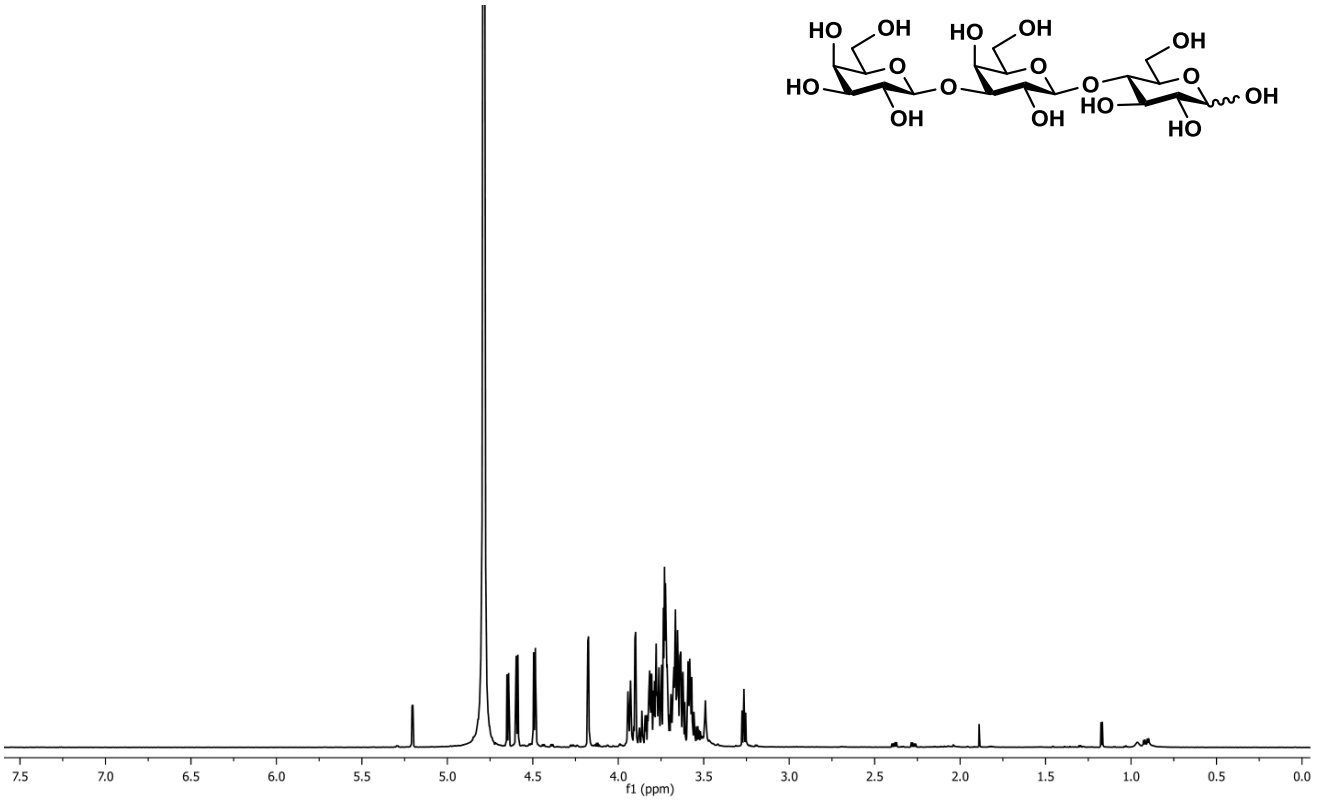
Residue	Carbon atom	Chemical shift (ppm)	
βGlc	C	Lac (α-isomer)	Galβ1–3Galβ1–4Glc (α-isomer) (**2**)
	1	91.68	91.70
	2	71.28	71.27
	3	71.01	71.03
	4	78.27	78.08
	5	69.97	68.43
	6	60.92	60.87
βGal(1–4)	1	102.75	102.39
	2	70.83	69.95
	3	**72.38**	**81.75**
	4	68.43	68.28
	5	75.22	74.65
	6	59.94	59.80
βGal(1–3)	1		104.21
	2		70.07
	3		72.36
	4		68.30
	5		74.85
	6		59.94

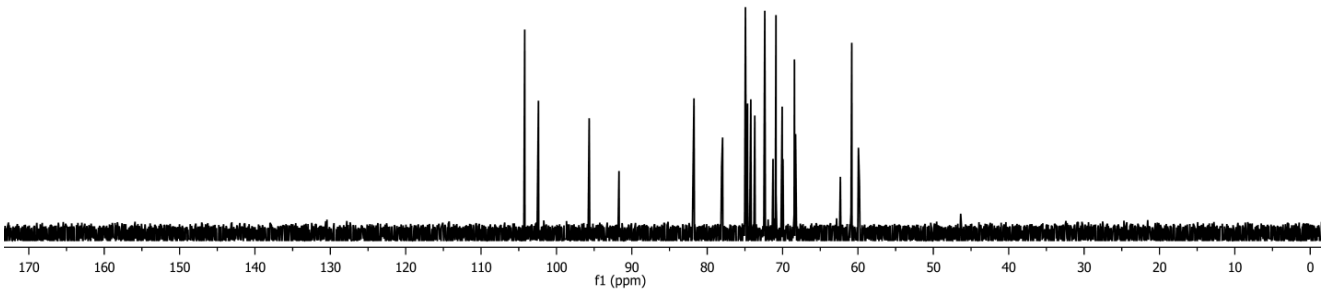
^{1}H and ^{13}C NMR spectra of GalNAcβ1–3Galβ1–4GlcβProN_3 (**1**)





^{1}H and ^{13}C NMR spectra of Galβ1–3Galβ1–4Glc (**2**)





References:

1. Chen, Y.; Thon, V.; Li, Y.; Yu, H.; Ding, L.; Lau, K.; Qu, J.; Hie, L.; Chen, X. *Chem. Commun.* **2011,** *47*, 10815.
2. Muthana, M. M.; Qu, J.; Li, Y.; Zhang, L.; Yu, H.; Ding, L.; Malekan, H.; Chen, X. *Chem. Commun.* **2012,** *48*, 2728.
3. Zhang, L.; Muthana, M. M.; Yu, H.; McArthur, J. B.; Qu, J.; Chen, X. *Carbohydr. Res.* **2016,** *419*, 18.
4. Muthana, M. M.; Qu, J.; Xue, M.; Klyuchnik, T.; Siu, A.; Li, Y.; Zhang, L.; Yu, H.; Li, L.; Wang, P. G.; Chen, X. *Chem. Commun.* **2015,** *51*, 4595.
5. Yu, H.; Yu, H.; Karpel, R.; Chen, X. *Bioorg. Med. Chem.* **2004,** *12*, 6427.
6. Zhang, L.; Lau, K.; Cheng, J.; Yu, H.; Li, Y.; Sugiarto, G.; Huang, S.; Ding, L.; Thon, V.; Wang, P. G.; Chen, X. *Glycobiology* **2010,** *20*, 1077.