Electronic Supplementary Material (ESI) for Molecular Omics.

Supplemental Figures

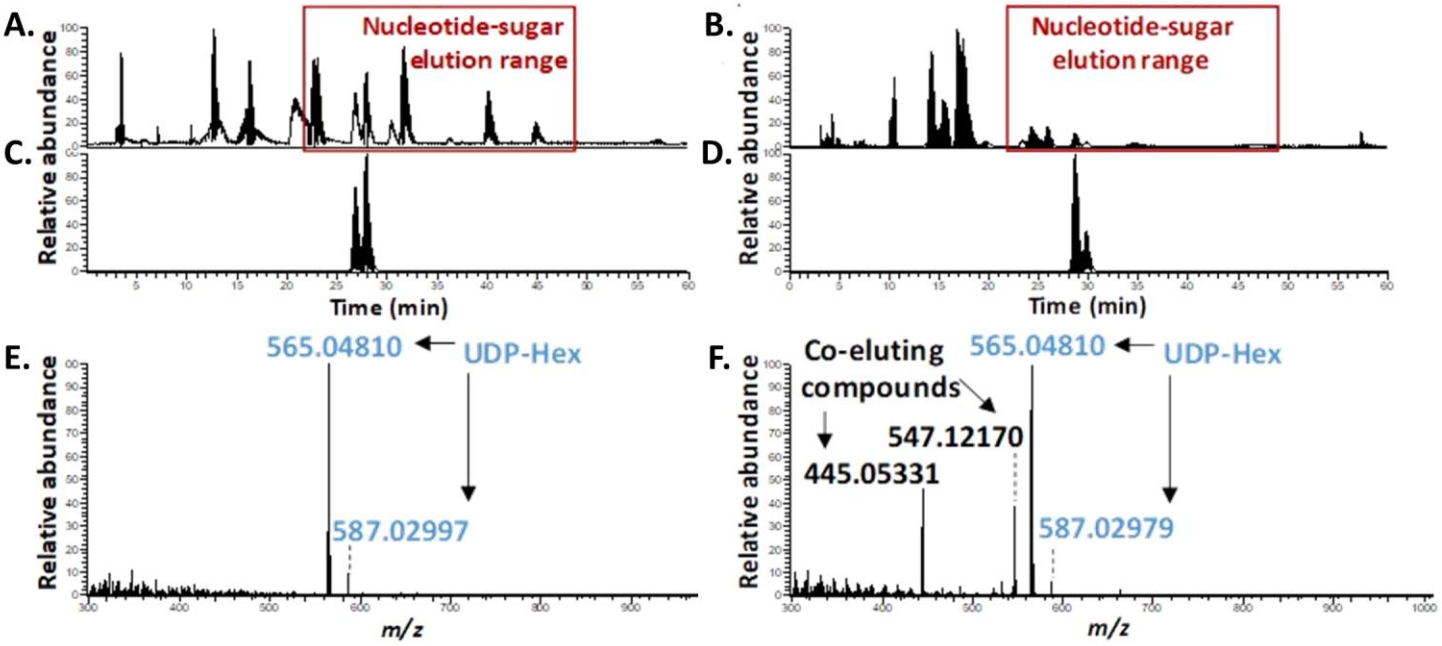
Table S1
Figures S1-S6

Table S1. Limit of detection (LoD) and limit of quantification (LoQ) of standard nucleotide sugars *

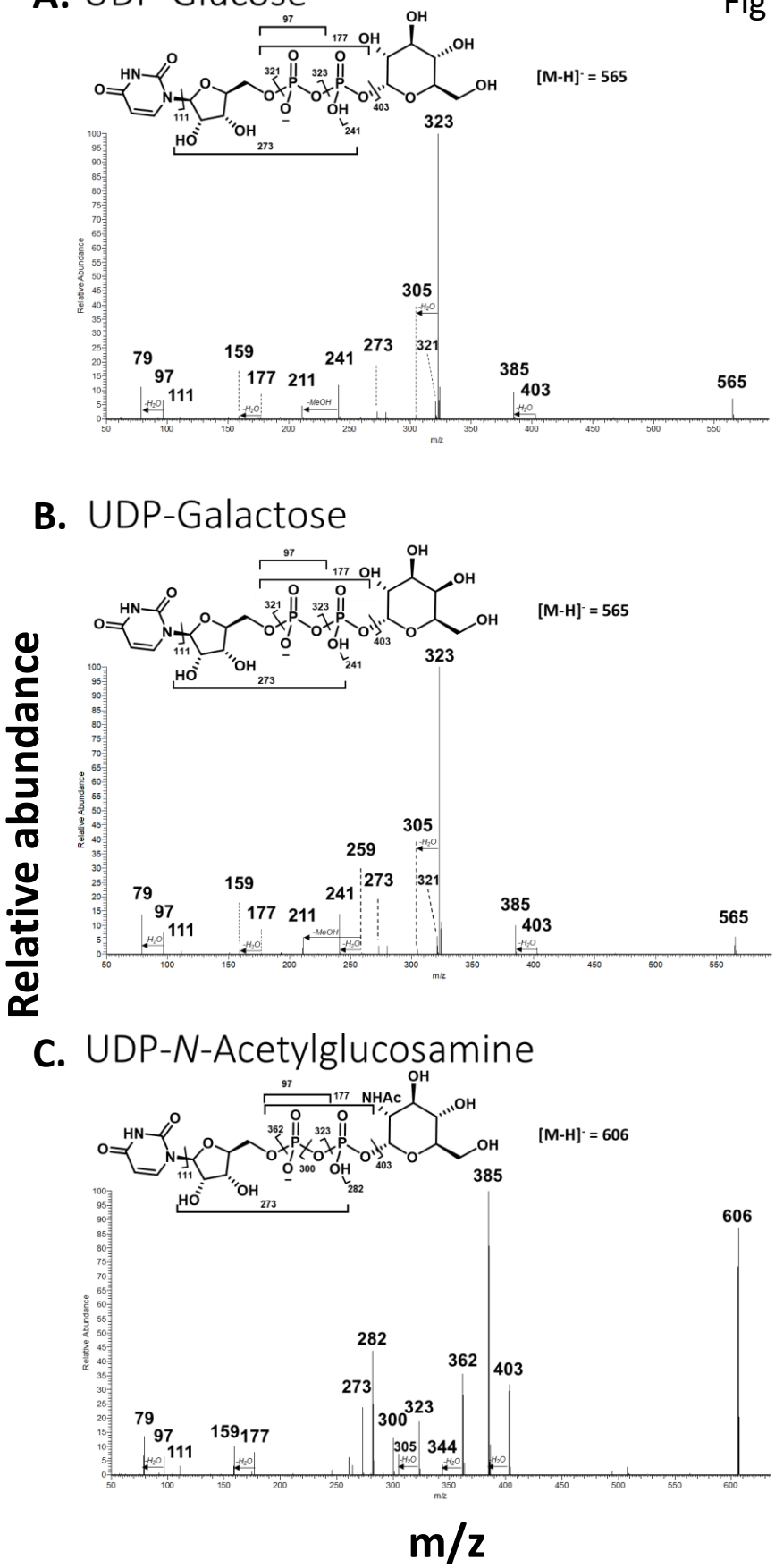
Nucleotide Sugar	LoD (pmol)	LoQ (pmol)	R^2
UDP-Glc	4.8	14.4	0.9997
UDP-Gal	1.6	4.9	0.9993
UDP-GlcA	7.4	22.4	0.9995
GDP-fuc	2.2	6.6	0.9999
GDP-Man	6.5	19.6	0.9998
UDP-GlcNAc	8.4	25.4	0.9994
UDP-GalNAc	2.9	8.6	0.9999
CMP-Neu5Ac	12.1	36.7	0.9987
UDP-Xyl	3.3	9.9	0.9996

* MS injection volume was 5µL. Thus, µM concentrations presented in main manuscript are converted to pmol units by multiplying by 5 (i.e 1µM is equivalent to 5pmol).

del Solar *et al.*
Fig S1



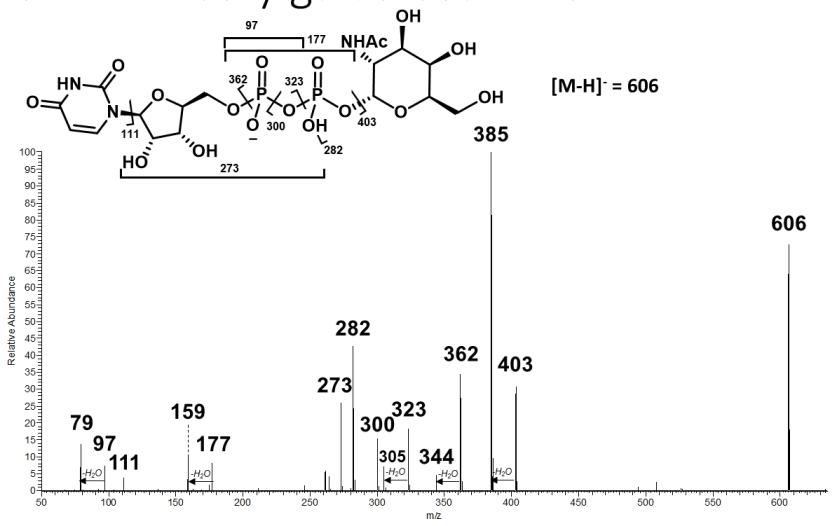
Supplemental Figure S1. Analysis of UDP-Hex in chemical standard run vs. cell lysate preparation. The elution profile of UDP-Hex in the chemical standard run (panels A, C, E) was compared with that of the same nucleotide-sugar in the HL60 vehicle control run (B, D, F). **A**, **B**: Complete high resolution MS1 ion current data for standard (A) and cell sample (B). **C**, **D**: Extracted Ion Chromatogram (EIC) for UDP-Hex for the standard (C) and cell samples (D). **E**, **F**: High resolution MS1 ion current data for two-minute window encompassing UDP-Hex for standard (E) and cell sample (F). In this time window, only two other compounds (m/z=547, 445) elute with UDP-Hex (m/z=565 [H^-], 587[Na^+H^-]) in the cell sample (panel F). Thus, the sample complexity is low in the time window where the nucleotide-sugars were identified.



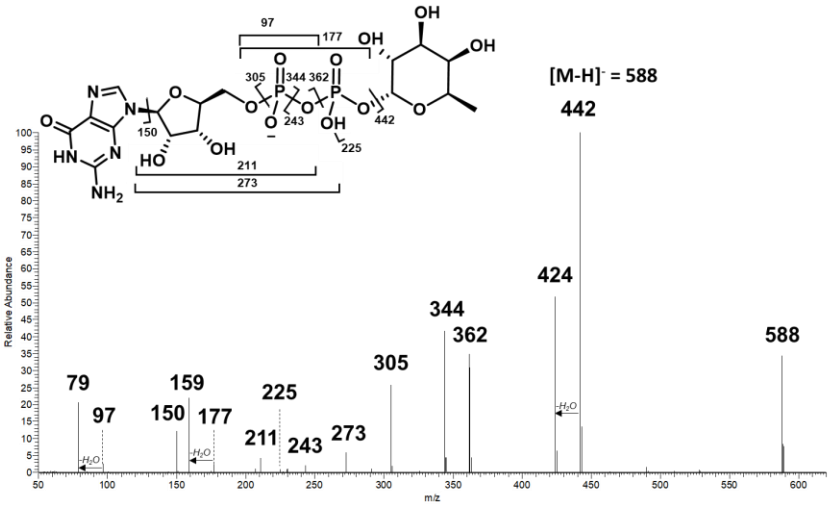
Supplemental Figure S2. A-O. MS^2 of nucleotide-sugars in cell extracts. Representative figures are presented. The MS/MS profile for each of the compounds was not very different in the different preparations, regardless of the sample.

del Solar *et al.*
Fig S2 (continued)

D. UDP-*N*-Acetylgalactosamine

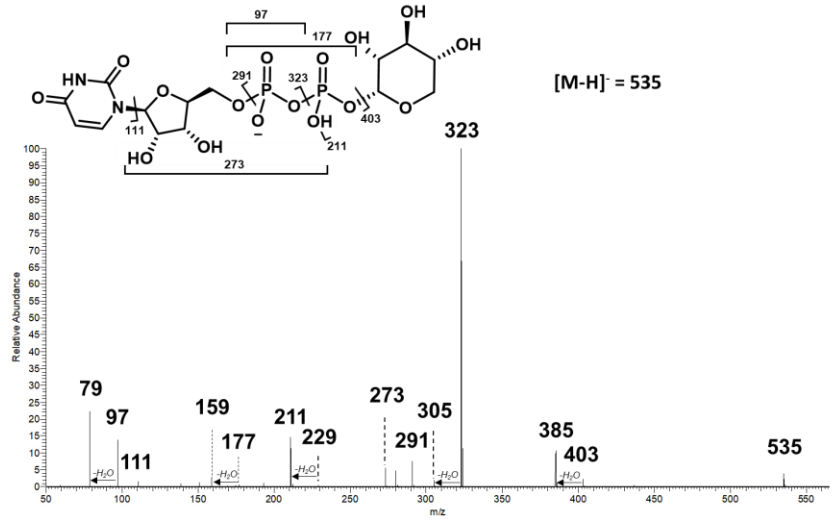


E. GDP-Fucose



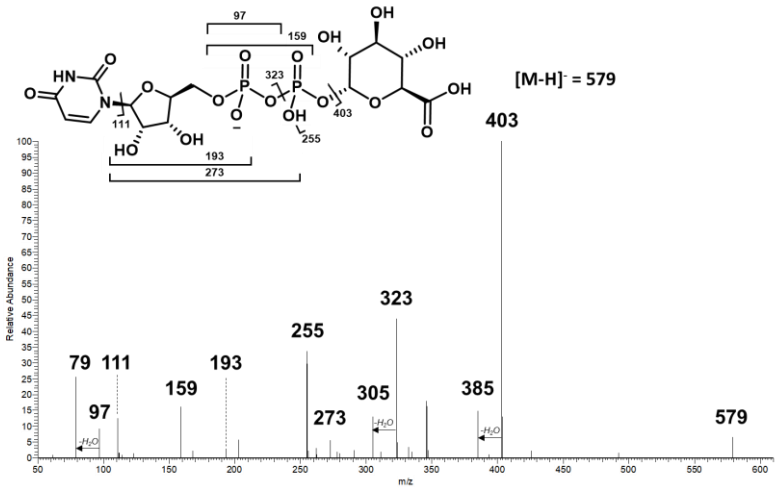
Relative abundance

F. UDP-Xylose

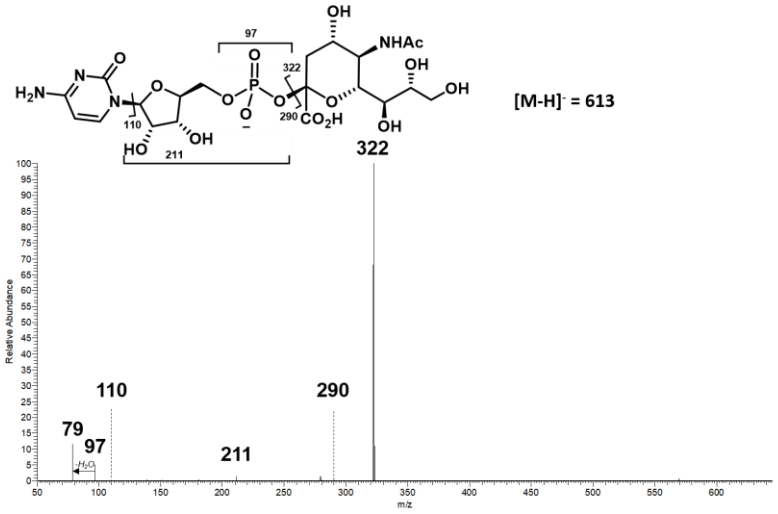


m/z

G. UDP-Glucuronic acid

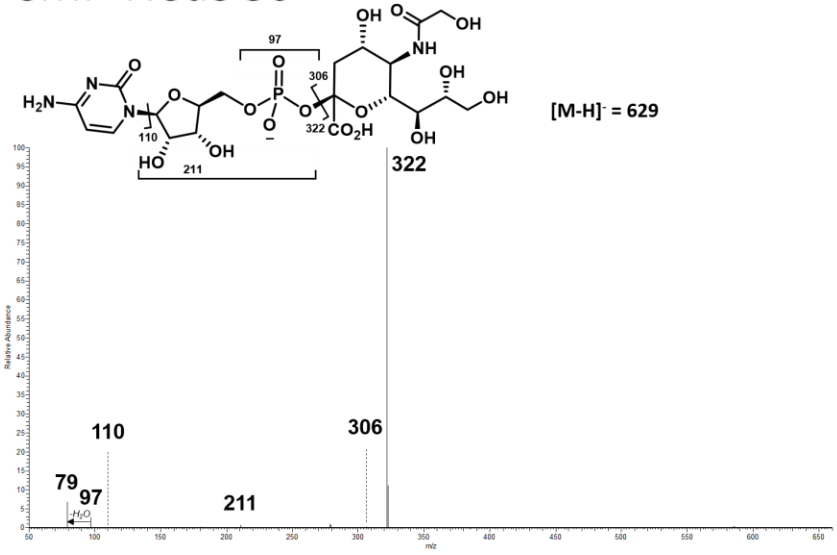


H. CMP-Neu5Ac



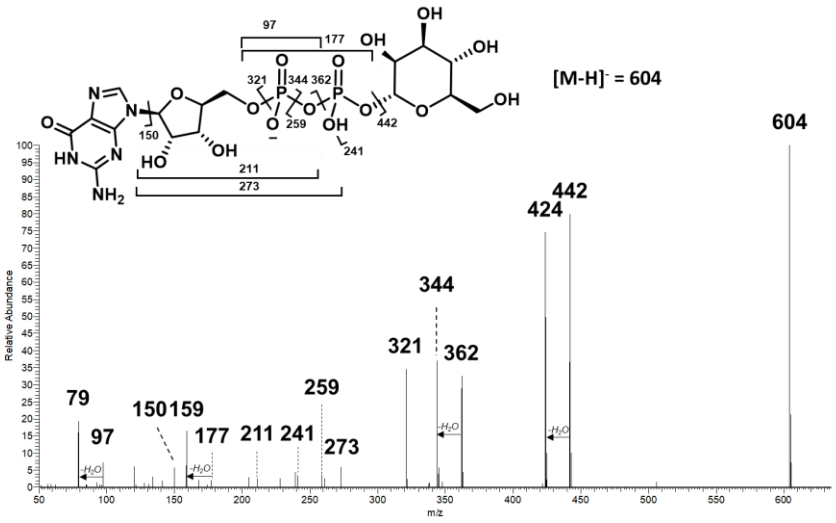
Relative abundance

I. CMP-Neu5Gc

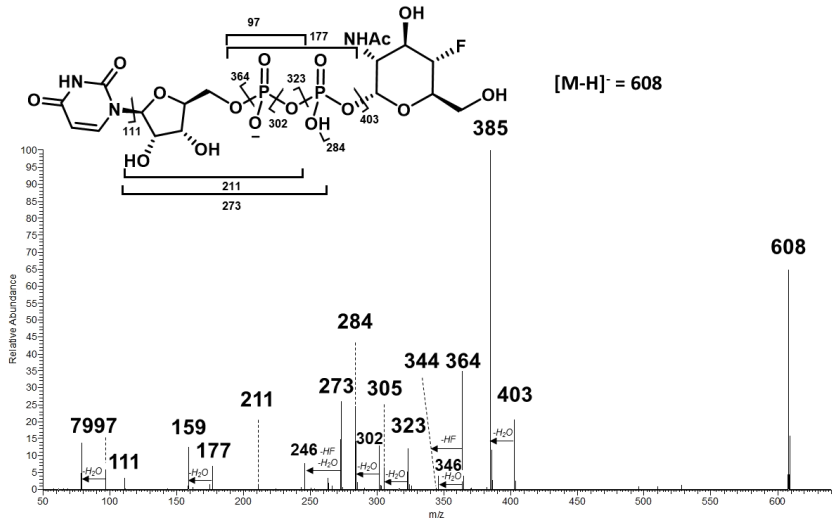


m/z

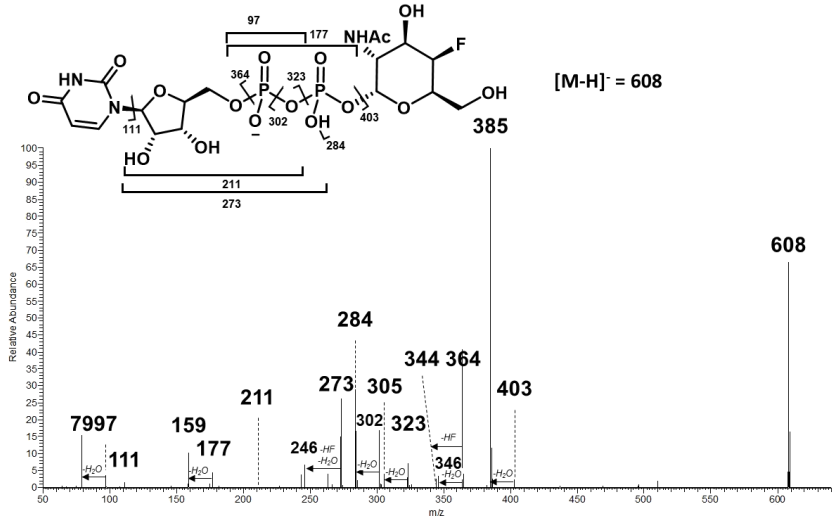
J. GDP-Mannose



K. UDP-4FGlcNAc



L. UDP-4FGalNAc

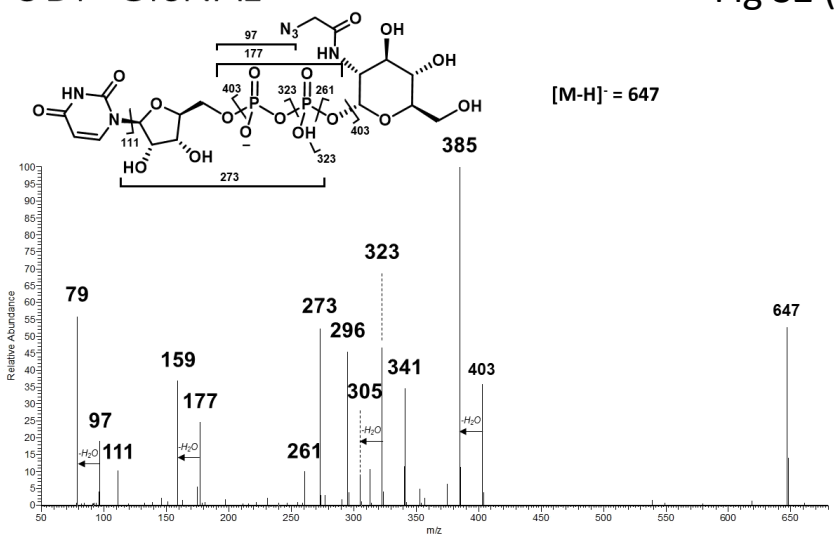


Relative abundance

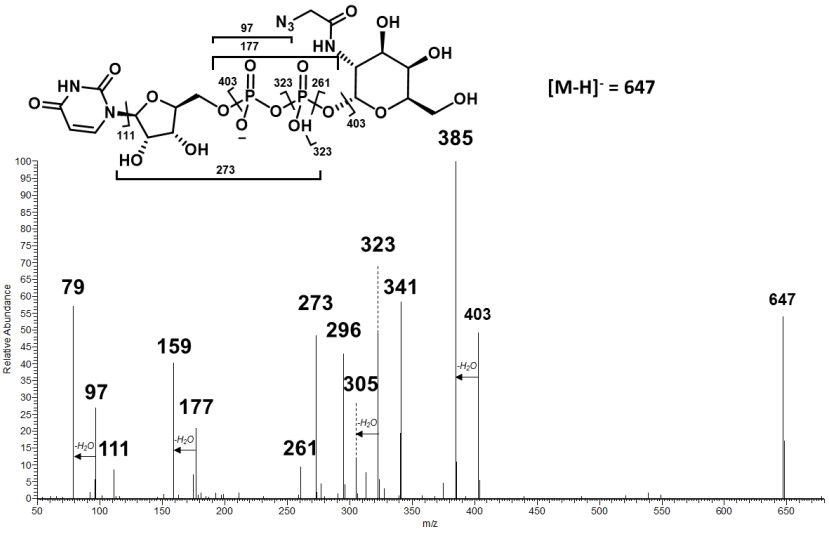
m/z

del Solar *et al.*
Fig S2 (continued)

M. UDP-GlcNAz

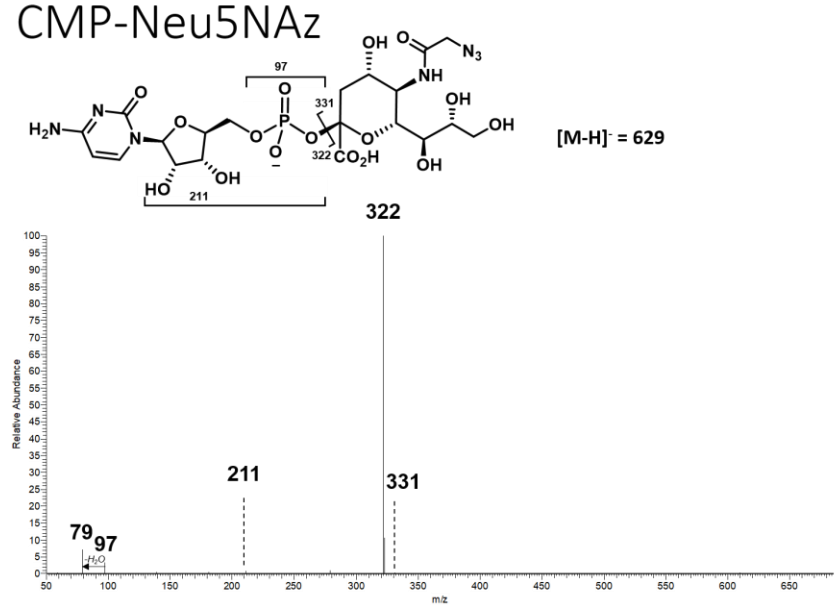


N. UDP-GalNAz

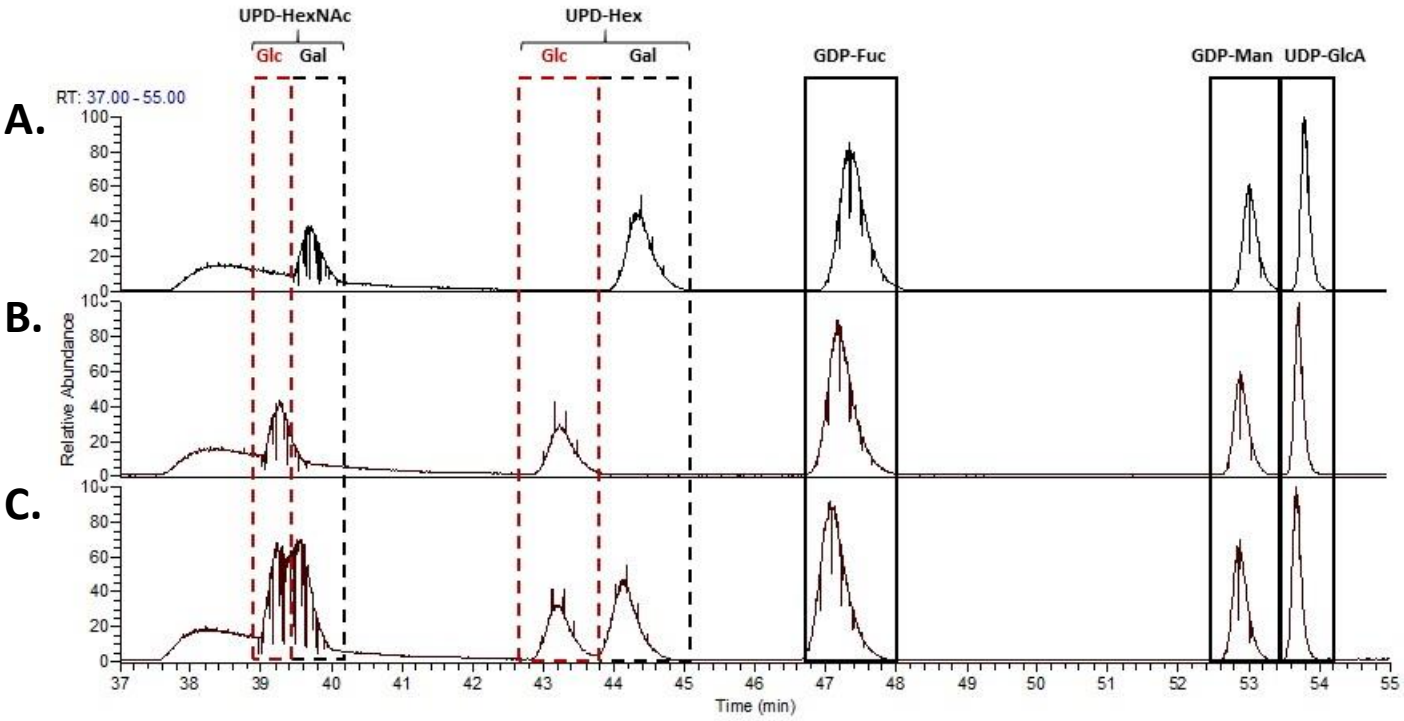


Relative abundance

O. CMP-Neu5NAz



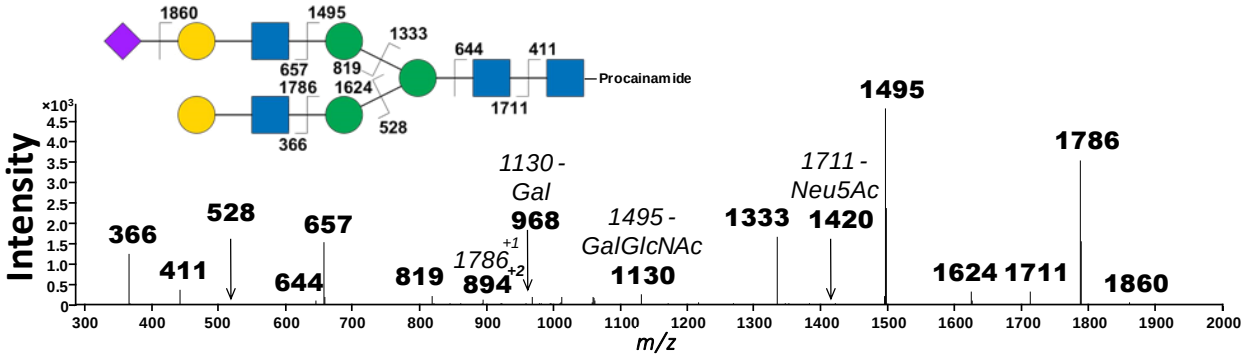
m/z



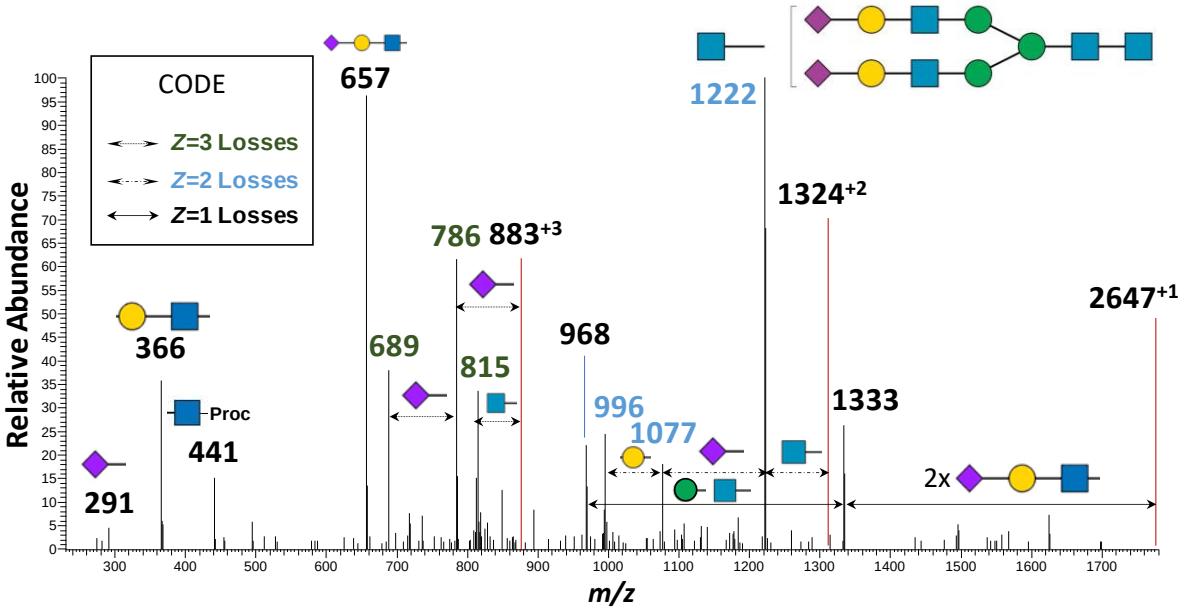
Supplemental Figure S3. Distinguishing Gal and Glc containing nucleotide-sugars. Three different standard mixture were prepared. All samples contained 10-12μM GDP-Man, 7μM GDP-Fuc and 5μM UDP-GlcA. In addition, the different panels contained: **A.** 3μM UDP-GlcNAc and 1μM UDP-Glc. **B.** 5μM UDP-GalNAc and 1μM UDP-Gal. **C.** both galactose (3-5μM each) and glucose (1μM each) based nucleotide-sugars. Glc based compounds elute first prior to Gal based molecules.

del Solar *et al.*
Fig S4

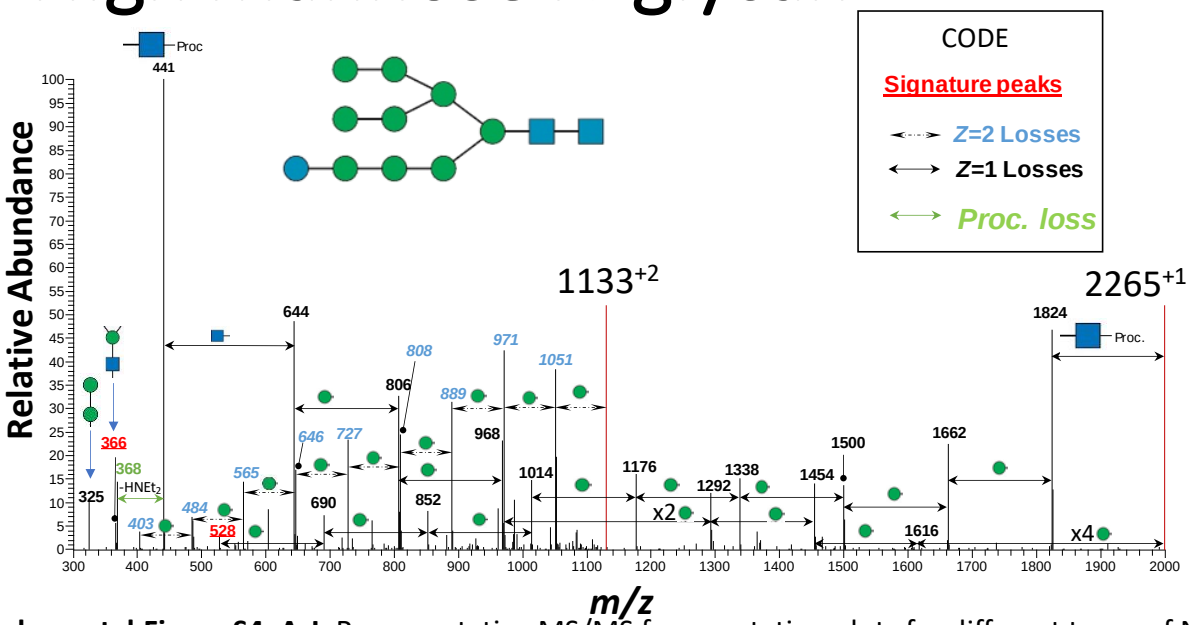
A. Monosialylated N-glycans



B. Biantennary sialyl N-glycan



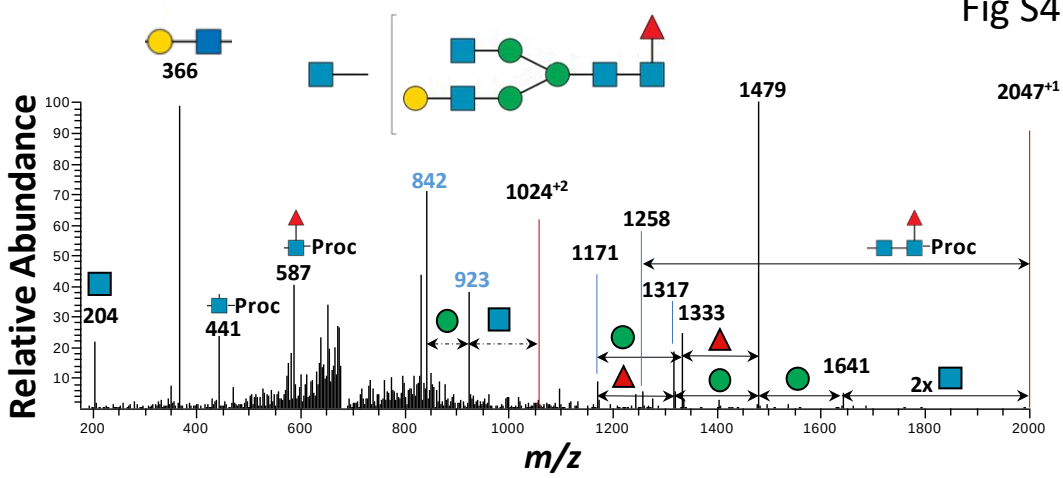
C. High Mannose N-glycan



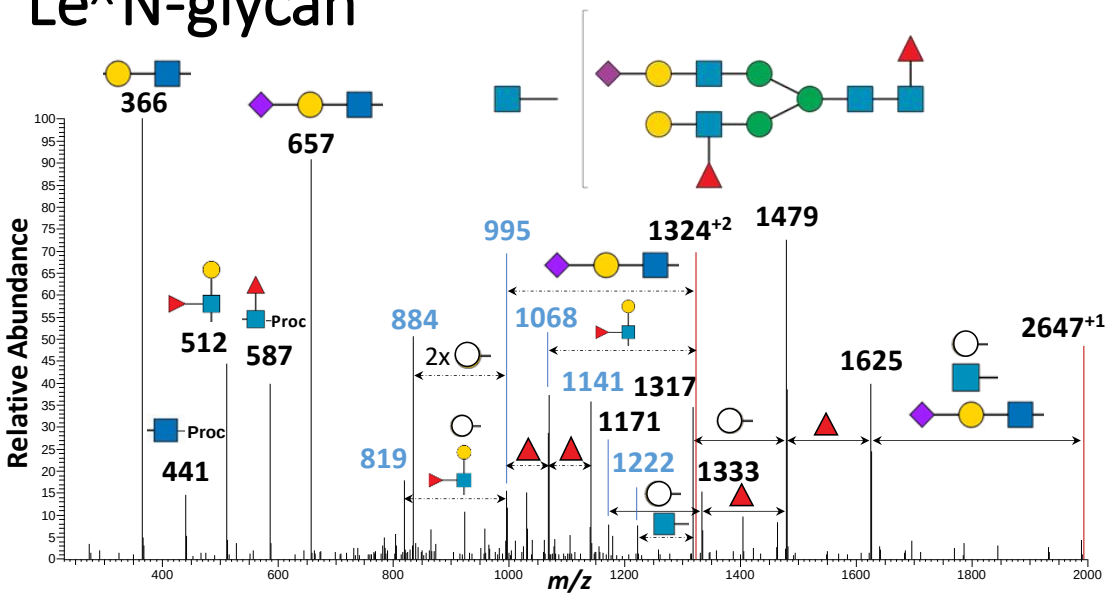
Supplemental Figure S4. A-J. Representative MS/MS fragmentation plots for different types of N-linked glycans. Initial glycan mass is shown using red line.

del Solar *et al.*
Fig S4 (continued)

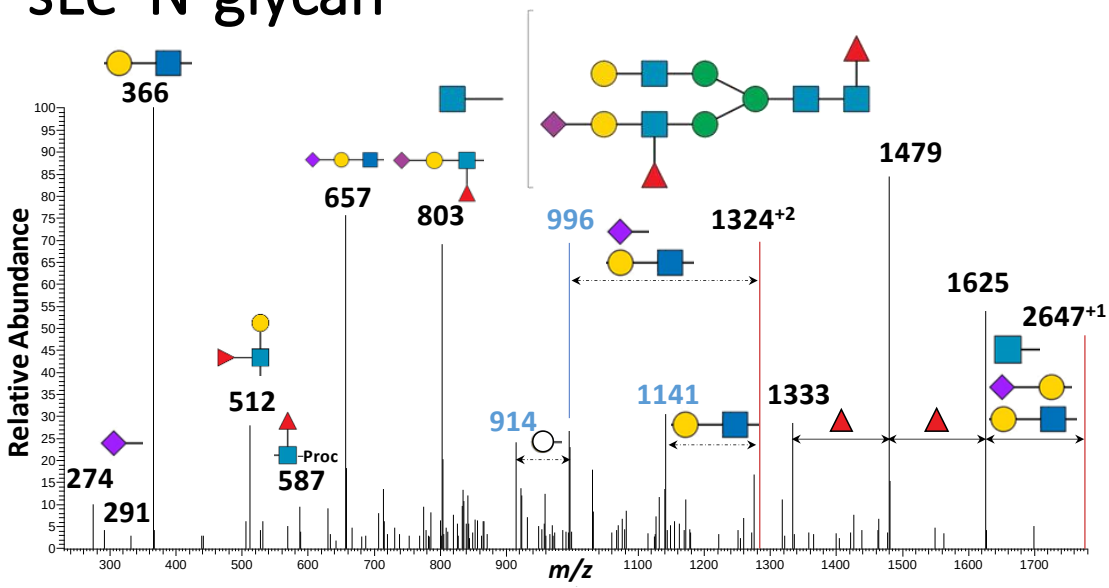
D. Fucosylated N-glycan



E. Le^X N-glycan

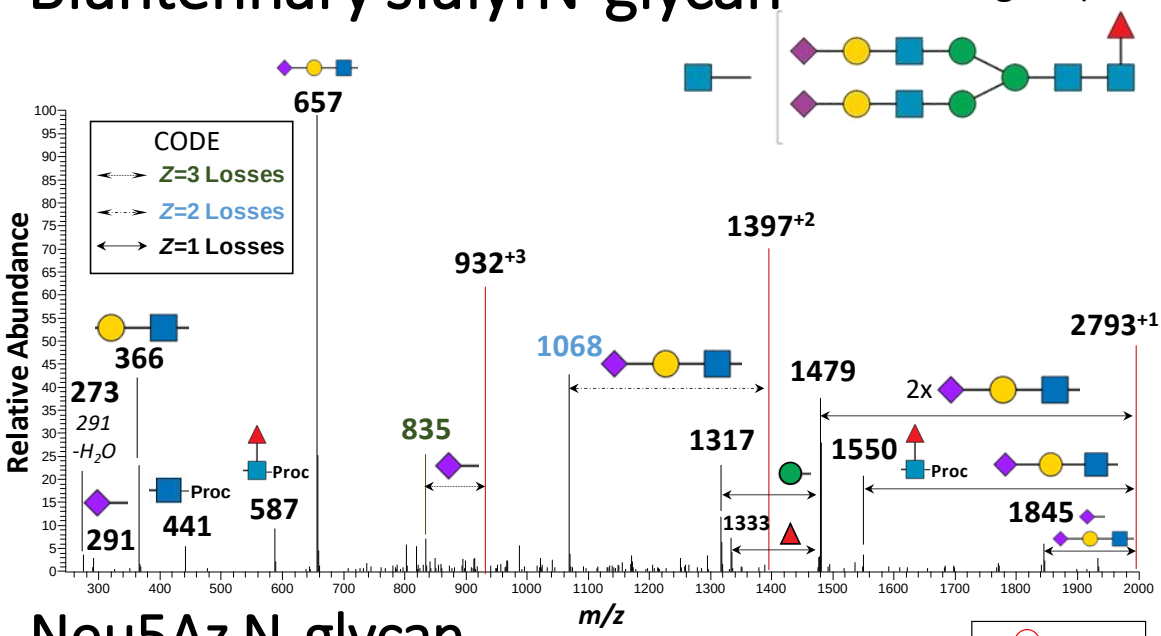


F. sLe^X N-glycan

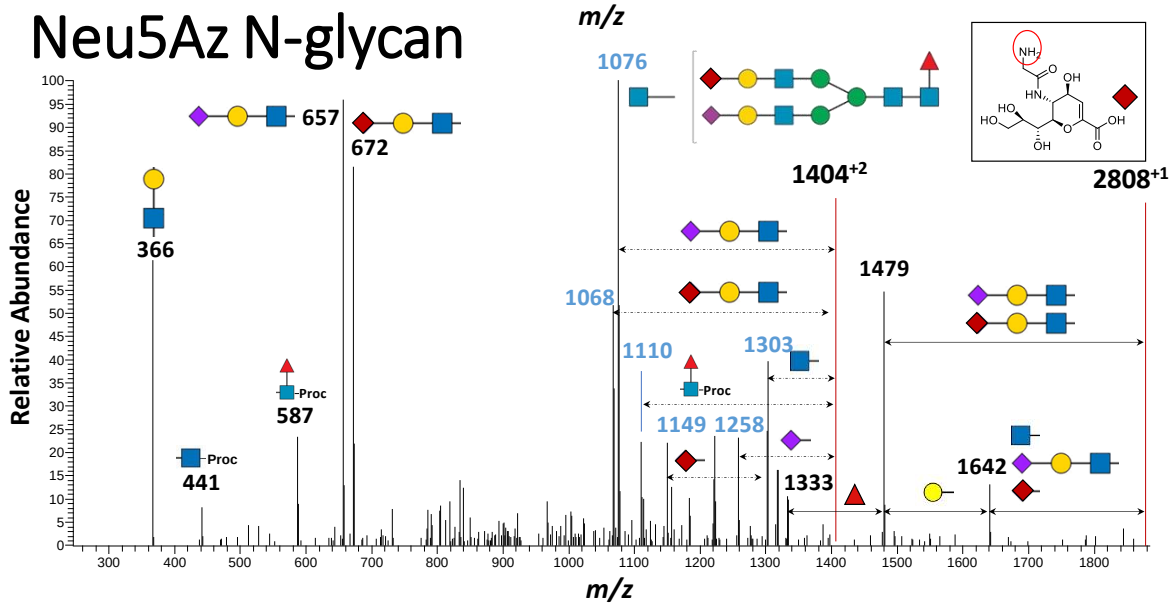


del Solar *et al.*
Fig S4 (continued)

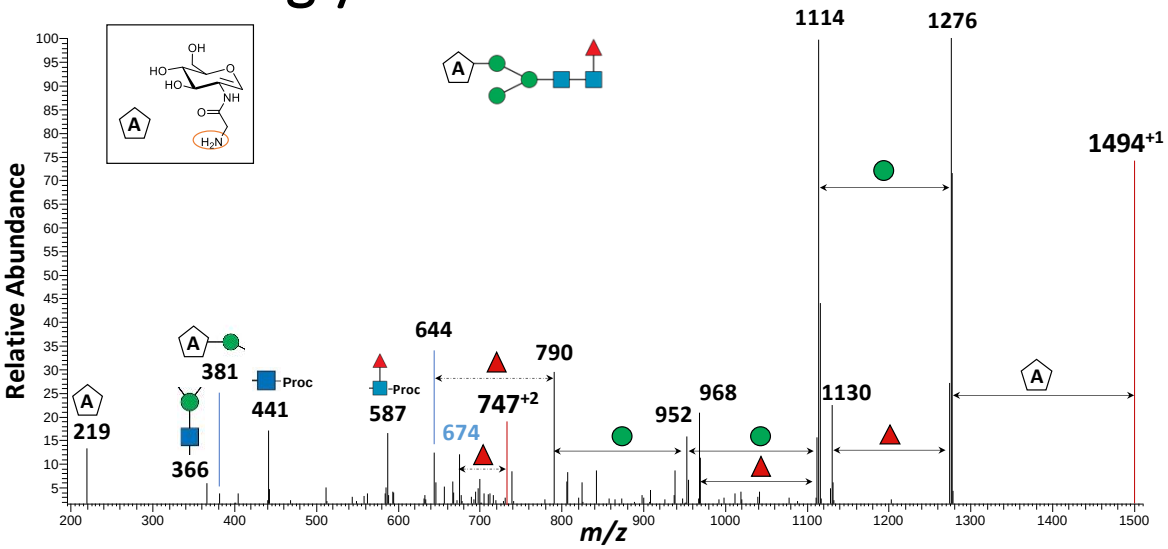
G. Biantennary sialyl N-glycan

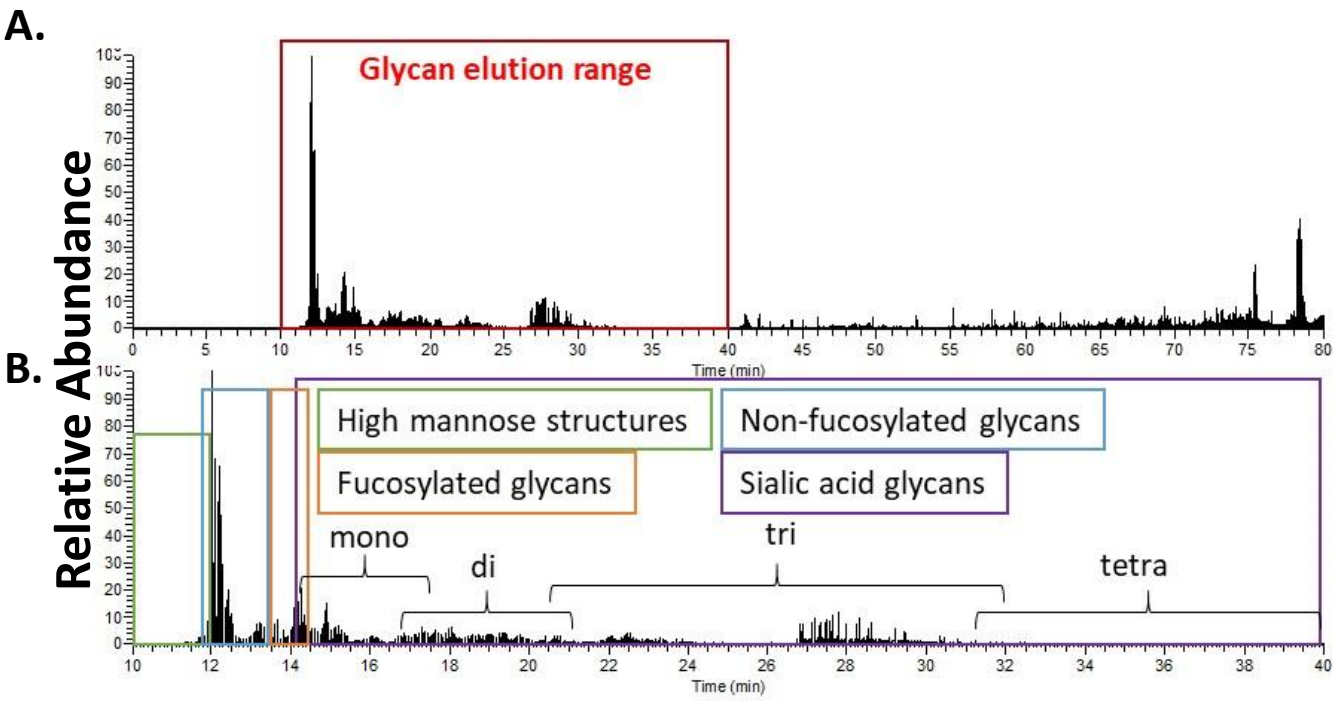


H. Neu5Az N-glycan

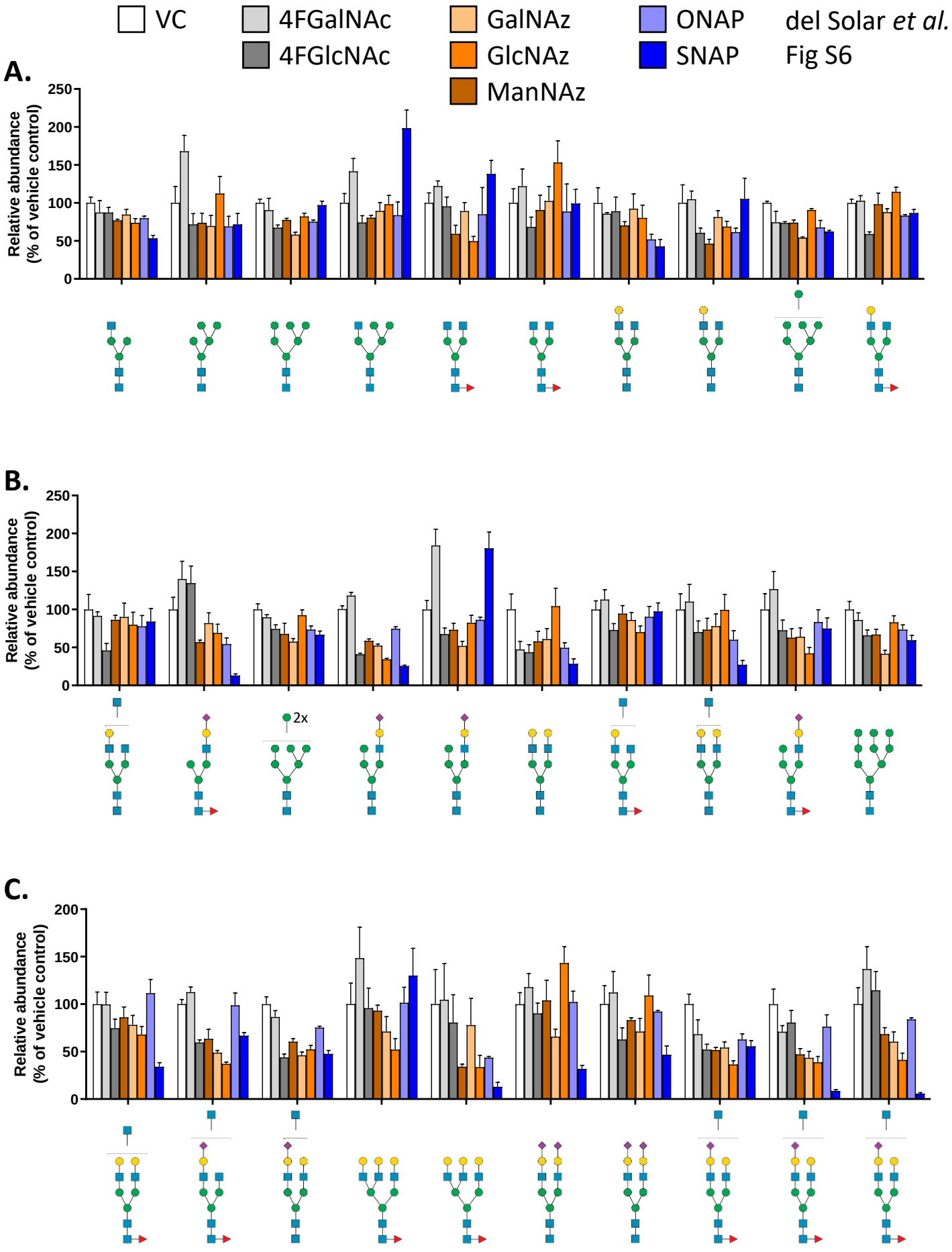


J. GalNAz N-glycan



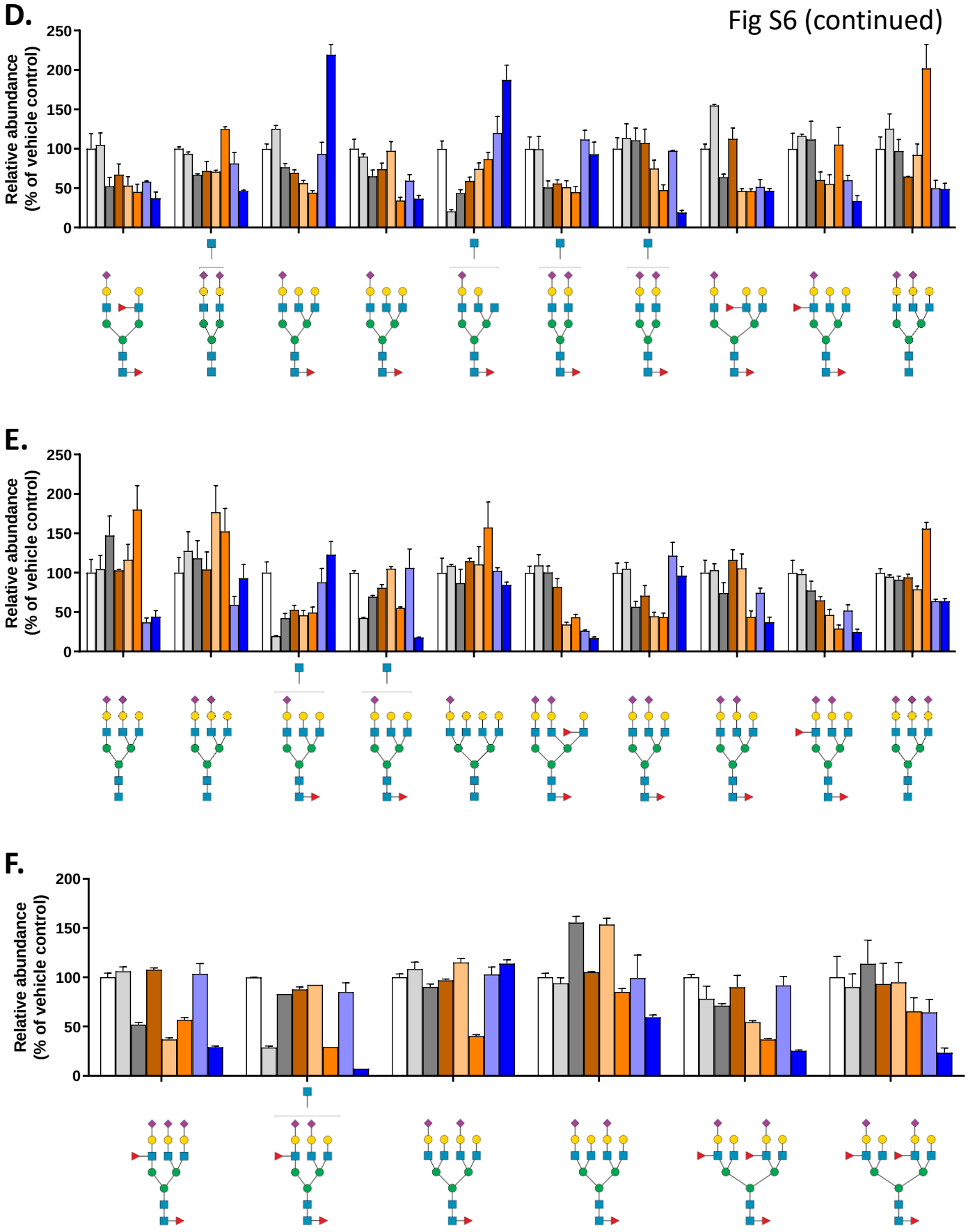


Supplemental Figure S5. Chromatogram and elution profile of N-linked glycans after treatment with vehicle **A.** Total ion count of N-glycans corresponding to VC sample. **B**. Elution window of N-glycans from vehicle-treated HL-60s (from 10 min to 40 min).



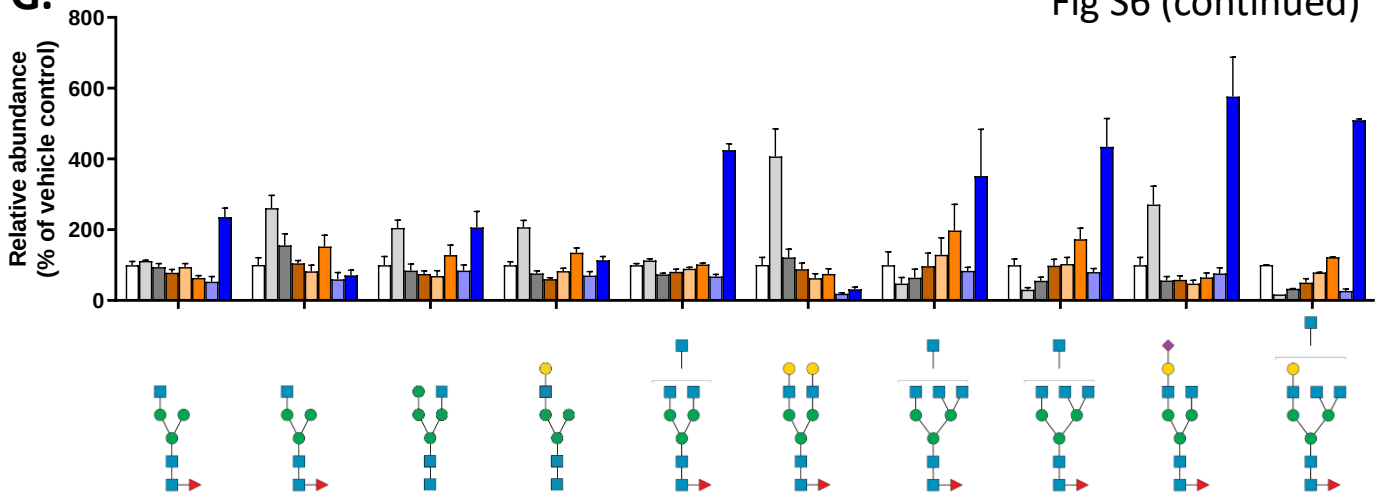
Supplemental Figure S6. Normalized relative area abundance of N-linked glycans after treatment with different sugar compounds. **A-F.** Plots show all glycans presenting relative abundance change lower than 200 (Vehicle=100 in all cases). **G-H.** Plots show some glycans presenting relative abundance change higher than 200. These represent the major glycans that are modified by small molecules.

del Solar *et al.*
Fig S6 (continued)



del Solar *et al.*
Fig S6 (continued)

G.



H.