

Supplementary Data

for

***N*-Acetyl- β -D-hexosaminidases mediate the generation of paucimannosidic proteins via a putative non-canonical truncation pathway in human neutrophils**

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Annotation and Fragmentation Key

● Mannose (162.0528 Da)

■ N-Acetylglucosamine (203.0794 Da)

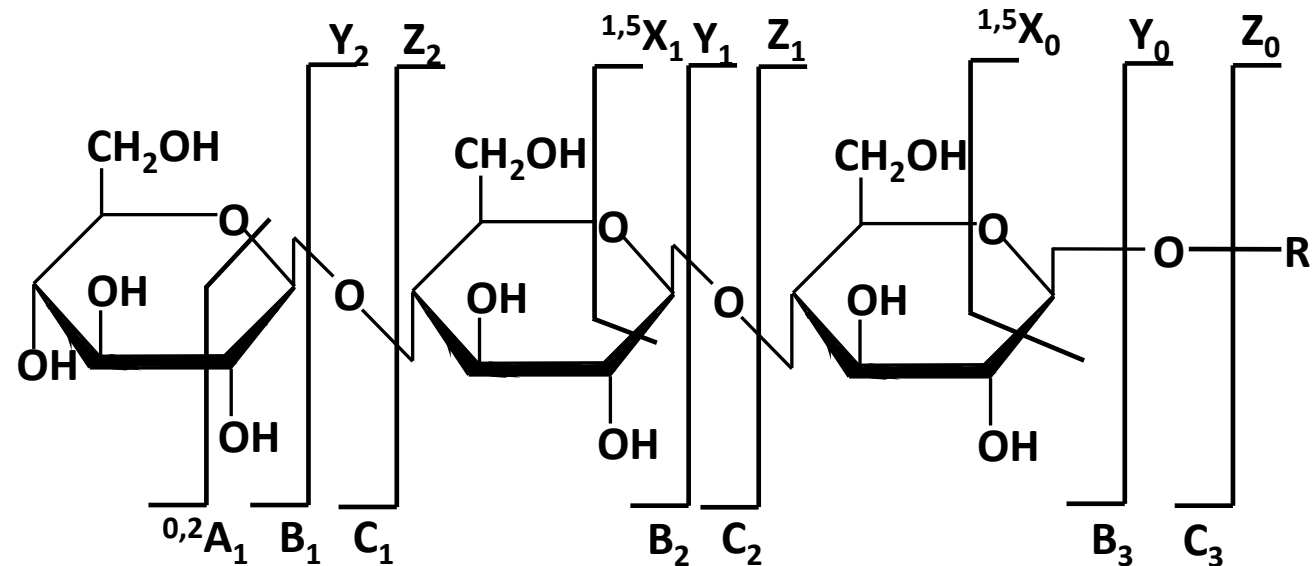
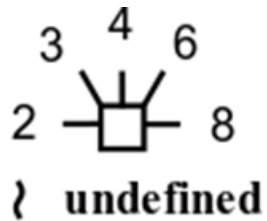
▲ Fucose (146.0579 Da)

Ac Acetyl group (42.0106 Da)

⌋ Indicates mostly Y ions (includes oxygen of glycosidic linkage)

⌋ Indicates mostly Z ions (excludes oxygen of glycosidic linkage)

⌋ Reduced reducing end

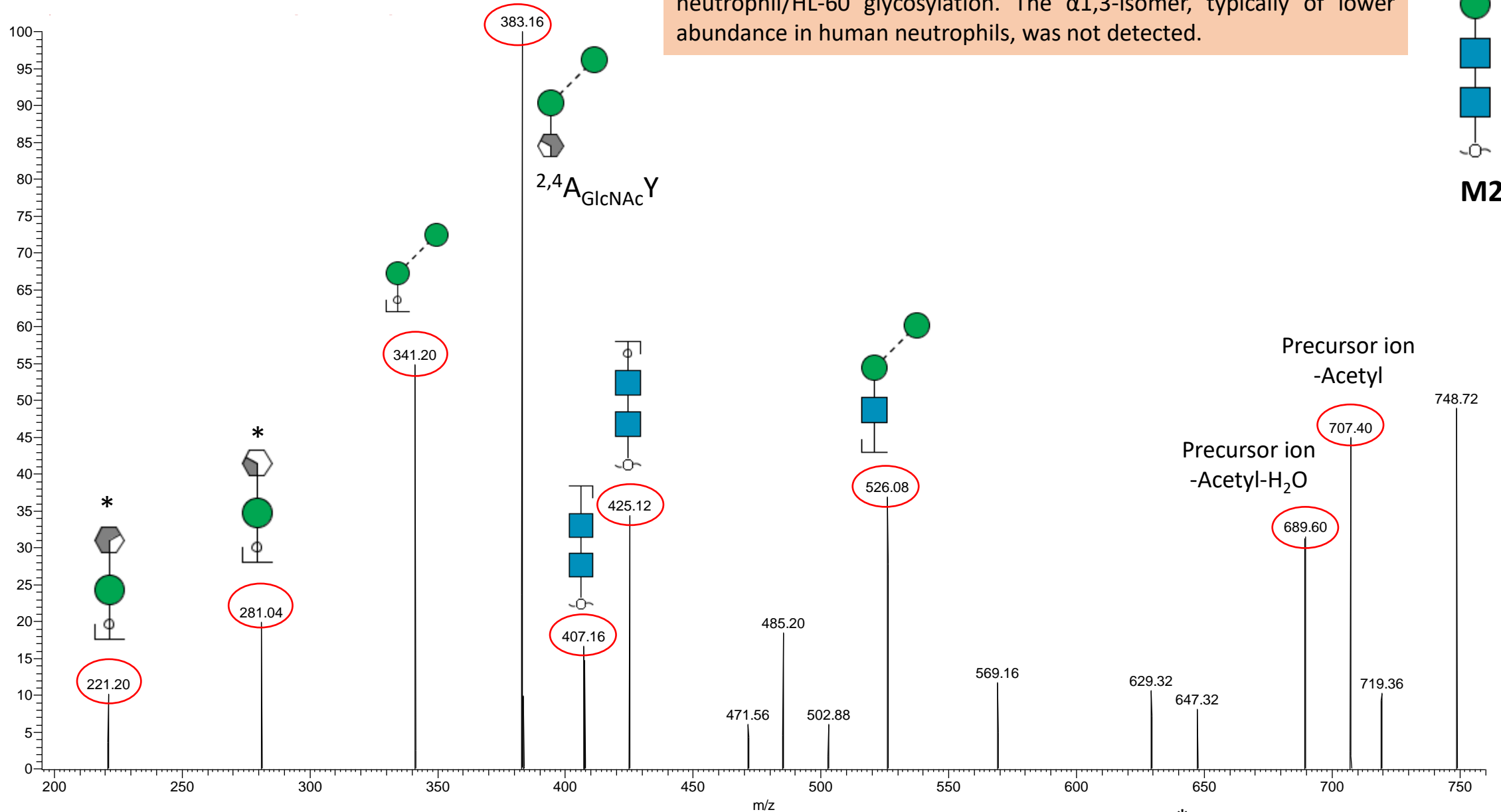


Supplementary Data S1

Manually annotated PGC-LC-ESI-CID-MS/MS (-) spectra of reduced paucimannosidic *N*-glycans released from wildtype and Hex-deficient HL-60 cells

Observed m/z 749.28 (1-), RT: ~13.0 min
[M-H]⁻ 749.28 Da

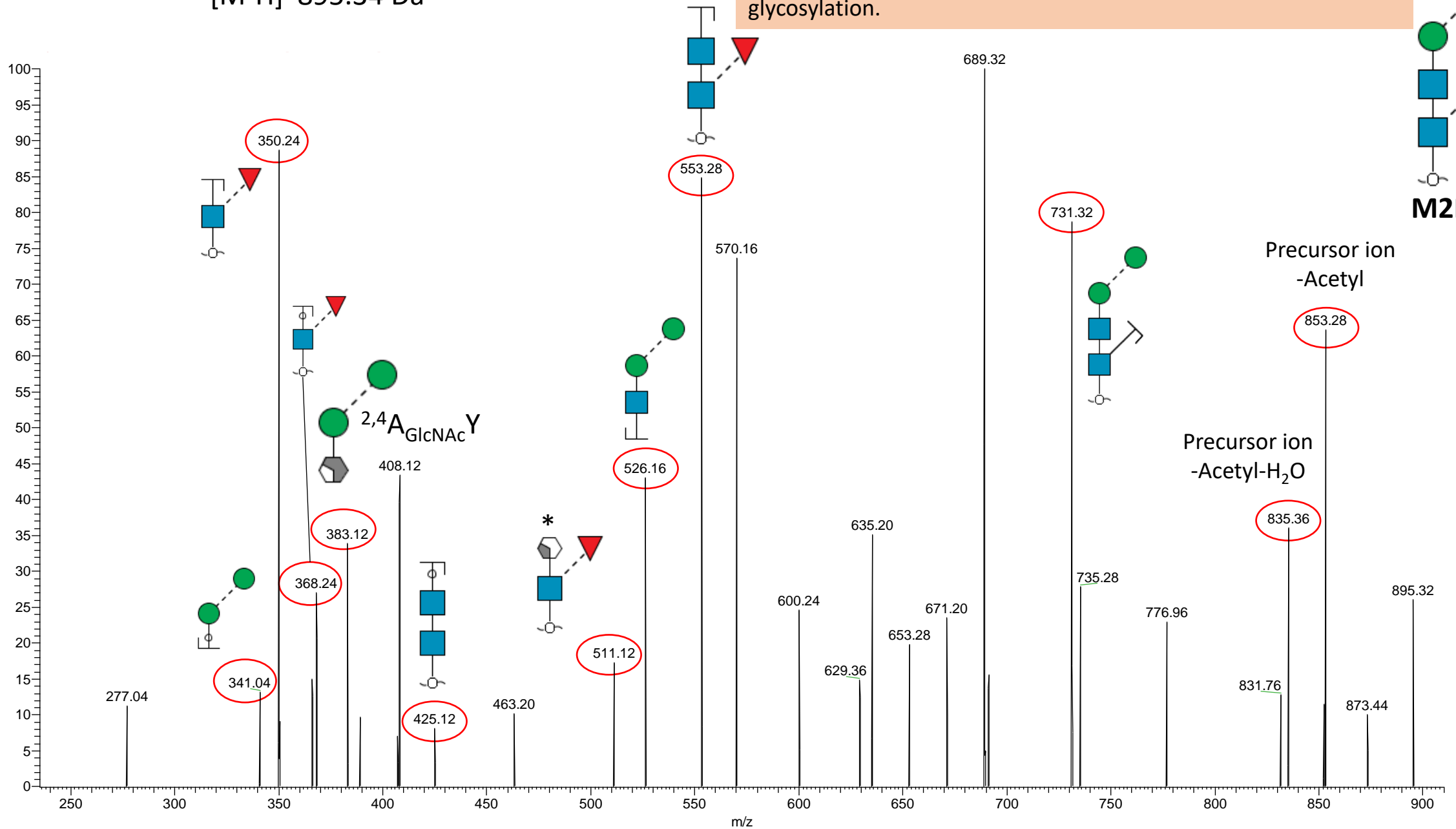
Note: Only one isomer was observed for M2, which is biosynthetically predicted to be α 1,6-linkage isomer based on experience with neutrophil/HL-60 glycosylation. The α 1,3-isomer, typically of lower abundance in human neutrophils, was not detected.



* Ambiguity of the cross-ring fragments

Observed m/z 895.36 (1-), RT: ~18.9 min
[M-H]⁻ 895.34 Da

Note: Identified as the α 1,6-Man isomer based on PGC LC elution time, its high abundance and experience with neutrophil/HL-60 N-glycosylation.

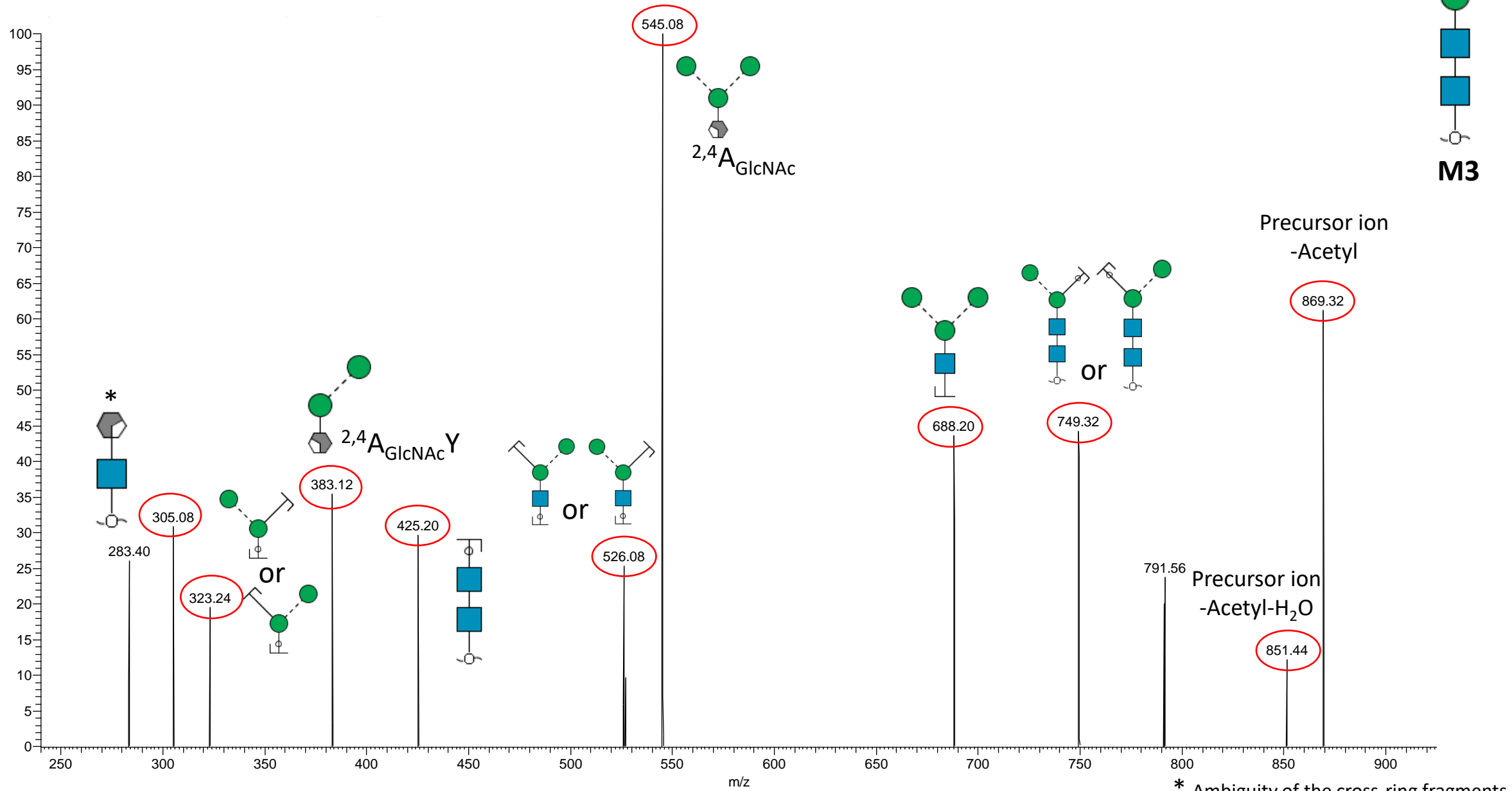


M2F

Precursor ion
-Acetyl

Precursor ion
-Acetyl-H₂O

Observed m/z 911.37 (1-), RT: ~17.8 min
 [M-H]⁻ 911.33 Da



Observed m/z 1057.41 (1-), RT: ~23.8 min
[M-H]⁻ 1057.39 Da

