Supplementary Data S1-S3

for

Serum *N*-Glycomics Stratifies Bacteremic Patients Infected with Different Pathogens

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Overview of Supplementary Data

Annotation and Fragmentation Key (slide 3)

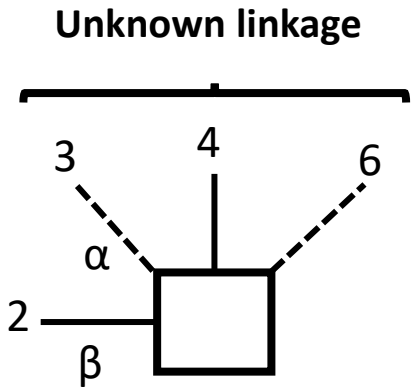
Supplementary Data S1: BPC, EICs and manually annotated PGC-LC-ESI-CID-MS (-) spectra of *N*-glycans (alditols) of key importance released from bacteremic sera (Slide 4 – Slide 19).

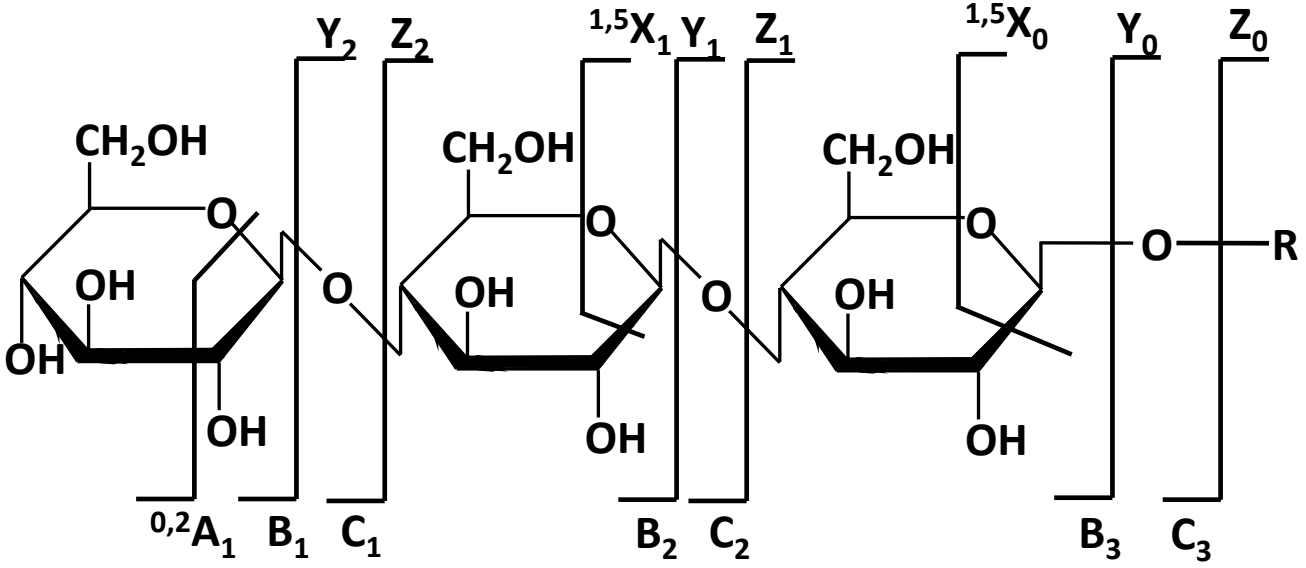
Supplementary Data S2: Examples of EICs, S/N and manually annotated PGC-LC-ESI-CID-MS (-) spectra of very low abundant *N*-glycans (alditols) released from bacteremic sera (0.01%-0.15% relative abundance levels) (Slide 20 – Slide 25).

Supplementary Data S3: Manually annotated PGC-LC-ESI-CID-MS/MS (-) spectra of all reported *N*-glycans (alditols) released from bacteremic and healthy sera (Slide 26 – Slide 88).

Annotation and Fragmentation Key

- Mannose (Man) (162.0528 Da)
- Galactose (Gal) (162.0528 Da)
- *N*-Acetylglucosamine (GlcNAc) (203.0794 Da)
- *N*-Acetylneuraminic acid (NeuAc) (291.0954 Da)
- Fucose (Fuc) (146.0579 Da)
- Cross-ring fragment (unspecified)
- Indicates mostly Y ions (includes oxygen of glyosidic linkage)
- Indicates mostly Z ions (excludes oxygen of glyosidic linkage)
- Reduced reducing end
- a-b-c-d Isomers





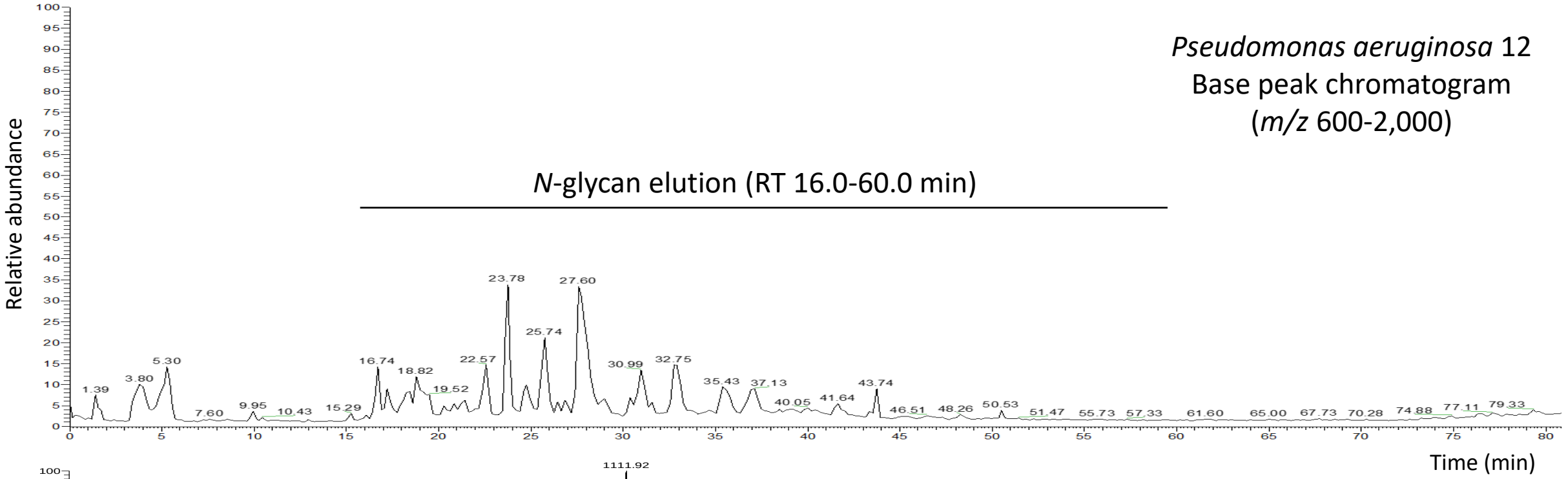
Han, L. & Costello, C.E. Mass spectrometry of glycans. *Biochemistry (Moscow)* 78, 710-720 (2013)

Supplementary Data S1

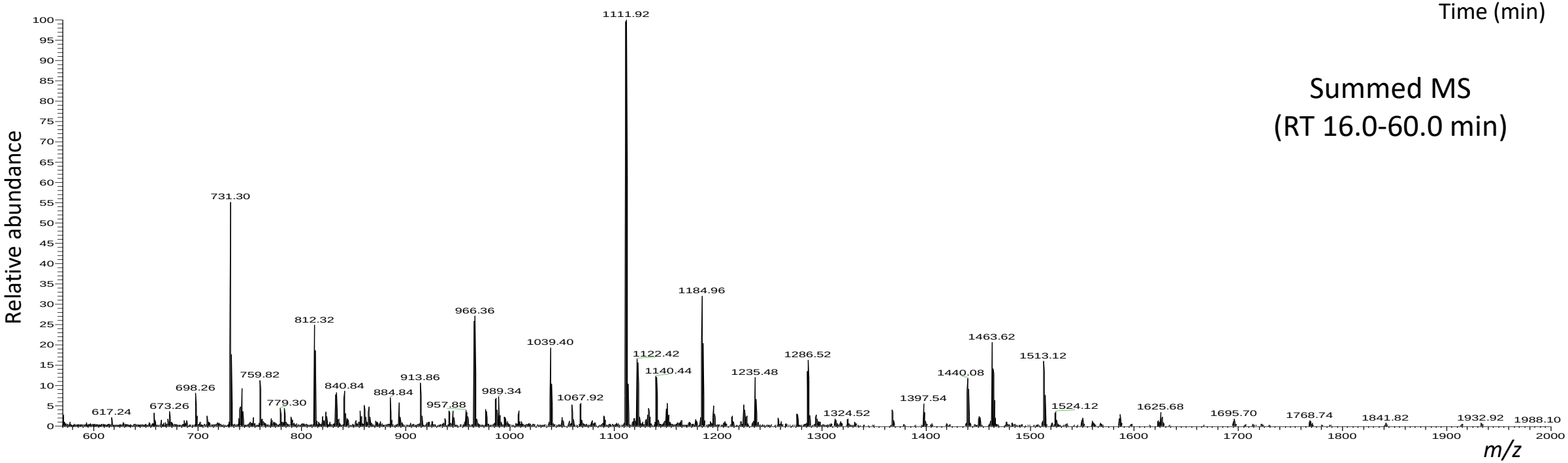
BPC, EICs and manually annotated PGC-LC-ESI-CID-MS (-) spectra of *N*-glycans (alditols) of key importance released from bacteremic sera

Pseudomonas aeruginosa 12
Base peak chromatogram
(*m/z* 600-2,000)

N-glycan elution (RT 16.0-60.0 min)

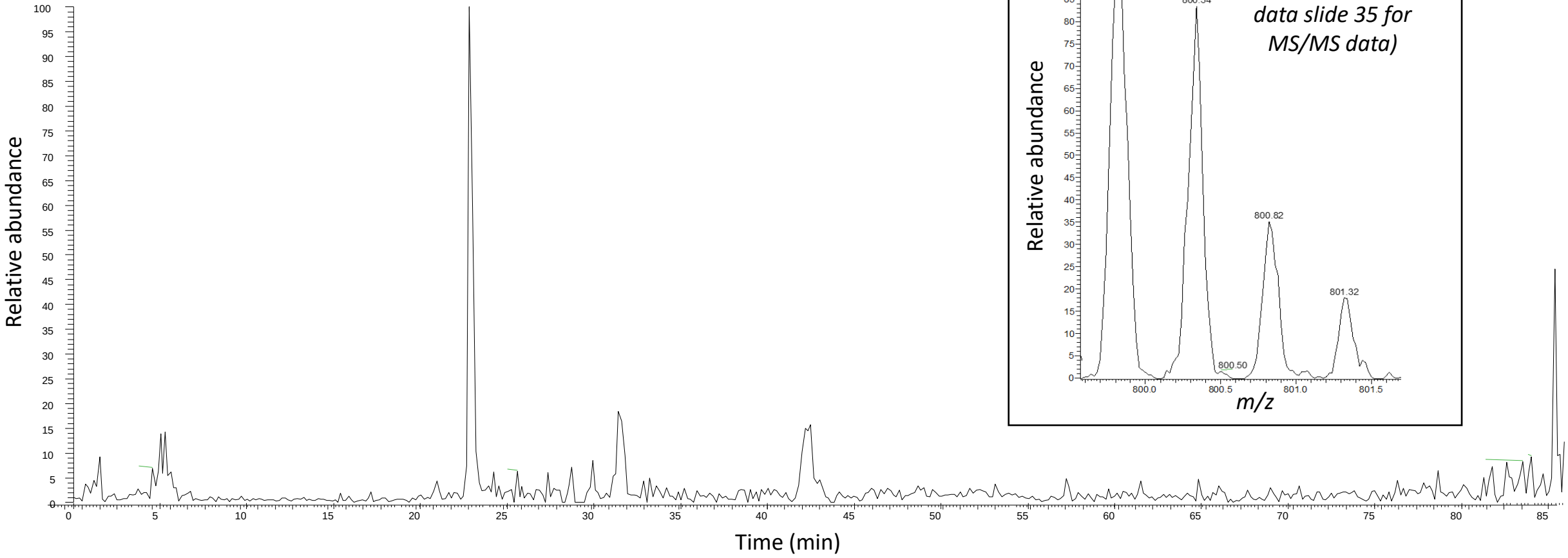


Summed MS
(RT 16.0-60.0 min)



Escherichia coli 6
Extracted ion chromatogram
(*m/z* 799.82)

Glycan #9
(RT 22.8 min)

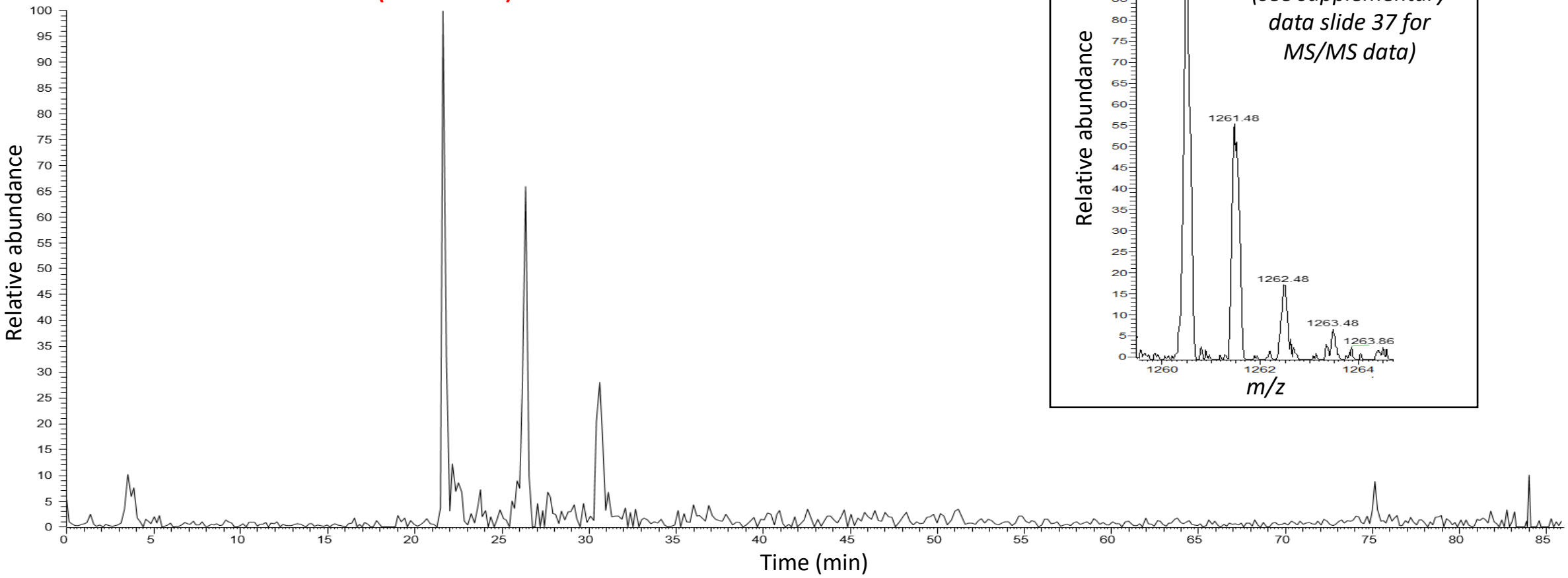


MS profile of glycan precursor (RT 22.8 min) *(see supplementary data slide 35 for MS/MS data)*

***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
(*m/z* 1260.48)

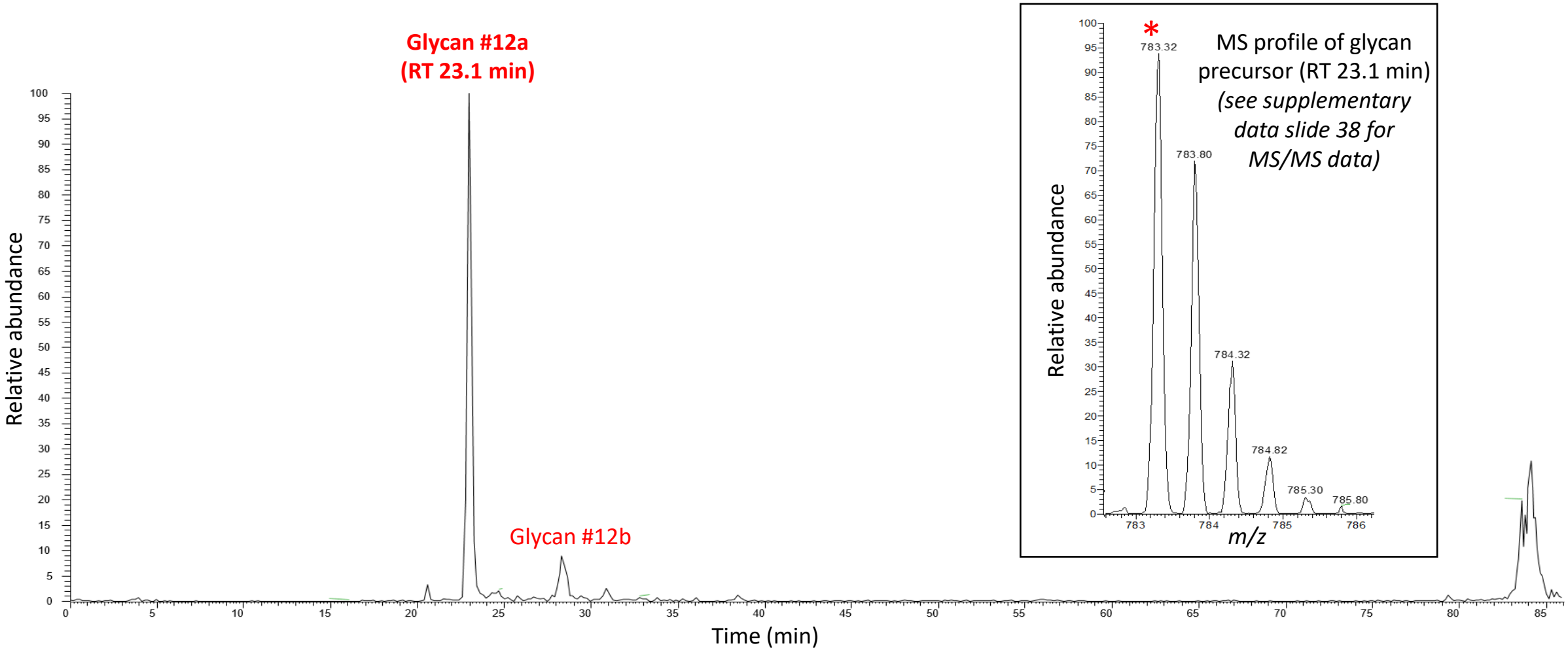
Glycan #11
(RT 21.7 min)



MS profile of glycan precursor (RT 21.7 min) *(see supplementary data slide 37 for MS/MS data)*

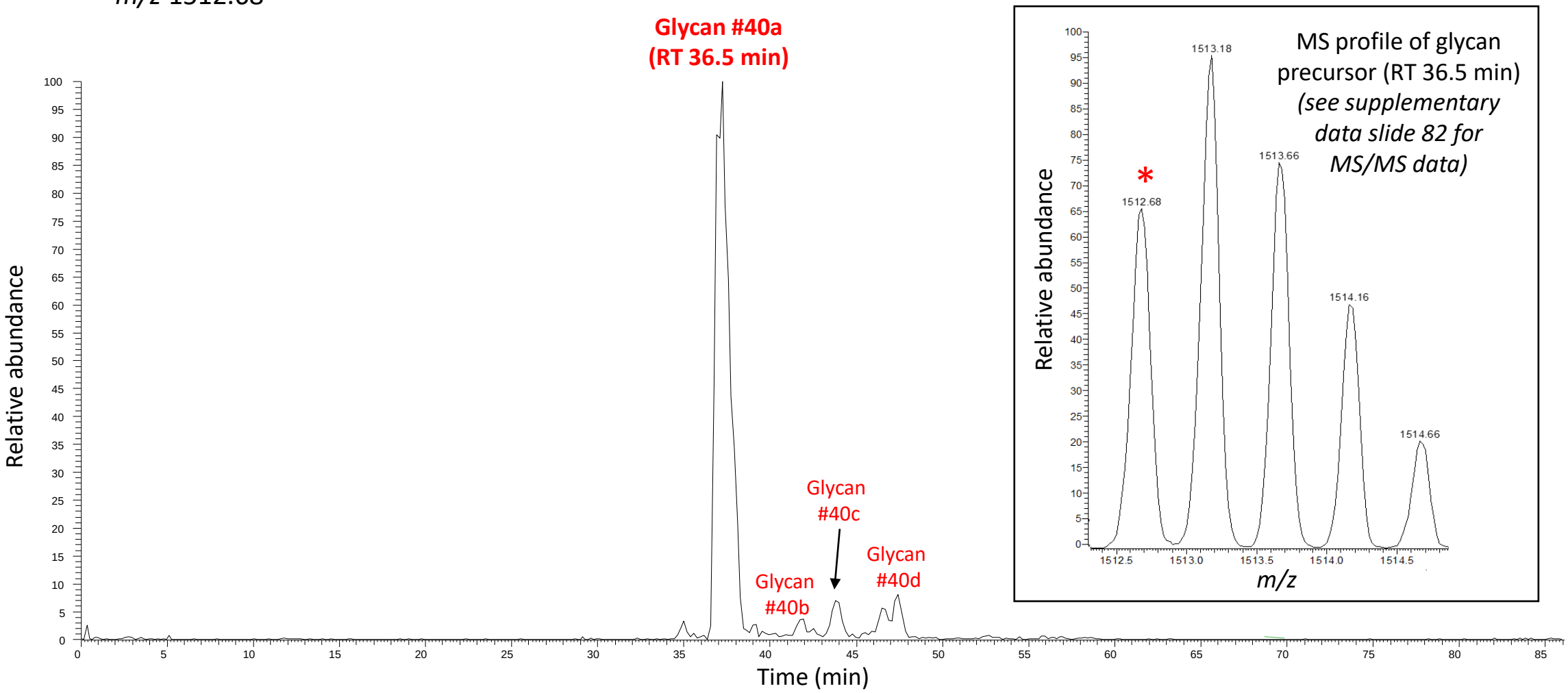
***Monoisotopic signal of identified glycan**

Staphylococcus aureus 19
Extracted Ion Chromatogram
m/z 783.32



***Monoisotopic signal of identified glycan**

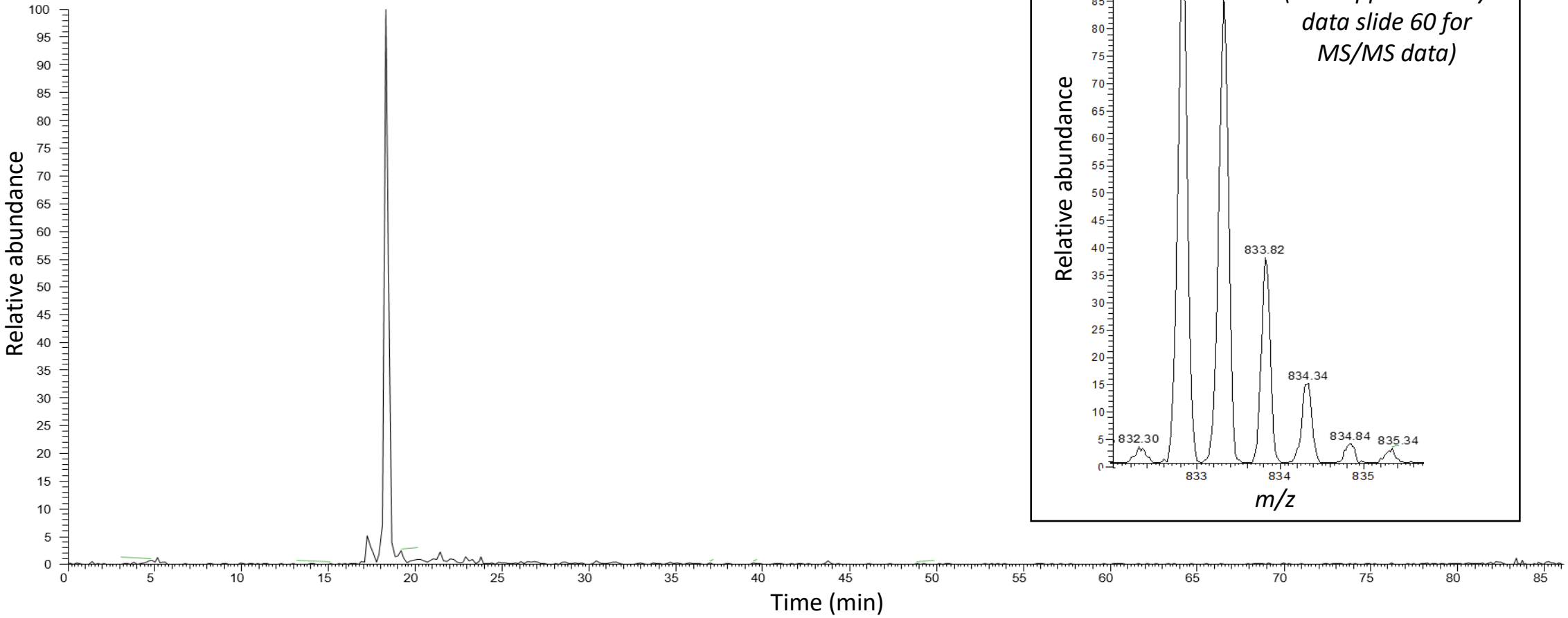
Streptococcus viridans 29
Extracted Ion Chromatogram
m/z 1512.68



***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 832.82

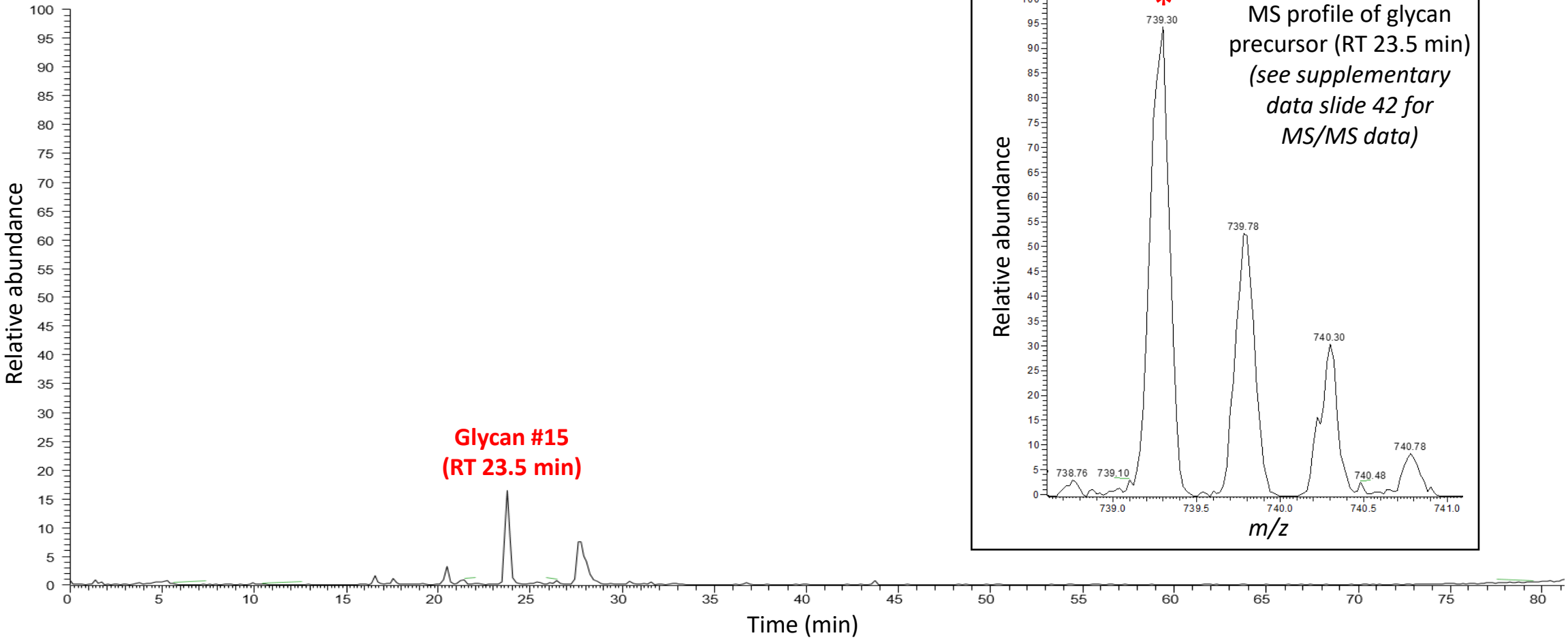
Glycan #26
(RT 18.3 min)



MS profile of glycan precursor (RT 18.3 min) *(see supplementary data slide 60 for MS/MS data)*

***Monoisotopic signal of identified glycan**

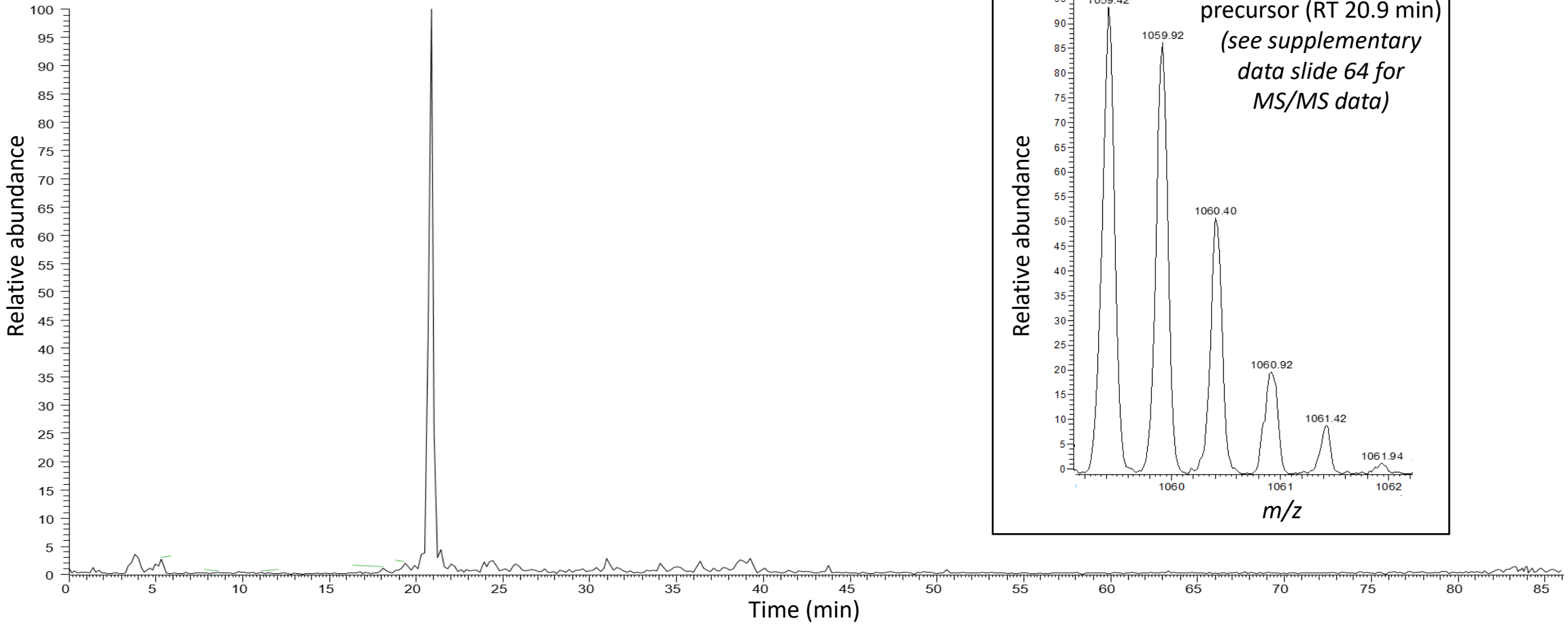
Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 739.30



***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 1059.42

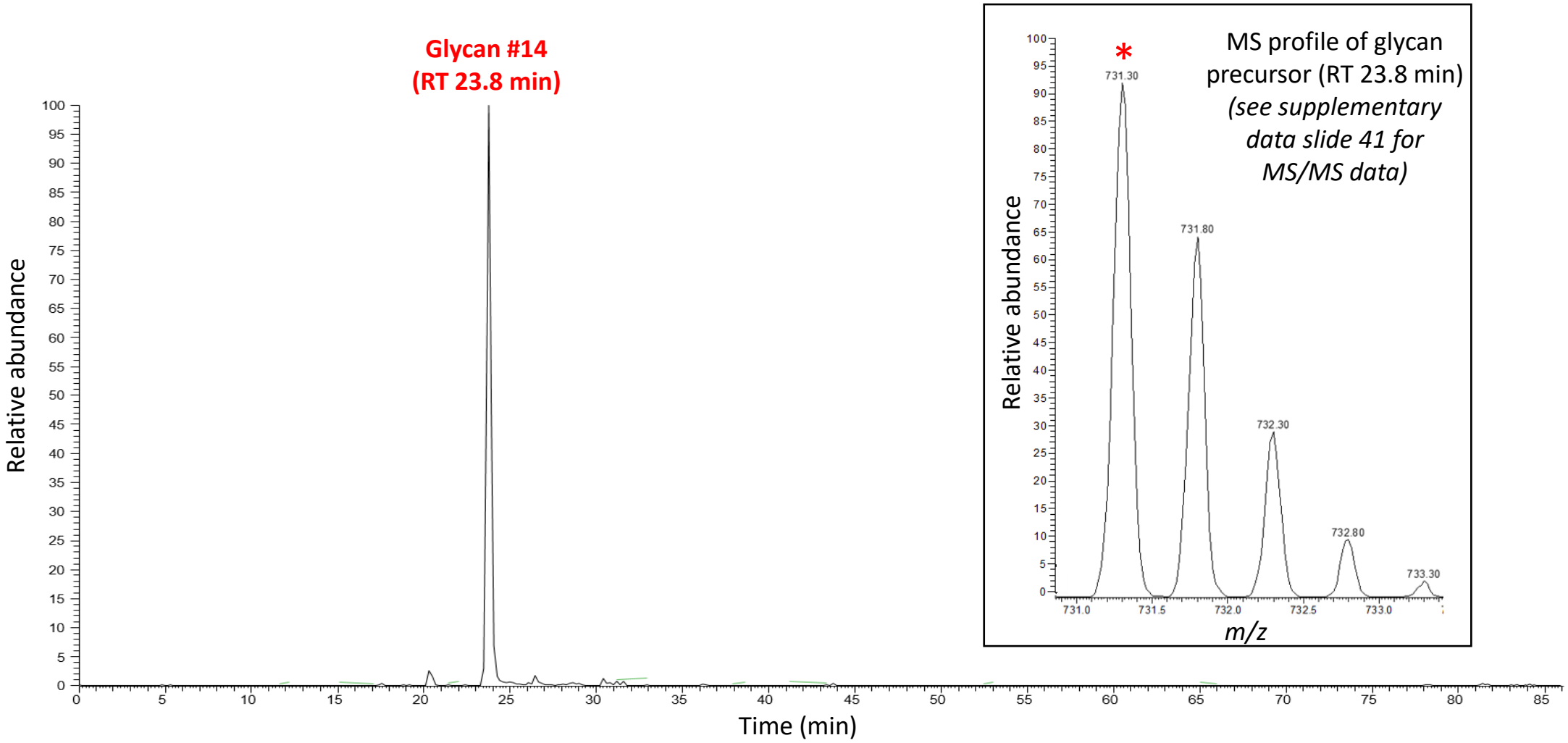
Glycan #30
(RT 20.9 min)



MS profile of glycan precursor (RT 20.9 min) *(see supplementary data slide 64 for MS/MS data)*

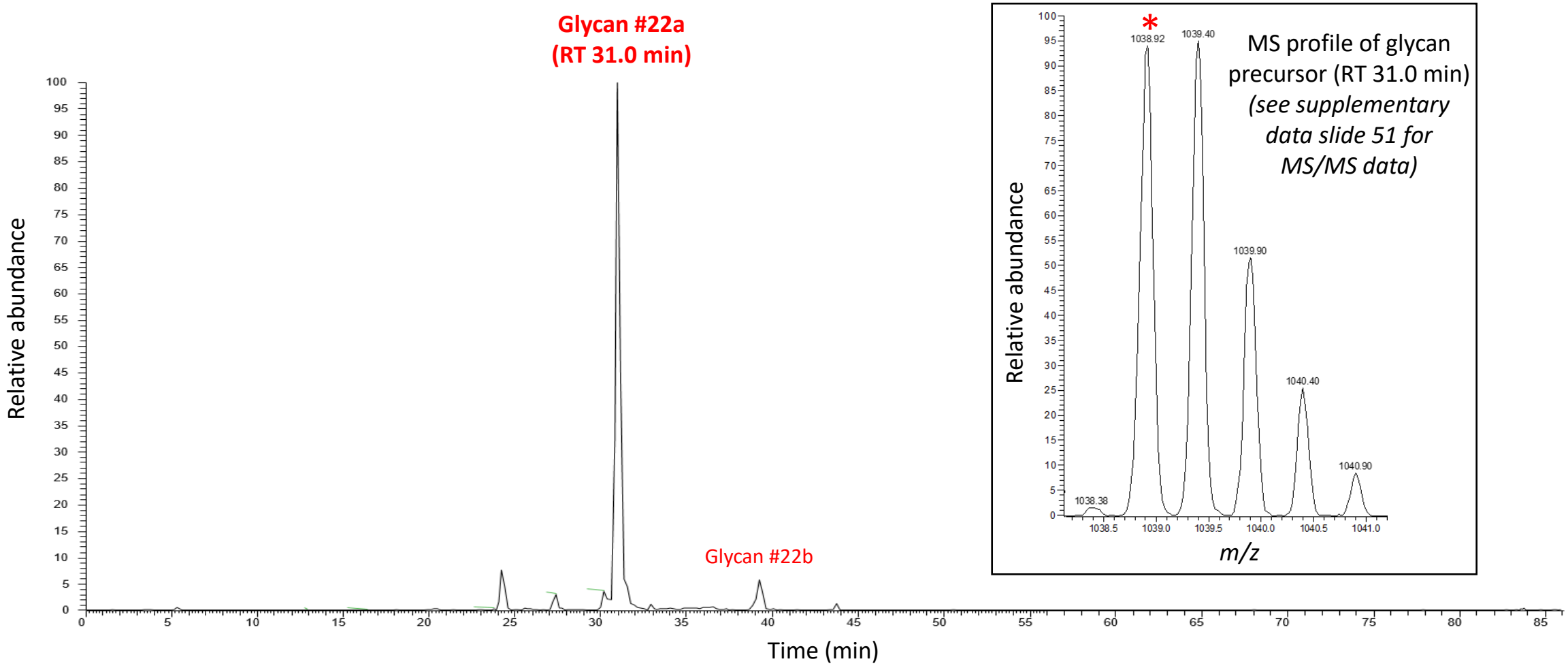
***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 731.30



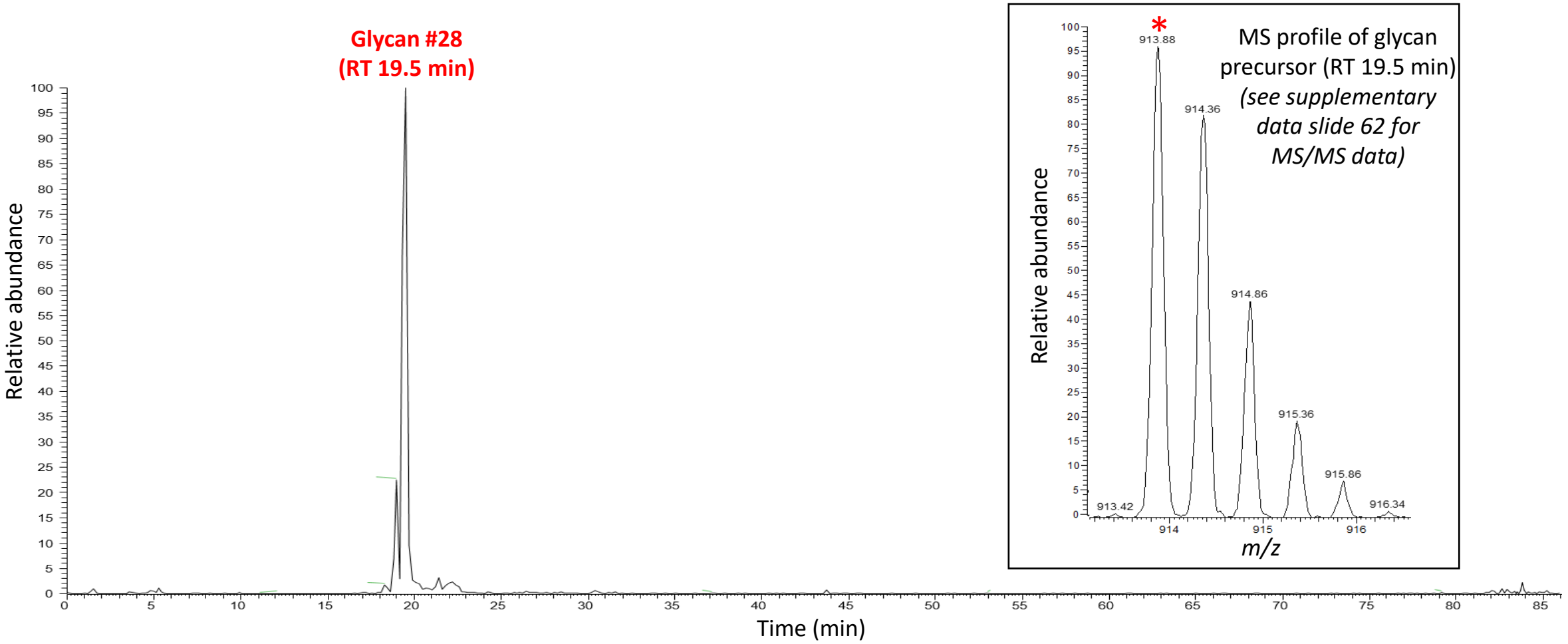
***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 1038.92



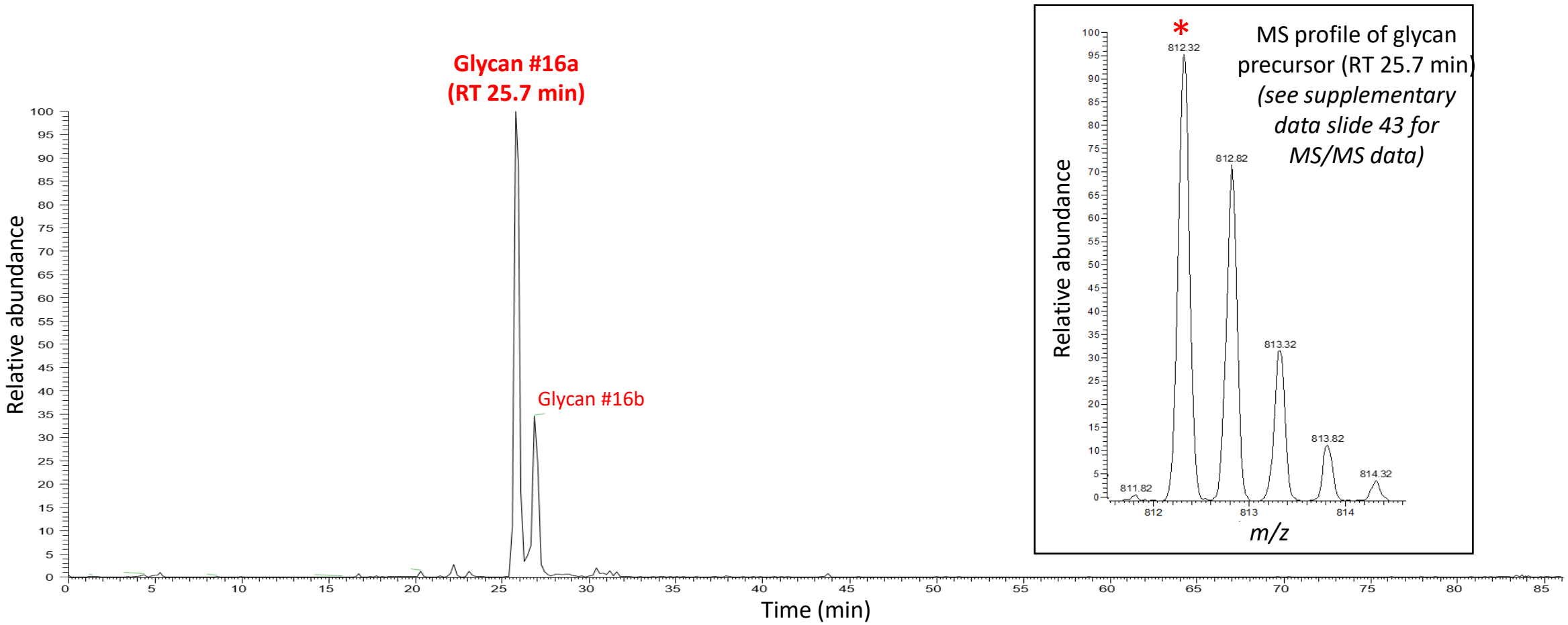
***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 913.88



***Monoisotopic signal of identified glycan**

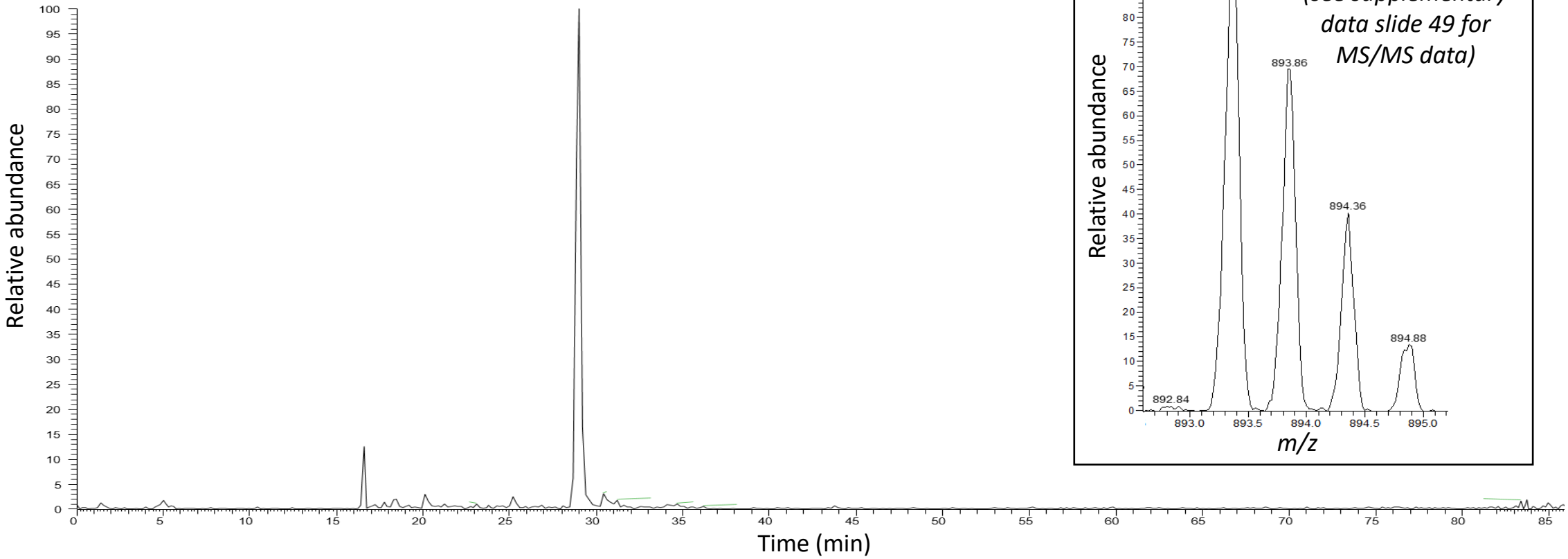
Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 812.32



***Monoisotopic signal of identified glycan**

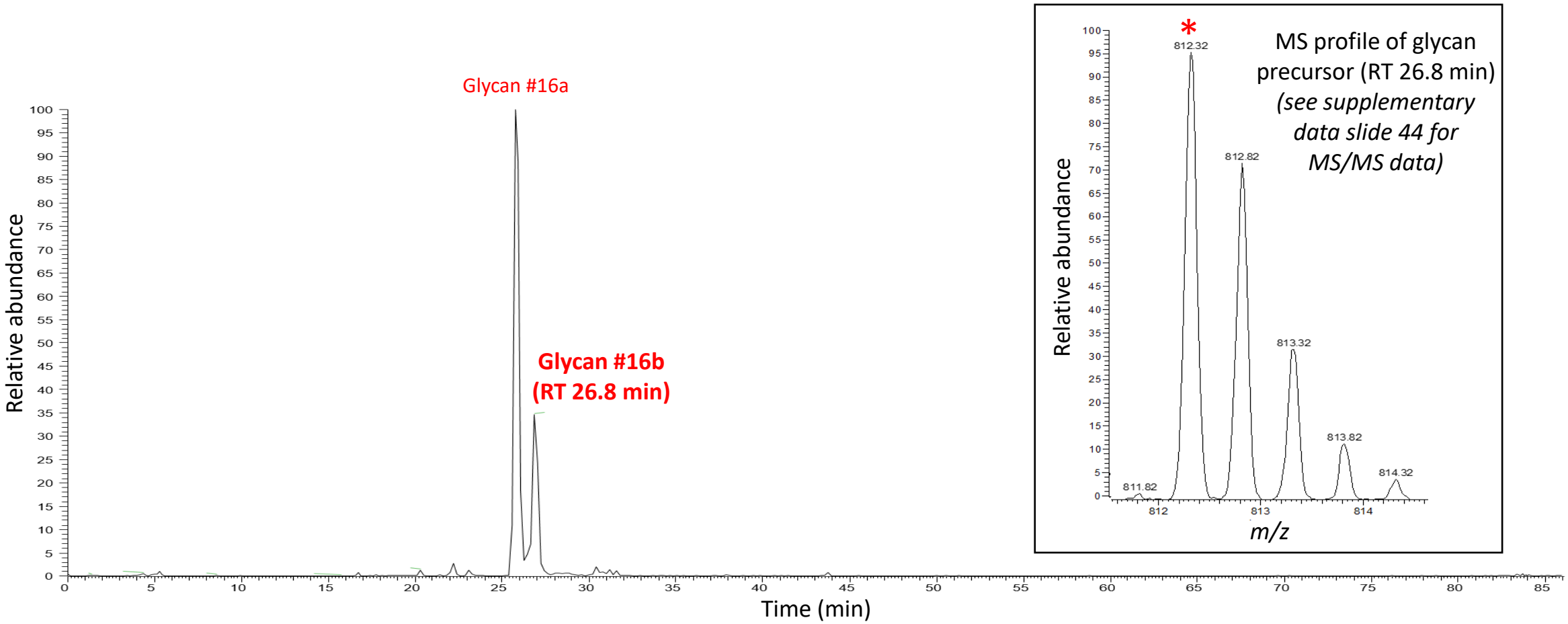
Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 893.36

Glycan #20
(RT 29.0 min)



***Monoisotopic signal of identified glycan**

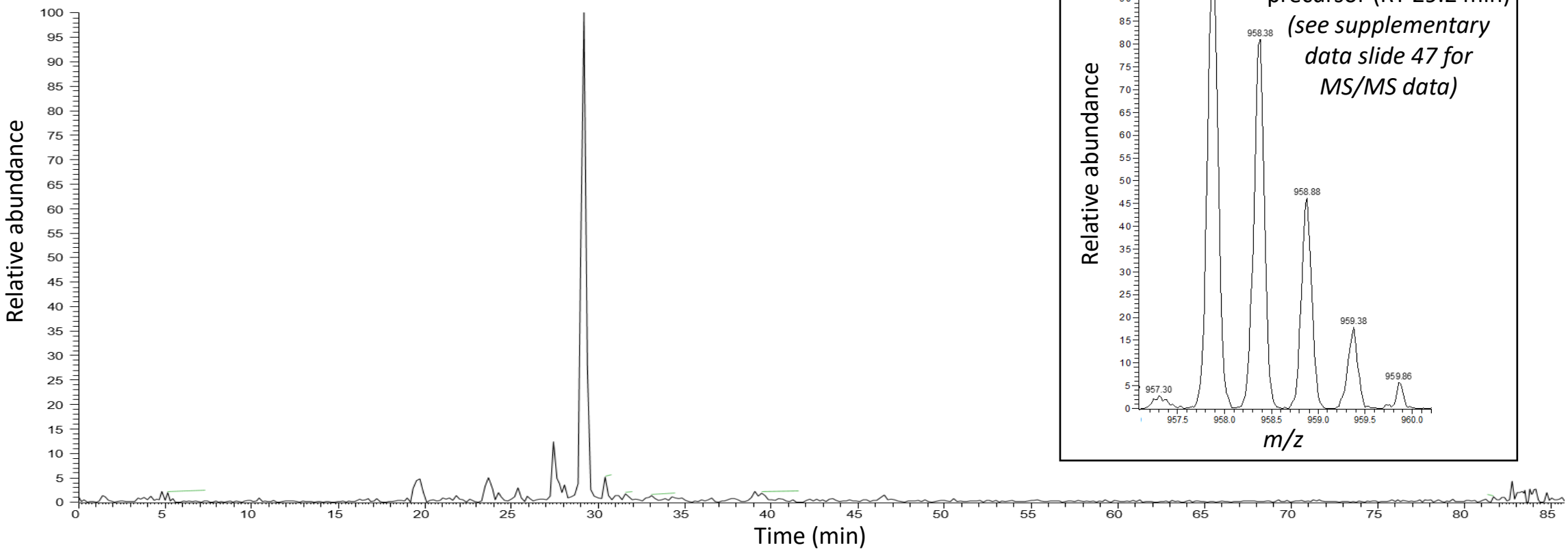
Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 812.32



***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 12
Extracted Ion Chromatogram
m/z 957.88

Glycan #18
(RT 29.2 min)

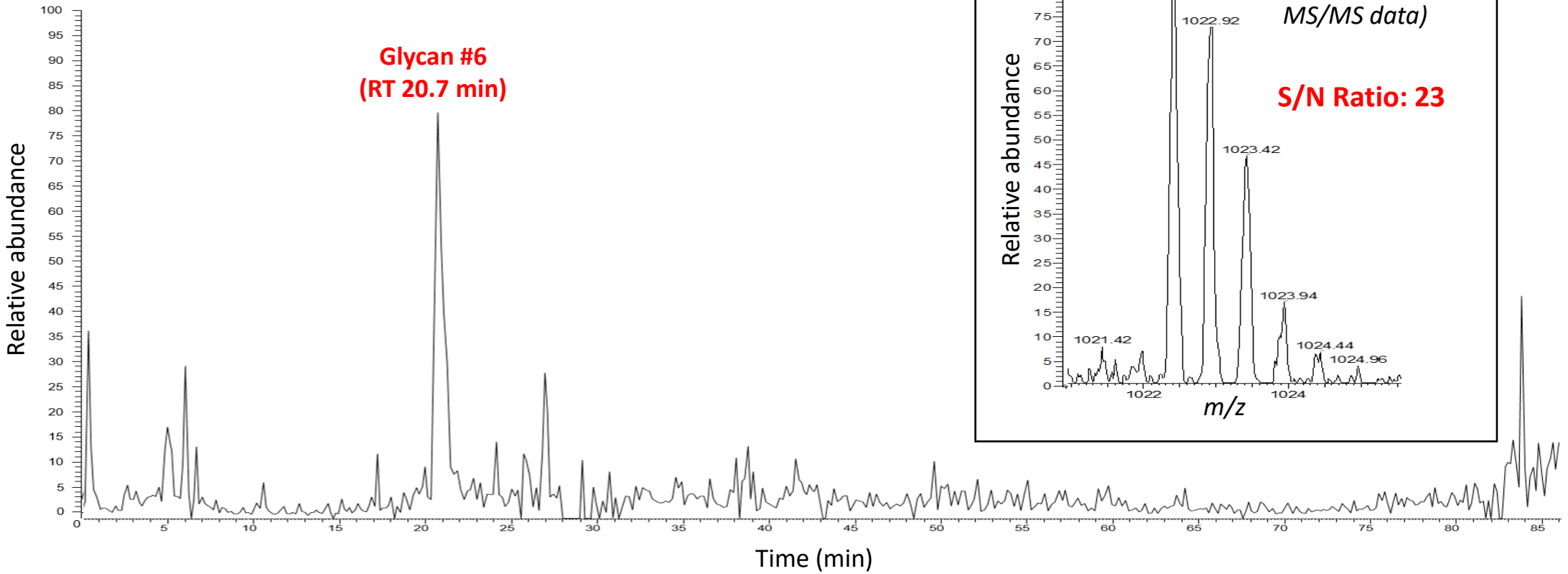


***Monoisotopic signal of identified glycan**

Supplementary Data S2

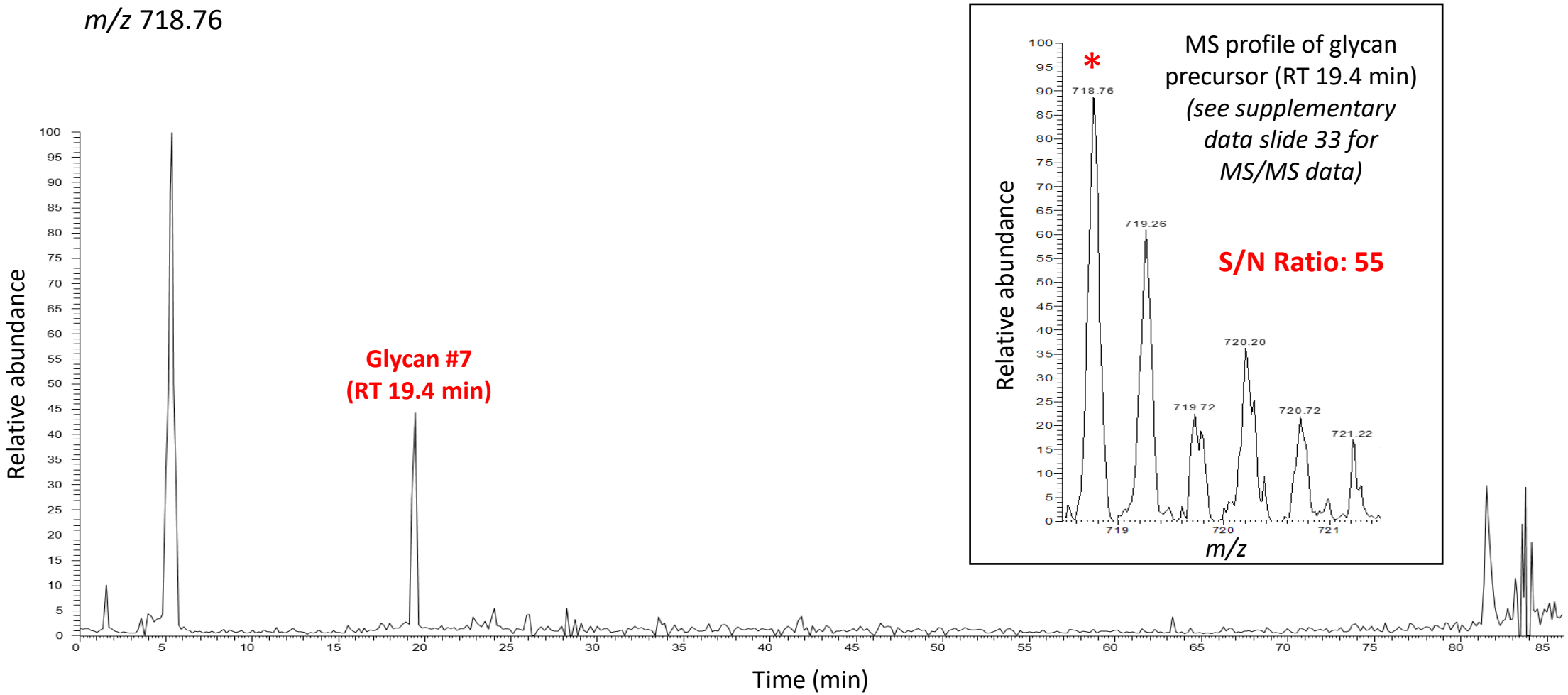
Examples of EICs, S/N and manually annotated PGC-LC-ESI-CID-MS (-) spectra of very low abundant *N*-glycans (alditols) released from bacteremic sera (0.01%-0.15% relative abundance levels)

Escherichia coli 8
Extracted Ion Chromatogram
m/z 1022.42



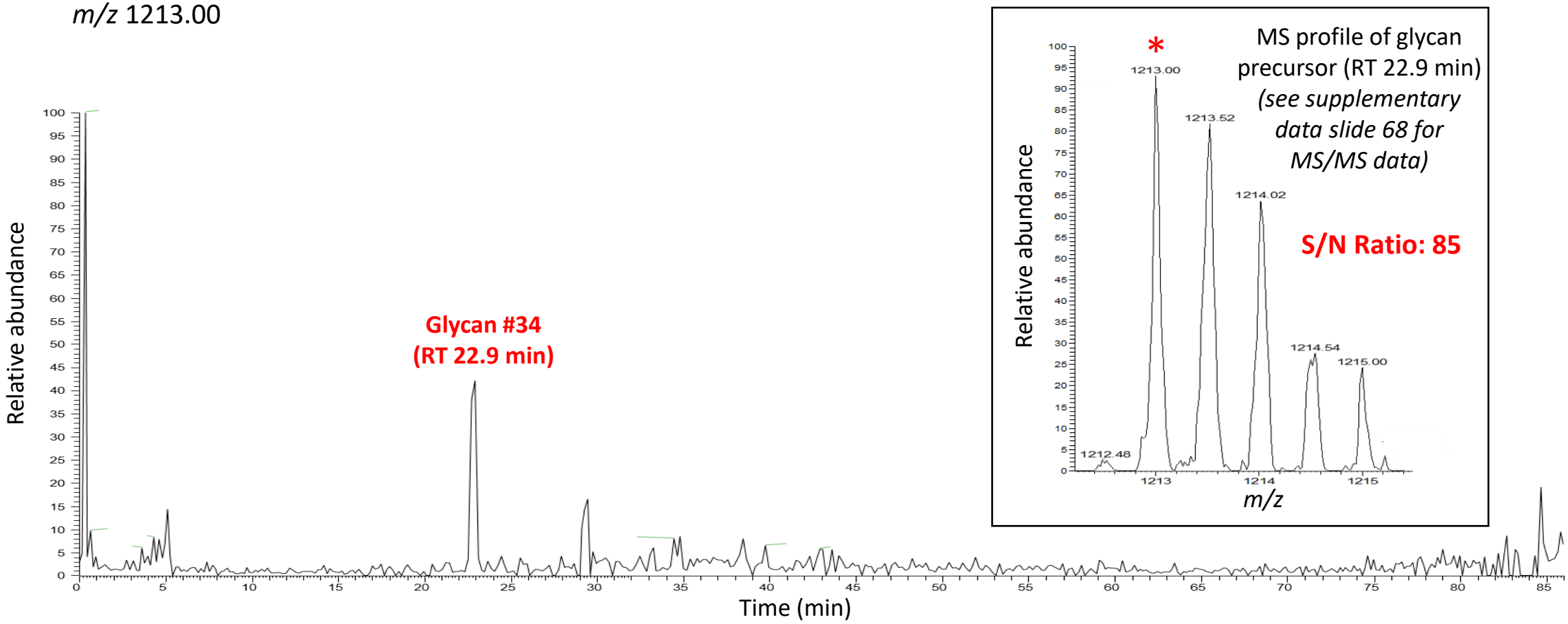
***Monoisotopic signal of identified glycan**

Staphylococcus aureus 17
Extracted Ion Chromatogram
m/z 718.76



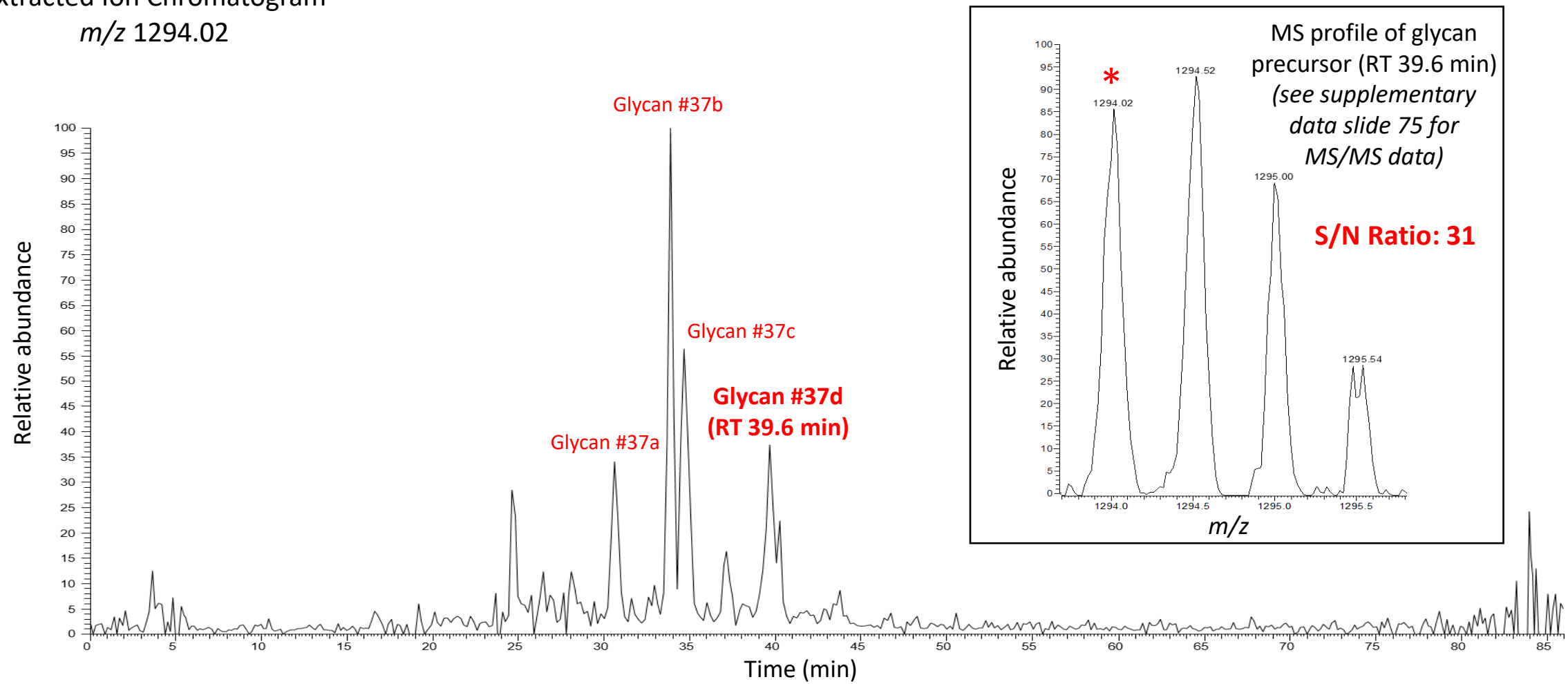
***Monoisotopic signal of identified glycan**

Streptococcus viridans 29
Extracted Ion Chromatogram
m/z 1213.00



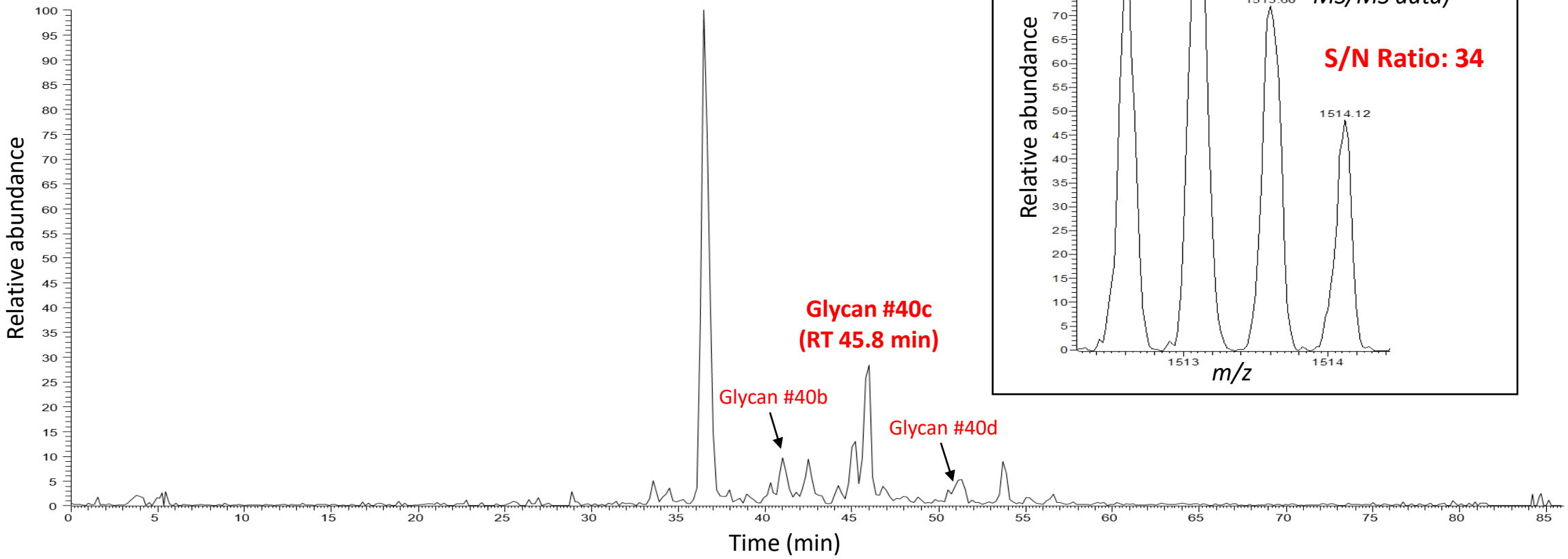
***Monoisotopic signal of identified glycan**

Pseudomonas aeruginosa 13
Extracted Ion Chromatogram
m/z 1294.02



***Monoisotopic signal of identified glycan**

Staphylococcus aureus 17
Extracted Ion Chromatogram
m/z 1512.60



***Monoisotopic signal of identified glycan**

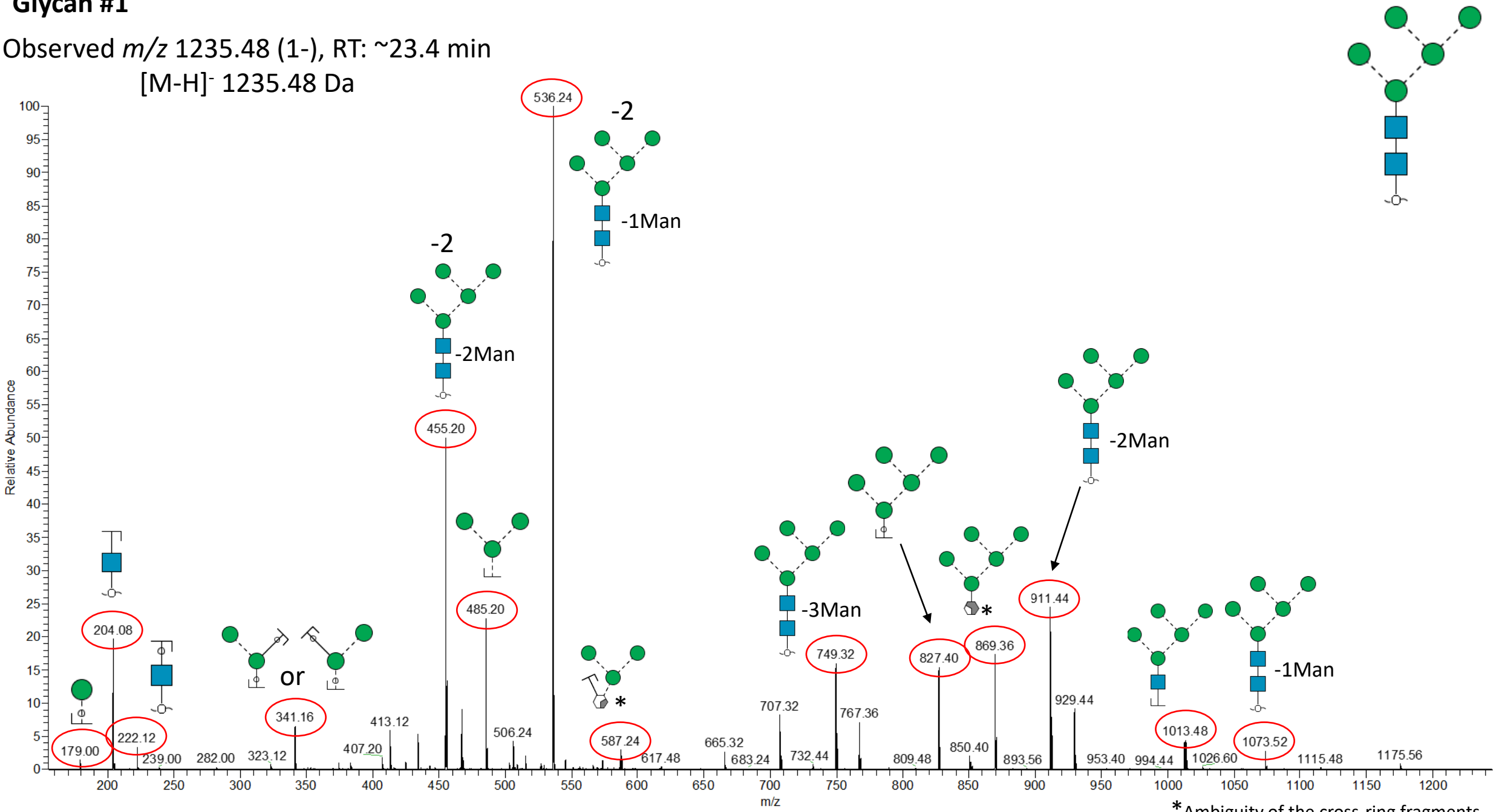
Supplementary Data S3

Manually annotated PGC-LC-ESI-CID-MS/MS (-) spectra of all reported *N*-glycans (alditols) released from bacteremic and healthy sera

Glycan #1

Observed *m/z* 1235.48 (1-), RT: ~23.4 min

$[M-H]^-$ 1235.48 Da

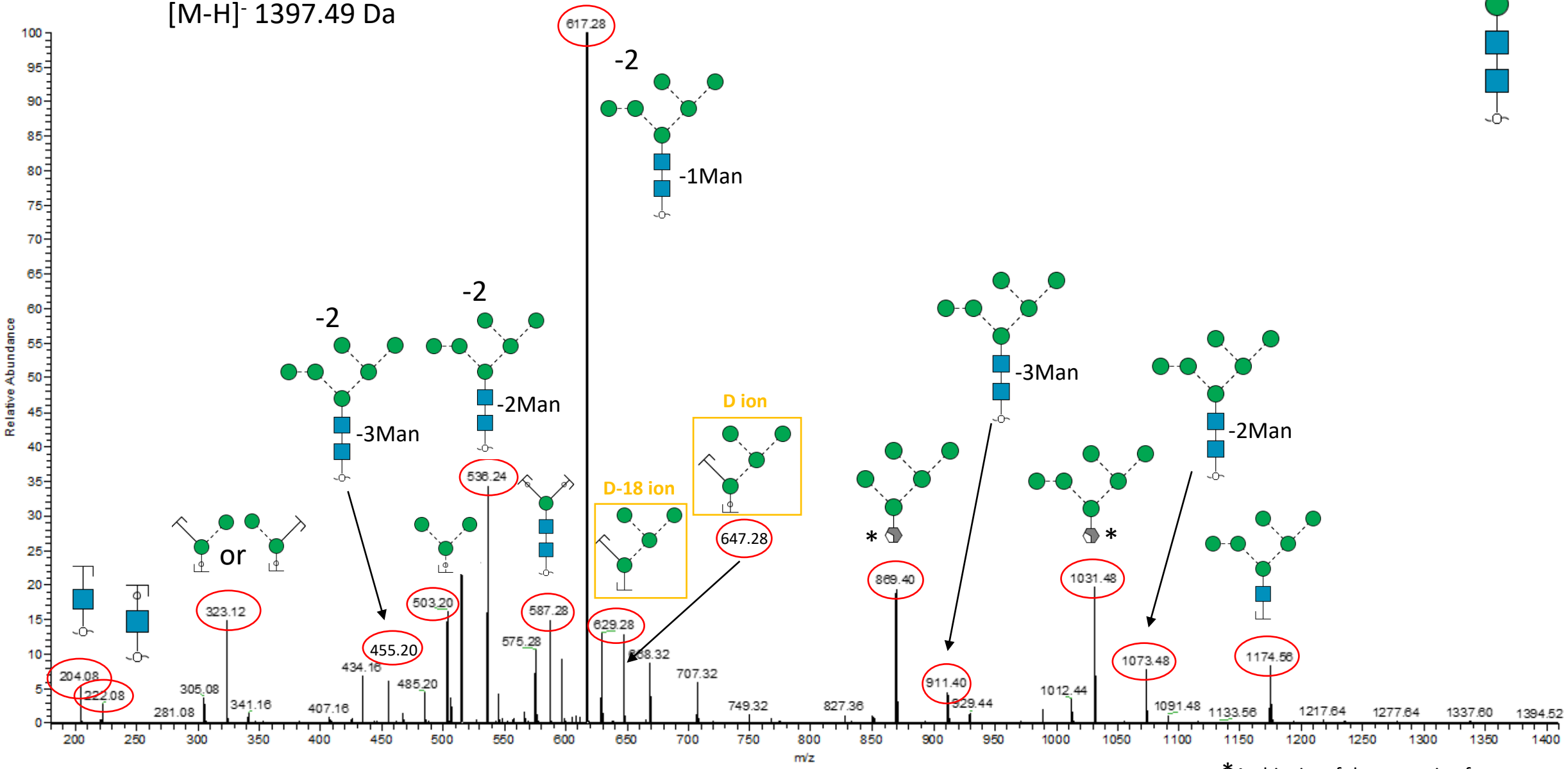


*Ambiguity of the cross-ring fragments

Glycan #2

Observed *m/z* 698.26 (2-), RT: ~19.3 min

$[M-H]^-$ 1397.49 Da

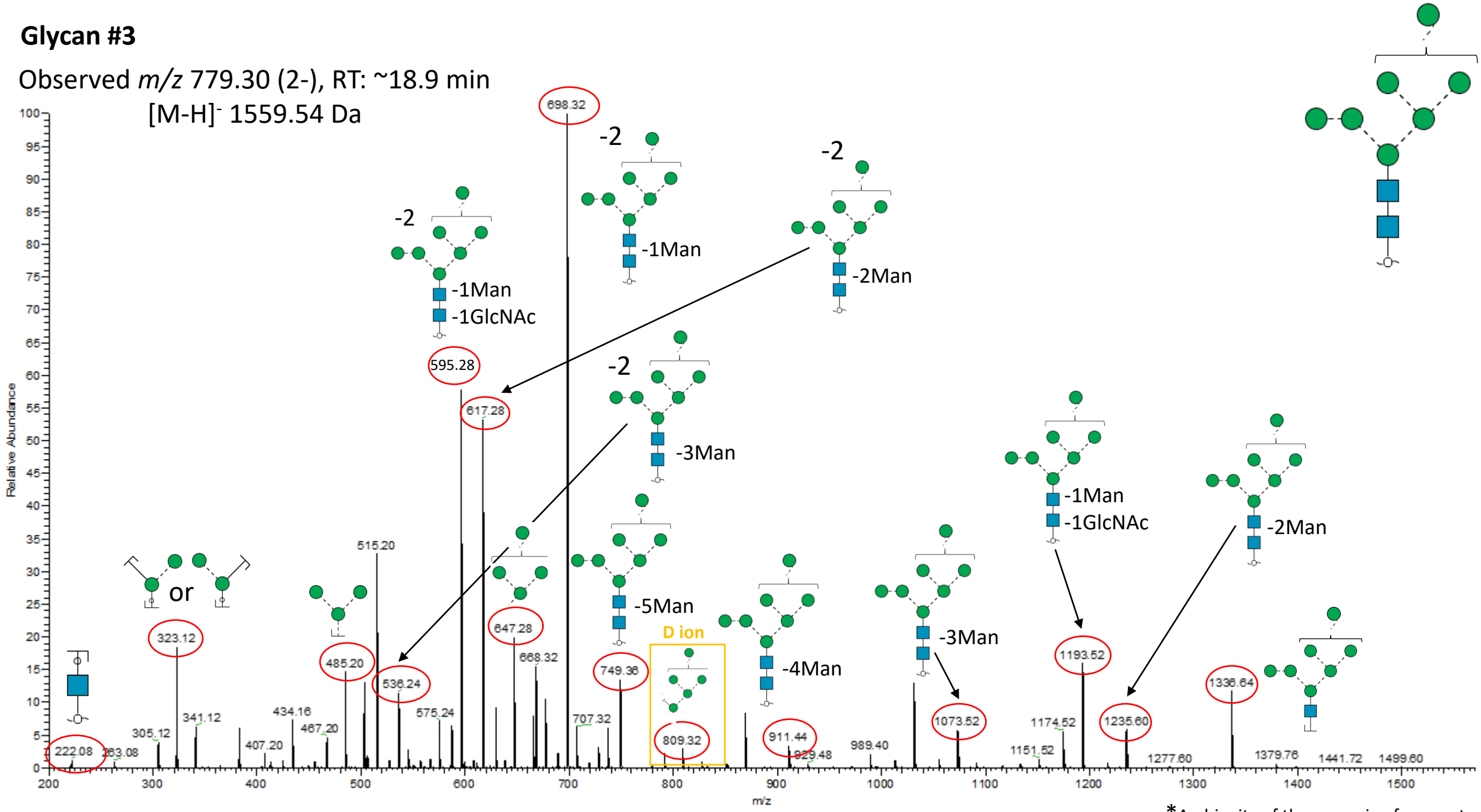


*Ambiguity of the cross-ring fragments

Glycan #3

Observed *m/z* 779.30 (2-), RT: ~18.9 min

$[M-H]^-$ 1559.54 Da

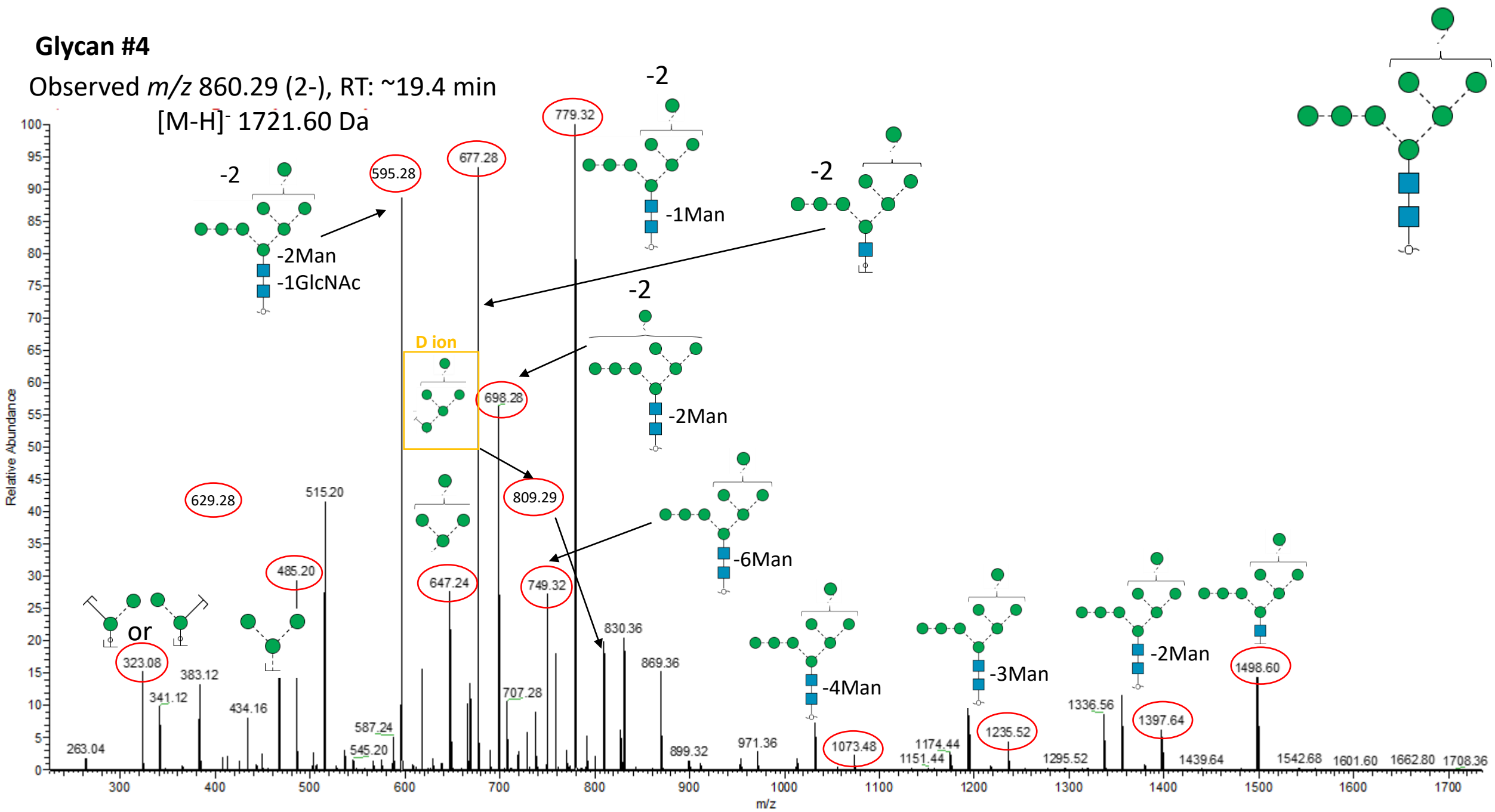


*Ambiguity of the cross-ring fragments

Glycan #4

Observed *m/z* 860.29 (2-), RT: ~19.4 min

$[M-H]^-$ 1721.60 Da

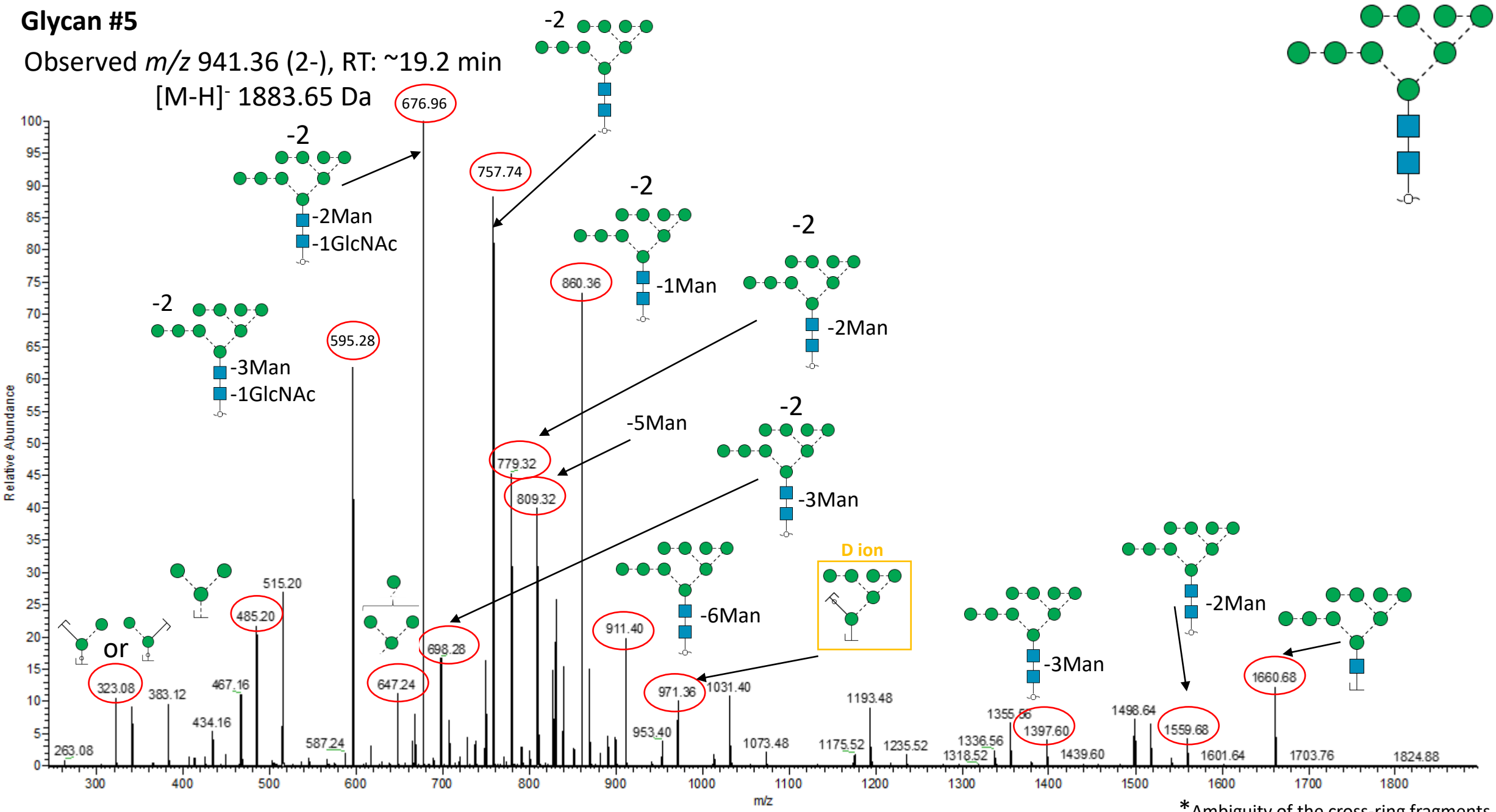


*Ambiguity of the cross-ring fragments

Glycan #5

Observed *m/z* 941.36 (2-), RT: ~19.2 min

$[M-H]^-$ 1883.65 Da

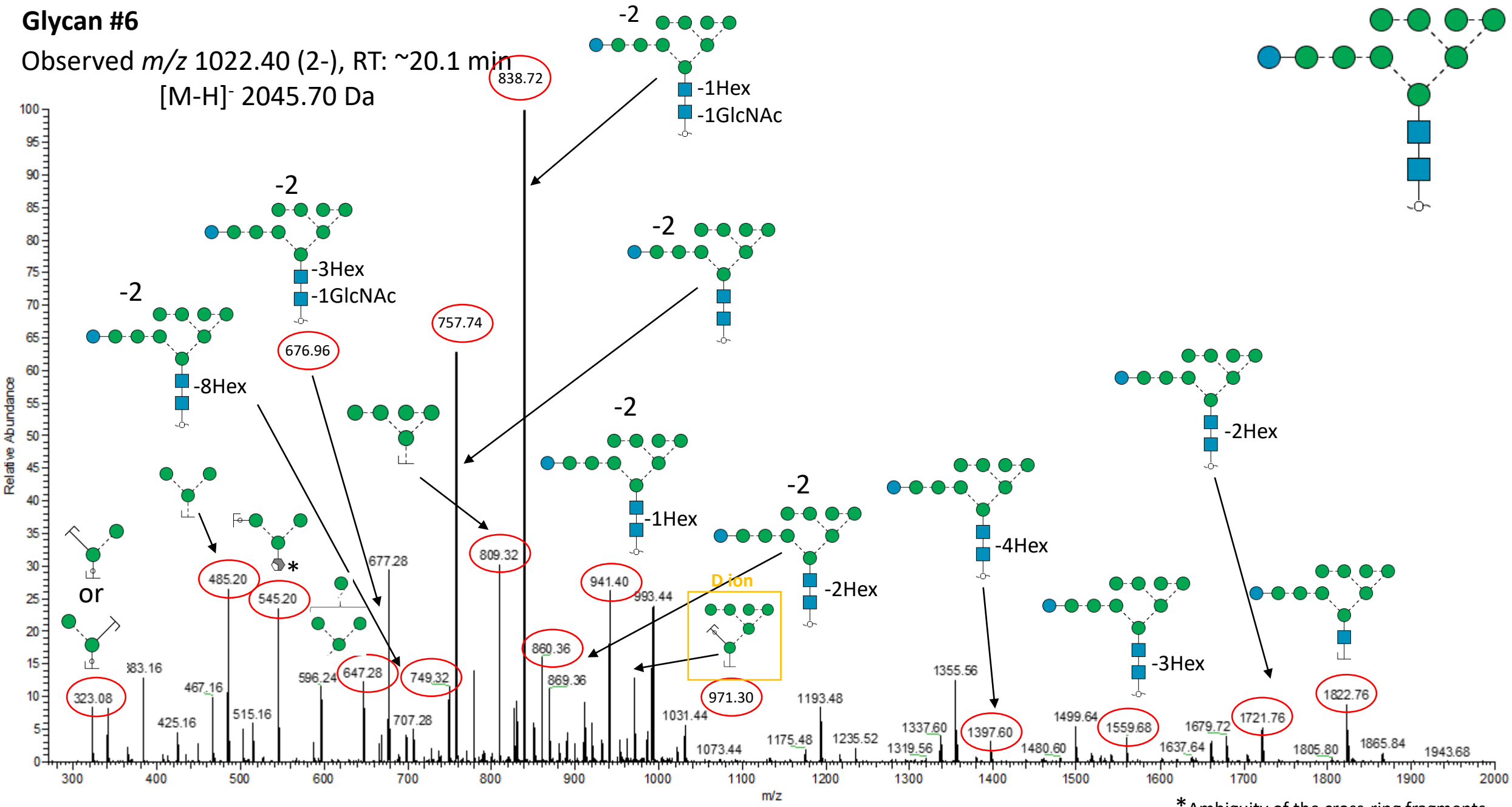


*Ambiguity of the cross-ring fragments

Glycan #6

Observed *m/z* 1022.40 (2-), RT: ~20.1 min

$[M-H]^-$ 2045.70 Da

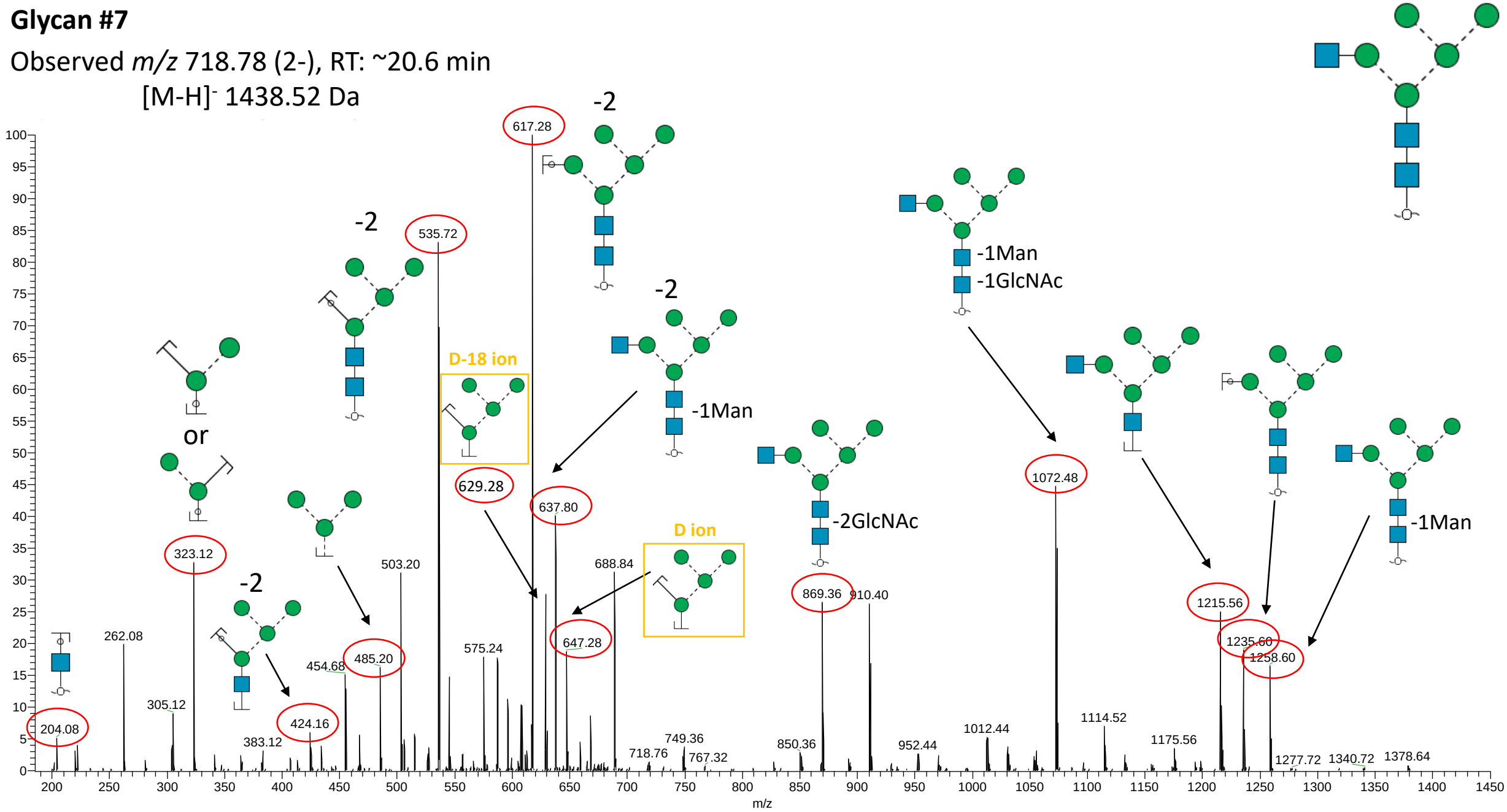


*Ambiguity of the cross-ring fragments

Glycan #7

Observed *m/z* 718.78 (2-), RT: ~20.6 min

$[M-H]^-$ 1438.52 Da



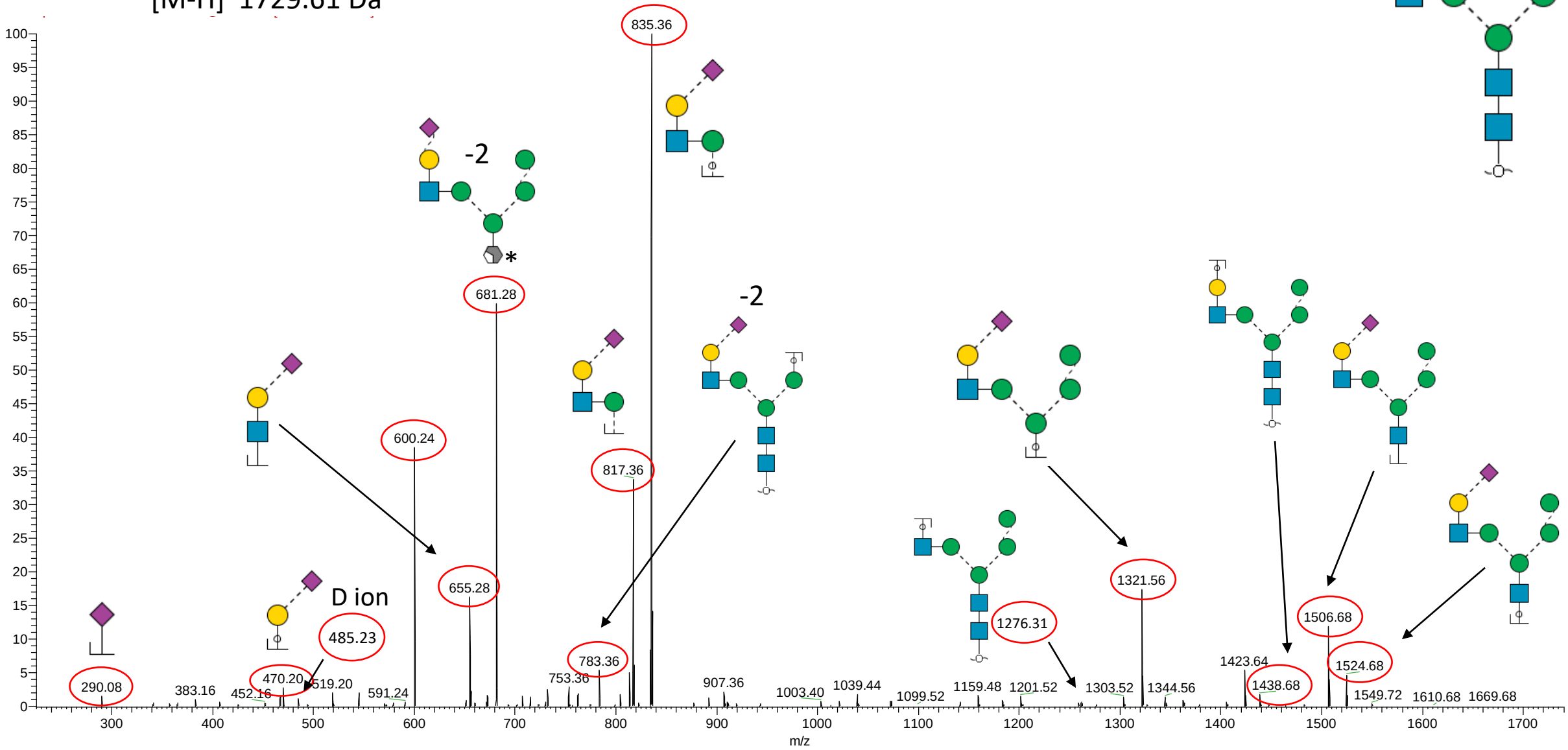
*Ambiguity in the cross-ring fragments

Glycan #8

Observed *m/z* 864.32 (2-), RT: ~22.5 min

$[M-H]^-$ 1729.61 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.

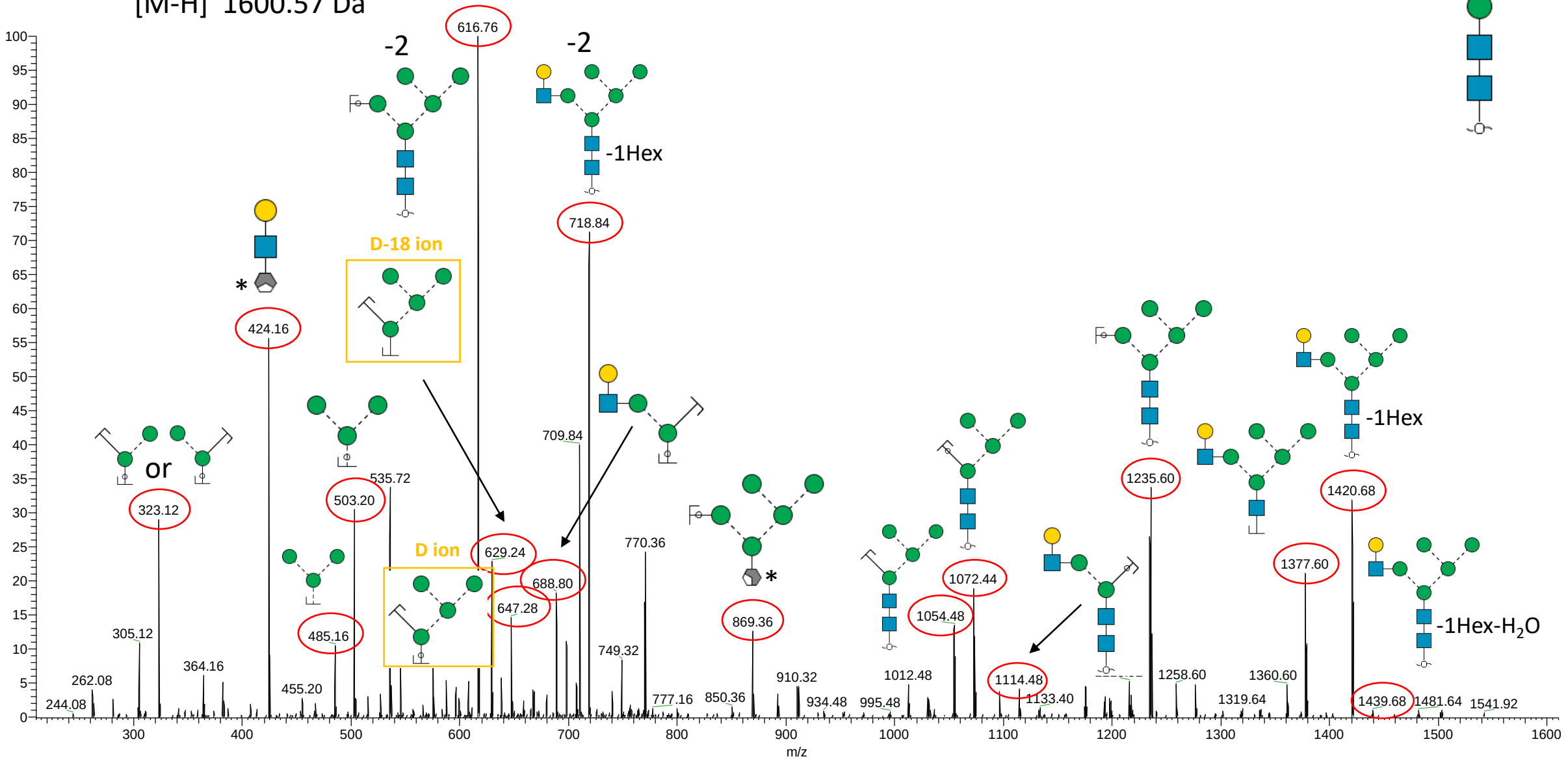


*Ambiguity in the cross-ring fragments

Glycan #9

Observed *m/z* 799.78 (2-), RT: ~23.2 min

$[M-H]^-$ 1600.57 Da



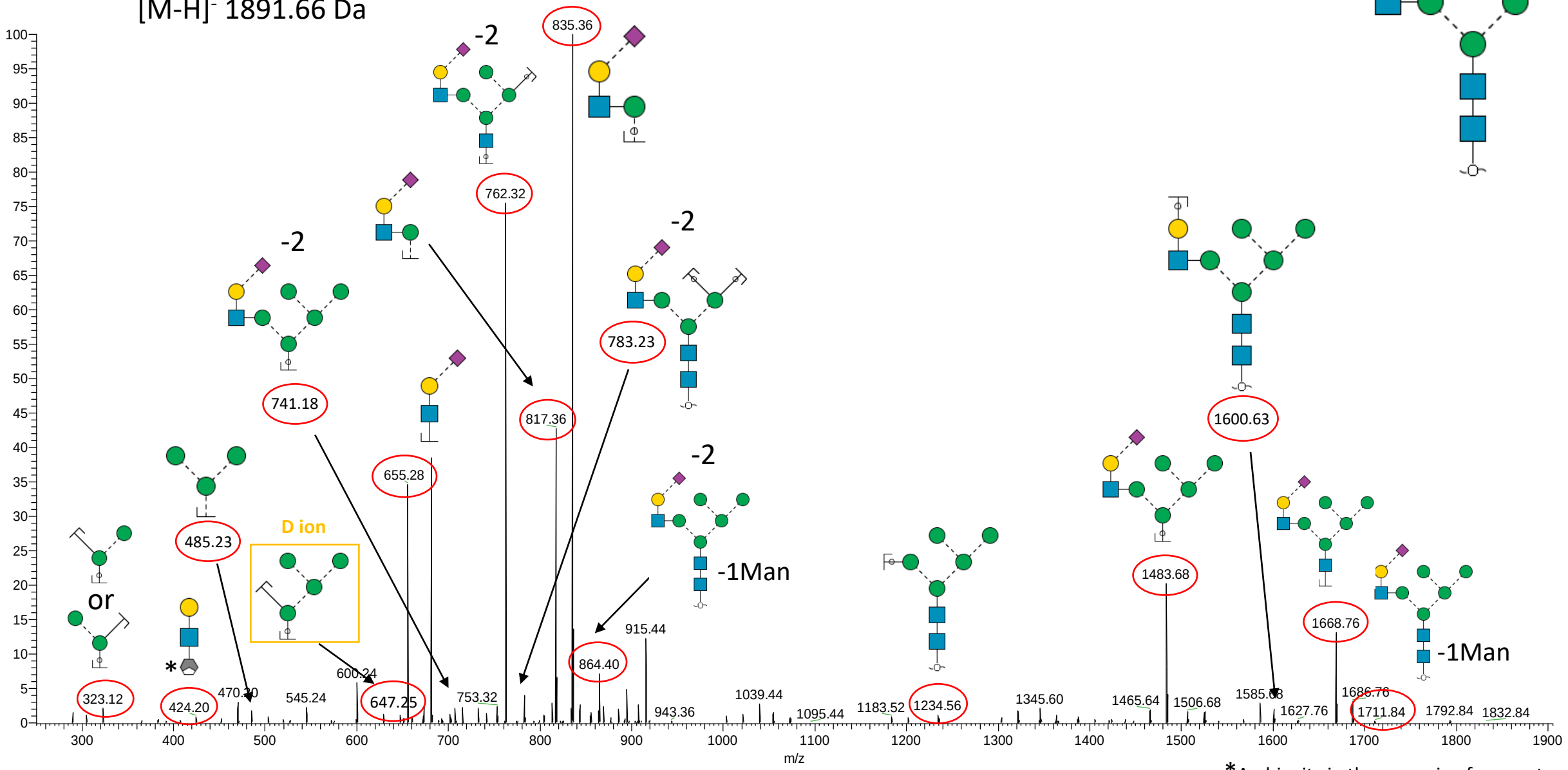
*Ambiguity in the cross-ring fragments

Glycan #10

Observed *m/z* 945.36 (2-), RT: ~25.1 min

$[M-H]^-$ 1891.66 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.

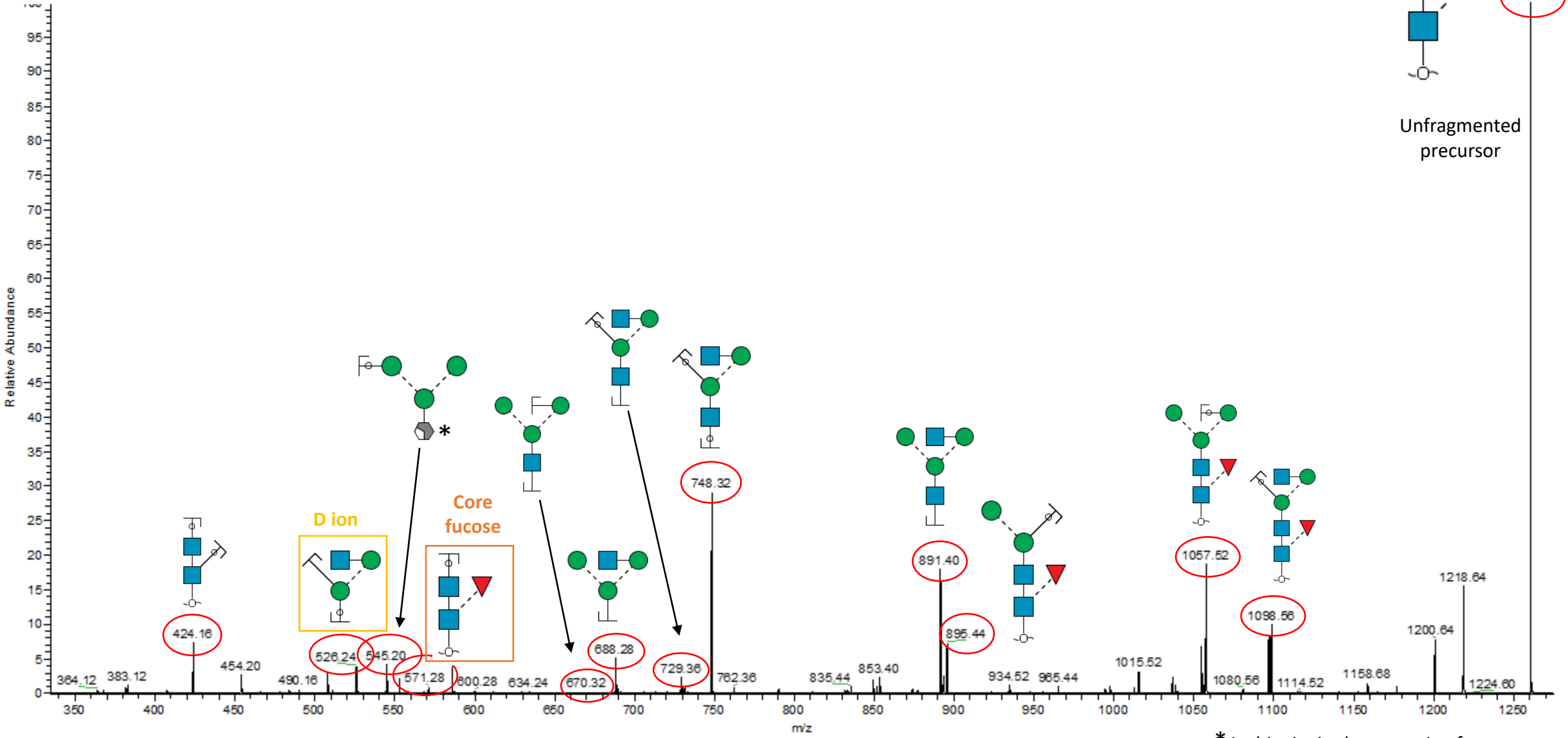


*Ambiguity in the cross-ring fragments

Glycan #11

Observed *m/z* 1260.48 (1-), RT: ~22.5 min

$[M-H]^-$ 1260.47 Da



Unfragmented precursor

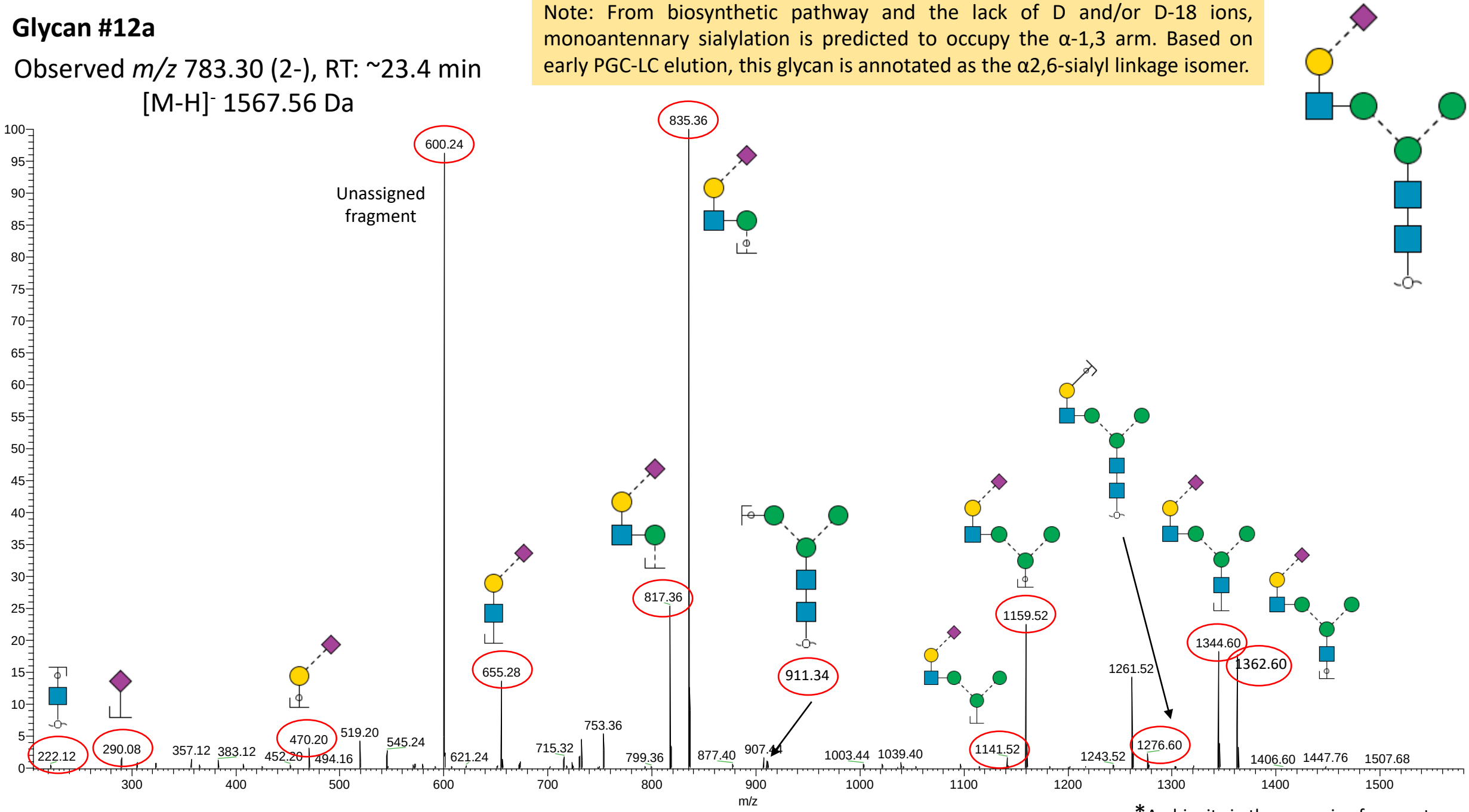
*Ambiguity in the cross-ring fragments

Glycan #12a

Observed *m/z* 783.30 (2-), RT: ~23.4 min

$[M-H]^-$ 1567.56 Da

Note: From biosynthetic pathway and the lack of D and/or D-18 ions, monoantennary sialylation is predicted to occupy the α-1,3 arm. Based on early PGC-LC elution, this glycan is annotated as the α2,6-sialyl linkage isomer.



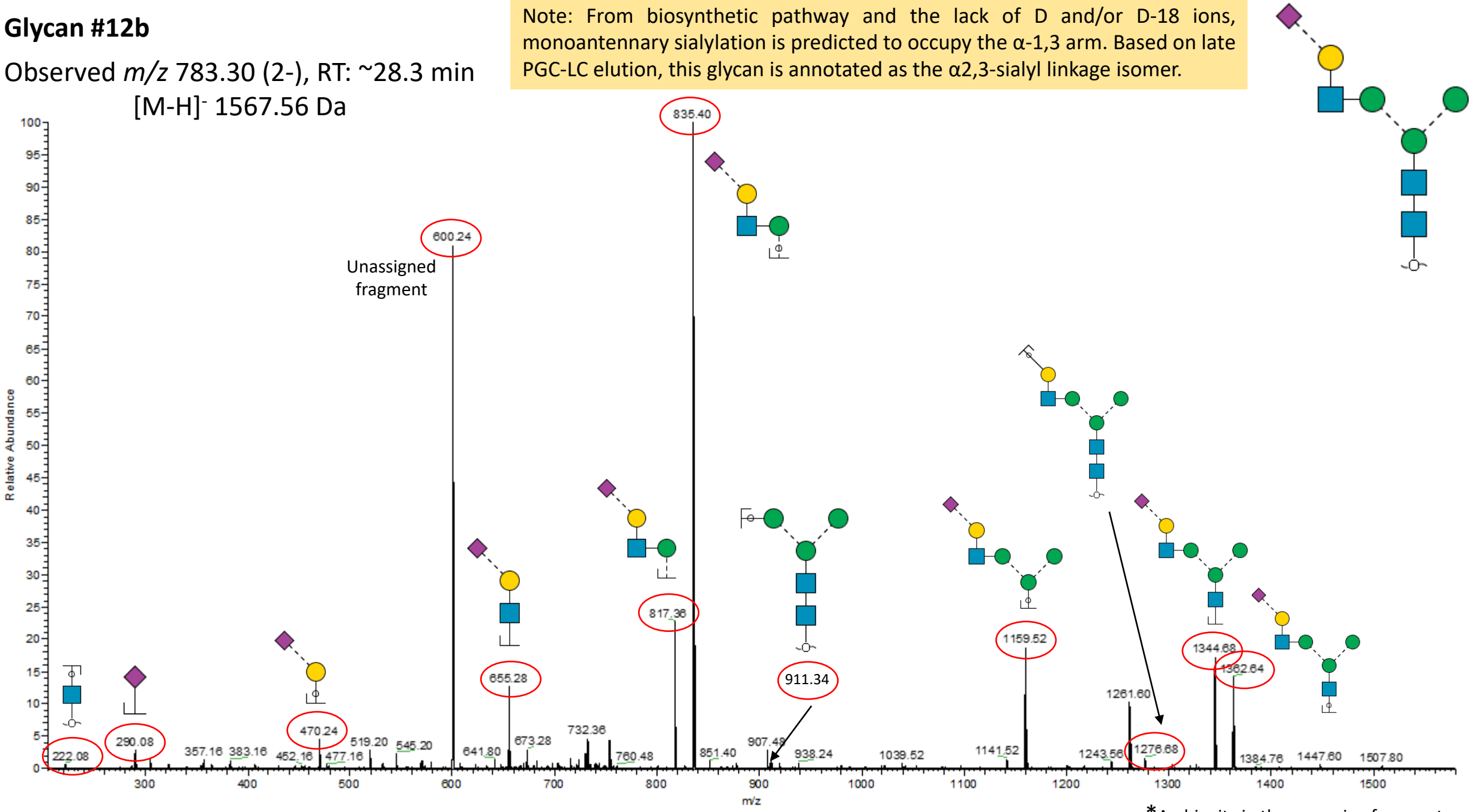
*Ambiguity in the cross-ring fragments

Glycan #12b

Observed *m/z* 783.30 (2-), RT: ~28.3 min

$[M-H]^-$ 1567.56 Da

Note: From biosynthetic pathway and the lack of D and/or D-18 ions, monoantennary sialylation is predicted to occupy the α-1,3 arm. Based on late PGC-LC elution, this glycan is annotated as the α2,3-sialyl linkage isomer.

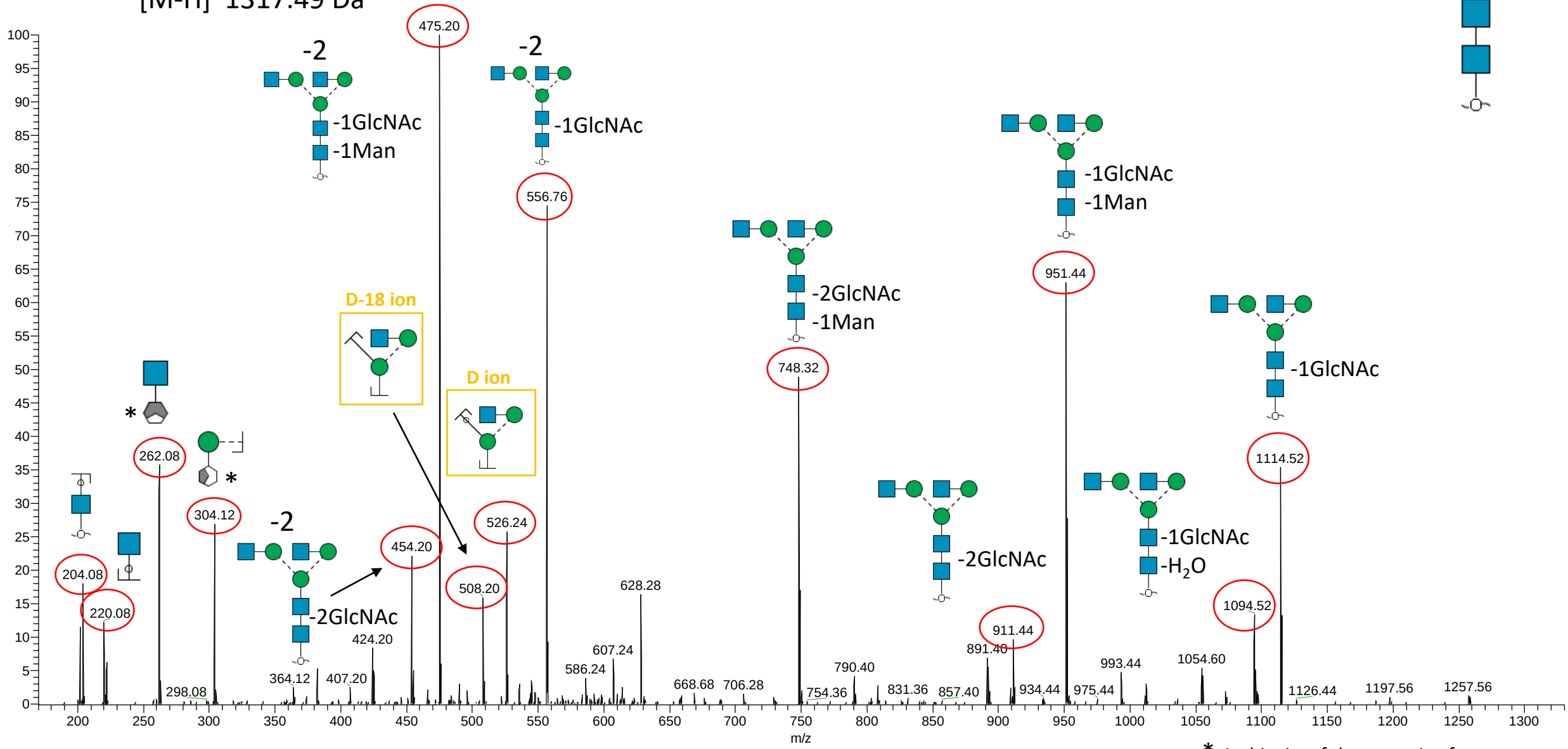


*Ambiguity in the cross-ring fragments

Glycan #13

Observed *m/z* 658.26 (2-), RT: ~20.5 min

[M-H]$^-$ 1317.49 Da

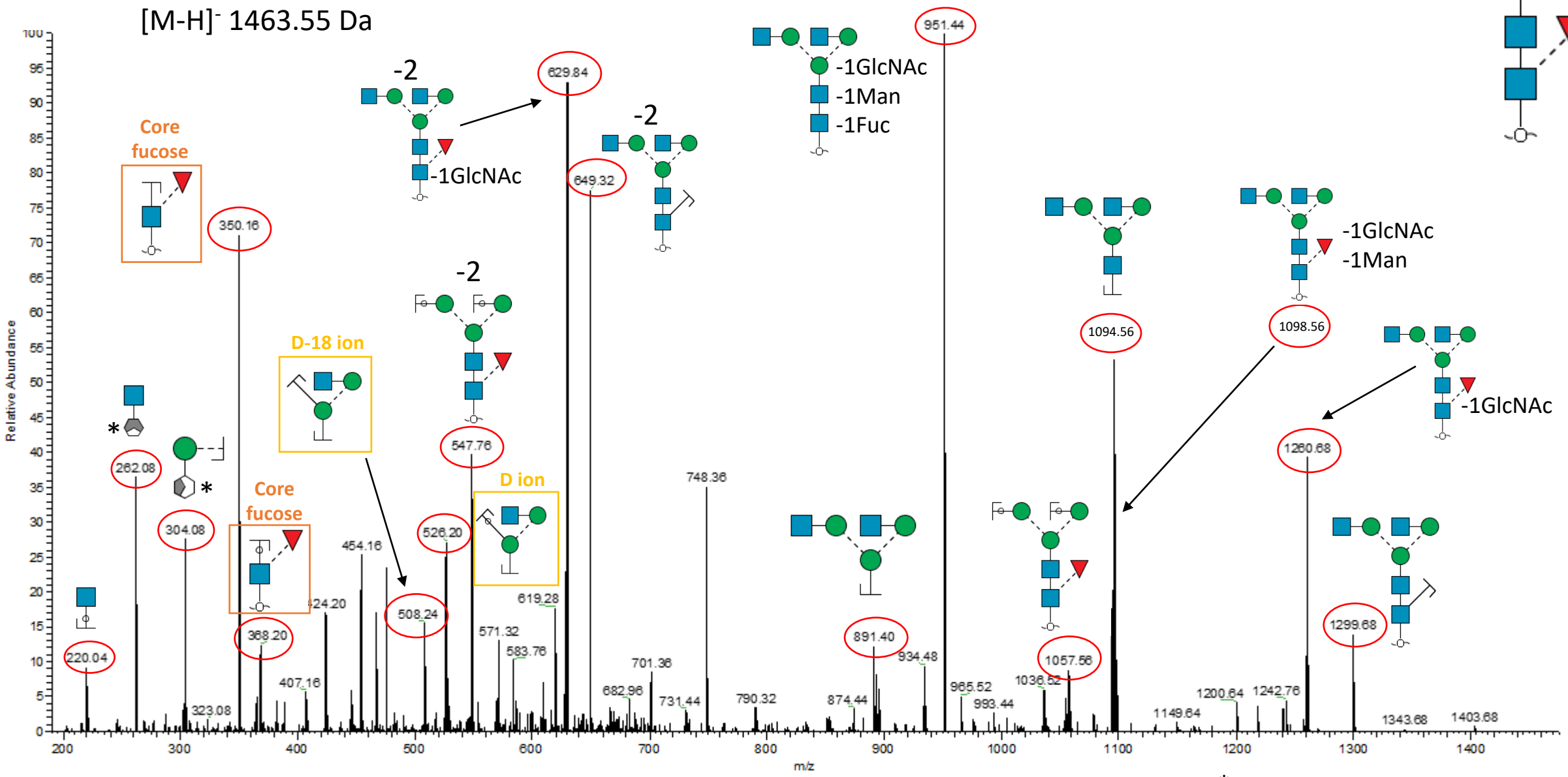


* Ambiguity of the cross-ring fragments

Glycan #14

Observed *m/z* 731.30 (2-), RT: ~24.7 min

$[M-H]^-$ 1463.55 Da

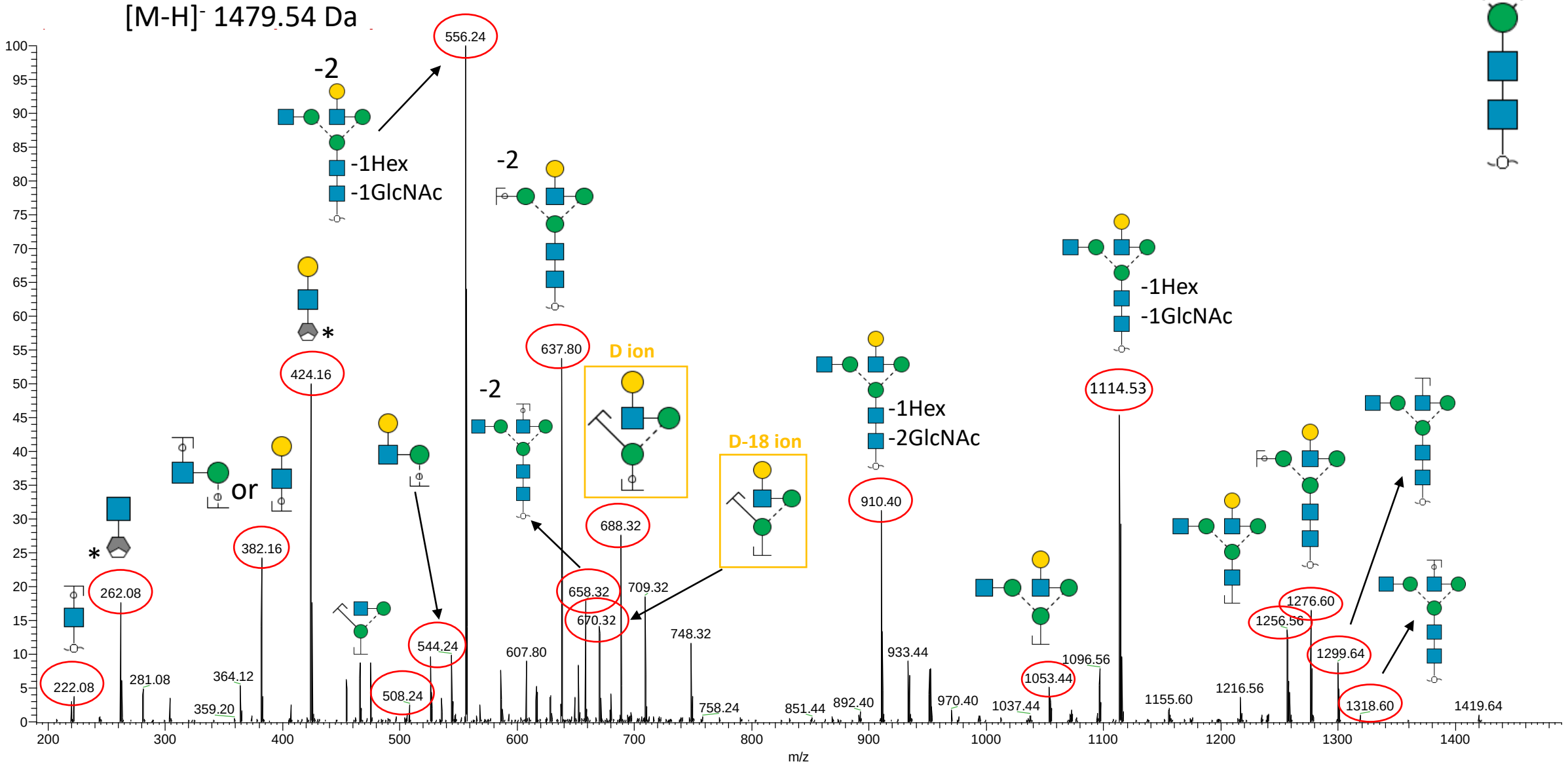


*Ambiguity of the cross-ring fragments

Glycan #15

Observed *m/z* 739.30 (2-), RT: ~22.3 min

$[M-H]^-$ 1479.54 Da

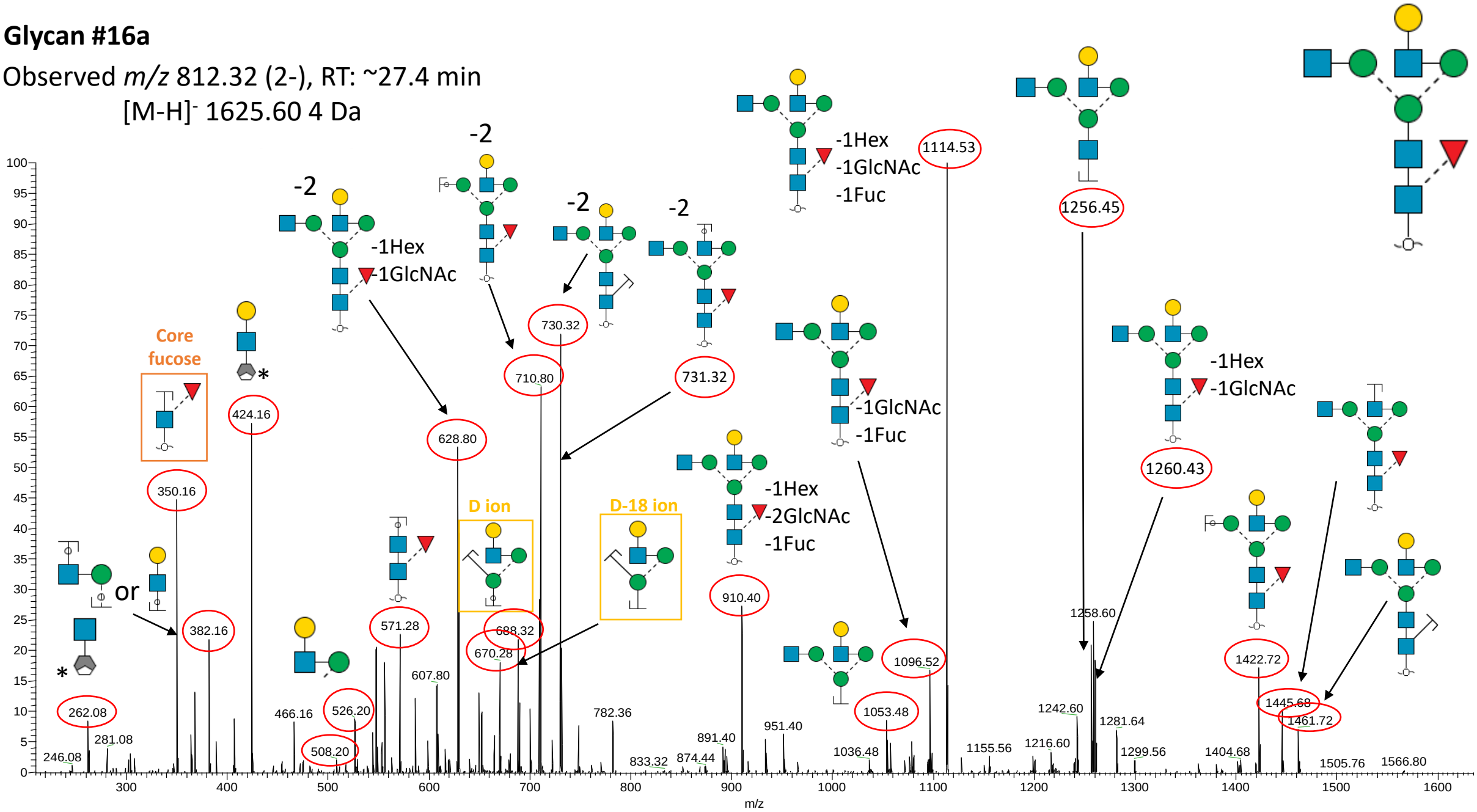


*Ambiguity of the cross-ring fragments

Glycan #16a

Observed *m/z* 812.32 (2-), RT: ~27.4 min

$[M-H]^-$ 1625.60 4 Da

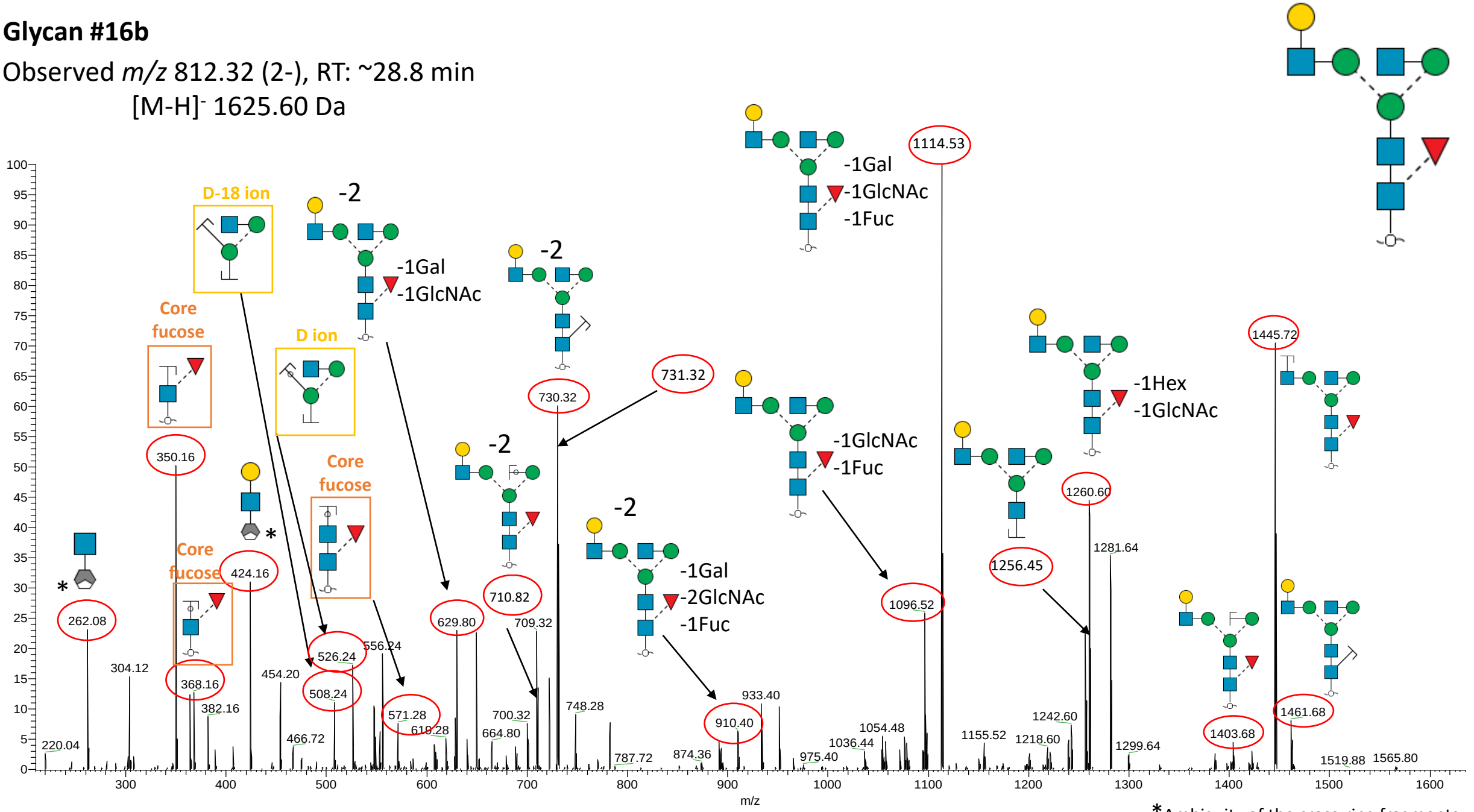


*Ambiguity of the cross-ring fragments

Glycan #16b

Observed *m/z* 812.32 (2-), RT: ~28.8 min

$[M-H]^-$ 1625.60 Da



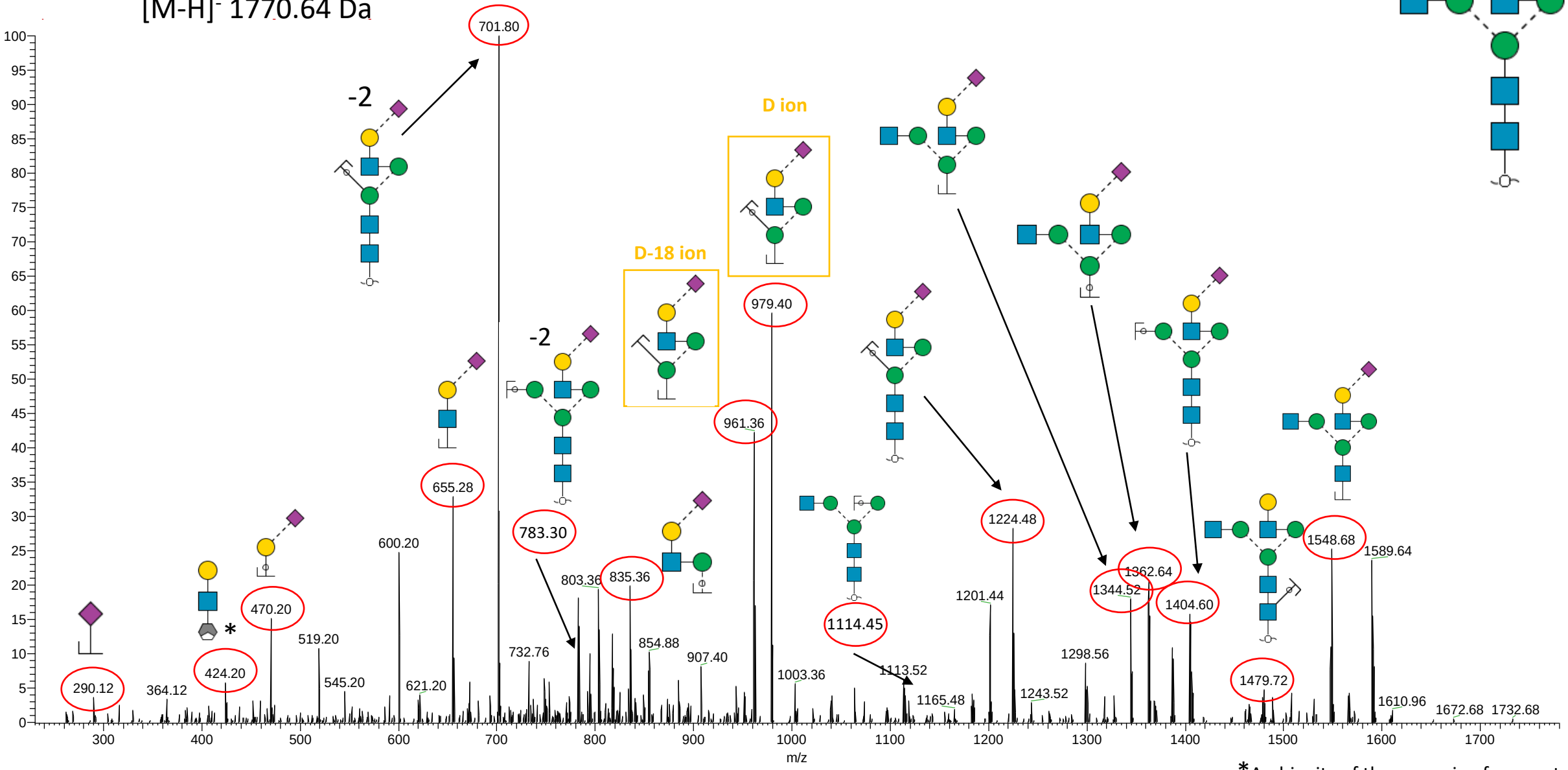
*Ambiguity of the cross-ring fragments

Glycan #17a

Observed *m/z* 884.84 (2-), RT: ~23.9 min

$[M-H]^-$ 1770.64 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



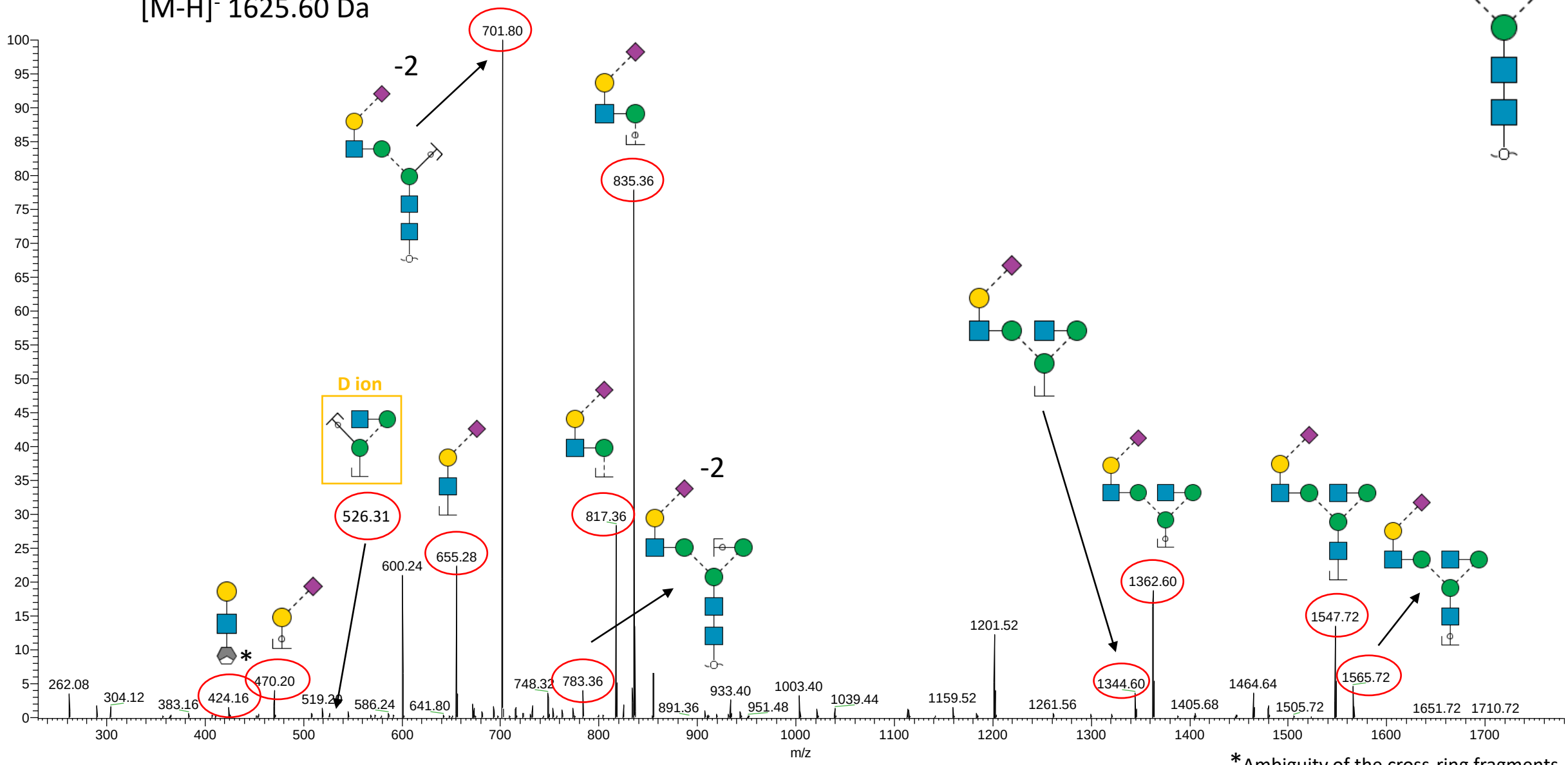
*Ambiguity of the cross-ring fragments

Glycan #17b

Observed *m/z* 884.84 (2-), RT: ~25.2 min

$[M-H]^-$ 1625.60 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



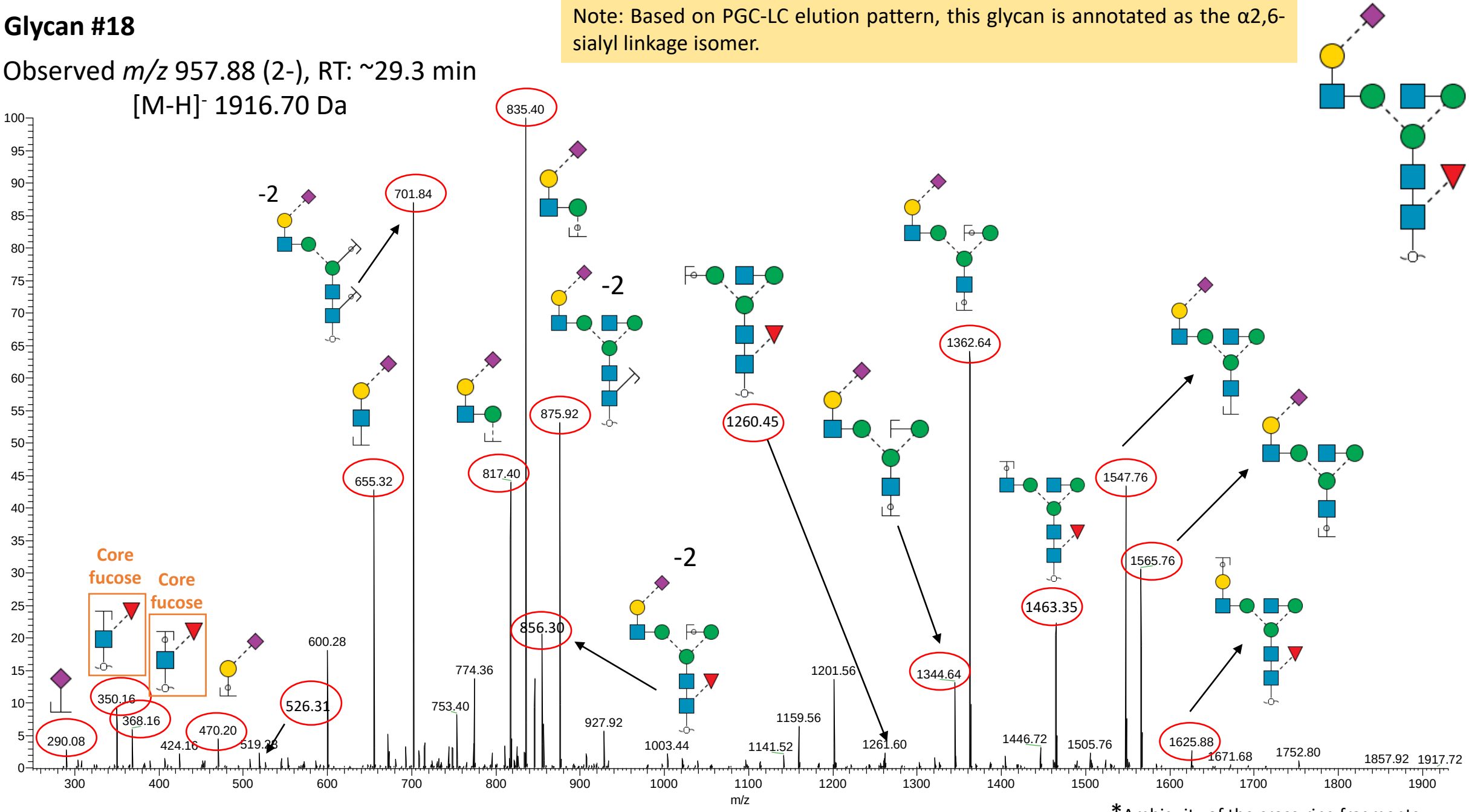
*Ambiguity of the cross-ring fragments

Glycan #18

Observed *m/z* 957.88 (2-), RT: ~29.3 min

$[M-H]^-$ 1916.70 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.

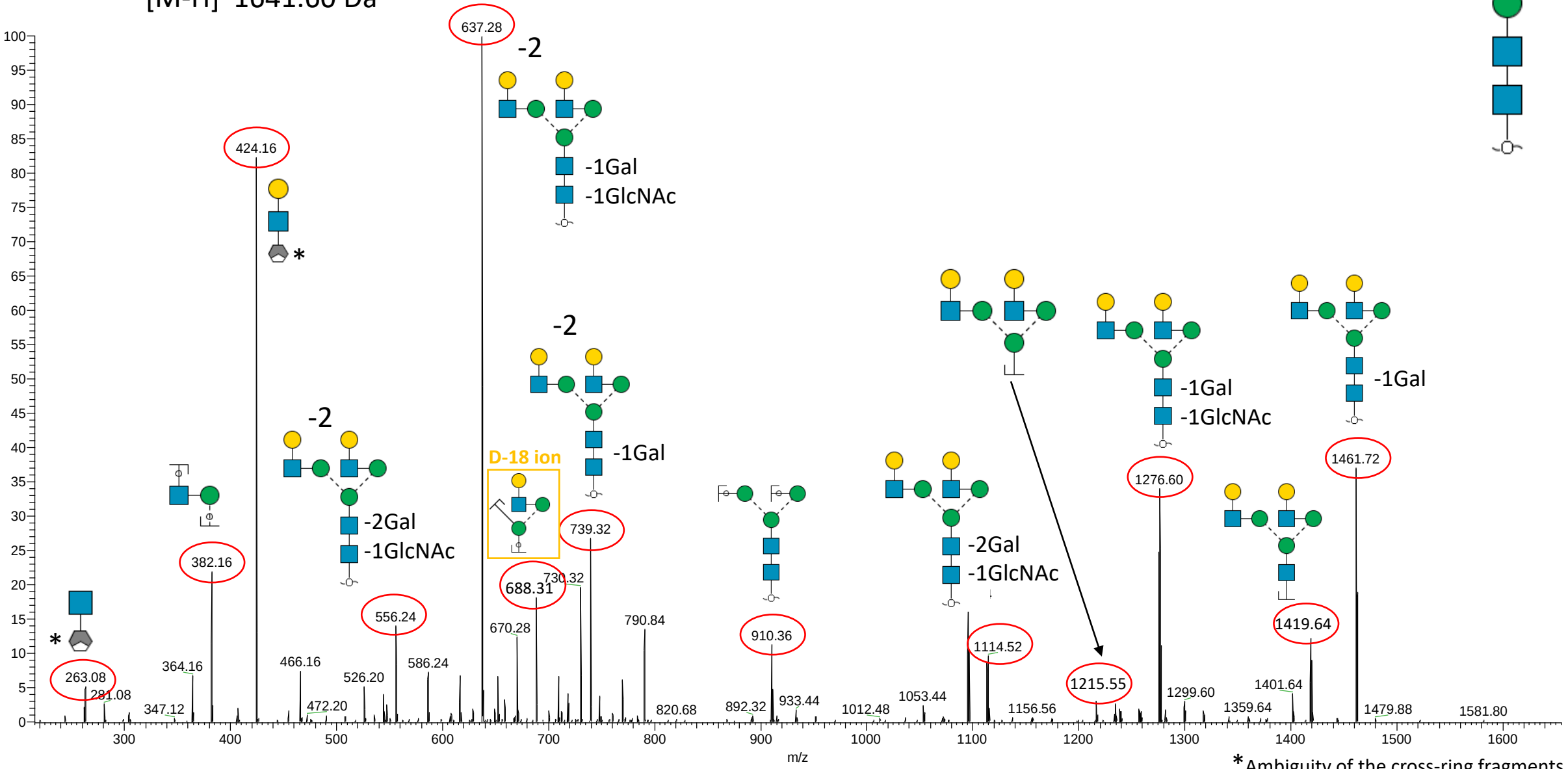


*Ambiguity of the cross-ring fragments

Glycan #19

Observed *m/z* 820.32 (2-), RT: ~25.6 min

$[M-H]^-$ 1641.60 Da

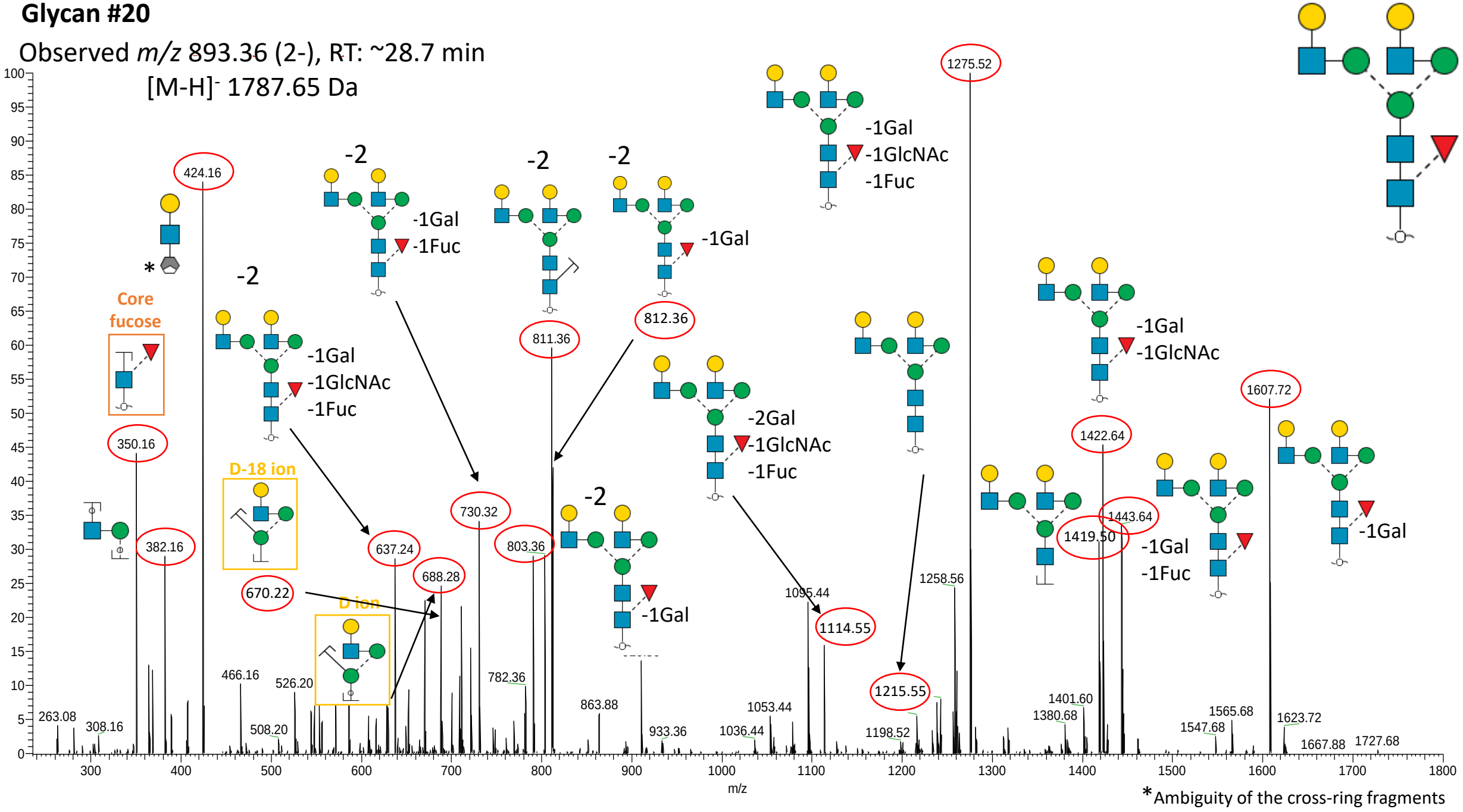


*Ambiguity of the cross-ring fragments

Glycan #20

Observed *m/z* 893.36 (2-), RT: ~28.7 min

$[M-H]^-$ 1787.65 Da



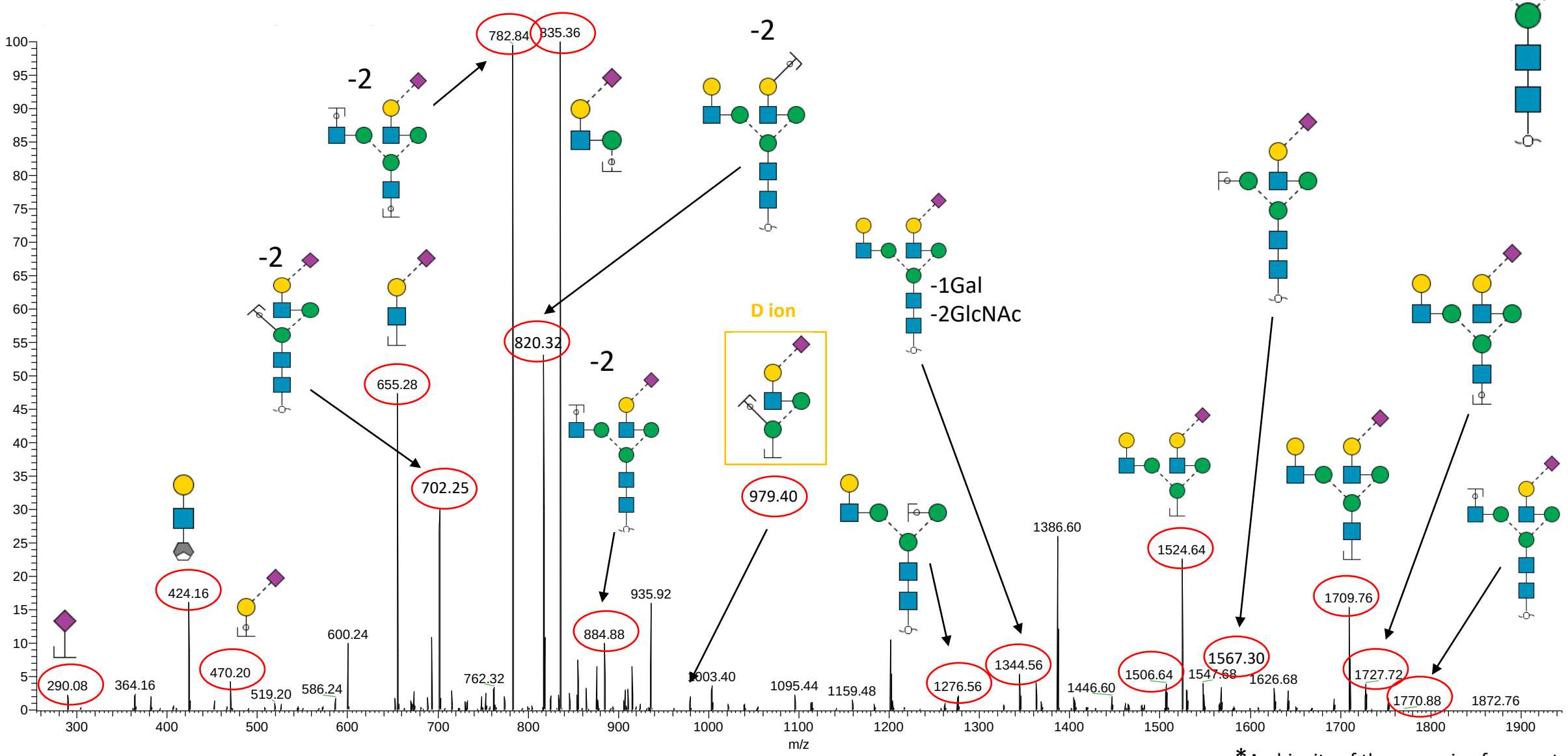
*Ambiguity of the cross-ring fragments

Glycan #21

Observed *m/z* 965.88 (2-), RT: ~25.2 min

[M-H]$^-$ 1932.69 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



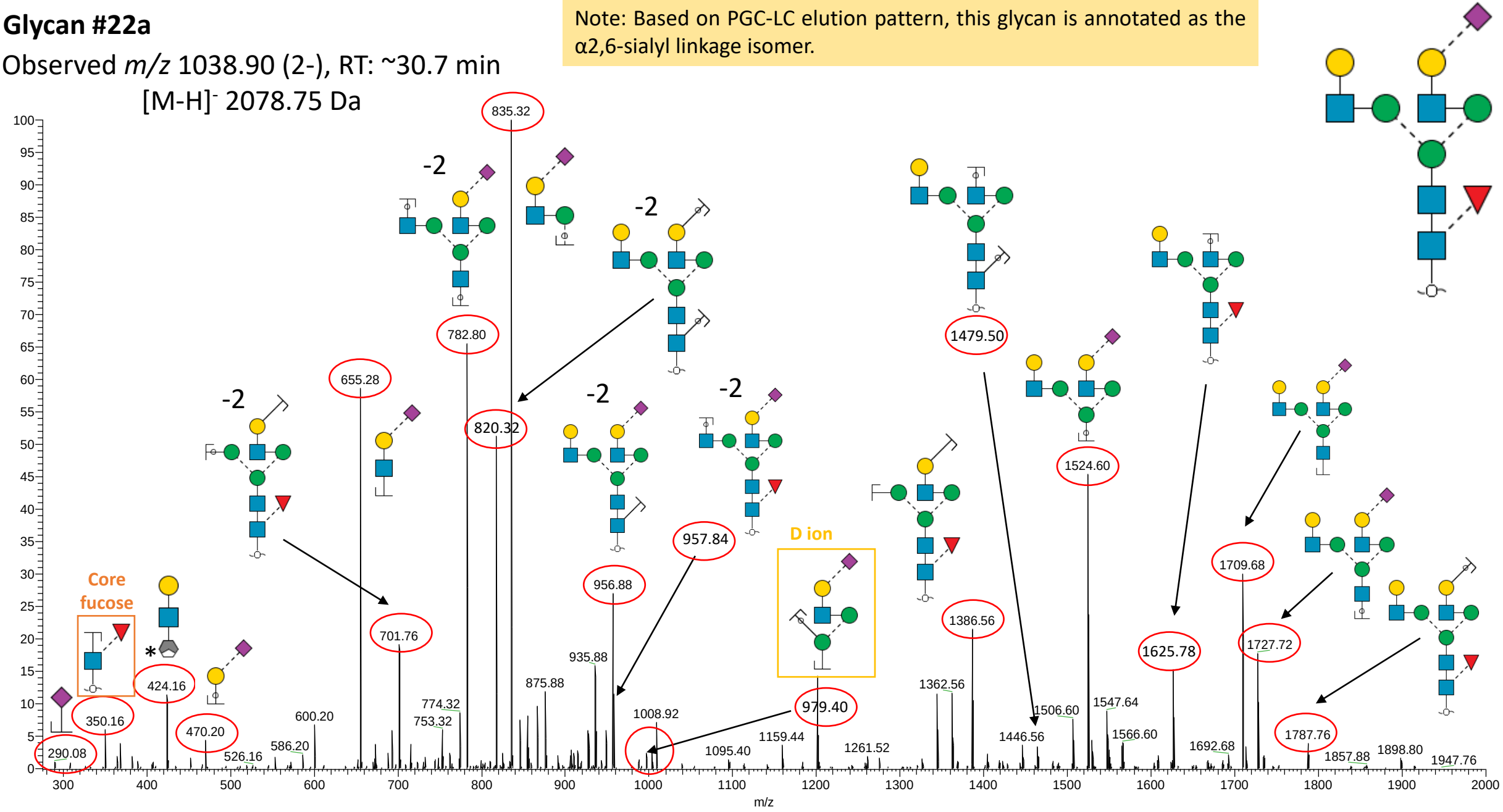
*Ambiguity of the cross-ring fragments

Glycan #22a

Observed *m/z* 1038.90 (2-), RT: ~30.7 min

$[M-H]^-$ 2078.75 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



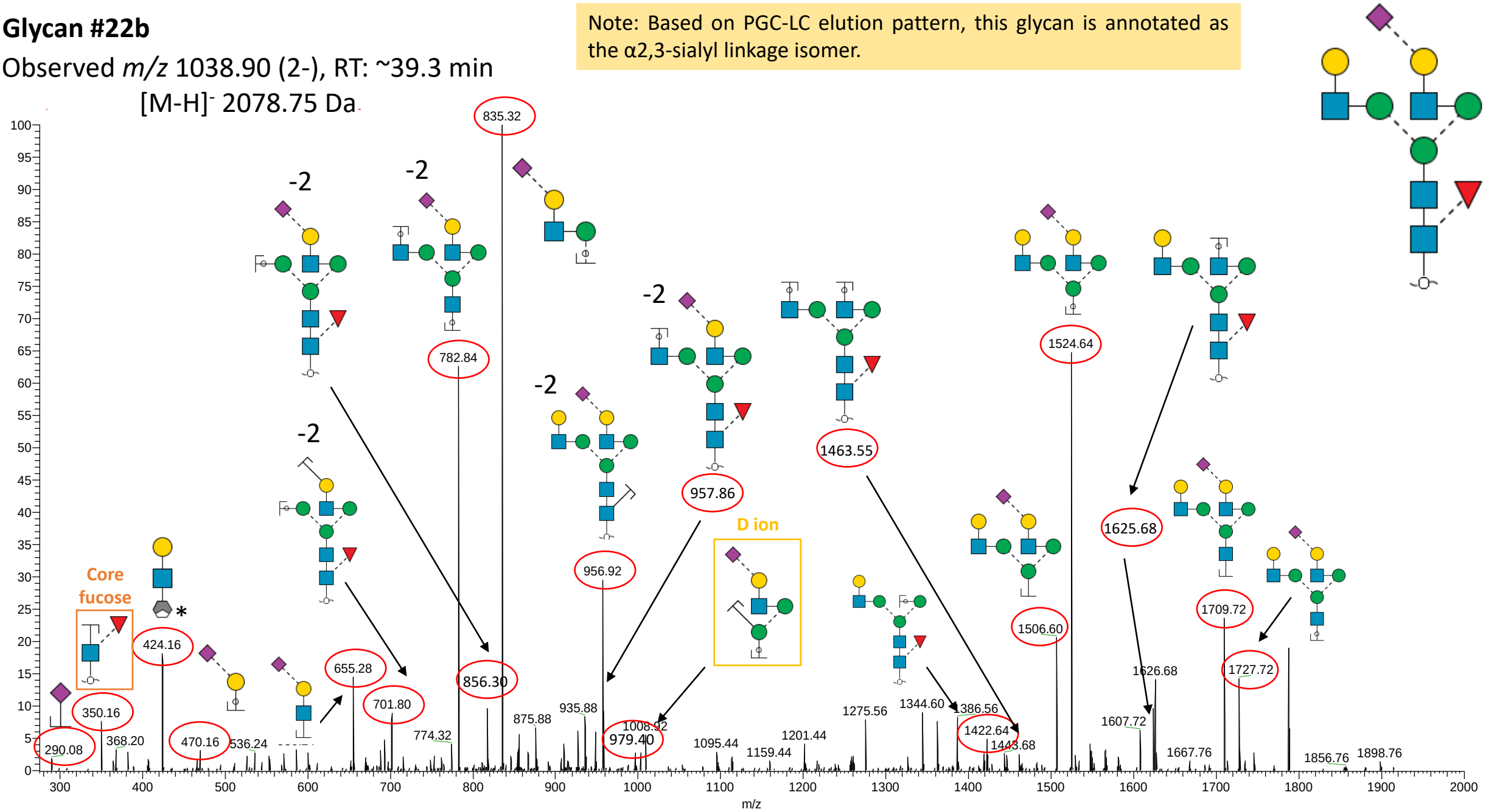
*Ambiguity of the cross-ring fragments

Glycan #22b

Observed *m/z* 1038.90 (2-), RT: ~39.3 min

$[M-H]^-$ 2078.75 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,3-sialyl linkage isomer.



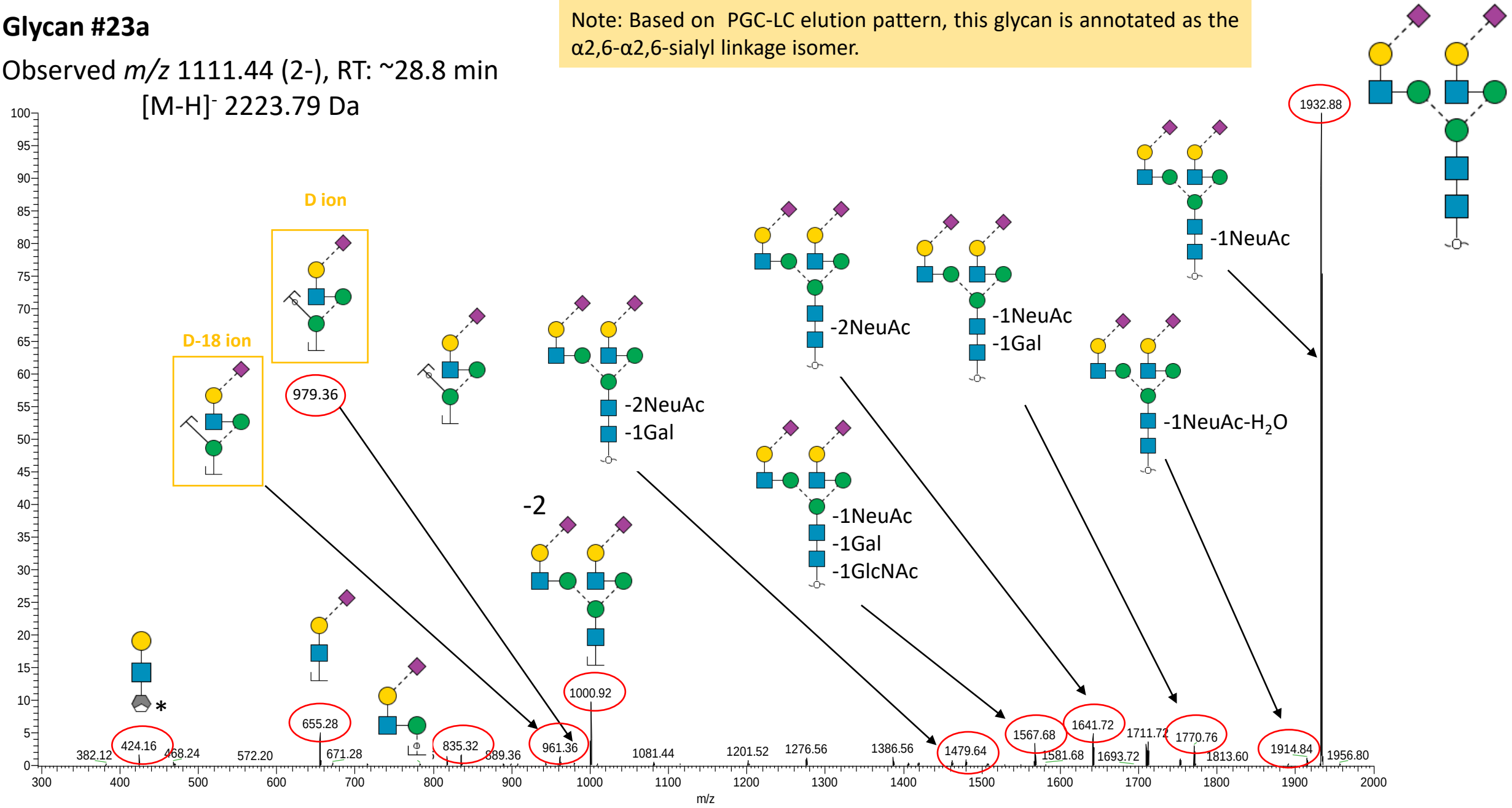
*Ambiguity of the cross-ring fragments

Glycan #23a

Observed *m/z* 1111.44 (2-), RT: ~28.8 min

$[M-H]^-$ 2223.79 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-sialyl linkage isomer.



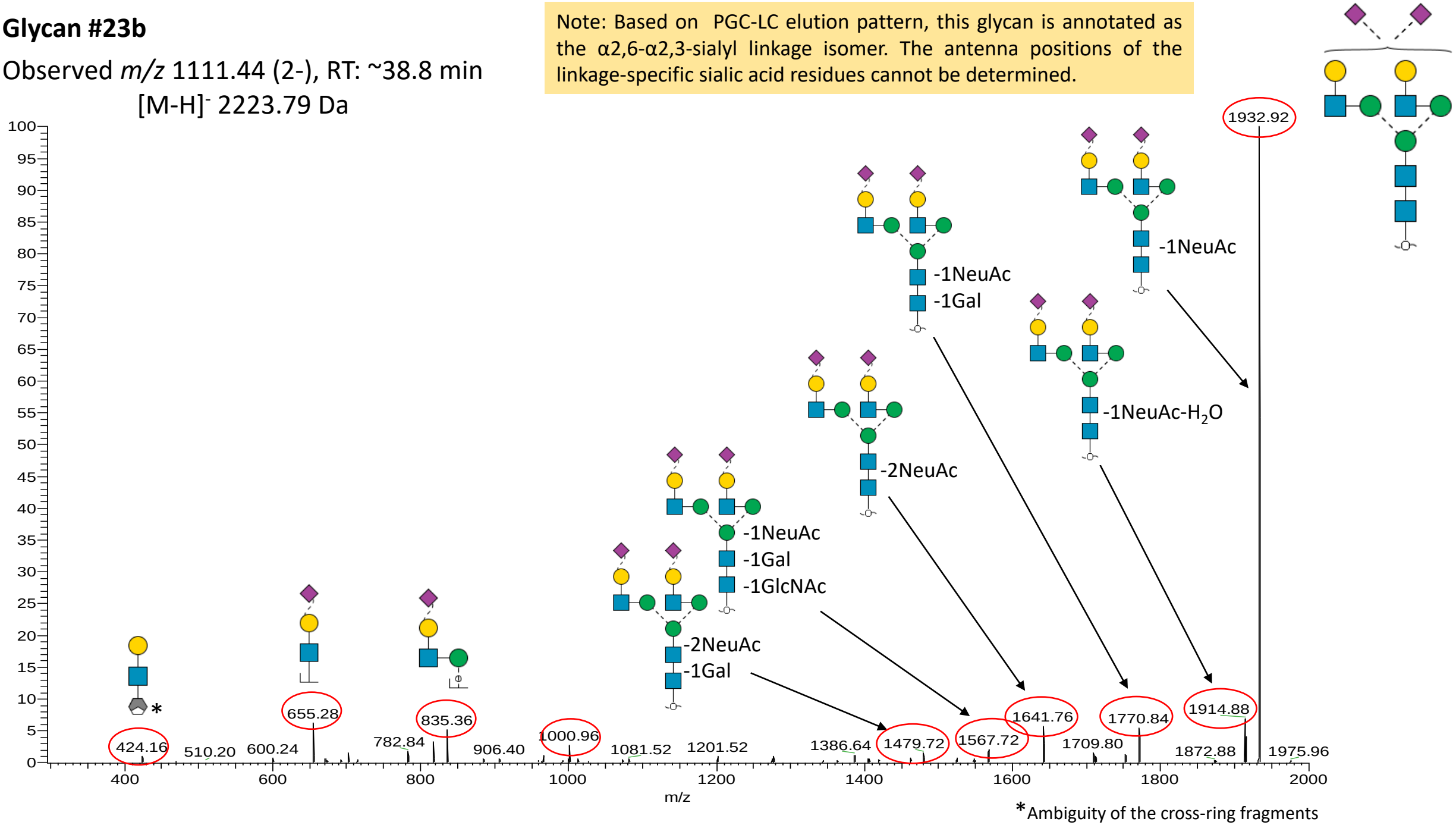
*Ambiguity of the cross-ring fragments

Glycan #23b

Observed *m/z* 1111.44 (2-), RT: ~38.8 min

$[M-H]^-$ 2223.79 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,3-sialyl linkage isomer. The antenna positions of the linkage-specific sialic acid residues cannot be determined.



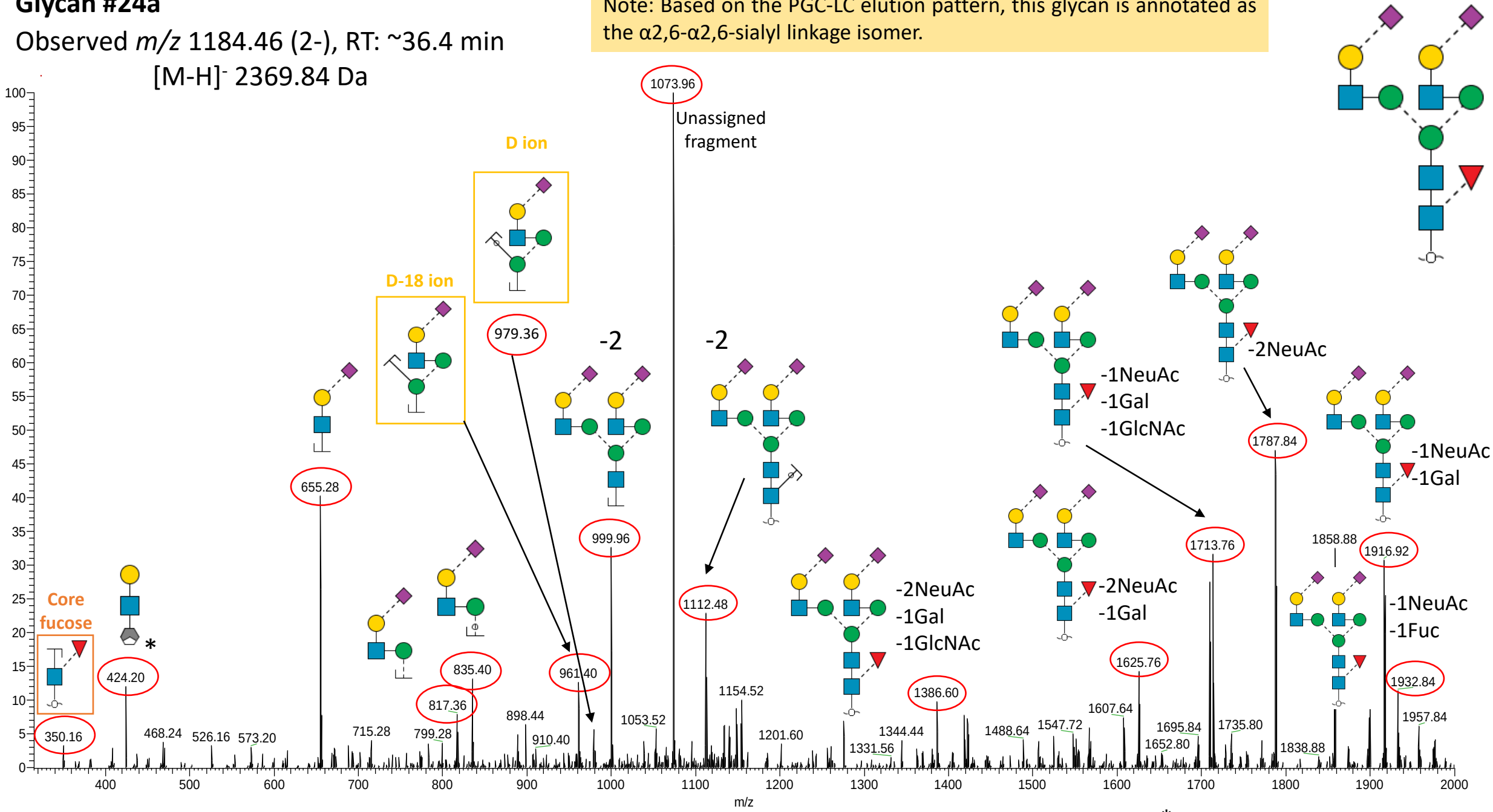
*Ambiguity of the cross-ring fragments

Glycan #24a

Observed *m/z* 1184.46 (2-), RT: ~36.4 min

[M-H]$^-$ 2369.84 Da

Note: Based on the PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-sialyl linkage isomer.



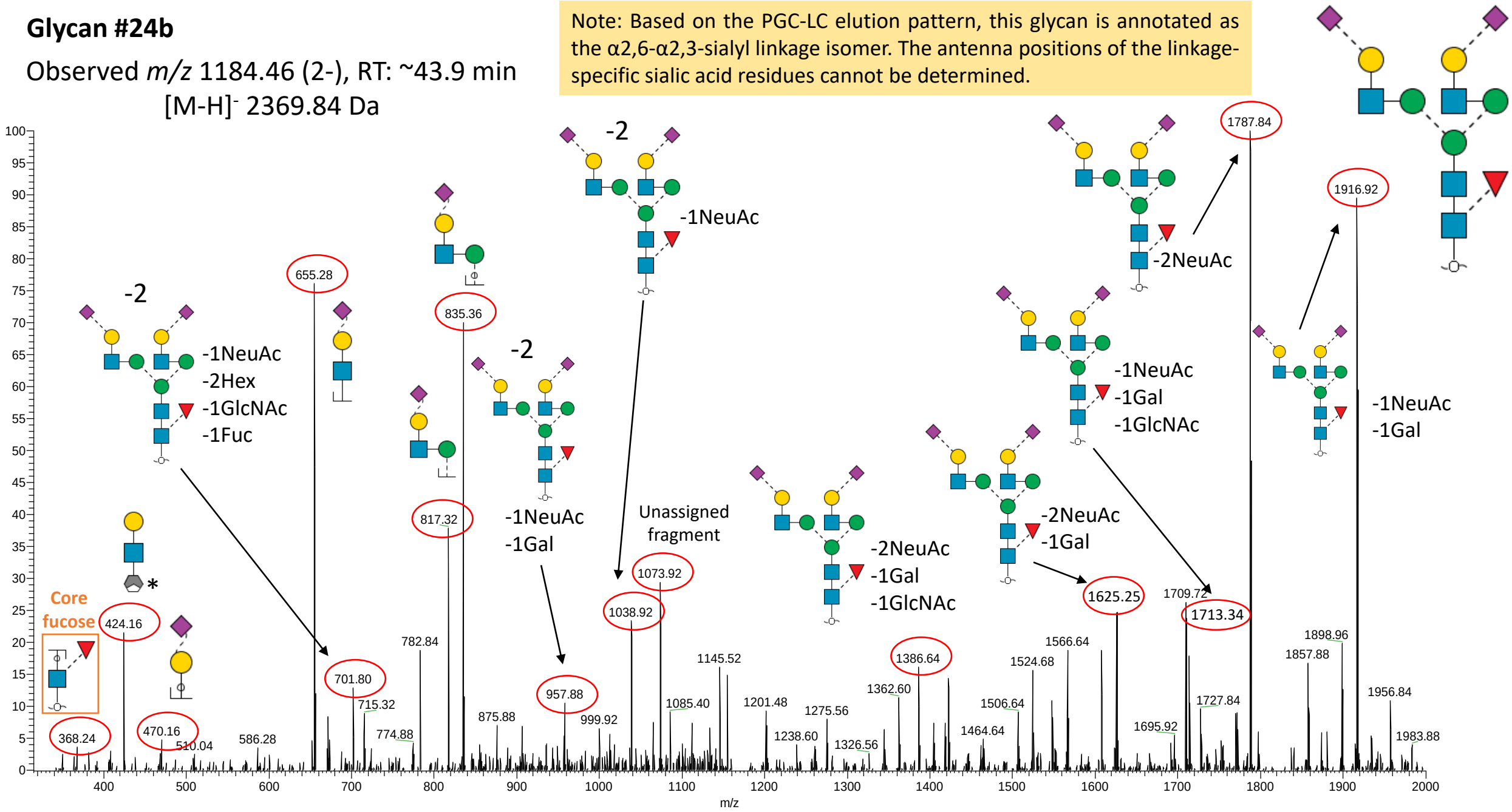
*Ambiguity of the cross-ring fragments

Glycan #24b

Observed *m/z* 1184.46 (2-), RT: ~43.9 min

$[M-H]^-$ 2369.84 Da

Note: Based on the PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,3-sialyl linkage isomer. The antenna positions of the linkage-specific sialic acid residues cannot be determined.



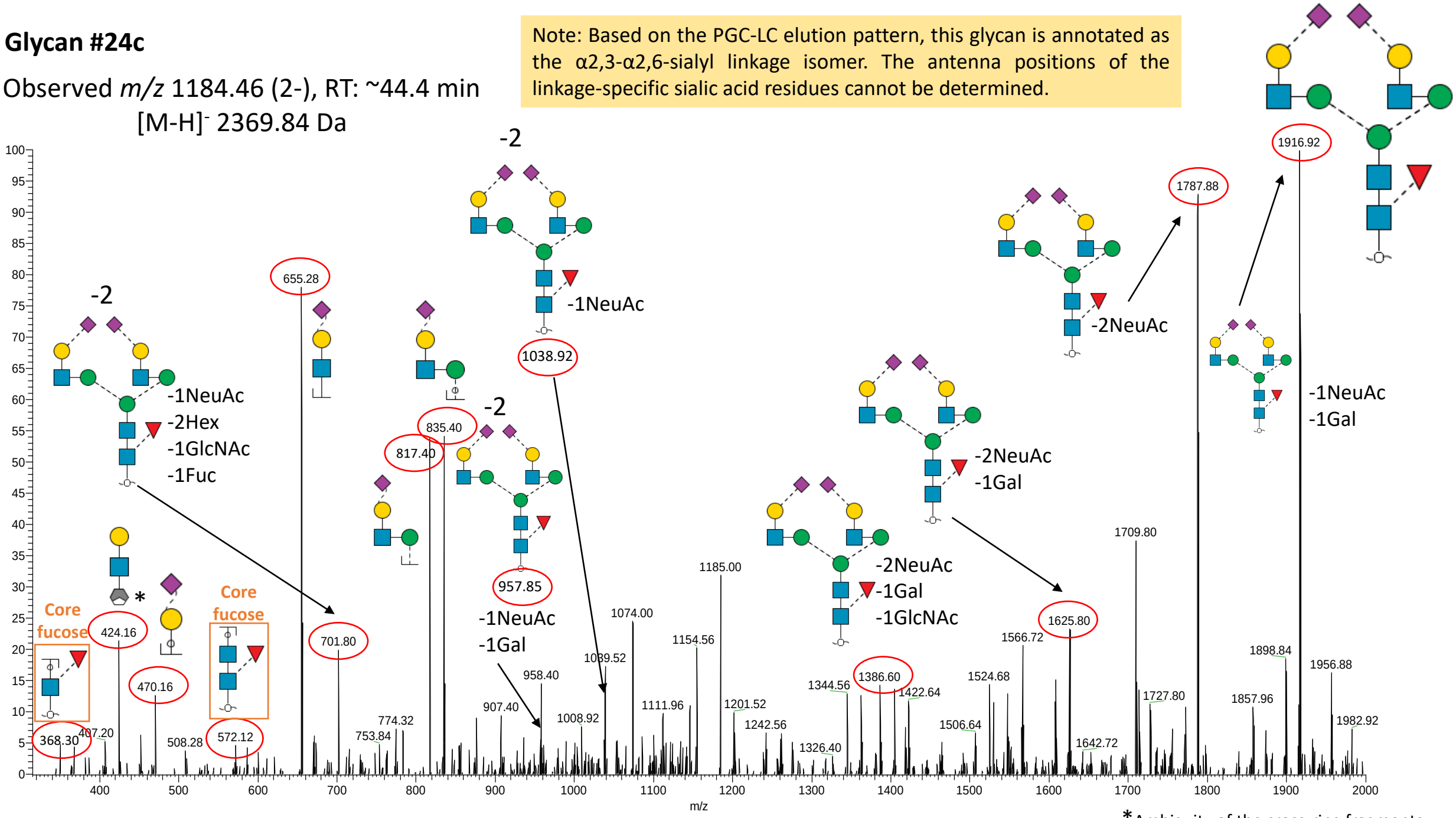
*Ambiguity of the cross-ring fragments

Glycan #24c

Observed *m/z* 1184.46 (2-), RT: ~44.4 min

$[M-H]^-$ 2369.84 Da

Note: Based on the PGC-LC elution pattern, this glycan is annotated as the α2,3-α2,6-sialyl linkage isomer. The antenna positions of the linkage-specific sialic acid residues cannot be determined.



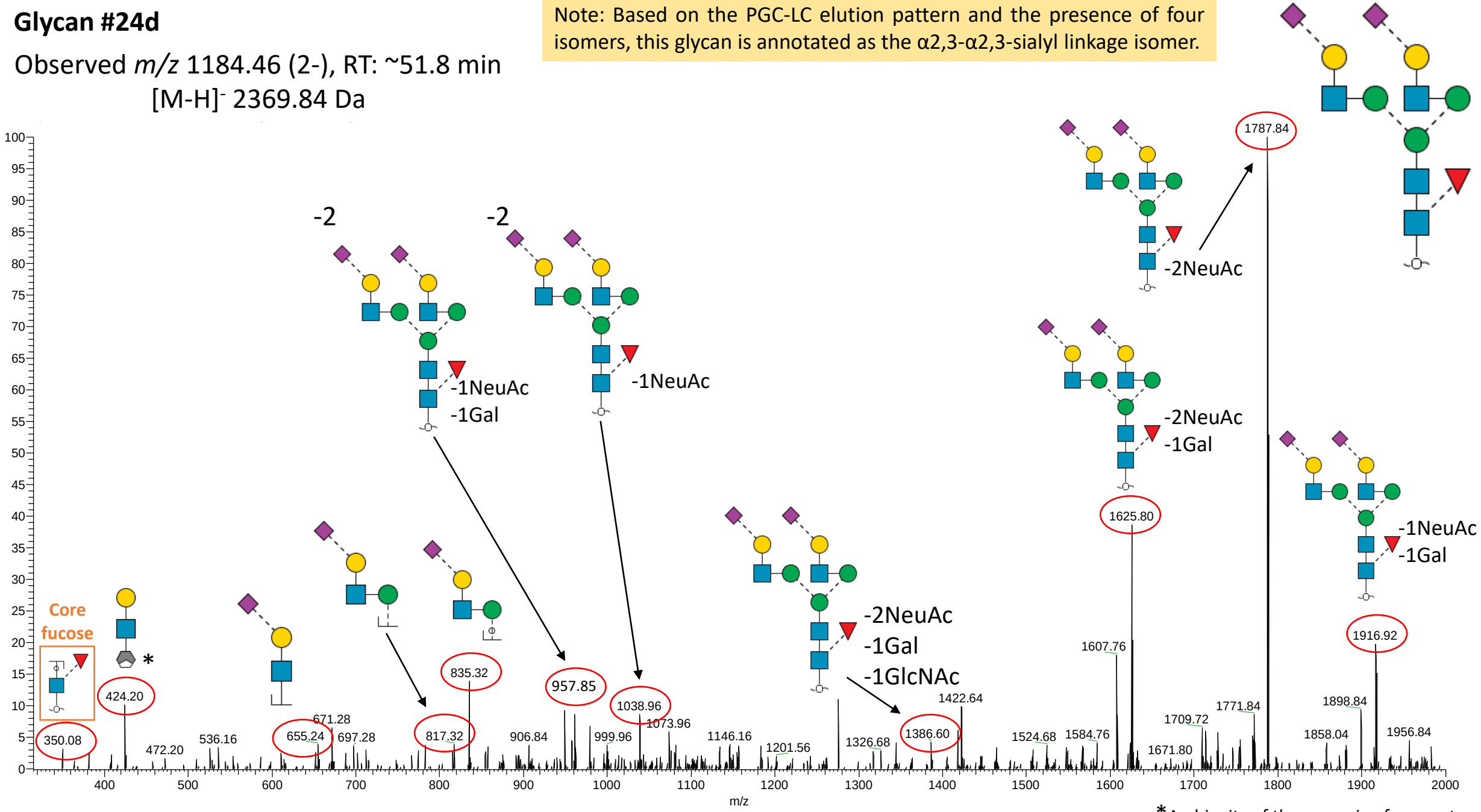
*Ambiguity of the cross-ring fragments

Glycan #24d

Observed *m/z* 1184.46 (2-), RT: ~51.8 min

$[M-H]^-$ 2369.84 Da

Note: Based on the PGC-LC elution pattern and the presence of four isomers, this glycan is annotated as the α2,3-α2,3-sialyl linkage isomer.



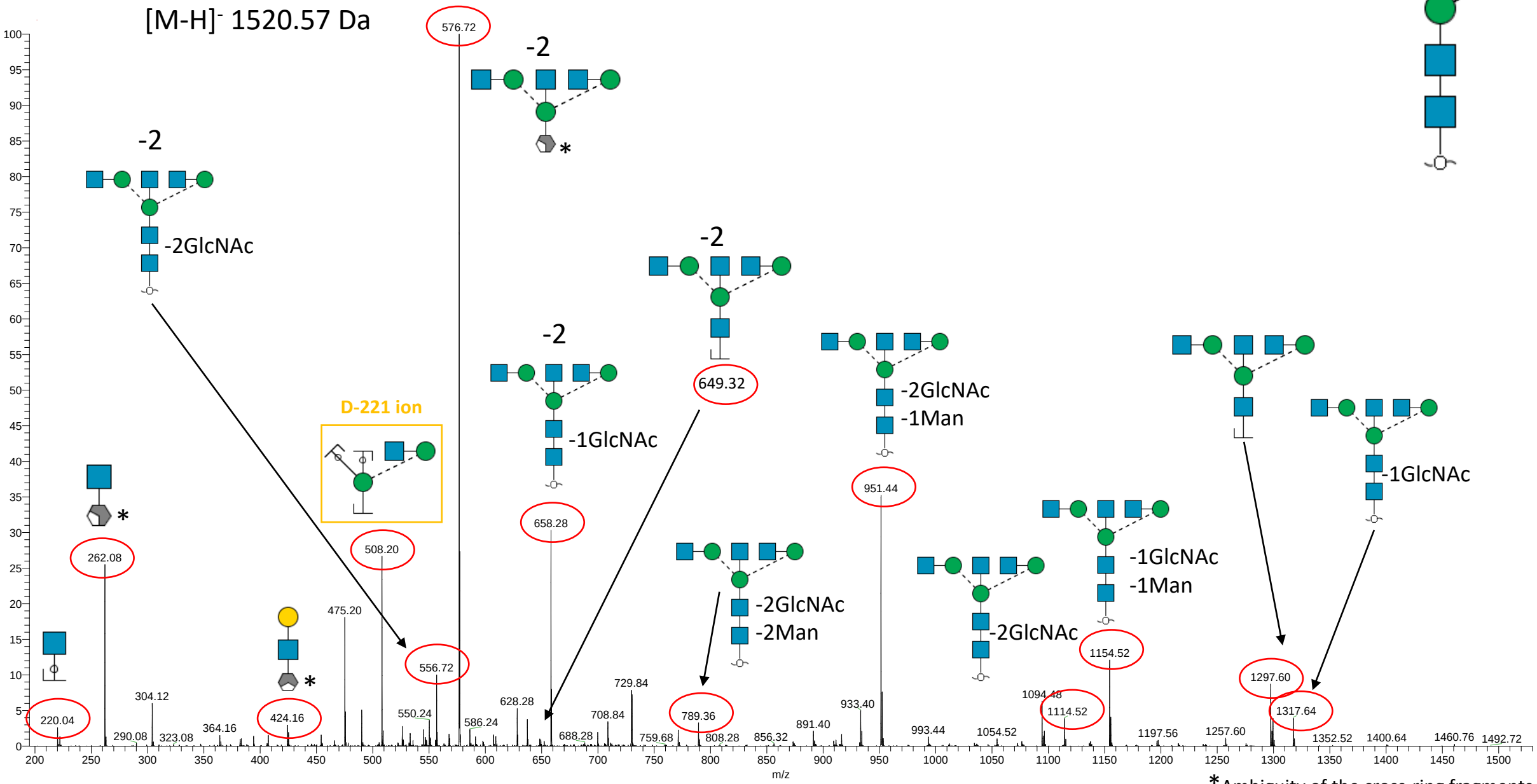
*Ambiguity of the cross-ring fragments

Glycan #25

Observed *m/z* 759.82 (2-), RT: ~16.8 min

$[M-H]^-$ 1520.57 Da

Note: Based on the very early PGC-LC elution time and the abundant D-ion, this structure is assigned as a bisecting GlcNAc.



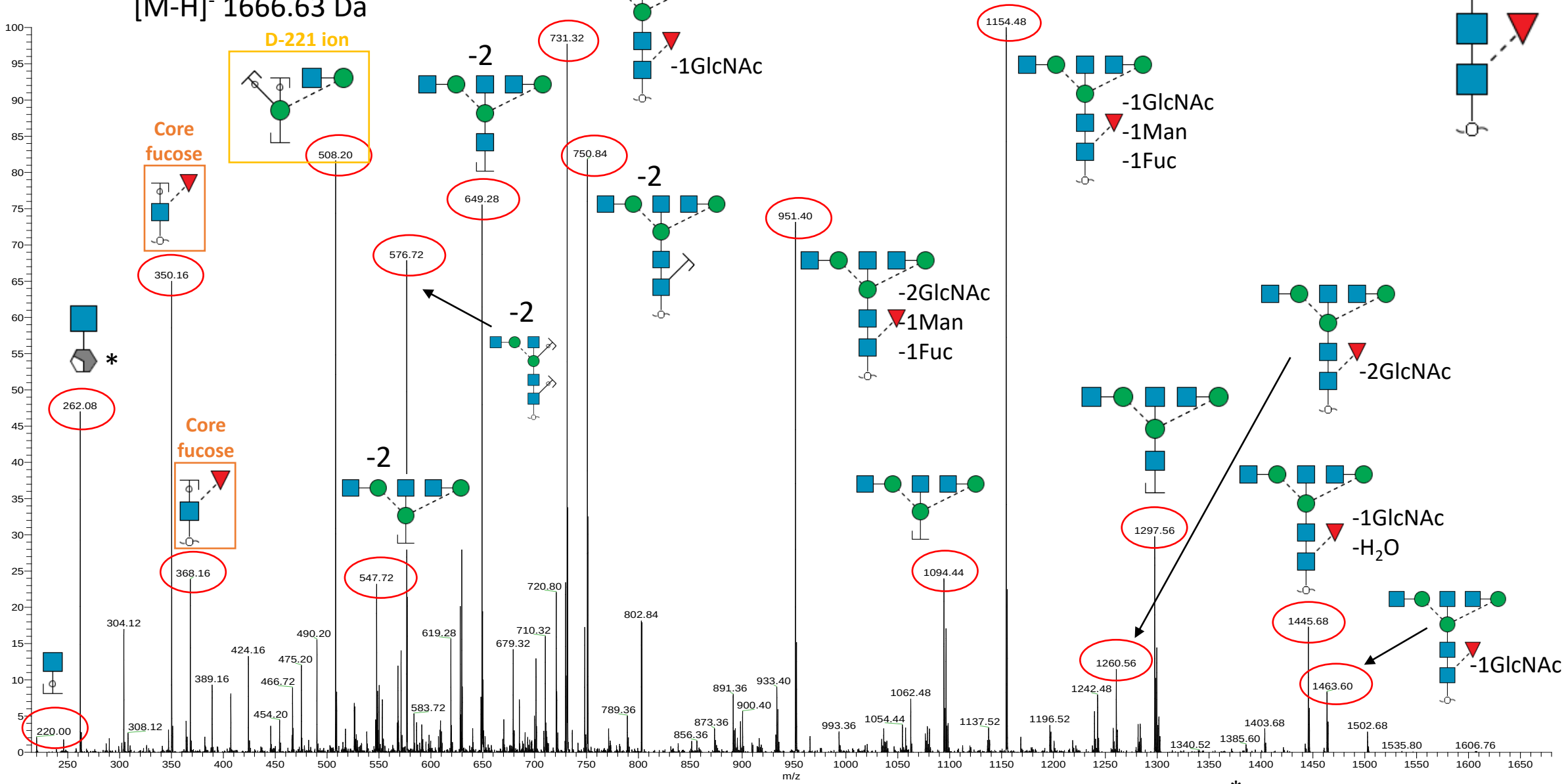
*Ambiguity of the cross-ring fragments

Glycan #26

Observed *m/z* 832.81 (2-), RT: ~19.7 min

$[M-H]^-$ 1666.63 Da

Note: Based on the very early PGC-LC elution time and the abundant D-ion, this structure is assigned as a bisecting GlcNAc.

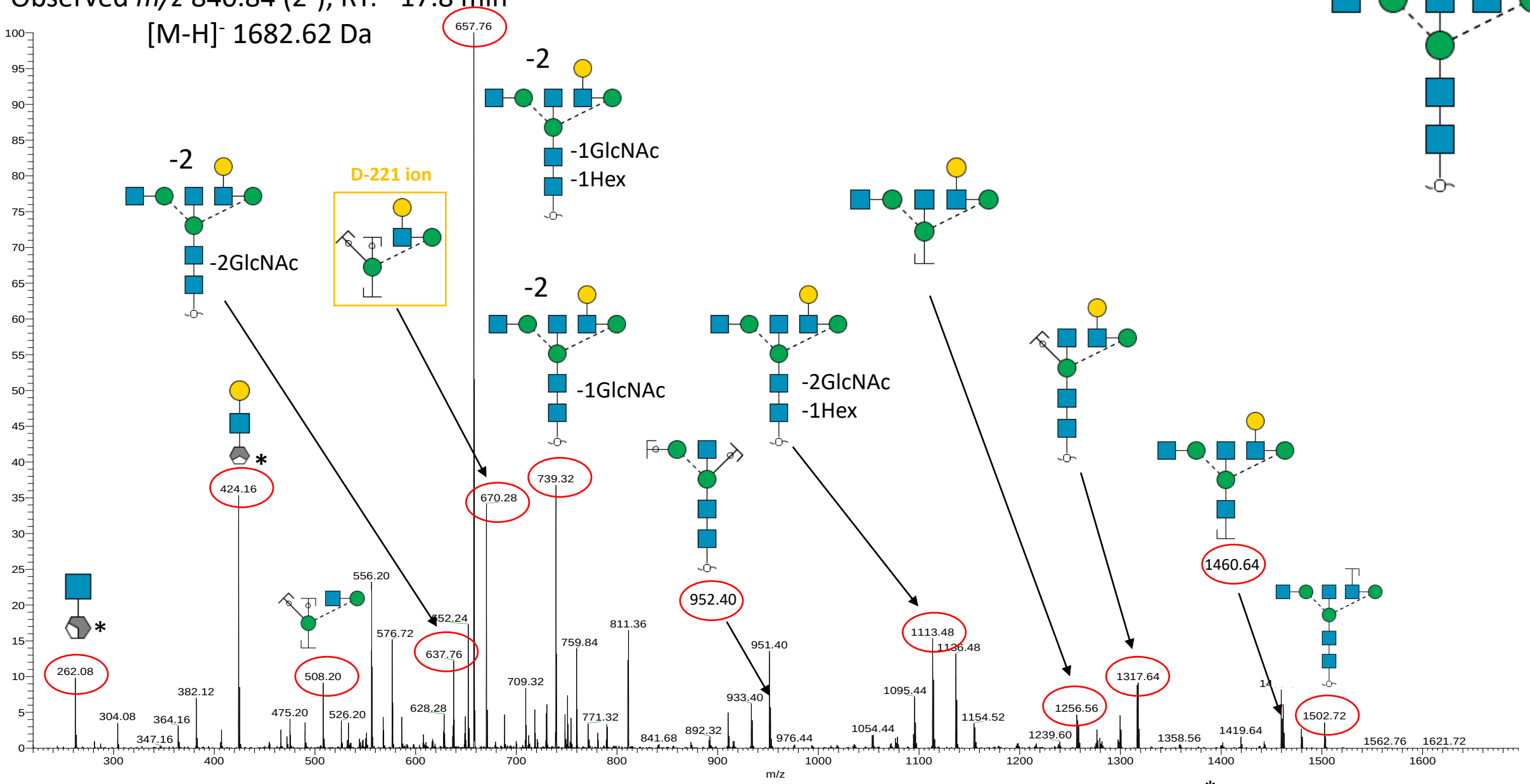


Glycan #27

Observed *m/z* 840.84 (2-), RT: ~17.8 min

$[M-H]^-$ 1682.62 Da

Note: Based on the very early PGC-LC elution time and the abundant D-ion, this structure is assigned as a bisecting GlcNAc.



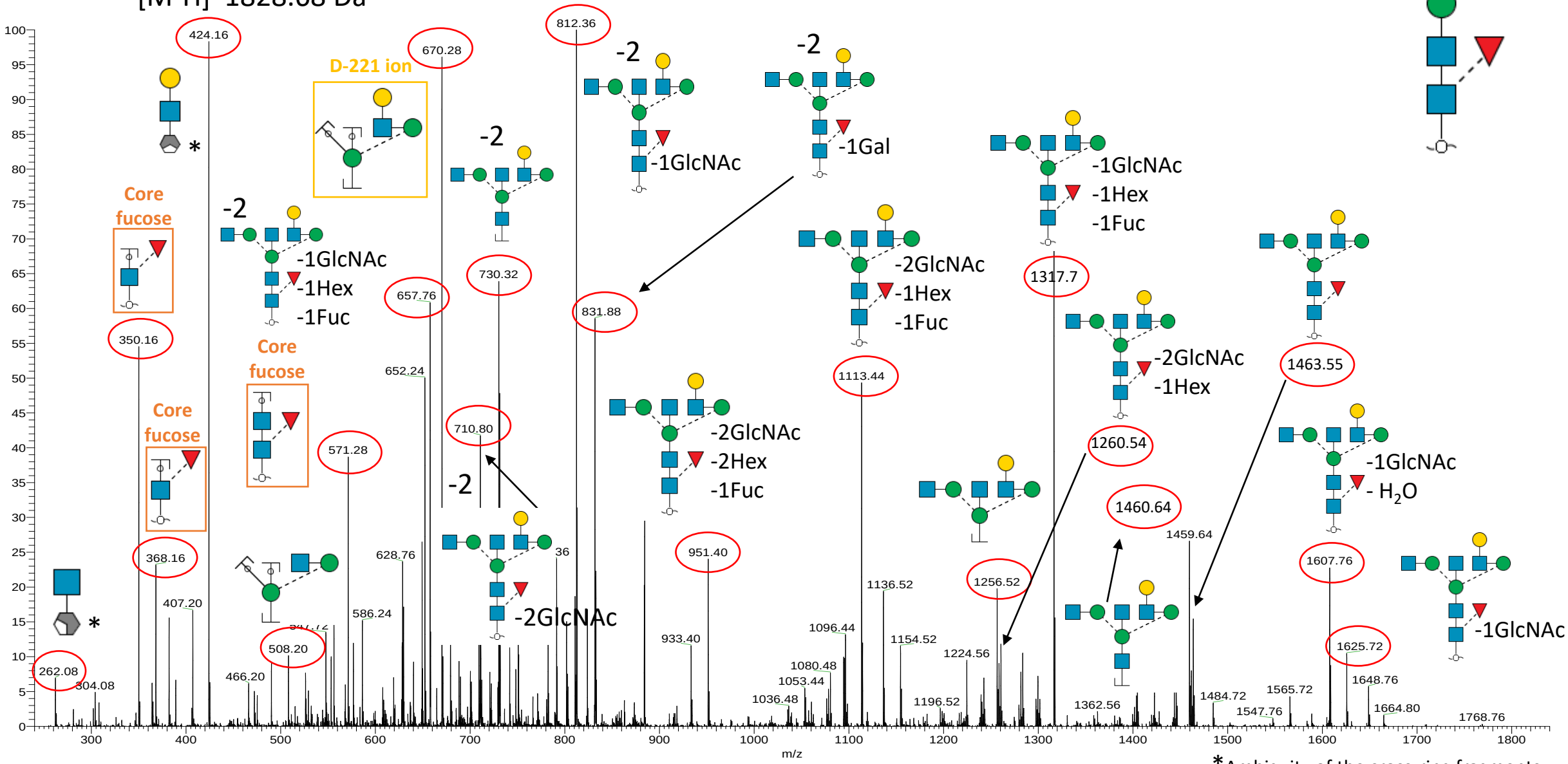
*Ambiguity of the cross-ring fragments

Glycan #28

Observed *m/z* 913.86 (2-), RT: ~20.8 min

$[M-H]^-$ 1828.68 Da

Note: Based on the very early PGC-LC elution time and the abundant D-ion, this structure is assigned as a bisecting GlcNAc.



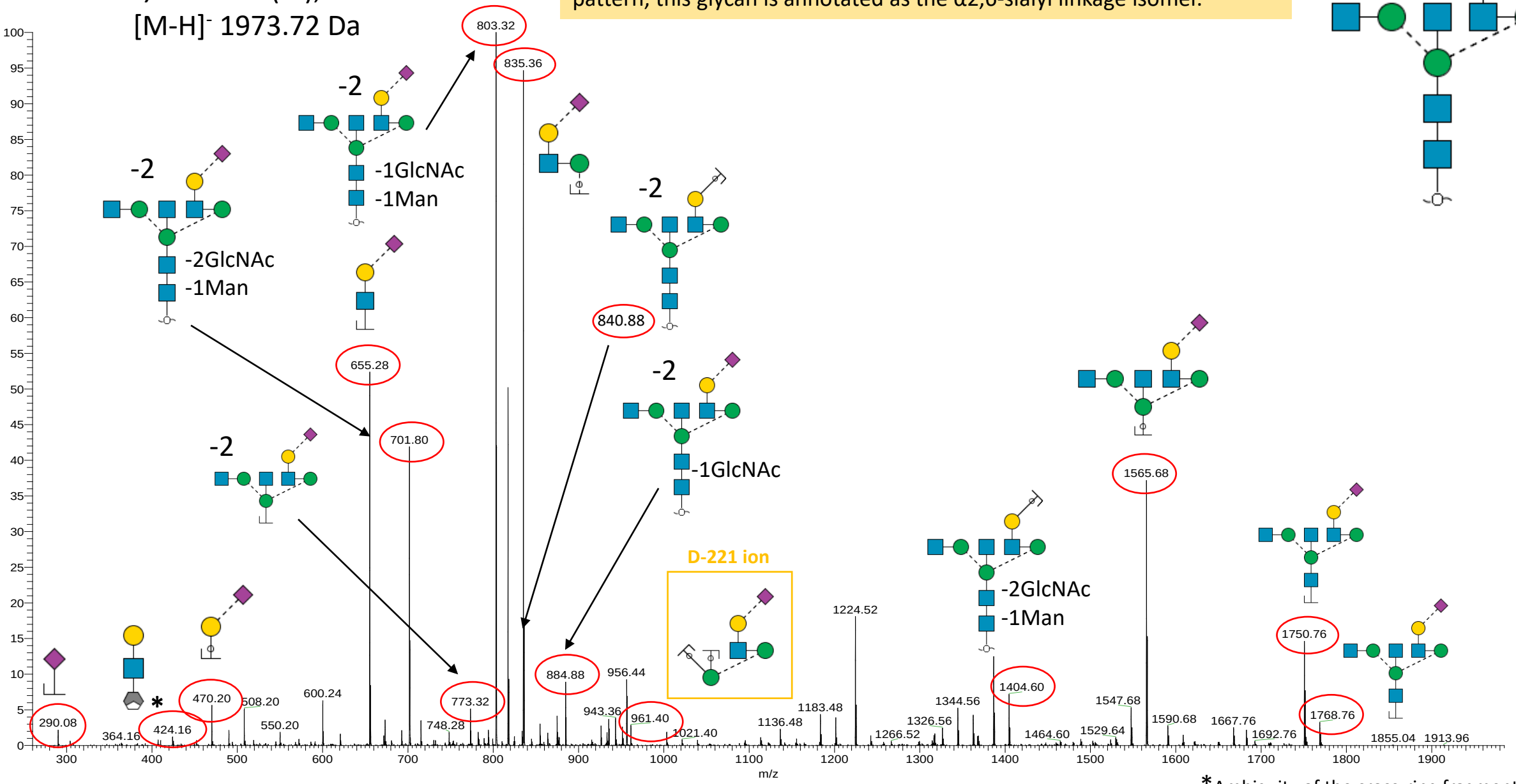
*Ambiguity of the cross-ring fragments

Glycan #29

Observed *m/z* 986.40 (2-), RT: ~18.9 min

$[M-H]^-$ 1973.72 Da

Note: Based on the very early PGC-LC elution time and the D-221 ion, this structure is assigned as a bisecting GlcNAc. Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



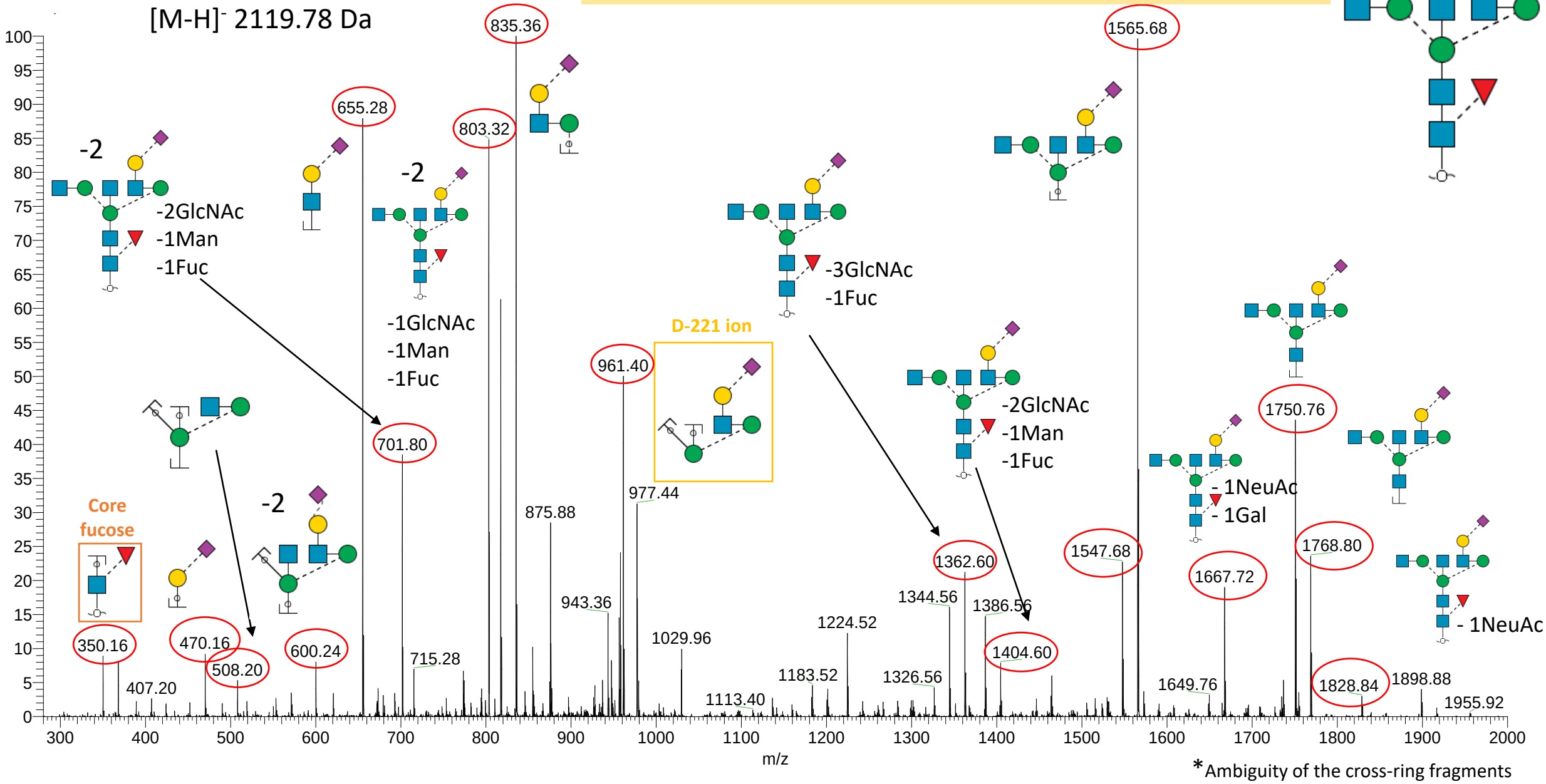
*Ambiguity of the cross-ring fragments

Glycan #30

Observed *m/z* 1059.42 (2-), RT: ~22.5 min

$[M-H]^-$ 2119.78 Da

Note: Based on the very early PGC-LC elution time and the abundant D-221 ion, this structure is assigned as a bisecting GlcNAc. Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



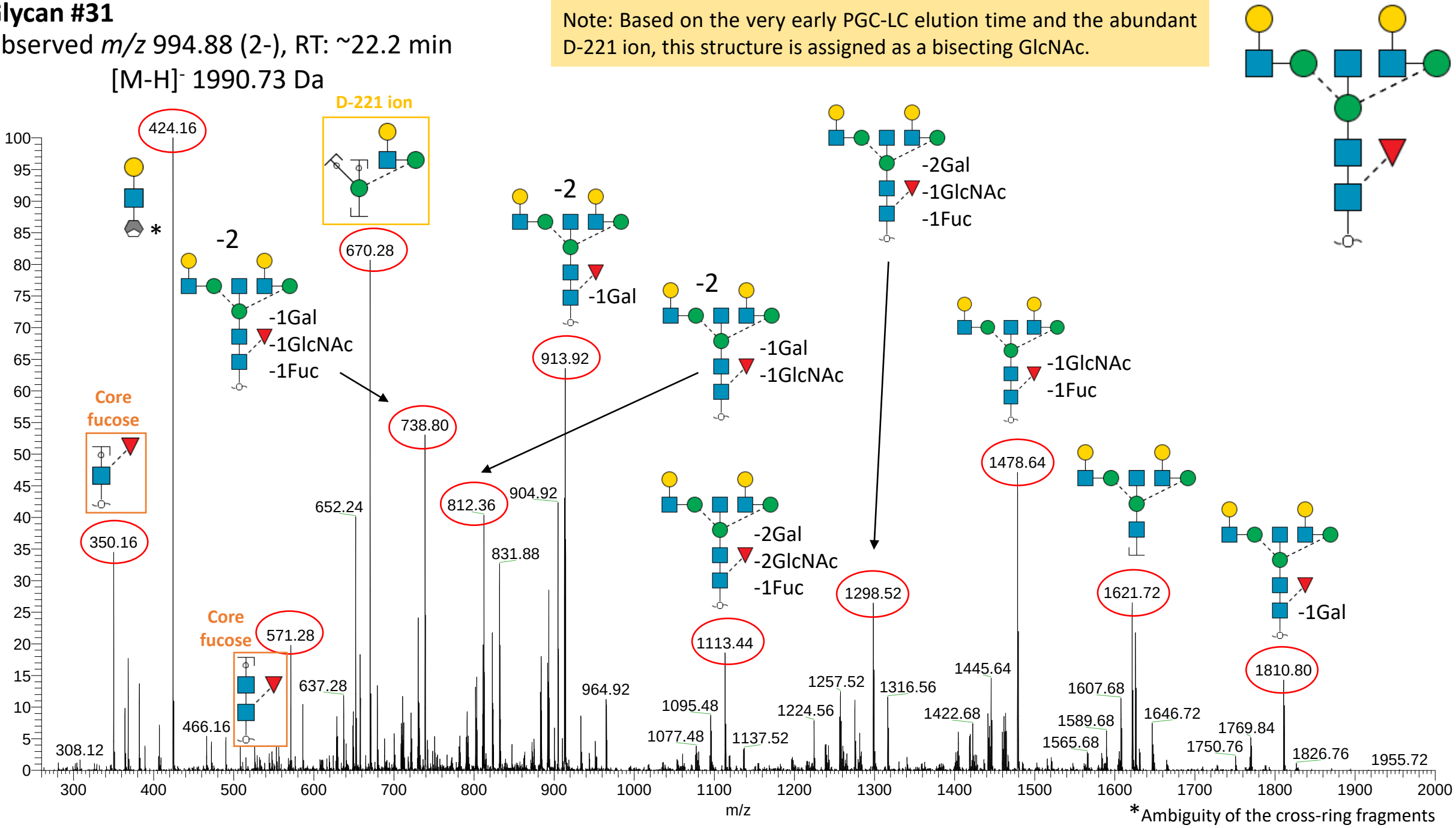
*Ambiguity of the cross-ring fragments

Glycan #31

Observed *m/z* 994.88 (2-), RT: ~22.2 min

$[M-H]^-$ 1990.73 Da

Note: Based on the very early PGC-LC elution time and the abundant D-221 ion, this structure is assigned as a bisecting GlcNAc.

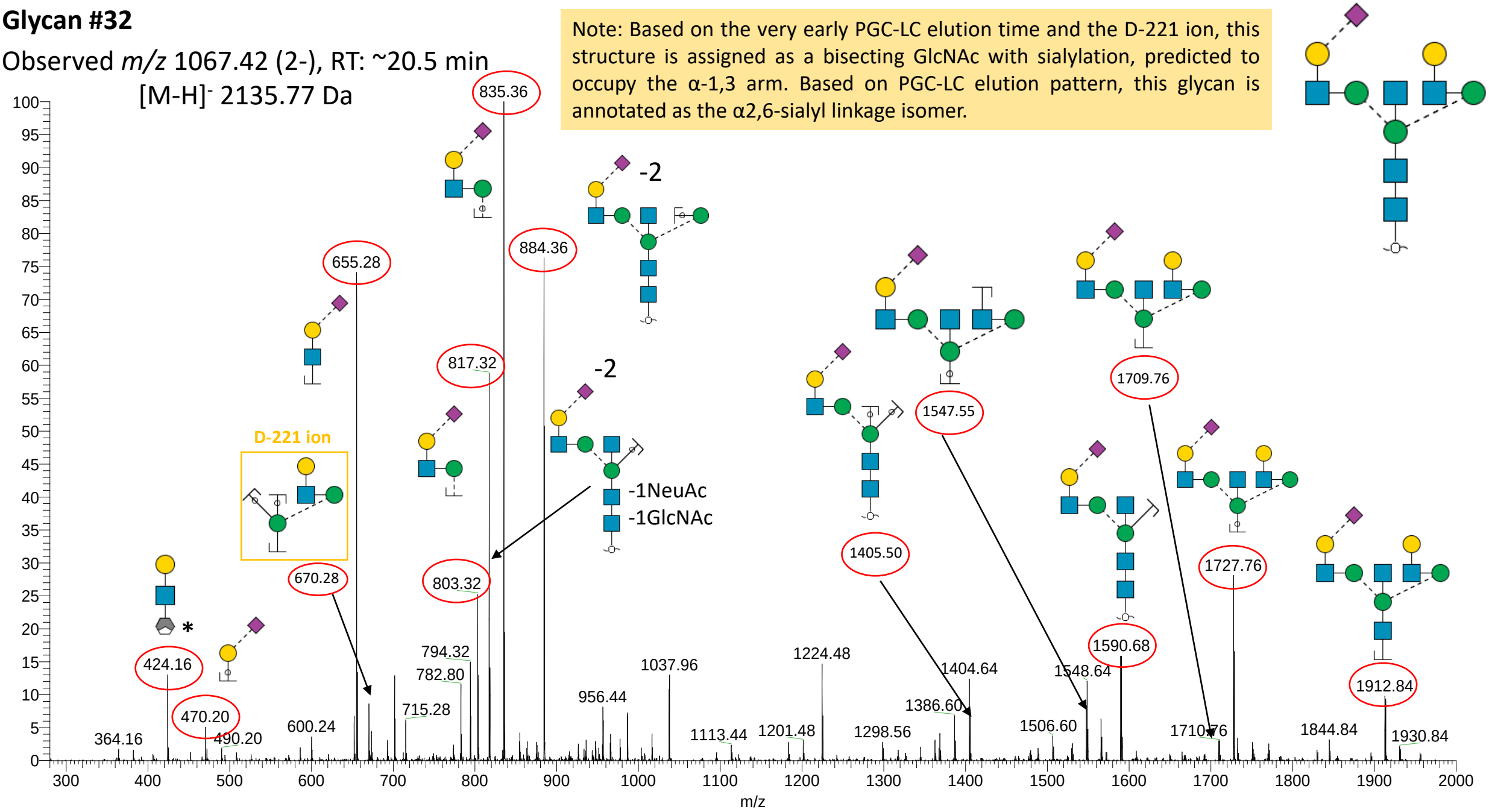


Glycan #32

Observed *m/z* 1067.42 (2-), RT: ~20.5 min

$[M-H]^-$ 2135.77 Da

Note: Based on the very early PGC-LC elution time and the D-221 ion, this structure is assigned as a bisecting GlcNAc with sialylation, predicted to occupy the α-1,3 arm. Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.



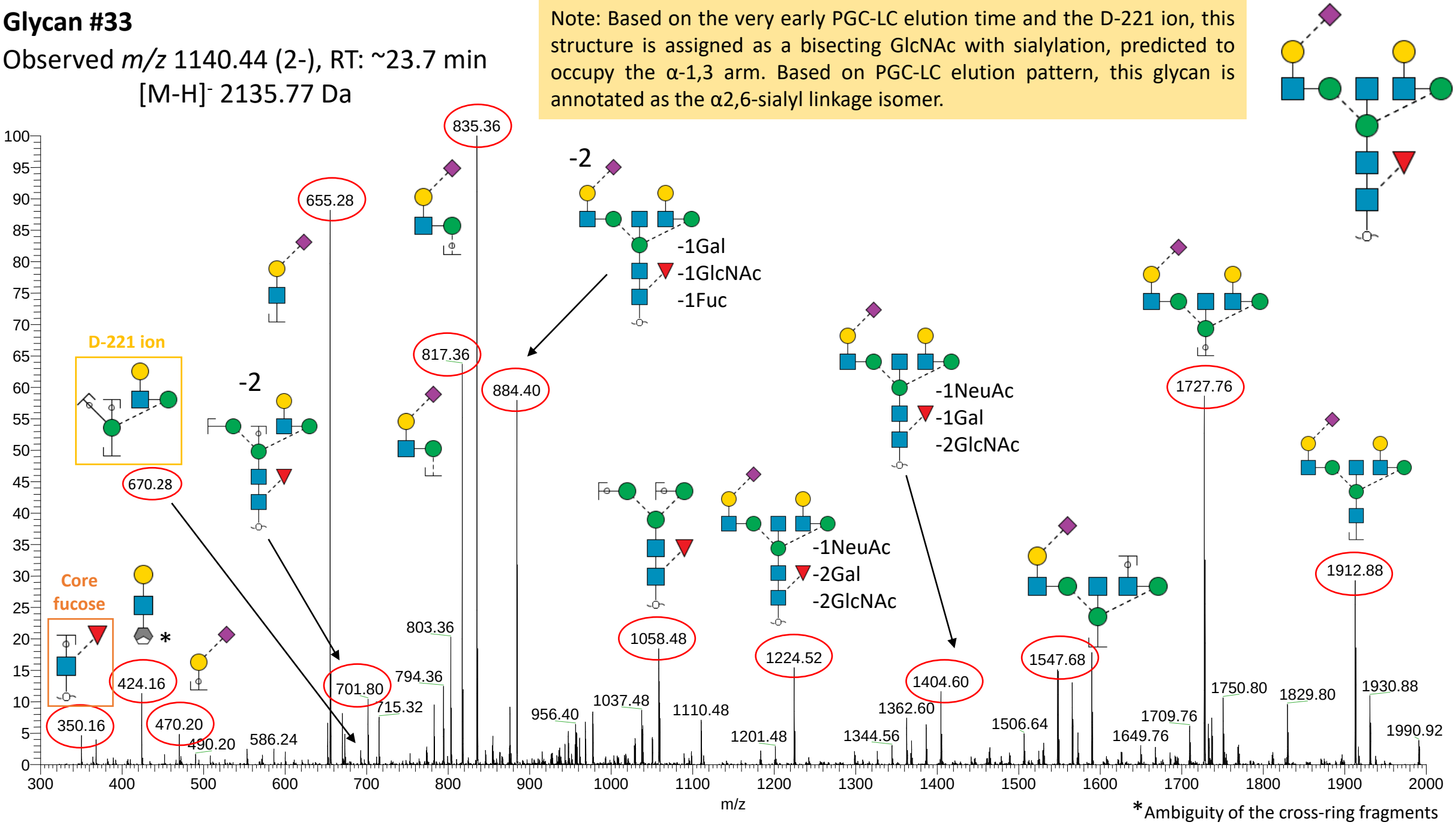
*Ambiguity of the cross-ring fragments

Glycan #33

Observed *m/z* 1140.44 (2-), RT: ~23.7 min

$[M-H]^-$ 2135.77 Da

Note: Based on the very early PGC-LC elution time and the D-221 ion, this structure is assigned as a bisecting GlcNAc with sialylation, predicted to occupy the α-1,3 arm. Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer.

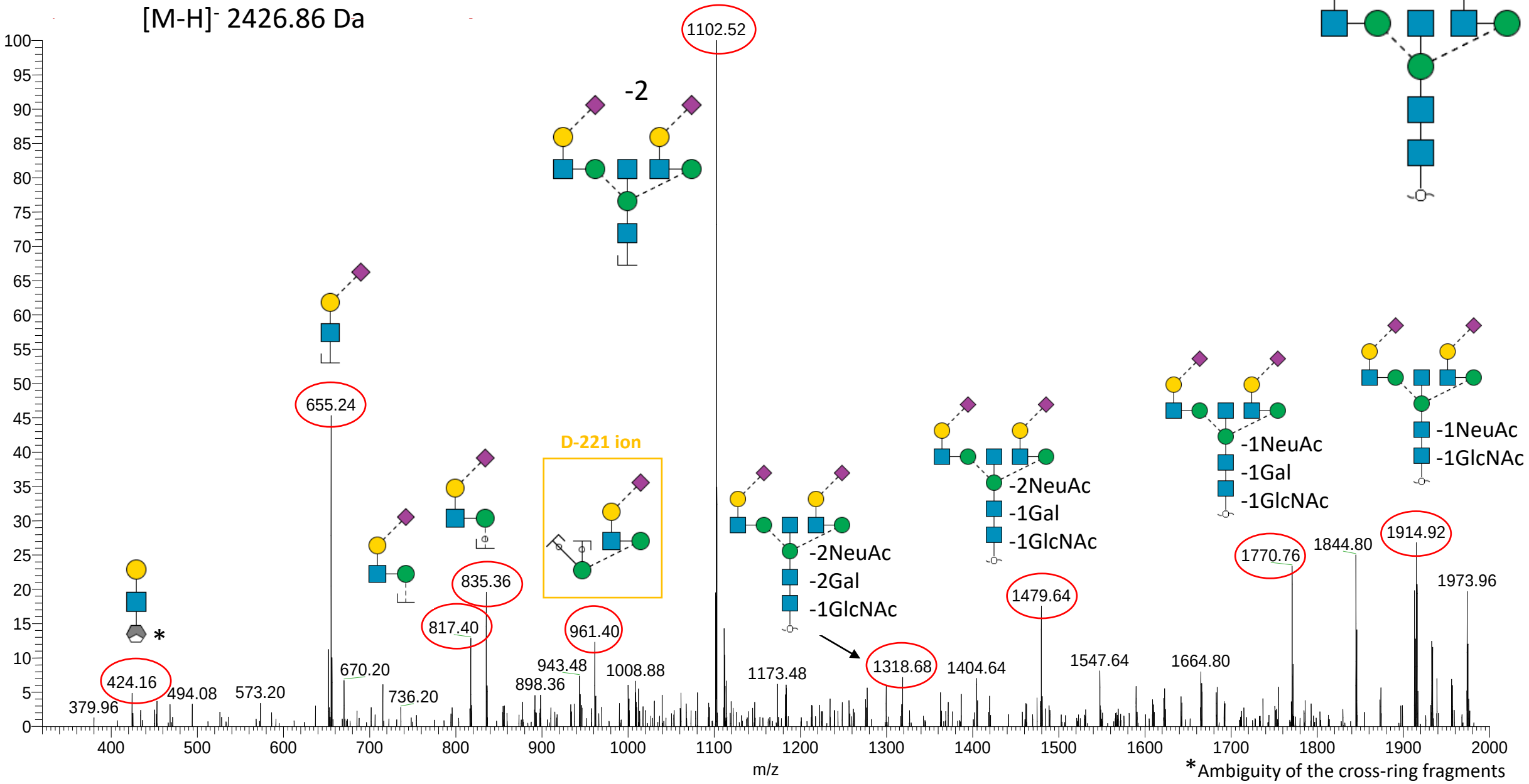


Glycan #34

Observed *m/z* 1212.96 (2-), RT: ~23.0 min

$[M-H]^-$ 2426.86 Da

Note: Based on the very early PGC-LC elution time and the D-221 ion, this structure is assigned as a bisecting GlcNAc. Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-sialyl linkage isomer.

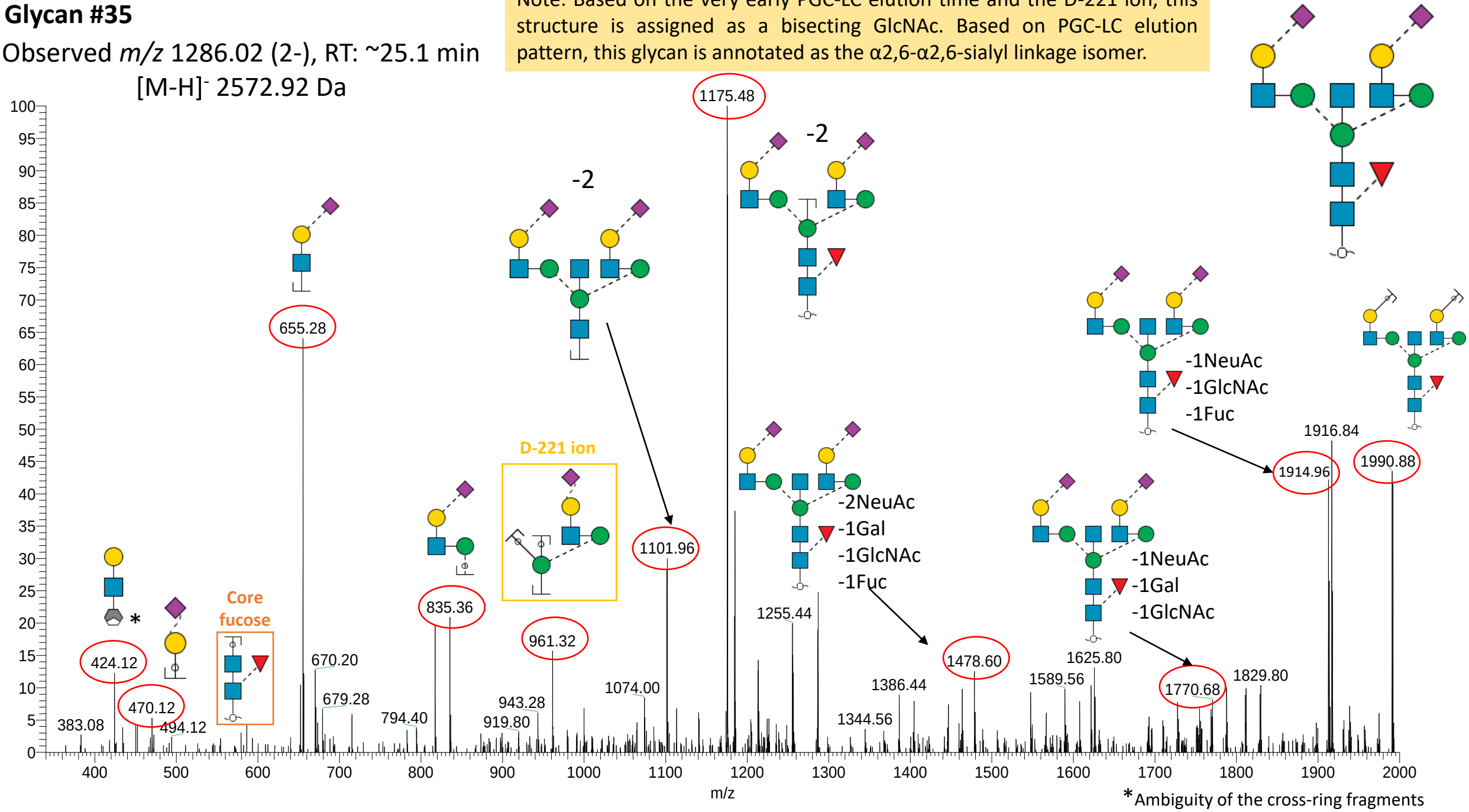


Glycan #35

Observed *m/z* 1286.02 (2-), RT: ~25.1 min

$[M-H]^-$ 2572.92 Da

Note: Based on the very early PGC-LC elution time and the D-221 ion, this structure is assigned as a bisecting GlcNAc. Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-sialyl linkage isomer.

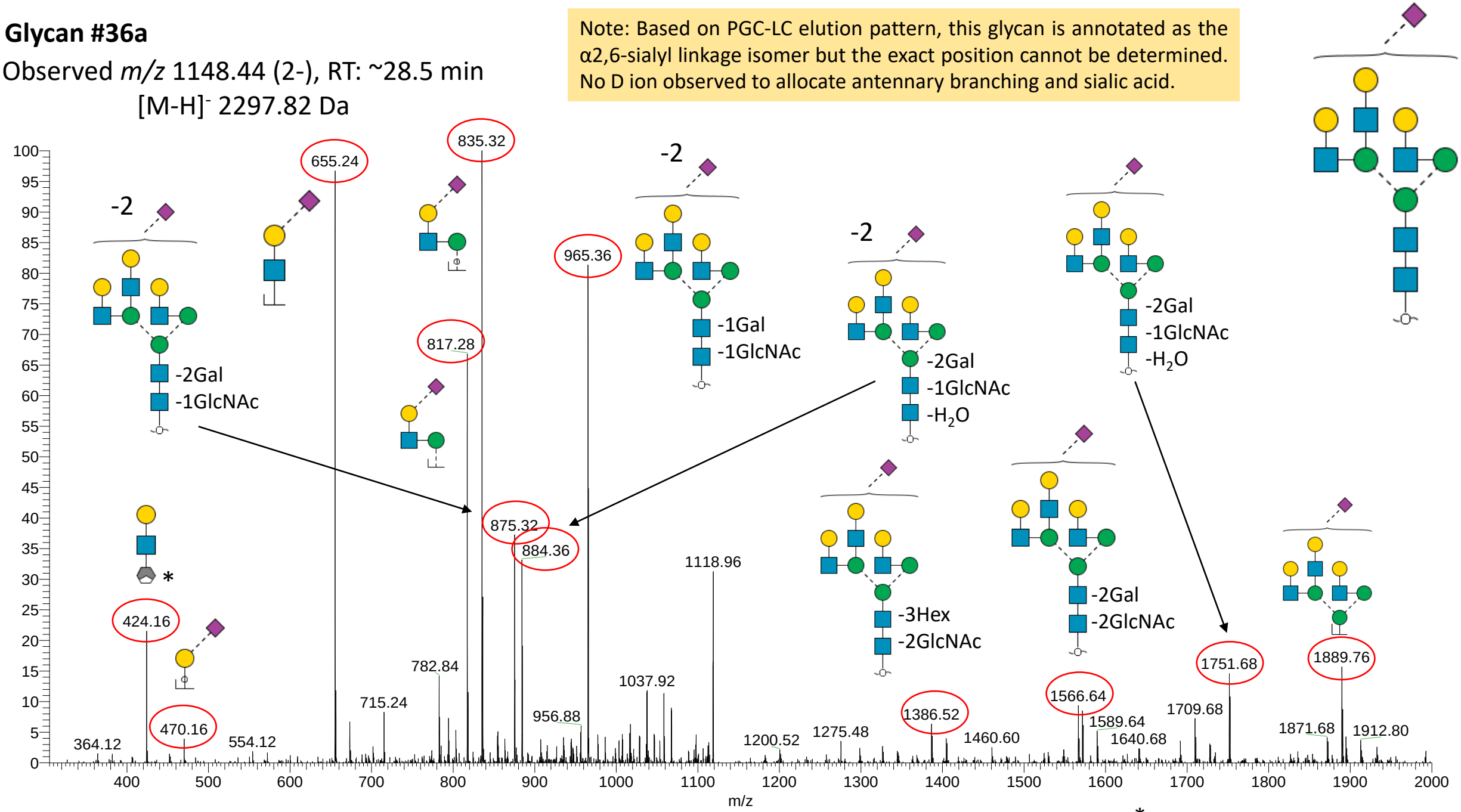


Glycan #36a

Observed *m/z* 1148.44 (2-), RT: ~28.5 min

$[M-H]^-$ 2297.82 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-sialyl linkage isomer but the exact position cannot be determined. No D ion observed to allocate antennary branching and sialic acid.



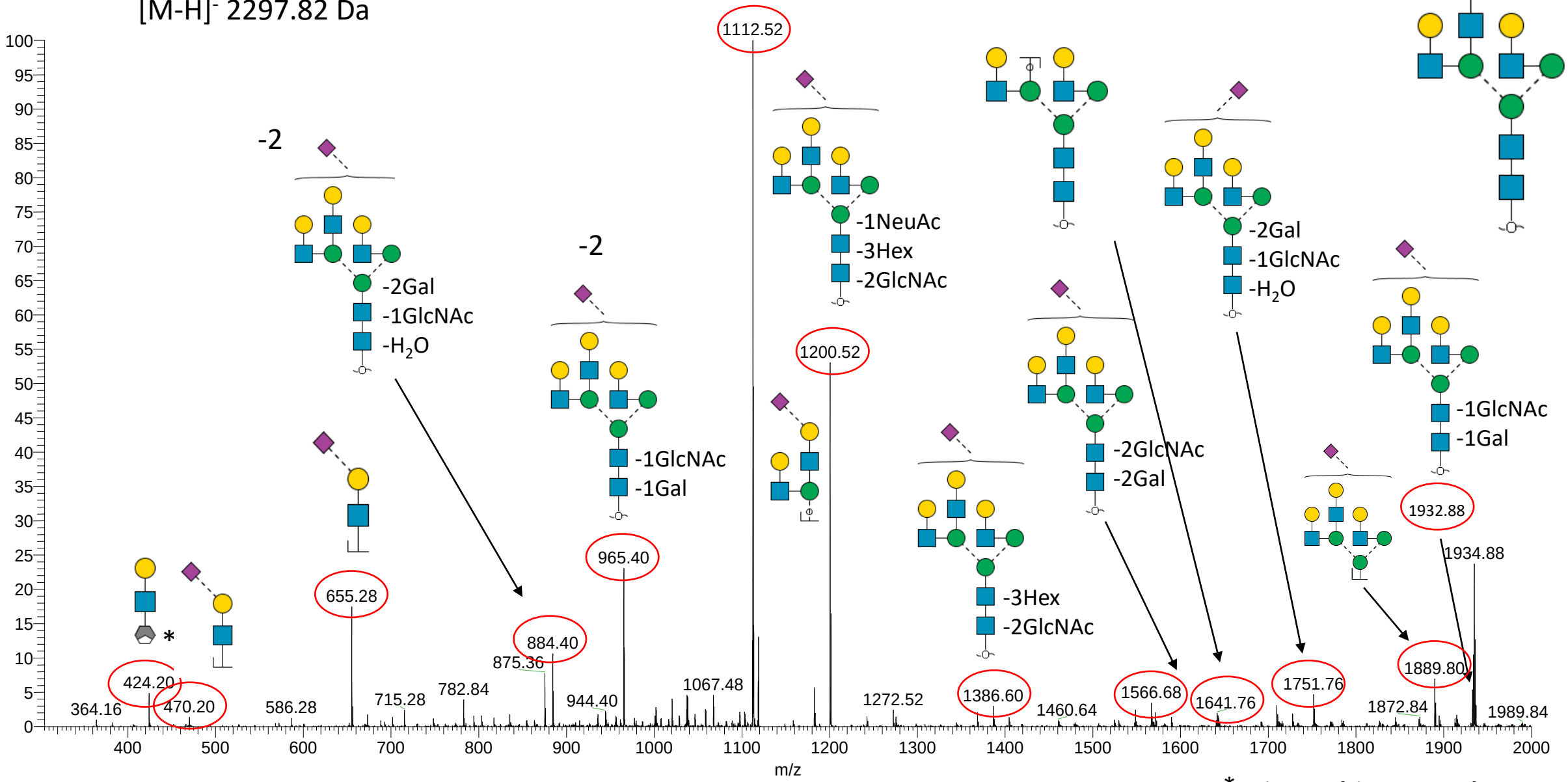
*Ambiguity of the cross-ring fragments

Glycan #36b

Observed *m/z* 1148.44 (2-), RT: ~33.6 min

$[M-H]^-$ 2297.82 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,3-sialyl linkage isomer but the exact position cannot be determined. No D ion observed to allocate antennary branching and sialic acid.



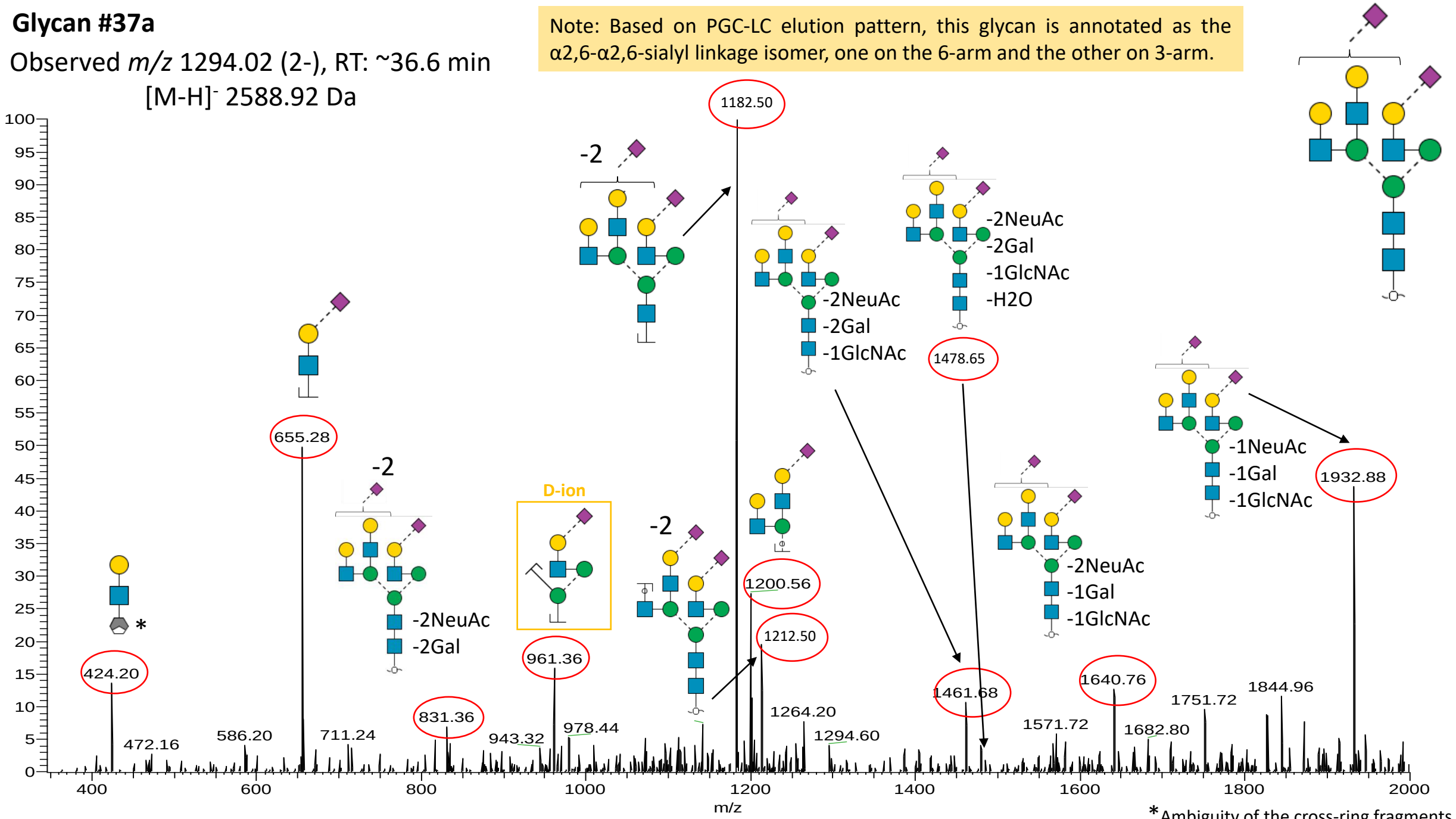
*Ambiguity of the cross-ring fragments

Glycan #37a

Observed *m/z* 1294.02 (2-), RT: ~36.6 min

$[M-H]^-$ 2588.92 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-sialyl linkage isomer, one on the 6-arm and the other on 3-arm.



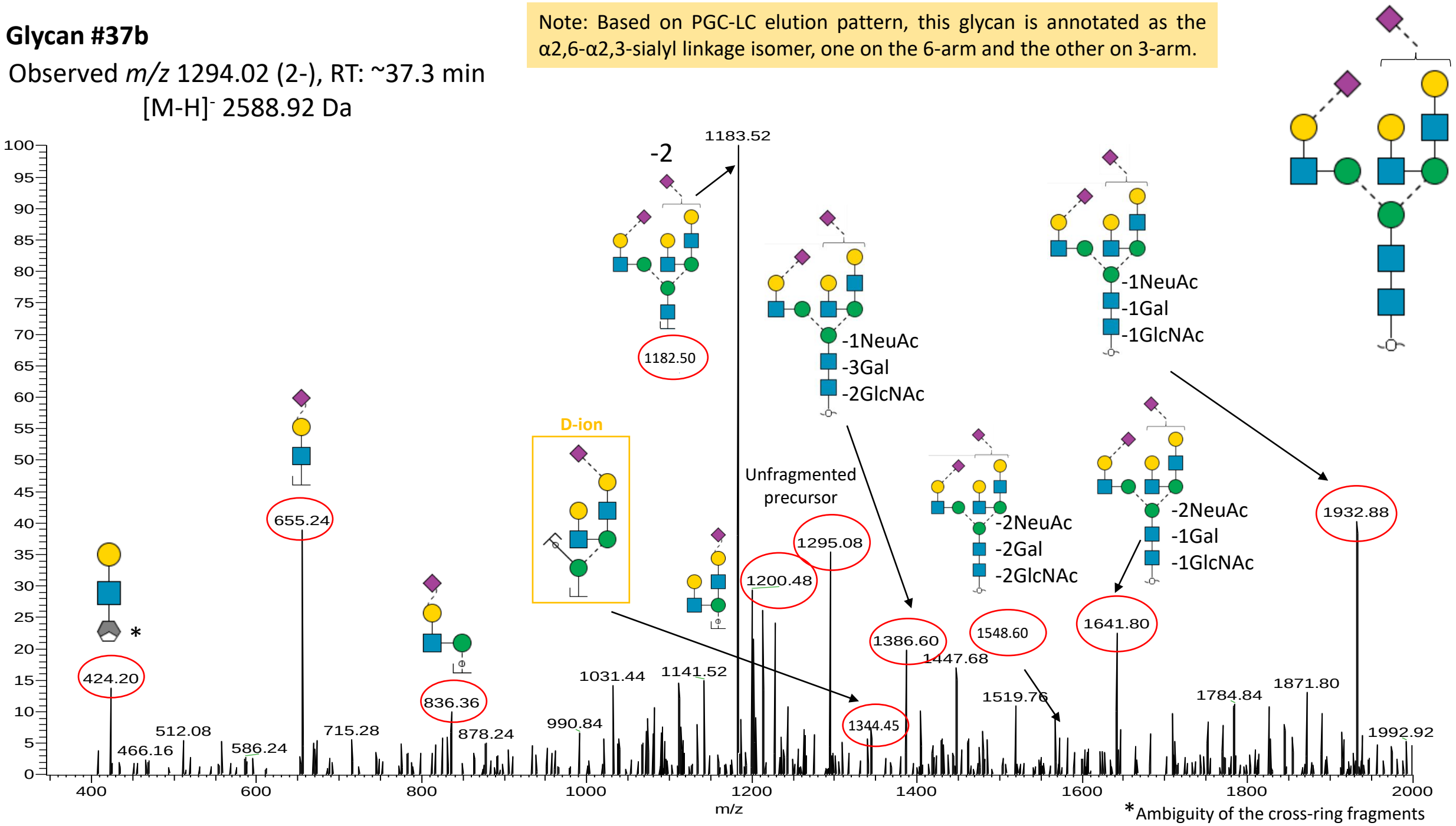
*Ambiguity of the cross-ring fragments

Glycan #37b

Observed *m/z* 1294.02 (2-), RT: ~37.3 min

$[M-H]^-$ 2588.92 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,3-sialyl linkage isomer, one on the 6-arm and the other on 3-arm.

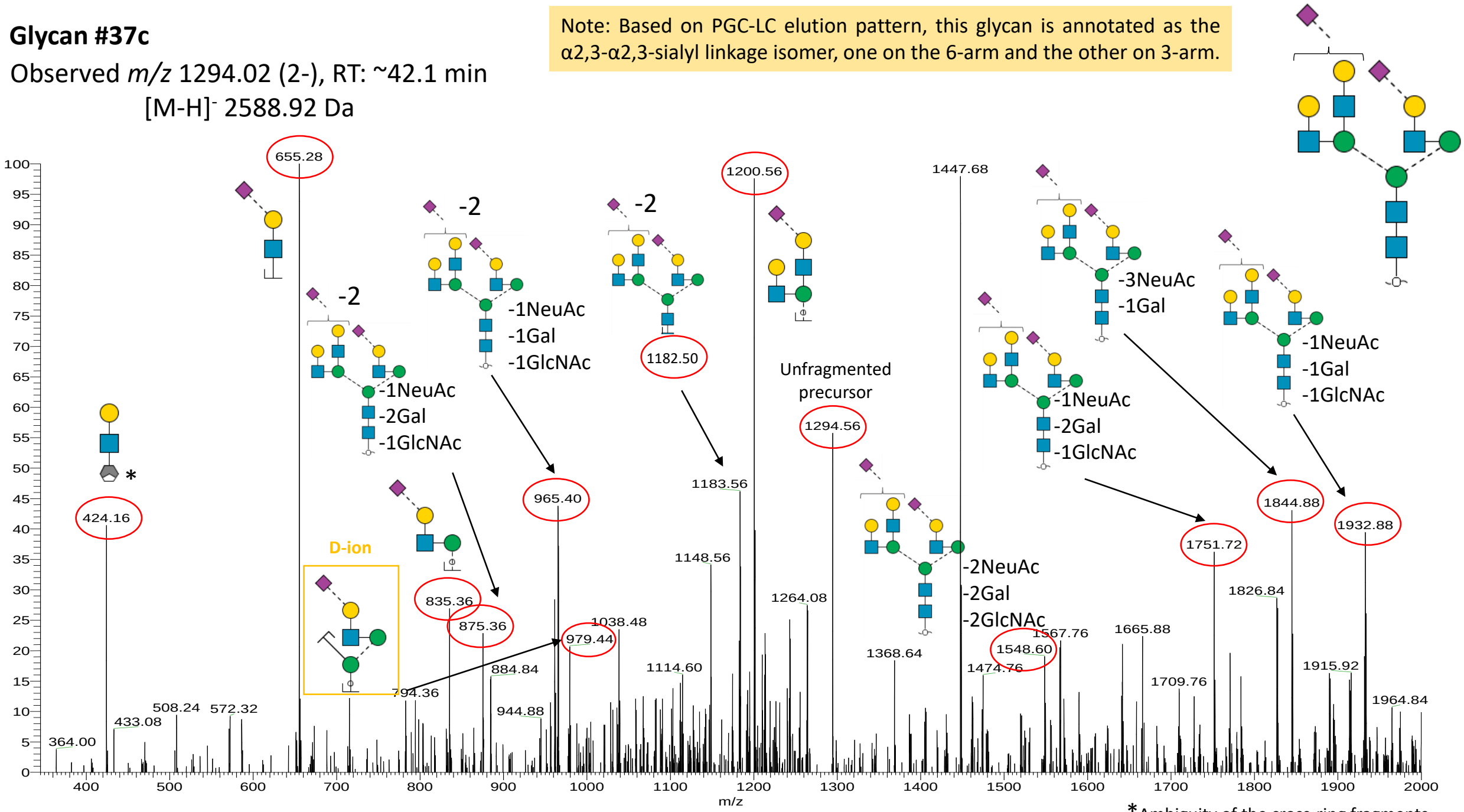


Glycan #37c

Observed *m/z* 1294.02 (2-), RT: ~42.1 min

$[M-H]^-$ 2588.92 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,3-α2,3-sialyl linkage isomer, one on the 6-arm and the other on 3-arm.



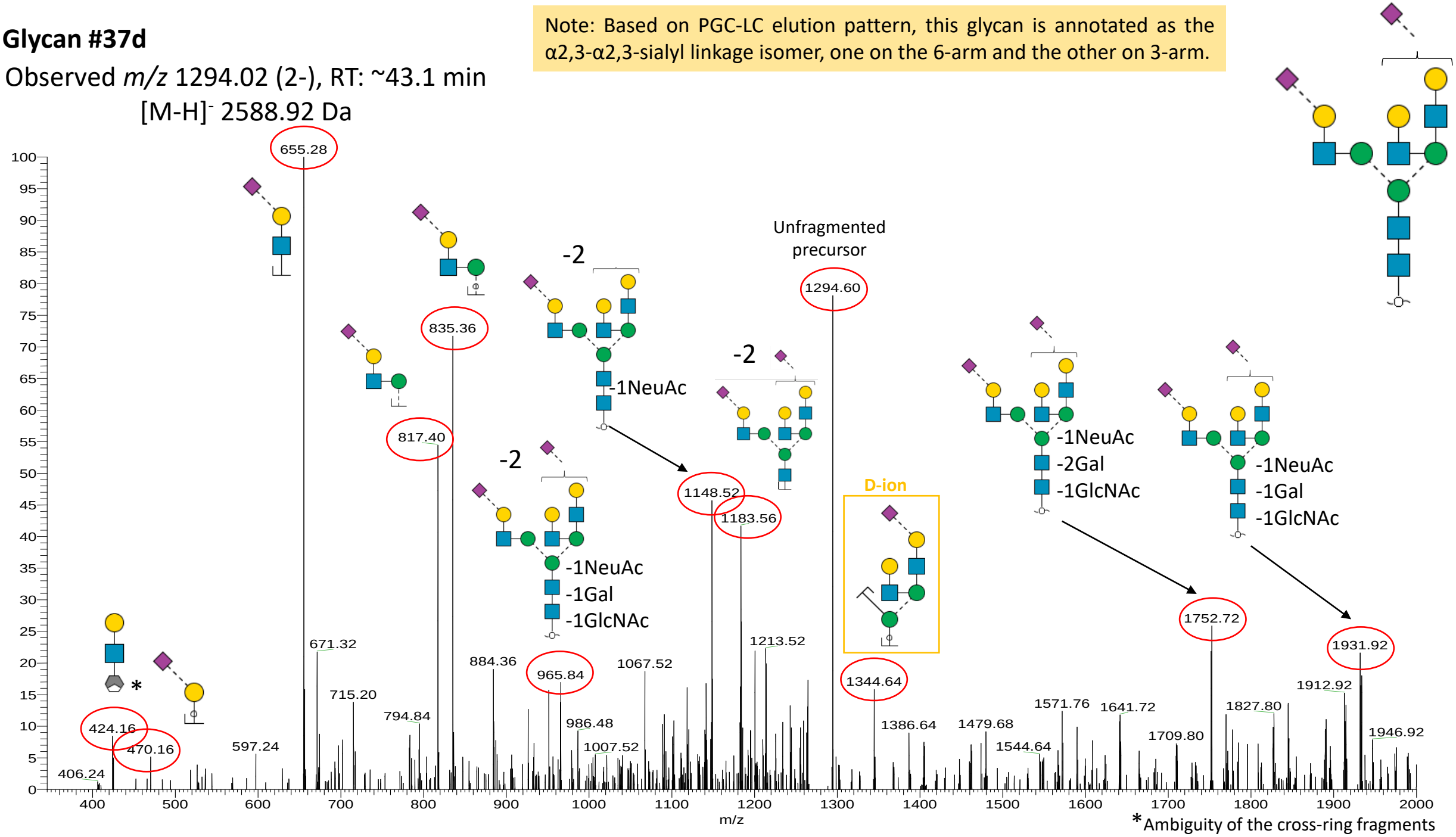
*Ambiguity of the cross-ring fragments

Glycan #37d

Observed *m/z* 1294.02 (2-), RT: ~43.1 min

$[M-H]^-$ 2588.92 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,3-α2,3-sialyl linkage isomer, one on the 6-arm and the other on 3-arm.

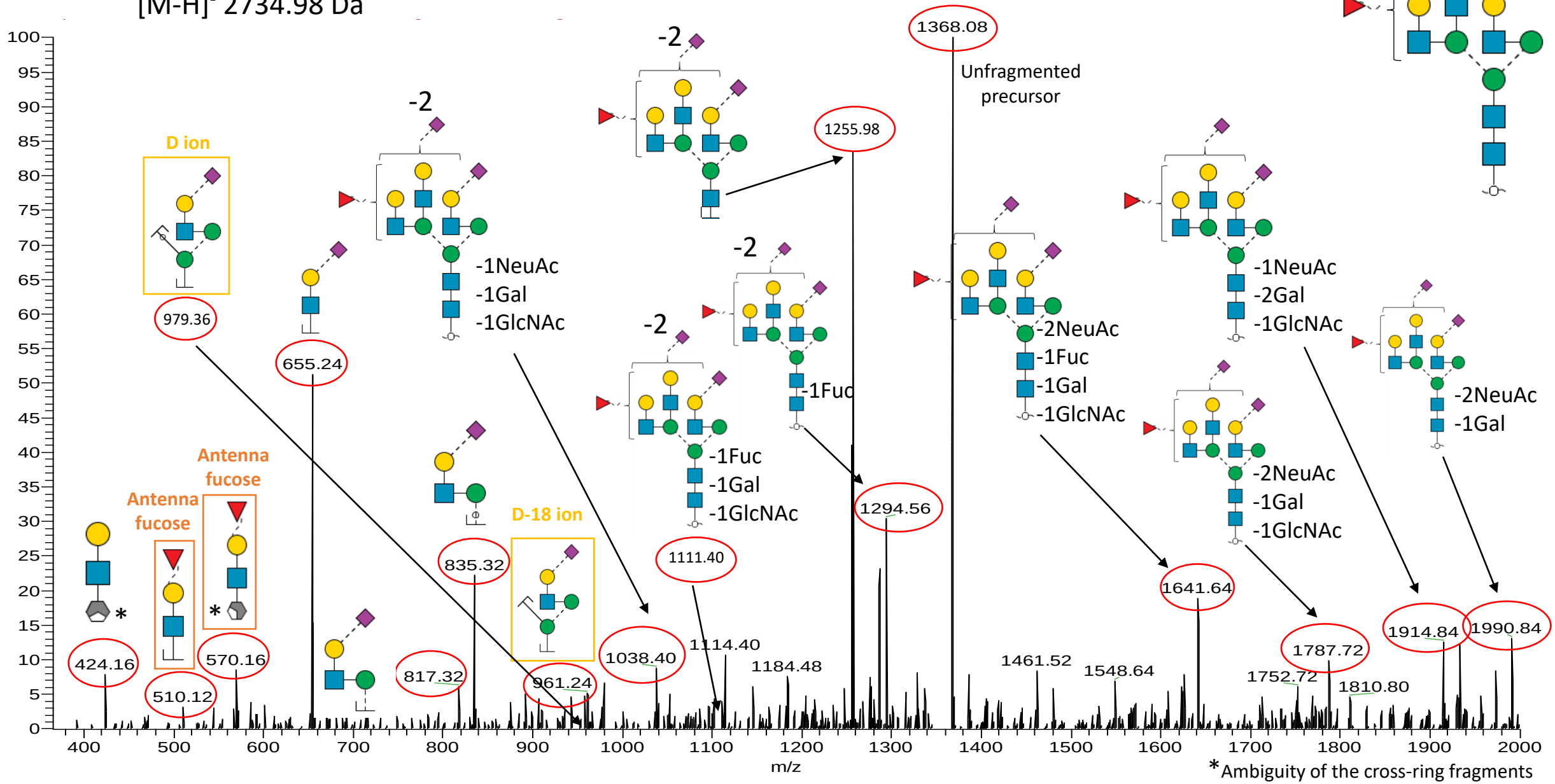


Glycan #38a

Observed *m/z* 1367.06 (2-), RT: ~31.7 min

$[M-H]^-$ 2734.98 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-sialyl linkage isomer but the exact position cannot be determined. Antenna fucose position and linkage also cannot be determined.

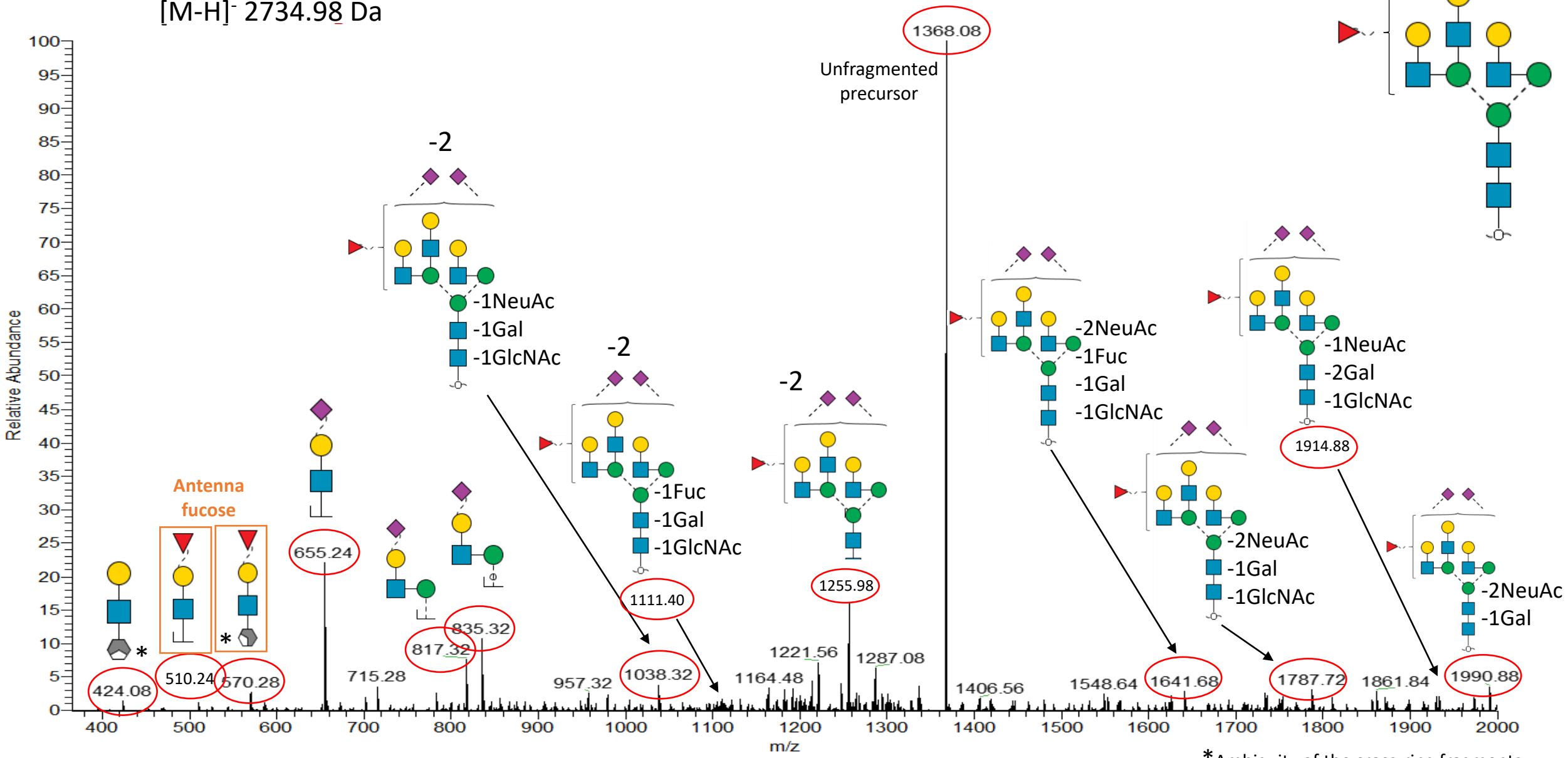


Glycan #38b

Observed *m/z* 1367.06 (2-), RT: ~33.8 min

$[M-H]^-$ 2734.98 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,3-sialyl linkage isomer but the exact position cannot be determined. Antenna fucose position and linkage also cannot be determined.



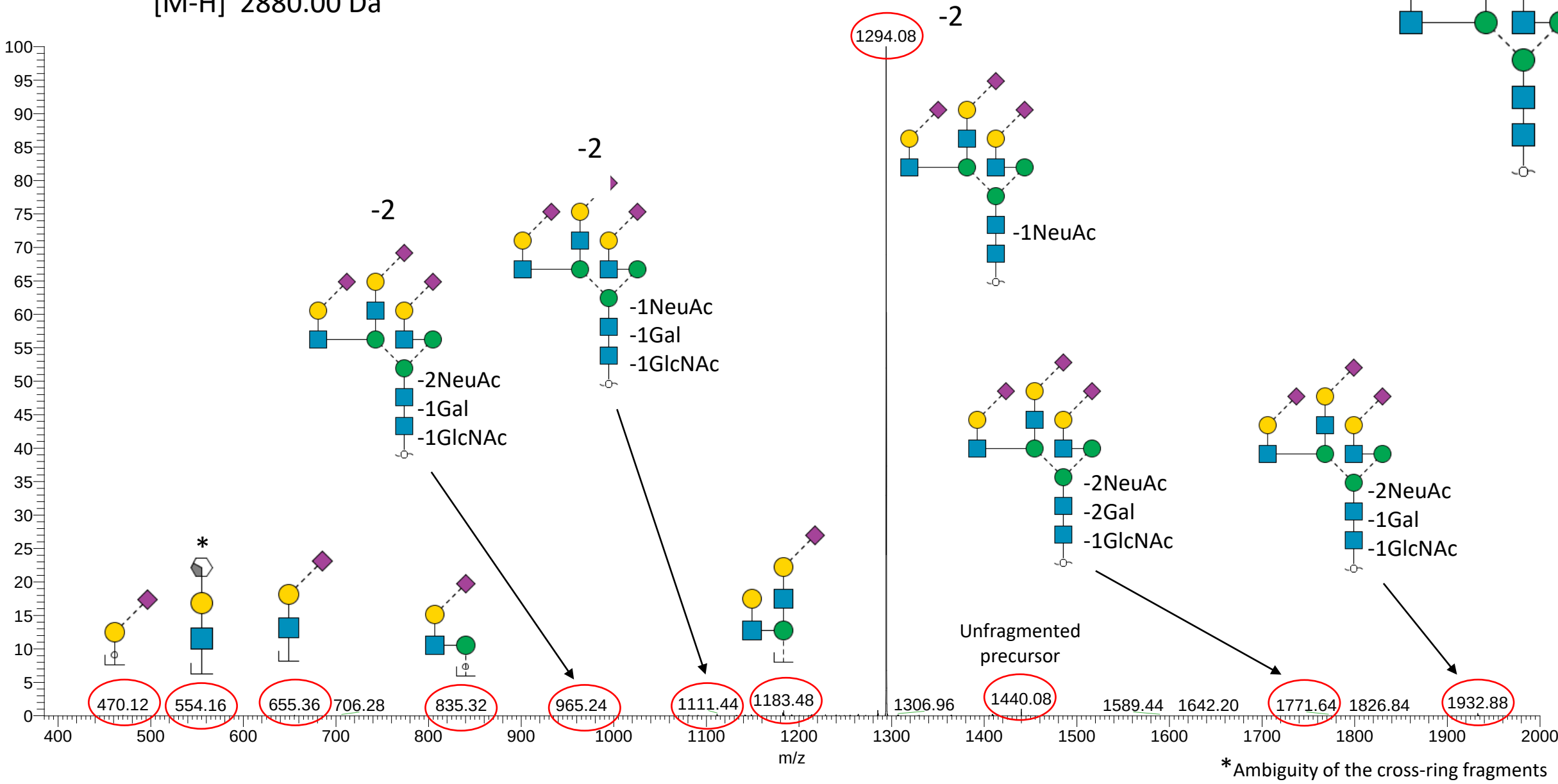
*Ambiguity of the cross-ring fragments

Glycan #39a

Observed *m/z* 1439.56 (2-), RT: ~37.5 min

$[M-H]^-$ 2880.00 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-α2,6-sialyl linkage isomer but the exact antennary branching cannot be determined.

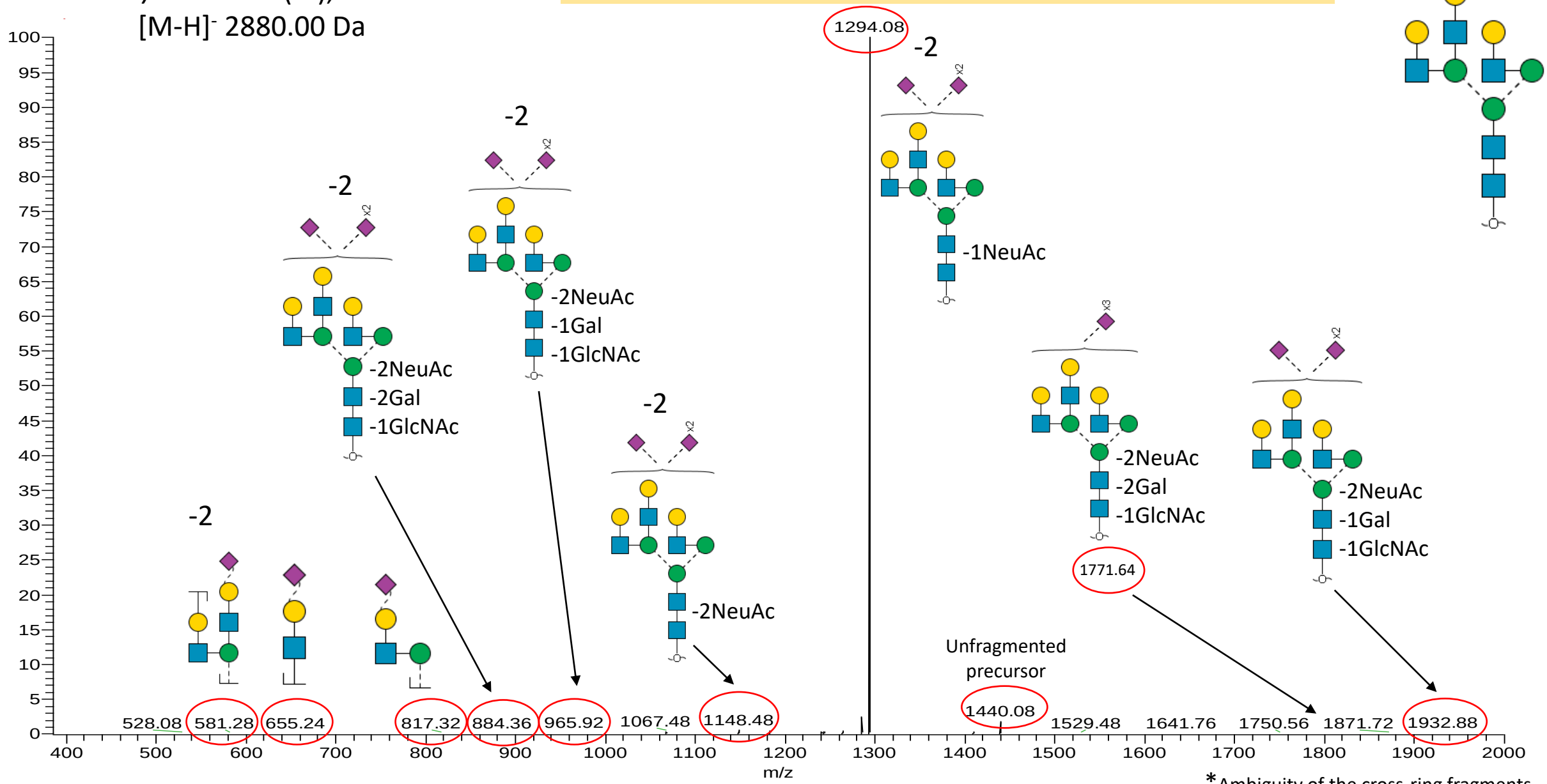


Glycan #39b

Observed *m/z* 1439.56 (2-), RT: ~41.2 min

$[M-H]^-$ 2880.00 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-α2,3-sialyl linkage isomer but the exact position and the antennary branching cannot be determined.



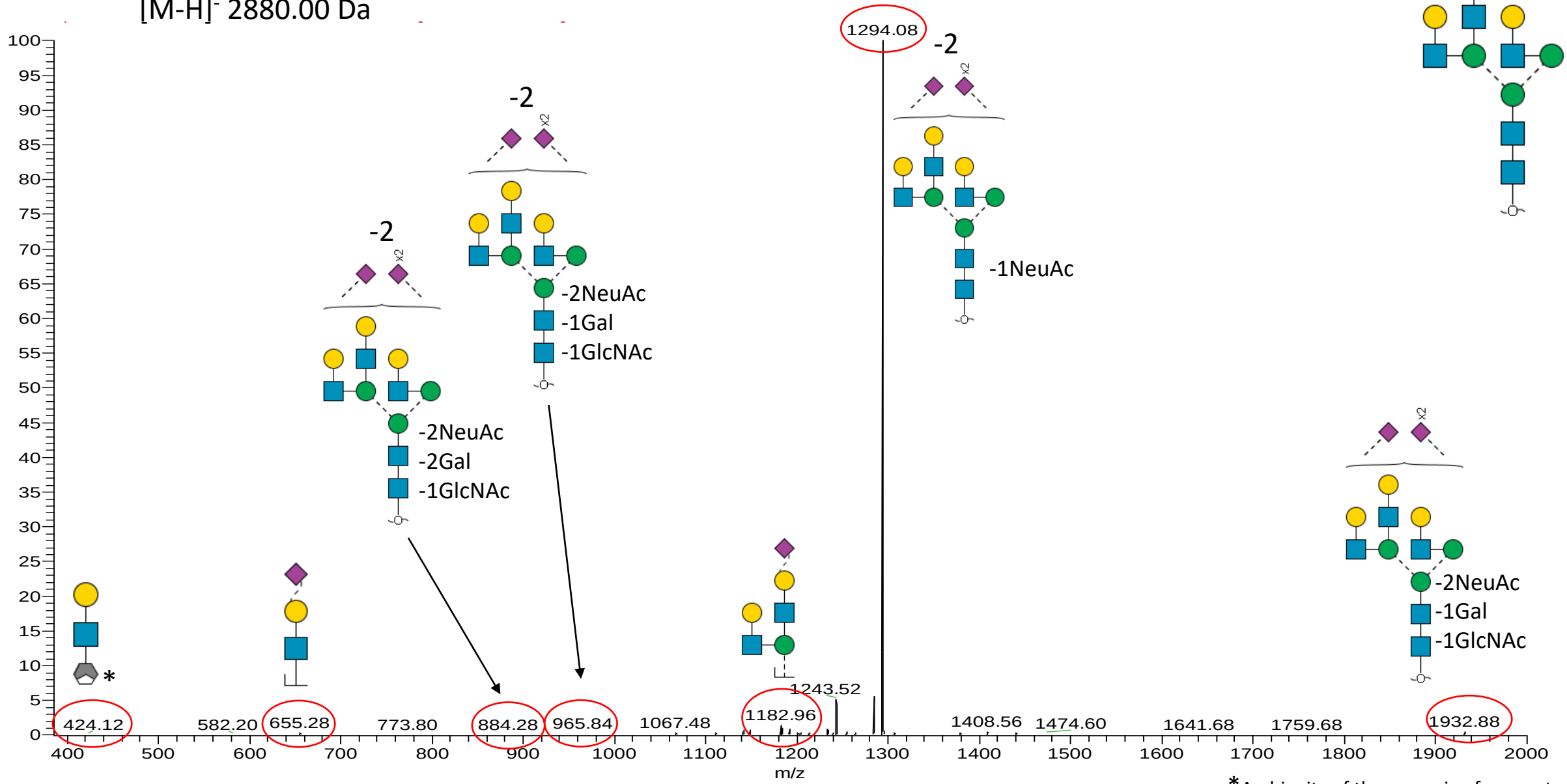
*Ambiguity of the cross-ring fragments

Glycan #39c

Observed *m/z* 1439.56 (2-), RT: ~45.8 min

$[M-H]^-$ 2880.00 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,3-α2,3-sialyl linkage isomer but the exact position and the antennary branching cannot be determined.



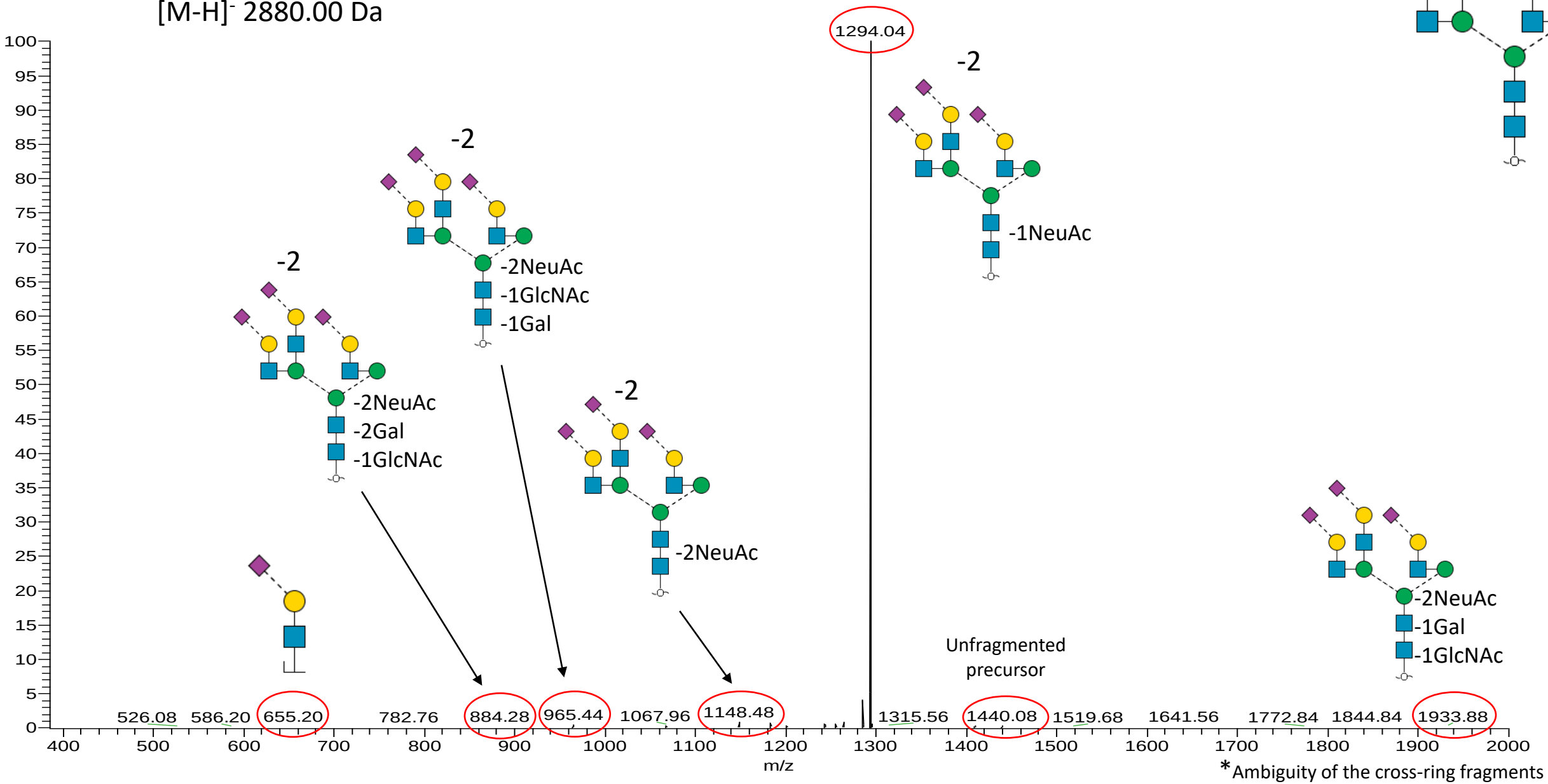
*Ambiguity of the cross-ring fragments

Glycan #39d

Observed *m/z* 1439.50 (2-), RT: ~49.7 min

$[M-H]^-$ 2880.00 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,3-α2,3-α2,3-sialyl linkage isomer but the antennary branching cannot be determined.

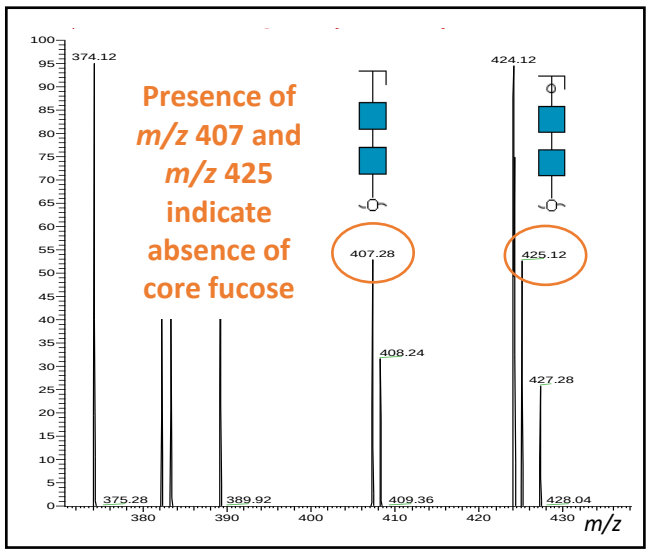


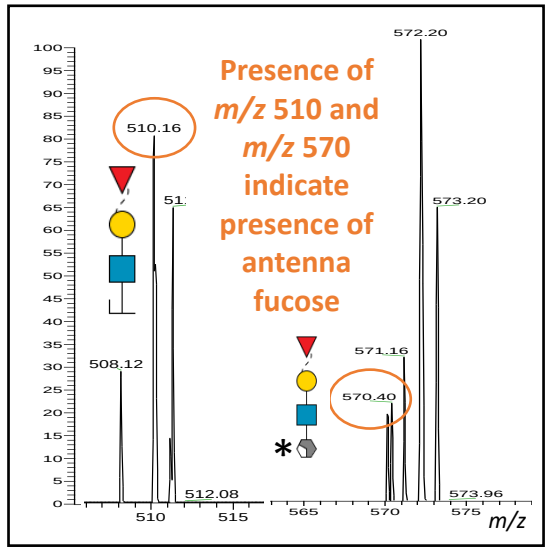
Glycan #40a

Observed *m/z* 1008.0 (3-), RT: ~32.3 min

$[M-H]^-$ 3026.06 Da, $[M-2H]^{2-}$ 1512.5 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-α2,6-sialyl linkage isomer but the antennary branching cannot be determined. Antenna fucose position and linkage also cannot be determined.





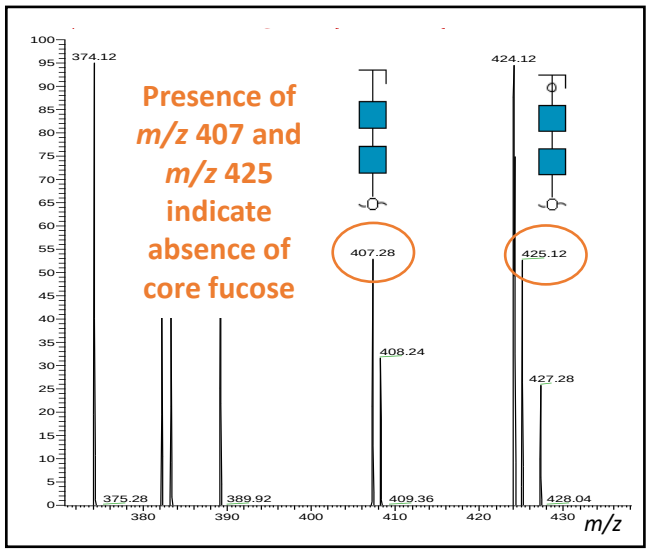
*Ambiguity of the cross-ring fragments

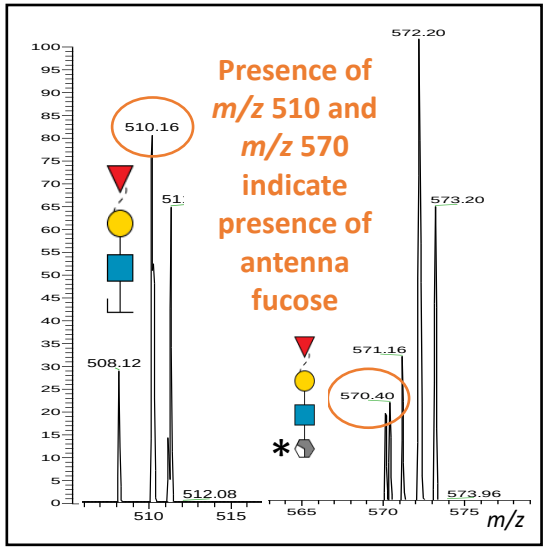
Glycan #40b

Observed *m/z* 1008.0 (3-), RT: ~39.3 min

$[M-H]^-$ 3026.06 Da , $[M-2H]^{2-}$ 1512.5 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,6-α2,3-sialyl linkage isomer but the exact position and antennary branching cannot be determined. Antenna fucose position and linkage also cannot be determined.



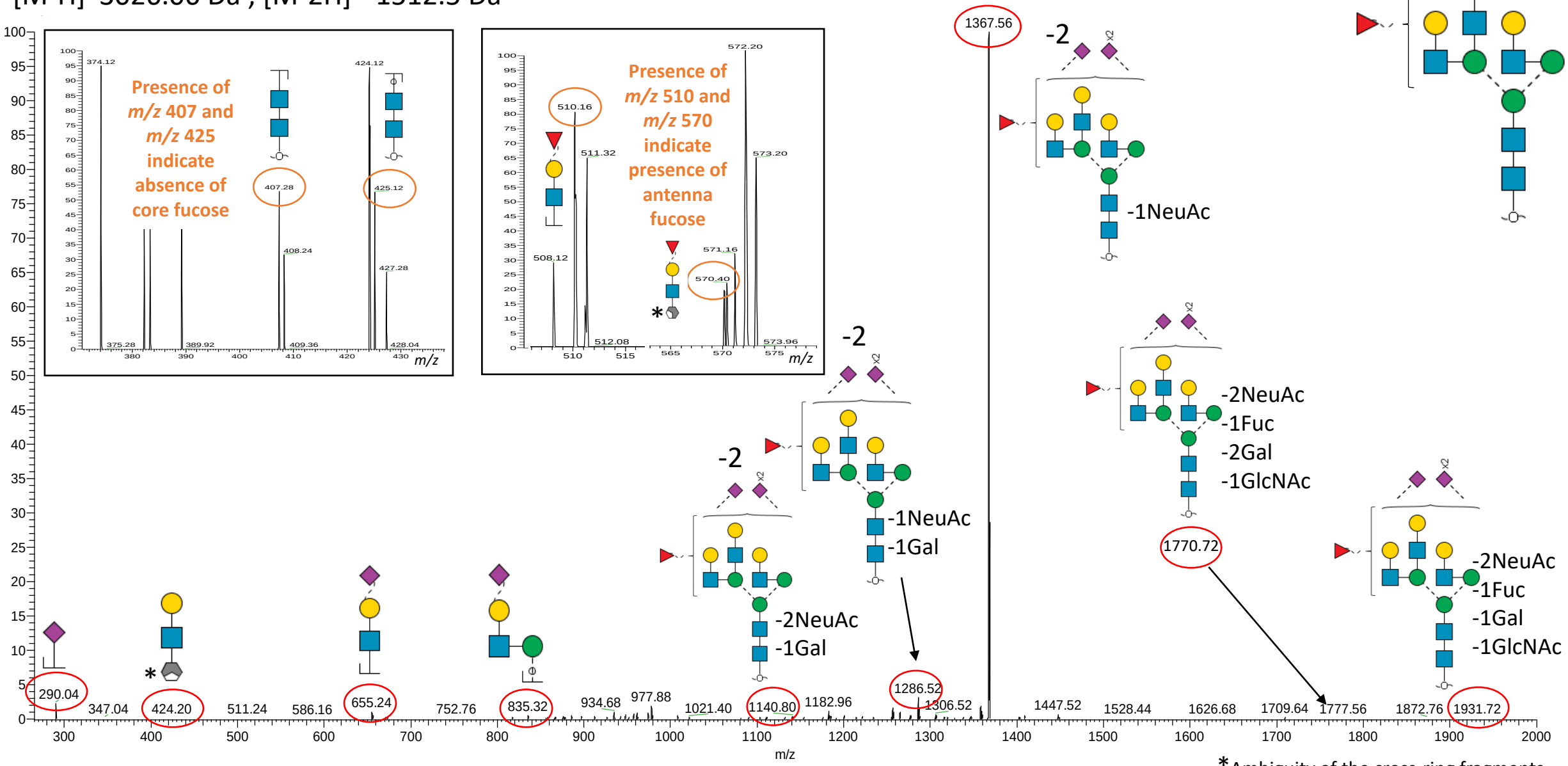


Glycan #40c

Observed *m/z* 1008.0 (3-), RT: ~42.2 min

$[M-H]^-$ 3026.06 Da , $[M-2H]^{2-}$ 1512.5 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,6-α2,3-α2,3-sialyl linkage isomer but the exact position and antennary branching cannot be determined. Antenna fucose position and linkage also cannot be determined.

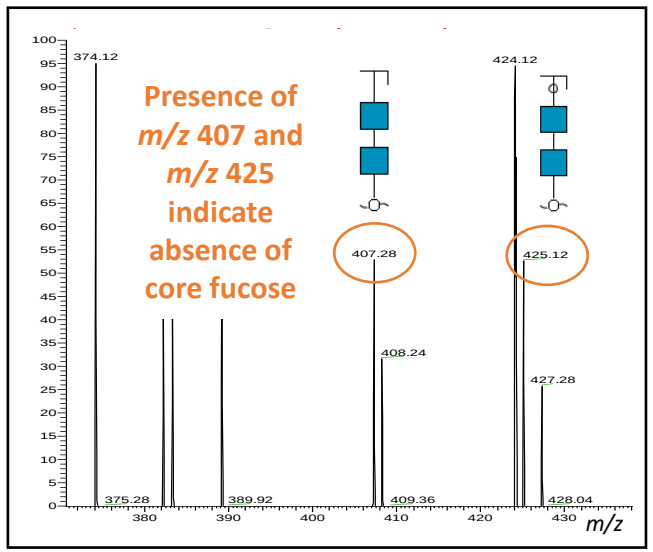


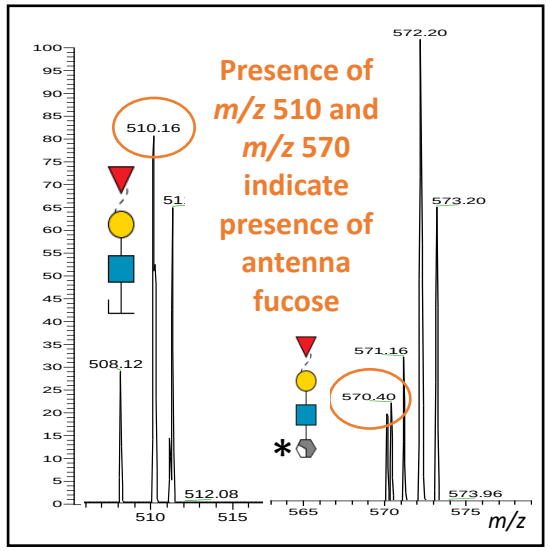
Glycan #40d

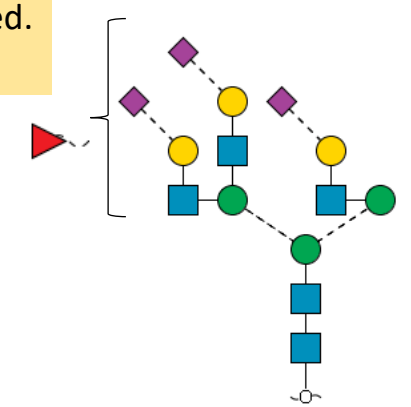
Observed *m/z* 1008.0 (3-), RT: ~53.0 min

$[M-H]^-$ 3026.06 Da , $[M-2H]^{2-}$ 1512.5 Da

Note: Based on PGC-LC elution pattern, this glycan is annotated as the α2,3-α2,3-α2,3-sialyl linkage isomer but the antennary branching cannot be determined. Antenna fucose position and linkage also cannot be determined.







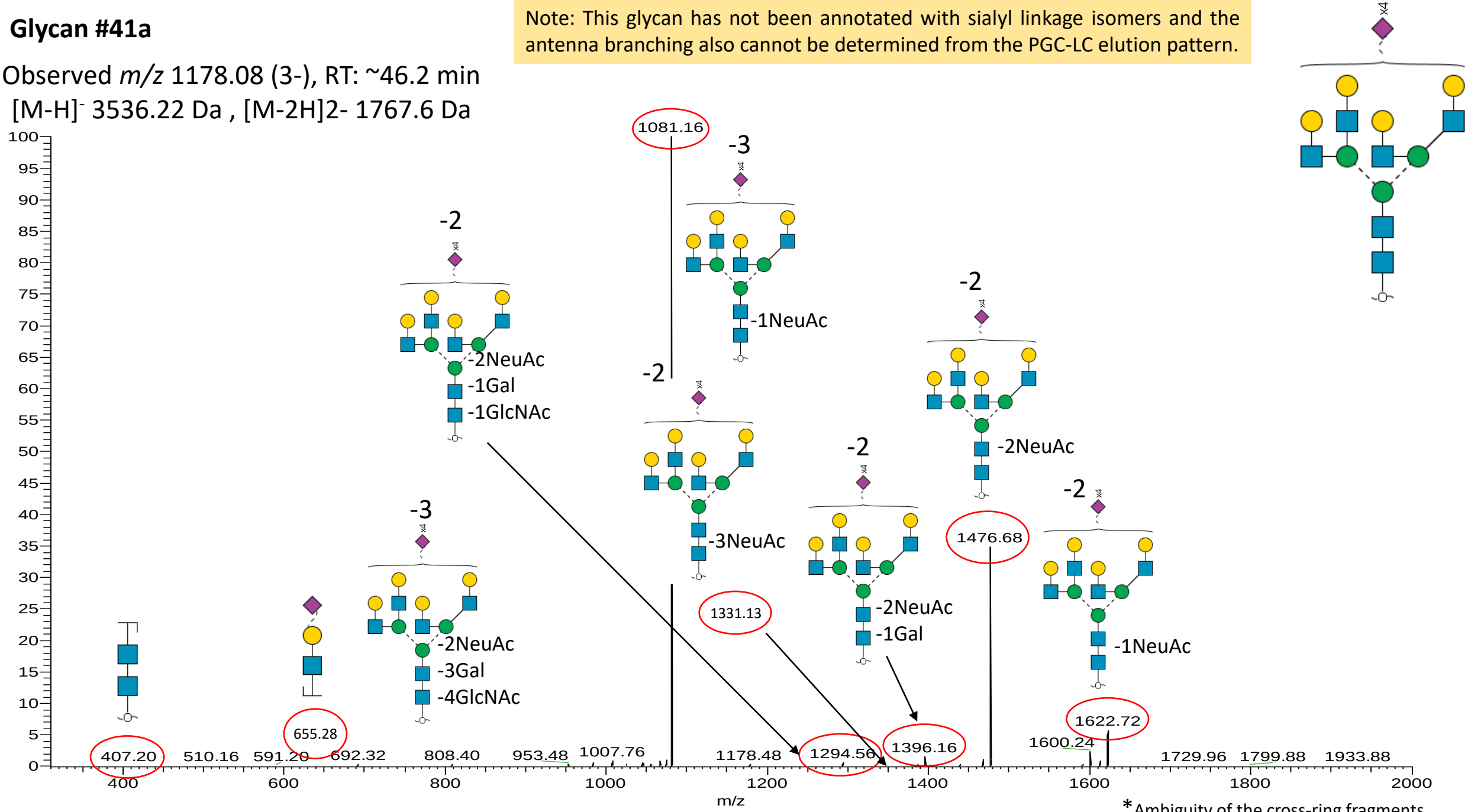
*Ambiguity of the cross-ring fragments

Glycan #41a

Observed *m/z* 1178.08 (3-), RT: ~46.2 min

$[M-H]^-$ 3536.22 Da , [M-2H]2- 1767.6 Da

Note: This glycan has not been annotated with sialyl linkage isomers and the antenna branching also cannot be determined from the PGC-LC elution pattern.



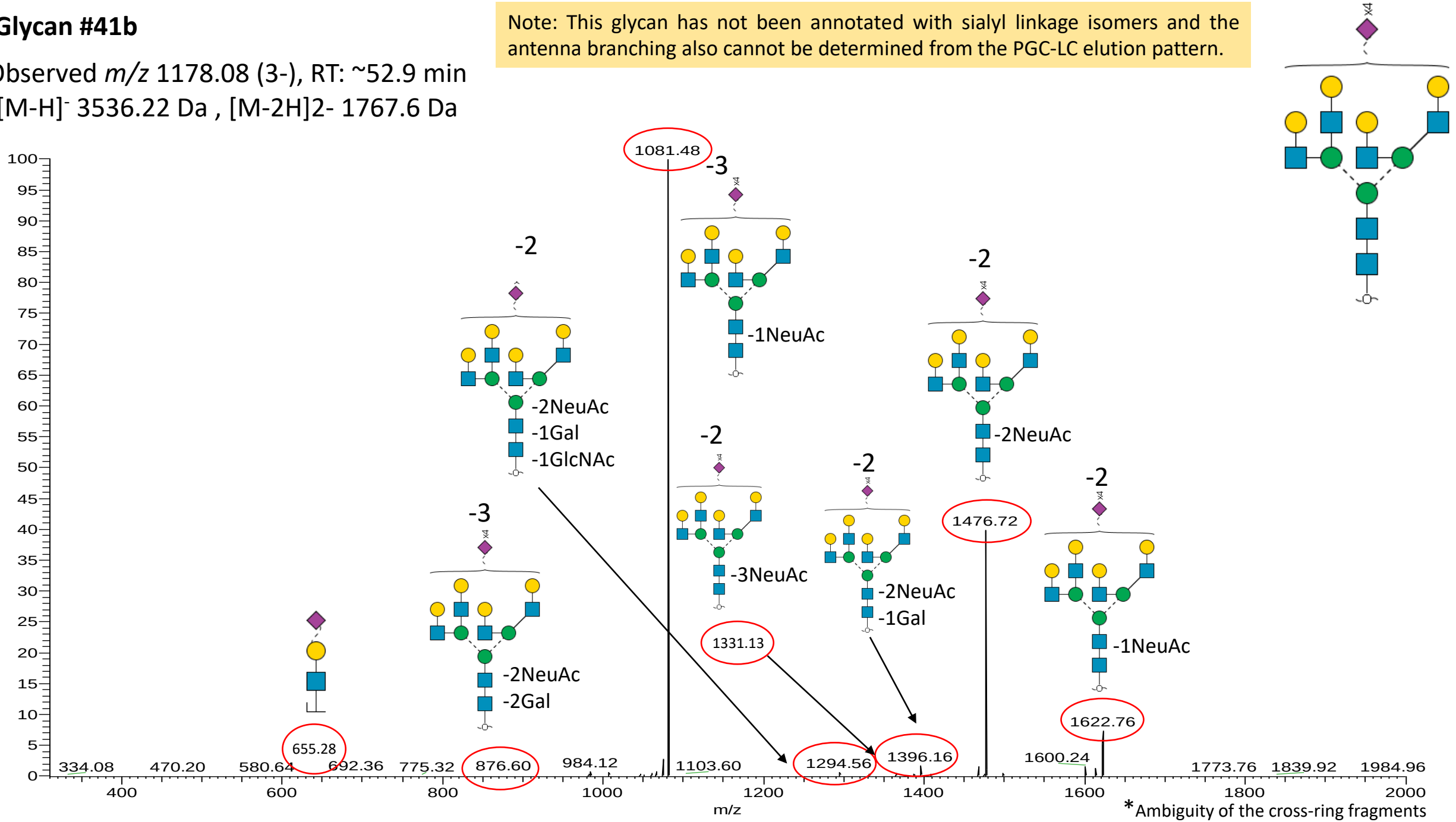
*Ambiguity of the cross-ring fragments

Glycan #41b

Observed *m/z* 1178.08 (3-), RT: ~52.9 min

$[M-H]^-$ 3536.22 Da , [M-2H]2- 1767.6 Da

Note: This glycan has not been annotated with sialyl linkage isomers and the antenna branching also cannot be determined from the PGC-LC elution pattern.



Glycan #41c

Observed *m/z* 1178.08 (3-), RT: ~56.2 min

$[M-H]^-$ 3536.22 Da, $[M-2H]2-$ 1767.6 Da

Note: This glycan has not been annotated with sialyl linkage isomers and the antenna branching also cannot be determined from the PGC-LC elution pattern.