

Supplementary Data

for

Hyper-truncated Asn355- and Asn391-glycans modulate the activity of neutrophil granule myeloperoxidase

Harry C. Tjondro^{a,b,1}, Julian Ugonotti^{a,b,1}, Rebeca Kawahara^{a,b,1}, Sayantani Chatterjee^{a,b,1}, Ian Loke^c, Siyun Chen^d, Fabian Soltermann^d, Hannes Hinneburg^{a,b}, Benjamin L. Parker^e, Vignesh Venkatakrisnan^f, Regis Dieckmann^f, Oliver C. Grant^g, Johan Bylund^h, Alison Rodger^{a,b}, Robert J. Woods^g, Anna Karlsson-Bengtsson^{f,i}, Weston B. Struwe^d, Morten Thaysen-Andersen^{a,b*}

^a*Department of Molecular Sciences, Macquarie University, Sydney, NSW, Australia*

^b*Biomolecular Discovery Research Centre, Macquarie University, Sydney, NSW, Australia*

^c*Cordlife Group Limited, Singapore, Singapore*

^d*Department of Chemistry, University of Oxford, Oxford, United Kingdom*

^e*Department of Physiology, University of Melbourne, Melbourne, VIC, Australia*

^f*Department of Rheumatology & Inflammation Research, Institute of Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

^g*Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia, United States*

^h*Department of Oral Microbiology and Immunology, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden*

ⁱ*Department of Biology and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden*

¹*Contributed equally*

***Corresponding author:**

Dr Morten Thaysen-Andersen, PhD

Department of Molecular Sciences - Macquarie University, NSW-2109, Sydney, Australia

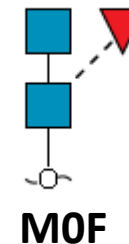
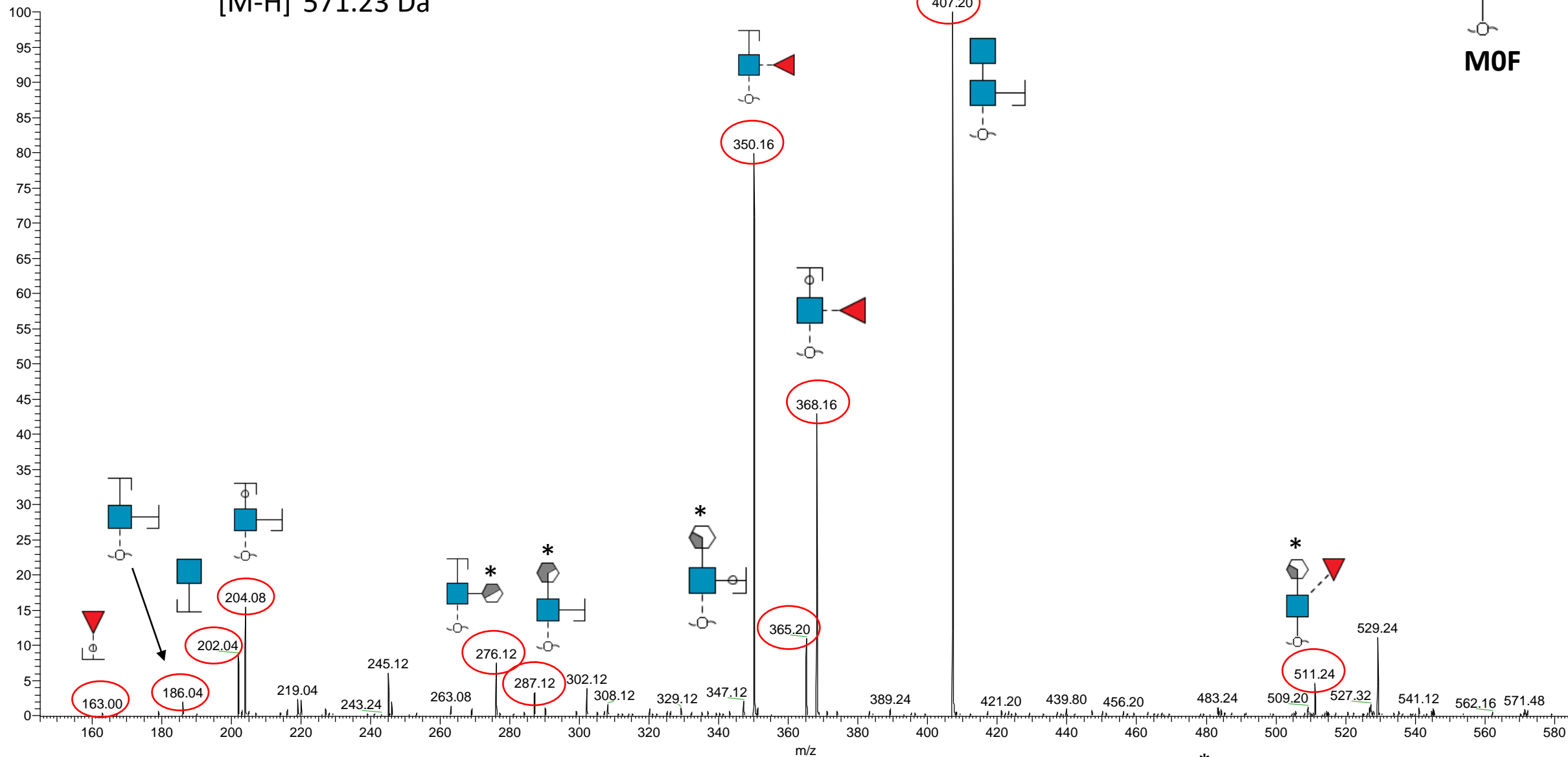
Ph: +61 2 9850 7487 - Email: morten.andersen@mq.edu.au

Supplementary Data S1

Manually annotated PGC-LC-ESI-CID-MS/MS (-) spectra of reduced *N*-glycans (alditols) released from nMPO

Glycan #1

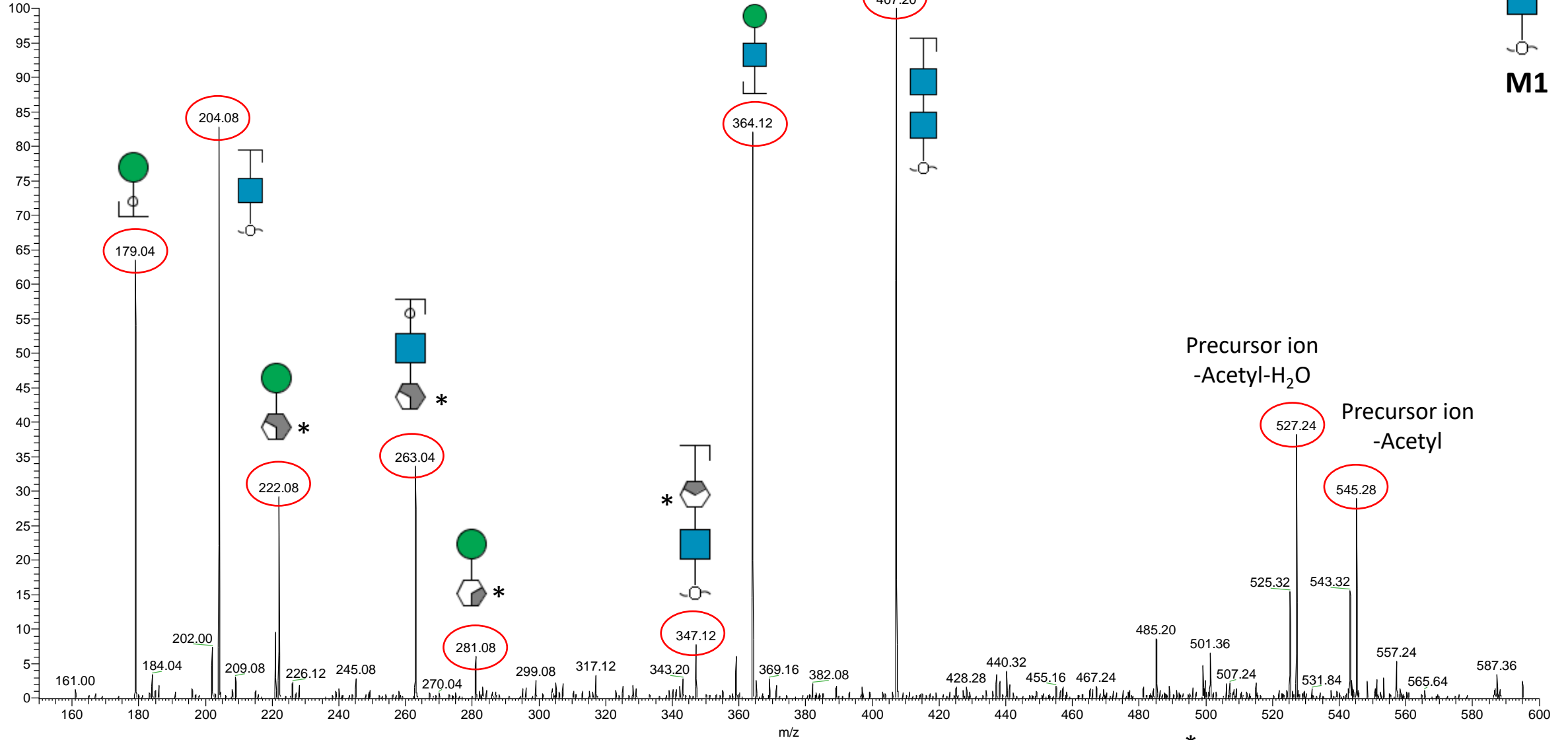
Observed m/z 571.24 (1-), RT: ~17.8 min
[M-H]⁻ 571.23 Da



* Ambiguity of the cross-ring fragments

Glycan #2

Observed m/z 587.30 (1-), RT: ~16.9 min
[M-H]⁻ 587.23 Da



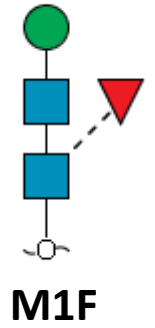
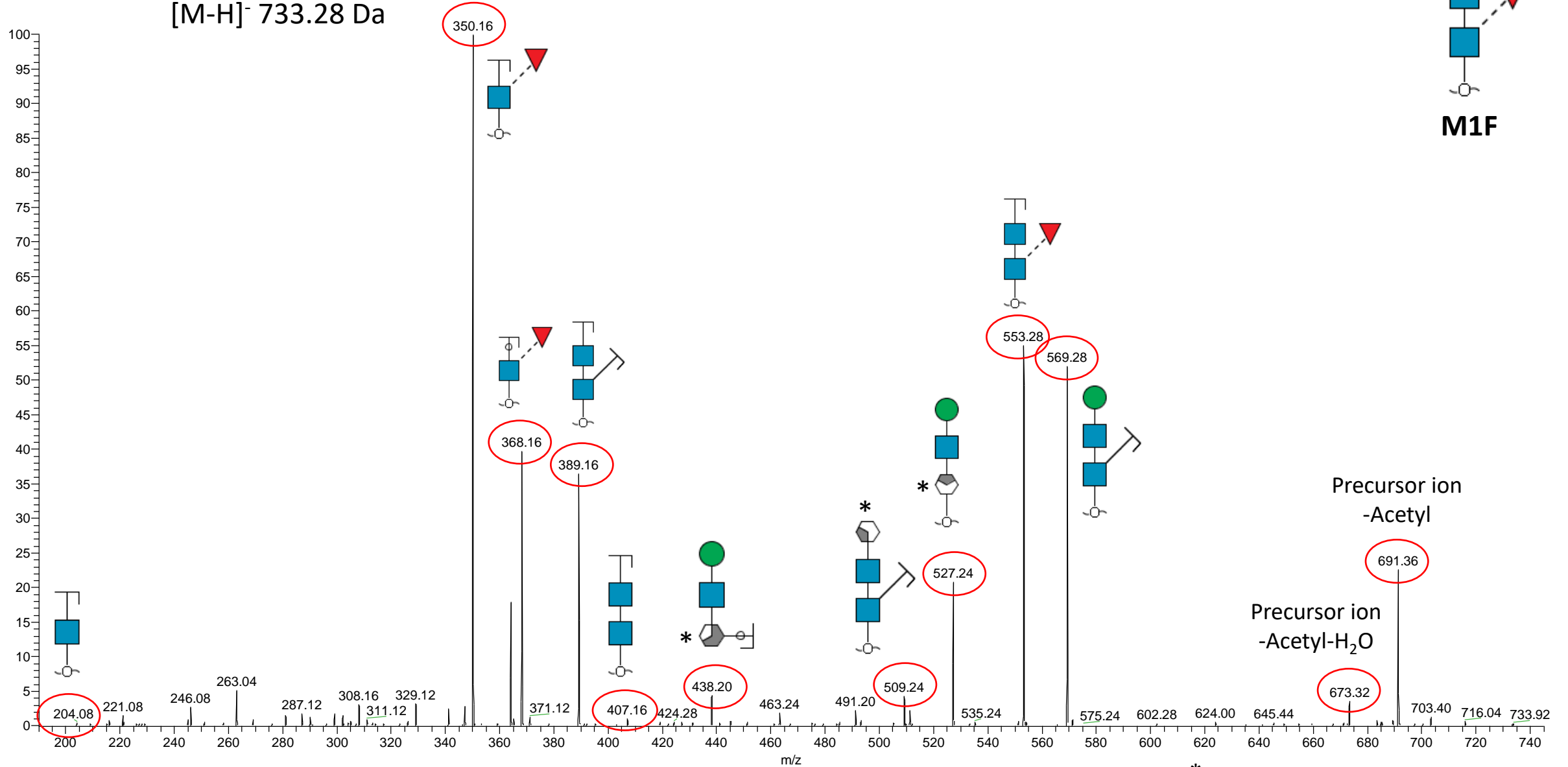
Precursor ion
-Acetyl-H₂O

Precursor ion
-Acetyl

* Ambiguity of the cross-ring fragments

Glycan #3

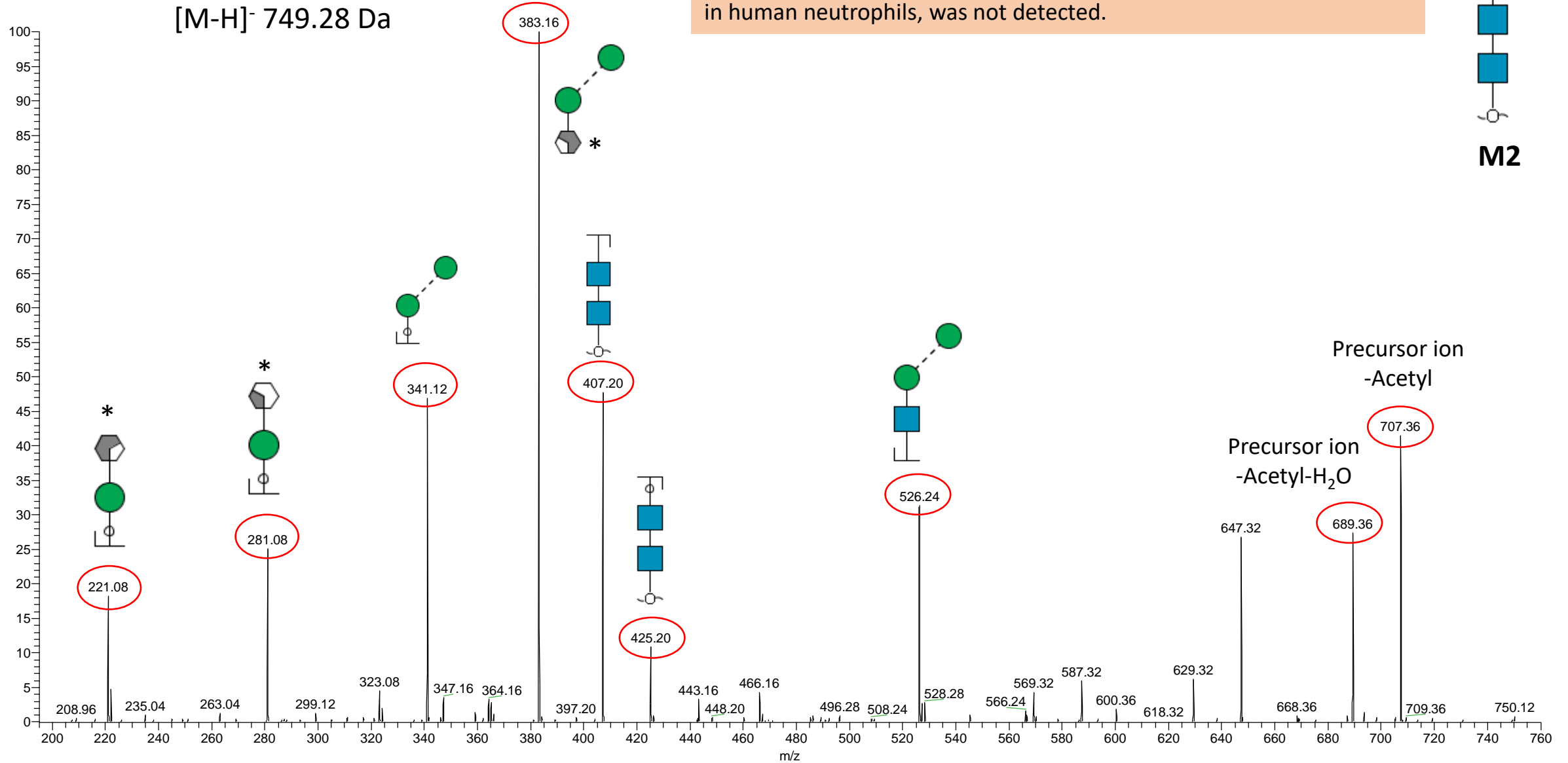
Observed m/z 733.36 (1-), RT: ~21.1 min
[M-H]⁻ 733.28 Da



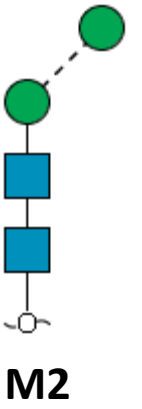
* Ambiguity of the cross-ring fragments

Glycan #4

Observed m/z 749.36 (1-), RT: ~18.4 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 glycosylation. The α 1,3-isomer, typically of lower abundance in human neutrophils, was not detected.

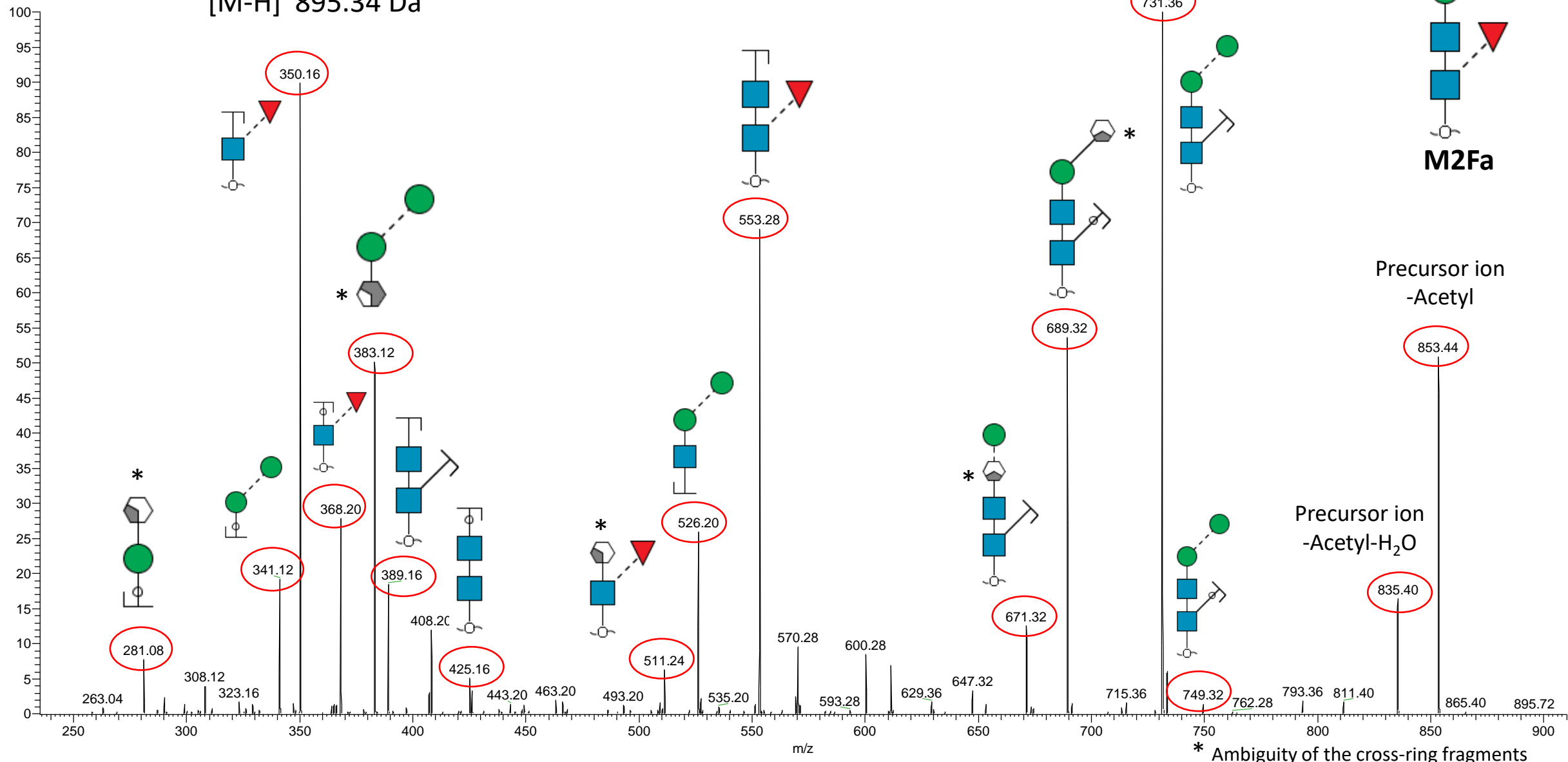


* Ambiguity of the cross-ring fragments

Glycan #5a

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

Observed m/z 895.46 (1-), RT: ~24.3 min
[M-H]⁻ 895.34 Da



M2Fa

Precursor ion
-Acetyl

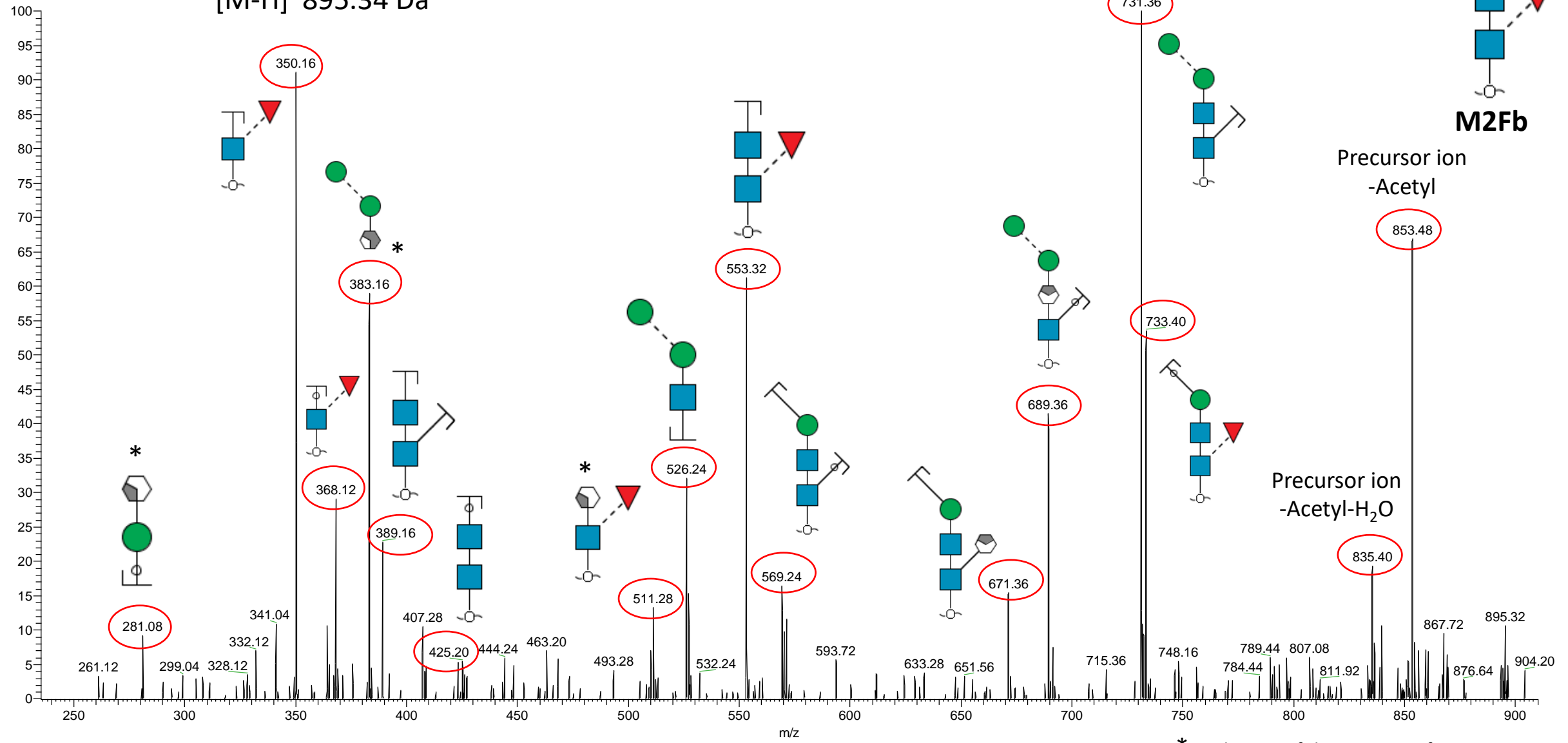
Precursor ion
-Acetyl-H₂O

* Ambiguity of the cross-ring fragments

Glycan #5b

Note: This low abundance glycan was annotated as the α 1,3-Man isomer based on PGC LC elution time and experience with neutrophil N-glycosylation.

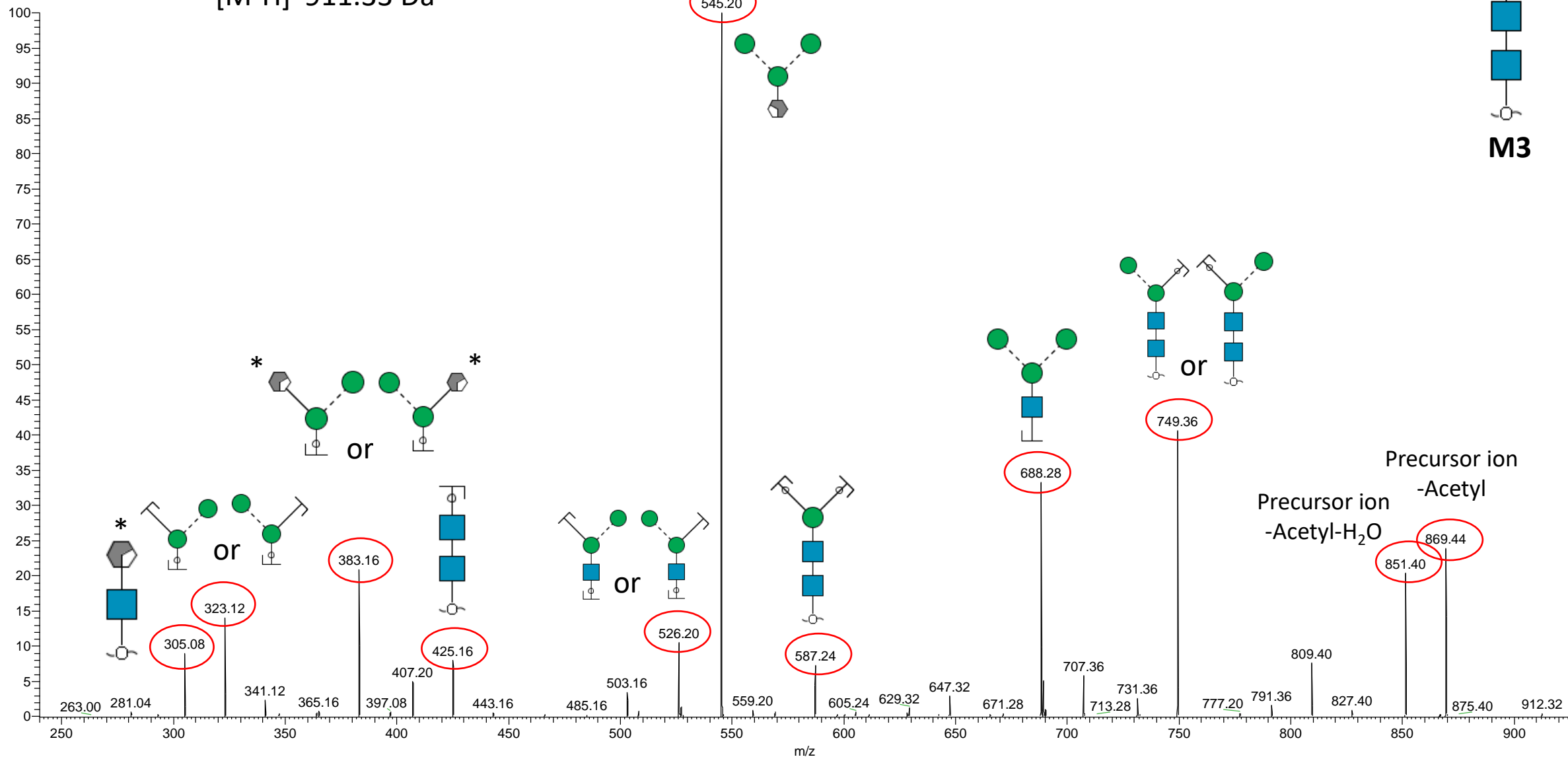
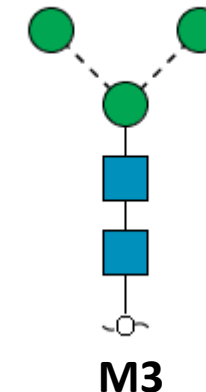
Observed m/z 895.46 (1-), RT: ~26.4 min
[M-H]⁻ 895.34 Da



* Ambiguity of the cross-ring fragments

Glycan #6

Observed m/z 911.44 (1-), RT: ~22.9 min
 $[M-H]^-$ 911.33 Da

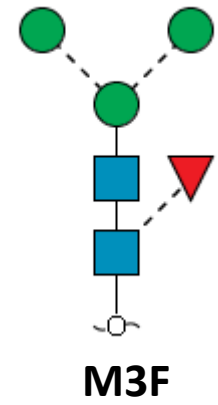
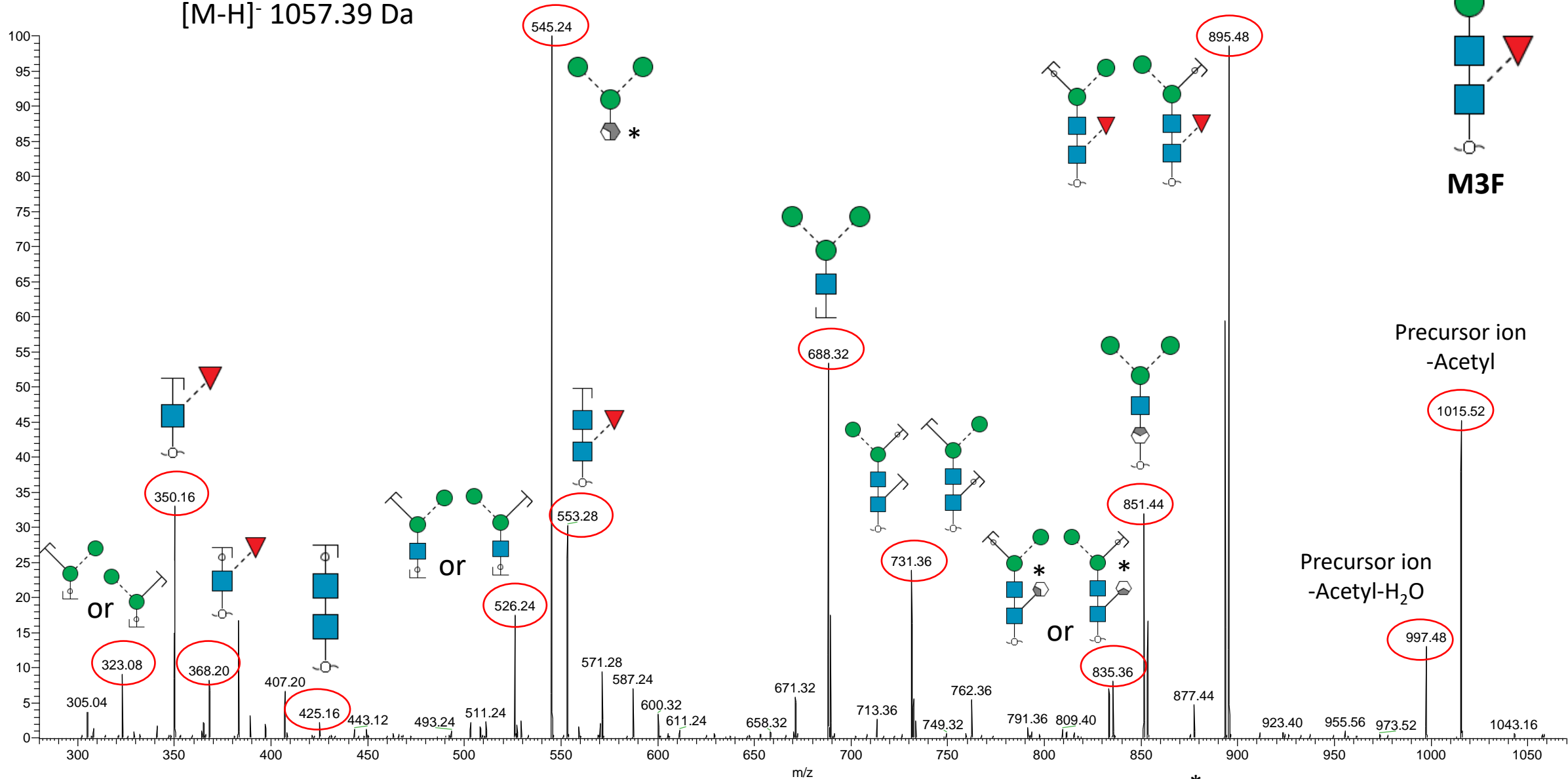


* Ambiguity of the cross-ring fragments

Glycan #7

Observed m/z 1057.54 (1-), RT: ~31.1 min

$[M-H]^-$ 1057.39 Da



Precursor ion
-Acetyl

1015.52

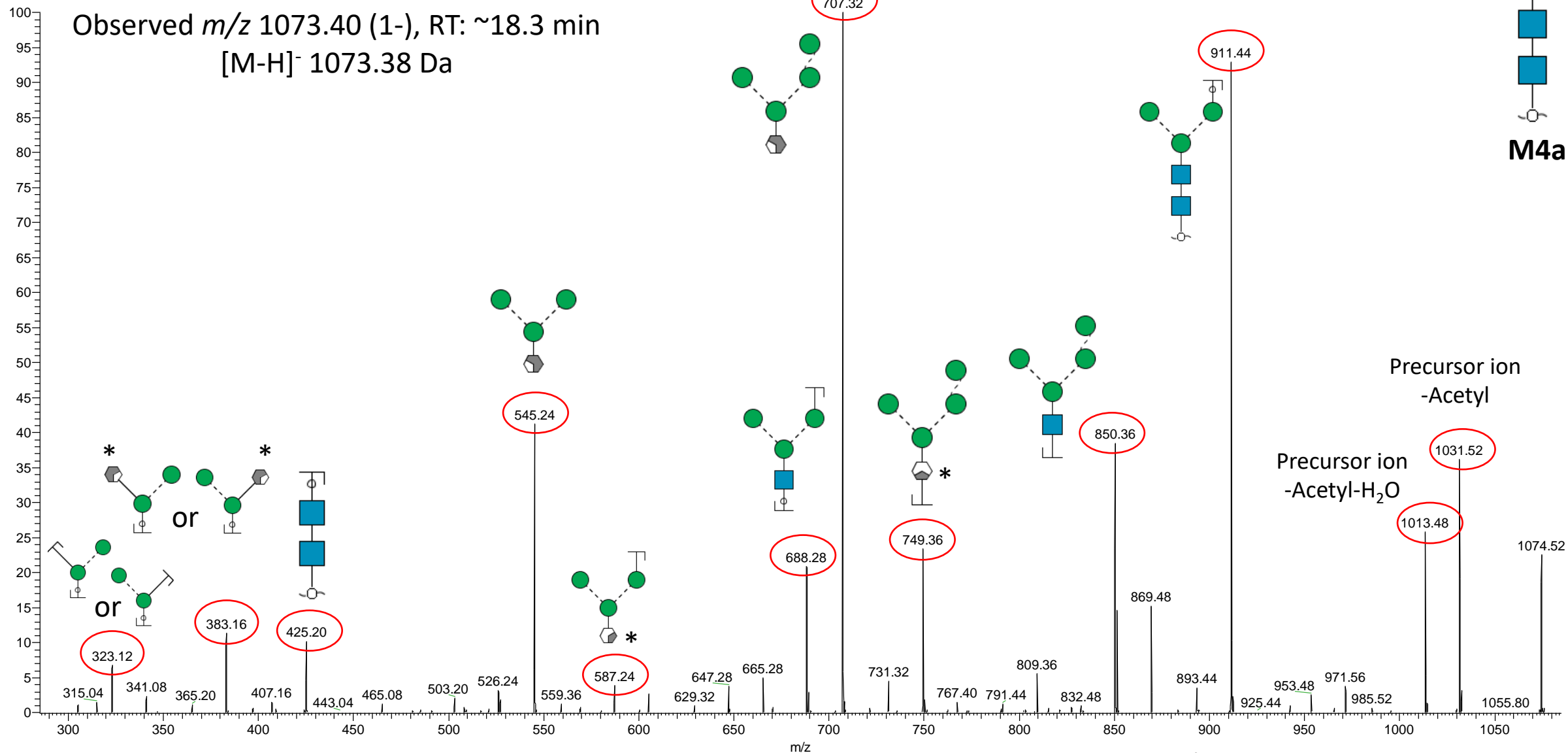
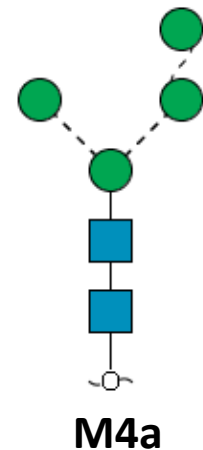
Precursor ion
-Acetyl-H₂O

997.48

* Ambiguity of the cross-ring fragments

Glycan #8a

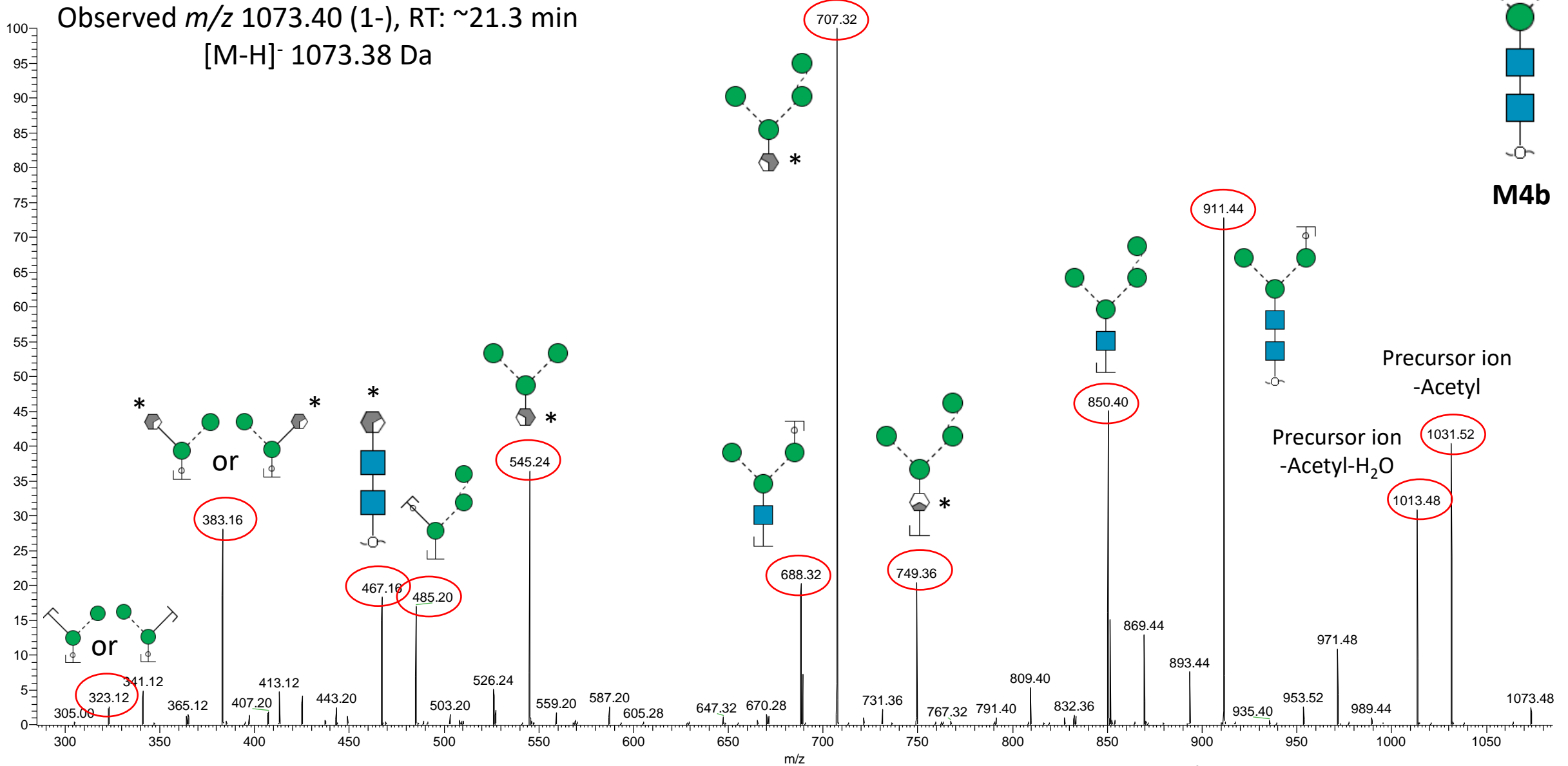
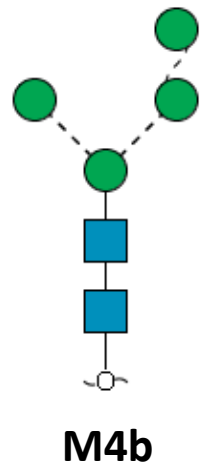
Note: The outer mannose residue has been placed on the α 1,6-Man arm based on biosynthetic rules and may be predicted to be α 1,6-linked based on PGC LC elution pattern (relative to the M4b isomer). However the mannosyl linkage of this residue was left unassigned since no MS/MS evidence is present to support this.



* Ambiguity of the cross-ring fragments

Glycan #8b

Note: The outer mannose residue has been placed on the α 1,6-Man arm based on biosynthetic rules and may be predicted to be α 1,3-linked based on PGC LC elution pattern (relative to the M4a isomer). However the mannosyl linkage of this residue was left unassigned since no MS/MS evidence is present to support this.

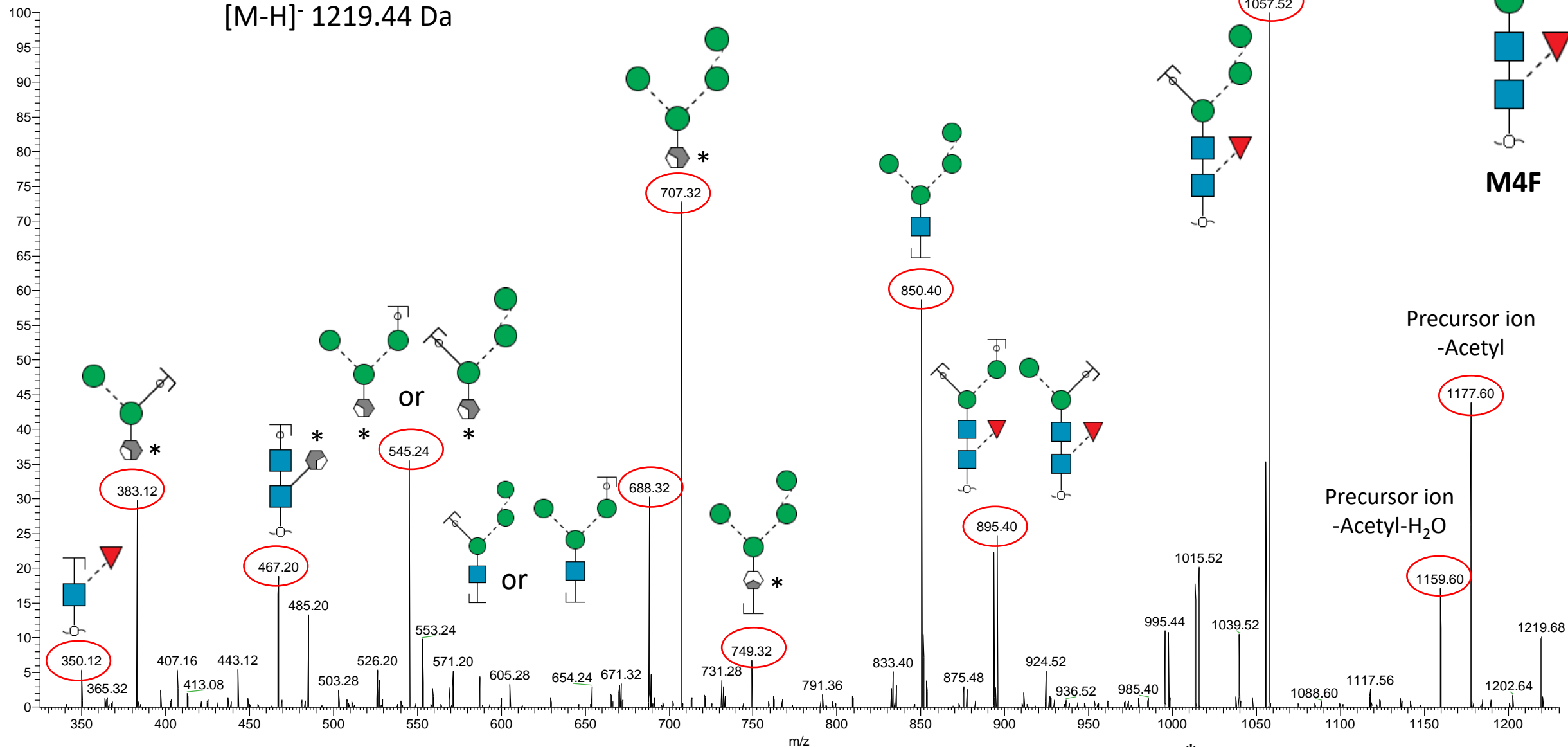


* Ambiguity of the cross-ring fragments

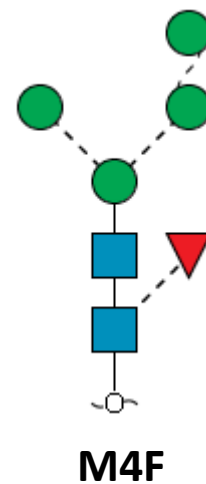
Glycan #9

Note: The outer mannose residue has been placed on the α 1,6-Man arm based on biosynthetic rules. Unknown if this residue is found in a α 1,3- or α 1,6-linkage since no MS/MS evidence to support either so left unassigned.

Observed m/z 1219.44 (1-), RT: ~28.5 min
[M-H]⁻ 1219.44 Da



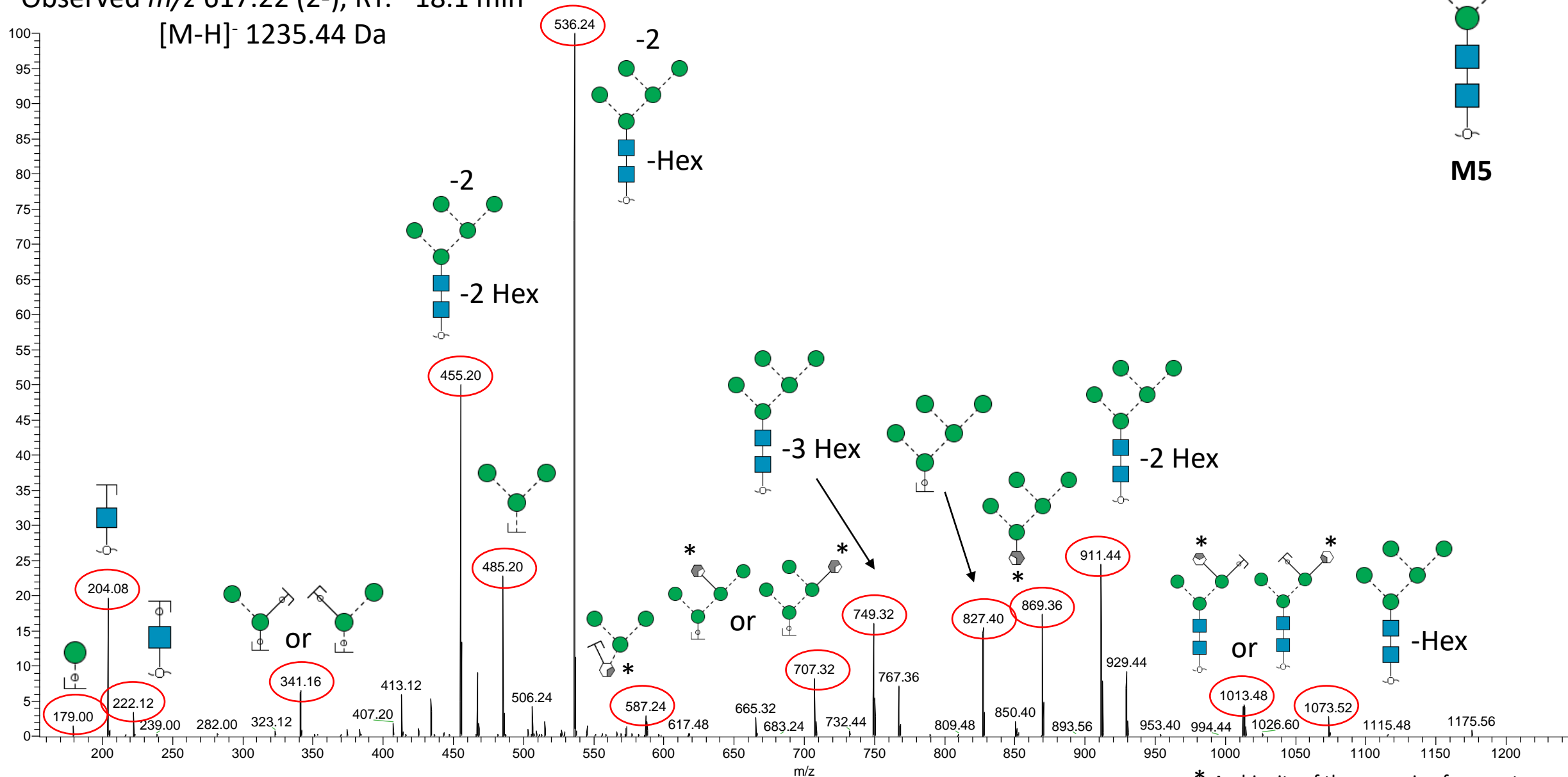
* Ambiguity of the cross-ring fragments



Glycan #10

Observed m/z 617.22 (2-), RT: ~18.1 min

$[M-H]^-$ 1235.44 Da

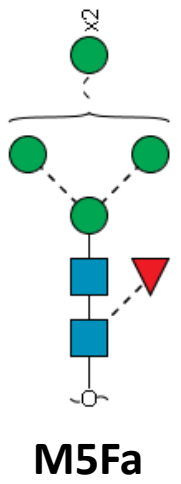
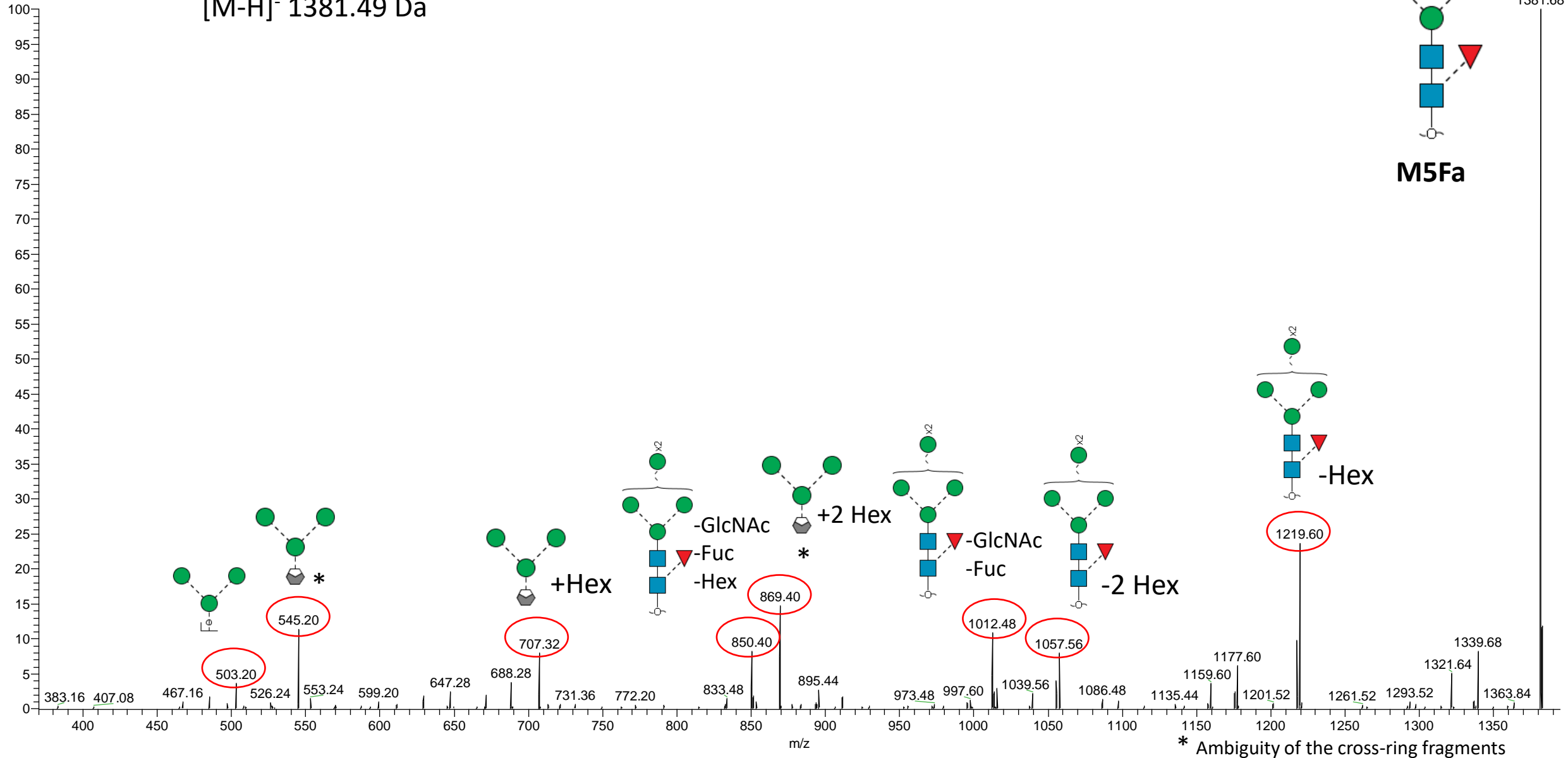


* Ambiguity of the cross-ring fragments

Glycan #11a

Note: Structure predicted from known biosynthetic rules, but no MS/MS support for exact topology of outer mannose residues.

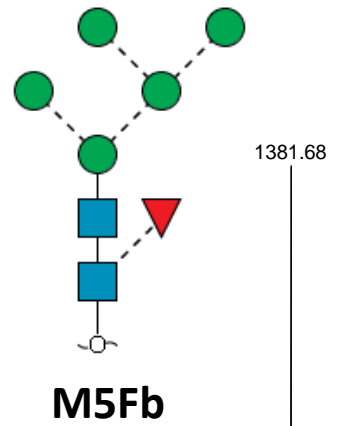
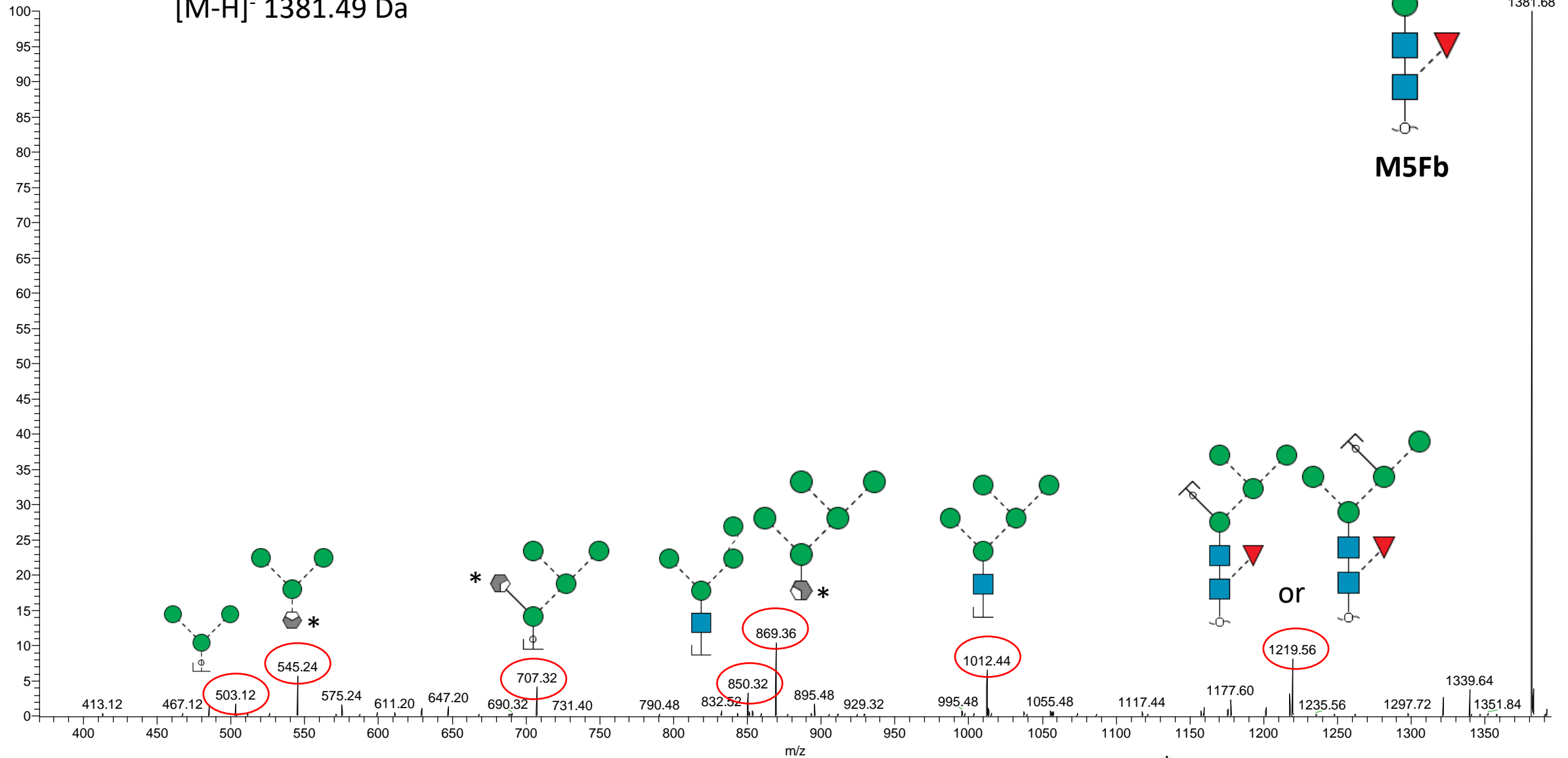
Observed m/z 1381.62 (1-), RT: ~30.4 min
[M-H]⁻ 1381.49 Da



Glycan #11b

Note: Structure predicted from known biosynthetic rules, but no MS/MS support for exact topology of outer mannose residues.

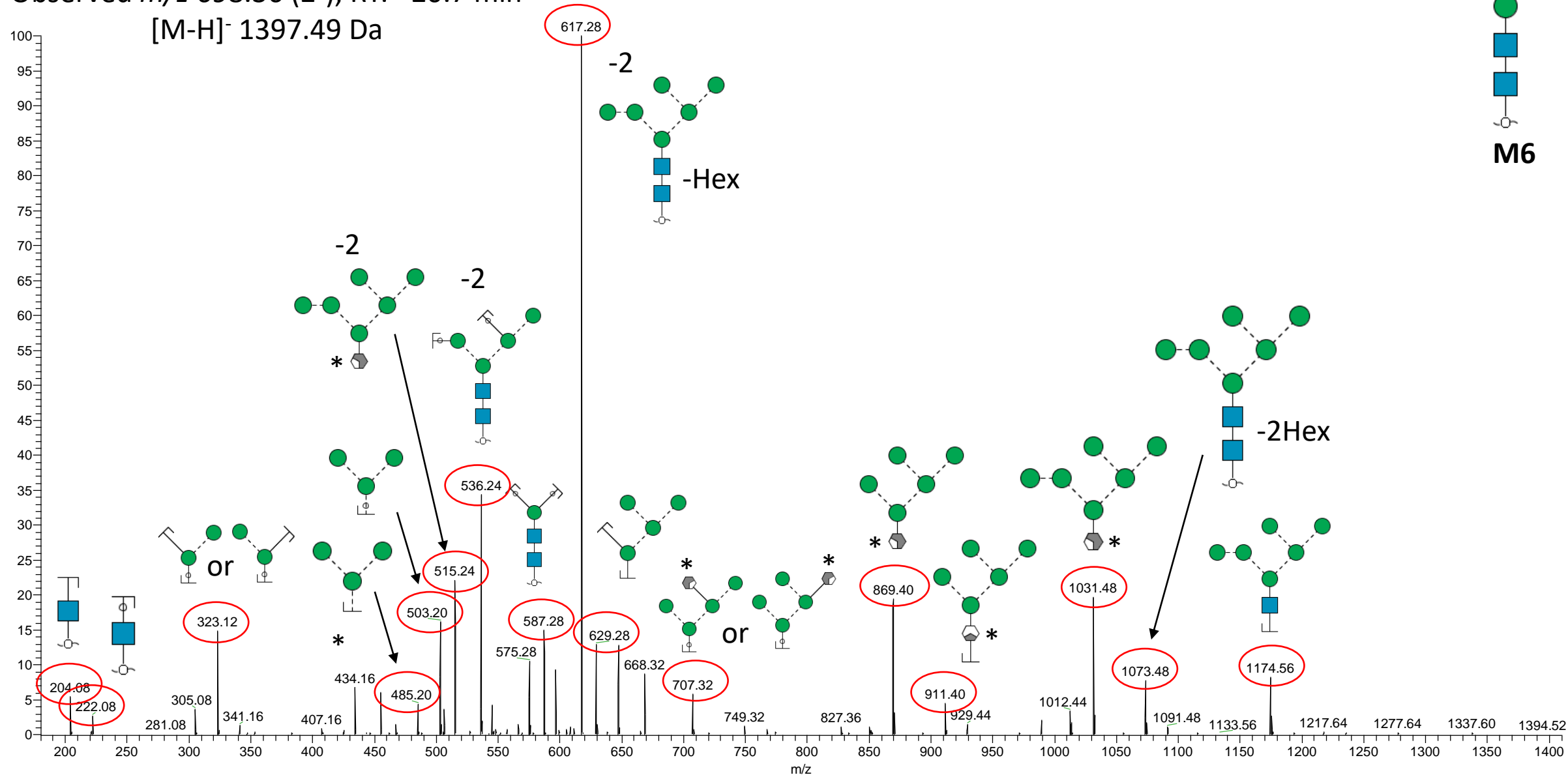
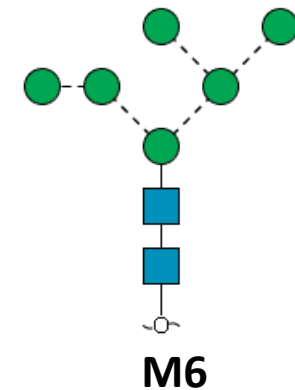
Observed m/z 1381.62 (1-), RT: ~33.2 min
[M-H]⁻ 1381.49 Da



* Ambiguity of the cross-ring fragments

Glycan #12

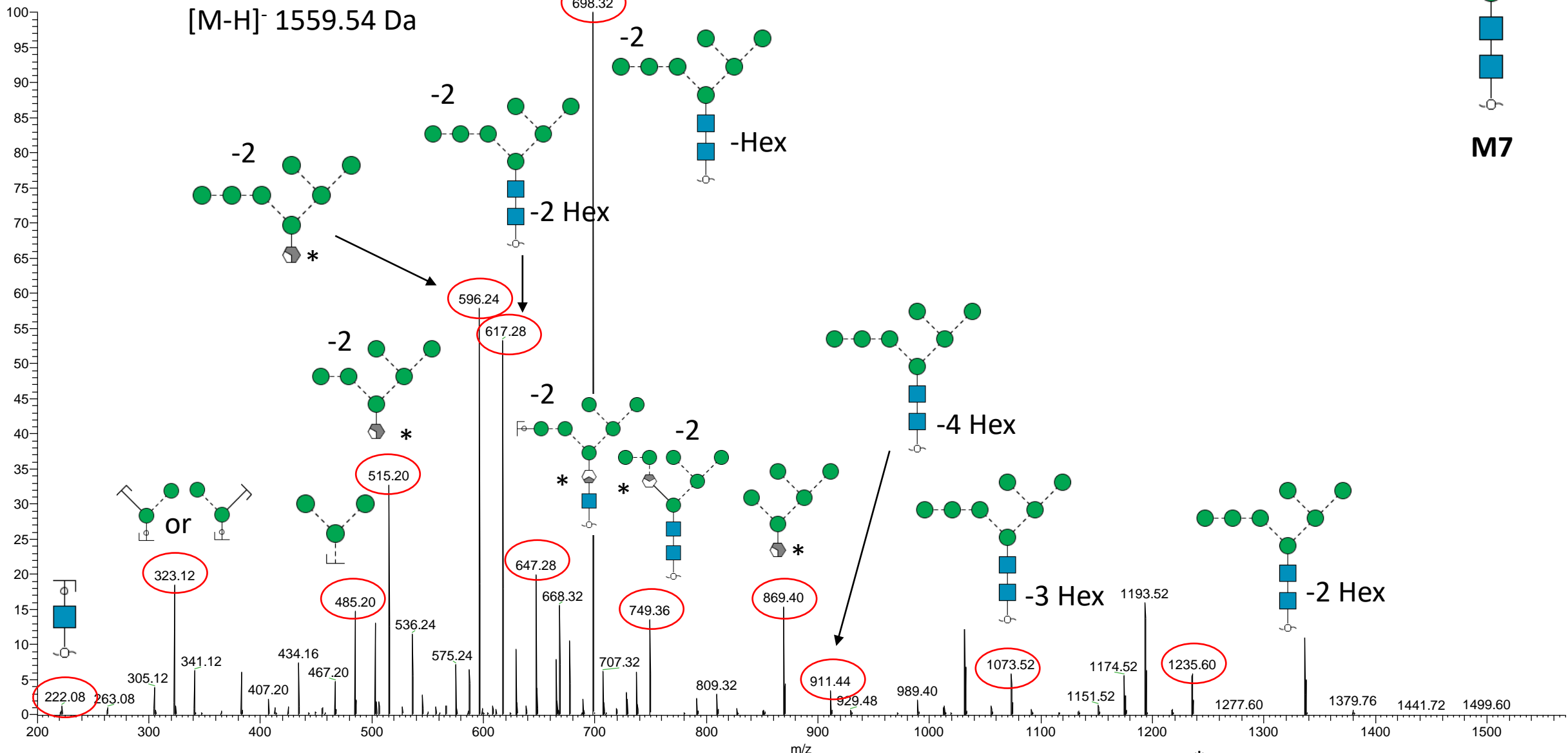
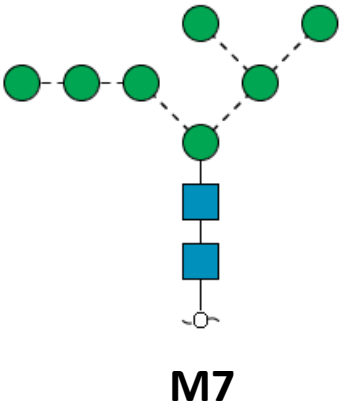
Observed m/z 698.36 (2-), RT: ~20.7 min
[M-H]⁻ 1397.49 Da



* Ambiguity of the cross-ring fragments

Glycan #13

Observed m/z 779.38 (2-), RT: ~20.9 min
 [M-H]⁻ 1559.54 Da



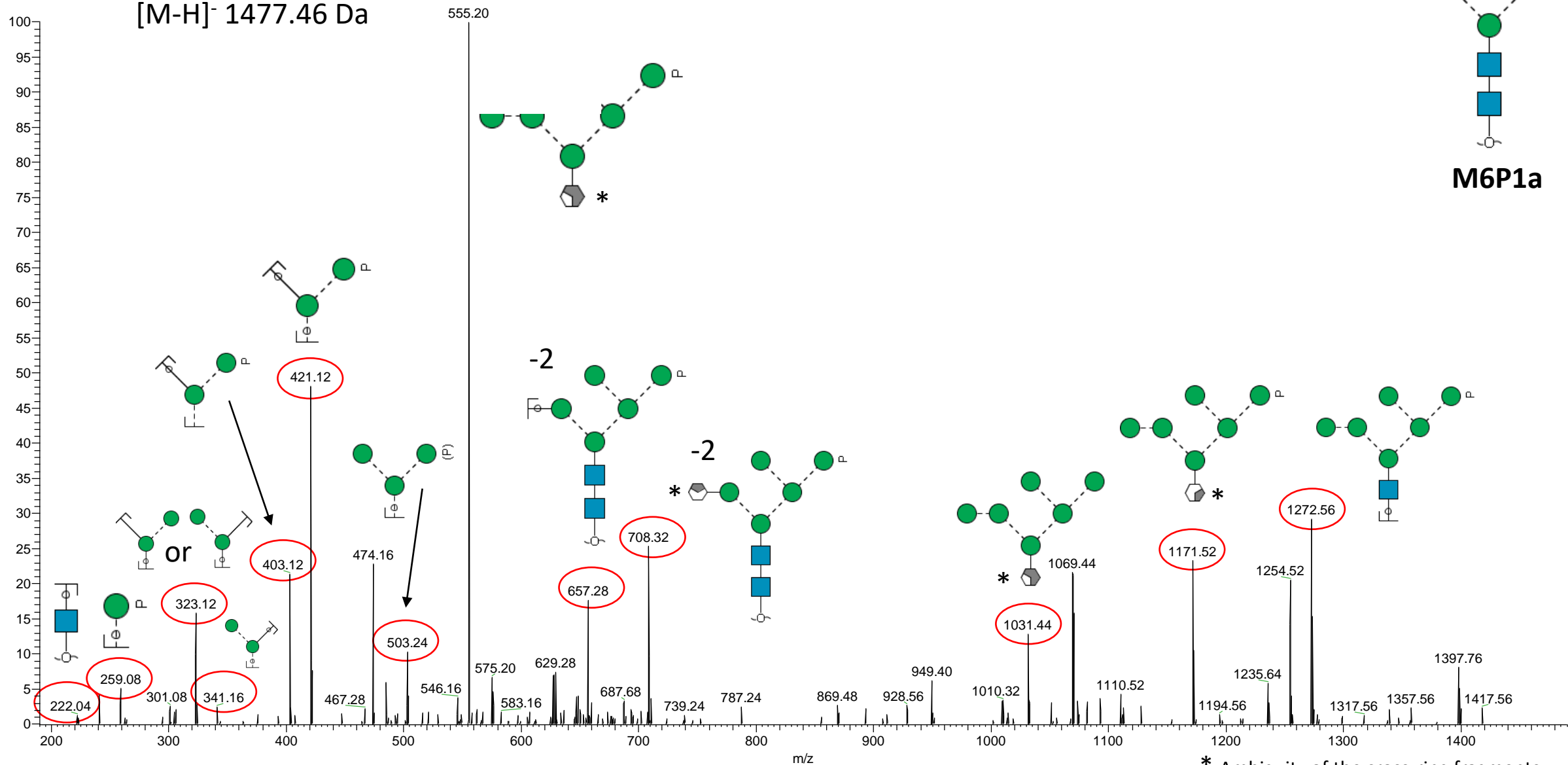
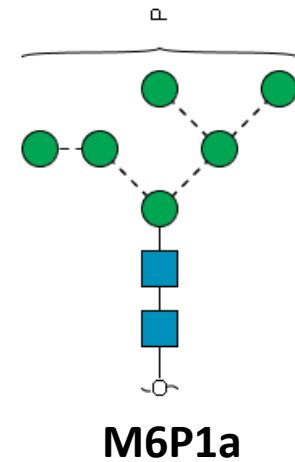
* Ambiguity of the cross-ring fragments

Glycan #14a

Observed m/z 738.23 (2-), RT: ~17.6 min

[M-H]⁻ 1477.46 Da

Note: Based on knowledge of the M6P pathway, the phosphate moiety appears on outer mannose residue, but no direct MS/MS evidence to support the exact position of the phosphate.

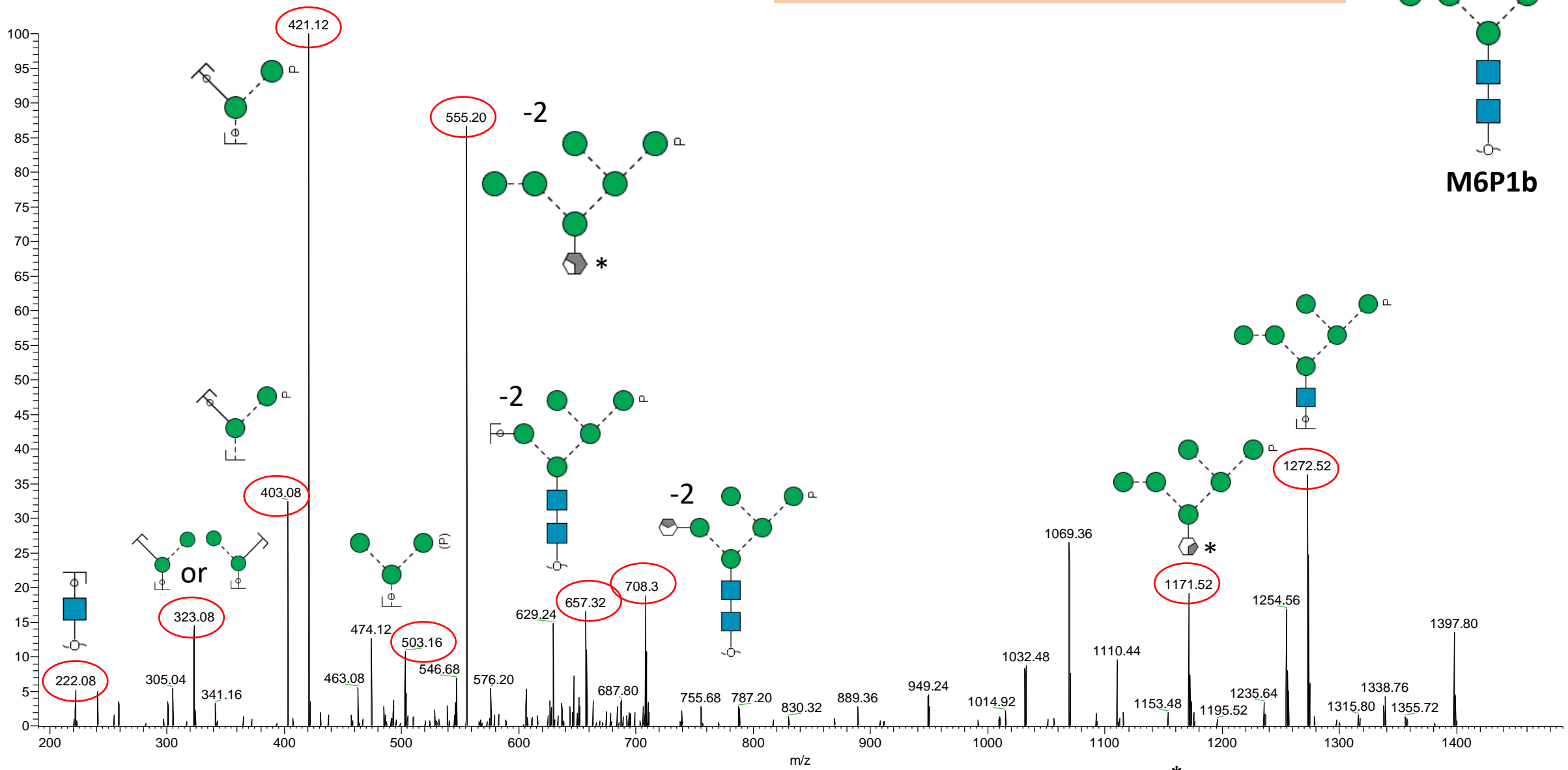
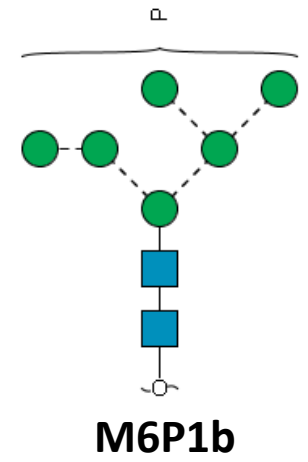


* Ambiguity of the cross-ring fragments

Glycan #14b

Observed m/z 738.23 (2-), RT: ~18.5 min
[M-H]⁻ 1477.46 Da

Note: Based on knowledge of the M6P pathway, the phosphate moiety appears on outer mannose residue, but no MS/MS evidence to support the exact position.



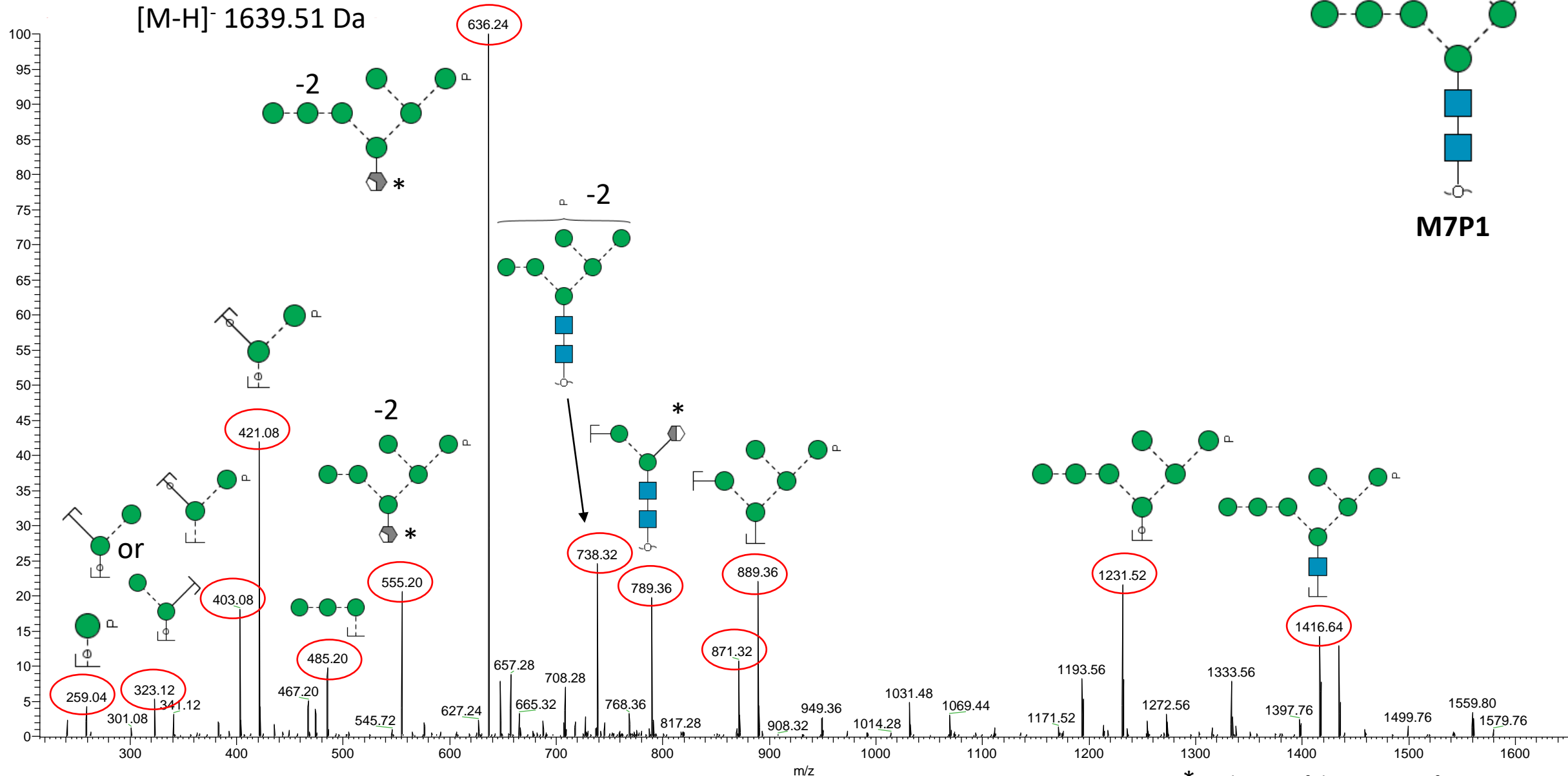
* Ambiguity of the cross-ring fragments

Glycan #15

Observed m/z 819.32 (2-), RT: ~17.8 min

$[M-H]^-$ 1639.51 Da

Note: Based on knowledge of the M6P pathway, the phosphate moiety appears on outer mannose residue, but no MS/MS evidence to support the exact position of the phosphate.

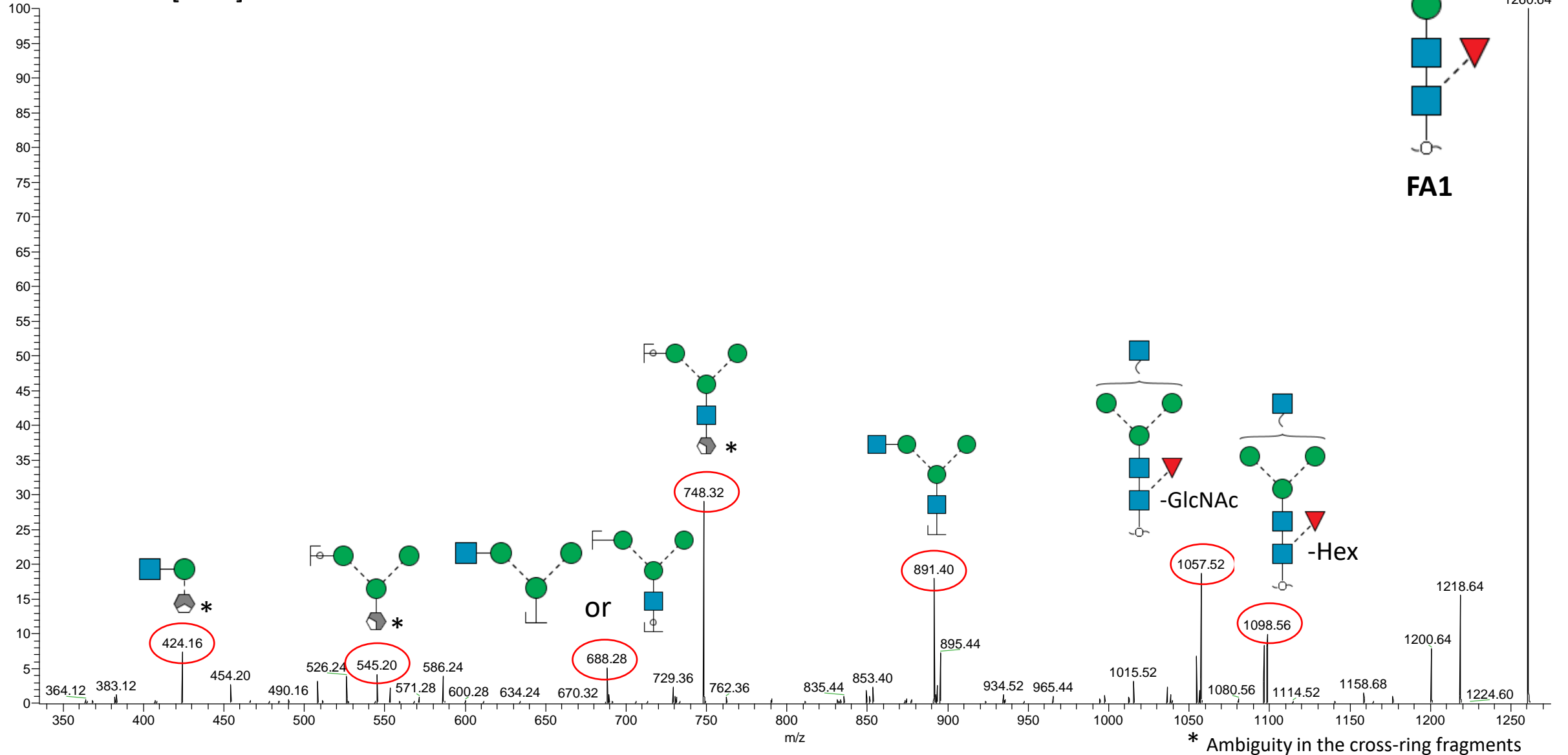


* Ambiguity of the cross-ring fragments

Glycan #16

Observed m/z 1260.60 (1-), RT: ~33.7 min
[M-H]⁻ 1260.47 Da

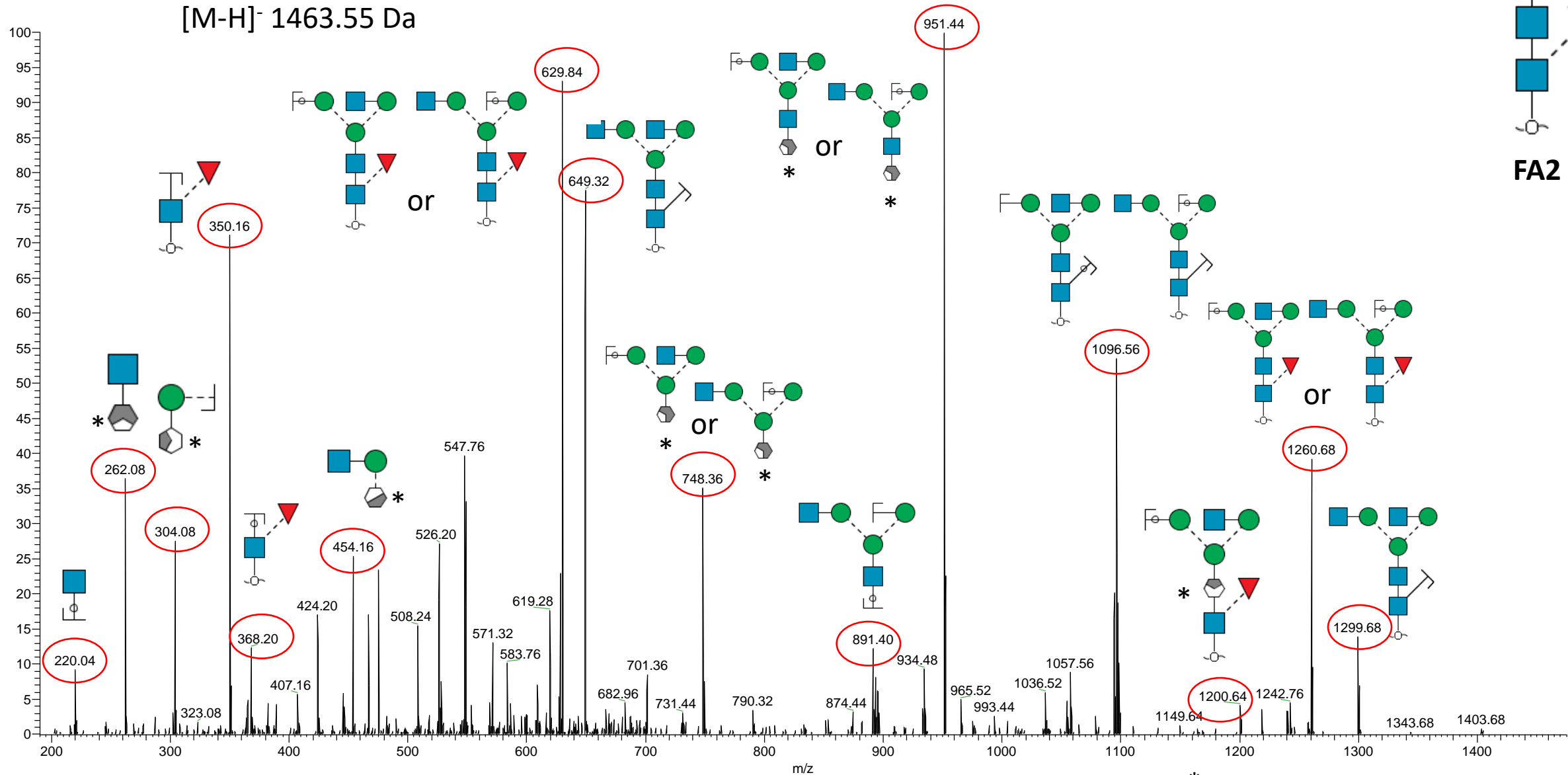
Note: From biosynthetic pathway, the single β 1,2-GlcNAc residue is predicted to occupy the α -1,3 arm but no MS/MS evidence to support this, so here left unassigned.



Glycan #17

Observed m/z 731.28 (2-), RT: ~26.1 min

$[M-H]^-$ 1463.55 Da

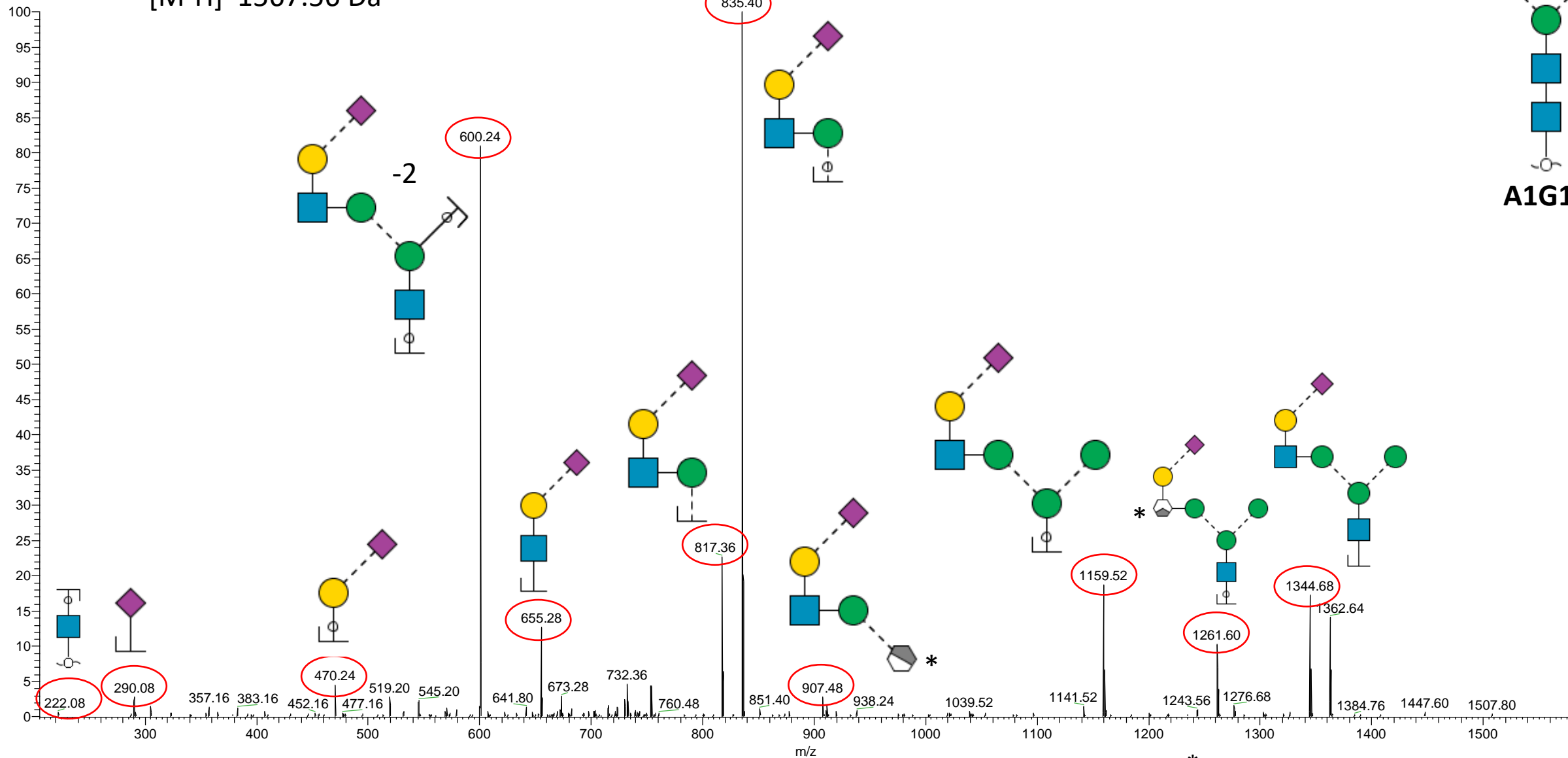
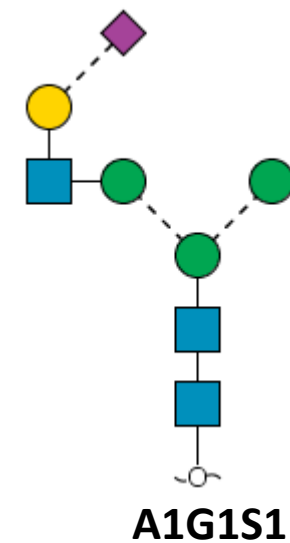


* Ambiguity of the cross-ring fragments

Glycan #18

Observed m/z 783.28 (2-), RT: ~22.3 min
[M-H]⁻ 1567.56 Da

Note: This glycan is annotated as the α 2,6-sialyl linkage isomer based on the early PGC-LC elution and experience with neutrophil N-glycan profiling. The elongated antenna is known to frequently occupy the α 1,3-arm rather than the α 1,6-arm of neutrophil N-glycans and have thus been assigned as such.

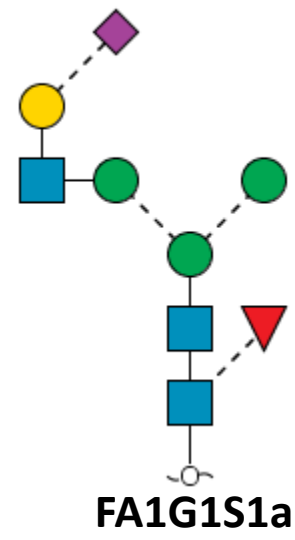
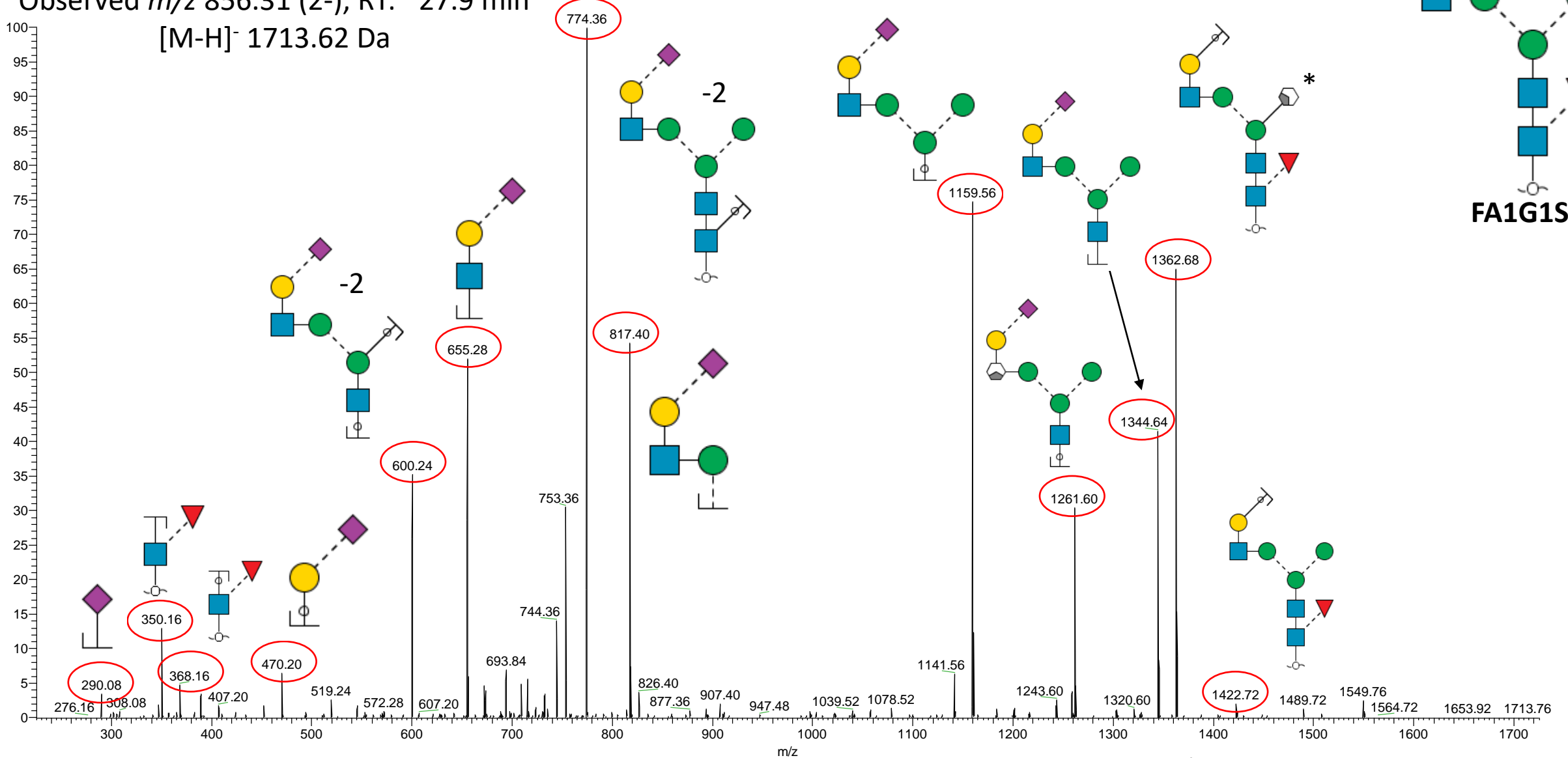


* Ambiguity in the cross-ring fragments

Glycan #19a

Note: This glycan is annotated as the α 2,6-sialyl linkage isomer based on the early PGC-LC elution and experience with neutrophil *N*-glycan profiling. The elongated antenna is known to frequently occupy the α 1,3-arm rather than the α 1,6-arm of neutrophil *N*-glycans and have thus been assigned as such.

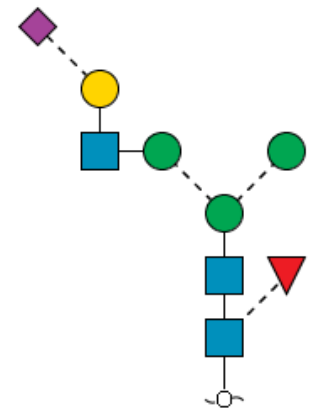
Observed m/z 856.31 (2-), RT: ~27.9 min
[M-H]⁻ 1713.62 Da



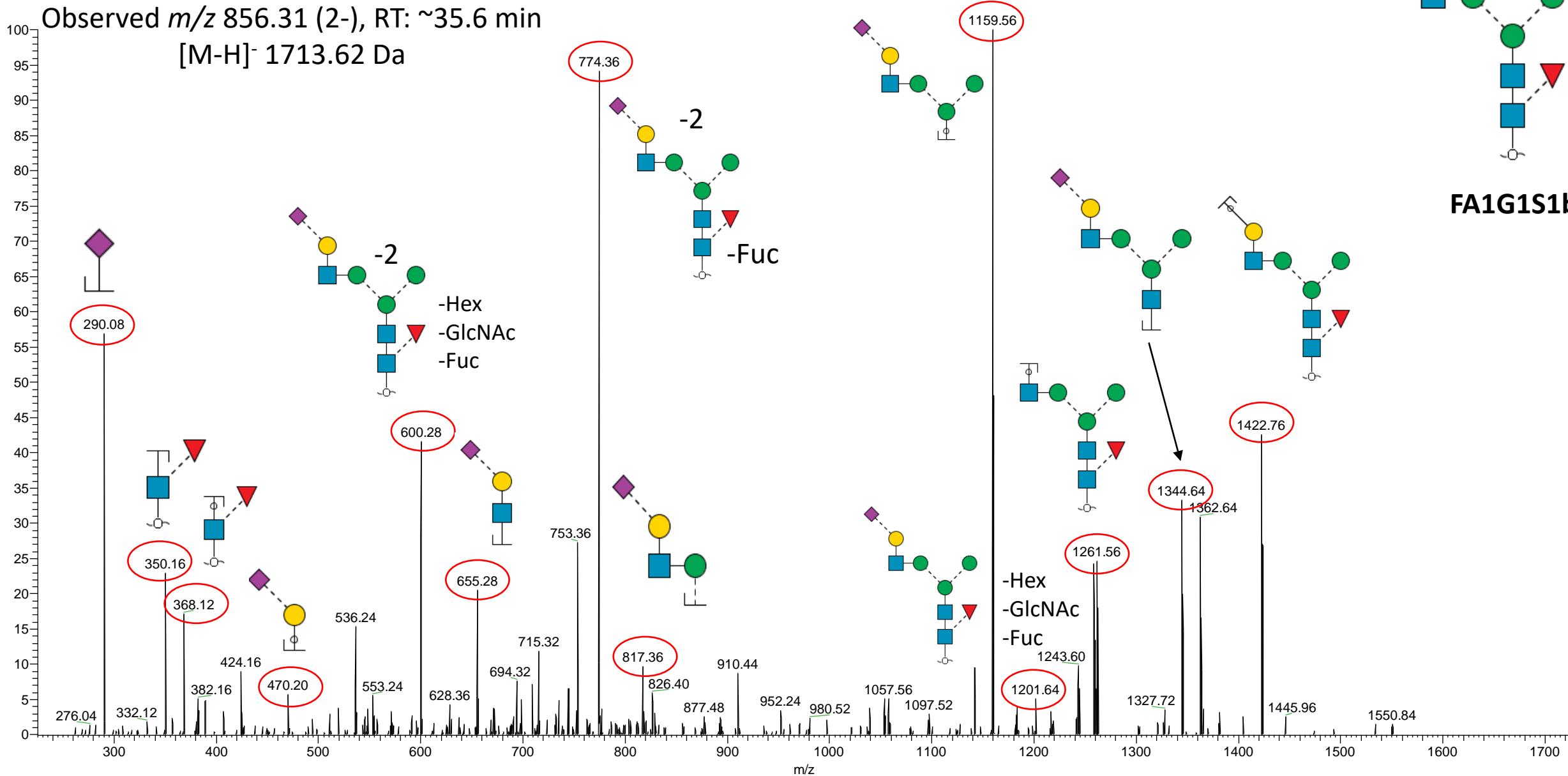
* Ambiguity of the cross-ring fragments

Glycan #19b

Note: This glycan is annotated as the α 2,3-sialyl linkage isomer based on the late PGC-LC elution and experience with neutrophil *N*-glycan profiling. The elongated antenna is known to frequently occupy the α 1,3-arm rather than the α 1,6-arm of neutrophil *N*-glycans and is thus assigned as such.



FA1G1S1b

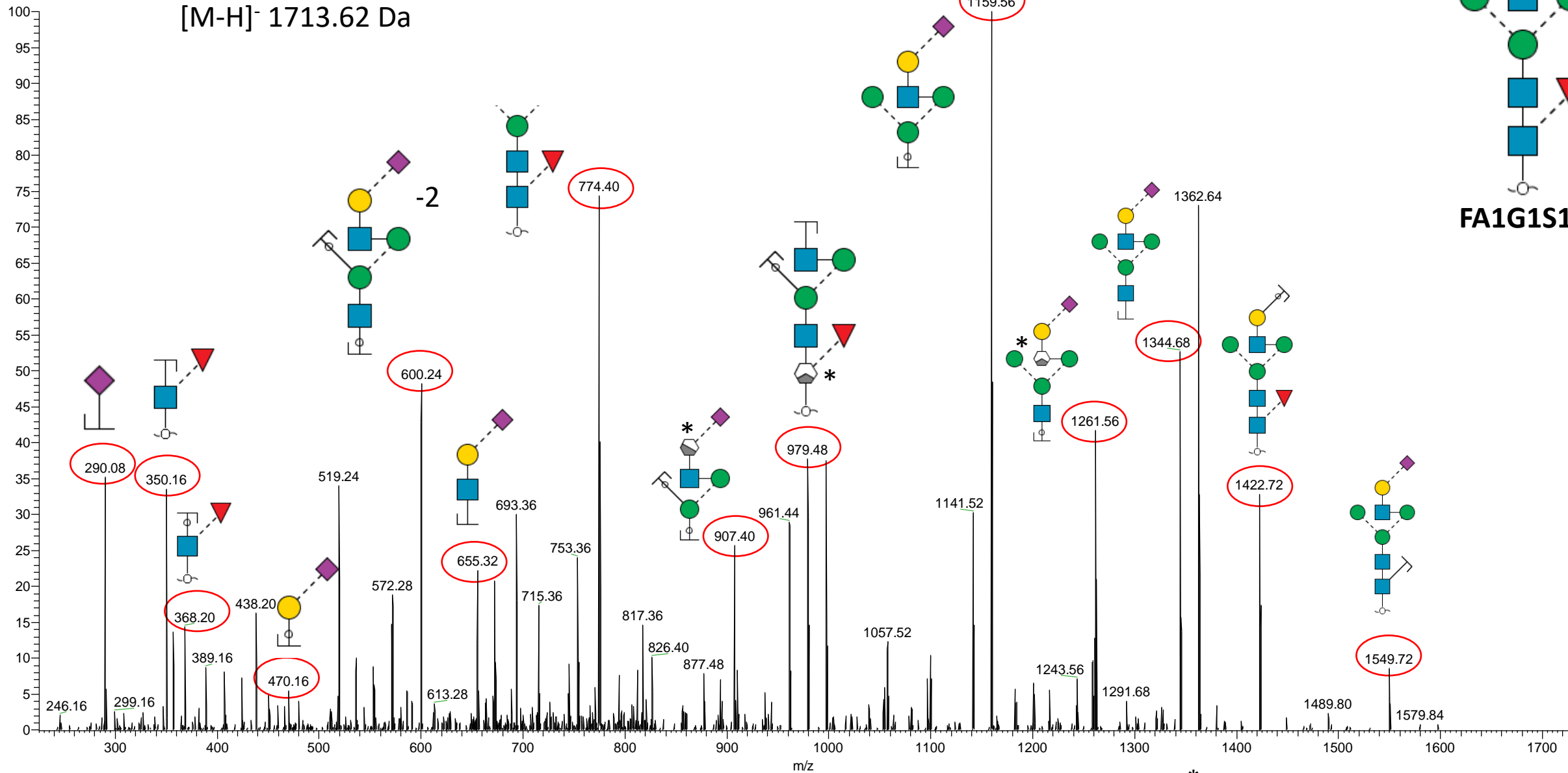


* Ambiguity of the cross-ring fragments

Glycan #19c

Note: This low abundance glycan is possibly the isomer where the Sia-Gal-GlcNAc antenna occupy the α 1,6-arm rather than the α 1,3-arm common for neutrophil *N*-glycans but no direct MS/MS evidence to support this

Observed m/z 856.31 (2-), RT: ~43.8 min
[M-H]⁻ 1713.62 Da



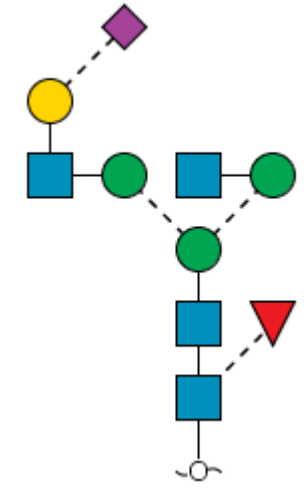
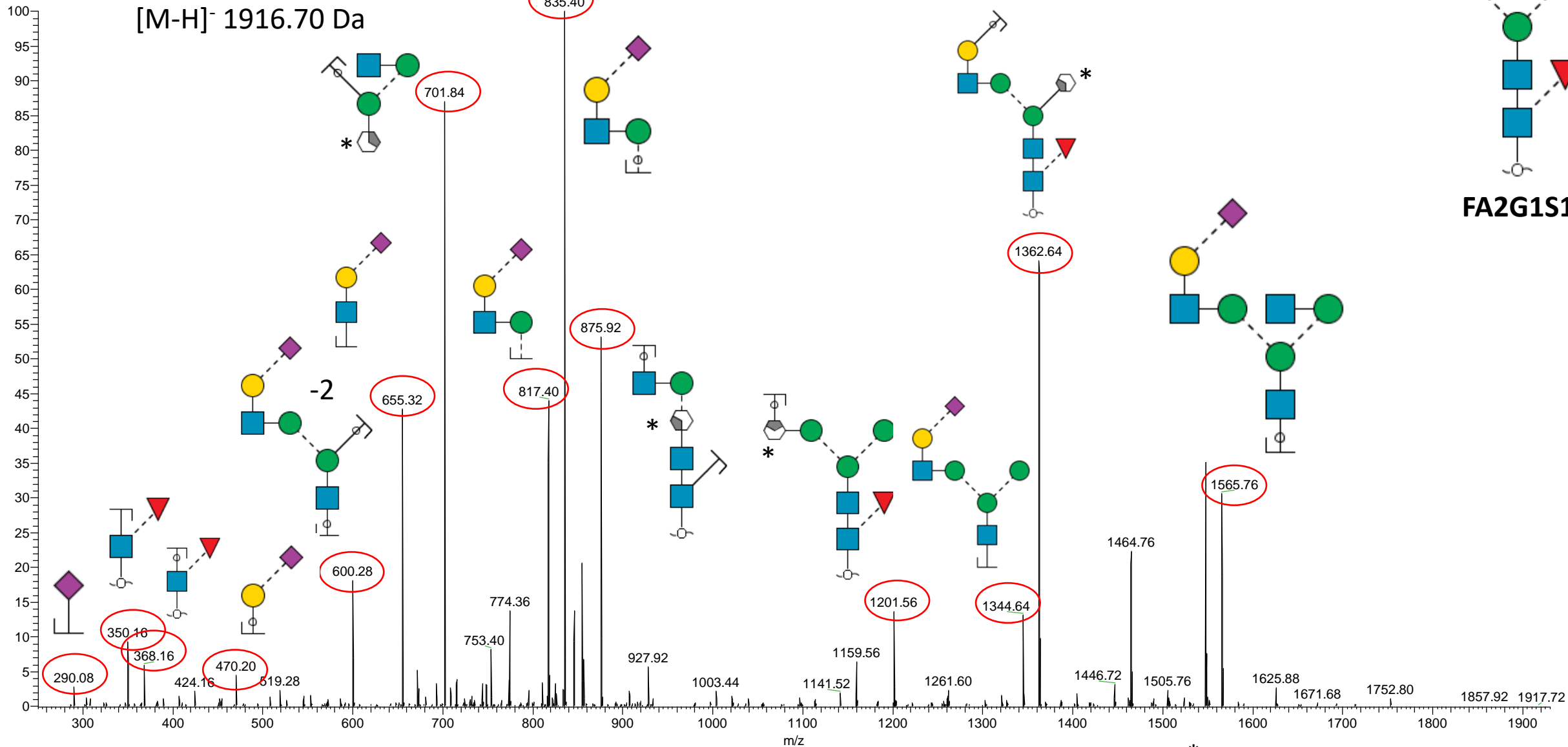
* Ambiguity of the cross-ring fragments

Glycan #20a

Note: This glycan is annotated as the α 2,6-sialyl linkage isomer based on the early PGC-LC elution and higher abundance (relative to Glycan #20b) and experience with neutrophil *N*-glycan profiling. The elongated antenna is known to frequently occupy the α 1,3-arm rather than the α 1,6-arm of neutrophil *N*-glycans and is thus represented as such.

Observed m/z 957.85 (2-), RT: ~29.3 min

[M-H]⁻ 1916.70 Da



FA2G1S1a

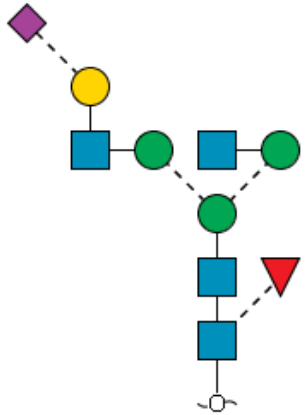
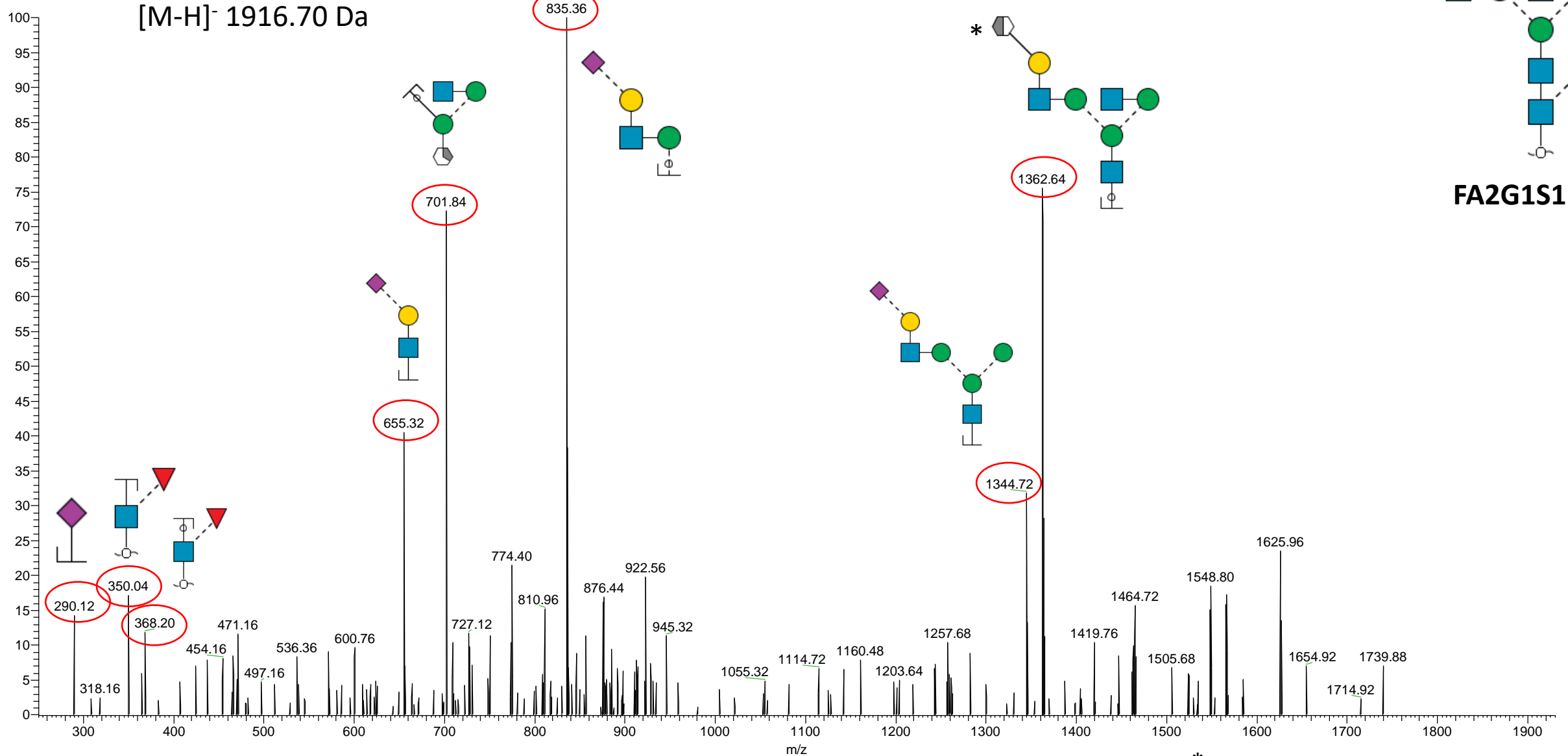
* Ambiguity of the cross-ring fragments

Note: This glycan is annotated as the α 2,3-sialyl linkage isomer based on the later PGC-LC elution and lower abundance (relative to Glycan #20a) and experience with neutrophil *N*-glycan profiling. The elongated antenna is known to frequently occupy the α 1,3-arm rather than the α 1,6-arm of neutrophil *N*-glycans and have thus been assigned as such.

Glycan #20b

Observed m/z 957.85 (2-), RT: ~37.3 min

$[M-H]^-$ 1916.70 Da



FA2G1S1b

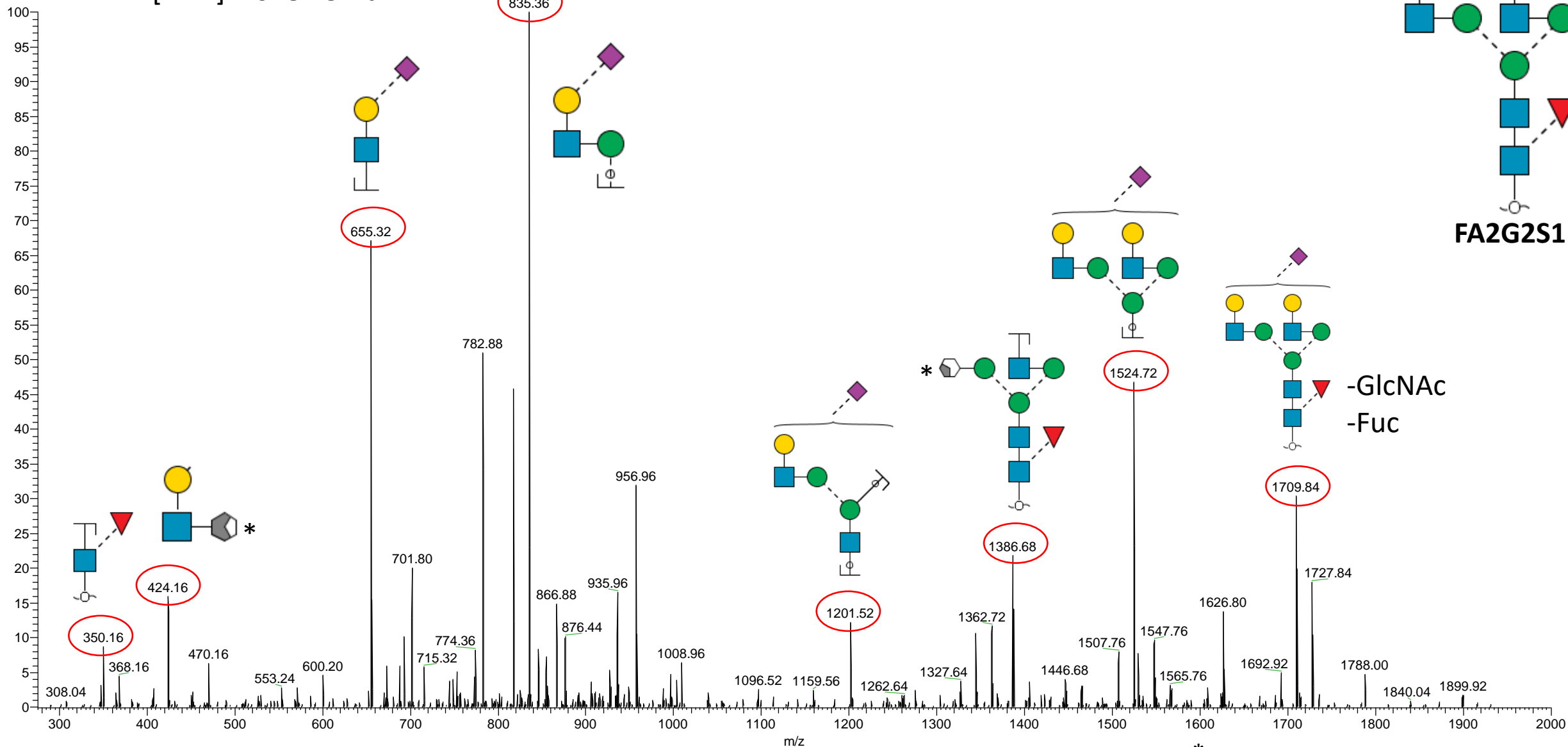
* Ambiguity in the cross-ring fragments

Glycan #21

Note: This glycan is annotated as the α 2,6-sialyl linkage isomer based on the early PGC-LC elution and experience with neutrophil *N*-glycan profiling. The arm position of the sialic acid could not be deduced.

Observed m/z 1038.88 (2-), RT: ~31.3 min

$[M-H]^-$ 2078.75 Da



-GlcNAc
-Fuc

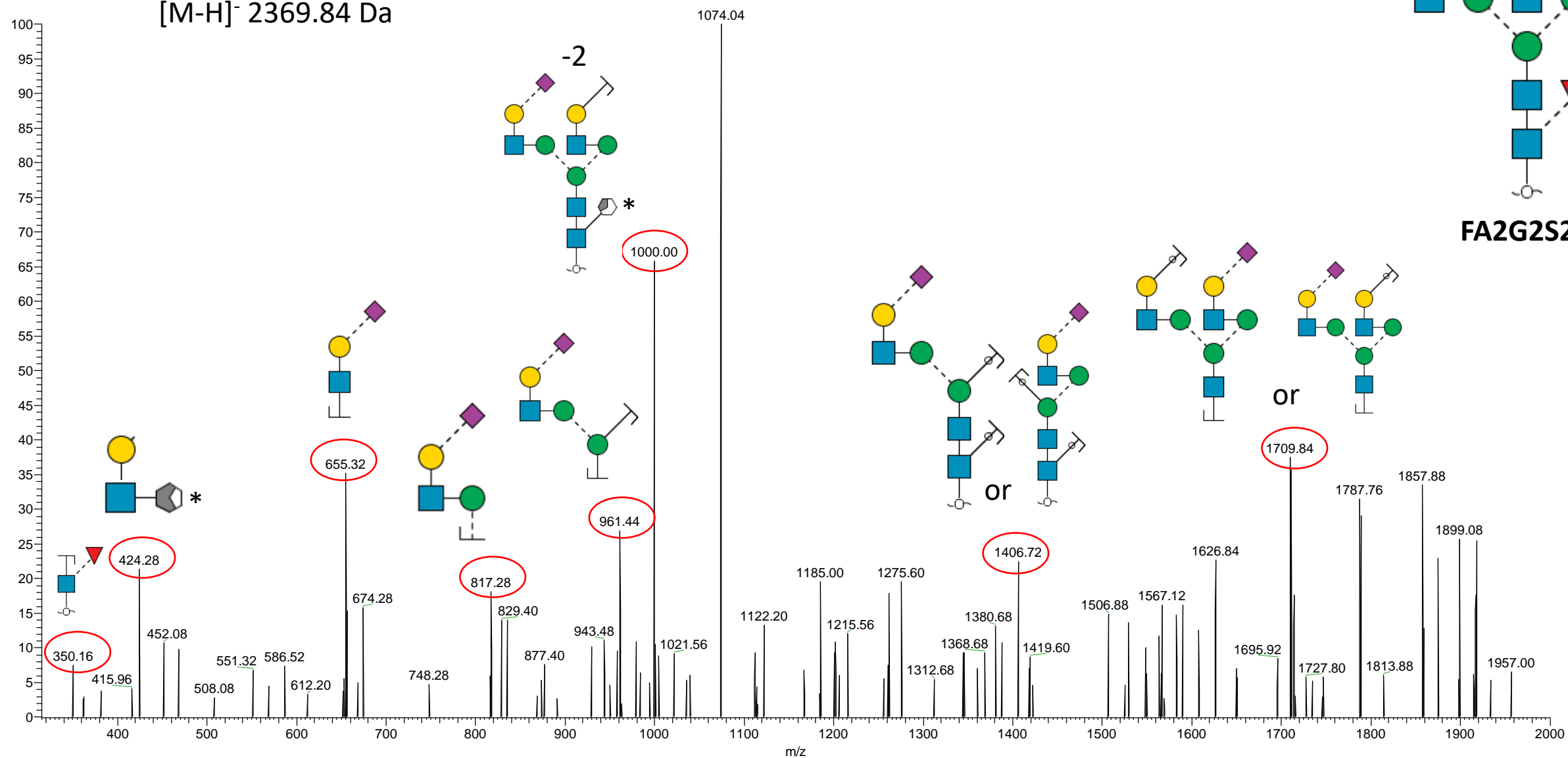
* Ambiguity in the cross-ring fragments

Note: This glycan has been annotated as the $\alpha 2,6$ -/ $\alpha 2,6$ -sialyl isomer based on the early PGC-LC elution time.

Glycan #22a

Observed m/z 1184.52 (2-), RT: ~ 31.1 min

$[M-H]^-$ 2369.84 Da



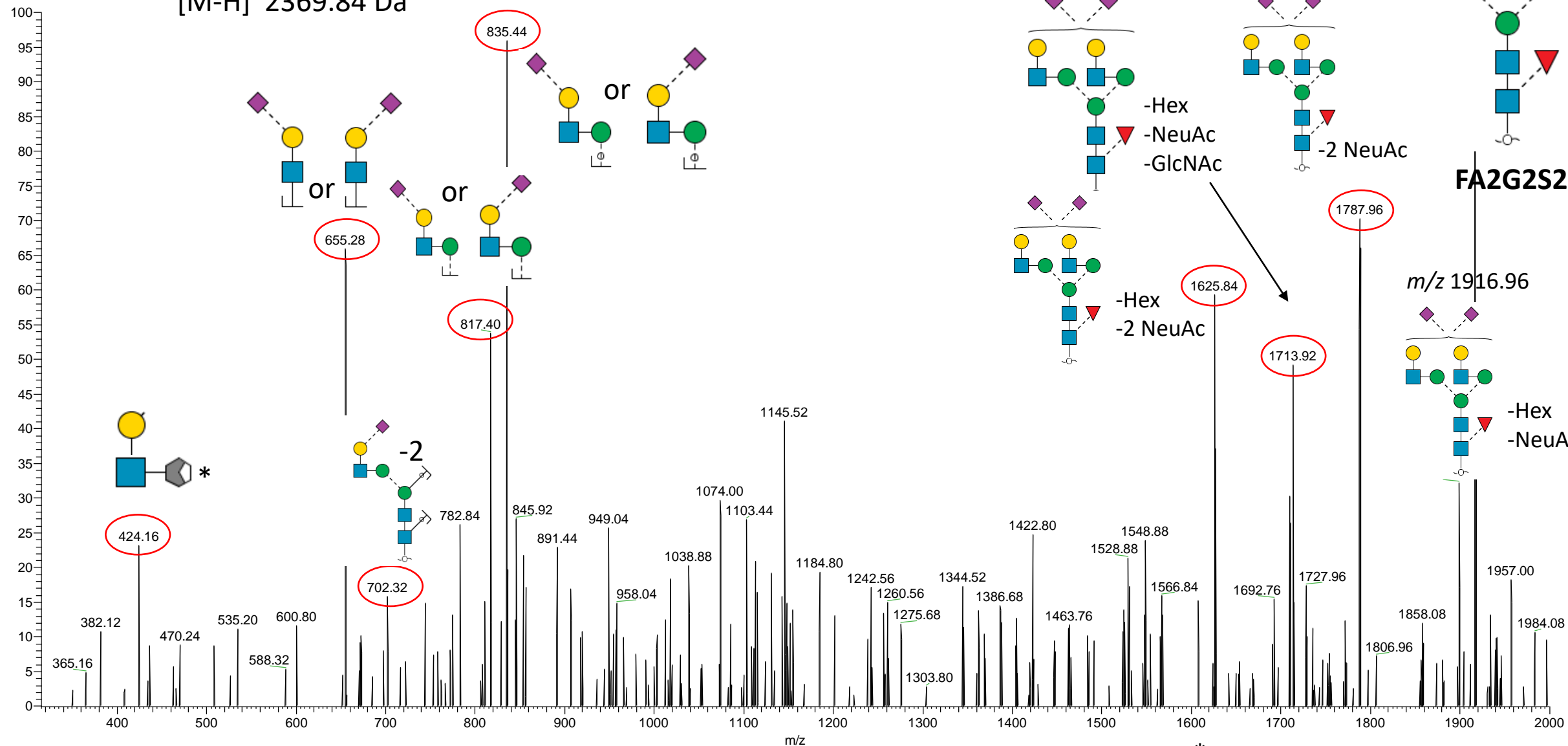
* Ambiguity of the cross-ring fragments

Glycan #22b

Note: This glycan has been annotated as the $\alpha 2,6$ -/ $\alpha 2,3$ -sialyl isomer based on the late PGC-LC elution time (relative to Glycan #22a) and experience with neutrophil *N*-glycosylation. Not possible to position the $\alpha 2,6$ - and $\alpha 2,3$ -sialic acid to specific arms.

Observed m/z 1184.52 (2-), RT: ~ 39.4 min

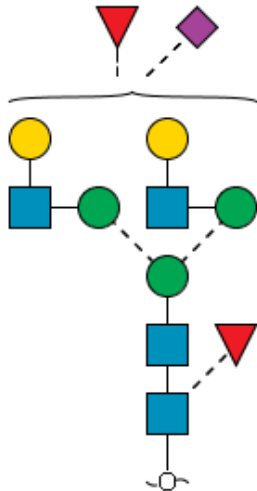
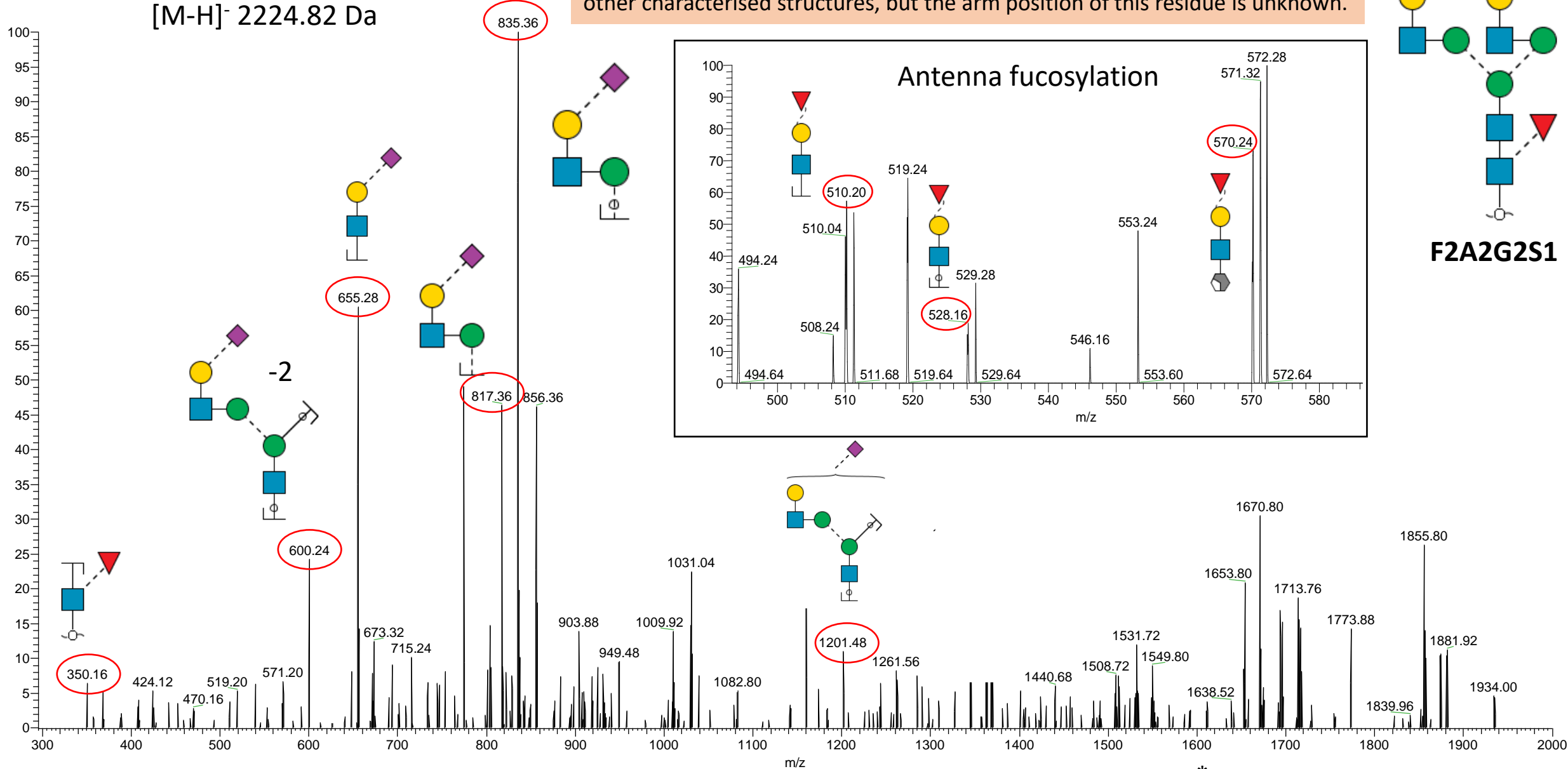
$[M-H]^-$ 2369.84 Da



Glycan #23

Observed m/z 1111.91 (2-), RT: ~29.9 min
[M-H]⁻ 2224.82 Da

Note: No MS/MS evidence to support fucosyl-linkage type or the position of this outer arm residue, hence these features relating to the fucosyl moiety have been left unassigned. The sialyl-linkage can be predicted to be α 2,6-sialyl from other characterised structures, but the arm position of this residue is unknown.



* Ambiguity of the cross-ring fragments

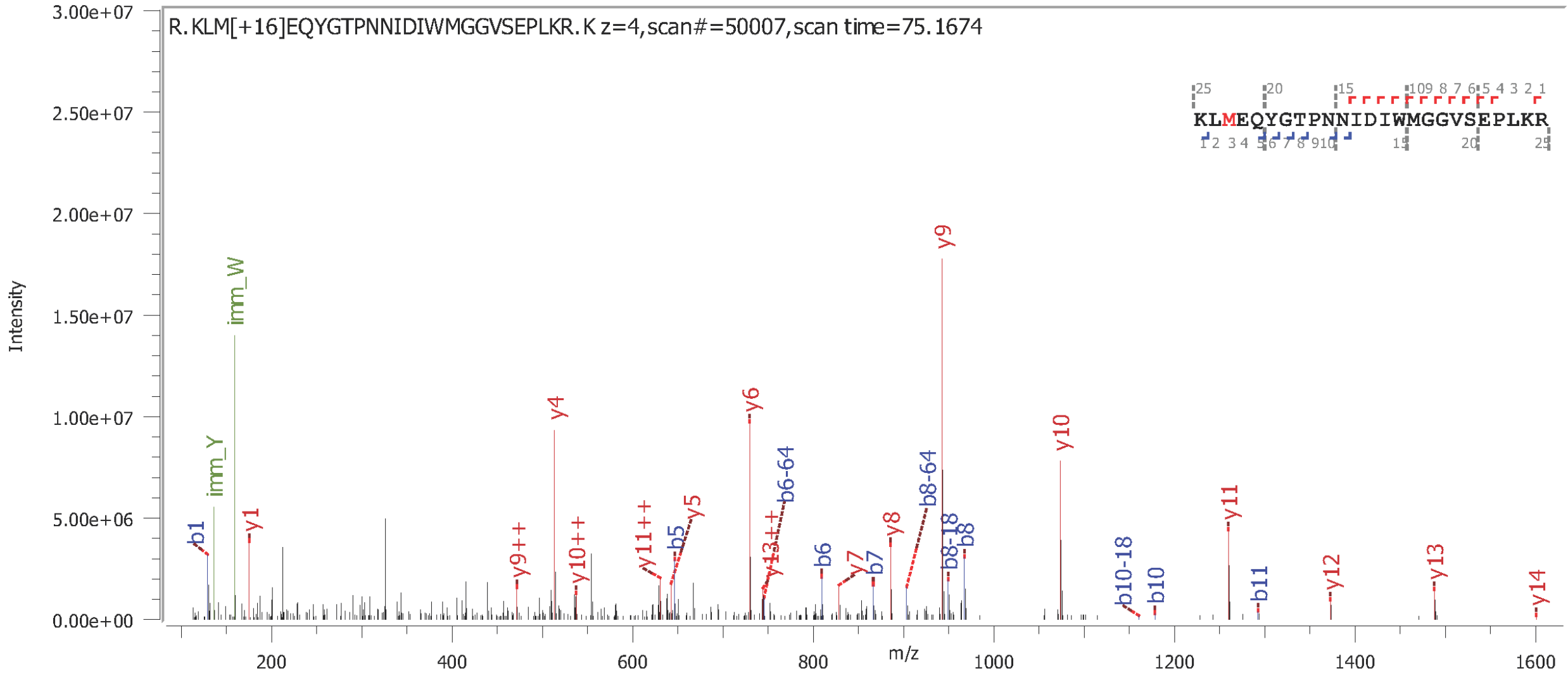
Supplementary Data S2A

Examples of Byonic-annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of peptides identified with non-glyco PTMs (i.e. Met and Trp mono- and di-oxidation and Tyr mono-chlorination) from the analysis of unenriched peptide mixtures of nMPO

Human myeloperoxidase (P05164)

Mono-oxidised methionine-containing peptide

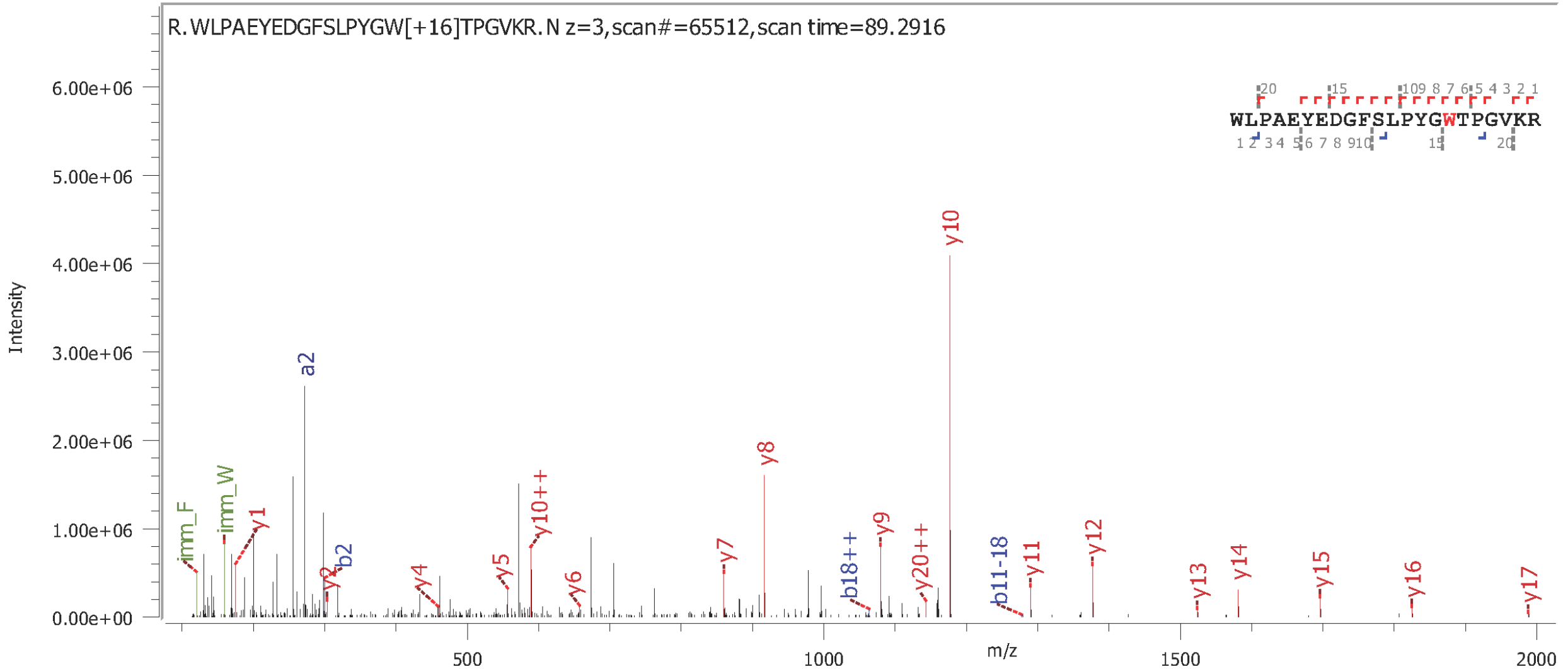
⁶²⁹KLMEQYGTPNNIDIWMGGVSEPLKR⁶⁴¹ (identification confidence level, PEP 2D: 2.4×10^{-20})



Human myeloperoxidase (P05164)

Mono-oxidised tryptophan-containing peptide

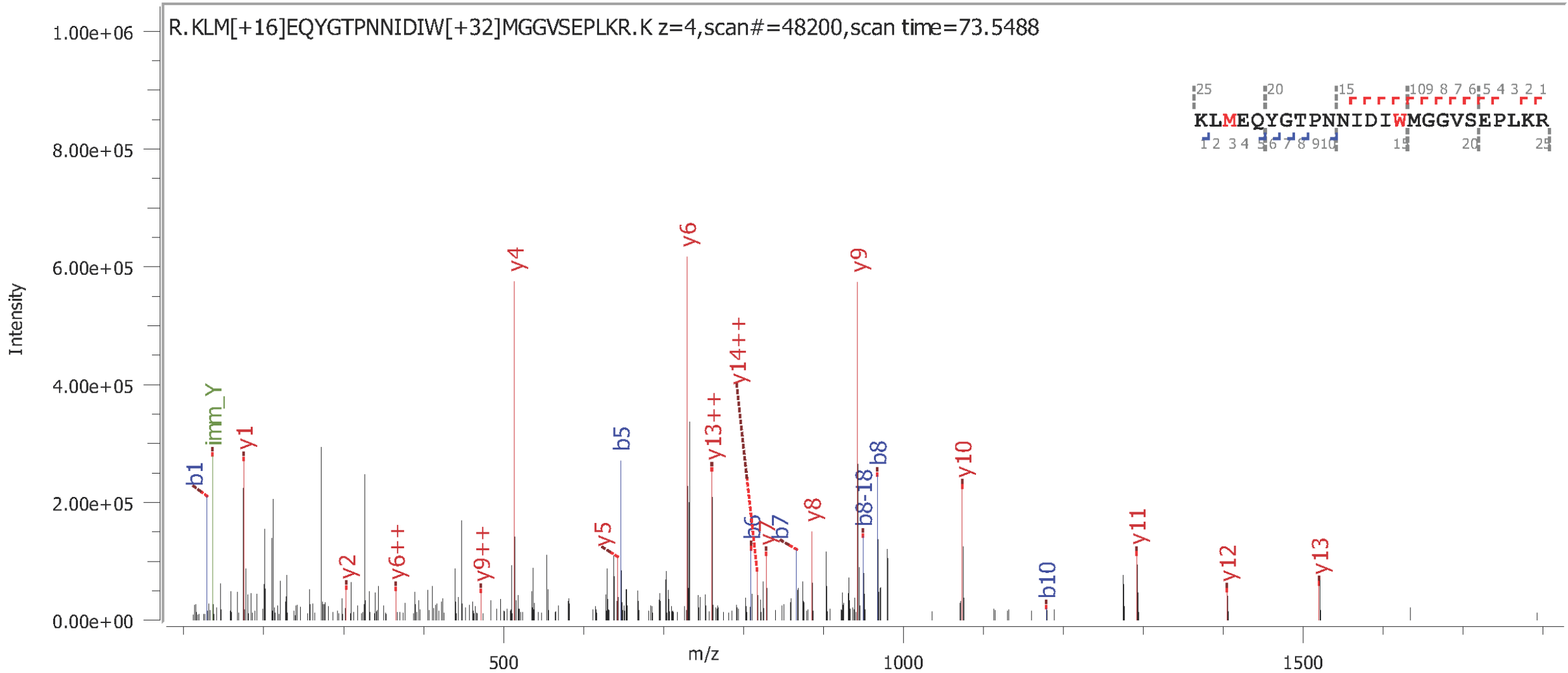
$^{198}\text{WLP AEYEDGFSLPYG}\underline{\text{W}}\text{TPGVKR}^{219}$ (identification confidence level, PEP 2D: 1.9×10^{-15})



Human myeloperoxidase (P05164)

Di-oxidised tryptophan-containing peptide

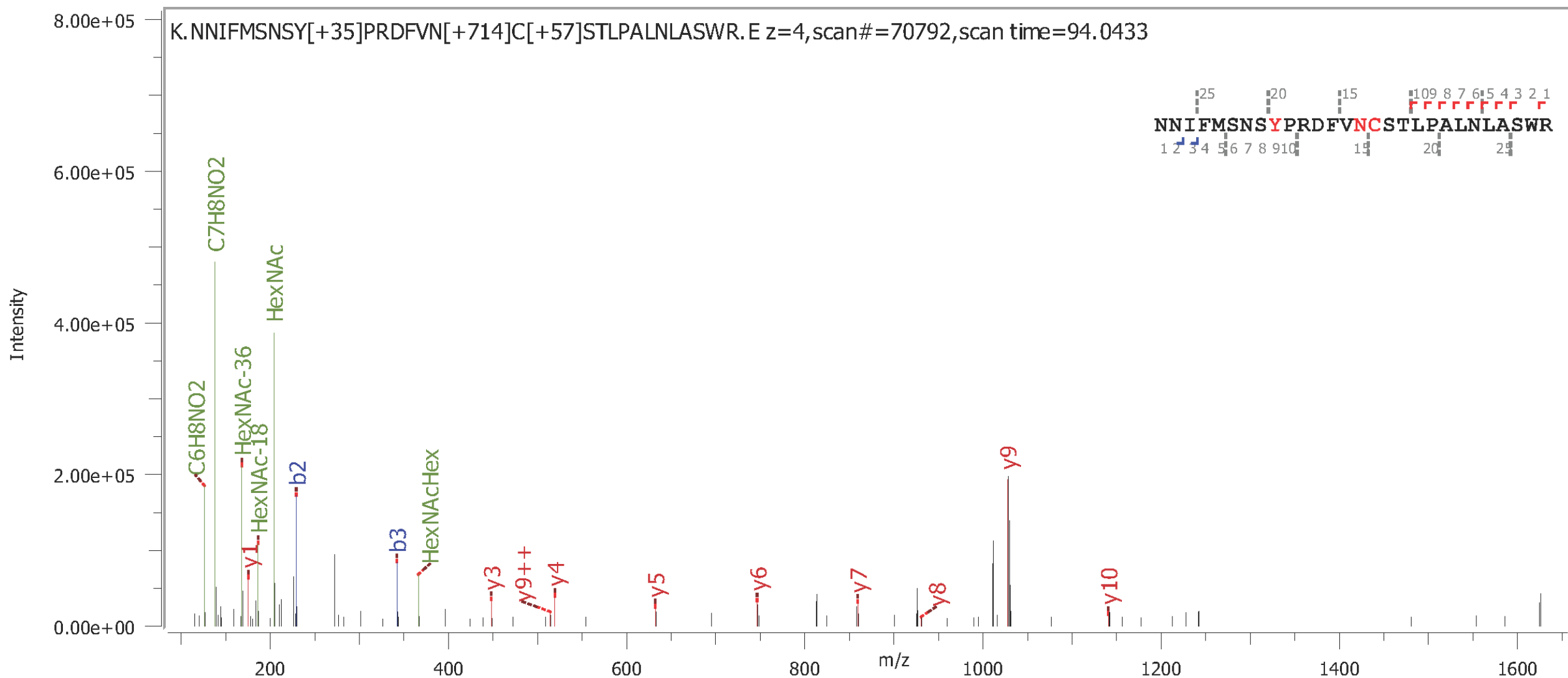
⁶²⁹KLMEQYGTPNNIDIWMGGVSEPLKR⁶⁴¹ (identification confidence level, PEP 2D: 1.9x10⁻¹⁸)



Human myeloperoxidase (P05164)

Mono-chlorinated tyrosine-containing peptide

715 NNIFMSNS**Y**PRDFVNCSTLPALNLSWR 742 (identification confidence level, PEP 2D: 3.3×10^{-8})



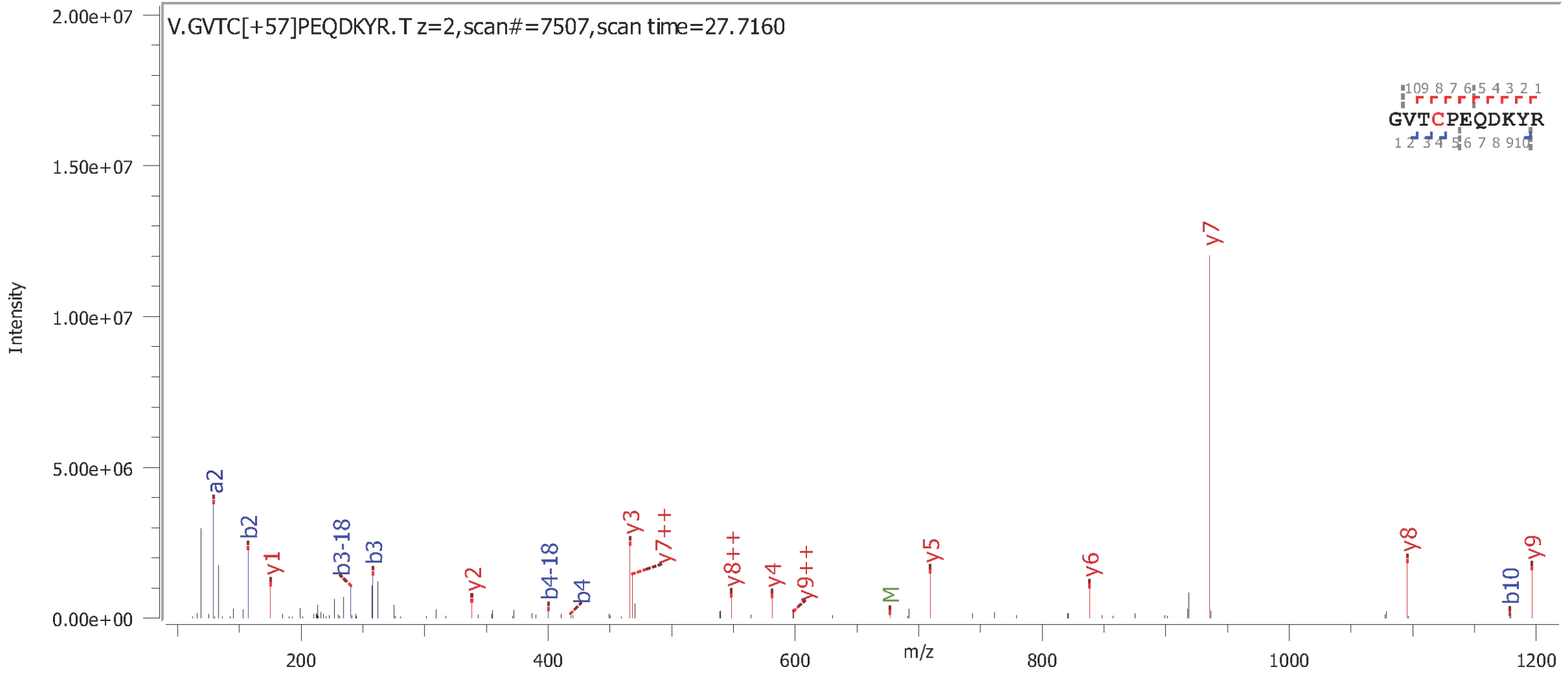
Supplementary Data S2B

Byonic-annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of N- and C-terminal truncation variants of the MPO α - and β -chain identified from the analysis of unenriched peptide mixtures of nMPO

Human myeloperoxidase (P05164)

α-chain N-terminal (truncation variant)

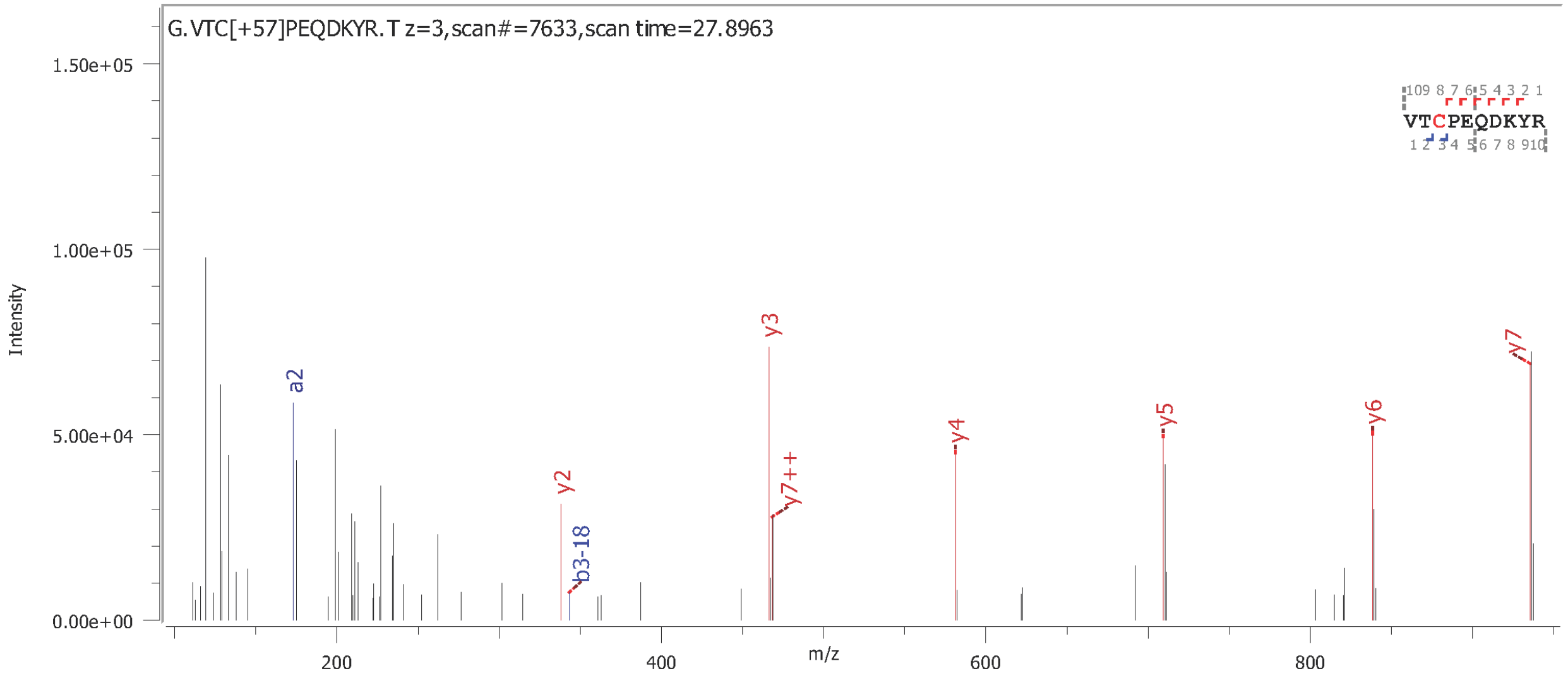
¹⁶⁴GVTCPEQDKYR (identification confidence level, PEP 2D: 4.0x10⁻¹¹)



Human myeloperoxidase (P05164)

α-chain N-terminal (truncation variant)

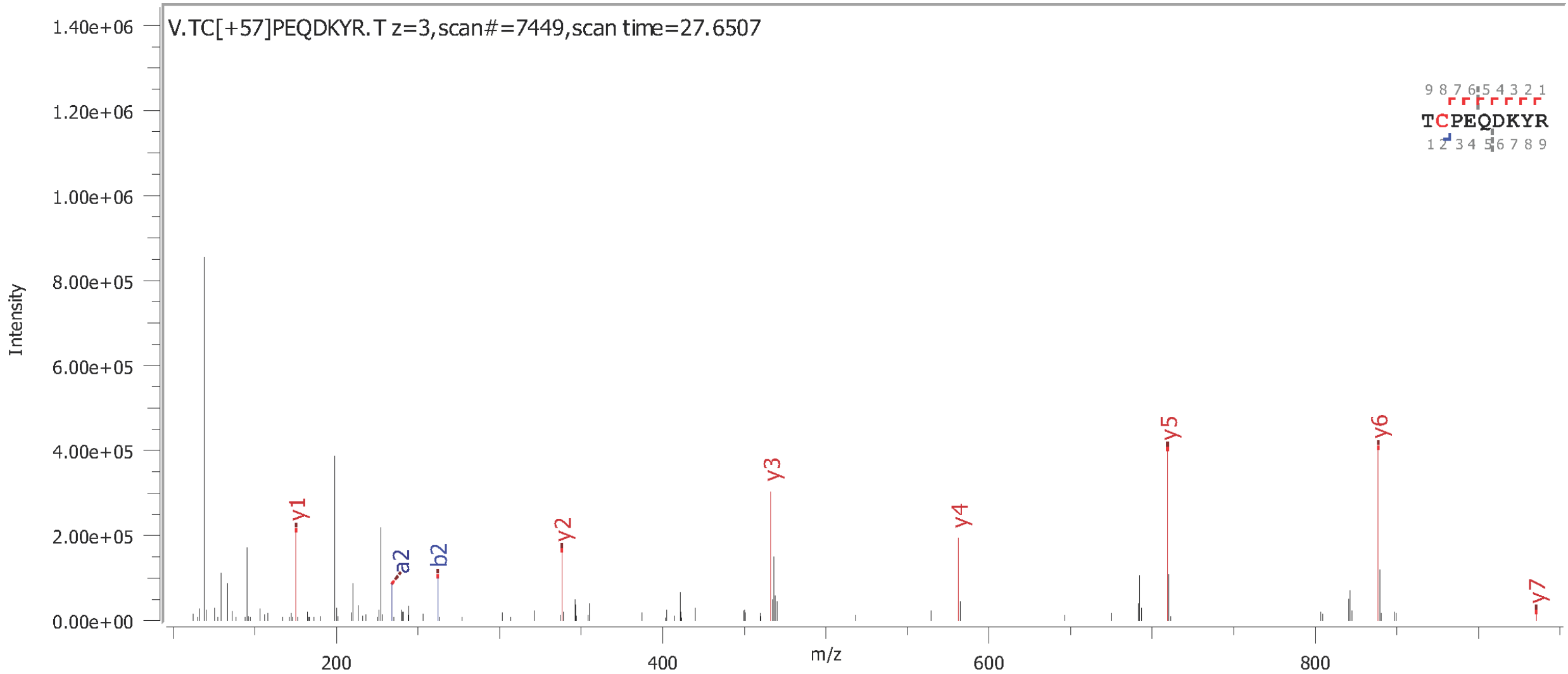
¹⁶⁵VTCPEQDKYR (identification confidence level, PEP 2D: 5.7 x 10⁻⁷)



Human myeloperoxidase (P05164)

α -chain N-terminal (truncation variant)

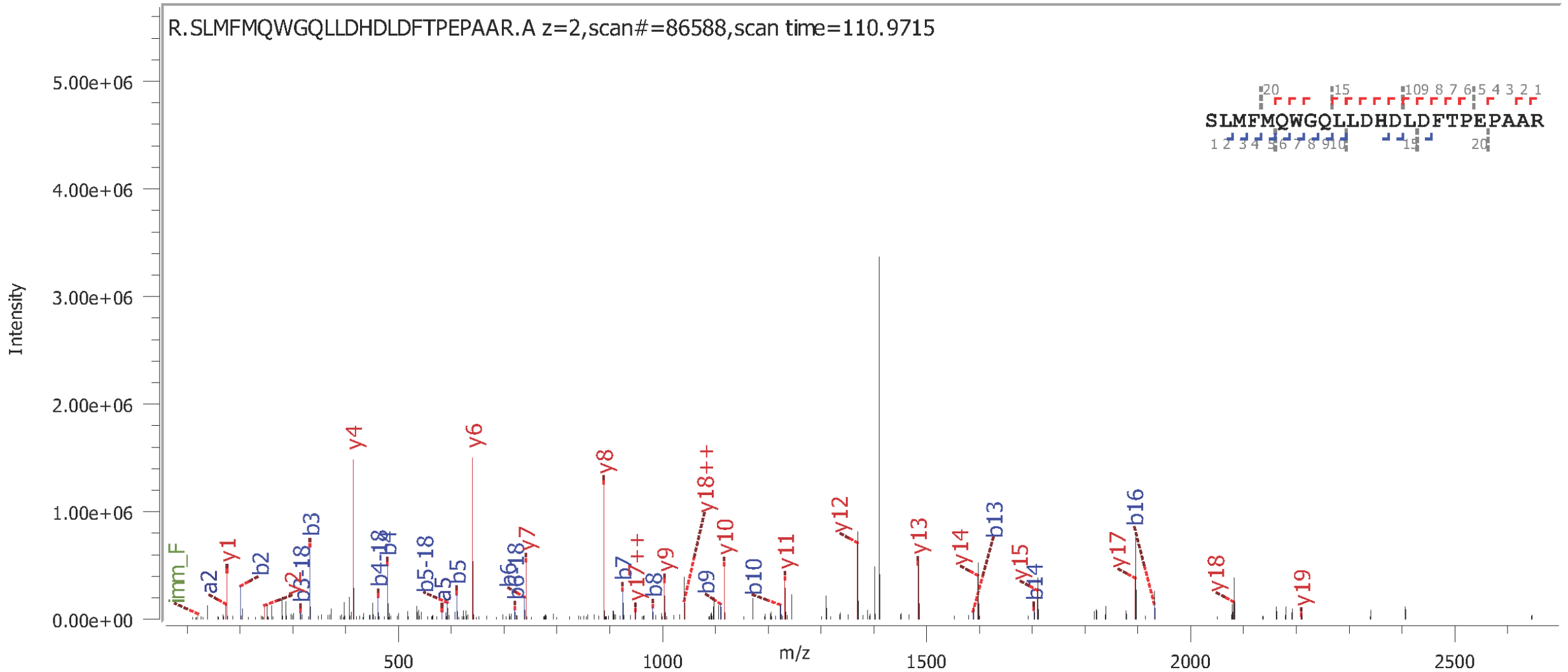
¹⁶⁶TCP¹⁶⁶EQDKYR (identification confidence level, PEP 2D: 3.3×10^{-8})



Human myeloperoxidase (P05164)

α -chain N-terminal (truncation variant)

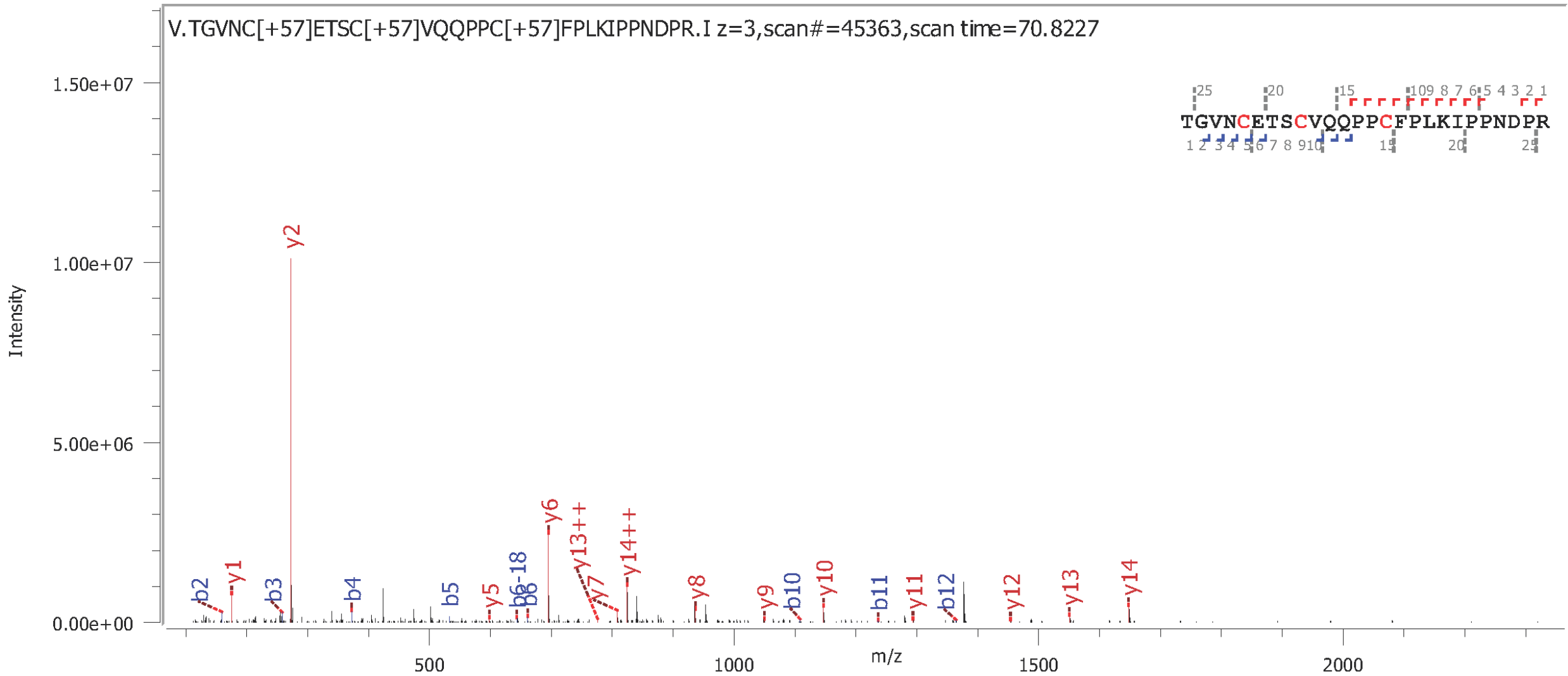
SLMFMQWGQLLDHLDFTPEPAAR^{R272} (identification confidence level, PEP 2D: 1.9×10^{-23})



Human myeloperoxidase (P05164)

β -chain N-terminal (truncation variant)

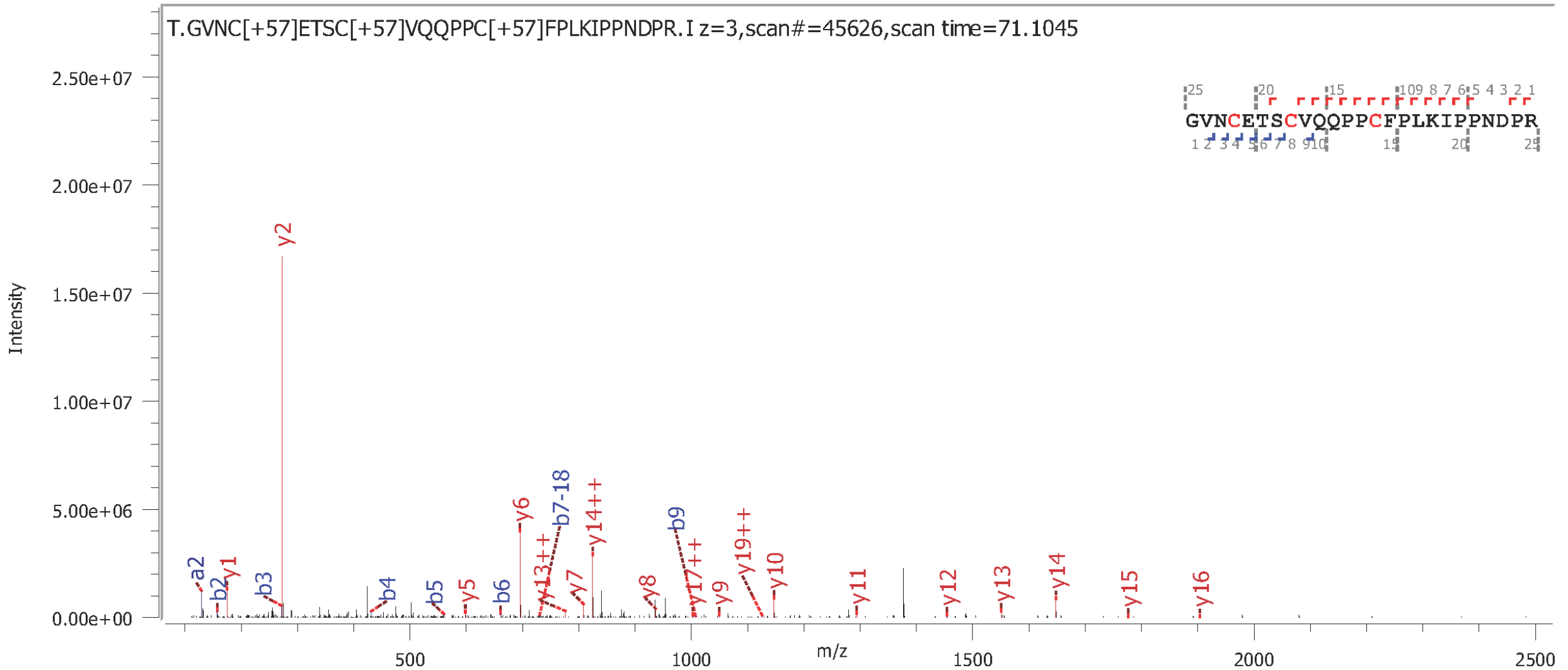
²⁷⁷TGVNCETSCVQQPPCFPLKIPPNDPR (identification confidence level, PEP 2D: 8.7×10^{-14})



Human myeloperoxidase (P05164)

β -chain N-termini (truncation variant)

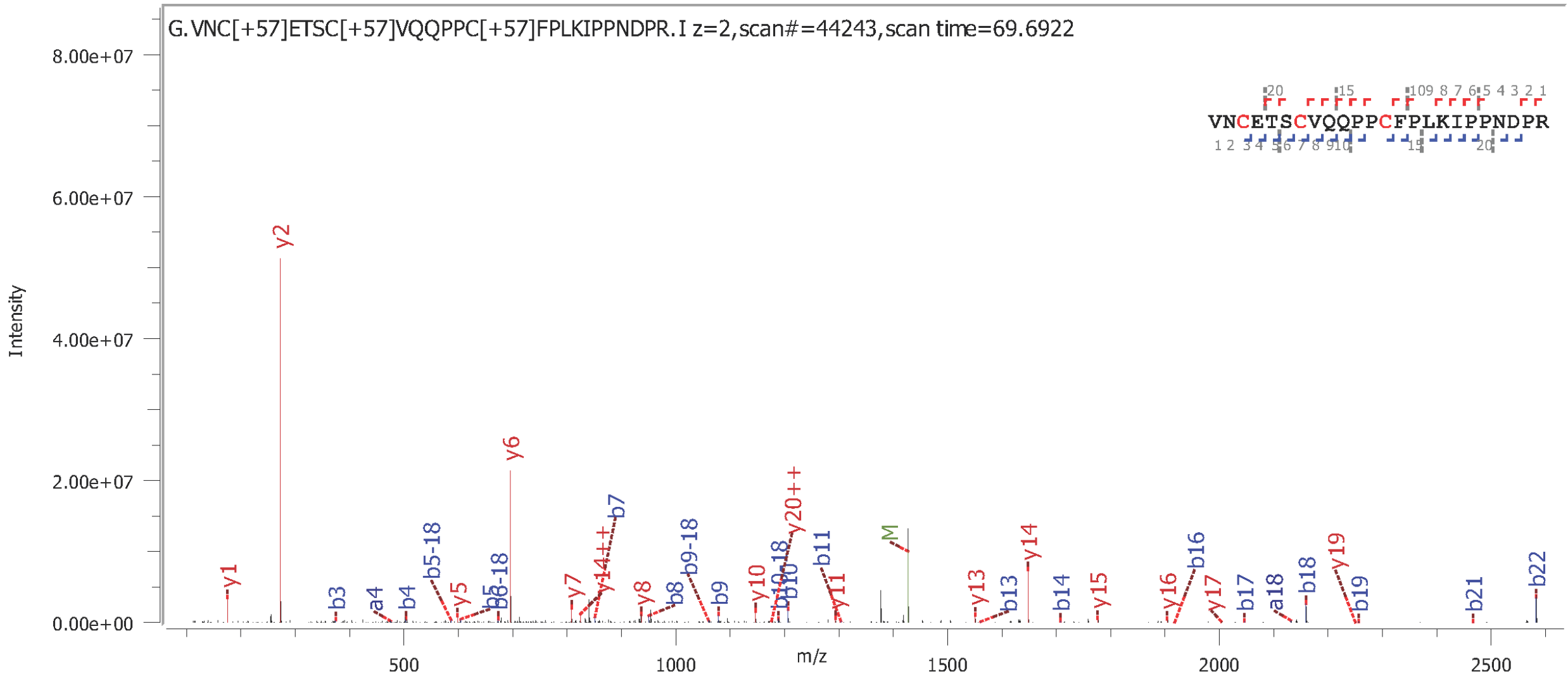
²⁷⁸G VNCETSCVQQPPCFPLKIPPNDPR (identification confidence level, PEP 2D: 1.1×10^{-15})



Human myeloperoxidase (P05164)

β -chain N-terminal (truncation variant)

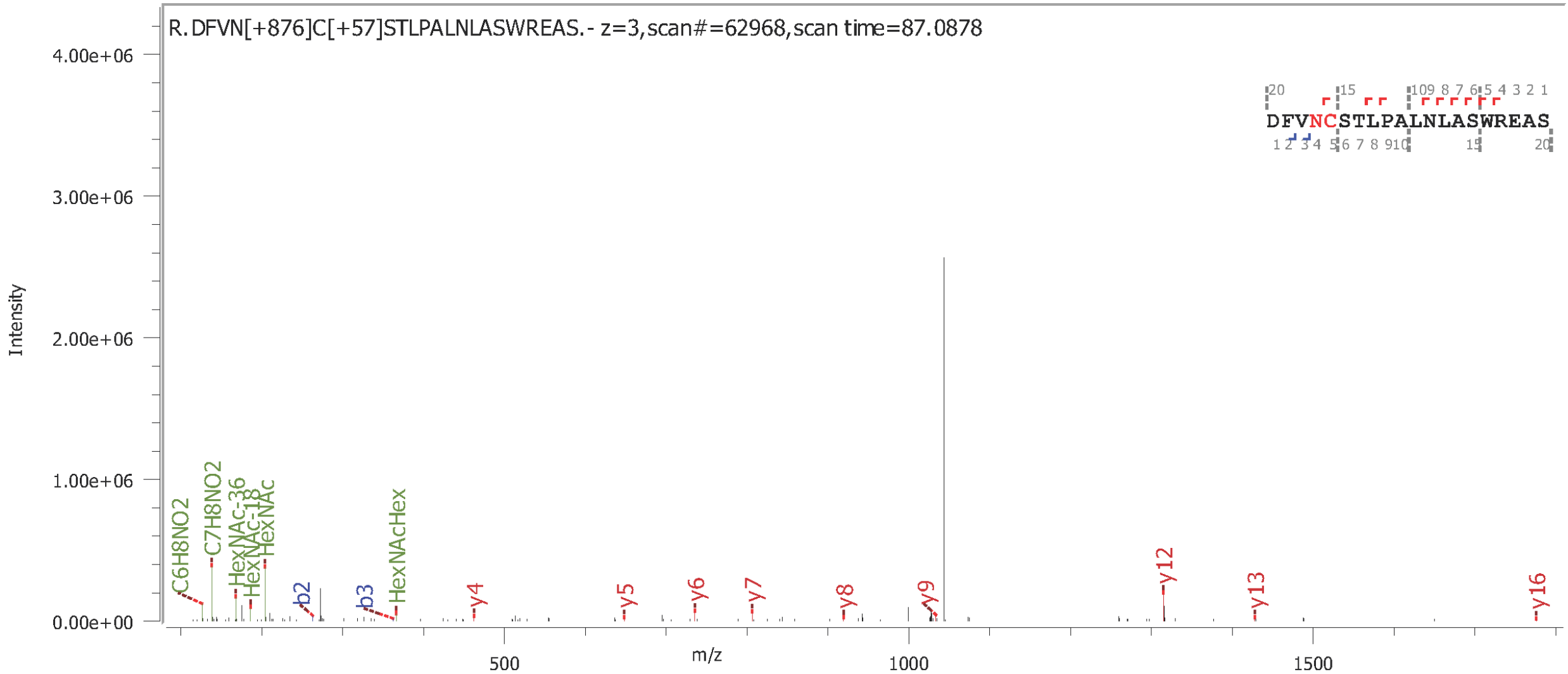
²⁷⁹VNCETSCVQQPPCFPLKIPPNDPR (identification confidence level, PEP 2D: 1.5×10^{-17})



Human myeloperoxidase (P05164)

β -chain C-terminal

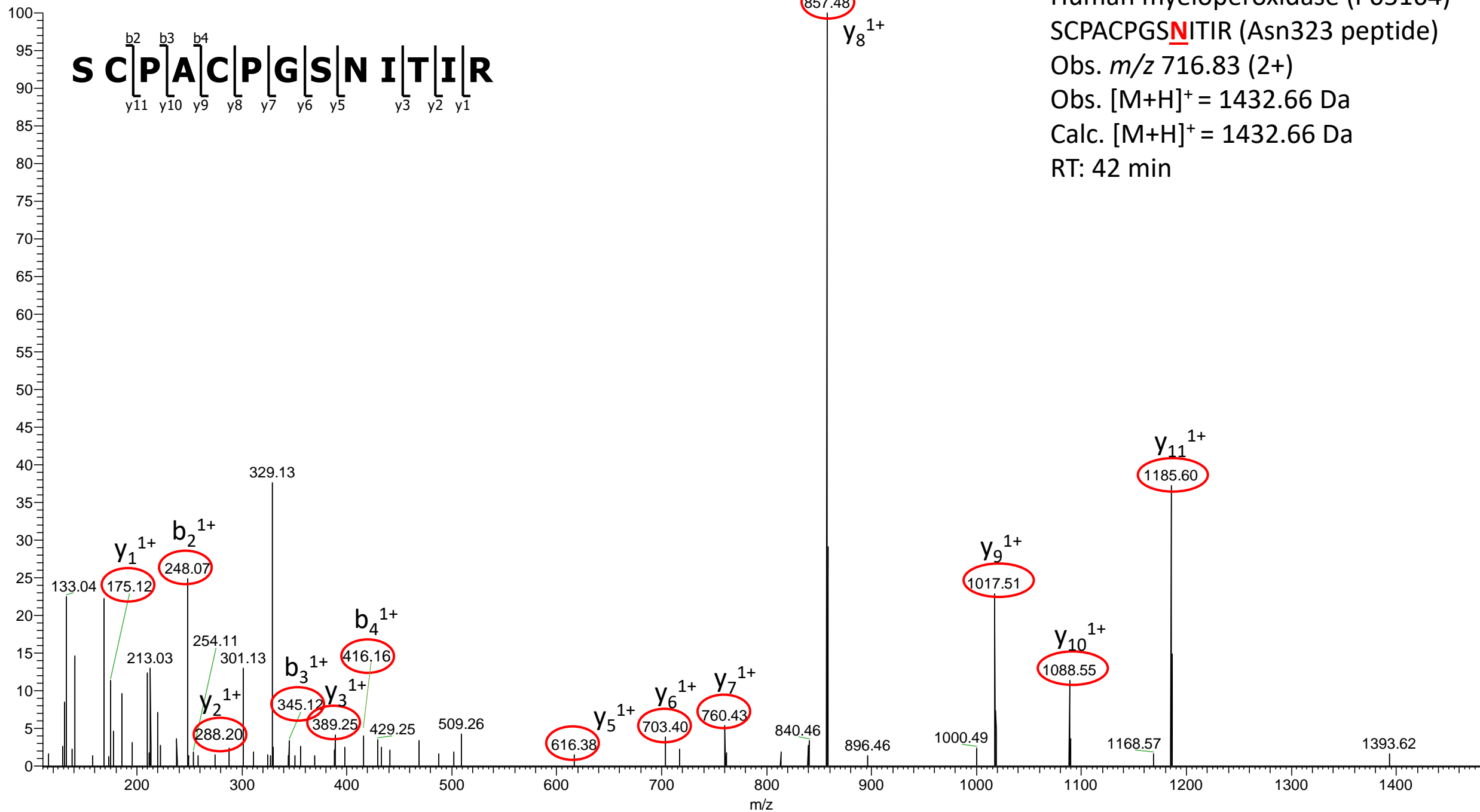
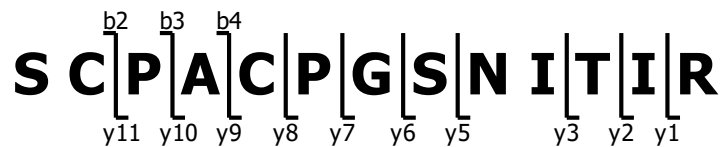
DFVNCSTLPALNLASWREAS^{S745} (identification confidence level, PEP 2D: 1.4×10^{-8})

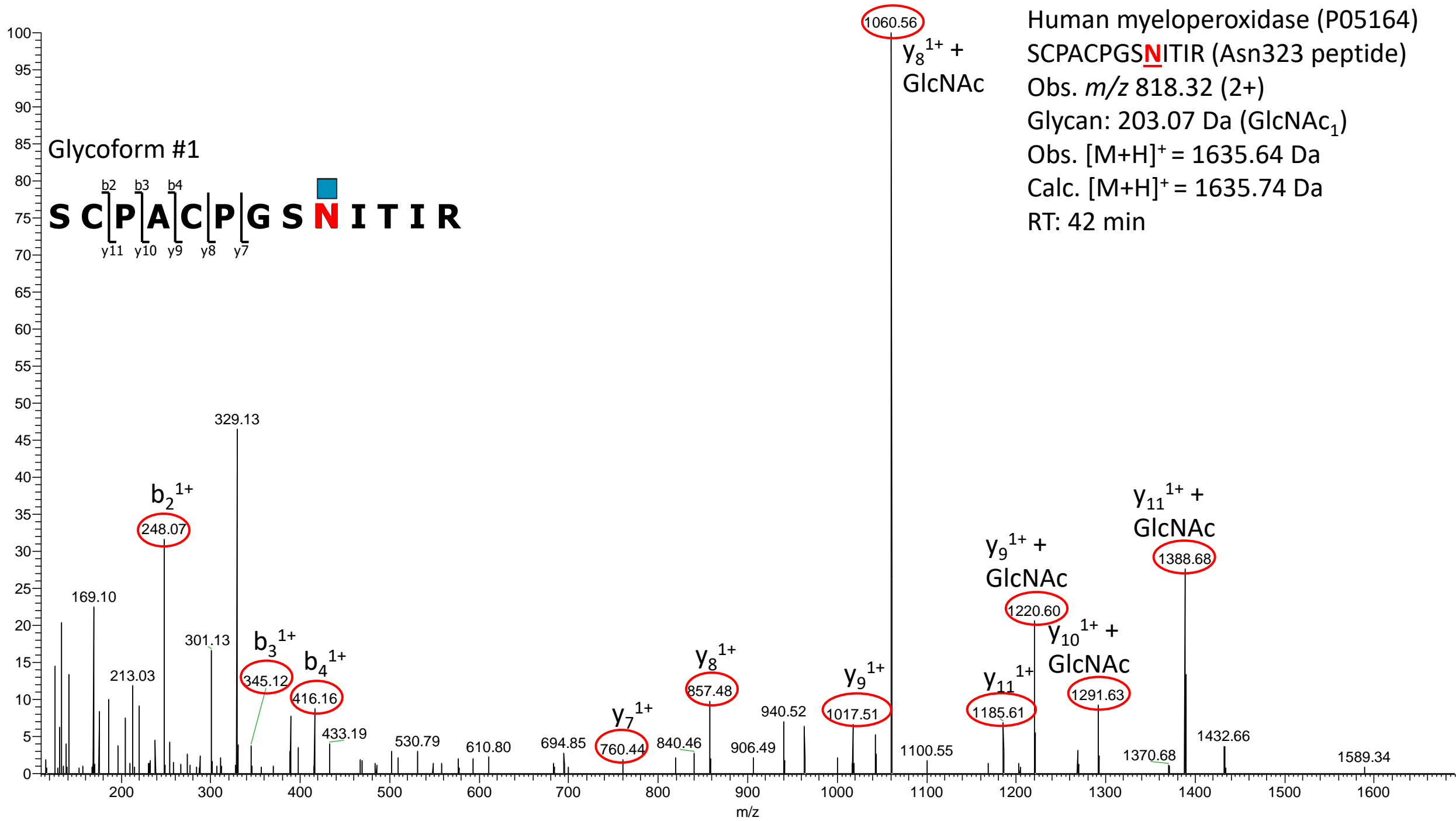


Supplementary Data S3A

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn323 identified from the analysis of the unenriched peptide mixtures of nMPO.

Non-glycosylated peptide





Human myeloperoxidase (P05164)

SCPACPGS**N**ITIR (Asn323 peptide)

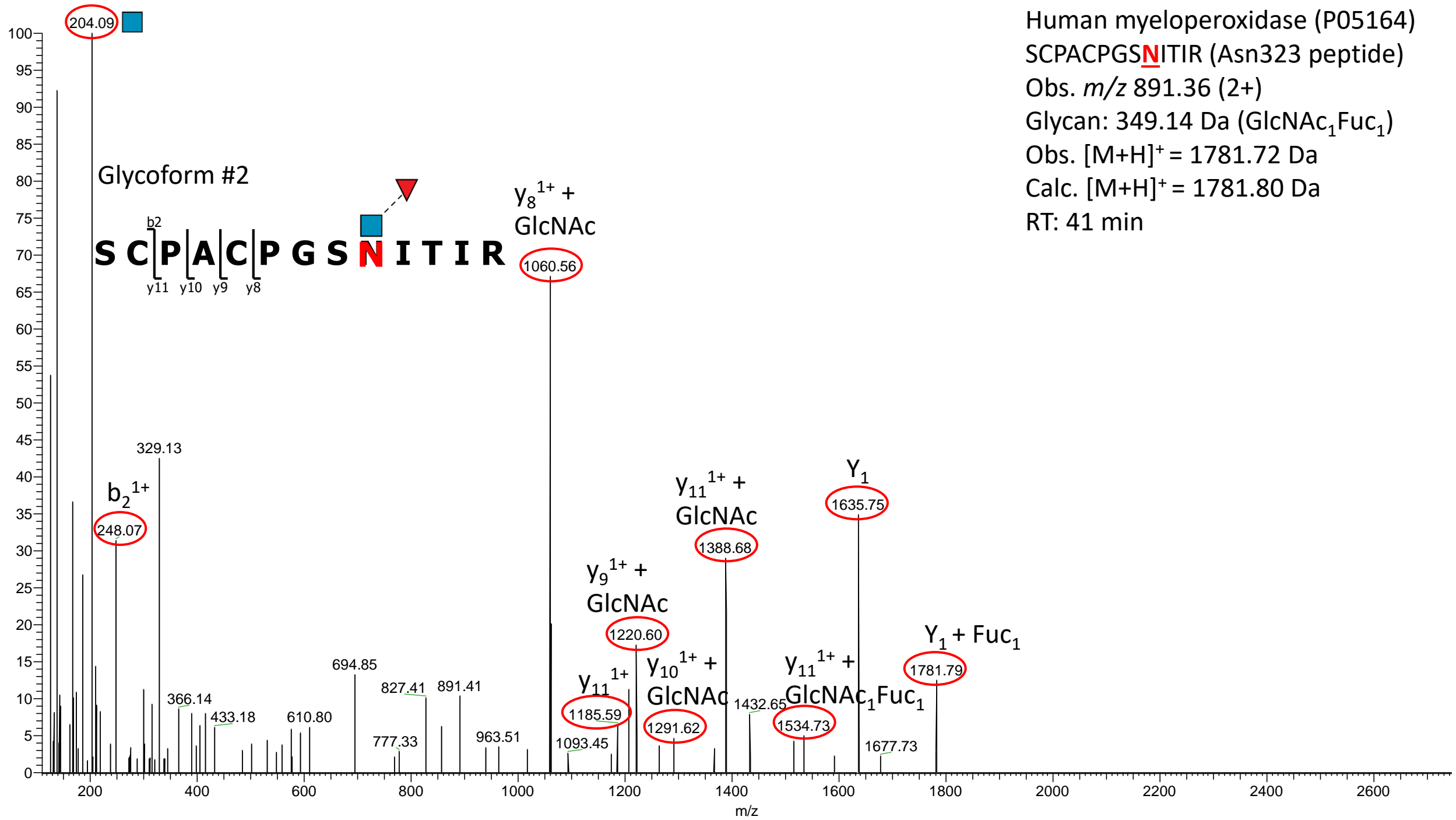
Obs. m/z 891.36 (2+)

Glycan: 349.14 Da (GlcNAc₁Fuc₁)

Obs. $[M+H]^+ = 1781.72$ Da

Calc. $[M+H]^+ = 1781.80$ Da

RT: 41 min



Human myeloperoxidase (P05164)

SCPACPGS**N**ITIR (Asn323 peptide)

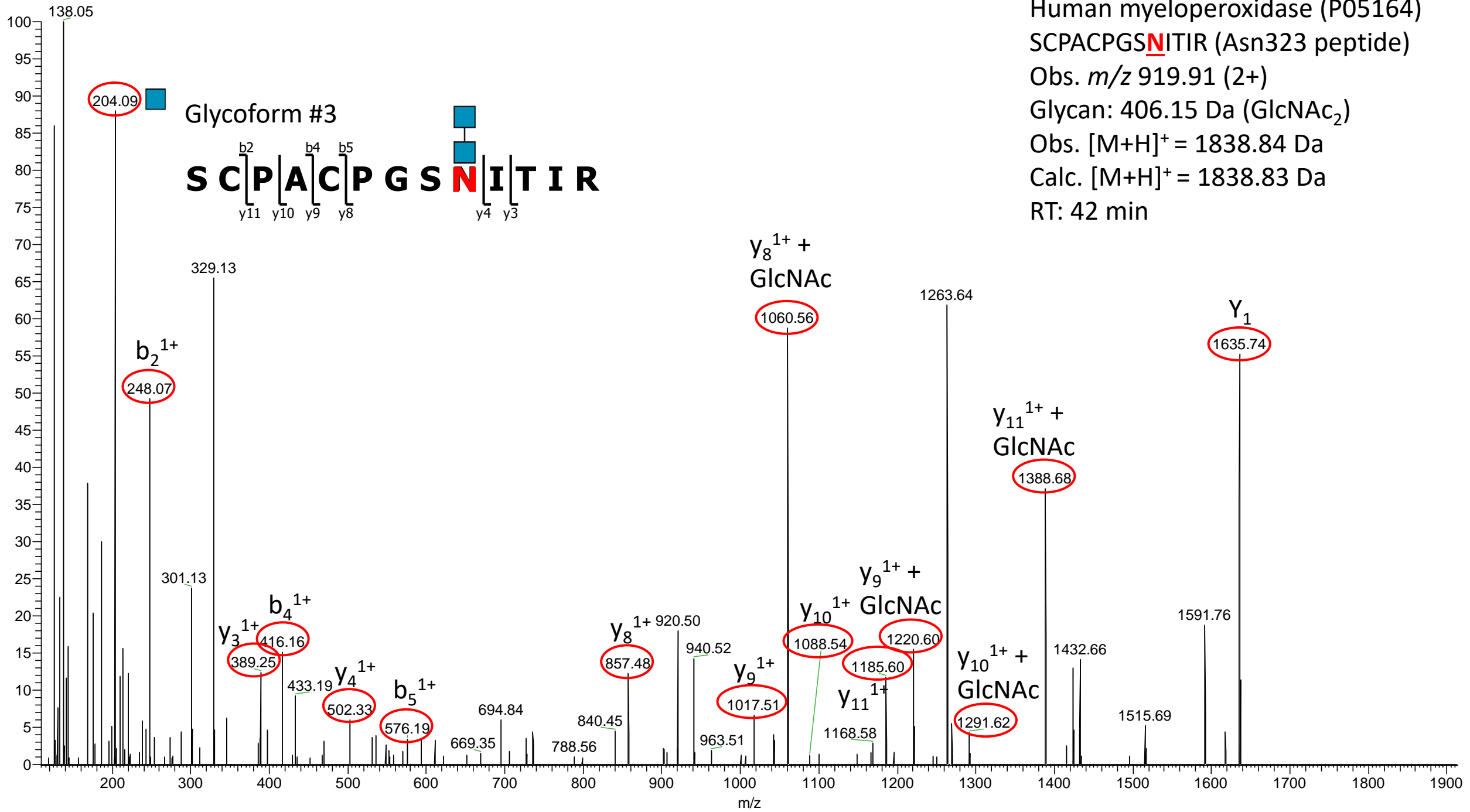
Obs. m/z 919.91 (2+)

Glycan: 406.15 Da (GlcNAc₂)

Obs. $[M+H]^+$ = 1838.84 Da

Calc. $[M+H]^+$ = 1838.83 Da

RT: 42 min



Human myeloperoxidase (P05164)

SCPACPGS**N**ITIR (Asn323 peptide)

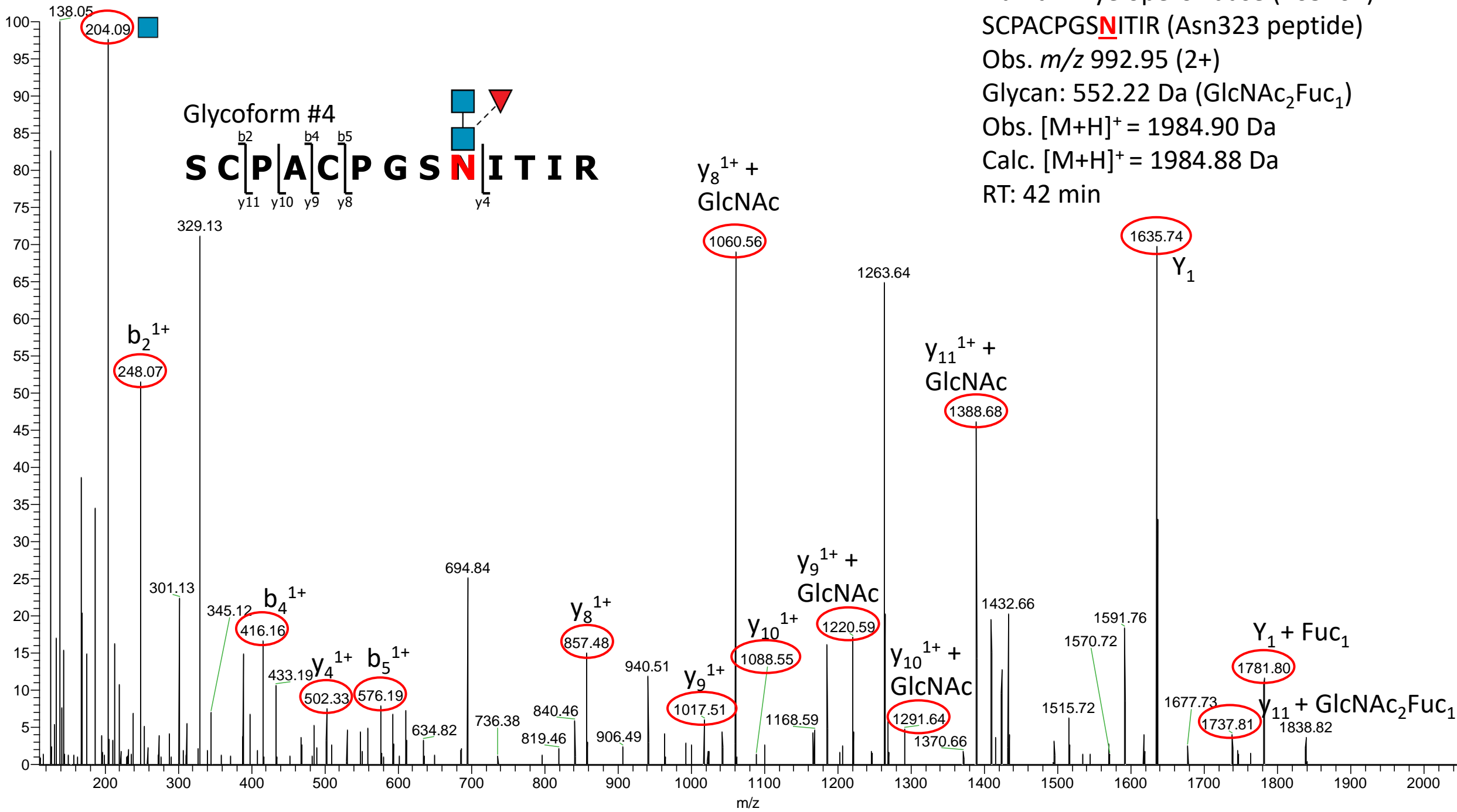
Obs. m/z 992.95 (2+)

Glycan: 552.22 Da (GlcNAc₂Fuc₁)

Obs. $[M+H]^+$ = 1984.90 Da

Calc. $[M+H]^+$ = 1984.88 Da

RT: 42 min



Human myeloperoxidase (P05164)

SCPACPGS**N**ITIR (Asn323 peptide)

Obs. m/z 1000.93 (2+)

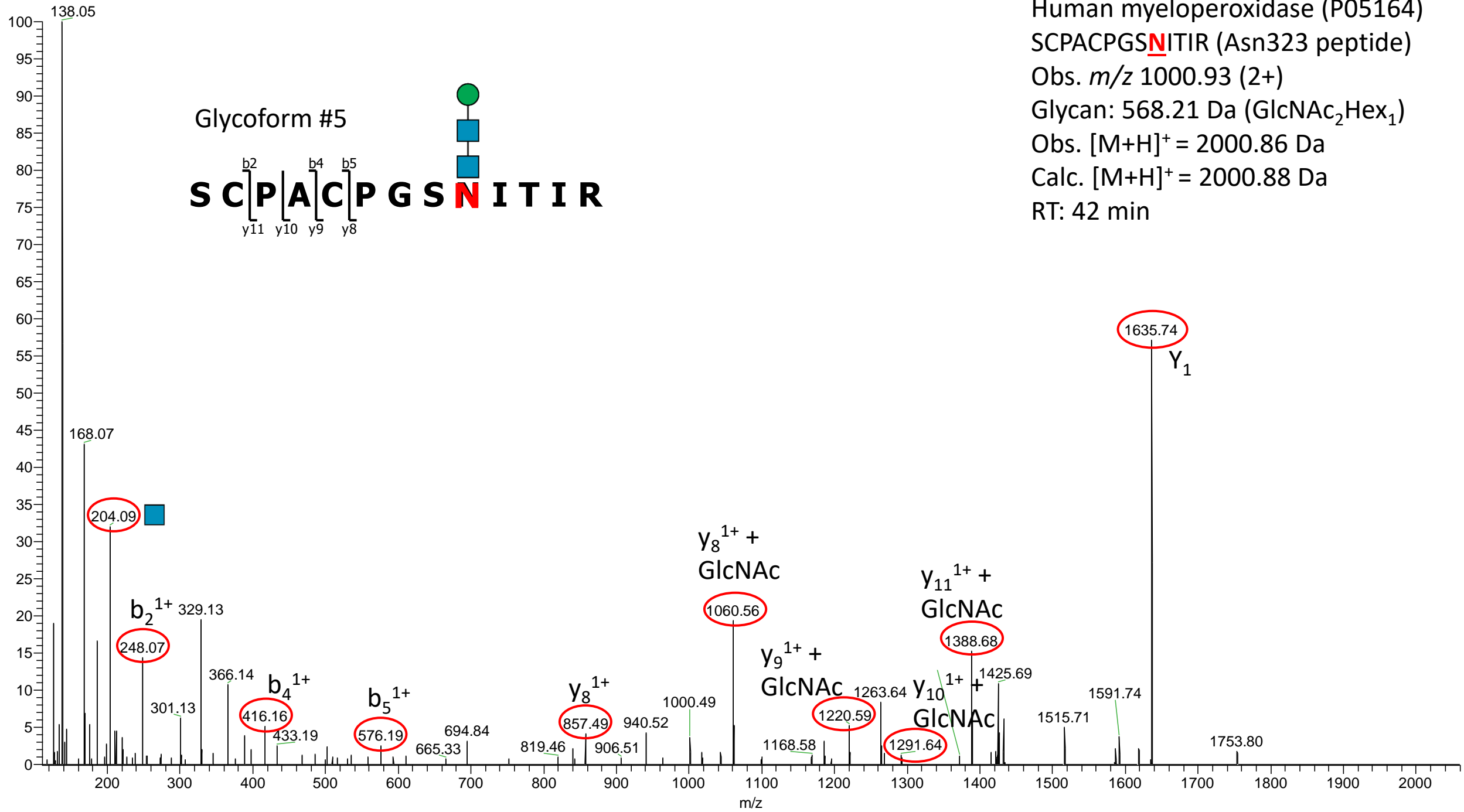
Glycan: 568.21 Da (GlcNAc₂Hex₁)

Obs. $[M+H]^+$ = 2000.86 Da

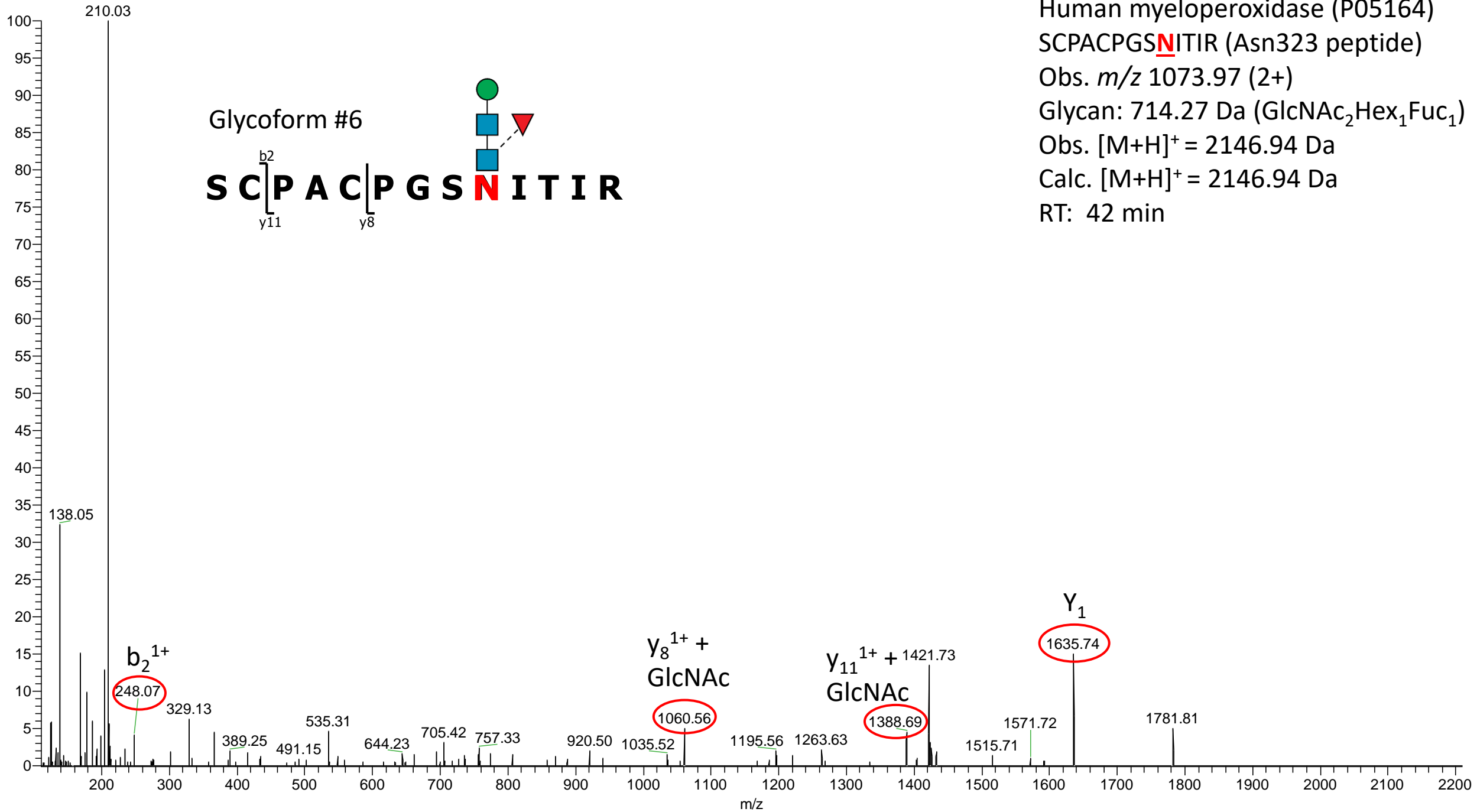
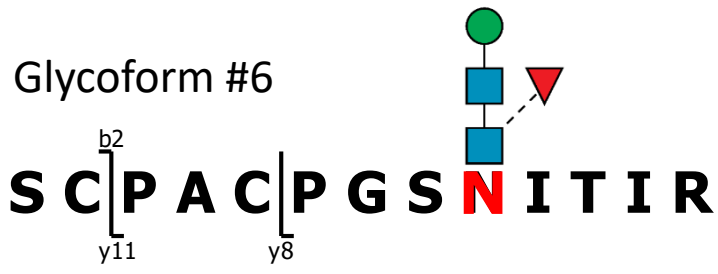
Calc. $[M+H]^+$ = 2000.88 Da

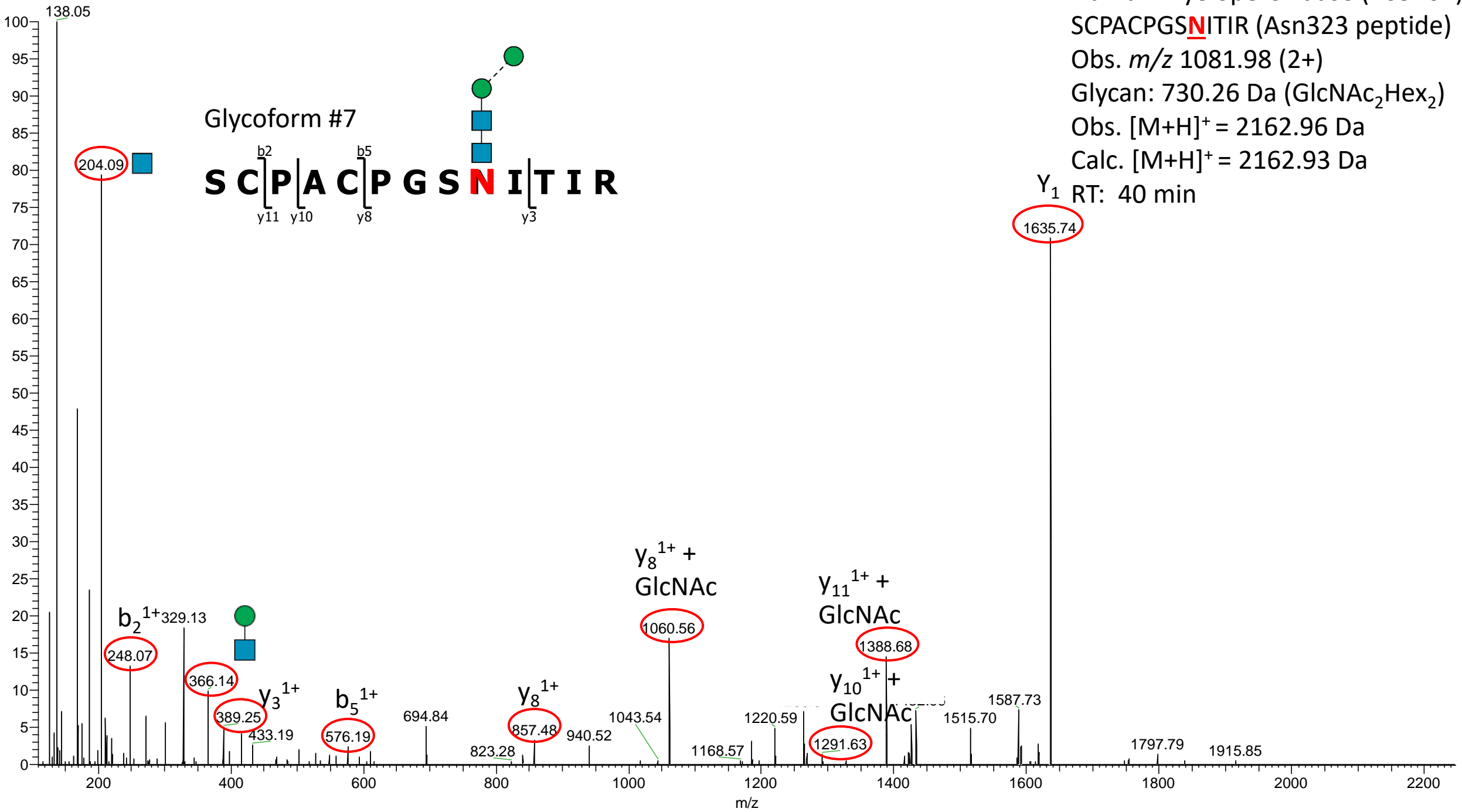
RT: 42 min

Glycoform #5



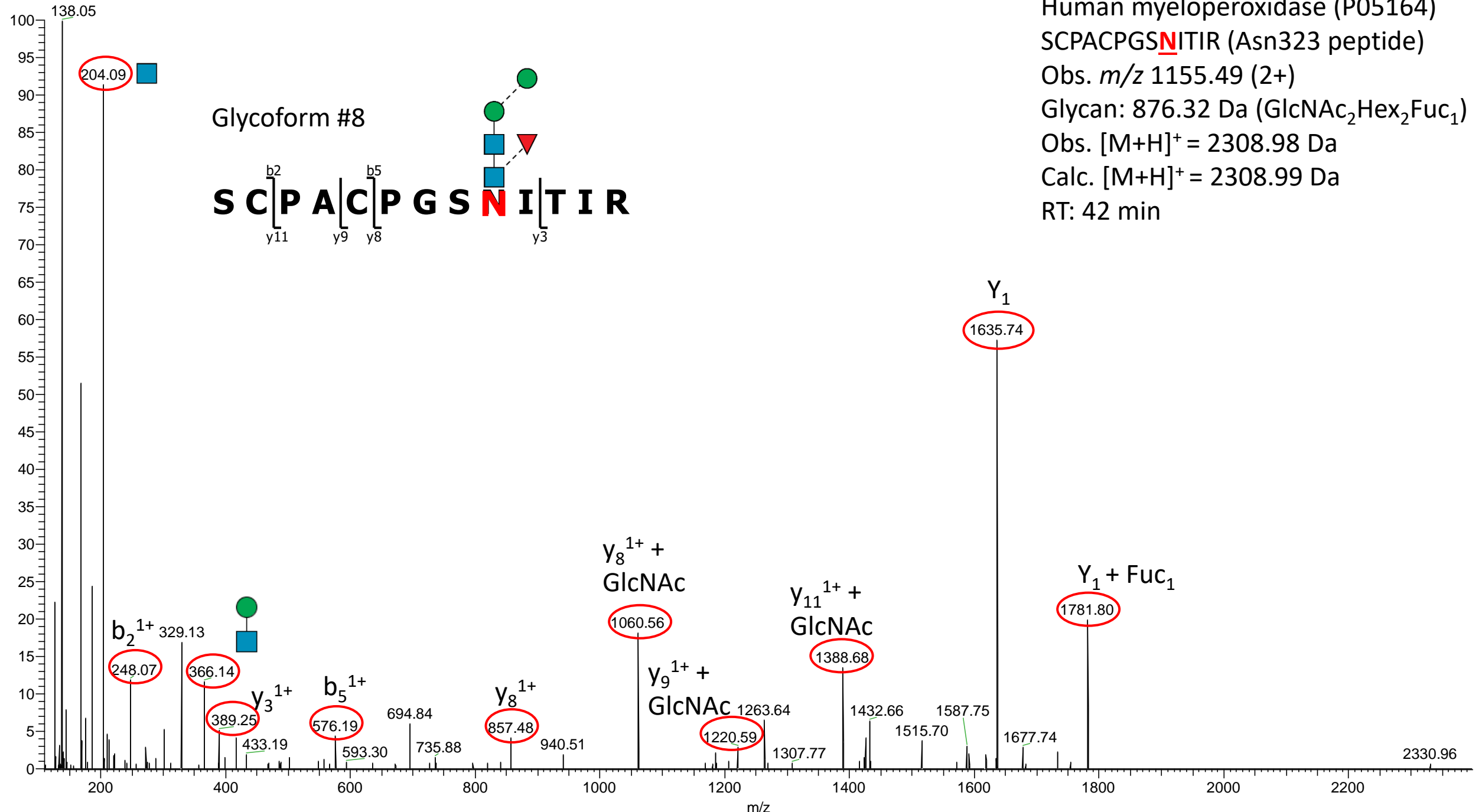
Human myeloperoxidase (P05164)
SCPACPGS**N**ITIR (Asn323 peptide)
Obs. m/z 1073.97 (2+)
Glycan: 714.27 Da (GlcNAc₂Hex₁Fuc₁)
Obs. $[M+H]^+$ = 2146.94 Da
Calc. $[M+H]^+$ = 2146.94 Da
RT: 42 min



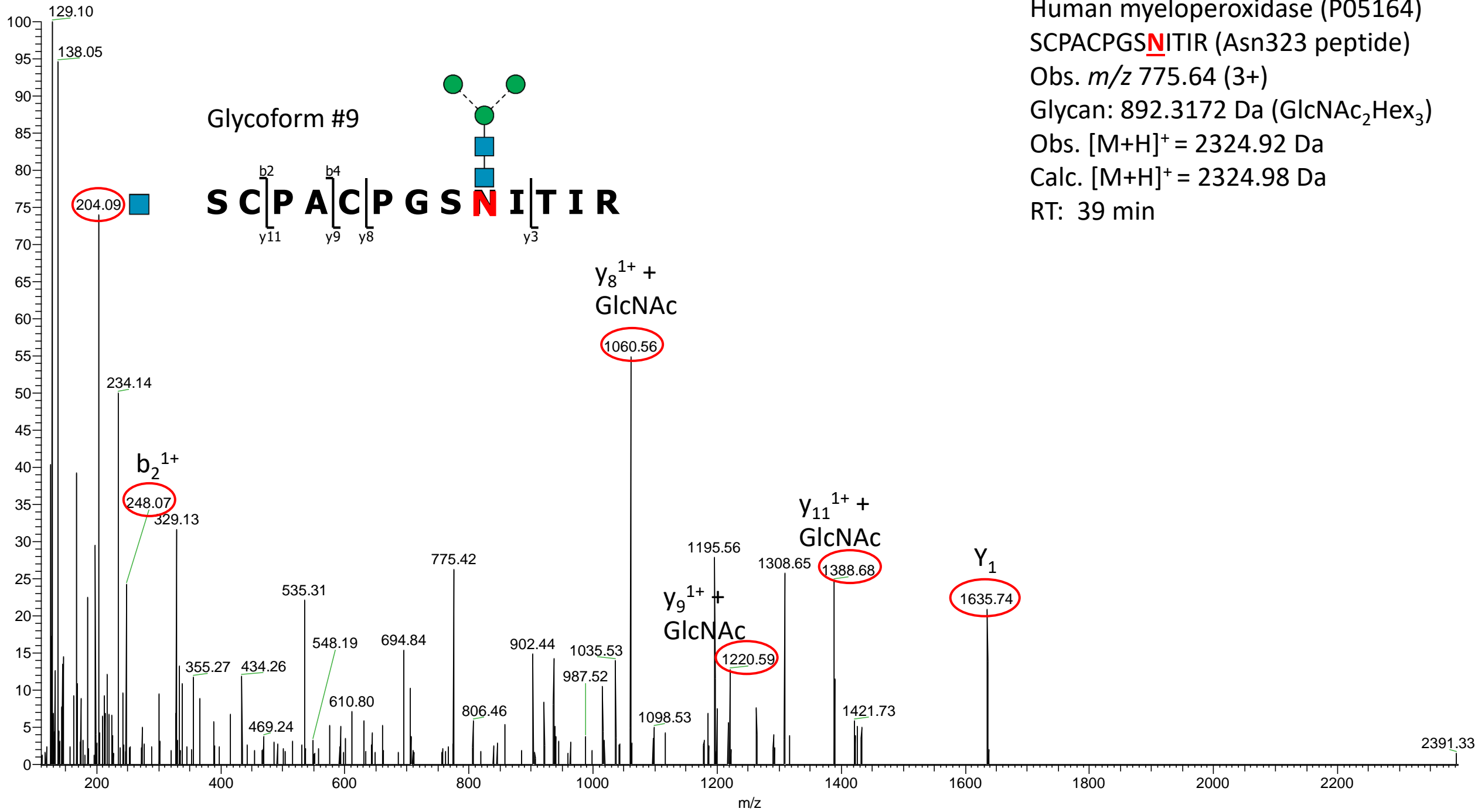


Human myeloperoxidase (P05164)
 SCPACPGS**N**ITIR (Asn323 peptide)
 Obs. m/z 1155.49 (2+)
 Glycan: 876.32 Da (GlcNac₂Hex₂Fuc₁)
 Obs. $[M+H]^+$ = 2308.98 Da
 Calc. $[M+H]^+$ = 2308.99 Da
 RT: 42 min

Glycoform #8

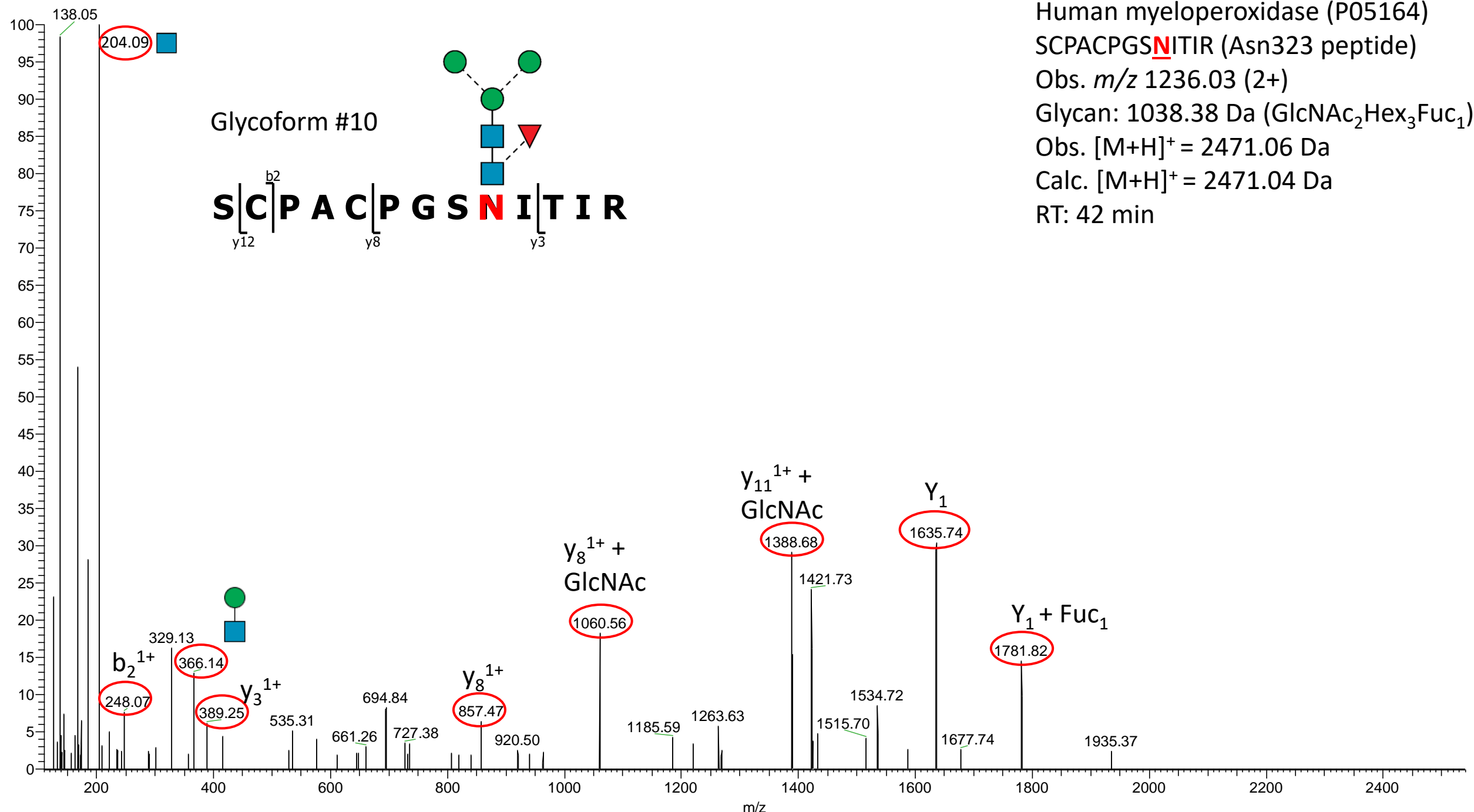
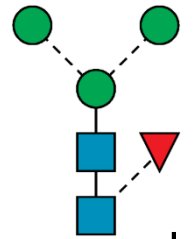


Human myeloperoxidase (P05164)
SCPACPGS**N**ITIR (Asn323 peptide)
Obs. m/z 775.64 (3+)
Glycan: 892.3172 Da (GlcNAc₂Hex₃)
Obs. $[M+H]^+$ = 2324.92 Da
Calc. $[M+H]^+$ = 2324.98 Da
RT: 39 min



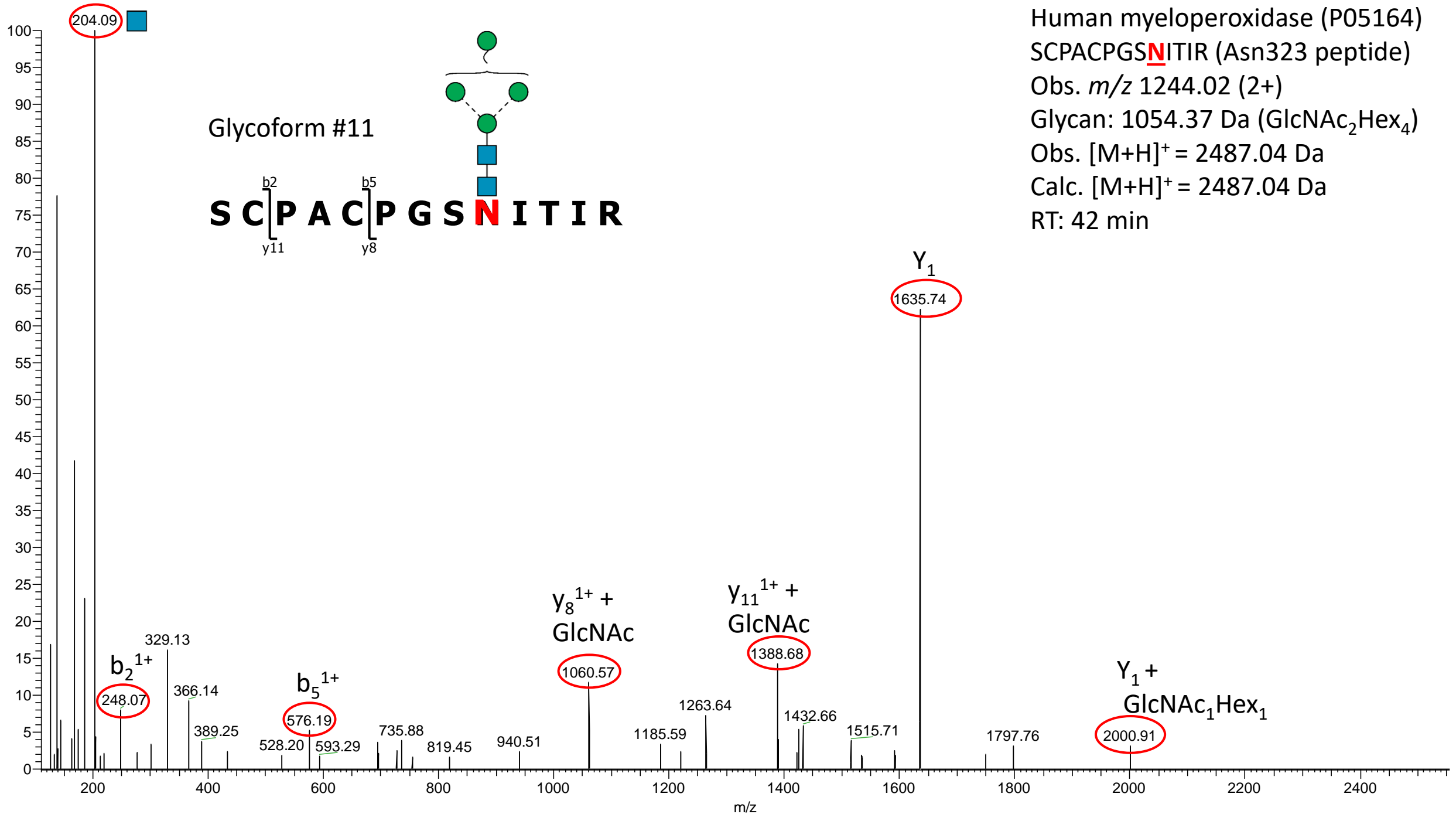
Human myeloperoxidase (P05164)
 SCPACPGS**N**ITIR (Asn323 peptide)
 Obs. m/z 1236.03 (2+)
 Glycan: 1038.38 Da (GlcNAc₂Hex₃Fuc₁)
 Obs. $[M+H]^+$ = 2471.06 Da
 Calc. $[M+H]^+$ = 2471.04 Da
 RT: 42 min

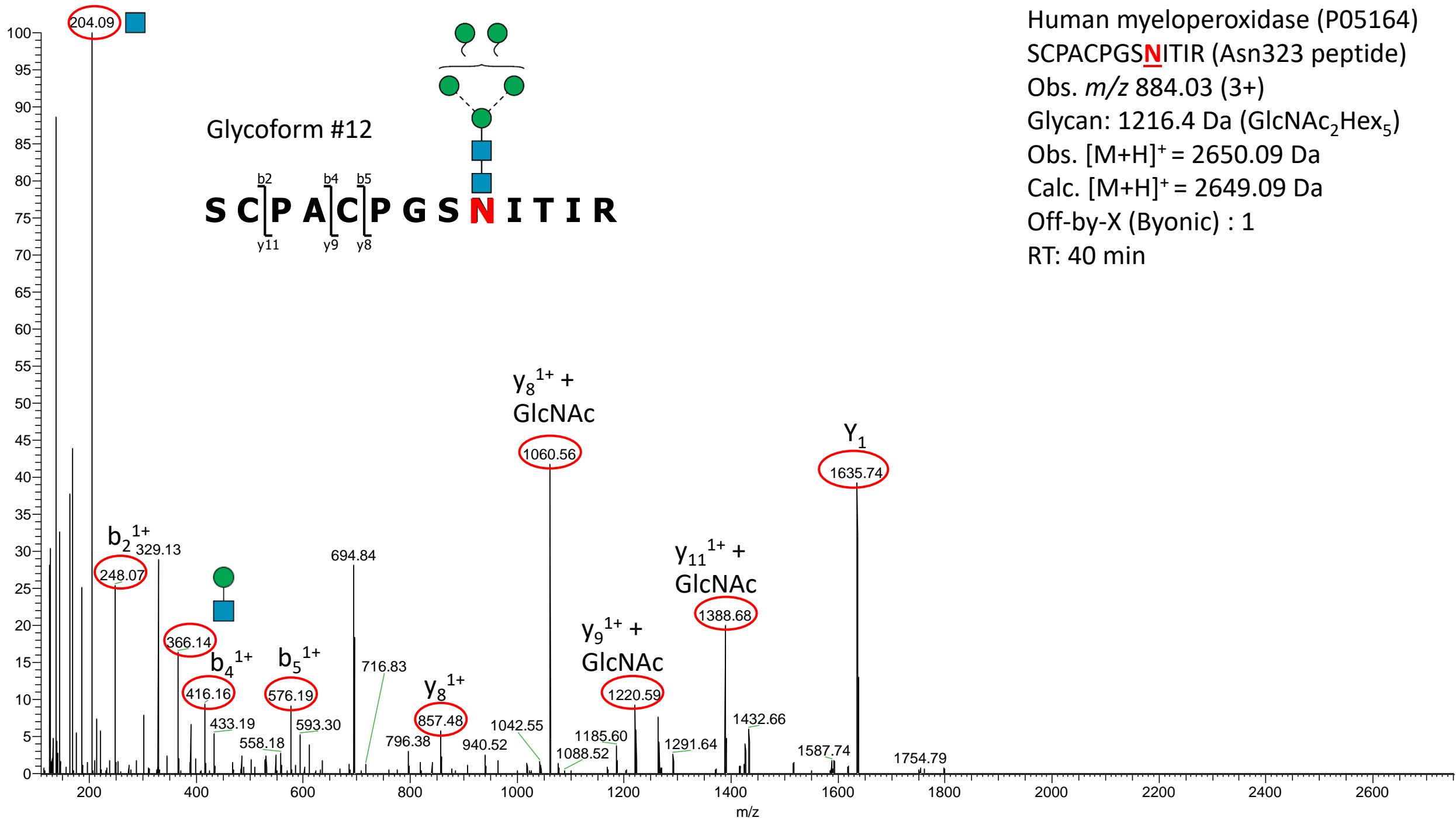
Glycoform #10

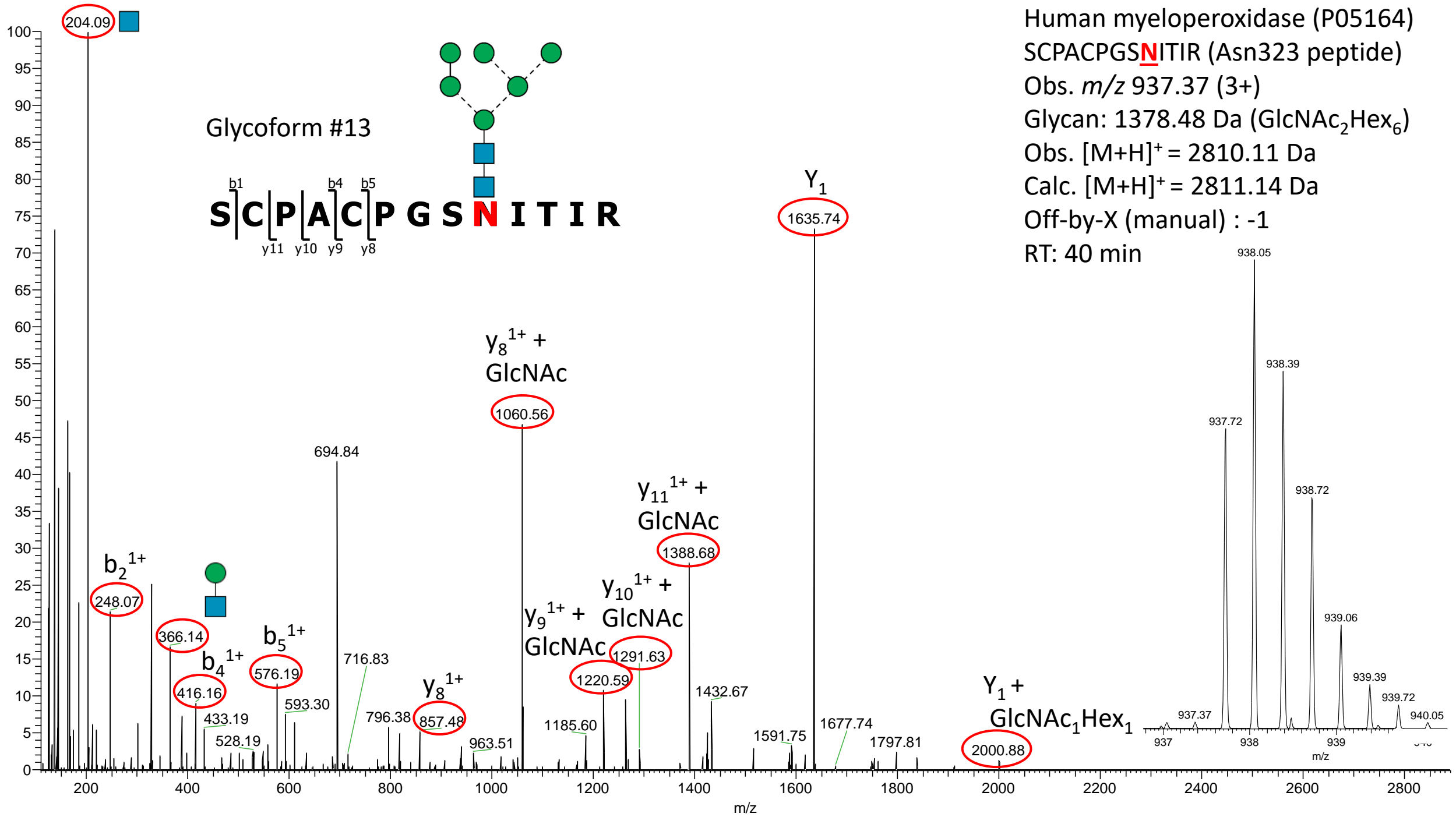


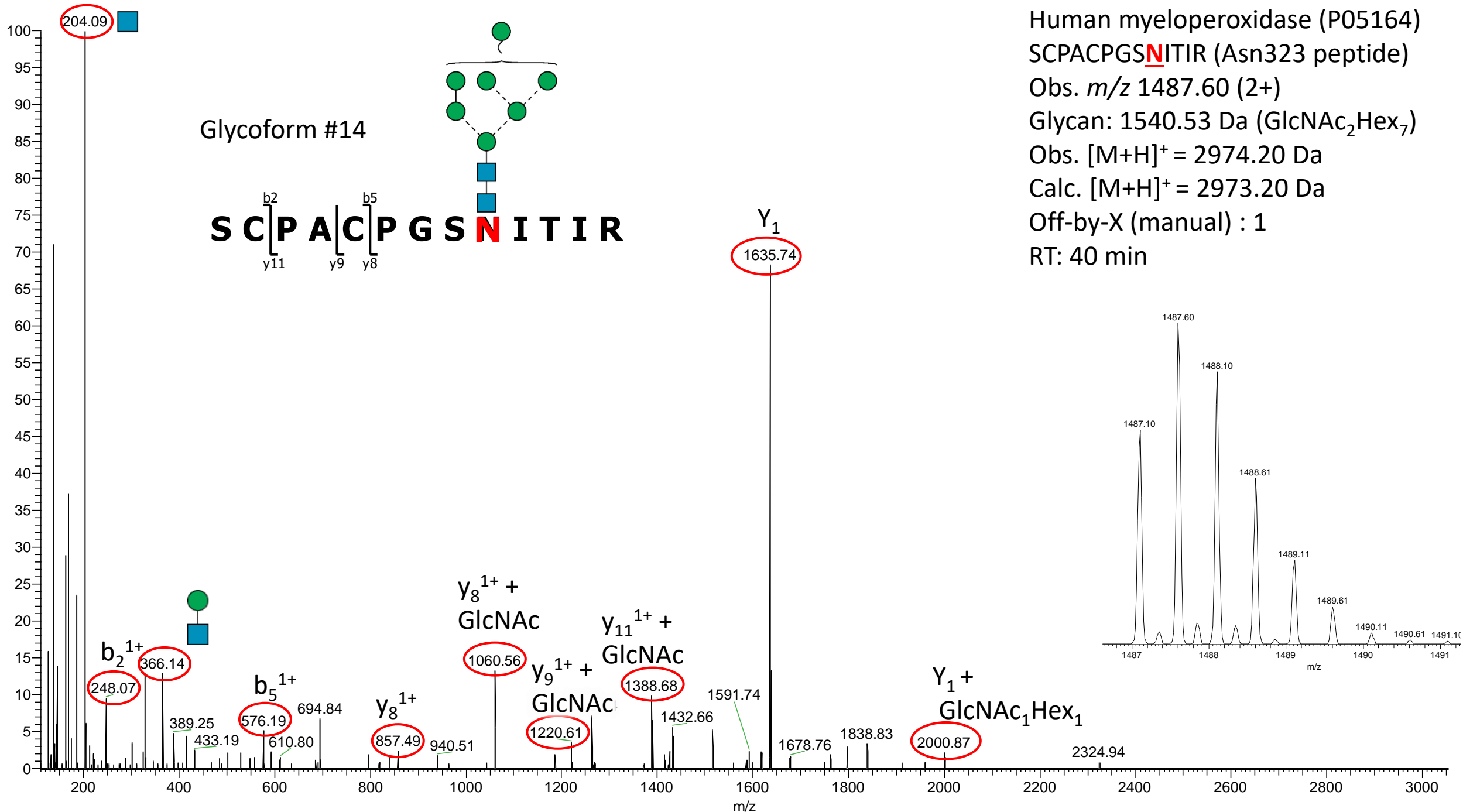
Human myeloperoxidase (P05164)
SCPACPGS**N**ITIR (Asn323 peptide)
Obs. m/z 1244.02 (2+)
Glycan: 1054.37 Da (GlcNAc₂Hex₄)
Obs. $[M+H]^+$ = 2487.04 Da
Calc. $[M+H]^+$ = 2487.04 Da
RT: 42 min

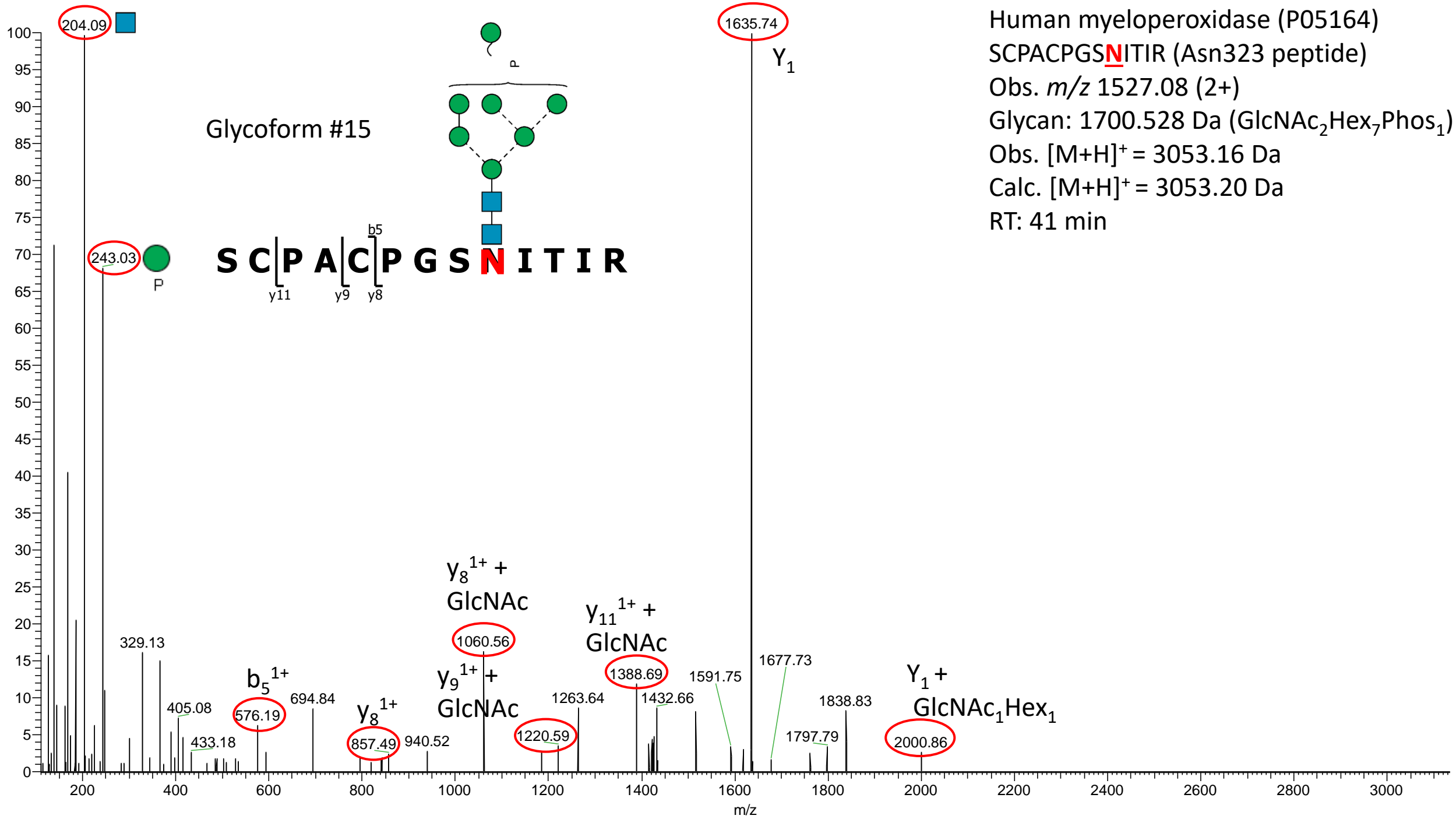
Glycoform #11

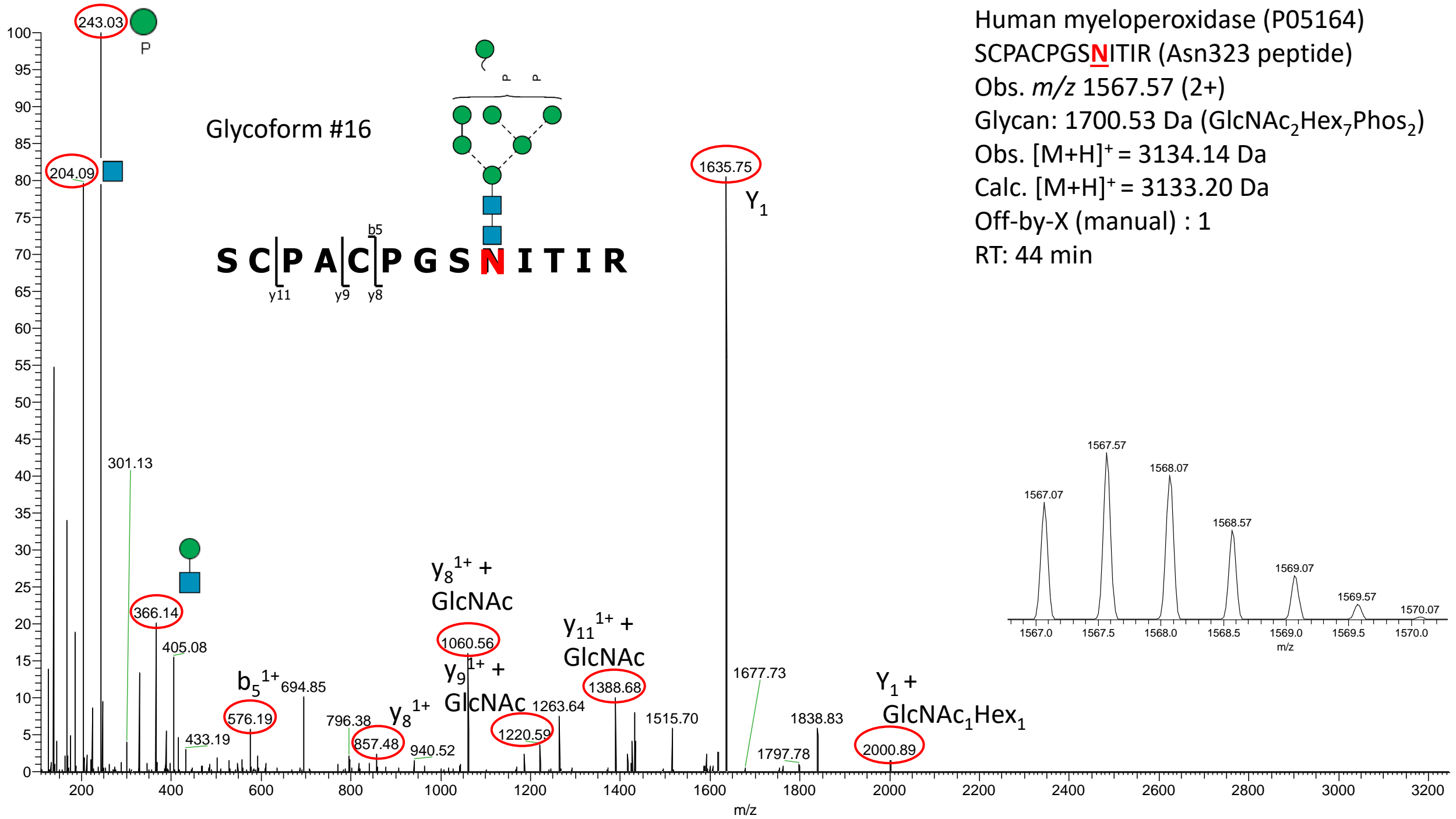


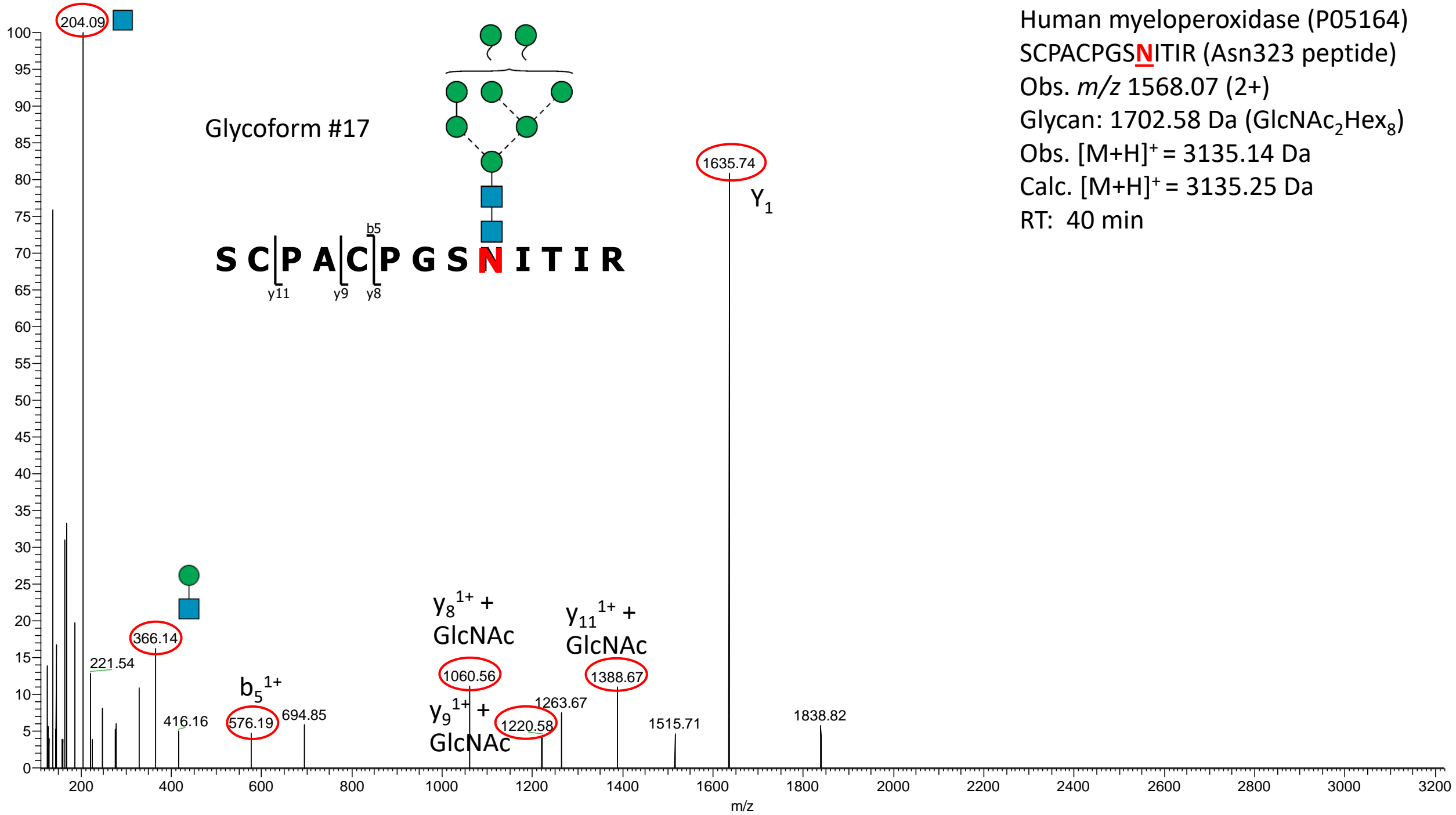












Human myeloperoxidase (P05164)

SCPACPGSNITIR (Asn323 peptide)

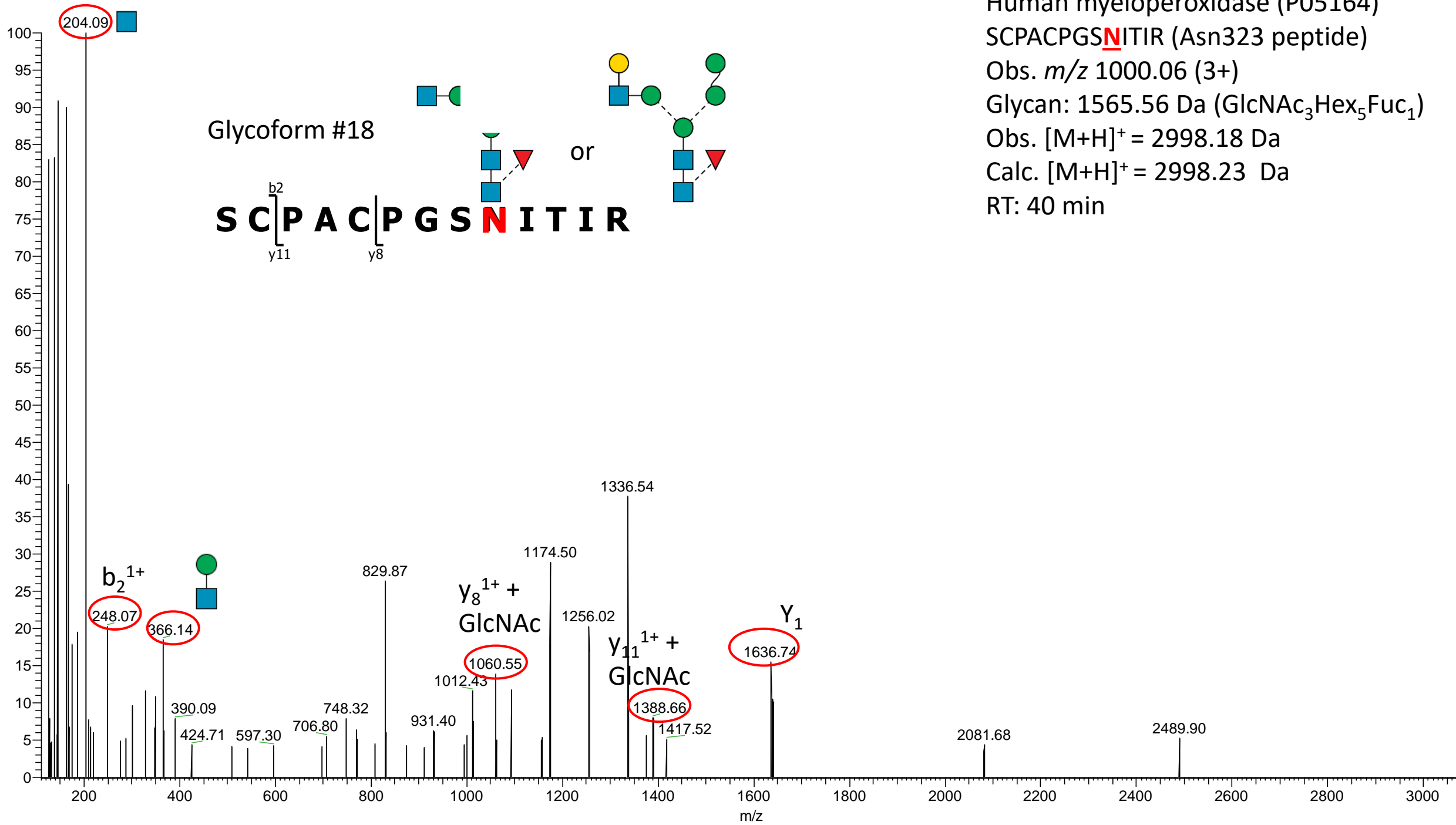
Obs. m/z 1000.06 (3+)

Glycan: 1565.56 Da (GlcNAc₃Hex₅Fuc₁)

Obs. $[M+H]^+$ = 2998.18 Da

Calc. $[M+H]^+$ = 2998.23 Da

RT: 40 min



Human myeloperoxidase (P05164)

SCPACPGSNITIR (Asn323 peptide)

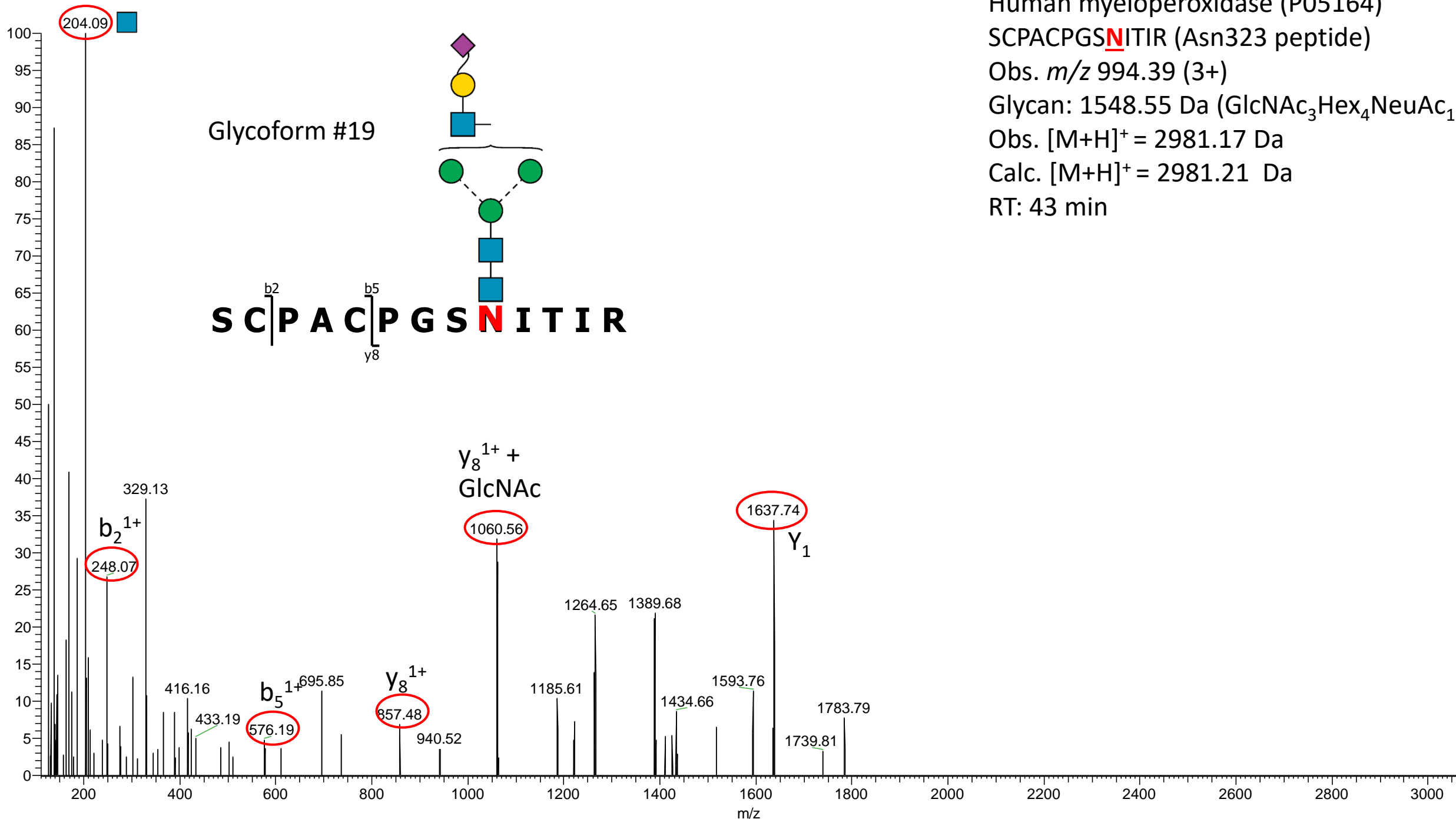
Obs. m/z 994.39 (3+)

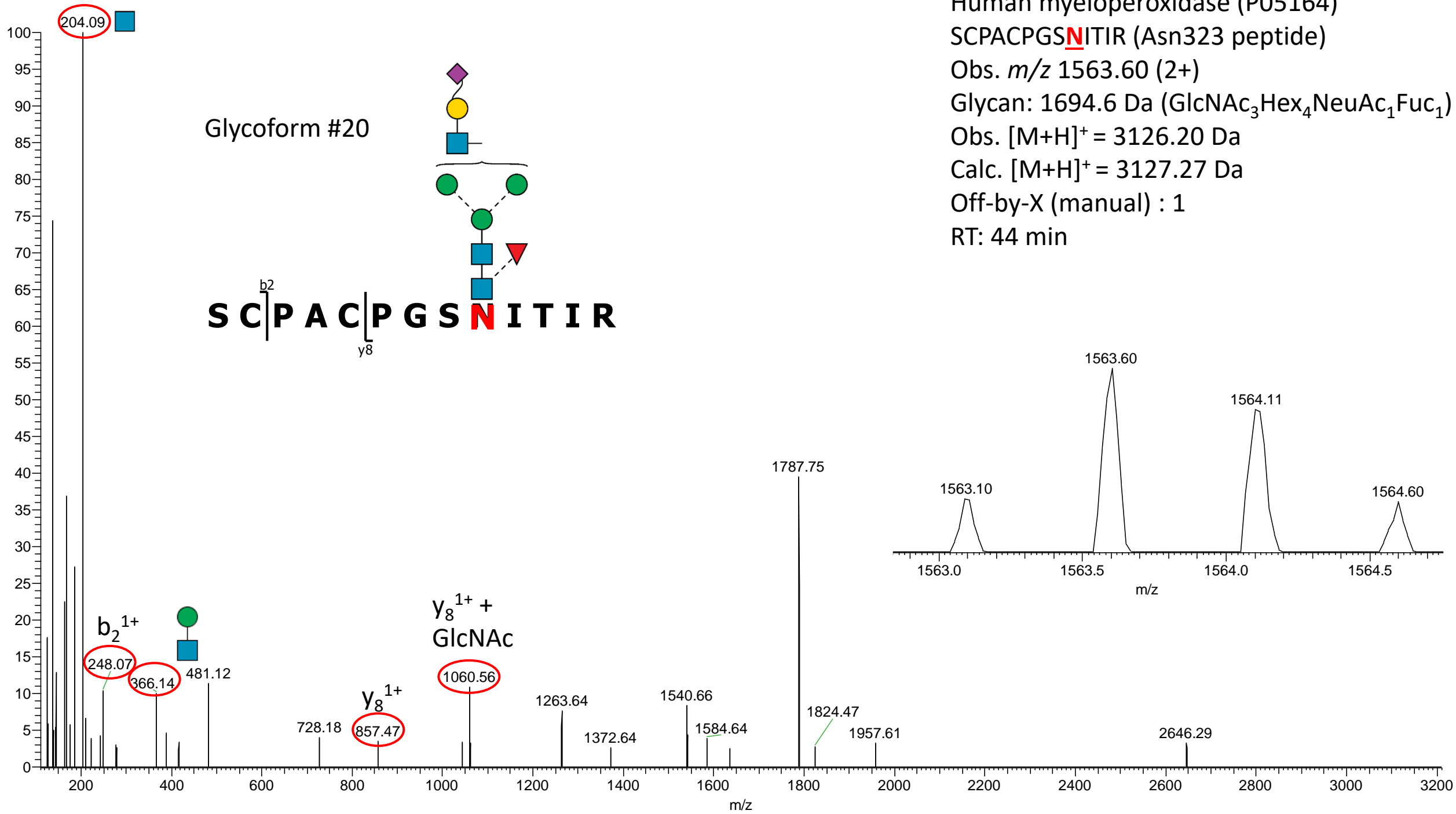
Glycan: 1548.55 Da (GlcNAc₃Hex₄NeuAc₁)

Obs. $[M+H]^+$ = 2981.17 Da

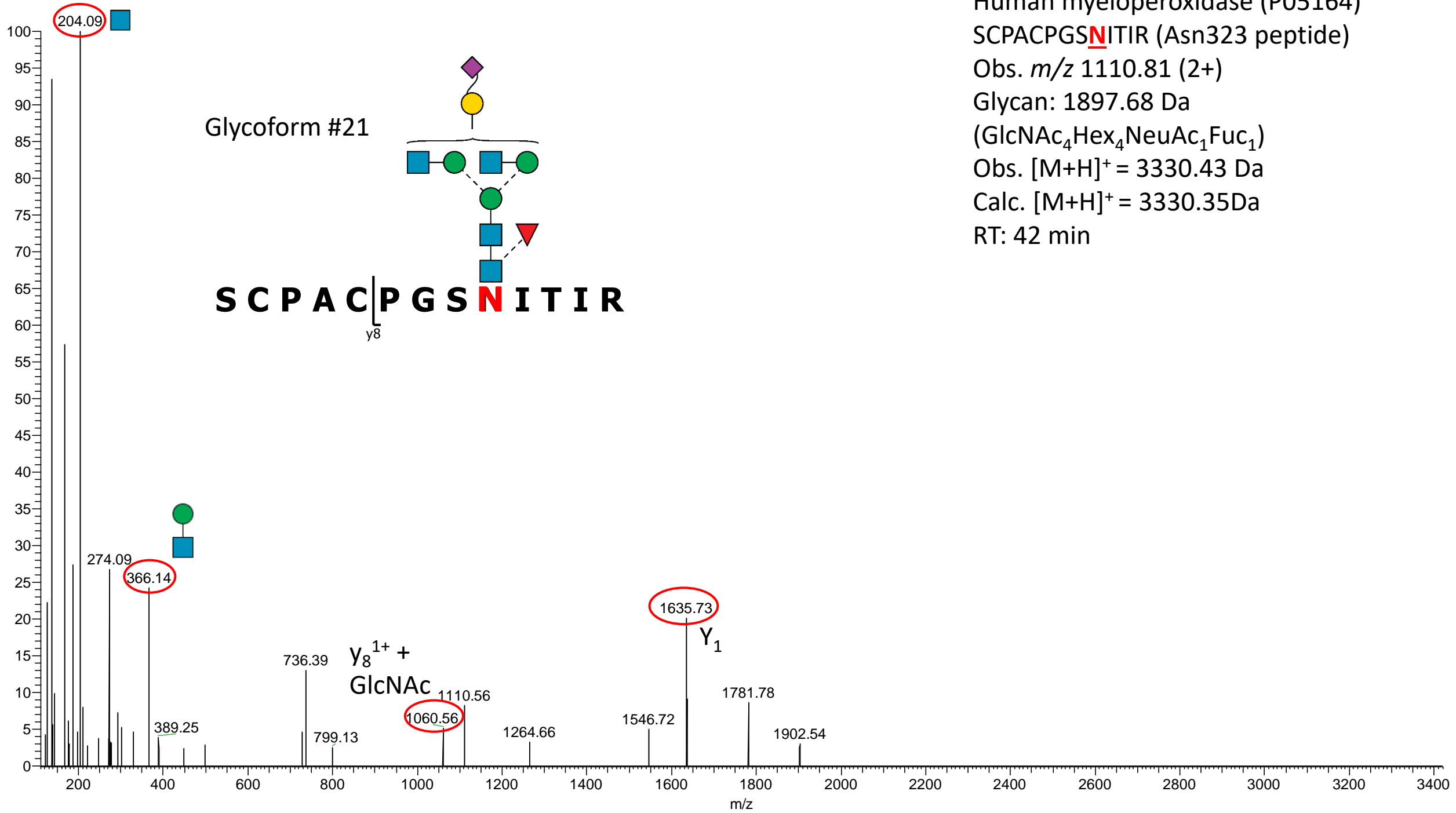
Calc. $[M+H]^+$ = 2981.21 Da

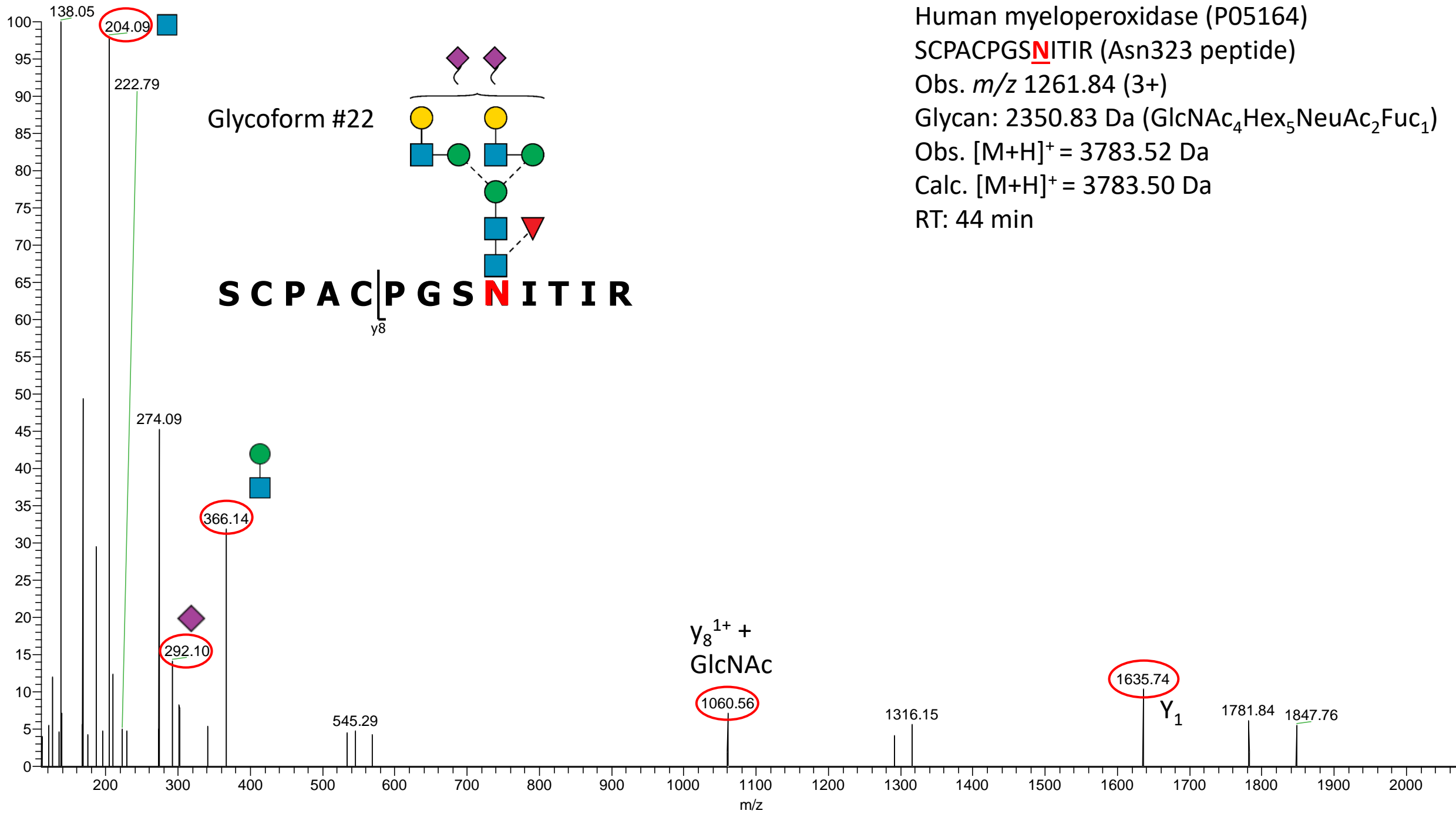
RT: 43 min





Human myeloperoxidase (P05164)
SCPACPGS**N**ITIR (Asn323 peptide)
Obs. m/z 1110.81 (2+)
Glycan: 1897.68 Da
(GlcNAc₄Hex₄NeuAc₁Fuc₁)
Obs. $[M+H]^+$ = 3330.43 Da
Calc. $[M+H]^+$ = 3330.35 Da
RT: 42 min



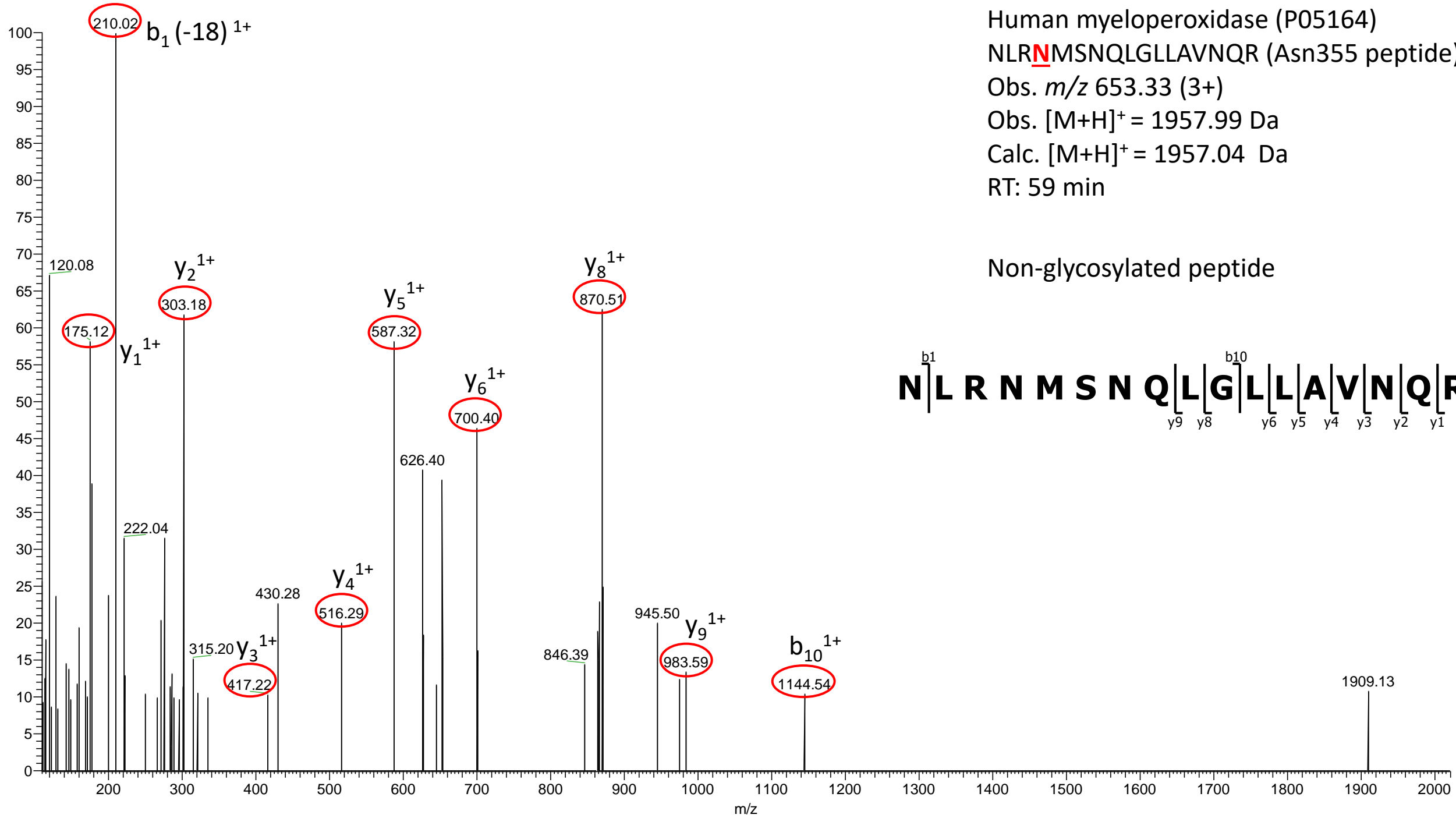


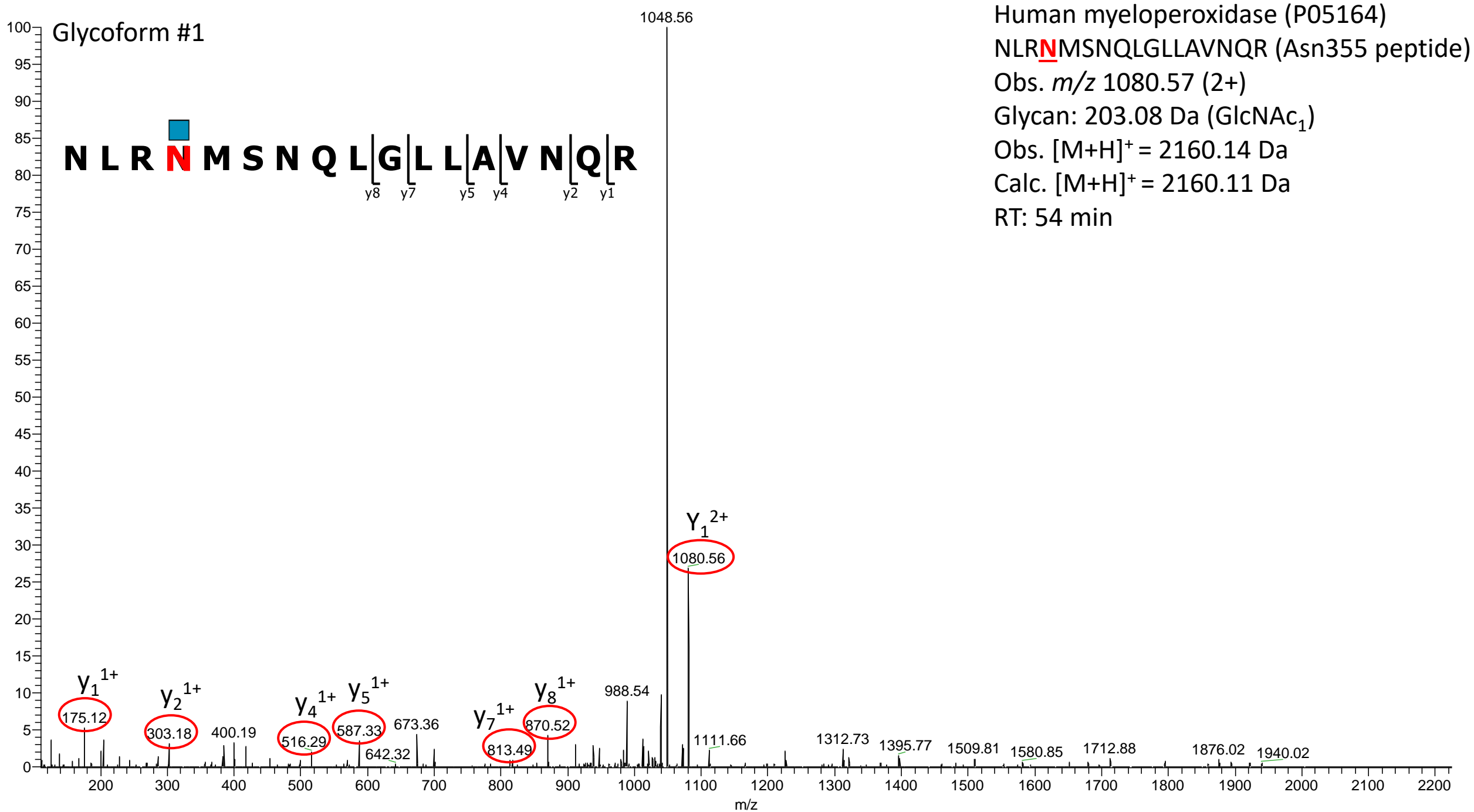
Supplementary Data S3B

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn355 identified from the analysis of the unenriched peptide mixtures of nMPO.

Human myeloperoxidase (P05164)
NLRNMSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 653.33 (3+)
Obs. $[M+H]^+ = 1957.99$ Da
Calc. $[M+H]^+ = 1957.04$ Da
RT: 59 min

Non-glycosylated peptide



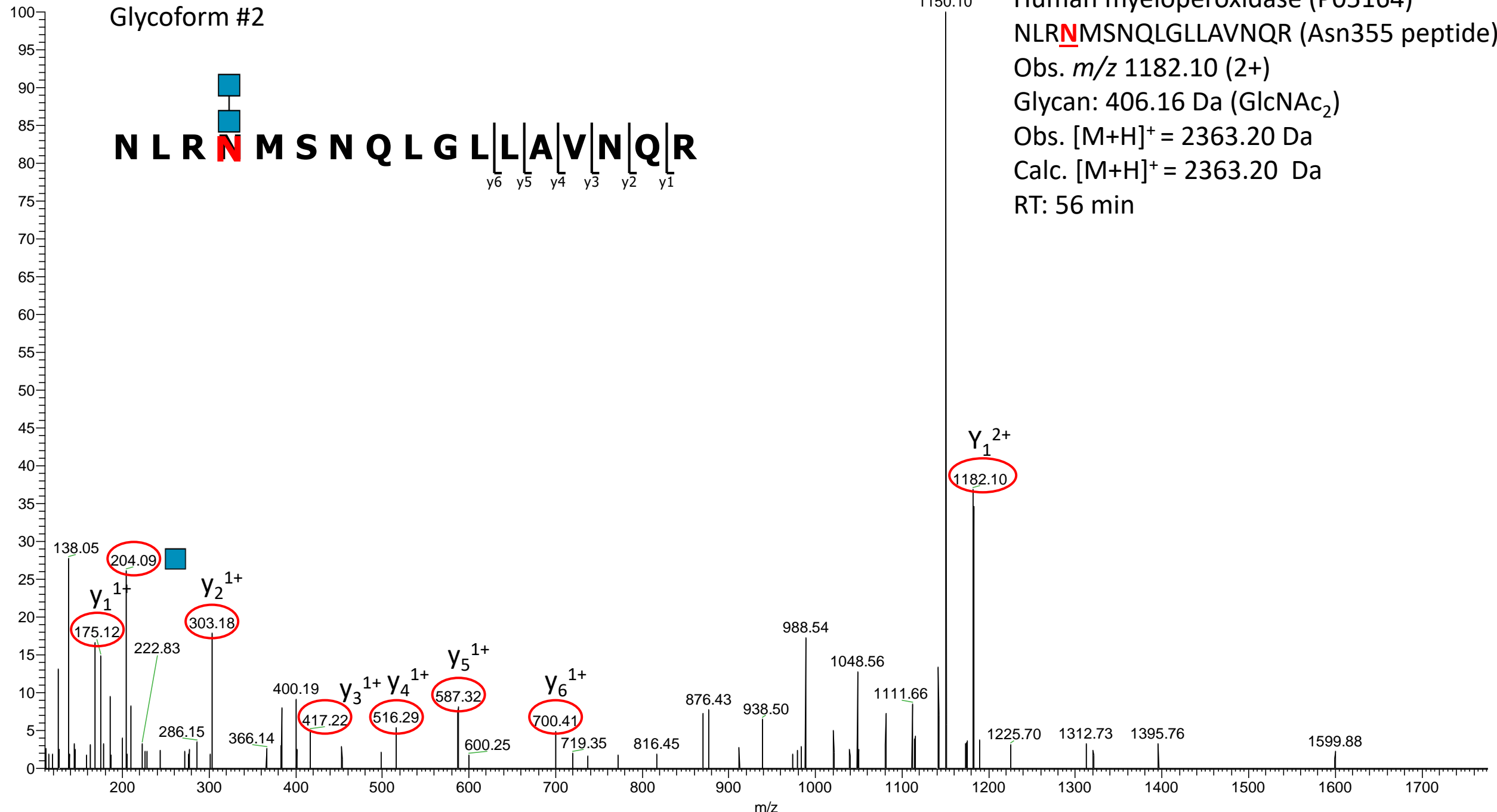


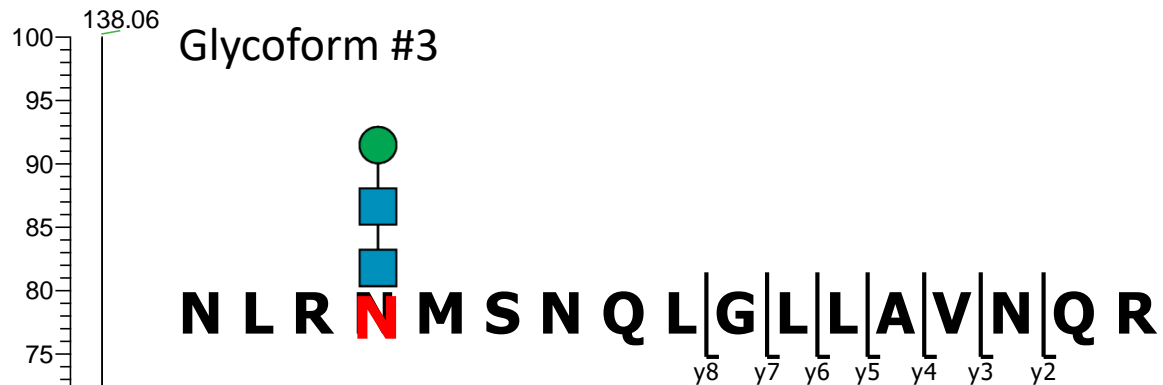
Glycoform #2



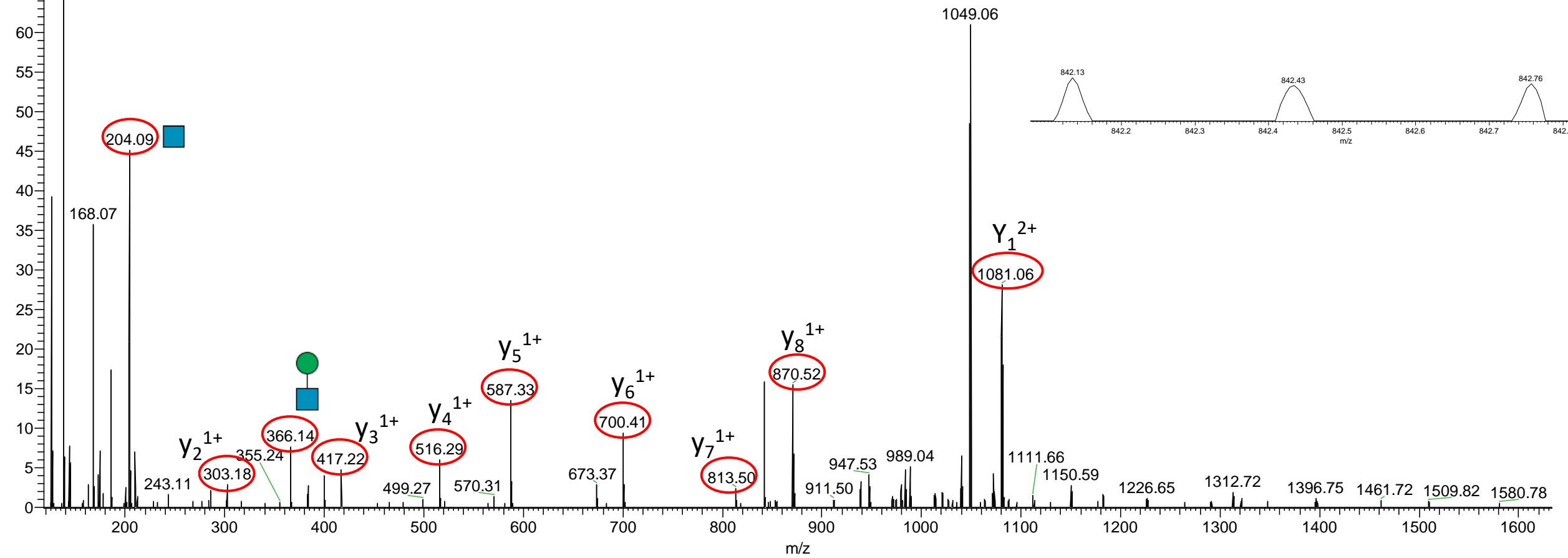
1150.10

Human myeloperoxidase (P05164)
NLR**N**MSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 1182.10 (2+)
Glycan: 406.16 Da (GlcNAc₂)
Obs. $[M+H]^+$ = 2363.20 Da
Calc. $[M+H]^+$ = 2363.20 Da
RT: 56 min

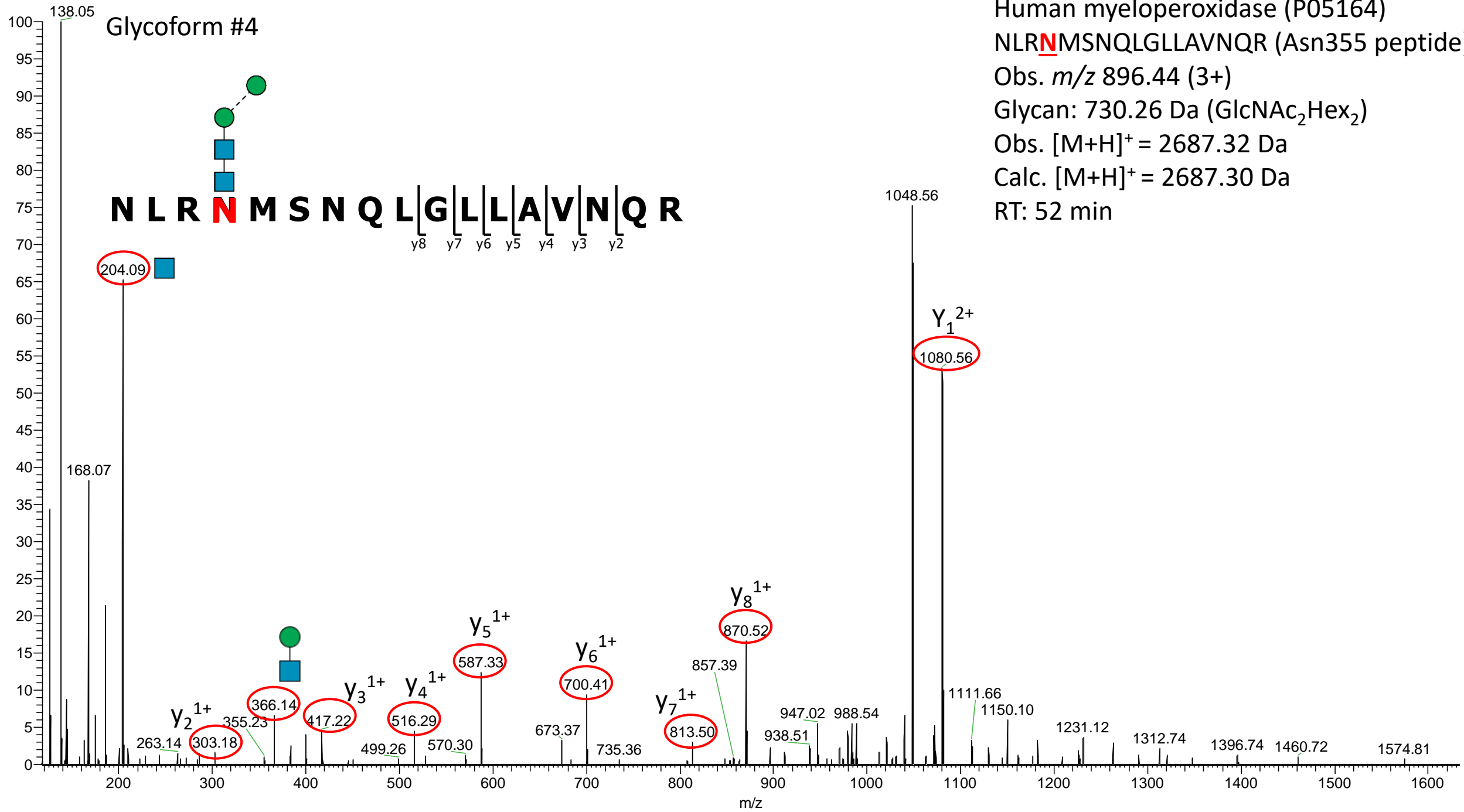


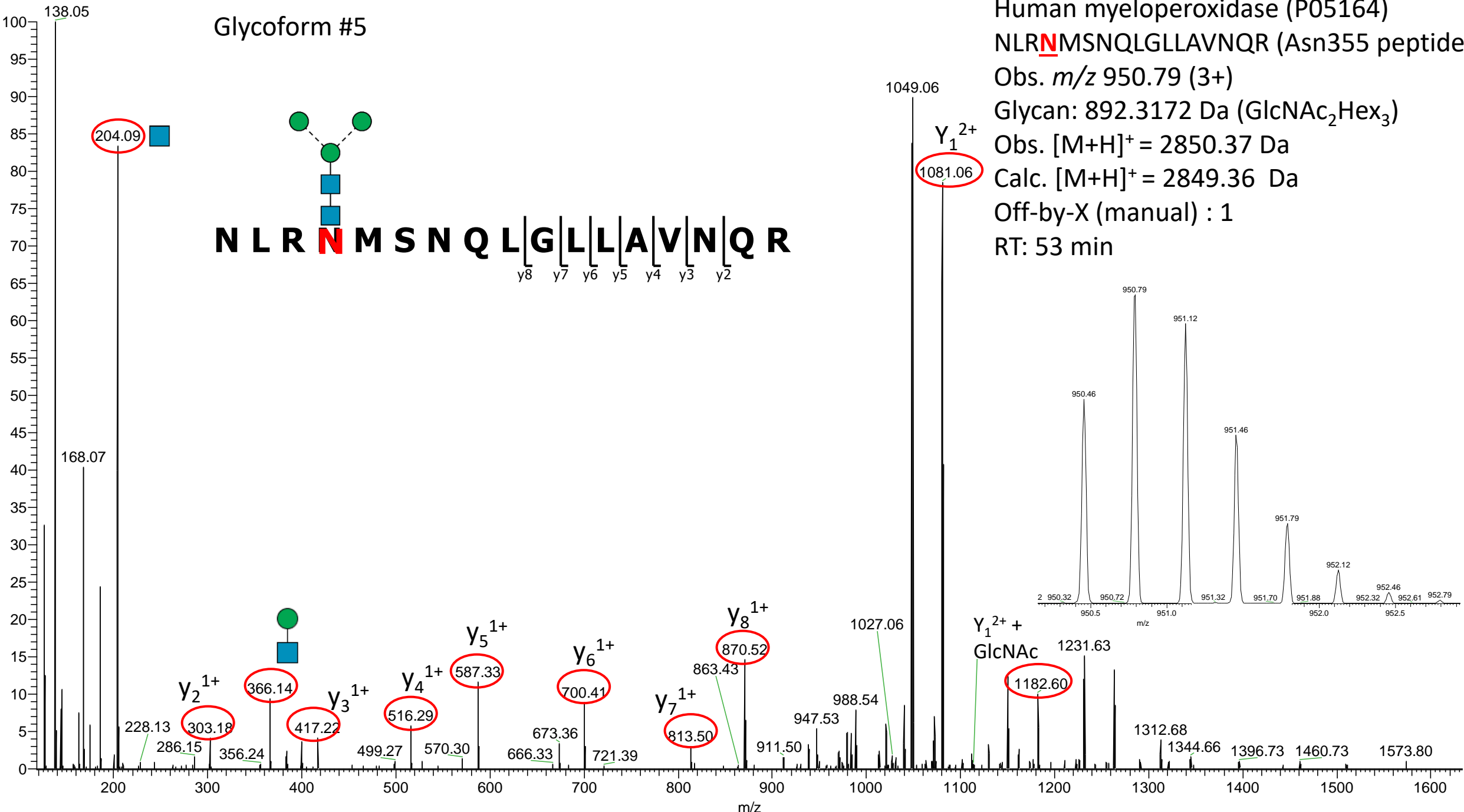


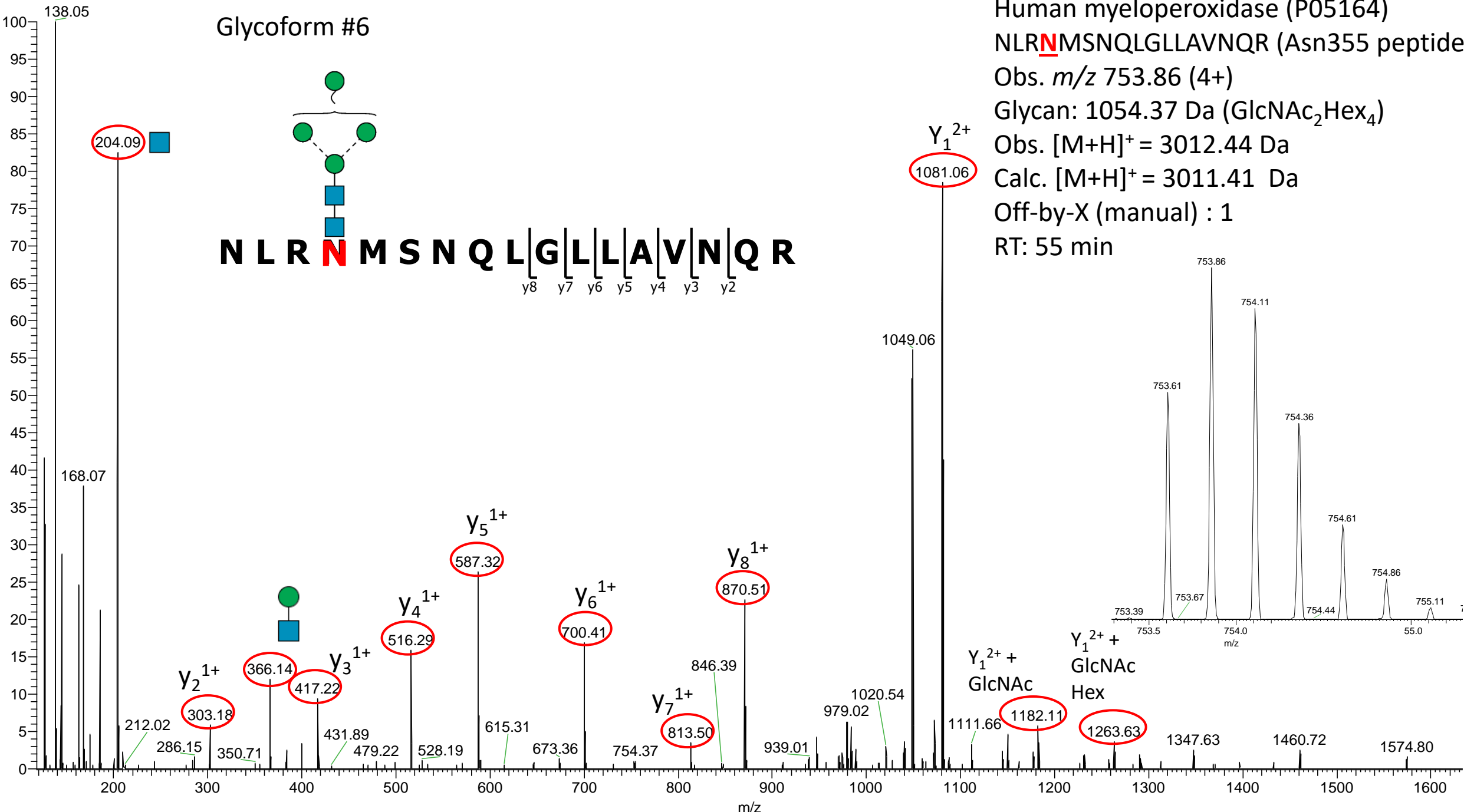
Human myeloperoxidase (P05164)
 NLR**N**MSNQLGLLAVNQR (Asn355 peptide)
 Obs. m/z 842.76 (3+)
 Glycan: 568.21 Da (GlcNAc₂Hex₁)
 Obs. $[M+H]^+$ = 2526.28 Da
 Calc. $[M+H]^+$ = 2525.25 Da
 Off-by-X (Manual) : 1
 RT: 53 min

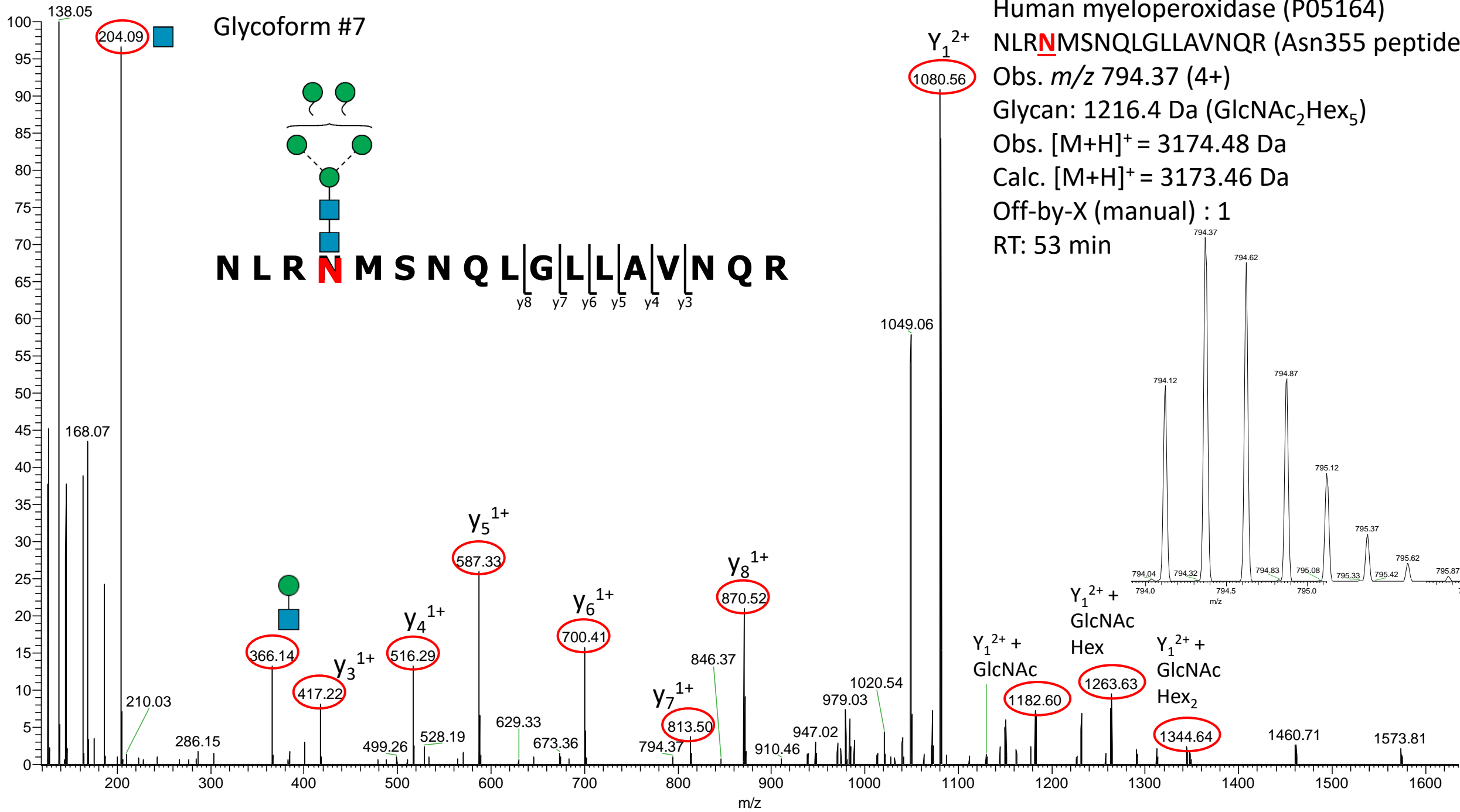


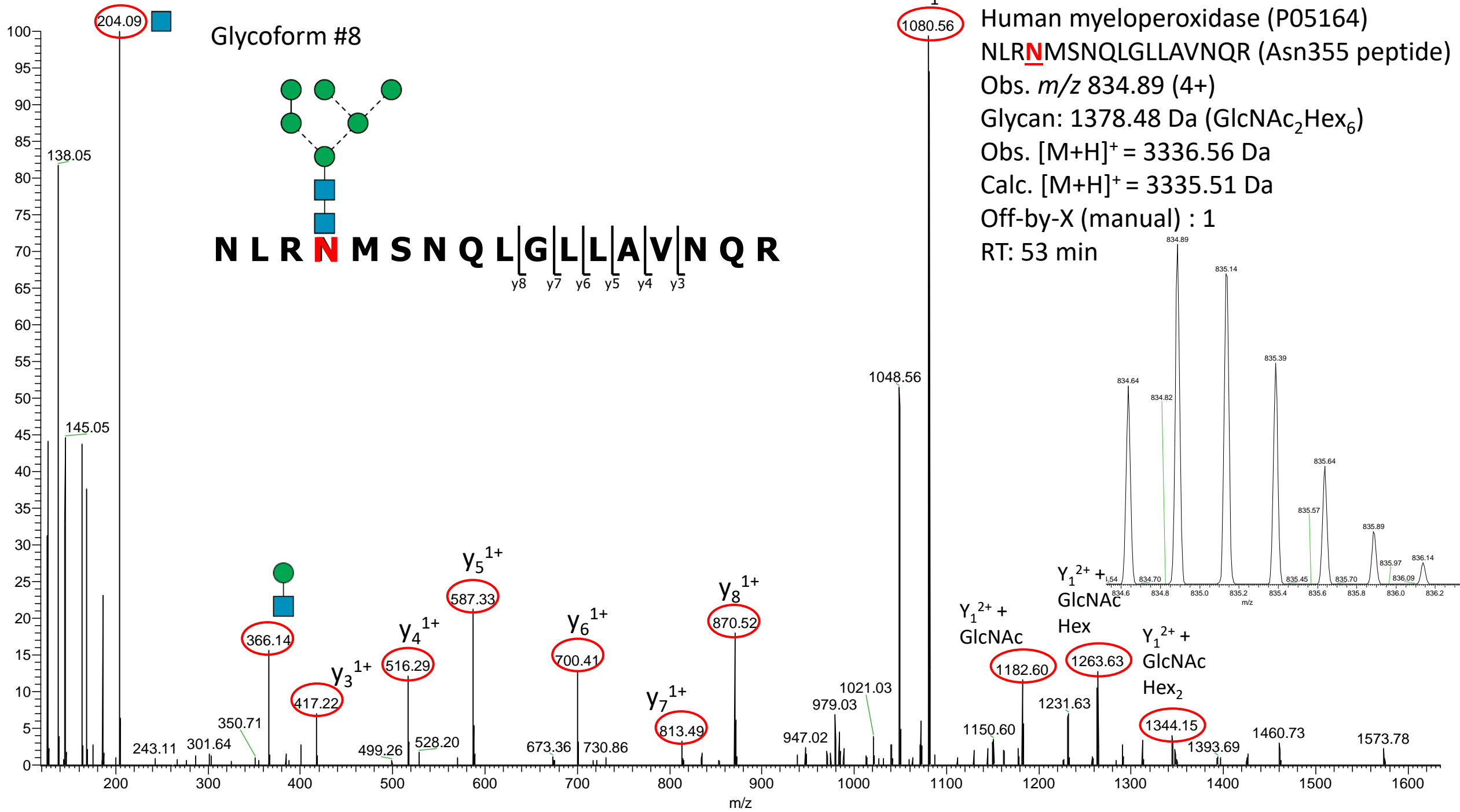
Human myeloperoxidase (P05164)
NLR**N**MSNQLGLLAVNQR (Asn355 peptide)
Obs. m/z 896.44 (3+)
Glycan: 730.26 Da (GlcNAc₂Hex₂)
Obs. $[M+H]^+$ = 2687.32 Da
Calc. $[M+H]^+$ = 2687.30 Da
RT: 52 min

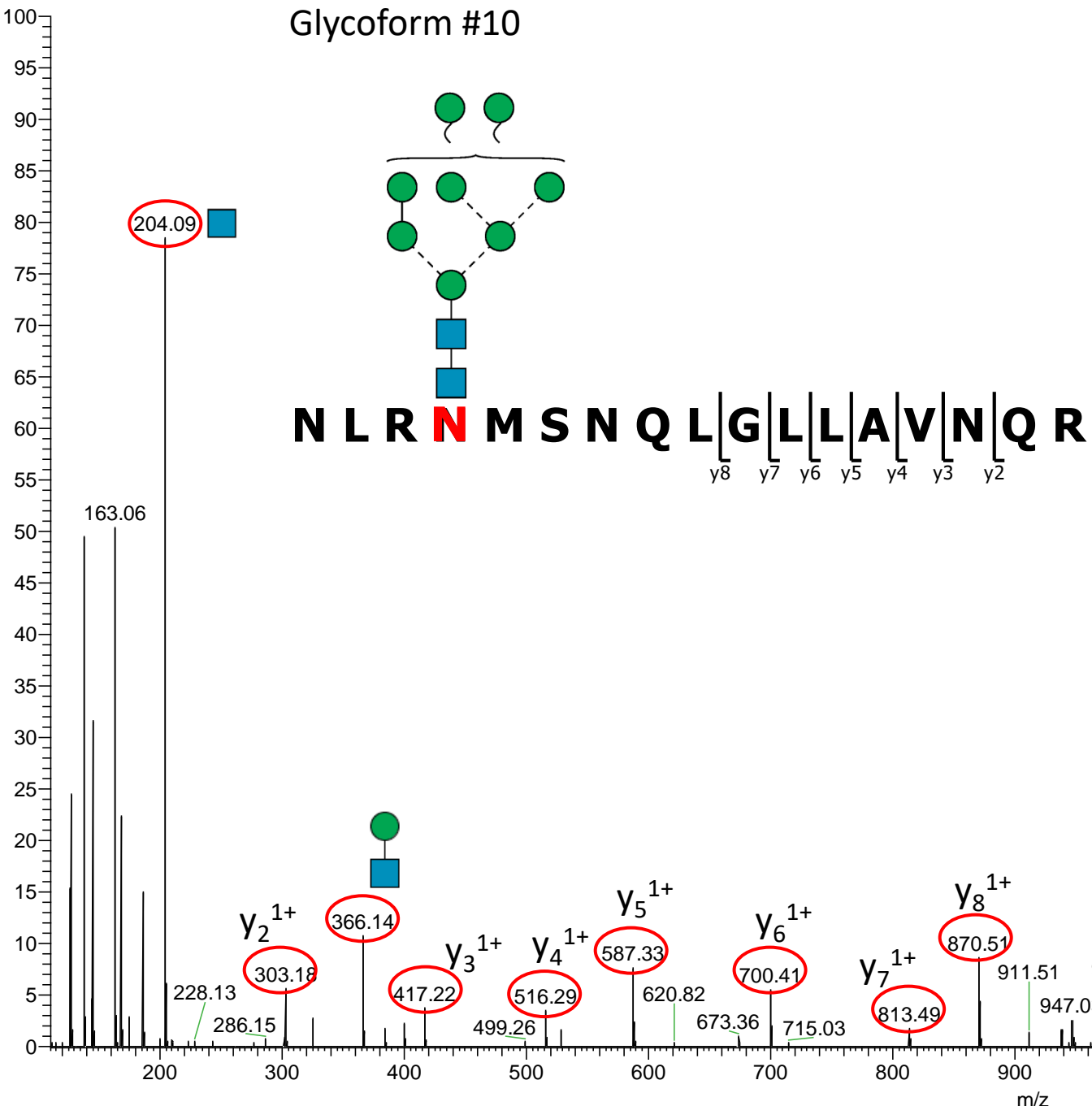






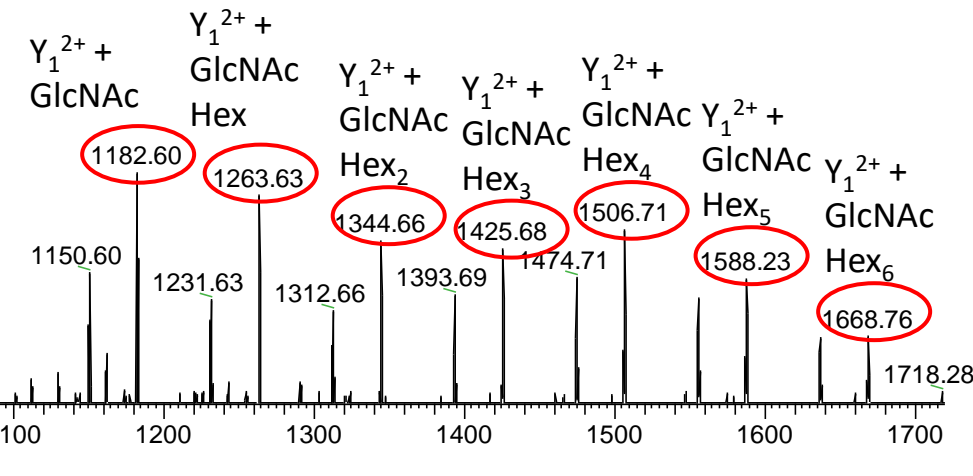
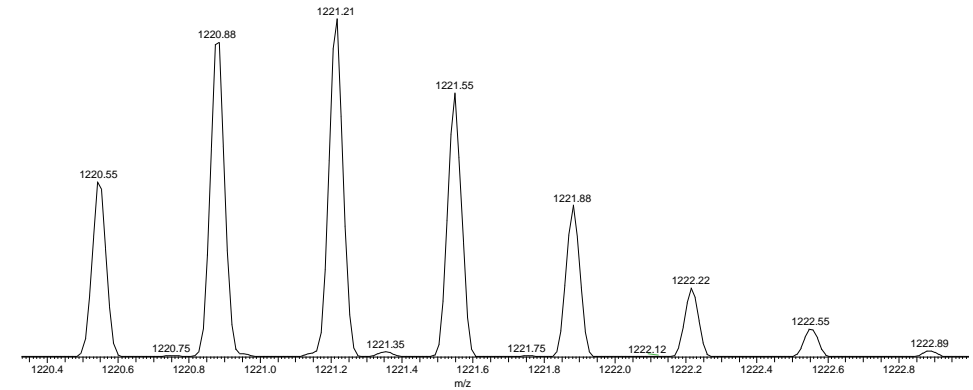






Y_1^{2+} 1081.06

Human myeloperoxidase (P05164)
 NLRNMSNQLGLLAVNQR (Asn355 peptide)
 Obs. m/z 1221.21 (3+)
 Glycan: 1702.58 Da (GlcNAc₂Hex₈)
 Obs. $[M+H]^+$ = 3661.63 Da
 Calc. $[M+H]^+$ = 3659.62 Da
 Off-by-X (manual) : 2
 RT: 53 min

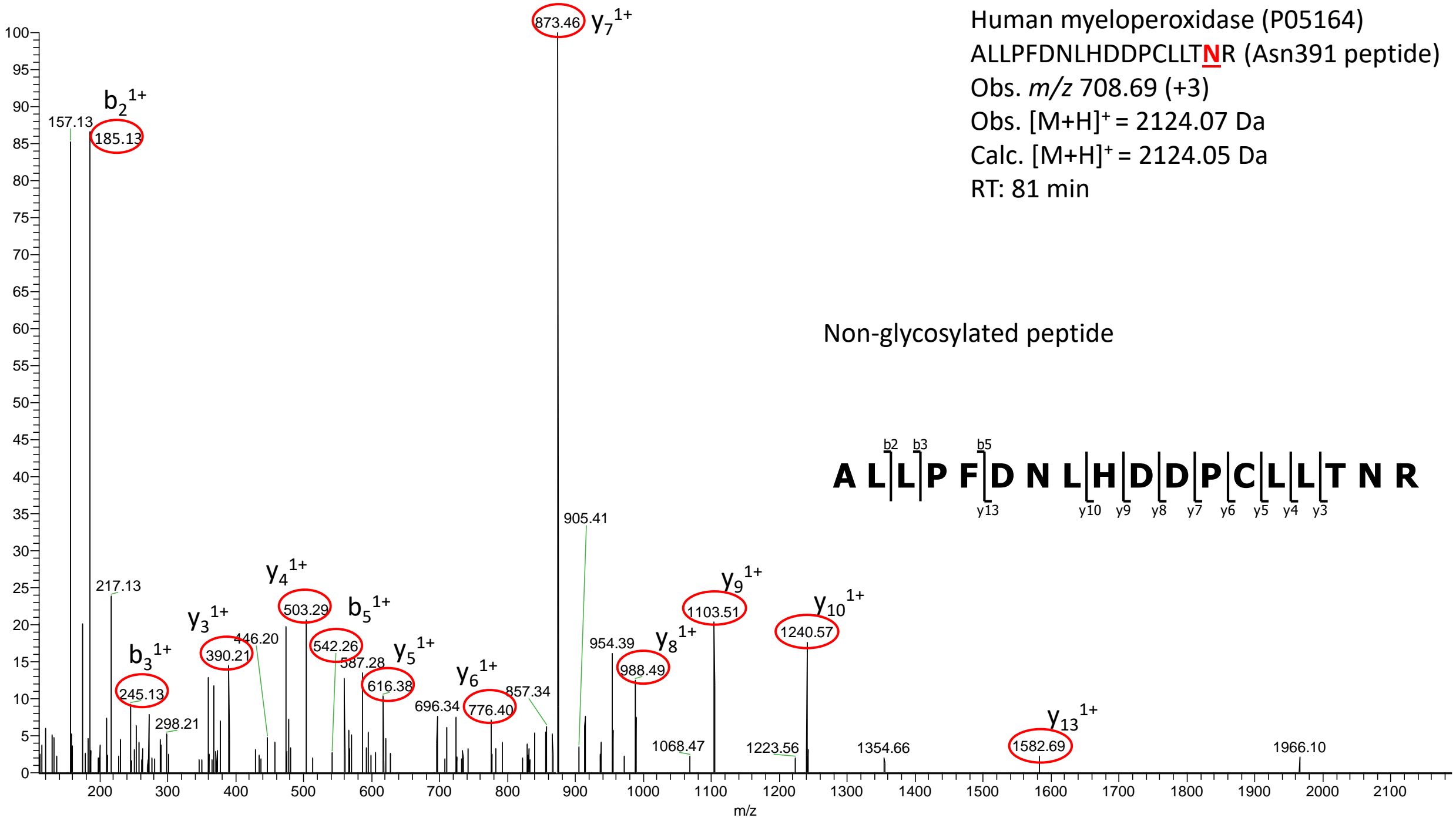
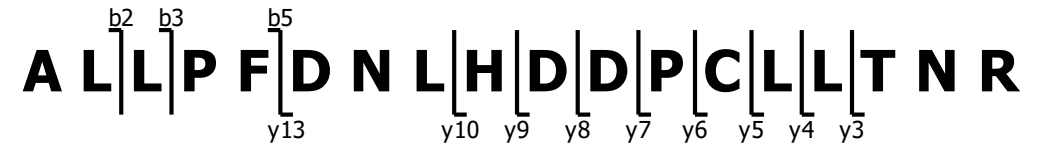


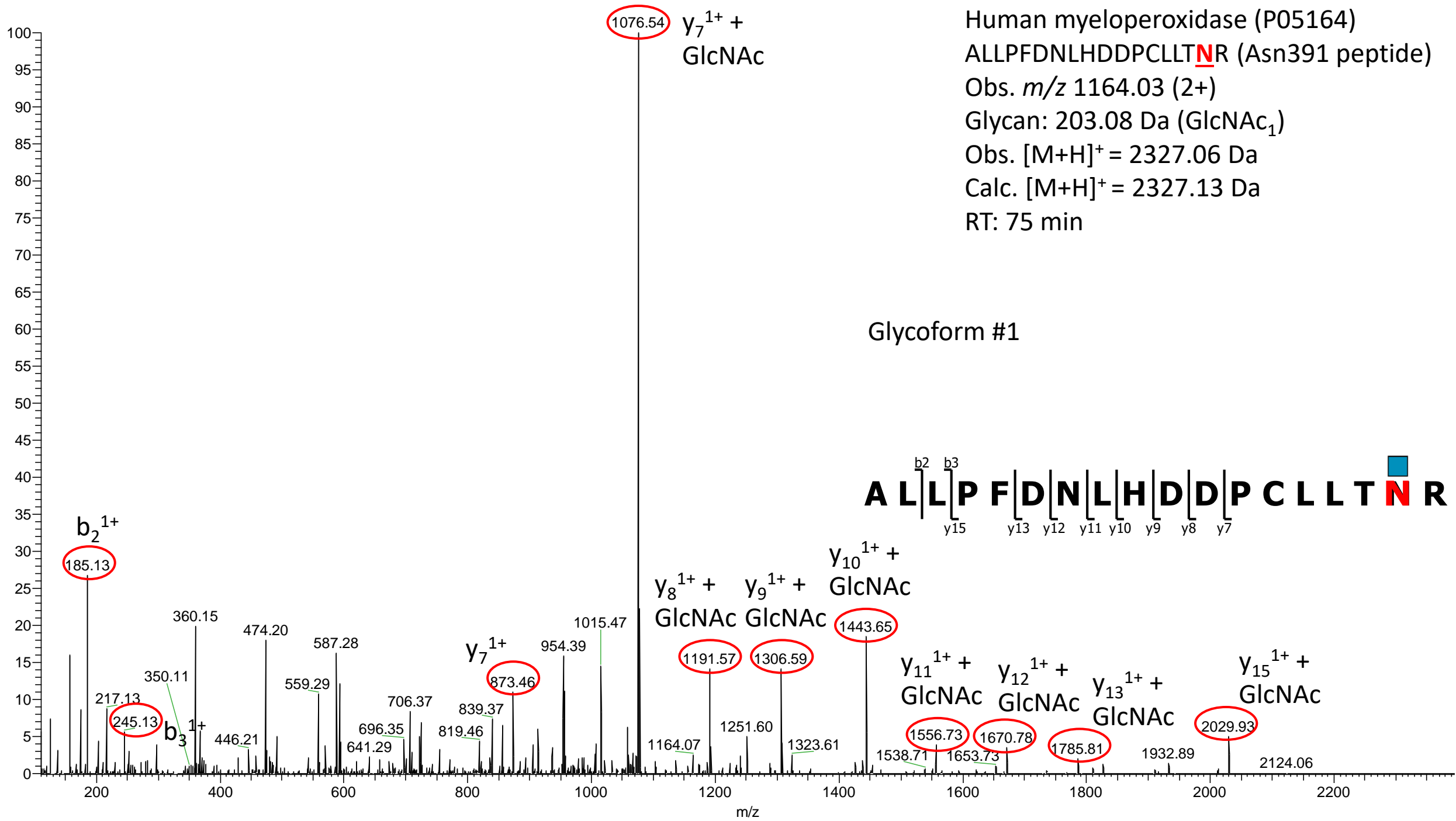
Supplementary Data S3C

Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn391 identified from the analysis of unenriched peptide mixtures of nMPO.

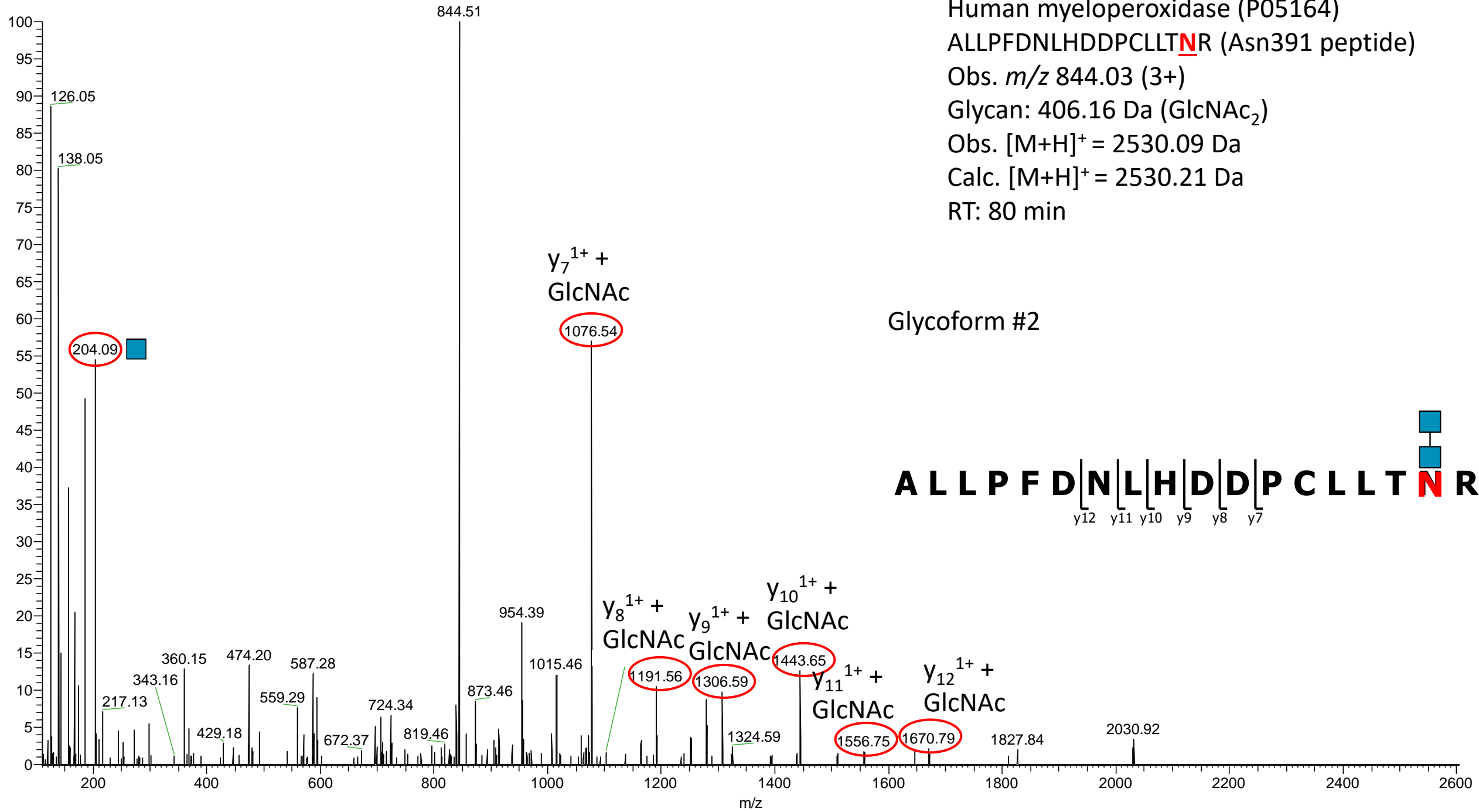
Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)
Obs. m/z 708.69 (+3)
Obs. $[M+H]^+ = 2124.07$ Da
Calc. $[M+H]^+ = 2124.05$ Da
RT: 81 min

Non-glycosylated peptide



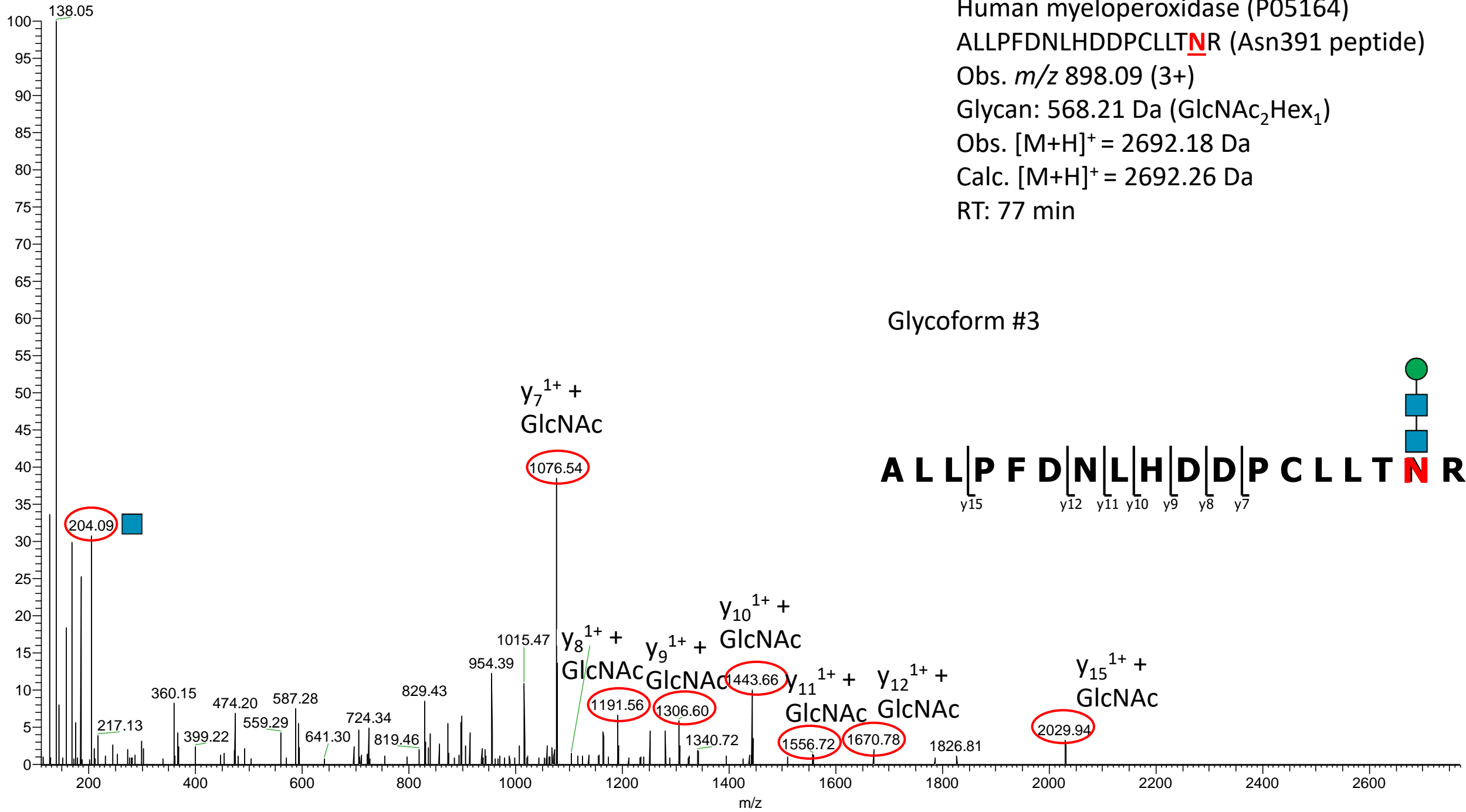


Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
Obs. m/z 844.03 (3+)
Glycan: 406.16 Da (GlcNAc₂)
Obs. $[M+H]^+$ = 2530.09 Da
Calc. $[M+H]^+$ = 2530.21 Da
RT: 80 min



Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 898.09 (3+)
 Glycan: 568.21 Da (GlcNAc₂Hex₁)
 Obs. $[M+H]^+$ = 2692.18 Da
 Calc. $[M+H]^+$ = 2692.26 Da
 RT: 77 min

Glycoform #3



Human myeloperoxidase (P05164)
ALLPFDNLHDDPCLLTNR (Asn391 peptide)

Obs. m/z 952.45 (3+)

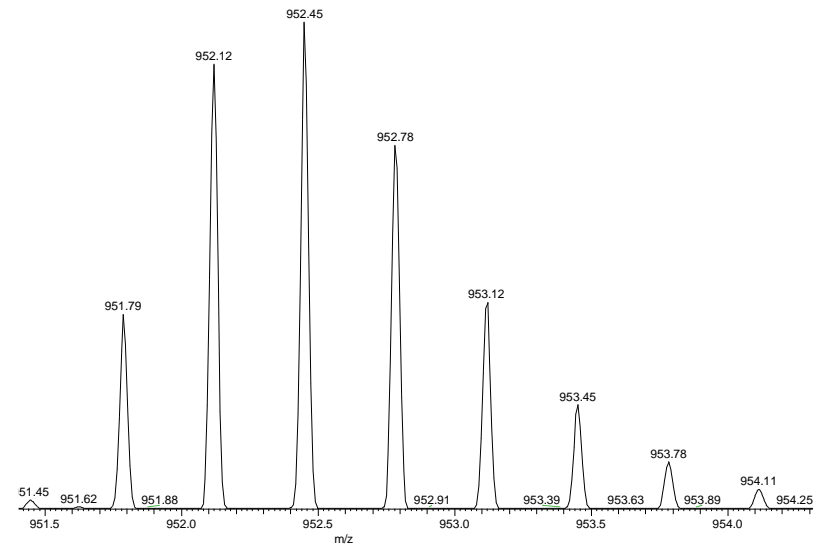
Glycan: 730.26 Da (GlcNAc₂Hex₂)

Obs. $[M+H]^+$ = 2855.35 Da

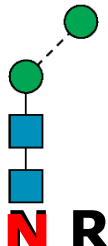
Calc. $[M+H]^+$ = 2854.31 Da

Off-by-X (manual) : 1

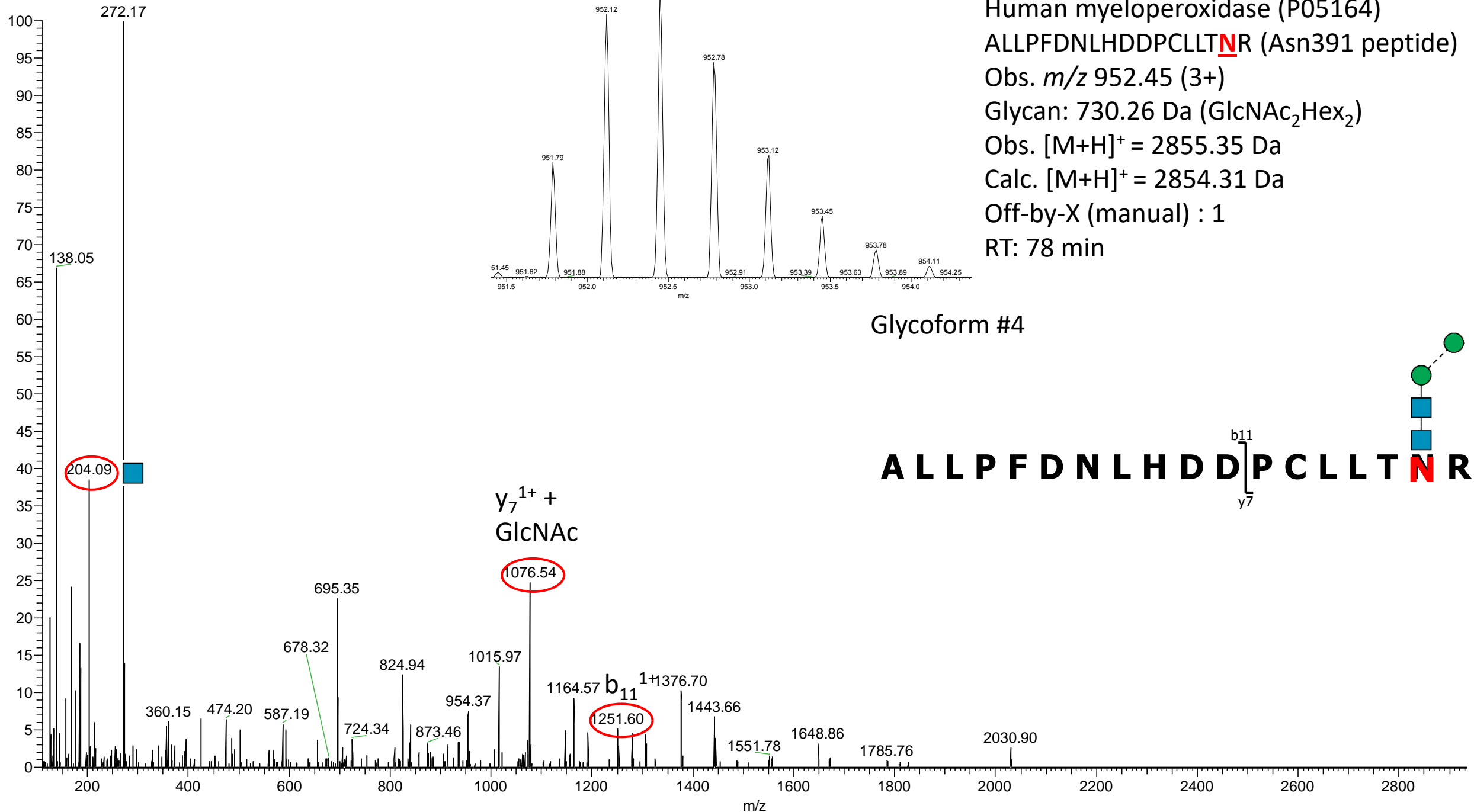
RT: 78 min



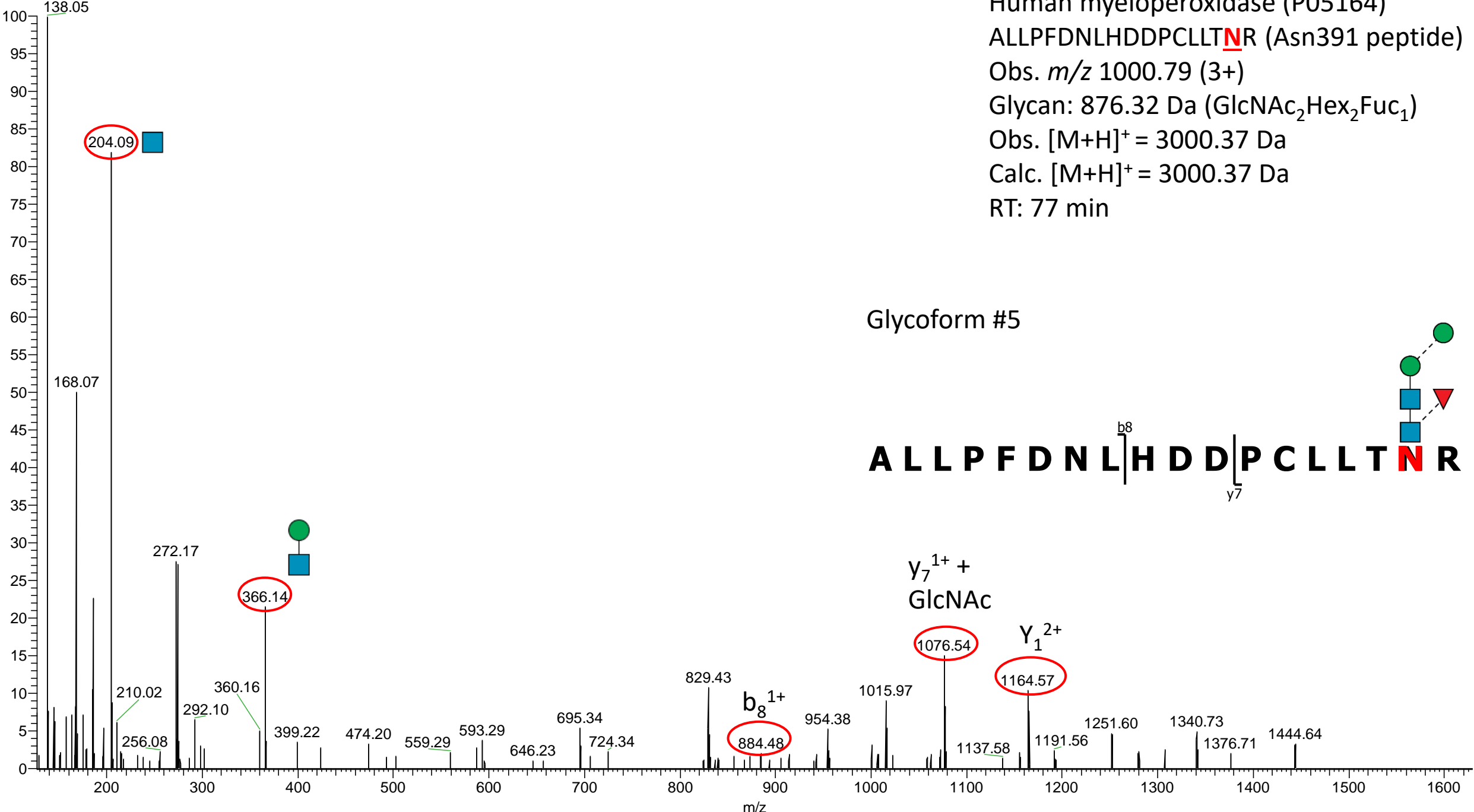
Glycoform #4



ALLPFDNLHDDPCLLTNR
b₁₁ |
y₇

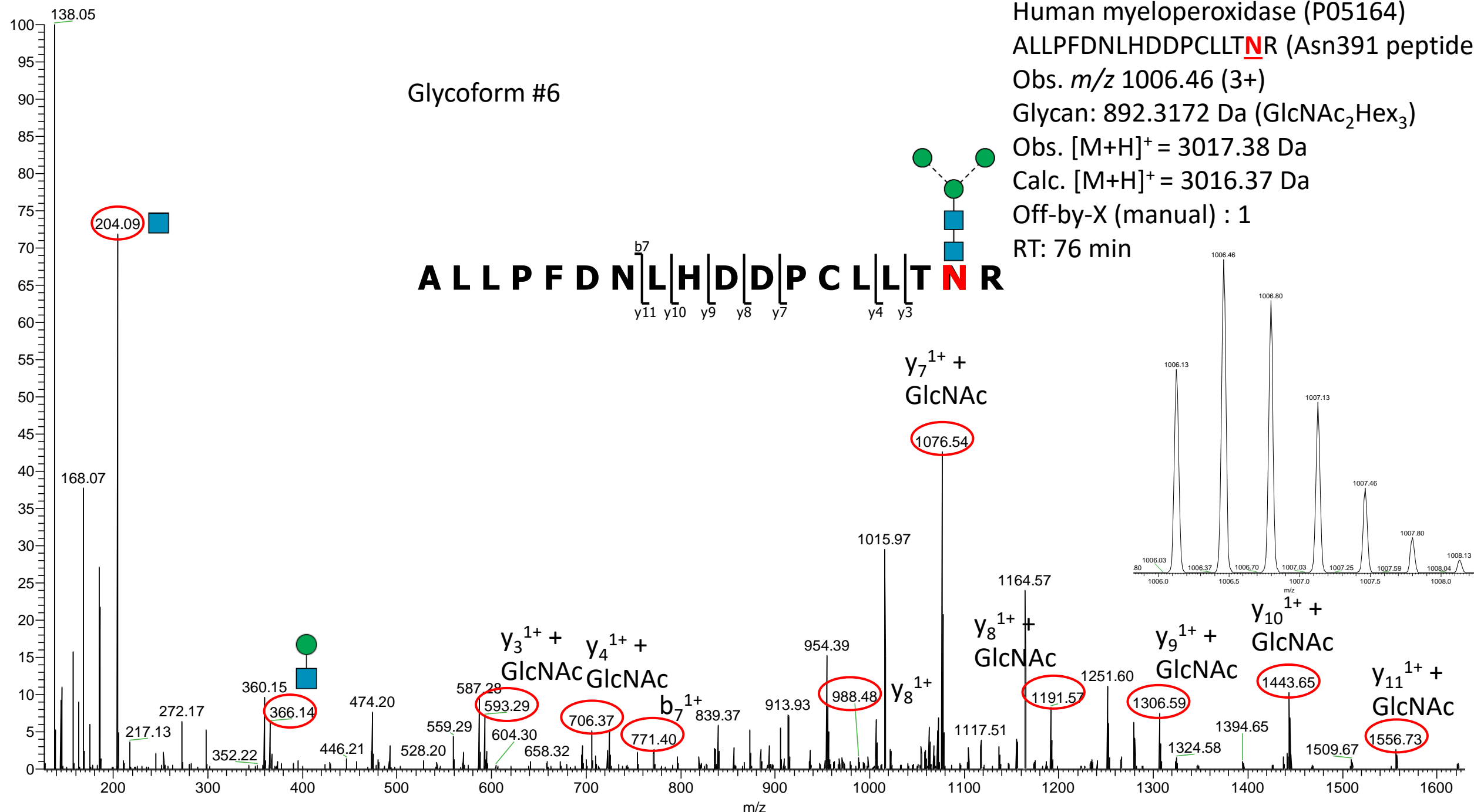
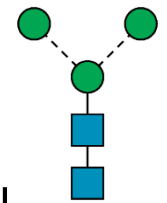
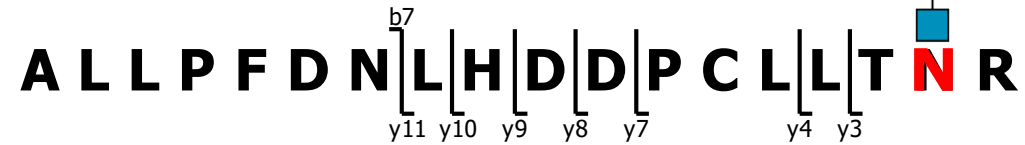


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1000.79 (3+)
 Glycan: 876.32 Da (GlcNAc₂Hex₂Fuc₁)
 Obs. $[M+H]^+$ = 3000.37 Da
 Calc. $[M+H]^+$ = 3000.37 Da
 RT: 77 min

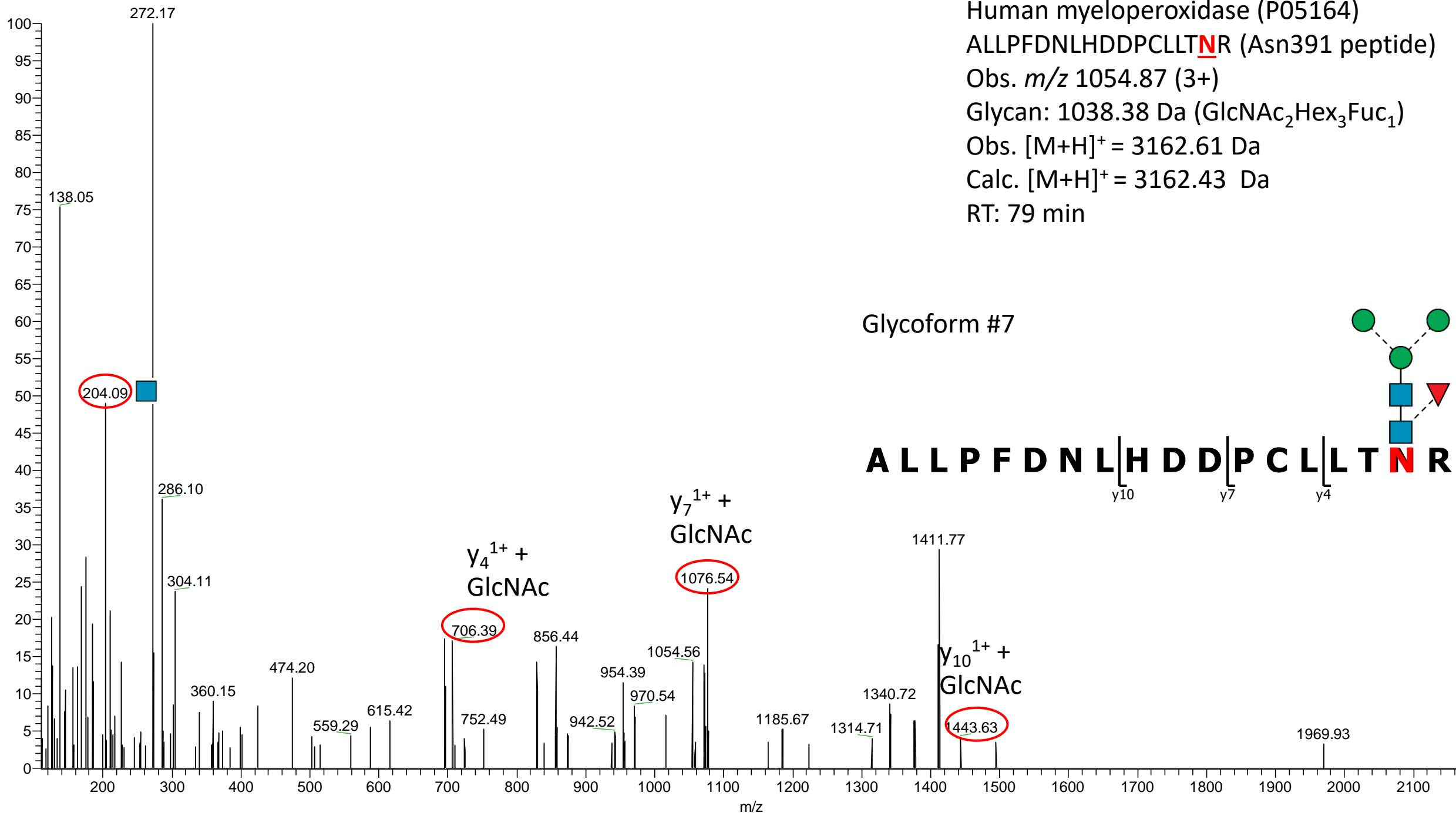


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1006.46 (3+)
 Glycan: 892.3172 Da (GlcNAc₂Hex₃)
 Obs. $[M+H]^+$ = 3017.38 Da
 Calc. $[M+H]^+$ = 3016.37 Da
 Off-by-X (manual) : 1
 RT: 76 min

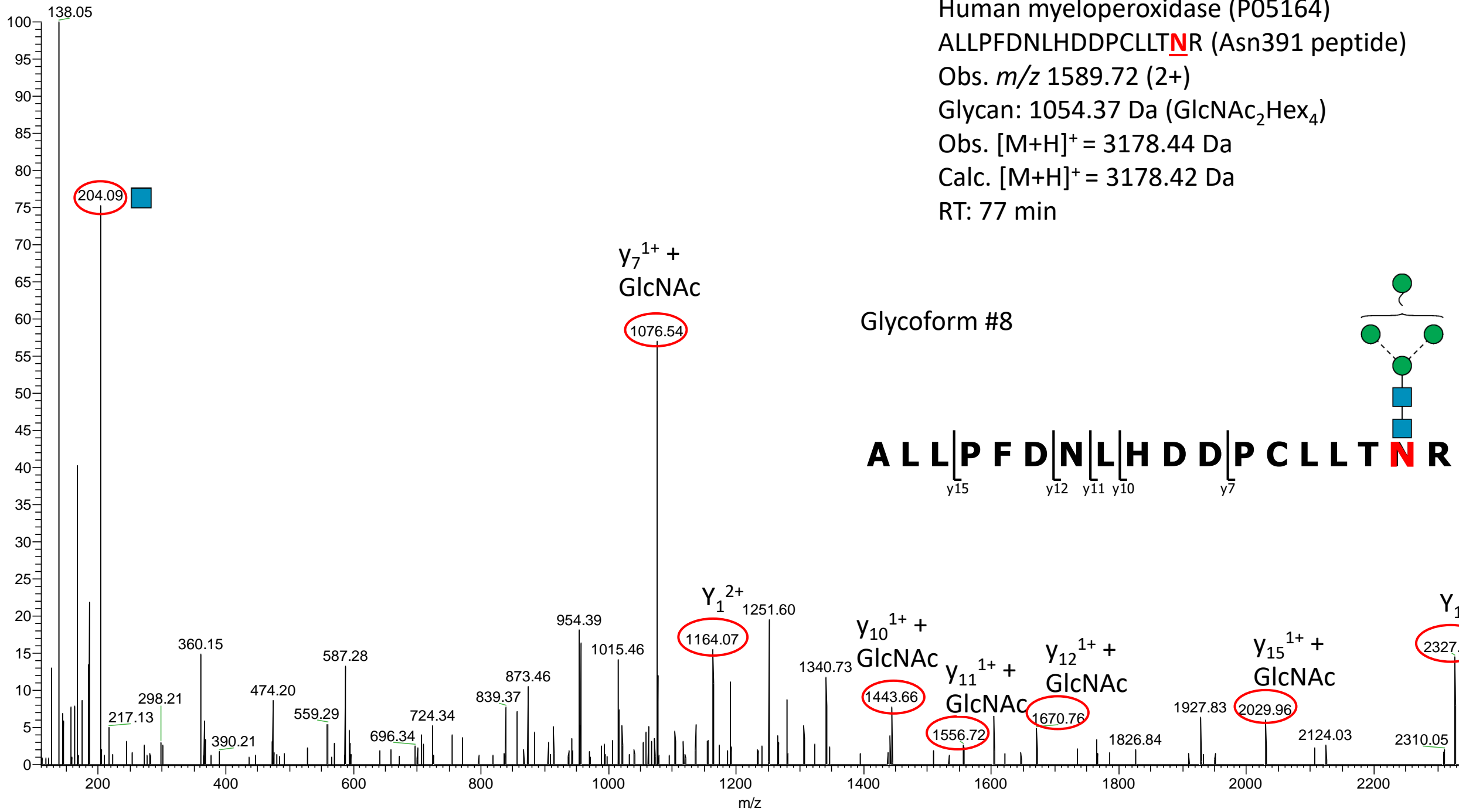
Glycoform #6



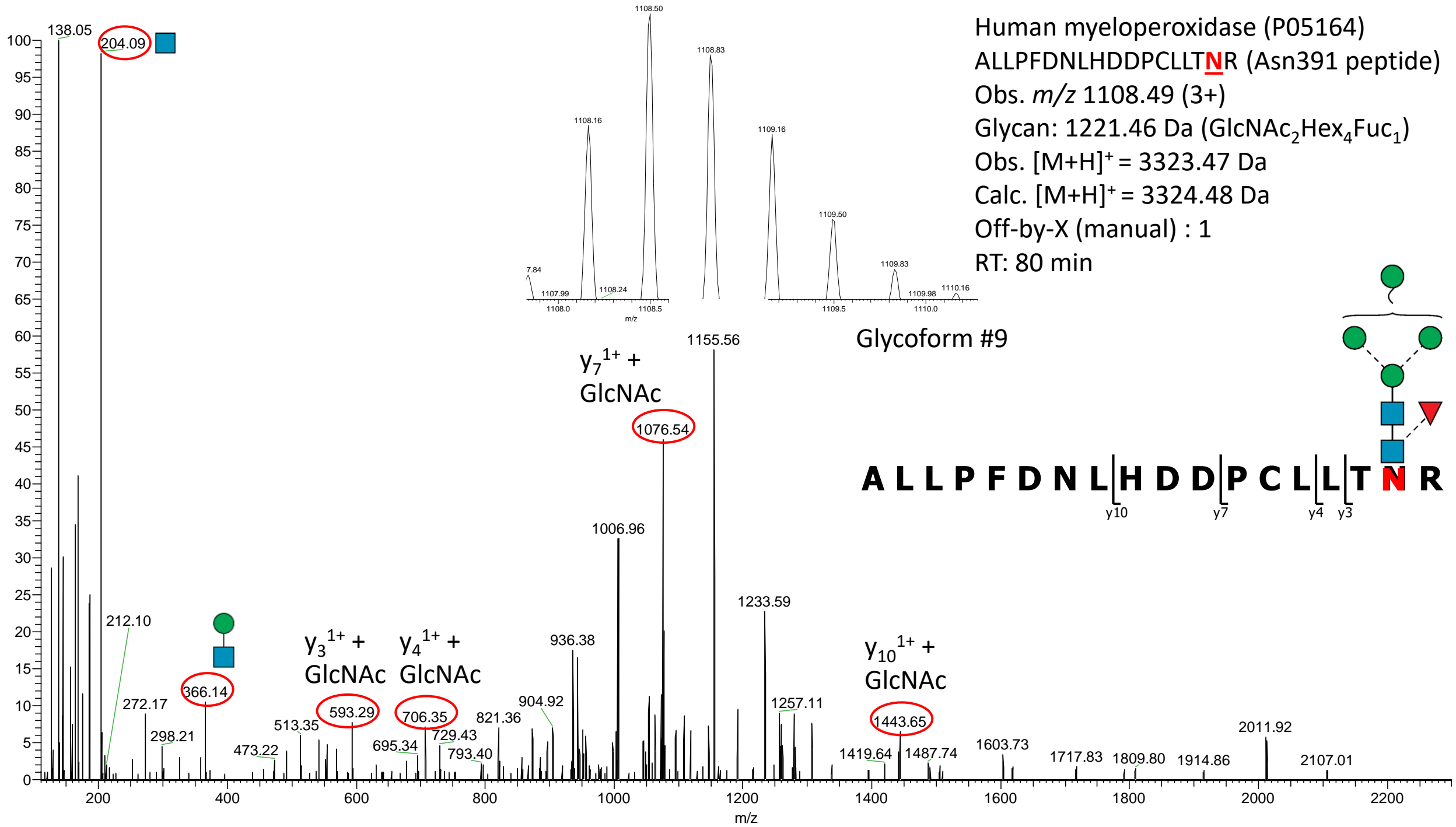
Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1054.87 (3+)
 Glycan: 1038.38 Da (GlcNAc₂Hex₃Fuc₁)
 Obs. $[M+H]^+$ = 3162.61 Da
 Calc. $[M+H]^+$ = 3162.43 Da
 RT: 79 min

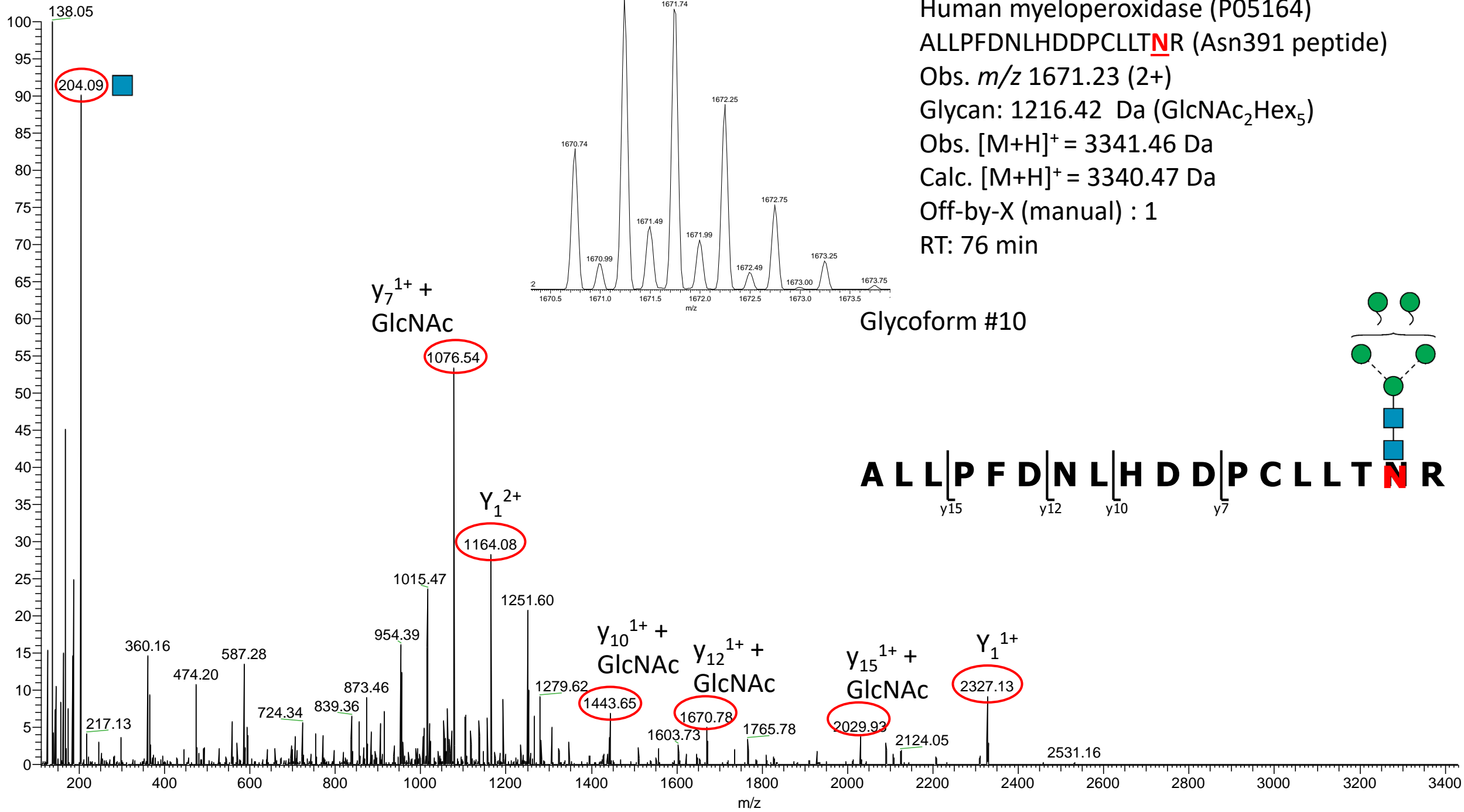


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1589.72 (2+)
 Glycan: 1054.37 Da (GlcNac₂Hex₄)
 Obs. $[M+H]^+$ = 3178.44 Da
 Calc. $[M+H]^+$ = 3178.42 Da
 RT: 77 min

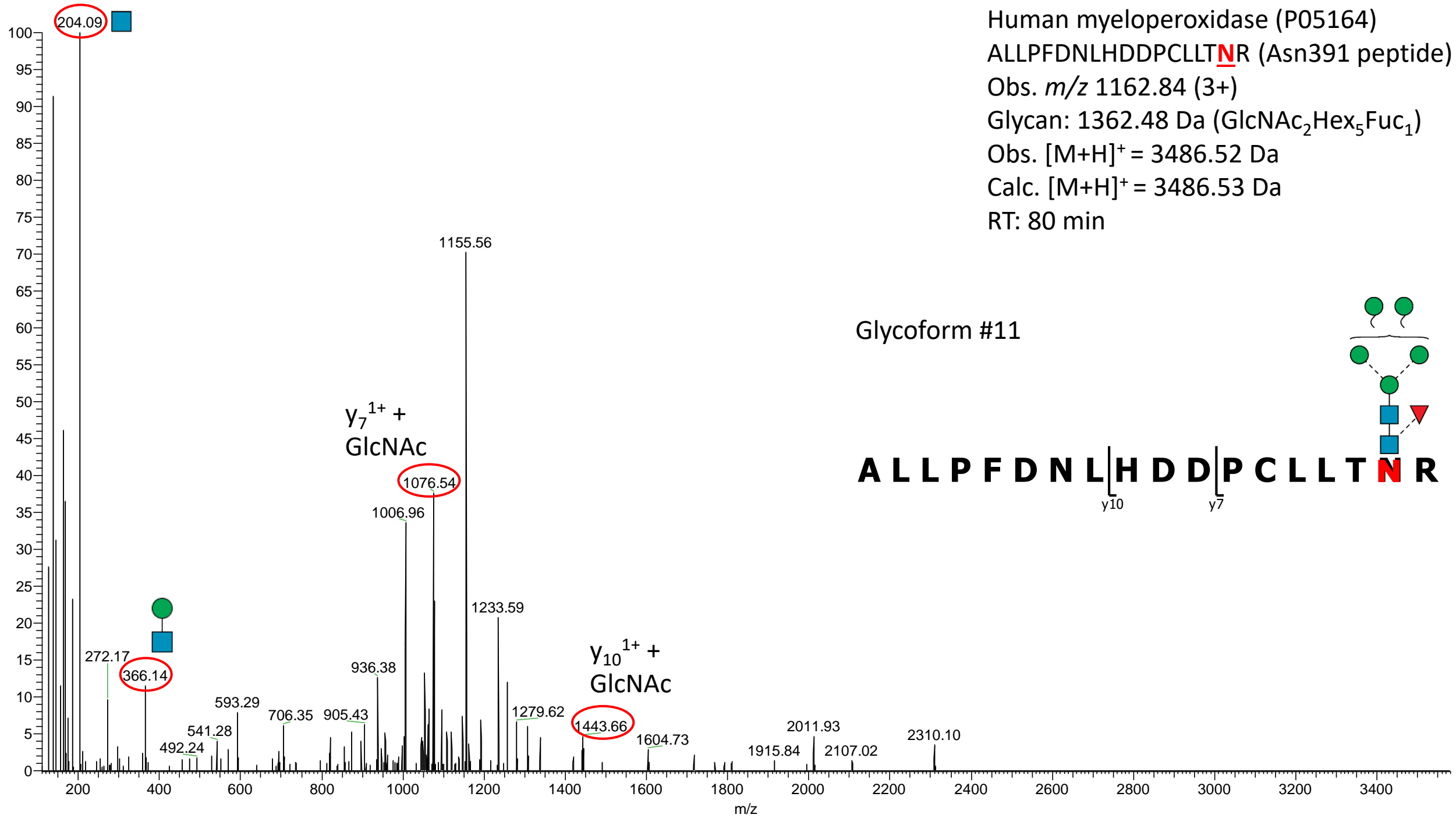


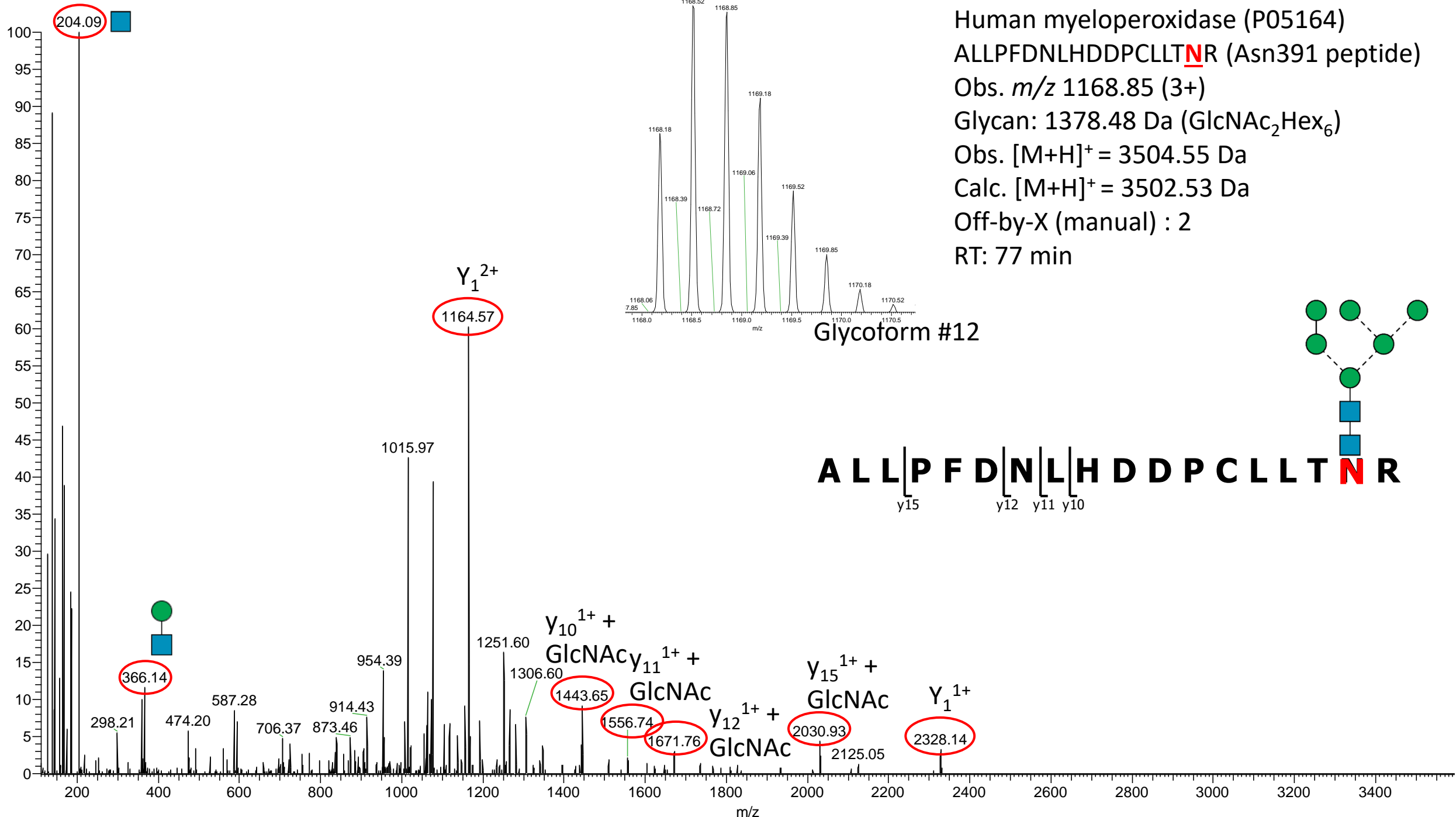
Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1108.49 (3+)
 Glycan: 1221.46 Da (GlcNAc₂Hex₄Fuc₁)
 Obs. $[M+H]^+$ = 3323.47 Da
 Calc. $[M+H]^+$ = 3324.48 Da
 Off-by-X (manual) : 1
 RT: 80 min

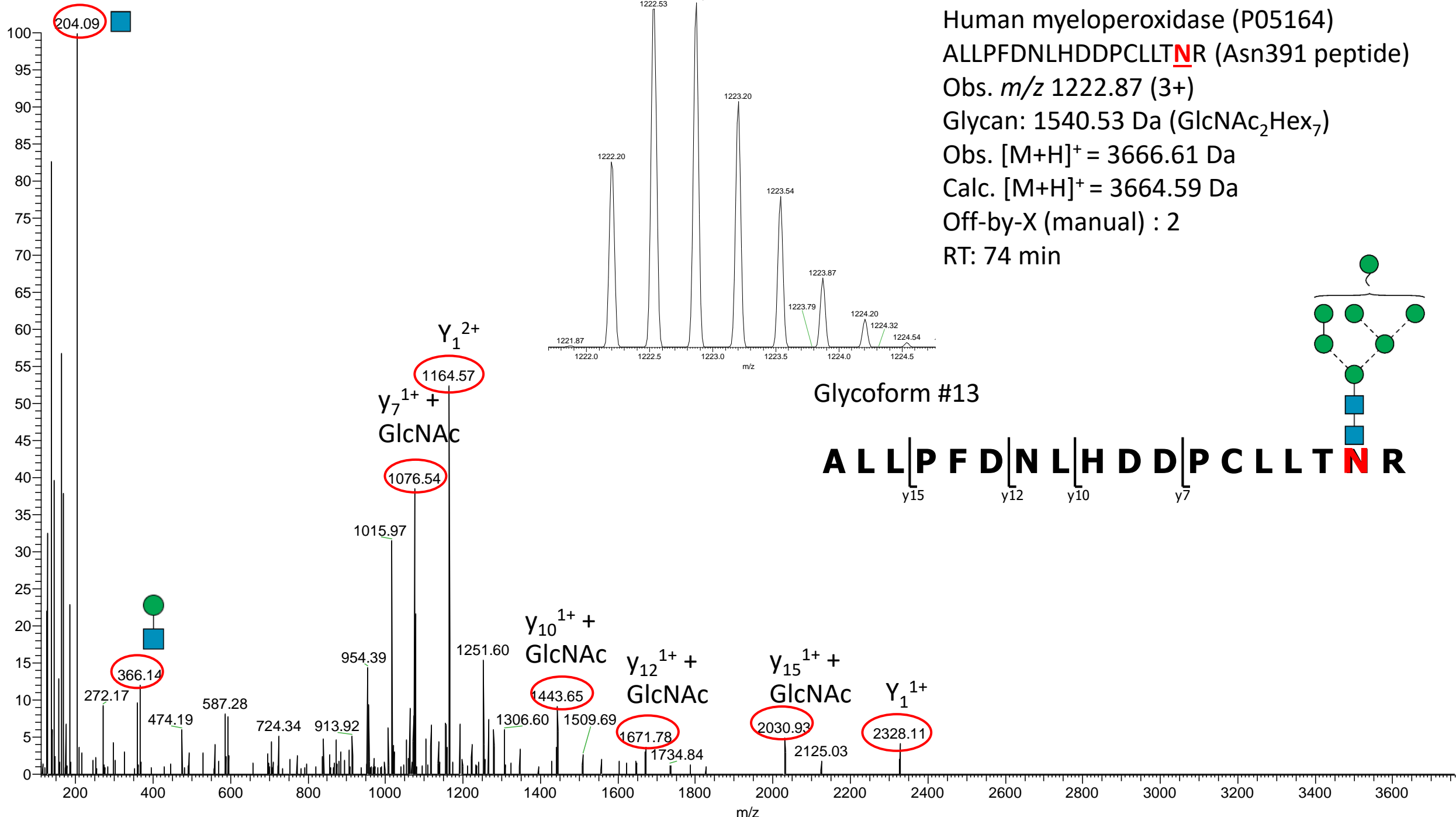


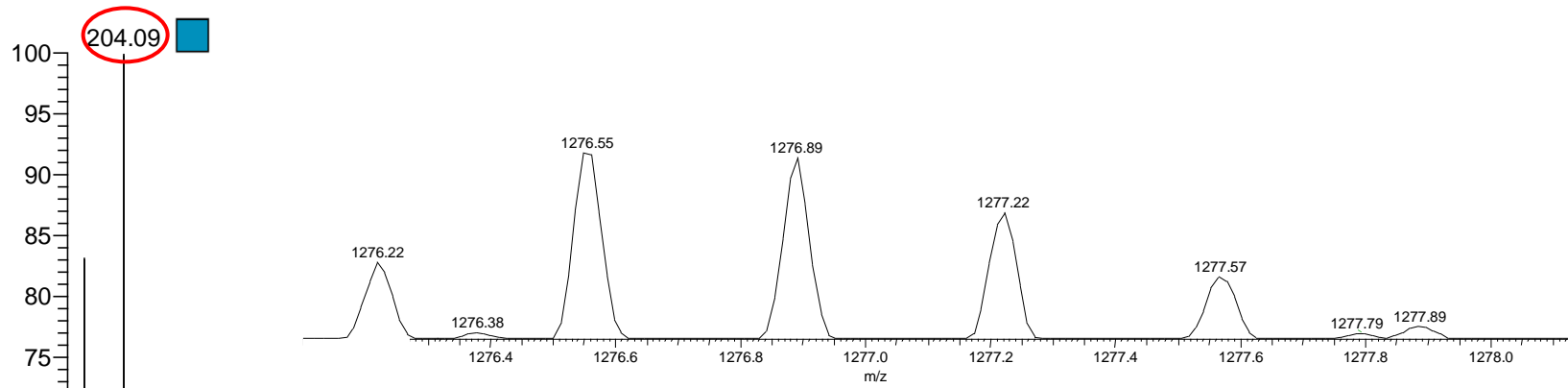


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1162.84 (3+)
 Glycan: 1362.48 Da (GlcNAc₂Hex₅Fuc₁)
 Obs. $[M+H]^+$ = 3486.52 Da
 Calc. $[M+H]^+$ = 3486.53 Da
 RT: 80 min

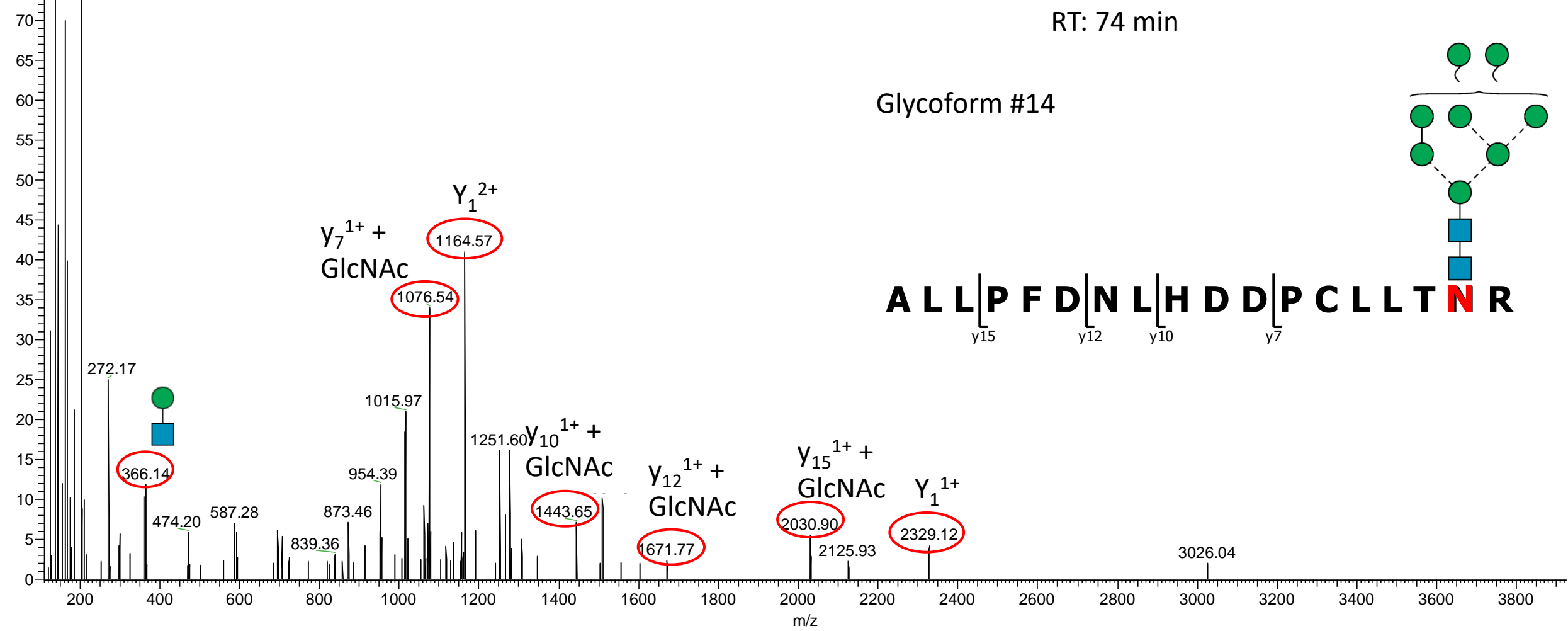




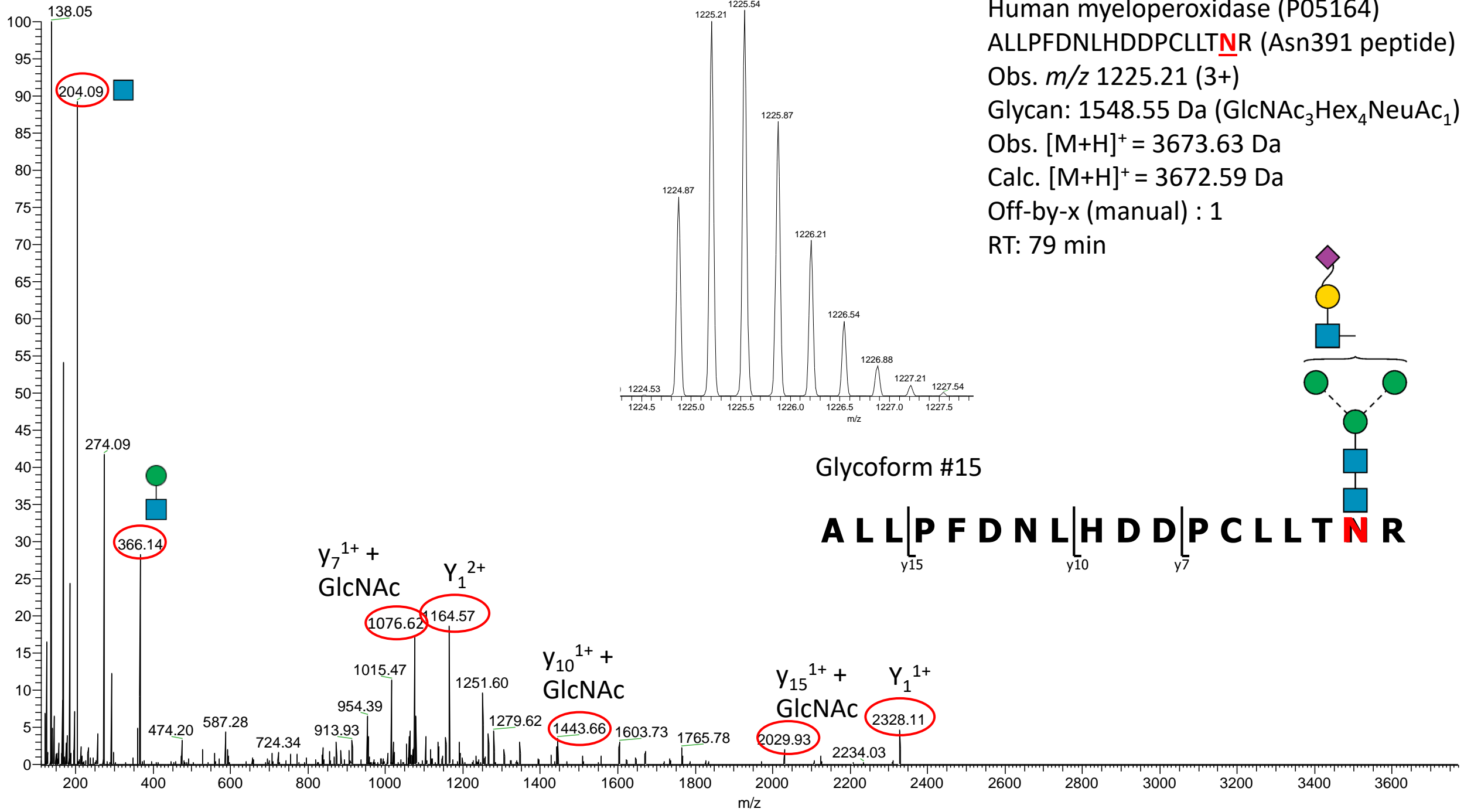


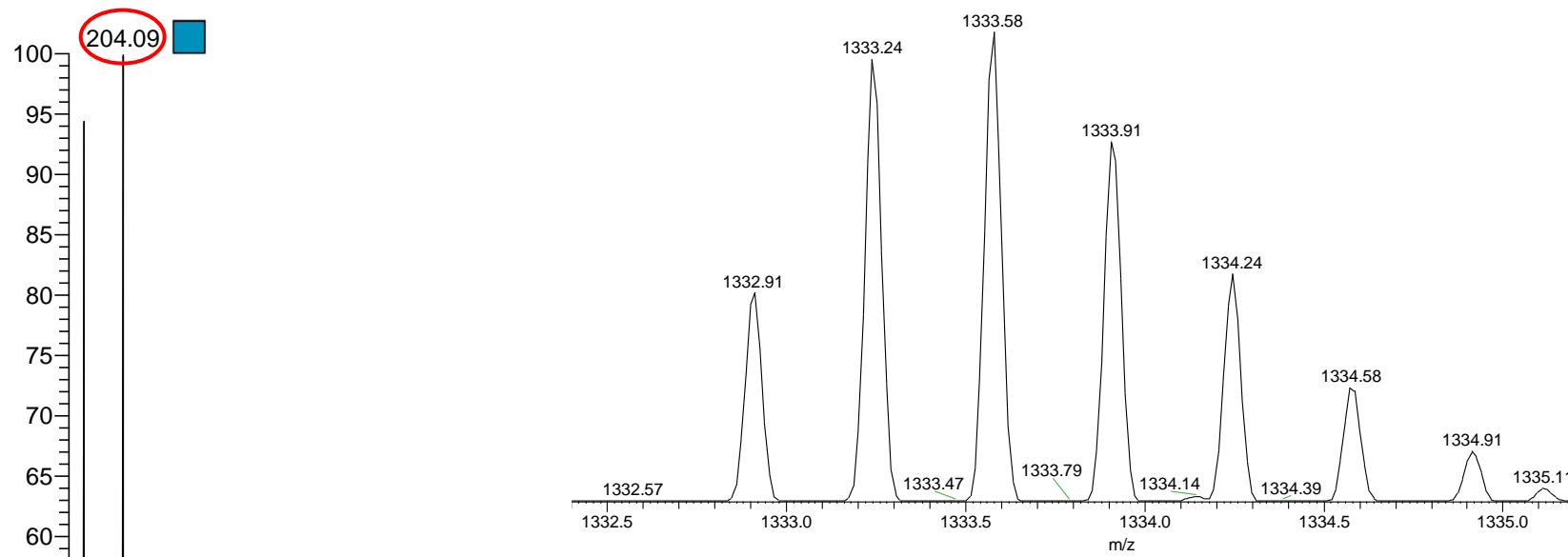


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLTNR (Asn391 peptide)
 Obs. m/z 1276.89 (3+)
 Glycan: 1702.58 Da (GlcNAc₂Hex₈)
 Obs. $[M+H]^+$ = 3828.67 Da
 Calc. $[M+H]^+$ = 3826.63 Da
 Off-by-X (manual) : 2
 RT: 74 min

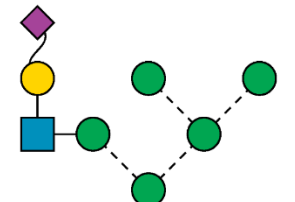


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1225.21 (3+)
 Glycan: 1548.55 Da (GlcNAc₃Hex₄NeuAc₁)
 Obs. $[M+H]^+$ = 3673.63 Da
 Calc. $[M+H]^+$ = 3672.59 Da
 Off-by-x (manual) : 1
 RT: 79 min

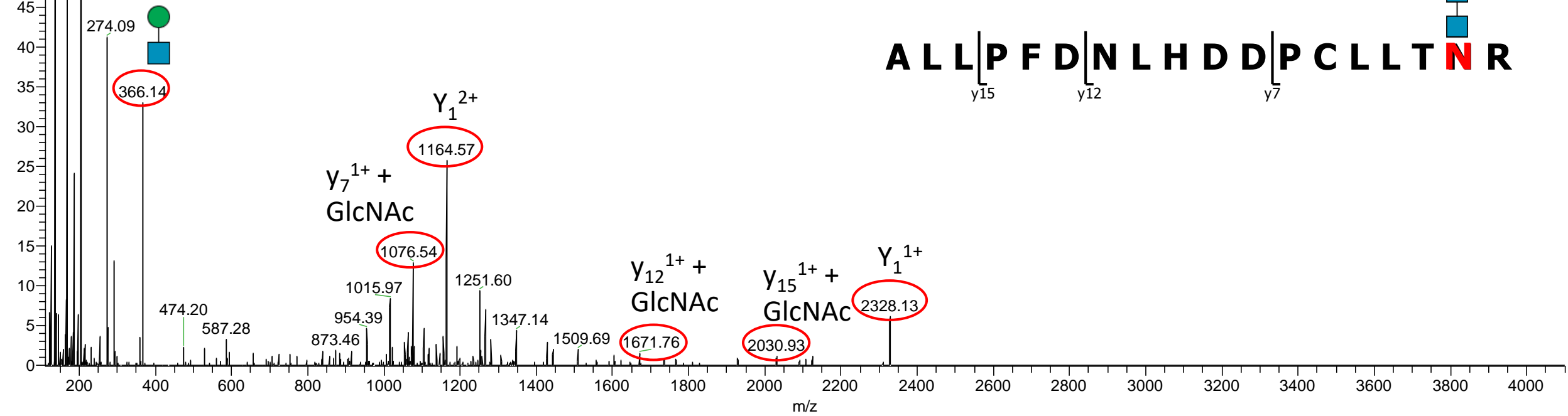


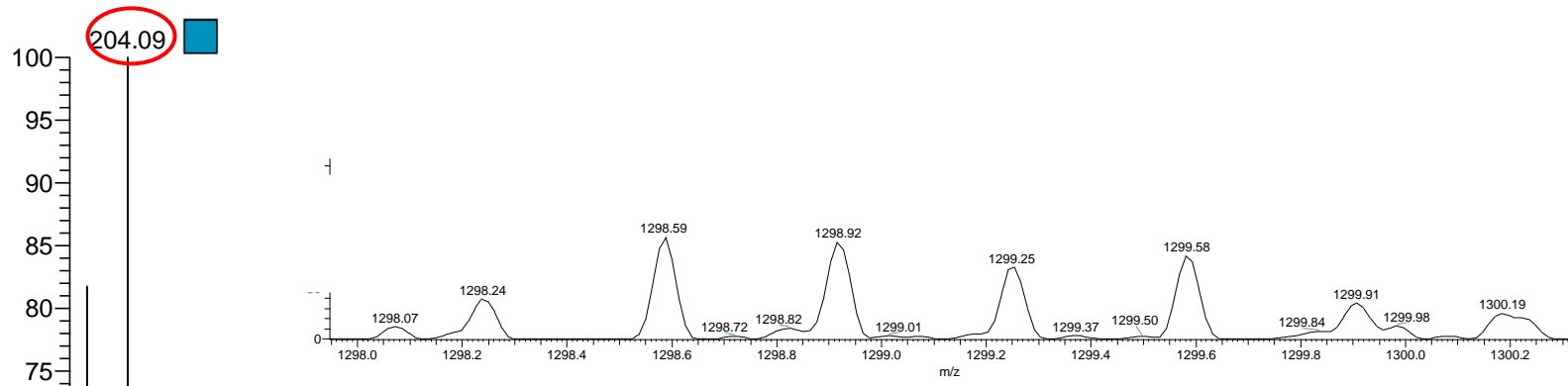


Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1333.58 (3+)
 Glycan: 1872.65 Da (GlcNAc₃Hex₆NeuAc₁)
 Obs. $[M+H]^+$ = 3998.74 Da
 Calc. $[M+H]^+$ = 3996.70 Da
 Off-by-X (Manual) : 2
 RT: 78 min

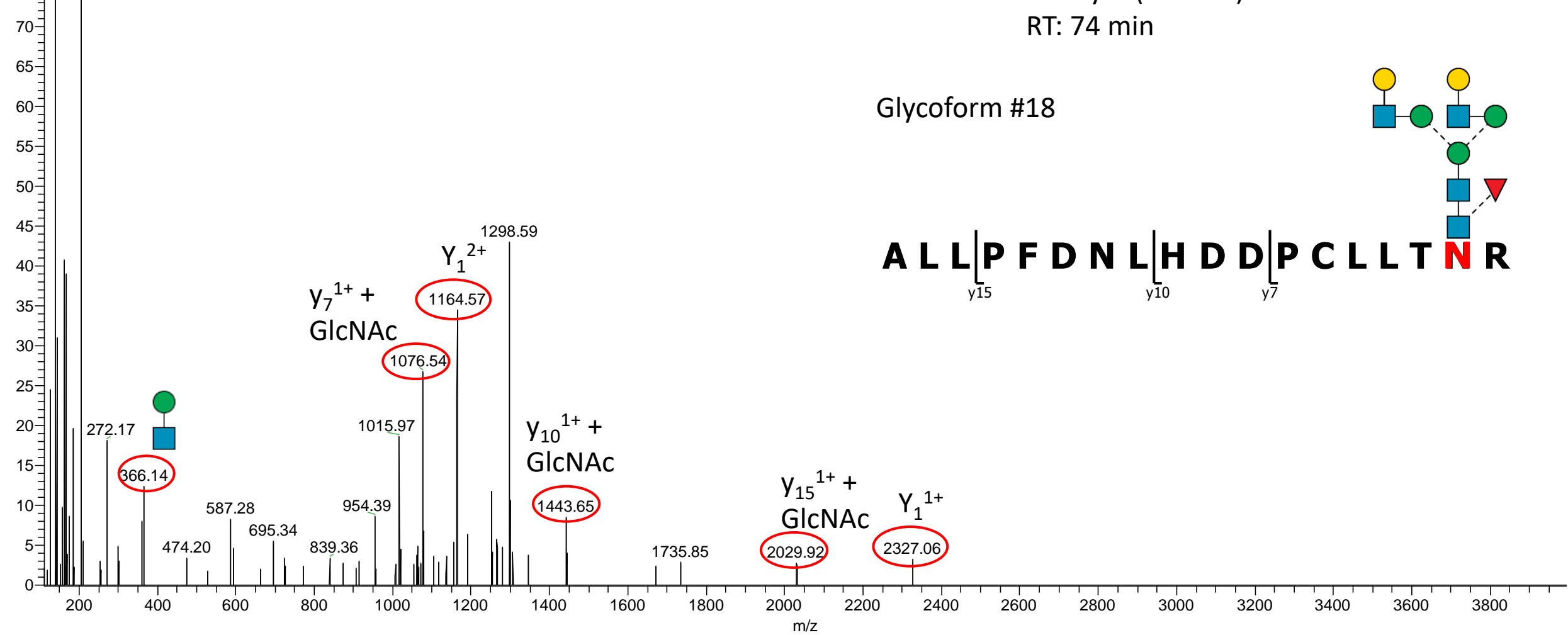


Glycoform #17

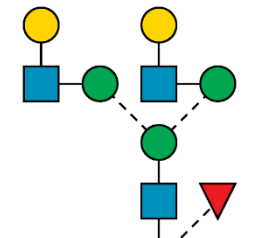




Human myeloperoxidase (P05164)
 ALLPFDNLHDDPCLLT**N**R (Asn391 peptide)
 Obs. m/z 1298.91 (3+)
 Glycan: 1768.64 Da (GlcNAc₄Hex₅Fuc₁)
 Obs. $[M+H]^+$ = 3894.73 Da
 Calc. $[M+H]^+$ = 3892.69 Da
 Off-by-X (manual) : 2
 RT: 74 min

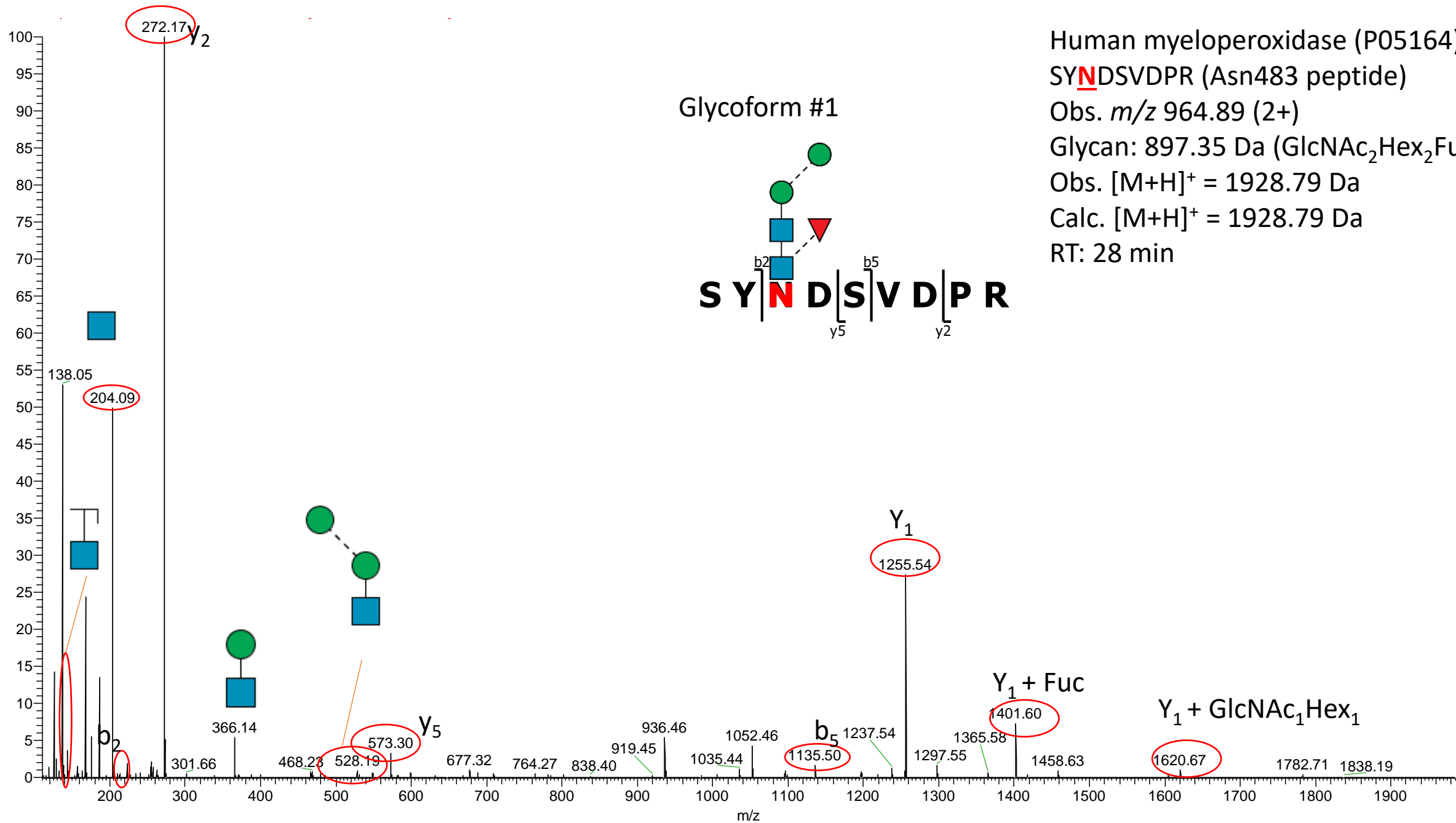


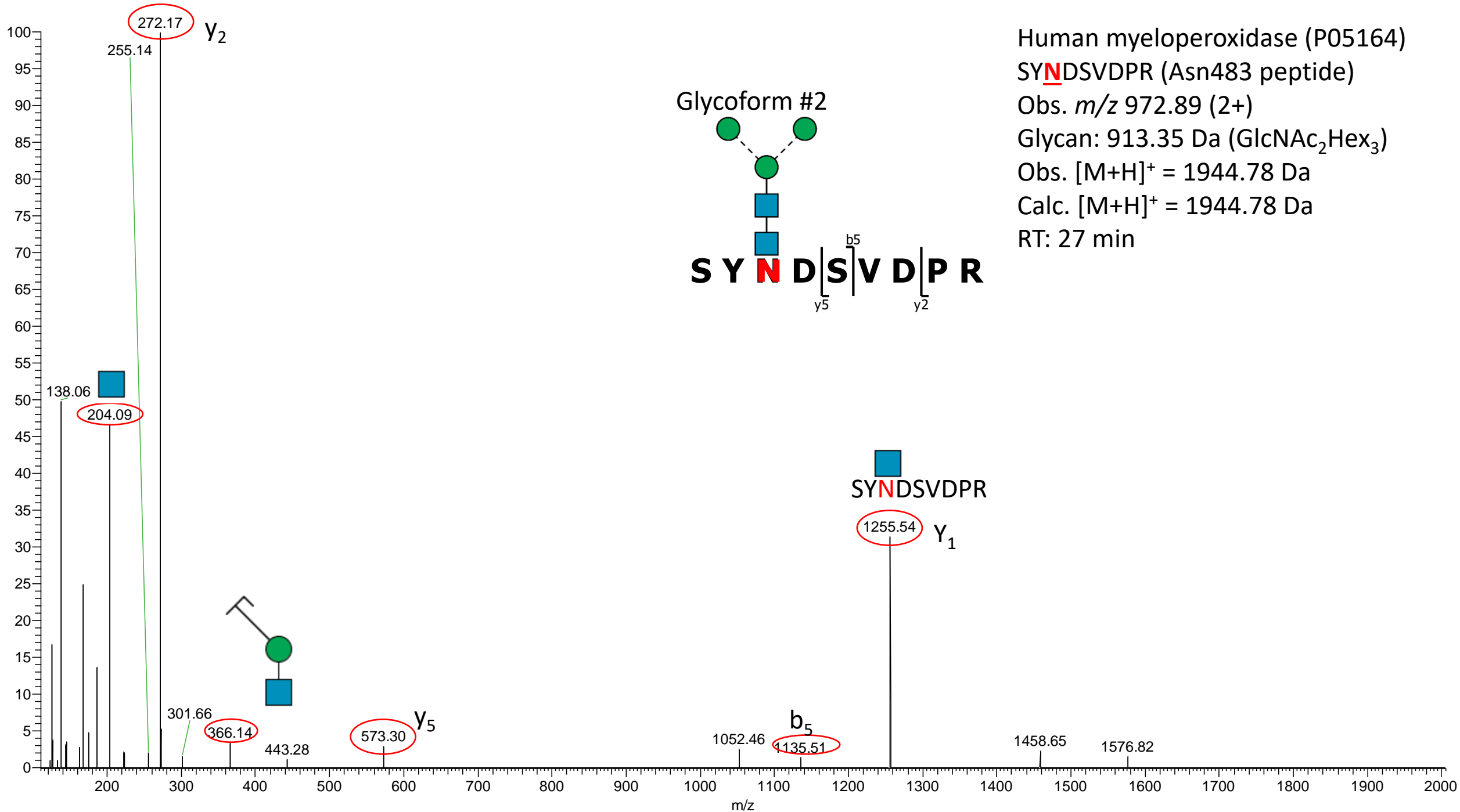
Glycoform #18

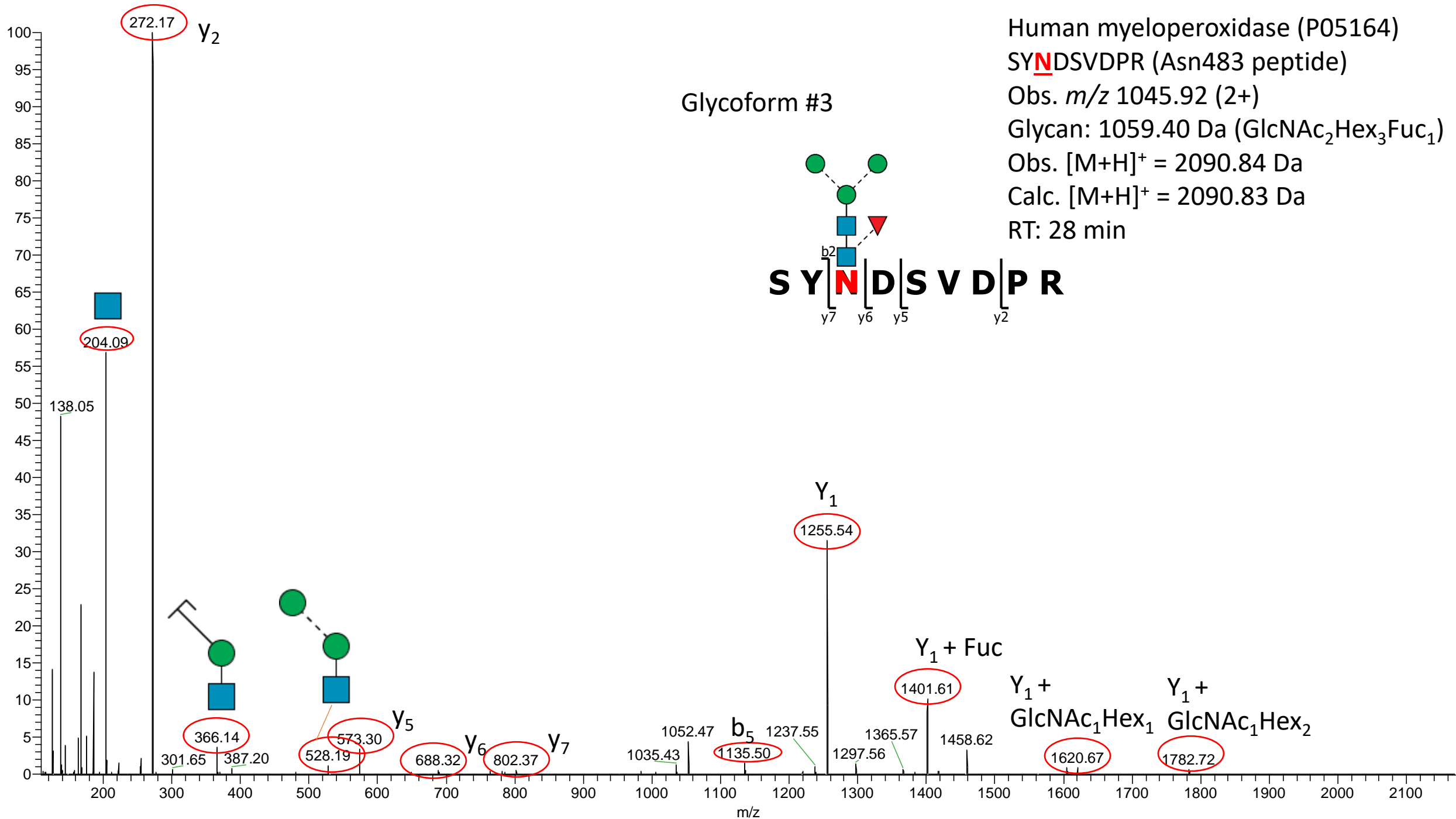


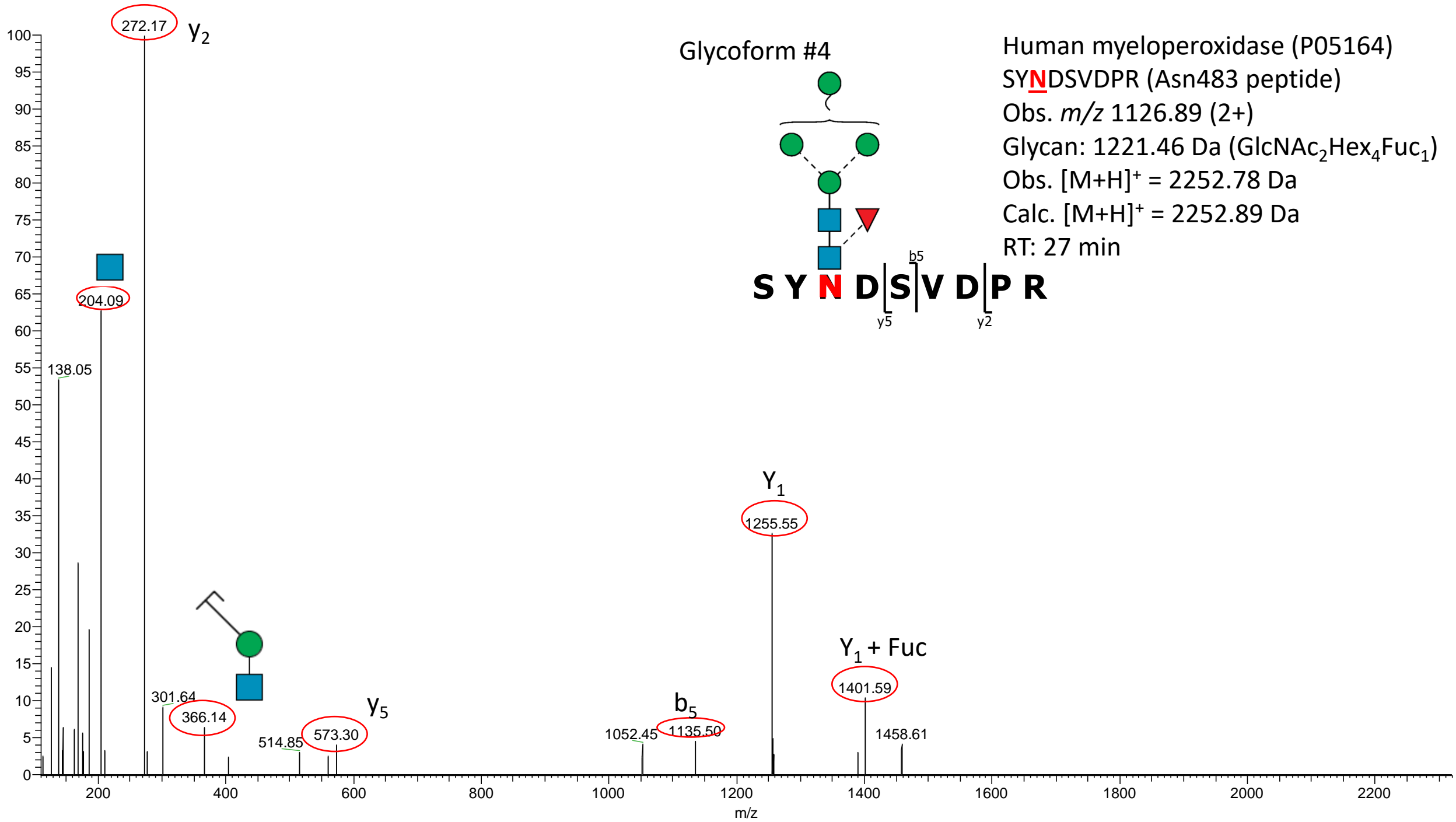
Supplementary Data S3D

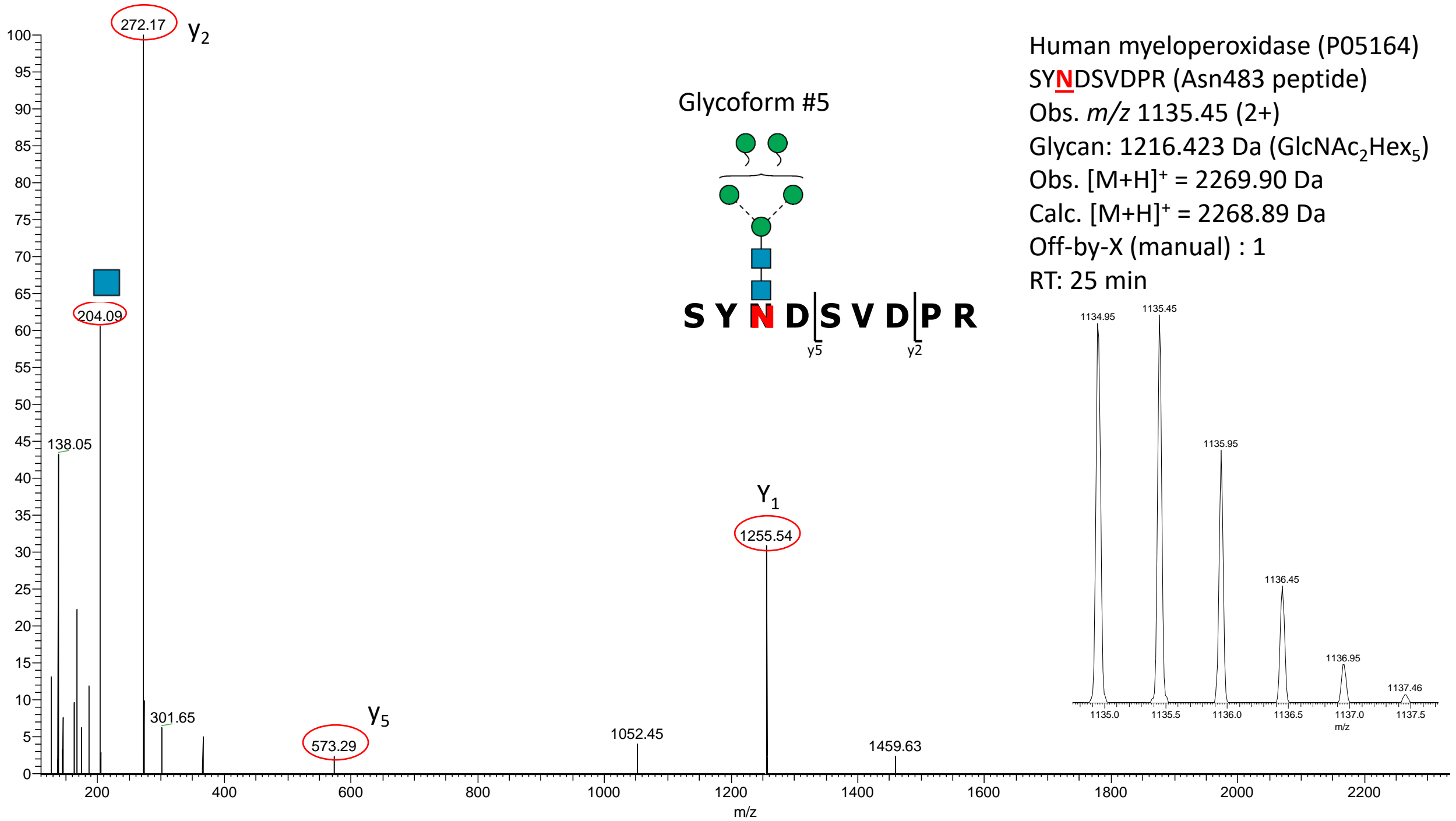
Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn483 identified from the analysis of the unenriched peptide mixtures of nMPO.

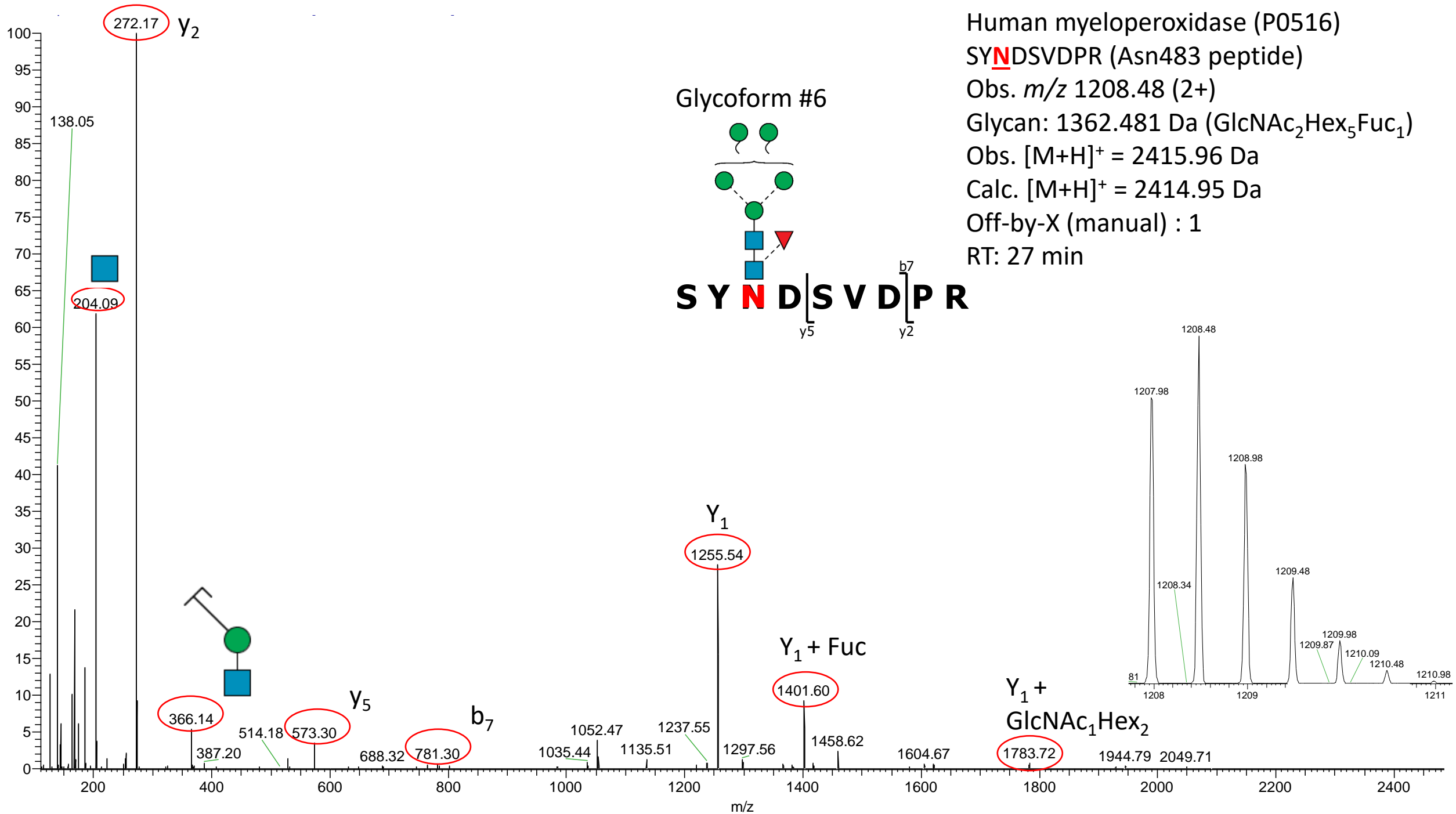


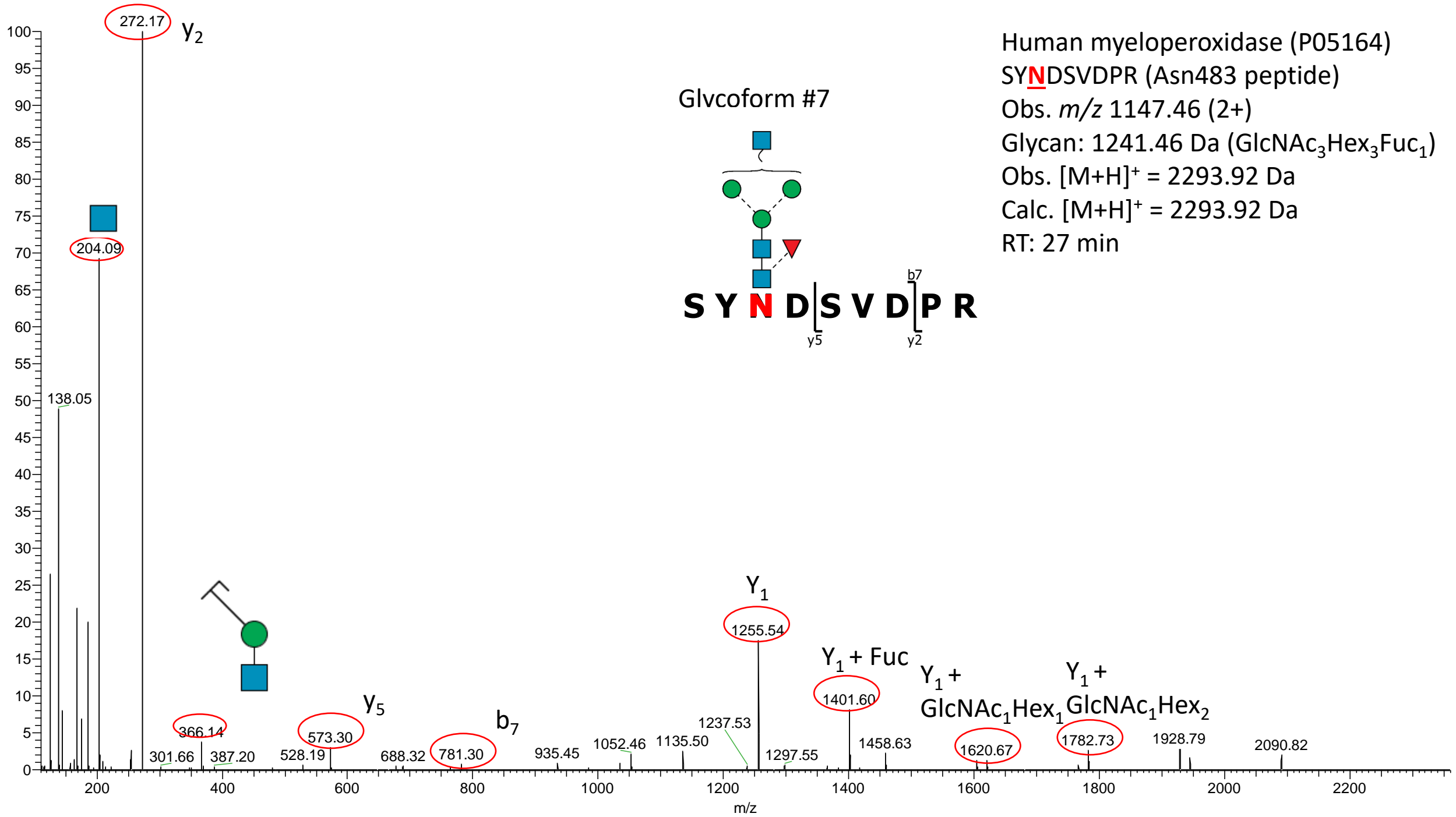


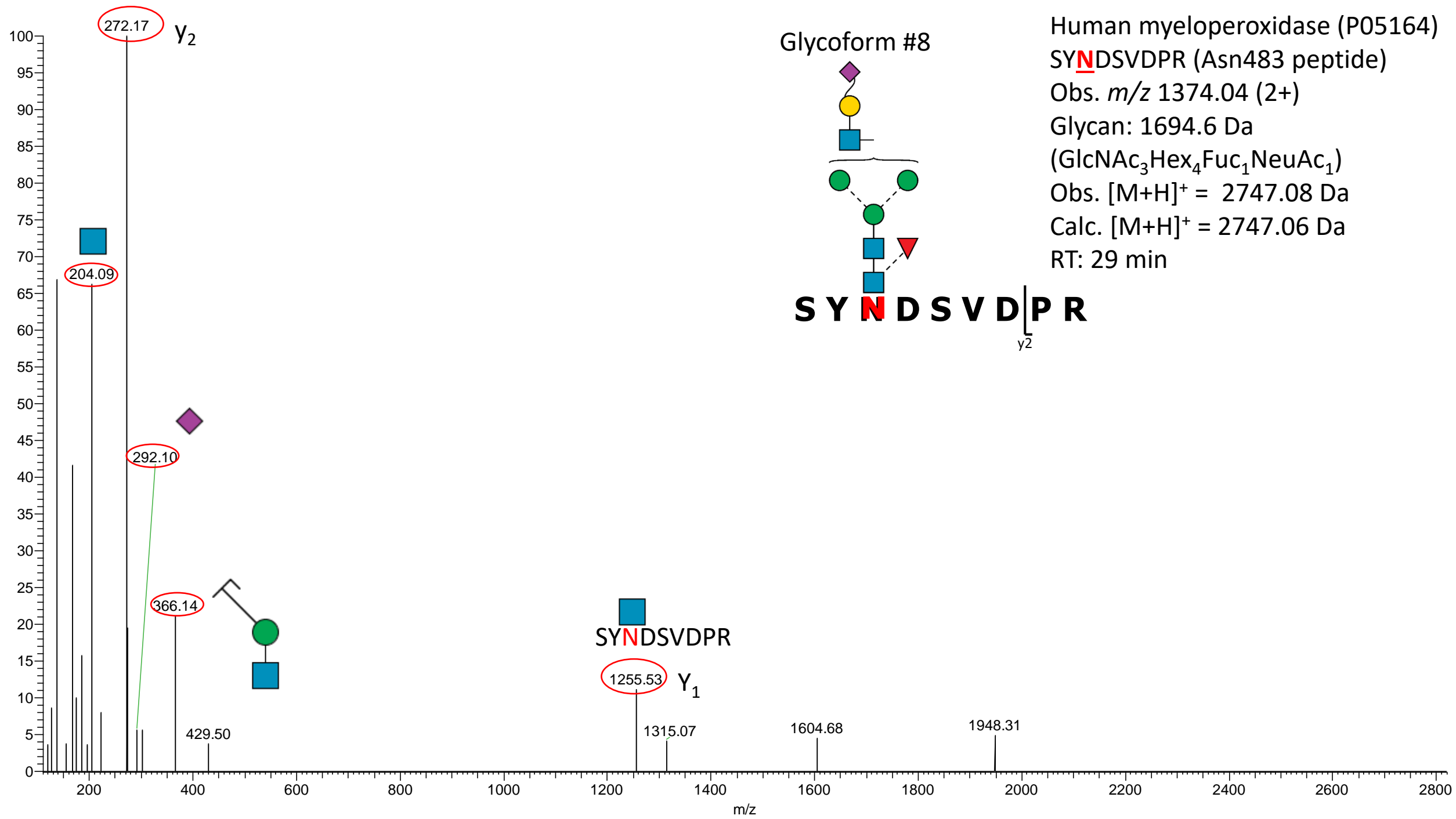


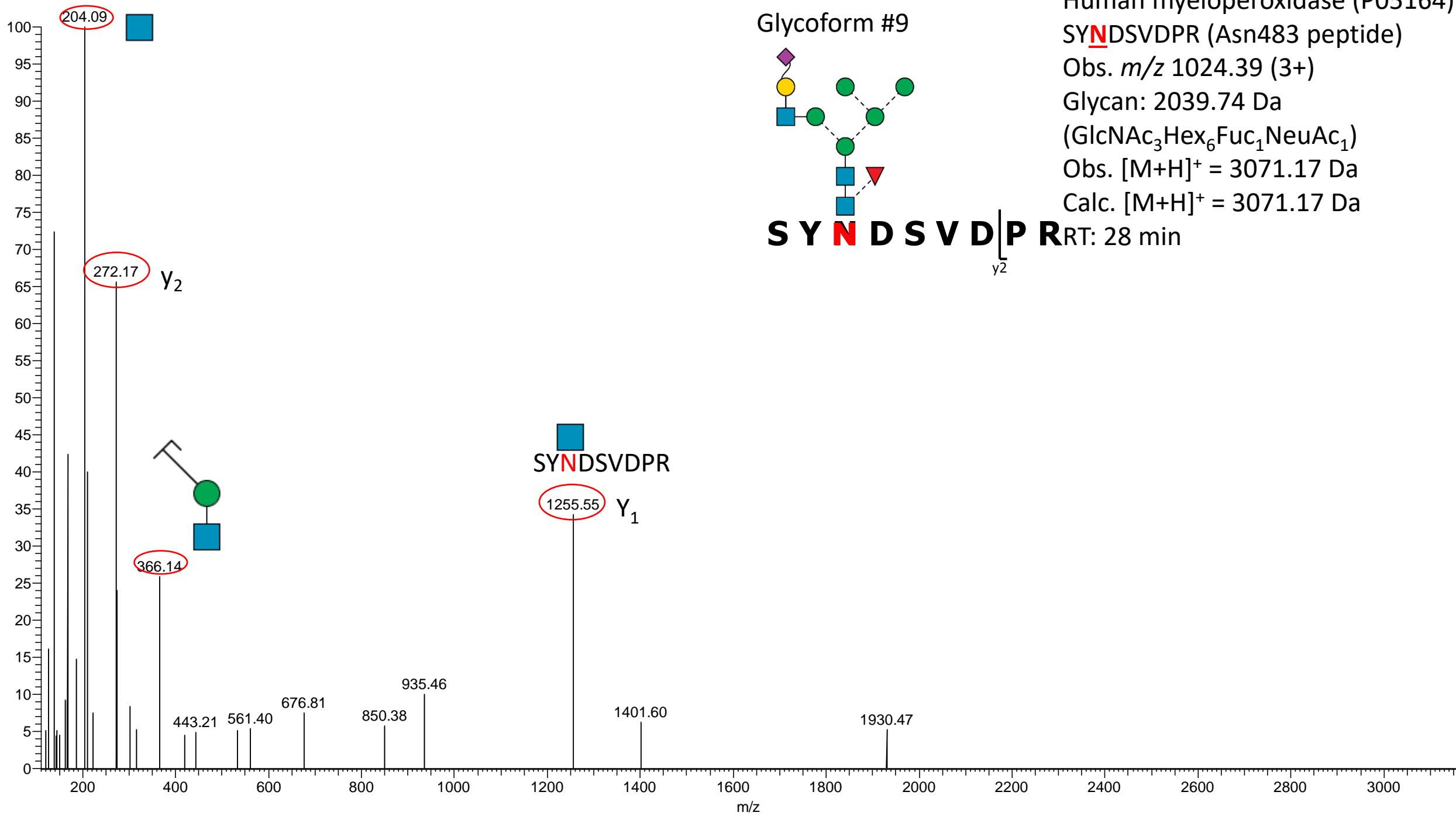


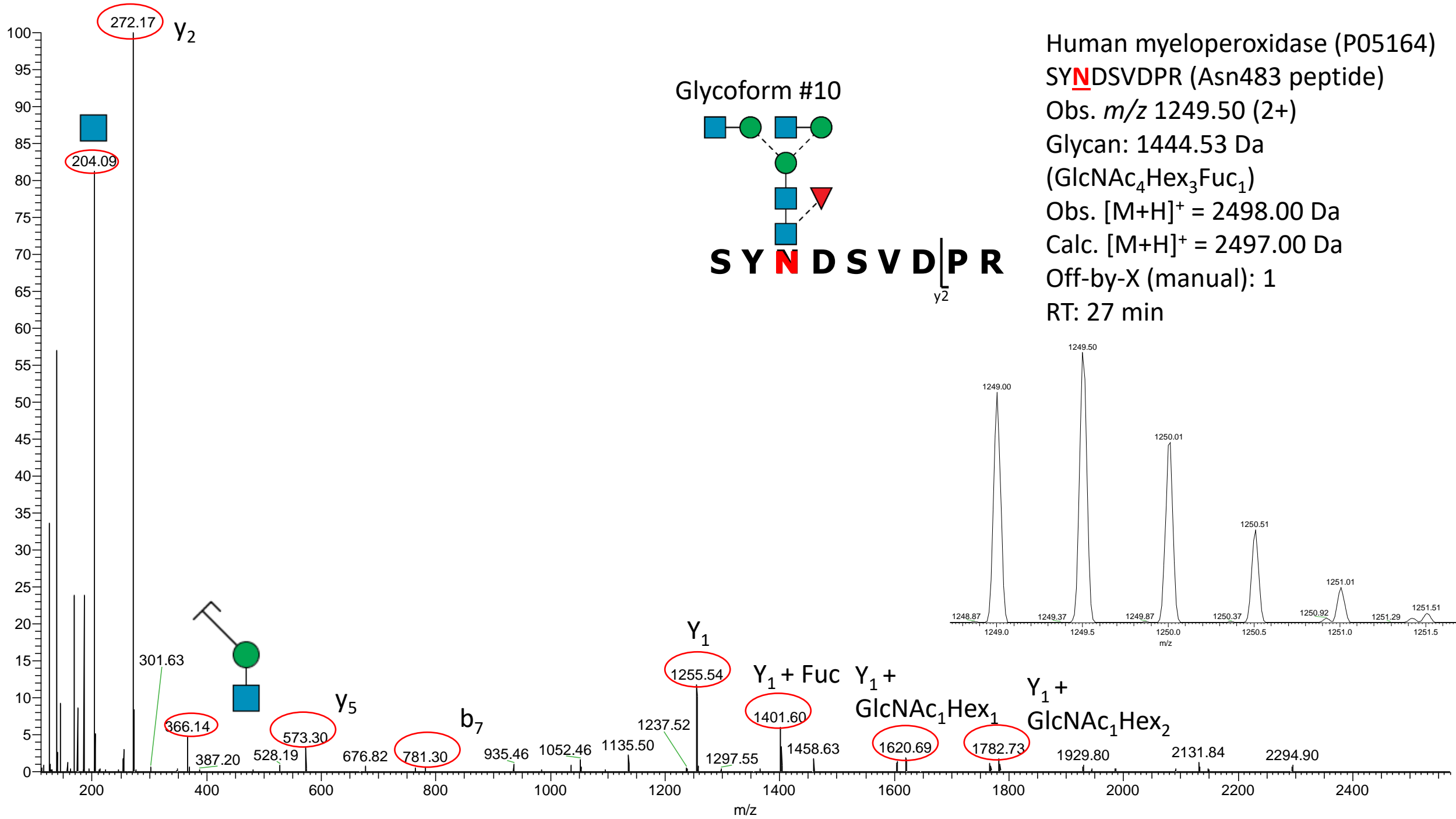


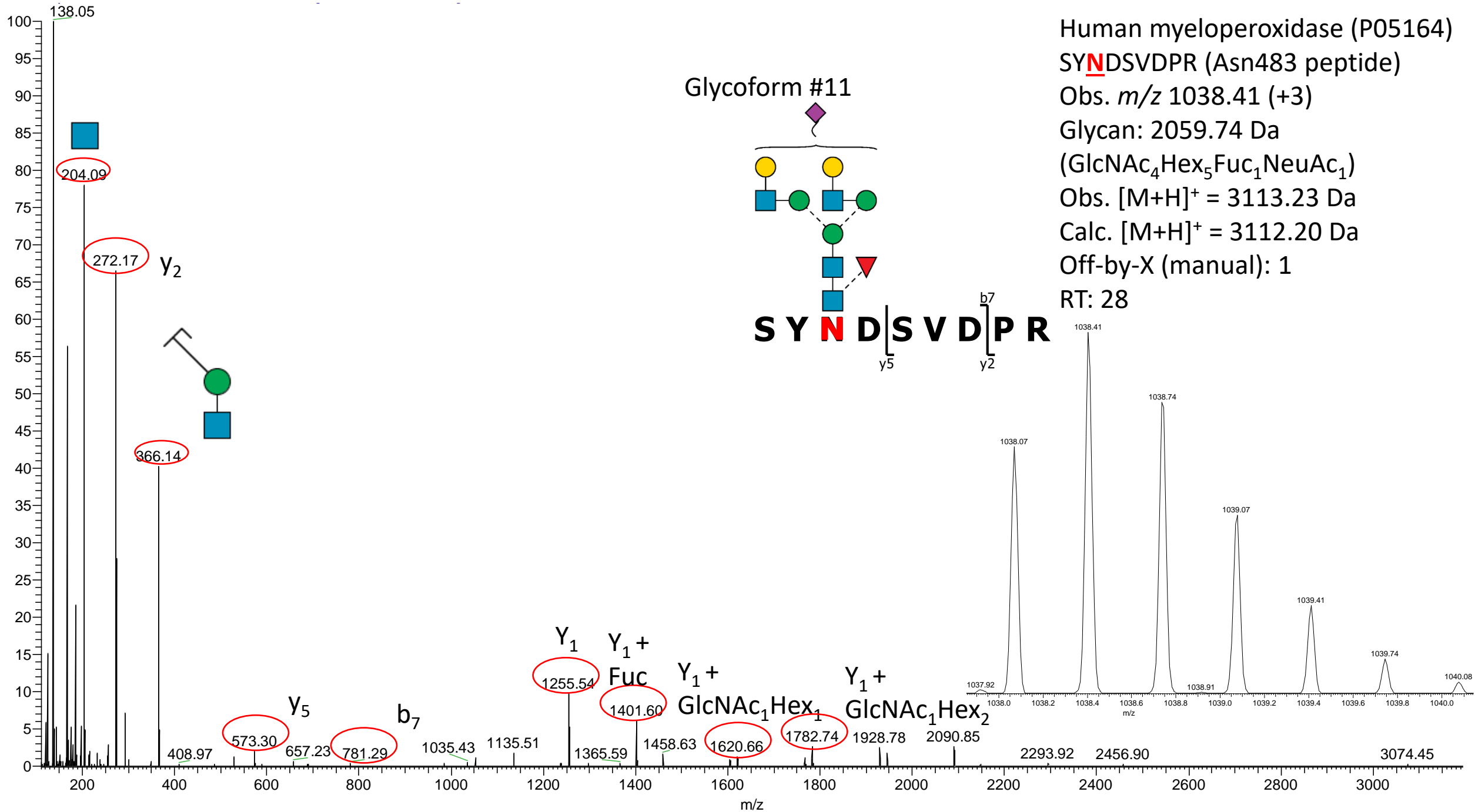


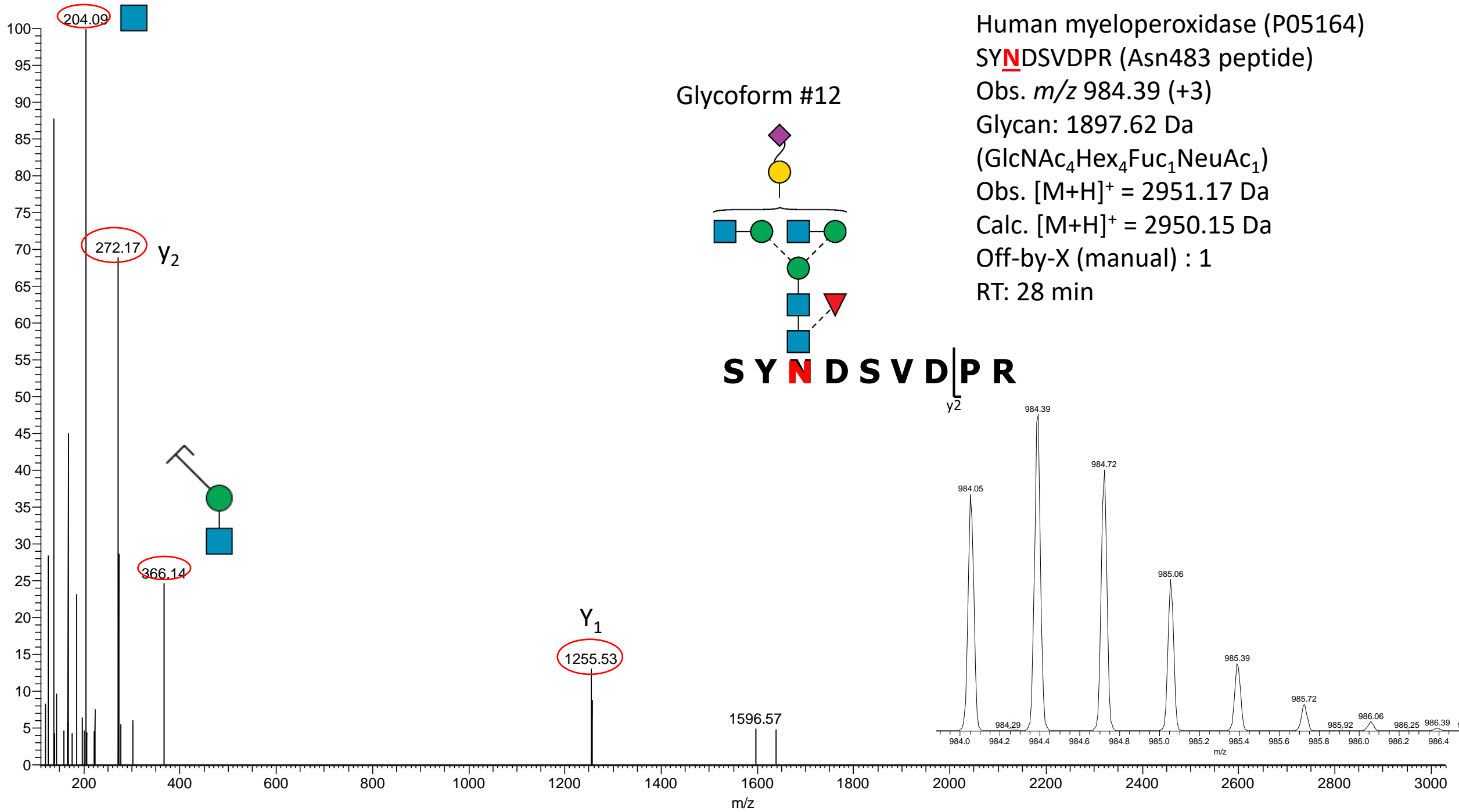










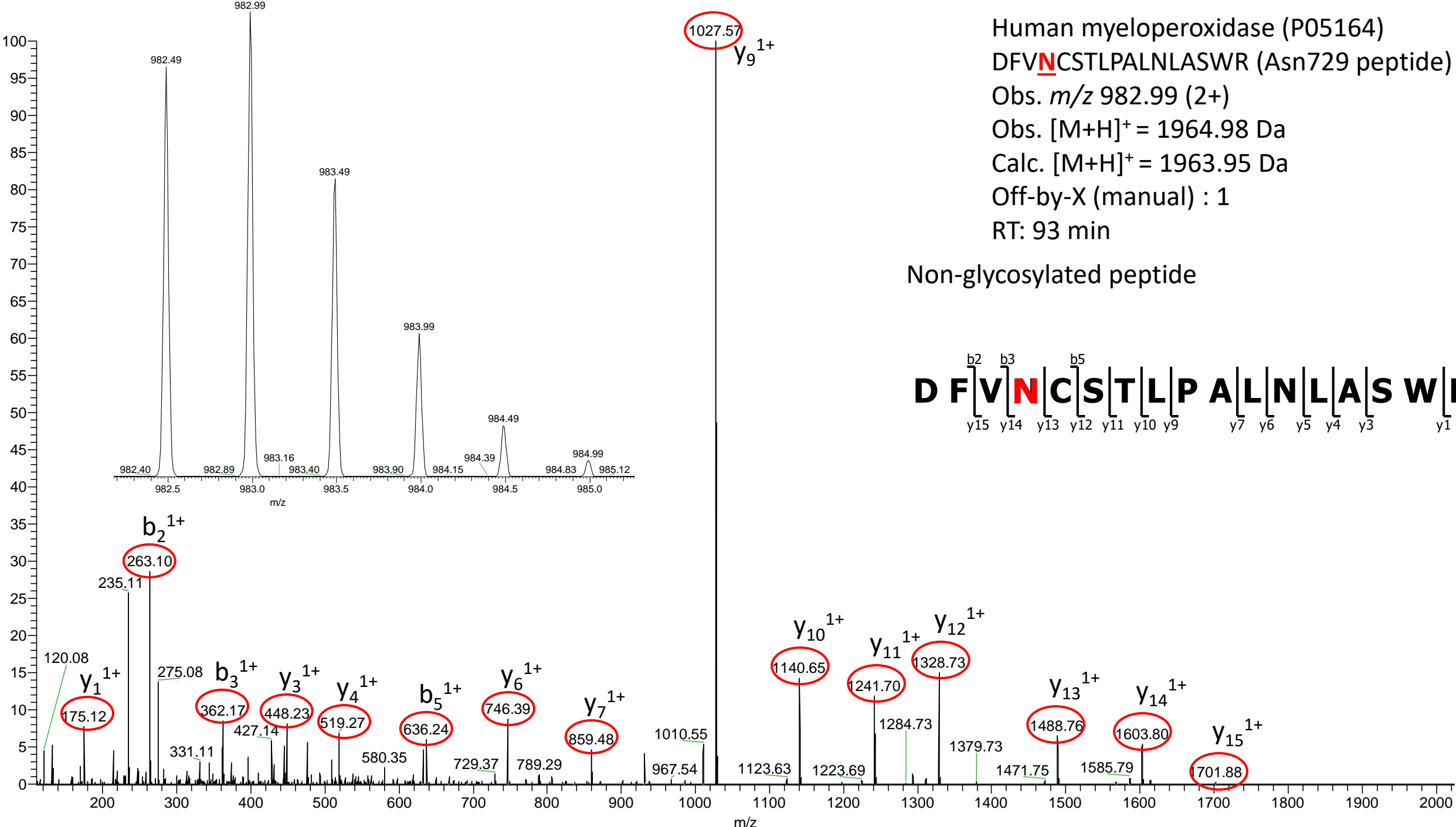


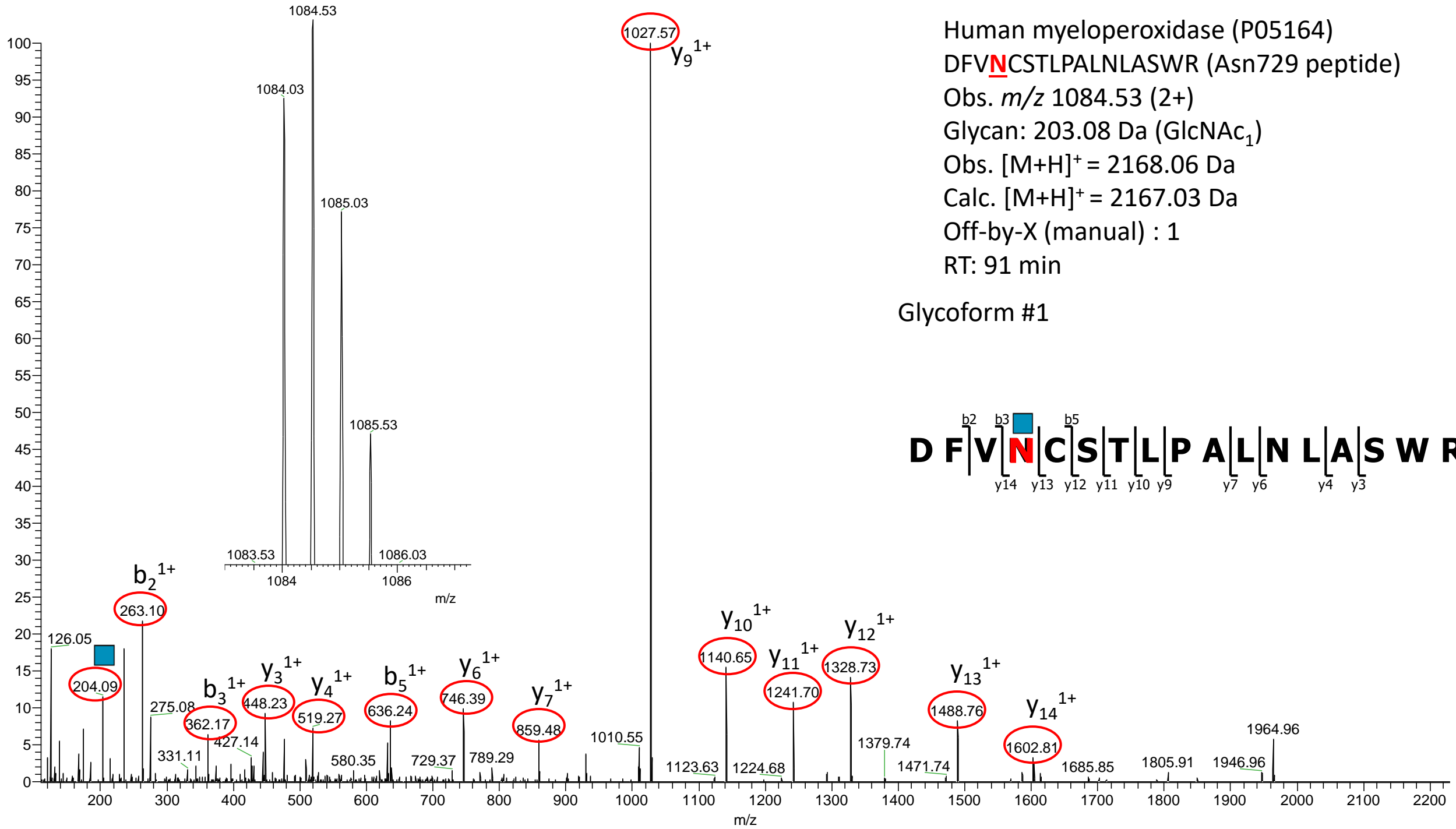
Supplementary Data S3E

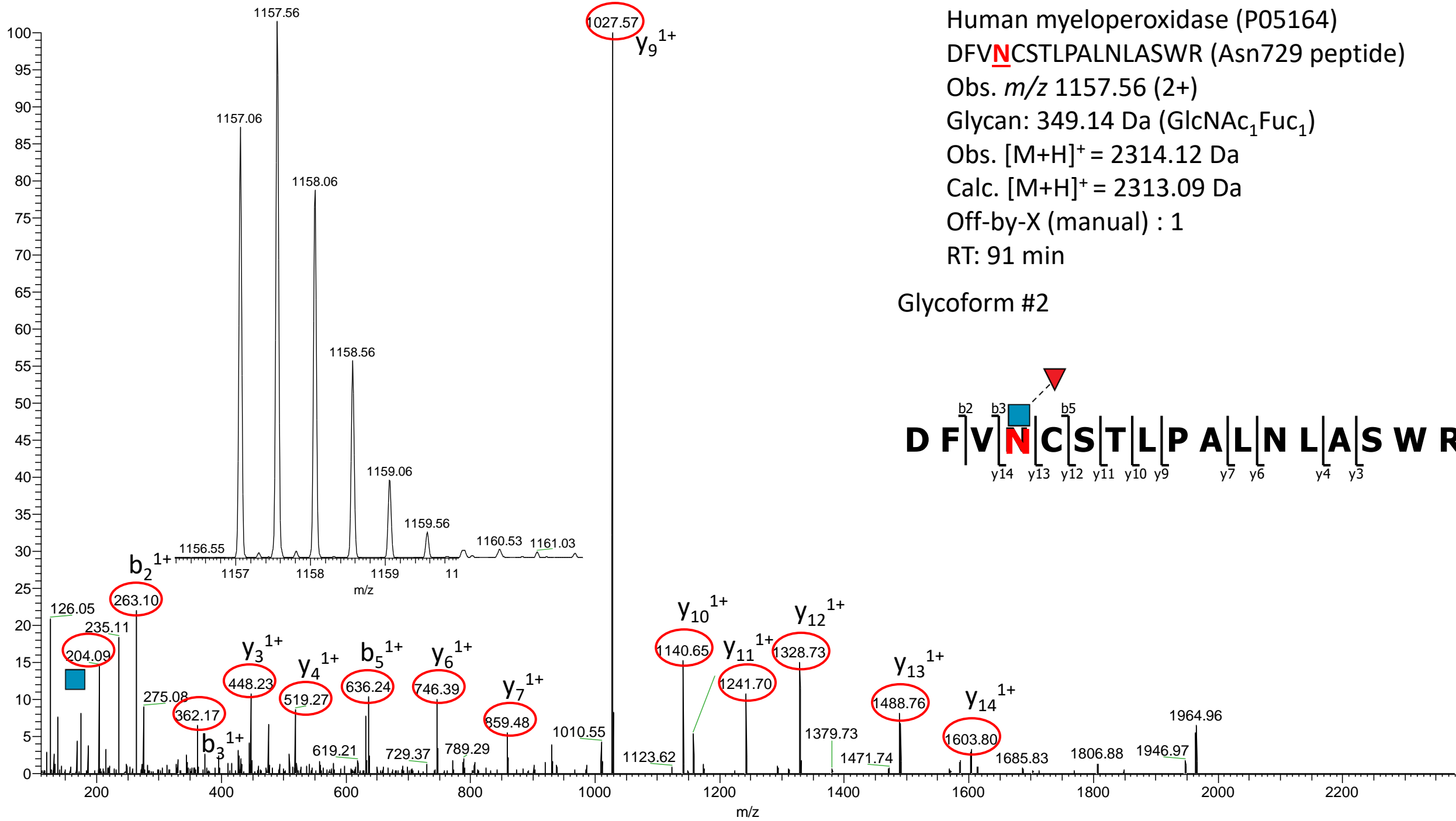
Manually annotated reversed phase-LC-ESI-HCD-MS/MS (+) spectra of all glycosylated and non-glycosylated peptides containing Asn729 identified from the analysis of unenriched peptide mixtures of nMPO.

Human myeloperoxidase (P05164)
 DFVNCSTLPALNLSWR (Asn729 peptide)
 Obs. m/z 982.99 (2+)
 Obs. $[M+H]^+ = 1964.98$ Da
 Calc. $[M+H]^+ = 1963.95$ Da
 Off-by-X (manual) : 1
 RT: 93 min

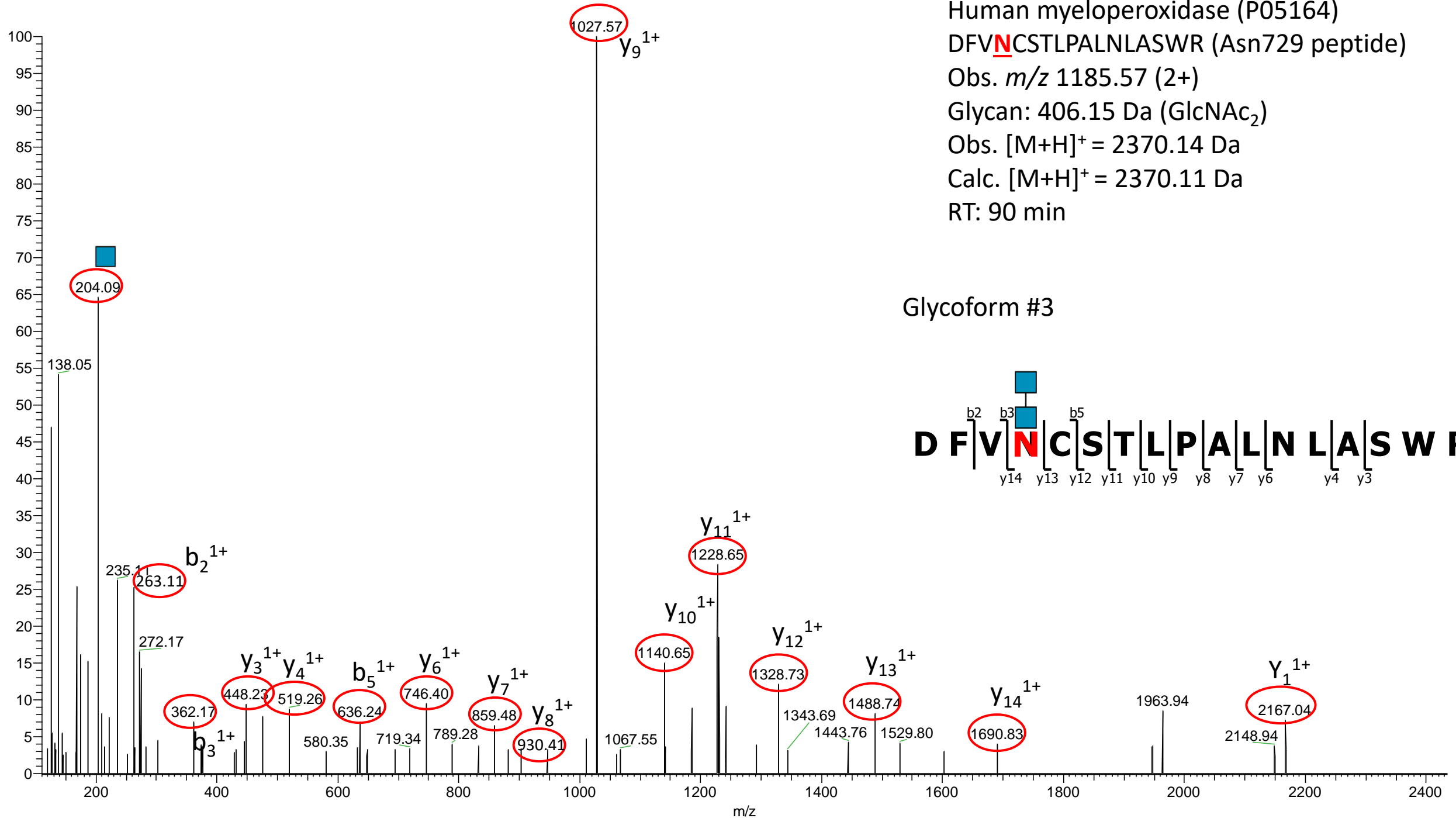
Non-glycosylated peptide







Human myeloperoxidase (P05164)
DFVNCSTLPALNLSWR (Asn729 peptide)
Obs. m/z 1185.57 (2+)
Glycan: 406.15 Da (GlcNAc₂)
Obs. $[M+H]^+$ = 2370.14 Da
Calc. $[M+H]^+$ = 2370.11 Da
RT: 90 min



Human myeloperoxidase (P05164)
DFVNCSTLPALNLSWR (Asn729 peptide)

Obs. m/z 1259.10 (2+)

Glycan: 552.22 Da (GlcNAc₂Fuc₁)

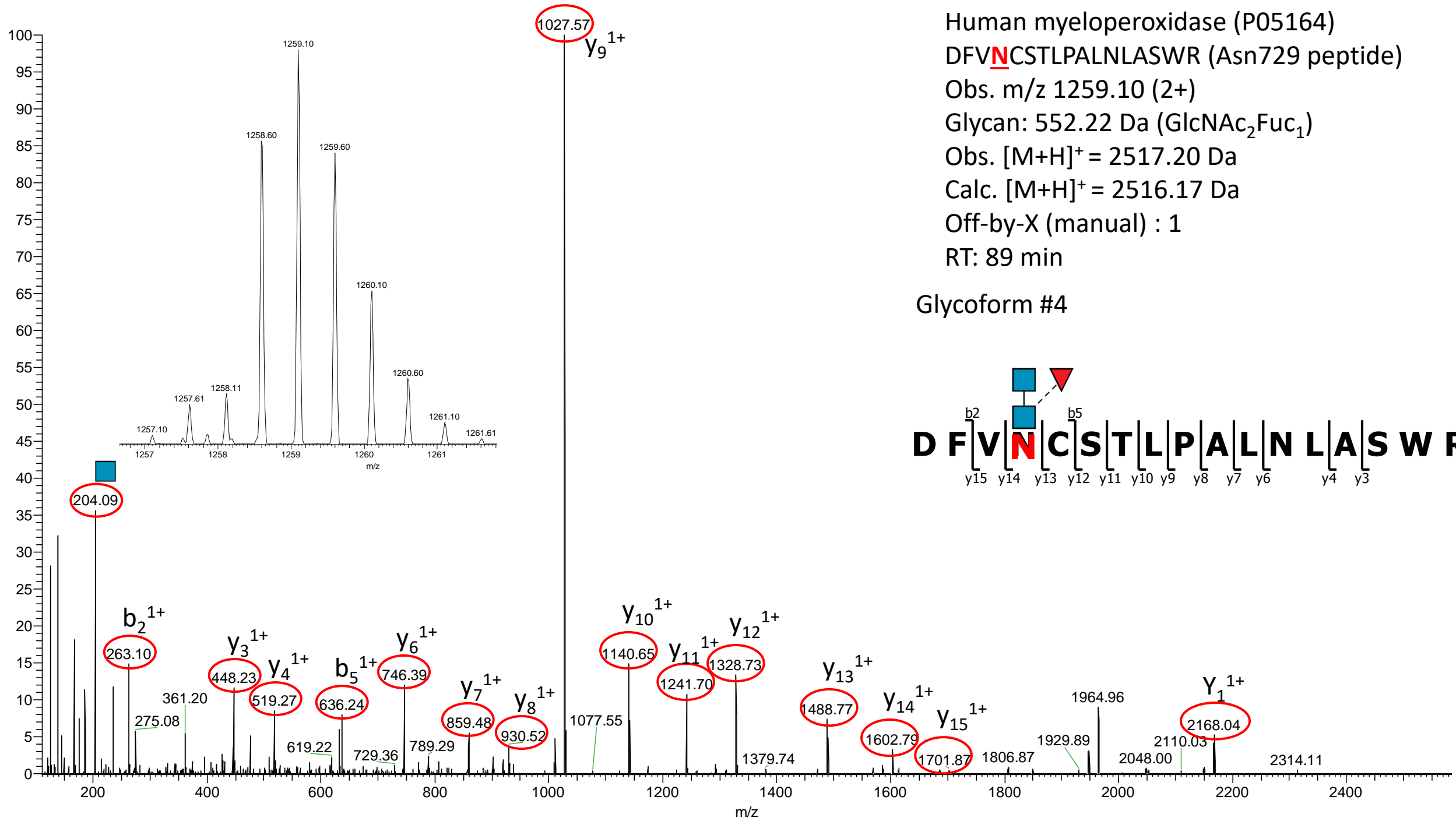
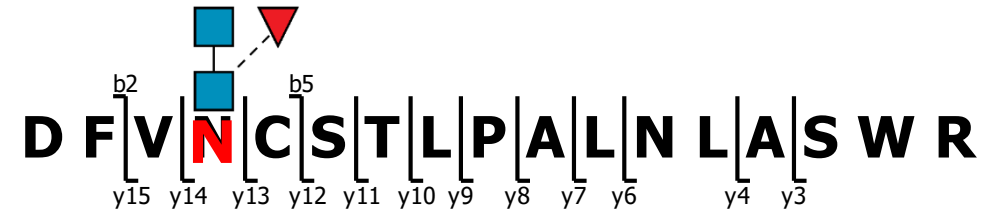
Obs. [M+H]⁺ = 2517.20 Da

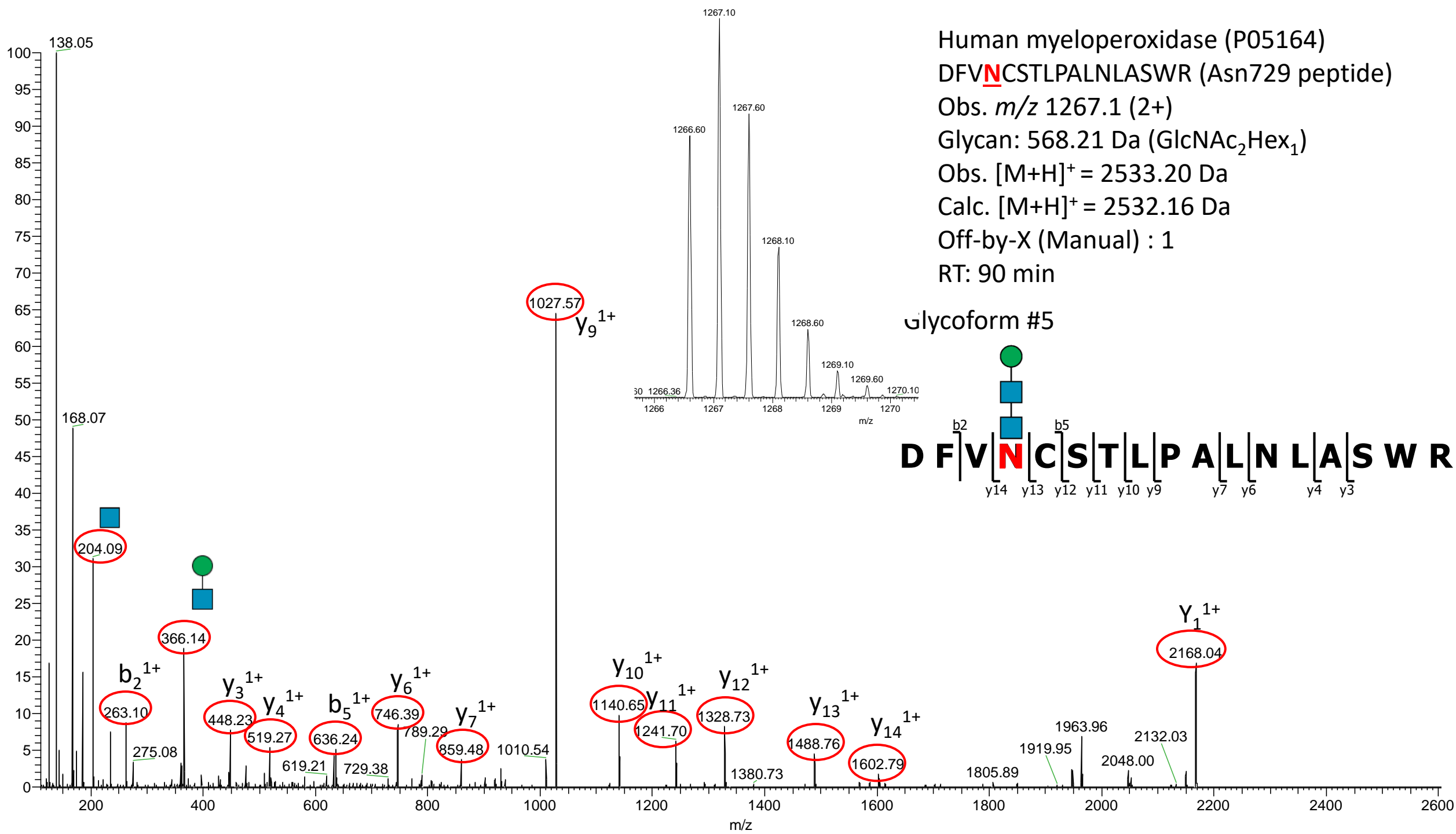
Calc. [M+H]⁺ = 2516.17 Da

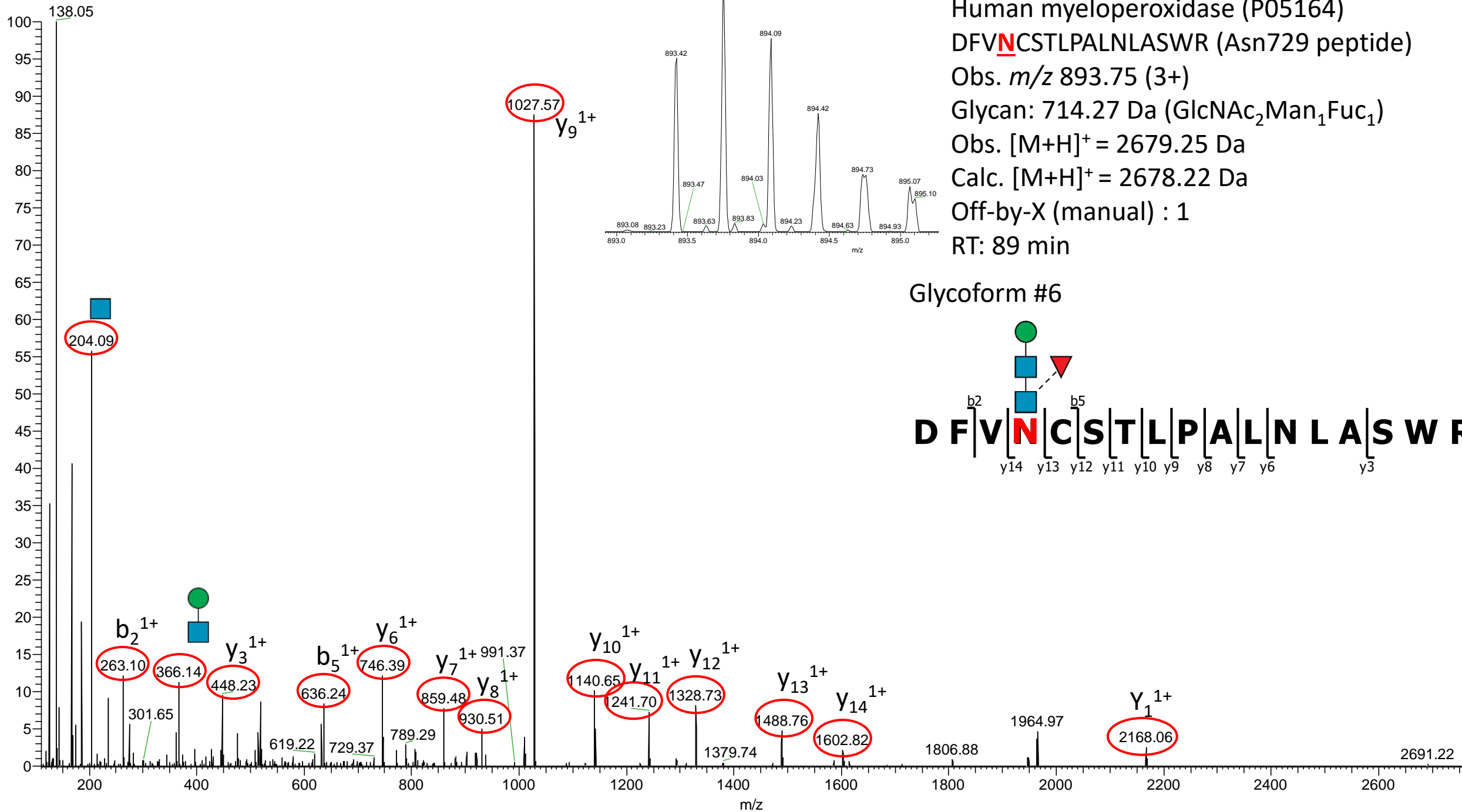
Off-by-X (manual) : 1

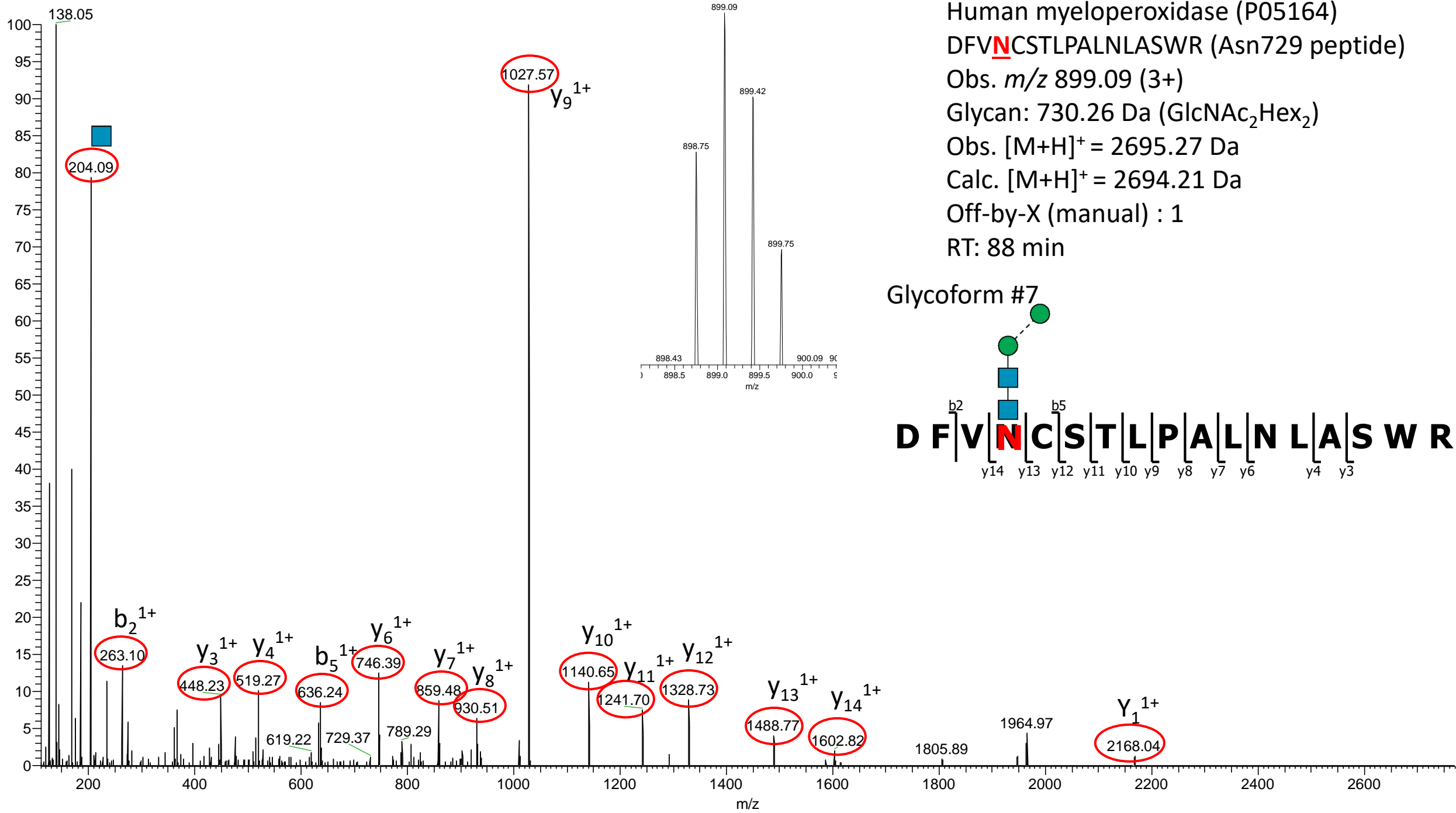
RT: 89 min

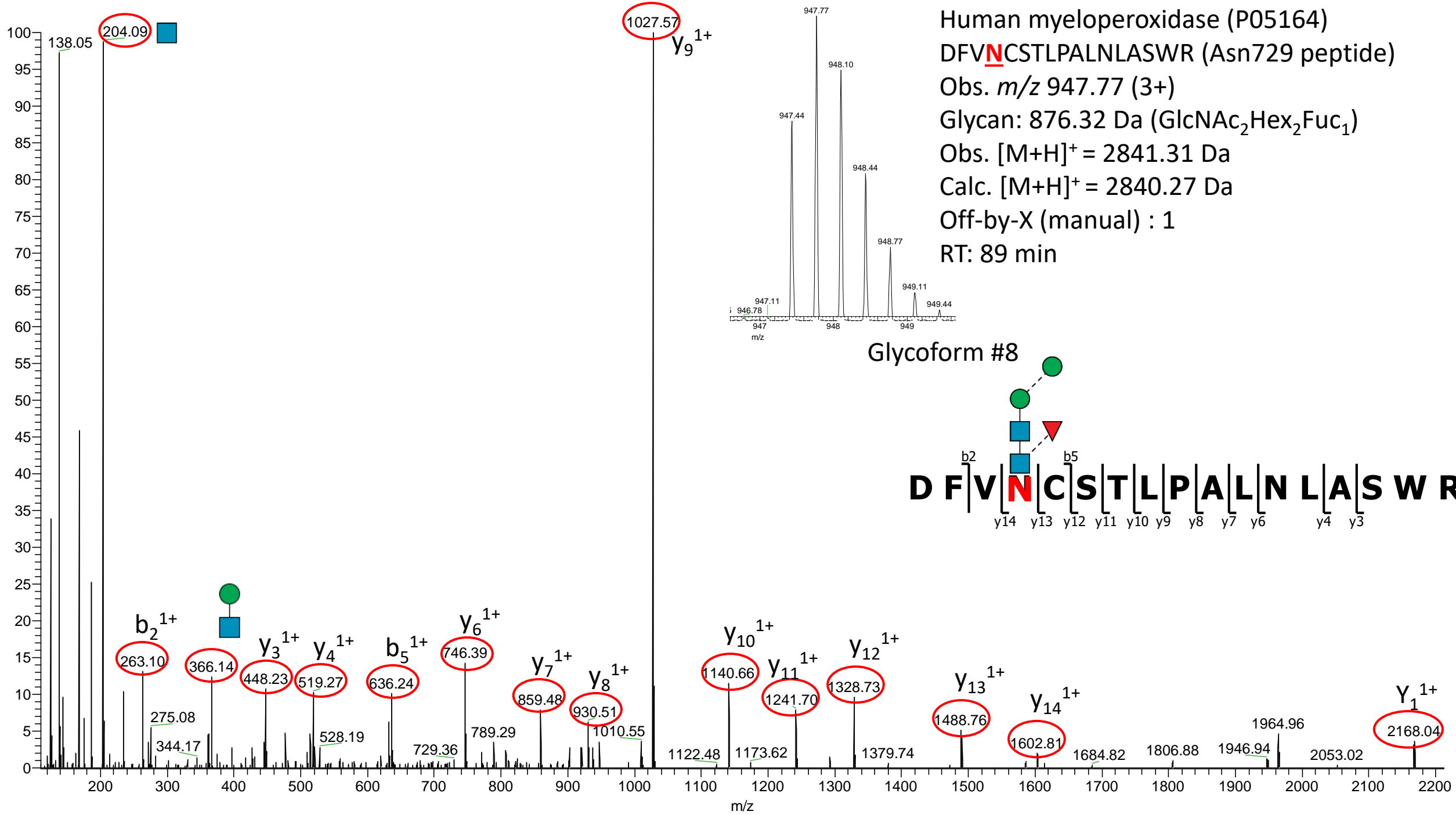
Glycoform #4

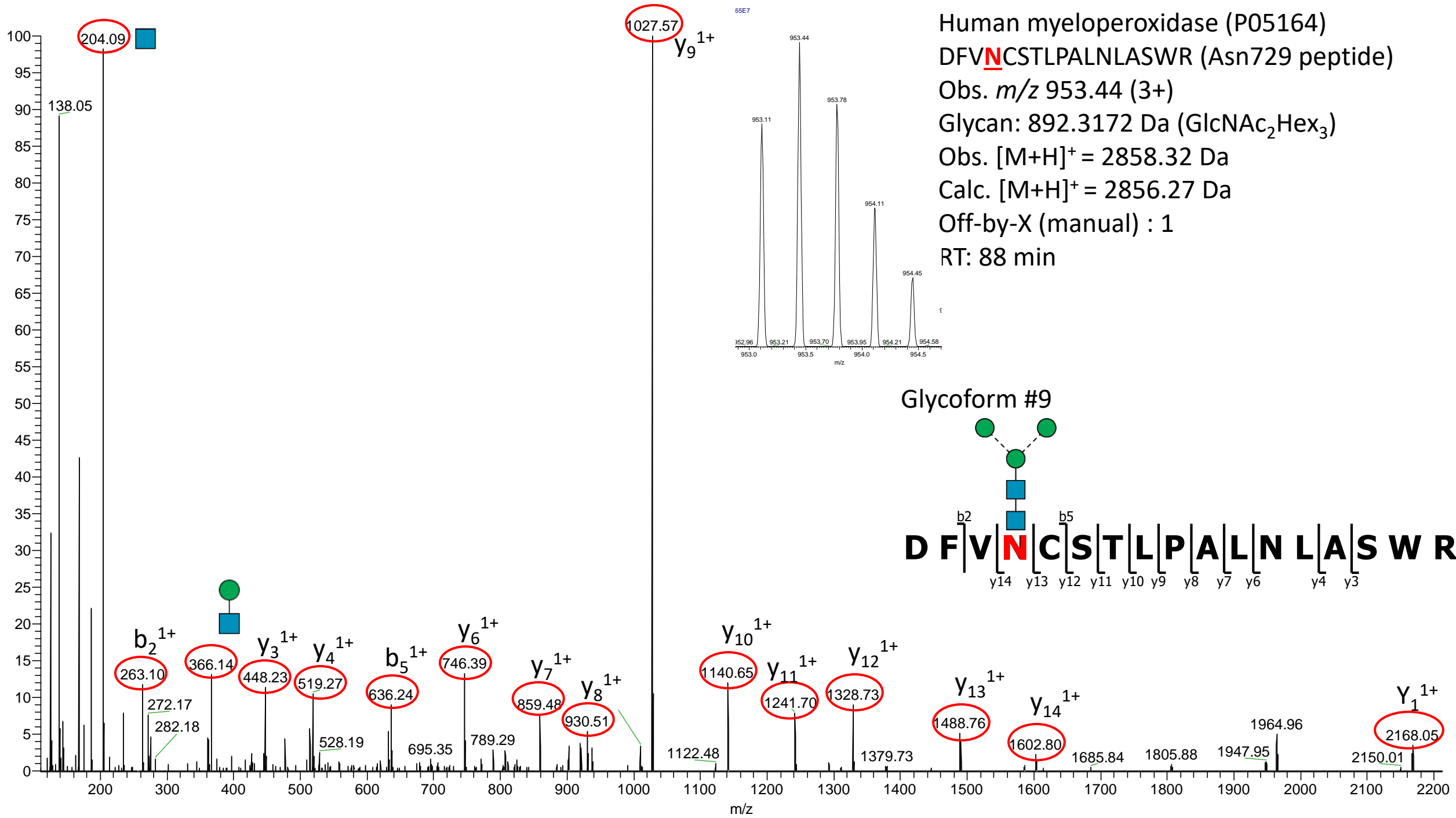


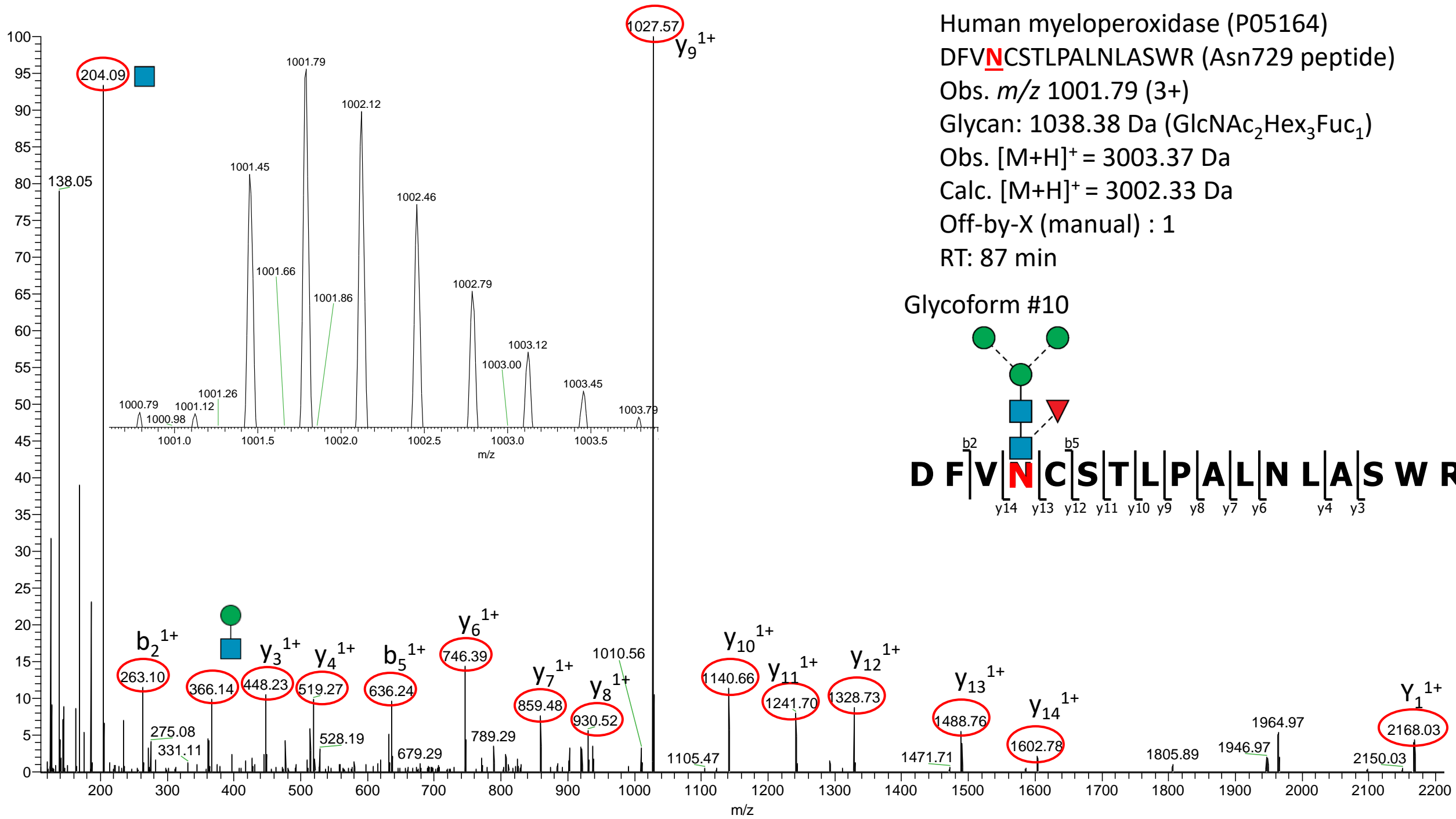


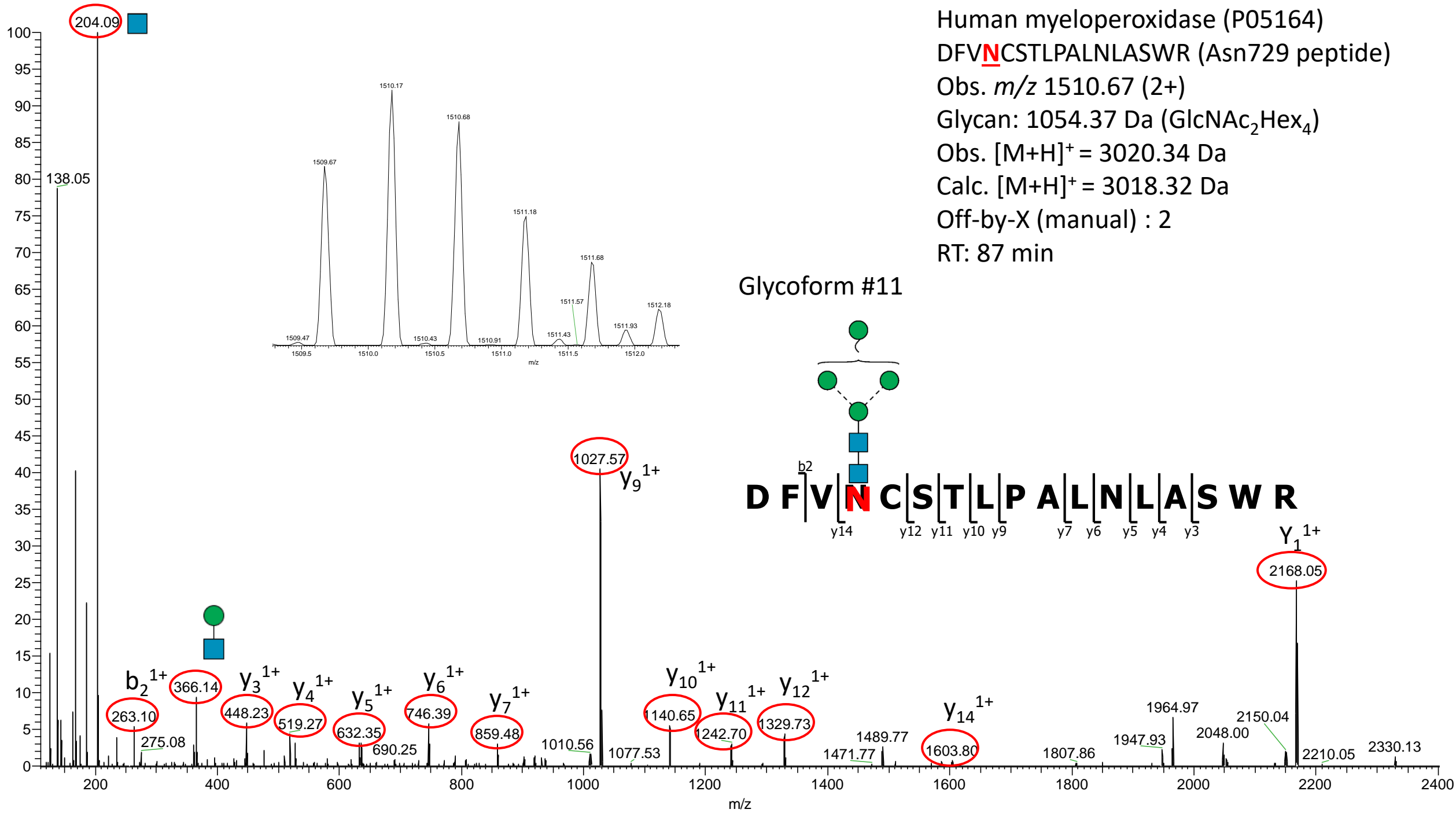


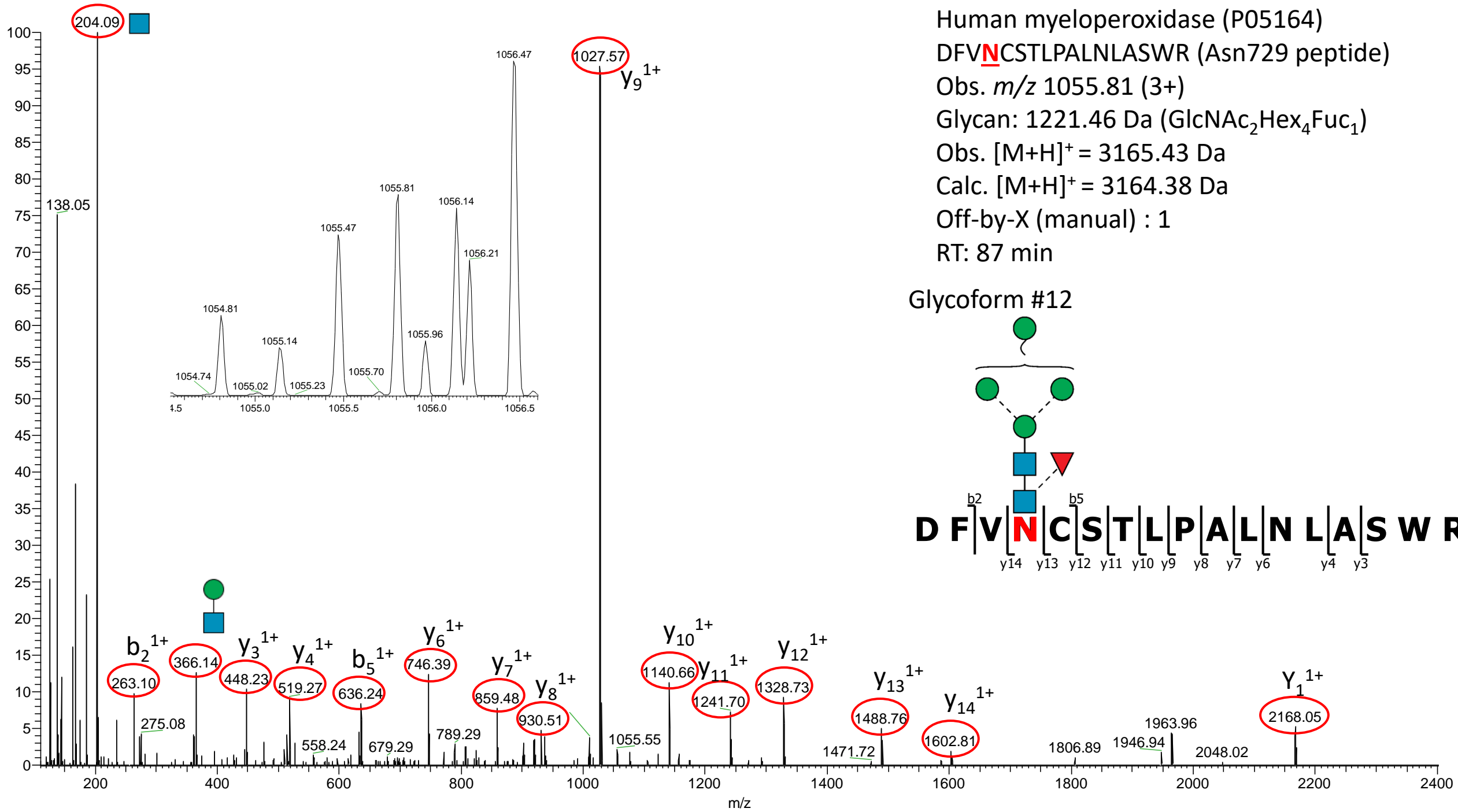


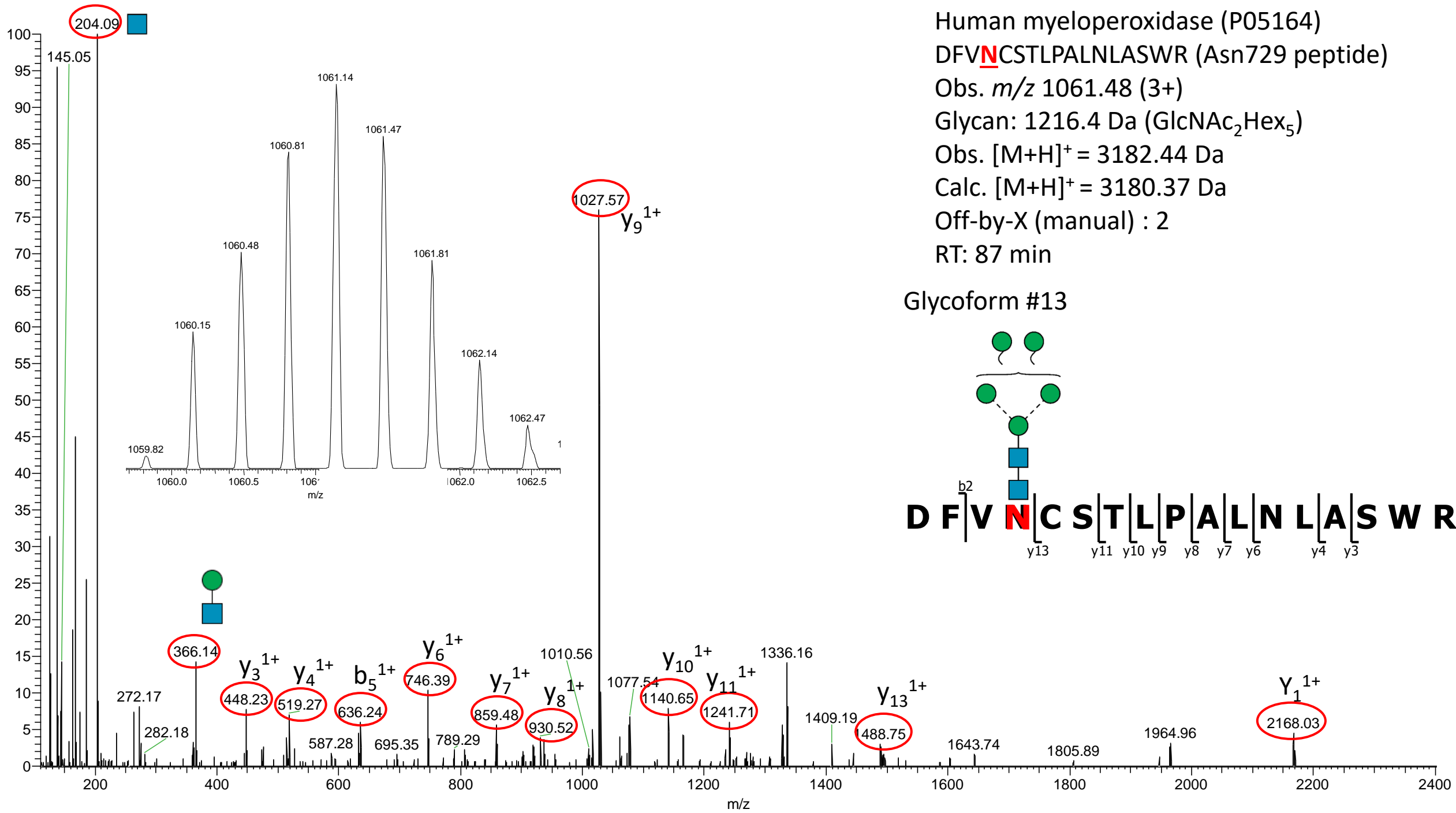


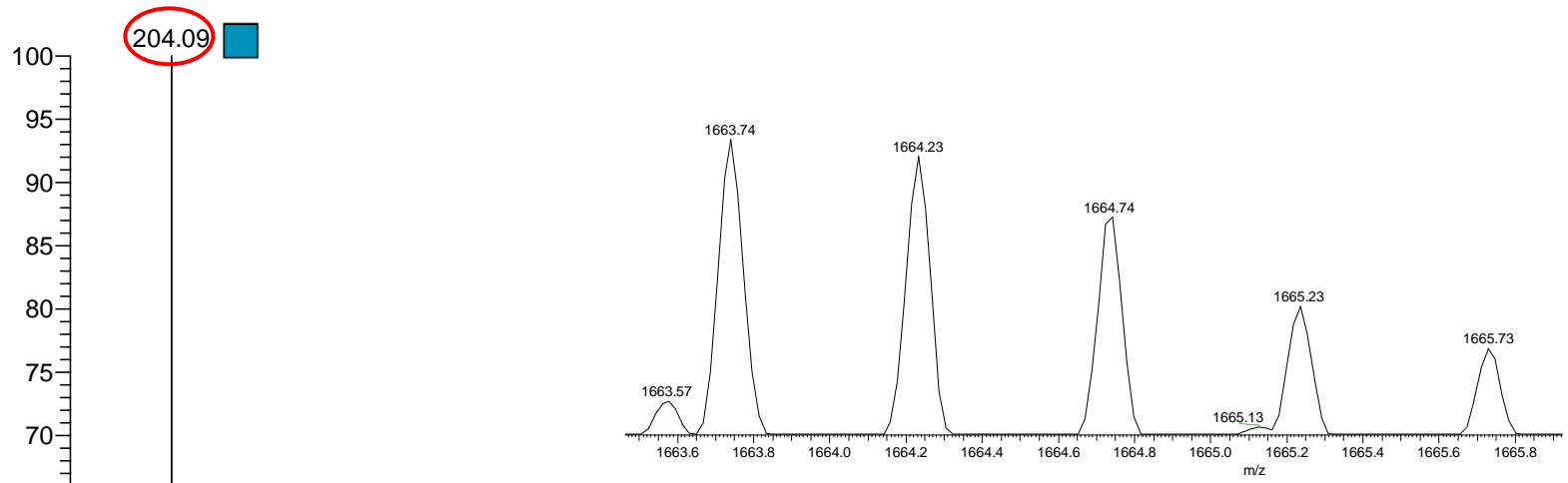




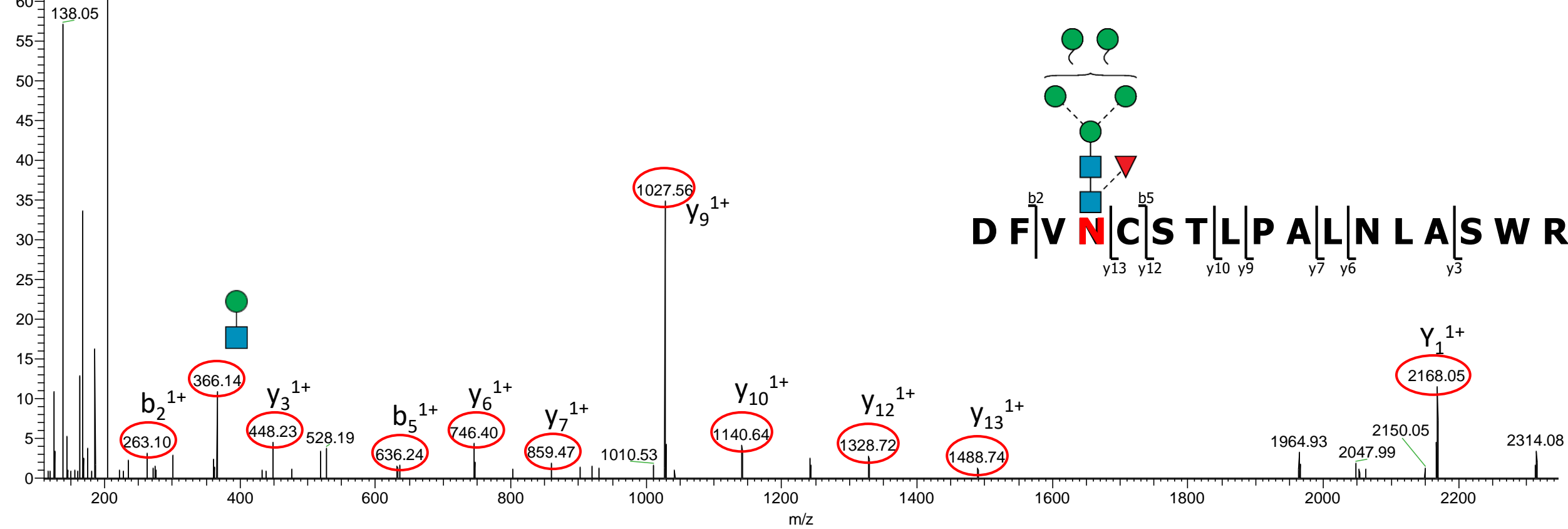




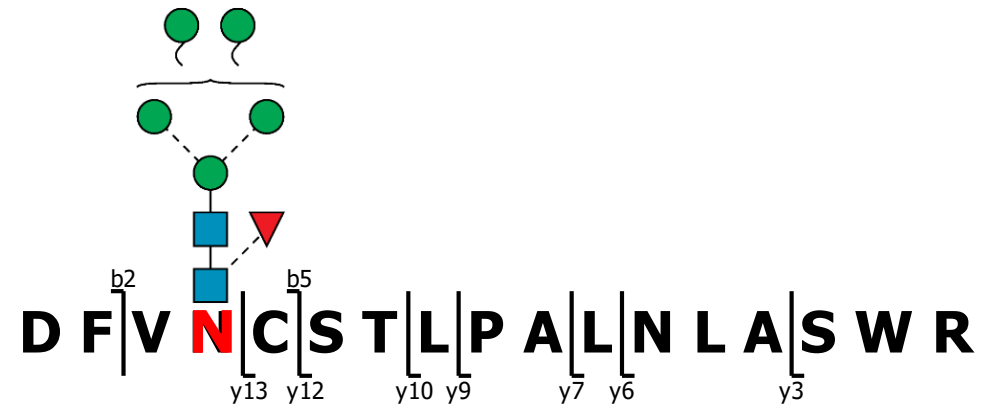


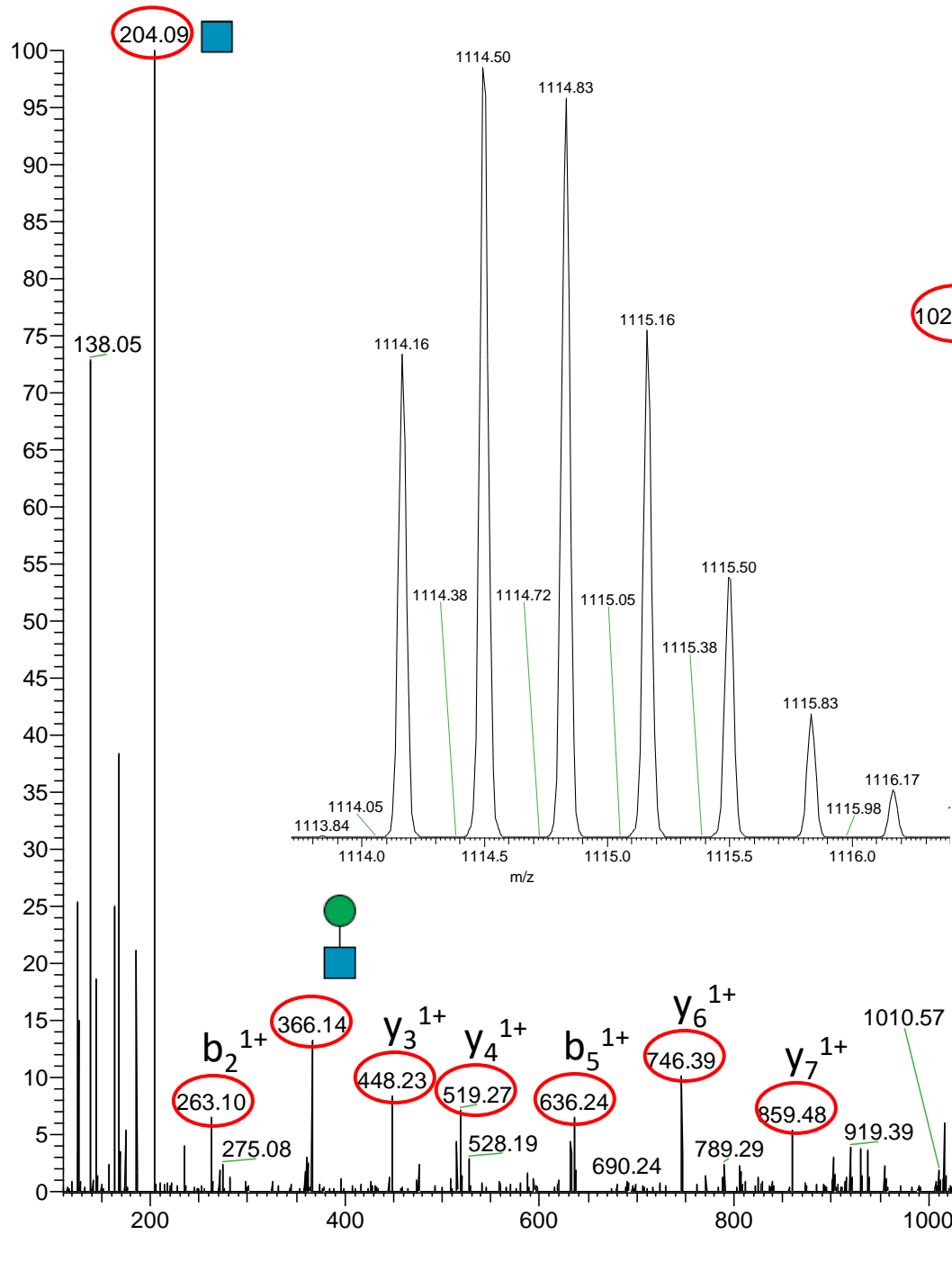


Human myeloperoxidase (P05164)
 DFVNCSTLPALNLSWR (Asn729 peptide)
 Obs. m/z 1664.73 (2+)
 Glycan: 1362.481 Da (GlcNAc₂Hex₅Fuc₁)
 Obs. $[M+H]^+$ = 3328.46 Da
 Calc. $[M+H]^+$ = 3326.43 Da
 Off-by-X (manual) : 2
 RT: 87 min



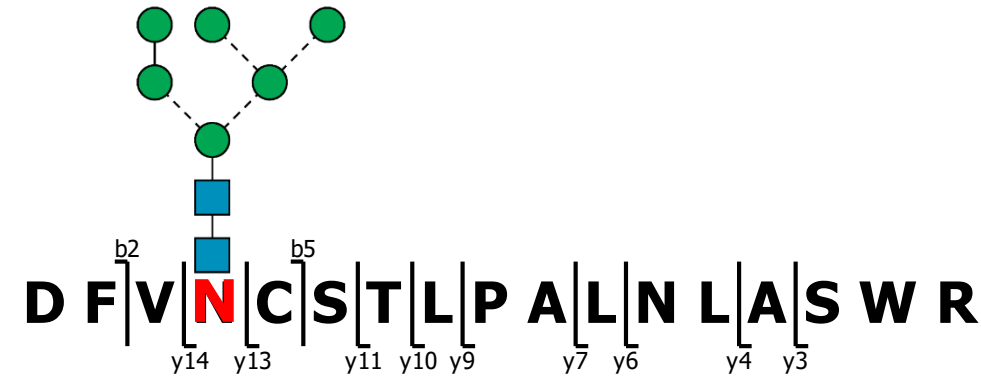
Glycoform #14



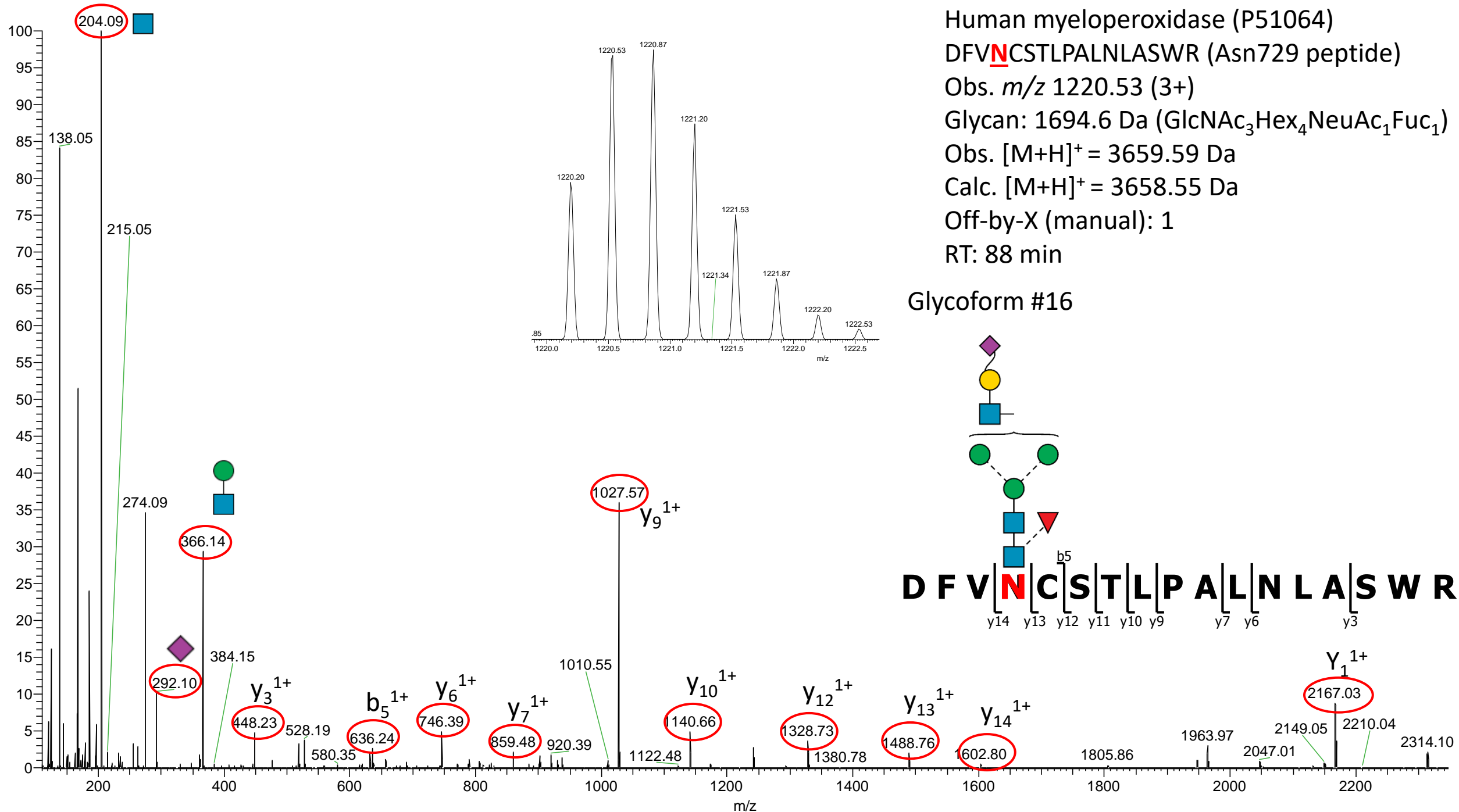


Human myeloperoxidase (P05164)
 DFVNCSTLPALNLSWR (Asn729 peptide)
 Obs. m/z 1115.50 (3+)
 Glycan: 1378.48 Da (GlcNAc₂Hex₆)
 Obs. $[M+H]^+$ = 3344.50 Da
 Calc. $[M+H]^+$ = 3342.43 Da
 Off-by-X (manual) : 2
 RT: 87 min

Glycoform #15



D F V N C S T L P A L N L A S W R
 y₁₄ y₁₃ y₁₁ y₁₀ y₉ y₇ y₆ y₄ y₃
 b₂ b₅



204.09

138.05

215.05

274.09

292.10

366.14

384.15

448.23

y₃¹⁺

528.19

b₅¹⁺

636.24

y₆¹⁺

746.39

y₇¹⁺

859.48

920.39

y₉¹⁺

1027.57

1010.55

1122.48

y₁₀¹⁺

140.66

y₁₂¹⁺

1328.73

y₁₃¹⁺

1488.76

y₁₄¹⁺

602.80

1805.86

1963.97

2047.01

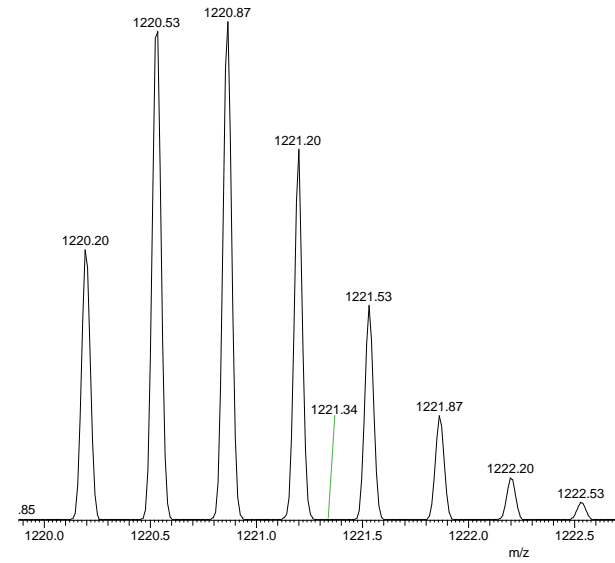
2149.05

Y₁¹⁺

2167.03

2210.04

2314.10



Glycoform #16

