

Fig. S1

LELAKQVILTLDDGTVK

MS1

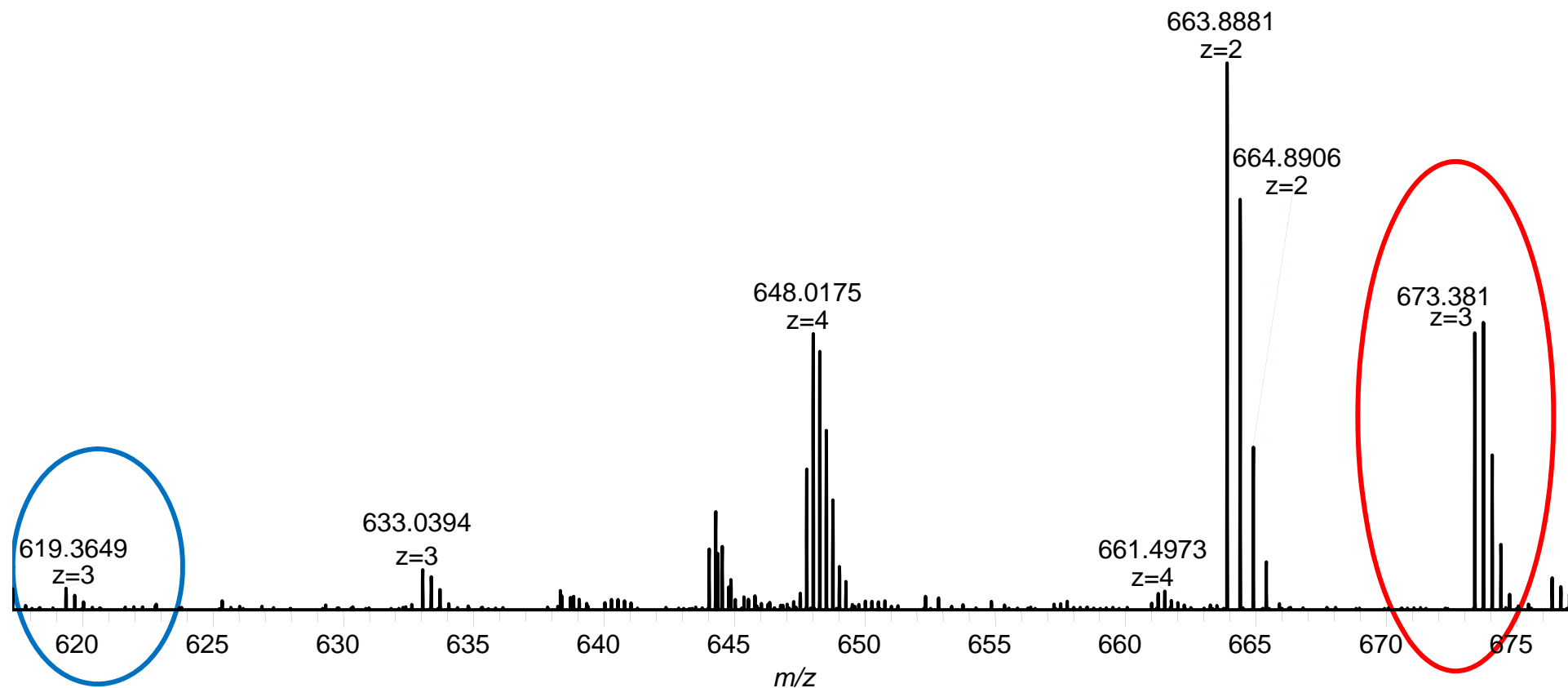


Fig. S1. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S2

LELAKQVILTLDDGTVK

LC MS/MS-CID

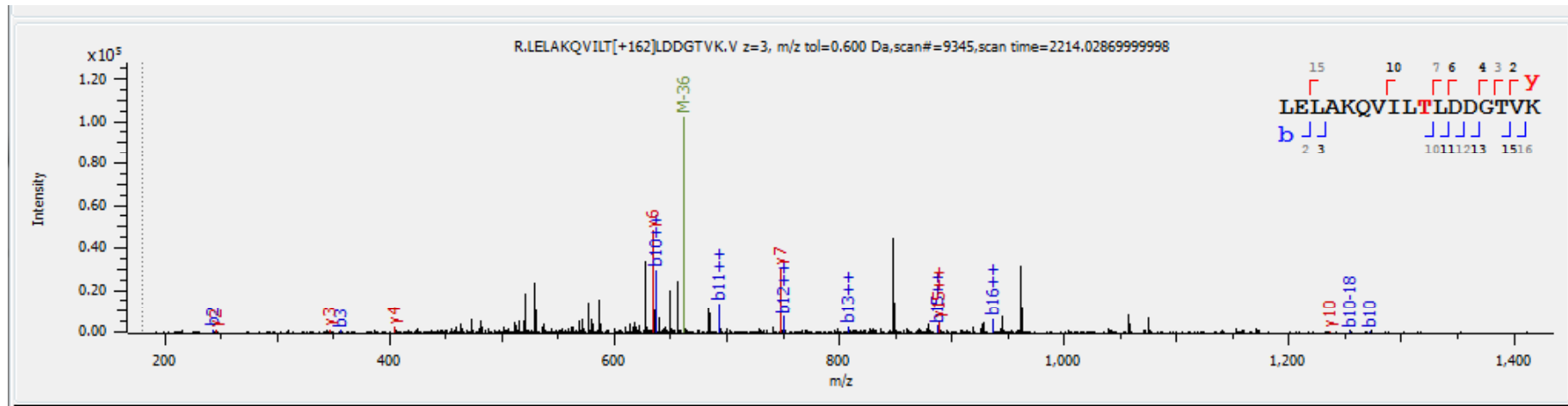


Fig. S2. LC MS/MS-CID of the glycosylation of the peptide LELAKQVILT¹¹⁷LDDGTVK of MARTH_403 showing the assigned b and y ions.

Fig. S3

LELAKQVILTLDDGTVK

¹³C

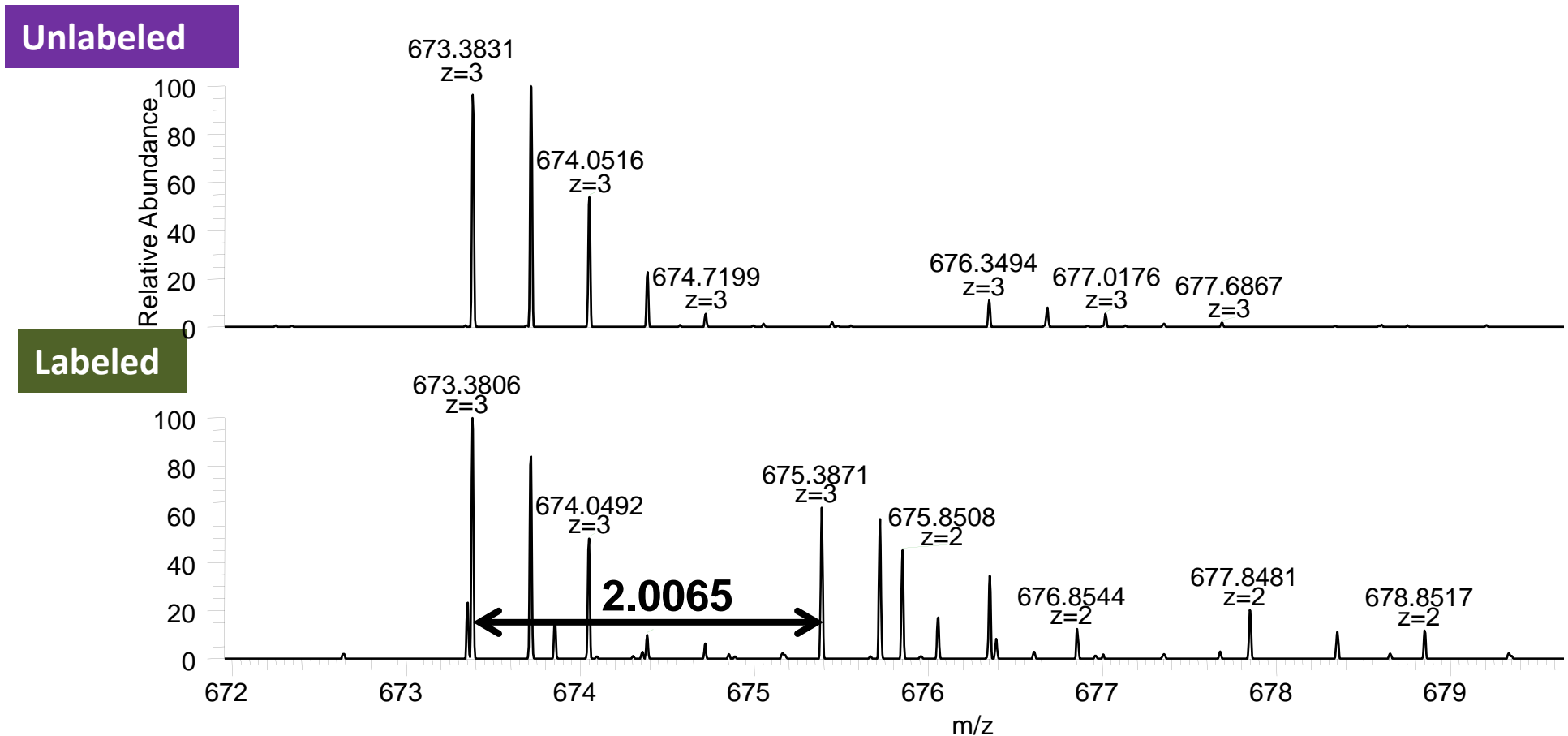


Fig. S3. Ions of glycosylated LELAKQVILT¹¹⁷LDDGTVK peptide of MARTH_403. Unlabeled spectrum shows triply-charged species grown in serum-free medium. Labeled spectrum shows triply-charged species grown in MB supplemented with ¹³C starch.

Fig. S4

MS1

RLELAKQVILTLDDGTVK

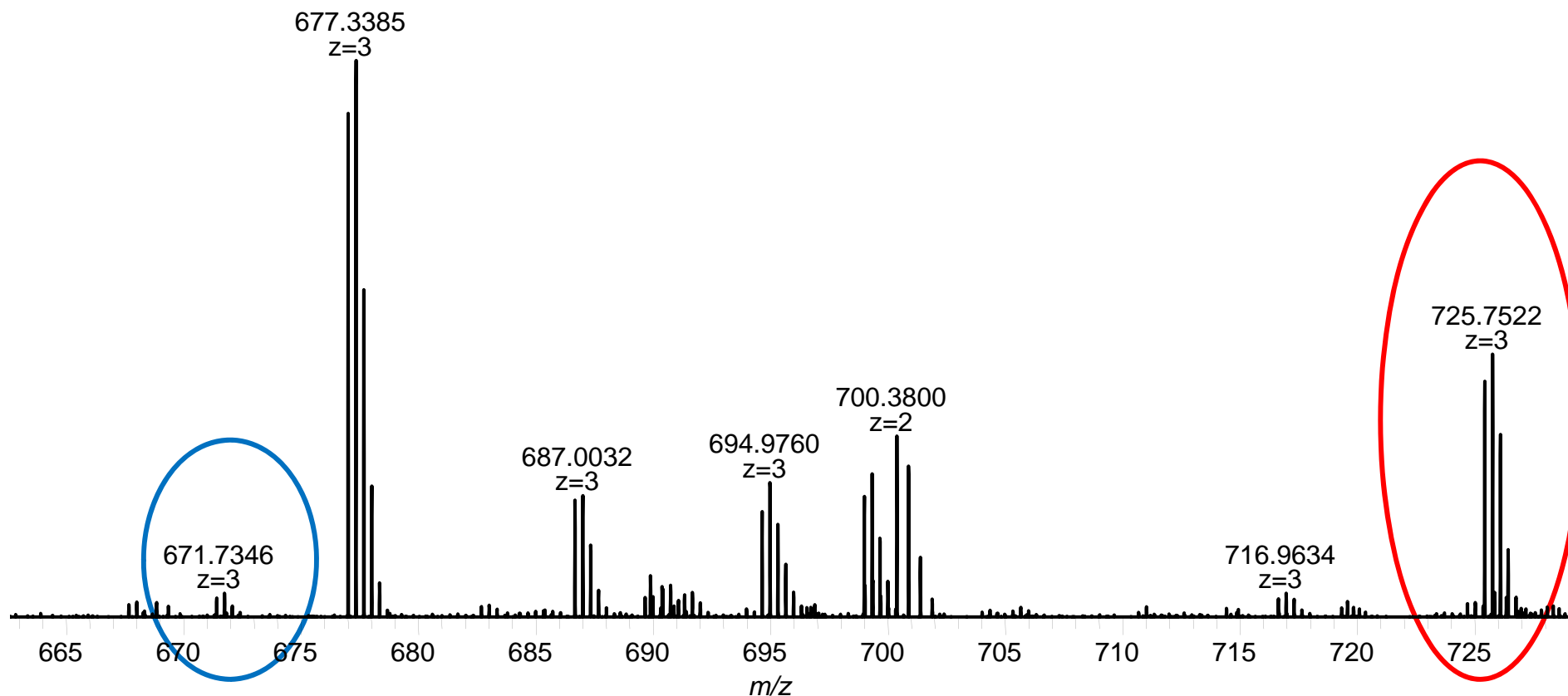


Fig. S4. LC-MS of non-glycosylated peptide ion (blue oval) and the glycosylated peptide ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S5

RLELAKQVILT₁₁₇LDDGTVK LC MS/MS-CID

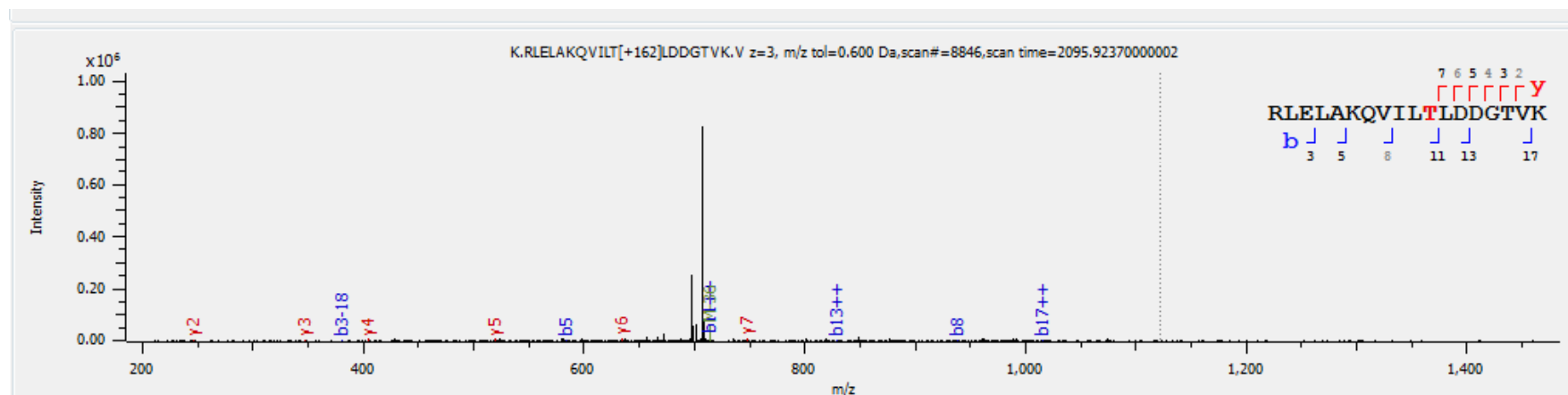


Fig. S5. LC MS/MS-CID of the glycosylation of the peptide RLELAKQVILT¹¹⁷LDDGTVK of MARTH_403 showing the assigned b and y ions.

Fig. S6

RLELAKQVILTLDDGTVK ¹³C

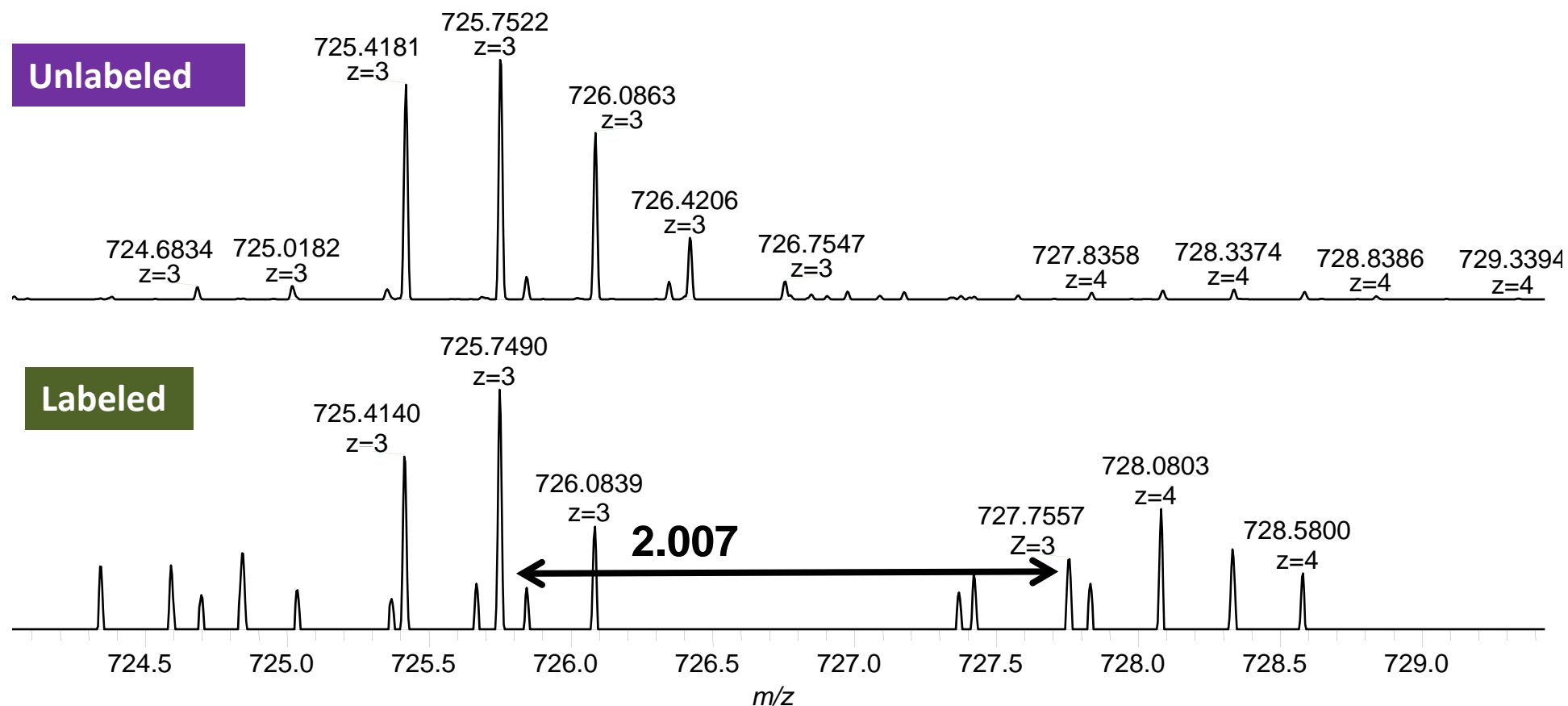


Fig. S6. Ions of glycosylated RLELAKQVILT¹¹⁷LDDGTVK peptide of MARTH_403. Unlabeled spectrum shows triply-charged species grown in serum-free medium. Labeled spectrum shows triply-charged species grown in MB supplemented with ¹³C starch.

Fig. S7

SINSKQFLEDLKK

MS1

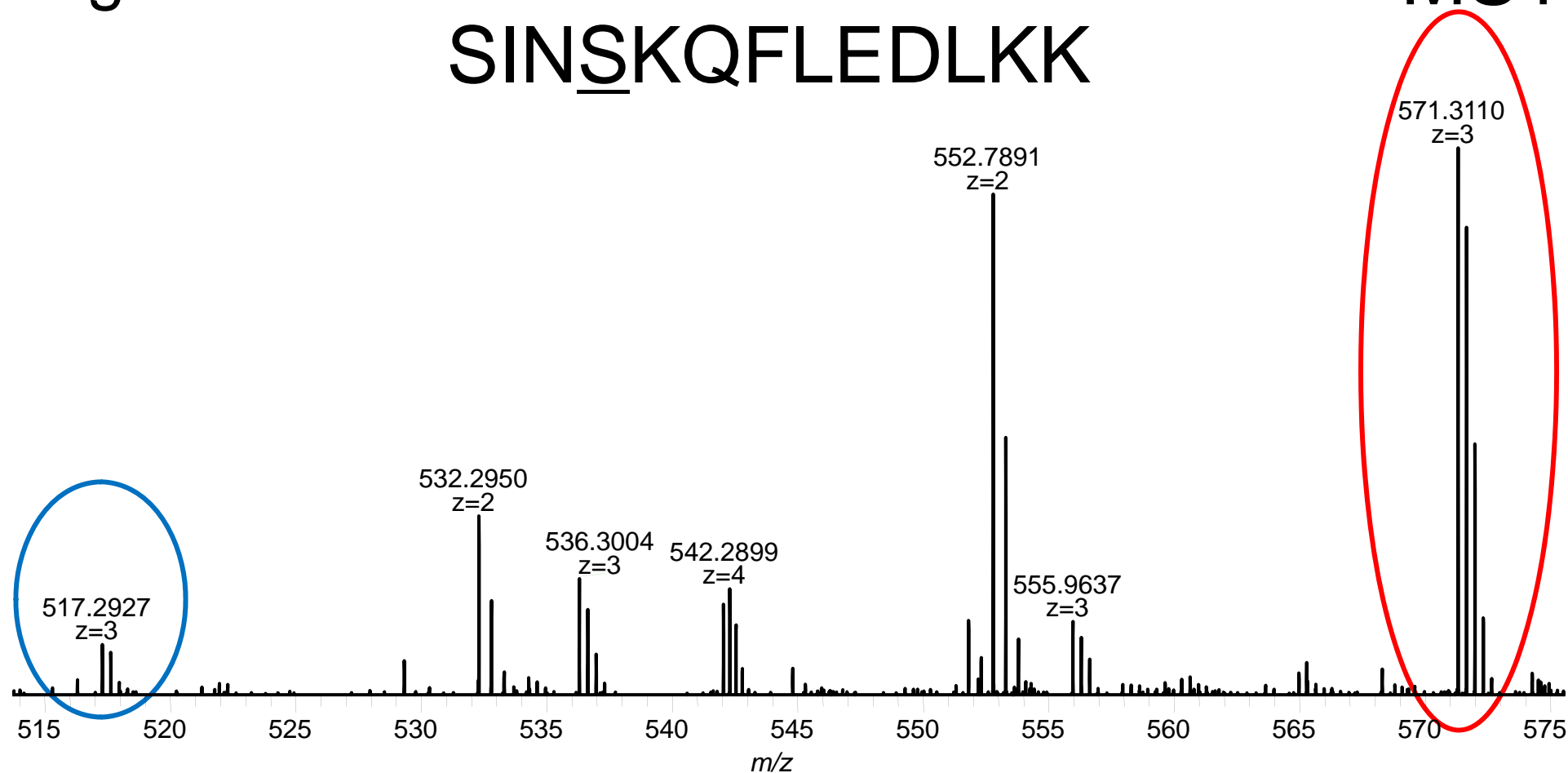


Fig. S7. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S8

SINSKQFLEDLKK

LC MS/MS-CID

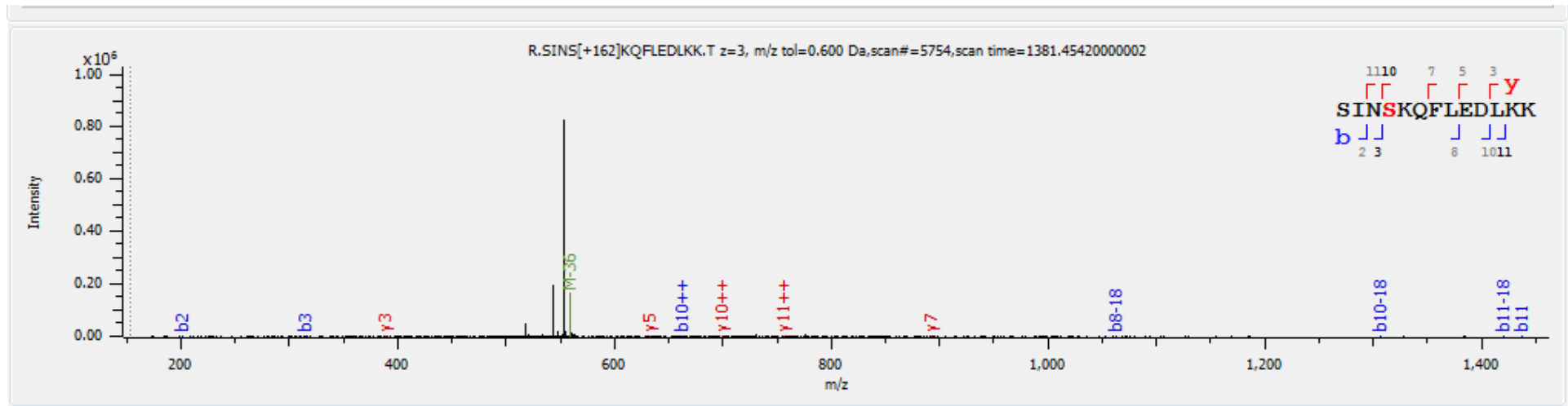


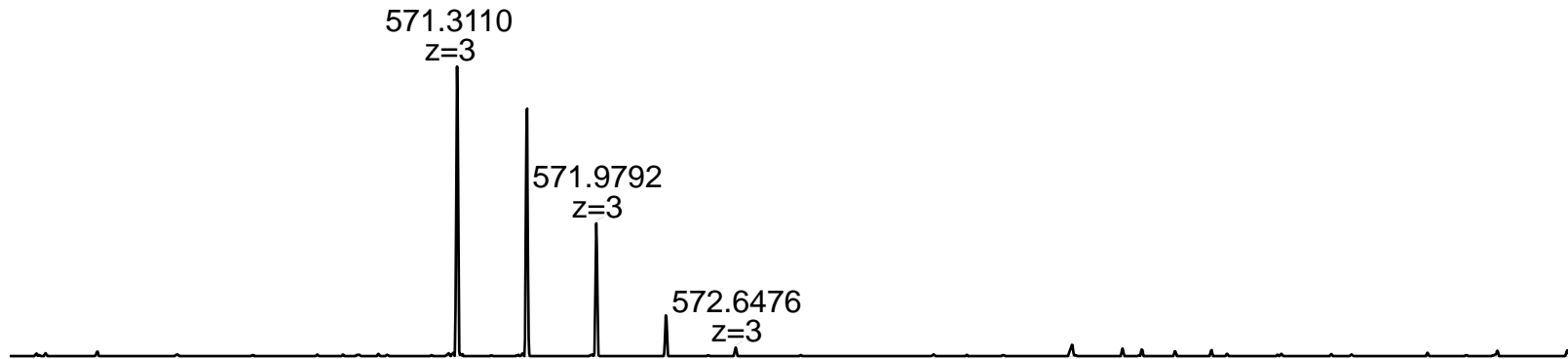
Fig. S8. LC MS/MS-CID of the glycosylation of the peptide SINS¹⁶⁴KQFLEDLKK of MARTH_403 showing the assigned b and y ions.

Fig. S9

SINSKQFLEDLKK

^{13}C

Unlabeled



Labeled

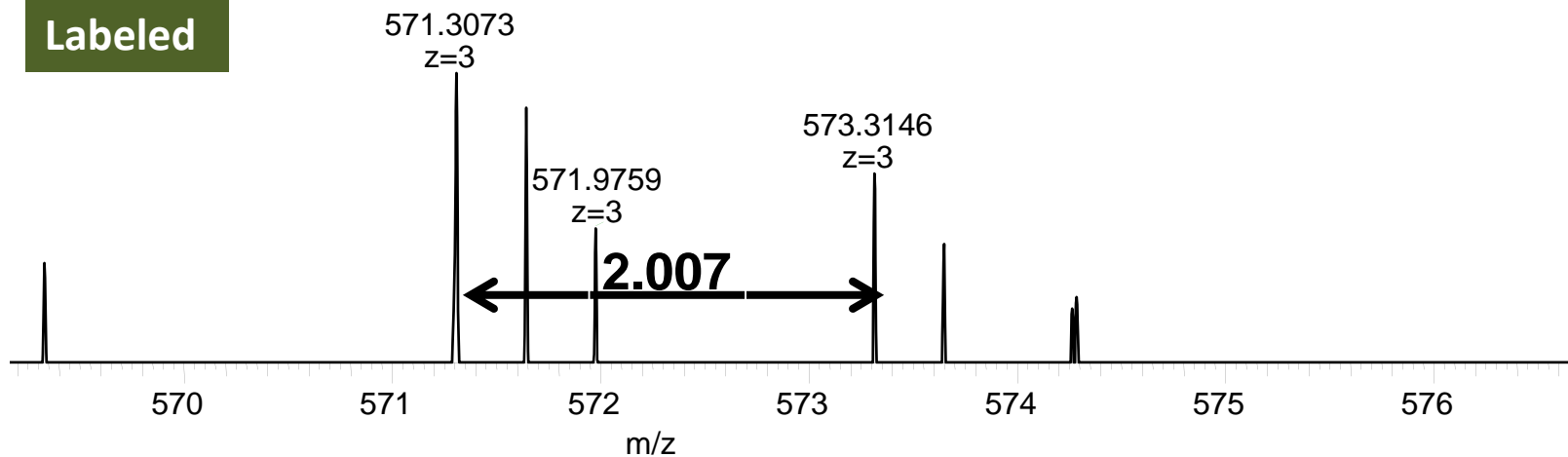


Fig. S9. Ions of glycosylated SINS 164 KQFLEDLKK peptide of MARTH_403. Unlabeled spectrum shows triply-charged species grown in serum-free medium. Labeled spectrum shows triply-charged species grown in MB supplemented with ^{13}C starch.

Fig. S10

YQQRPQEKEIFSTR

MS1

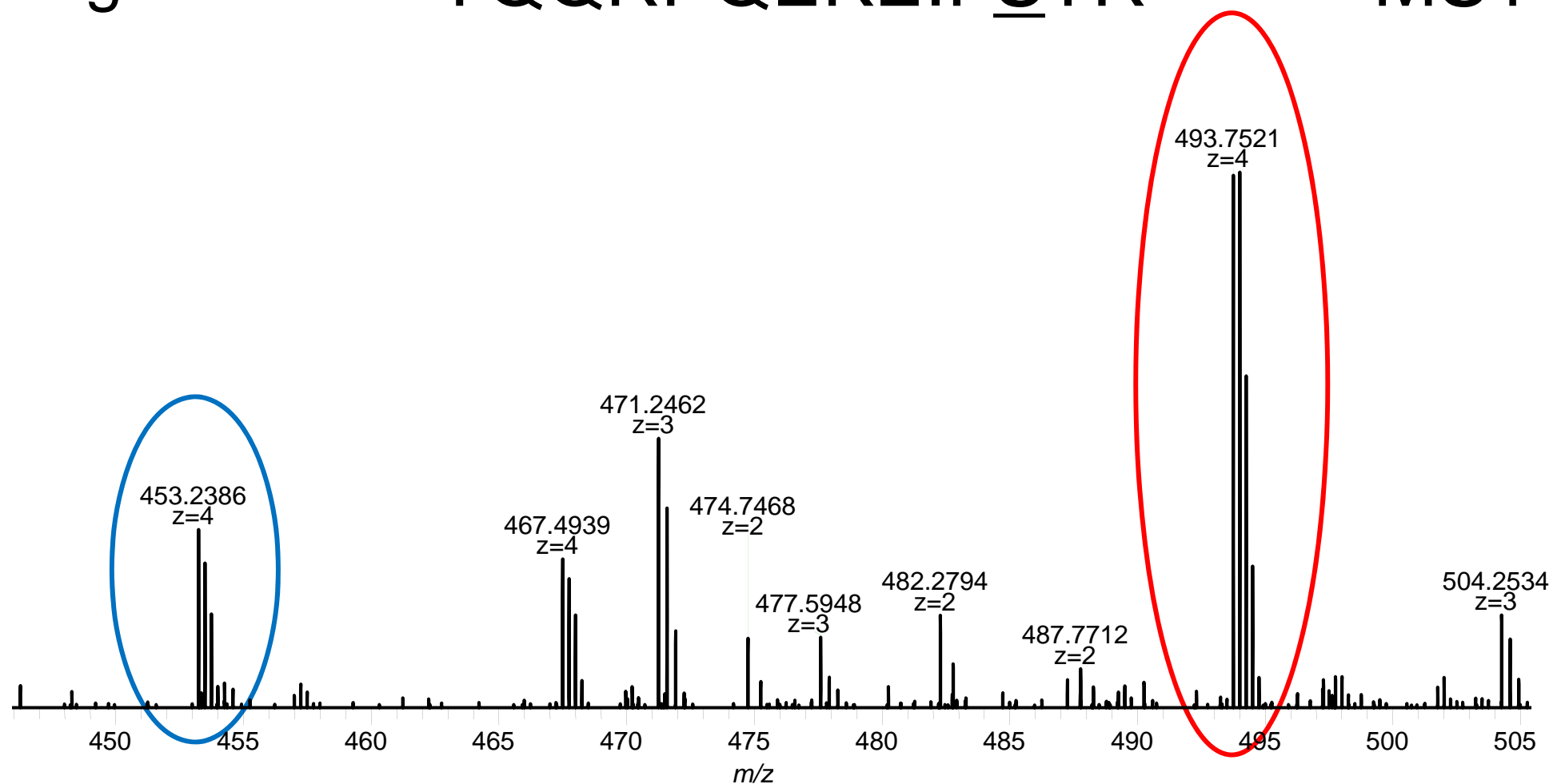


Fig. S10. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S11

YQQR**P**QEKEIFSTR

LC MS/MS-CID

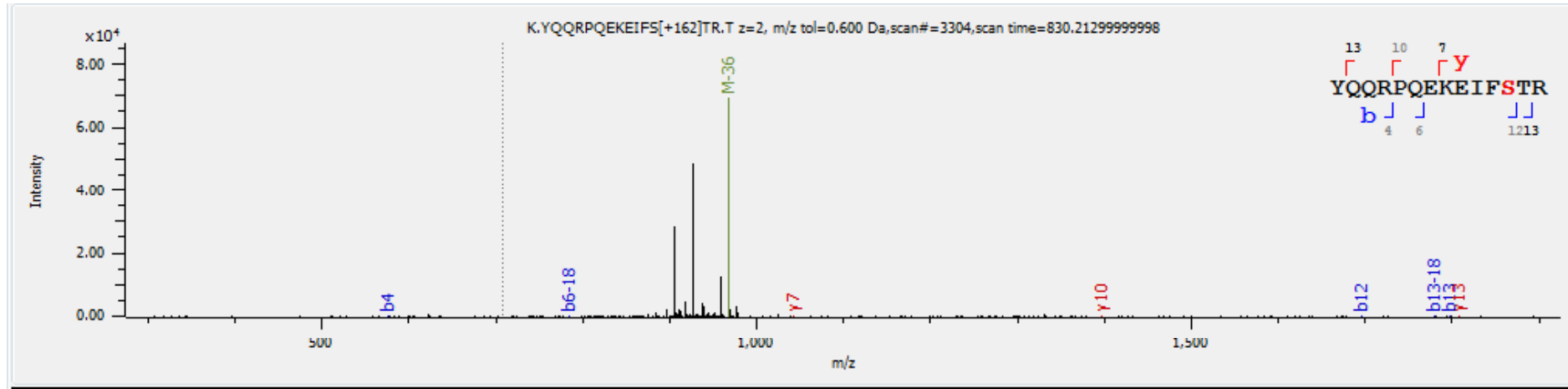


Fig. S11. LC MS/MS-CID of the glycosylation of the peptide YQQRPEKEIFS⁴²⁷TR of MARTH_403 showing the assigned b and y ions.

Fig. S12

MS1

TWNLKQGQLSSIPFSTLTQAQQQEAIK

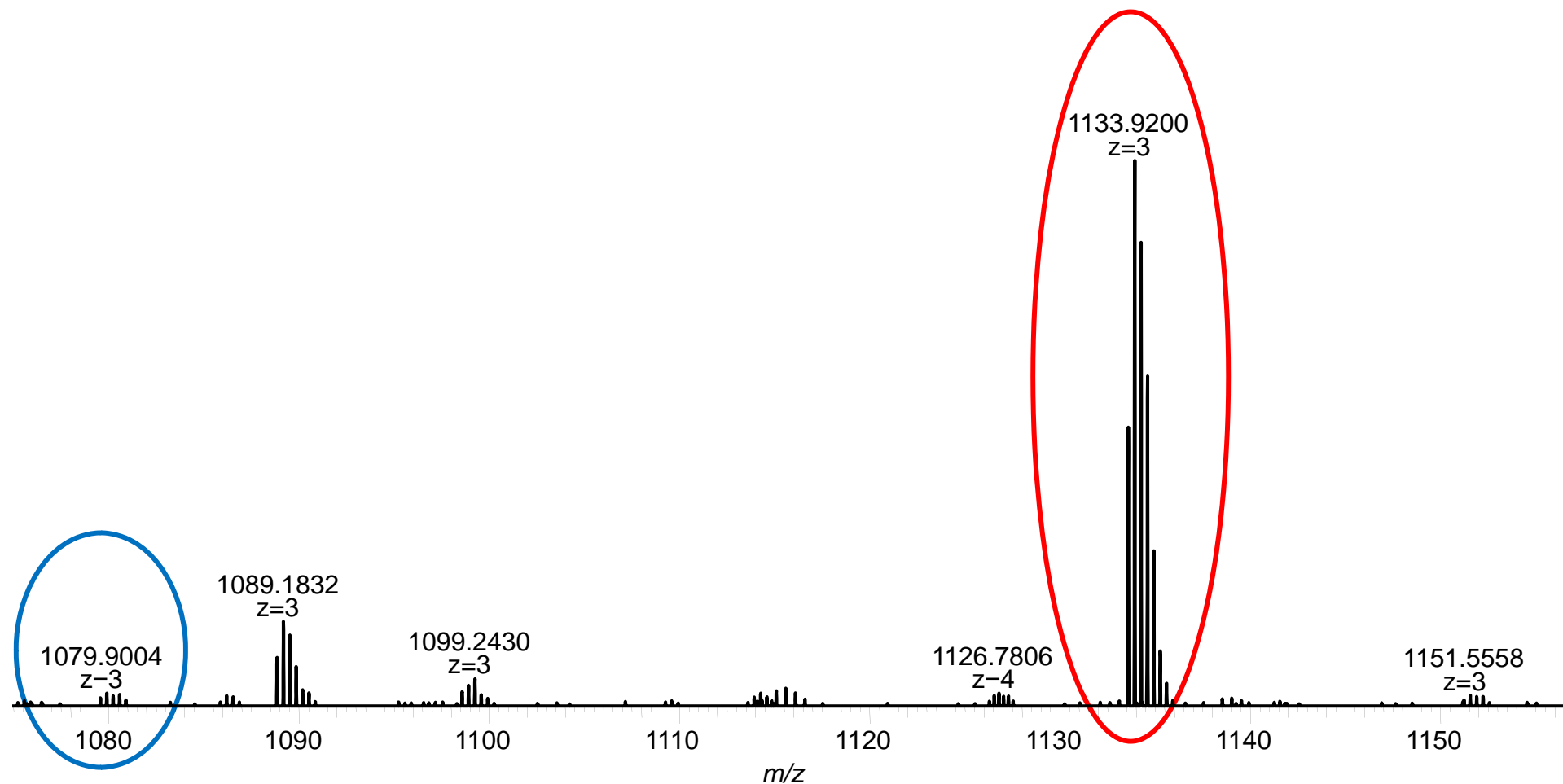


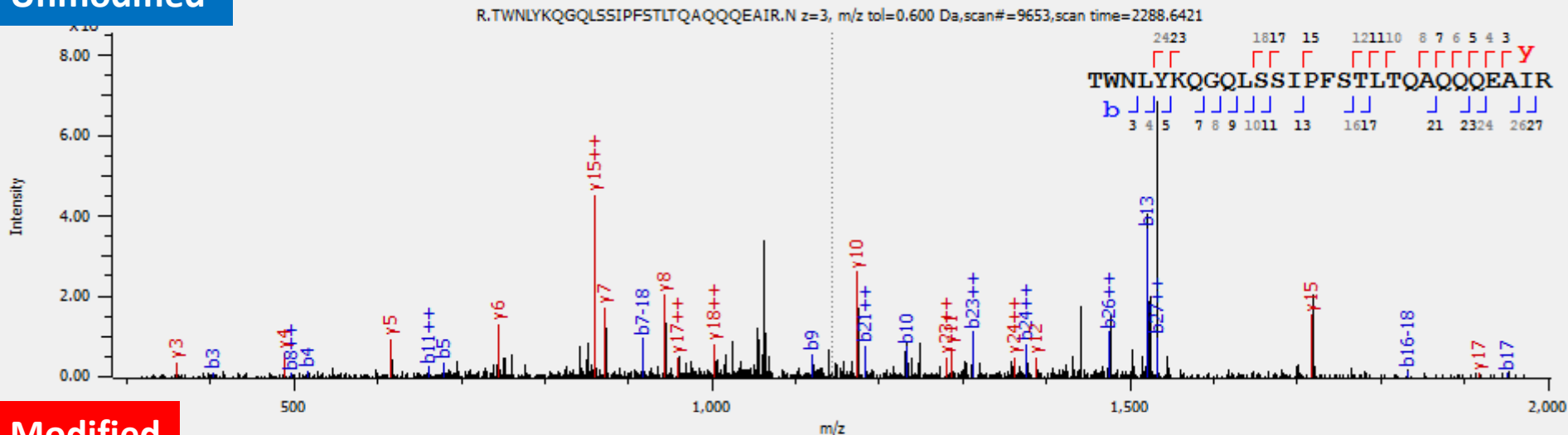
Fig. S12. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S13

TWNLYKQGQLSSIPFSTLTQAQQQEAIR

LC MS/MS-CID

Unmodified



Modified

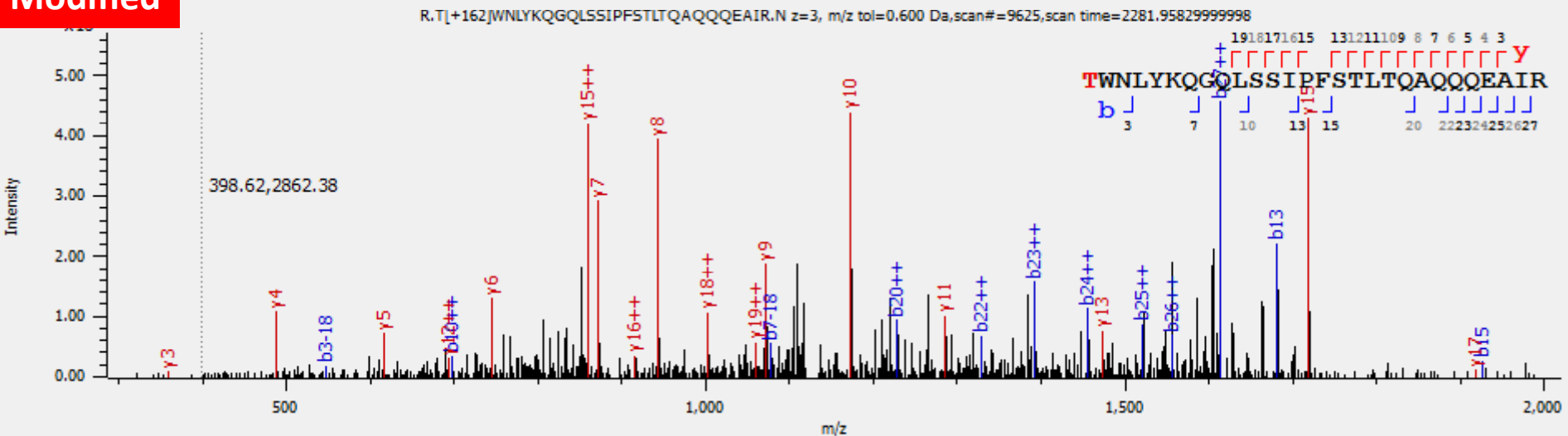


Fig. S13. LC MS/MS-CID of the glycosylation of the peptide T⁴³⁰WNLYKQGQLSSIPFSTLTQAQQQEAI R of MARTH_403 showing the assigned b and y ions.

Fig. S14

L S E L A E D L A K Y E E S H K MS1

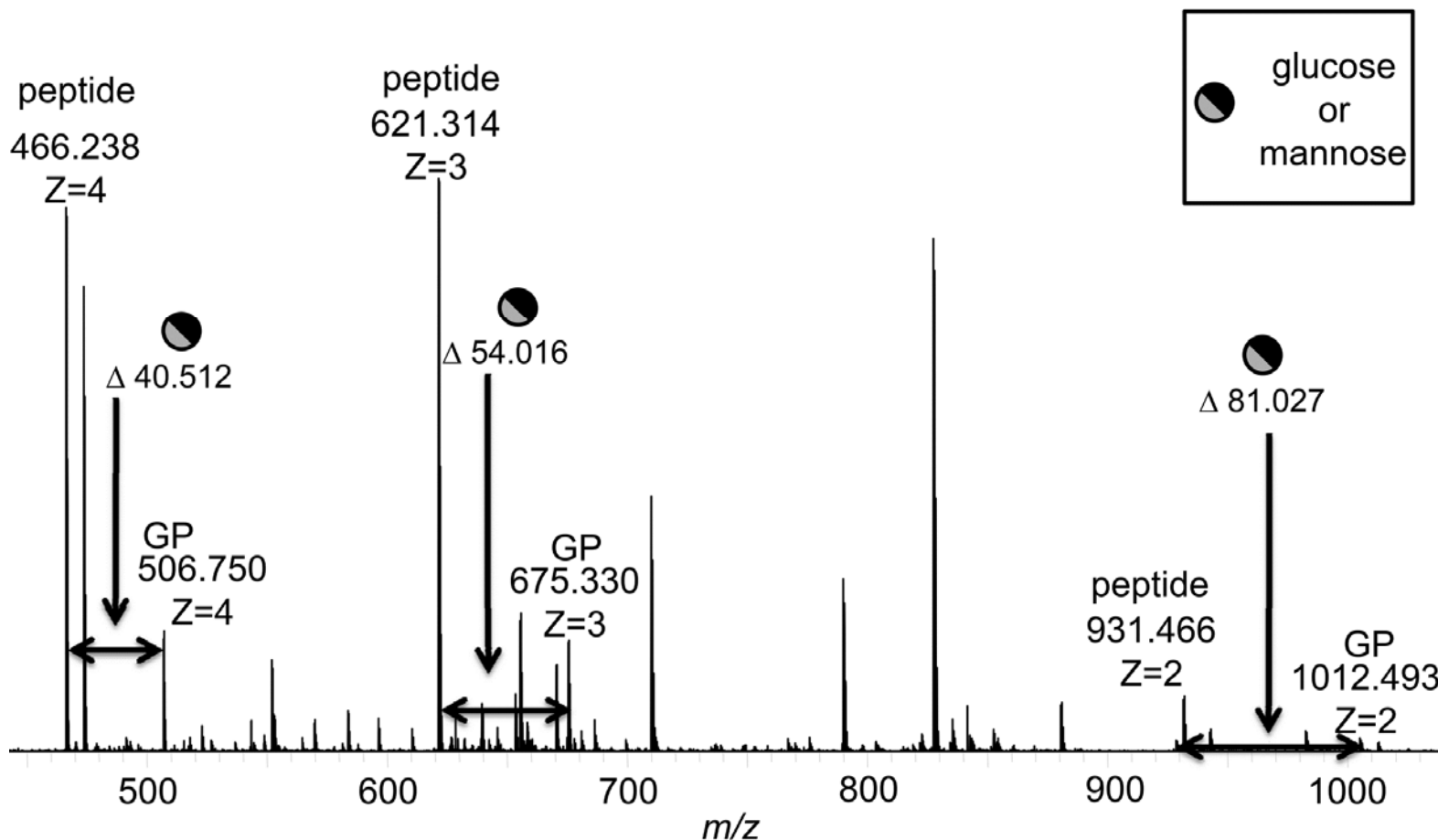


Fig. S14. Glycosylation of Ser⁷⁷⁹ in the peptide LSELAEDLAKYEESHK of MARTH_403. Orbitrap MS1 showing mass shift of the quadruply-, triply- and doubly-charged ions. The 40.512 shift for $z = 4$ equates to a mass shift of 162.048 Da. The 54.016 shift for $z = 3$ equates to a mass shift of 162.048 Da. The 81.027 shift for $z = 2$ equates to a mass shift of 162.054 Da. These mass shifts correspond to a hexose.

Fig. S

LC MS/MS-CID

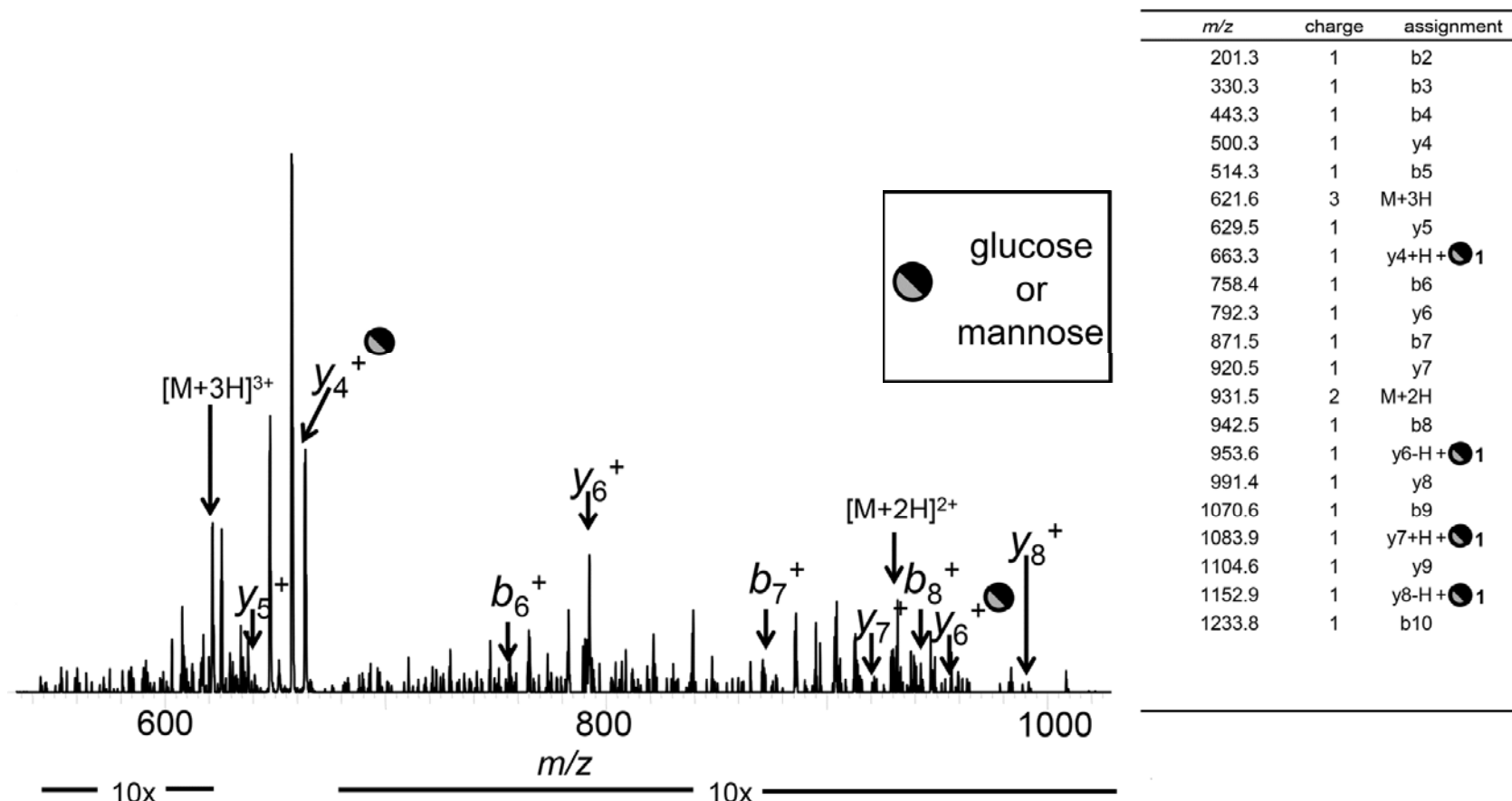
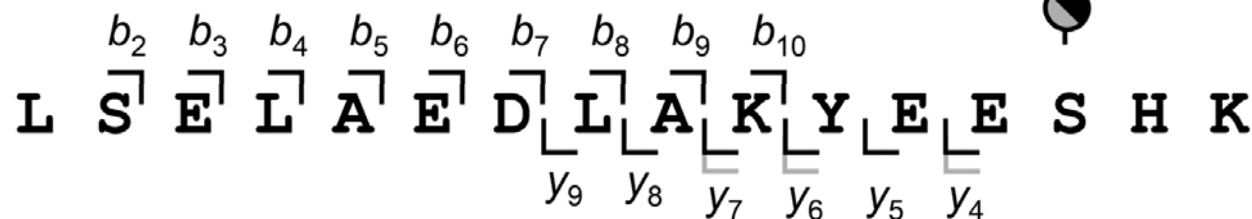
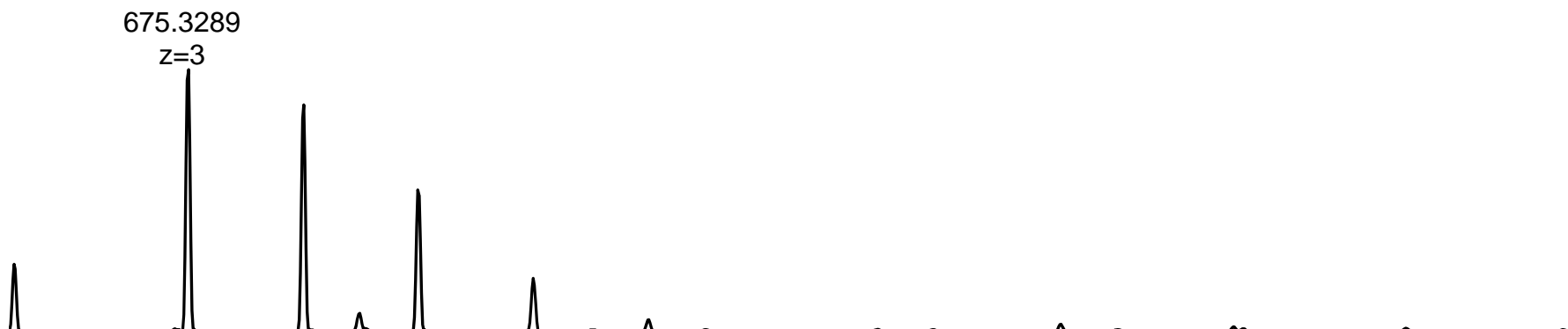


Fig. S15. LC MS/MS-CID of the glycosylation of the peptide LSELAEDLAKYEES⁷⁷⁹HK of MARTH_403 showing the assigned b and y ions. Ion dividers above and below the peptide sequence are gray for glycosylated fragments and black for nonglycosylated fragments.

Fig. S16

LSELAEDLAKYEESSHK ¹³C

Unlabeled



Labeled

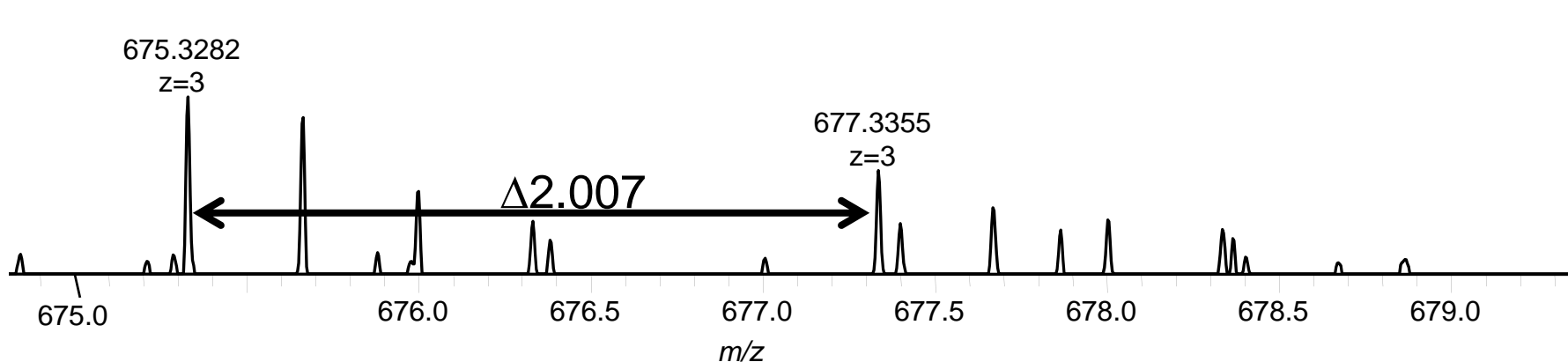


Fig. S16. Ions of glycosylated LSELAEDLAKYEES⁷⁷⁹HK peptide of MARTH_403. Unlabeled spectrum shows triply-charged species grown in serum-free medium. Labeled spectrum shows triply-charged species grown in MB supplemented with ¹³C starch.

Fig. S17

LSELAEDLAKYEESHK ¹³C

Unlabeled

506.7492
z=4

Labeled

506.7482
z=4

508.2532
z=4

Δ1.505

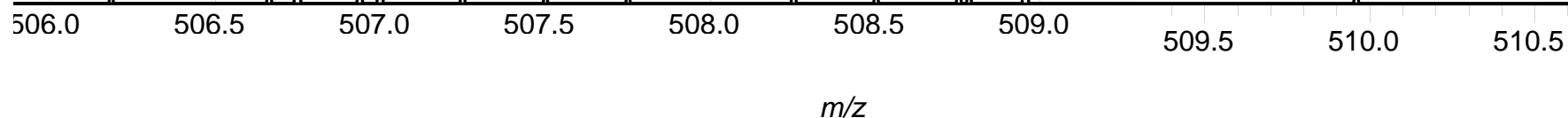


Fig. S17. Ions of glycosylated LSELAEDLAKYEES⁷⁷⁹HK peptide of MARTH_403. Unlabeled spectrum shows quadruply-charged species grown in serum-free medium. Labeled spectrum shows quadruply-charged species grown in MB supplemented with ¹³C starch.

Fig. S18 YEESHKIIGSIPFGDFDK

MS1

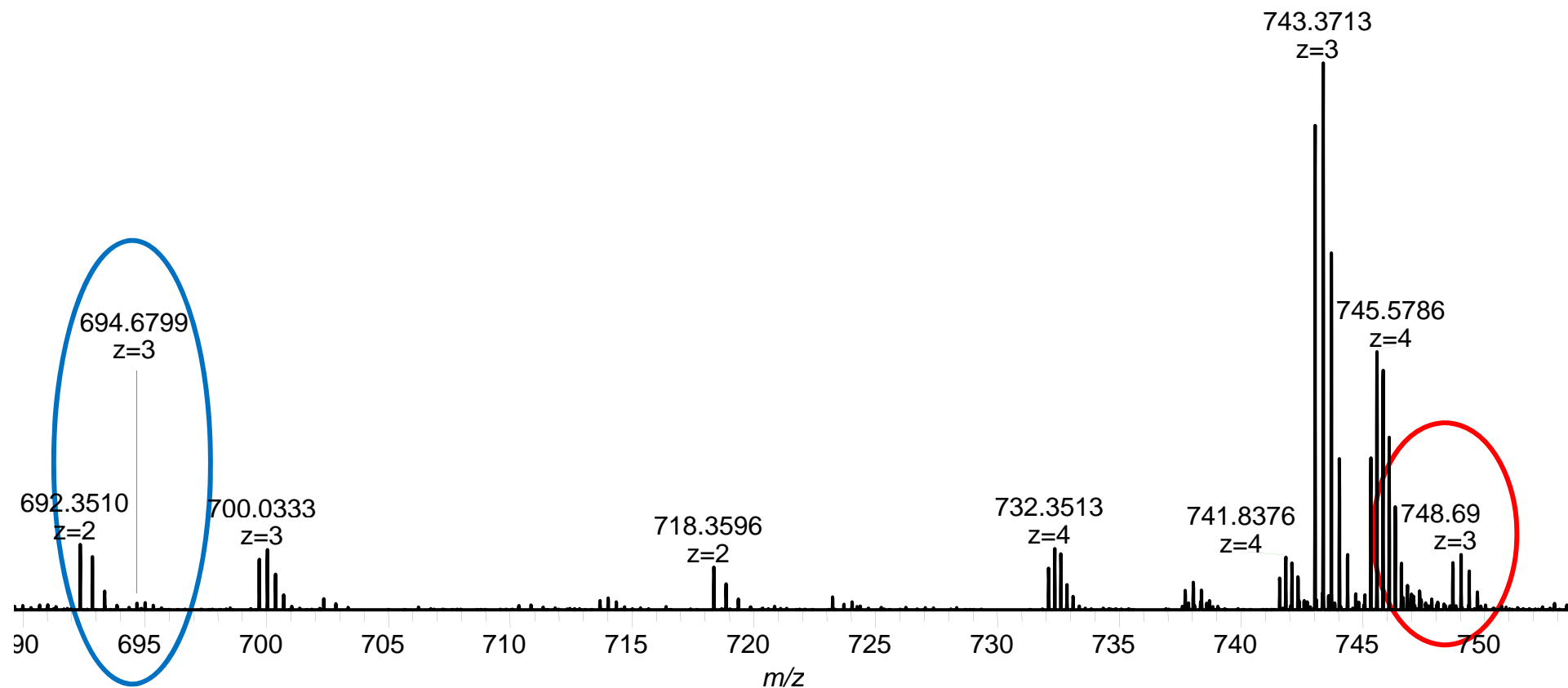
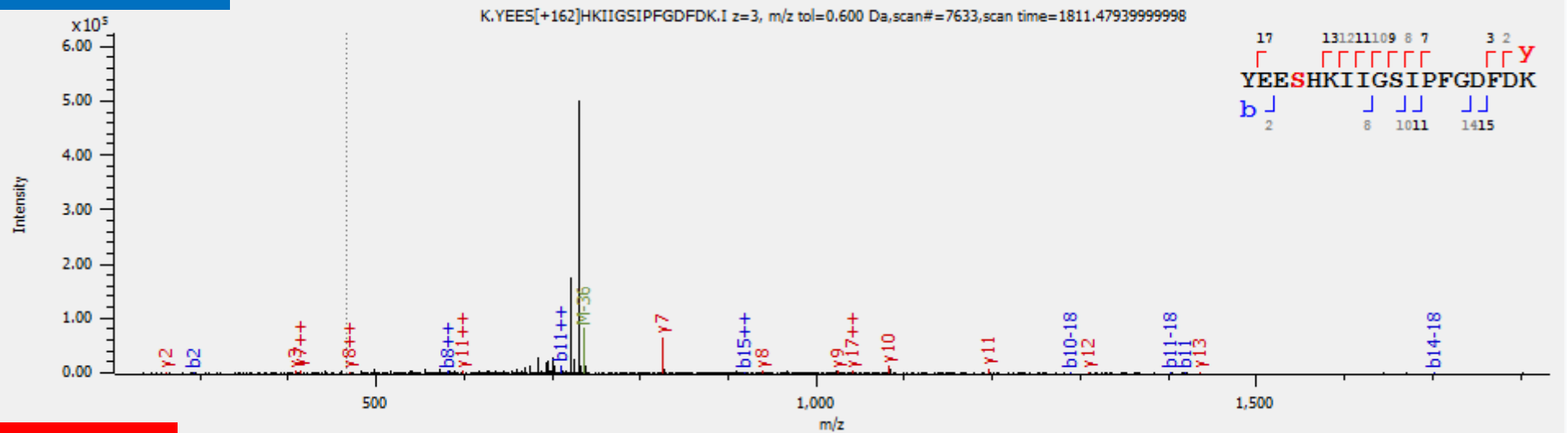


Fig. S18. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S19 YEESHKIIGSIPFGDFDK

LC MS/MS-CID

Unmodified



Modified

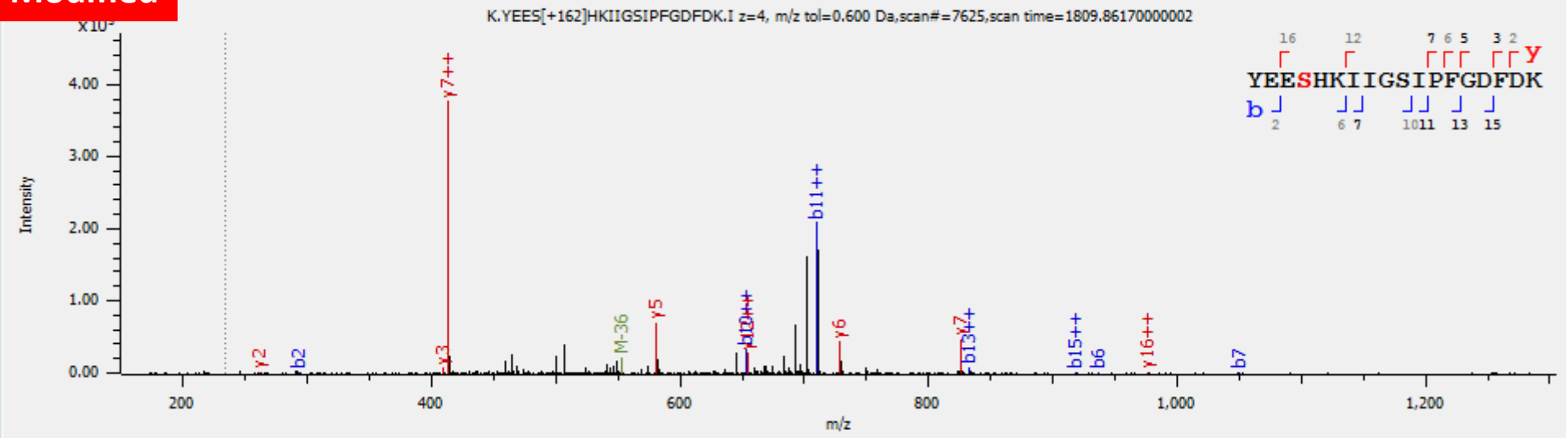


Fig. S19. LC MS/MS-CID of the glycosylation of the peptide YEE^{S779}HKIIGSIPFGDFDK of MARTH_403 showing the assigned b and y ions.

Fig. S20

DILENKDDSLSTQGK

MS1

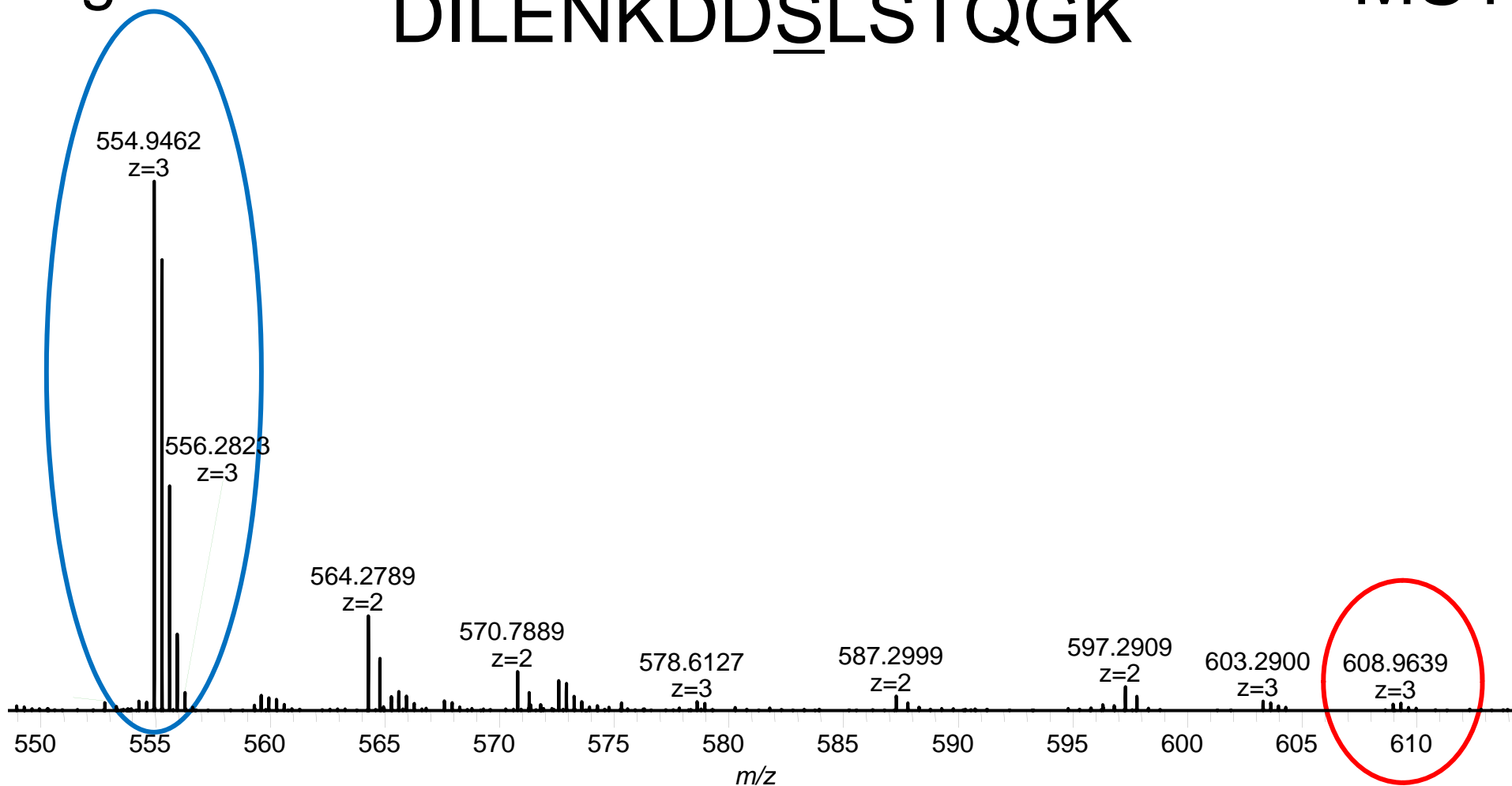
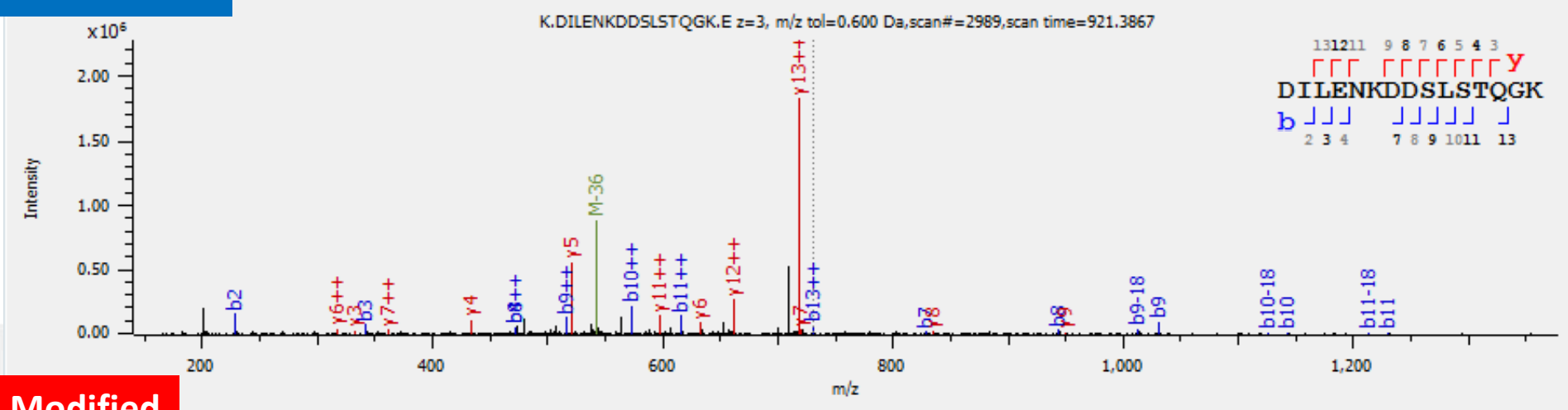


Fig. S20. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S21 DILENKDDSLSTQ GK LC MS/MS-CID

Unmodified



Modified

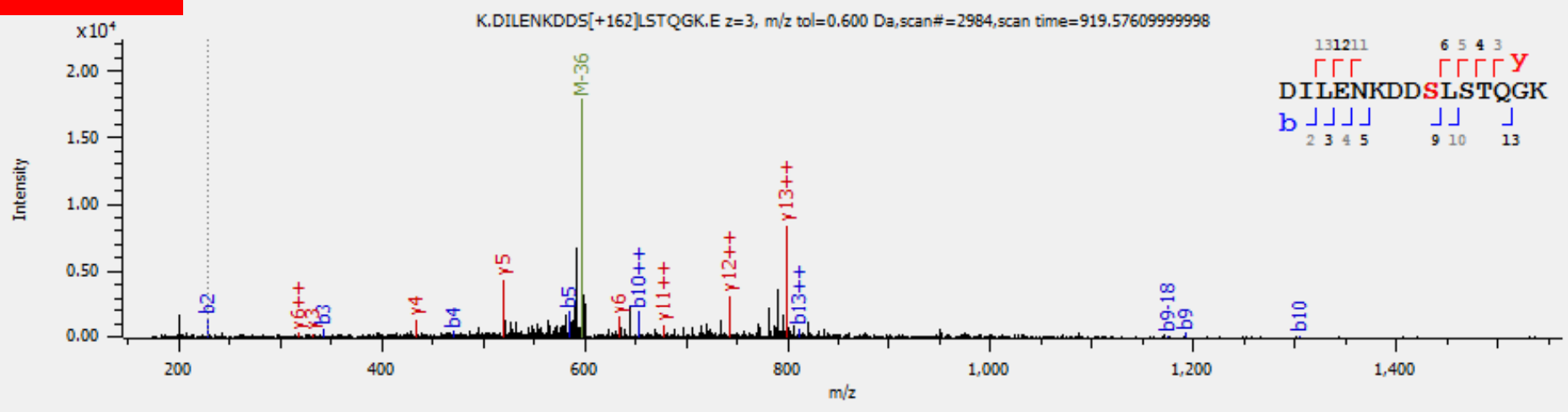


Fig. S21. LC MS/MS-CID of the glycosylation of the peptide DILENKDD⁷⁹⁵SLSTQ GK of MARTH_455 showing the assigned b and y ions.

Fig. S22

MS1

YINKLEALDENDLTPDSLAWAR

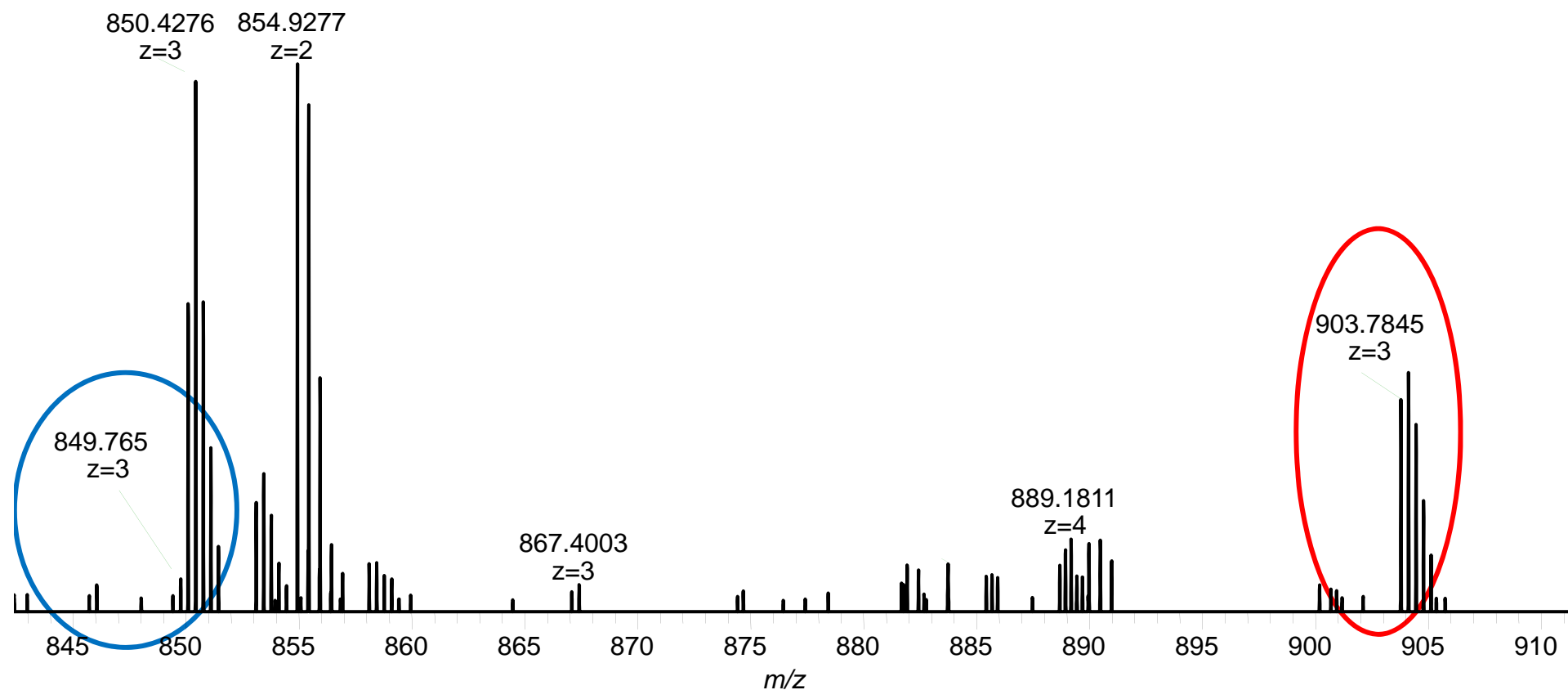


Fig. S22. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S23 YINKLEALDENDLT₁₆₈PDSLAWAR LC MS/MS-CID

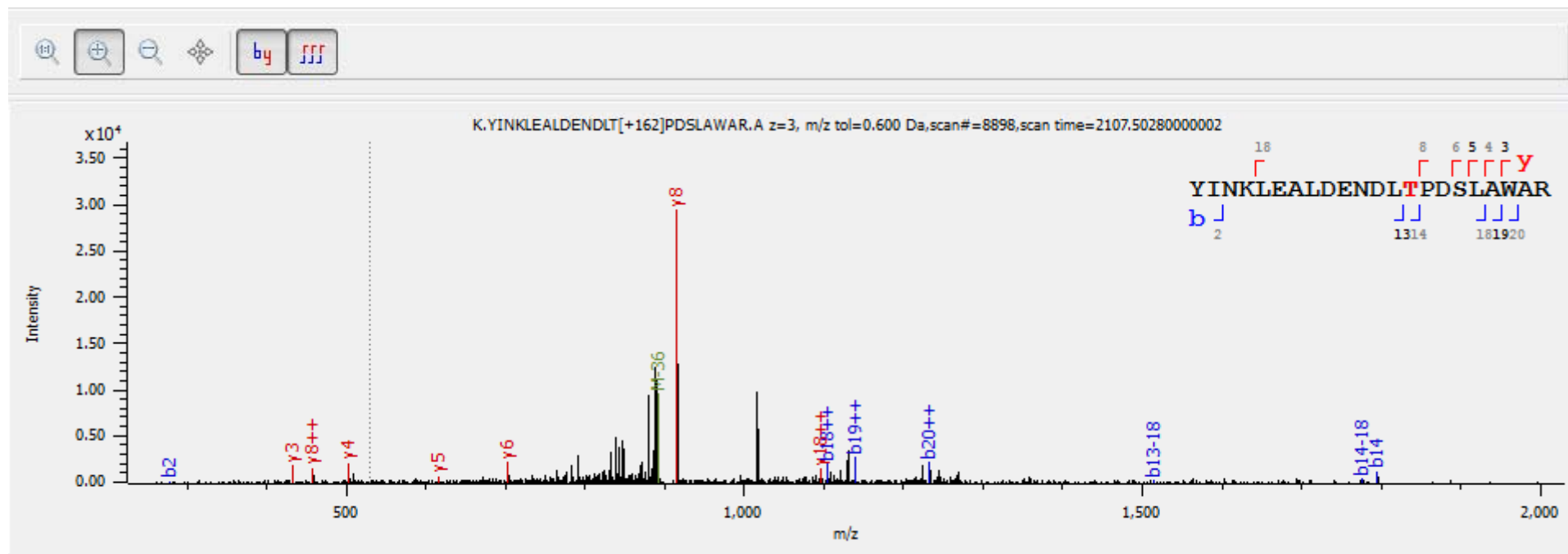


Fig. S23. LC MS/MS-CID of the glycosylation of the peptide YINKLEALDENDLT¹⁶⁸PDSLAWAR of MARTH_665 showing the assigned b and y ions.

Fig. S24

MS1

TAVITDGGDINDISFNQSAWEGVLNFM^uEQVKAPIQK

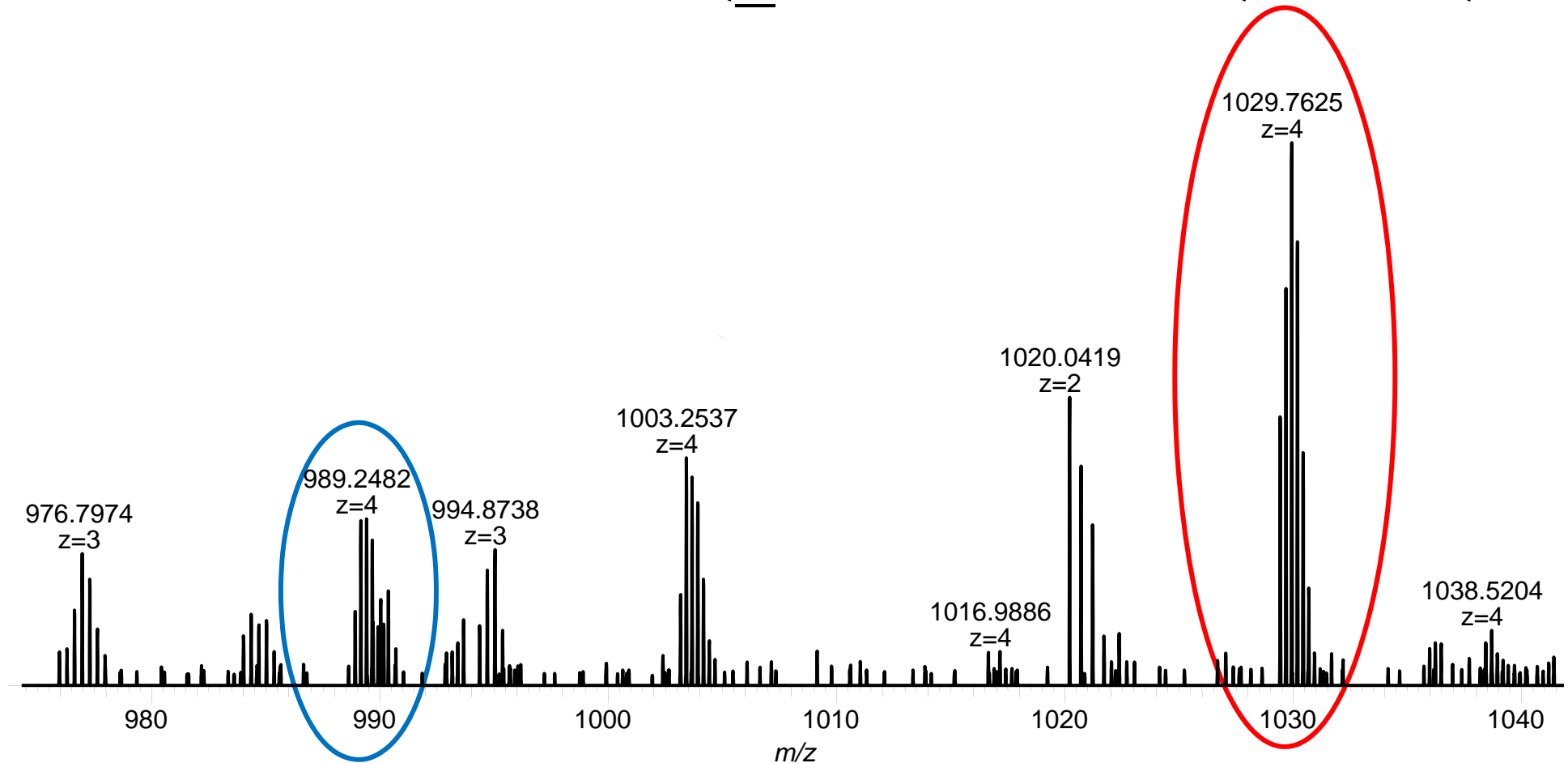


Fig. S24. LC-MS of nonglycosylated peptide ion (blue oval) and the glycosylated ion (red oval) corresponding to a hexose O-linked to the peptide.

Fig. S25 TAVITDGGDINDISFNQSAWEGVLNFMEQVKAPIQK LC MS/MS-CID

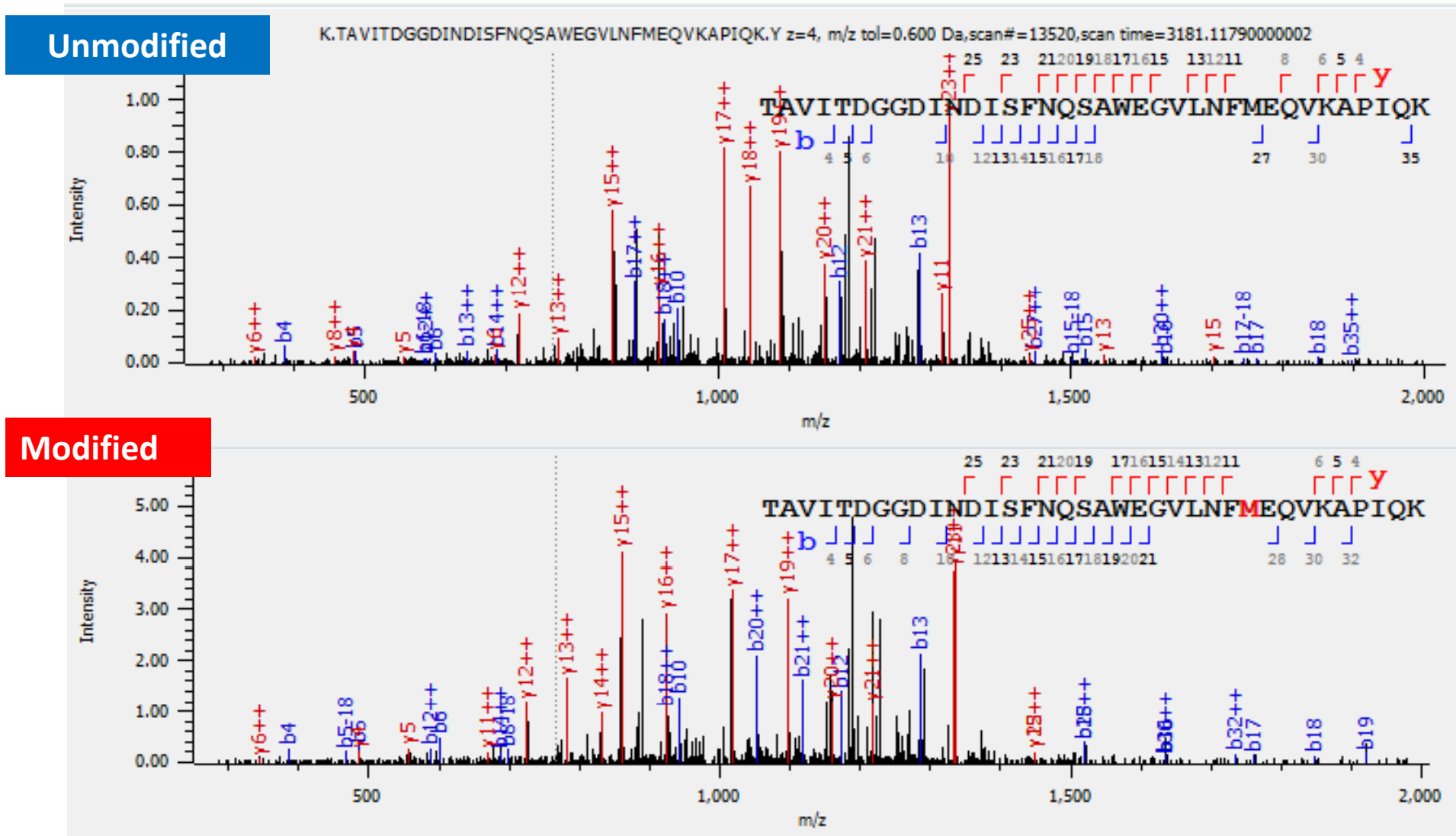


Fig. S25. LC MS/MS-CID of the glycosylation of the peptide TAVITDGGDINDISFNQ⁷⁹AWEGVLNFMEQVKAPIQK of MARTH_819 showing the assigned b and y ions.

Fig. S26 TAVITDGGDINDISFNQSAWEGVLNFMEQVKAPIQK LC MS/MS-CID

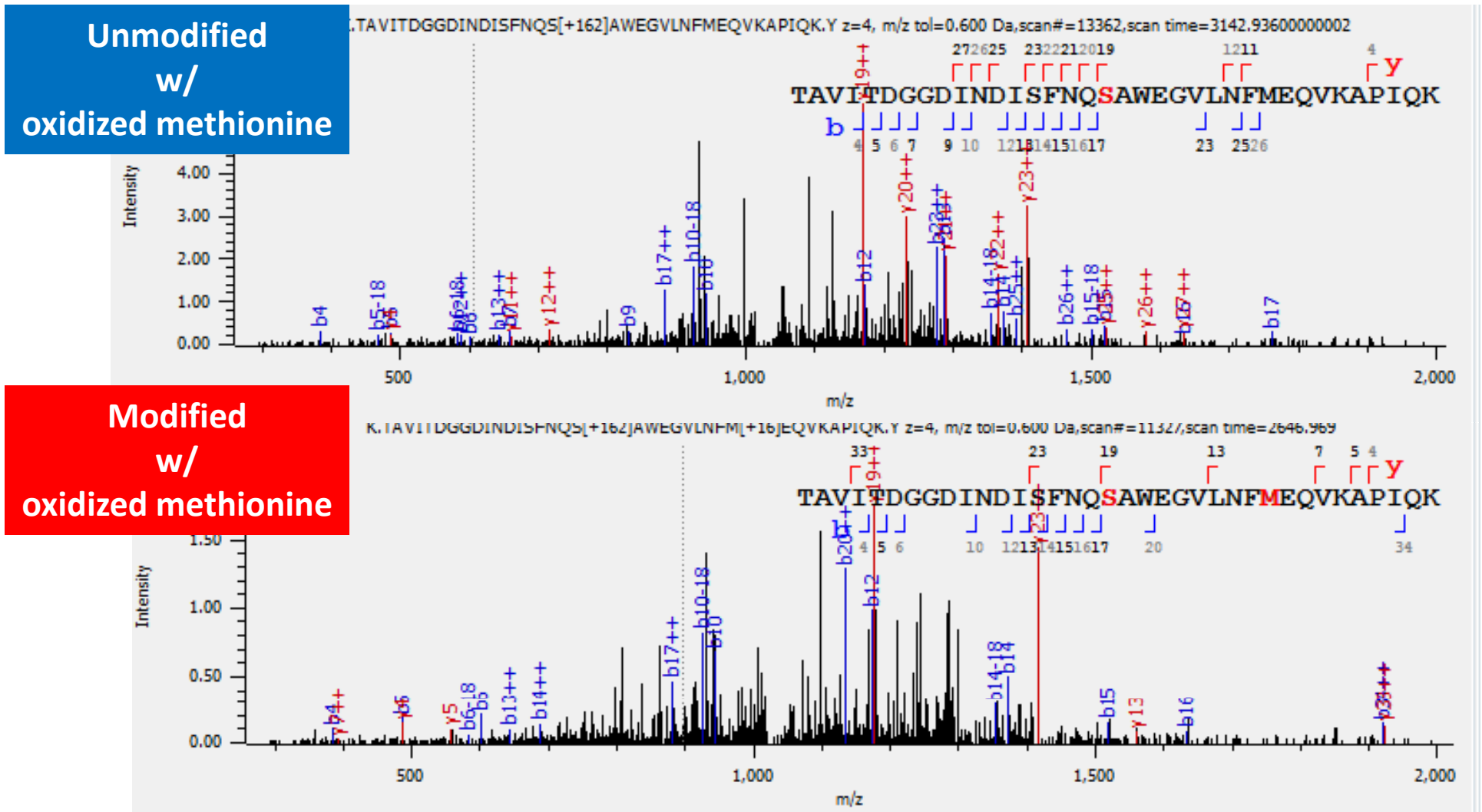


Fig. S26. LC MS/MS-CID of the glycosylation and methionine oxidation of the peptide TAVITDGGDINDISFNQ⁷⁹AWEGVLNFMEQVKAPIQK of MARTH_819 showing the assigned b and y ions.

Fig. S27

V I D G T F Q E A Y K R

MS1

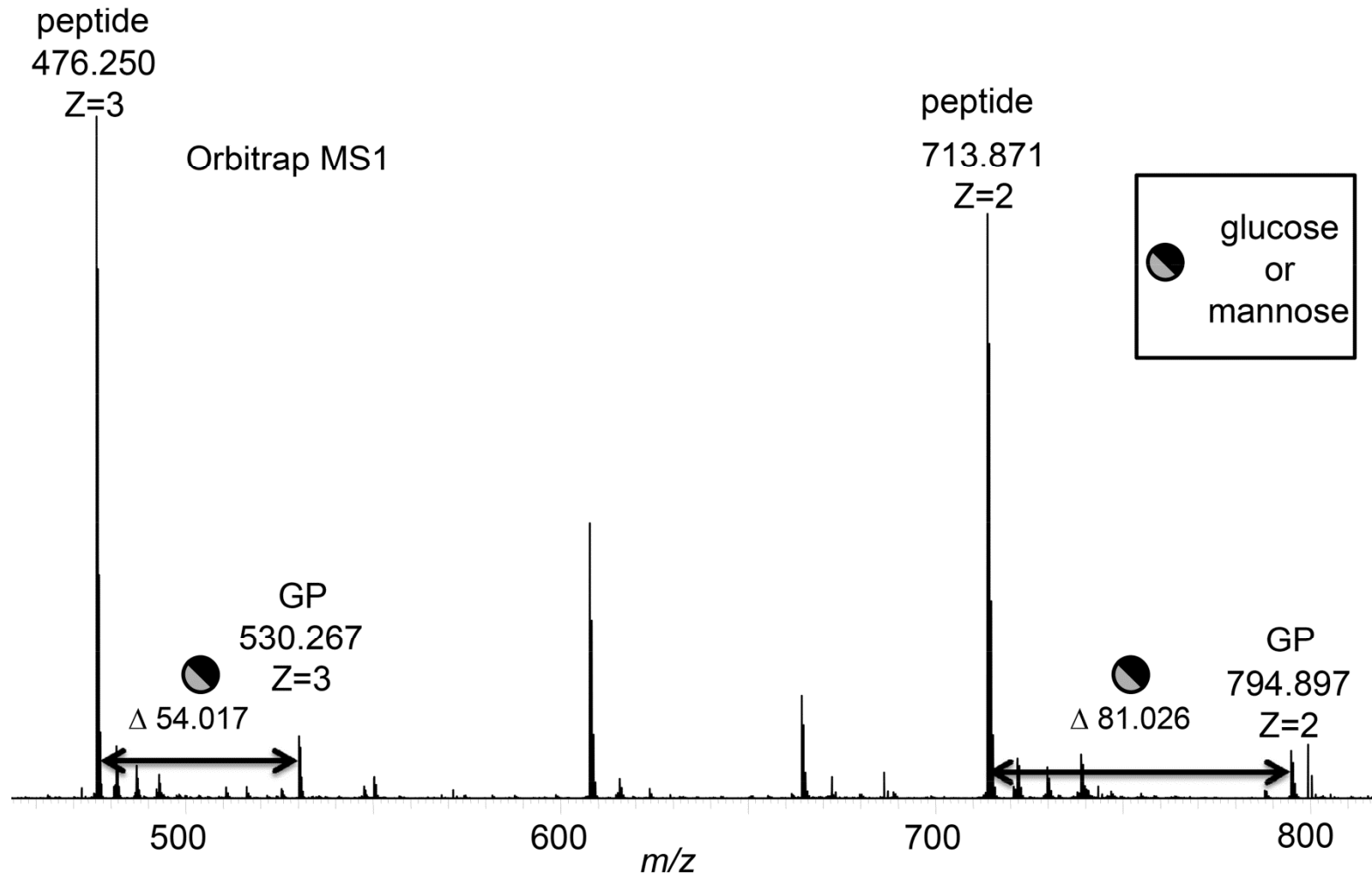
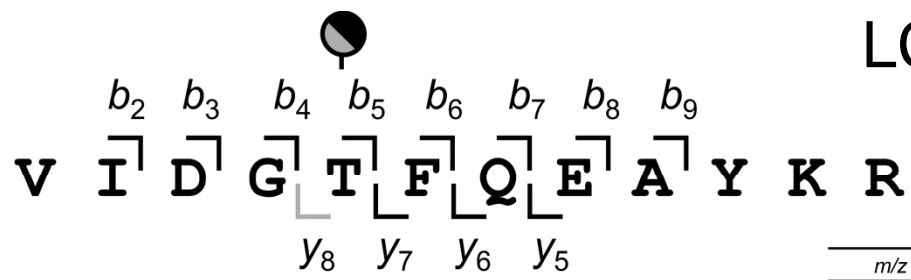


Fig. S27. Glycosylation of Thr¹⁰⁷ in the peptide VIDGTFQEAYKR of MARTH_819. Orbitrap MS1 showing mass shift of the triply- and doubly-charged ions. The 54.017 shift for $z = 3$ equates to a mass shift of 162.051 Da. The 81.026 shift for $z = 2$ equates to a mass shift of 162.052 Da. These mass shifts correspond to a hexose.

Fig. S28

LC MS/MS-CID



<i>m/z</i>	charge	assignment
213.1	1	b2
328.3	1	b3
385.4	1	b4
476.3	3	M+3H
486.3	1	b5
518.3	3	M-H ₂ O+3H+ 1
633.4	1	b6
666.3	1	y6
761.5	1	b7
793.4	1	y6
890.6	1	b8
941.4	1	y7
961.4	1	b9
1203.5	1	y8+ 1

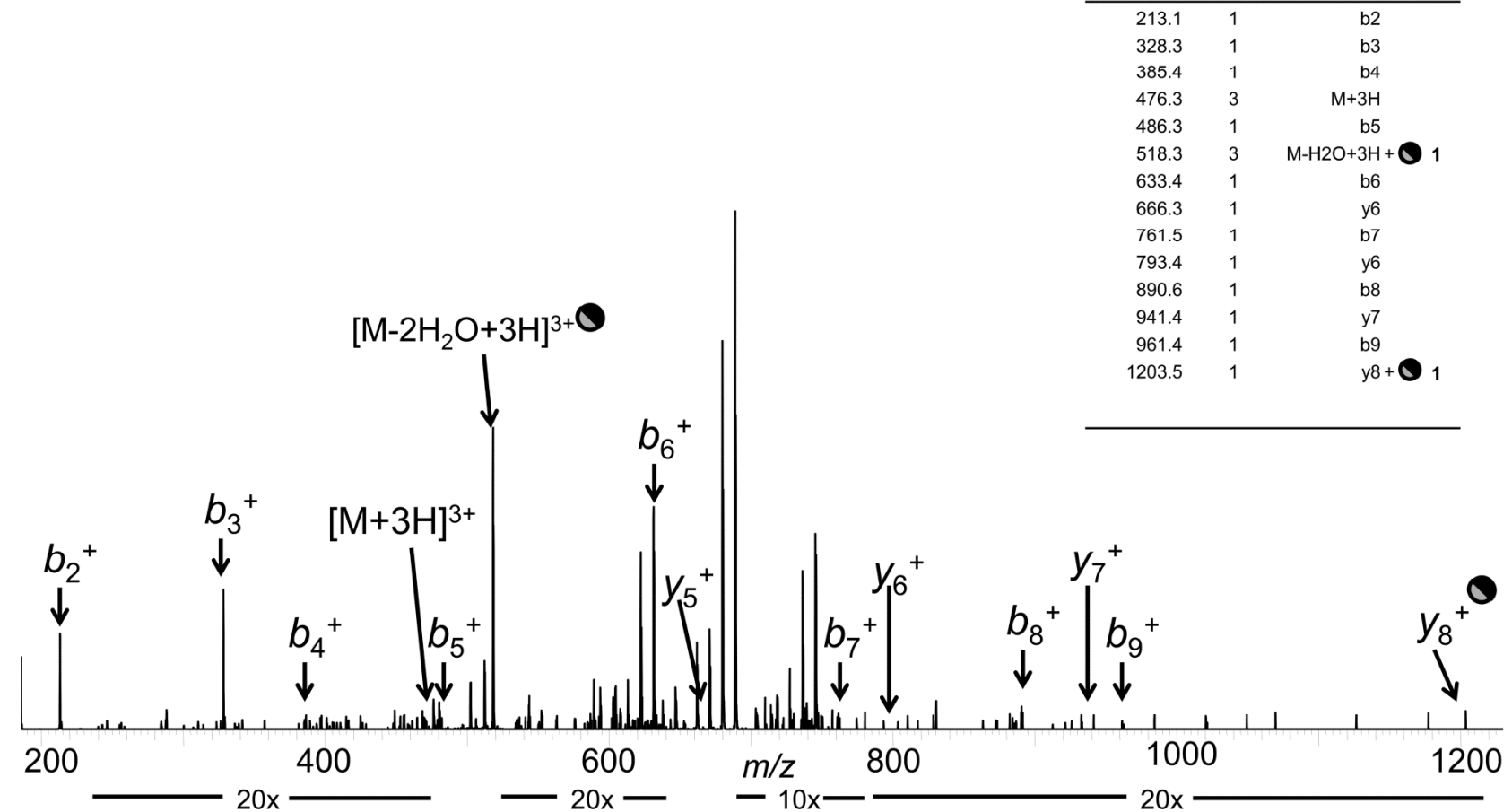


Fig. S28. LC MS/MS-CID of the glycosylated peptide VIDGT¹⁰⁷FQEAYKR of MARTH_819 showing the assigned b and y ions. Ion dividers above and below the peptide sequence are gray for glycosylated fragments and black for non-glycosylated fragments.

Fig. S29

¹³C

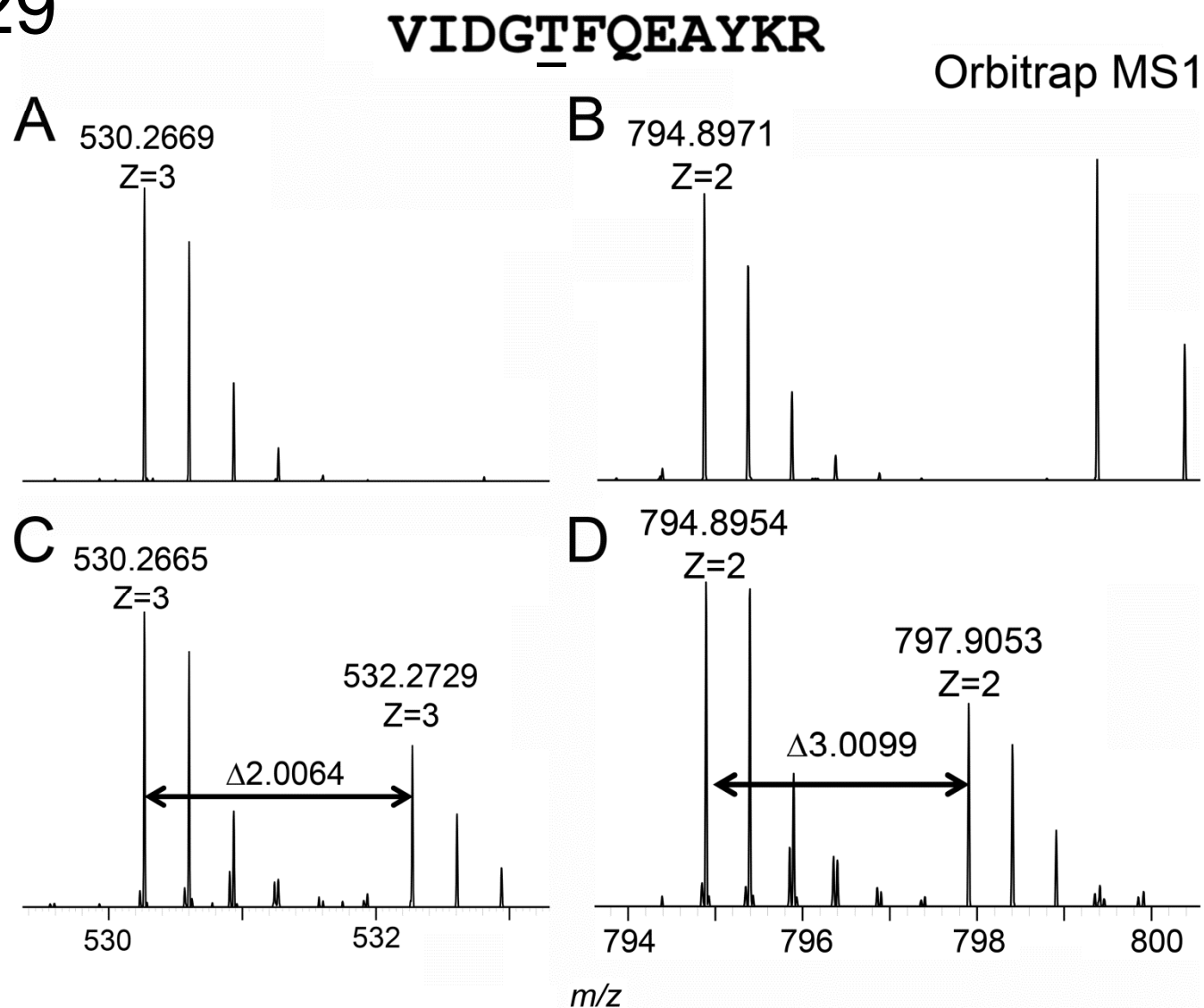


Fig. S29. Ions of glycosylated VIDGT¹⁰⁷FQEAYKR peptide of MARTH_819. (A) triply-charged species grown in serum-free medium, (B) doubly-charged species grown in serum-free medium, (C) triply-charged species grown in MB supplemented with ¹³C starch, and (D) doubly-charged species grown in MB supplemented with ¹³C starch.