

Appendix S3: Annotated Spectra of Identified HPG sites in T7

1. Description of spectra

The annotated spectra below show the best-identified spectrum for each identified HPG site. The precursor ion m/z error was less than 4.5 ppm after recalibration and the fragment ion m/z error was less than 20 ppm and the peptide-spectrum match false discovery rate was less than 1%. Methionines (M) annotated with a “me” indicate the methionines that were replaced with HPG.

Page 2: T7 - P03716, position 279.

Page 3: T7 - P03728, position 179.

Page 4: T7 - P03800, position 1.

Page 5: T7 - P19726; P19727, position 293.

Page 6: T7 - P03726, position 150.

Page 7: T7 - P19726; P19727, position 10.

Page 8: T7 - P00969, position 288.

Page 9: T7 - P03728, position 118.

Page 10: T7 - P19726, position 327.

Page 11: T7 - P03696, position 137.

Page 12: T7 - P03728, position 112.

Page 13: T7 - P03693, position 22.

Page 14: EhV - G4YBV8 at M23.

Page 15: EhV - Q4G3F4 at M216.

Page 16: EhV - G4YBL4 at M97.

Page 17: EhV - G4YBX3 at M97.

Page 18: EhV - G4YCD5 at M91.

Page 19: EhV - R1D6F2 at M234.

Page 20: EhV - G4YCE6 at M39.

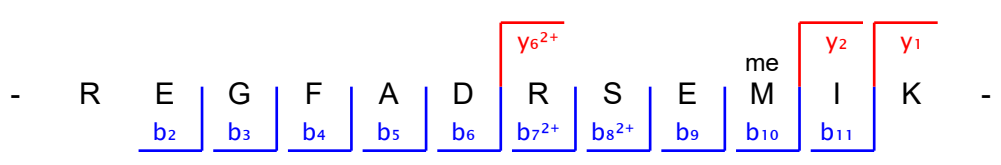
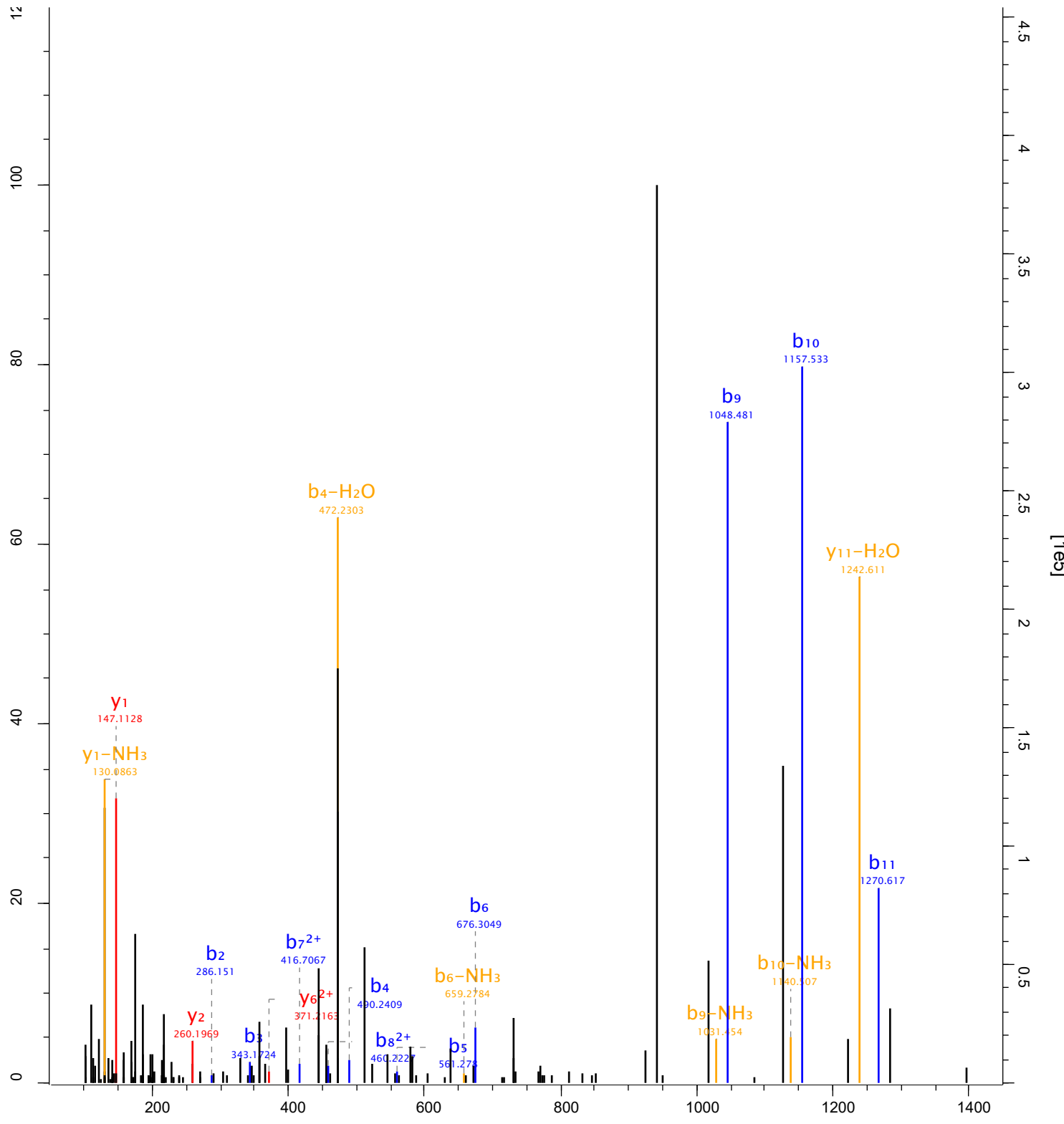
Page 21: EhV - G4YBJ4 at M105.

Page 22 (false positive): EhV - R1B4L2 at M306.

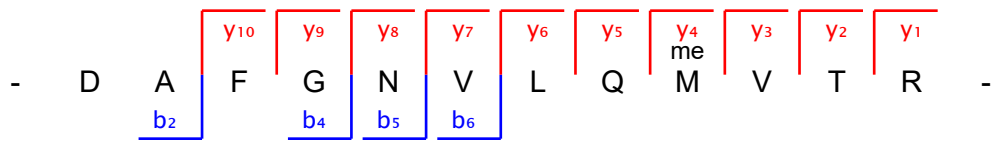
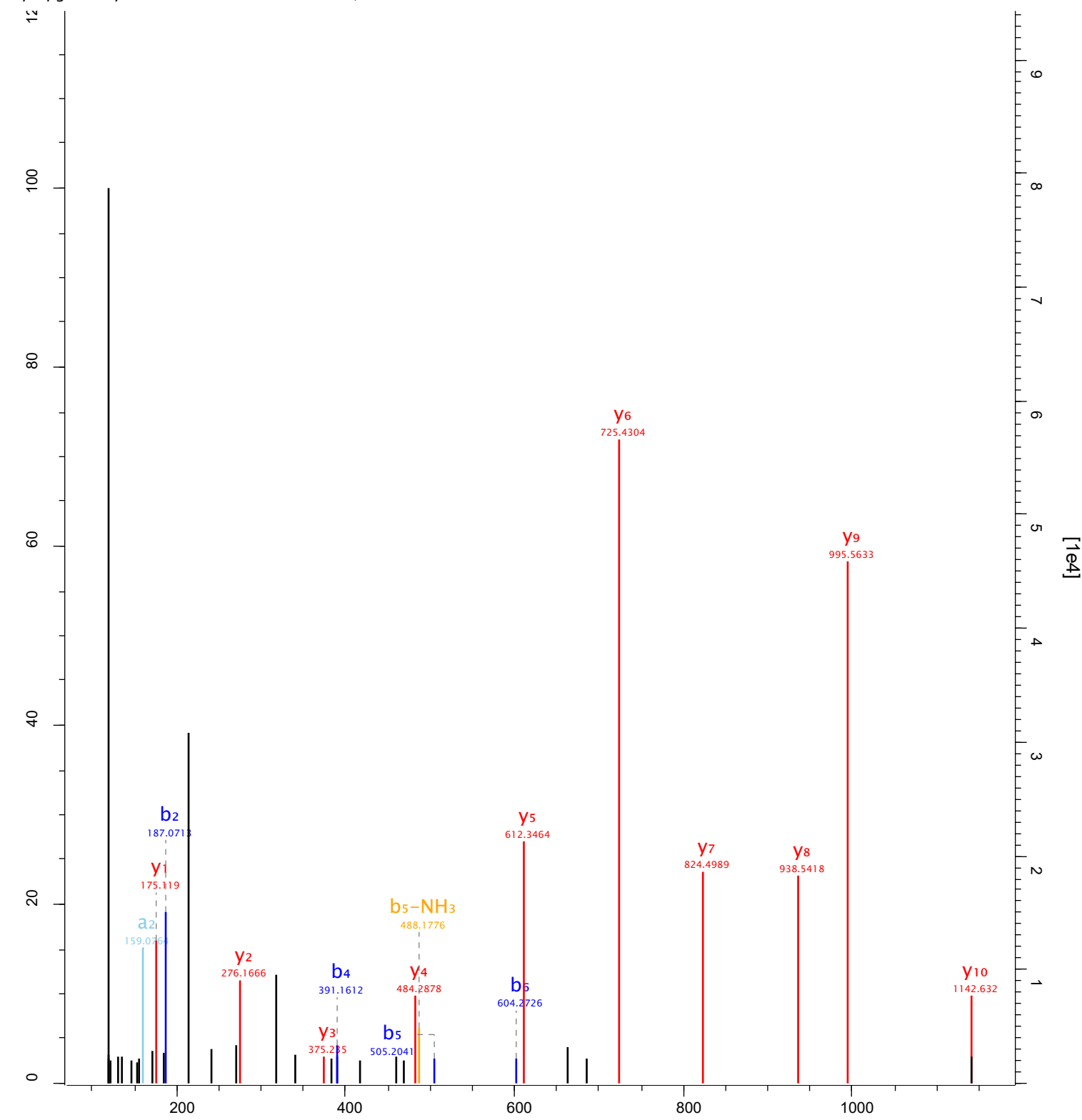
2. Summary of HPG incorporation

Of the 268 MS2 spectra from T7 that had at least one genetically encoded Met, 15 (5.6%) had the Met replaced with HPG. For EhV, 9 of the 159 MS2 spectra (5.7%) had the Met replaced with HPG. However, this is only a semi-quantitative measure of HPG incorporation due to the fact that we did not confirm the difference, if any, in ionization efficiency of peptides with HPG vs Met. Furthermore, spectral counting cannot be interpreted quantitatively without comparison to specific standards.

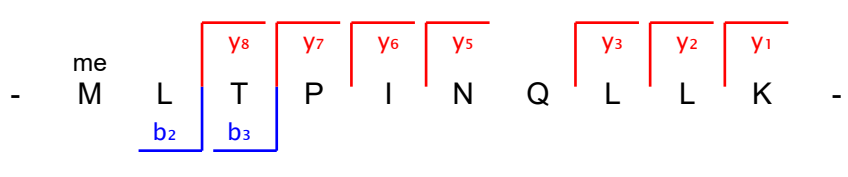
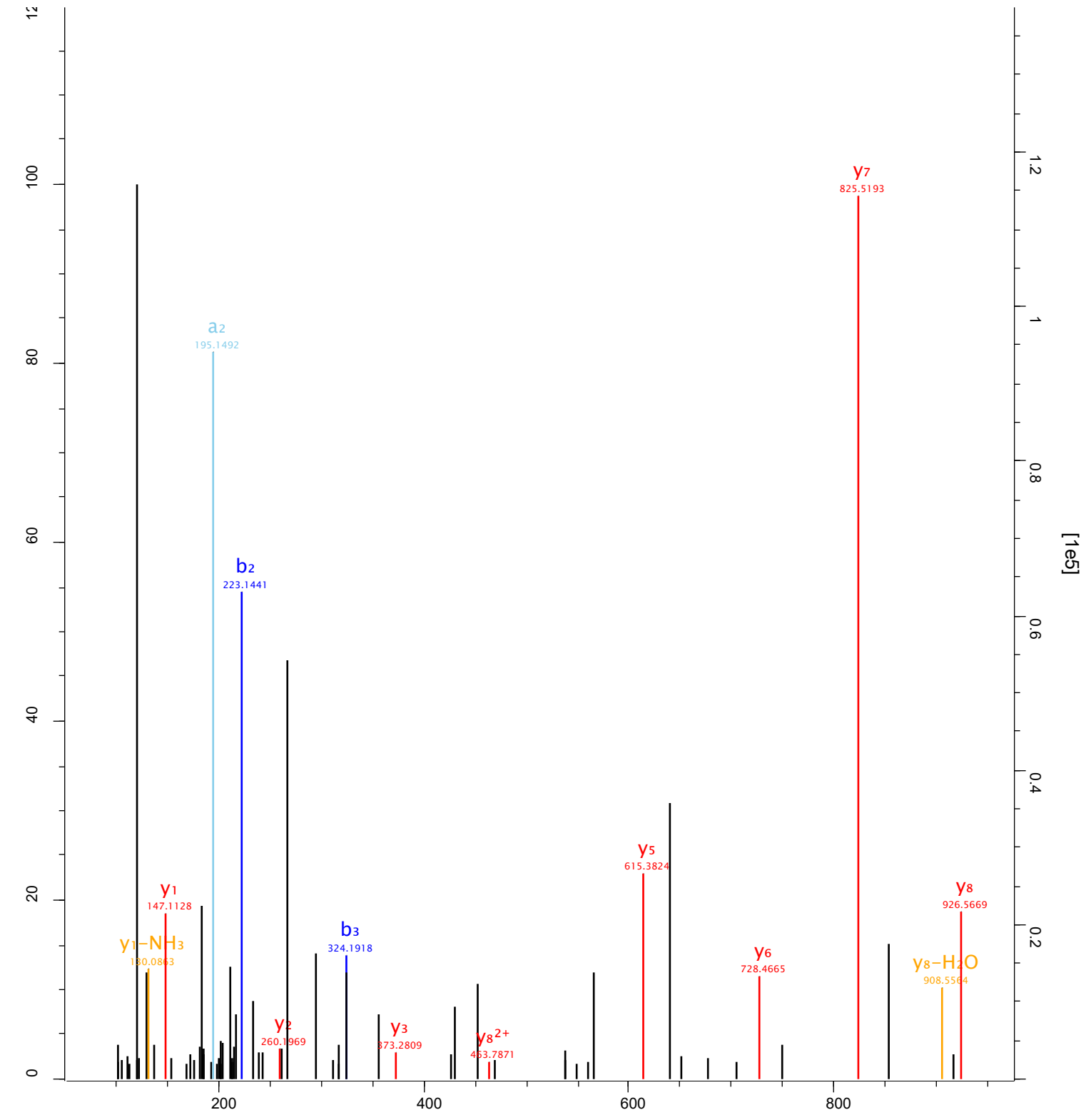
3. Spectra

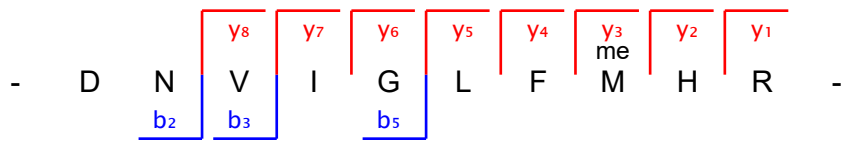
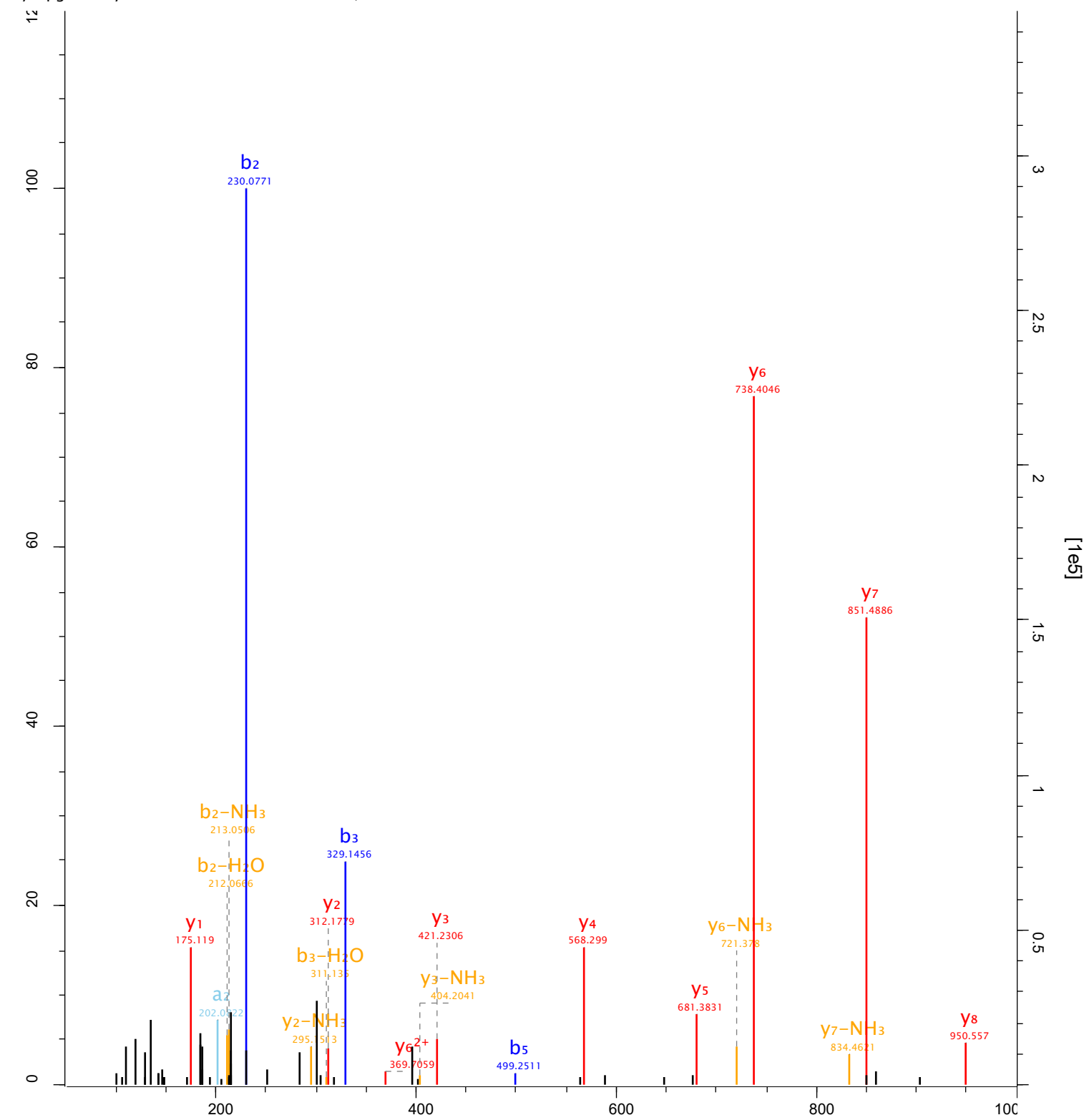


Raw file ally_hpg_19May16 Scan 59235 Method FTMS; HCD Score 76.73 m/z 664.85

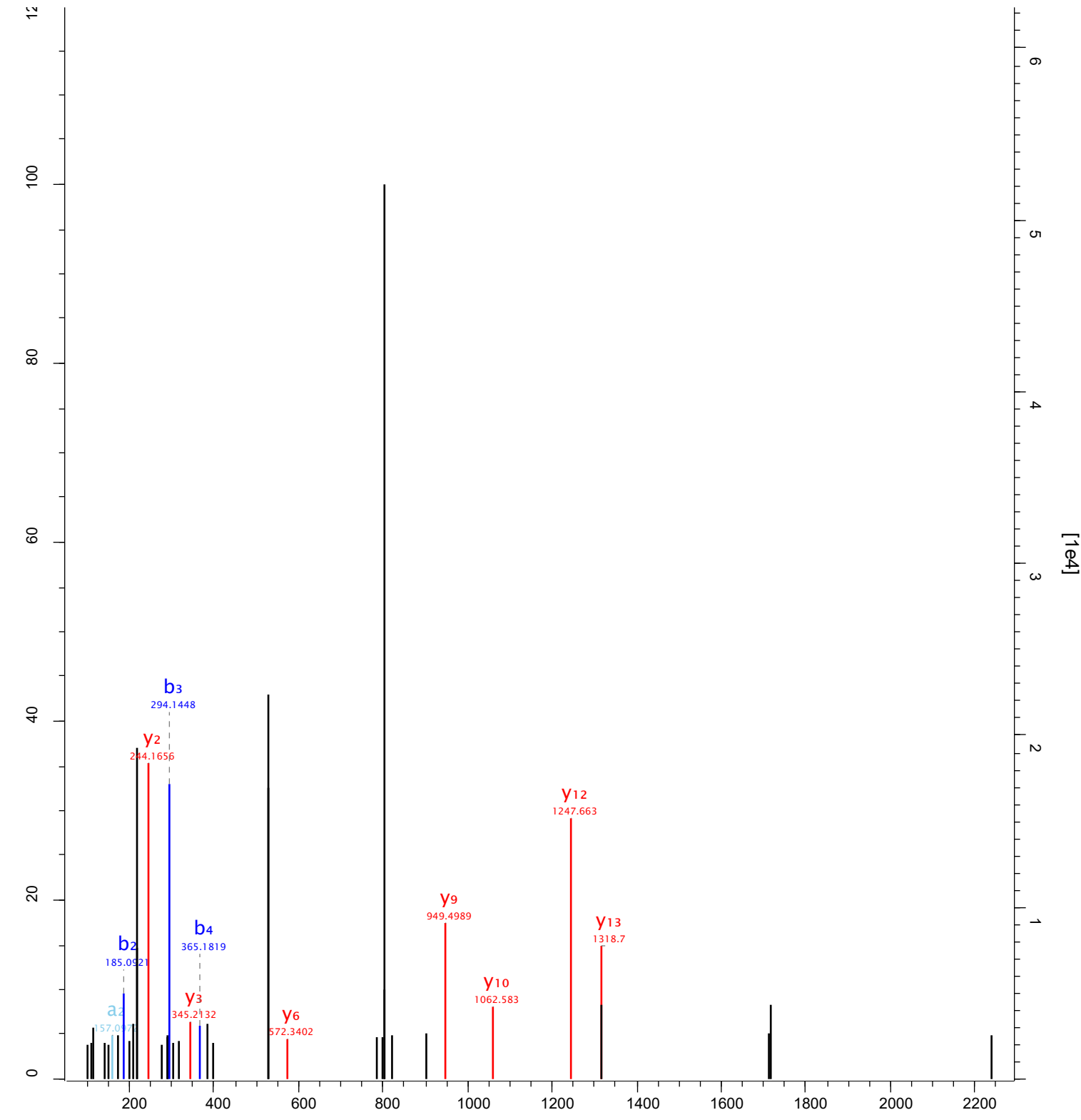


Raw file ally_hpg_19May16 Scan 51883 Method FTMS; HCD Score 71.61 m/z 574.86





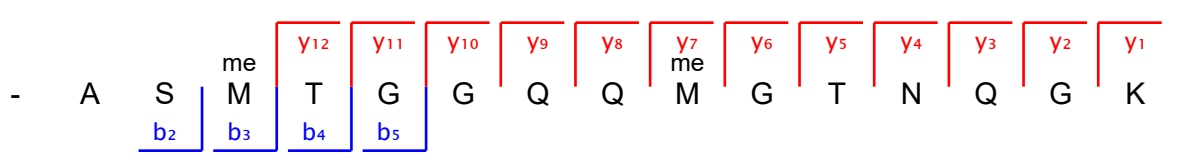
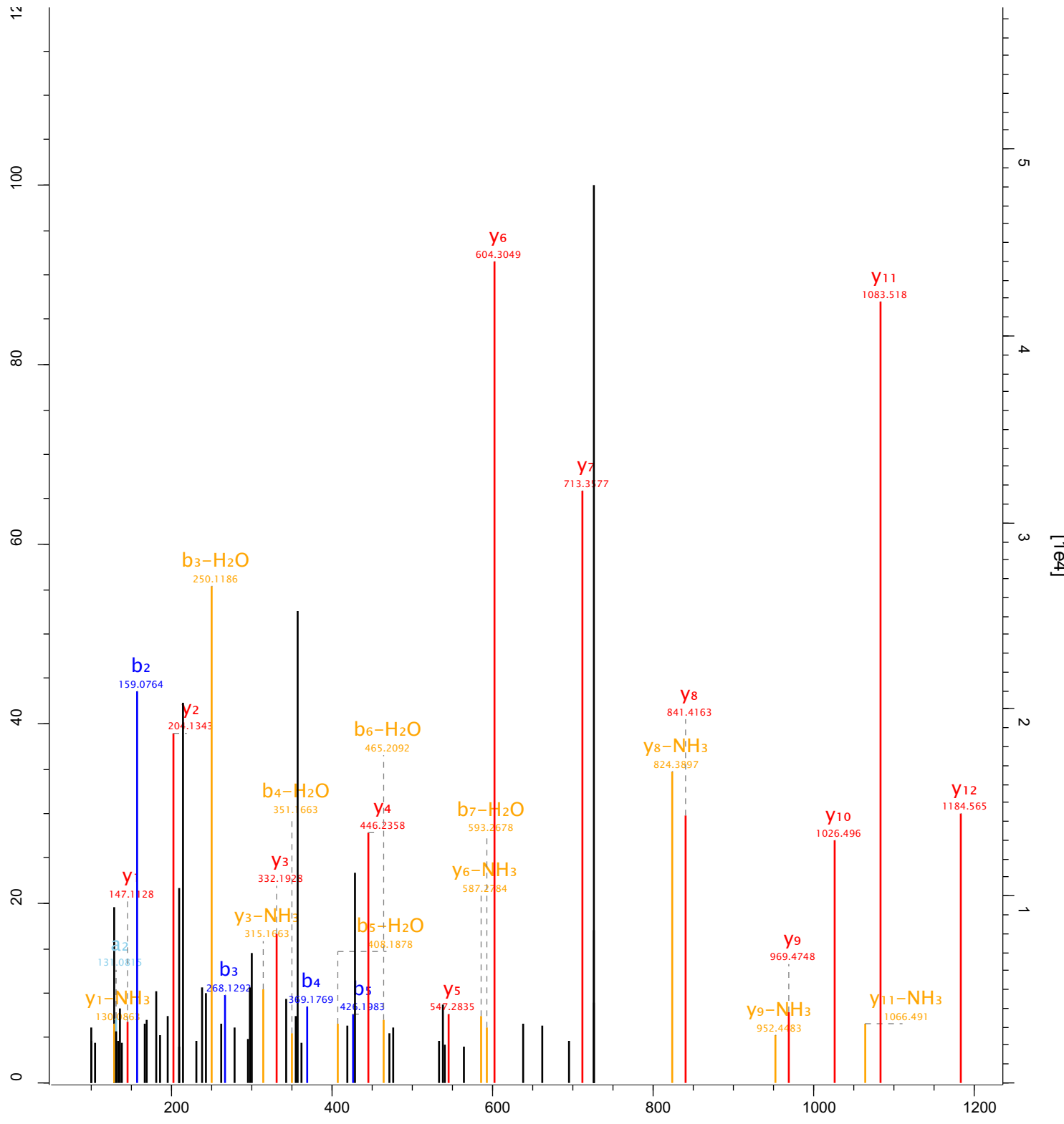
Raw file ally_hpg_19May16 Scan 44181 Method FTMS; HCD Score 42.31 m/z 806.42

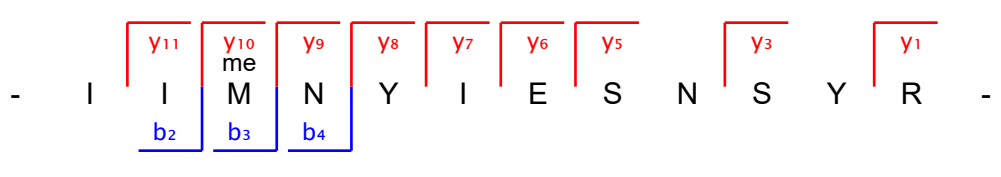
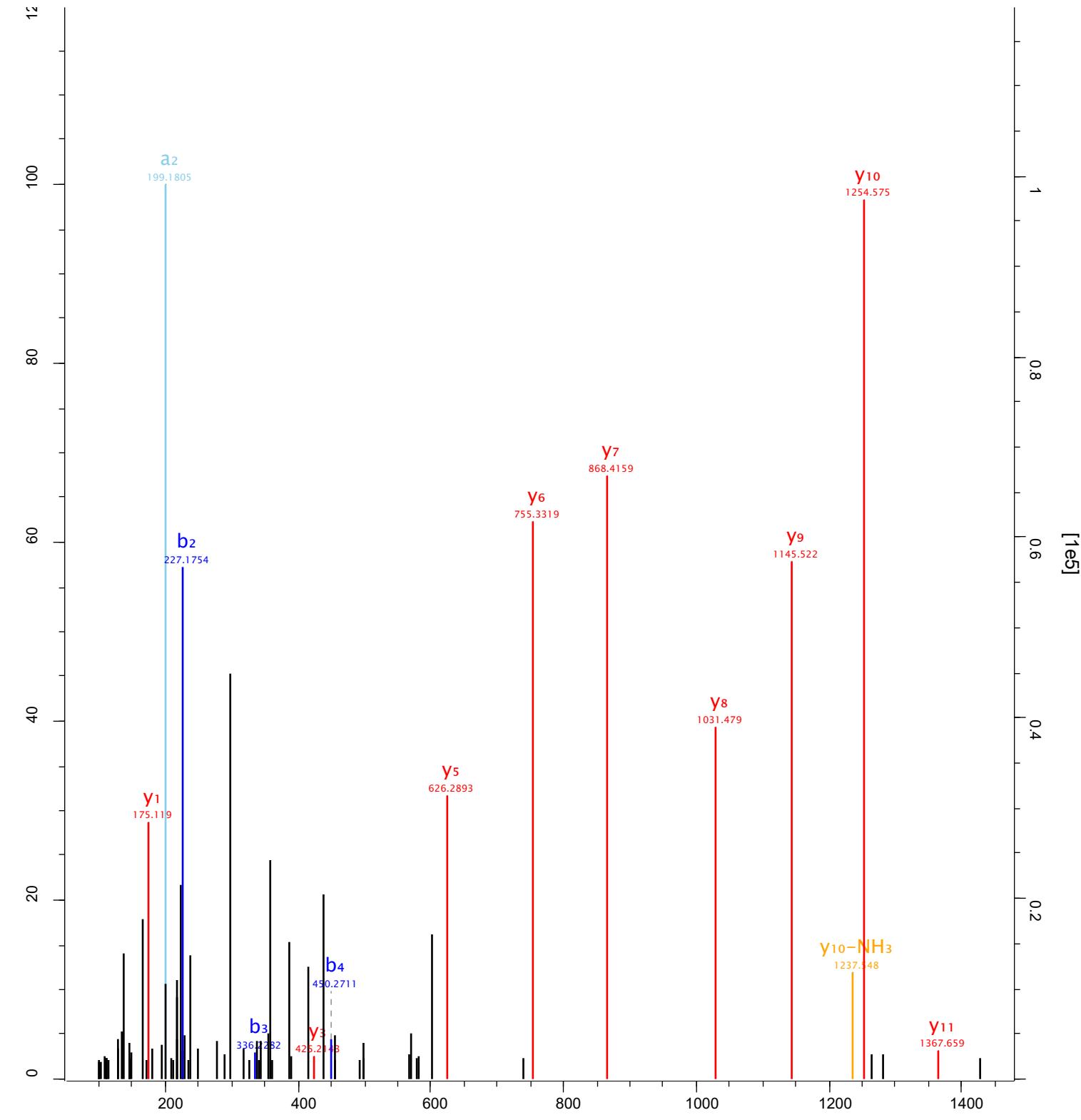


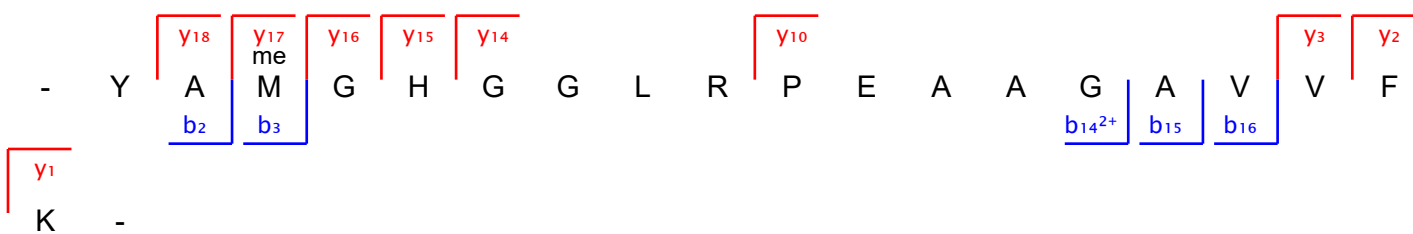
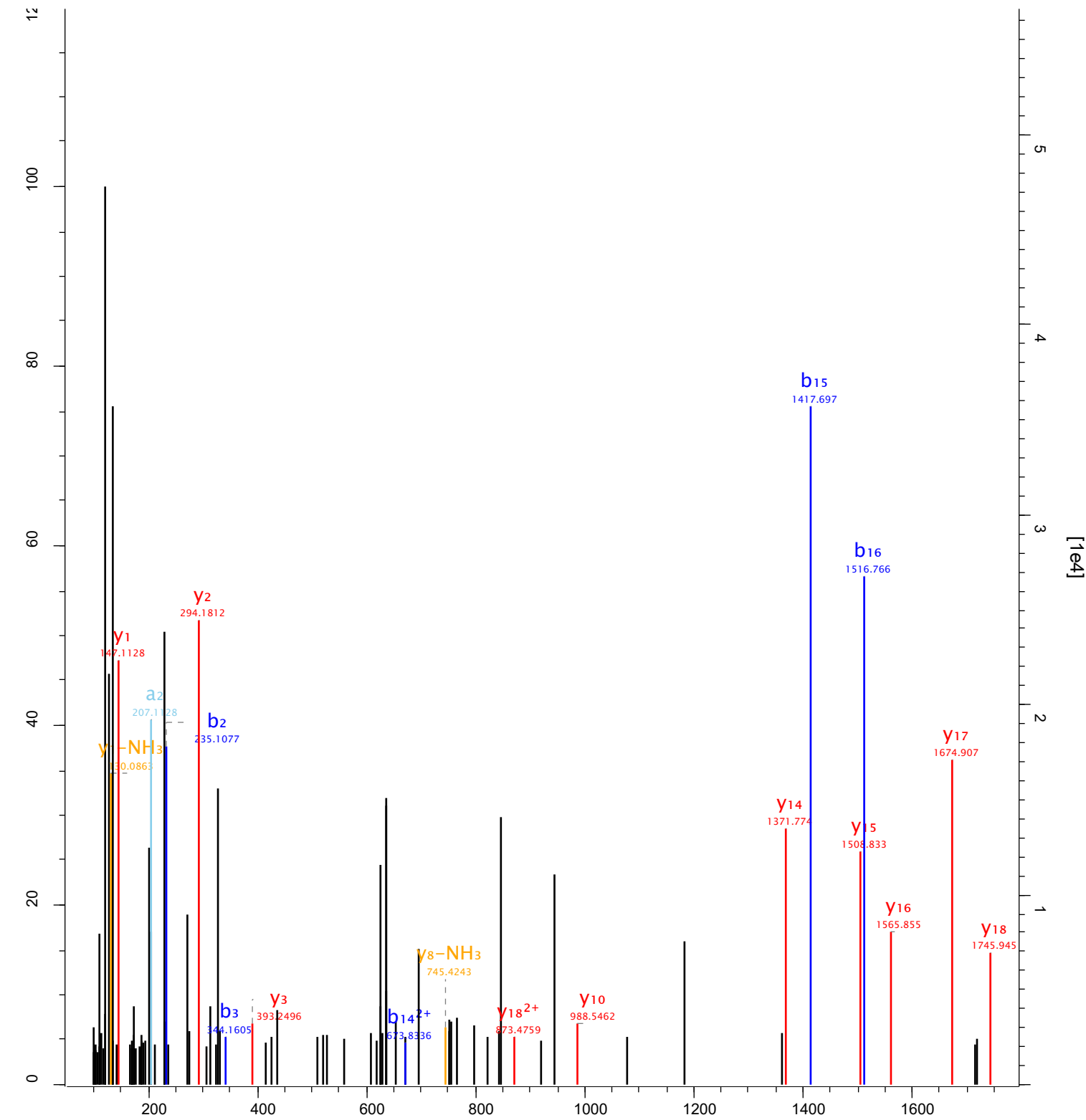
- S P M A G Q L E T F G G I T P K -

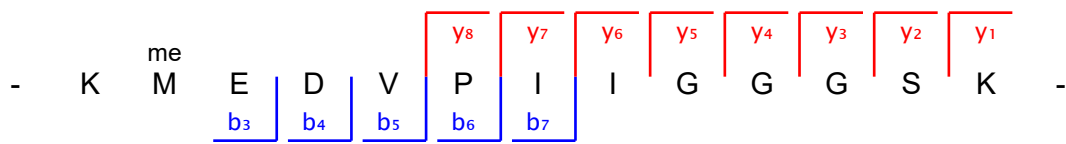
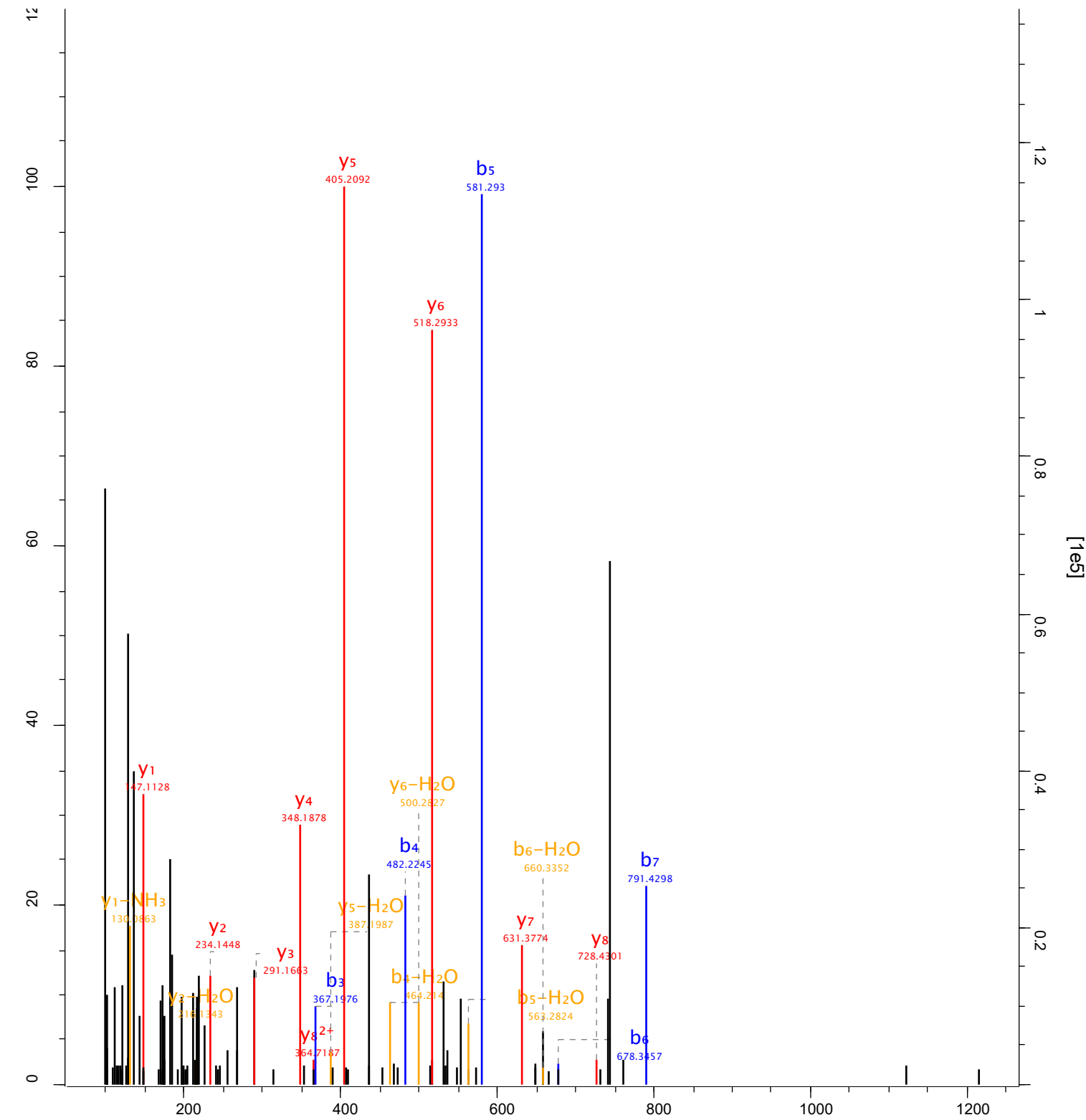
Fragmentation mapping (b-ions in blue, y-ions in red):

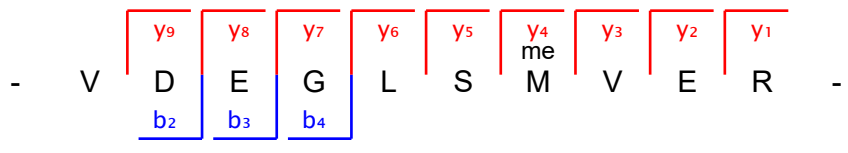
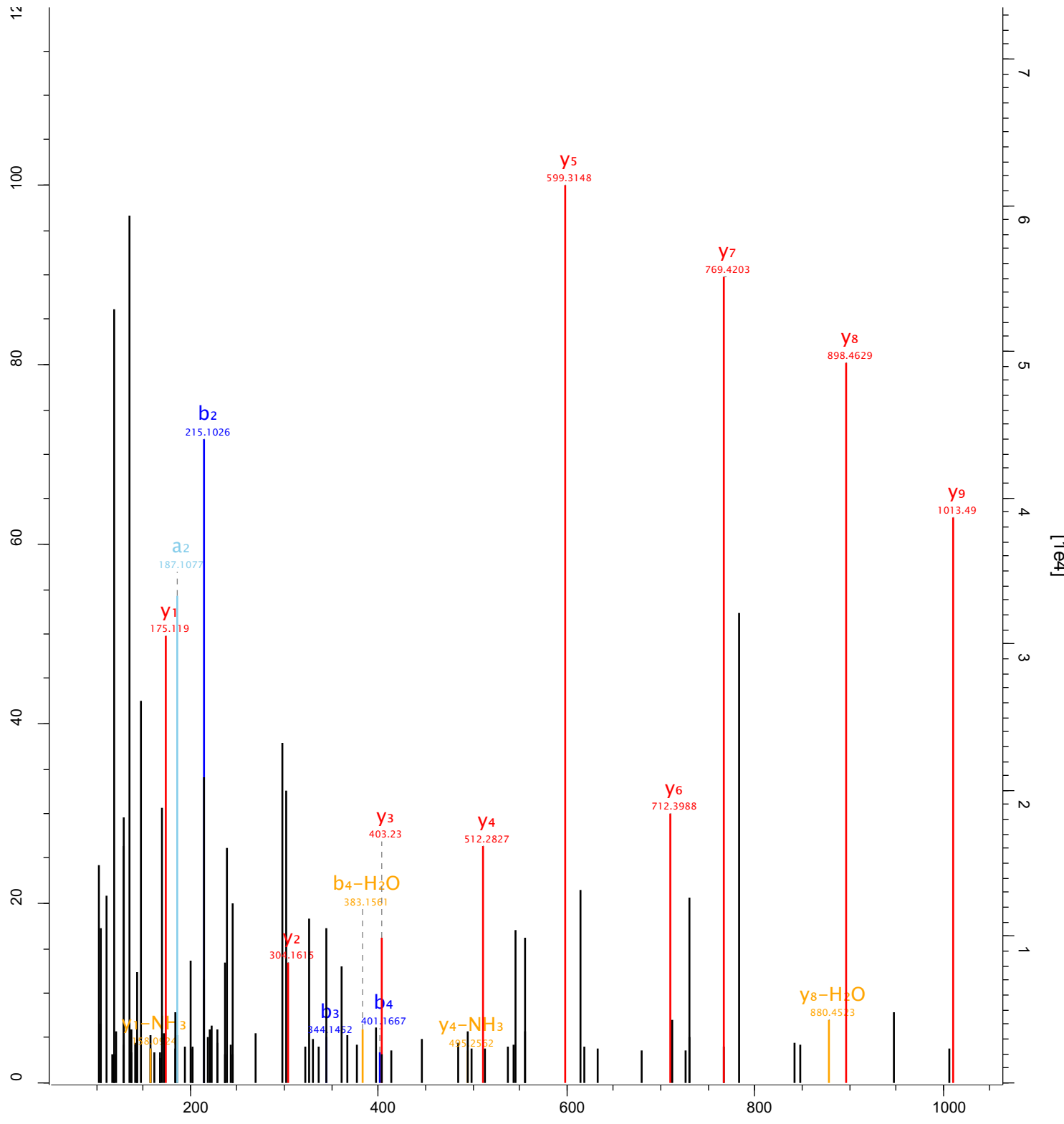
- b₂ (P)
- b₃ (M)
- b₄ (A)
- y₁₃ (A)
- y₁₂ (G)
- y₁₀ (L)
- y₉ (E)
- y₆ (F)
- y₃ (I)
- y₂ (T)

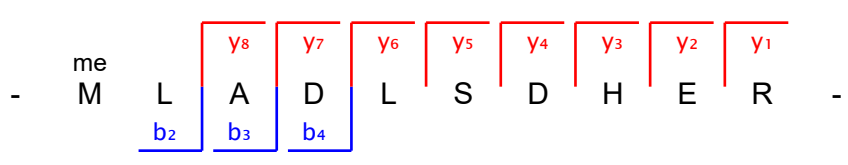
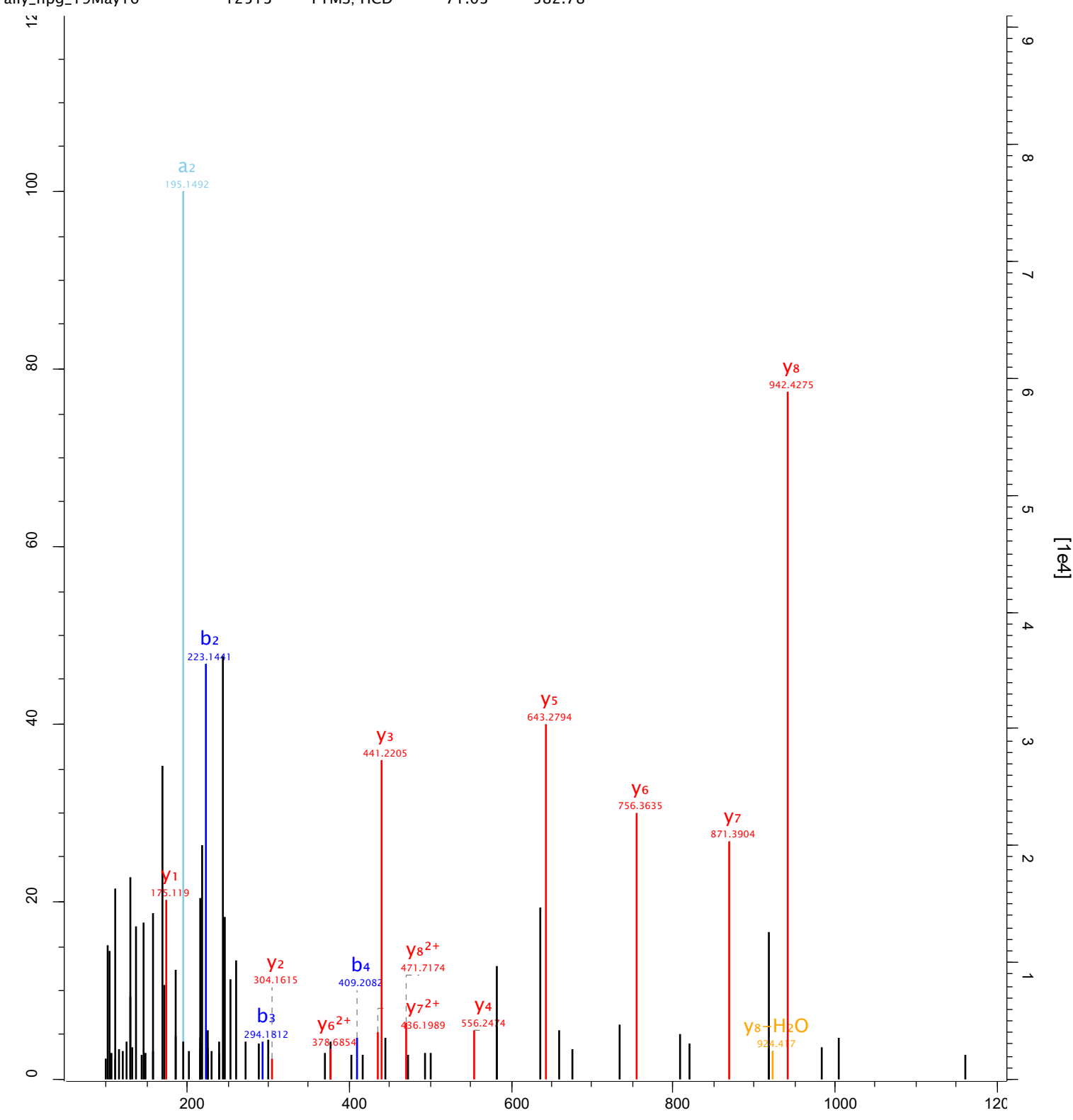


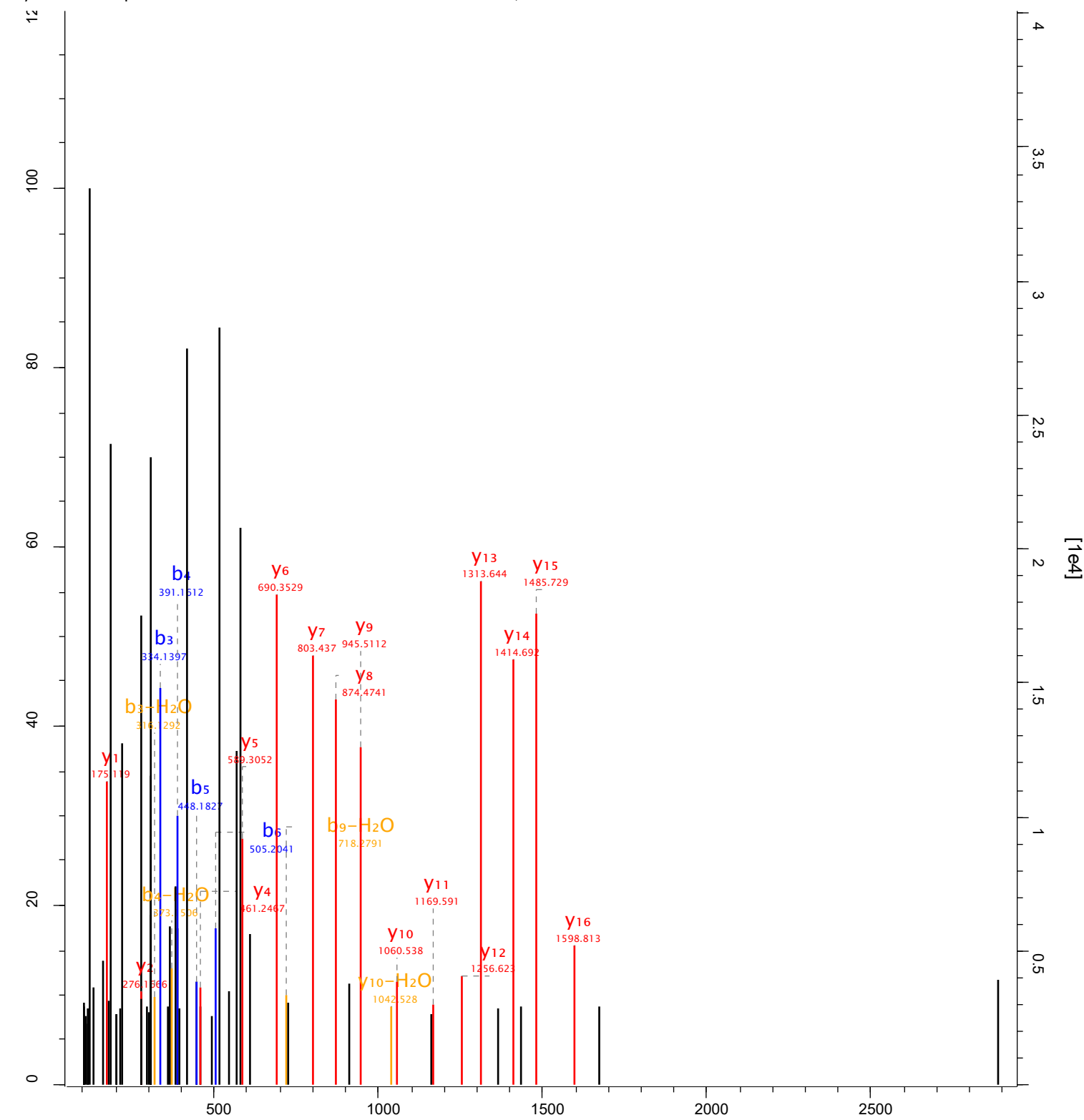












ac
 - S G F G G G S S G A G S L T Q L L A
 b3 b4 b5 b6
 y14 y13 y12 y11 y10 y9 y8 y7 y6 y5 y4
 T G S M D A A L T Q N A T R -
 y16 y15

