

Supplemental document to

## Early Pleistocene enamel proteome sequences from Dmanisi resolve *Stephanorhinus* phylogeny

General remark.

This document contains a selection of MS/MS spectra, and the related b and y fragment ion series, supporting the identification of the peptides covering the positions of phylogenetic interest (SAPs) in the enamel proteome of *Stephanorhinus* specimen Dm.5/157-16635. Such positions are marked by gray bars, with amino acid coordinates provided at the top. Note that for each position many other peptides/spectrum matches in either MaxQuant or PEAKS were observed (data not shown).

For each tandem mass spectrum we also provide the "stagetip number - scan number" for easy referral to the raw data. For several spectra we also confirmed the peptide identification through manual annotation and/or synthetic peptides. The manual and automatically annotated spectra are displayed on the same page (top=MaxQuant, bottom>manual/synthetic).

Synthetic peptides and their corresponding ancient spectra are shown at the end of the document. Annotation of the synthetic and ancient peptides is performed manually through the Interactive Peptide Spectral Annotator freeware.

For manual annotation, annotation abbreviations are as follows:

$y/b^{\circ} = y/b-H_2O$

$y/b^* = y/b-NH_3$

$y^{\Delta} = y-H_3PO_4$

$y^{\Delta\Delta} = y-2H_3PO_4$

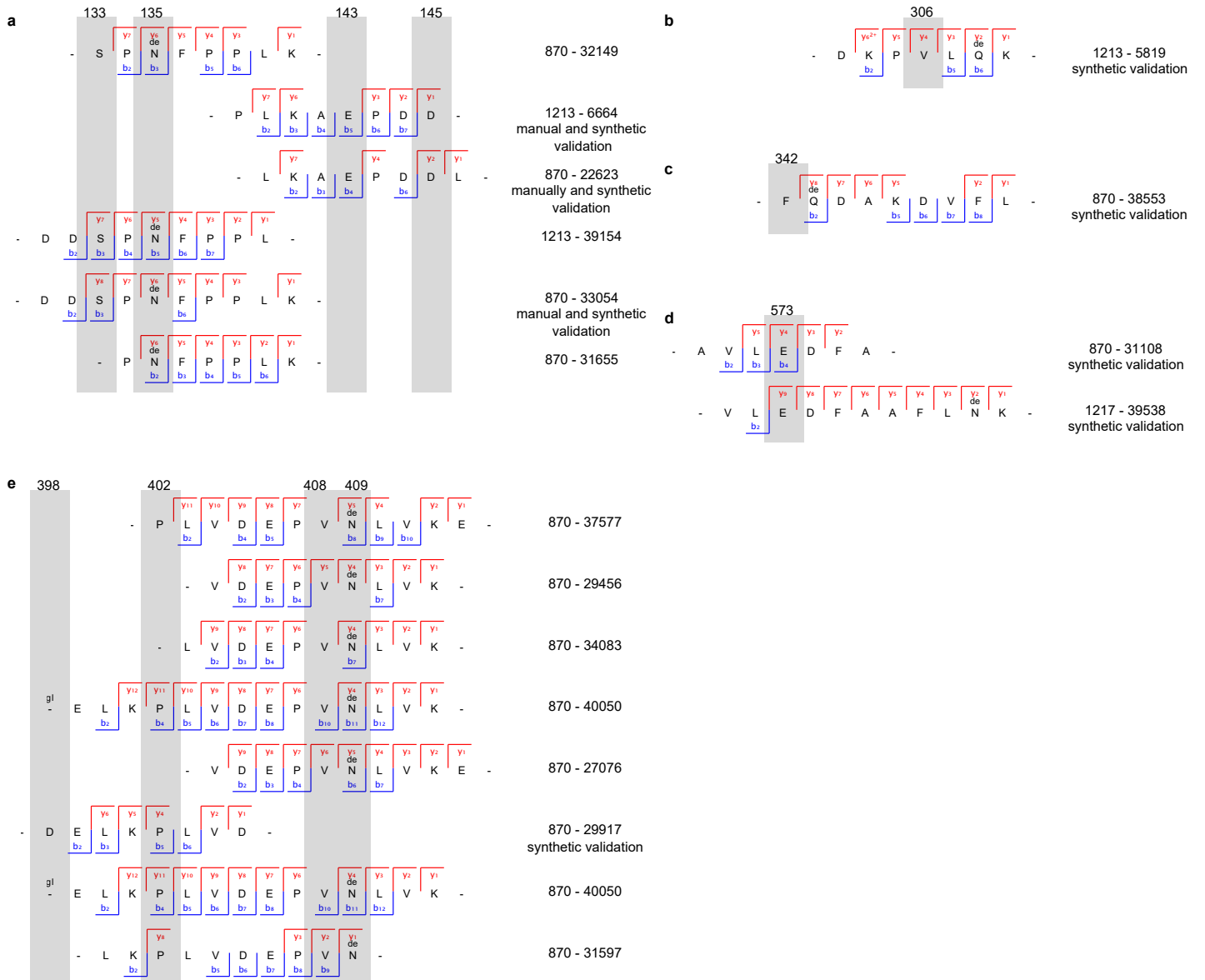
For MQ annotation, annotation abbreviations including a \* indicate the presence of a neutral phosphate loss on the fragment ion (-98Da,  $-H_3PO_4$ ).

Several spectra, including two manually verified, indicate the presence of endogenous phosphorylation.

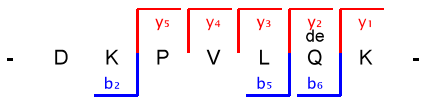
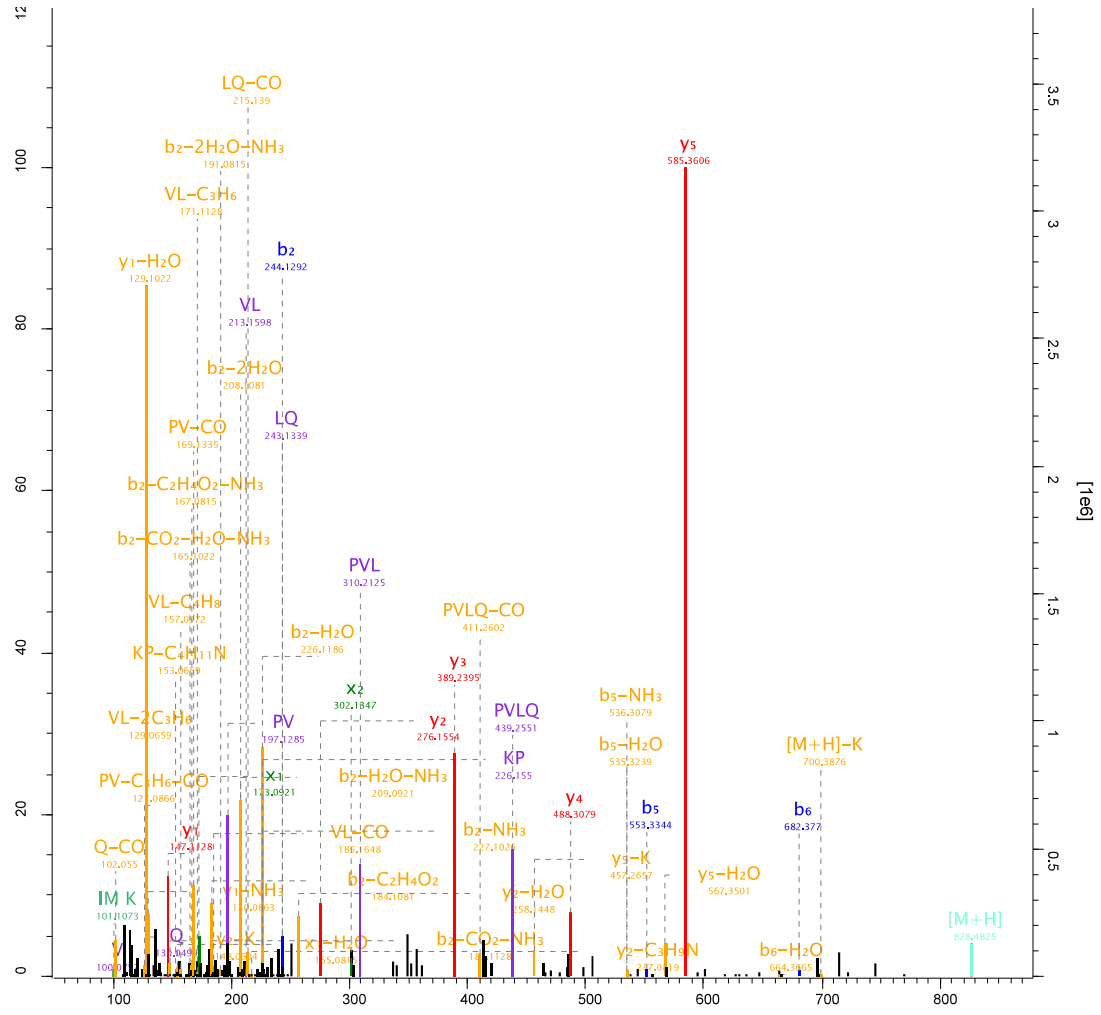
Leucines and isoleucines cannot be differentiated from each other in our MS set-up.

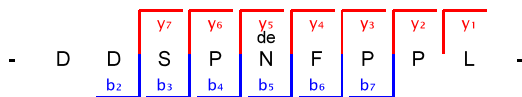
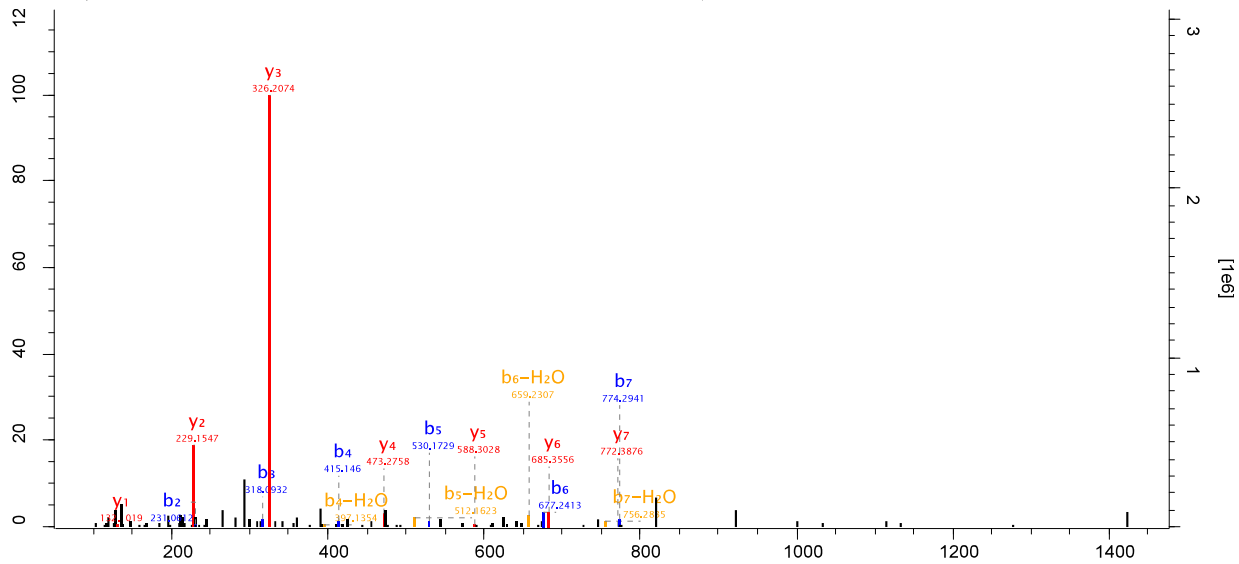
(Stagetip - scan number)

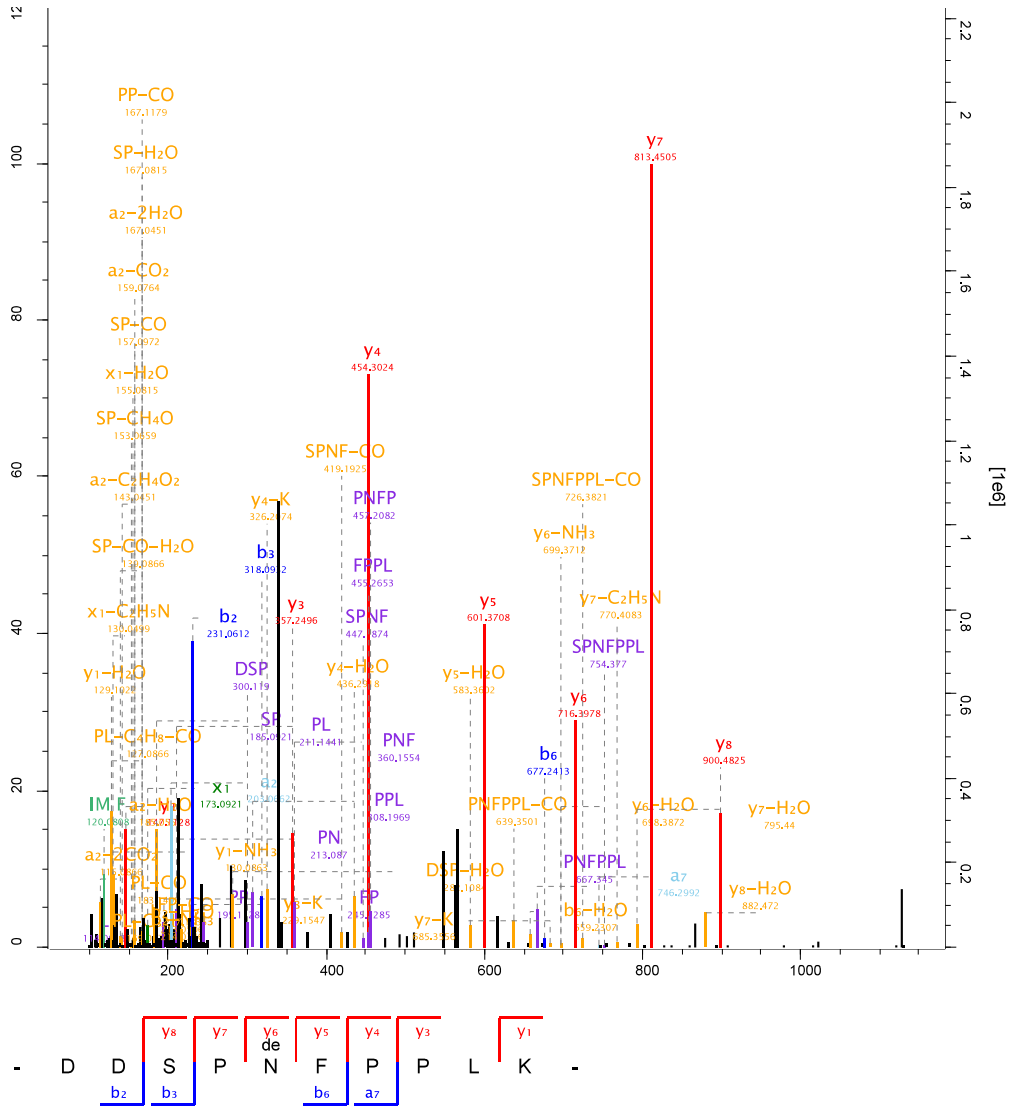
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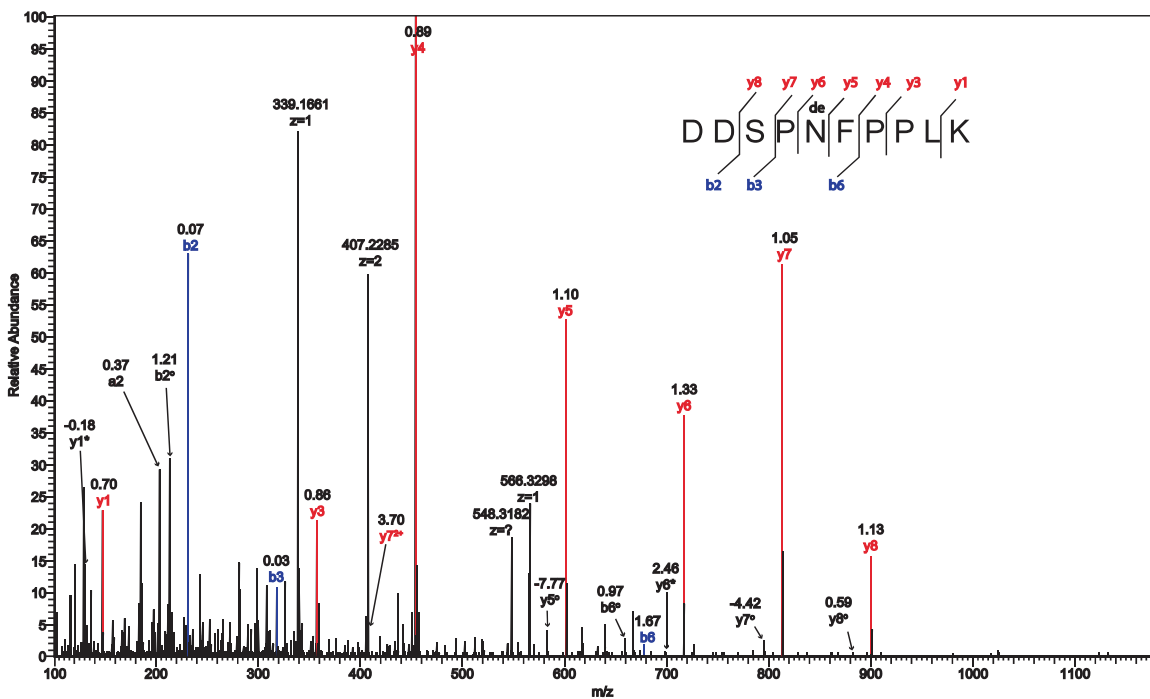
**ALB.** Peptide matches to phylogenetically informative positions 133, 135, 143, 145 (a), 306 (b), 342 (c), 573 (d), and 398, 402, 408, 409 (e). Coordinates in reference to XP\_004419220.

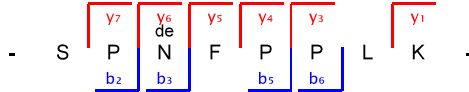
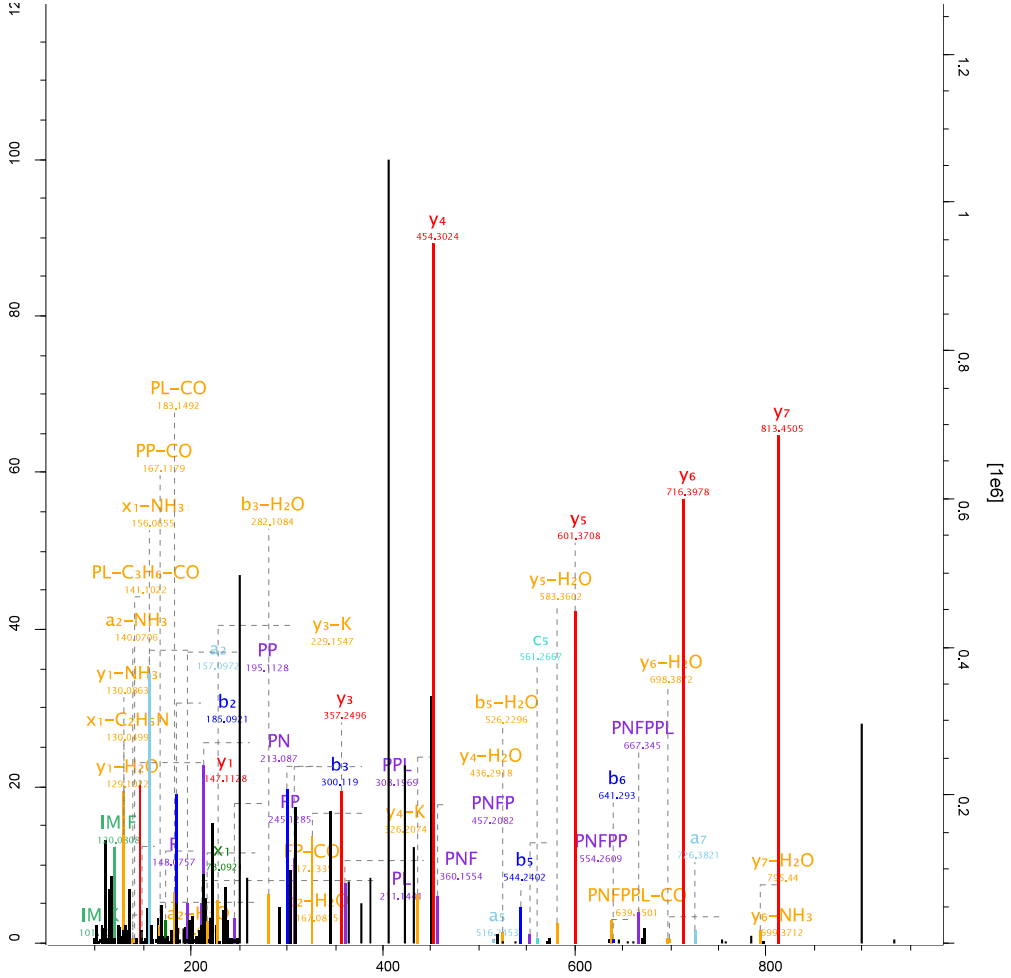


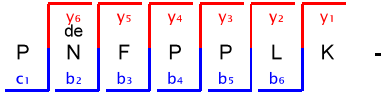
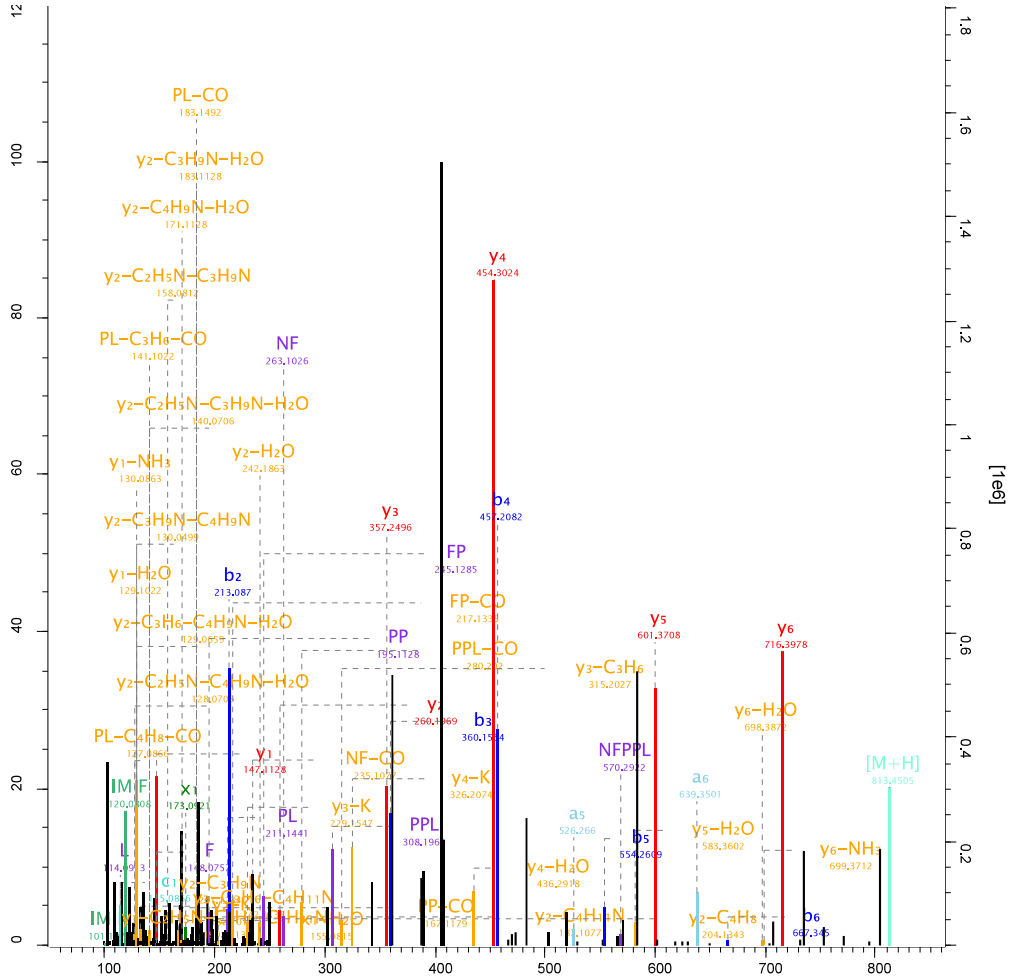


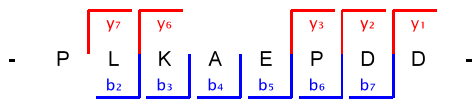
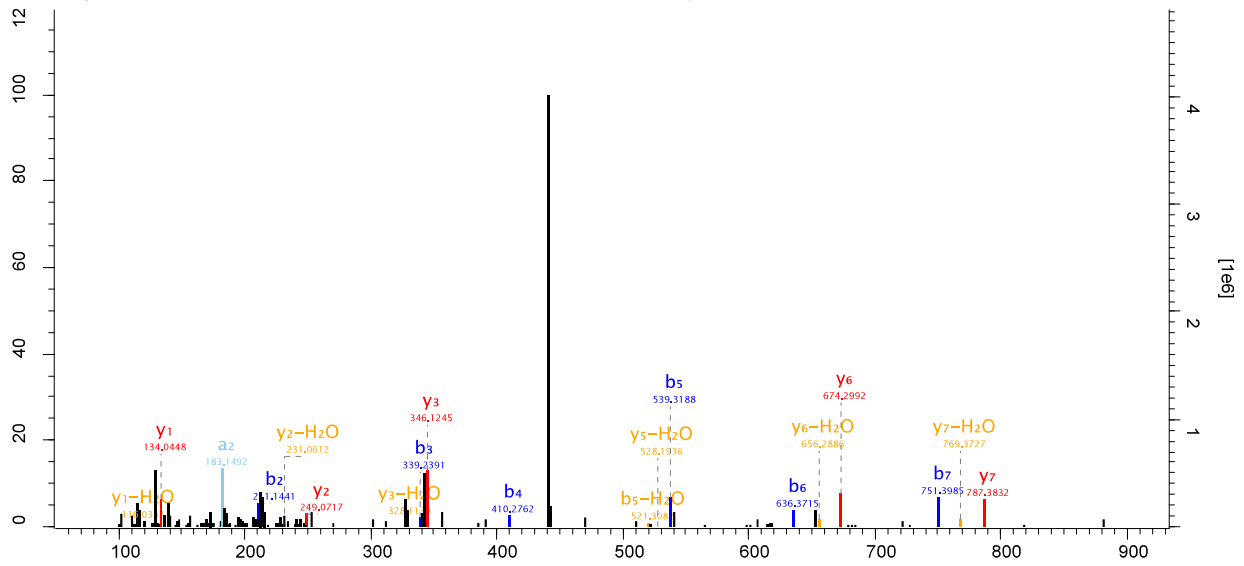


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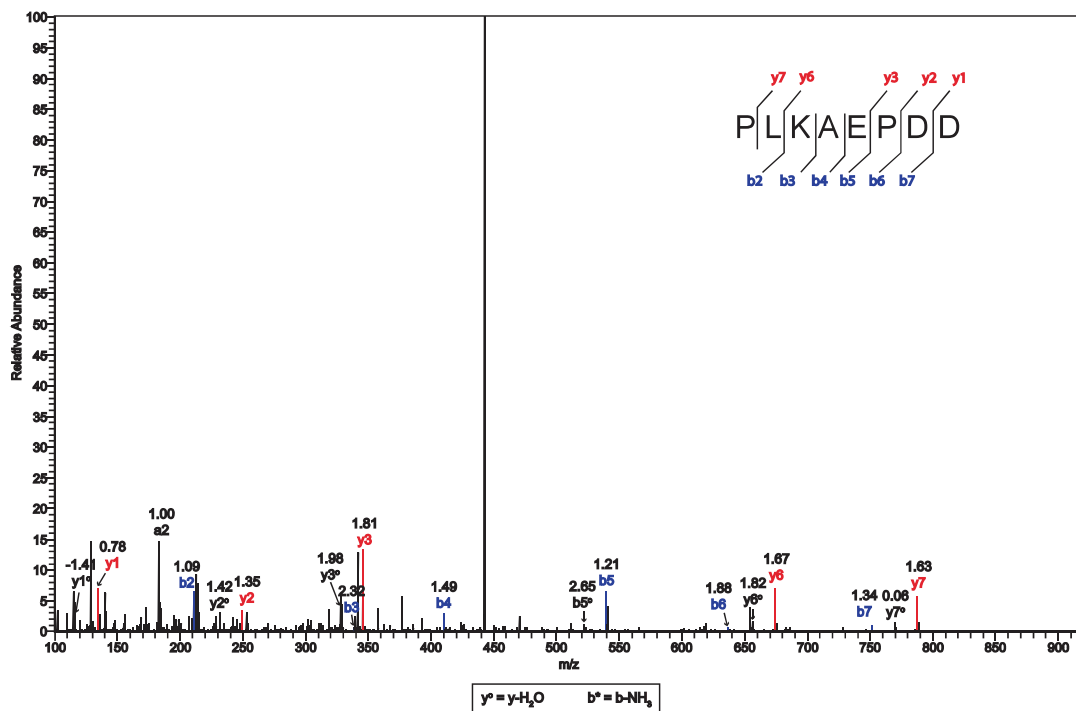




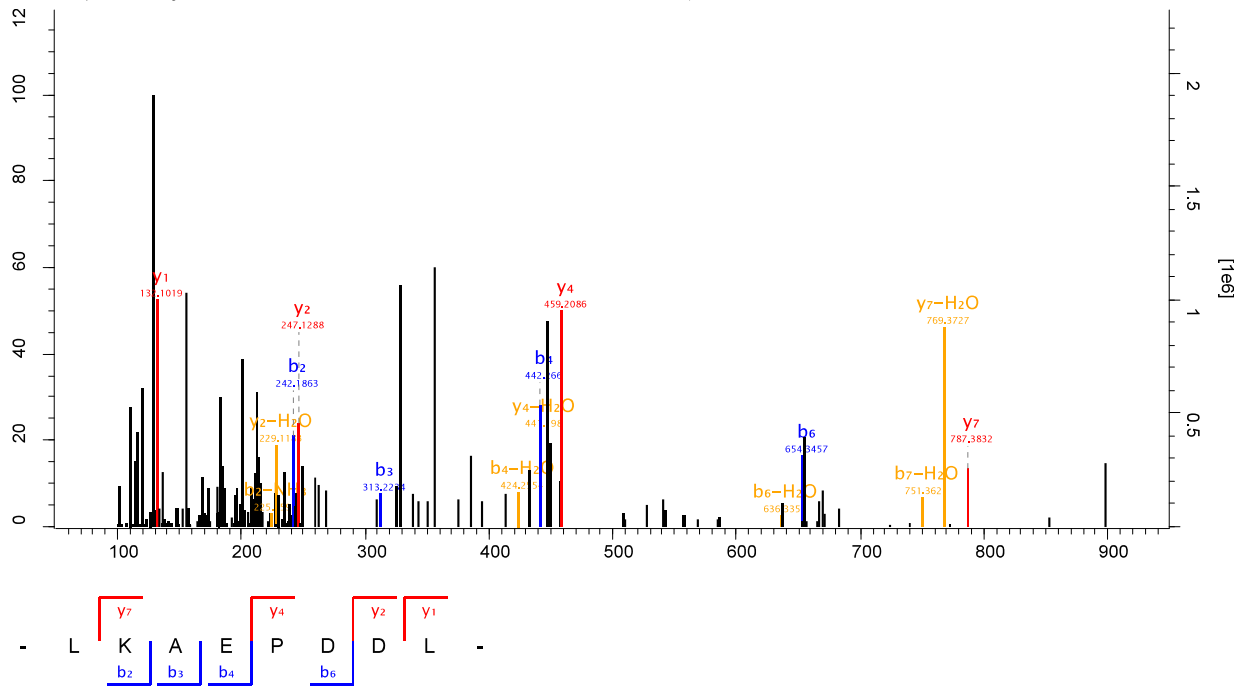




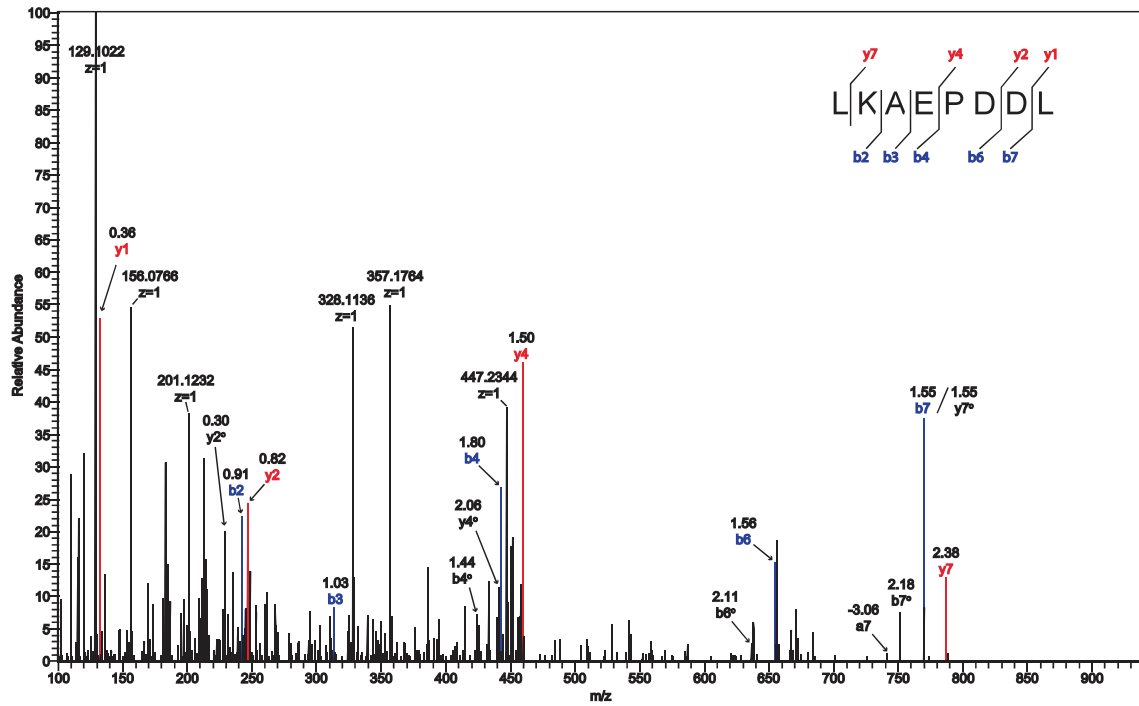
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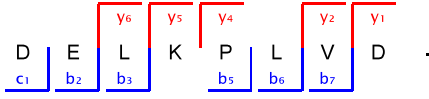
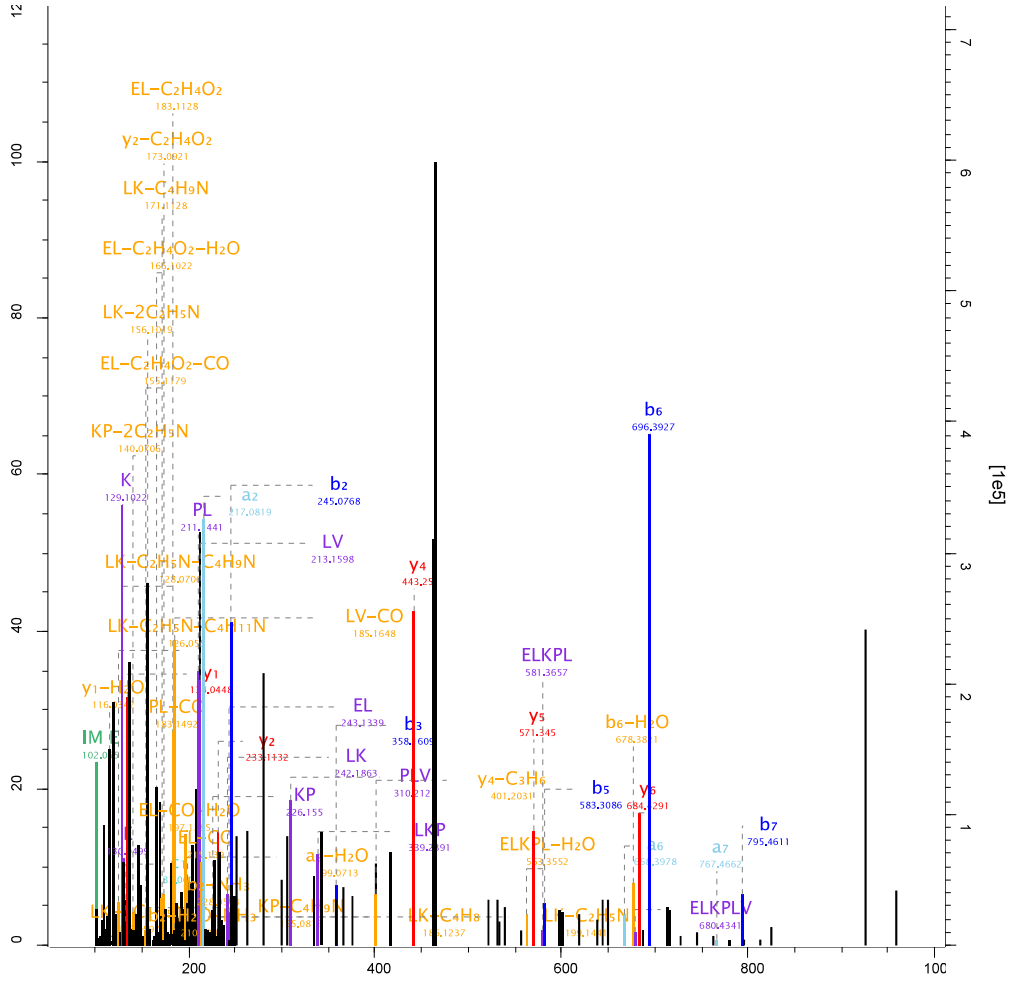


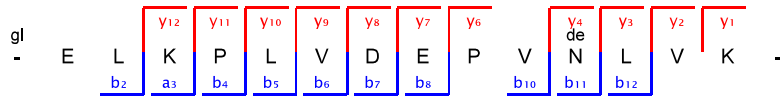
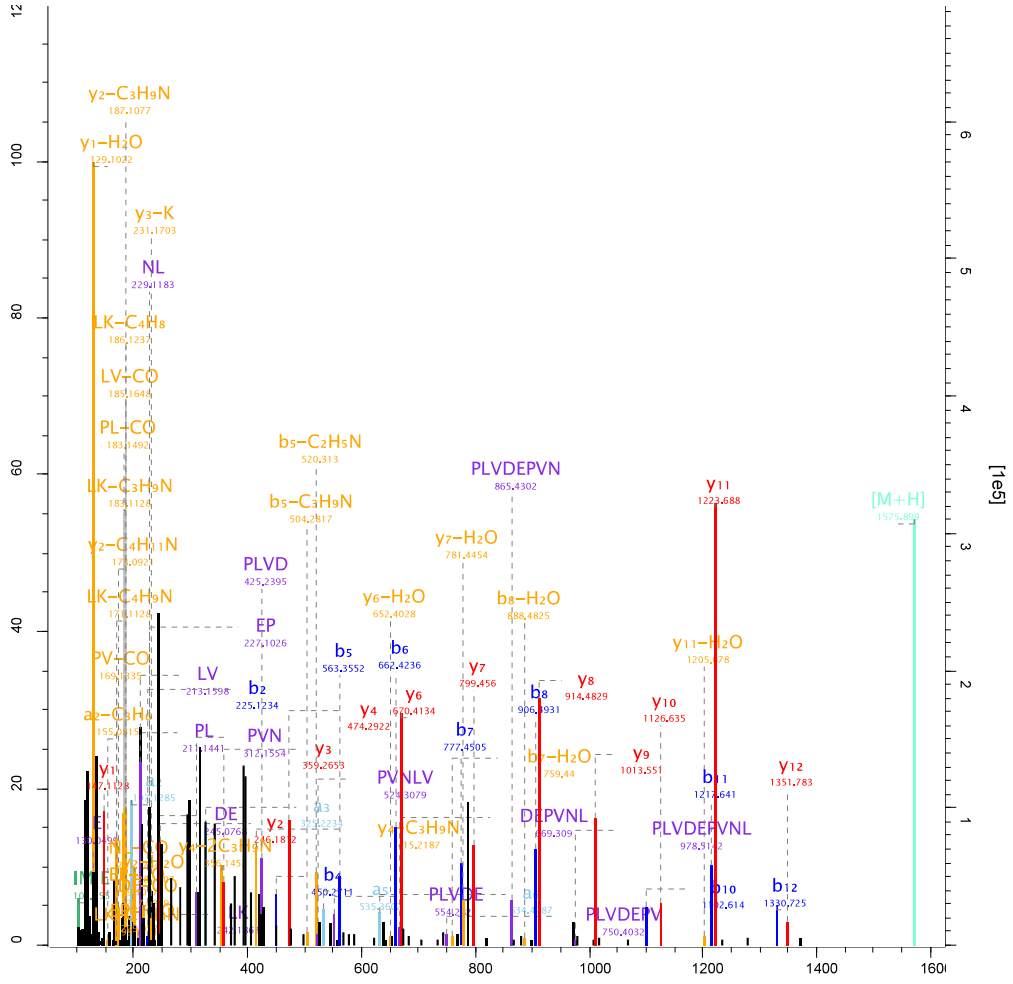


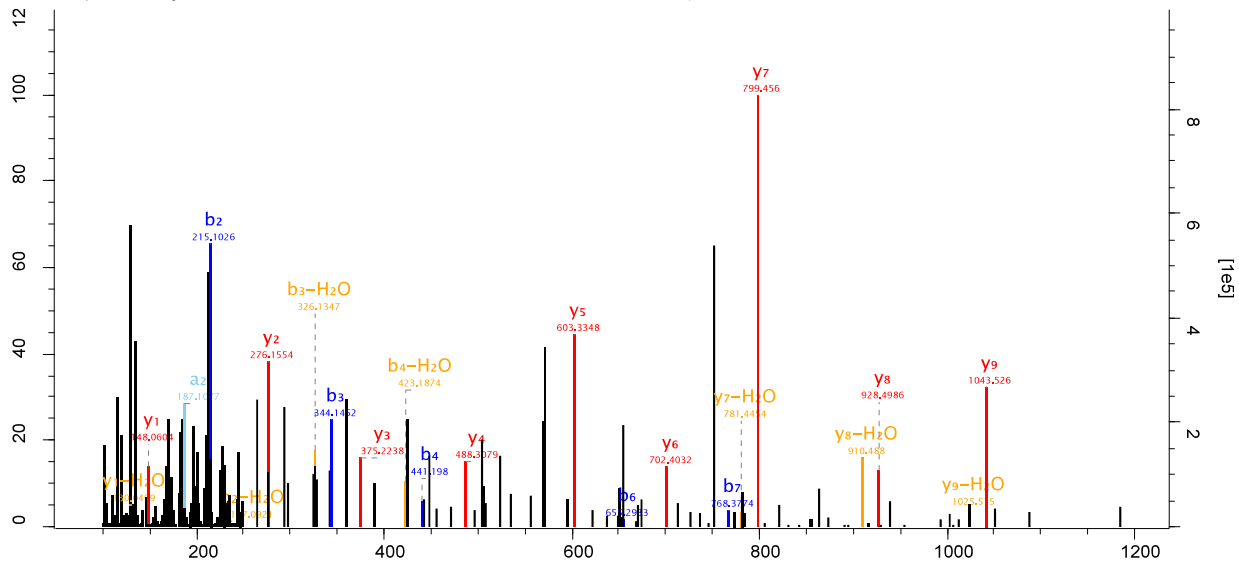


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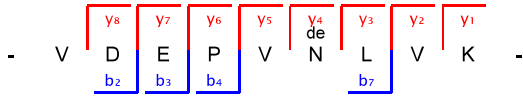
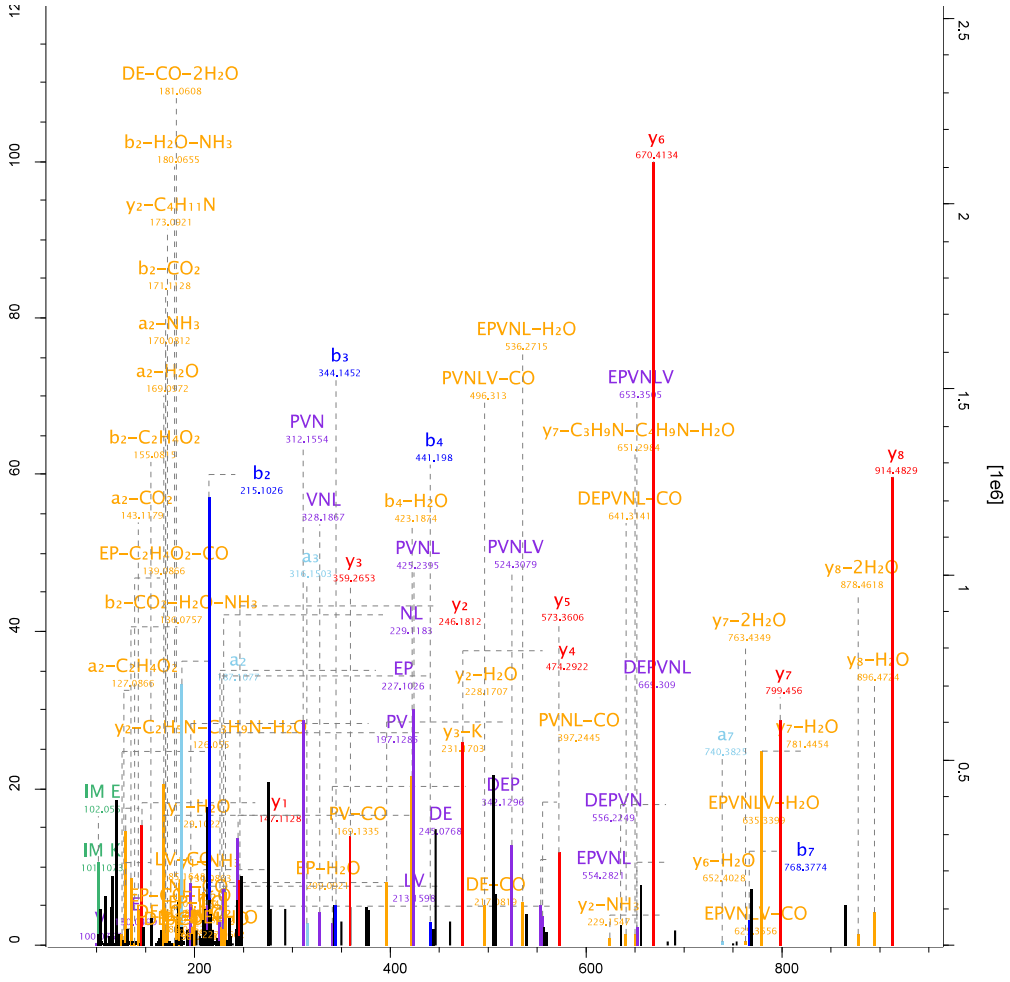


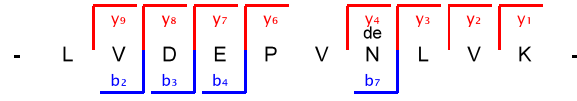
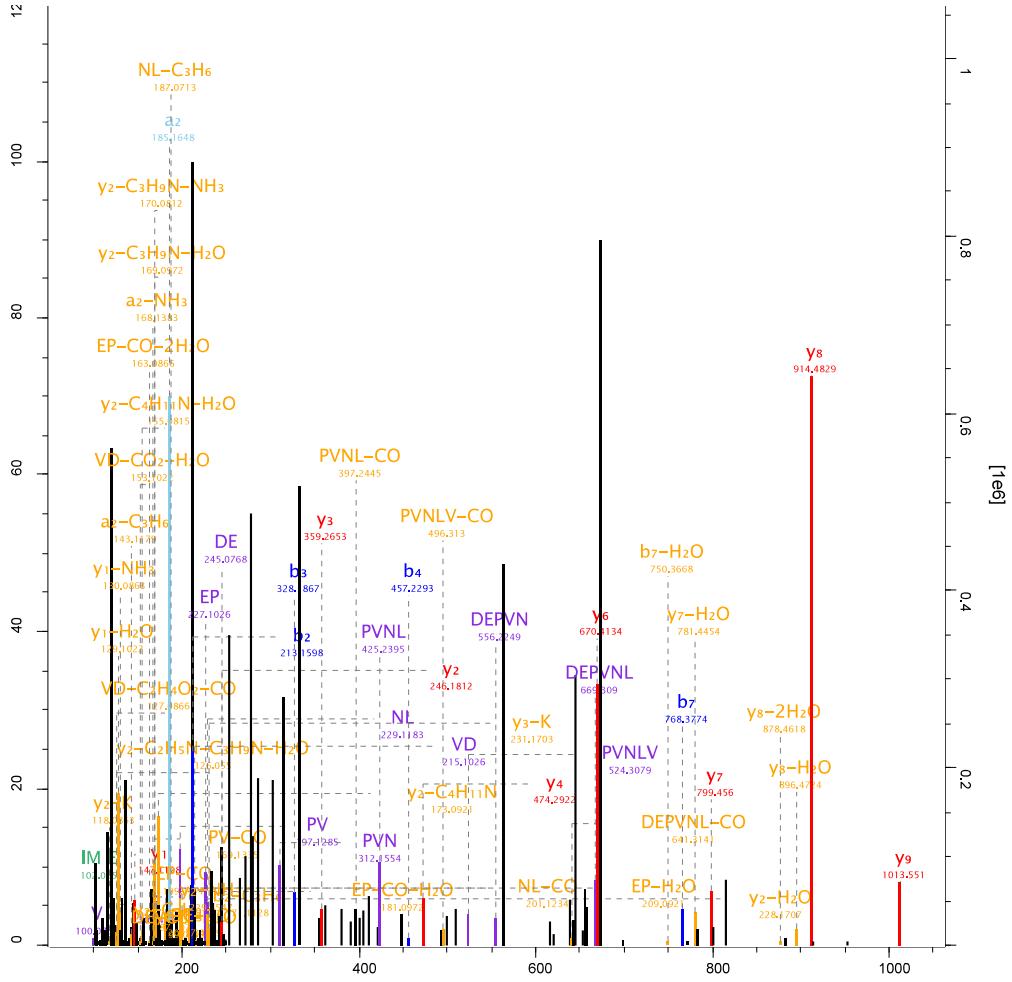


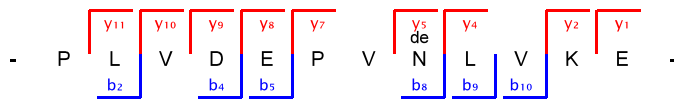
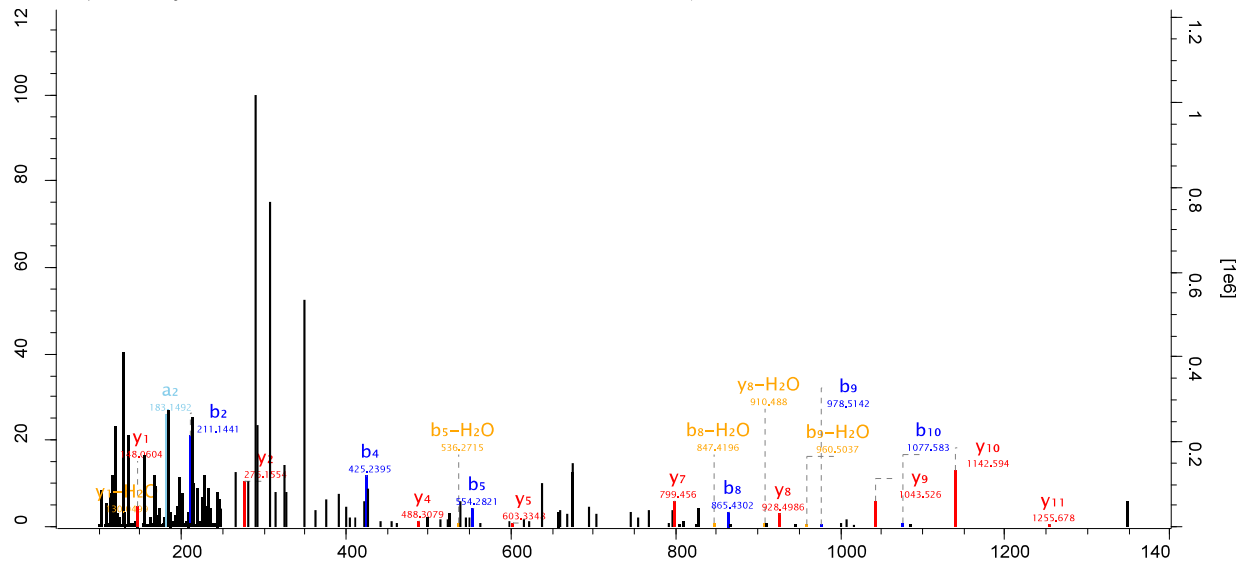


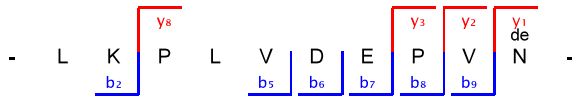
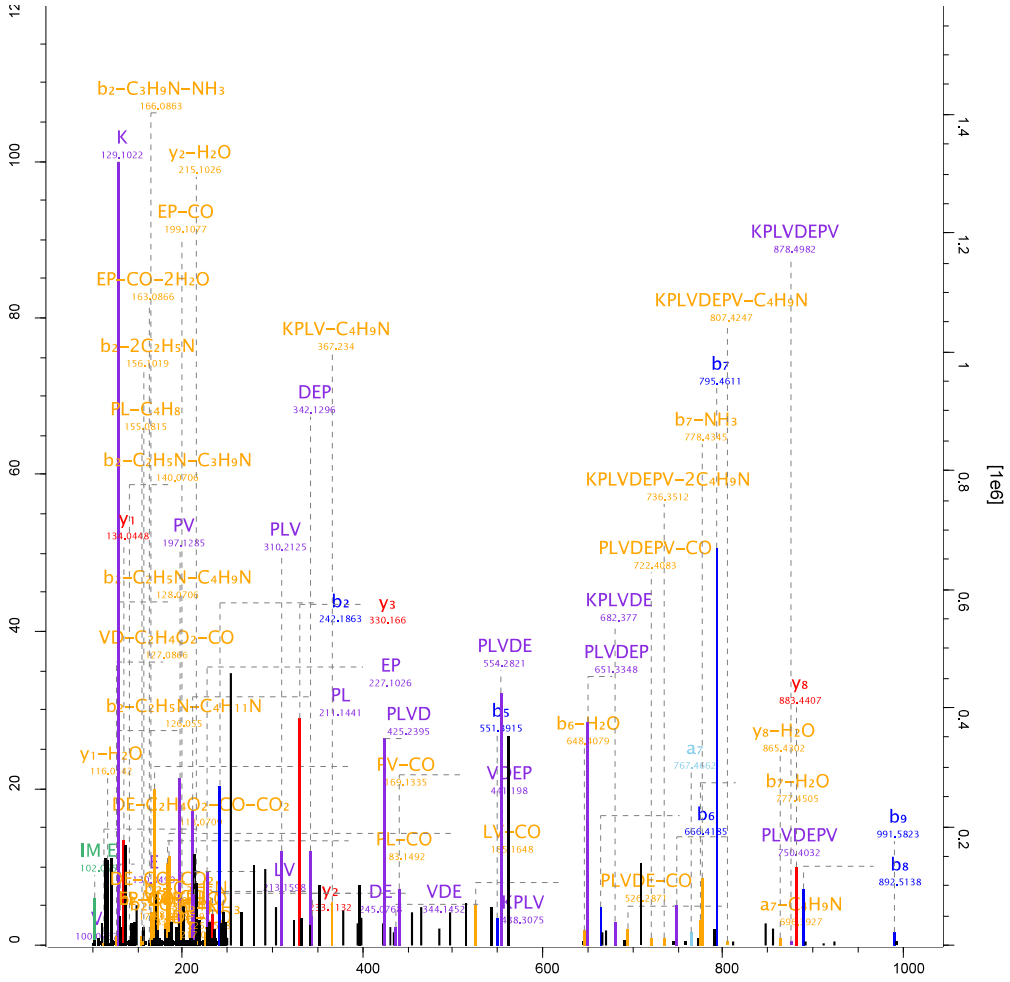


- V D E P V N L V K E -  
b2 b3 b4 b6 b7

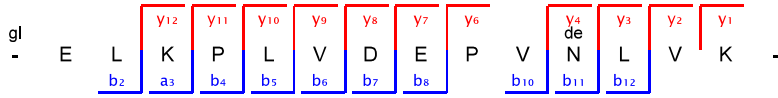
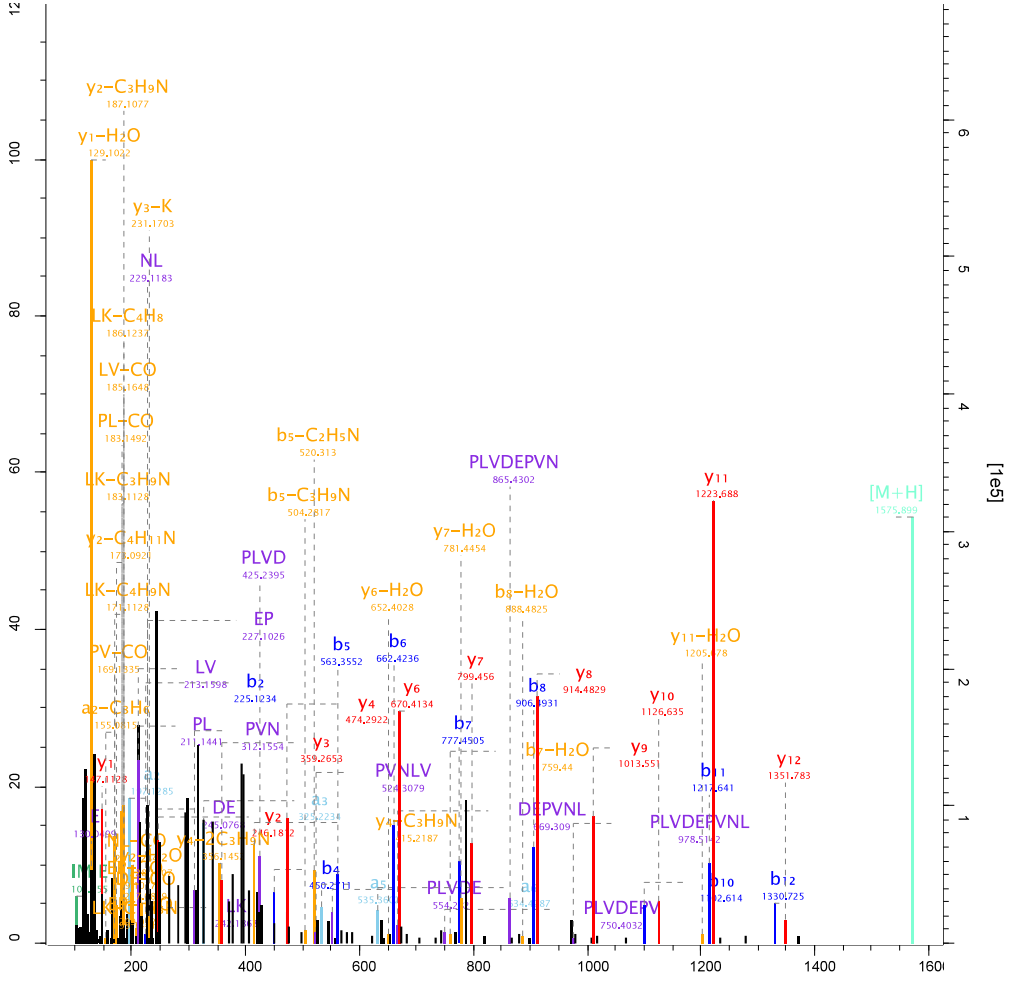


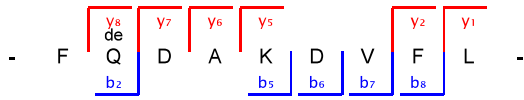
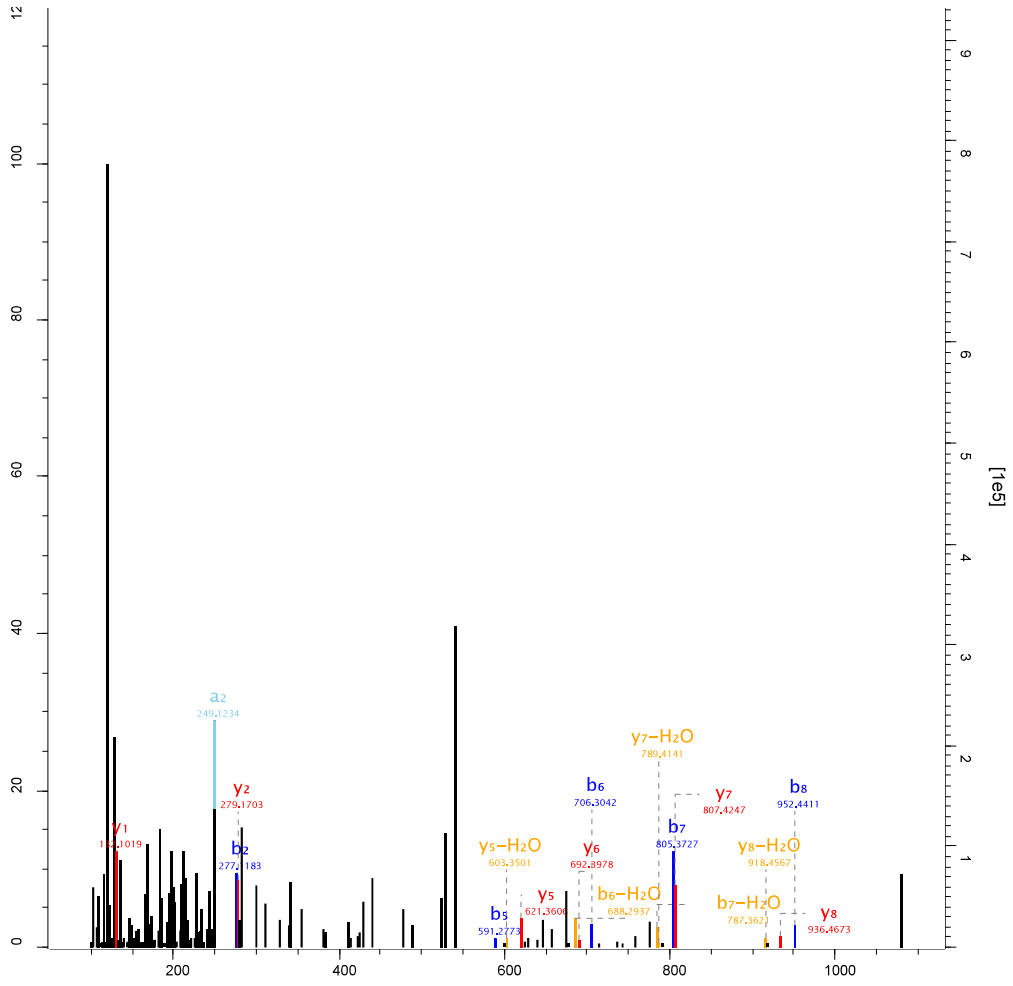


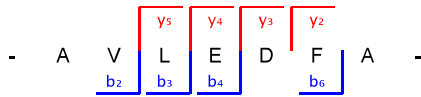
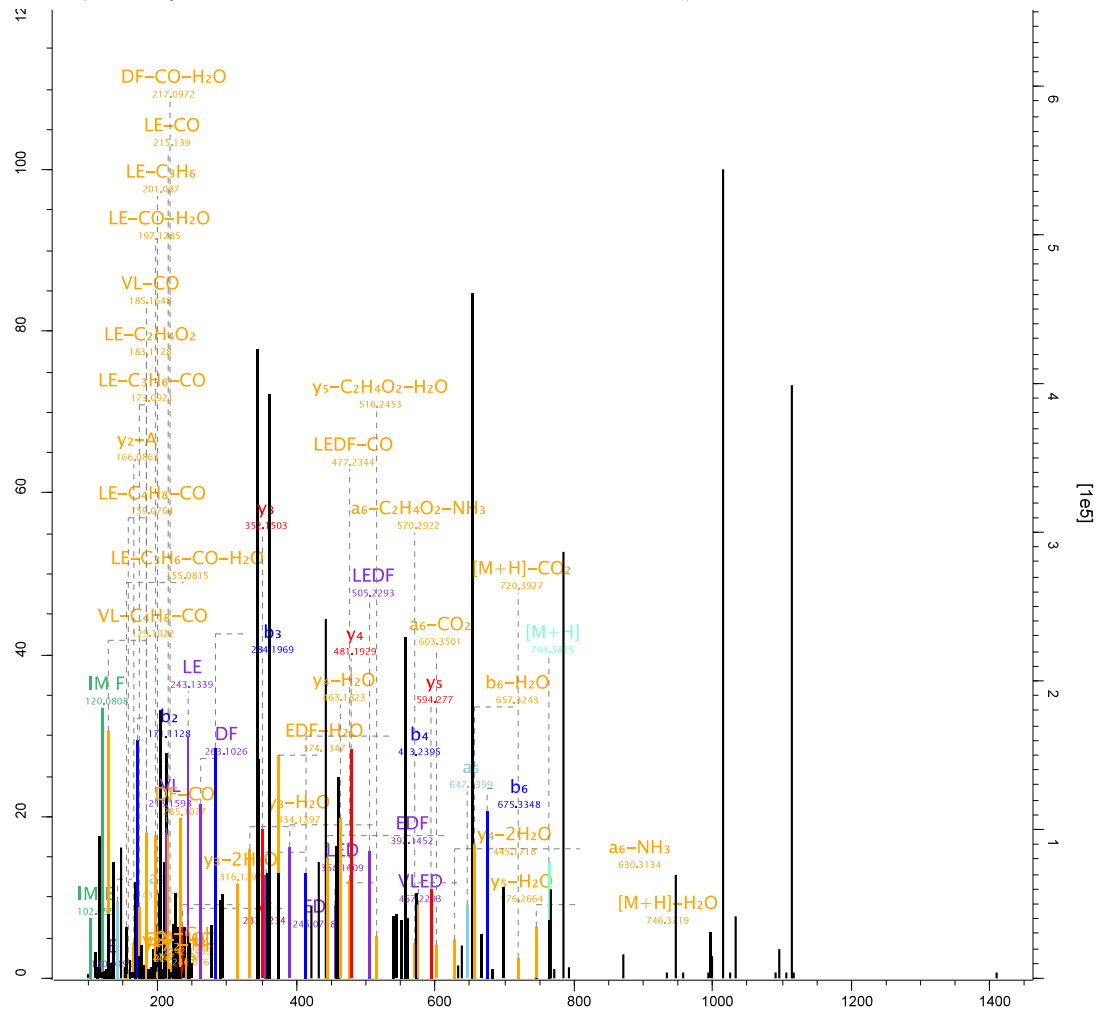


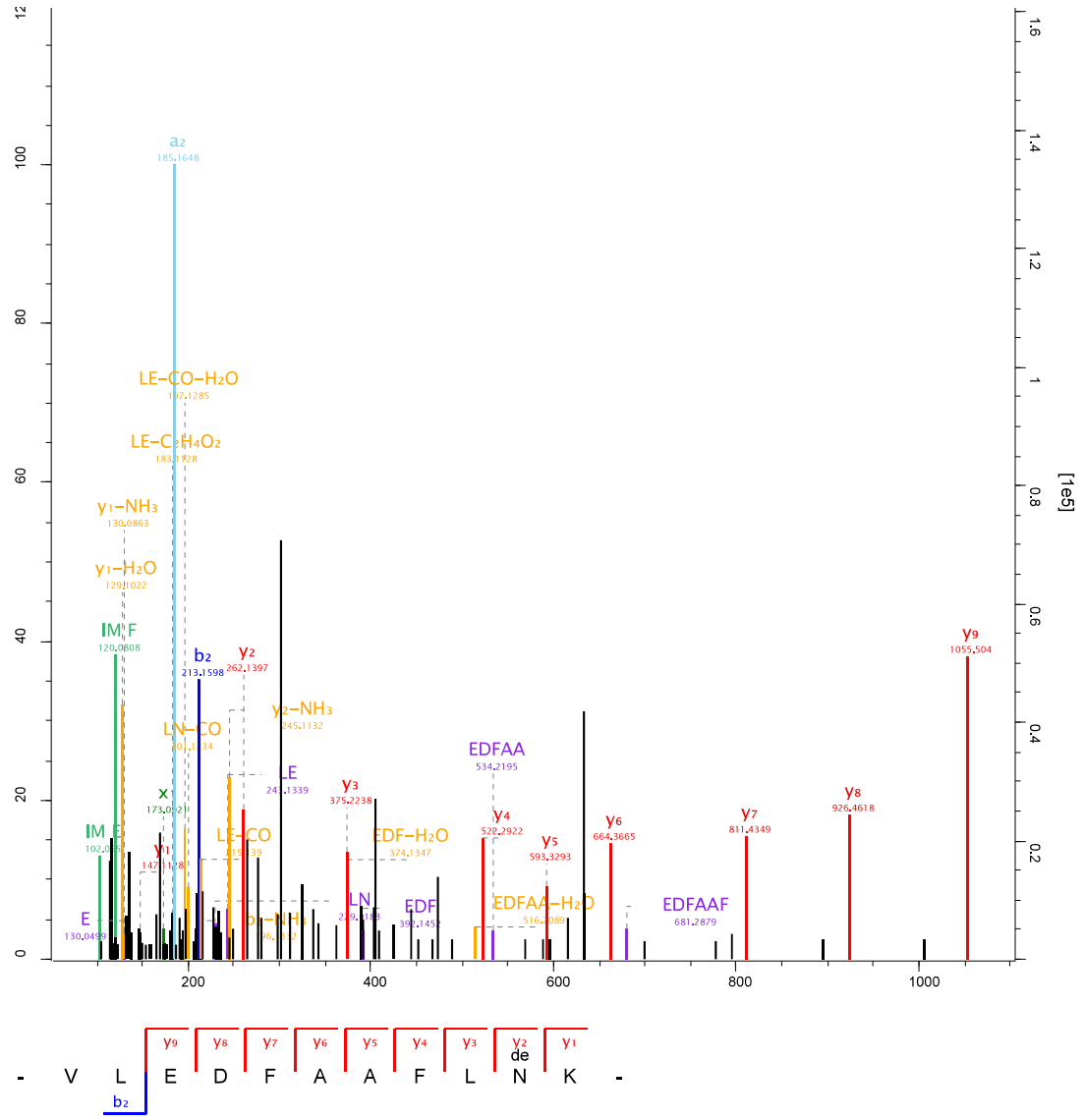








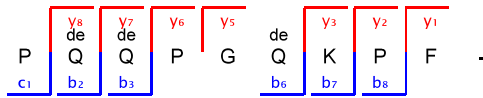
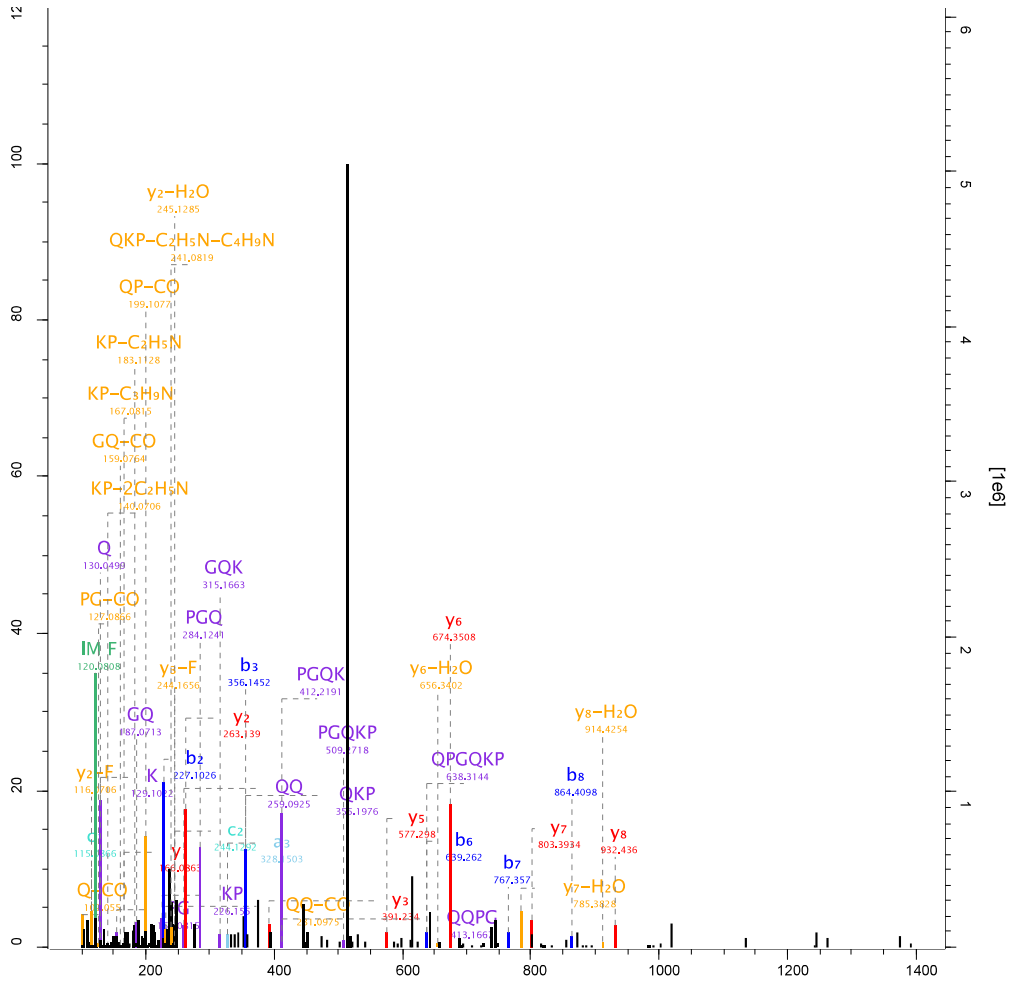


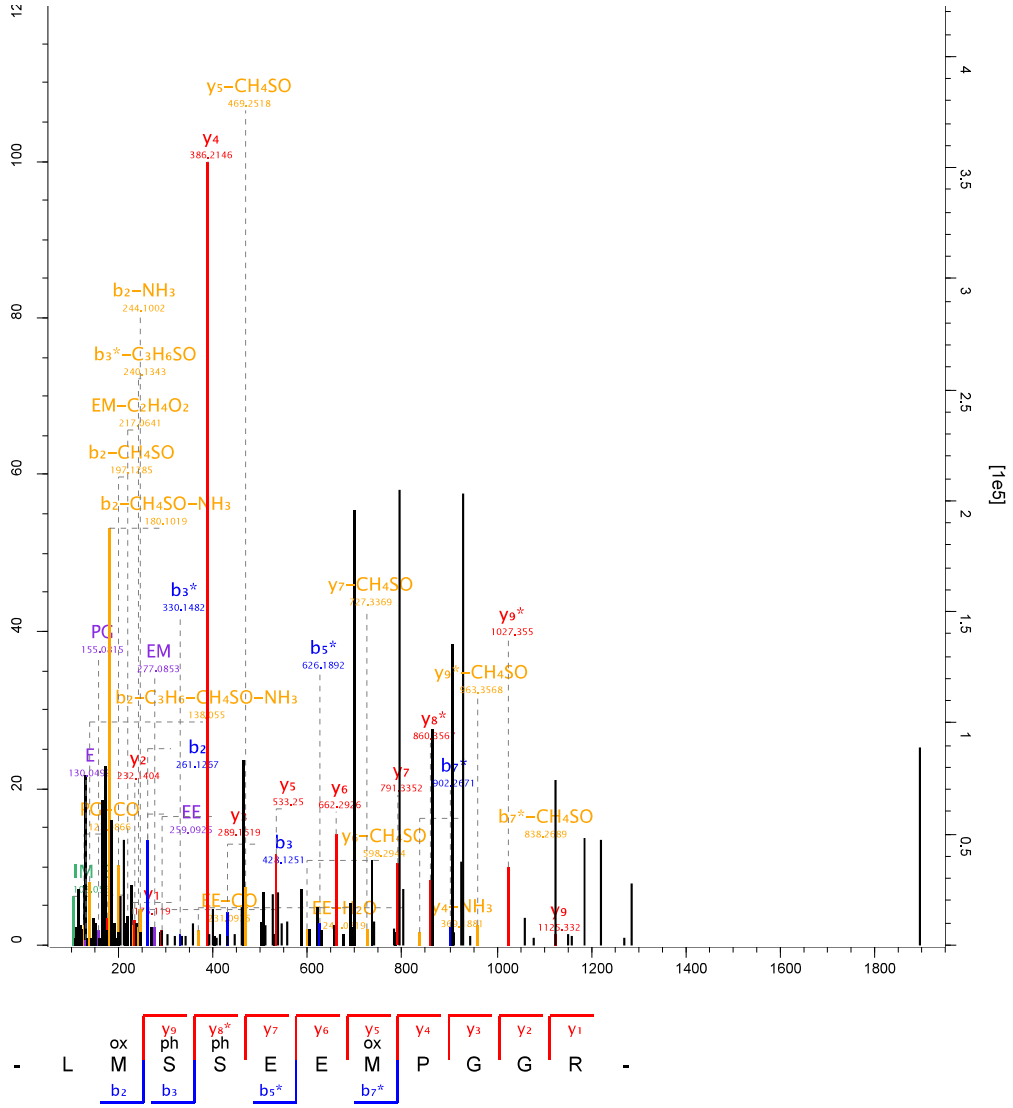


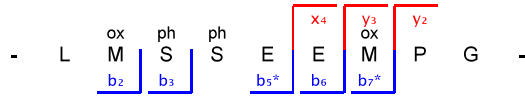
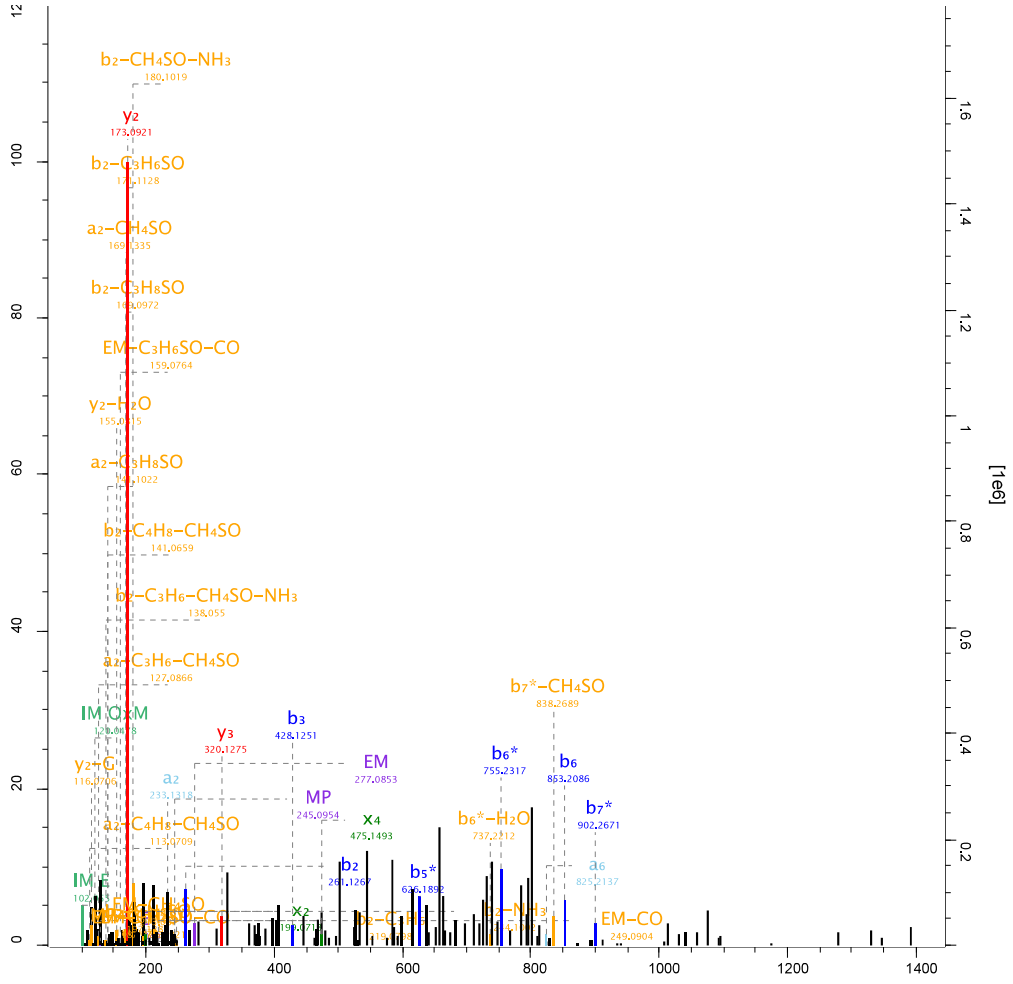
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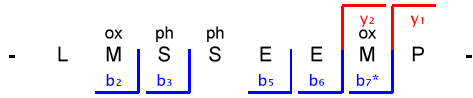
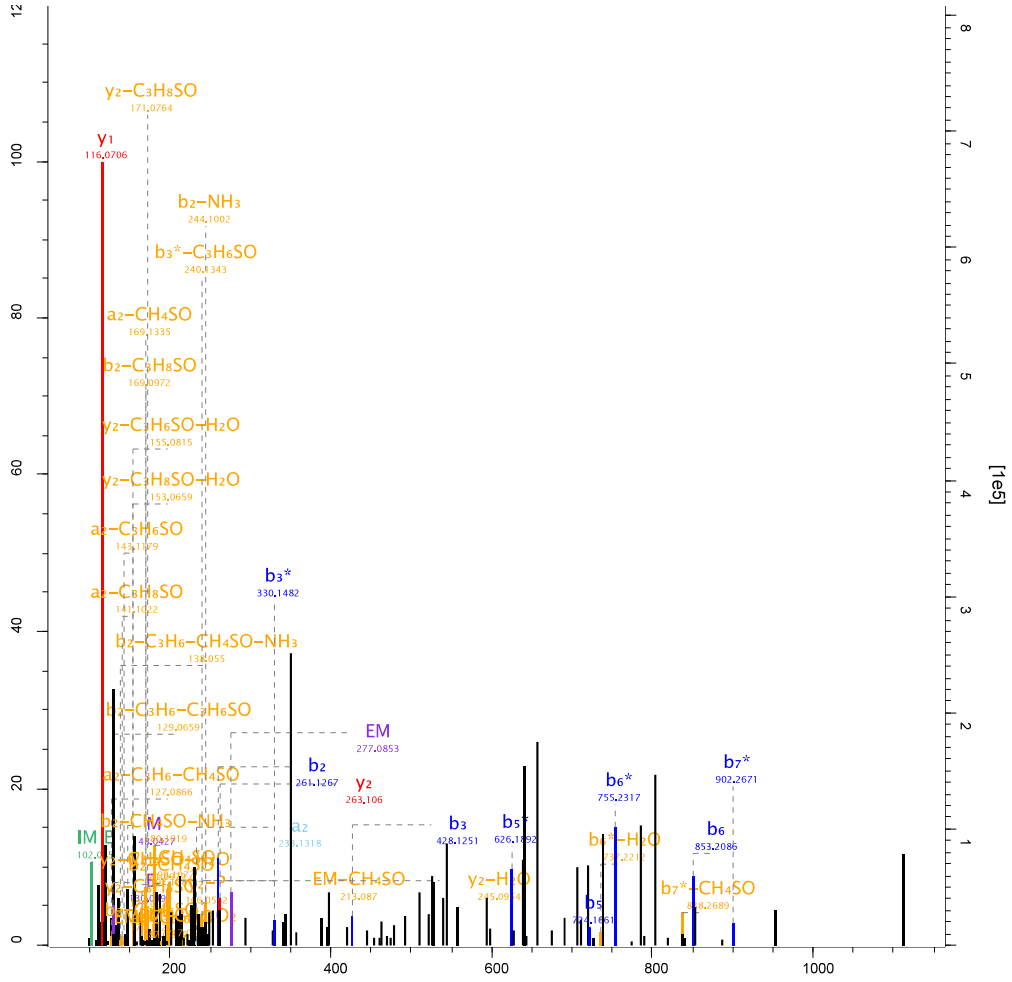
**AMBN.** Peptide matches to phylogenetically informative positions 125 (a), and 241 (b). Coordinates in reference to XP\_004419200.

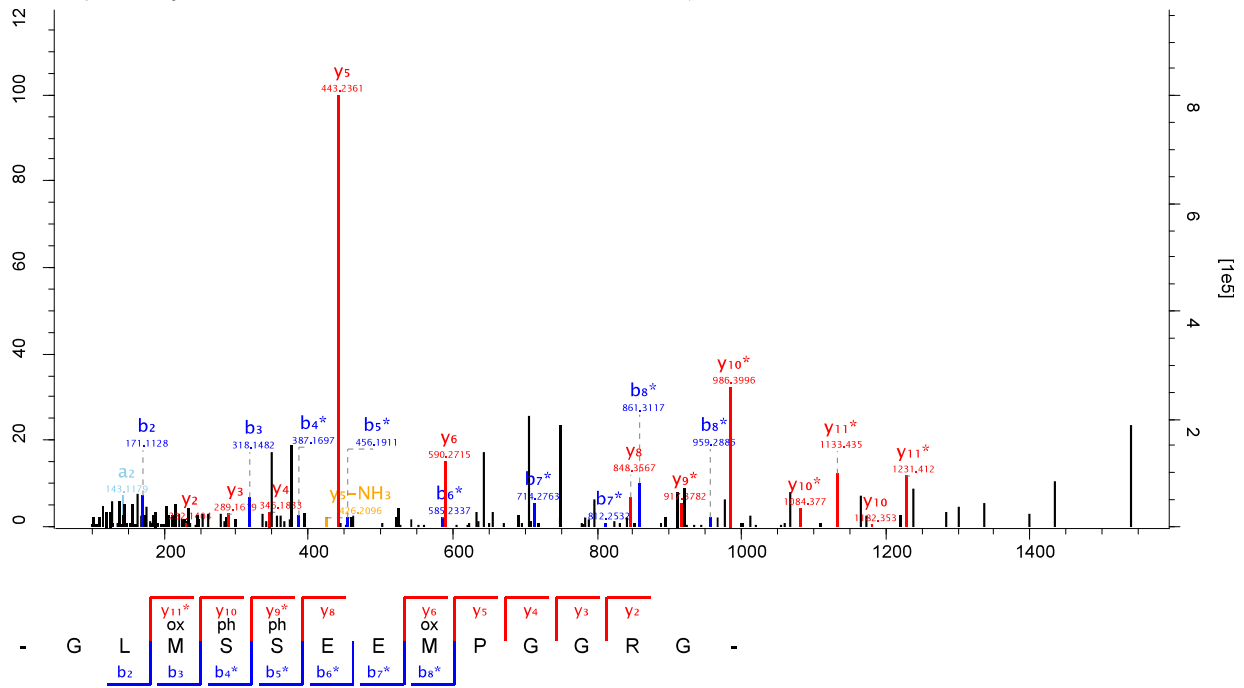




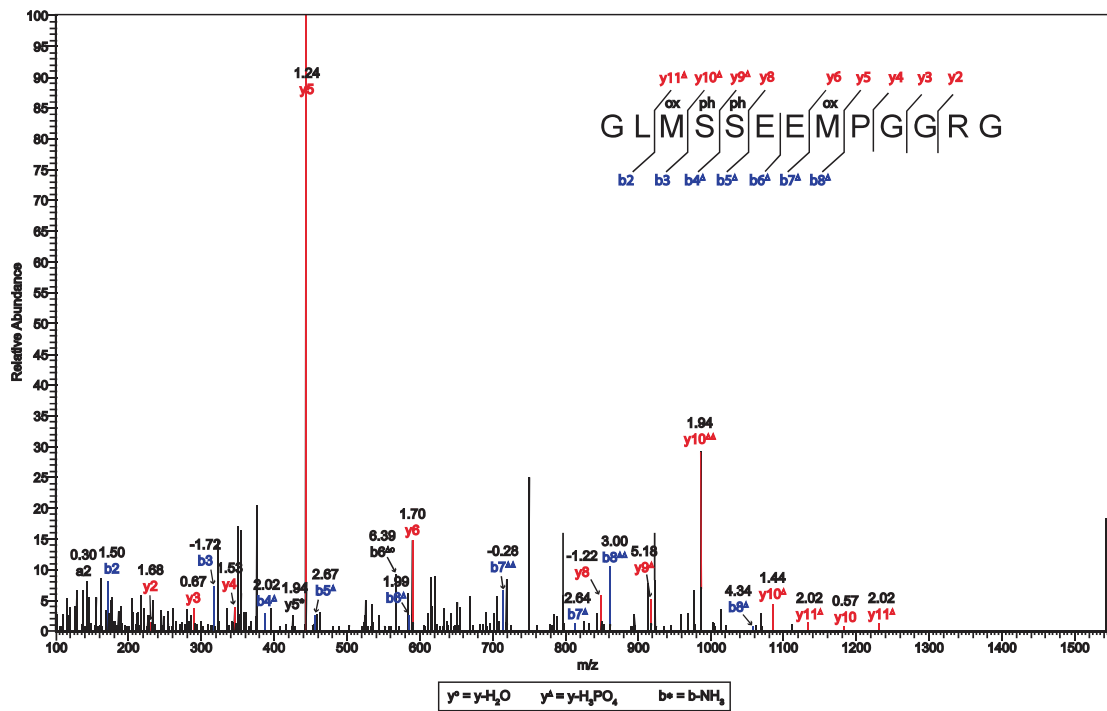


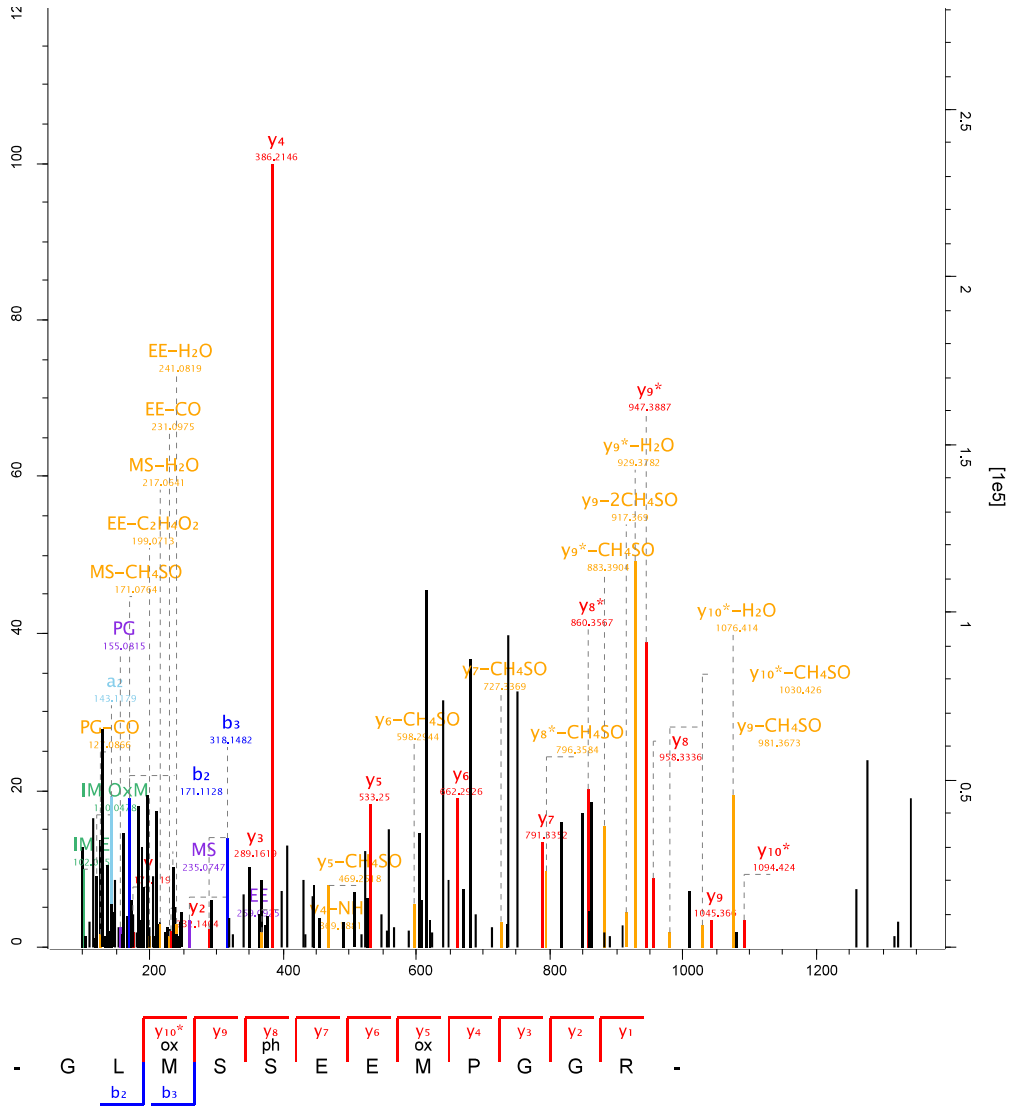




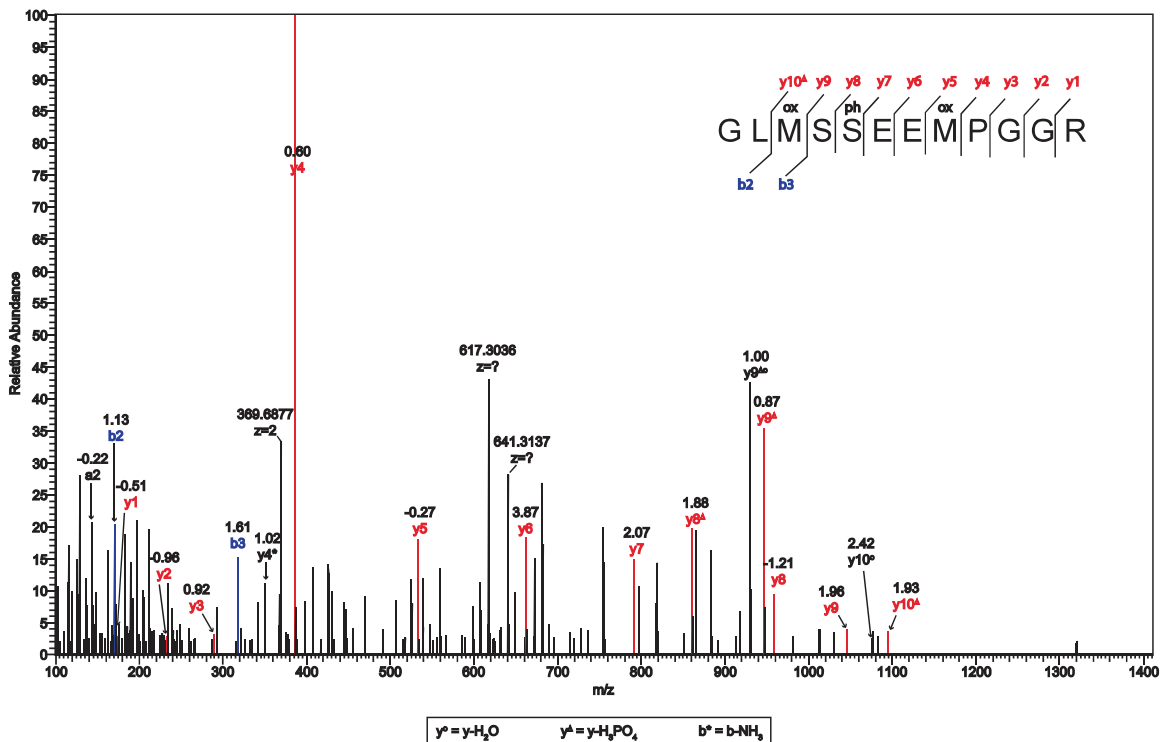


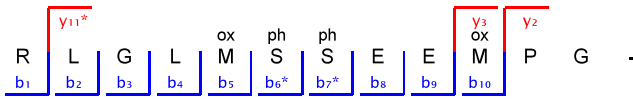
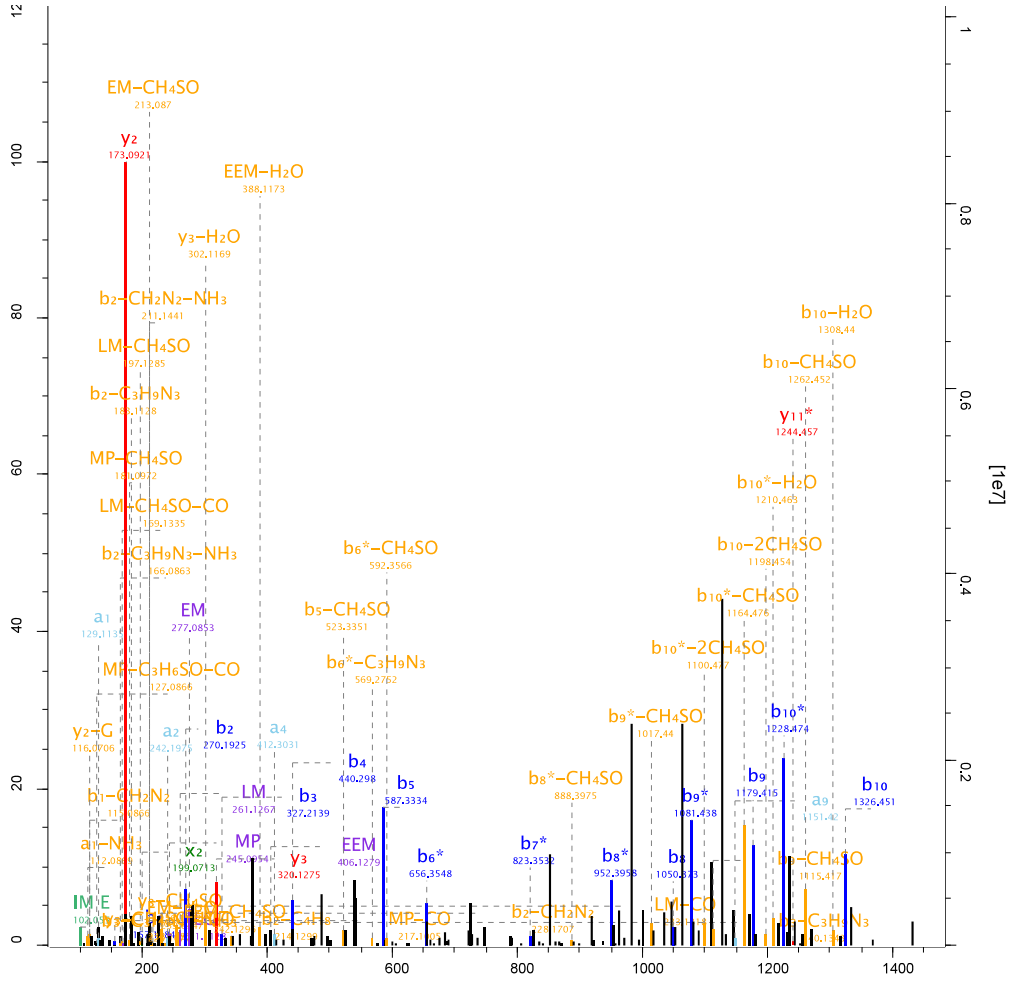
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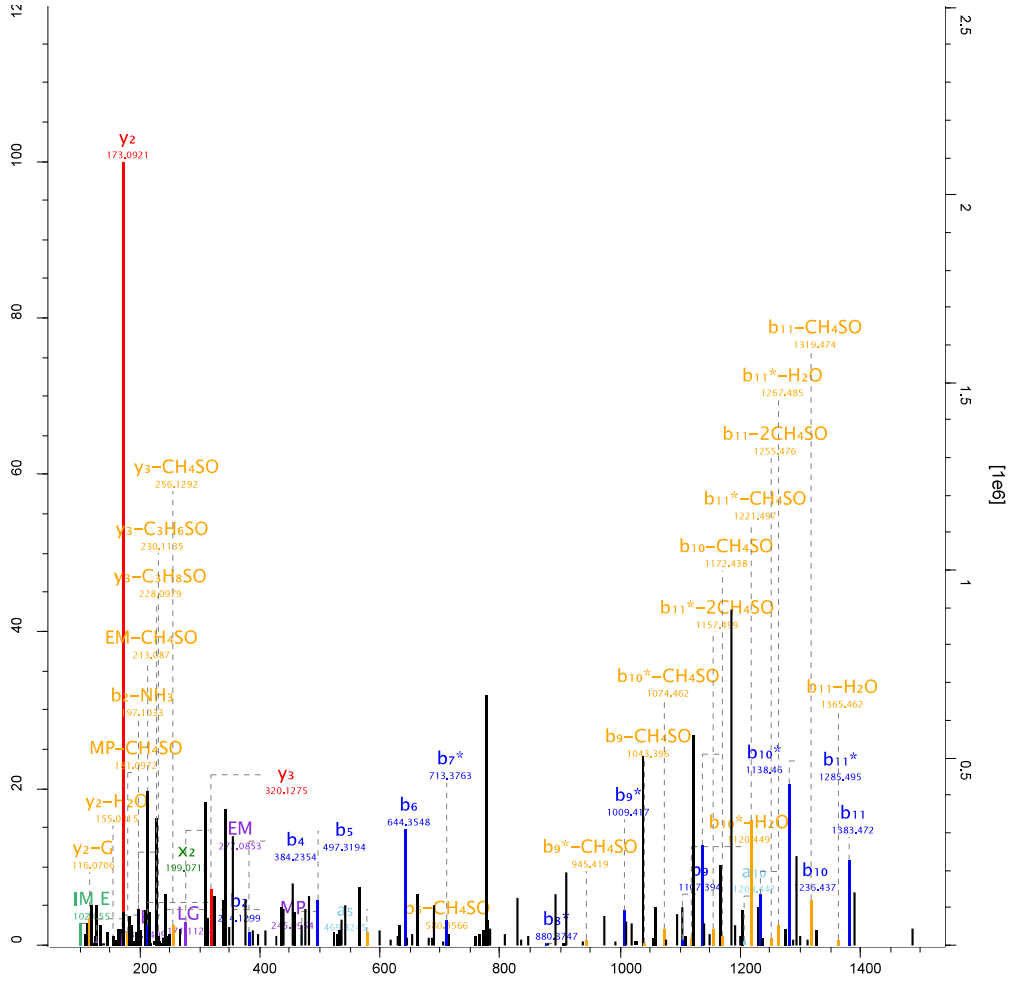




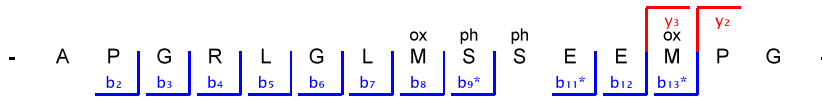
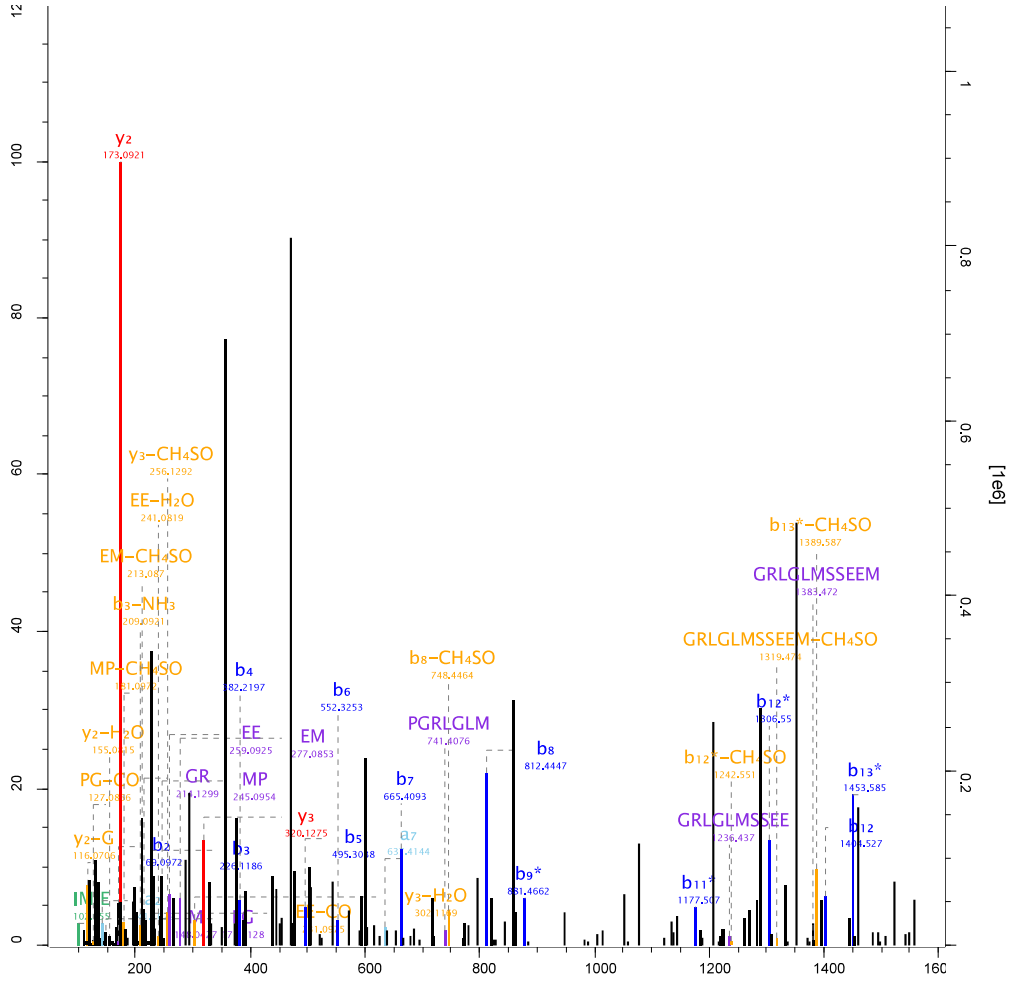
20151231\_QE5\_UPLC1\_RJC\_COLLAB\_GeoG\_870\_01#12271 RT: 48.29 AV: 1 NL: 5.70E4  
T: FTMS + p NSI d Full ms 2 681.76 @ hcd 28.00 [100.00-1410.00]

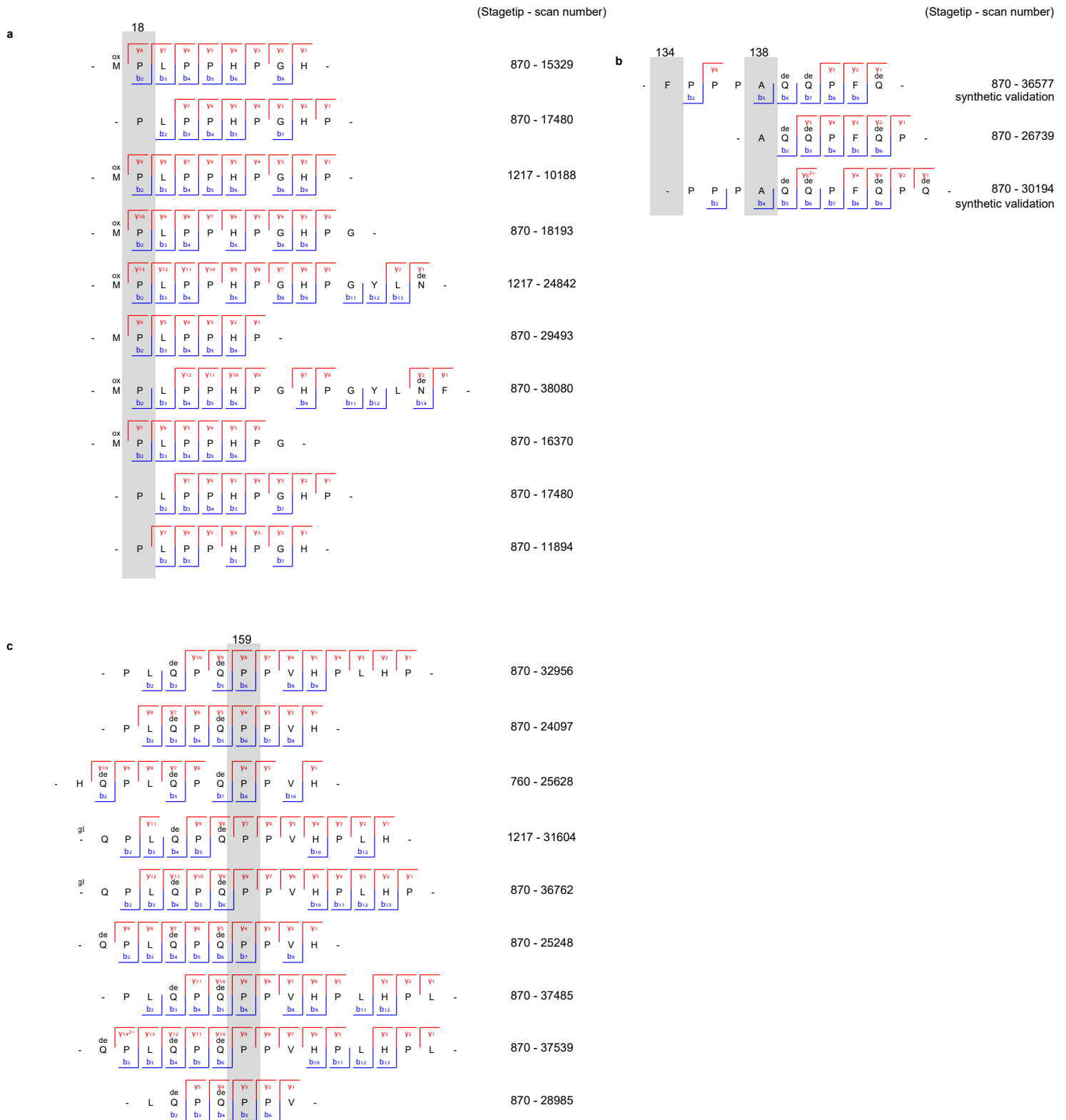




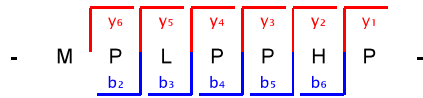
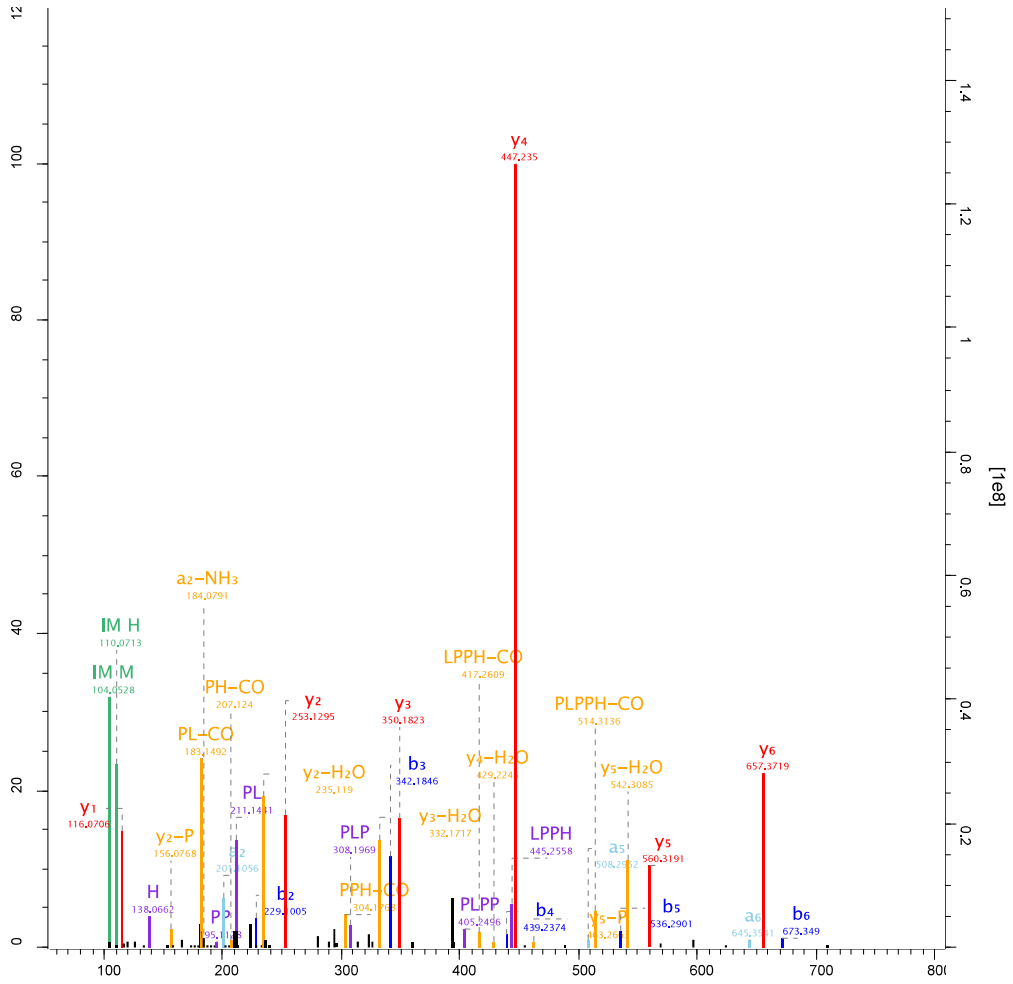


- G R L G L ox ph ph Y3 Y2  
 b2 b4 b5 b6 b7\* b8\* b9 b10 b11 ox M P G -

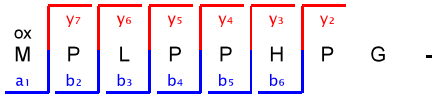
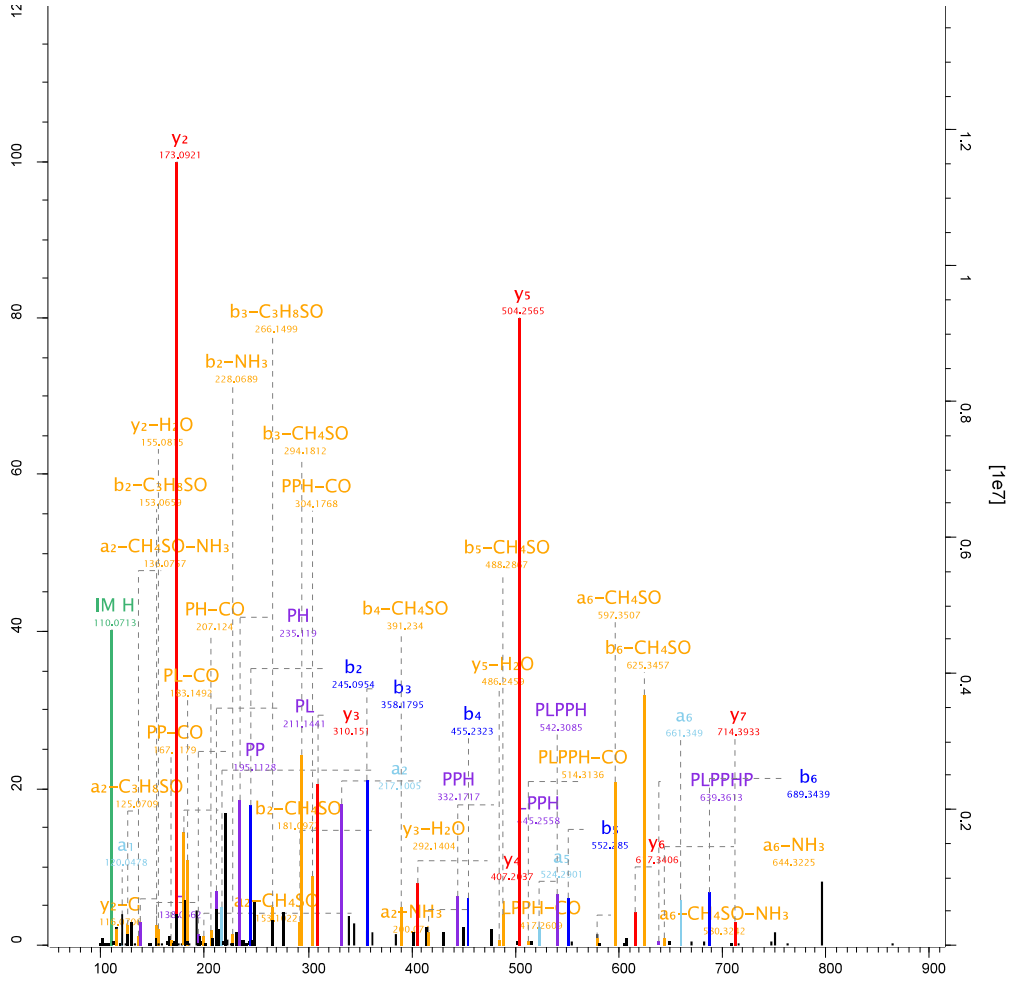


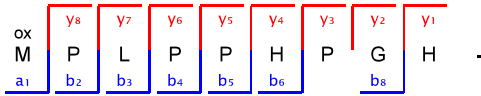
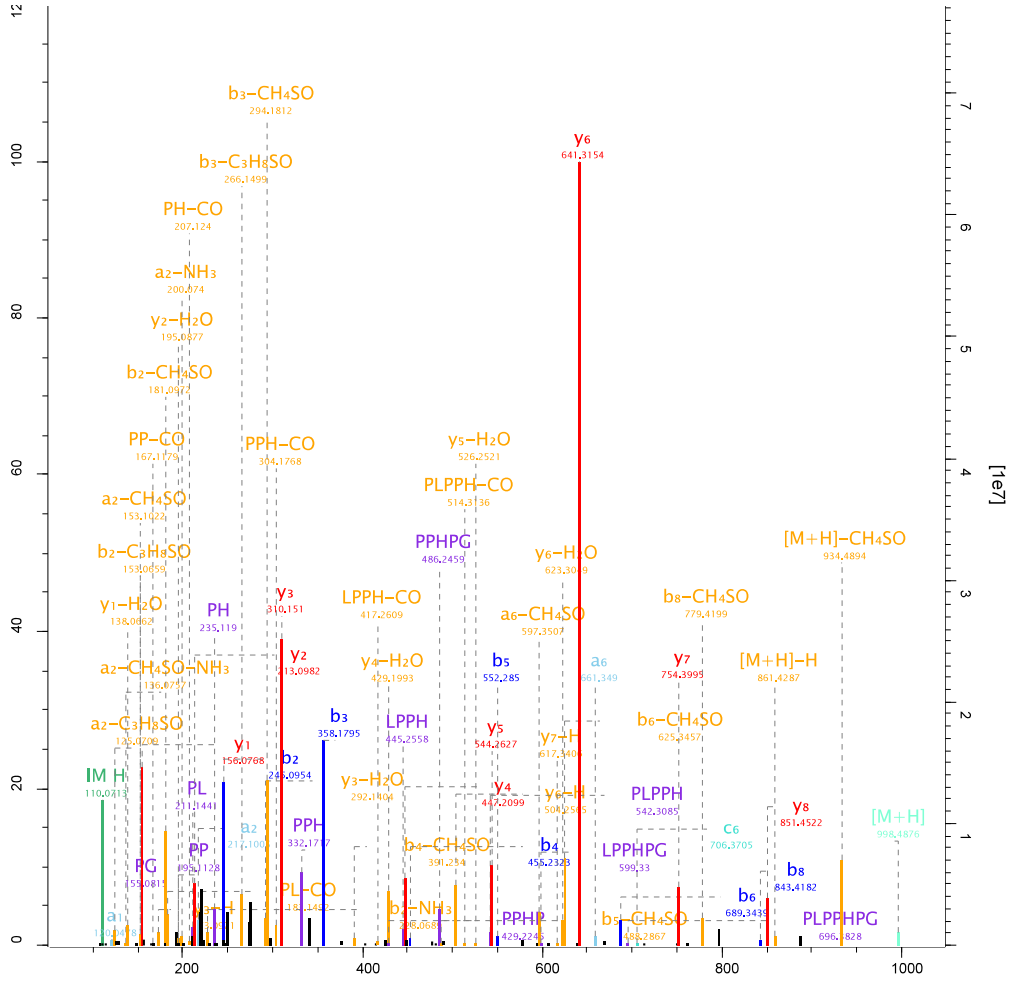


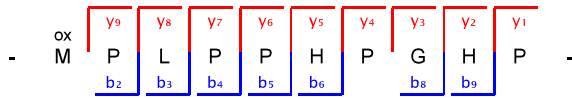
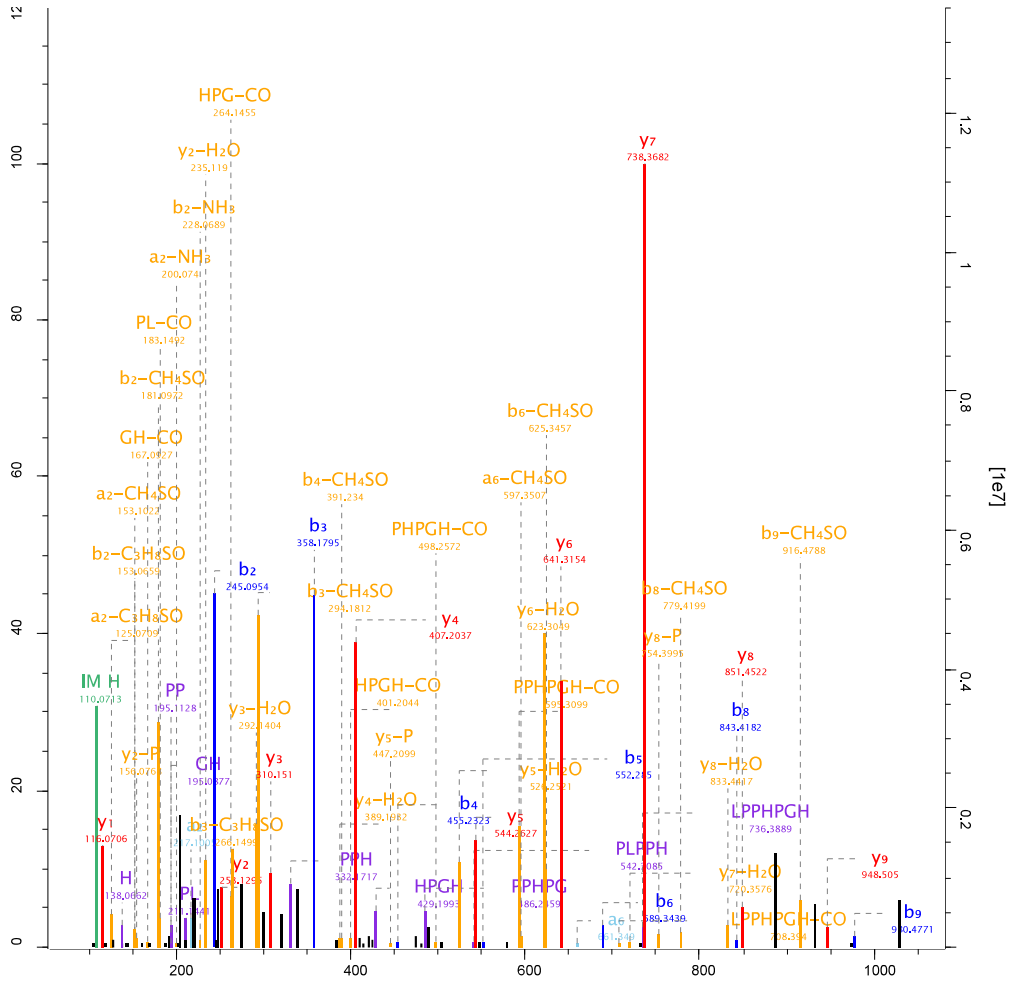
**AMELX.** Peptide matches to phylogenetically informative positions 18 (a), 134, 138 (b), and 159 (c). Coordinates in reference to XP\_004435161.

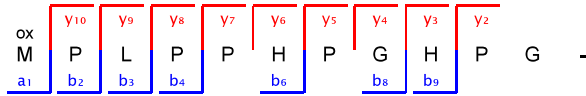
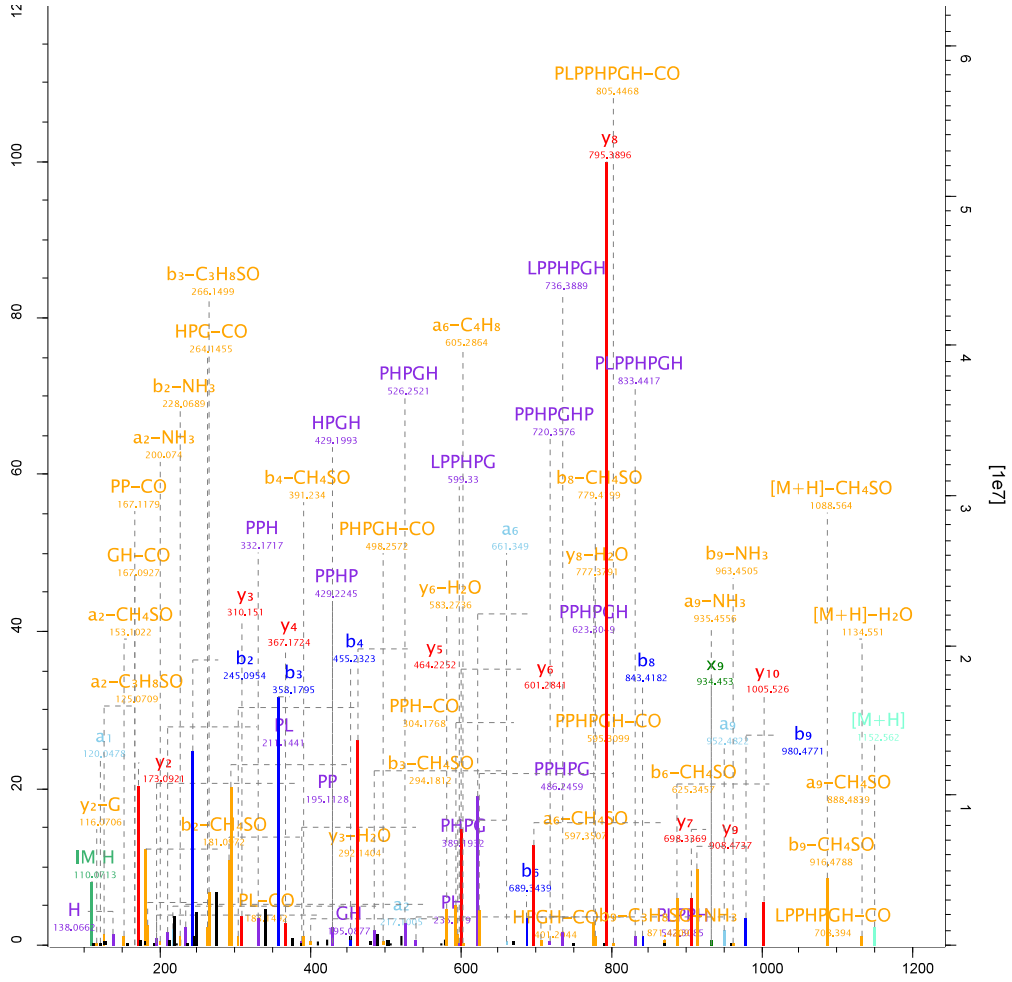


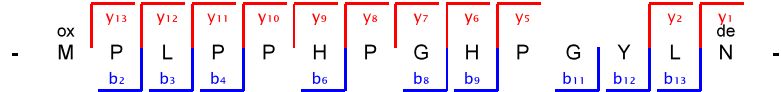
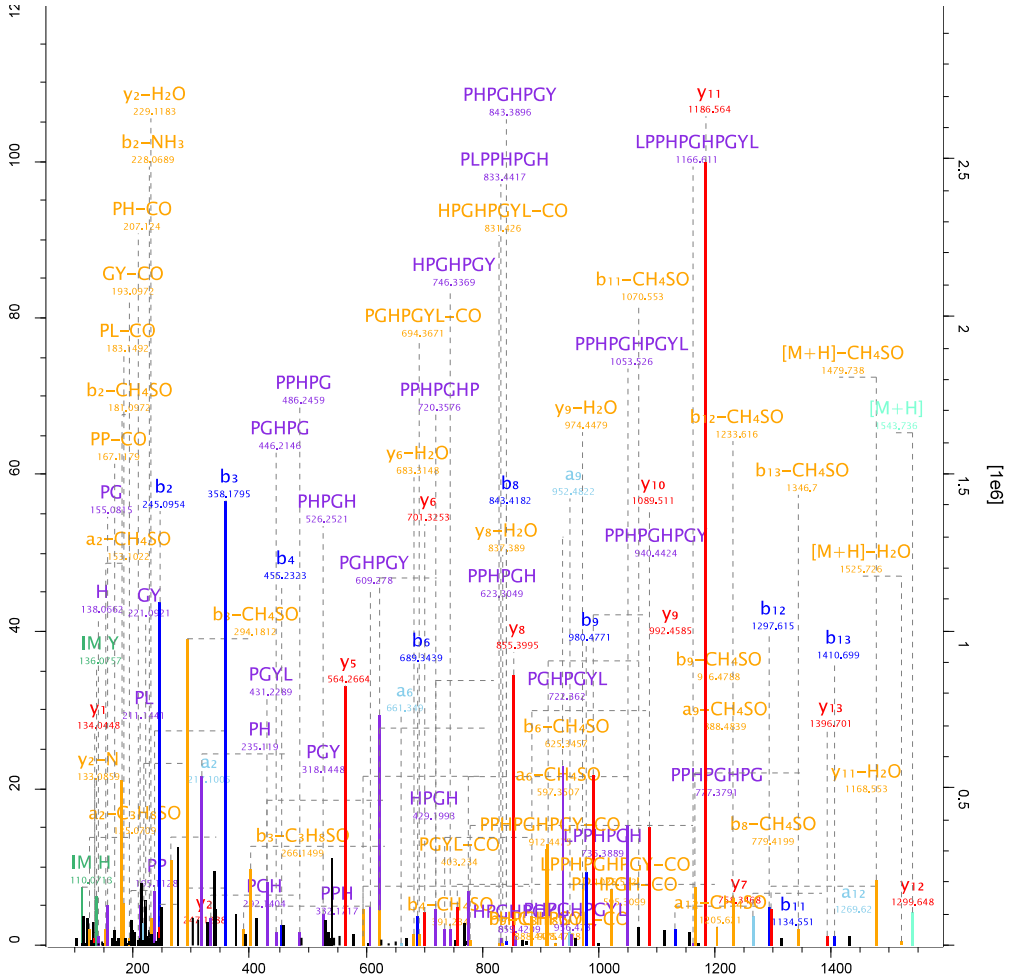


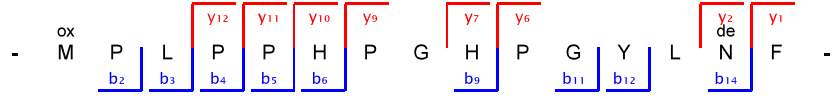
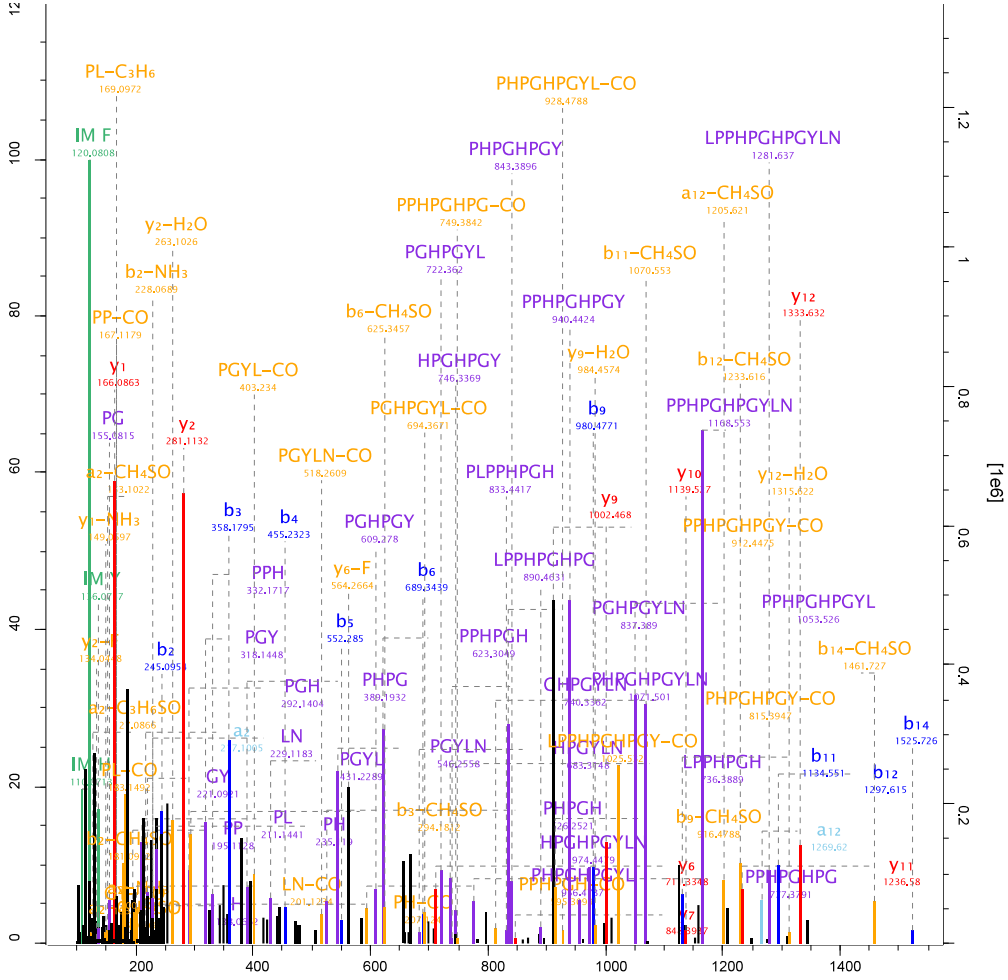


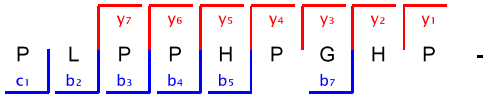
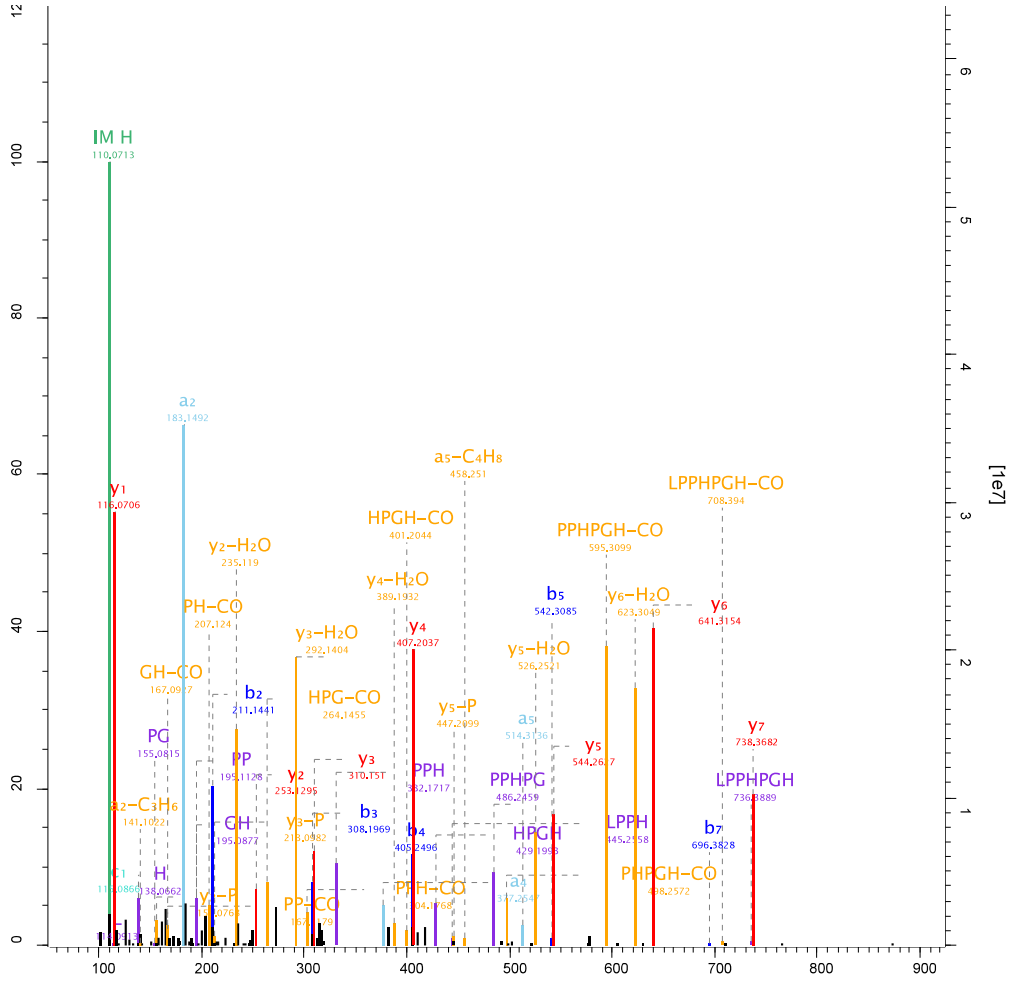


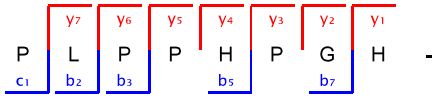
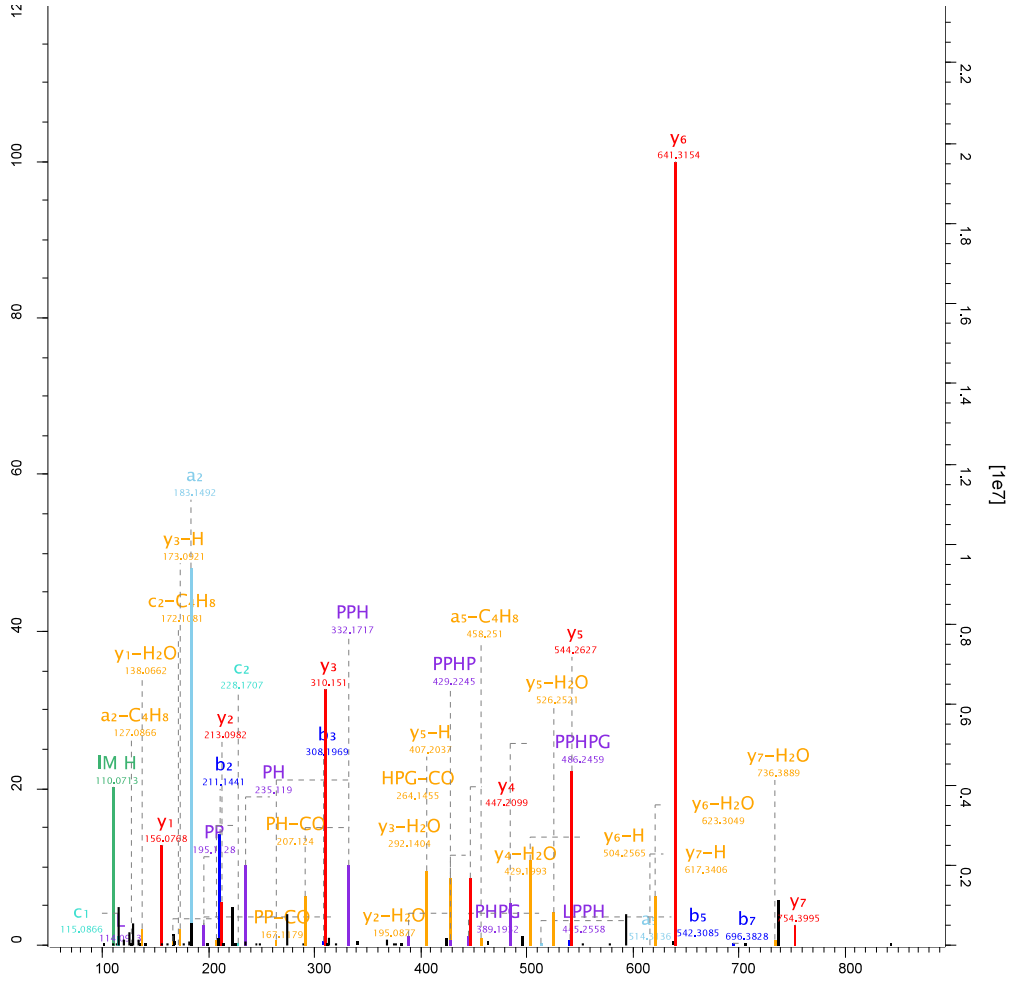




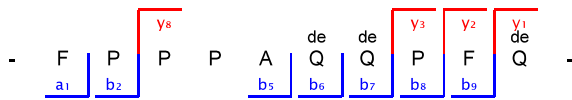
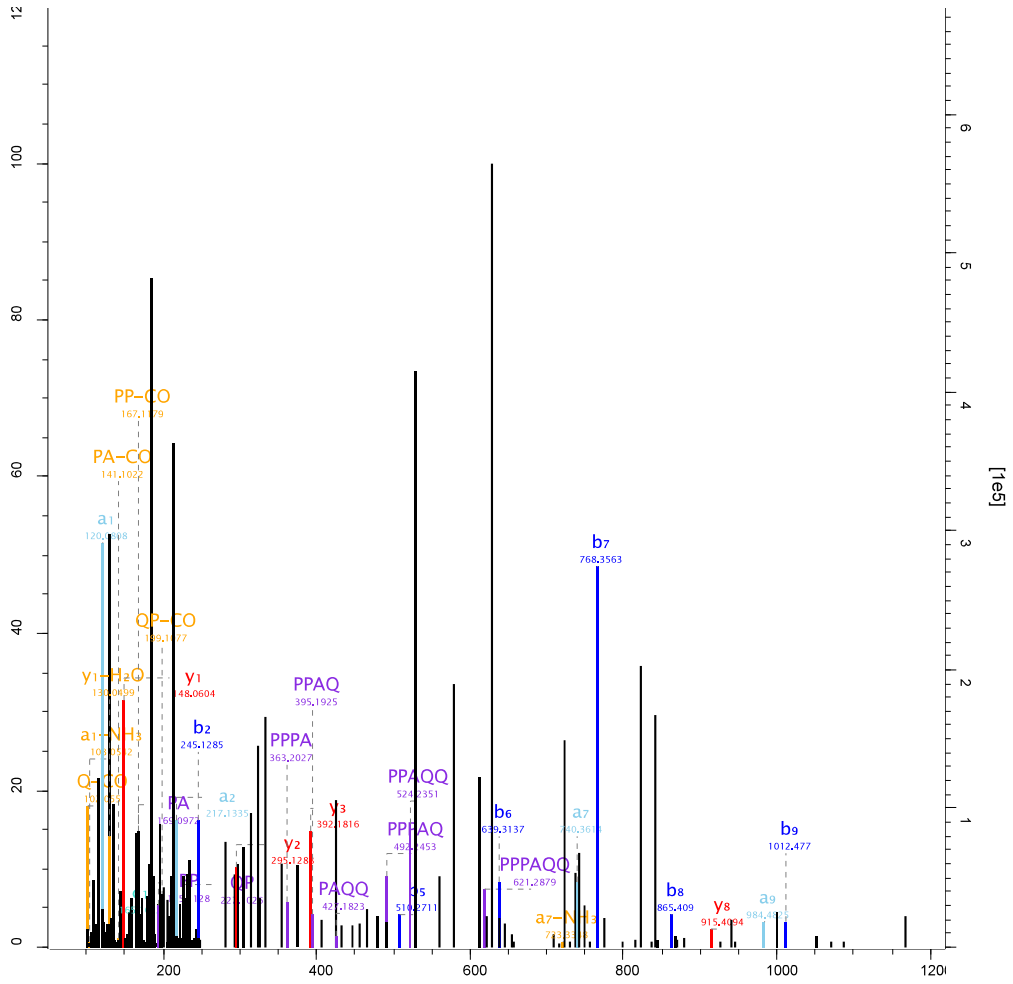


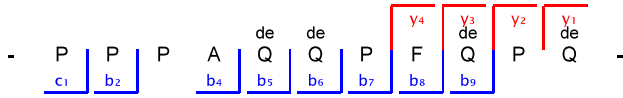
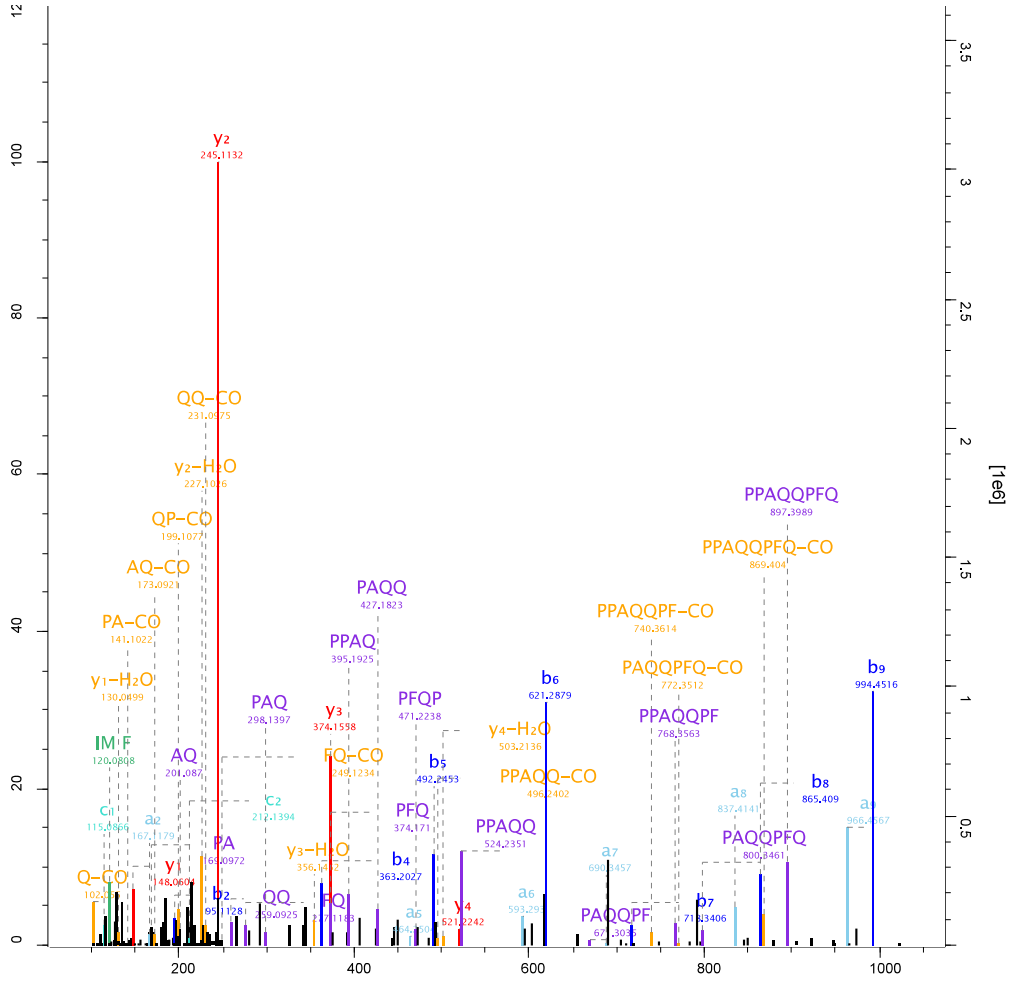


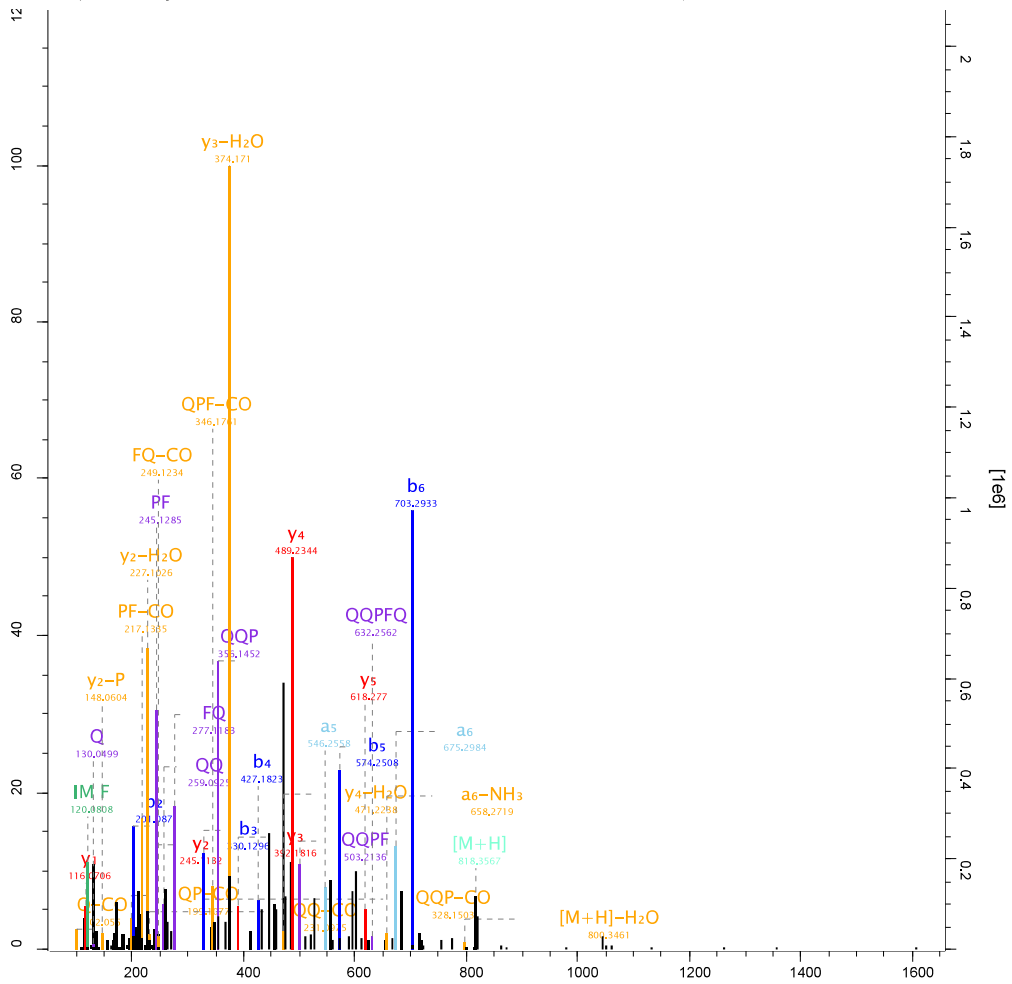


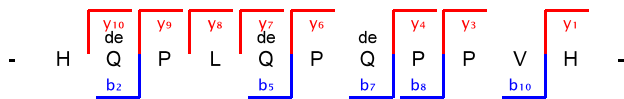
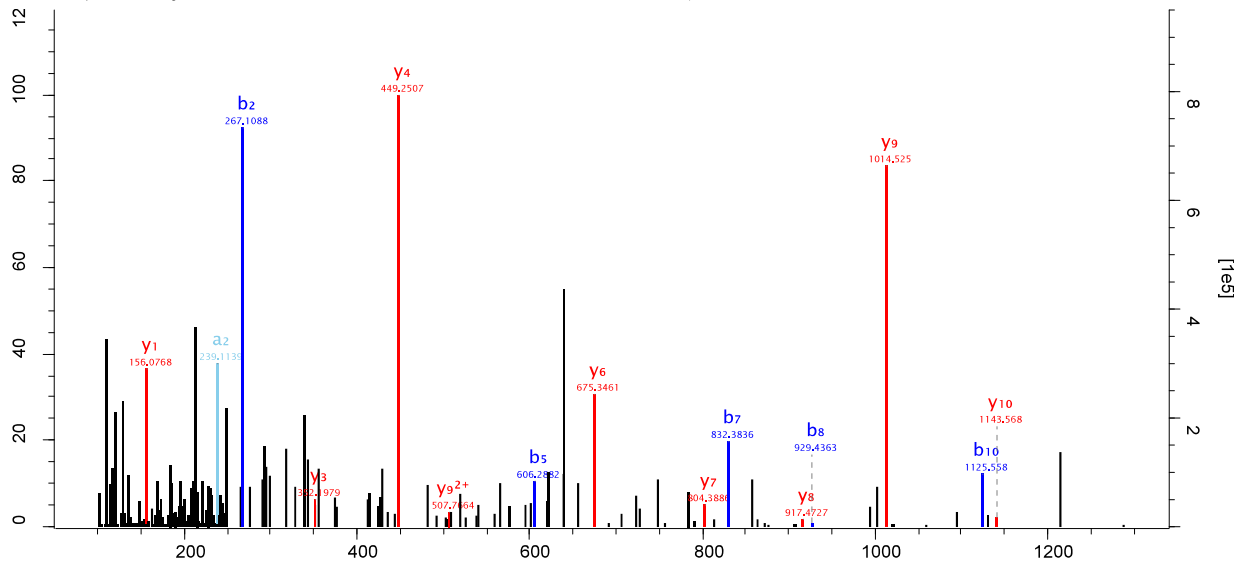


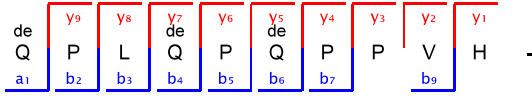
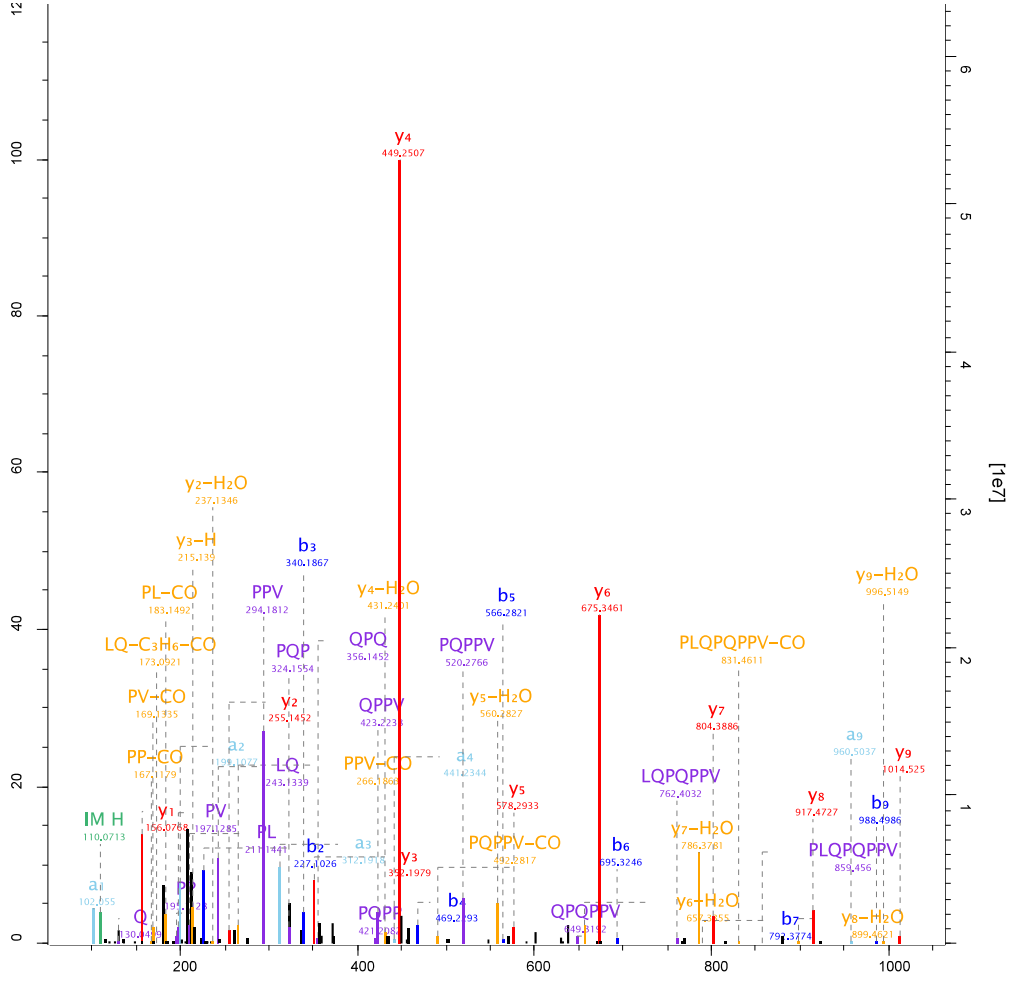


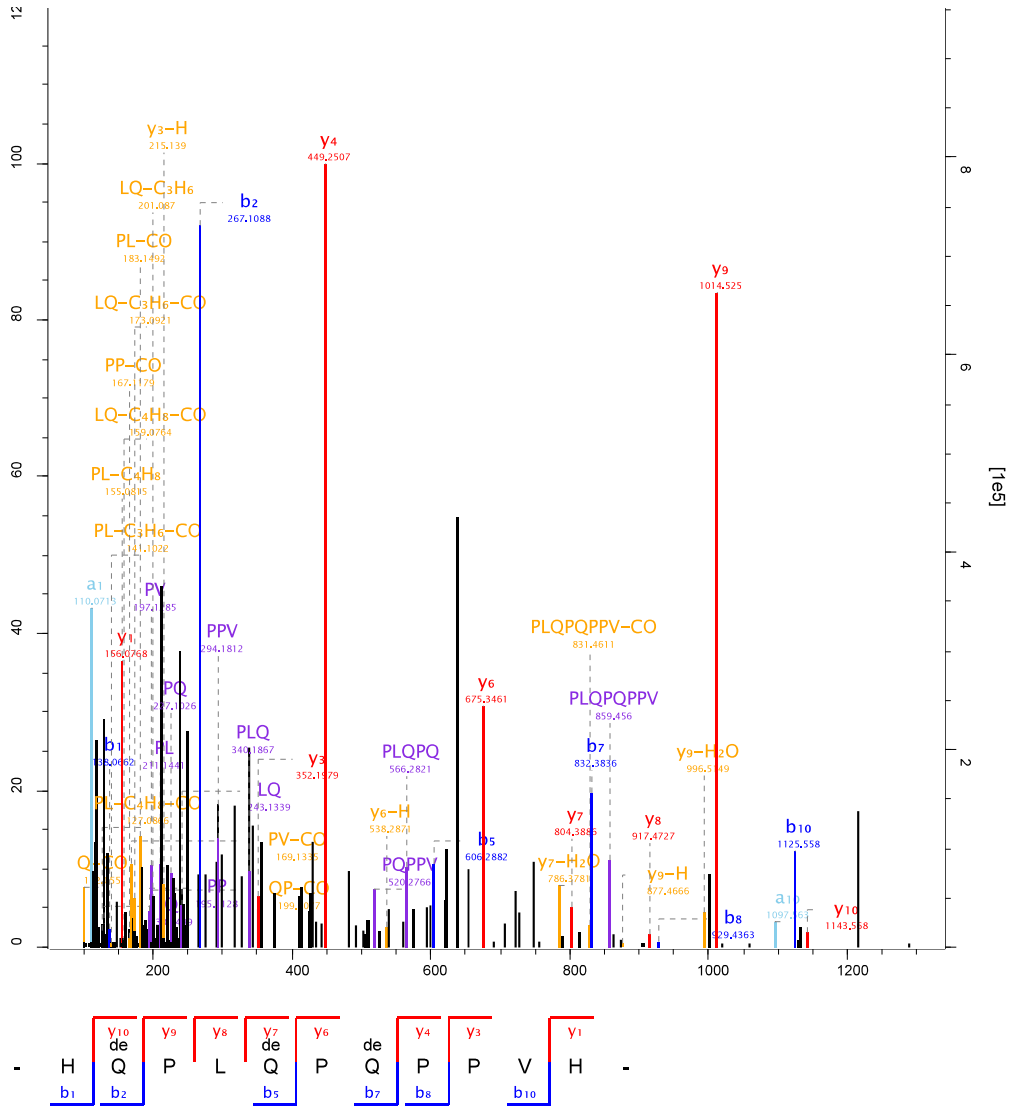


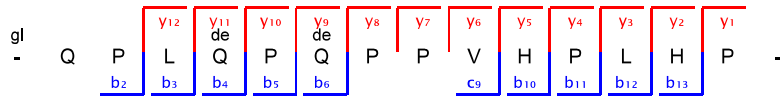
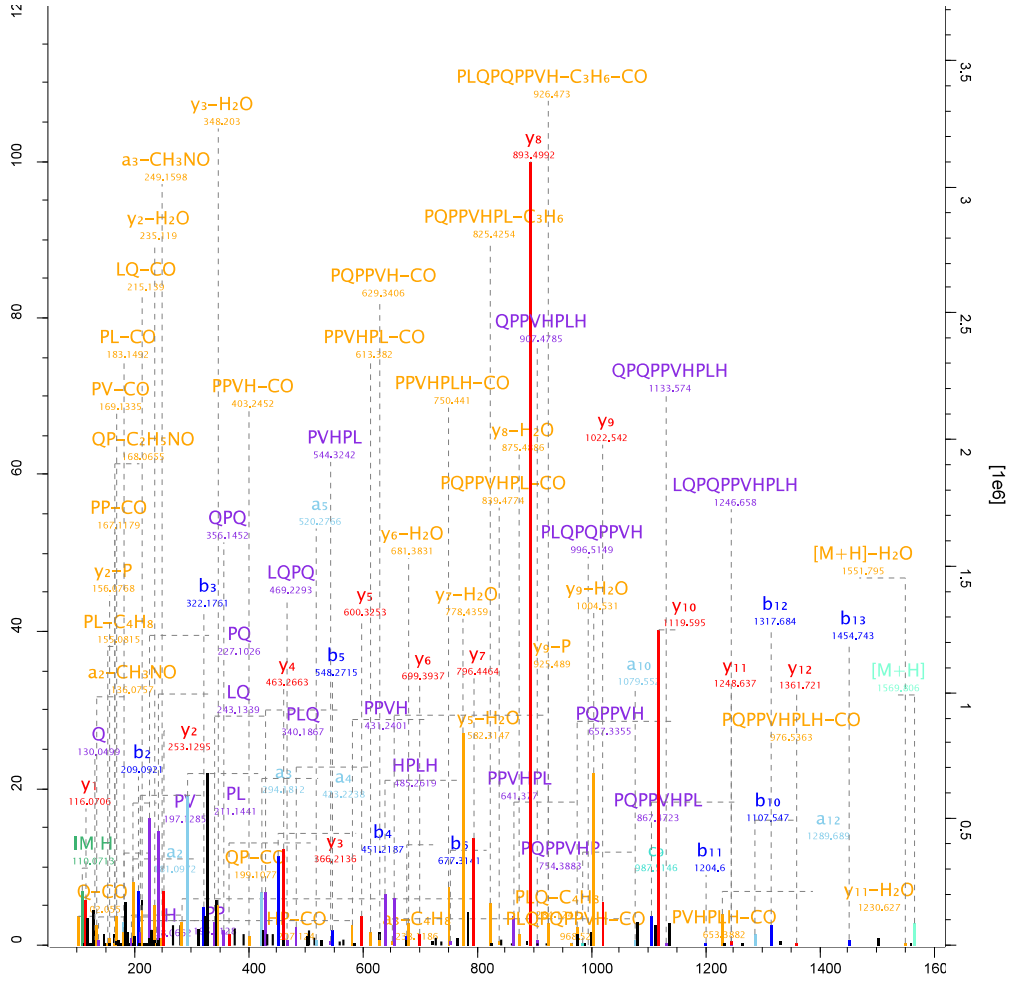


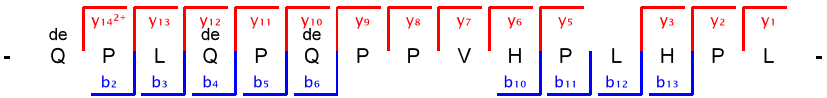
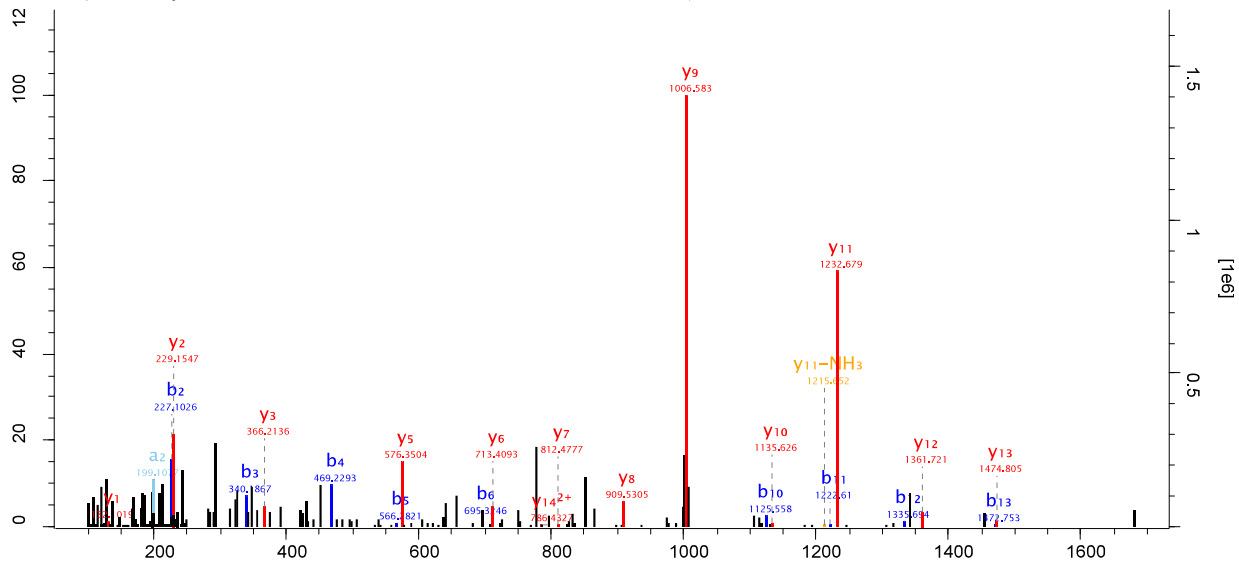




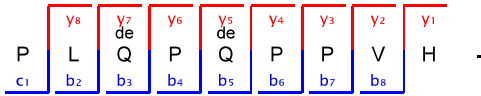
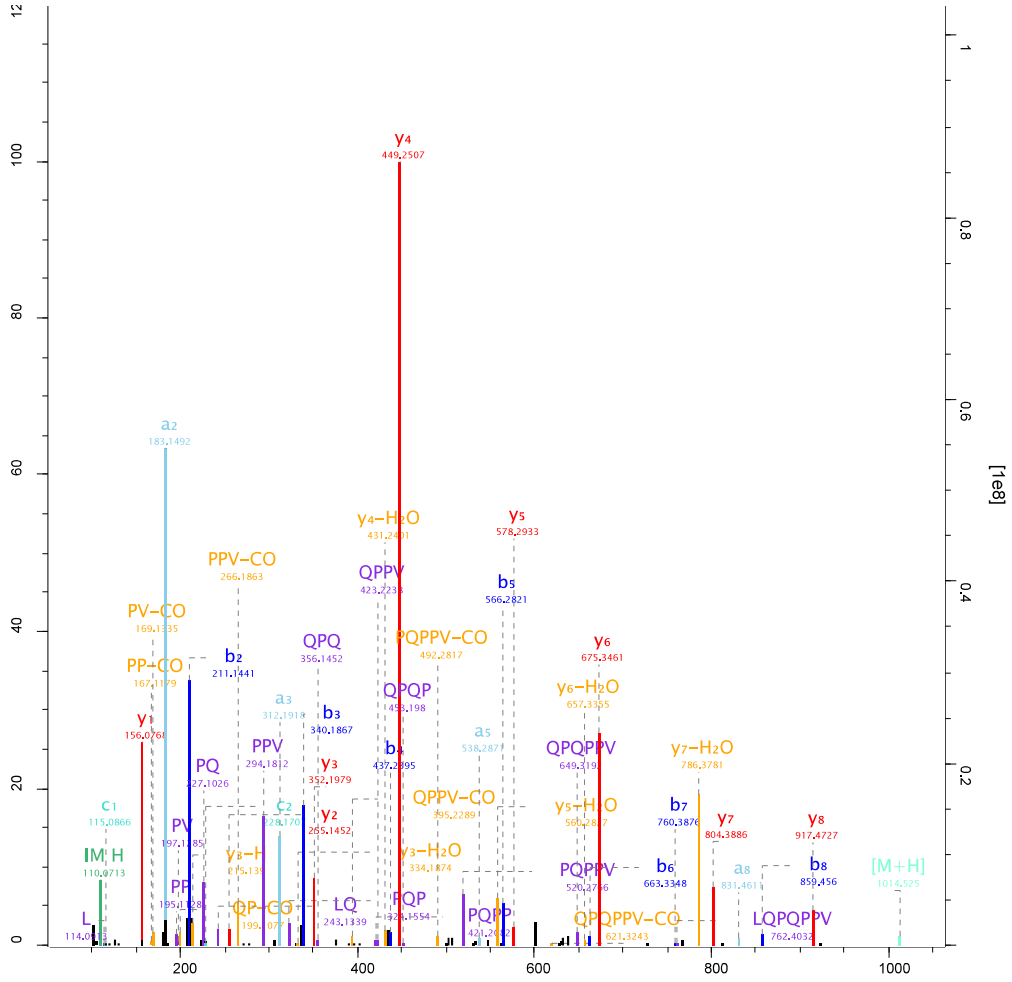


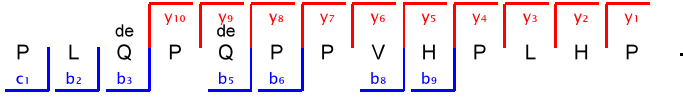
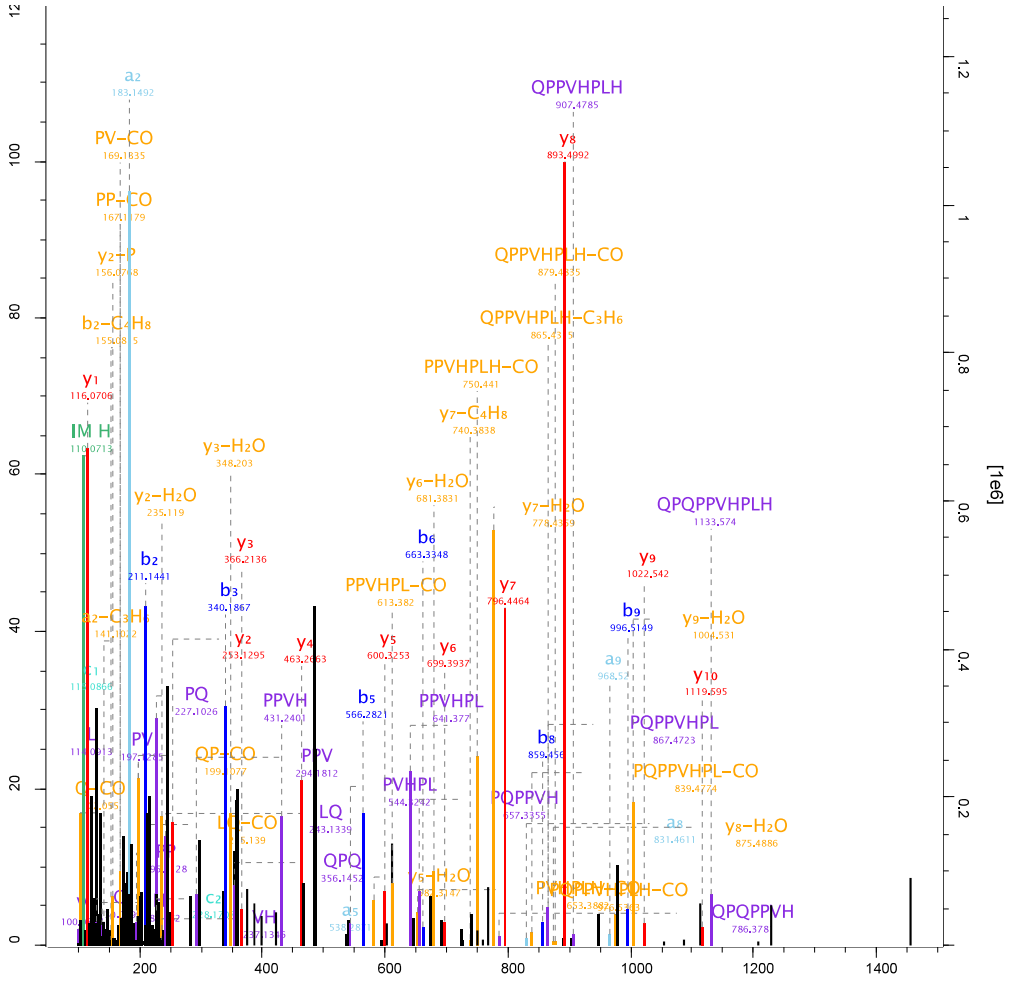


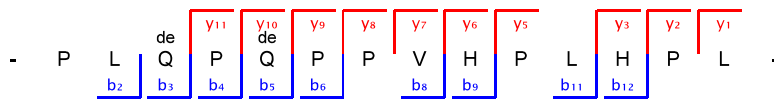
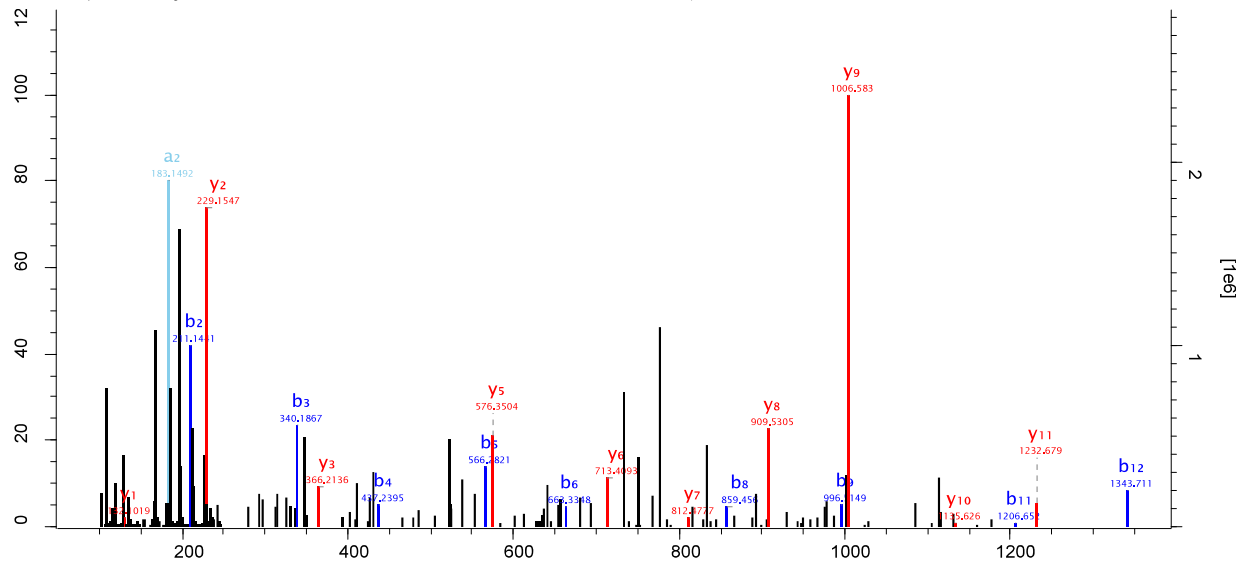


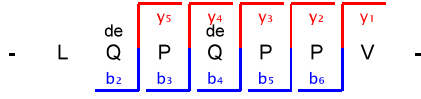
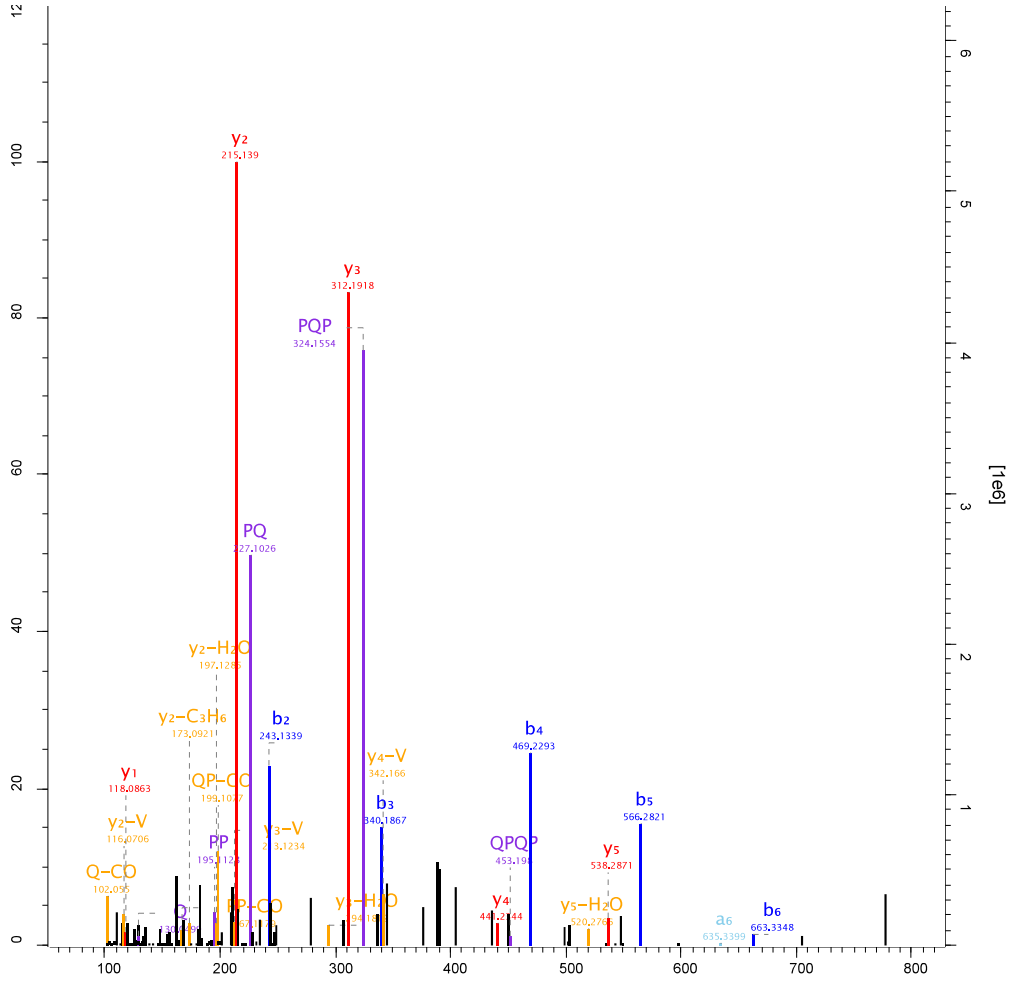






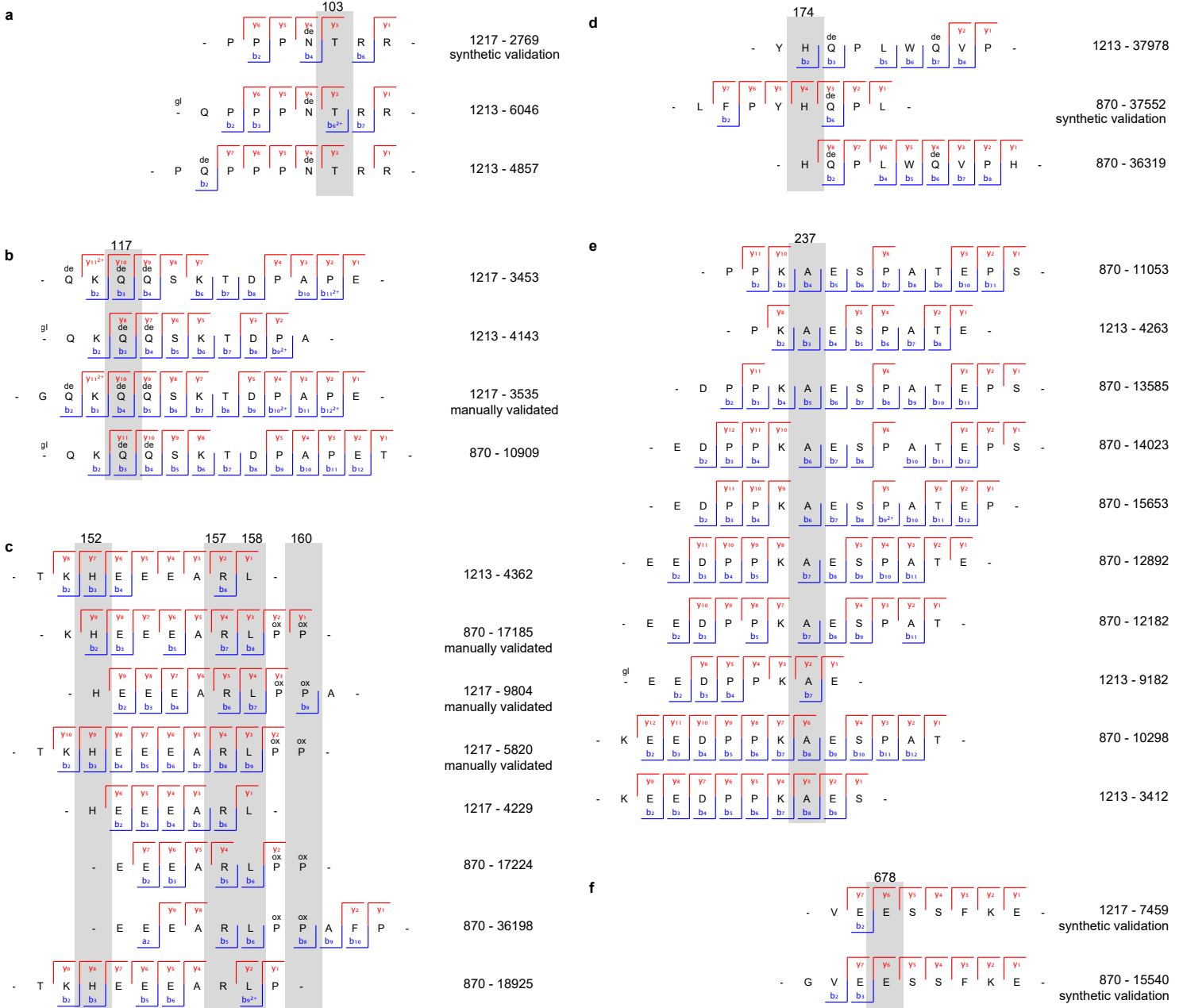




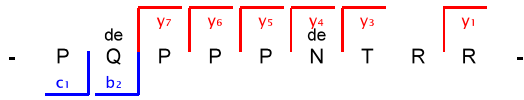
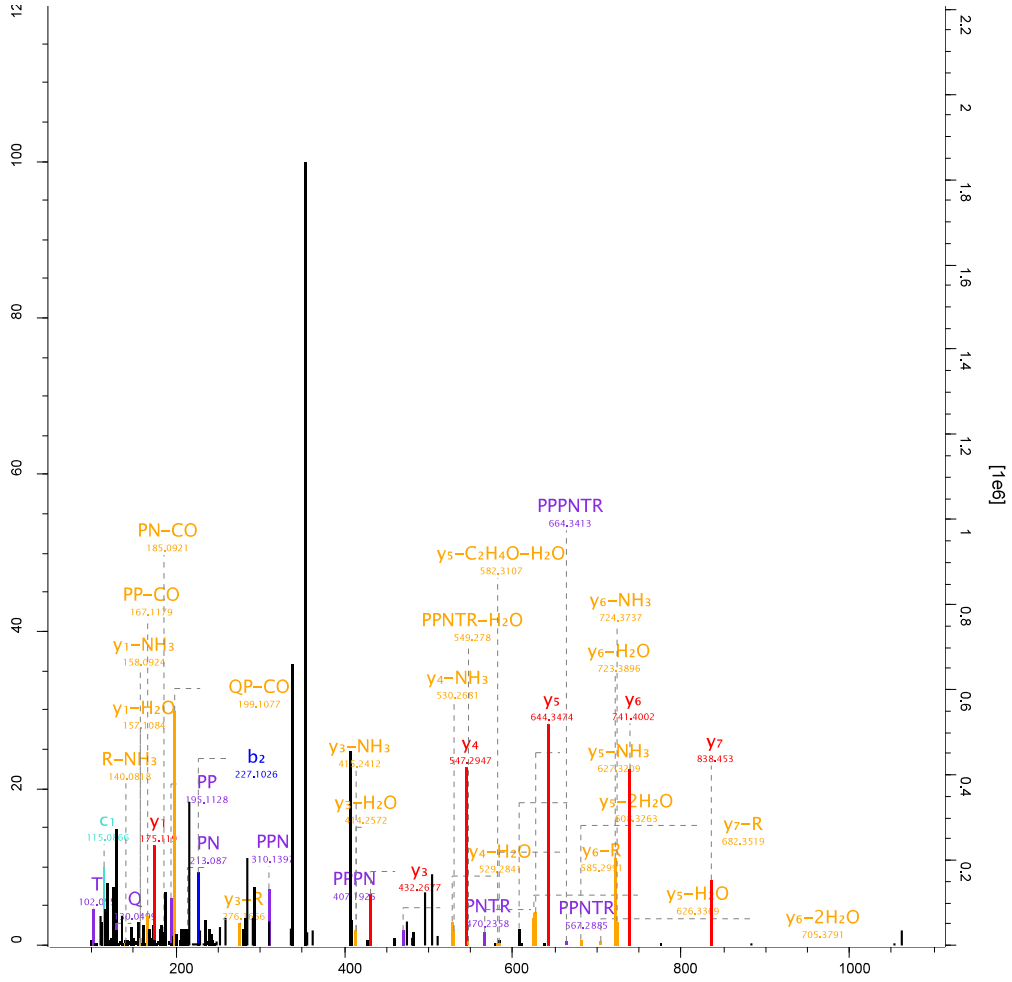


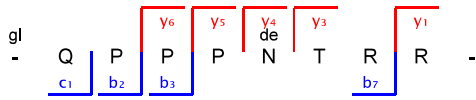
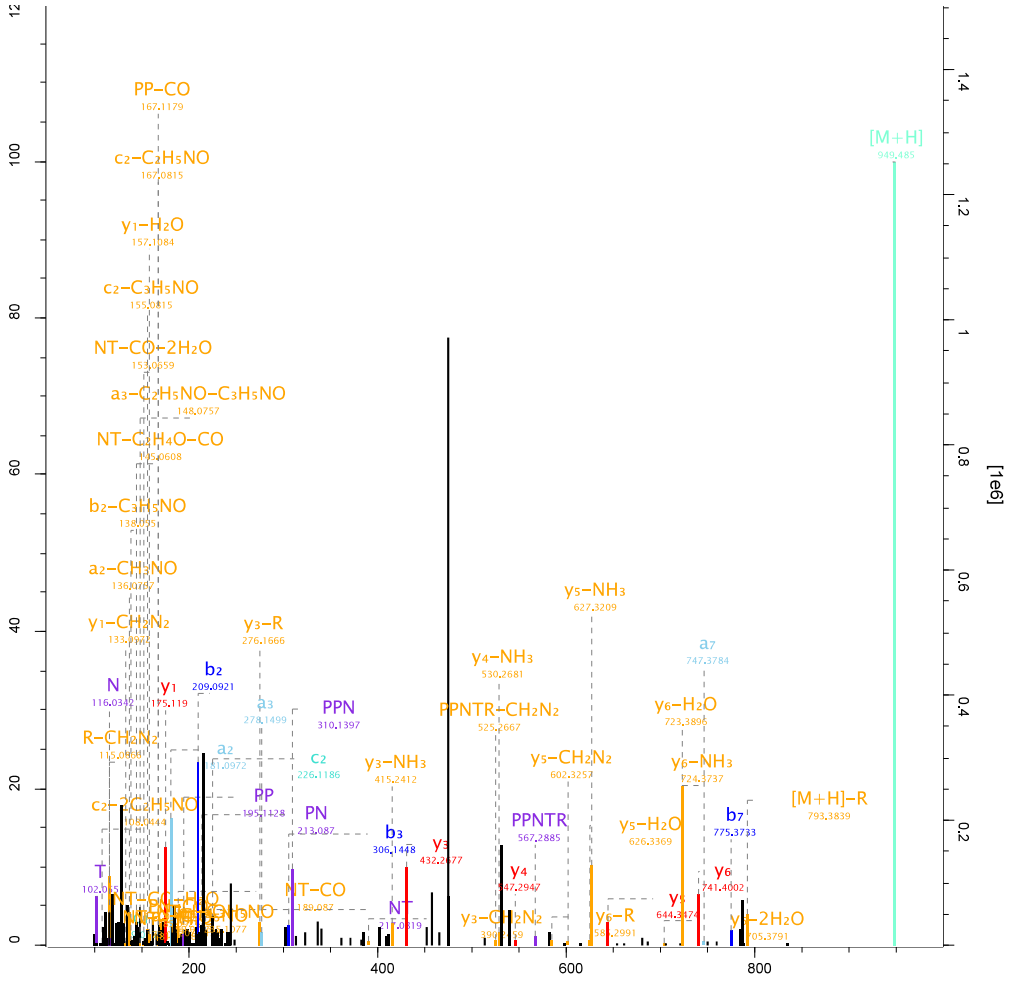
(Stagetip - scan number)

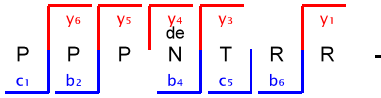
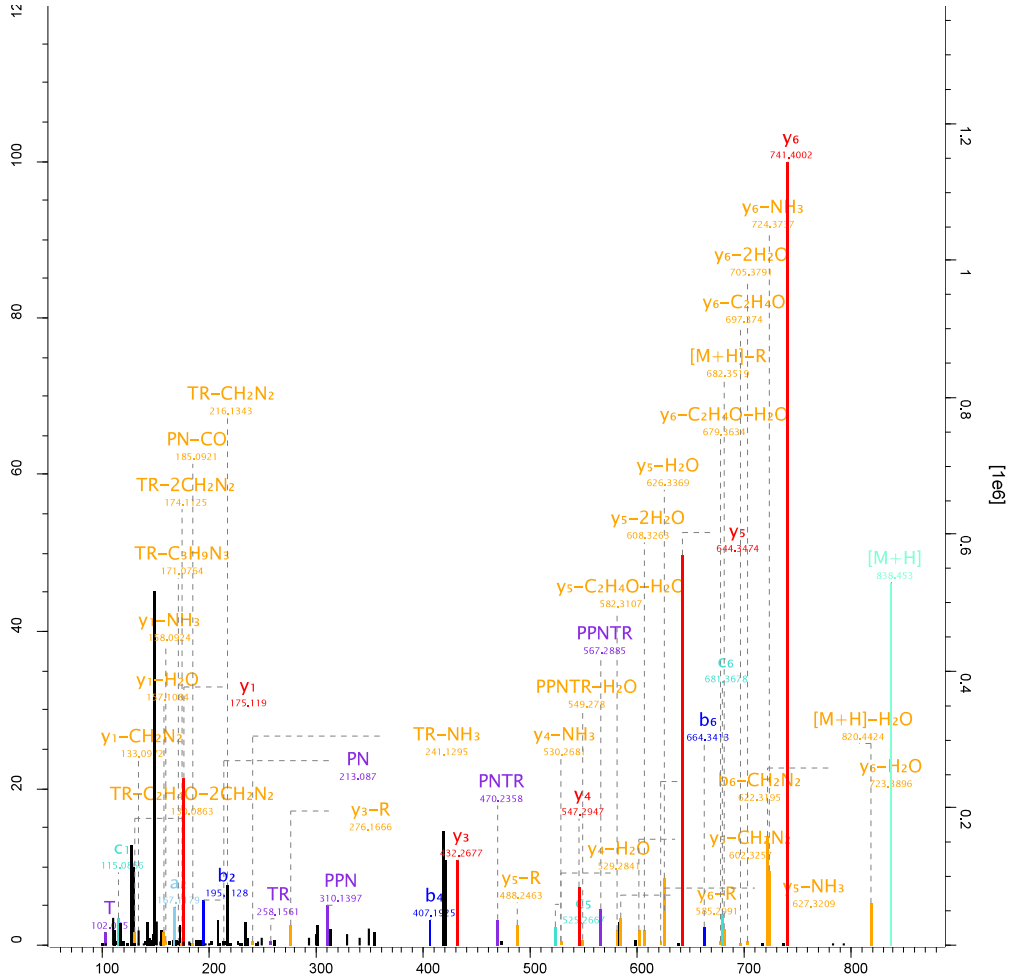
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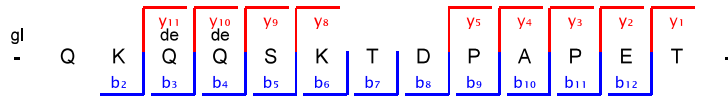
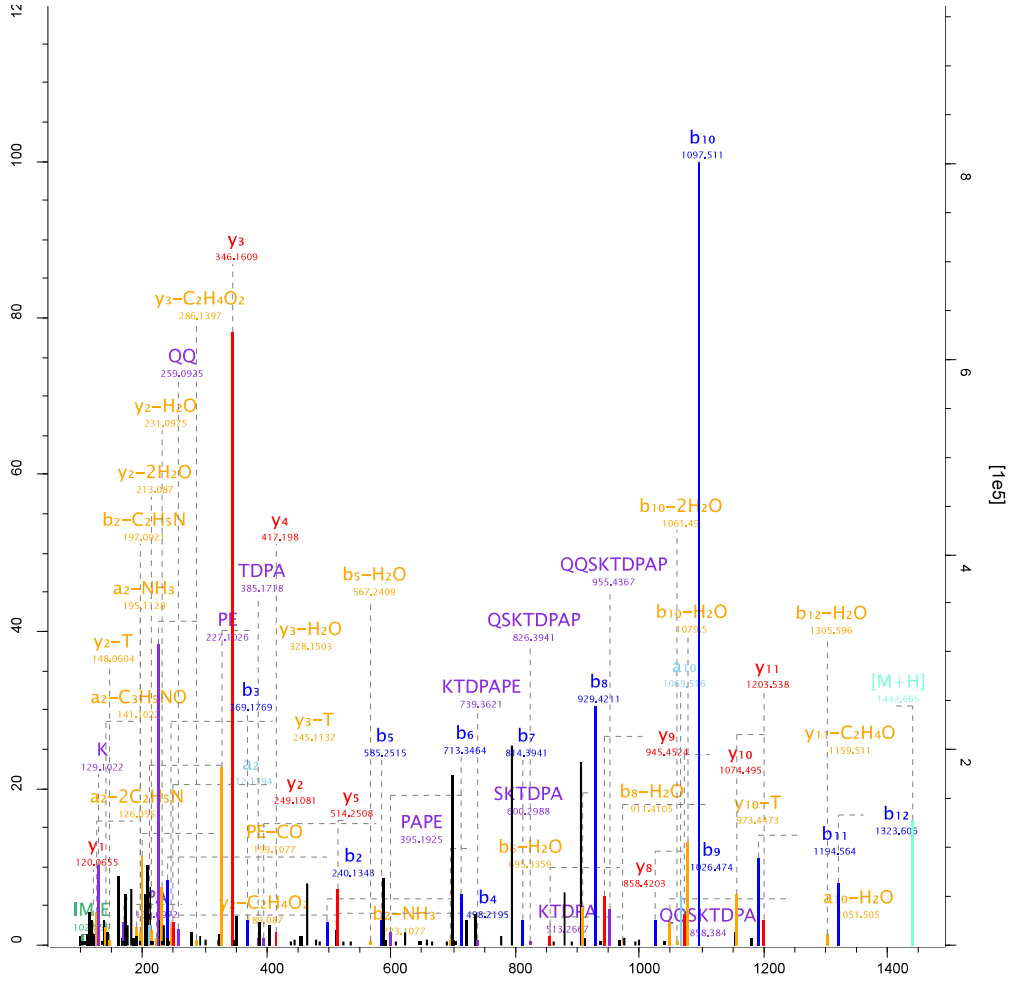
**ENAM.** Peptide matches to phylogenetically informative positions 103 (a), 117 (b), 152, 157, 158, and 160 (c), 174 (d), 237 (e), and 678 (f). Coordinates in reference to XP\_004419201.

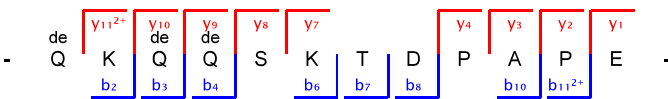
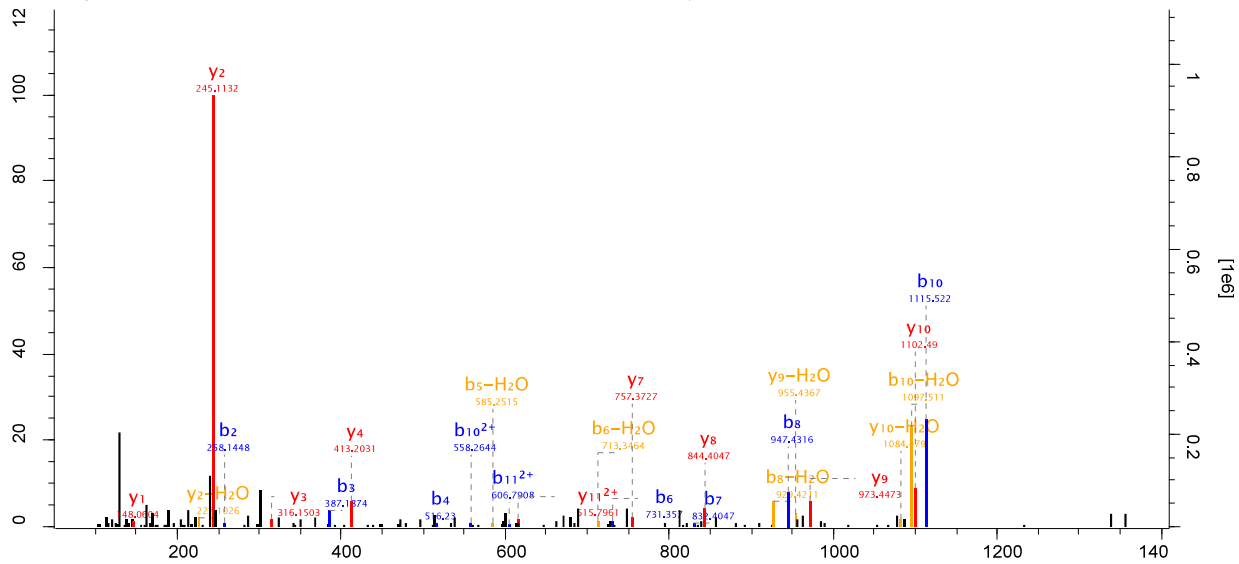


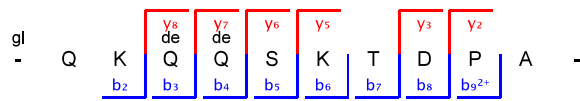
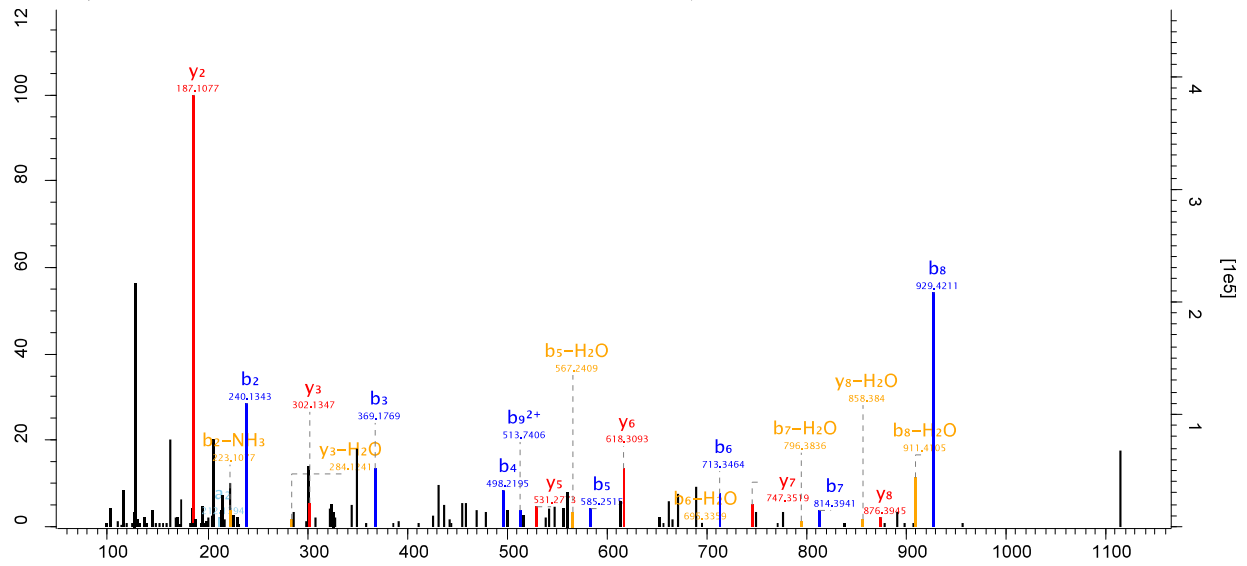


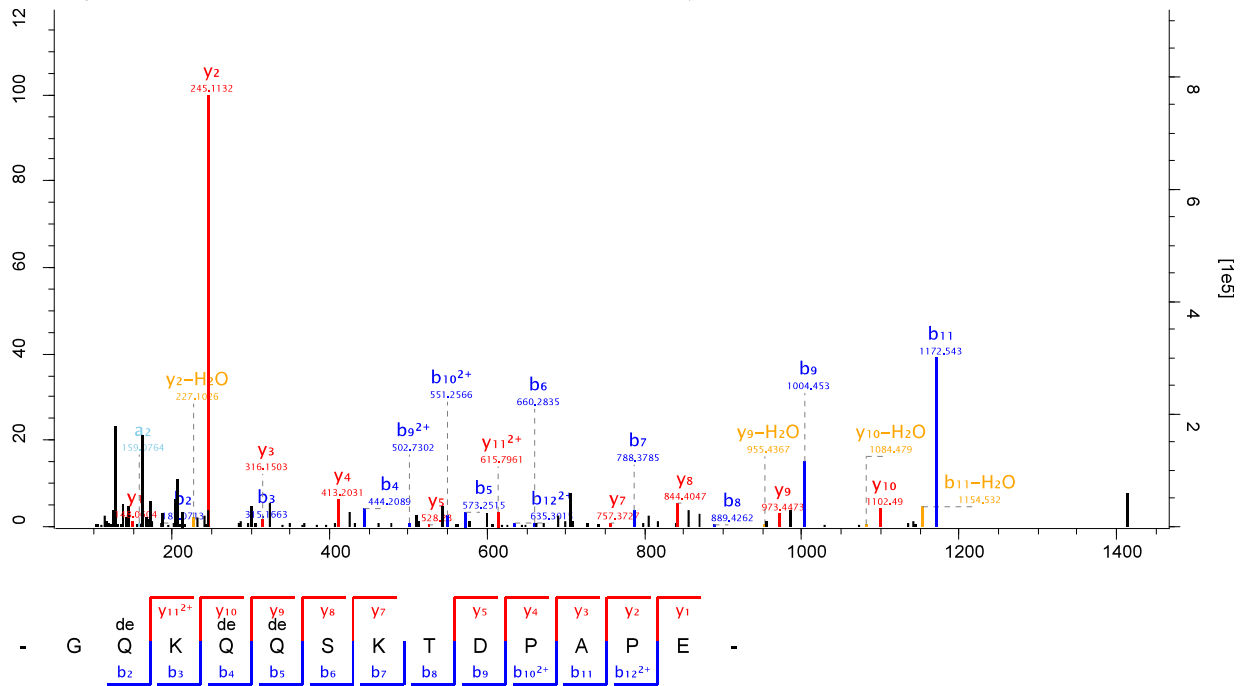




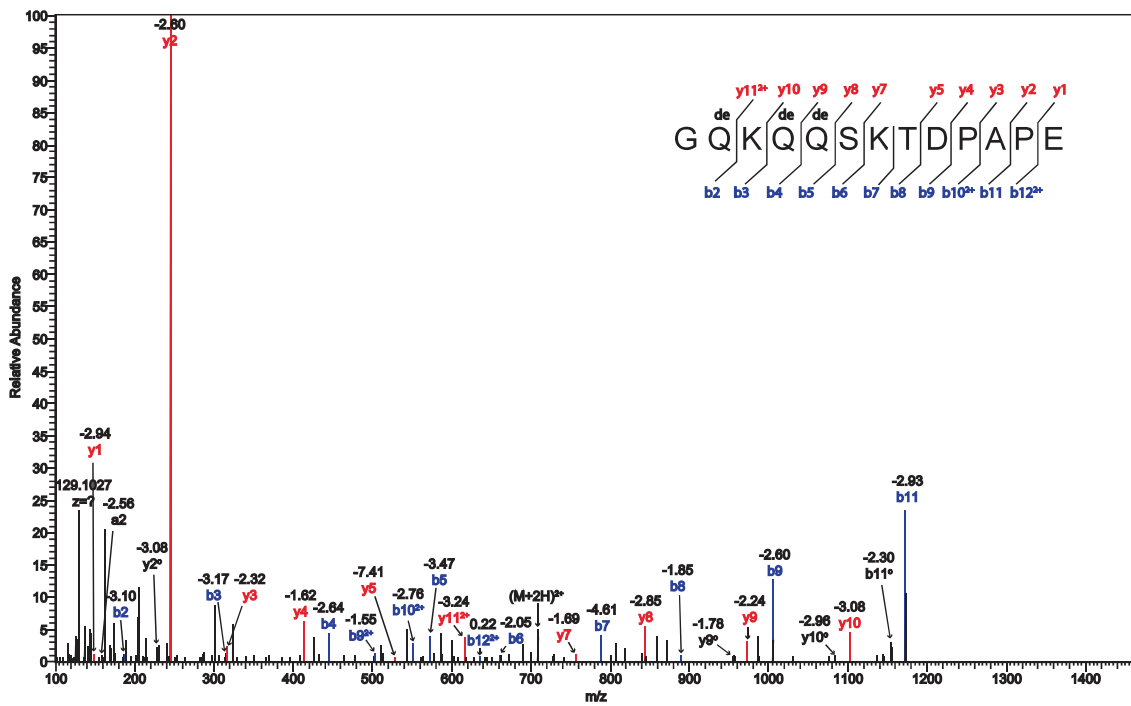


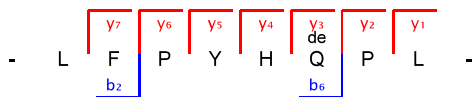
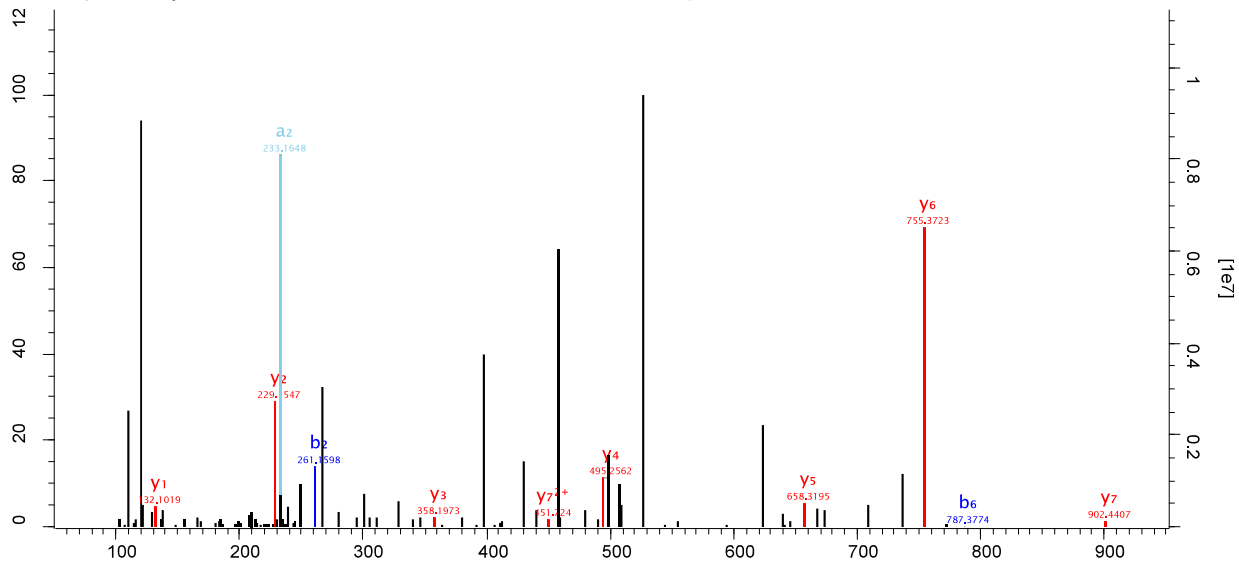


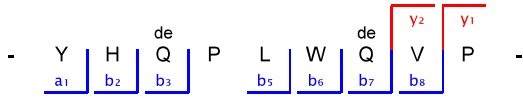
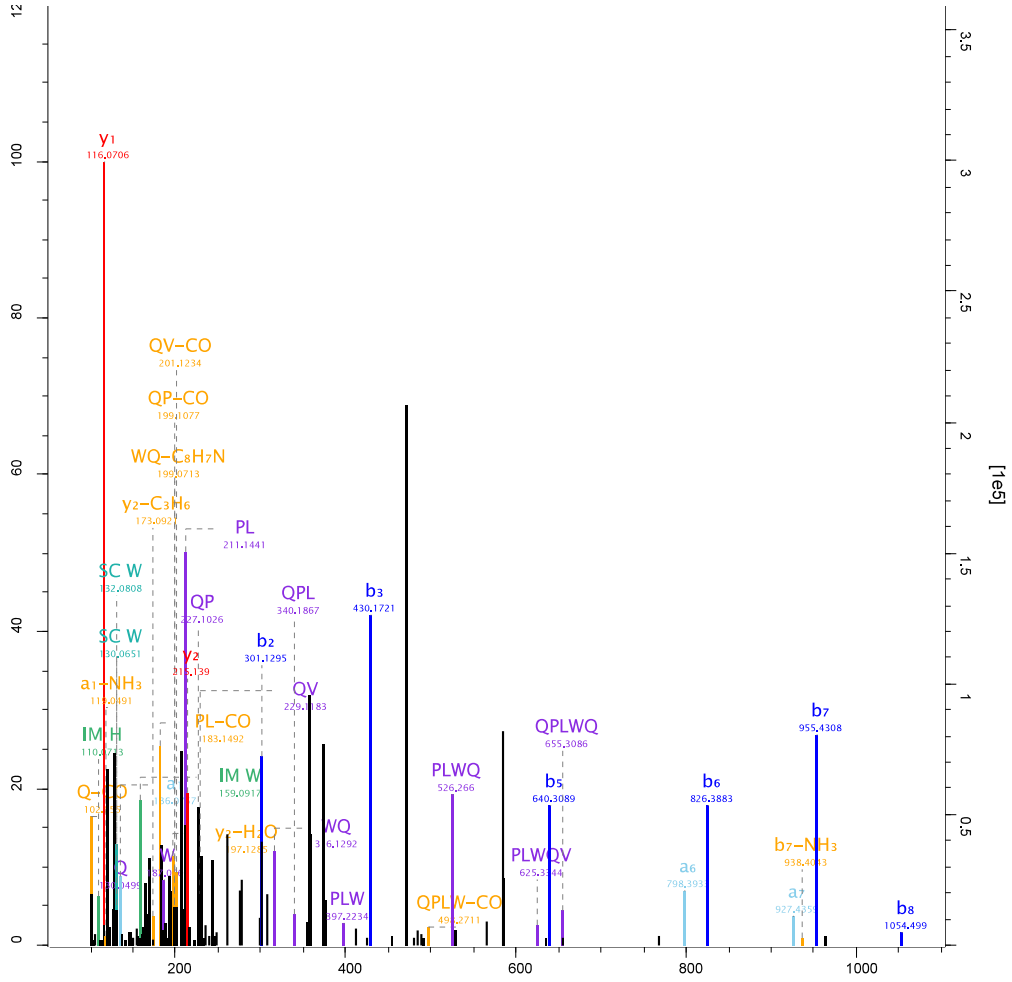


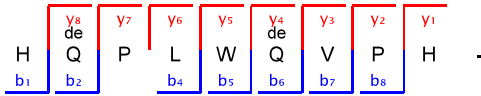
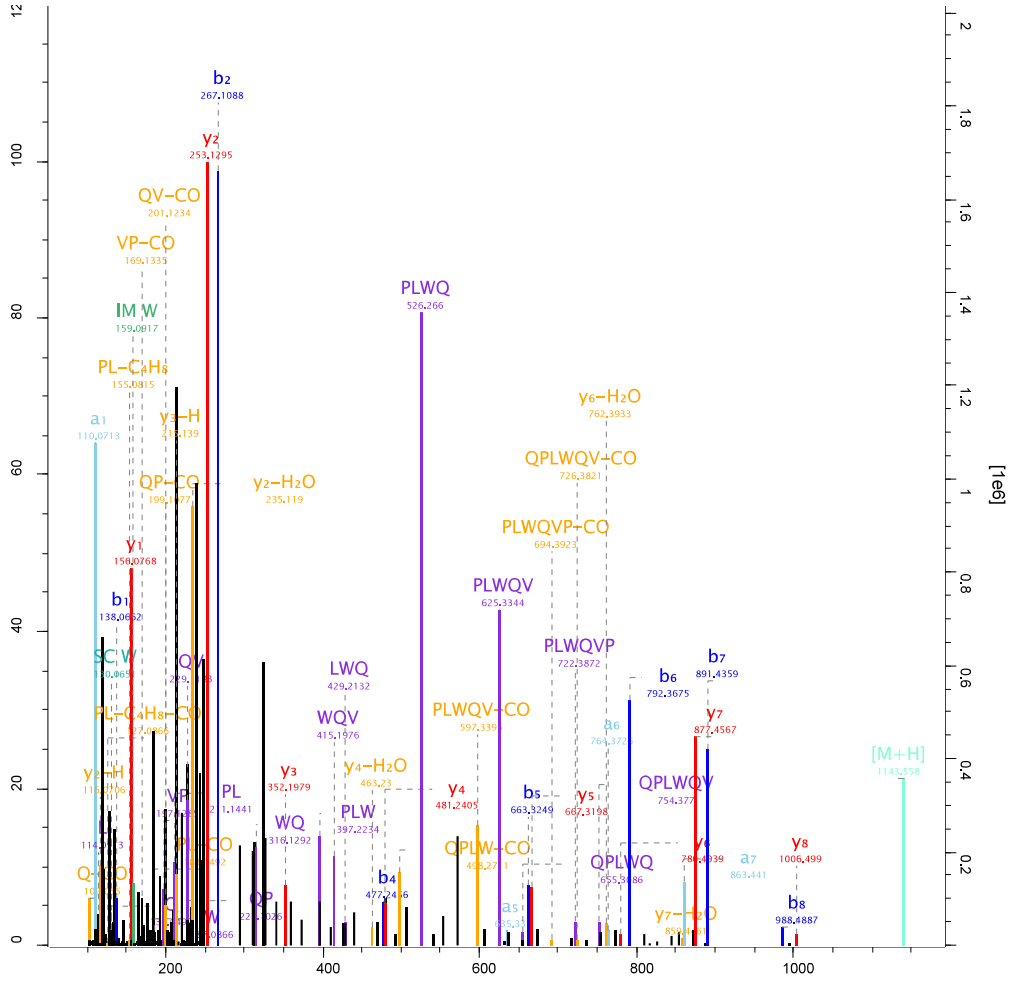


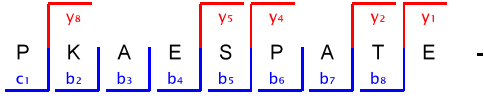
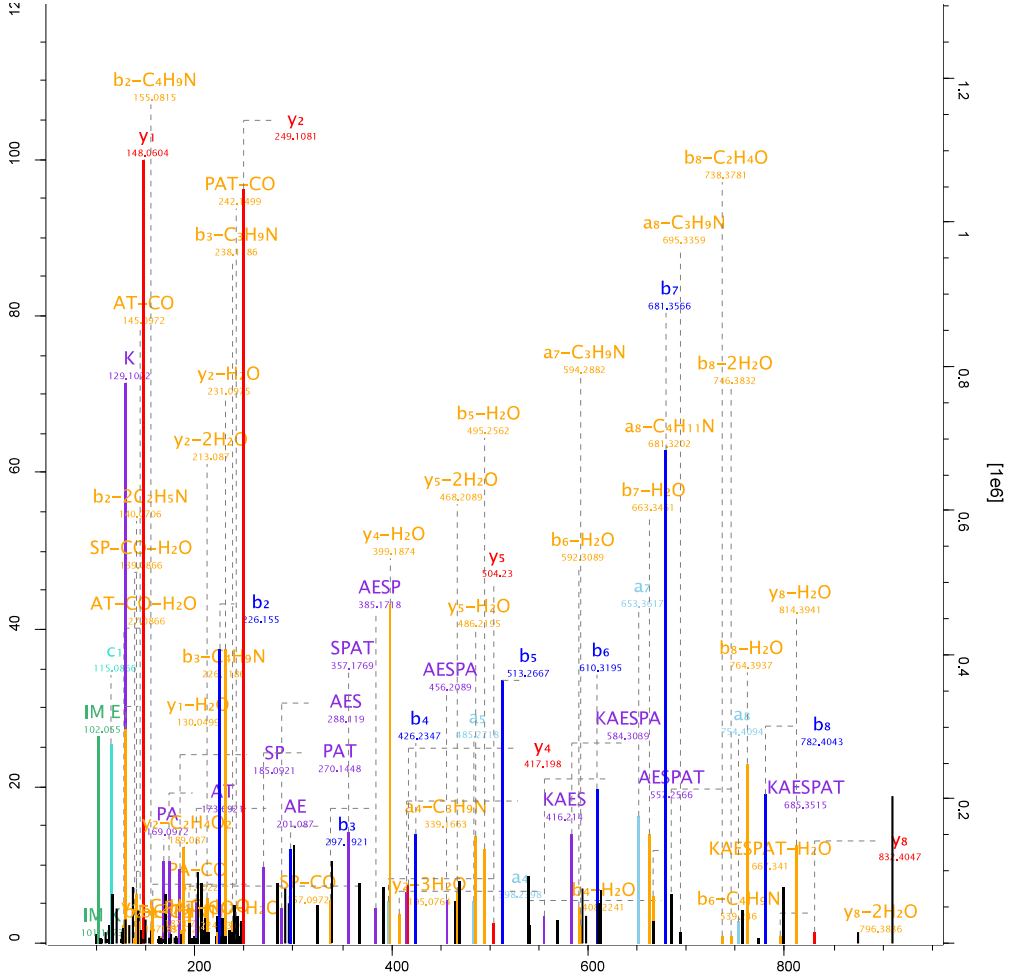
20170509\_QE3\_nLC5\_MEM\_COLLAB\_GeoG\_1217\_01#3535 RT: 15.61 AV: 1 NL: 1.91E5  
T: FTMS + p NSI d Full ms2 708.83@hcd28.00 [100.00-1465.00]



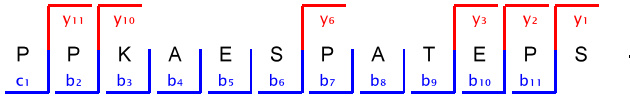
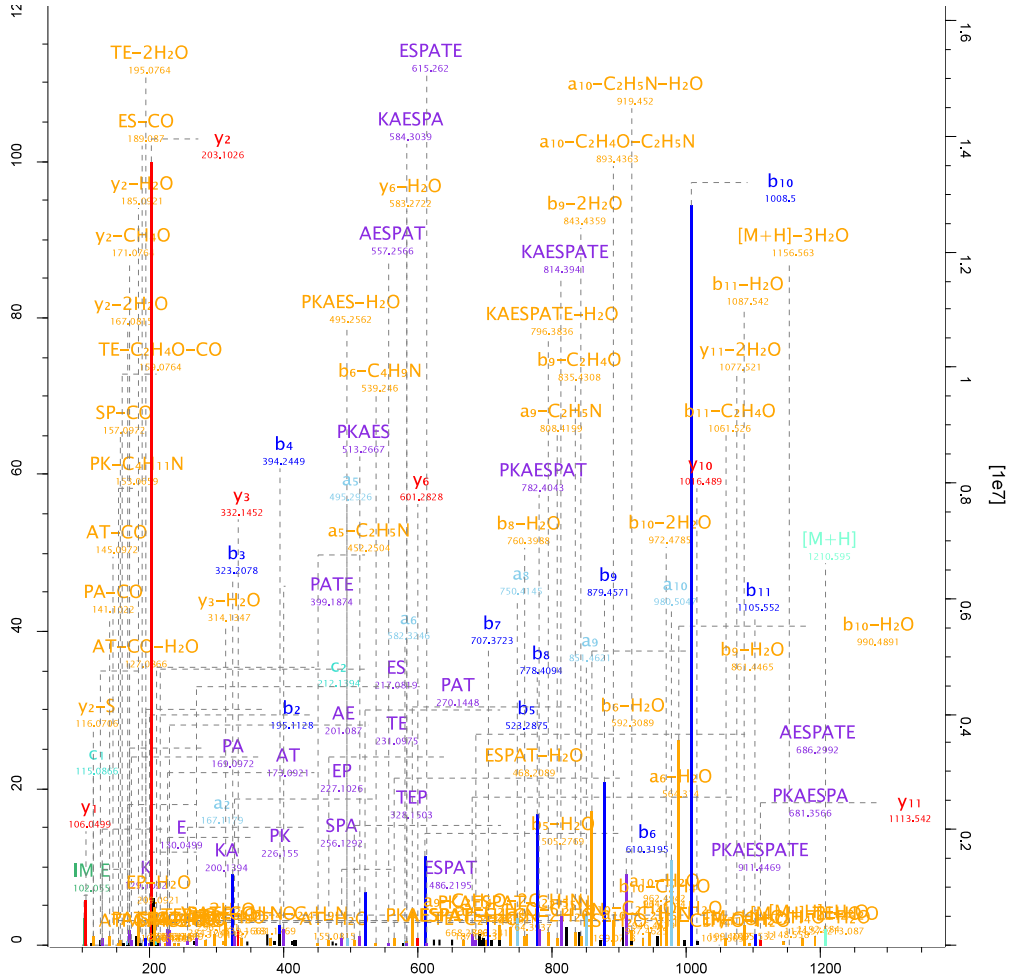


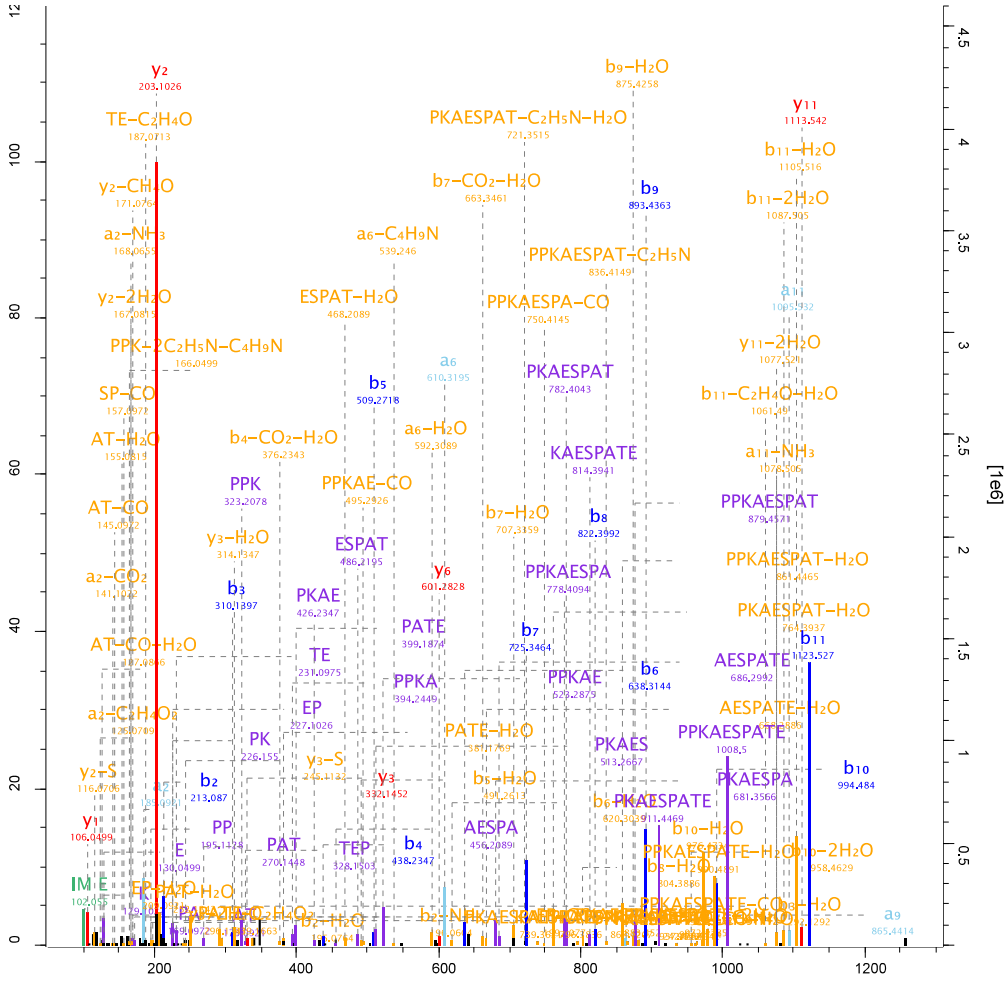






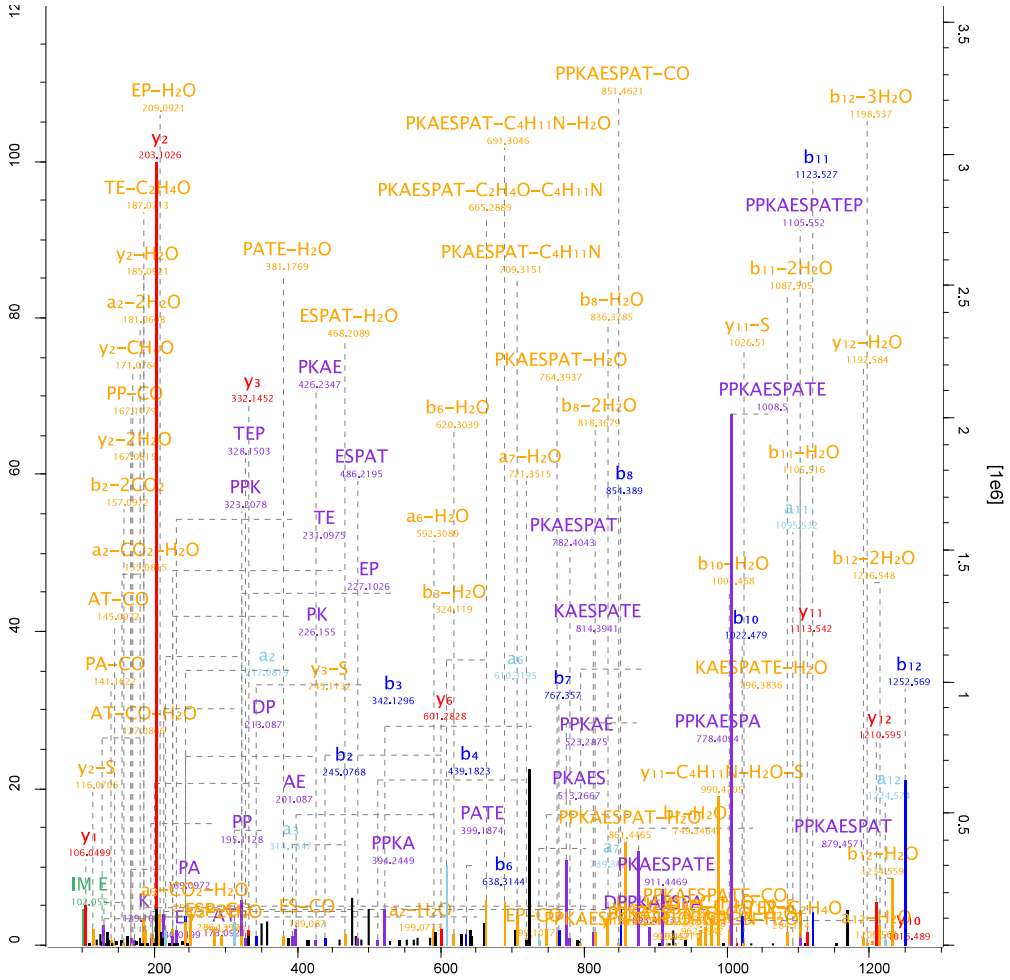




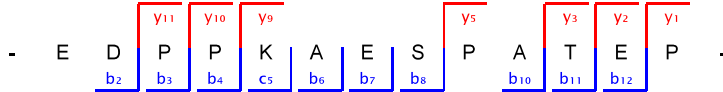
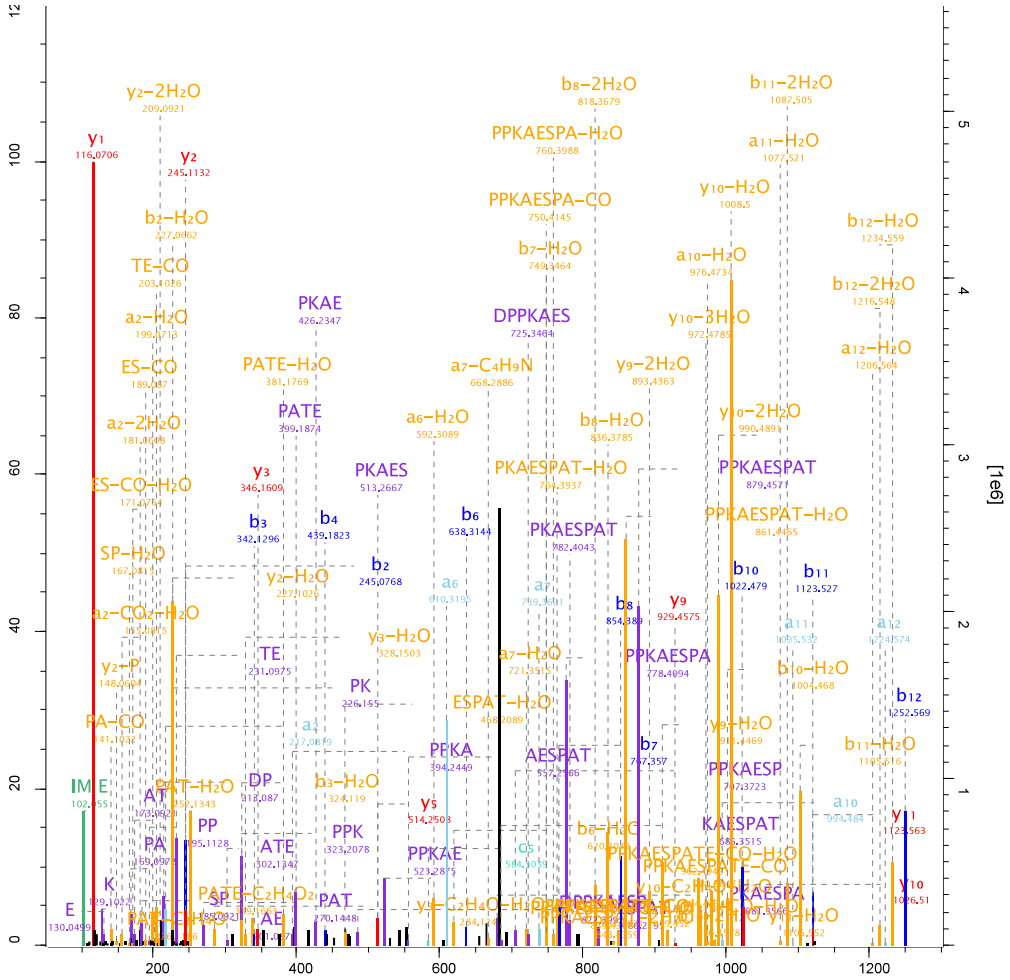


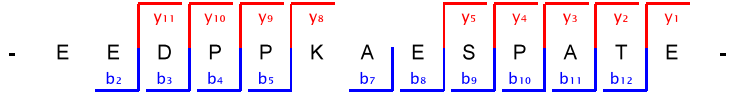
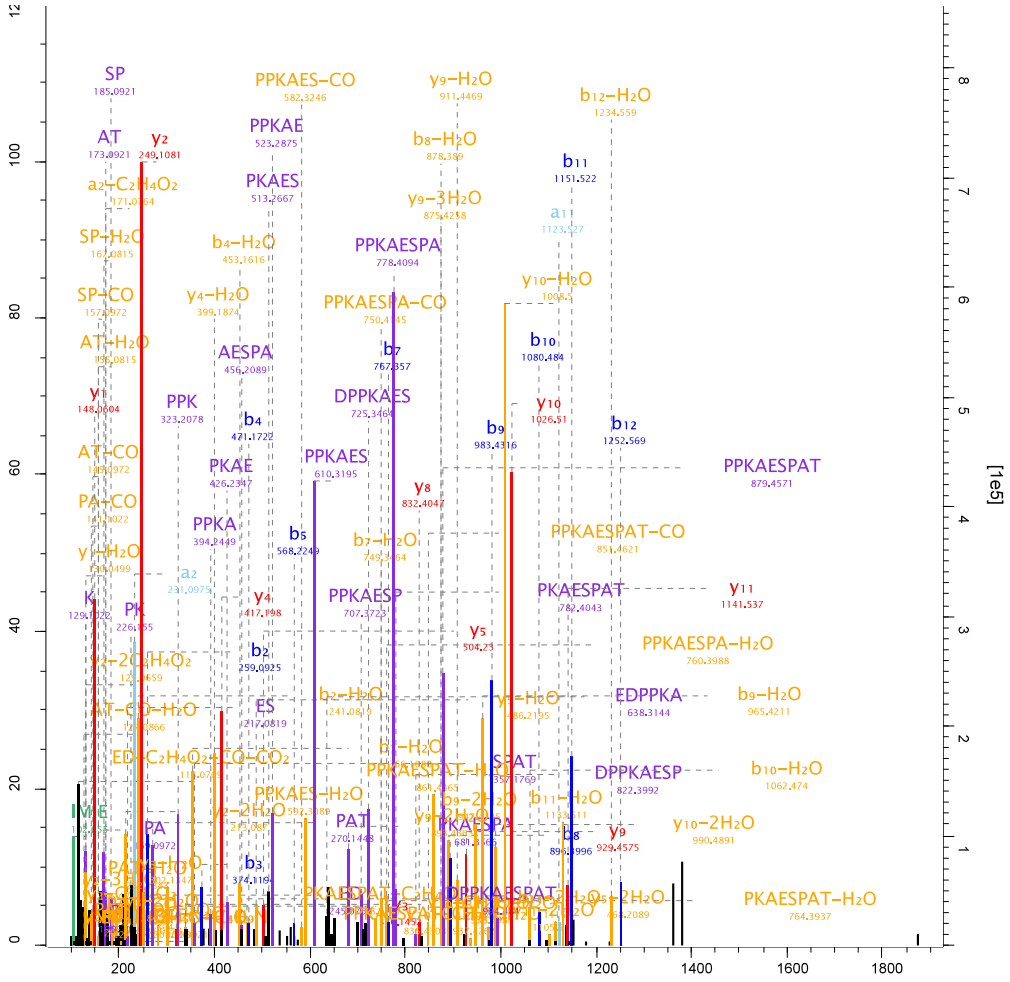
- D P P K A E S P A T E P S -

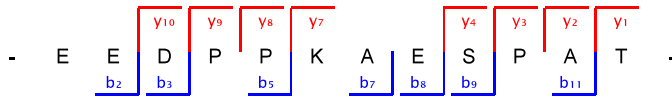
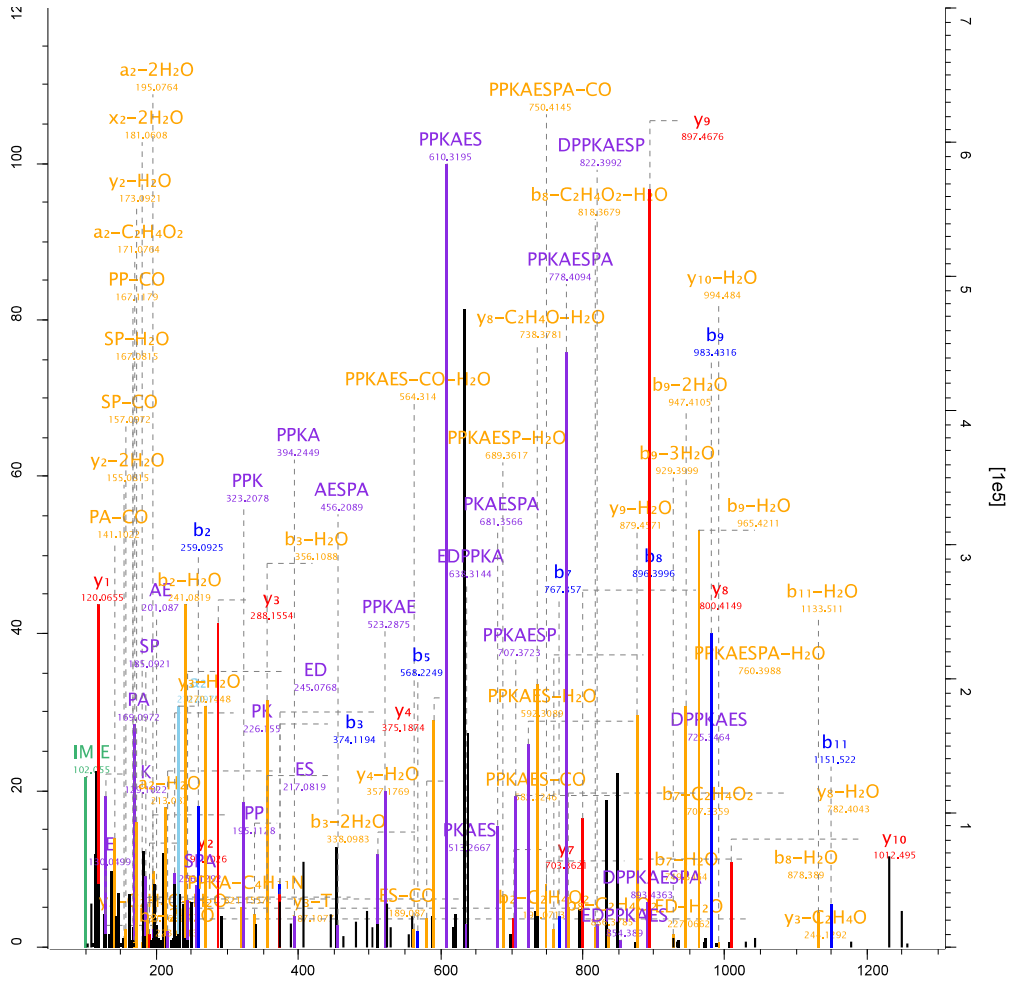
y11  
b2
b3
b4
b5
b6
b7
b8
b9
b10
b11

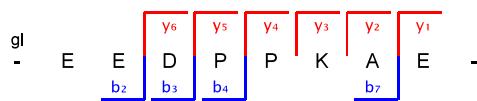
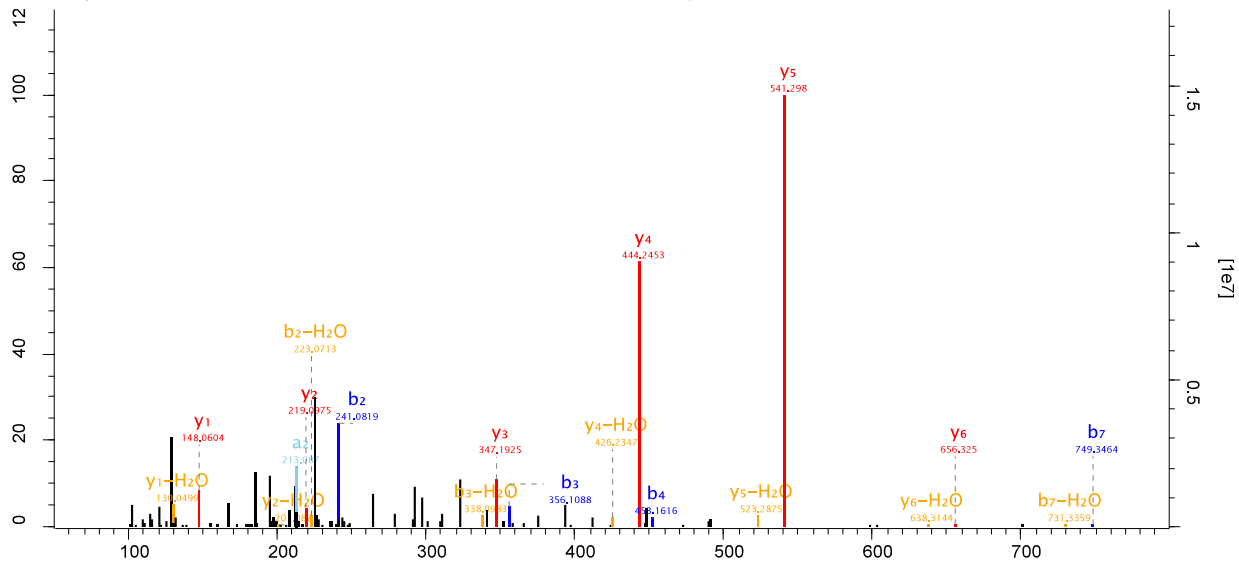


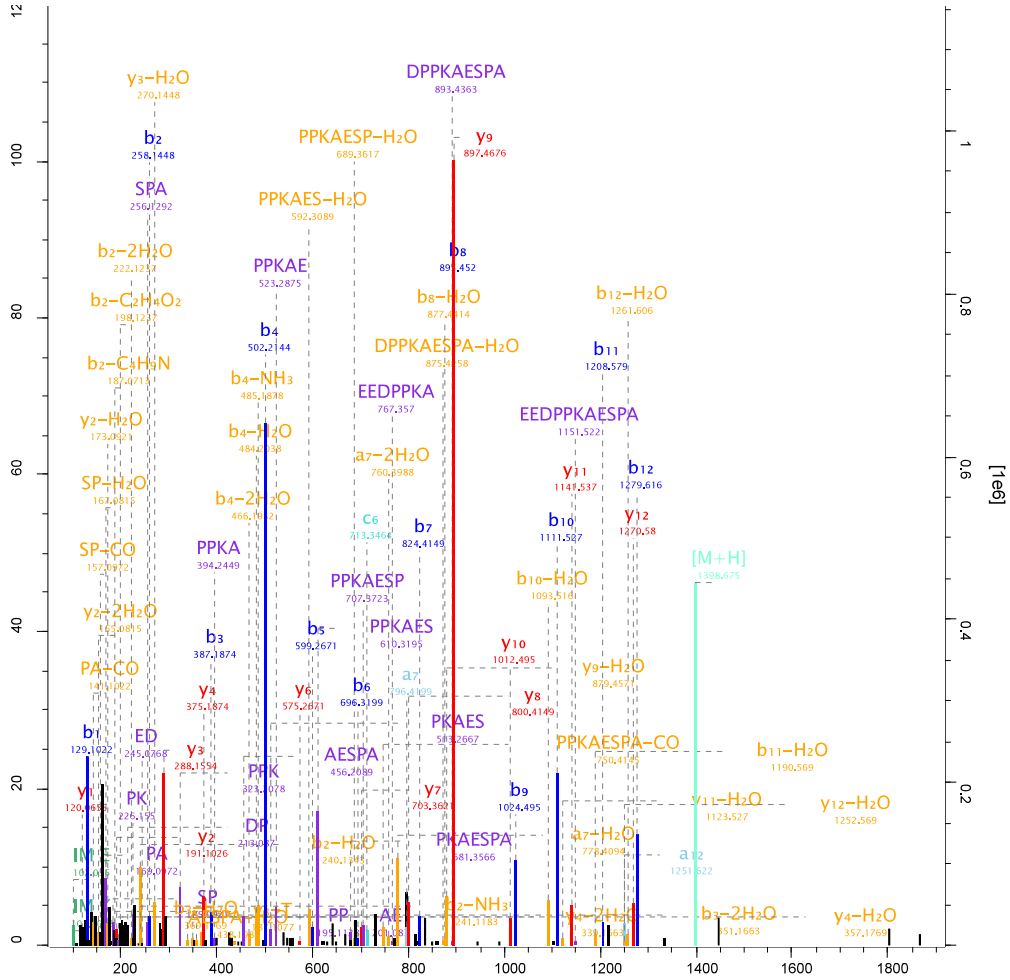
- E D P P K A E S P A T E P S -  
 b<sub>2</sub> b<sub>3</sub> b<sub>4</sub> b<sub>6</sub> b<sub>7</sub> b<sub>8</sub> b<sub>10</sub> b<sub>11</sub> b<sub>12</sub> y<sub>6</sub> y<sub>3</sub> y<sub>2</sub> y<sub>1</sub>





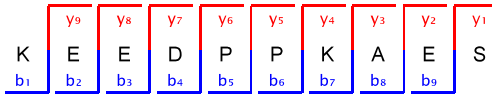
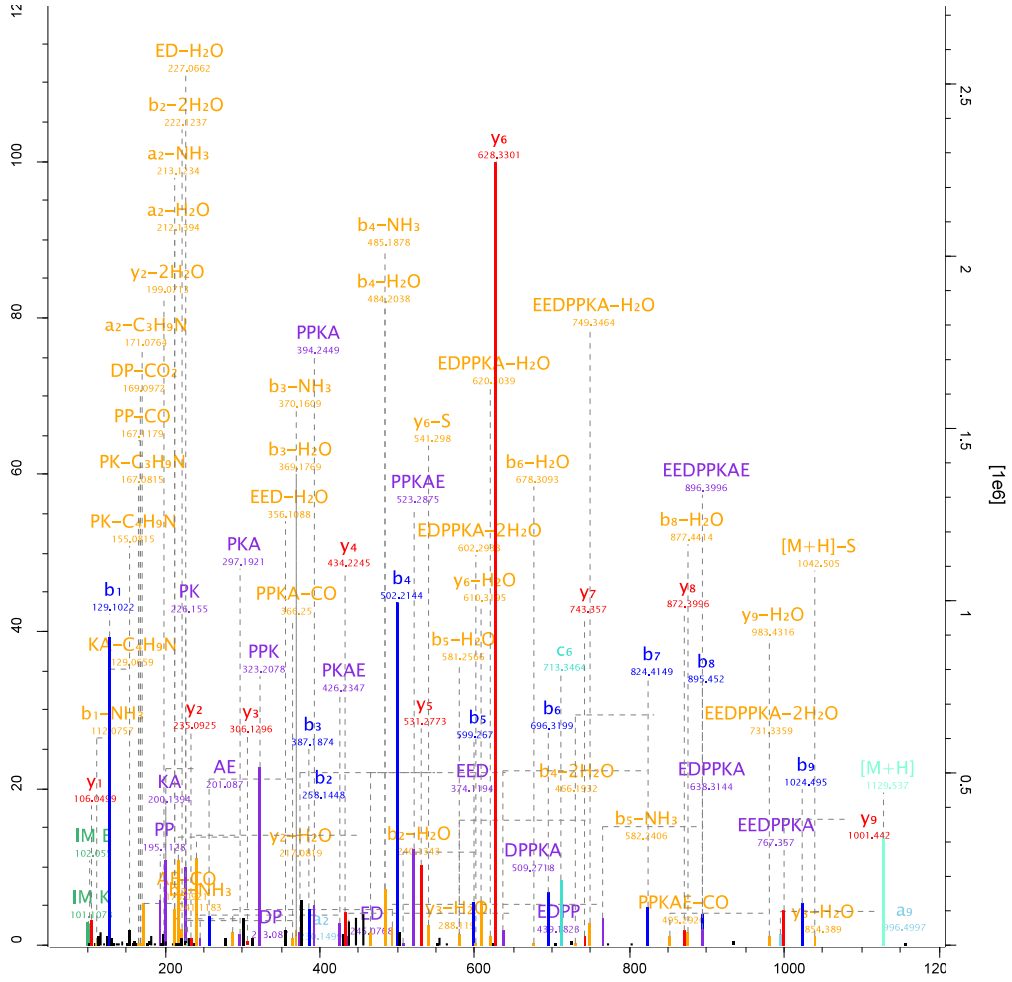


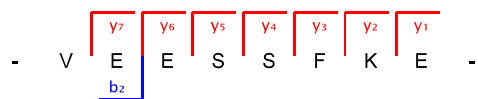
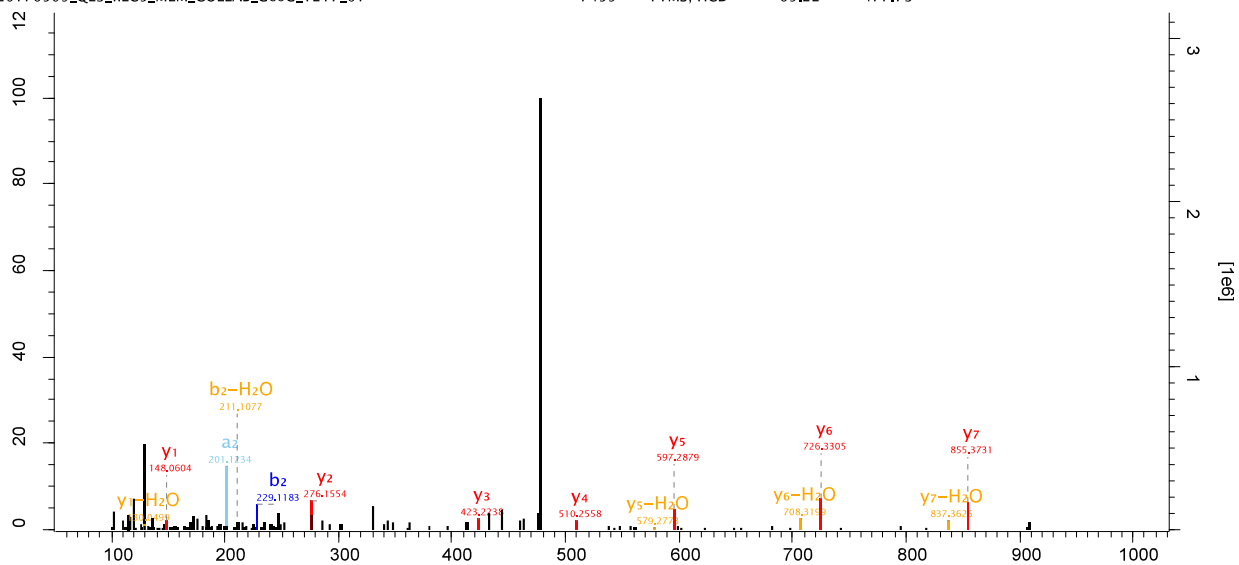


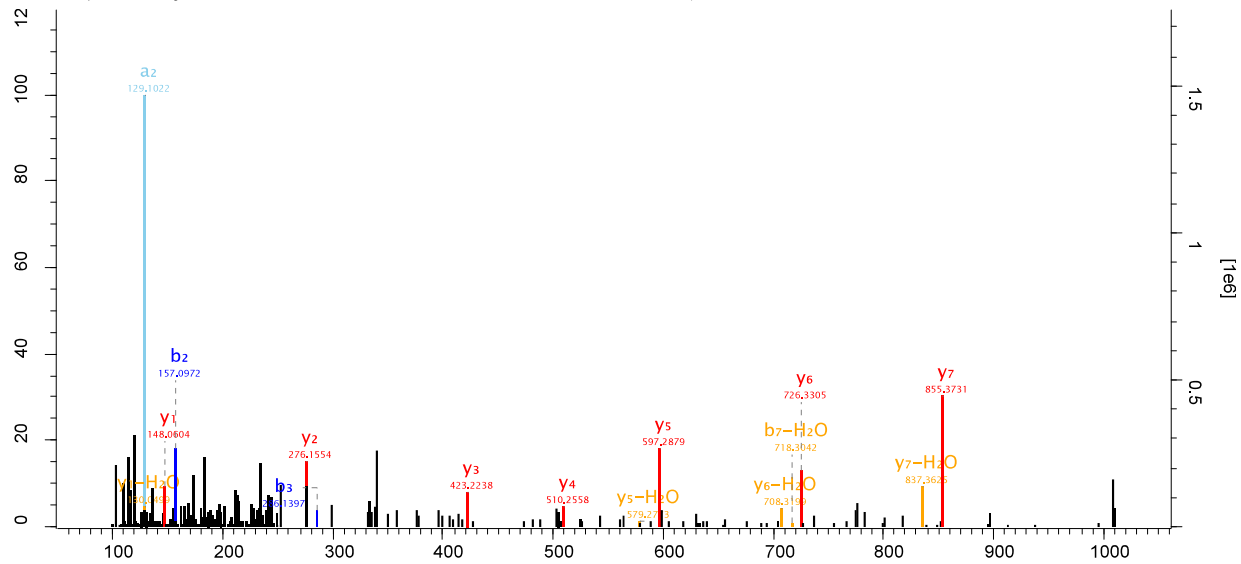


- K E E D P P K A E S P A T -  
 b<sub>1</sub> b<sub>2</sub> b<sub>3</sub> b<sub>4</sub> b<sub>5</sub> b<sub>6</sub> b<sub>7</sub> b<sub>8</sub> b<sub>9</sub> b<sub>10</sub> b<sub>11</sub> b<sub>12</sub>

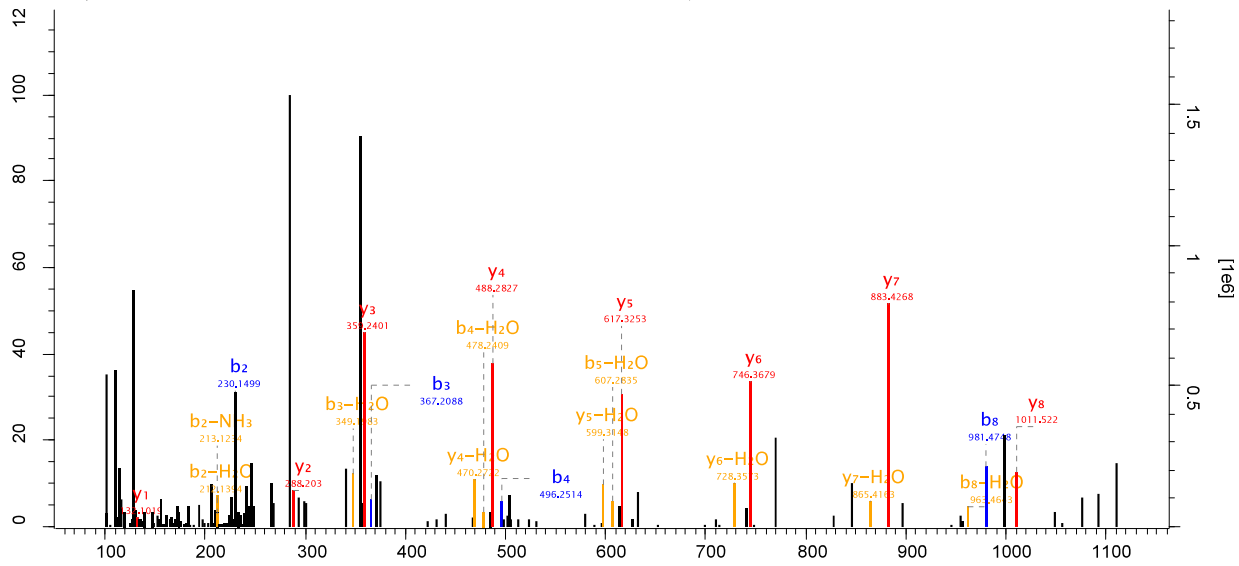




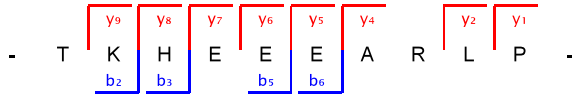
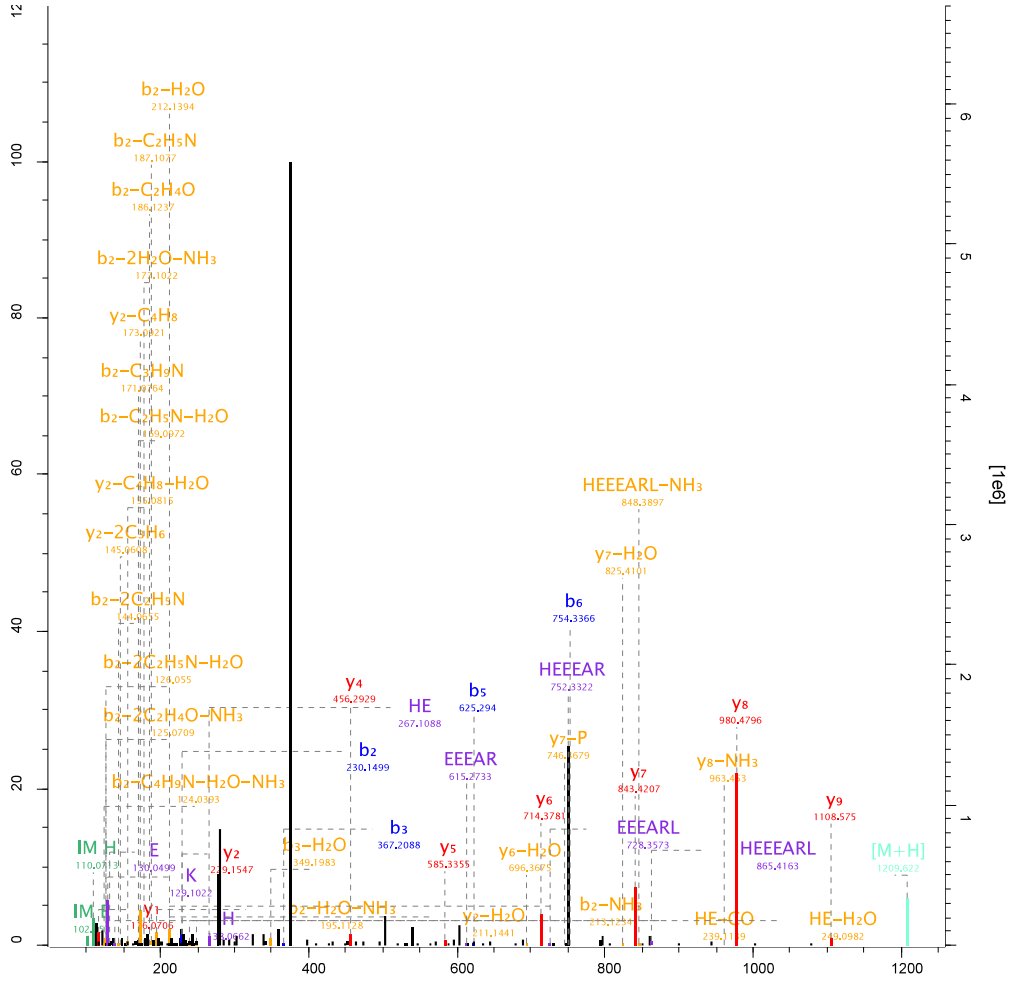


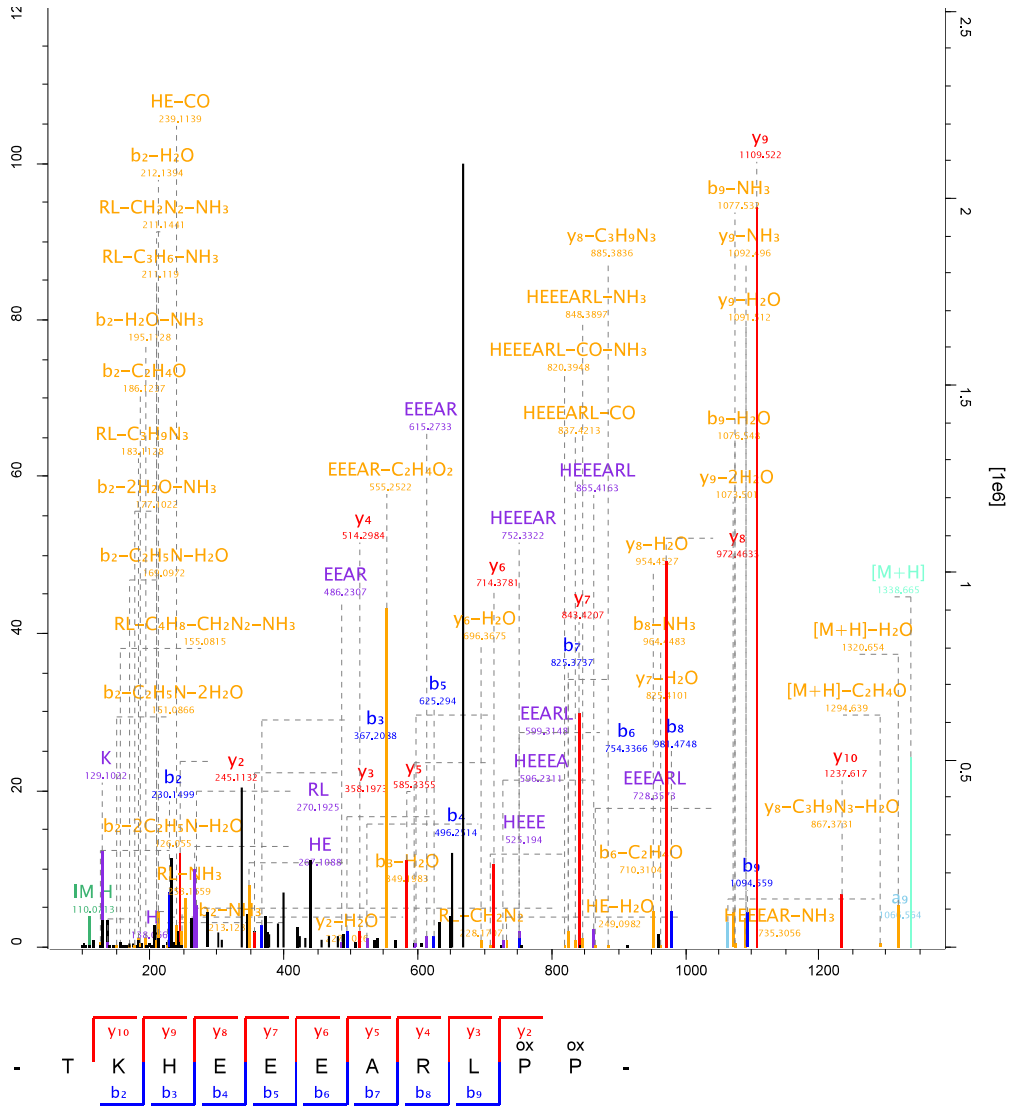


- G V E E S S F K E -  
b2 b3 y7 y6 y5 y4 y3 y2 y1

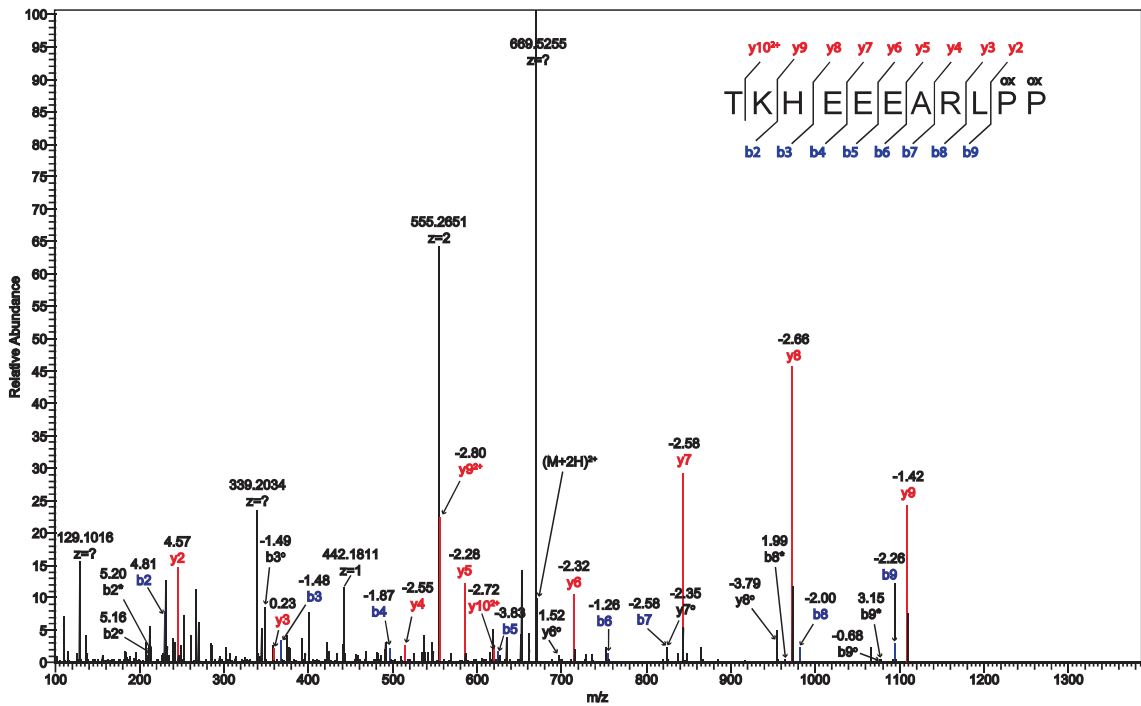


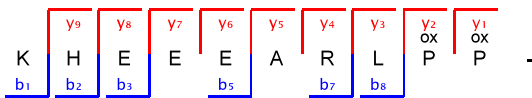
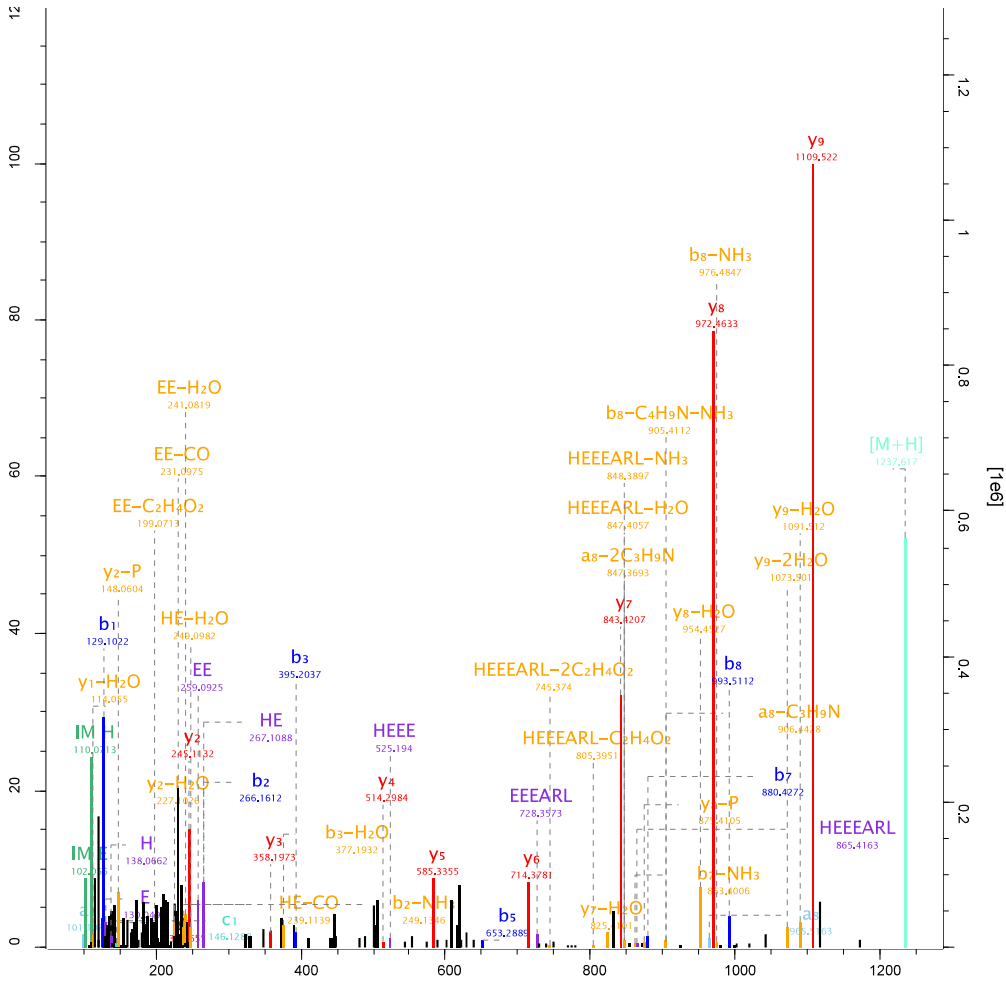
- T K H E E E A R L -  
          b2 b3 b4                  b8



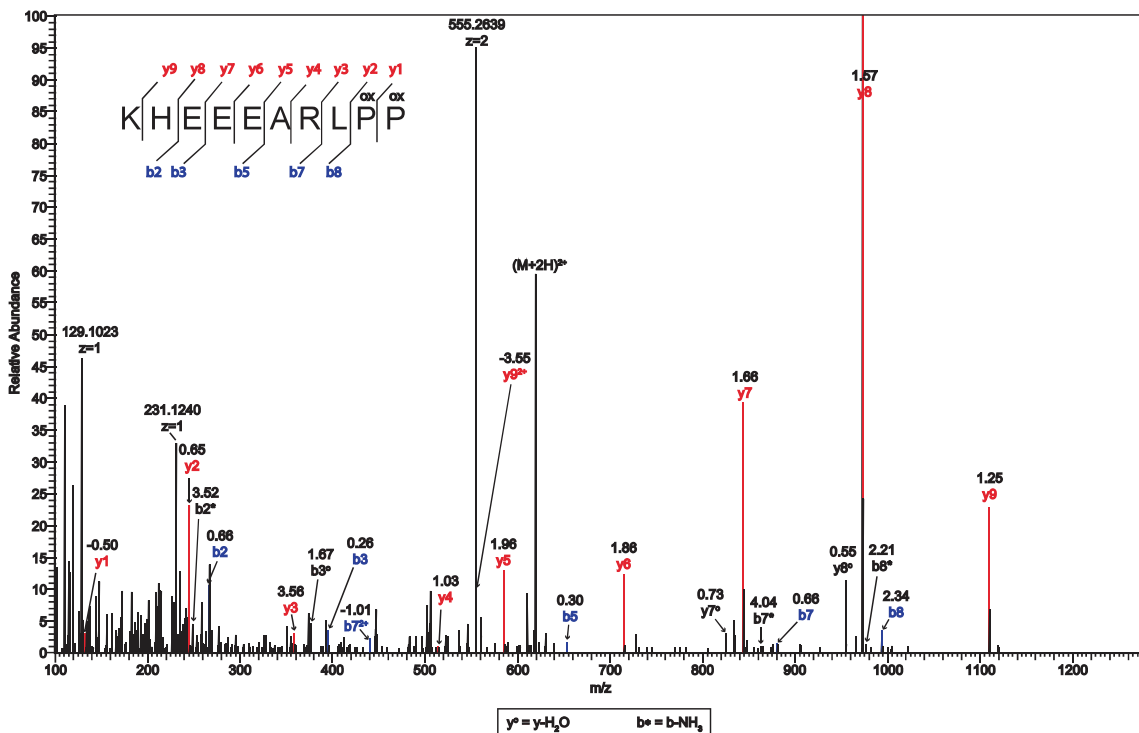


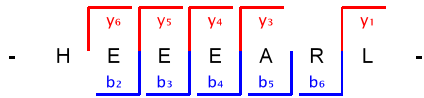
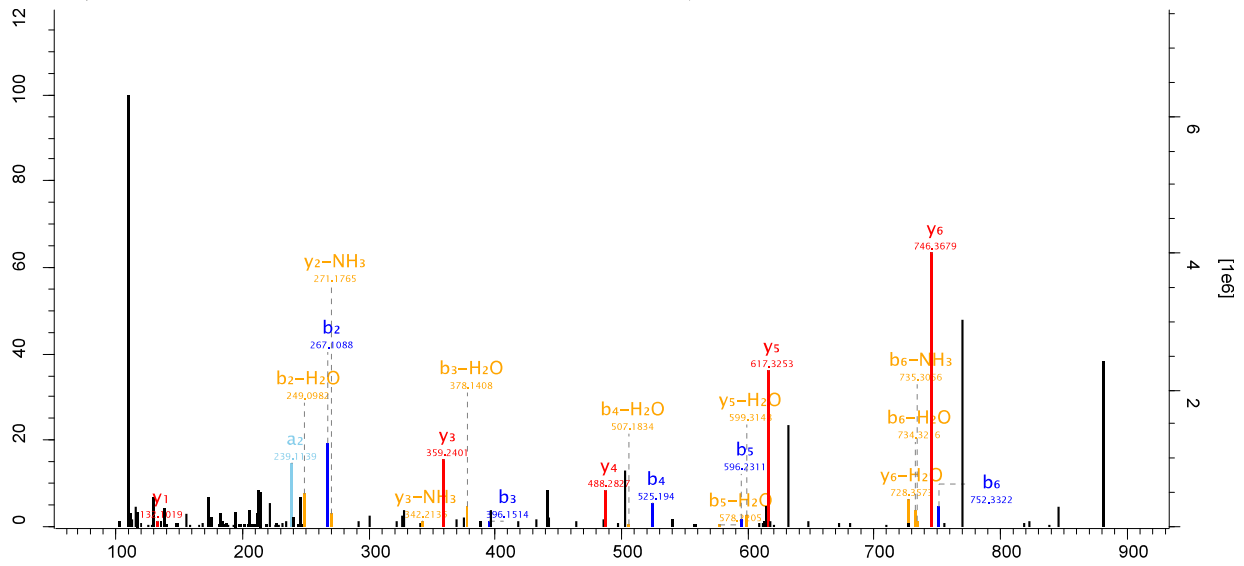
20170509\_QE3\_nLC5\_MEM\_COLLAB\_GeoG\_1217\_01 #5820 RT: 22.17 AV: 1 NL: 4.63E5  
T: FTMS + p NSI(d Full ms2 669.84@)ms228.00 [100.00-1386.00]



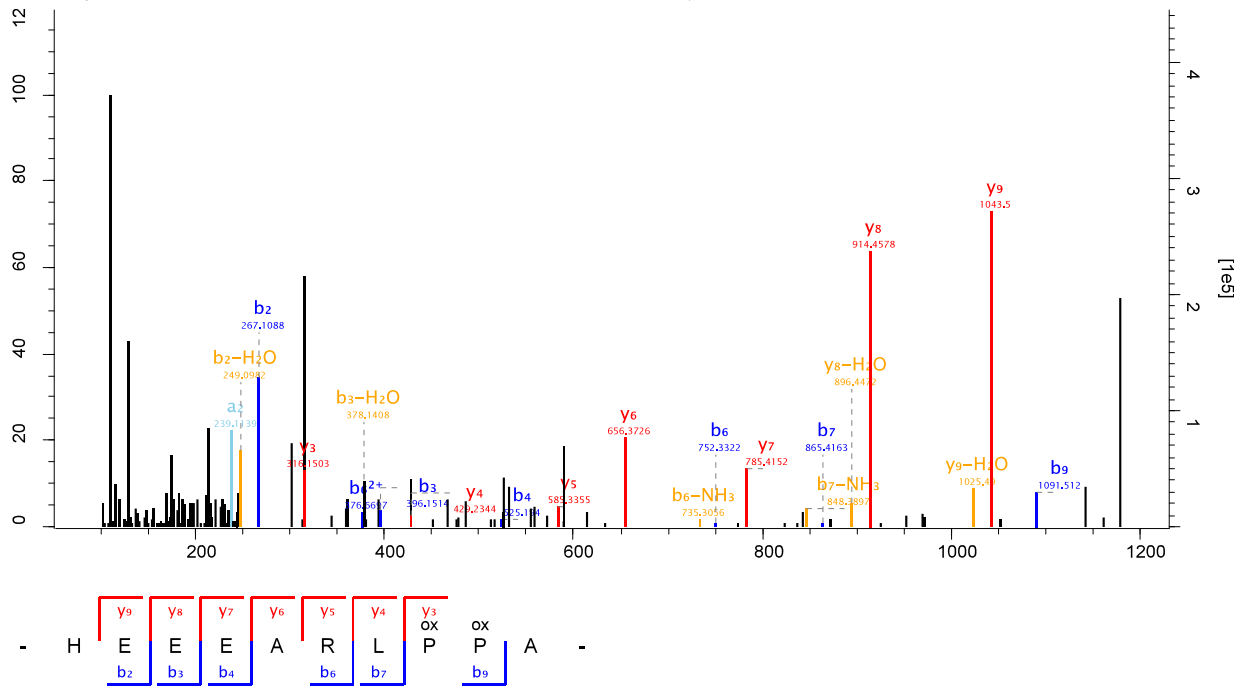


20151231\_QE5\_UPLC1\_RJC\_COLLAB\_GeoG\_870\_01 #17185 RT: 61.55 AV: 1 NL: 1.73E5  
T: FTMS + p NSI d Full ms2 619.32@hcd28.00 [100.00-1280.00]

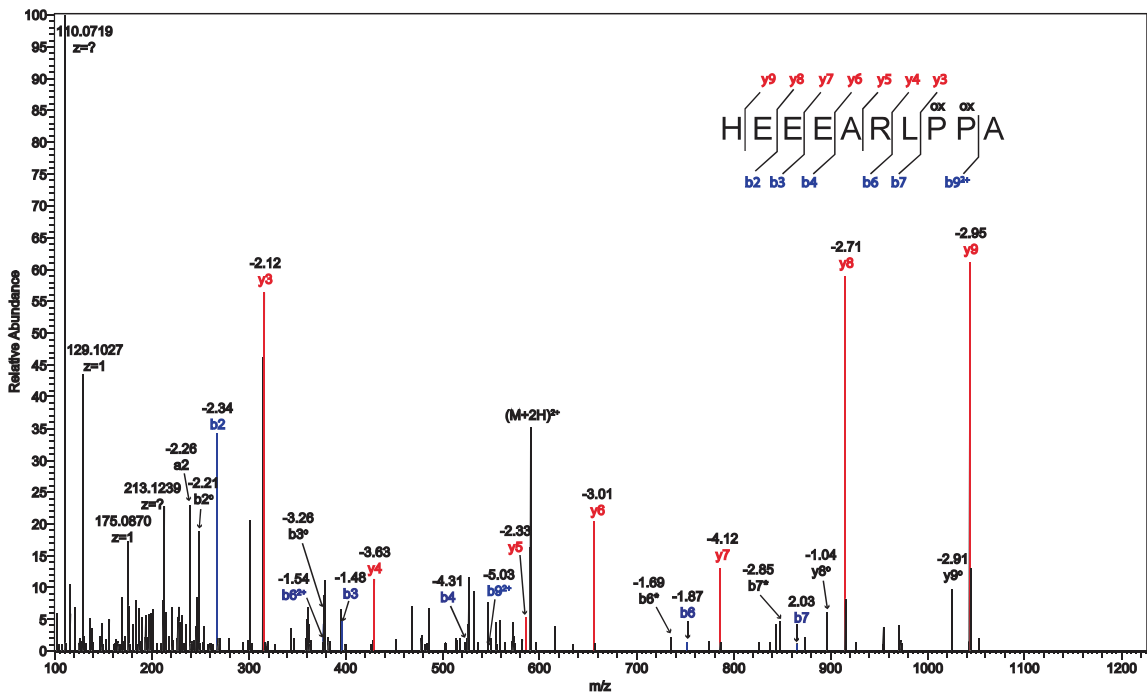


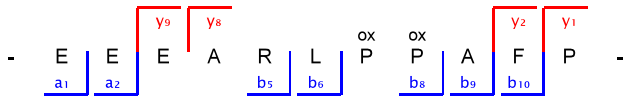
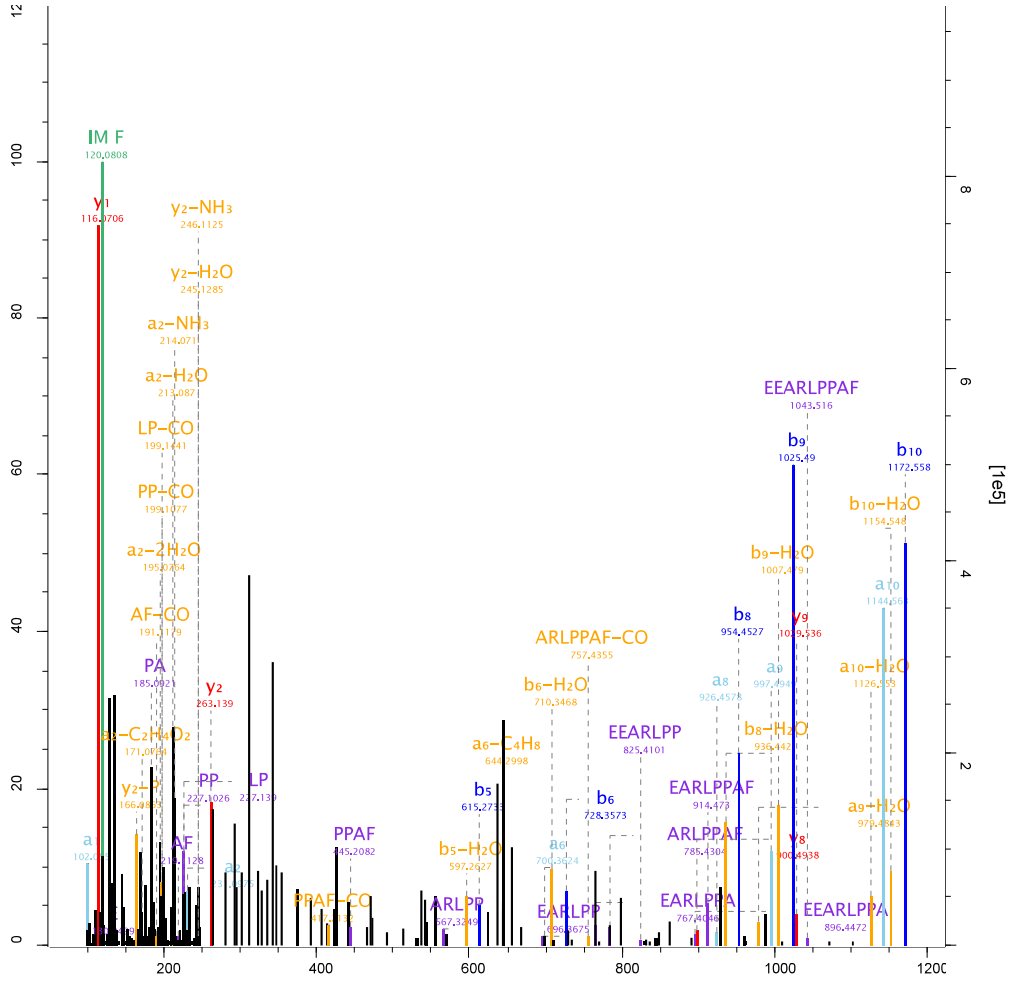


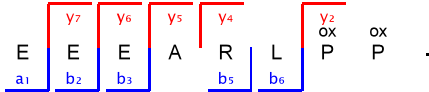
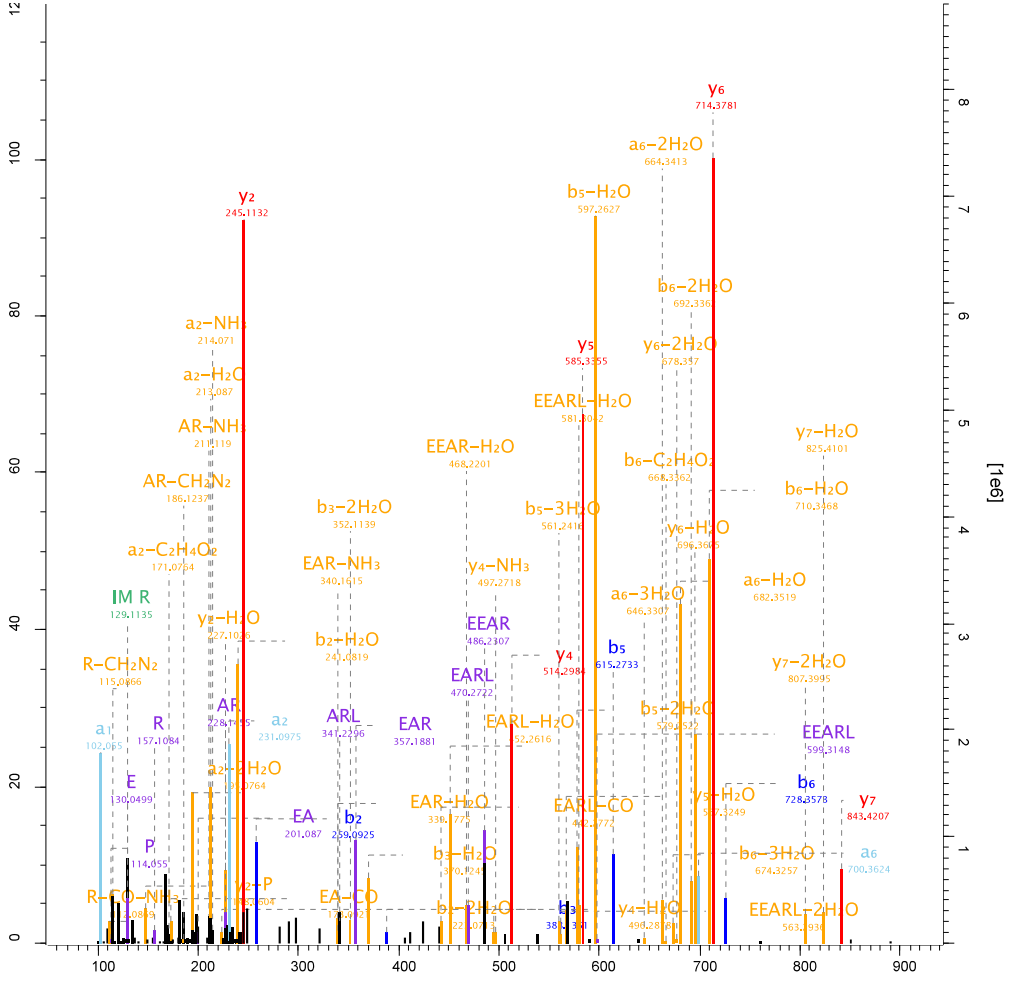


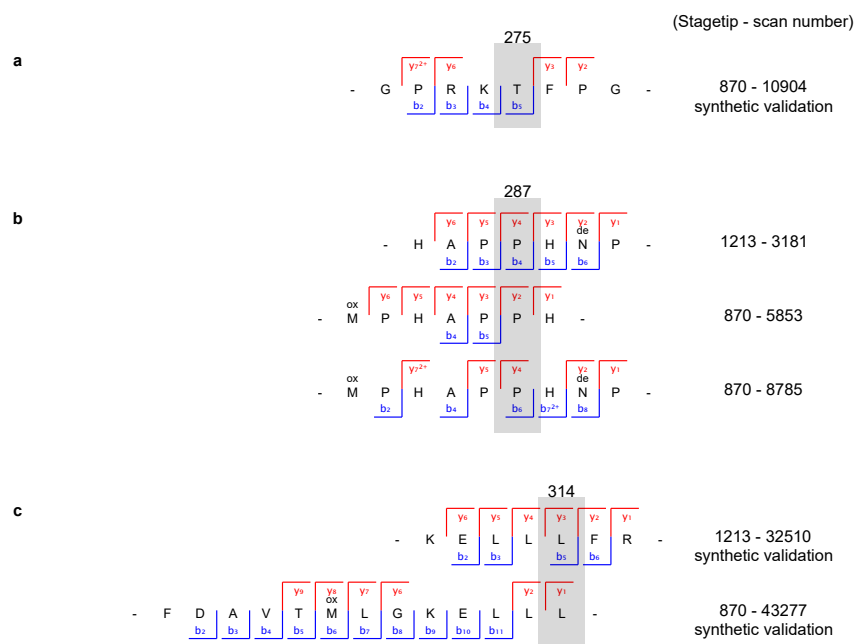


20170509\_QE3\_nLC5\_MEM\_COLLAB\_GeoG\_1217\_01 #9804 RT: 34.30 AV: 1 NL: 9.18E4  
T: FTMS + p NSI'd Full ms2 590.78@hcd28.00 [100.00-1225.00]

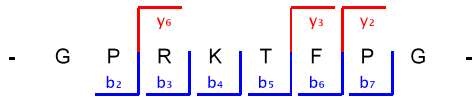
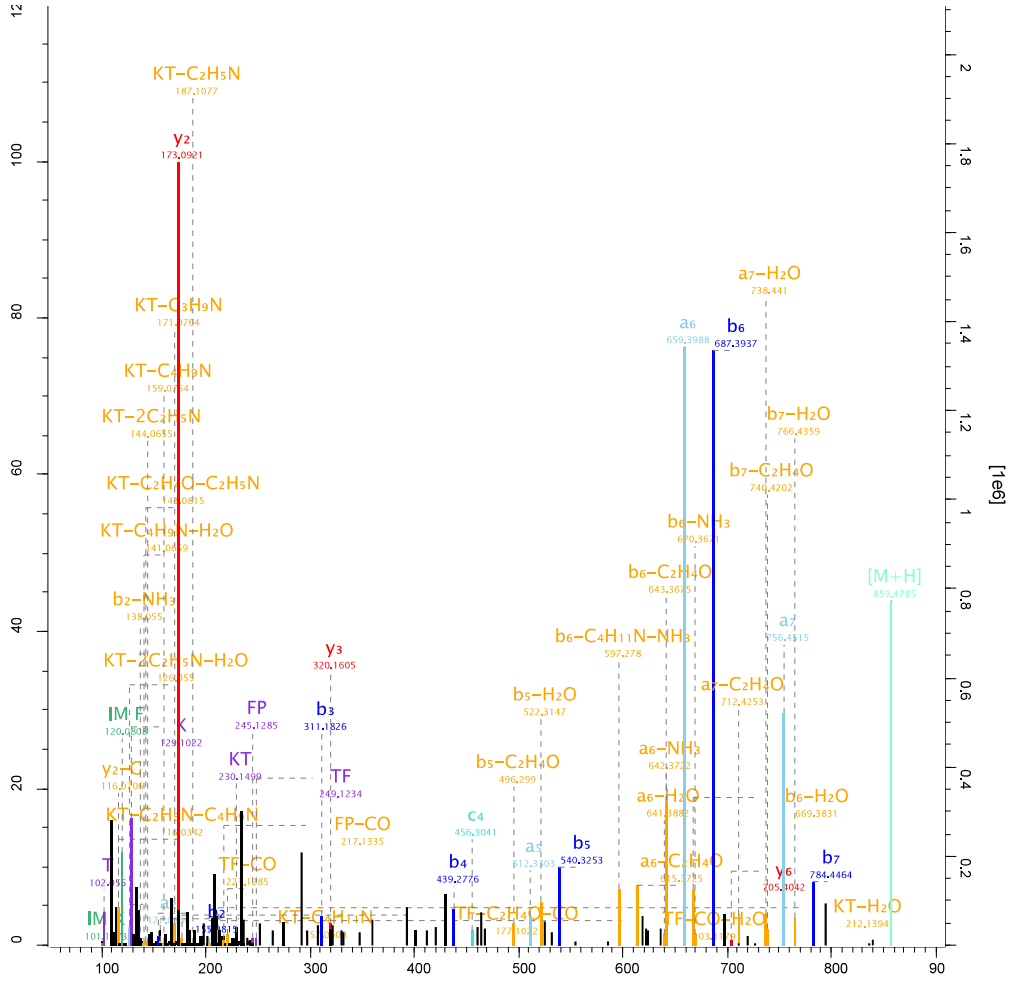


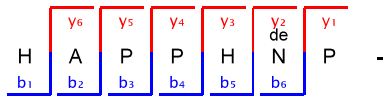
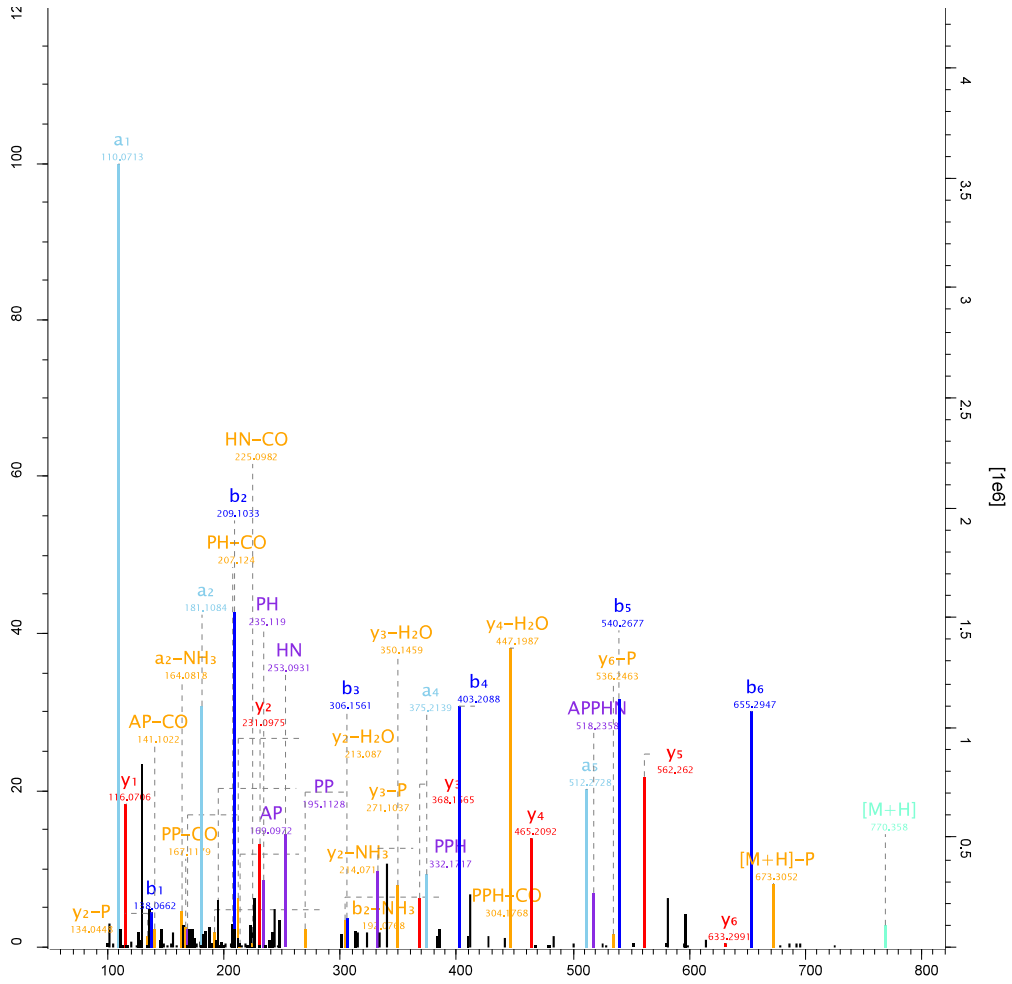


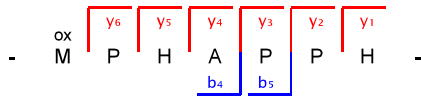
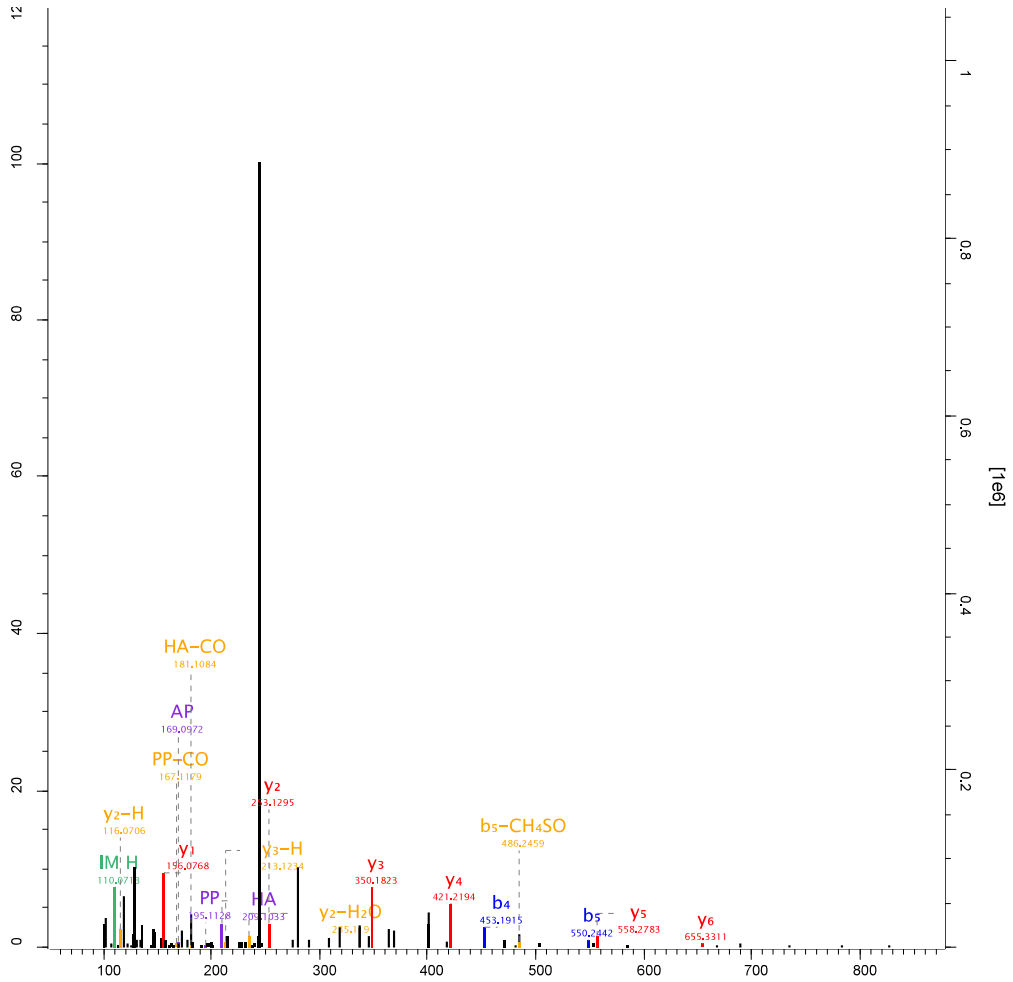


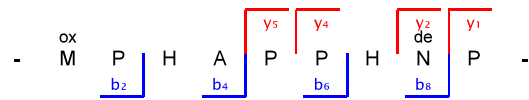
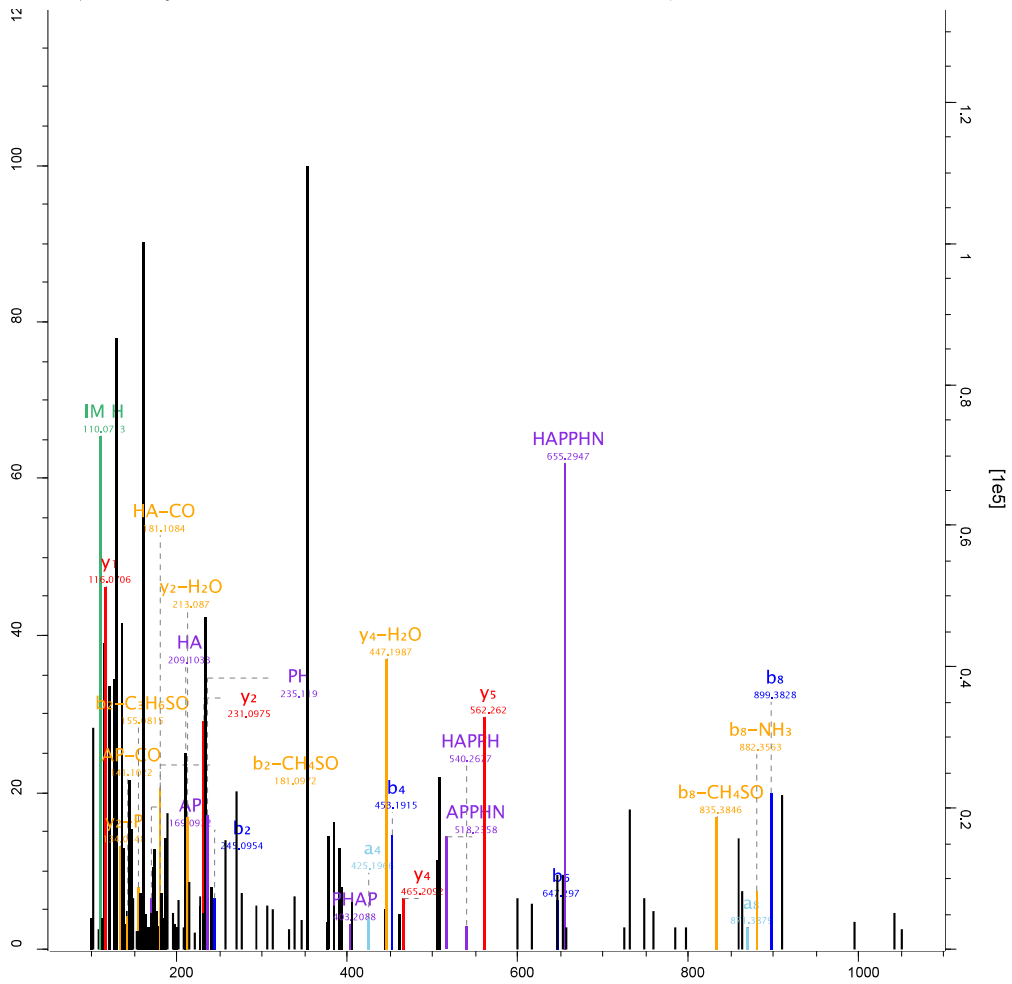


**MMP20.** Peptide matches to phylogenetically informative positions 275 (a), 287 (b), and 314 (c). Coordinates in reference to XP\_004427457.

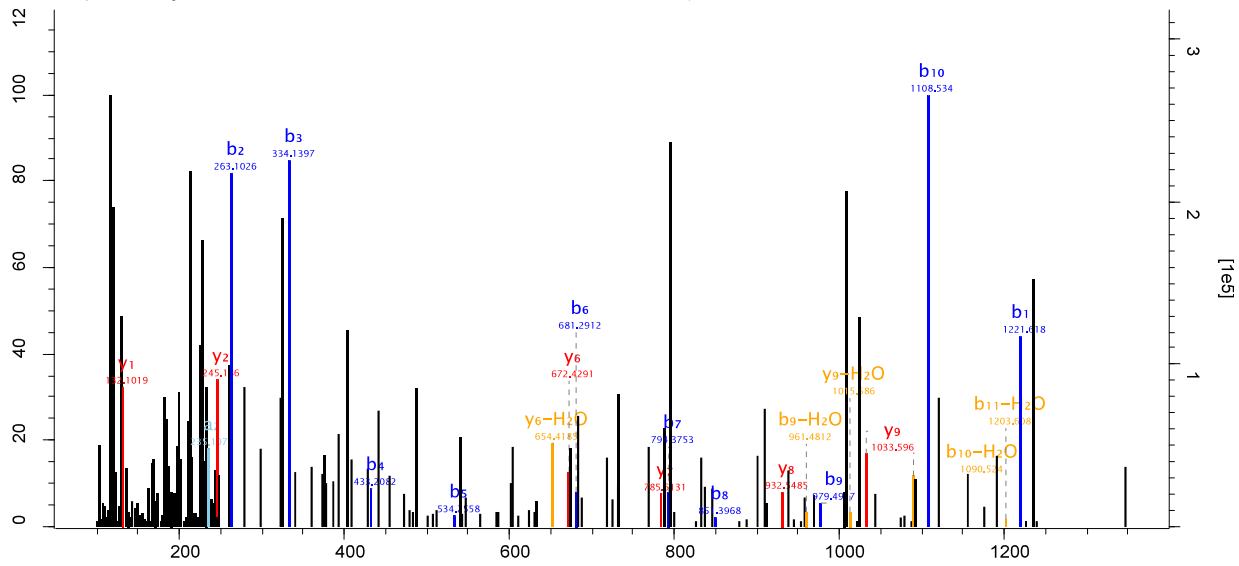


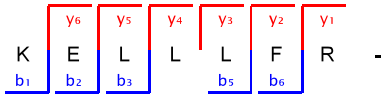
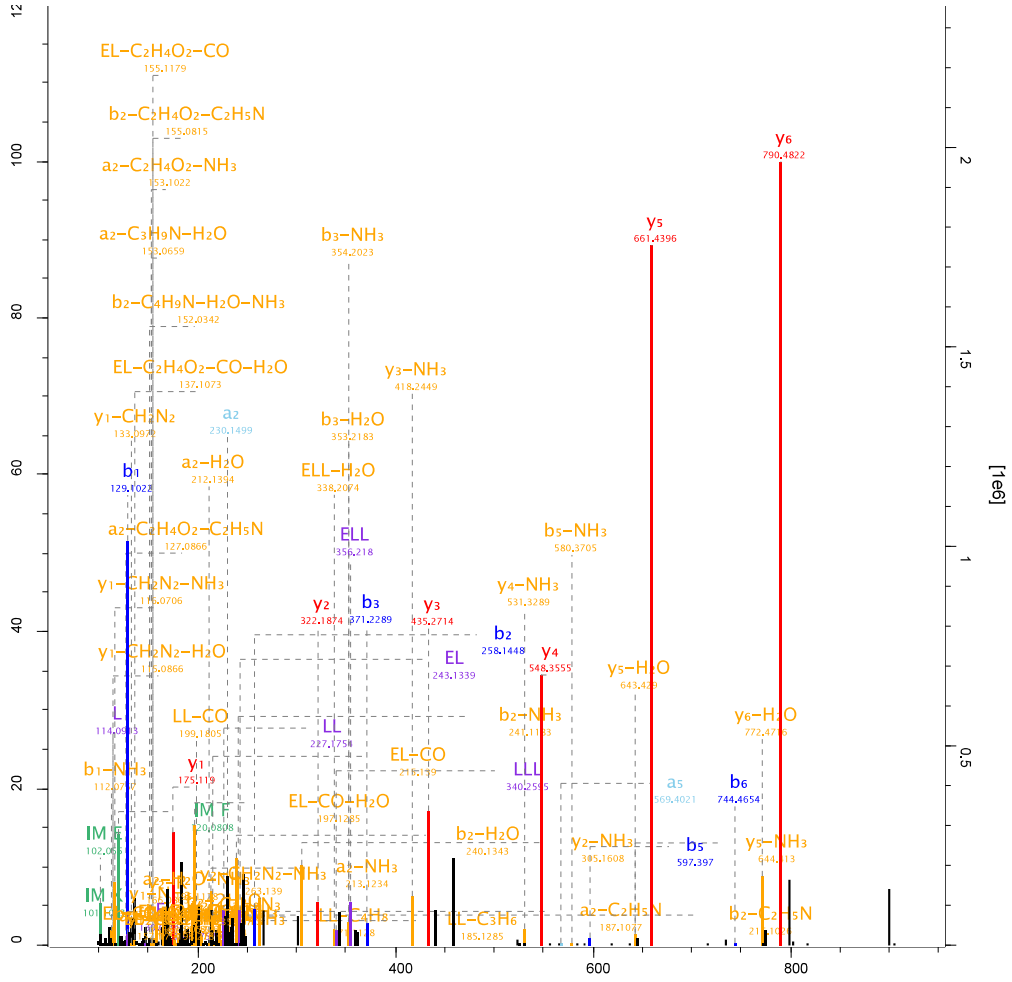












## Synthetic Peptides

The following pages contain the manual annotation of phylogenetically informative peptides (n=18) and phosphopeptides (n=3) confirmed through synthetic production of the peptide sequences identified through MaxQuant. Both synthetic and ancient peptides are manually annotated through the Interactive Peptide Spectral Annotator (IPSA). These annotations, and associated mass errors, indicate almost complete correspondence between the two peptide datasets. This includes the phosphopeptides.

For each peptide, the MS2 spectrum of the synthetic peptide is shown at the top of the page, and the corresponding MS2 spectrum of the ancient peptide is shown at the bottom of the page. MaxQuant fragment ion annotation (automated) for the latter spectra can be found earlier in the document.

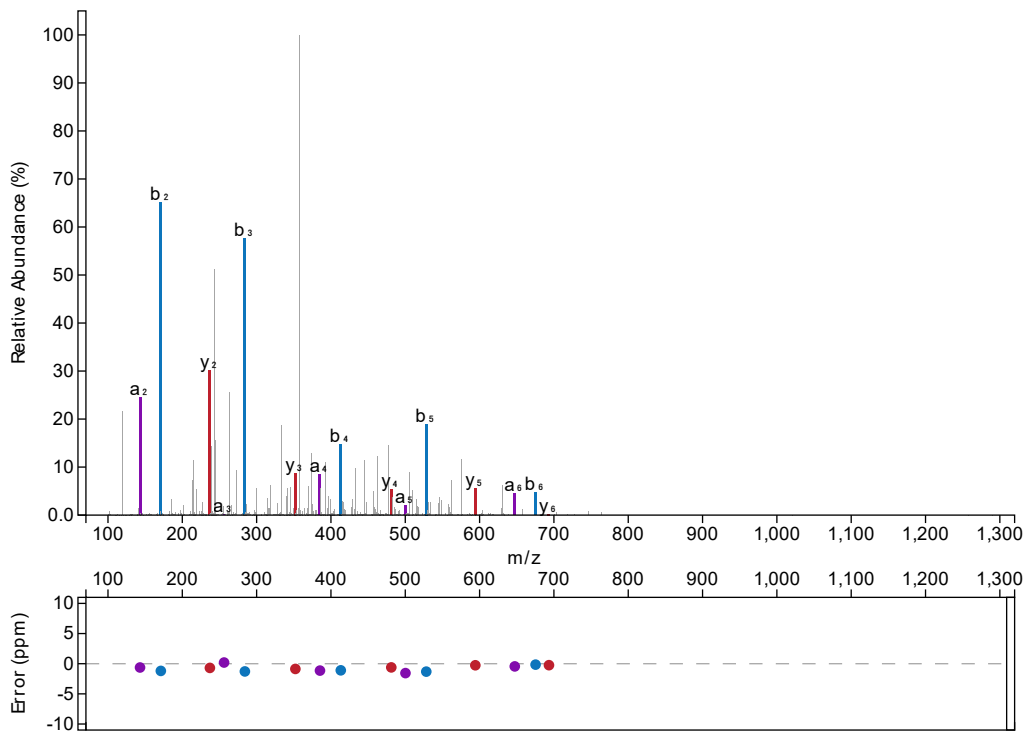
Note that some spectra contain additional high-intensity fragment ions that are not identified as y- or b-ions. This is likely due to the cofragmentation of impurities present in the synthetic and/or ancient peptide mixture.



Precursor m/z: 764.3824

Charge: +1

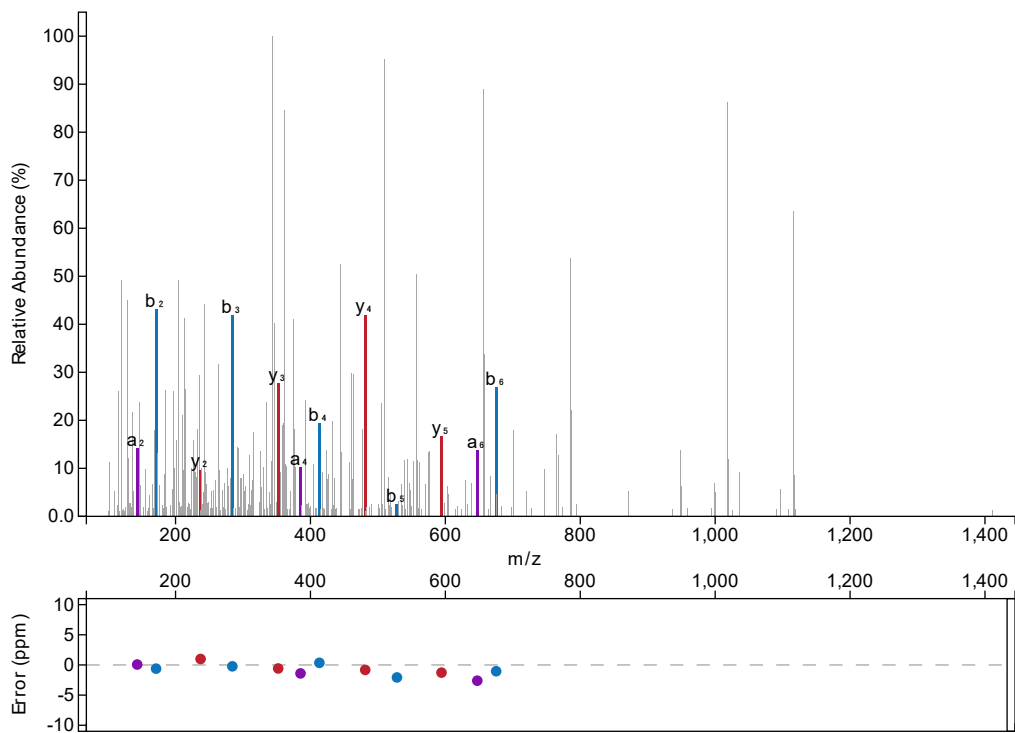
Fragmented Bonds: 6/6



Precursor m/z: 764.3824

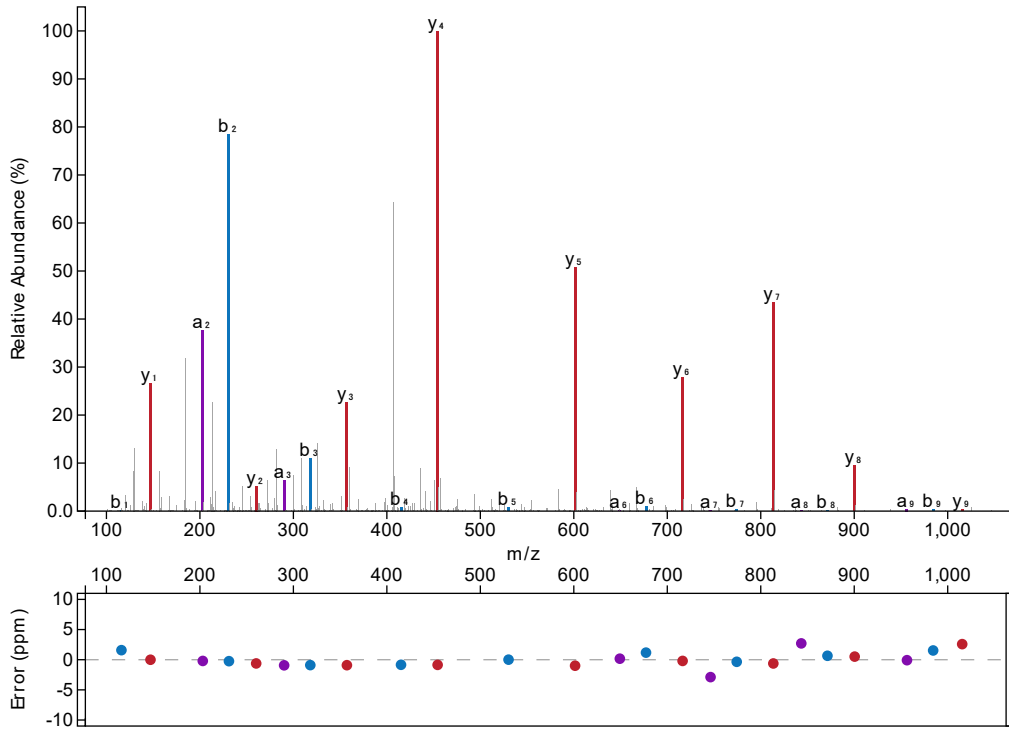
Charge: +1

Fragmented Bonds: 5/6

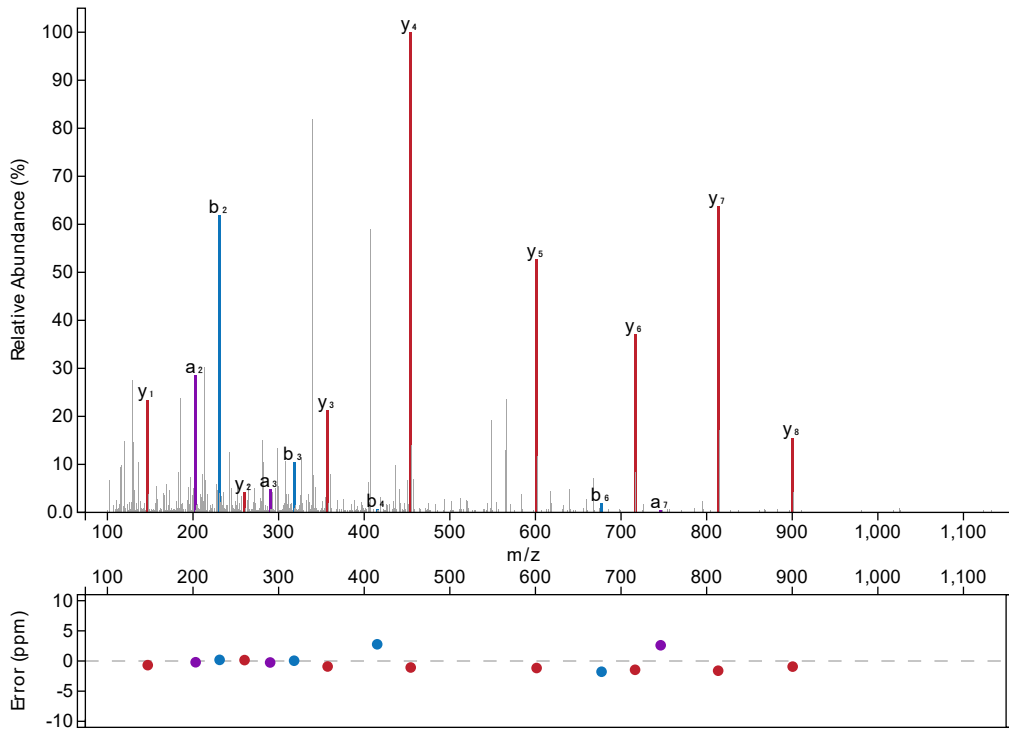




Precursor m/z: 565.7718 Charge: +2 Fragmented Bonds: 9/9



Precursor m/z: 565.7718 Charge: +2 Fragmented Bonds: 8/9

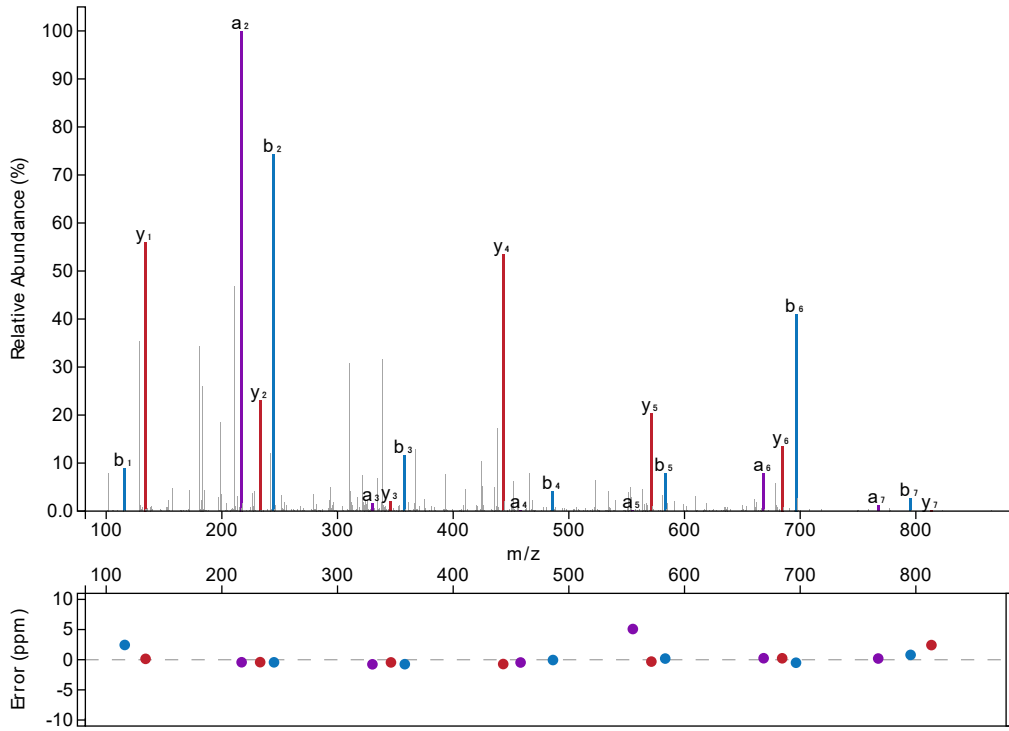




Precursor m/z: 464.7529

Charge: +2

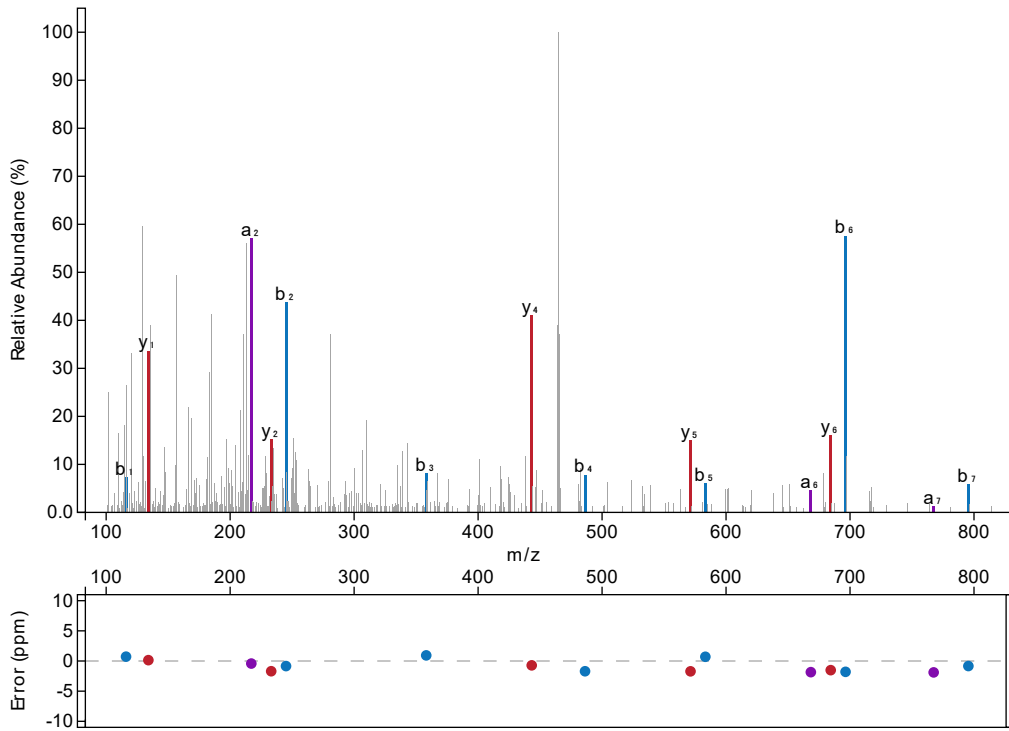
Fragmented Bonds: 7/7



Precursor m/z: 464.7529

Charge: +2

Fragmented Bonds: 7/7

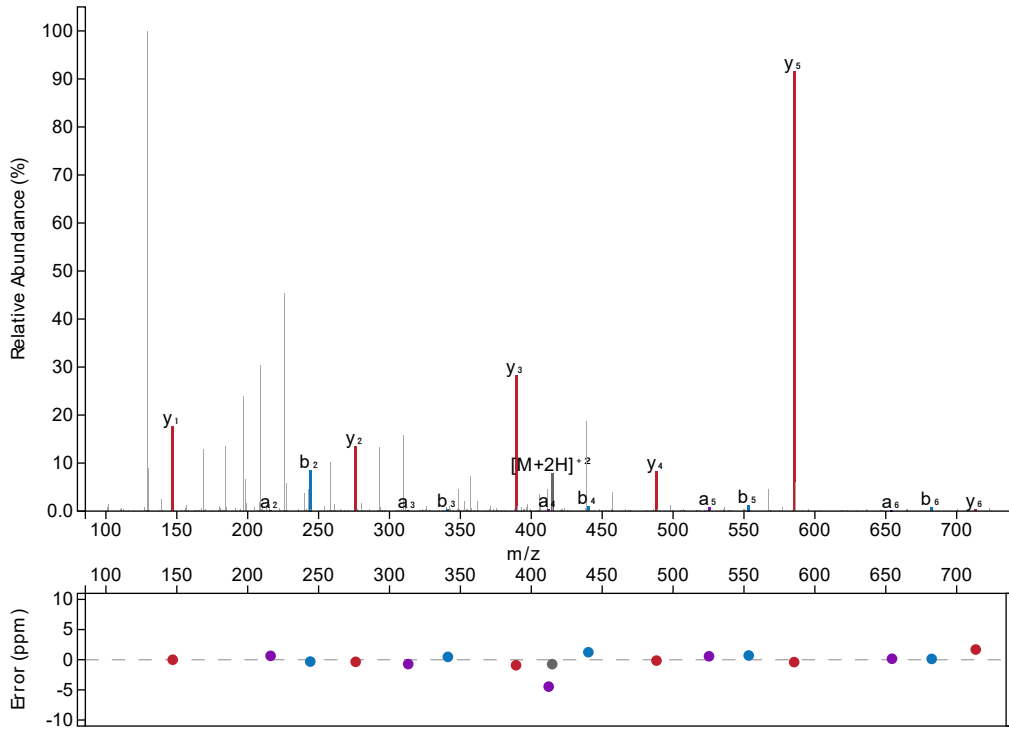




Precursor m/z: 414.7449

Charge: +2

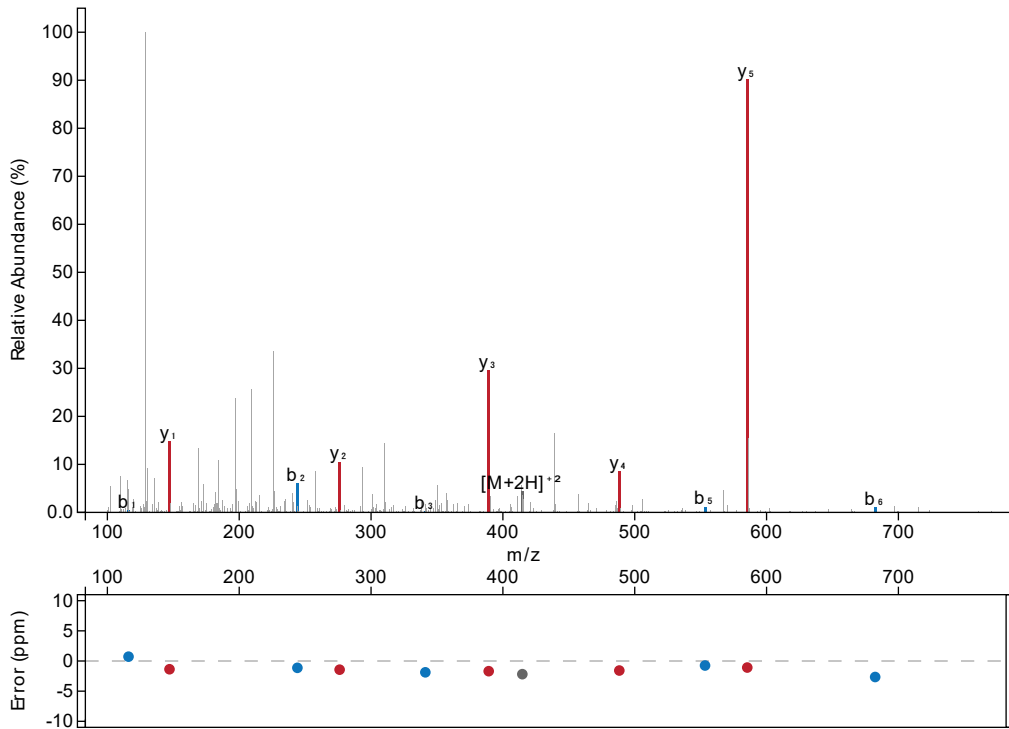
Fragmented Bonds: 6/6



Precursor m/z: 414.7449

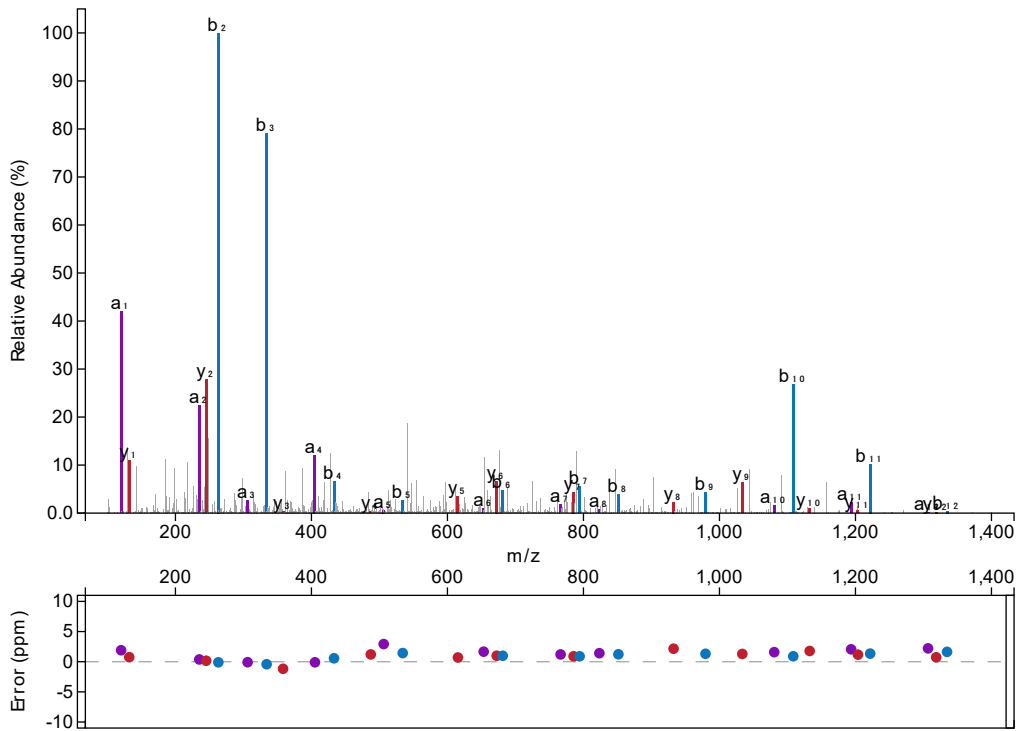
Charge: +2

Fragmented Bonds: 6/6

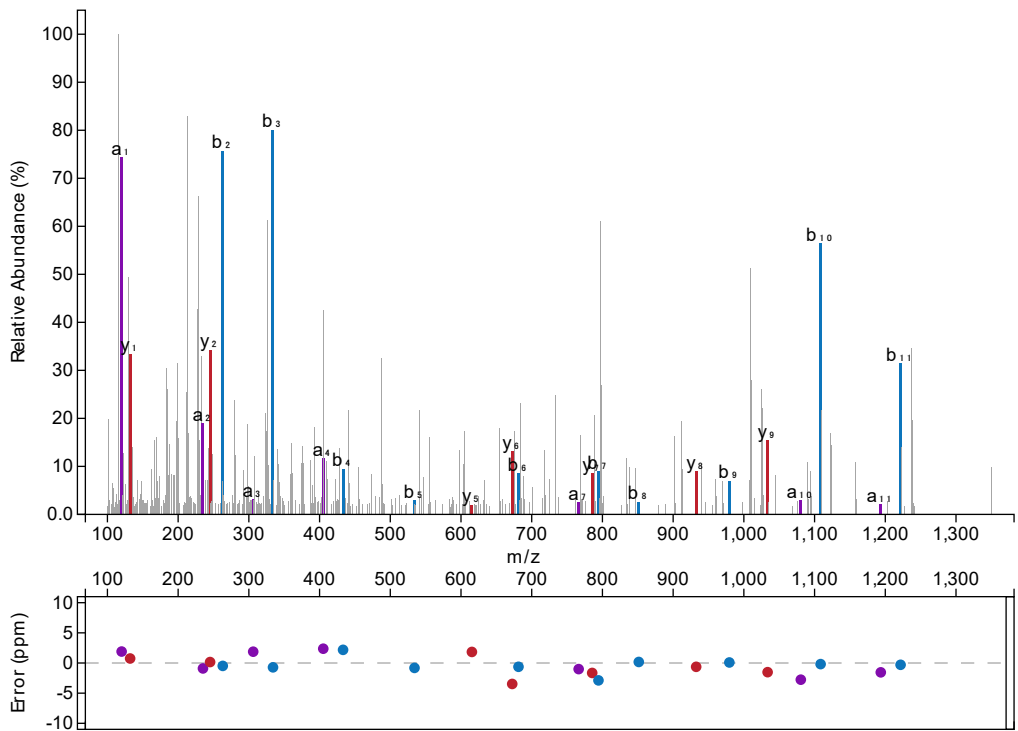




Precursor m/z: 733.4022 Charge: +2 Fragmented Bonds: 12/12



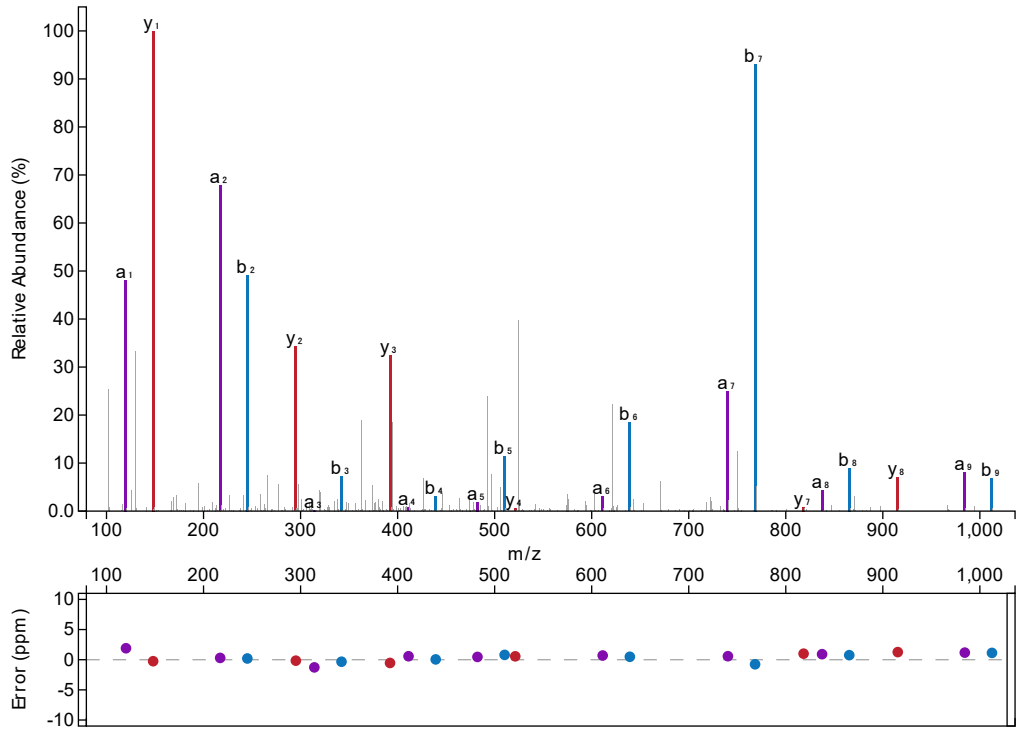
Precursor m/z: 733.4022 Charge: +2 Fragmented Bonds: 12/12



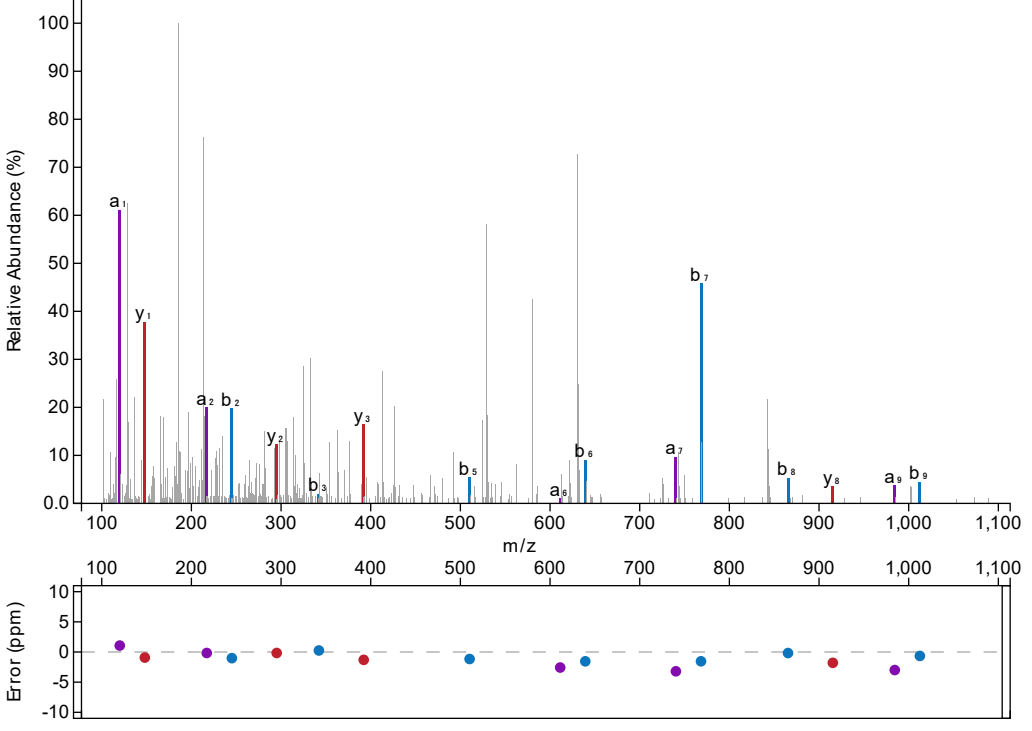




Precursor m/z: 580.2689 Charge: +2 Fragmented Bonds: 9/9

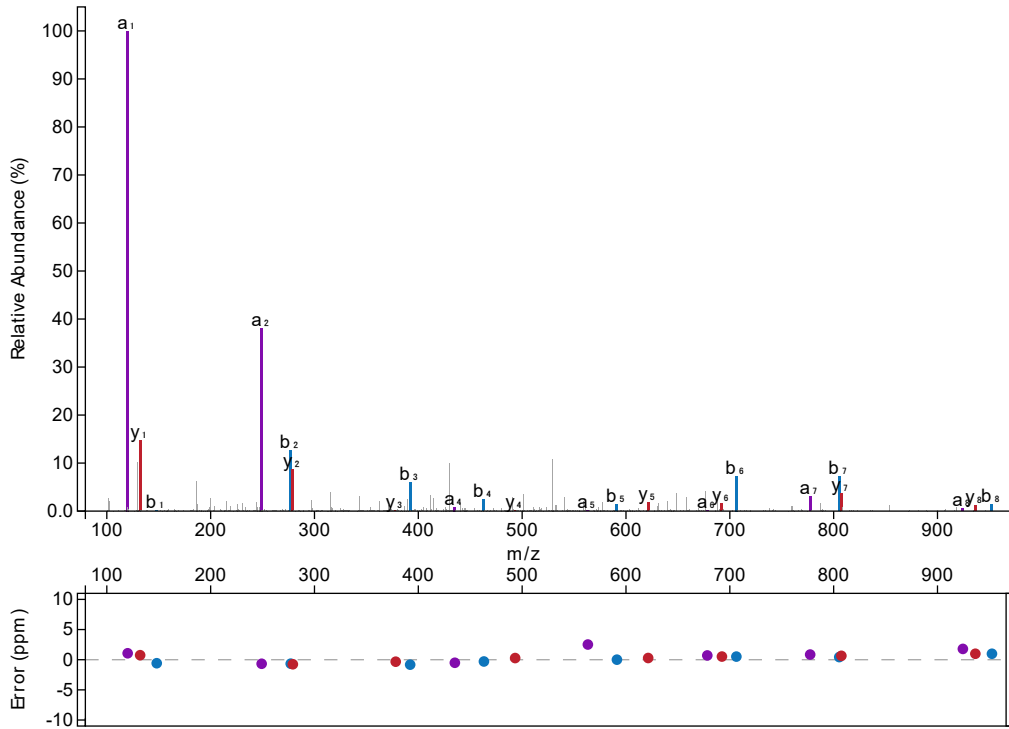


Precursor m/z: 580.2689 Charge: +2 Fragmented Bonds: 8/9

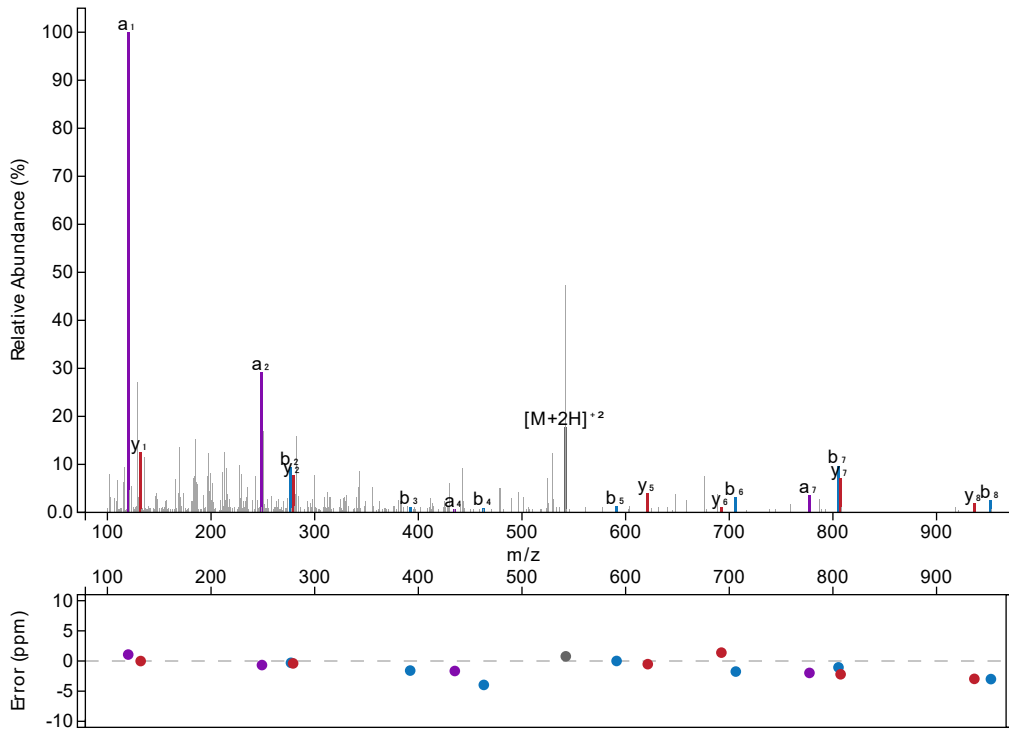




Precursor m/z: 542.2715 Charge: +2 Fragmented Bonds: 8/8

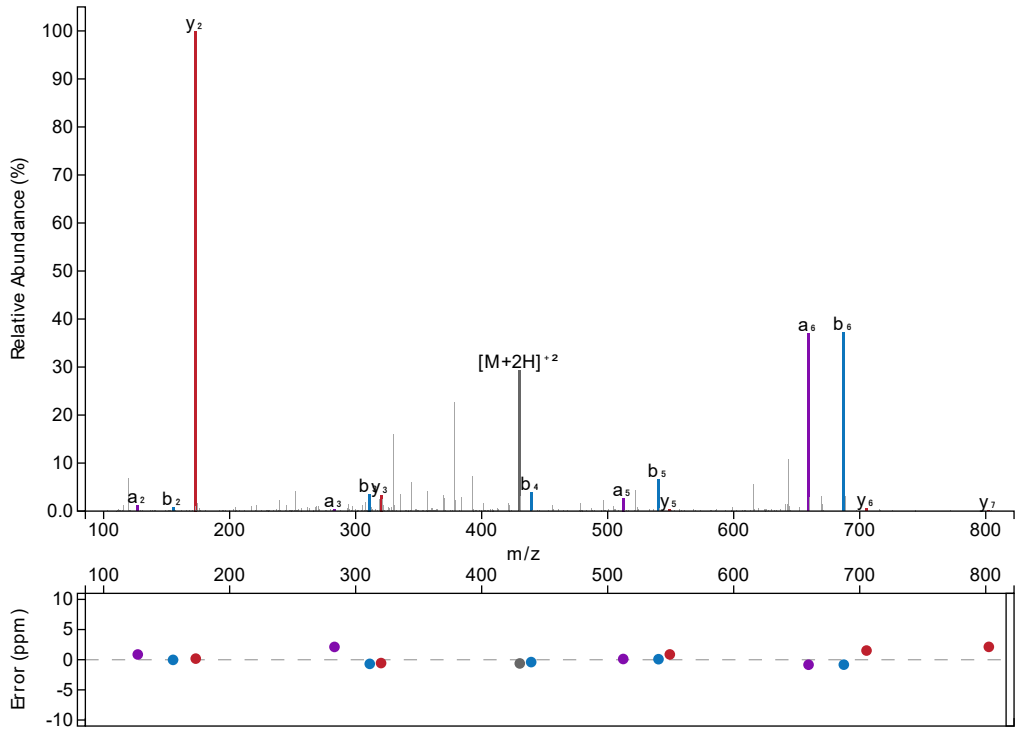


Precursor m/z: 542.2715 Charge: +2 Fragmented Bonds: 8/8

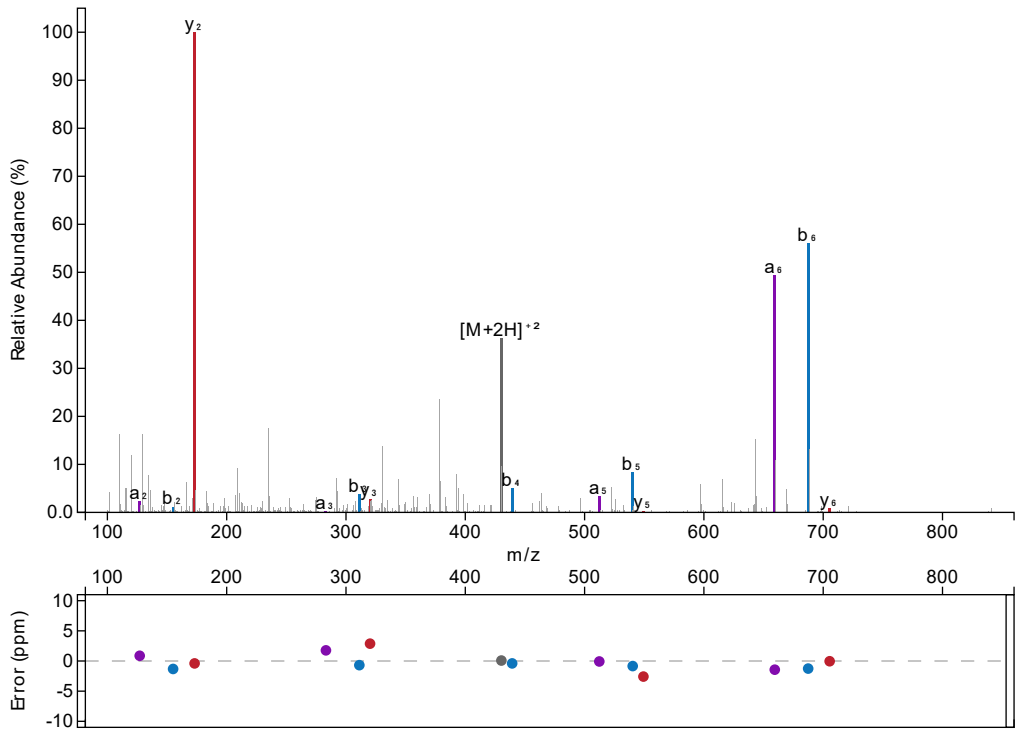




Precursor m/z: 430.2429 Charge: +2 Fragmented Bonds: 6/7

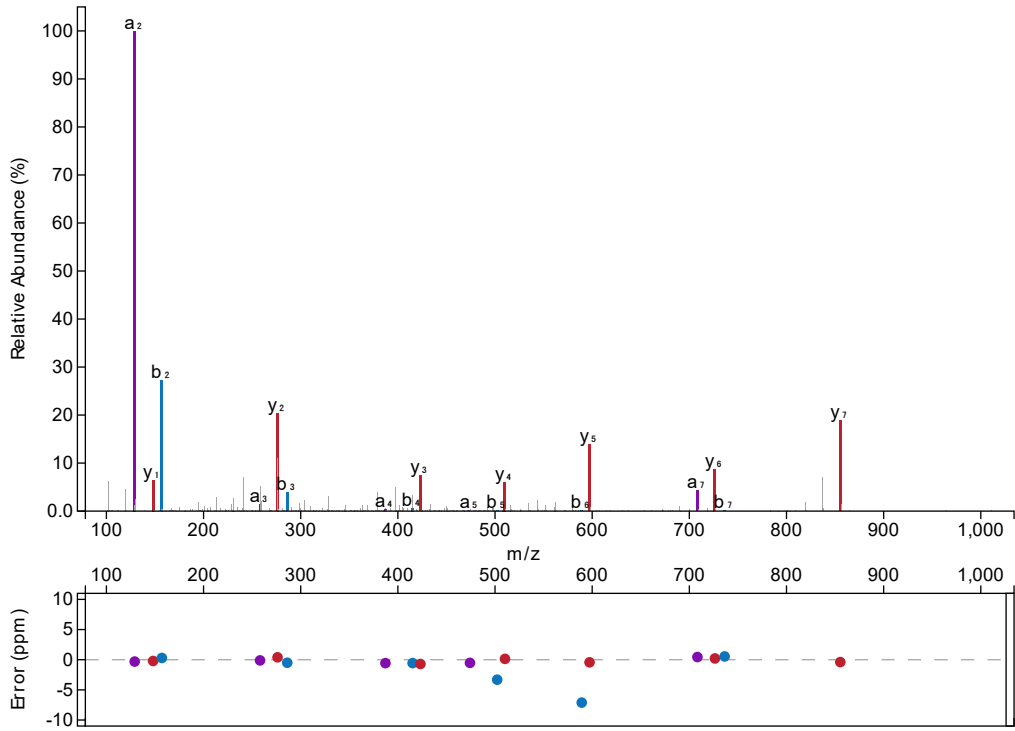


Precursor m/z: 430.2429 Charge: +2 Fragmented Bonds: 5/7

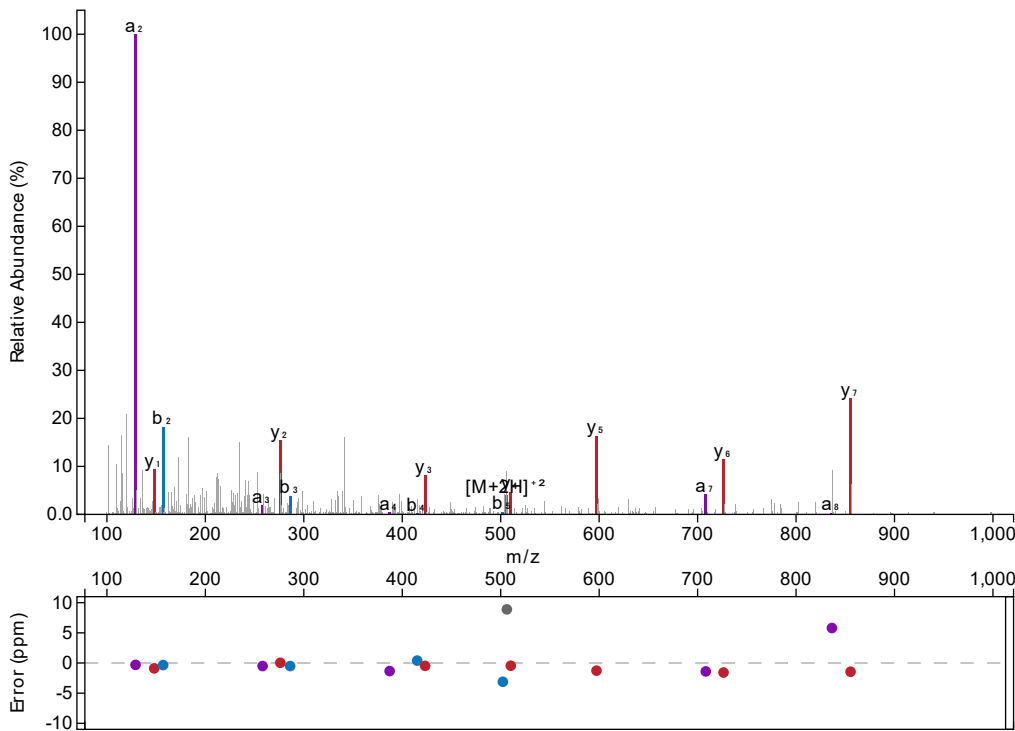




Precursor m/z: 506.2351 Charge: +2 Fragmented Bonds: 7/8

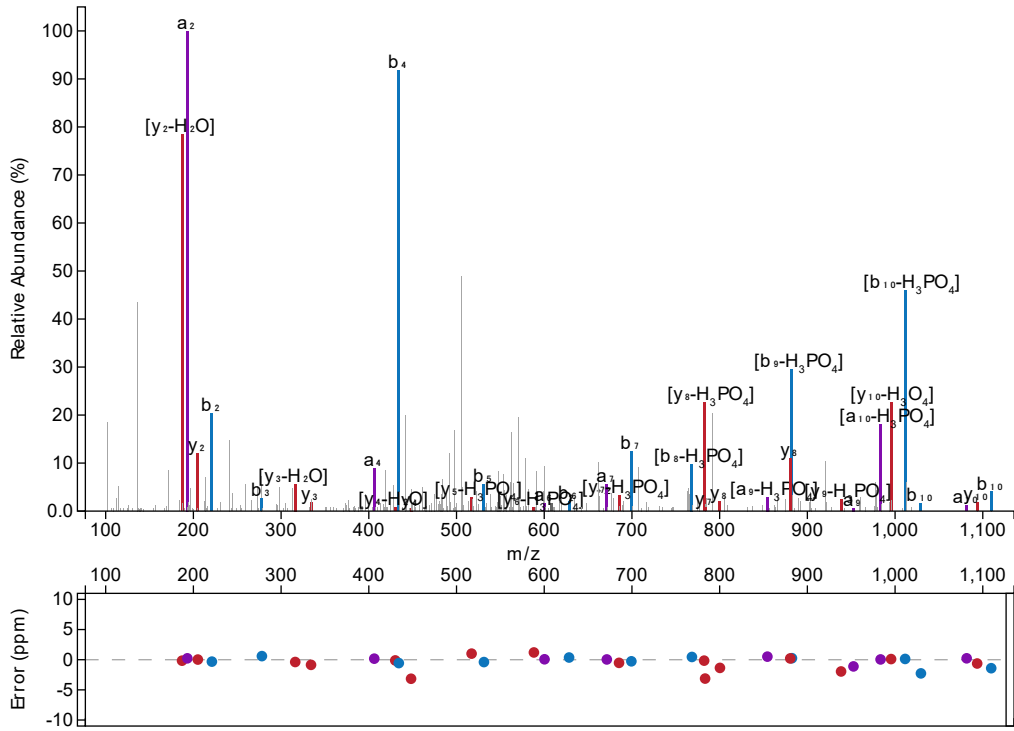


Precursor m/z: 506.2351 Charge: +2 Fragmented Bonds: 7/8



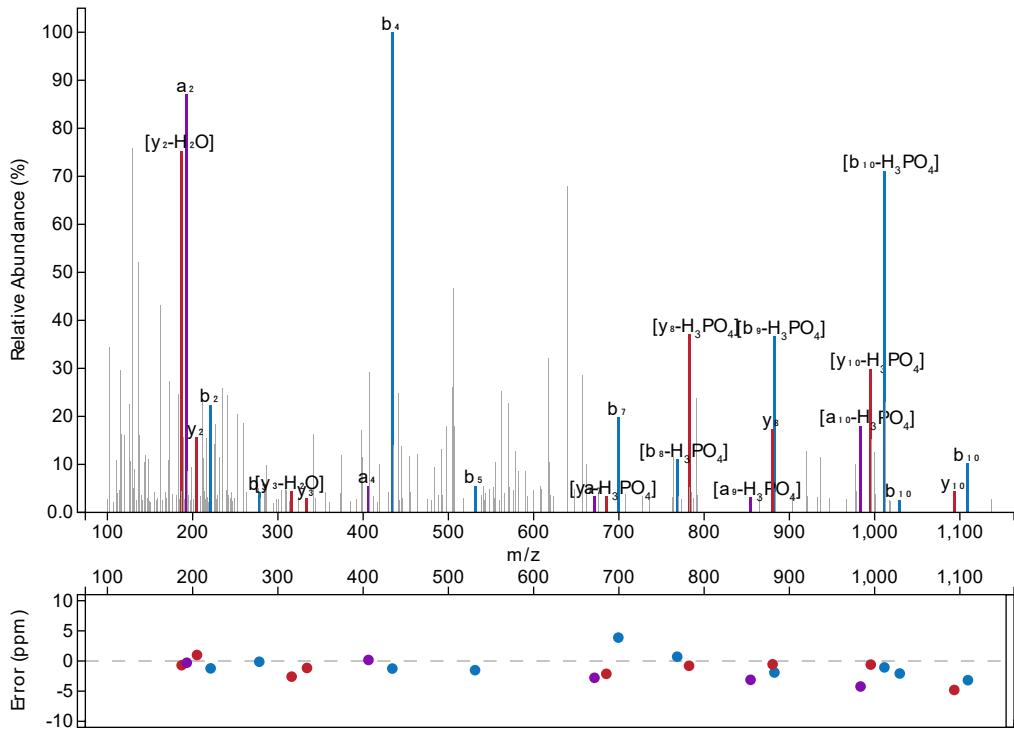
G Y G R P P A s N E E G

Precursor m/z: 657.2615 Charge: +2 Fragmented Bonds: 9/11



G Y G R P P A s N E E G

Precursor m/z: 657.2615 Charge: +2 Fragmented Bonds: 8/11

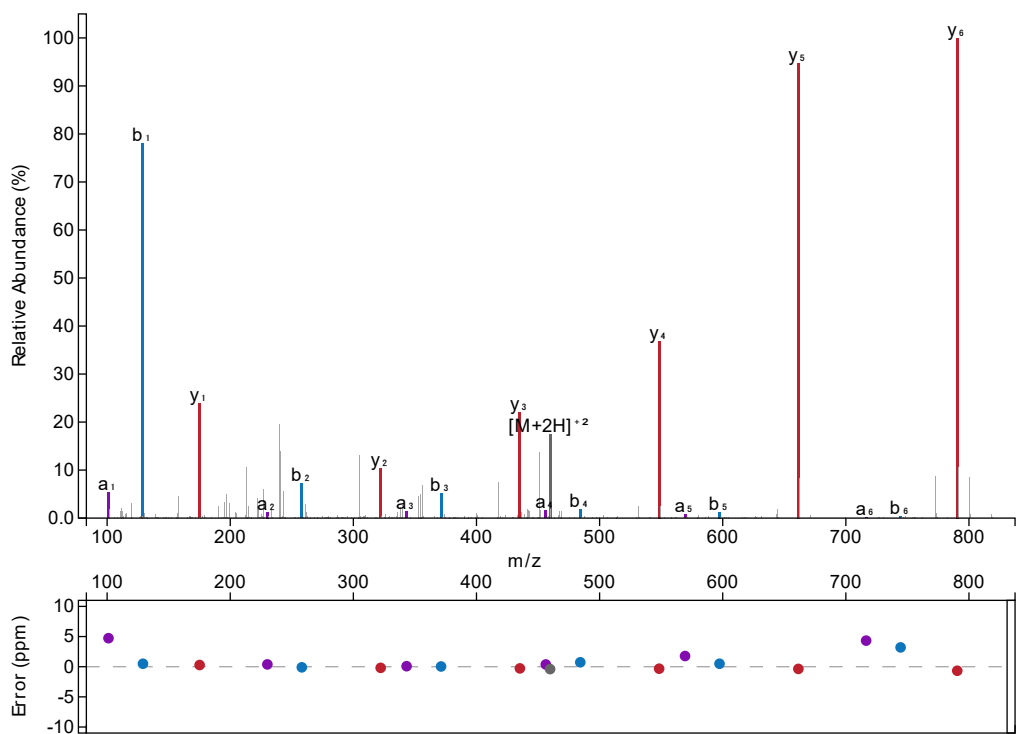




Precursor m/z: 459.7922

Charge: +2

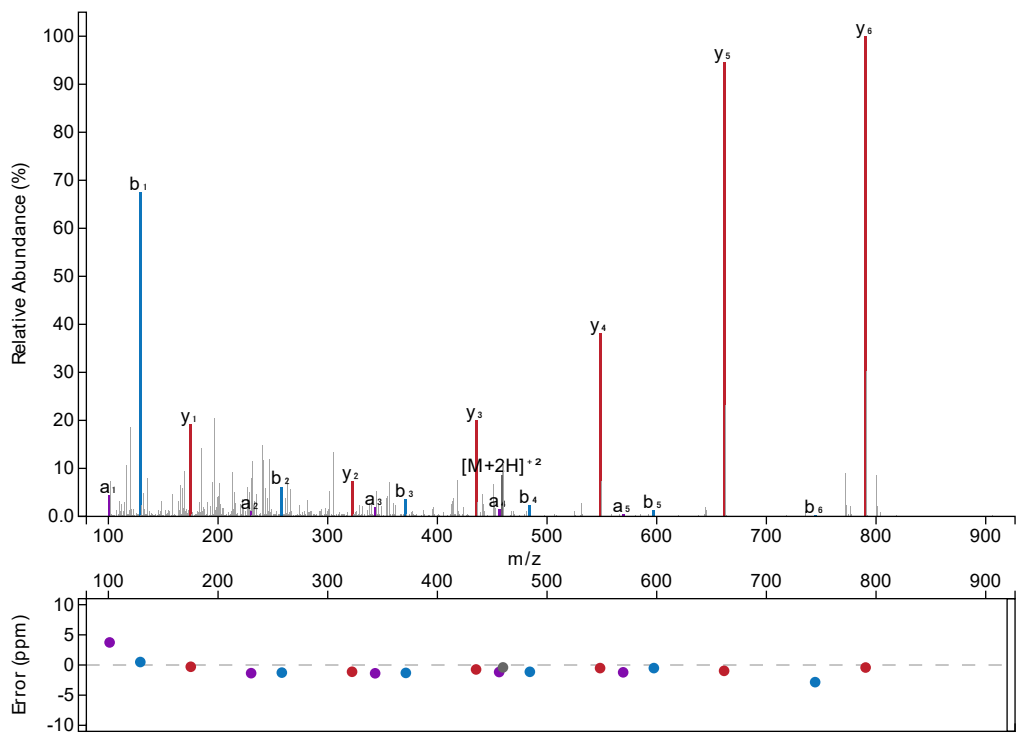
Fragmented Bonds: 6/6



Precursor m/z: 459.7922

Charge: +2

Fragmented Bonds: 6/6

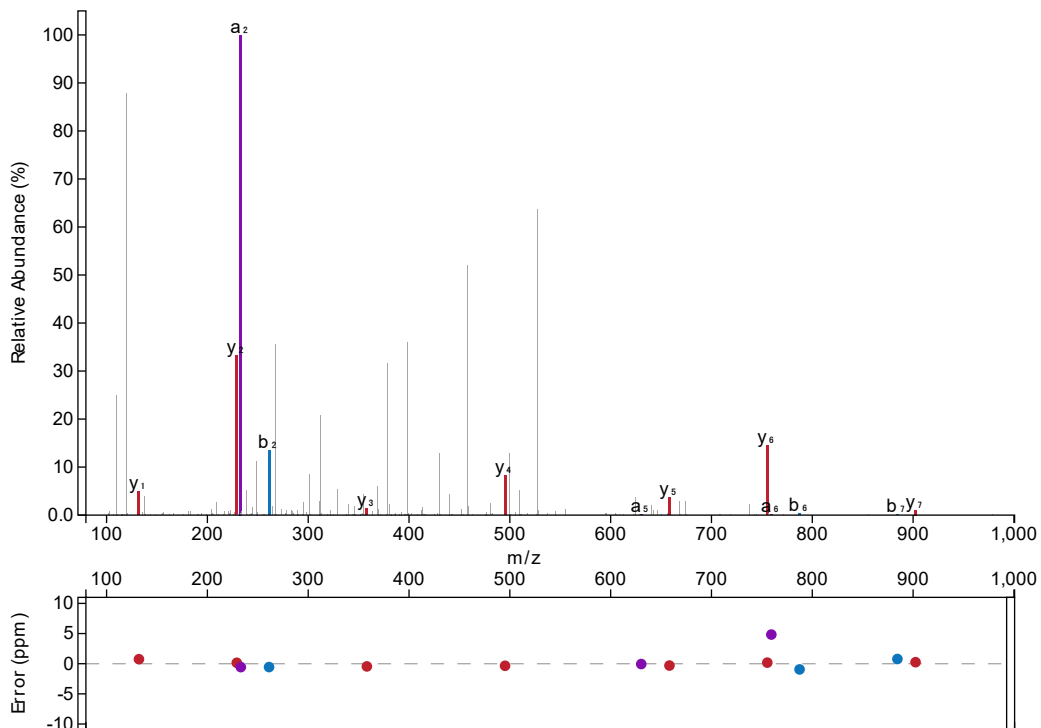




Precursor m/z: 508.2660

Charge: +2

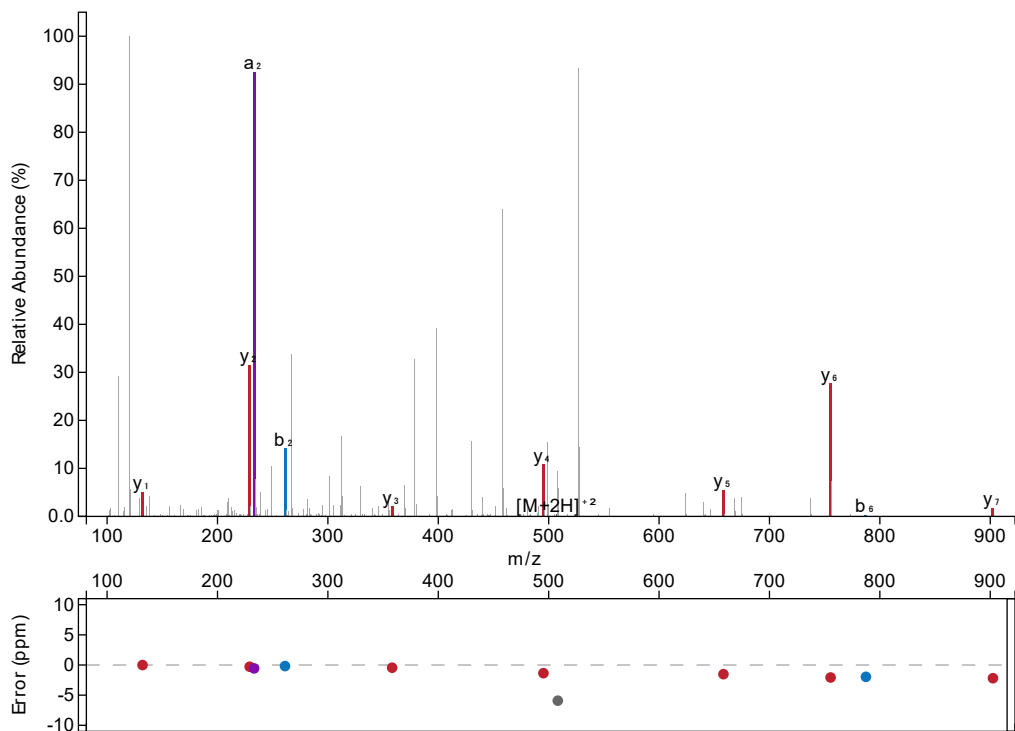
Fragmented Bonds: 7/7



Precursor m/z: 508.2660

Charge: +2

Fragmented Bonds: 7/7

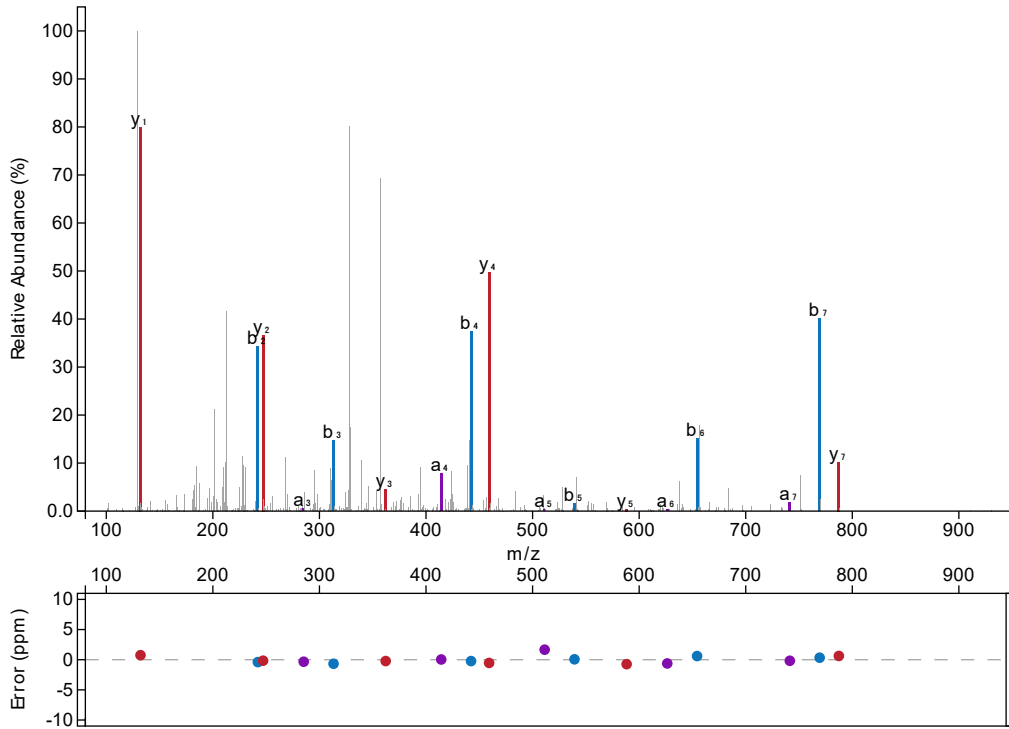




Precursor m/z: 450.7373

Charge: +2

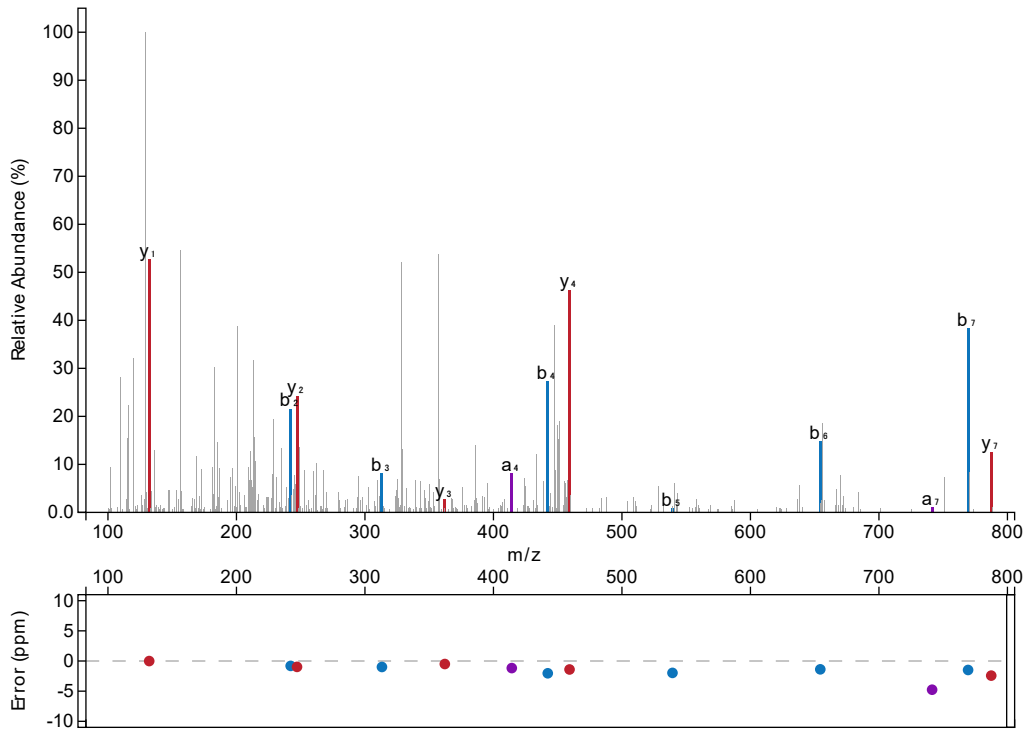
Fragmented Bonds: 7/7



Precursor m/z: 450.7373

Charge: +2

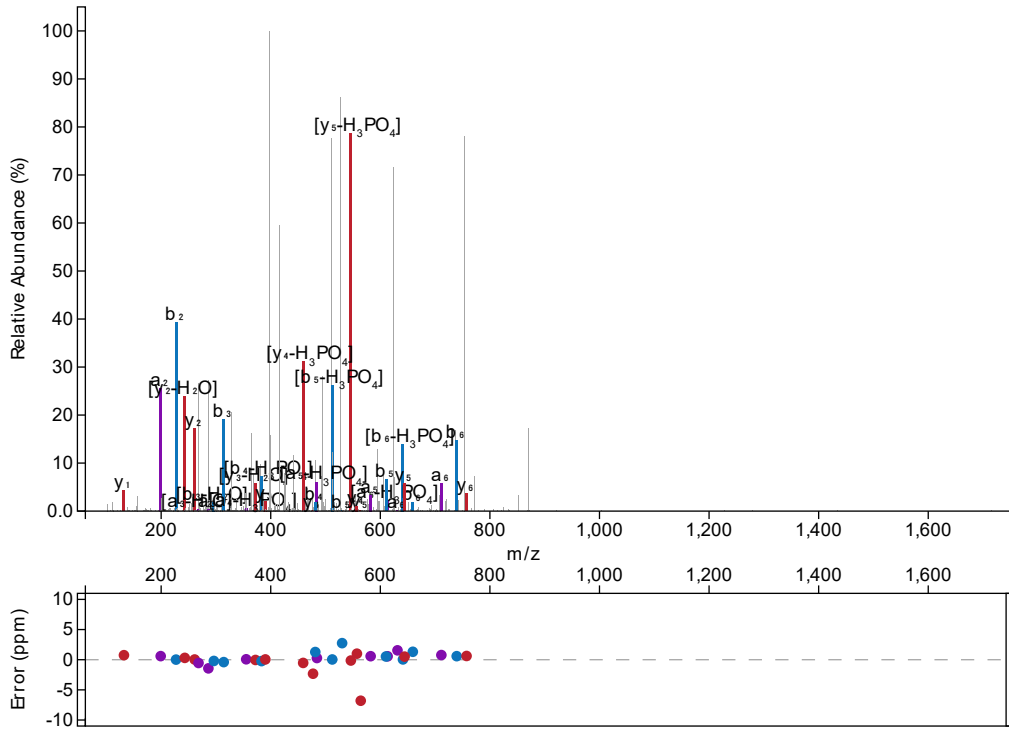
Fragmented Bonds: 7/7



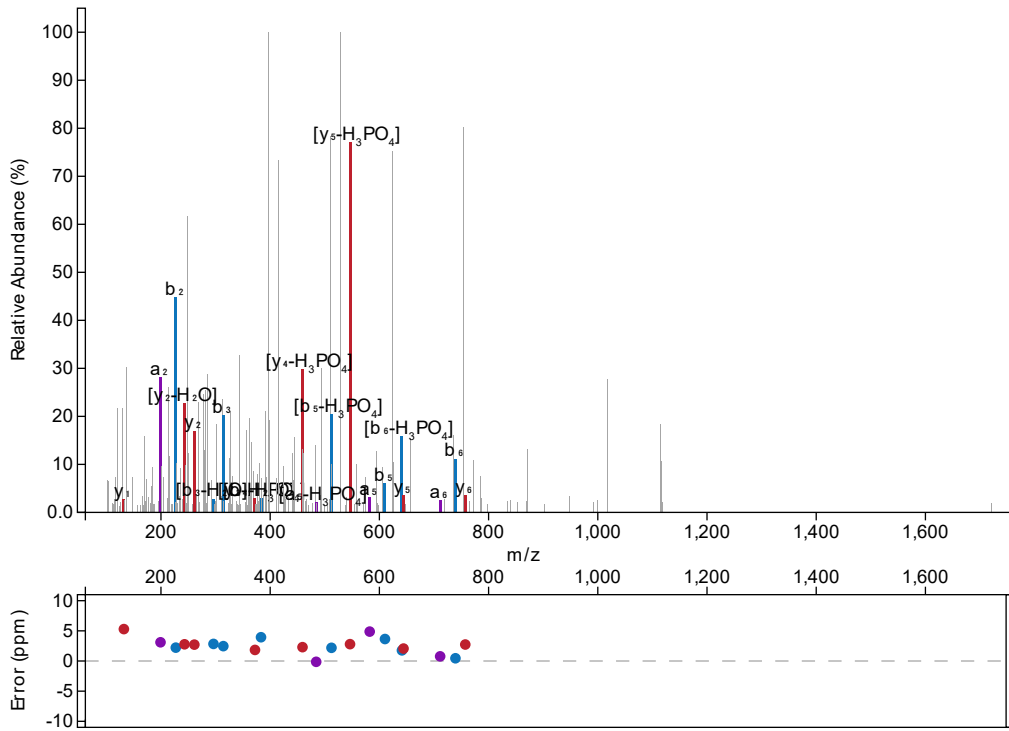




Precursor m/z: 870.3856 Charge: +1 Fragmented Bonds: 5/6

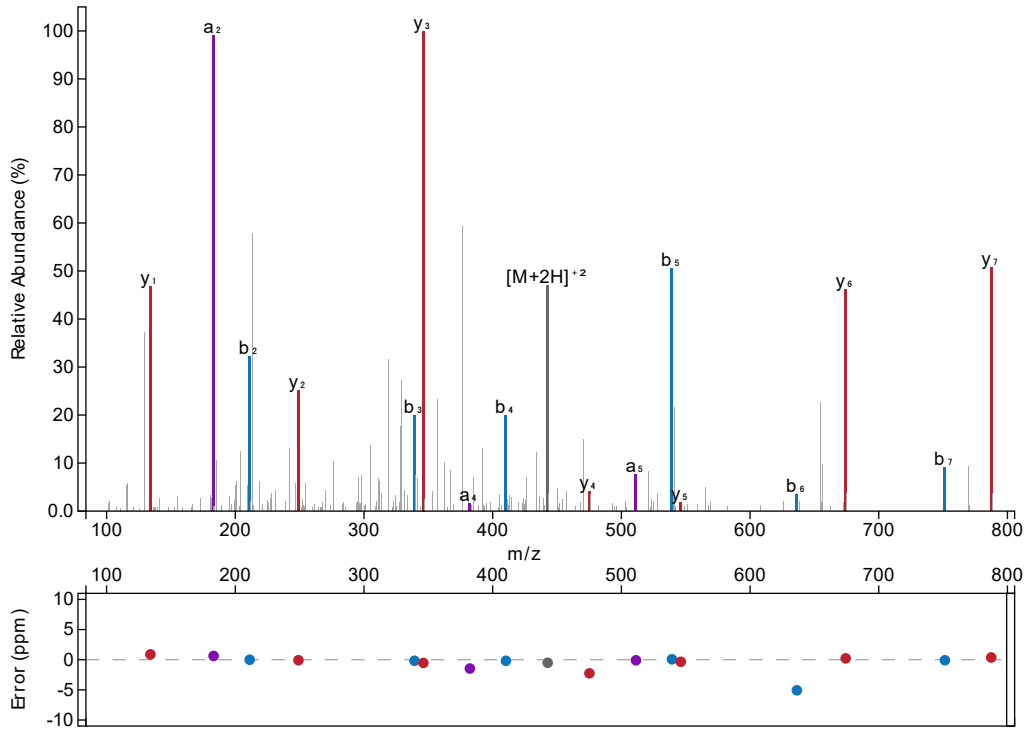


Precursor m/z: 870.3856 Charge: +1 Fragmented Bonds: 5/6

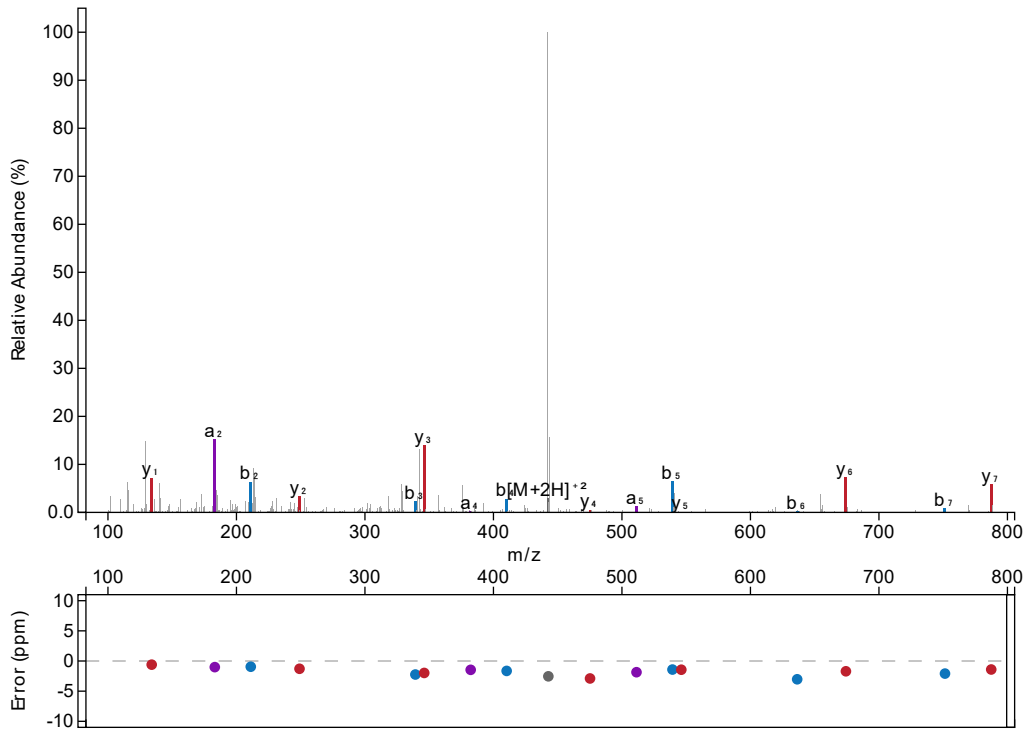




Precursor m/z: 442.7216 Charge: +2 Fragmented Bonds: 7/7

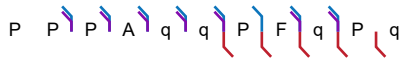
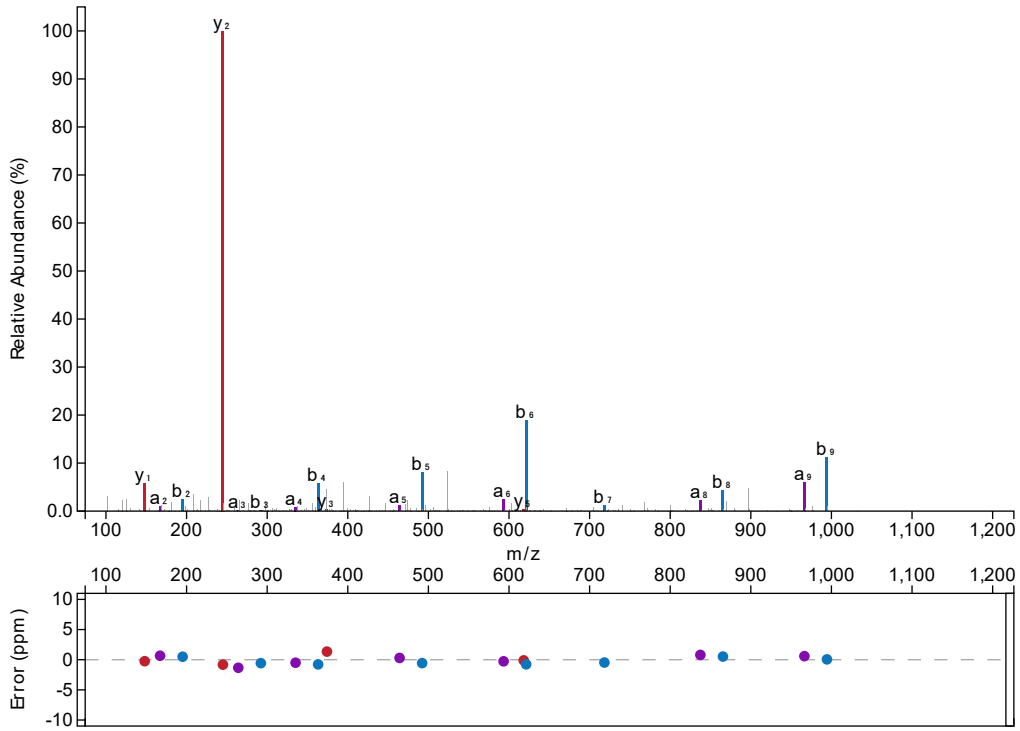


Precursor m/z: 442.7216 Charge: +2 Fragmented Bonds: 7/7

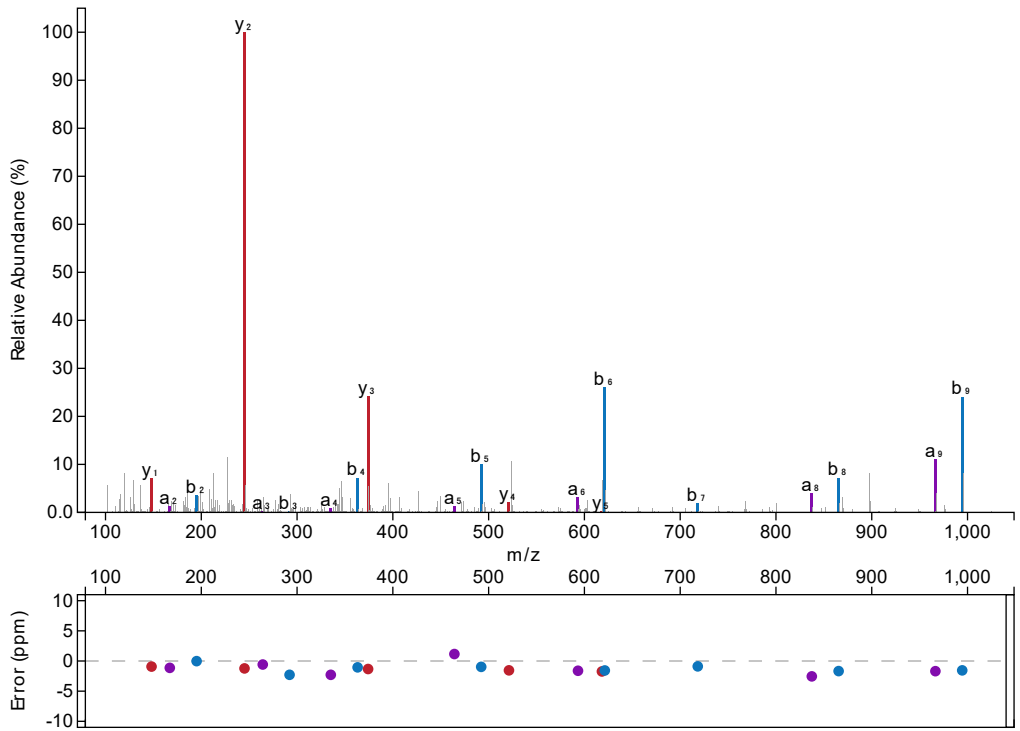




Precursor m/z: 619.7824 Charge: +2 Fragmented Bonds: 9/10



Precursor m/z: 619.7824 Charge: +2 Fragmented Bonds: 9/10

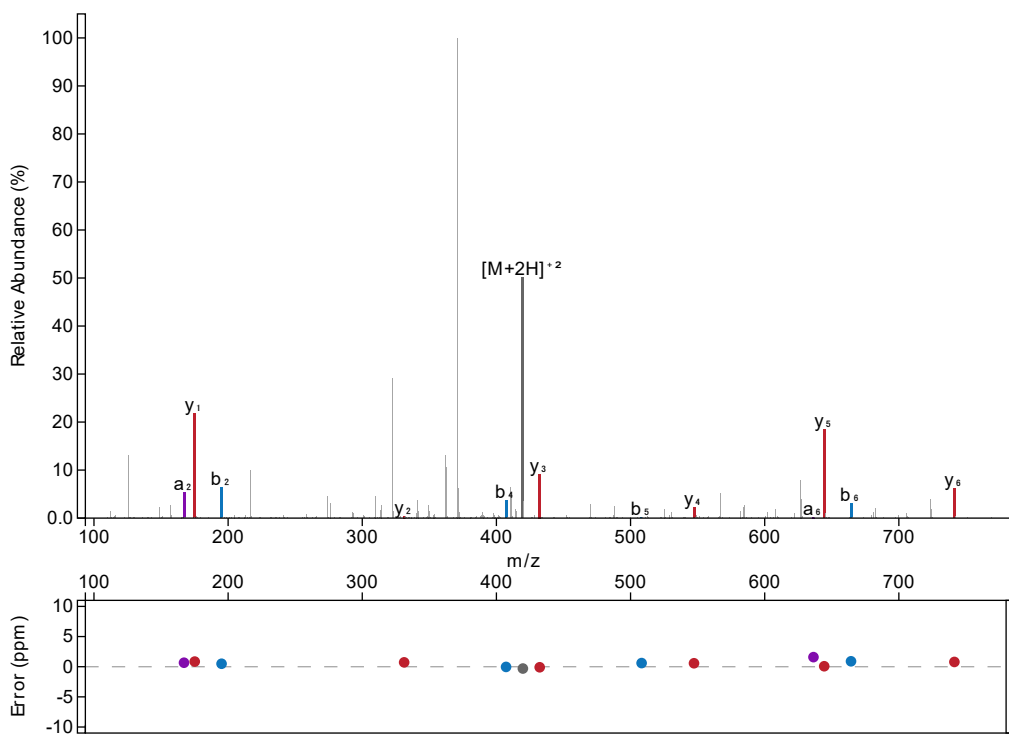




Precursor m/z: 419.7301

Charge: +2

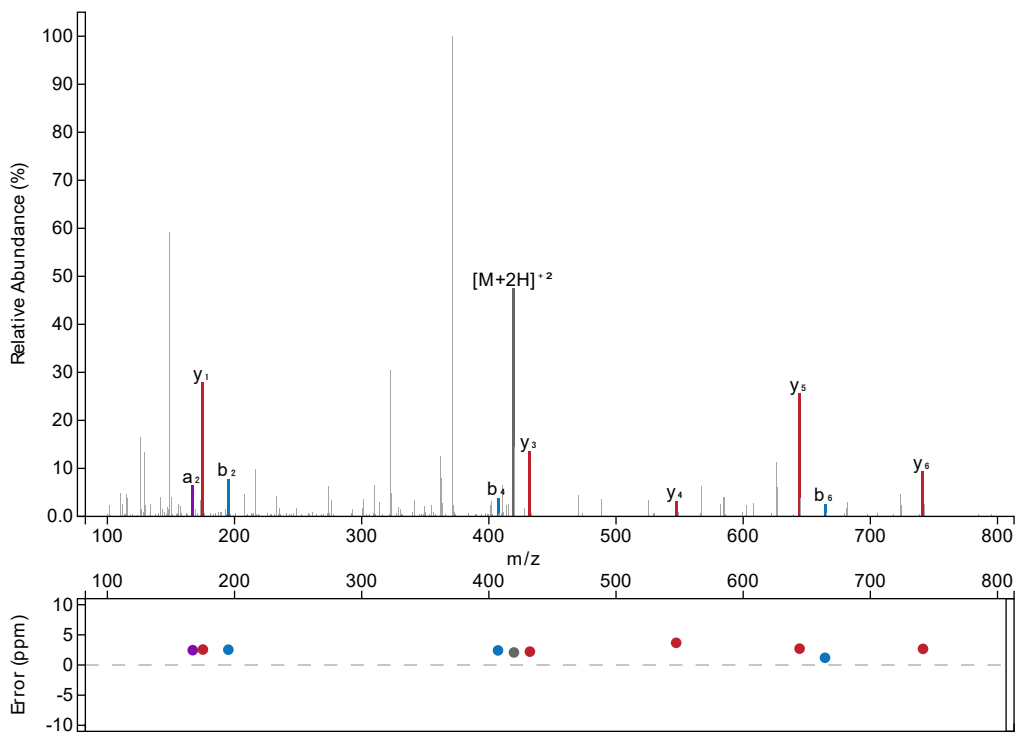
Fragmented Bonds: 6/6



Precursor m/z: 419.7301

Charge: +2

Fragmented Bonds: 5/6

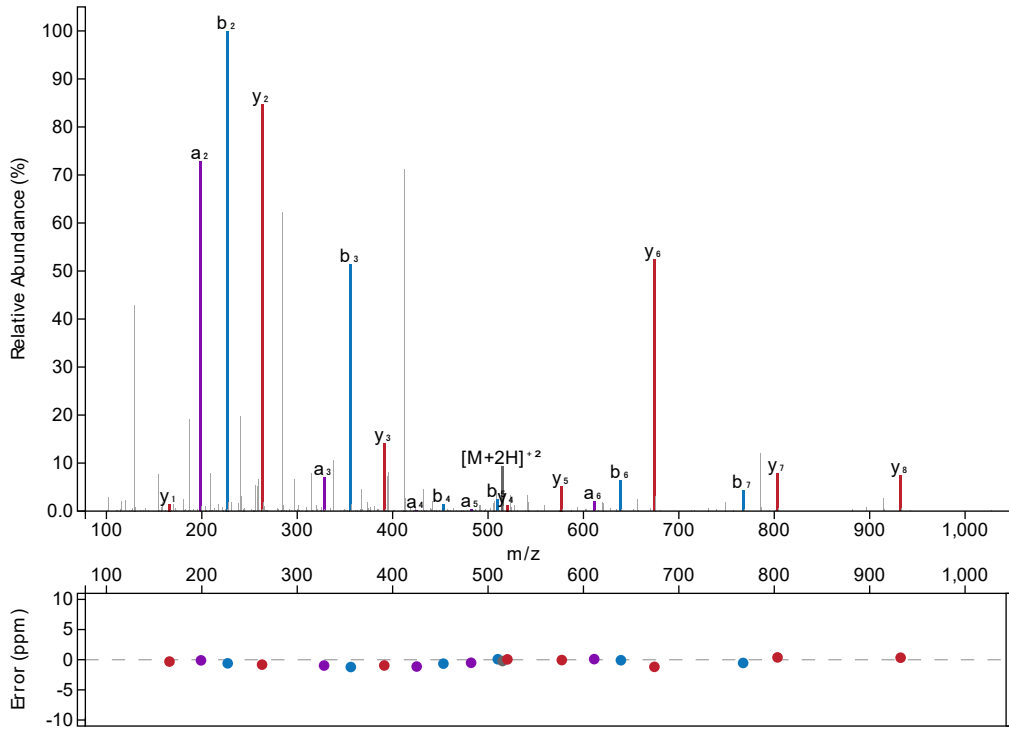




Precursor m/z: 515.2480

Charge: +2

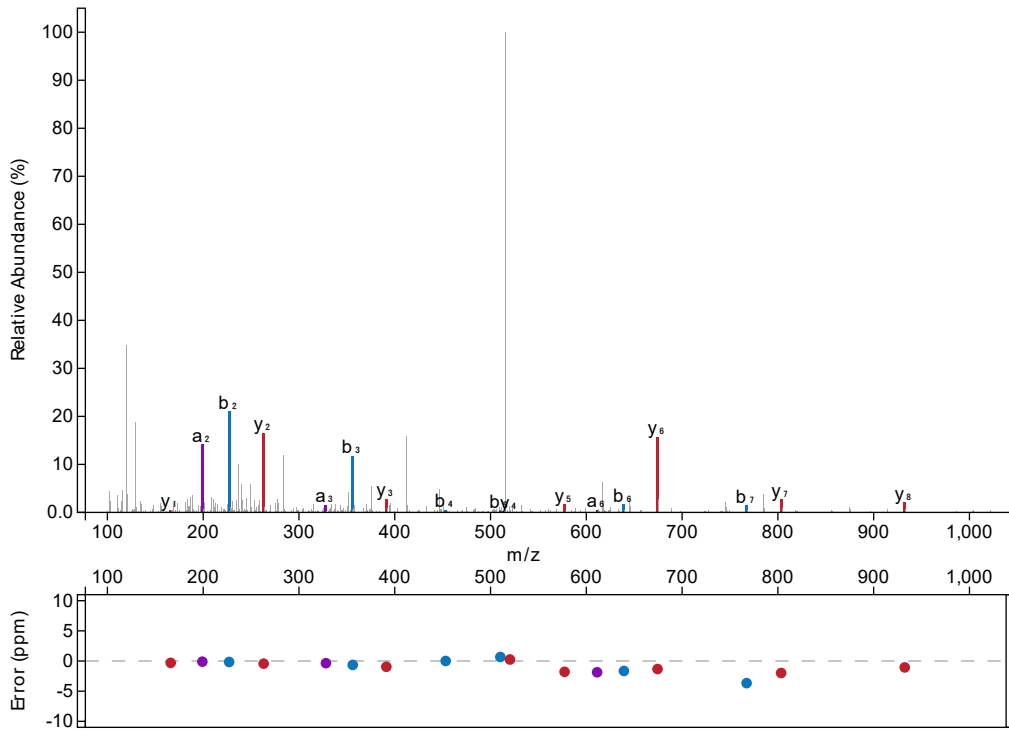
Fragmented Bonds: 8/8

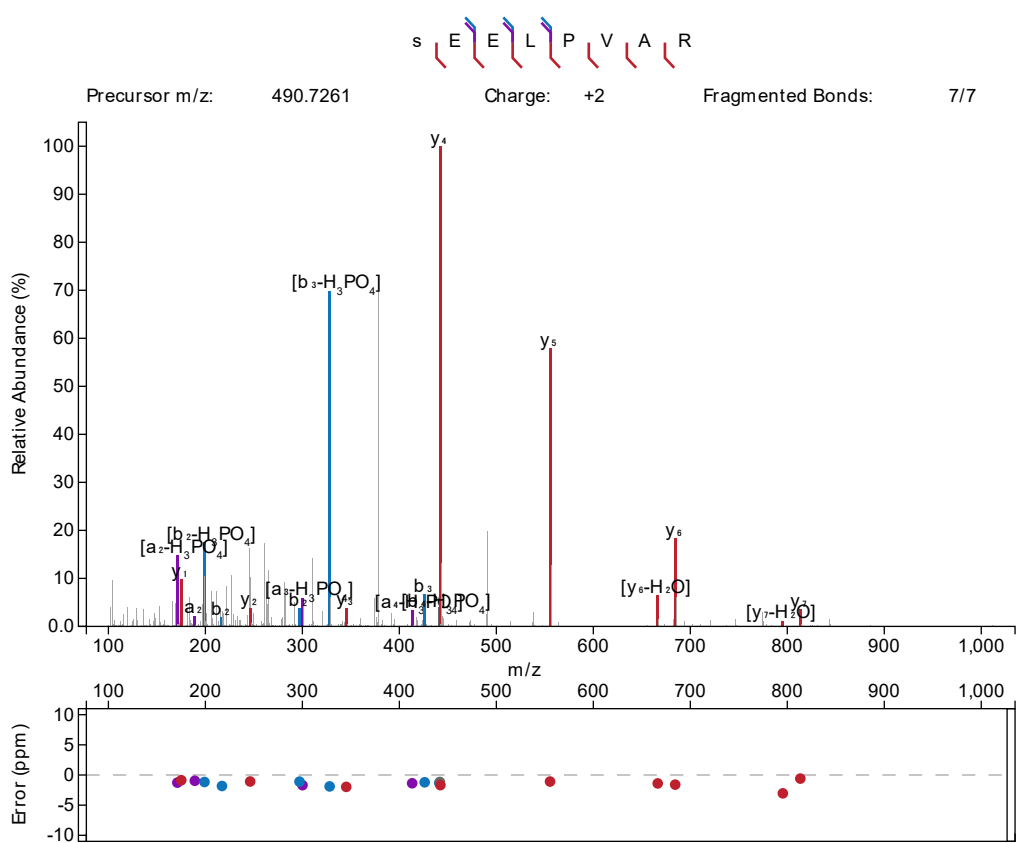
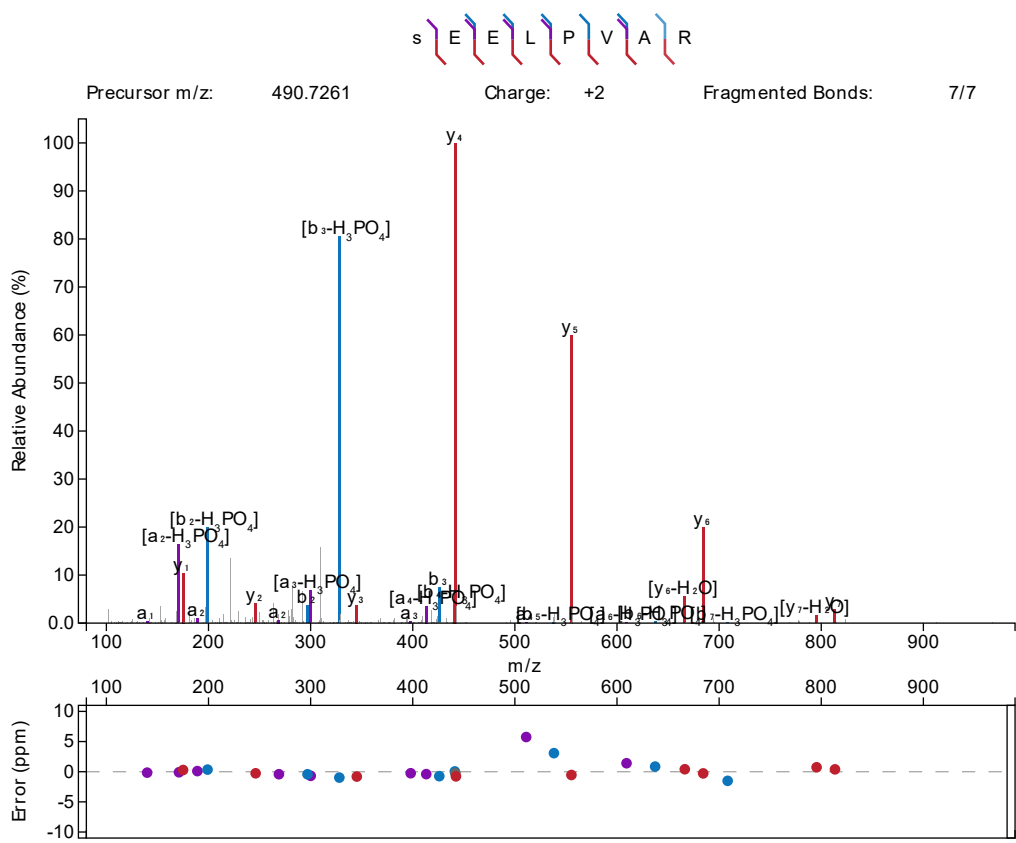


Precursor m/z: 515.2480

Charge: +2

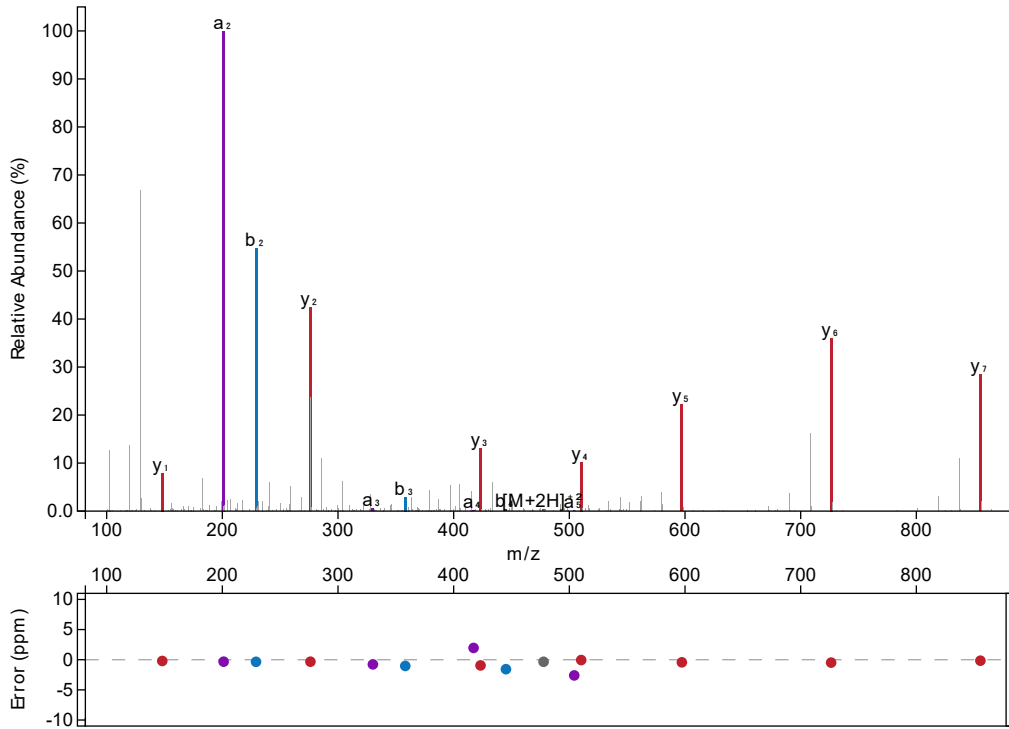
Fragmented Bonds: 8/8



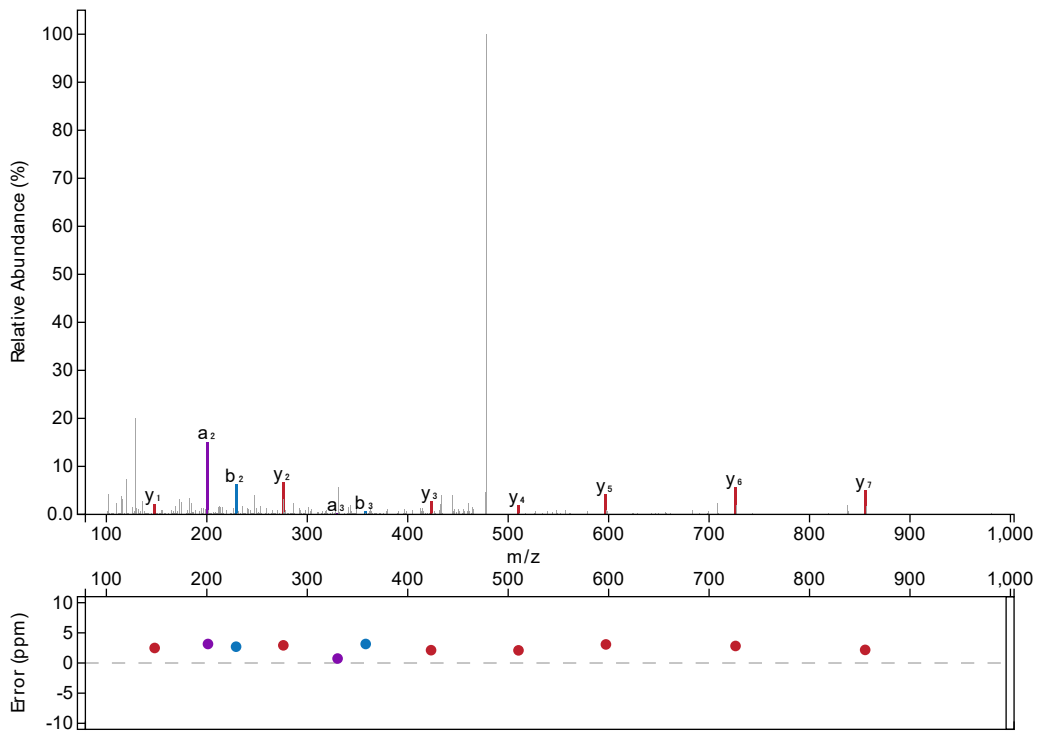




Precursor m/z: 477.7244 Charge: +2 Fragmented Bonds: 7/7

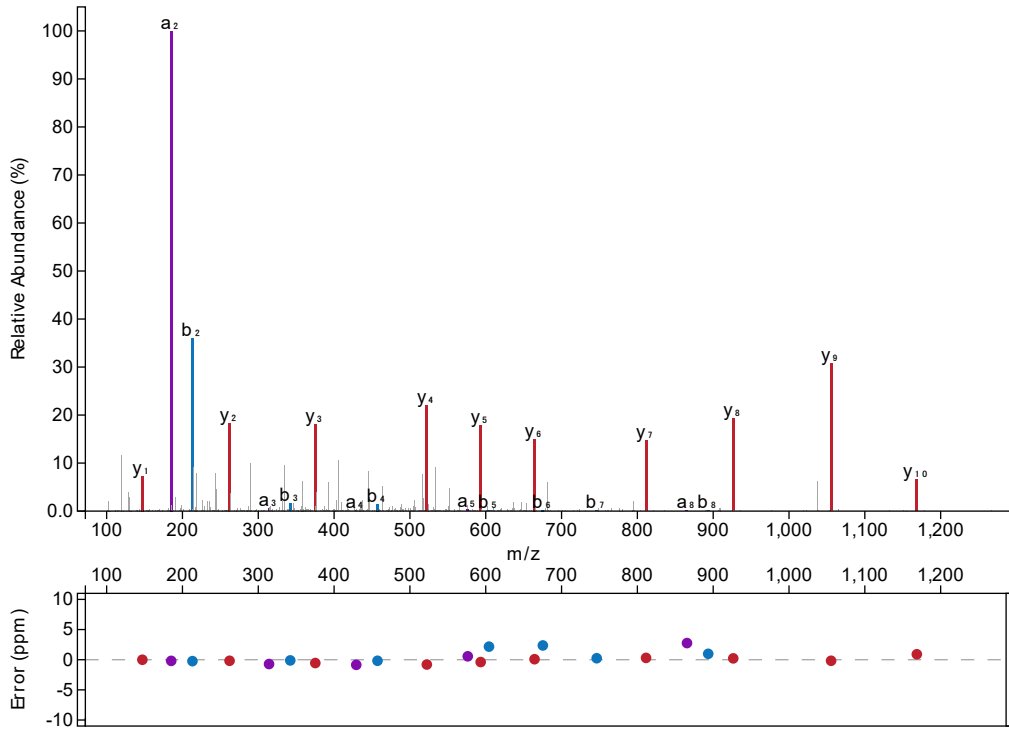


Precursor m/z: 477.7244 Charge: +2 Fragmented Bonds: 7/7





Precursor m/z: 634.3321 Charge: +2 Fragmented Bonds: 10/10



Precursor m/z: 634.3321 Charge: +2 Fragmented Bonds: 9/10

