

Supplementary Material

AminoxyTMT: A novel multi-functional reagent for proteomic characterization of protein carbonylation

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²Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, Minneapolis, MN, USA

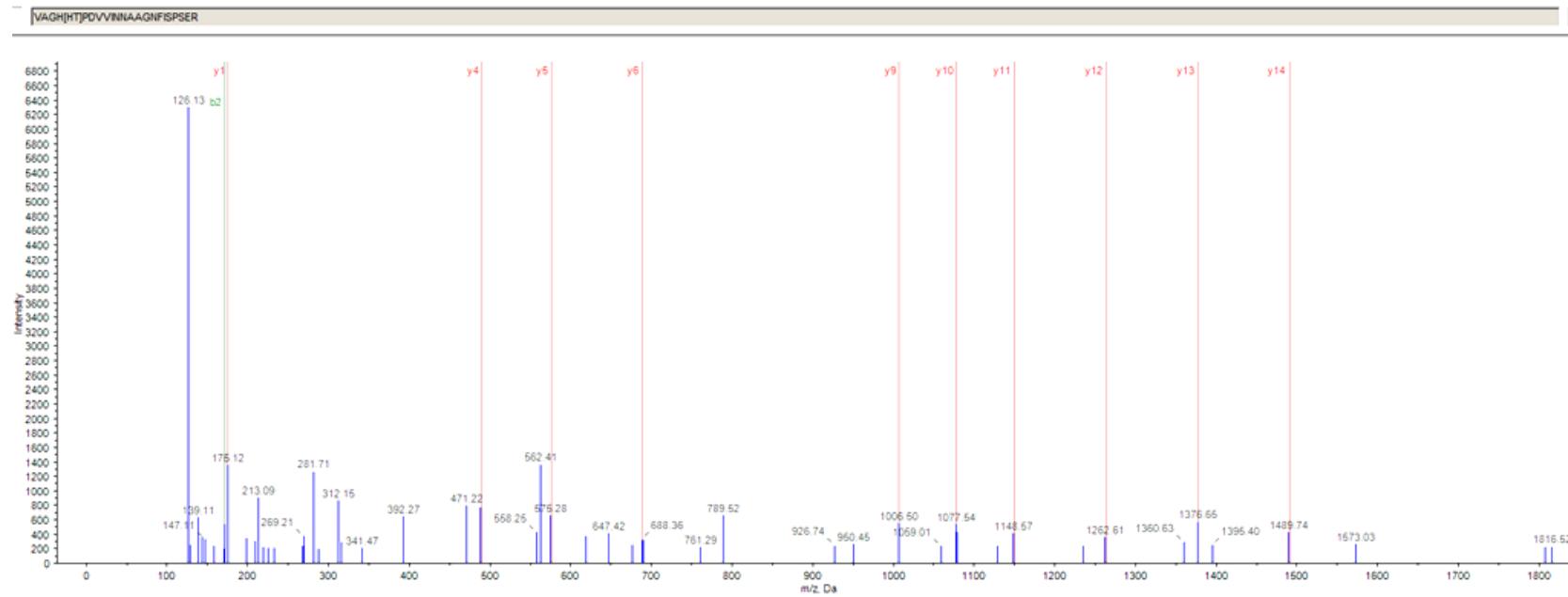
³Thermo Fisher Scientific, Rockford, IL, USA

*Corresponding author: Dr Timothy J Griffin

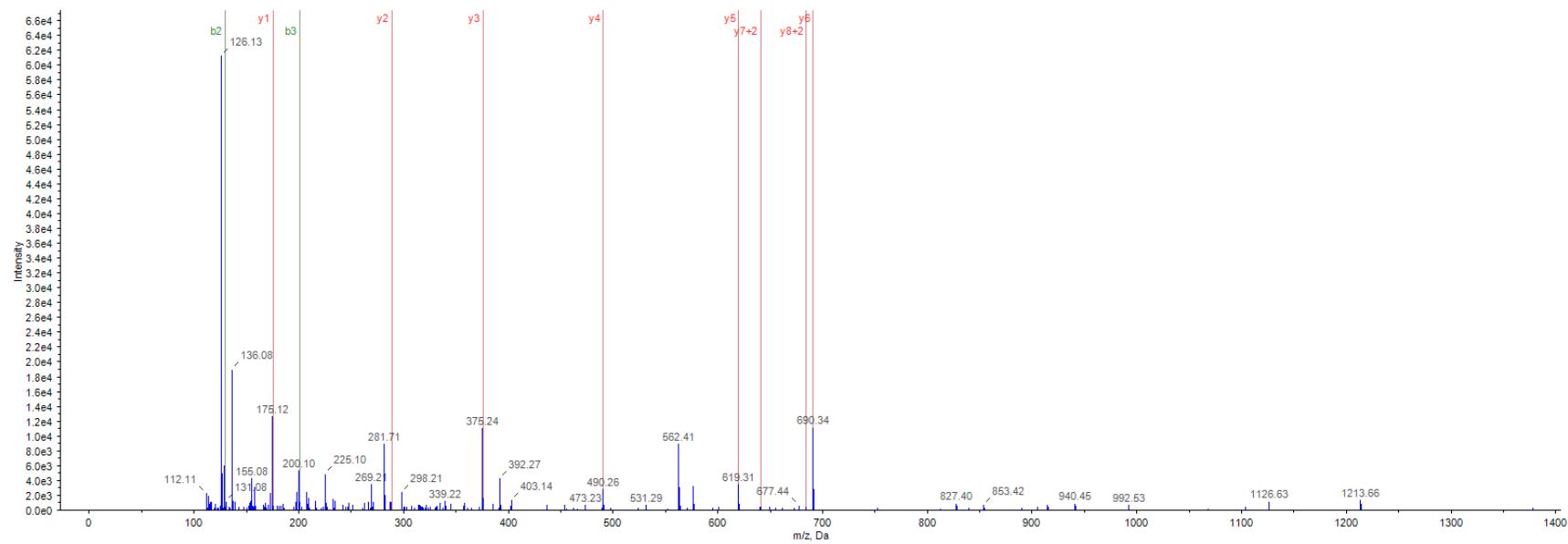
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| Figure S-1. Peptide sequence match spectra of aminoxyTMT labeled, HNE-modified peptides [HT] in HNE-treated liver mitochondria samples labeled with aminoxyTMT after enrichment with antiTMT resin | 4 |

| | | | |
|--|---|---------|--------|
| EGYL <u>H</u> IGGTTQQAQR | Expressed sequence AU018778 | Ces1f | Q91WU0 |
| IAD <u>QCPSSLAIQENANALAR</u> | Fructose-bisphosphate aldolase B | Aldob | Q91Y97 |
| DSNY <u>HLLMSVQESLER</u> ; <u>HGGTIPVVPTAEFQDR</u> | Glutamate dehydrogenase 1, mitochondrial | Glud1 | P26443 |
| TQVE <u>QCEPTNDAVR</u> | Glycogen [starch] synthase, liver | Gys2 | Q8VCB3 |
| STAGDT <u>H</u> LGGEDFDNR | Heat shock cognate 71 kDa protein | Hspa8 | P63017 |
| NANC <u>SIEESFQR</u> | Hydroxymethylglutaryl-CoA lyase, mitochondrial | Hmgcl | P38060 |
| VIGH <u>DEDASAPTVGVR</u> | Immunity-related GTPase family M member 1 (Fragment) | Irgm1 | Q5NCB5 |
| TVEAAAH <u>GTVTR</u> | Isocitrate dehydrogenase [NADP] cytoplasmic | Idh1 | O88844 |
| ADGT <u>VLECS</u> ESSNADVQAR; VVA <u>HYPVEVR</u> | L-gulonolactone oxidase | Gulo | P58710 |
| VIGSG <u>CNLDSAR</u> | L-lactate dehydrogenase | Ldha | G5E8N5 |
| NHNDLN <u>NSVPQEPR</u> | Lysosomal alpha-glucosidase (Fragment) | Gaa | F6R5R5 |
| DE <u>ECS</u> ELSMVADK; INGEW <u>H</u> TIILASDK | Major urinary protein 1 | Mup7 | Q58EV3 |
| DFTPV <u>CTTELGR</u> | MCG48959; Peroxiredoxin 6 | Prdx6b | Q8BG37 |
| WIDI <u>HNPATNEVVG</u> R | Methylmalonate-semialdehyde dehydrogenase [acylating] | Aldh6a1 | Q9EQ20 |
| EIAGATPH <u>ITAAEGR</u> ; GIEQAVQS <u>HAVAEEEAR</u> | Mitochondrial inner membrane protein | Immt | E9Q800 |
| NYPEG <u>H</u> QAAAVLPVLDAQR | NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial | Ndufv2 | Q9D6J6 |
| EVHP <u>DIDSER</u> | Peroxisomal acyl-coenzyme A oxidase 2 | Acox2 | Q9QXD1 |
| HVLQQ <u>FADNDVSR</u> | Peroxisomal multifunctional enzyme type 2 | Hsd17b4 | P51660 |
| ED <u>CTQP</u> DYEATSR | Probable proline dehydrogenase 2 | Prodh2 | Q8VCZ9 |
| GHP <u>DLQGQPADDIF</u> ESIGDR; | Protein ERGIC-53 | Lman1 | Q9D0F3 |
| NQQEGV <u>CPEGSIDN</u> SPVK | Protein Gm20425; Peroxiredoxin 6 | Gm2042 | E9Q035 |
| ADFA <u>QA</u> C <u>QDAGVR</u> ; DA <u>H</u> QSLLATR; | Pyruvate carboxylase | Pcx | G5E8R3 |
| NIDE <u>H</u> ANEDVER | Ras-related protein Rab-10 | Rab10 | P61027 |
| YLDED <u>TVYH</u> LQPSGR | S-adenosylmethionine synthase isoform type-1 | Mat1a | Q91X83 |
| D <u>H</u> DASVYLR; NYSVVFP <u>H</u> DEPLAGR | Sarcosine dehydrogenase, mitochondrial | Sardh | Q99LB7 |
| SGYQQAA <u>SEH</u> GLVVIA <u>PDTSPR</u> | S-formylglutathione hydrolase | Esd | H3BLJ9 |
| SGGGGGSSGAGGGP <u>SC</u> GTSSR | Signal peptidase complex subunit 2 | Spc52 | Q9CYN2 |
| SA <u>H</u> AGTYEV <u>R</u> | Signal sequence receptor, delta | Ssr4 | Q9D8L3 |
| <u>H</u> VLGVPLDDR | Solute carrier family 2, facilitated glucose transporter member 2 | Slc2a2 | E9PXR7 |
| NDIS <u>H</u> PPVEGSYAPR; S <u>SH</u> YDELLAAE <u>AR</u> | Staphylococcal nuclease domain-containing protein 1 | Snd1 | Q78PY7 |
| GAAA <u>H</u> PDSEEQQQR | Talin-1 | Tln1 | P26039 |
| D <u>H</u> DTFLAVR | Vesicular integral-membrane VIP36 | Lman2 | Q9DBH5 |

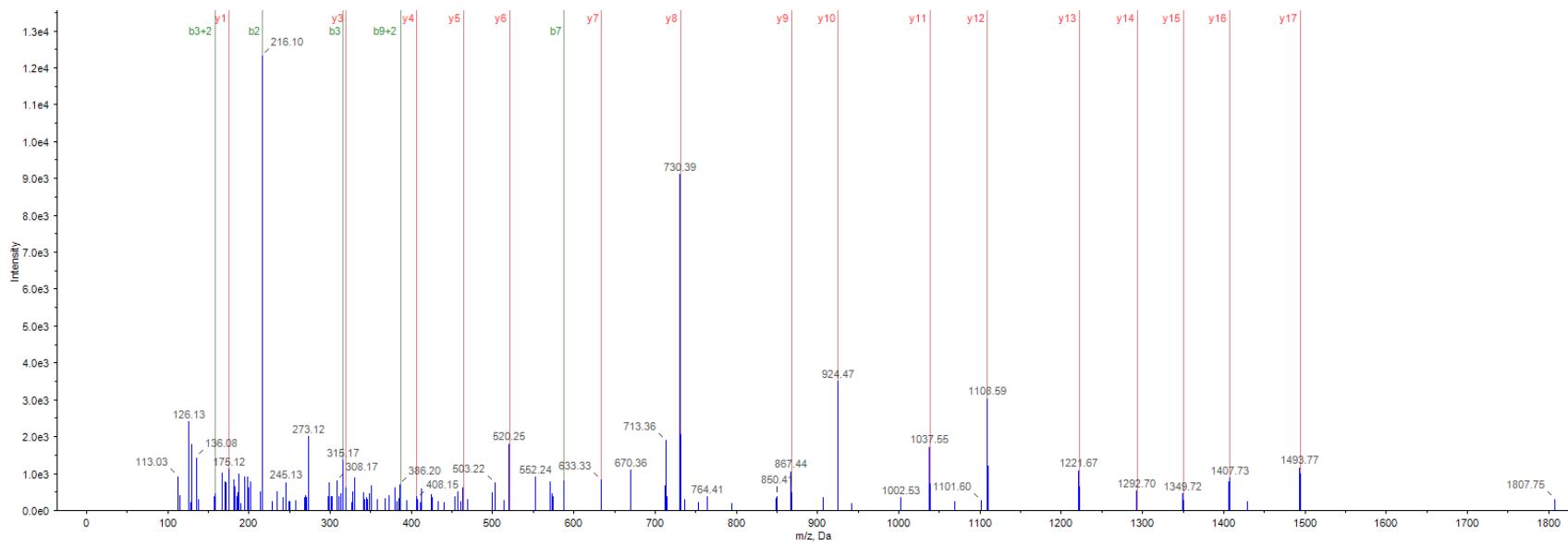
*Underlined amino acid in each peptide sequence are modified with HNE and labeled with amidoxyTMT. All peptides shown were identified at >90% Confidence using the ProteinPilot sequencing database searching program.

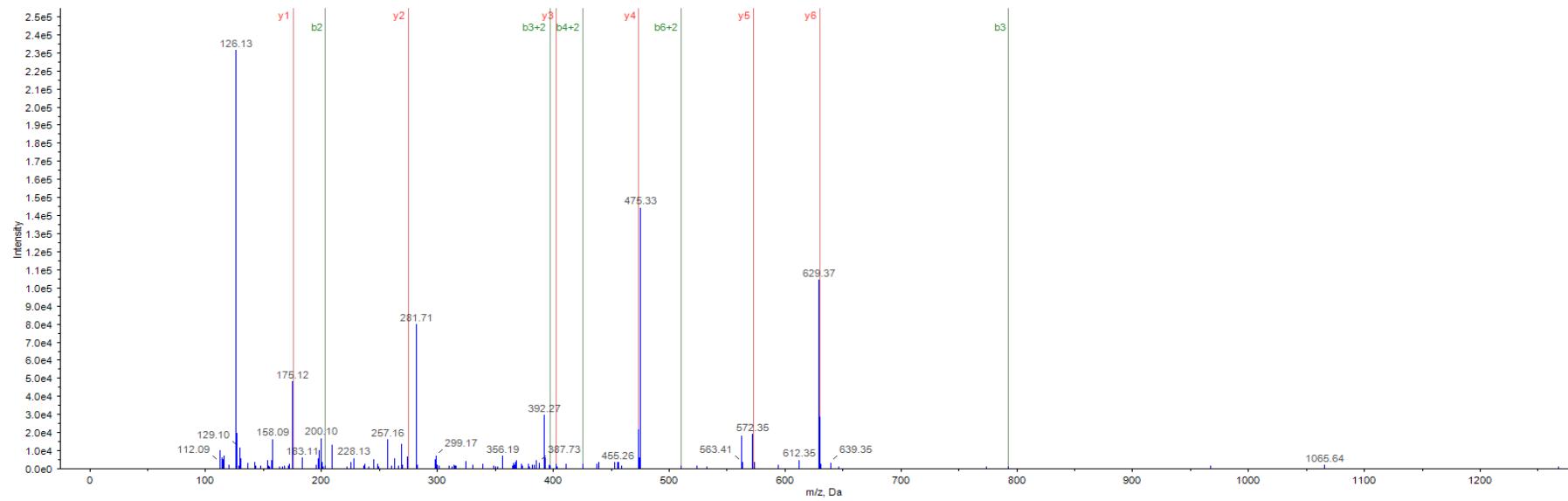


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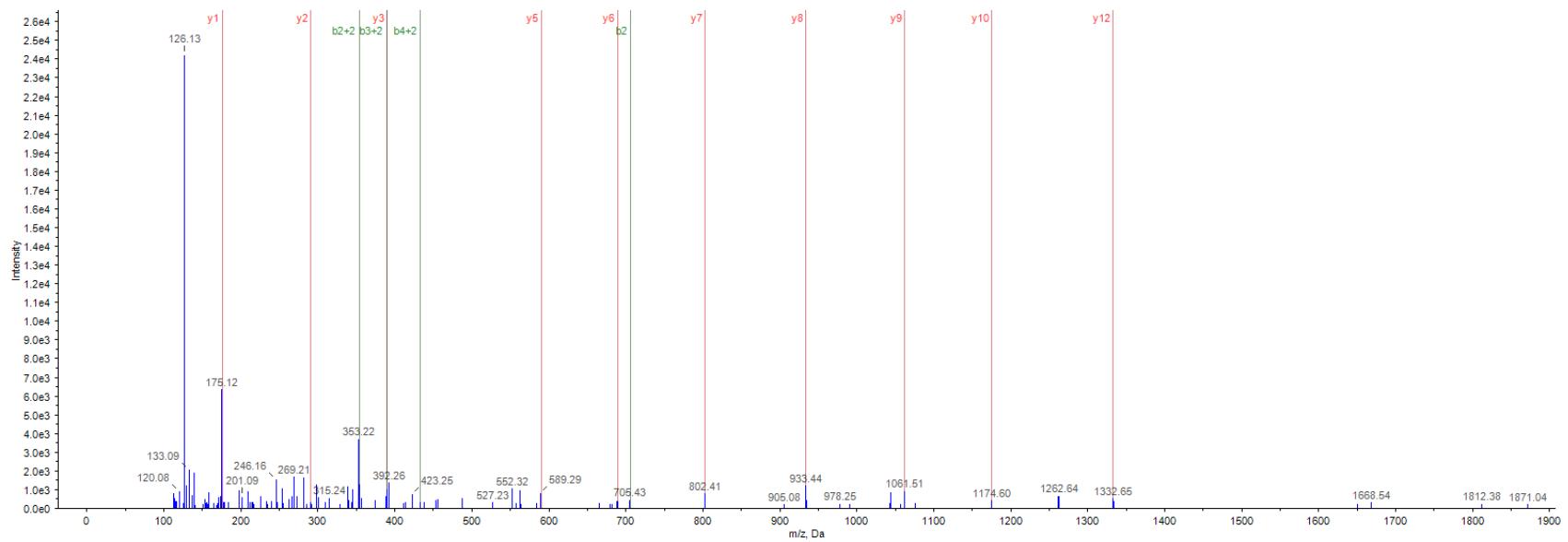


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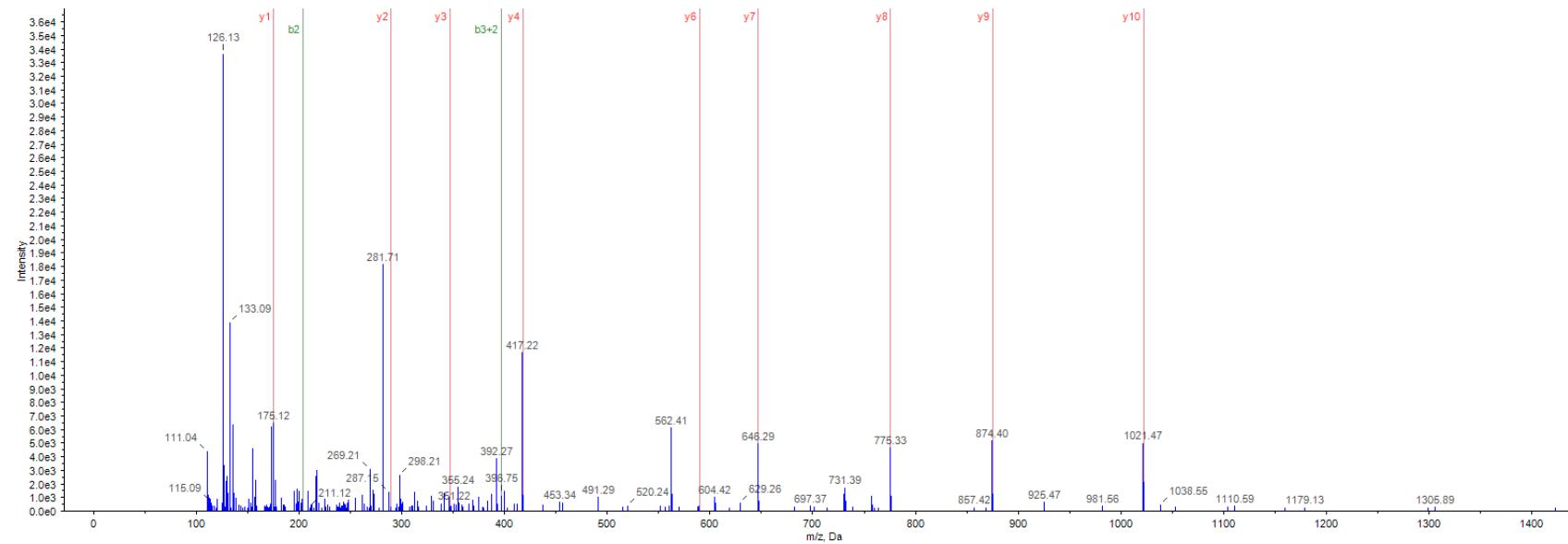




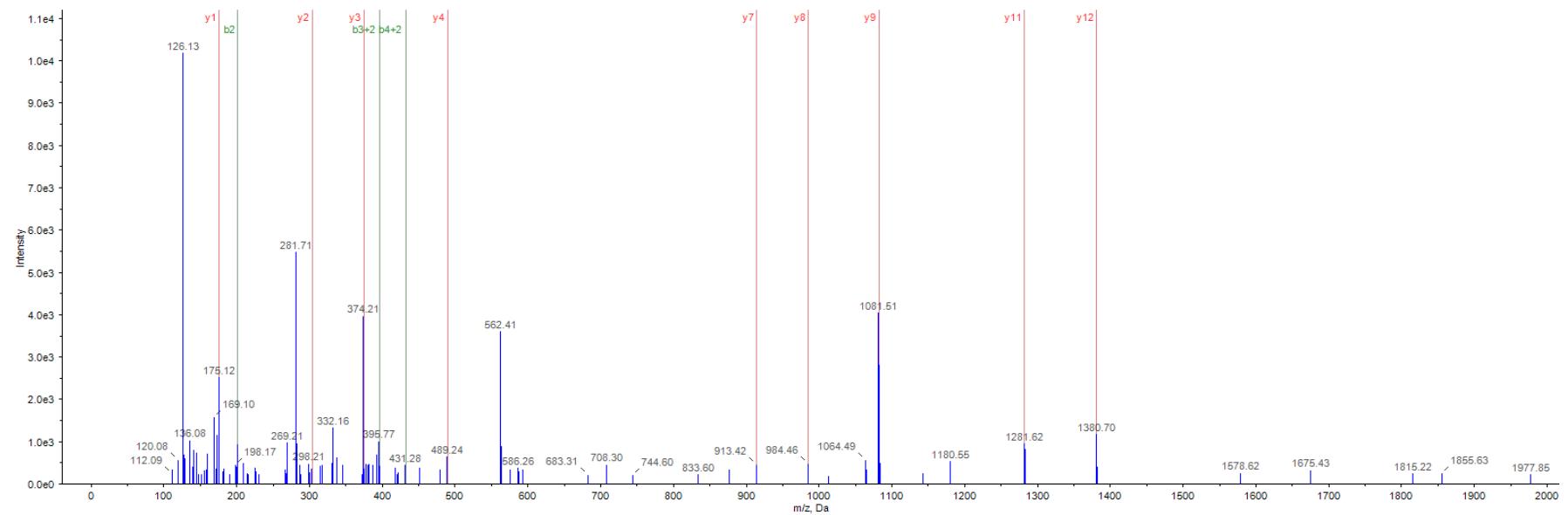
DH[HT]ASIQMNVAEVDR

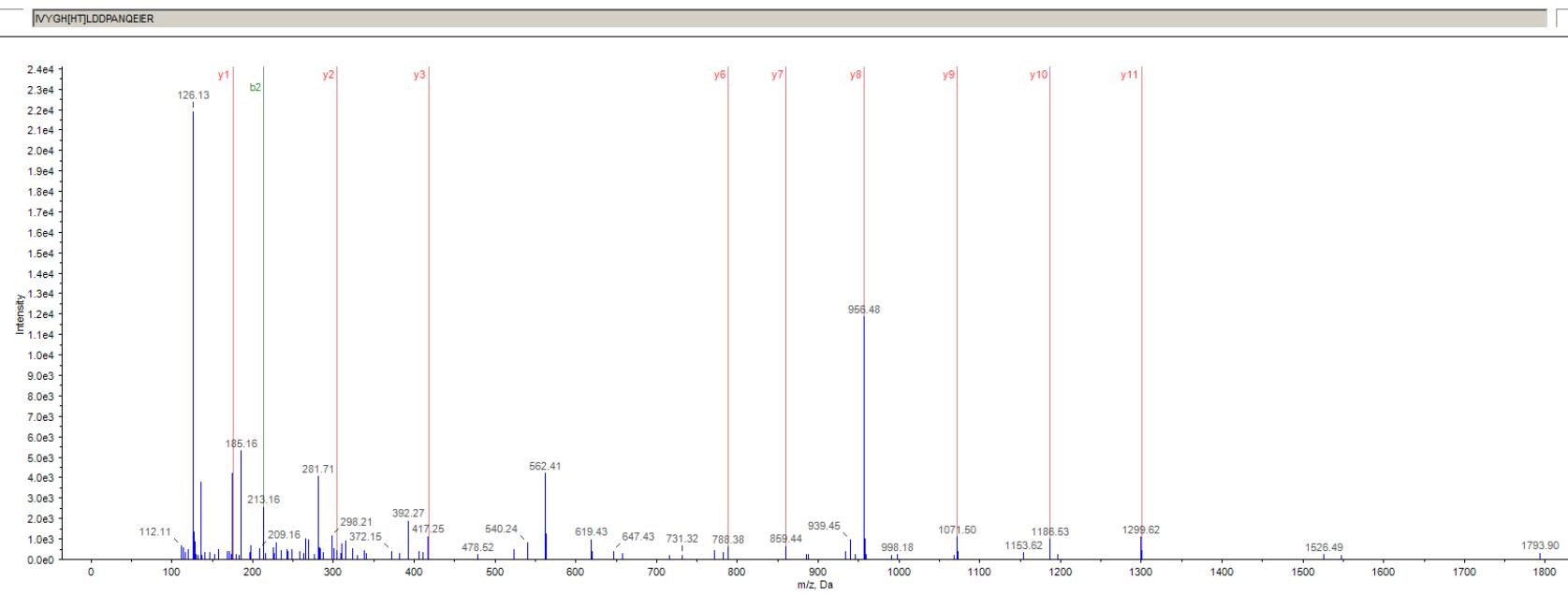


TTH[HT]FVEGGDAGNR

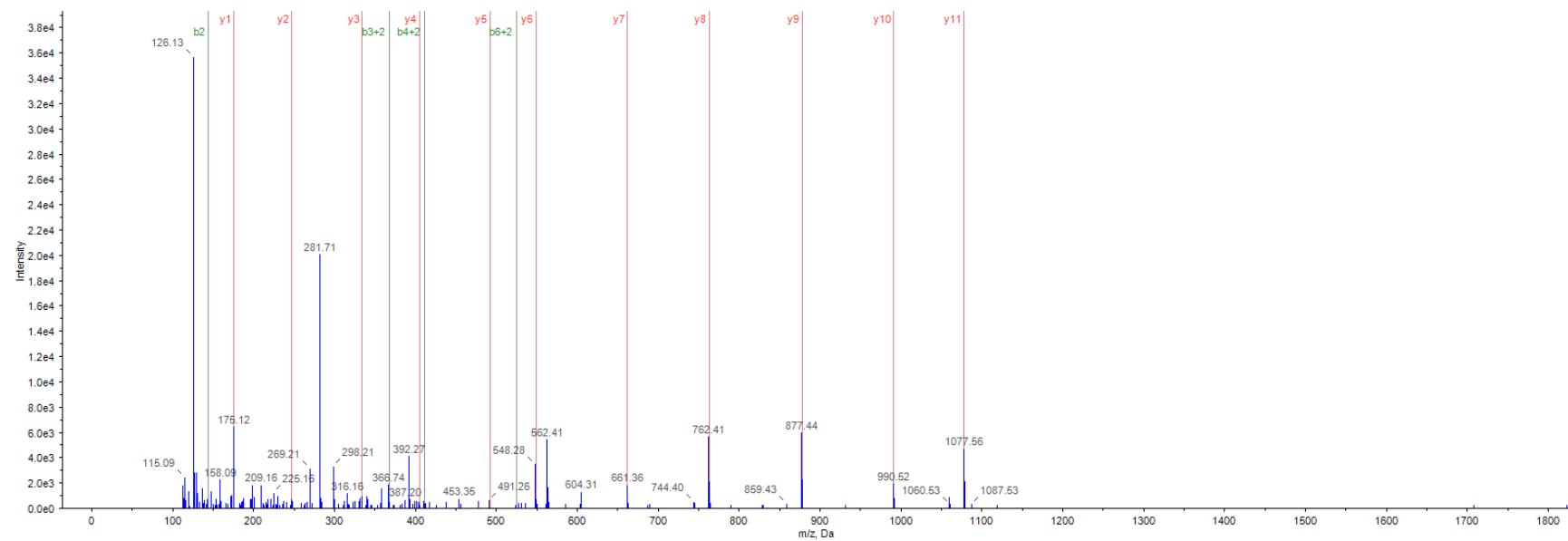


[MTH[HT]AVVTVPAYFNDAQR

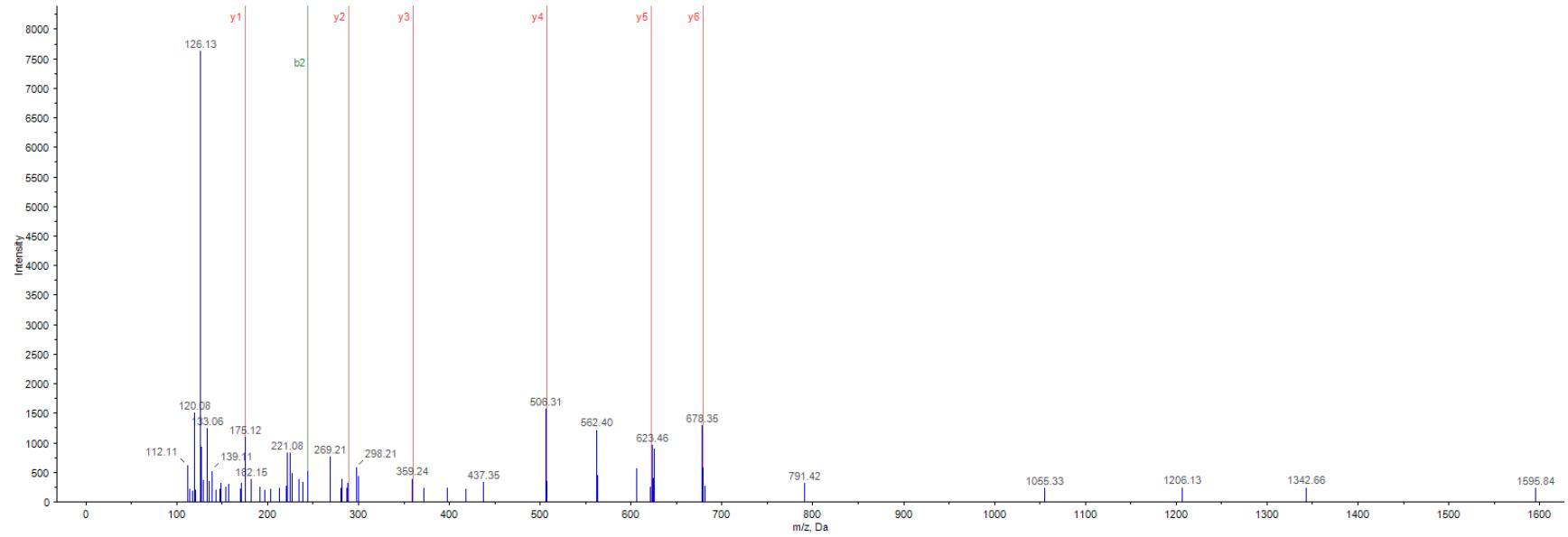




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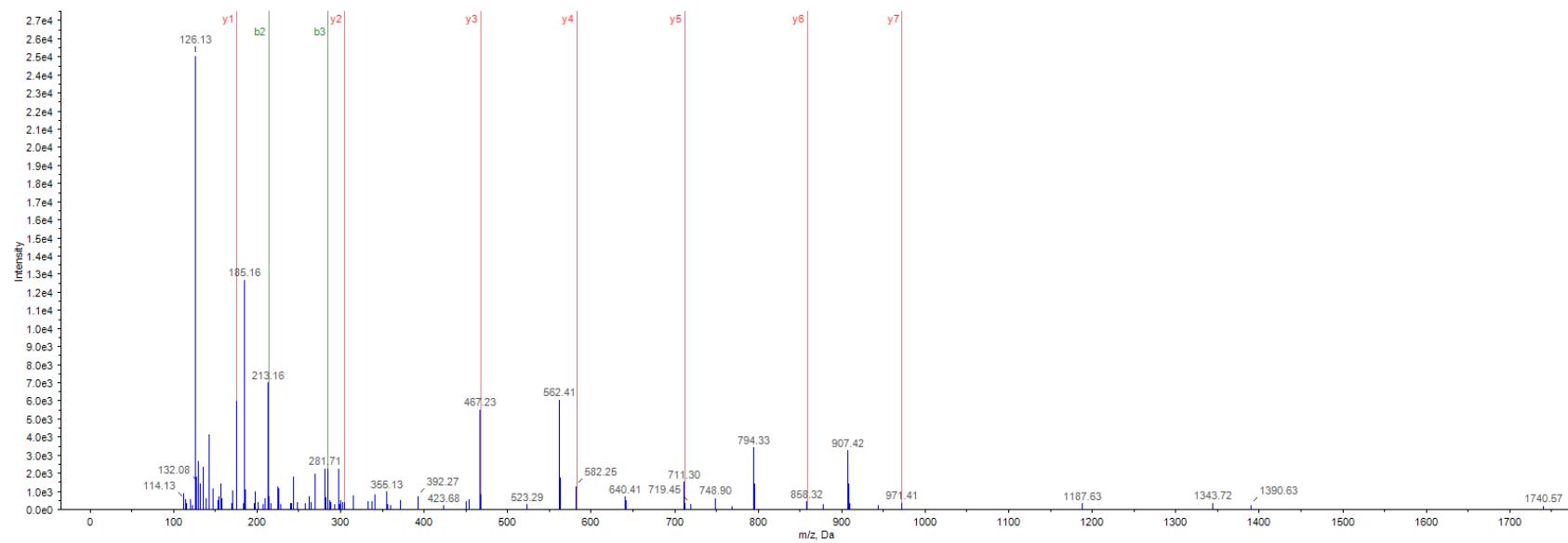


DQSPASH[HT]EATNLGDFALR

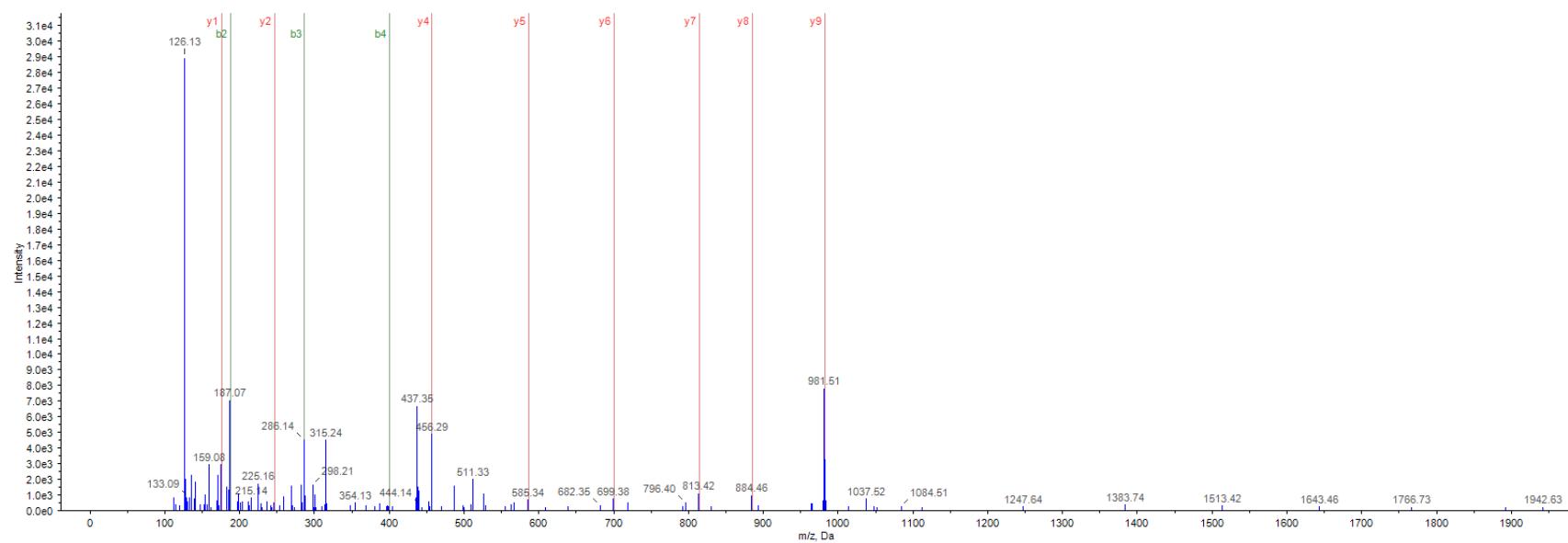


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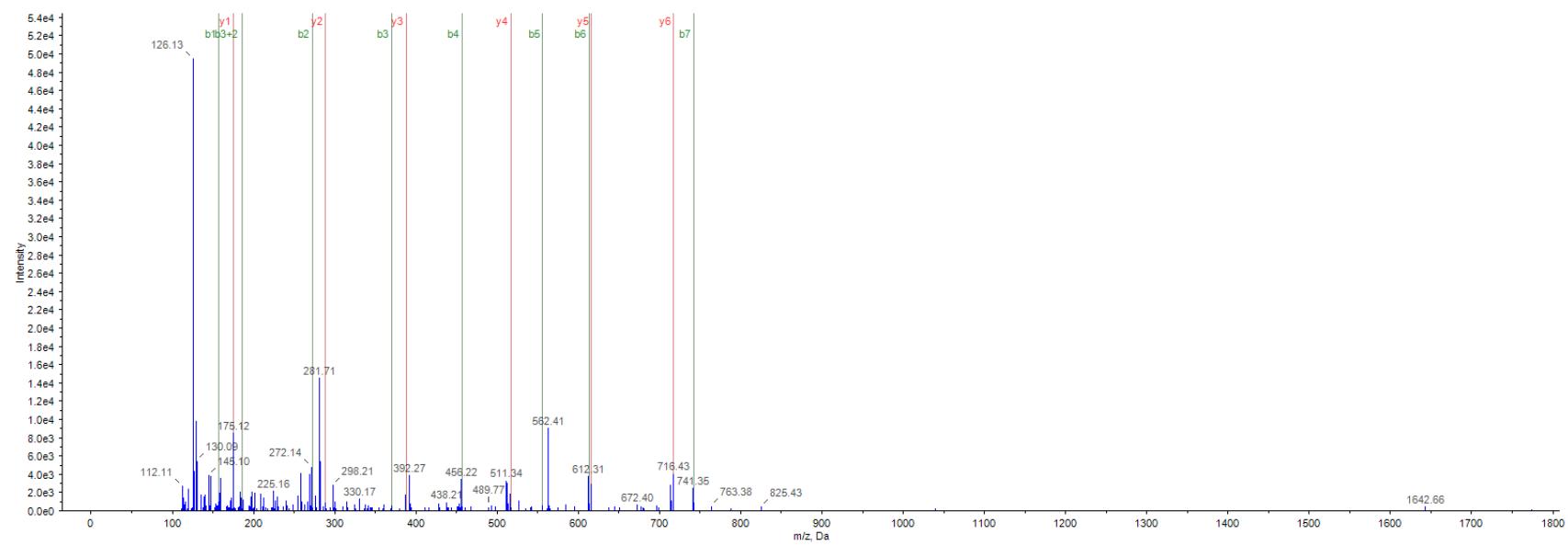
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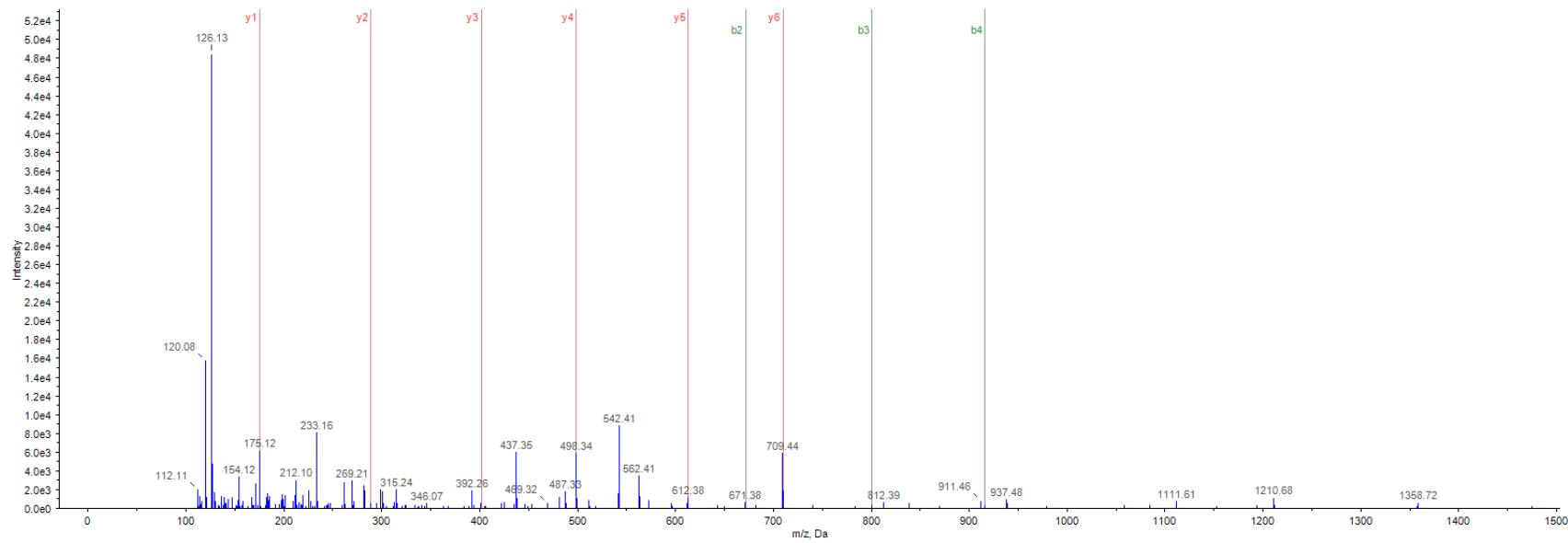


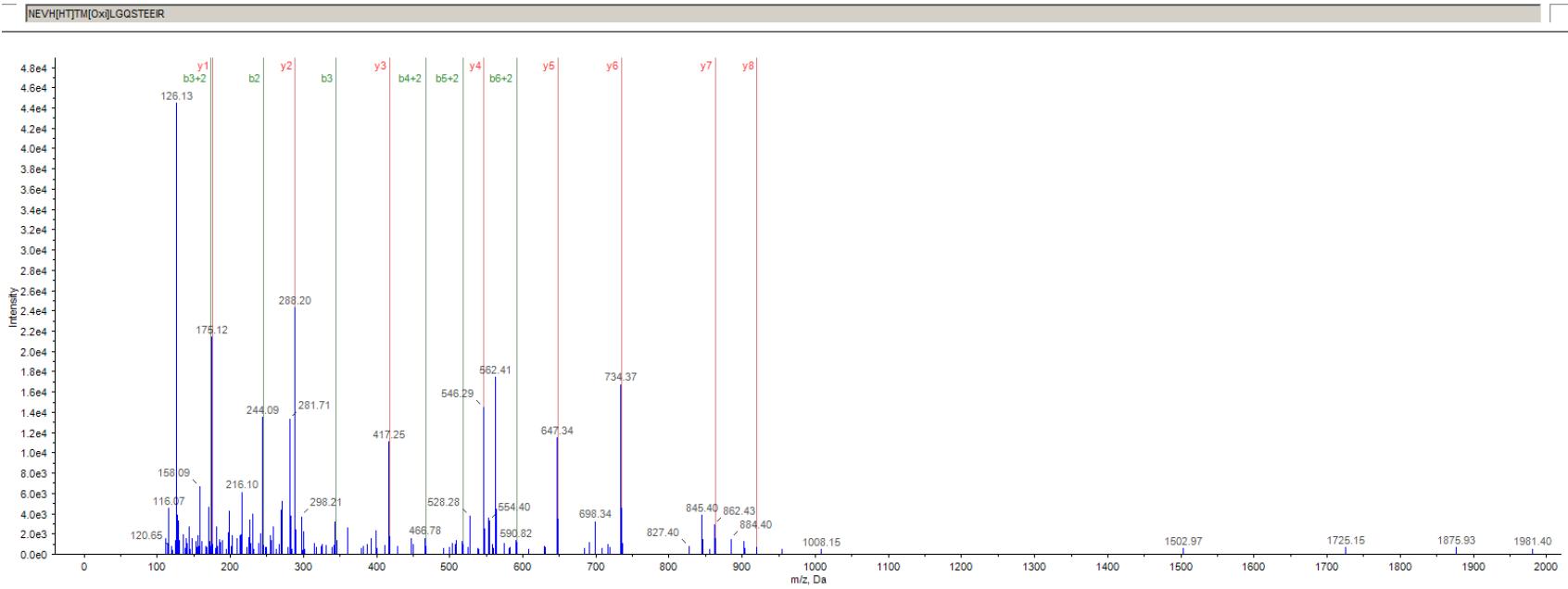
GEVITYC[HT]PANNEPAR



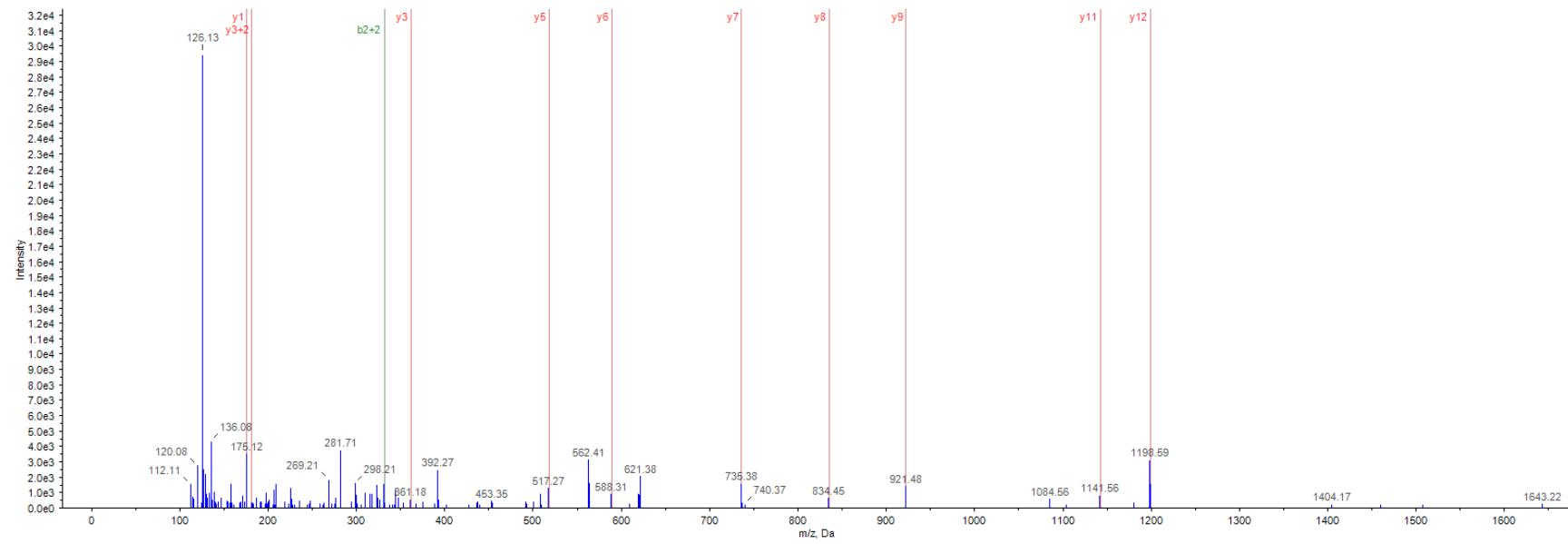
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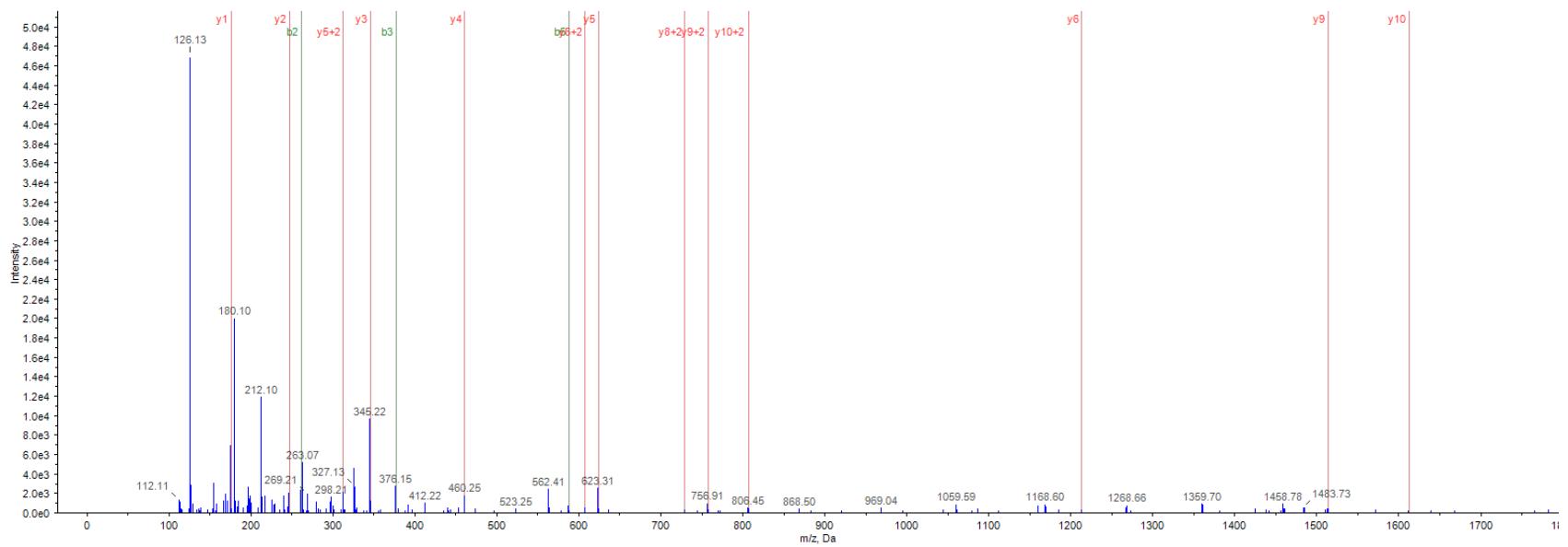




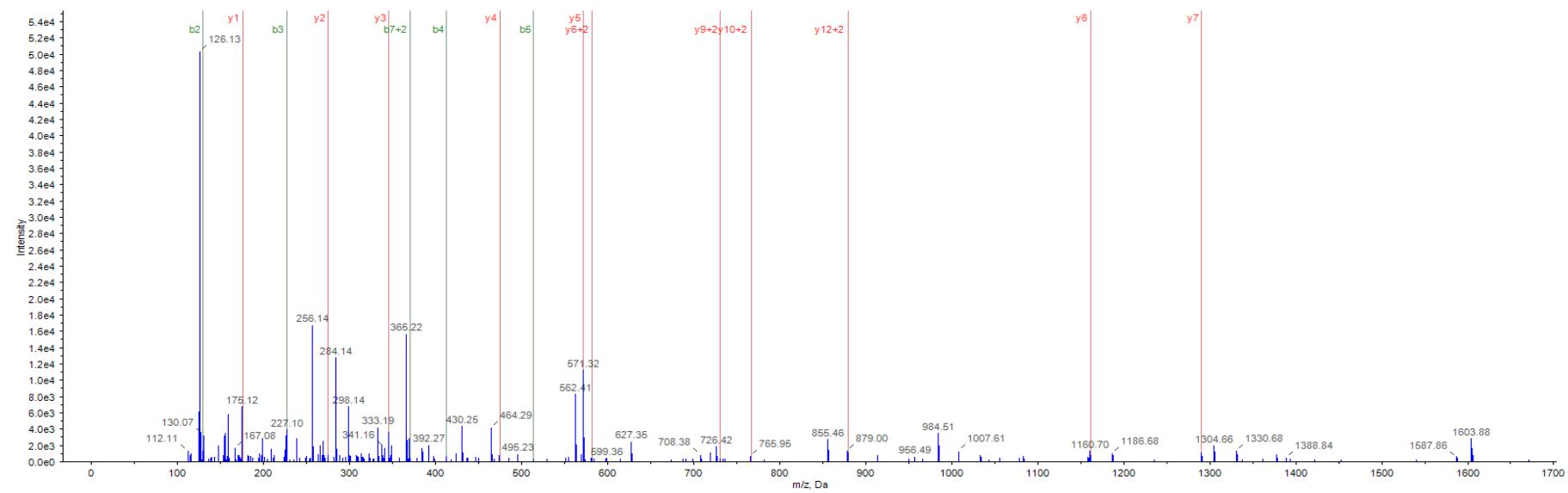
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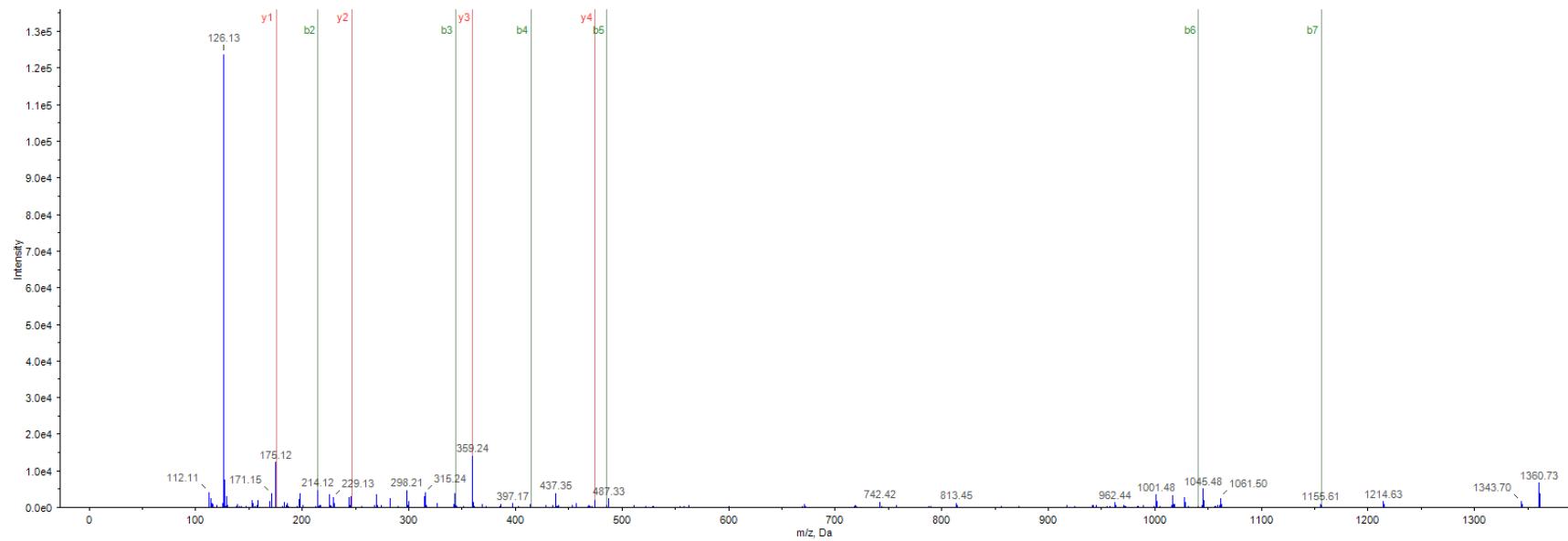


[M+Ox]DPNIVGNEH[HT]YDVAR

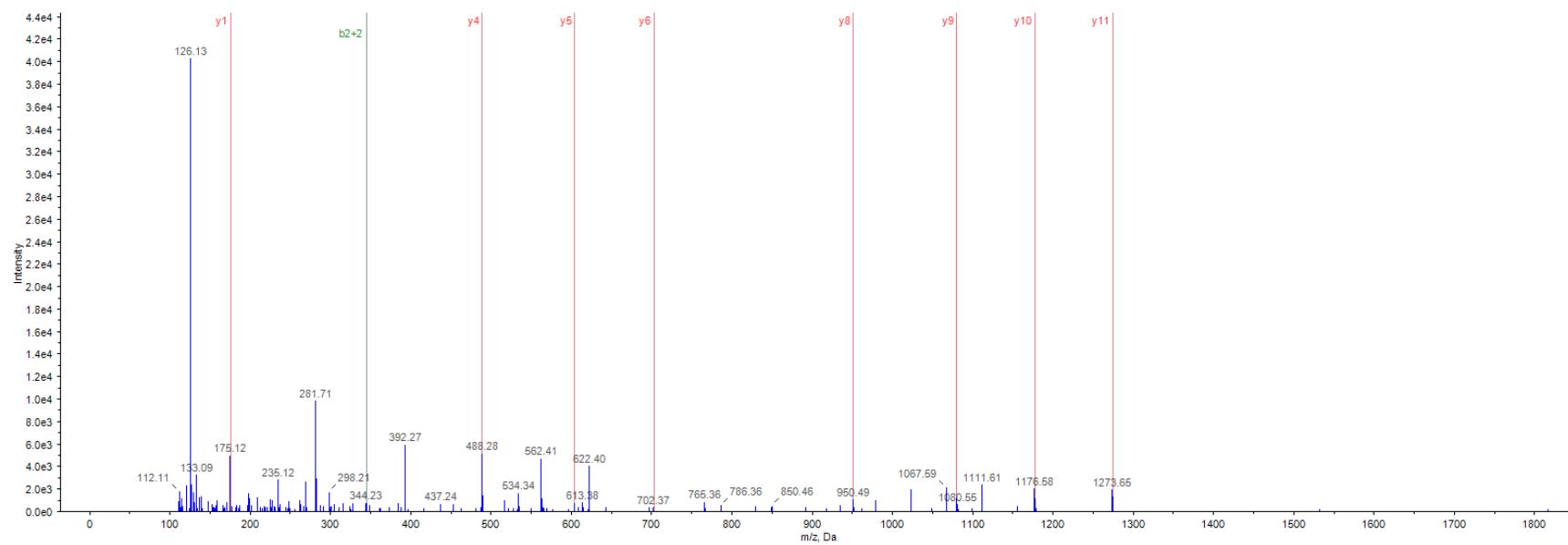


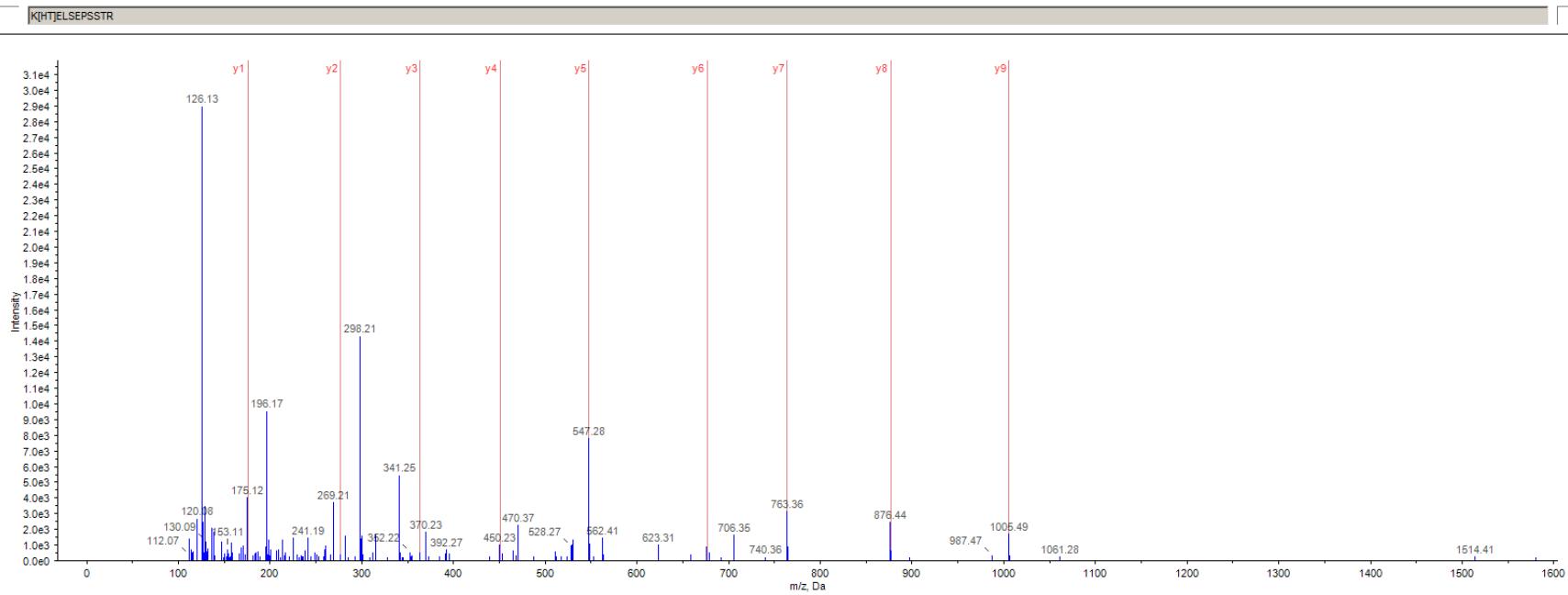
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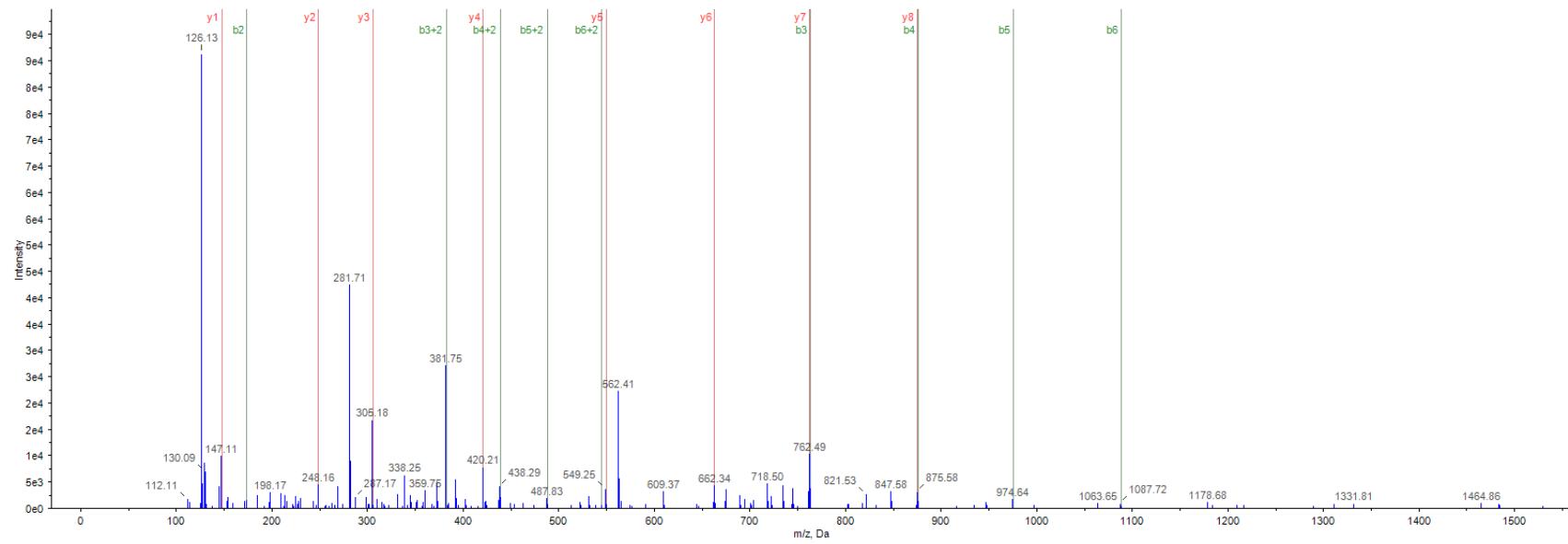


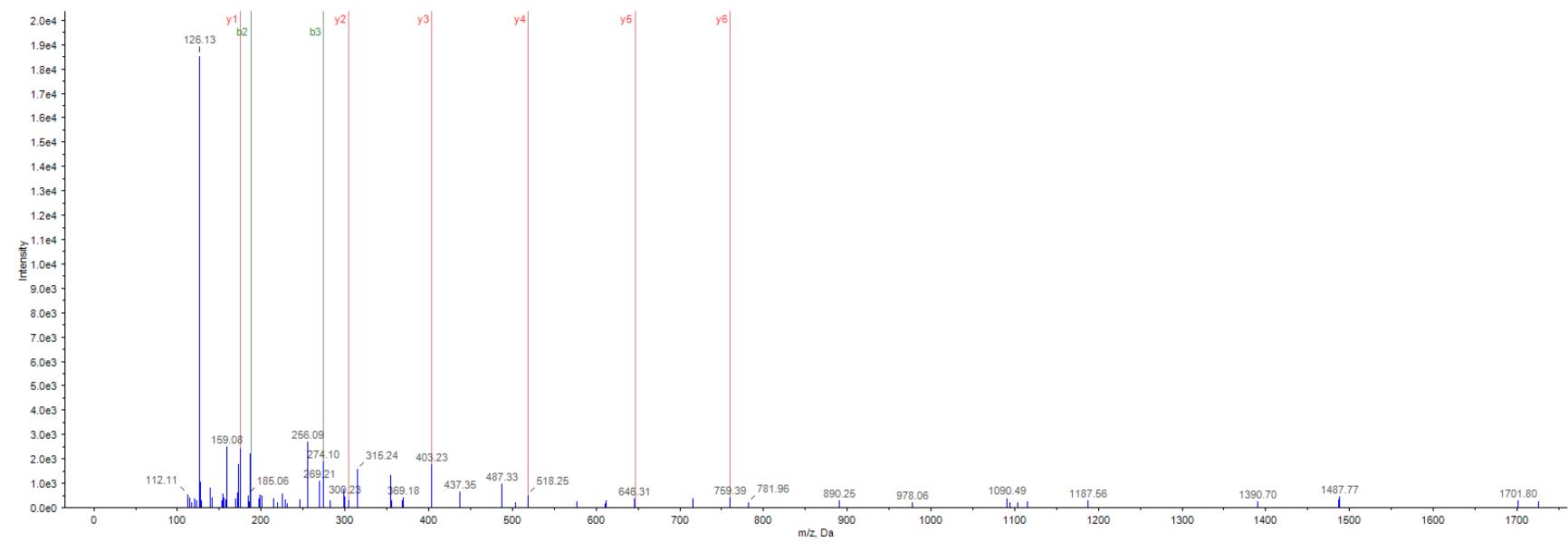


H[HT]PPETFVDALEK

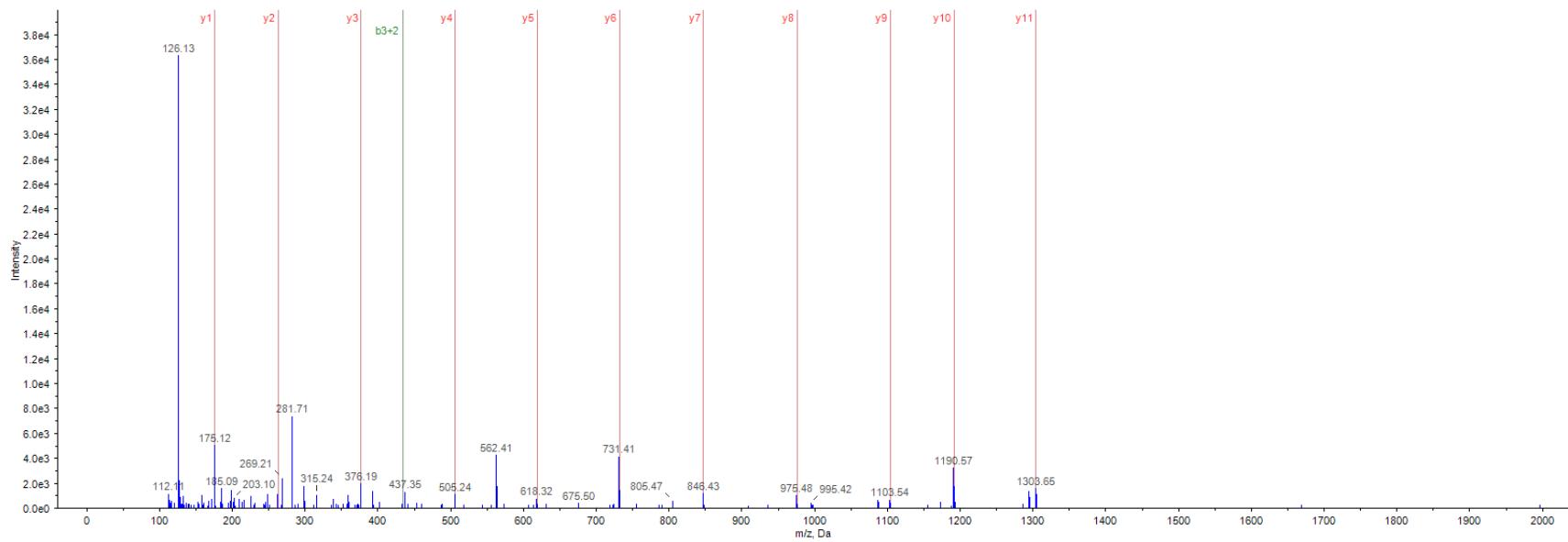




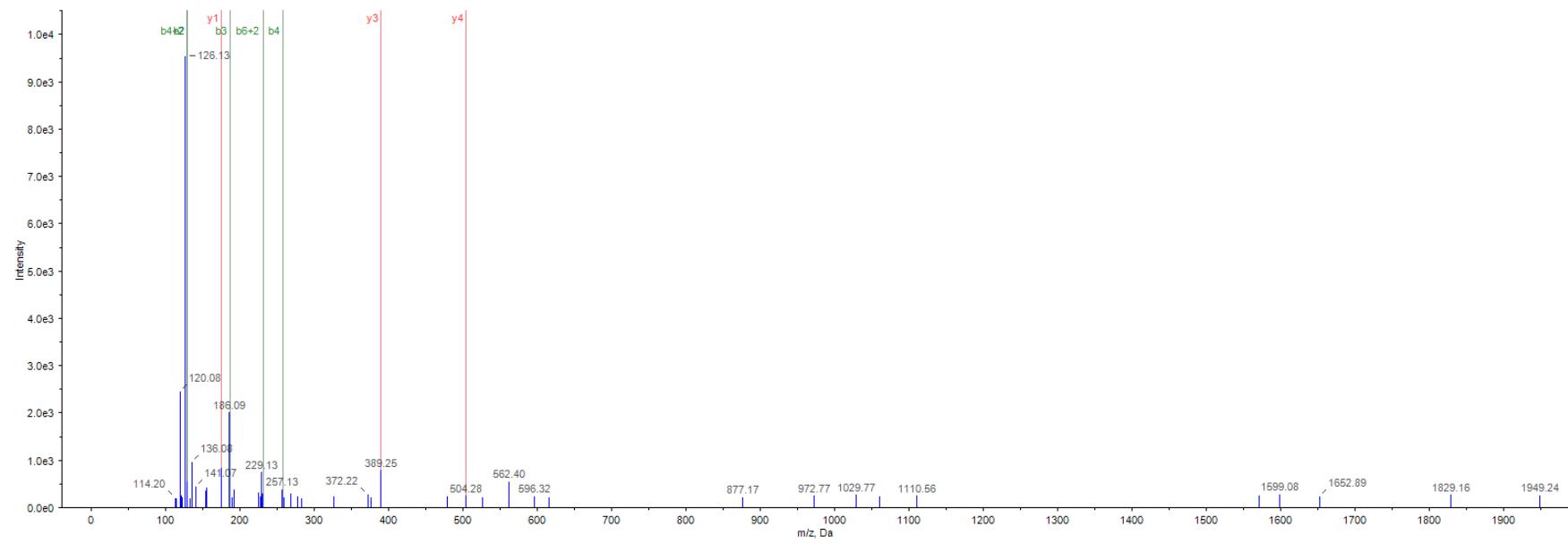




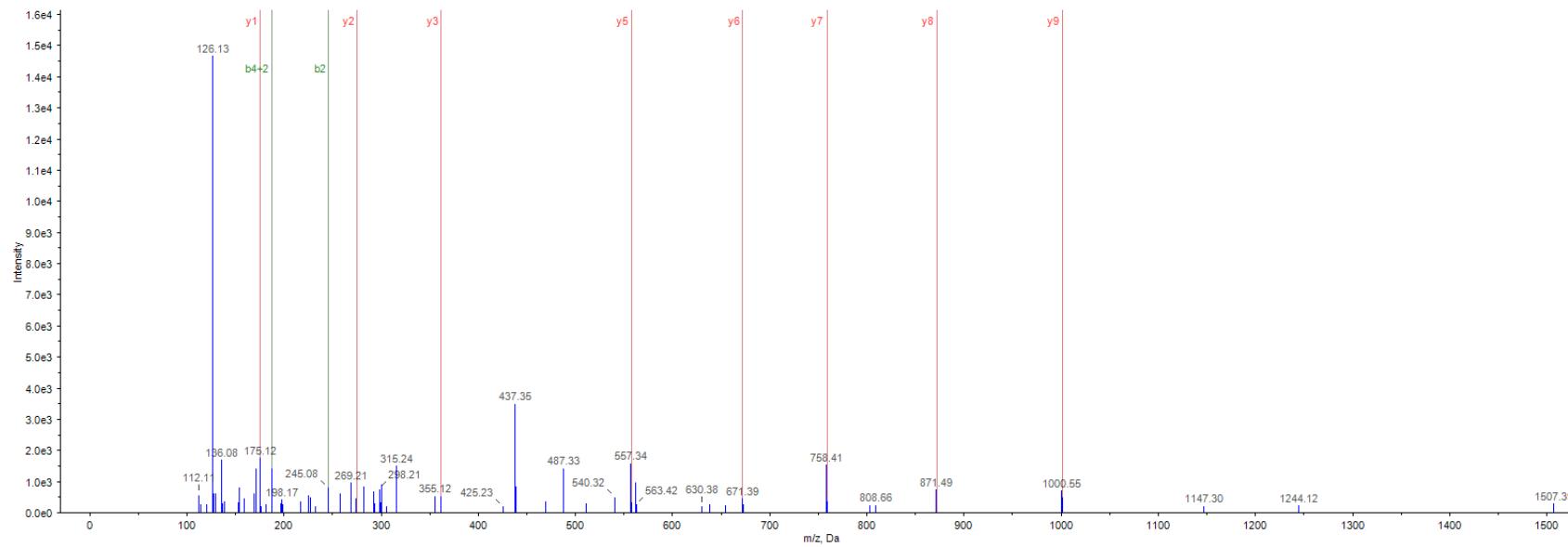
[M+Ox][E][H][HT]LSQEDLLNSR



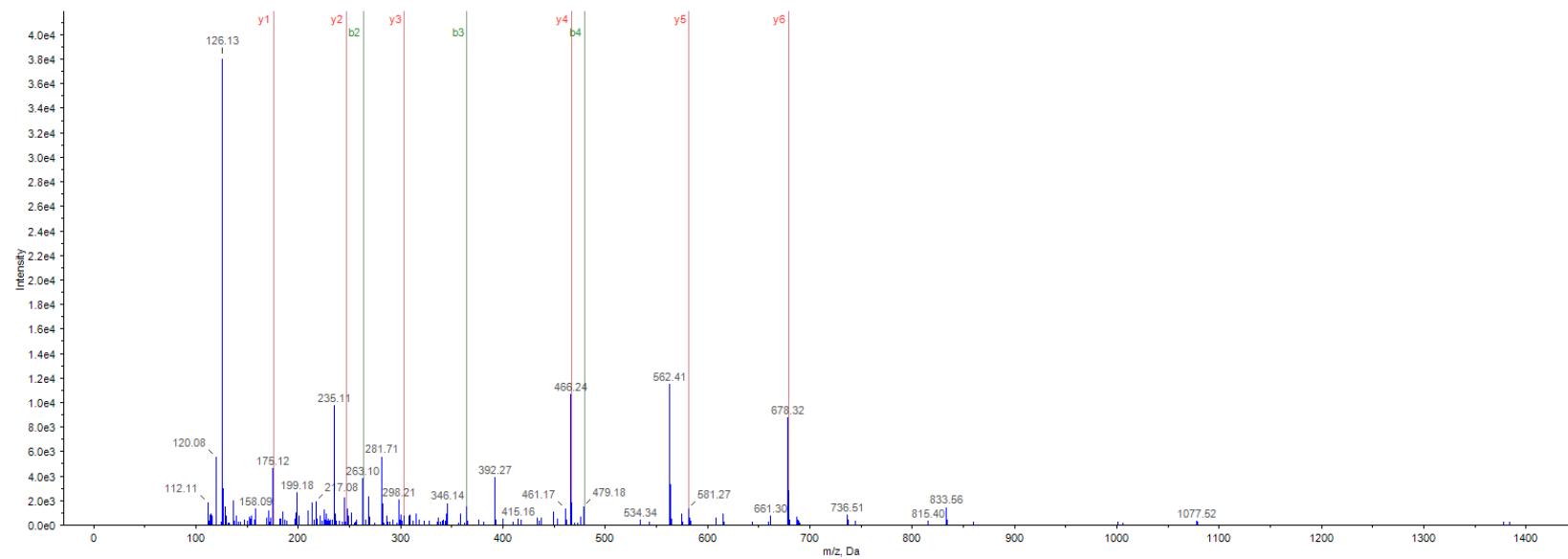
GAGAGFGYFEVTH[HT]DITR



[EDAGEYQC[HT]EISNPVSVR

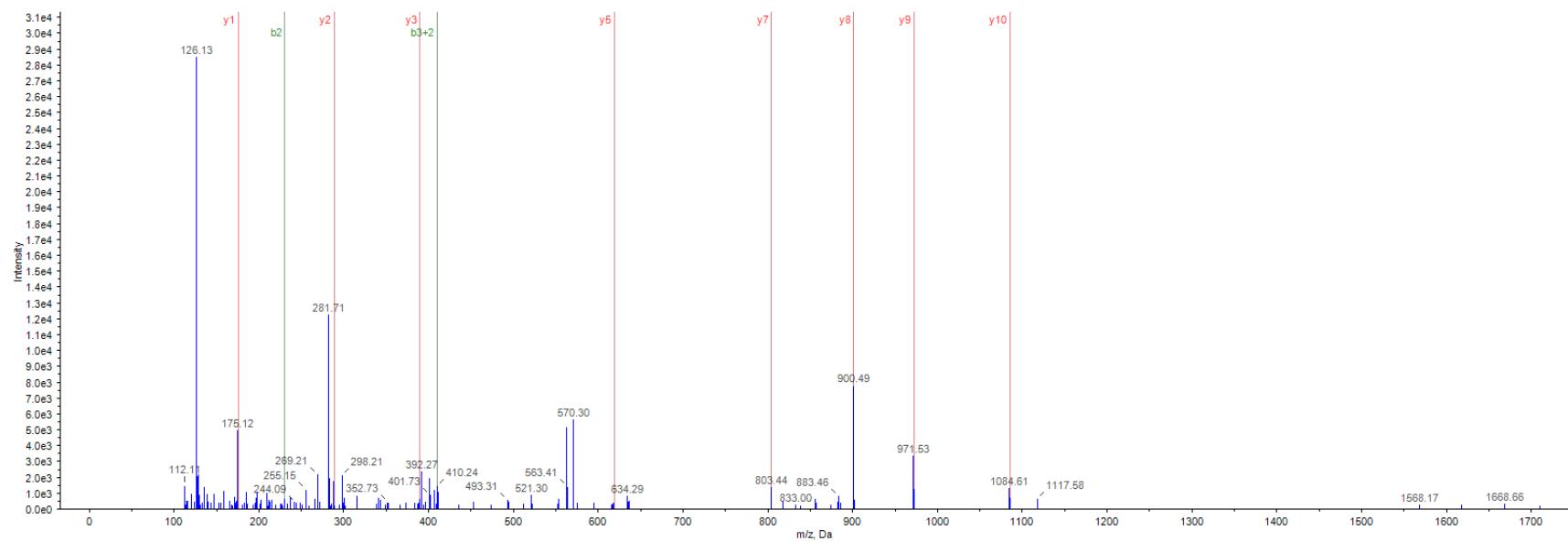


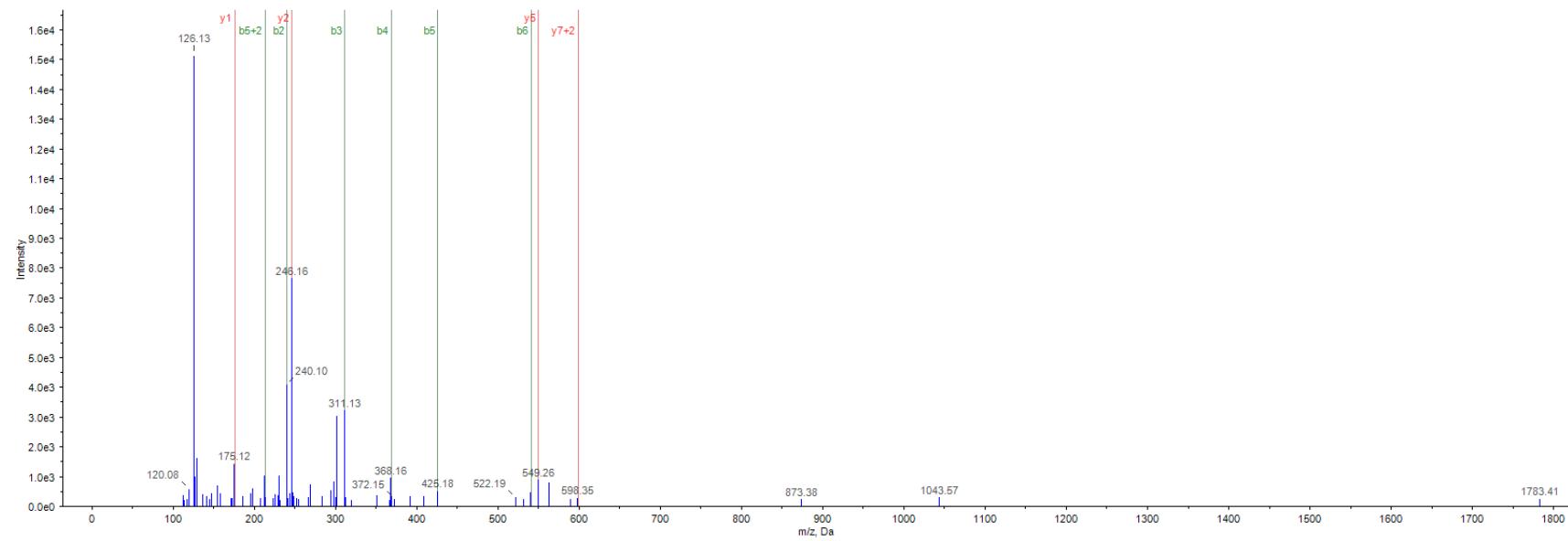
[N]DeaFTDVH[HT]PDY GAR

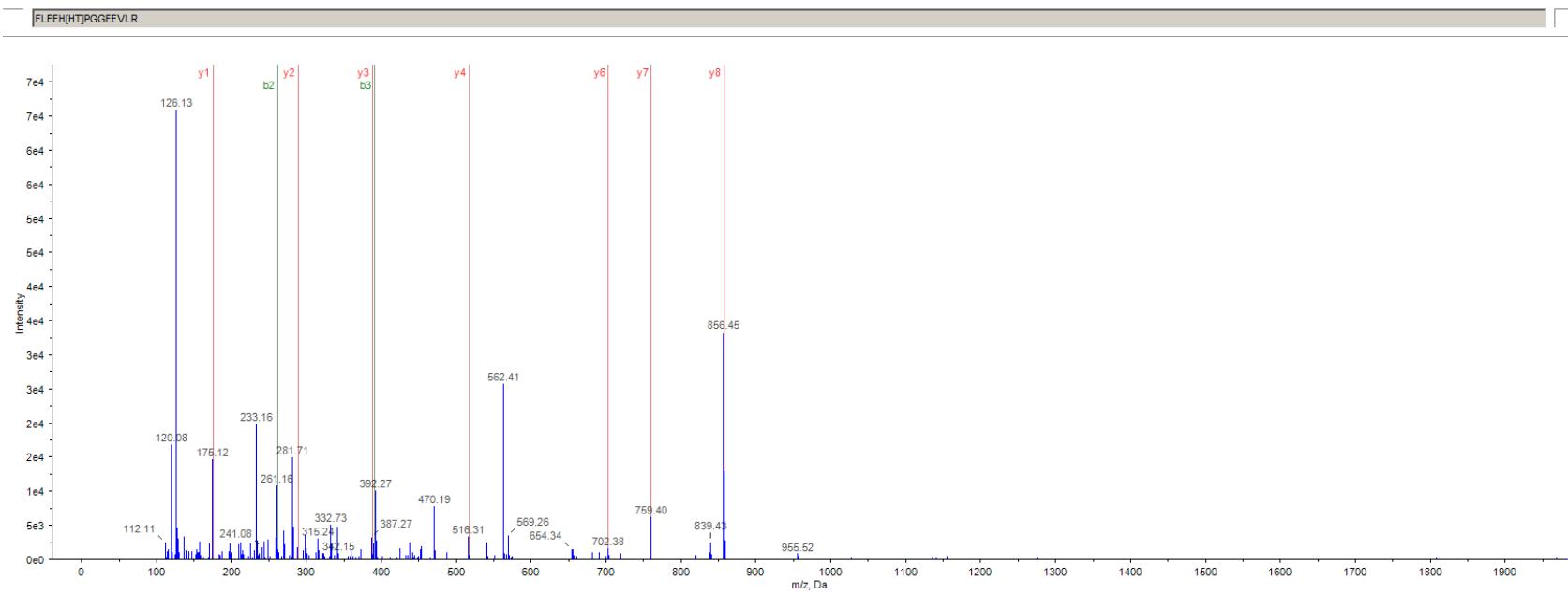


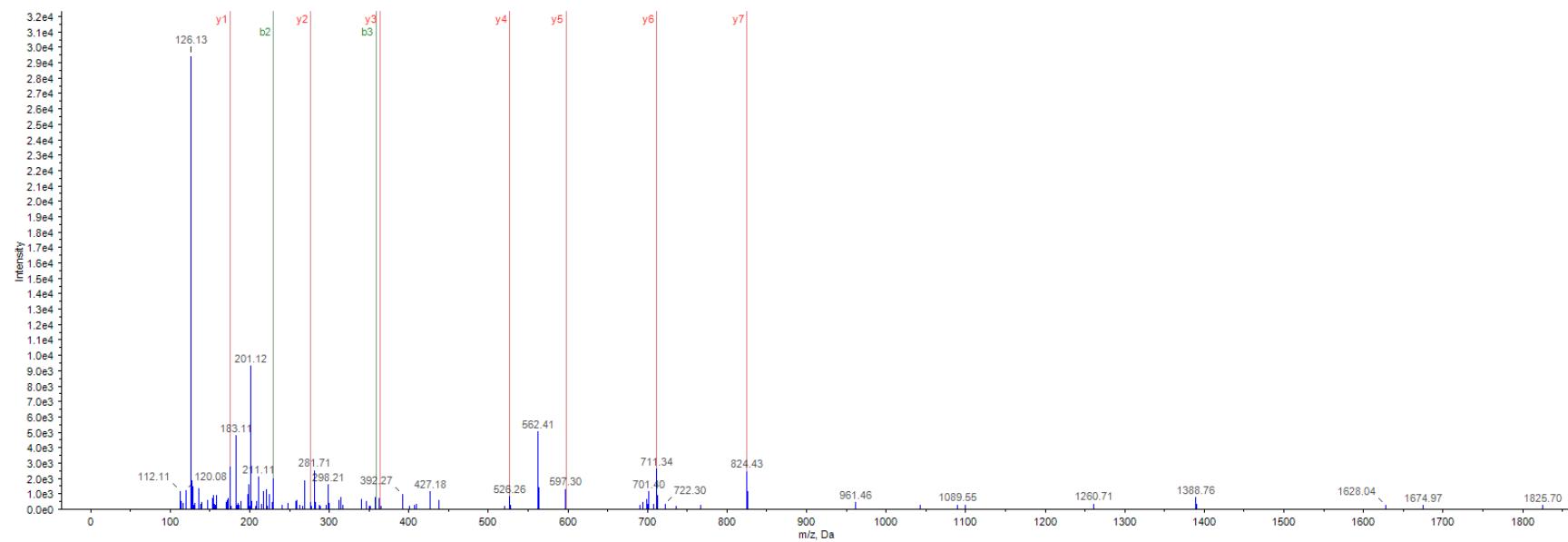
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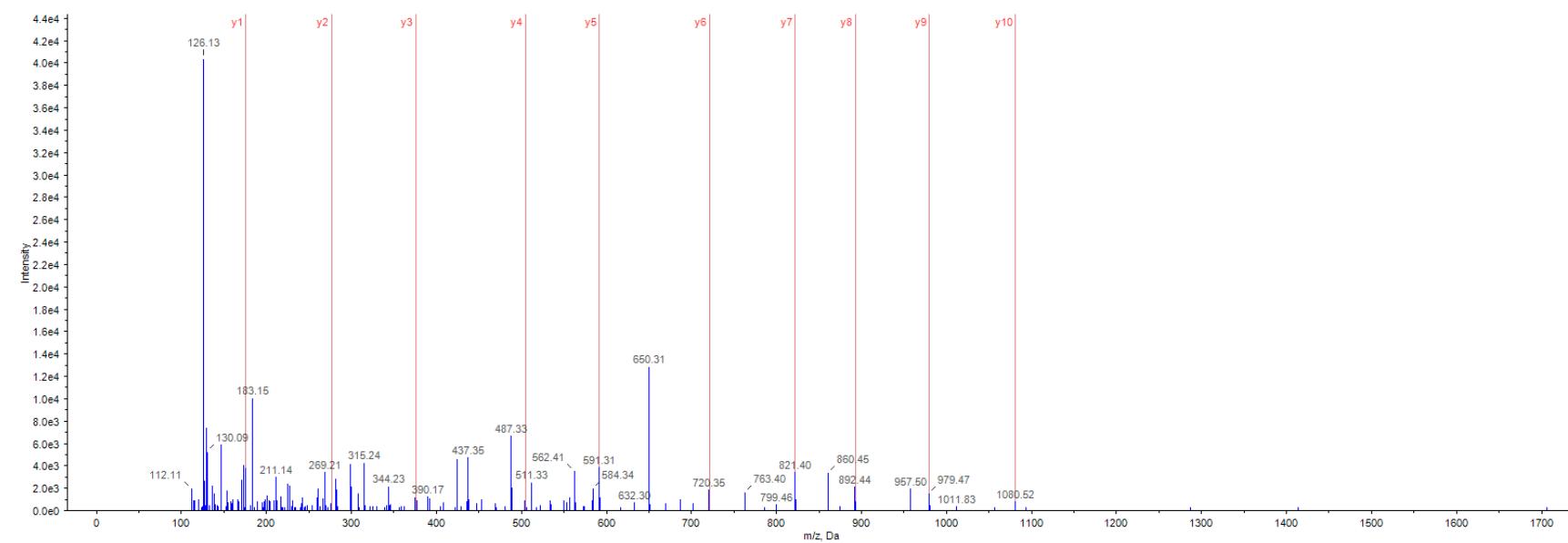
[DNH][HT]LAPGQQTTLR



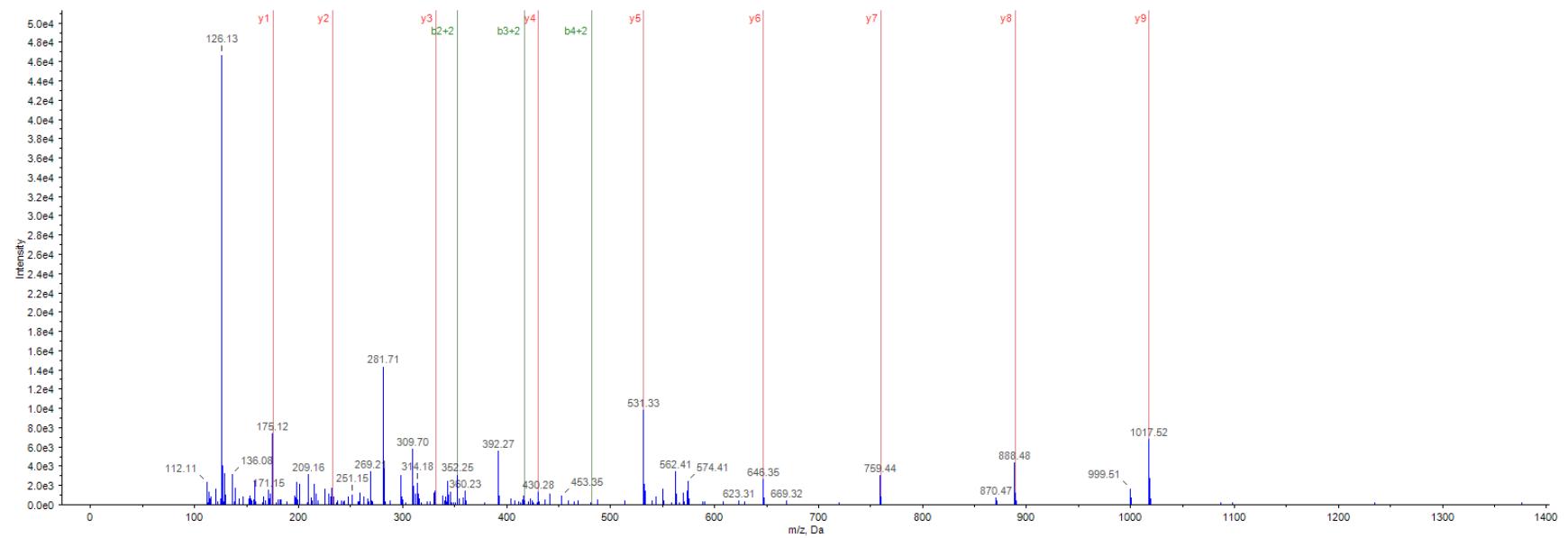




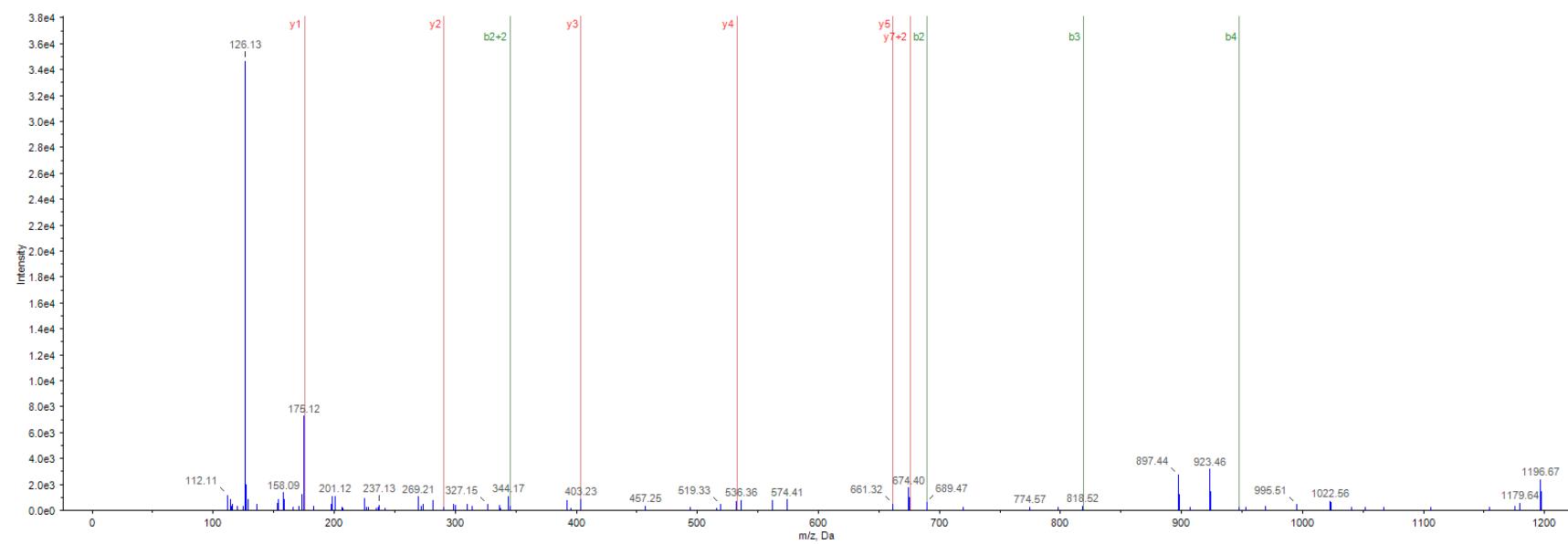




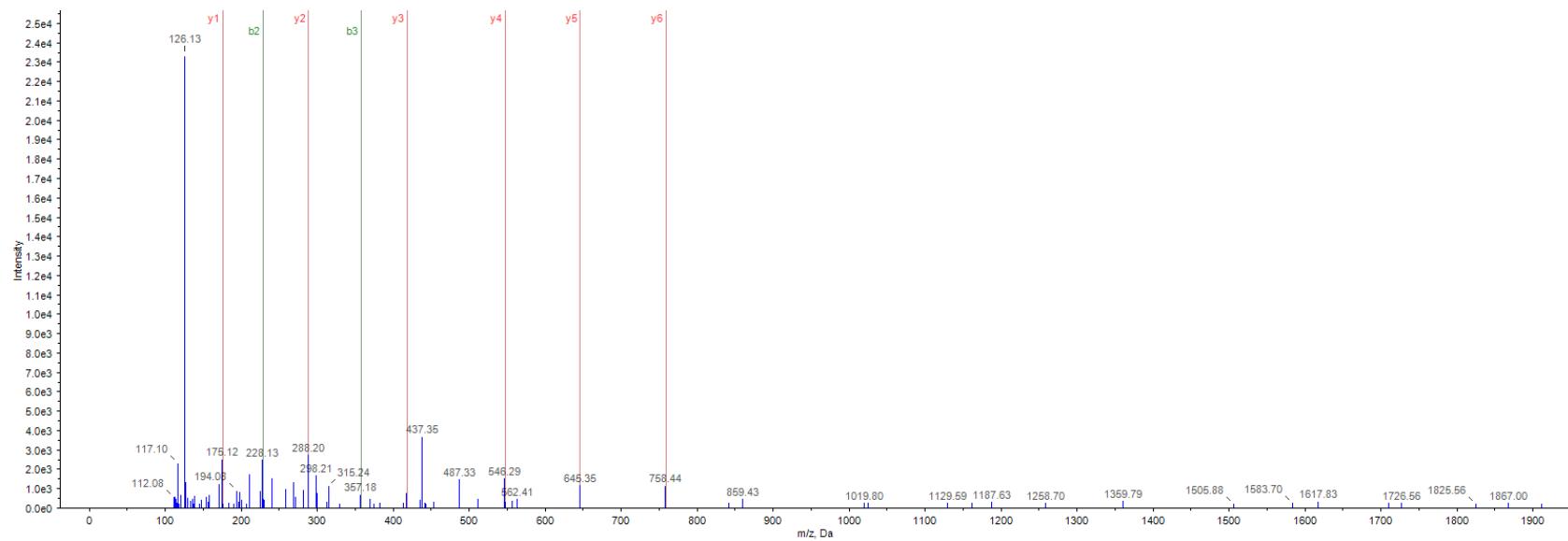
[H][HT]EELDTV[VGR]



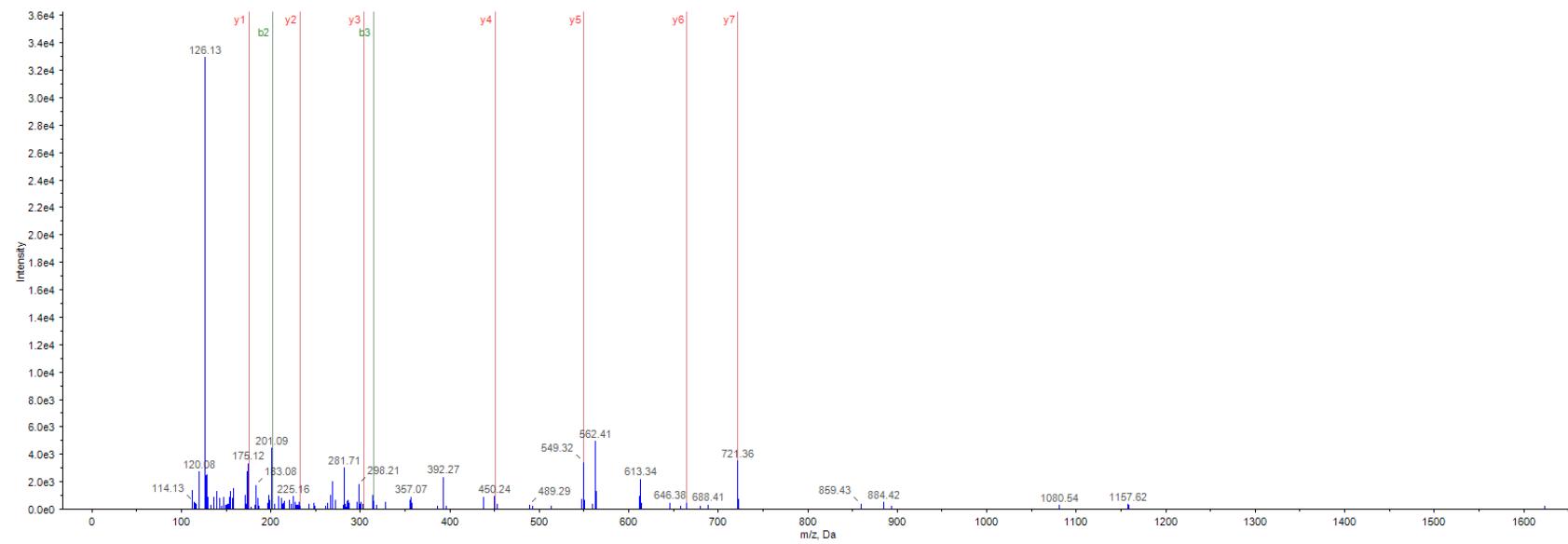
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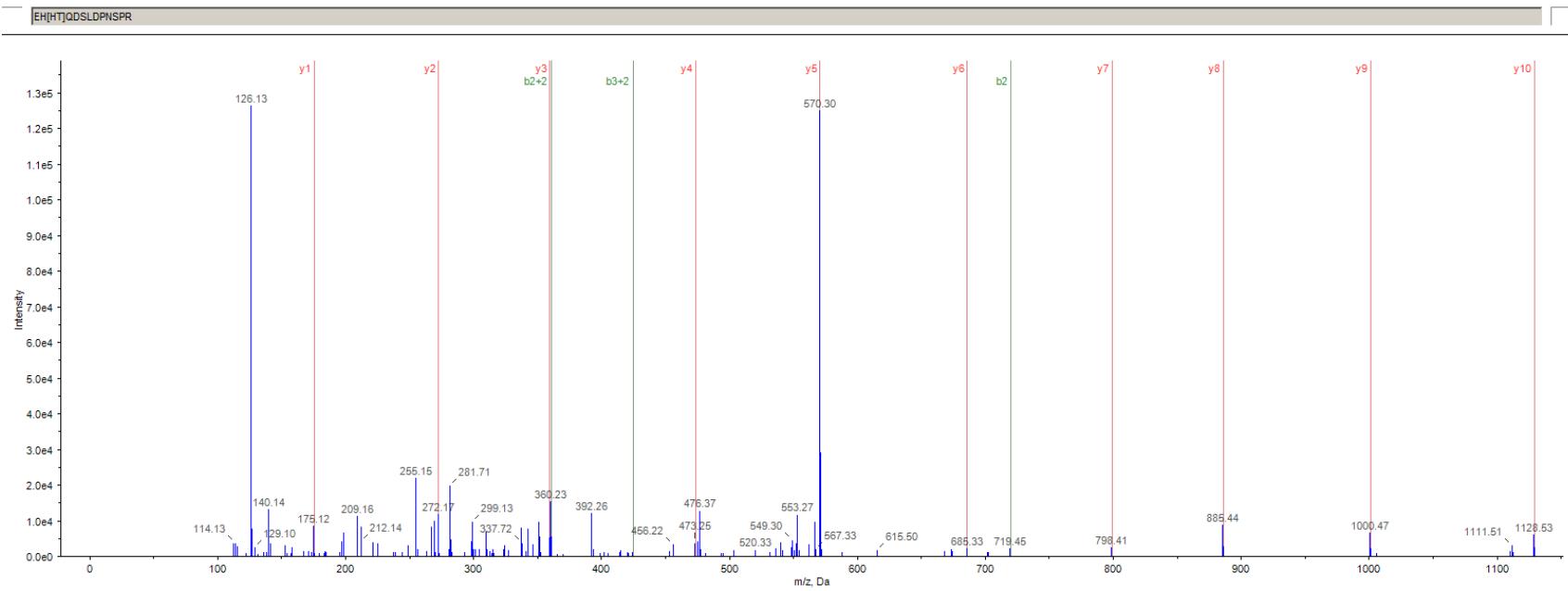


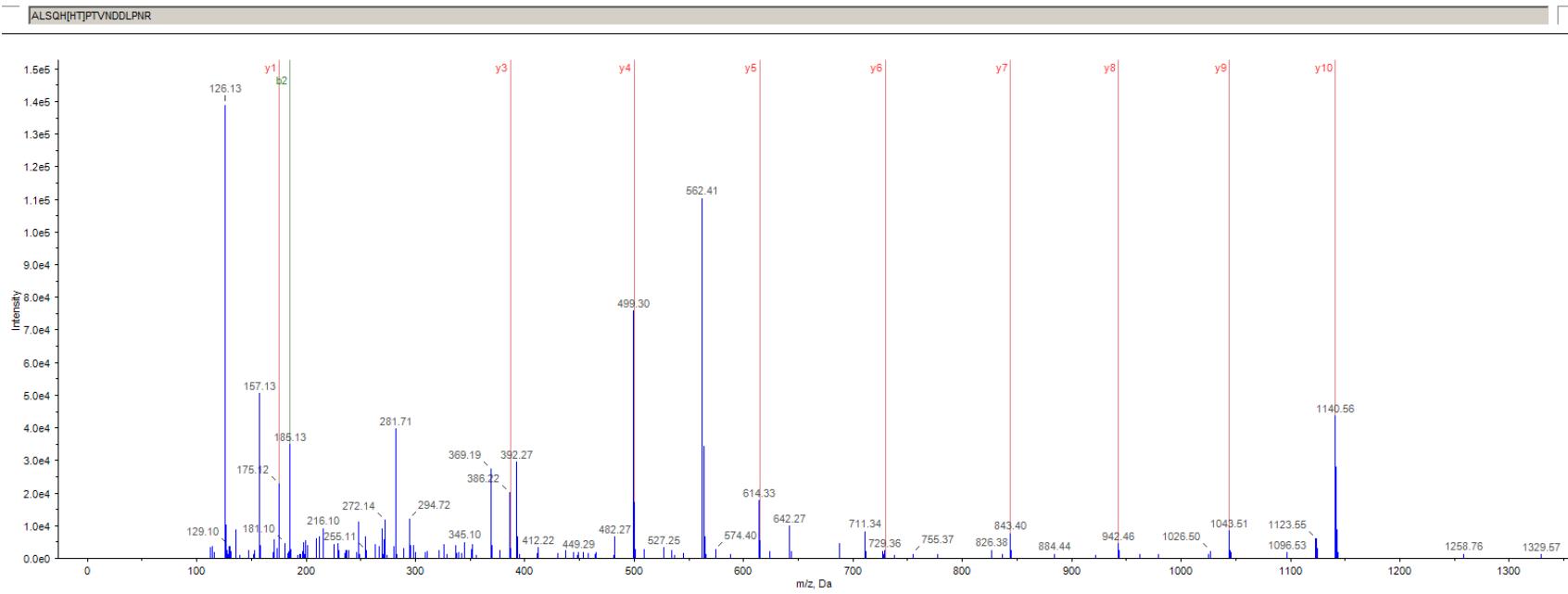
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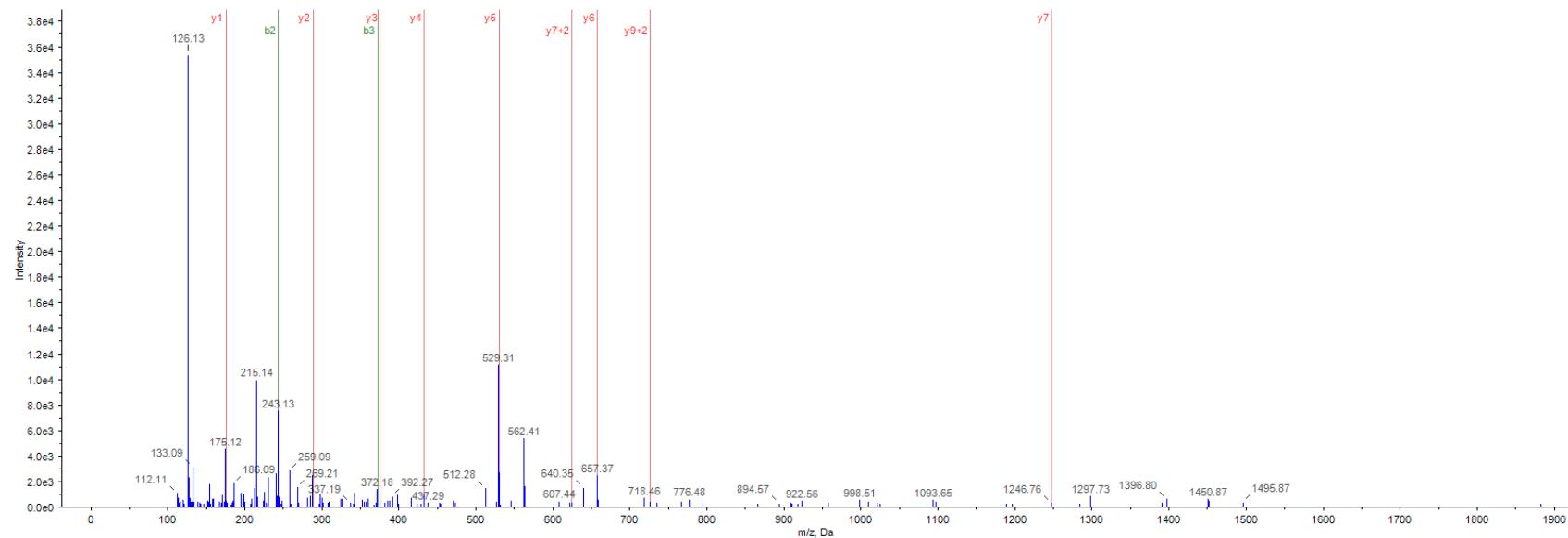
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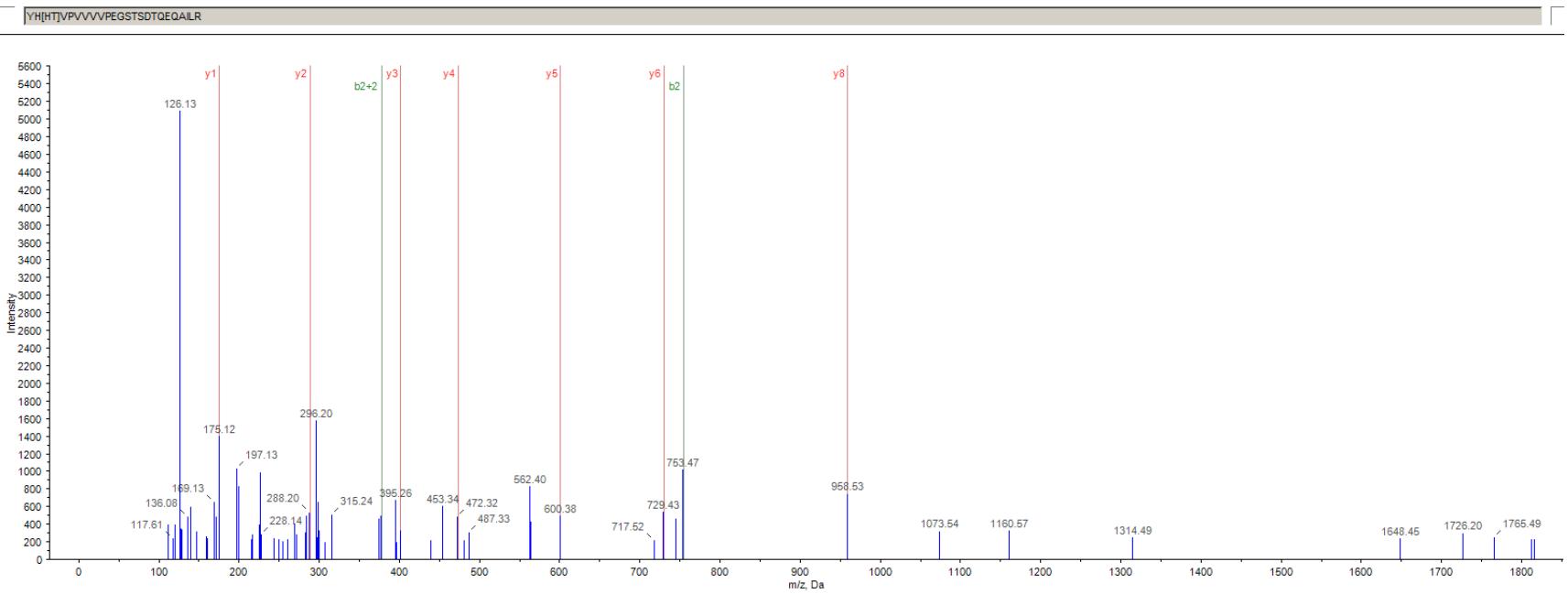




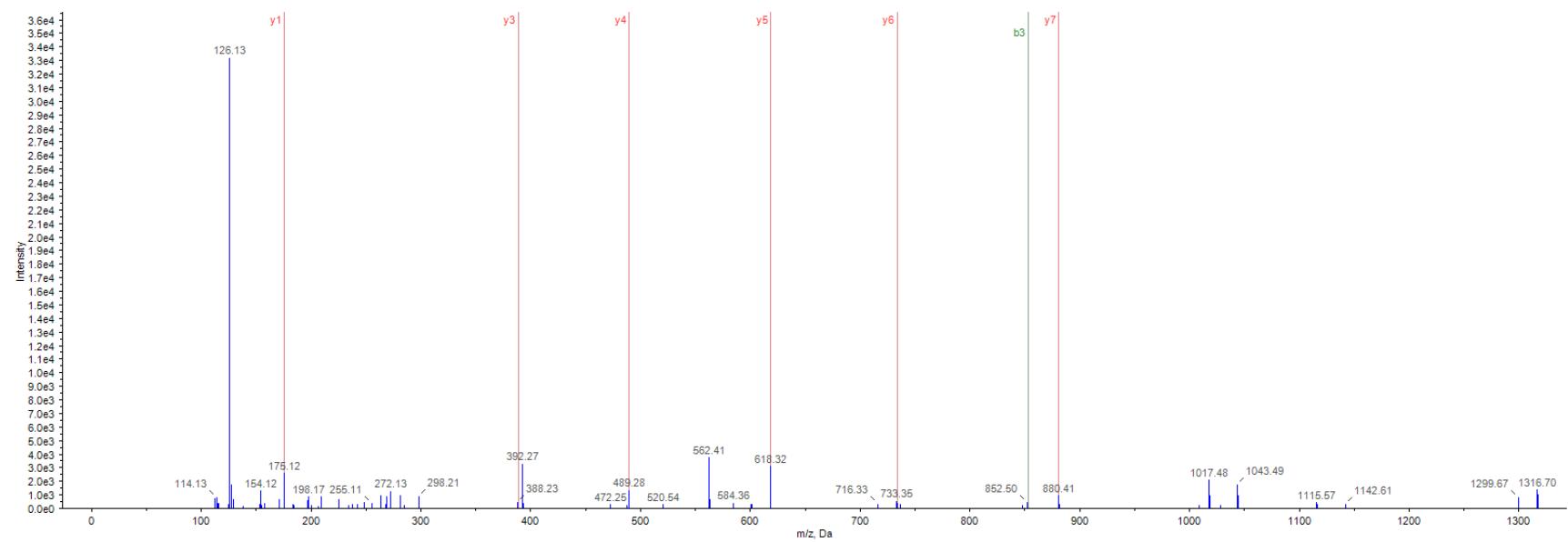


[LEEEETGQVVVGFH[HT]QPGSIR

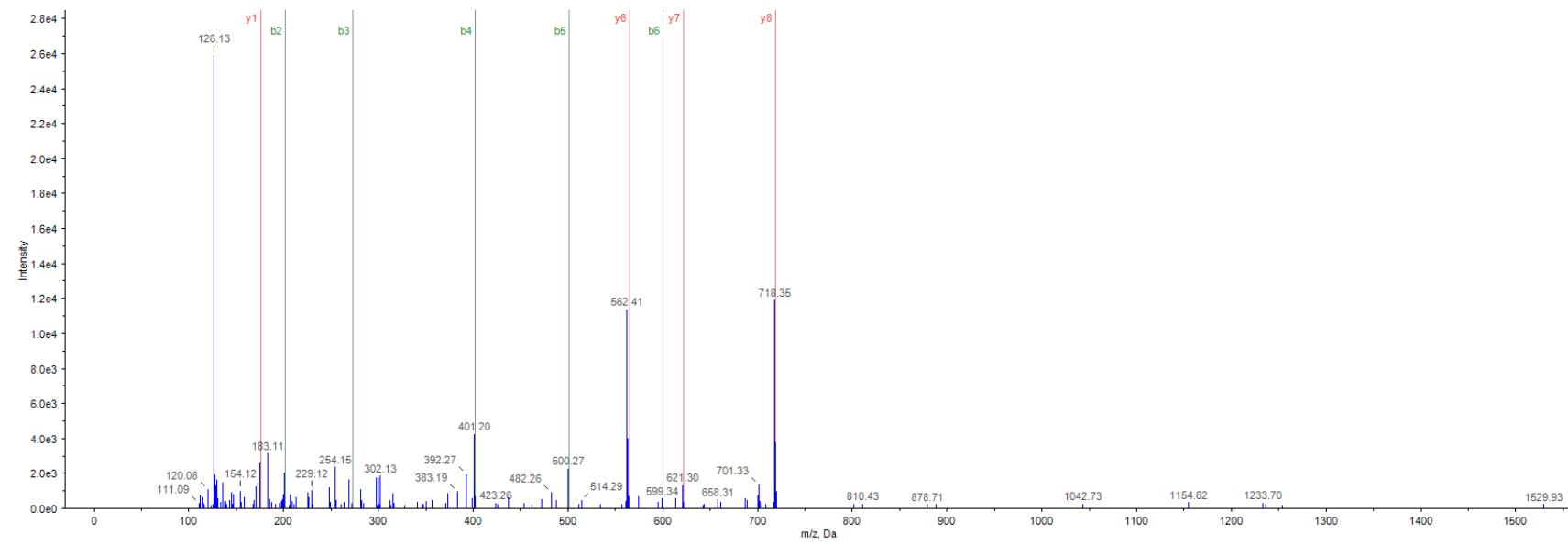




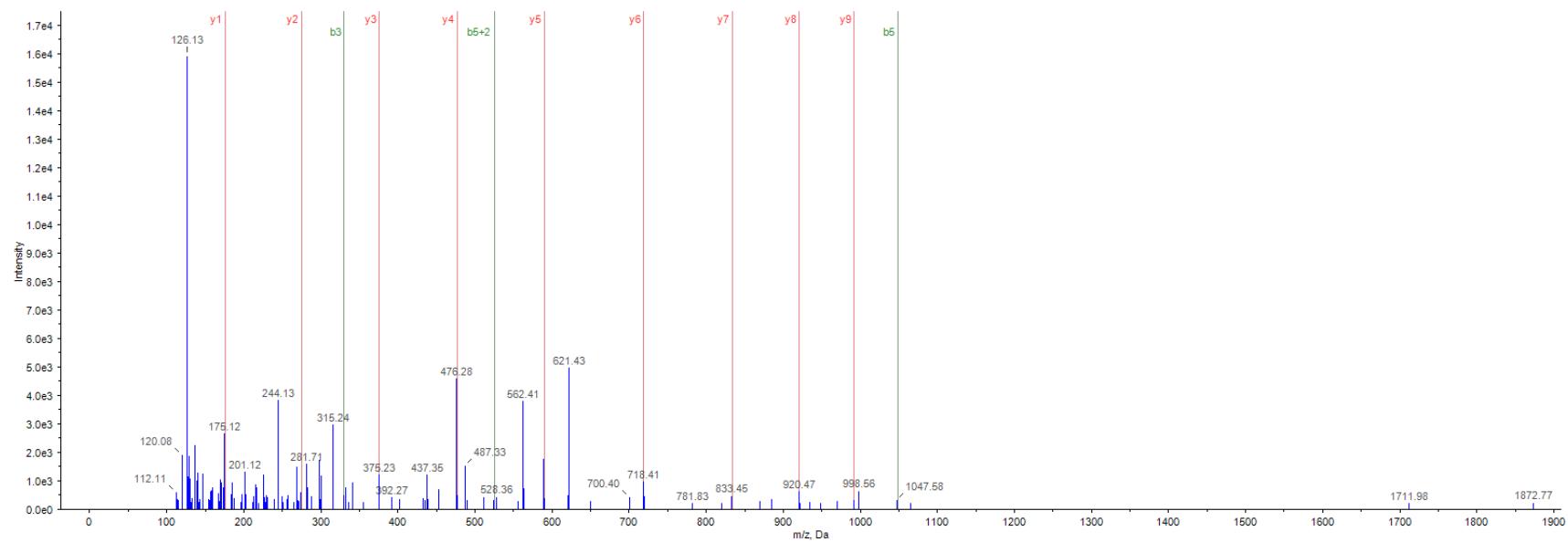
H[HT]FDETVNR



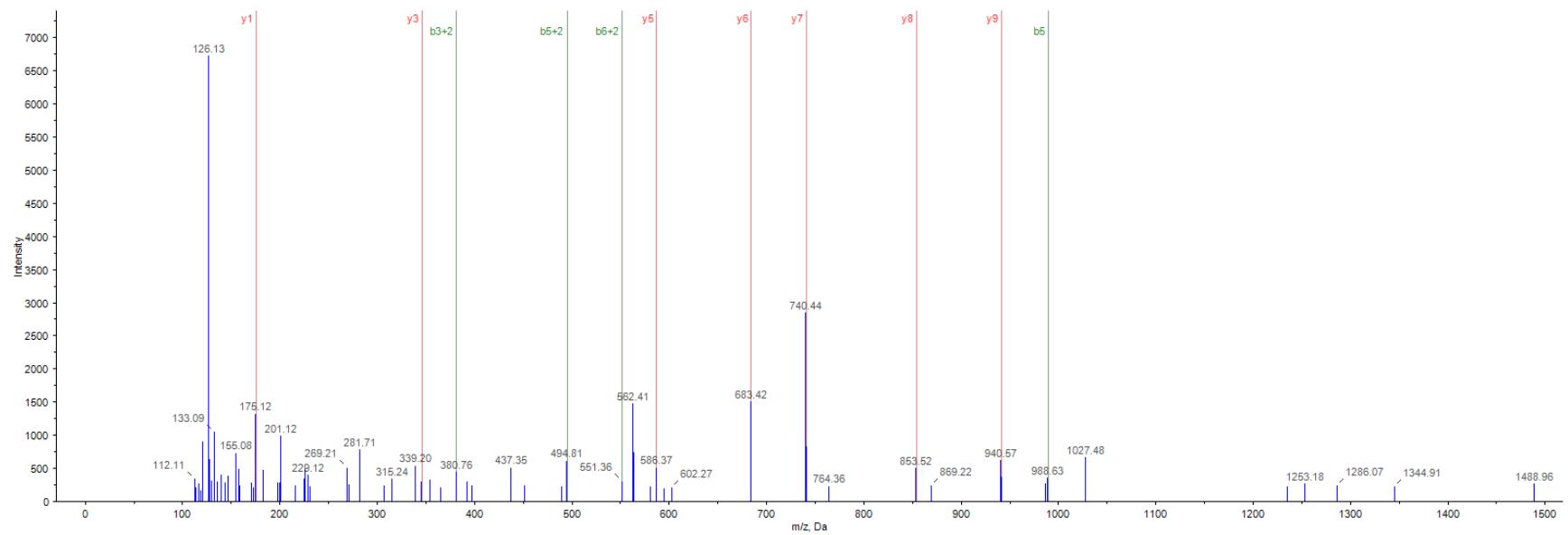
|VTAEVVVLVH[HT]PGGGSTSR|



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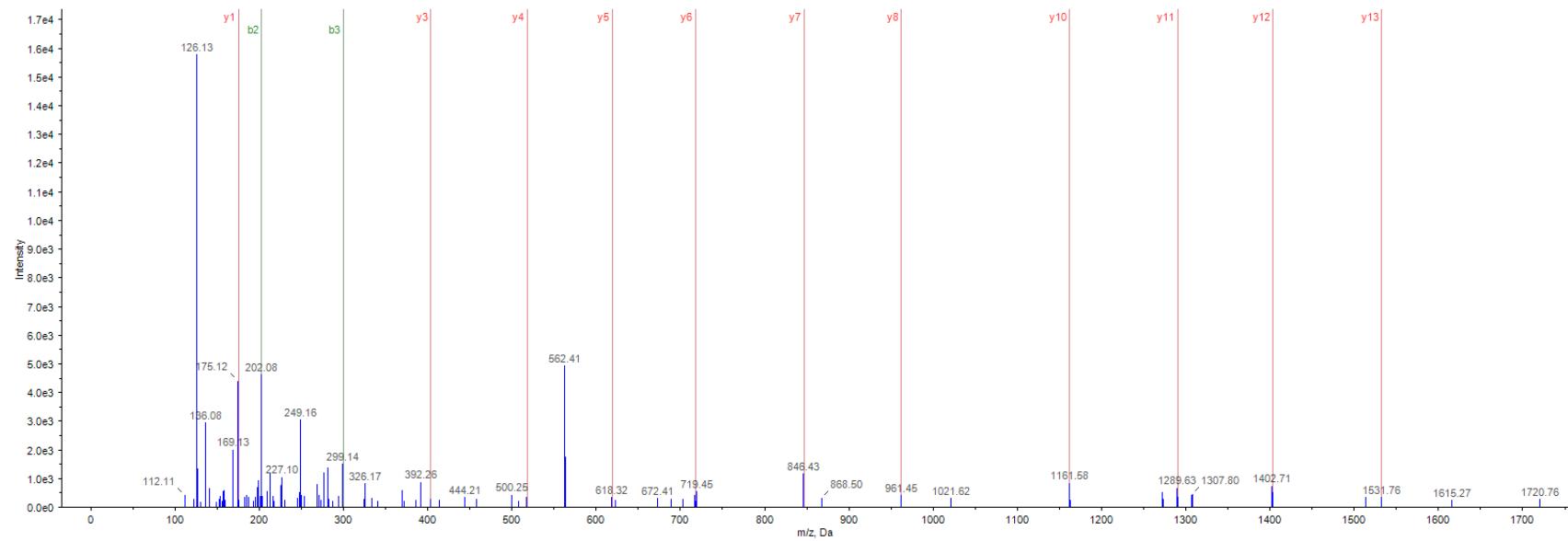


|G|H|H|T|V|E|P|G|A|Q|A|E|S|L|G|P|L|Q|V|R|

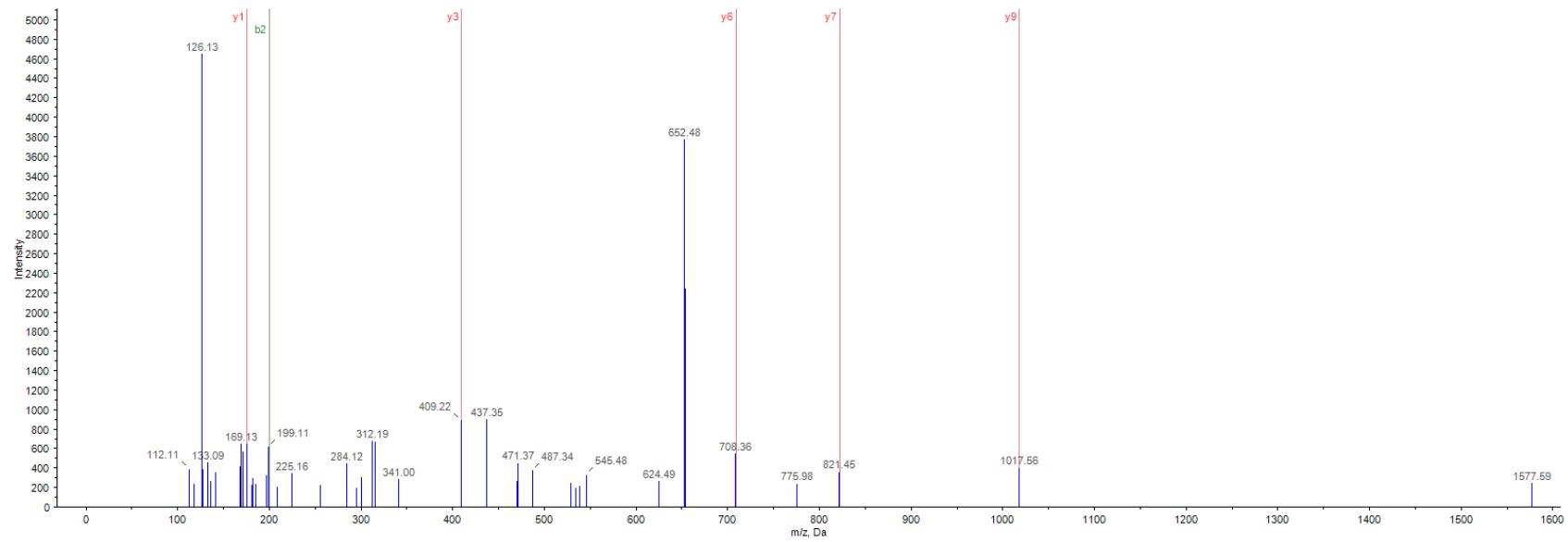


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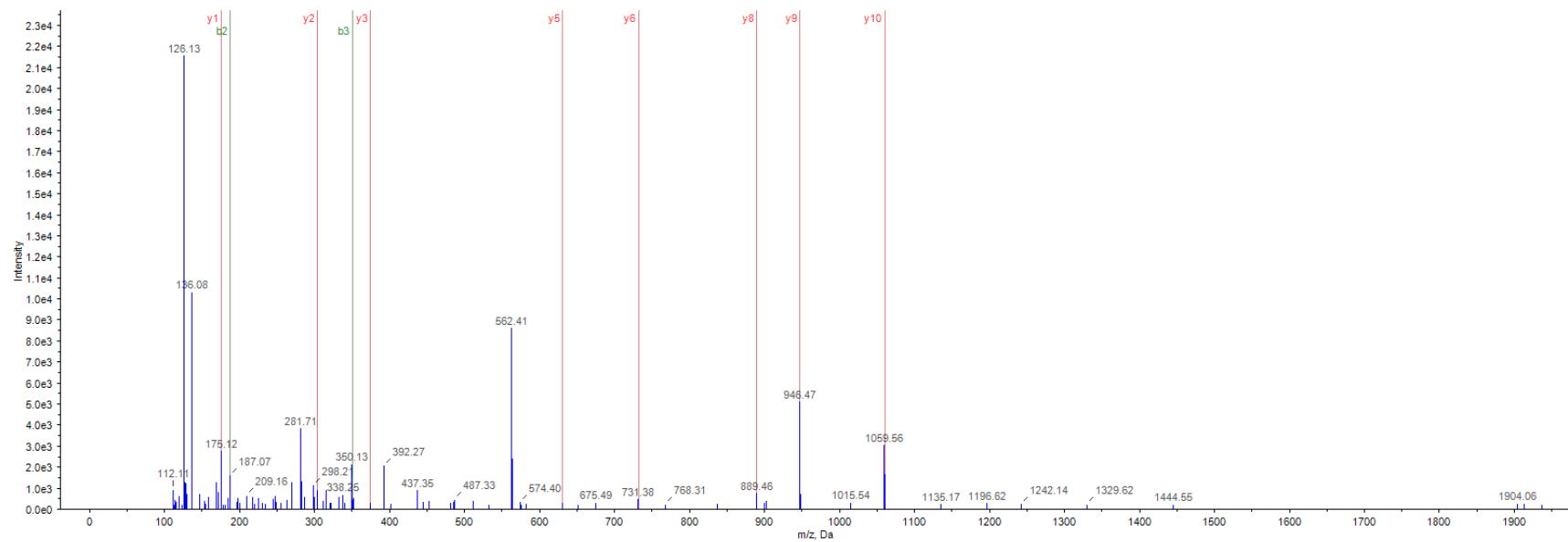


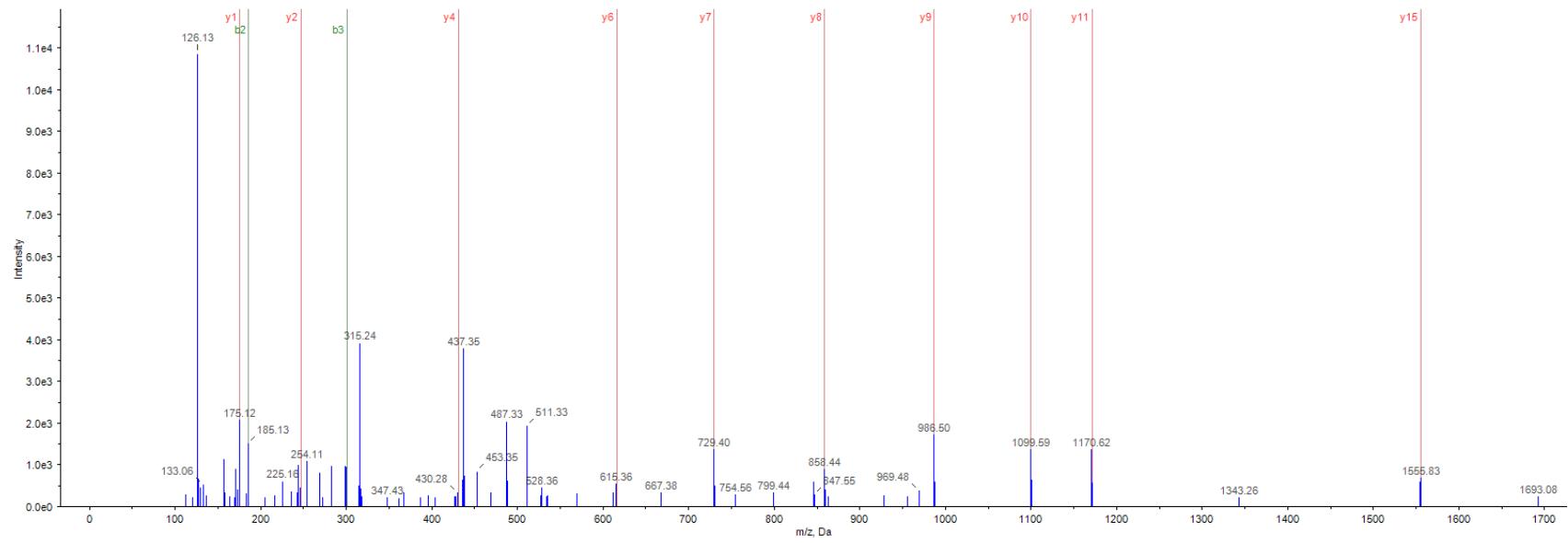
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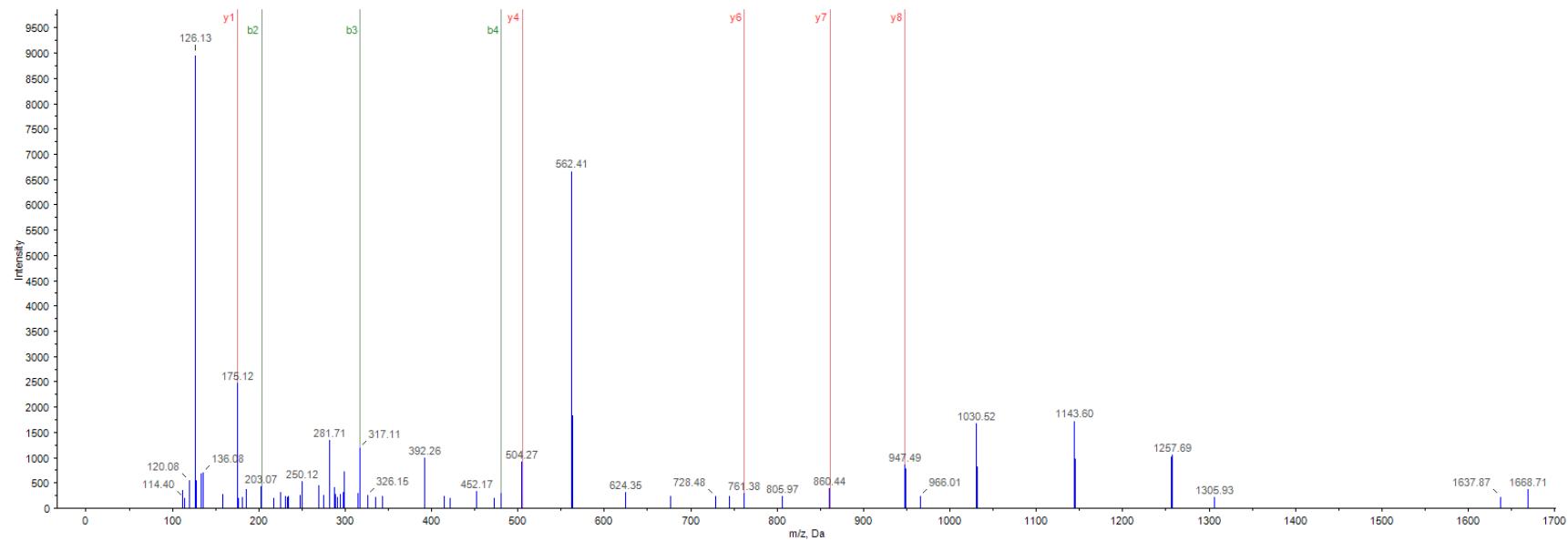


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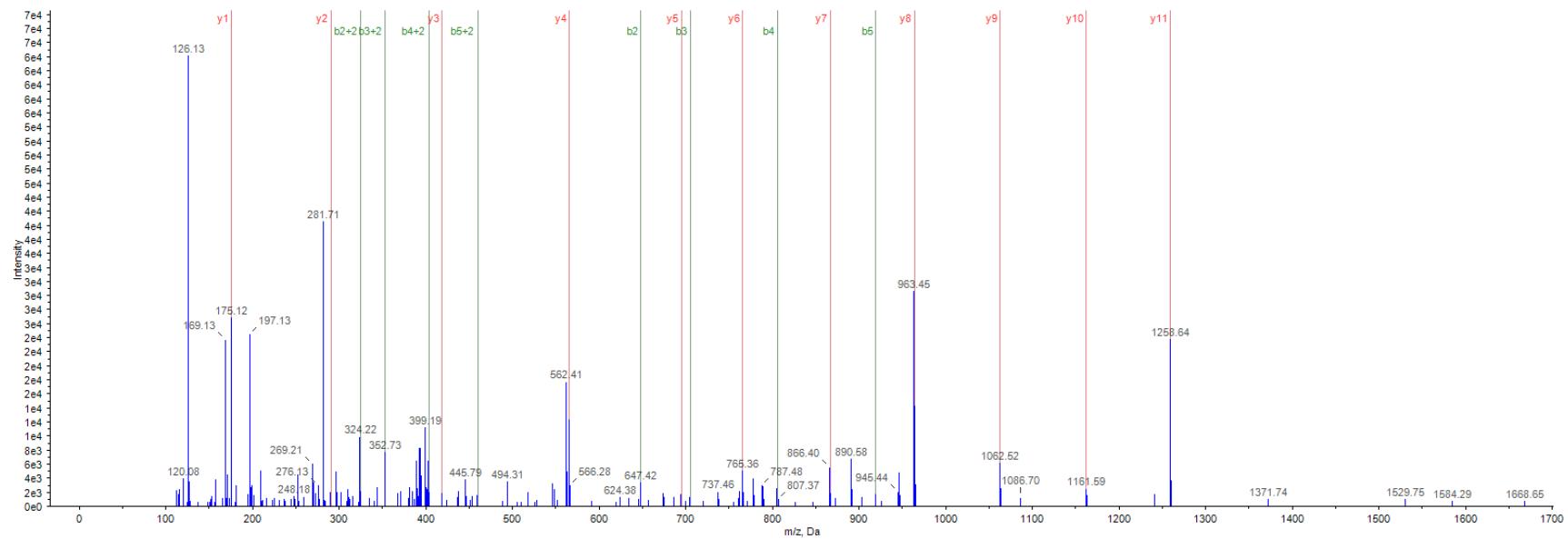
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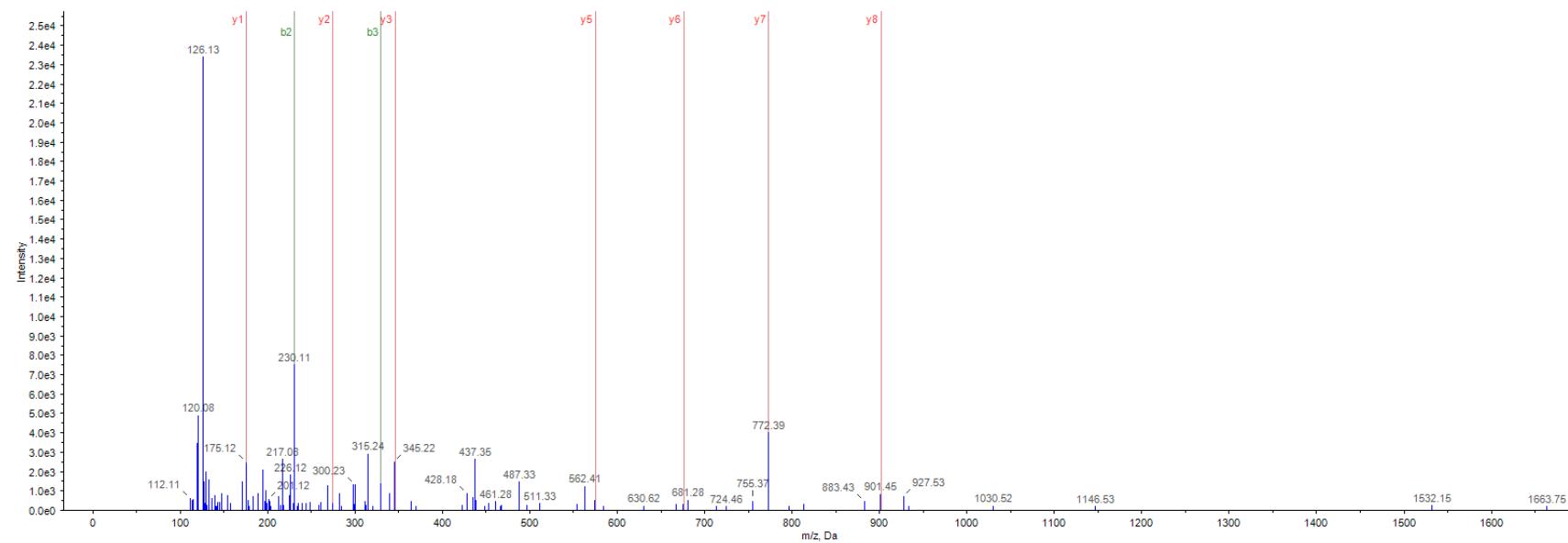




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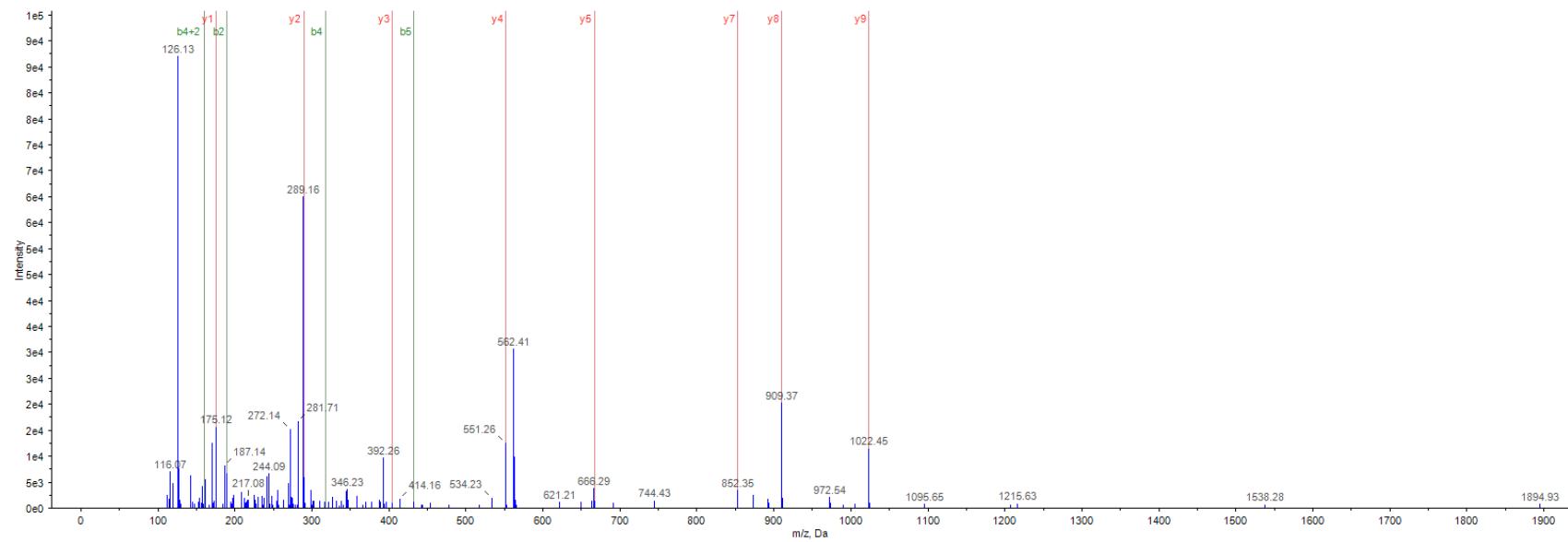


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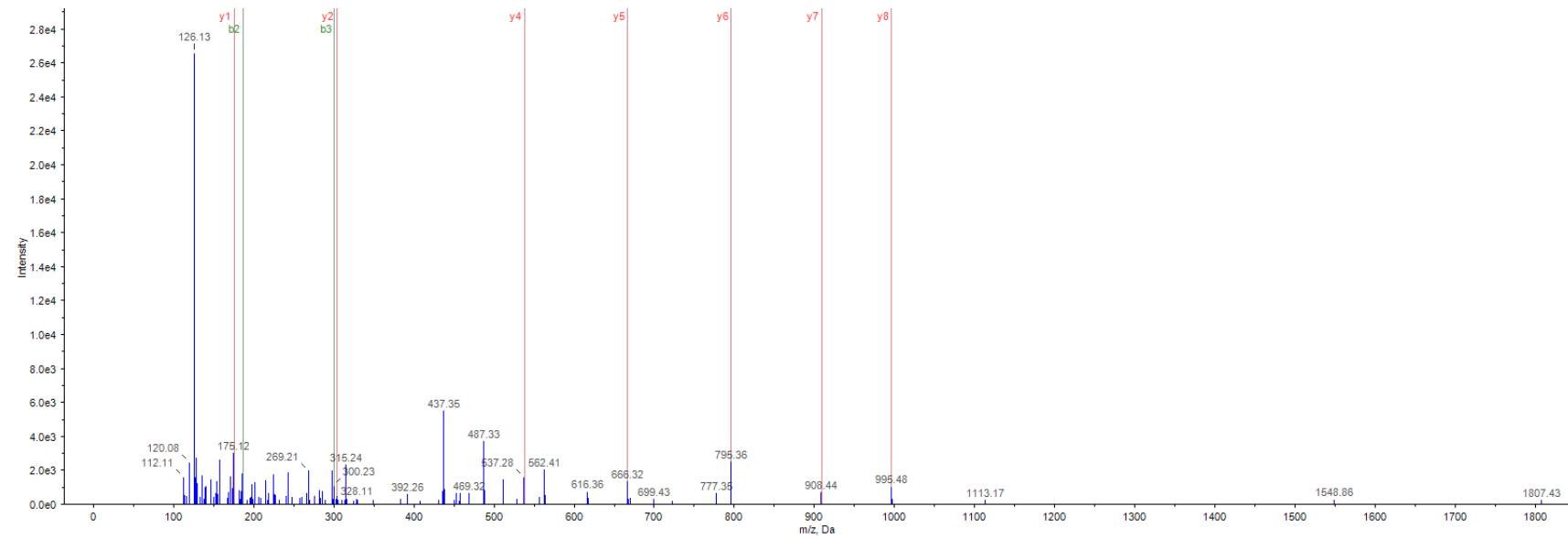
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STAGDTH[HT]LGGEDFDNR



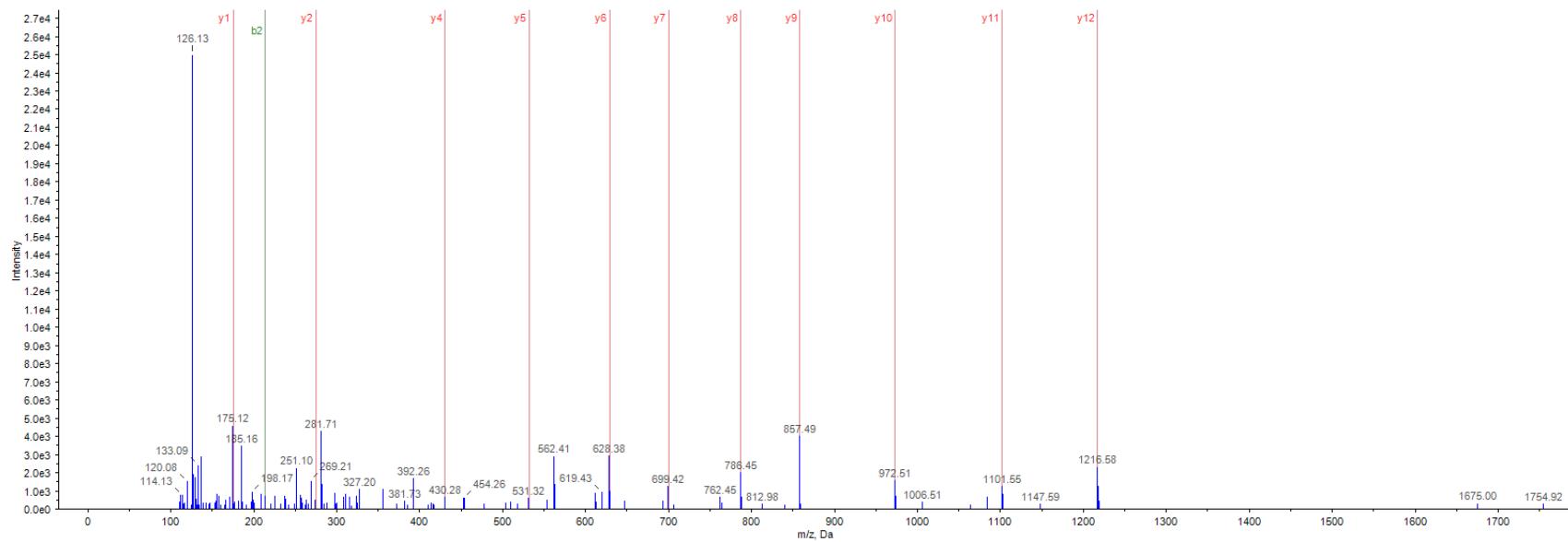
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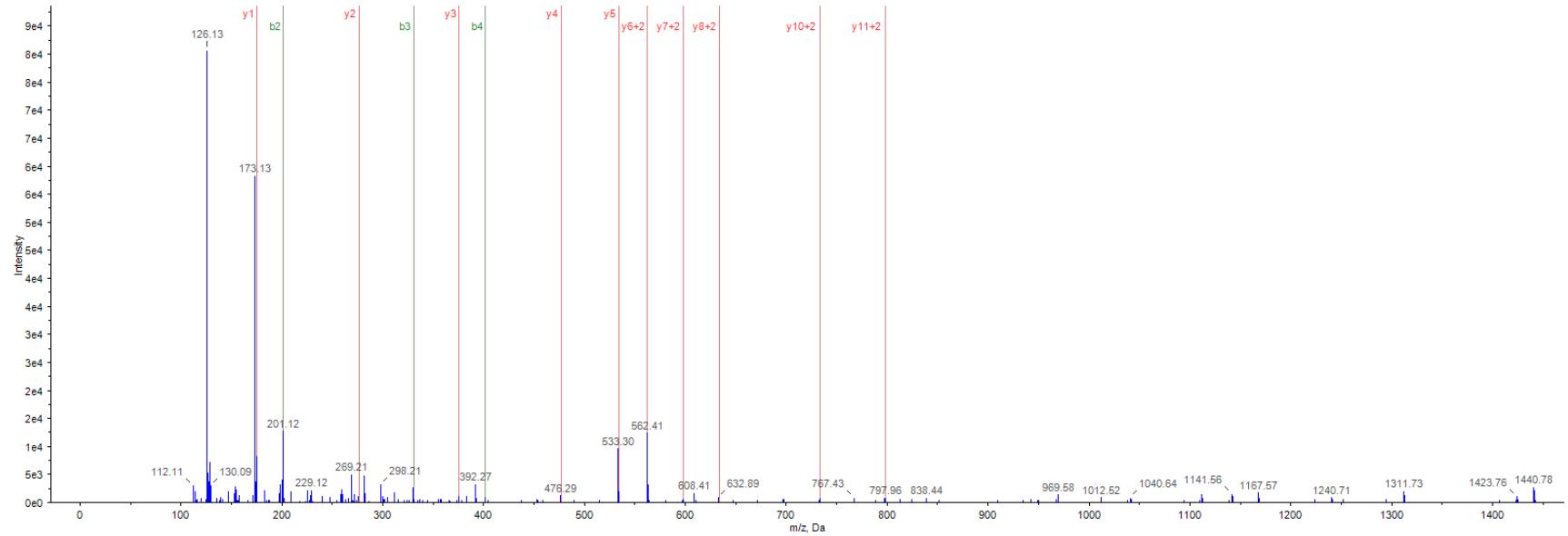


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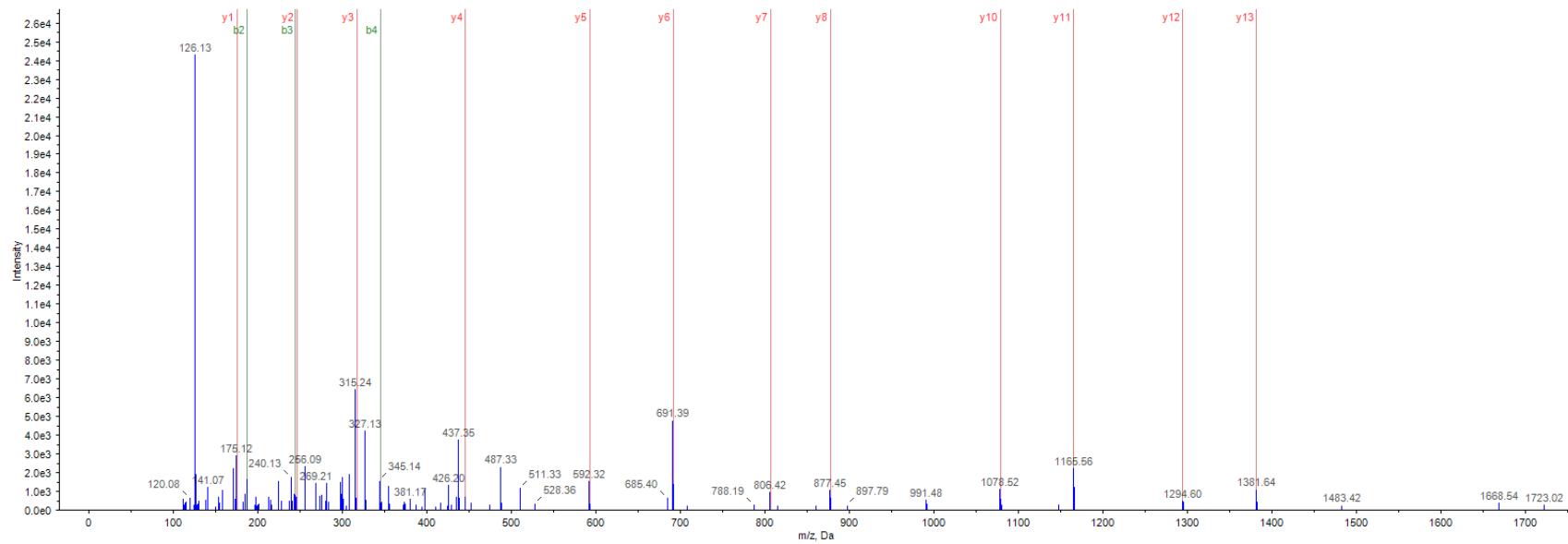
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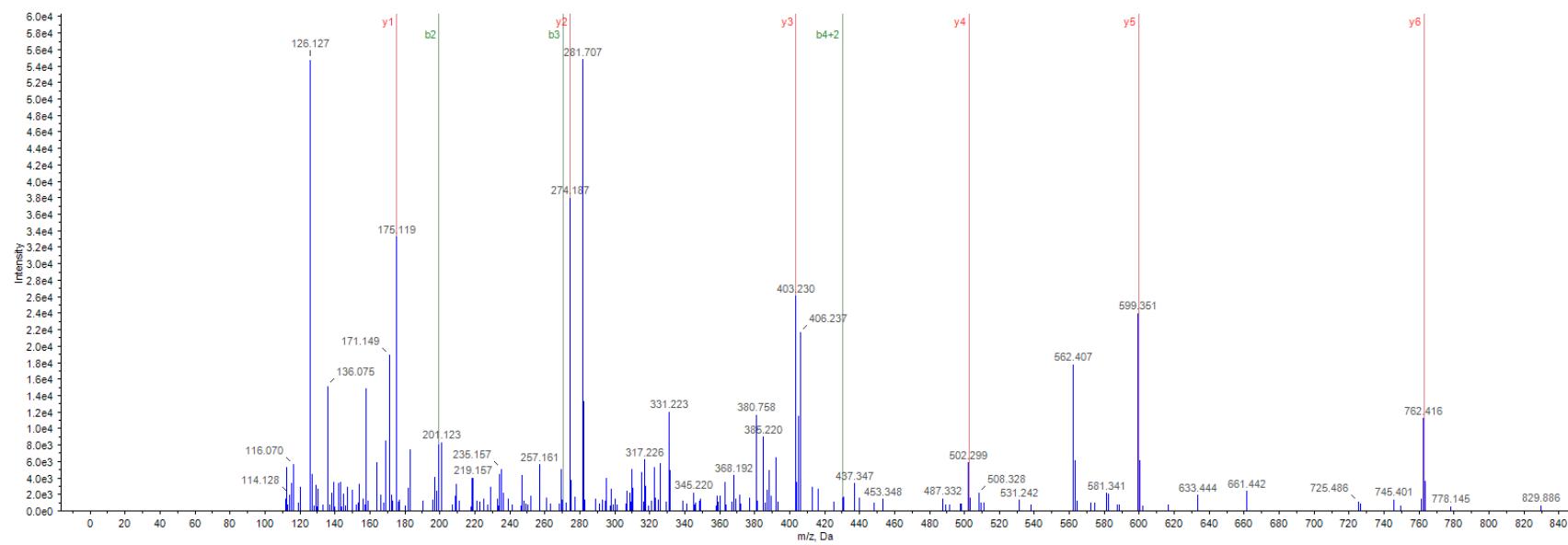


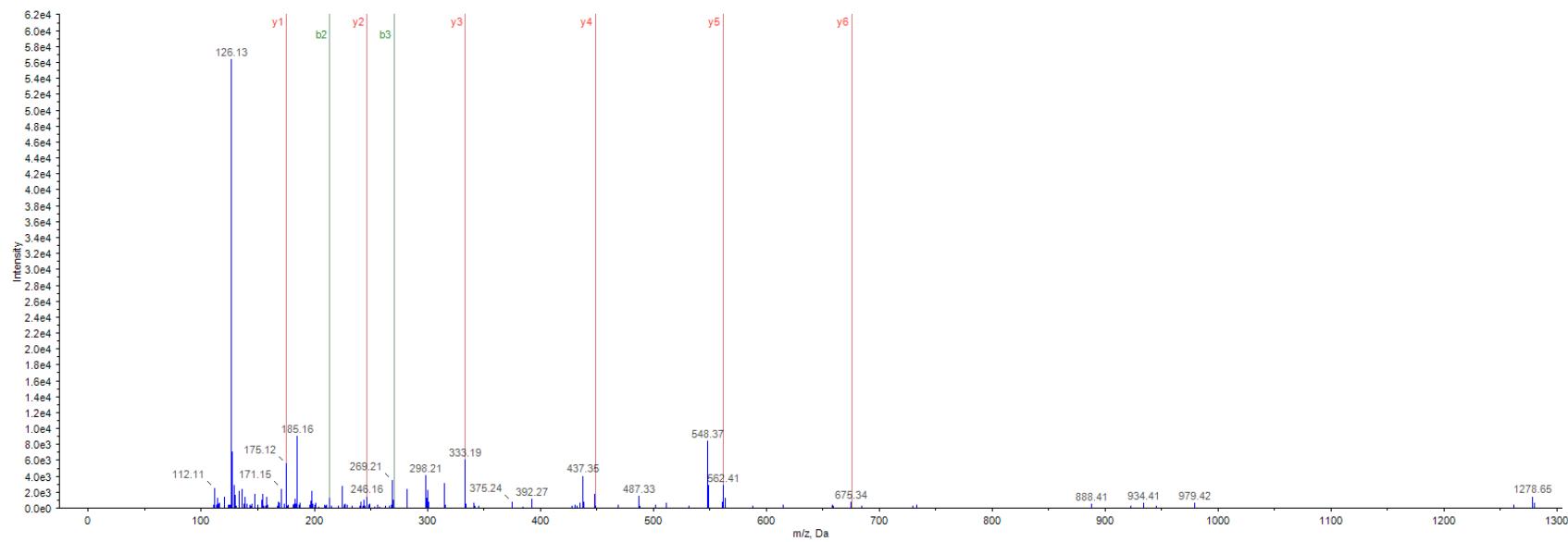
ADGTIVLEC[HT]SESSNADVFOAR



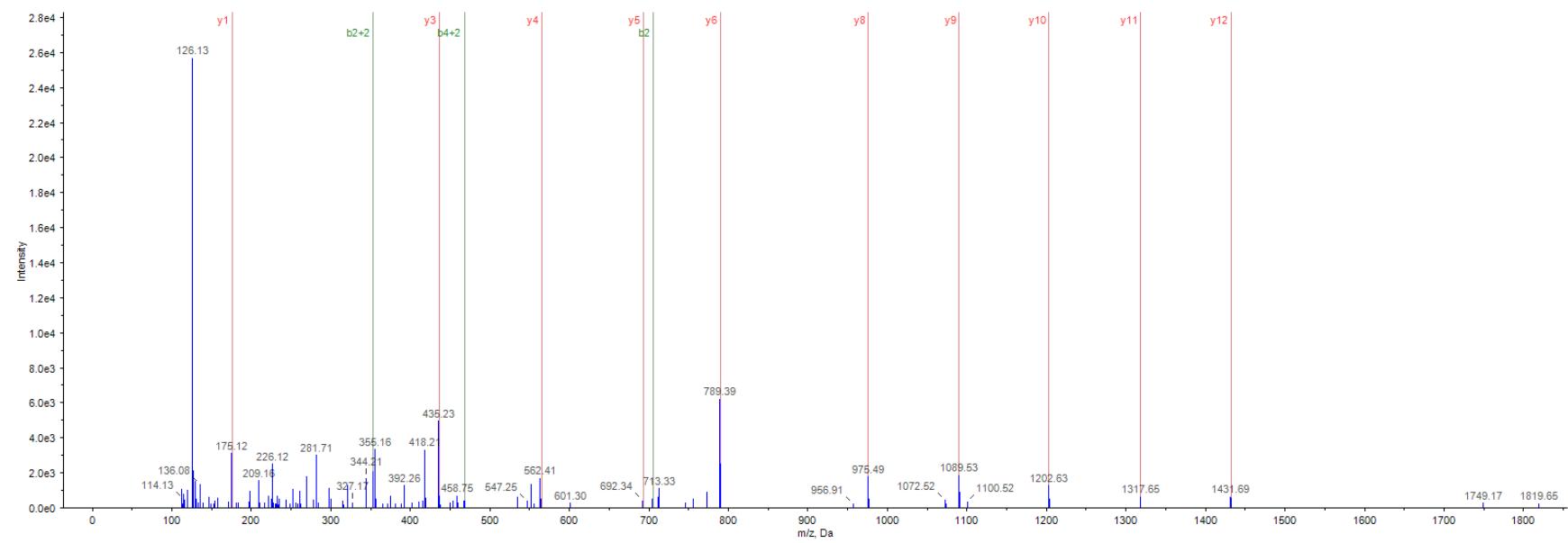
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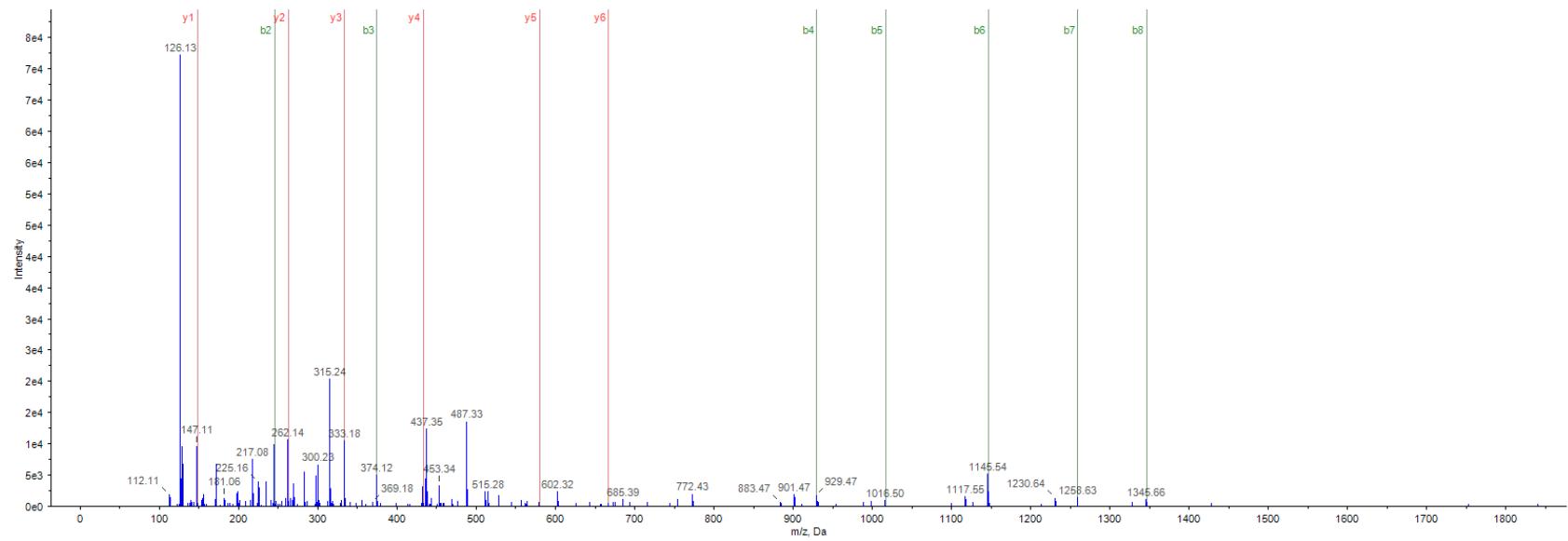




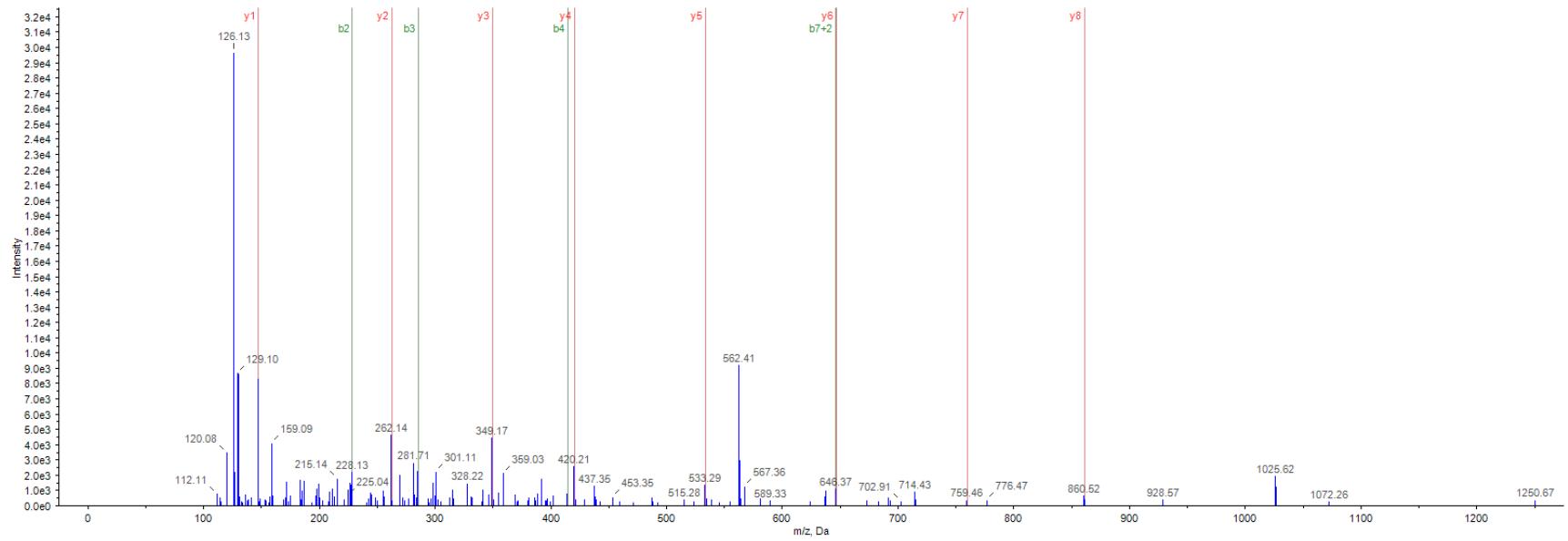
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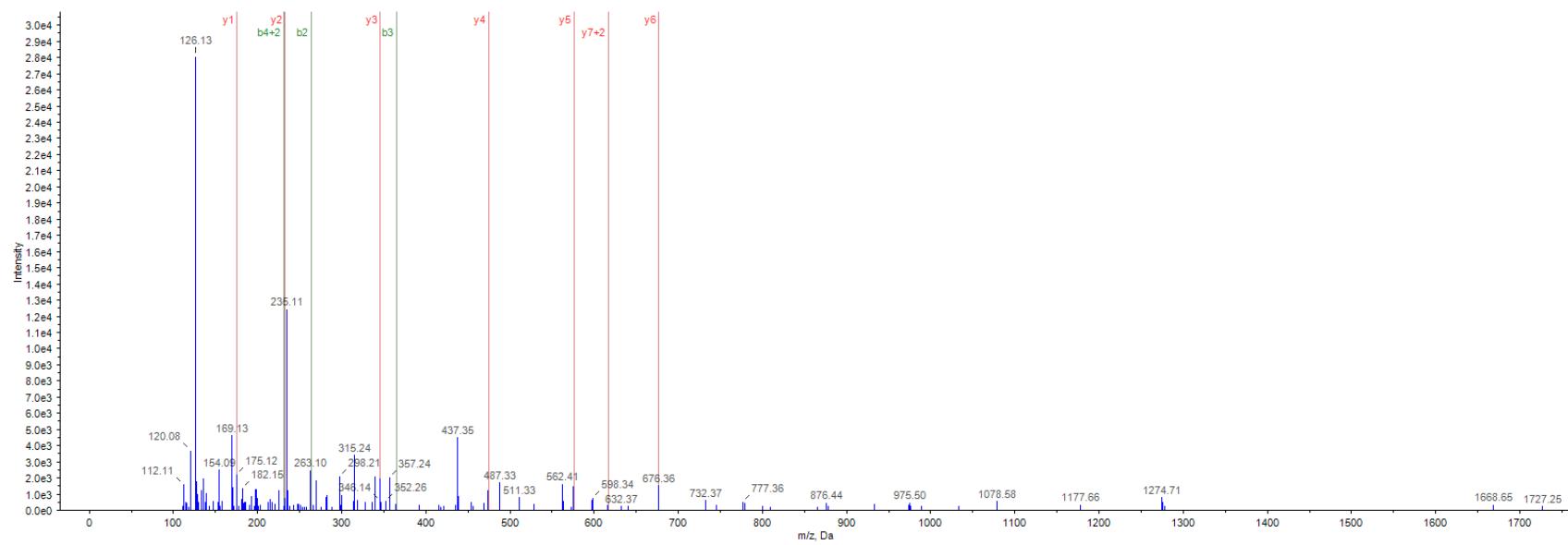


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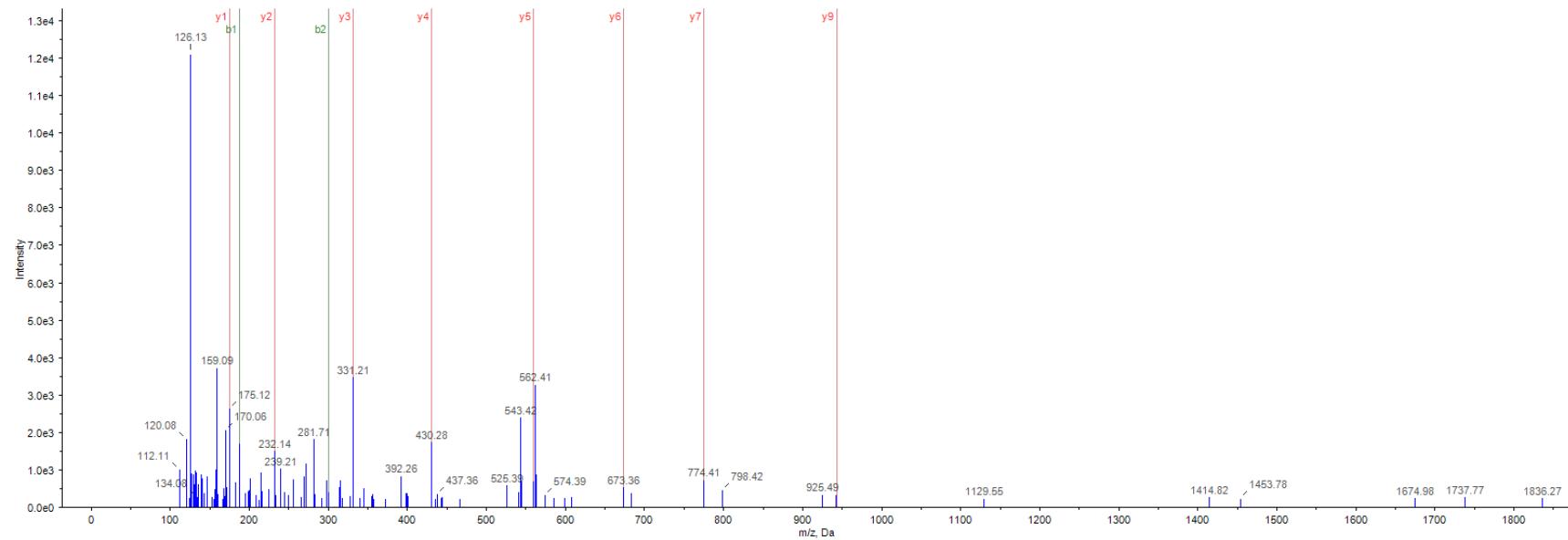


INGEWH[HT]TILASDK

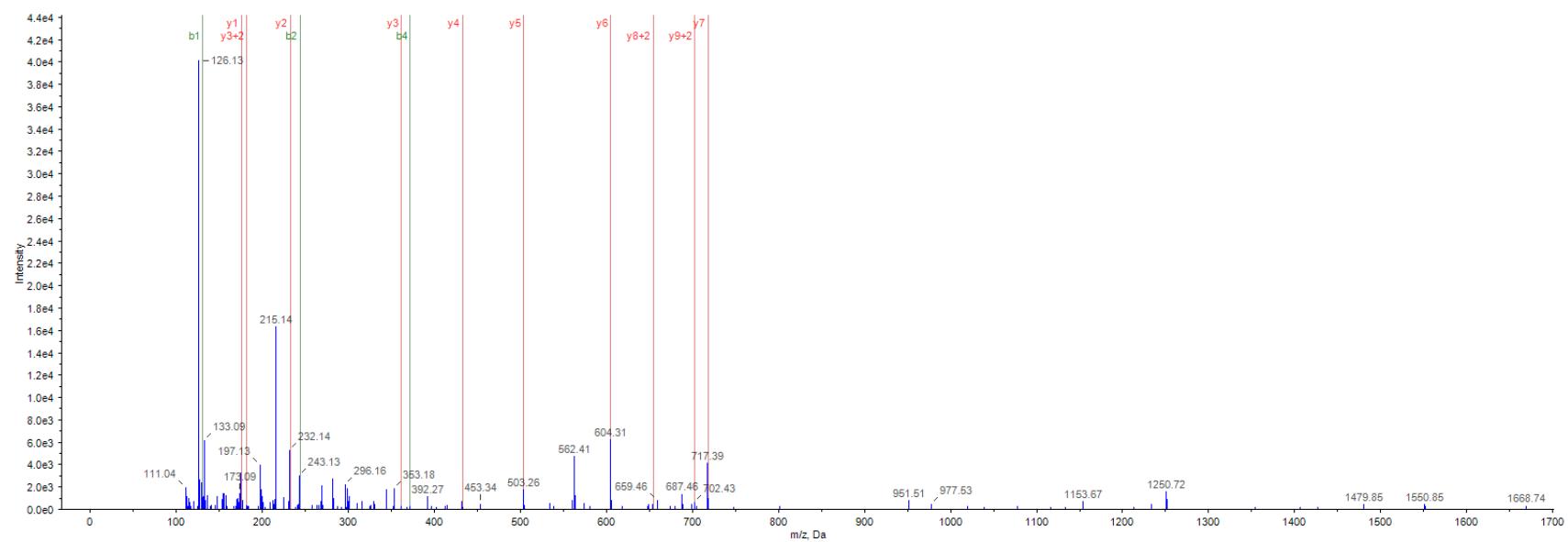




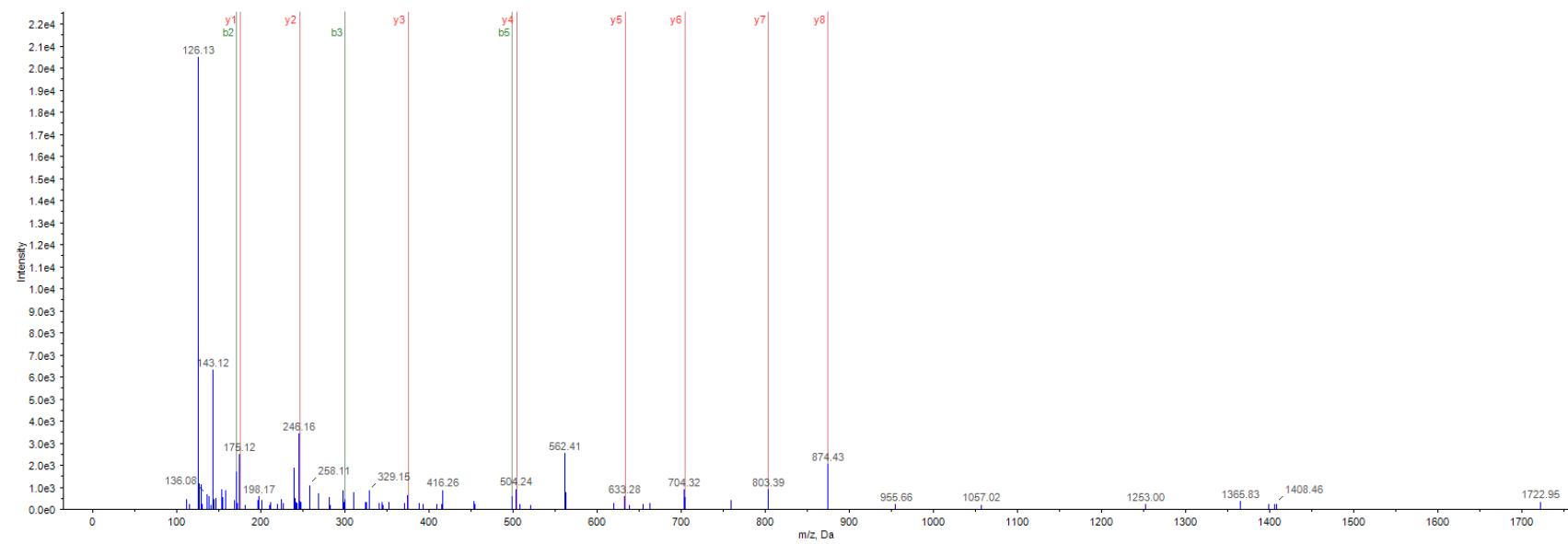
[WIDIH[HT]NPATNEVVGR]



EIAGATPH[HT]TAAEGR

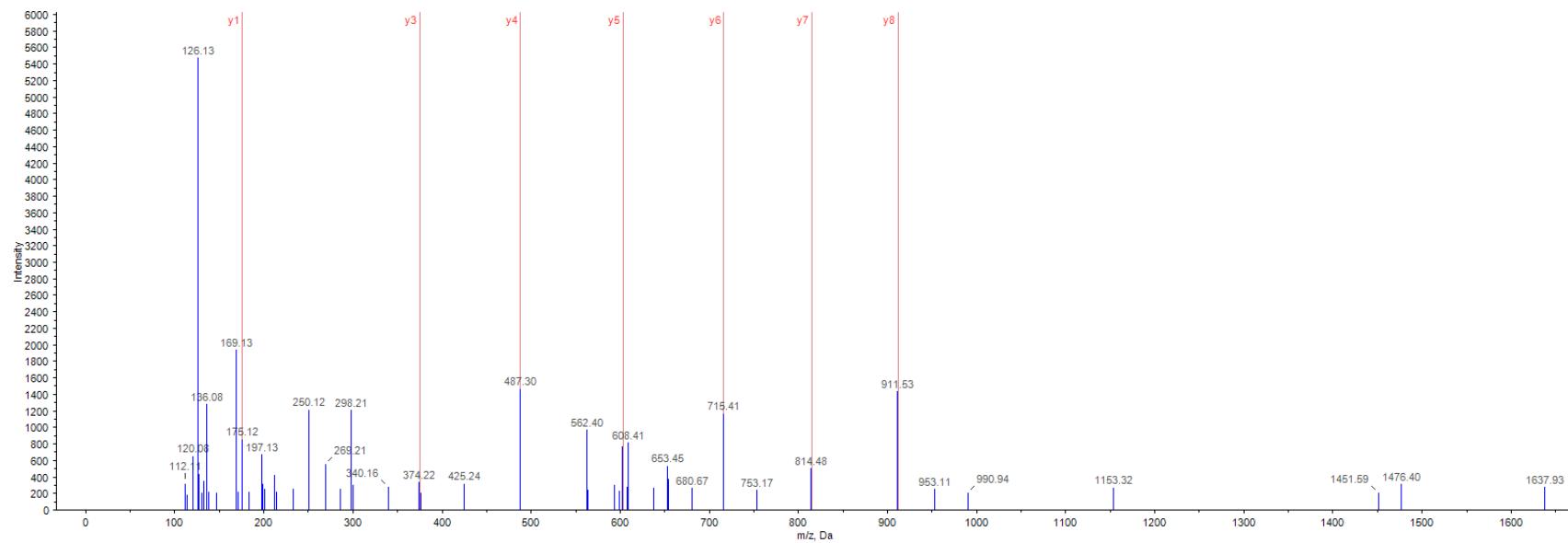


GIEQAVQSH[HT]AVAEEAR



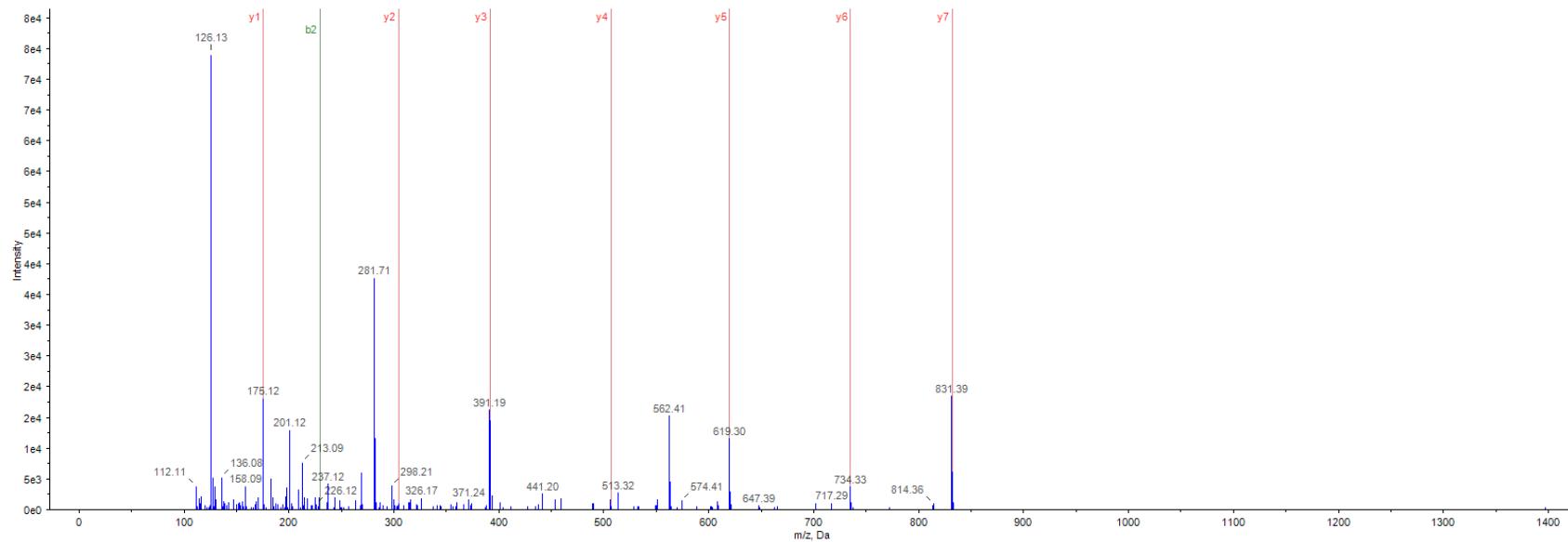
S-1 68

[NYPFGH[HT]QAAAVLPVLDAQR



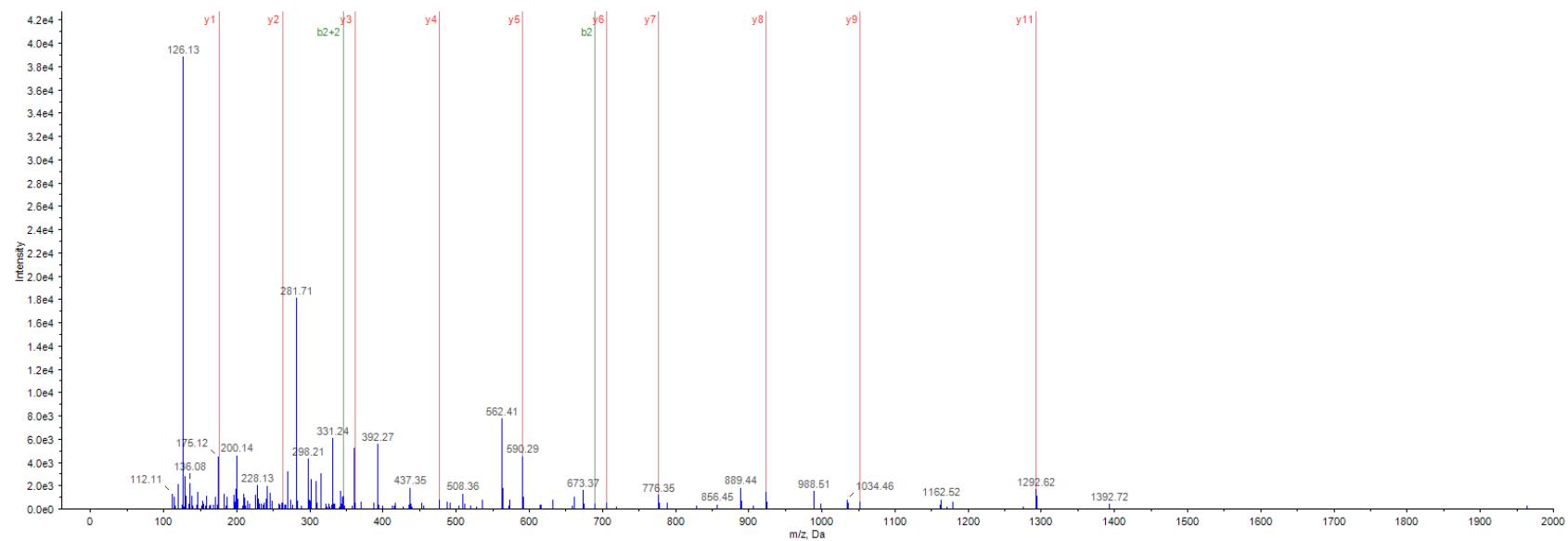
S-1 69

[EVH[HT]PODSER

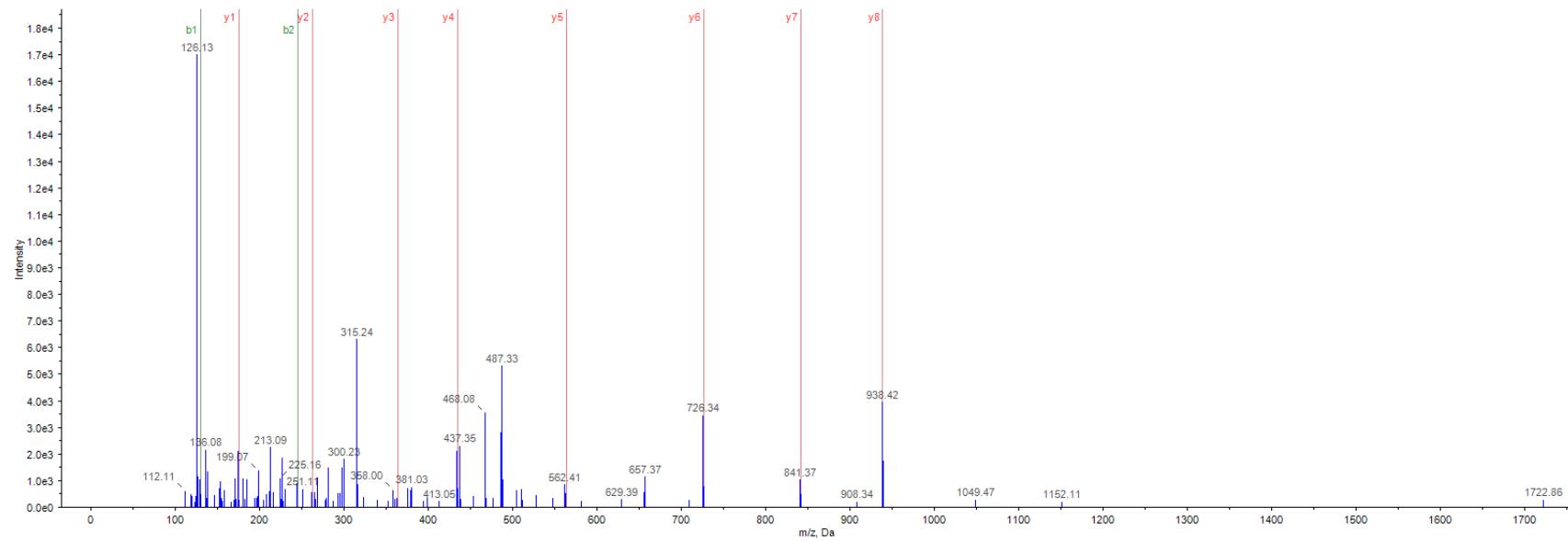


S-1 70

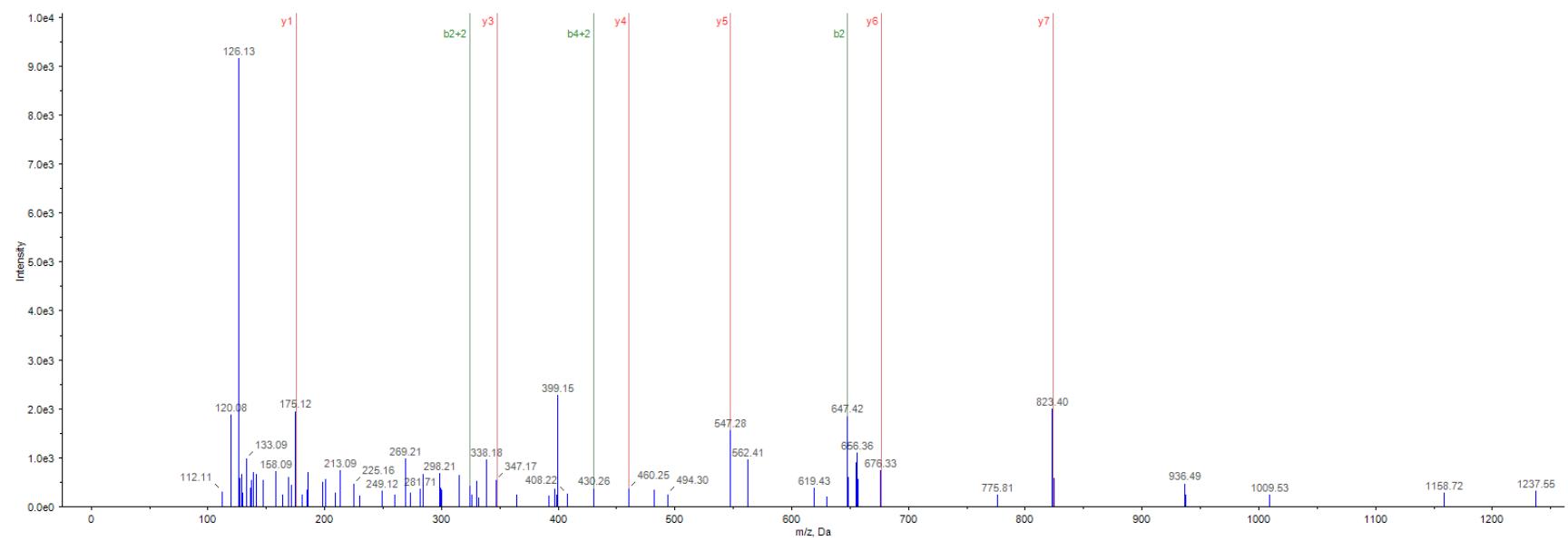
H[HT]VLQQFADNDVSR



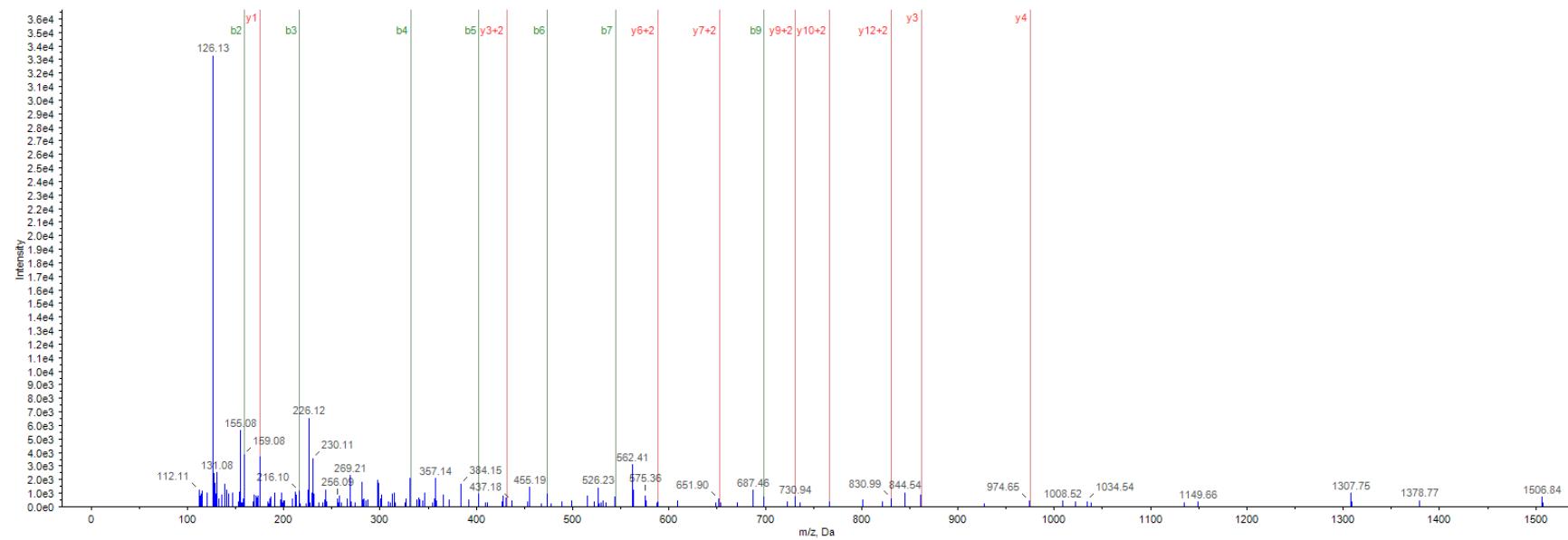
[EDC[HT]TQPDYEATSR]



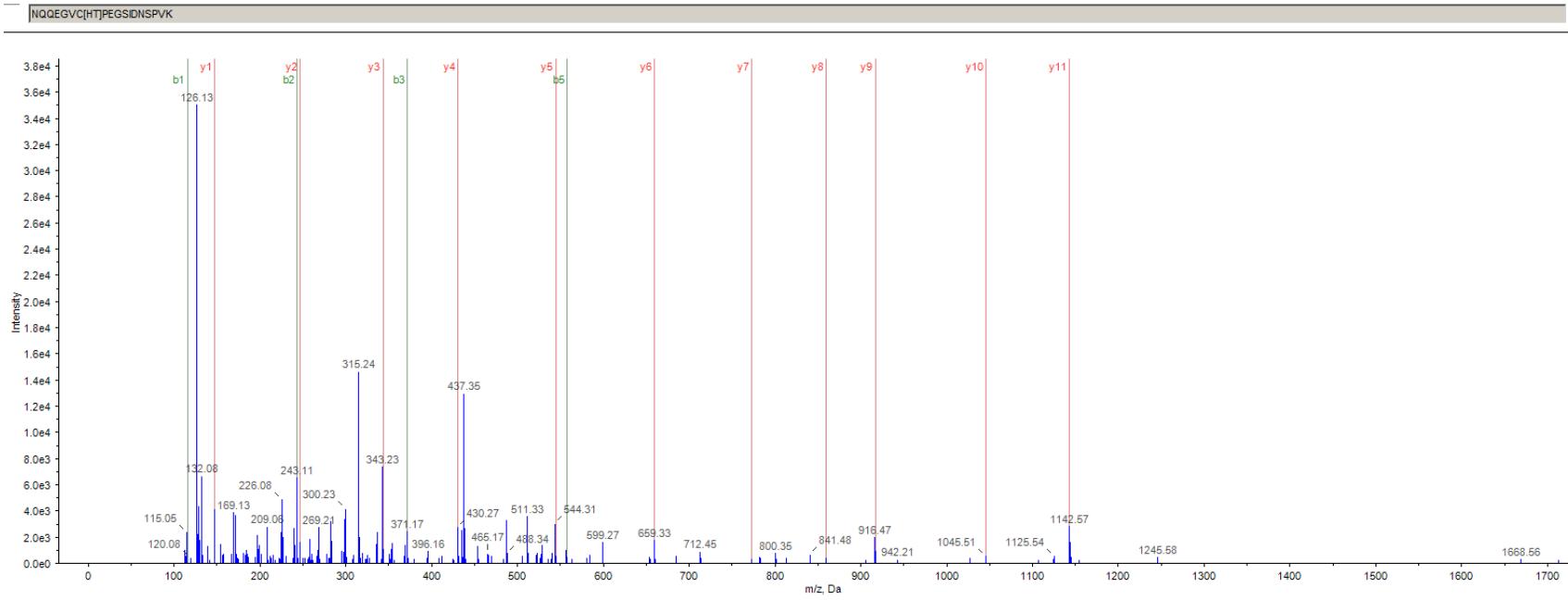
GH[HT]PDLQQQ[Dea]PADDIFESIGDR



TGGDAAAPGAAGTQAEELPH[HT]R

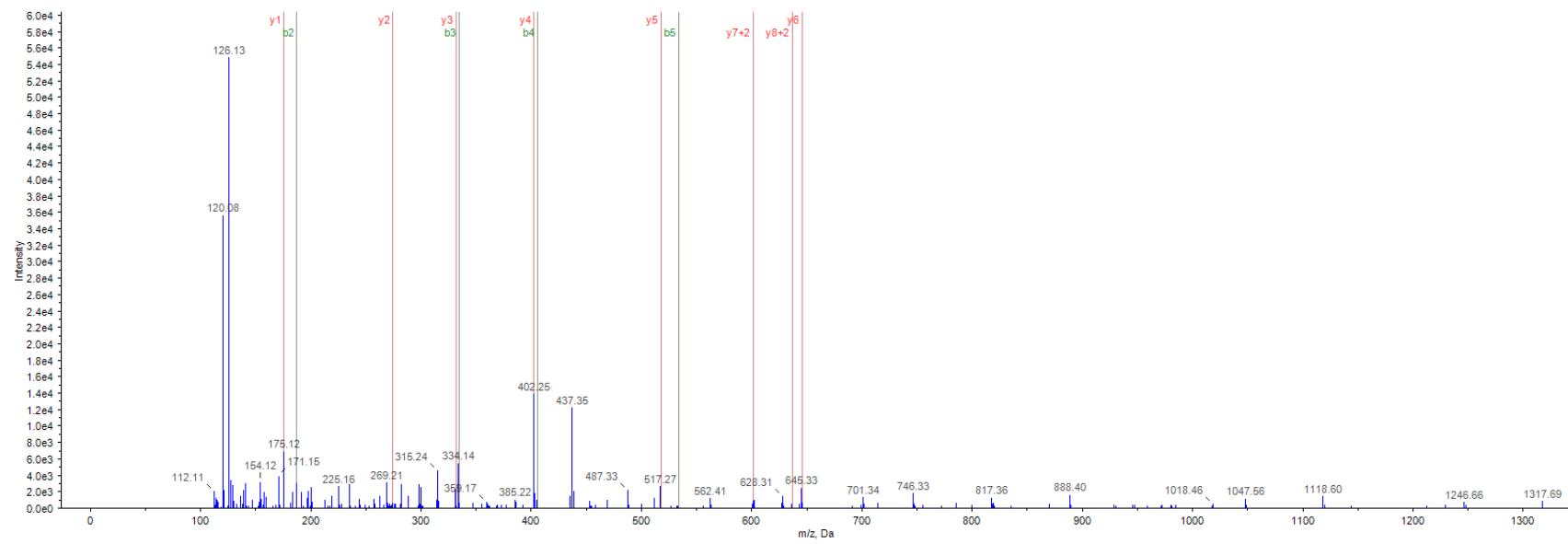


S-1 74

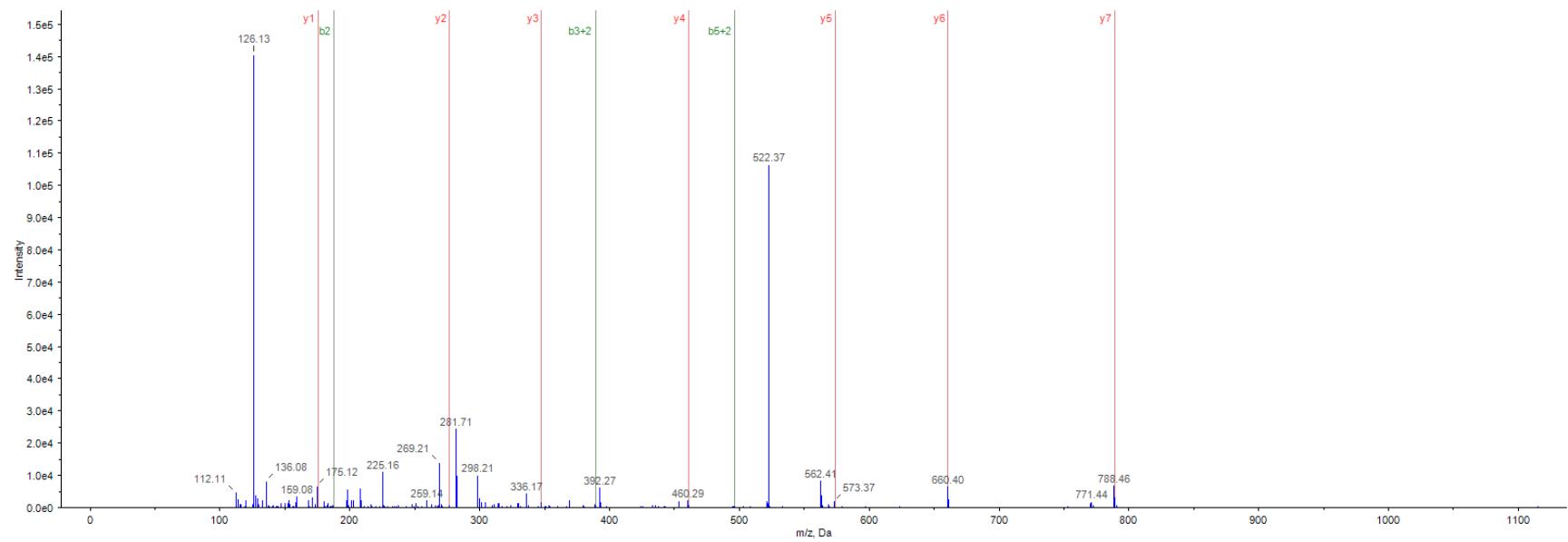


S-1 75

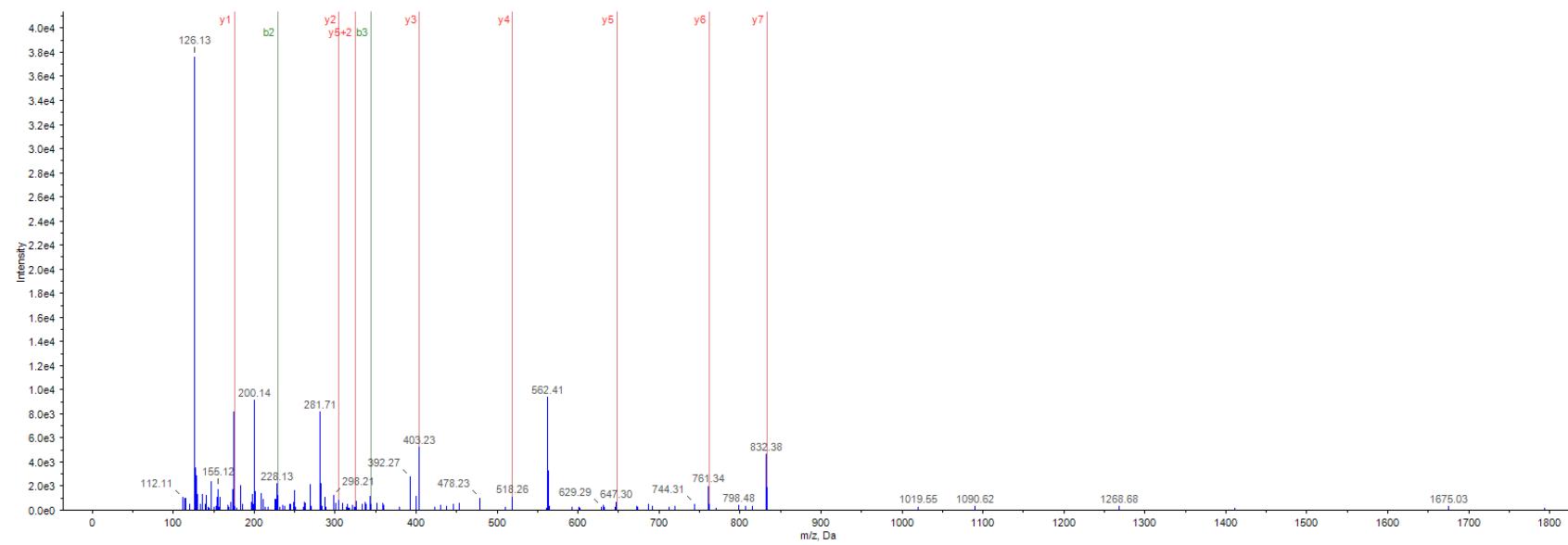
[ADFAQAC[HT]QDAGVR



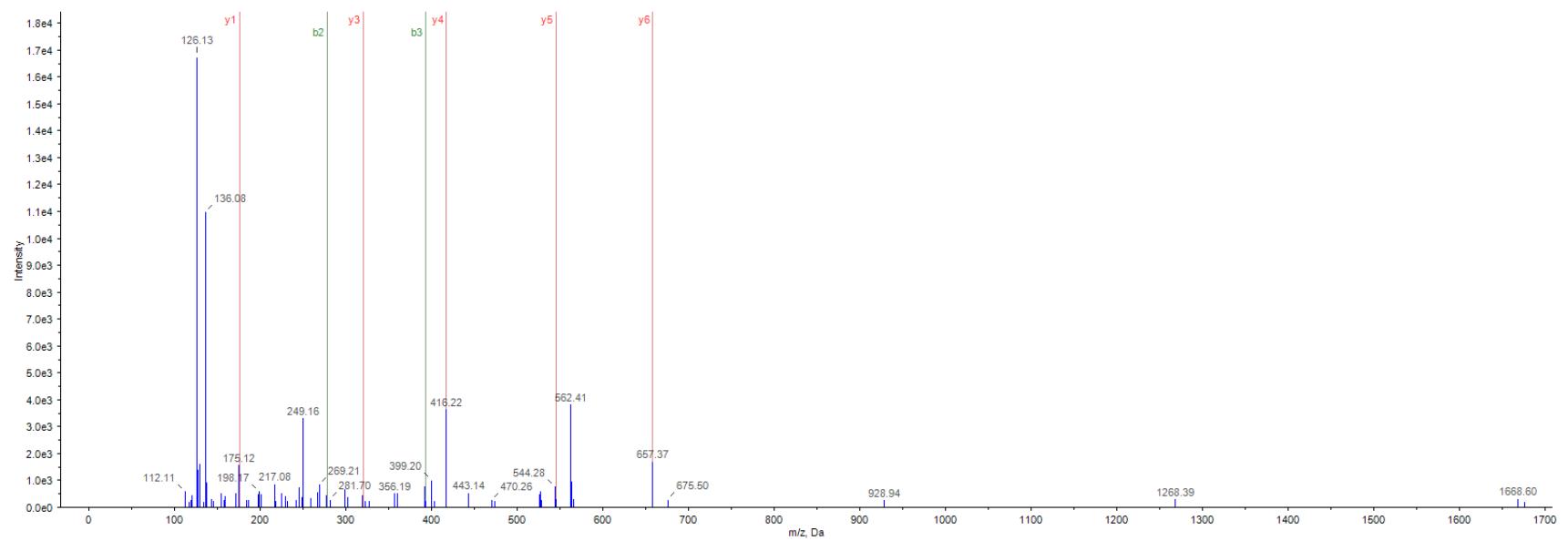
[DAH[HT]QSLLATR]



S-1 77

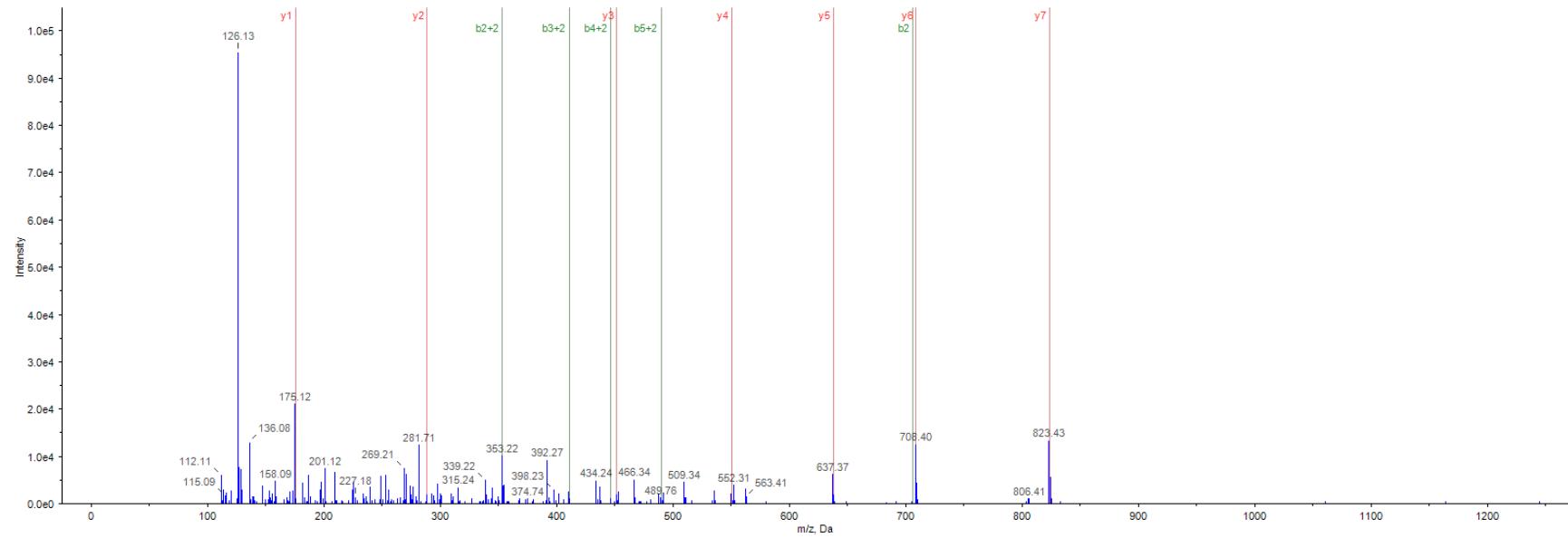


[YLDDEDTVYH[HT]LQPSGR]

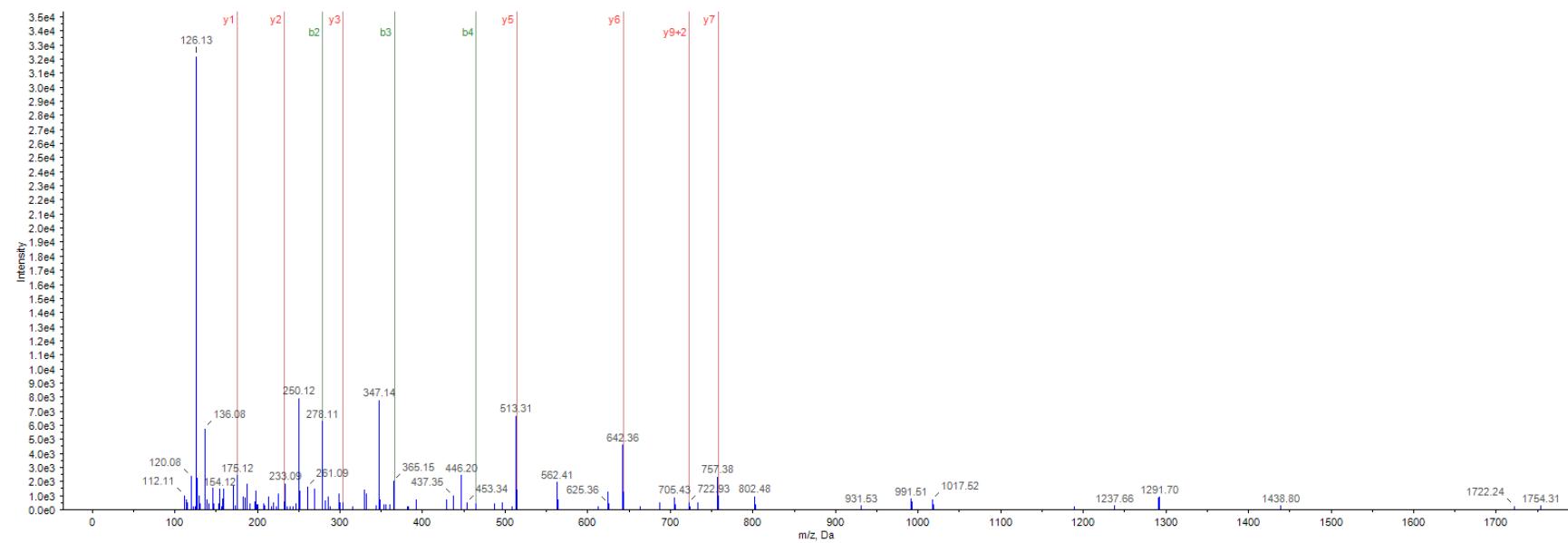


S-1 79

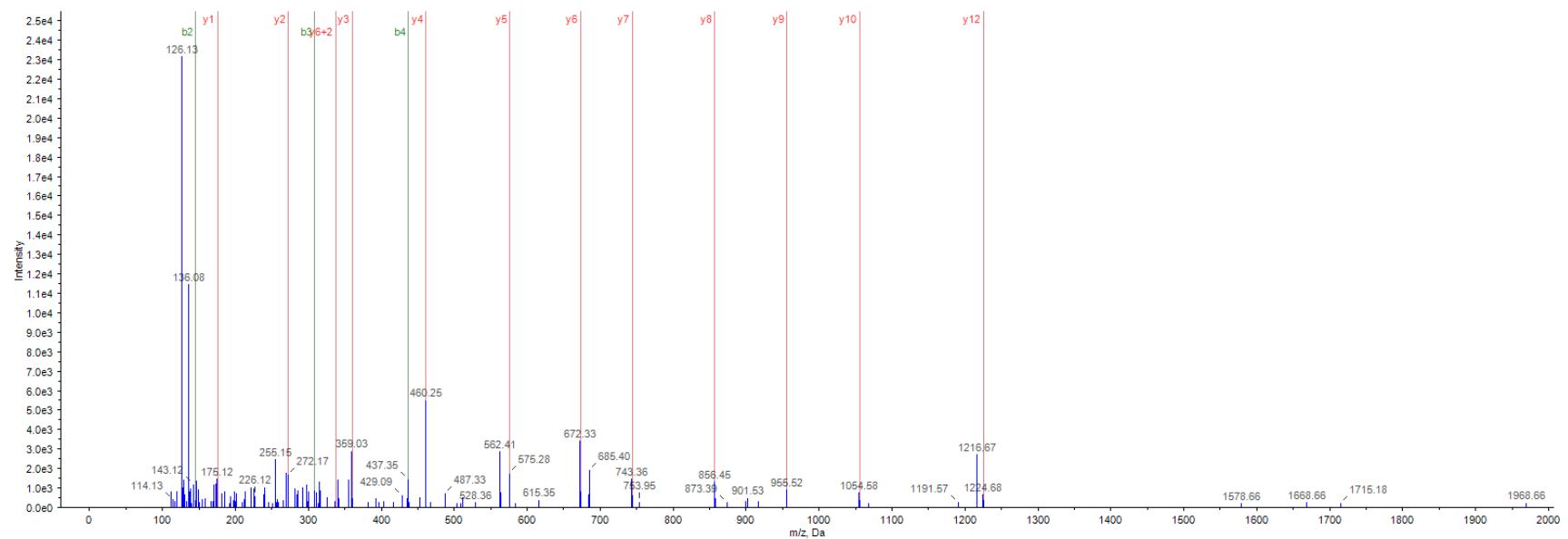
DH[HT]DASVYLR



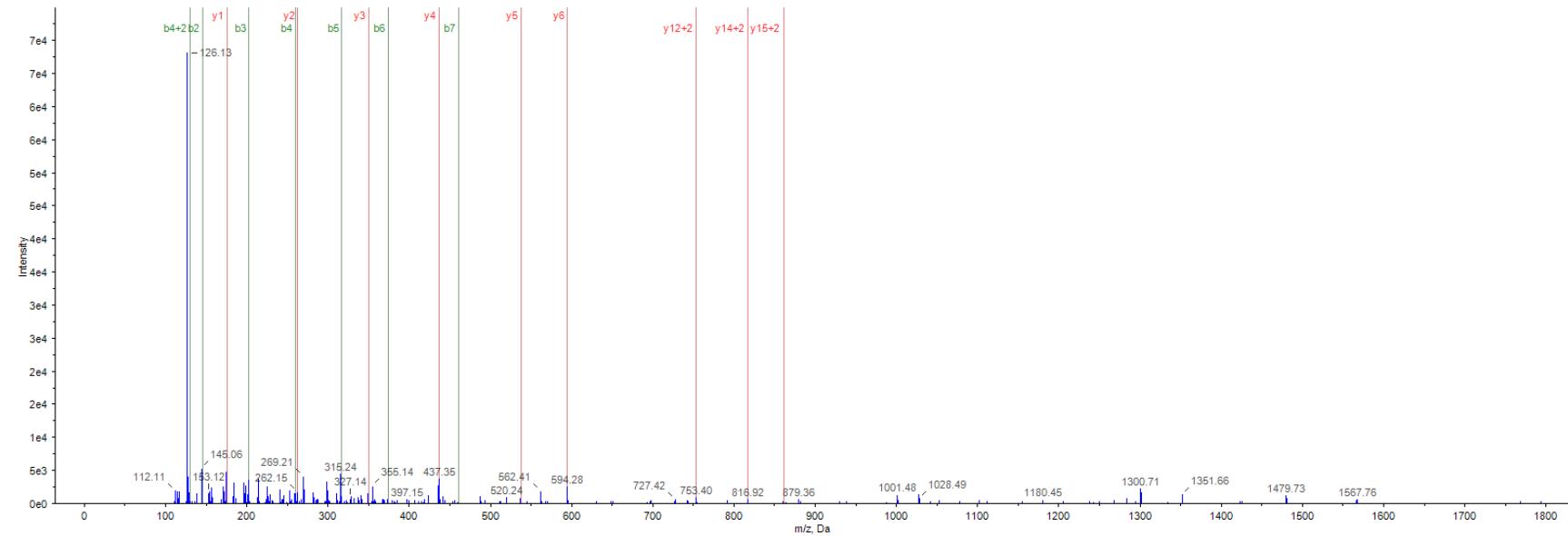
[NYSVVFPH[HT]DEPLAGR]



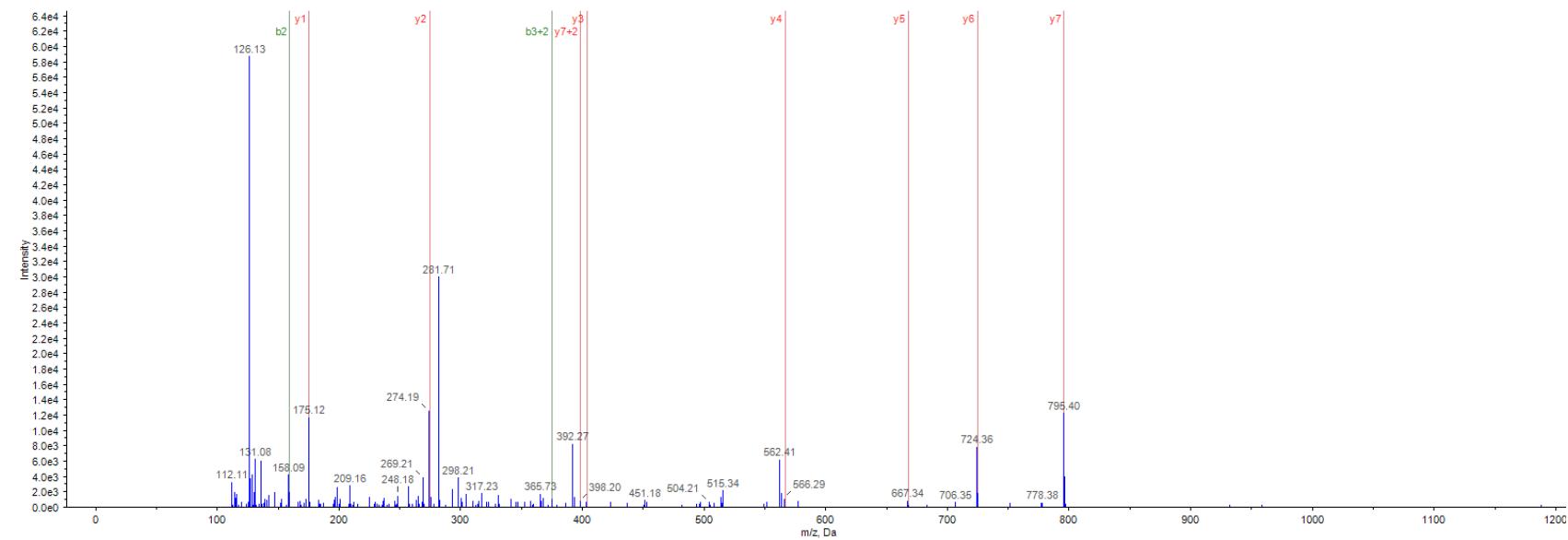
[SGYQQAASEH[HT]GLVVIAPDTSPR]



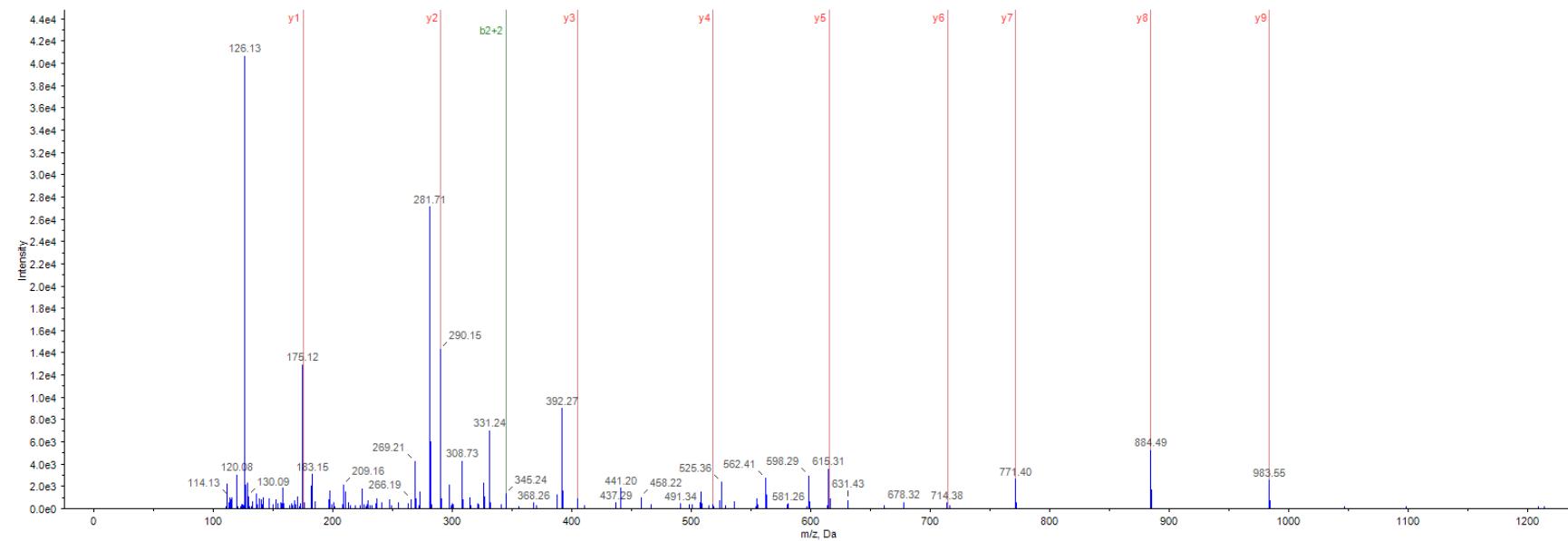
[SGGGGGSSGAGGGPSC[HT]GTSSSR



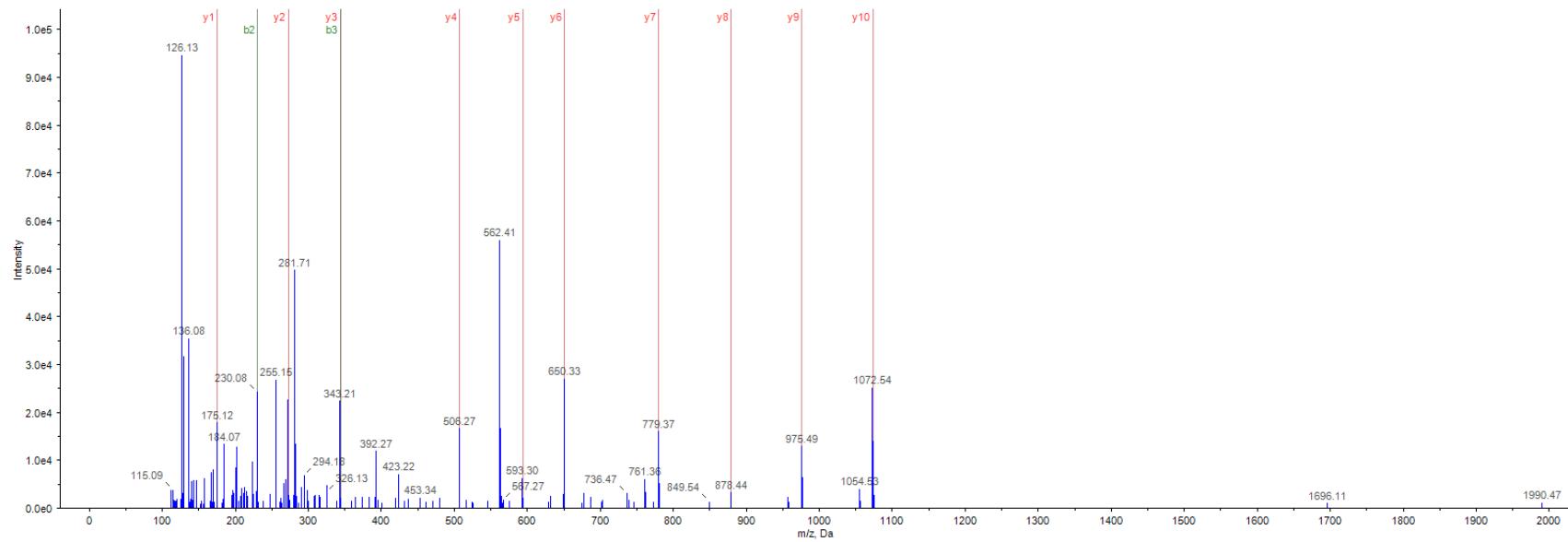
SAH[HT]AGTYEVR



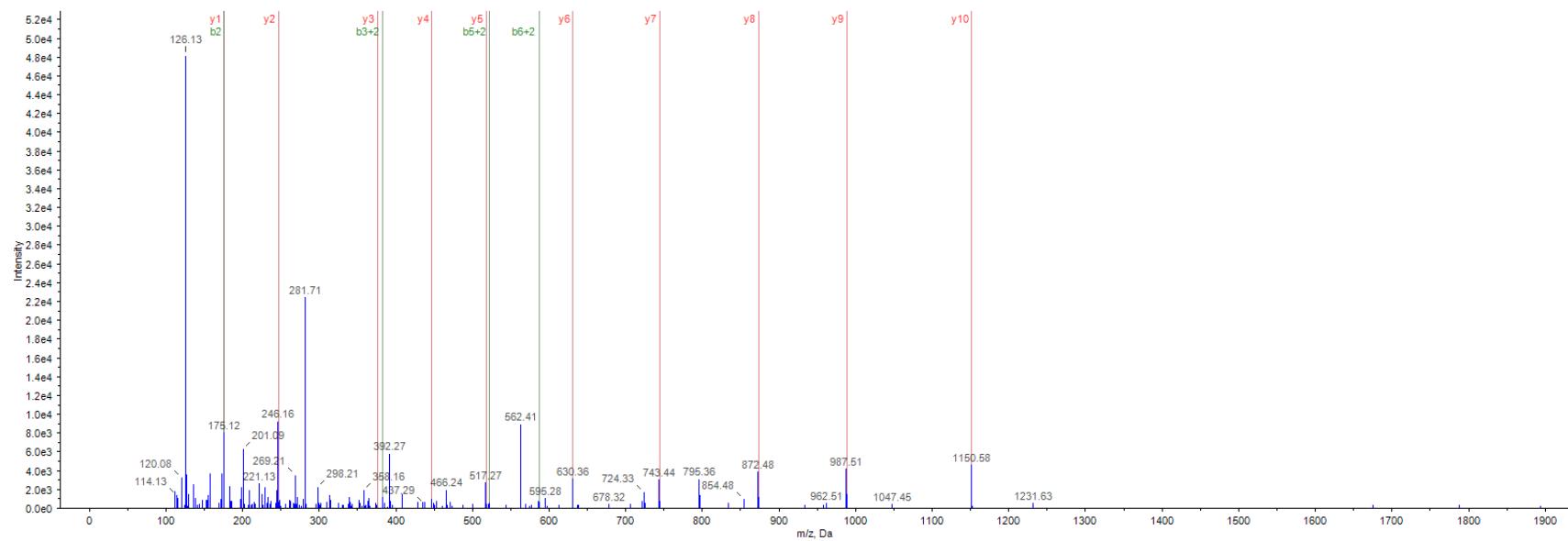
S-1 84



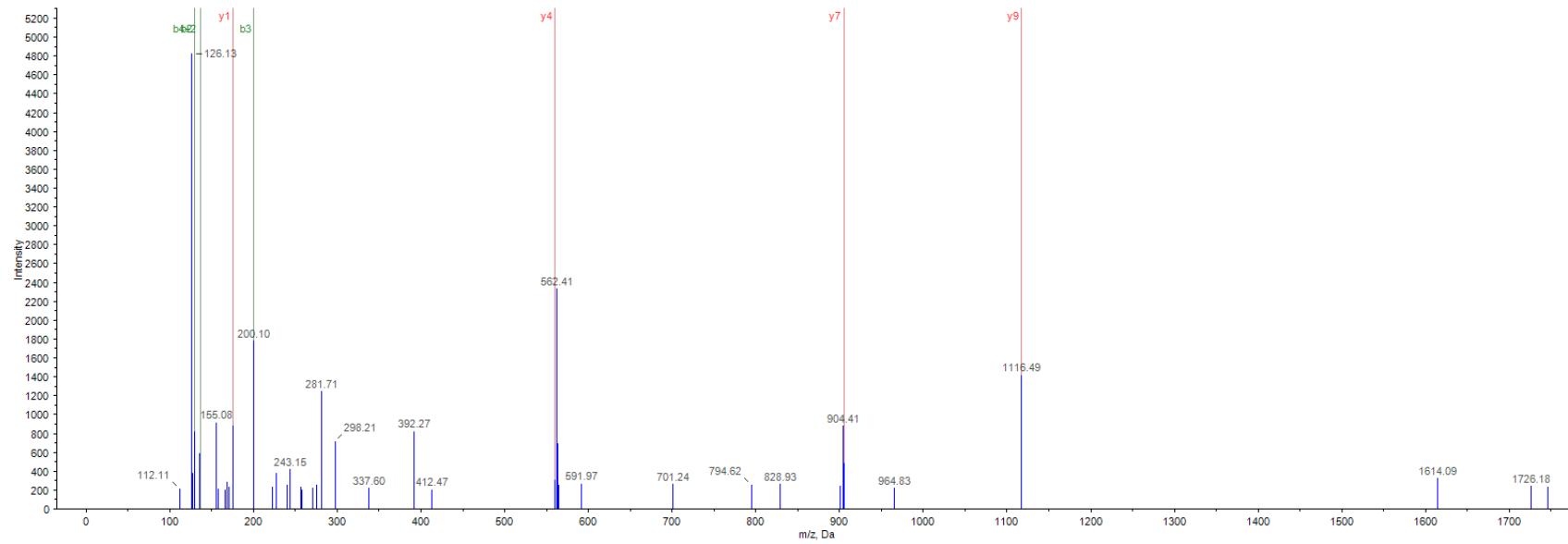
NDISSH[HT]PPVEGSYAPR



SSH[HT]YDELLAAEAR



GAAAH[HT]PDSEEEQQQR



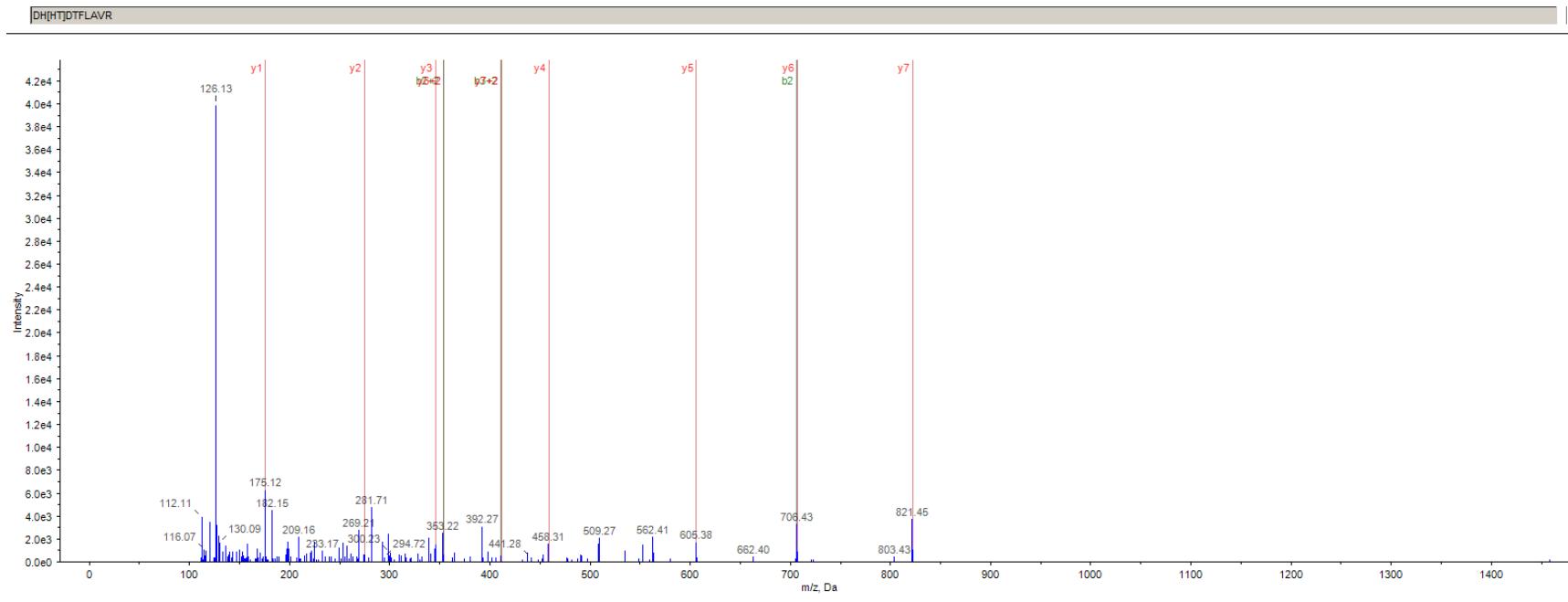


Figure S-1. Peptide sequence match spectra of aminoxyTMT labeled, HNE-modified peptides [HT] in HNE-treated liver mitochondria samples labeled with aminoxyTMT after enrichment with antiTMT resin