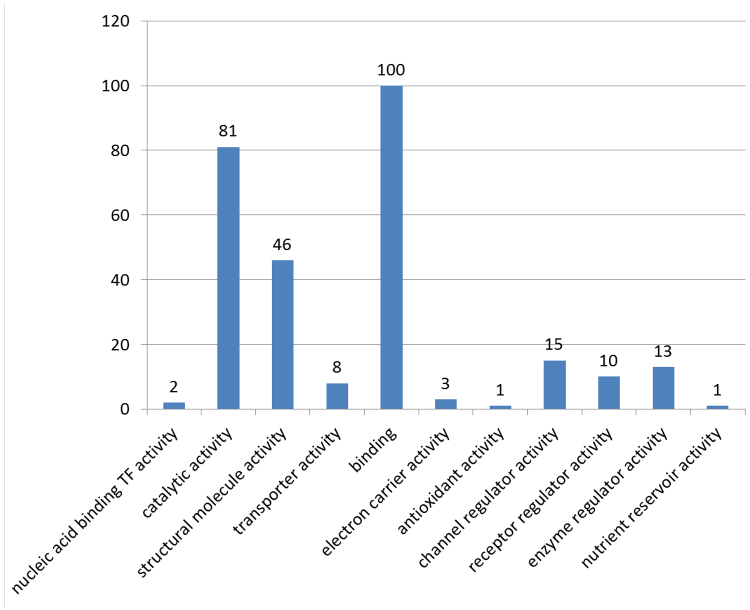
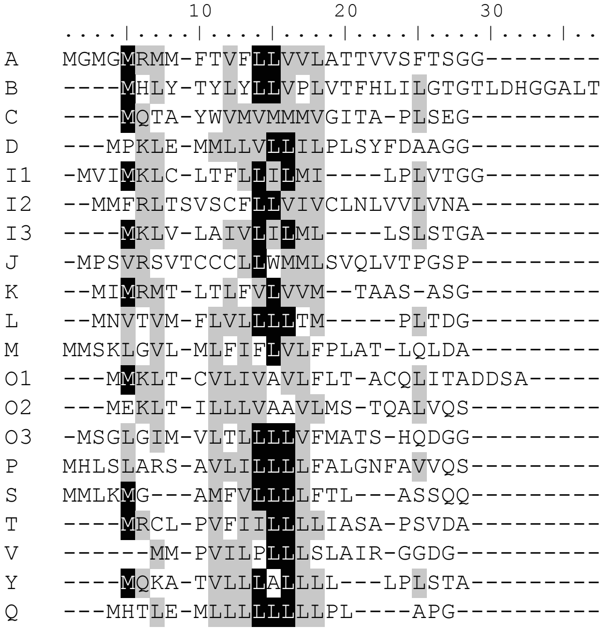


Figure S1. Go annotation on molecular functions of small peptides identified in the cDNA library of *C. flavidus* venom duct.



```
              10        20        30
     ....|....|....|....|....|....|....|..
A    MGMGMRMM-FTVFLLVVLATTVVSFTSGG--------
B    ----MHLY-TYLYLLVPLVTFHLILGTGTLDHGGALT
C    ----MQTA-YWVMVMMMVGITA-PLSEG---------
D    ---MPKLE-MMLLVLLILPLSYFDAAGG---------
I1   -MVIMKLC-LTFLLILMI----LPLVTGG--------
I2   --MMFRLTSVSCFLLVIVCLNLVVLVNA---------
I3   ----MKLV-LAIVLILML----LSLSTGA--------
J    -MPSVRSVTCCCLLWMMLSVQLVTPGSP---------
K    --MIMRMT-LTLFVLVVM--TAAS-ASG---------
L    --MNVTVM-FLVLLLLTM-----PLTDG---------
M    MMSKLGVL-MLFIFLVLFPLAT-LQLDA---------
O1   ---MMKLT-CVLIVAVLFLT-ACQLITADDSA-----
O2   ---MEKLT-ILLLVAAVLMS-TQALVQS---------
O3   -MSGLGIM-VLTLLLLVFMATS-HQDGG---------
P    MHLSLARS-AVLILLLLFALGNFAVVQS---------
S    MMLKMG---AMFVLLLLFTL---ASSQQ---------
T    ----MRCL-PVFIILLLLIASA-PSVDA---------
V    ------MM-PVILPLLLSLAIR-GGDG----------
Y    ----MQKA-TVLLLALLLL---LPLSTA---------
Q    ---MHTLE-MLLLLLLLLPL----APG----------
```

Figure S2. Alignment of consensus signal peptide sequences of different superfamilies. The consensus sequences were composed by most frequently appeared amino acid residues in each position.

SEQ: CCSDPPCRHKHQDLC, Carb(C1) Carb (C2) Carb(C7) Carb (C15) Amidation (C-term), Charge:3, MH+: 1968.7996, Score: 4.03e-011

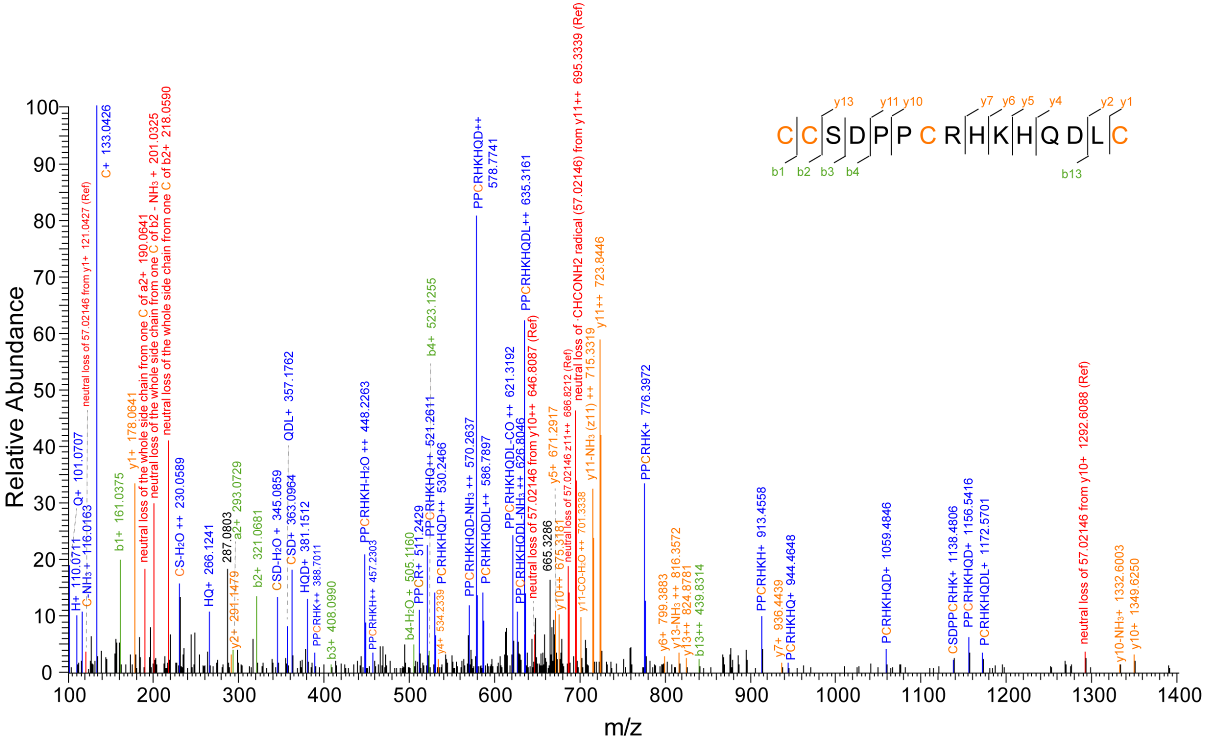


Figure S3. MS/MS spectrum of fla1a, CCSDPPCRHKHQDLC (C-term amidation). The red peaks are speculated assignments with neutral loss of iodoacetamide radical (CHCONH$_2$, 57.02146) from the side chain of C-terminal iodoacetamide cysteine, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

SEQ: GCCSDPPCRHKHQDLC, Carb(C2) Carb (C3) Carb(C8) Carb (C16) Amidation (C-term), Charge:3, MH+: 2025.8185, Score: 3.92e-011

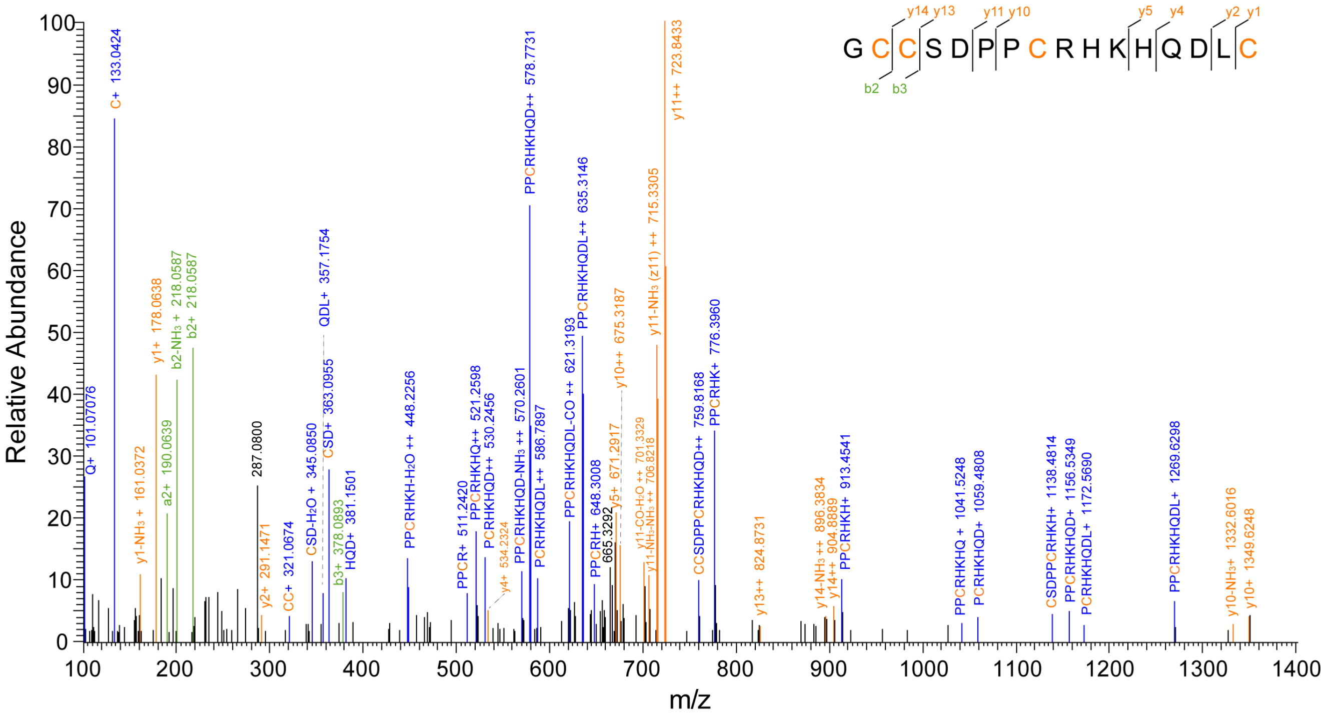


Figure S4. MS/MS spectrum of fla1b, GCCSDPPCRHKHQDLC (C-term amidation).

SEQ: ACNPPCSDILTCLHGTCKHLGI, Carb(C2) Carb (C6) Carb(C12) Carb (C17) Amidation (C-term), Charge:4, MH+: 2523.1657, Score: 8.90e-043

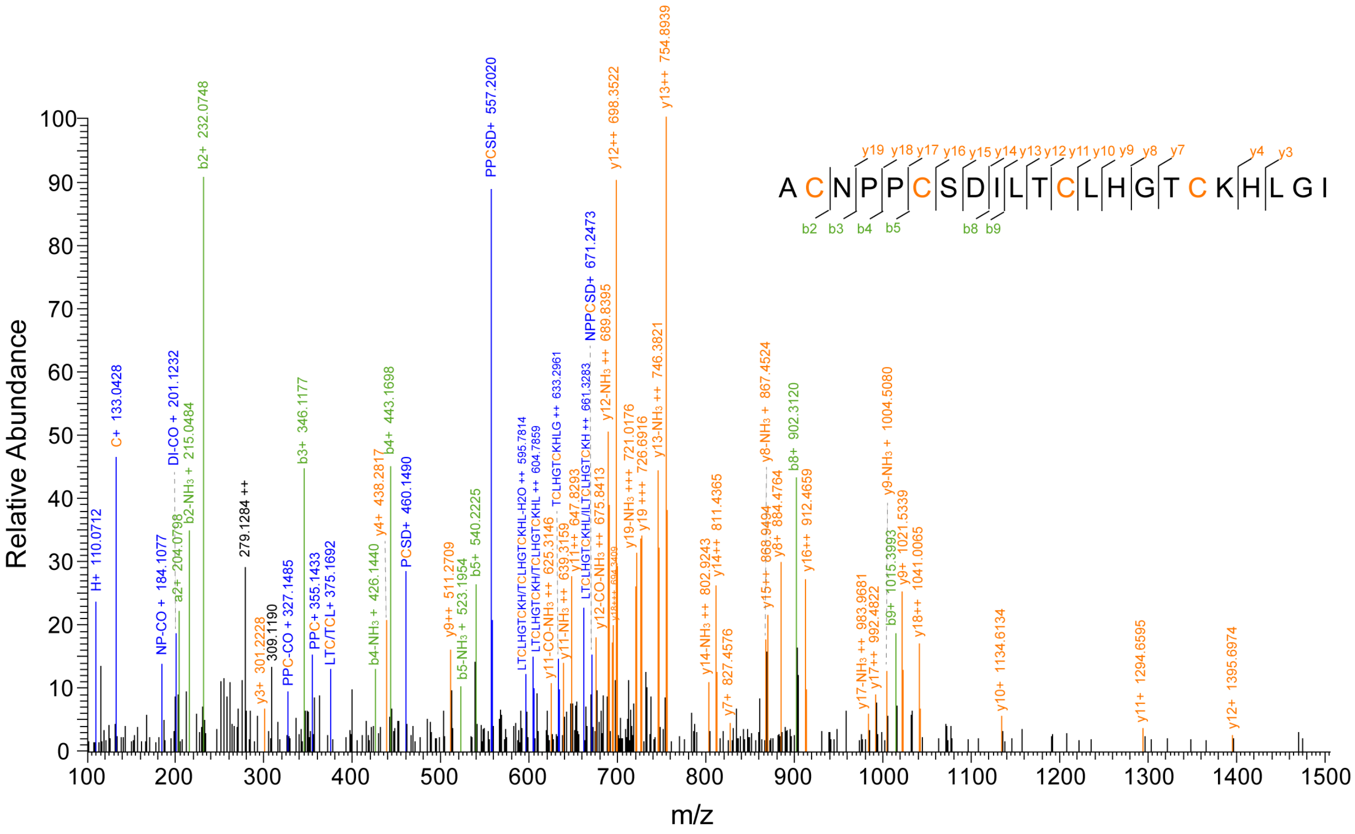


Figure S5. MS/MS spectrum of fla14a, ACNPPCSDILTCLHGTCKHLGI (C-term amidation).

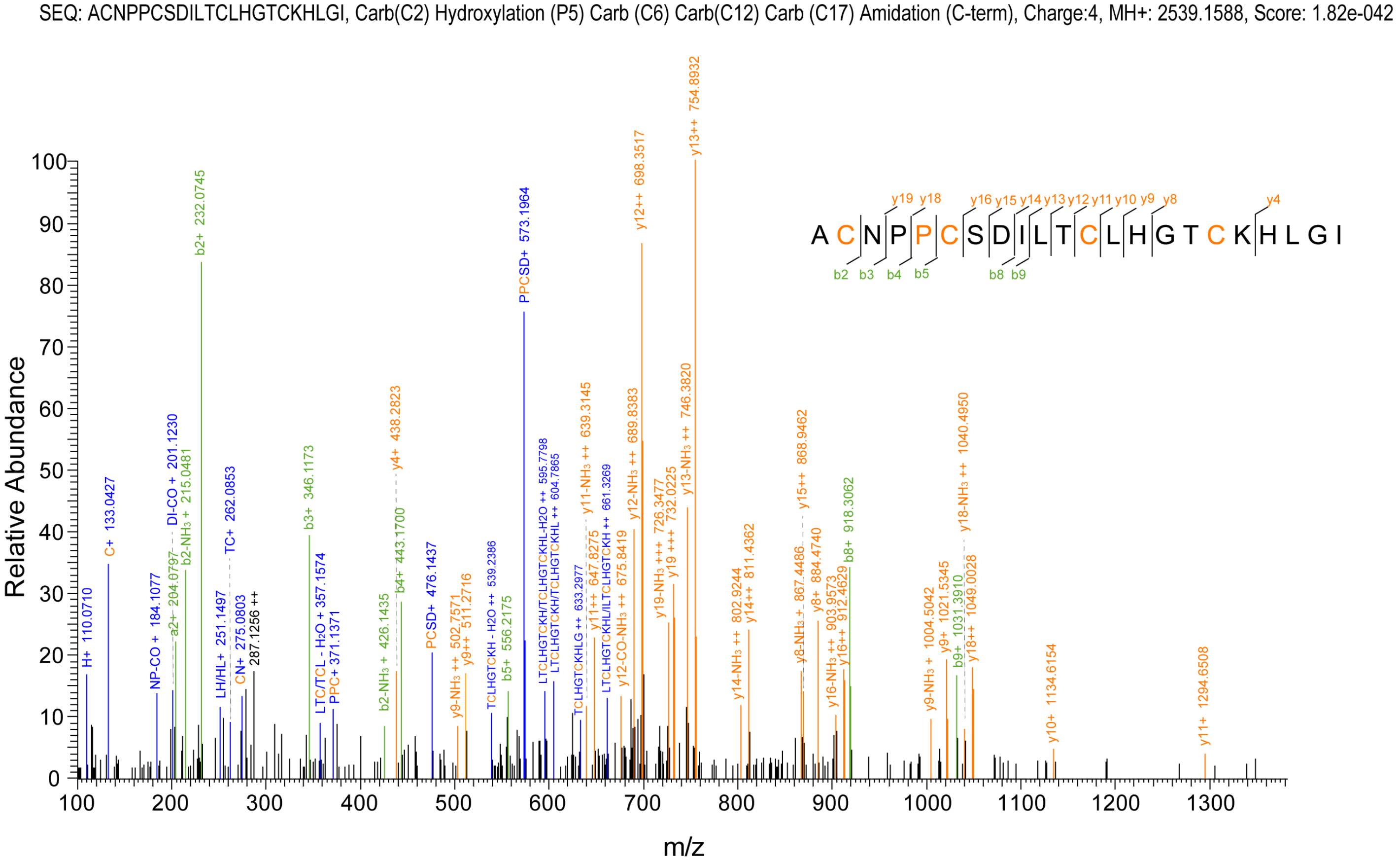
 SEQ: ACNPPCSDILTCLHGTCKHLGI, Carb(C2) Hydroxylation (P5) Carb (C6) Carb(C12) Carb (C17) Amidation (C-term), Charge:4, MH+: 2539.1588, Score: 1.82e-042

Figure S6. MS/MS spectrum of fla14b, ACNPPCSDILTCLHGTCKHLGI (hydro(P5), C-term amidation), orange “P” indicates P with hydroxylation.

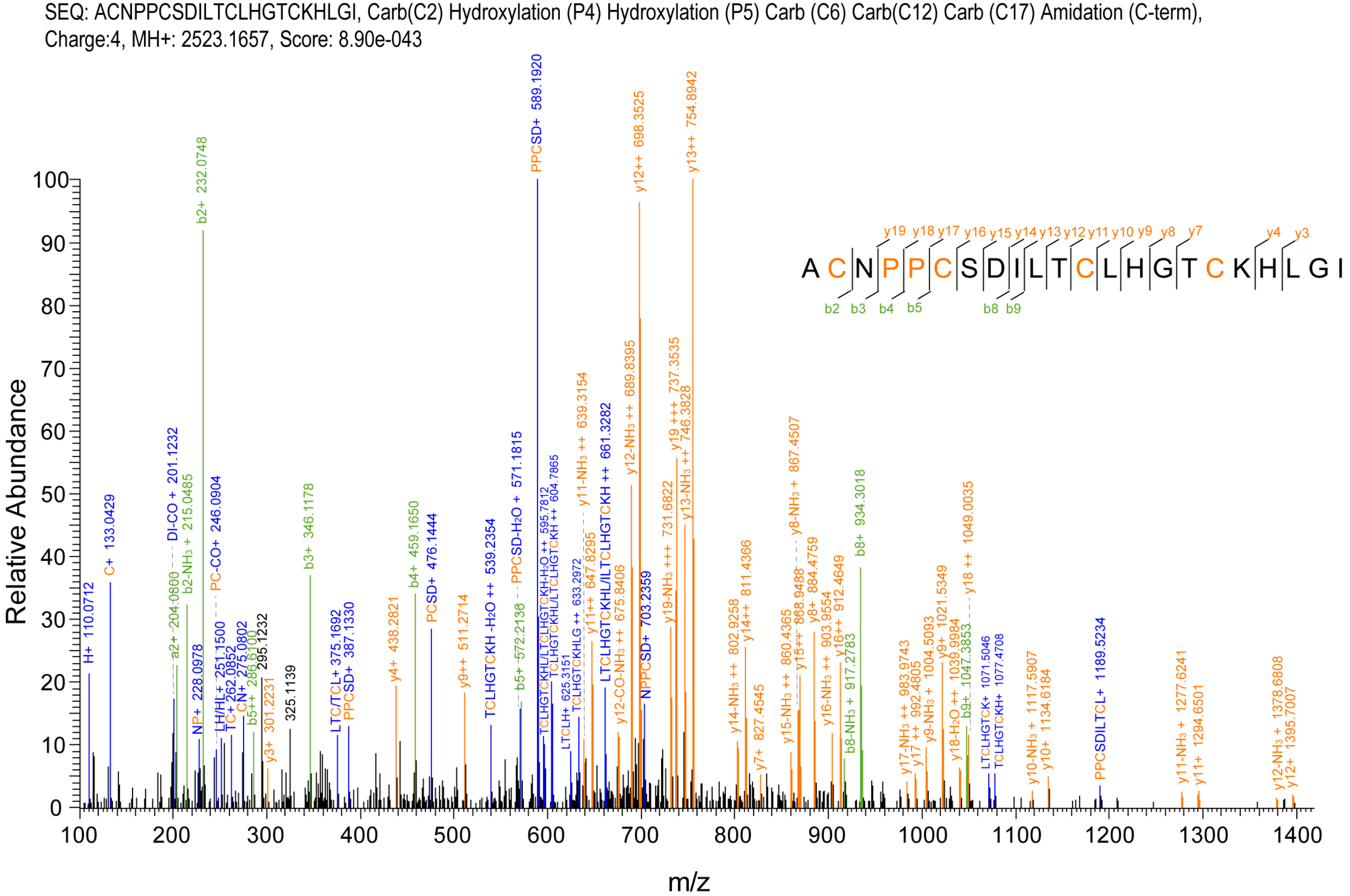


Figure S7. MS/MS spectrum of fla14c, ACNPPCSDILTCLHGTCKHLGI (hydro(P4, P5), C-term amidation), orange “P” indicates P with hydroxylation.

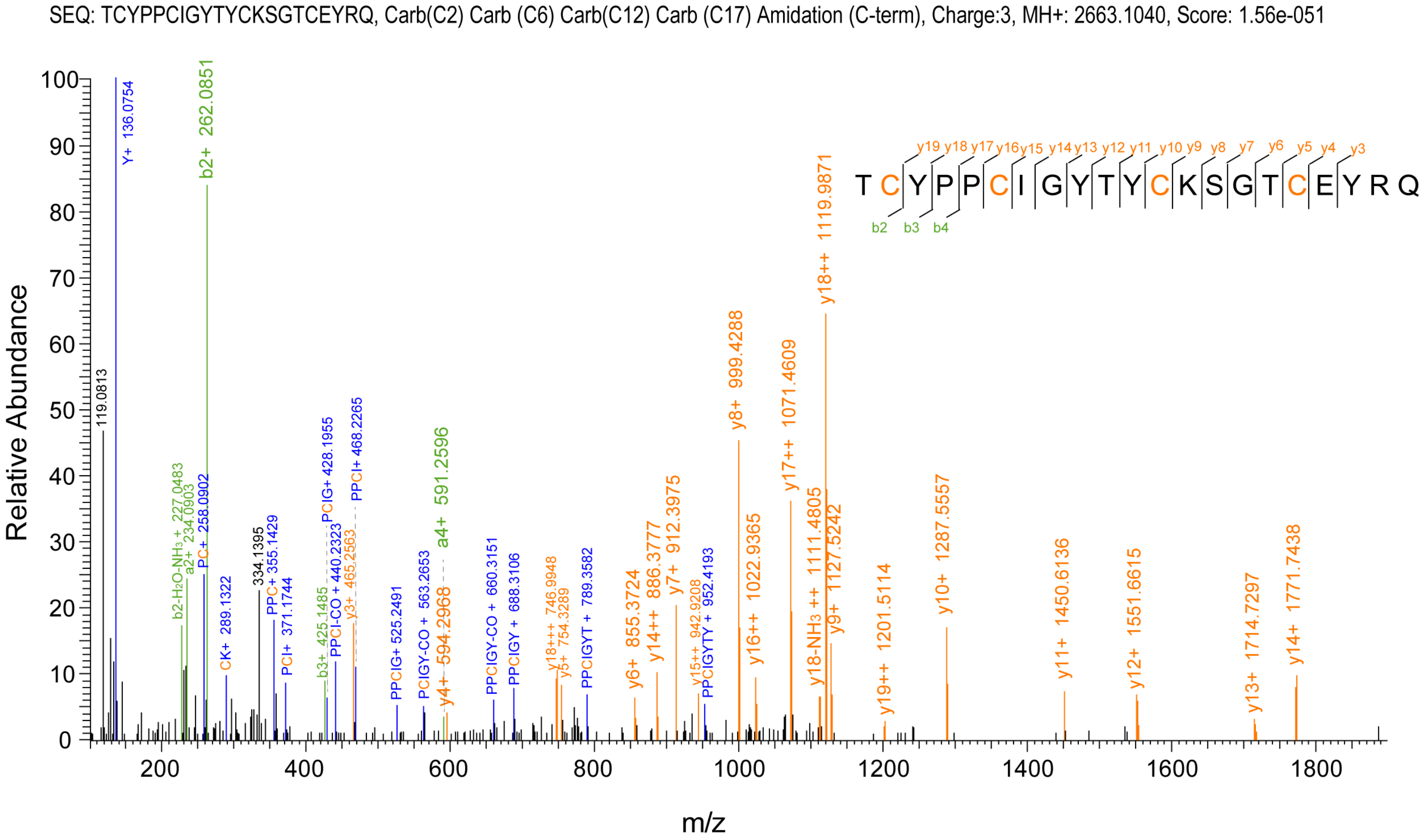


Figure S8. MS/MS spectrum of fla14d, TCYPPCIGYTYCKSGTCEYRQ (C-term amidation).

SEQ: CCSKYCWECTPCCPYSS, Carb(C1) Carb (C2) Carb(C6) Carb (C9) Hydrxoylation(P11) Carb(C12) Carb (C13) Amidation (C-term),
Charge:2, MH+: 2319.7952, Score: 2.28e-022

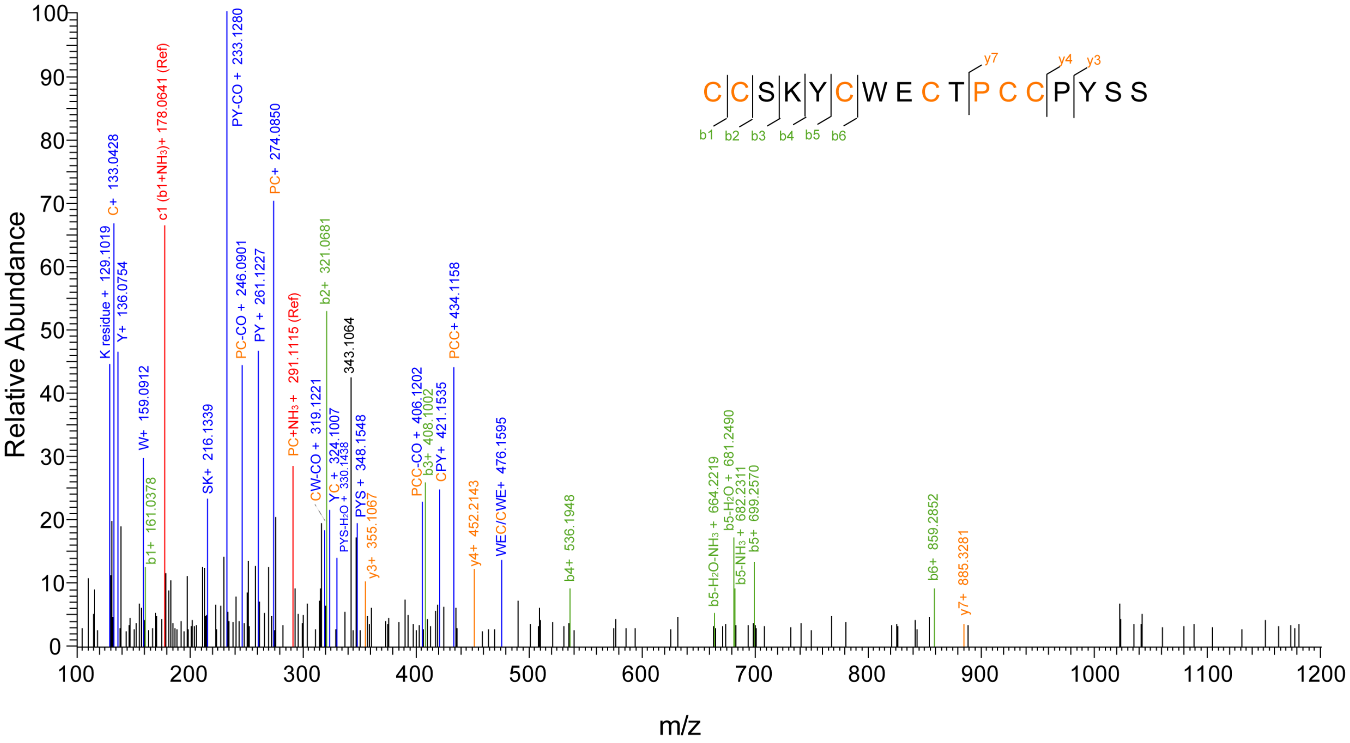


Figure S9. MS/MS spectrum of fla3a, CCSKYCWECTPCCPYSS (hydro(P11), C-term amidation), orange “P” indicates P with hydroxylation. The red peaks are speculated assignments to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

SEQ: RCCLWPACWGCVCCY, Carb(C2) Carb (C3) Carb(C8) Carb (C11) Carb(C13) Carb (C14), Charge:2, MH+: 2107.7830, Score: 6.19e-026

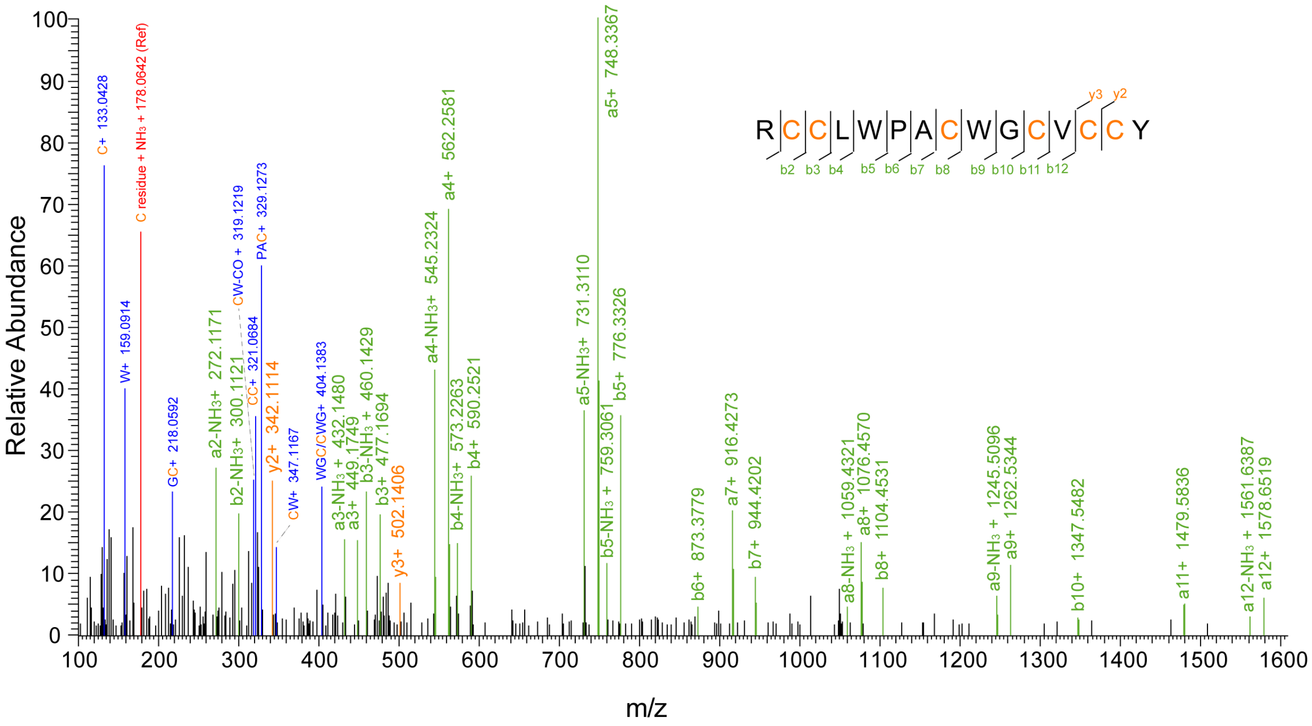


Figure S10. MS/MS spectrum of fla3b, RCCLWPACWGCVCCY. The red peak is speculated assignment to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

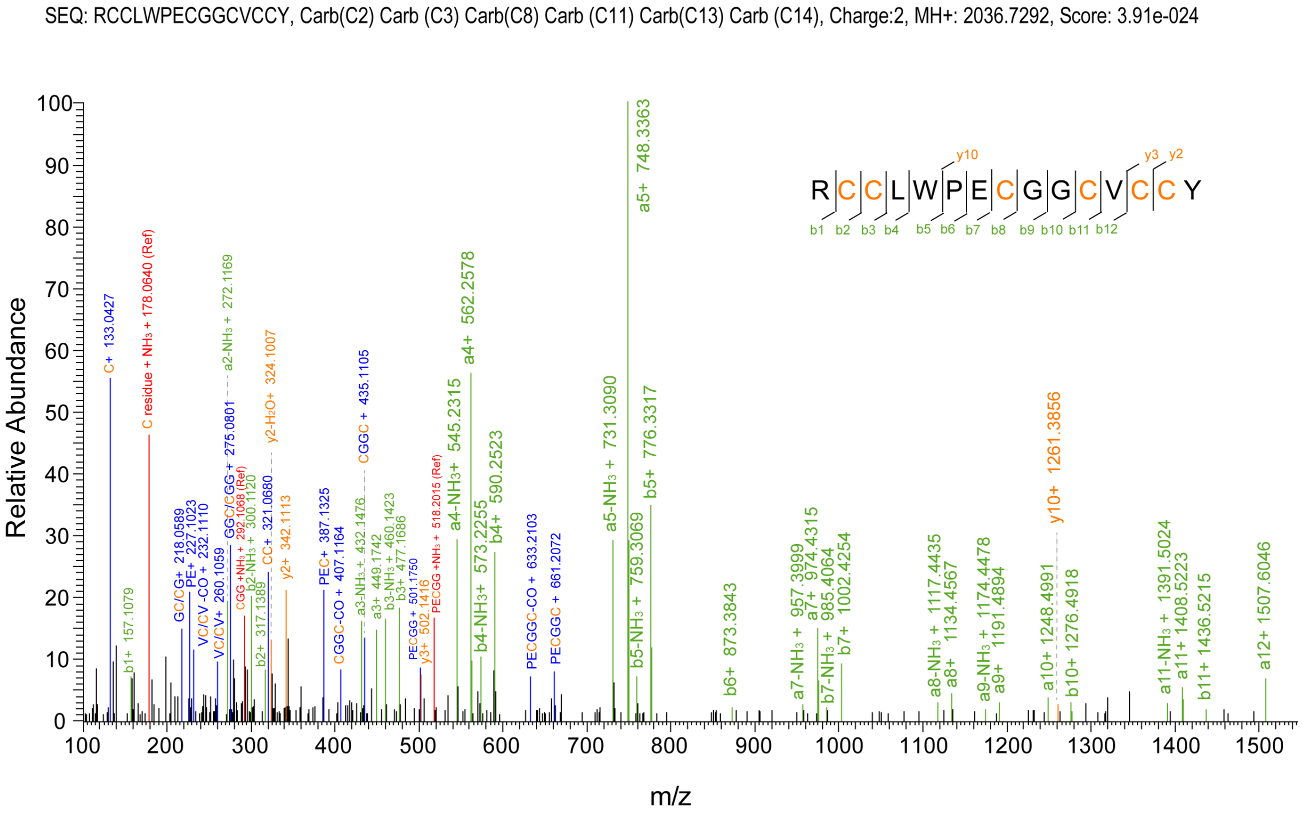


Figure S11. MS/MS spectrum of fla3c, RCCLWPECGGCVCCY. The red indicated peaks are speculated assignments to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

SEQ: RCCLWPECGGCVCCY, Carb(C2) Carb (C3) Gala (E7) Carb(C8) Carb (C11) Carb(C13) Carb (C14), Charge:2, MH+: 2080.7210, Score: 5.38e-016

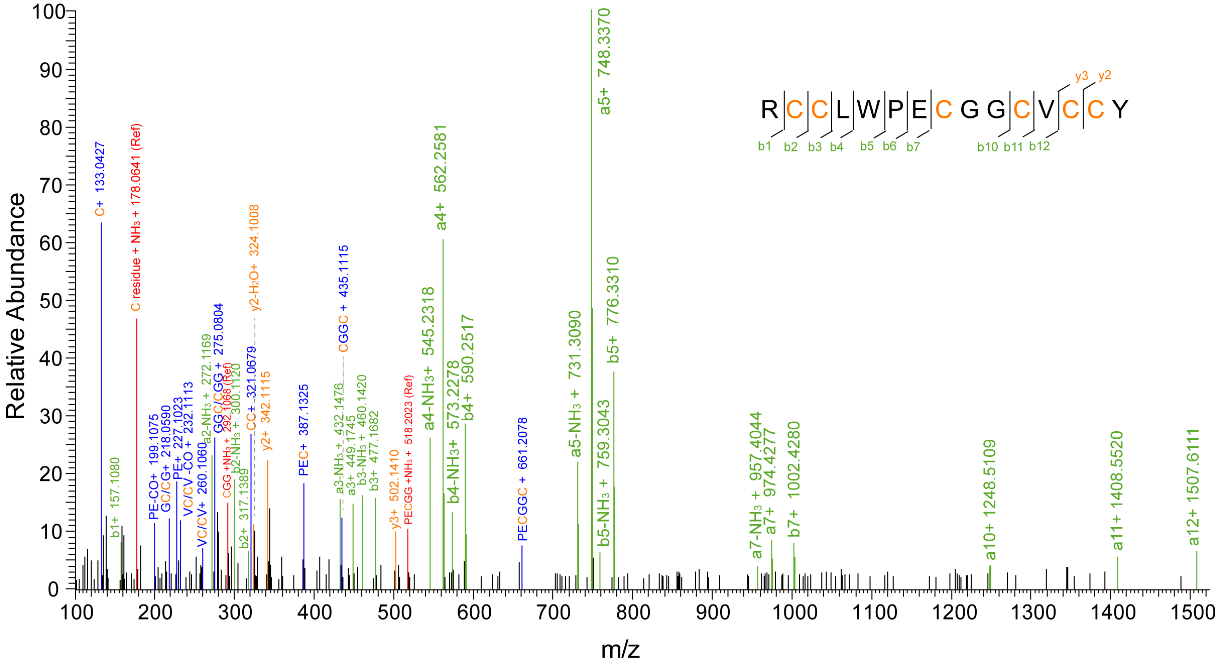


Figure S12. MS/MS spectrum of fla3d, RCCLWPECGGCVCCY (Gla(E7)). The red peaks are speculated assignments to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

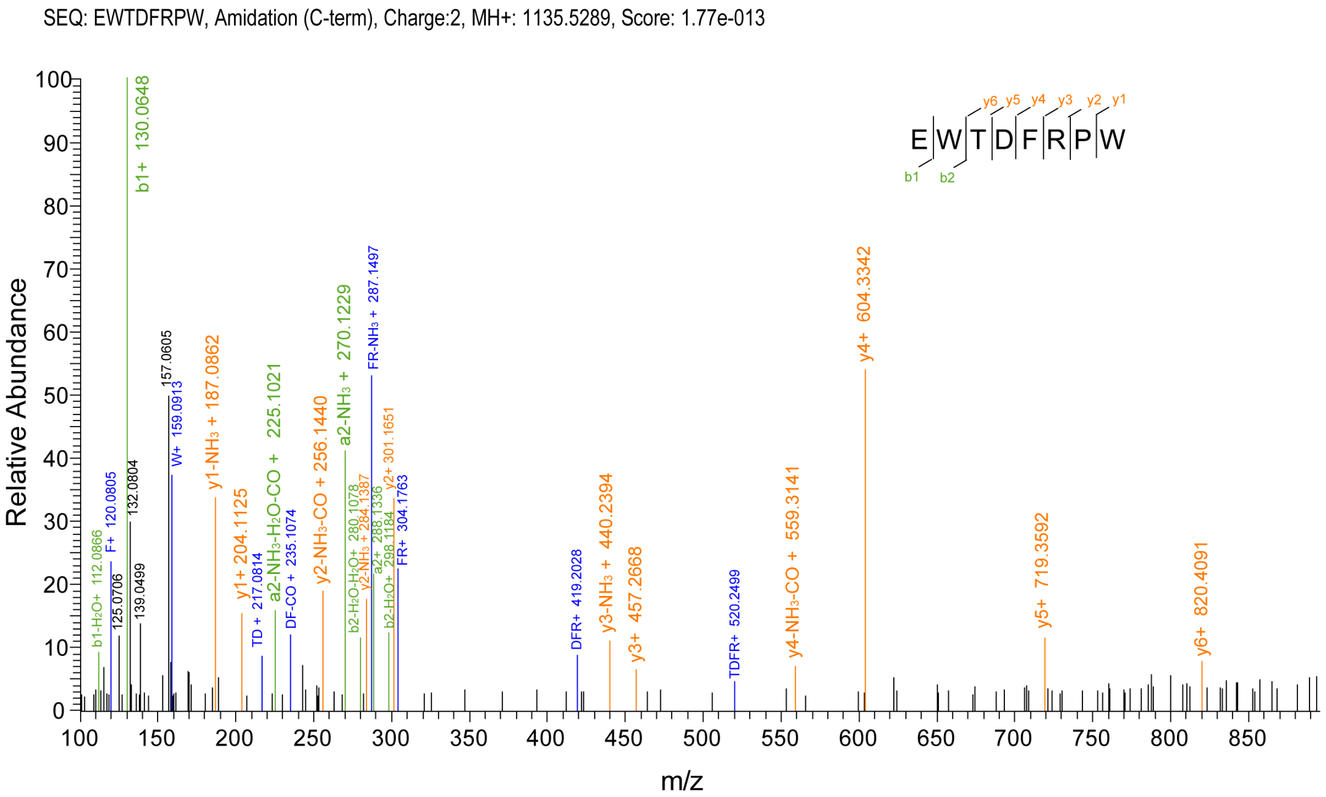


Figure S13. MS/MS spectrum of fla01, EWTDFRPW (C-term amidation).

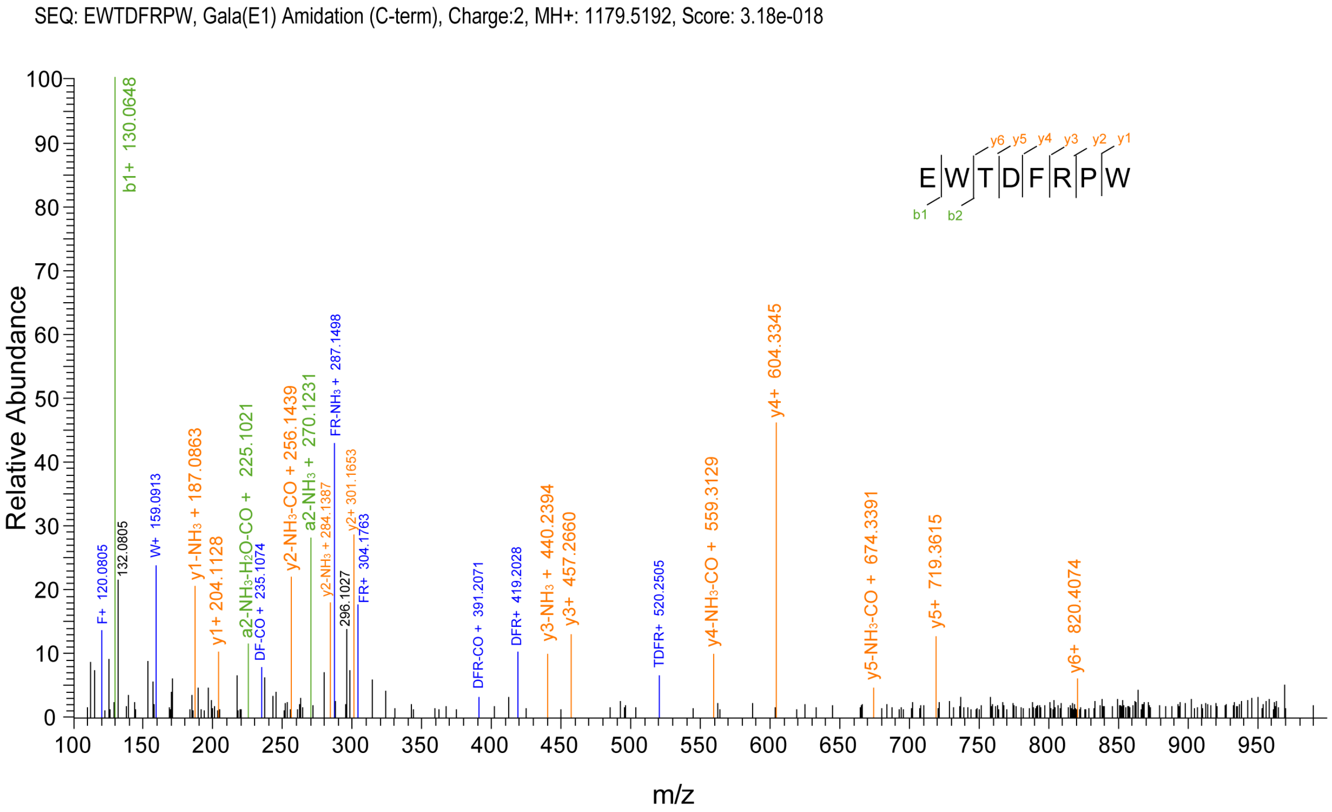


Figure S14. MS/MS spectrum of fla02, EWTDFRPW (Gla(E1), C-term amidation). There is a neutral loss of CO_2 from gamma carboxyglutamic acid Gla1.

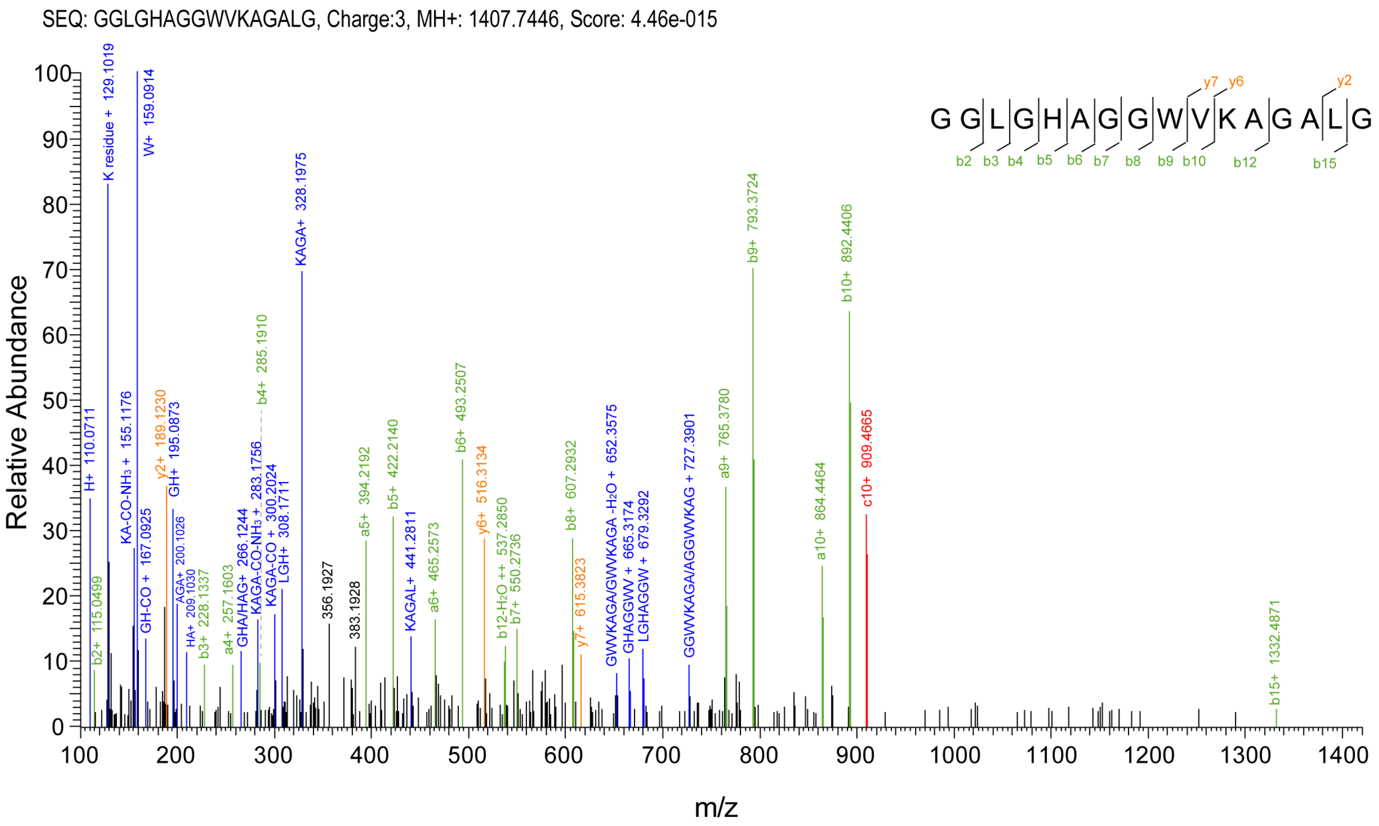


Figure S15. MS/MS spectrum of fla03, GGLGHAGGWVKAGALG. The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.

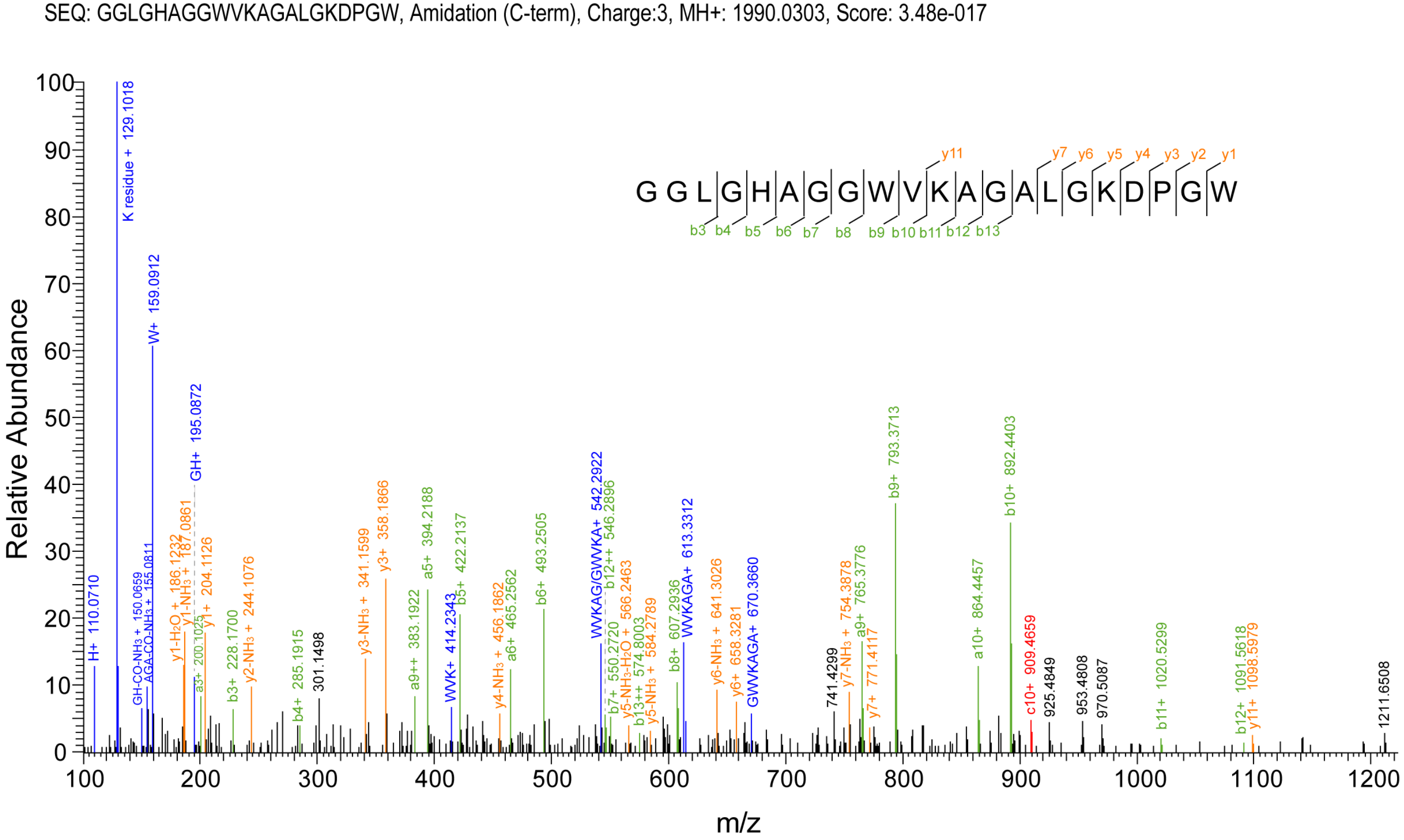


Figure S16. MS/MS spectrum of fla04, GGLGHAGGWVKAGALGKDPGW (C-term amidation). The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.

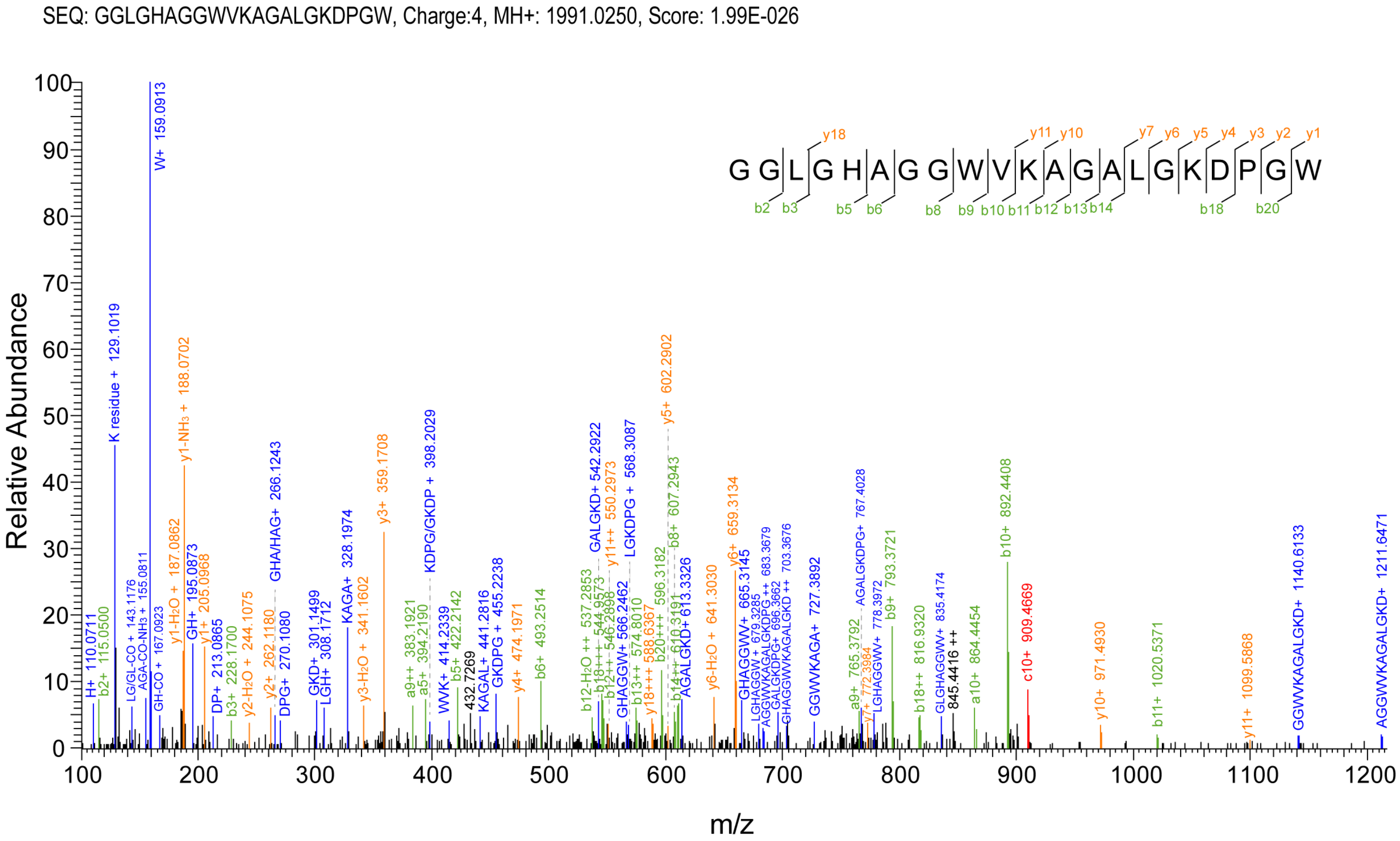


Figure S17. MS/MS spectrum of fla05, GGLGHAGGWVKAGALGKDPGW. The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.

SEQ: LGHAGGWVKAGALGKDPGW, Amidation (C-term), Charge:4, MH+: 1875.9901, Score: 5.49e-012

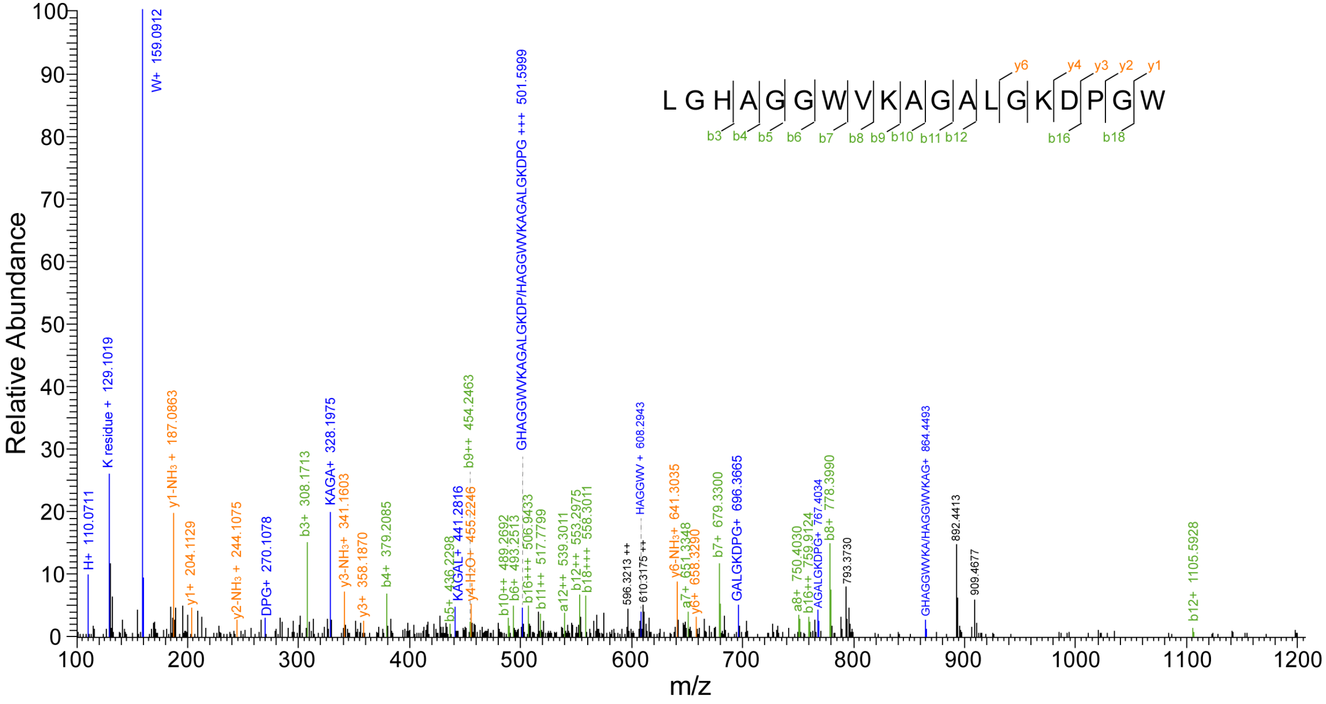


Figure S18. MS/MS spectrum of fla06, LGHAGGWVKAGALGKDPGW (C-term amidation).

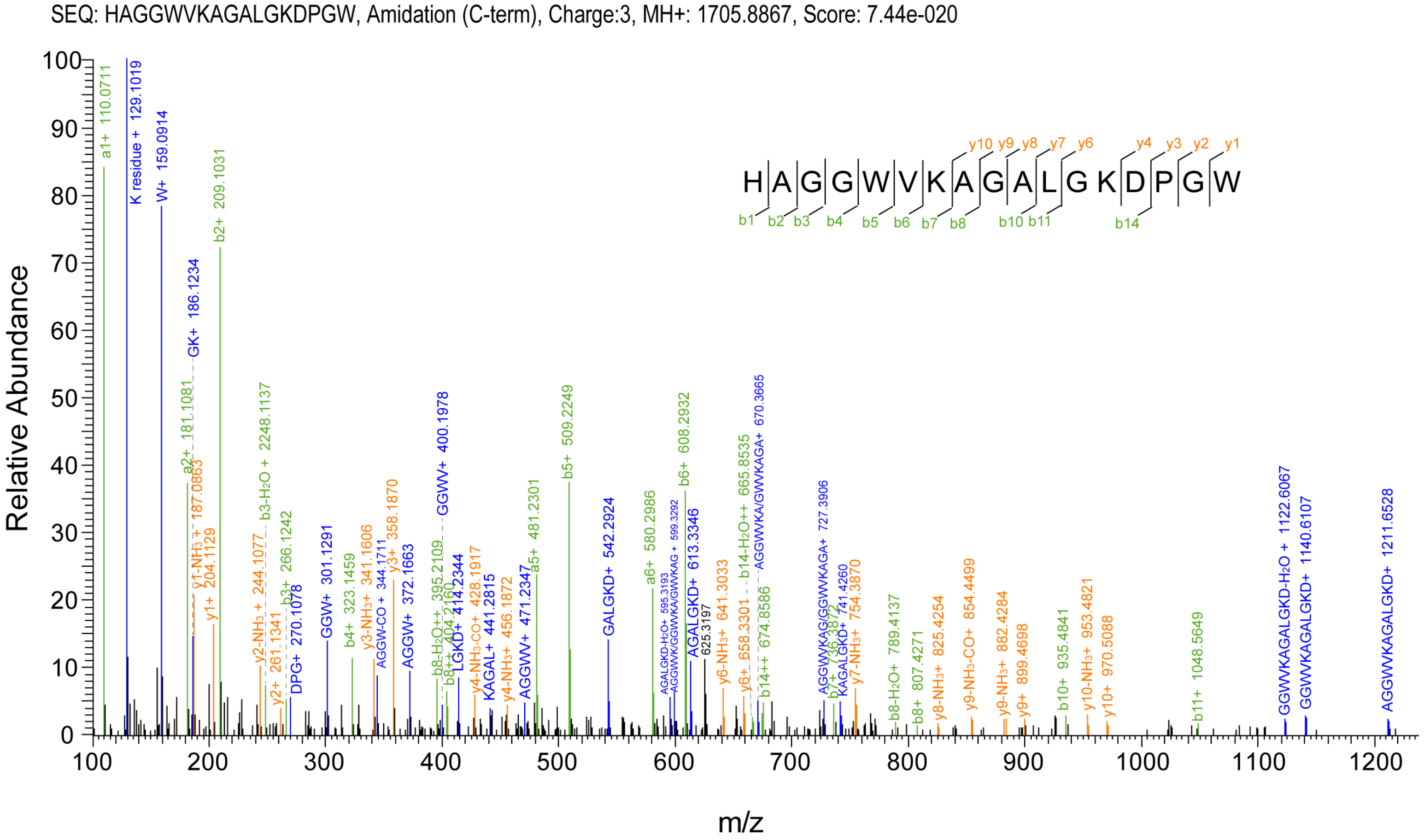


Figure S19. MS/MS spectrum of fla07, HAGGWVKAGALGKDPGW (C-term amidation).

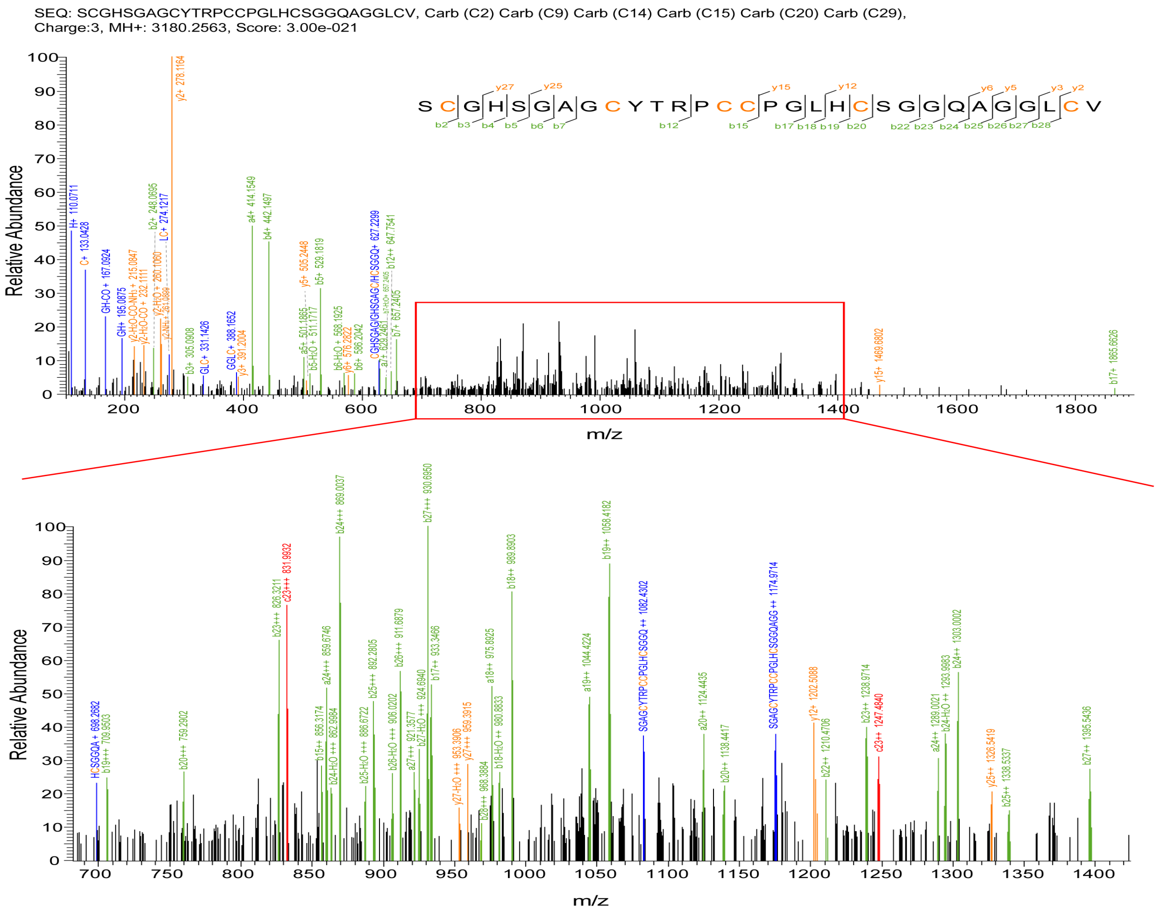


Figure S20. MS/MS spectrum of fla6a, SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV. The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.

SEQ: SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV, Carb (C2) Carb (C9) Hydroxylation (P13) Carb (C14) Carb (C15) Carb (C20) Carb (C29), Charge:3, MH+: 3196.2557, Score: 6.22e-018

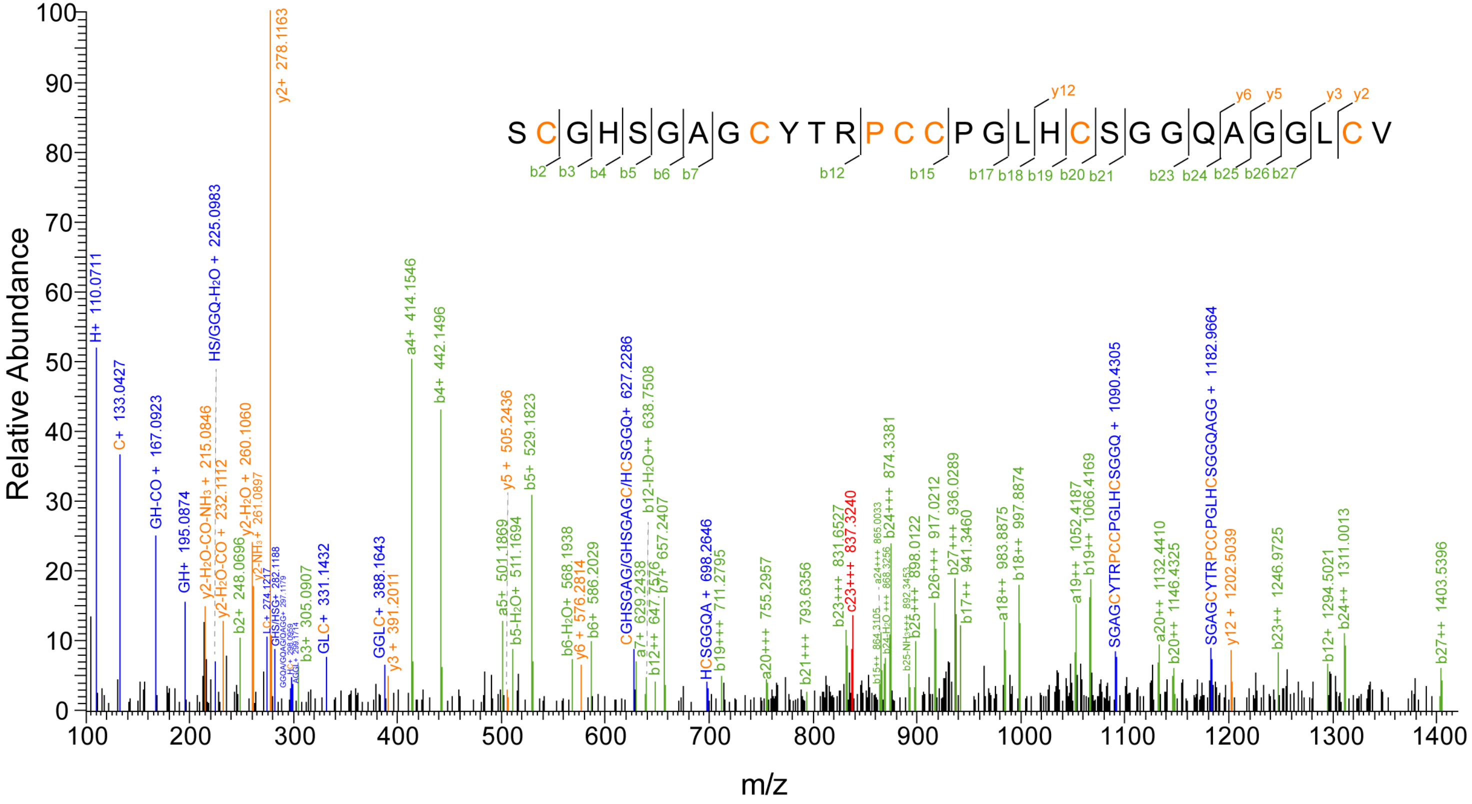


Figure S21. MS/MS spectrum of fla6b, SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV (Hydroxylation of P13). The c ion formation in CID experiment was previously reported by reference: Farrugia JM, O'Hair RAJ, Reid GE (2001). Do all b2 ions have oxazolone structures? Multistage mass spectrometry and ab initio studies on protonated N-acyl amino acid methyl ester model systems. Int. J. Mass Spectrom. 210, 71-87.

SEQ: SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV, Carb (C2) Carb (C9) Hydroxylation (P13) Carb (C14) Carb (C15) Hydroxylation (P16) Carb (C20) Carb (C29),
Charge:3, MH+: 3212.2467, Score: 5.08e-009

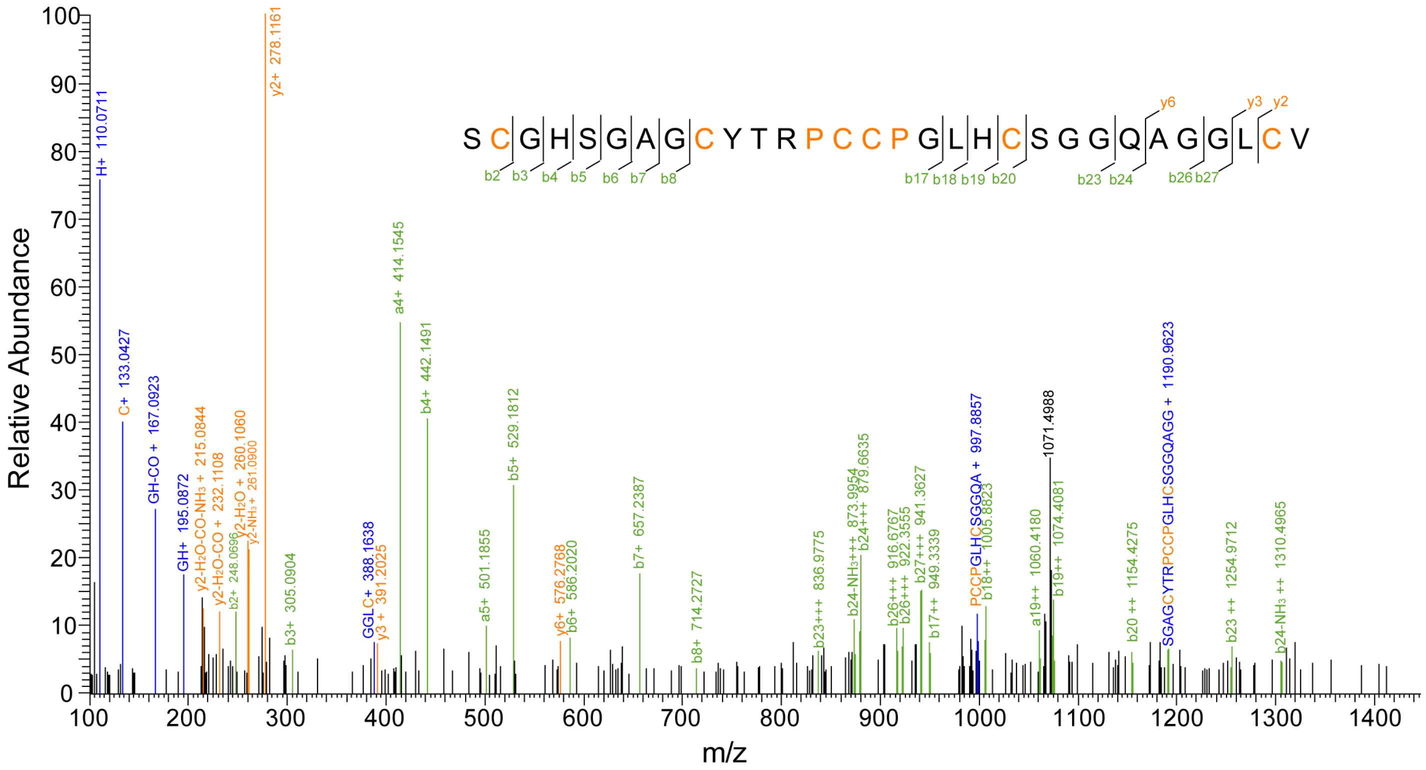


Figure S22. MS/MS spectrum of fla6c, SCGHSGAGCYTRPCCPGLHCSGGQAGGLCV (hydroxylation of P13 and P16).

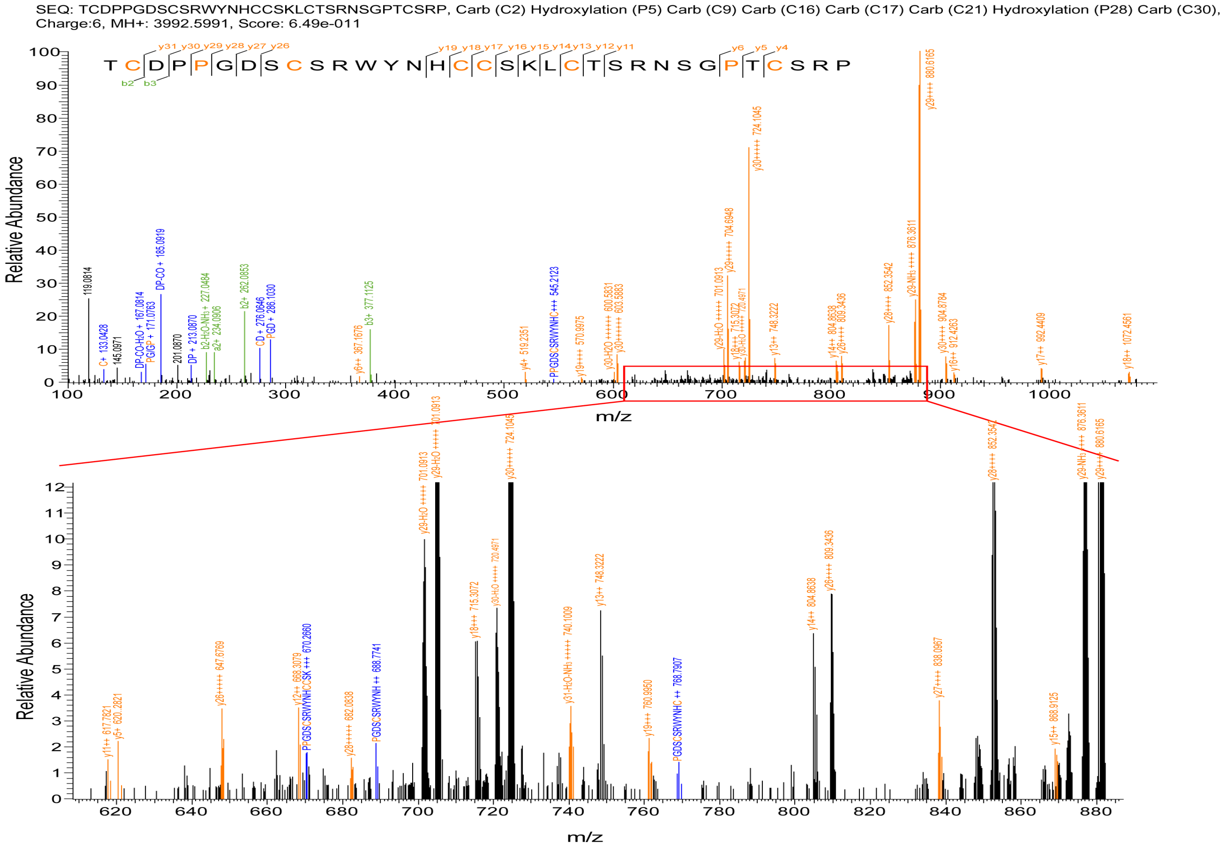


Figure S23. MS/MS spectrum of fla6d, TCDPPGDSCSRWYNHCCSKLCTSRNSGPTCSRP (hydroxylation of P5 and P28), P marked in red indicates hydroxylated proline.

SEQ: ALCCYGYAFCCRL, Carb (C3) Carb (C4) Carb (C10) Carb (C11) Amidation (C-term), Charge:2, MH+: 1712.7104, Score: 1.24e-031

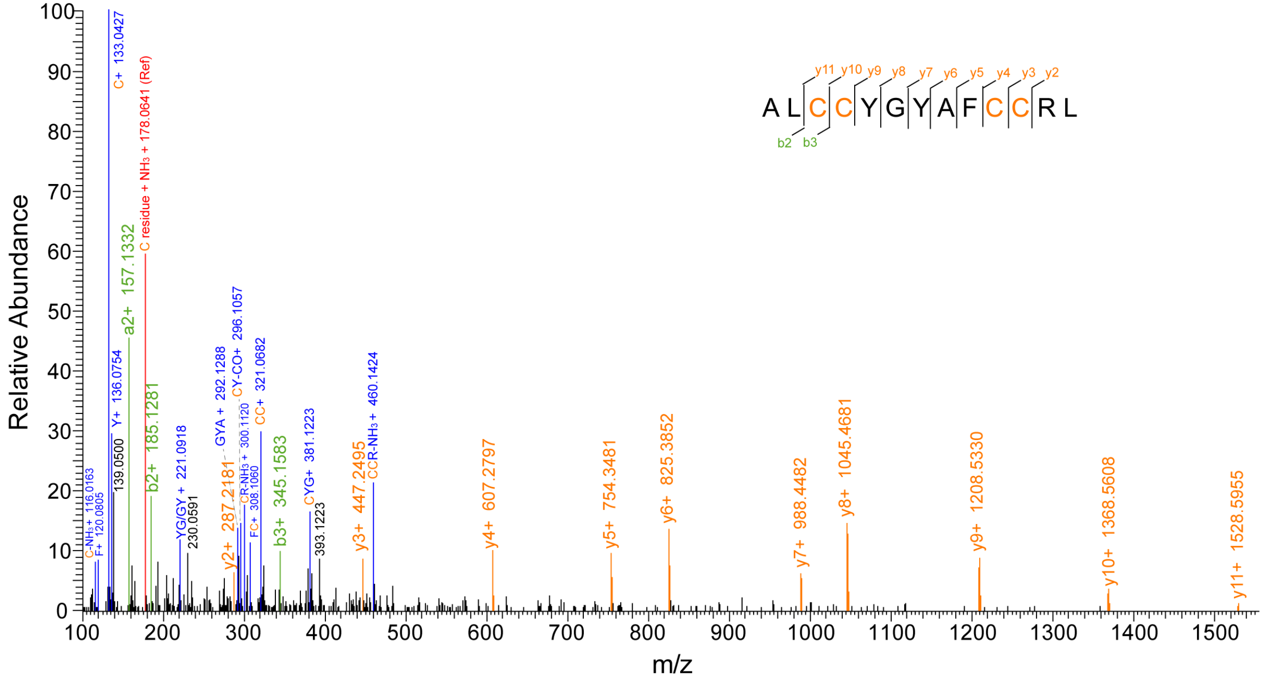


Figure S24. MS/MS spectrum of fla5a, ALCCYGYAFCCRL (C-term amidation). The red peak is speculated assignment to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

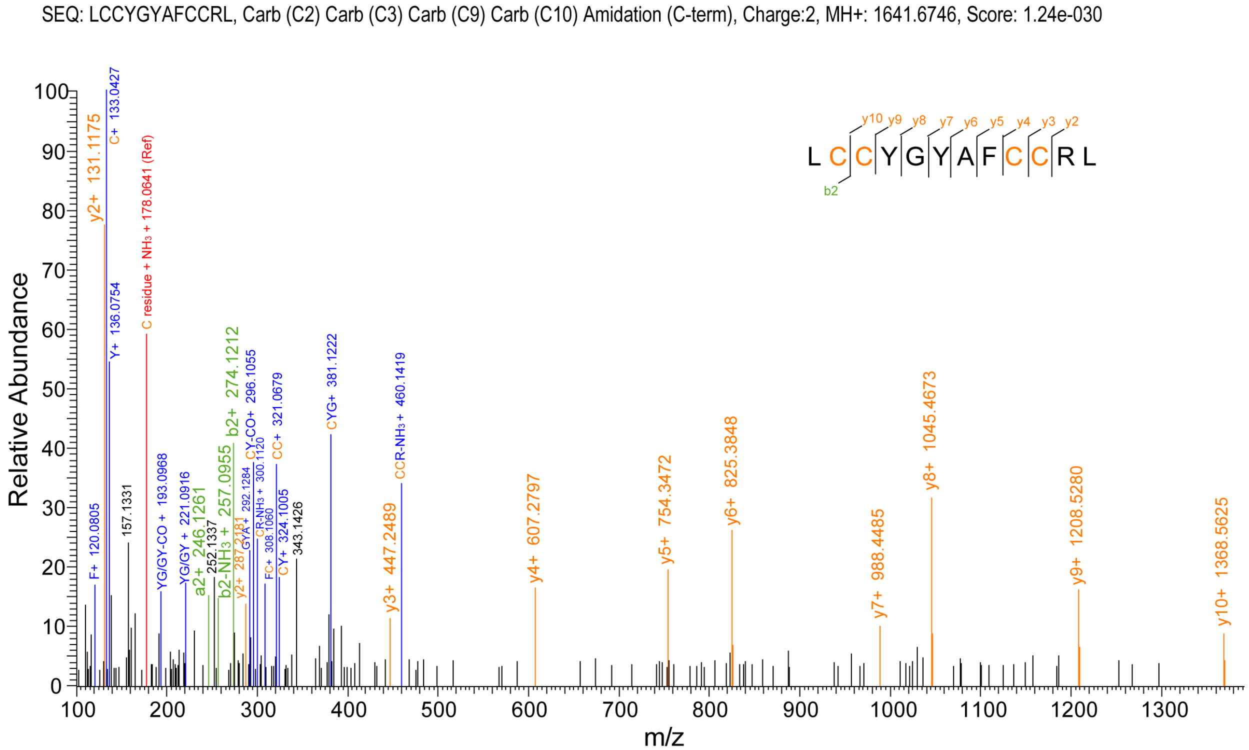


Figure S25. MS/MS spectrum of fla5b, LCCYGYAFCCRL (C-term amidation). The red peak is speculated assignment to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

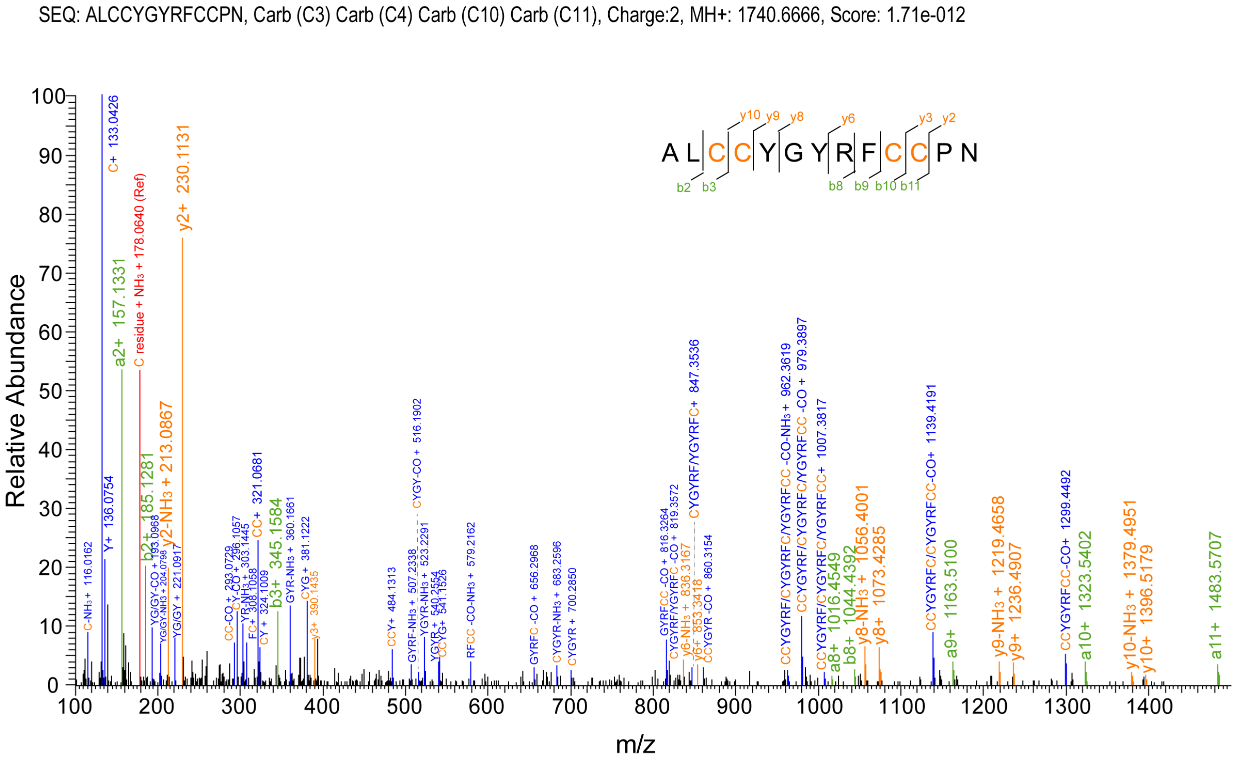


Figure S26. MS/MS spectrum of fla5d, ALCCYGYRFCCPN. The red peak is speculated assignment to the breakdown of N-Cα of cysteine residue, which was reported previously by reference: Asakawa D, Smargiasso N, Quinton L, De Pauw E. (2013) Peptide backbone fragmentation initiated by side-chain loss at cysteine residue in matrix-assisted laser desorption/ionization in-source decay mass spectrometry. J Mass Spectrom. 48(3): 352-360.

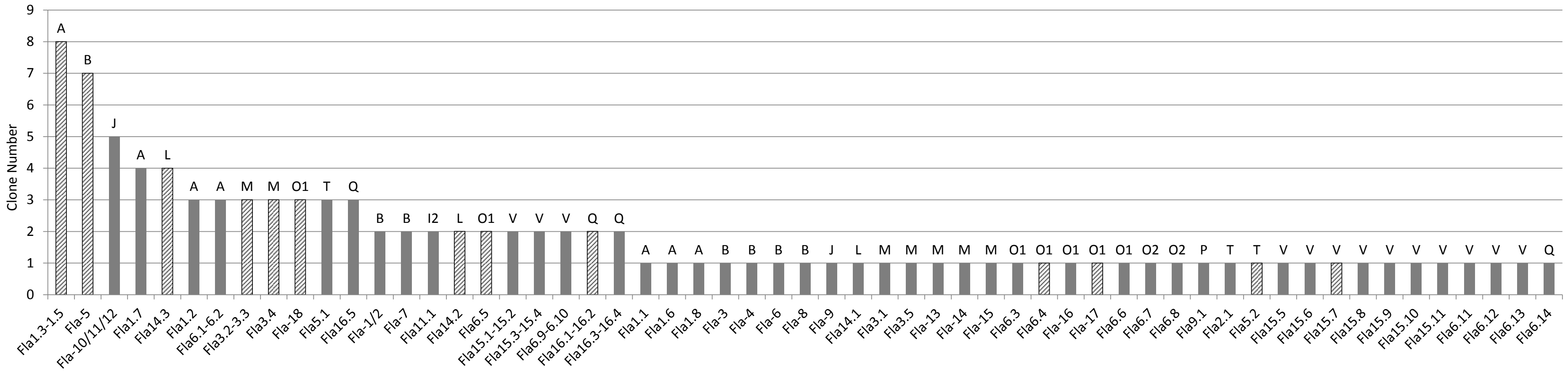


Figure S27. Clone numbers of 57 non-redundant mature peptides identified in the cDNA libraray of *C. flavidus*. The sequences with corresponding peptide components being identified in MS/MS analysis are shown in columns with meshy pattern.

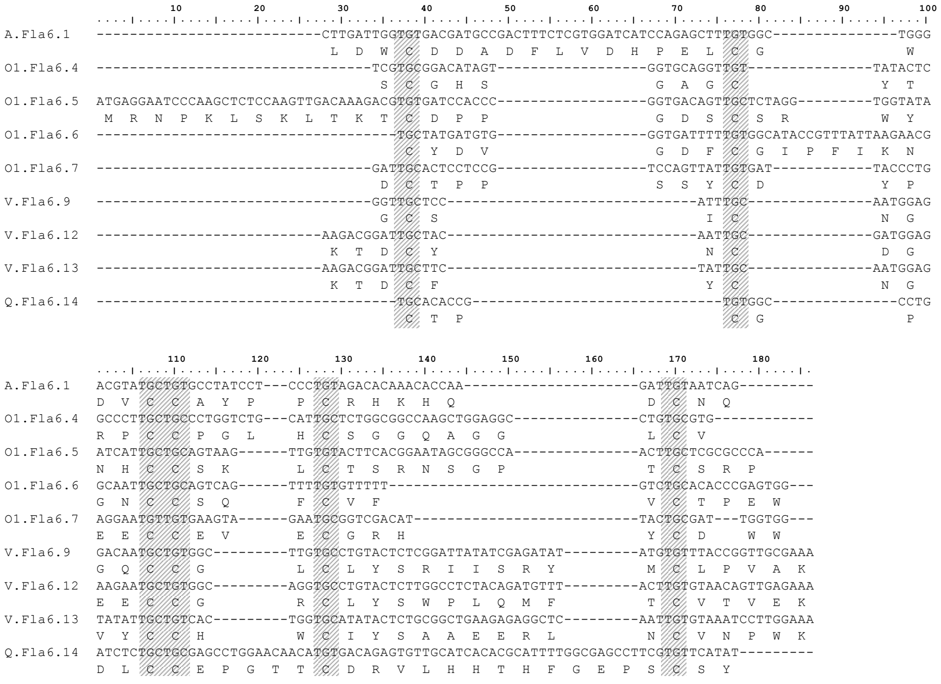


Figure S28. Alignment of conotoxins with cysteine framework VI/VII from different superfamilies of *C. flavidus*.