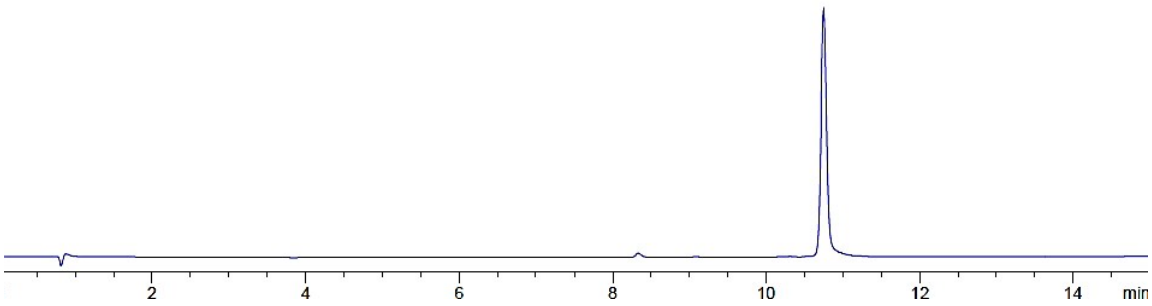
Supplementary Information

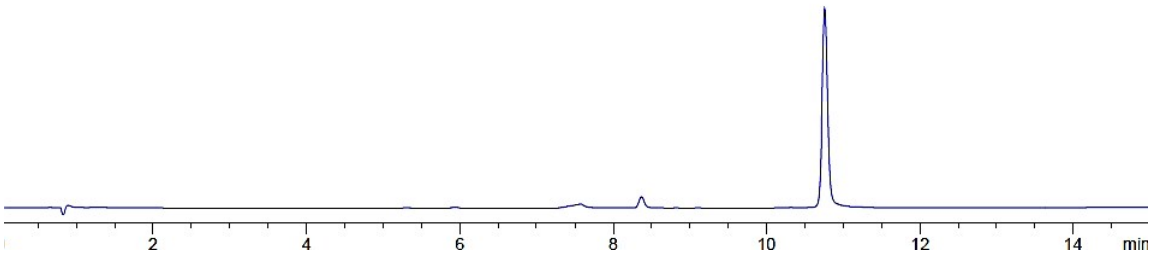
Cleaving Protected Peptides from 2-Chlorotrityl Chloride Resin. Moving Away from Dichloromethane

Mahama Alhassan,[1,2] Othman Al Musaimi,[1,2] Jonathan M. Collins,[3] Fernando Albericio,[1,4,5*] Beatriz G. de la Torre,[2*]

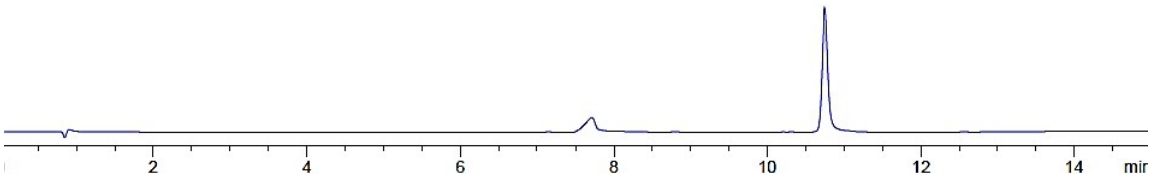
[1]Peptide Science Laboratory, School of Chemistry and Physics, University of KwaZulu-Natal, Durban 4001, South Africa; [2]KwaZulu-Natal Research Innovation and Sequencing Platform (KRISP), School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4041, South Africa; [3]CEM Corporation, 3100 Smith Farm Road, Matthews, North Carolina 28104, United States; [4]CIBER-BBN, Networking Centre on Bioengineering, Biomaterials and Nanomedicine, Barcelona Science Park, University of Barcelona, 08028 Barcelona, Spain; [5]Department of Organic Chemistry, University of Barcelona, 08028 Barcelona, Spain



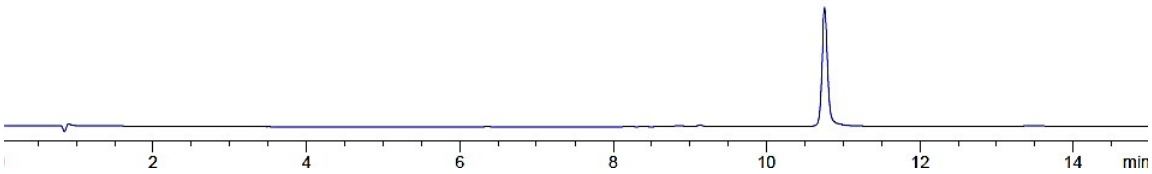
Supplementary Figure 1. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in DCM. 5−95% gradient elution in 15 min. λ = 300 nm. Mobile phase A: 0.1% TFA in H_2O; mobile phase B: 0.1% TFA in CH_3CN; Phenomenex Luna 5 µm C_5, (50 × 4.6) mm, 100 Å column.



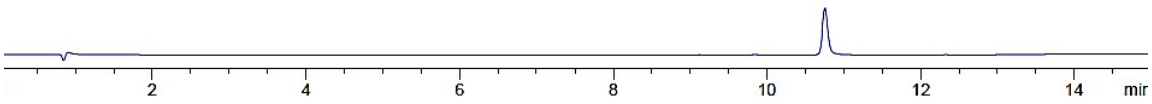
Supplementary Figure 2. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in anisole. See legend of Supplementary Figure 1 for chromatographic conditions.



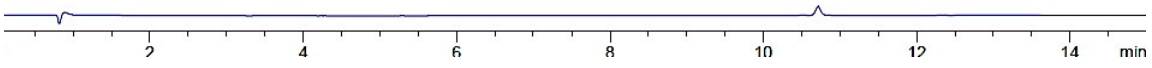
Supplementary Figure 3. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in 1,3-dimethoxy benzene. See legend of Supplementary Figure 1 for chromatographic conditions.



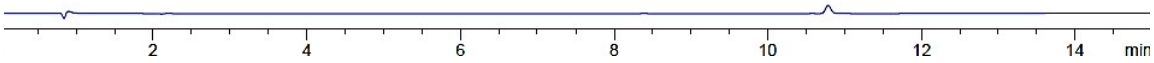
Supplementary Figure 4. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in toluene. See legend of Supplementary Figure 1 for chromatographic conditions.



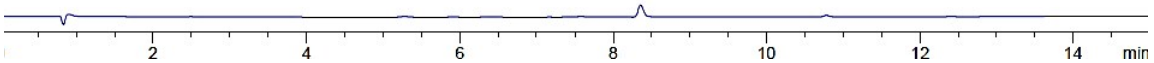
Supplementary Figure 5. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in EtOAc-CH_3CN (1:1). See legend of Supplementary Figure 1 for chromatographic conditions.



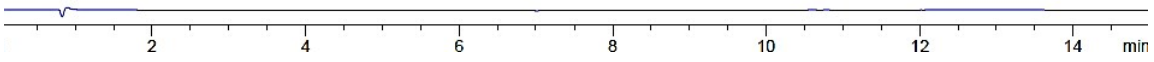
Supplementary Figure 6. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in 2-MeTHF. See legend of Supplementary Figure 1 for chromatographic conditions.



Supplementary Figure 7. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in GVL. See legend of Supplementary Figure 1 for chromatographic conditions.



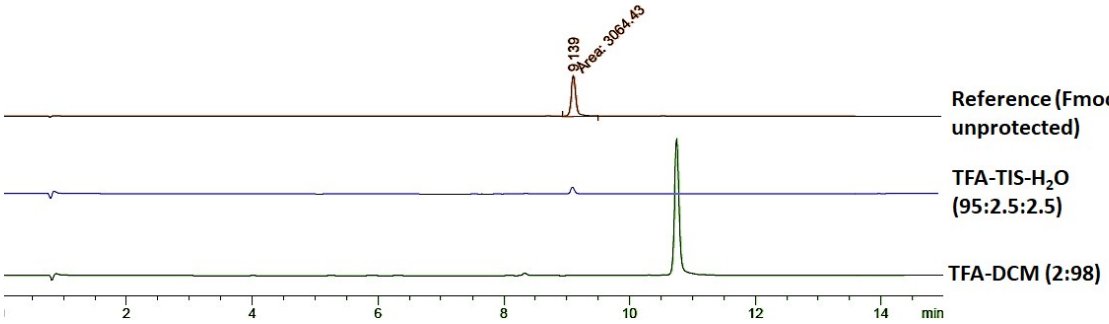
Supplementary Figure 8. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in Isoamyl acetate. See legend of Supplementary Figure 1 for chromatographic conditions.



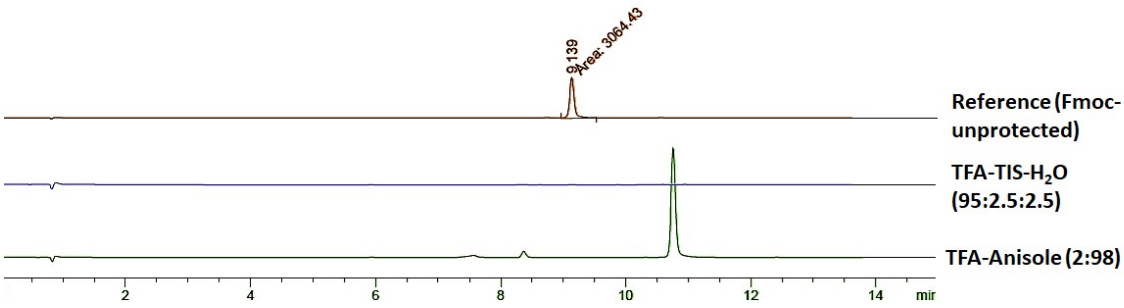
Supplementary Figure 9. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in Isobutyl acetate. See legend of Supplementary Figure 1 for chromatographic conditions.

Supplementary Figure 10. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in Cyrene. See legend of Supplementary Figure 1 for chromatographic conditions.

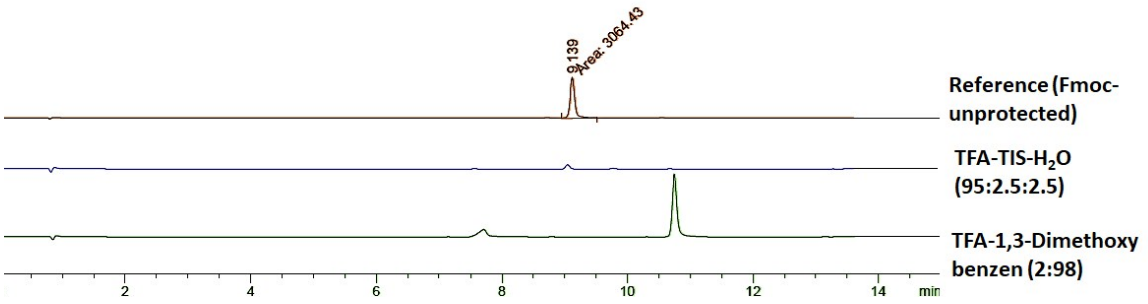
Supplementary Figure 11. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in NBP. See legend of Supplementary Figure 1 for chromatographic conditions.



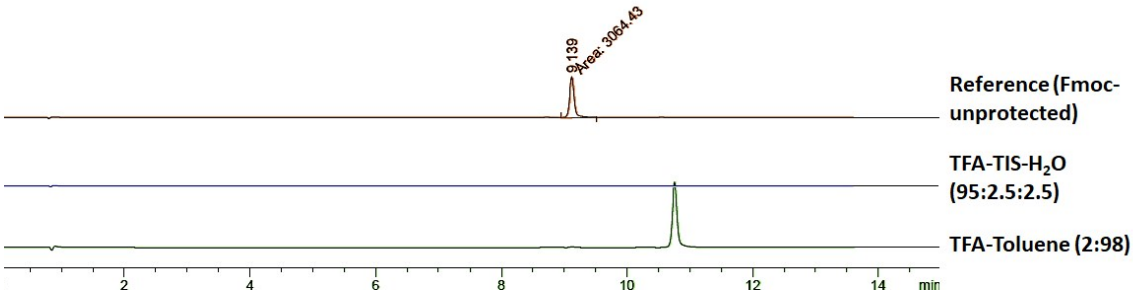
Supplementary Figure 12. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in DCM vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



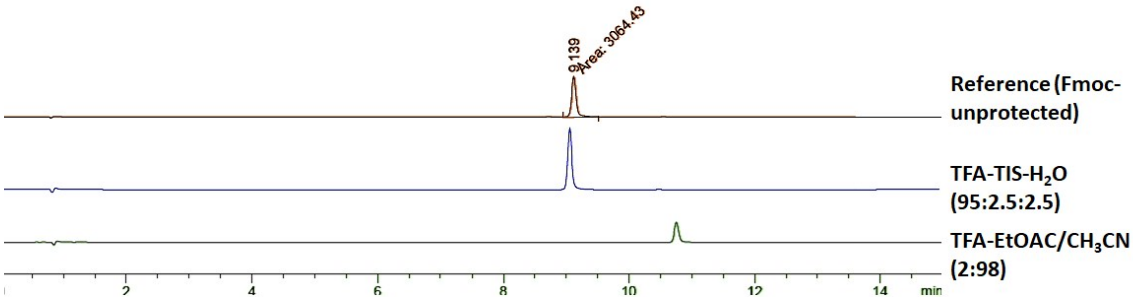
Supplementary Figure 13. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in anisole vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



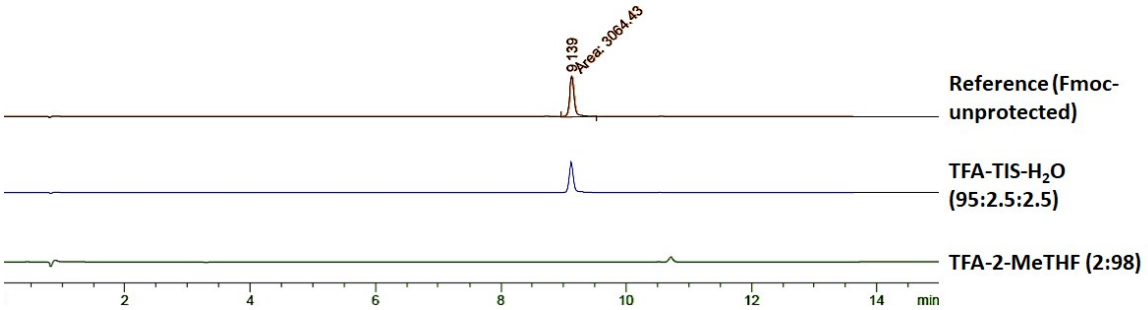
Supplementary Figure 14. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in 1,3-diemthoxy benzene vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



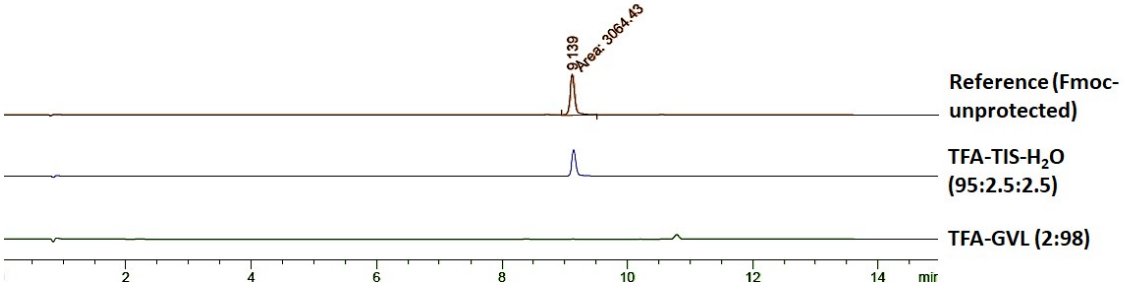
Supplementary Figure 15. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in toluene vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



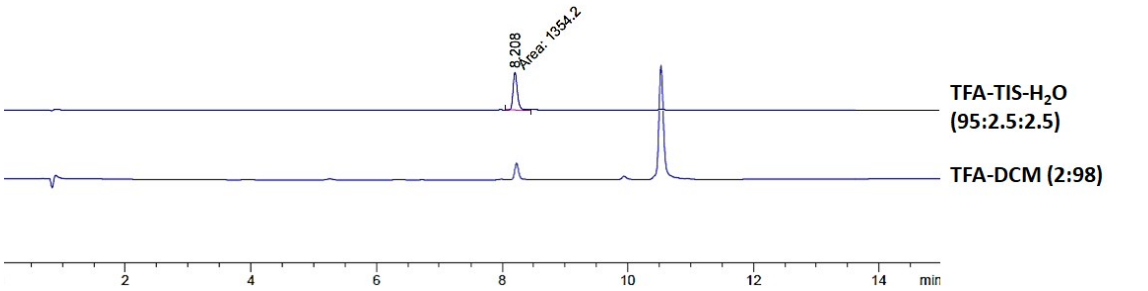
Supplementary Figure 16. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in EtOAc-CH_3CN (1:1) vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



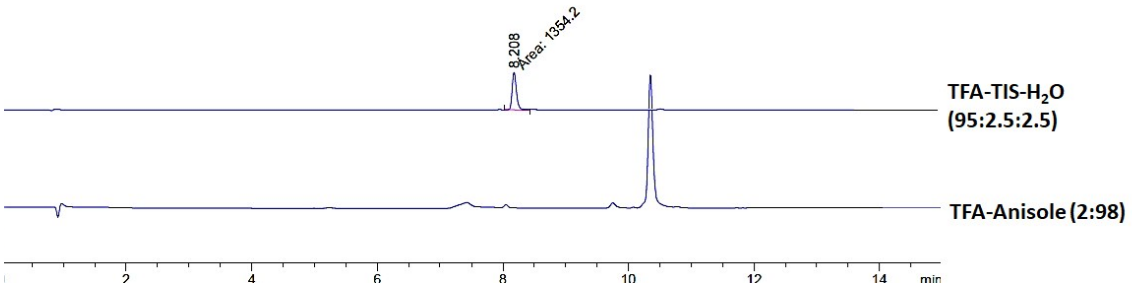
Supplementary Figure 17. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in 2-MeTHF vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



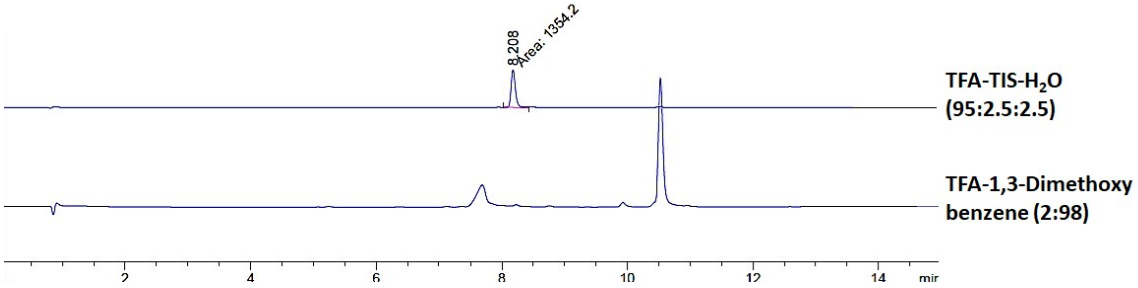
Supplementary Figure 18. Cleaved Fmoc-Tyr(*t*Bu)-Gly-Gly-Phe-Leu-OH using 2% TFA in GVL vs. cleaved Fmoc-Tyr-Gly-Gly-Phe-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



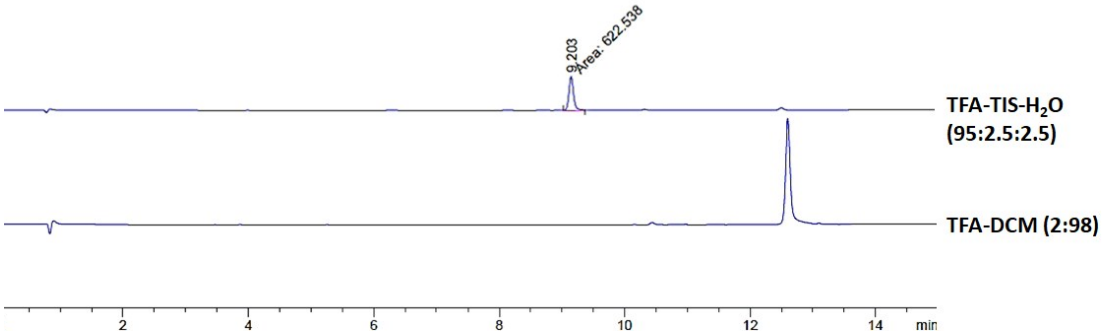
Supplementary Figure 19. Cleaved Fmoc-Leu-His(Trt)-Leu-OH using 2% TFA in DCM vs. cleaved Fmoc-Leu-His-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



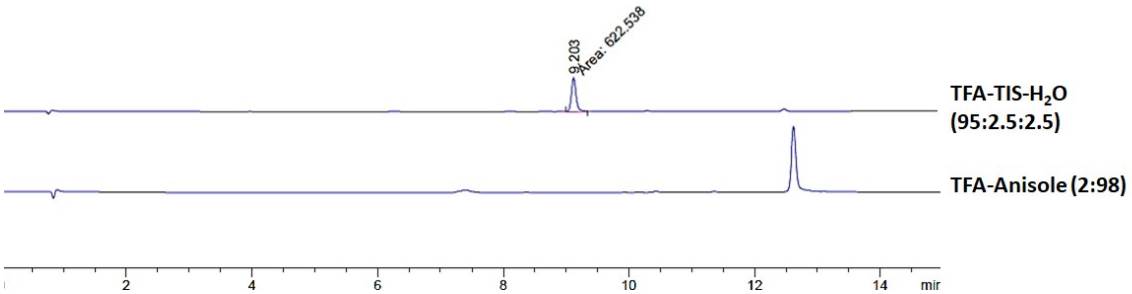
Supplementary Figure 20. Cleaved Fmoc-Leu-His(Trt)-Leu-OH using 2% TFA in anisole vs. cleaved Fmoc- Leu-His-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



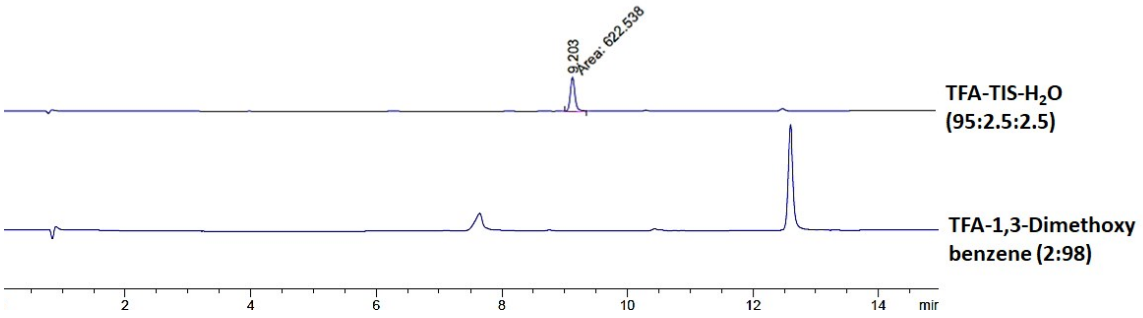
Supplementary Figure 21. Cleaved Fmoc-Leu-His(Trt)-Leu-OH using 2% TFA in 1,3-dimethoxy benzene vs. cleaved Fmoc- Leu-His-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



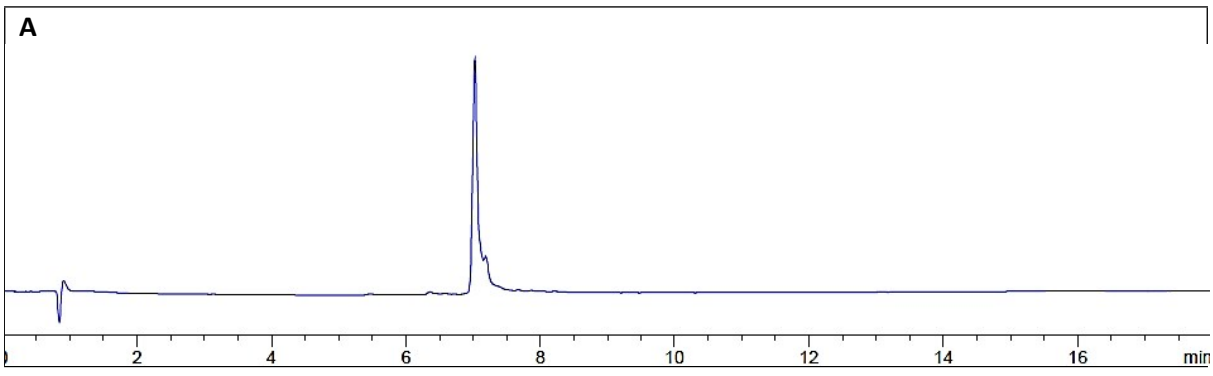
Supplementary Figure 22. Cleaved Fmoc-Leu-Asn(Trt)-Leu-OH using 2% TFA in DCM vs. cleaved Fmoc- Leu-Asn-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.

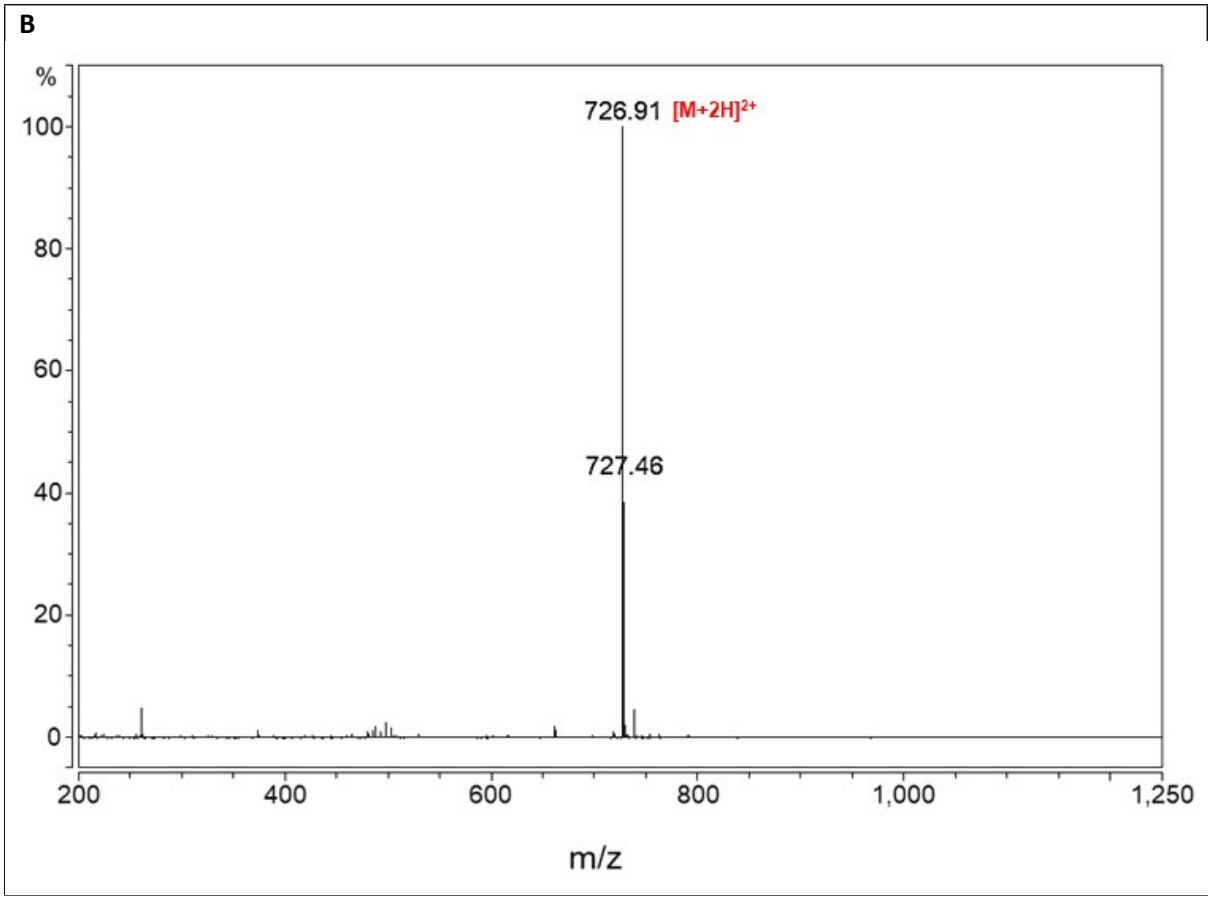


Supplementary Figure 23. Cleaved Fmoc-Leu-Asn(Trt)-Leu-OH using 2% TFA in anisole vs. cleaved Fmoc- Leu-Asn-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.

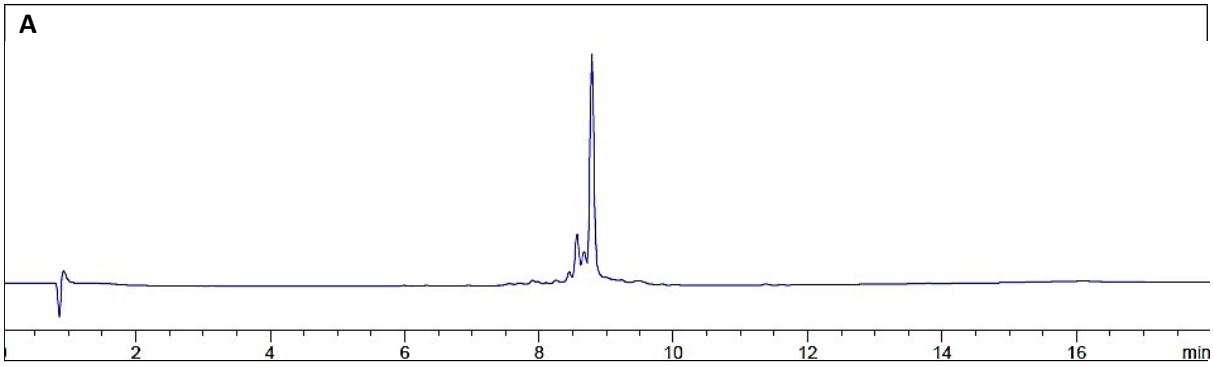


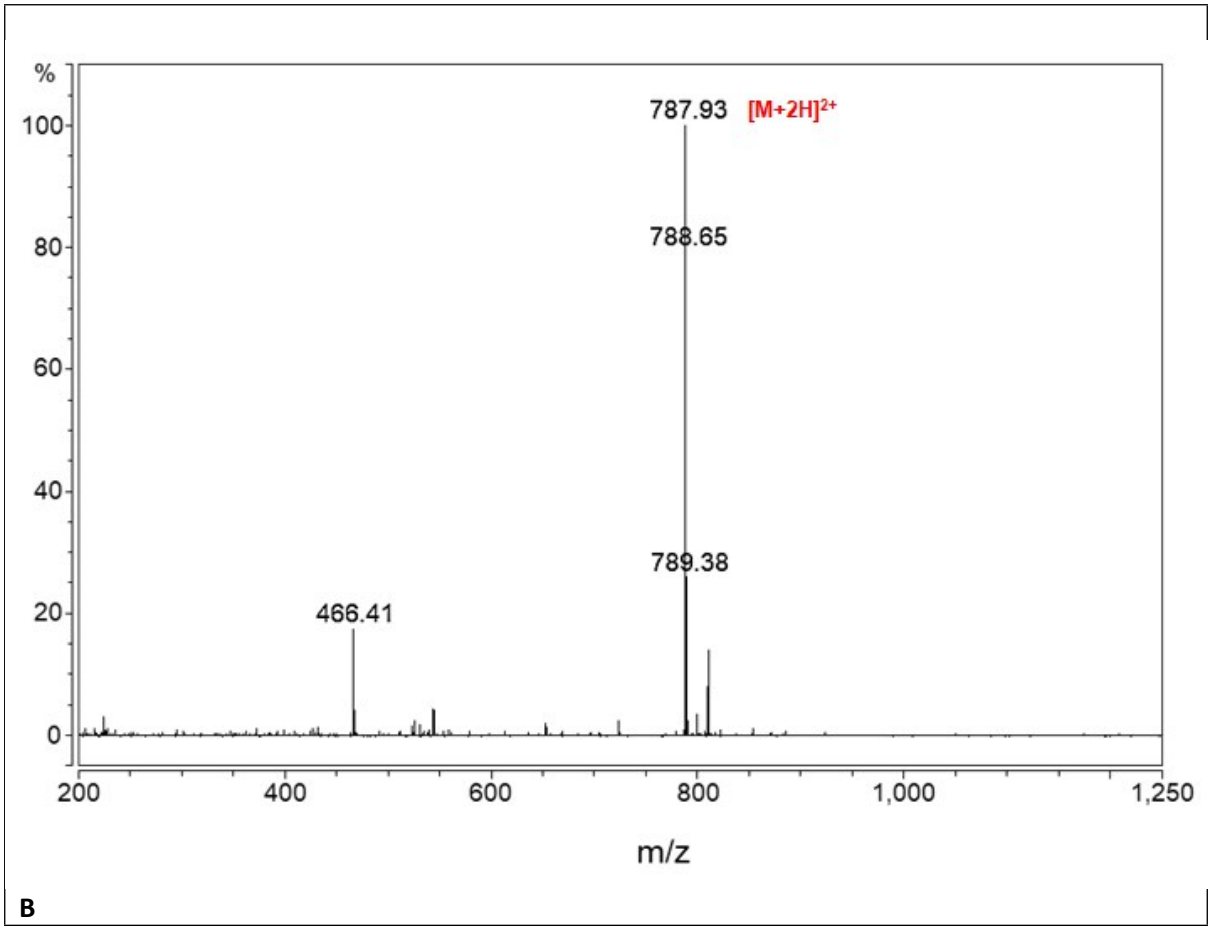
Supplementary Figure 24. Cleaved Fmoc-Leu-Asn(Trt)-Leu-OH using 2% TFA in 1,3-dimethoxy benzene vs. cleaved Fmoc- Leu-Asn-Leu -OH using TFA-TIS-H_2O (95:2.5:2.5) (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



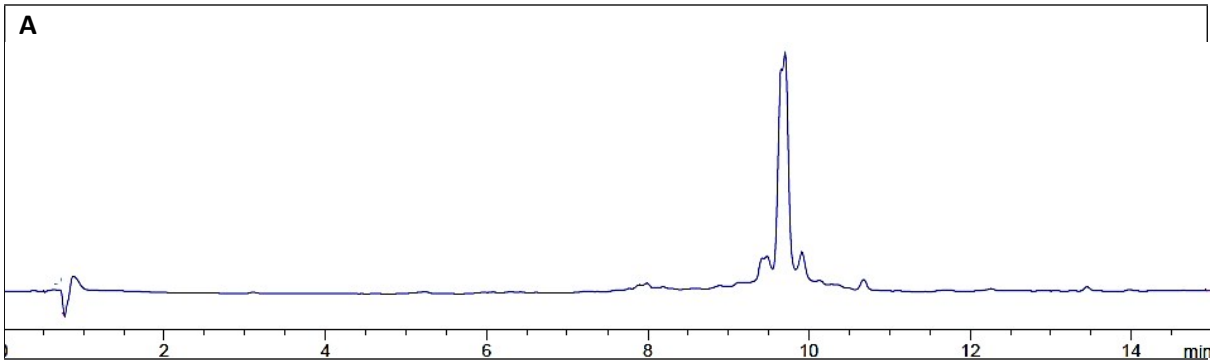


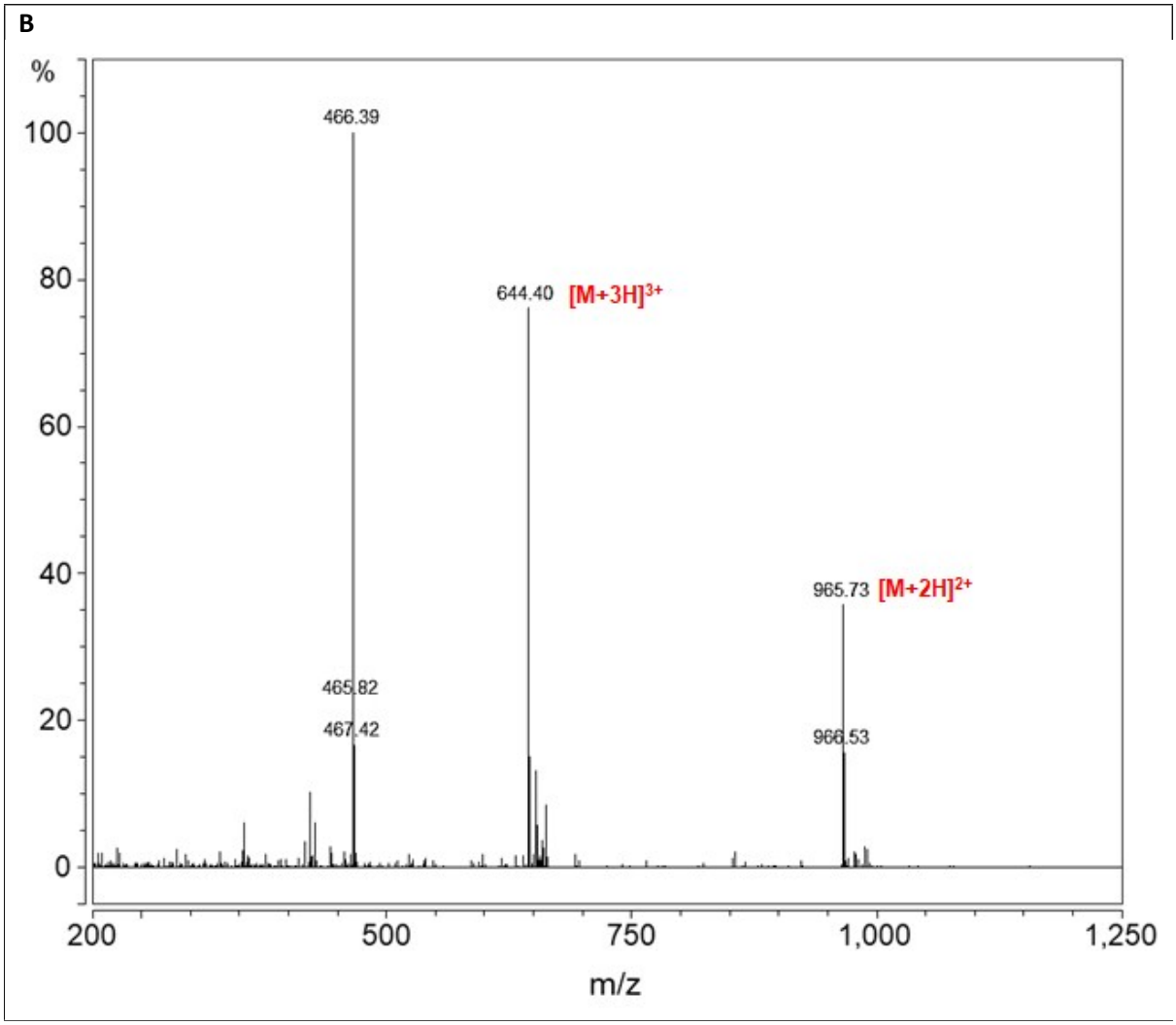
Supplementary Figure 25. **A.** Cleaved Fmoc-Glu-Lys-Asn-Glu--Asn-Glu-Leu-Leu-Glu-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5), see legend of Supplementary Figure 1 for chromatographic conditions. **B.** Mass for Fmoc-Glu-Lys-Asn-Glu--Asn-Glu-Leu-Leu-Glu-Leu-OH.



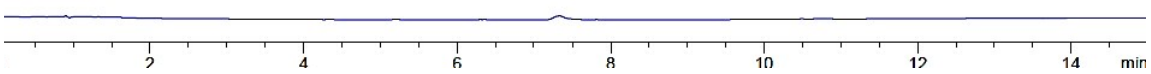


Supplementary Figure 26. **A.** Cleaved Fmoc-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5), see legend of Supplementary Figure 1 for chromatographic conditions. **B.** Mass for Fmoc-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH.

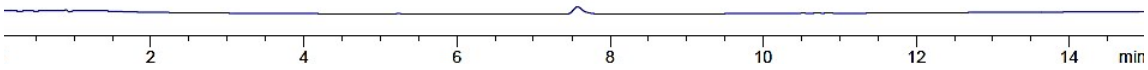




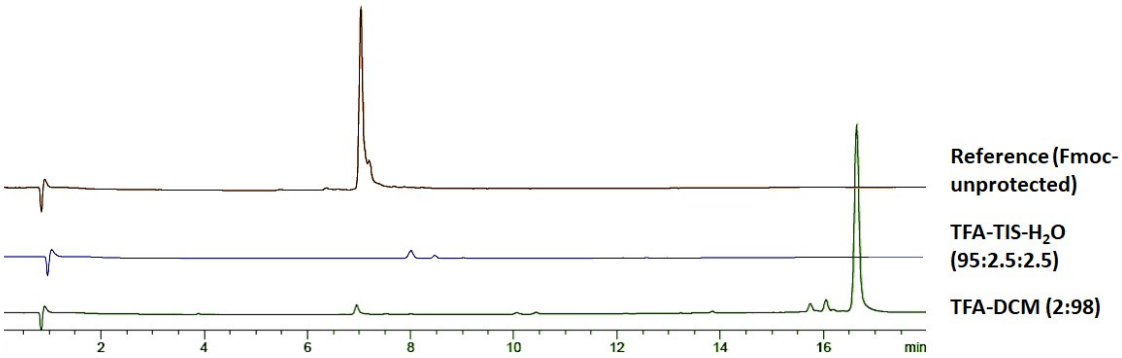
Supplementary Figure 27. **A.** Cleaved Fmoc-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5), see legend of Supplementary Figure 1 for chromatographic conditions. **B.** Mass for Fmoc-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH.



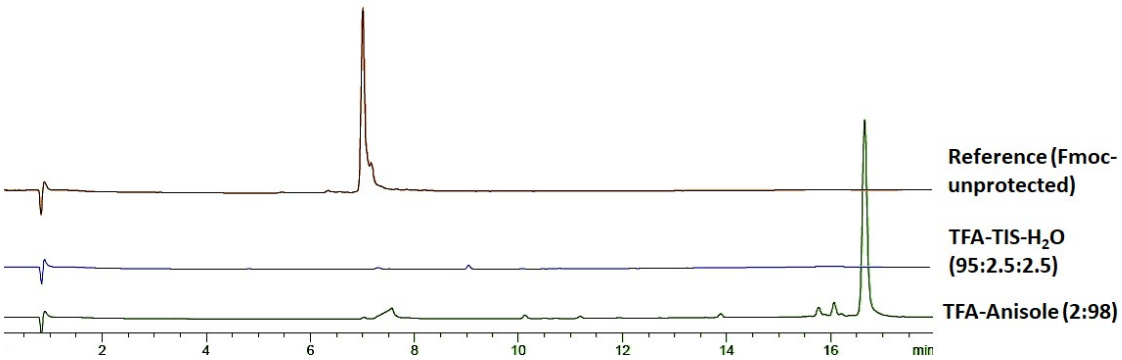
Supplementary Figure 28. Supernatant injection (2% TFA in anisole + H_2O) after precipitating the peptide over water. See legend of Supplementary Figure 1 for chromatographic conditions.



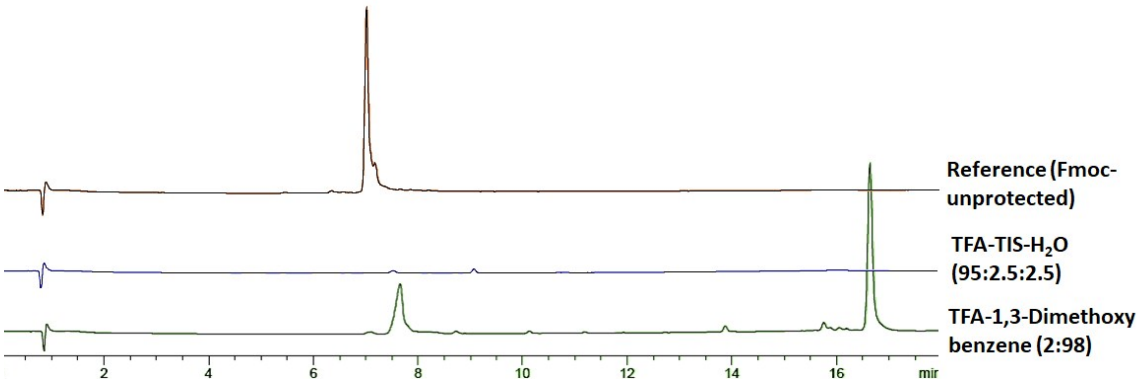
Supplementary Figure 29. Supernatant injection (2% TFA in 1,3-dimethoxy benzene + H_2O) after precipitating the peptide over water. See legend of Supplementary Figure 1 for chromatographic conditions.



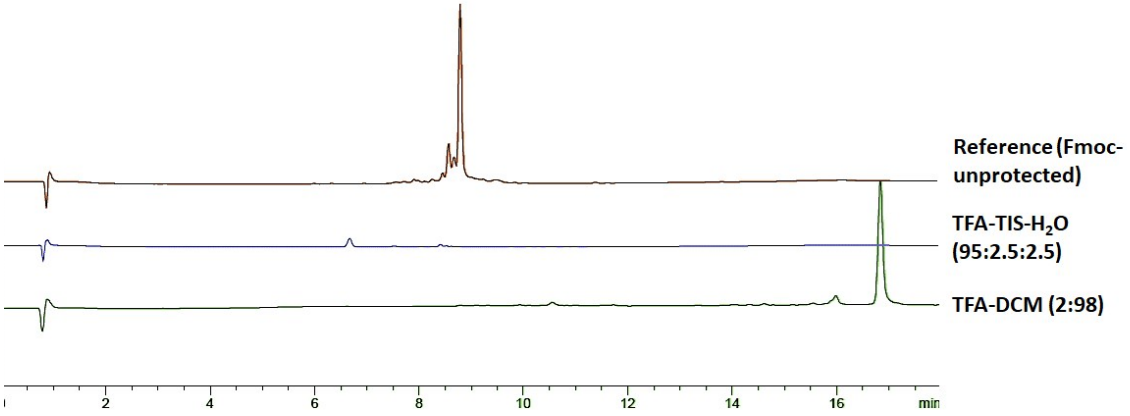
Supplementary Figure 30. Cleaved Fmoc-Glu(*t*Bu)-Lys(Boc)-Asn(Trt)-Glu-(*t*Bu)-Asn(Trt)-Glu(*t*Bu)-Leu-Leu-Glu-(*t*Bu)-Leu-OH using 2% TFA in DCM vs. cleaved Fmoc-Glu-Lys-Asn-Glu--Asn-Glu-Leu-Leu-Glu-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



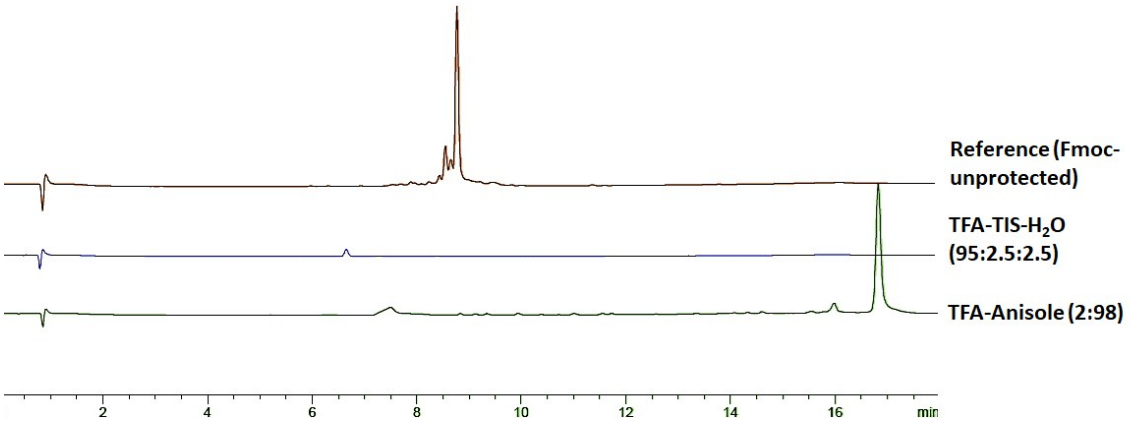
Supplementary Figure 31. Cleaved Fmoc-Glu(*t*Bu)-Lys(Boc)-Asn(Trt)-Glu-(*t*Bu)-Asn(Trt)-Glu(*t*Bu)-Leu-Leu-Glu-(*t*Bu)-Leu-OH using 2% TFA in anisole vs. cleaved Fmoc-Glu-Lys-Asn-Glu--Asn-Glu-Leu-Leu-Glu-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



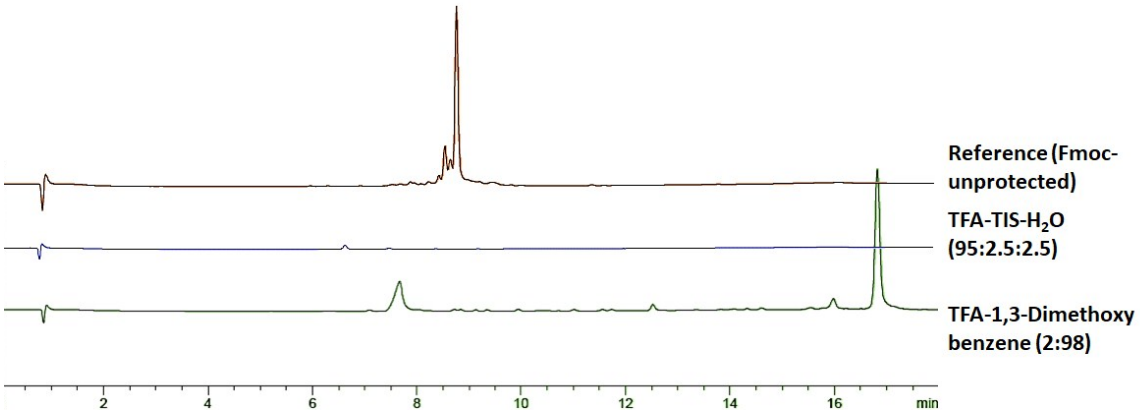
Supplementary Figure 32. Cleaved Fmoc-Glu(*t*Bu)-Lys(Boc)-Asn(Trt)-Glu-(*t*Bu)-Asn(Trt)-Glu(*t*Bu)-Leu-Leu-Glu-(*t*Bu)-Leu-OH using 2% TFA in 1,3-dimethoxy benzene vs. cleaved Fmoc-Glu-Lys-Asn-Glu-Asn-Glu-Leu-Leu-Glu-Leu-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



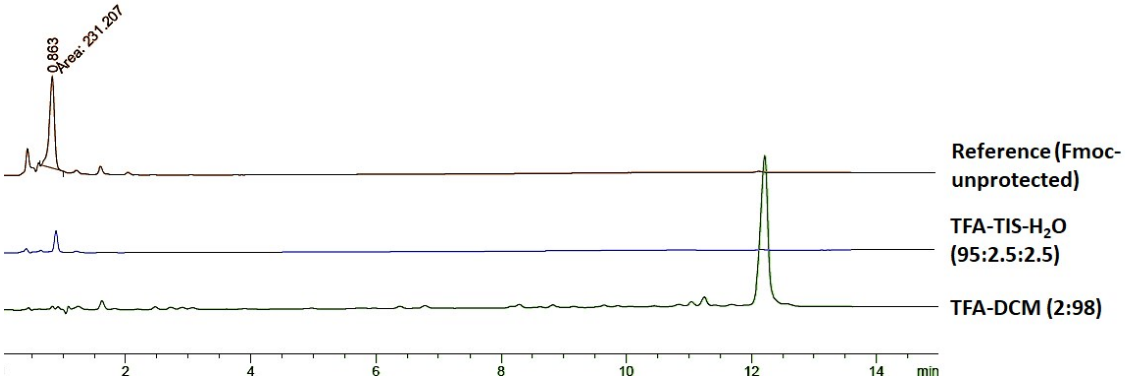
Supplementary Figure 33. Cleaved Fmoc-Asp(*t*Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*Bu)-Leu-Trp(Boc)-Asn(Trt)-Trp(Boc)-Phe-OH using 2% TFA in DCM vs. cleaved Fmoc-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



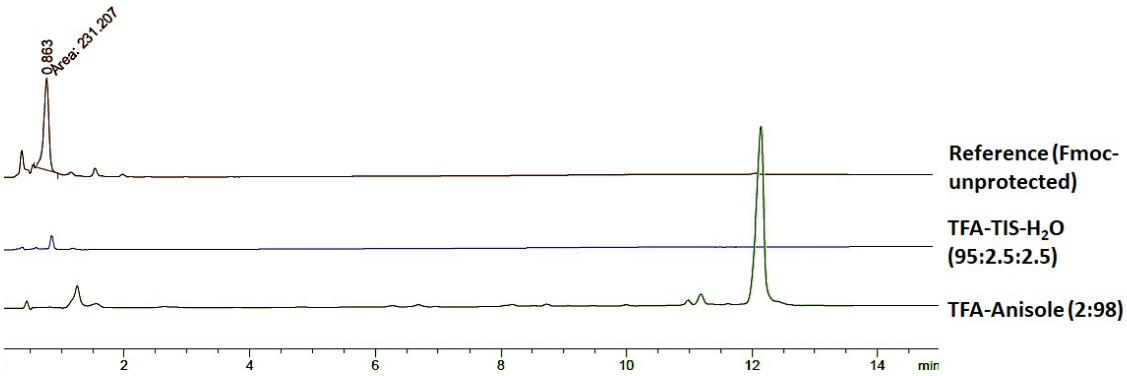
Supplementary Figure 34. Cleaved Fmoc-Asp(*t*Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*Bu)-Leu-Trp(Boc)-Asn(Trt)-Trp(Boc)-Phe-OH using 2% TFA in anisole vs. cleaved Fmoc-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



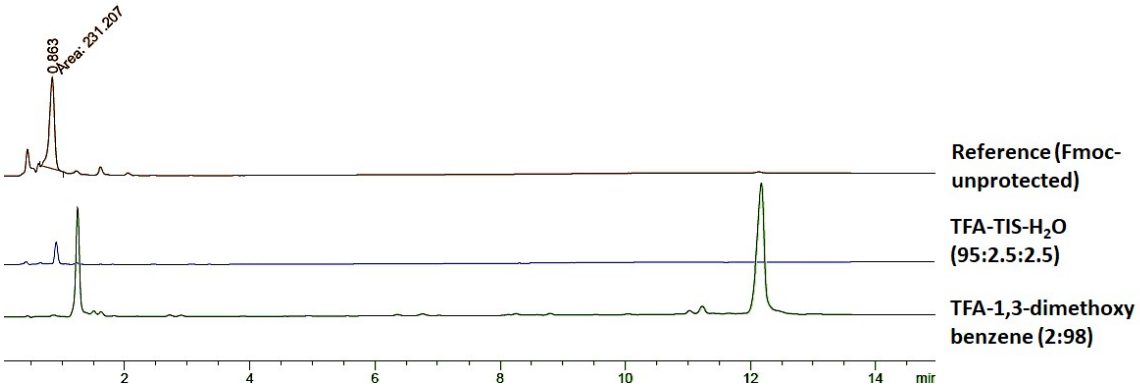
Supplementary Figure 35. Cleaved Fmoc-Asp(*t*Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*Bu)-Leu-Trp(Boc)-Asn(Trt)-Trp(Boc)-Phe-OH using 2% TFA in 1,3-dimethoxy benzene vs. cleaved Fmoc-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 1 for chromatographic conditions.



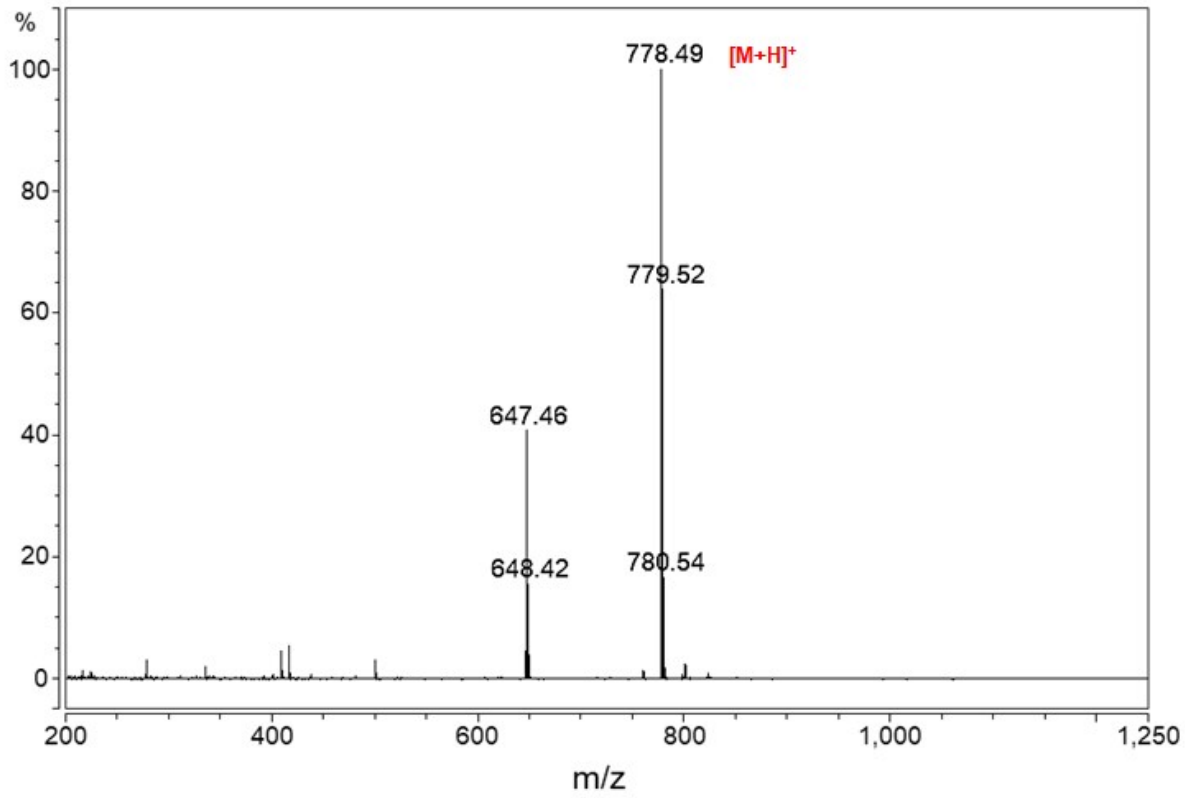
Supplementary Figure 36. Cleaved Fmoc-Leu-Glu-(*t*Bu)-Leu-Asp(*t*Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*Bu)-Leu-Trp(Boc)-Asn(Trt)-Trp(Boc)-Phe-OH using 2% TFA in DCM vs. cleaved Fmoc-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). Gradient elution: 60−100% (B) in 10 min and then extra 10 min at 100%.



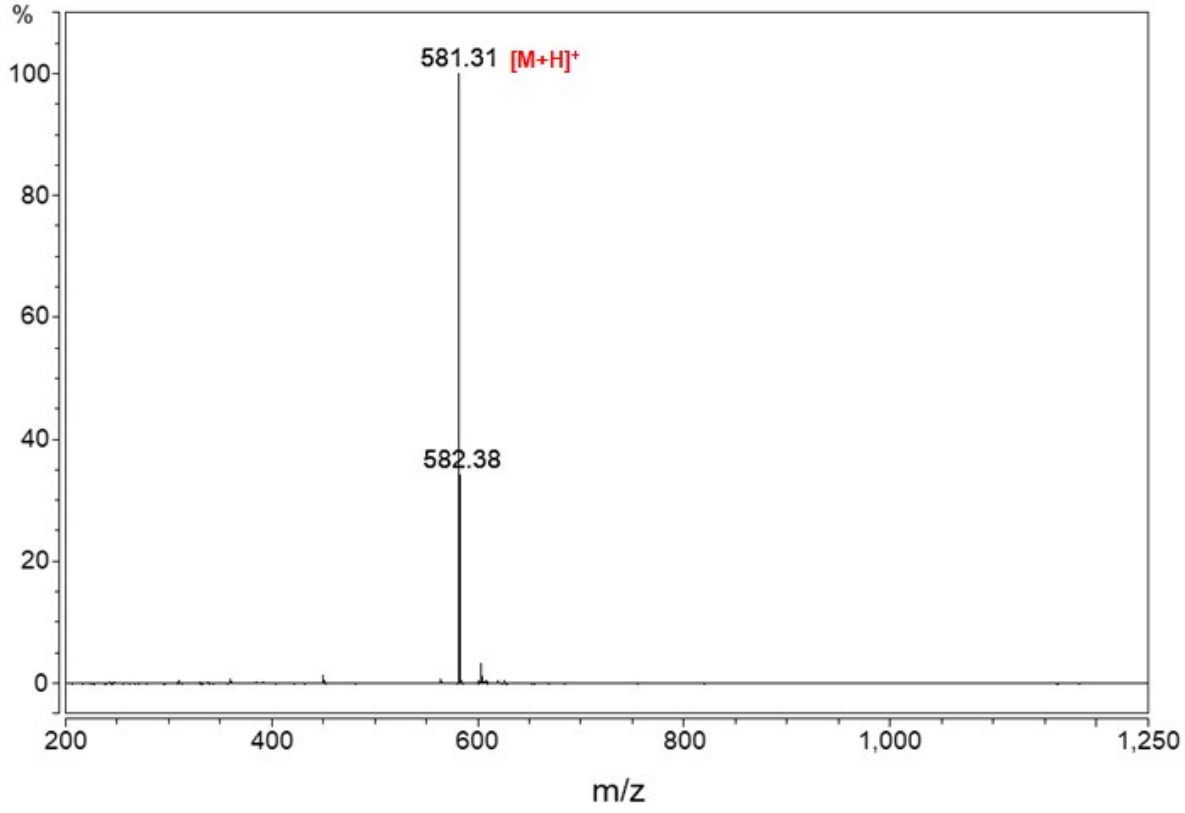
Supplementary Figure 37. Cleaved Fmoc-Leu-Glu-(*t*Bu)-Leu-Asp(*t*Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*Bu)-Leu-Trp(Boc)-Asn(Trt)-Trp(Boc)-Phe-OH using 2% TFA in anisole vs. cleaved Fmoc-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 36 for chromatographic conditions.



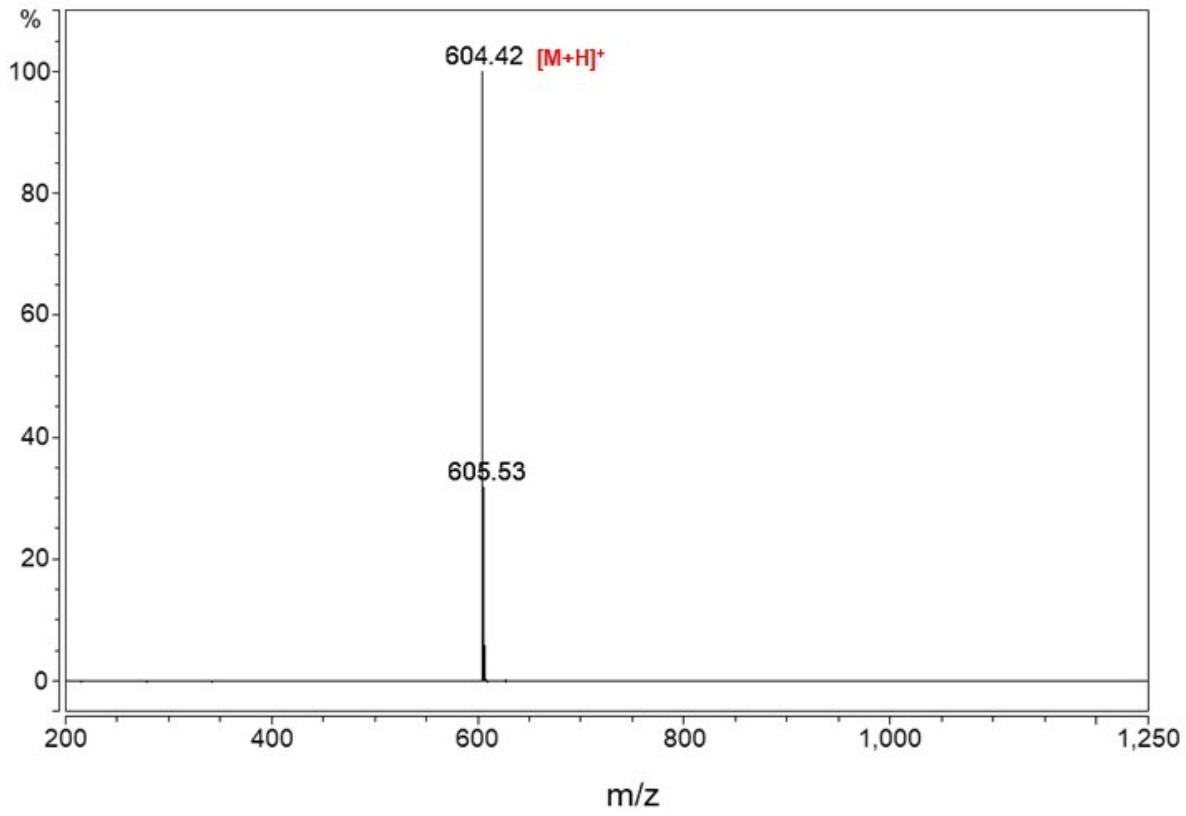
Supplementary Figure 38. Cleaved Fmoc-Leu-Glu-(*t*Bu)-Leu-Asp(*t*Bu)-Lys(Boc)-Trp(Boc)-Ala-Ser(*t*Bu)-Leu-Trp(Boc)-Asn(Trt)-Trp(Boc)-Phe-OH using 2% TFA in 1,3-dimethoxy benzene vs. cleaved Fmoc-Leu-Glu-Leu-Asp-Lys-Trp-Ala-Ser-Leu-Trp-Asn-Trp-Phe-OH using TFA-TIS-H_2O (95:2.5:2.5). (Protected vs. unprotected). See legend of Supplementary Figure 36 for chromatographic conditions.



Supplementary Figure 39. Mass for Fmoc-Tyr-Gly-Gly-Phe-Leu-OH.



Supplementary Figure 40. Mass for Fmoc-Leu-Asn-Leu-OH.



Supplementary Figure 41. Mass for Fmoc-Leu-His-Leu-OH.