Towards Green, Scalable Peptide Synthesis: Leveraging DEG-Crosslinked Polystyrene Resins to Overcome Hydrophobicity Challenges

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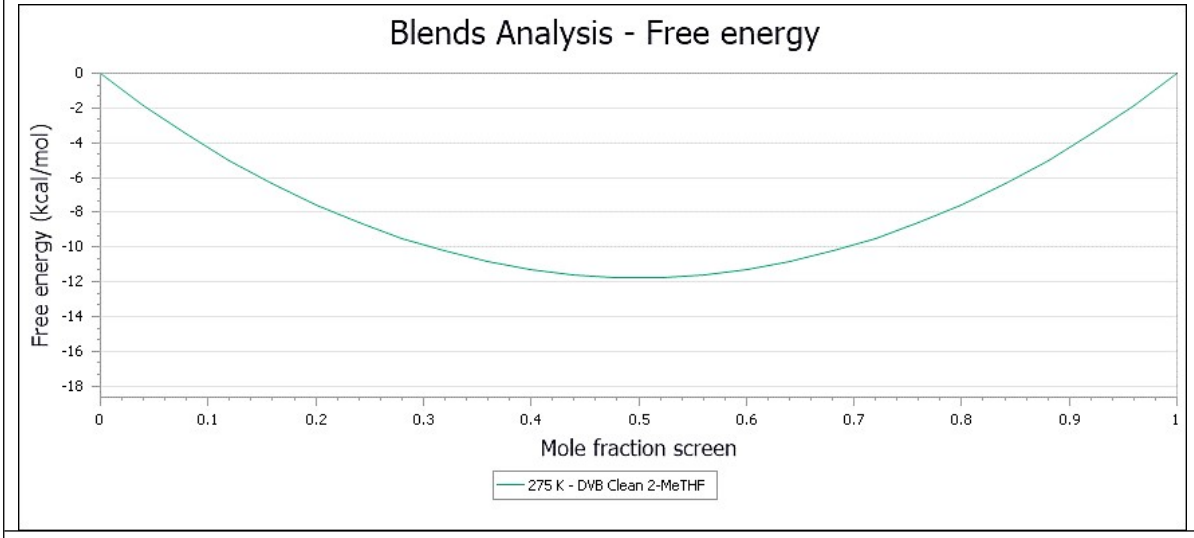
Supplemental Tables

Supplemental Table 1. Peptides investigated in this study.

Peptide	Sequence	Formula	Mass
Leu-Enkephalin	H-Tyr-Gly-Gly-Ohe-Leu-OH	$C_{28}H_{37}N_5O_7$	555.27
β (34-42)	H-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-OH	$C_{39}H_{71}N_9O_{10}S$	858.11
ACP	H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH	$C_{47}H_{74}N_{12}O_{16}$	1063.18
Aib-ACP	H-Val-Gln-Aib-Aib-Ile-Asp-Tyr-Ile-Asn-Gly-OH	$C_{49}H_{78}N_{12}O_{16}$	1091.23
JR	H-Trp-Phe-Thr-Thr-Leu-Ile-Ser-Thr-Ile-Met-OH	$C_{58}H_{89}N_{11}O_{15}S$	1212.47
ABRF 1992	H-Gly-Val-Arg-Gly-Asp-Lys-Gly-Asn-Pro-Gly-Trp-Pro-Gly-Ala-Pro-Tyr-NH_2	$C_{73}H_{106}N_{22}O_{21}$	1627.78
ABC	H-Val-Tyr-Trp-Thr-Ser-Pro-Phe-Met-Lys-Leu-Ile-His-Glu-Gln-Cys-Asn-Arg-Ala-Asp-Gln-OH	$C_{110}H_{164}N_{30}O_{31}S_2$	2466.82
Thymosin	H-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-NH_2	$C_{127}H_{213}N_{33}O_{54}$	3066.28

Supplemental Figures

A



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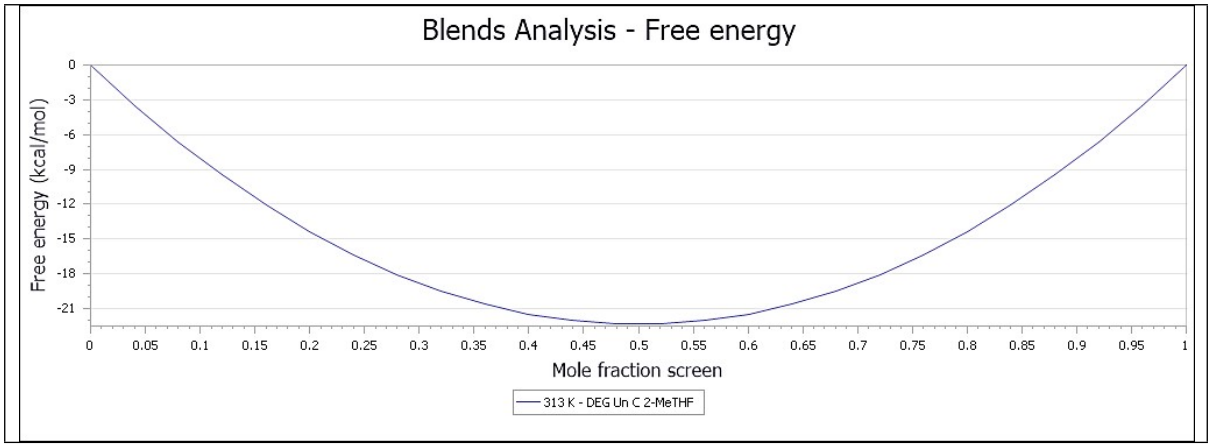
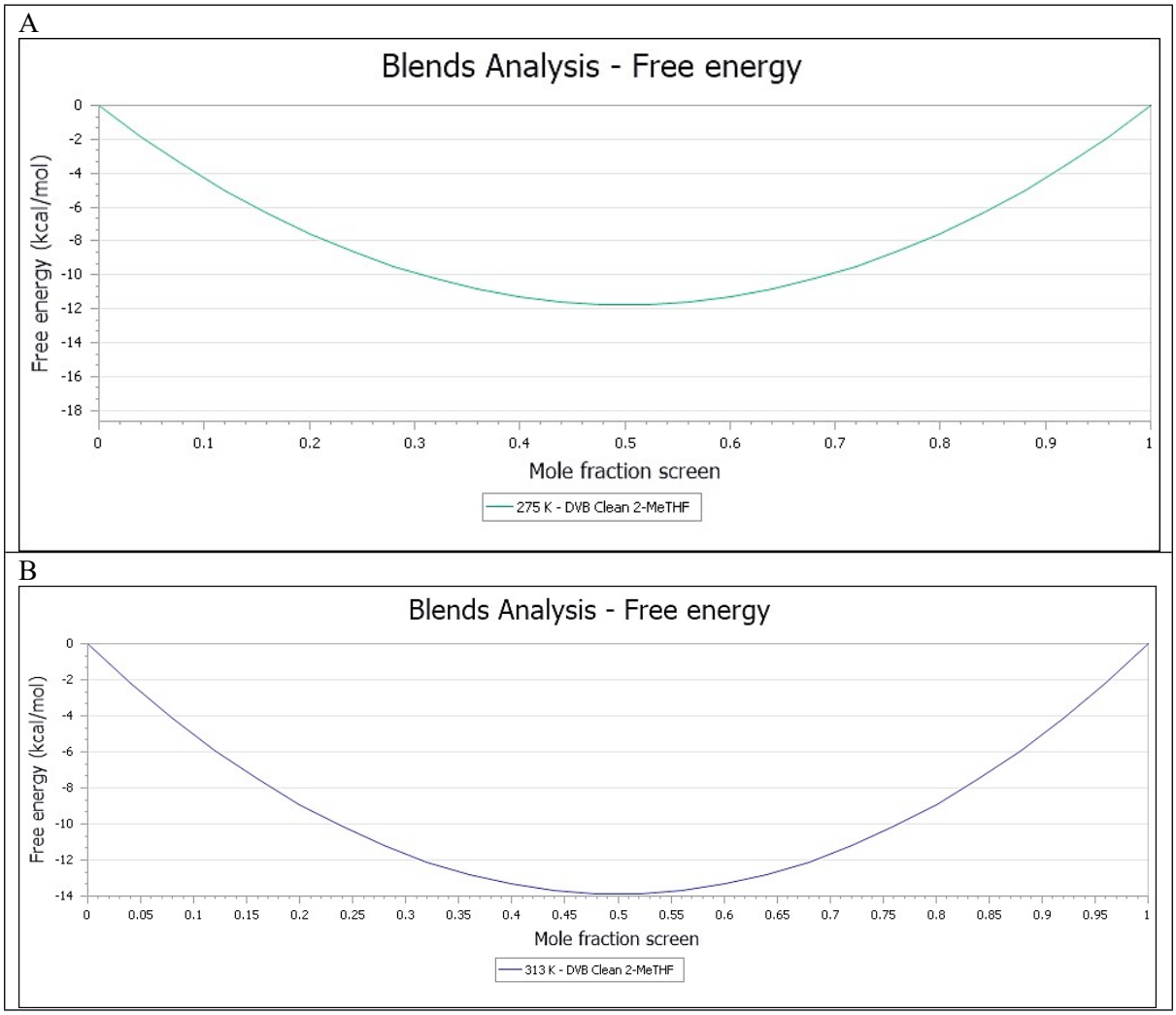
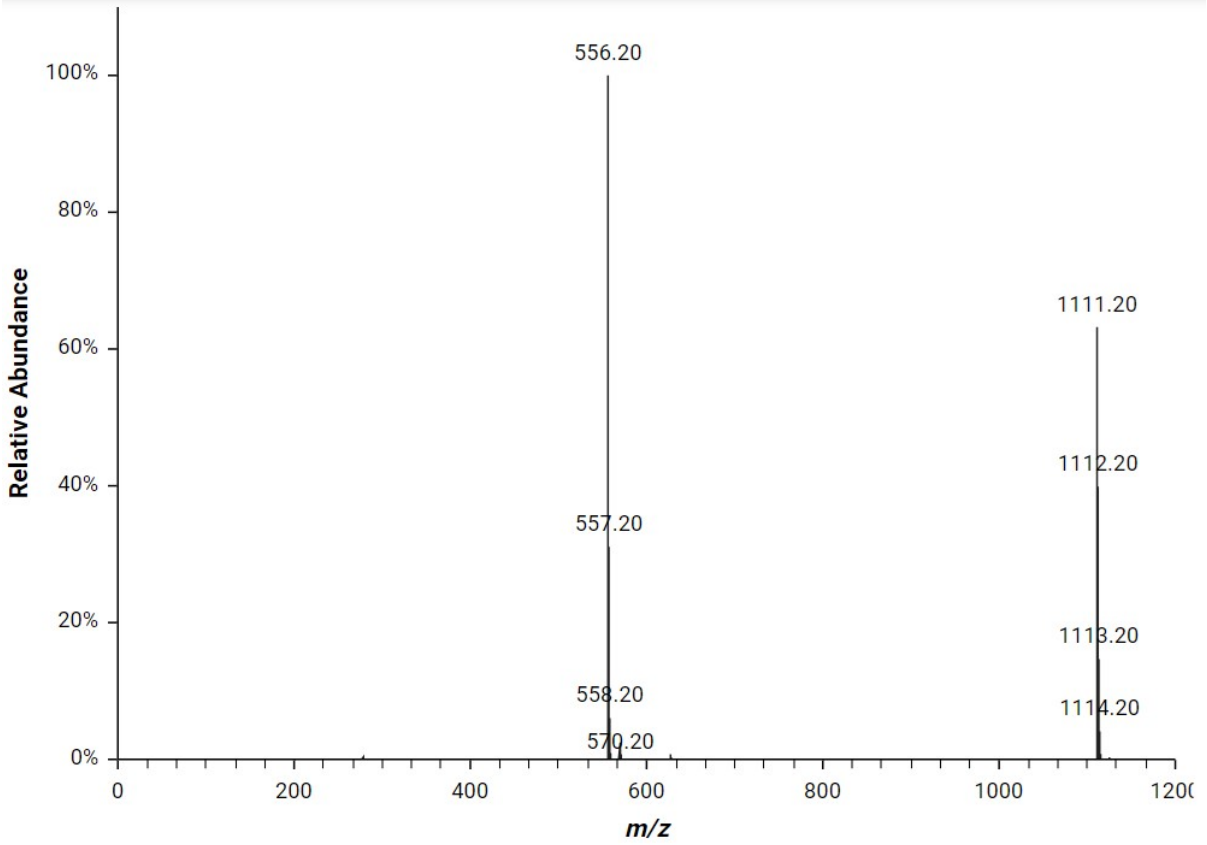


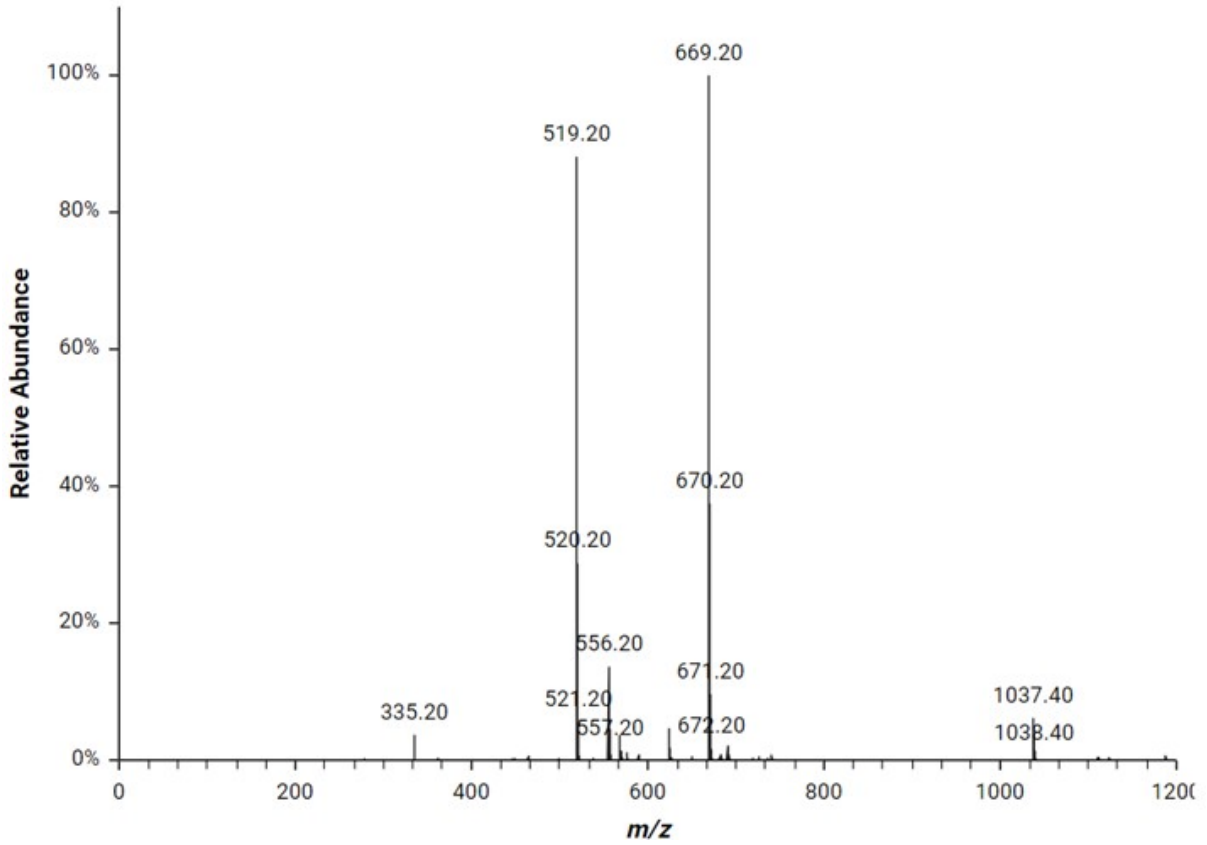
Supplementary Figure 1. Free energy of DEG-PS. A. at RT. B. At 40 °C.



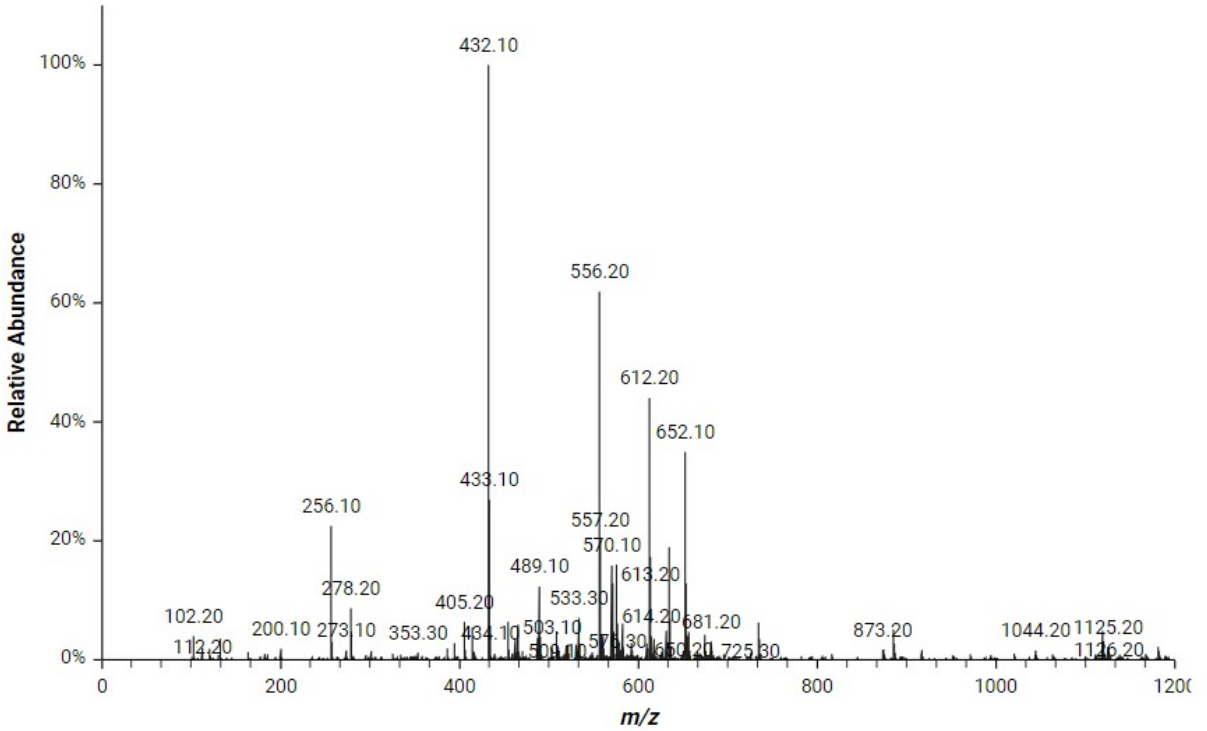
Supplementary Figure 2. Free energy of DVB-PS. A. at RT. B. At 40 °C.



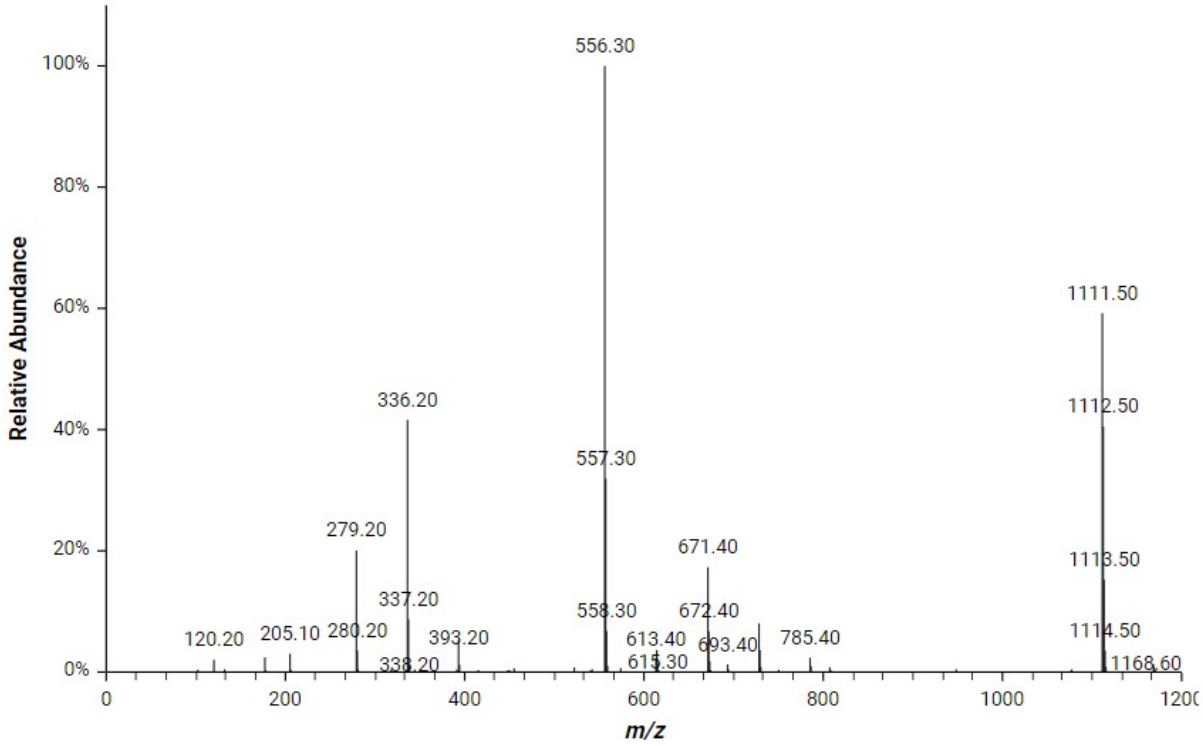
Supplementary Figure 3. Mass spectrum of crude Leu-Enkephalin peptide on DVB-PS using DMF at RT. Calculated for $C_{28}H_{37}N_5O_7$: 555.6 Da, found: 556.2 $[M + H]^+$, 1111.2 $[2M + H]^+$ (non-covalent dimer). Experimental conditions 1.



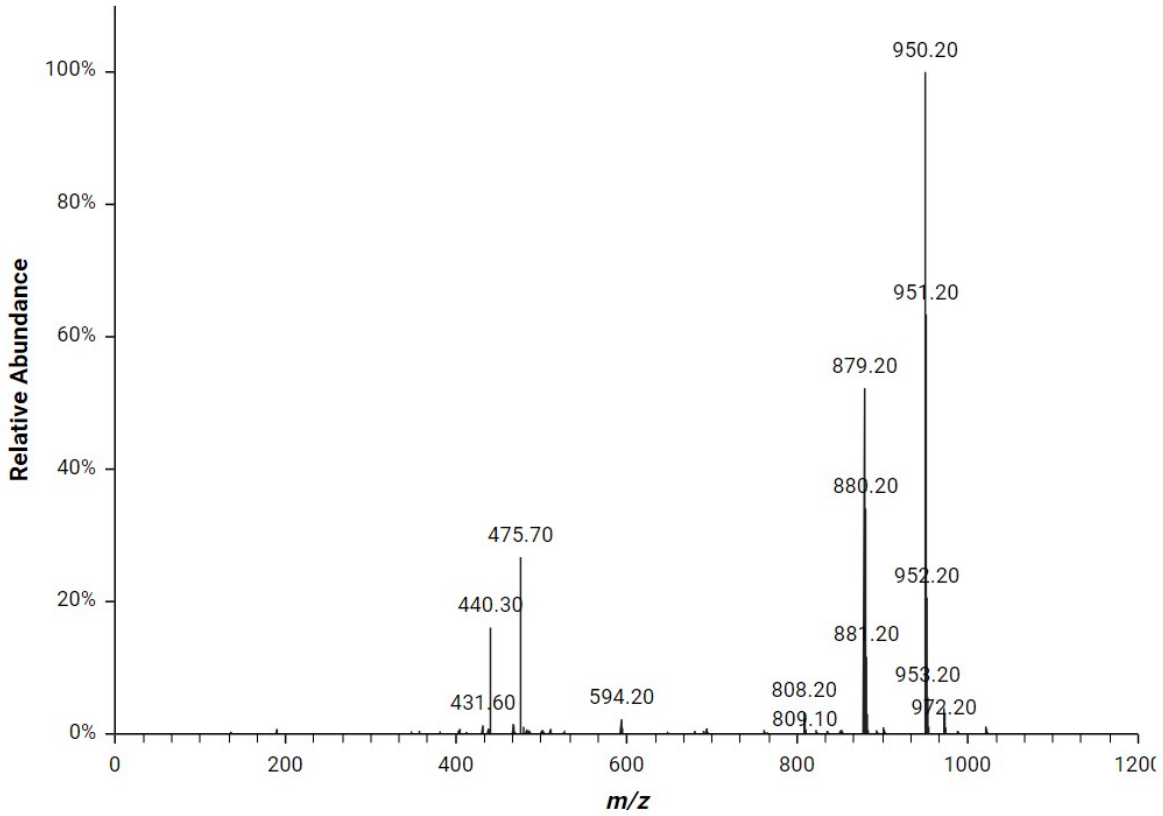
Supplementary Figure 4. Mass spectrum of crude double-incorporated Leu of the Leu-Enkephalin synthesis on DVB-PS using DMF at RT. Calculated for $C_{34}H_{48}N_6O_8$: 668.8 Da, found: 669.2 $[M + H]^+$, 1037.4 $[2M + H]^{2+}$ (non-covalent dimer). Experimental conditions 1.



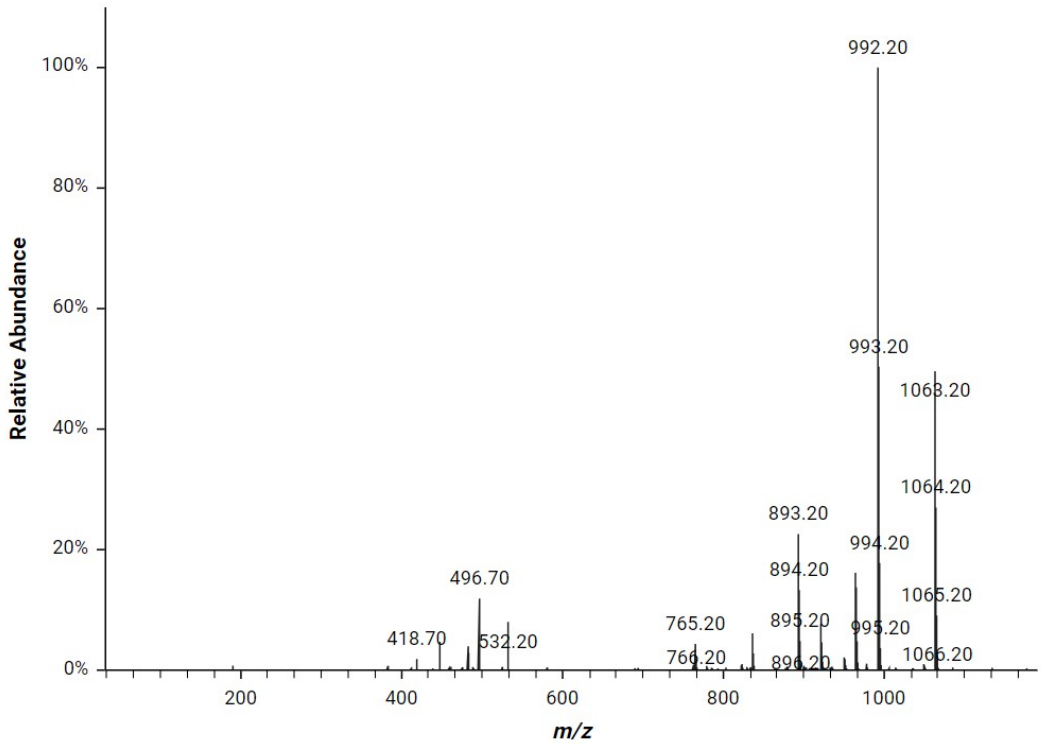
Supplementary Figure 5. Mass spectrum of Des-Gly-Gly in the crude double-incorporated Leu of the Leu-Enkephalin synthesis on DVB-PS using DMF at RT. Calculated for $C_{30}H_{42}N_4O_6$: 554.7 Da, found: 556.2 $[M + H]^+$. Experimental conditions 1.



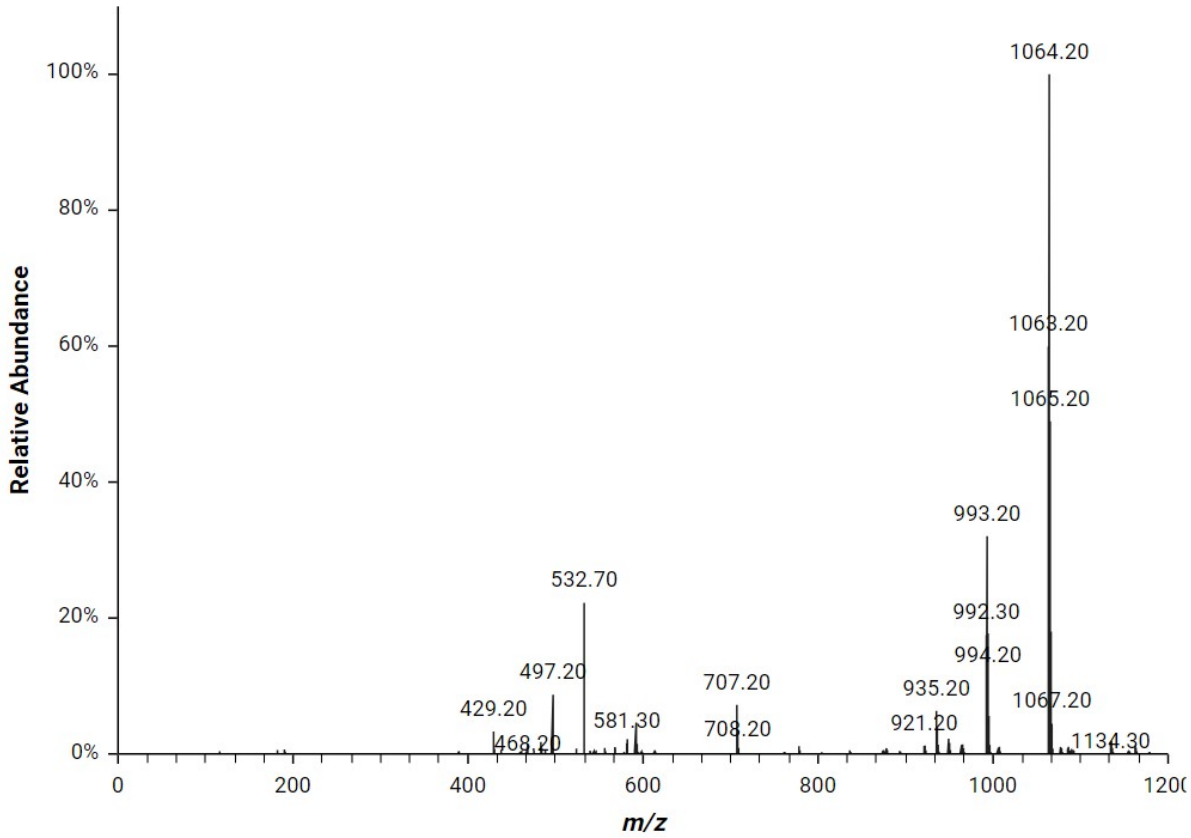
Supplementary Figure 6. Mass spectrum of crude Leu-Enkephalin peptide on DEG-PS using DMF at RT. Calculated for $C_{28}H_{37}N_5O_7$: 555.3 Da, found: 556.2 $[M + H]^+$, 1111.5 $[2M + H]^+$ (non-covalent dimer). Experimental conditions 1.



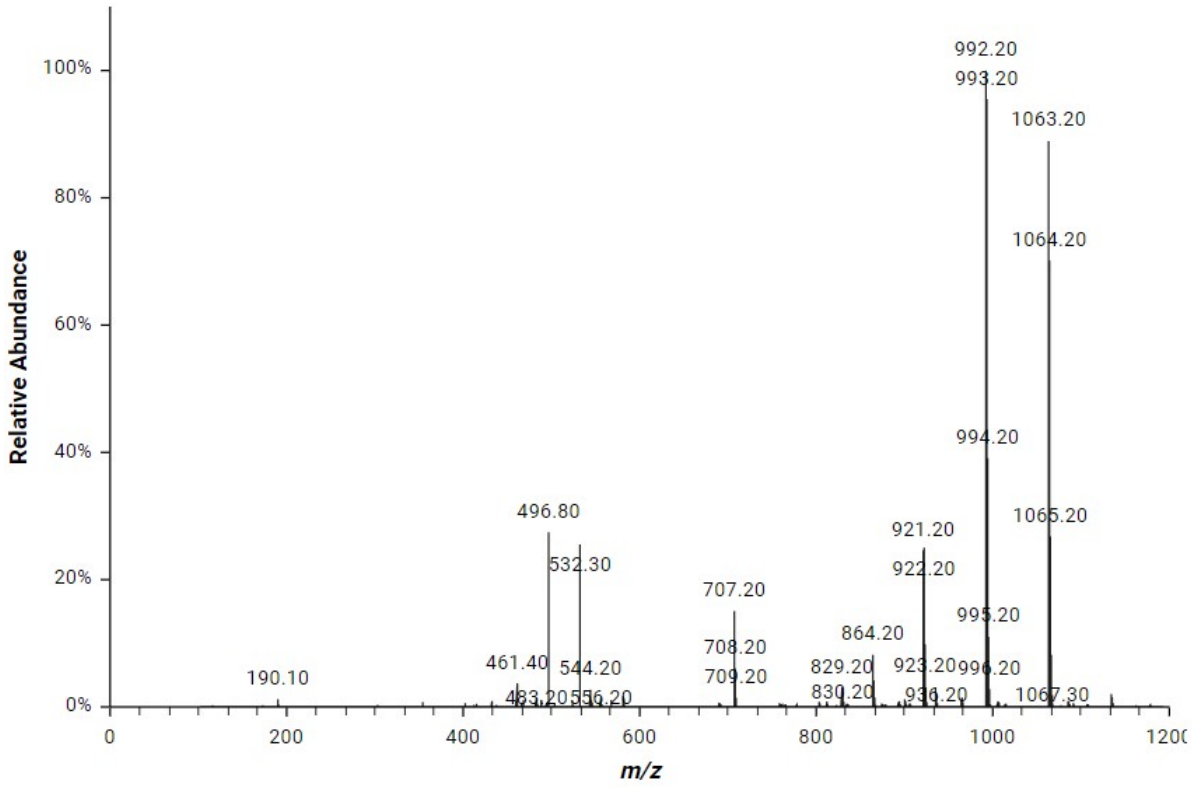
Supplementary Figure 7. Mass spectrum of Des-Ile in crude ACP synthesis on DVB-PS using DMF at RT. Calculated for $C_{47}H_{74}N_{12}O_{16}$: 950.0 Da, found: 951.2 $[M + H]^+$, 475.7 $[M + 2H]^{2+}$. Experimental conditions 1.



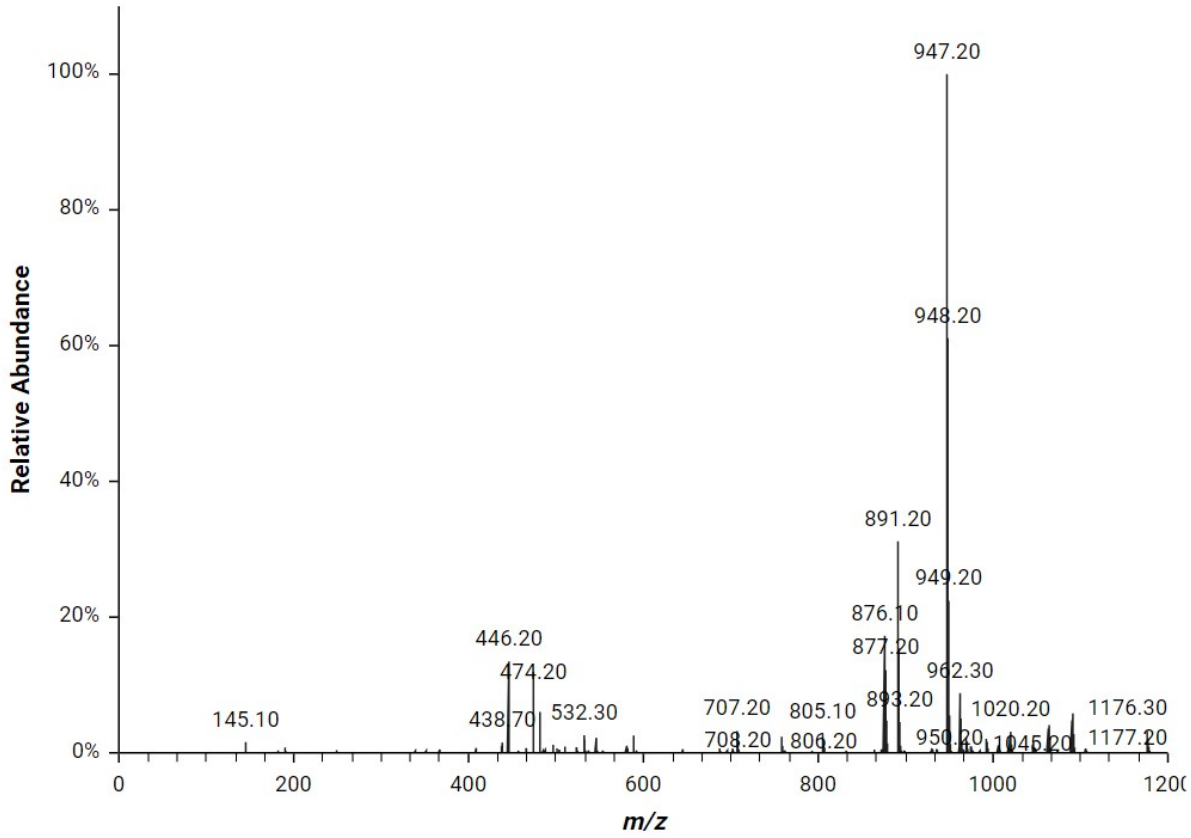
Supplementary Figure 8. Mass spectrum of Des-Ala in crude ACP synthesis on DVB-PS using DMF at RT. Calculated for $C_{44}H_{69}N_{11}O_{15}$: 992.1 Da, found: 993.2 $[M + H]^+$. Experimental conditions 1.



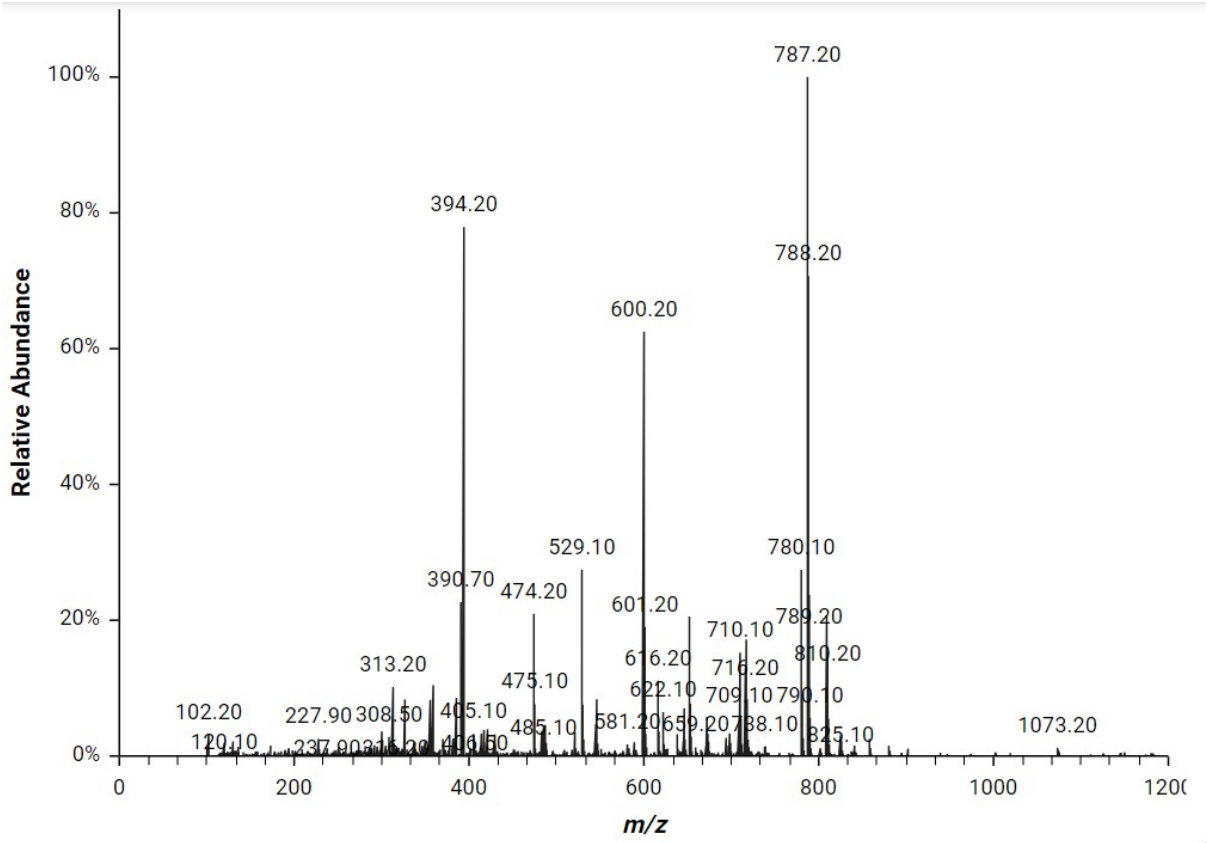
Supplementary Figure 9. Mass spectrum of crude ACP peptide on DVB-PS using DMF at RT. Calculated for $C_{47}H_{74}N_{12}O_{16}$: 1063.2 Da, found: 1064.2 $[M + H]^+$, 532.7 $[M + 2H]^{2+}$. Experimental conditions 1.



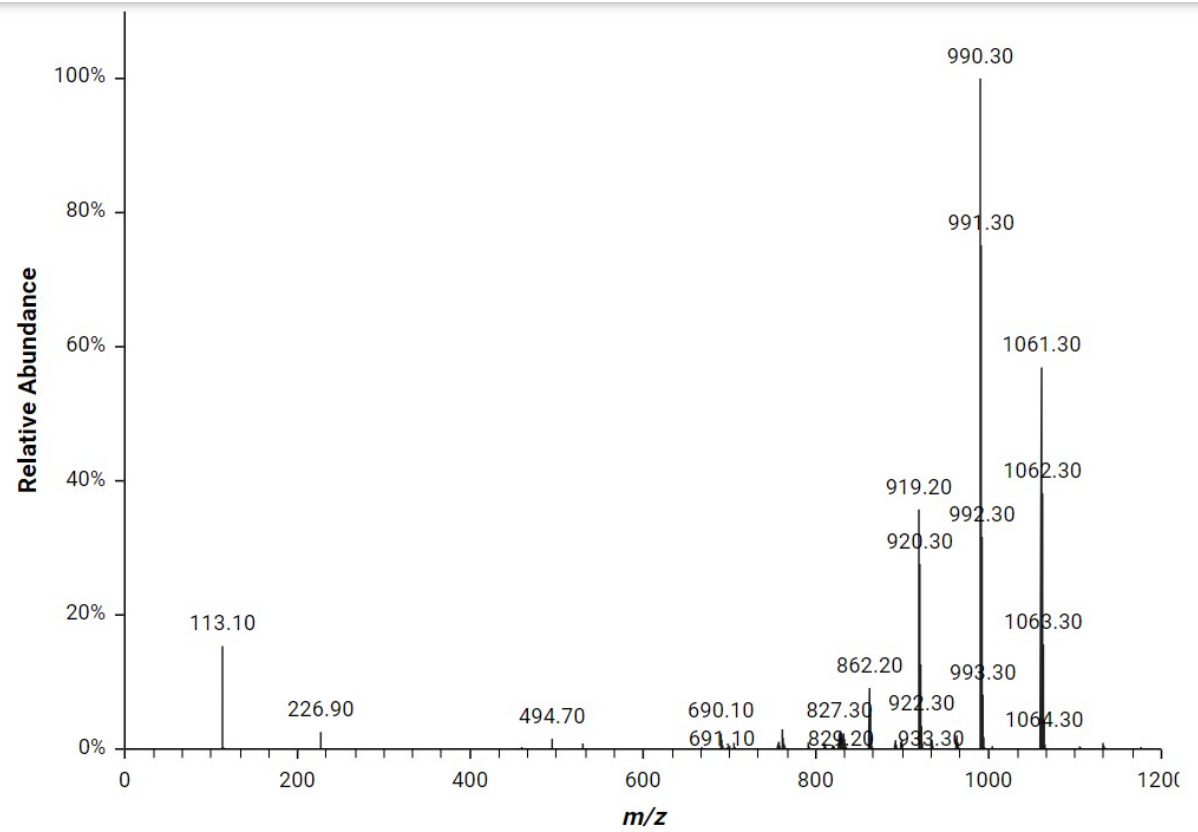
Supplementary Figure 10. Mass spectrum of crude ACP peptide on DEG-PS using DMF at RT. Calculated for $C_{47}H_{74}N_{12}O_{16}$: 1063.2 Da, found: 1064.2 $[M + H]^+$, 532.3 $[M + 2H]^{2+}$. Experimental conditions 1.



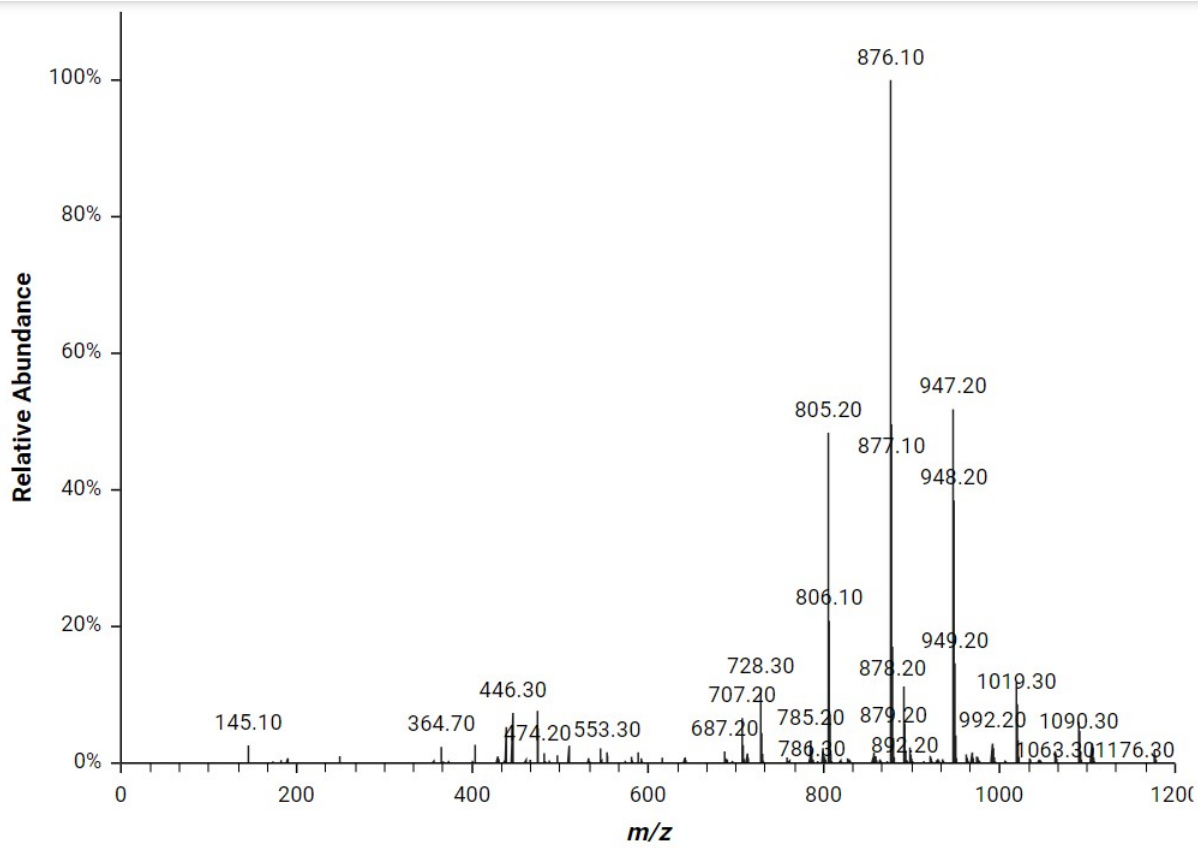
Supplementary Figure 11. Mass spectrum of Des-Asp in crude ACP synthesis on DVB-PS using DMF at RT. Calculated for $C_{43}H_{69}N_{11}O_{13}$: 948.1 Da, found: 949.2 $[M + H]^+$. Experimental conditions 1.



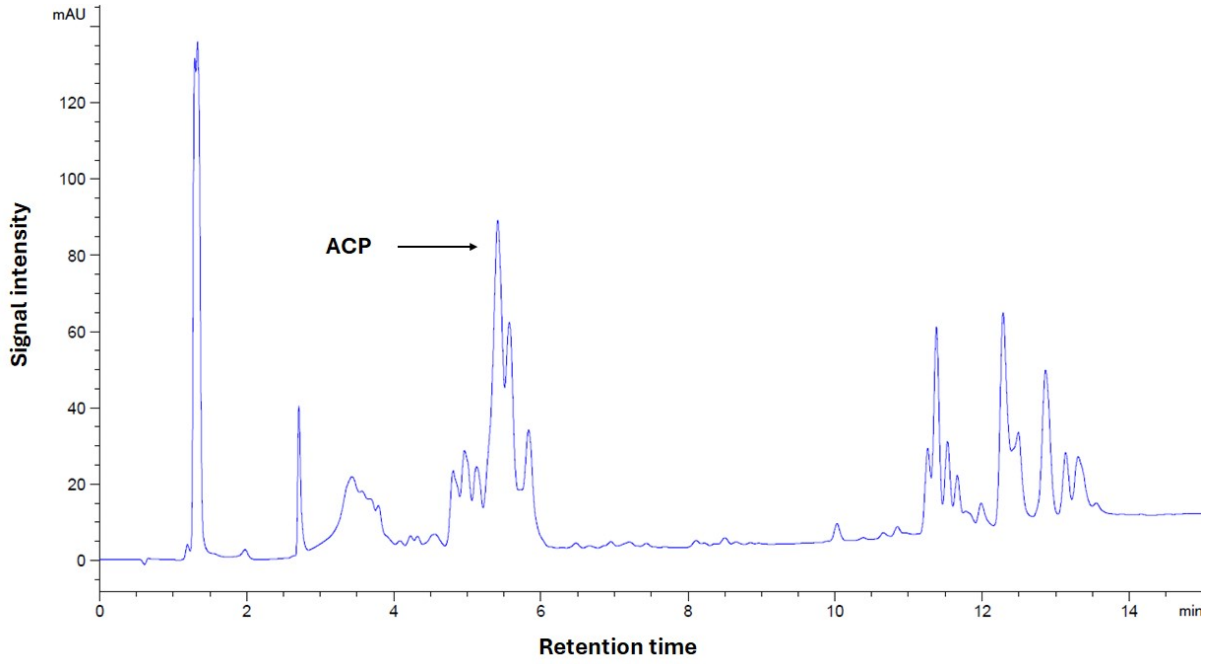
Supplementary Figure 12. Mass spectrum of Des-Tyr-Ile in crude ACP synthesis on DEG-PS using DMF at RT. Calculated for $C_{32}H_{54}N_{10}O_{13}$: 786.8 Da, found: 787.2 $[M + H]^+$, 394.2 $[M + 2H]^{2+}$. Experimental conditions 1.



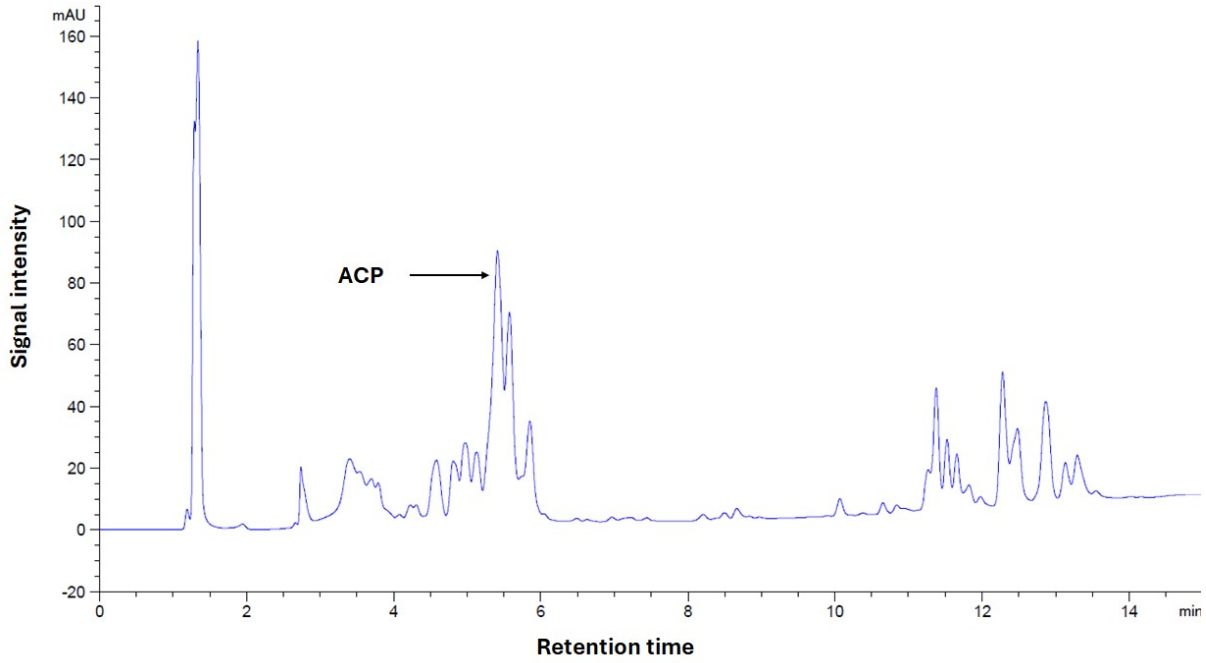
Supplementary Figure 13. Mass spectrum of Des-Ala in crude ACP synthesis on DEG-PS using DMF at RT. Calculated for $C_{44}H_{69}N_{11}O_{15}$: 992.1 Da, found: 993.3 $[M + H]^+$. Experimental conditions 1.



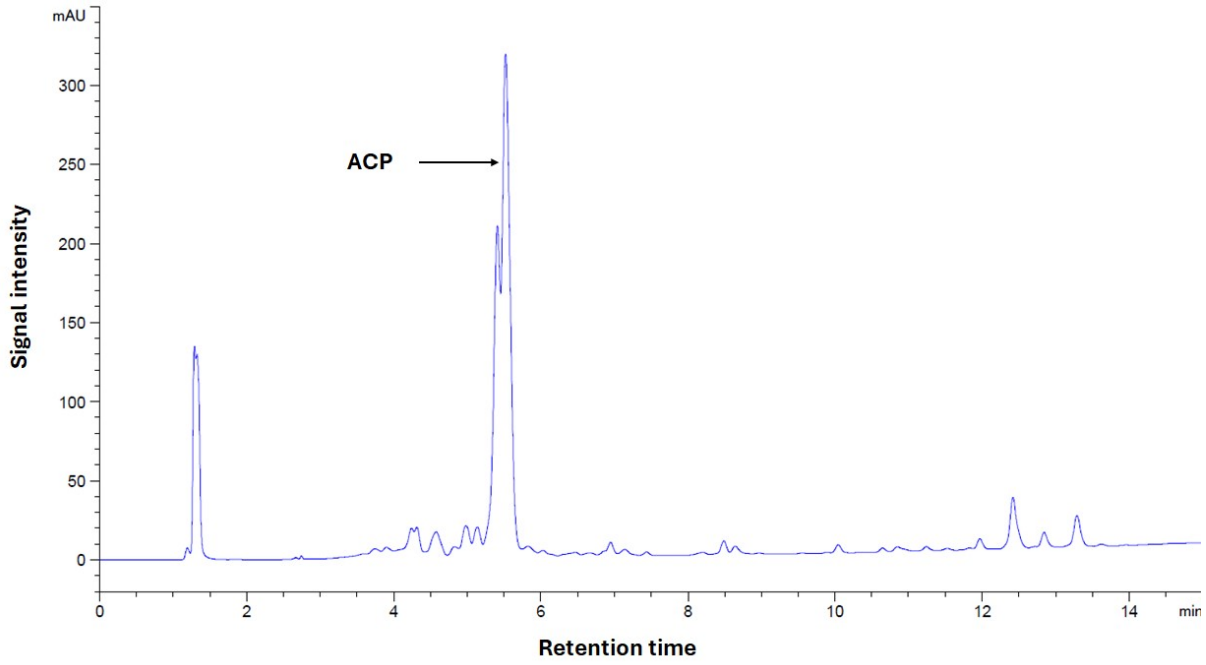
Supplementary Figure 14. Mass spectrum of Des-Ala-Asp in crude ACP crude synthesis on DEG-PS using DMF at RT. Calculated for $C_{40}H_{64}N_{10}O_{12}$: 877.0 Da, found: 878.2 $[M + H]^+$. Experimental conditions 1.



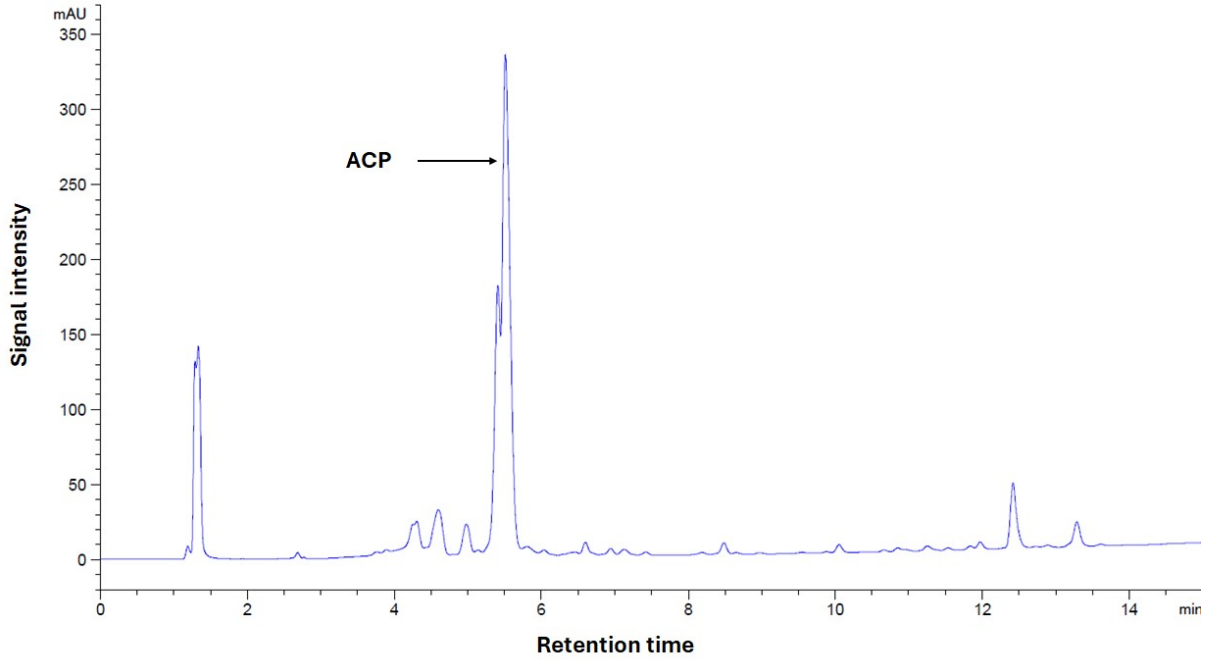
Supplementary Figure 15. HPLC chromatogram (220 nm) of crude ACP peptide synthesis on DVB-PS Wang resin, in 2-MeTHF, at RT. Refer to legend of Figure 8 for chromatographic conditions. Experimental conditions 1.



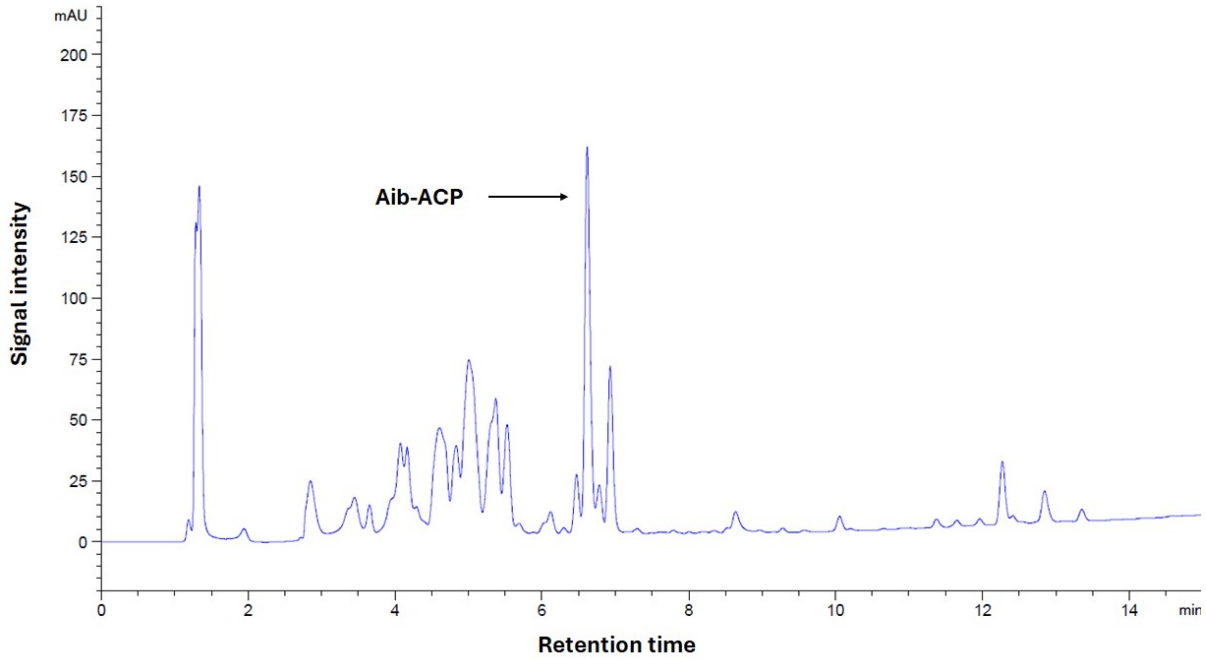
Supplementary Figure 16. HPLC chromatogram (220 nm) of crude ACP peptide synthesis on DEG-PS Wang resin, in 2-MeTHF, at RT. Experimental conditions 1.



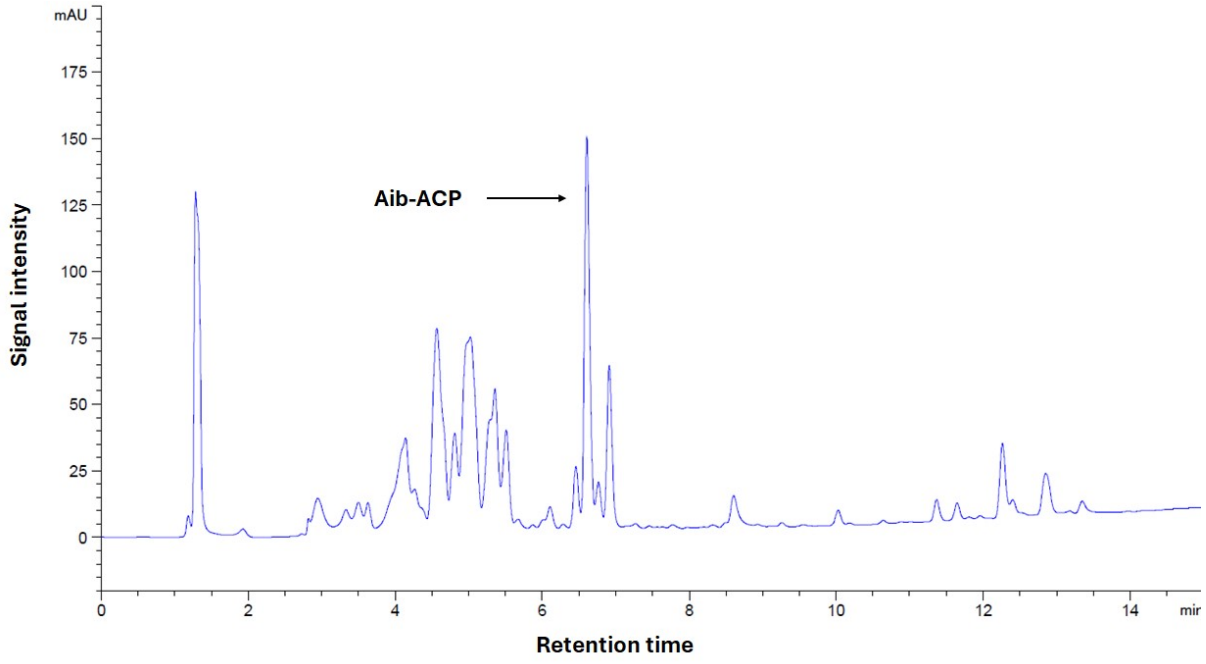
Supplementary Figure 17. HPLC chromatogram (220 nm) of crude ACP peptide on DVB-PS Wang resin, in 2-MeTHF, at 40 °C. Experimental conditions 1.



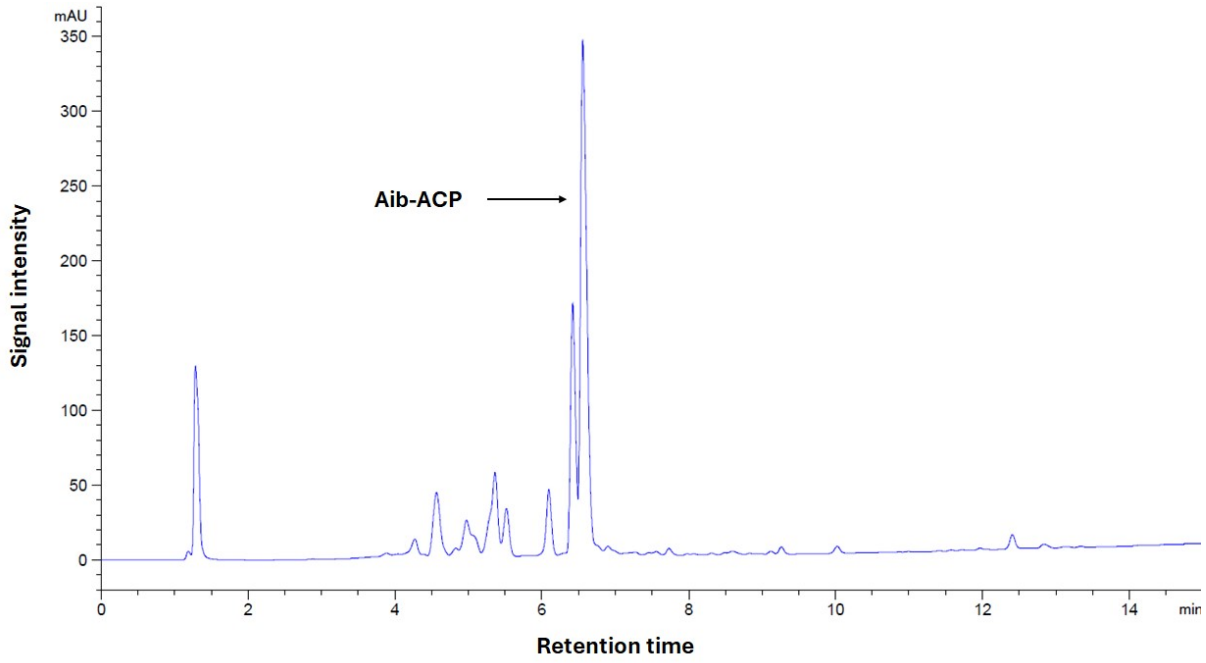
Supplementary Figure 18. HPLC chromatogram (220 nm) of crude ACP peptide on DEG-PS Wang resin, in 2-MeTHF, at 40 °C. Experimental conditions 1.



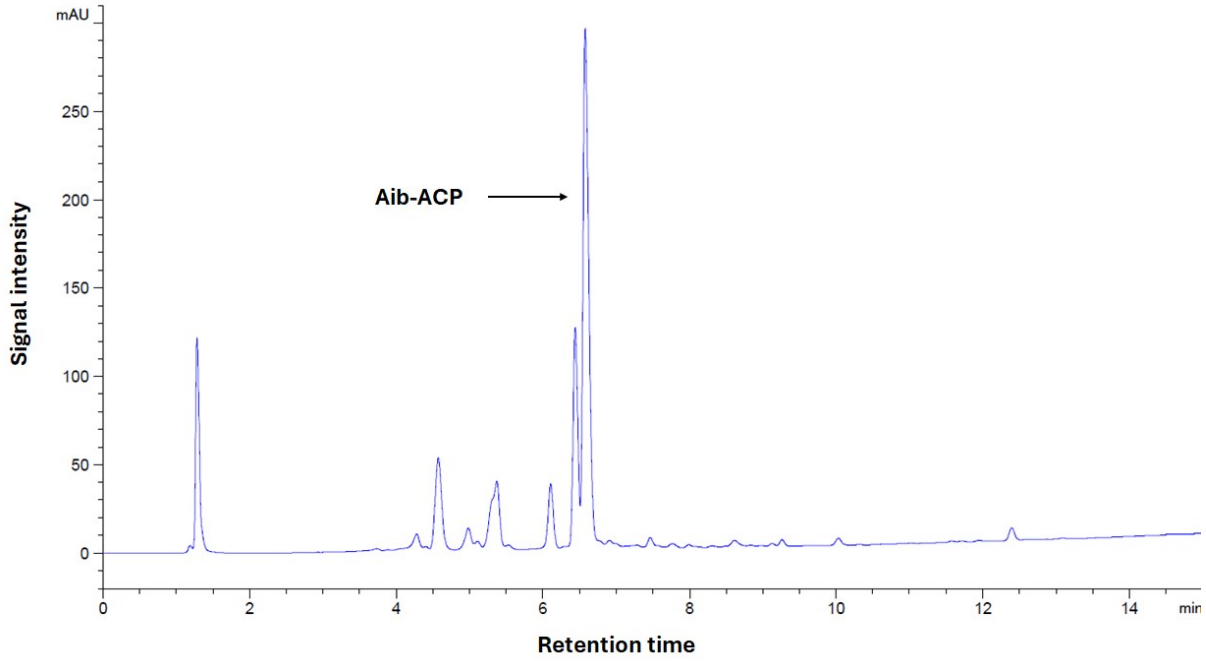
Supplementary Figure 19. HPLC chromatogram (220 nm) of crude Aib-ACP peptide on DVB-PS Wang resin, in 2-MeTHF, at RT. Experimental conditions 1.



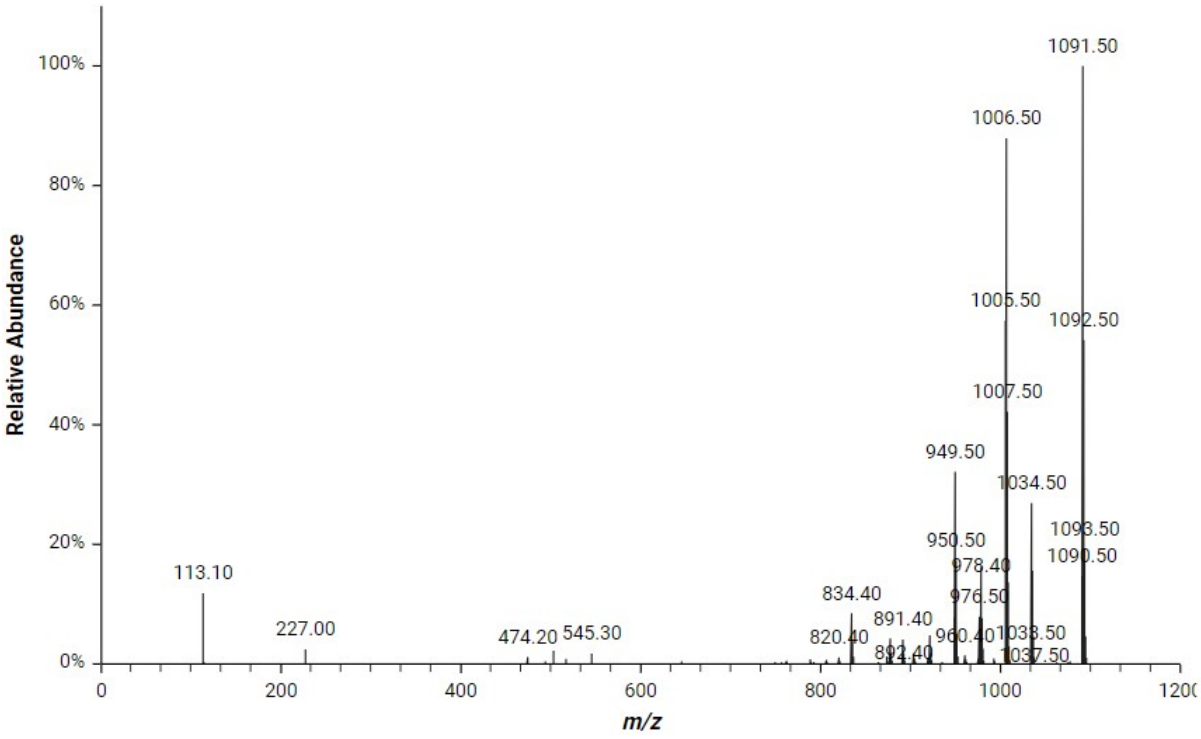
Supplementary Figure 20. HPLC chromatogram (220 nm) of crude Aib-ACP peptide on DEG-PS Wang resin, in 2-MeTHF, at RT. Experimental conditions 1.



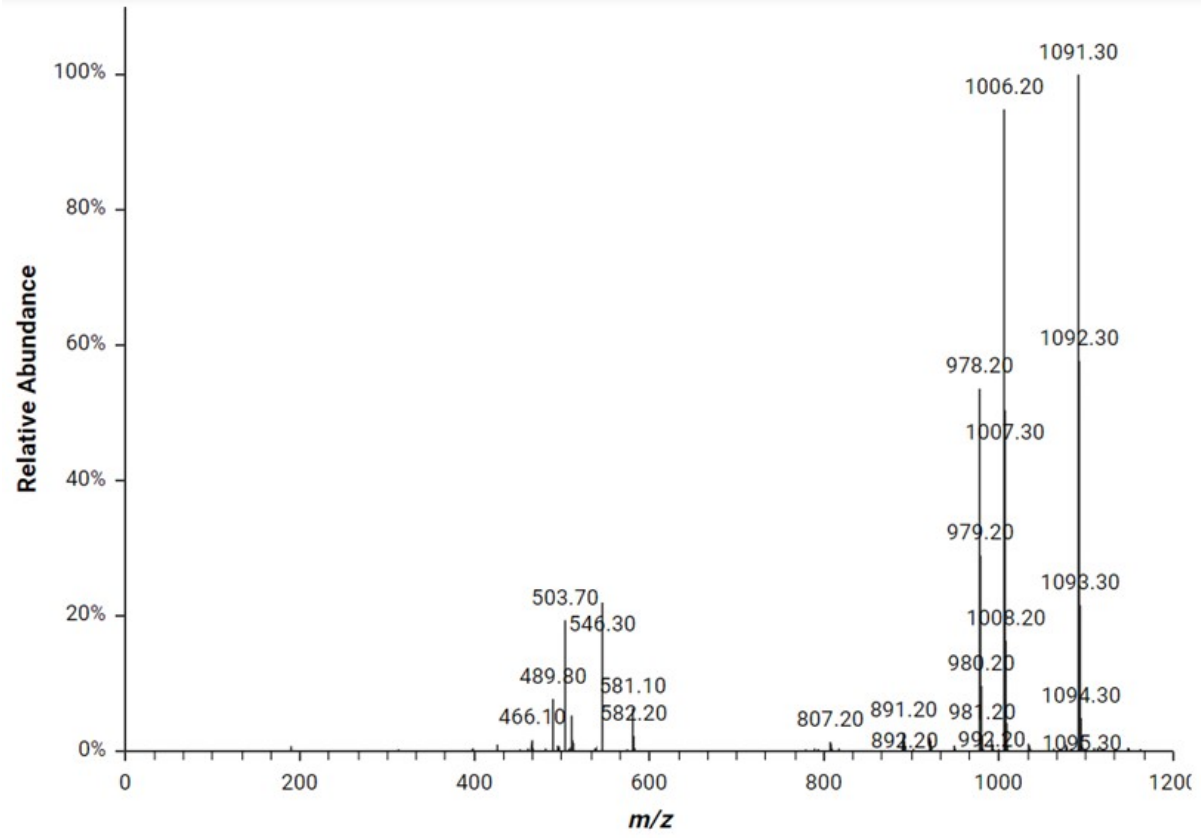
Supplementary Figure 21. HPLC chromatogram (220 nm) of crude Aib-ACP peptide on DVB-PS Wang resin, in 2-MeTHF, at 40 °C. Experimental conditions 1.



Supplementary Figure 22. HPLC chromatogram (220 nm) of crude Aib-ACP peptide on DEG-PS Wang resin, in 2-MeTHF, at 40 °C. Experimental conditions 1.

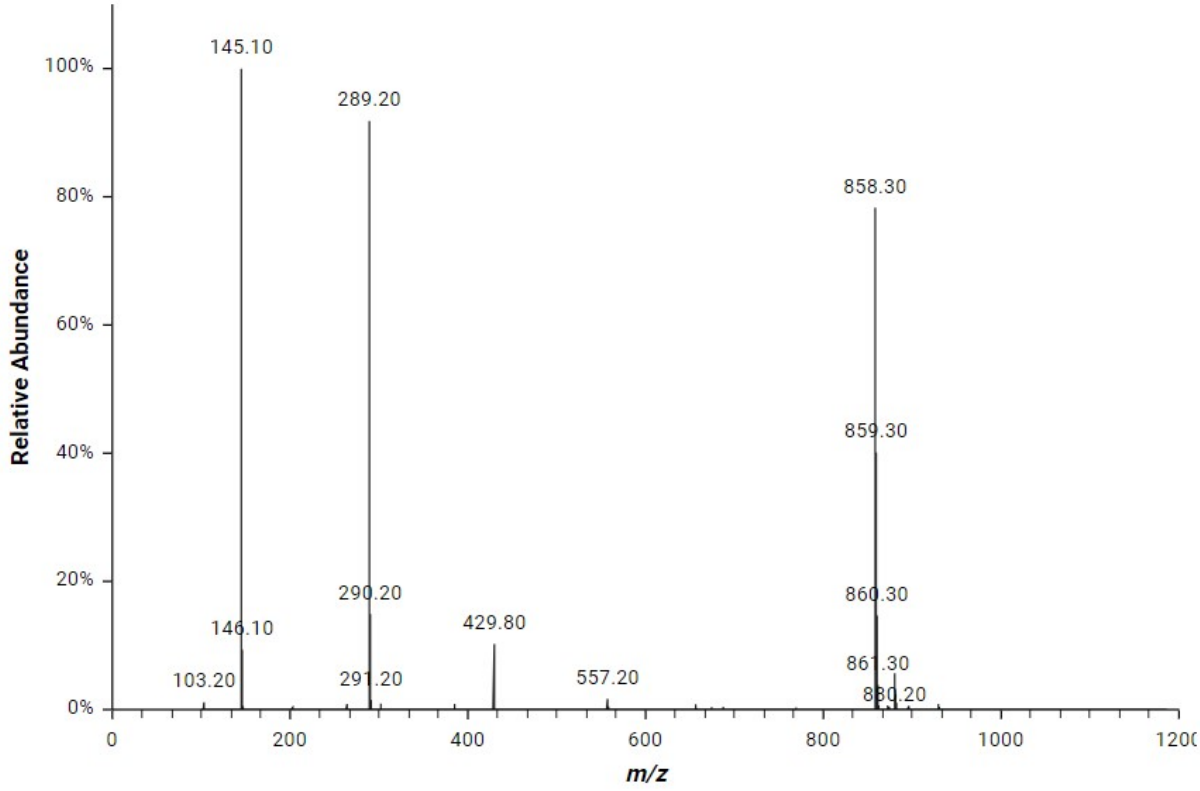


Supplementary Figure 23. Mass spectrum of crude Aib-ACP peptide on DVB-PS using DMF at RT. Calculated for $C_{49}H_{83}N_{12}O_{16}$: 1091.2 Da, found: 1092.5 $[M + H]^+$. Des-Aib, calculated: 1006.1, found: 1006.5 $[M + H]^+$. Experimental conditions 1.

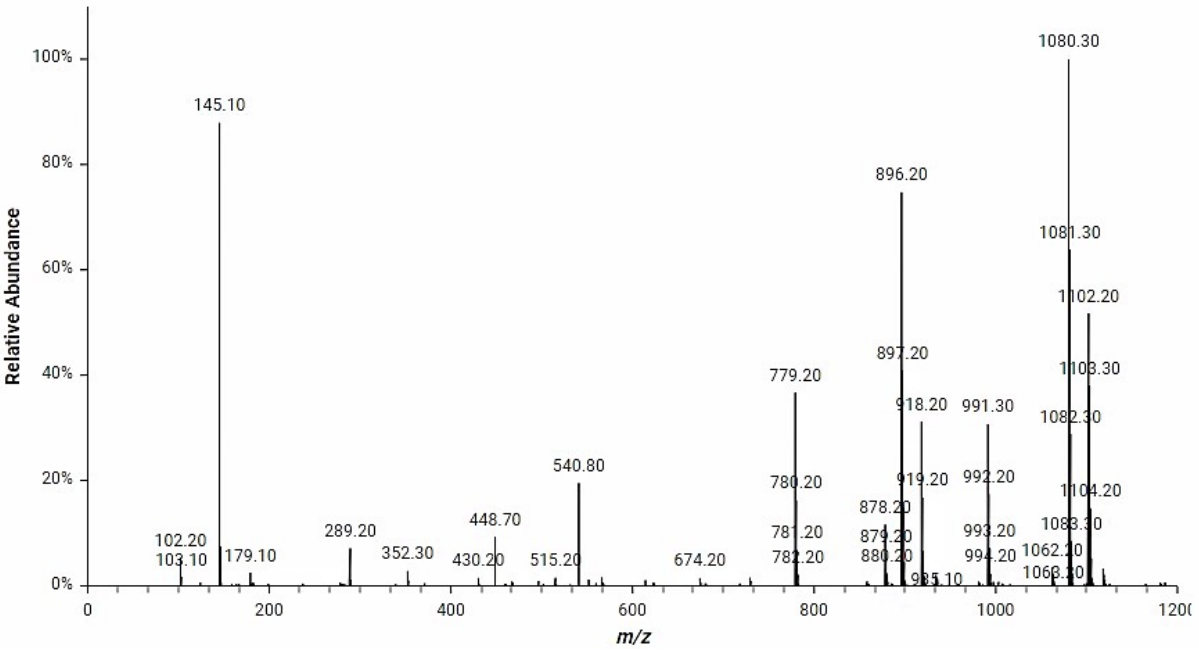


Supplementary Figure 24. Mass spectrum of crude Aib-ACP peptide on DEG-PS using DMF at RT.

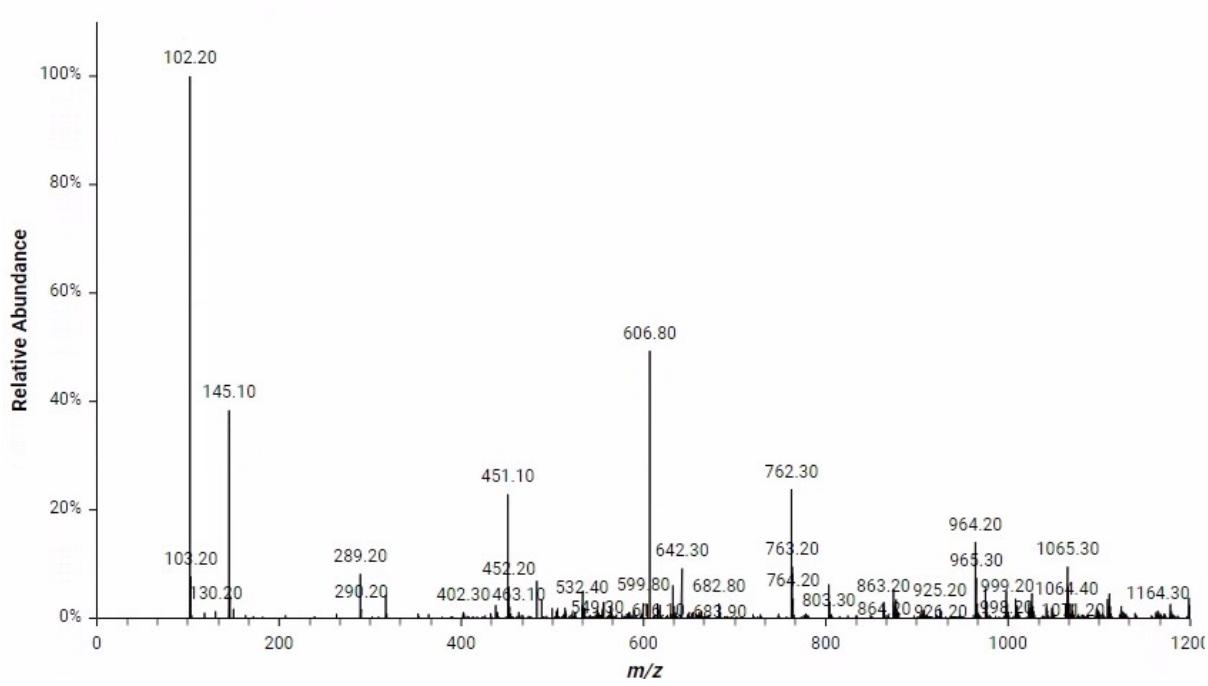
Calculated for $C_{49}H_{83}N_{12}O_{16}$: 1091.2 Da, found: 1092.3 $[M + H]^+$, 546.3 $[M + 2H]^{2+}$. Des-Aib, calculated: 1006.1, found: 1007.3 $[M + H]^+$, 503.7 $[M + 2H]^{2+}$. Experimental conditions 1.



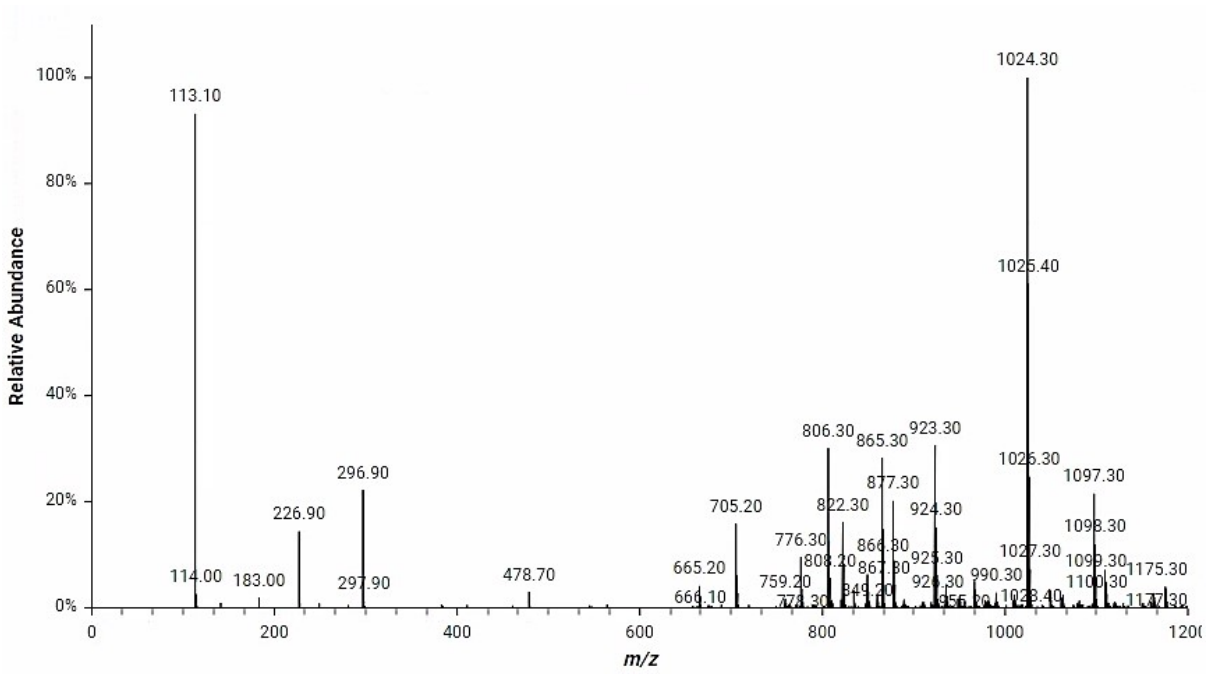
Supplementary Figure 25. Mass spectrum of crude β (34–42) peptide on DEG-PS using 2-MeTHF at 40 °C. Calculated for $C_{39}H_{71}N_9O_{10}S$: 858.11 Da, found: 858.30 $[M + H]^+$, 429.80 $[M + 2H]^{2+}$. Experimental conditions 1.



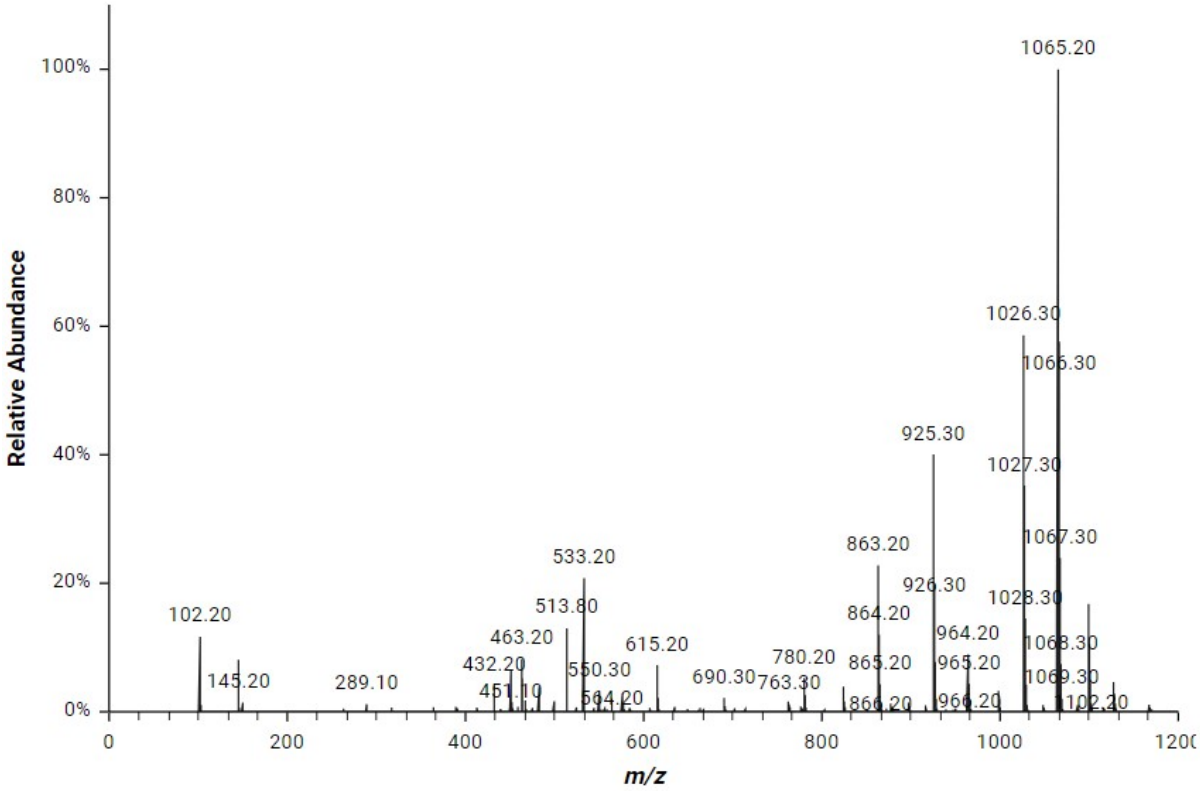
Supplementary Figure 26. Mass spectrum of crude Fmoc-β (34–42) peptide on DEG-PS using 2-MeTHF at 40 °C. Calculated for $C_{54}H_{81}N_9O_{12}S$: 1080.4 Da, found: 1081.3 $[M + H]^+$, 540.8 $[M + 2H]^{2+}$. Experimental conditions 1.



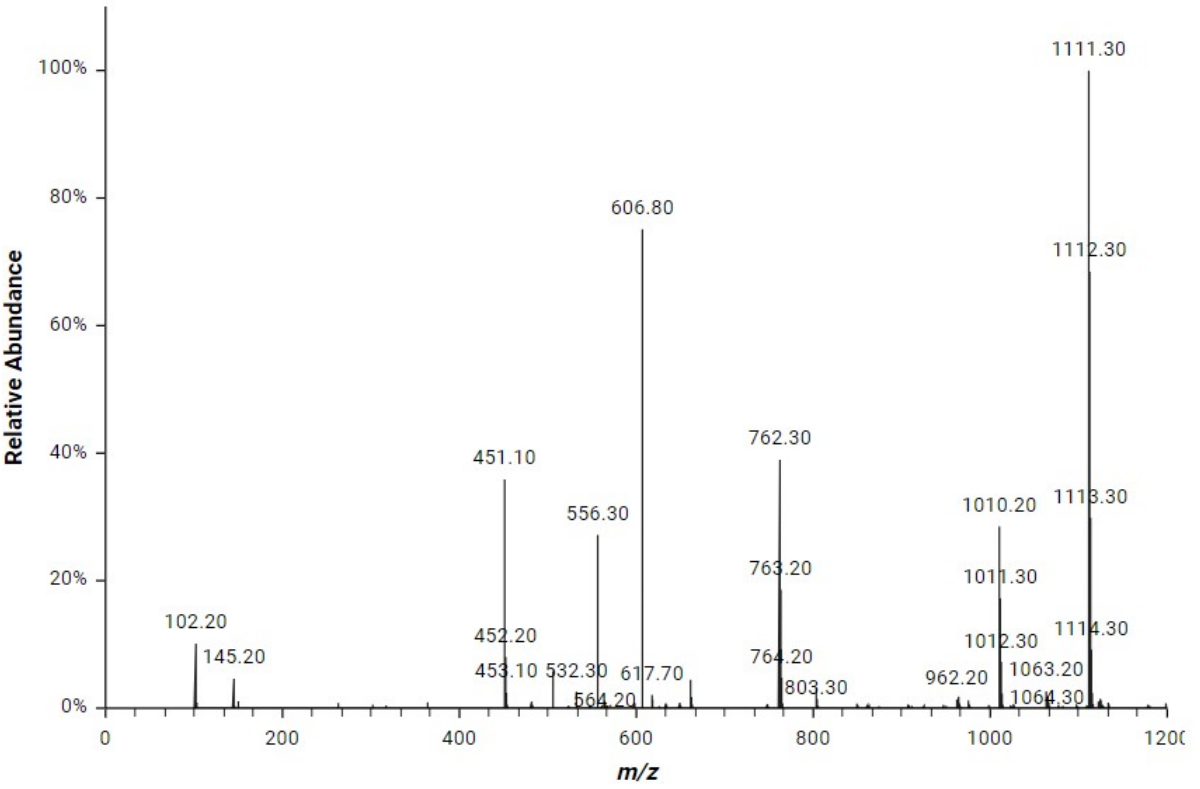
Supplementary Figure 27. Mass spectrum of crude JR peptide on DEG-PS using 2-MeTHF at 40 °C. Calculated for $C_{58}H_{89}N_{11}O_{15}S$: 1212.47 Da, found: 606.80 $[M + 2H]^{2+}$. Experimental conditions 1.



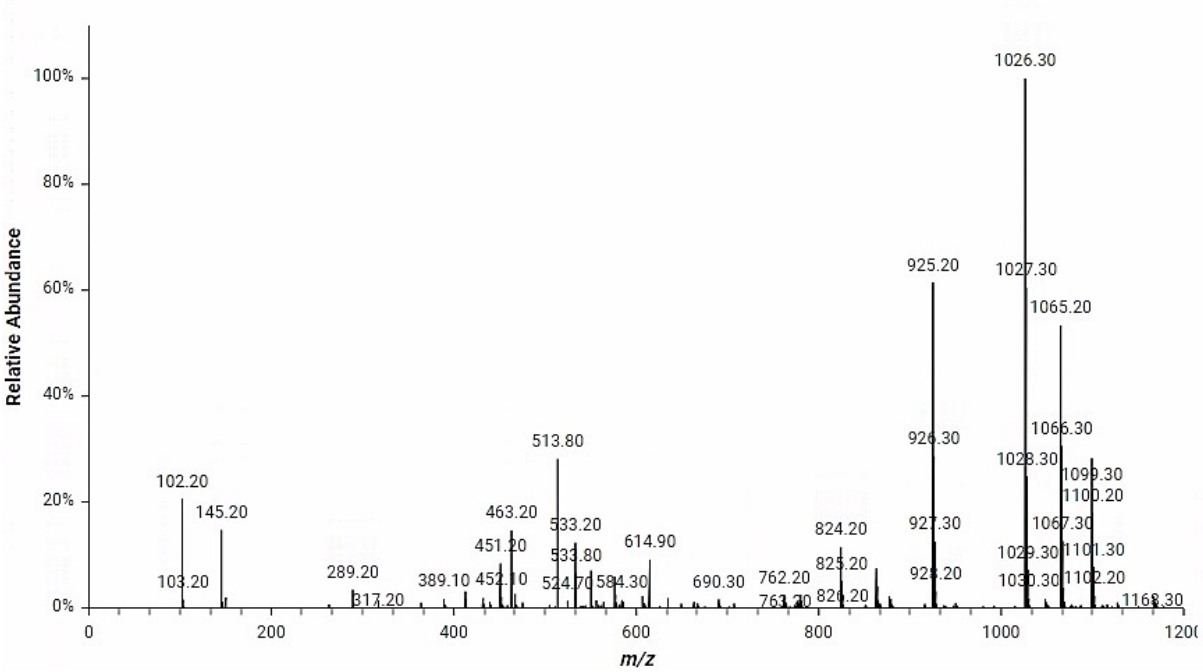
Supplementary Figure 28. Mass spectrum of des-Trp in crude JR peptide on DEG-PS using 2-MeTHF at 40 °C. Calculated for $C_{47}H_{79}N_9O_{14}S$: 1026.26 Da, found: 1027.30 $[M + H]^+$. Experimental conditions 1.



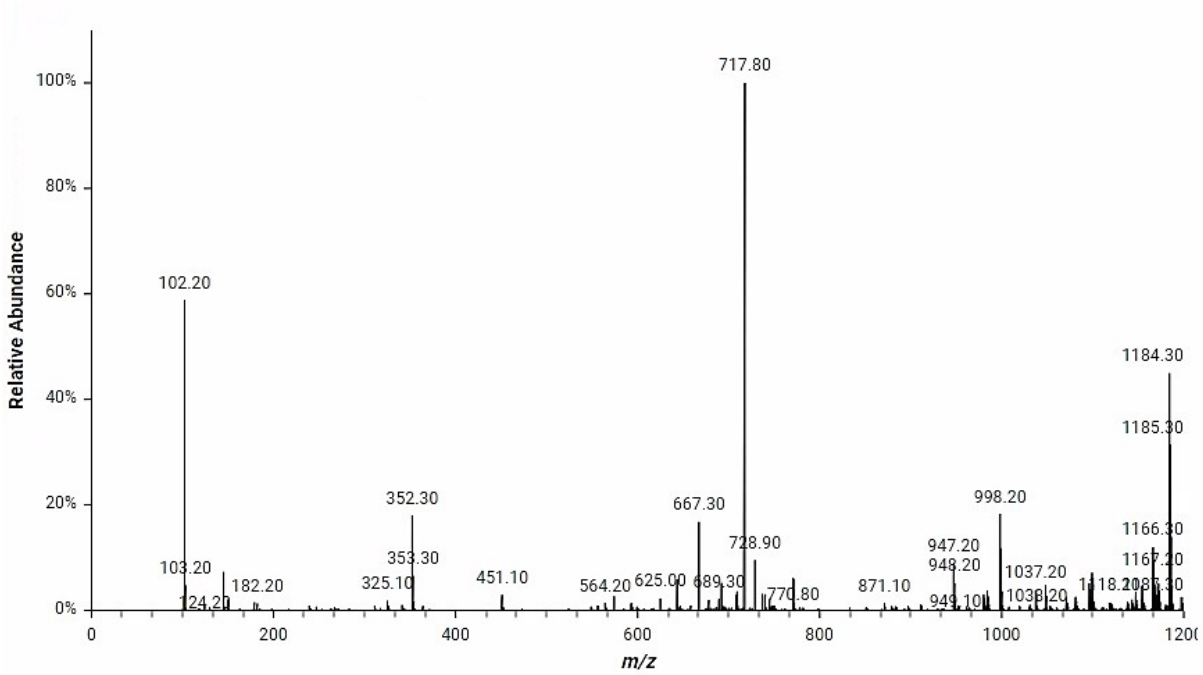
Supplementary Figure 29. Mass spectrum of des-Phe in crude JR peptide on DEG-PS using 2-MeTHF at 40 °C. Calculated for $C_{49}H_{80}N_{10}O_{14}S$: 1065.30 Da, found: 1065.30 $[M + H]^+$, 533.2 $[M + 2H]^{2+}$ Experimental conditions 1.



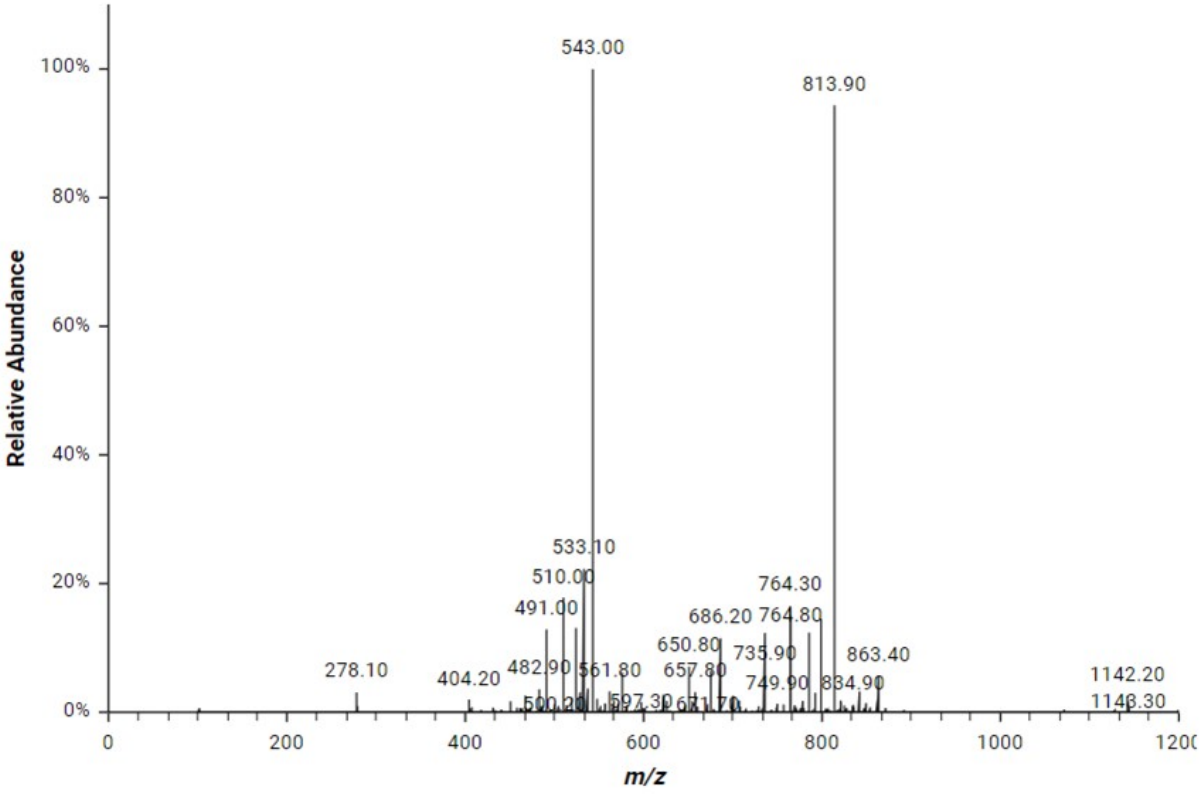
Supplementary Figure 30. Mass spectrum of des-Thr in crude JR peptide on DEG-PS using 2-MeTHF at 40 °C. Calculated for $C_{54}H_{82}N_{10}O_{13}S$: 1111.37 Da, found: 1112.30 $[M + H]^+$, 556.30 $[M + 2H]^{2+}$. Experimental conditions 1.



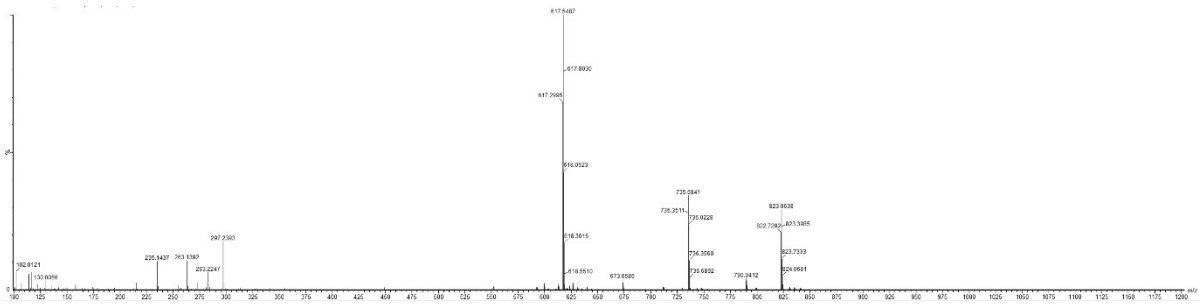
Supplementary Figure 31. Mass spectrum of des-Trp-Thr in crude JR peptide on DEG-PS using 2-MeTHF at 40°C. Calculated for $C_{43}H_{72}N_8O_{12}S$: 925.15 Da, found: 926.30 $[M + H]^+$. Experimental conditions 1.



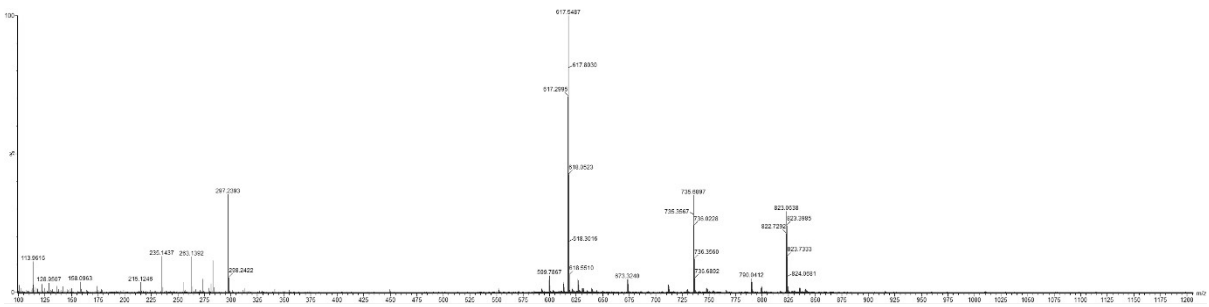
Supplementary Figure 32. Mass spectrum of crude Fmoc-JR peptide on DEG-PS using 2-MeTHF at 40°C. Calculated for $C_{73}H_{99}N_{11}O_{17}S$: 1434.72 Da, found: 717.80 $[M + 2H]^{2+}$. Experimental conditions 1.



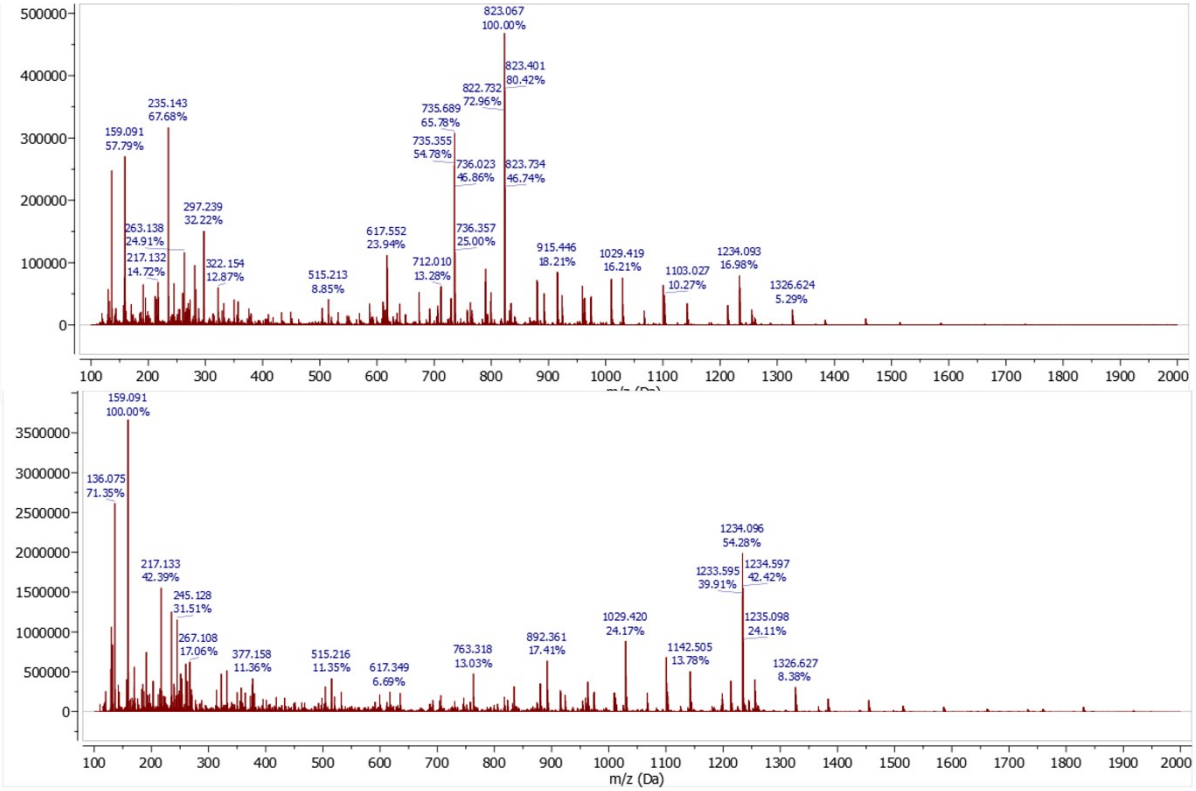
Supplementary Figure 33. Mass spectrum of crude ABRF1992 peptide on DEG-PS using 2-MeTHF at 40°C. Calculated for $C_{73}H_{106}N_{22}O_{21}$: 1627.78 Da, found: 813.90 $[M + 2H]^{2+}$, 543.00 $[M + 3H]^{3+}$. Des-Aib, calculated: 1006.1, found: 1007.3 $[M + H]^{+}$, 503.7 $[M + 2H]^{2+}$. Experimental conditions 1.



Supplementary Figure 34. High resolution mass spectrum of crude ABC analogue peptide on DEG-PS resin. $[M+3H]^{3+}$ calculated for $C_{110}H_{164}N_{30}O_{31}S_2$: 822.7285 Da, found: 822.7292 Da, Δ = 0.85 ppm, $[M+4H]^{4+}$ calculated: 617.2983 Da, found: 617.2995 Da Δ = 1.95 ppm. Experimental conditions 2.



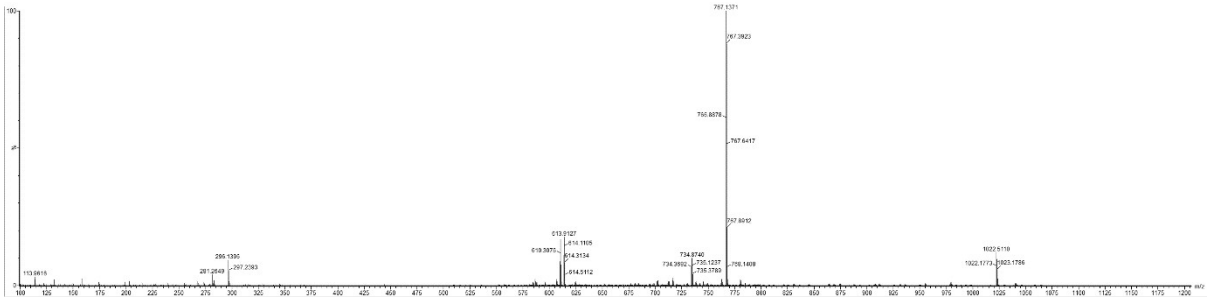
Supplementary Figure 35. High resolution mass spectrum of crude ABC analogue peptide on DVB-PS resin. $[M+3H]^{3+}$ calculated for $C_{110}H_{164}N_{30}O_{31}S2$: 822.7285 Da, found: 822.7292 Da, Δ = 0.85 ppm, $[M+4H]^{4+}$ calculated: 617.2983 Da, found: 617.2995 Da Δ = 1.95 ppm. Experimental conditions 2.



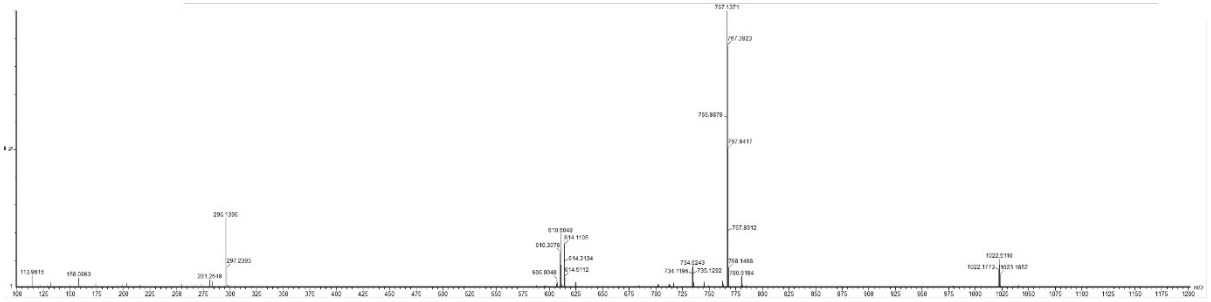
Supplementary Figure 36. MS^E spectra of ABC analogue peptide on DEG-PS resin, generated using conditions 2 with a collision voltage ramp of 15 V to 30 V (top) and 30 V to 60 V (bottom).

Supplementary Table 2: Fragment ions identified within the MS^E spectra obtained for the crude ABC analogue peptide.

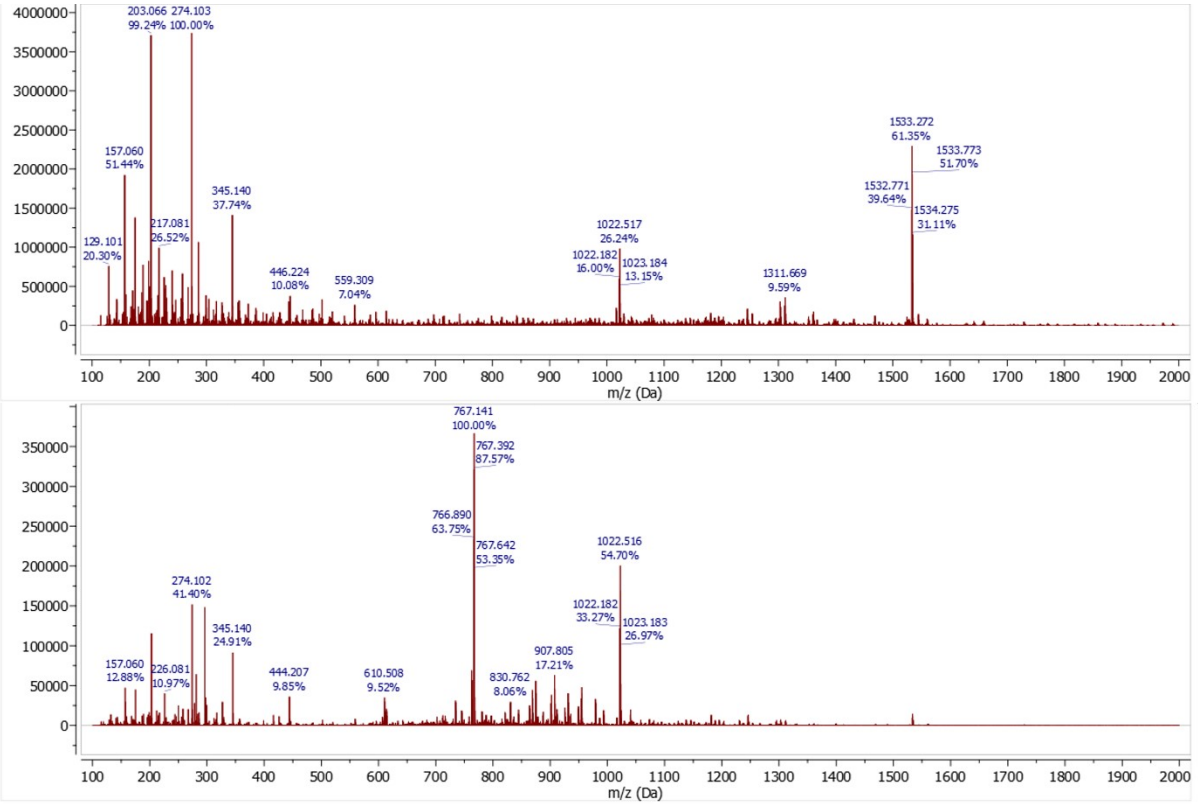
Fragment ion	Calculated m/z	Observed m/z
B_2^+	263.139	263.138
B_3^+	449.218	449.218
Y_4^{++}	1009.483	1009.486
Y_5^+	1916.911	1916.927
Y_6^+	1829.879	1829.875
Y_7^+	1732.826	1732.845
Y_8^+	1585.757	1585.751
Y_9^+	1454.717	1454.722
Y_{10}^+	1326.622	1326.624
Y_{11}^+	1213.538	1213.539
Y_{12}^+	1100.454	1100.455
Y_{13}^+	963.395	963.397
Y_{14}^+	834.352	834.354
Y_{15}^+	706.294	706.296
Y_{17}^{++}	245.124	245.128



Supplementary Figure 37. High resolution mass spectrum of crude Thymosin peptide on DEG-PS resin. $[M+3H]^{3+}$ calculated for $C_{127}H_{214}N_{34}O_{53}$: 1022.1777 Da, found: 1022.1773 $[M + 3H]^{3+}$, $\Delta = 0.39$ ppm. $[M+4H]^{4+}$ calculated: 766.8852 Da, found: 766.8878 $[M + 4H]^{4+}$ + $\Delta = 3.39$ ppm, $[M+5H]^{5+}$ calculated: 613.7097 Da, found: 613.7099, $\Delta = 0.33$ ppm. Experimental conditions 2.



Supplementary Figure 38. High resolution mass spectrum of crude Thymosin peptide on DVB-PS resin. $[M+3H]^{3+}$ calculated for $C_{127}H_{214}N_{34}O_{53}$: 1022.1777 Da, found: 1022.1773 $[M + 3H]^{3+}$, $\Delta = 0.39$ ppm. $[M+4H]^{4+}$ calculated: 766.8852 Da, found: 766.8878 $[M + 4H]^{4+}$ + $\Delta = 3.39$ ppm, $[M+5H]^{5+}$ calculated: 613.7097 Da, found: 613.7099, $\Delta = 0.33$ ppm. Experimental conditions 2.



Supplementary Figure 39. High resolution MS^E spectra of crude Thymosin peptide on DEG-PS resin, generated using conditions 2 with a collision voltage ramp of 15 V to 30 V (top) and 30 V to 60 V (bottom).

Supplementary Table 3: Fragment ions identified within the MS^E spectra obtained for the crude Thymosin peptide.

Fragment ion	Calculated m/z	Observed m/z
B_2^+	203.066	203.066
B_3^+	274.103	274.103
B_4^+	345.141	345.140
B_5^+	444.209	444.208
B_6^+	559.236	559.237
Y_7^{++}	1254.140	1254.144
Y_{15}^{++}	830.425	830.433
B_{24}^+	1302.666	1302.659