

Supporting information for:

Design of Transmembrane Mimetic Structural Probes to Trap Different Stages of γ -Secretase-Substrate Interaction

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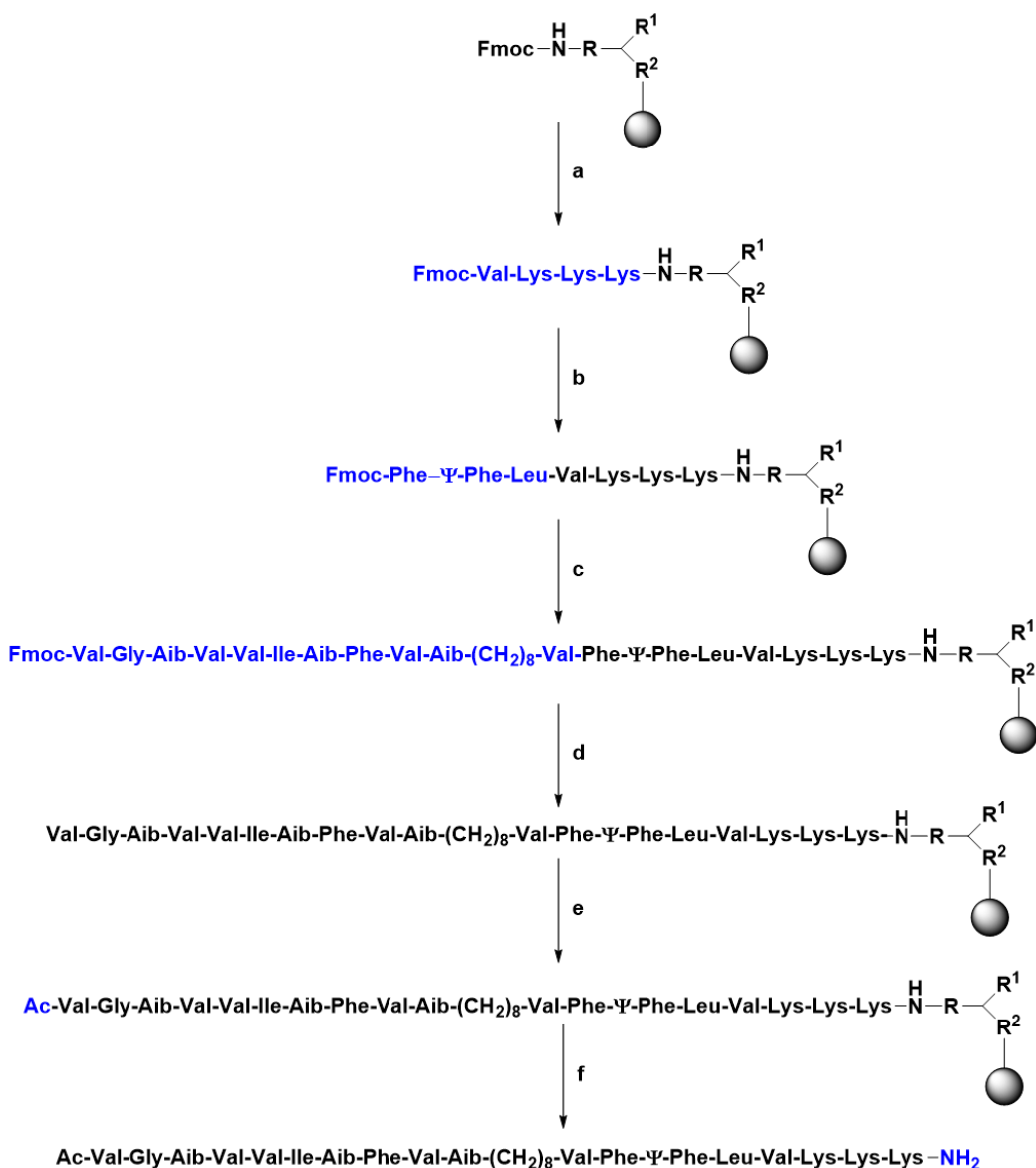
[§]These authors contributed equally to the work

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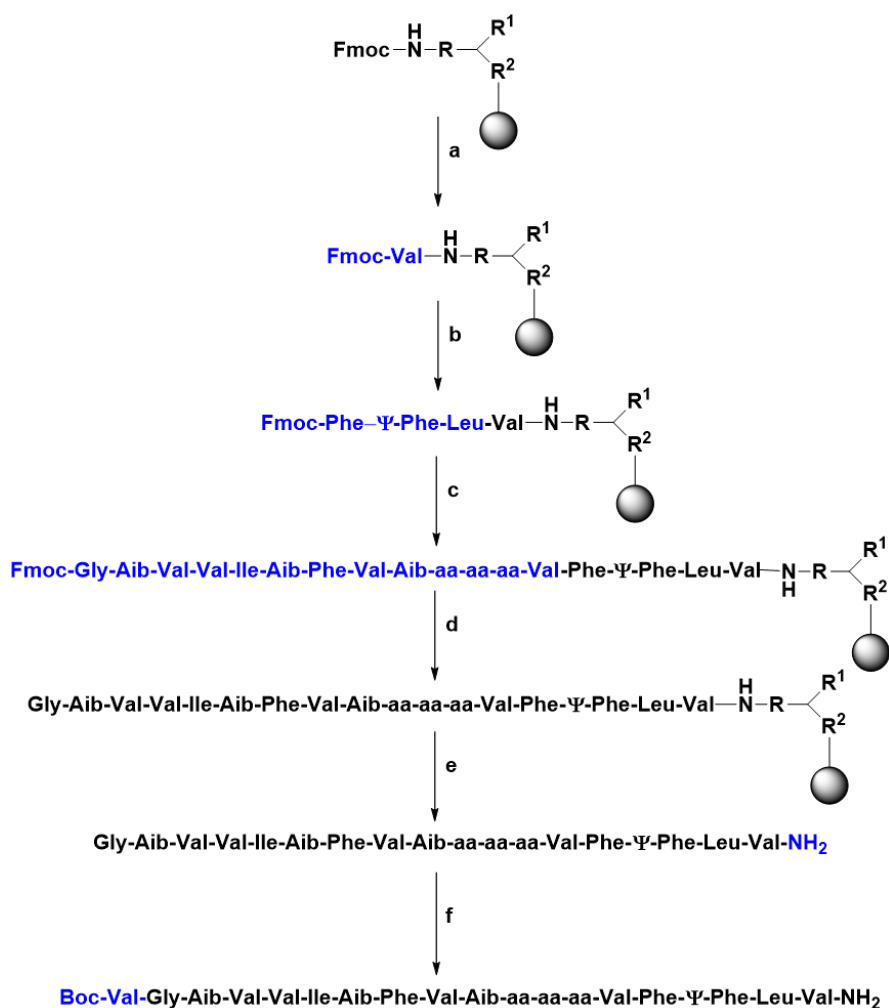
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Scheme S1. Solid-phase synthesis of *N*-acetylated, C-amide L-peptidomimetics using Rink amide resin (for peptides **20**, **21**, **30**, **31**).^a



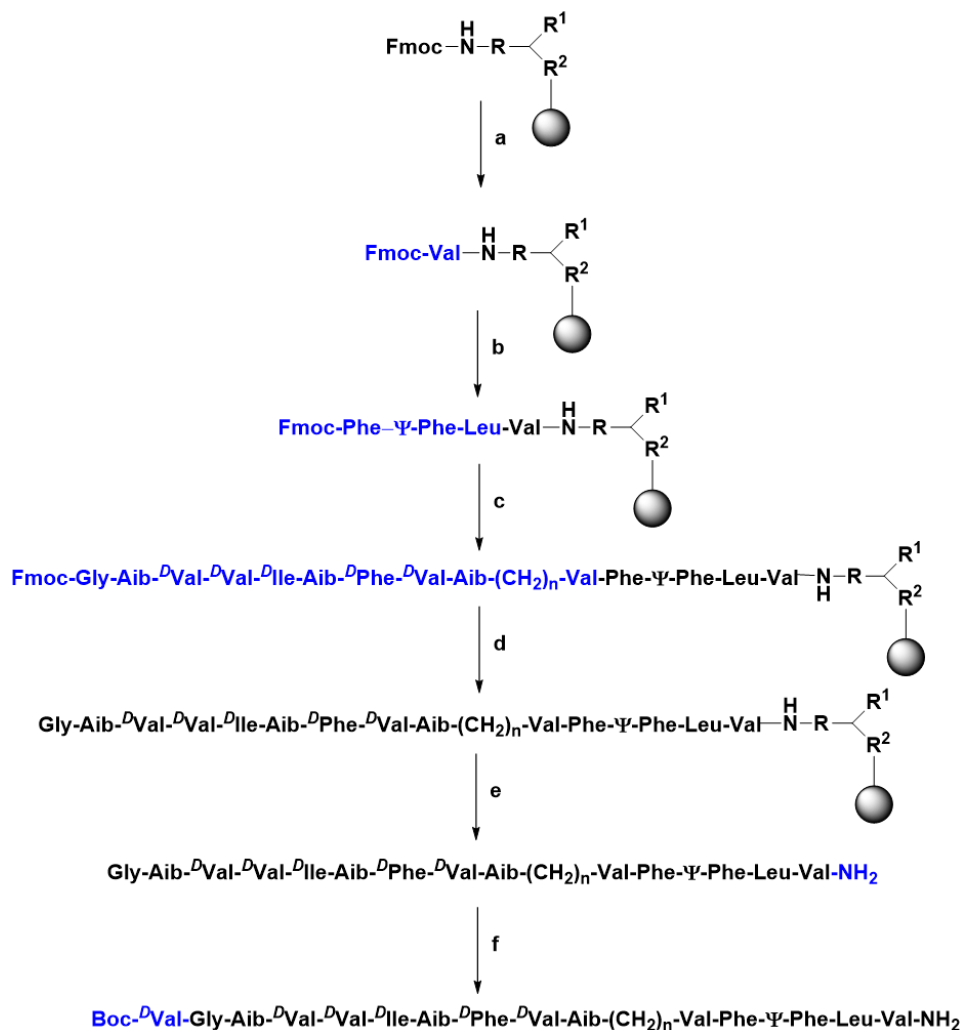
^aReagents and conditions: (a) Iteratively: i. 20% piperidine in DMF; ii. 0.2 M Fmoc-protected amino acid (3 x ϵ -*N*-Boc-Lys, then Val), 0.2 M DIC (*N,N'*-diisopropylcarbodiimide), and 0.2 M OXYMA (ethyl cyano(hydroxyimino)acetate) in DMF, 70 °C, 8 min, double coupling; (b) i. 20% piperidine in DMF; (ii) 0.2 M **11**, 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling; (c) Iteratively: i. 20% piperidine in DMF; ii. 0.2 M Fmoc-protected L-amino acids (Val, Fmoc-NH(CH₂)₈CO₂H for peptide **20** and **21** or 3 x Gly for peptide **30** or 4 x Gly for peptide **31**, Aib, Val, Phe, Aib, Ile, Val, Val, Aib, Gly, Val), 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling; (d) 20% piperidine in DMF; (e) Ac₂O, 7% DIPEA in DMF, 60 min (f) TFA:TIPS (triisopropylsilane): H₂O: DoDt (2,2'-(ethylenedioxy)diethanethiol):: 92.5:2.5:2.5:2.5, r.t., 2 h. (Note: for the synthesis of peptide **21** in step a, the amino acid residue coupled was only valine).

Scheme S2. Solid-phase synthesis of *N*-Boc, C-amide L-peptidomimetics using Rink amide resin (for peptide **22-29**).^a



^aReagents and conditions: (a) (i) 20% piperidine in DMF; (ii) 0.2 M Fmoc-valine, 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling; (b) (i) 20% piperidine in DMF; (ii) 0.2 M **11**, 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling; (c) Iteratively: i. 20% piperidine in DMF; ii. 0.2 M Fmoc-amino acids (Val, 3 amino acids replacing 10-atom linker region, Aib, Val, Phe, Aib, Ile, Val, Val, Aib, Gly), 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling (d) 20% piperidine in DMF (e) TFA:TIPS:H₂O:DoDt :: 92.5:2.5:2.5:2.5, rt, 2 h; (f) 1.0 eq. Boc-valine, 0.9 eq. HCTU, 2.0 eq. DIPEA, 3 mL DMF, rt, 24 h, yield 45-50%. (Note: for the synthesis of peptide **22**, **23**, **24**, **25**, **26**, **27**, **28**, **29** three amino acids replacing linker region were VIV, VIG, VGG, IVI, IVG, IGG, GGG, and GGI, respectively. All the peptides were cleaved from resin at their penultimate length, and the terminal Boc-Val-OH was attached in solution phase using ~0.1 mmol penultimate peptide precursor).

Scheme S3. Solid-phase synthesis of *N*-Boc, C-amide D-peptidomimetics using Rink amide resin (for peptide **33-39**).^a



^aReagents and conditions: (a) (i) 20% piperidine in DMF; (ii) 0.2 M Fmoc-valine, 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling; (b) Iteratively: (i) 20% piperidine in DMF; (ii) 0.2 M **11**, 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling; (c) Iteratively: i. 20% piperidine in DMF; ii. 0.2 M Fmoc-amino acids (Val, Fmoc-NH(CH₂)_nCO₂H, Aib, ^DVal, ^DPhe, Aib, ^DIle, ^DVal, ^DVal, Aib, Gly), 0.2 M DIC, and 0.2 M OXYMA in DMF, 70 °C, 8 min, double coupling (d) 20% piperidine in DMF (e) TFA:TIPS:H₂O:DoDt :: 92.5:2.5:2.5:2.5, rt, 2h; (f) 1.0 eq. Boc-^Dvaline, 0.9 eq. HCTU, 2.0 eq. DIPEA, 3 mL DMF, rt, 24 h, yield 45-50%. (Note: All peptides were cleaved from the resin at their penultimate length, and the terminal Boc-^DVal-OH was attached in solution phase using ~0.1 mmol penultimate peptide precursor. Alkyl spacers n = 0, 2, 4, 8, 10, and 11 were used for HPI-TSAs **33-39**, respectively).

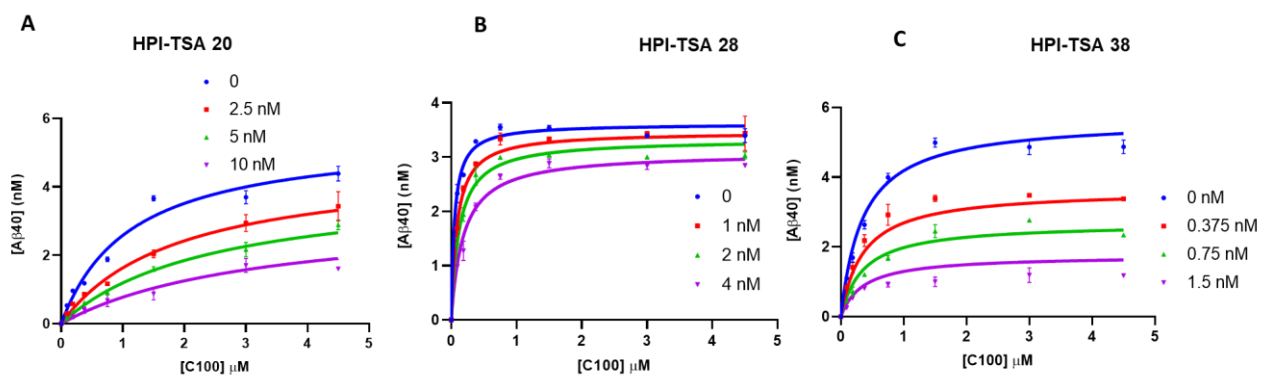


Figure S1. Michaelis-Menten plot of top inhibitors of γ -secretase designed for each stage of substrate recognition. **A)** Compound **20**, designed to trap the endoproteolysis transition state ($K_i = 2.64 \pm 0.18$ nM). **B)** Compound **28**, designed to capture helix unwinding ($K_i = 1.19 \pm 0.10$ nM). **C)** Compound **38**, designed to capture lateral gating ($K_i = 0.65 \pm 0.02$ nM).



Figure S2: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **20**. calcd for $C_{112}H_{187}N_{23}O_{20}Na$: 2197.4221; found: 2197.4341.

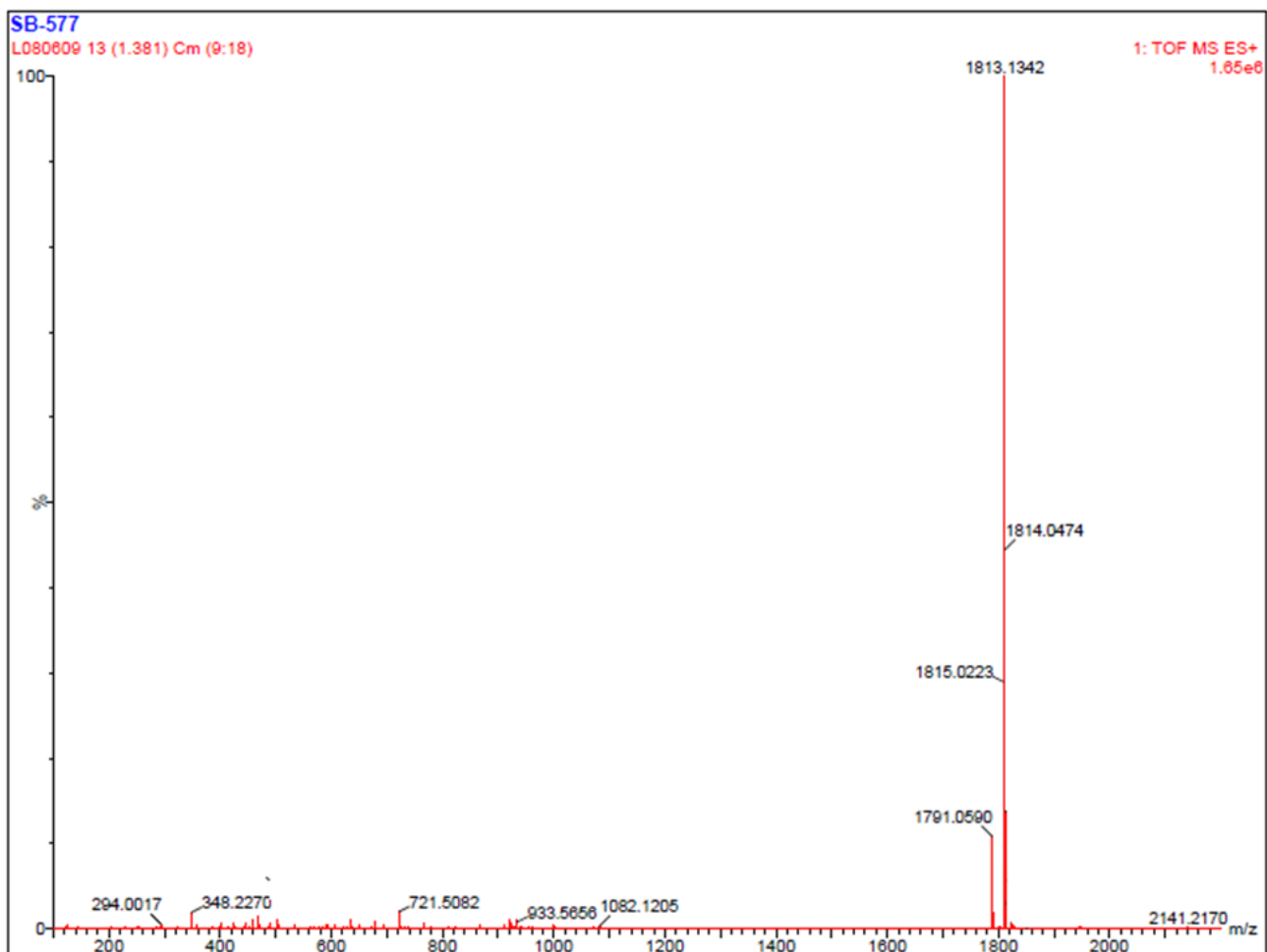


Figure S3: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **21**. calcd for $C_{112}H_{187}N_{23}O_{20}Na$: 1813.1372; found: 1813.1342.

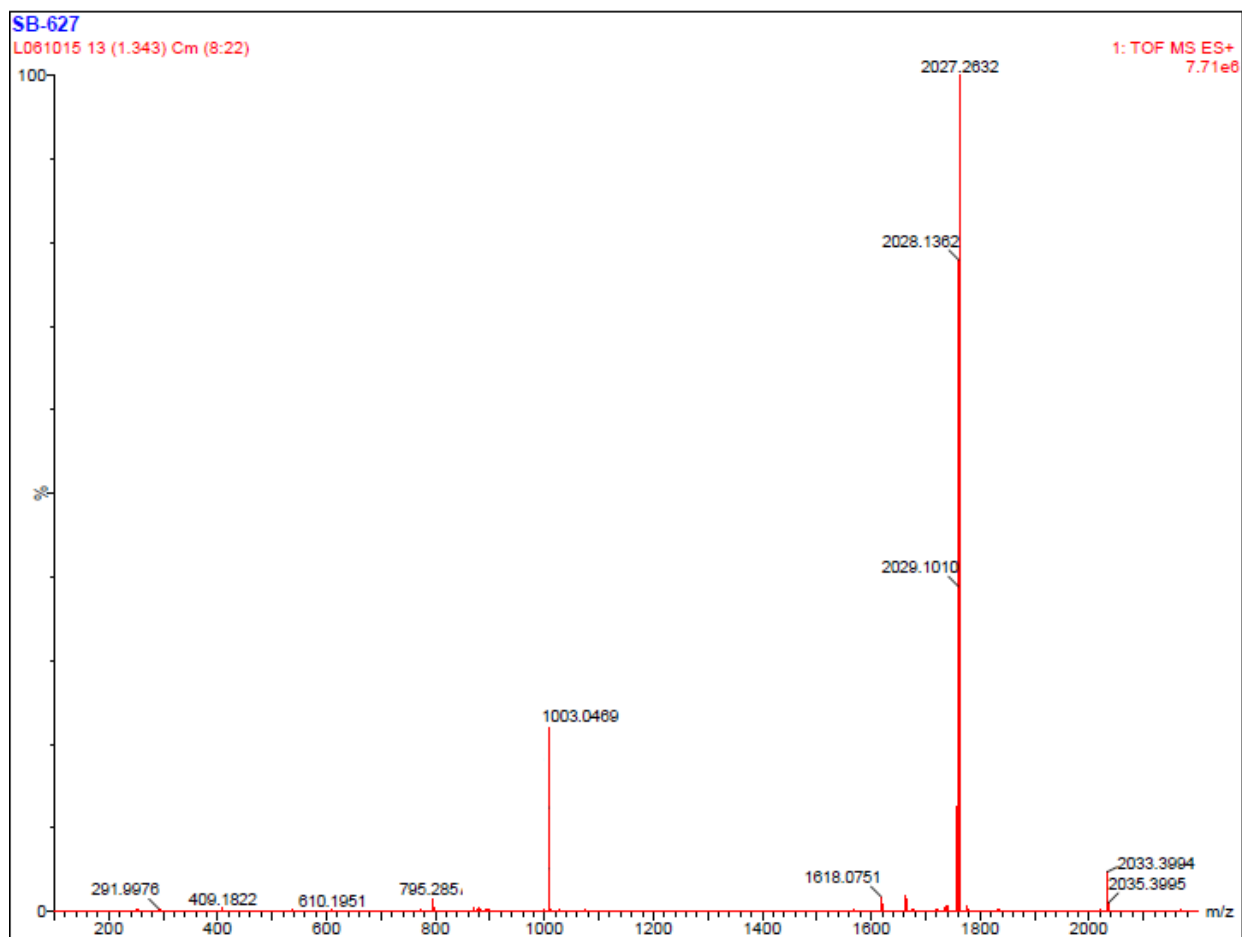


Figure S4: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **22**. calcd for $C_{104}H_{169}N_{19}O_{20}Na$: 2027.2689; found: 2027.2632.

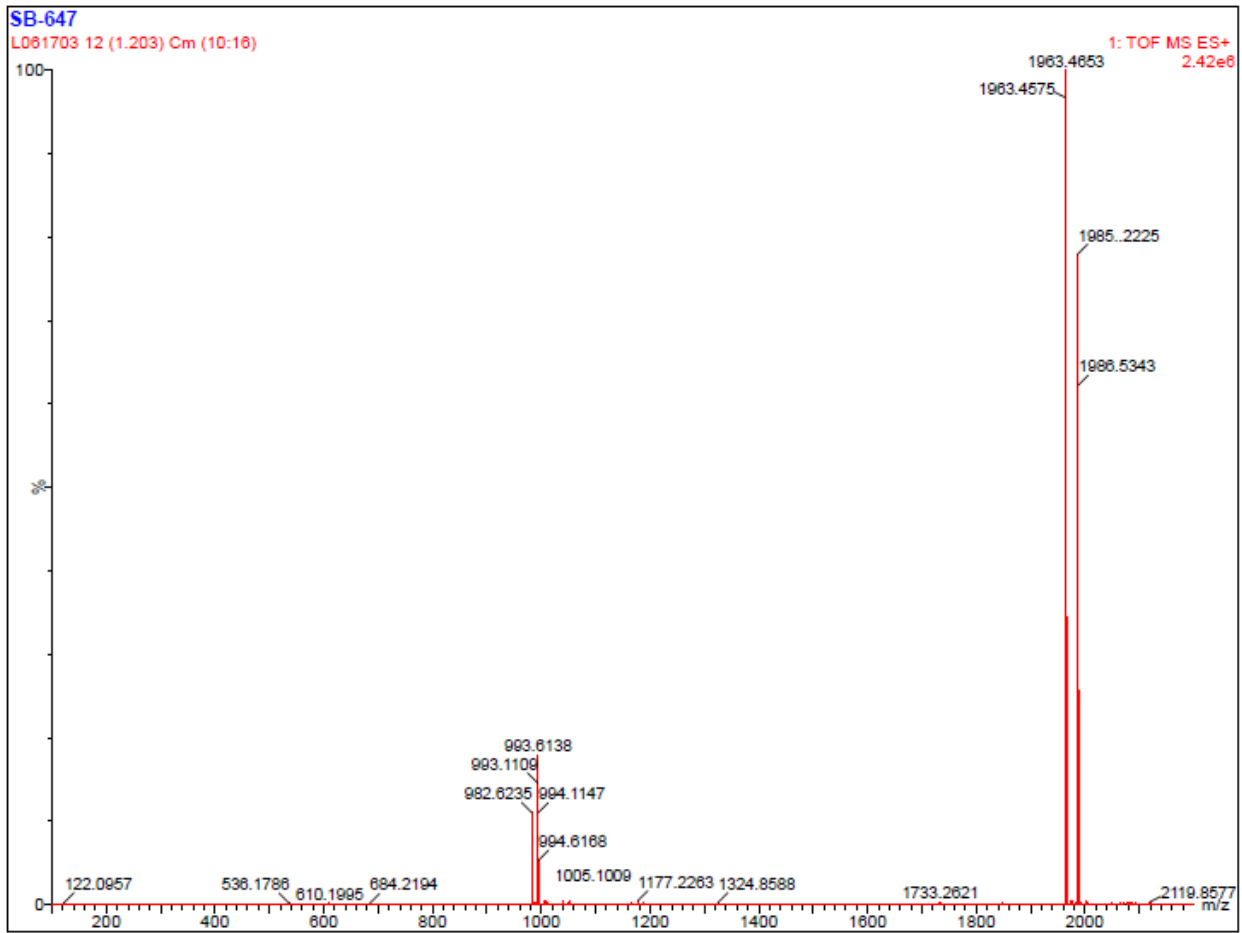


Figure S5: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **23**. calcd for $C_{101}H_{163}N_{19}O_{20}Na$: 1985.2220; found: 1985.2225.

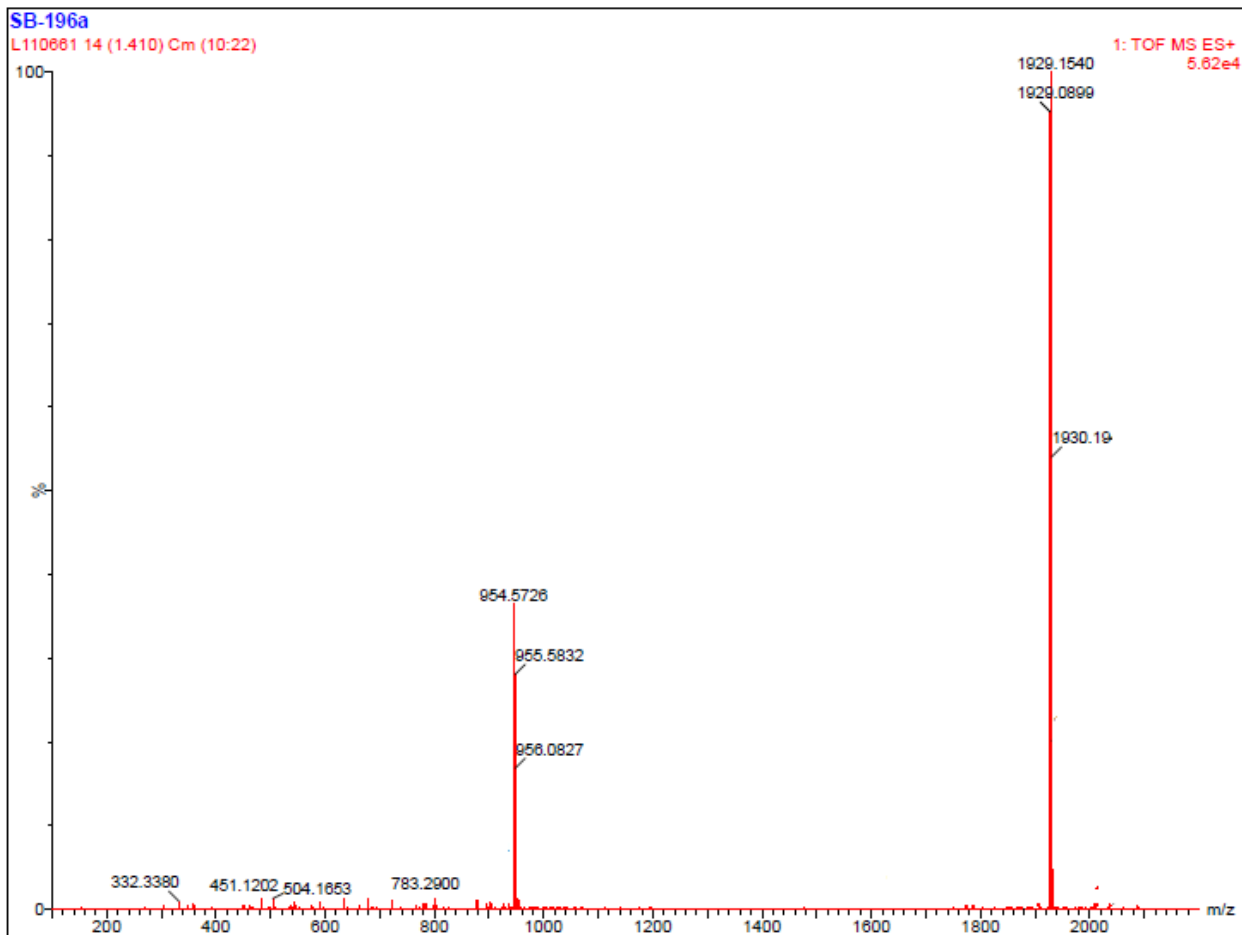


Figure S6: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **24**. calcd for $C_{97}H_{155}N_{19}O_{20}Na$: 1929.1594; found: 1929.1540.



Figure S7: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **25**. calcd for $C_{105}H_{171}N_{19}O_{20}Na$: 2041.2846; found: 2041.2835.

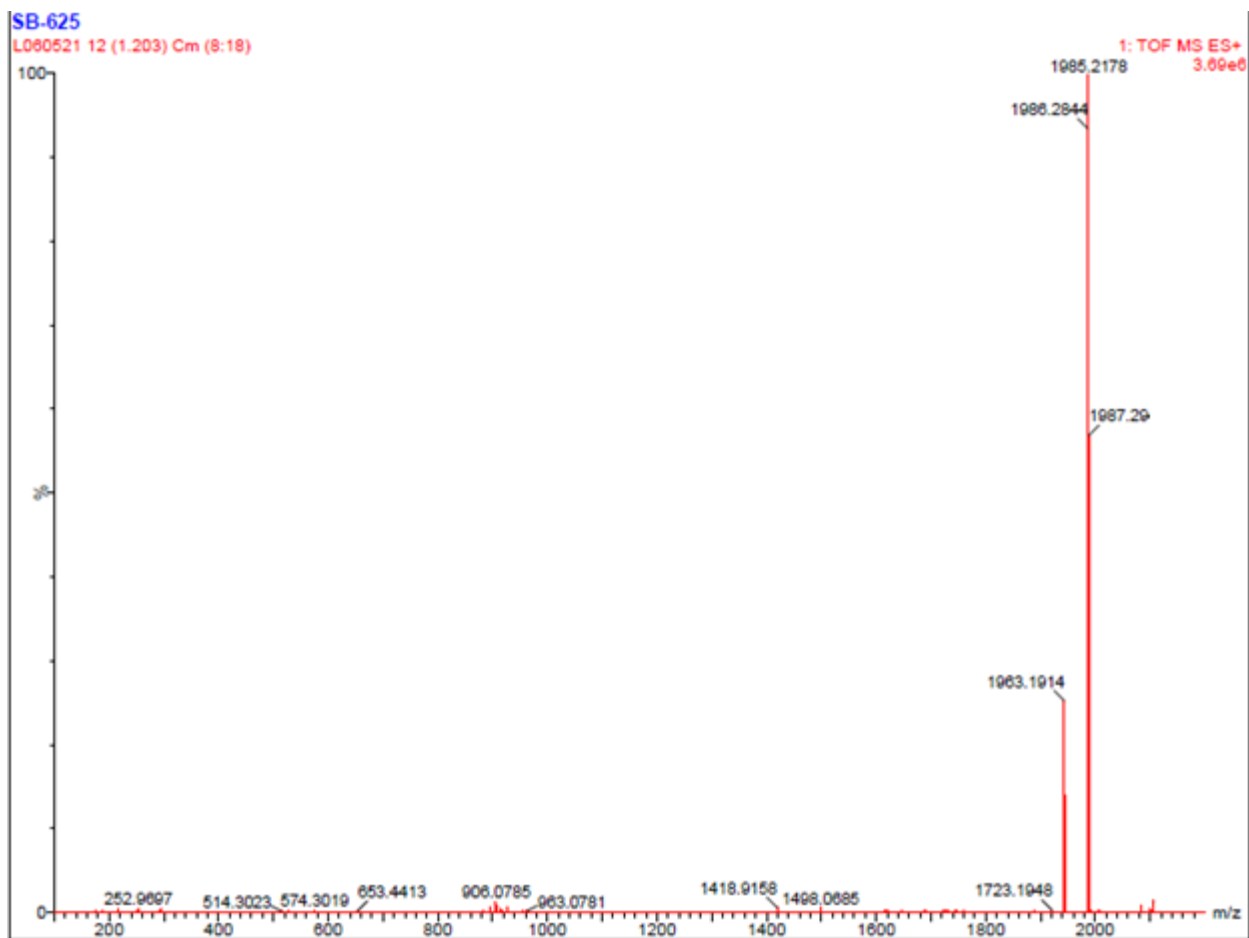


Figure S8: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **26**. calcd for $C_{101}H_{163}N_{19}O_{20}Na$: 1985.2220; found: 1985.2178.



Figure S9: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **27**. calcd for $C_{98}H_{157}N_{19}O_{20}Na$: 1943.1750; found: 1943.2187.

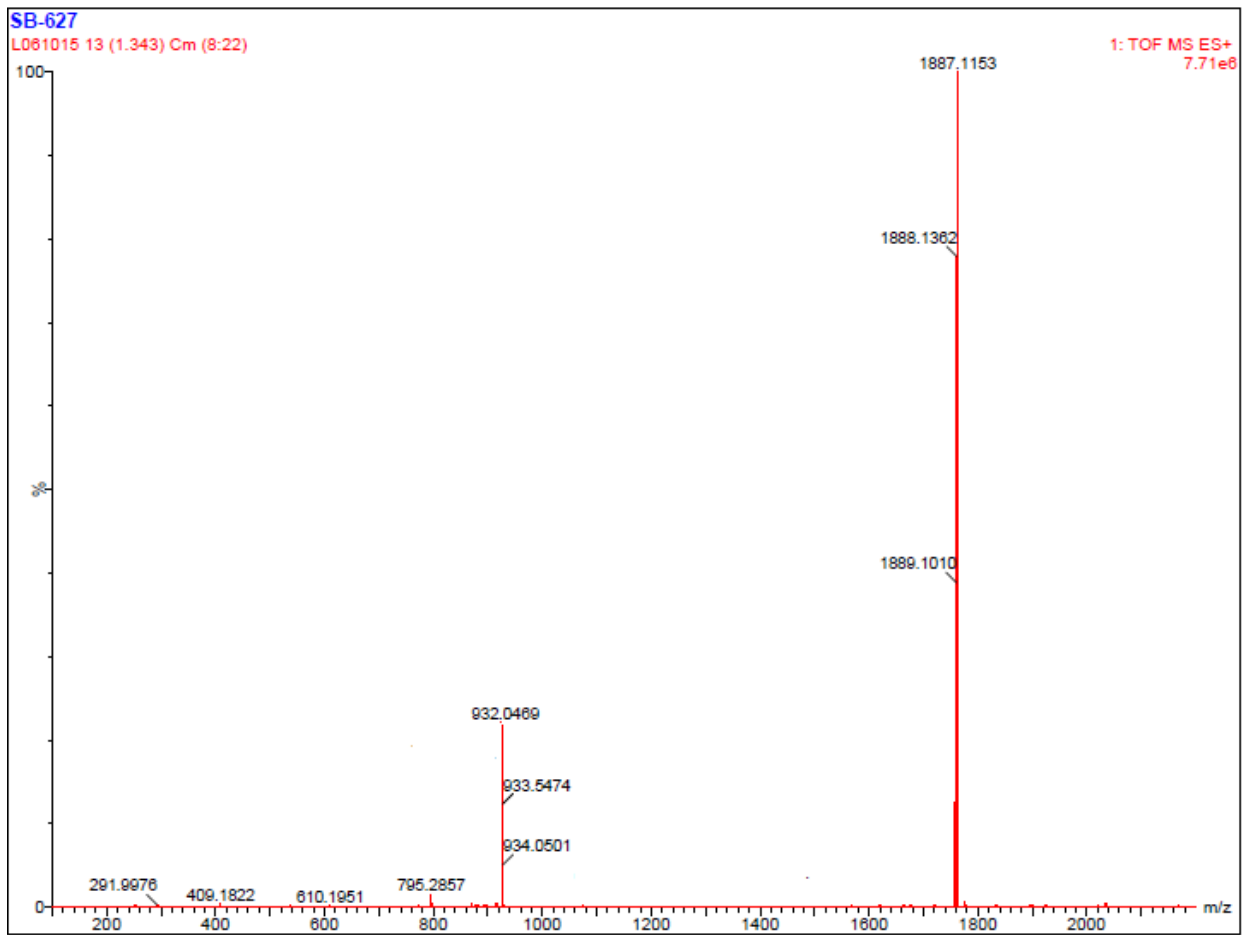


Figure S10: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **28**. calcd for $C_{94}H_{149}N_{19}O_{20}Na$: 1887.1124; found: 1887.1153.

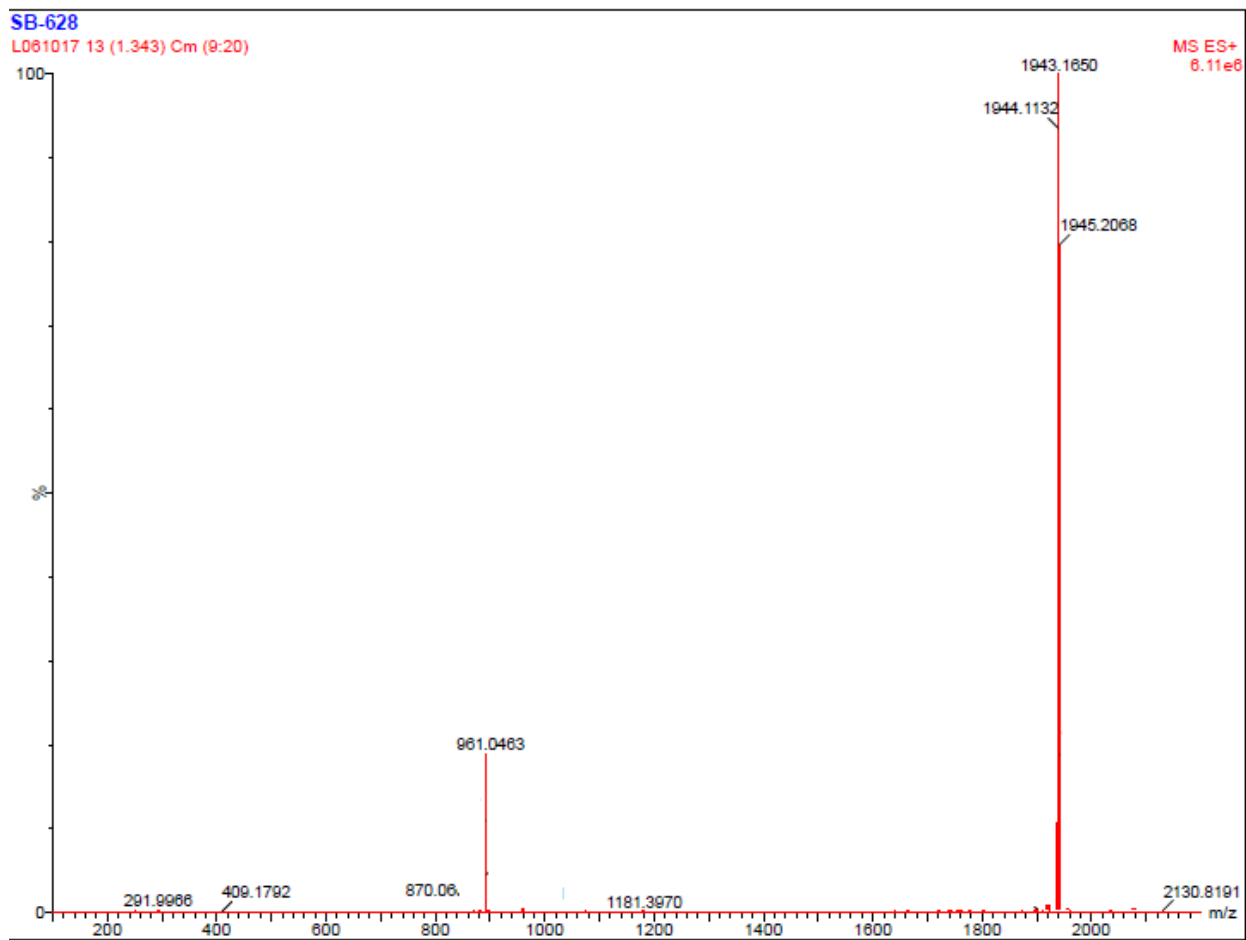


Figure S11: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **29**. calcd for $C_{98}H_{157}N_{19}O_{20}Na$: 1943.1750; found: 1943.1650.

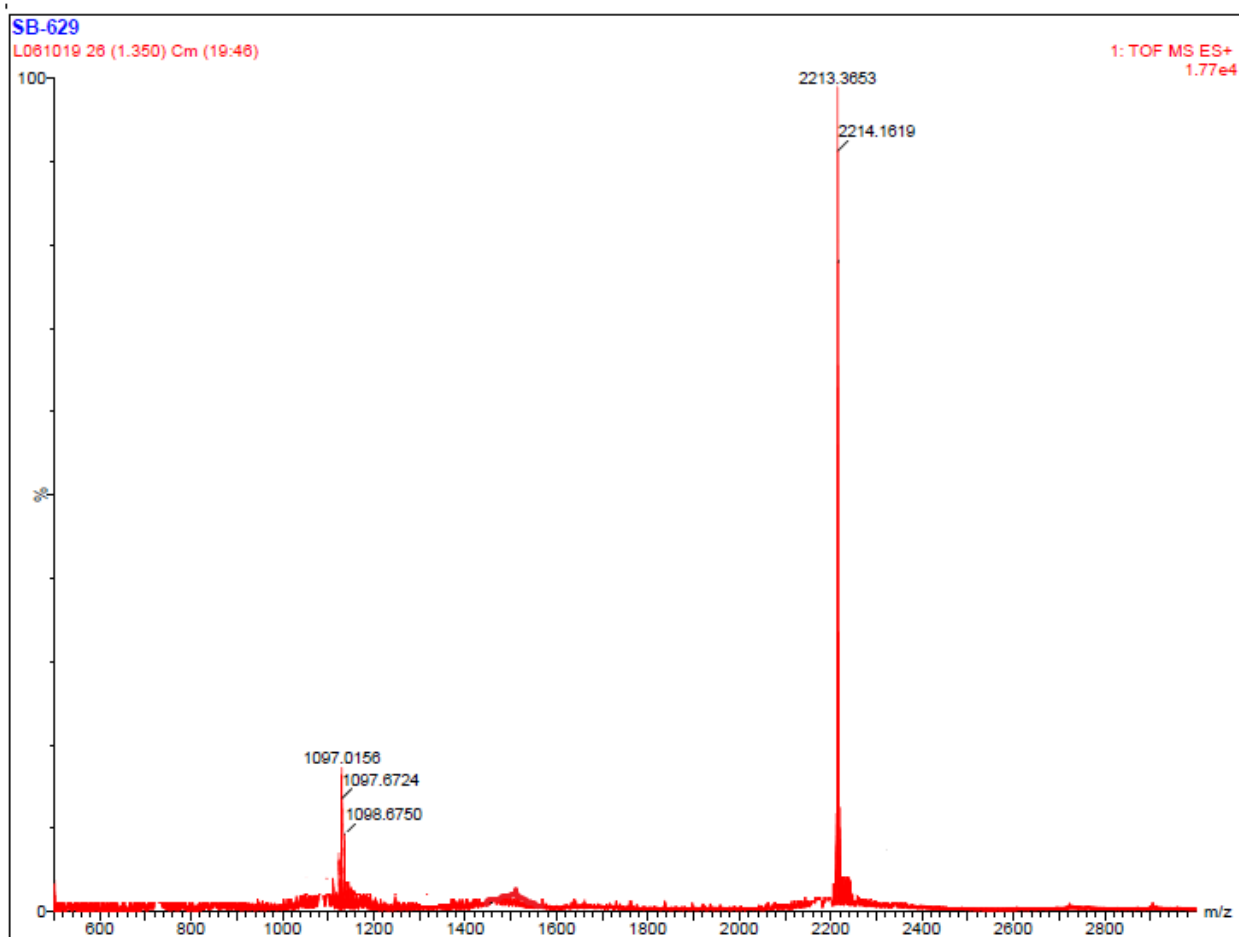


Figure S12: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **30**. calcd for $C_{109}H_{179}N_{25}O_{22}Na$: 2213.3555; found: 2213.3653.

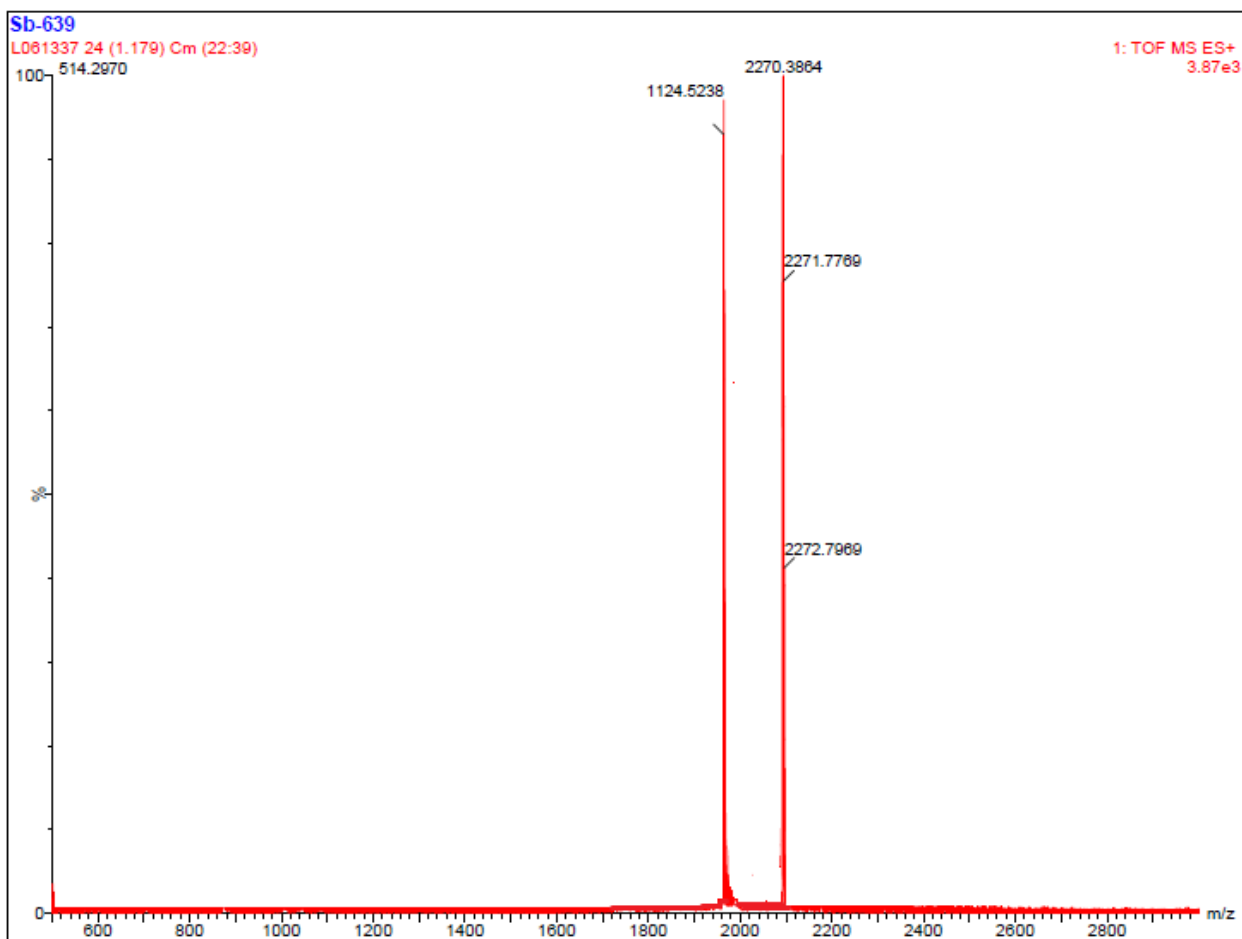


Figure S13: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **31**. calcd for $C_{111}H_{182}N_{26}O_{23}Na$: 2270.3769; found: 2270.3864.

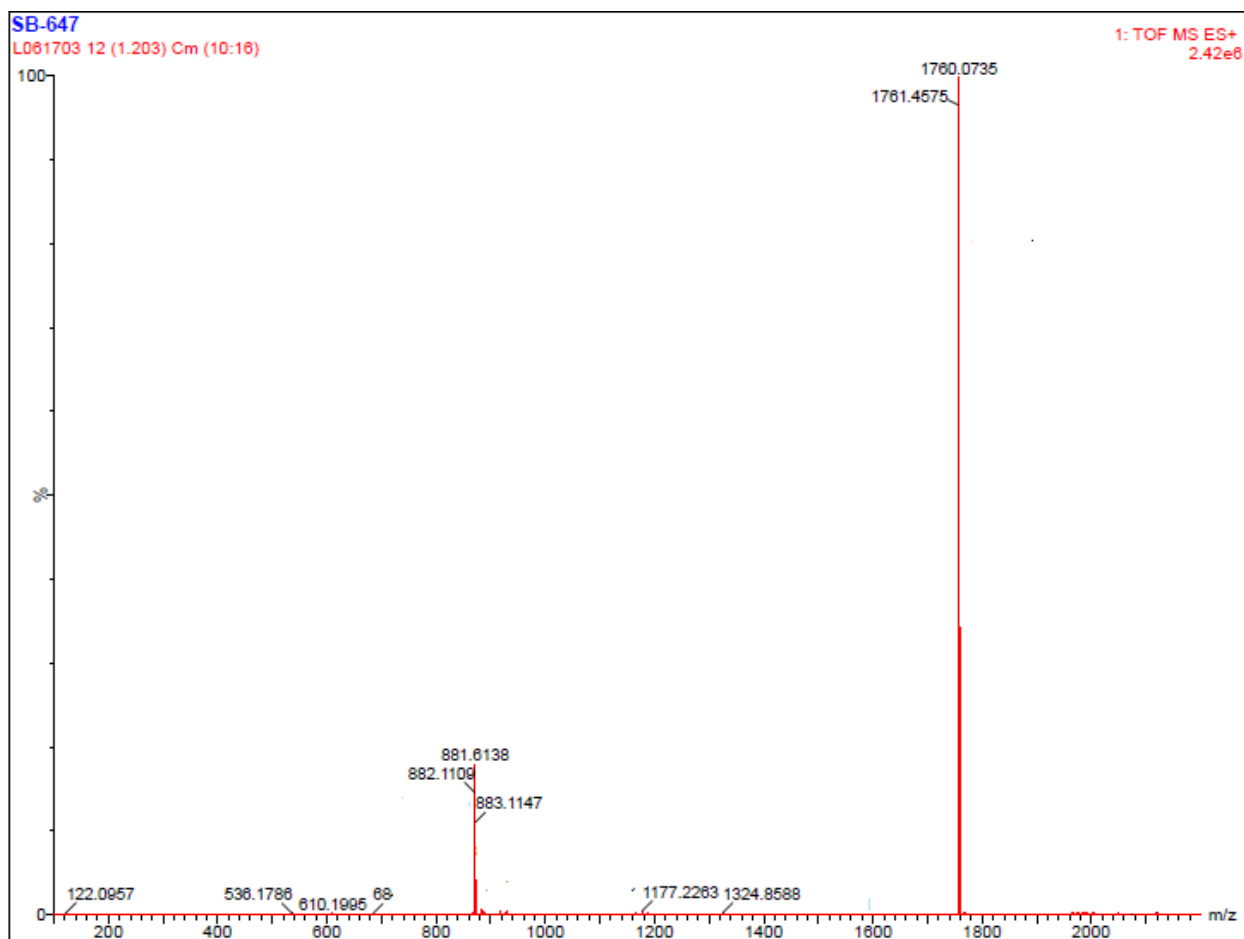


Figure S14: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **33**. calcd for $C_{90}H_{144}N_{16}O_{18}Na$: 1760.0743; found: 1760.0735.

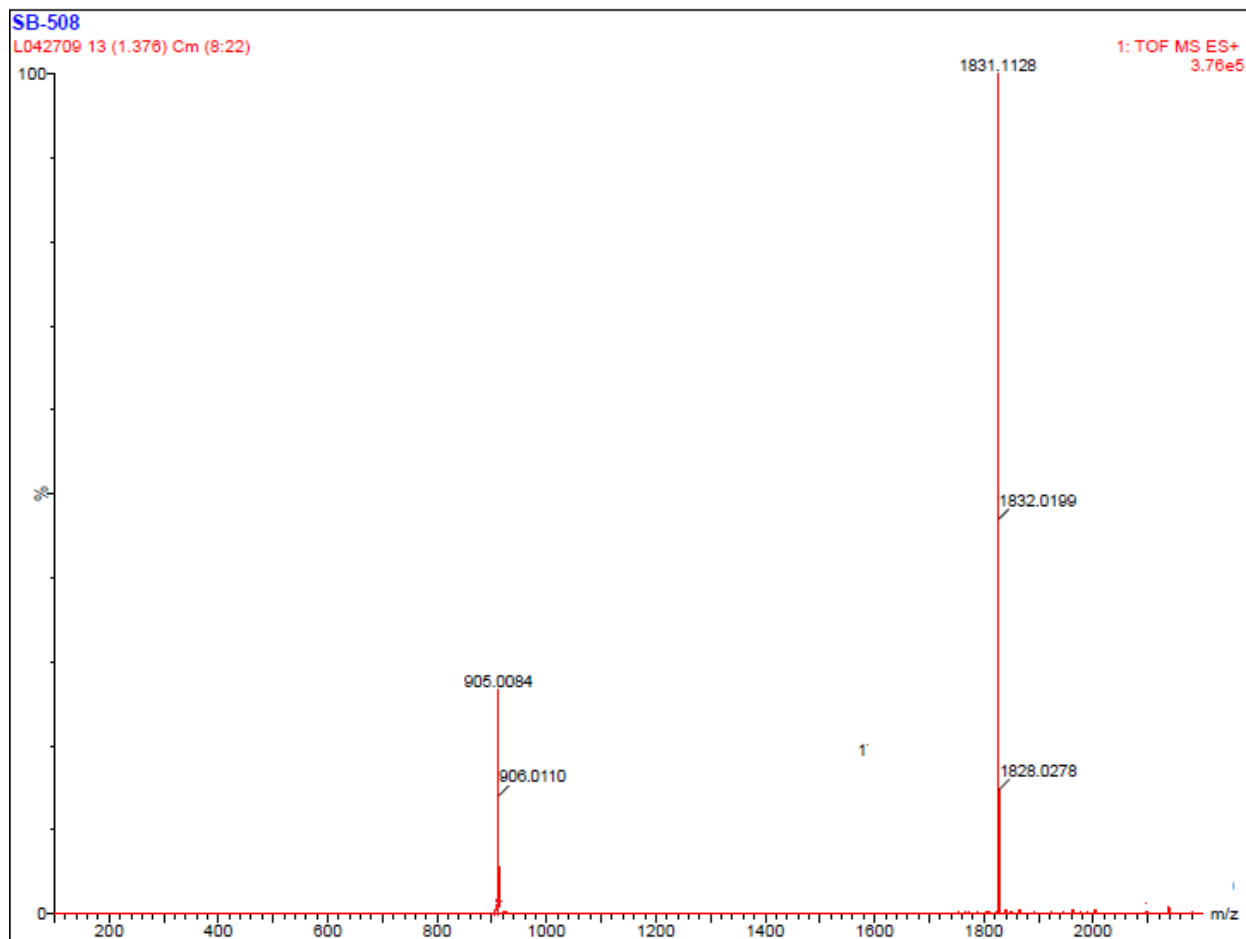


Figure S15: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **34**. calcd for $C_{93}H_{149}N_{17}O_{19}Na$: 1831.1114; found: 1831.1128.

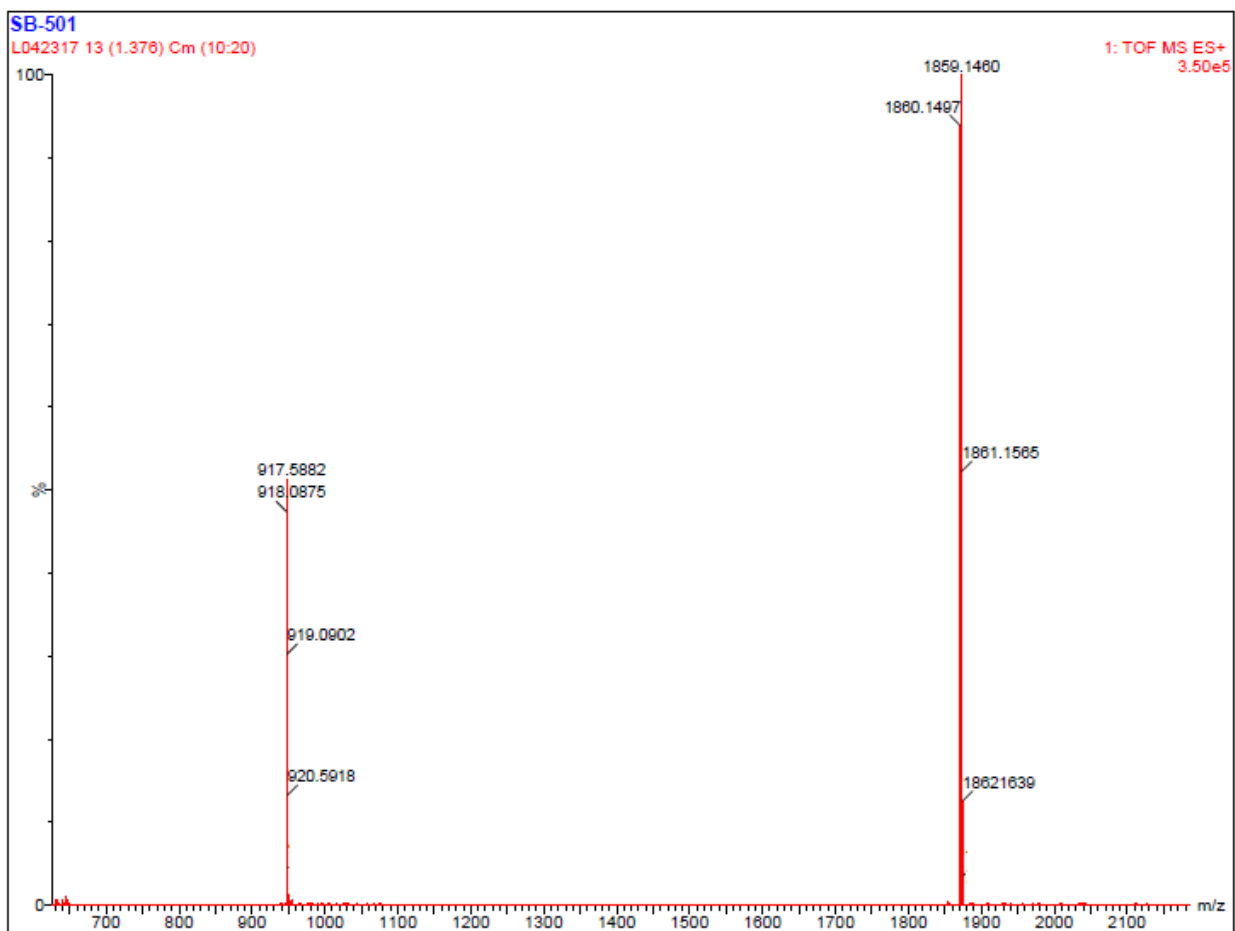


Figure S16: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **35**. calcd for $C_{95}H_{153}N_{17}O_{19}Na$: 1859.1427; found: 1859.1460.

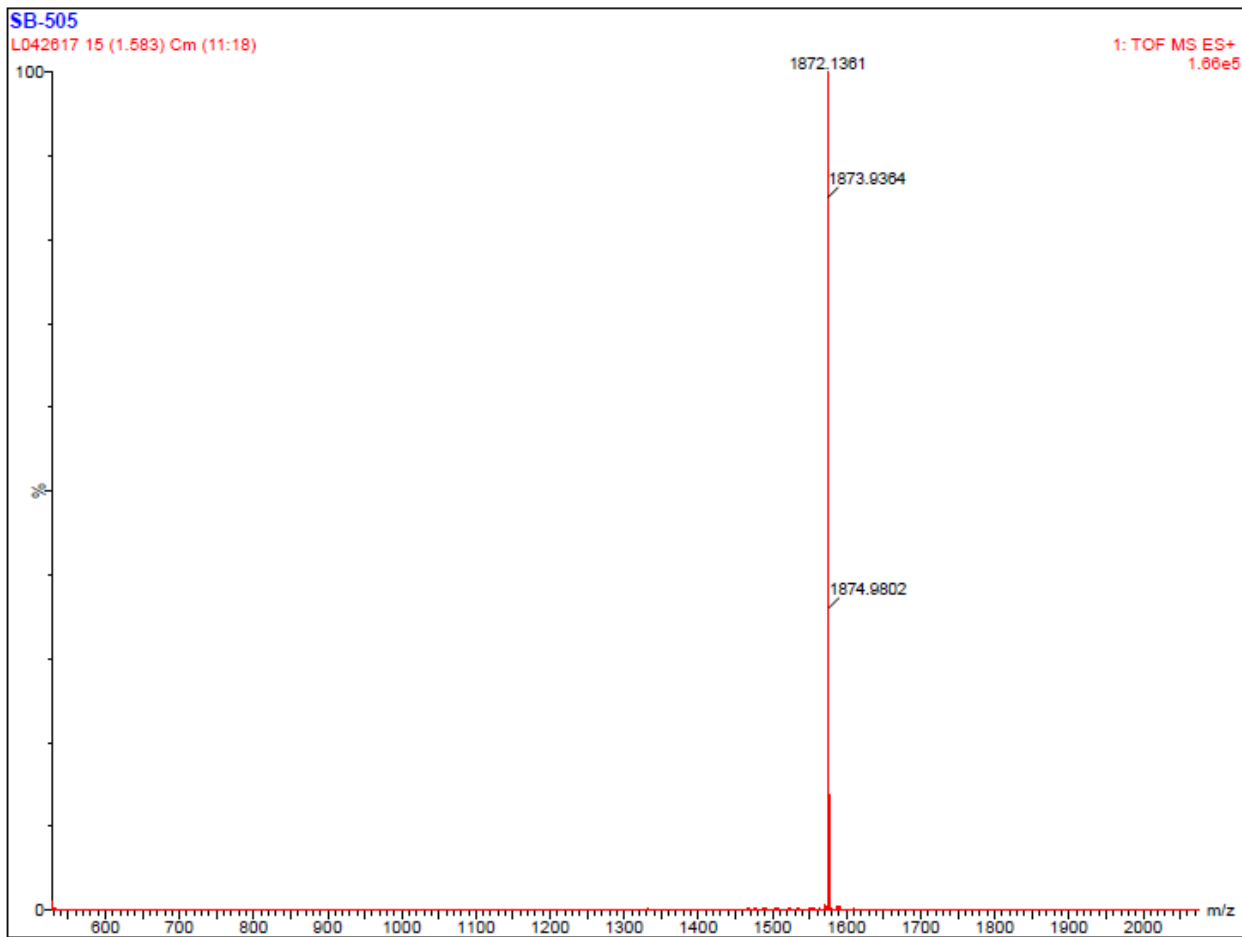


Figure S17: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **36**. calcd for $C_{95}H_{153}N_{17}O_{19}Na$: 1872.1427; found: 1872.1361.

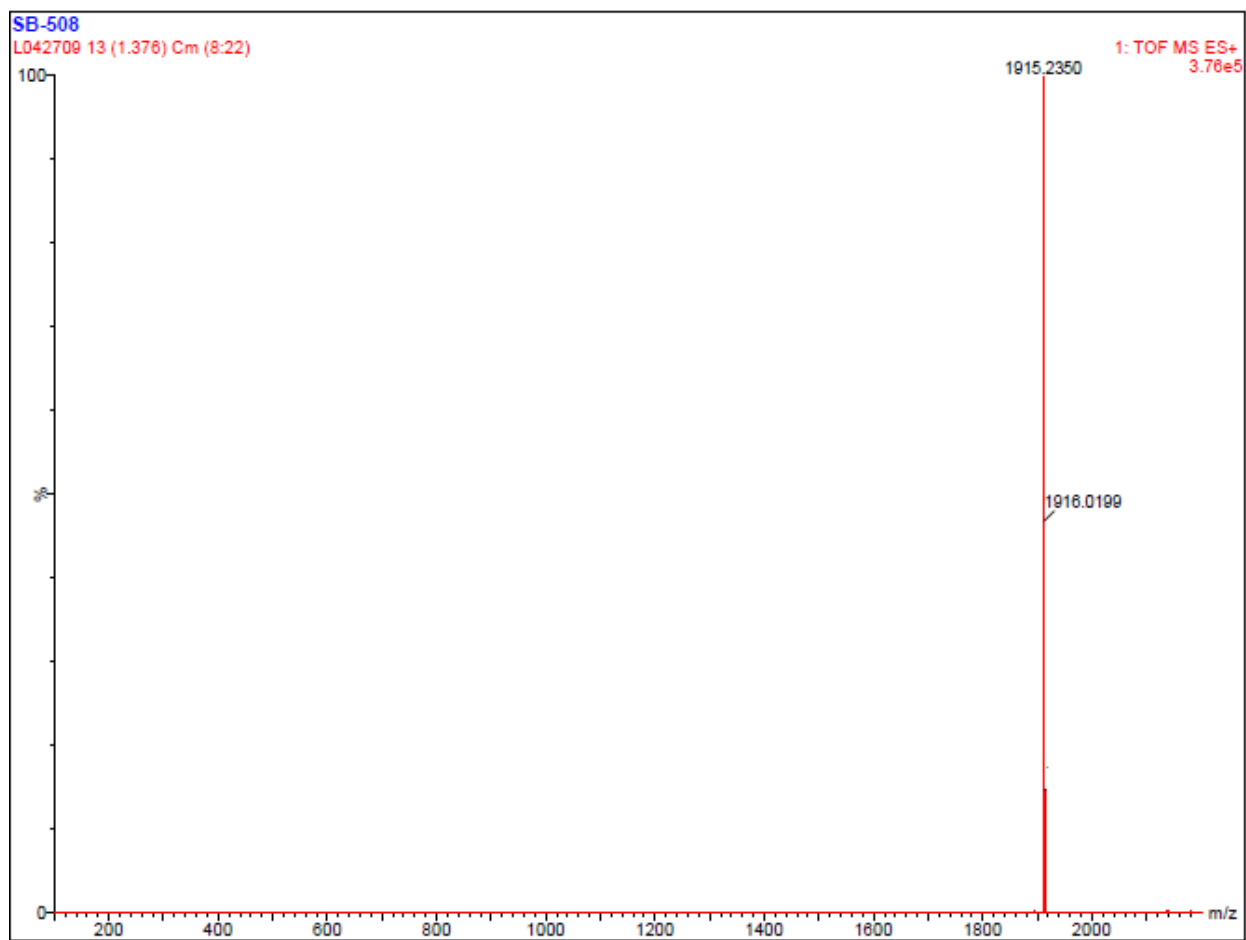


Figure S18: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **37**. calcd for $C_{99}H_{161}N_{17}O_{19}Na$: 1915.2053; found: 1915.2350.



Figure S19: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **38**. calcd for $C_{101}H_{165}N_{17}O_{19}Na$: 1943.2366; found: 1943.2636.

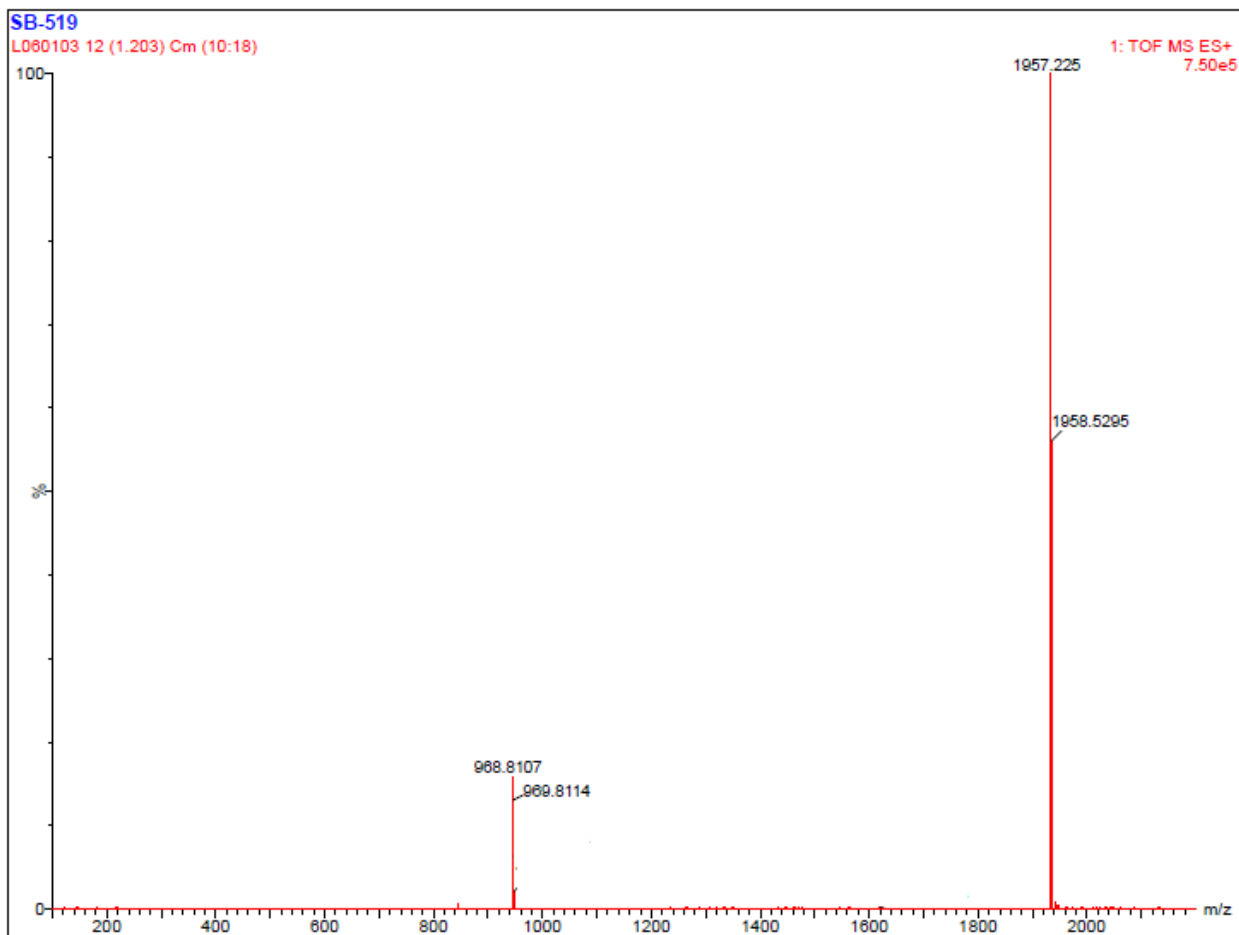


Figure S20: Section of HRMS (ESI): m/z $[M + Na]^+$ spectra for the synthesized peptide **39**. calcd for $C_{102}H_{163}N_{17}O_{19}Na$: 1957.2245; found: 1957.2225.

Sample Summary:

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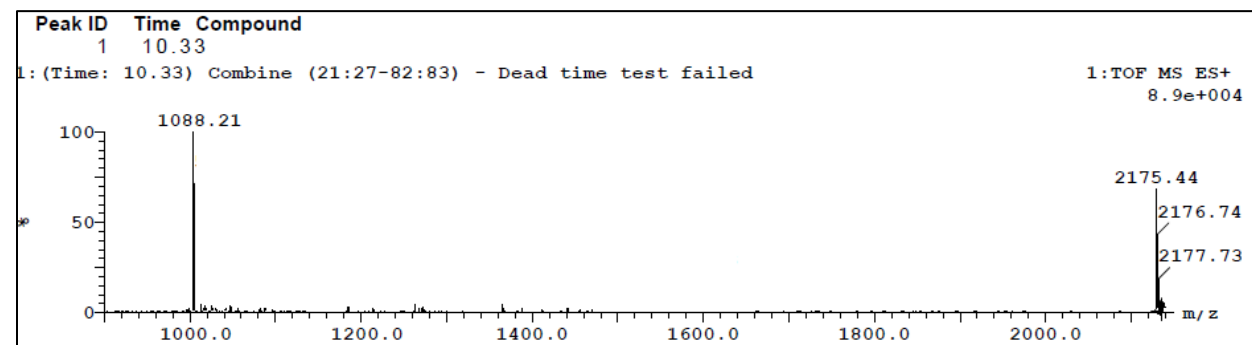
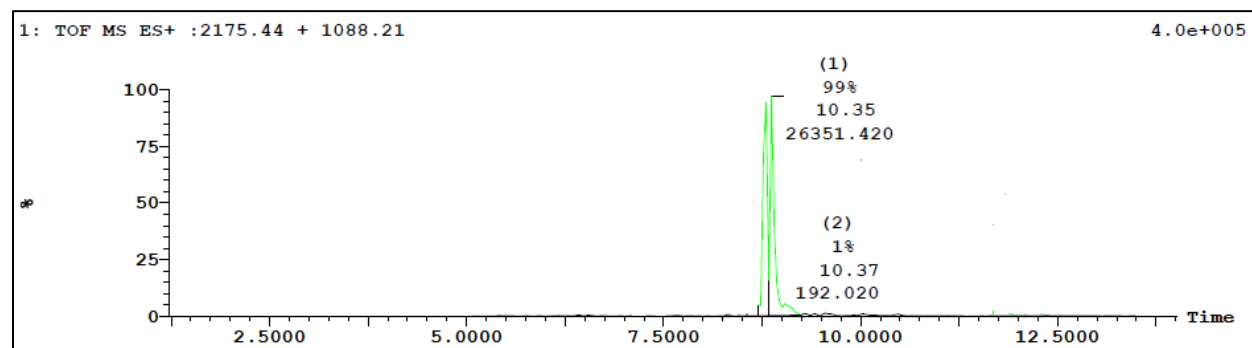
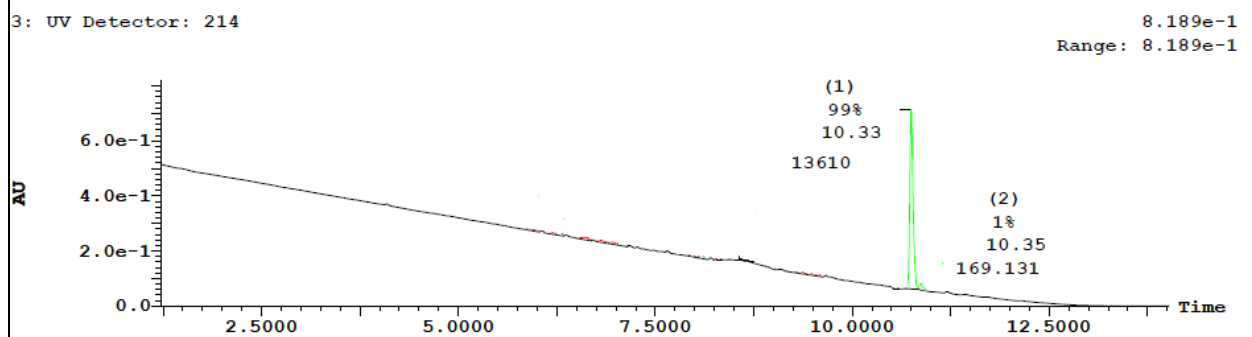


Figure S21. LC/TOF-ES-MS spectra of the synthesized peptide **20** is shown in positive mode and the peak at 10.33 min belongs to the peptide **20** ($m/z = 2175$). The purity of peptide **20** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 99%.

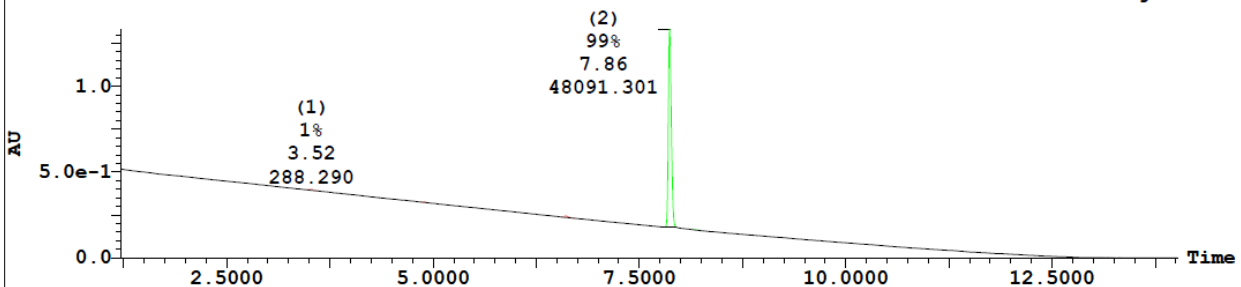
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ID Sanjay-171030-01 File SB171030WT012 Date 01-Nov-2017 Time 16:37:13 Description MDF031171

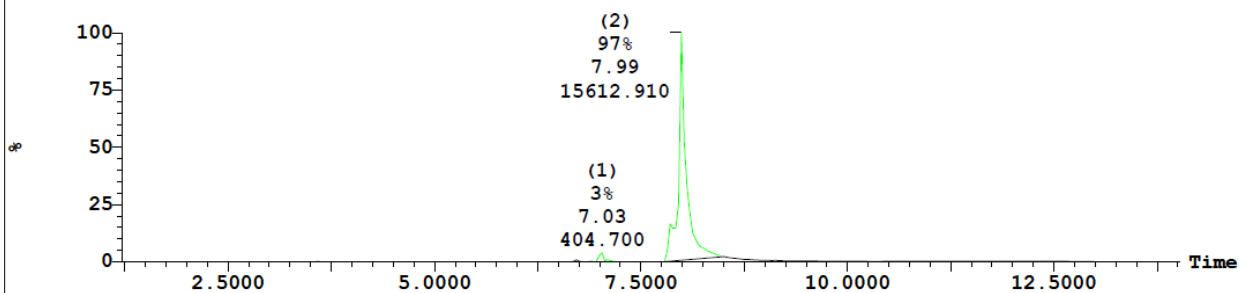
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1.329
Range: 1.329



1: TOF MS ES+ : 1791.18 + 896.09

1.4e+005



Peak ID Time Compound

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1: TOF MS ES+
5.7e+003

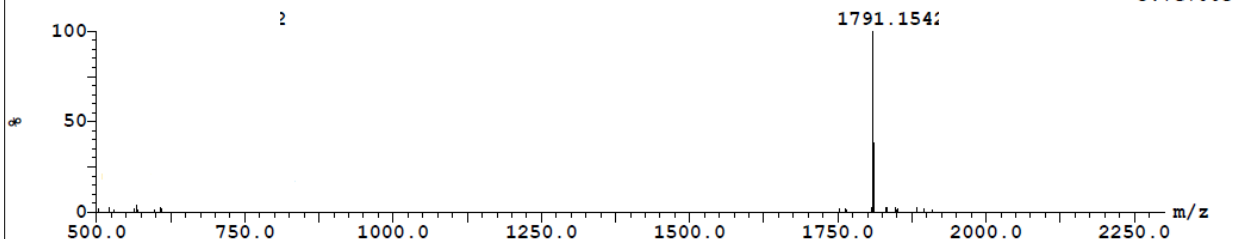


Figure S22. LC/TOF-ES-MS spectra of the synthesized peptide **21** is shown in positive mode and the peak at 7.86 min belongs to the peptide **21** ($m/z = 1791$). The purity of peptide **21** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

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ID Sanjay-171030-03 File SB171030WT014 Date 01-Nov-2017 Time 17:08:42 Description MDF031176

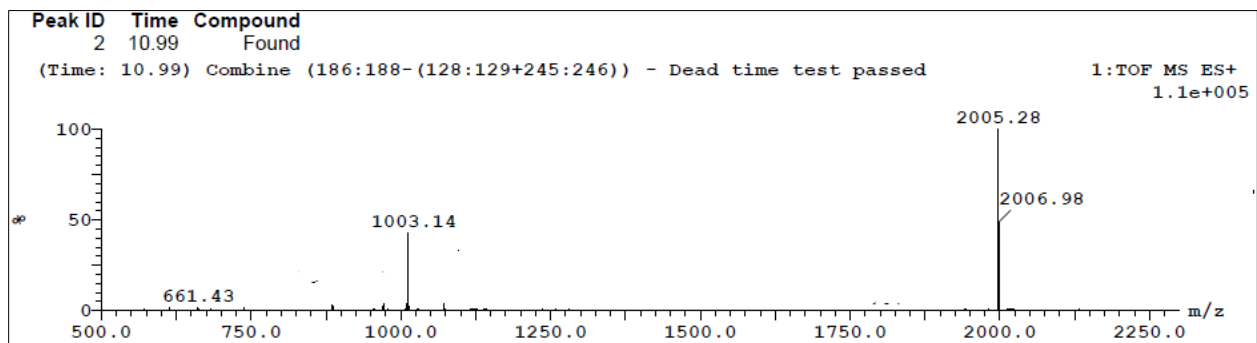
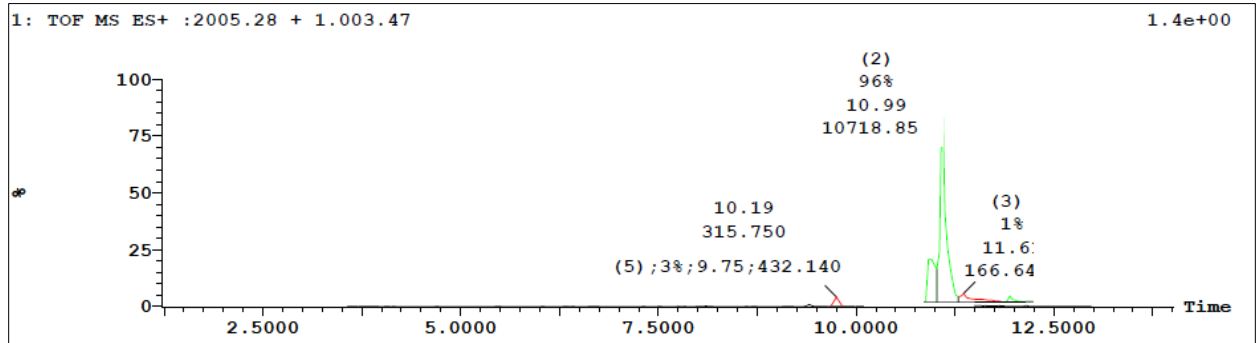
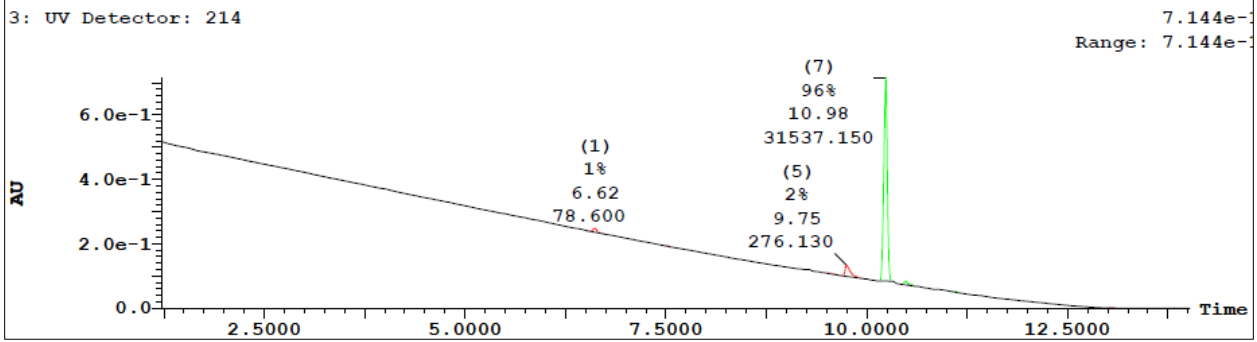


Figure S23. LC/TOF-ES-MS spectra of the synthesized peptide **22** is shown in positive mode and the peak at 10.33 min belongs to the peptide **22** ($m/z = 2005$). The purity of peptide **22** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

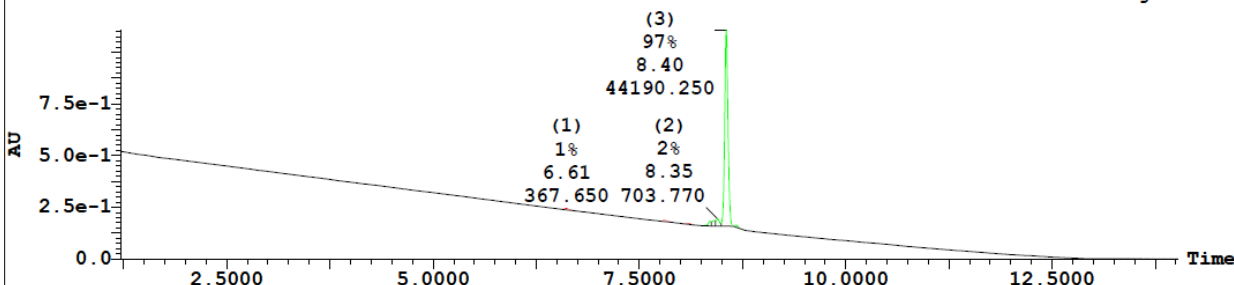
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								565.0199	0.0000	

ID Sanjay-171107-01 File SB171107WT002 Date 07-Nov-2017 Time 11:54:42 Description SCCT009900

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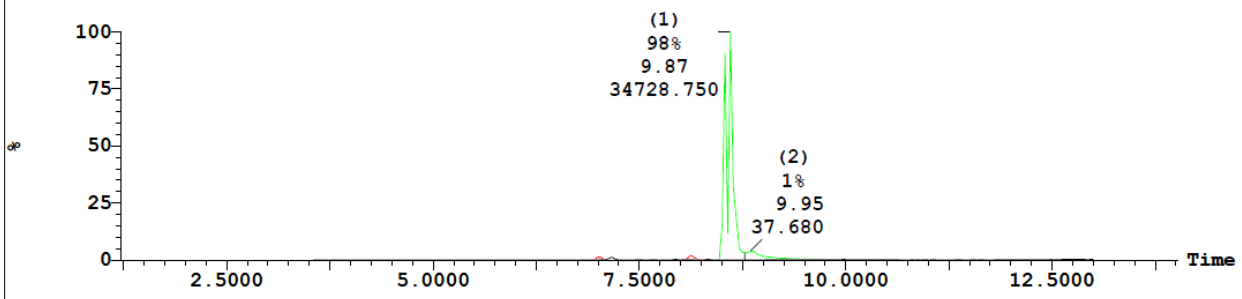
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Range: 1.105



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3.6e+005



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1:TOF MS ES+

5.9e+00

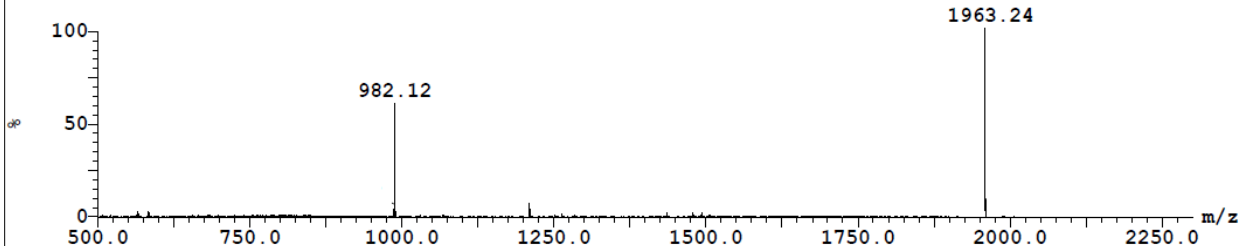


Figure S24. LC/TOF-ES-MS spectra of the synthesized peptide **23** is shown in positive mode and the peak at 10.33 min belongs to the peptide **23** ($m/z = 1963$). The purity of peptide **23** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 97%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
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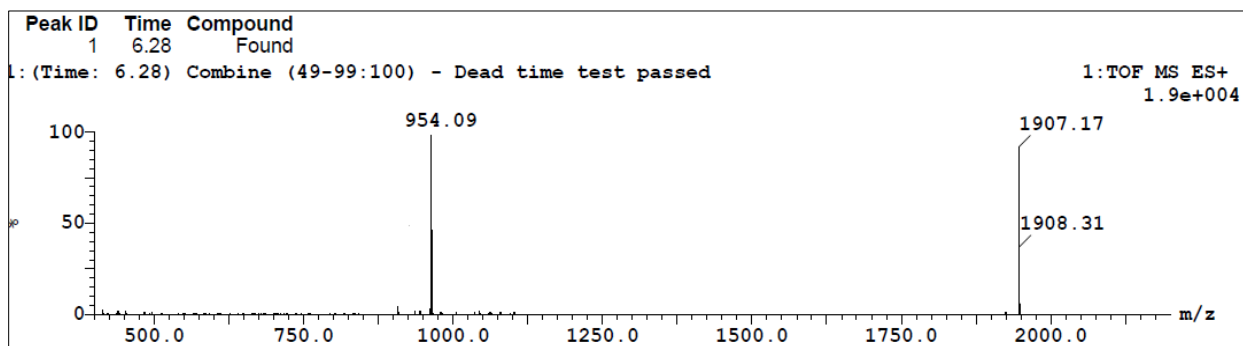
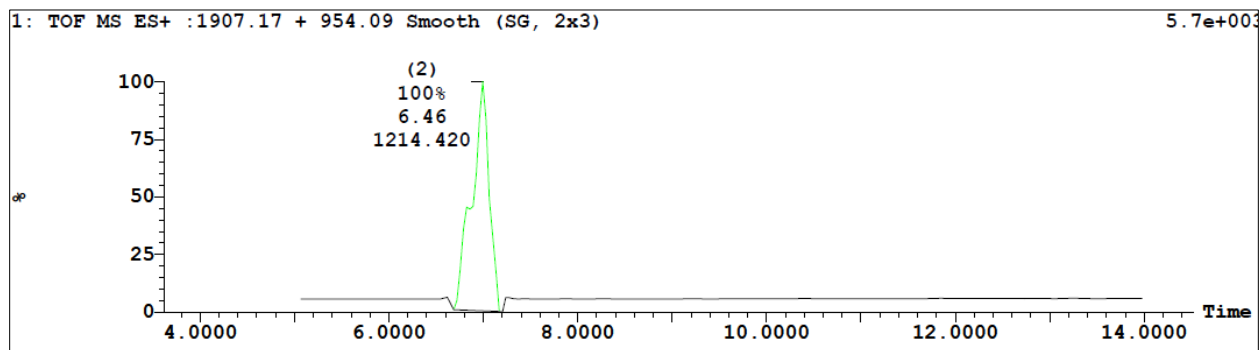
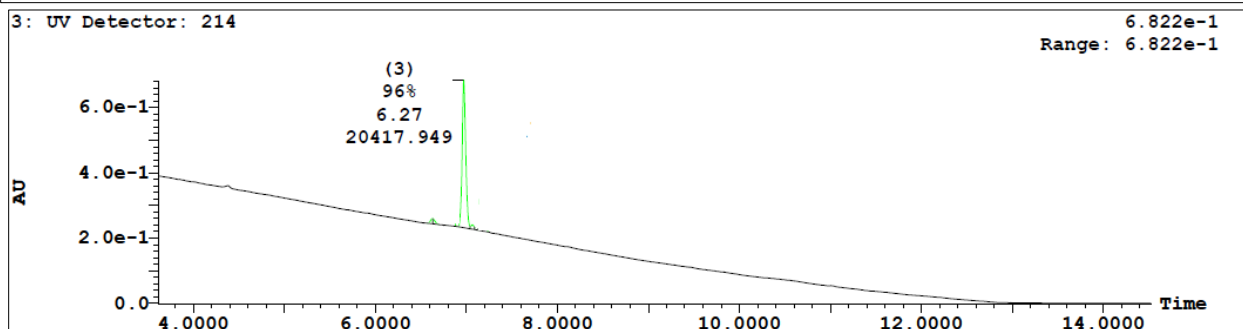


Figure S25. LC/TOF-ES-MS spectra of the synthesized peptide **24** is shown in positive mode and the peak at 6.28 min belongs to the peptide **24** ($m/z = 1907$). The purity of peptide **24** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
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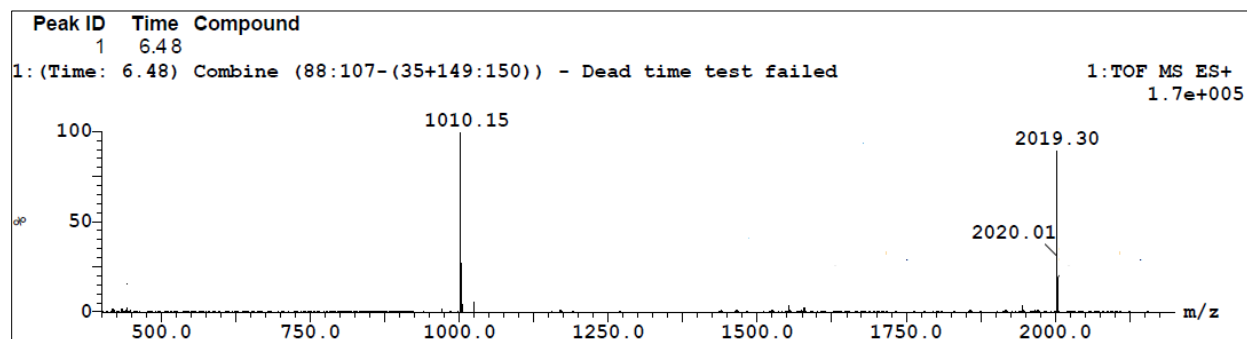
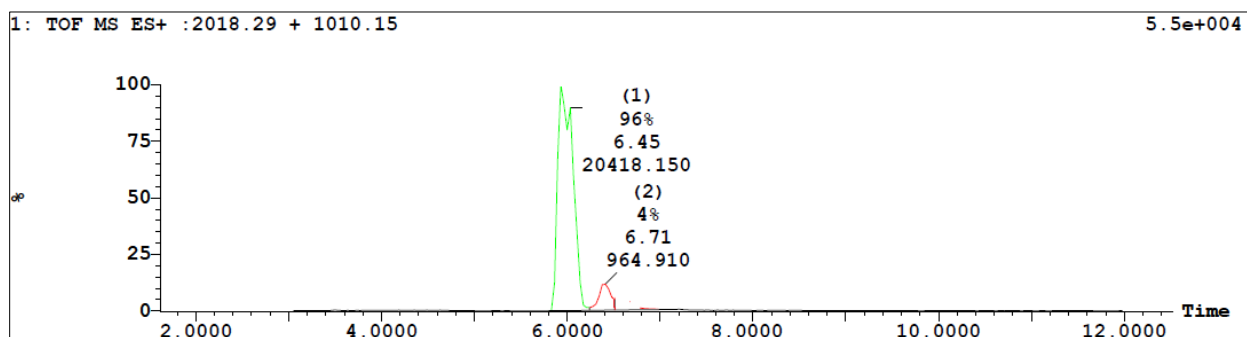
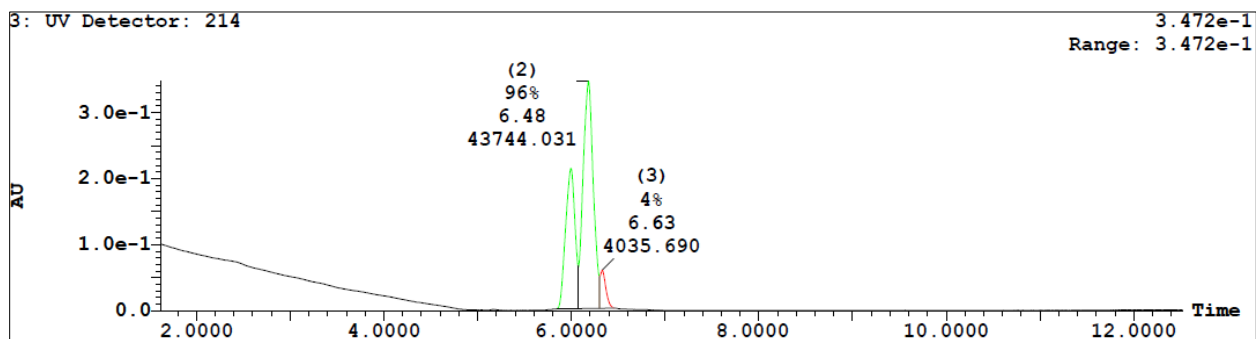


Figure S26. LC/TOF-ES-MS spectra of the synthesized peptide **25** is shown in positive mode and the peak at 6.48 min belongs to the peptide **25** ($m/z = 2019$). The purity of peptide **25** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs	Peak	Calc Mass	Result Mass	Error mDa
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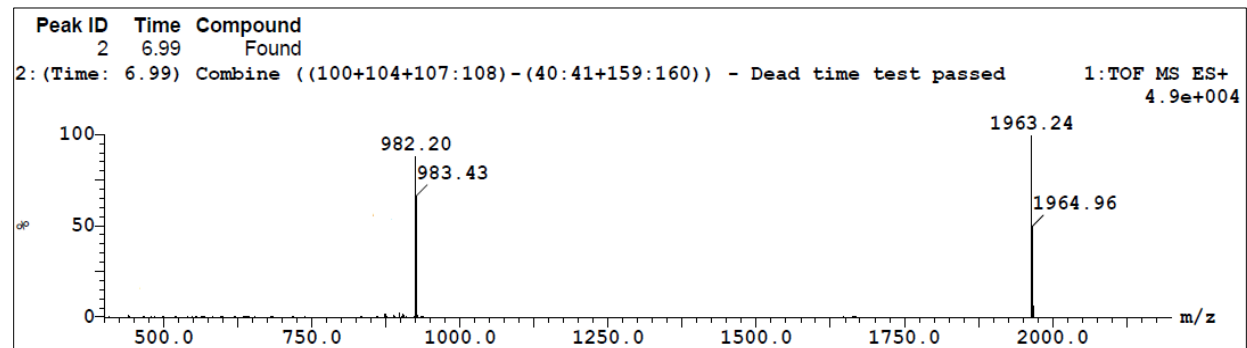
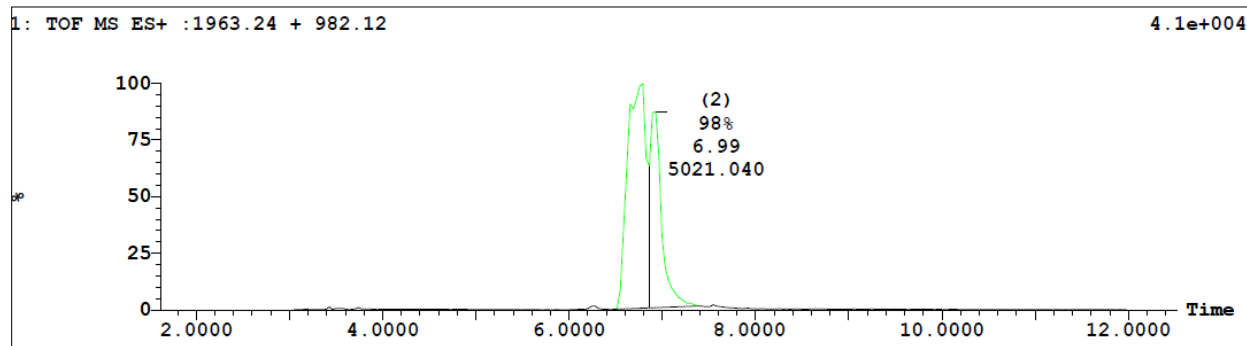
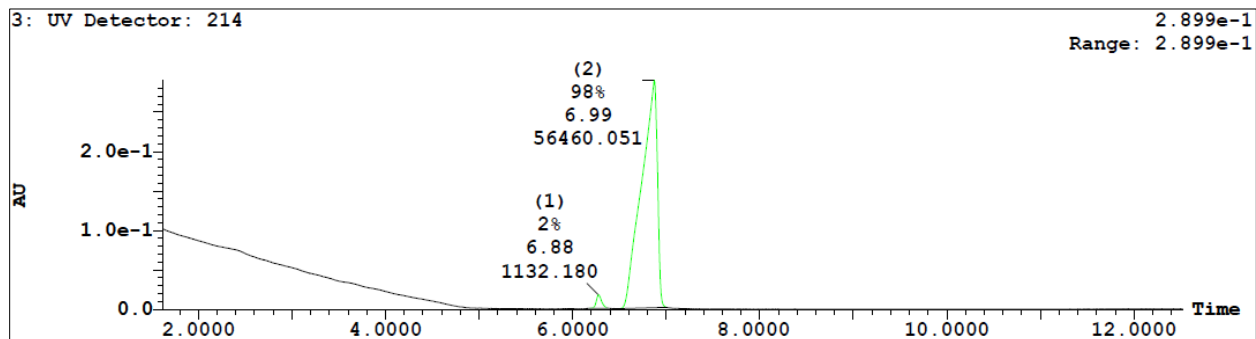


Figure S27. LC/TOF-ES-MS spectra of the synthesized peptide **26** is shown in positive mode and the peak at 6.99 min belongs to the peptide **26** ($m/z = 1963$). The purity of peptide **26** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 98%.

Sample Summary:

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								961.0981	961.0488	-2.70
								531.9971	0.0000	

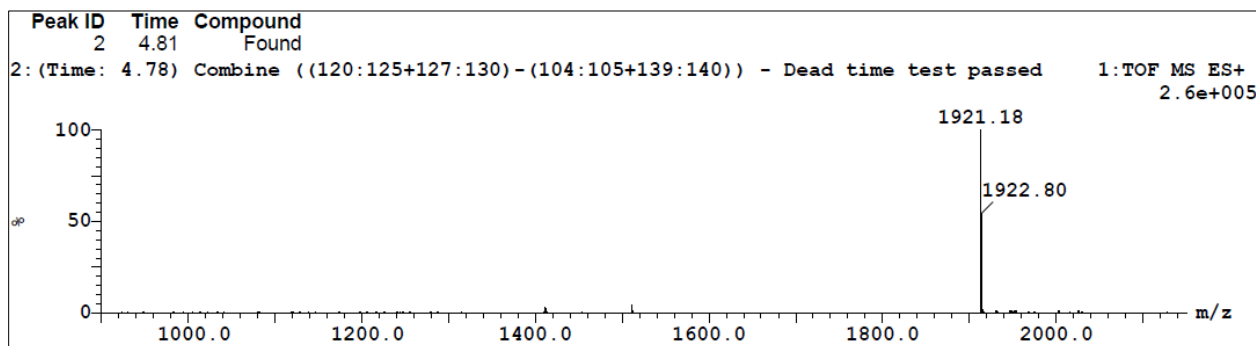
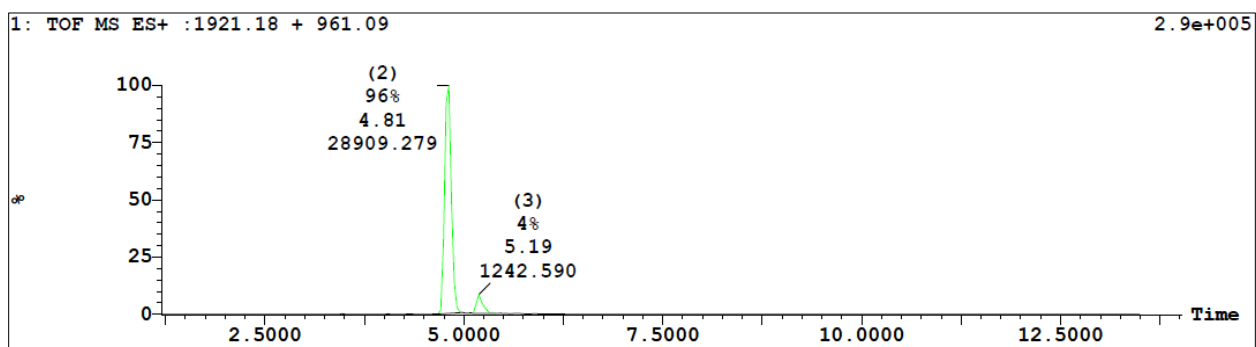
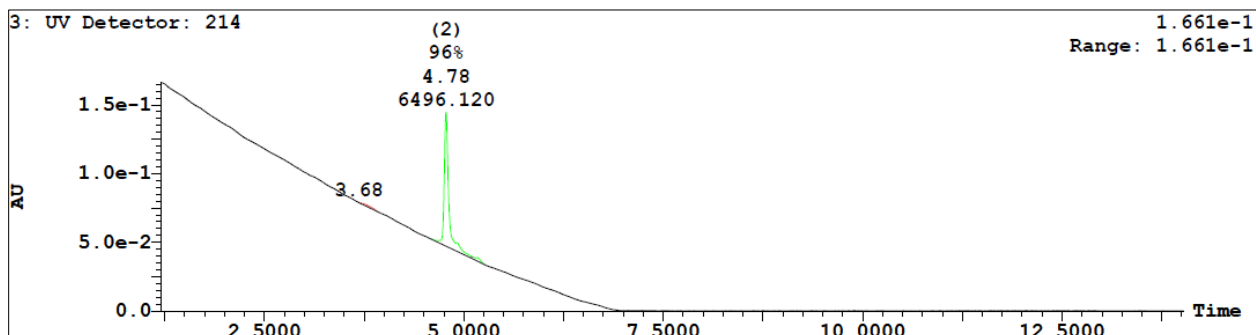


Figure S28. LC/TOF-ES-MS spectra of the synthesized peptide **27** is shown in positive mode and the peak at 4.78 min belongs to the peptide **27** ($m/z = 1921$). The purity of peptide **27** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
anjay-180117-04	B180118WT005	8:4	YES	8.03	10108	2	1865.1306 933.0693	1865.1284 933.0332	0.70 -3.10

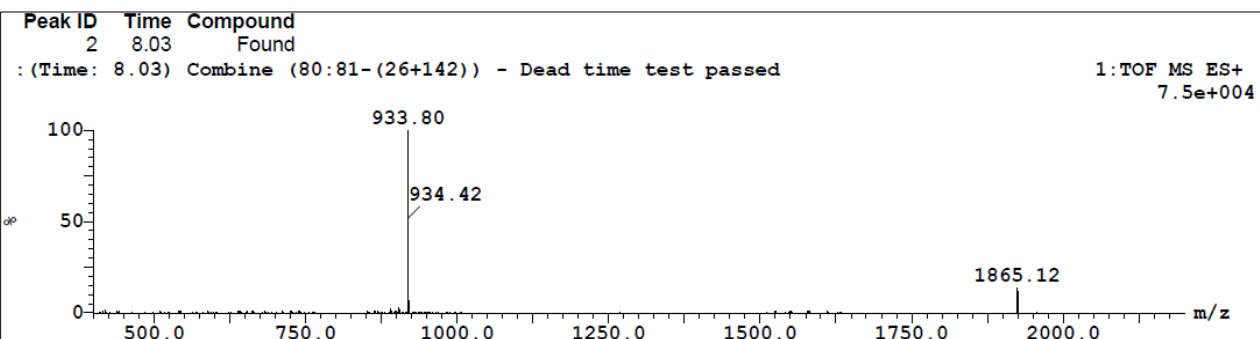
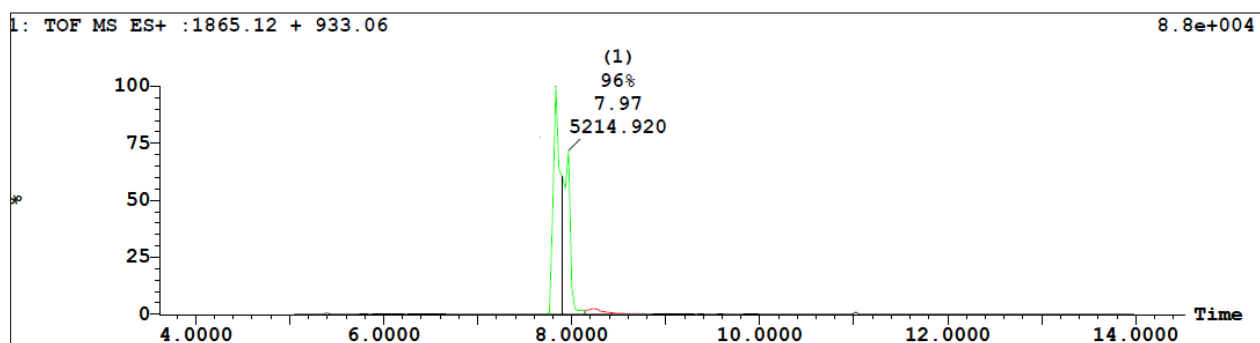
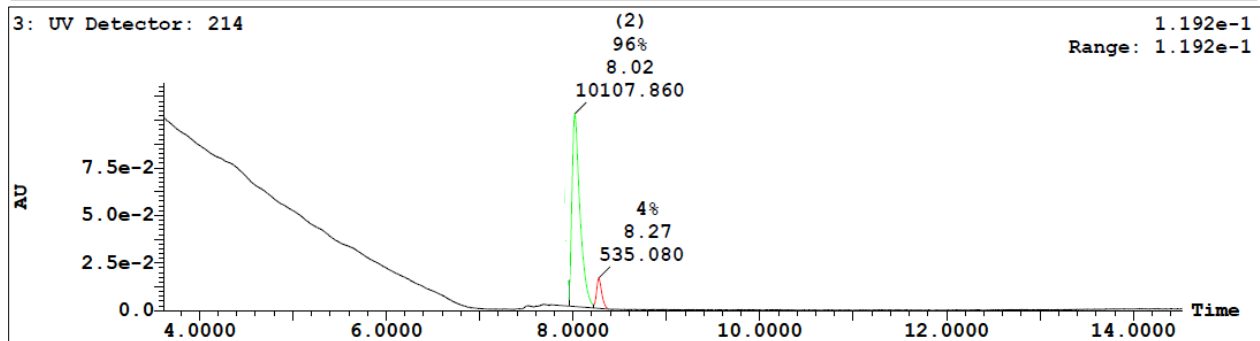


Figure S29. LC/TOF-ES-MS spectra of the synthesized peptide **28** is shown in positive mode and the peak at 8.03 min belongs to the peptide **28** ($m/z = 1865$). The purity of peptide **28** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
anjay-180117-02	B180118WT003	8:2	YES	8.55	44559	2	1921.1932 961.0050	1921.1939 961.0062	1.20 1.00

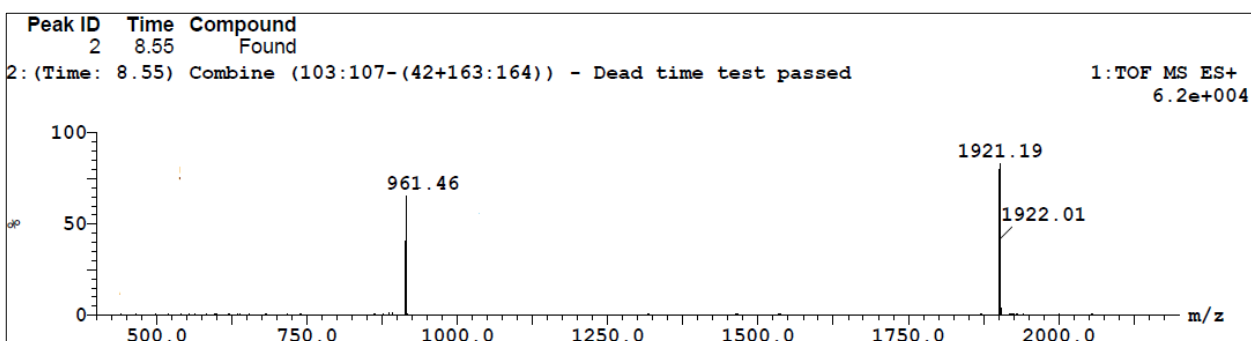
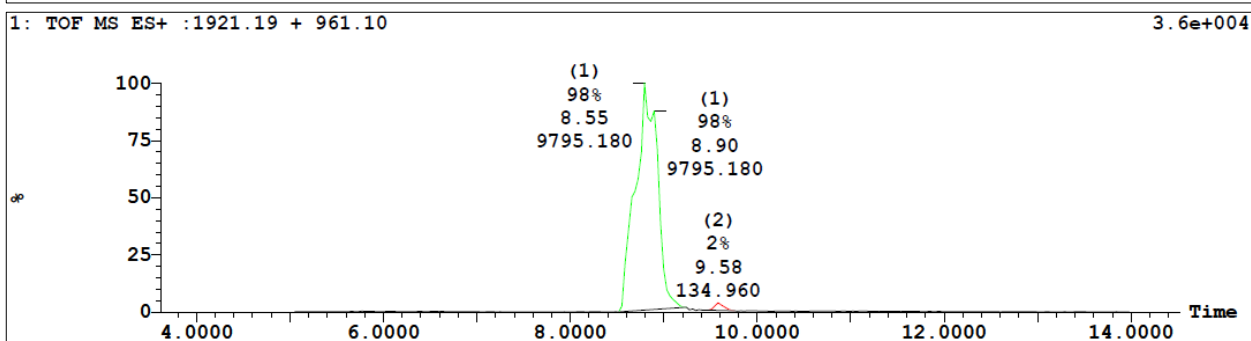
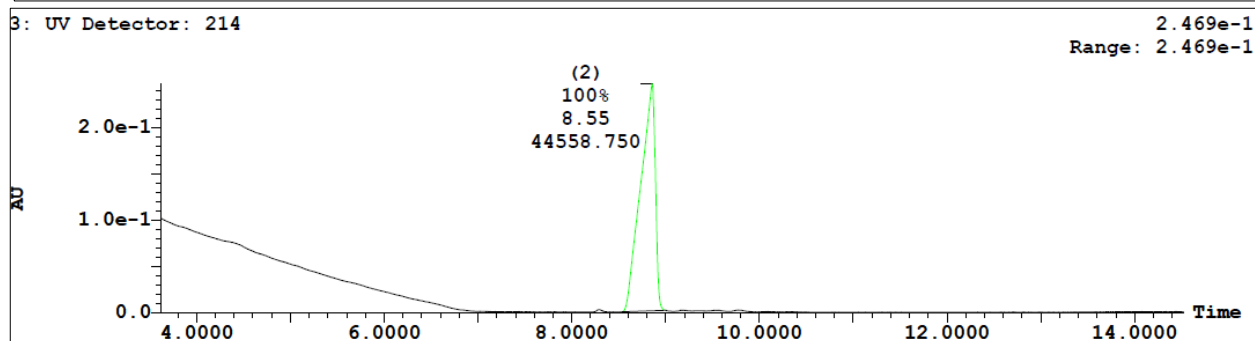


Figure S30. LC/TOF-ES-MS spectra of the synthesized peptide **29** is shown in positive mode and the peak at 8.55 min belongs to the peptide **29** ($m/z = 1921$). The purity of peptide **29** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 98%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs	Peak	Calc Mass	Result Mass	Error mDa
anjay-171113-01	B171113WT002	8:13	YES	7.31	63218		3	2191.3737 1095.1828	2191.3842 1095.1828	1.90

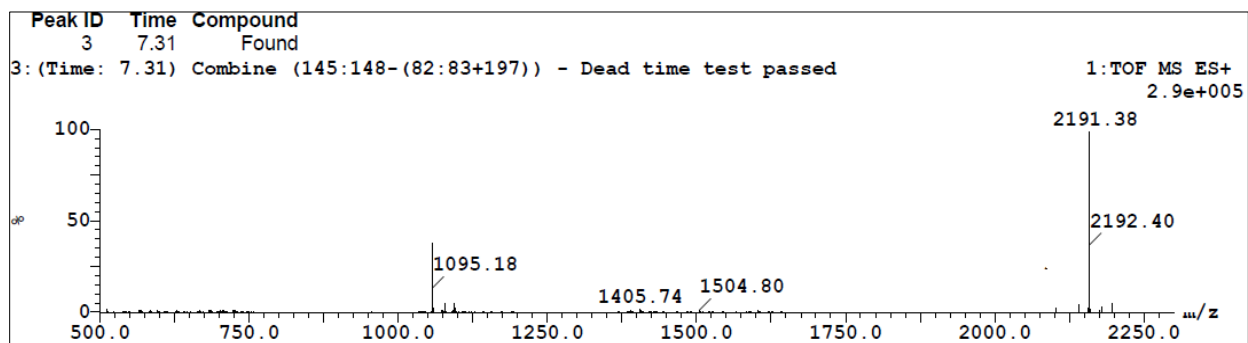
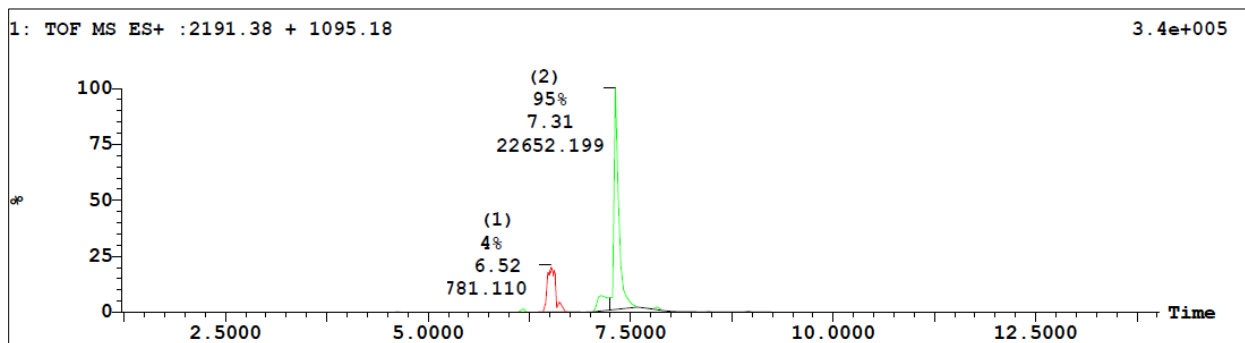
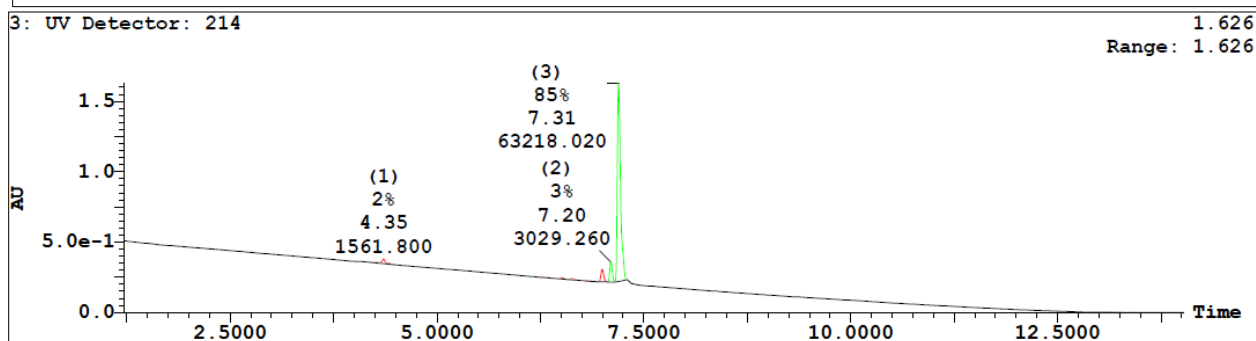


Figure S31. LC/TOF-ES-MS spectra of the synthesized peptide **30** is shown in positive mode and the peak at 7.31 min belongs to the peptide **30** ($m/z = 2191$). The purity of peptide **30** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 95%.

Sample Summary:										
ID	File	Vial	Found	Time	Area	Abs	Peak	Calc Mass	Result Mass	Error mDa
-Anal-180413-03	B180413WT009	8:15	YES	10.49	26084		2	1738.0925 869.5502	1738.1045 869.5504	2.00

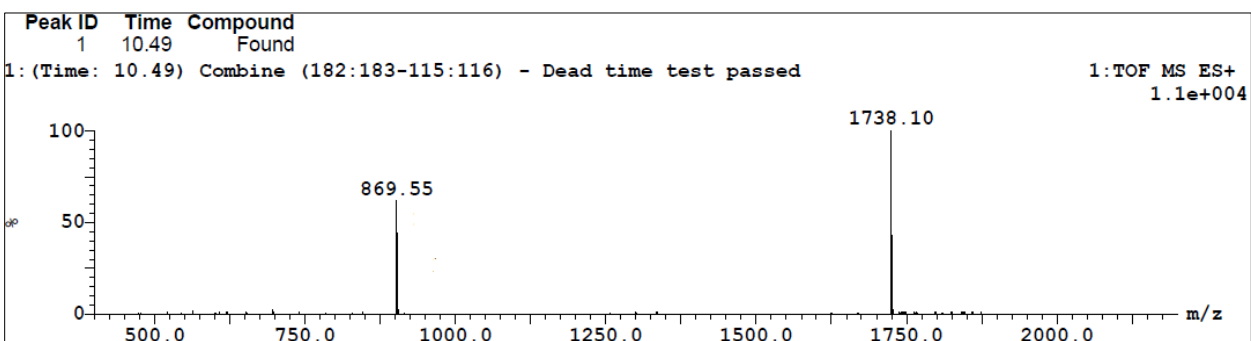
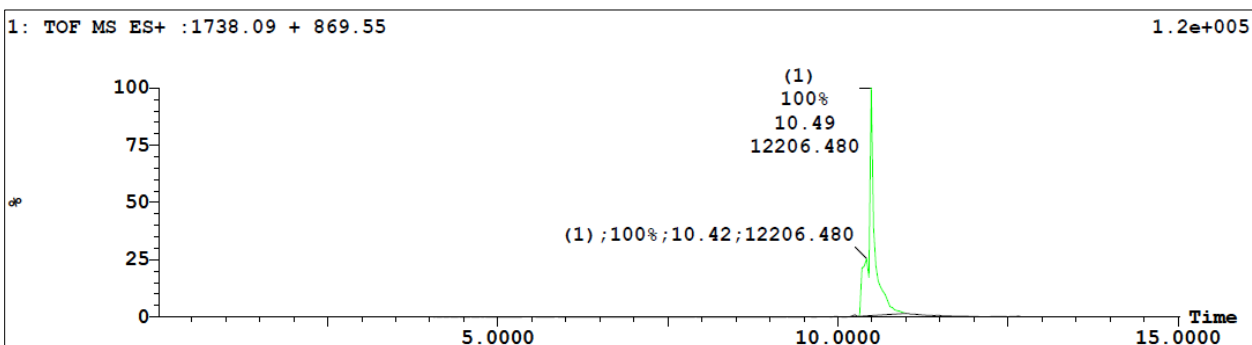
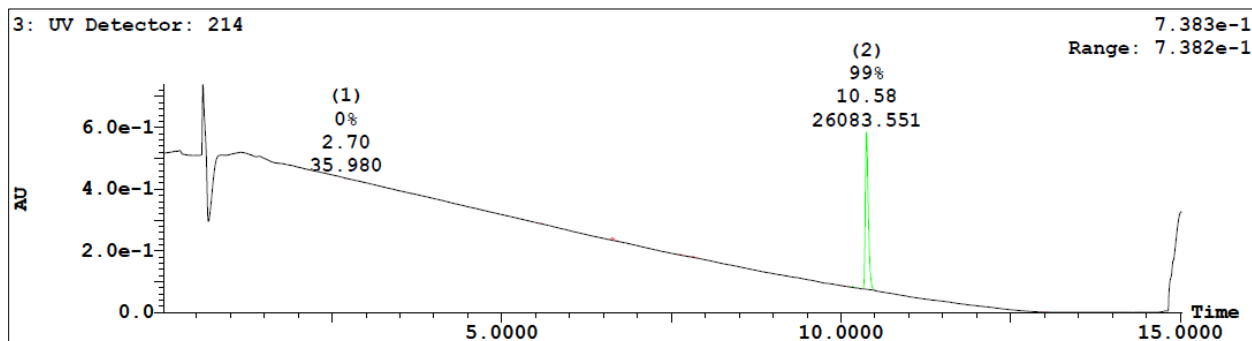


Figure S33. LC/TOF-ES-MS spectra of the synthesized peptide **33** is shown in positive mode and the peak at 10.49 min belongs to the peptide **33** ($m/z = 1738$). The purity of peptide **33** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 99%.

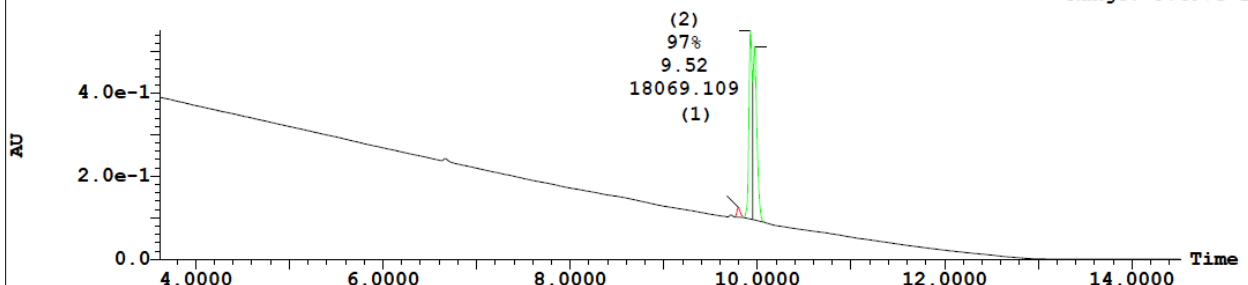
Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
anjay-180213-02	B180213WT005	8:8	YES	9.52	18069	2	1809.1296 905.0688	1809.1274 0.0000	-4.50

ID Sanjay-180213-02 File SB180213WT005 Date 15-Feb-2018 Time 14:35:27 Description SCCT109999

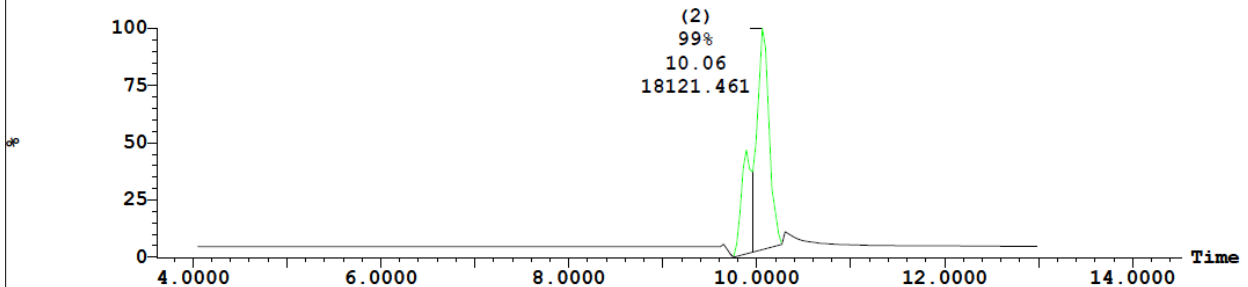
3: UV Detector: 214

5.497e-1
Range: 5.497e-1



1: TOF MS ES+ :1809.12 + 905.06

1.3e+005



Peak ID Time Compound
2 9.52 Found

2:(Time: 9.52) Combine (165-(101:102+216:217)) - Dead time test passed

:TOF MS ES+
2.6e+005

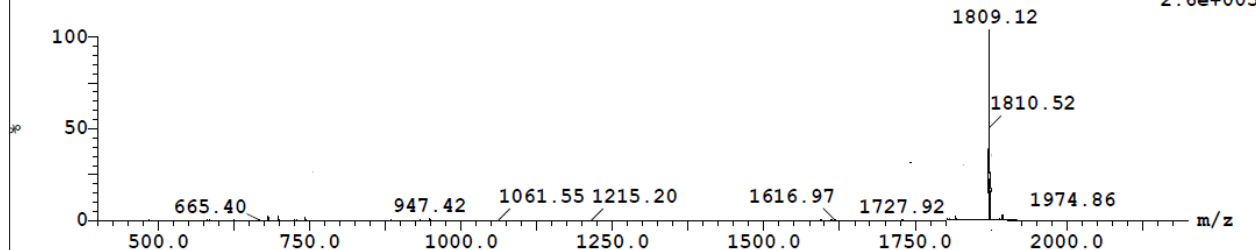


Figure S34. LC/TOF-ES-MS spectra of the synthesized peptide **34** is shown in positive mode and the peak at 9.52 min belongs to the peptide **34** ($m/z = 1809$). The purity of peptide **34** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 97%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs	Peak	Calc Mass	Result Mass	Error mDa
anjay-180117-01	B180118WT002	8:1	YES	9.30	56460		3	1837.1609 919.0844	1837.1654 919.0028	4.10 -2.20

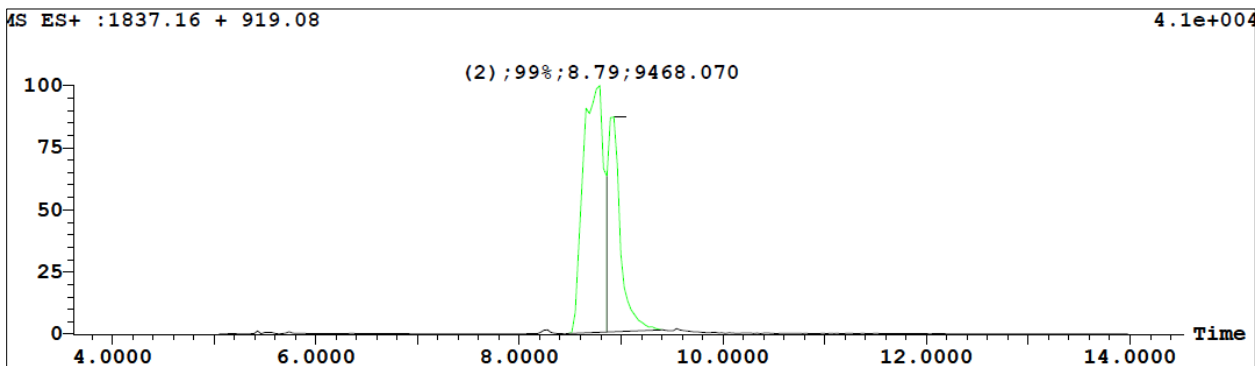
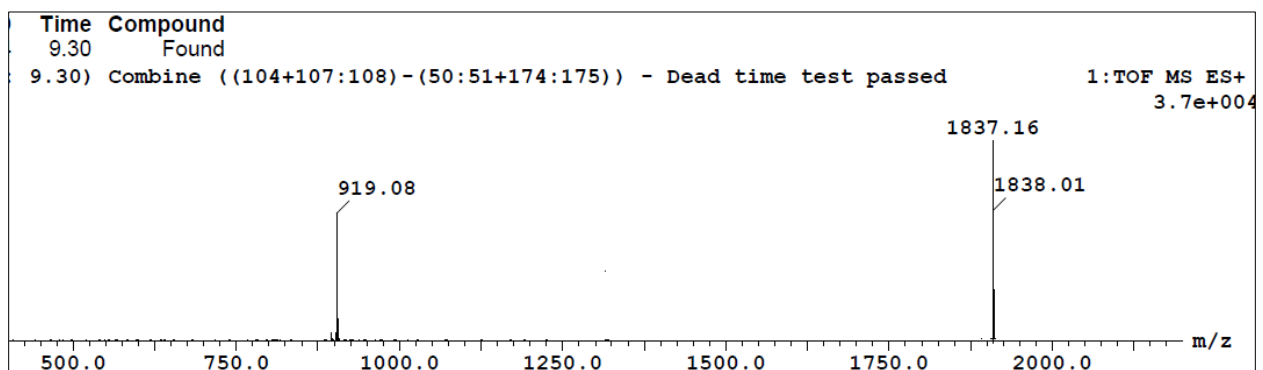
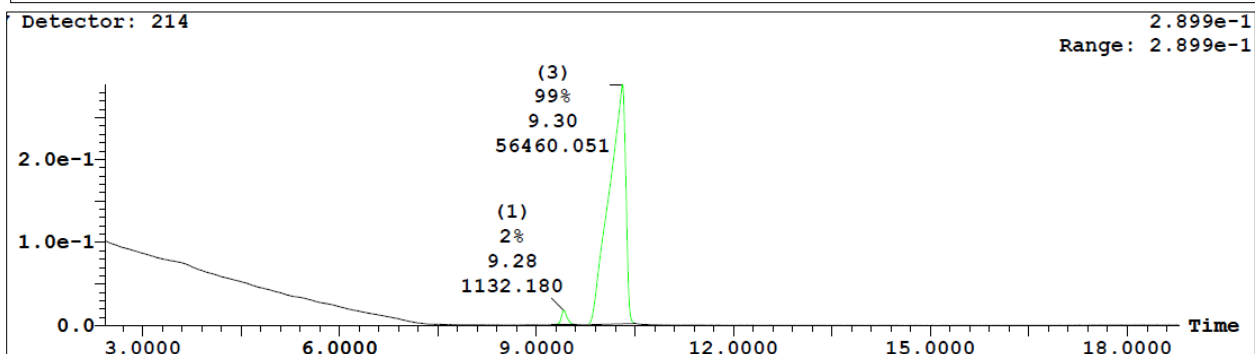


Figure S35. LC/TOF-ES-MS spectra of the synthesized peptide **35** is shown in positive mode and the peak at 9.30 min belongs to the peptide **35** ($m/z = 1837$). The purity of peptide **35** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 99%.

Sample Summary:

ID	File	Vial	Found	Time	Area Abs	Peak	Calc Mass	Result Mass	Error mDa
anjay-180116-04	B180117WT005	8:4	YES	8.40	43744	3	1850.1813	1850.1381	5.20
							925.5946	925.6842	4.20

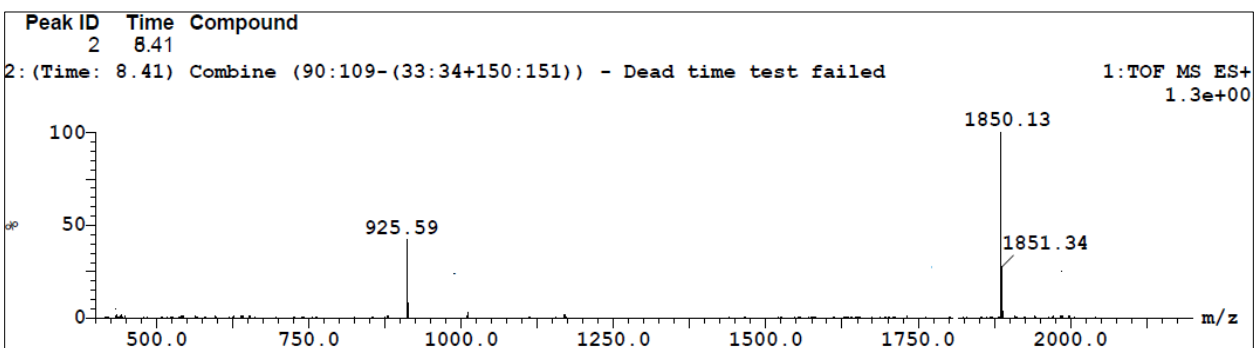
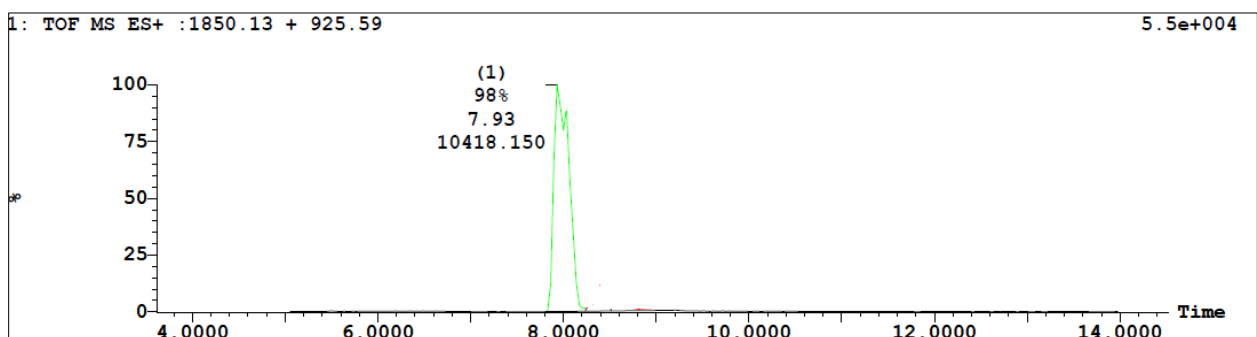
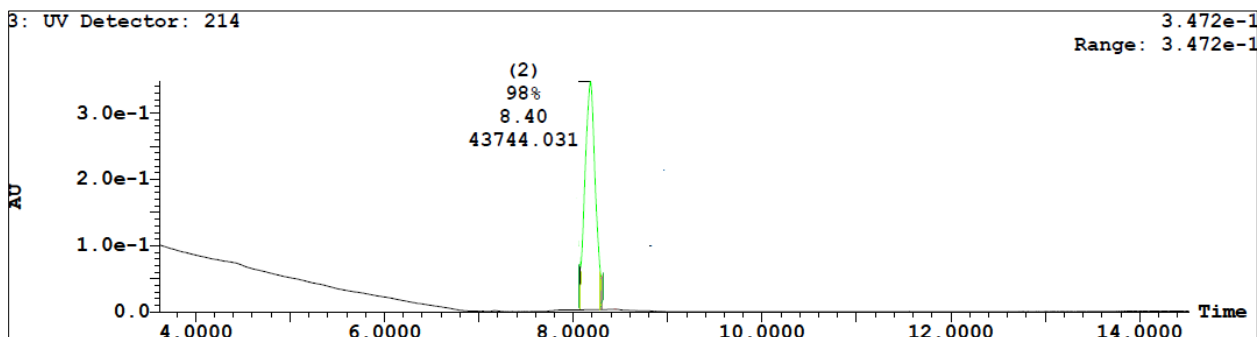


Figure S36. LC/TOF-ES-MS spectra of the synthesized peptide **36** is shown in positive mode and the peak at 8.41 min belongs to the peptide **36** ($m/z = 1850$). The purity of peptide **36** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 98%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
Sanjay-190326-4	.190326WT004b	8:10	YES	8.24	21466	2	1893.2235 947.1157	1893.2325 947.0673	1.40

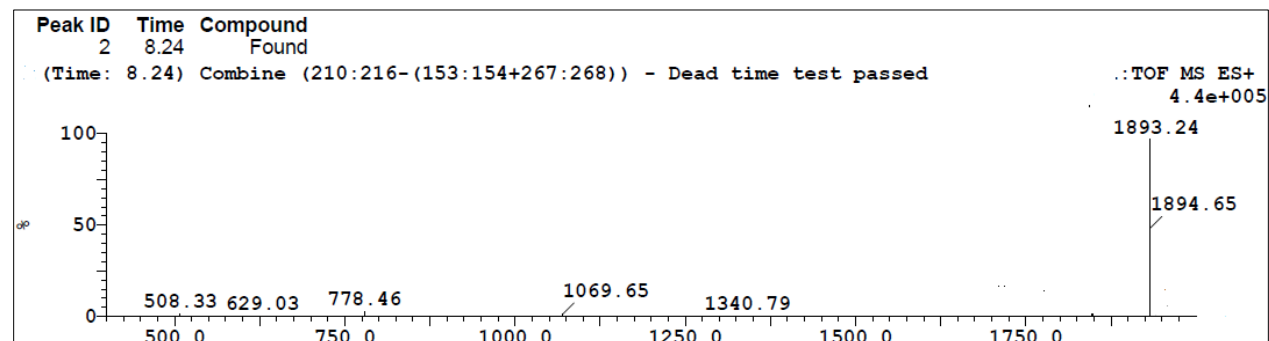
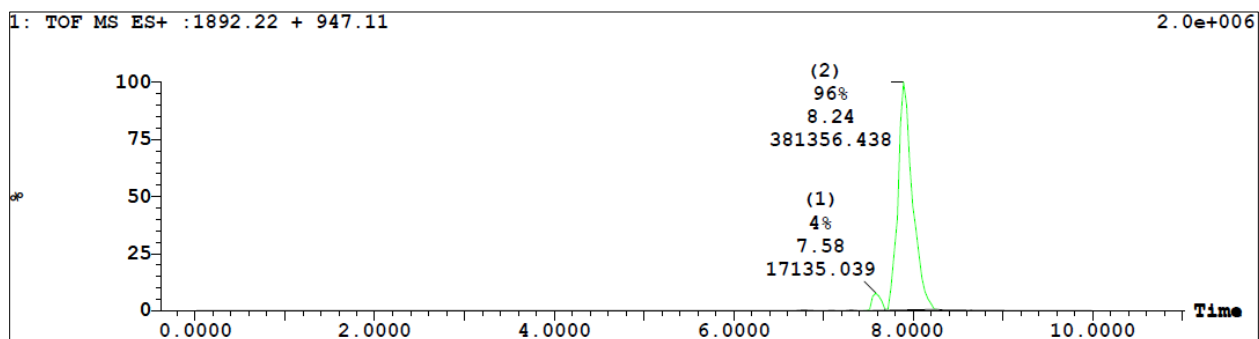
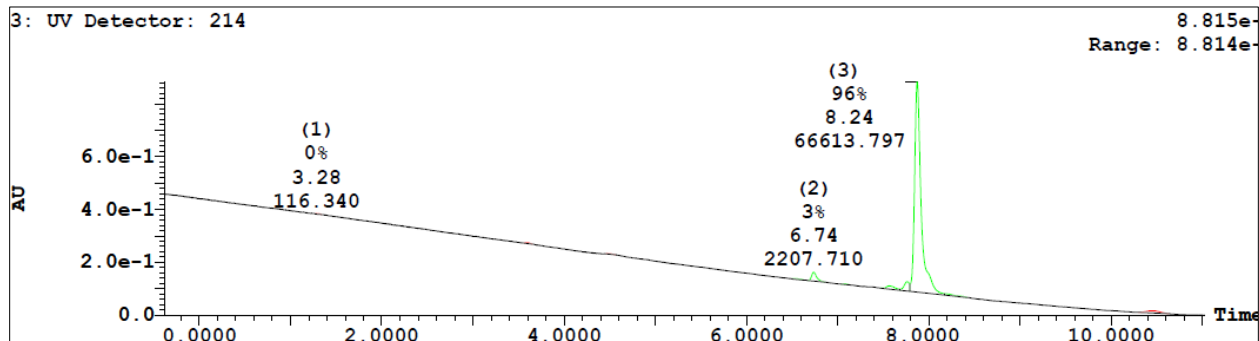


Figure S37. LC/TOF-ES-MS spectra of the synthesized peptide **37** is shown in positive mode and the peak at 8.24 min belongs to the peptide **37** ($m/z = 1893$). The purity of peptide **37** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 96%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
anjay-181212-01	B181212WT002	8:27	YES	8.24	24175	2	1921.2548 961.1314	1921.2845 961.6888	2.30 11.30

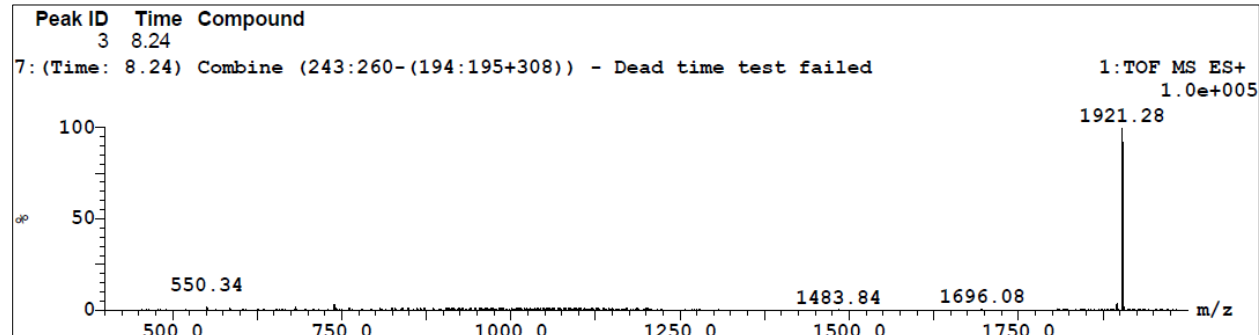
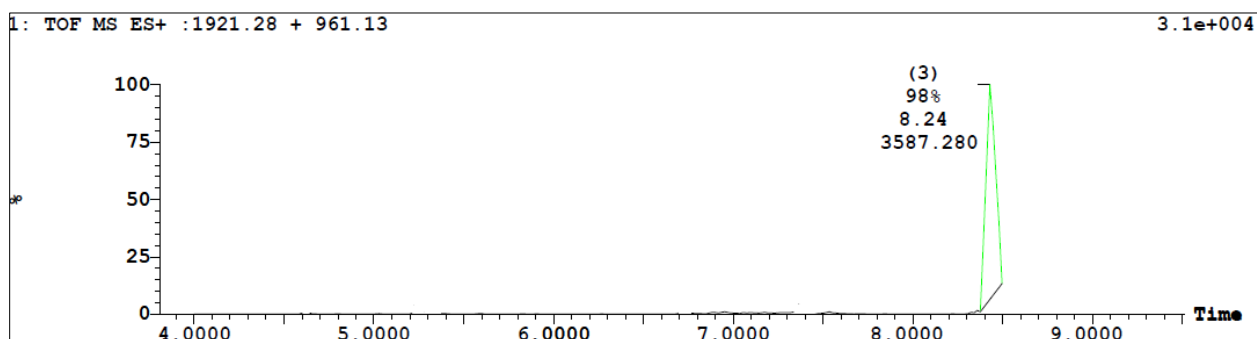
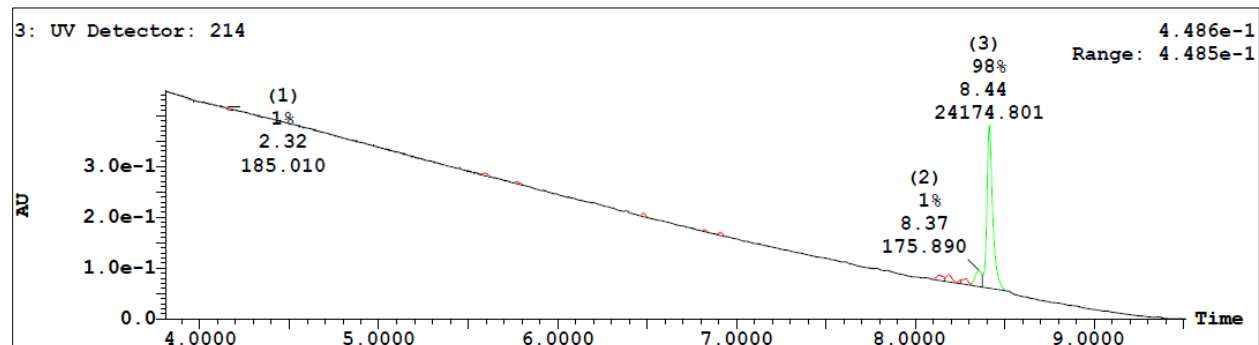


Figure S38. LC/TOF-ES-MS spectra of the synthesized peptide **38** is shown in positive mode and the peak at 8.24 min belongs to the peptide **38** ($m/z = 1921$). The purity of peptide **38** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 98%.

Sample Summary:

ID	File	Vial	Found	Time	Area	Abs Peak	Calc Mass	Result Mass	Error mDa
anjay-180618-02	B180618WT007	8:26	YES	8.35	60891	1	1935.2704 968.1352	1935.2407 968.1095	-7.40 -1.50

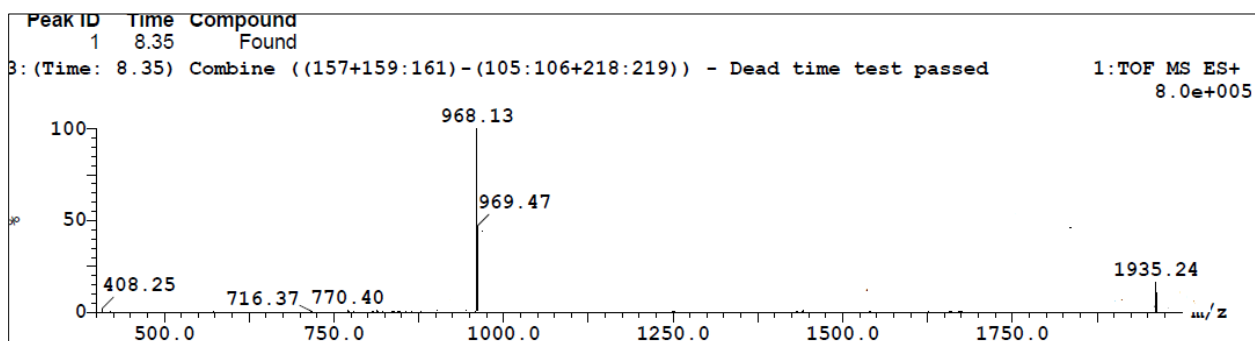
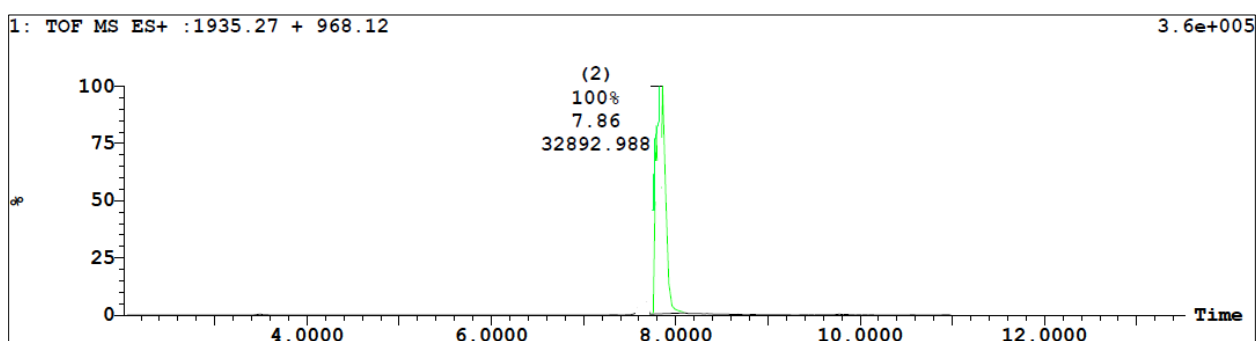
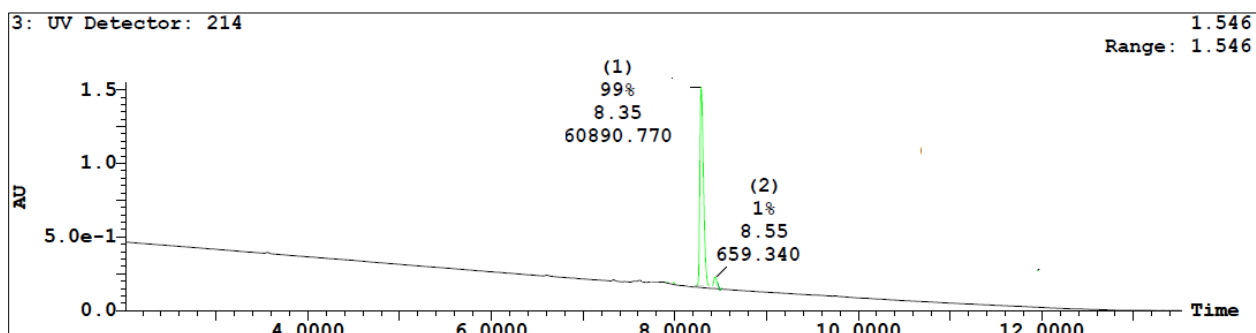


Figure S39. LC/TOF-ES-MS spectra of the synthesized peptide **39** is shown in positive mode and the peak at 8.35 min belongs to the peptide **39** ($m/z = 1935$). The purity of peptide **39** was also determined by HPLC-UV (214 nm)-ESI-MS and was found to be 99%.