SUPPORTING INFORMATION

Lactoferrin-derived Peptides Active towards Influenza: Identification of Three Potent Tetrapeptide Inhibitors

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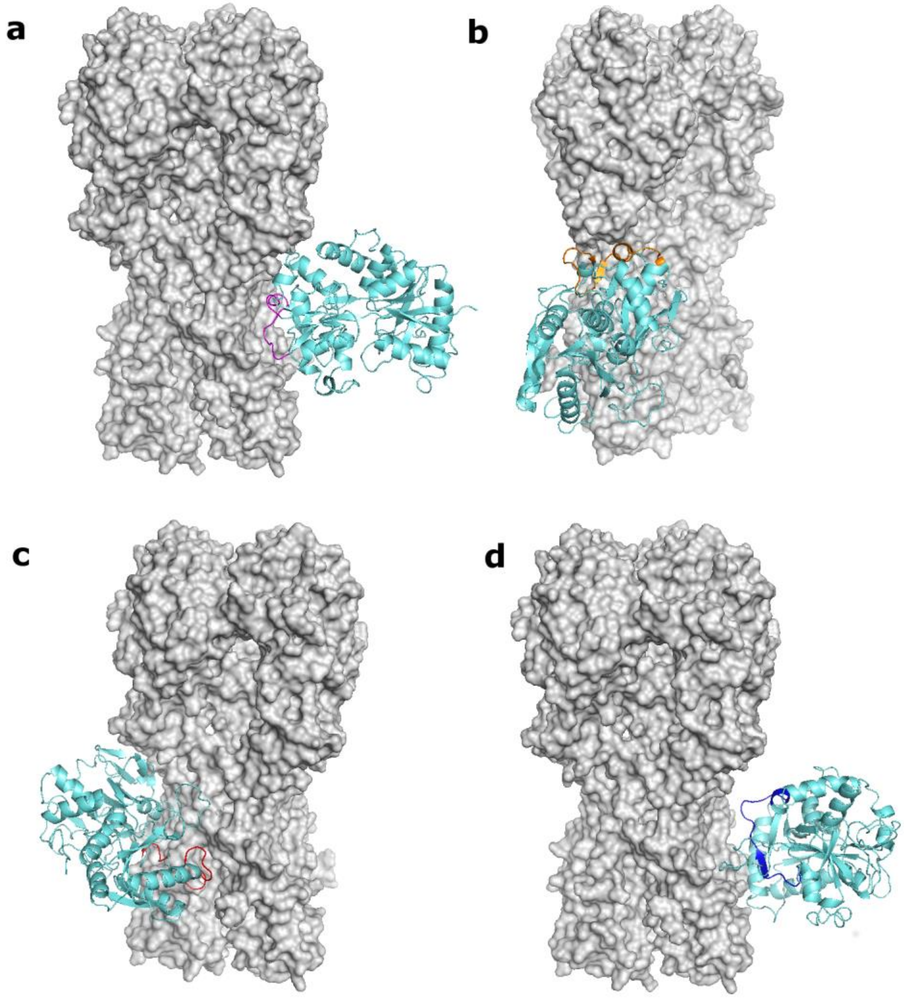


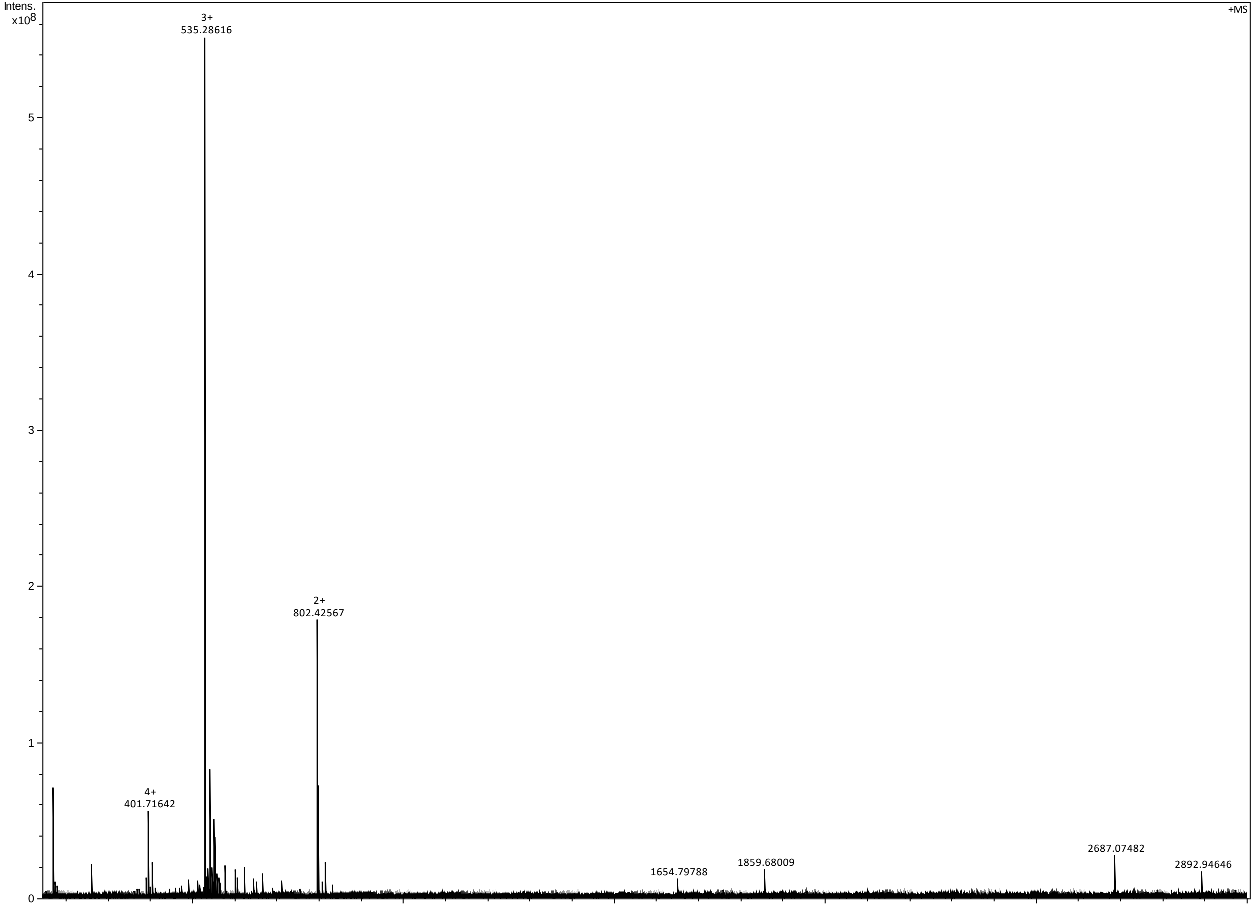
Figure S1. Docked poses of the bLf C-lobe (cyan cartoon) on the HA (grey surface) highlighting the potential role of selected loop sequences. Selected sequences are colored: a) peptide **4** (purple); b) peptide **5** (orange); c) peptides **7** and **8** (red); d) peptide **9** (blue).

Table S1. Analytical data of peptides **4-17**.

Pep.	**Sequence**	**HPLC k'^{a}**	**HRMS**
4	KANEGLTWNSLKDK	6.47	1602.85134
5	TGSCAFDEFFSQSCAPGADPKSR	6.89	2408.02628
6	TNGESTADWAKN	5.07	1292.58779
7	GKNGKNCPDKFC	4.26	1308.58370
8	KSETKN	5.22	706.37420
9	NDNTECLAKLGGRPTYEE	5.76	2009.92731
10	NRKSSKHSSLDCVLRPTEGY	6.08	2278.15056
11	NRKSSKHSSLDCVLRP	5.71	1825.98013
12	SKHSSLDCVLRPTEGY	2.45	1791.87152
13	SLDCVLRP	6.67	901.49417
14	VLRP	5.70	525.35082
15	SLDC	3.93	477.18970
16	SKHSSLDC	4.14	876.38864
17	SKHS	4.68	499.26235

[a] k'=[(peptide retention time-solvent retention time)/solvent retention time].

Supplementary figures of Mass spectrometry and HPLC of peptides used in the study



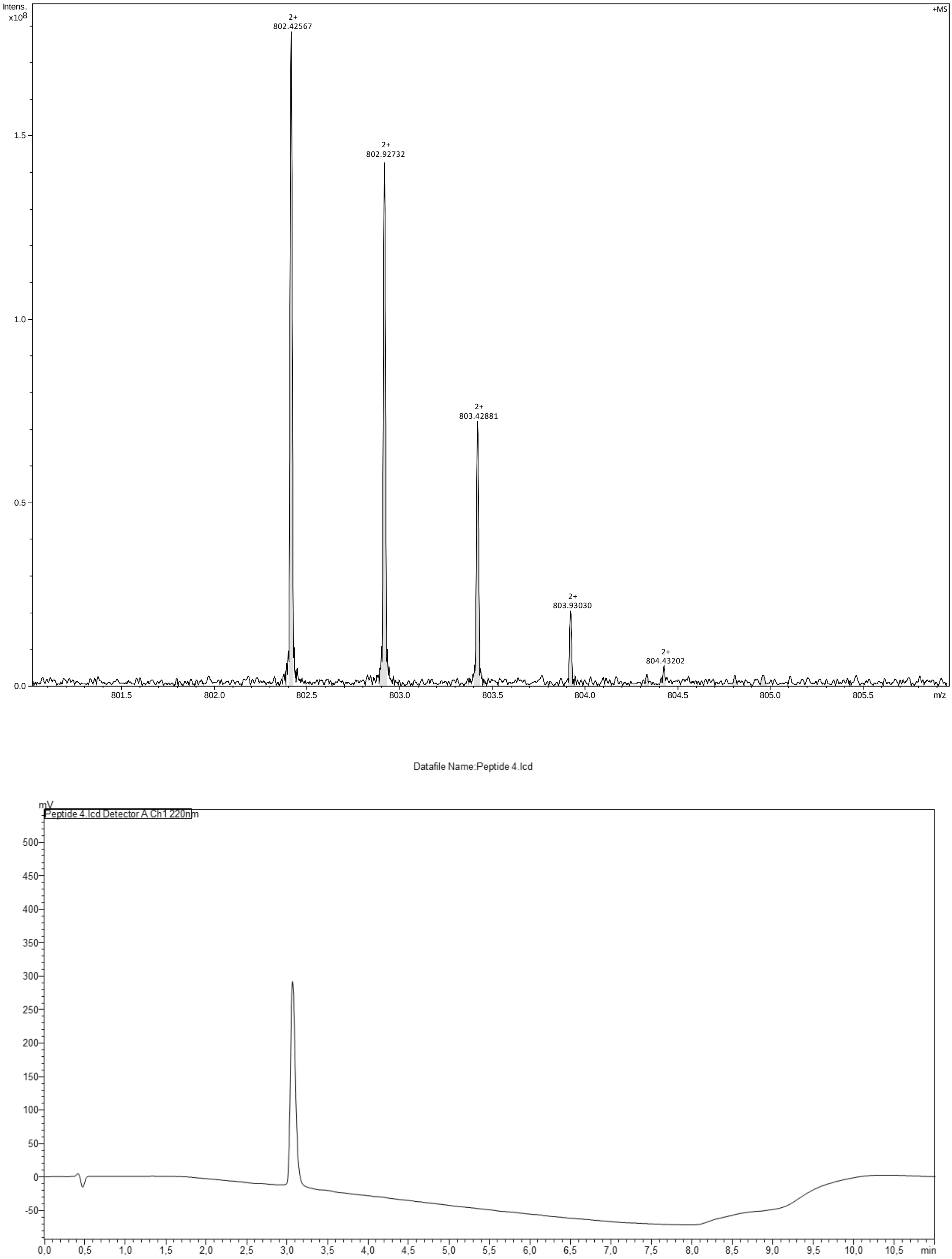
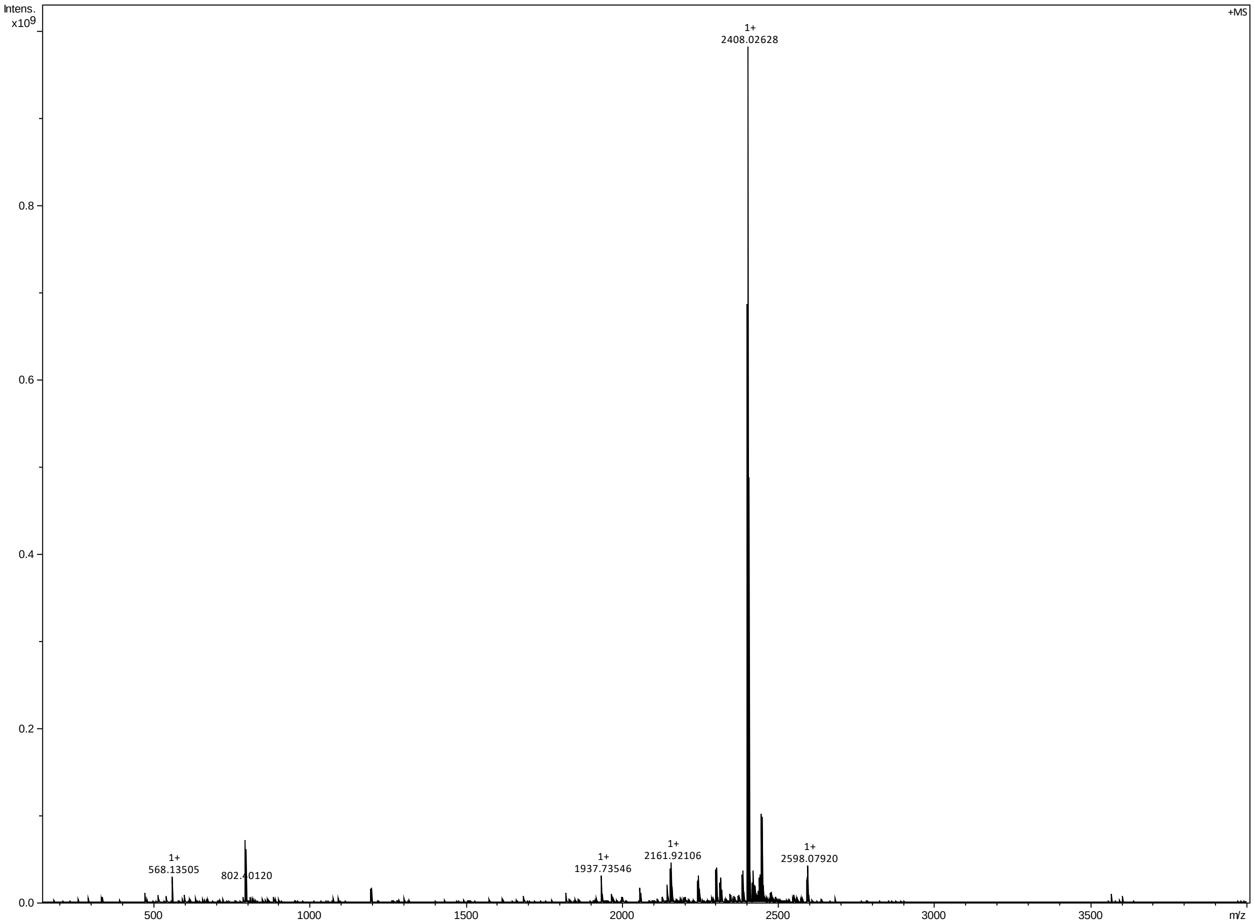


Figure S2. HR-ESI-MS of Peptide **4** ion $[M+H]^{2+}$ and analytical HPLC trace at 220 nm.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0.4
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2500
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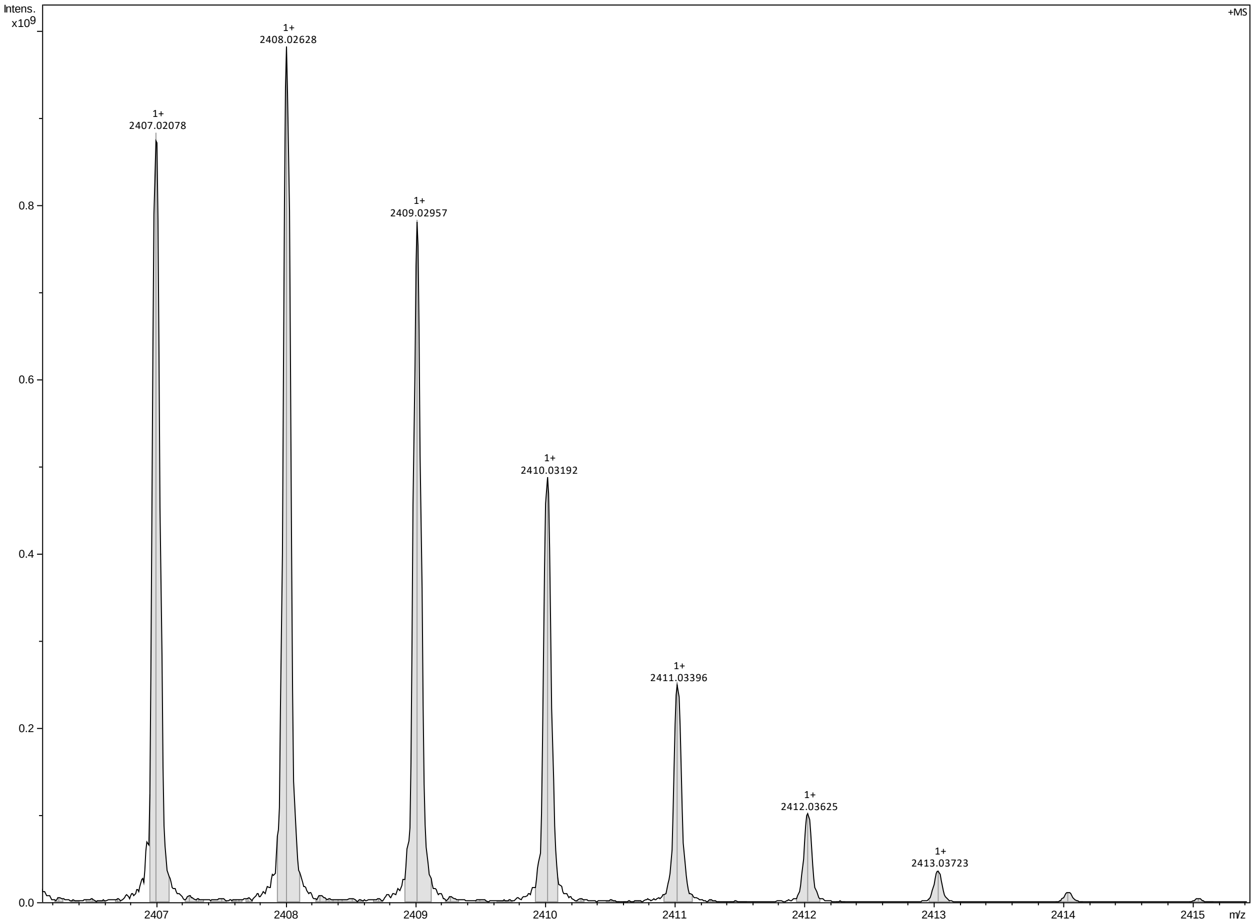
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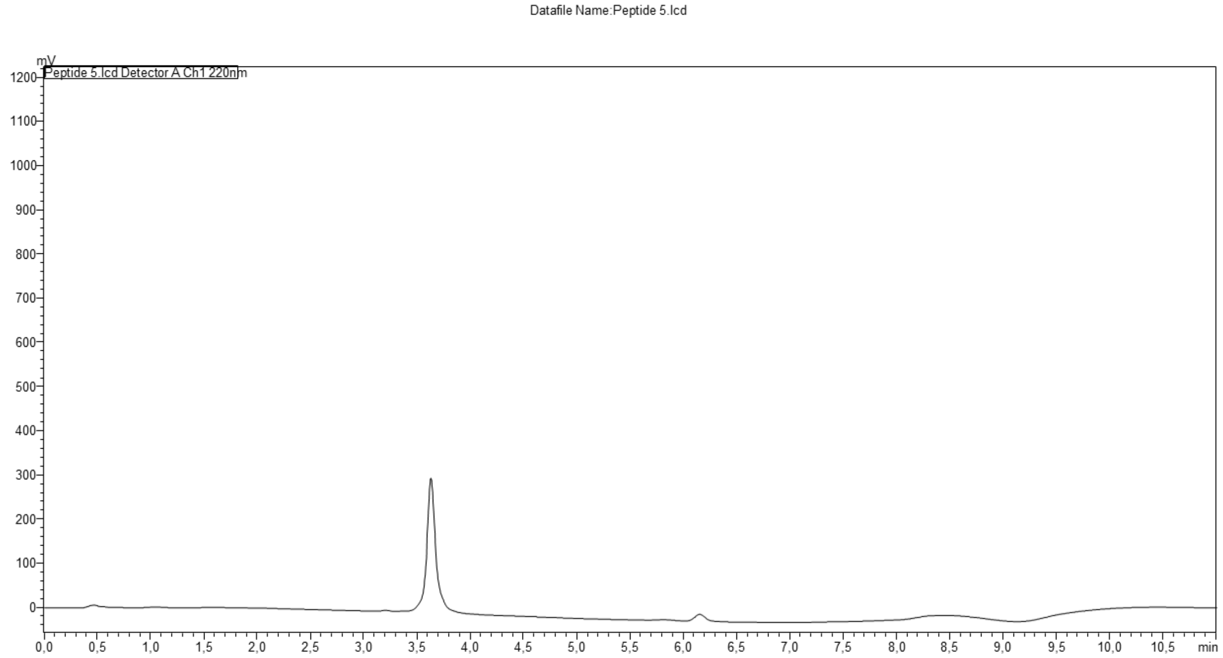
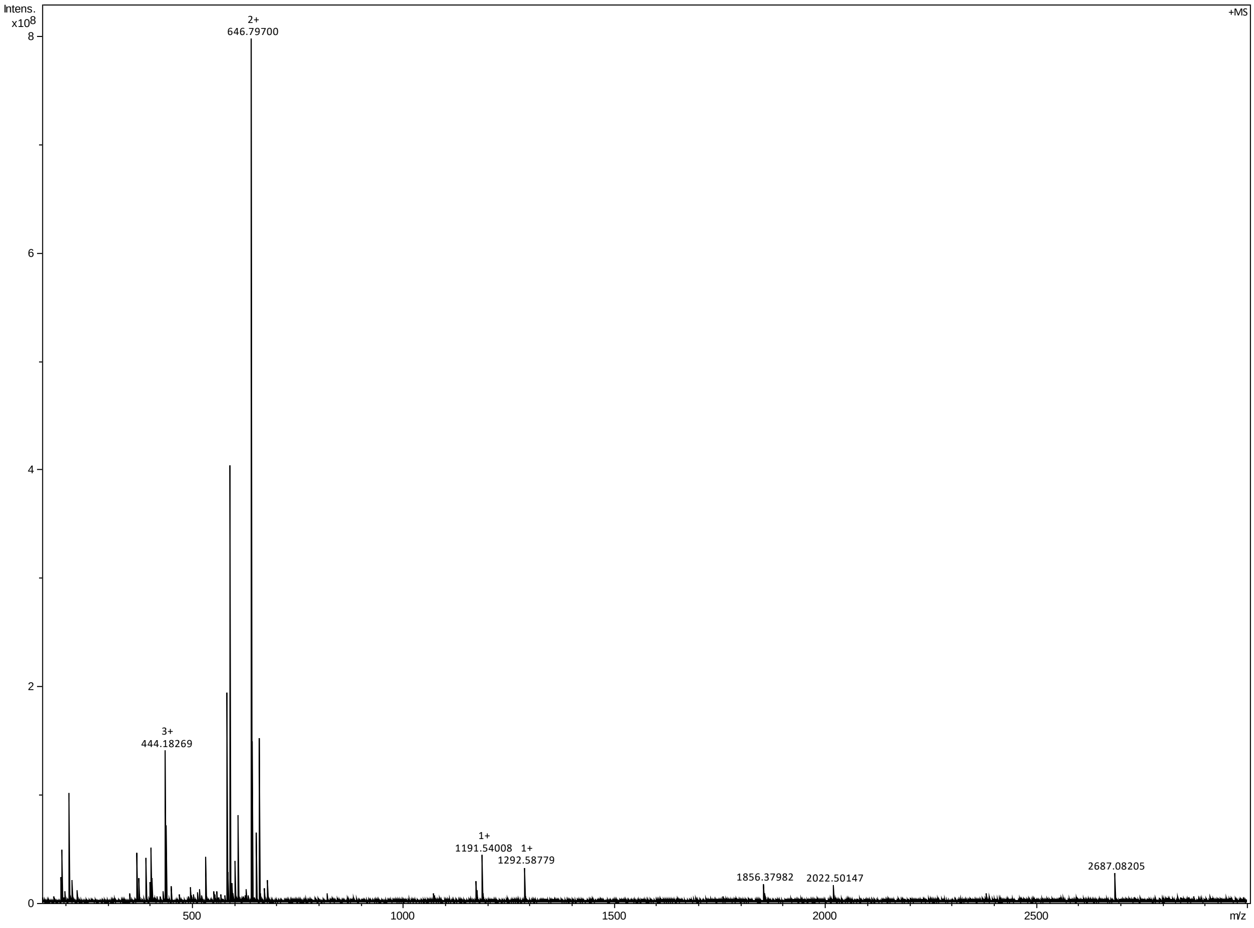


Figure S3. HR-MALDI-MS of Peptide **5** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



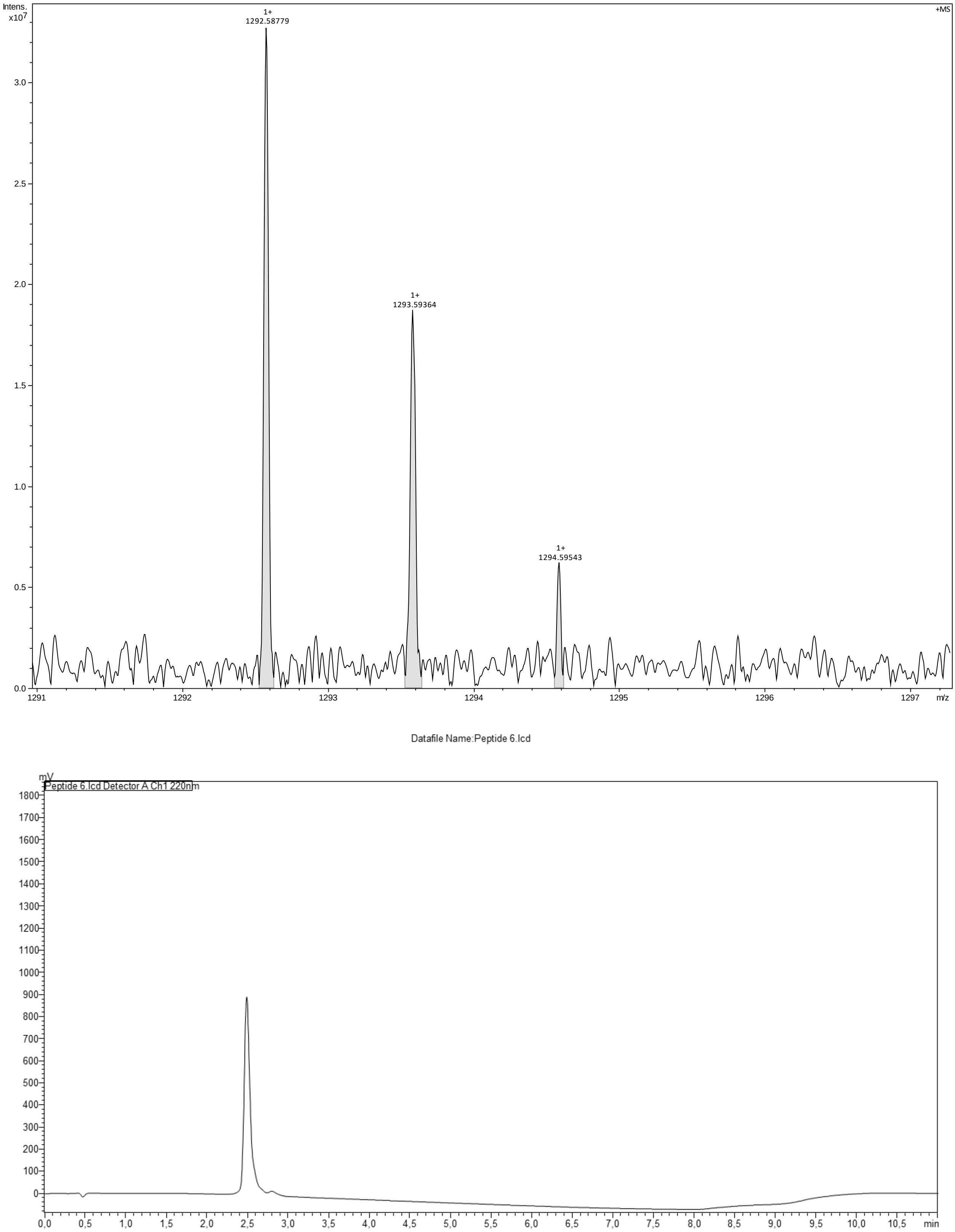
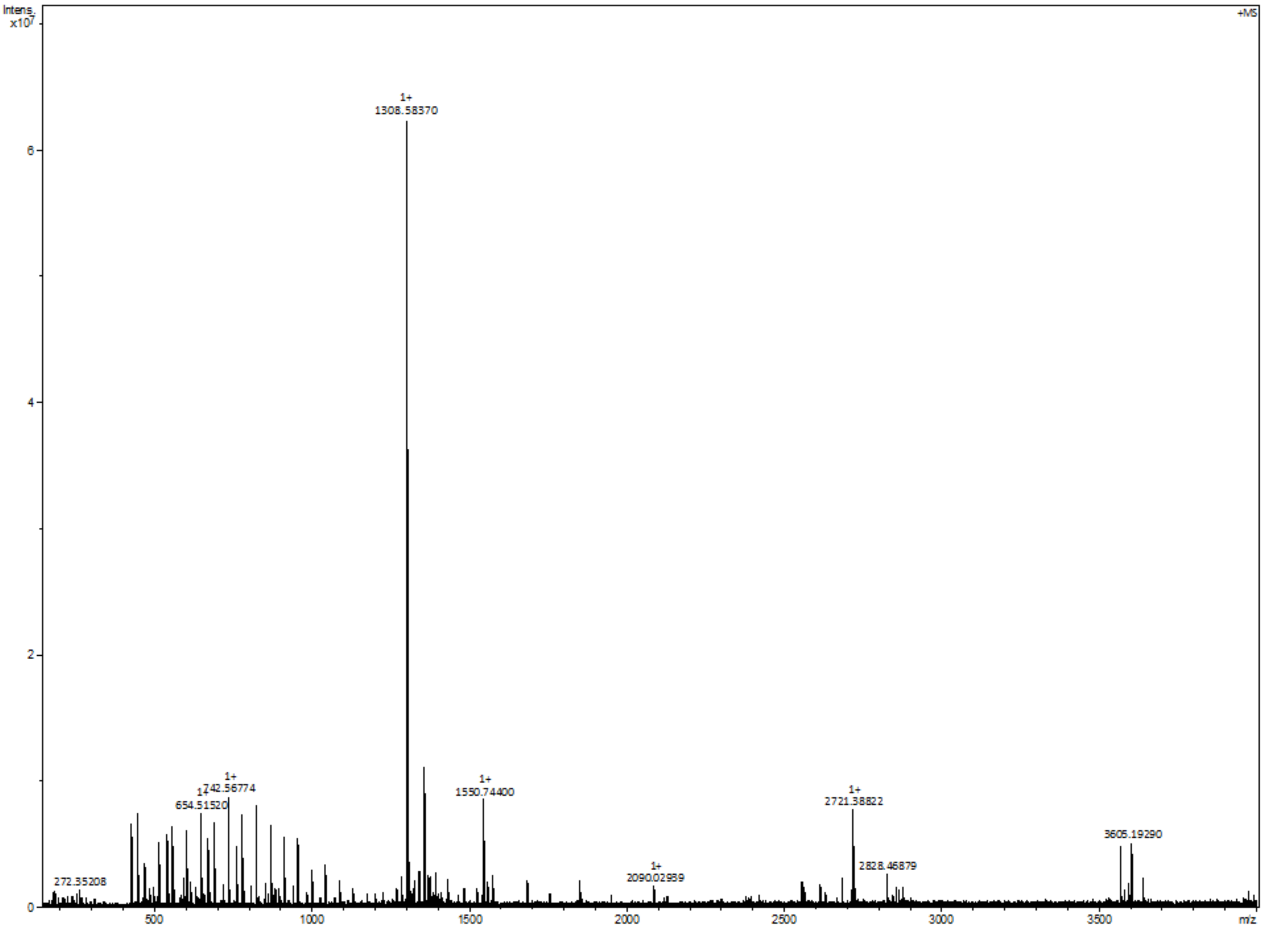


Figure S4. HR-ESI-MS of Peptide **6** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.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2721.38822
3605.19290
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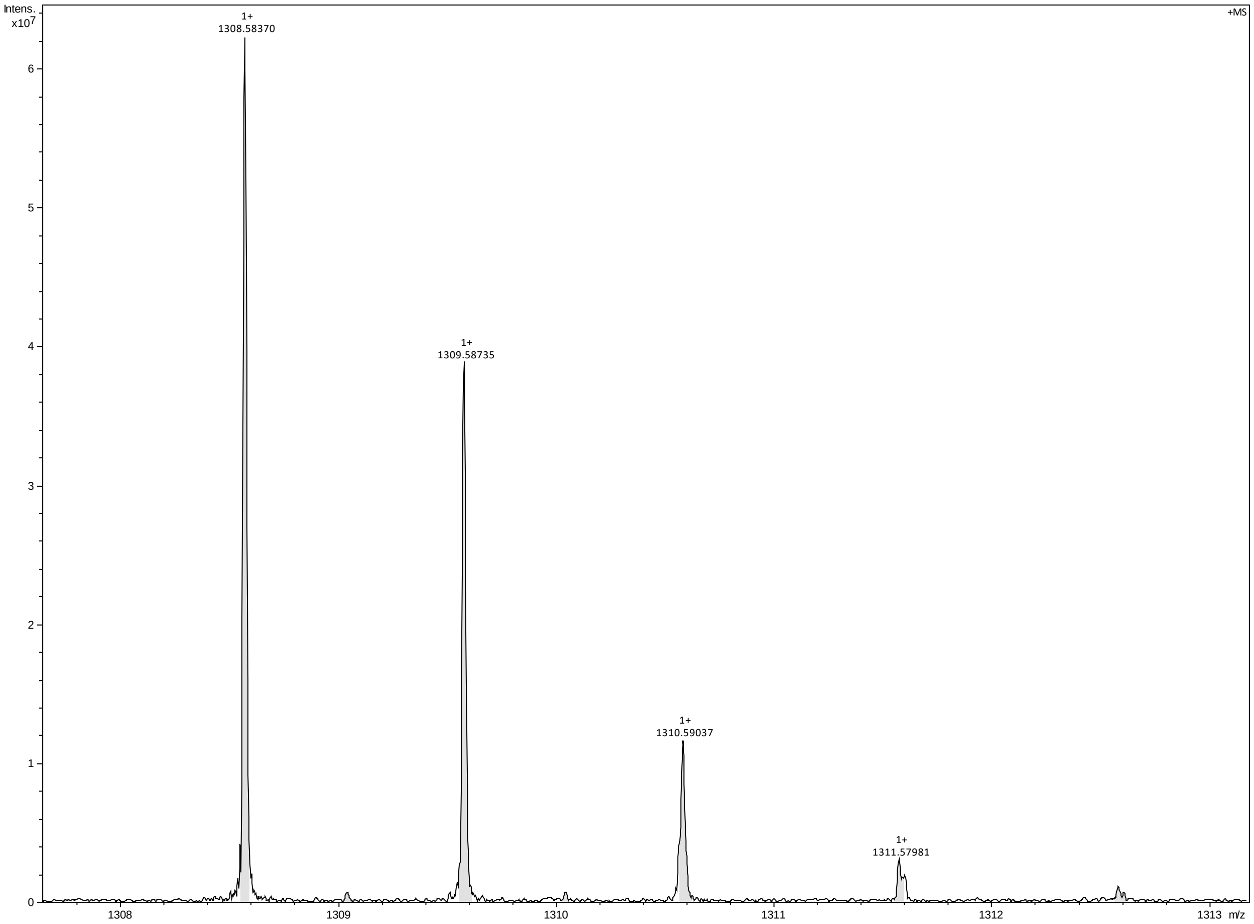
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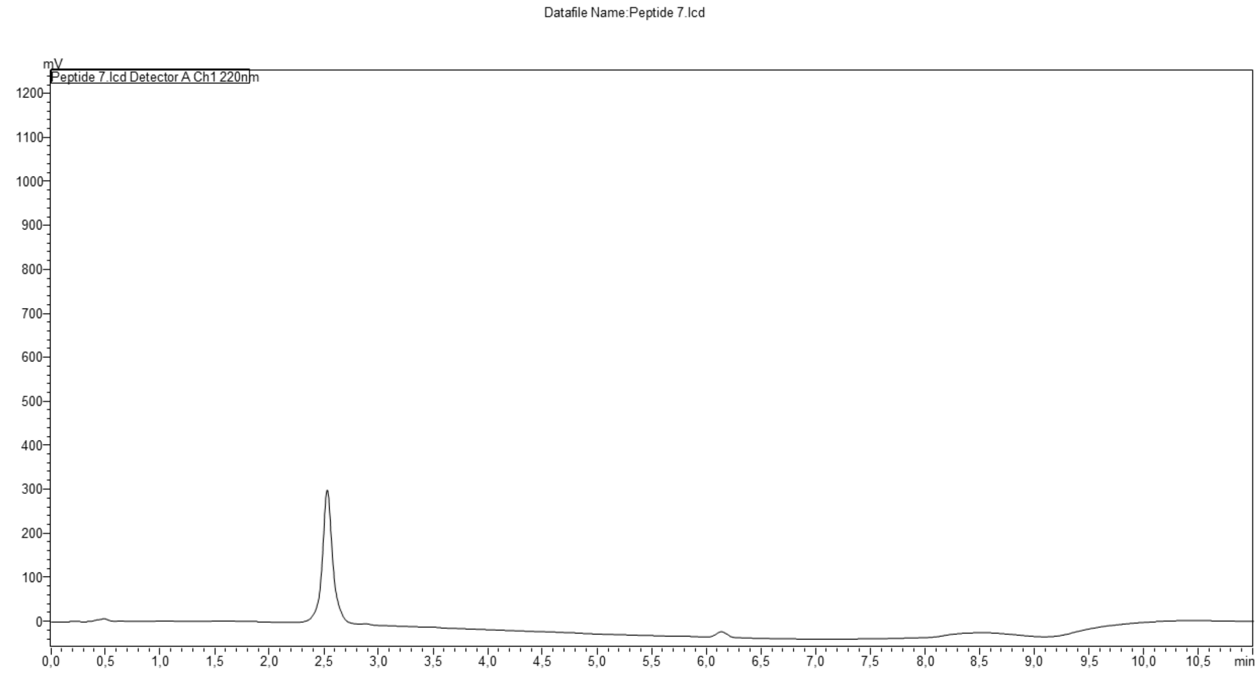
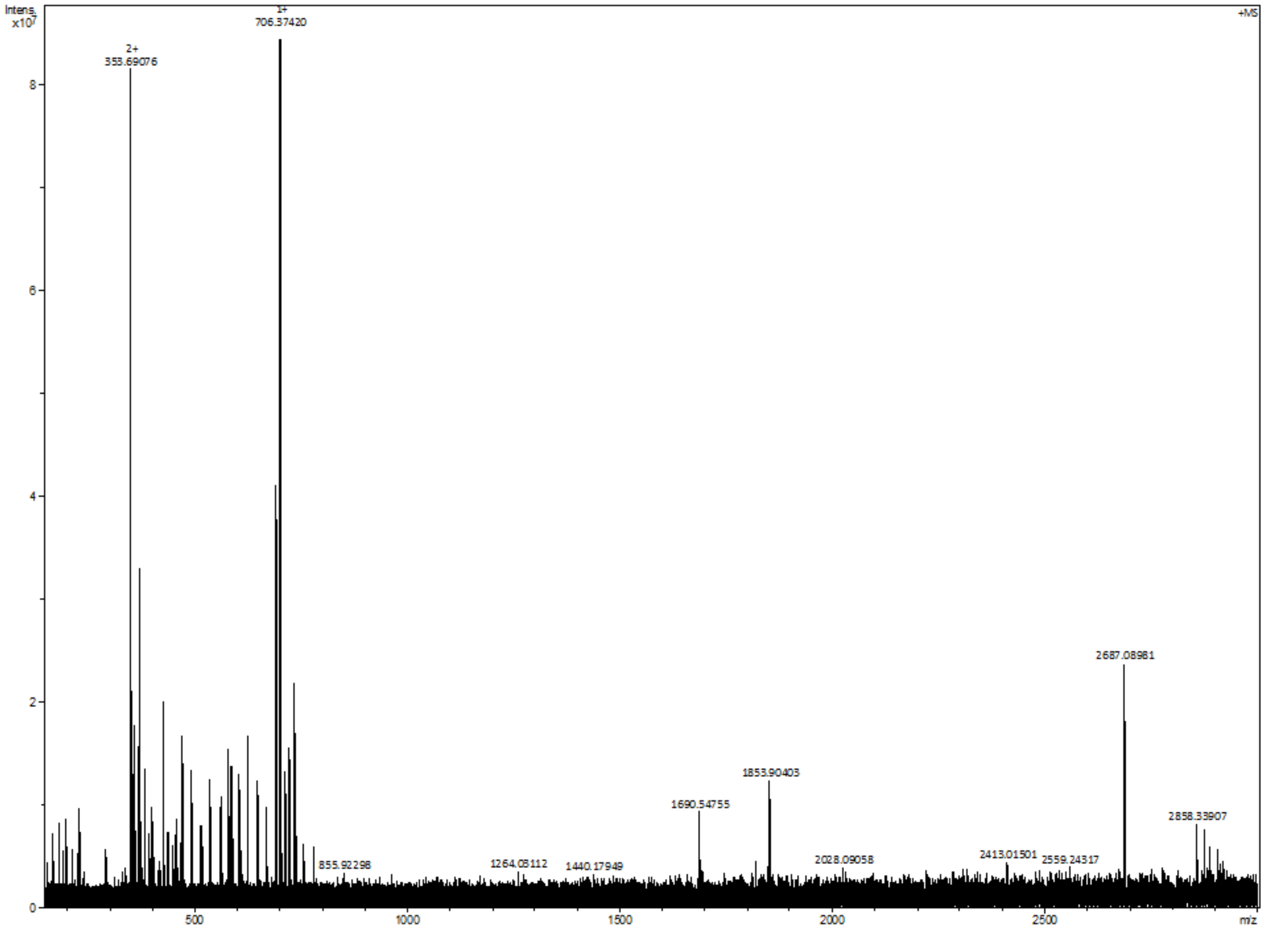


Figure S5. HR-MALDI-MS of Peptide **7** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



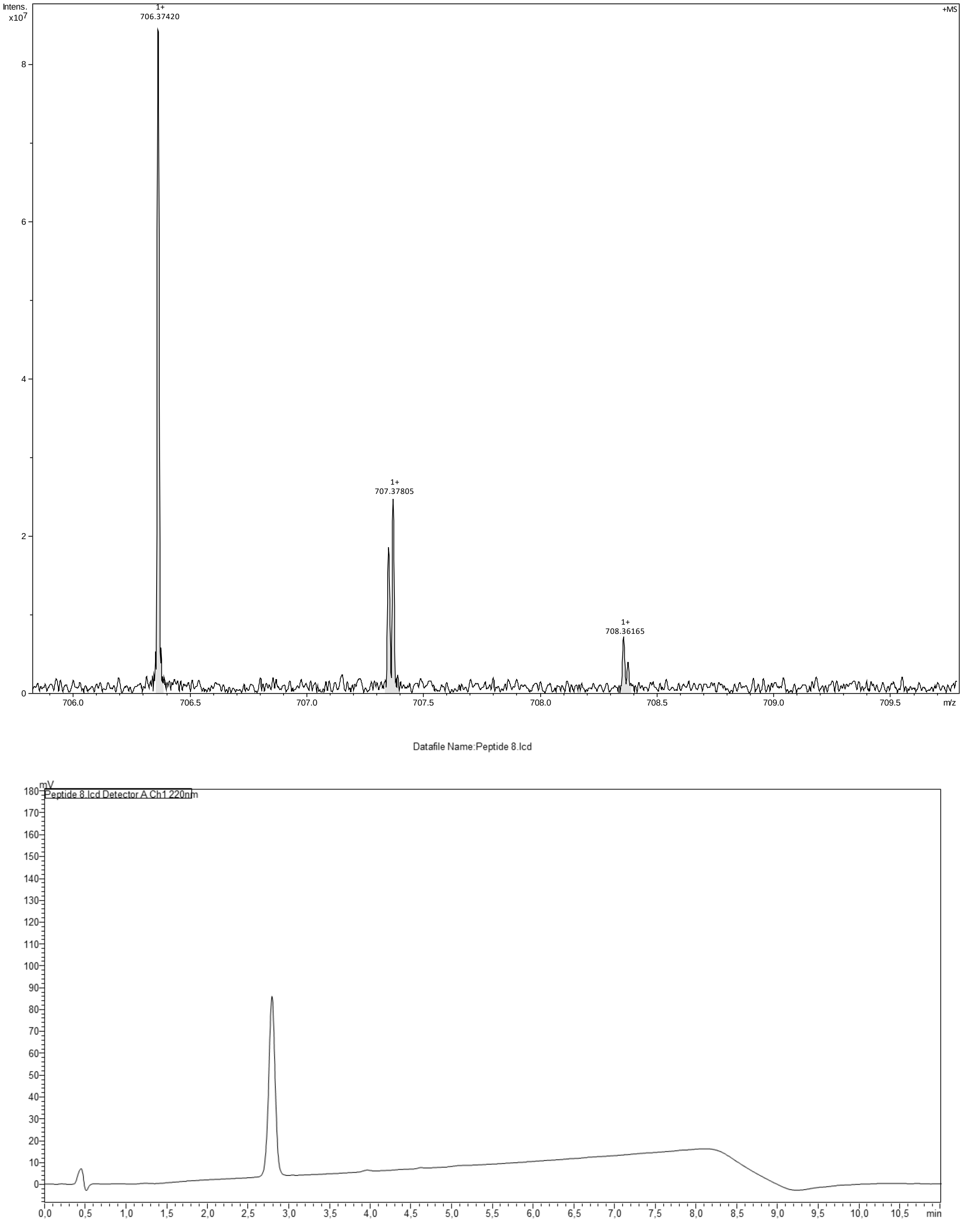
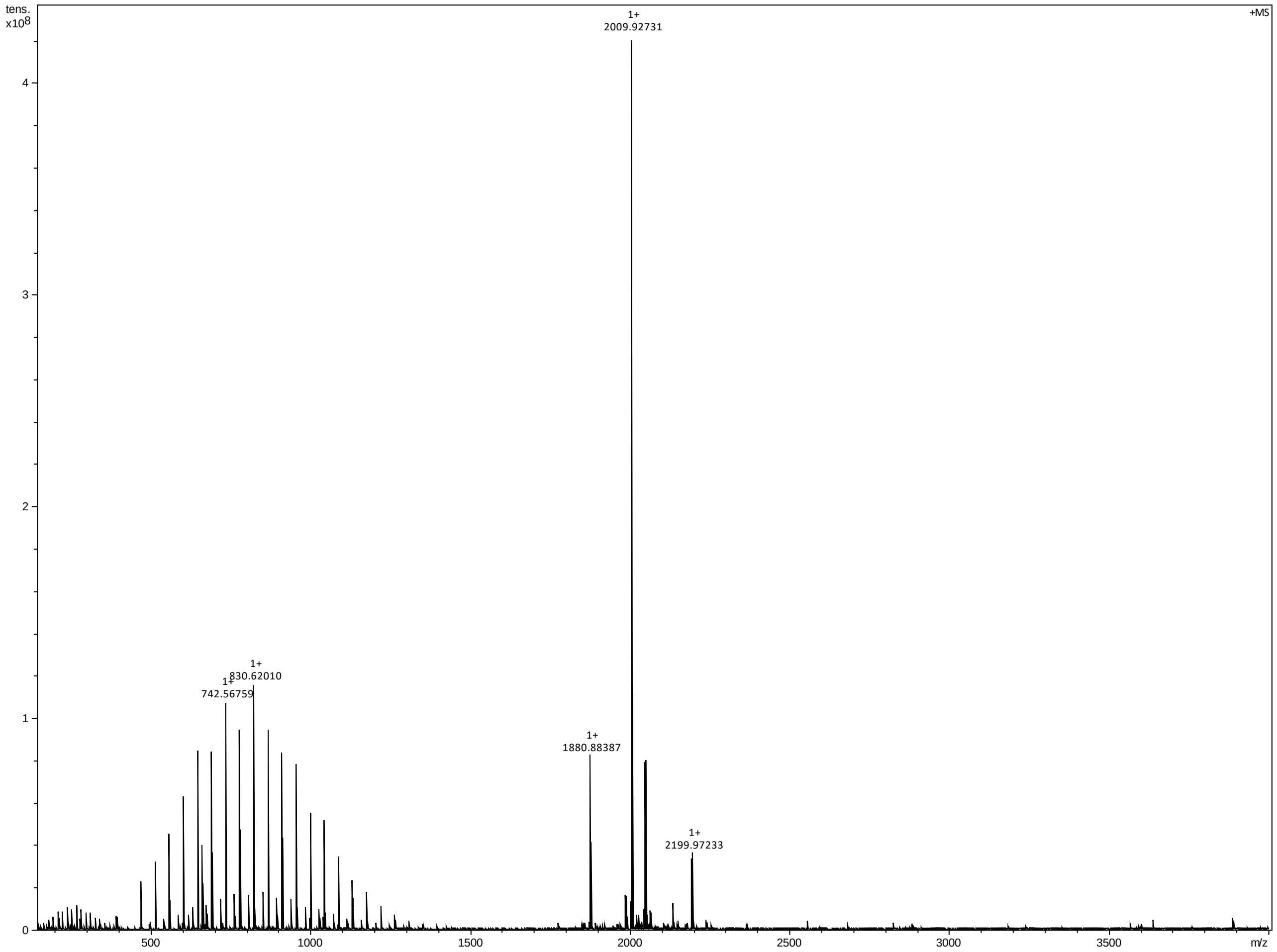


Figure S6. HR-ESI-MS of Peptide **8** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.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1+
830.62010
1+
742.56759
1+
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m/z

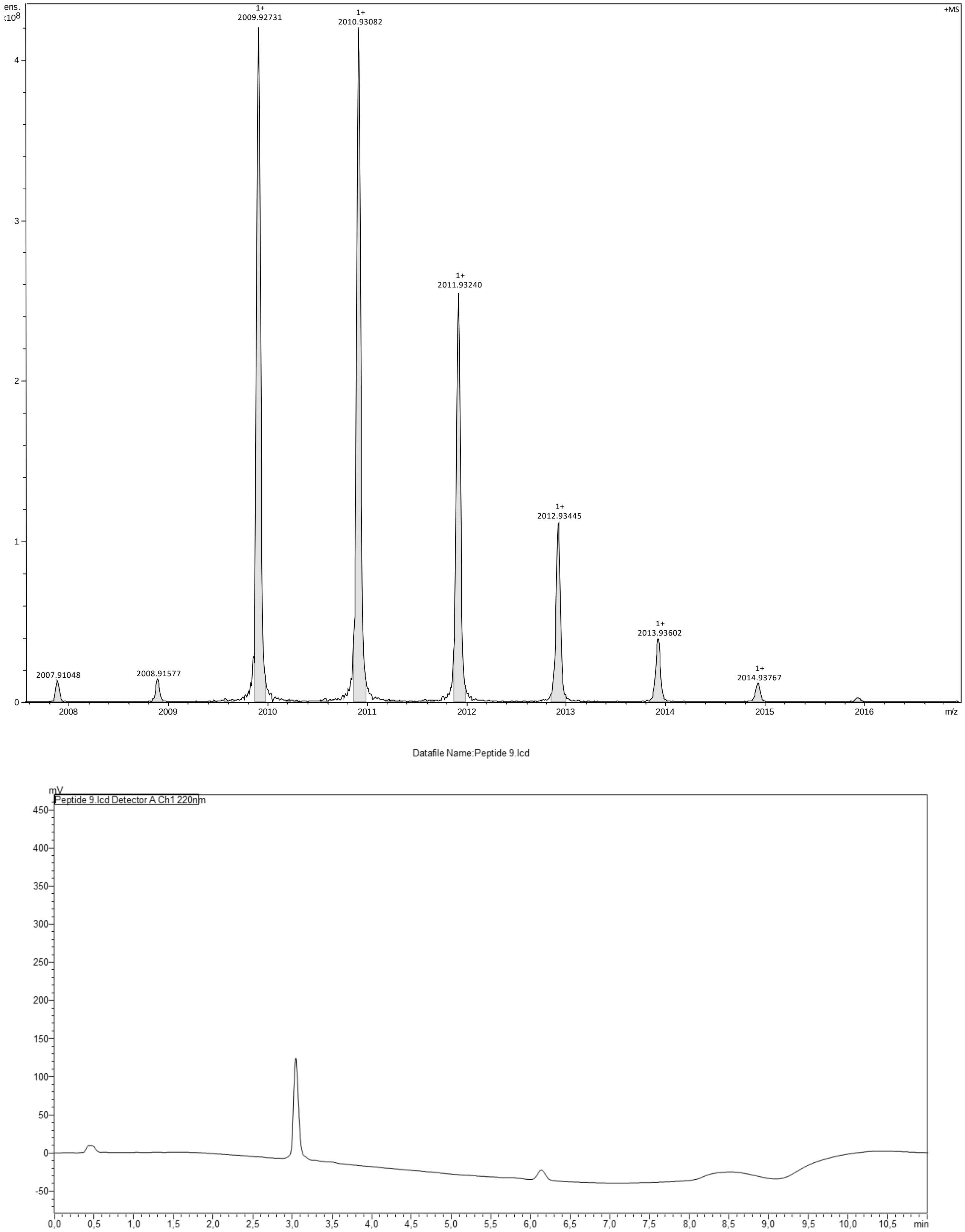
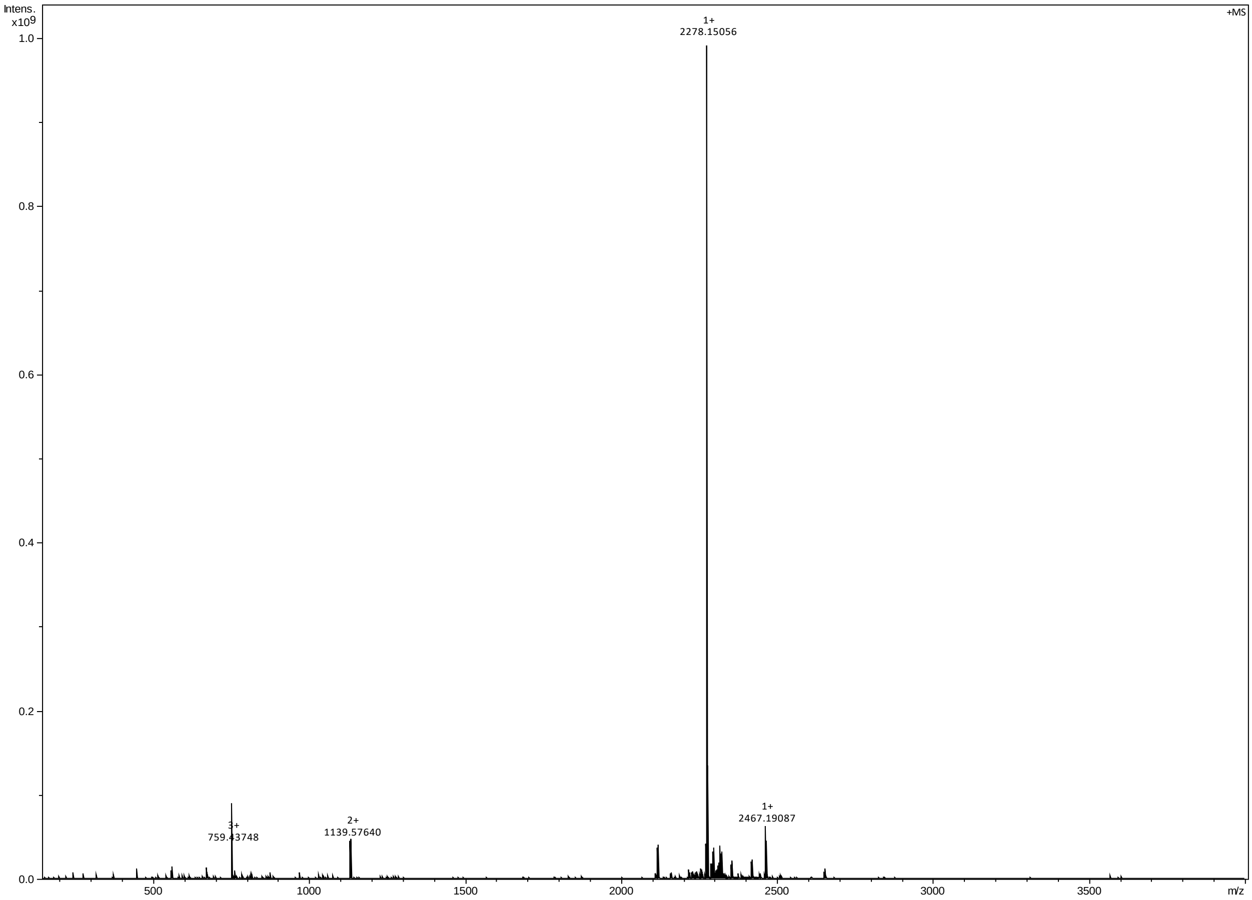


Figure S7. HR-MALDI-MS of Peptide **9** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.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759.43748
2+
1139.57640
1+
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1000
1500
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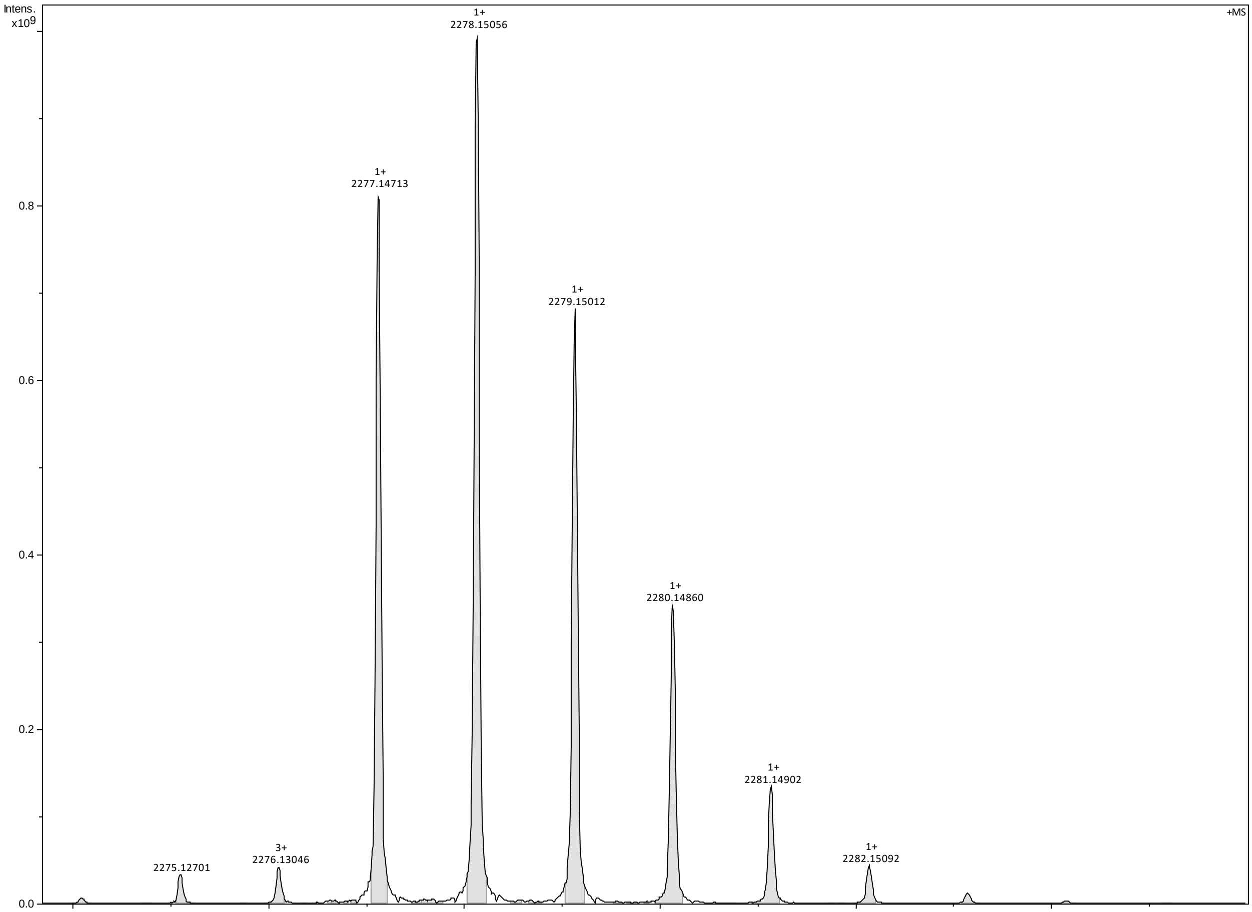
2500
3000
3500
m/z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1+
2277.14713
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1+
2281.14902
1+
2282.15092
3+
2276.13046
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2280
2282
2284
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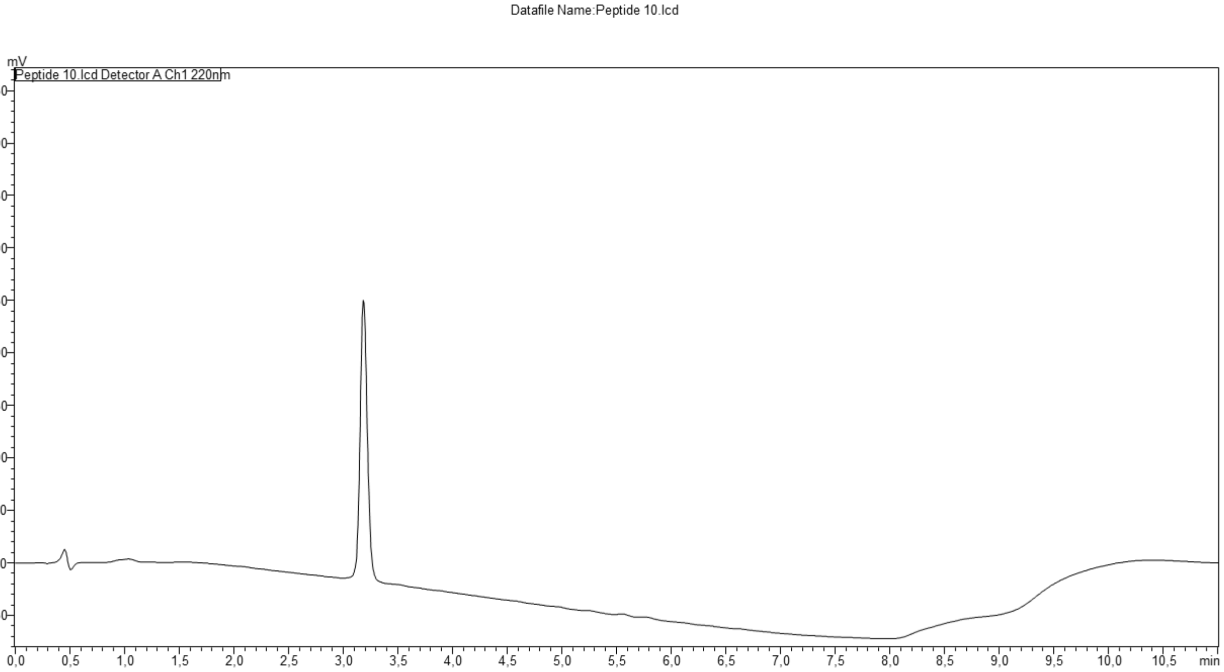
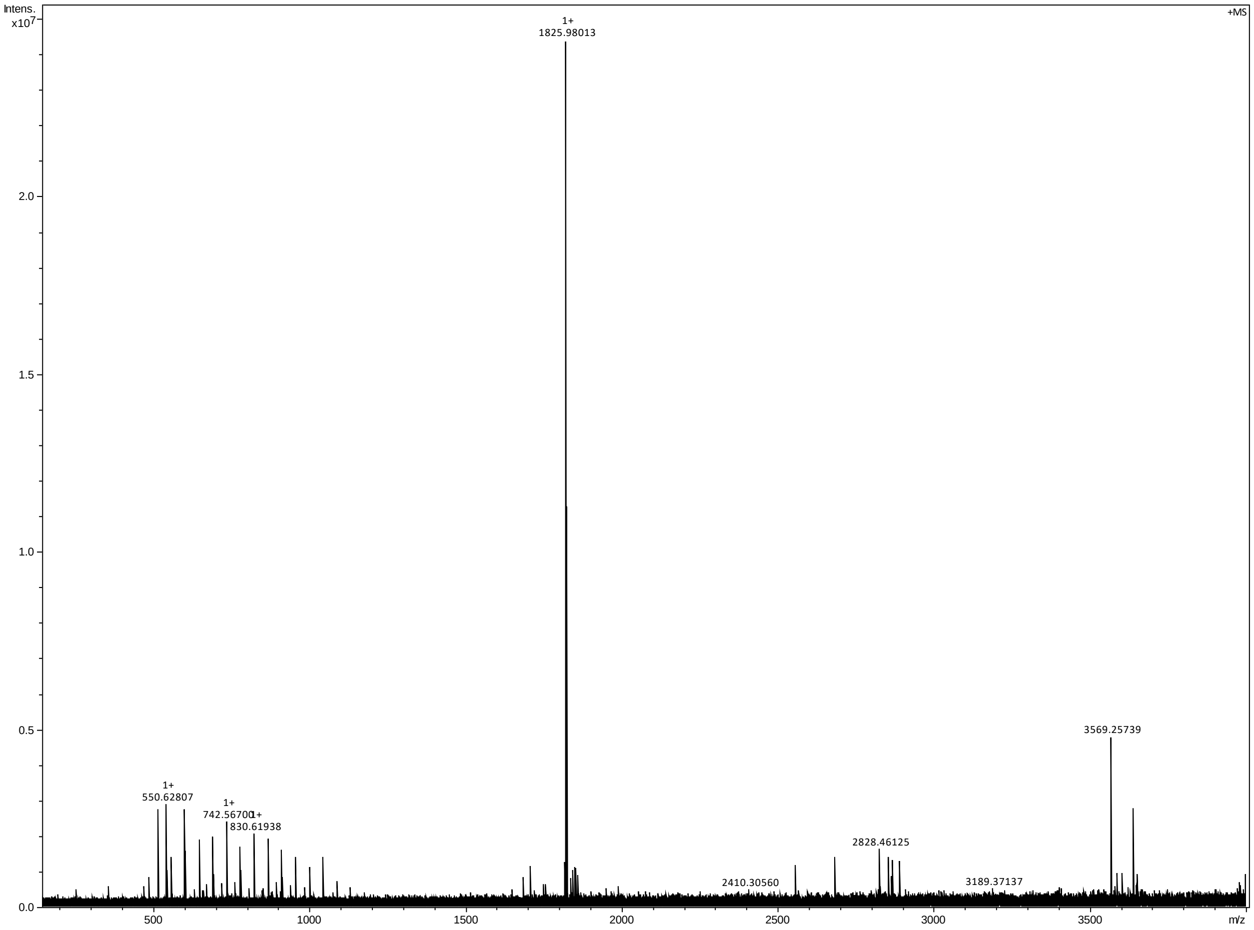


Figure S8. HR-MALDI-MS of Peptide **10** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



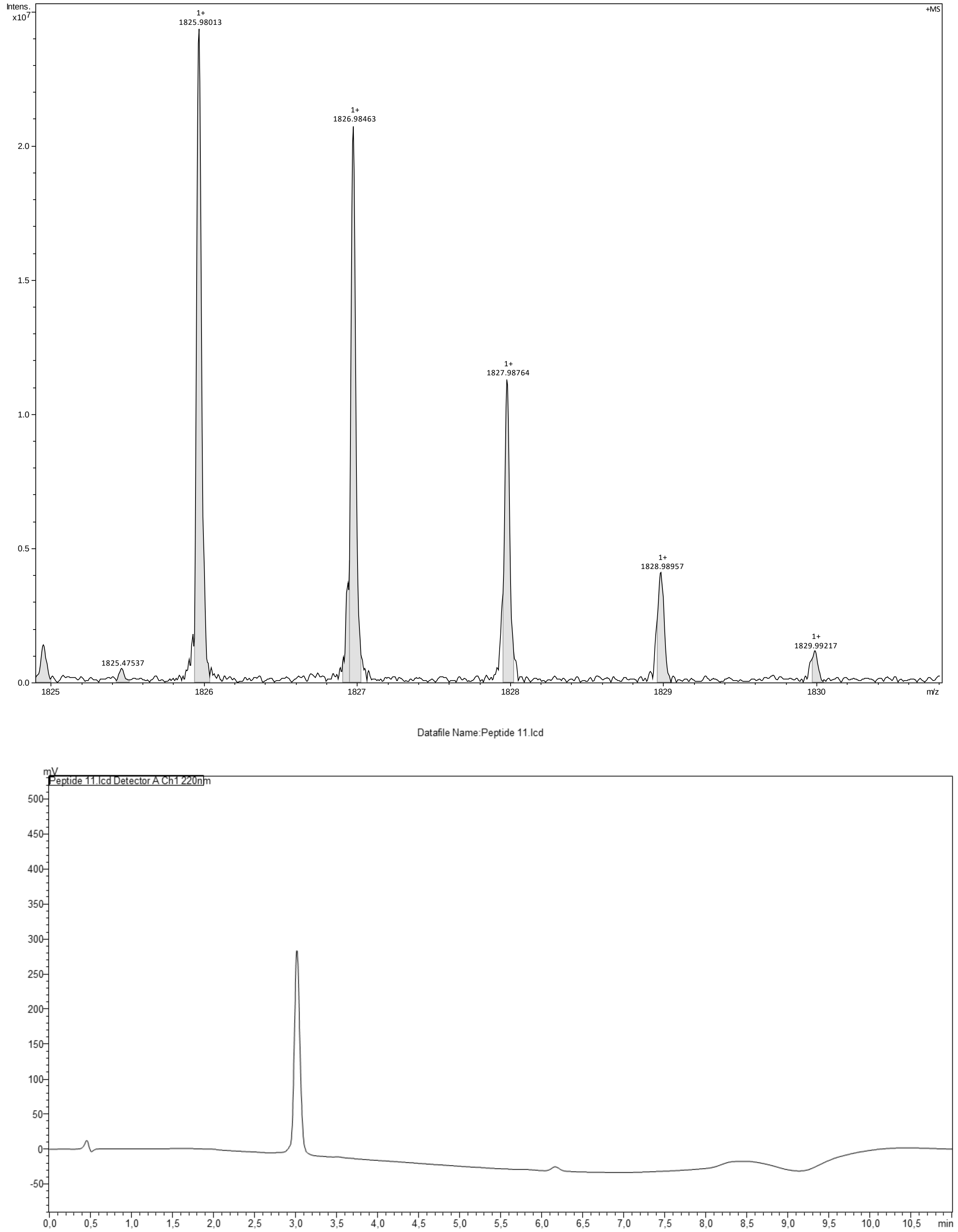
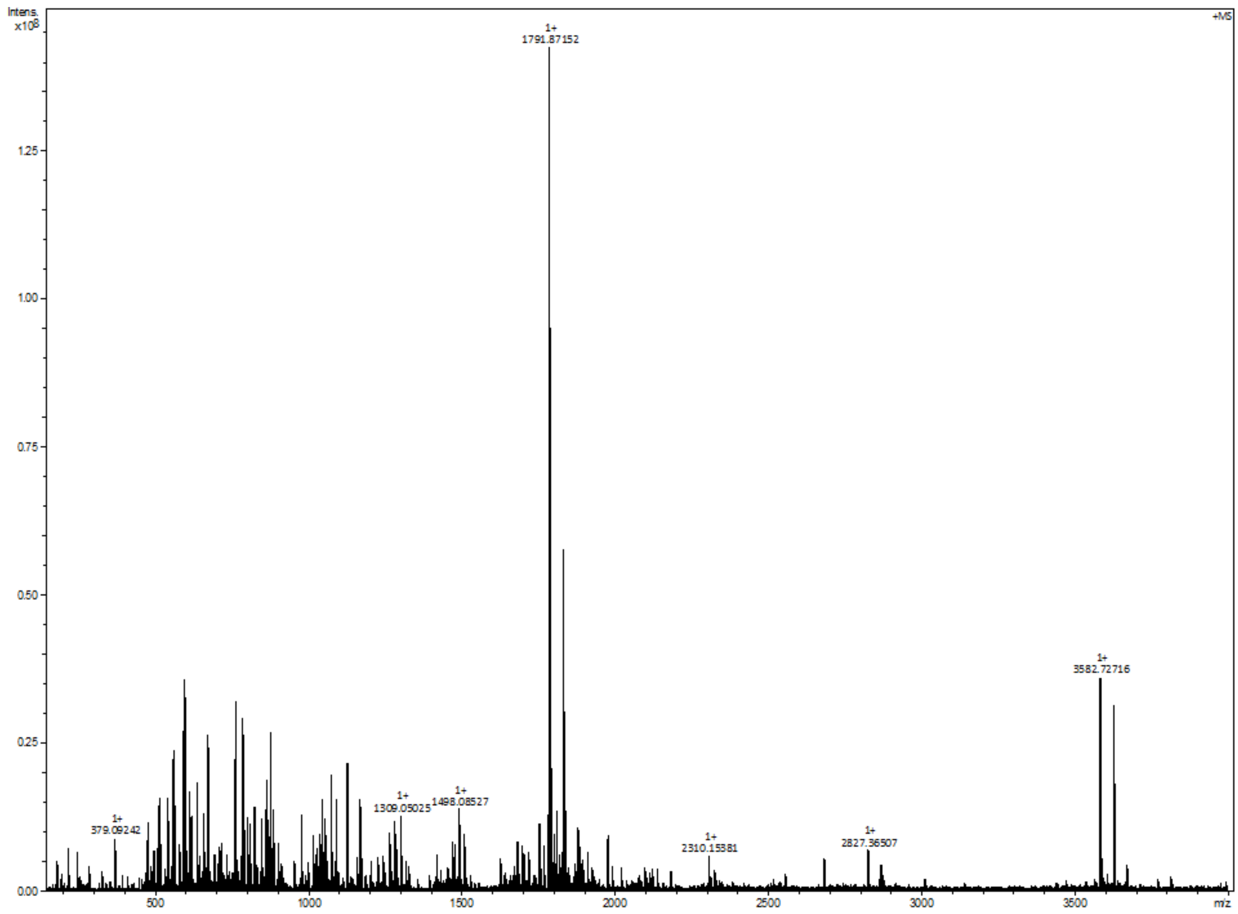


Figure S9. HR-MALDI-MS of Peptide **11** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.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1+
1309.05025
1+
1498.08527
1+
2310.15381
1+
2827.36507
1+
3582.72716
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3500
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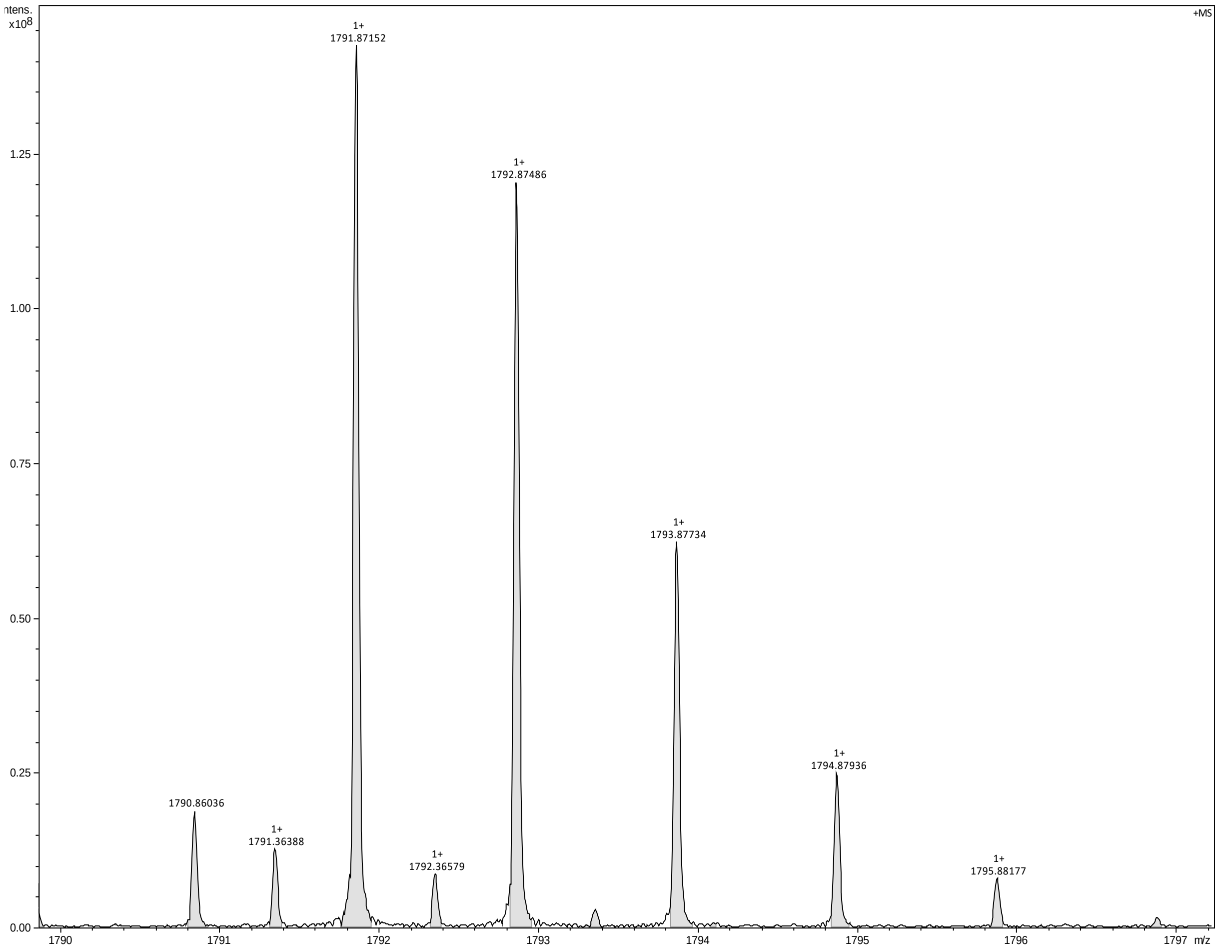
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1790.86036
1+
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1+
1791.87152
1+
1792.36579
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1792.87486
1+
1793.87734
1+
1794.87936
1+
1795.88177
0.00
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0.50
0.75
1.00
1.25
1790
1791
1792
1793
1794
1795
1796
1797
m/z

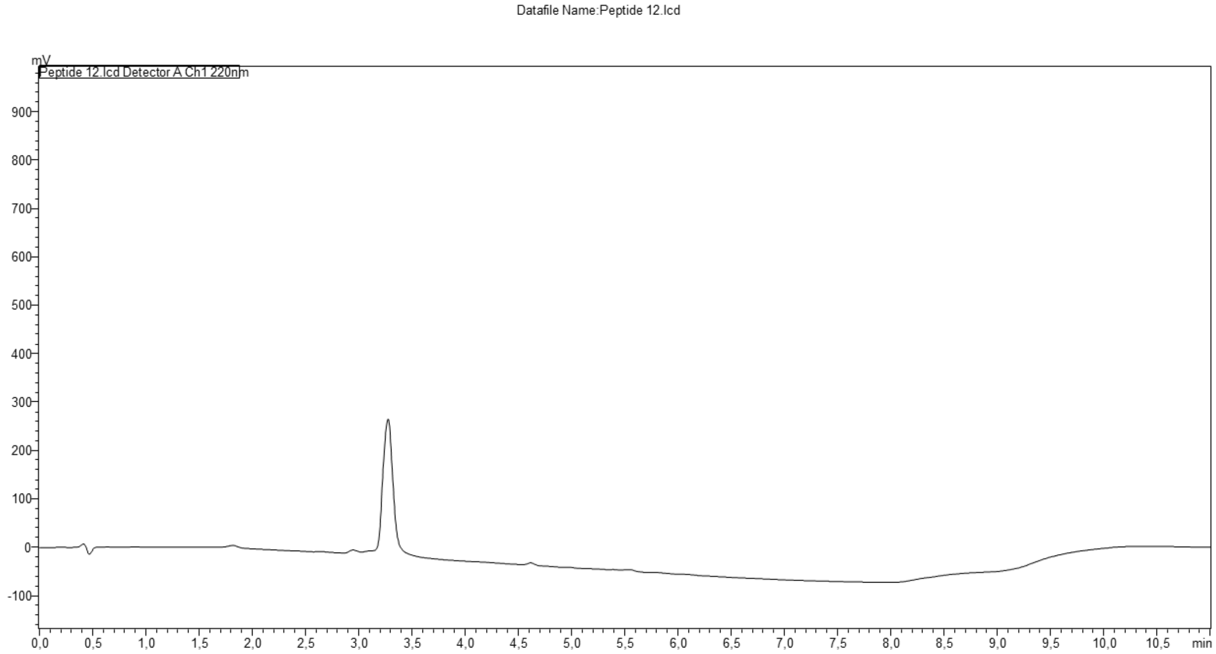
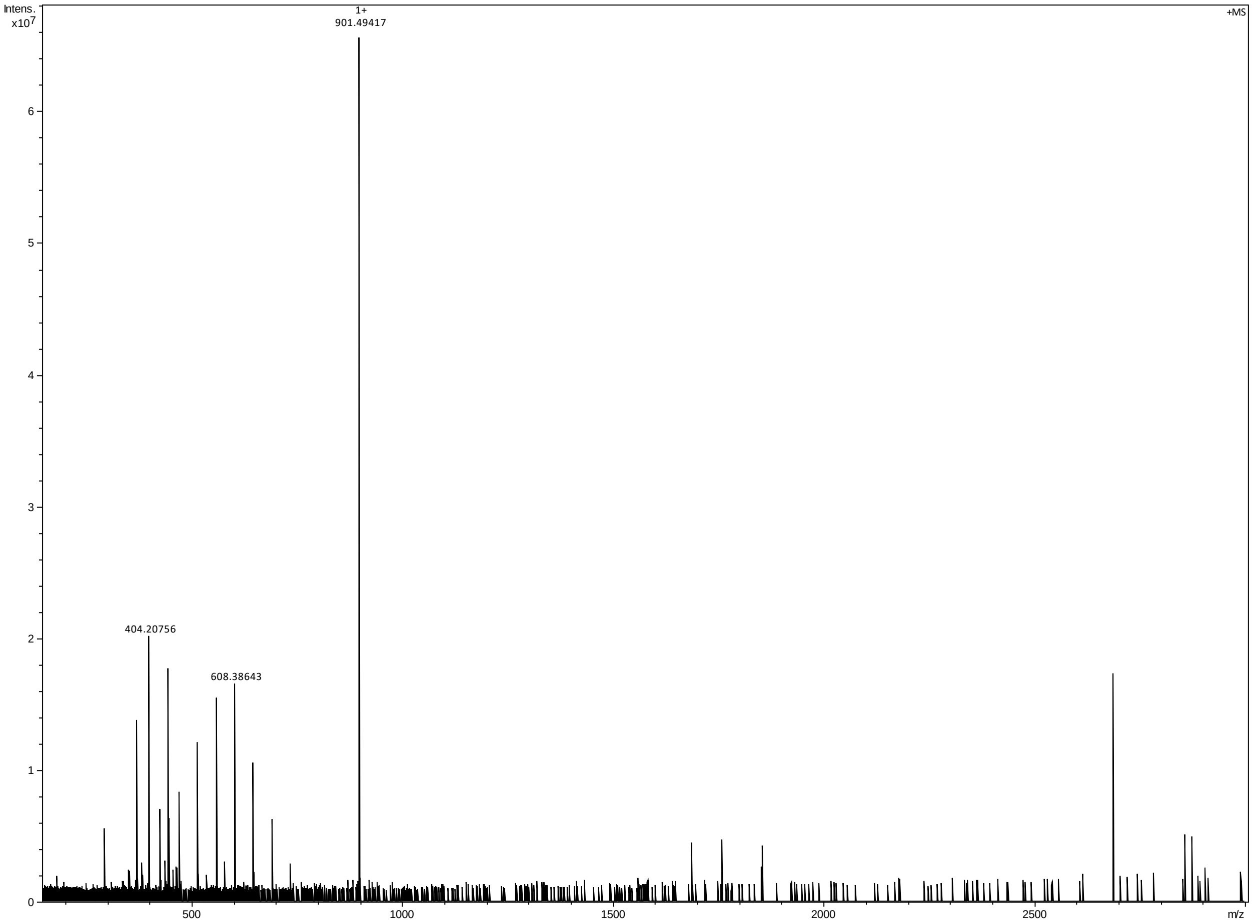


Figure S10. HR-MALDI-MS of Peptide **12** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



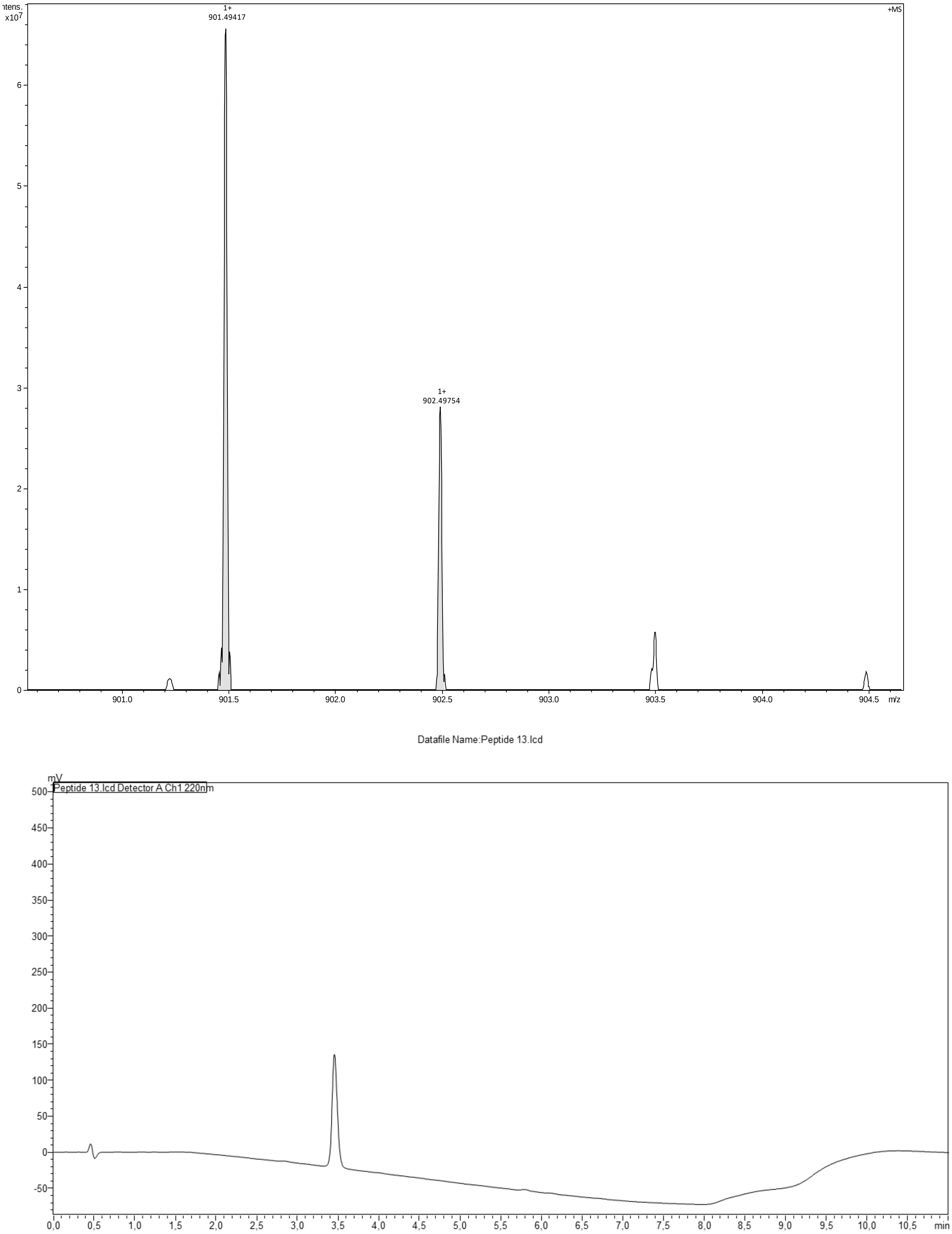


Figure S11. HR-ESI-MS of Peptide **13** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.

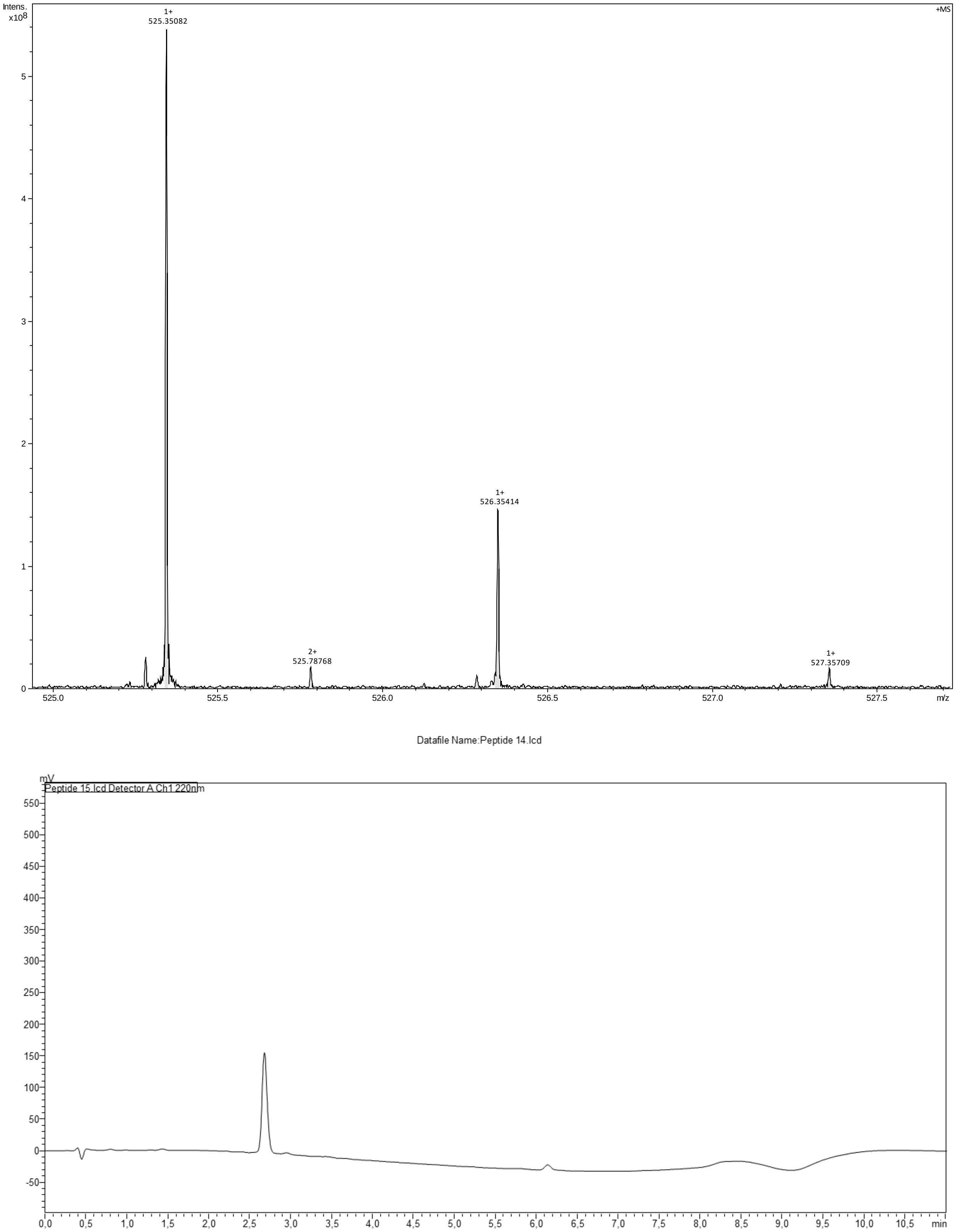
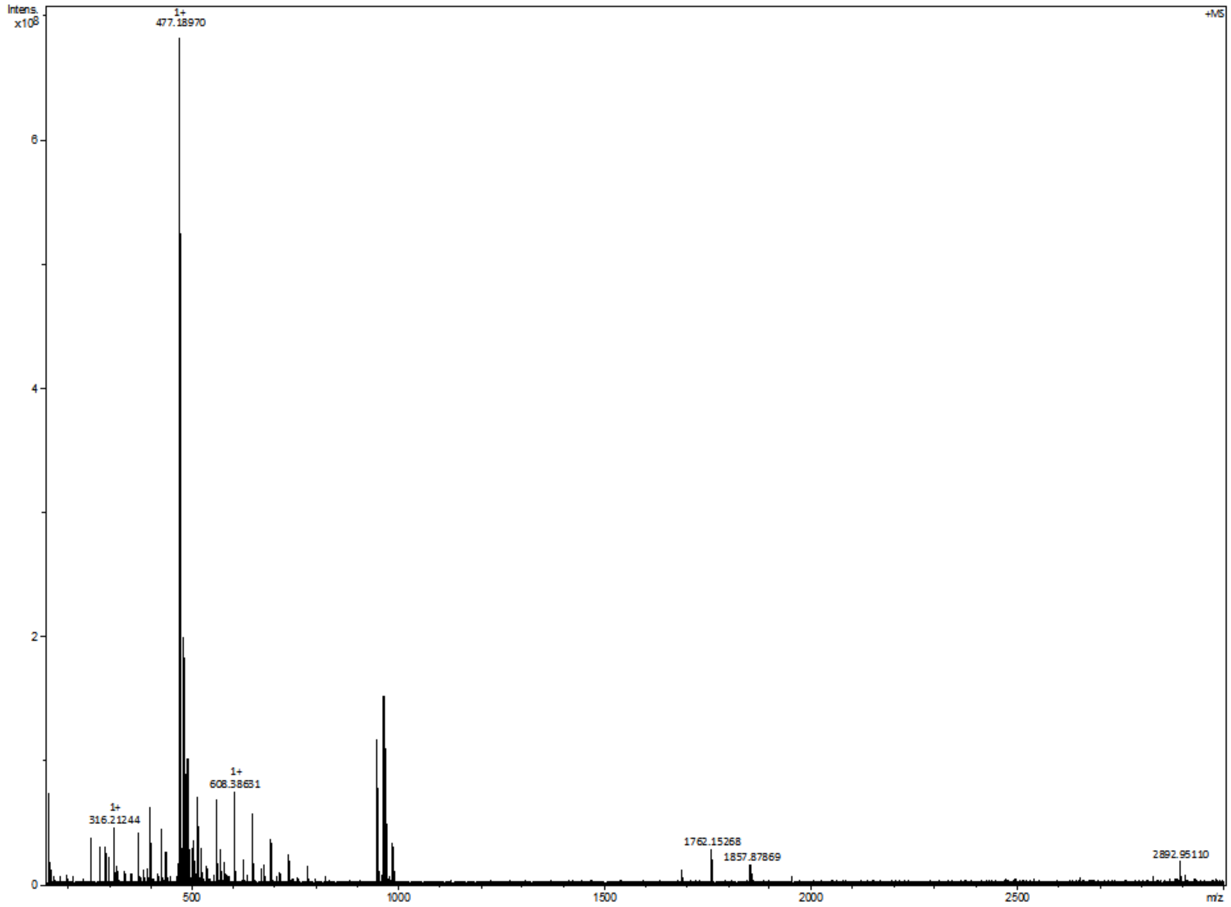


Figure S12. HR-ESI-MS of Peptide **14** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.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1+
316.21244
1+
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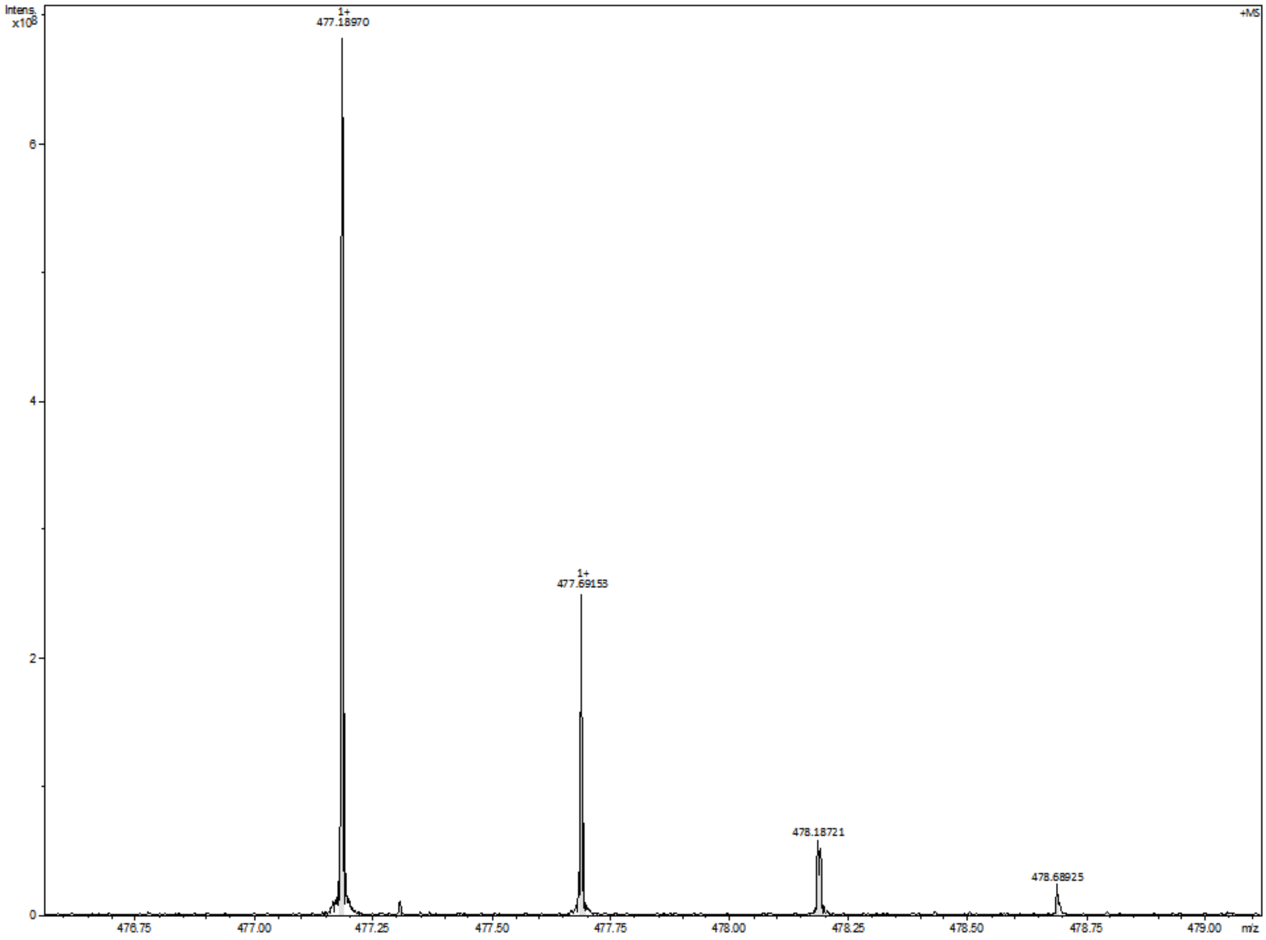
1857.87869
2892.95110
0
2
4
6
500
1000
1500
2000
2500
m/z


Intens.
x10^8
+MS
1+
477.18970
1+
477.69153
478.18721
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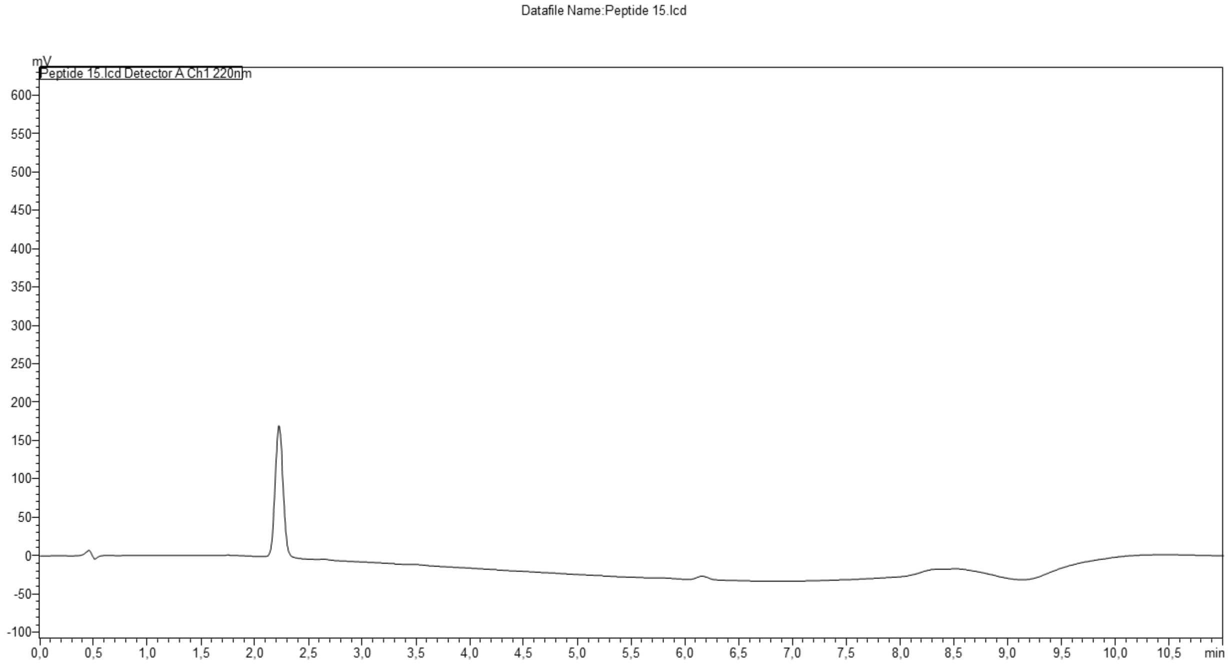
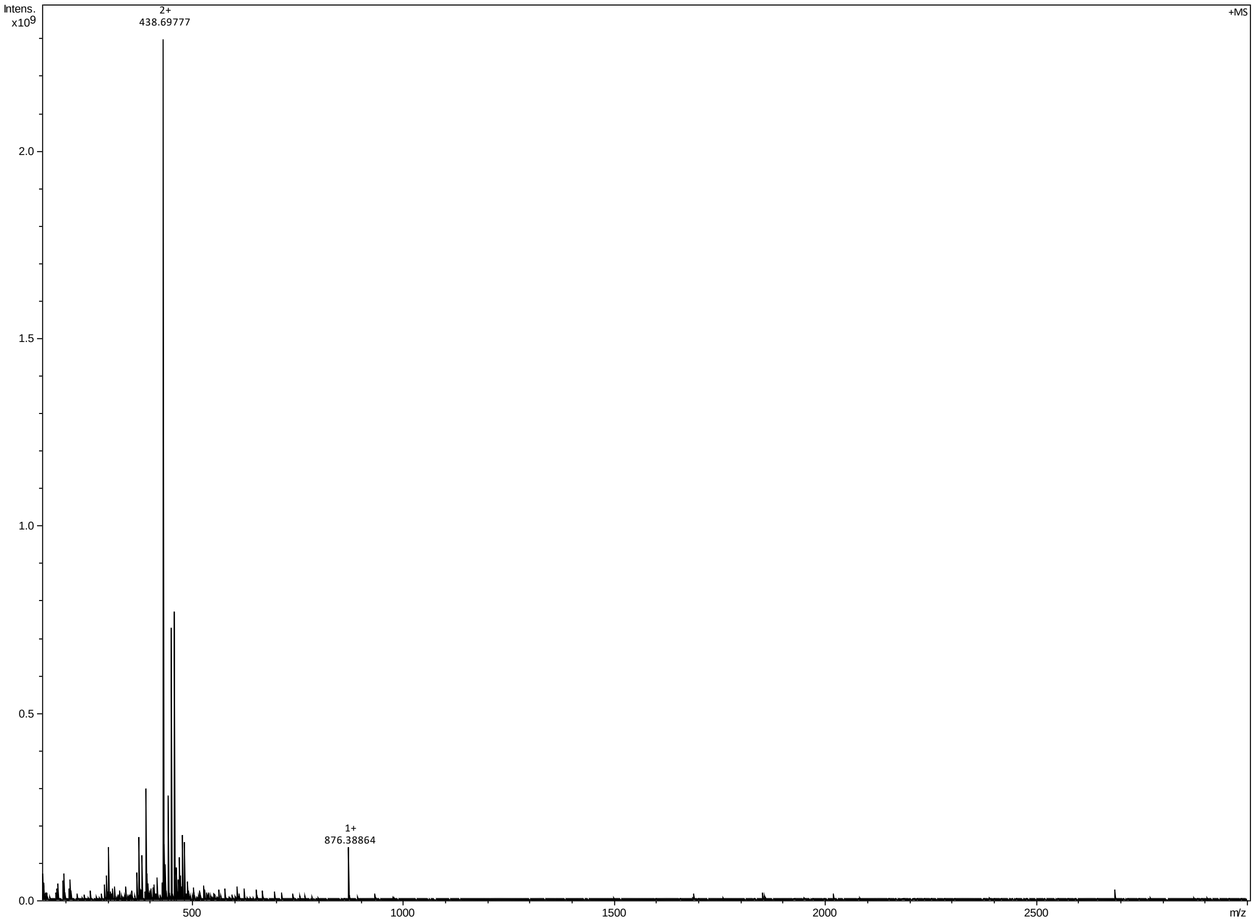


Figure S13. HR-ESI-MS of Peptide **15** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.



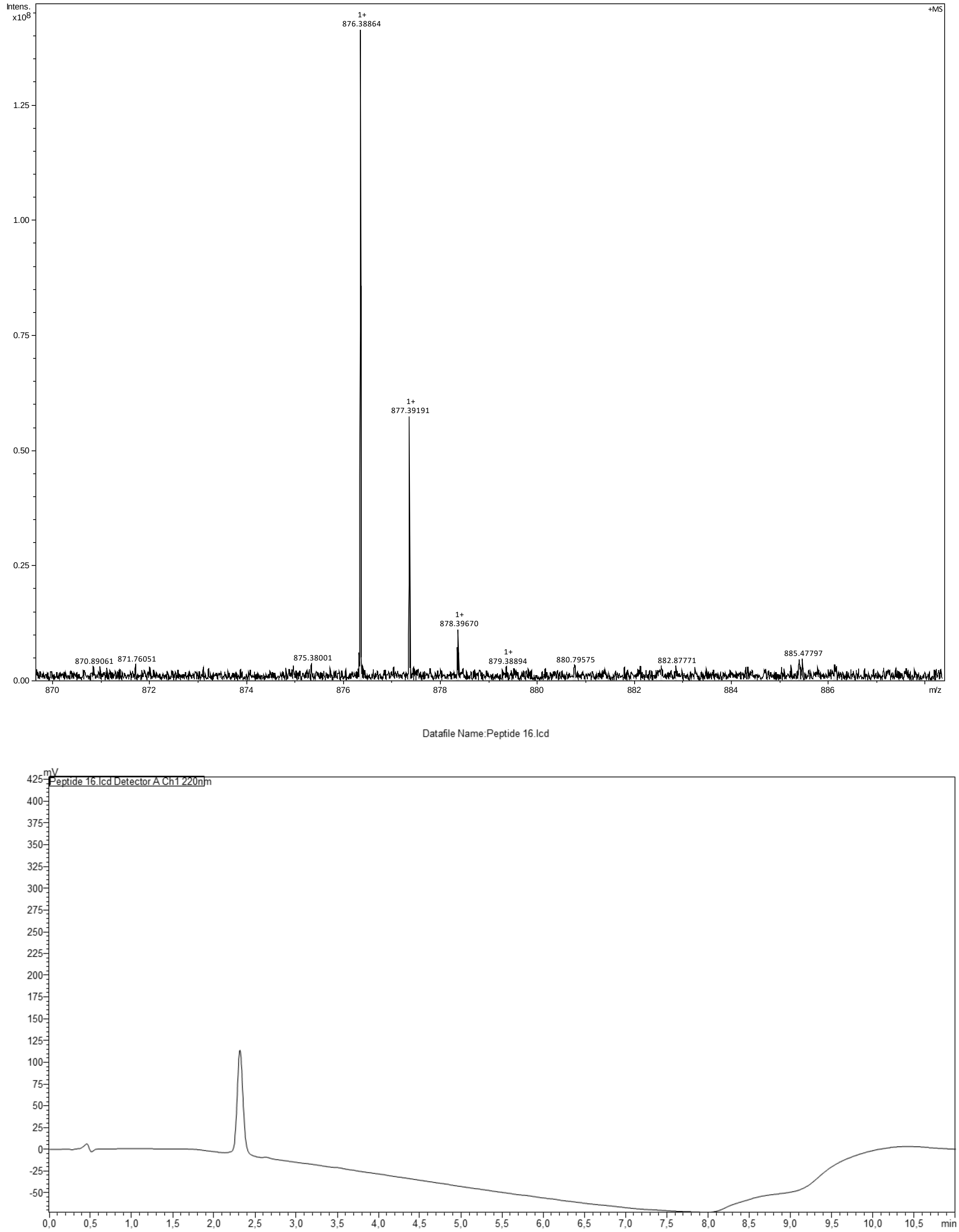


Figure S14. HR-ESI-MS of Peptide **16** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.

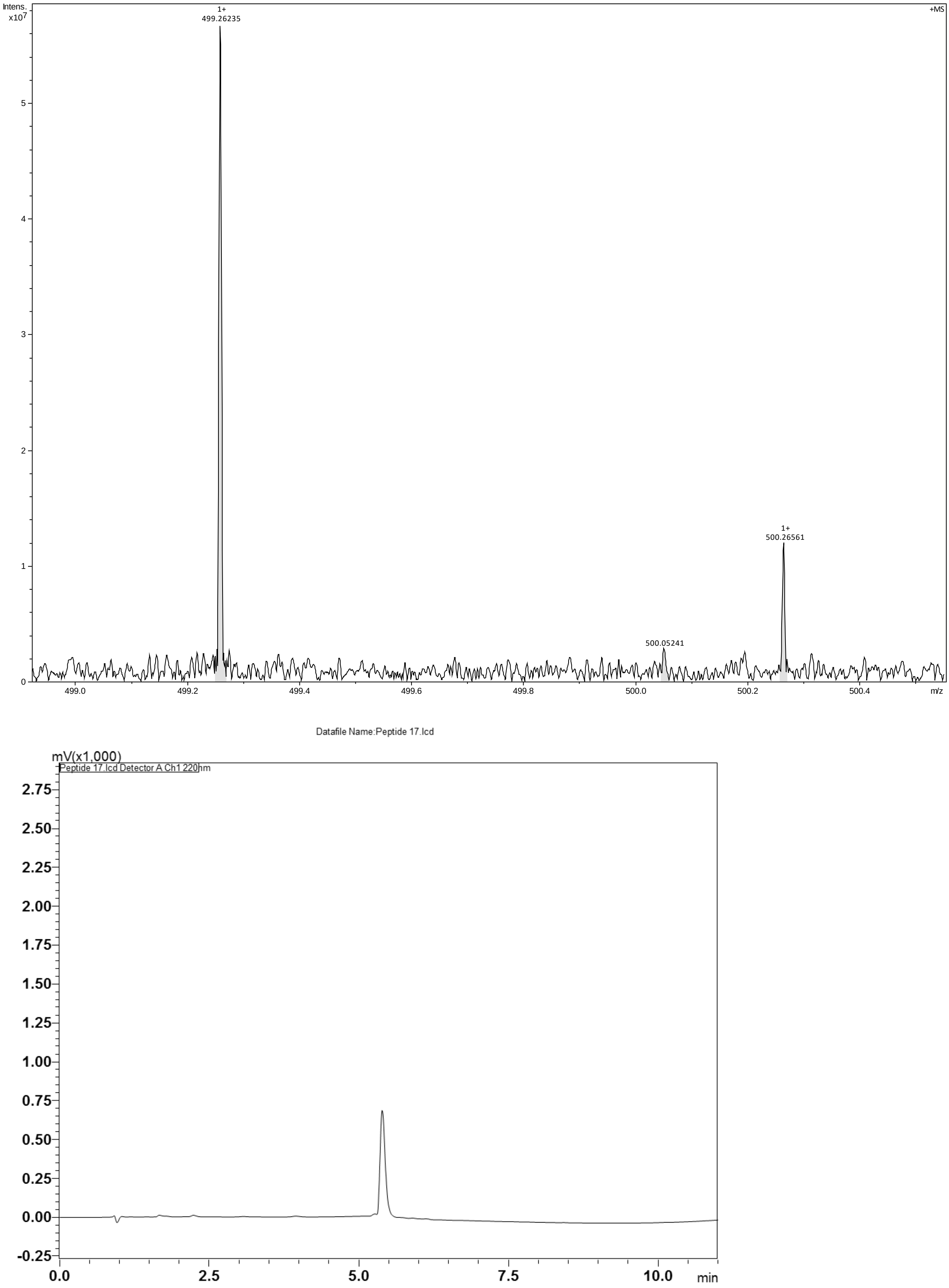
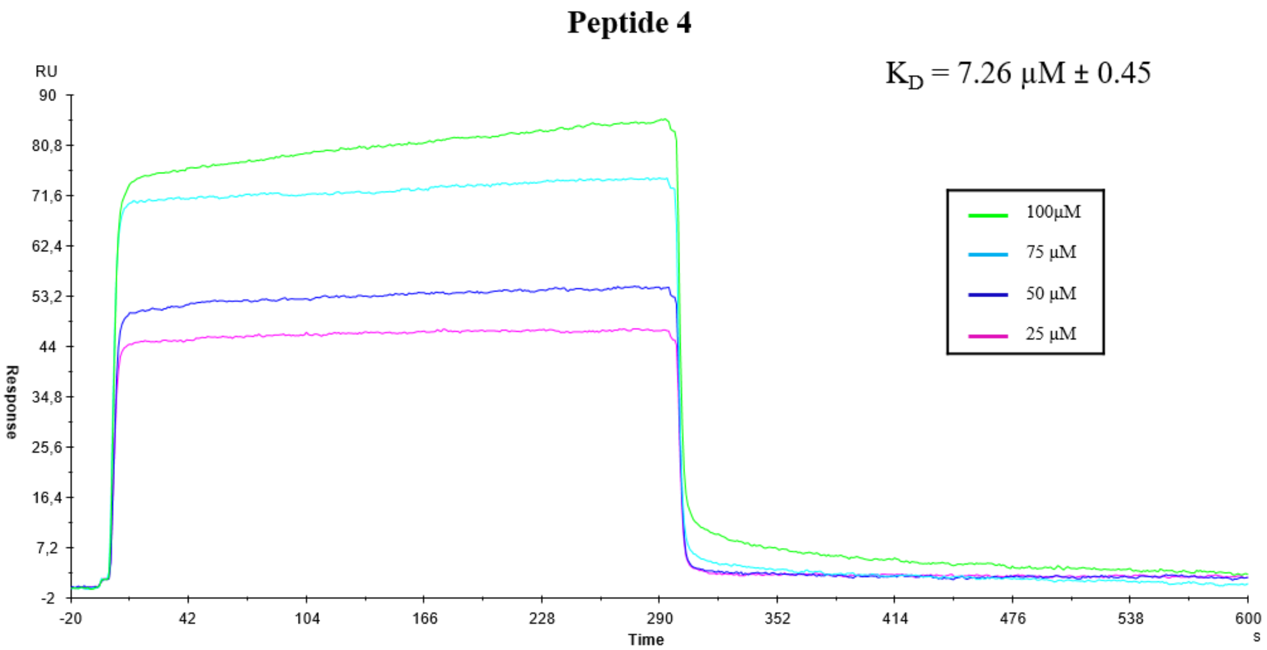


Figure S15. HR-ESI-MS of Peptide **17** ion $[M+H]^+$ and analytical HPLC trace at 220 nm.


Peptide 4

K_D = 7.26 µM ± 0.45

Figure S16. Sensorgram obtained from SPR interaction analysis of compound **4** binding to immobilized HA. This compound was injected at four different concentrations (from 25 µM to 100 µM).

Table S2. ^{1}H chemical shifts (ppm) of **1** in HFA/H_2O (600 MHz, 300 K).

residue	NH	αH	βH	γH	δH	εH	Others
Ser1	7.83	4.12	3.63	-	-	-	-
Lys2	7.45	3.96	1.47	1.12	1.34	2.65	-
			1.43	1.07			
His3	7.87	4.37	2.95	-	6.89 (2H)	-	-
			2.84		8.08 (4H)	-	-
Ser4	7.80	4.22	3.66	-	-	-	-
			3.54	-	-	-	-
Ser5	7.89	4.12	3.61				
Leu6	7.35	4.06	1.29	1.22	0.61	-	-
					0.51	-	-
Asp7	7.59	4.14	2.43	-	-	-	-
Cys8	7.40	4.06	2.69	-	-	-	-
			2.65				
Val9	7.25	3.76	1.81	0.61	-	-	-
Leu10	7.31	3.99	1.30	1.22	0.53	-	-
					0.51		
Arg11	7.21	4.33	1.53	1.54	2.85		6.609 (NHE)
			1.44		2.77		7.526 (NHZ)
Pro12	-	4.04	1.93	1.61	3.39	-	-
			1.71		3.25		

Table S3. ^{1}H chemical shifts (ppm) of **13** in HFA/H_2O (600 MHz, 300 K).

residue	NH	αH	βH	γH	δH	εH	Others
Ser1	7.48	4.28	3.78	-	-	-	
Leu2	8.11	4.06	1.37	1.27	0.60 0.64		
Asp3	7.98	4.19	2.42				
Cys4	7.54	4.18	2.69				
Val5	7.41	3.82	1.87	0.66			
Leu6	7.37	4.06	1.28	1.37	0.57 0.61		
Arg7	7.37	4.37	1.47 1.57	1.36	2.89	-	6.71(NHE)
Pro8	-	4.08	1.76 1.99	1.67	3.31 3.44	-	-

Table S4. ^{1}H chemical shifts (ppm) of **14** in [D_6]DMSO (600 MHz, 300 K).

residue	NH	αH	βH	γH	δH	εH	Others
Acetyl	-	-	-	-	-	-	1.88
Val1	7.94	4.12	1.97	0.84	-	-	-
Leu2	8.01	4.31	1.48 1.59	1.45	0.82 0.87	-	-
Arg3	7.91	4.49	1.55 1.77	1.53	3.09	-	7.83
Pro4	-	4.20	1.92 2.03	1.79	3.59 3.73	-	6.88 7.32

Table S5. ^{1}H chemical shifts (ppm) of **15** in [D$_6$]DMSO (600 MHz, 300 K).

residue	NH	αH	βH	γH	δH	εH	Others
Acetyl	-	-	-	-	-	-	1.88
Ser1	7.95	4.30	3.54 3.57				
Leu2	8.03	4.27	1.62	1.49	0.85 0.88		
Asp3	8.20	4.52	2.53 2.77				
Cys4	7.75	4.28	2.76 2.83				7.24

Table S6. ^{1}H chemical shifts (ppm) of **17** in [D$_6$]DMSO (600 MHz, 300 K).

residue	NH	αH	βH	γH	δH	εH	Others
Acetyl	-	-	-	-	-	-	1.87
Ser1	8.12	4.33	3.62 3.57	-	-	-	-
Lys2	8.34	4.17	1.65 1.51	1.27	1.51	2.73	-
His3	8.06	4.41	2.96 2.85	-	6.82 (2H)	7.51 (4H)	-
Ser4	7.86	4.14	3.63	-	-	-	-

Table S7. Mean values of φ, ψ and χ1 angles and αC distances relative to the most representative conformers of **1**.

peptide	sequence	i+1			i+2			αC distance	
		φ	ψ	χ1	φ	ψ	χ1	i to i+2	i to i+3
1	Lys2- Ser5	-52.9	-29.7	-83.7	-138.1	-42.8	-140.3	-	4.8
	Ser4-Asp7	-76.3	166.1	-124.7	69.9	8.5	-67.0	-	6.2
	Leu6-Val9	-108.6	75.2	-95.6	64.6	27.2	-120.6	-	5.7
	Asp7-Leu10	64.6	27.2	-120.6	71.1	42.9	-164.2	-	5.3
	Val9-Arg11	-76.1	91.0	-147.5	-	-	-	5.9	-

Table S8. Mean values of φ, ψ and χ1 angles and αC distances relative to the most representative conformers of **13**.

		i+1			i+2			αC distance
peptide	sequence	φ	ψ	χ1	φ	ψ	χ1	i to i+3
13	Cys4-Arg7	-51.9	-29.7	-164.4	-152.9	20.8	-116.0	4.4

Table S9. ^{1}H chemical shifts (ppm) of **4** in HFA/H_2O (600 MHz, 300 K).

residue	NH	αH	βH	γH	δH	εH	Others
Lys1		3.74	1.67	2.76	1.21		
Ala2	8.22	4.01	1.15				
Asn3	7.89	4.34	2.53		6.34 6.98		
Glu4	8.01	3.95	1.71 1.79	2.05			
Gly5	7.92	3.65					
Leu6	7.45	4.04	1.37	1.30	0.60		
Thr7	7.54	3.95		0.90			
Trp8	7.70	4.22	3.05				6.85 7.17 7.29 6.93 9.35
Asn9	7.63	4.10	2.31 2.36		6.27 6.91		
Ser10	7.63	4.05	3.64 3.70				
Leu11	7.45	4.56	1.42	1.28	0.58		
Lys12	7.38	3.89	1.41	1.06	1.30	2.66	
Asp13	7.66	4.41	2.45 2.53				
Lys14	7.25	3.92	1.49 1.61	1.14	1.40	2.71	

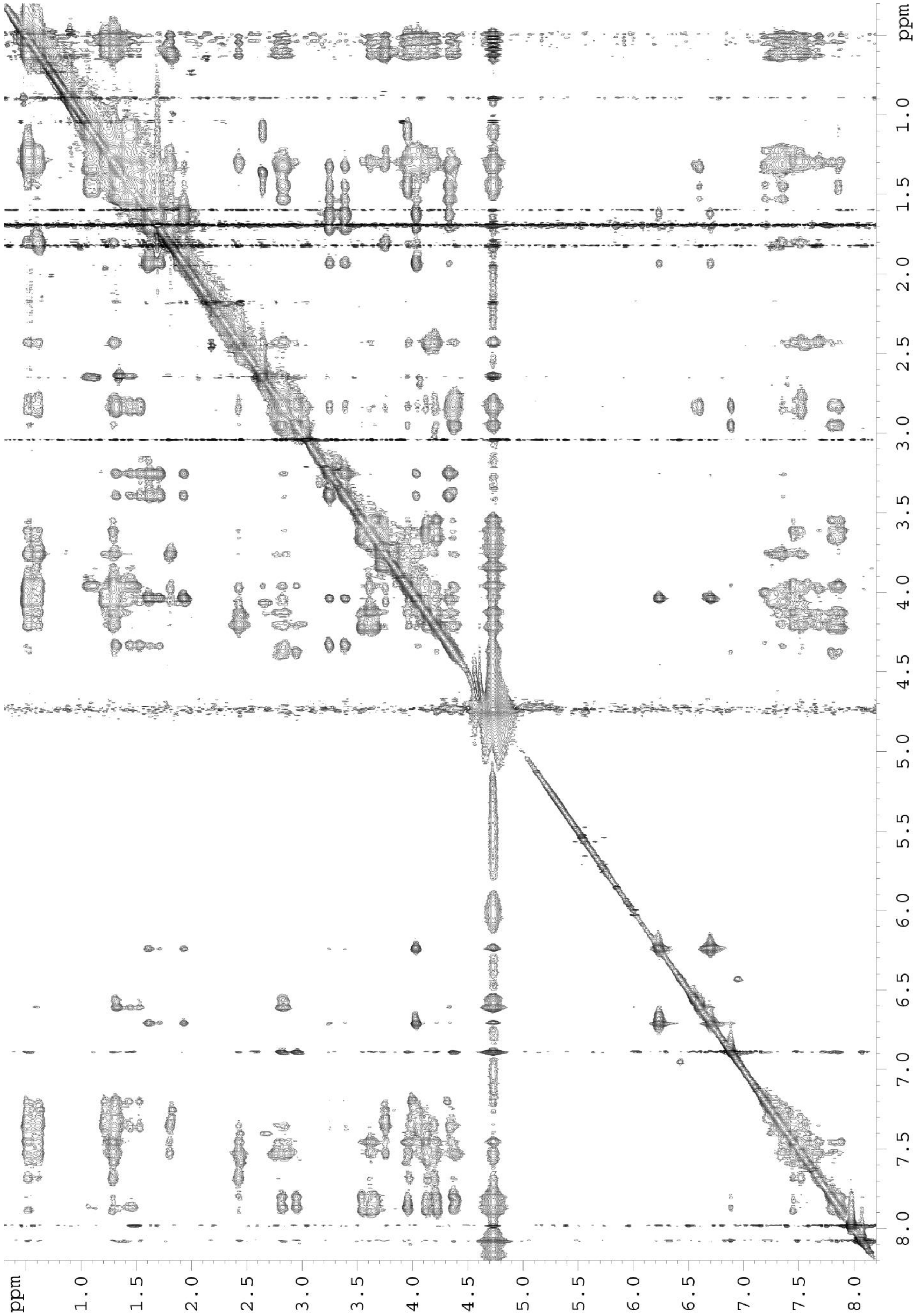


Figure S17. 2D-NOESY spectrum of **1** HFA/H_2O solution (600 MHz, 300 K, t_{mix} = 400 ms).

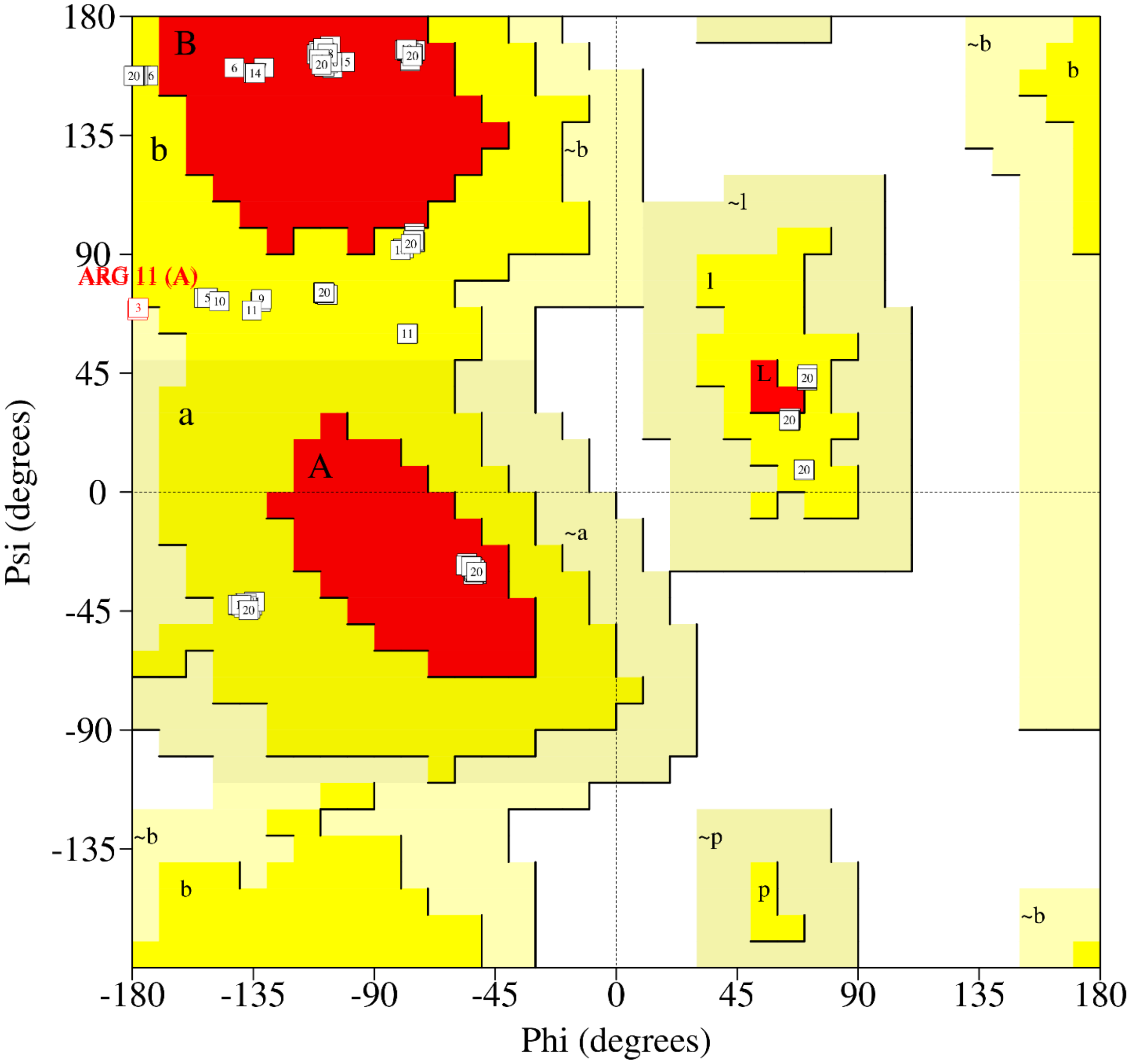


Figure S18. Ramachandran plot of NMR derived bundle of **1**, calculated by PROCHECK[i] software.

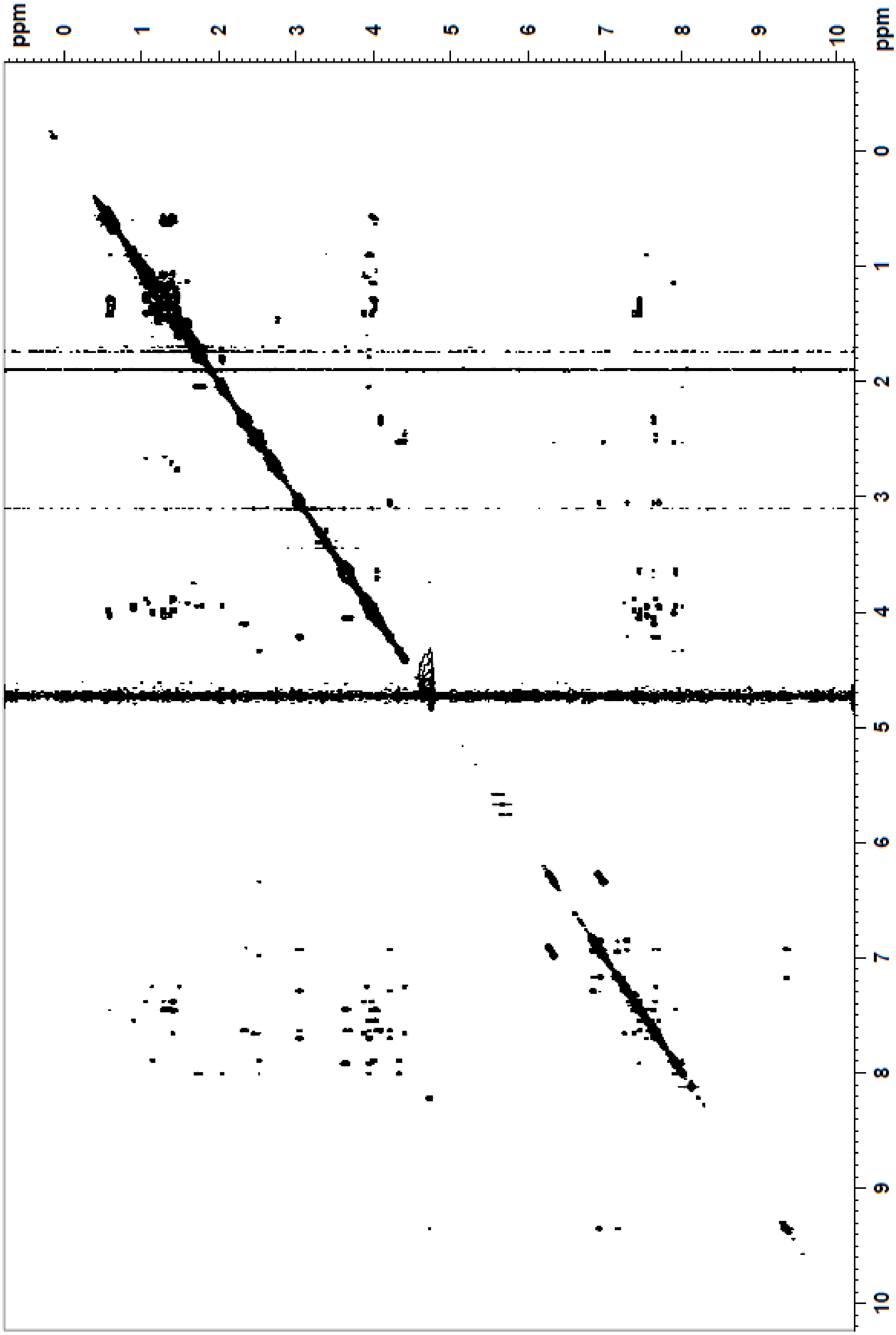


Figure S19. 2D-NOESY spectrum of **4** HFA/H_2O solution (600 MHz, 300 K, t_{mix} = 300 ms).

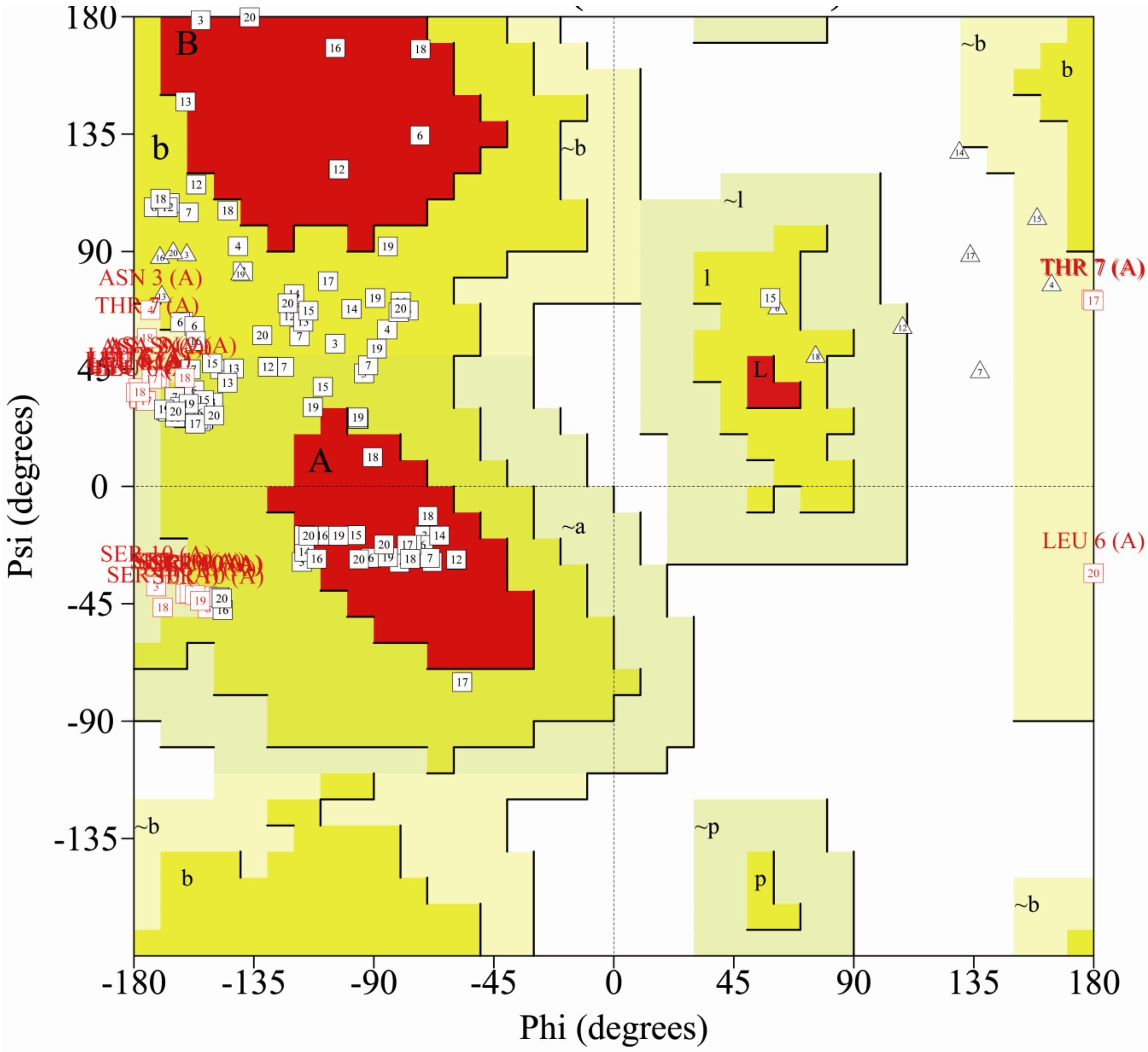


Figure S20. Ramachandran plot of NMR derived bundle of **4**, calculated by PROCHECK[i] software.

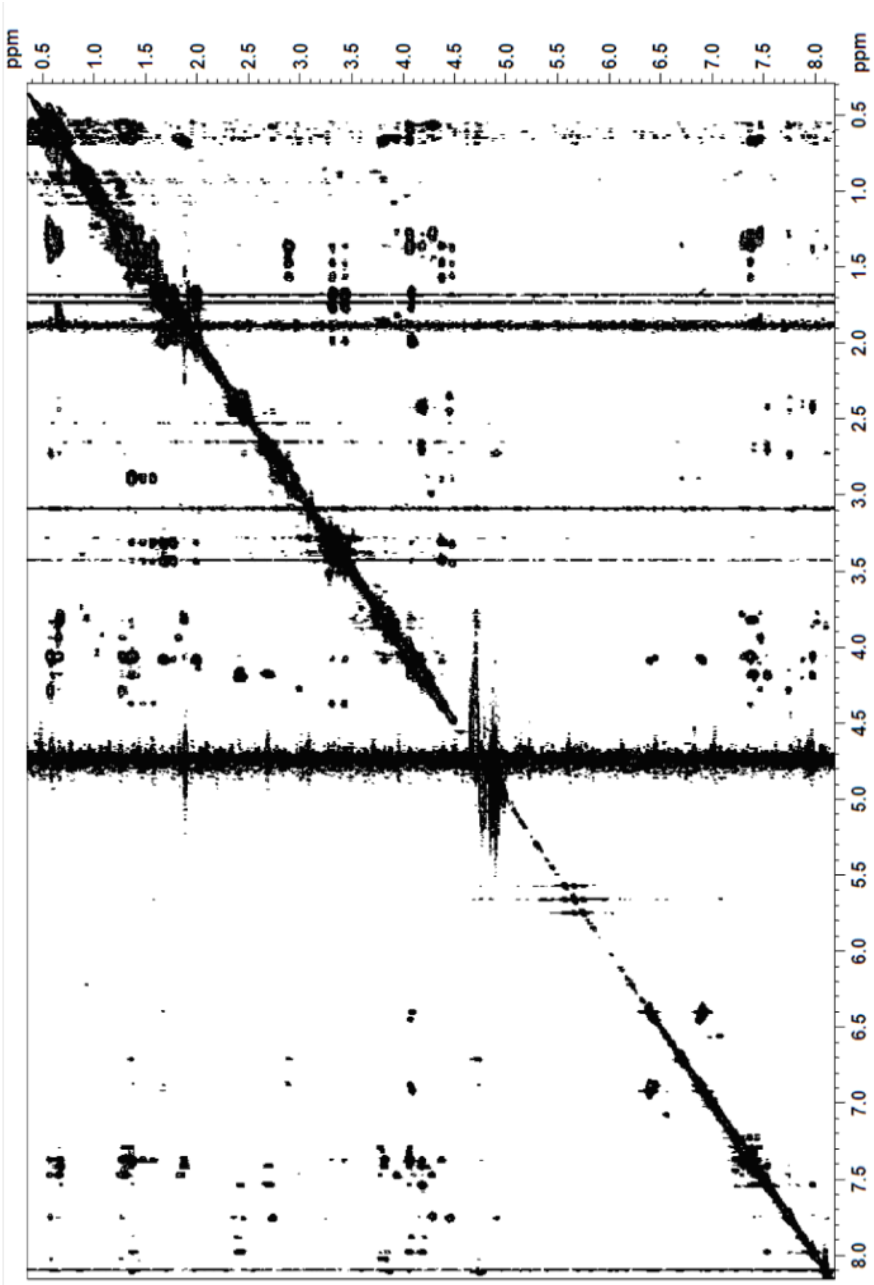


Figure S21. 2D-NOESY spectrum of **13** HFA/H_2O solution (600 MHz, 300 K, t_{mix} = 500 ms).

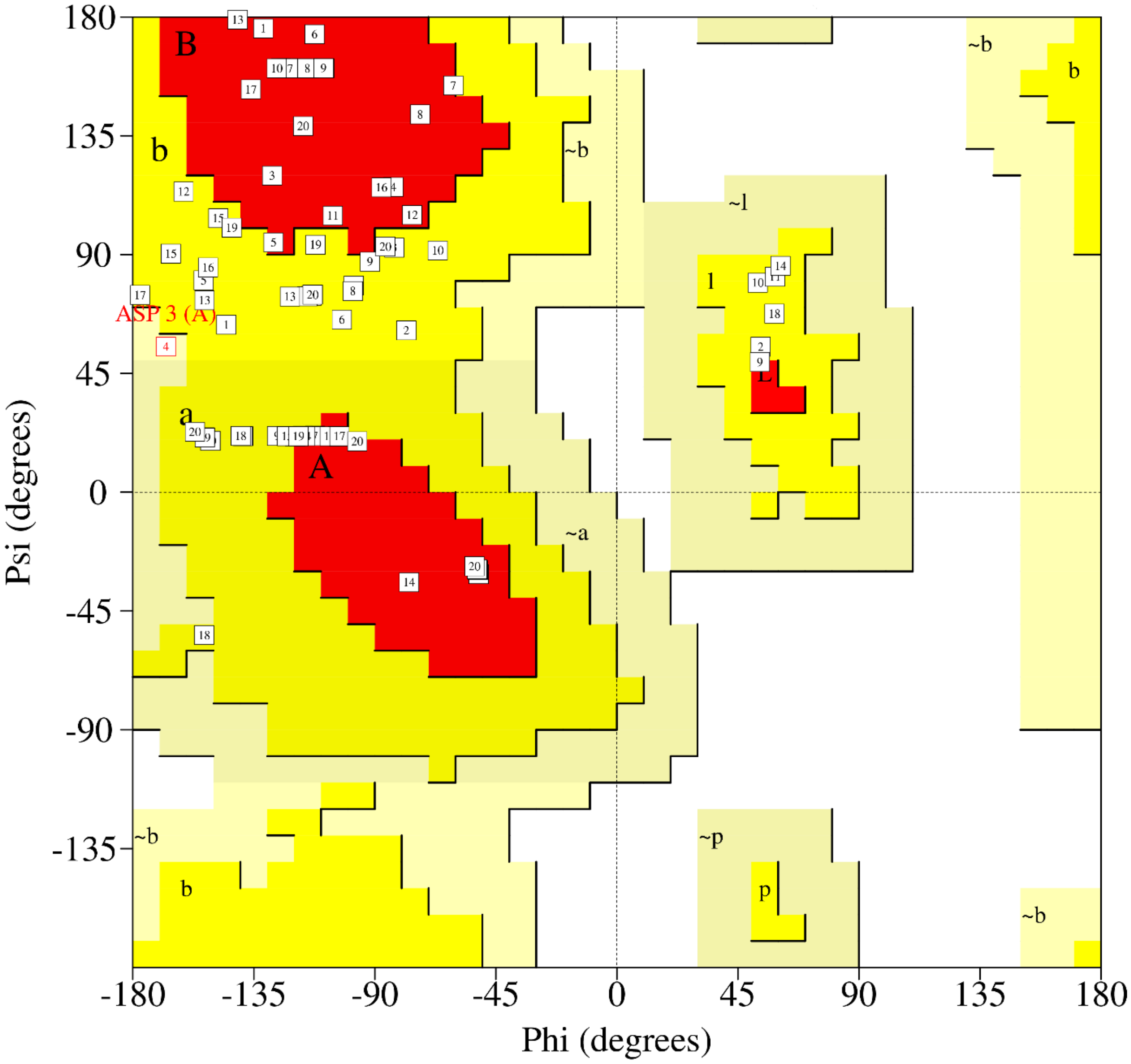


Figure S22. Ramachandran plot of NMR derived bundle of **13**, calculated by PROCHECK[i] software.

References

[i] Laskowski, R. A., Rullmannn, J. A., MacArthur, M. W., Kaptein, R., Thornton, J. M. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *J. Biomol. NMR.* **8**, 477-486 (1996).