

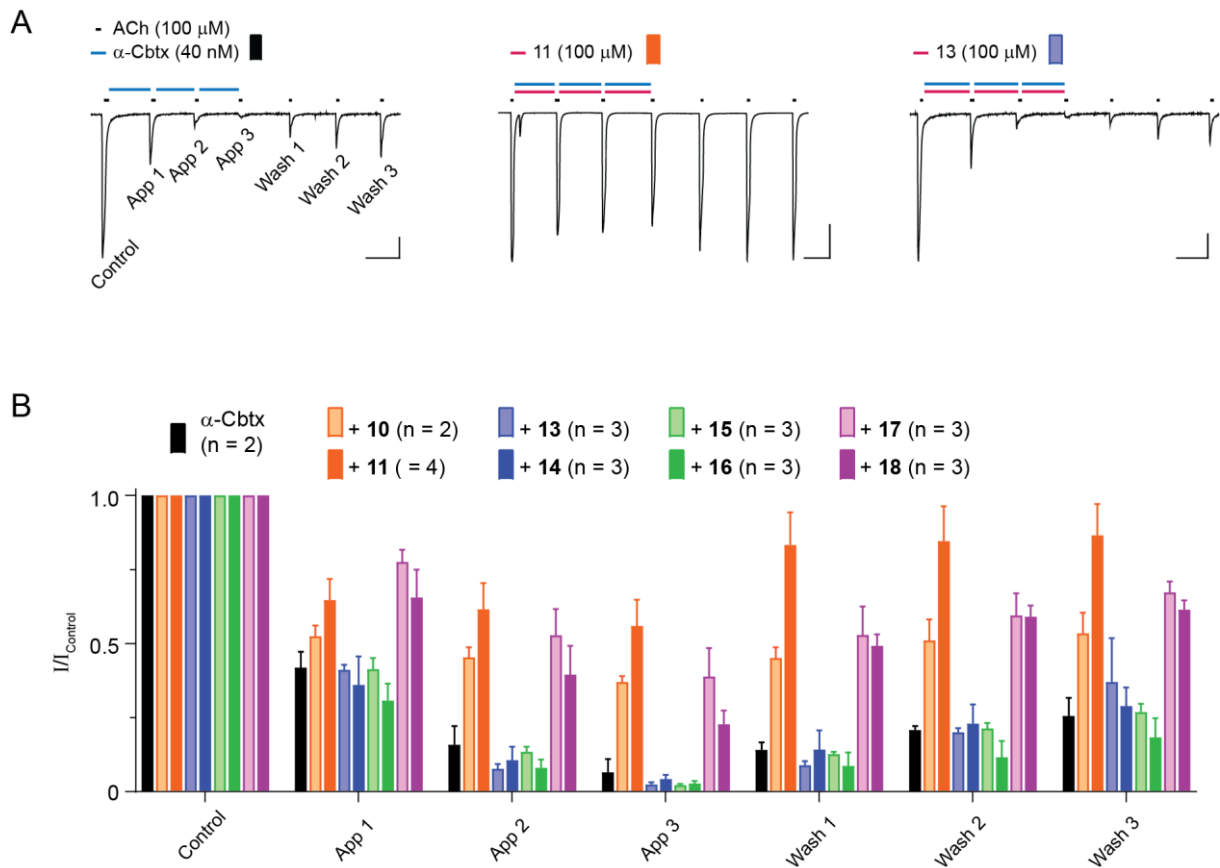
## Supporting Information

### Peptide inhibitors of the $\alpha$ -cobratoxin–nicotinic acetylcholine receptor interaction

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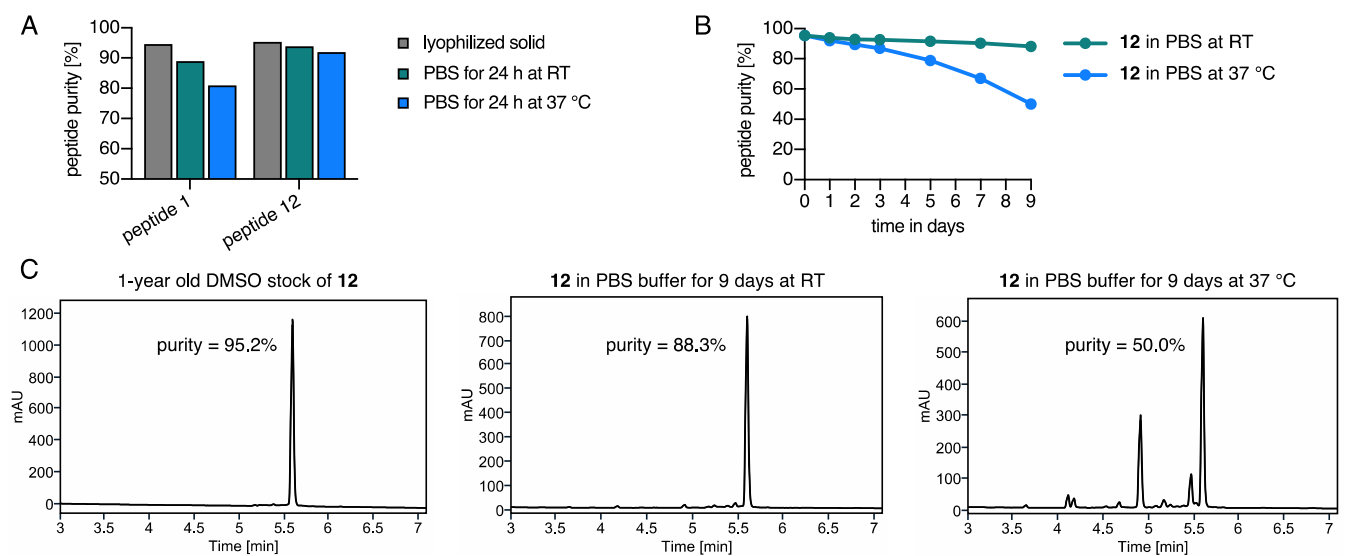
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**Figure S1. Effects of additional peptides on  $\alpha$ -cobratoxin inhibition of nAChRs. Related to Table 1.**

(A) Example two electrode voltage clamp recordings of acetylcholine (ACh)-gated currents in *Xenopus laevis* oocytes expressing L247V mutant  $\alpha 7$  ACh receptors and RIC-3 (see *Experimental Section, Electrophysiological Assays*). Scale bars: x, 1 min; y, 1  $\mu$ A. ACh was applied for 10 s in regular conditions (“x” or “control”) and then three times after the application of either  $\alpha$ -Cbtx (blue bars) or  $\alpha$ -Cbtx with peptide (blue and magenta bars). After this, ACh was applied three more times to observe washout of any effects.

(B) Normalized, mean (n = 2-4) data from experiments illustrated in (A) for various peptides (see Table 1 for peptide sequences).

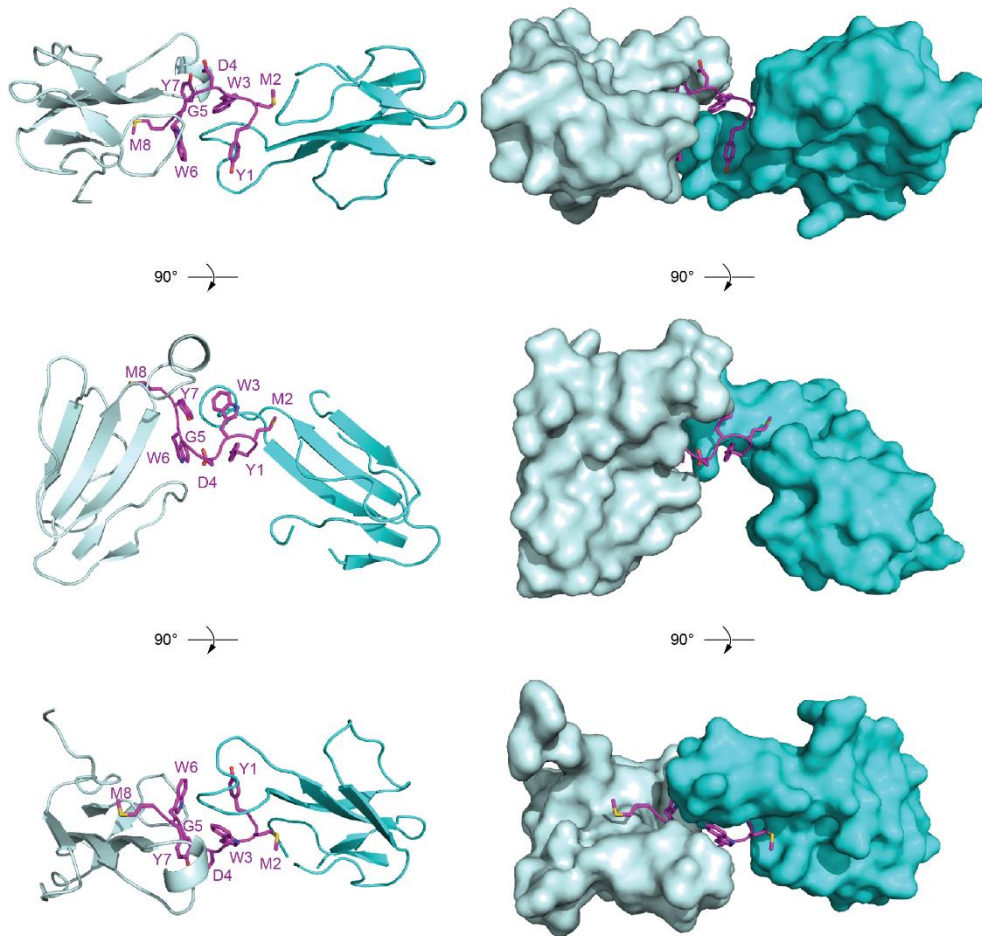


**Figure S2. Chemical stability of peptide 1 and 12.**

(A) Purities of peptide **1** and **12** after 24 h incubation in phosphate-buffered saline (PBS) buffer (pH = 7.4) at room temperature (RT) and 37 °C determined by HPLC ( $\lambda = 215$  nm). Peptide **1** precipitated after 24 h from the buffer and could not be analyzed for longer than 24 h.

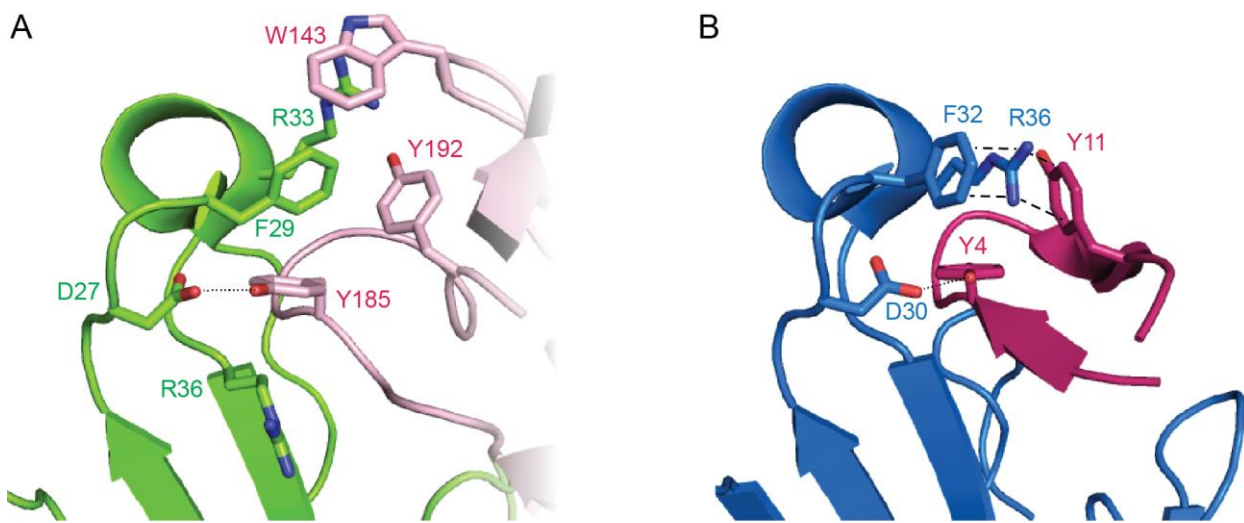
(B) Stability of peptide **12** over 9 days in PBS buffer at RT and 37 °C determined by HPLC ( $\lambda = 215$  nm).

(C) HPLC chromatograms of **12** before and after 9 days of incubation in PBS buffer at RT and 37 °C.



**Figure S3. Architecture of the 12:α-Cbtx complex observed in the crystal structure (PDBID 6ZFM).**

Two α-Cbtx molecules (light and dark cyan) bind one **12** ligand (magenta), which results in the formation of an asymmetrical dimer and two distinct binding interfaces. Top, side and bottom views are shown with α-Cbtx in cartoon (left) and surface (right) representation.



**Figure S4. Structurally homologous  $\alpha$ -neurotoxin binding interactions with other proteins.**

(A) Interaction of  $\alpha$ -Cbtx (green) loop II with acetylcholine binding protein (AChBP, pink) principal face (PDB 1Y15). Amino acid side chains shown as sticks, key interacting residues are labeled. Y-D hydrogen bond indicated by dashed line.

(B) Interaction of  $\alpha$ -Bgtx (blue) loop II with an inhibitory peptide (magenta) bearing structural homology to the  $\alpha$  subunit of the muscle nAChR (PDBID 1HC9). Cation- $\pi$  interactions of  $\alpha$ -Bgtx<sup>R36</sup> and the  $\alpha$ -Bgtx<sup>D30</sup>–peptide<sup>Y4</sup> hydrogen bond are highlighted by dashed and dotted lines, respectively.

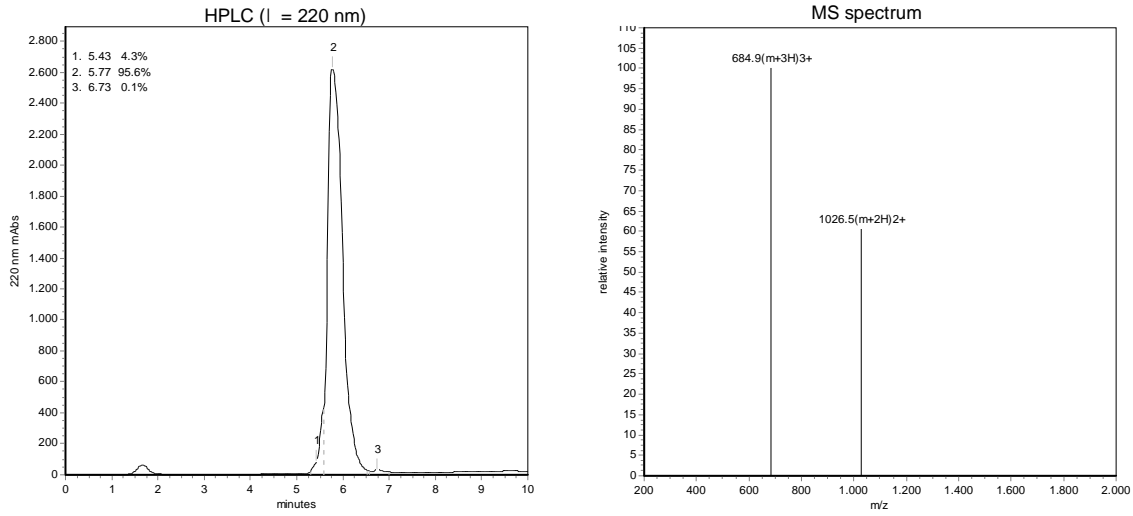
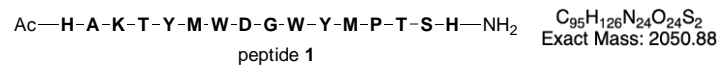


Figure S5. HPLC chromatogram and MS spectrum, peptide 1 (purchased from Schafer-N).

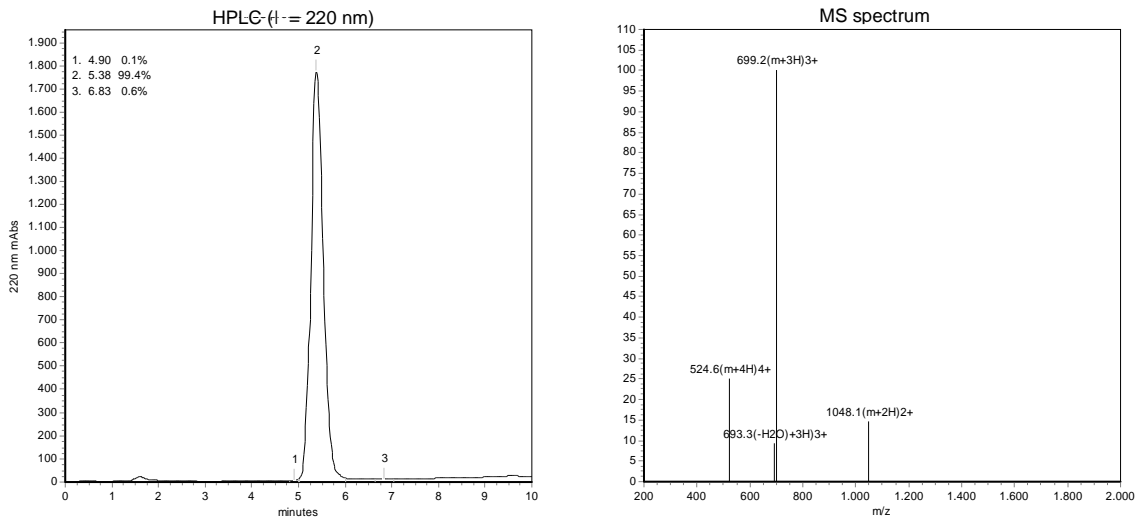
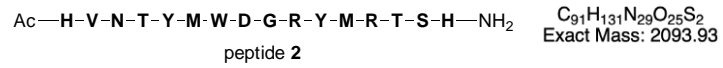


Figure S6. HPLC chromatogram and MS spectrum, peptide 2 (purchased from Schafer-N).

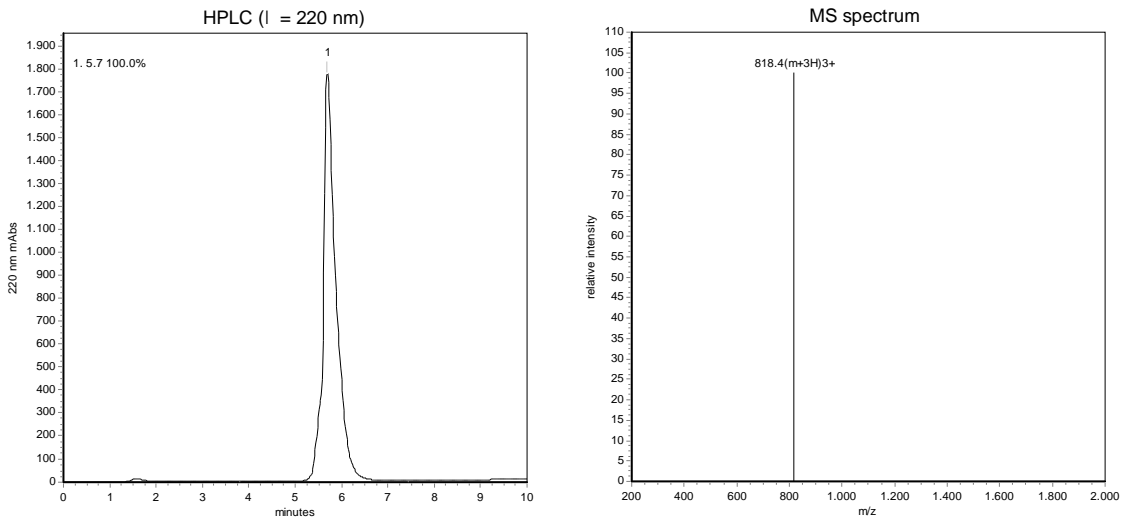
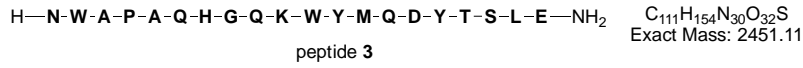


Figure S7. HPLC chromatogram and MS spectrum, peptide 3 (purchased from Schafer-N).

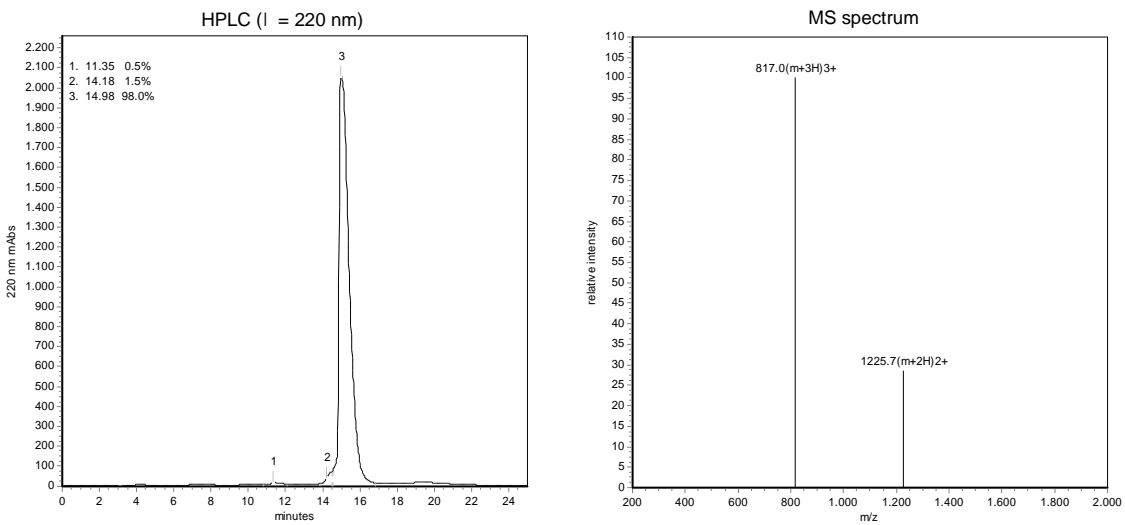
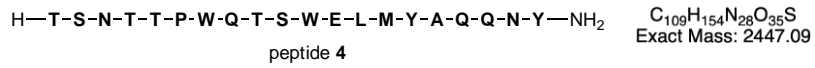


Figure S8. HPLC chromatogram and MS spectrum, peptide 4 (purchased from Schafer-N).

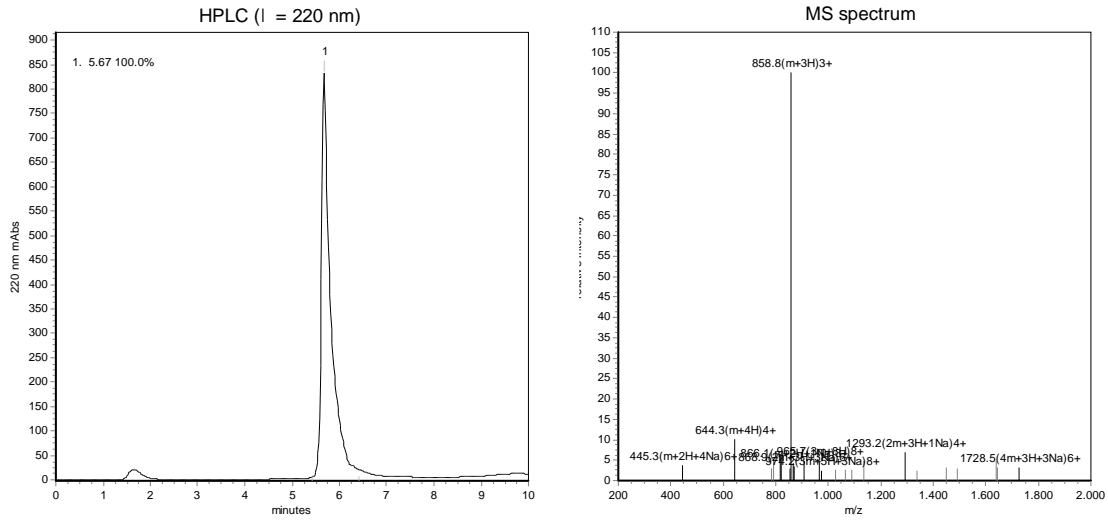
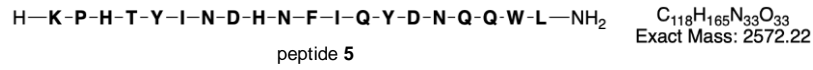


Figure S9. HPLC chromatogram and MS spectrum, peptide 5 (purchased from Schafer-N).

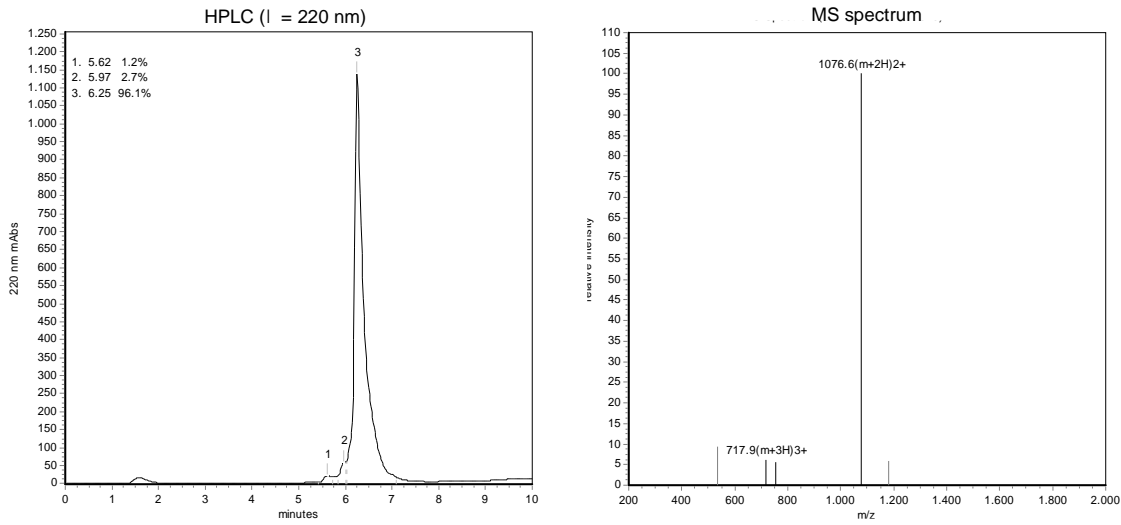
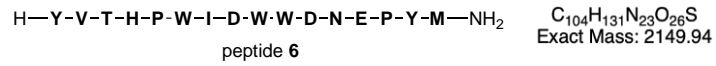


Figure S10. HPLC chromatogram and MS spectrum, peptide 6 (purchased from Schafer-N).



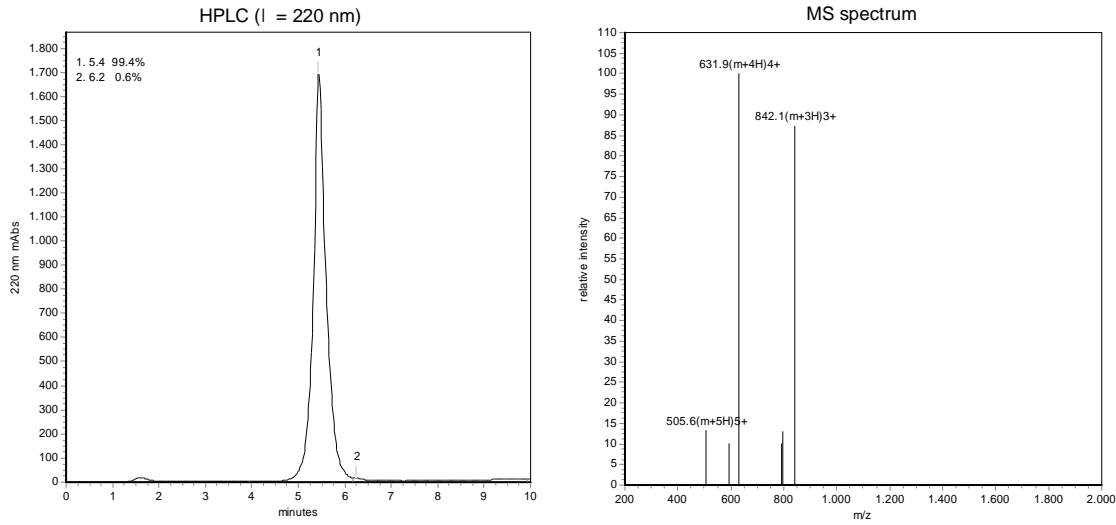
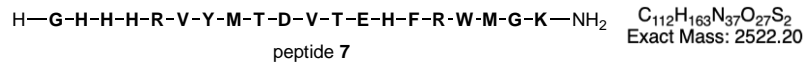


Figure S11. HPLC chromatogram and MS spectrum, peptide 7 (purchased from Schafer-N).

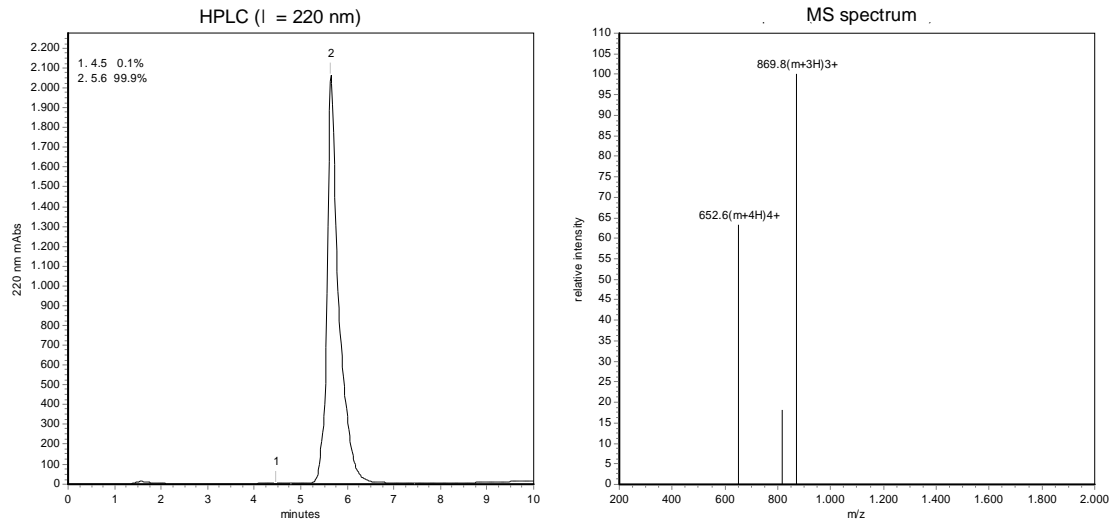
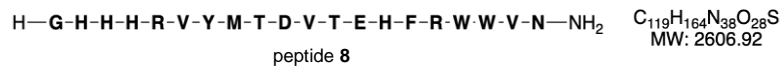


Figure S12. HPLC chromatogram and MS spectrum, peptide 8 (purchased from Schafer-N).

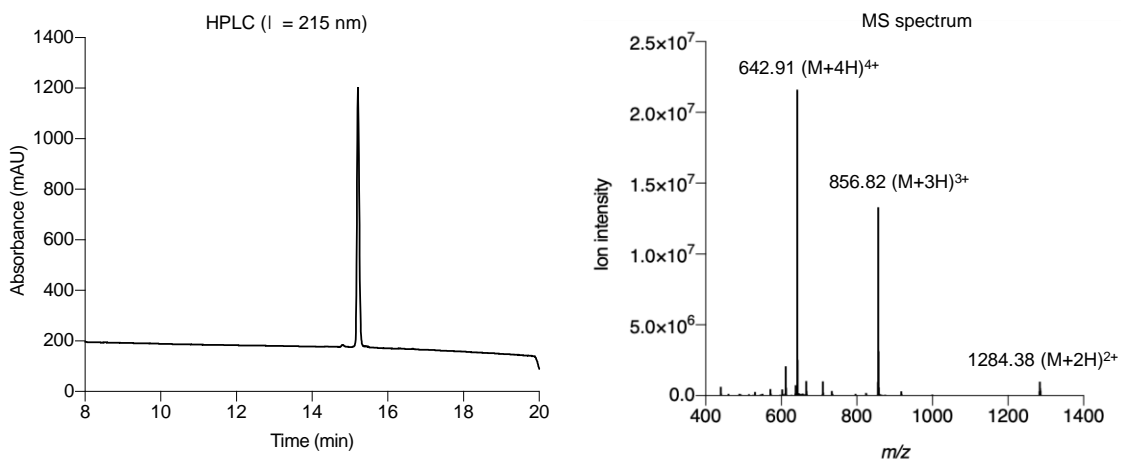
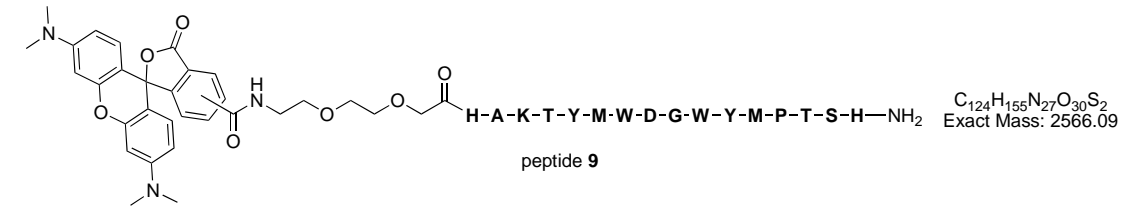
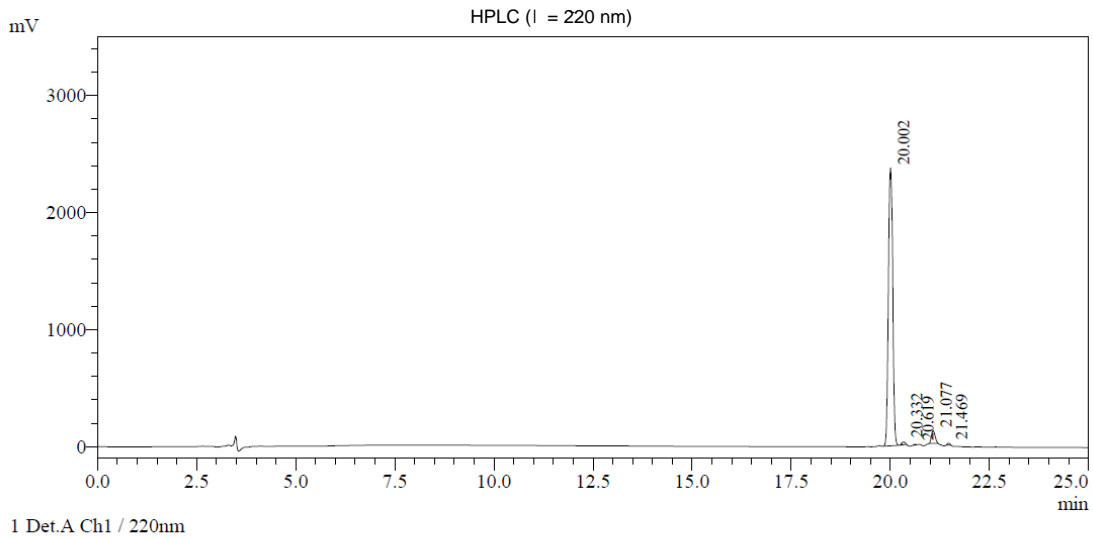
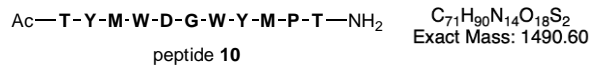


Figure S13. HPLC chromatogram and MS spectrum, peptide 9.



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	20.002	18178852	2372993	95.072
2	20.332	139272	25091	0.728
3	20.619	27270	6120	0.143
4	21.077	708124	108524	3.703
5	21.469	67705	15994	0.354
Total		19121223	2528721	100.000

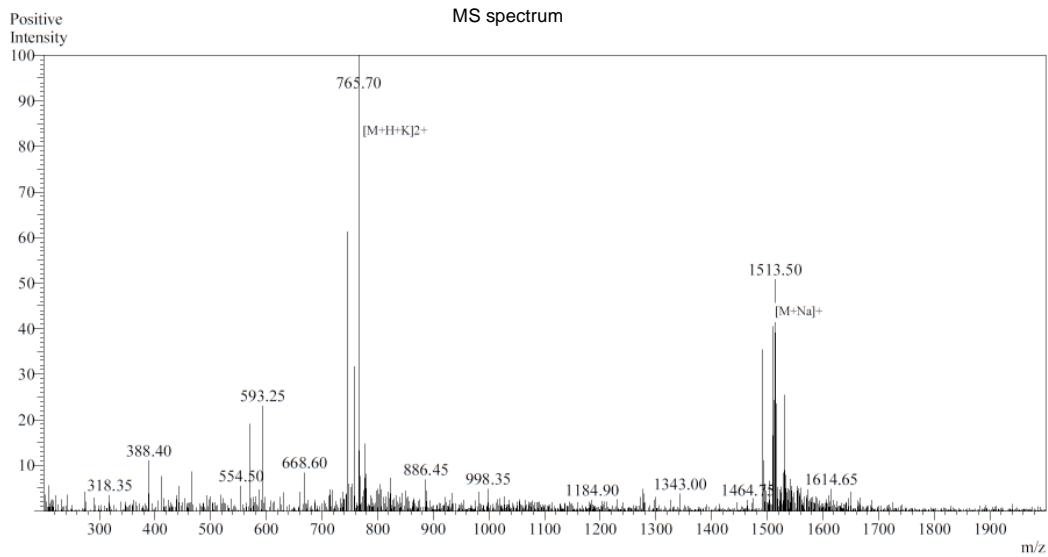
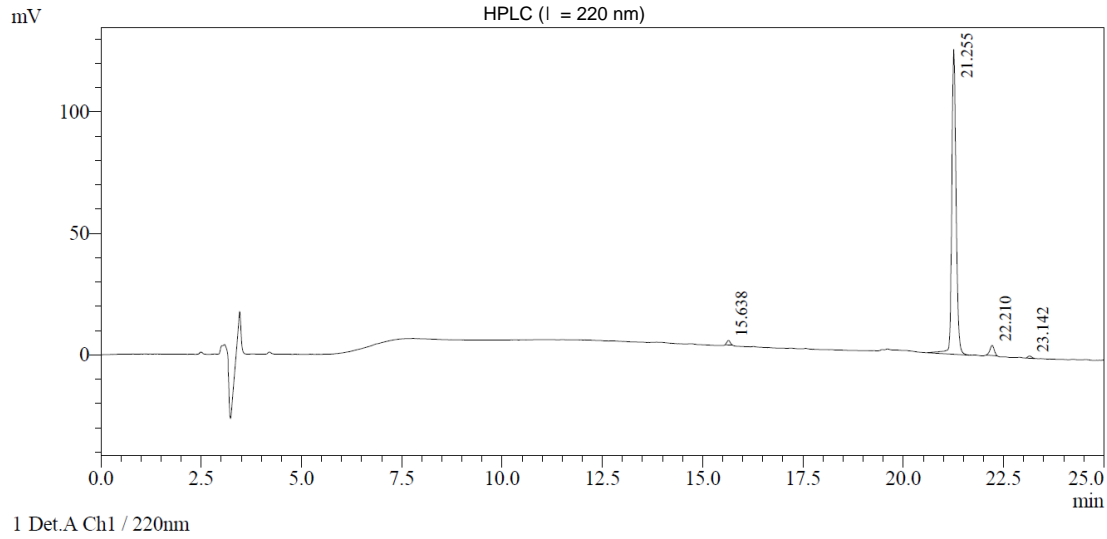
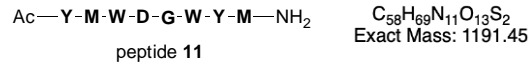


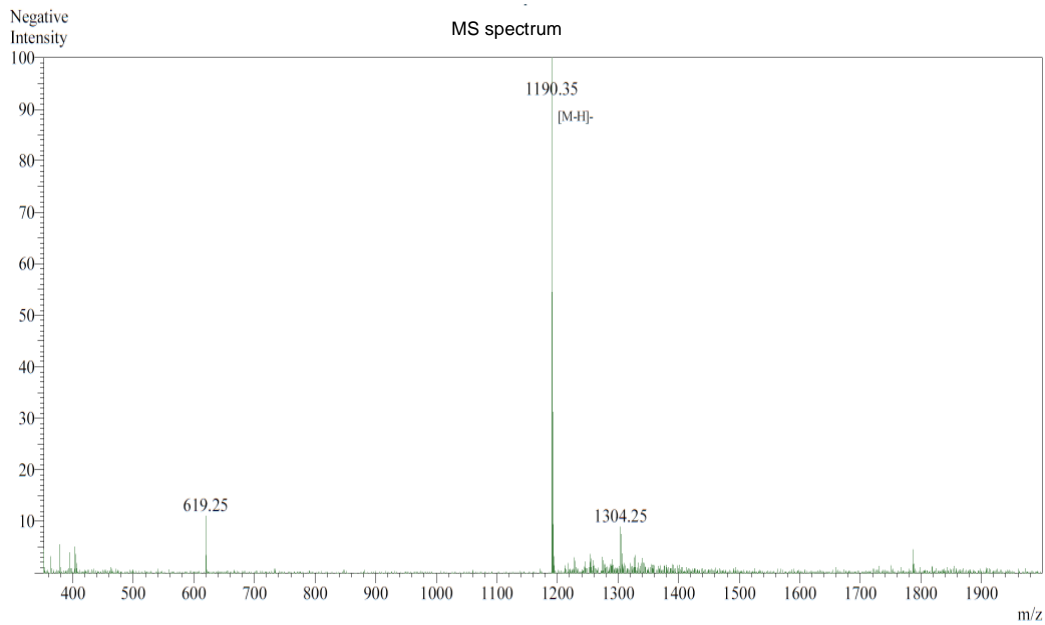
Figure S14. HPLC chromatogram and MS spectrum, peptide 10 (purchased from Genscript).



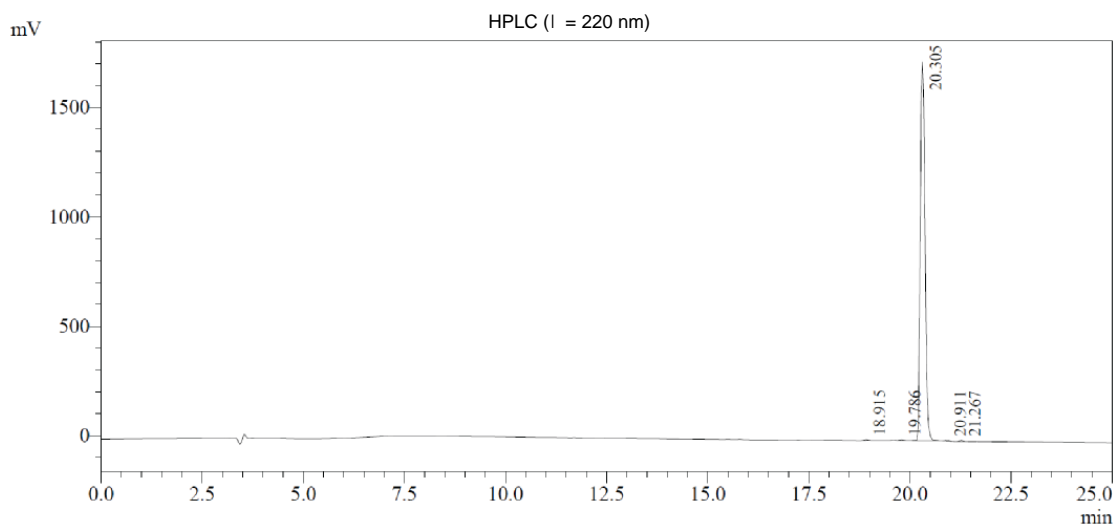
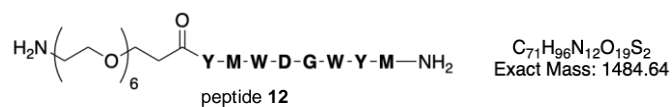
Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	15.638	10715	1852	1.085
2	21.255	939786	125408	95.122
3	22.210	30947	4168	3.132
4	23.142	6536	913	0.662
Total		987983	132341	100.000



**Figure S15. HPLC chromatogram and MS spectrum, peptide 11 (purchased from Genscript).**



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	18.915	29718	4555	0.209
2	19.786	29396	4238	0.207
3	20.305	14085870	1731230	99.164
4	20.911	29628	3942	0.209
5	21.267	29949	4302	0.211
Total		14204560	1748266	100.000

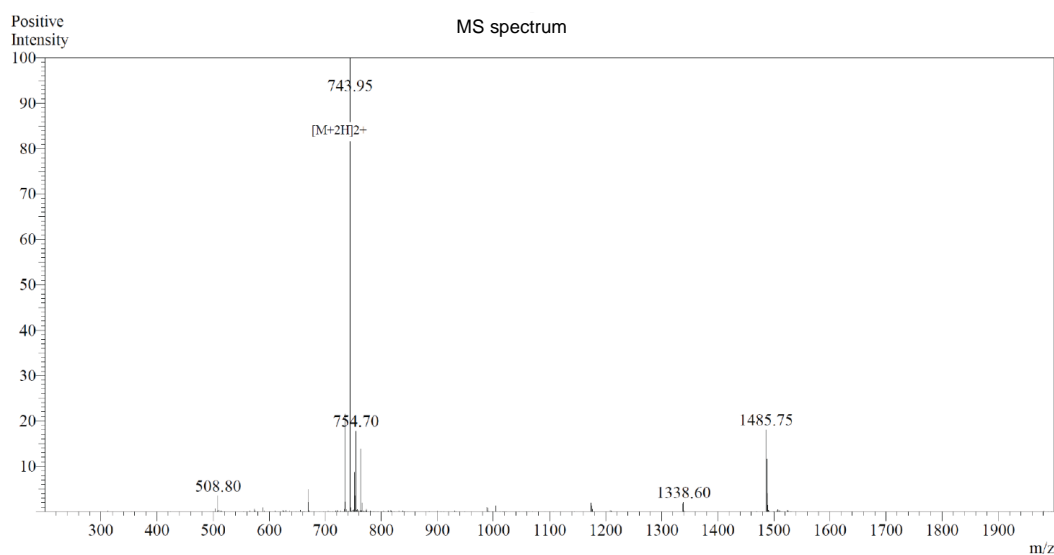
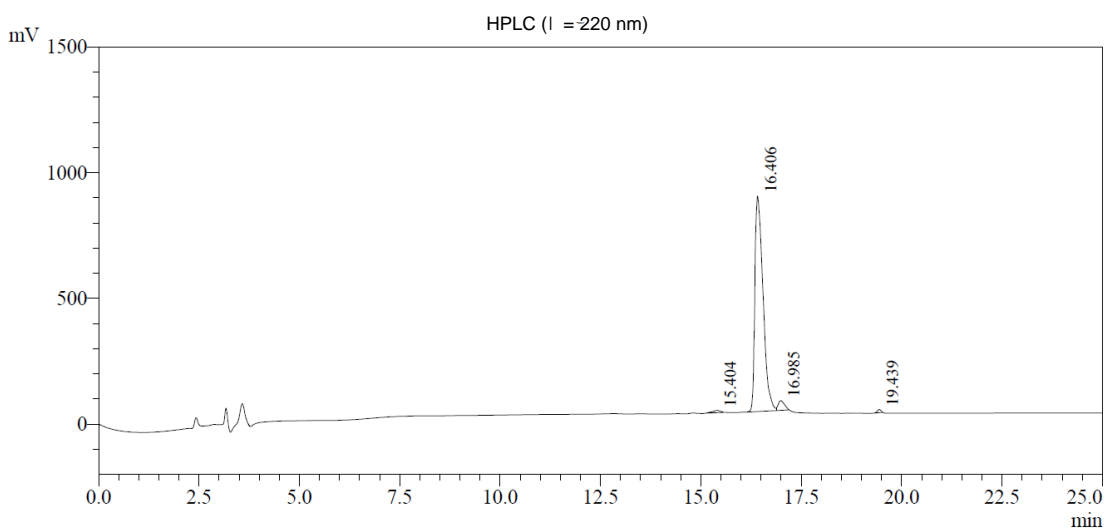
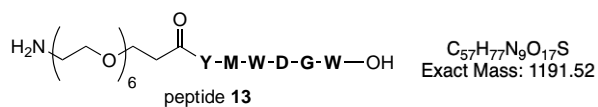


Figure S16. HPLC chromatogram and MS spectrum, peptide 12 (purchased from Genscript).



1 Det.A Ch1 / 214nm

Peak Table

Detector A Ch1 214nm

Peak#	Ret. Time	Area	Height	Area %
1	15.404	119943	9089	0.944
2	16.406	12079842	857140	95.117
3	16.985	418153	38831	3.293
4	19.439	82068	12879	0.646
Total		12700006	917939	100.000

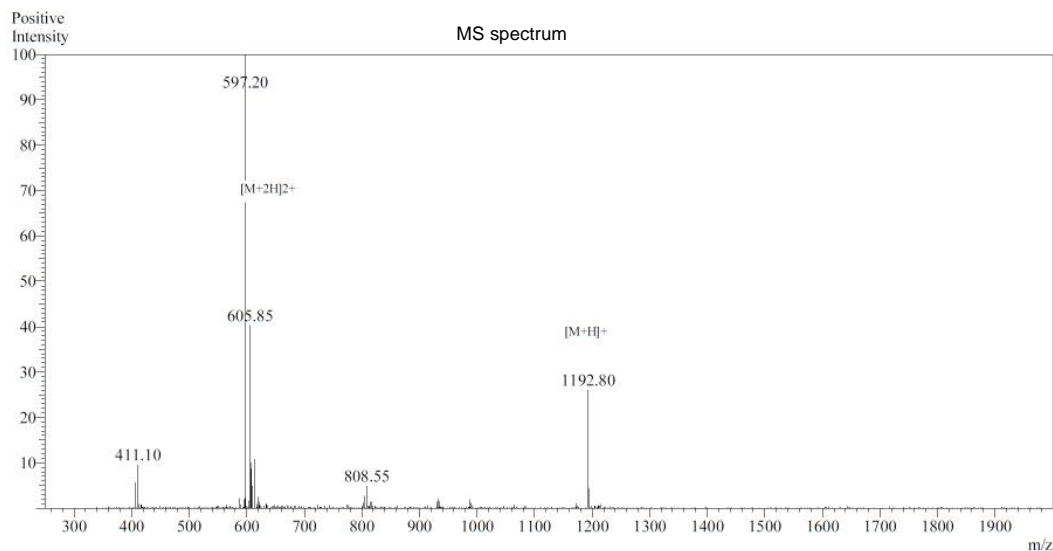
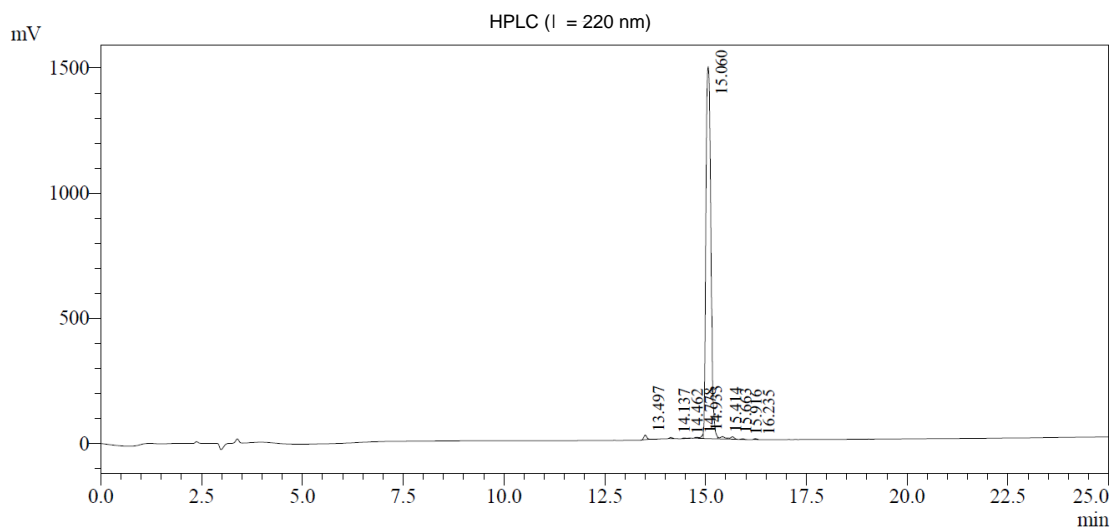
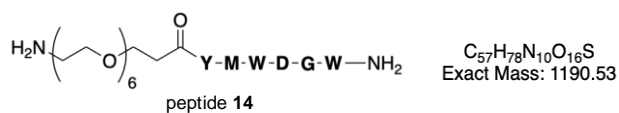


Figure S17. HPLC chromatogram and MS spectrum, peptide 13 (purchased from Genscript).



1 Det.A Ch1 / 220nm

Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	13.497	93187	18325	0.716
2	14.137	22272	4489	0.171
3	14.462	17574	2324	0.135
4	14.778	26005	3772	0.200
5	14.933	49818	15961	0.383
6	15.060	12616304	1485741	96.935
7	15.414	85398	9360	0.656
8	15.663	74460	9636	0.572
9	15.916	13429	2479	0.103
10	16.235	16816	3662	0.129
Total		13015262	1555748	100.000

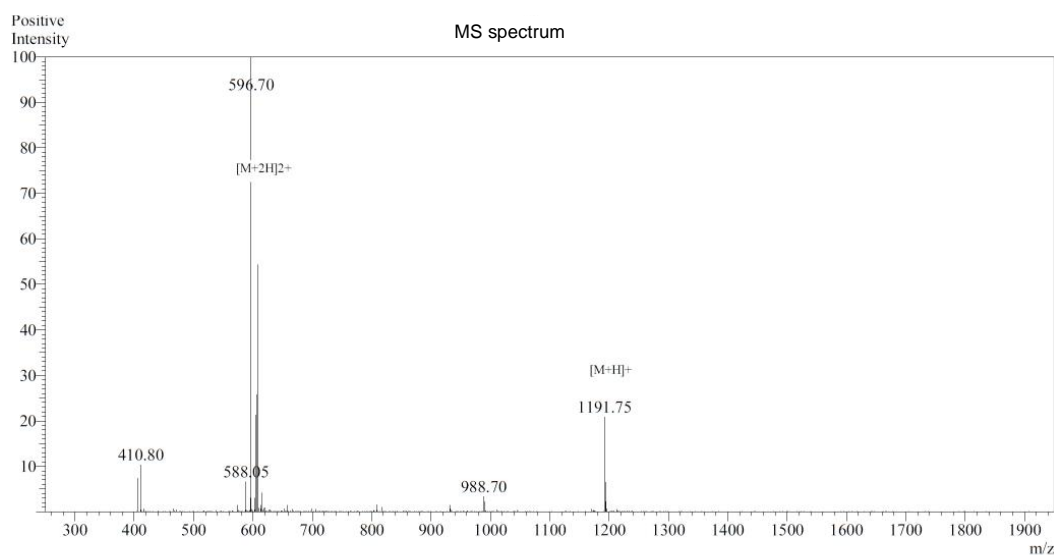
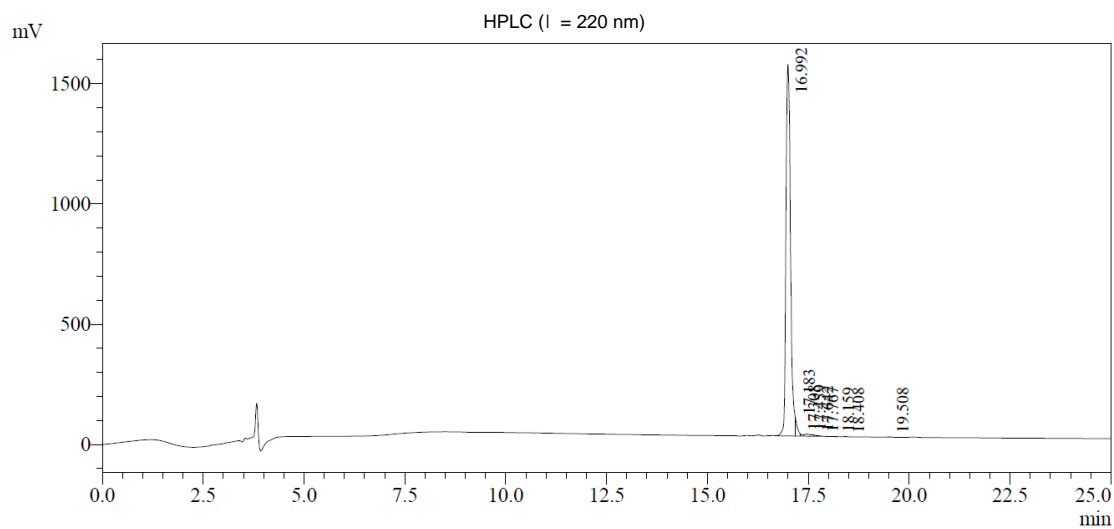
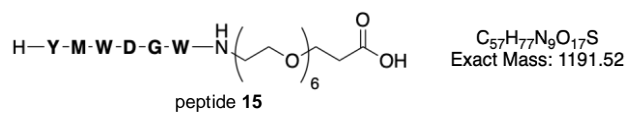


Figure S18. HPLC chromatogram and MS spectrum, peptide 14 (purchased from Genscript).



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	16.992	12109564	1543874	96.565
2	17.183	244954	71675	1.953
3	17.308	33001	8093	0.263
4	17.459	98778	8590	0.788
5	17.642	26744	5310	0.213
6	17.767	9108	2471	0.073
7	18.159	3307	653	0.026
8	18.408	5457	888	0.044
9	19.508	9406	1619	0.075
Total		12540319	1643174	100.000

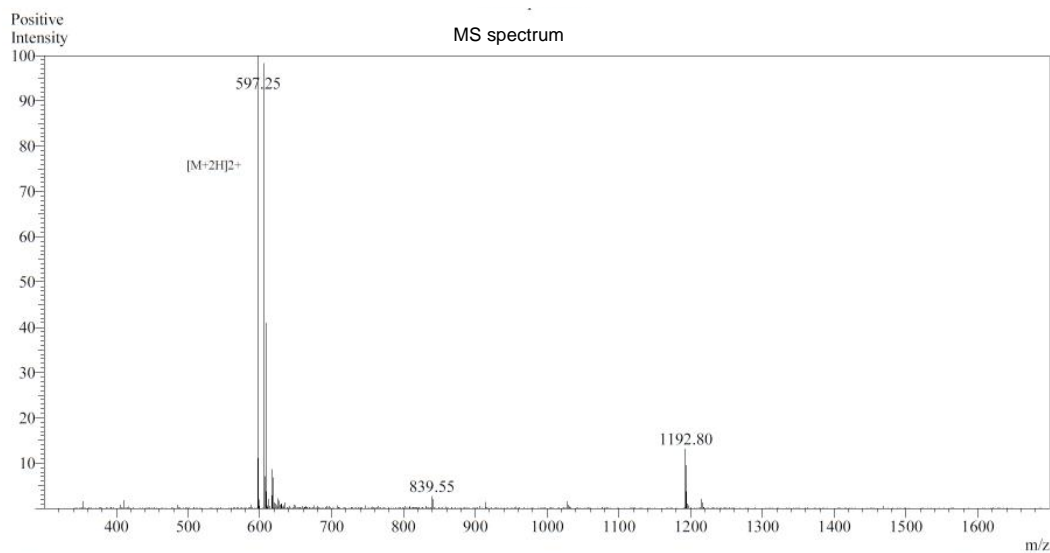
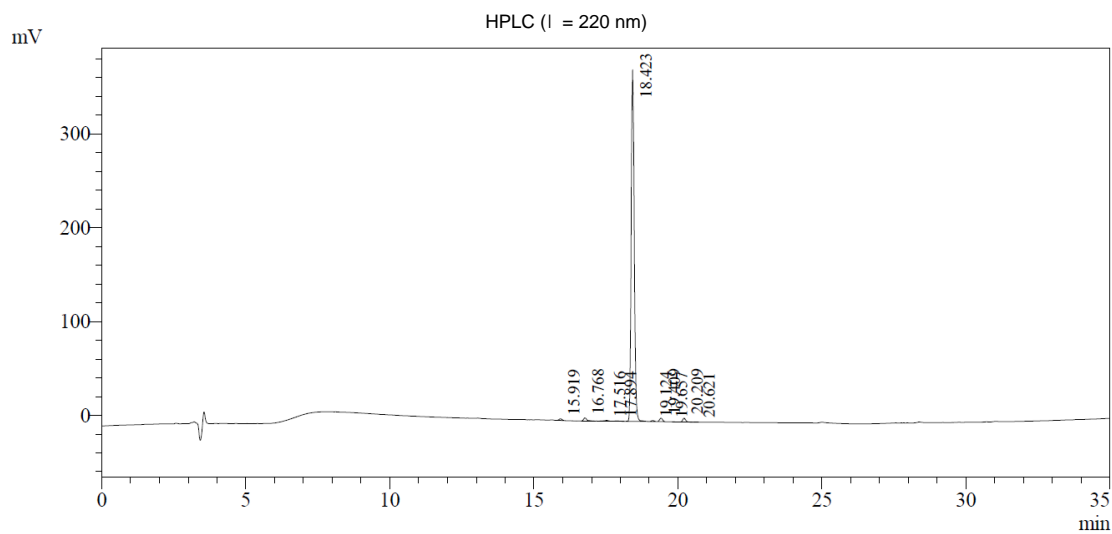
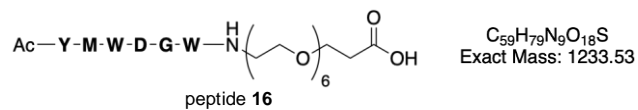


Figure S19. HPLC chromatogram and MS spectrum, peptide 15 (purchased from Genscript).

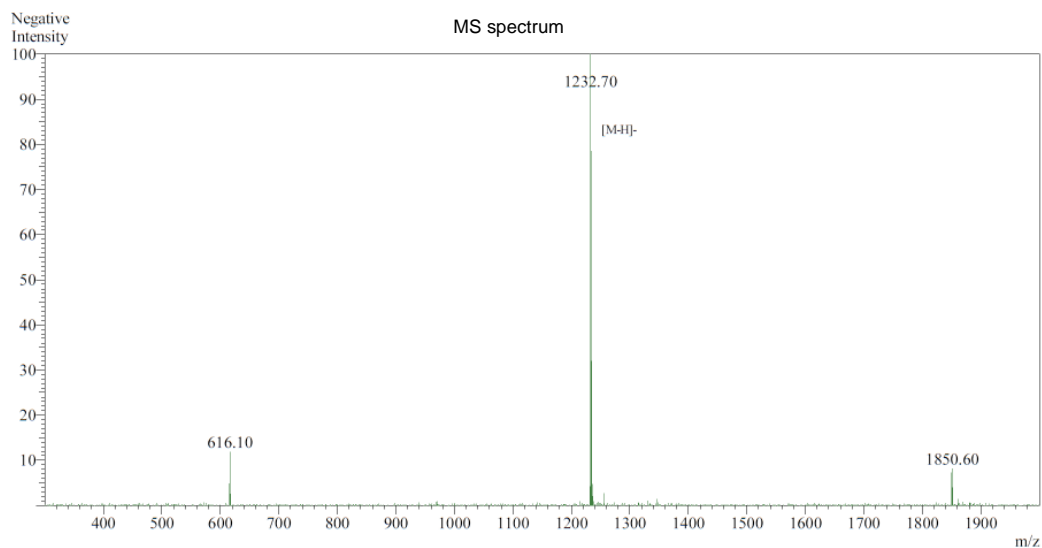




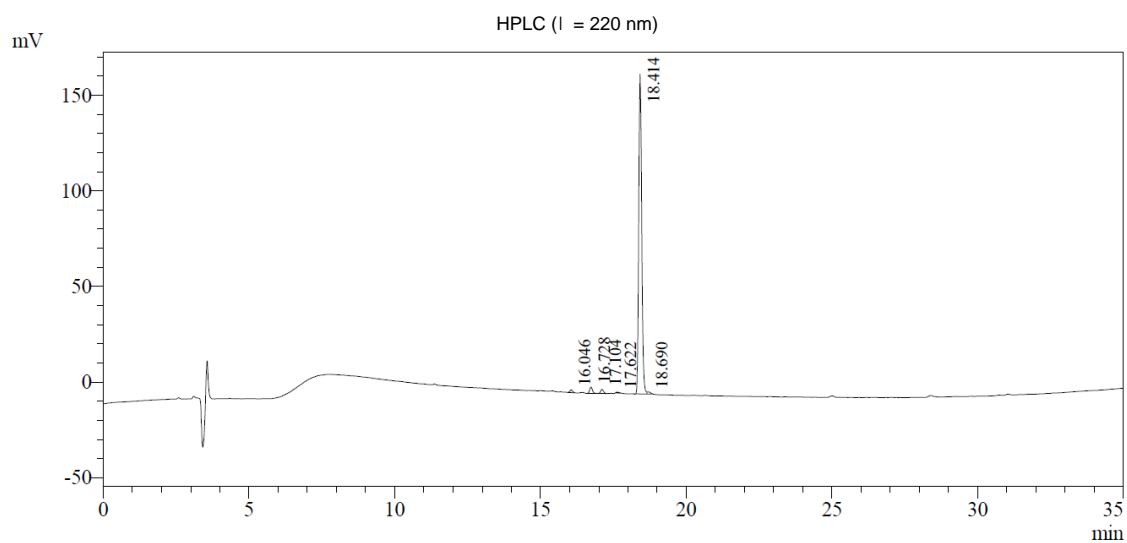
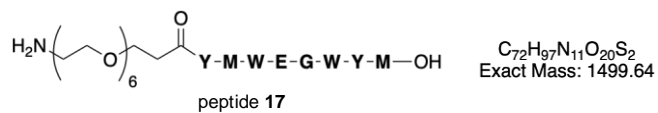
Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	15.919	12390	1755	0.428
2	16.768	26747	3353	0.924
3	17.516	7358	719	0.254
4	17.894	2891	327	0.100
5	18.423	2779212	374885	95.992
6	19.124	7545	1082	0.261
7	19.409	27108	3749	0.936
8	19.657	1080	105	0.037
9	20.209	29691	4094	1.025
10	20.621	1243	223	0.043
Total		2895266	390291	100.000



**Figure S20. HPLC chromatogram and MS spectrum, peptide 16 (purchased from Genscript).**



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	16.046	9285	1432	0.743
2	16.728	21771	3273	1.742
3	17.104	14496	2101	1.160
4	17.622	4131	558	0.331
5	18.414	1192636	167296	95.419
6	18.690	7579	1079	0.606
Total		1249898	175739	100.000

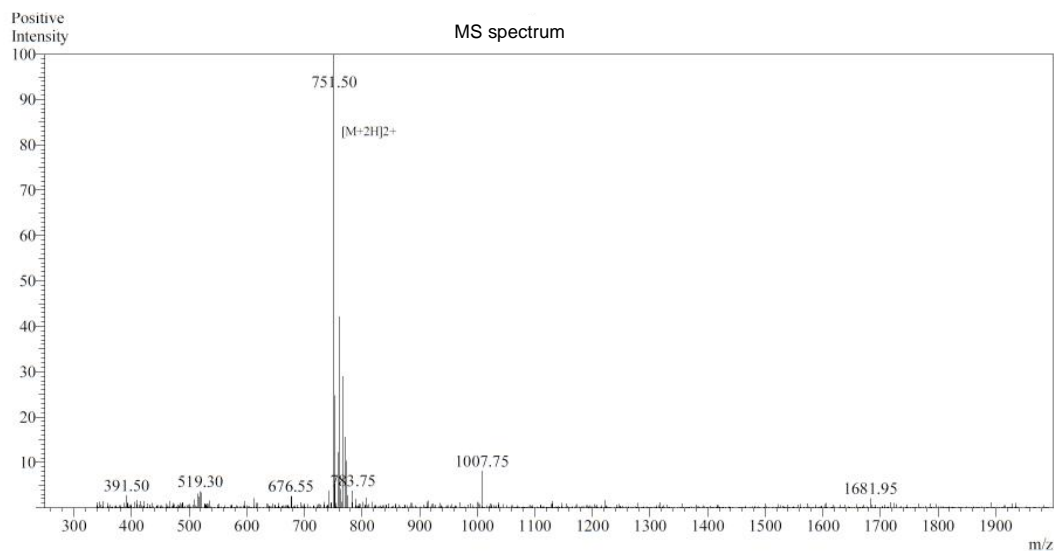
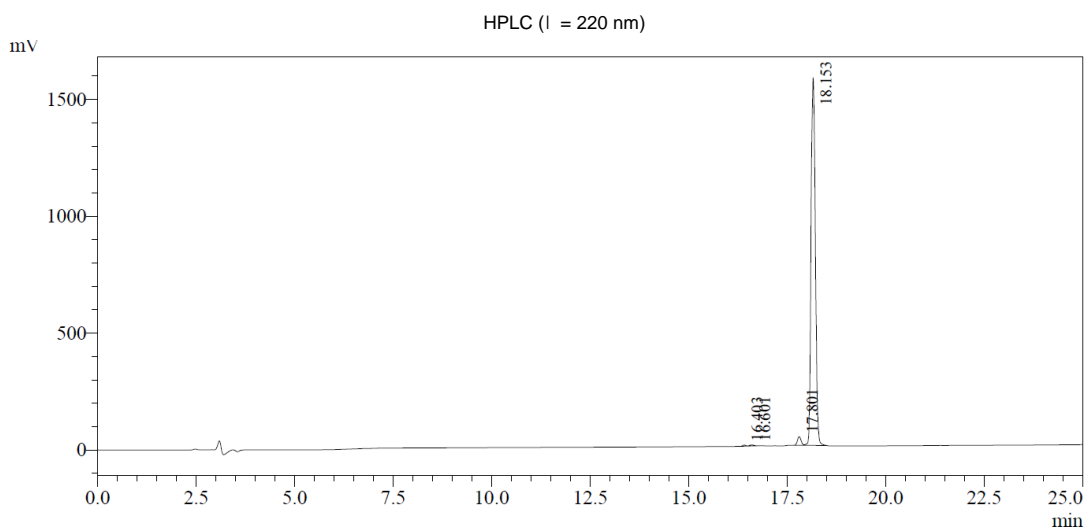
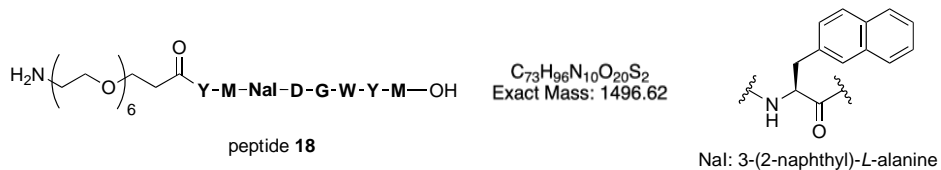


Figure S21. HPLC chromatogram and MS spectrum, peptide 17 (purchased from Genscript).



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	16.403	31290	5200	0.255
2	16.601	37047	4631	0.302
3	17.801	242968	37311	1.982
4	18.153	11945427	1573392	97.460
Total		12256731	1620534	100.000

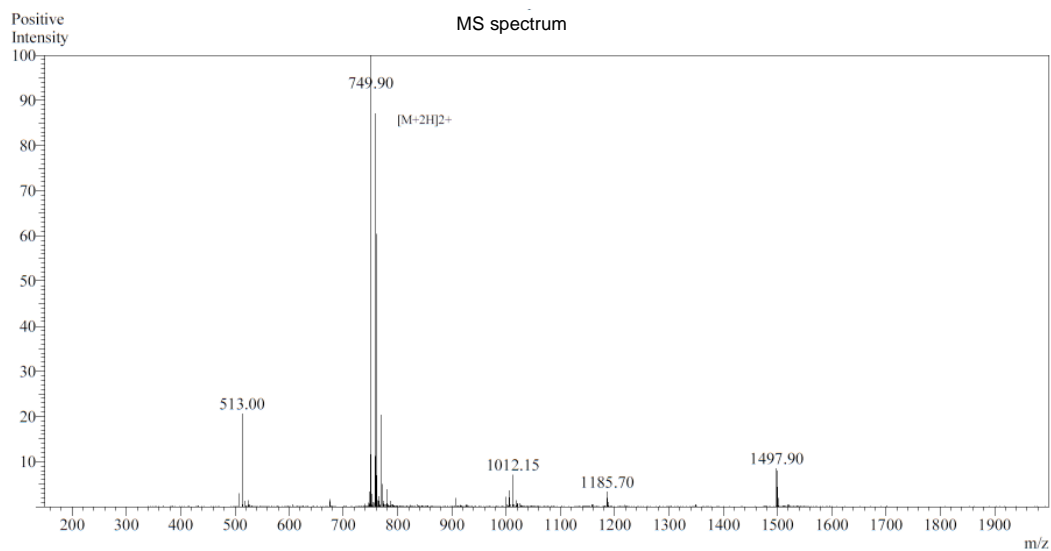


Figure S22. HPLC chromatogram and MS spectrum, peptide 18 (purchased from Genscript).

**Table S1. Data processing and refinement statistics for the structure determination of the 12: $\alpha$ -Cbtx complex.**

		<b>12:<math>\alpha</math>-Cbtx (PDBID 6ZFM)</b>	
Space group		P6 <sub>1</sub>	
Wavelength [Å]		0.9762	
Unit cell	a [Å]	49.4	
	b [Å]	49.4	
	c [Å]	289.6	
	$\alpha$ [°]	90.0	
	$\beta$ [°]	90.0	
	$\gamma$ [°]	120.0	
Resolution [Å] <sup>a</sup>		29.75-1.68	(1.71-1.68)
Unique reflections <sup>a</sup>		45,445	(2,353)
Multiplicity <sup>a</sup>		9.6	(9.8)
I/ $\sigma$ <sup>a</sup>		9.2	(1.7)
Completeness [%] <sup>a</sup>		100	(100)
R <sub>meas</sub> [%] <sup>a</sup>		10.4	(160,6)
CC(1/2) <sup>a</sup>		96.9	(51.5)
Wilson B factor [Å <sup>2</sup> ]		38.3	
Refinement [Å]		27.65-1.90	
Reflections		31,224	
R <sub>work</sub> / R <sub>free</sub> [%]		20.1/23.0	
rmsd bond distances [Å]		0.006	
rmsd bond angles [°]		0.938	
Ramachandran diagram [%]			
	favored	97.06	
	allowed	2.94	
	outlier	0.00	
B value [Å <sup>2</sup> ]			
	protein	53.7	
	ligand	58.6	
	waters	53.7	
	overall	53.8	

<sup>a</sup> Values in parenthesis refer to outer shell of reflections