

## Supporting Information

### **Self-assembling Modified Neuropeptide S Enhances Nose-to-Brain Penetration and Exerts a Prolong Anxiolytic-like Effect**

Shu Li<sup>a</sup>, Chen Guo<sup>a</sup>, Xingjiao Zhang<sup>a</sup>, Xiaojing Liu<sup>a</sup>, Jing Mu<sup>a</sup>, Chunxia Liu<sup>a</sup>, Yali Peng<sup>a</sup>, Min Chang<sup>\*a</sup>

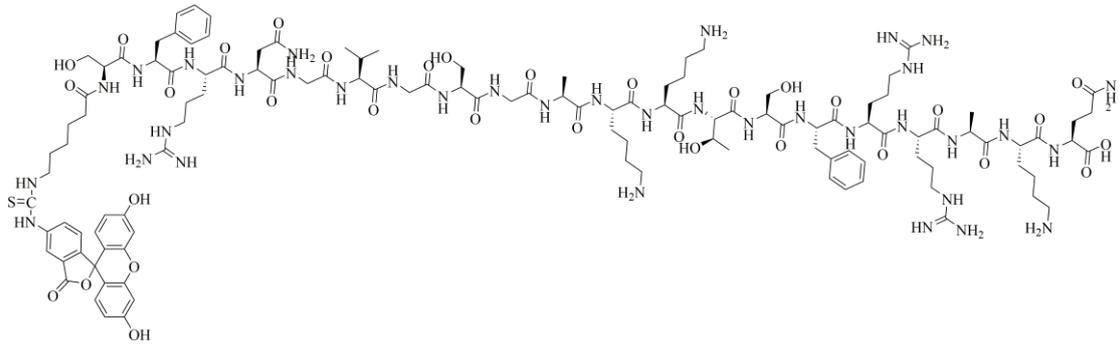
<sup>a</sup> Institute of Biochemistry and Molecular Biology, School of Life Sciences, Lanzhou University, Lanzhou 730000, China.

**\*Corresponding author:**

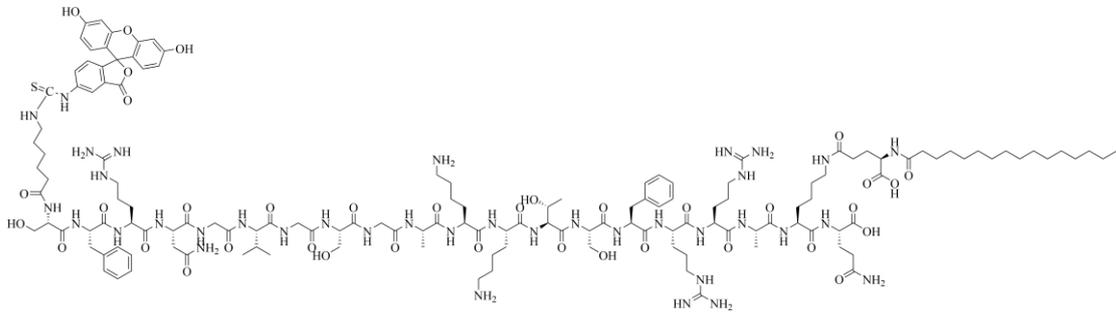
Min Chang

E-mail addresses: changmin@lzu.edu.cn

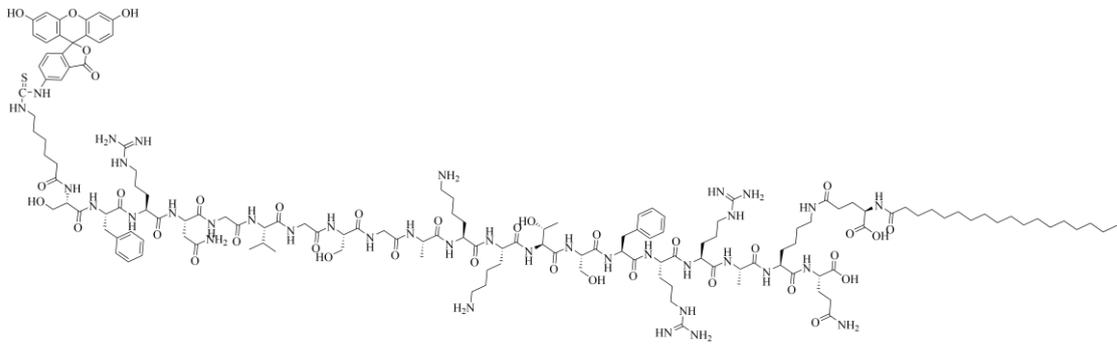
A



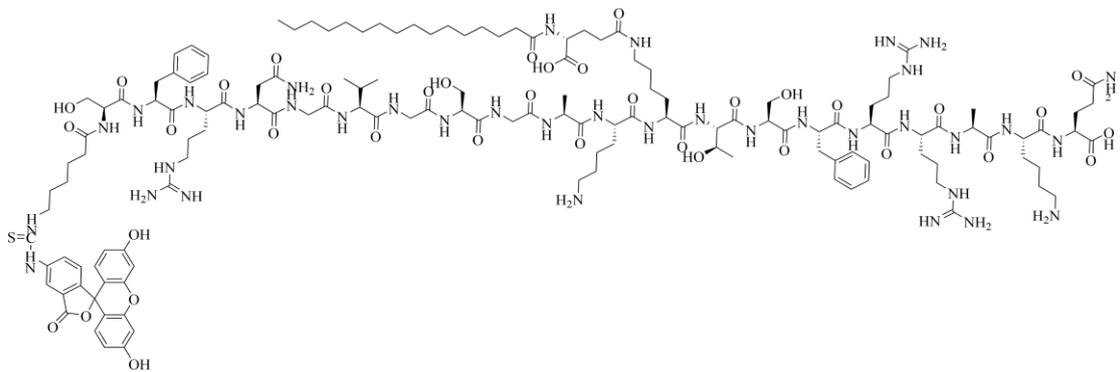
FITC-mNPS



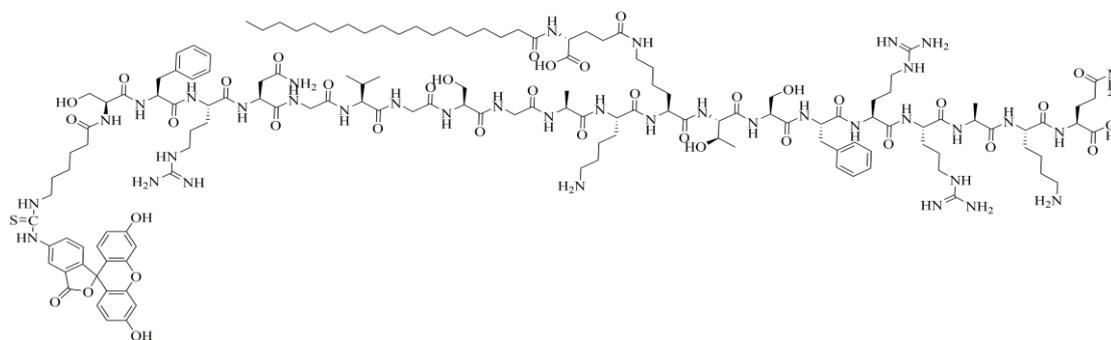
FITC-M-1



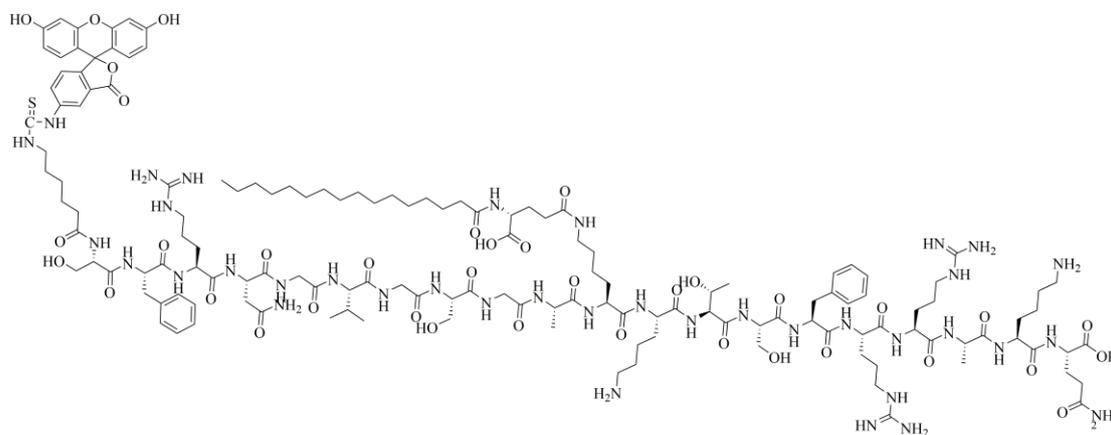
FITC-M-2



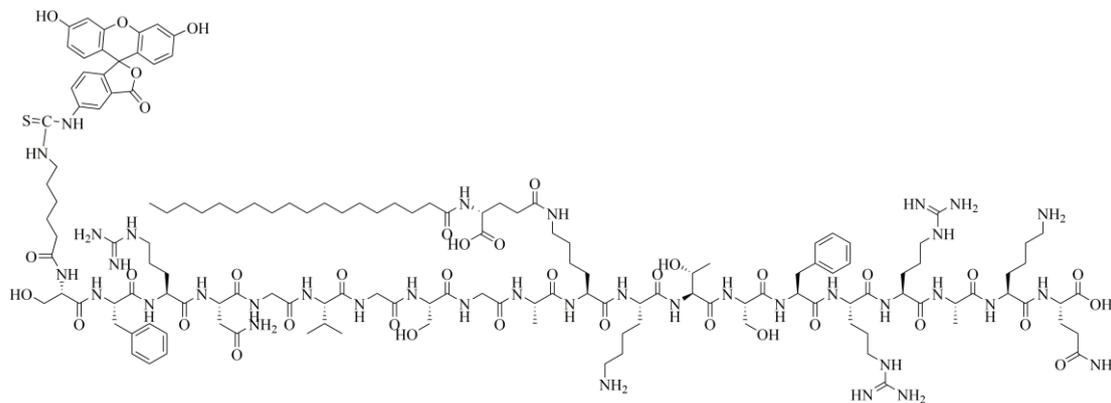
FITC-M-3



FITC-M-4

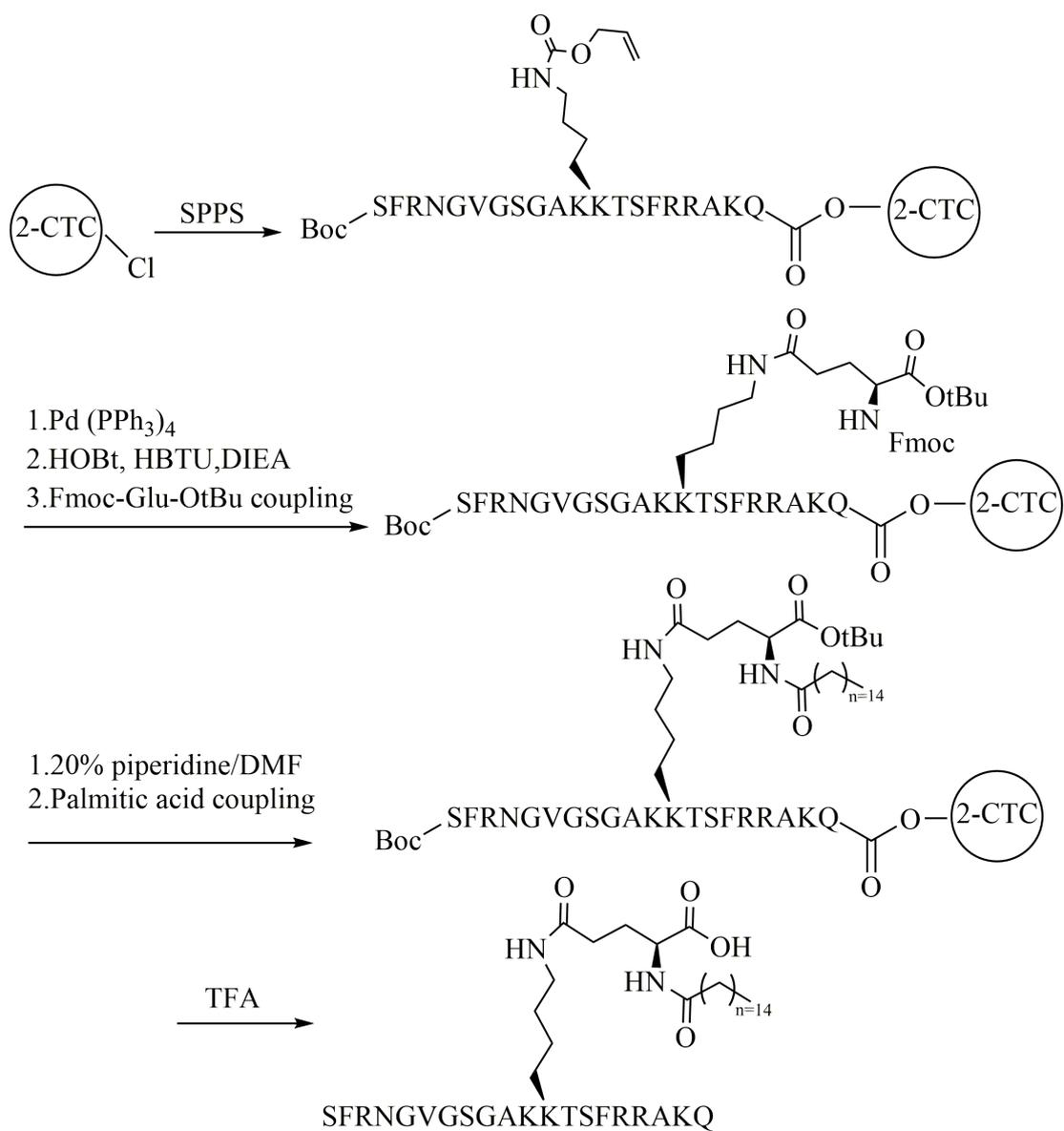


FITC-M-5

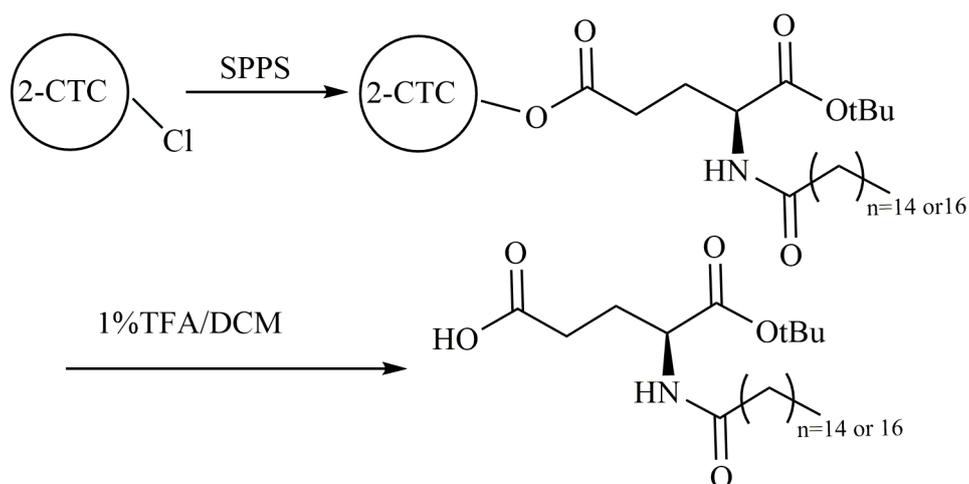


FITC-M-6

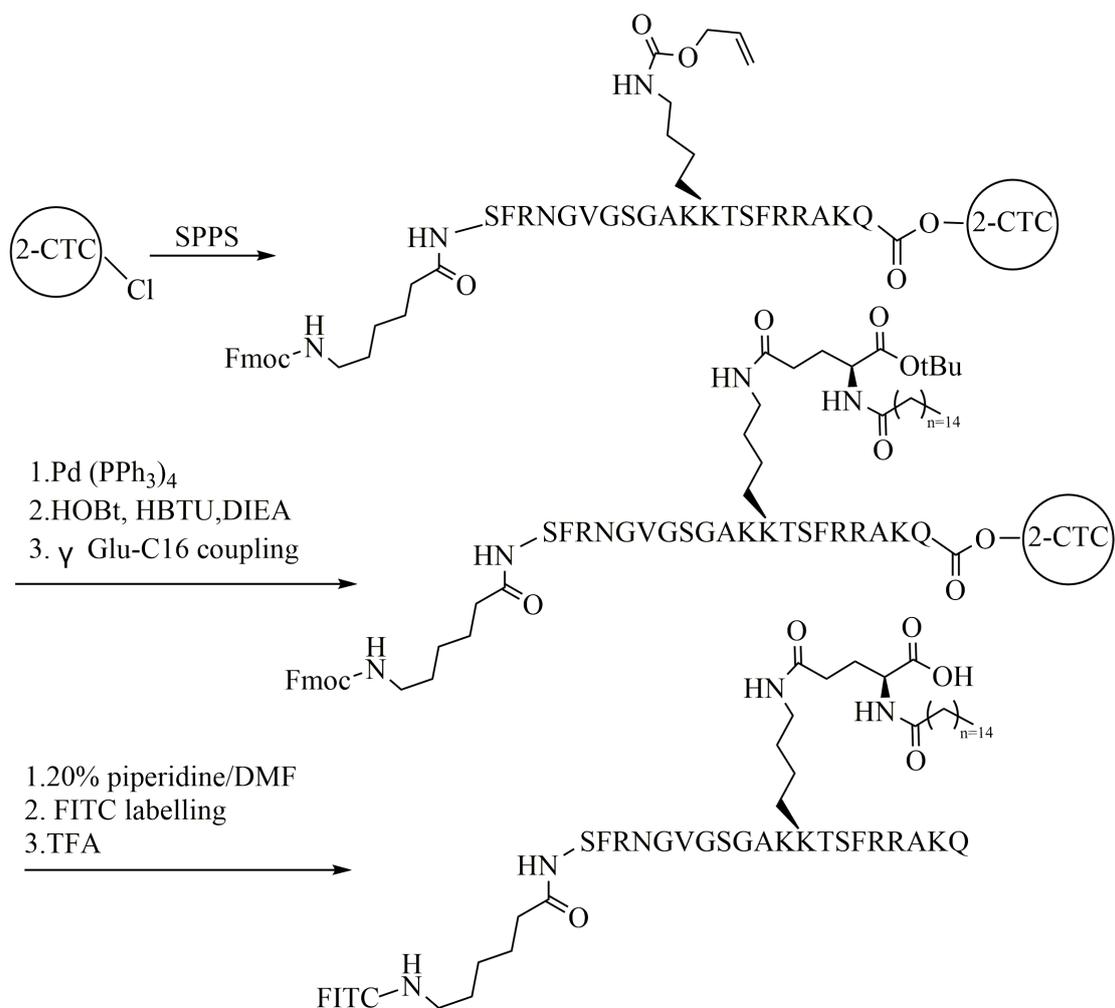
**Fig. S1** Chemical structures of FITC labelled peptides.



**Scheme S1.** Synthesis procedures of M-3.



**Scheme S2.** Synthesis procedures of  $\gamma$ Glu-C16 or  $\gamma$ Glu-C18.



**Scheme S3.** Synthesis procedures of FITC labelling M-3.

**Table S1.** Analytical Data for mNPS and the new peptides.

Peptides ID	$t_R$ [min.]	analyt. HPLC MS	
		Found [M+H] <sup>+</sup>	Calcd [M] <sup>+</sup>
mNPS	12.612	2182.1501	2181.19
M-1	22.574	2551.2058	2550.48
M-2	23.959	2578.4410	2577.51
M-3	21.417	2550.4480	2549.48
M-4	22.177	2578.5093	2577.51
M-5	20.852	2550.4499	2549.48
M-6	22.946	2578.5118	2577.51

**Table S2.** Physical characterization of the mNPS and the new peptides.

Each value represents average and standard deviation (n=3).

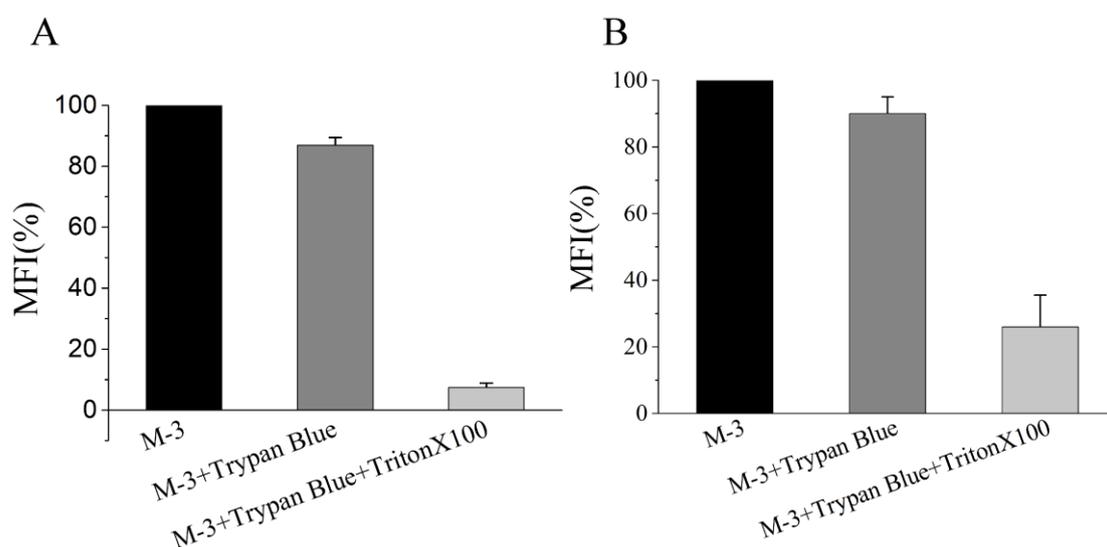
Peptides ID	Size (nm)	Zeta potential (mV)
mNPS	-	-
M-1	-	-
M-2	283±7.9	43±2.4
M-3	86±4.9	47±2.3
M-4	160±10	50±4.8
M-5	-	-
M-6	180±4.7	34±3.1

**Table S3:** Stability of mNPS and the new peptides in brain homogenate

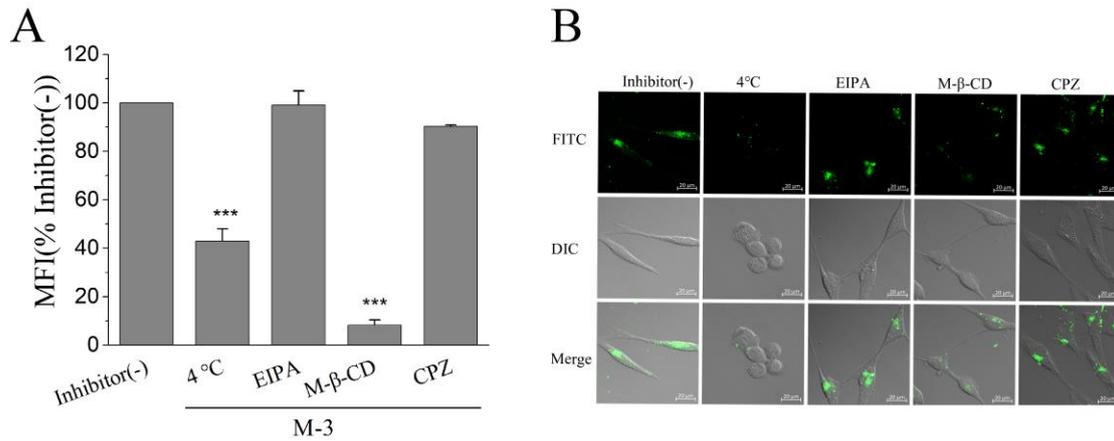
Peptides ID	$t_{1/2}^{[a]} \pm \text{sem}^{[b]}$ (Min)
mNPS	11.8 $\pm$ 4.0
M-1	50.3 $\pm$ 5.1
M-2	48 $\pm$ 9.4
M-3	525 $\pm$ 31.2
M-4	1193.9 $\pm$ 23.6
M-5	39.7 $\pm$ 6.4
M-6	52.6 $\pm$ 6.8

[a]=Time of the remaining 50% when peptides degradation;

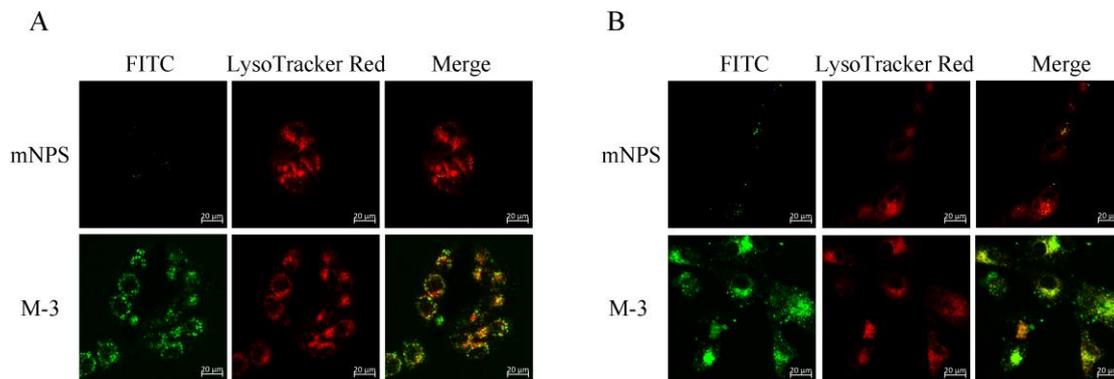
[b]=standard error of the mean.



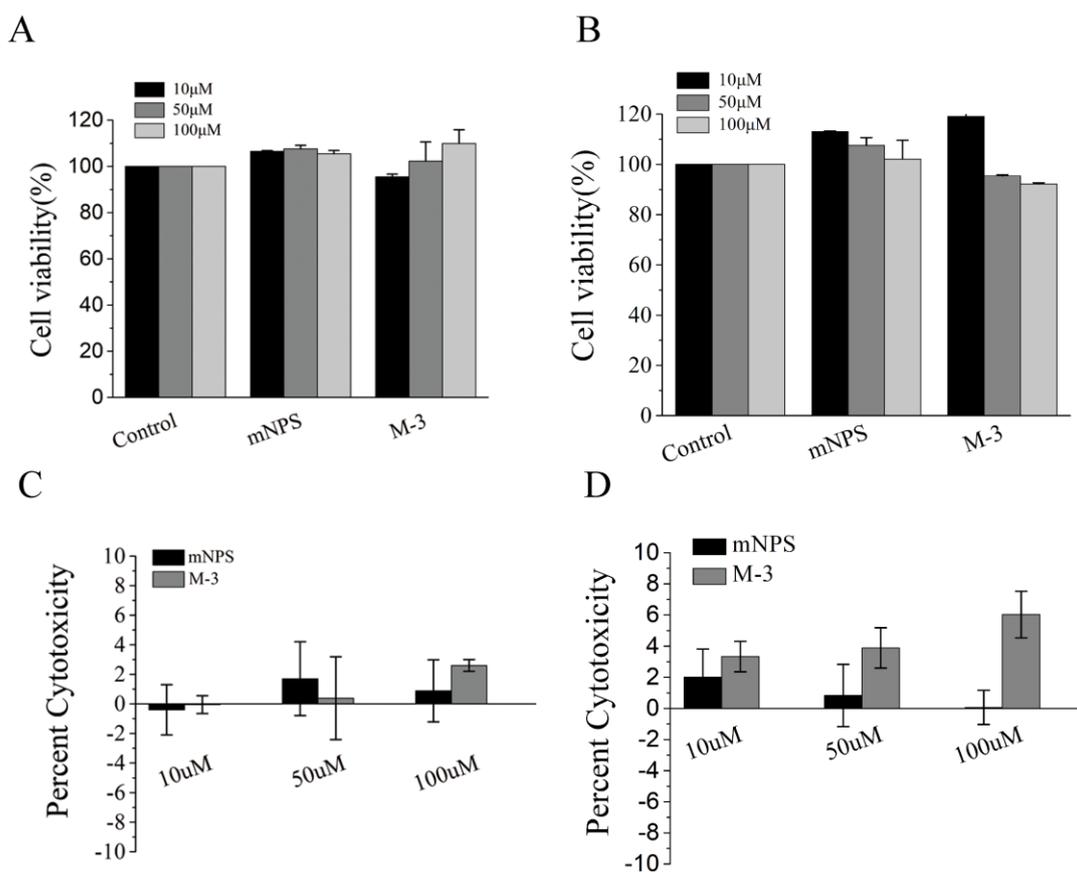
**Fig. S2** Relative mean fluorescence intensity of 5 μM FITC labeled M-3 in the presence and absence of Trypan blue and Triton-X100 in HeLa cells (A) and U87 cells (B) measured by a flow cytometer.



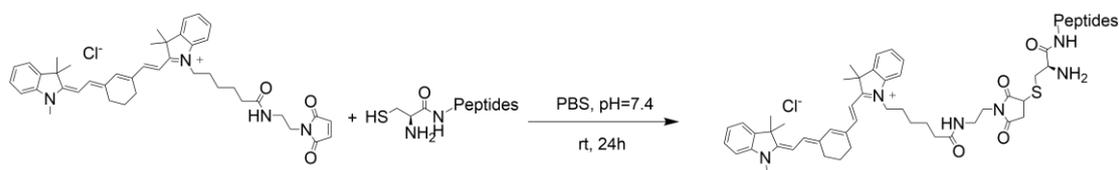
**Fig. S3** Relative mean fluorescence intensity(A) of 5  $\mu$ M FITC labeled M-3 in U87 cells in the presence of endocytosis inhibitors or at 4  $^{\circ}$ C and Confocal images of cellular uptake of 5  $\mu$ M FITC labeled M-3 by U87 cells in the presence of endocytosis inhibitors or at 4  $^{\circ}$ C (B). \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ , versus inhibitor (-) group respectively. Bar = 20  $\mu$ m.



**Fig. S4** Co-localization of mNPS or M-3 and lysosome in HeLa cells (A) and U87 cells (B). HeLa cells and U87 cells were incubated with 5  $\mu$ M mNPS or M-3 for 2 h respectively, followed by LysoTracker Red staining. The images were obtained by confocal microscope. Bar = 20  $\mu$ m.

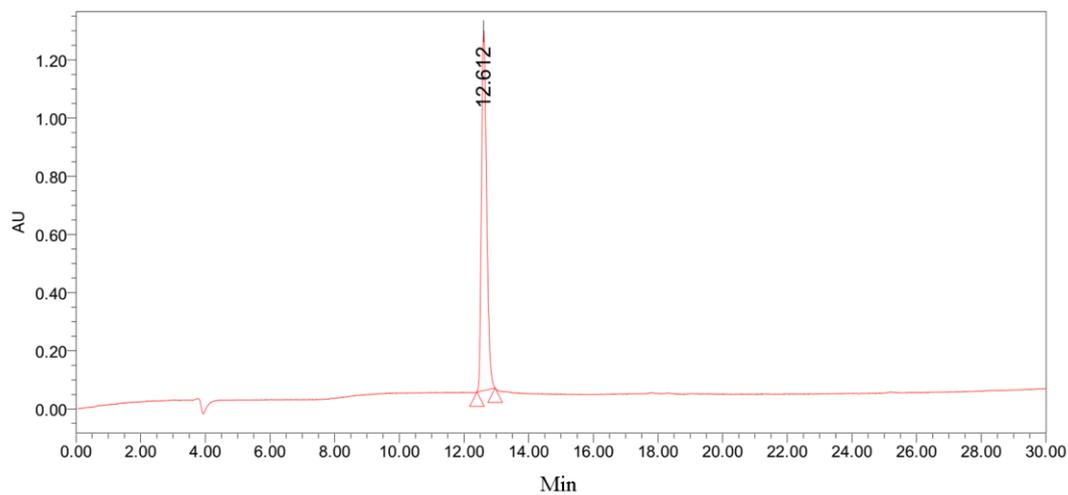
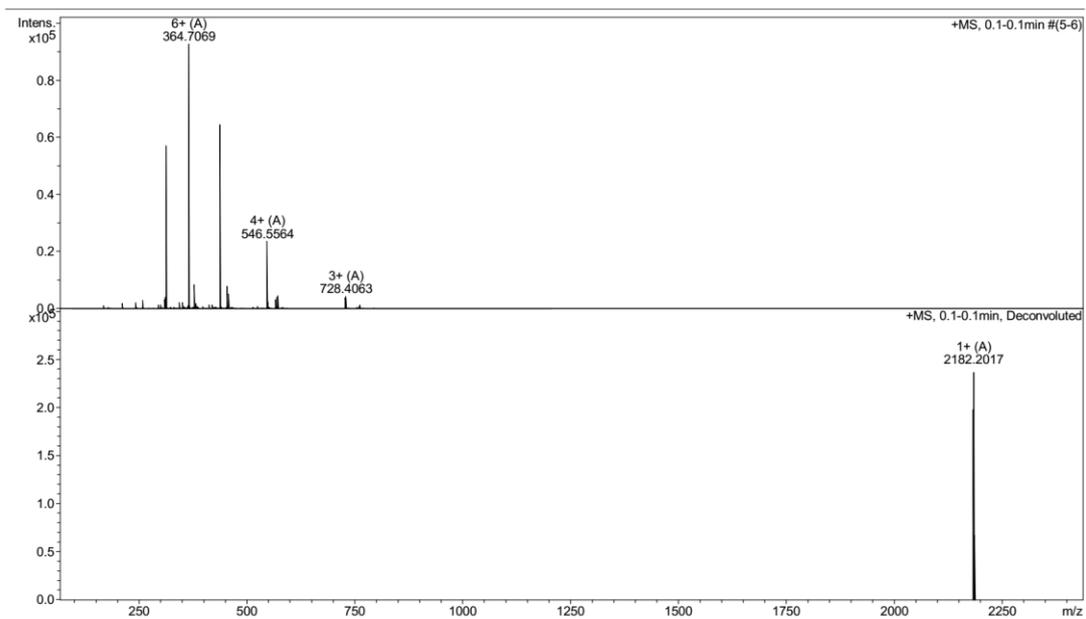
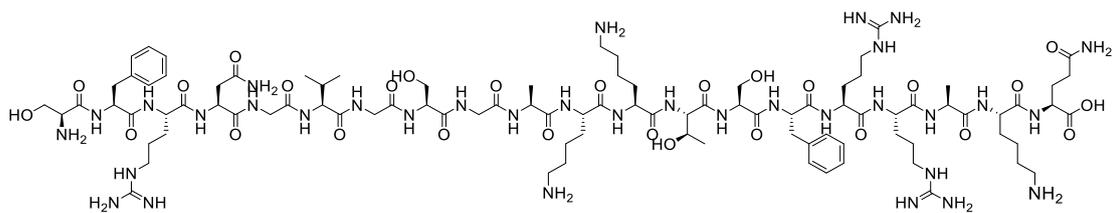


**Fig. S5** Cytotoxicity of mNPS and M-3 in U87 and HeLa cells. The cells were treated with DMSO (control), mNPS and M-3 at 10, 50, and 100  $\mu\text{M}$  for 24 h in U87 cells(A) and HeLa cells (B). LDH assay data of mNPS and M-3 in U87 cells (C) and HeLa cells (D) for 2 h.



**Scheme S4.** Synthetic scheme for Cyanine7 maleimide labeled peptides.

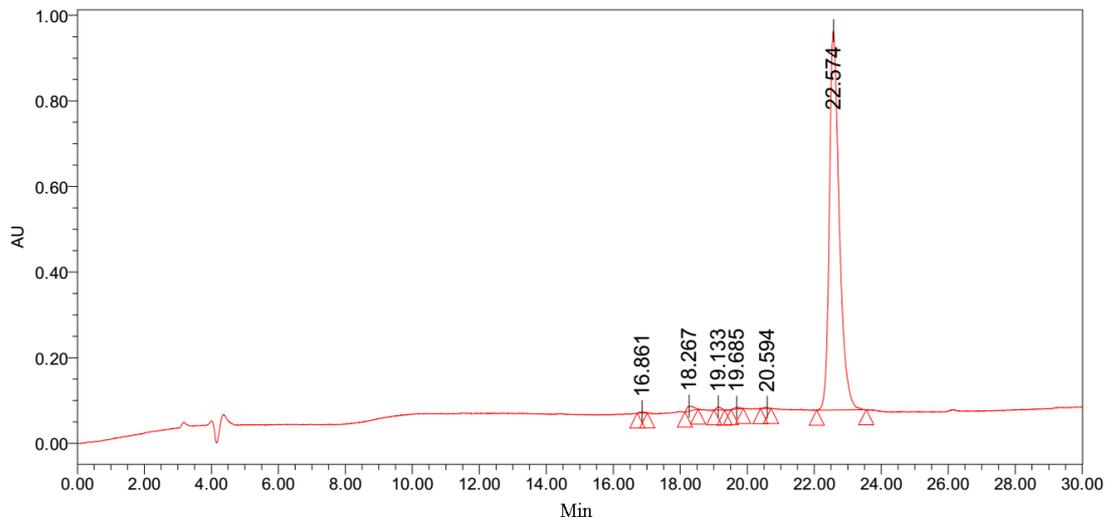
# mNPS



Peak results:

Index	Name	Time (Min)	Area ( $\mu\text{V}^*\text{s}$ )	Height ( $\mu\text{V}$ )	Area% (%)
1		12.612	13612237	1236204	100

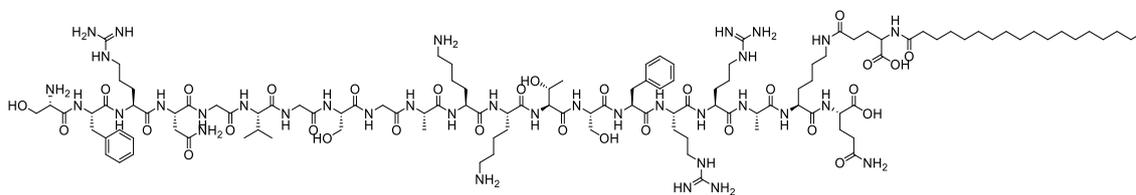


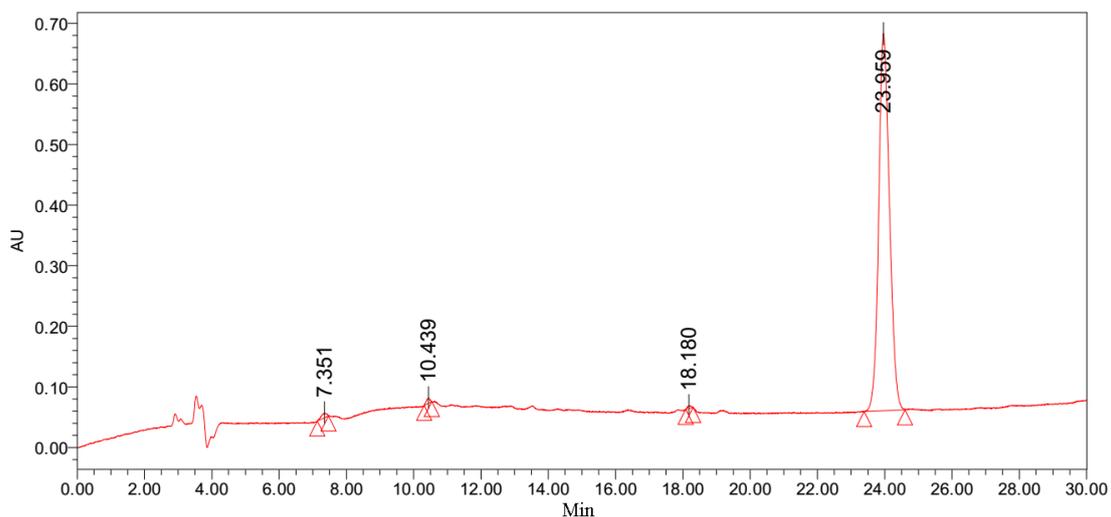
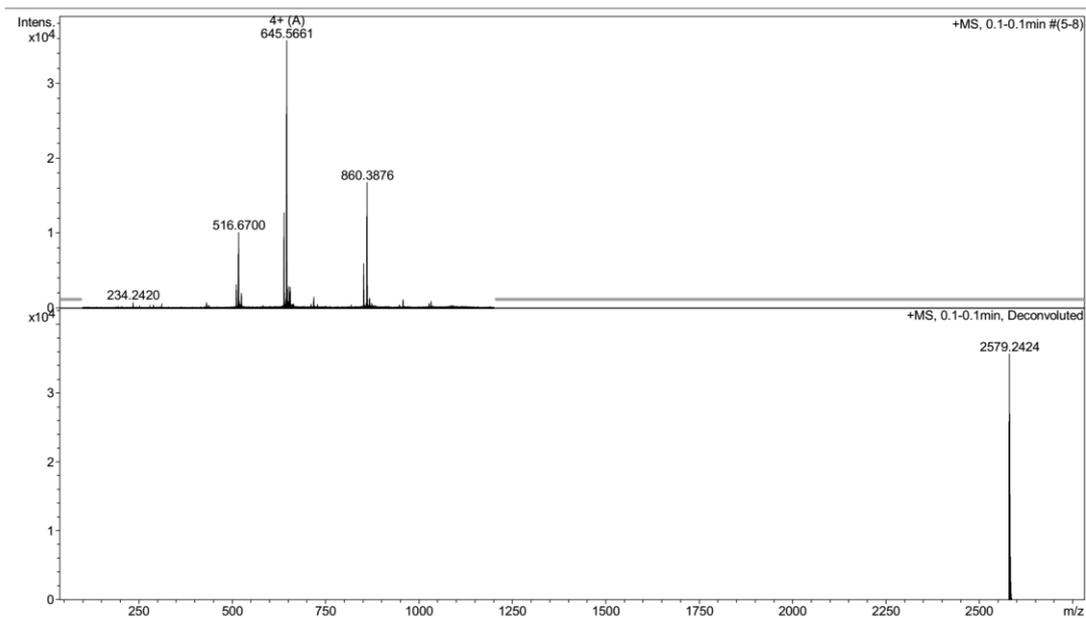


Peak results:

Index	Name	Time (Min)	Area (μV.S)	Height (μV)	Area% (%)
1		16.861	30998	3370	0.16
2		18.267	156400	11669	0.85
3		19.133	71280	7715	0.39
4		19.685	50461	4685	0.27
5		20.594	27668	3467	0.15
6		22.574	18164320	886213	98.18

M-2

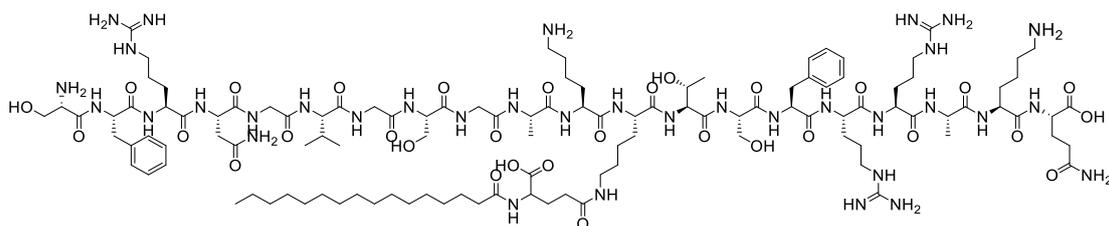


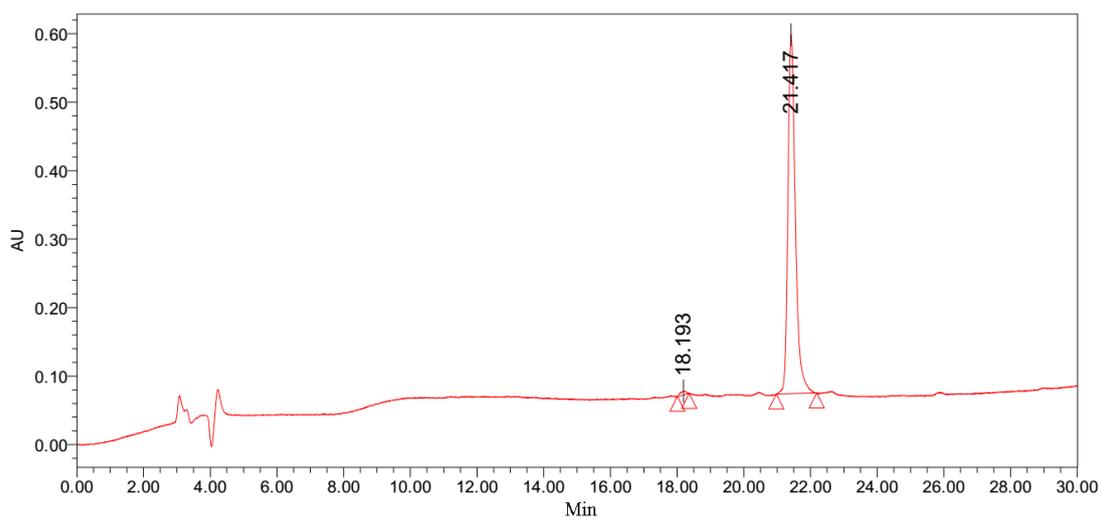
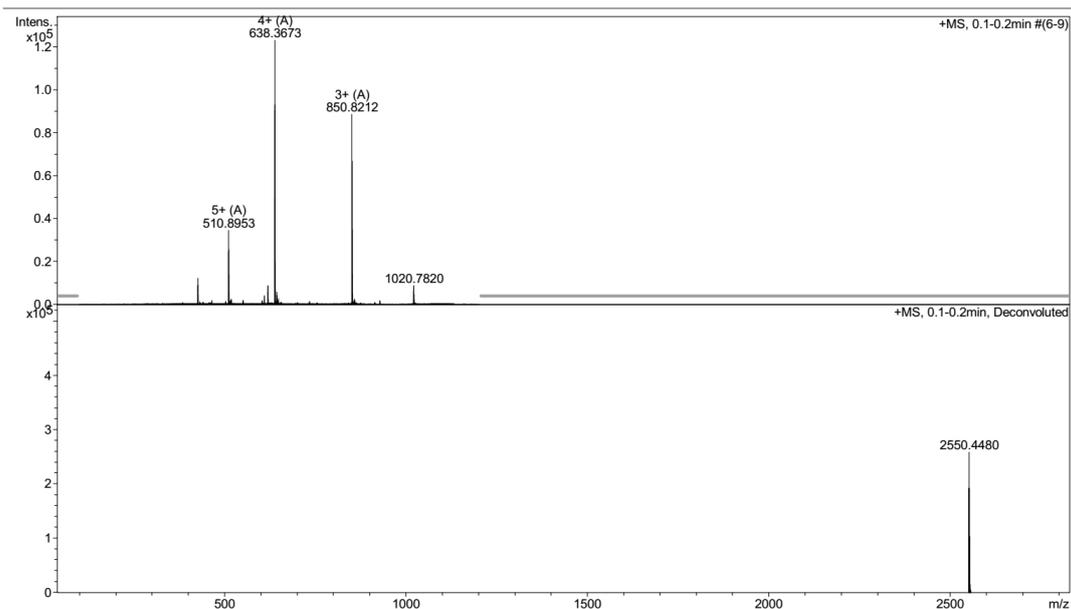


Peak results:

Index	Name	Time (Min)	Area ( $\mu$ V.S)	Height ( $\mu$ V)	Area% (%)
1		7.351	86589	8036	0.62
2		10.439	63975	9185	0.46
3		18.180	34673	4617	0.25
4		23.959	13776568	622433	98.67

M-3

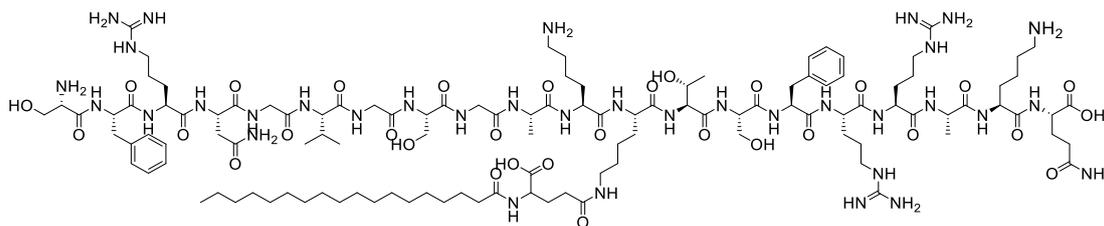




Peak results:

Index	Name	Time (Min)	Area ( $\mu$ V.S)	Height ( $\mu$ V)	Area% (%)
1		18.193	68531	5768	0.84
2		21.417	8114376	524195	99.16

M-4



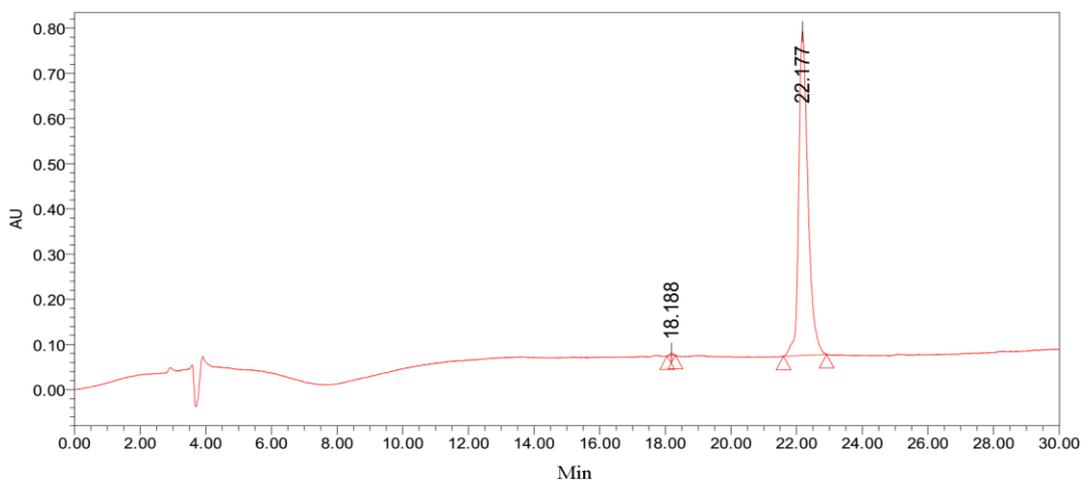
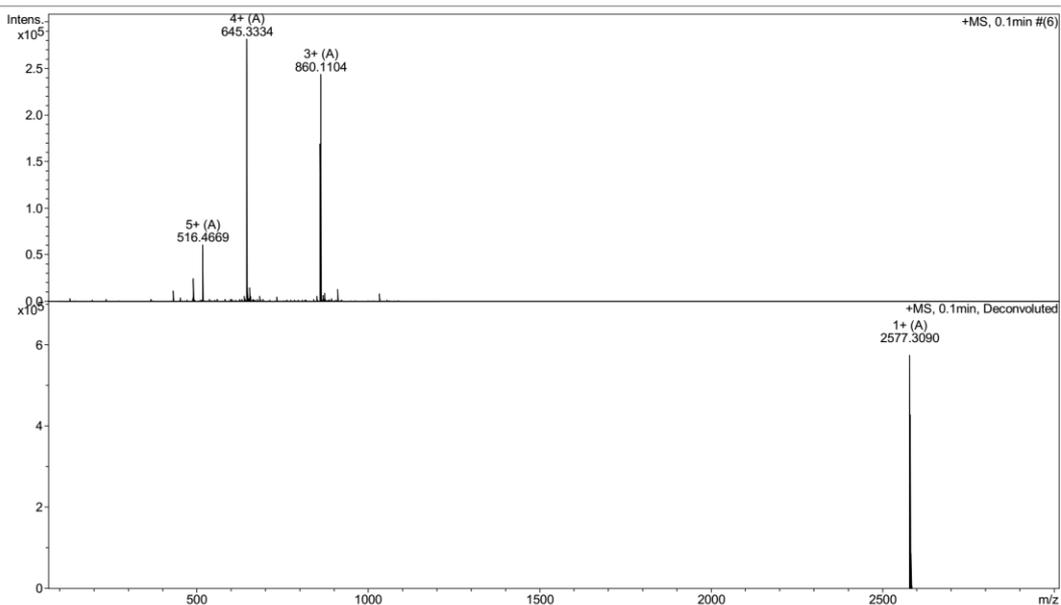
## Generic Display Report

**Analysis Info**

Analysis Name: D:\Data\201903\20190325guochen04.d  
 Method: POS\_TuneLow\_NaTFCal\_100-1200.m  
 Sample Name: < No Sample >  
 Comment:

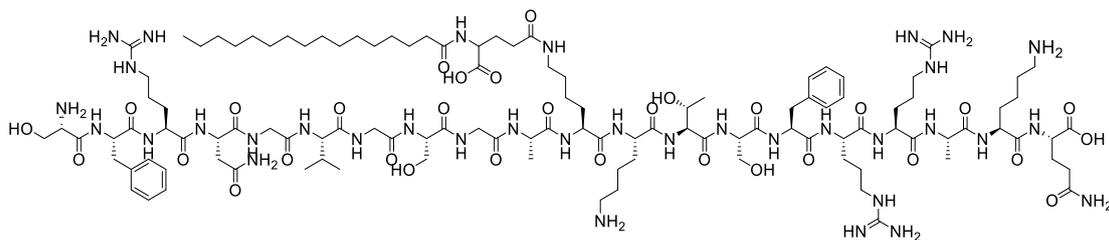
Acquisition Date: 3/25/2019 3:24:51 PM

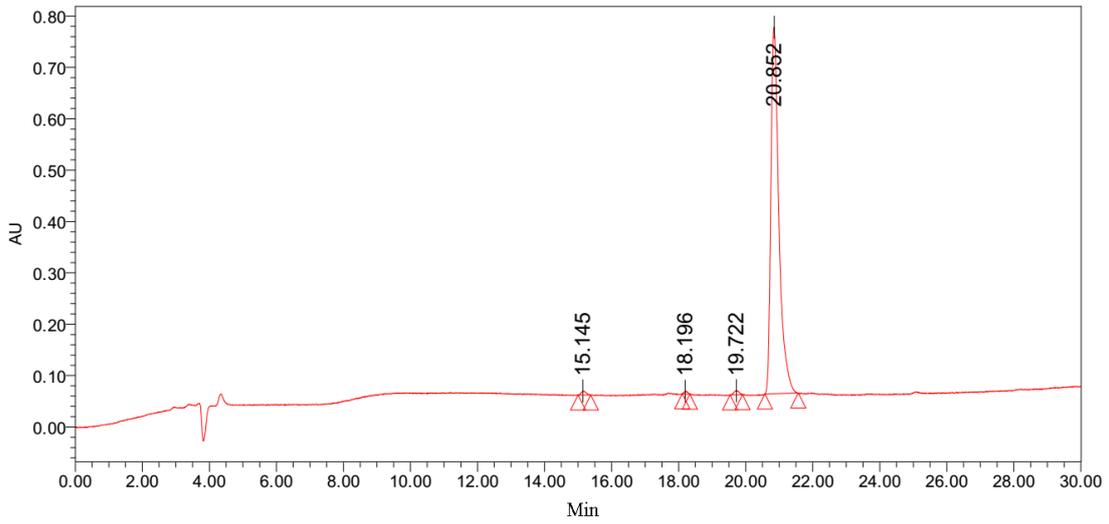
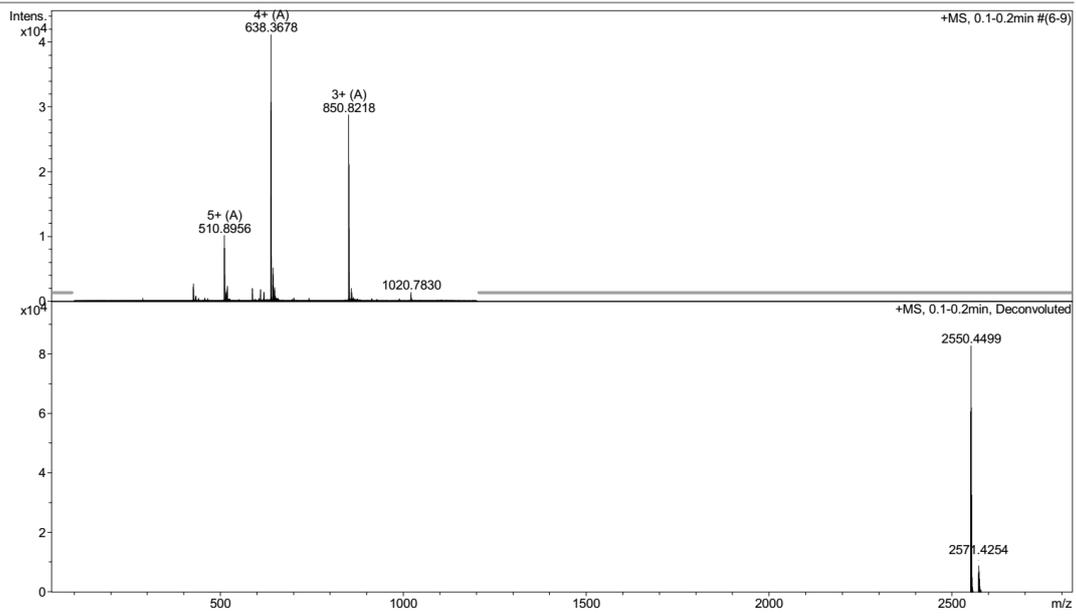
Operator: BDAL@DE  
 Instrument: maXis 4G


**Peak results:**

Index	Name	Time (Min)	Area (μV.S)	Height (μV)	Area% (%)
1		18.188	38793	5498	0.28
2		22.177	13933158	717230	99.72

M-5

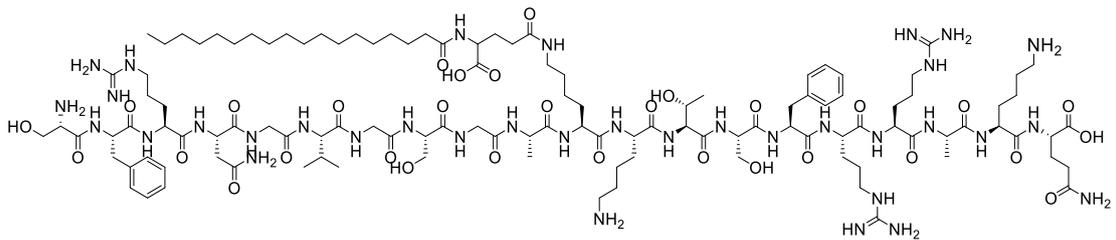


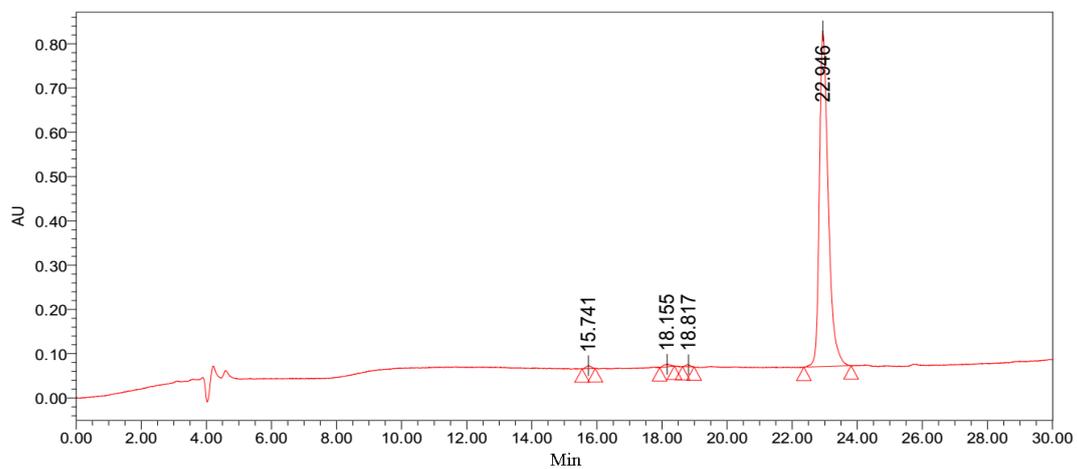
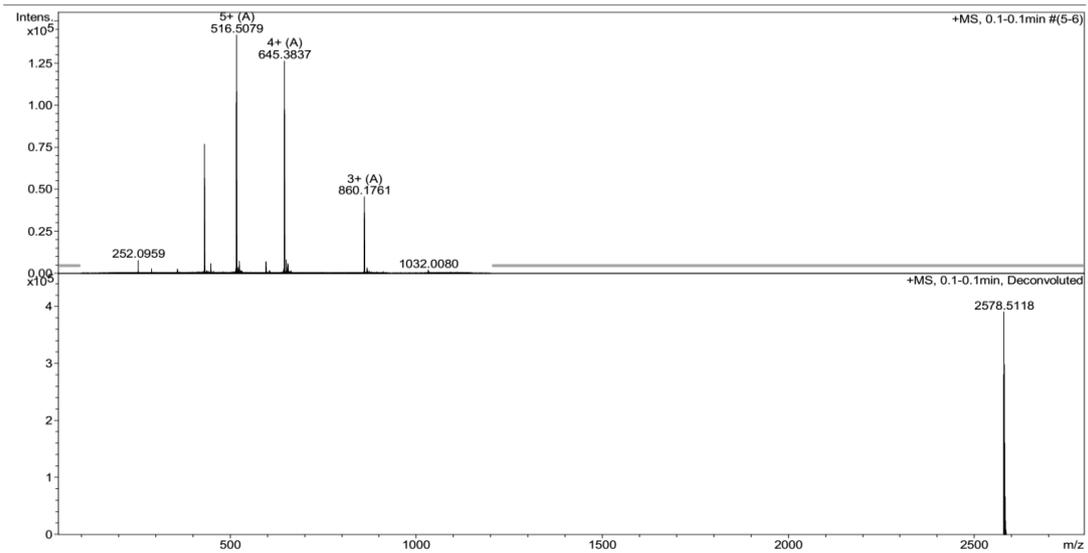


Peak results:

Index	Name	Time (Min)	Area (μV.S)	Height (μV)	Area% (%)
1		15.145	76884	7668	0.64
2		18.196	40687	5115	0.34
3		19.722	88457	8066	0.73
4		20.852	11875438	713631	98.29

M-6

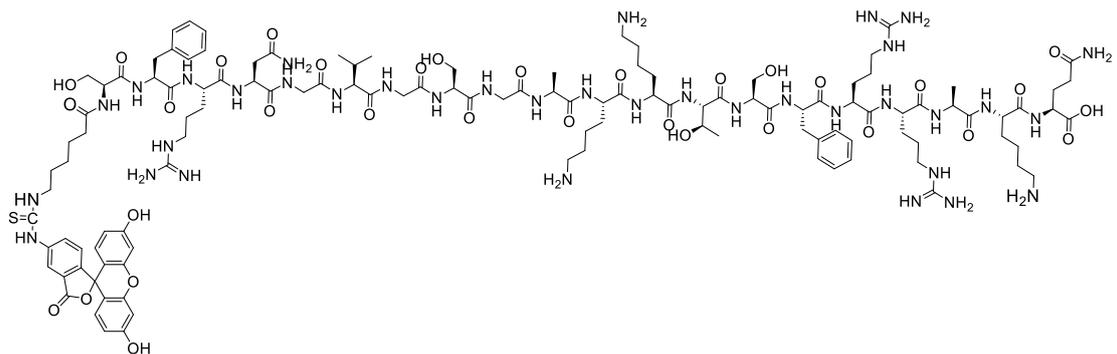




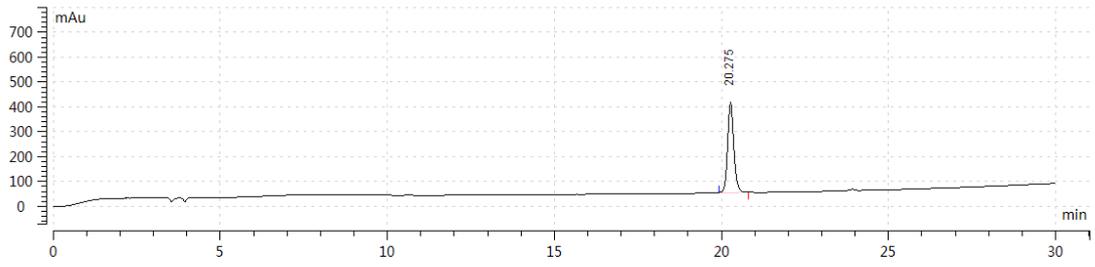
Peak results:

Index	Name	Time (Min)	Area (μV.S)	Height (μV)	Area% (%)
1		15.741	76164	6745	0.52
2		18.155	77225	5646	0.53
3		18.817	40198	4409	0.28
4		22.946	14353494	758043	98.67

## FITC-mNPS







## FITC-M-2

