Electronic Supporting Information

An in-tether sulfilimine chiral center induces β turn conformation in short peptides

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General information

1. Abbreviations

NMP, N-methylpyrolidone; DCM, dichloromethane; DMF, dimethylformamide; DIPEA , diisopropylethylamine; TFA, trifluoroacetic acid; TIPS, triisopropylsilane; DMPA, 2,2-dimethoxy-2-phenylacetophenone; Fmoc, 9-fluorenylmethyloxycarbonyl; HATU, 2-(7-Aza-1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HCTU, 2-(1H-6-chlorobenzotriazol-1-yl)-1,1,3,3-tetramethyl uranium hexafluorophosphate; SPPS, solid-phase peptide synthesis; NMR, nuclear magnetic resonance; ESI-MS, electrospray ionization mass spectrometry; LC-MS, liquid chromatography mass spectrometry; HPLC, high performance liquid chromatography; CD, circular dichroism; CTC resin, 2-chlorotritylchloride resin.

2. Materials

The materials used for solid phase peptide synthesis were purchased from GL Biochem. 2-chlorotritylchloride resin (loading value ~0.65 mmol/g) was purchased from Nankai Synthesis. Other reagents and solvents were purchased from Energy Chemical, Aladdin Chemical and J&K. All reagents were used without further purification.

3. HPLC and LC-MS

The reverse phase high performance liquid chromatography (HPLC) was performed on Shimadzu prominence LC-20AT instrument equipped with C18 analytic column (Agilent ZORBAX SB-Aq, 4.6 ×250 mm, 5 μm, flow rate 1.0 mL/min). Filtered water/ acetonitrile (both containing 0.1% TFA) were used as the eluent condition. LC-MS was recorded on Shimadzu LC-MS 8030 instrument equipped with electrospray ionization.

4. NMR spectroscopy

1D-NMR spectra were recorded on Bruker Avance-III 400MHz spectrometer in $CDCl_3$. 2D NMR spectra were recorded on Bruker Avance-III 600 MHz spectrometer with a TXI probe in H_2O:D_2O=9:1.

5. Circular dichroism spectroscopy

All CD spectra were recorded on Chirascan Plus Circular Dichroism Spectrometer (Applied photophysics). All peptide samples were dissolved in 400 μl dd H_2O. CD data were collected at a scan speed of 20 nm/sec with wavelength from 250 to 190 nm and 0.1 cm path length. Each sample was measured for 2 times.

For peptide **7B**, temperature-dependent CD experiment was performed at varied temperature from 30 to 75 °C in 5 °C increments. For peptide **9B**, temperature-dependent CD experiment was performed at varied temperature from 25 to 75 °C in 5 °C increments.

Experimental section

1. Synthesis of S_5 and S_6

The synthesis of S_5 was showed below as an example, and S_6 was synthesized in the similar route.

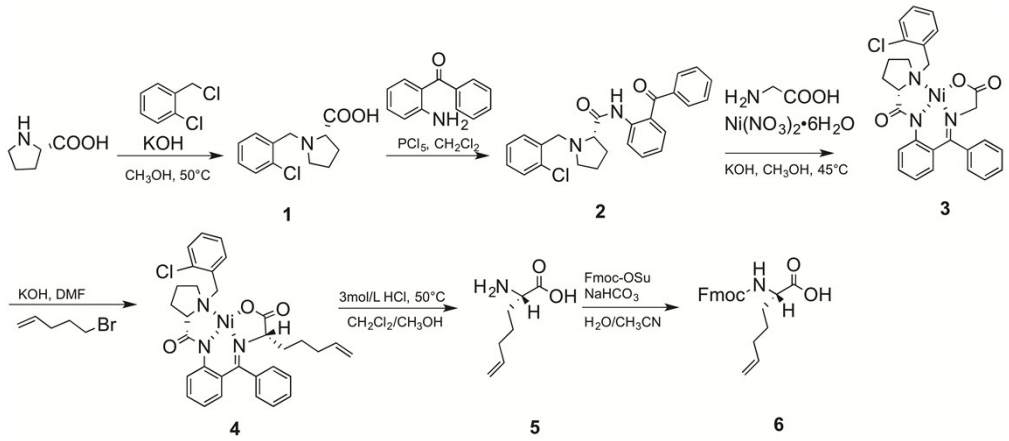
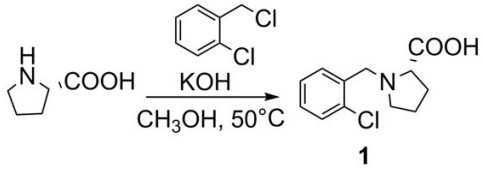


Figure S1 The synthetic route of S_5

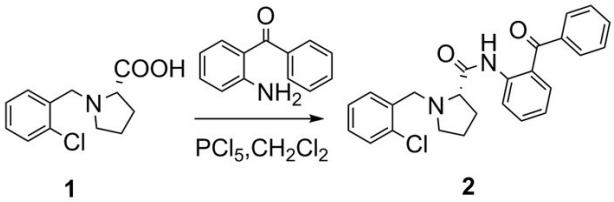
1.1 Synthesis of compound **1**



Potassium hydroxide (38.4 g, 0.7 mol) was added into methanol (150 ml) and then heated to 50 °C until the complete dissolution, followed by the slow addition of L-proline (23 g, 0.2 mol) and 2-chlorobenzyl chloride (32.8 ml, 0.26 mol). The mixture was stirred for 24h and followed by the addition of CH_2Cl_2 (100 ml). Then the reaction mixture stood for another 4h. After 4h, the mixture was filtered out and the resulting residue was washed with CH_2Cl_2. The filtrate was gathered and concentrated in vacuo.

The crude product was crystallized in acetone to produce compound **1** (40.3 g, yield: 80%) without further purification.

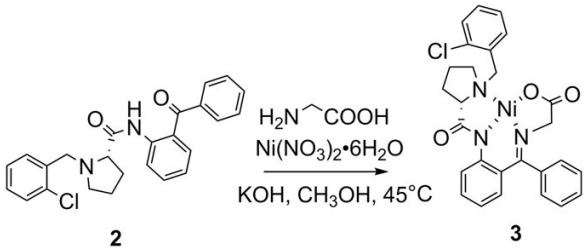
1.2 Synthesis of compound **2**



Compound **1** (12.4 g, 0.05 mol) was dissolved in CH_2Cl_2 (100 ml) and followed by the slow addition of phosphorus pentachloride (15.1 g, 0.075 mol) at 0 °C. Then the reaction mixture was stirred for 1h. After 1h, 2-aminobenzophenone (10.0 g, 0.05 mol) was added into the reaction and the mixture was stirred at r.t. for another 4h.

After the reaction completion, the reaction mixture was concentrated in vacuo and followed by the addition of acetone for further crystallization to produce compound **2** (11.8 g, yield: 52%).

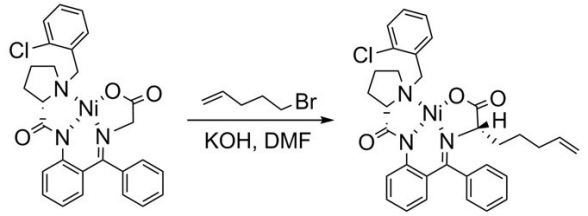
1.3 Synthesis of compound **3**



2 3

The potassium hydroxide (12.5 g, 0.235 mol) in methanol (100 ml) was added dropwise to an anhydrous methanol (150 ml) solution of compound **2** (12.5 g, 0.0325 mol), nickel (II) nitrate hexahydrate (15.8 g, 0.055 mol) and glycine (10.25 g, 0.135 mol) at 50 °C. The mixture was stirred for 4h and followed by the addition of acetic acid to quench the reaction. The mixture was concentrated to dryness in vacuo and followed by the addition of water (500 ml). Then the mixture was stirred at r.t. overnight to produce red precipitates. The residue was filtered out and washed with water to afford compound **3** (11 g, yield: 74%).

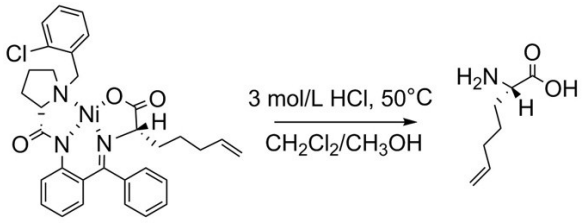
1.4 Synthesis of compound **4**



3 4

Powdered potassium hydroxide (10.55 g, 0.2 mol) was slowly added to a DMF (100 ml) solution of compound **3** (10.0 g, 0.02 mol). The reaction mixture was stirred at r.t. for 1h under nitrogen protection. The the reaction was cooled to 0 °C and then 5-bromo-1-pentene (2.35 ml, 0.021 mol) was slowly added into the solution. Then the reaction mixture was stirred for another 4h and followed by the addition of 5% (v/v) acetic acid aqueous solution for quenching and stirred for another 6h for the precipitation. Then the resulting residue was filtered out, gathered and washed with water for 3 times to afford compound **4** without further purification (9.85 g, yield: 85%).

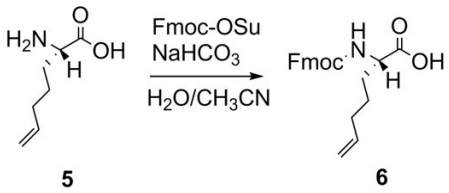
1.5 Synthesis of compound **5**



4 **5**

3M hydrochloric acid aqueous solution (50 ml) was added into a solution of compound **4** (9.85 g, 0.0175 mol) in mixed methanol/dichloromethane (v/v = 25 ml/50 ml). Then the reaction mixture was stirred overnight at 60 °C. The reaction completion was indicated by the appearance of yellow/green color. Methanol/dichloromethane were removed under reduced pressure and the product was extracted with chloroform for 3 times. The combined water layer was gathered and used for the next step without further purification.

1.6 Synthesis of compound **6**



5 **6**

Sodium bicarbonate was added into an aqueous solution of EDTA-Na (9.3 g, 0.025 mol) to adjust the pH of the mixture to pH 8. The acetonitrile solution of Fmoc-OSu (5.85 g, 0.0175 mol) was added dropwise to the mixture at 0 °C. Then the reaction was stirred for 24 h at room temperature. Acetonitrile was removed in vacuo and citric acid solid was added into aqueous solution to adjust the pH of the mixture to pH 2-3. Then the reaction was extracted with ethyl acetate for 3 times. The combined organic layer was gathered, dried with Na_2SO_4 and concentrated under reduced pressure. The final compound **6** was obtained after the purification of flash column chromatography (SiO_2, CH_2Cl_2:CH_3OH = 80:1) (2.5 g, yield: 38%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.59 (d, *J* = 7.4 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.31 (td, *J* = 7.4, 0.9 Hz, 2H), 5.75 (s, 1H), 5.57 (s, 1H), 4.98 (dd, *J* = 27.6, 13.4 Hz, 2H), 4.40 (s, 2H), 4.21 (t, *J* = 6.5 Hz, 1H), 2.34 – 2.16 (m, 1H), 2.00 (d, *J* = 35.3 Hz, 3H), 1.62 (s, 2H).

2. Represent example for synthesis of cyclic thioether peptides

The synthesis of cyclic thioether peptide Ac-(cyclo-1,4)-[S_5AAC]-NH_2 was showed as an example below, and thioether peptides **2**-**6** were synthesized in a similar way.

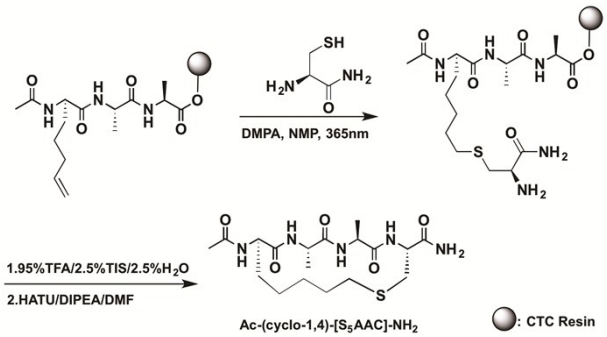


Figure S2 The synthetic route of cyclic thioether peptide **1**

2.1 Synthesis of linear peptide before thiol-ene reaction

Peptide was synthesized on 2-chlorotritylchloride resin (loading value 0.65 mmol/g) based on solid-phase peptide synthesis. Firstly, the weighed resin was swelled with NMP and bubbled with N_2 for 30 minutes. Then Fmoc-Ala-OH (5 equiv according to the dosage of the resin) and DIPEA (10 equiv) were dissolved in NMP and added to the resin with N_2 bubbling for 3h, followed by sequential wash with DCM and NMP for 3 times. 50% morpholine in NMP sulotion was added to remove the Fmoc group of Ala for 1h. Then the second Fmoc-Ala-OH (5 equiv), HCTU (4.9 equiv) and DIPEA (10 equiv) were sequentially added into NMP and the mixture solution was added to the resin for 2h, followed by the sequential wash with DCM/NMP and Fmoc group removal with 50% morpholine in NMP. The third Fmoc-S_5-OH was coupled to the resin in a similar way as the second Fmoc-Ala-OH. The free amino group of S_5 was acetylated with a solution of acetic anhydride and DIPEA in NMP (1:4: 20 in volume) for 1h.

2.2 Intermolecular thiol-ene reaction

The resin from the previous step was swelled in NMP for 20 min. Then Cys (1 equiv according to the dosage of the resin) and DMPA (1 equiv) were added to the resin. The reaction mixture was stirred at 365 nm UV irradiation for 2h under nitrogen protection, followed by the wash with DCM for 3 times.

2.3 Macrocyclization

The crude linear peptide was cleaved from the resin by treating it with a mixture of TFA/H2O /TIS (95/2.5/2.5) for 2 h, followed by the stream of nitrogen for concentration. Et_2O was added for precipitation of the crude peptide and the supernatant was removed after centrifugation. Then the resulting peptide was dissolved in anhydrous DMF, followed by the addition of HATU (1 equiv) and DIPEA (1 equiv) at 0 °C. The reaction mixture was stirred at room temperature for 24h under nitrogen protection. After the reaction completion, DMF was removed in vacuo and 30% (v/v) water/acetonitrile was added to dissolve the crude peptide product, which was further purified by reversed phase HPLC and analyzed by LC-MS.

Gradient elution condition:
2 % acetonitrile constant gradient from 0 to 10 min, then 2 % to 30 % acetonitrile linear gradient from 10 to 60 min, and then 30 % to 95 % acetonitrile linear gradient from 60 to 70 min

3. Synthesis of cyclic sulfilimine peptides

Cyclic thioether peptide (1 equiv) and chloramine-T (1.2 equiv) were dissolved in acetonitrile and the reaction mixture was stirred at room temperature for 24h. Then acetonitrile was removed under reduced pressure, followed by the addition of 30% (v/v) water/acetonitrile for further reversed phase HPLC purification.
Gradient elution condition:
2 % to 25 % acetonitrile linear gradient from 0 to 50 min, and then 25 % to 95 % acetonitrile linear gradient from 50 to 60 min

4. 2D-NMR experiment

2D NMR spectra of peptide **14B** were collected on a Bruker Avance III 600MHz spectrometer with a TXI probe. Watergate pulse sequence with gradients was used for water suppression. 80 ms and 350 ms mixing time were set in 2D 1H-1H TOCSY and 2D 1H-1H ROESY, respectively. A 10 ppm spectra width and 2048×256 complex points were used for 2D 1H-1H spectra, followed by the data process with TopSpin® and analyzation with CCPNMR software. Temperature dependence for amide NH chemical shifts of peptide **14B** was measured in 2D TOCSY at varied temperature from 288 to 313 K in 5 K increments and with an equilibration time of 15 min.

5. Crystallization and data collection

Peptide **7B** and its mirror image **14B** at a ratio of 1:1 were dissolved in methanol/water (v/v=1:1) and crystallized at 4 °C using sitting drop vapor diffusion method. Crystals were screened, reserved in liquid nitrogen and cryo-protected in buffer solution (methanol : water : glycerol=5:3:2). Data collection of co-crystals **7B**/**14B** was performed at 113 K on the X-ray diffraction system equipped with high-intensity sealed copper tube X-ray generator (Rigaku® MicroMax-002+), an AFC11 goniometer, a Saturn 944+ CCD detector (Rigaku®) and an Oxford Cryo-system.

Figures and tables

Table S1 MS data for peptides **1~14**.

peptide	$[M+H]^+$ (m/z)	
	calculated	observed
1	430.2	430.3
2	472.3	472.4
3	501.3	501.4
4	543.3	543.5
5	501.3	501.4
6	444.2	444.4
7A	599.2	599.2
7B	599.2	599.2
8A	641.3	641.4
8B	641.3	641.4
9A	670.1	670.2
9B	670.1	670.3
10A	712.3	712.5
10B	712.3	712.4
11A	670.1	670.4
11B	670.1	670.3
12A	613.3	613.4
12B	613.3	613.4
13A	446.2	446.3
13B	446.2	446.3
14A	599.2	599.4
14B	599.2	599.4

Table S2 Amide NH chemical shift of peptide **14B** in 90% H_2O:10% D_2O at 288K, 293K, 298K, 303K, 308K and 313K.

K	S_5	Ala	Ala	Cys
	HN	HN	HN	HN
288	8.483	8.513	8.013	8.243
293	8.432	8.472	7.992	8.222
298	8.395	8.435	7.975	8.205
303	8.352	8.392	7.962	8.192
308	8.308	8.358	7.948	8.178
313	8.265	8.325	7.925	8.155

$^3J_{NH\text{-}CH\alpha}$	4.4Hz	3.3Hz	5.8Hz	5.3Hz

Calculation of temperature coefficient Δδ/T

For S_5, Δδ/T = (Amide proton chemical shift at 313 K - Amide proton chemical shift at 288 K) * 1000 / (313 K – 288 K) = (8.265 – 8.483) * 1000 / 25 = - 8.72 ppb/K

For the second Ala, Δδ/T = (Amide proton chemical shift at 313 K - Amide proton chemical shift at 288 K) * 1000 / (313 K – 288 K) = (8.325 – 8.513) * 1000 / 25 = - 7.52 ppb/K

For the third Ala, Δδ/T = (Amide proton chemical shift at 313 K - Amide proton chemical shift at 288 K) * 1000 / (313 K – 288 K) = (7.925 – 8.013) * 1000 / 25 = - 3.52 ppb/K

For Cys, Δδ/T = (Amide proton chemical shift at 313 K - Amide proton chemical shift at 288 K) * 1000 / (313 K – 288 K) = (8.155 – 8.243) * 1000 / 25 = - 3.52 ppb/K

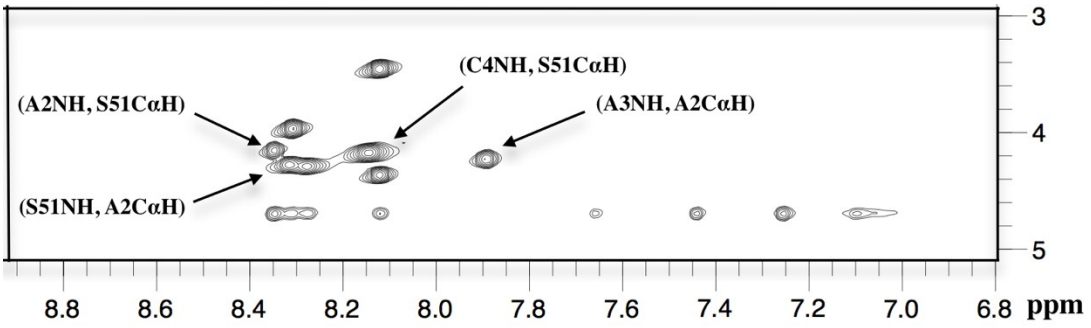


Figure S3 NH-CαH region of 2D-ROESY spectrum for peptide **14B** (H_2O:D_2O=9:1, 298K, 600 MHz). NH-CαH cross-peaks were indicated and labelled by one letter amino acid codes and their sequential numbers from N to C terminal in **14B**.

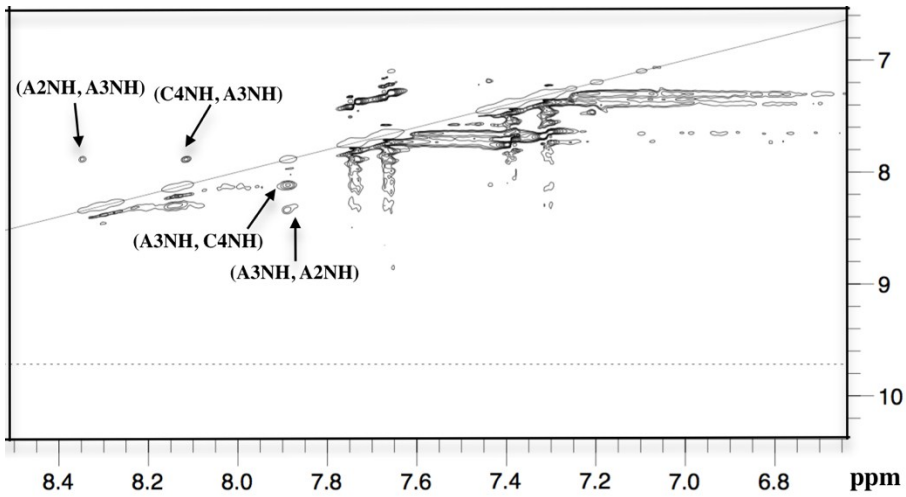


Figure S4 NH-NH region of ROESY spectrum for peptide **14B** (H_2O:D_2O=9:1, 298K, 600 MHz).

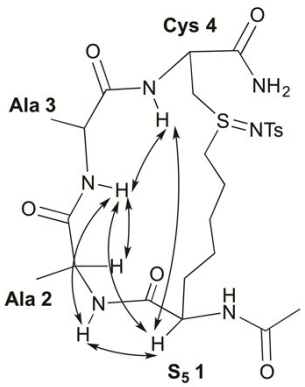


Figure S5 Region of 600-MHz NOESY spectrum of peptide **14B** at 25 °C in H_2O ($S_5$1 CαH-Cys4 NH; $S_5$1 CαH-Ala2 NH; $S_5$1 CαH-Ala3 NH; Ala2 NH-Ala3 NH; Ala2 CαH-Ala3 NH; Ala3 NH-Cys4 NH).

Table S3 Summary of data collection and structure refinement for peptide **7B**.

Crystal name	Ac-(cyclo-1,4)-[S_5AAC(NTs)]-NH_2 (**7B**)
Data collection	Cu Kα
Chemical formula	C25H38N6O7S2
Formula weight	598.73
Crystal system	Monoclinic
Temperature (K)	113
Crystal dimensions (mm)	0.20 X 0.20 X 0.20
Unit-cell volume (Å^3)	3050.5
Unit-cell dimensions	
a, b, c (Å)	16.53, 9.15, 21.56
α, β, γ (°)	90, 110, 90
Space group	P21/c
Z value	4
Radiation wavelength (Å)	1.54187
Monochromator	Graphite
Calculated density (g/cm^3)	1.304
Number of parameters	387
Number of reflections	
Total	54106
Unique	5495
R_{int}	0.151
F_{000}	1272.00
$2q_{max}$ (°)	136.3
Data completeness	0.988
Refinement	
Structure solution	Direct methods (SHELX97)
Refinement method	Full-matrix least-squares on F^2
Reflection/parameter ratio	14.20
Corrections	Lorentz-polarization
Residuals: R1	0.1171
Residuals: R	0.1422
Residuals: wR2	0.3475
Goodness of fit indicator	1.948
Max shift/error in final cycle	0.000
Diffraction density range (e$^-$/Å^3)	-1.180~0.940
	16
Number of restraints	Mixed
Hydrogen treatment	-19~19
h range	-9~10
k range	-25~25
l range	

Table S4 Summary of data collection and structure refinement for peptide **14B**.

Crystal name	D-Ac-(cyclo-1,4)-[S_5AAC(NTs)]-NH_2 (**14B**)
Data collection	Cu Kα
Chemical formula	C25H38N6O7S2
Formula weight	598.73
Crystal system	Monoclinic
Temperature (K)	113
Crystal dimensions (mm)	0.20 X 0.20 X 0.20
Unit-cell volume (Å^3)	3050.5
Unit-cell dimensions	
a, b, c (Å)	16.53, 9.15, 21.56
α, β, γ (°)	90, 110, 90
Space group	P21/c
Z value	4
Radiation wavelength (Å)	1.54187
Monochromator	Graphite
Calculated density (g/cm^3)	1.304
Number of parameters	387
Number of reflections	
Total	54106
Unique	5495
R_{int}	0.151
F_{000}	1272.00
$2q_{max}$ (°)	136.3
Data completeness	0.988
Refinement	
Structure solution	Direct methods (SHELX97)
Refinement method	Full-matrix least-squares on F^2
Reflection/parameter ratio	14.20
Corrections	Lorentz-polarization
Residuals: R1	0.1171
Residuals: R	0.1422
Residuals: wR2	0.3475
Goodness of fit indicator	1.948
Max shift/error in final cycle	0.000
Diffraction density range (e$^-$/Å^3)	-1.180~0.940
	16
Number of restraints	Mixed
Hydrogen treatment	-19~19
h range	-9~10
k range	-25~25
l range	

HPLC separation spectra of sulfilimine peptides 7~14

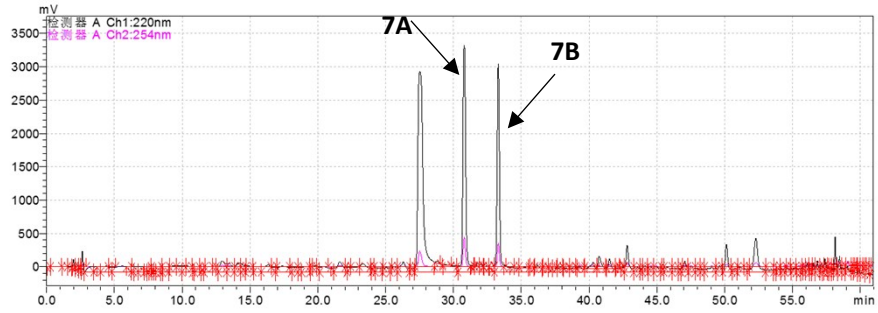


Figure S6 HPLC separation spectrum of **7A** and **7B**

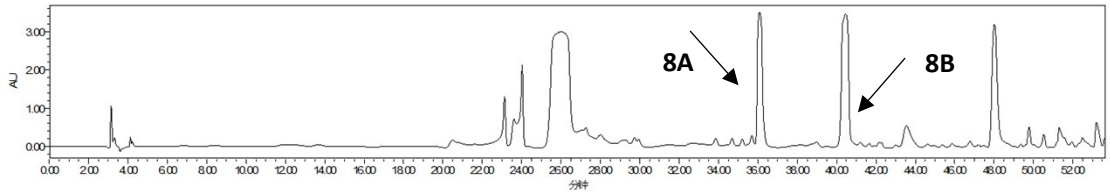


Figure S7 HPLC separation spectrum of **8A** and **8B**

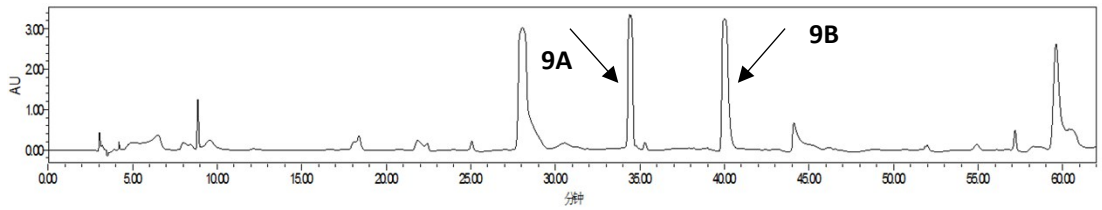


Figure S8 HPLC separation spectrum of **9A** and **9B**

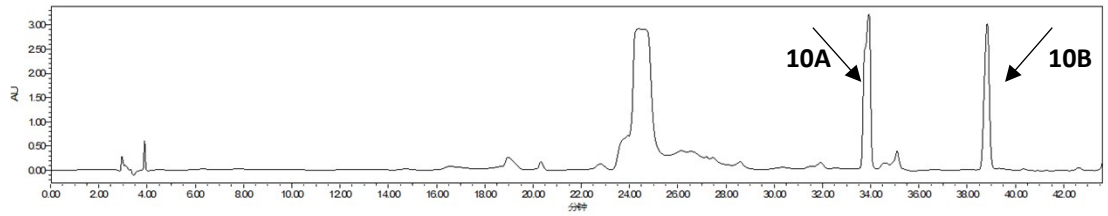


Figure S9 HPLC separation spectrum of **10A** and **10B**

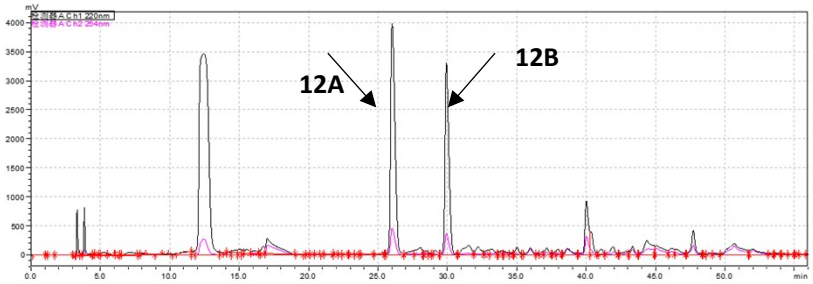


Figure S10 HPLC separation spectrum of **12A** and **12B**

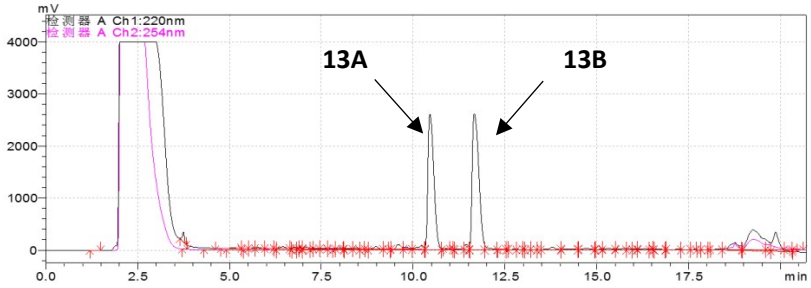


Figure S11 HPLC separation spectrum of **13A** and **13B**

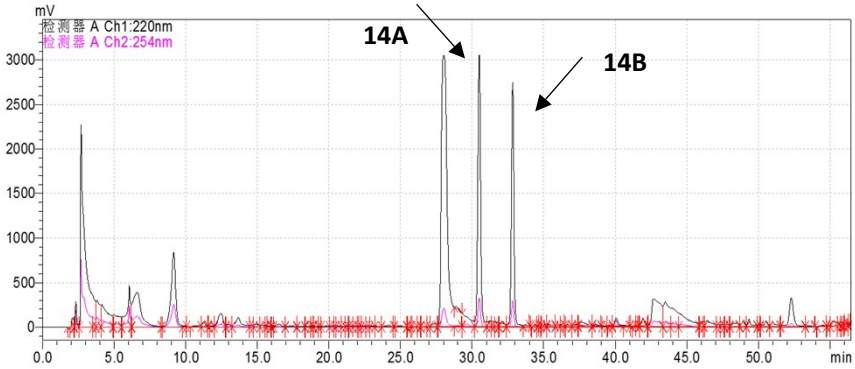
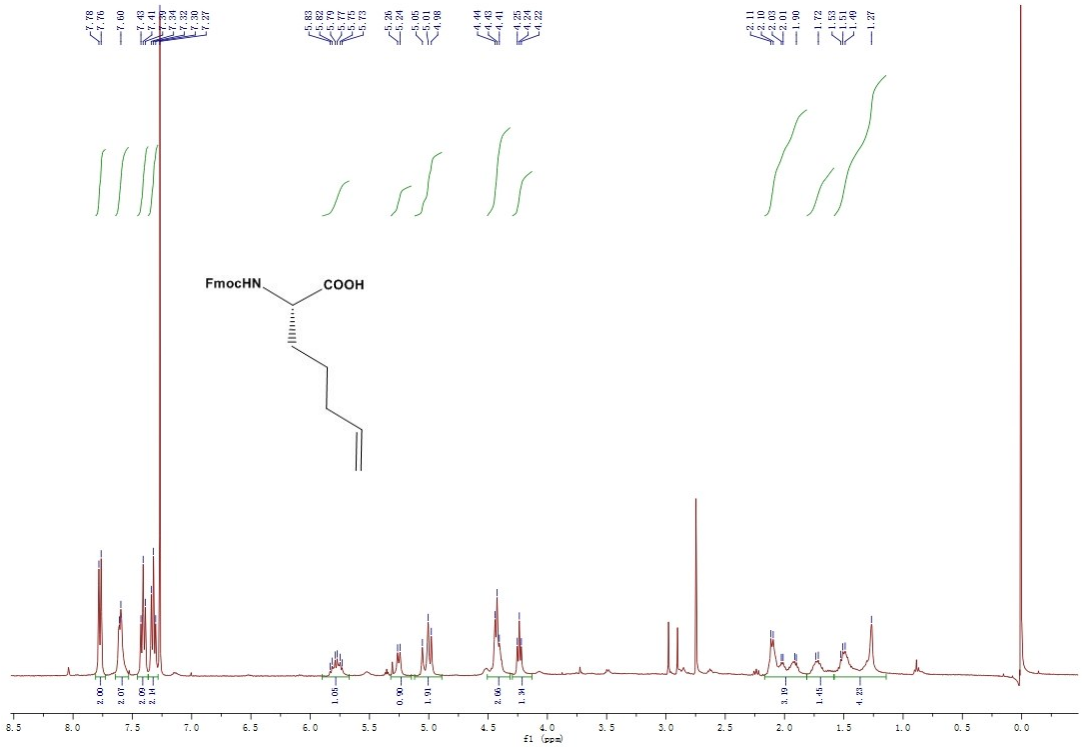


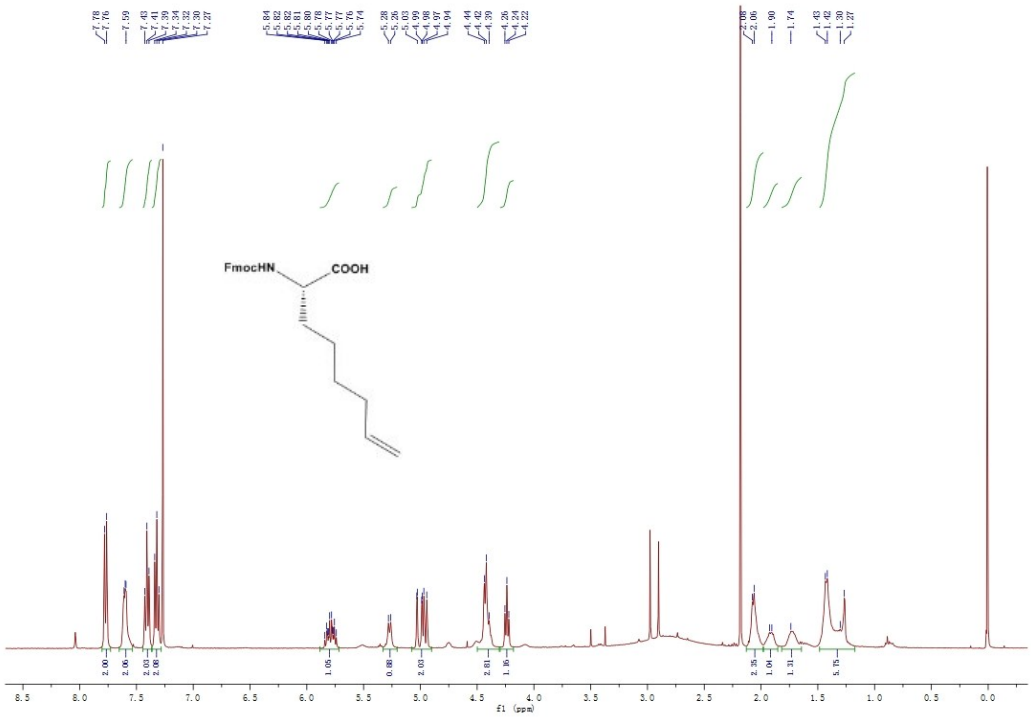
Figure S12 HPLC separation spectrum of **14A** and **14B**

^{1}H NMR spectra of S_5 and S_6

(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)hept-6-enoic acid

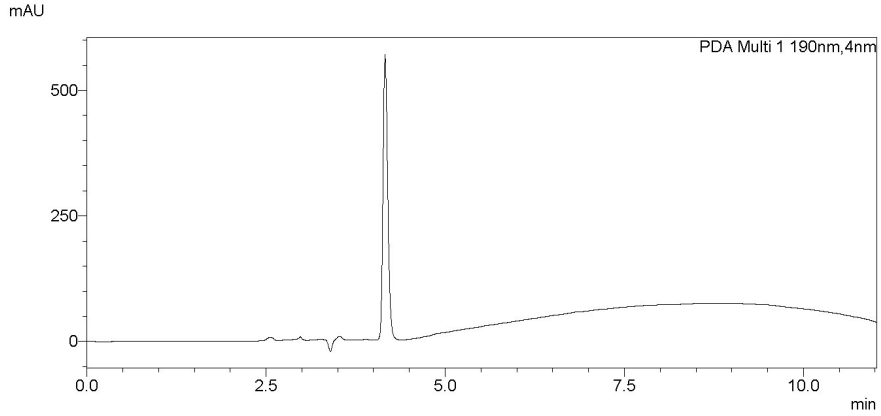


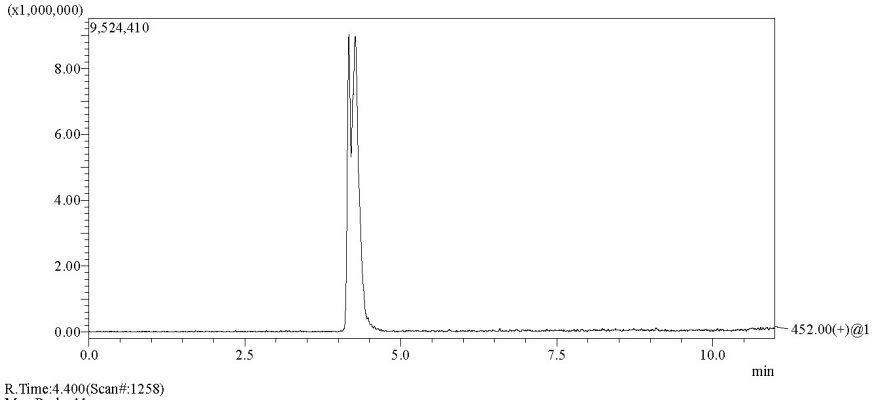
(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)oct-7-enoic acid

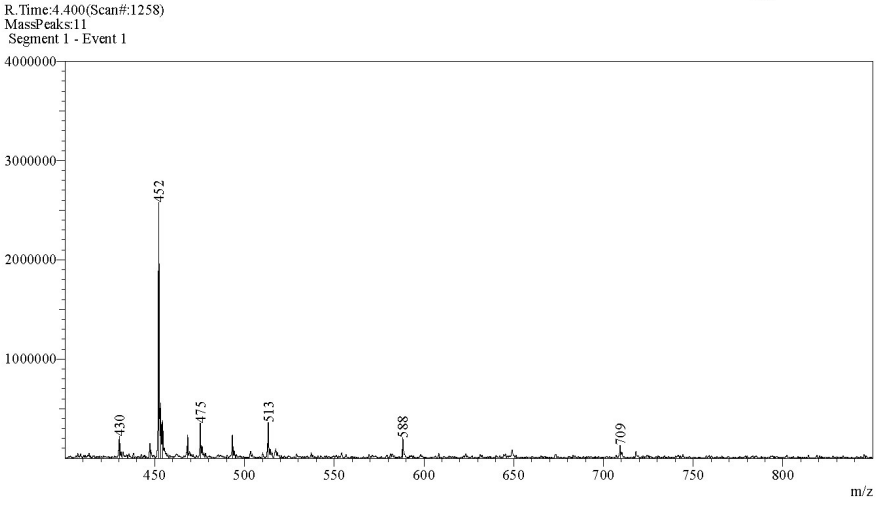


LC-MS spectra of peptides 1~14

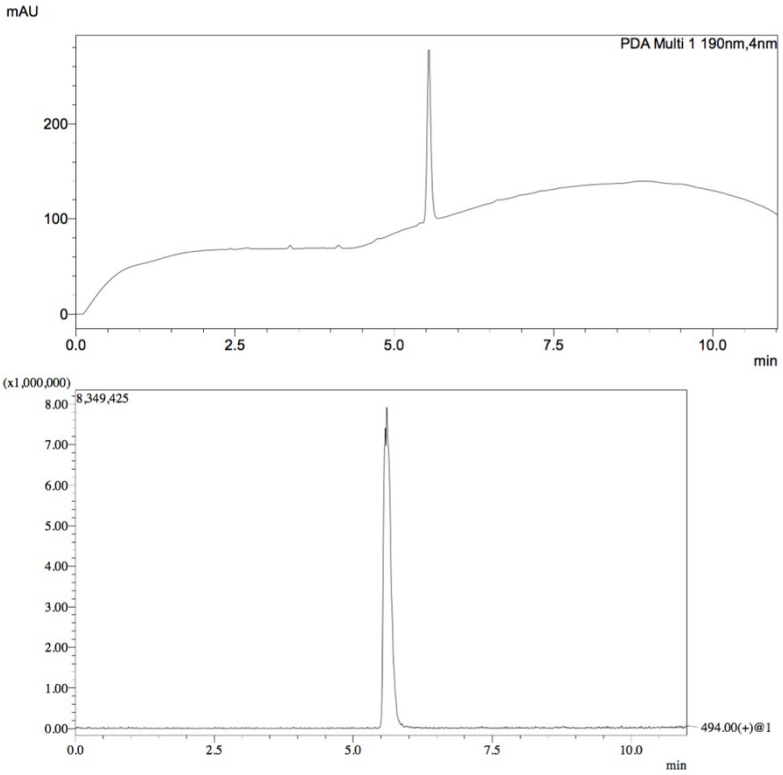
Ac-(cyclo-1,4)-[S_5AAC]-NH_2 **1**

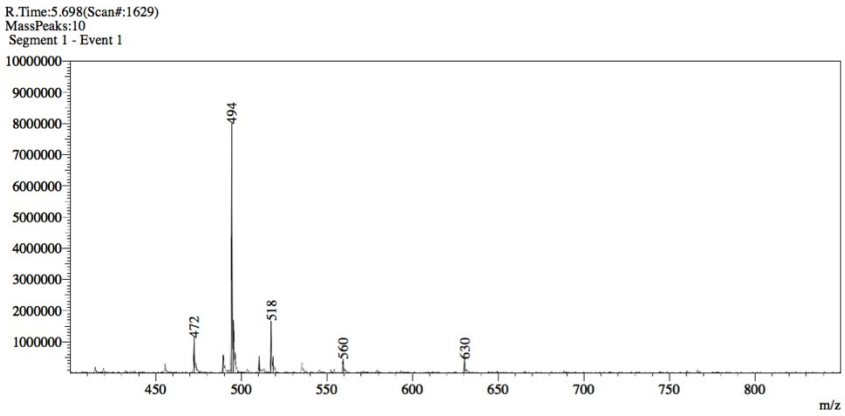




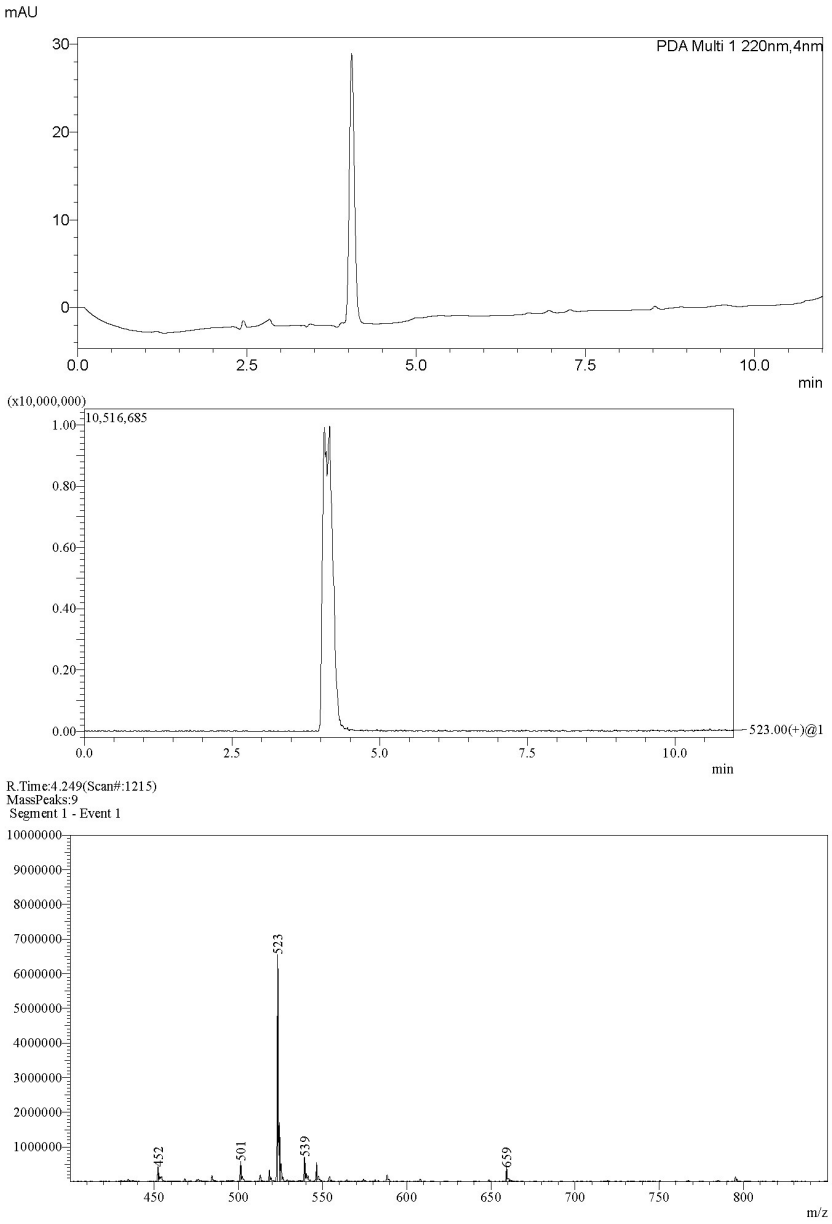


Ac-(cyclo-1,4)-[S_5IAC]-NH_2 **2**

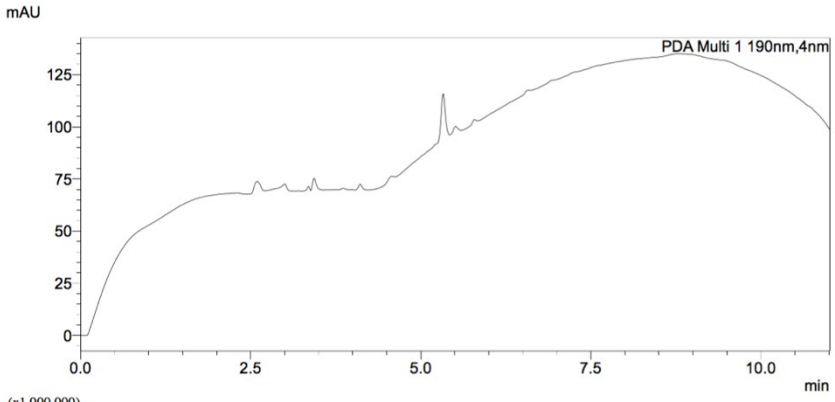


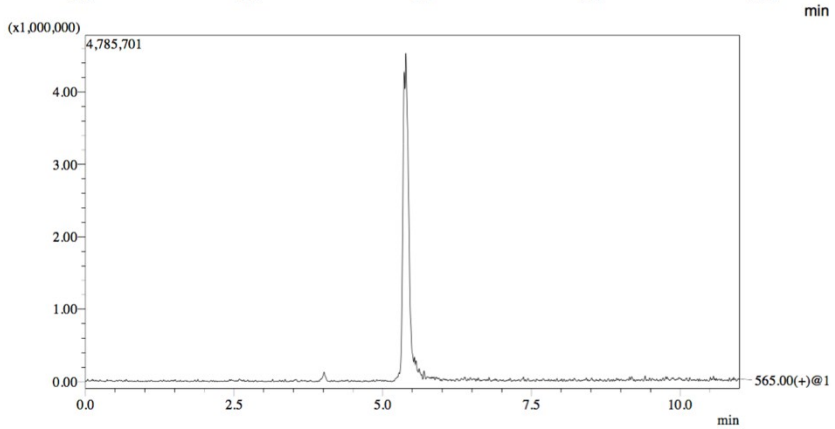


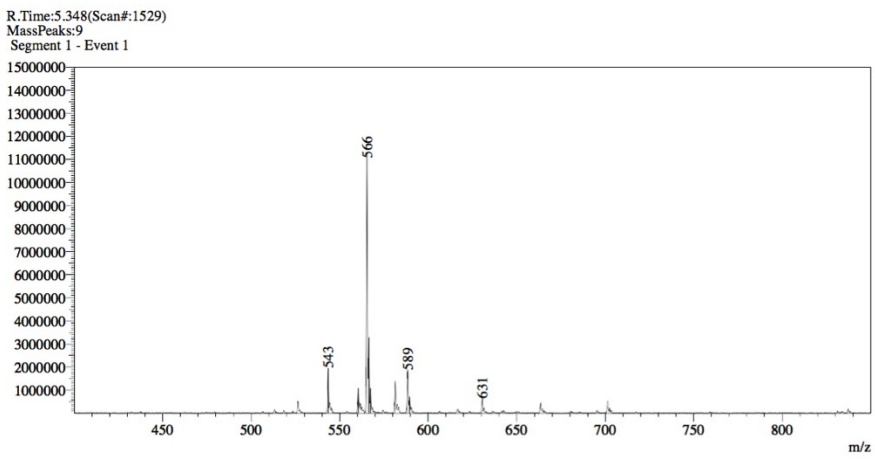
Ac-A-(cyclo-2,5)-[S_5AAC]-NH_2 **3**



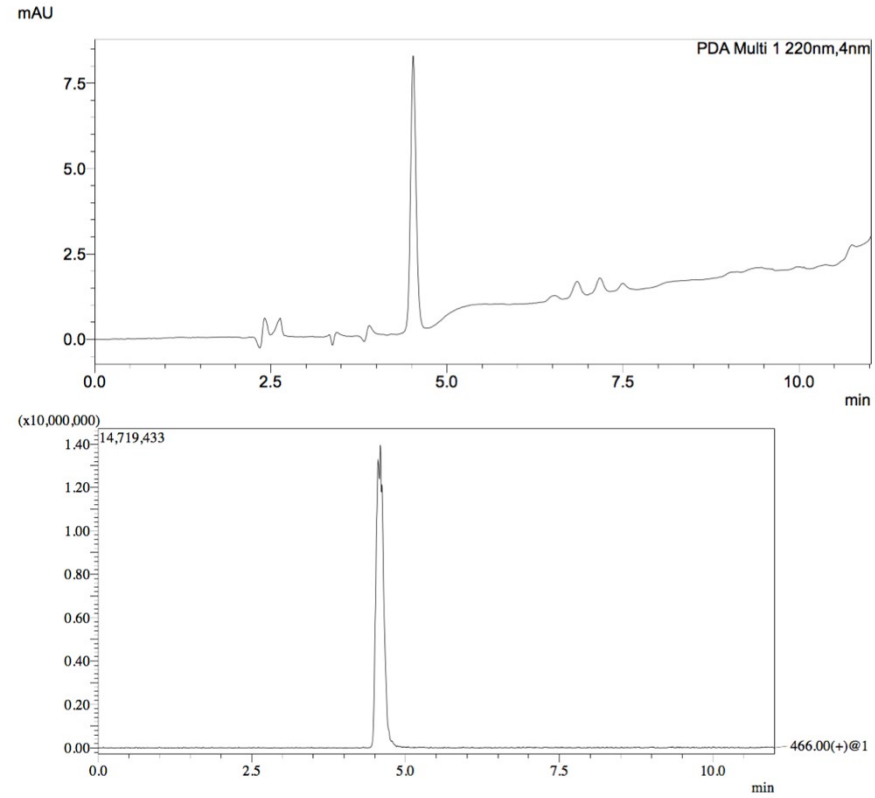
Ac-A-(cyclo-2,5)-[S_5IAC]-NH_2 **4**

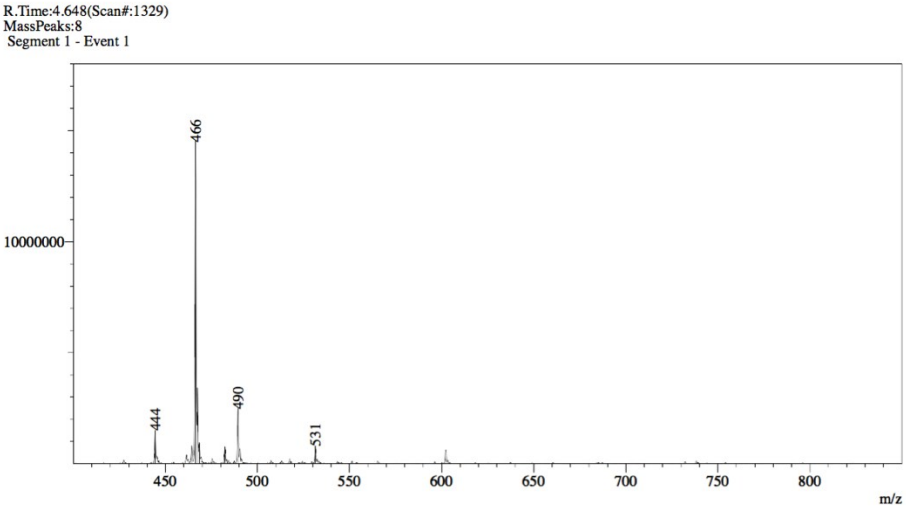




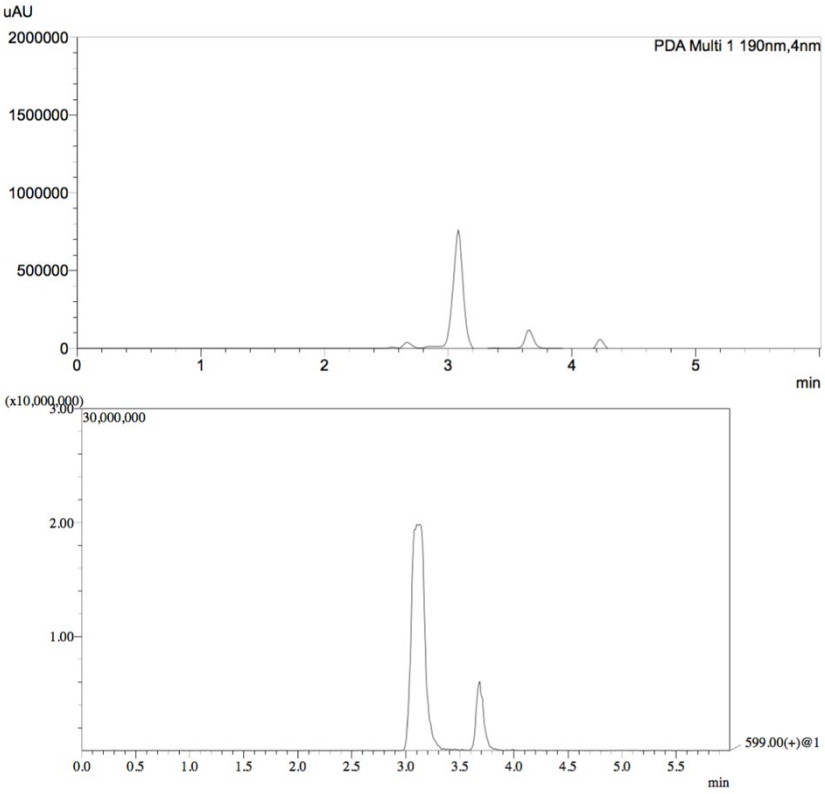


Ac-(cyclo-1,4)-[S_6AAC]-NH_2 **6**





Ac-(cyclo-1,4)-[S_5AAC(NTs)]-NH_2 **7A**



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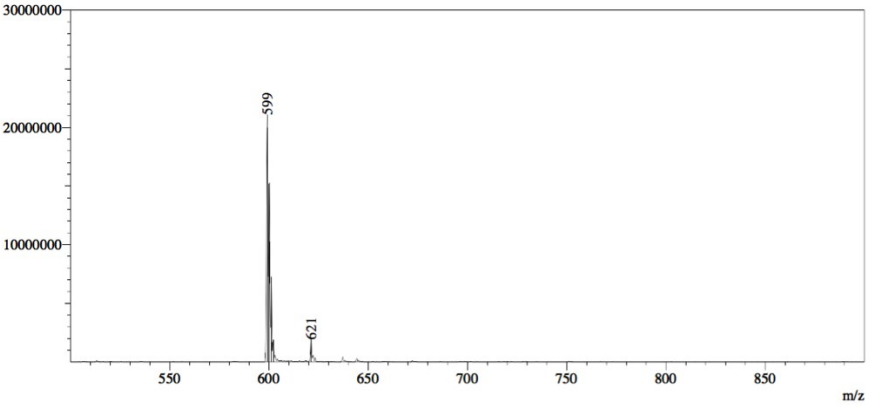
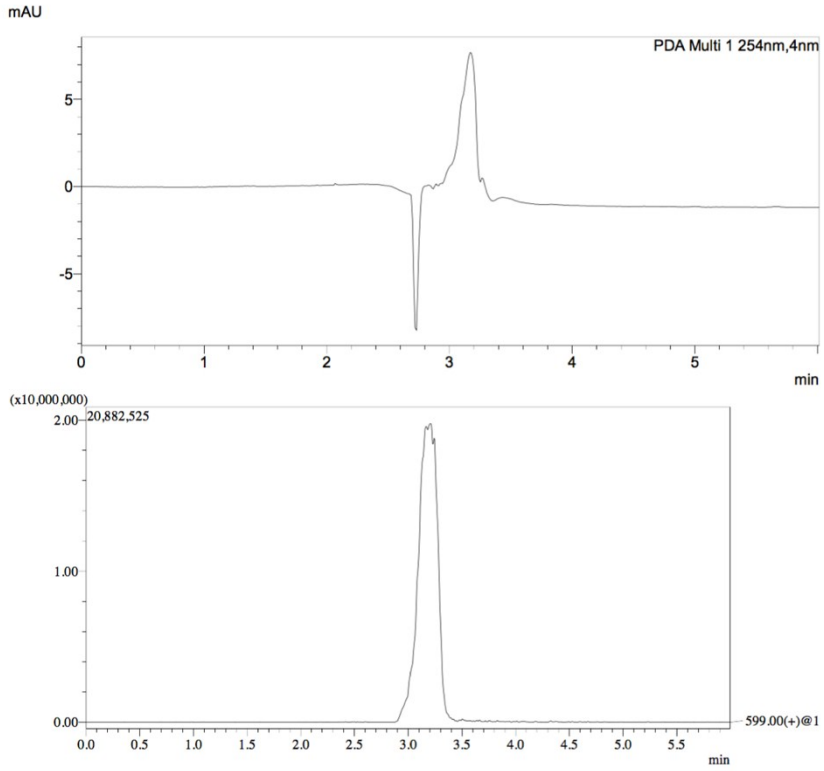
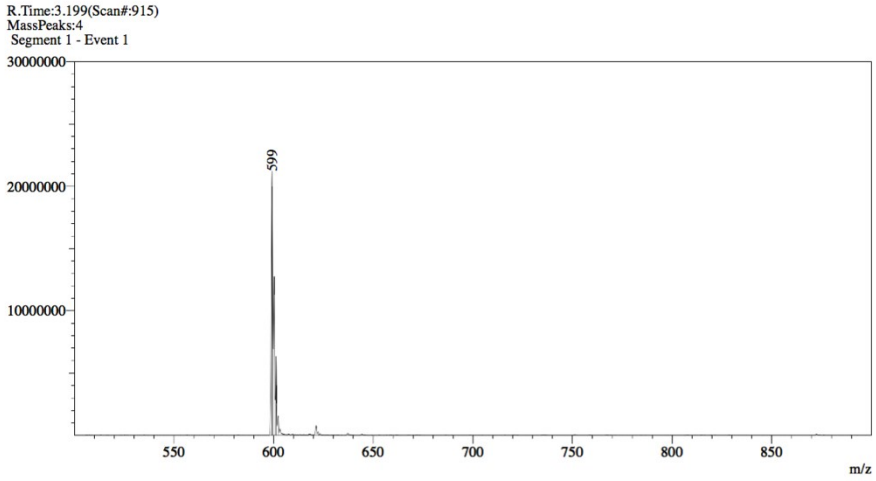
MassPeaks:5
Segment 1 - Event 1

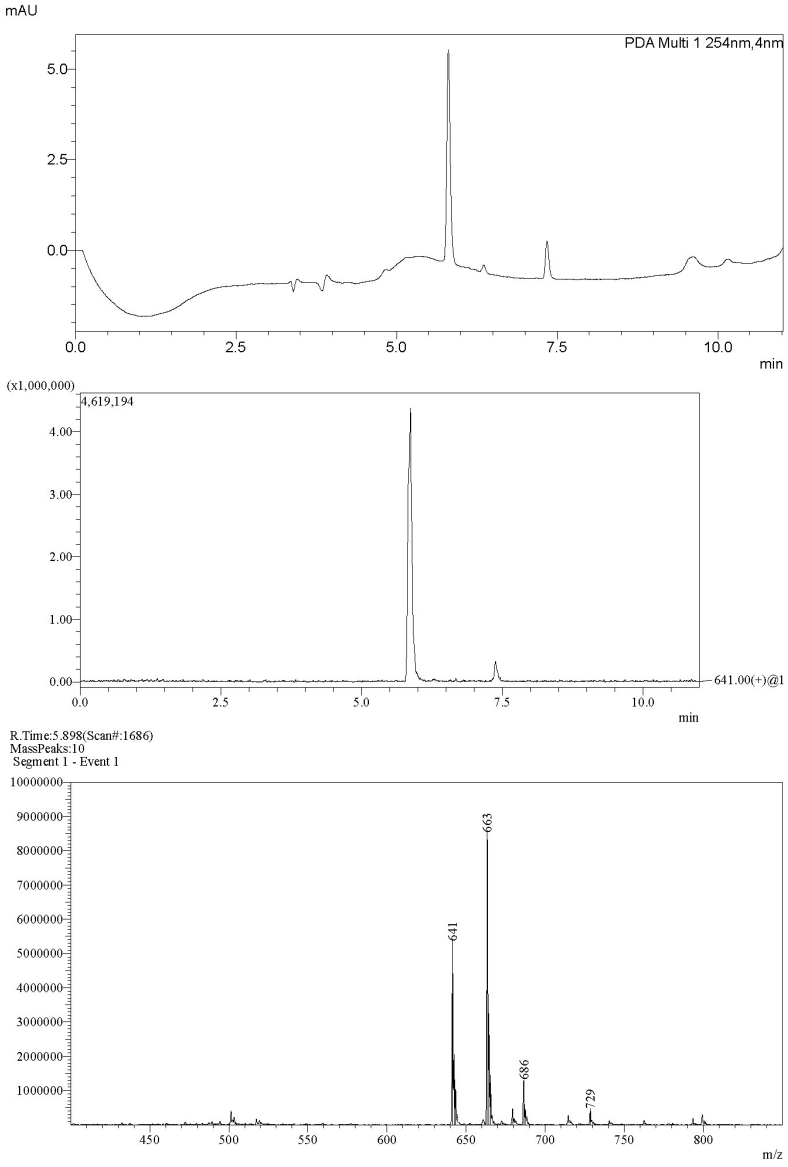


Ac-(cyclo-1,4)-[S_5AAC(NTs)]-NH_2 **7B**

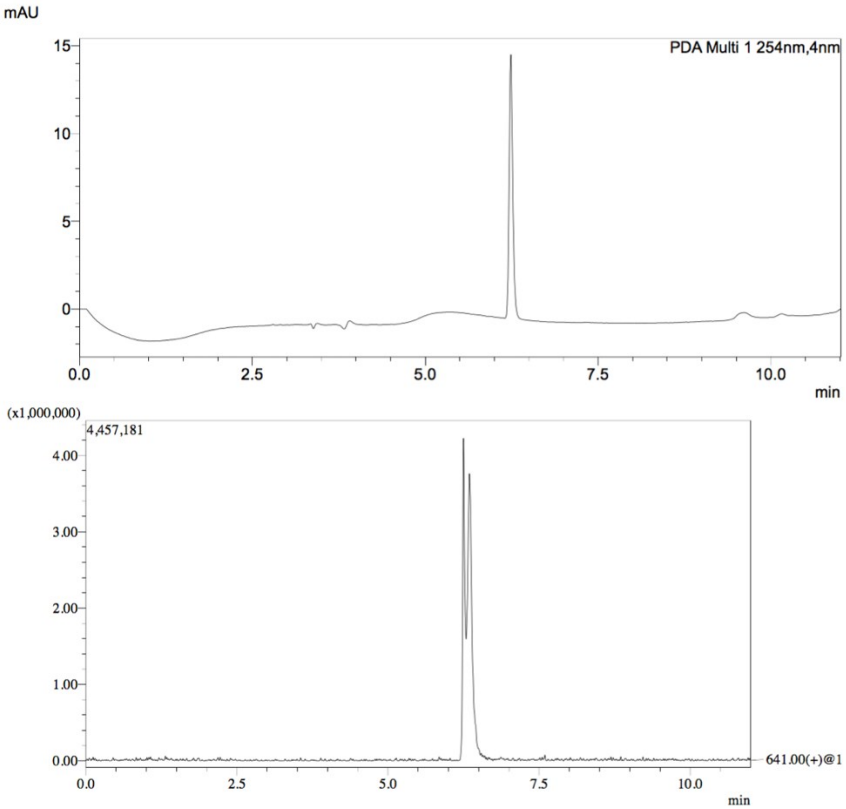


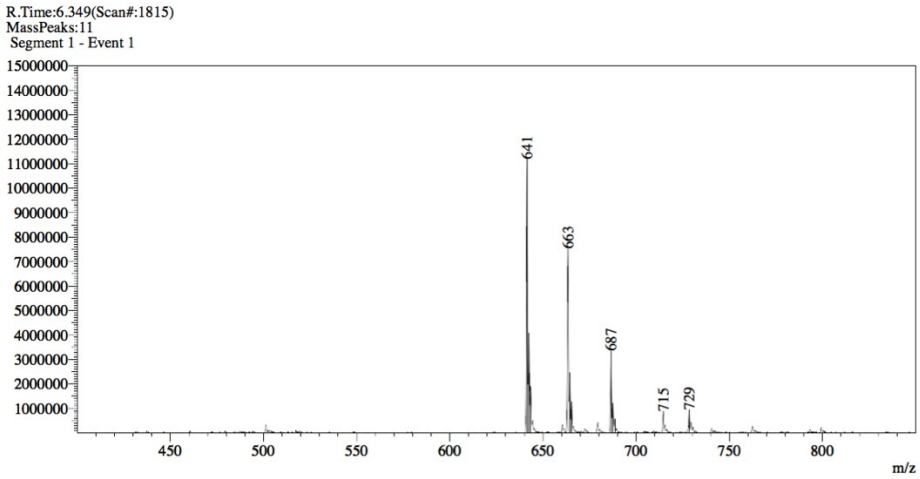


Ac-(cyclo-1,4)-[S_5IAC(NTs)]-NH_2 **8A**

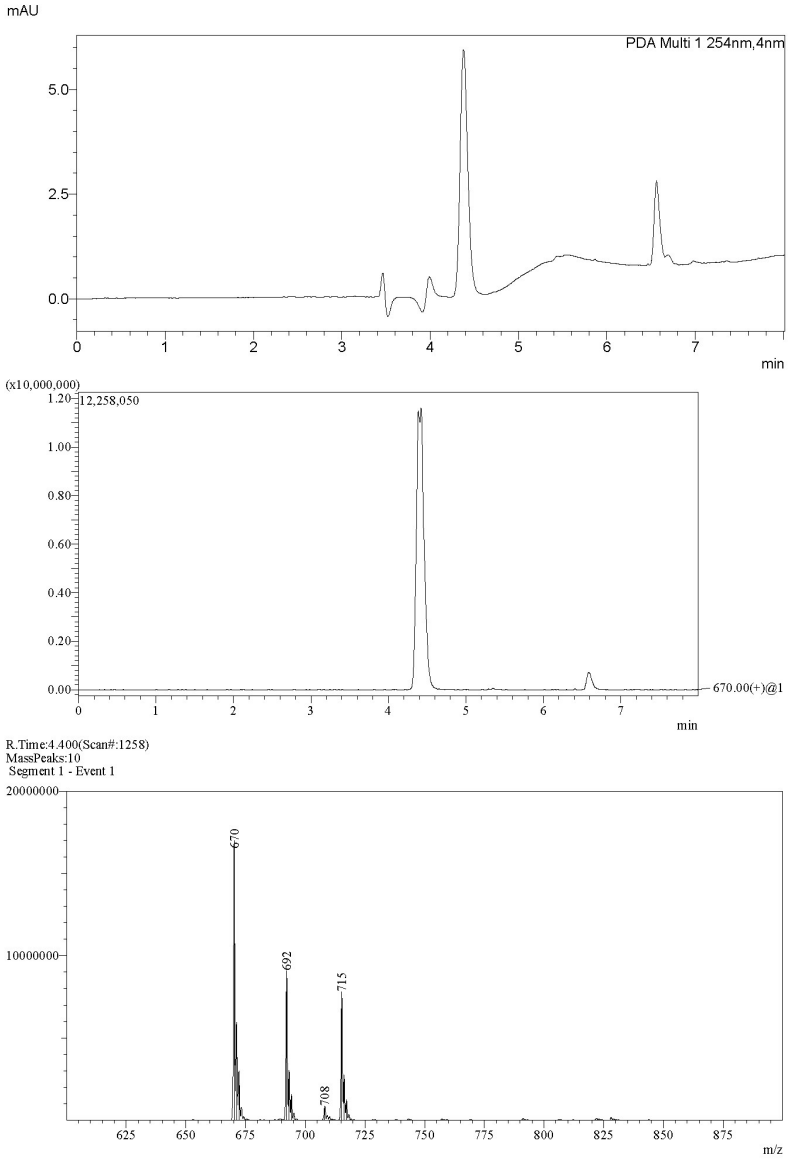


Ac-(cyclo-1,4)-[S_5IAC(NTs)]-NH_2 **8B**

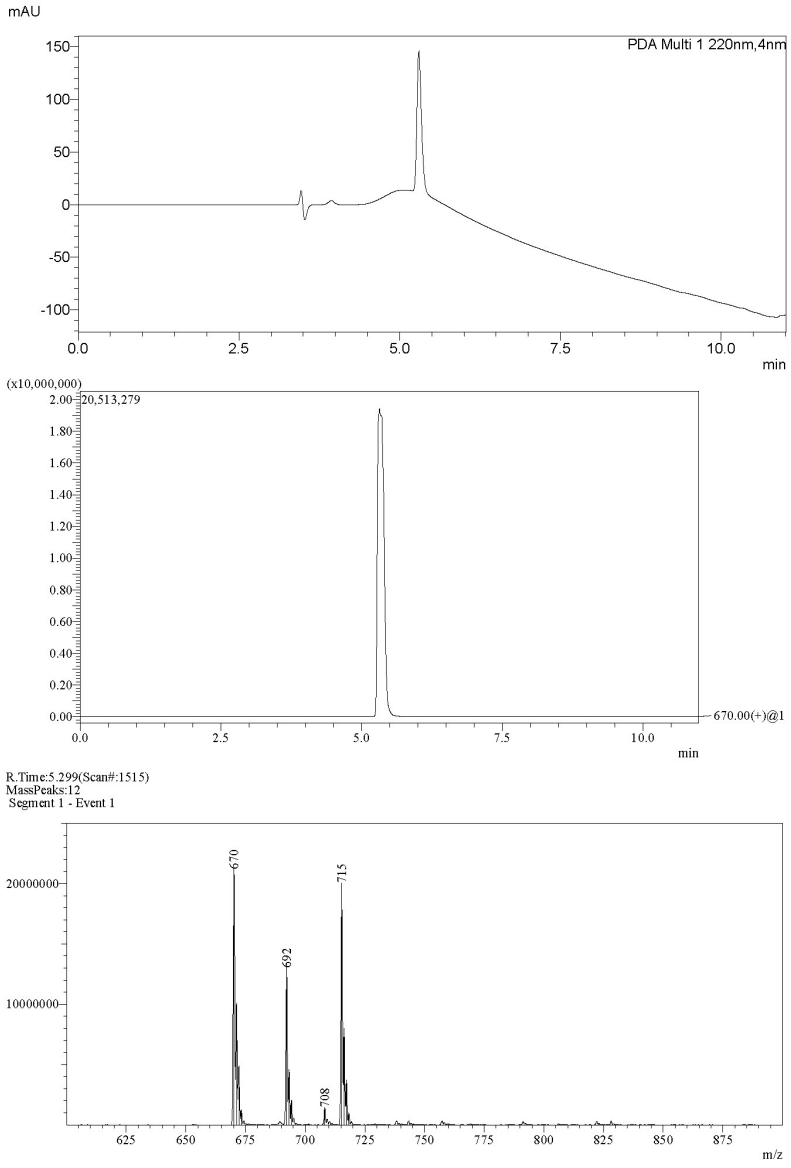




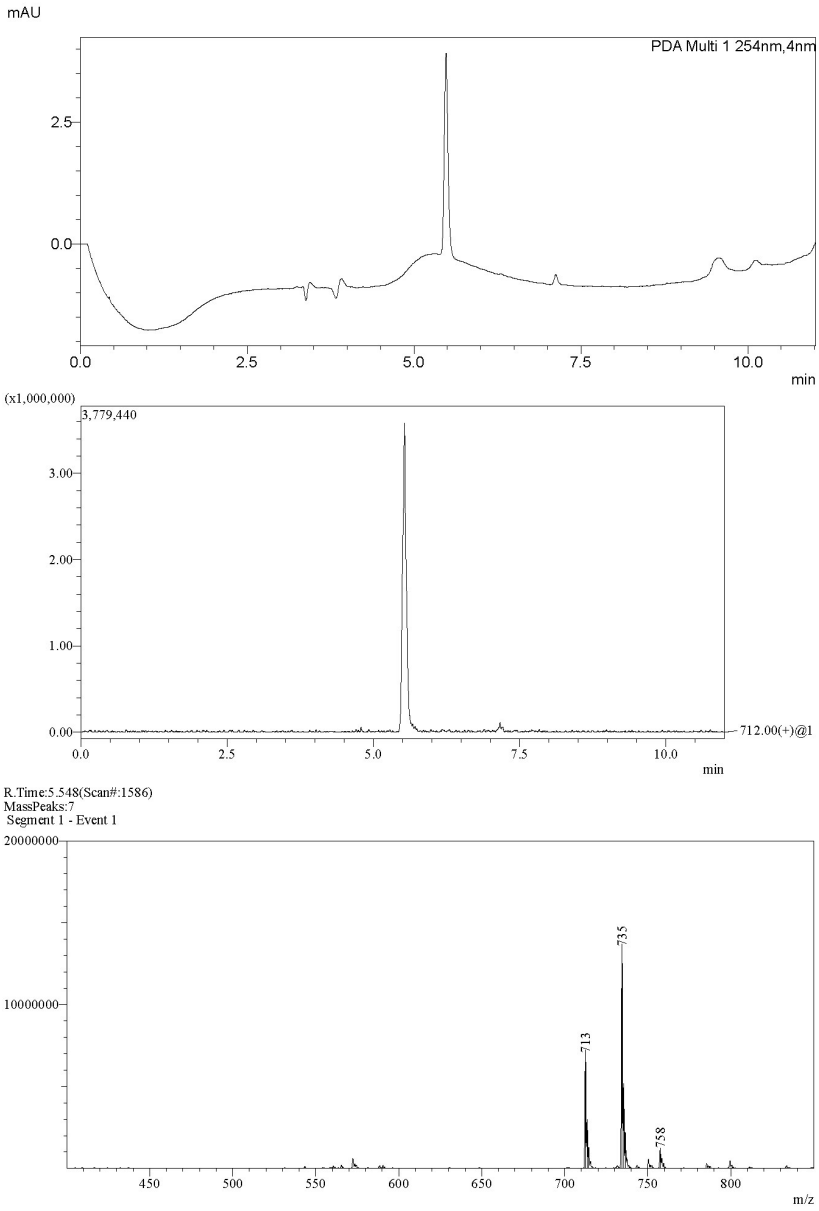
Ac-A-(cyclo-2,5)-[S_5AAC(NTs)]-NH_2 **9A**



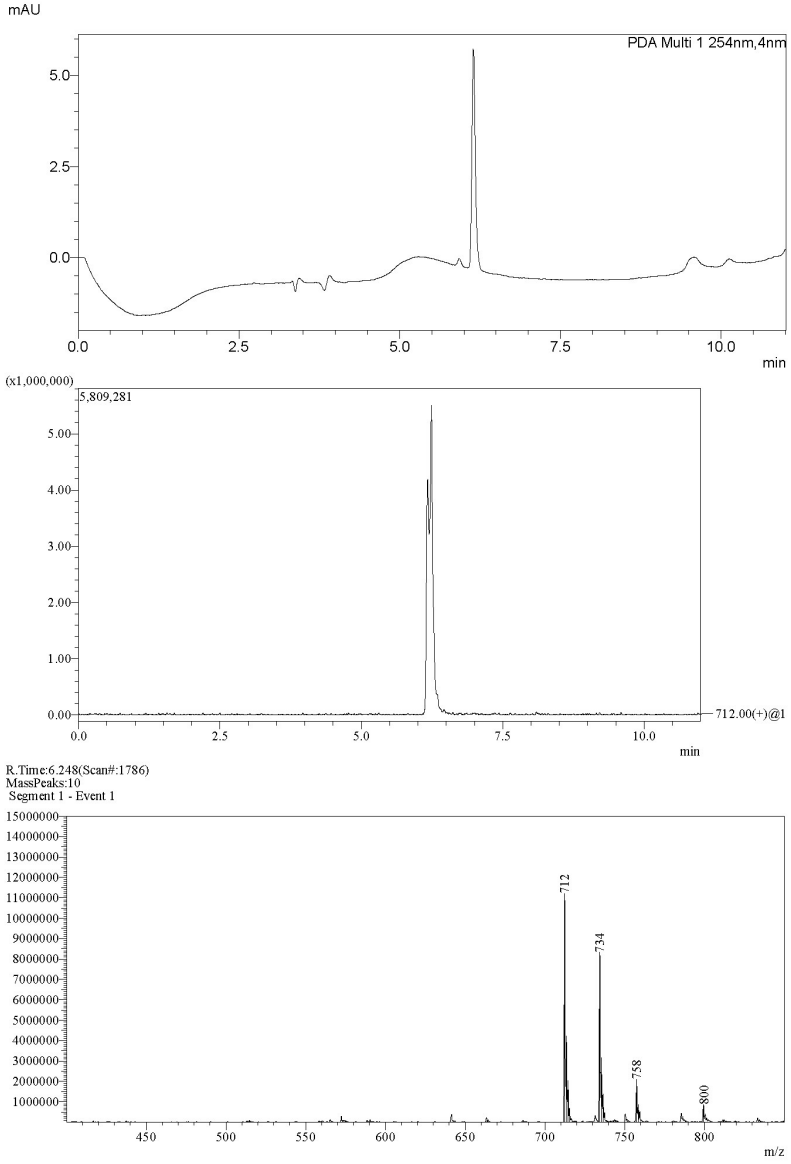
Ac-A-(cyclo-2,5)-[S_5AAC(NTs)]-NH_2 **9B**



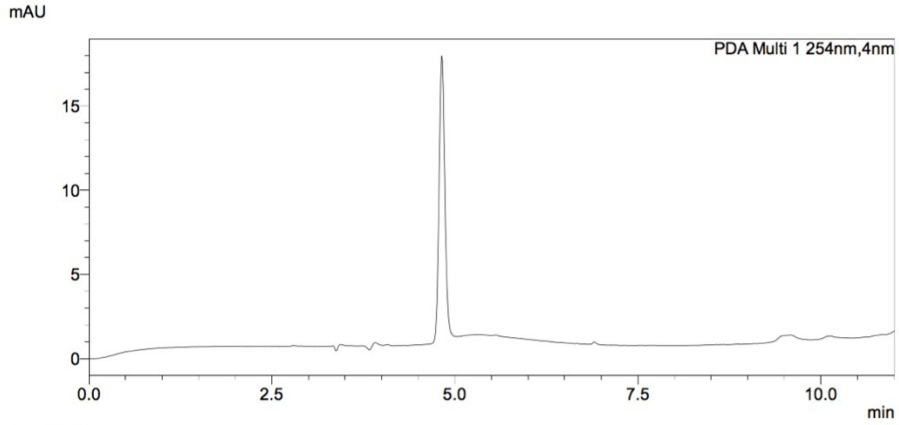
Ac-A-(cyclo-2,5)-[S_5IAC(NTs)]-NH_2 **10A**

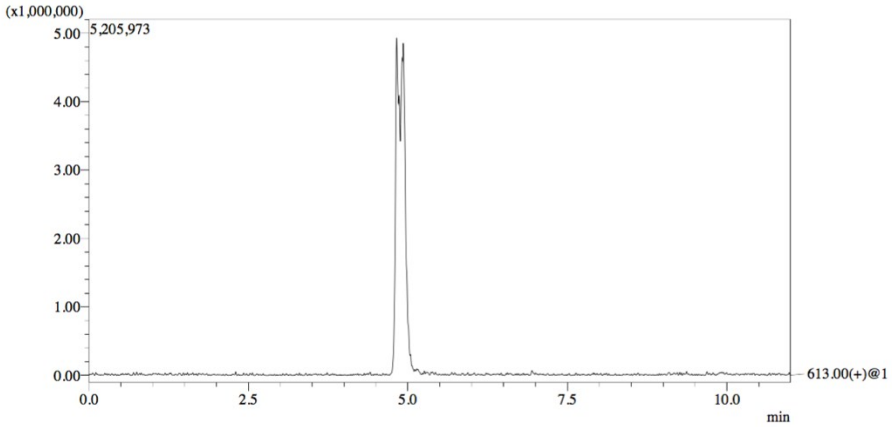


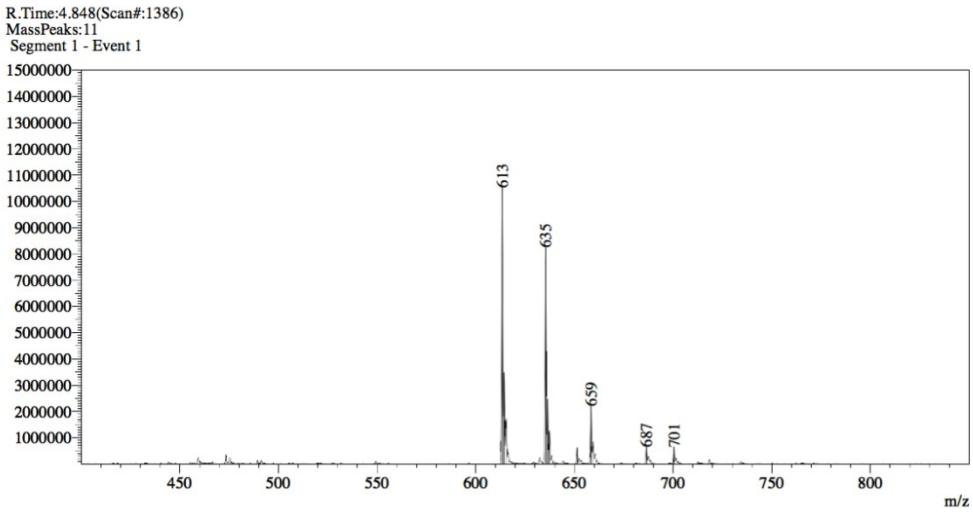
Ac-A-(cyclo-2,5)-[S_5IAC(NTs)]-NH_2 **10B**



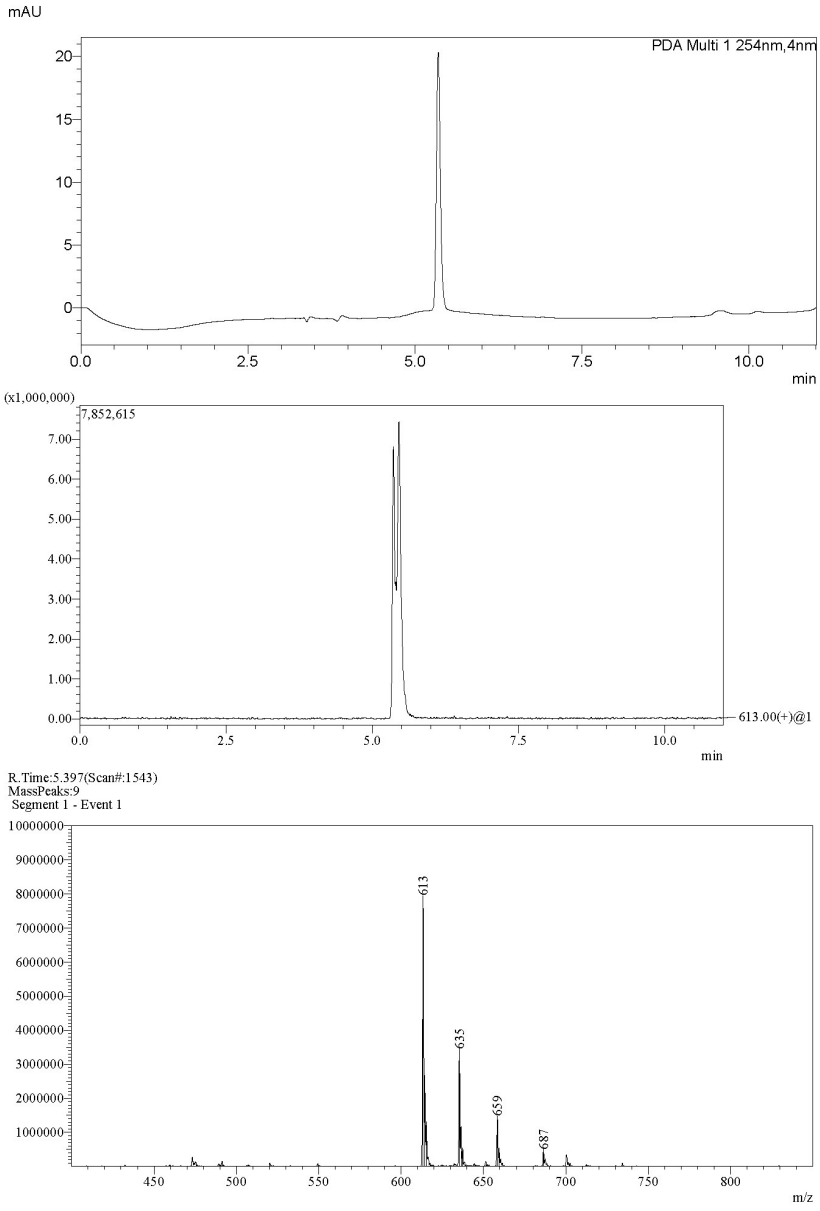
Ac-(cyclo-1,4)-[S_6AAC(NTs)]-NH_2 **12A**



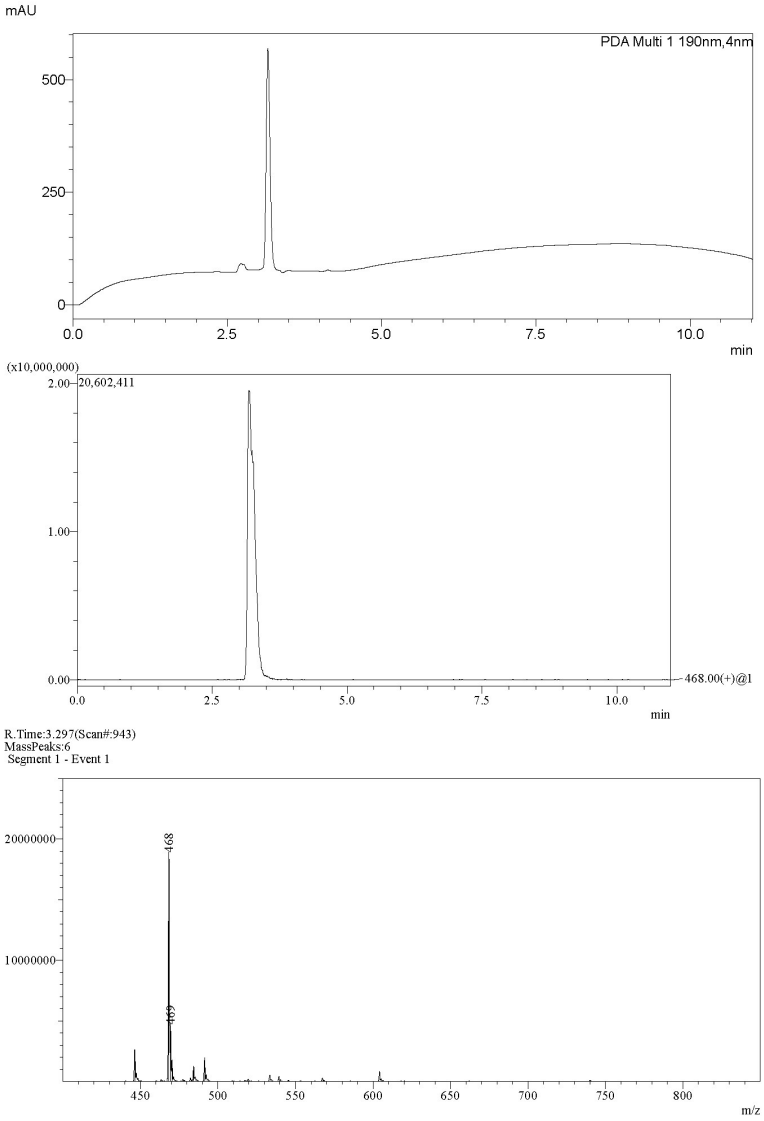




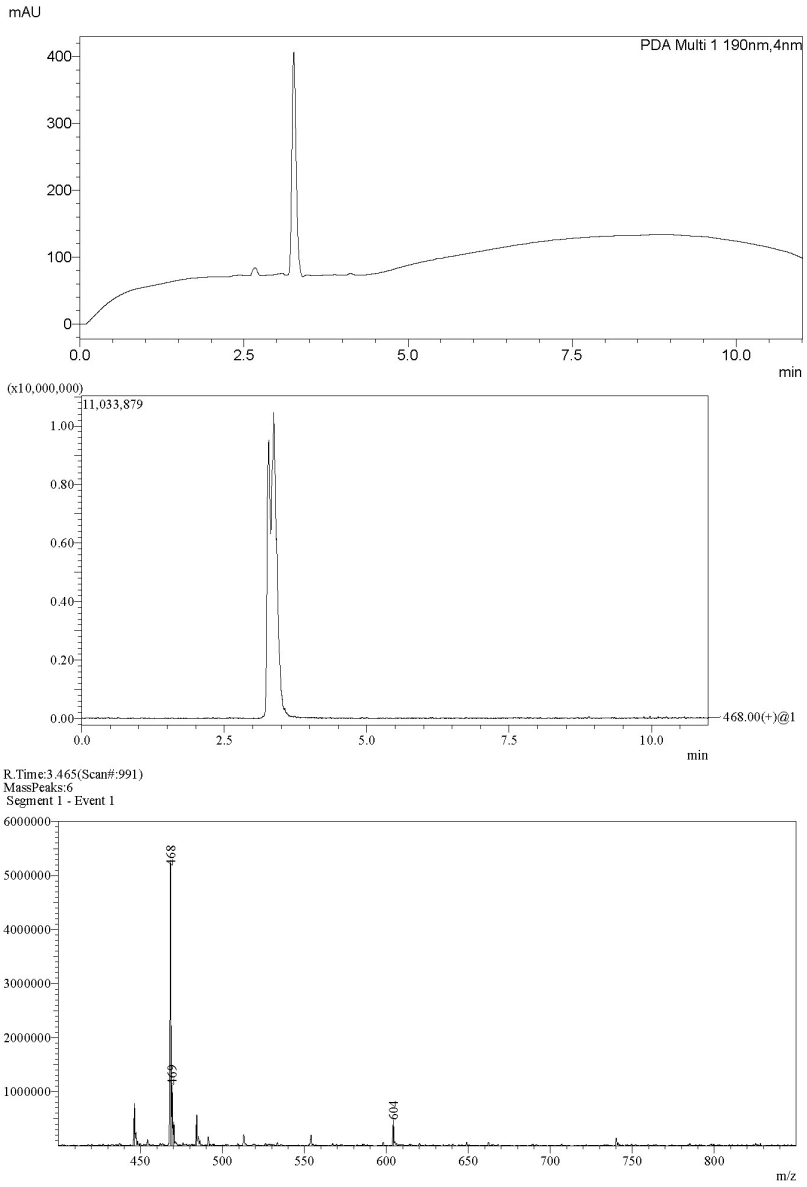
Ac-(cyclo-1,4)-[S_6AAC(NTs)]-NH_2 **12B**



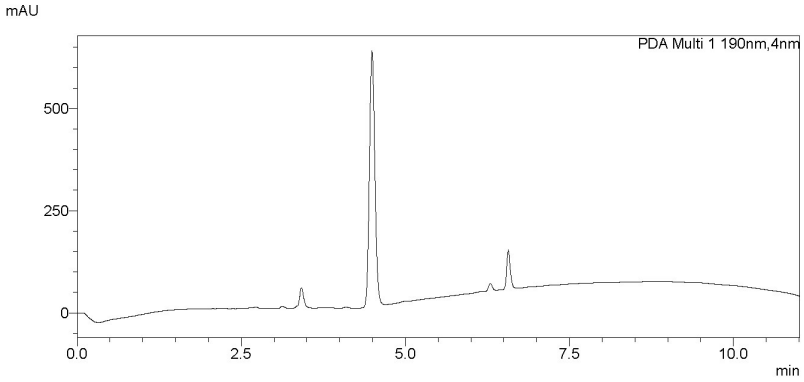
Ac-(cyclo-1,4)-[S_5AAC(O)]-NH_2 **13A**

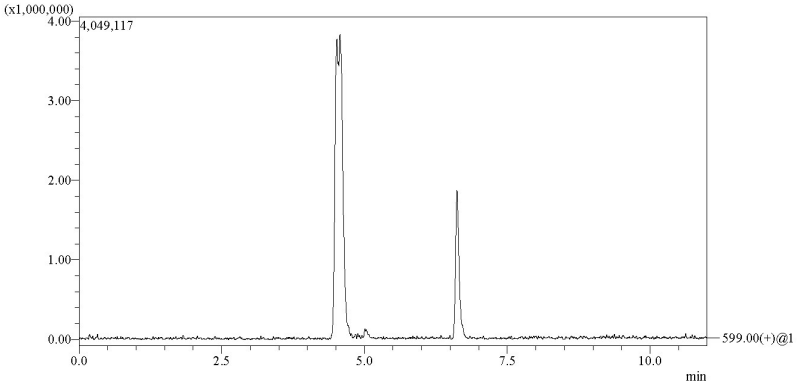


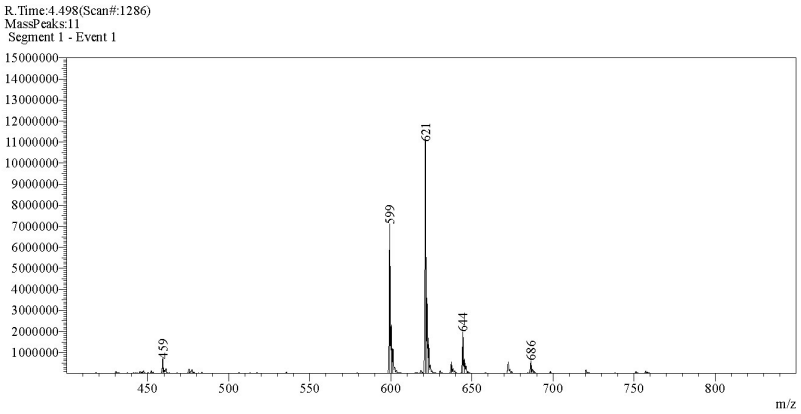
Ac-(cyclo-1,4)-[S_5AAC(O)]-NH_2 **13B**



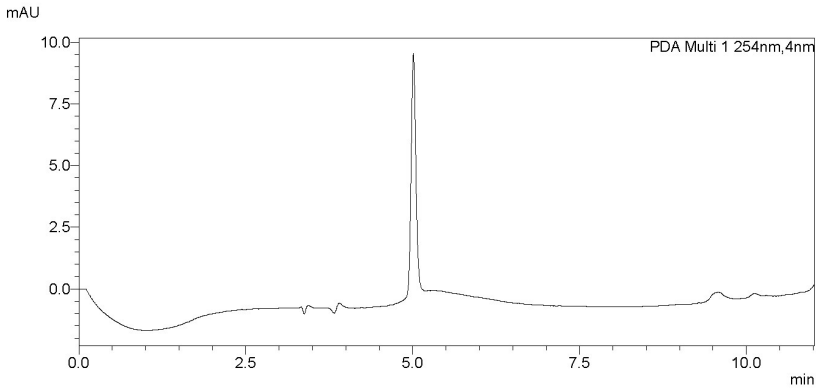
D-Ac-(cyclo-1,4)-[S_5AAC(NTs)]-NH_2 **14A**

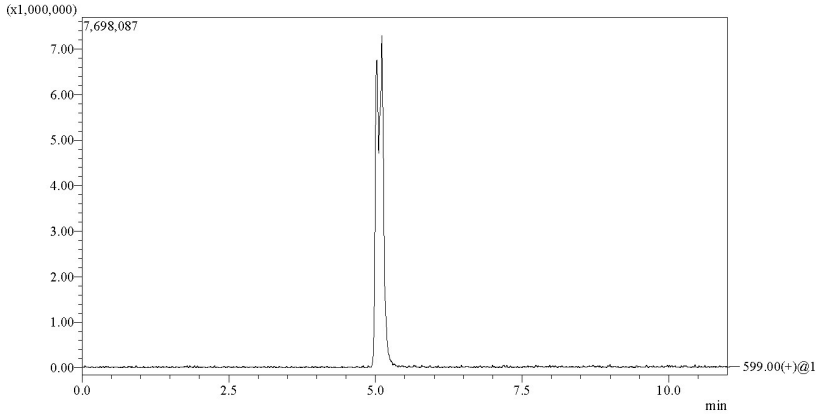






D-Ac-(cyclo-1,4)-[S_5AAC(NTs)]-NH_2 **14B**





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MassPeaks:9
Segment 1 - Event 1