Supporting Information

Facile Chemoselective Modification of Thio-Ethers Generates Chiral Center-Induced Helical Peptides

Zhanfeng Hou, Chengjie Sun, Hao Geng, Kuan Hu, Mingsheng Xie, Yue Ma, Fan Jiang,* Feng Yin* and Zigang Li*

E-mails: jiangfan@pku.edu.cn; yinfeng@pkusz.edu.cn; lizg@pkusz.edu.cn

State Key Laboratory of Chemical Oncogenomics, School of Chemical Biology and Biotechnology, Peking University Shenzhen Graduate School, Shenzhen, 518055 (China).

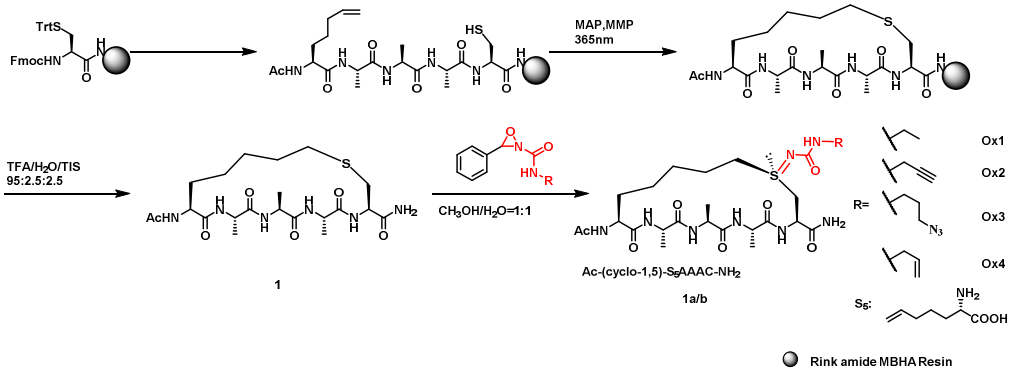
Reagents and materials

For peptides synthesis, all amino acids and resins were purchased from GL Biochem (Shanghai). N-methylpyrolidone (NMP), dichloromethane (DCM), dimethylformamide (DMF), diisopropylethylamine (DIPEA), trifluoroacetic acid (TFA), triisopropylsilane (TIS), Morpholine 1- (4-Methoxyphenyl) ethenone (MAP) 2-hydroxy-1-(4- (2-hydroxyethoxy) phenyl) -2-methyl-1-propanon (MMP) were purchased from Energy Chemical.

NMRs were measured on nuclear magnetic resonance (NMR) spectroscopy (Bruker AVANCE-III 300, 400 and 500). The reverse phase high performance liquid chromatography (HPLC) was performed on Shimadzu prominence LC-20AT instrument equipped with C18 column (Kromasil 100-5-C18 4.6×250mm) and acetonitrile/water (0.1%TFA) as the eluent condition. HPLC fractions containing product (screened by ESI) were combined and lyophilized. Molecular weights were measured on SHIMAZU-SPD2020; CD spectra were measured on Chirascan Circular Dichroism Spectrometer.

Solid phase peptide synthesis

The synthesis of cyclic pentapeptide sulfimide Ac- (cyclo-1,5) - [S_5AAAC] -NH_2 was showed as an example, and other peptide sulfimides were synthesized in the similar route. All peptides were prepared by standard Fmoc solid-phase synthesis on Rink amide MBHA resin.

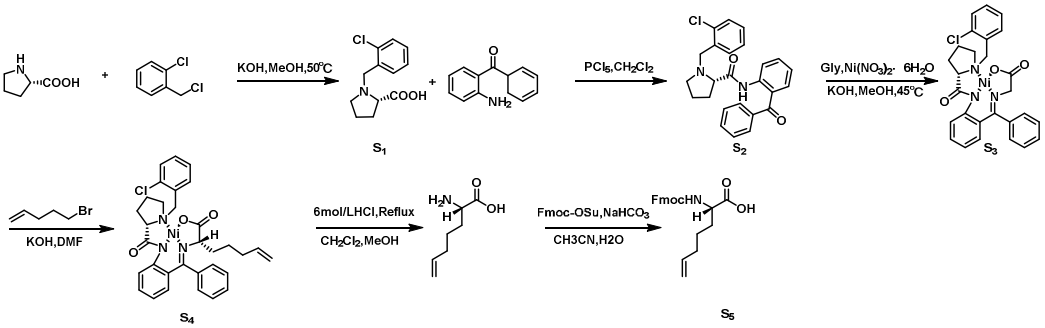


Scheme 1 The synthetic route of cyclic peptide sulfimide.

Preparation of cyclic pentapeptide sulfilimides

Cyclic peptide sulfimide (1 eq.) and oxaziridine (1 eq.) were dissolved in CH3OH: H2O=1:1 (0.5 ml), and the reaction was stirred at room temperature for 5min. Then the solution was removed under vacuum and H2O/CH3CN were added to dissolve the crude for HPLC purification.

Synthesis of unnatural amino acids



Scheme 2 Flow chart for unnatural amino acids synthesis.[1]

Circular dichroism spectroscopy[2]

All peptide samples were dissolved in deionized H_2O for CD measurements. CD scans were performed at wavelength from 190 nm to 250 nm with the 0.1 cm path 4 length for twice. Every sample was scanned twice,

and the final CD spectrum was averaged and smoothed. The α-helical content of each peptide was calculated as reported previously.[3] Variable temperature CD scans for peptide **6**, **7-Ac-b**,**8-Ac-b** and **9-Ac-b** were collected from 20°C to 70°C at 10°C intervals.

Guanidine hydrochloride denaturation experiment

Cyclic peptide sulfimide **7-Ac-b** (~1 mg) and precursor peptide **6** were added into varied concentration of guanidine hydrochloride (0.5 ml) 0, 1.0, 2.0, 4.0,8.0 M. The helicity was monitored by molar ellipticity at 215 nm.

NMR spectroscopy

NMR data were recorded on a Bruker AVANCE III 300, 400 or 500 MHz spectrometer. For this experiment, the peptides were dissolved in 9:1 H_2O: D_2O. 2D NMR data were collected on a Bruker Avance III 500MHz spectrometer with a TXI probe. Watergate pulse sequence with gradients were used for water suppression in 1D and 2D ^{1}H spectrum. 2D ^{1}H-^{1}H TOCSY and NOESY spectra were acquired with mixing time of 100ms and 300ms, respectively. The TOCSY and NOESY spectra were acquired with a width of 10ppm and ^{13}C spectra width of 100ppm, and size of 1024×400 complex points. All the 2D NMR spectra were processed by Topspin® to final 2048×1024 complex points and analyzed by MestreNova software. $^{3}J_{(NH\text{-}H\alpha)}$ couplings were measured from 1D-1H spectrum.

Fluorescence polarization

ER-α peptide Fluorescence polarization experiments were performed in 96-well plates (Perkin Elmer Optiplate-96F) on plate reader (Perkin Elmer, Envision, 2104 multilabel reader). Concentrations of the peptides were determined by 495 nm absorption of FITC. Purified ER-LBD (at increasing concentrations, 20μL) and fluorescein-labeled peptides (10 nM, 80L) inassay buffer (10 μM 17-β-estradiol, 20 mM Tris-HCl pH 8.0, 25 mM NaCl, 10% glycerol, 10 μMbeta-estradiol and 1 mM TCEP) were mixed. The fluorescence polarization of the labeled peptides was measured at 20°C with excitation at 485nm and emission at 520 nm and then plotted against the concentrations of the ER-LBD. The data points were fitted by GraphPad Prism6.

Flow cytometry

Hela cells were grown in DMEM medium with 10% FBS (v/v) in imaging dishes (50000cells/well) in 37°C, 5% CO_2 incubator for two days (50,000 cells per well). Cells were treated with fluoresceinated peptides (5 μM) for up to 2 hours at 37°C. After washing with media, the cells were exposed to trypsin (0.25%; Gibco) digestion (5 min, 37°C), washed with PBS, and resuspended in PBS. Cellular fluorescence was analyzed using a BD FACSCalibur flow cytometer (Becton Dickinson) and CFlow plus.

Molecular cloning, protein expression and purification

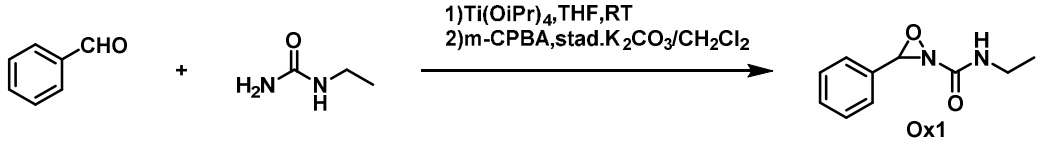
Cloning, Expression and Purification of ER-α LBD4. Human ER-α LBD 301-553 was cloned into pET23b via NdeI and XhoI generating untagged constructs. Expression was carried out in *E. coli*BL21 (DE3) without IPTG induce. Cultures were grown in 2YT medium at 37°C to OD_{600} of 0.8 and then transferred to 20°C for 18h. Cells were harvested by centrifugation and flush frozen. Harvested cells were lysed via sonication in 100mM lysis buffer (Tris-Cl pH 8.1, 300 mM KCl, 5mM EDTA, 4Mm DTT and 1 mmol/L PMSF). Cell debris were removed by centrifugation and the supernatant ran over a 1mL estradiol affinity column (PDI technology) and the column was then eluted with elution buffer (100 μM estradiol, 20mM Tris pH 8.1 and 0.25M NaSCN). High molecular weight species and excess salts were removed on a Superdex 200 column equilibrated in buffer of 50 mM Tris pH 7.4, 150 mM NaCl, 10% glycerol and 1mM DTT.

Primer sequence:

ER-alpha-NdeI-301: GTGTACACATATGtctaagaagaacagcctggccttgt

ER-alpha-NdeI-553: Ccctcgagttaagtgggcgcatgtaggcggt

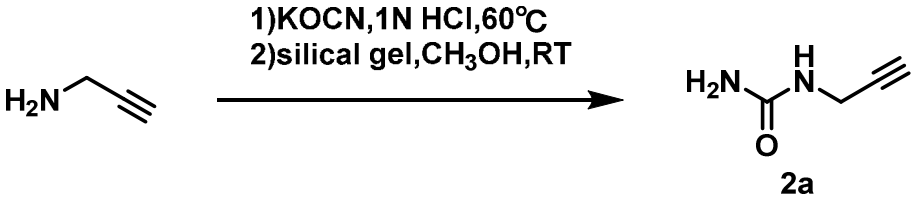
Chemical synthesis of oxaziridine probes[4]



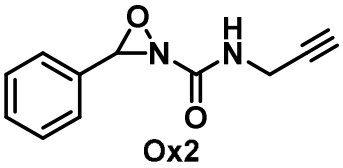
Ox1

N-ethyl-3-phenyl-1,2-oxaziridine-2-carboxamide (Ox1) To a solution of benzaldehyde (0.69 mL, 6.73 mmol) and urea (500 mg, 5.61 mmol) in THF (20 mL) was added Ti $(OiPr)_4$ (2.0 mL, 6.73 mmol) at room temperature. After stirring overnight, the mixture was concentrated under vacuum to afford a residue.

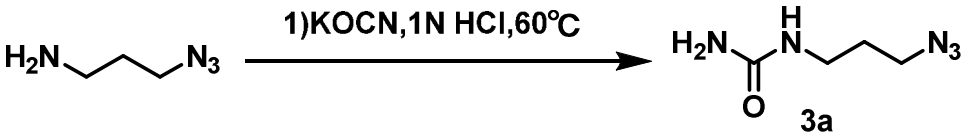
To a mixture solution of satd. K_2CO_3 (30 mL) and DCM (30 mL) was added 3-Chlorobenzene-1-carboperoxoic acid (mCPBA, 3.4 g, 85% purity, 16.84 mmol) at room temperature. After stirring for 10min, a solution of the above residue in DCM (30 mL) was added slowly into the mixture at room temperature. After stirring for overnight, water (100 mL) was added and the mixture was extracted with DCM for three time. The combined organic layer was then wash with brine, dried over $NaSO_4$, filtered and concentrated under vacuum to give a residue which was purified by column chromatography (DCM/Et_2O, 100:1) to afford the Ox1 as a white solid (647mg, 60%). ^{1}H NMR (500MHz, $CDCl_3$) δ7.48-7.39 (m, 5H),6.05 (br,1H),5.00 (s,1H),3.36-3.31 (m,2H),1.21(t,3H).^{13}C NMR (126MHz, $CDCl_3$) δ162.28, 132.64, 131.11, 128.76, 128.10, 79.50, 35.23, 15.08.



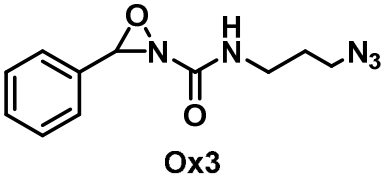
N-2-Propyn-1-ylurea (2a) To a solution of propargylamine (1.6 mL, 25 mmol) in aqueous HCl soulution (1.0 N, 25 mL) was added KOCN (8 g, 100 mmol) at room temperature. After stirring overnight at 60 °C, the mixture was cooled to 0°C to give a white precipitate. After filtration, the solid was dissolved in CH3OH (75mL) and stirred with silica gel (12.5g) for 6h. The mixture was then filtered and concentrated under vacuum to give the desired urea **2a** as a white solid (1.5g, 61%) without further purification. ^{1}H NMR (500MHz, DMSO) δ6.24 (brs, 1H), 5.56 (br, 2H), 3.75 (dd, J=2.5Hz, 2H), 3.02 (t, J=2.5Hz, 1H). ^{13}C NMR (126MHz, MeOD) δ161.53, 81.14, 71.21, 30.22.



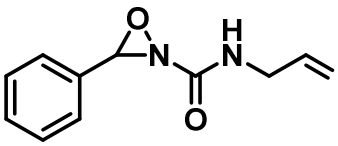
3-phenyl-N-(prop-2-yn-1-yl)-1,2-oxaziridine-2-carboxamide (Ox2) was synthesized with the same procedure using **2a** as substrate in 53% yield. ^{1}H NMR (300MHz, $CDCl_3$) δ7.47-7.41 (m,5H), 6.36 (br,1H), 5.03 (s,1H), 4.09-4.06 (m,2H), 2.30 (t, J=2.7Hz,1H). ^{13}CNMR (75MHz, $CDCl_3$) δ162.04, 132.13, 131.29, 128.80, 128.12, 79.63, 72.74, 30.34.



N-(3-Azidopropyl)urea (3a) To a solution of 3-Azido-1-propanamine (700mg,7mmol) in 1 N HCl (10mL) was added KOCN (2.27g, 28mmol). After stirring for 15h at 60°C, the mixture was cooled to room temperature. The reaction mixture was then concentrated under reduced pressure, and give the desired 3a (910mg, 91% yield) crystallized from the remaining solution. ^{1}H NMR (500MHz, DMSO) δ5.99 (br,1H), 5.40 (br,2H), 3.34 (q,2H), 3.01 (q,2H), 1.64-1.58 (m,2H). ^{13}C NMR (126MHz, DMSO) δ168.08, 57.94, 45.98, 38.68.



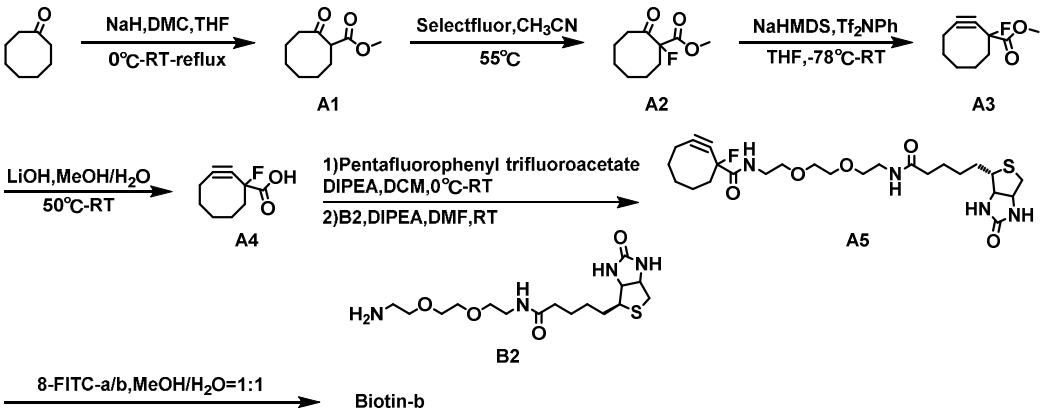
N-(3-azidopropyl)-3-phenyl-1,2-oxaziridine-2-carboxamide (Ox3) was synthesized with the same procedure using **3a** as substrate in 48% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ7.48-7.42 (m,5H), 6.25 (br,1H), 5.01 (s,1H), 3.44-3.40 (m,4H), 1.87-1.84 (m,2H). ^{13}C NMR (101MHz, $CDCl_3$) δ162.55, 132.38, 131.22, 128.80, 128.11, 79.63, 49.31, 38.25, 28.77.



Ox4

N-allyl-3-phenyl-1,2-oxaziridine-2-carboxamide (Ox4) was synthesized with the same procedure using **N-2-Propen-1-ylurea** as substrate in 56% yield. ^{1}H NMR (400MHz, $CDCl_3$) δ7.49-7.39 (m,5H), 6.15 (br,1H), 5.89-5.83 (m,1H), 5.28-5.19 (m,2H), 5.03 (s,1H), 3.93-3.89 (m,2H). ^{13}C NMR (101MHz, $CDCl_3$) δ162.17, 133.01, 132.25, 130.81, 128.43, 127.80, 116.79, 79.12, 42.47.

Chemical synthesis of compound Biotin-a/b[5, 6]



Methyl-2-oxocyclooctane-1-carboxylate (A1) To a solution of dimethyl carbonate (0.67 mL, 7.92 mmol) in dry THF (13 mL) was added sodium hydride (443.7 mg, 11.09 mmol) by portion at 5-10°C. The resulting mixture was stirred at this temperature for 30 min and then was added a solution of cyclooctanone (500 mg, 3.96 mmol) in THF (2 mL) dropwise over 3 min. The resultant mixture was stirred at refluxed for 4 h before cooling to room temperature. The reaction mixture was poured into saturated $NaHCO_3$ solution (5 mL) and ice (10 g), and then the mixture was extracted with PE/EA (4:1, 10 mL x 2). The organic layer was washed with brine (20 mL), dried over Na_2SO_4 and concentrated to give crude product. Then the target product (700 mg, 95%) was purified by column chromatography (PE/EA=20:1).

Methyl 1-fluoro-2-oxocyclooctane-1-carboxylate (A2) To a stirred solution of methyl-2-oxocyclooctane-1-carboxylate (500 mg, 2.71 mmol) in dry acetonitrile cooled to 0°C was added Selectfluor (1.15 g, 3.26 mmol). The resulting mixture was then heated in a 55°C for 8 h. After cooling to room temperature, the reaction was quenched with water (6.5 mL) and extracted with ethyl acetate. The combined organic layer was dried (anhydrous Na_2SO_4), filtered and concentrated in vacuo yield clear oil which was then dissolved in DCM and filtered through a plug of silica gel to give **A2** as white solid (490 mg, 89%).

Methyl 1-fluorocyclooct-2-yne-1-carboxylate (A3) A solution of NaHMDS (2.0 M in THF, 1.11 mL, 2.22 mmol) was added dropwise to a stirred solution of **A2** (200 mg, 0.99 mmol) in THF (13 mL) at -78°C. After the addition was complete the reaction mixture was maintained for 30 min and then Tf_2NPh (388.6 mg, 1.09 mmol) in THF (2.5 mL) was added slowly via syringe. The reaction was stirred at -78°C for one hour, and then allowed to warm to room temperature and stirred an additional five hour. Methanol was then added and reaction mixture was concentrated under vacuum. The crude residue was purified by flash column chromatography on silica gel using PE/EA=20:1 to afford **A3** as pale yellow liquid (120 mg, 70%).

1-fluorocyclooct-2-yne-1-carboxylic acid (A4) LiOH (132 mg, 3.15 mmol) and A3 (290 mg, 1.57 mmol) were combined in 5 mL of 50% aqueous MeOH. This mixture was heated in a 50°C for 20 min. After this time the reaction was allowed to cool to room temperature and stirred an additional 2 h. The reaction mixture was cooled to 0°C, diluted with water, and acidified to pH 2 with dilute aq. HCl solution. The mixture was extracted with ethyl acetate and the combined organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated. The crude product was purified by silica gel flash column chromatography (PE/EA=1:1) to afford **A4** as a yellow oil (190 mg, 71%).

MFCO(A5) Pentafluorophenyl trifluoroacetate (138 mg, 0.49 mmol) and cyclooctyne acid **A4** (70 mg, 0.41

mmol) were combined in dichloromethane (2 mL) at 0°C. Diisopropylethylamine (86 μℓ, 0.49 mmol) was added and the resulting mixture was warmed to room temperature and stirred for 1.5 h. The reaction mixture was evaporated to give crude cyclooctyne-PFP ester which was used directly in the next step.

Diisopropylethylamine (35 μL, 0.20 mmol) was added to a stirred solution of **B2**[7] (80 mg, 0.21 mmol) and cyclooctyne-PFP ester (53 mg, 0.17 mmol) in DMF (2 mL) at 0°C. The reaction was warmed to room temperature and stirred for 2 h. The solvent was then removed under vacuum and the residue purified by HPLC to afford **A5** (74 mg, 83%) as a pale yellow liquid. ^{1}H NMR (400MHz, MeOD) δ4.50 (dd, J=7.4,4.9Hz,1H), 4.31 (dd, J=7.5,4.5Hz,1H), 3.65-3.52 (m, 8H), 3.50-3.40 (m, 2H), 3.37-3.34 (m,2H), 3.25-3.17 (m, 1H), 2.97-2.89 (m, 1H), 2.75-2.66 (m, 2H), 2.32 (dt, J=8.0,5.0Hz, 2H), 2.28-2.17 (m, 3H), 1.86-1.80 (m, 1H), 1.80-1.53 (m, 8H), 1.50-1.39 (m, 3H). ^{13}C NMR (101MHz, MeOD) δ 208.11, 175.87, 166.00, 137.22, 132.78, 110.18 (J=10.1Hz), 95.21(J=187.4Hz), 88.32 (J=31.4Hz), 71.29, 70.62, 70.20, 63.36, 61.62, 56.99, 47.66 (J=25.0Hz), 41.04, 40.30, 36.74, 34.98, 30.13, 29.75, 29.49, 26.83, 21.07.

Biotin-b A5 (0.5mM, 0.1mL) and **8-FITC-βA-b (0.5mM, 0.1mL)** were stirred at room temperature in CH_3OH/H_2O=1:1 for 2h. The reaction mixture purified by HPLC to yield **Biotin-b** as a yellow solid. HRMS (ESI): calculated for $C_{111}H_{162}FN_{29}O_{27}S_3$ $[M+H]^+$, 2448.13, found $[M/2+H]^+$ 1225.65.

Supplementary Figures

Entry	Peptide
1b	Ac-(cyclo-1,5)-S_5AAAC-NH_2
2b	Ac-(cyclo-1,5)-S_5AGAC-NH_2
3b	Ac-(cyclo-1,5)-S_5AQAC-NH_2
4b	Ac-(cyclo-1,5)-S_5AAIC-NH_2
5b	Ac-(cyclo-1,5)-S_5AEAC-NH_2

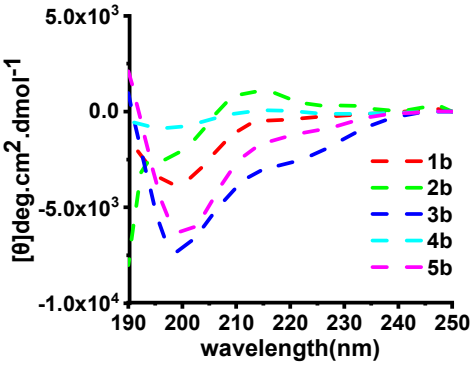


Figure S1. CD spectra of ***S*** epimers of variable sequences **1b-5b** in H_2O at 25°C. Concentrations of samples are about 0.5mM.

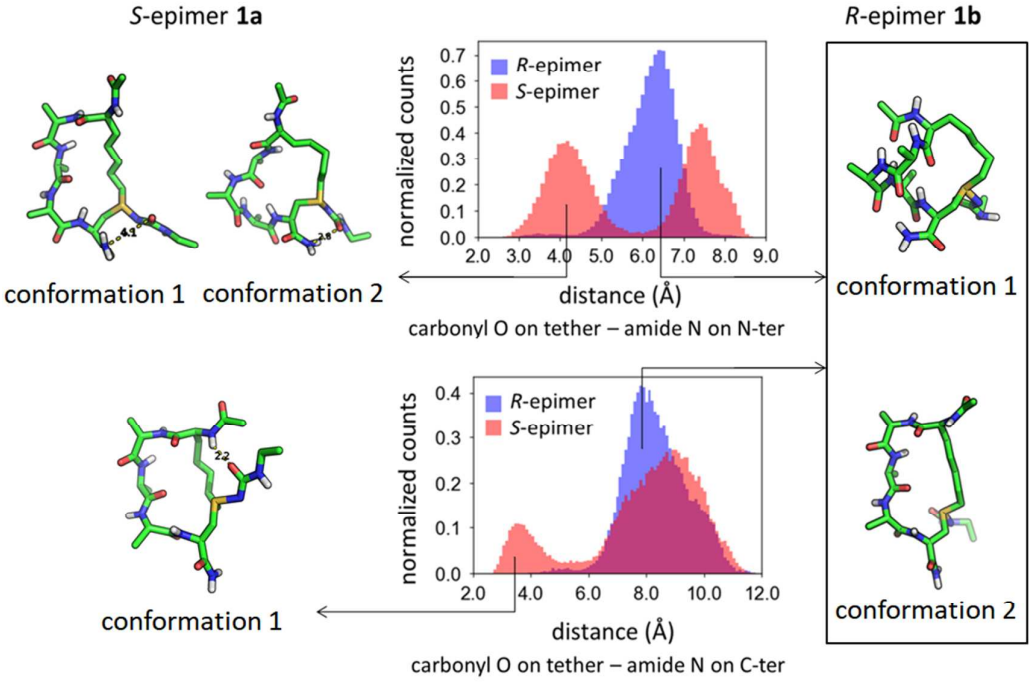


Figure S2 Two histograms in the middle show the distance distributions of the carbonyl oxygen in on-tether substitution group to amide nitrogen on two terminals of two peptides. They were draw using all structures from trajectory at room temperature (300 K) with bin size of 0.1 Å. The representative structures of the main conformations of the corresponding peak in distribution histogram of two peptides are shown on two sides.

Computational Methods

All simulations were carried out using the Gromacs 4.5.4[8], Each starting structure was initially constructed using the HyperChem software and the two amide bonds on the tether were set as *trans* conformation. Two peptides were solvated in a truncated octahedron boxes (31 Å in length) with 713 TIP3P[9] water molecules.

Energy minimization was carried out using steepest descent method. Then, the initial periodic box

volume was equilibrated using a 3 ns MD simulation in an NPT ensemble near 300 K and 1 atm. Replica exchange molecular dynamics (REMD) was used for all simulations to increase the sampling efficiency. The initial structures for REMD were obtained at regular intervals from a 30 ns NVT MD trajectory at 600 K. For each REMD simulation, 24 replicas of the system were simulated for 200 ns (a total of 4.8μs simulation) with temperature range from 300 K to 600 K. The intermediate temperatures were chosen following a recent study to obtain uniform exchange rate and exchanges were attempted among conformations of the neighboring replicas every 1.0 ps.

The electrostatics were treated using the particle-mesh Ewald (PME)[10] method with a real-space cutoff of 0.9 nm and van der Waals interaction cutoff at 0.9 nm with the long-range dispersion correction for energy and pressure in all simulations. A velocity rescaling thermostat[11] with τT = 0.2 ps and a Berendsen barostat[12] with τP = 0.5 ps were used to maintain constant temperature and constant pressure (for NPT simulations), respectively. All bonds involving hydrogen were constrained using LINCS,[13] and a time step of 2 fs was used. At the same time, the mass of water oxygen atom was reduced from 16 to 2 amu to increase the sampling efficiency[14] without altering the thermodynamics equilibrium properties.

Clustering analysis was performed on 10,000 snapshots sampled at 300 K, using the 'gromos'[15] method with 1Å cutoff based on RMSD of the non-H atoms of peptides.

For each peptide, the Ramachandran plot was draw using the ϕ, ψ values of all five residues together, from 10000 snapshots sampled in 300 K trajectory. To draw the plot, the whole 360°x 360° ϕ, ψ space is divided into 300 x 300 small bins, and the relative probability of each bin was calculated from the counts. A logarithmic color scale is used to visualize the probability distribution, and the bins not sampled are left in white.

Calculated helicity (helical content) was defined as the percentage of the structures with all residues in the right-handed-helix conformation (-160° <ϕ<-40° , -80° <ψ<40°).

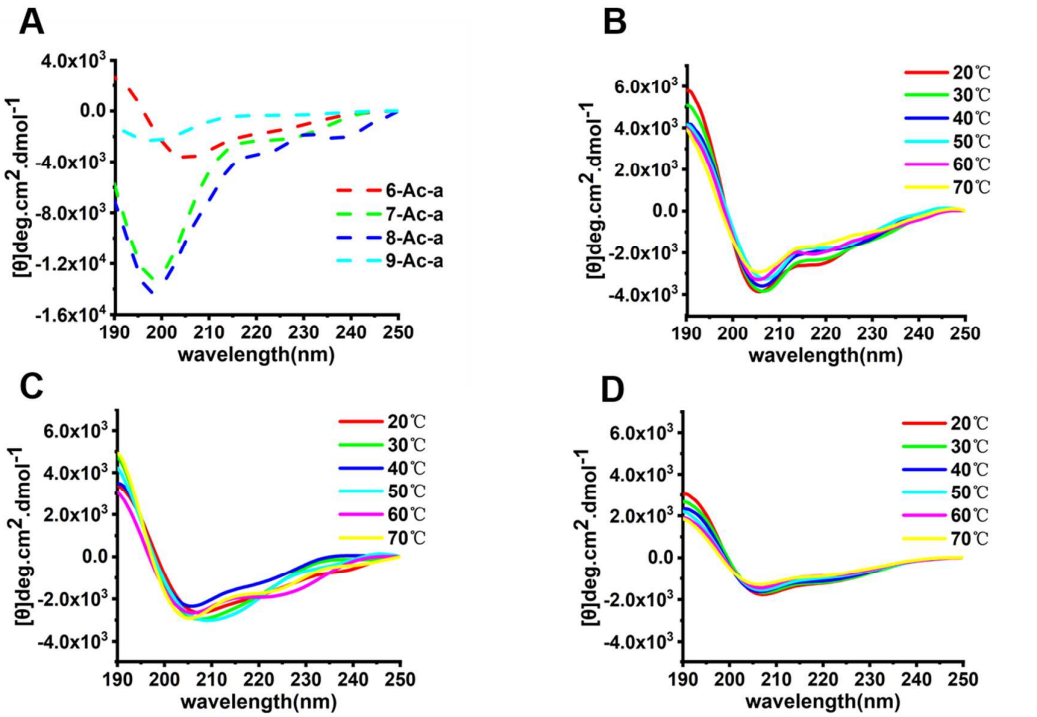


Figure S3 (A) CD spectrums of peptides **6-Ac-a** to **9-Ac-a**. The CD spectroscopy measurements were performed in H_2O at 25°C. (B-D) Dependence on temperature (20-70°C) of full CD spectrums of **7-Ac-b**, **8-Ac-b, 9-Ac-b** in H_2O. Concentrations of samples are about 1.5mM.

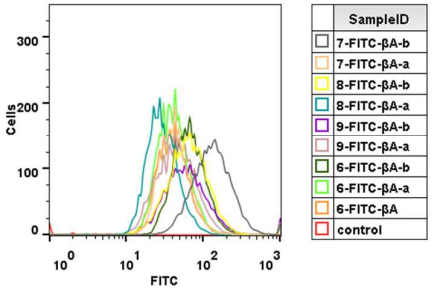


Figure S4 Flow cytometry measurement of **6-FITC-βA**, **6-FITC-βA-a/b** to **9-FITC-βA-a/b**, positive control peptide after incubation with Hela cells at 37˚C for 2 hours.

Mass statistics data for the peptides.

Calculated and Founded m/z are presented as $[M+H]^+$ /$[M/2+H]^+$/$[M/3+H]^+$

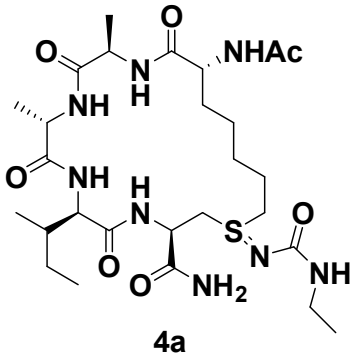
Peptide	Sequence	Calculated Mass	Found Mass	Retention Time (min)
1a	Ac-(cyclo-1,5)-S_5AAAC-NH_2	586.29	587.31	6.07
1b	Ac-(cyclo-1,5)-S_5AAAC-NH_2	586.29	587.26	7.76
2a	Ac-(cyclo-1,5)-S_5AGAC-NH_2	572.27	573.36	4.12
2b	Ac-(cyclo-1,5)-S_5AGAC-NH_2	572.27	573.26	4.26
3a	Ac-(cyclo-1,5)-S_5AQAC-NH_2	643.31	644.36	4.08
3b	Ac-(cyclo-1,5)-S_5AQAC-NH_2	643.31	644.31	6.73
4a	Ac-(cyclo-1,5)-S_5AAIC-NH_2	628.34	629.41	6.23
4b	Ac-(cyclo-1,5)-S_5AAIC-NH_2	628.34	629.36	6.76
5a	Ac-(cyclo-1,5)-S_5AEAC-NH_2	644.30	645.40	6.09
5b	Ac-(cyclo-1,5)-S_5AEAC-NH_2	644.30	645.35	6.32
6-Ac-a	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1448.79	725.75	12.72
6-Ac-b	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1448.79	725.70	12.96
7-Ac-a	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1458.78	730.70	12.27
7-Ac-b	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1458.78	730.50	12.88
8-Ac-a	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1503.81	753.25	12.04
8-Ac-b	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1503.81	753.35	13.08
9-Ac-a	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1460.79	731.65	12.08
9-Ac-b	Ac-(cyclo-2,6)-RS_5ILHCLLQDS-NH_2	1460.79	731.55	16.50
6-FITC-a	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1866.85	934.85	12.38
6-FITC-b	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1866.85	934.75	12.69
7-FITC-a	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1876.84	939.90	12.54
7-FITC-b	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1876.84	939.85	13.31
8-FITC-a	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1921.87	962.35	13.34
8-FITC-b	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1921.87	962.25	13.85
9-FITC-a	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1878.85	627.70	13.04
9-FITC-b	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	1878.85	627.65	13.81
Biotin-b	FITC-(cyclo-3,7)-βAlaRS_5ILHCLLQDS-NH_2	2448.13	1225.65	12.89

Table S1. HPLC traces of the purified peptides. HPLC method: Inertsil ODS-SP HPLC C18 column, 5 µm, 4.6 X 250 mm(GL Sciences Inc.), flow rate: 0.8 mL/min; solvent A 0.1%TFA+Water, solvent B: 100% Acetonitrile; gradient (A:B): 80:20(0 min) to 20:80(20 min) to 2:98(25 min).

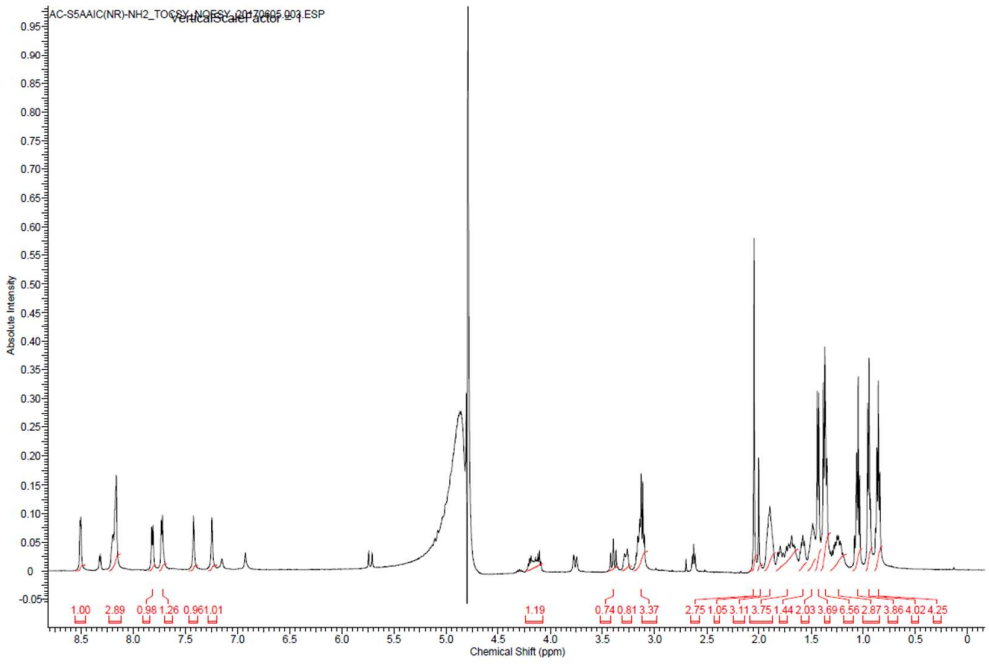
Entry	Residue	NH	Hα	Hβ	H(side chain)	$^{3}J_{NH-Hα}$
1	S_5	8.09	4.1	1.62	1.24,0.83	3.89
2	A	8.41	4.05	1.26		2.91
3	A	7.63	4.07	1.31		5.14
4	I	7.71	3.99	1.81	0.83,1.37	6.35
5	C	8.06	4.59	3.28		——

Table S2. ^{1}H NMR chemical shifts (δ) and $^{3}J_{NH\text{-}\alpha H}$(Hz) for peptide **4a** in H_2O with 10% D_2O at 298K.

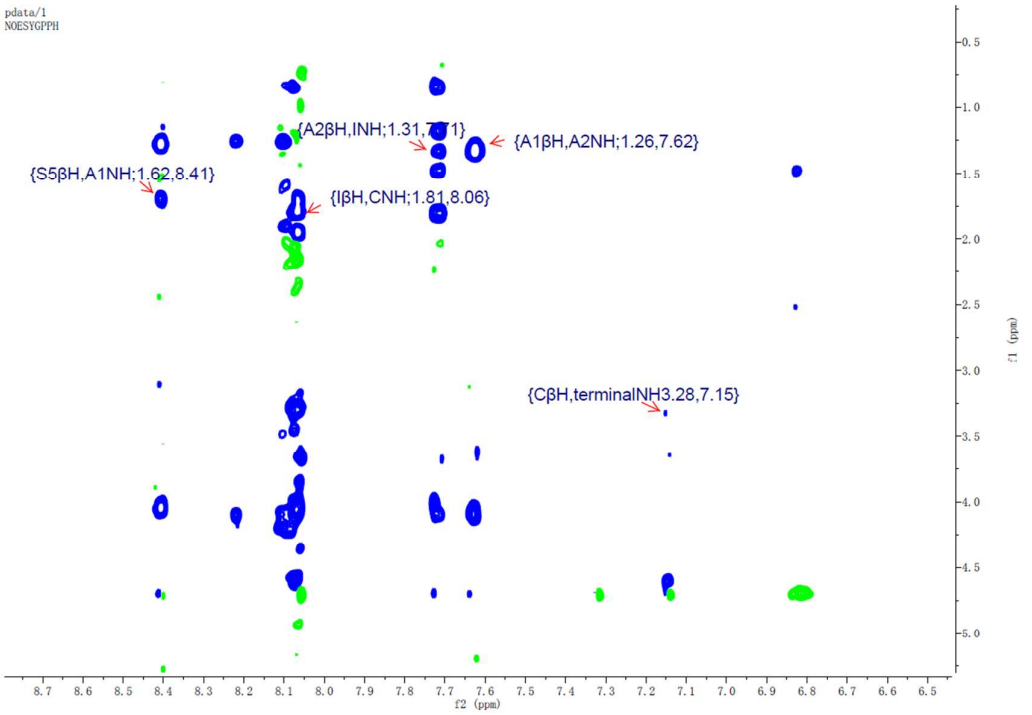
1D and 2D NMR spectra for **4a**



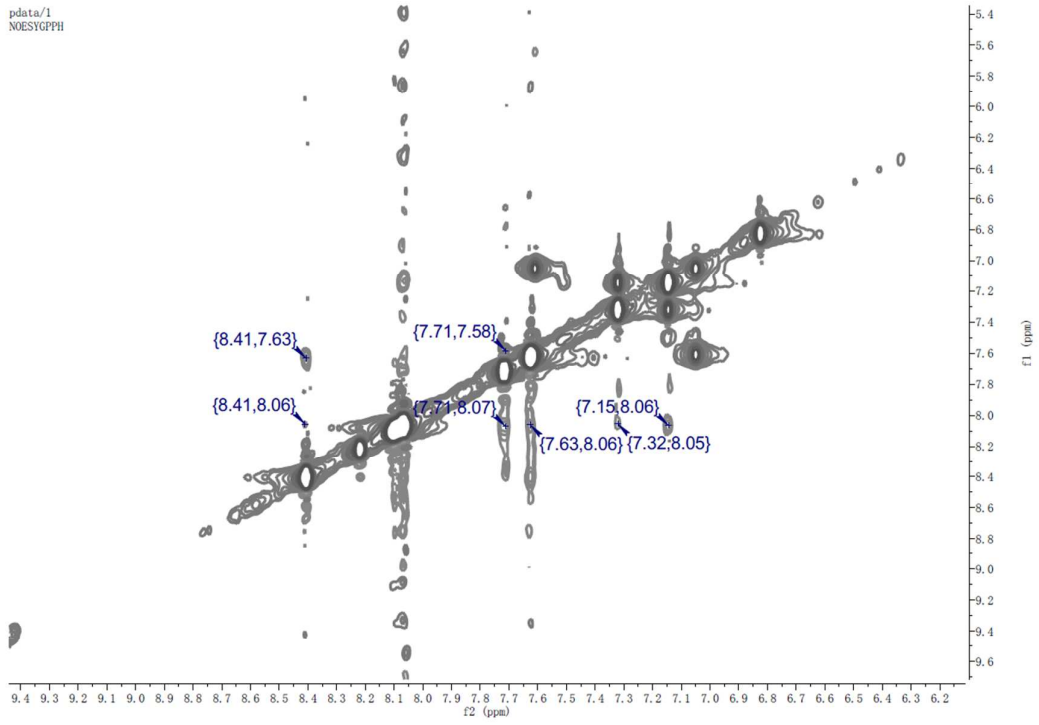
4a



1H NMR spectrum of **4a** (500MHz in H_2O with 10% D_2O at 298K)



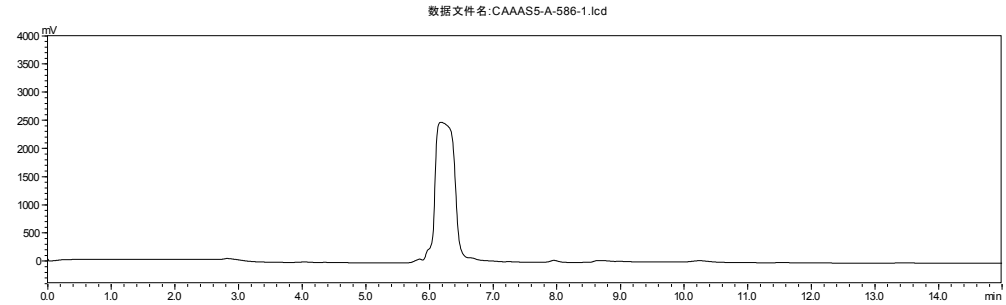
TOCSY spectrum of **4a** (500MHz in H_2O with 10% D_2O at 298K)

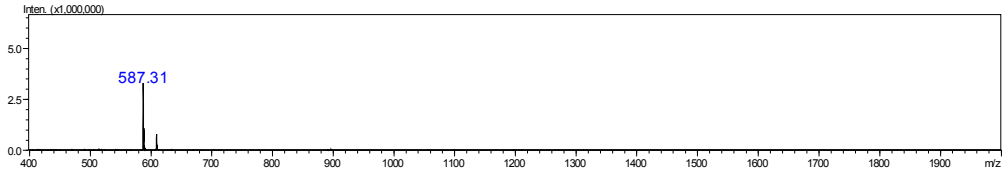


NH region of NOESY spectrum of **4a** (500MHz in H_2O with 10% D_2O at 298K)

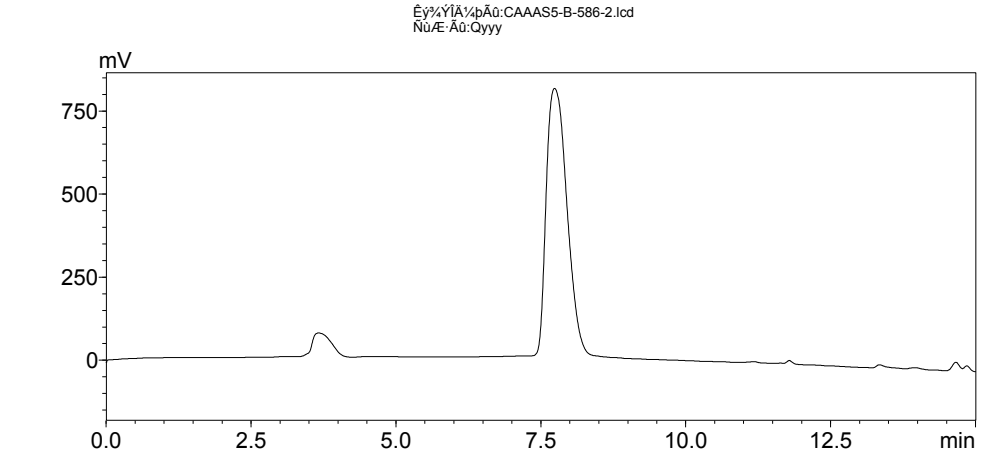
HPLC traces and MS spectra

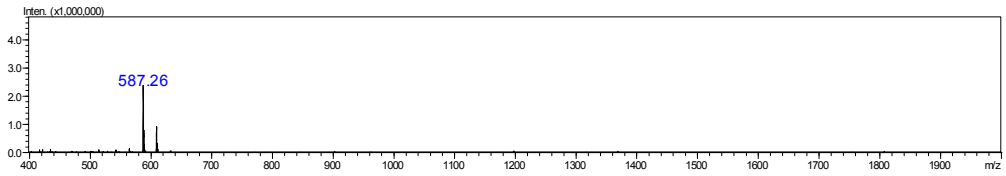
Peptide **1a**



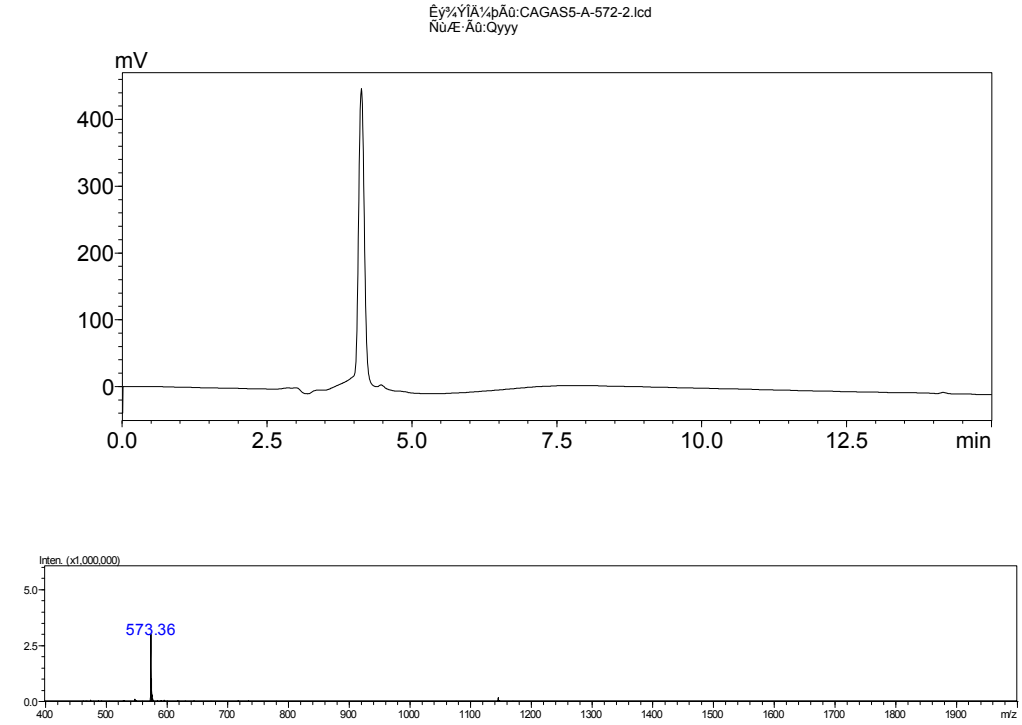


Peptide **1b**

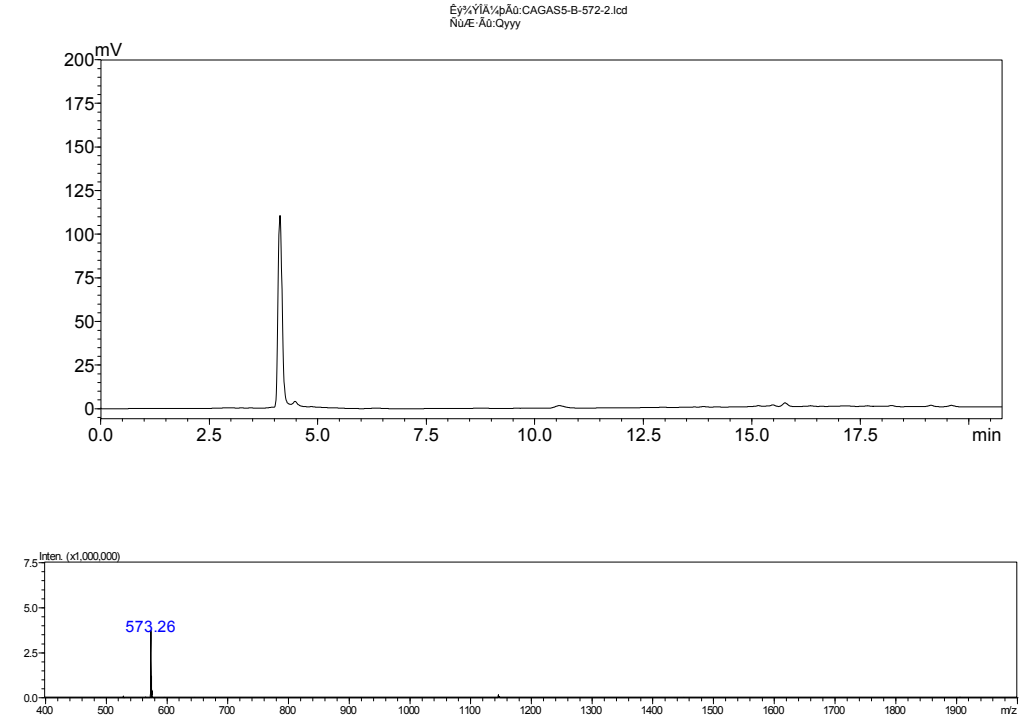




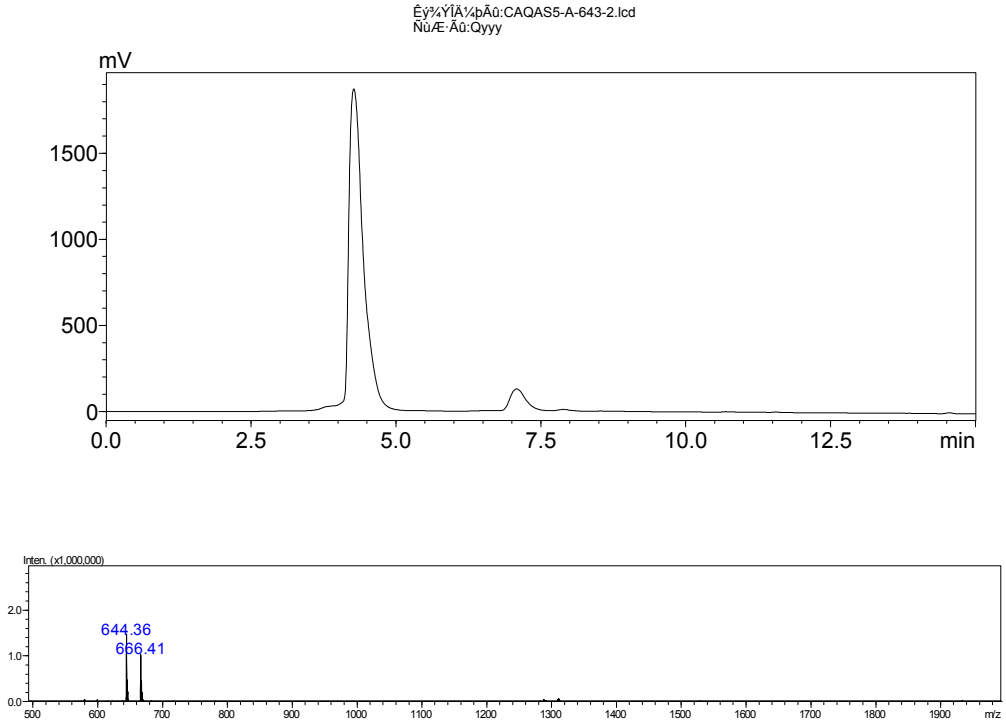
Peptide **2a**



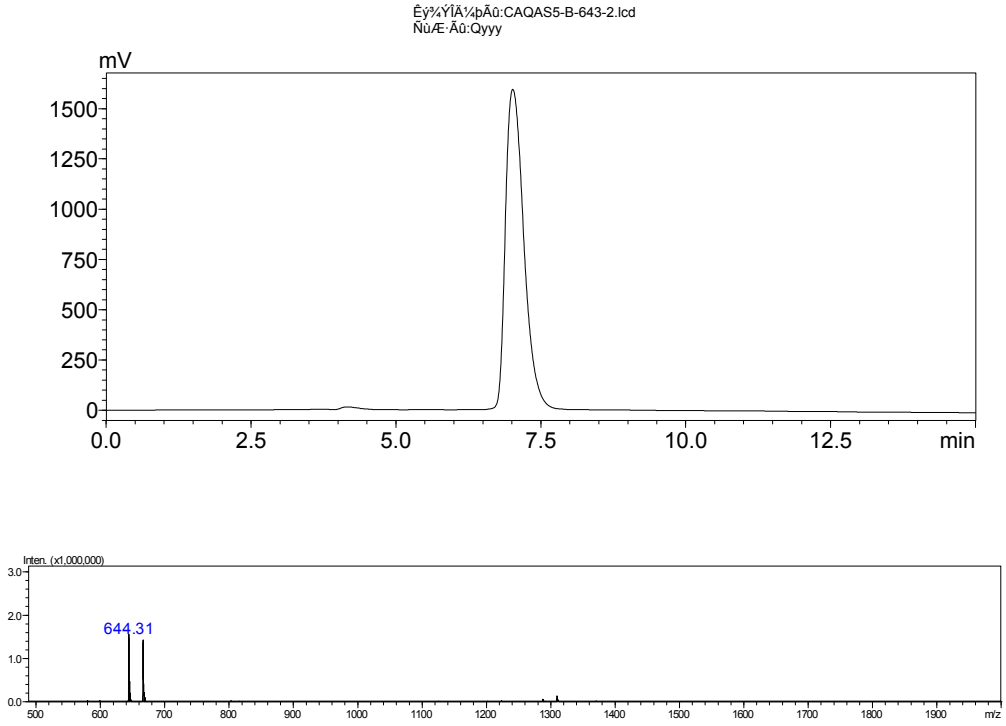
Peptide **2b**



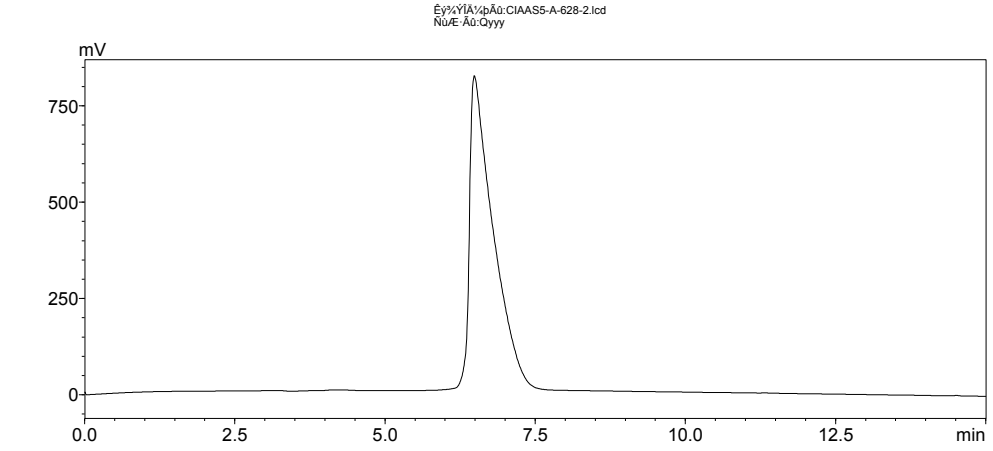
Peptide **3a**

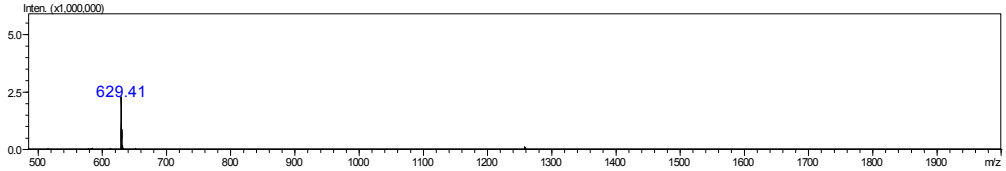


Peptide **3b**

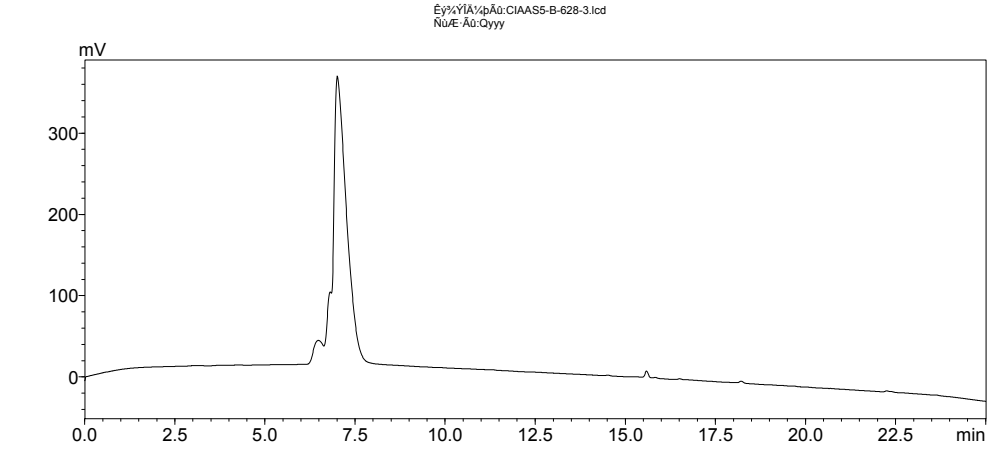


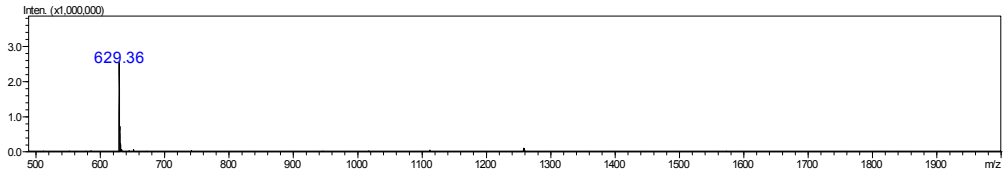
Peptide **4a**



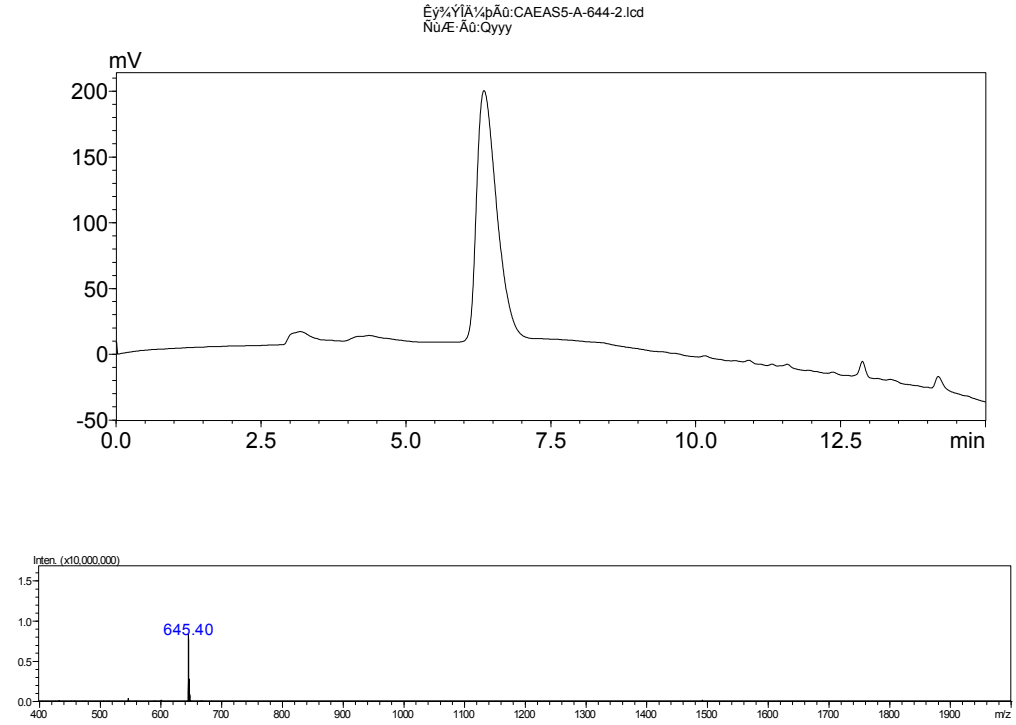


Peptide **4b**

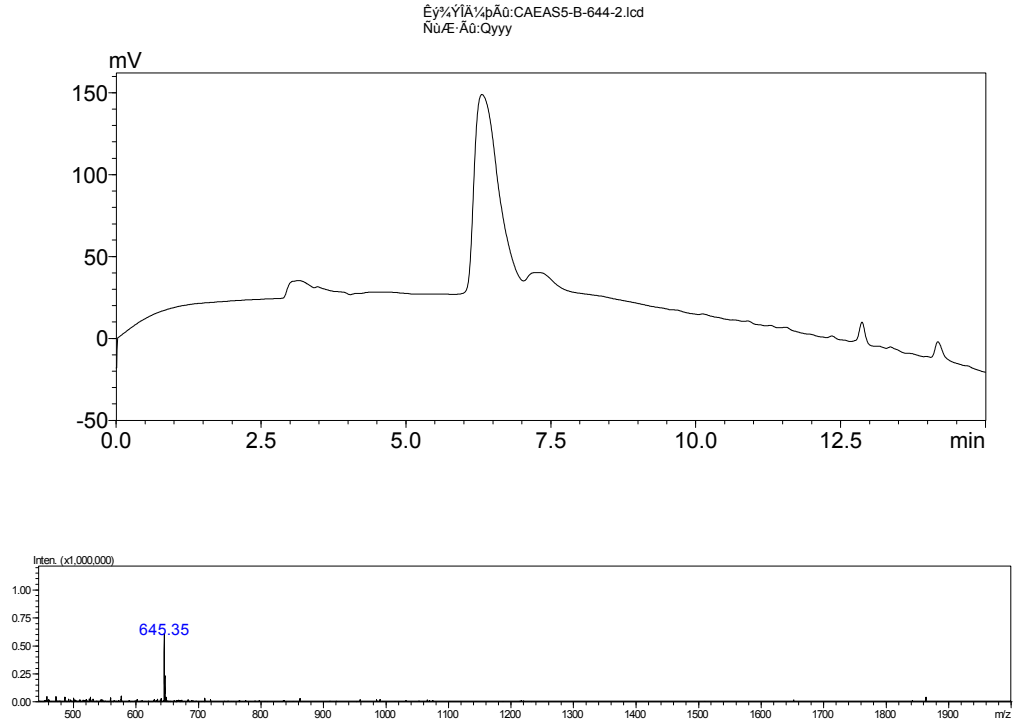




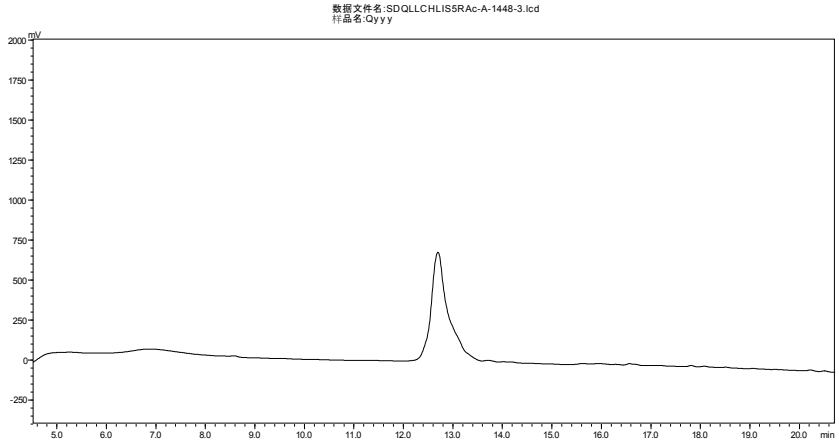
Peptide **5a**

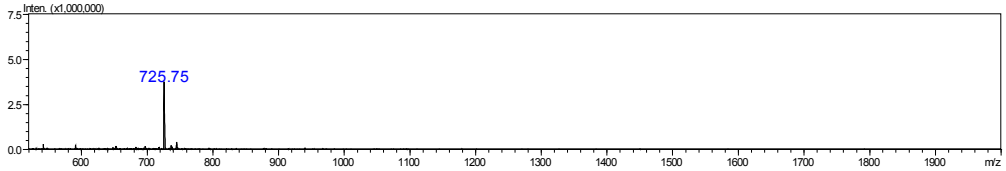


Peptide **5b**

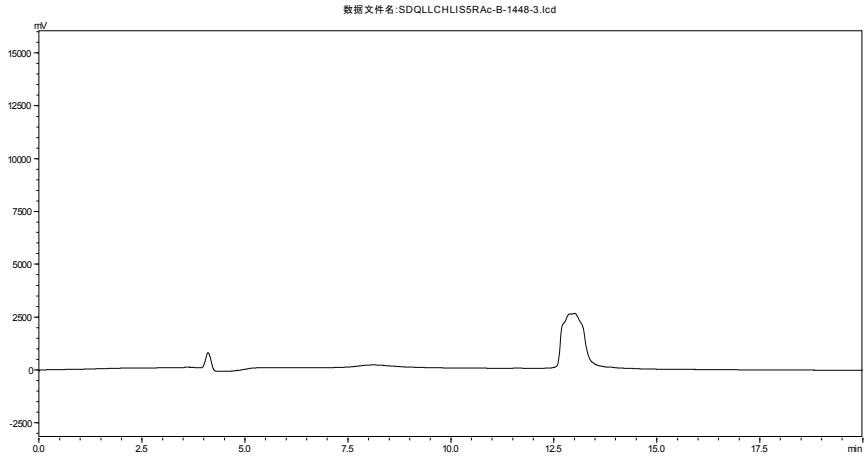


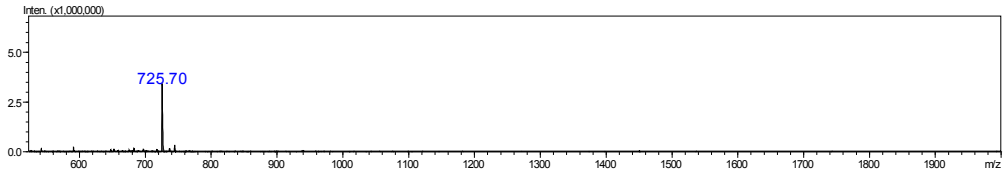
Peptide **6-Ac-a**



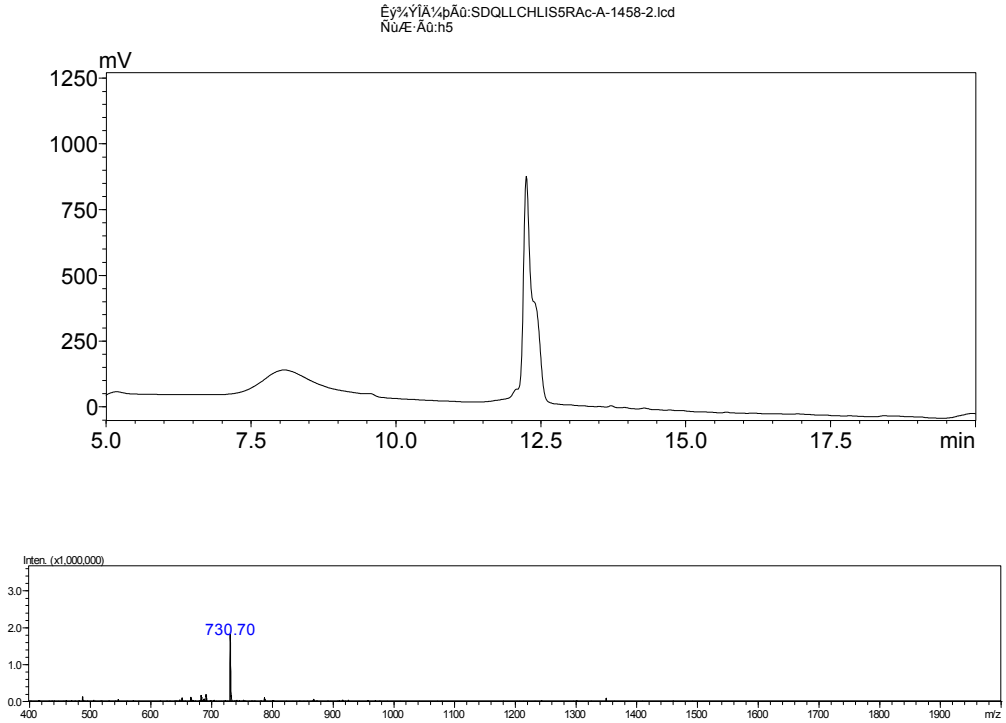


Peptide **6-Ac-b**

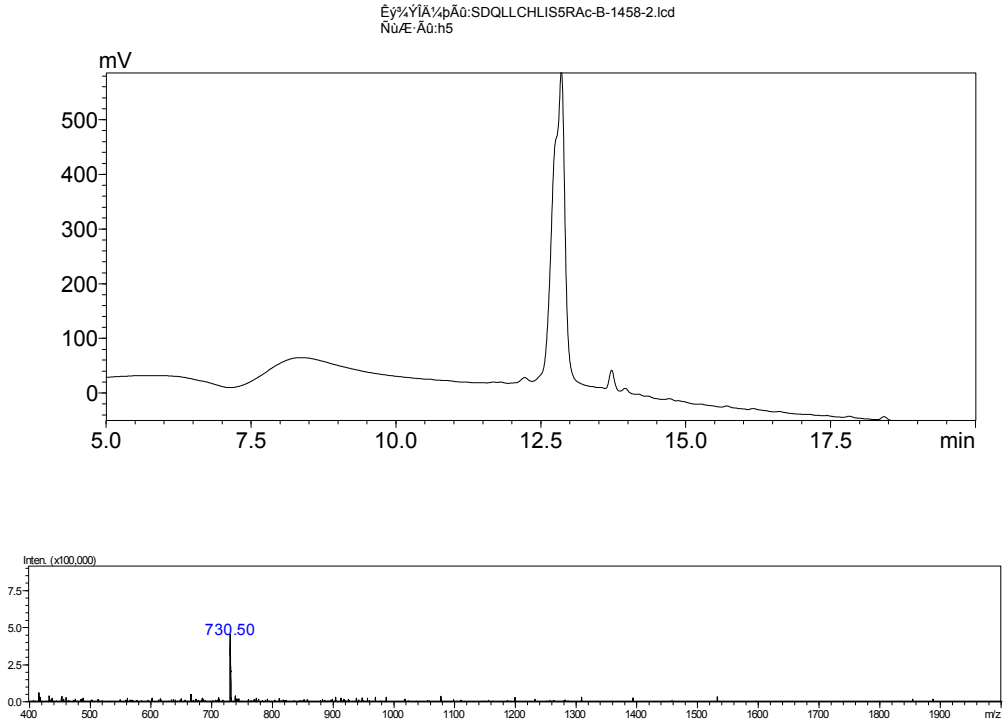




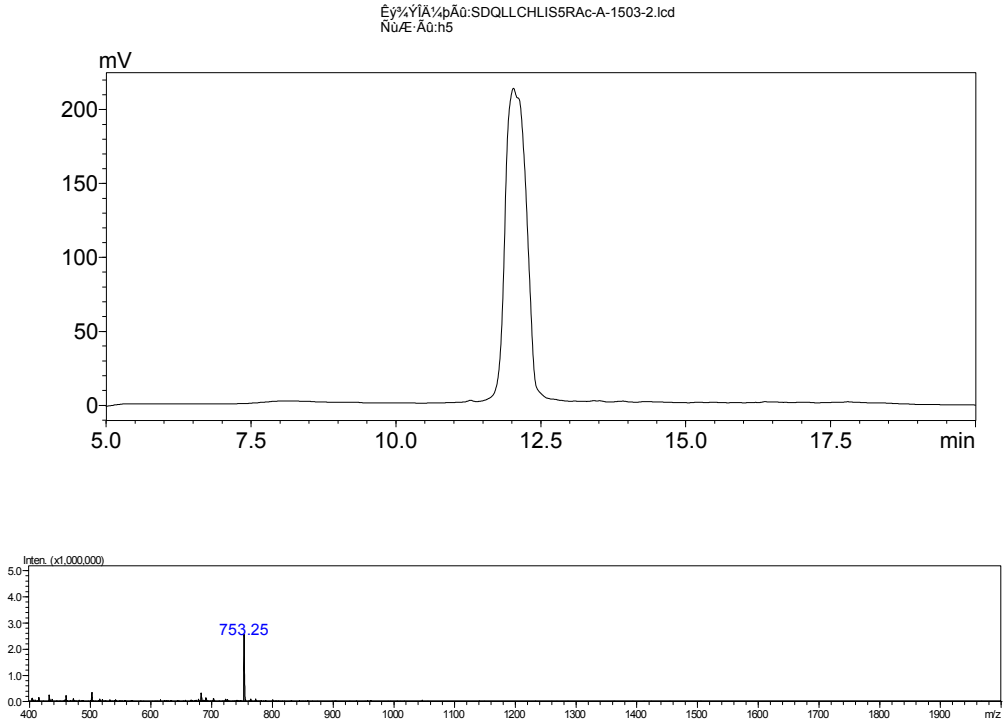
Peptide **7-Ac-a**



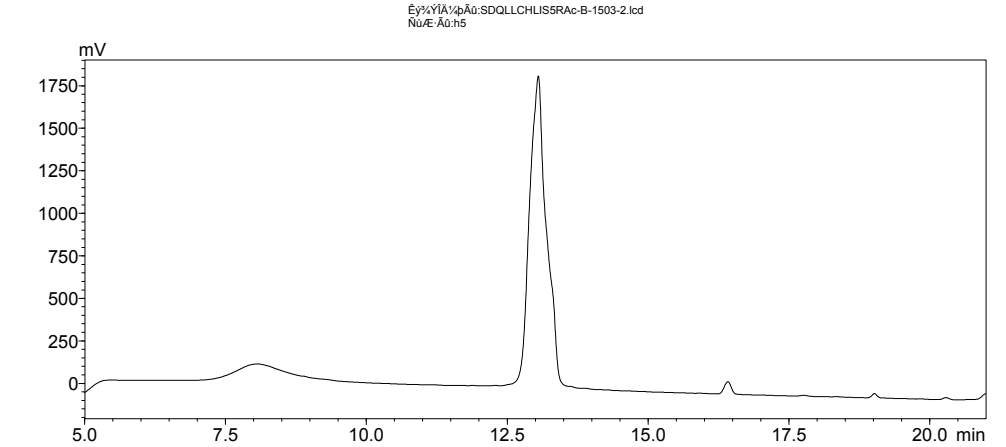
Peptide **7-Ac-b**

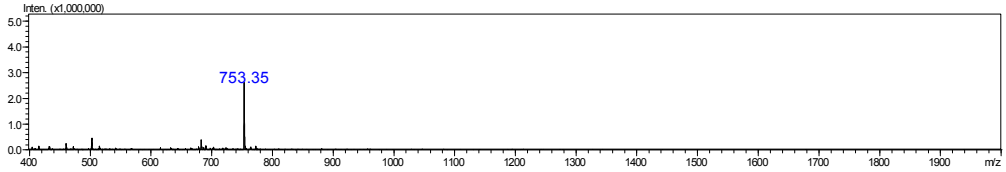


Peptide **8-Ac-a**

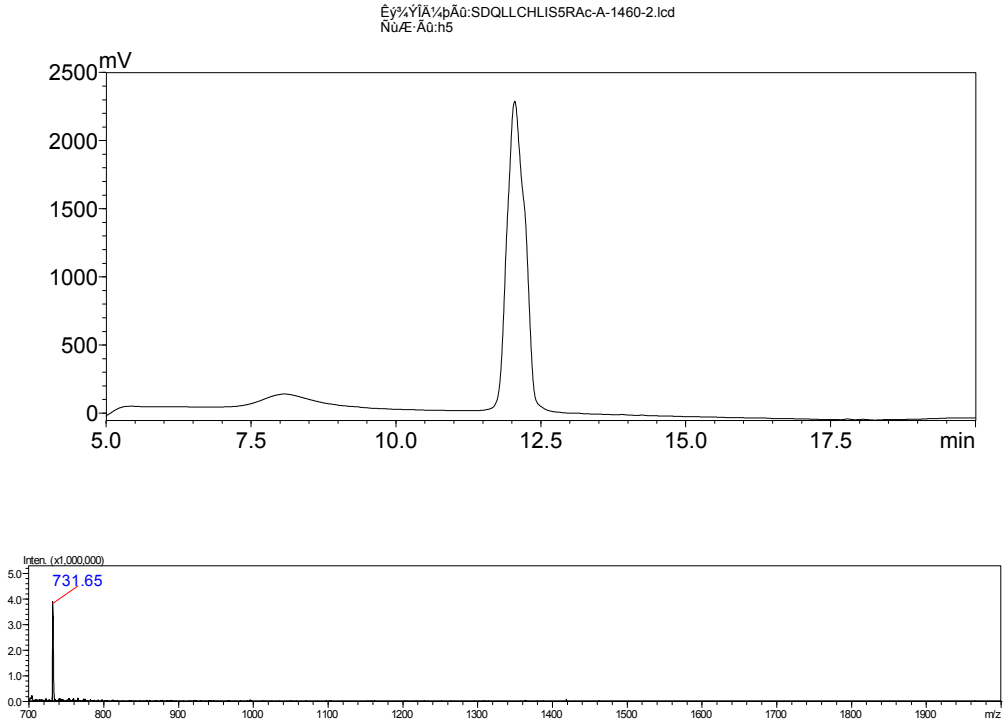


Peptide **8-Ac-b**

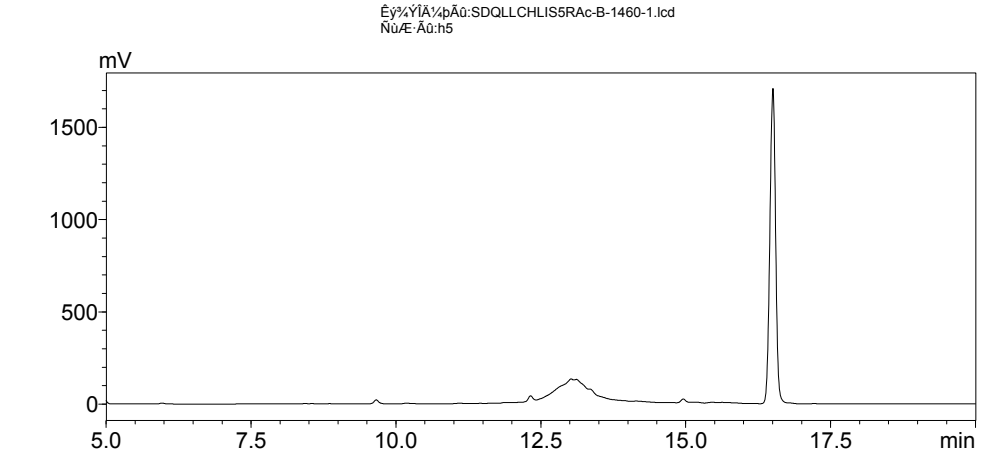


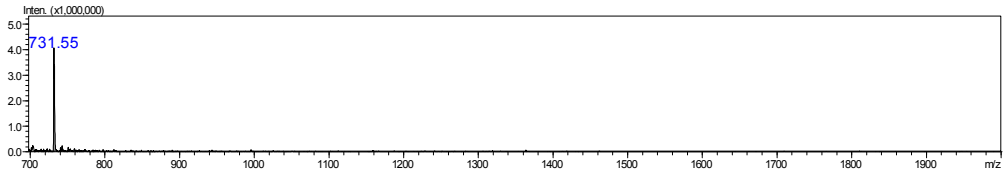


Peptide **9-Ac-a**

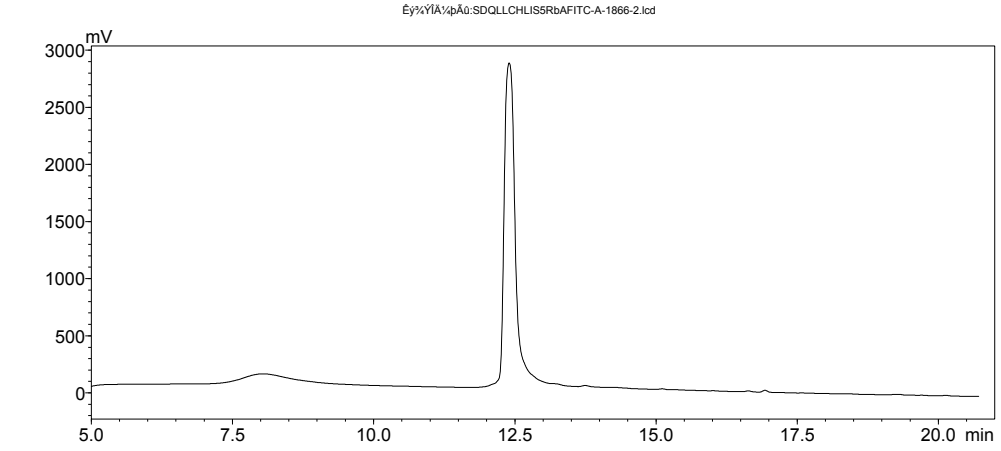


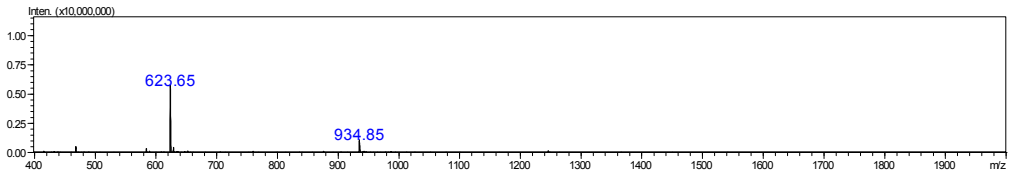
Peptide **9-Ac-b**



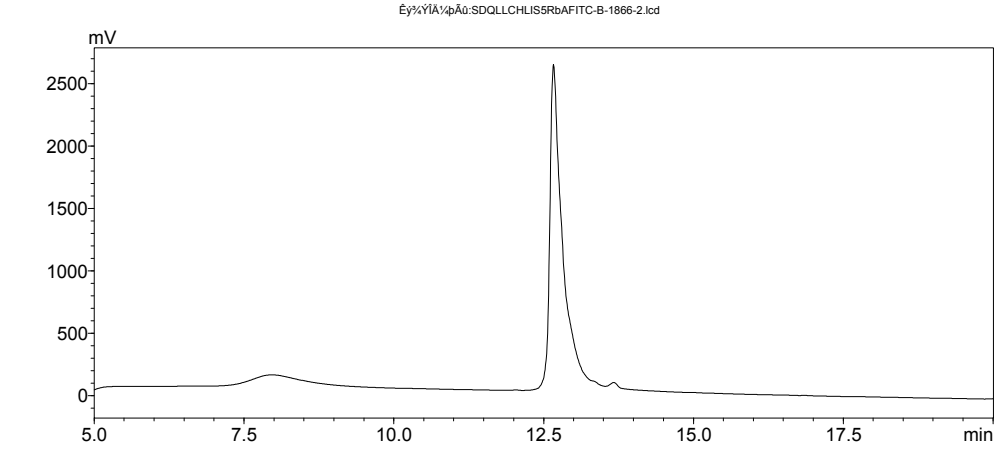


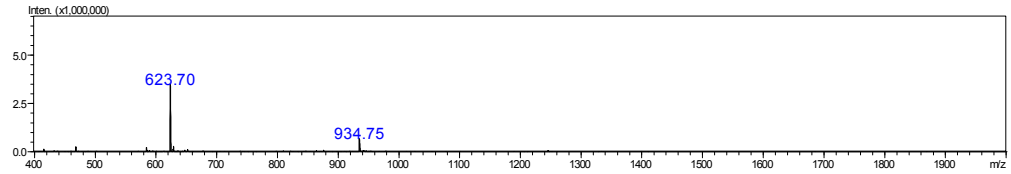
Peptide **6-FITC-βA-a**



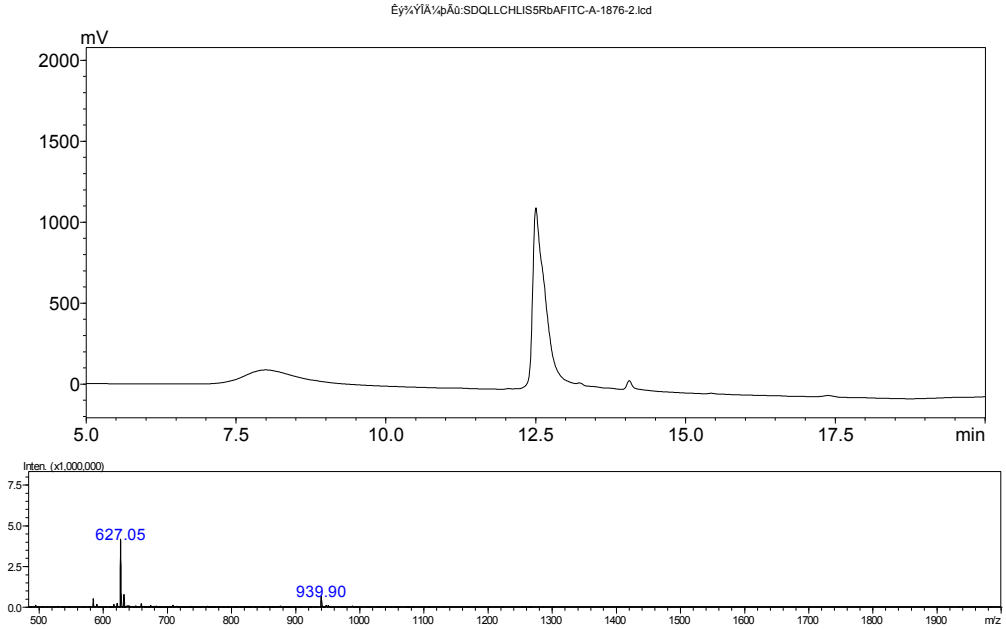


Peptide **6-FITC-βA-b**

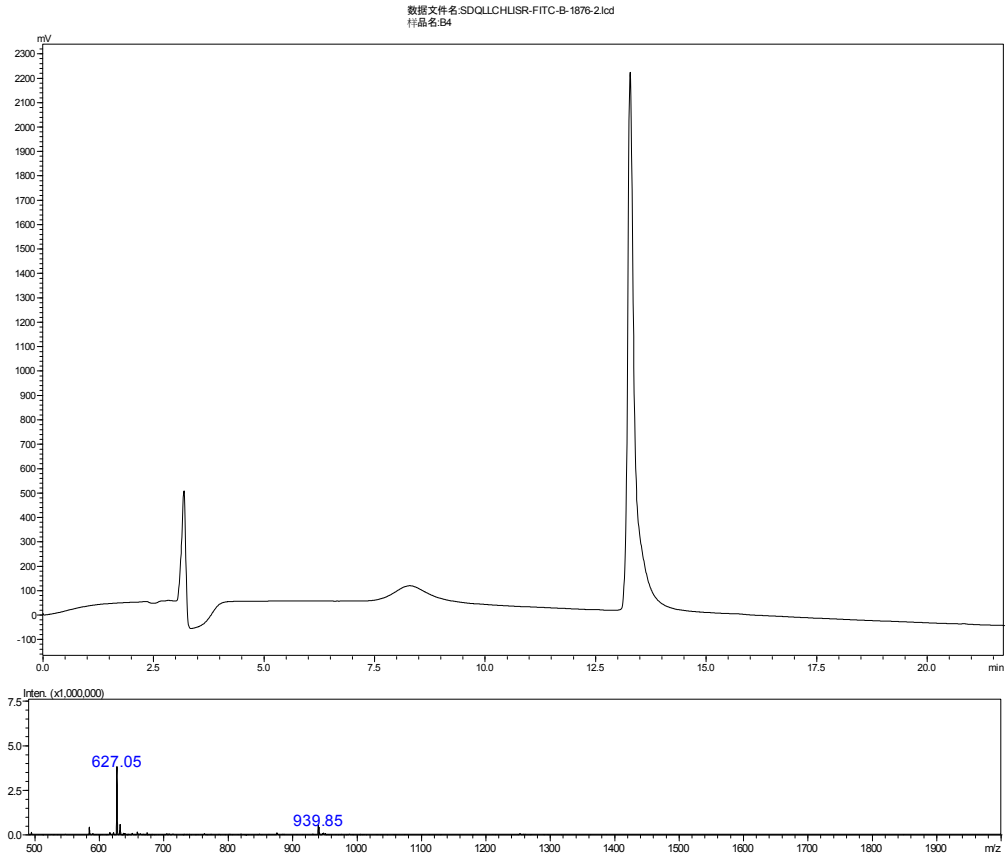




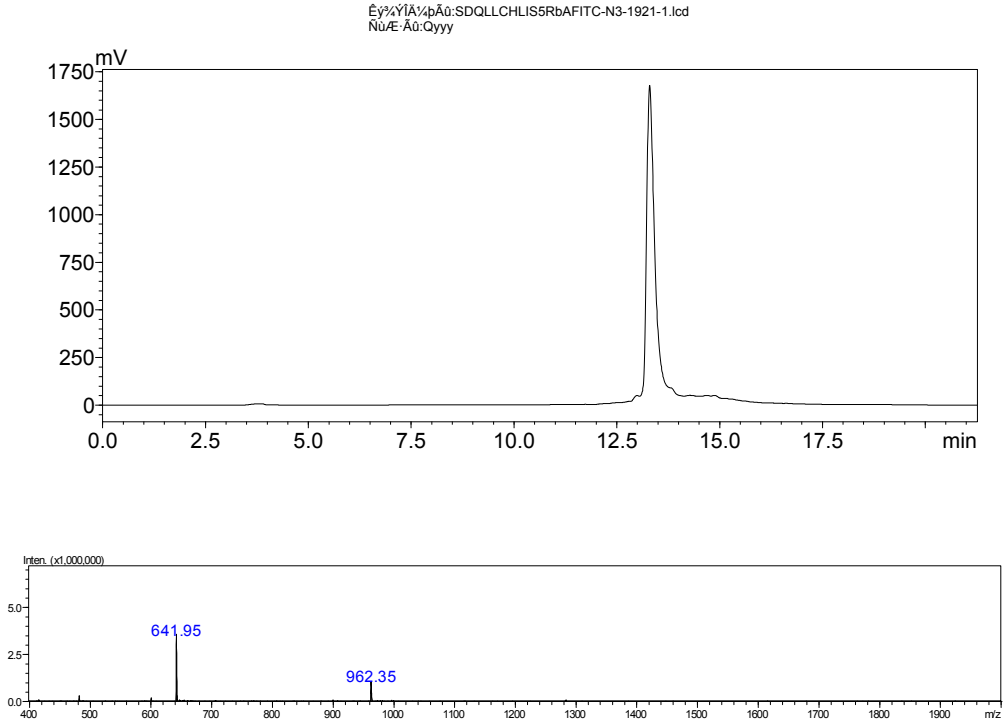
Peptide **7-FITC-βA-a**



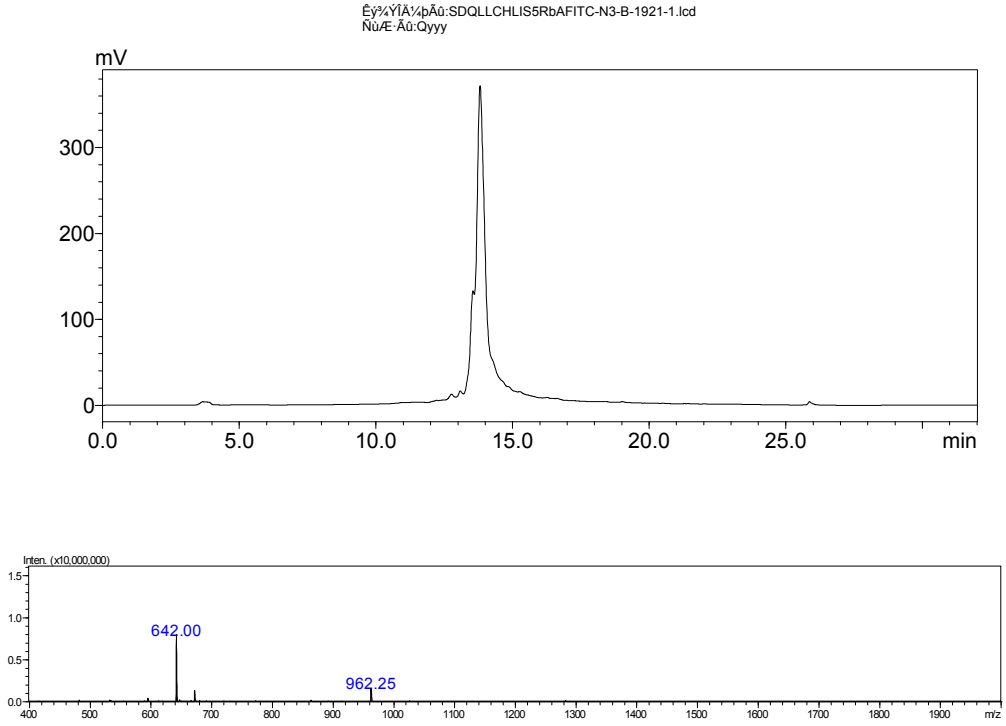
Peptide **7-FITC-βA-b**



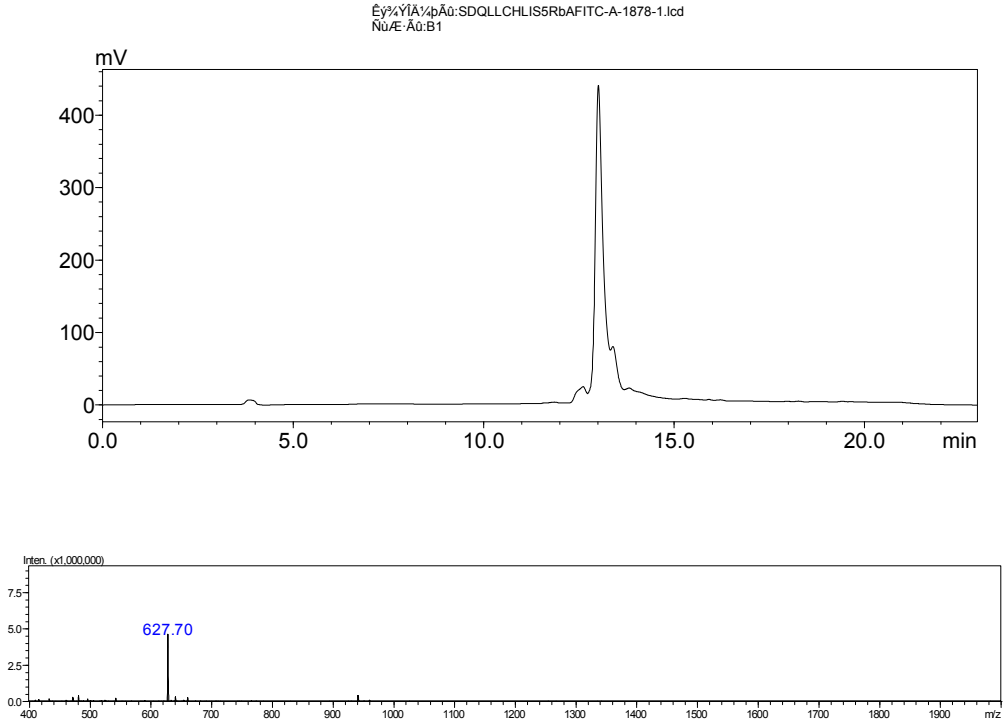
Peptide **8-FITC-βA-a**



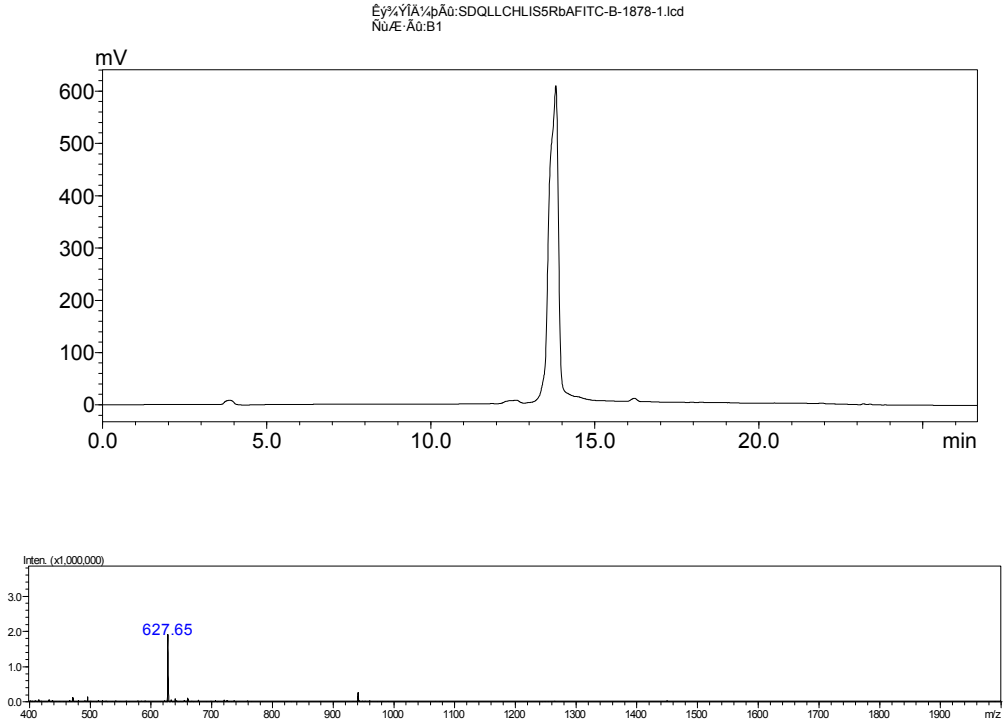
Peptide **8-FITC-βA-b**



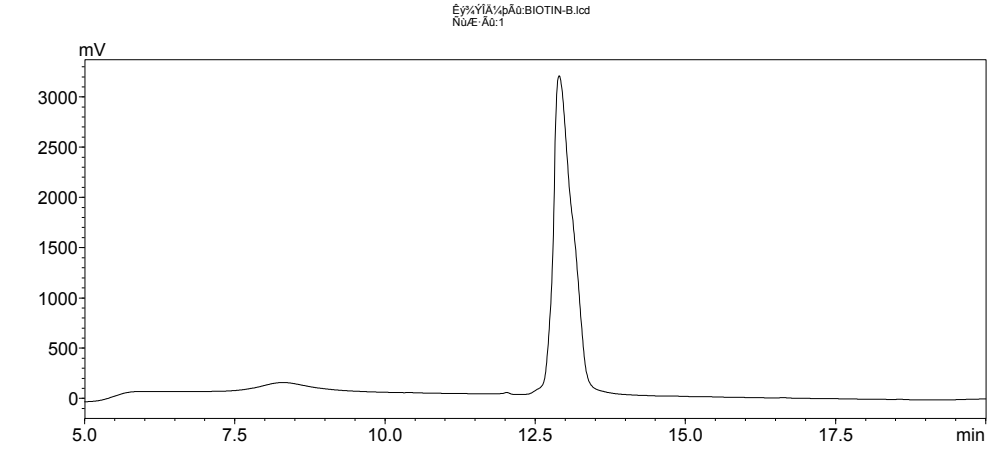
Peptide **9-FITC-βA-a**

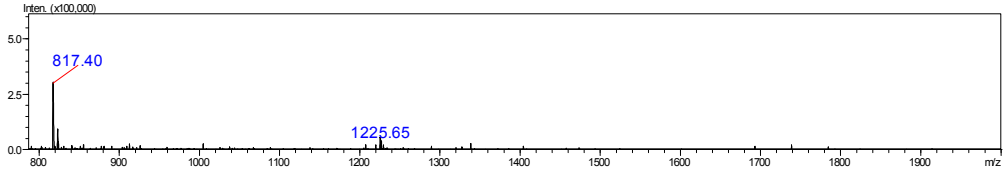


Peptide **9-FITC-βA-b**

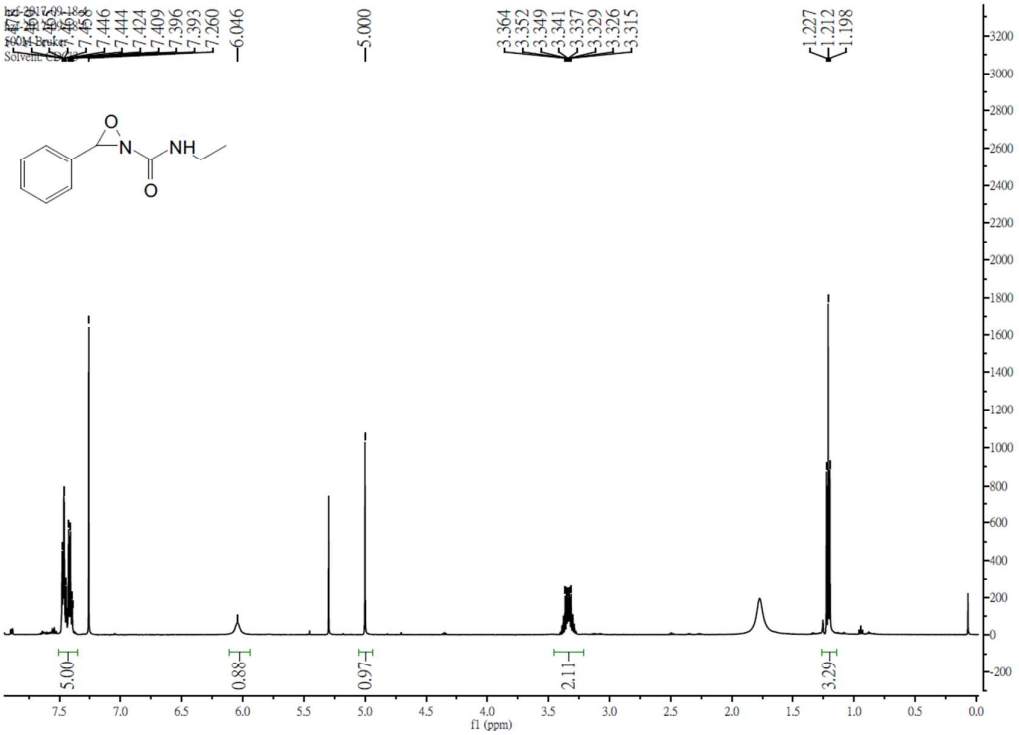


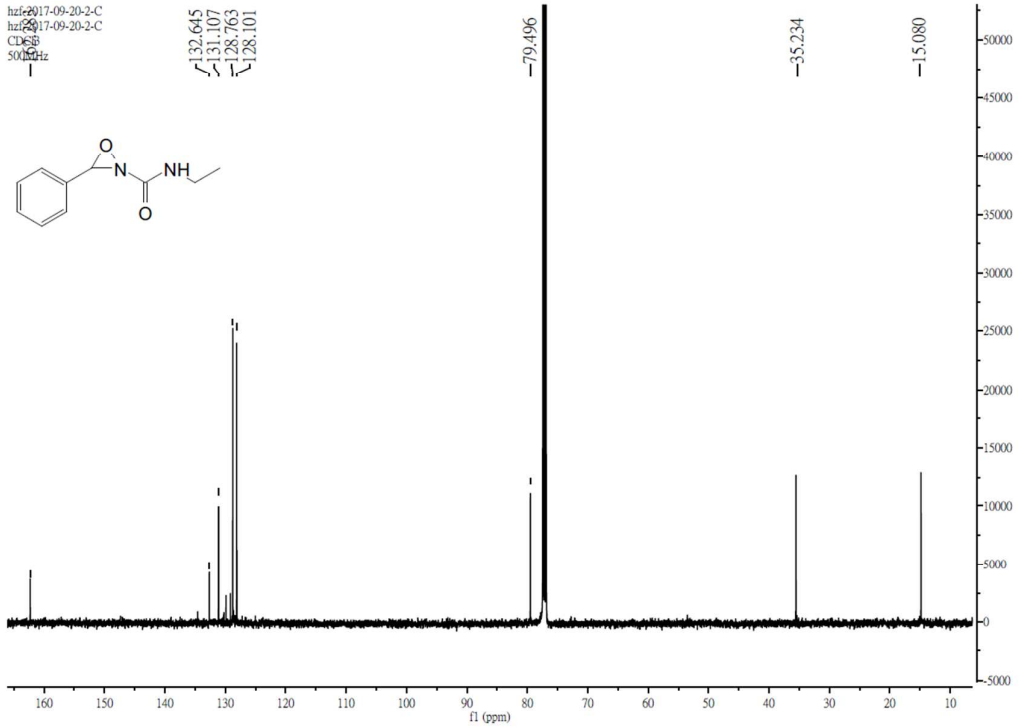
Biotin-b

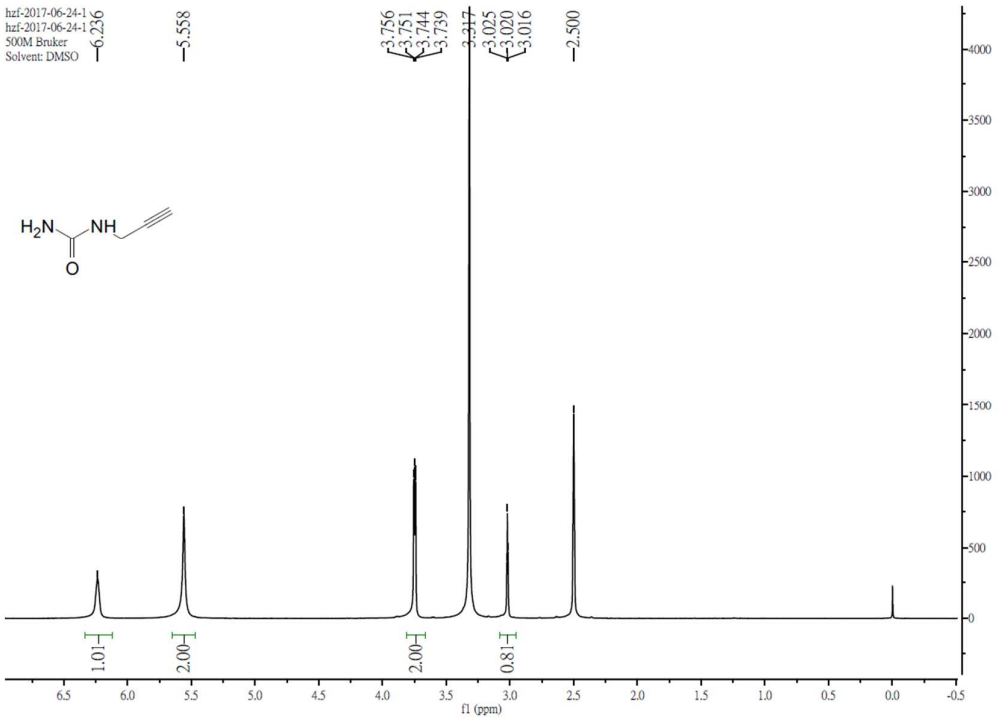

ÊÿÝ¼ÝÎÄ¼þÃû:BIOTIN-B.lcd
Ñû¼Æ:Ã0:1
mV
3000
2500
2000
1500
1000
500
0
5.0
7.5
10.0
12.5
15.0
17.5
min

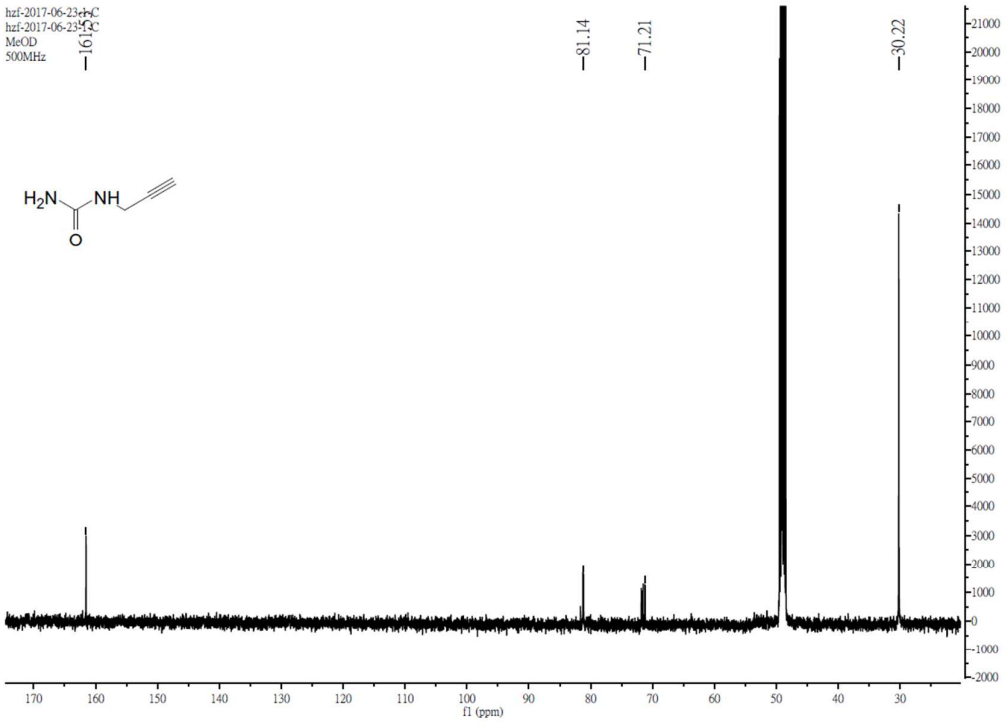

Inten. (x100,000)
5.0
2.5
0.0
817.40
1225.65
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
m/z

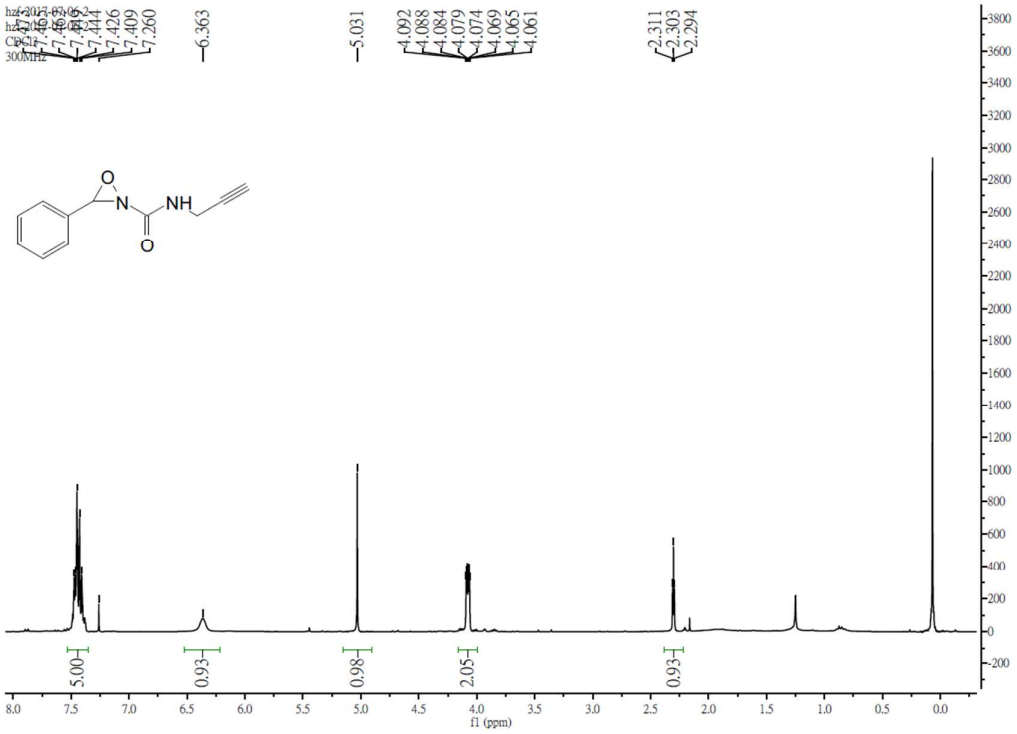
NMR

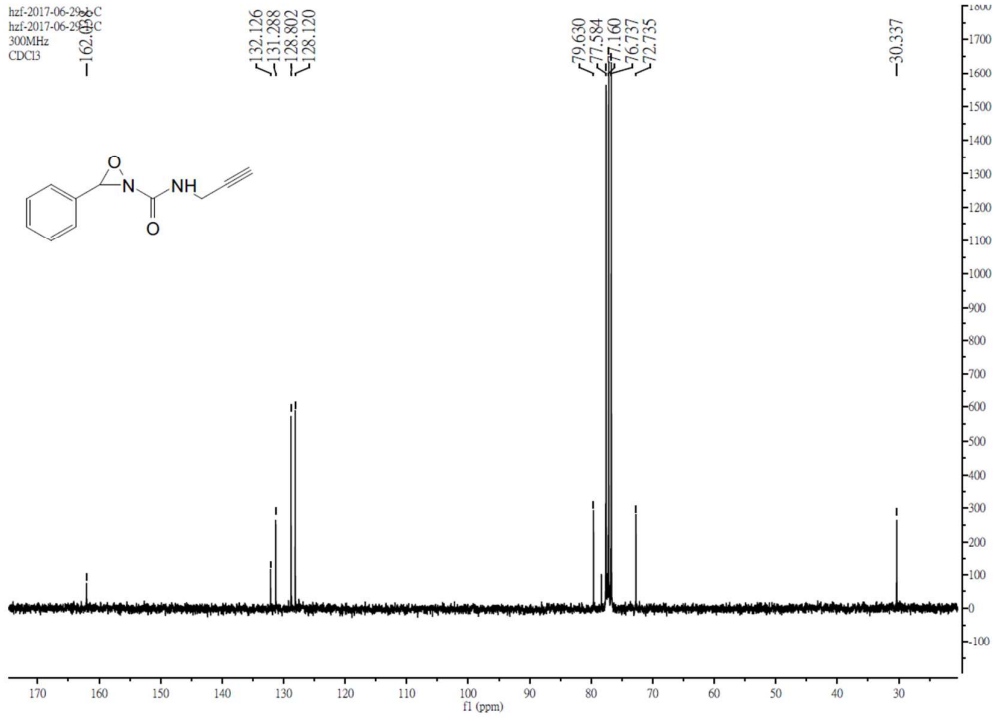

7.458
7.446
7.444
7.424
7.409
7.396
7.393
7.260
6.046
5.000
3.364
3.352
3.349
3.341
3.337
3.329
3.326
3.315
1.227
1.212
1.198
O
N
NH
O
5.00
0.88
0.97
2.11
3.29
f1 (ppm)

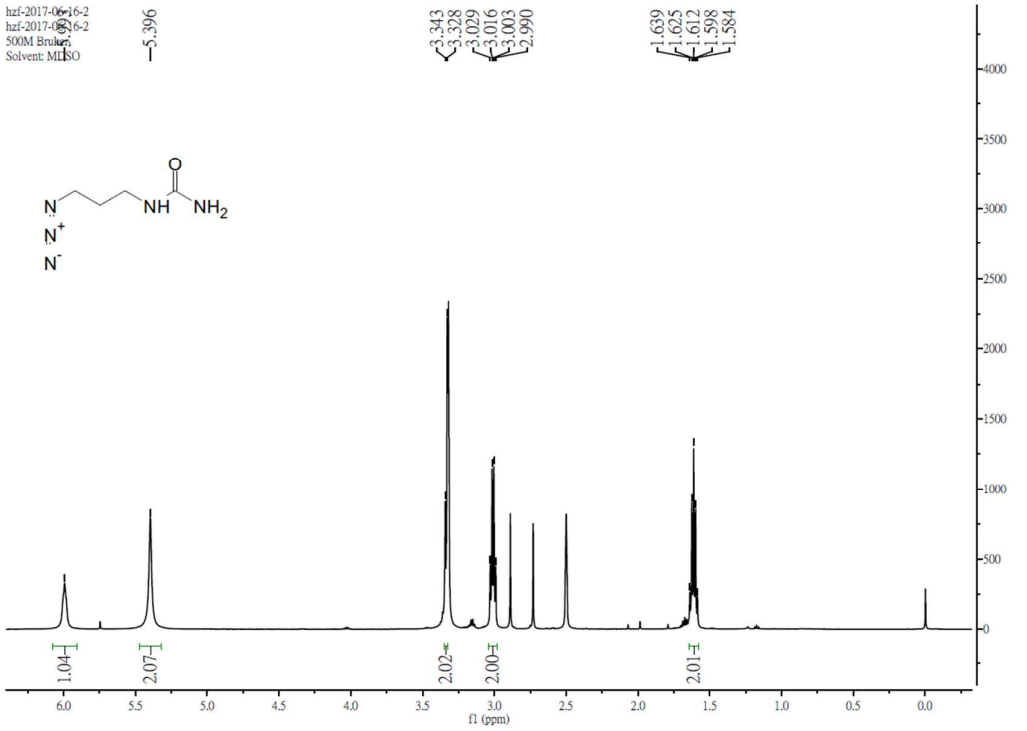

132.645
131.107
128.763
128.101
79.496
35.234
15.080
O
N
NH
O
f1 (ppm)

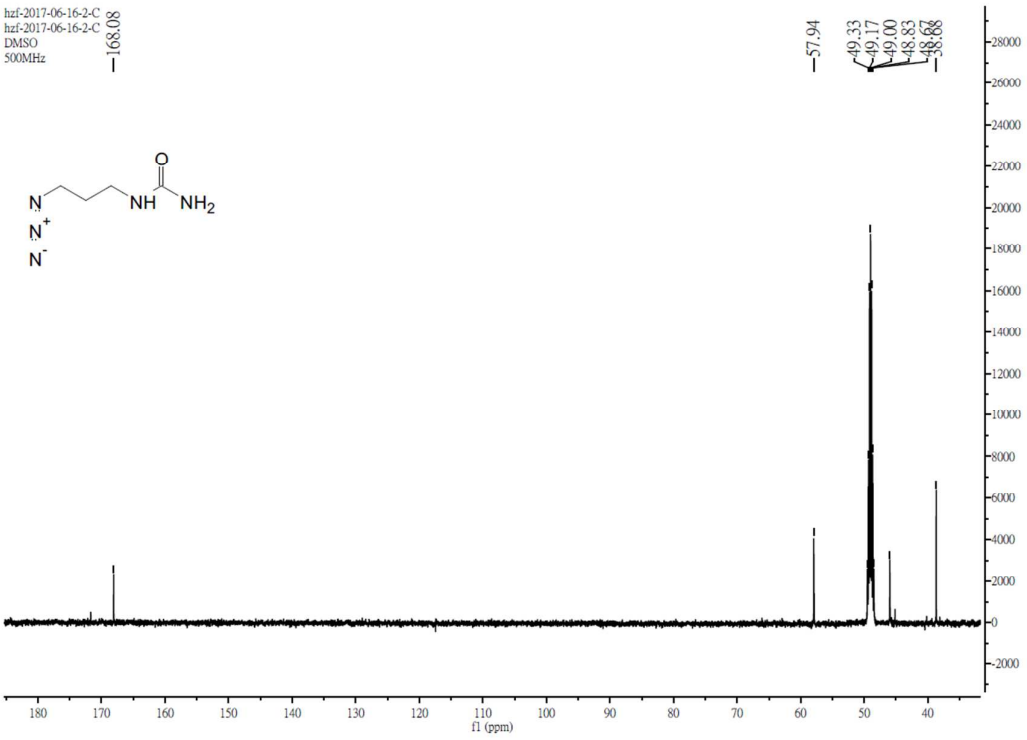

hzf-2017-06-24-1
hzf-2017-06-24-1
500M Bruker
Solvent: DMSO
6.236
5.558
3.756
3.751
3.744
3.739
3.217
3.025
3.020
3.016
2.500
1.01
2.00
2.00
0.81
f1 (ppm)

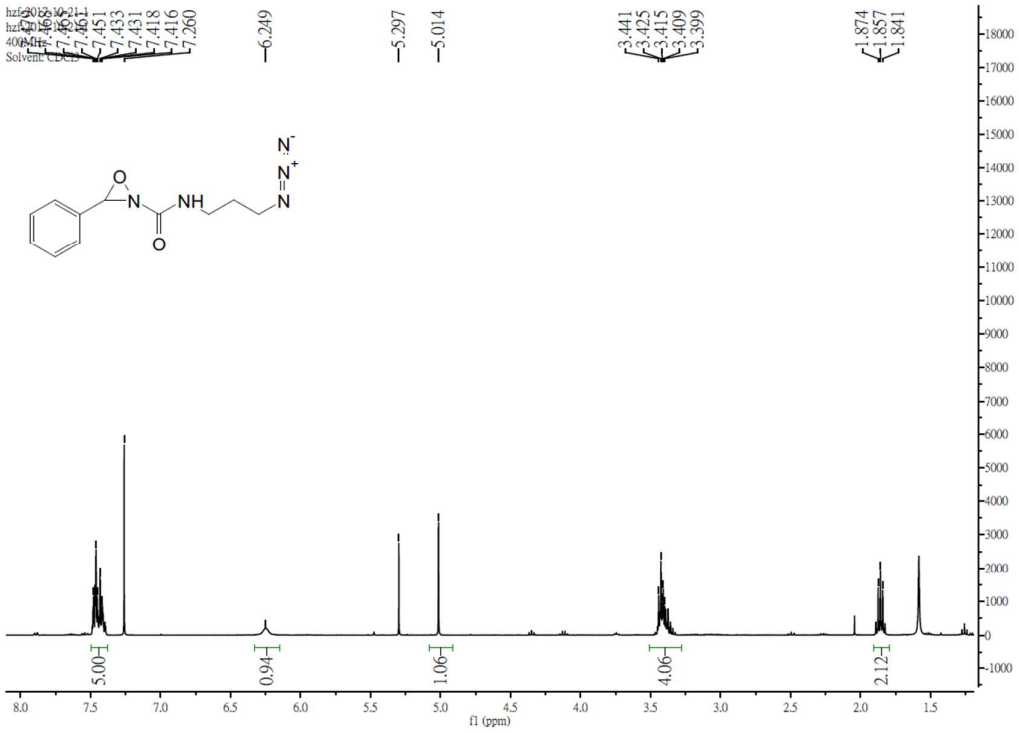

hzf-2017-06-23-13C
hzf-2017-06-23-13C
MeOD
500MHz
161.53
81.14
71.21
30.22
f1 (ppm)

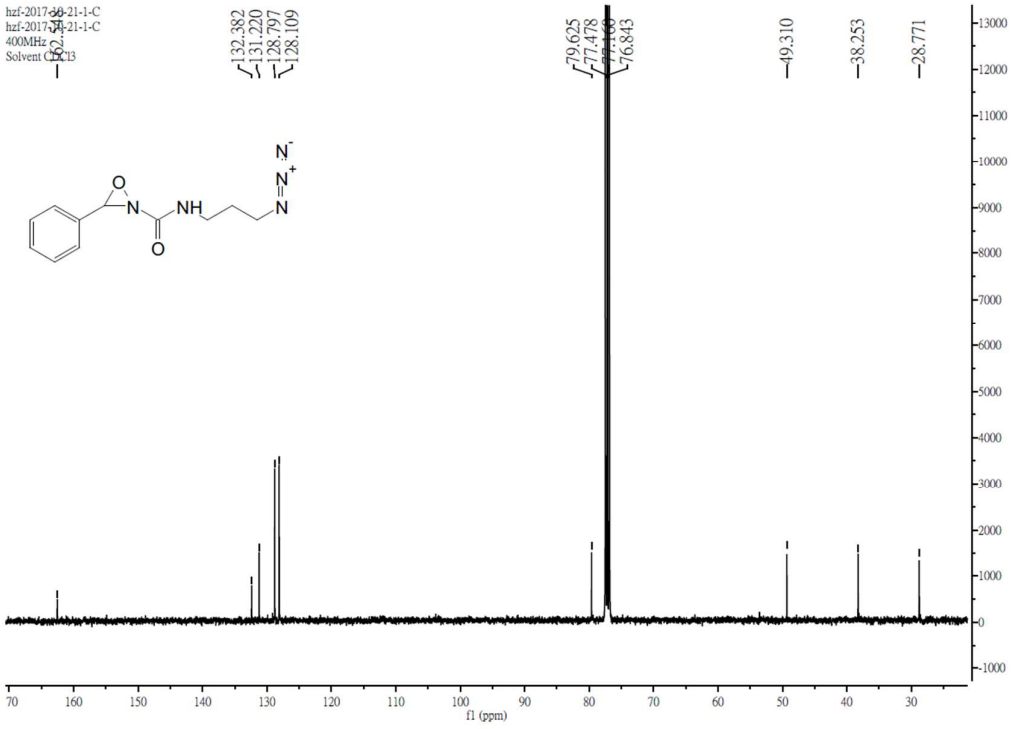

7.426
7.409
7.260
6.363
5.031
4.092
4.088
4.084
4.079
4.074
4.069
4.065
4.061
2.311
2.303
2.294
O
N
NH
O
5.00
0.93
0.98
2.05
0.93
f1 (ppm)

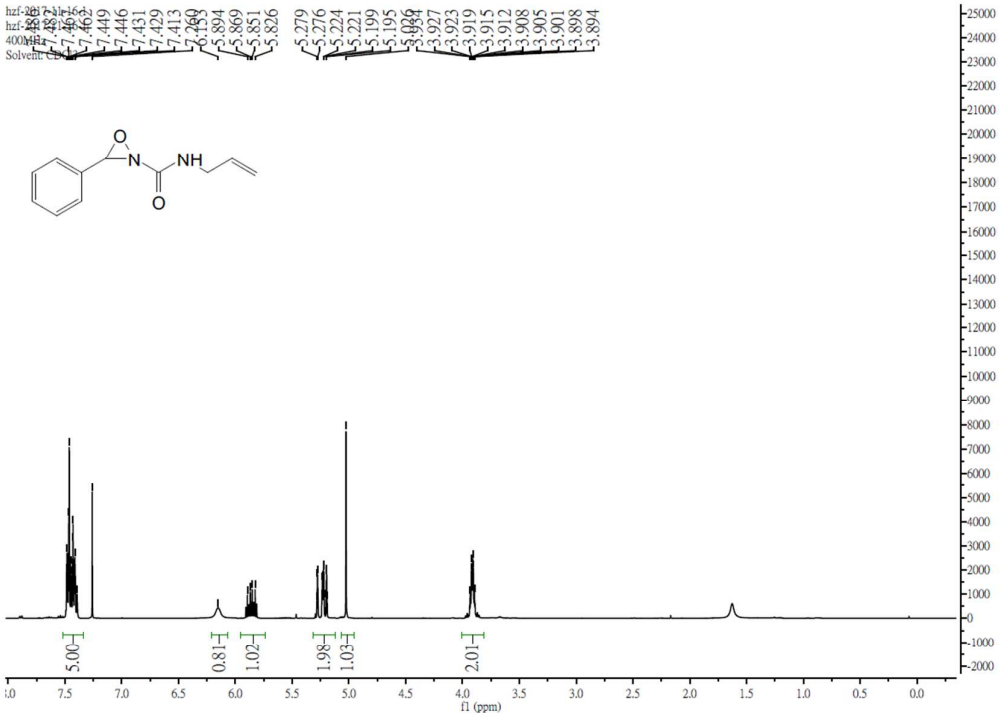

300MHz
CDCl3
162.066
132.126
131.288
128.802
128.120
79.630
77.584
77.160
76.737
72.735
30.337
O
N
NH
O
f1 (ppm)

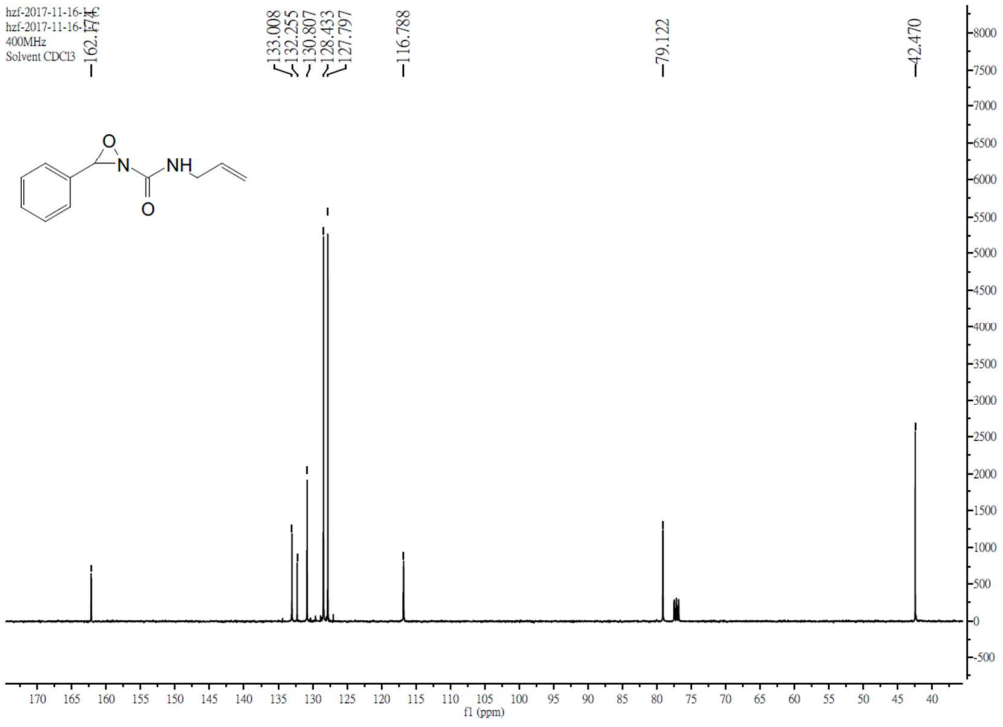

hzf-2017-06-16-2
hzf-2017-06-16-2
500M Bruker
Solvent: MDSO
5.396
3.343
3.328
3.029
3.016
3.003
2.990
1.639
1.625
1.612
1.598
1.584
1.04
2.07
2.02
2.00
2.01
f1 (ppm)

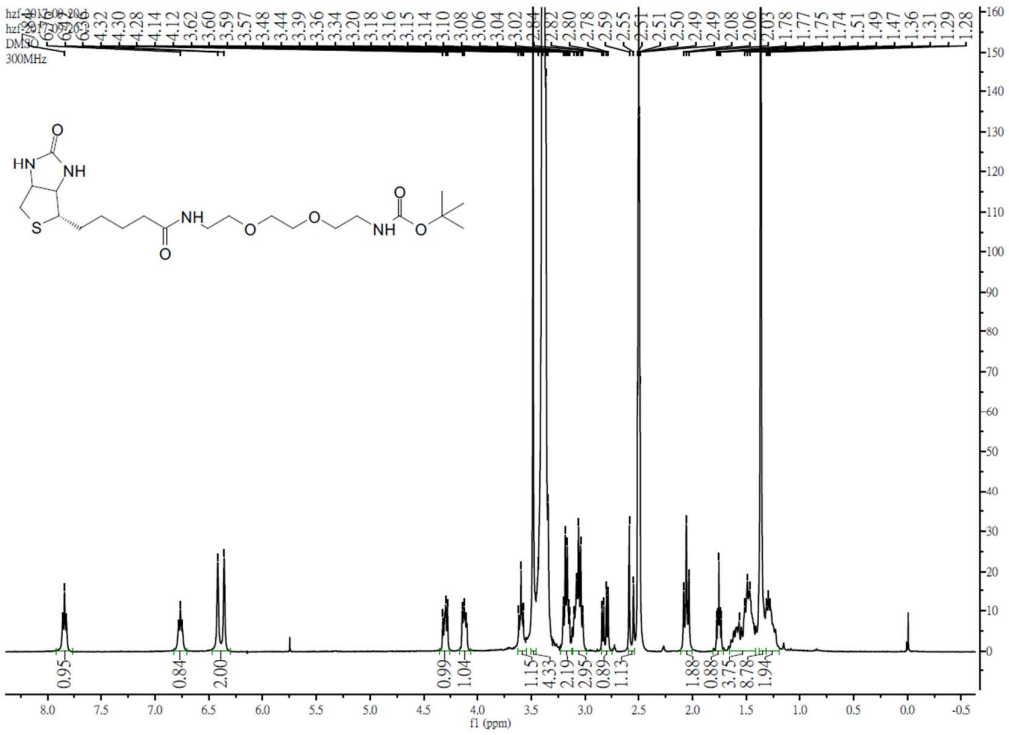

hzf-2017-06-16-2-C
hzf-2017-06-16-2-C
DMSO
500MHz
168.08
57.94
49.33
49.17
49.00
48.83
48.67
38.68
f1 (ppm)

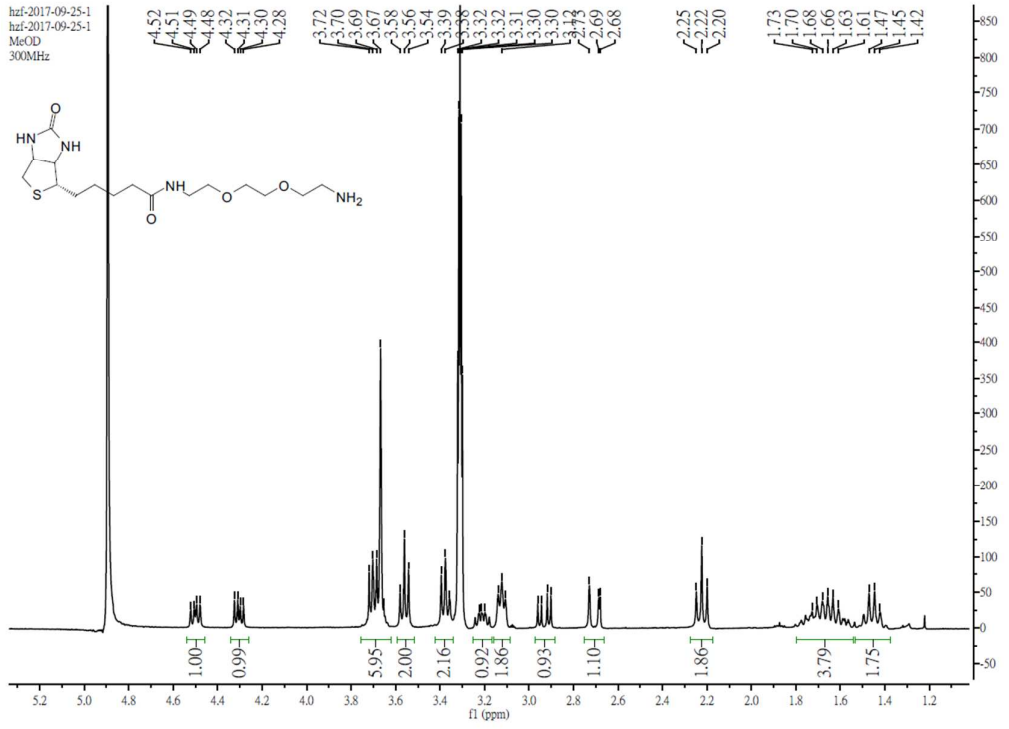

7.461
7.451
7.433
7.431
7.418
7.416
7.260
6.249
5.297
5.014
3.441
3.425
3.415
3.409
3.399
1.874
1.857
1.841
5.00
0.94
1.06
4.06
2.12
f1 (ppm)

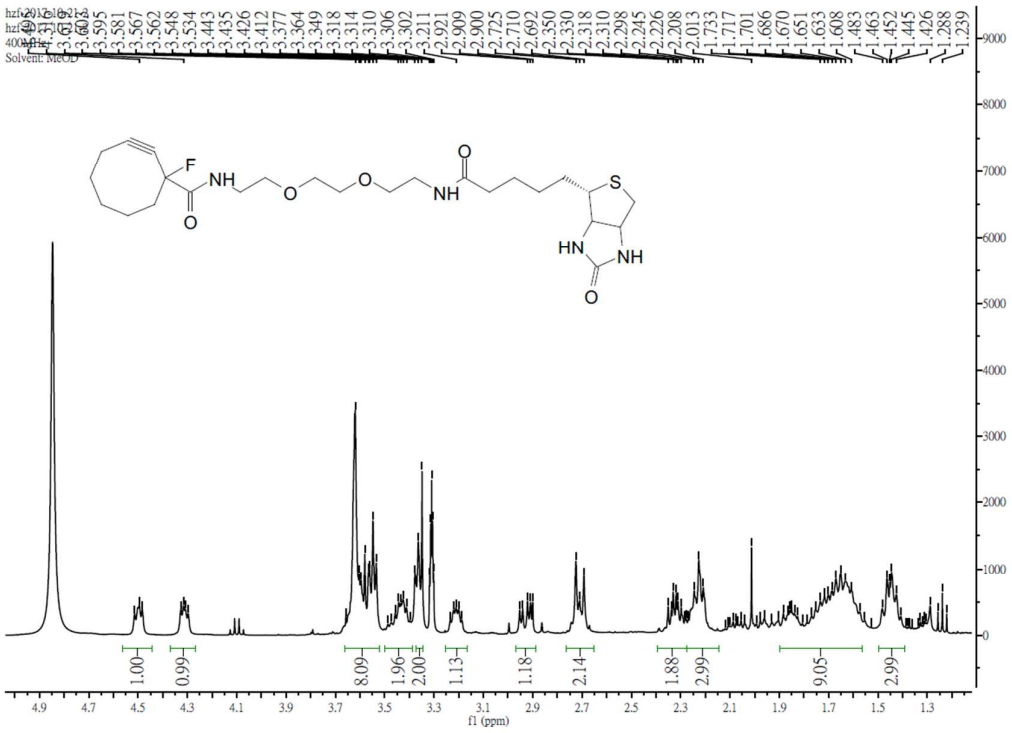

162.
132.382
131.220
128.797
128.109
79.625
77.478
77.160
76.843
49.310
38.253
28.771
f1 (ppm)

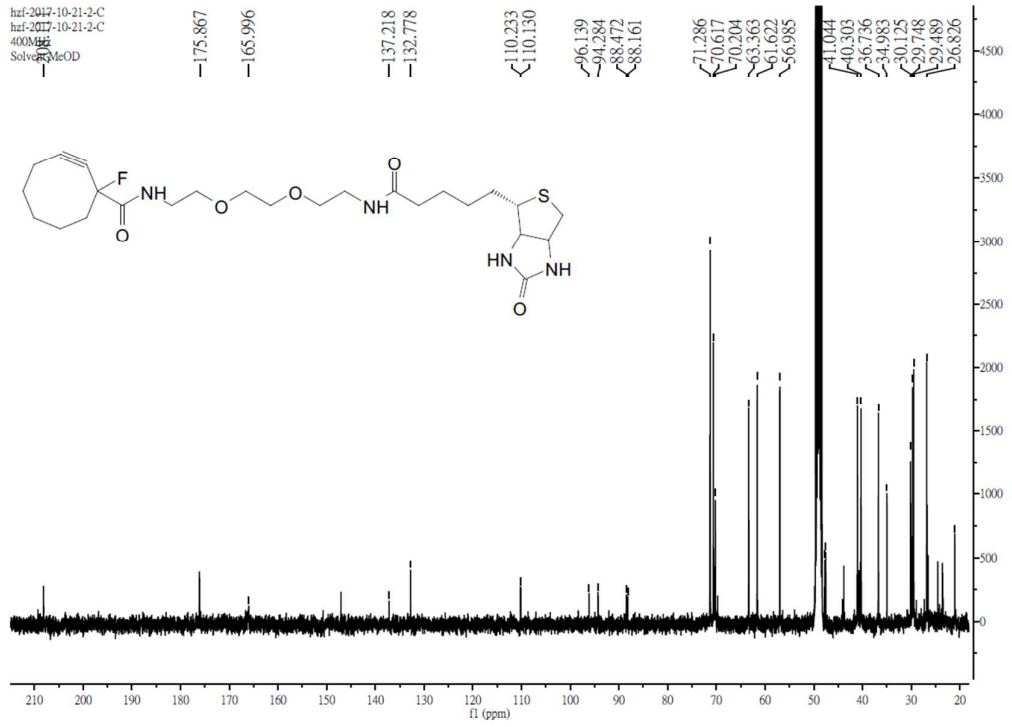

7.462
7.449
7.446
7.431
7.429
7.413
7.260
5.894
5.869
5.851
5.826
5.279
5.276
5.224
5.221
5.199
5.195
3.954
3.927
3.923
3.919
3.915
3.912
3.908
3.905
3.901
3.898
3.894
O
N
NH
O
5.00
0.81
1.02
1.98
1.03
2.01
f1 (ppm)


hzf-2017-11-16-1
hzf-2017-11-16-2
400MHz
Solvent CDCl3
162.174
133.008
132.255
130.807
128.433
127.797
116.788
79.122
42.470
O
N
NH
O
f1 (ppm)


DMSO
300MHz
f1 (ppm)


hzf-2017-09-25-1
hzf-2017-09-25-1
MeOD
300MHz
f1 (ppm)


Solvent: MeOD
F
NH
O
O
O
NH
O
S
HN
NH
O
1.00
0.99
8.09
1.96
2.00
1.13
1.18
2.14
1.88
2.99
9.05
2.99
f1 (ppm)


Solvent: MeOD
175.867
165.996
137.218
132.778
110.233
110.130
96.139
94.284
88.472
88.161
71.286
70.617
70.204
63.363
61.622
56.985
41.044
40.303
36.736
34.983
30.125
29.748
29.489
26.826
F
NH
O
O
O
NH
O
S
HN
NH
O
f1 (ppm)

References

1. H. Lin, Y. Jiang, Q. Zhang, K. Hu, Z. Li, *Chem. Commun.*, **2016**, *52*, 10389-10391.
2. Y.-H. Chen, J. T. Yang, K. H. Chau, *Biochemistry*, **1974**, *13*, 3350-3359.
3. N. E. Shepherd, H. N. Hoang, G. Abbenante, D. P. Fairlie, *J. Am. Chem. Soc.*, **2005**, *127*, 2974-2983.
4. S. Lin, X. Yang, S. Jia, A. M. Weeks, M. Hornsby, P. S. Lee, R. V. Nichiporuk, A. T. Iavarone, A. M. Weeks, F. D. Toste, C. J. Chang, *Science*, **2017**, *355*, 597-602.
5. L. E. Smeenk, N. Dailly, H. Hiemstra, J. H. van Maarseveen, P. Timmerman, *Org. Lett.*, **2012**, *14*, 1194-1197.
6. M. K. Schultz, S. G. Parameswarappa, F. C. Pigge, *Org. Lett.*, **2010**, *12*, 2398-2401.
7. K. Chiba, M. Asanuma, M. Ishikawa, Y. Hashimoto, K. Dodo, M. Sodeoka, T. Yamaguchi, *Chem. Commun.*, **2017**, *53*, 8751-8754.
8. S. Pronk, S. Páll, R. Schulz, P. Larsson, P. Bjelkmar, R. Apostolov, M. R. Shirts, J. C. Smith, P. M. Kasson D. Van Der Spoel, *Bioinformatics*, **2013**, *29*, 845-854.
9. W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, M. L. Klein, *J. Chem. Phys.*, **1983**, *79*, 926-935.
10. T. Darden, D. York, L. Pedersen, *J. Chem. Phys.*, **1993**, *98*, 10089-10092.
11. G. Bussi, D. Donadio, M. Parrinello, *J. Chem. Phys.*, **2007**, *126*, 014101.
12. H. J. Berendsen, J. v. Postma, W. F. Van Gunsteren, A. DiNola, J. Haak, *J. Chem. Phys.*, **1984**, *81*, 3684-3690.
13. B. Hess, H. Bekker, H. J. Berendsen and J. G. Fraaije, *J. Comput. Chem.*, **1997**, *18*, 1463-1472.
14. I.-C. Lin, M. E. Tuckerman, *J. Phys. Chem. B*, **2010**, *114*, 15935-15940.
15. X. Daura, K. Gademann, B. Jaun, D. Seebach, W. F. Van Gunsteren, A. E. Mark, *Angew. Chem. Int. Ed.*, **1999**, *38*, 236-240.