Supporting Information

Photo-induced synthesis of chiral Galaxamide analogs and the biological activities against human tumor cells

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Contents

1. Experimental

General

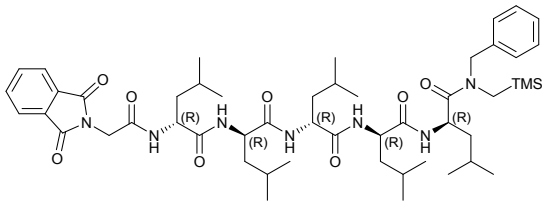
L-leucine (61-90-5), Di-tert-butyl dicarbonate (24424-99-5), N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinline (EEDQ, 16357-59-8), N-[(Trimethylsilyl)methyl]benzylamine (53215-95-5), Phthalylglycyl chloride (6780-38-7) and Trifluoroacetic acid (TFA, 76-05-1) were purchased from Energy Chemical. Dichloromethane, methanol, ethyl acetate, petroleum ether, 1,4-Dioxane were analytical reagent. Dulbecco's modied eagle medium (DMEM), penicillin, fetal bovine serum (FBS), and streptomycin were purchased from Beijing Dingguo Biotechnology Co. Phosphatebuffered saline (PBS) purchased from Invitrogen (10010) was used as a balanced salt solution in cell culture. All the solvents were distilled and puried by standard procedures. All the above chemicals reagents were used without further purification. ^{1}H and ^{13}C-NMR spectra were recorded at 400 and 100 MHz, respectively, on an AMX400 spectrometer (Bruker, Bremen, Germany) with tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a JEOL JMS-700 spectrometer using the fast atom bombardment (FAB) or electron impact (EI) mode.

Preparation of trimethylsilylbenzylamide dipeptide

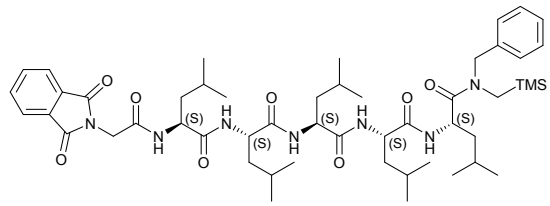
Boc-leucine (1.5 g, 6.2 mmol) and N-[(trimethylsilyl)methyl]benzylamine (1.93 g, 10 mmol) were dissolved in 30 mL anhydrous dichloromethane, EEDQ (2.4 g, 9.7 mmol , in 10 mL of dichloromethane). It was added dropwise with stirring at room temperature. The Bi drops were continued to stir for 24 hours. After the reaction, the reaction solution was washed twice with a 16 mL aqueous solution of sodium chloride, and the organic layer was dried over anhydrous sodium sulfate and concentrated, and the residue was purified by silica gel column chromatography (mobile phase VEA / VPE = 1:4) N-Boc-Leu-Si$(CH_3)_3$ (a solid). N-Boc-Leu-Si$(CH_3)_3$ was dissolved in 20 mL of anhydrous dichloromethane, and 10 mL of trifluoroacetic acid was added dropwise, followed by stirring for 3 hours. After removing the trifluoroacetic acid, the methylene chloride was evaporated to dryness, and the residue was crystallised eluted eluted eluted with 20 $(CH_3)_3$ 2.53g (butter). Leu-Si$(CH_3)_3$ (2.53 g, 6 mmol) and Boc-leucine (1.32 g, 7 mmol) were dissolved in 20 mL of anhydrous dichloromethane. EEDQ (2.59 g, 10.5 mmol in 10 ml THF) was added dropwise with stirring at room temperature for 72 hours. After completion of the reaction, the reaction solution was washed twice with 10 mL of aq. sodium chloride, and the organic layer was dried over anhydrous sodium sulfate and evaporated. 3 hours. After removing the trifluoroacetic acid dichloromethane, the residue was dissolved in 10 mL of dichloromethane, washed twice with 10 mL of sodium chloride aqueous solution, dried over anhydrous sodium sulfate and concentrated to give Leu-Leu-Si$(CH_3)_3$ 2.05 g (also solid)). The same method was used to synthesize Leu-Leu-Leu-Si$(CH_3)_3$, Leu-Leu-Leu-Leu-Si$(CH_3)_3$, Leu-Leu-Leu-Leu-Leu Si$(CH_3)_3$.

Preparation of N-phthaloyl-Gly-Leu-Leu-Leu-Leu-Leu-Si$(CH_3)_3$

Leu-Leu-Leu-Leu-Leu-Si$(CH_3)_3$ (3.57 g, 0.01 mol) and triethylamine (1 mL) were dissolved in anhydrous dichloromethane, then phthalimide acetyl chloride was added (2.14 g, 0.01 mol, 10 mL of 1,4-dioxane was added dropwise. After stirring at room temperature for 30 minutes, the reaction solution was washed twice with 20 mL of water. The organic layer was dried with anhydrous sodium sulfate and then evaporated and evaporated to silica gel.



N-Phthaloyl-Gly-*D*-Leu-*D*-Leu-*D*-Leu-*D*-Leu-*D*-Leu-*D*-Leu-Si(CH_3)$_3$.(1a) while solid (yield 65%). ^{1}HNMR($CDCl_3$) δ : 0.01~0.11(m, 9H, $SiMe_3$), 0.84~0.91 (m, 30H, CH_3), 1.21~1.28 (m, 5H, **CH**(CH_3)$_2$), 1.30~1.54 (m, 10H, CH**CH$_2$**CH), 2.80~3.01 (m, 2H, **CH$_2$**$SiMe_3$), 4.11~4.19 (m, 2H, **CH$_2$**Ph), 4.33~4.58 (m, 5H, CH_2**CH**NH(CO)), 4.64~ 4.88(m, 2H, NH**CH2**CON), 7.10~7.34 (m, 5H, ArH) 7.60~7.85 (m, 4H, Phthaloyl); ^{13}CNMR($CDCl_3$) δ : -2.3, -2.1, 21.9, 22.0, 22.2, 23.9, 24.1, 24.2, 39.6, 41.0, 40.4, 41.1, 42.0, 42.9, 43.1, 43.5, 48.1, 48.2, 48.3, 48.5, 48.6, 48.8, 50.0, 50.3, 50.7, 50.8, 122.6, 125.9, 126.9, 127.8, 128.0, 131.5, 131.6, 133.1, 166.6, 166.7, 166.9, 170.8, 170.9. HRMS (ESI) m/z calcd for $C_{51}H_{80}N_7O_8^+$ $(M+H)^+$ 945.5759, $C_{51}H_{82}N_7O_8^+$ $(M+3H)^+$ 948.5996, $C_{51}H_{83}N_7O_8^+$ $(M+4H)^+$ 949.6075, found 946.5801, 948.5970, 949.6045.



N-Phthaloyl-Gly-*L*-Leu-*L*-Leu-*L*-Leu-*L*-Leu-*L*-Leu-*L*-Leu-Si(CH_3)$_3$ (**2a**) while solid (yield 65%). ^{1}HNMR($CDCl_3$) δ : 0.01~0.14(m, 9H, $SiMe_3$), 0.92~0.95 (m, 30H, CH_3), 1.27~1.34 (m, 5H, **CH**(CH_3)$_2$), 1.40~1.62 (m, 10H, CH**CH$_2$**CH), 2.50~3.09 (m, 2H, **CH$_2$**$SiMe_3$), 4.22~4.25 (m, 2H, **CH$_2$**Ph), 4.39~4.90 (m, 5H, CH_2**CH**NH(CO)) , 5.11~ 5.25(m, 2H, NH**CH$_2$**CON), 7.13~7.33 (m, 5H, ArH), 7.68~7.89 (m, 4H, Phthaloyl); ^{13}CNMR($CDCl_3$) δ :-2.4, -2.2, 20.8, 21.5, 21.9, 22.0, 22.5, 23.7, 23.8, 24.1, 39.5, 41.0, 41.3, 41.4, 41.6, 41.7, 46.0, 49.7, 50.6, 50.7, 51.0, 52.3, 122.6, 125.9, 126.4, 127.0, 127.8, 128.1, 131.4, 133.1, 135.6, 165.2, 166.8, 170.4, 170.6, 171.0, 171.3. HRMS (ESI) m/z calcd for $C_{51}H_{80}N_7O_8^+$ $(M+H)^+$ 945.5759, found 956.5845; calcd for $C_{51}H_{81}N_7O_8^+$ $(M+2H)^+$ 947.5817, found 947.5878; calcd for $C_{51}H_{79}N_7O_8Na^+$ $Na(M+Na)^+$ 968.5657, found 968.5658.

2. Determine the absolute configurations (ACs) of 1 and 2

Due to the more hydrogen-bonds interaction existed in compound 1 compared to that in compound 2, compound 1 present more γ-ture features, showing strong CE around 230 nm. The origin of the CEs at 238 in the experimental ECD spectra of 1 could be explained by molecular orbital (MO) analysis at the same level as the ECD calculation, as shown in Figure S2. The negative CE at 296 nm might be caused by the transition from MO175 to MO178 attributed to the transitions from the peptides skeleton to isoindolinone and phenyl grooup. The transition from MO171 to MO177 also contributed the absorption, caused by the electrons distributed in the peptides skeleton transferring to the isoindolinone structure.

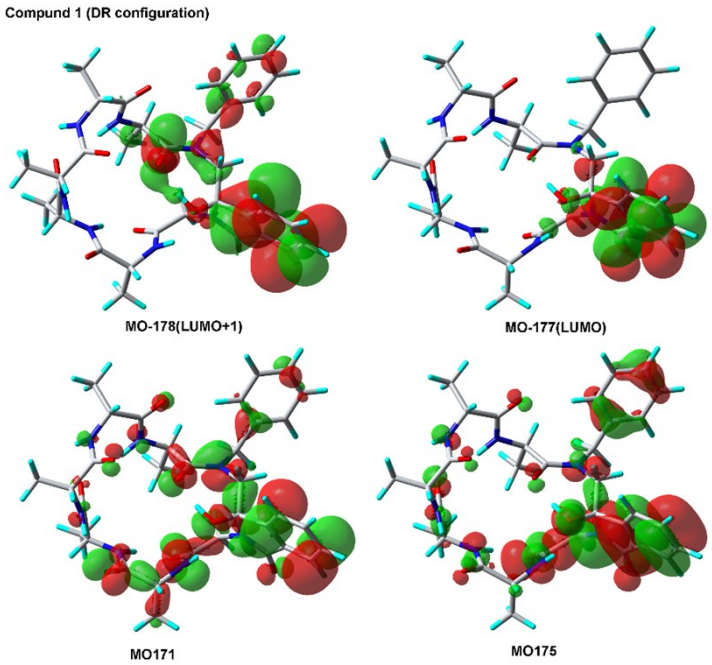


Figure S1. The molecular orbital (MO) involved of the CEs at 238 in the experimental ECD spectra of 1

The molecular orbital (MO) involved of the CEs at 234 in the experimental ECD spectra of 2 were shown in Figure S3. The negative CE at 232 nm for compound 2 was caused by the transition from MO166, MO167 to MO177, and from MO170 to MO178. Similar to that of compound 1 these transitions were also attributed to electron transfer from the cyclic peptide skeleton to isoindolinone and phenyl group.

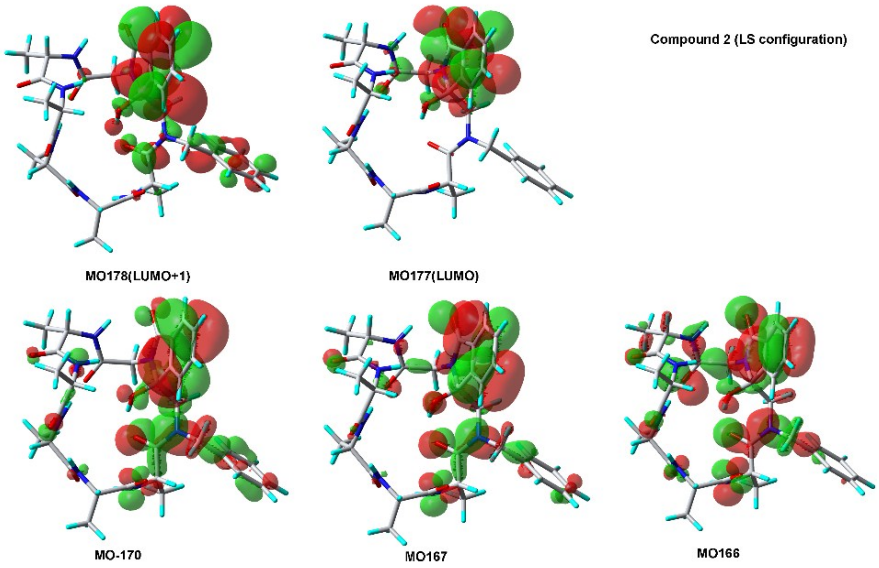


Figure S2. The molecular orbital (MO) involved of the CEs at 234 in the experimental ECD spectra of 2

3. In vitro antitumor activities

The test was divided into a blank control group and a cyclic peptide experimental group. Human cervical cancer HepG-2 cells in logarithmic growth phase (4×10^3 cells/mL) were trypsinized, and then cell suspension with

a cell concentration of 6000 cells/mL was prepared with 10% by mass of calf serum medium. , inoculated in a 96-well culture plate. The experimental group was given different concentrations of samples. Six wells were repeated for each dose. After administration for 48 h, add MTT solution (100 μL, 0.5 mg/mL) to each well, continue to incubate at 37 ° C for 4 h, discard the supernatant, add 150 μL of DMSO to dissolve the formazan precipitate, and mix with a shaker. Thereafter, the optical density (OD) value was measured at a wavelength of 490 nm on a microplate reader. Cell viability (%) is calculated using the following formula:

Survival rate (%) = A_{490} (sample) / A_{490} (control) × 100%

The A_{490} (sample) represents the absorbance value of the cells treated with different concentrations of sample, and A_{490} (control) represents the absorbance value of DMEM + 10% fetal bovine serum cells.

Figure 2 shows the dose-response curves for 1 and 2 for HepG-2 cells. The black histogram represents the cell viability of the 1 experimental group, and the red histogram represents the cell viability of the 2 experimental group. The concentrations of the two groups were selected at 18.75, 25, 37.5, 50, 75, 100, 150, 200 and 300 μM for study. The results clearly show that as the drug concentration increases, cell viability decreases. When the concentration of 1 reached 300 μM, the cell viability decreased to 4%, indicating that 1 has a good cell growth

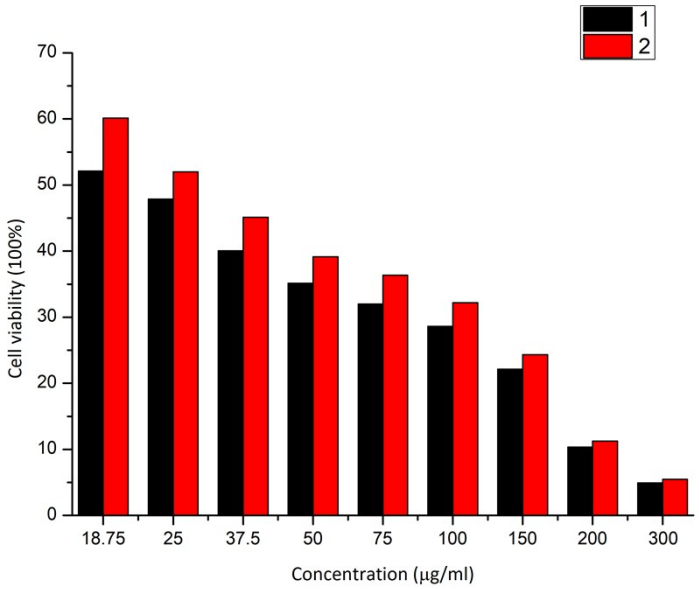


Figure S3. Fig.3 Cell viability of two groups: experiment groups (1, black columns), experimental groups (2, red columns).

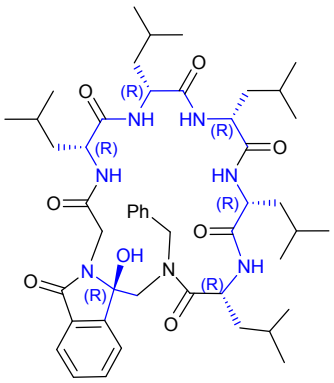
inhibitory ability.

4. Cell morphological changes after treatment of samples

According to the results of the MTT experiment, 1 has the best biological activity. This paper selects 1 to study the morphological changes of cells. When the cells were treated with 1 for 0 hours, 12 hours, 24 hours, and 48 hours, the morphology of the cells was observed under a bright field using a fluorescence inverted microscope (FIM). HepG-2 cells were seeded on 6-well plates, cultured for 24 hours, and compounds were added for 16 hours, and changes were observed using a fluorescence inverted microscope (FIM).

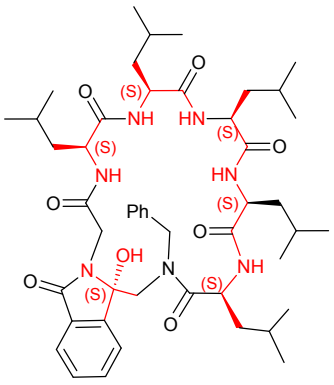
5. Irradiation of 1a and 2a to obtain 1 and 2

Nitrogen purged solutions of the substrates in the indicated solvents were irradiated by using Pyrex glass filtered light in an water cooled immersion reactor for time periods required to to give >90% coversion (examined by TLC). Concentration of the photoproducts were followed by column chromatography to yield the pure products listed below. In brief, 0.5 g of compound 7 in 200 mL of anhydrous methanol were placed in a reactor, then ventilated nitrogen flow for 30 min. Upon maintaining the ventilation of nitrogen, the solutions were irradiated by ultraviolet light (Pyrex tube filtered-light λ > 290 nm).



Compound 1

Isoindolinone-cyclo-*D*-Leu-*D*-Leu-*D*-Leu-*D*-Leu-*D*-Leu (**1**): while solid (yield 30%). ^{1}HNMR($CDCl_3$) δ : 0.84~0.92 (m, 30H, CH_3), 1.15~1.23 (m, 5H, **CH**(CH_3)$_2$), 1.56~1.60 (m, 10H, CH**CH$_2$**CH), 3.91~3.93 (m, 2H, CO**CH2**C(OH)N), 4.11~ 4.73(m, 2H, **CH$_2$**Ph and m, 5H, CH_2**CH**NH(CO)), 4.88~ 5.04(m,2H, NH**CH$_2$**CON),7.14~7.55 (m, 9H, ArH); ^{13}CNMR($CDCl_3$) δ : 21.2, 21.3, 21.8, 22.0, 22.1, 22.2, 22.3, 22.4, 22.5, 22.6, 22.8, 24.2, 24.3, 40.6, 41.0, 41.1, 42.6, 51.0, 51.1, 51.3, 52.0, 60.7, 84.7, 122.8, 126.2, 128.1, 128.2, 128.3, 131.7, 133.2, 133.4, 158.7, 165.7, 167.0, 167.1, 171.1, 171.2, 171.3, 171.4, 171.8. HRMS (ESI) m/z calcd for $C_{48}H_{72}N_7O_8^+$ $(M+H)^+$ 874.5443, $C_{48}H_{73}N_7O_8^+$ $(M+2H)^+$ 875.5530, found 874.5453, 875.5535.



Compound 2

Isoindolinone-cyclo-*L*-Leu-*L*-Leu-*L*-Leu-*L*-Leu-*L*-Leu-*L*-Leu (**2**): while solid (yield 30%). 1HNMR($CDCl_3$) δ : 0.0.7~0.93 (m, 30H, CH_3), 1.21~1.24 (m, 5H, **CH**(CH_3)$_2$), 1.38~1.55 (m, 10H, CH**CH$_2$**CH), 3.68~3.74 (m, 2H, CO**CH$_2$**C(OH)N), 3.85~ 4.69(m, 2H, **CH$_2$**Ph and m, 5H, CH_2**CH**NH(CO)), 4.92~ 5.13(m,2H, NH**CH$_2$**CON),7.05~7.67 (m, 9H, ArH); ^{13}CNMR($CDCl_3$) δ :21.2, 21.7, 22.4, 22.5, 22.6, 22.7, 22.9, 23.3, 23.9, 24.8, 24.9, 40.6, 41.9, 42.9, 51.0, 51.5, 51.8, 52.6, 60.1, 83.9, 126.2, 126.3, 126.7, 127.7, 128.6, 128.8, 129.8, 160.5, 165.5, 167.0, 167.3, 171.4, 171.6, 171.7, 171.8,. HRMS (ESI) m/z calcd for $C_{48}H_{72}N_7O_8^+$ $(M+H)^+$ 874.5443, $C_{48}H_{73}N_7O_8^+$ $(M+2H)^+$ 875.5530, $C_{48}H_{71}N_7O_8Na^+$ $(M+Na)^+$ 896.5262, found 874.5447, 875.5530, 896.5264

Computational details:

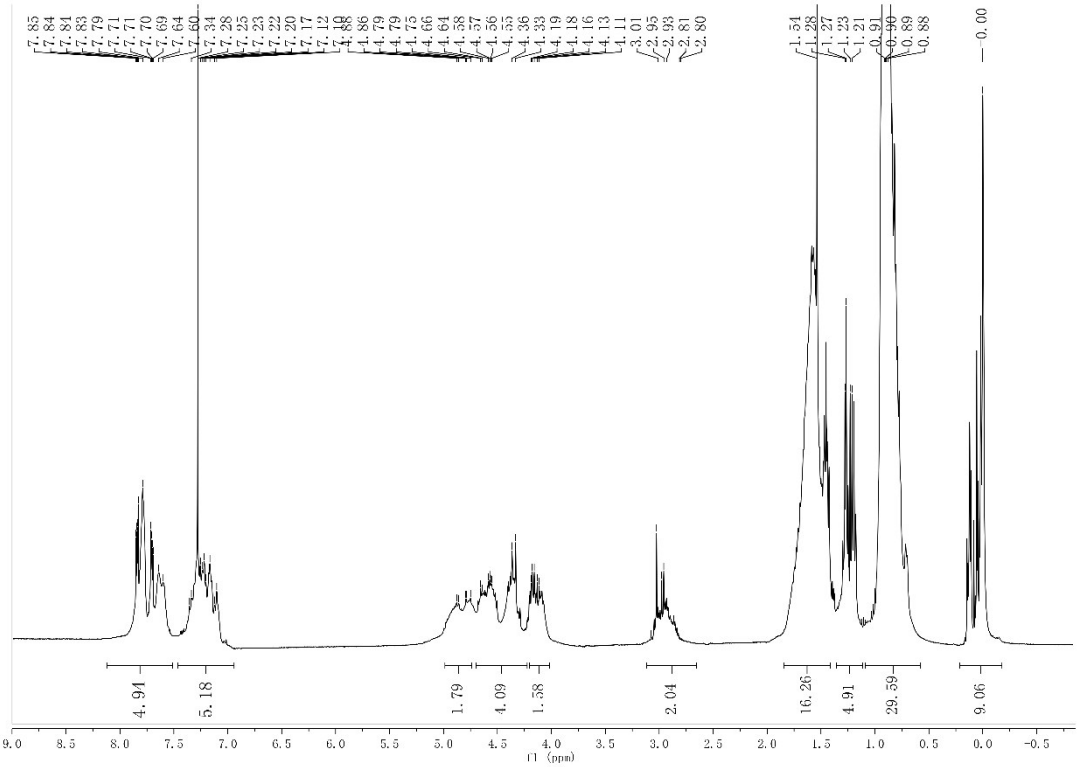
The conformational analysis was performed by arbitrarily fixing the absolute configuration of C-3 for compound 1 and 2, using the Spartan 08 package[1] with the MMFF94 molecular mechanics force field and Monte Carlo searching. The obtained conformers within 1 kcal/mol were geometrically optimized at the level of DFT/B3LYP/6-31G** in the program package Gaussina 09.[2] Then the conformers with ΔG 4 kcal/mol were used for further ECD simulations. TD-DFT/CAM-B3LYP/TZVP was employed to calculate excitation energy (denoted by wavelength in nm) and rotatory strength R. ECD curves were calculated based on rotatory strengths using half bandwidth of 0.3 eV with conformers.

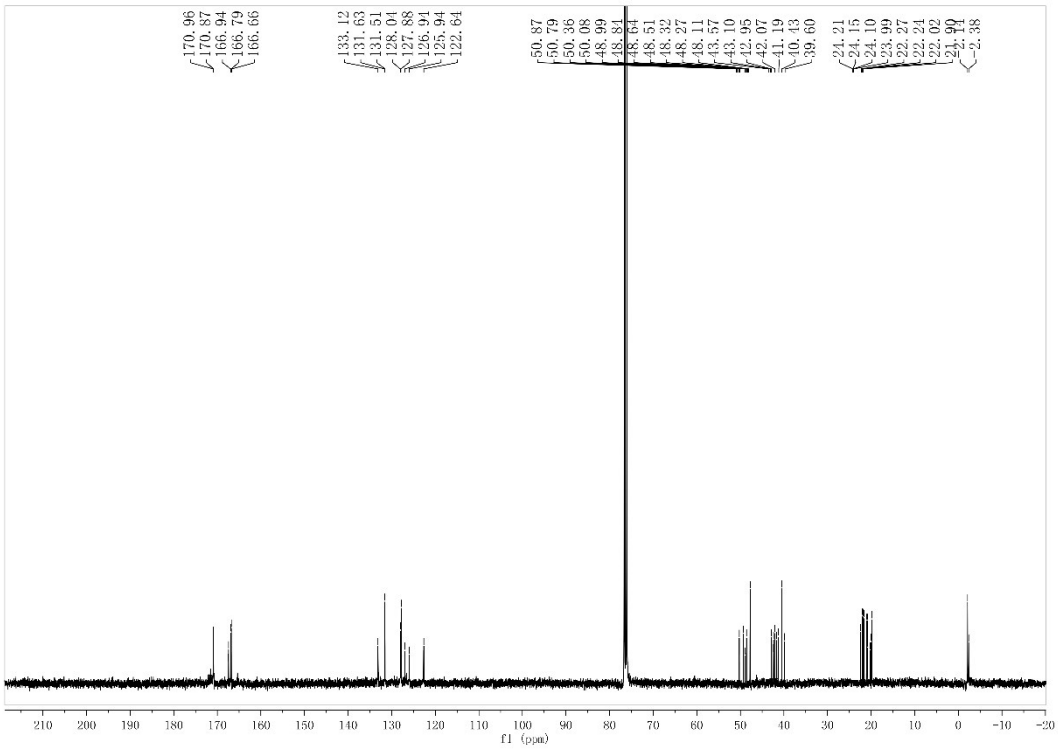
References:

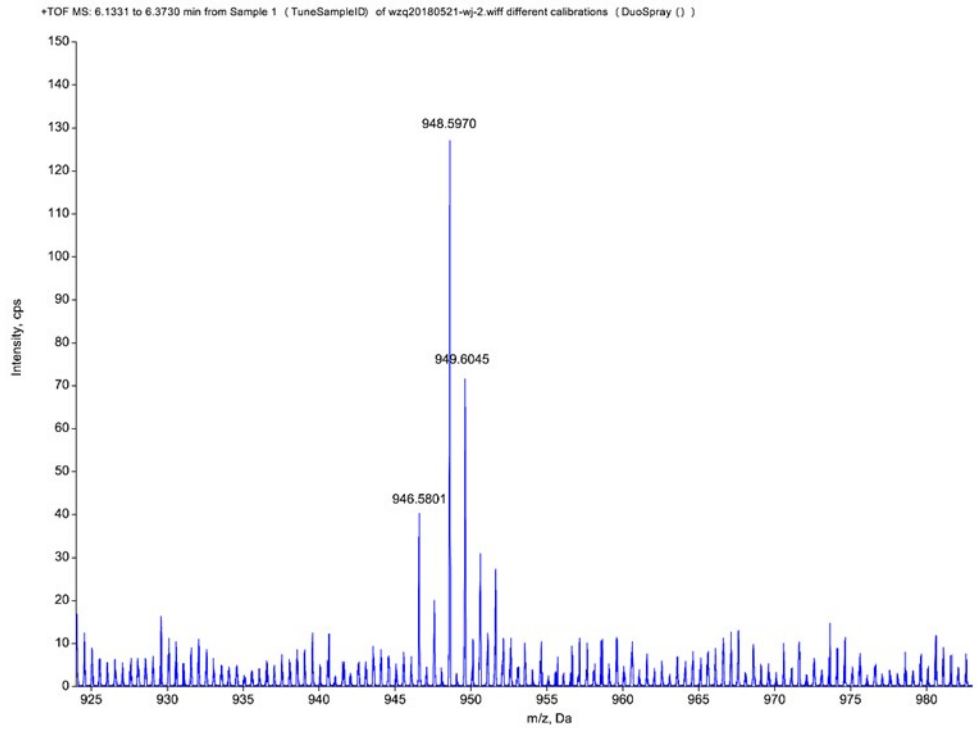
1. Spartan'08, Wavefunction, Inc. Irvine, CA

2. Gaussian 09, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.

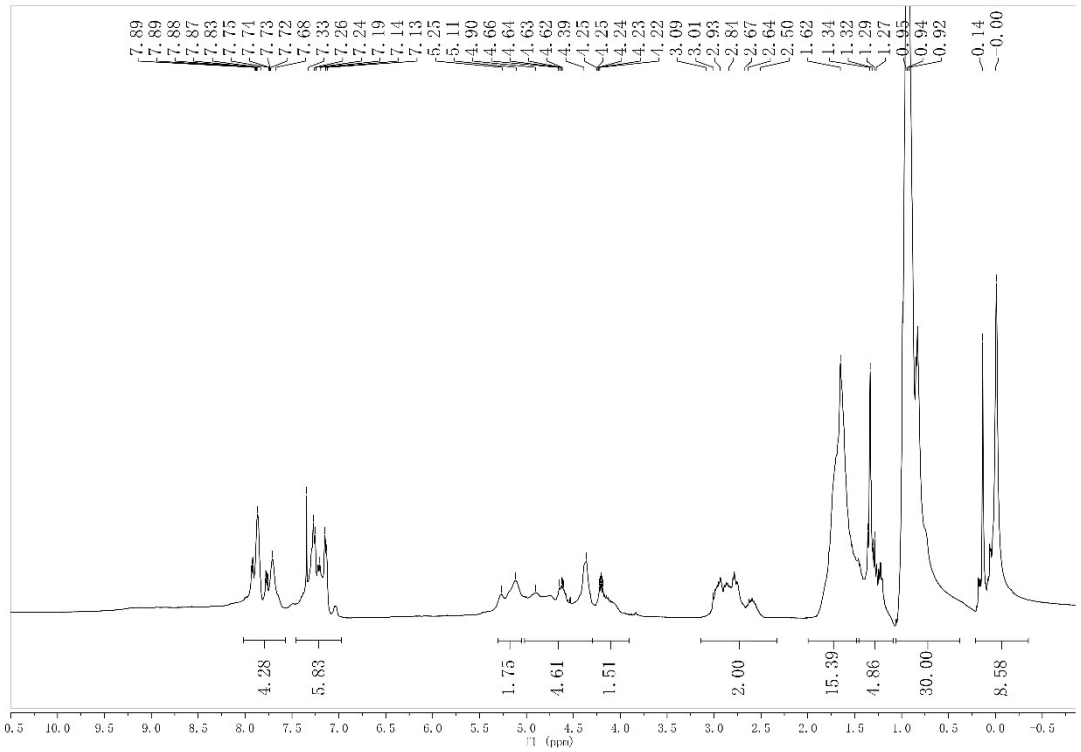
6. ^{1}H, ^{13}C-NMR and HRMS of linear peptide precursor **1a** (D-Leu)

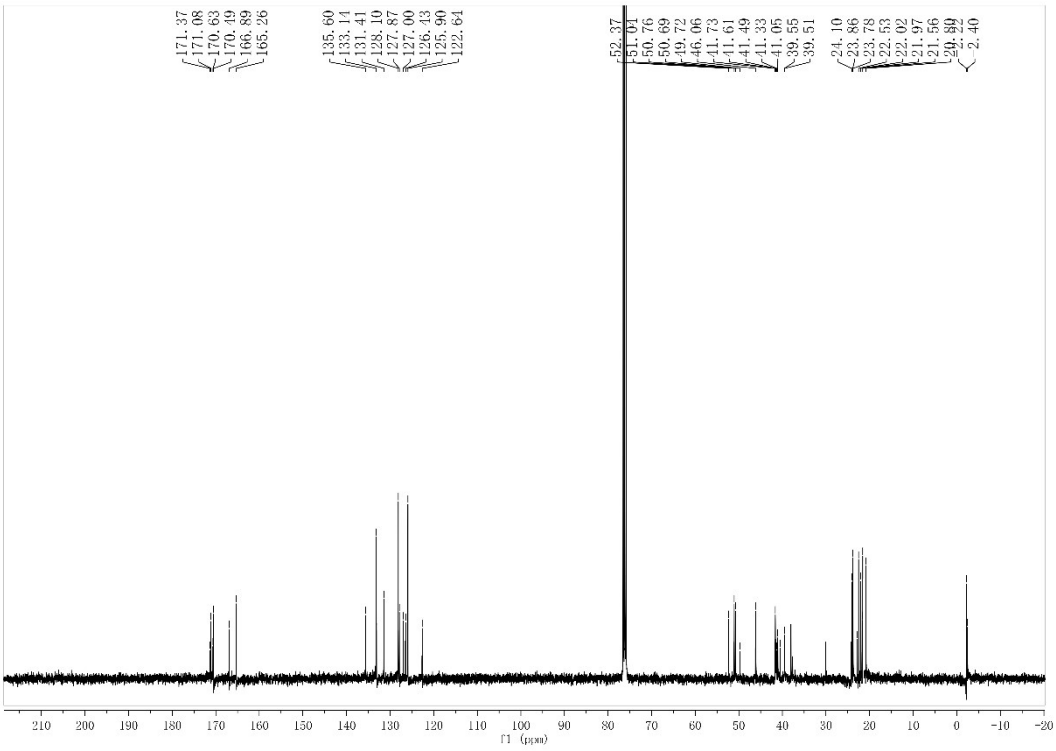






7. ^{1}H, ^{13}C-NMR and HRMS of linear peptide precursor **2a** (L-Leu)

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52.37
51.01
50.76
50.69
49.72
46.06
41.73
41.61
41.49
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21.97
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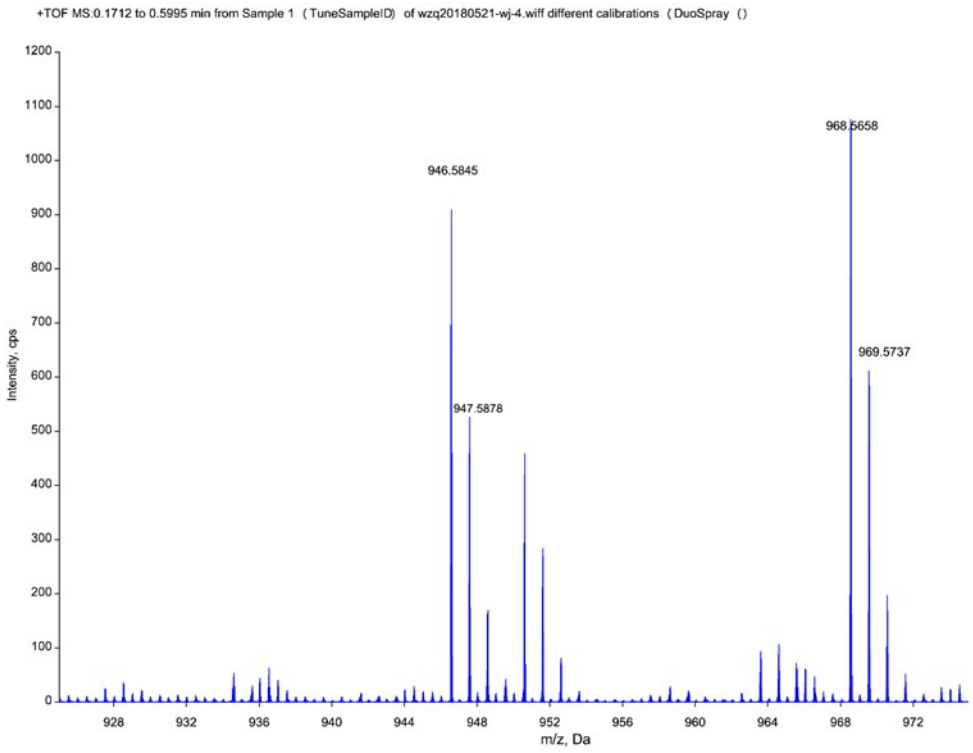
2.40
f1 (ppm)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946.5845
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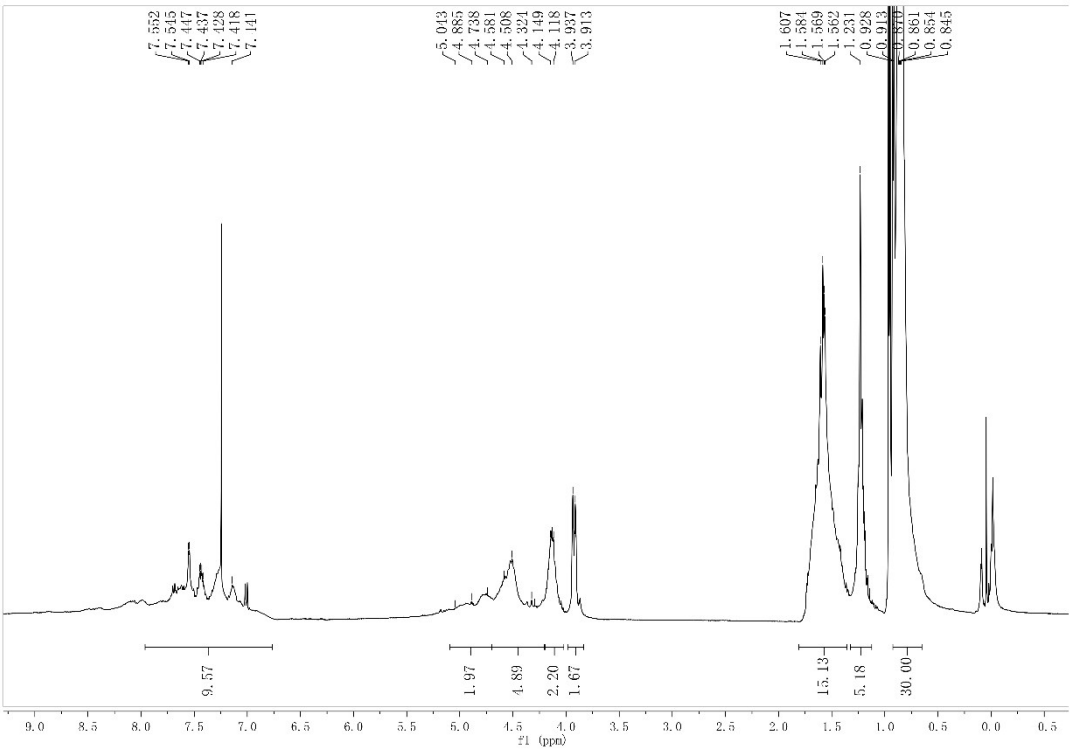
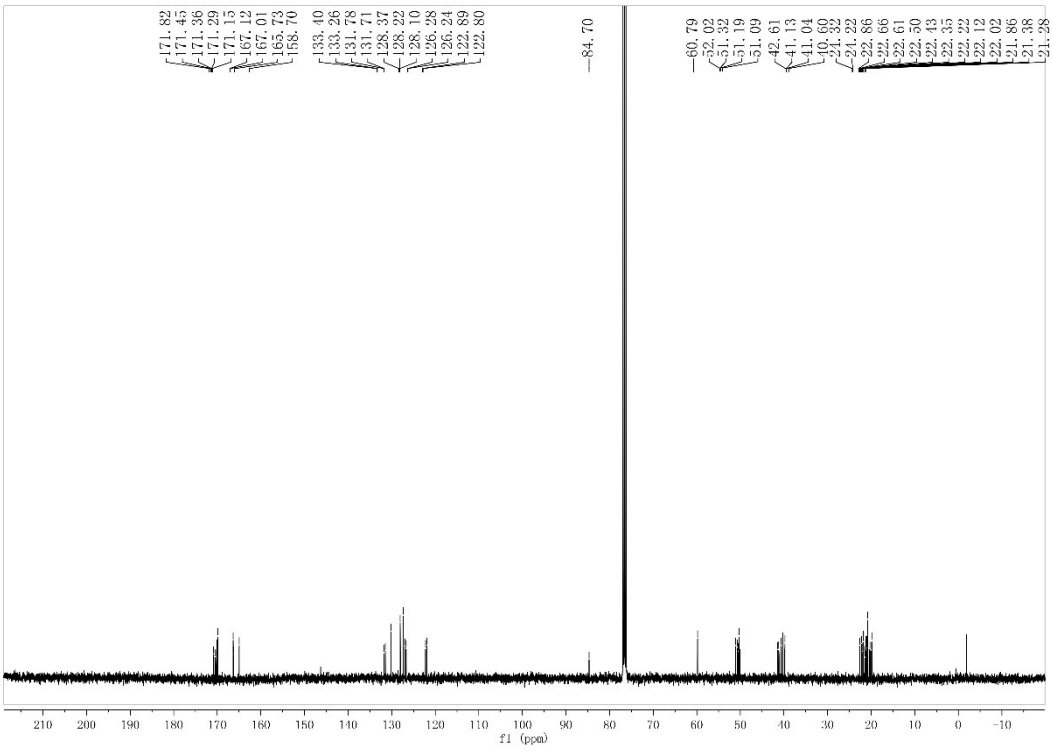
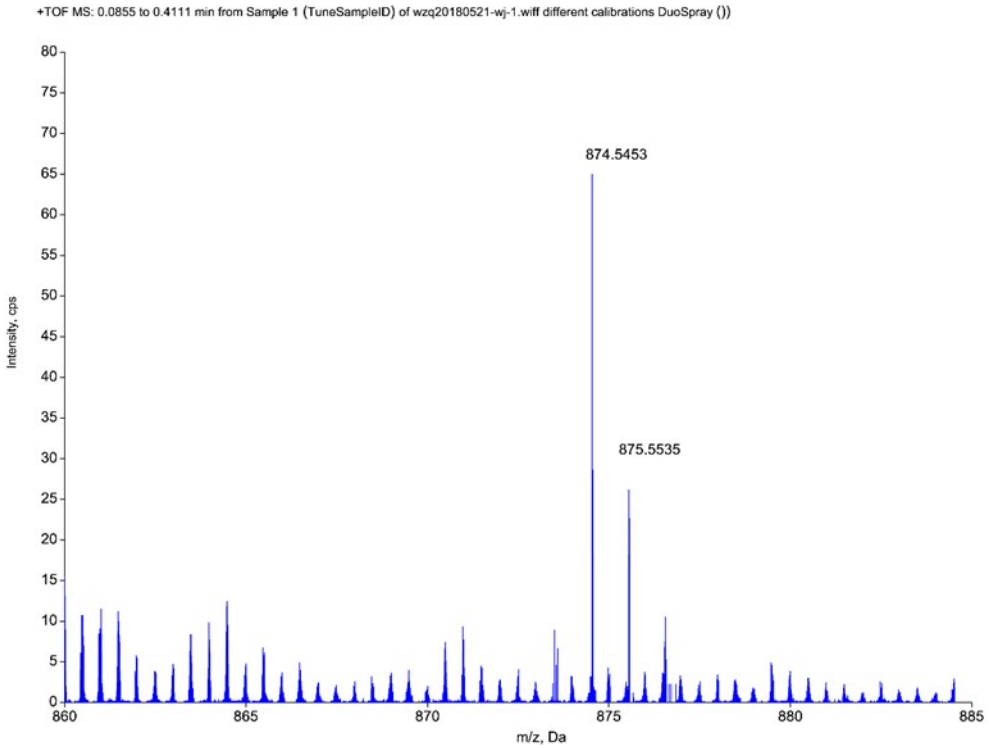
947.5878
Intensity, cps
m/z, Da

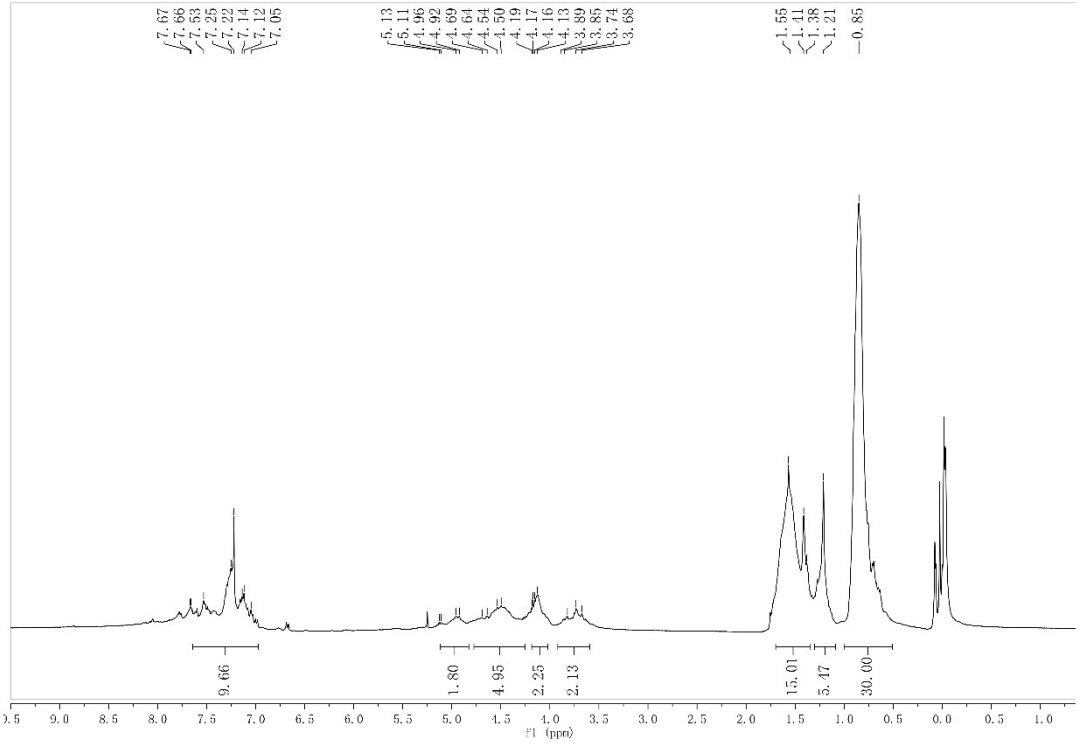
8.^{1}H, ^{13}C-NMR and HRMS of cyclopeptide **1**

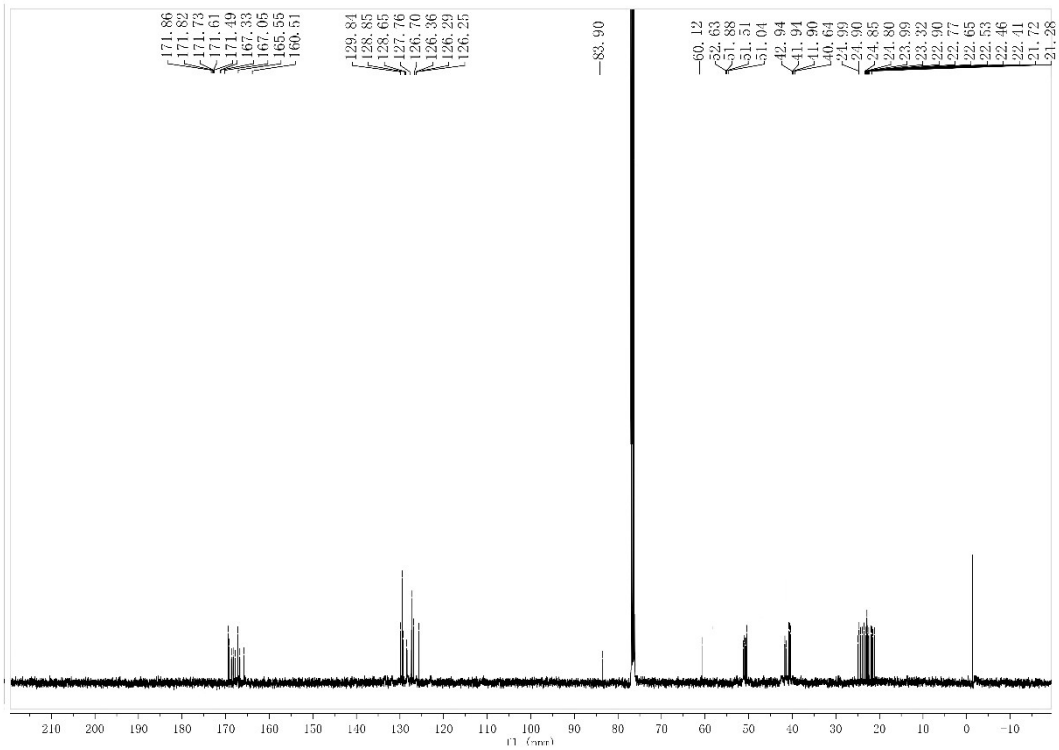






9.^{1}H, ^{13}C-NMR and HRMS of cyclopeptide **2**

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24.90
24.85
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23.99
23.32
22.90
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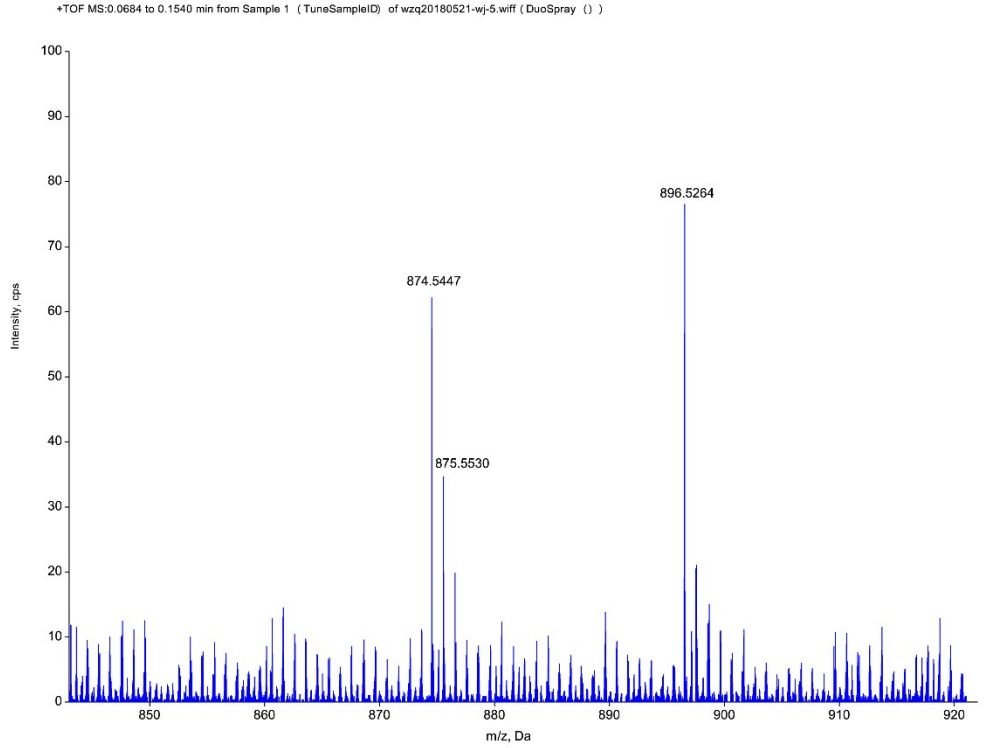
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40
30
20
10
0
850
860
870
880
890
900
910
920