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Supporting Information

for

Photo-induced synthesis, structure and *in vitro* bioactivity of a natural cyclic peptides Yunnanin A analog

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Experimental

General

Boc-L-proline(15761-39-4), Glycine (56-40-6), Boc-L-phenylalanine(13734-34-4), 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 modified eagle medium (DMEM), penicillin, fetal bovine serum (FBS), and streptomycin were purchased from Beijing Dingguo Biotechnology Co. Phosphate buffered saline (PBS) purchased from Invitrogen (10010) was used as a balanced salt solution in cell culture. All the solvents were distilled and purified 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. A 450 W Hanovia medium-pressure mercury lamp surrounded by a Pyrex glass filter (λ > 290 nm) was used for electronic excitation.

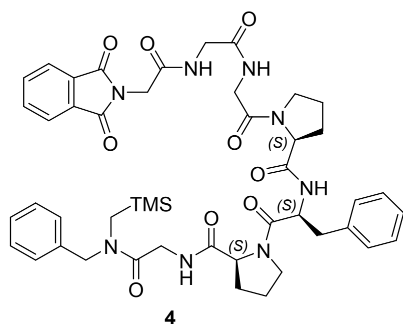
Preparation of trimethylsilylbenzylamido hexapeptides

The Boc-Glycine (1.5 g, 8.57 mmol) and N-[(Trimethylsilyl) methyl]-benzylamine (1.65 g, 8.57 mmol) was dissolved in anhydrous dichloromethane (DCM; 20 mL), EEDQ (2.54 g, 10.28 mmol in 5 mL DCM) was added dropwise with stirring at room temperature. After that, continue stirring 48 h. After completion of the reaction, the solution was washed with 15% NaCl (20 mL×2), the organic layer was dried over anhydrous sodium sulfate (Na_2SO_4) and concentrated, the residue was purified by silica gel column chromatography (mobile phase V_{EA}/V_{PE} = 1:4) to obtain N-Boc-Gly-Si(CH_3)$_3$ (yellow oil, yield: 92 %). The N-Boc-Gly-Si(CH_3)$_3$ was dissolved in anhydrous DCM (20 mL), and trifluoroacetic acid (TFA; 10 mL) added dropwise, then the mixture was stirred for 3 h. After the reaction, Saturated sodium carbonate (Na_2CO_3) was added to remove TFA, then the solution washed with 15% NaCl (20 mL×2), dried over anhydrous Na_2SO_4 and concentrated to give a chemically pure Gly-Si(CH_3)$_3$ 1.84 g (yellow oil, yield: 86 %). The Gly-Si(CH_3)$_3$ (1.84 g, 7.35 mmol) and Boc-L-proline (1.58 g, 7.35 mmol) was dissolved in anhydrous DCM (20 mL), EEDQ (2.18 g, 8.82 mmol in 5 ml DCM) was added dropwise with stirring at room temperature for 48 h. After completion of the reaction, the solution was washed with 15% NaCl (20 mL×2), the organic layer was dried over anhydrous Na_2SO_4 and concentrated, the residue was purified by silica gel column chromatography (mobile phase V_{EA}/V_{PE} = 1:3) to obtain N-Boc-Pro-Gly-Si(CH_3)$_3$ (a white solid, yield: 87 %). The N-Boc-Pro-Gly-Si(CH_3)$_3$ was dissolved in anhydrous DCM (10 mL), and trifluoroacetic acid (TFA; 3 mL) added dropwise, then the mixture was stirred for 3 h. After removal of TFA, the residue washed with 15% NaCl (20 mL×2), dried over anhydrous Na_2SO_4 and concentrated to give Pro-Gly-Si(CH_3)$_3$ (a white solid, yield: 84 %). The same method was used for synthesis of Phe-Pro-Gly-Si(CH_3)$_3$, Pro-Phe-Pro-Gly-Si(CH_3)$_3$, Gly-Pro-Phe-Pro-Gly-Si(CH_3)$_3$, Gly-Gly-Pro-Phe-Pro-Gly-Si(CH_3)$_3$.

Preparation of *N*-Phthalimido-Gly-Gly-Gly-Pro-Phe-Pro-Gly-Si(CH_3)$_3$ （4）

Gly-Gly-Pro-Phe-Pro-Gly-Si(CH_3)$_3$ (0.71 g, 1 mmol) and triethylamine (TEA; 2 mL) were dissolved in anhydrous DCM (10 mL), then phthalimide acetyl chloride (0.22 g, 1 mmol, in 3 mL of 1,4-dioxane) was added dropwise. After stirring at room temperature for 24 h, the solution washed with 15% NaCl (20 mL×2). The organic layer was dried over anhydrous Na_2SO_4 and concentrated, the residue was purified by silica gel column chromatography (mobile phase

V_{EA}/V_{MeOH} = 5:1) to obtain *N*-Phthalimido-Gly-Gly-Gly-Pro-Phe-Pro-Gly-$Si(CH_3)_3$ (a white solid).



N-Phthalimido-Gly-Gly-Gly-Pro-Phe-Pro-Gly-$Si(CH_3)_3$ (4): while solid (yield: 67.5%, after purification).

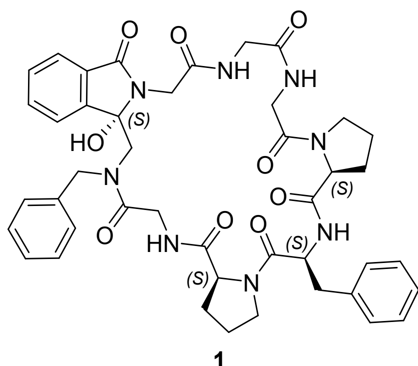
^{1}H-NMR($CDCl_3$) δ : -0.10~0.18(m, 9H, $SiMe_3$), 1.73~2.30(m, 8H, $(NCH_2\mathbf{CH_2}CH_2CH)_2$ and $(NCH_2CH_2\mathbf{CH_2}CH)_2$), 2.58~2.77(m, 2H, N$\mathbf{CH_2}$$SiMe_3$), 2.82~3.00(m, 2H, NH$\mathbf{CH_2}$CO), 3.19~3.43(m, 2H, NH$\mathbf{CH_2}$CO), 3.44~3.61(m, 2H, NH$\mathbf{CH_2}$CO), 3.81~4.14(m, 6H, $(N\mathbf{CH_2}CH_2CH_2CH)_2$ and N$\mathbf{CH_2}$CO), 4.29~4.56(m, 5H, $(\mathbf{CH_2}Ph)_2$ and **CH**CH_2Ph), 4.58~4.82(m, 1H, $NCH_2CH_2CH_2\mathbf{CH}$), 4.83~5.02(m, 1H, $NCH_2CH_2CH_2\mathbf{CH}$), 7.08~7.89(m, 14H, Ar**H**);

^{13}C-NMR($CDCl_3$) δ : -0.001, 25.4, 26.2, 30.0, 30.9, 33.3, 40.0, 41.8, 42.5, 43.4, 44.1, 47.9, 48.7, 53.4, 61.4, 61.7, 68.4, 124.8, 127.8, 128.9, 129.1, 129.5, 130.0, 130.2, 130.5, 130.8, 133.4, 135.3, 137.0, 137.4, 137.7, 137.9, 168.3, 169.2, 169.9, 170.6, 171.3, 171.9, 172.4, 173.4.

HRMS (ESI) m/z calcd for $C_{46}H_{56}N_8O_9^+$ $(M+H)^+$ 893.39400, found 893.40173.

Irradiation of 4 to obtain 1

Nitrogen purged solutions of the substrate in the indicated solvents was irradiated by using Pyrex glass filtered light in a water-cooled immersion reactor for time periods required. Concentration of the photoproduct was followed by silica gel column chromatography to yield the pure product. In brief, 0.5 g of compound **4** in anhydrous MeOH (200 mL) was placed in a reactor, then ventilated nitrogen flow for 40 min. Upon maintaining the ventilation of nitrogen, the solutions were irradiated by ultraviolet light for 40 min (Pyrex tube filtered-light, λ > 290 nm).

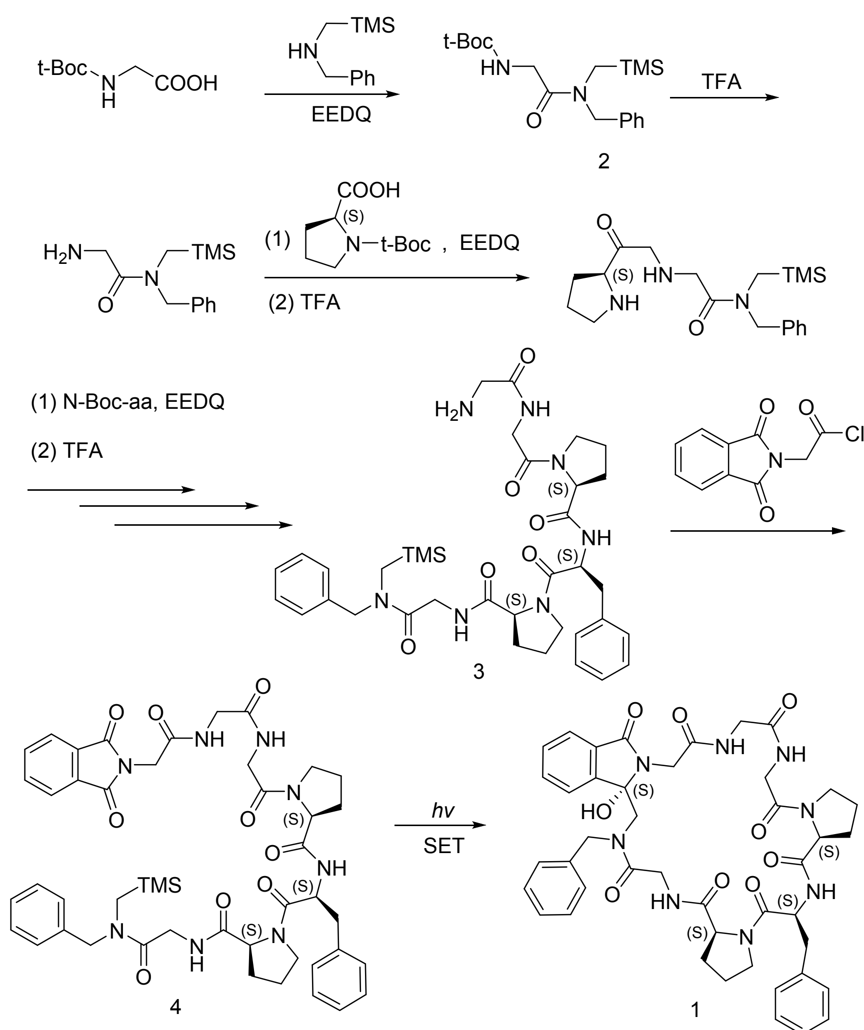


3-Hydroxy-isoindolinone-cyclo-Gly-Gly-Gly-Pro-Phe-Pro-Gly (**1**) : white solid (yield: 32.8%, after purification).

^{1}H-NMR($CDCl_3$) δ : 1.61~2.29(m, 8H, $(NCH_2\mathbf{CH_2}CH_2CH)_2$ and $(NCH_2CH_2\mathbf{CH_2}CH)_2$), 2.85~3.29(m, 6H, $(NH\mathbf{CH_2}CO)_3$), 3.50~3.73(m, 4H, $(N\mathbf{CH_2}CH_2CH_2CH)_2$), 3.79~3.96(m, 2H, $\mathbf{CH_2}$Ph), 4.06~4.39(m, 4H, N$\mathbf{CH_2}$CO and N$\mathbf{CH_2}$C(OH)), 4.40~4.71(m, 4H, N$\mathbf{CH_2}$Ph and $(NCH_2CH_2CH_2\mathbf{CH})_2$), 4.84~5.20(m, 1H, **CH**CH_2Ph), 6.75~8.02(m, 14H, Ar**H**);

^{13}C-NMR($CDCl_3$) δ : 23.7, 25.0, 28.8, 29.6, 37.4, 41.1, 41.8, 42.6, 43.7, 46.6, 47.7, 51.0, 51.6, 60.2, 60.6, 89.1, 122.1, 123.8, 125.9, 126.7, 127.9, 128.3, 129.1, 129.4, 130.0, 130.8, 132.5, 135.4, 167.8, 169.9, 170.2, 170.4, 170.5, 170.6, 171.2, 171.6.

HRMS (ESI) m/z calcd for $C_{43}H_{48}N_8O_9^+$ $(M+H)^+$ 821.35440, found 821.36151.



Scheme S1. The synthesis of target cyclopeptide.

Cell culture

HepG-2 cells, HeLa cells and L929 cells were maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10 % fetal bovine serum (FBS), penicillin G (100 U/mL), and streptomycin (100 g/mL). These cell lines were received from Harbin engineering University. Cells were cultured at 37 °C in a humidified incubator with 5 % CO_2. Cells were grown on plates and were subcultured after 0.25 % trypsin treatment. The following MTT experiments were performed when the ratio of cell fusion reached 80 %.

MTT assay for cell viability

The HepG-2 cells ,HeLa cells and L929 cells were seeded in a 96-well plate at an initial density of 4×10^3 cells per well in DMEM complete medium and incubated at 37 °C in 5 % CO_2 for 24 h. Then, they were treated with various concentrations (300, 200, 150, 100, 75, 50, 37.5, 25 and 18.75 g/mL) of samples, the control groups were set without treatment. Each dosage was replicated in six wells. After 48 h incubation, MTT dyes (100 µL, 0.5 mg/mL) were added to the wells and incubated for 4 h. The MTT solutions were then removed and dimethyl sulfoxide (DMSO; 150 µL) was added to dissove the generated formazan crystals. Then microplate reader was used to detect the absorbance of each well at 490 nm. Cell viability (%) was calculated by the following formula:

Cell viability (%) = $A_{490\ (sample)}/A_{490\ (control)} \times 100\%$

Here $A_{490\ (sample)}$ represents values of the wells treated with various concentrations of samples, and $A_{490\ (control)}$ represents those wells treated with DMEM+10% FBS, without any samples. Statistical analyses were performed using the SPSS statistical software version (SPSS Inc., Chicago, IL, USA).

Cell morphological changes of HepG-2 Cells after treatment of sample

After being cultured with compound **1** for 0h, 12h, 24h, 48h, respectively, the cell phenotype in bright field was analyzed by Leica DM IL LED Fluorescence inverted microscope (FIM). HepG-2 cell lines were incubated on 6 well plates for 24 h. The compound **1** (1 mL, 40 μg/mL) was added to each well and then incubated for additional 6 h. Then nucleus's morphological variation was immediately observed under FIM.

Computational details:

The conformational analysis was performed by arbitrarily fixing the absolute configuration of C-3 for compound **1**, using the Spartan 08 package [1] with the MMFF94 molecular mechanics force field and Monte Carlo searching. The obtained conformers were geometrically optimized at the DFT/B3LYP/6-31G(d, p) level of theory in the program package Gaussina 09.[2] TDDFT/ B3LYP/6311++(2d,2p) 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 0.25 eV with conformers by Specdis 1.61.[3]

References:

1. Spartan'08, Wavefunction, Inc. Irvine, CA
2. Gaussian 09, Revision C.01; Gaussian, Inc.: Wallingford, CT, 2010.
3. T. Bruhn, A. Schaumloffel, Y. Hemberger, G. Bringmann, *Chirality,* 2013, **25**, 243.

1. HPLC of linear peptide **4** and compound **1.**

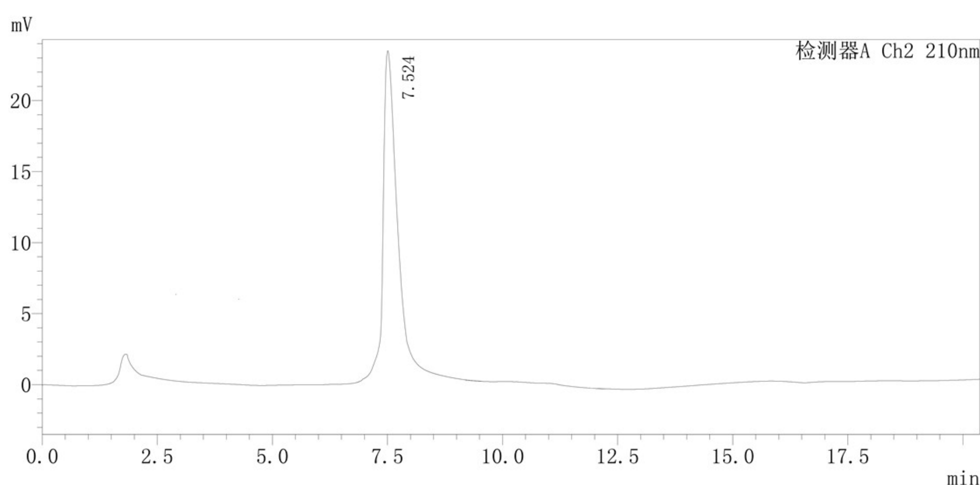


Figure S1. HPLC of linear peptide **4.**

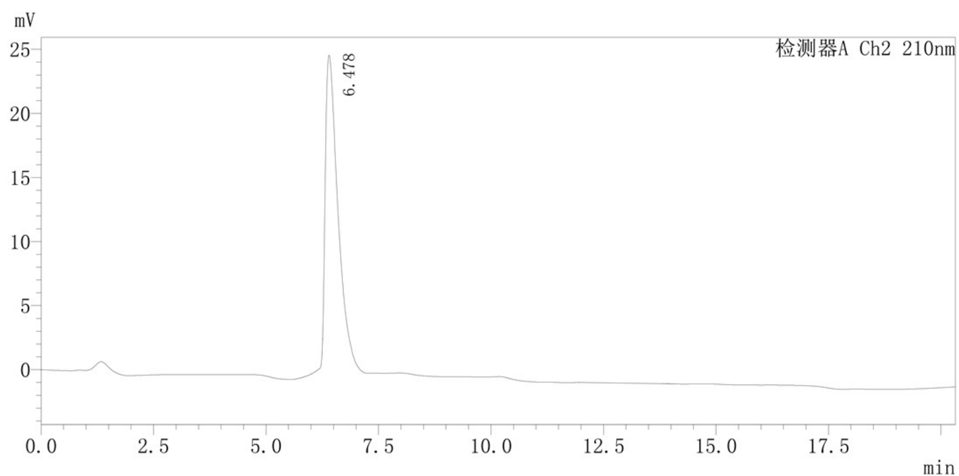


Figure S2. HPLC of compound **1.**

2. UV of compound **1.**

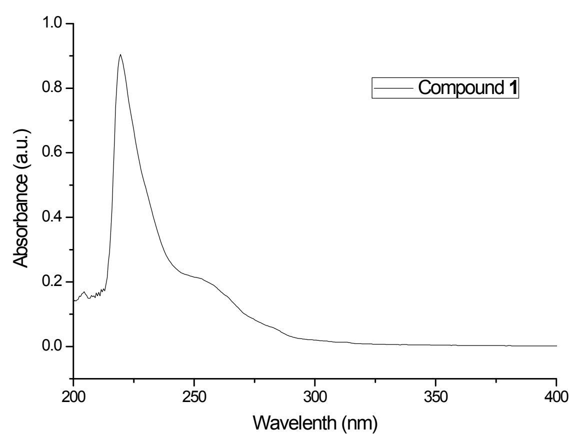


Figure S3. UV spectrum of compound **1**.

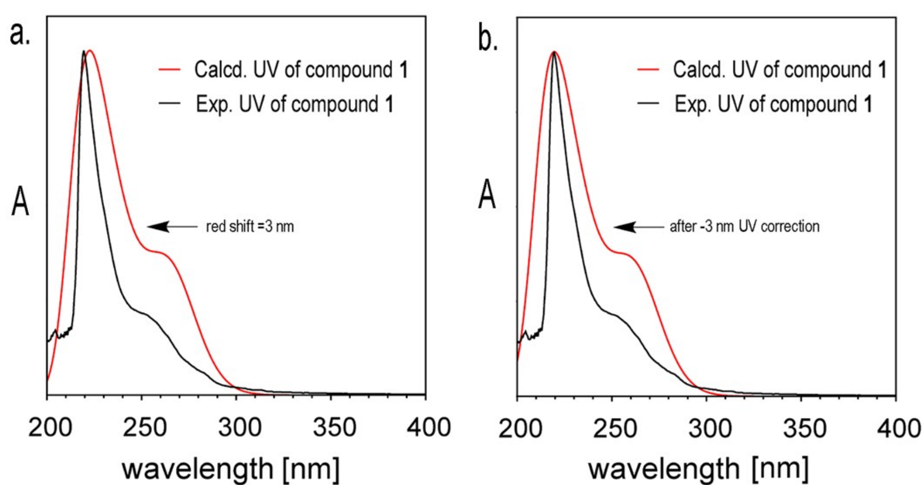


Figure S4. a). Experimental and calculated UV spectra of compound **1**; b). Corrected experimental and calculated UV spectra of compound **1**.

3. ¹H, ¹³C-NMR and HRMS of linear peptide **4.**

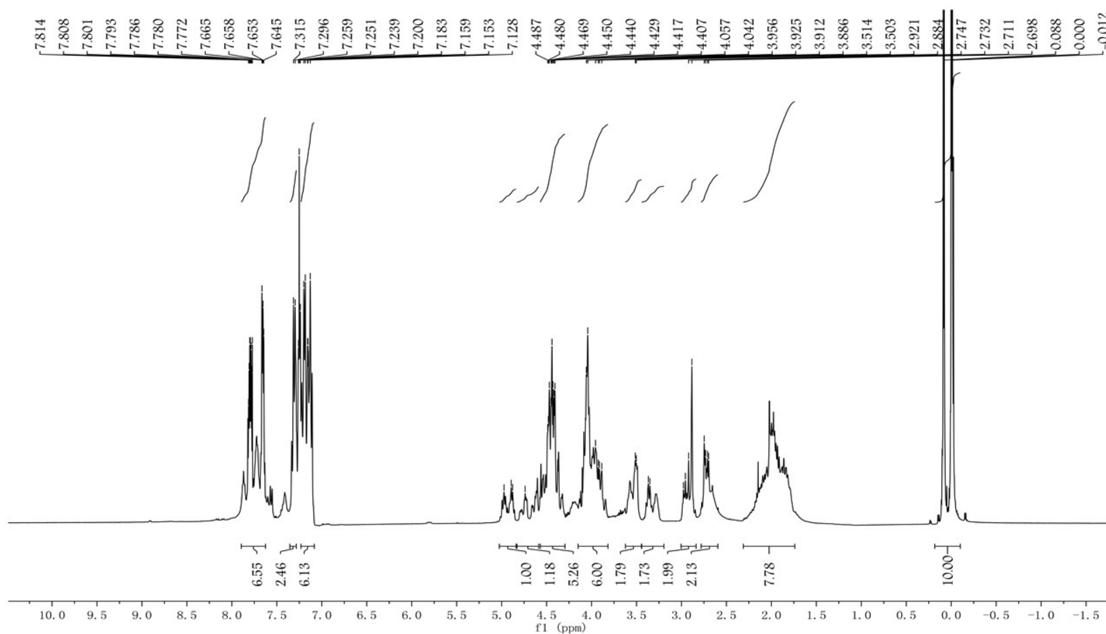


Figure S5. The 400 MHz ¹H-NMR spectrum of linear peptide **4** ($CDCl_3$ as solvent)

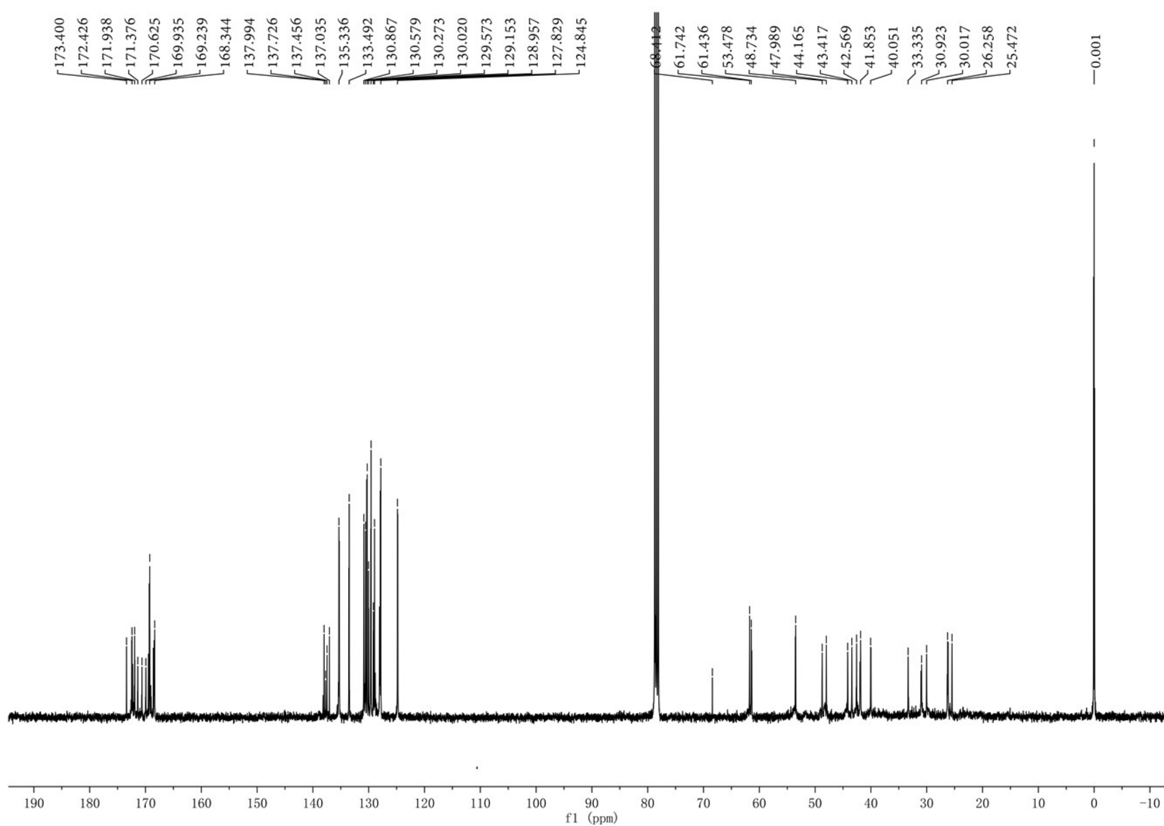


Figure S6. The 400 MHz ¹³C-NMR spectrum of linear peptide **4** ($CDCl_3$ as solvent)

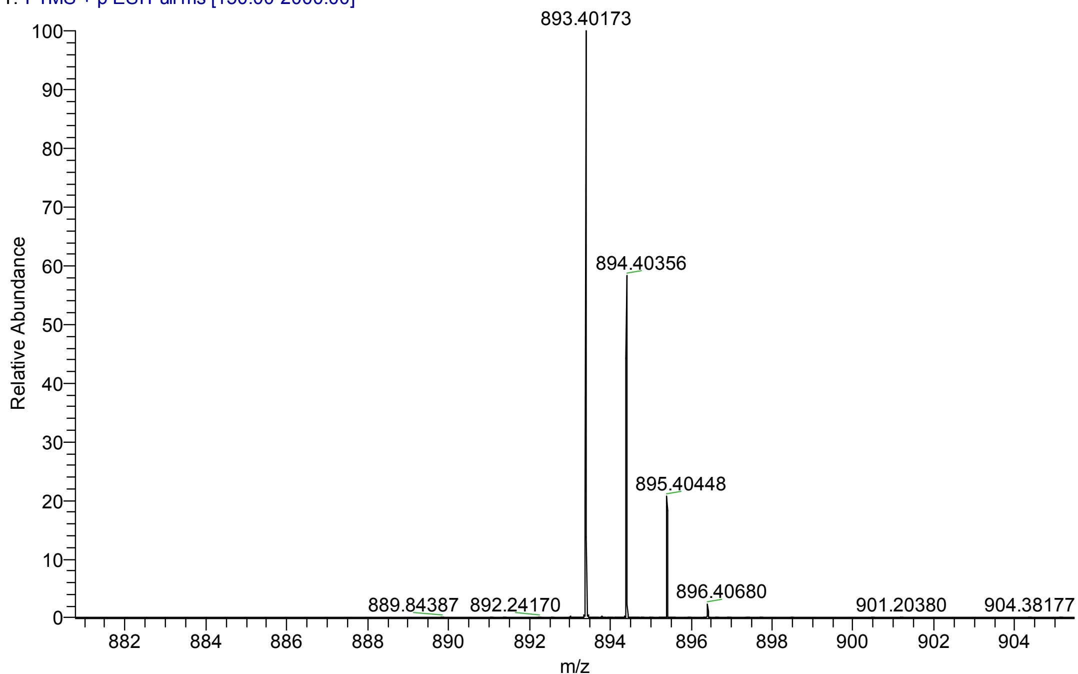


Figure S7. The HRMS of linear peptide **4** (ESI as ionization source)

4. ^{1}H, ^{13}C-NMR and HRMS of cyclic peptide **1.**

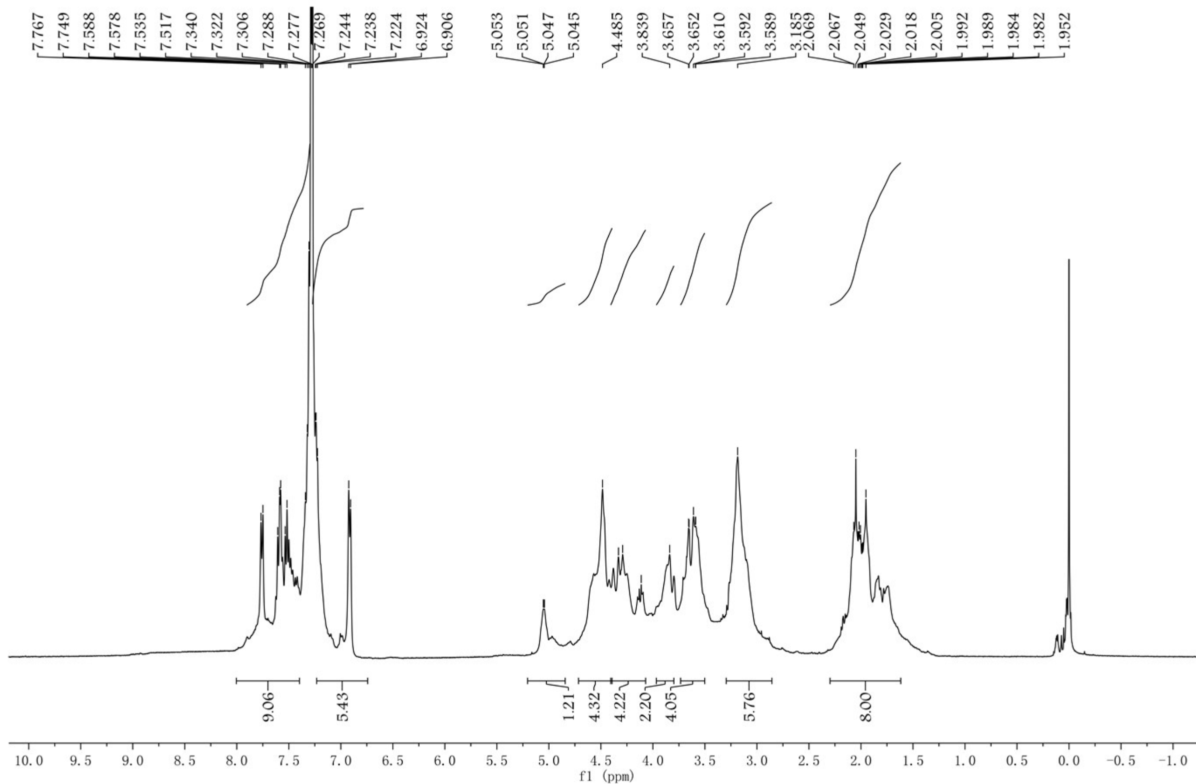


Figure S8. The 400 MHz ^{1}H-NMR spectrum of compound **1** ($CDCl_3$ as solvent)

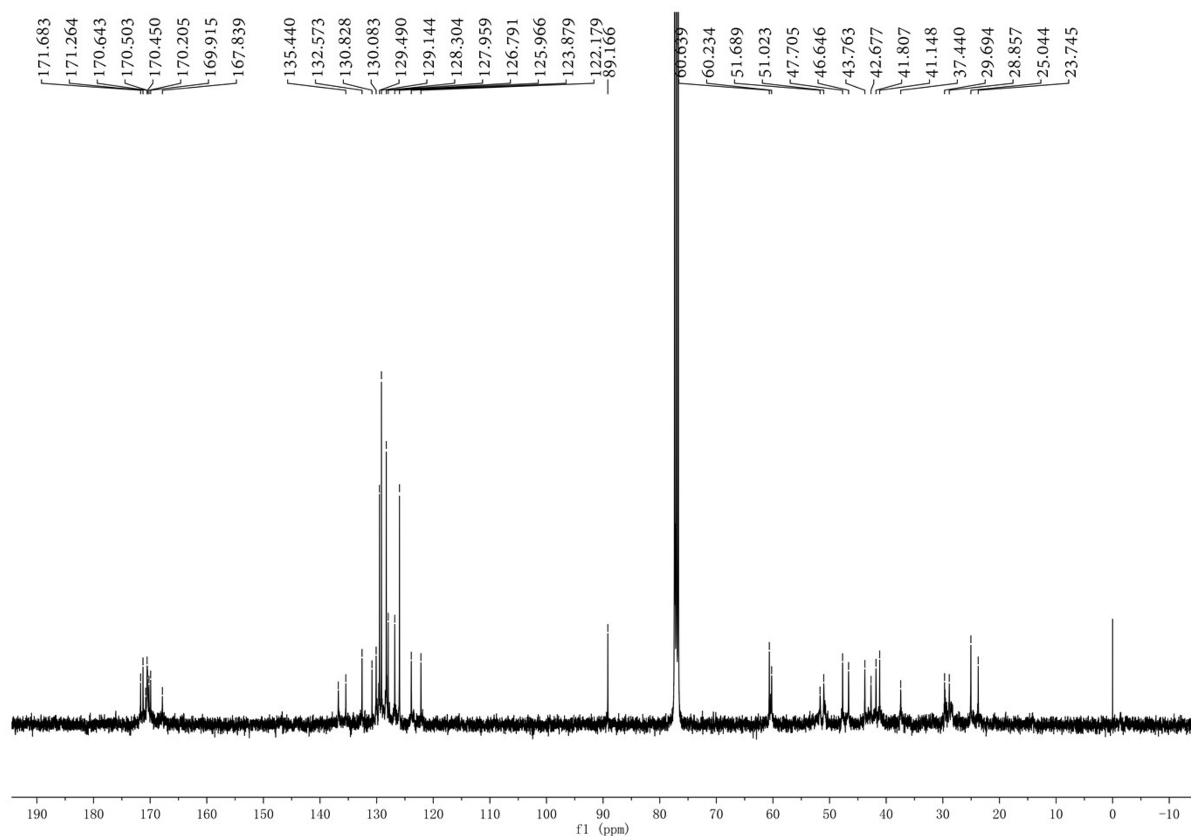


Figure S9. The 400 MHz ^{13}C-NMR spectrum of compound **1** ($CDCl_3$ as solvent)

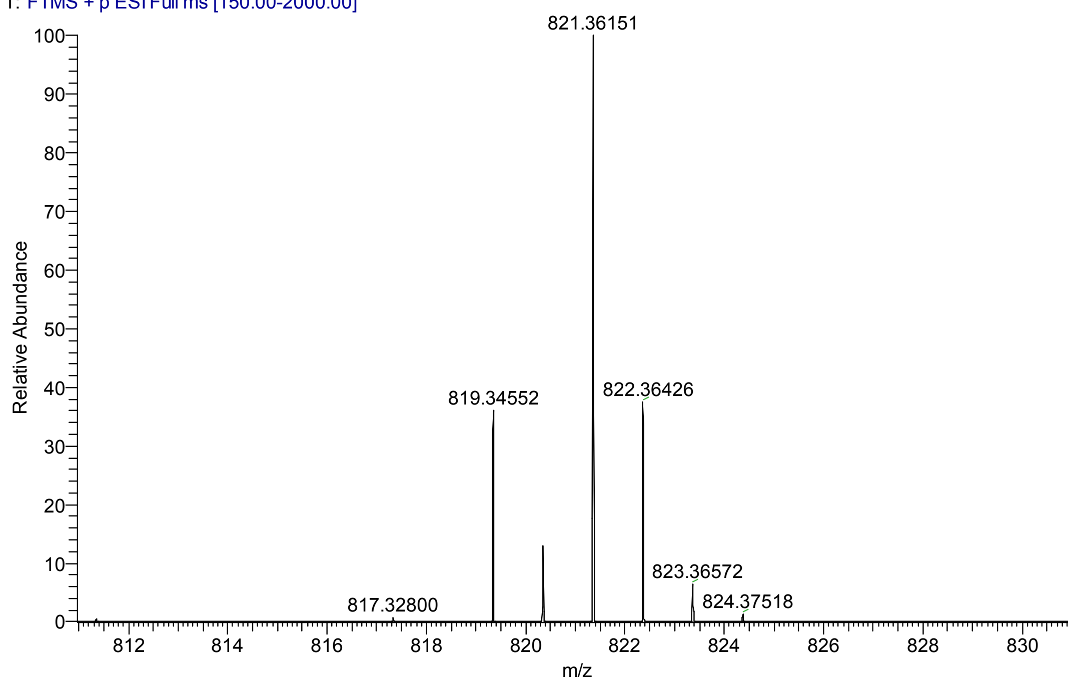


Figure S10. The HRMS of compound **1** (ESI as ionization source)

5. VT ^{1}H-NMR of compound **1.**

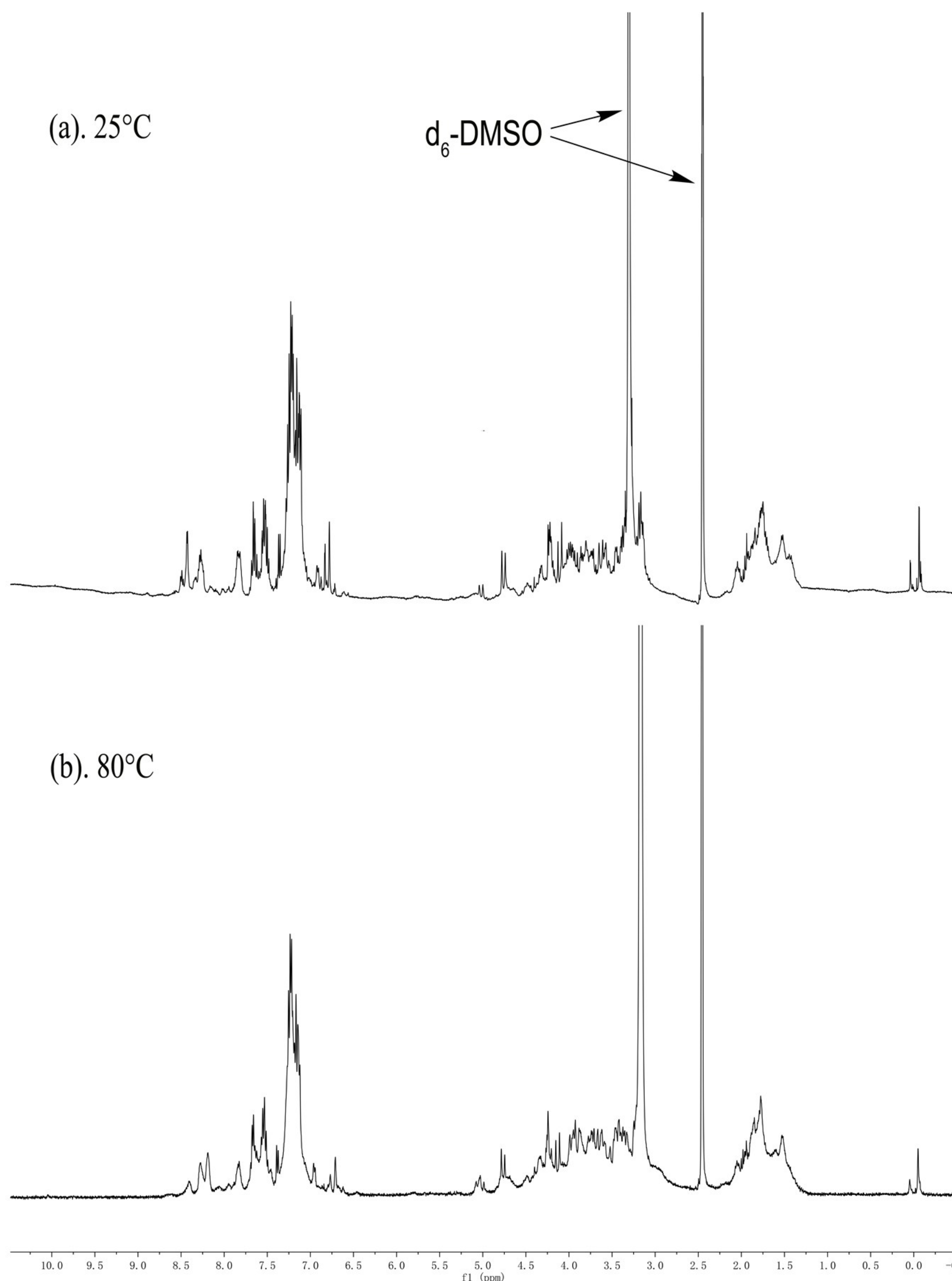


Figure S11. The 400 MHz ^{1}H-NMR spectrum of compound **1** measured at 25°C and 80°C (d_6-DMSO as solvent)

As can be seen from the obtained spectrum, when the temperature raised from 25°C to 80°C, the ^{1}H-NMR signal peaks were observed with a slight coalesce in some places. For example, at the chemical shift δ 8.2~8.6, there were no obvious peaks measured at 25°C and $CDCl_3$ as solvent (**Figure S7**). While measured at

25°C in d_6-DMSO, there emerged triple peaks. Yet when the temperature raised to 80°C, the ratio of the triple peaks changed obviously combined with less splitting and coalesced peaks (**Figure S10**). This result showed the existence of other different conformers except the predominate conformer and they exchanges slowly on the NMR timescale. The same phenomenon also observed in δ 7.4~7.9, 3.8~4.0 and 3.0~3.5. When temperature increases, signals were eventually coalesced when the rate of conformations exchange becomes fast on the timescale. This proved the consistency of NMR determination and molecular modelling.

6. Relatively stable conformers of compound **1** in methanol calculated at the DFT/B3LYP/6-31G** level of theory.

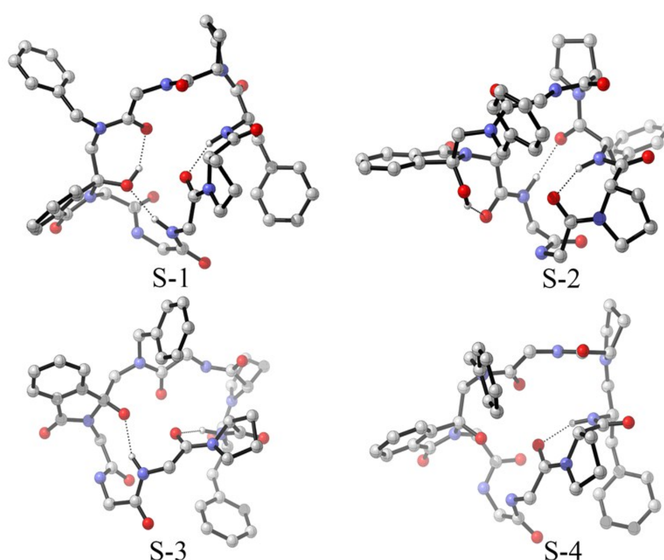


Figure S12. Relatively stable conformers of compound **1** in methanol.

The two conformers S-1, S-2 both present three intramolecular hydrogen bonds (HBs) (black dotted line in **Fig.S11**), yet there are two HBs around C3-OH of S-1 making the backbone more rigid and stable at room temperature, resulting a higher Boltzmann weighting factor. In the case of S-3, there exist two intramolecular HBs. In the case of S-4, only one intramolecular HBs between CO (Gly^3) and NH (Phe^5) was observed. the large steric hindrance between the phenyl group and isoindolinone gives them higher energy and makes them less stable at room temperature.