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Chemical synthesis and biological activity of peptides incorporating an ether bridge as a surrogate for a disulfide bond

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1. General information

a. Materials and Reagents

Rink amide AM resin was bought from CS Bio, GL Biochem (Shanghai, China). HCTU, HATU, PyAOP, HOAt, DIEA were bought from Adamas (Shanghai, China). Dimethylformamide (DMF), dichloromethane (DCM), dimethyl sulfoxide (DMSO), and anhydrous diethyl ether were purchased from Sinopharm Chemical Reagent. Thioanisole and trifluoroacetic acid (TFA, HPLC grade) were purchased from J&K Scientific (Beijing, China). Thin-layer chromatography (TLC) was performed on plates pre-coated with silica gel 60 F254 (250 layer thickness). Flash column chromatography was carried out by forced-flow chromatography using Silica Gel (200-300 mesh on small-scale or 300-400 mesh on large-scale). Manual peptide synthesis was performed in a peptide synthesis vessel under a constant temperature shaker (30 ℃).

b. HPLC

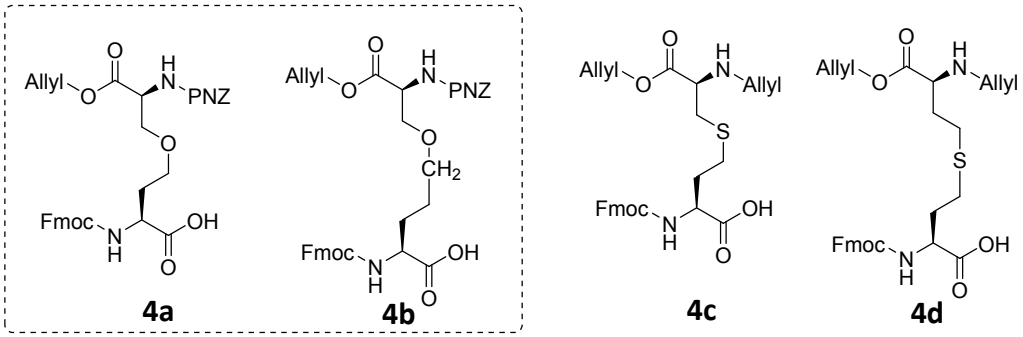
Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using analytical column (Grace Vydac "Protein & Peptide C18", 250 × 4.6 mm, 5 μm particle size, flow rate 1.0 mL/min, R.T.) solution A (0.08 % trifluoroacetic acid in acetonitrile) and solution B (0.1 % trifluoroacetic acid in ddH_2O) in a linear gradient. Analytical samples were monitored at 214 nm

and 254 nm. Semi-preparative HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using a semi preparative column (Grace Vydac "Peptide C18", 250 × 10 mm, 10 µm particle size, flow rate 4.0 mL/min, rt), and solution A (0.1 % trifluoroacetic acid in ddH_2O) and solution B (0.08 % trifluoroacetic acid in acetonitrile) in a linear gradient (with a flow rate of 4.0 mL/min).

c. Mass spectrometry and NMR

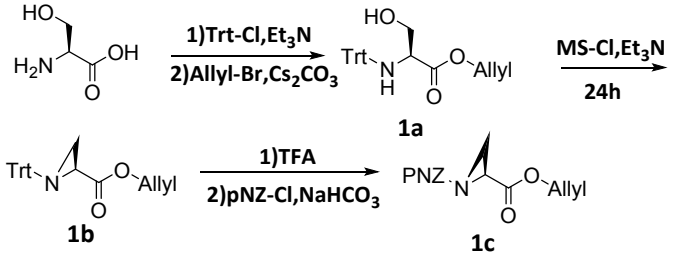
ESI-MS spectra were recorded on a Finnigan LCQ Advantage MAX ion trap mass spectrometer (Thermo Fisher Scientific. USA) equipped with a standard ESI ion source. Data acquisition and analysis were done with the Xcalibur (version 2.0, Thermo quest Finnigan) software package. ^{1}H NMR spectra were recorded on a Bruker 400 MHz spectrometer using deuteriochloroform ($CDCl_3$) as the solvent ($CDCl_3$: 7.26 ppm, as internal reference) unless otherwise stated. ^{13}C-NMR spectra were recorded with ^{1}H-decoupling on a Brucker 101 MHz spectrometer.

2. Synthesis of ether-bond diaminodiacids and thioether diaminodiacids



Scheme S1: Structure of ether-bond diaminodiacids and thioether diaminodiacids.

a. Synthesis of pNZ-aziridinocarboxylate ester 1c



Scheme S2

Allyl 3-hydroxy-2-(tritylamino)propanoate 1a

To a suspension of L-Serine (1 g, 9.5 mmol) in dichloromethane (14 mL) chlorotrimethylsilane (3.8 mL, 30 mmol) was added dropwise followed by stirred at reflux temperature for 20 min. Then 4 mL triethylamine was dissolved in 14 mL dichloromethane and added to the reaction system reflow for another 40 min. After cooled to 0℃, 0.5 mL MeOH was added followed by 1.4 mL triethylamine and TriphenylMethyl chloride (2.7 g，9.5 mmol). The reaction mixture was stirred for 20 h and diluted with EtOAc (50 mL). The organic layer was washed with 5% aqueous citric acid (3 × 100 mL), dried over Na_2SO_4, and concentrated in vacuo to give trityl homserine without further purification. Then trityl homserine and cesium carbonate (1 g, 30 mmol) was dissolved in 33 mL MeOH stirred at room temperature for 1 h and concentrated in vacuo, then dissolved in 20 mL DMF and allyl bromide (0.63 mL, 7.3 mmol) was added dropwise at room temperature. The reaction mixture was stirred overnight and diluted with EtOAc (50 mL) and the organic layer was washed with 5% aqueous citric acid (3 × 100 mL), dried over Na_2SO_4, and the crude product was purified by flash column chromatography to provide **1a** (1.5 g, two-steps yield 40%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.55 – 7.49 (m, 6H), 7.34 – 7.27 (m, 6H), 7.25 – 7.20 (m, 3H), 5.73 (m, 1H), 5.26 – 5.16 (m, 2H), 4.18 (m, 2H), 3.75 (m, 1H), 3.64 – 3.55 (m, 2H).

Allyl 1-tritylaziridine-2-carboxylate 1b

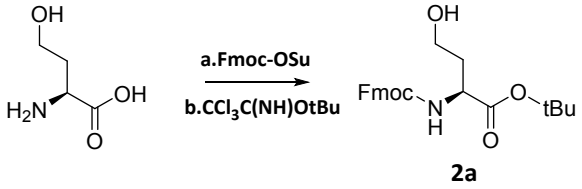
To a stirred solution of 1a (1.2 g, 3.1 mmol) in 25 mL THF at 0℃, triethylamine 1 mL was added followed by dropwise addition of methanesulfonyl chloride (1.2 mL, 15.5 mmol) and the solution was stirred at 0℃ for 30 min and at reflux temperature for 24 h. After solvent was removed under reduced pressure the resulting residue was dissolved in EtOAc (30 mL) and sequentially washed with 10% citric acid (3 × 20 mL), H_2O (2 × 20 mL), saturated Na_2CO_3 (3 × 20 mL) and H_2O (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo，and the crude product was purified by flash column chromatography to provide **1b** (0.64 g, yield 56%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.52 – 7.48 (m, 6H), 7.34 – 7.27 (m, 6H), 7.25 – 7.20 (m, 3H), 6.00 – 5.87 (m, 1H), 5.37 – 5.22 (m, 2H), 4.67 (m, 2H), 2.27 (dd, 1H), 1.91 (dd, 1H), 1.42 (dd, 1H).

2-Allyl 1-(4-nitrobenzyloxycarbonyl)aziridine-2-carboxylate 1c

To a stirred solution of **1b** (1 g, 2.7 mmol) in 3 mL dichloromethane and 3 mL methanol，2.5 mL trifluoroacetic acid was added dropwise and stirred at 0 °C for 30 min. Trifluoroacetic acid was removed by azeotroping with Et_2O (5 × 3 mL) then 8 ml saturated sodium bicarbonate

aqueous and 10 mL EtOAc were added followed by pNZ-Cl (0.67g, 3.1 mml) and stirred overnight at room temperature. After completion of the reaction, the two layers were separated and aqueous layer was extracted with EtOAc (3 × 20 mL). The combined organic layers were washed with brine (3 × 50 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was further purified by flash chromatography to provide **1c** (0.4g, yield 48%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.23 (d, 2H), 7.53 (d, 2H), 6.07 – 5.77 (m, 1H), 5.48 – 5.11 (m, 4H), 4.65 (m, 2H), 3.18 (m, 1H), 2.74 – 2.49 (m, 2H).

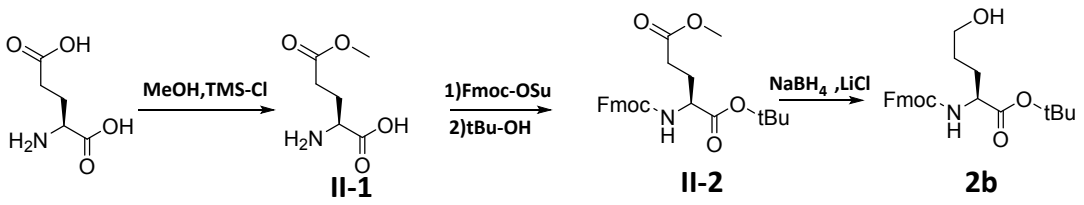
b. Synthesis of Fmoc-homoserine-tBu 2a [1]



Scheme S3

Homoserine (1.0 g, 8.3 mmol) and sodium carbonate (1 g, 9.4 mmol) were dissolved in 30 mL water/1, 4-dioxane (5:1, v:v), at 0℃and Fmoc-OSu (3.5 g, 10.4 mmol) was added. The reaction mixture was stirred at room temperature overnight. Then the residue was washed with EtOAc and the aqueous phase was acidified to pH 2 with HCl. The aqueous suspension was extracted by EtOAc, dried over Na_2SO_4, filtrated and evaporated. The residue was dissolved in DCM/THF (40 mL/10 mL). Then, tert-butyl 2, 2, 2-trichloroacetimidate (4.35 mL, 24 mmol, CAS: 98946-18-0) was added. The reaction mixture was stirred overnight then concentrated in vacuo, followed by addition of EtOAc. The combined organic phase was washed with saturated sodium bicarbonate and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to afford compound **2a** (1.5 g, 3.78 mmol, two-steps yield 45%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.77 (d, 2H), 7.60 (d, 2H), 7.40 (t, 2H), 7.32 (t, 2H), 5.64 (d, 1H), 4.52 – 4.35 (m, 3H), 4.22 (t, 1H), 3.77 – 3.50 (m, 2H), 2.30 – 2.11 (m, 2H), 1.48 (m, 9H).

c. Synthesis of Fmoc-homohomoserine-tBu (2b)

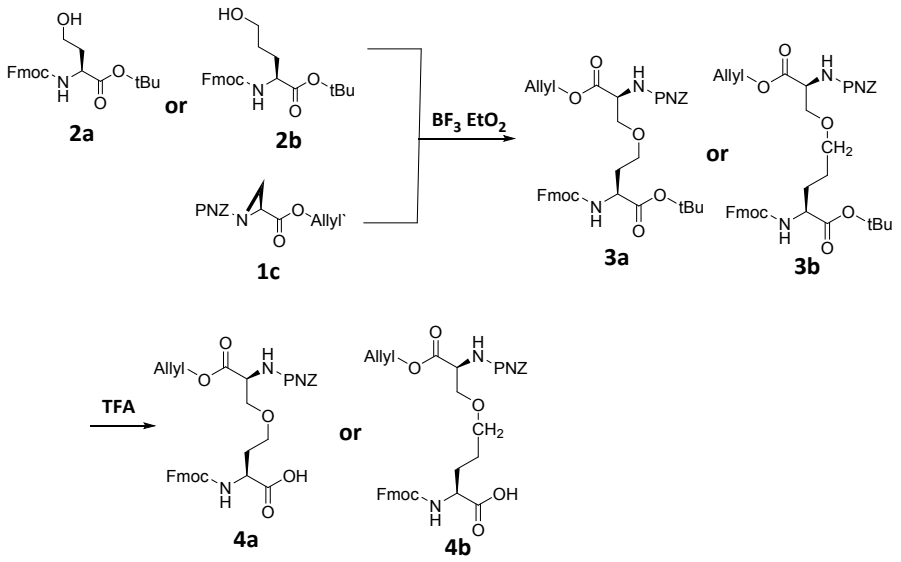


Scheme S4

Glutamic acid (6 g, 40.8 mmol) were added to 100 mL MeOH at 30℃. The reaction mixture was stirred for 5 min, then TMS-Cl (11.4 mL, 89.8 mmol) was added dropwise to the reaction system and stirred for 20 min. MeOH was removed under vacuum to afford compound **II-1**. The residue **II-1** was dissolved in 150 mL of water/Dioxane (1:2, v:v), at 0℃ and sodium bicarbonate (10.5 g, 102 mmol), Fmoc-OSu (20.6 g, 61.2 mmol) was added. The reaction mixture was stirred overnight followed by washing with EtOAc and the aqueous phase was acidified to pH 2 with HCl. The aqueous suspension was extracted by EtOAc, dried over Na_2SO_4, filtrated and evaporated. Then the residue and DCC (10.1 g, 48.96 mmol), DMAP (0.5 g, 4.08 mmol) were dissolved in 150 mL DCM at 0℃, tBu-OH (37.3 mL, 408 mmol) was added to the reaction system and stirred for 1h, then the reaction mixture was gradually heated to room temperature and stirred overnight. The reaction mixture was filtrated and evaporated, and then the crude product was purified by chromatography to afford compound **II-2** (8.9 g, three-steps yield 49%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76 (d, 2H), 7.60 (d, 2H), 7.40 (t, 2H), 7.34 – 7.29 (t, 2H), 5.45 (d, 1H), 4.38 (d, 2H), 4.30 (m, 1H), 4.22 (m, 1H), 3.67 (s, 3H), 2.48 – 2.29 (m, 2H), 2.21 (d, 1H), 2.03 – 1.93 (m, 1H), 1.48 (s, 9H).

LiCl (1.6 g, 37.6 mmol), $NaBH_4$ (1.4 g, 37.6 mmol) were added to 60 mL of EtOH/THF (1:1, v:v), at -5℃ (30 mL/30 mL). The mixture was stirred for 5 min. Compound **II-2** (4.15 g, 9.4 mmol) was dissolved in 20 mL of EtOH/THF (1:1, v:v) and was added dropwise. The reaction mixture was gradually heated to room temperature and stirred overnight. Then, the mixture was filtrated and concentrated. The crude product was purified by chromatography to afford compound **2b** (1.16 g, 2.82 mmol, yield 30%) as a colorless oil. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.69 (d, 2H), 7.53 (d, 2H), 7.33 (t, 2H), 7.24 (t, 2H), 5.43 (d, 1H), 4.32 (d, 2H), 4.24 (q, 1H), 4.15 (t, 1H), 3.61 (t, 2H), 1.67 (m, 2H), 1.54 (m, 2H), 1.40 (s, 9H).

d. Synthesis of ether-bond diaminodiacid 4a、4b



Scheme S5

To a stirred solution of aziridine-2-carboxylate **1c** (200 mg, 1.0 eq) and **2a** (260 mg, 1.0 eq.) in 5ml chloroform BF_3•OEt_2 (0.1 eq.) was added at 0 ˚C. The resulting reaction mixture was warmed to 40 ˚C and stirred for 24 h. The solvent was removed under reduced pressure and the crude product was purified by flash column chromatography to provide the corresponding product 3**a** (138mg, yield 30%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.06 (dd, 2H), 7.74 (dd, 2H), 7.60 (dd, 2H), 7.38 (dt, 4H), 7.31 – 7.25 (m, 2H), 6.62 (d, 1H), 5.91 (ddt, 1H), 5.73 (d, 1H), 5.39 – 5.04 (m, 4H), 4.73 – 4.63 (m, 2H), 4.55 – 4.26 (m, 4H), 4.17 (q, 1H), 4.02 (dd, 1H), 3.62 – 3.36 (m, 3H), 2.14 (td, 1H), 1.89 (ddd, 1H), 1.48 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.85, 170.06, 156.22, 156.00, 147.33, 144.00(2C), 143.88(2C), 141.21, 131.59, 127.72(2C), 127.63(2C), 127.08, 127.05, 125.19, 125.13, 123.62, 123.55, 119.97(2C), 118.51, 82.36, 71.00, 67.62, 67.21, 66.14, 65.20, 54.66, 51.94, 47.15, 32.28, 28.04(3C). HRMS calcd for $C_{37}H_{41}N_3O_{11}$ 703.28139, found $[M+H]^+$ 704.28198.

The **3b** was prepared in a similar manner to that described above for **3a** by using **1c** and **2b**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.15 (d, 2H), 7.75 (d, 2H), 7.58 (d, 2H), 7.48 – 7.37 (m, 4H), 7.30 (t, 2H), 5.96 (d, 1H), 5.88 (dq, 1H), 5.40 (m, 1H), 5.45 – 5.07 (m, 5H), 4.67 (q, 2H), 4.51 (d, 1H), 4.38 (d, 2H), 4.25 (dt, 2H), 3.89 (dd, 1H), 3.70 – 3.62 (m, 1H), 3.47 (d, 2H), 1.63 (dd, 4H), 1.47 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 171.57, 169.85, 155.91, 155.72, 147.52, 143.92(2C), 143.77(2C), 141.28, 131.53, 127.95(2C), 127.71(2C), 127.05(2C), 125.08(2C), 123.69(2C), 119.99(2C), 118.65, 82.24, 70.89, 70.49, 66.98, 66.16, 65.38, 54.63, 53.94, 47.16, 28.02(3C), 27.22, 24.99. HRMS calcd for $C_{38}H_{43}N_3O_{11}$ 717.28976, found $[M+H]^+$ 718.29755.

Then **3a** (138 mg) was dissolved in 1.5 mL dichloromethane and 500 uL trifluoroacetic acid was added and stirred overnight at room temperature then trifluoroacetic acid was removed by azeotroping with Et_2O (5 × 3 mL) and concentrated without further purification to afford compound **4a** (120 mg, yield 95%). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.02 (dd, 2H), 7.74 – 7.63 (m, 2H), 7.52 (dd, 2H), 7.42 – 7.30 (m, 3H), 7.22 (d, 3H), 6.63 (d, 1H), 5.95 (d, 1H), 5.85 (ddd, 1H), 5.39 – 4.94 (m, 4H), 4.67 – 4.01 (m, 7H), 3.90 (d, 1H), 3.68 – 3.38 (m, 3H), 2.20 – 1.93 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.64, 170.09, 156.28, 155.97, 147.38, 143.75(2C), 141.24, 131.51, 129.92, 129.88, 127.74(2C), 127.07(2C), 125.10, 125.07, 123.67, 123.58, 119.98(2C), 118.69, 77.25, 70.84, 67.50, 67.19, 66.29, 65.31, 54.64, 54.61, 51.63, 47.19, 35.99. HRMS calcd for $C_{33}H_{33}N_3O_{11}$ 647.21151, found $[M+H]^+$ 648.28157.

Diaminodiacid **4b** was prepared in a similar manner to that described above for **4a**. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.07 (d, 2H), 7.71 (d, 2H), 7.53 (t, 2H), 7.41 – 7.32 (m, 4H), 7.24 (s, 2H), 6.16 (d, 1H), 5.84 (ddt, 1H), 5.72 (s, 1H), 5.30 – 5.03 (m, 4H), 4.61 (dq, 2H), 4.50 (d, 1H), 4.31 (dt, 3H), 4.14 (t, 1H), 3.83 (d, 1H), 3.64 (d, 1H), 3.43 (s, 2H), 2.03 – 1.86 (m, 1H), 1.74 – 1.54 (m, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.16, 168.92, 156.32, 155.80, 147.48, 143.72(2C), 143.67(2C), 141.24, 131.43, 127.88(2C), 127.72(2C), 127.04(2C), 125.03(2C), 123.63(2C), 119.97(2C), 118.76, 70.92, 70.50, 67.03, 66.28, 65.37, 54.66, 53.83, 47.11, 27.21, 22.67.MS calcd for $C_{34}H_{35}N_3O_{11}$ 661.23, found $[M+Na]^+$ 684.76.

e. Synthesis of thioether diaminodiacid 4c、4d[2,3]

Thioether diaminodiacids **4c** and **4d** were prepared according to previous works.

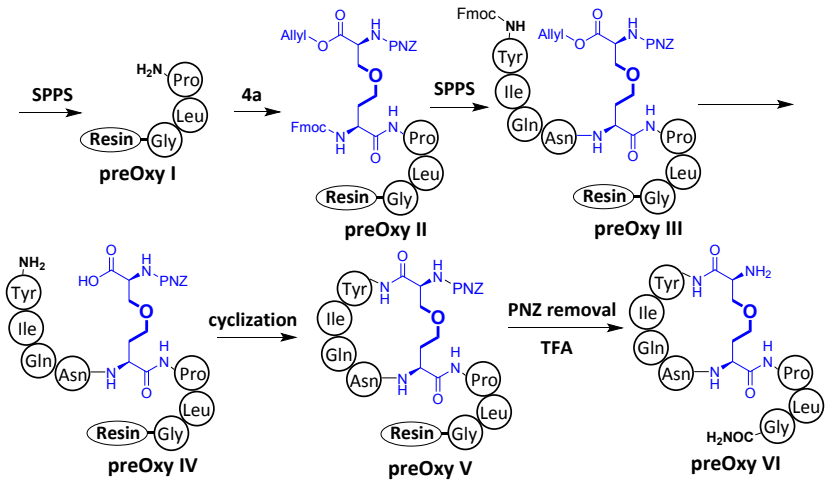
3. Solid-phase peptide synthesis of disulfide peptide mimics

a. Fmoc-based solid phase peptide synthesis

First of all, the Rink amide AM resin was swelled with DCM/DMF (1/1, v/v) for 30 min. The first amino acid (4.0 equiv to resin loading) was pre-activated with HCTU (4.0 eq) and DIEA (8.0 eq) in DMF for 0.5-1 min. Then, the mixture was added to the resin for coupling. After 40 min, the resin was washed with DMF (3 times), DCM (3 times) and DMF (3 times). The Fmoc group was removed with piperidine (20% in DMF, 5 min+10 min). Again, the resin was washed with DMF (3 times), DCM (3 times) and DMF (3 times). The following amino acid residues were coupled to the resin with the same procedure. After the solid phase amino acid assembly, the completed peptide was cleaved from the resin with a mixture of TFA/water/phenol/TIPS (88/5/5/2, v/v/v/v). After 2 h, the TFA-containing solution was collected, and concentrated by blowing with

N_2. The crude peptide was obtained by precipitation with cold ether and centrifugation. The residue was dissolved in water/acetonitrile (1:1, 0.1% TFA), purified by HPLC and analyzed by ESI mass spectra.

b. Synthesis and characterization of oxytocin mimic



Scheme S6: Solid-phase peptide synthesis of oxytocin mimic

100 umol Rink amide AM resin (300 mg, 0.3 mmol/g) was used. Fmoc-Gly-OH, Fmoc-Leu-OH, Fmoc-Pro-OH were assembled to amino group of the resin by the standard Fmoc-based SPPS protocol. For diaminodiacid coupling, ether-bond diaminodiacid **4a** (1.5 equiv.) was pre-activated with PyAOP (2 equiv.), HOAt (2 equiv) and NMM (4 equiv) in DMF for 1 min, then reacted with the resin-bound peptide **preOxy I** overnight and deprotected by the standard protocol. Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile and Fmoc-Tyr(tBu)-OH were successively coupled to N-terminal of **preOxy II**. The Allyl and Fmoc protecting groups of **preOxy III** were selectively removed by using a solution of $Pd(PPh_3)_4$ (28.0 mg, 2.0 equiv) and $PhSiH_3$ (0.12 mL, 10 equiv) in DMF/CH_2Cl_2(2.5 mL/2.5 mL) for 2 h and piperidine (20% in DMF, 5 min+10 min), respectively. Then the resin was washed with DMF (3 mL×3 times), DCM (3 mL×3 times) and DMF (3 mL×3 times). Cyclization was promoted by adding a solution of PyAOP (5.0 equiv.), HOAt (5.0 equiv.) and NMM (10.0 equiv.) in DMF to the resin and reacted for 12 h. The pNZ protecting groups were removed by adding a solution of $SnCl_2$ (6 M) and HCl/dioxane (5 mM) in DMF (5 mL) to resin-bound intermediate **preOxy V** and then retreat for 1 h. The resin was washed with CH_2Cl_2 (3 mL×5 times), DMF/H_2O (6 mL×5 times), THF/H_2O (6 mL×5 times) and DMF (3 mL×5 times). The peptide was cleaved from resin with TFA/water/phenol/TIPS (88/5/5/2, v/v/v/v) for 3 h. The cleaved resin was removed by filtration, and the filtrate was concentrated under a stream of N_2. The crude peptide was precipitated with cold Et_2O and then was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4

mL/min).

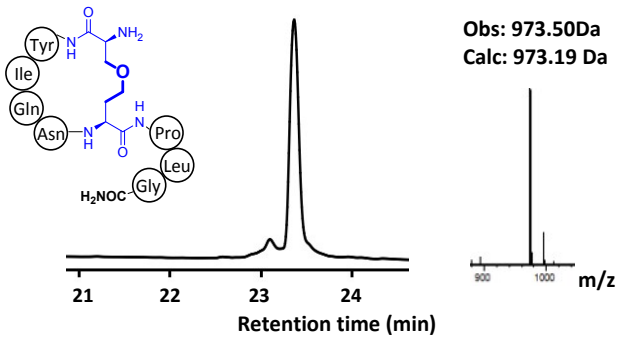
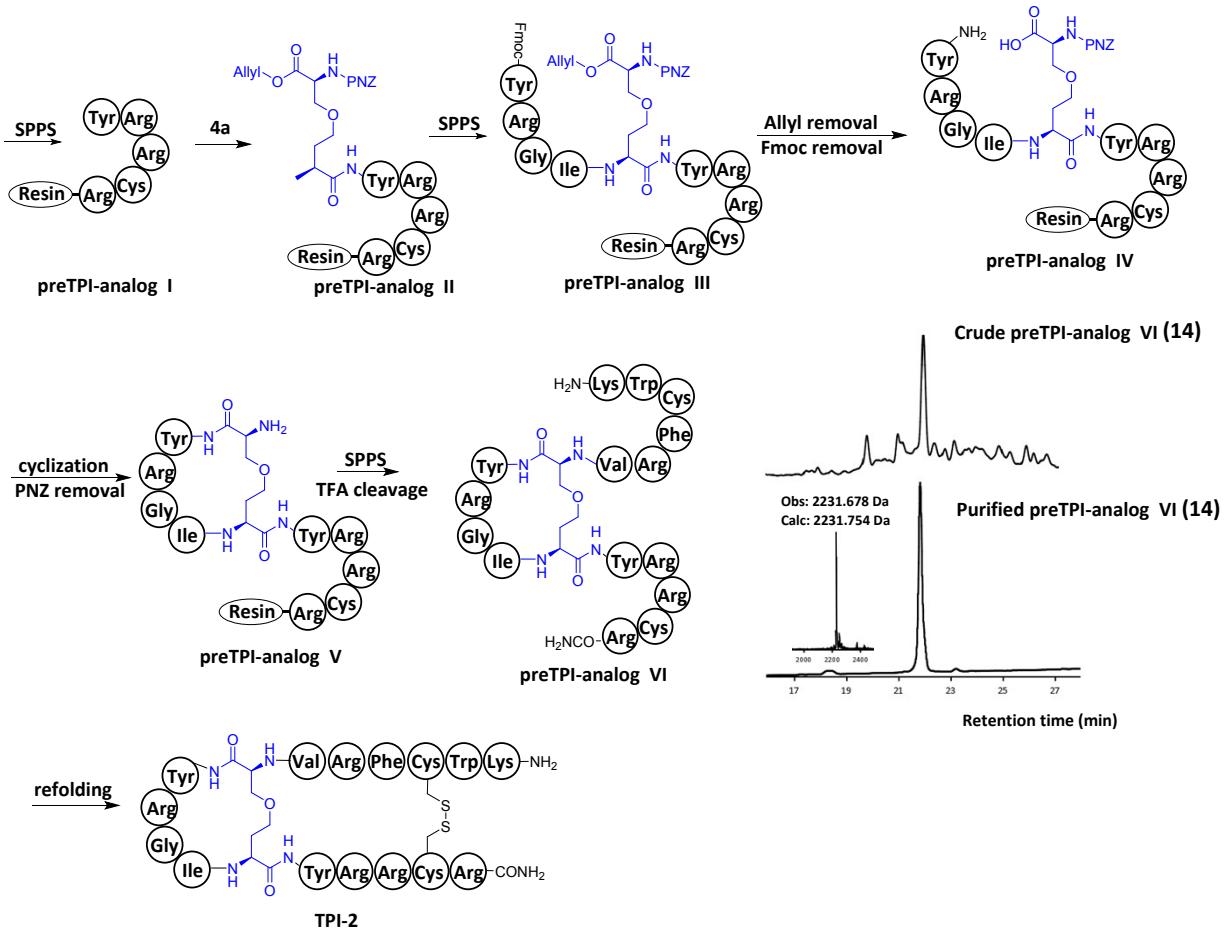


Figure S1. HPLC traces and MS analysis of purified oxytocin analog

c. Synthesis and characterization of TPI mimics



Scheme S7: Solid-phase peptide synthesis of TPI-2

Rink amide AM resin (300 mg, initial substitution 0.3 mmol/g) was used and Fmoc-Arg(Pbf)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Tyr(tBu)-OH was coupled/deprotected to resin by the standard protocol. Diaminodiacid **4a** (2 equiv.) was pre-activated with PyAop (2 equiv.), HoAt (2 equiv.) and NMM (4.0 equiv.) in DMF for 1 min, then

reacted with the **preTPI-analog I** for 12 h. After Fmoc group was removed, Fmoc-Ile-OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf)-OH and Fmoc-Tyr(tBu)-OH were coupled to resin-bound peptide **preTPI-analog II** to yield **preTPI-analog III** by the standard protocol. The Allyl and Fmoc protecting groups were selectively removed by using 2.0 equiv $Pd(PPh_3)_4$ 10.0 equiv $PhSiH_3$ for 2h and piperidine (20% in DMF, 5 min+10 min) respectively to yield **preTPI-analog IV**. Cyclization was promoted by adding a solution of PyAOP (5.0 equiv.), HOAt (5.0 equiv.) and NMM (10.0 equiv.) in DMF to the resin and reacted for 12h. The pNZ protecting groups were removed by adding a solution of $SnCl_2$ (6 M) and HCl/dioxane (5 mM) in DMF (5 mL) retreat for 1 h to yeild **preTPI-analog IV**. The resin was washed with CH_2Cl_2 (3 mL×5 times), DMF/H_2O (6 mL×5 times), THF/H_2O (6 mL×5 times) and DMF (3 mL×5 times). Then Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Phe-OH, Fmoc-Cys(Trt)-OH, Fmoc-Trp(tBu)-OH and Fmoc-Lys(Boc)-OH were coupled to resin-bound **preTPI-analog V** and deprotected by the standard protocol. The peptide was cleaved from resin with TFA/water/phenol/TIPS (88/5/5/2, v/v/v/v) for 3 h. The cleaved resin was removed by filtration, and the filtrate was concentrated under a stream of N_2. The crude peptide was precipitated with cold Et_2O then purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4 mL/min) and acquired as white powder.

The peptide **preTPI-analog VI** (1.0 equiv., 10mg) and glutathione mixture (1:1, glutathione oxidized (4 equiv.)/glutathione reduced (4 equiv.) was added to 100 mL solution (33% acetonitrile, pH 7.5) . After 6 h, the **TPI-2** was purified using HPLC showed almost a single peak, in which the main peak was further confirmed by ESI-MS as the correct target product. (Fig. S2 for HPLC and ESI-MALDI-TOF-MS) calc. 2229.754, found 2229.071

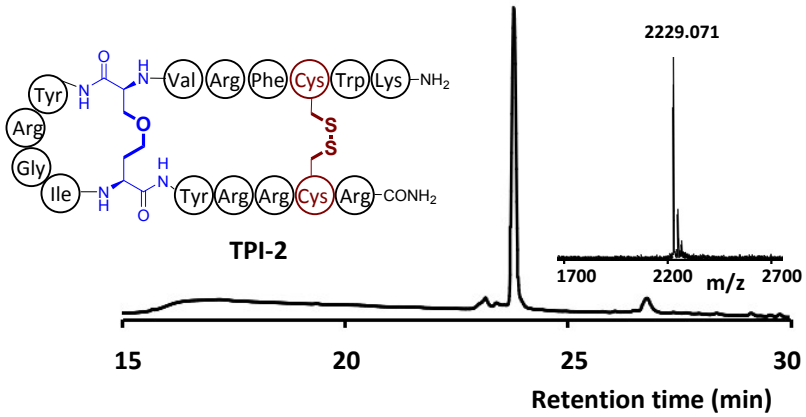


Figure S2. HPLC traces and ESI-MS analysis of purified TPI-2

TPI-3 was prepared by the same standard protocol as **TPI-2**. **TPI-3** was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4 mL/min) and acquired as white powder. (Fig. S3 for HPLC and ESI-MALDI- TOF-MS)

calc. 2244.763, found 2244.736

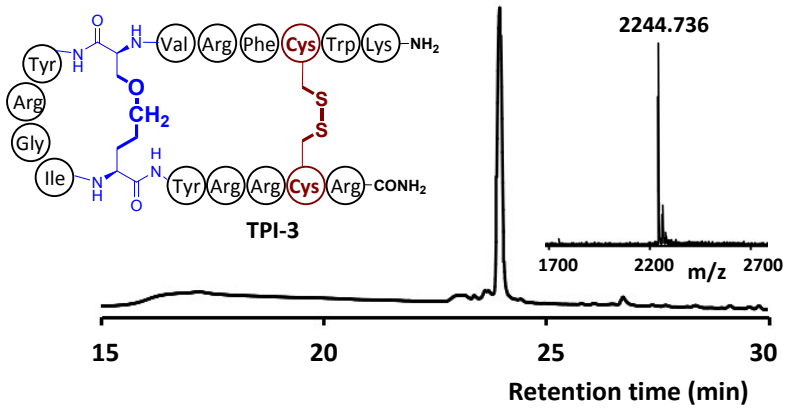


Figure S3. HPLC traces and ESI-MS analysis of purified TPI-3

TPI-4 was prepared in a similar manner to **TPI-2**. **TPI-4** was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4 mL/min) and acquired as white powder. (Fig. S4 for HPLC and ESI-MALDI-TOF-MS) calc. 2245.763, found 2245.462.

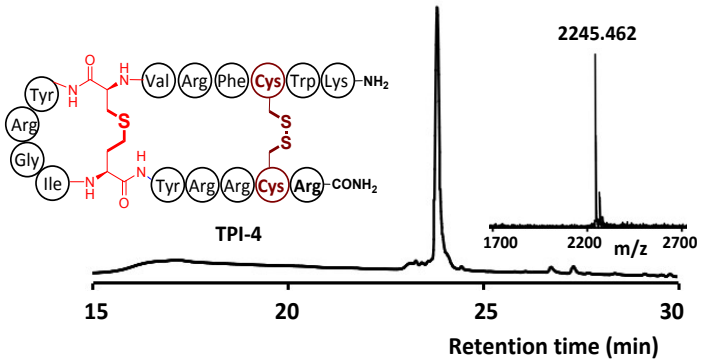


Figure S4. HPLC traces and ESI-MS analysis of purified TPI-4

TPI-5 was prepared in a similar manner to **TPI-2**. **TPI-5** was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4 mL/min) and acquired as white powder. (Fig. S5 for HPLC and ESI-MALDI- TOF-MS) calc. 2259.765, found 2259.579

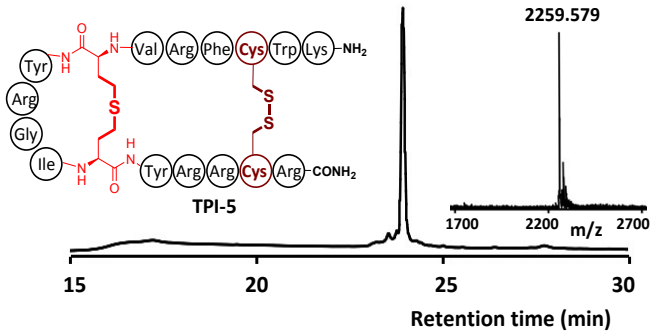


Figure S5. HPLC traces and ESI-MS analysis of purified TPI-5

TPI-1 was prepared in standard protocol. **TPI-1** was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4 mL/min) and acquired as white powder. (Fig. S5 for HPLC and ESI-MALDI-TOF-MS) calc. 2263.765, found 2264.246

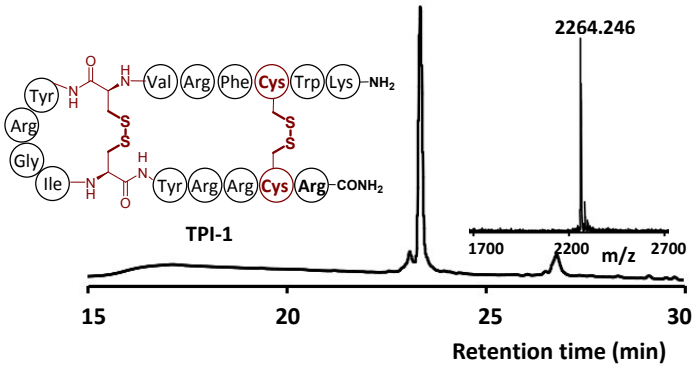
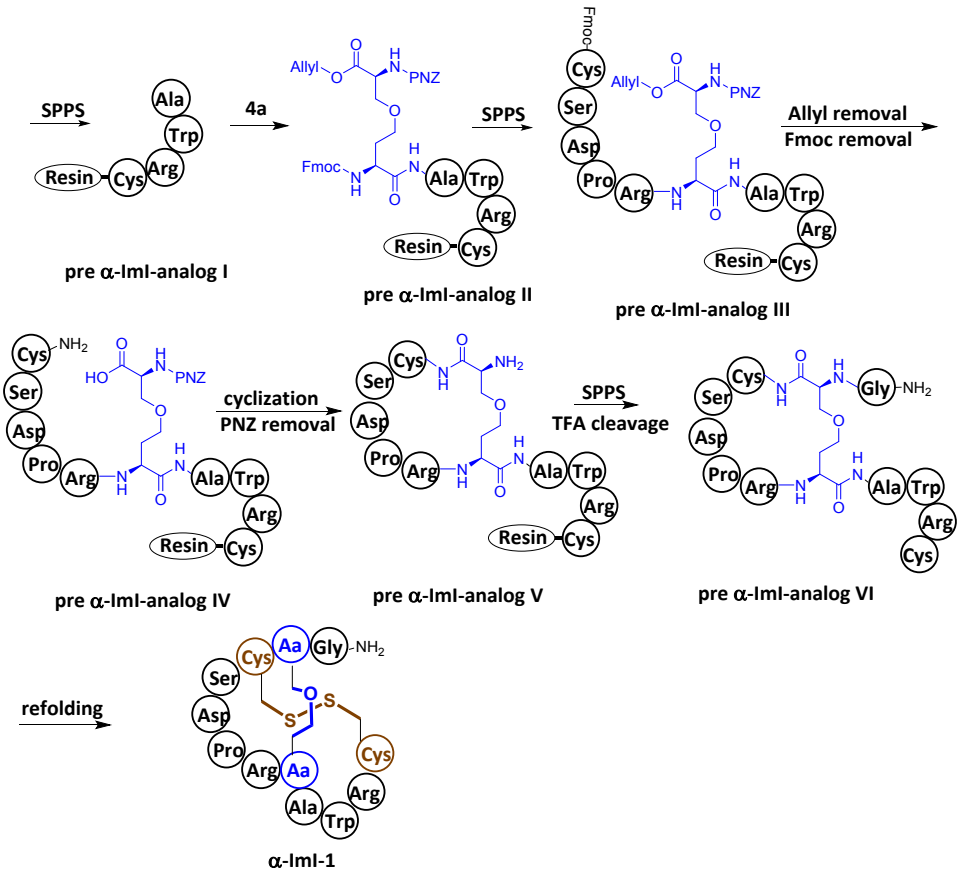


Figure S6. HPLC traces and ESI-MS analysis of purified TPI-1

d. Synthesis and characterization of α-ImI mimics



Scheme S8: Solid-phase peptide synthesis of α-ImI-1

α-ImI-1 was synthesized and refolded in a similar manner to **TPI-2**. **α-ImI-1** was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic acid, 30 min, 4 mL/min) and acquired as white powder. (Fig. S6 for HPLC and ESI-MALDI-TOF-MS) calc. 1317.651, found 1317.501

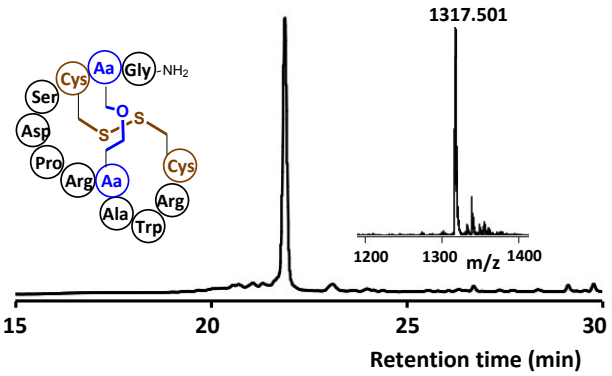


Figure S7. HPLC traces and ESI-MS analysis of purified α-ImI-1

α-ImI-2 was synthesized and refolded in a similar manner to **TPI-2**. **α-ImI-2** was purified by semi-preparative HPLC (a linear gradient from 1% to 90% acetonitrile in 0.1 % trifluoroacetic

acid, 30 min, 4 mL/min) and acquired as white powder. (Fig. S67 for HPLC and ESI-MALDI-TOF-MS) calc. 1333.535, found 1333.356

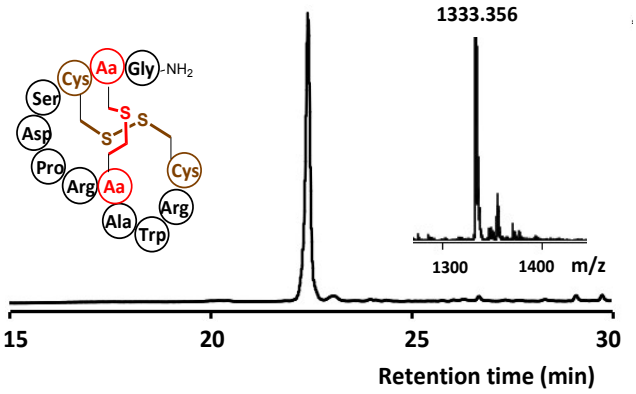


Figure S8. HPLC traces and ESI-MS analysis of purified α-ImI-2

4. The antimicrobial assays of the TPI analogs

Several bacterial strains, including two Gram-positive bacteria (Bacillus subtilis and Staphylococcus epidermidis) and one Gram-negative bacteria (Escherichia coli) were cultured to 10^8 CFU at 37 ℃ using LB medium. Bacteria inocula were added to the two-fold diluted samples. After incubation for bacteria 18-24 h at 37℃, the last tube with no growth of microorganism was recorded to represent the MIC value expressed in μg/mL.

5. The reduction and oxidation stability of disulfide peptide mimics

a. Reduction stability of TPI-1 and TPI-2

First, the 50 μL TPI-1(2.56 mg/mL) and 50 μL aqueous dithiothreitol (DTT, 1 mm) solution was add to 100 μL of phosphate buffer (1 mmol, pH 7.2). Every 20 minutes, 30 μL reaction solution was add to 70 μL buffer (1 mM NaCl, 1% TFA, water: acetonitrile=1:1) and monitored by HPLC.

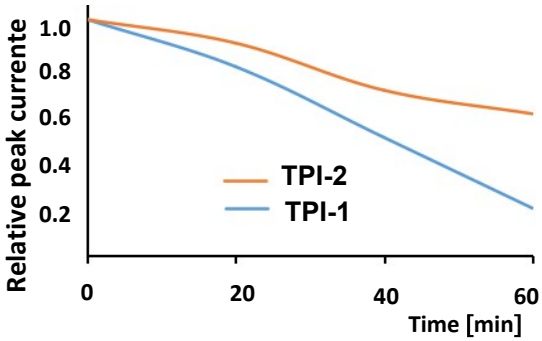


Figure S9. Stability of TPI-1 and TPI-2 in reducing environment.

b. Oxidation stability of α-ImI-1 and α-ImI-2

α-ImI analogs aqueous solution (1 mM) and 0.2% H_2O_2, 0.2% TFA aqueous were mixed in equal volume. Then the mixture was stirred at room temperature and analyzed after 4 h、12 h. After 12 hours, almost all α-ImI-2 is oxidized. Meanwhile, no oxidation by-products of α-ImI-1 were observed after 12 hours under the same oxidation conditions

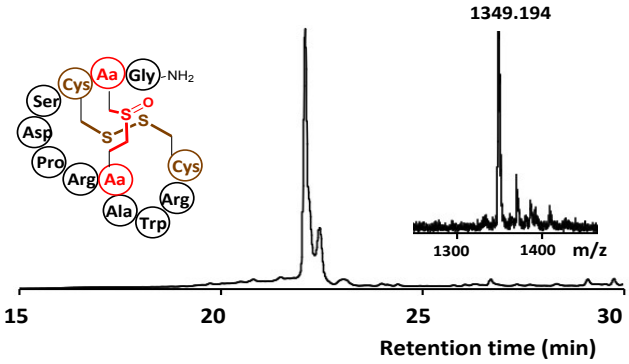


Figure S10. HPLC traces and ESI-MS analysis of oxidized α-ImI-2

6. NMR structure determination of oxytocin mimic and TPI-2

Samples were prepared by dissolving dry peptide in 90% (vol/vol) H_2O / 10% (vol/vol) D_2O at a concentration of 2~3 mM, and the pH value was 6.5. NMR experiments, including 1D, total correlation spectroscopy (TOCSY), nuclear Overhauser effect spectroscopy (NOESY), double quantum filtered correlation spectroscopy (DQFCOSY), rotating-frame nuclear Overhauser effect correlation spectroscopy (ROESY), heteronuclear single quantum coherence spectroscopy (13C-HSQC, 15N-HSQC), were recorded on a Bruker Avance-600 MHz spectrometer at 298K. TOCSY and NOESY experiments were carried out with a mixing time of 80 ms and 500 ms, respectively. Spectra were analyzed and integrated with SPARKY software.[4] Initial amino acid assignment was conducted based on TOCSY and NOESY/ROESY spectra. Distance restraints were derived from NOESY spectra for TPI-2 and ROESY spectra for Oxytocin. Backbone phi- and psi-dihedral angle restraints were generated from backbone chemical shifts using the program TALOS+ (Shen, Delaglio, Cornilescu, and Bax). Structures were calculated based on distance restraints obtained from NOE peak intensities using Xplor-NIH.[5] The 20 lowest energy structures were selected out of 100 structures. The quality of structures was assessed using PROCHECK-NMR and MOLMOL.[6,7]

Table S1. Structural statistics for the final 20 conformers of Oxytocin

Structural statistics	58
Intraresidue	23
Sequential	23
Medium-range（2≤|i-j|≤4）	3
Long-range（|i-j|≥5）	3
Dihedral restraints	6
	Backbone/ heavy atoms
RMSD to the mean（1-8）	0.824/1.476

Table S2. Structural statistics for the final 20 conformers of TPI-2

Structural statistics	158
Intraresidue	60
Sequential	62
Medium-range（2≤|i-j|≤4）	6
Long-range（|i-j|≥5）	8
Dihedral restraints	22
	Backbone/ heavy atoms
RMSD to the mean（5-14）	0.389/1.317

Table S3. Chemical shifts Assignment of Oxytocin

Residue	Group	Atom	Shift
1	CYS/DAD	CA	56.083
1	CYS/DAD	HA	3.904
1	CYS/DAD	HB#	3.726
2	TYR	CB	38.519
2	TYR	HA	4.72
2	TYR	HB2	3.206
2	TYR	HB3	2.936
2	TYR	HN	7.16
2	TYR	N	117.618
3	ILE	CB	40.003
3	ILE	CG1	26.843
3	ILE	HA	4.256
3	ILE	HB	1.878
3	ILE	HD1#	0.854
3	ILE	HG1#	1.261
3	ILE	HG2#	1.013
3	ILE	HN	7.472
3	ILE	N	117.618
4	GLN	CA	57.657
4	GLN	CG	33.783

4	GLN	HA	4.05
4	GLN	HB#	1.99
4	GLN	HG#	2.365
4	GLN	HN	8.252
4	GLN	N	120.897
5	ASN	CB	37.916
5	ASN	HA	4.58
5	ASN	HB2	2.869
5	ASN	HB3	2.786
5	ASN	HN	8.414
5	ASN	N	116.456
6	CYS/DAD	CB	32.406
6	CYS/DAD	HA	4.448
6	CYS/DAD	HB2	2.031
6	CYS/DAD	HB3	1.972
6	CYS/DAD	HG2	3.563
6	CYS/DAD	HG3	3.48
6	CYS/DAD	HN	7.732
6	CYS/DAD	N	120.447
7	PRO	HA	4.398
8	LEU	CB	42.148
8	LEU	CG	26.993
8	LEU	HA	4.277
8	LEU	HB2	1.652

8	LEU	HB3	1.575
8	LEU	HD1#	0.914
8	LEU	HD2#	0.864
8	LEU	HG	1.653
8	LEU	HN	8.463
8	LEU	N	122.808
9	GLY	CA	44.961
9	GLY	HA2	3.891
9	GLY	HA3	3.849
9	GLY	HN	8.4
9	GLY	N	110.602

Table S4. Chemical shifts Assignment of TPI-2

Residue	Group	Atom	Shift
1	LYS	HA	4.101
2	TRP	CB	30.632
2	TRP	HA	4.968
2	TRP	HB#	3.151
2	TRP	HD1	7.182
2	TRP	HE1	10.07
2	TRP	HN	8.974
2	TRP	HZ2	7.431
3	CYS	HA	4.948
3	CYS	HB#	3.141

3	CYS	HN	8.821
3	CYS	N	124.07
4	PHE	HA	5.105
4	PHE	HB2	2.897
4	PHE	HB3	2.809
4	PHE	HN	8.623
4	PHE	N	124.621
5	ARG	HA	4.722
5	ARG	HB#	1.667
5	ARG	HD2	2.915
5	ARG	HD3	2.858
5	ARG	HG#	1.437
5	ARG	HN	8.563
5	ARG	N	121.003
6	VAL	CB	33.113
6	VAL	CG#	20.744
6	VAL	HA	4.115
6	VAL	HB	1.884
6	VAL	HG#	0.925
6	VAL	HN	8.461
6	VAL	N	122.65
7	CYS/DAD	HA	5.143
7	CYS/DAD	HB2	3.549
7	CYS/DAD	HB3	3.445

7	CYS/DAD	HN	8.425
7	CYS/DAD	N	122.262
8	TYR	CB	39.207
8	TYR	HA	4.496
8	TYR	HB#	3.007
8	TYR	HD#	7.153
8	TYR	HN	8.441
8	TYR	N	122.805
9	ARG	CA	56.387
9	ARG	HA	3.976
9	ARG	HG#	1.592
9	ARG	HN	8.52
10	GLY	CA	45.322
10	GLY	HA2	4.086
10	GLY	HA3	3.702
10	GLY	HN	8.293
11	ILE	CB	38.659
11	ILE	CG2	17.625
11	ILE	HA	4.316
11	ILE	HB	1.851
11	ILE	HD1#	0.734
11	ILE	HG12	1.417
11	ILE	HG13	1.125
11	ILE	HG2#	0.734

11	ILE	HN	8.014
11	ILE	N	122.214
12	CYS/DAD	HA	4.986
12	CYS/DAD	HB2	1.832
12	CYS/DAD	HB3	1.741
12	CYS/DAD	HG2	3.635
12	CYS/DAD	HG3	3.34
12	CYS/DAD	HN	8.607
12	CYS/DAD	N	125.081
13	TYR	CB	40.469
13	TYR	HA	4.895
13	TYR	HB2	2.921
13	TYR	HB3	2.737
13	TYR	HD#	6.891
13	TYR	HN	8.489
13	TYR	N	120.343
14	ARG	CB	32.344
14	ARG	HA	4.684
14	ARG	HB2	1.757
14	ARG	HB3	1.669
14	ARG	HD#	3.082
14	ARG	HG#	1.426
14	ARG	HN	8.665
14	ARG	N	120.936

15	ARG	CB	31.411
15	ARG	HA	4.353
15	ARG	HB2	1.569
15	ARG	HB3	1.443
15	ARG	HD#	3.028
15	ARG	HG#	1.321
15	ARG	HN	8.632
15	ARG	N	121.959
16	CYS	HA	4.971
16	CYS	HB2	3.068
16	CYS	HB3	2.994
16	CYS	HN	8.565
16	CYS	N	120.321
17	ARG	CB	29.531
17	ARG	HA	4.349
17	ARG	HB#	1.863
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17	ARG	HN	8.691
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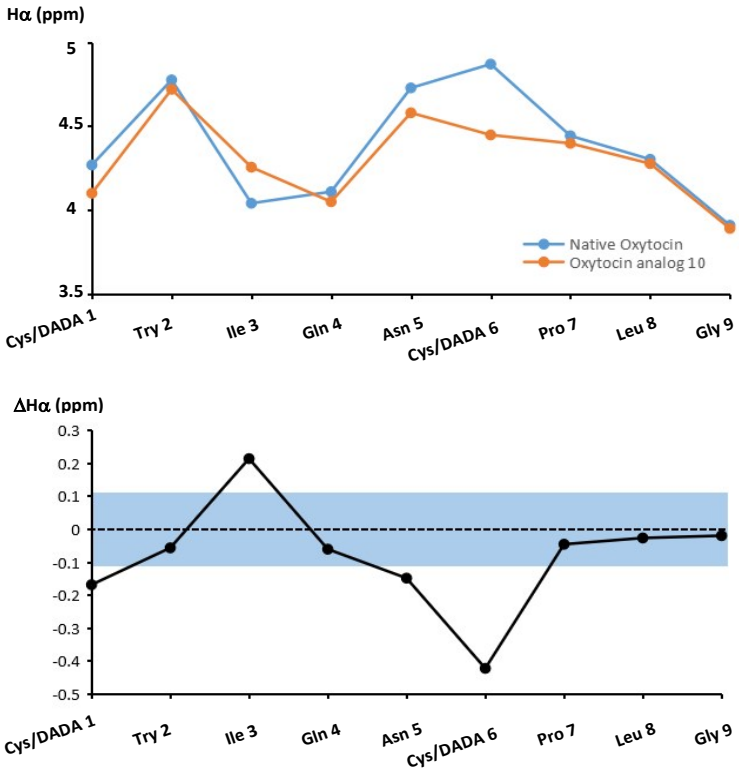


Figure S11. The chemical shift differences of backbone Ha between 10 and native oxytocin

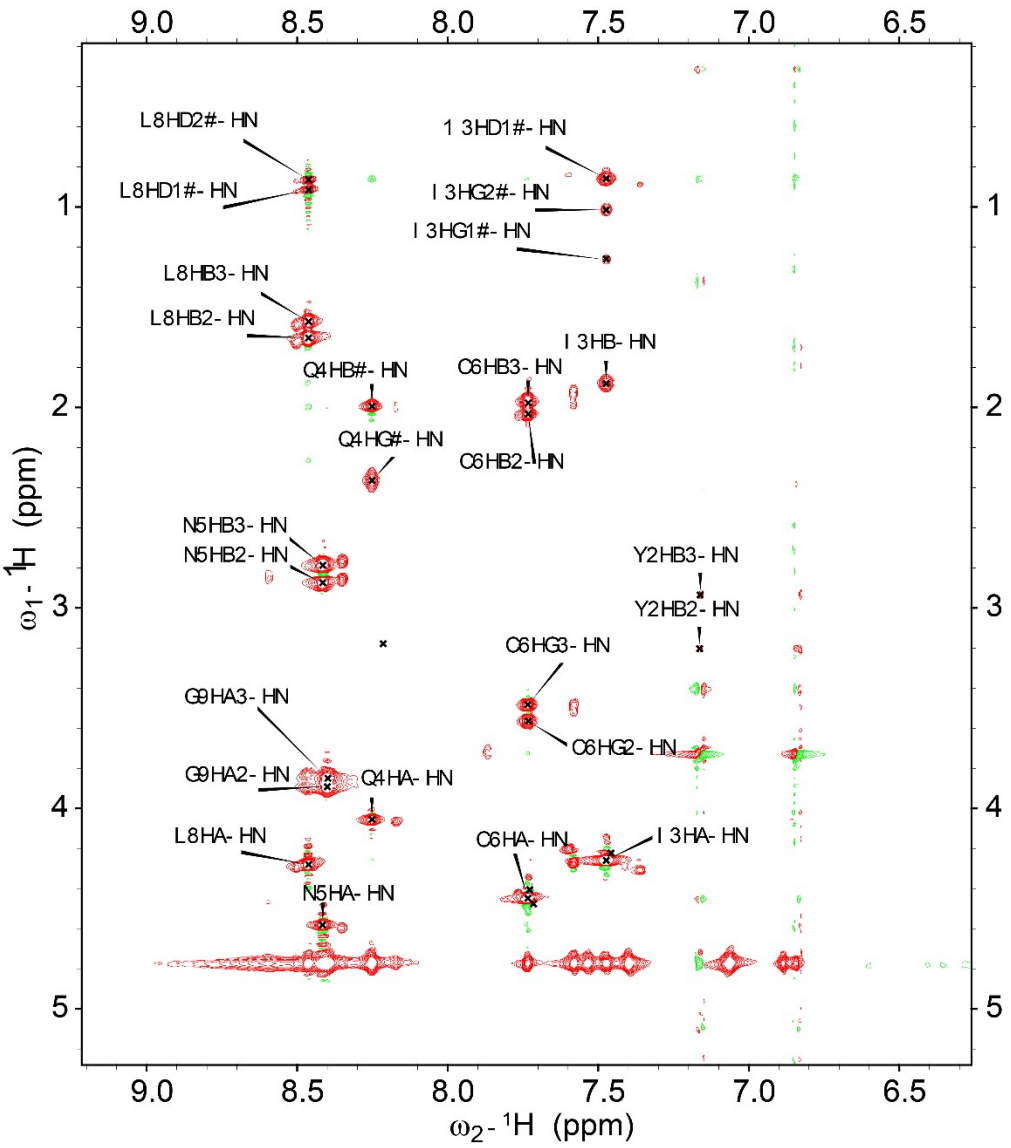


Figure S12. HN region of the synthetic 10 (Oxytocin) TOCSY spectra.

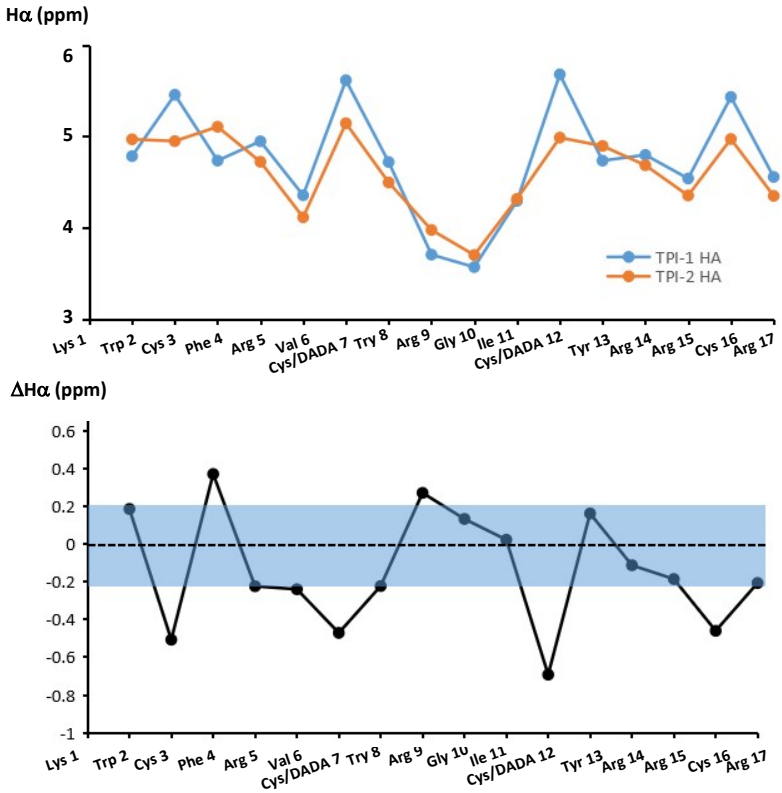


Figure S13. The chemical shift differences of backbone Ha between TPI-2 and TPI-1

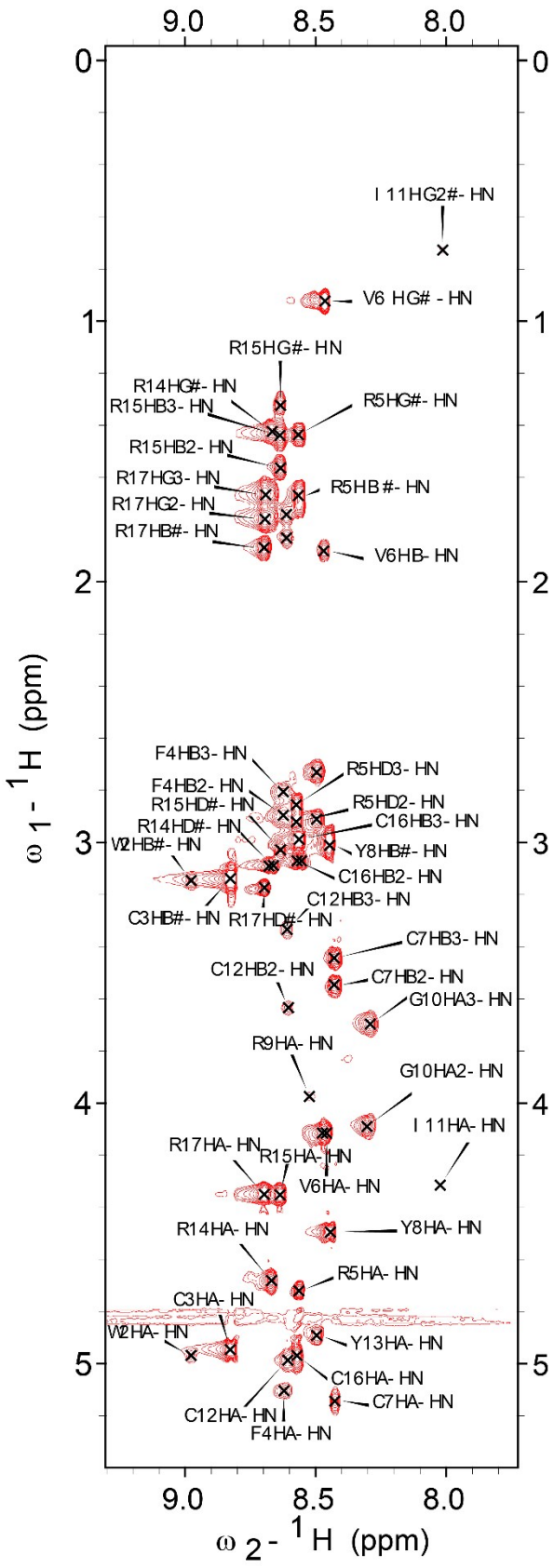


Figure S14. HN region of the synthetic TPI-2 TOCSY spectra.

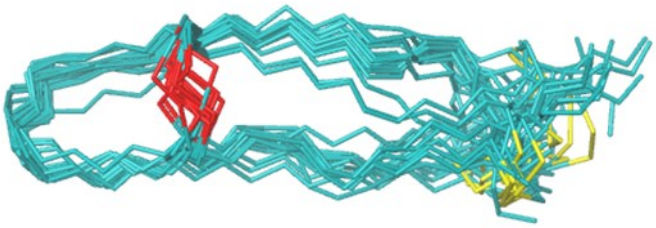
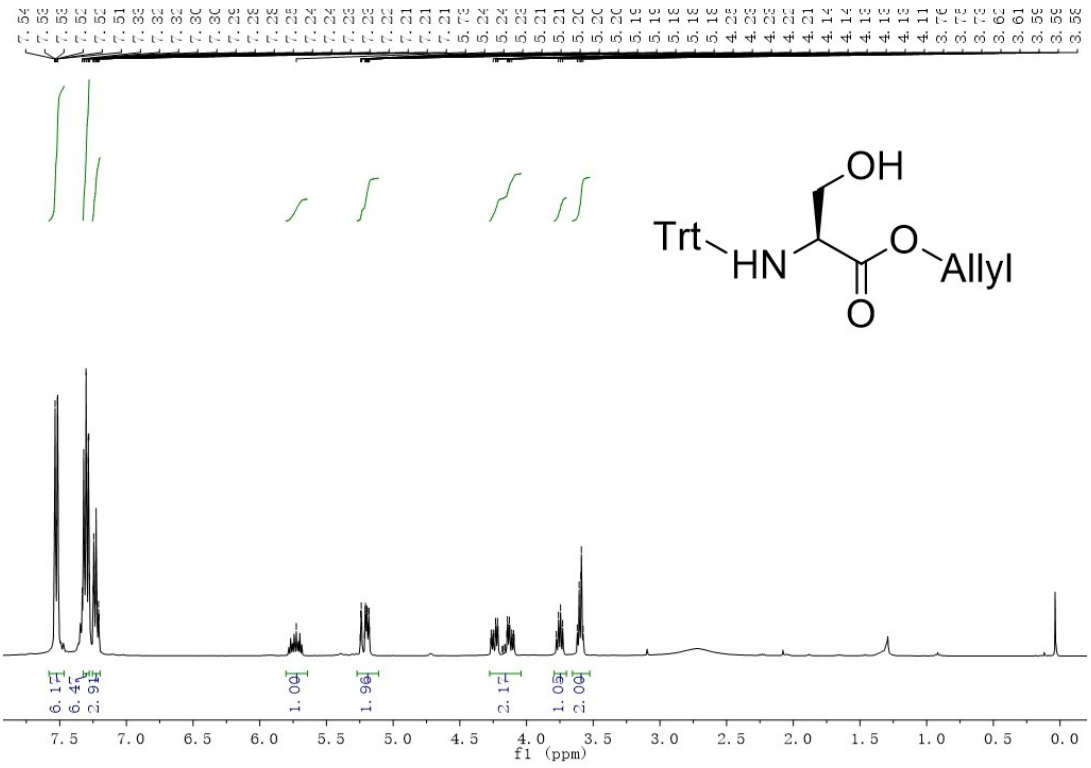
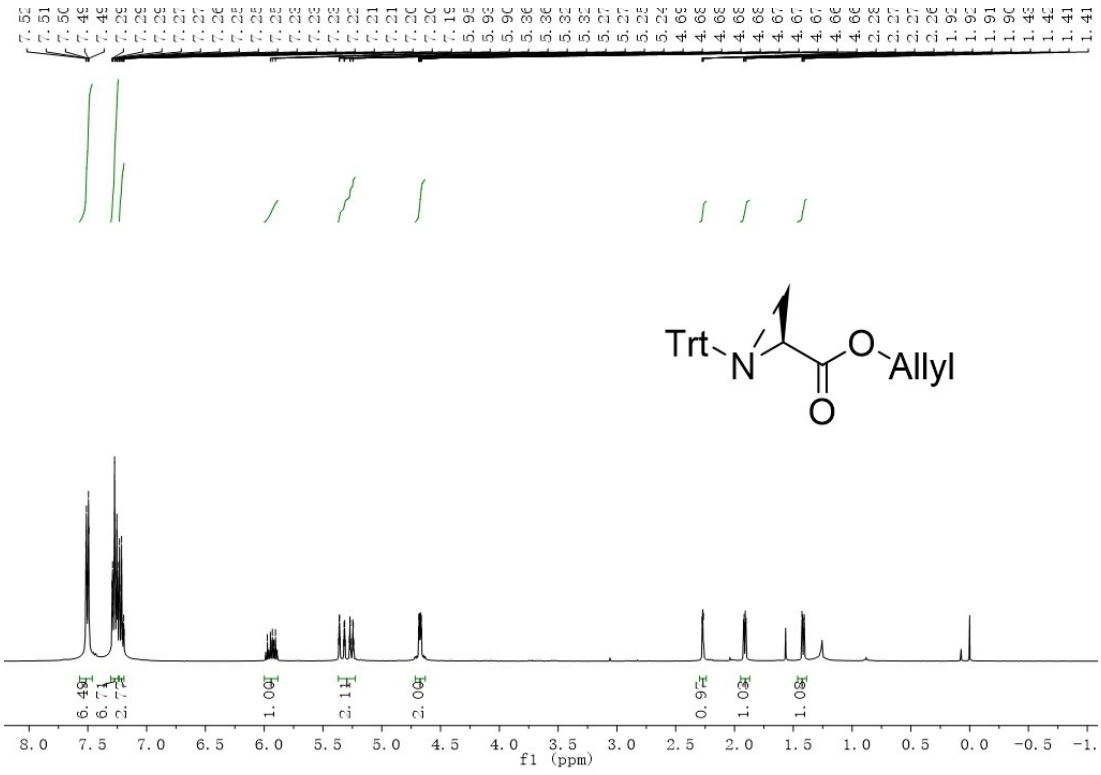


Figure S15. Backbone ensembles of 20 lowest-energy NMR structures of TPI-2

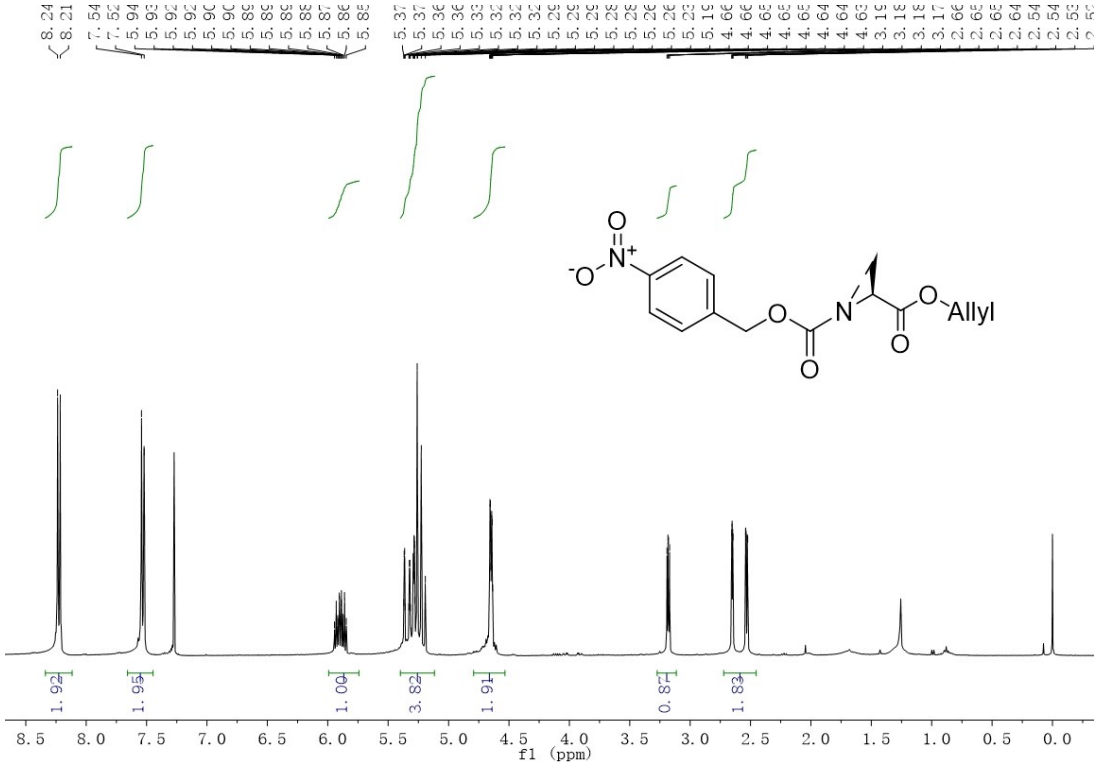
7. NMR and MASS Data for ether-bond diaminodiacids



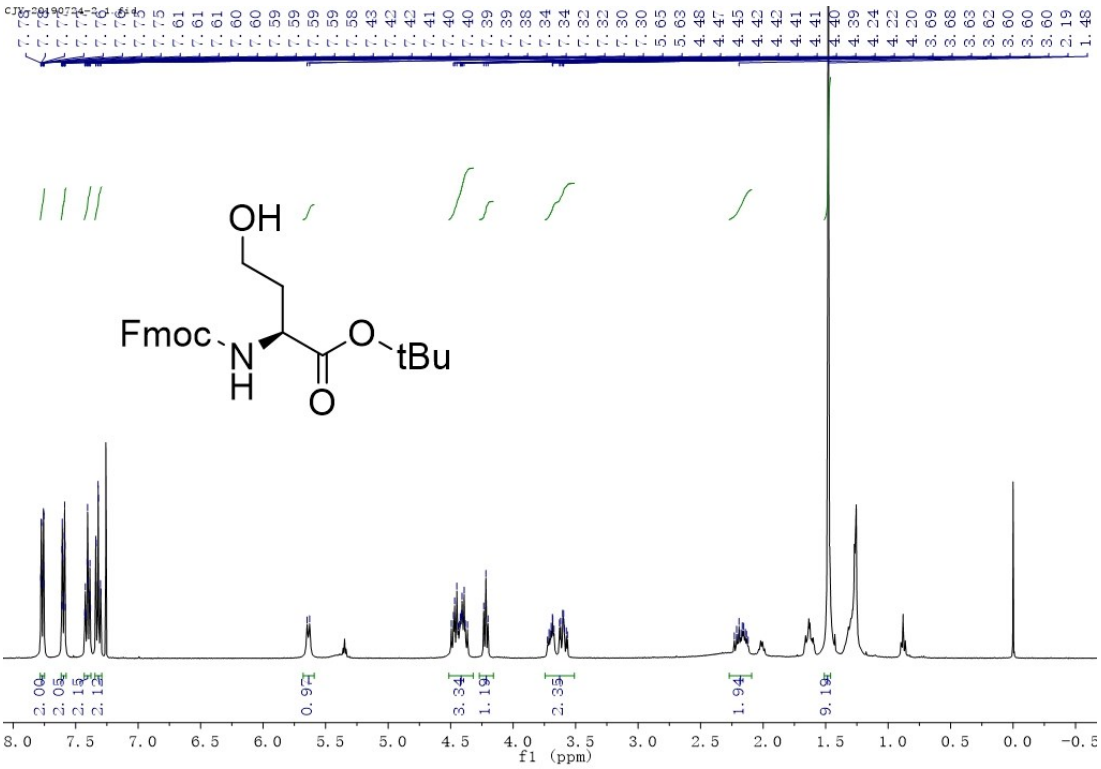
^{1}H NMR spectrum of 1a.



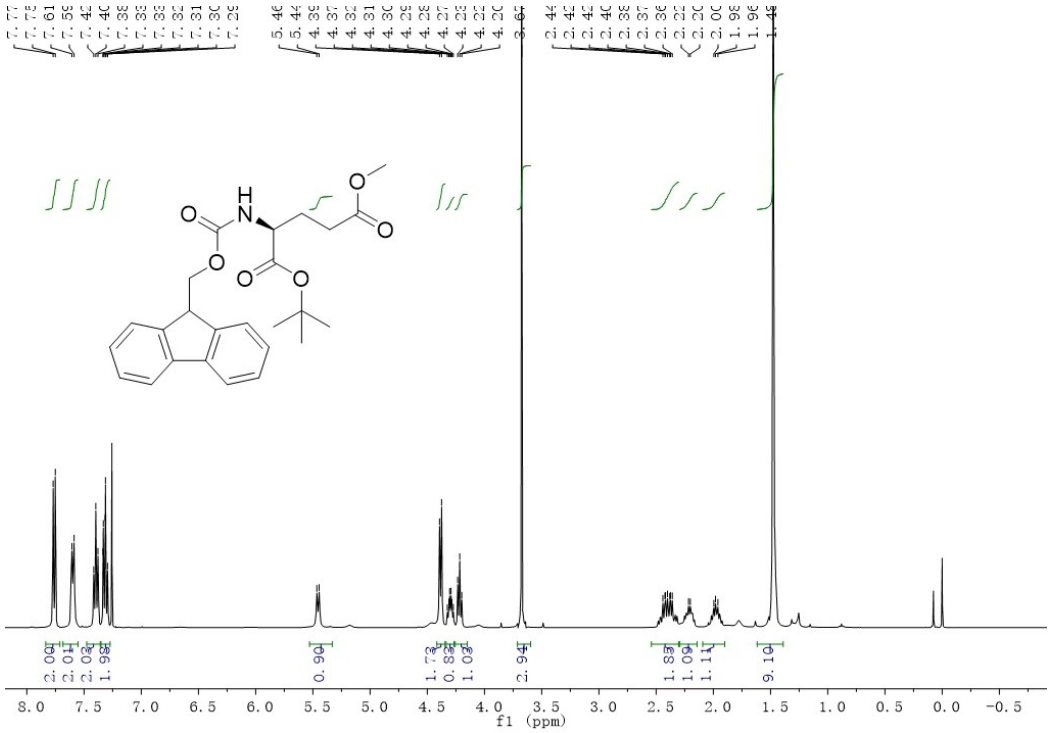
^{1}H NMR spectrum of 1b.



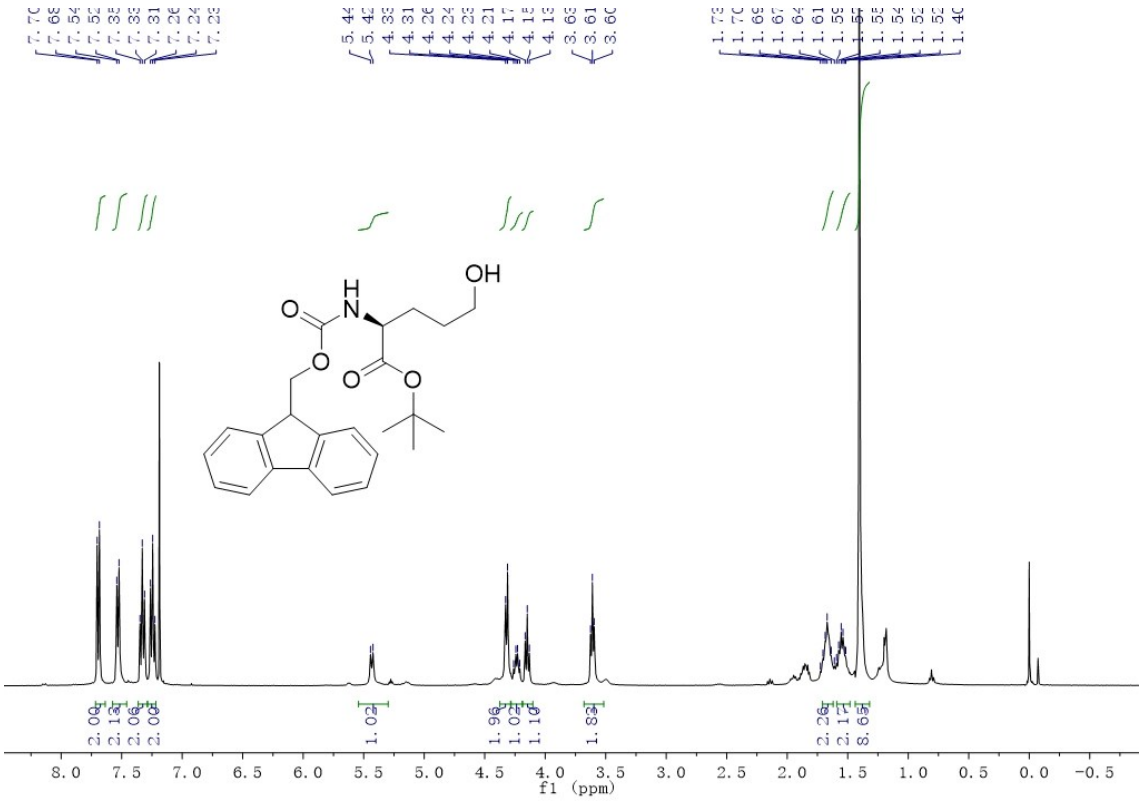
[1]H NMR spectrum of 1c.



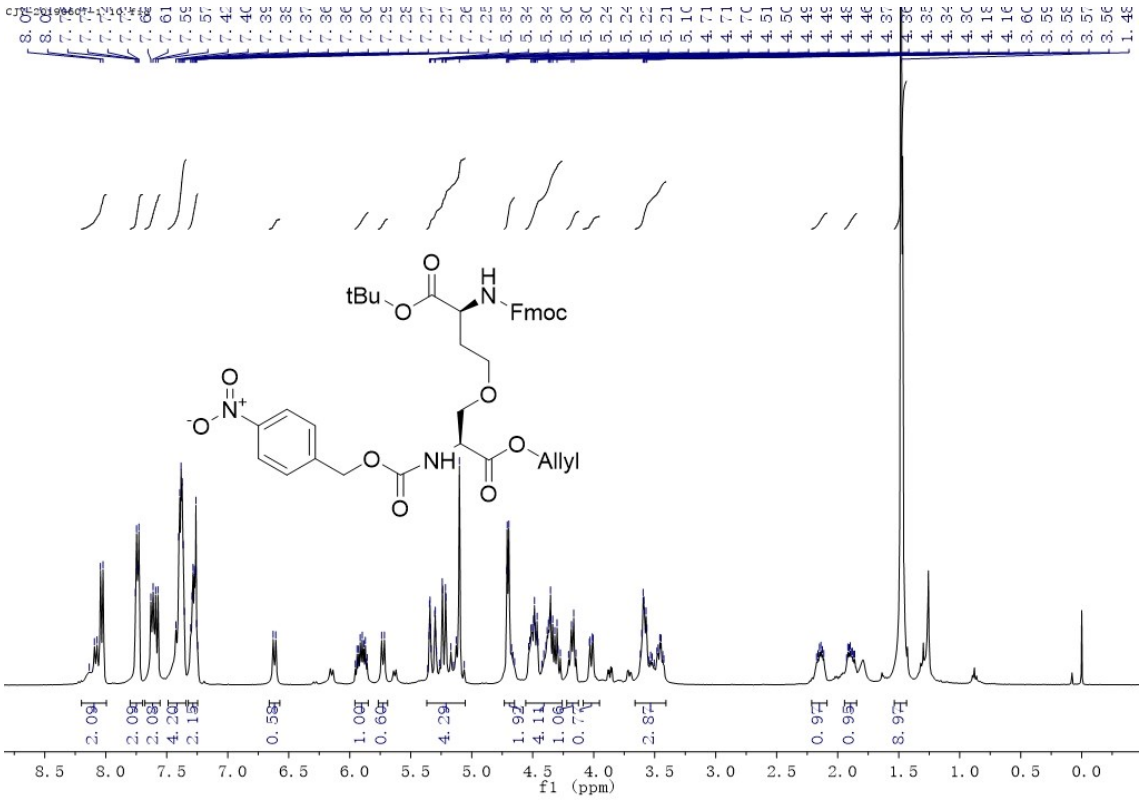
[1]H NMR spectrum of 2a.



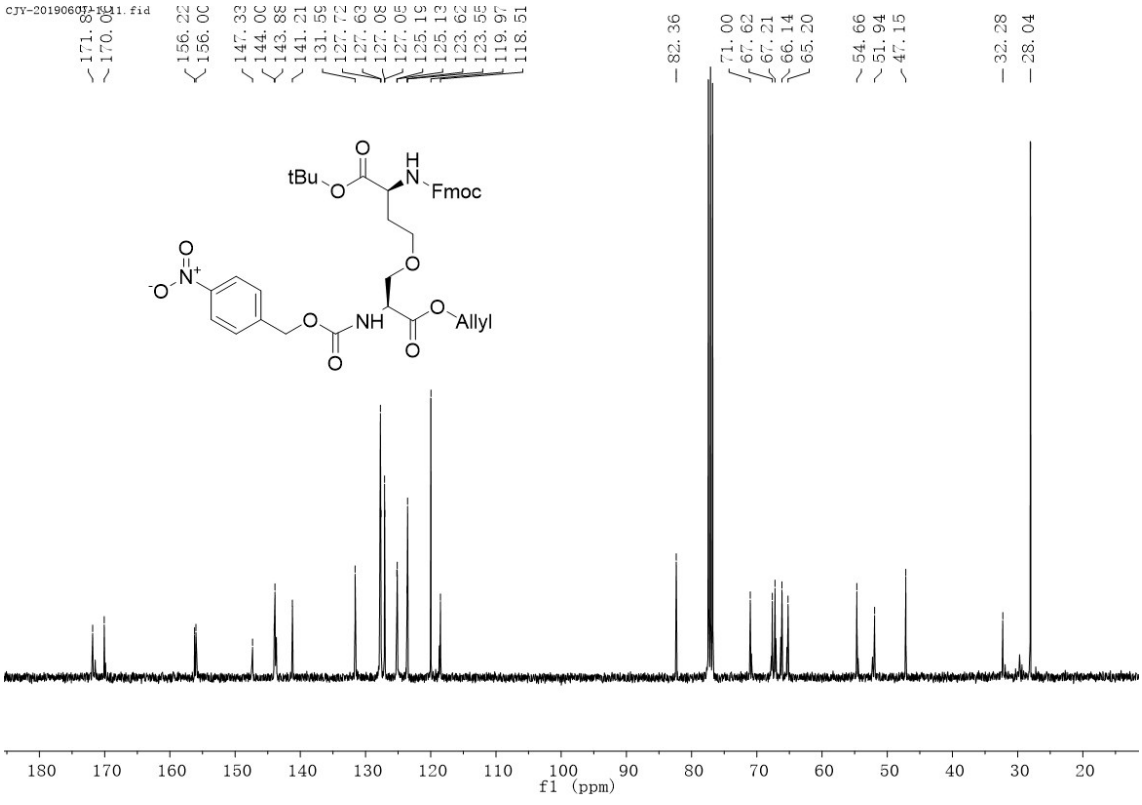
^{1}H NMR spectrum of II-2.



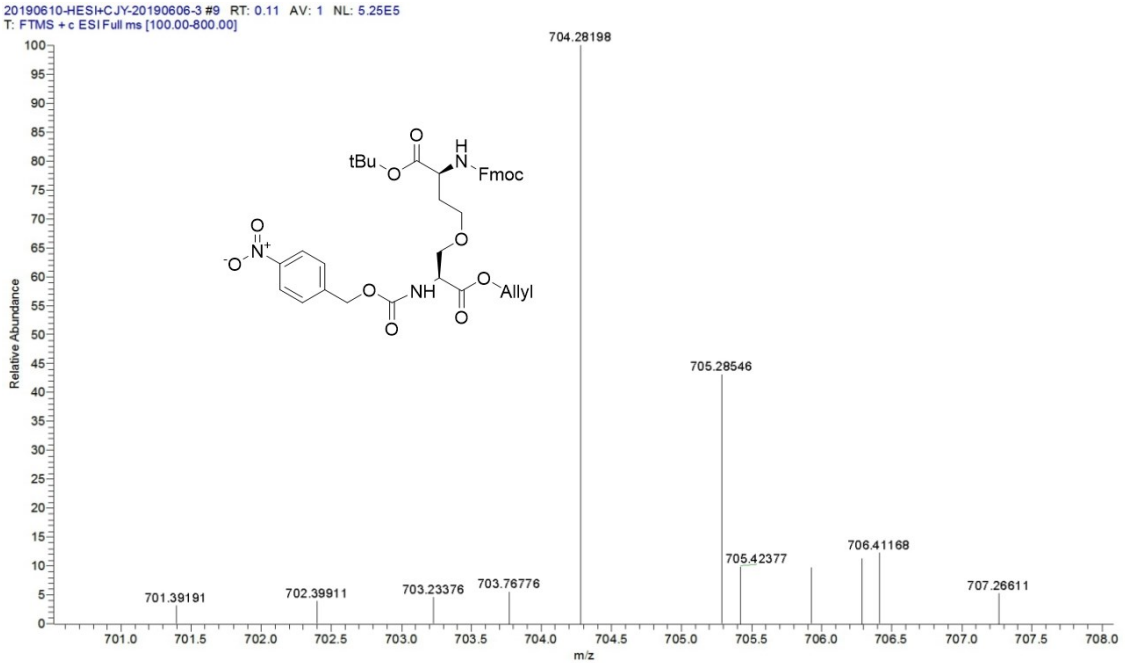
^{1}H NMR spectrum of 2b.



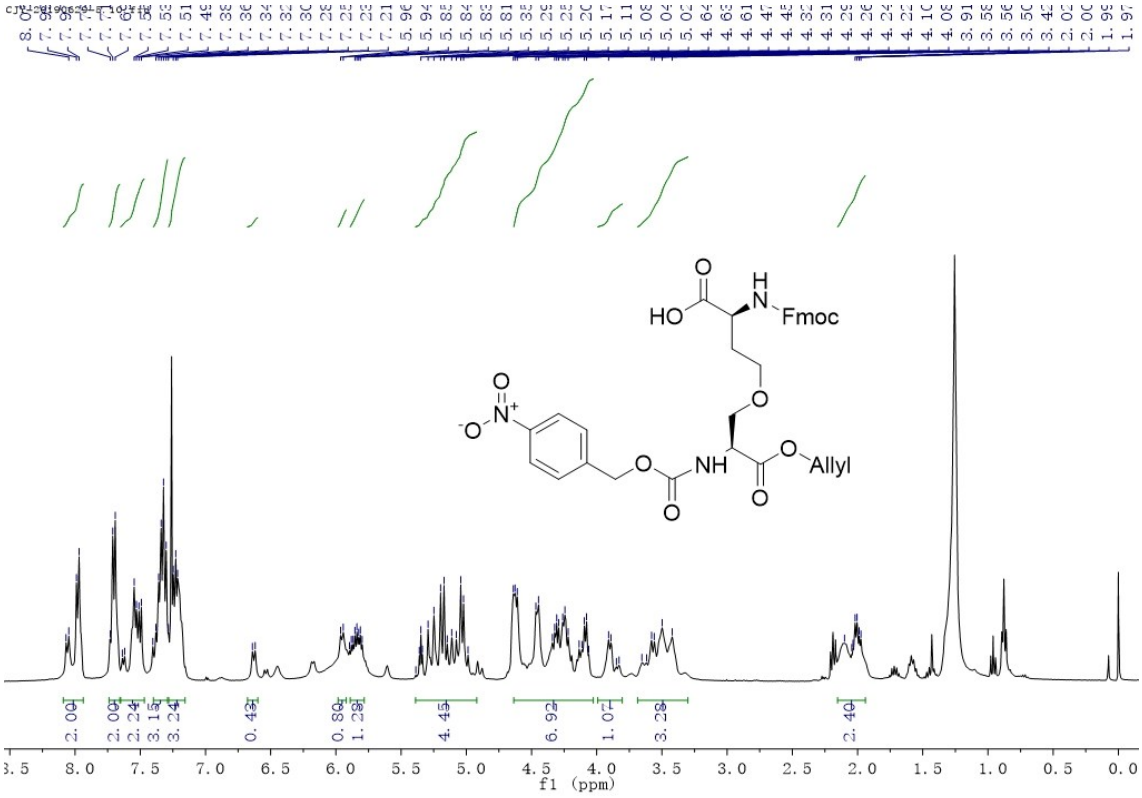
^{1}H NMR spectrum of 3a.



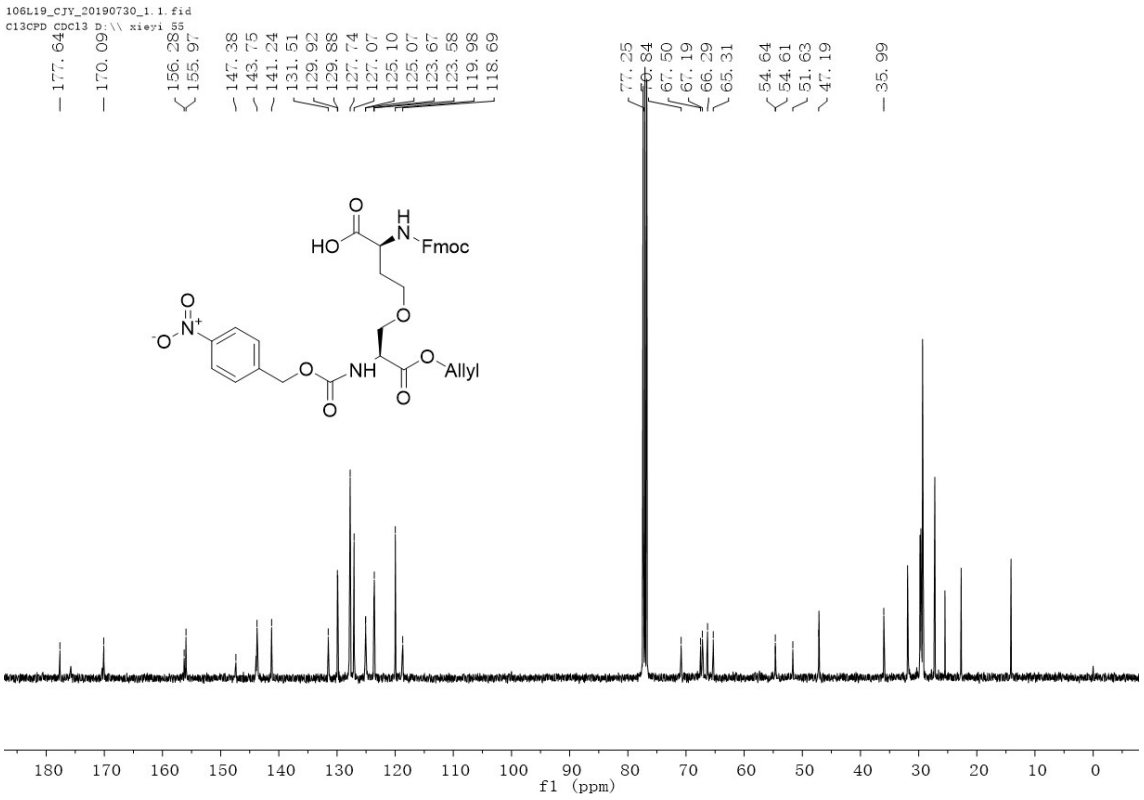
^{13}C NMR spectrum of 3a.



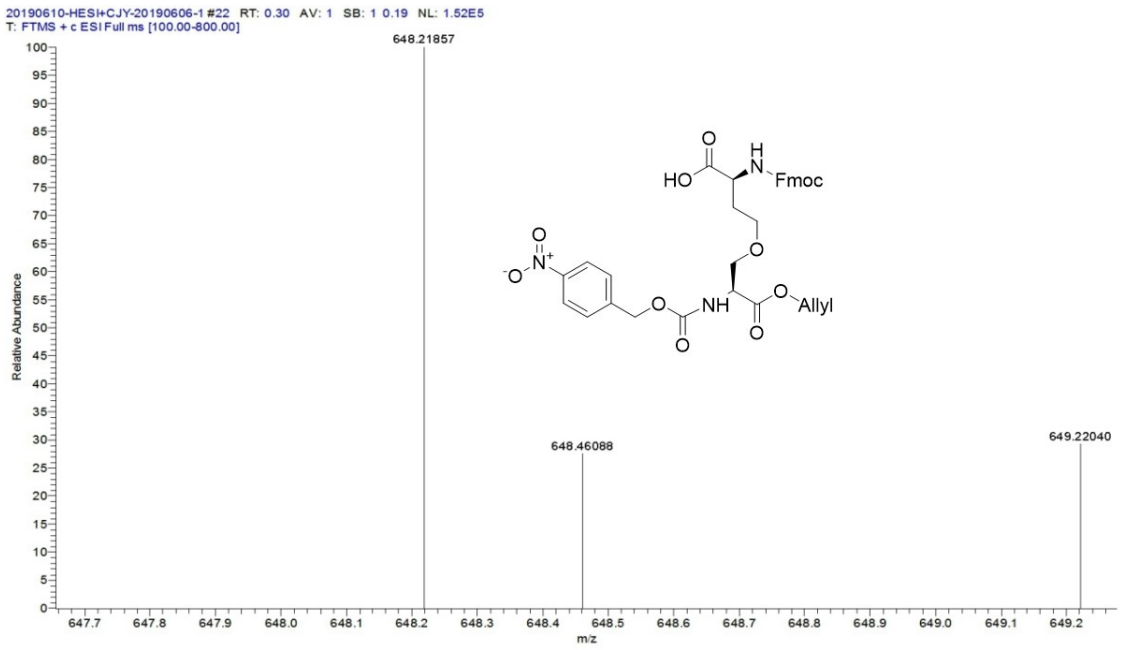
HRMS of compound 3a.



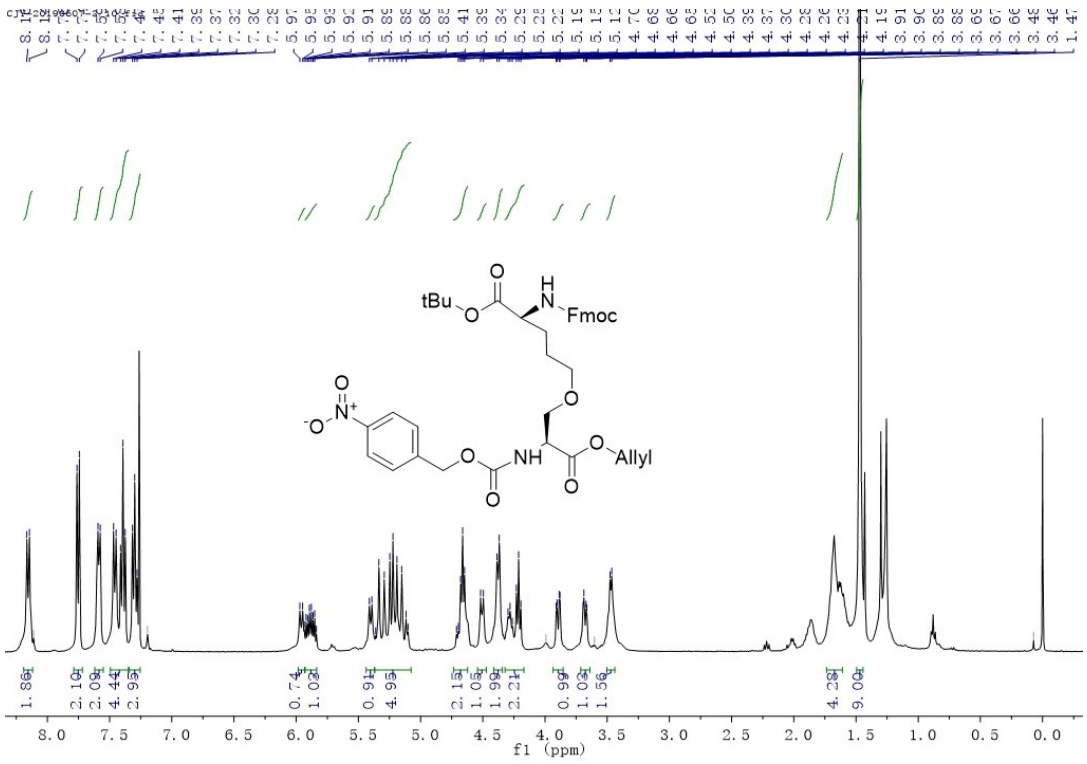
^{1}H NMR spectrum of 4a.



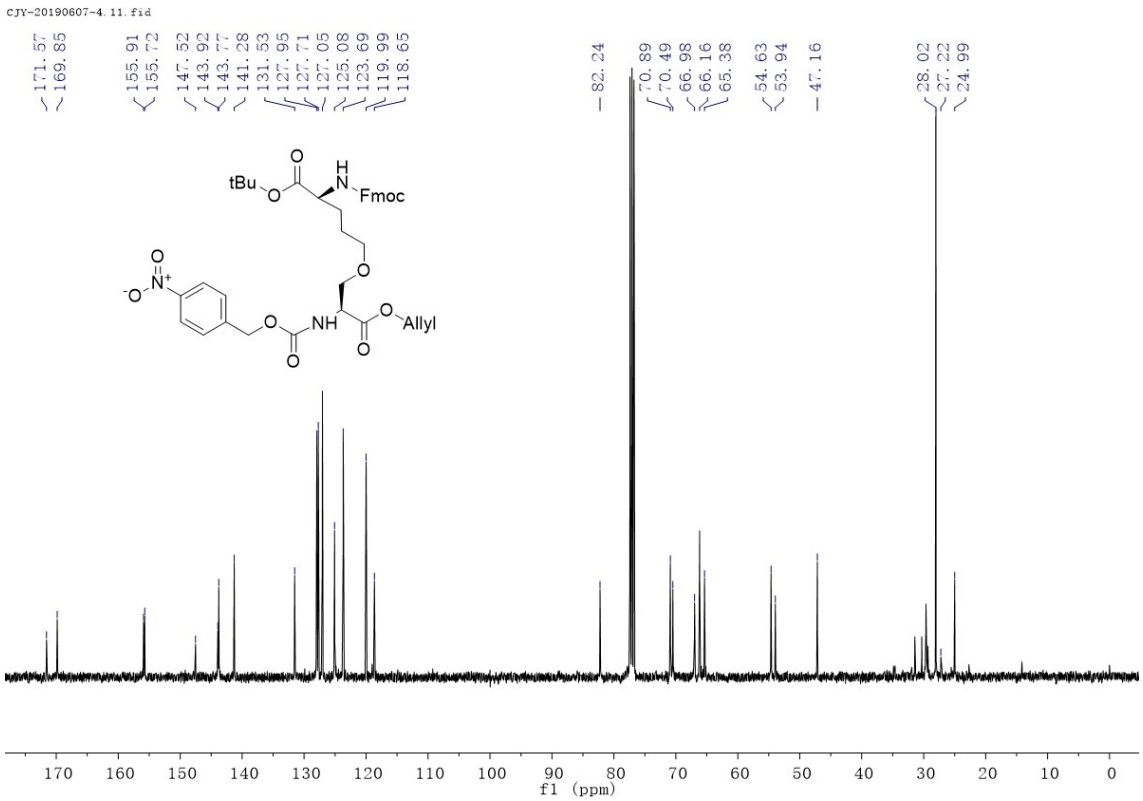
^{13}C NMR spectrum of 4a.



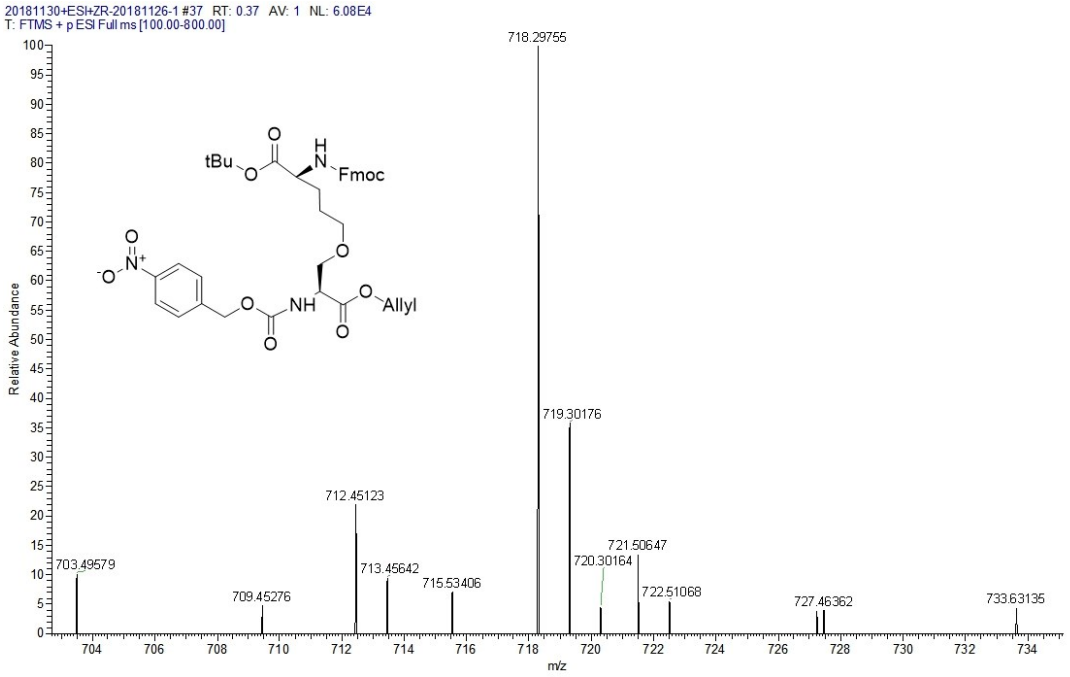
HRMS of compound 4a.



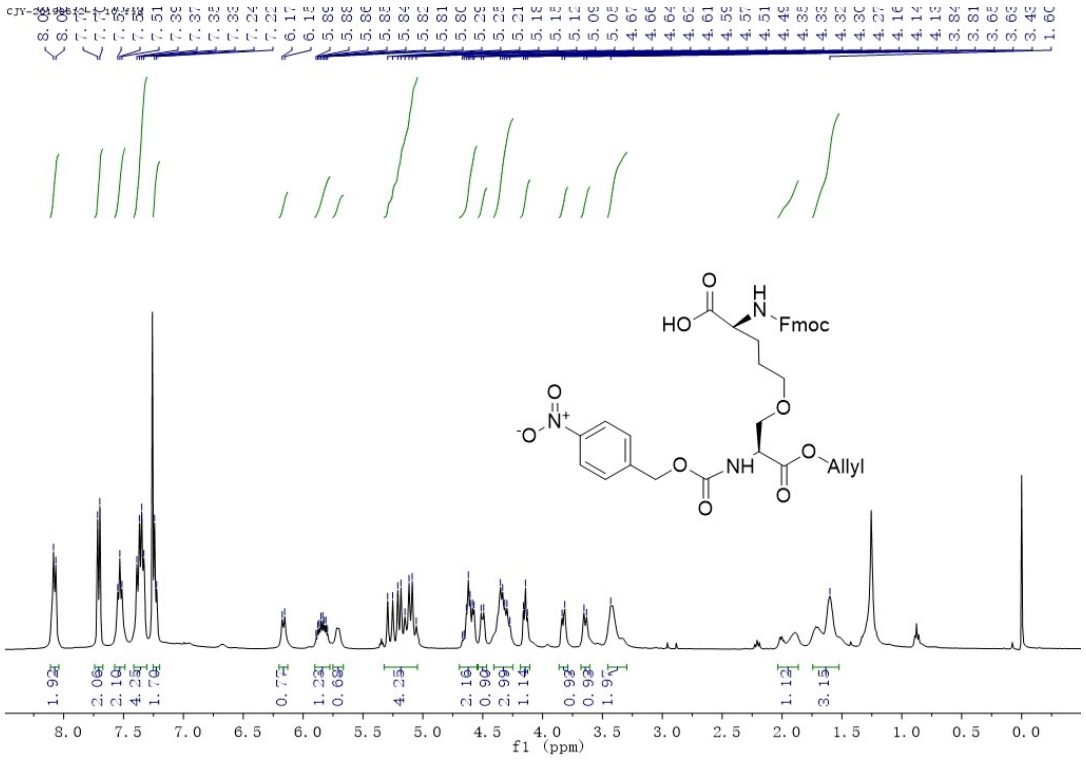
^{1}H NMR spectrum of 3b.



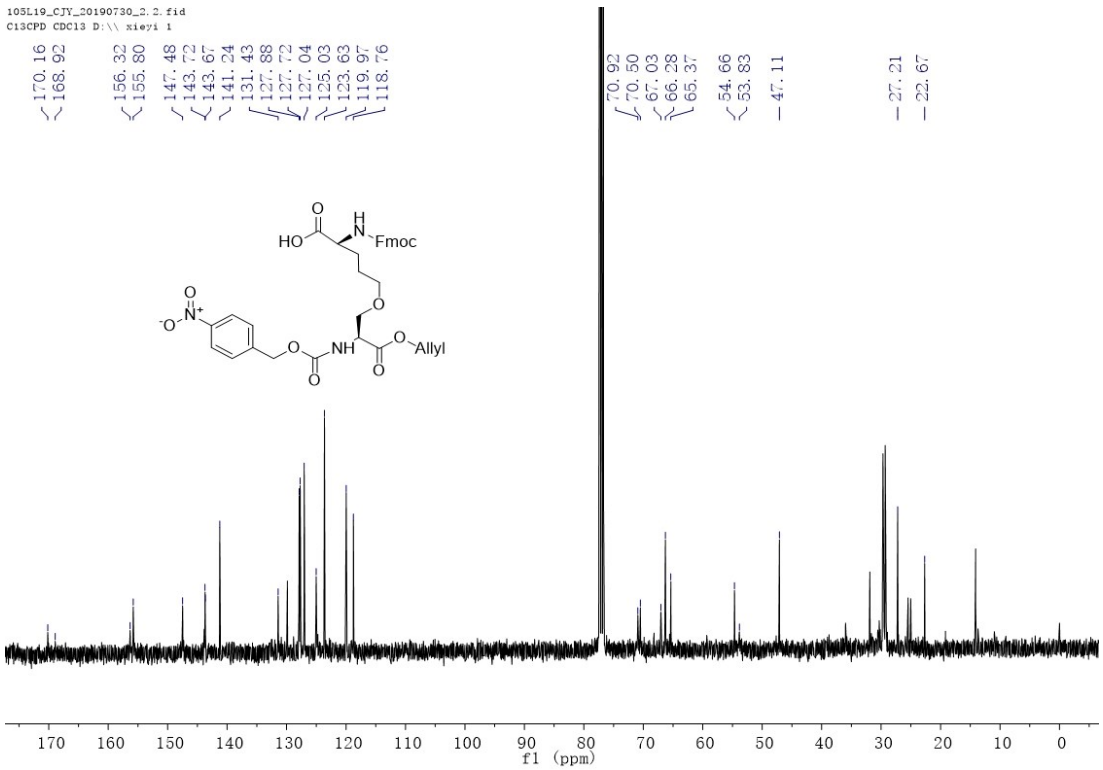
^{13}C NMR spectrum of 3b.



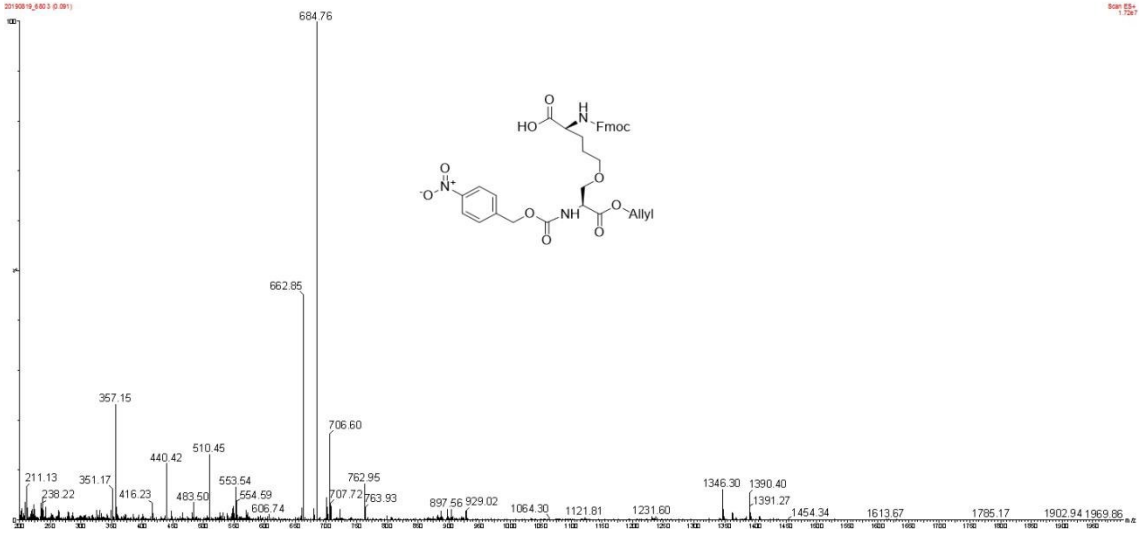
HRMS of compound 3b.



^{1}H NMR spectrum of 4b.



^{13}C NMR spectrum of 4b.



MS of compound 4b.

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