Synthesis of peptide disulfide bond mimics by using fully orthogonally protected diaminodiacids

Tao Wang, [‡a,c] Jian Fan, [‡b] Xiao-Xu Chen,[a] Rui Zhao,[b] Yang Xu,[b] Donald Bierer,[e] Lei Liu,[d] Yi-Ming Li, [c] Jing Shi, [b*] Ge-Min Fang [a*]

[a] *School of Life Science, Institute of Physical Science and Information Technology, Anhui University, Hefei 230601, PR China.*

[b] *Department of Chemistry, University of Science and Technology of China, Hefei 230026, China.*

[c] *School of Biological and Medical Engineering, Hefei University of Technology, Hefei, Anhui 230009, China.*

[d] *Tsinghua University, Beijing 100084, PR China*

[e] *Bayer AG, Department of Medicinal Chemistry, Aprather Weg 18A, 42096 Wuppertal, Germany*

Email：fanggm@ahu.edu.cn

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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), N-methyl-2-pyrrolidone (NMP) 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

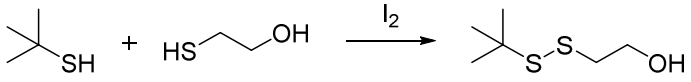
Analytical HPLC was run on a SHIMADZU (Prominence LC-20AT) instrument using analytical column (Grace Vydac "Protein & Peptide C18", 250 × 4.6 mm, 5 μm particle size, flow rate 1.0 mL/min, R.T.) solution A (0.08 % trifluoroacetic acid in acetonitrile) and solution B (0.1 % trifluoroacetic acid in ddH_2O) 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). The chiral column used is Ultimate®Amy-D (4.6x250 mm, 5 μm particle size, UV 254 nm), with a flow rate of 0.9 mL/min and a constant solution of n-hexane/ethanol (1:1).

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. Chemical synthesis of Fmoc/Mtt/Tbe protected diaminodiacids

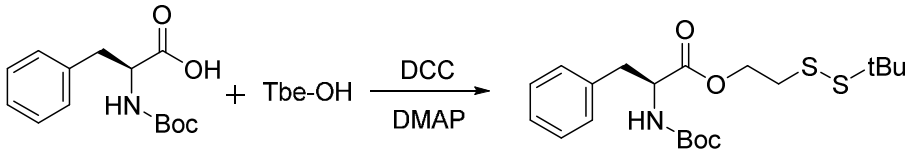
a. Synthesis of Tbe-OH



To a solution of 2-mercaptoethanol (8 mmol, 0.624 g) in 95% EtOH (10 mL) was added 2-methyl-2-propanethiol (80 mmol, 7.2 g). Then, a solution of iodine (30 mmol, 7.62 g) in 95%

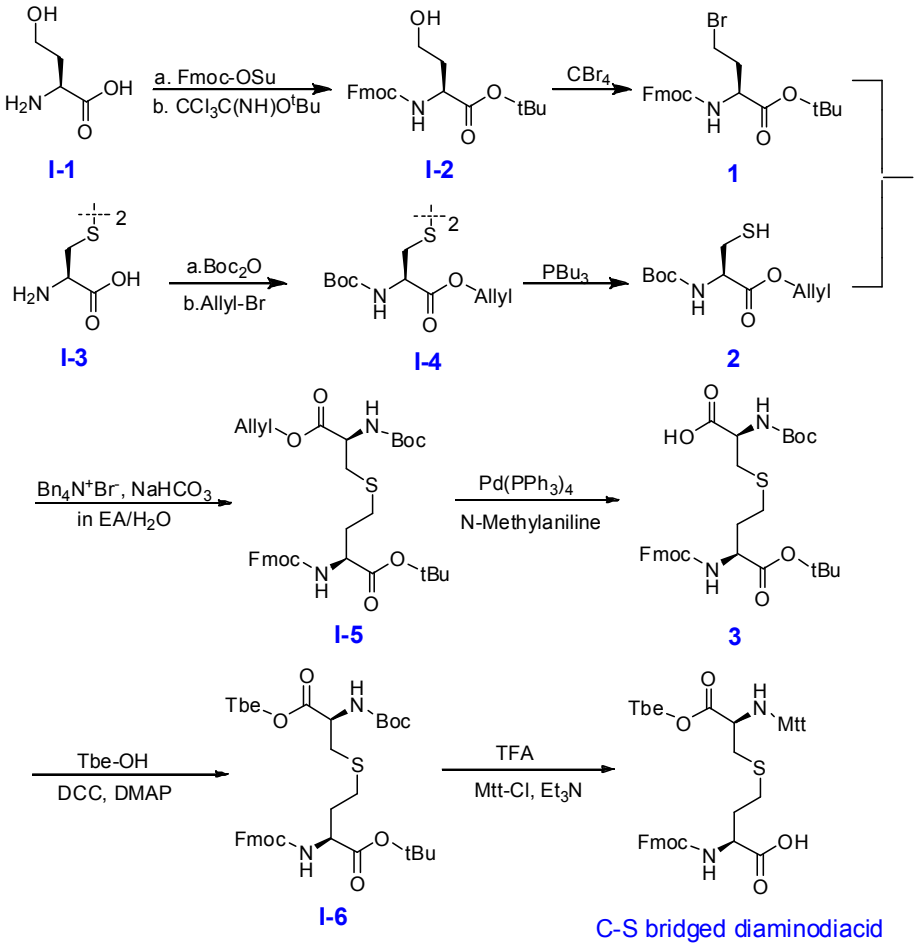
EtOH (40 mL) was added dropwise under ice-water bath until the color of the system changed from colorless to brown. After 90 min, at 0□, saturated aqueous $NaHCO_3$ was added until pH＞7. The solution was concentrated in vacuo. Ethyl acetate was added to the residue and the organic layer was washed with 10% Na_2SO_4 (3×) and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated in vacuo. The disulfide was purified by flash column chromatography to afford the desired product with a yield of 75.3% (1 g, 6.03 mmol). 1H NMR (400 MHz, $CDCl_3$): δ =3.93 (t, J=5.8Hz, 2H), 2.91 (t, J=5.8Hz, 2H), 2.06 (s, 1H), 1.45 (s, 9H). 13C NMR (400 MHz, CDCl3) δ = 60.67, 48.03, 42.68, 29.88.

b. Synthesis of Boc-Phe-OTbe



To a solution of Boc-Phe-OH (0.27 g, 1 mmol) in DCM (2 mL) were added Tbe-OH (0.33 g, 2 mmol), DCC (0.25 g, 1.2 mmol in 1 mL DCM) and DMAP (0.024 g, 0.2 mmol). The reaction mixture was stirred at room temperature for 12 h. Then, the mixture was filtered to remove the formed DCU. The combined organic phase was washed with brine, dried over Na_2SO_4. The crude product was purified by chromatography to afford Boc-Phe-Tb with a yield of 42% (170 mg, 0.416 mmol). R_f 0.65 (5:1 petroleum ether/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.29 – 7.14 (m, 5H)) 4.97 (br, 1H), 4.59 (br, 1H), 4.33 (t, *J*=6.7Hz, 2H), 3.09 (m, 2H), 2.83 (t, *J*=6.7Hz, 2H), 1.42 (s, 9H), 1.34 (s, 9H).^{13}C NMR (400 MHz, $CDCl_3$): δ = 171.70, 155.08, 135.96, 129.39, 128.57, 127.05, 63.54, 54.44, 48.07, 38.36, 38.07, 29.86, 28.31.

c. Synthesis of C-S bridged diaminodiacid



Scheme S1. Synthetic route for C-S bridged diaminodiacid 1

Synthesis of compound I-2

L-Homoserine (1.0 g, 8.4 mmol), sodium carbonate (0.89 g, 8.4 mmol) and Fmoc-OSu (2.84 g 8.4 mmol) were dissolved in 30 mL of water/1, 4-dioxane (2:1, v:v), at 0°C. The reaction mixture was gradually heated to room temperature and stirred for 10 h. Then, 1, 4-dioxane was removed under vacuum. The residue was washed with EtOAc and acidified to pH 2 with HCl. The aqueous suspension was extracted by EtOAc, dried over Na_2SO_4, filtrated and evaporated. The residue was used for the next step without further purification.

The residue was dissolved in DCM/THF (50 mL/12 mL). Then, tert-butyl 2, 2, 2-trichloroacetimidate (3.37 mL, 19 mmol, CAS: 98946-18-0) was added. The reaction mixture was stirred overnight. The reaction was 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 (2:1 petroleum ether/EtOAc) to afford compound I-2 (1.33 g, 3.36 mmol, 40%). R_f 0.35 (2:1 petroleum ether/EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 2H), 7.32 (t, J = 7.4 Hz, 2H), 5.62 (br, 1H), 4.54 – 4.33 (m, 3H), 4.22

(t, J = 6.8 Hz, 1H), 3.73 – 3.50 (m, 2H), 2.17 (m, 2H), 1.48 (s, 9H).ESI-MS (positive): 398.19598 (observed, M+H); 397.1889 (calculated, M).

Synthesis of compound 1

Compound □-2 (1.10 g, 2.77 mmol) and carbon tetrabromide (1.10 g, 3.32 mmol) were dissolved in DCM (10 mL). Under 0^{o}C, triphenylphosphine (0.87 g, 3.32 mmol) in DCM (5 mL) was added dropwise. The reaction mixture was gradually heated to room temperature and stirred for 2 h. Then, the mixture was diluted with DCM. The organic phase was washed with water and brine, dried over Na_2SO_4, filtrated and concentrated to ~10 mL. Excess of petroleum ether/EtOAc (1:1) was added to precipitate phosphine oxide byproducts, followed by filtration through Celite. The filtrate was concentrated under vacuum. The crude product was purified by chromatography (5:1 petroleum ether/EtOAc) to afford compound 1 (0.82 g, 1.79 mmol, 64.5%) as colorless oil. R_f 0.5 (5:1 petroleum ether/EtOAc). 1H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 7.5 Hz, 2H), 7.59 (d, J = 7.4 Hz, 2H), 7.40 (t, J = 7.4 Hz, 2H), 7.31 (t, J =7.5 Hz, 2H), 5.68 (br, 1H), 4.51 – 4.34 (m, 3H), 4.22 (t, J = 6.8 Hz, 1H), 3.69 – 3.52 (m, 2H), 2.41 (m, 1H), 1.65 (m, 1H), 1.48 (s, 9H). ESI-MS (positive): 482.0937 (observed, M+Na); 459.1045 (calculated, M).

Synthesis of compound I-4

L-Cystine (2.4g, 10 mmol) and $NaHCO_3$ (6.72g, 80 mmol) were dissolved in water/1, 4-dioxane (80 mL/20 mL). Then, Boc_2O (6.55g, 30 mmol) was added under an ice bath. The reaction mixture was stirred at room temperature overnight. Afterwards, the mixture was diluted with water, and washed with diethyl ether twice. The aqueous layer was neutralized to pH 2 with 2 M HCl, and extracted with EtOAc. The combined organic phase was washed with water and brine, dried over Na_2SO_4, evaporated to dryness. The residue was used for the next step without further purification.

The residue was dissolved in DMF (40 mL), followed by addition of sodium bicarbonate (5.04g, 60 mmol) and allyl bromide (2.9g, 24 mmol). After 12 h, the mixture was extracted with EtOAc, washed with water (many times to remove remaining DMF), brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to afford compound I-4 (3.5 g, 6.73 mmol, 67.3%). 1H NMR (400 MHz, $CDCl_3$) δ = 5.93 (m, J_{dc}=17.2Hz, J_{db}=10.4Hz, J_{da}=5.8Hz, $2H_d$,CH_a-CH_d=CH_bH_c), 5.45-5.40 (br, 2NH), 5.40-5.32 (m, J_{cd}=17.2Hz, J_{cb}=1.32Hz $2H_c$, CH_a-CH_d=CH_bH_c), 5.30-5.25 (m, J_{bd}=10.4Hz, J_{bc}=1.32Hz, $2H_b$, CH_a-CH_d=CH_bH_c), 4.66 (m, J_{ad}=5.8Hz, J_{ab}=1.24Hz, $4H_a$, CH_a-CH_d=CH_bH_c), 4.62 (m, 2H), 3.2 (d, J=5Hz, 4H),1.45 (s, 18H).

Synthesis of compound 2

Compound I-4 (1.04 g, 2 mmol) was dissolved in THF (15 mL), followed by dropwise adding tributylphosphine (0.61 g, 3 mmol). The reaction mixture was stirred for 30 min under nitrogen. Then, water (2.5mL) was added to the solution. The mixture was stirred at room temperature for another 2 h. Then, the reaction was concentrated in vacuo, dissolved in EtOAc. The organic phase was washed with citric acid (w/w 10%), brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography (4:1 petroleum ether/EtOAc) to afford compound 2 (0.83 g, 3.2 mmol, 80%). 1H NMR (400 MHz, CDCl3) δ = 5.93 (m, 1H, CH=CH_2), 5.32 (m, 3H), 4.86 – 4.26 (m, 3H), 3.00 (m, 2H), 1.46 (s, 9H).

Synthesis of compound I-5

Compound 1 (2.2 mmol) and compound 2 (2.1 mmol) were dissolved in EtOAc (50 mL). Then, tetrabutylammonium bromide (2.71 g, 8.4 mmol) in saturated $NaHCO_3$ (10 mL) was added. After vigorously stirring overnight, the reaction mixture was diluted with EtOAc. The combined organic phase was washed with water and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to afford compound □-5 (1.36 mmol, 65%) as colorless oil. R_f 0.25 (4:1 petroleum ether/EtOAc).1H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J*=7.5, 2H), 7.61 (d, *J*=7.3, 2H), 7.40 (t, *J*=7.4, 2H), 7.31 (t, *J*=7.4, 2H), 5.90 (m, J_{dc}=17.2 Hz, J_{db}=10.2Hz, J_{da}=5.6Hz, $1H_d$, CH_a-CH_d=CH_bH_c), 5.45-5.40 (br, 2NH), 5.40-5.32 (d, J_{cd}=17.2Hz, H_c, CH_a-CH_d=CH_bH_c), 5.30-5.25 (d, J_{bd}=10.2Hz, H_b, CH_a-CH_d=CH_bH_c), 4.66 (d, J_{ad}=5.6Hz, $2H_a$, CH_a-CH_d=CH_bH_c), 4.5 (br, 1H), 4.45 (m, 3H), 4.20 (m, 1H), 2.98 (br, 2H), 2.57 (br, 2H), 2.10(br, 1H), 1.90 (br, 1H), *J*=5Hz, 4H), 1.47 (s, 9H), 1.44 (s, 9H). ^{13}CNMR (400 MHz, CDCl3): δ = 170.94, 170.73, 155.93, 155.17, 143.89, 143.76, 141.30, 131.40, 127.73, 127.08, 125.15, 120.00, 119.10, 82.60, 80.24, 67.03, 66.30, 53.53, 53.34, 47.17, 34.65, 32.72, 29.72, 28.31, 28.02.

Synthesis of compound 3

To a solution of compound □-5 (0.64g 1 mmol) in THF (10 mL) was added $Pd(PPh_3)_4$ (0.23g 0.2 mmol). Then, N-methylaniline (0.21g, 2 mmol) was added dropwise. After vigorously stirring 30 min, the reaction mixture was diluted with EtOAc. The combined organic phase was washed with 1M HCl and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to afford compound 3 (0.48 g, 0.8 mmol, 80%).1H NMR (400 MHz, $CDCl_3$) δ = 7.69 (d, *J*=7.5, 2H), 7.54 (d, *J*=7.4, 2H), 7.33 (t, *J*=7.4, 2H), 7.24 (t, *J*=7.4, 2H), 5.67 (br, 1H), 5.19 (br, 1H), 4.31 (m, 4H), 4.16 (m, 1H), 2.90 (m, 2H), 2.55 (br, 2H), 2.04 (br, 1H), 1.90 (br, 1H), 1.41 (s, 9H), 1.35 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$): 13C NMR (400 MHz, CDCl3) δ = 173.82, 171.13, 156.23, 143.73, 141.31, 134.27, 127.75, 127.12, 125.13, 120.01, 82.82, 80.49, 67.15, 60.52, 53.62, 47.10, 34.49, 32.92, 29.74, 28.31, 28.00. ESI-MS (positive): 623.23889 (observed, M+Na); 600.25 (calculated, M).

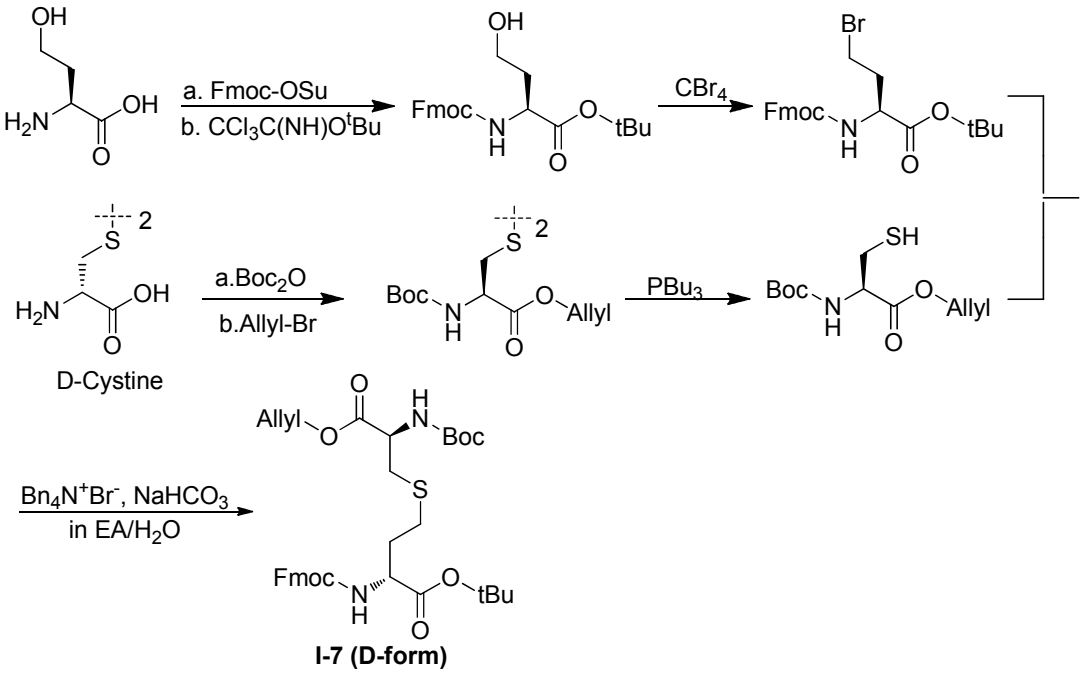
Synthesis of compound I-6

To a solution of compound 3 (0.3g, 0.5 mmol) in DCM (2 mL) were added Tbe-OH (0.17g, 1 mmol), DCC (0.12g, 0.6 mmol in 1 mL DCM) and DMAP (0.012g, 0.1 mmol). After vigorously stirring at room temperature for 12 h, the mixture was filtered to remove the formed DCU. The combined organic phase was washed with brine, dried over Na_2SO_4. The crude product was purified by chromatography to afford compound I-6 (0.449g, 0.3 mmol, 60%). R_f 0.5 (5:1 petroleum ether/EtOAc). 1H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, *J*=7.5, 2H), 7.61 (d, *J*=7.4, 2H), 7.41 (t, *J*=7.4, 2H), 7.32 (t, *J*=7.5, 2H), 5.48 (m, 1H), 5.40 (m, 1H), 4.55 (br, 1H), 4.38 (m, 5H), 4.23 (t, 1H), 3.0-2.84 (br, 2H), 2.90 (t, 2H), 2.59 (br, 2H), 2.16 (m, 1H), 1.95 (m, 1H), 1.48 (s, 9H), 1.44 (s, 9H), 1.32 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 170.98, 170.84, 155.96, 155.17, 143.80, 141.31, 127.73, 127.10, 125.19, 120.01, 82.59, 80.26, 67.06, 63.82, 60.69, 53.55, 48.11, 47.18, 42.71, 38.13, 29.89, 29.85, 28.32, 28.04. ESI-MS (positive): 749.29504 (observed, M+H); 748.29 (calculated, M)

Synthesis of C-S bridged diaminodiacid

To a solution of compound I-6 (8 mmol) in DCM (10 mL) was added TFA (10 mL). After vigorously stirring overnight at room temperature, the solvent was evaporated under high vacuum. The remaining residue was dissolved in dry DCM (150 mL). Under nitrogen atmosphere and room temperature, trimethylsilyl chloride (24.8 mmol, 3.15 mL) and DIPEA (24.8 mmol, 1.4 mL) were added. After 1 h reflux, the reaction mixture became homogeneous. Then, the mixture was cooled to 0 □, followed by addition of DIPEA (24.8 mmol, 4.1 mL) and 4-methyltrityl chloride (8.4 mmol, 2.5 g). After stirring at room temperature for another 16 h, methanol (25 mL) was added to quench the reaction. After 15 min, the solvent was evaporated under vacuum at 30 °C, and the remaining residue was partitioned between DCM and pH 5 acetate buffer. The combined organic phase was dried, and concentrated under vacuum to 10 mL. C-S bridged diaminodiacid was obtain by precipitation with hexane, as a light yellow solid and purified by silica gel chromatography (4.14 g, 6.87 mmol, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.59 (d, 2H), 7.40 (m, 6H), 7.23 (m, 4H), 7.09 (t, 6H), 7.01 (d, 2H), 6.91 (d, 2H), 4.15 (br, 1H), 4.11 (br, 1H), 4.00 (br, 2H), 3.69 (br, 1H), 3.61(br, 1H), 3.29 (br, 1H), 2.70 (br, 1H), 2.59 (br, 1H), 2.47 (br, 2H), 2.27 (br, 2H), 2.15 (s, 3H), 1.74 (br, 1H), 1.52 (m, 1H), 1.17 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 169.41, 156.01, 147.02, 144.06, 143.83, 141.24, 136.93, 129.93, 129.89, 128.63, 127.90, 127.88, 127.17, 125.31, 119.93, 81.89, 67.24, 64.72, 60.40, 52.12, 48.13, 47.12, 37.95, 35.94, 31.92, 29.83, 27.22, 21.02. ESI-MS (positive): 849.30497 (observed, M+H); 848.14 (calculated, M).

d. Synthesis of D-form compound I-7



Scheme S2. Chemical synthesis of D-form of compound I-7

To check the potential racemization during the the synthesis, we first prepared the D-form of compound I-7 by using D-Cystine instead of L-Cystine as the starting material. The synthetic route is showed in Figure 1. With both D- and L-form compound I-7 in hand, we then analyzed the purity of the compound by running a chiral column. The retention time of the L-form of compound I-7 is at 11.8 min, and the retention time of D form is at 20 min. We concluded that the chiral center of synthesized compound I-7 should not be racemized under our reaction conditions ($Bn_4N^+Br^-$, $NaHCO_3$). In addition, our synthetic route for compound I-7 is based on the previous paper (*Angew. Chem. Int. Ed.*, **2015**, 54, 14276-14281) which, by analyzing the crystal structure of C-S bridged EETI-II derivatives, actually already confirmed that the synthesized diaminodiacid is chiral pure.

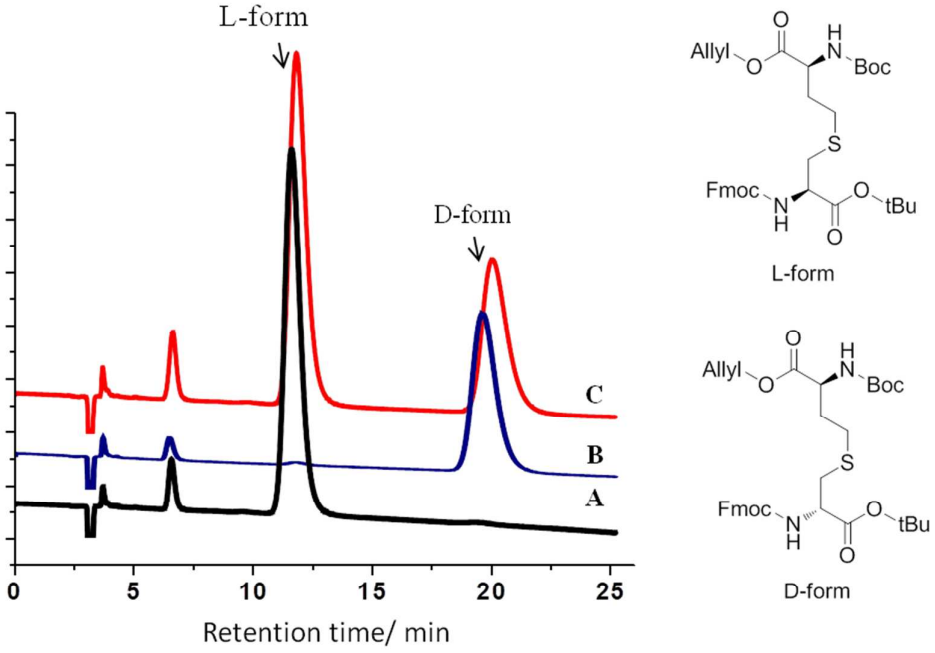
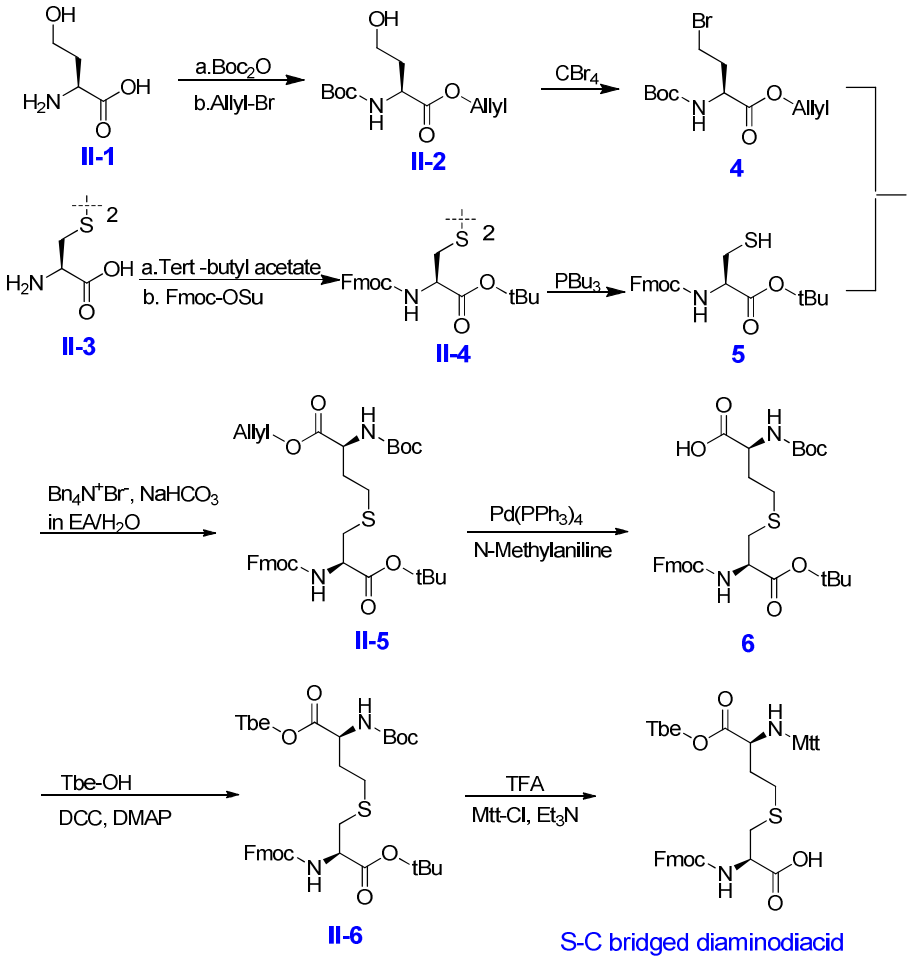


Figure S1. HPLC analysis of L-form and D-form of compound I-7 using a chiral column (Ultimate®Amy-D, 4.6x250 mm, 5 μm particle size, UV 254 nm, flow rate 0.9 mL/min, elution solution: 1:1 n-hexane / ethanol.) A: L-form of compound I-7; B: D-form of compound I-7; C: co-injection of L-form and D-form.

e. Synthesis of S-C bridged Diaminodiacid 2



Scheme S3. Synthetic route for S-C bridged diaminodiacid

Synthesis of compound 4

L-Homoserine (1.19 g, 10 mmol), $NaHCO_3$ (3.36 g, 40 mmol) were dissolved in water/1, 4-dioxane (40 mL/10 mL), followed by addition of Boc_2O (3.27g, 15 mmol) under an ice bath. After 10 h, the mixture was washed with diethyl ether twice. The aqueous layer was neutralized to pH 2 with 2 M HCl, and extracted with EtOAc. The combined organic phase was washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated to dryness.

The remaining residue was dissolved in DMF (20 mL), followed by addition of sodium bicarbonate (2.02 g, 24 mmol) and allyl bromide (1.45 g, 12 mmol). After vigorously stirring overnight, the mixture was diluted with EtOAc. The combined organic phase was washed with water (many times to remove DMF), brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to afford compound II-2 (1.22 g, 4.7 mmol, 47%).

Compound II-2 (1.30 g, 5 mmol) and carbon tetrabromide (1.99 g, 6 mmol) were dissolved in DCM (25 mL). Under 0 ^{o}C, triphenylphosphine (1.57 g, 6 mmol) in DCM (10 mL) was added dropwise. The reaction mixture was gradually heat to room temperature. After 2 h, the reaction mixture was diluted with DCM, washed with water and brine, dried over Na_2SO_4, filtrated and concentrated to ~10 mL. Excess of petroleum ether/EtOAc (1:1) was added to precipitate

phosphine oxide byproduct. The solid byproduct was removed by filtration through Celite. The filtrate was evaporated to dryness. The crude product was purified by chromatography to give compound 4 (0.684 g, 2.13 mmol, 42.6%). R_f 0.6 (5:1 petroleum ether/EtOAc) 1H NMR (400 MHz, $CDCl_3$) δ = 5.85 (m, $1H_a$, CH_a=CH_2,), 5.35 – 5.12 (m, 2H), 5.05 (br, 1H), 4.59 (d, *J*=5.8Hz, 2H), 4.38 (br, 1H), 3.38 (t, *J*=7, 2H), 2.37 (m, 1H), 2.18 (m, 1H), 1.38 (s, 9H).

Synthesis of compound II-4

L-Cystine (2.4g, 10 mmol) was dissolved in 70% perchloric acid (5mL), followed by dropwise adding tert -butyl acetate (30 mL). After 24 h, the reaction solution was chilled on ice, diluted with water. The pH of the aqueous layer was adjusted to 10 with 10 M NaOH. The mixture was extracted with EtOAc. Combined organicc layers were washed with water and brine, dried over anhydrous Na_2SO_4, evaporated to dryness. The remaining residue was used for the next step without further purification.

The residue was dissolved in THF (40 mL), followed by addition of N-methylmorpholine (1.54 mL, 14 mmol). Then, Fmoc-OSu (4.71 g, 14 mmol) was added dropwise under an ice-water bath. The reaction was gradually warmed to room temperature. After 24 h, the mixture was concentrated, resuspended in EtOAc, washed with water and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to give compound II-4 (2.57 g, 2.9 mmol, 29%). R_f0.5 (5:1 petroleum ether/EtOAc). 1H NMR (400 MHz, CDCl3) δ = 7.68 (t, J=8, 4H), 7.51 (d, J=7.9,4H), 7.30 (d, J=7.6, 4H), 7.22 (d, J=7.8, 4H), 5.67 (d, J=7.8, 2H), 4.50 (d, J=4, 2H), 4.34 (m, 4H), 4.13 (d, J=4, 2H), 3.14 (m, 4H), 1.41 (s,18H).

Synthesis of compound II- 5

Compound II-4 (1.59 g, 2 mmol) was dissolved in THF (15 mL), followed by dropwise adding tributylphosphine (0.61 g, 3 mmol), under nitrogen atmosphere. After 30 min, water (2.5mL) was added, at room temperature. After 2 h, the reaction mixture was concentrated in vacuo, suspended in EtOAc, washed with citric acid (w/w 10%) and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to afford compound 5 (1.52 g, 3.8 mmol, 95%). R_f0.45 (5:1 petroleum ether/EtOAc).

Compound 4 (2.2 mmol) and compound 5 (2.1 mmol) were dissolved in EtOAc (50 mL). Then, tetrabutylammonium bromide (2.71 g, 8.4 mmol) in saturated $NaHCO_3$ solution (10 mL) was added. After stirring vigorously overnight, the mixture was diluted with EtOAc, washed with water and brine, dried over Na_2SO_4, filtrated and concentrated. The crude product was purified by chromatography to give compound II-5 (1.17g, 1.83 mmol, 87%) as colorless oil. R_f 0.3 (5:1 petroleum ether/EtOAc).^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.69 (d, 2H), 7.55 (d, 2H), 7.33 (t, 2H), 7.24 (t, 2H), 5.83 (m, J_{dc}=19.3Hz, J_{db}=10.5Hz, J_{dc}=5.6Hz, 1H, CH_a-CH_d=CH_bH_c), 5.60 (d, J_{cd}=19.3Hz, 1H), 5.25 (d, J_{bd}=10.5Hz, 1H), 5.17 (d, 1H), 5.01 (br, 1H), 4.56 (d, J_{ad}=5.6Hz, 2H), 4.41 (br, 1H), 4.35 (br, 1H), 4.31 (d, 2H),4.17 (t, 1H), 2.90 (m, 2H), 2.53 (m, 2H), 2.01 (m, 1H) 1.86 (m, 1H), 1.42 (s, 9H), 1.35 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 171.93, 169.70, 155.72, 155.36, 143.81, 141.30, 131.50, 127.74, 127.10, 125.19, 120.00, 119.04, 82.98, 80.11, 67.19, 66.12, 54.18, 52.67, 47.12, 34.76, 32.59, 28.76, 28.31, 28.02. ESI-MS (positive): 663.26886 (observed, M+Na); 640.28 (calculated, M).

Synthesis of compound 6

To a solution of compound II-5 (0.64 g 1 mmol) in THF (10 mL) was added $Pd(PPh_3)_4$ (0.23 g, 0.2 mmol), followed by dropwise adding N-methylaniline (0.21 g, 2 mmol). After 30 min, the reaction mixture was diluted with EtOAc, washed with 1M HCl and brine, dried over Na_2SO_4, filtrated and concentrated. Then the crude product was purified by chromatography to give compound 6 (0.51 g, 0.85 mmol, 85%). R_f 0.4 (5:1 petroleum ether/EtOAc with 0.5% AcOH).1H NMR (400 MHz, $CDCl_3$): δ = 7.69 (d, 2H), 7.54 (d, 2H), 7.33 (t, 2H), 7.24 (t, 2H), 5.68 (d, 1H), 5.19 (d, 1H), 4.42 (br, 1H), 4.32 (br, 1H), 4.30 (d, 2H),4.16 (t, 1H), 2.90 (m, 2H), 2.65 (m, 2H), 2.03(m, 1H), 1.89(m, 1H), 1.41 (s, 9H), 1.39 (s, 9H). ^{1}13C NMR (400 MHz, CDCl3) δ = 174.16, 168.72, 154.84, 153.84, 142.73, 140.26, 126.72, 126.08, 124.16, 118.98, 82.06, 66.26, 53.21, 46.05, 33.57, 28.69, 27.27, 26.98. ESI-MS. ESI-MS (positive): 623.23724 (observed, M+Na); 600.25 (calculated, M).

Synthesis of compound II-6

To a solution of compound 6 (0.3 g, 0.5 mmol) in DCM (2 mL) were added Tbe-OH (0.17 g, 1 mmol), DCC (0.12 g, 0.6 mmol in 1 mL DCM) and DMAP (0.012 g, 0.1 mmol). After vigorously stirring overnight at room temperature, the mixture was filtered to remove DCU. The filtrate was washed with brine, dried over Na_2SO_4. The crude product was purified by chromatography to give compound II-6 (0.225g, 0.3 mmol, 60%). R_f 0.5 (5:1 petroleum ether/EtOAc). 1H NMR (400 MHz, $CDCl_3$): δ = 7.70 (d, 2H), 7.55 (d, 2H), 7.34 (t, 2H), 7.25 (t, 2H), 5.62 (d, H),5.09 (d, 1H), 4.41 (m, 1H),4.30 (m, 5H), 4.17(t, 1H), 2.90 (m, 2H), 2.83 (m, 2H), 2.55 (t, 2H), 2.05 (m, 1H), 1.85 (m, 1H), 1.43 (s, 9H), 1.36 (s, 9H), 1.25 (s, 9H). 13C NMR (400 MHz, CDCl3) δ = 170.98, 170.84, 155.96, 155.17, 143.80, 141.31, 127.73, 127.10, 125.19, 120.01, 82.59, 80.26, 67.06, 63.82, 60.69, 53.55, 48.11, 47.18, 42.71, 38.13, 29.89, 29.85, 28.32, 28.04. ESI-MS (positive): 771.27509 (observed, M+Na); 748.29 (calculated, M)

Synthesis of S-C bridged diaminodiacid

Compound II-6 (8 mmol) was dissolved in DCM (20 mL), followed by addition of TFA (20 mL). After vigorously stirring overnight at room temperature, the reaction mixture was evaporated to dryness used directly in the next step without further purification. The remaining residue was dissolved in dry DCM (150 mL) under nitrogen at room temperature, followed by addition of trimethylsilyl chloride (24.8 mmol, 3.15 mL) and DIPEA (24.8 mmol, 1.4 mL). After 1 h reflux, the reaction mixture became homogeneous. After cooling to 0 °C, DIPEA (24.8 mmol, 4.1 mL) was added, followed by addition of 4-methyltrityl chloride (8.4 mmol, 2.5 g). After vigorously stirring at room temperature for 16 h, methanol (25 mL) was added to quench the reaction. After 15 min, the solvent was evaporated under vacuum at 30 °C. The remaining residue was partitioned between DCM and pH 5 acetate buffers. The organic phase was dried over Na_2SO_4, concentrated under vacuum to 10 mL. The S-C bridged diaminodiacid was obtained by precipitation with hexane, as a light yellow solid (4.14 g, 6.87 mmol, 85%).^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.57 (d, 2H), 7.42 (m, 2H), 7.34 (d, 4H), 7.25 (m, 4H), 7.17–7.08 (t, 6H), 6.99 (t, 2H), 6.89 (d, 2H), 4.16 (br, 1H), 4.0 2 (br, 2H), 3.91 (t, 1H), 3.54 (br, 2H), 3.28 (br, 1H), 2.83 (m, 4H), 2.49 (m, 1H), 2.40 (m, 3H), 2.13 (s, 3H), 1.94 (m, 1H), 1.50 (m, 1H), 1.15 (s, 9H). ^{13}C NMR (400 MHz, $CDCl_3$) δ = 170.14, 156.04, 148.59, 144.90, 142.89, 141.90, 134.83, 128.89, 128.86, 127.75, 127.67, 127.50, 126.78, 126.52, 126.06, 125.33, 124.18, 118.77, 83.77, 69.71, 62.64, 62.31, 54.67, 46.95, 45.97,

36.93, 34.91, 30.88, 28.75, 26.55, 21.58. ESI-MS (positive): 849.30426 (observed, M+H); 848.14 (calculated, M).

4. Stability of Boc-Phe-OTbe to 25% piperidine in DMF

Table S1. The stability of Boc-Phe-OTbe to 25% piperidine

Entry	Time	Stability	New product
1	5 min	stable	No
2	10 min	stable	No
3	30 min	stable	No
4	1 h	stable	No
5	2 h	stable	No
6	5 h	stable	No
7	12 h	stable	No
8	24 h	stable	No

Boc-Phe-OTbe (0.28g, 1mmol) was treated with piperidine (20% in DMF). The mixture was stirred at room temperature. The stability of Boc-Phe-oTbe was confirmed by thin-layer chromatography (TLC). We did not observe any decomposed Boc-Phe-OTbe within 24 h.

Note: We purified it by silica gel chromatography and confirmed its correctness by NMR.

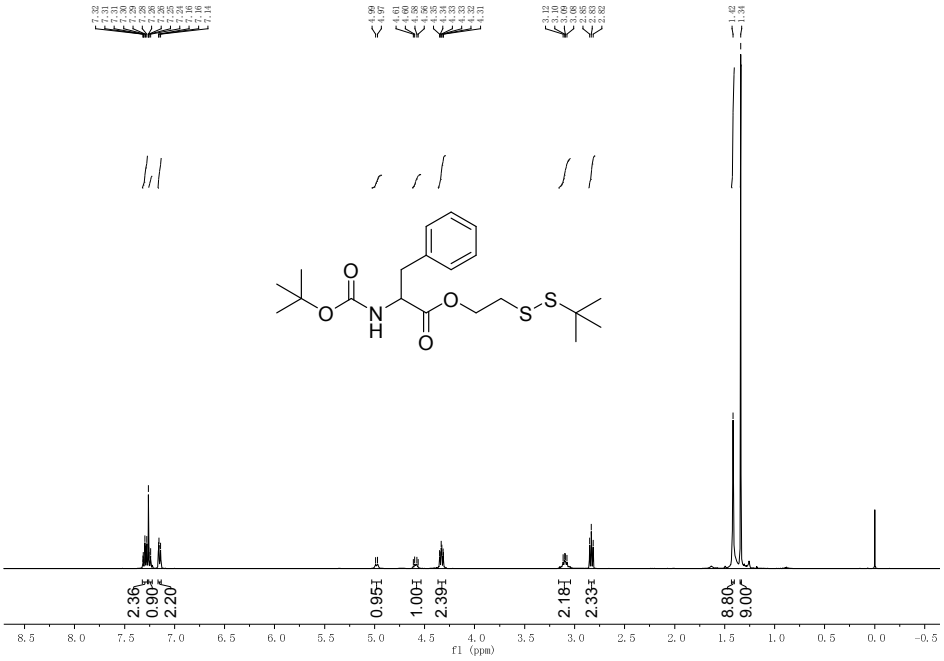
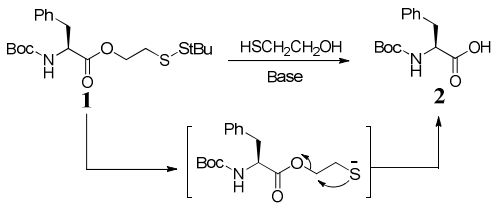


Figure S2. NMR data of the covered Boc-Phe-OTbe

4. Optimization of the Tbe ester deprotection conditions

Table S2. Deprotection of Tbe ester by 2-mercaptoethanol in the presence of different organic bases



Entry	Organic base	Time	Yield of 2 (%)
1	DBU (1.0 M)	10 min	99
2	DBU (1.0 M)	4 h	99
3	Et_3N (1.0 M)	10 min	98
4	Et_3N (1.0 M)	4 h	99
5	DIPEA (1.0 M)	10 min	98
6	DIPEA (1.0 M)	4 h	99
7	No base	2h	0

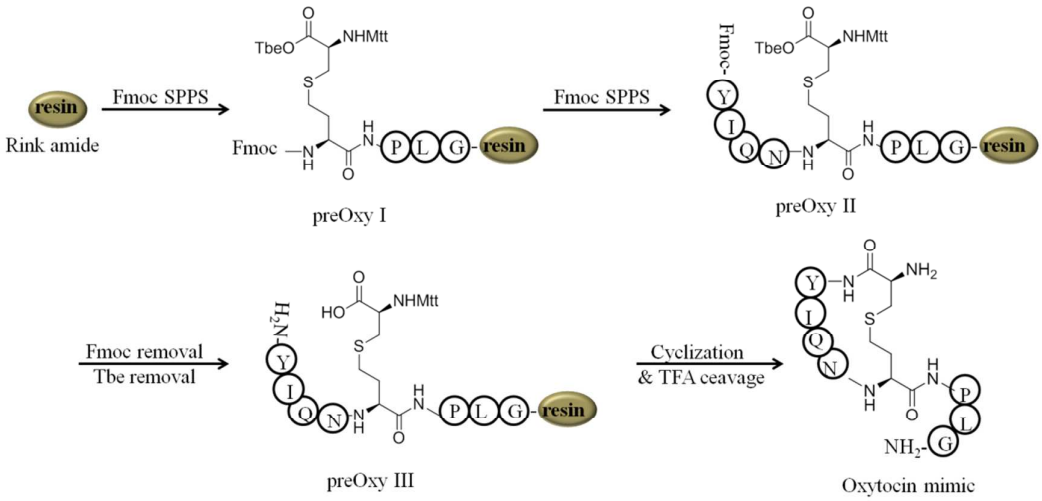
[a]Conditions: 1 (5 mM), 2-mercaptoethanol (0.5 M) in NMP, 20-25 ºC

5. 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 (4.0eq) and DIEA (8.0eq) in DMF for 0.5-1min. Then, the mixture was added to the resin for coupling. After 30 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 high-resolution ESI mass spectra.

b. Synthesis and characterization of CS oxytocin mimic



Scheme S4. Solid-phase peptide synthesis of CS Oxytocin mimic

RP-HPLC trace of preOxy I

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, C-S brideged diaminodiacid (1.5 equiv.) was pre-activated with PyAOP (5 equiv.), HOAt (5 equiv) and NMM (8.0 equiv) in DMF for 1 min, then transferred to the resin. After 2 h, the resulting preOxy I was cleaved from resin and analyzed by RP-HPLC, shown as below (gradient: 15-99% B in 30 min, 1 mL/min).

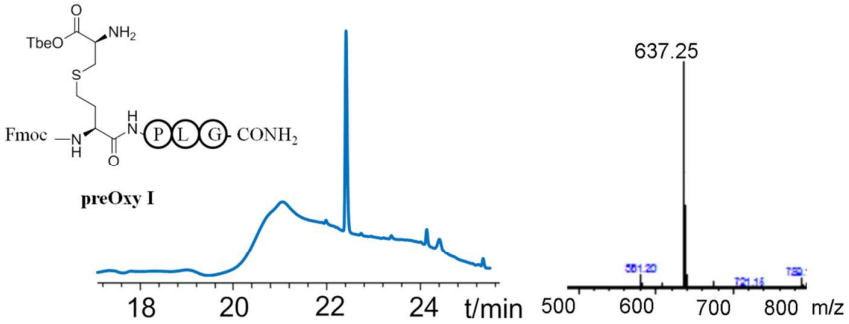


Figure S3. HPLC trace of crude preOxy I after TFA cleavage

RP-HPLC trace of preOxy II

Fmoc-Asn(Trt)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ile and Fmoc-Tyr(tBu)-OH were successively coupled to N-terminal of preOxy I. The resulting preOxy II was cleaved from the resin and analyzed by RP-HPLC (gradient: 15-99% B in 30 min, 1 mL/min)..

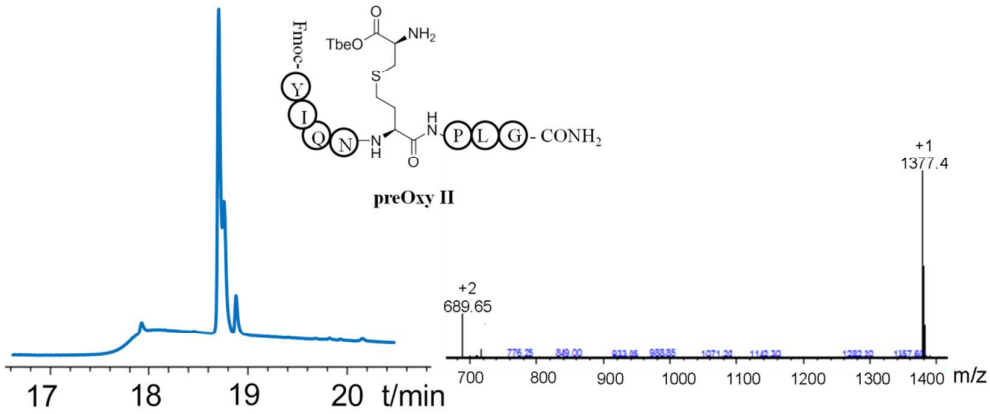


Figure S4. HPLC trace of crude preOxy II after TFA cleavage

RP-HPLC trace of preOxy III

The Tbe protecting groups were removed by treatment with a solution of 2-mercaptoethanol (0.005M)/DIEA (1.25M) in NMP (4ml), for 2 h x 2. Then the resin was washed with DMF (6 mL×5), DCM (3 mL×5) and DMF (3 mL×5). After Fmoc deprotection, the preOxy III was cleaved from the resin and analyzed by RP-HPLC (gradient: 1-80% B in 30 min, 1 mL/min).

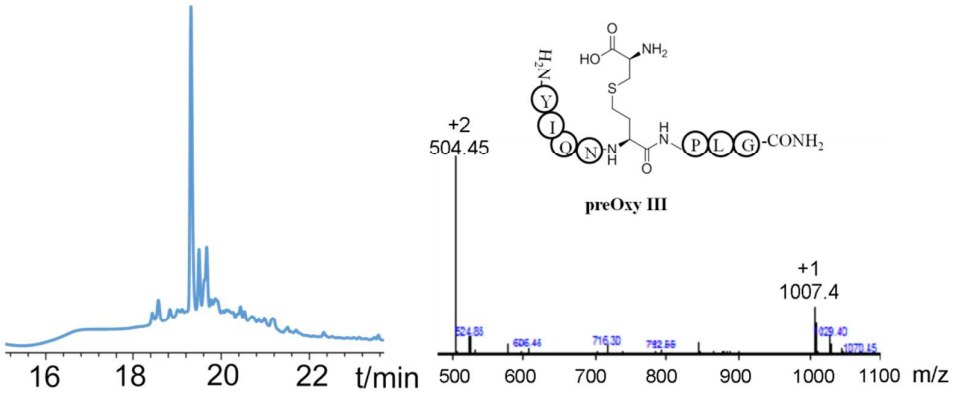


Figure S5. HPLC trace of crude preOxy III after TFA cleavage

RP-HPLC trace of Oxytocin mimic

Cyclization conditions: PyAOP (5.0 equiv), HOAt (5.0 equiv) and NMM (10.0 equiv) in DMF, RT, 4 h. The peptide was cleaved from resin with TFA/water/phenol/TIPS (88/5/5/2, *v/v/v/v*) for 3 h. The TFA cleavage solution was collected and concentrated under a stream of N_2. The peptide was precipitated with cold Et_2O. The crude oxytocin disulfide mimic was analyzed by RP-HPLC (gradient: 1-90% B in 30 min, 1 mL/min) and purified by semi-preparative HPLC (gradient: 1-90% B in 30 min, 4 mL/min). (Calcd: 989.2).

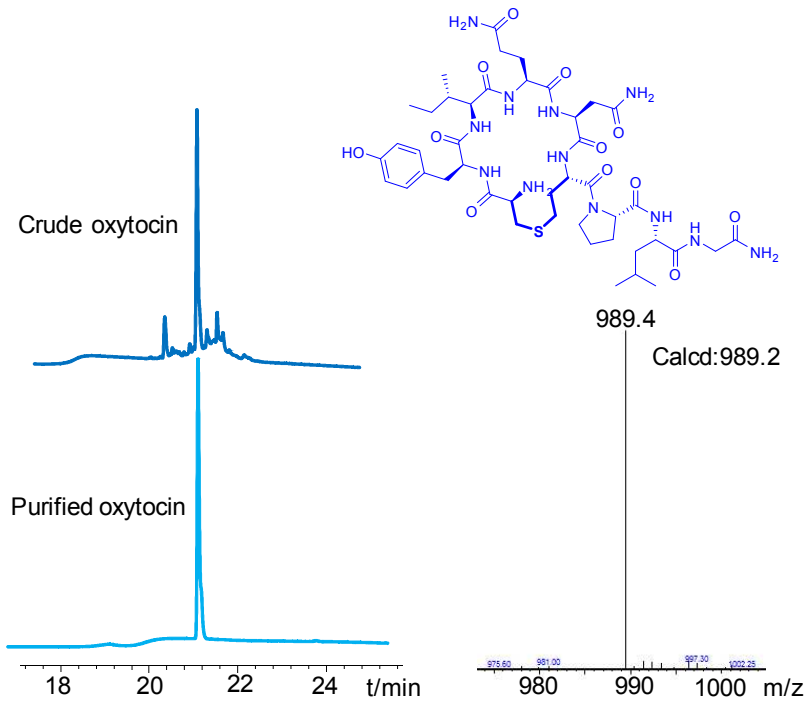


Figure S6. HPLC trace of crude and purified CS oxytocin mimic

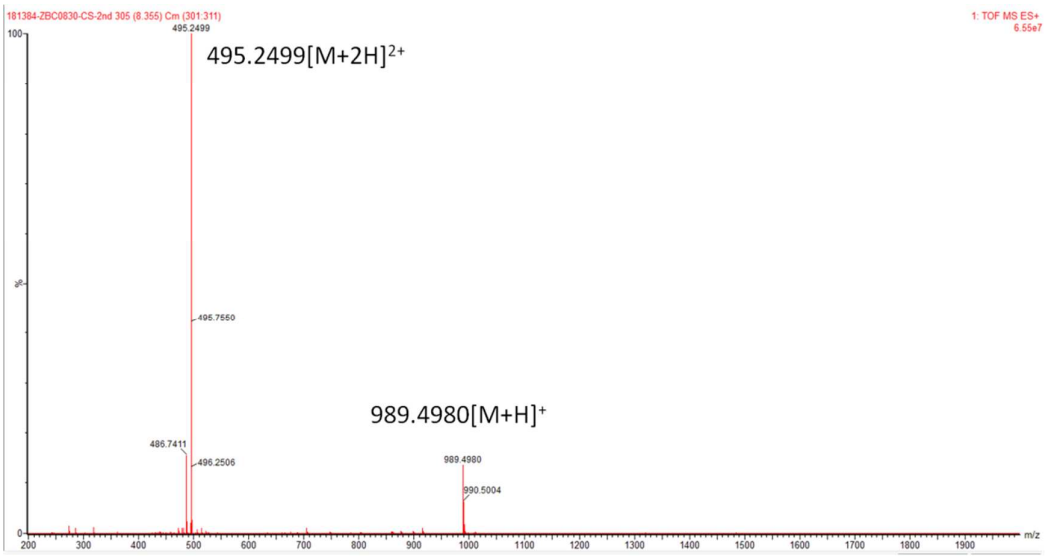
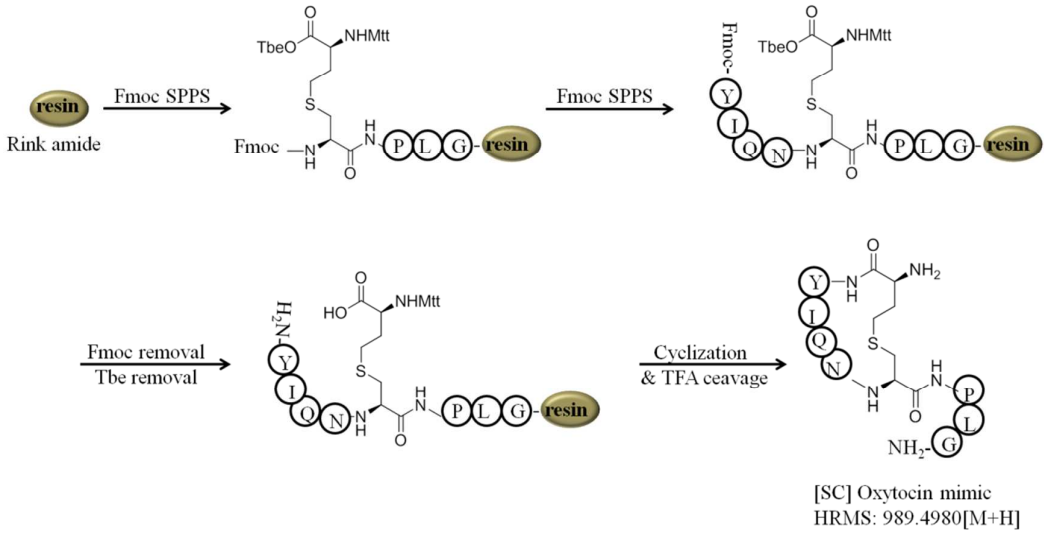


Figure S7. HRMS of [CS] oxytocin mimic. HRMS(EI) $C_{44}H_{68}N_{12}O_{12}S$ m/z calcd: 988.4800; Found: 495.2499 $[M+2H]^{2+}$, 989.4980 $[M+H]^{+}$.

b. Synthesis and characterization of SC oxytocin mimic



Scheme S5. Solid-phase peptide synthesis of SC oxytocin mimic

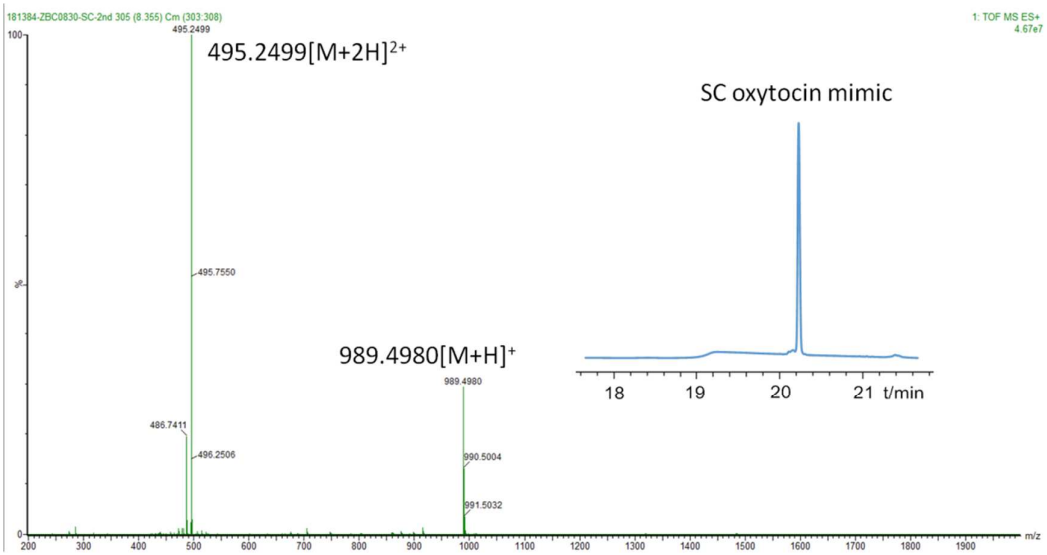
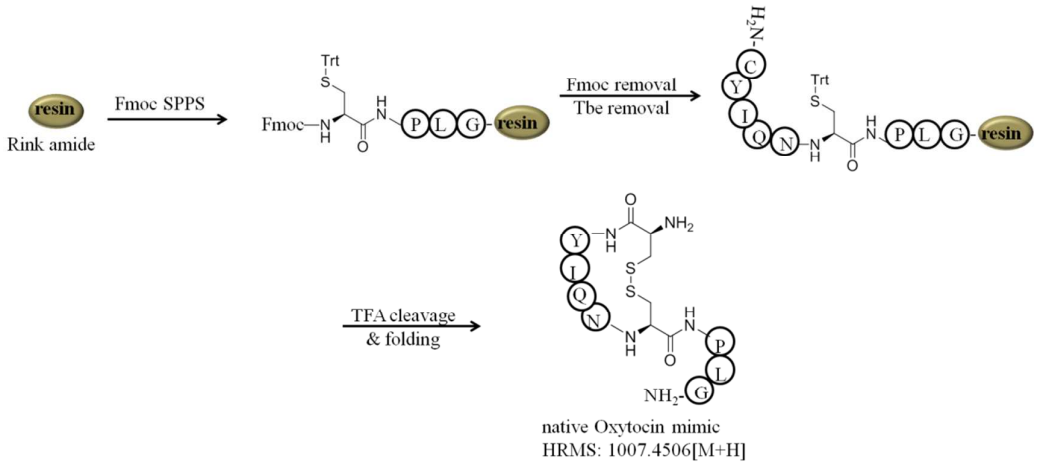


Figure S8. HRMS of [SC] oxytocin mimic. HRMS(EI) $C_{44}H_{68}N_{12}O_{12}S$ m/z calcd: 988.4800; 495.2499 $[M+2H]^{2+}$, 989.4980 $[M+H]^{+}$.

d. Synthesis and characterization of native oxytocin



Scheme S6. Solid-phase peptide synthesis of native oxytocin

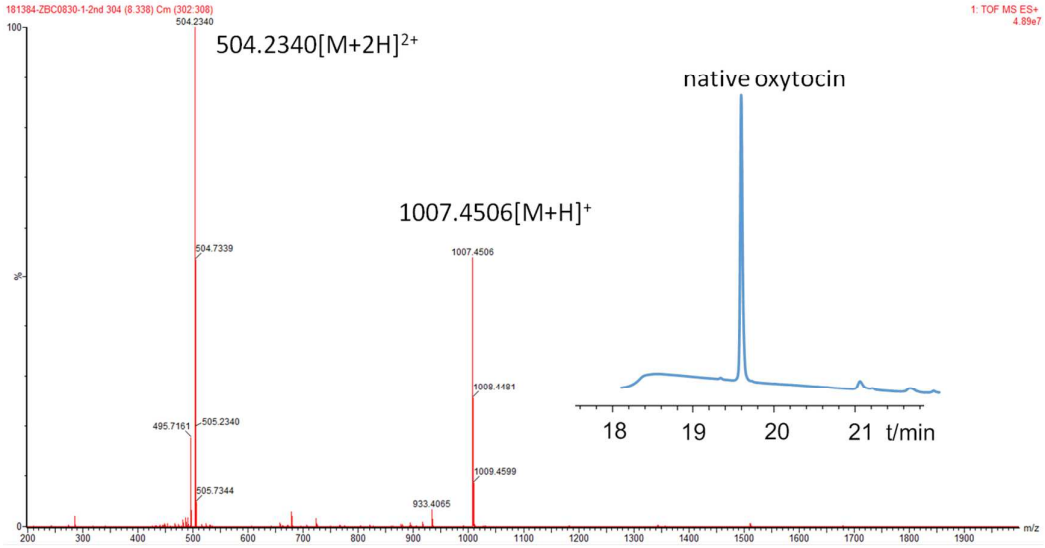


Figure S9. HRMS of native oxytocin. HRMS(EI) $C_{43}H_{66}N_{12}O_{12}S_2$ m/z calcd: 1006.4365; 504.2340 $[M+2H]^{2+}$, 1007.4506 $[M+H]^{+}$.

e. CD spectra of oxytocin and its mimics

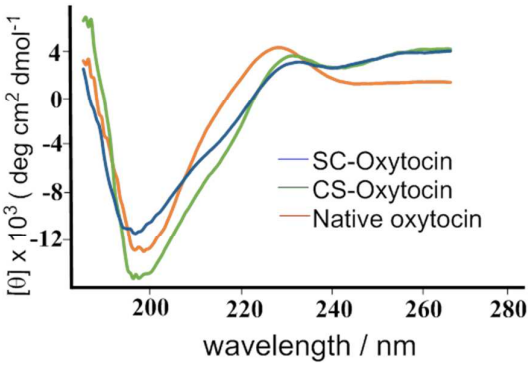
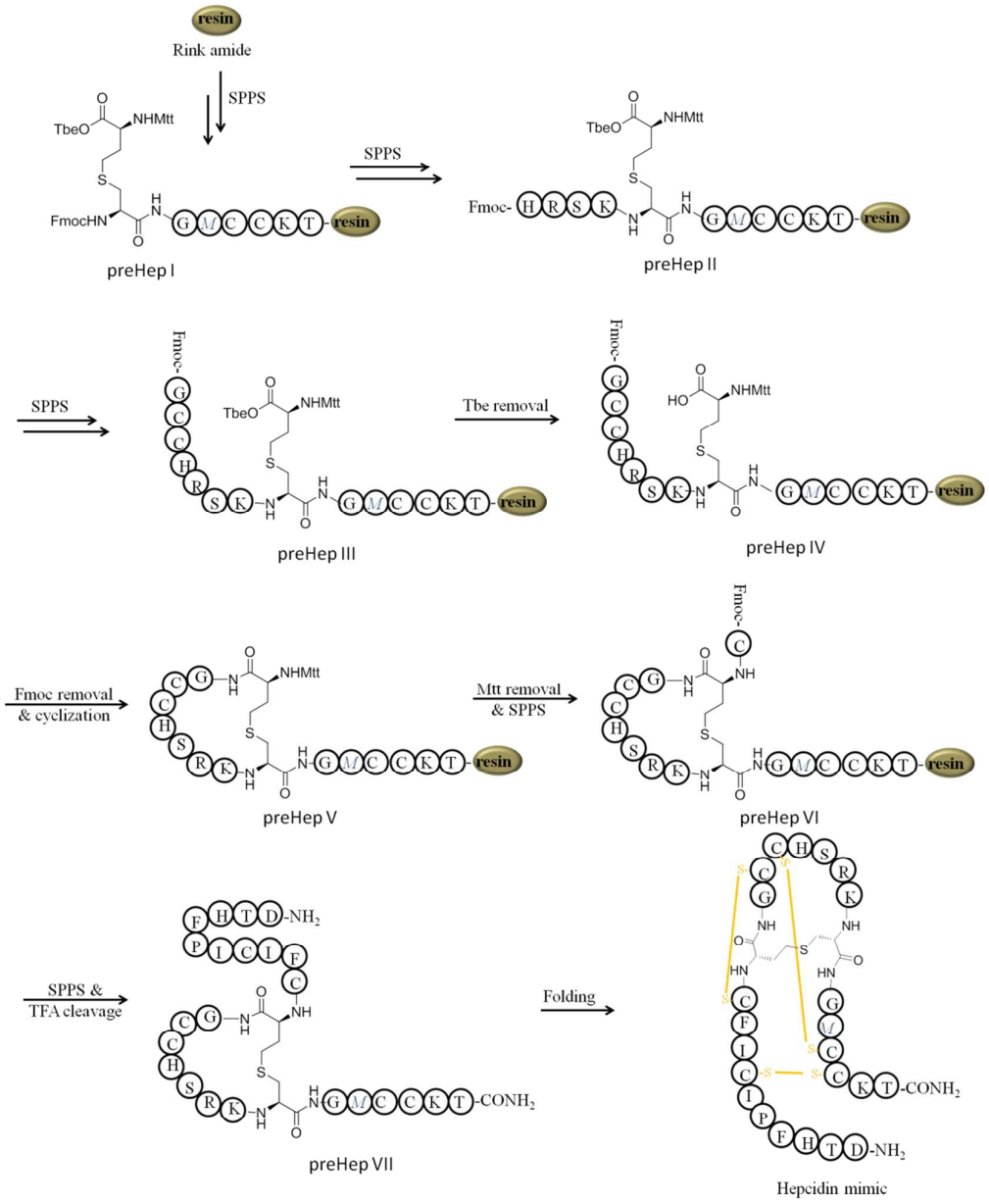


Figure S10. CD spectra of [SC] oxytocin, [CS] oxytocin and native oxytocin

f. Synthesis and characterization of hepcidin mimic



Scheme S7. Solid-phase peptide synthesis of Hepcidin disulfide mimic

RP-HPLC trace of preHep I

Fmoc-Thr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Cys(Trt)-OH, Fmoc-Nle-OH (marked as *M* in Scheme S4) and Fmoc-Gly-OH were coupled to amino group of Rink amide resin by Fmoc-based SPPS. For diaminodiacid coupling, S-C bridged diaminodiacid (1.5 equiv.) was preactivated with PyAOP (5 equiv.), HOAt (5 equiv) and NMM (8.0 equiv) in DMF for 1 min, then transferred to the resin for 2 h. The resulting preHep I was cleaved from the resin and analyzed by RP-HPLC, shown as below (gradient: 15-99% B in 30 min, 1 mL/min).

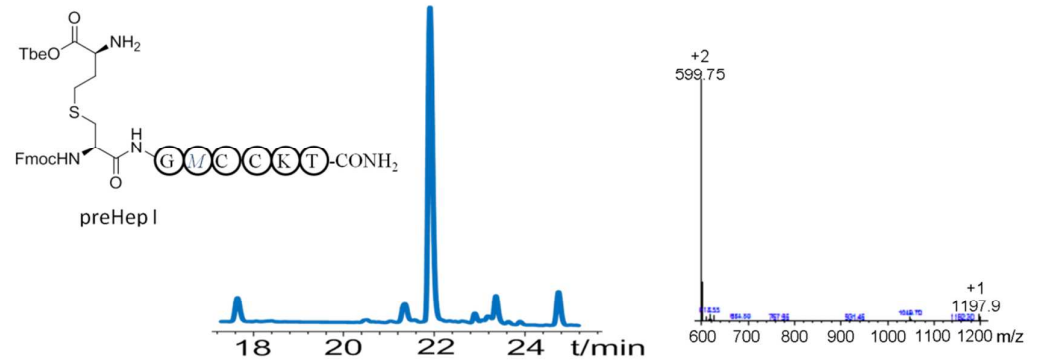


Figure S11. HPLC trace of crude preHep I after TFA cleavage

RP-HPLC trace of preHep II

Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pbf)-OH and Fmoc-His(Trt)-OH were successively coupled to N-terminal of preHep I. The resulting preHep II was cleaved from the resin and analyzed by RP-HPLC (gradient: 15-99% B in 30 min, 1 mL/min).

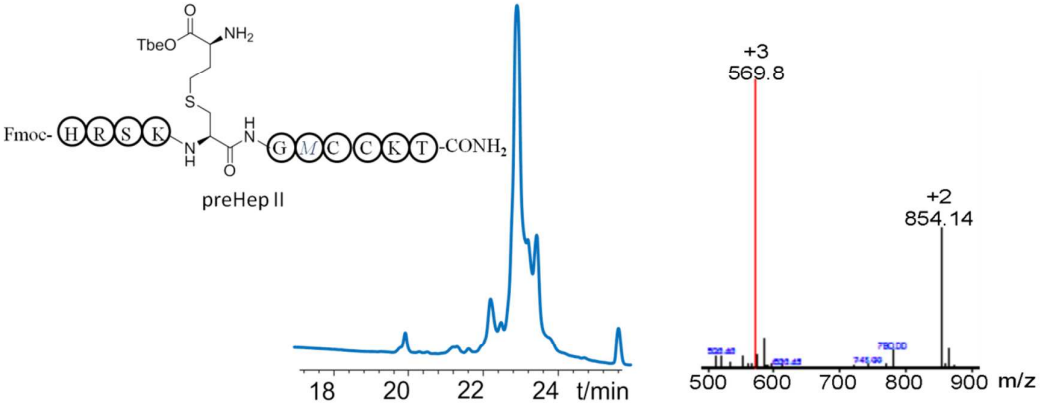


Figure S12. HPLC trace of crude preHep II after TFA cleavage

RP-HPLC trace of preHep III

Fmoc-Cys(Trt)-OH, Fmoc-Cys(Trt)-OH and Fmoc-Gly-OH were successively coupled to N-terminal preHep II. The resulting preHep III was cleaved from the resin and analyzed by RP-HPLC (gradient: 15-99% B in 30 min, 1 mL/min).

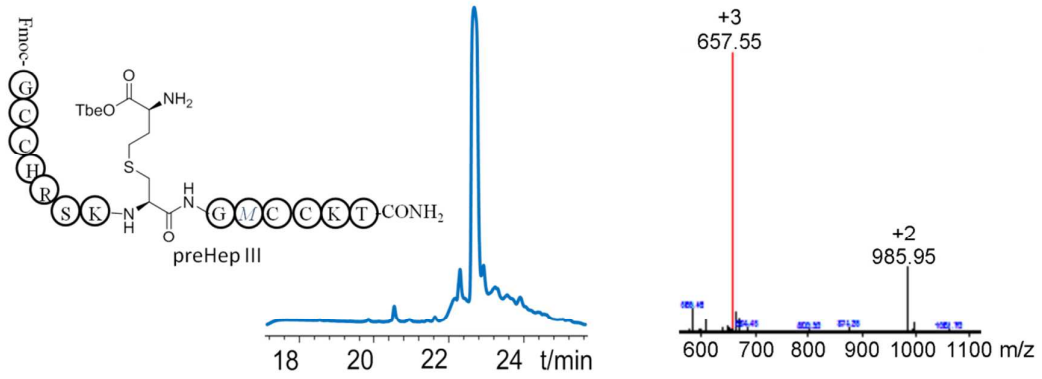


Figure S13. HPLC trace of crude preHep III after TFA cleavage

RP-HPLC trace of preHep IV

The Tbe protecting group was removed by treatment with a solution of 2-mercaptoethanol (0.005M)/DIEA (1.25M) in NMP (4ml), for 2 hour x 2. The resulting preHep IV was cleaved from the resin and analyzed by RP-HPLC (gradient: 1-90% B in 30 min, 1 mL/min).

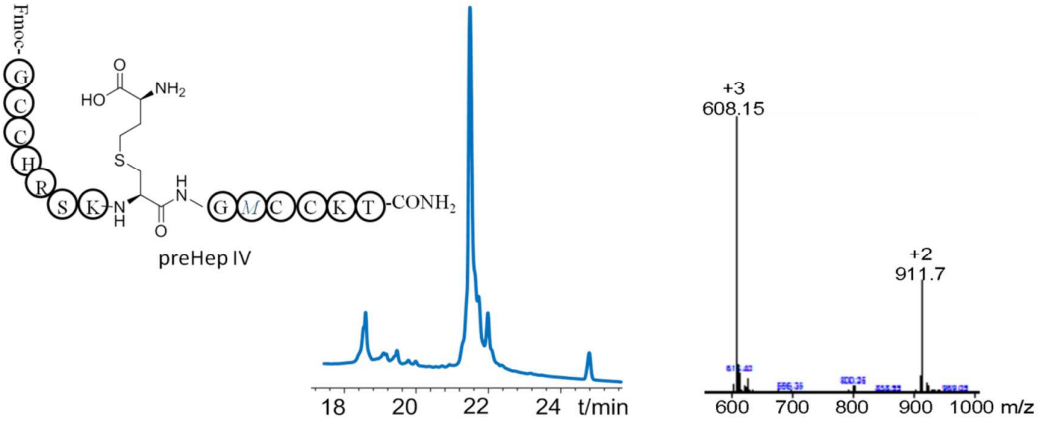


Figure S14. HPLC trace of crude preHep IV after TFA cleavage

RP-HPLC trace of preHep V

Cyclization conditions: PyAOP (5.0 equiv), HOAt (5.0 equiv) and NMM (8.0 equiv) in DMF, RT, 4 h. The resulting preHep V was cleaved from the resin and analyzed by RP-HPLC (gradient: 1-90% B in 30 min, 1 mL/min).

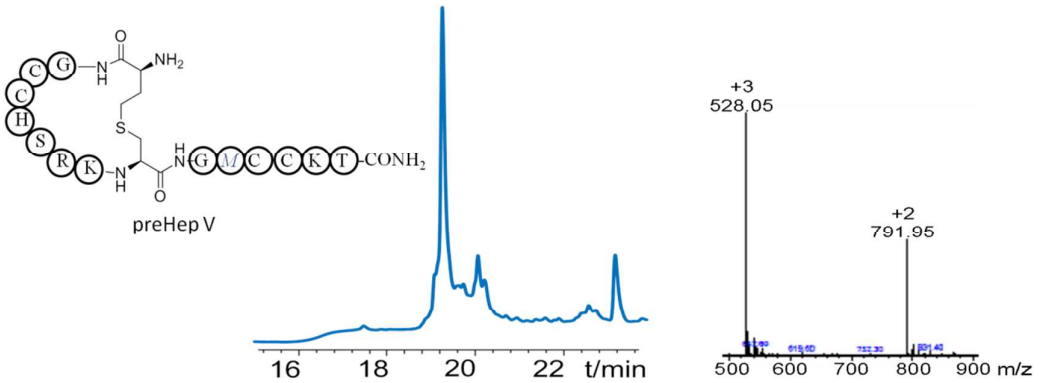


Figure S15. HPLC trace of crude preHep V after TFA cleavage

RP-HPLC trace of preHep VI

Initially, we used 1% TFA in DCM to deprotect Mtt protecting group. To our disappointment, we can't find any correct product. We encountered the problem of partial removal of Trt protecting group of Cys residues as shown in Fig. S13 (gradient: 15-99% B in 30 min, 1 mL/min).

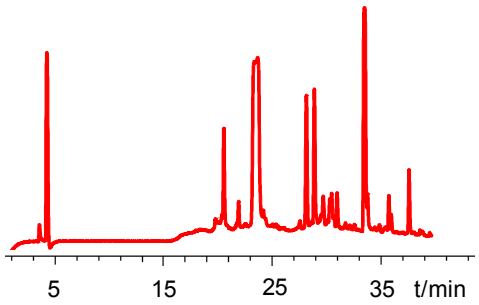


Figure S16. HPLC trace of crude VI though 1% TFA cleavage

Then, we turned to a HFIP-containing reagent for Mtt removal. To 10 mg of resin (0.2 mmol, 1.0 equiv) was added a 200 uL mixture solution (122 mg HOBt, 5 mL hexafluoroisopropanol, 5 mL DCE). After 3 min, the HFIP-containing reagent was refreshed, and this step repeated for 4 times. In the following step, Fmoc-Cys(Trt)-OH was coupled to the solid-anchored peptide, and the resulting preHep VI was cleaved from the resin and analyzed by RP-HPLC (gradient: 15-99% B in 30 min, 1 mL/min).

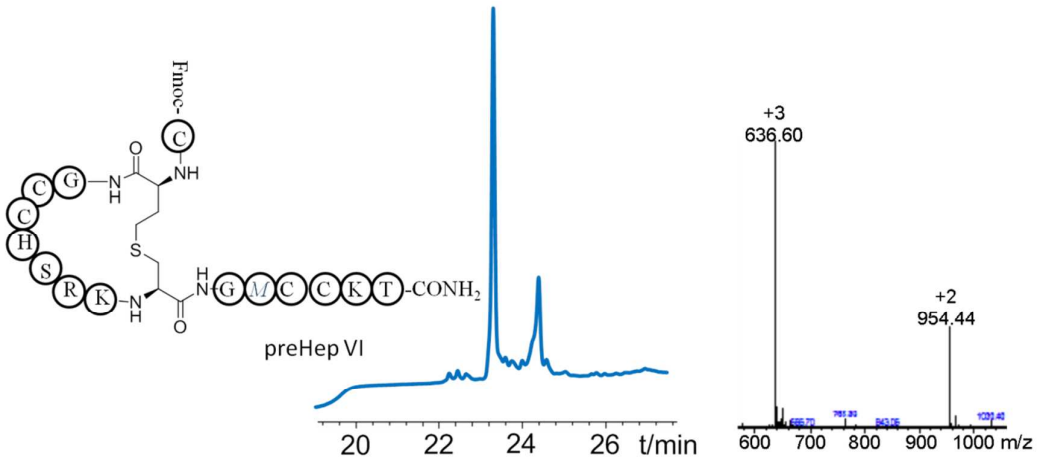


Figure S17. HPLC trace of crude preHep VI after TFA cleavage

RP-HPLC trace of linear hepcidin: preHep VII

Fmoc-Phe-OH, Fmoc-Ile-OH, Fmoc-Cys(Trt)-OH, Fmoc-Ile-OH, Fmoc-Pro-OH, Fmoc-Phe-OH, Fmoc-His(Trt)-OH, Fmoc-Thr(tBu)-OH, and Fmoc-Asp(tBu)-OH were successively coupled to N-terminal preHep VI. The completed peptides was cleaved from resin with a mixture of TFA/water/phenol/TIPS (88/5/5/2, *v/v/v/v*). After 3 h, the combined TFA solution was collected and concentrated by blowing with N_2. The crude peptide was obtained by precipitation with cold

ether and centrifugation. The remaining residue was dissolved with 50% acetonitrile, analyzed and purified by HPLC (gradient: 1-80% B in 30 min, 1 mL/min), and confirmed by high-resolution ESI mass spectra.

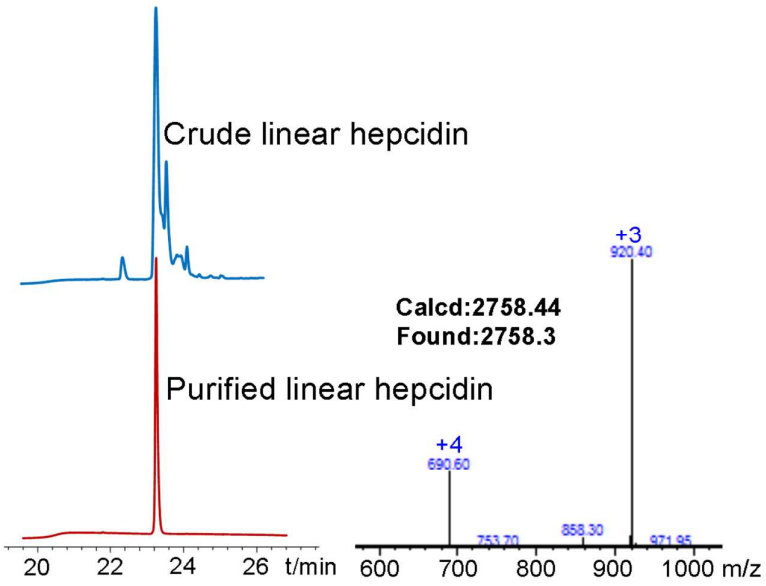


Figure S18. HPLC trace of crude linear hepcidin after TFA cleavage

In vitro folding of synthetic hepcidin mimic

15 mg of pure sample was dissolved in a mixed solution of 66 ml water/acetonitrile (66 mL/28 mL), followed by addition of 13.8 mg of GSSG and 7.05 mg of GSH. The pH of the solution was adjusted to about 7.5. After 14 h on a shaker at 70 rpm at 37 ^{o}C, adjust the pH of the solution to 2, The solution was then freeze-dried with liquid nitrogen and concentrated using a freeze dryer, the final folded hepcidin was lyophilized and purified by RP-HPLC (gradient: 1-80% B in 30 min, 1 mL/min).

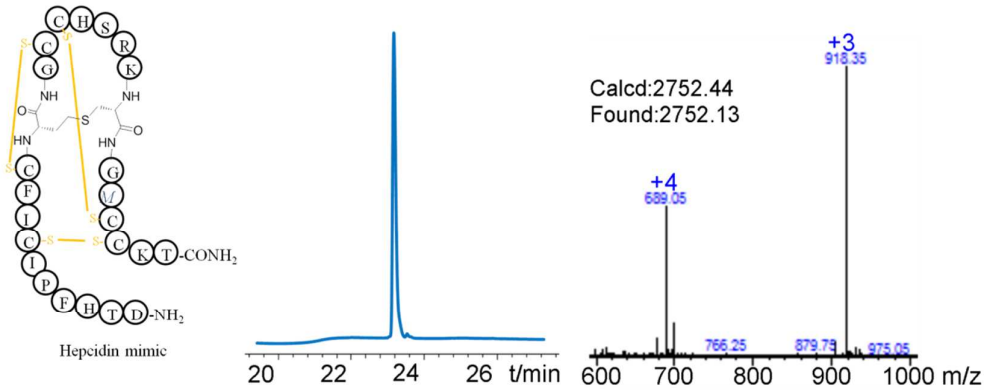


Figure S19. HPLC trace of folded hepcidin mimic

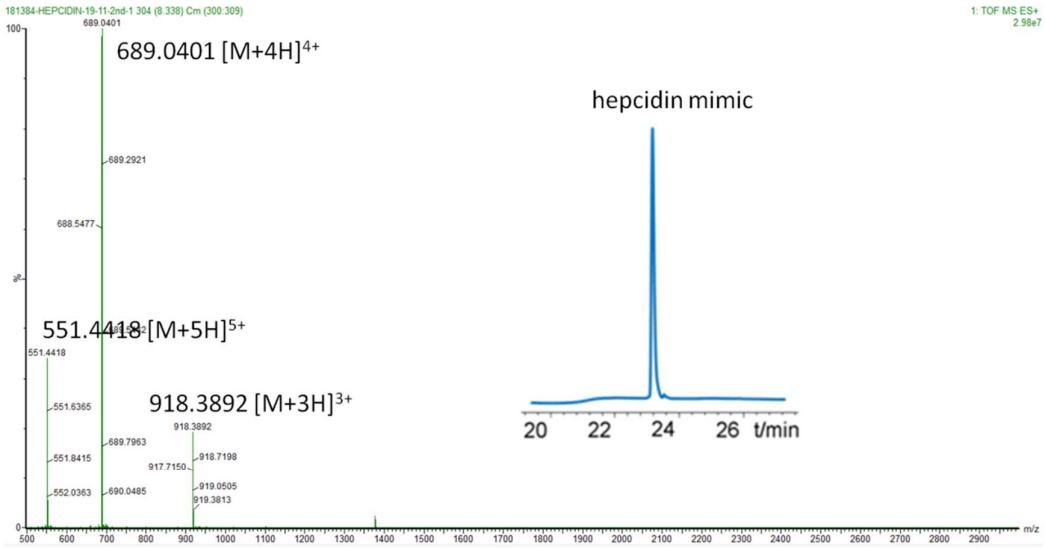
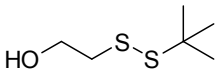
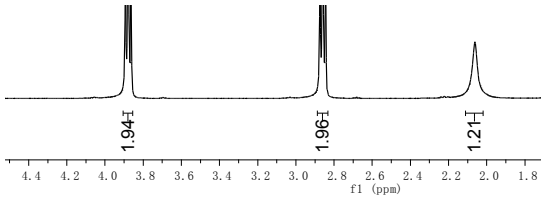


Figure 20. HRMS of hepcidin mimic. HRMS(EI) $C_{115}H_{175}N_{35}O_{30}S_7$ m/z calcd: 2751.1323; Found: 551.4418 $[M+5H]^{5+}$, 689.0401 $[M+4H]^{4+}$, 918.3892 $[M+3H]^{3+}$.

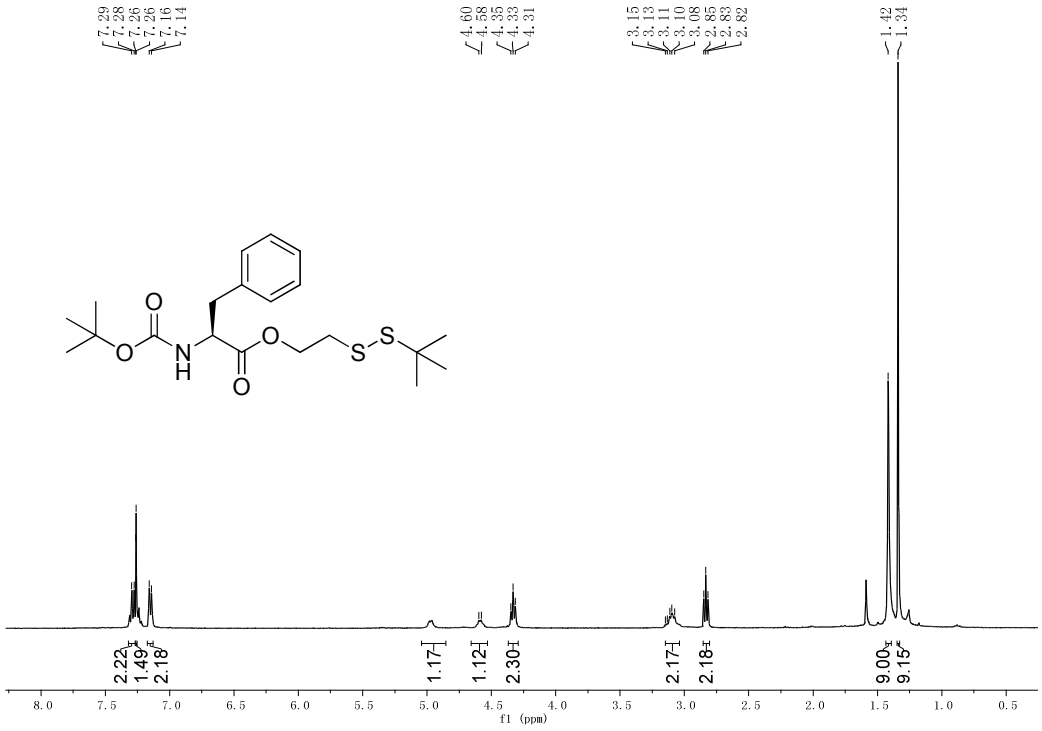
6. NMR and MASS Data for Fmoc/Mtt/Tbe diaminodiacids



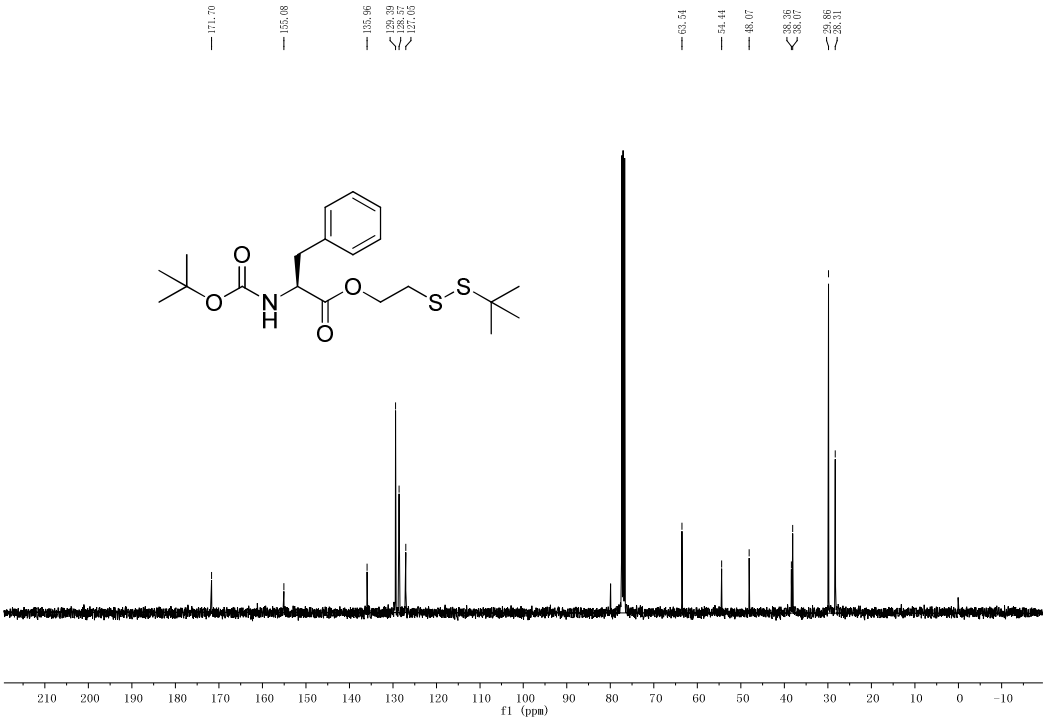


The ^{1}HNMR spectrum of Tbe-OH

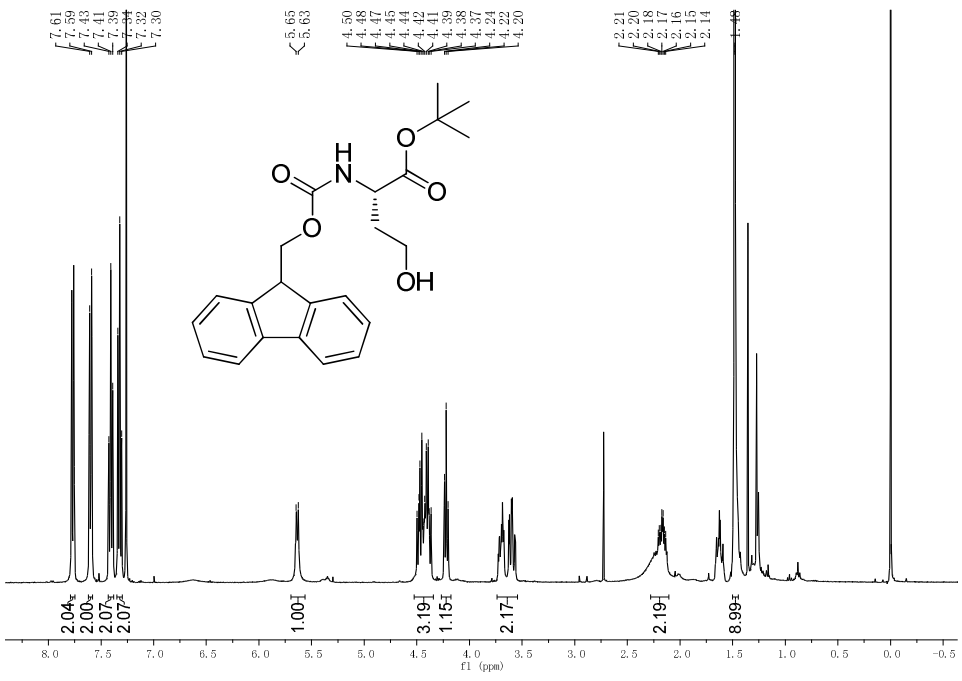
The ^{13}CNMR spectrum of Tbe-OH



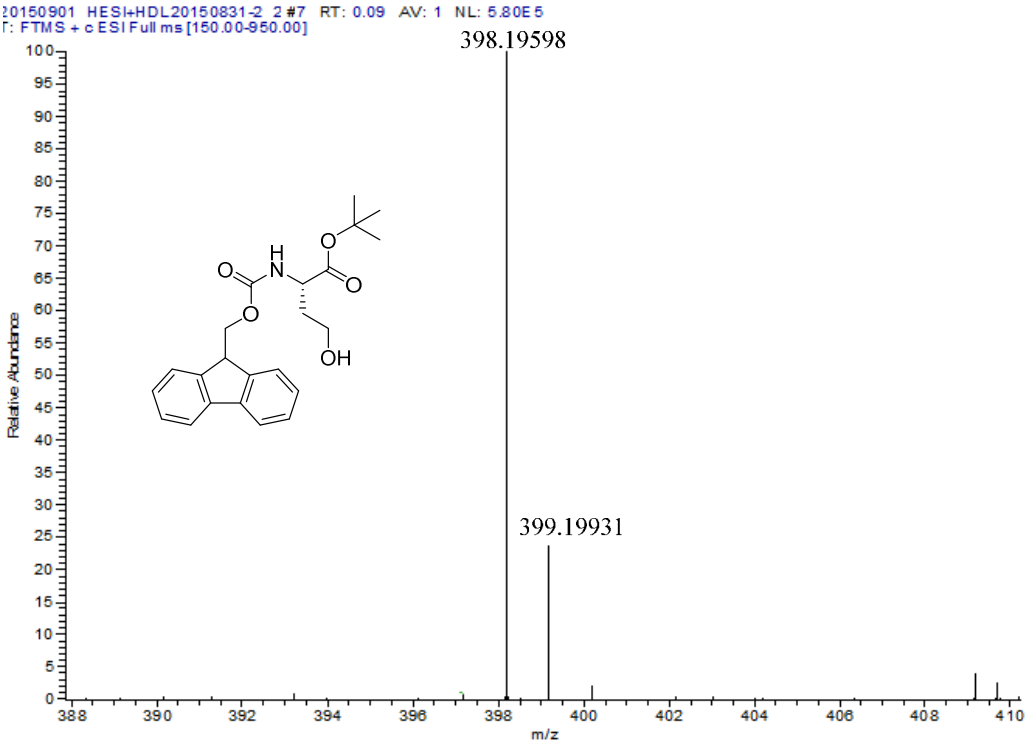
The ^{1}HNMR spectrum of Boc-Phe-OTbe



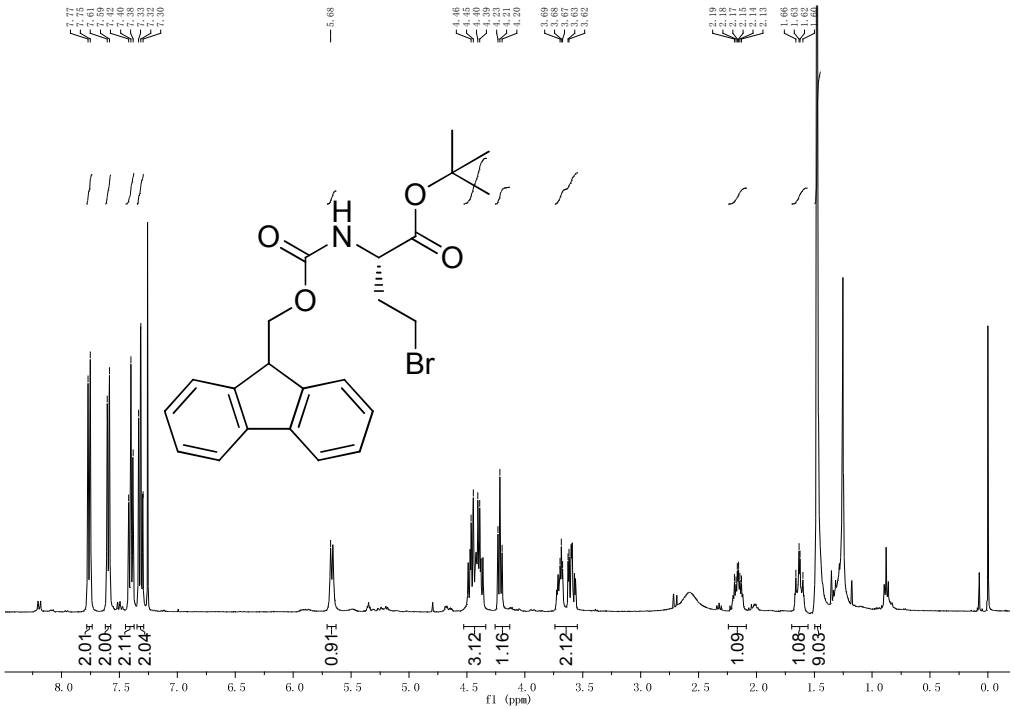
The ^{13}CNMR spectrum of Boc-Phe-OTbe



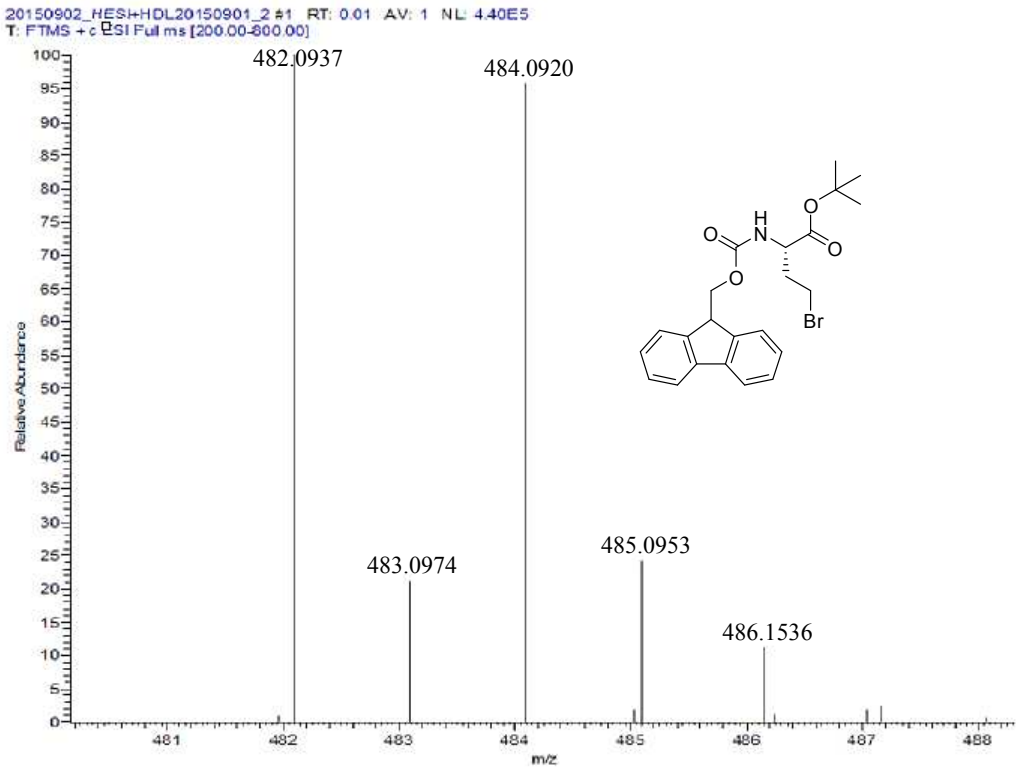
The ^{1}HNMR spectrum of I-2



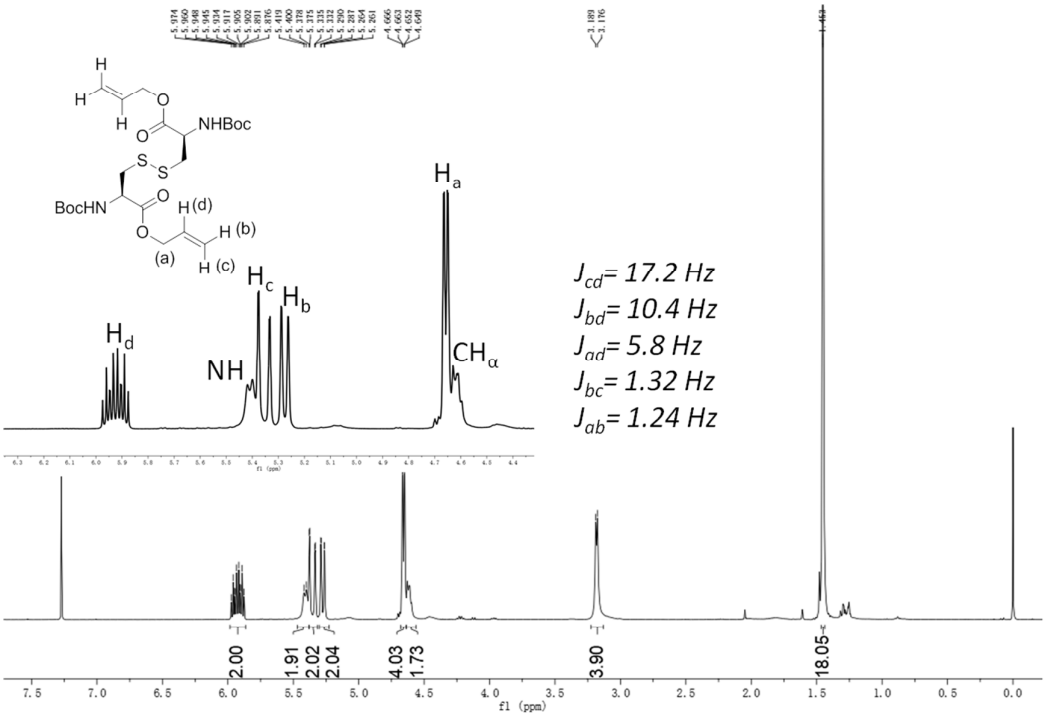
HRMS (ESI) mass spectrum of I-2



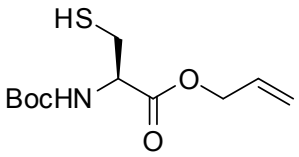
The ^{1}HNMR spectrum of 1

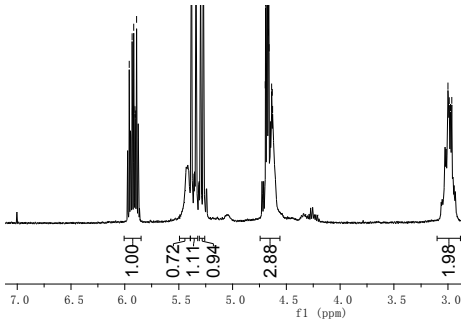


HRMS (ESI) mass spectrum of 1

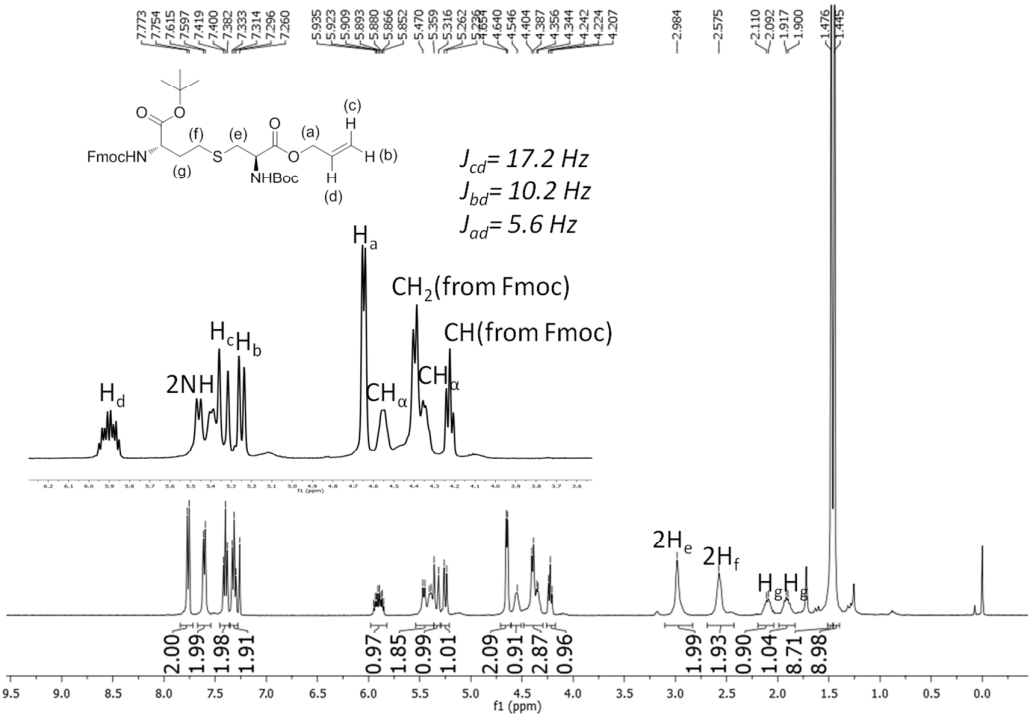


The ^{1}HNMR spectrum of I-4

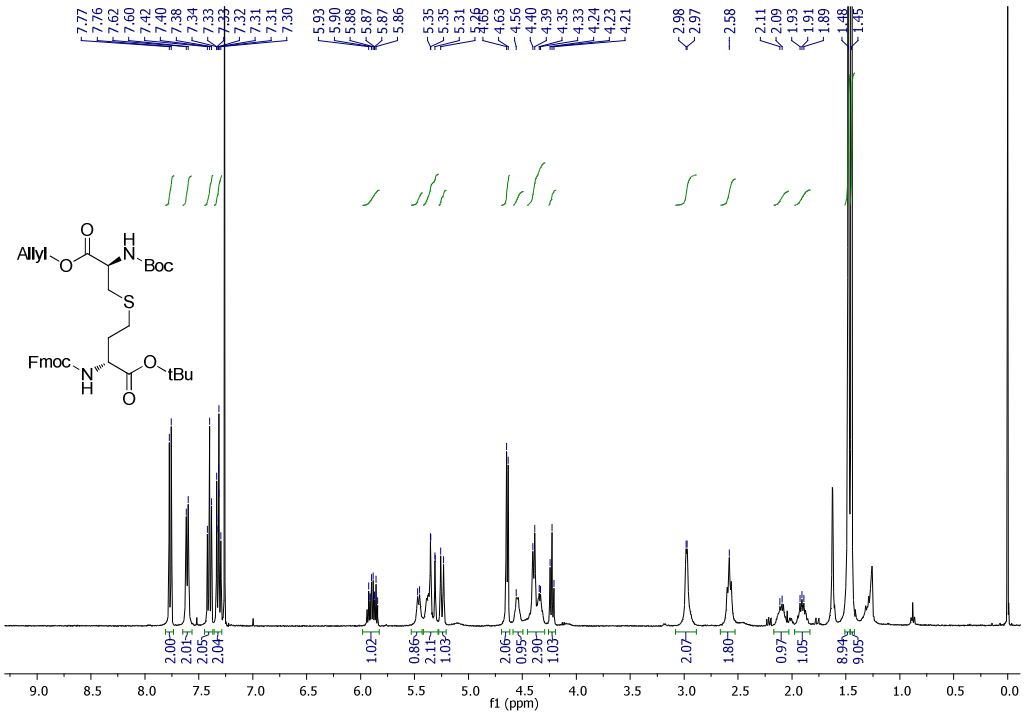




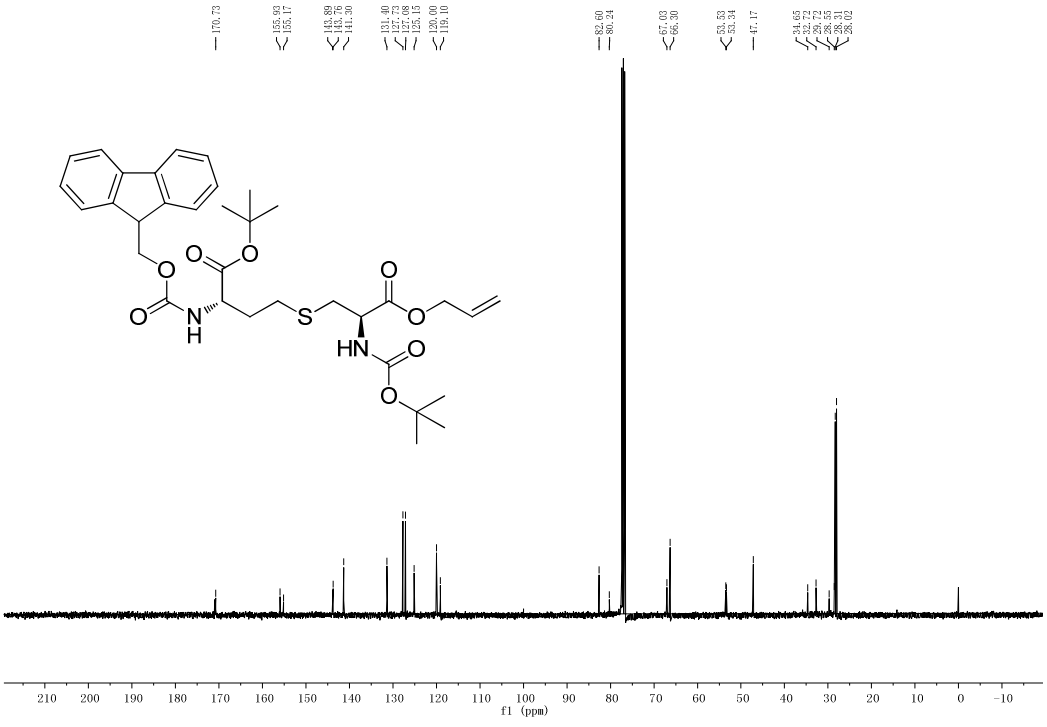
The [1]HNMR spectrum of 2



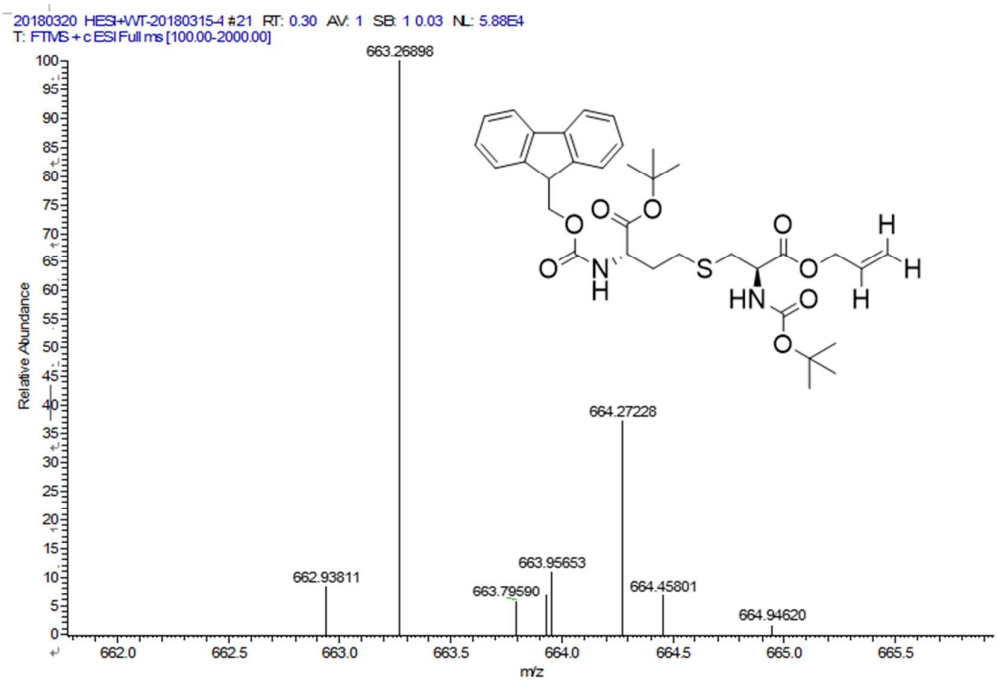
The [1]HNMR spectrum of I-5



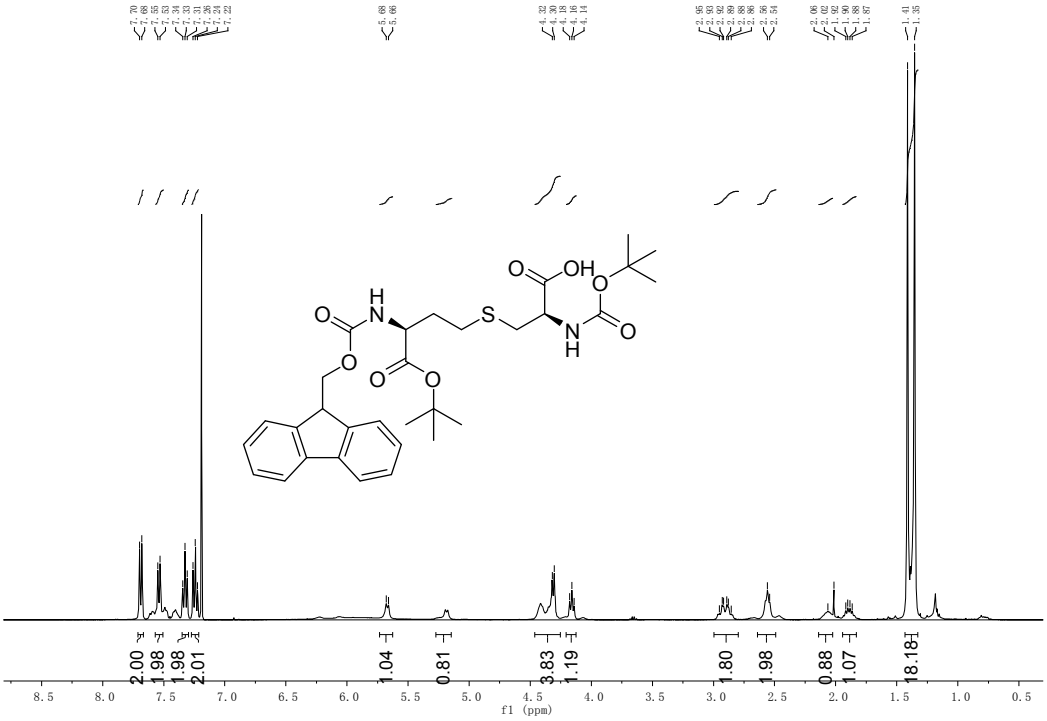
The ^{1}HNMR spectrum of D-form I-7



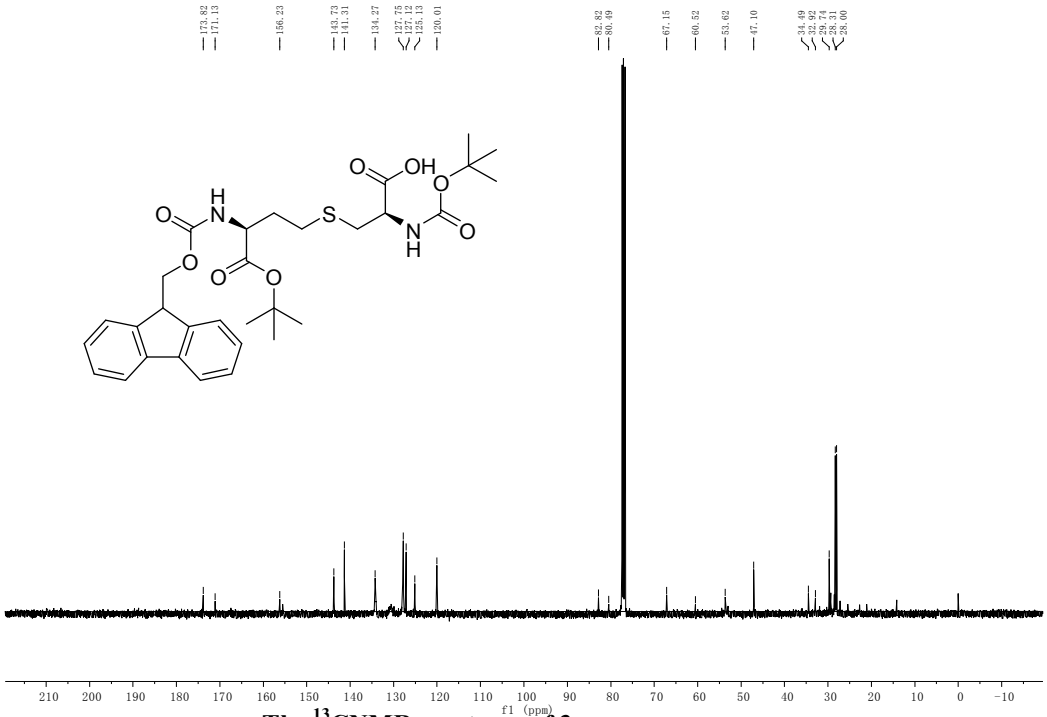
The ^{13}HNMR spectrum of I-5



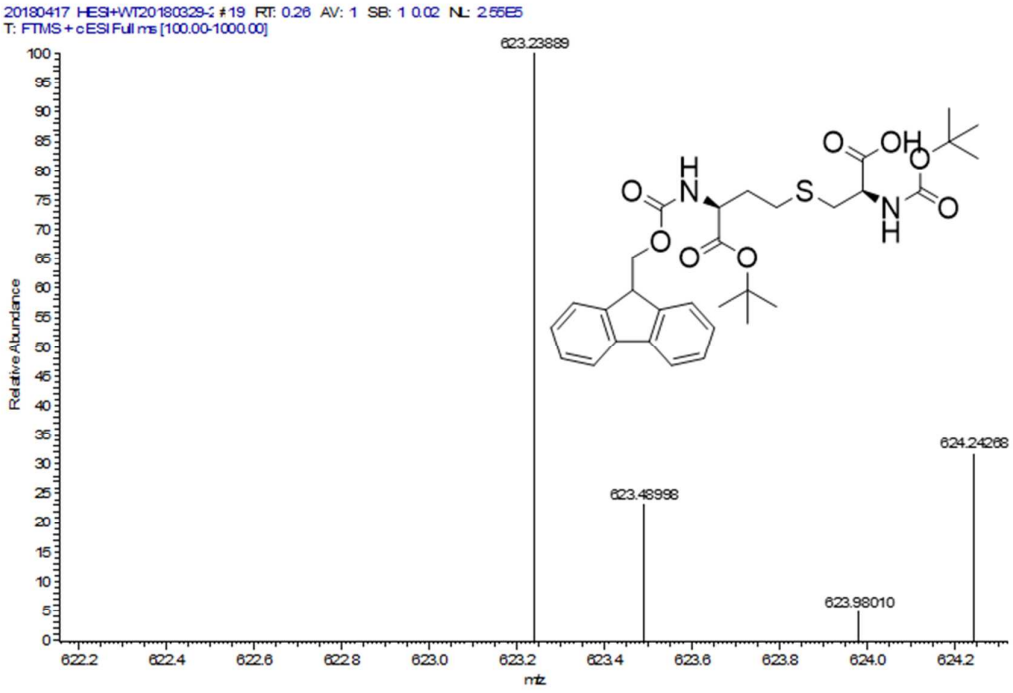
HRMS (ESI) mass spectrum of I-5



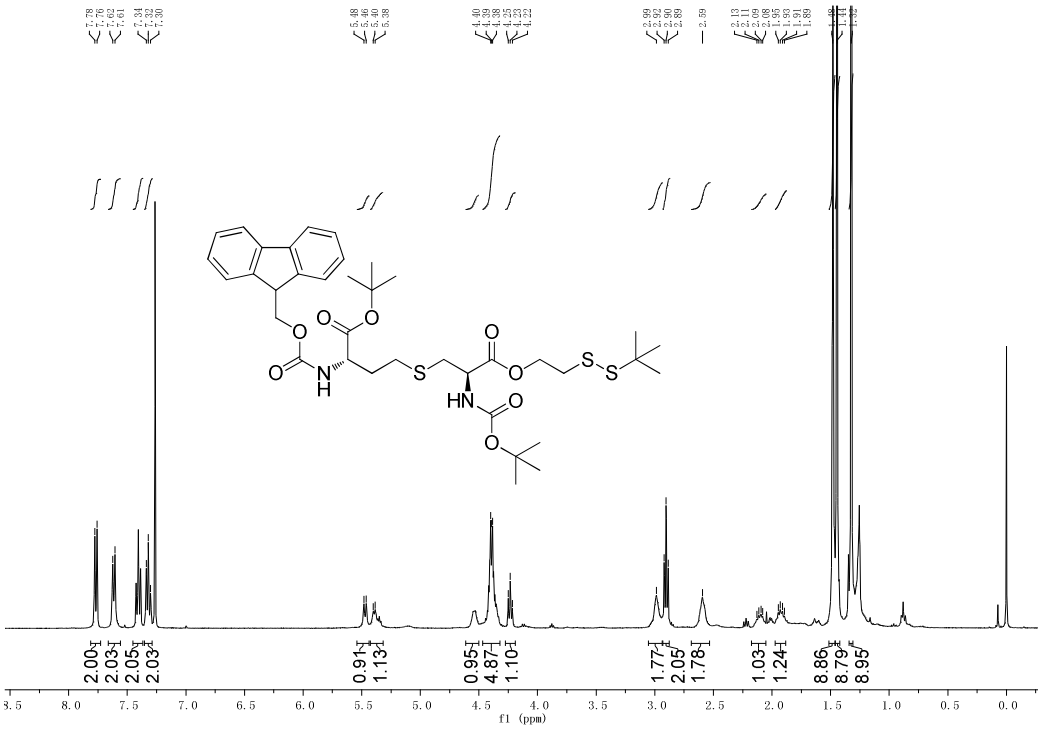
The ^{1}HNMR spectrum of 3



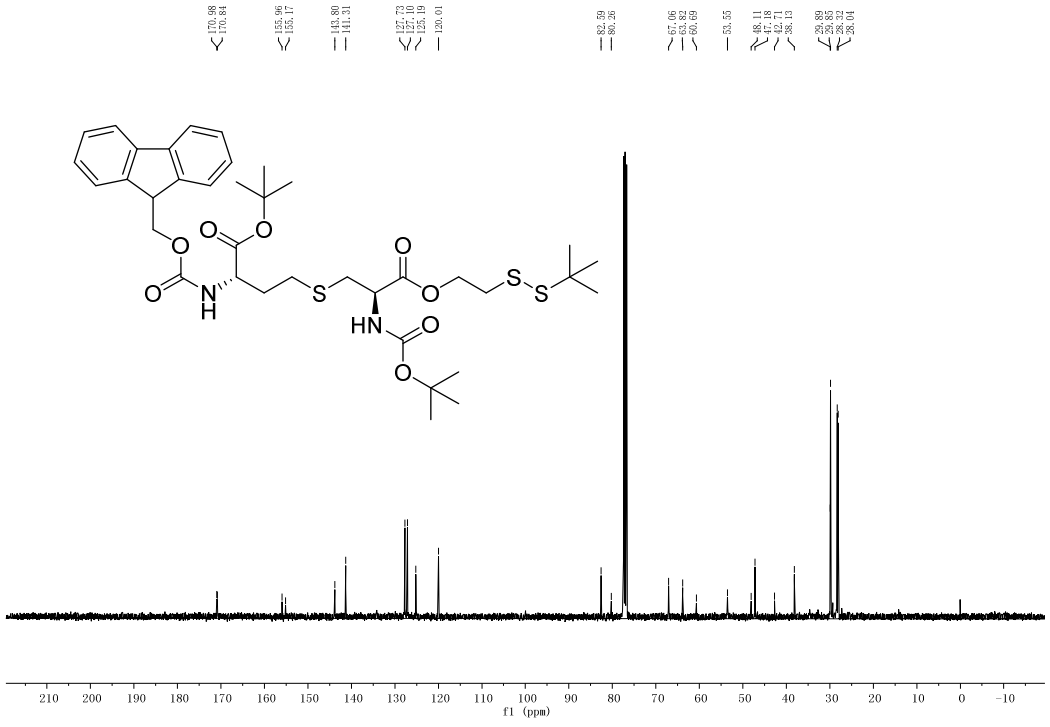
The ^{13}CNMR spectrum of 3



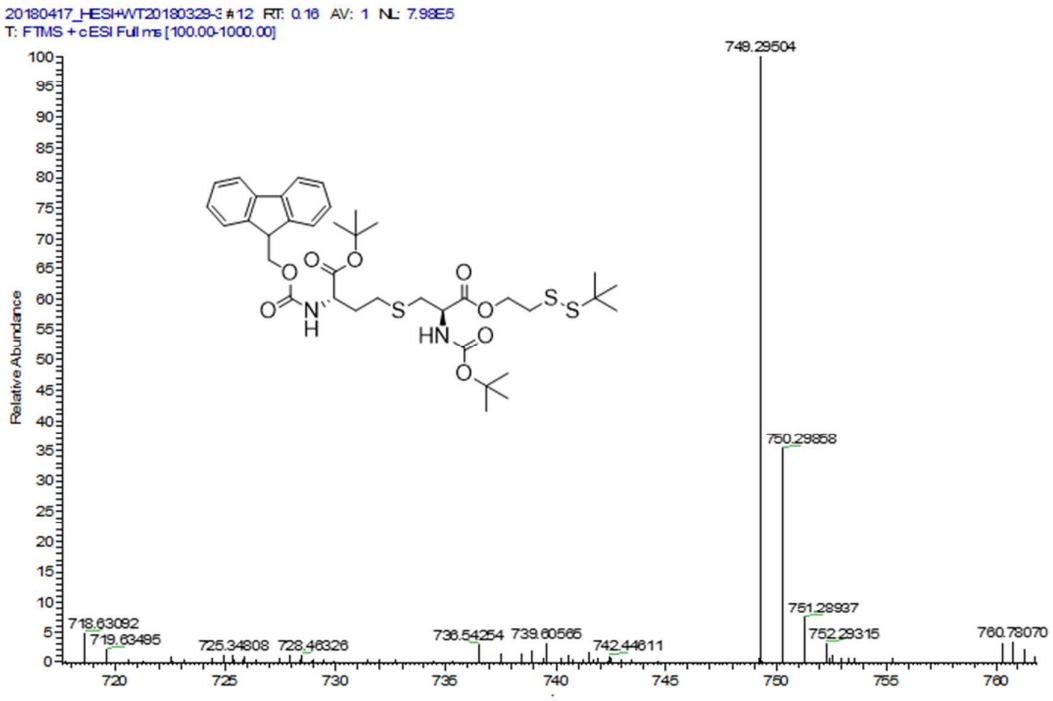
HRMS (ESI) mass spectrum of 3



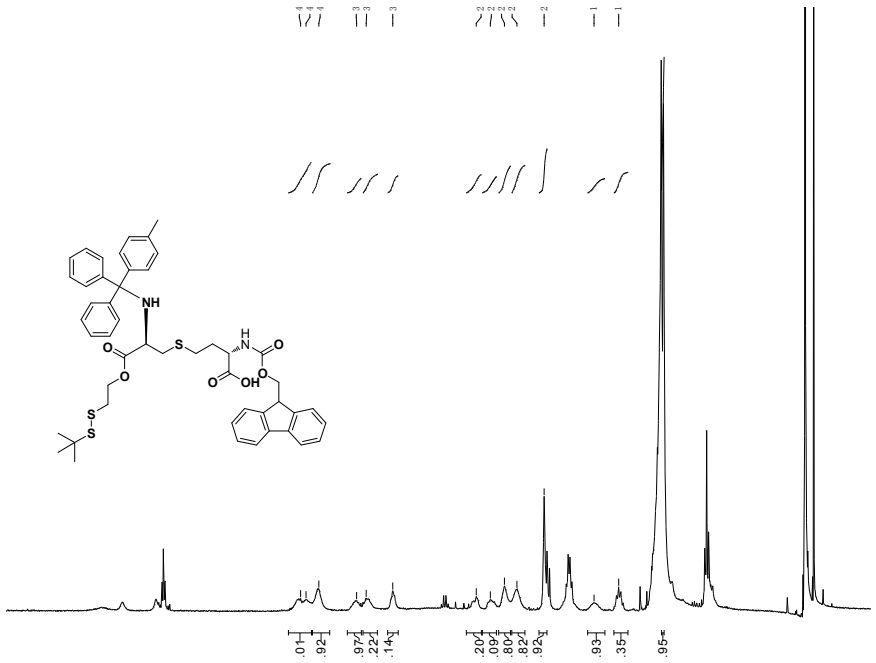
The ^{1}HNMR spectrum of I-6



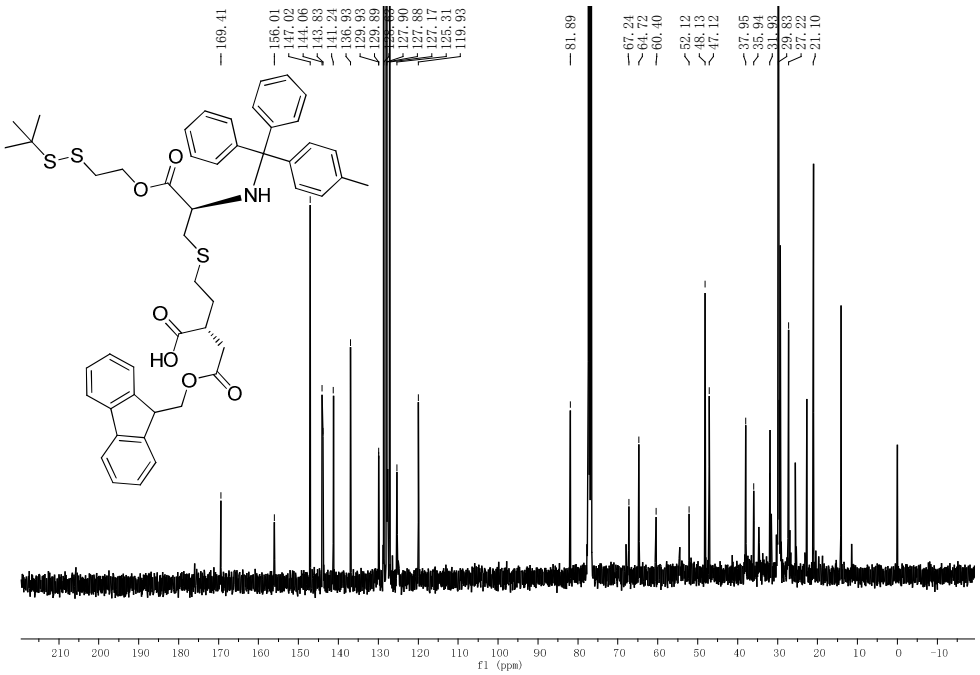
The ^{13}CNMR spectrum of I-6



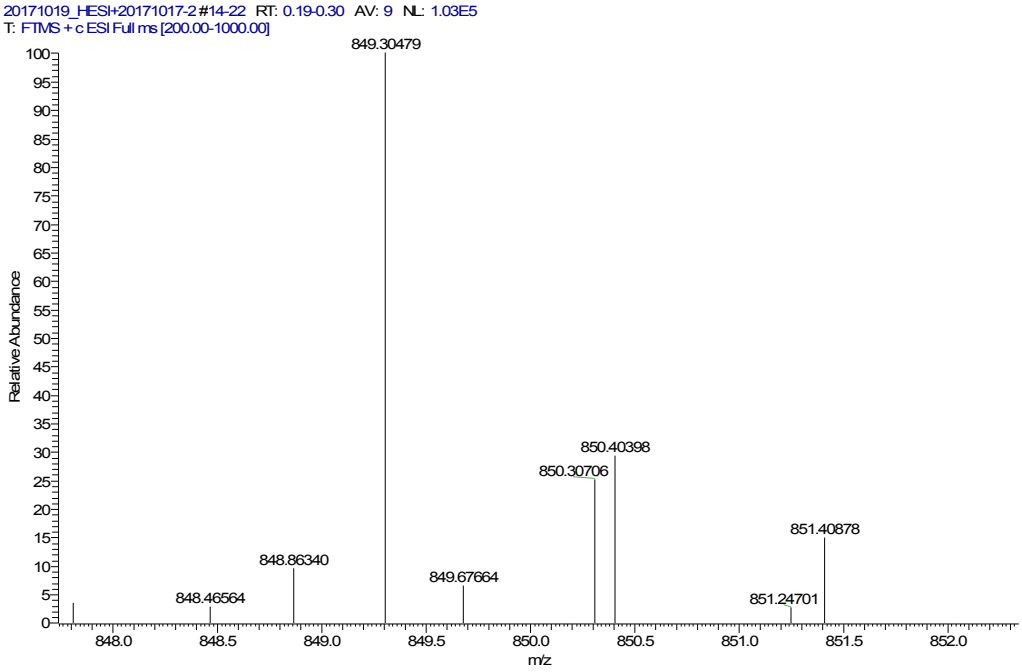
HRMS (ESI) mass spectrum of I-6



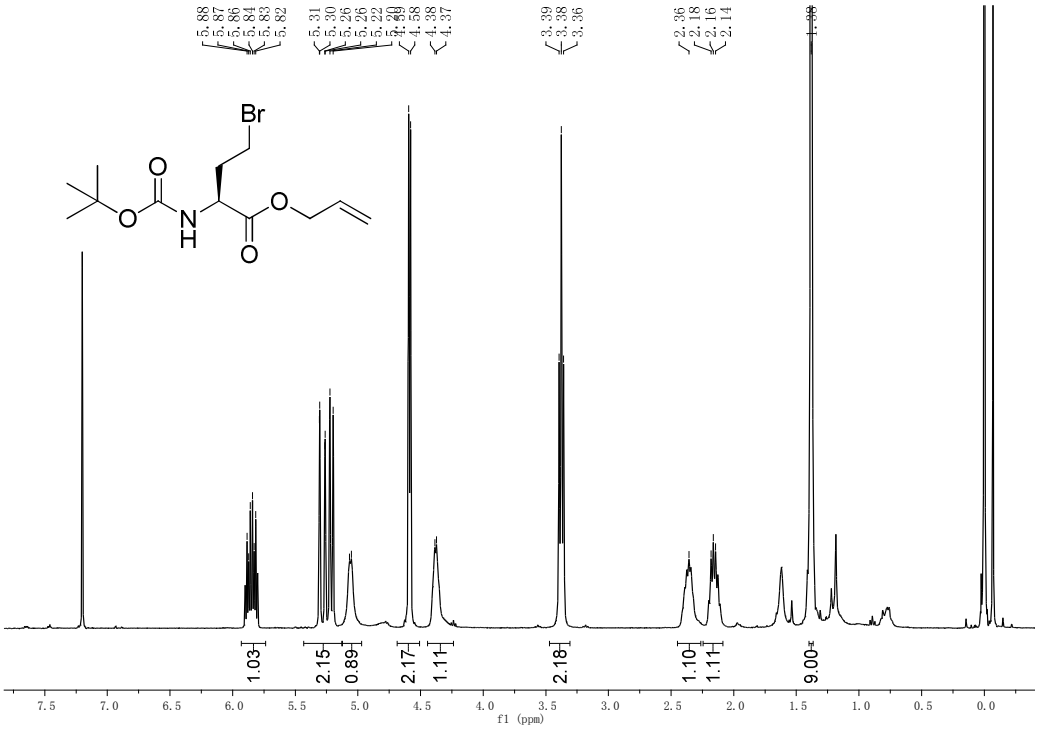
The ^{1}HNMR spectrum of C-S bridged diaminodiacid



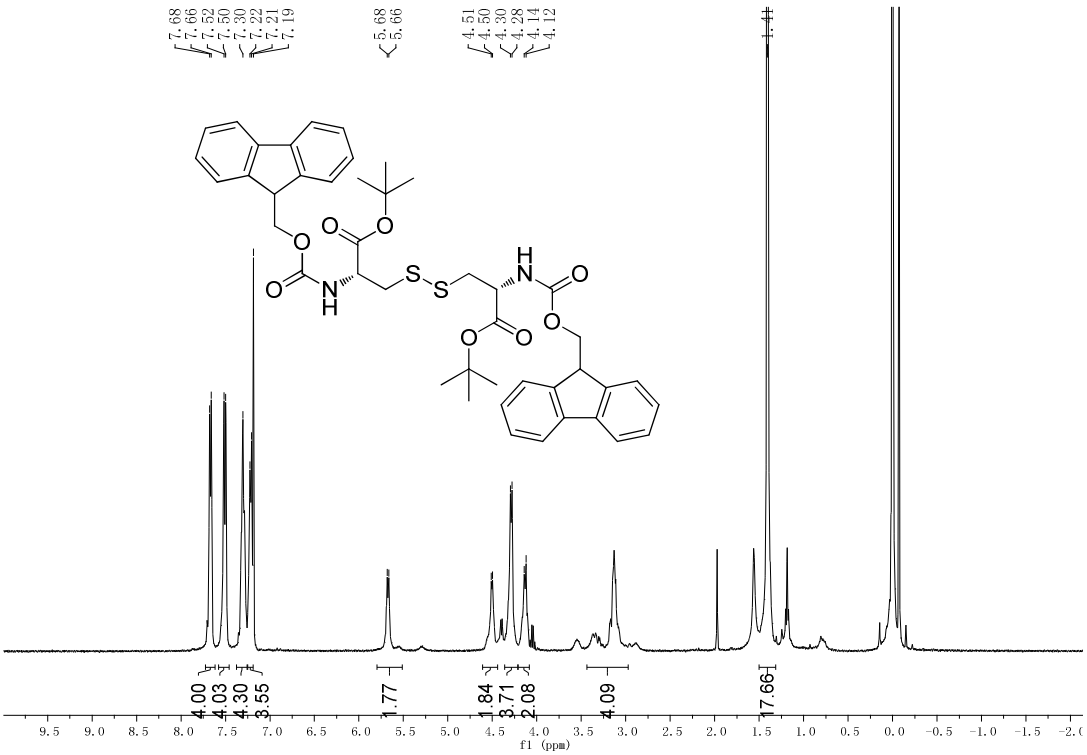
The ^{13}CNMR spectrum of C-S bridged diaminodiacid



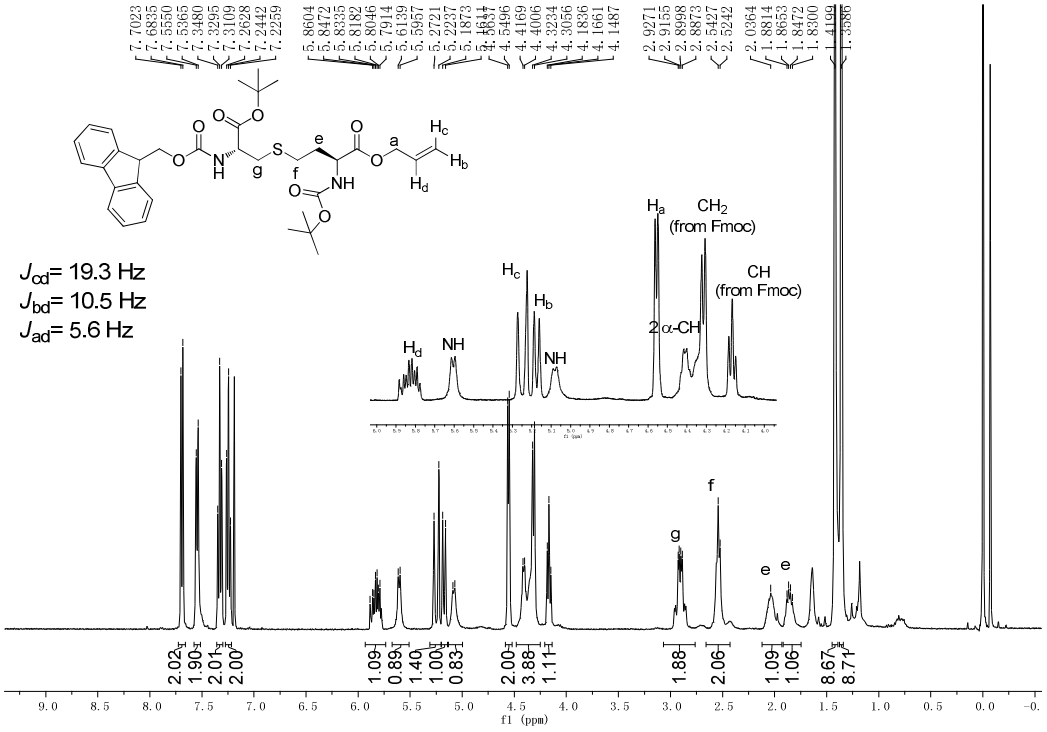
HRMS (ESI) mass spectrum of C-S bridged diaminodiacid



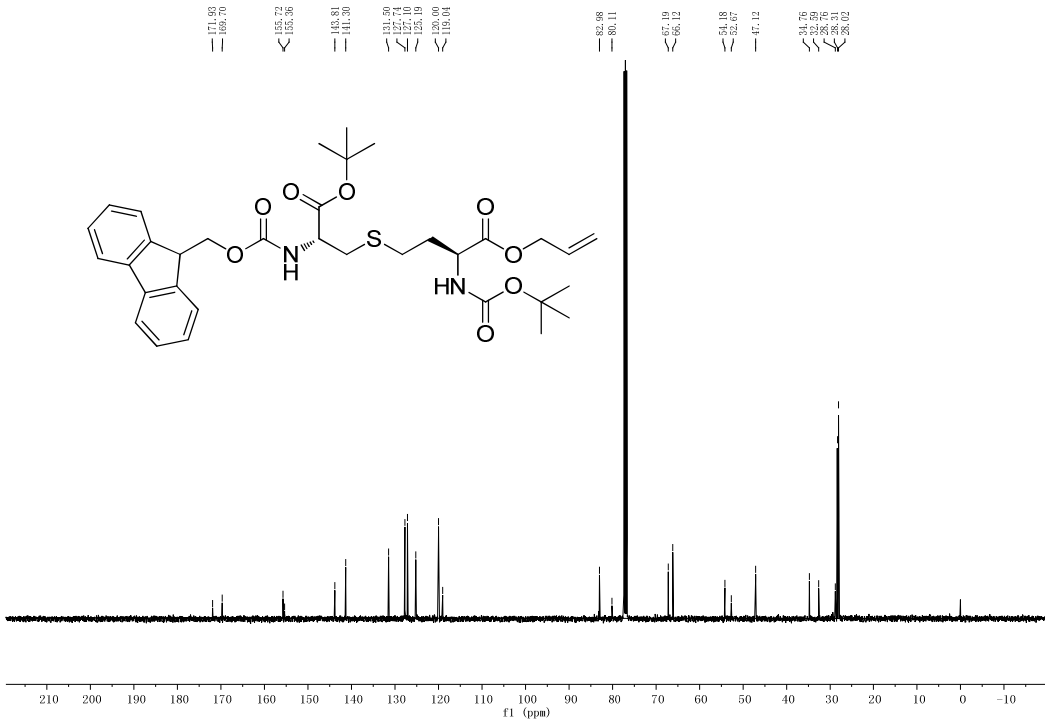
The ^{1}HNMR spectrum of 4



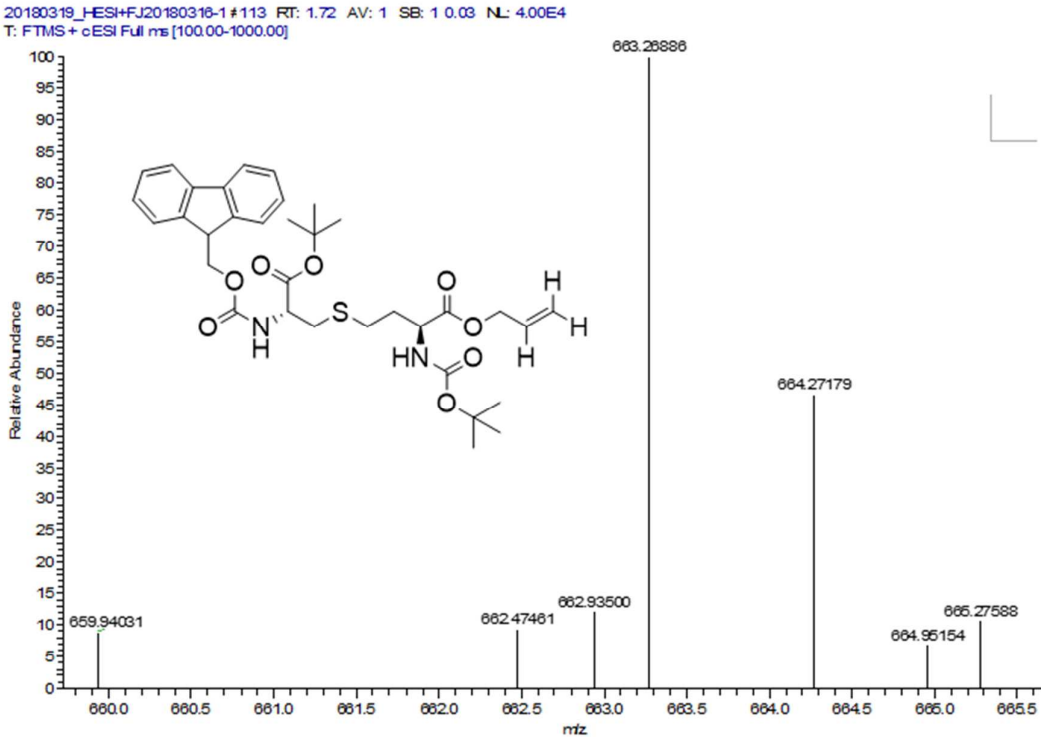
The ^{1}HNMR spectrum of II-4



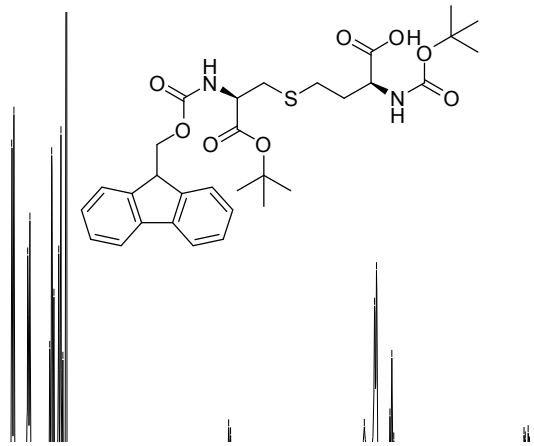
The ^{1}HNMR spectrum of II-5



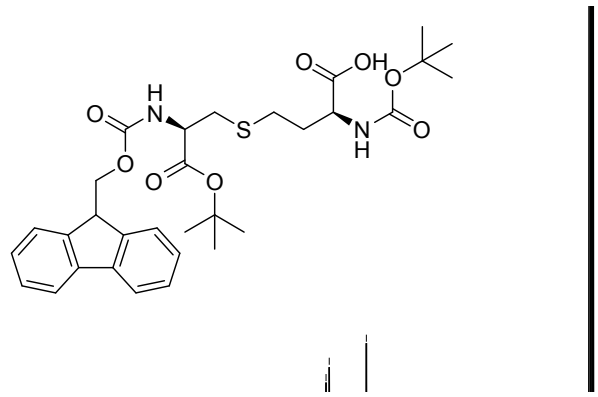
The ^{13}CNMR spectrum of II-5



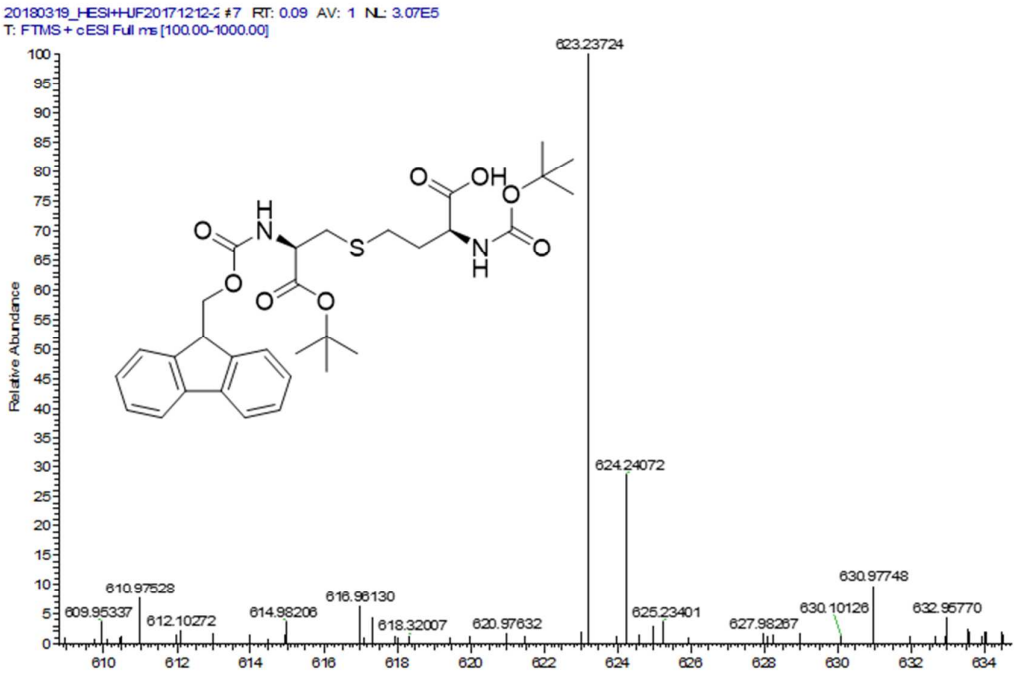
HRMS (ESI) mass spectrum of II-5



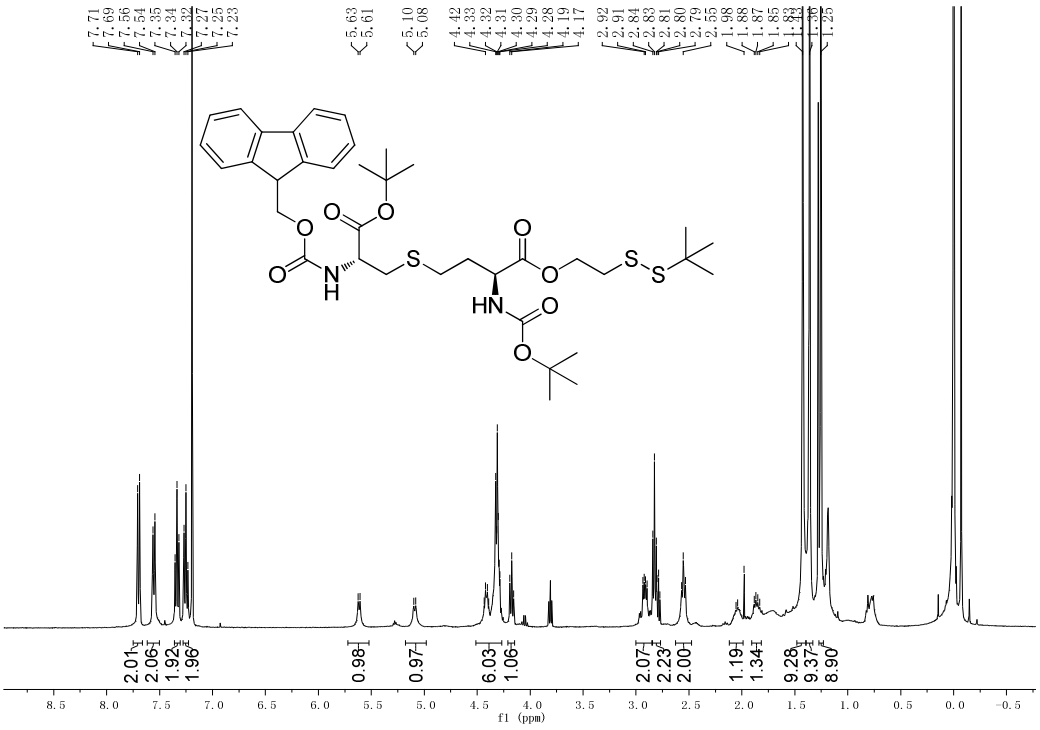
The ^{1}HNMR spectrum of 6



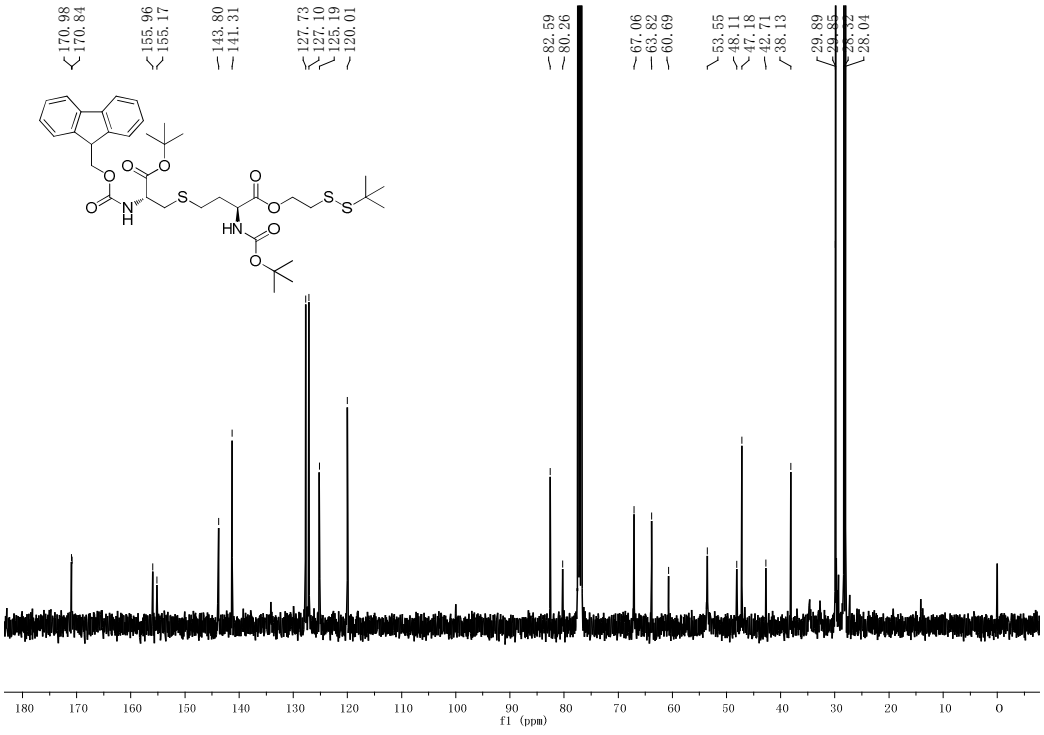
The ^{13}CNMR spectrum of 6



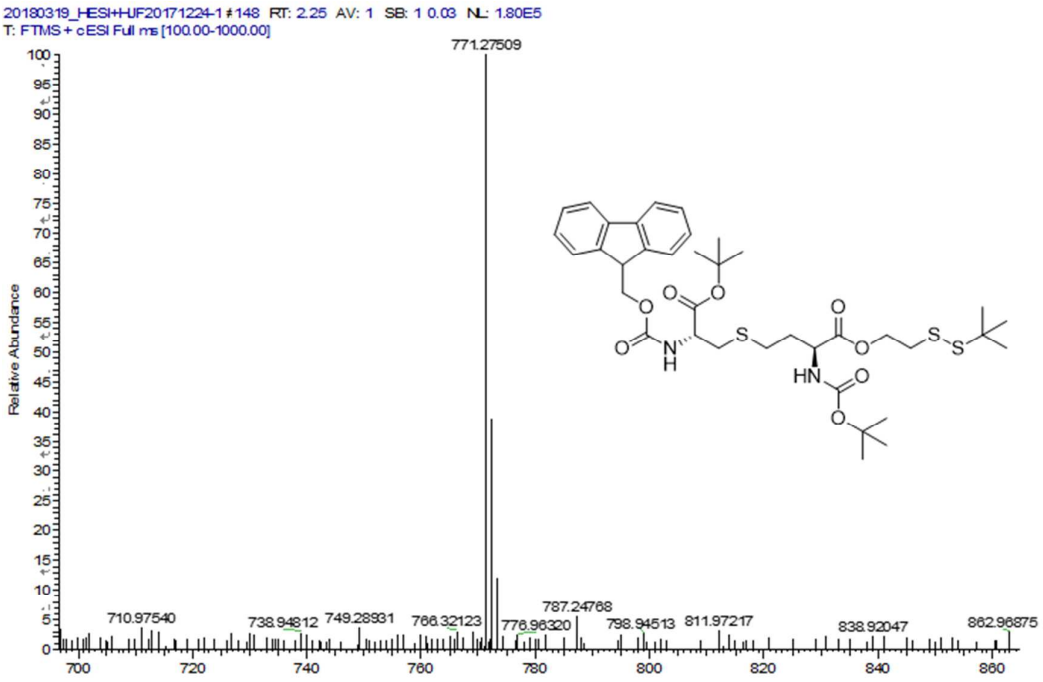
HRMS (ESI) mass spectrum of 6



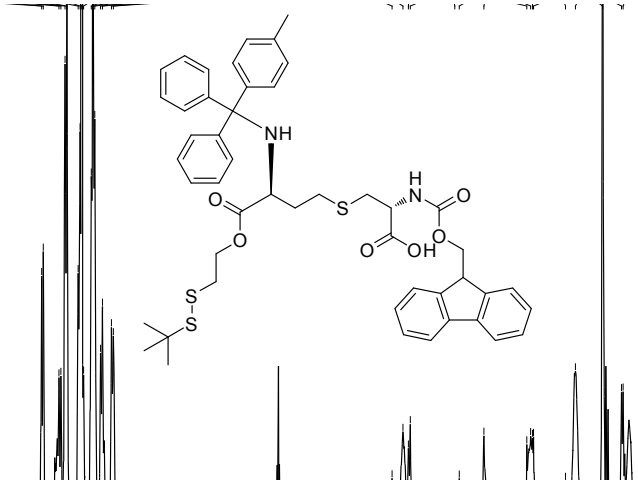
The ^{1}HNMR spectrum of II-6



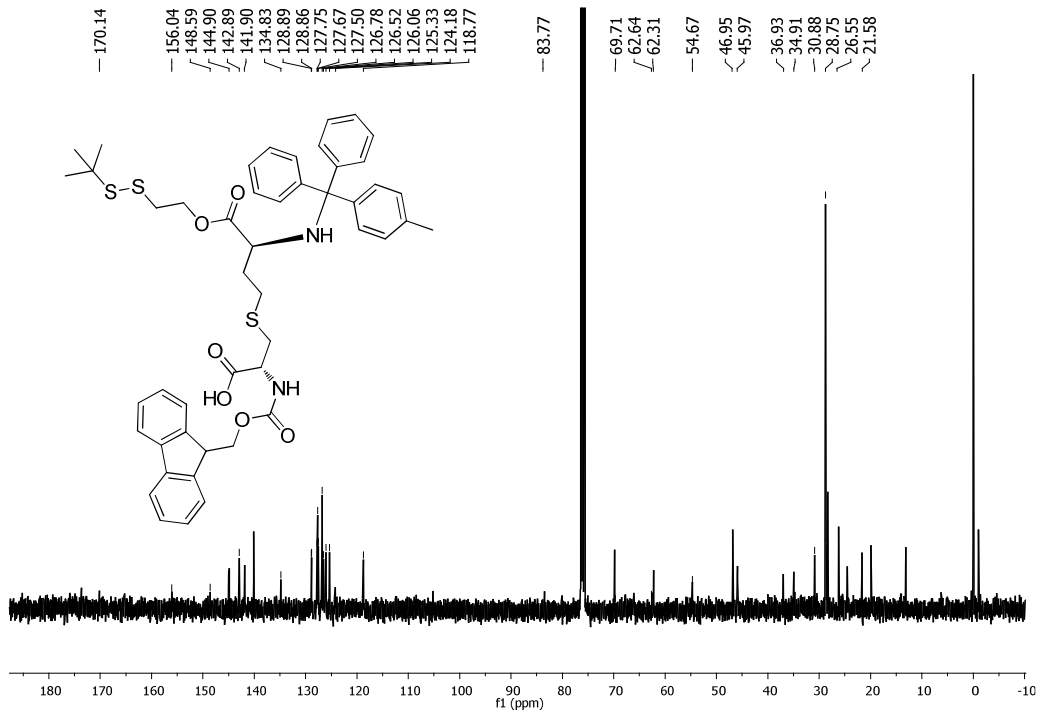
The ^{13}CNMR spectrum of II-6



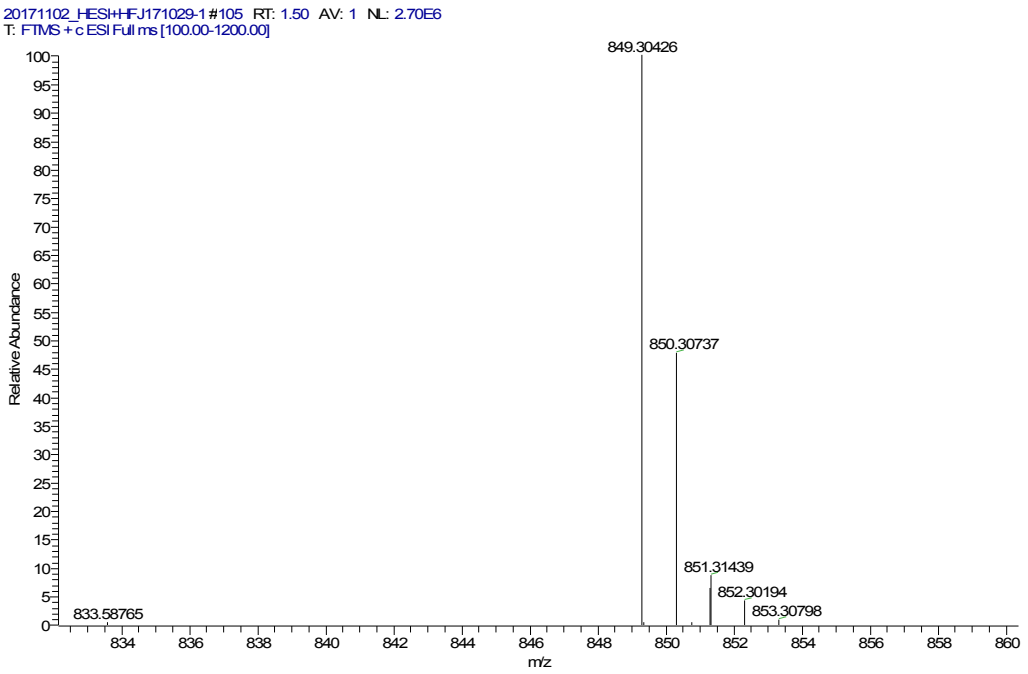
HRMS (ESI) mass spectrum of II-6



The ^{1}HNMR spectrum of S-C bridged diaminodiacid



The ^{13}CNMR spectrum of S-C bridged diaminodiacid



The HRMS(ESI)-mass spectrum of S-C bridged diaminodiacid