Supporting Information

Diaminodiacid-Based Synthesis of Macrocyclic Peptides by Using 1,2,3-Triazole as Disulfide Bond Mimetics

Ye Guo,‡[ab] Chao Liu,‡[a] Hui Song[c], Feng-Liang Wang[b], Yan Zou[a], Qiu-Ye Wu*[a], and Hong-Gang Hu*[a]

Email: wuqysmmu@hotmail.com; huhonggang_fox@msn.com

a) Department of Organic Chemistry, College of Pharmacy, Second Military Medical University, Shanghai 200433, China

b) Department of Pharmacy, Baotou Medical College, Baotou, Inner Mongolia, 014060, China.

c) College of Pharmacy, Weifang Medical University, Weifang, Shandong, 261053, China.

1. General Information

1.1 Materials

All reagents were purchased from Acros, Sigma-Aldrich, Alfa Aesar, Adamas and Innochem Chemical Reagent. Amino acids were commercial available from GL Biochem Shanghai Co. Ltd. All solvents used were bought from Sinopharm Chemical Reagent Co. Ltd. Dichloromethane (DCM) and N,N-Dimethylformamide (DMF) were distilled over calcium hydride (CaH_2) under argon atmosphere and stored in flask containing 4 Å molecular sieves. All reactions vessels were oven-dried before use. Reactions were monitored by thin-layer chromatography (TLC) and visualized by UV analyzer (254 nm), ninhydrin and/or phosphomolybdic acid.

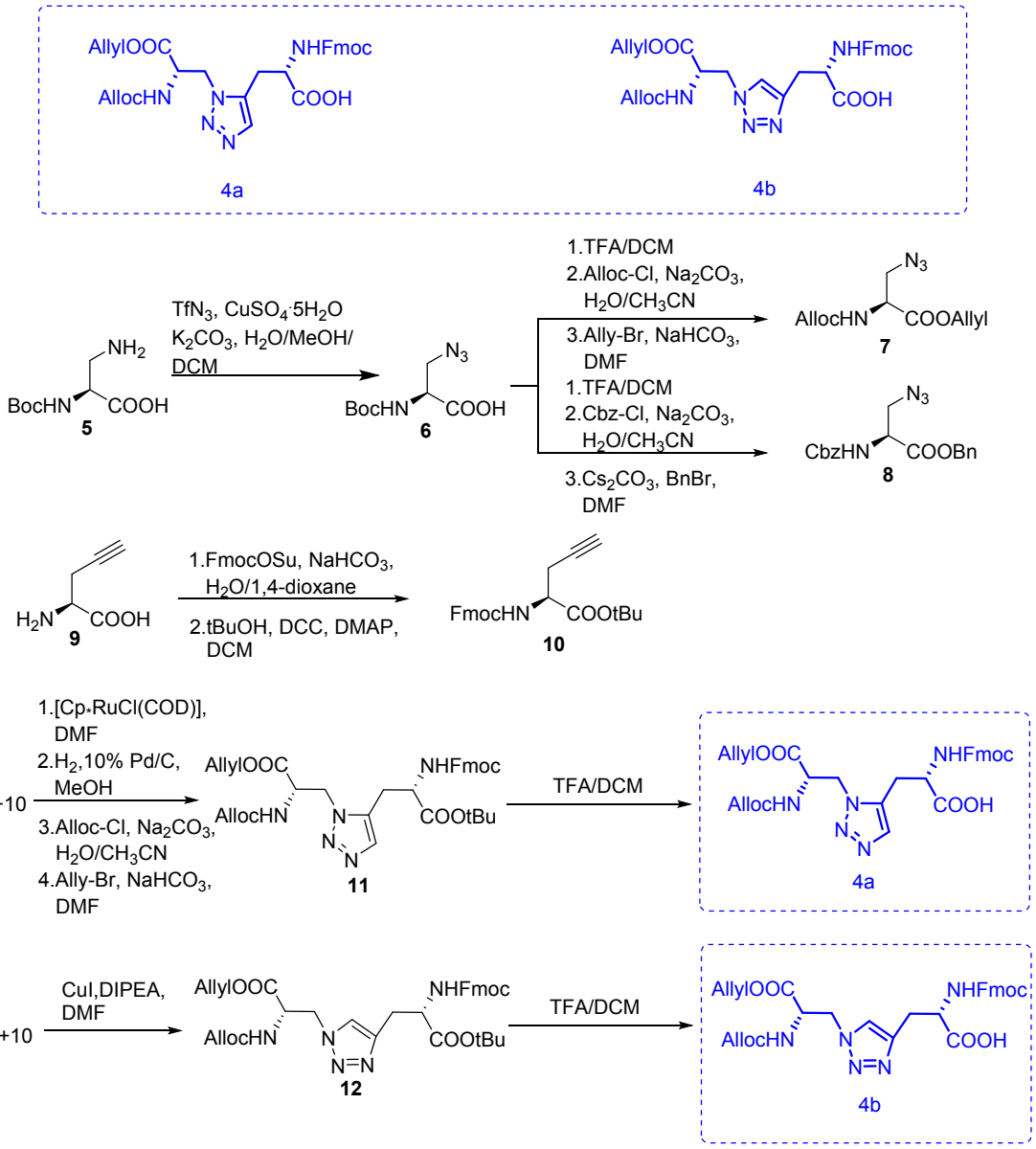
1.2 HPLC, Mass spectrometry and NMR

Peptides were analyzed and purified by reverse phase HPLC. A C18 analytic column (SHIMAZU Shim-pack VP-ODS, 4.6×250 mm, 5 μm particle size, flow rate 1 mL/min) was used for analytical RP-HPLC, and a C18 column (SHIMAZU Shim-pack PRC-ODS, 50×250 mm, 15 μm particle size, flow rate 13 mL/min) was used for semi-preparative RP-HPLC. The solvents systems were buffer A (0.1% TFA in CH_3CN) and buffer B (0.1% TFA in water). Data were recorded and analyzed using the software system LC Solution. High resolution mass spectra were measured on a Waters Xevo G2 QTOF mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance 300 MHz instrument. Chemical shifts (δ) were reported relative to TMS (0 ppm) for ^{1}H-NMR and ^{13}C-NMR spectra. The coupling constants (*J*) were displayed in Hertz (Hz) and the splitting patterns were defined as follows: singlet (s); broad singlet (s, br); doublet (d); doublet of doublet (dd); triplet (t); quartet (q); multiplet (m).

2. Experimental Section

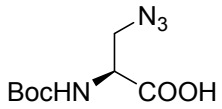
2.1 Synthesis and characterization of diaminodiacids:

To use diaminodiacids for SPPS of peptidomimetics with disulfide surrogates, we synthesized the 1,5 and 1,4-disubstituted 1,2,3-triazole diaminodiacids building blocks (**4a** and **4b**) shown in Scheme S1. Synthetic route was also shown in Scheme S1.



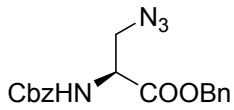
Scheme S1. Structures of diaminodiacids and the general synthetic route of 1,5- and 1,4-disubstituted 1,2,3-triazole diaminodiacids (**4a** and **4b**).

2.1.1 Synthesis and characterization data of 4a



(S)-3-azido-2-((tert-butoxycarbonyl)amino)propanoic acid (6): To a solution of NaN_3 (9.75 g, 154.00 mmol) in DCM/H_2O (2:1, 75.00 mL) was added Tf_2O (5.00 mL, 31.00 mmol) dropwise over 15 min at 0 ºC. The reaction mixture was stirred vigorously at 0 ºC for 2 hours. The organic phase was separated, washed with saturated Na_2CO_3 (3×100.00 mL) and used without further purification. To a mixture of commercially available Boc-Dap-OH (**5,** 3.18g, 15.6 mmol), K_2CO_3 (4.30 g, 31.20 mmol) and $CuSO_4{\cdot}5H_2O$ (25.00 mg, 0.10 mmol) in H_2O/MeOH (2:1, 135.00 mL) was added freshly prepared TfN_3 solution dropwise at 0 ºC, then more MeOH was added to homogeneity. The mixture was allowed to warm to room temperature and stirred overnight. The DCM and MeOH was removed under vacuum and the aqueous solution was acidified by 10 % HCl to pH 2. After dilution with EtOAc (100.00 mL), the organic layer was separated, washed with brine (3×100.00 mL), dried over Na_2SO_4, concentrated and purified by column chromatography (80:1-20:1, DCM/MeOH) to give 6 as a colorless oil (2.62 g, 73.0 %).

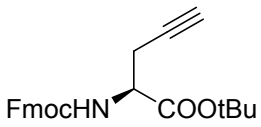
^{1}H-NMR (300 MHz, DMSO): δ 7.24 (d, *J*= 6.0 Hz, 1H), 4.14 (s, 1H), 3.60-3.51 (m, 2H), 1.39 (s, 9H).
^{13}C-NMR (300 MHz, DMSO): δ 171.32, 155.37, 78.53, 53.51, 50.94, 28.20.
HR-MS, *m/z* calcd for $C_8H_{14}N_4O_4$ 230.1015; found $[M-H]^-$ 229.2424.



benzyl (S)-3-azido-2-(((benzyloxy)carbonyl)amino)propanoate (8): 6 (2.62 g, 11.40 mmol) was dissolved in 4M HCl/1,4-dioxane (30.00 mL) at 0 ºC. The reaction mixture was stirred for 3 h at room temperature. Then the resulting mixture was filtrated and the solid was washed with EtOAc twice. After dried over vacuum, the solid was used directly in the next step. A mixture of the resulting amino acid hydrochloride in acetonitrile (100.00 mL) was cooled to 0 ºC and a solution of Na_2CO_3 (3.62 g, 34.2 mmol) in H_2O (20.00 mL) was added. Benzyloxycarbonyl chloride (1.77 mL, 12.62 mmol) in acetonitrile (10.00 mL) was then added dropwise to the reaction mixture. The reaction mixture was stirred at 0 ºC for 2 h, then warmed up to room temperature and stirred overnight. After quenching with H_2O, the organic

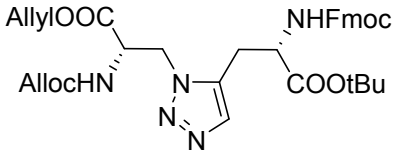
phase was removed by vacuum and the reaction was acidified with 10 % HCl to pH 1-2 and diluted with EtOAc (3×100.00 mL). The organic layers were washed with brine (3×100.00 mL) ,dried over Na_2SO_4 and concentrated to afford the Cbz-protected amino acid which was used without further purification. A solution of the resulting Cbz-protected amino acid in MeOH/H_2O (9:1, 30.00 mL) was titrated to pH 7 with 20% aqueous Cs_2CO_3 and the solvent was removed under reduced pressure. To a solution of the residue in DMF (20.00 mL) was added benzyl bromide (1.50 mL, 12.62 mmol) dropwise under Ar atmosphere at 0 ºC. The reaction mixture was stirred at 0 ºC for 2h and then allowed to warm to room temperature and stirred overnight. The turbid mixture was evaporated to dryness and diluted with ether and H_2O (100.00 mL each). The organic layer was successively washed with saturated $NaHCO_3$ (3×100.00 mL), brine (3×100.00 mL), dried over Na_2SO_4, filtered, concentrated and purified by column chromatography (30:1-10:1, petro ether/EtOAc) to afford **8** as a white powder (2.50 g, 62.0 %, 3 steps).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.39-7.38 (m, 10H), 5.71-5.69 (m, 1H), 5.24 (s, 2H), 5.16 (s, 2H), 4.61-4.59 (m, 1H), 3.78 (s, 2H).
^{13}C-NMR (300 MHz, $CDCl_3$): δ 169.33, 155.68, 135.98, 134.80, 128.73, 128.59, 128.41, 128,31, 128.12, 67.93, 67.31, 54.03, 52.60.
HR-MS, *m/z* calcd for $C_{18}H_{18}N_4O_4$ 354.1328; found $[M+Na]^+$ 377.4421.



tert-butyl (S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)pent-4-ynoate (10): To a solution of commercial available H-Pra-OH (**9**, 5.00 g, 44.24 mmol) in H_2O/1,4-
dioxane (1:1, 500.00 mL) was added $NaHCO_3$ (18.58 g, 221.20 mmol) and FmocOSu (74.61 g, 221.20 mmol) successively at 0 ºC. Then the mixture was stirred overnight at room temperature. After the 1,4-dioxane was removed under vacuum, the aqueous phase was acidified with 1 M HCl to pH=1-2 and extracted with EtOAc (2×100.00 mL). The organic layer was washed with brine (3×100.00 mL), dried over Na_2SO_4 and concentrated to provided crude Fmoc-Pra-OH which was used directly without further purification. To a solution of the crude Fmoc-Pra-OH, tert-Butanol (8.10 ml, 88.48 mmol) and DMAP (1.08 g, 8.85 mmol) in DCM (500.00 mL) was added DCC (10.03 g, 48.66 mmol, solved in 500.00 mL DCM) dropwise at 0 ºC. The mixture was stirred for 1 h at 0 ºC and 4 h at room temperature. Then, the reaction was filtrated and the filtrates was washed successively with 1 M HCl (3×100.00 mL), saturated $NaHCO_3$ (3×100.00 mL) and brine (3×100.00 mL). The organic phase was dried overNa_2SO_4,filtered,concentrated and purified by column chromatography (50:1-10:1, petro ether/EtOAc) to give **10** as a white powder (12.11 g, 70.0 %, 2 steps).
^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.80-7.78 (m, 2H), 7.43-7.40 (m, 2H), 7.40-7.37 (m,

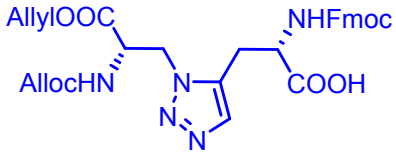
2H), 7.34-7.32 (m, 2H), 5.70 (d, *J*= 6.0 Hz, 1H), 4.46-4.40 (m, 3H), 4.29-4.24 (m, 1H), 2.79 (s, 2H), 1.53 (s, 9H).
^{13}C-NMR (300 MHz, $CDCl_3$): δ 169.31, 155.61, 143.87, 143.80, 141.30, 127.73, 127.08, 125.17, 120.00, 78.56, 82.88, 71.54, 67.20, 52.65, 47.14, 27.98, 22.99.
HR-MS, *m/z* calcd for $C_{24}H_{25}NO_4$ 391.1784; found $[M+H]^+$ 392.3519.



allyl (S)-3-(5-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-butoxy)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)-2-(((allyloxy)carbonyl)amino)propanoate (11):
To a solution of **10** (100.00 mg, 0.255 mmol) and **8** (90.27 mg, 0.255 mmol) in dry DMF (1.40 mL) was added Cp*RuCl(COD) (1% mol) under Ar atmosphere. The reaction mixture is stirred for 48 h at room temperature. The resulting mixture is diluted with EtOAc (100.00 mL) and H_2O (100.00 mL). The organic phases are separated, washed with brine (25.00 mL), dried over Na_2SO_4, filtered and concentrated. The residue was solved in MeOH (2.00 mL). After addition of 10% Pd/C (20.00 mg) under H_2 conditions, the reaction mixture was stirred overnight at room temperature. The solid was filtered using celite and the filtrate was concentrated and used without further purification. To a solution of the residue and sodium carbonate (27.03 mg, 0.255 mmol) in H_2O (1.00 mL) and acetonitrile (0.50 mL) was added allyl chloroformate (28.00 µL, 0.255 mmol) dropwise at 0 ºC. The reaction was allowed to warm to room temperature and stirred under Ar for 18 h. The solvents was removed and the residue was diluted with dimethylformamide (DMF, 20.00 mL). Then sodium bicarbonate (21.50 mg, 0.255 mmol) was added, followed by allyl bromide (22.00 µL, 0.255 mmol). The heterogeneous mixture was stirred for 48 h at room temperature (Additional allyl bromide (11 µL, 0.125 mmol) was added at 22 h). The reaction was concentrated, diluted with EtOAc (10.00 mL) and washed with saturated $NaHCO_3$, 0.1 M $KHSO_4$ and brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and purified by column chromatography (10:1-2:1, petro ether/EtOAc) to give **11** as a white powder (90.00 mg, 54.7 %, 4 steps).

^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.78 (d, J = 6.0 Hz, 2H), 7.61 (d, J = 6.0 Hz, 2H), 7.43-7.40 (m, 3H), 7.35-7.31 (m, 2H), 5,93-5.88 (m, 3H), 5.89-5.81 (m, 2H), 5.37-5.29 (m, 4H), 4.82-4.76 (m, 3H), 4.69-4.68 (m, 3H), 4.67-4.66 (m, 3H), 4.65-4.55 (m, 2H), 4.39-4.21 (m, 1H), 3.35-3.27 (m, 2H), 1.47 (s, 9 H).
^{13}C-NMR (300 MHz, $CDCl_3$): δ 169.86, 168.21, 159.52, 159.10, 156.02, 155.68, 143.86, 143.75, 141.70, 133.30, 133.11, 130.94, 127.74, 127.13, 125.19, 123.93, 119.99, 119.82, 118.28, 116.08, 82.96, 67.23, 67.18, 67.16, 66.31, 54.09, 53.92, 47.09, 27.94.

HR-MS, *m/z* calcd for $C_{34}H_{39}N_5O_8$ 645.2799; found $[M+H]^+$ 646.3848.

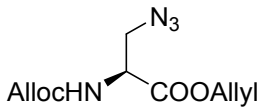


(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-((S)-2-(((allyloxy)carbonyl)amino)-3-oxo-3-(((E)-prop-1-en-1-yl)oxy)propyl)-1H-1,2,3-triazol-5-yl)propanoic acid (4a): 11 (90.00 mg, 0.14 mmol) was dissolved in TFA/DCM (1:1, 5.00 mL) and stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* to yield **4a** as a white powder (78.00 mg, 95.0 %) which was used directly in SPPS without further purification.

^{1}H-NMR (300 MHz, d-DMSO): δ 7.89 (d, J = 6.0 Hz, 2H), 7.69 (s, 1H), 7.44-7.39 (m, 2H), 7.34 (t, J = 6.0 Hz, 2H), 7.31 (t, J = 6.0 Hz, 2H), 5.92-5.81 (m, 3H), 5.33-5.14 (m, 5H), 4.76-4.73 (m, 1H), 4.70 (s, 4H), 4.45 (s, 2H), 4.27-4.21 (m, 4H), 3.15-2.94 (m, 2H).
^{13}C-NMR (300 MHz, d-DMSO): δ 173.42, 169.52, 156.44, 156.12, 144.20, 143.47, 141.15, 133.67, 132.48, 128.10, 127.53, 125.72, 124.23, 120.57, 118.42, 117.55, 66.17, 65.90, 54.65, 54.32, 49.60, 47.03, 27.62.
HR-MS, *m/z* calcd for $C_{30}H_{31}N_5O_8$ 589.2173; found $[M-H]^-$ 588.1908.

.

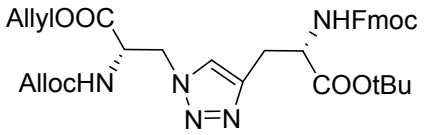
2.1.2 Synthesis and characterization data of 4b



allyl (S)-2-(((allyloxy)carbonyl)amino)-3-azidopropanoate (7): 6 (3.00 g, 13.00 mmol) was dissolved in 4M HCl/1,4-dioxane (30.00 mL) at 0 ºC. The reaction mixture was stirred for 3 h at room temperature. Then the resulting mixture was filtrated and the solid was washed with EtOAc twice. After dried over vacuum, the solid was used directly in the next step. To a solution of the resulting amino acid hydrochloride and sodium carbonate (2.75 g, 26.00 mmol) in H_2O/acetonitrile (2:1, 75.00 mL) was added allyl chloroformate (1.37 mL, 13.00 mmol) dropwise at 0 ºC. The reaction was allowed to warm to room temperature and stirred under Ar for 18 h. The solvents was removed and the residue was diluted with dimethylformamide (DMF, 20.00 mL). Then sodium bicarbonate (1.09 g, 13.00 mmol) and allyl bromide (1.12 mL, 13.00 mmol) were added. The heterogeneous mixture was stirred for 48 h

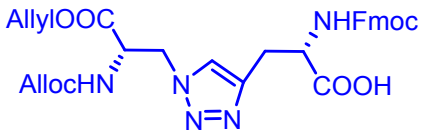
at room temperature (Additional allyl bromide (0.56 mL, 6.50 mmol) was added at 22 h). The reaction was concentrated, diluted with EtOAc (100.00 mL), washed with saturated $NaHCO_3$, 0.1 M $KHSO_4$, brine. The organic layer was dried over Na_2SO_4, filtered, concentrated and purified by column chromatography (50:1-10:1, petro ether/EtOAc) to give **7** as a yellow oil (2.43 g, 73.6 %, 3 steps).

^{1}H-NMR (400 MHz, CDCl3): δ 6.00-5.87 (m, 2H), 5.62 (d, J= 6.0 Hz, 1H), 5.40-5.27 (m, 4H), 4.65 (d, J = 6.0 Hz, 2H), 4.63-4.55 (m, 3H), 3.79 (d, J = 3.0 Hz, 2H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 169.15, 155.53, 132.33, 131.06, 119.45, 118.08, 66.71, 66.11, 53.95, 52.63.
HR-MS, *m/z* calcd for $C_{10}H_{14}N_4O_4$ 254.1015; found $[M+H]^+$ 255.2640.



allyl (S)-3-(4-((S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(tert-buto xy)-3-oxopropyl)-1H-1,2,3-triazol-1-yl)-2-(((allyloxy)carbonyl)amino)propanoate (12): To a solution of **10** (100.00 mg, 0.255 mmol), **7** (64.77 mg, 0.255 mmol) and CuI (72.20 mg, 0.38 mmol) in dry DMF (1.40 mL) was added DIPEA (0.44 mL, 2.55 mmol) under Ar atmosphere. Protected from light, the reaction mixture was stirred for 14 h at room temperature. The resulting mixture is diluted with EtOAc (100.00 mL) and H_2O (100.00 mL). The organic phases are filtered, washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by column chromatography (10:1-2:1, petro ether/EtOAc) to give **12** as a white powder (138.00 mg, 84.1 %)**.**

^{1}H-NMR (300 MHz, CDCl3): δ7.76 (d, J = 6.0 Hz, 2H), 7.61 (d, J = 6.0 Hz, 2H), 7.40 (t, J = 3.0 Hz, 2H), 7.38-7.31 (m, 3H), 5.91-5.77 (m, 2H), 5.75 (d, J = 6.0 Hz, 1H), 5.66 (d, J = 6.0 Hz, 1H), 5.34-5.30 (m, 3H), 5.29-5.27 (m, 1H), 4.84-4.77 (m, 3H), 4.76-4.73 (m, 2H), 4.66 (d, J = 6.0 Hz, 3H), 4.37 (d, J = 6.0 Hz, 2H), 4.23-4.21 (m, 1H), 3.27-3.22 (m, 2H), 1.45 (s, 9H).
^{13}C-NMR (400 MHz, $CDCl_3$): δ 170.12, 168.43, 155.90, 155.65, 143.92, 143.84, 143.03, 141.31, 132.22, 131.06, 127.72, 127.11, 125.21, 123.40, 123.27, 119.98, 119.65, 118.17, 82.64, 67.01, 66.22, 54.15, 53.84, 50.95, 50.81, 47.16, 29.71, 28.58, 27.98.
HR-MS, *m/z* calcd for $C_{34}H_{39}N_5O_8$ 645.2799; found $[M+Na]^+$ 668.5172.



(S)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-3-(1-((S)-3-oxo-2-(((tert-pentyloxy)carbonyl)amino)-3-(((E)-prop-1-en-1-yl)oxy)propyl)-1H-1,2,3-triazol-4-yl)
propanoic acid (4b): 12 (138 mg, 0.21 mmol) was dissolved in TFA/DCM (1:1, 5 mL) and stirred for 2 h at room temperature. The reaction mixture was concentrated *in vacuo* to yield **4b** as a white powder (121.64 mg, 96.3 %) which was used directly in SPPS without further purification.

^{1}H-NMR (300 MHz, CD_3OD): δ7.78 (d, J = 6.0 Hz, 2H), 7.76-7.74 (m, 1H), 7.64-7.62 (m, 2H), 7.38 (t, J = 6.0 Hz, 2H), 7.29 (t, J = 6.0 Hz, 2H), 5.95-5.83 (m, 2H), 5.33 (d, J = 6.0 Hz, 1H), 5.32-5.31 (m, 2H), 5.24-5.22 (m, 1H), 4.83-4.82 (m, 3H), 4.73-4.72 (m, 2H), 4.70-4.64 (m, 2H), 4.63-4.48 (m, 3H), 4.35-4.26 (m, 2H), 4.25-4.20 (m, 1H), 3.32-3.29 (m, 1H).
^{13}C-NMR (300 MHz, CD_3OD): δ 174.42, 174.32, 170.28, 158.45, 158.06, 145.26, 144.81, 144.49, 142.61, 134.08, 133.03, 128.84, 128.22, 126.33, 125.40, 120.97, 119.12, 117.78, 68.10, 67.50, 66.86, 55.61, 55.27, 55.11, 51.50, 28.82.
HR-MS, *m/z* calcd for $C_{30}H_{31}N_5O_8$ 589.2173; found $[M-H]^-$ 588.1905.

2.2 Synthesis and characterization of peptides

2.2.1 General procedures for the Fmoc solid phase peptide synthesis

The amino acid residues were attached to the resin with a single coupling procedure (30 min at 35 ºC). All peptides were synthesized with a scale of 0.10 mmol.

(a) Standard pre-activation of resin protocol: The Rink amide AM resin (0.3 mmol/g loading capacity) was swollen in DCM/DMF mixture solvent for 10 min.

(b) Standard Fmoc-deprotection protocol: After treatment with 20% piperidine/DMF (15 min twice) the resin was washed with DMF (5×), DCM (5×), and DMF (5×).

(c) Standard coupling of natural amino acids protocol: After pre-activation of 4 equiv of Fmoc-protected amino acid in DMF for 5 min using 3.80 equiv of HCTU and 8.00 equiv of DIEA, the solution was added to the resin. After 30 min, the resin was washed with DMF (5×), DCM (5×), and DMF (5×). The coupling reaction was monitored with the ninhydrin test.

(d) Standard coupling of diaminodiacids protocol: After pre-activation of 1.50 equiv of Fmoc-protected diaminodiacid in DMF for 1 min using 2.00 equiv of HATU and 8.00 equiv of DIPEA, the solution was added to the resin. After 2 h, the resin was washed with DMF (5×), DCM (5×), and DMF (5×). The coupling reaction was monitored with the ninhydrin test.

(e) Standard deprotection of Alloc/Allyl protocol: To the peptide resin was

added a solution of $Pd(PPh_3)_4$ (2.00 equiv) and $PhSiH_3$ (10.00 equiv) in DMF/DCM (2.50 mL/2.50 mL) in the presence of argon. The resin was stirred for 3h prevented from light. Then, the resin was washed with DCM (5×), 0.50 % sodium diethyldithiocarbamate in DMF (5×) and DMF (5×).

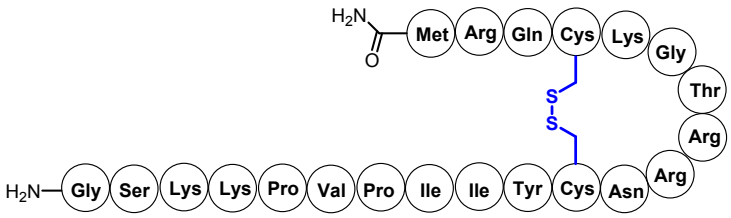
(f) Standard cyclization protocol: After removal of Alloc/Allyl and N-terminus Fmoc successively, a solution of PyAOP (5.00 equiv), HOAt (5.00 equiv) and NMM (10.00 equiv) in NMP was added to the resin. After overnight reaction, the resin was washed with DMF (5×), DCM (5×), and DMF (5×).

(g) Standard capping protocol: Ac_2O/DIEA/DMF (1:1:8) was added to the resin. After mechanically stirring for 15 min, the resin was washed with DMF (5×) and DCM (10×).

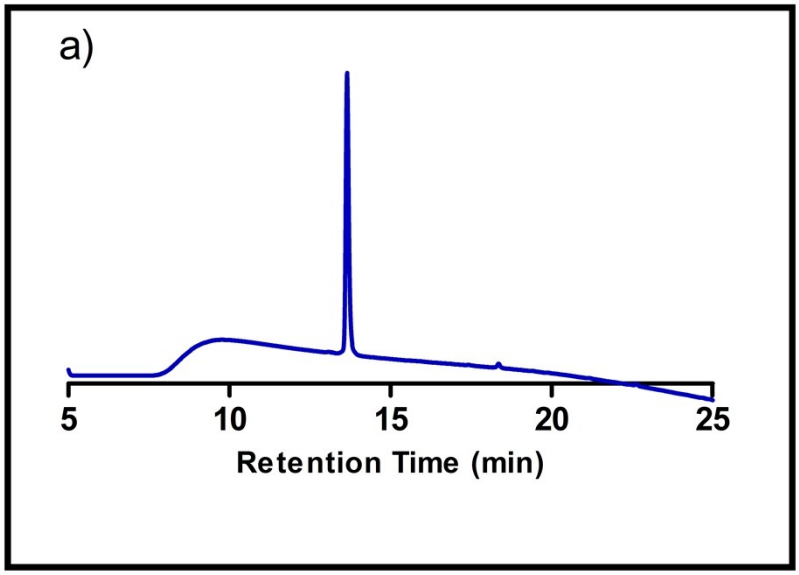
(h) Standard cleavage protocol: The cleavage cocktail (TFA: TIPS: H_2O= 95: 2.5: 2.5, v/v/v) was added to the resin at 35 ºC. After stirring for 2h, the cleavage cocktail was collected. The solution were bubbled with argon for concentration and the chilled diethyl ether was added to precipitate the crude peptides. The peptide suspensions were centrifuged for 3 min at 3000 rpm and then the clear solution was decanted. The step of precipitation, centrifugation and decantation operations was repeated three times. The resulting white residues were dissolved in CH_3CN/H_2O, analyzed and purified by RP-HPLC.

(i) Standard oxidative folding protocol: RP-HPLC purified peptide in the reduced form was dissolved in the oxidation buffer [0.50 mg/mL peptide in 6.00 M guanidine hydrochloride and 100.00 mM sodium dihydrogen phosphate PBS buffer, pH=7.40, with 10.00 % dimethylsulfoxide (DMSO)]. This mixture was allowed to stir for 24 h at room temperature. Then it was analyzed and purified by RP-HPLC.

2.2.2 HPLC traces and MS spectrums of purified peptides



Thanatin (1)



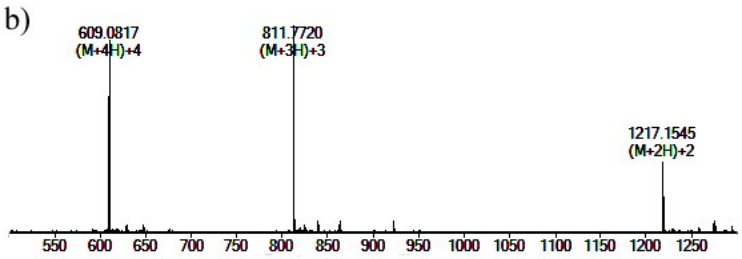
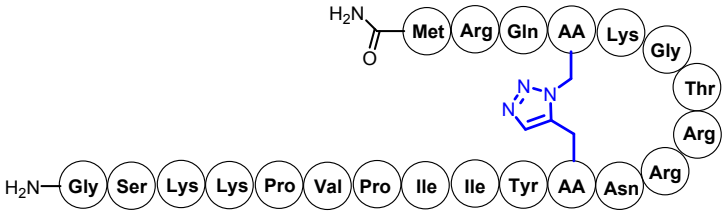
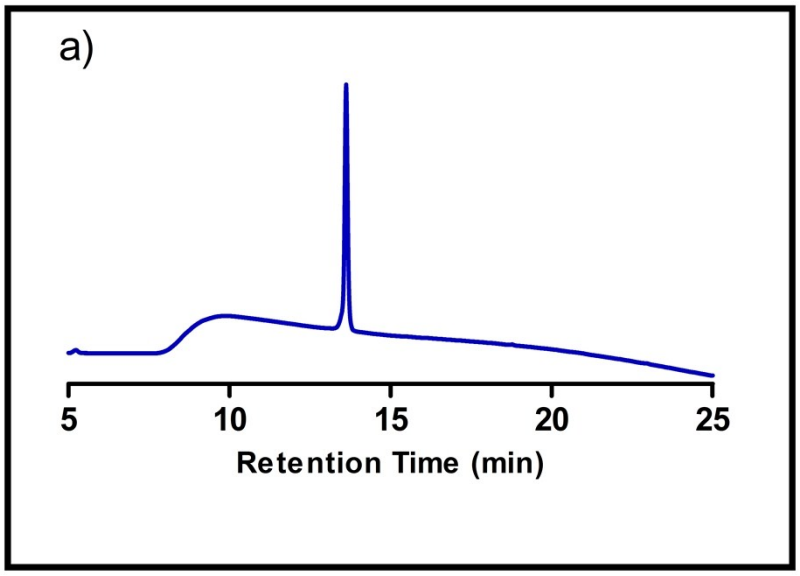


Figure S1. Thanatin (1) was obtained as a white powder (95.71 mg, 39.4 % yield according to initial resin load). a) HPLC trace of purified **Thanatin (1)**. Gradient: 90-0% of buffer B in 25 min with C18 column (5 μm, 4.6×250 mm). b) HR-MS

spectrum of **Thanatin (1)** (calc. for $C_{103}H_{178}N_{36}O_{26}S_3$ 2431.2875, found $[M+2H]^{2+}$ 217.1545, $[M+3H]^{3+}$ 811.7720, $[M+4H]^{4+}$ 609.0817).



2



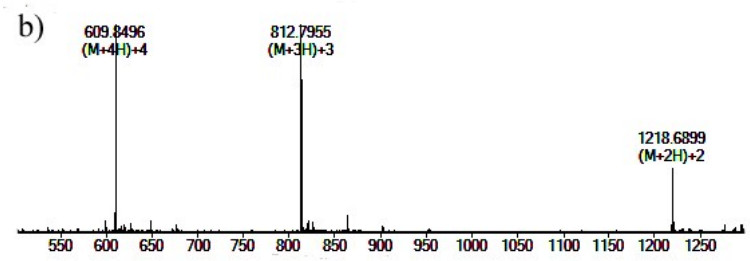
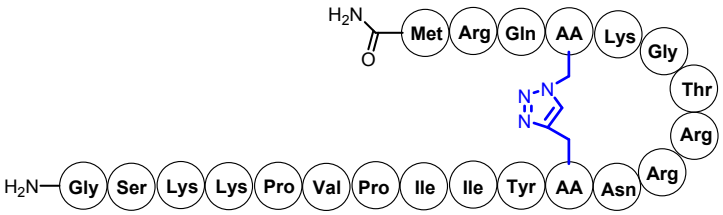
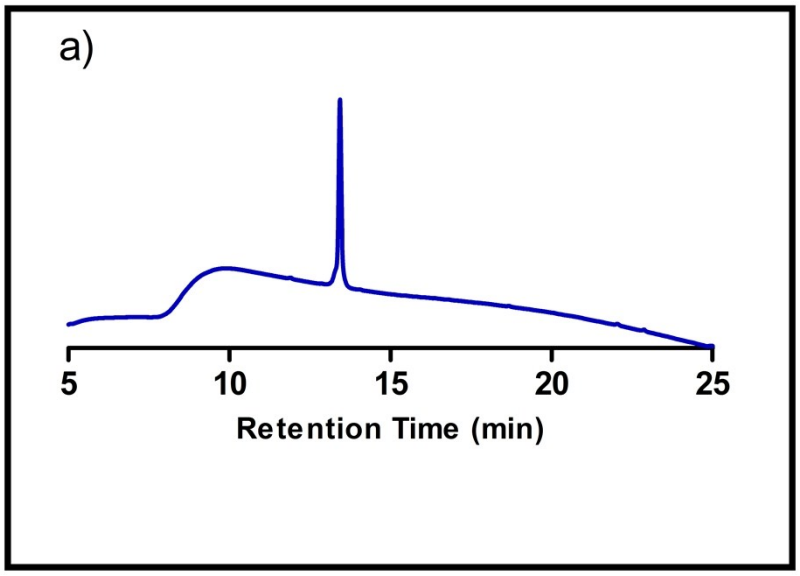


Figure S2. 2 was obtained as a white powder (72.24 mg, 29.7 % yield according to initial resin load). a) HPLC trace of purified **2**. Gradient: 90-0% of buffer B in 25 min with C18 column (5 µm, 4.6×250 mm). b) HR-MS spectrum of **2** (calc. for $C_{105}H_{179}N_{39}O_{26}S$ 2434.3693, found $[M+2H]^{2+}$ 1218.6899, $[M+3H]^{3+}$ 812.7966,

$[M+4H]^{4+}$ 609.8496).



3



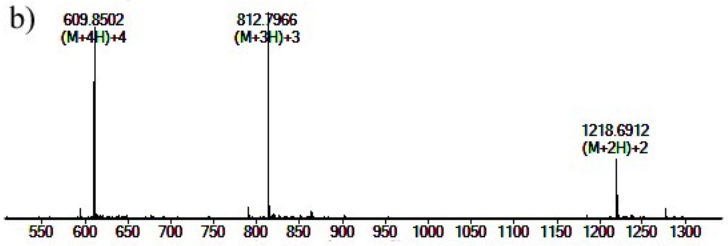


Figure S3. 3 was obtained as a white powder (83.90 mg, 34.5 % yield according to initial resin load). a) HPLC trace of purified **3**. Gradient: 90-0% of buffer B in 25 min with C18 column (5 µm, 4.6×250 mm). b) HR-MS spectrum of **3** (calc. for $C_{105}H_{179}N_{39}O_{26}S$ 2434.3675, found $[M+2H]^{2+}$ 1218.6912, $[M+3H]^{3+}$ 812.7955, $[M+4H]^{4+}$ 609.8502).

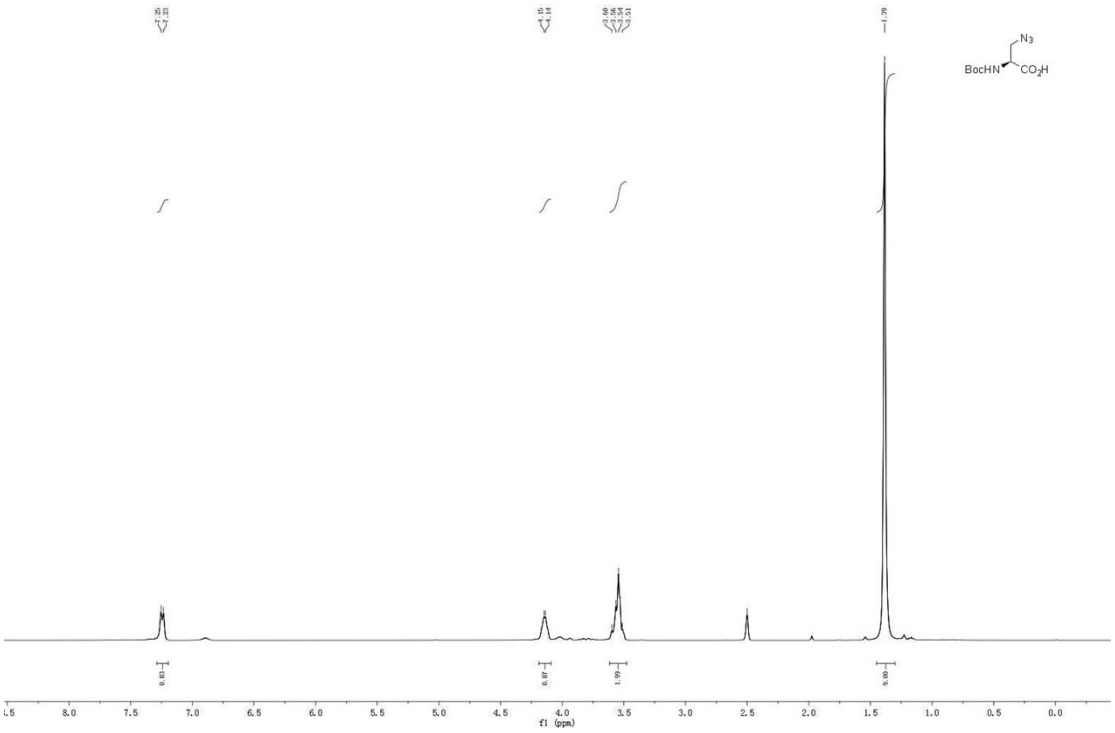
2.3 Determination of minimal inhibitory concentration (MIC)

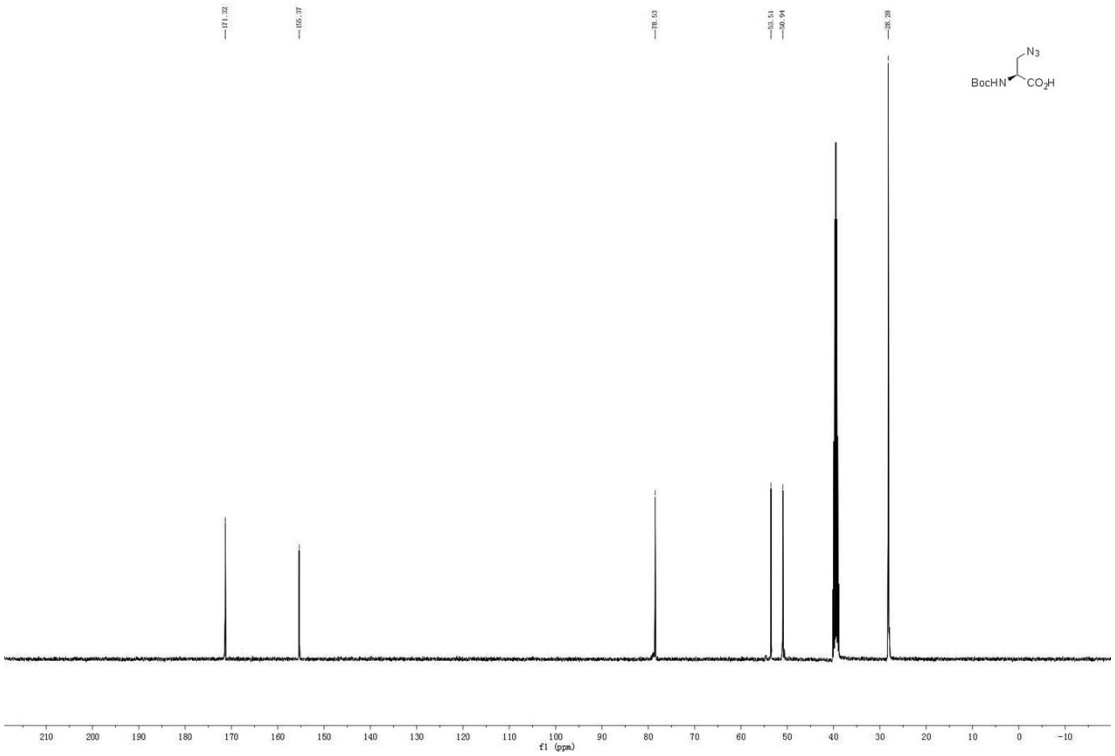
The MIC of the peptides were determined by the method reported previously.[1] Briefly, Gram-negative bacteria (*Escherichia coli, Salmonella typhimurium, klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) were purchased from the American type culture collection (ATCC) and cultured in a nutrient broth at 37 ºC overnight. The cultures were diluted to 1:20 in the same broth, cultured at 37 ºC until absorbance 0.4-0.5 at 650 nm and diluted in a basal medium of 1% bactopeptone to 1:200. 100 uL of the serially 2 fold diluted peptides (0.31 to 20 mM) in 1% bactopeptone were mixed with 50 ml of the above bacterial suspension, then incubated overnight at 37 ºC in a shaking incubator. The MIC was defined as the lowest concentration of the peptide at which there was no growth of bacteria.

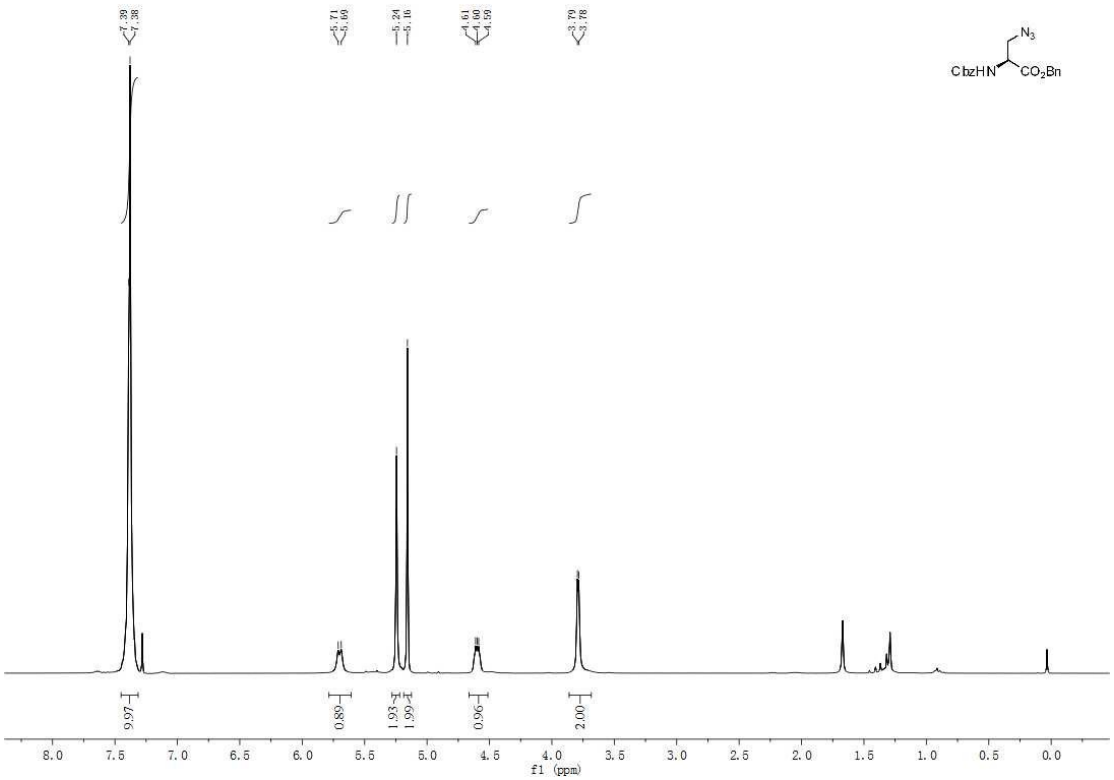
Reference:

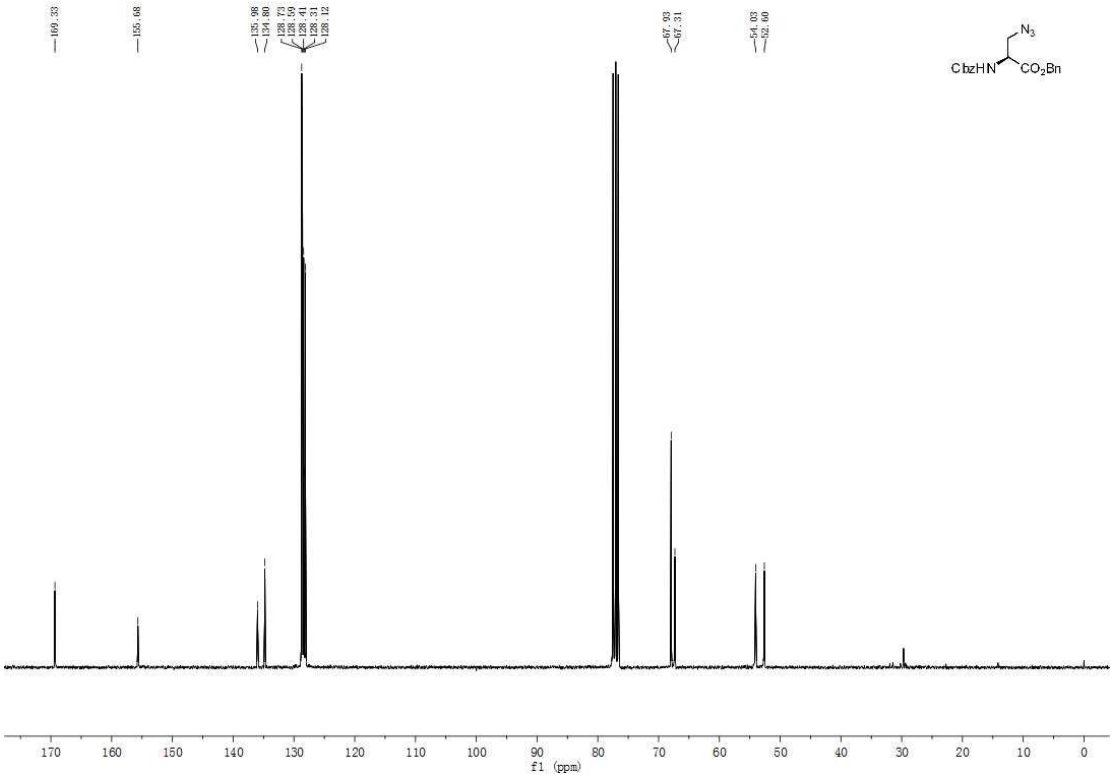
1 M. K. Lee, L. Cha, S. H. Lee and K. S. Hahm, *J. Biochem. Mol. Biol.*, 2002, **35**, 291-296.

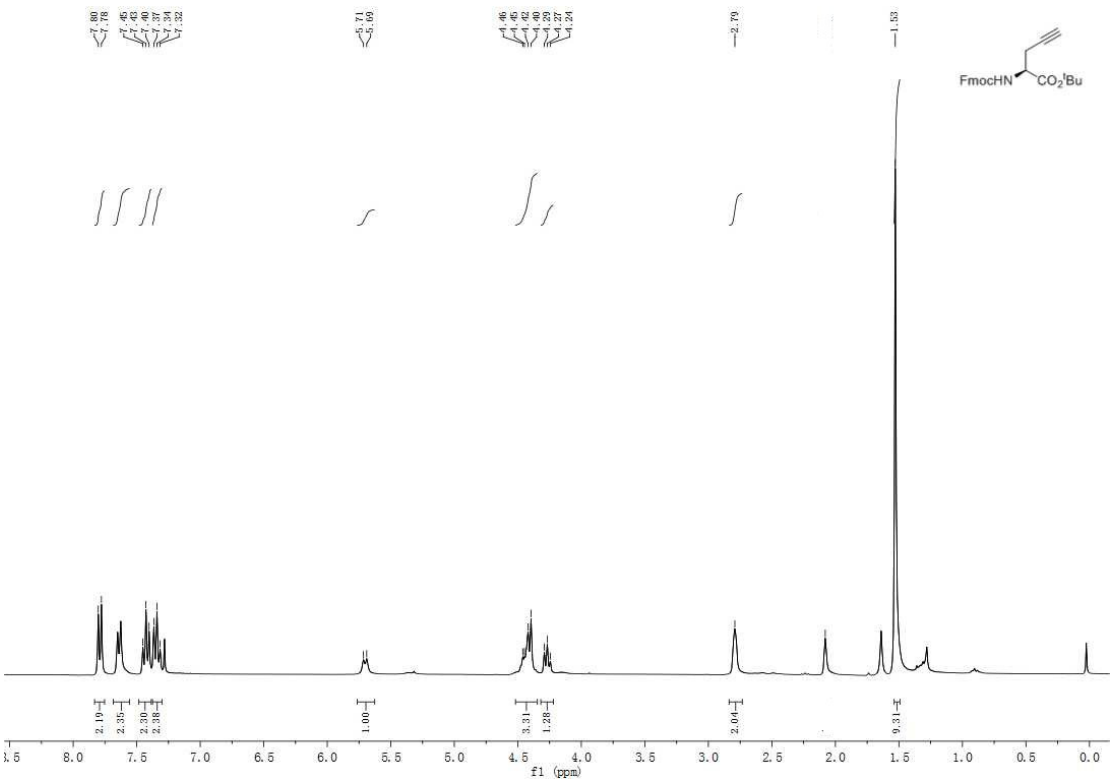
^{1}H and ^{13}C NMR spectra of compounds

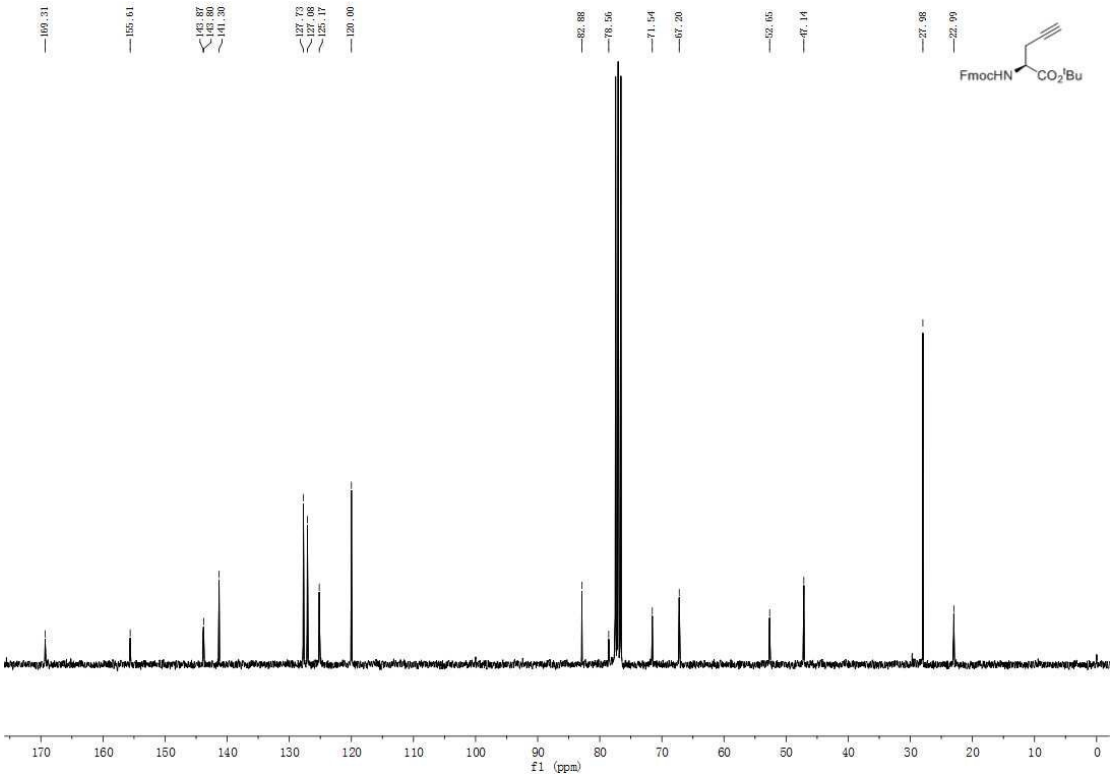


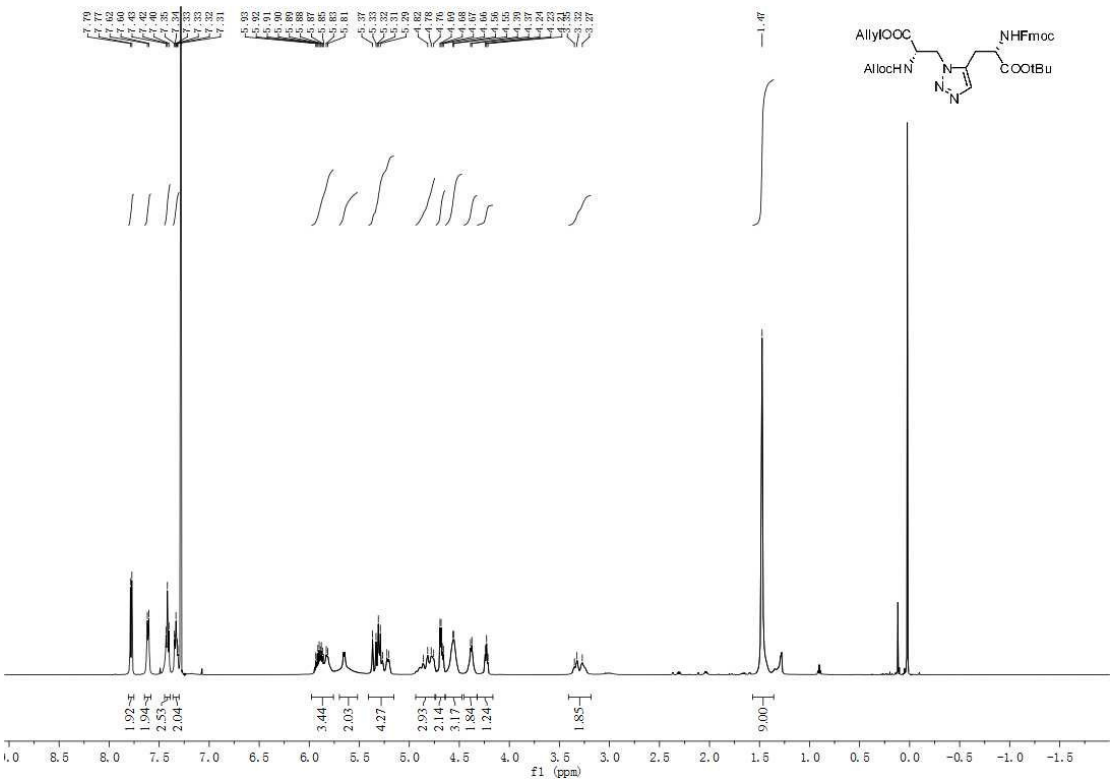


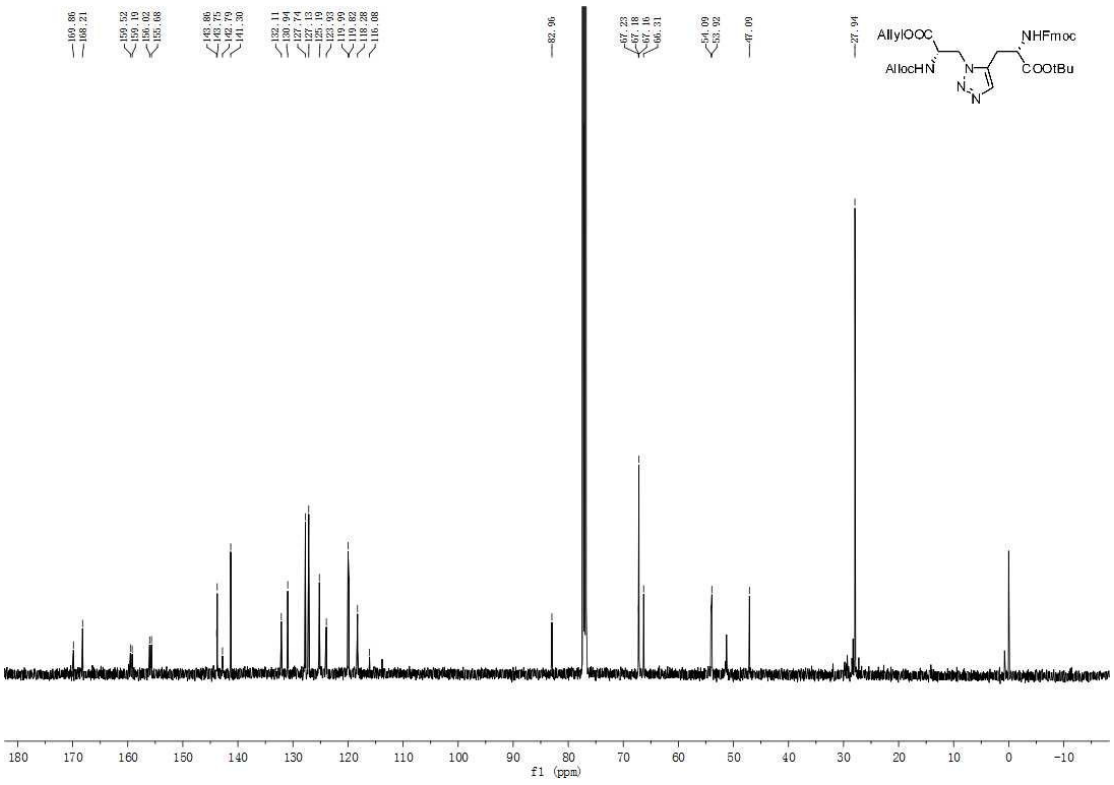

7.39
7.38
5.71
5.69
5.24
5.16
4.61
4.60
4.59
3.79
3.78
N3
CbzHN
CO2Bn
9.97
0.89
1.93
1.99
0.96
2.00
f1 (ppm)

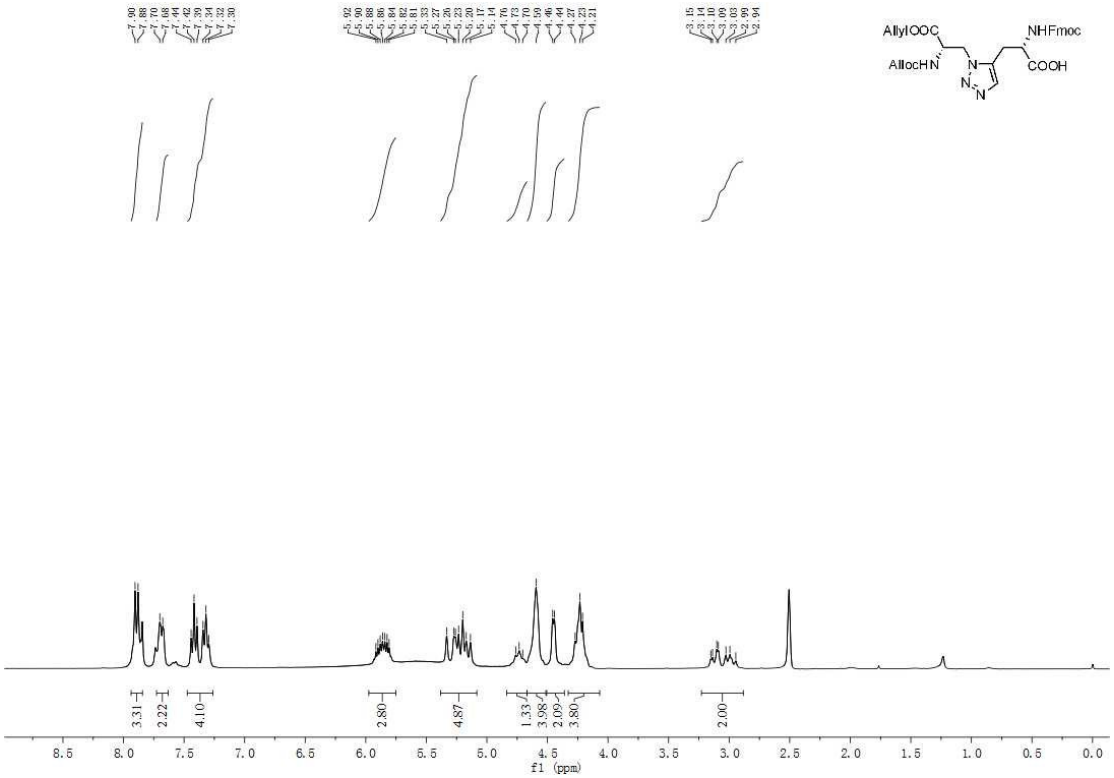

169.33
155.68
135.98
134.80
128.73
128.59
128.41
128.31
128.12
67.93
67.31
54.03
52.60
N3
CbzHN
CO2Bn
f1 (ppm)

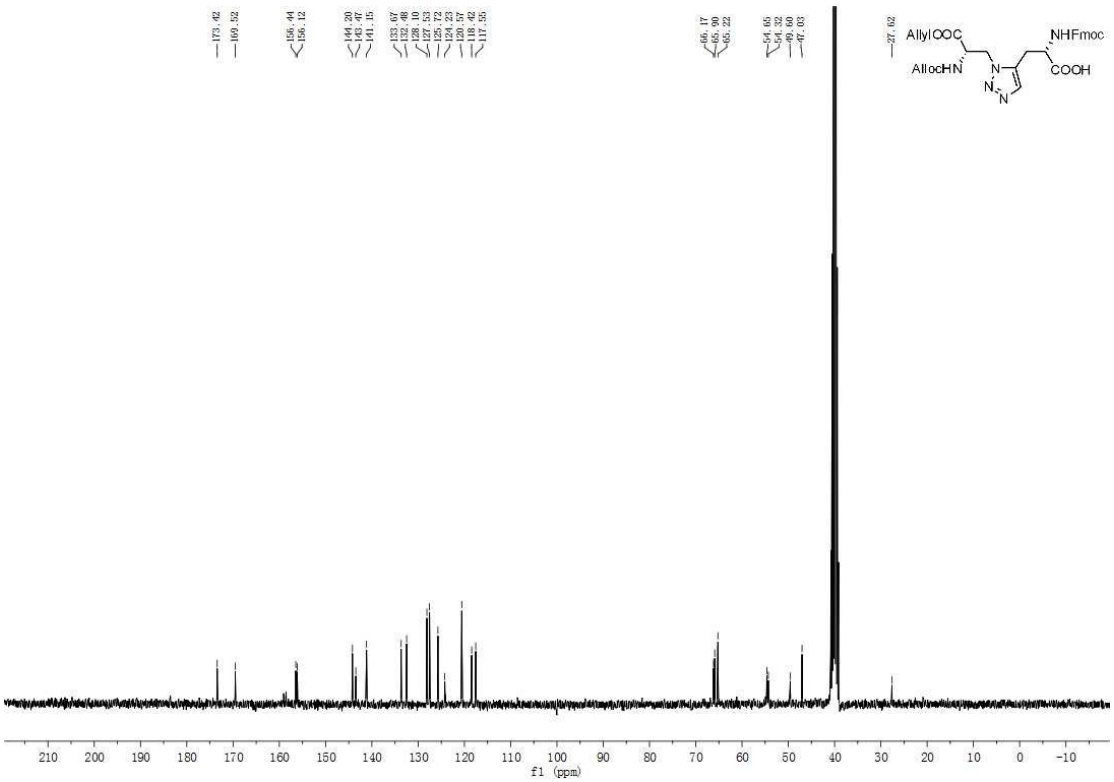

7.80
7.78
7.45
7.43
7.40
7.37
7.34
7.32
5.71
5.69
4.46
4.45
4.42
4.40
4.29
4.27
4.24
2.79
1.53
FmocHN
CO2tBu
2.19
2.35
2.30
2.38
1.00
3.31
1.28
2.04
9.31
f1 (ppm)

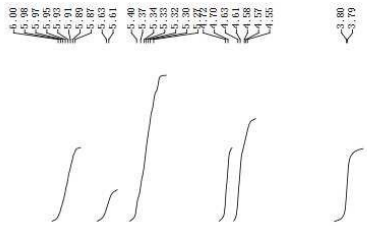

169.31
155.61
143.87
143.80
141.30
127.73
127.08
125.17
120.00
82.88
78.56
71.54
67.20
52.65
47.14
27.98
22.99
FmocHN
CO2tBu
f1 (ppm)

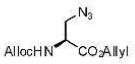

AllylOOC
AllocHN
NHFmoc
COOtBu
f1 (ppm)

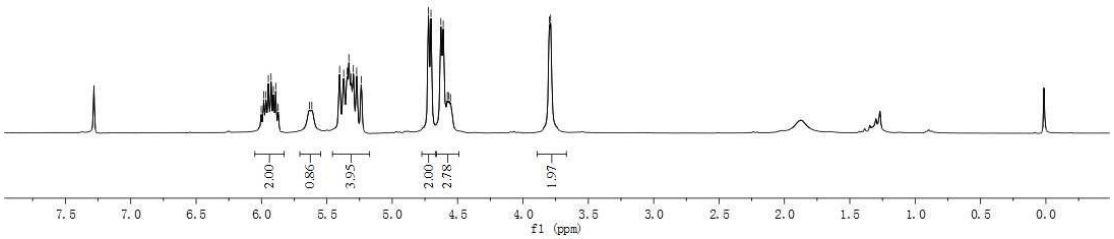

AllylOOC
AllocHN
NHFmoc
COOtBu
f1 (ppm)

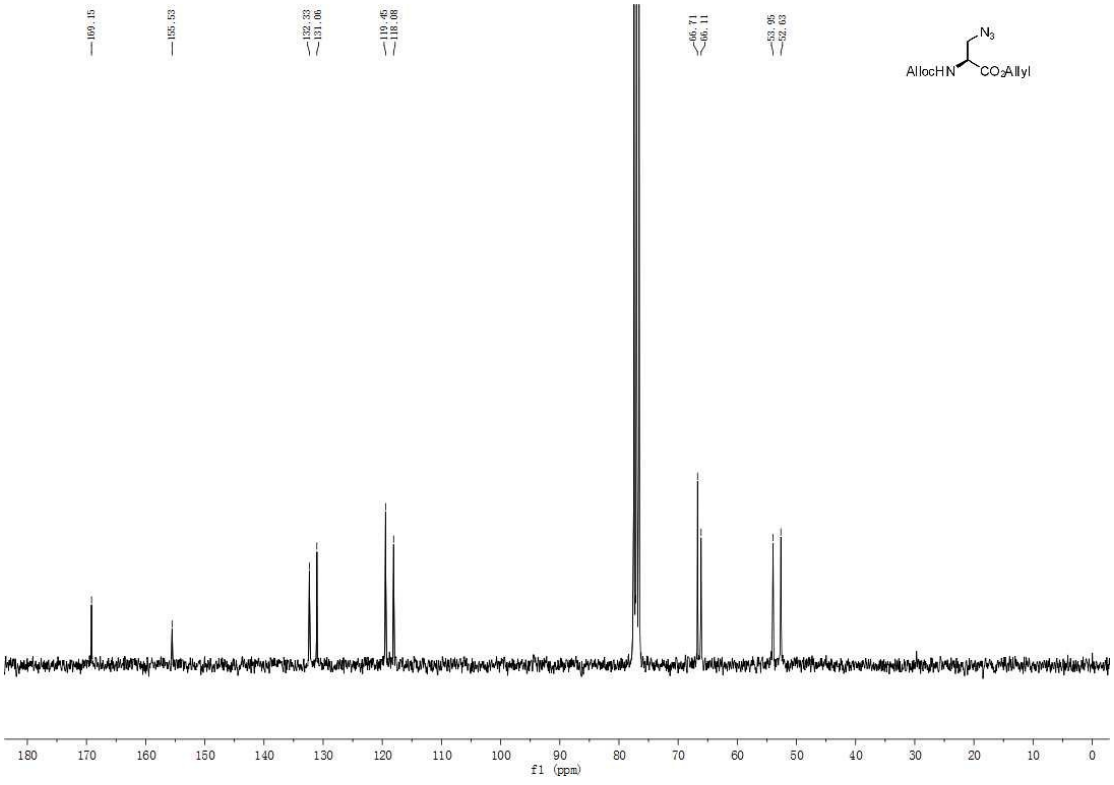

AllylOOC
AllocHN
NHFmoc
COOH
3.31
2.22
4.10
2.80
4.87
1.33
3.98
2.09
3.80
2.00
f1 (ppm)


AllylOOC
AllocHN
NHFmoc
COOH
f1 (ppm)

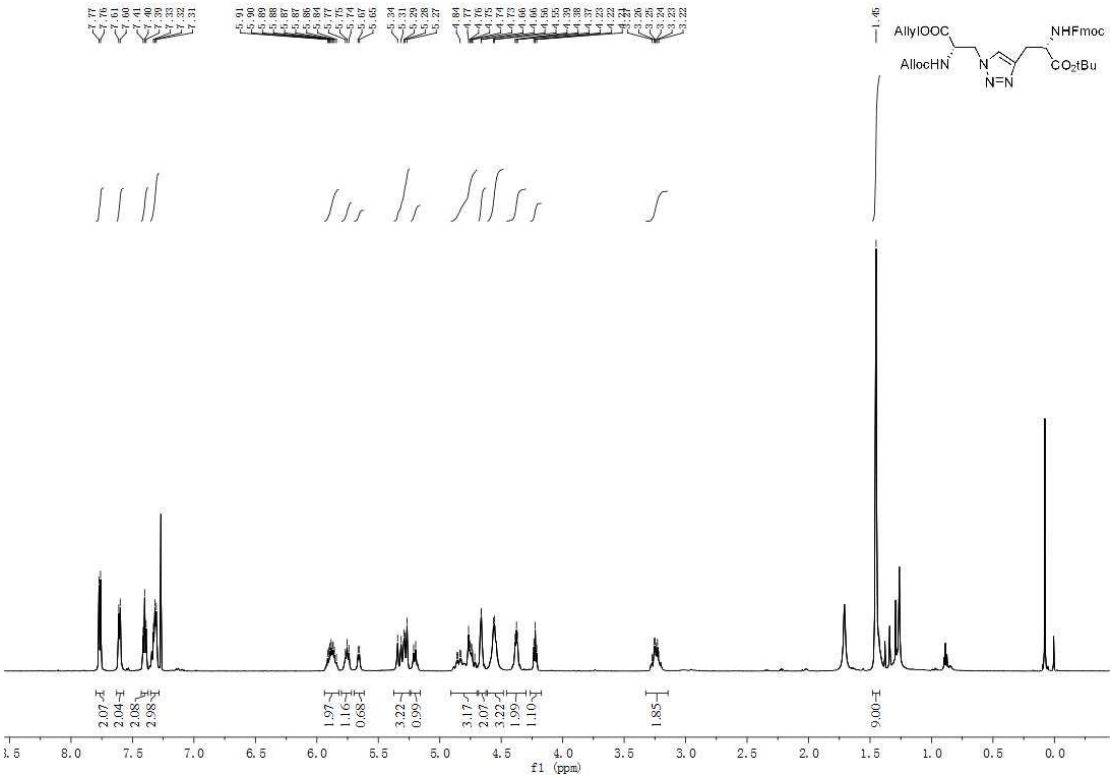

6.00
5.98
5.97
5.95
5.93
5.91
5.89
5.87
5.65
5.61
5.40
5.37
5.34
5.33
5.32
5.30
5.27
5.22
5.20
4.63
4.61
4.58
4.57
4.55
3.80
3.79

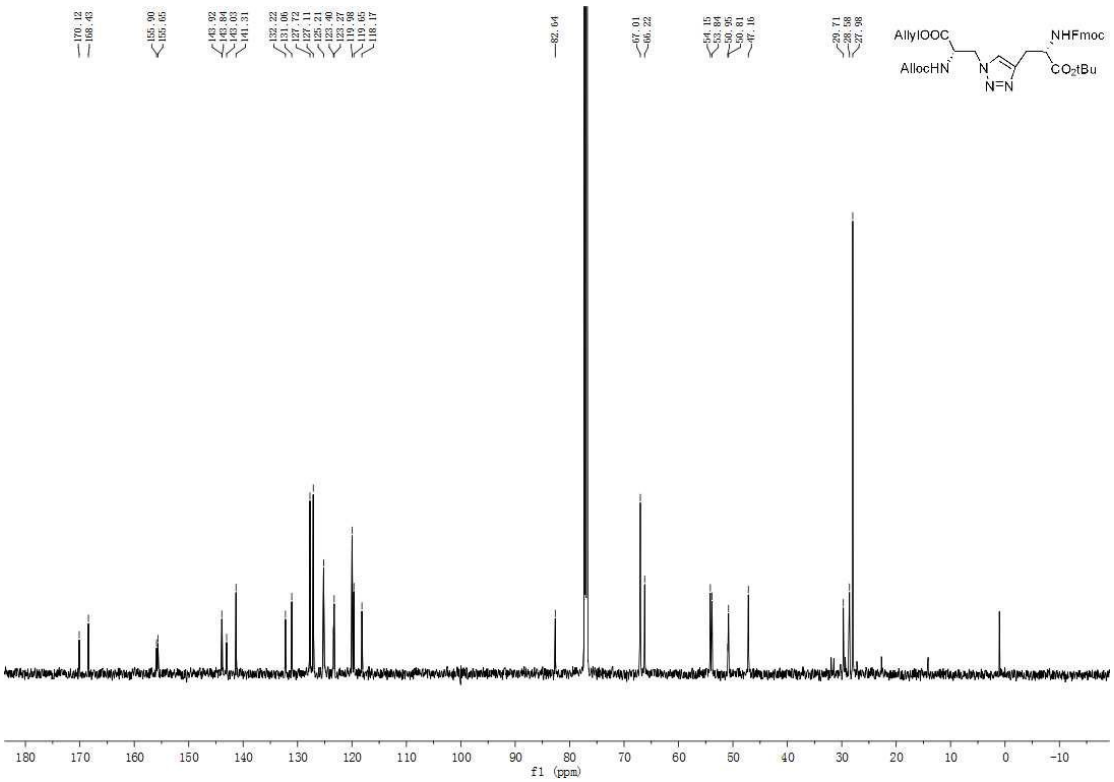

N3
AllocHN
CO2Allyl

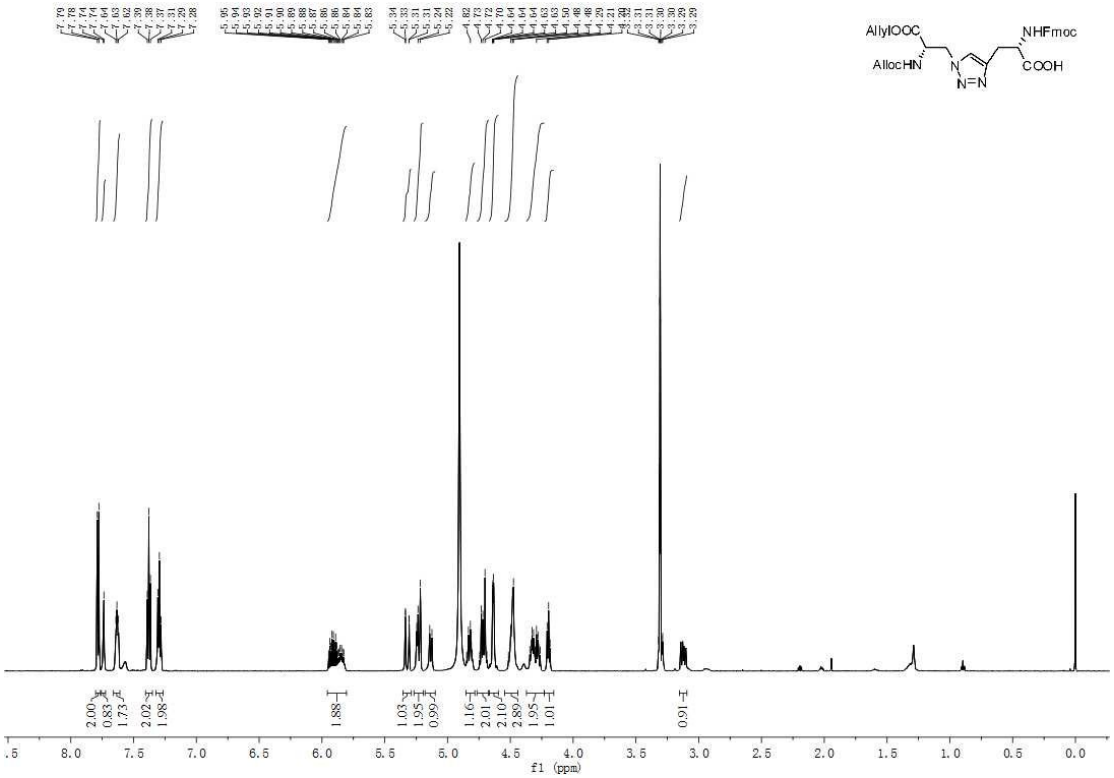

2.00
0.86
3.95
2.00
2.78
1.97
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

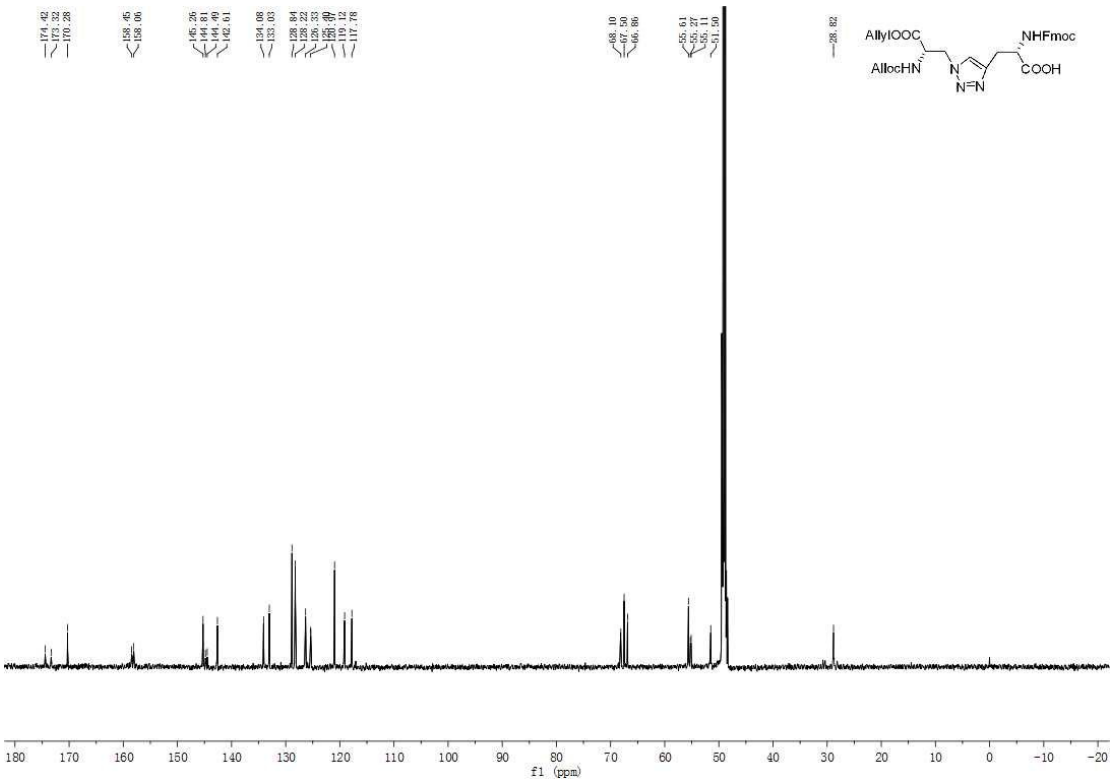

169.15
155.53
132.33
131.06
119.45
118.08
66.71
66.11
53.95
52.63
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

N3
AllocHN
CO2Allyl


AllylOOC
NHFmoc
AllocHN
CO2tBu
N=N
f1 (ppm)


AllylOOC
NHFmoc
AllocHN
CO2tBu
N=N
f1 (ppm)


AllylOOC
AllocHN
NHFmoc
COOH
N=N
f1 (ppm)


AllylOOC
AllocHN
NHFmoc
COOH
N=N
f1 (ppm)