Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.


# Supplementary Information

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# 1. General information

## 1.1 Materials and Reagents

Rink amide AM resin, Fmoc amino acids, PyAOP, HATU, HCTU, HOAT, N,N'-Diisopropyl-carbodiimide (DIC), 4-Methylmorpholine (NMM), DIEA, Ethyl cyanoglyoxylate-2-oxime (Oxyma), trifluoroacetic acid (TFA), Dichloromethane (DCM), dimethylformamide (DMF), Triisopropylsilane, Piperidine and anhydrous diethyl ether ($Et_2O$), Acetonitrile (HPLC grade) were purchased as described in the previous work.[1] Thin-layer chromatography (TLC) and Flash column chromatography were performed as previously described.[2]

## 1.2 HPLC

Semi-preparative HPLC and Analytical HPLC were performed as previous work.[3]

## 1.3 Mass spectrometry and NMR

$^1H$ NMR spectra, $^{13}C$-NMR spectra and ESI-MS spectra, were recorded as previous work description.[2]

# 2. Synthesis of amide-bonded diaminodiacid 1.

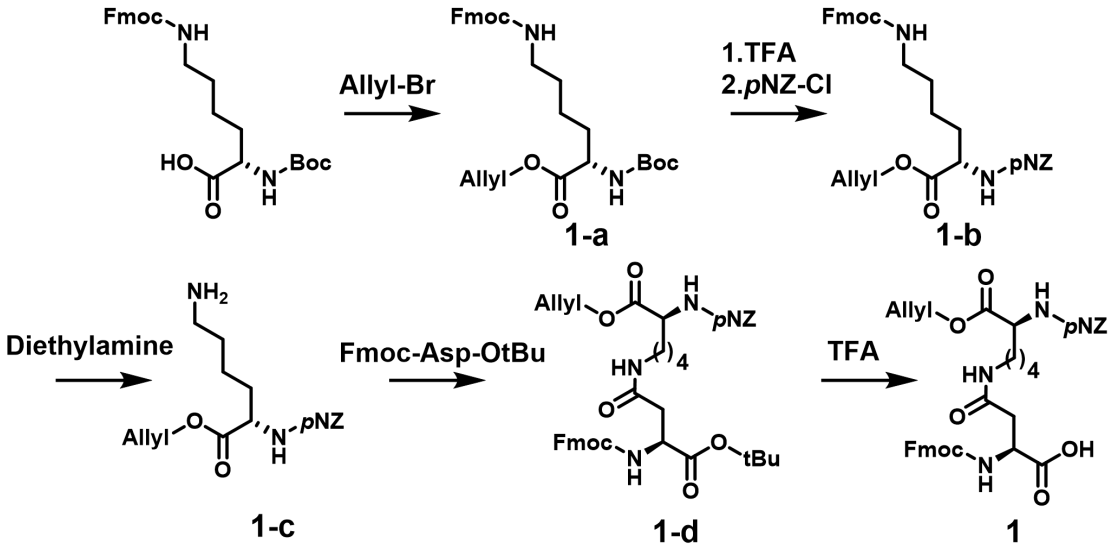



**Figure S1**: Synthetic route to amide-bonded diaminodiacid **1**.

## 2.1 Synthesis of compound 1-a：

Boc-Lys(Fmoc)-OH (1 g, 2.13 mmol) and $NaHCO_3$ (0.43 g, 5.12 mmol) were dissolved in 30 mL DMF, followed by addition of allyl bromide (444 μL, 5.12 mmol). The reaction mixture was

stirred at room temperature overnight. Followed by diluted with water and extracted with EtOAc, the combined organic phase was washed with water, dried over $Na_2SO_4$, filtrated and concentrated in vacuo. The crude product was purified by chromatography to afford compound **1-a**. (0.680 g, 1.34 mmol, 63%). Rf = 0.42 (4: 1, petroleum ether/EtOAc). $^1$H NMR (400 MHz, $CDCl_3$) δ 7.77 (dd, *J* = 7.5, 1.1 Hz, 2H), 7.59 (d, *J* = 7.5 Hz, 2H), 7.43 – 7.38 (m, 2H), 7.32 (td, *J* = 7.4, 1.2 Hz, 2H), 5.90 (ddt, *J* = 16.5, 10.4, 5.8 Hz, 1H), 5.36 – 5.23 (m, 2H), 5.08 (d, *J* = 8.5 Hz, 1H), 4.84 (s, 1H), 4.69 – 4.57 (m, 2H), 4.50 – 4.28 (m, 3H), 4.21 (t, *J* = 6.9 Hz, 1H), 3.19 (q, *J* = 6.6 Hz, 2H), 2.25 – 1.78 (m, 2H), 1.60 (dq, *J* = 47.7, 6.6 Hz, 4H), 1.44 (s, 9H).

## 2.2 Synthesis of compound 1-b：

Compound **1-a** (0.58 g, 1.28 mmol) was dissolved in DCM (6 mL) and trifluoroacetic acid (6 mL) and stirred for 0.5 h at room temperature. The mixture is then used for the next step without further purification after the trifluoroacetic acid was removed. The residue was dissolved in 6.25 mL EtOAc and 5 mL saturated $NaHCO_3$ aqueous solution, followed by PNZ-Cl (368 mg, 1.7 mmol) added and stirred overnight at room temperature. After completion of the reaction, the mixture was diluted with water and extracted with EtOAc for three times. The combined organic phase was dried over with $Na_2SO_4$, filtered and concentrated in vacuo. The crude product was purified by flash column chromatography to afford compound **1-b**. (0.45 g, 0.765 mmol, 59%). Rf = 0.33 (1: 1, petroleum ether/EtOAc). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, *J* = 8.6 Hz, 2H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.57 (d, *J* = 7.4 Hz, 2H), 7.45 – 7.35 (m, 4H), 7.30 (t, *J* = 7.4 Hz, 2H), 5.90 (ddt, *J* = 16.4, 11.3, 5.8 Hz, 1H), 5.55 (d, *J* = 8.5 Hz, 1H), 5.37 – 5.09 (m, 4H), 4.85 (t, *J* = 6.0 Hz, 1H), 4.64 (d, *J* = 5.1 Hz, 2H), 4.45 – 4.32 (m, 3H), 4.20 (t, *J* = 6.9 Hz, 1H), 3.27 – 3.12 (m, 2H), 1.93 – 1.69 (m, 2H), 1.54 (dt, *J* = 14.1, 7.1 Hz, 2H), 1.44 – 1.36 (m, 2H).

## 2.3 Synthesis of compound 1-c：

Compound **1-b** (0.15 g, 0.26 mmol) was stirred in a solution of diethylamine/DCM (2 mL, v: v, 1: 1) at room temperature for 2 h. The residue was evaporated to dryness in vacuo and used for the next step without purification.

## 2.4 Synthesis of compound 1-d：

Fmoc-Asp-OtBu (0.186 g, 0.45 mmol) was dissolved in 2 mL DMF, followed by the addition of HATU (0.187 g, 0.49 mmol), HOAT (0.067 g, 0.49 mmol), and DIEA (163 μL, 0.99 mmol) to the solution. The solution was shaken for 1 minute, the compound **1-c** (0.15 g, 0.41 mmol) was

added to the solution. The reaction mixture was stirred at room temperature for 2 h, monitored by TLC. After diluting with water and extracting with EtOAc, the combined organic phase was washed with water, dried over $Na_2SO_4$, filtrated and concentrated in vacuo. The crude product was purified by chromatography to give compound **1-d**. (0.13 g, 0.166 mmol, 41%). Rf = 0.33 (1: 2, petroleum ether/EtOAc). $^1$H NMR (400 MHz, $CDCl_3$) δ 8.11 (d, *J* = 8.5 Hz, 2H), 7.68 (d, *J* = 7.4 Hz, 2H), 7.52 (dd, *J* = 7.1, 2.9 Hz, 2H), 7.41 (d, *J* = 8.3 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 7.22 (t, *J* = 7.5 Hz, 2H), 6.04 (d, *J* = 7.7 Hz, 1H), 5.81 (dq, *J* = 16.7, 11.0, 8.2 Hz, 2H), 5.52 (d, *J* = 7.9 Hz, 1H), 5.28 – 5.18 (m, 2H), 5.17 – 5.07 (m, 2H), 4.56 (d, *J* = 5.3 Hz, 2H), 4.45 – 4.35 (m, 1H), 4.35 – 4.19 (m, 3H), 4.15 – 4.11 (m, 1H), 3.18 – 3.11 (m, 2H), 2.82 – 2.53 (m, 2H), 2.01 – 1.86 (m, 2H), 1.78 (s, 2H), 1.59 (dq, *J* = 22.1, 6.9, 6.2 Hz, 2H), 1.39 (s, 9H). $^{13}$C NMR (101 MHz, $CDCl_3$) δ 174.68, 170.90, 168.92, 155.20, 154.59, 146.53, 142.83, 140.21, 130.37, 127.03, 126.69, 126.04, 124.16, 122.71, 118.96, 118.07, 81.41, 66.12, 65.10, 64.38, 52.71, 50.48, 46.05, 37.98, 37.03, 28.67, 28.29, 26.88, 21.66. HRMS calcd for $C_{40}H_{36}N_4O_{11}$ 758.31631, found $[M+Na]^+$ 781.30499.

## 2.5 Synthesis of compound 1:

Compound **1-d** (0.06 g, 0.077 mmol) was dissolved in a solution containing DCM and TFA (v: v, 1: 1) and reacted overnight. After subsequent removal of trifluoroacetic acid, the mixture was purified by chromatography to give compound **1**. Rf = 0.32(10: 1, DCM/$CH_3OH$). $^1$H NMR (400 MHz, DMSO) δ 8.24 (d, *J* = 8.7 Hz, 2H), 8.08 (q, *J* = 5.8 Hz, 1H), 7.96 (d, *J* = 7.7 Hz, 1H), 7.88 (d, *J* = 7.6 Hz, 2H), 7.70 (d, *J* = 6.1 Hz, 2H), 7.61 (d, *J* = 8.7 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 7.23 (d, *J* = 6.0 Hz, 1H), 5.93 – 5.82 (m, 1H), 5.36 – 5.26 (m, 2H), 5.23 – 5.12 (m, 3H), 4.57 (d, *J* = 4.4 Hz, 2H), 4.27 – 4.12 (m, 4H), 4.03 (td, *J* = 9.2, 5.0 Hz, 1H), 3.02 (d, *J* = 5.6 Hz, 2H), 2.05 – 1.93 (m, 2H), 1.76 – 1.58 (m, 2H), 1.47 (q, *J* = 7.0 Hz, 2H), 1.40 (d, *J* = 2.8 Hz, 2H). $^{13}$C NMR (101 MHz, DMSO) δ 174.76, 172.49, 170.05, 156.36, 147.39, 145.40, 144.32, 141.14, 132.84, 128.58, 128.06, 127.53, 125.72, 123.99, 120.55, 118.15, 66.05, 65.24, 64.75, 54.49, 47.08, 32.00, 31.75, 29.51, 29.05, 25.58, 22.57. HRMS calcd for $C_{36}H_{38}N_4O_{11}$ 725.24293, found $[M+Na]^+$ 725.24202.

# 3. Solid-phase peptide synthesis of peptides

## 3.1 Fmoc-based solid-phase peptide synthesis

Peptide synthesis began with swelling of the Rink Amide AM resin in DMF/DCM (1: 1, v/v) for 30 min. Amino acid coupling and Fmoc deprotection were included in each coupling cycle. Before coupling to the resin, 10 eq of Fmoc-protected amino acid was pre-activated with 10 eq Oxyma and 20 eq DIC for 30s, single coupling was performed at 75°C for 20 min, except for Fmoc-His(Trt)-OH and Fmoc-Arg(Pbf)-OH, which were pre-activated with 5 eq coupling reagent (HCTU) and 10 eq DIEA in DMF for 30s and then coupled to the resin at 37°C for 30 min. Fmoc protecting group was removed by 20% piperidine in DMF (5 min). After every reaction, the resin was washed by DMF (3 times), DCM (3 times) and DMF (3 times). After finishing the assembly of peptides, the resin was washed by DCM and treated with 8 mL cleavage cocktails (TFA: phenol: $H_2O$: Triisopropylsilane, 85/5/5/5, v/v/v/v) for 2 h. The TFA was blown by $N_2$ and then cold $Et_2O$ was added. The crude peptides were purified by semi-preparative HPLC and analyzed by ESI-MS.

## 3.2 Synthesis and characterization of cyclo [Lys9, Asp13] KIIIA7-14 containing amide-bonded DADA

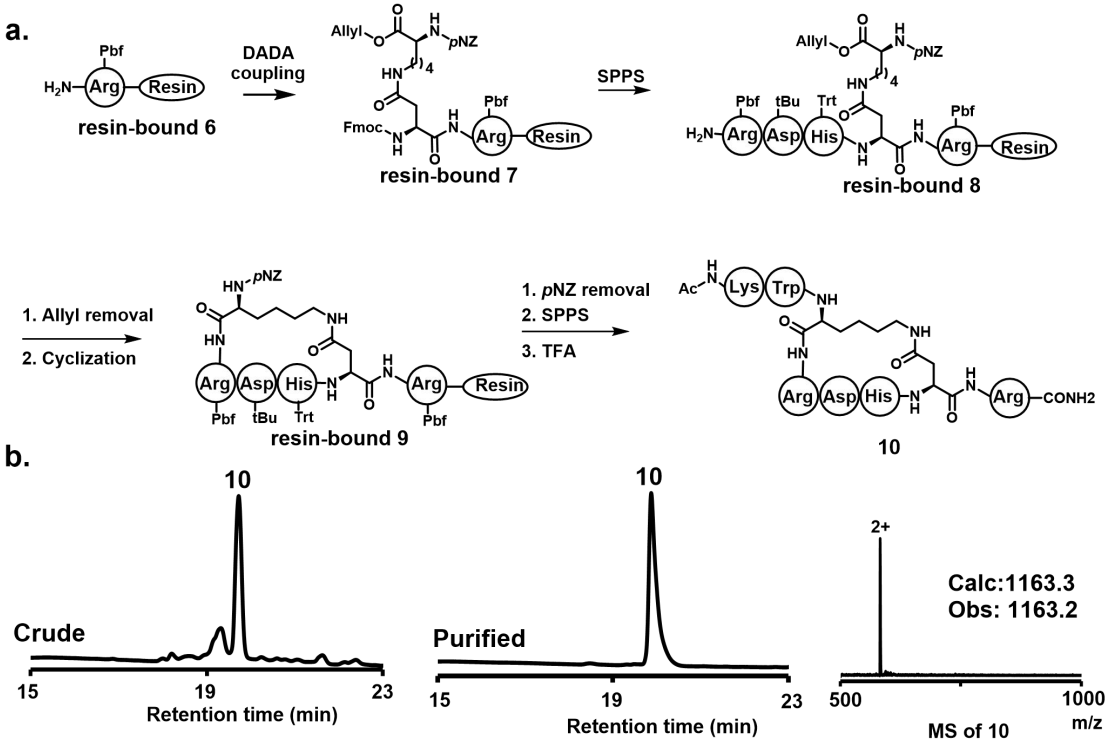


**Figure S2**: (a) Synthesis route of cyclo [Lys9, Asp13] KIIIA7-14 using amide-bonded **DADA**. (b) RP-HPLC and ESI-MS of cyclo [Lys9, Asp13] KIIIA7-14.

0.02 mmol Rink amide AM resin (63 mg, 0.32 mmol/g) was used for the synthesis of cyclo [Lys9, Asp13] KIIIA7-14. At first, Fmoc-Arg(Pbf)-OH was coupling to the resin according to standard Fmoc-based solid-phase peptide synthesis to give resin-bound **6**. Then, pre-activated **DADA** (2 equiv.) was coupled to the resin by PyAOP/HOAT/NMM. After shaking overnight to give resin-bound **7**, Fmoc-His(Trt)-OH, Fmoc-Asp(tBu)-OH and Fmoc-Arg(Pbf)-OH were coupled to the resin in sequence to give resin-bound **8.** Then the allyl group on the **DADA** moiety of resin-bound **8** was removed using $Pd(PPh_3)_4/PhSiH_3$, and the resulting carboxyl group was efficiently cyclized with the *N*-terminal amino group of Arg(Pbf) by PyAOP/HOAT/NMM to give resin-bound **9.** Next, the PNZ group was efficiently removed using a solution of 4M HCl in 1,4-dioxane (6.5 μL) and $SnCl_2$ (4.55 g) in 5 mL DMF for 2 h. Then Fmoc-Trp(Boc)-OH and Ac-Lys(Boc)-OH were sequentially coupled to the resin to give compound **10.** The resin was treated with a mixture of TFA/phenol/water/Triisopropylsilane (85/5/5/5, v/v/v/v, 4 mL) for 2h. The crude peptide was purified by semi-preparative RP-HPLC (a linear gradient from 5% to 80% acetonitrile, 30 min, 4 mL/min) and lyophilized to give 6 mg purified **10** (13% isolated yield).

### 3.3 Resin-bound 7 incubation with 20% piperidine

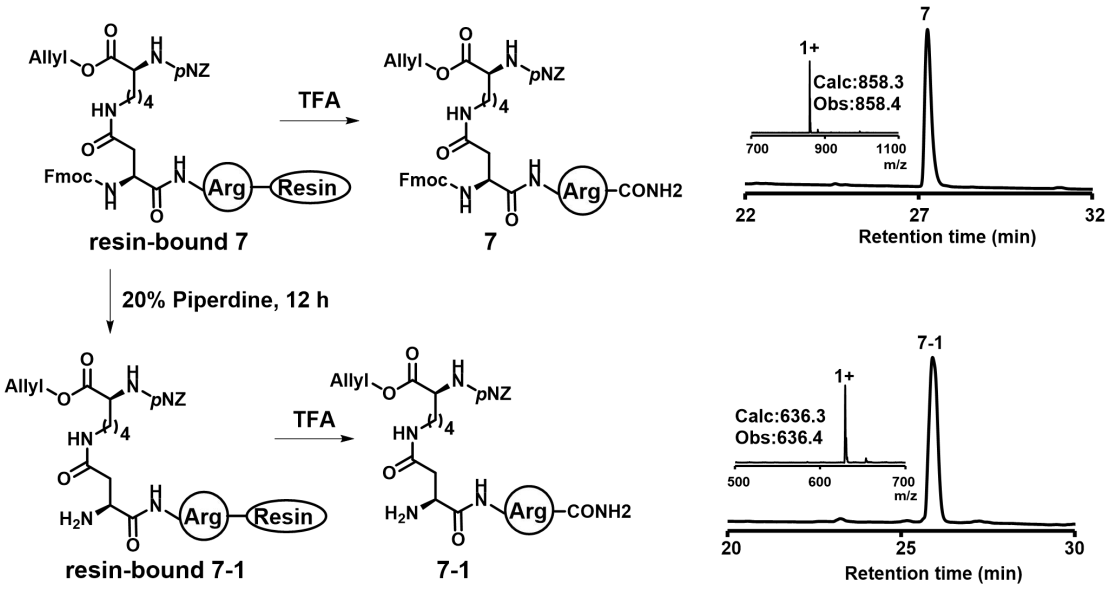



**Figure S3**: Resin-bound **7** incubated with 20% piperidine for 12 h. RP-HPLC and ESI-MS of **7** and **7-1**.

### 3.4 Synthesis and characterization of 1Y containing amide-bonded DADA

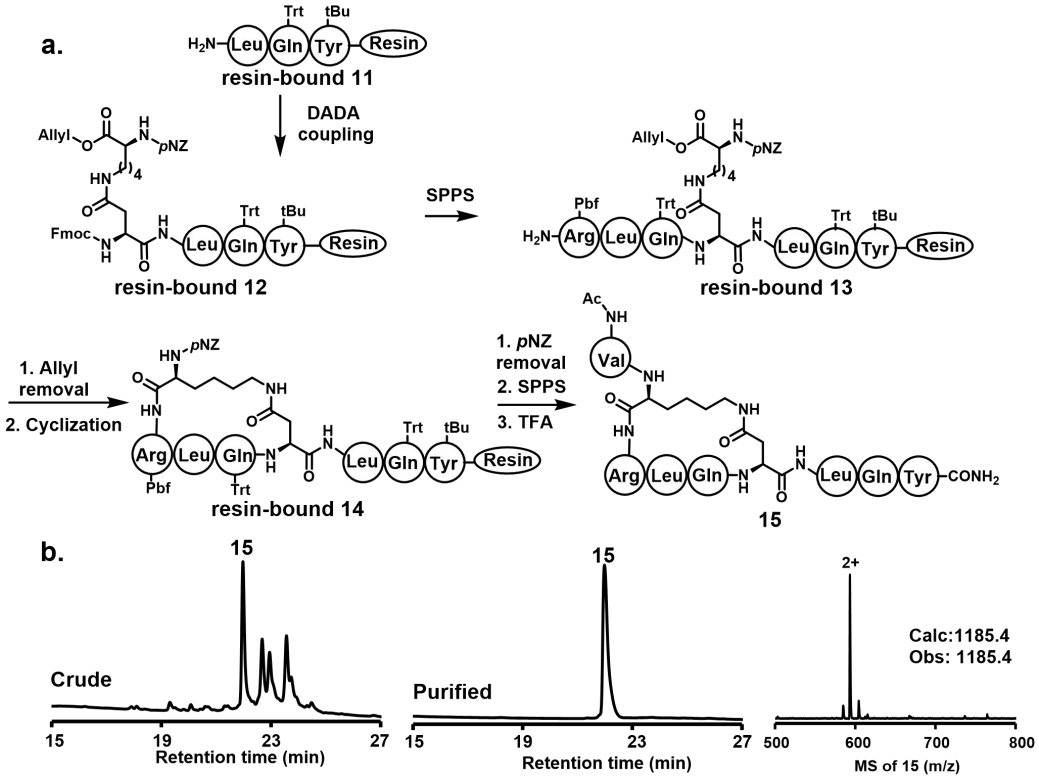



**Figure S4**: (a) Synthesis route of 1Y using amide-bonded **DADA**. (b) RP-HPLC and ESI-MS of 1Y.

The synthetic route for 1Y follows a similar approach to that of cyclo [Lys9, Asp13] KIIIA7-14. The crude peptide was purified by semi-preparative RP-HPLC (a linear gradient from 10% to 80% acetonitrile, 30 min, 4 mL/min), which was then lyophilized to yield 2.88 mg of purified **15** with a 10% isolated yield.

### 3.5 Resin-bound 12 incubation with 20% piperidine

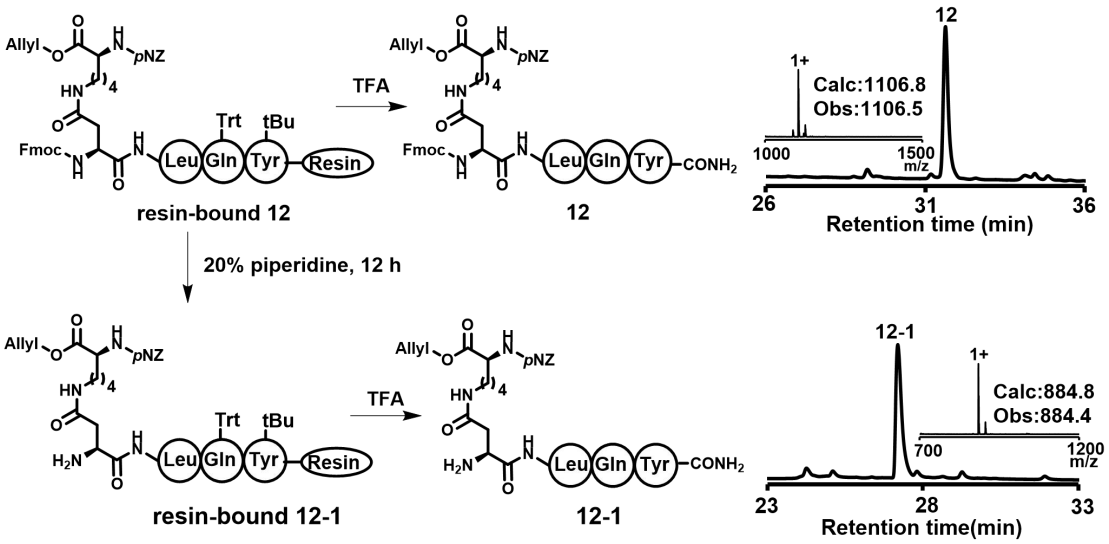



**Figure S5**: Resin-bound **12** was incubated in the same way in 20% piperidine as resin-bound **7**. RP-HPLC and ESI-MS of **12** and **12-1**.

### 3.6 The synthesis of cyclic peptide cyclo [Lys9, Asp 13] KIIIA7-14 using Fmoc-Asp(OAll)-OH:

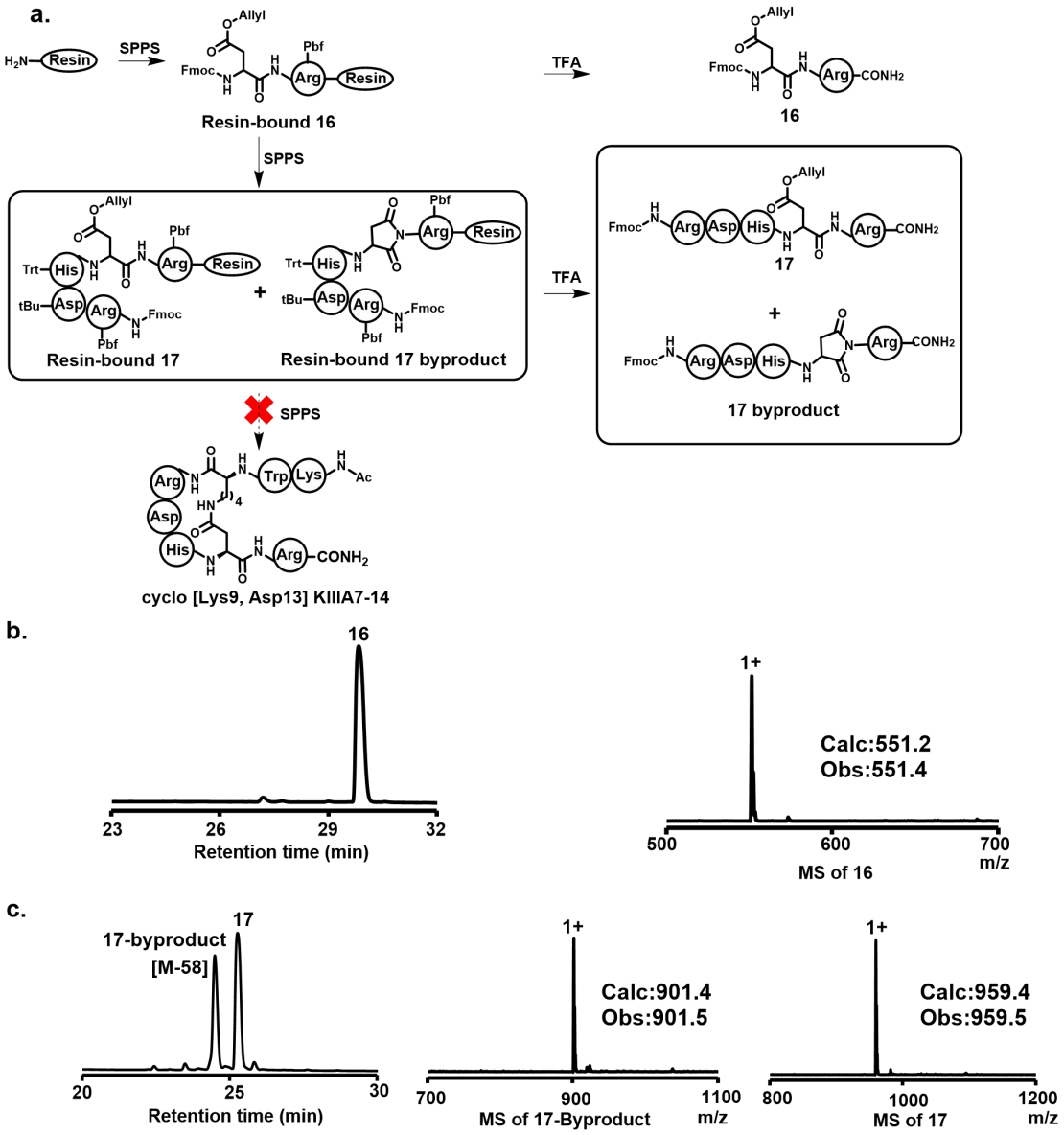



**Figure S6**: (a) Synthesis route of cyclo [Lys9, Asp 13] KIIIA7-14 using Fmoc-Asp(OAll)-OH. (b) RP-HPLC and ESI-MS of **16**. (c) RP-HPLC and ESI-MS of **17** and **17**-byproduct.

0.02 mmol Rink amide AM resin (63 mg, 0.32 mmol/g) was swelled in DCM/DMF (1/1, V/V) for 30 minutes. Fmoc-Arg(Pbf)-OH, Fmoc-Asp(OAll)-OH, Fmoc-His(Trt)-OH, Fmoc-Asp(tBu)-OH and Fmoc-Arg(Pbf)-OH were assembled to the resin according to the protocol described in Fmoc-based solid-phase peptide synthesis.

### 3.7 The synthesis of cyclic peptide 1Y using Fmoc-Asp(OAll)-OH：

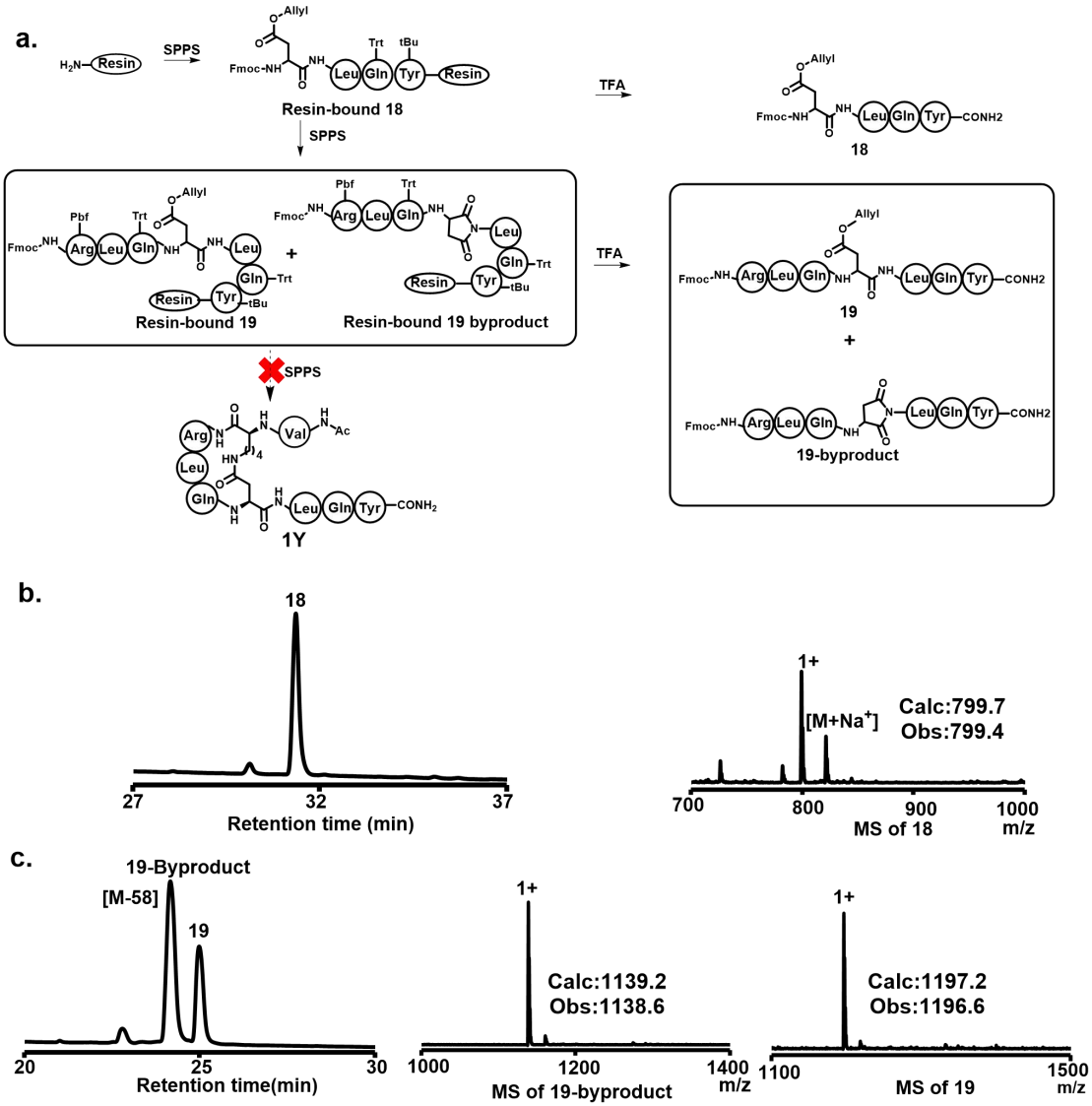
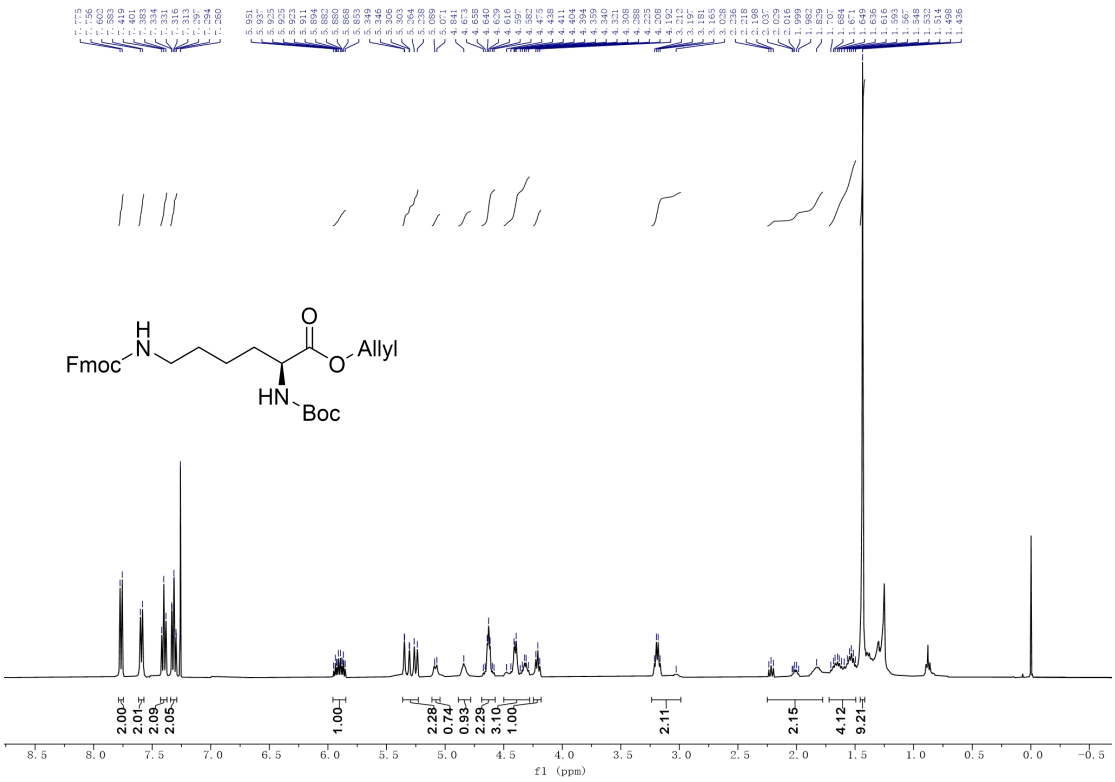



**Figure S7**: (a) Synthesis route of 1Y using Fmoc-Asp(OAll)-OH. (b) RP-HPLC and ESI-MS of **18**. (c) RP-HPLC and ESI-MS of **19** and **19**-byproduct.

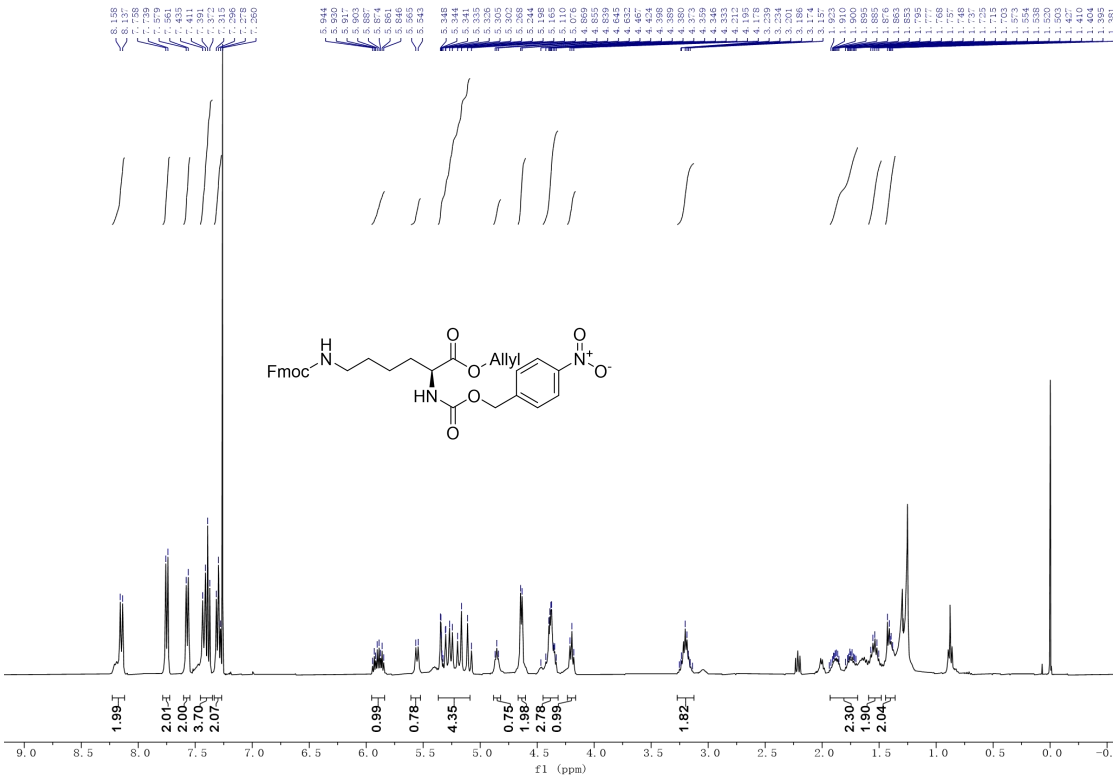
0.02 mmol Rink amide AM resin (63 mg, 0.32 mmol/g) was swelled in DCM/DMF (1/1, V/V) for 30 minutes. Fmoc-Tyr(tBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH, Fmoc-Asp(OAll)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Leu-OH and Fmoc-Arg(Pbf)-OH were assembled to the resin according to the protocol described in Fmoc-based solid-phase peptide synthesis.

# 4. NMR and MASS Data for amide-bonded DADA

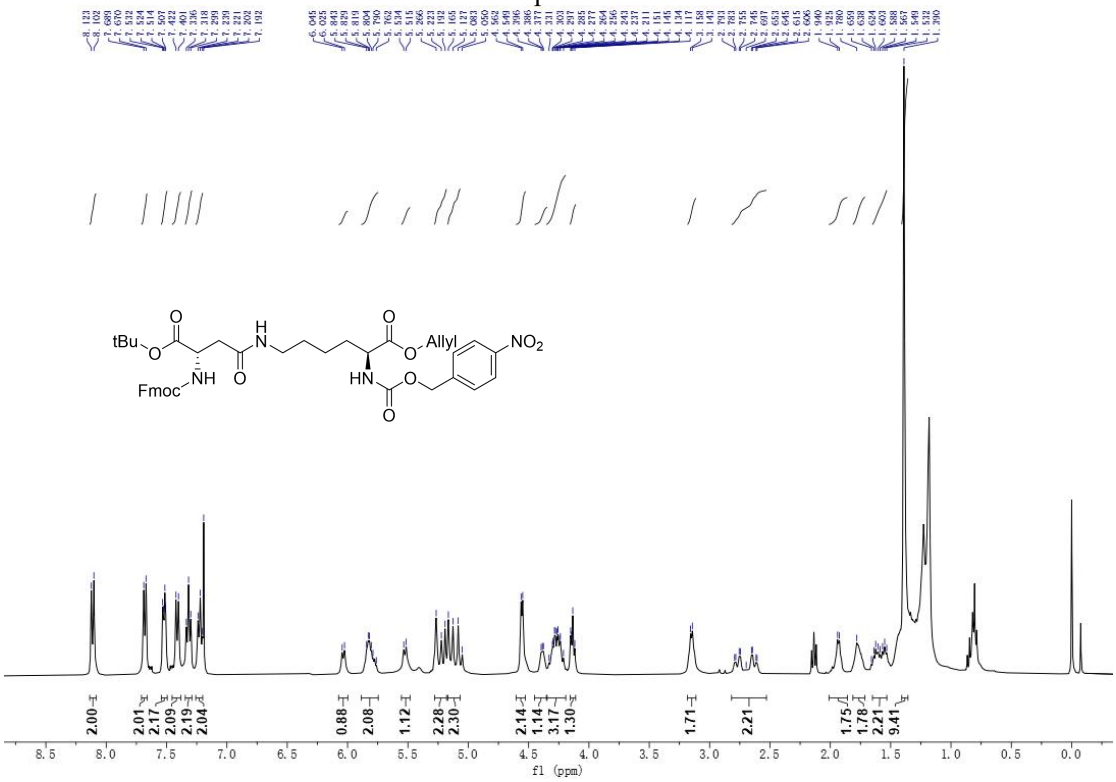
$^1$H NMR spectrum of 1-a



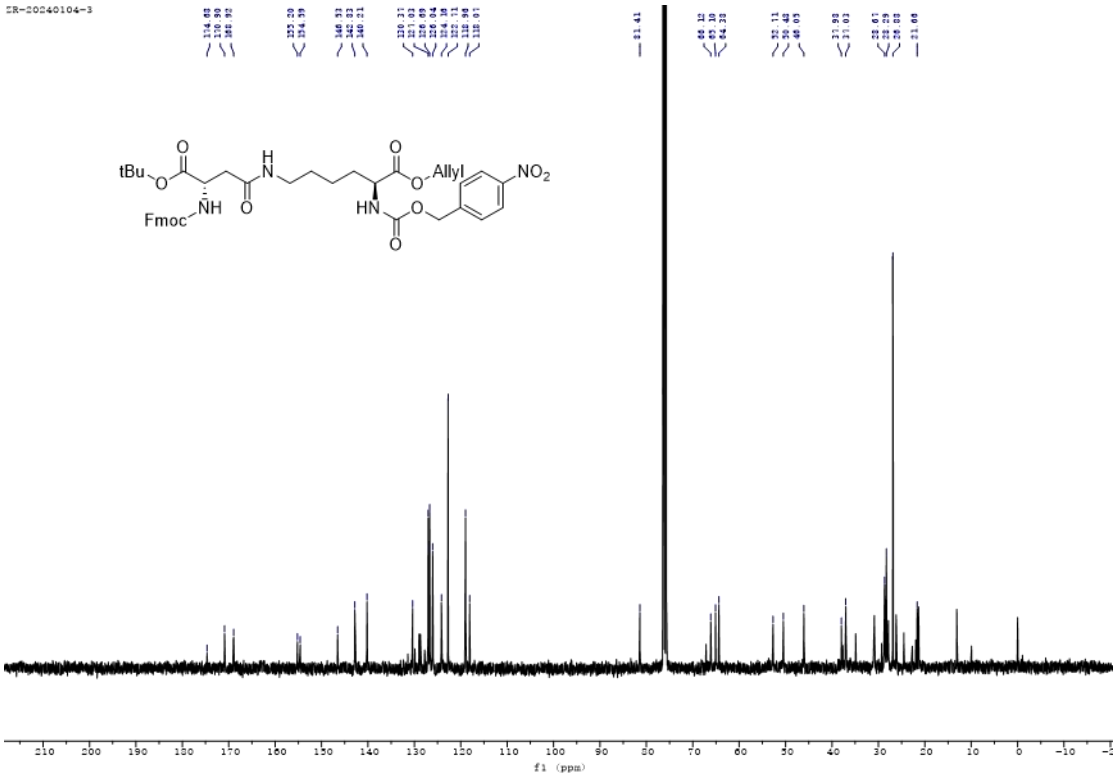

$^1$H NMR spectrum of 1-b



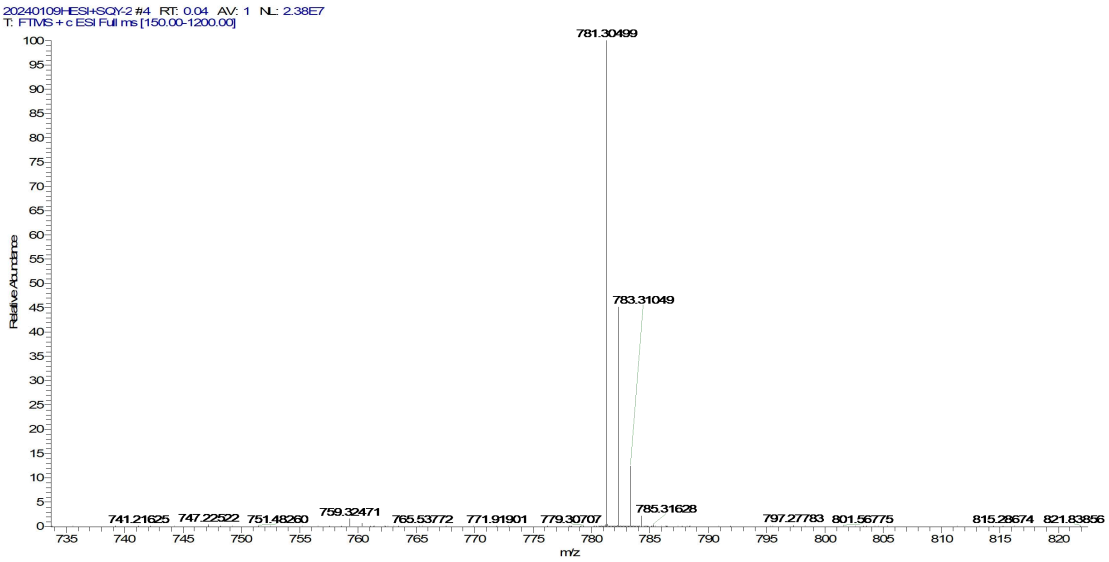
$^{1}$H NMR spectrum of 1-d



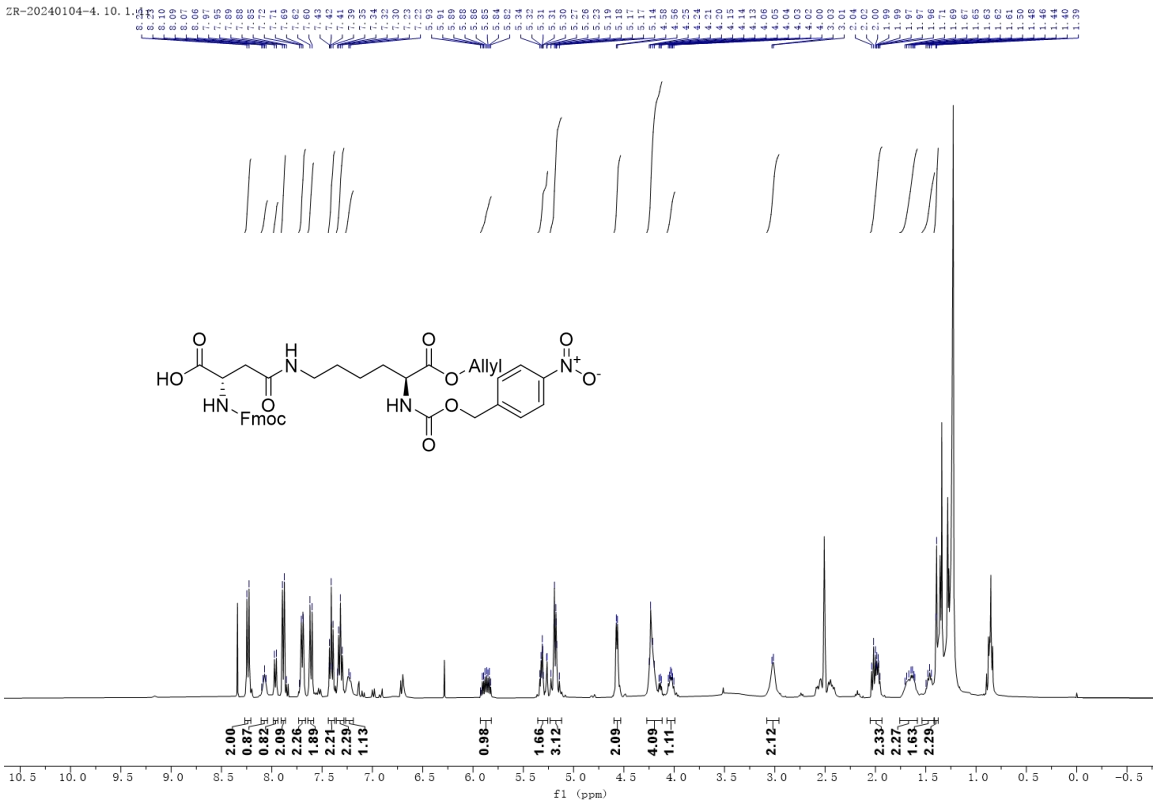

$^{13}$C NMR spectrum of 1-d.



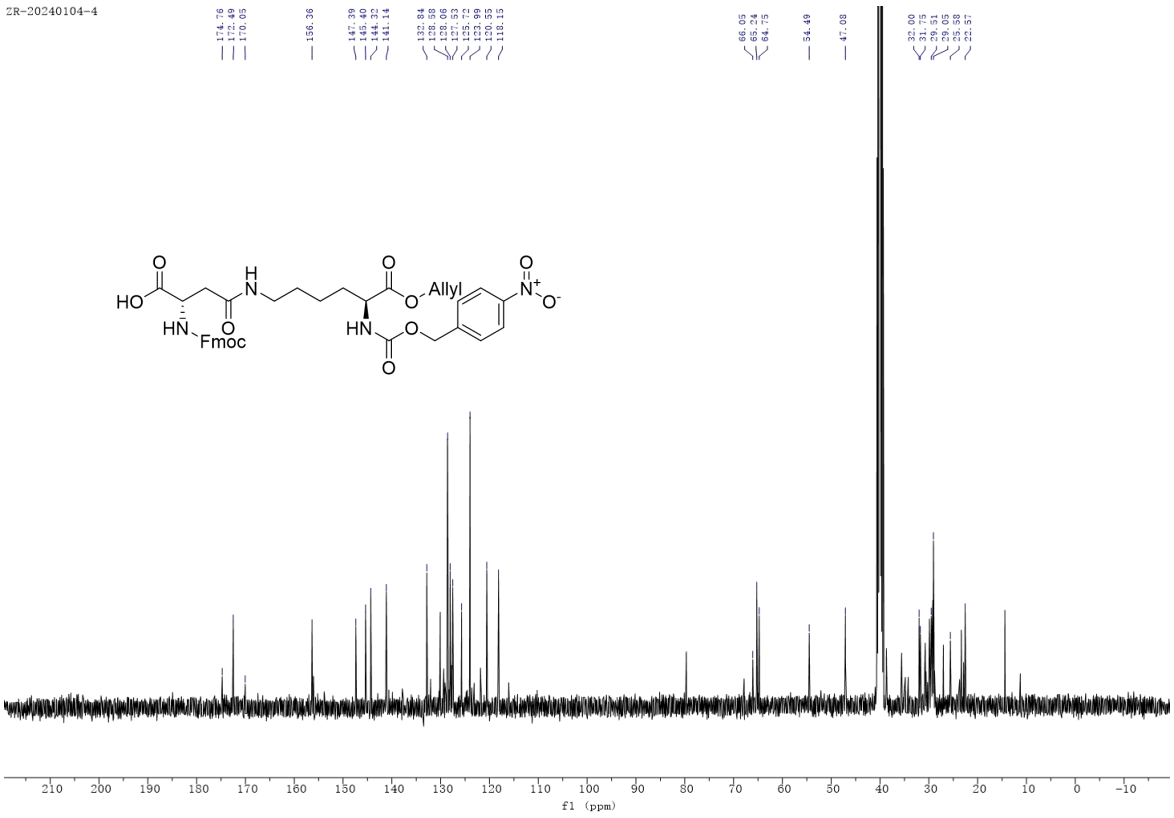
HRMS of 1-d



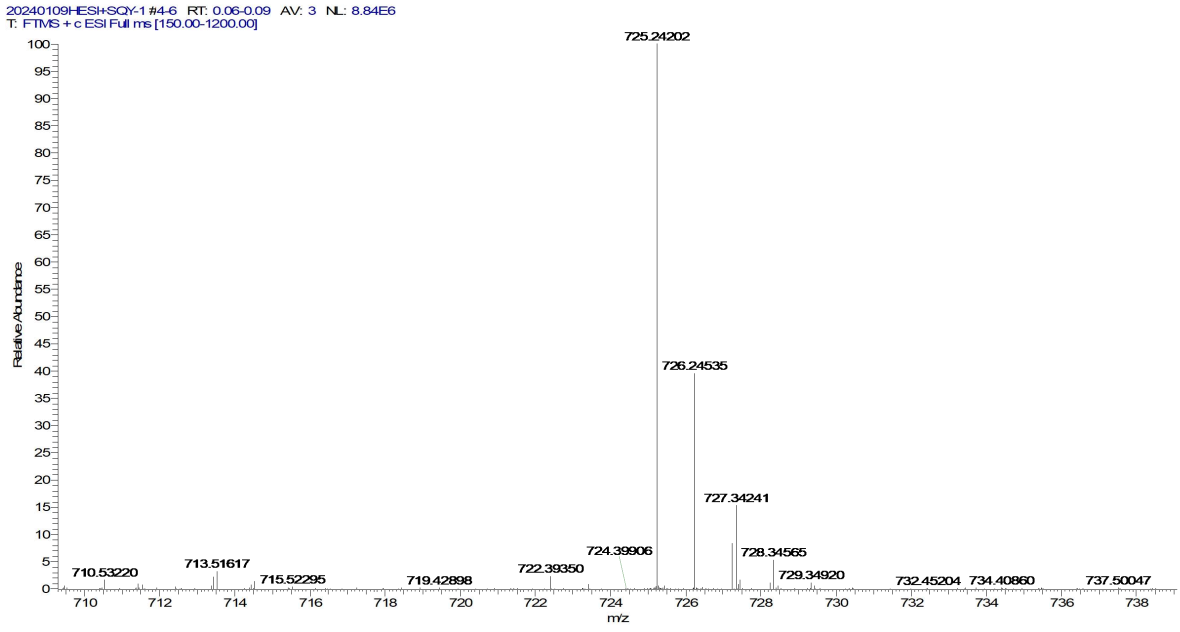

$^{1}$H NMR spectrum of 1



$^{13}$C NMR spectrum of 1.




HRMS of 1



## References


1. R. Zhao, P. Shi, X.-X. Wei, Z. Xia, C. Shi and J. Shi, *Org. Lett.*, 2023, **25**, 6544-6548.
2. X. X. Wei, J. B. Cui, R. Zhao, J. Luo, Y. M. Li, D. Bierer and J. Shi, *New J. Chem.*, 2023, **47**, 4213-4217
3. J.-H. Tang, R.-J. Yuan, J. Luo, N. Wang, J. Wang and Y.-M. Li, *Org. Lett.*, 2023, **25**, 2939-2943.