Supporting Information

Photocatalytic Acylation of Lysine Screened by a Microfluidic-Based Chemical Robotic System

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Table of Contents

1. Supporting Information for instrument and machine learning

Figure S1. The major structure of the high-throughput microfluidic-based chemical robotic system.

The system design, configuration and application method of high-throughput microfluidic-based chemical robotic system are shown in the unpublished manuscript: Heming Jiang, Ying Chen, Meirong Huang, Tingjun Liu, Yun-Dong Wu, Xinhao Zhang. Exploring unknown chemical space with an accessible high throughput screening (OPEN-HTS) chemical robotic system.

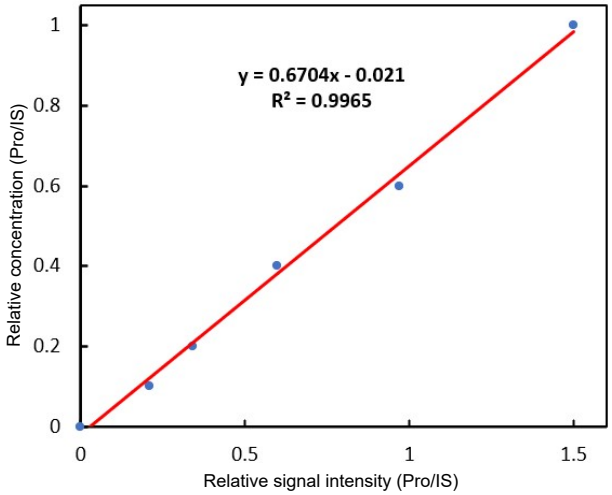


Figure S2. Correlation between the relative concentration (pro/IS) of reaction mixture and the relative signal intensity detected by LC-MS.

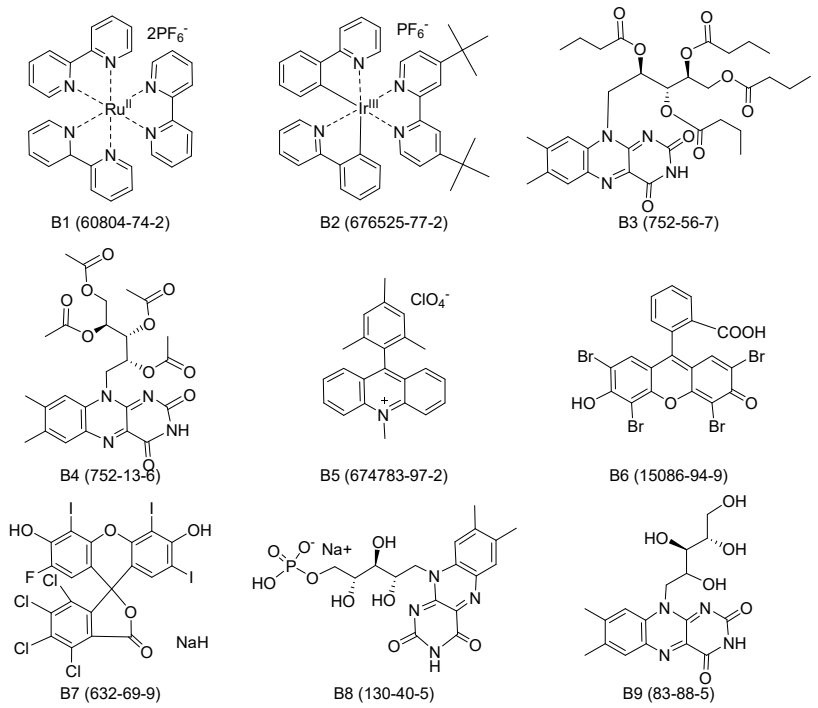


Figure S3. Nine different photocatalysts (B1-B9).

Table S1. The Screening Results of The Total 18 Conditions.

Entry	Catalyst loading	AcSK equivalent	Time (min)	Temperature (°C)	Yield (%)
1	**0.1**	**4**	**2**	**15**	**18.2**
2	**0.2**	**4**	**2**	**15**	**14.0**
3	**0.4**	**4**	**2**	**15**	**21.8**
4	**0.1**	**6**	**2**	**15**	**16.6**
5	**0.2**	**6**	**2**	**15**	**25.1**
6	**0.4**	**6**	**2**	**15**	**44.6**
7	**0.1**	**8**	**2**	**15**	**11.3**
8	**0.2**	**8**	**2**	**15**	**21.2**
9	**0.4**	**8**	**2**	**15**	**46.1**
10	**0.4**	**6**	**1**	**5**	**39.9**
11	**0.4**	**6**	**2**	**5**	**44.6**
12	**0.4**	**6**	**4**	**5**	**46.8**
13	**0.4**	**6**	**1**	**15**	**39.8**
14	**0.4**	**6**	**2**	**15**	**30.3**
15	**0.4**	**6**	**4**	**15**	**34.6**
16	**0.4**	**6**	**1**	**25**	**19.4**
17	**0.4**	**6**	**2**	**25**	**34.1**
18	**0.4**	**6**	**4**	**25**	**13.5**

Procedure: the substrate of peptide (0.001 mmol, 1.0 equiv.), substrate AcSK (4.0, 6.0 or 8.0 equiv.) and Dibenzyl sulfoxide (0.0001 mmol, 0.2 equiv.) are added to a 4 ml clear glass vial, and then the co-solvent

of 1.0 mL (MeCN:H_2O =1:1, PBS buffer, pH 7.4) is added. The catalyst B9 (0.1, 0.2 or 0.4 equiv.) is added to a 4 mL clear glass vial, and then the co-solvent of 1.0 mL (MeCN:H_2O =1:1, PBS buffer, pH 7.4) is added. 9 different samples were prepared in this process. Then these 9 samples are transferred to the sample pool of the chemical robot, and reaction screening process started with sample injection and irradiation under blue LED (4.8 W) modules(5, 15 or 25 °C). In this experiments, 18 reactions with 4 variables are conducted, and the results are shown in **Table S1**.

Table S2. The Screening Results of The Total 9 Conditions.

Entry	Catalyst loading	AcSK equivalent	Time (min)	Temperature (°C)	Yield (%)
1	**0.2**	**6.5**	**1**	**10**	**49.6**
2	**0.2**	**6.5**	**2**	**10**	**41.1**
3	**0.2**	**6.5**	**4**	**10**	**59.8**
4	**0.4**	**6.5**	**1**	**10**	**44.9**
5	**0.4**	**6.5**	**2**	**10**	**69.3**
6	**0.4**	**6.5**	**4**	**10**	**68.4**
7	**0.6**	**6.5**	**2**	**10**	**77.1**
8	**0.6**	**6.5**	**4**	**10**	**74.5**
9	**0.6**	**6.5**	**2.5**	**10**	**83.5**

Procedure: the substrate of peptide (0.001 mmol, 1.0 equiv.), substrate AcSK (0.0065 mmol, 6.5 equiv.) and Dibenzyl sulfoxide (0.0001 mmol, 0.2 equiv.) are added to a 4 mL clear glass vial, and then the co-solvent of 1.0 mL (MeCN:H_2O =1:1, PBS buffer, pH 7.4) is added. The catalyst B9 (0.2, 0.4 or 0.6 equiv.) is added to a 4 mL clear glass vial, and then the co-solvent of 1.0 mL (MeCN:H_2O =1:1, PBS buffer, pH 7.4) is added. Then these 4 samples are transferred to the sample pool of the chemical robot, and reaction screening process started with sample injection and irradiation under blue LED (4.8 W) modules(10 °C). In this experiments, 8 reactions with 2 variables are conducted, and the results are shown in **Table S2**.

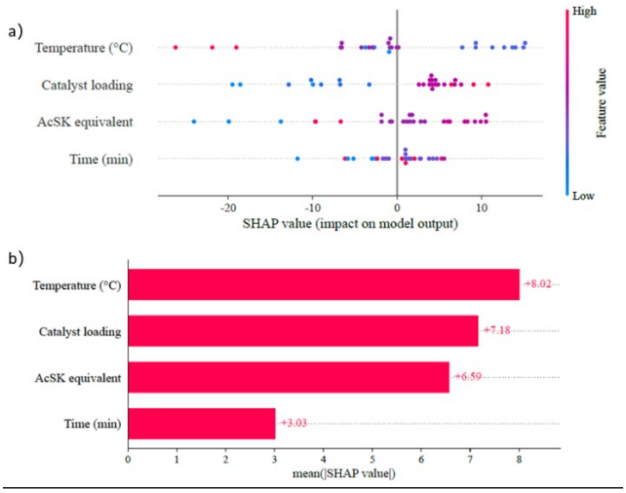


Figure S4. Feature importance analyzed by SHAP (with Gaussian Process Regressor model and 26 data sets in Table S1 and Table S2). a) SHAP value of 4 features: Temperature, Catalyst loading, AcSK equivalent and Time. b) mean absolute SHAP valve of above 4 features.

2. Supporting information for organic chemistry

2.1 General information

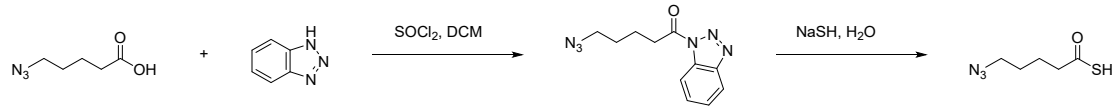
All chemical reagents are commercially available from *Energy Chemical* without purification. The reactions were monitored by TLC (silica gel-G). **Nuclear Magnetic Resonance (NMR)** spectra were recorded on Qone 400 MHz spectrometer using trimethylsilane (TMS) as internal standard under ambient temperature (20 °C). **High-Resolution Mass Spectrometry (HRMS)** were measured on a Q_Exactive_Focus. **Mass Spectrometry** (**MS**) to screen the molecular weight of HPLC fractions were carried out on SHIMAZU LC-MS 8030 in positive ion mode.

General method for preparative LC

Preparative LC were performed on a Shimadzu LC-6AD system equipped with Shimadzu Shim-pack GIST C18 column (20 × 250 mm, 5 μm; room temperature). Water (containing 0.1% TFA) and pure CH_3CN were used as solvents in linear gradient mixtures at a flow rate of 8 mL·min^{-1}.

2.2 Synthesis of thioacids

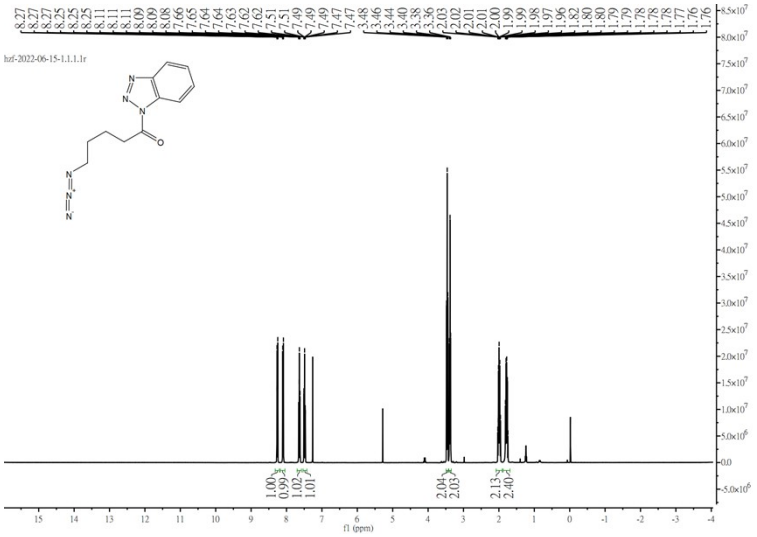
The synthesis of thioacid 1e was followed the similar procedure of the previous reports.



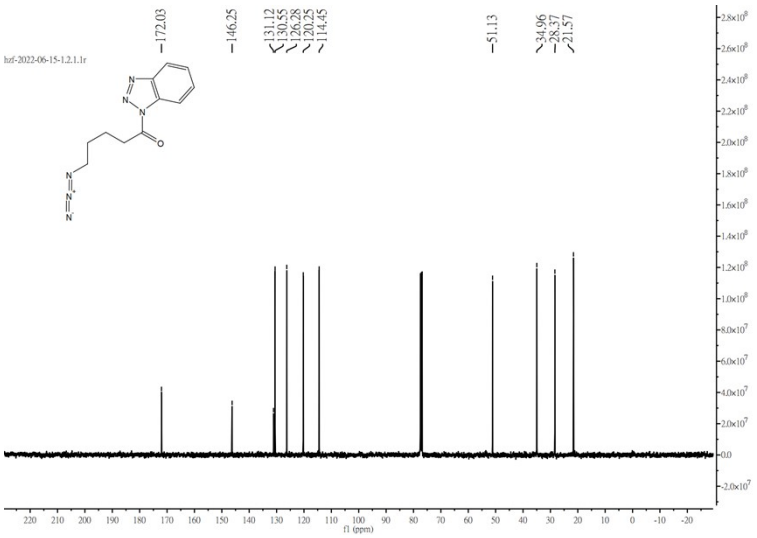
To a stirred solution of benzotriazole (1.5 g, 4.0 equiv., 12.6 mmol) in DCM (15 mL) was added $SOCl_2$ (328 mg, 1.1 equiv., 2.75 mmol) at room temperature, and the mixture was kept for 30 min. A solution of 5-azido carboxylic acid (358 mg, 1.0 equiv., 2.5 mmol) in DCM (2.5 mL) was added, and precipitation of white solid was observed from the reaction mixture after 1h. The mixture was filtered and washed with DCM, and the combined filtrate was washed with 10% NaOH aqueous solution and brine, then dried over Na_2SO_4 and concentrated. The residue was purified by flash chromatography (eluent: EtOAc/PE = 1/20) to give the 5-azidopentanoic acid-Bt as a colorless oil (513 mg, 84% yield). ^{1}H NMR (500 MHz, Chloroform-*d*). δ 8.26 (dt, *J* = 8.2, 1.0 Hz, 1H), 8.10 (dt, *J* = 8.2, 1.0 Hz, 1H), 7.64 (ddd, *J* = 8.3, 7.1, 1.0 Hz, 1H), 7.49 (ddd, *J* = 8.2, 7.1, 1.0 Hz, 1H), 3.46 (t, *J* = 7.3 Hz, 2H), 3.38 (t, *J* = 6.7 Hz, 2H), 2.11 – 1.90 (m, 2H), 1.87 – 1.69 (m, 2H). ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.0, 146.2, 131.1, 130.6, 126.3, 120.3, 114.5, 51.1, 35.0, 28.4, 21.6.

To a stirred solution of 5-azidopentanoic acid-Bt (100 mg, 1.0 equiv., 0.4 mmol) in H_2O (1 mL) was added NaSH (67 mg, 3 equiv., 1.2 mmol) at room temperature. The mixture was stirred at room temperature overnight. The mixture was added to a mixture of ethyl acetate and an aqueous solution of 1 M HCl. The organic layer was separated and the aqueous layer was extracted with ethyl acetate. The organic layers were washed with brine, dried Na_2SO_4 and concentrated in vacuo to give the crude product **1e**. The residue was purified by flash chromatography (eluent: DCM/MeOH = 50/1) to give the **1e** as a colorless oil (32 mg, 51% yield). **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 3.12 (ddd, *J* = 6.4, 5.0, 1.0 Hz, 1H), 2.75 – 2.45 (m, 1H), 2.11 – 1.87 (m, 2H). **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 201.5, 53.5, 41.3,

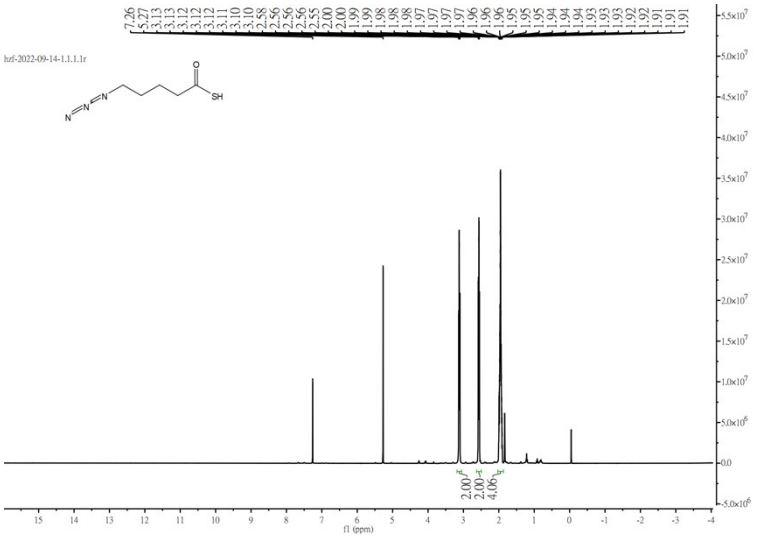
30.5, 23.0, 22.8. **HRMS (ESI-TOF)** Calc. for $C_5H_8N_3OS$ $[M-H]^+$, 158.0383, found, 158.0385.



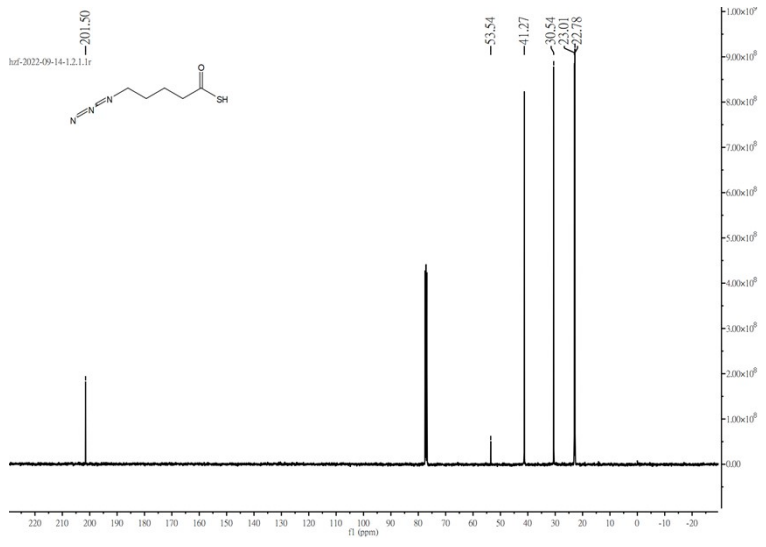
^{1}H NMR of 5-azido carboxylic acid-Bt



^{13}C NMR of 5-azido carboxylic acid-Bt

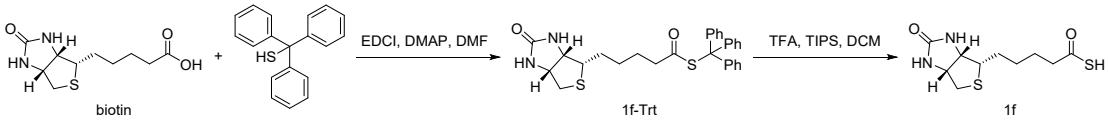


^{1}H NMR of **1e**



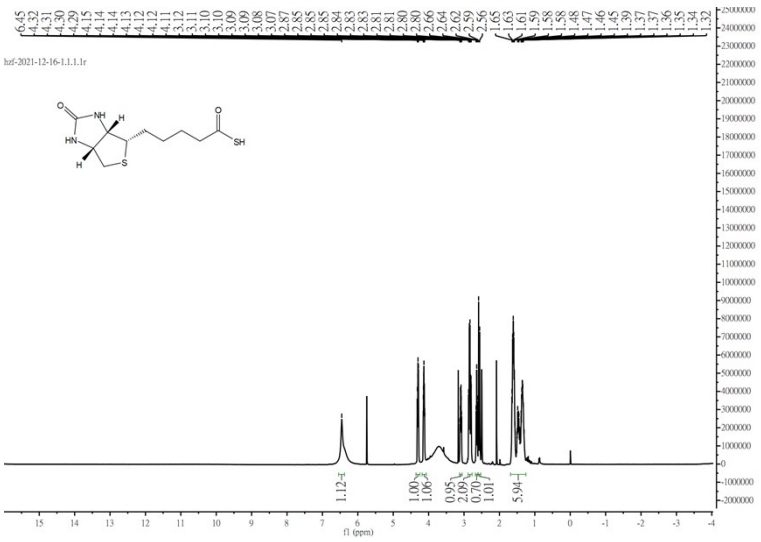
^{13}C NMR of **1e**

The synthesis of thioacid 1f was followed the similar procedure of the previous reports.

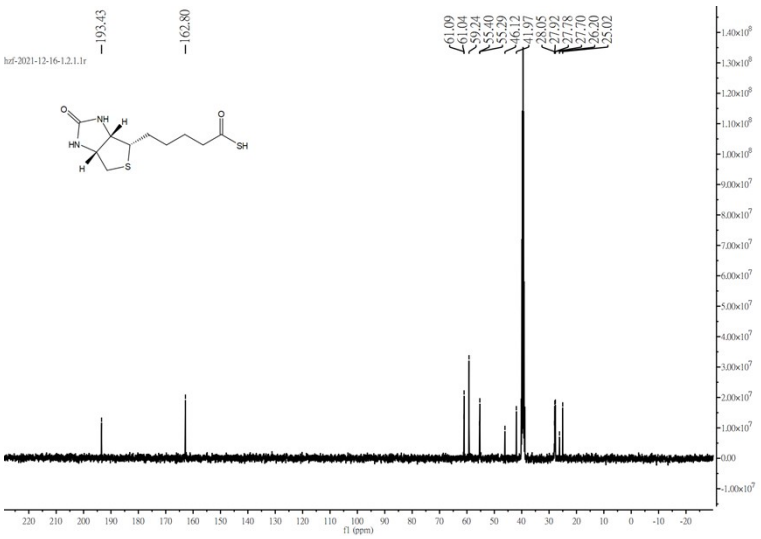


To a glass flask was added biotin (2.44 g, 1.0 equiv., 10 mmol), triphenylmethanethiol (3.04 g, 1.1 equiv., 11 mmol), EDCI (2.11 g, 1.1 equiv., 11 mmol), DMAP (122 mg, 0.1 equiv., 1 mmol) and 25 mL DMF. The mixture was stirred at room temperature overnight. After completion of the reaction, water was added and the desired product was filtered. The product biotin-Trt (4.37 g, 87%) as a while solid, was used without further purification.

To a stirred solution of biotin-Trt (503 mg, 1.0 equiv., 1 mmol) in CH_2Cl_2 (3.0 mL/mmol) in a 25 mL round-bottomed flask was added TFA (1 mL), followed immediately by the addition of TIPS (317 mg, 2.0 equiv., 2 mmol). The reaction was stirred for 15 min, and the stirring bar, solvent, TFA and TIPS were removed in vacuo, maintaining the temperature below 30 °C. After completion of the reaction, ether was added and the desired product was filtered. The product 1f (237 mg, 91%) as a while solid. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 6.45 (s, 1H), 4.30 (dd, J = 7.7, 4.9 Hz, 1H), 4.13 (ddd, J = 7.6, 4.4, 2.8 Hz, 1H), 3.09 (ddd, J = 8.4, 6.2, 4.3 Hz, 1H), 2.95 – 2.76 (m, 2H), 2.74 – 2.53 (m, 2H), 1.73 – 1.18 (m, 6H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 193.4, 162.8, 61.1, 61.0, 59.2, 55.4, 55.3, 46.1, 42.0, 28.0, 27.9, 27.8, 27.7, 26.2, 25.0. **HRMS (ESI-TOF)** Calc. for $C_{10}H_{15}N_2O_2S_2$ $[M\text{-}H]^+$, 259.0569, found, 259.0578.



^{1}H NMR of **1f**



^{13}C NMR of **1f**

2.3 General procedure A for the light promoted amidation by thioacids

To a glass flask was added thioacid **1** (1 mmol), amine compound **2** (0.5 mmol), RFTA (5% mol), Na_3PO_4 (1 mmol) and 8 mL water. The flask was then sealed and equipped with magnetic bar. The reaction was stirred and irradiated with 450 nm blue LED light (16 W) for 30 min. For the substrate **2a**-**2d**, the resulting aqueous solution was extracted by EtOAc (3×10 mL). The organic phase was collected, and dried by Na_2SO_4. After concentration under vacuum, the crude product was purified by flash column chromatography using eluent solution Hexane/EtOAc (10:1). For the substrate **2e**-**2j**, the resulting solution was purified directly via preparative HPLC after filtration. Desired distillates were identified by MS and lyophilized to obtain target products **3**.

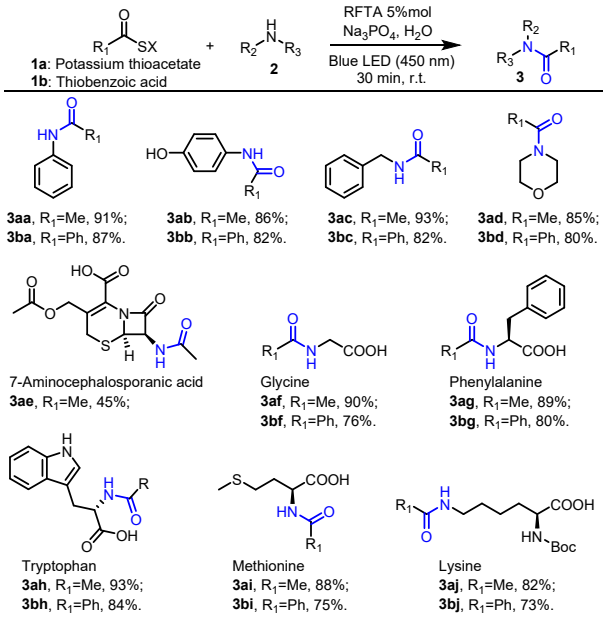
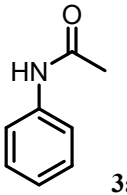
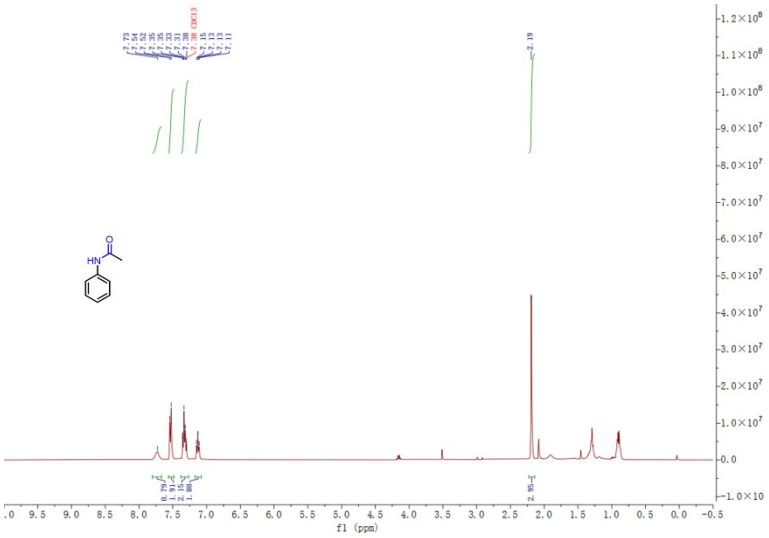


Figure S5 Scope of the acylation between thioacids and micromolecules.

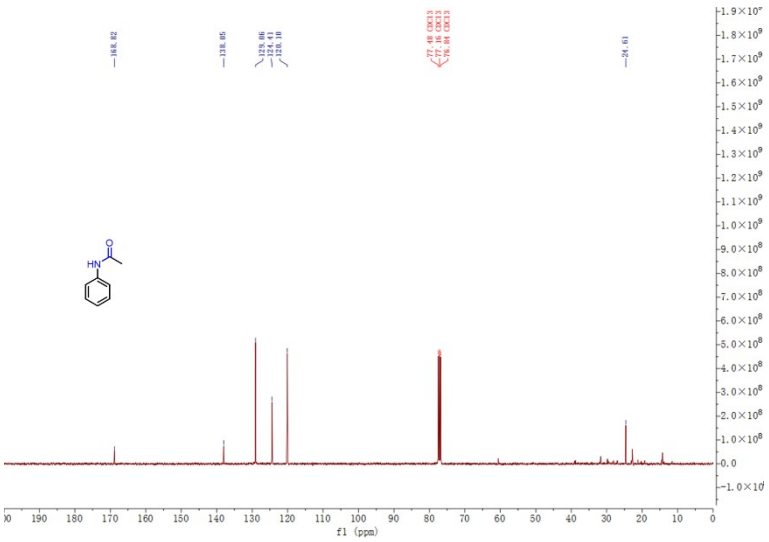
2.3.1 NMR spectra of acylation of amino compounds



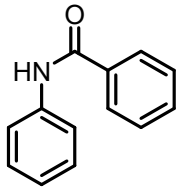
3aa, yellow oil, 91%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.38 – 7.27 (m, 2H), 7.17 – 7.08 (m, 1H), 2.19 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 168.8, 138.1, 129.1, 124.4, 120.1, 24.6.



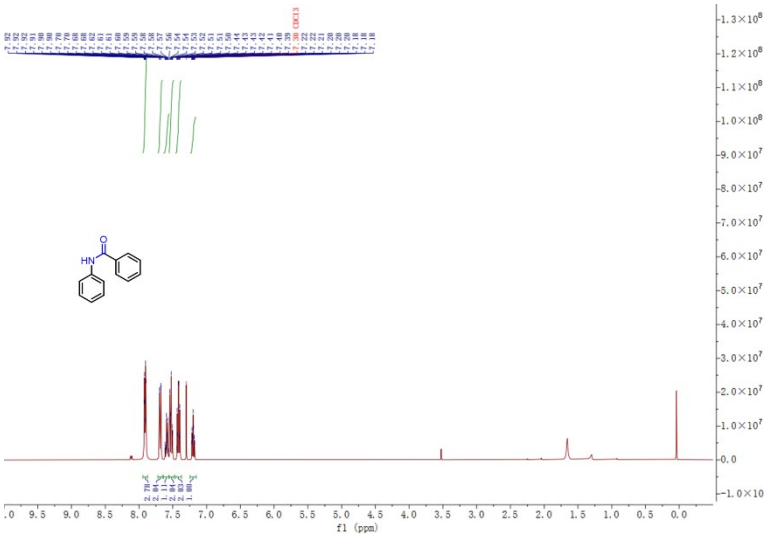
^{1}H NMR spectrum of **3aa**



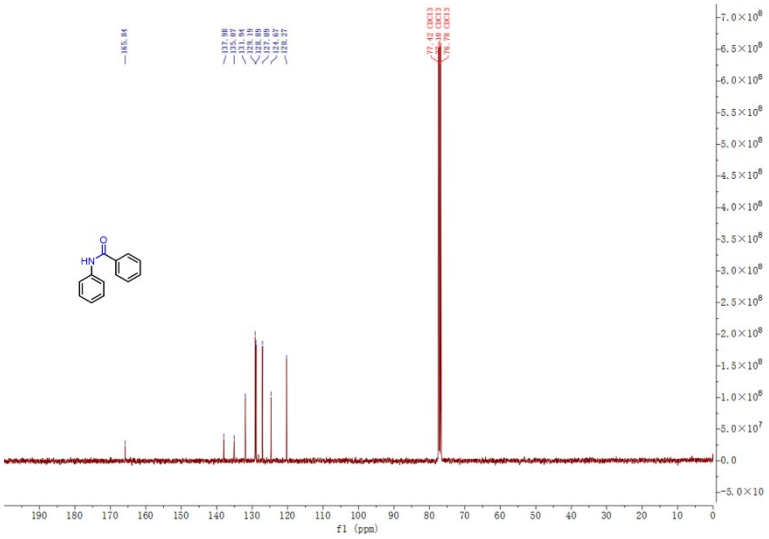
^{13}C NMR spectrum of **3aa**



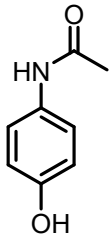
3ba, yellow solid, 87%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.91 (dd, *J* = 7.0, 1.7 Hz, 3H), 7.72 – 7.65 (m, 2H), 7.64 – 7.56 (m, 1H), 7.56 – 7.48 (m, 2H), 7.46 – 7.37 (m, 2H), 7.20 (td, *J* = 7.4, 1.2 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 165.8, 138.0, 135.1, 131.9, 129.2, 128.9, 127.1, 124.7, 120.3.



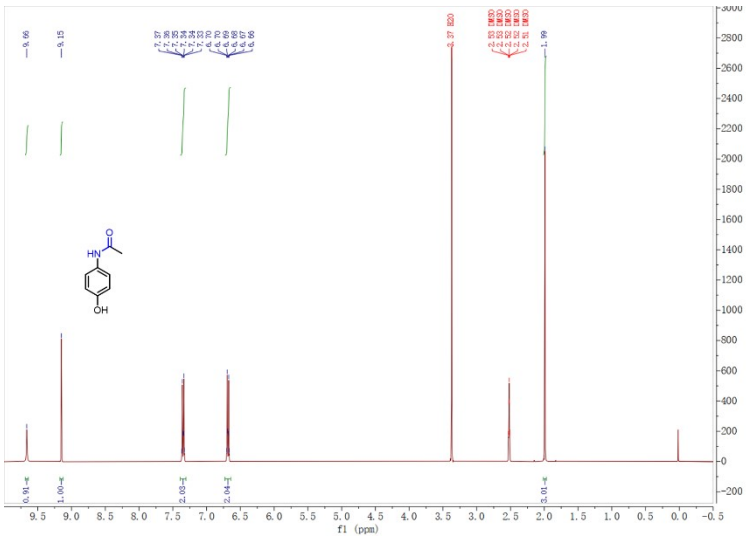
^{1}H NMR spectrum of **3ba**



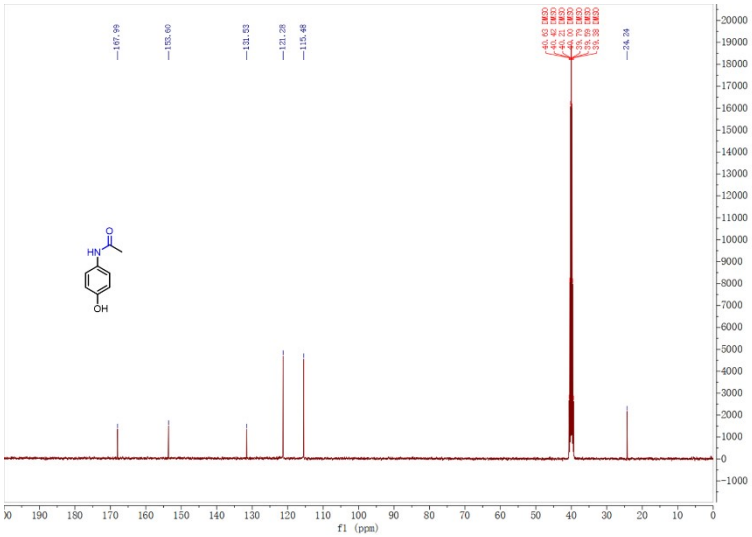
^{13}C NMR spectrum of **3ba**



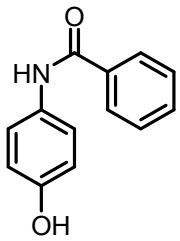
3ab, white solid, 86%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 9.66 (s, 1H), 9.15 (s, 1H), 7.39 – 7.31 (m, 2H), 6.73 – 6.64 (m, 2H), 1.99 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 168.0, 153.6, 131.5, 121.3, 115.5, 24.2.



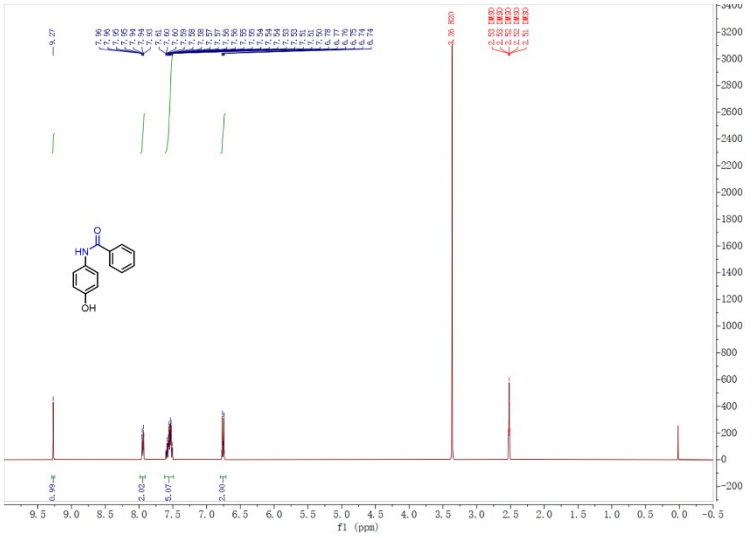
^{1}H NMR spectrum of **3ab**



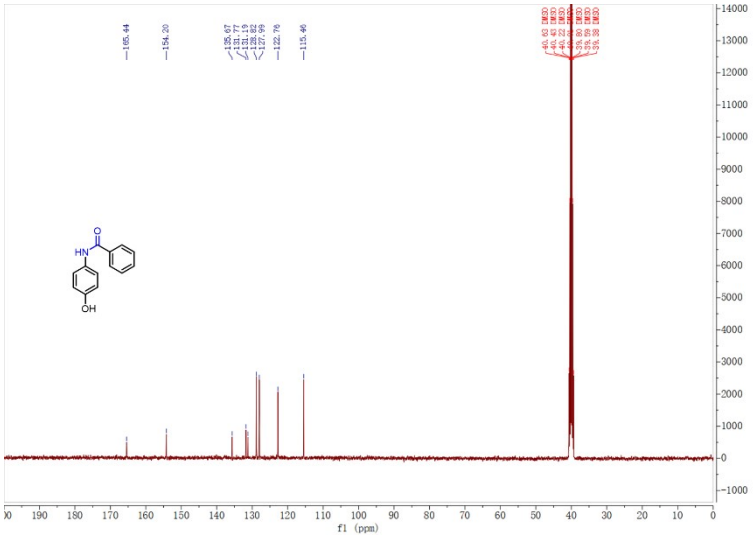
^{13}C NMR spectrum of **3ab**



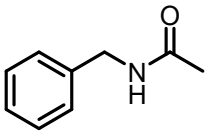
3bb, gray solid, 82%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.04 (s, 1H), 9.27 (s, 1H), 7.98 – 7.91 (m, 2H), 7.62 – 7.49 (m, 5H), 6.80 – 6.71 (m, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 165.4, 154.2, 135.7, 131.8, 131.2, 128.8, 128.0, 122.8, 115.5.



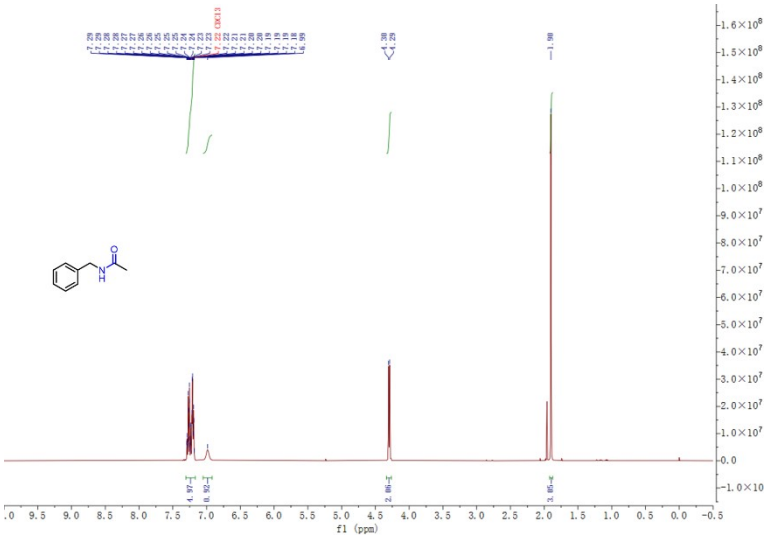
^{1}H NMR spectrum of **3bb**



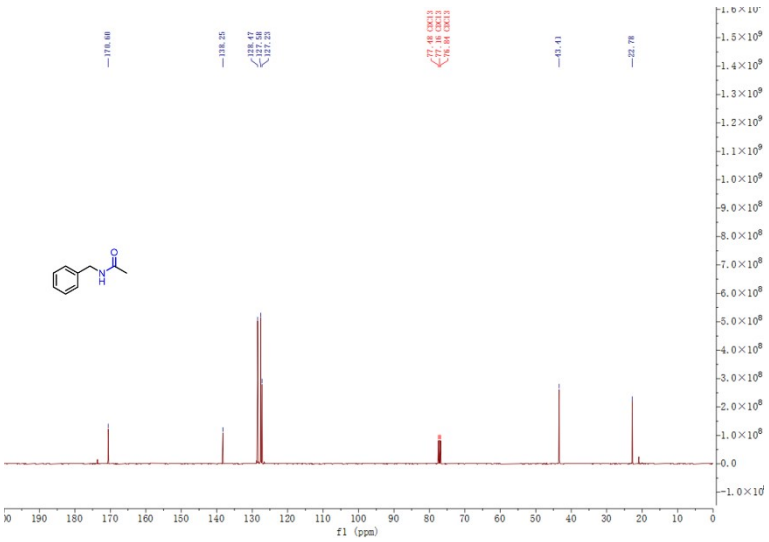
^{13}C NMR spectrum of **3bb**



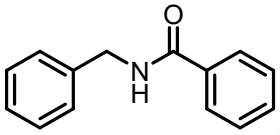
3ac, yellow oil, 93%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.31 – 7.17 (m, 5H), 6.99 (s, 1H), 4.30 (d, *J* = 5.8 Hz, 2H), 1.90 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.6, 138.3, 128.5, 127.6, 127.2, 43.4, 22.8.



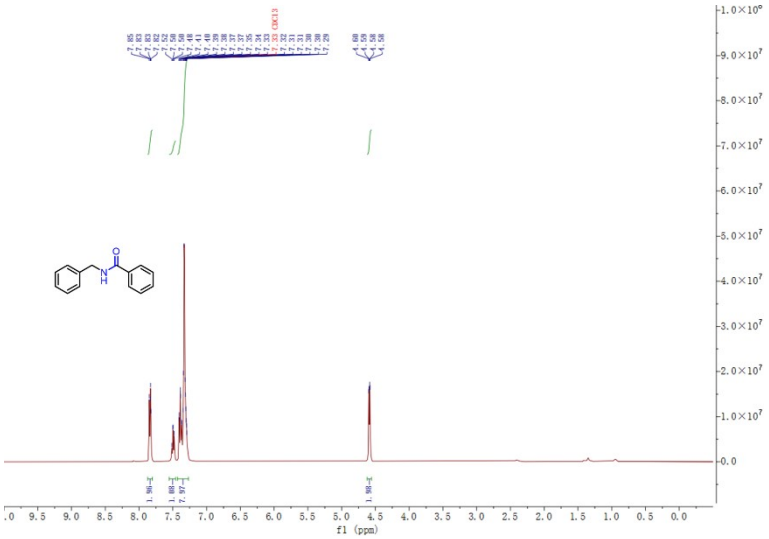
^{1}H NMR spectrum of **3ac**



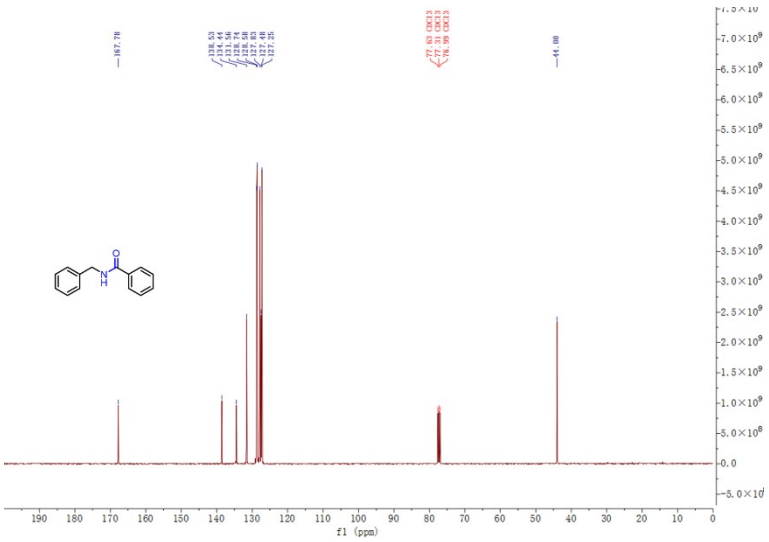
^{13}C NMR spectrum of **3ac**



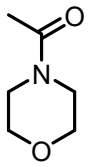
3bc, yellow solid, 82%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87 – 7.80 (m, 2H), 7.55 – 7.46 (m, 1H), 7.43 – 7.27 (m, 8H), 4.59 (dd, *J* = 5.9, 1.7 Hz, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 167.8, 138.5, 134.4, 131.6, 128.7, 128.6, 127.8, 127.5, 127.3, 44.0.



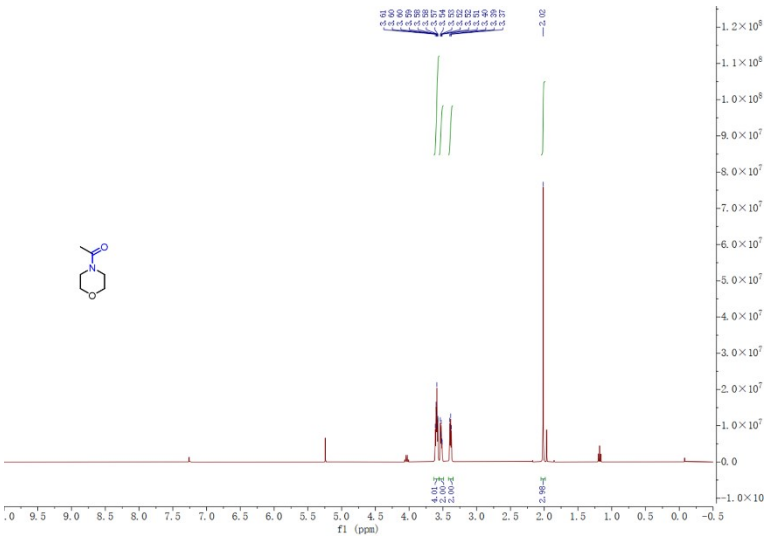
^{1}H NMR spectrum of **3bc**



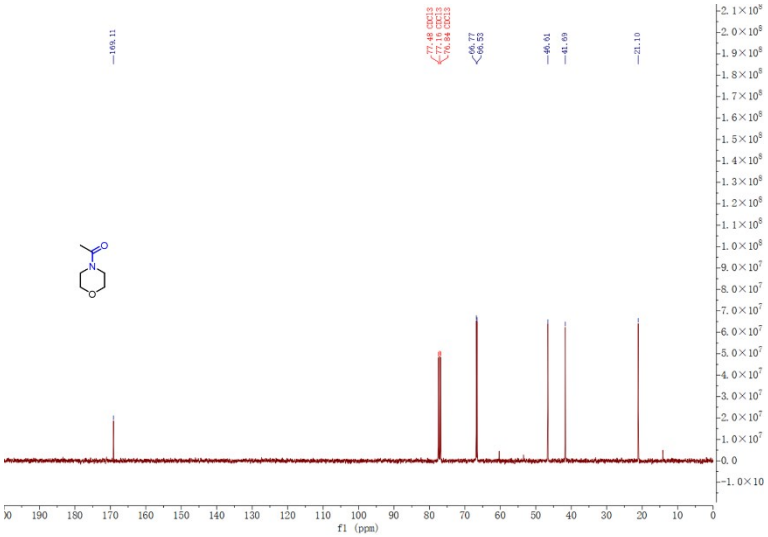
^{13}C NMR spectrum of **3bc**



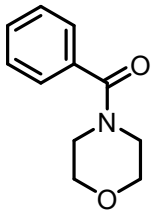
3ad, yellow oil, 85%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 3.63 – 3.55 (m, 4H), 3.53 (dd, *J* = 6.3, 4.2 Hz, 2H), 3.42 – 3.35 (m, 2H), 2.02 (s, 3H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.1, 66.8, 66.5, 46.6, 41.7, 21.1.



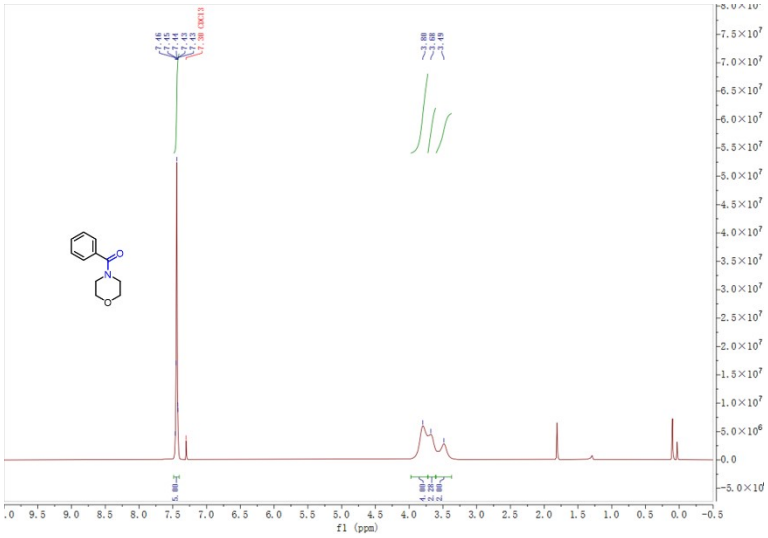
^{1}H NMR spectrum of **3ad**



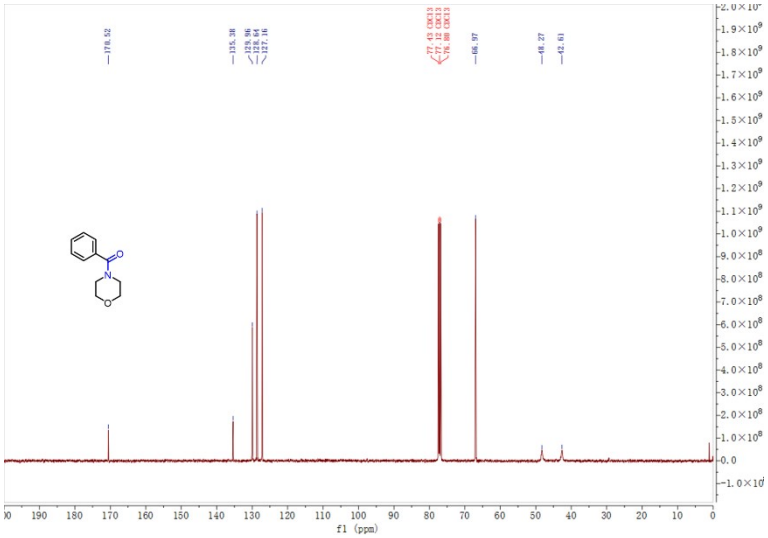
^{13}C NMR spectrum of **3ad**



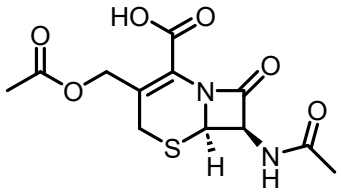
3bd, yellow solid, 80%. **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.45 (d, *J* = 3.9 Hz, 5H), 3.80 (s, 4H), 3.68 (s, 2H), 3.49 (s, 2H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 170.5, 135.4, 130.0, 128.6, 127.2, 67.0, 48.3, 42.6.



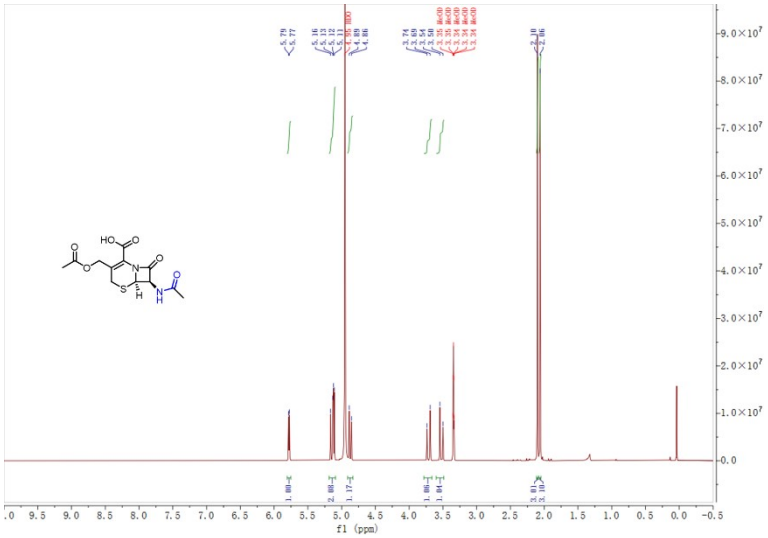
^{1}H NMR spectrum of **3bd**



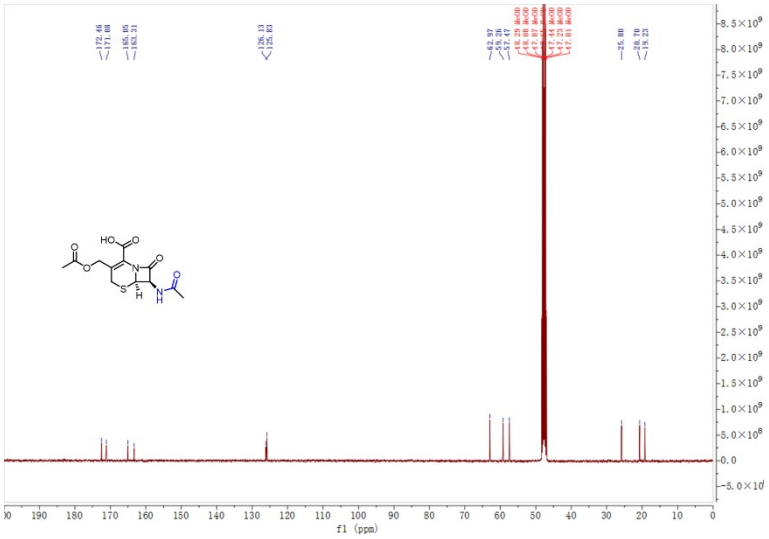
^{13}C NMR spectrum of **3bd**



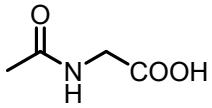
3ae, white powder, 45%. **^{1}H NMR** (400 MHz, Methanol-d_4) δ 5.78 (d, J = 4.8 Hz, 1H), 5.19 – 5.09 (m, 2H), 4.87 (d, J = 13.1 Hz, 1H), 3.71 (d, J = 18.3 Hz, 1H), 3.52 (d, J = 18.3 Hz, 1H), 2.10 (s, 3H), 2.06 (s, 3H). **^{13}C NMR** (101 MHz, Methanol-d_4) δ 172.5, 171.1, 165.1, 163.3, 126.1, 125.8, 63.0, 59.3, 57.5, 25.8, 20.7, 19.2. **HRMS** m/z (ESI-TOF): $[M+H]^+$ calcd for $C_{12}H_{14}O_6N_2SNa^+$ 337.0465, found 337.0466.



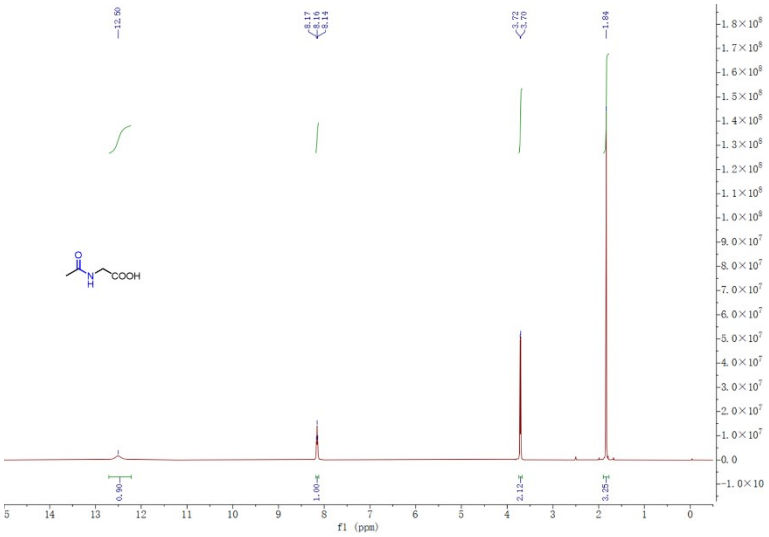
^{1}H NMR spectrum of **3ae**



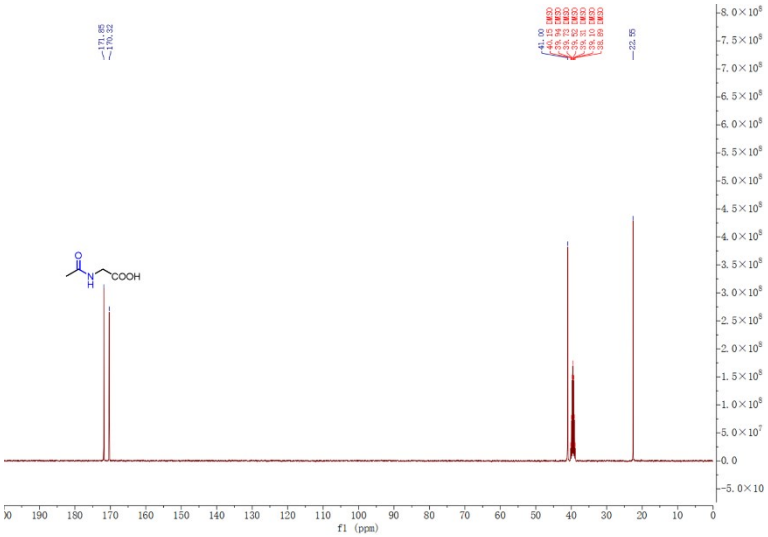
^{13}C NMR spectrum of **3ae**



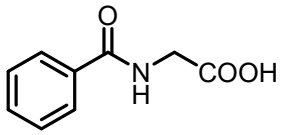
3af, white powder, 90%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.50 (s, 1H), 8.16 (t, J = 5.9 Hz, 1H), 3.71 (d, J = 6.0 Hz, 2H), 1.84 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.9, 170.3, 41.0, 22.6.



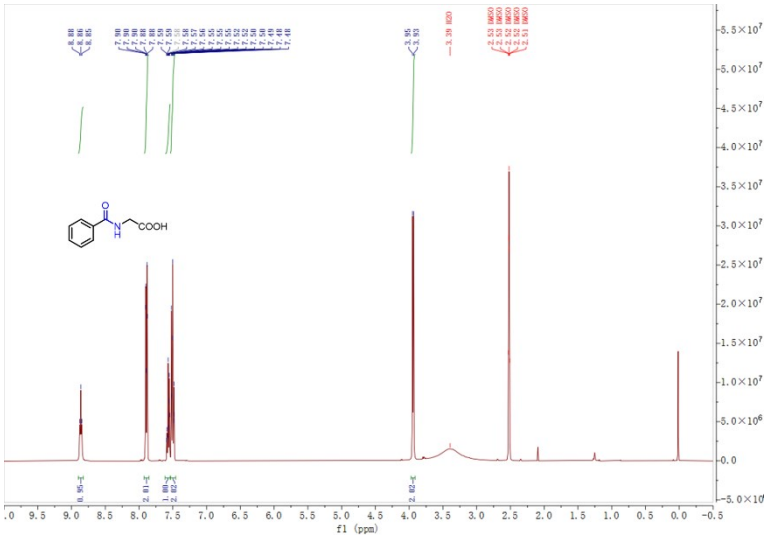
^{1}H NMR spectrum of **3af**



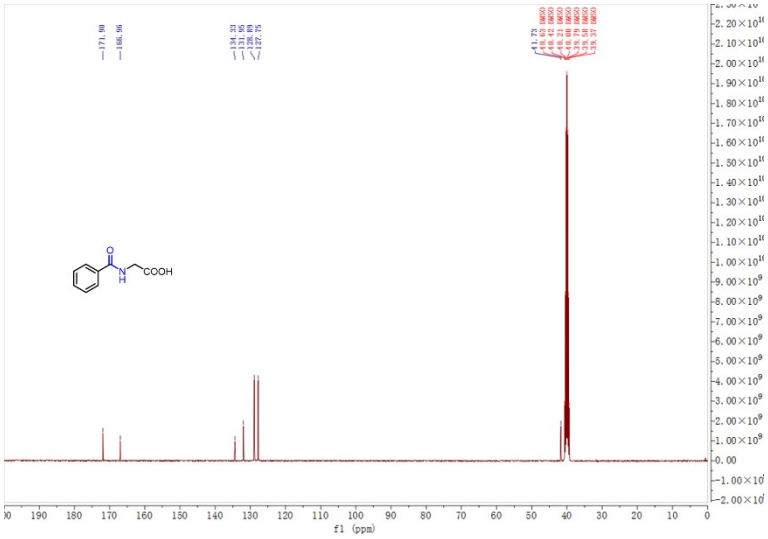
^{13}C NMR spectrum of **3af**



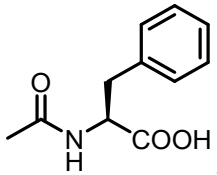
3bf, white powder, 76%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.86 (t, J = 5.9 Hz, 1H), 7.92 – 7.85 (m, 2H), 7.61 – 7.54 (m, 1H), 7.50 (dd, J = 8.2, 6.6 Hz, 2H), 3.94 (d, J = 5.9 Hz, 2H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 171.9, 167.0, 134.3, 132.0, 128.9, 127.8, 41.7.



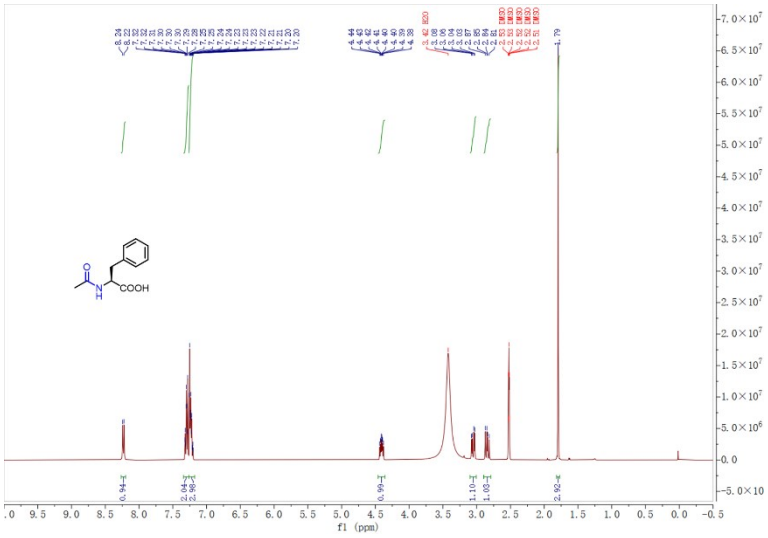
^{1}H NMR spectrum of **3bf**



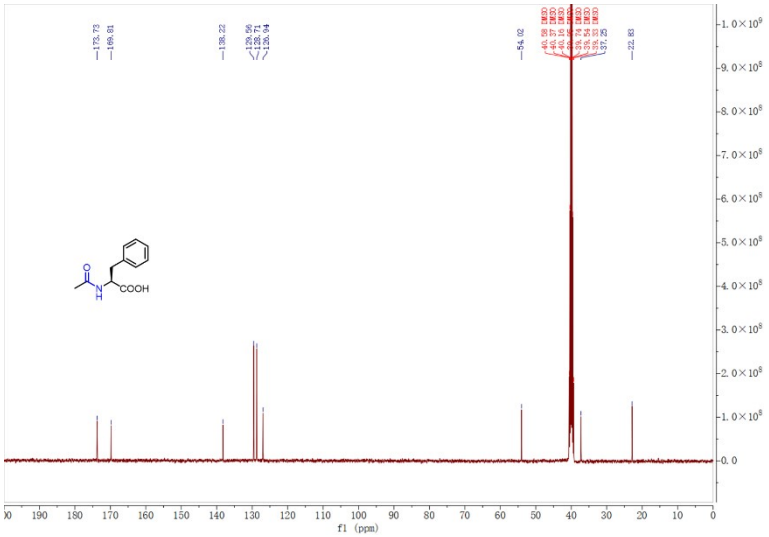
^{13}C NMR spectrum of **3bf**



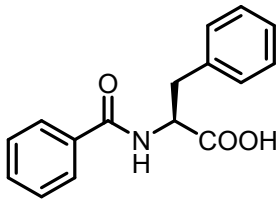
3ag, white powder, 89%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.23 (d, J = 8.1 Hz, 1H), 7.34 – 7.26 (m, 2H), 7.26 – 7.18 (m, 3H), 4.41 (ddd, J = 9.5, 8.0, 4.9 Hz, 1H), 3.05 (dd, J = 13.8, 4.9 Hz, 1H), 2.84 (dd, J = 13.8, 9.6 Hz, 1H), 1.79 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.7, 169.8, 138.2, 129.6, 128.7, 126.9, 54.0, 37.3, 22.8.



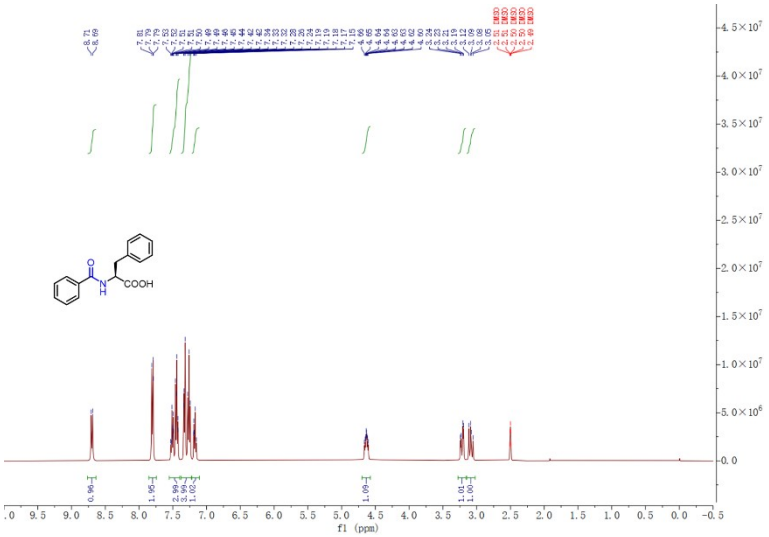
^{1}H NMR spectrum of **3ag**



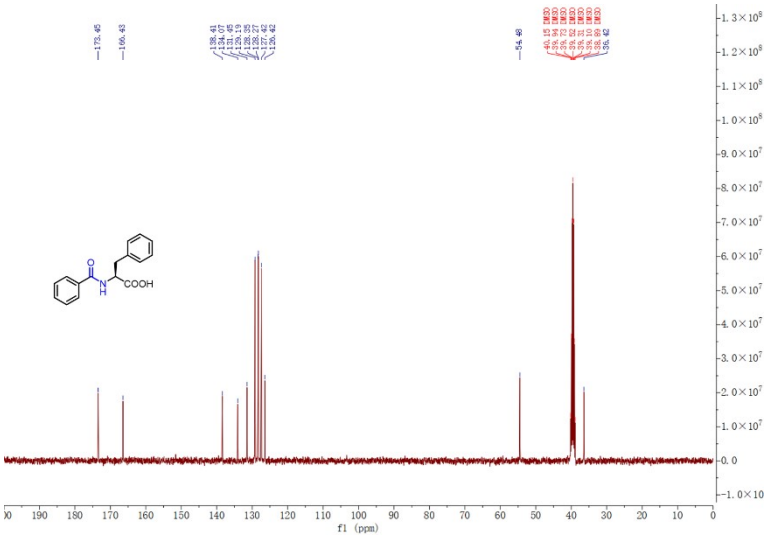
^{13}C NMR spectrum of **3ag**



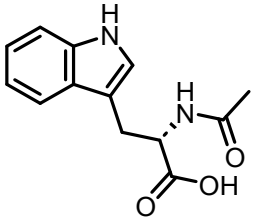
3bg, white powder, 80%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.70 (d, J = 8.2 Hz, 1H), 7.86 – 7.74 (m, 2H), 7.55 – 7.40 (m, 3H), 7.38 – 7.22 (m, 4H), 7.17 (t, J = 7.2 Hz, 1H), 4.63 (ddd, J = 10.4, 8.0, 4.3 Hz, 1H), 3.22 (dd, J = 13.8, 4.4 Hz, 1H), 3.09 (dd, J = 13.8, 10.6 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 173.5, 166.4, 138.4, 134.1, 131.5, 129.2, 128.4, 128.3, 127.4, 126.4, 54.5, 36.4.



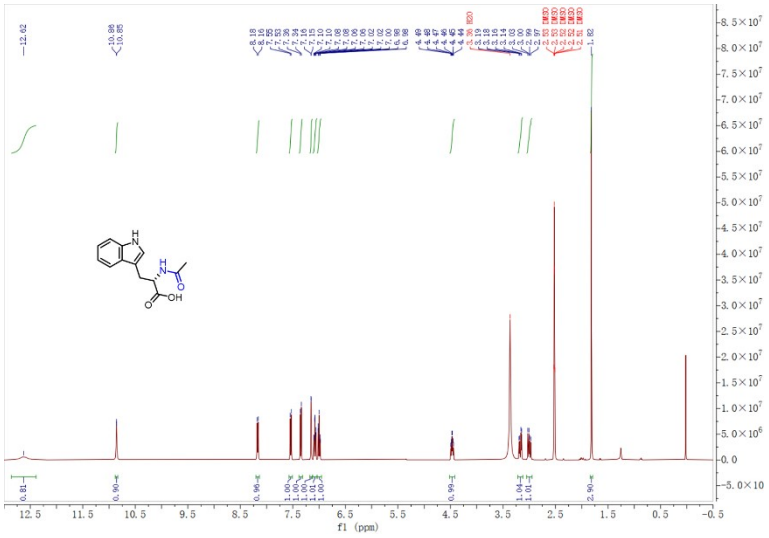
^{1}H NMR spectrum of **3bg**



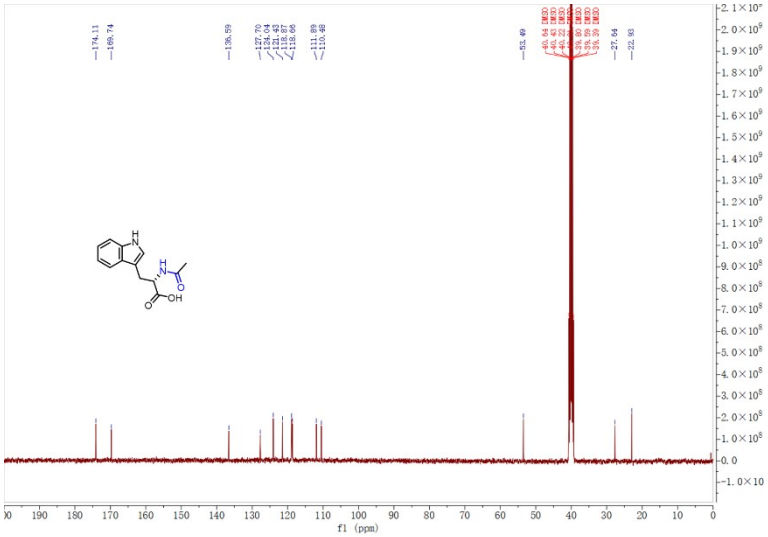
^{13}C NMR spectrum of **3bg**



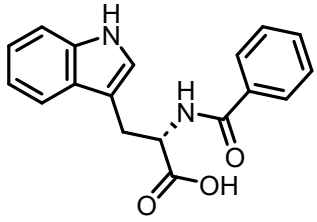
3ah, white powder, 93%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 12.62 (s, 1H), 10.88 – 10.83 (m, 1H), 8.17 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 8.0 Hz, 1H), 7.15 (d, J = 2.3 Hz, 1H), 7.12 – 7.04 (m, 1H), 7.04 – 6.96 (m, 1H), 4.47 (td, J = 8.3, 5.1 Hz, 1H), 3.17 (dd, J = 14.6, 5.1 Hz, 1H), 3.00 (dd, J = 14.6, 8.8 Hz, 1H), 1.82 (s, 3H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.1, 169.7, 136.6, 127.7, 124.0, 121.4, 118.9, 118.7, 111.9, 110.5, 53.5, 27.6, 22.9.



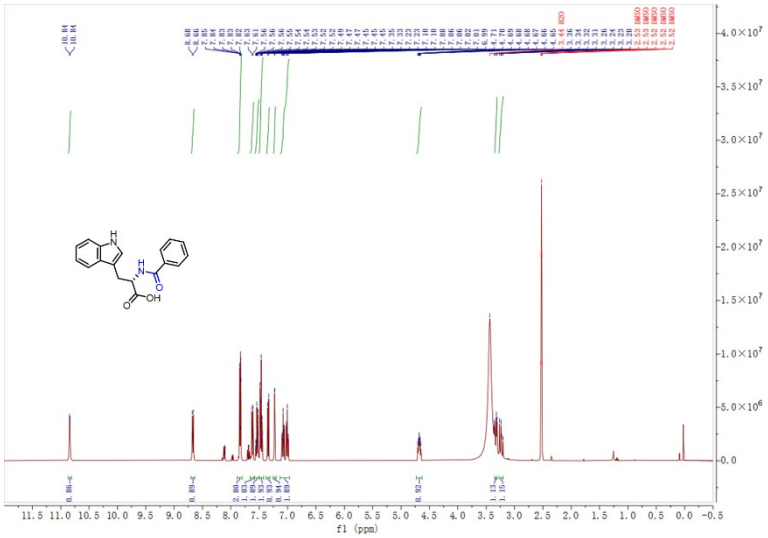
^{1}H NMR spectrum of **3ah**



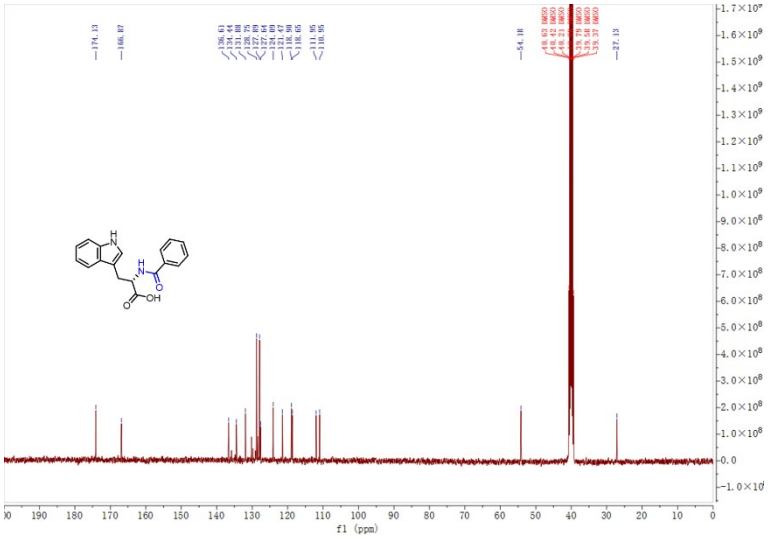
^{13}C NMR spectrum of **3ah**



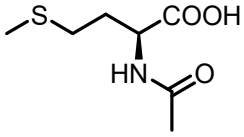
3bh, white powder, 84%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 10.84 (d, J = 2.6 Hz, 1H), 8.67 (d, J = 7.9 Hz, 1H), 7.89 – 7.80 (m, 2H), 7.62 (d, J = 7.9 Hz, 1H), 7.58 – 7.50 (m, 1H), 7.47 (dd, J = 8.2, 6.6 Hz, 2H), 7.34 (d, J = 8.0 Hz, 1H), 7.23 (d, J = 2.3 Hz, 1H), 7.13 – 6.97 (m, 2H), 4.68 (ddd, J = 9.9, 7.8, 4.5 Hz, 1H), 3.35 – 3.31 (m, 1H), 3.23 (dd, J = 14.7, 9.9 Hz, 1H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.1, 166.9, 136.6, 134.4, 131.9, 128.8, 127.9, 127.6, 124.1, 121.5, 118.9, 118.7, 112.0, 111.0, 54.2, 27.1.



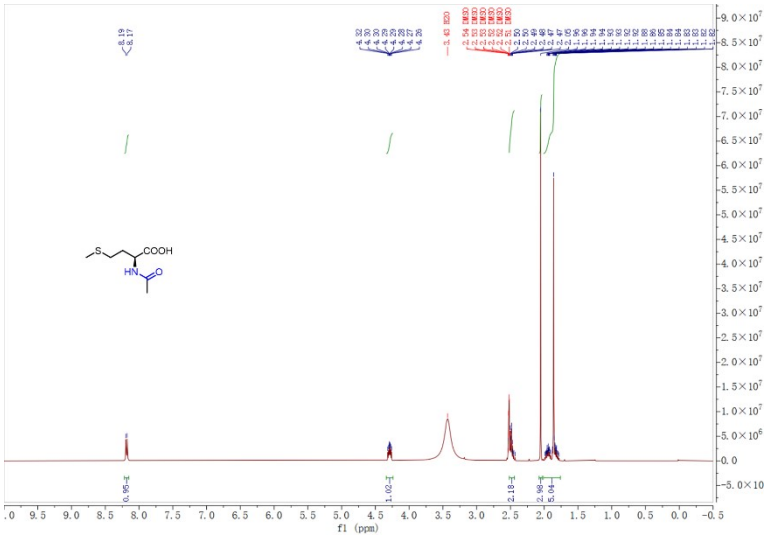
^{1}H NMR spectrum of **3bh**



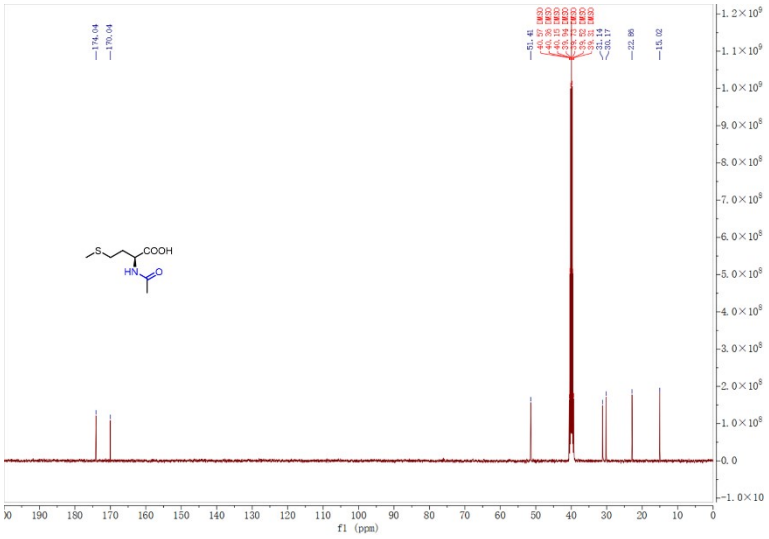
^{13}C NMR spectrum of **3bh**



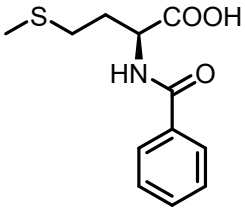
3ai, yellow powder, 88%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.18 (d, J = 7.8 Hz, 1H), 4.29 (ddd, J = 9.3, 7.8, 4.6 Hz, 1H), 2.52 – 2.44 (m, 2H), 2.05 (s, 3H), 2.01 – 1.76 (m, 5H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.0, 170.0, 51.4, 31.1, 30.2, 22.9, 15.0.



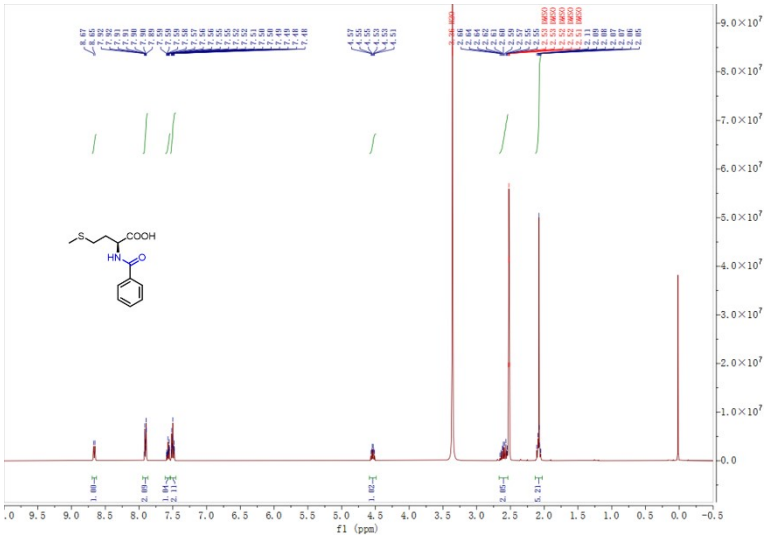
^{1}H NMR spectrum of **3ai**



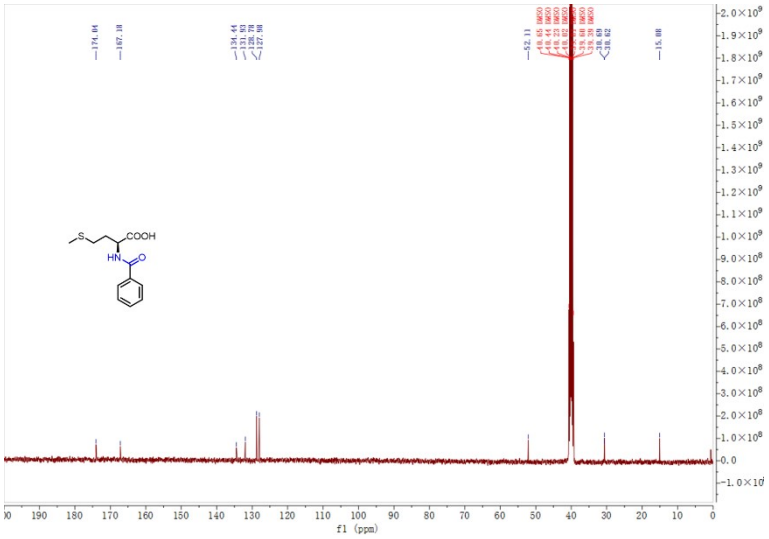
^{13}C NMR spectrum of **3ai**



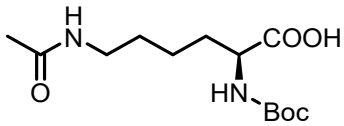
3bi, yellow powder, 75%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 8.66 (d, J = 7.8 Hz, 1H), 7.94 – 7.87 (m, 2H), 7.61 – 7.54 (m, 1H), 7.54 – 7.46 (m, 2H), 4.54 (td, J = 7.8, 6.2 Hz, 1H), 2.67 – 2.54 (m, 2H), 2.13 – 2.03 (m, 5H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.0, 167.2, 134.4, 131.9, 128.8, 128.0, 52.1, 30.7, 30.6, 15.1.



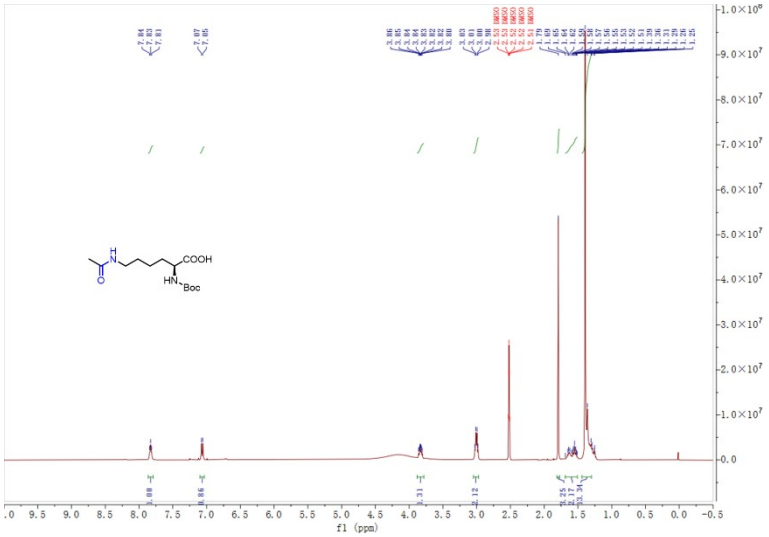
^{1}H NMR spectrum of **3bi**



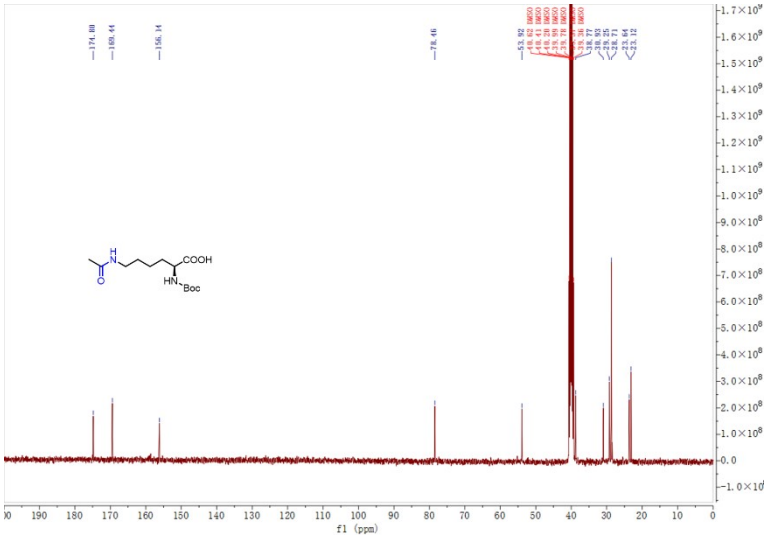
^{13}C NMR spectrum of **3bi**



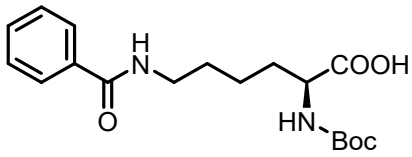
3aj, white powder, 82%. **^{1}H NMR** (400 MHz, DMSO-d_6) δ 7.83 (t, J = 5.6 Hz, 1H), 7.06 (d, J = 8.0 Hz, 1H), 3.83 (ddd, J = 9.4, 7.9, 4.7 Hz, 1H), 3.00 (q, J = 6.4 Hz, 2H), 1.79 (s, 3H), 1.69 – 1.51 (m, 2H), 1.39 (s, 13H). **^{13}C NMR** (101 MHz, DMSO-d_6) δ 174.8, 169.4, 156.1, 78.5, 53.9, 38.8, 30.9, 29.3, 28.7, 23.6, 23.1.



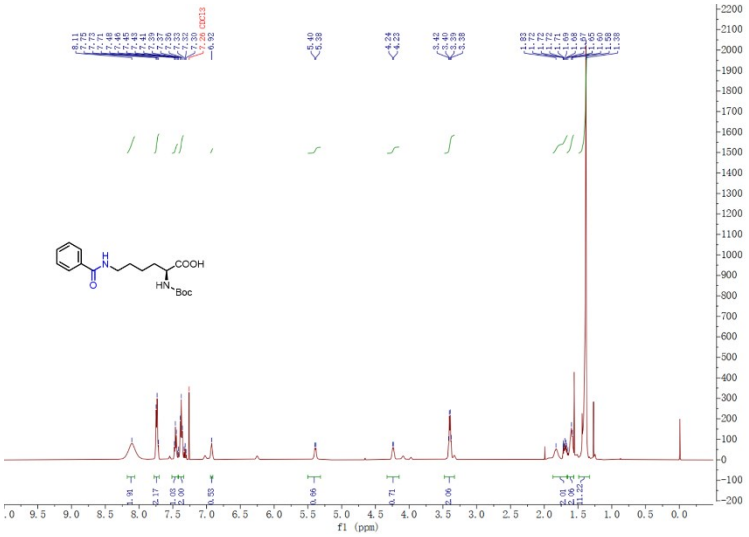
^{1}H NMR spectrum of **3aj**



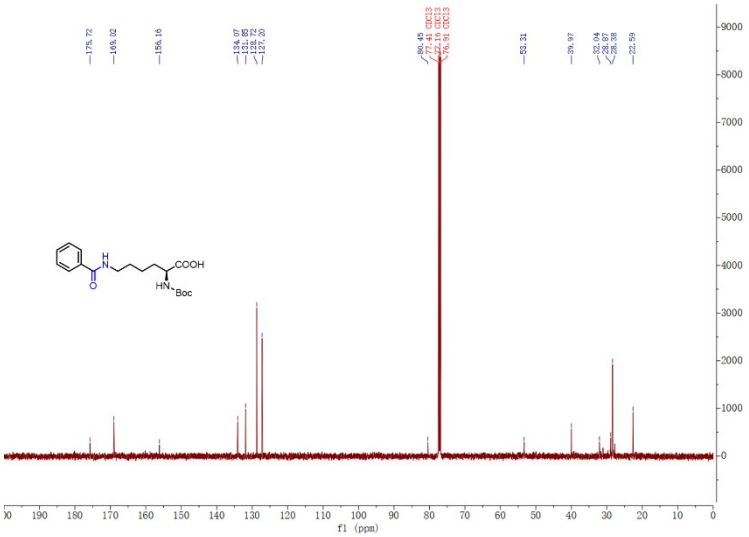
^{13}C NMR spectrum of **3aj**



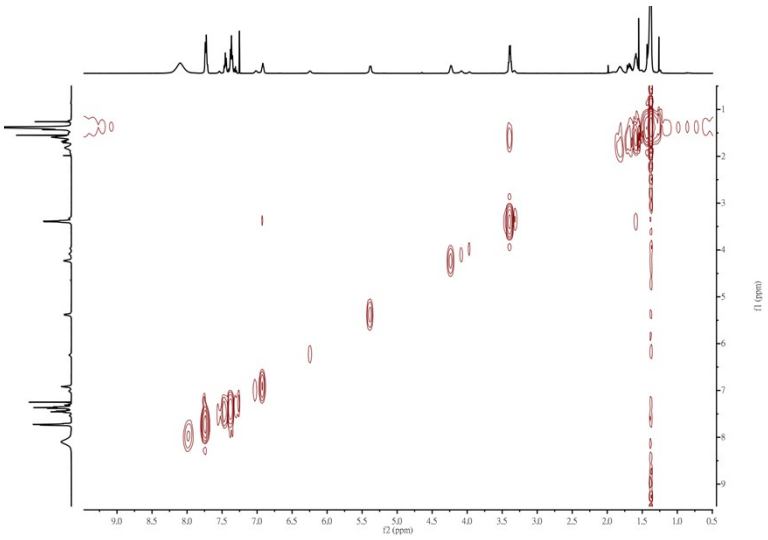
3bj, white powder, 73%. **^{1}H NMR** (500 MHz, $CDCl_3$) δ 8.11 (s, 2H), 7.73 (t, *J* = 8.5 Hz, 2H), 7.45 (q, *J* = 9.0, 8.1 Hz, 1H), 7.37 (t, *J* = 7.5 Hz, 2H), 6.92 (s, 1H), 5.39 (d, *J* = 6.9 Hz, 1H), 4.33 – 4.15 (m, 1H), 3.40 (q, *J* = 6.5 Hz, 2H), 1.87 – 1.66 (m, 2H), 1.66 – 1.56 (m, 2H), 1.38 (s, 11H). **^{13}C NMR** (126 MHz, $CDCl_3$) δ 175.7, 169.0, 156.2, 134.1, 131.9, 128.7, 127.2, 80.5, 53.3, 40.0, 32.0, 28.9, 28.4, 22.6.



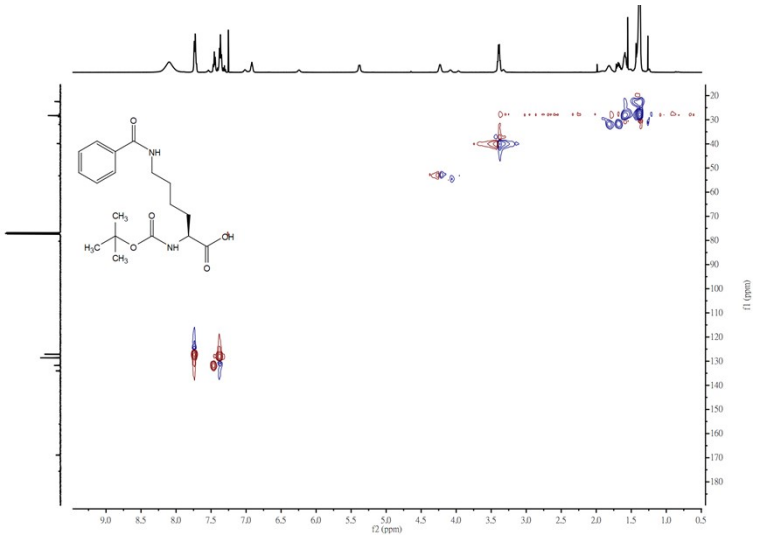
^{1}H NMR spectrum of **3bj**



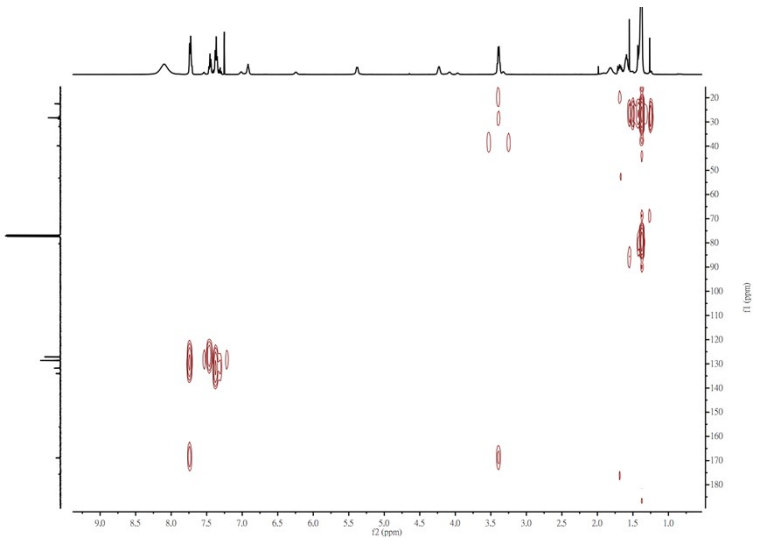
^{13}C NMR spectrum of **3bj**



^{1}H-^{1}H COSY spectrum of **3bj**



^{1}H-^{13}C HSQC spectrum of **3bj**



^{1}H-^{13}C HMBC spectrum of **3bj**

2.4 Mechanistic studies

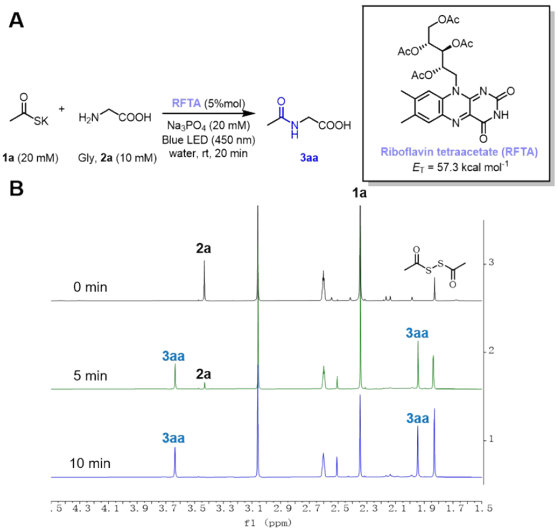


Figure S6 Visible-light-induced amidation of thioacid. (**A**) Screening of optimized conditions. Standard conditions: **1a** (20 mM), **2** (10 mM) and photocatalysts (5% mol) under blue LED (450 nm) for 20 min in D_2O at rt. Yield was determined by ^{1}H NMR with dimethyl sulfone (MSM) as internal standard. (**B**) Assignment for ^{1}H NMR analysis of the reaction.

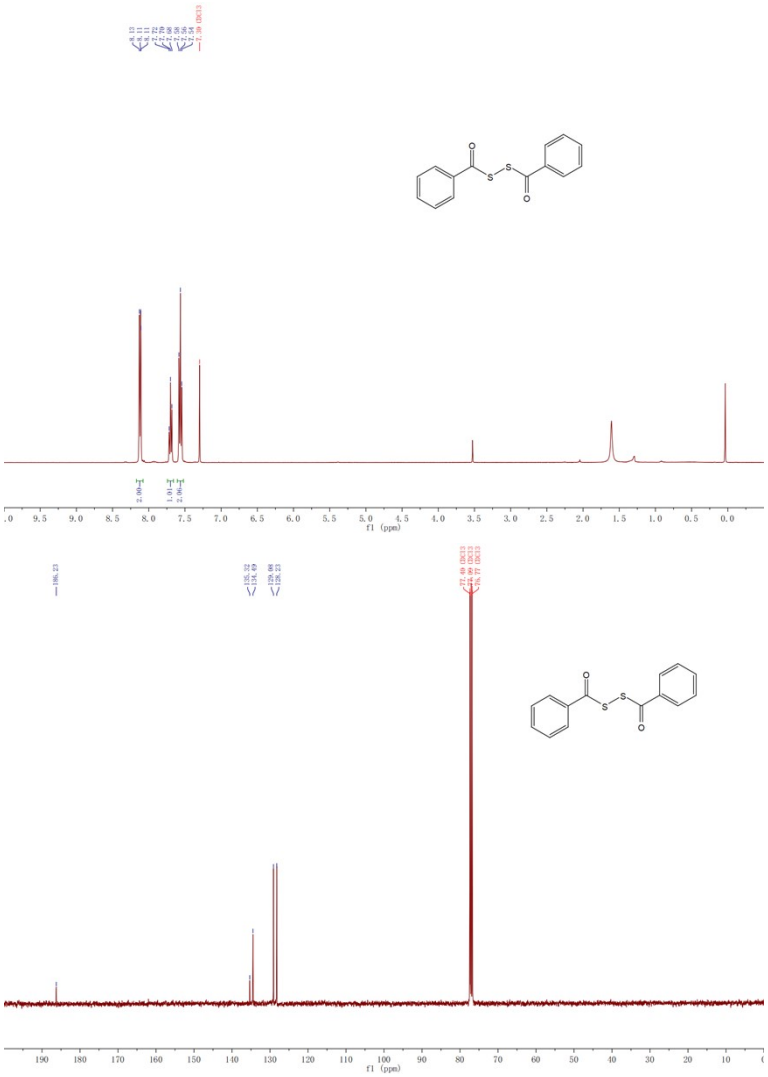


Figure S7 ^{1}H and ^{13}C NMR of isolated 1c intermediates.

2.4.1 Luminescence screening

The luminescence screening was performed by following a similar procedure of previous report. In order to consistent with the reaction conditions, PBS buffer (pH 7.4, 25%MeCN) was used as the solvent for luminescence experiments. A photocatalyst concentration of 20 μM was used throughout the experiments along with substrate concentrations of 20 mM, which equates to 1000 equivalents of each potential quencher relative to the photocatalyst. The luminescence emission spectrum of each sample excited at 450 nm was measured twice and an average was taken. The emission intensity (I) at a predefined wavelength was noted and compared with that of the photocatalyst in isolation (I_0). The amount of decrease in the emission intensity was then quantified as a "quenching percentage" (F) defined by the following formula:

$$F(\%) = 100\left(1 - \frac{I}{I_0}\right)\% \quad (1)$$

The structure of the photocatalysts employed in this study are shown in **Figure S3**. The results were shown in **Figure S8**.

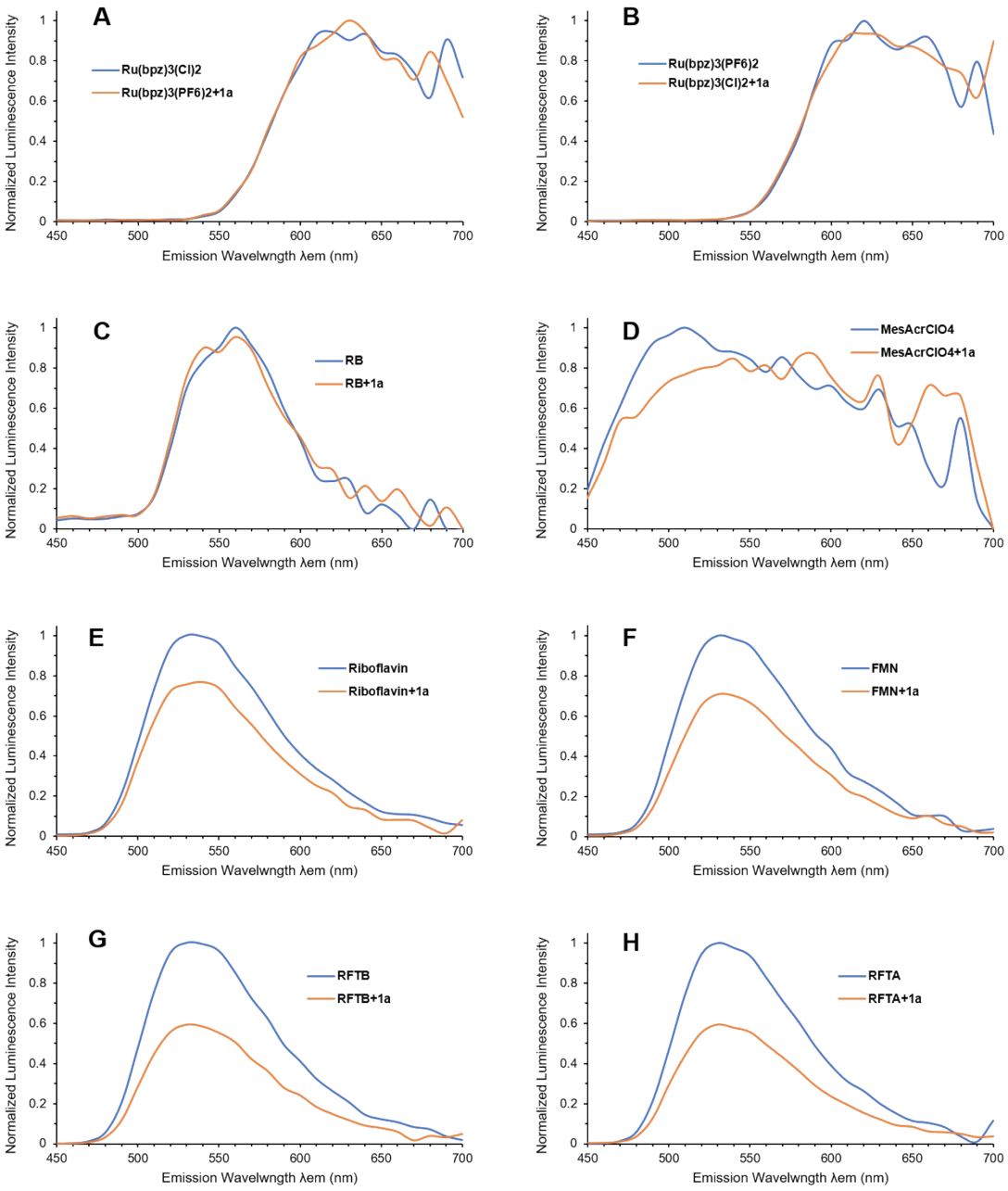


Figure S8. Luminescence spectra of the screening of the photocatalysts with thioacid **1a**.

2.4.2 Stern-Volmer luminescence quenching studies

Stern-Volmer luminescence quenching studies were carried out using a 20 μM solution of RFTA and variable concentrations of **1a** in PBS buffer (pH 7.4, 25% MeCN). Two independent duplications were performed for two substrates. The solutions were irradiated at 420 nm and the luminescence was measured at 530 nm (I_0 = emission intensity of the photocatalyst in isolation at the specified wavelength; I = observed intensity as a function of the quencher concentration). The luminescence spectra and were summarized in **Figure S9**.

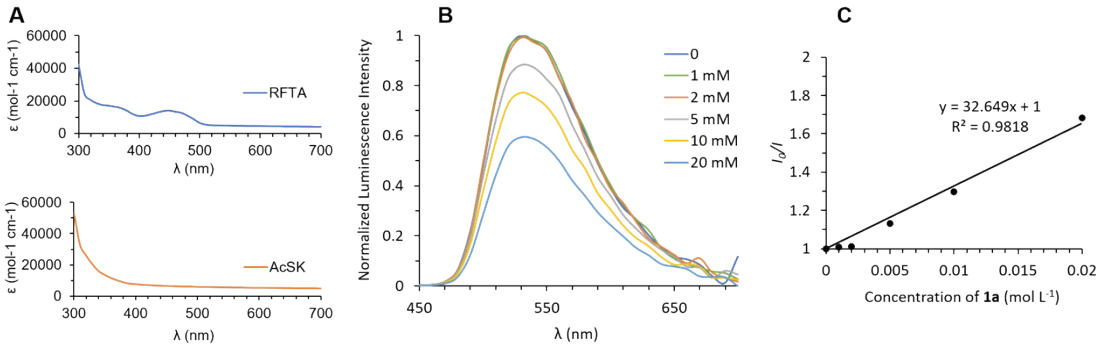


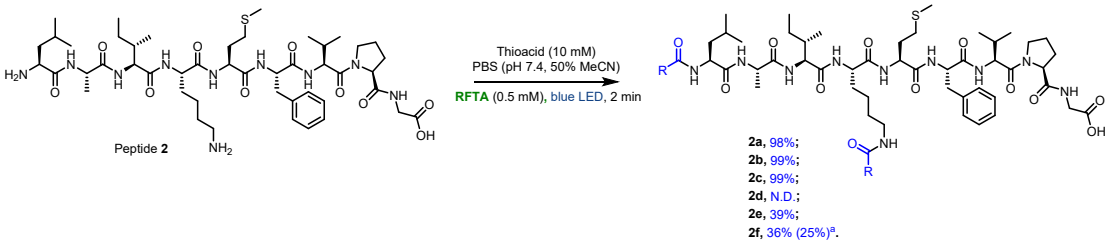
Figure S9. Stern-Volmer luminescence quenching analysis for RFTA with thioacid **1a**.

2.5 General procedure B for the reaction between thioacids and peptides

LC-MS yields were estimated by UV absorption at 220 nm of the peak corresponding to the thioacetal adducted product versus the internal standard (diphenyl sulfoxide): % yield = $(A_p/A_{st}) \times k$. A_p is the peak area of thioacetal-adducted products; A_{st} is the peak area of the internal standard; k is the quantity coefficient between specific peptide and standard. The quantity coefficients were measured by the correlation of a gradient concentration.

2.6 Characterization of the reaction between thioacids and peptides

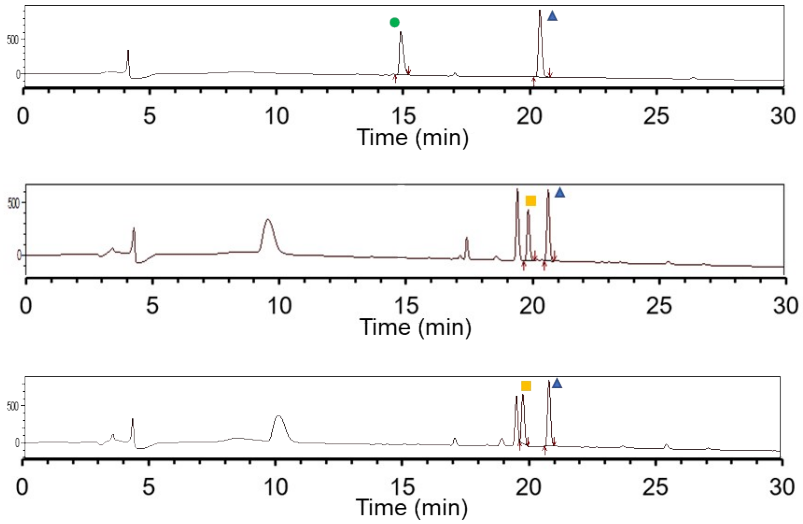
2.6.1 Characterization of the reaction of peptide 2



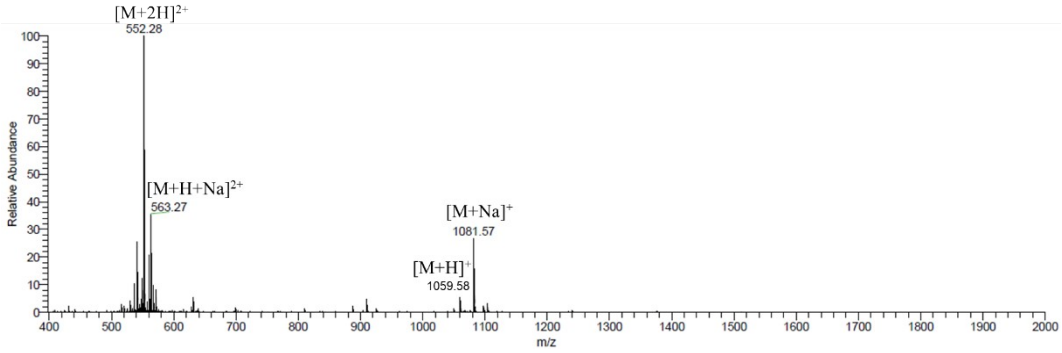
The reaction was followed **General Procedure B** using peptide **2** (1 mM), sequence: **<u>NH$_2$</u>**-

LAI**K**MFVPG-OH, and thioacid **1a-1f**. A stock solution of peptide **2** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1 mg peptide **2**.

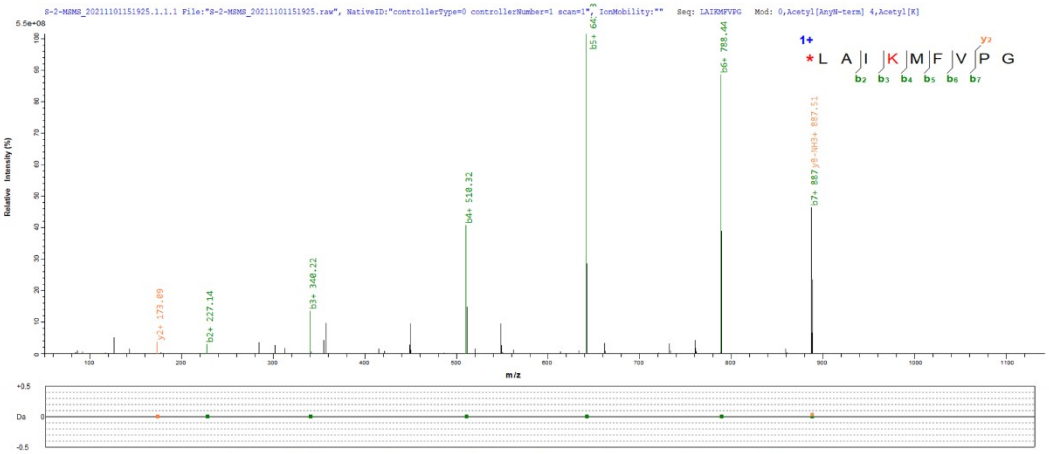
Characterization data of 2a and 2b



HPLC traces of the quantity relationship of matter and crude reaction of **2a** and **2b**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

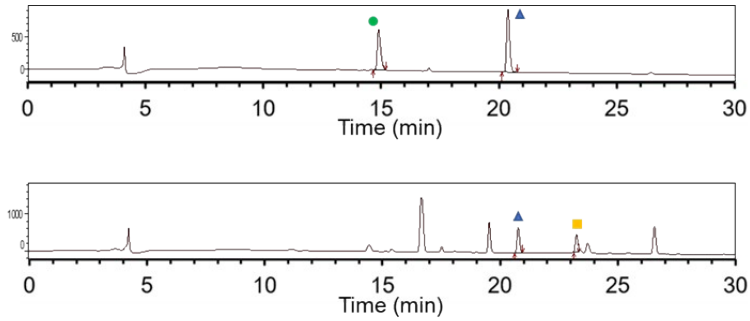


ESI Mass spectrum of purified product **2a**. Calculated Mass $[M+H]^+$: 1059.58, $[M+2H]^{2+}$: 552.28; Mass Found (ESI+) $[M+H]^+$: 1059.58, $[M+2H]^{2+}$: 552.28.

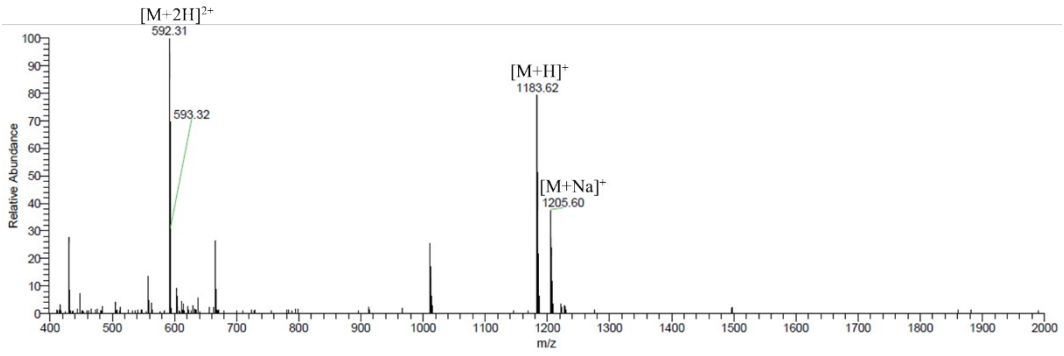


#	L(1/*)	A(2/8)	I(3/7)	K(4/6)	M(5/5)	F(6/4)	V(7/3)	P(8/2)	G(*/1)
b+	156.1019	227.1390	340.2231	510.3286	641.3691	788.4375	887.5059	984.5587	*
b++	78.5546	114.0731	170.6152	255.6679	321.1882	394.7224	444.2566	492.7830	*
y+	*	904.4961	833.4590	720.3749	550.2694	419.2289	272.1605	173.0921	76.0393
y++	*	452.7517	417.2331	360.6911	275.6383	210.1181	136.5839	87.0497	38.5233

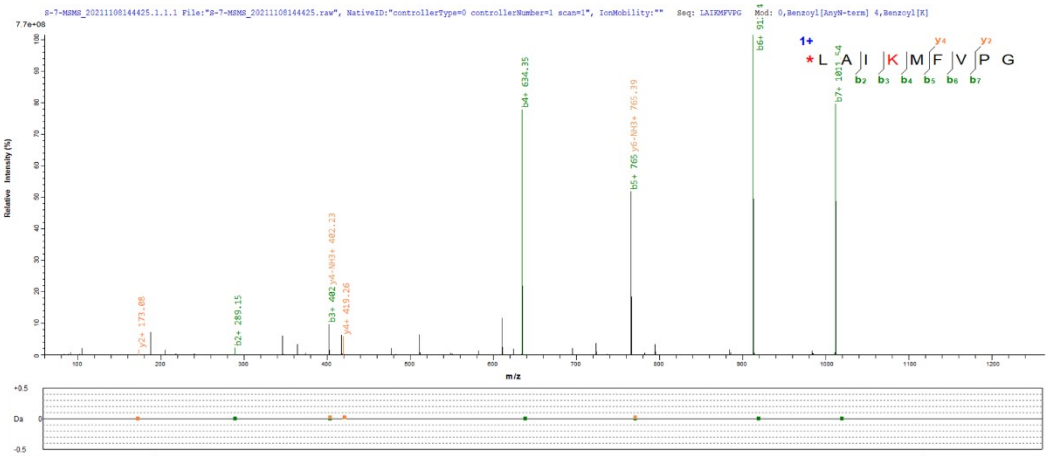
MS/MS analysis of **2a**



HPLC traces of the quantity relationship of matter and crude reaction of **2c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

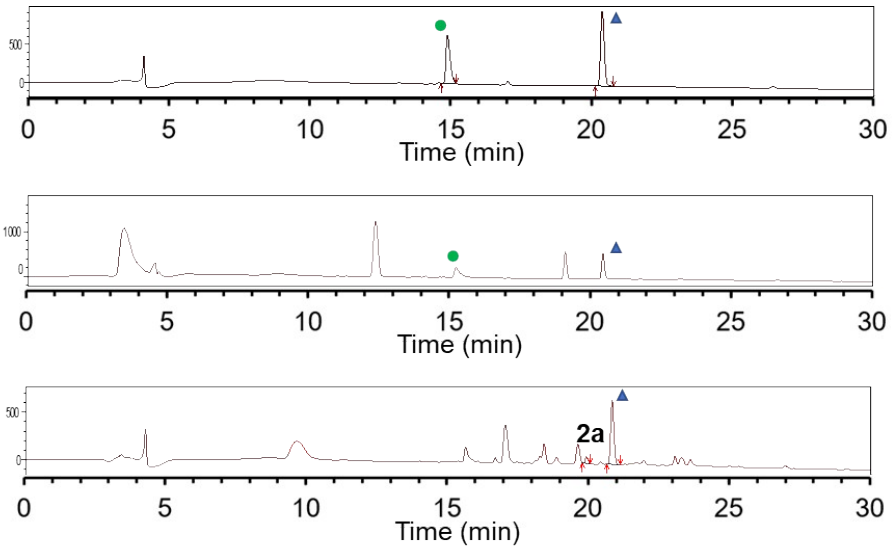


ESI Mass spectrum of purified product **2c**. Calculated Mass $[M+H]^+$: 1183.62, $[M+2H]^{2+}$: 592.31; Mass Found (ESI+) $[M+H]^+$: 1183.62, $[M+2H]^{2+}$: 592.31.

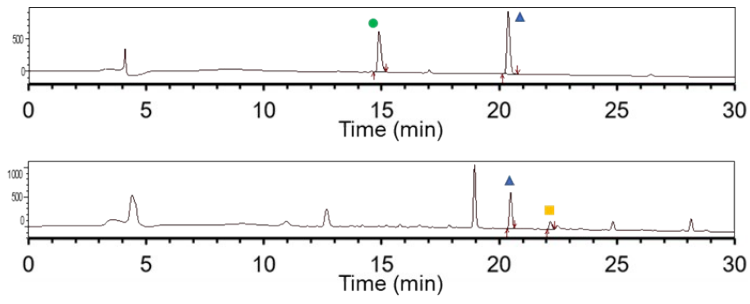


#	L(1/*)	A(2/8)	I(3/7)	K(4/6)	M(5/5)	F(6/4)	V(7/3)	P(8/2)	G(*/1)
b+	218.1176	289.1547	402.2387	634.3599	765.4004	912.4688	1011.5372	1108.5900	*
b++	109.5624	145.0810	201.6230	317.6836	383.2038	456.7380	506.2722	554.7986	*
y+	*	966.5117	895.4746	782.3905	550.2694	419.2289	272.1605	173.0921	76.0393
y++	*	483.7595	448.2409	391.6989	275.6383	210.1181	136.5839	87.0497	38.5233

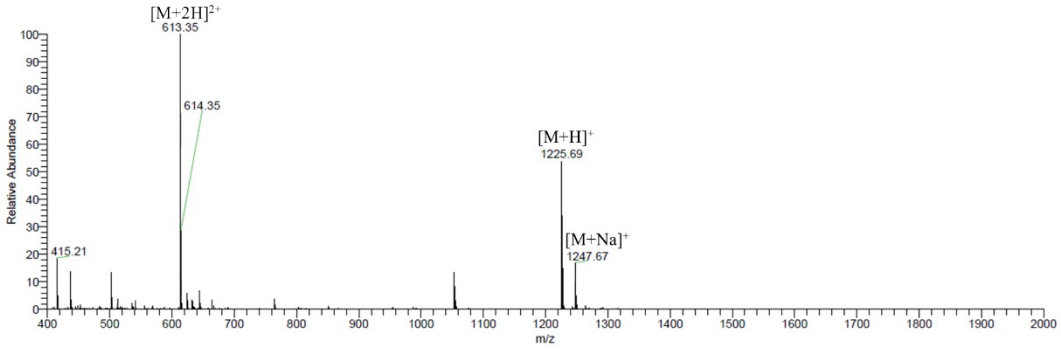
MS/MS analysis of **2c**



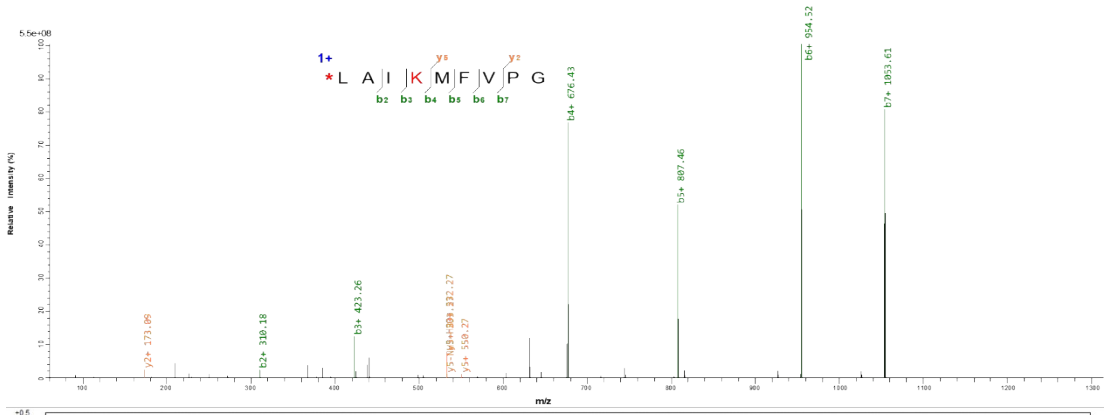
HPLC traces of the quantity relationship of matter, crude reaction of **2d** and a control reaction between peptide **2**, potassium thioacetate **1a** and alkynyl compound (pentynyl acid). (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle and blue triangle and orange square refer to the starting peptide and internal standard. No target product was detected in the reaction between **2** and **1d**. And, the adding of the alkynyl compound (pentynyl acid) decreased the conversion of the **2a** to around 10%.



HPLC traces of the quantity relationship of matter and crude reaction of **2e**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

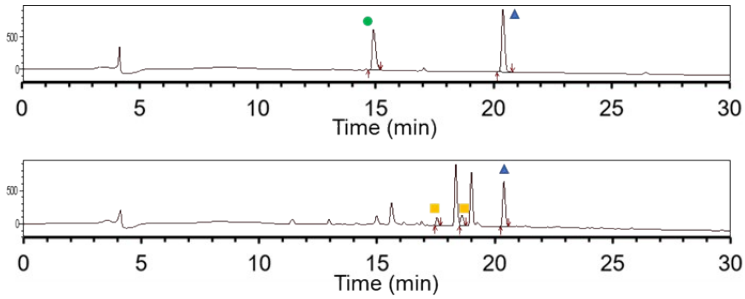


ESI Mass spectrum of purified product **2e**. Calculated Mass $[M+H]^+$: 1225.69, $[M+2H]^{2+}$: 613.35; Mass Found (ESI+) $[M+H]^+$: 1225.69, $[M+2H]^{2+}$: 613.35.



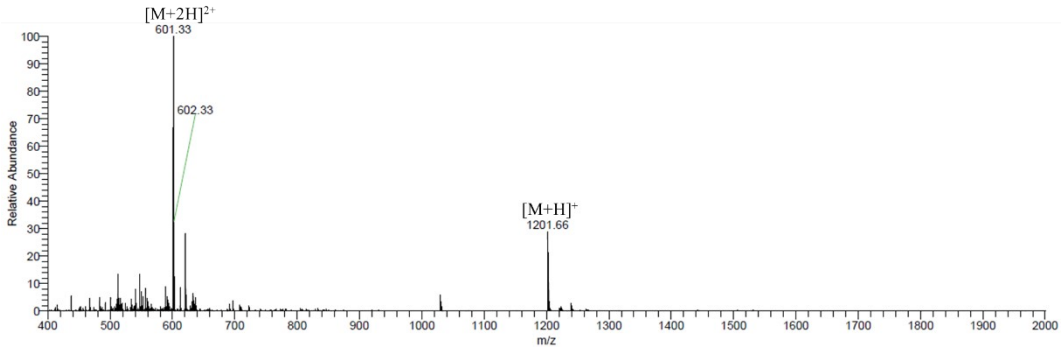
#	L(1/*)	A(2/8)	I(3/7)	K(4/6)	M(5/5)	F(6/4)	V(7/3)	P(8/2)	G(*/1)
b+	239.1502	310.1873	423.2714	676.4253	807.4658	954.5342	1053.6026	1150.6553	*
b++	120.0788	155.5973	212.1393	338.7163	404.2365	477.7707	527.3049	575.8313	*
y+	*	987.5444	916.5073	803.4232	550.2694	419.2289	272.1605	173.0921	76.0393
y++	*	494.2758	458.7573	402.2153	275.6383	210.1181	136.5839	87.0497	38.5233

MS/MS analysis of **2e**

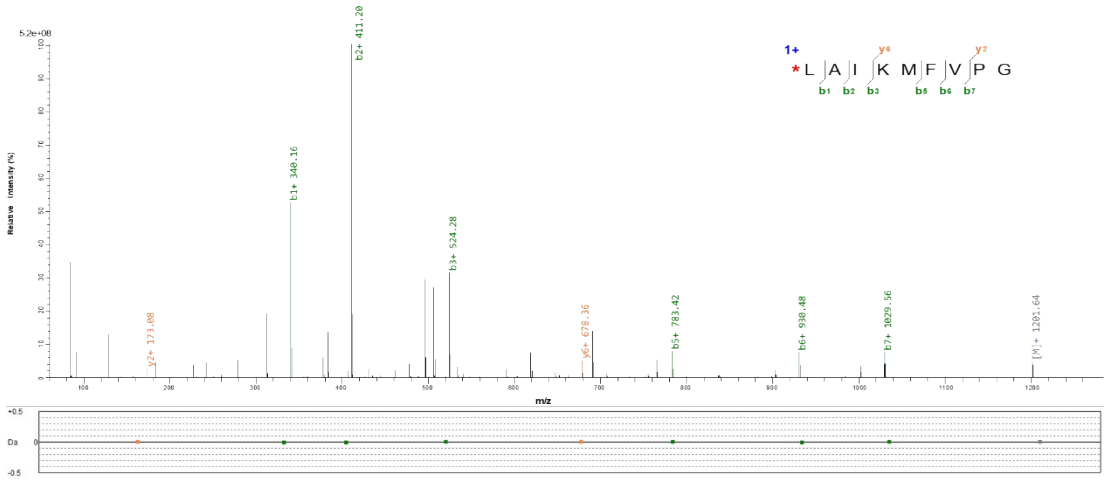


HPLC traces of the quantity relationship of matter and crude reaction of single-amidated and double-amidated **2f**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil

100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

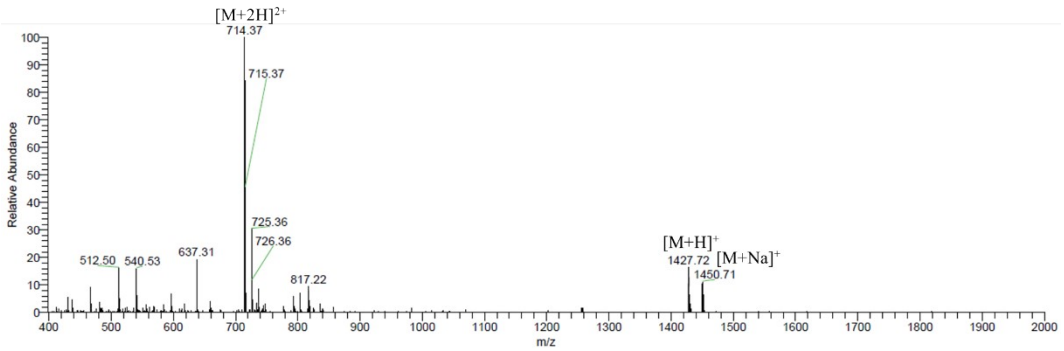


ESI Mass spectrum of purified single-amidated product **2f**. Calculated Mass $[M+H]^+$: 1201.66, $[M+2H]^{2+}$: 601.33; Mass Found (ESI+) $[M+H]^+$: 1201.66, $[M+2H]^{2+}$: 601.33.

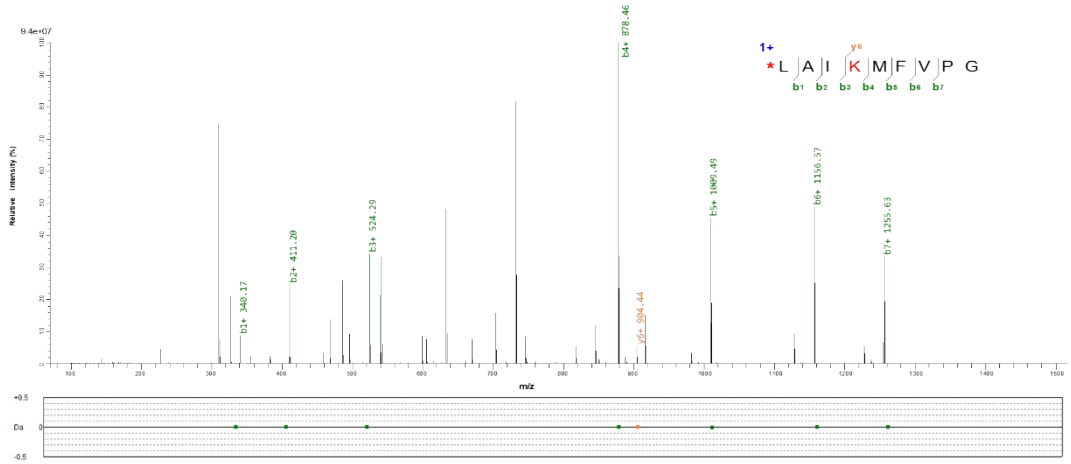


#	L(1/*)	A(2/8)	I(3/7)	K(4/6)	M(5/5)	F(6/4)	V(7/3)	P(8/2)	G(*/1)
b+	340.1689	411.2060	524.2901	652.3851	783.4256	930.4940	1029.5624	1126.6151	*
b++	170.5881	206.1067	262.6487	326.6962	392.2164	465.7506	515.2848	563.8112	*
y+	*	862.4855	791.4484	678.3643	550.2694	419.2289	272.1605	173.0921	76.0393
y++	*	431.7464	396.2278	339.6858	275.6383	210.1181	136.5839	87.0497	38.5233

MS/MS analysis of single-amidated **2f**



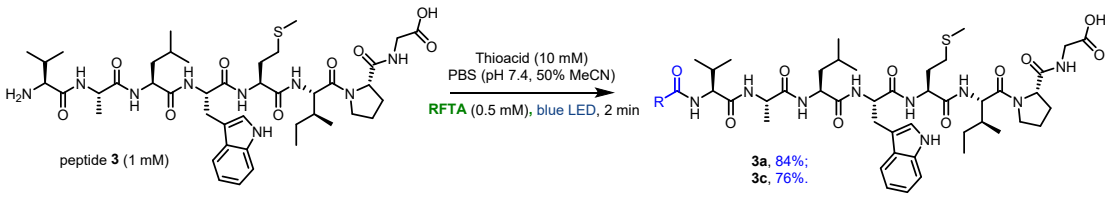
ESI Mass spectrum of purified double-amidated product **2f**. Calculated Mass $[M+H]^+$: 1427.72, $[M+2H]^{2+}$: 714.37; Mass Found (ESI+) $[M+H]^+$: 1427.72, $[M+2H]^{2+}$: 714.37.



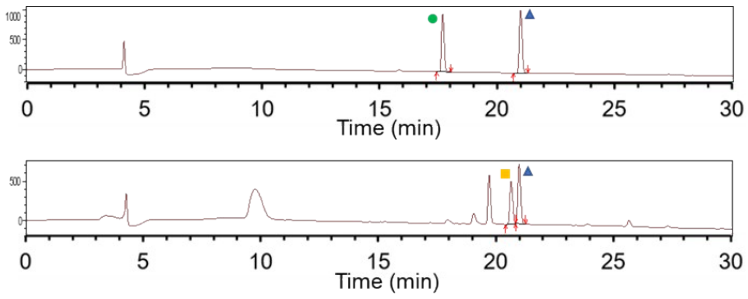
#	L(1/*)	A(2/8)	I(3/7)	K(4/6)	M(5/5)	F(6/4)	V(7/3)	P(8/2)	G(*/1)
b+	340.1689	411.2060	524.2901	878.4627	1009.5032	1156.5716	1255.6400	1352.6927	*
b++	170.5881	206.1067	262.6487	439.7350	505.2552	578.7894	628.3236	676.8500	*
y+	*	1088.5631	1017.5260	904.4419	550.2694	419.2289	272.1605	173.0921	76.0393
y++	*	544.7852	509.2666	452.7246	275.6383	210.1181	136.5839	87.0497	38.5233

MS/MS analysis of double-amidated **2f**

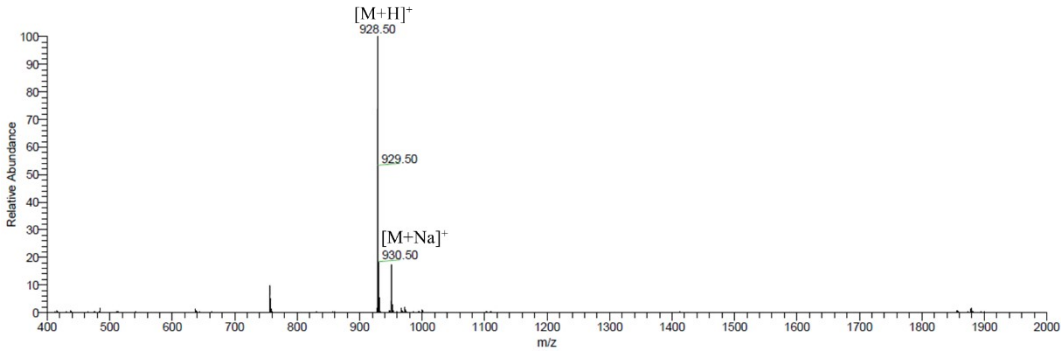
2.6.2 Characterization of the reaction of peptide 3



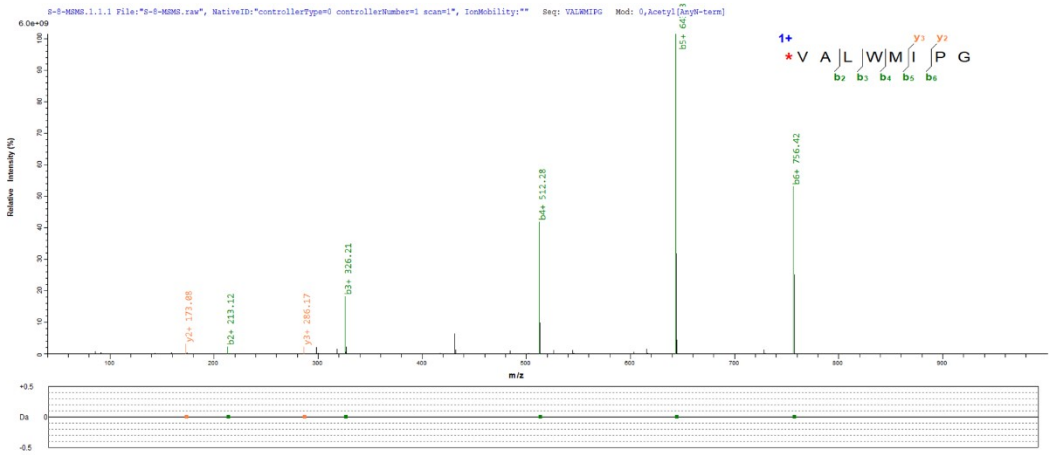
The reaction was followed **General Procedure B** using peptide **3** (1 mM), sequence: **$\underline{NH_2}$**-VALWMIPG-OH, and thioacid **1a** and **1c**. A stock solution of peptide **3** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 0.9 mg peptide **3**.



HPLC traces of the quantity relationship of matter and crude reaction of **3a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

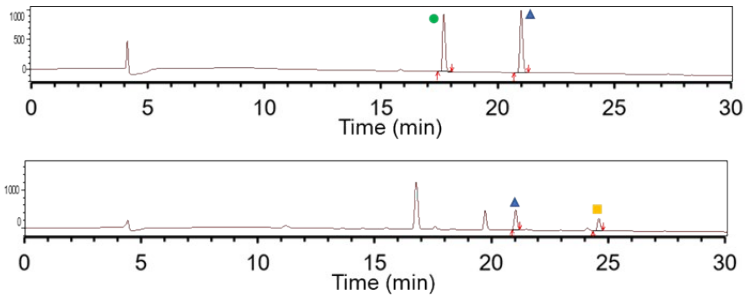


ESI Mass spectrum of purified product **3a**. Calculated Mass $[M+H]^+$: 928.50; Mass Found (ESI+) $[M+H]^+$: 928.50.

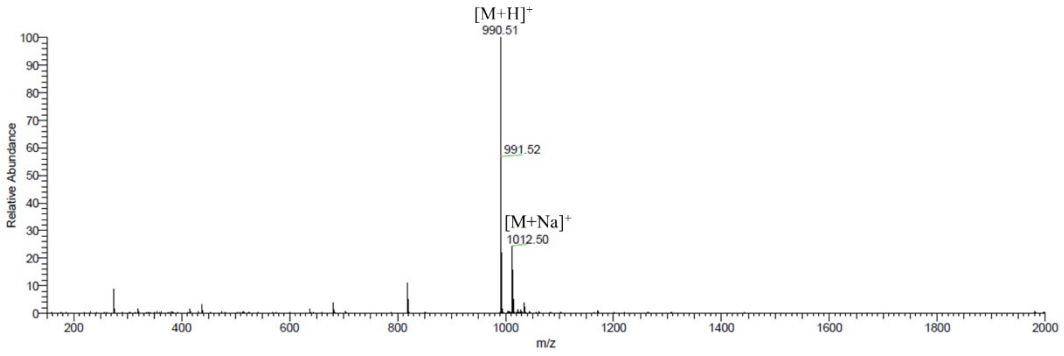


#	V(1/*)	A(2/7)	L(3/6)	W(4/5)	M(5/4)	I(6/3)	P(7/2)	G(*/1)
b+	142.0863	213.1234	326.2074	512.2867	643.3272	756.4113	853.4641	*
b++	71.5468	107.0653	163.6074	256.6470	322.1673	378.7093	427.2357	*
y+	*	787.4171	716.3800	603.2959	417.2166	286.1761	173.0921	76.0393
y++	*	394.2122	358.6936	302.1516	209.1119	143.5917	87.0497	38.5233

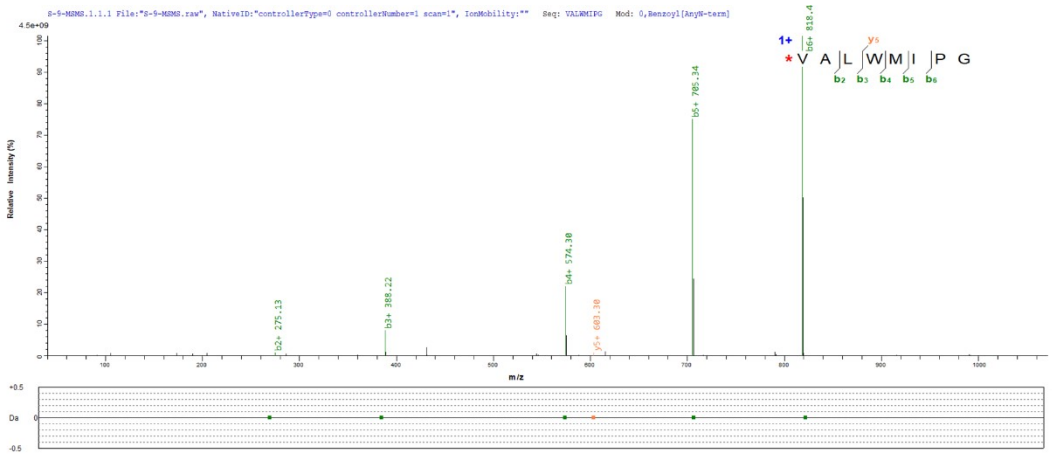
MS/MS analysis of **3a**



HPLC traces of the quantity relationship of matter and crude reaction of **3c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.



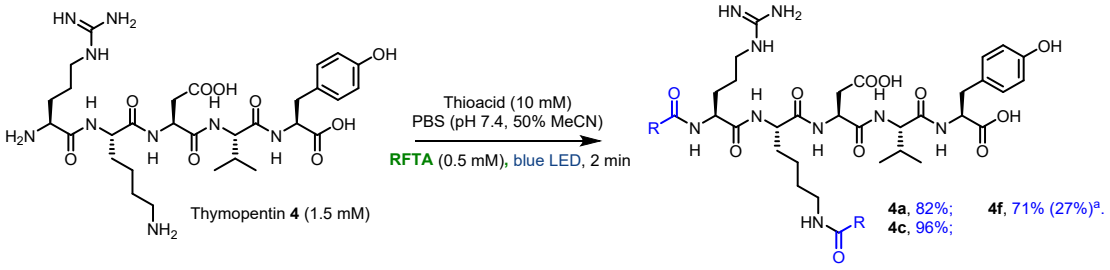
ESI Mass spectrum of purified product **3c**. Calculated Mass $[M+H]^+$: 990.51; Mass Found (ESI+) 990.51.



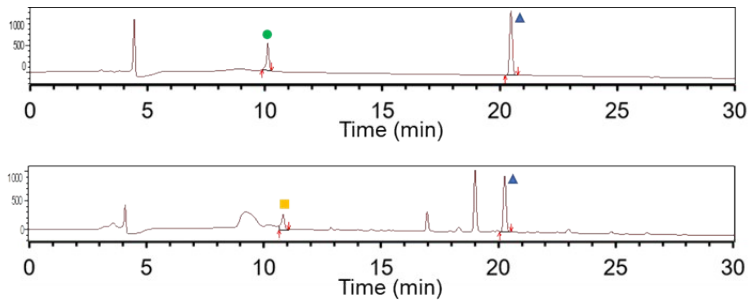
#	V(1/*)	A(2/7)	L(3/6)	W(4/5)	M(5/4)	I(6/3)	P(7/2)	G(*/1)
b+	204.1019	275.1390	388.2231	574.3024	705.3429	818.4269	915.4797	*
b++	102.5546	138.0731	194.6152	287.6548	353.1751	409.7171	458.2435	*
y+	*	787.4171	716.3800	603.2959	417.2166	286.1761	173.0921	76.0393
y++	*	394.2122	358.6936	302.1516	209.1119	143.5917	87.0497	38.5233

MS/MS analysis of **3c**

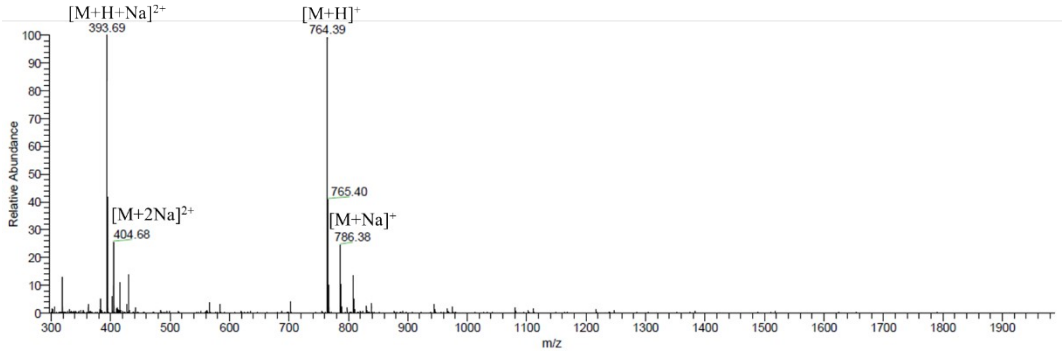
2.6.3 Characterization of the reaction of peptide 4



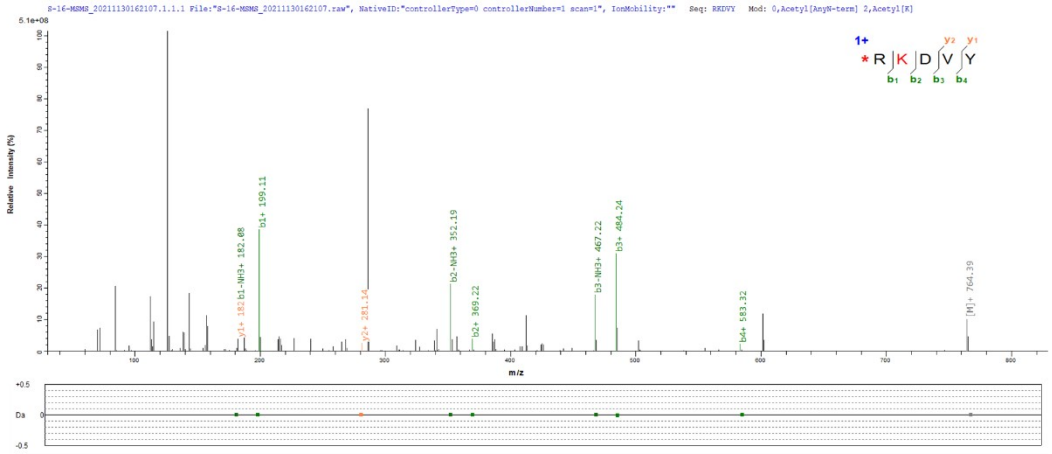
The reaction was followed **General Procedure B** using peptide **4** (1.5 mM), sequence: **$\underline{NH_2}$**-R**$\underline{K}$**DVY-OH, and thioacid **1a**, **1c** and **1f**. A stock solution of peptide **4** (1.5 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1 mg peptide **4**.



HPLC traces of the quantity relationship of matter and crude reaction of **4a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

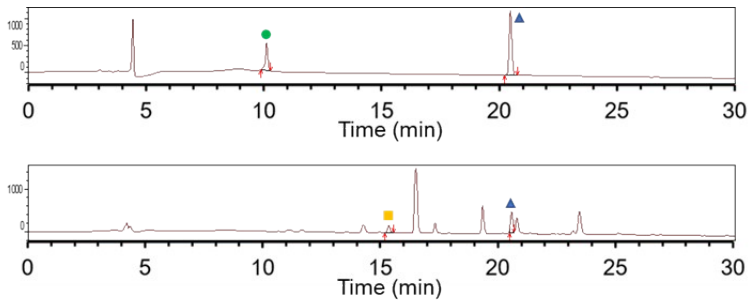


ESI Mass spectrum of purified product **4a**. Calculated Mass $[M+H]^+$: 764.39; Mass Found (ESI+) $[M+H]^+$: 764.39.

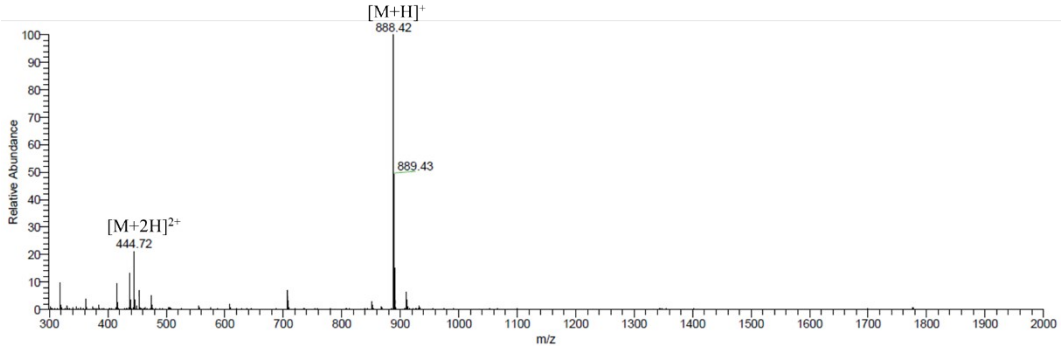


#	R(1/*)	K(2/4)	D(3/3)	V(4/2)	Y(*/1)
b+	199.1189	369.2245	484.2514	583.3198	*
b++	100.0631	185.1159	242.6293	292.1635	*
y+	*	566.2820	396.1765	281.1496	182.0812
y++	*	283.6447	198.5919	141.0784	91.5442

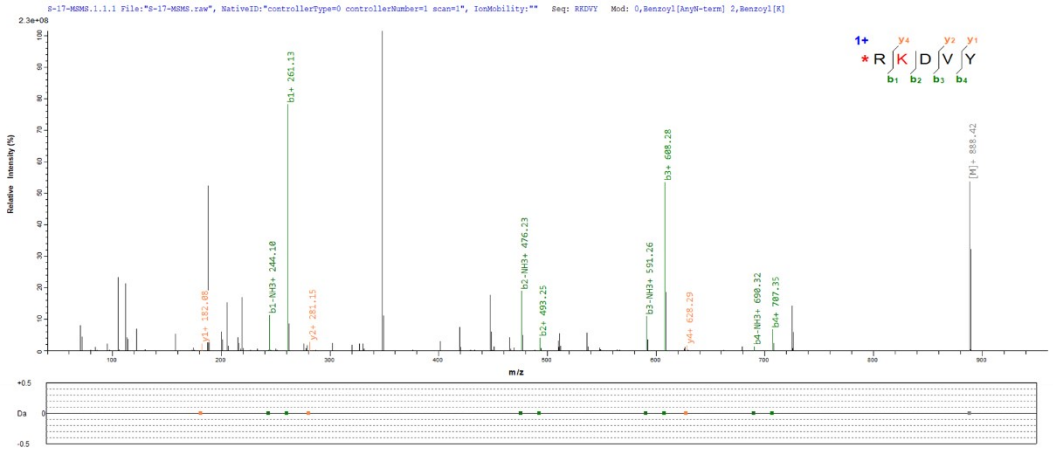
MS/MS analysis of **4a**



HPLC traces of the quantity relationship of matter and crude reaction of **4c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

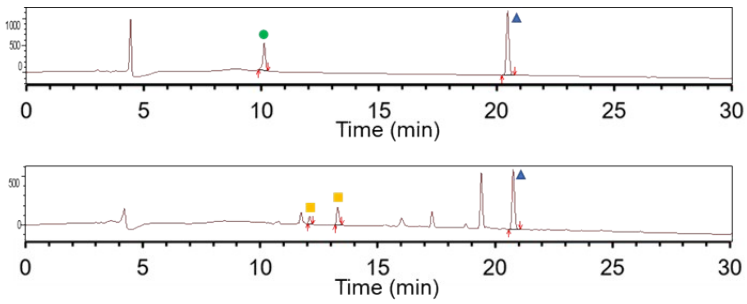


ESI Mass spectrum of purified product **4c**. Calculated Mass $[M+H]^+$: 888.42; Mass Found (ESI+) $[M+H]^+$: 888.42.

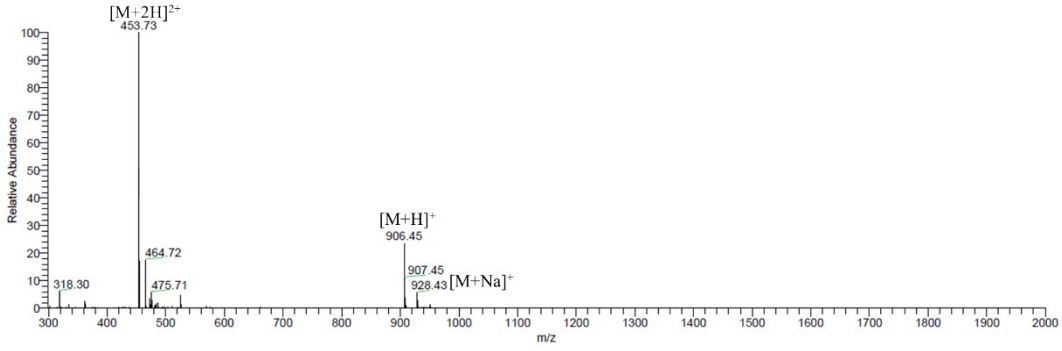


#	R(1/*)	K(2/4)	D(3/3)	V(4/2)	Y(*/1)
b+	261.1346	493.2558	608.2827	707.3511	*
b++	131.0709	247.1315	304.6450	354.1792	*
y+	*	628.2977	396.1765	281.1496	182.0812
y++	*	314.6525	198.5919	141.0784	91.5442

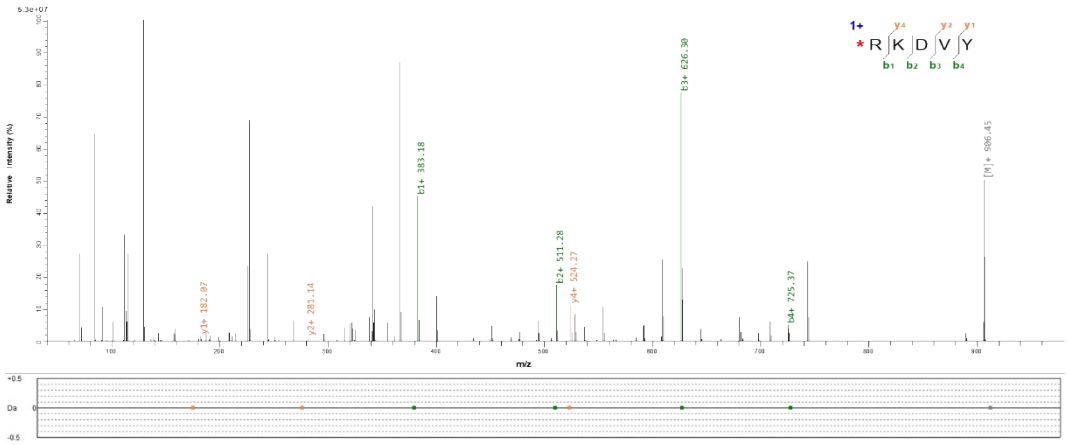
MS/MS analysis of **4c**



HPLC traces of the quantity relationship of matter and crude reaction of single-amidated and double-amidated **4f**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

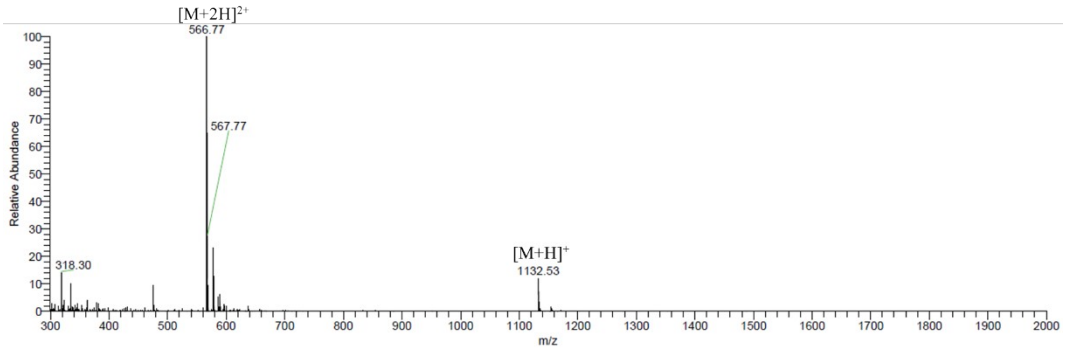


ESI Mass spectrum of purified single-amidated product **4f**. Calculated Mass $[M+H]^+$: 906.45, $[M+2H]^{2+}$: 453.73; Mass Found (ESI+) $[M+H]^+$: 906.45, $[M+2H]^{2+}$: 453.73.

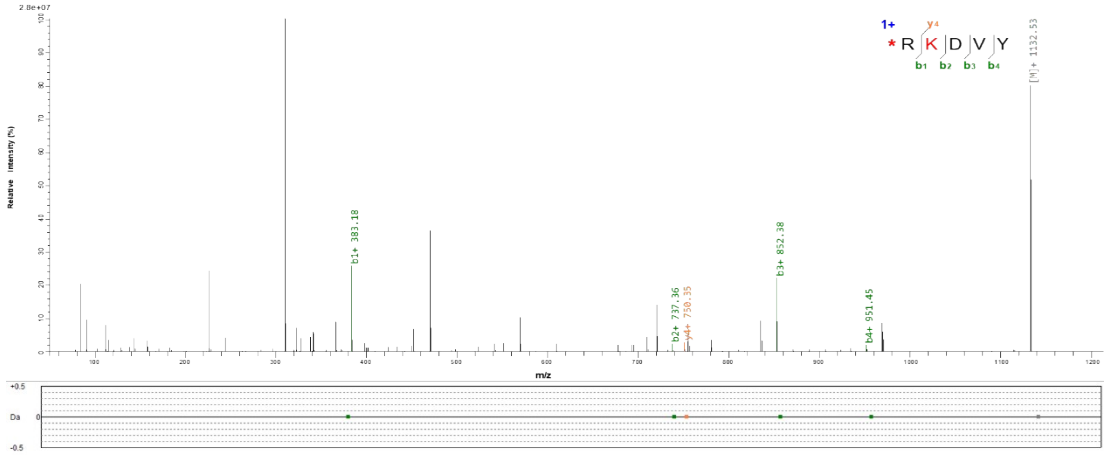


#	R(1/*)	K(2/4)	D(3/3)	V(4/2)	Y(*/1)
b+	383.1860	511.2809	626.3079	725.3763	*
b++	192.0966	256.1441	313.6576	363.1918	*
y+	*	524.2715	396.1765	281.1496	182.0812
y++	*	262.6394	198.5919	141.0784	91.5442

MS/MS analysis of single-amidated **4f**



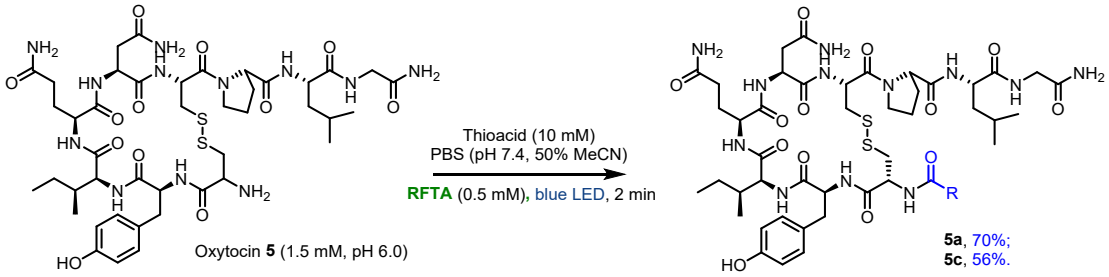
ESI Mass spectrum of purified double-amidated product **4f**. Calculated Mass $[M+H]^+$: 1132.53, $[M+2H]^{2+}$: 566.77; Mass Found (ESI+) $[M+H]^+$: 1132.53, $[M+2H]^{2+}$: 566.77.



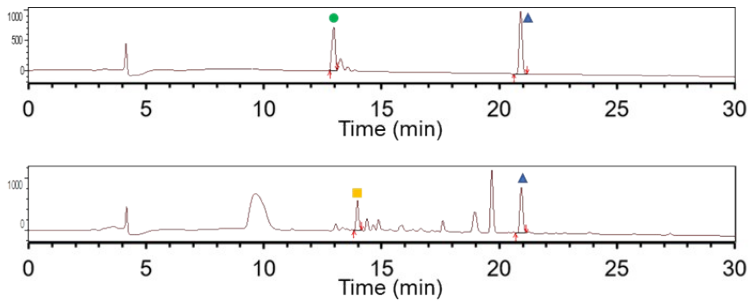
#	R(1/*)	K(2/4)	D(3/3)	V(4/2)	Y(*/1)
b+	383.1860	737.3585	852.3855	951.4539	*
b++	192.0966	369.1829	426.6964	476.2306	*
y+	*	750.3491	396.1765	281.1496	182.0812
y++	*	375.6782	198.5919	141.0784	91.5442

MS/MS analysis of double-amidated **4f**

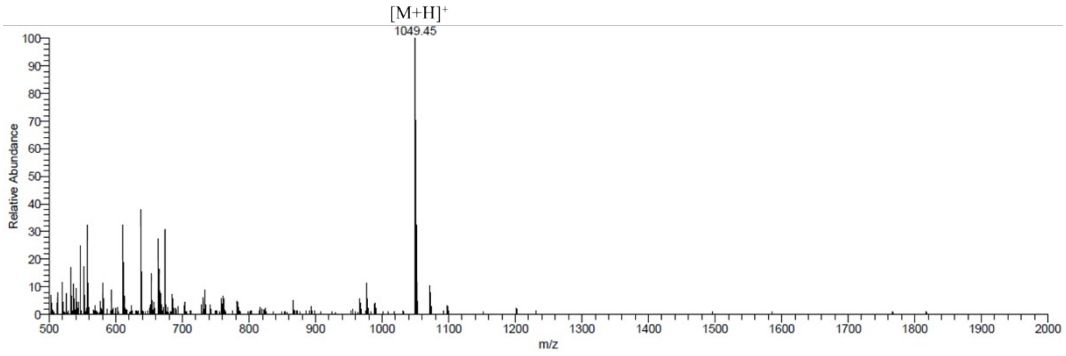
2.6.4 Characterization of the reaction of peptide 5



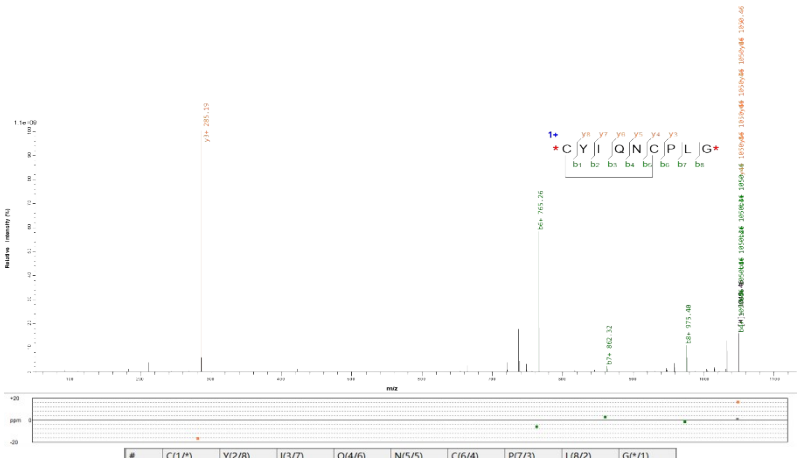
The reaction was followed **General Procedure B** using peptide **5** (1.5 mM), sequence: **$\underline{NH_2}$**-CYIQNCPLG-NH_2 (disulfide: Cys1-Cys6), and thioacid **1a** and **1c**. A stock solution of peptide **5** (1.5 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1.5 mg peptide **5**.



HPLC traces of the quantity relationship of matter and crude reaction of **5a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

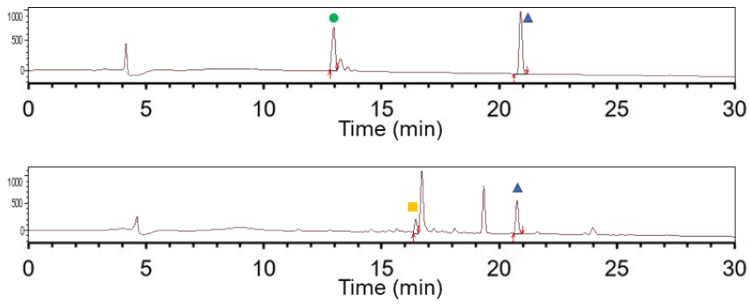


ESI Mass spectrum of purified product **5a**. Calculated Mass $[M+H]^+$: 1049.45; Mass Found (ESI+) $[M+H]^+$: 1049.45.

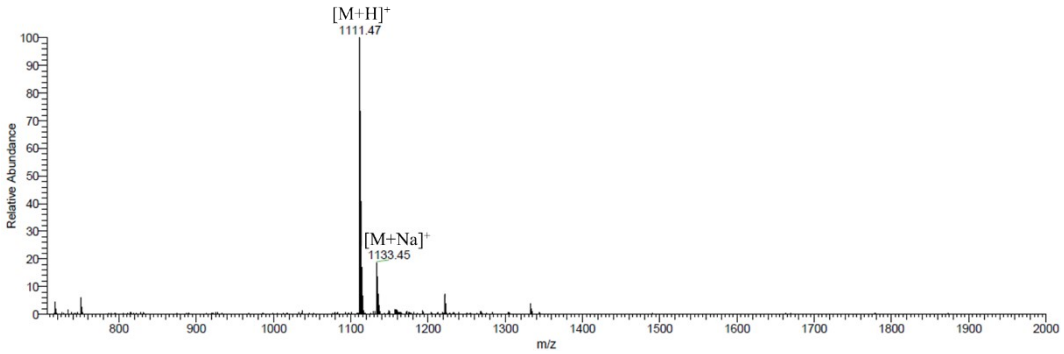


#	C(1/*)	Y(2/8)	I(3/7)	Q(4/6)	N(5/5)	C(6/4)	P(7/3)	L(8/2)	G(*/1)
b+	0.0000	0.0000	0.0000	0.0000	0.0000	765.2691	862.3219	975.4059	*
b++	0.0000	0.0000	0.0000	0.0000	0.0000	383.1382	431.6646	488.2066	*
y+	*	0.0000	0.0000	0.0000	0.0000	0.0000	285.1921	188.1393	75.0553
y++	*	0.0000	0.0000	0.0000	0.0000	0.0000	143.0997	94.5733	38.0313

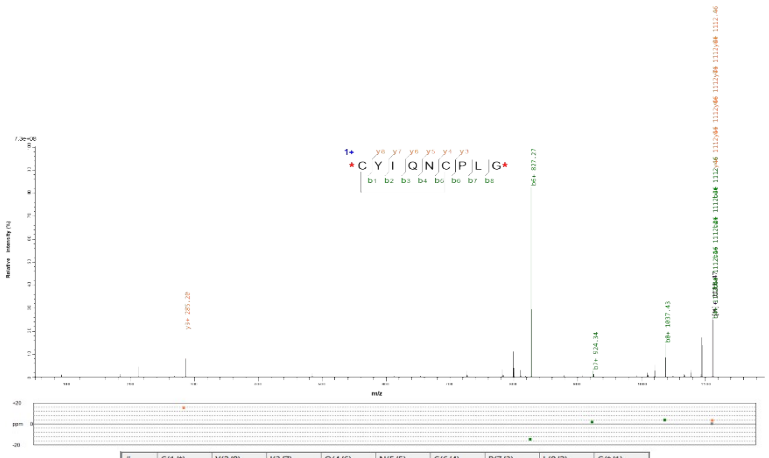
MS/MS analysis of **5a**



HPLC traces of the quantity relationship of matter and crude reaction of **5c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.



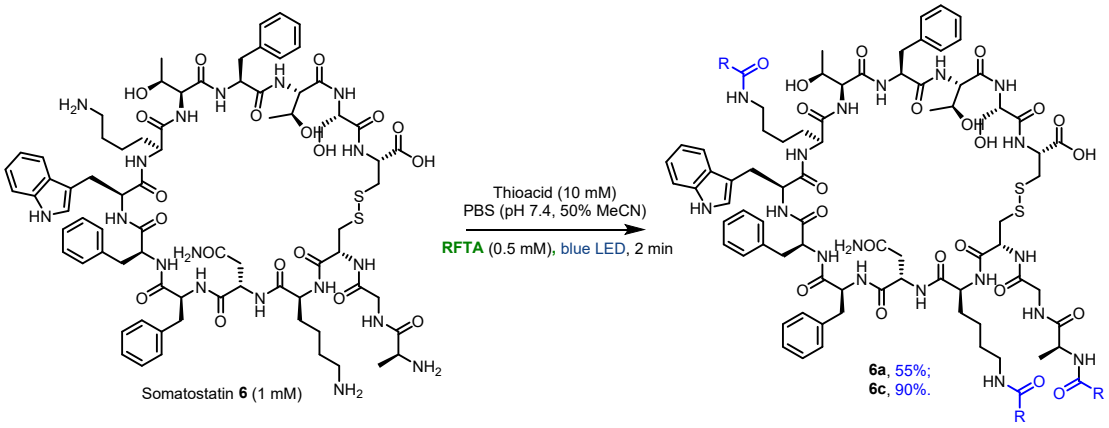
ESI Mass spectrum of purified product **5c**. Calculated Mass $[M+H]^+$: 1111.47; Mass Found (ESI+) $[M+H]^+$: 1111.47.



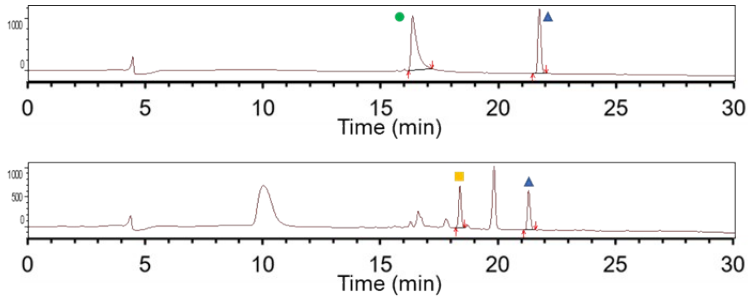
#	C(1/*)	Y(2/8)	I(3/7)	Q(4/6)	N(5/5)	C(6/4)	P(7/3)	L(8/2)	G(*/1)
b+	0.0000	0.0000	0.0000	0.0000	0.0000	827.2848	924.3375	1037.4216	*
b++	0.0000	0.0000	0.0000	0.0000	0.0000	414.1460	462.6724	519.2144	*
y+	*	0.0000	0.0000	0.0000	0.0000	0.0000	285.1921	188.1393	75.0553
y++	*	0.0000	0.0000	0.0000	0.0000	0.0000	143.0997	94.5733	38.0313

MS/MS analysis of **5c**

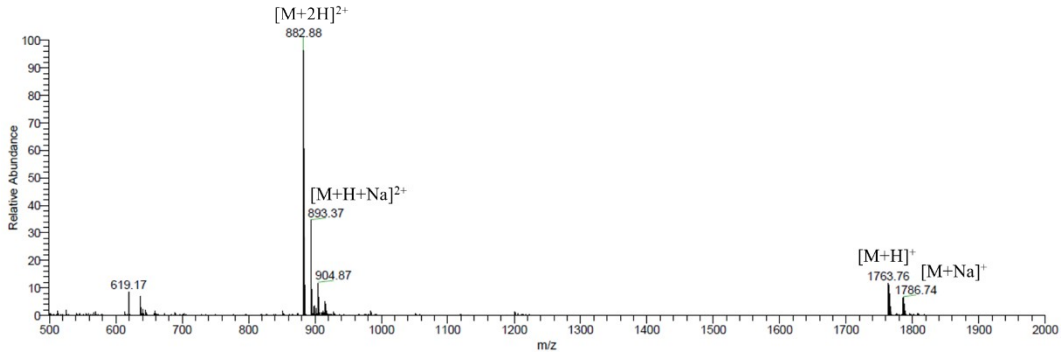
2.6.5 Characterization of the reaction of peptide 6



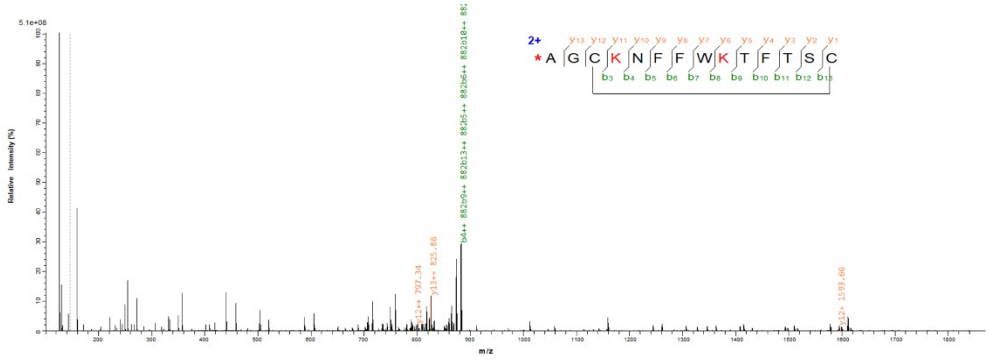
The reaction was followed **General Procedure B** using peptide **6** (1 mM), sequence: **NH$_2$**-AGC**K**NFFW**K**TFTSC-OH (disulfide: Cys3-Cys14), and thioacid **1a** and **1c**. A stock solution of peptide **6** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1.6 mg peptide **6**.



HPLC traces of the quantity relationship of matter and crude reaction of **6a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

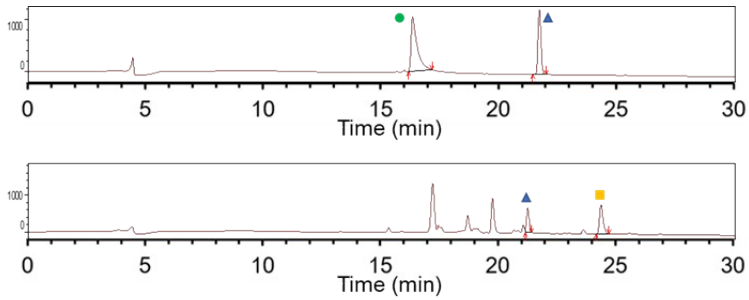


ESI Mass spectrum of purified product **6a**. Calculated Mass $[M+H]^+$: 1763.76, $[M+2H]^{2+}$: 882.88; Mass Found (ESI+) $[M+H]^+$: 1763.76, $[M+2H]^{2+}$: 882.88.

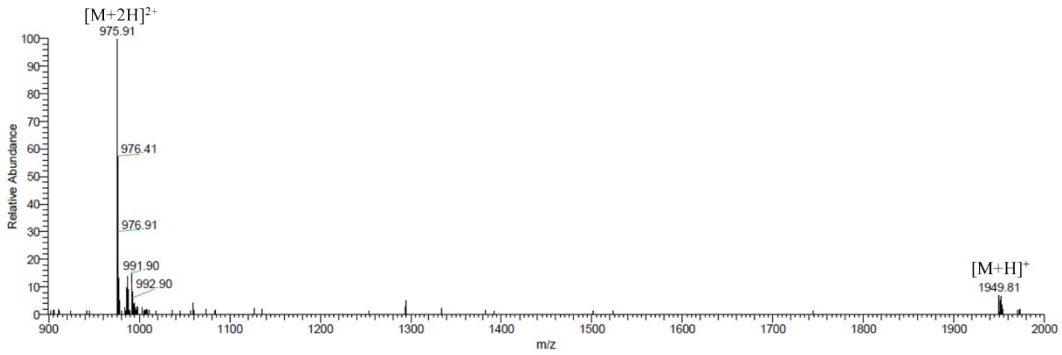


#	A(1/*)	G(2/13)	C(3/12)	K(4/11)	N(5/10)	F(6/9)	F(7/8)	W(8/7)	K(9/6)	T(10/5)	F(11/4)	T(12/3)	S(13/2)	C(*/1)
b+	114.0550	171.0764	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*
b++	57.5311	86.0418	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*
y+	*	1650.7076	1593.6861	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
y++	*	825.8574	797.3467	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

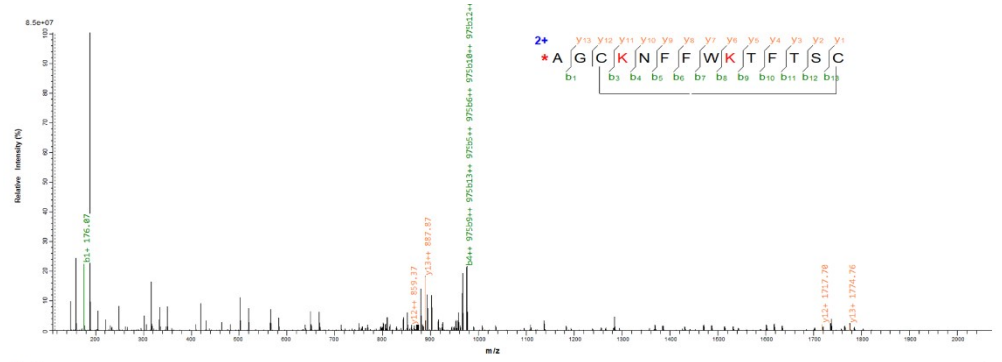
MS/MS analysis of **6a**



HPLC traces of the quantity relationship of matter and crude reaction of **6c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.



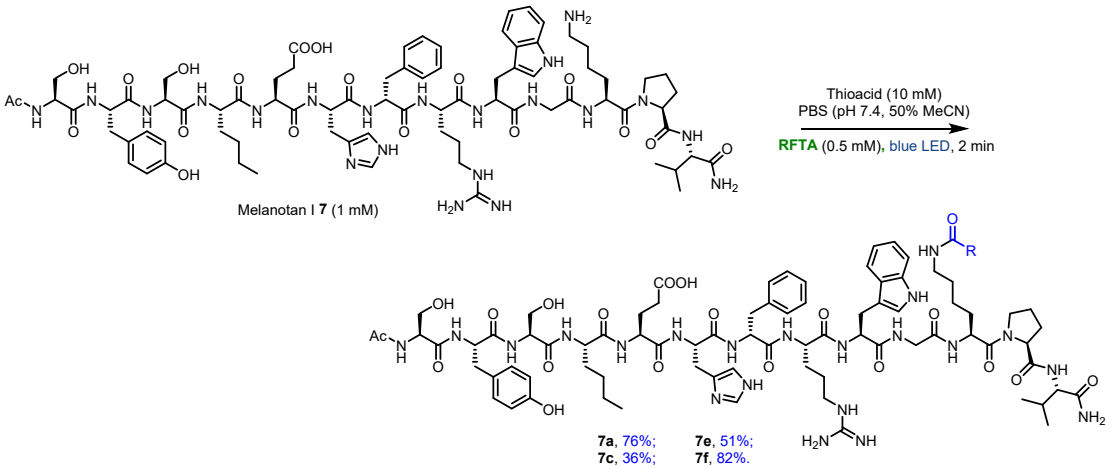
ESI Mass spectrum of purified product **6c**. Calculated Mass $[M+H]^+$: 1949.81, $[M+2H]^{2+}$: 975.91; Mass Found (ESI+) $[M+H]^+$: 1949.81, $[M+2H]^{2+}$: 975.91.



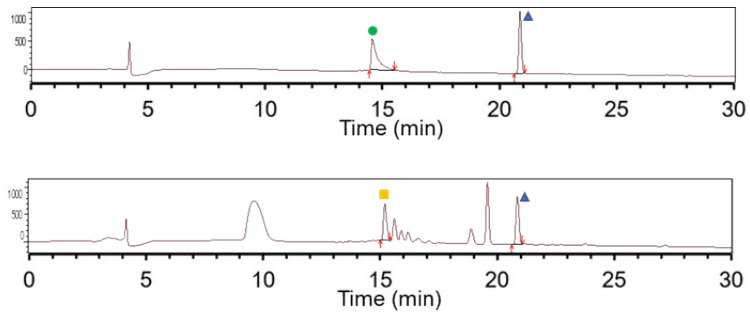
#	A(1/*)	G(2/13)	C(3/12)	K(4/11)	N(5/10)	F(6/9)	F(7/8)	W(8/7)	K(9/6)	T(10/5)	F(11/4)	T(12/3)	S(13/2)	C(*/1)
b+	176.0706	233.0921	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*
b++	88.5389	117.0497	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	*
y+	*	1774.7389	1717.7174	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
y++	*	887.8731	859.3624	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

MS/MS analysis of **6c**

2.6.6 Characterization of the reaction of peptide 7

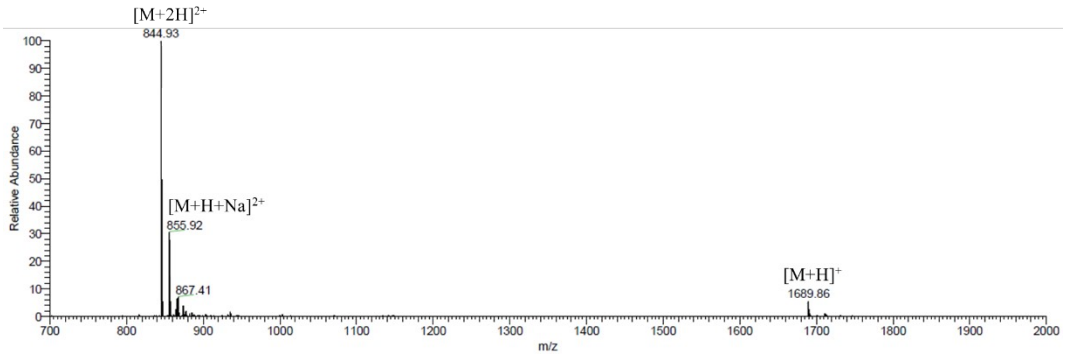


The reaction was followed **General Procedure B** using peptide **7** (1 mM), sequence: Ac-SYS(Nle)EHFRWG**K**PV-NH_2, and thioacid **1a**, **1b**, **1e** and **1f**. A stock solution of peptide **7** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1.6 mg peptide **7**.

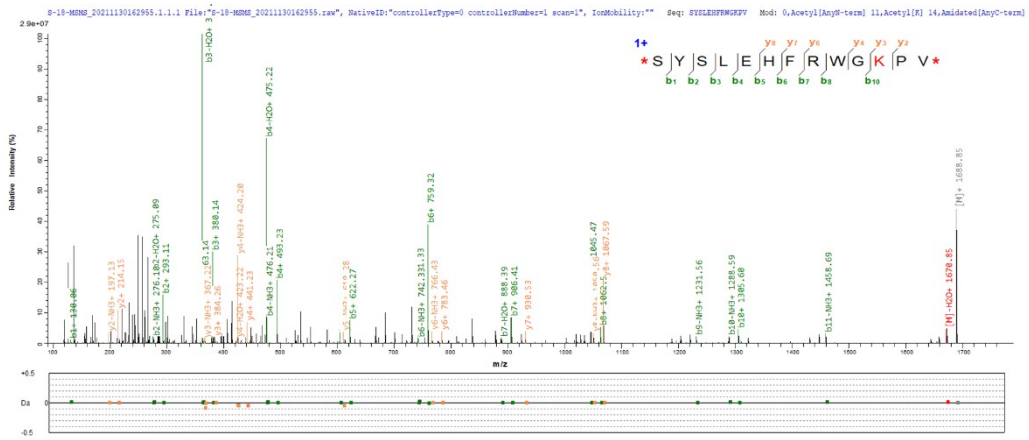


HPLC traces of the quantity relationship of matter and crude reaction of **7a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target

product.

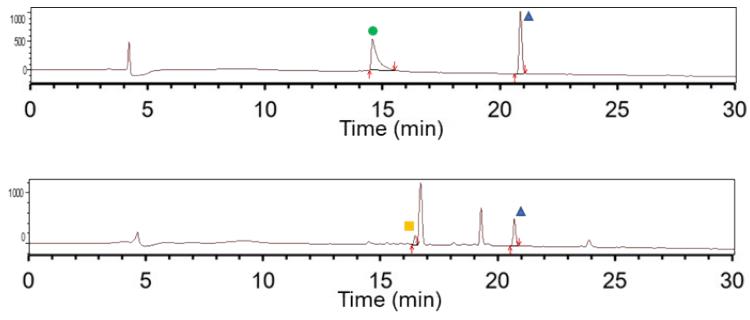


ESI Mass spectrum of purified product **7a**. Calculated Mass $[M+H]^+$: 1689.86, $[M+2H]^{2+}$: 844.93; Mass Found (ESI+) $[M+H]^+$: 1689.86, $[M+2H]^{2+}$: 844.93.

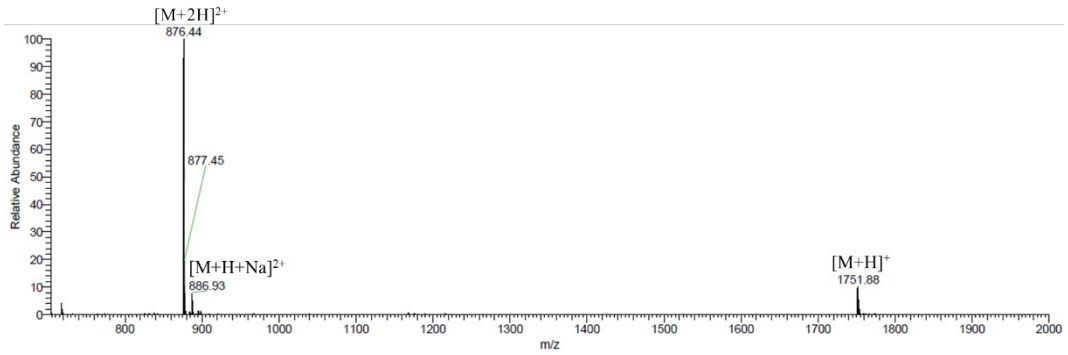


#	S(1/*)	Y(2/12)	S(3/11)	L(4/10)	E(5/9)	H(6/8)	F(7/7)	R(8/6)	W(9/5)	G(10/4)	K(11/3)	P(12/2)	V(*/1)
b+	130.0499	293.1132	380.1452	493.2293	622.2719	759.3308	906.3992	1062.5003	1248.5796	1305.6011	1475.7066	1572.7593	*
b++	65.5286	147.0602	190.5762	247.1183	311.6396	380.1690	453.7032	531.7538	624.7934	653.3042	738.3569	786.8833	*
y+	*	1559.8117	1396.7484	1309.7164	1196.6323	1067.5897	930.5308	783.4624	627.3613	441.2820	384.2605	214.1550	117.1022
y++	*	780.4095	698.8778	655.3618	598.8198	534.2985	465.7690	392.2348	314.1843	221.1446	192.6339	107.5811	59.0548

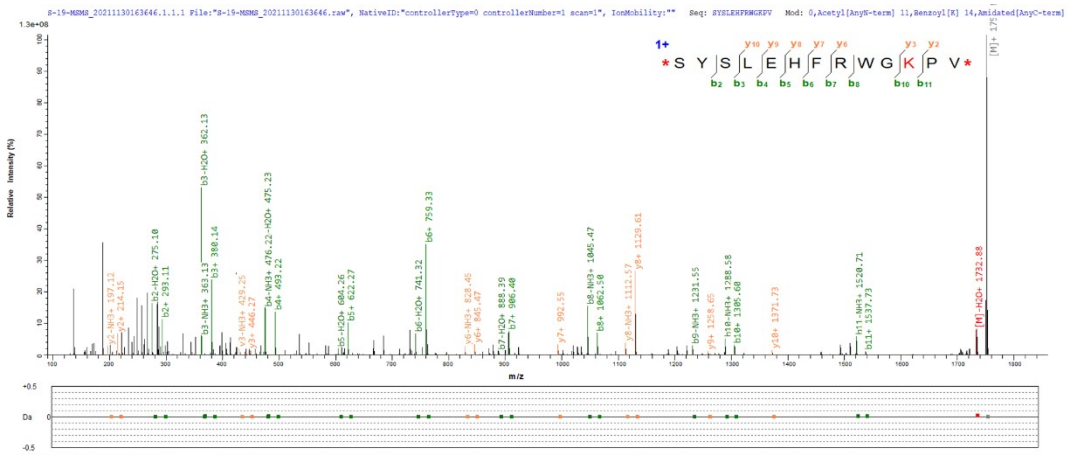
MS/MS analysis of **7a**



HPLC traces of the quantity relationship of matter and crude reaction of **7c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

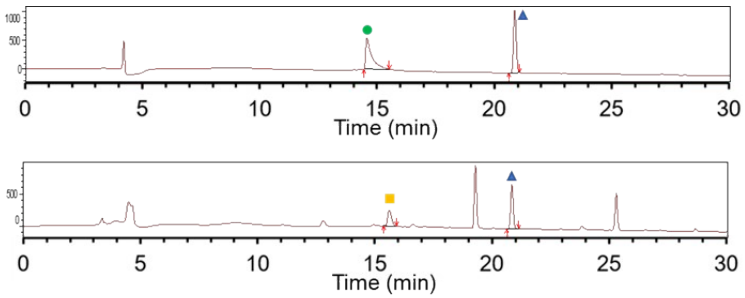


ESI Mass spectrum of purified product **7c**. Calculated Mass $[M+H]^+$: 1751.88, $[M+2H]^{2+}$: 876.44; Mass Found (ESI+) $[M+H]^+$: 1751.88, $[M+2H]^{2+}$: 876.44.

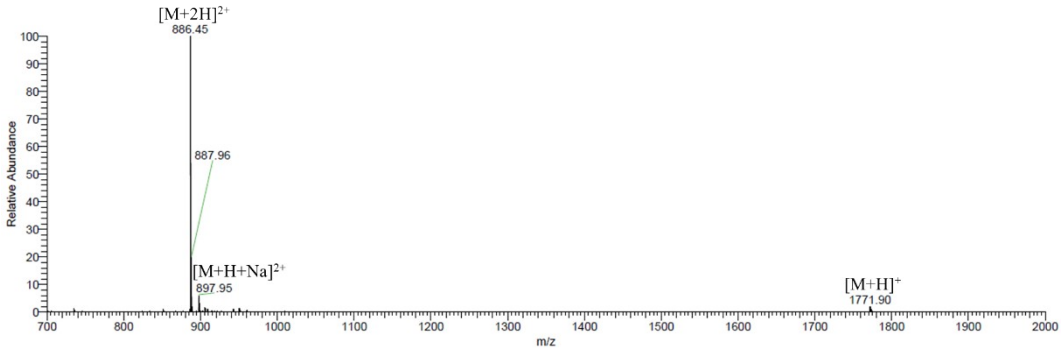


#	S(1/*)	Y(2/12)	S(3/11)	L(4/10)	E(5/9)	H(6/8)	F(7/7)	R(8/6)	W(9/5)	G(10/4)	K(11/3)	P(12/2)	V(*/1)
b+	130.0499	293.1132	380.1452	493.2293	622.2719	759.3308	906.3992	1062.5003	1248.5796	1305.6011	1537.7222	1634.7750	*
b++	65.5286	147.0602	190.5762	247.1183	311.6396	380.1690	453.7032	531.7538	624.7934	653.3042	769.3648	817.8911	*
y+	*	1621.8274	1458.7640	1371.7320	1258.6480	1129.6054	992.5465	845.4780	689.3769	503.2976	446.2762	214.1550	117.1022
y++	*	811.4173	729.8857	686.3696	629.8276	565.3063	496.7769	423.2427	345.1921	252.1525	223.6417	107.5811	59.0548

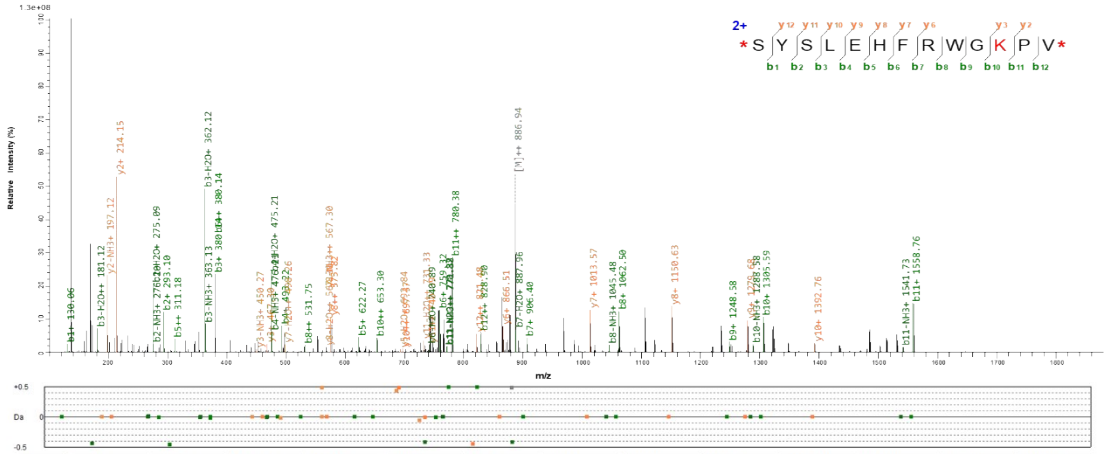
MS/MS analysis of **7c**



HPLC traces of the quantity relationship of matter and crude reaction of **7e**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

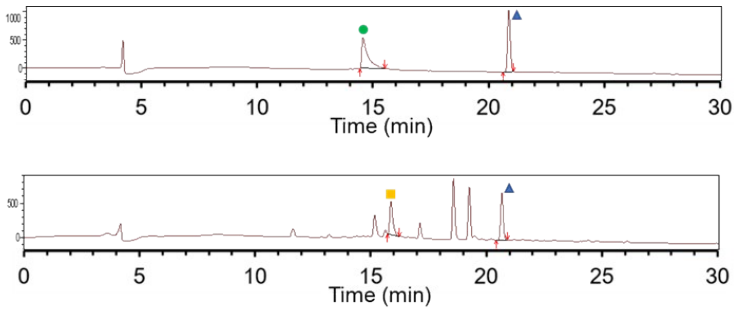


ESI Mass spectrum of purified product **7e**. Calculated Mass $[M+H]^+$: 1771.90, $[M+2H]^{2+}$: 886.45; Mass Found (ESI+) $[M+H]^+$: 1771.90, $[M+2H]^{2+}$: 886.45.

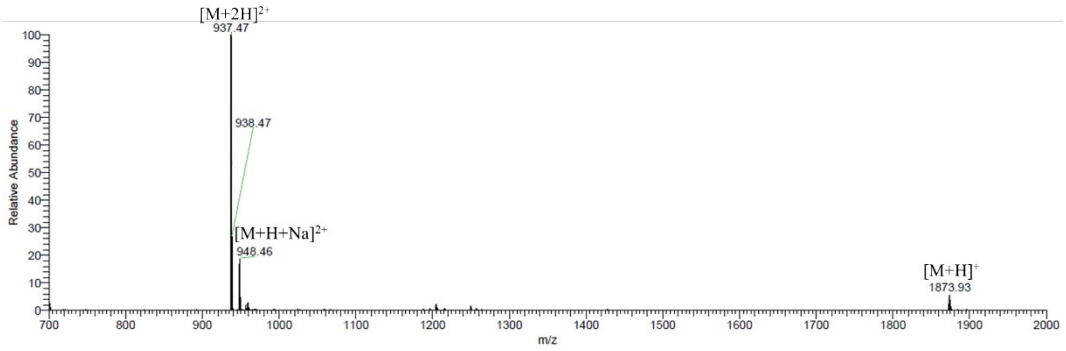


#	S(1/*)	Y(2/12)	S(3/11)	L(4/10)	E(5/9)	H(6/8)	F(7/7)	R(8/6)	W(9/5)	G(10/4)	K(11/3)	P(12/2)	V(*/1)
b+	130.0499	293.1132	380.1452	493.2293	622.2719	759.3308	906.3992	1062.5003	1248.5796	1305.6011	1558.7549	1655.8077	*
b++	65.5286	147.0602	190.5762	247.1183	311.6396	380.1690	453.7032	531.7538	624.7934	653.3042	779.8811	828.4075	*
y+	*	1642.8601	1479.7967	1392.7647	1279.6806	1150.6381	1013.5791	866.5107	710.4096	524.3303	467.3089	214.1550	117.1022
y++	*	821.9337	740.4020	696.8860	640.3440	575.8227	507.2932	433.7590	355.7085	262.6688	234.1581	107.5811	59.0548

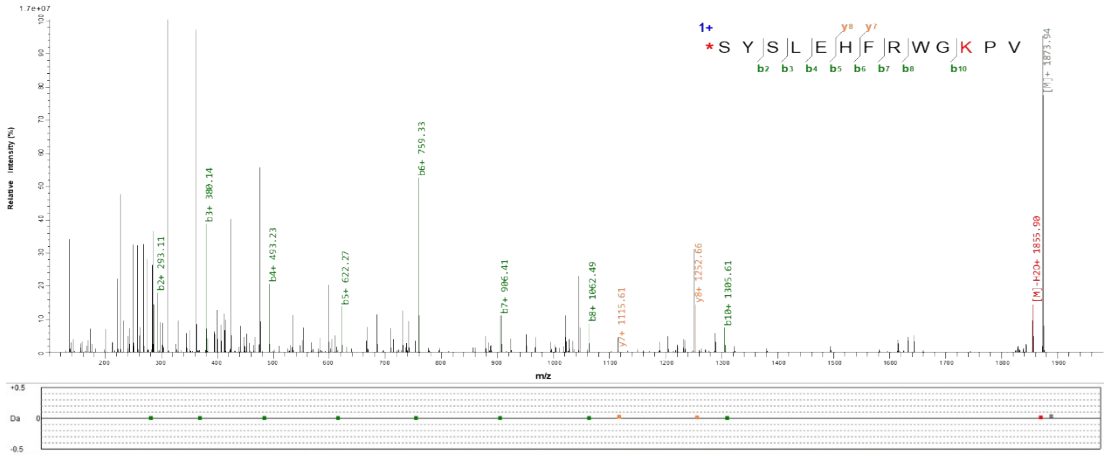
MS/MS analysis of **7e**



HPLC traces of the quantity relationship of matter and crude reaction of **7f**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.



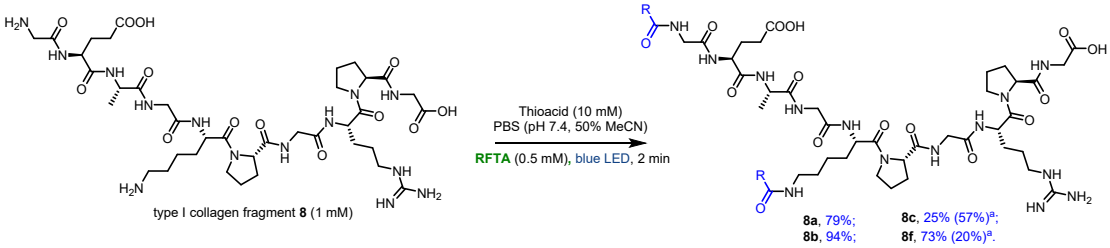
ESI Mass spectrum of purified product **7f**. Calculated Mass $[M+H]^+$: 1873.93, $[M+2H]^{2+}$: 937.47; Mass Found (ESI+) $[M+H]^+$: 1873.93, $[M+2H]^{2+}$: 937.47.



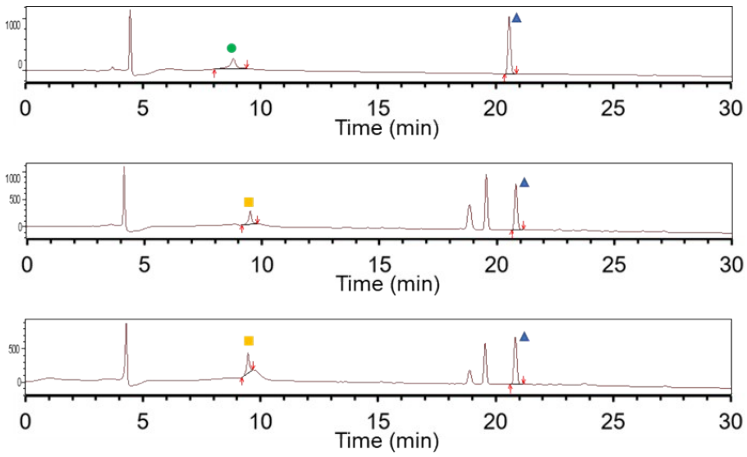
#	S(1/*)	Y(2/12)	S(3/11)	L(4/10)	E(5/9)	H(6/8)	F(7/7)	R(8/6)	W(9/5)	G(10/4)	K(11/3)	P(12/2)	V(*/1)
b+	130.0499	293.1132	380.1452	493.2293	622.2719	759.3308	906.3992	1062.5003	1248.5796	1305.6011	1659.7736	1756.8264	*
b++	65.5286	147.0602	190.5762	247.1183	311.6396	380.1690	453.7032	531.7538	624.7934	653.3042	830.3905	878.9168	*
y+	*	1744.8628	1581.7994	1494.7674	1381.6834	1252.6408	1115.5819	968.5134	812.4123	626.3330	569.3116	215.1390	118.0863
y++	*	872.9350	791.4034	747.8873	691.3453	626.8240	558.2946	484.7604	406.7098	313.6702	285.1594	108.0731	59.5468

MS/MS analysis of **7f**

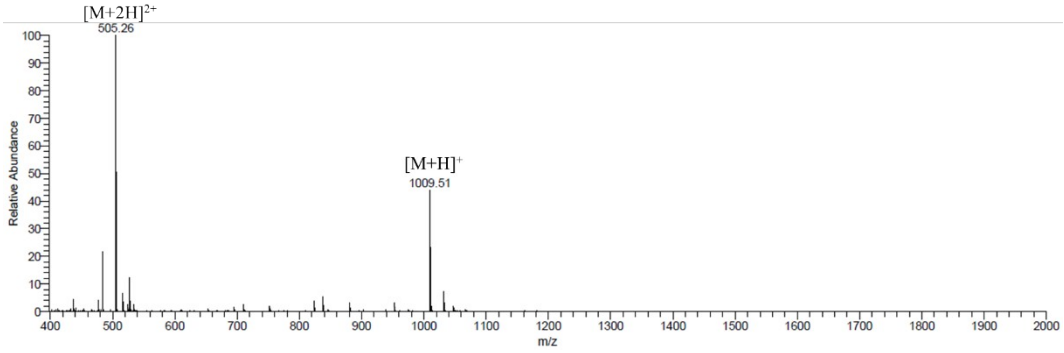
2.6.7 Characterization of the reaction of peptide 8



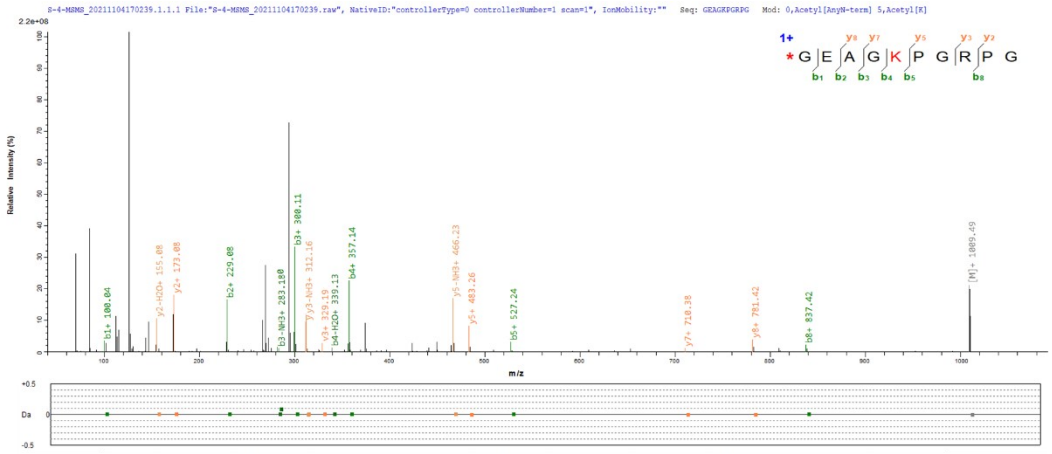
The reaction was followed **General Procedure B** using peptide **8** (1 mM), sequence: **$\underline{NH_2}$**-GEAG**$\underline{K}$**PGRPG-OH, and thioacid **1a**, **1b**, **1c** and **1f**. A stock solution of peptide **8** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 0.9 mg peptide **8**.



HPLC traces of the quantity relationship of matter and crude reaction of **8a** and **8b**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

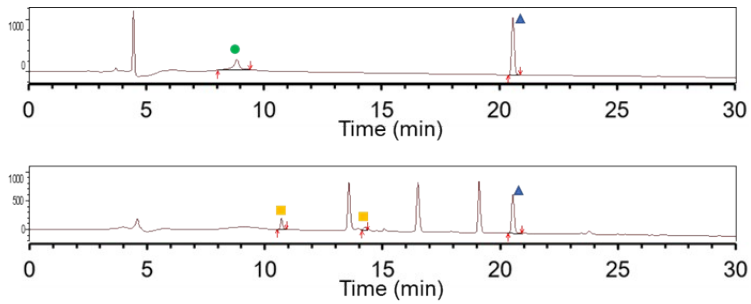


ESI Mass spectrum of purified product **8a**. Calculated Mass $[M+H]^+$: 1009.51, $[M+2H]^{2+}$: 505.26; Mass Found (ESI+) $[M+H]^+$: 1009.51, $[M+2H]^{2+}$: 505.26.

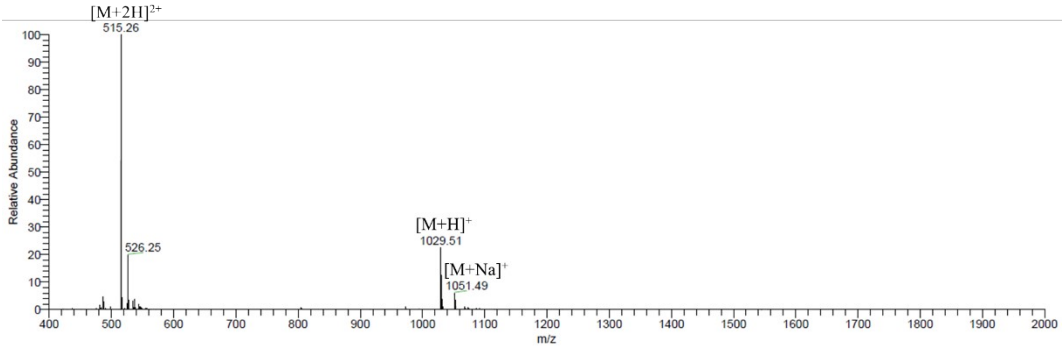


#	G(1/*)	E(2/9)	A(3/8)	G(4/7)	K(5/6)	P(6/5)	G(7/4)	R(8/3)	P(9/2)	G(*/1)
b+	100.0393	229.0819	300.1190	357.1405	527.2460	624.2987	681.3202	837.4213	934.4741	*
b++	50.5233	115.0446	150.5631	179.0739	264.1266	312.6530	341.1637	419.2143	467.7407	*
y+	*	910.4741	781.4315	710.3944	653.3729	483.2674	386.2146	329.1932	173.0921	76.0393
y++	*	455.7407	391.2194	355.7008	327.1901	242.1373	193.6110	165.1002	87.0497	38.5233

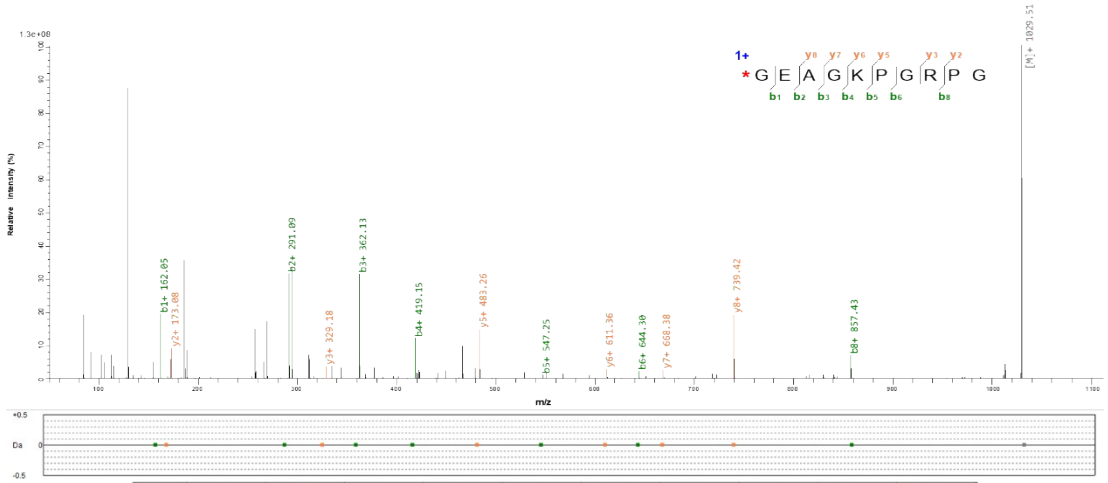
MS/MS analysis of **8a**



HPLC traces of the quantity relationship of matter and crude reaction **8c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

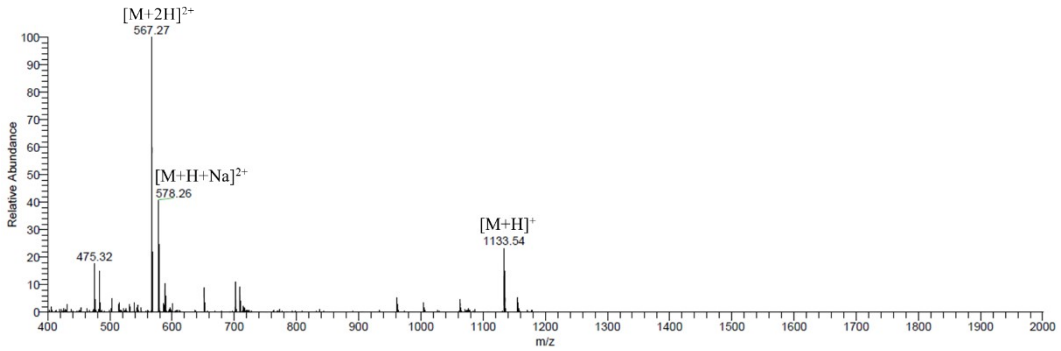


ESI Mass spectrum of purified single-amidated product **8c**. Calculated Mass $[M+H]^+$: 1029.51, $[M+2H]^{2+}$: 515.26; Mass Found (ESI+) $[M+H]^+$: 1029.51, $[M+2H]^{2+}$: 515.26.

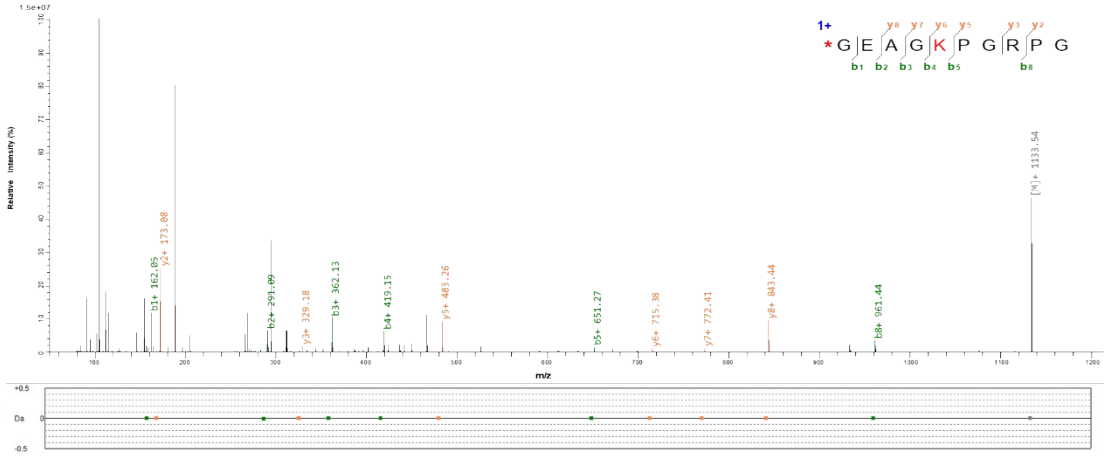


#	G(1/*)	E(2/9)	A(3/8)	G(4/7)	K(5/6)	P(6/5)	G(7/4)	R(8/3)	P(9/2)	G(*/1)
b+	162.0550	291.0975	362.1346	419.1561	547.2511	644.3038	701.3253	857.4264	954.4791	*
b++	81.5311	146.0524	181.5710	210.0817	274.1292	322.6556	351.1663	429.2168	477.7432	*
y+	*	868.4635	739.4209	668.3838	611.3623	483.2674	386.2146	329.1932	173.0921	76.0393
y++	*	434.7354	370.2141	334.6955	306.1848	242.1373	193.6110	165.1002	87.0497	38.5233

MS/MS analysis of single-amidated **8c**

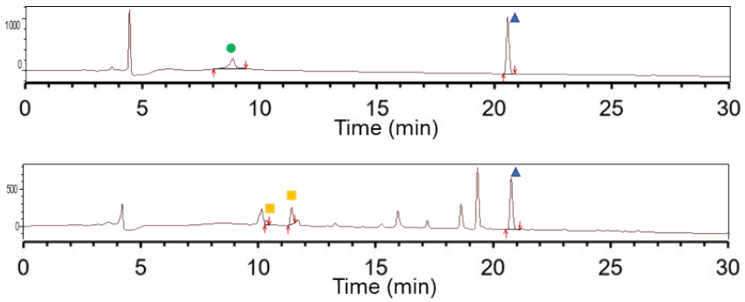


ESI Mass spectrum of purified double-amidated product **8c**. Calculated Mass $[M+H]^+$: 1133.54, $[M+2H]^{2+}$: 567.27; Mass Found (ESI+) $[M+H]^+$: 1133.54, $[M+2H]^{2+}$: 567.27.

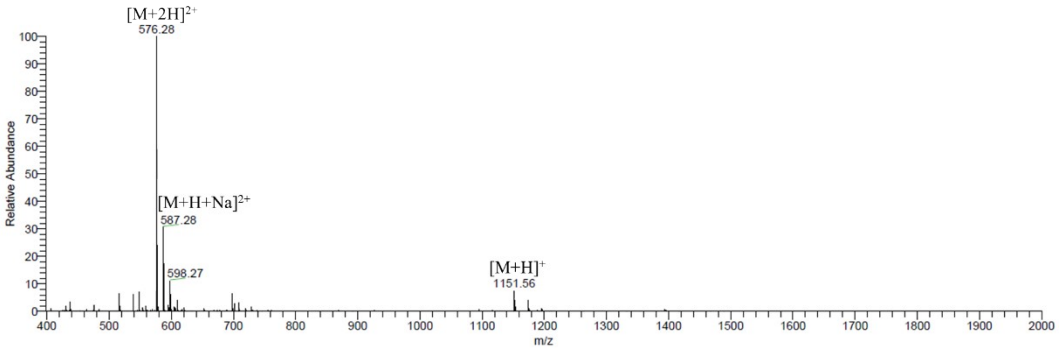


#	G(1/*)	E(2/9)	A(3/8)	G(4/7)	K(5/6)	P(6/5)	G(7/4)	R(8/3)	P(9/2)	G(*/1)
b+	162.0550	291.0975	362.1346	419.1561	651.2773	748.3300	805.3515	961.4526	1058.5054	*
b++	81.5311	146.0524	181.5710	210.0817	326.1423	374.6687	403.1794	481.2299	529.7563	*
y+	*	972.4897	843.4471	772.4100	715.3886	483.2674	386.2146	329.1932	173.0921	76.0393
y++	*	486.7485	422.2272	386.7086	358.1979	242.1373	193.6110	165.1002	87.0497	38.5233

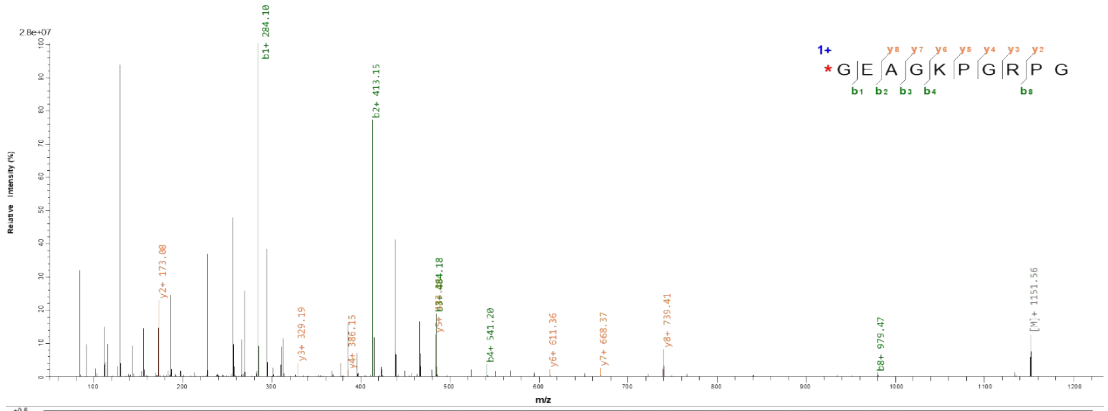
MS/MS analysis of double-amidated **8c**



HPLC traces of the quantity relationship of matter and crude reaction **8f**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

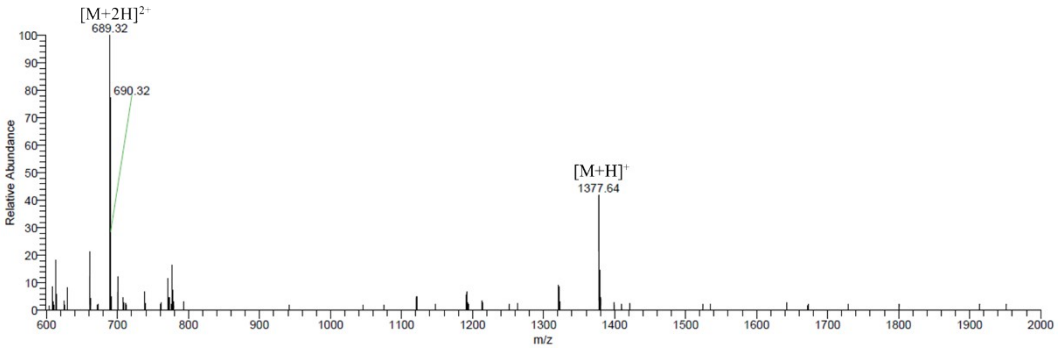


ESI Mass spectrum of purified single-amidated product **8f**. Calculated Mass $[M+H]^+$: 1151.56, $[M+2H]^{2+}$: 576.28; Mass Found (ESI+) $[M+H]^+$: 1151.56, $[M+2H]^{2+}$: 576.28.

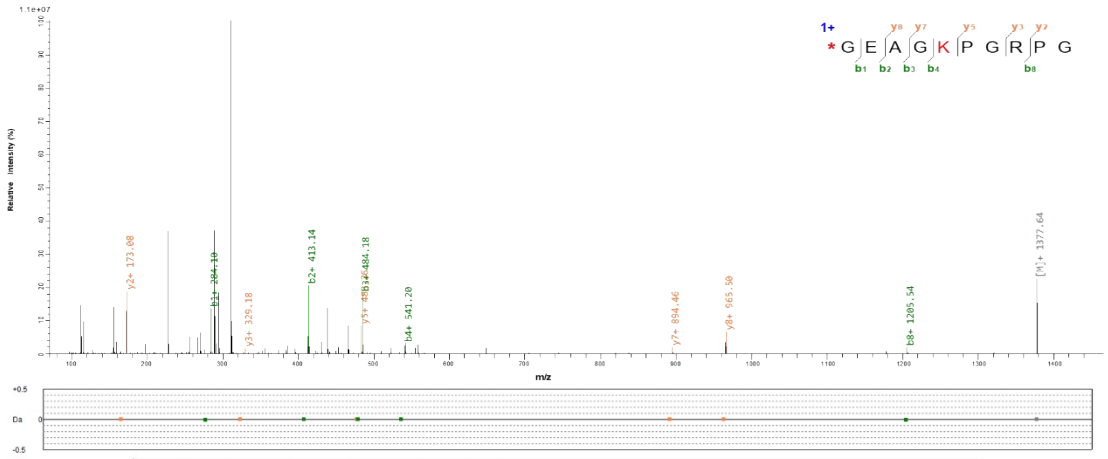


#	G(1/*)	E(2/9)	A(3/8)	G(4/7)	K(5/6)	P(6/5)	G(7/4)	R(8/3)	P(9/2)	G(*/1)
b+	284.1063	413.1489	484.1860	541.2075	669.3024	766.3552	823.3767	979.4778	1076.5305	*
b++	142.5568	207.0781	242.5967	271.1074	335.1549	383.6812	412.1920	490.2425	538.7689	*
y+	*	868.4635	739.4209	668.3838	611.3623	483.2674	386.2146	329.1932	173.0921	76.0393
y++	*	434.7354	370.2141	334.6955	306.1848	242.1373	193.6110	165.1002	87.0497	38.5233

MS/MS analysis of single-amidated **8f**



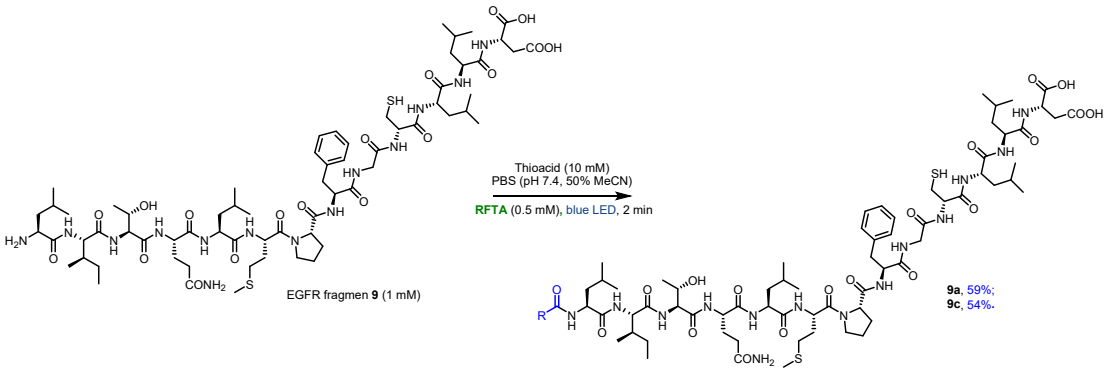
ESI Mass spectrum of purified double-amidated product **8f**. Calculated Mass $[M+H]^+$: 1377.64, $[M+2H]^{2+}$: 689.32; Mass Found (ESI+) $[M+H]^+$: 1377.64, $[M+2H]^{2+}$: 689.32.



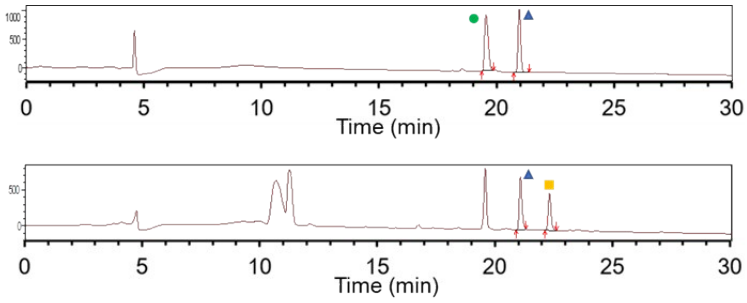
#	G(1/*)	E(2/9)	A(3/8)	G(4/7)	K(5/6)	P(6/5)	G(7/4)	R(8/3)	P(9/2)	G(*/1)
b+	284.1063	413.1489	484.1860	541.2075	895.3800	992.4328	1049.4543	1205.5554	1302.6081	*
b++	142.5568	207.0781	242.5967	271.1074	448.1937	496.7200	525.2308	603.2813	651.8077	*
y+	*	1094.5411	965.4985	894.4614	837.4399	483.2674	386.2146	329.1932	173.0921	76.0393
y++	*	547.7742	483.2529	447.7343	419.2236	242.1373	193.6110	165.1002	87.0497	38.5233

MS/MS analysis of double-amidated **8f**

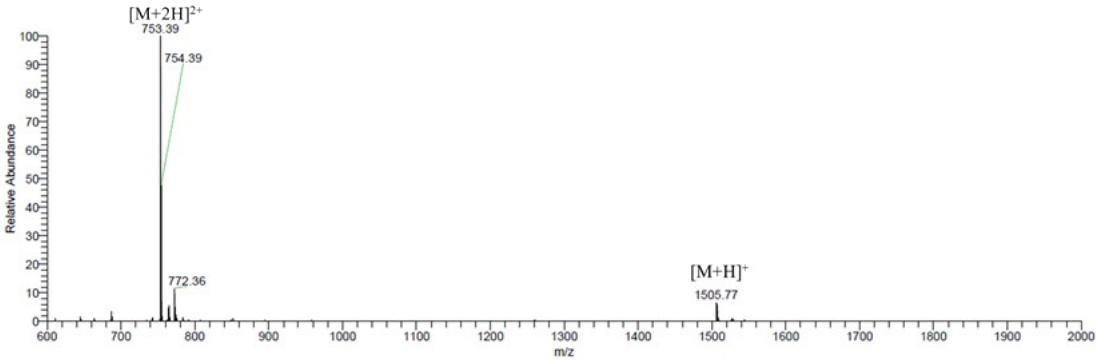
2.6.8 Characterization of the reaction of peptide 9



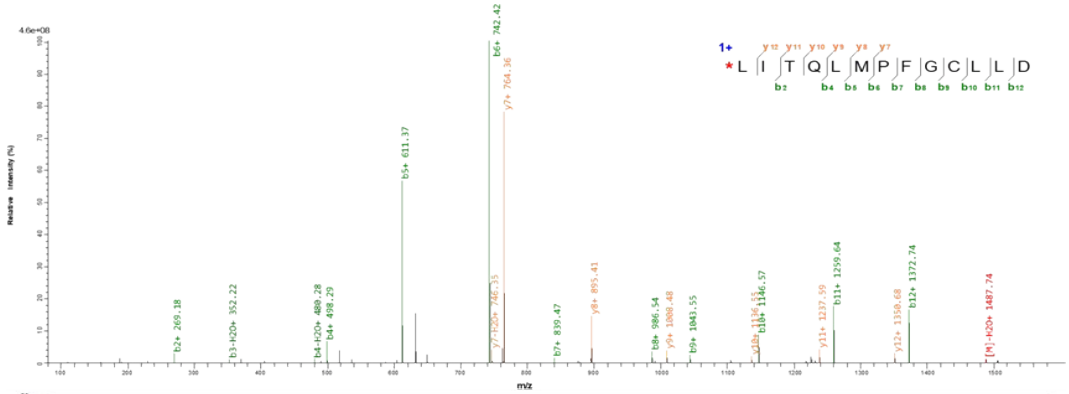
The reaction was followed **General Procedure B** using peptide **9** (1 mM), sequence: **$\underline{NH_2}$**-LITQLMPFGCLLD-OH, and thioacid **1a** and **1c**. A stock solution of peptide **9** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1.5 mg peptide **9**.



HPLC traces of the quantity relationship of matter and crude reaction **9a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

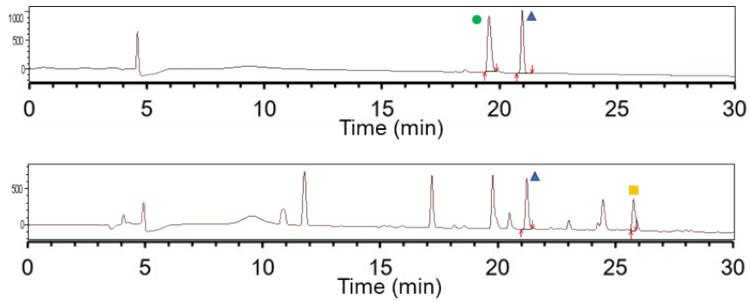


ESI Mass spectrum of purified product **9a**. Calculated Mass $[M+H]^+$: 1505.77, $[M+2H]^{2+}$: 753.39; Mass Found (ESI+) $[M+H]^+$: 1505.77, $[M+2H]^{2+}$: 753.39.

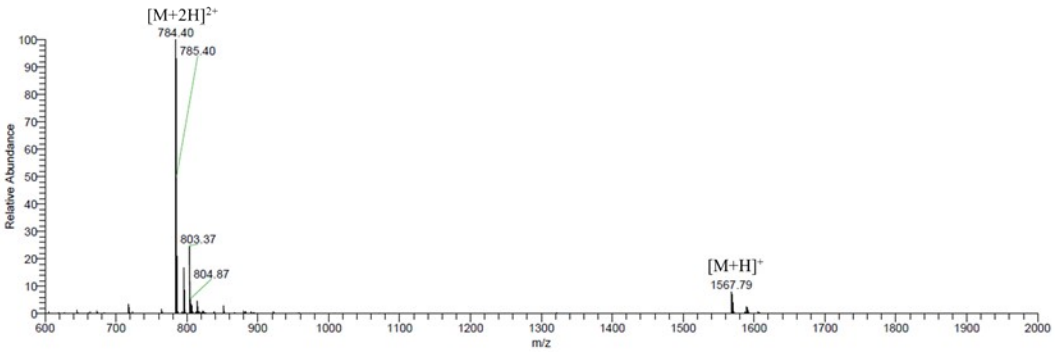


#	L(1/*)	I(2/12)	T(3/11)	Q(4/10)	L(5/9)	M(6/8)	P(7/7)	F(8/6)	G(9/5)	C(10/4)	L(11/3)	L(12/2)	D(*/1)
b+	156.1019	269.1860	370.2336	498.2922	611.3763	742.4168	839.4695	986.5379	1043.5594	1146.5686	1259.6527	1372.7367	*
b++	78.5546	135.0966	185.6205	249.6498	306.1918	371.7120	420.2384	493.7726	522.2833	573.7879	630.3300	686.8720	*
y+	*	1350.6796	1237.5955	1136.5479	1008.4893	895.4052	764.3647	667.3120	520.2436	463.2221	360.2129	247.1288	134.0448
y++	*	675.8434	619.3014	568.7776	504.7483	448.2062	382.6860	334.1596	260.6254	232.1147	180.6101	124.0681	67.5260

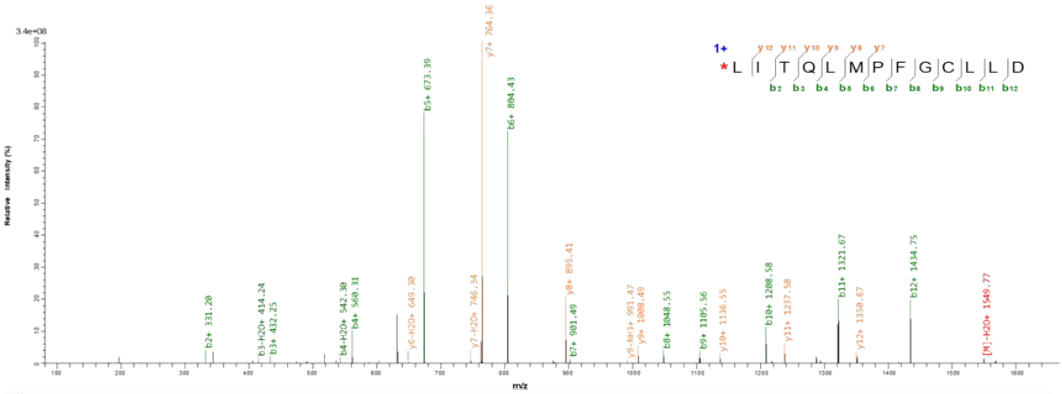
MS/MS analysis of **9a**



HPLC traces of the quantity relationship of matter and crude reaction **9c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.



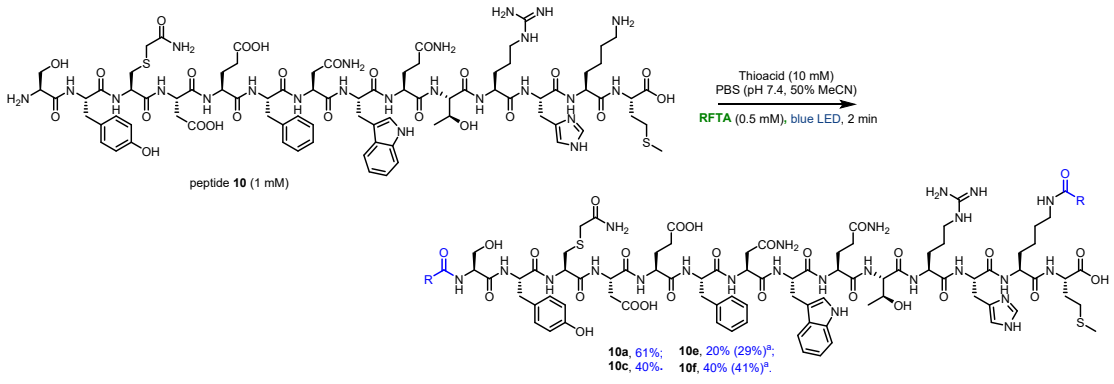
ESI Mass spectrum of purified product **9c**. Calculated Mass $[M+H]^+$: 1567.79, $[M+2H]^{2+}$: 784.40; Mass Found (ESI+) $[M+H]^+$: 1567.79, $[M+2H]^{2+}$: 784.40.



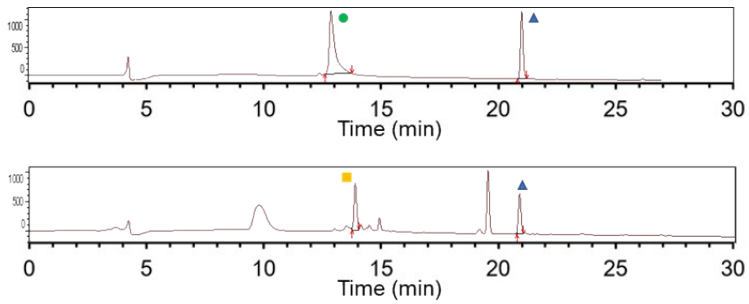
#	L(1/*)	I(2/12)	T(3/11)	Q(4/10)	L(5/9)	M(6/8)	P(7/7)	F(8/6)	G(9/5)	C(10/4)	L(11/3)	L(12/2)	D(*/1)
b+	218.1176	331.2016	432.2493	560.3079	673.3919	804.4324	901.4852	1048.5536	1105.5751	1208.5842	1321.6683	1434.7524	*
b++	109.5624	166.1044	216.6283	280.6576	337.1996	402.7199	451.2462	524.7804	553.2912	604.7958	661.3378	717.8798	*
y+	*	1350.6796	1237.5955	1136.5479	1008.4893	895.4052	764.3647	667.3120	520.2436	463.2221	360.2129	247.1288	134.0448
y++	*	675.8434	619.3014	568.7776	504.7483	448.2062	382.6860	334.1596	260.6254	232.1147	180.6101	124.0681	67.5260

MS/MS analysis of **9c**

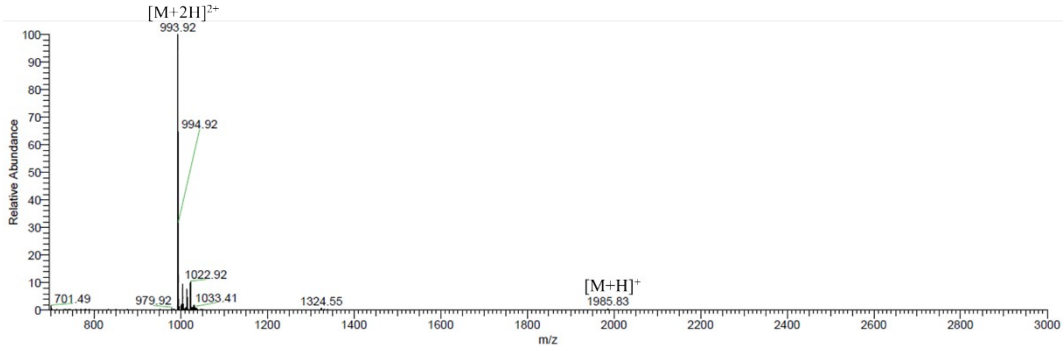
2.6.9 Characterization of the reaction of peptide 10



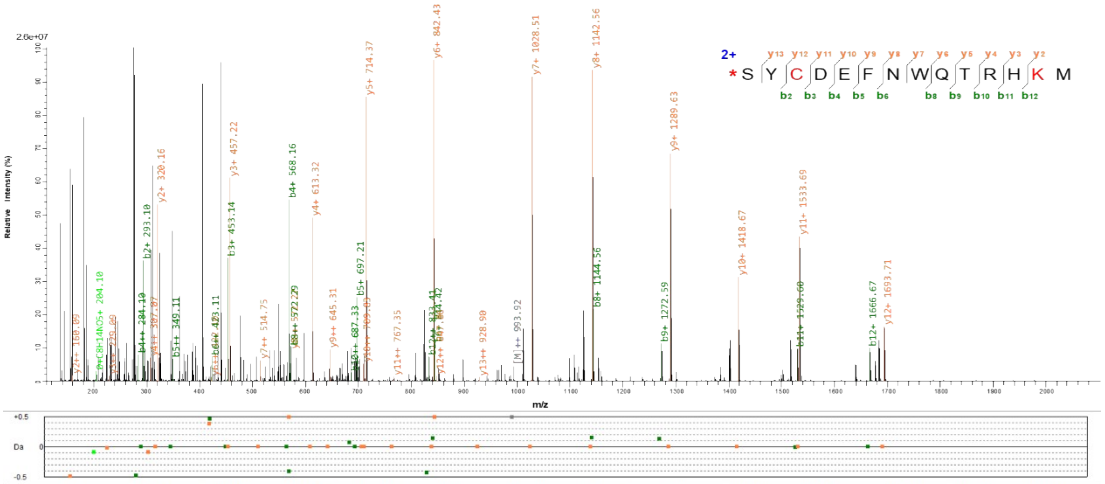
The reaction was followed **General Procedure B** using peptide **10** (1 mM), sequence: **<u>NH$_2$</u>**-SYC(AMI)DEFNWQTRH**<u>K</u>**M-OH, and thioacid **1a**, **1c**, **1e** and **1f**. A stock solution of peptide **10** (1 mM) was made up by 1 mL PBS buffer (pH 7.4, 50% MeCN) and 1.9 mg peptide **10**.



HPLC traces of the quantity relationship of matter and crude reaction **10a**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 μm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

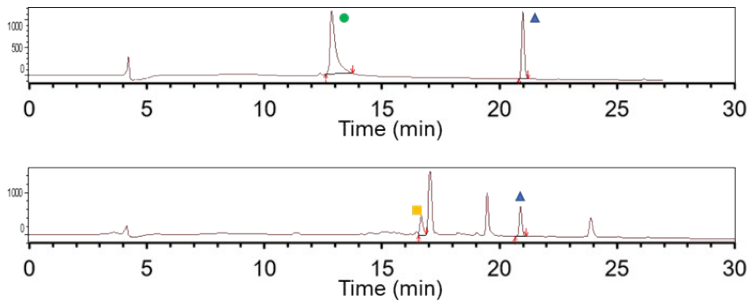


ESI Mass spectrum of purified product **10a**. Calculated Mass $[M+H]^+$: 1985.83, $[M+2H]^{2+}$: 993.92; Mass Found (ESI+) $[M+H]^+$: 1985.83, $[M+2H]^{2+}$: 993.92.

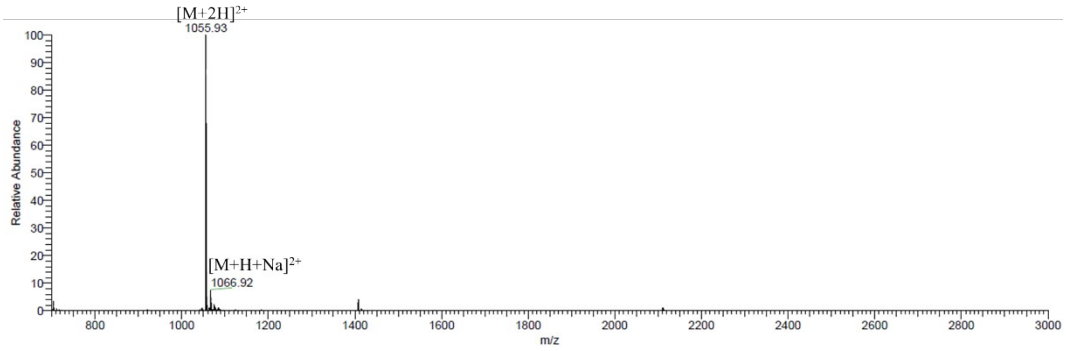


#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	130.0499	293.1132	453.1439	568.1708	697.2134	844.2818	958.3247	1144.4041	1272.4626	1373.5103	1529.6114	1666.6703	1836.7759	*
b++	65.5286	147.0602	227.0756	284.5891	349.1103	422.6445	479.6660	572.7057	636.7350	687.2588	765.3094	833.8388	918.8916	*
y+	*	1856.7843	1693.7210	1533.6903	1418.6634	1289.6208	1142.5524	1028.5095	842.4301	714.3716	613.3239	457.2228	320.1639	150.0583
y++	*	928.8958	847.3641	767.3488	709.8353	645.3140	571.7798	514.7584	421.7187	357.6894	307.1656	229.1150	160.5856	75.5328

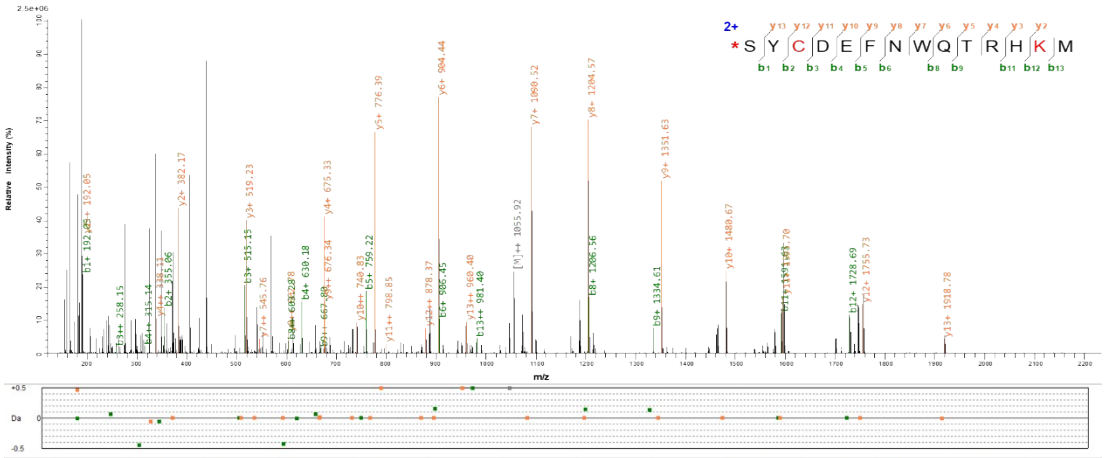
MS/MS analysis of **10a**



HPLC traces of the quantity relationship of matter and crude reaction of **10c**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

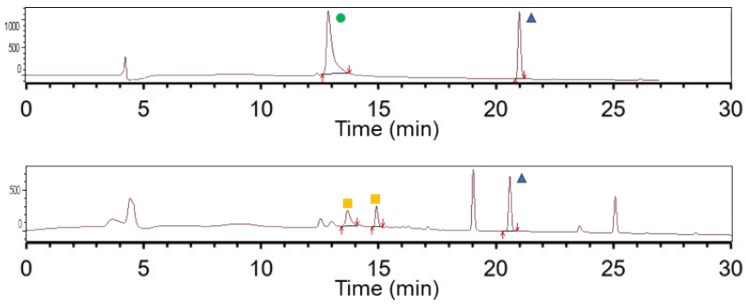


ESI Mass spectrum of purified product **10c**. Calculated Mass $[M+2H]^{2+}$: 1055.93; Mass Found (ESI+) $[M+2H]^{2+}$: 1055.93.

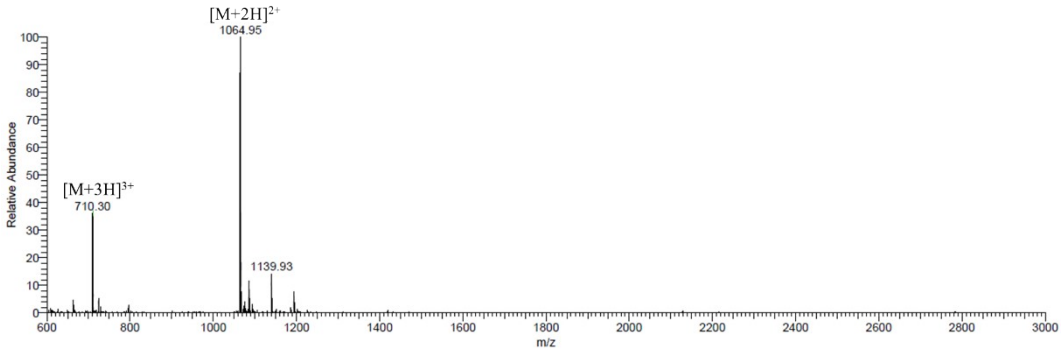


#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	192.0655	355.1288	515.1595	630.1865	759.2291	906.2975	1020.3404	1206.4197	1334.4783	1435.5260	1591.6271	1728.6860	1960.8072	*
b++	96.5364	178.0681	258.0834	315.5969	380.1182	453.6524	510.6738	603.7135	667.7428	718.2666	796.3172	864.8466	980.9072	*
y+	*	1918.8000	1755.7367	1595.7060	1480.6790	1351.6364	1204.5680	1090.5251	904.4458	776.3872	675.3395	519.2384	382.1795	150.0583
y++	*	959.9036	878.3720	798.3566	740.8432	676.3219	602.7877	545.7662	452.7265	388.6972	338.1734	260.1228	191.5934	75.5328

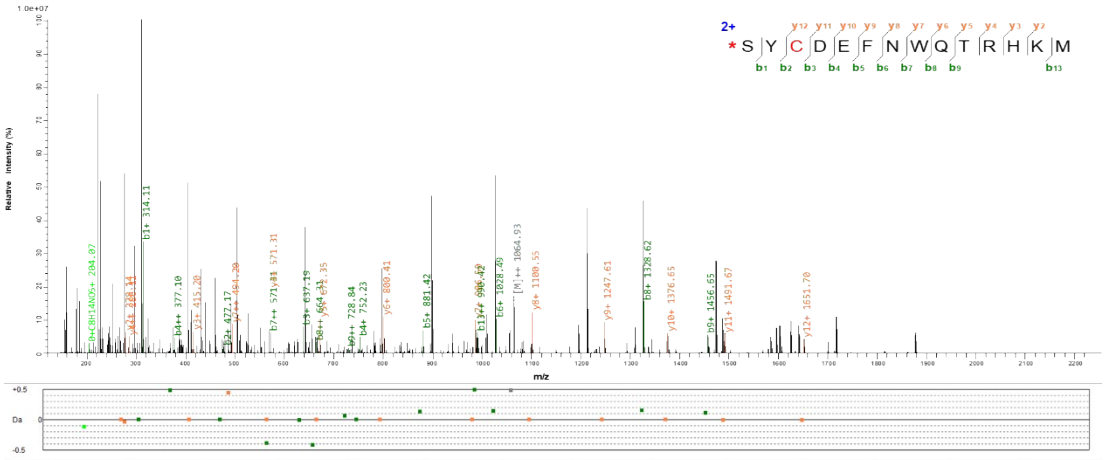
MS/MS analysis of **10c**



HPLC traces of the quantity relationship of matter and crude reaction of **10e**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

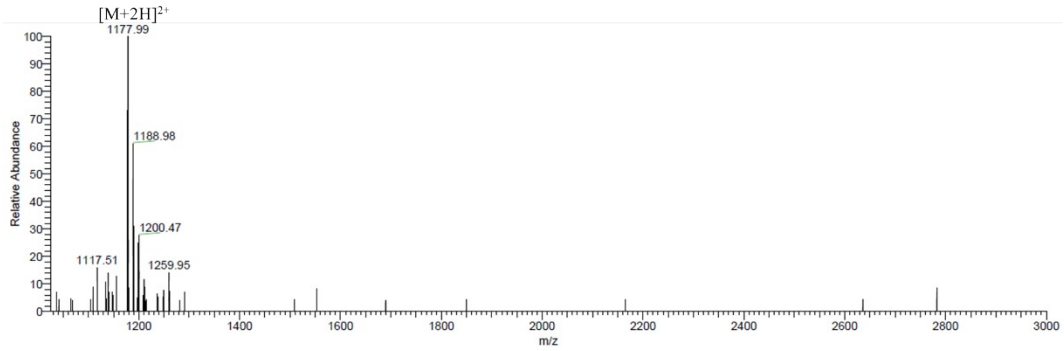


ESI Mass spectrum of purified single-amidated product **10e**. Calculated Mass $[M+2H]^{2+}$: 1064.95, $[M+3H]^{3+}$: 710.30; Mass Found (ESI+) $[M+2H]^{2+}$: 1064.95, $[M+3H]^{3+}$: 710.30.

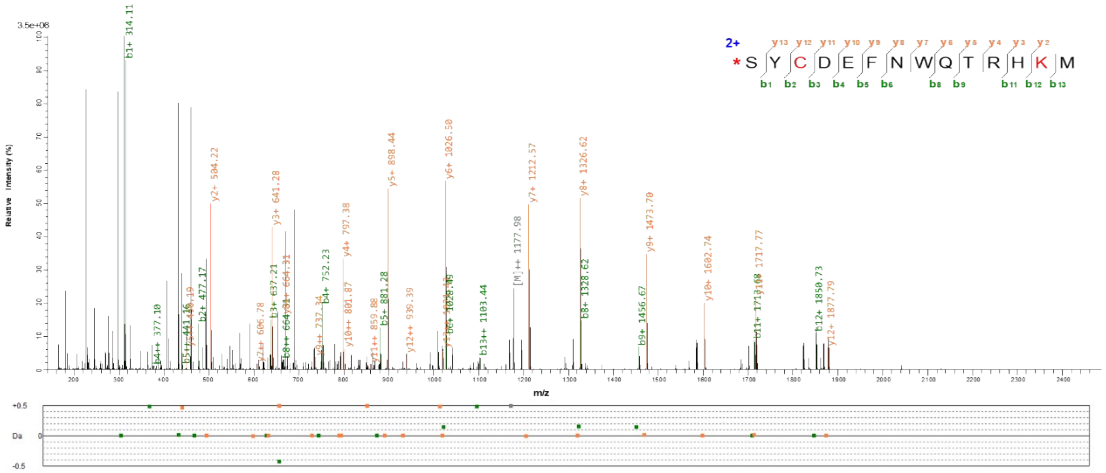


#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	314.1169	477.1802	637.2109	752.2379	881.2804	1028.3489	1142.3918	1328.4711	1456.5297	1557.5774	1713.6785	1850.7374	1978.8323	*
b++	157.5621	239.0938	319.1091	376.6226	441.1439	514.6781	571.6995	664.7392	728.7685	779.2923	857.3429	925.8723	989.9198	*
y+	*	1814.7738	1651.7104	1491.6798	1376.6528	1247.6102	1100.5418	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	907.8905	826.3589	746.3435	688.8300	624.3088	550.7745	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

MS/MS analysis of single-amidated **10e**

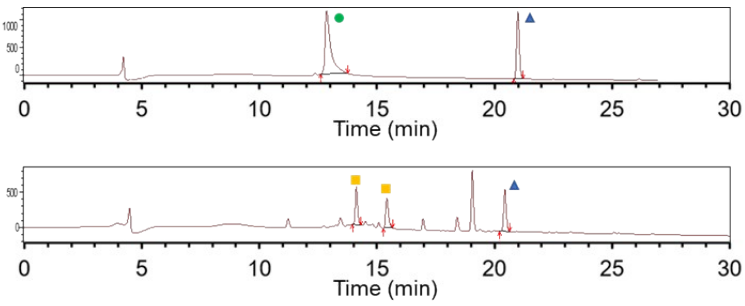


ESI Mass spectrum of purified double-amidated product **10e**. Calculated Mass $[M+2H]^{2+}$: 1177.99; Mass Found (ESI+) $[M+2H]^{2+}$: 1177.99.

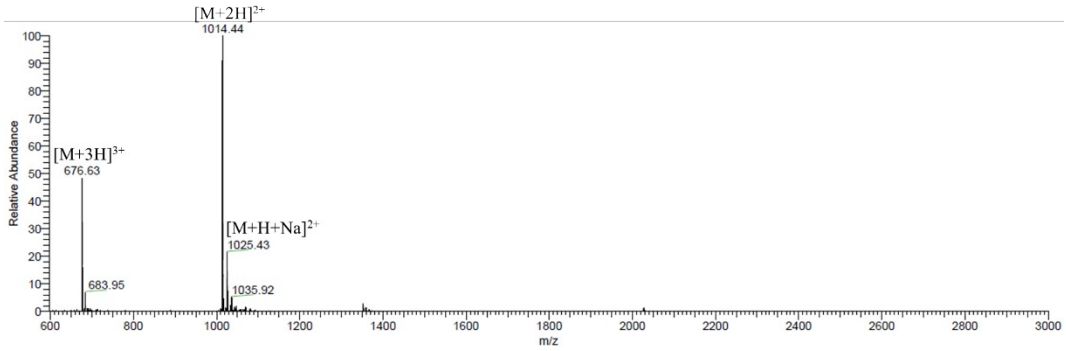


#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	314.1169	477.1802	637.2109	752.2379	881.2804	1028.3489	1142.3918	1328.4711	1456.5297	1557.5774	1713.6785	1850.7374	2204.9099	*
b++	157.5621	239.0938	319.1091	376.6226	441.1439	514.6781	571.6995	664.7392	728.7685	779.2923	857.3429	925.8723	1102.9586	*
y+	*	2040.8514	1877.7880	1717.7574	1602.7304	1473.6878	1326.6194	1212.5765	1026.4972	898.4386	797.3909	641.2898	504.2309	150.0583
y++	*	1020.9293	939.3977	859.3823	801.8688	737.3475	663.8133	606.7919	513.7522	449.7229	399.1991	321.1485	252.6191	75.5328

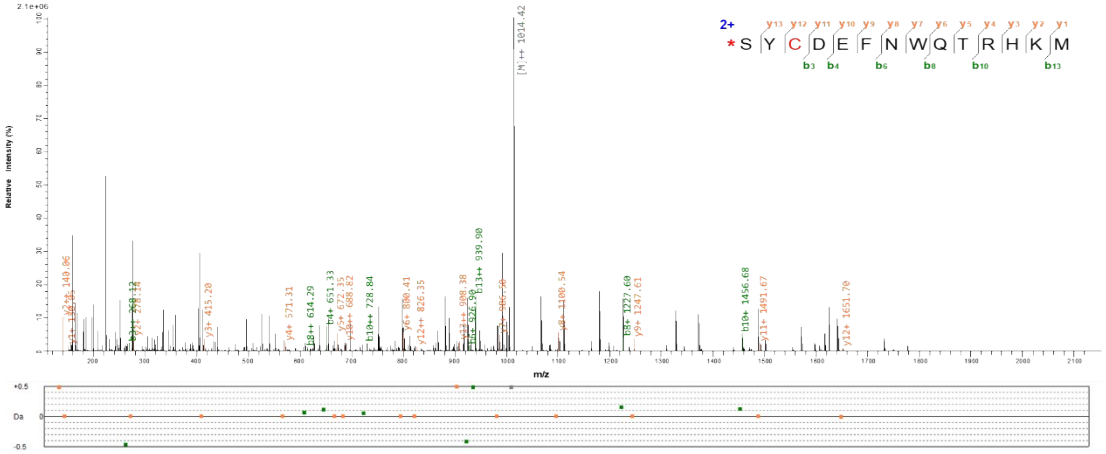
MS/MS analysis of double-amidated **10e**



HPLC traces of the quantity relationship of matter and crude reaction of **10f**. (10-80% B phase over 25 min, 1 mL·min^{-1} flow rate, 0.1% TFA, λ = 220 nm, Kromasil 100-5-C18 4.6 × 250 mm, 5 µm column). The green circle, blue triangle and orange square refer to the starting peptide, internal standard and target product.

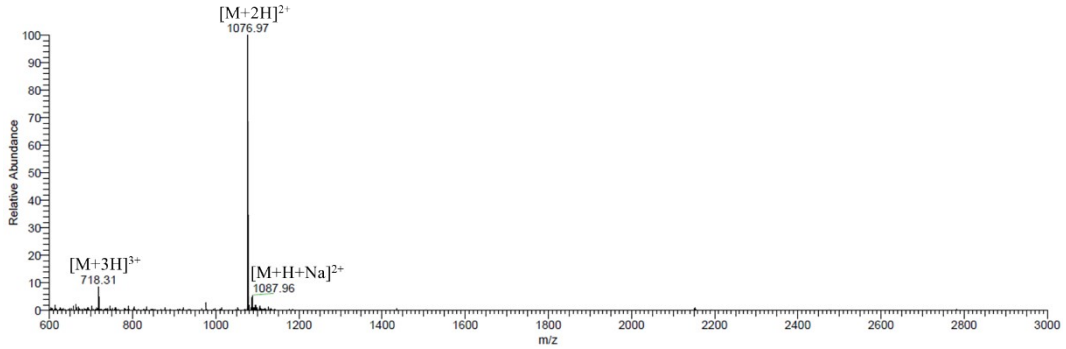


ESI Mass spectrum of purified single-amidated product **10f**. Calculated Mass $[M+2H]^{2+}$: 1014.44, $[M+3H]^{3+}$: 676.63; Mass Found (ESI+) $[M+2H]^{2+}$: 1014.44, $[M+3H]^{3+}$: 676.63.

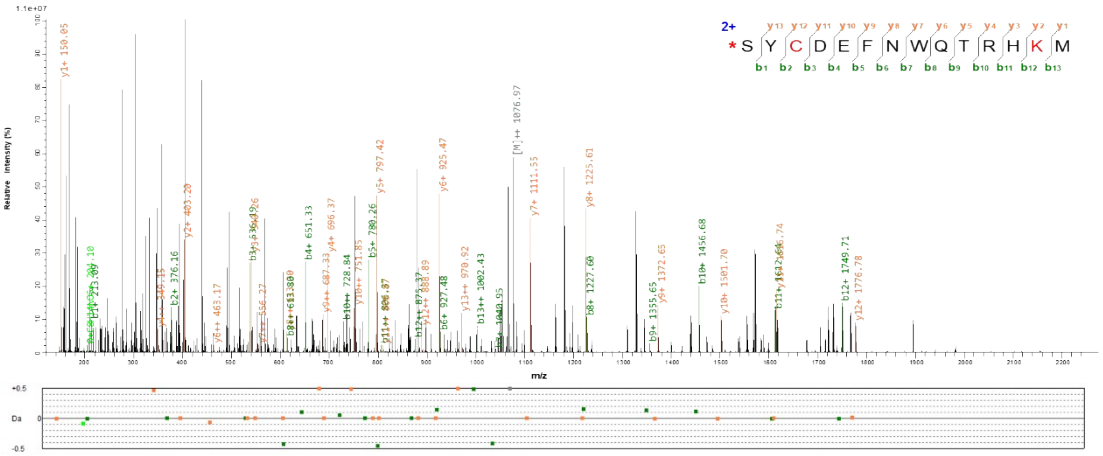


#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	213.0982	376.1615	536.1922	651.2192	780.2617	927.3302	1041.3731	1227.4524	1355.5110	1456.5587	1612.6598	1749.7187	1877.8136	*
b++	107.0527	188.5844	268.5997	326.1132	390.6345	464.1687	521.1902	614.2298	678.2591	728.7830	806.8335	875.3630	939.4105	*
y+	*	1814.7738	1651.7104	1491.6798	1376.6528	1247.6102	1100.5418	986.4989	800.4196	672.3610	571.3133	415.2122	278.1533	150.0583
y++	*	907.8905	826.3589	746.3435	688.8300	624.3088	550.7745	493.7531	400.7134	336.6841	286.1603	208.1097	139.5803	75.5328

MS/MS analysis of single-amidated **10f**



ESI Mass spectrum of purified single-amidated product **10f**. Calculated Mass $[M+2H]^{2+}$: 1076.97, $[M+3H]^{3+}$: 718.31; Mass Found (ESI+) $[M+2H]^{2+}$: 1076.97, $[M+3H]^{3+}$: 718.31.



#	S(1/*)	Y(2/13)	C(3/12)	D(4/11)	E(5/10)	F(6/9)	N(7/8)	W(8/7)	Q(9/6)	T(10/5)	R(11/4)	H(12/3)	K(13/2)	M(*/1)
b+	213.0982	376.1615	536.1922	651.2192	780.2617	927.3302	1041.3731	1227.4524	1355.5110	1456.5587	1612.6598	1749.7187	2002.8725	*
b++	107.0527	188.5844	268.5997	326.1132	390.6345	464.1687	521.1902	614.2298	678.2591	728.7830	806.8335	875.3630	1001.9399	*
y+	*	1939.8327	1776.7693	1616.7387	1501.7117	1372.6691	1225.6007	1111.5578	925.4785	797.4199	696.3722	540.2711	403.2122	150.0583
y++	*	970.4200	888.8883	808.8730	751.3595	686.8382	613.3040	556.2825	463.2429	399.2136	348.6897	270.6392	202.1097	75.5328

MS/MS analysis of single-amidated **10f**

3. Supporting information for protein conjugates

3.1 Antibody modifications at different light time

We analyzed the molecular weight of trastuzumab conjugates at different light time (10s and 60s) by protein mass spectrometry. The trastuzumab was de-glycosylated with PNGase F only, and was not reduced.

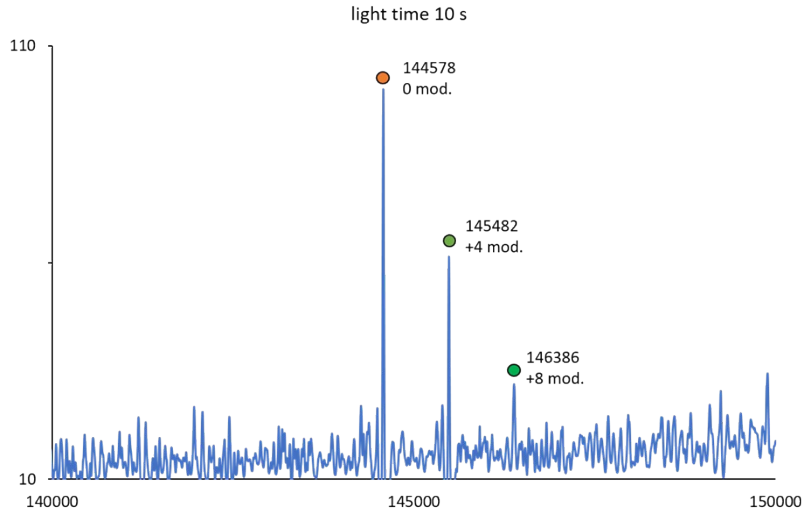


Figure S10 Trastuzumab was illuminated for 10s.

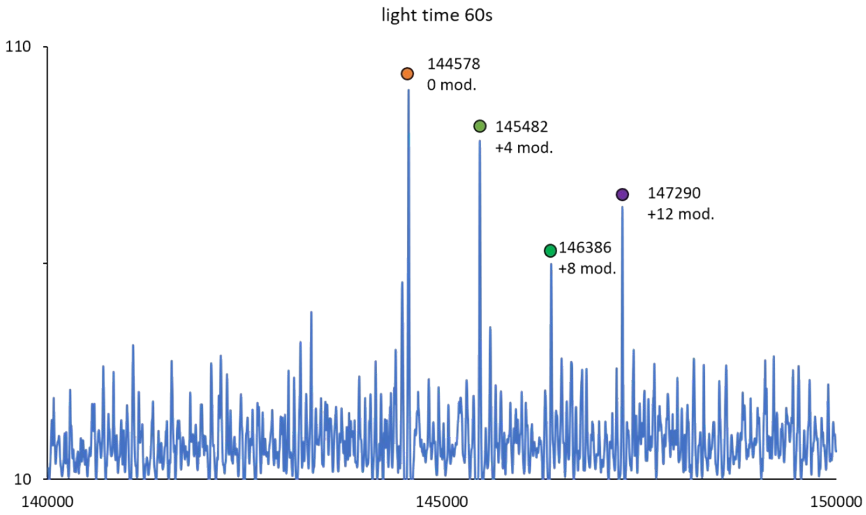
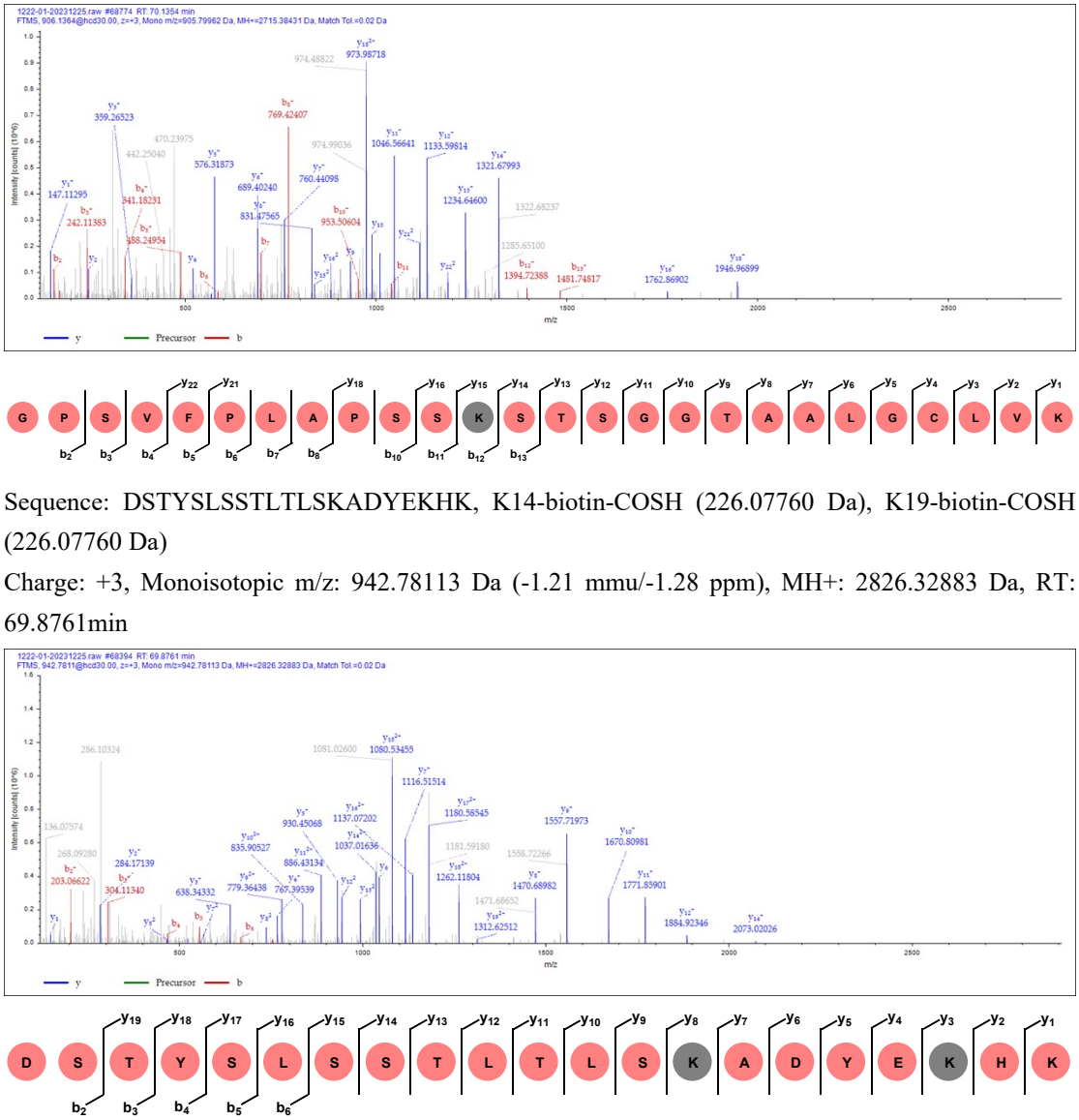


Figure S11 Trastuzumab was illuminated for 60s.

Sequence: GPSVFPLAPSSKSTSGGTAALGCLVK, C23-Carbamidomethyl (57.02146 Da), K12-biotin-COSH (226.07760 Da)

Charge: +3, Monoisotopic m/z: 905.79962 Da (-0.04 mmu/-0.05 ppm), MH+: 2715.38431 Da, RT: 70.1354 min,



Sequence: DSTYSLSSTLTLSKADYEKHK, K14-biotin-COSH (226.07760 Da), K19-biotin-COSH (226.07760 Da)

Charge: +3, Monoisotopic m/z: 942.78113 Da (-1.21 mmu/-1.28 ppm), MH+: 2826.32883 Da, RT: 69.8761min

Figure S12 MS/MS analysis of trastuzumab-biotin

3.2 Antibody aggregation assay

Trastuzumab (0.4 mg/mL) and trastuzumab-biotin (40 μg/mL) were analyzed with Superdex 200 increase 10 300 GL.

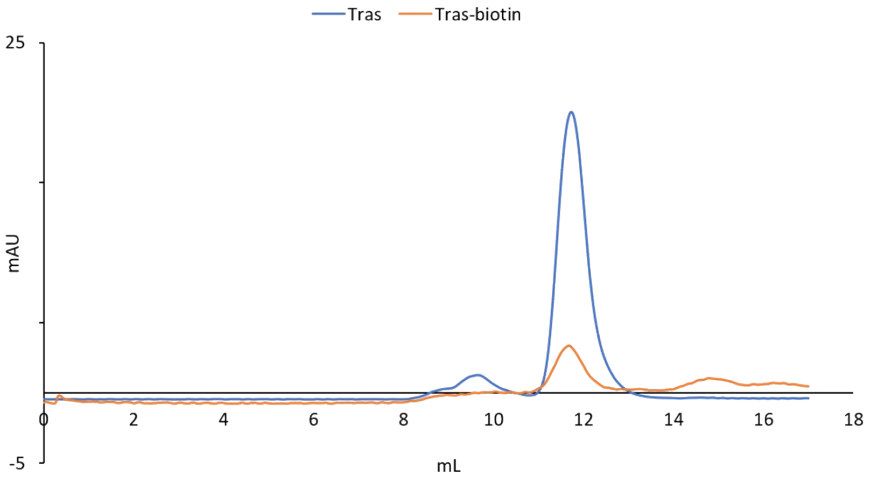


Figure S13 Comparison antibody aggregation of Tras and Tras-biotin with SEC.

3.3 Sample processing of LC-MS/MS to determine protein modification site

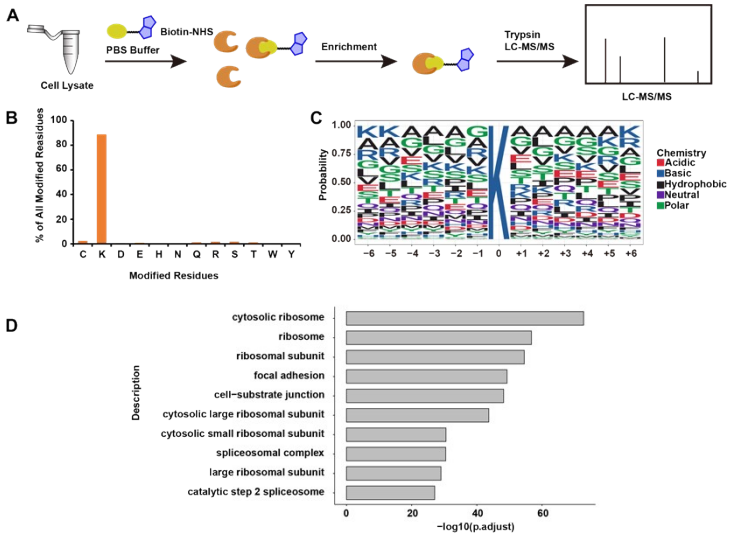


Figure S14 (A) Schematic representation of the labeling proteome. (B) **Biotin-NHS** preferentially labels lysine in SK-BR-3 cells. (E) Frequency plots showing the conserved motifs from SK-BR-3 cells using **Biotin-NHS**. (D) GO-Cellular Component (GO-CC) annotation of highly enriched protein targeted by **Biotin-NHS**.