ELECTRONIC SUPPLEMENTARY INFORMATION

Light-Initiated 1,3-Dipolar Cycloaddition between Dehydroalanines and Tetrazoles: Application to Late-stage Peptide and Protein Modifications

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General information

Materials

2-tert-Butoxycarbonylamino-acrylic acid methyl ester, biotin-PEG_4-amine and dansylcadaverine were purchased from Bidepharm. Biotin-PEG_3-azide was purchased from Energy Chemical. Fluorescein-PEG_2-N_3 was purchased from Aladdin. Thiostrepton was purchased from Macklin. Fetal bovine serum (FBS) was purchased from Gibco Life Technologies. Penicillin-streptomycin and trypsin-EDTA were purchased from Sigma-Aldrich. Phosphate-buffered saline (PBS, pH 7.4) and RPMI-1640 were purchased from Corning. Cell Counting Kit-8 (CCK-8) was purchased from Dojindo. All the reagents obtained from commercial companies were used directly without further purification. All solvents used in the experiments were reagent/HPLC grade. Light source: a 8 W hand-held UV lamp equipped with two 302-nm wavelength bulbs (Taobao, China).

General Methods

Time-course analysis of photo-initiated 1,3-dipolar cycloaddition between small molecule dehydroalanine derivative (Boc-Dha-OMe) and tetrazole was performed in the 24-well plate on BioTek Synergy 4 Multi-Mode Microplate Reader.

Fluorescence measurements were conducted using 1-cm pathlength cuvettes on HORIBA FluoroMax Spectrofluorometer at 20 °C.

Circular dichroism (CD) spectra measurements were performed using a 1-mm pathlength cuvette on Applied Photophysics Chirascan Plus CD spectrometer.

Cytotoxicity assay was conducted in the 96-well plate on the BioTek Synergy 4 Multi-Mode Microplate Reader. Cell labeling rates and fluorescence intensity were analyzed by the Beckman Coulter's CytoFLEX LX Flow Cytometer. Confocal experiment was conducted on the inverted Zeiss LSM780 laser scanning confocal microscope.

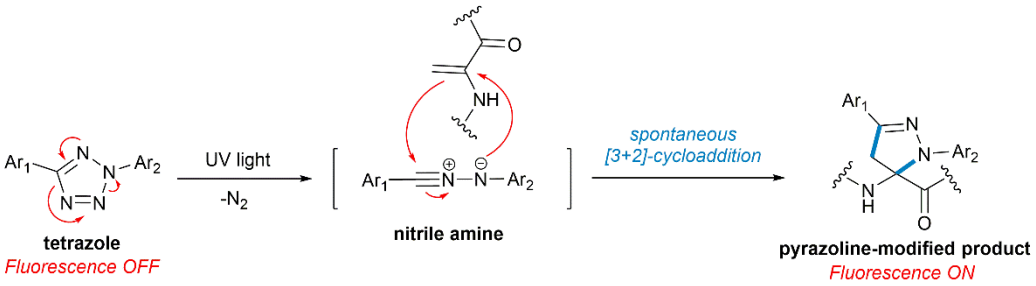
The time-course analysis of peptide substrate was performed on Shimadzu LC-2010A HPLC (YMC analytic C18 column, Japan, 10 μm, 4.5×150 mm) at a flow rate of 0.7 mL/min and UV detection was set at 215 and 254 nm. The purifications of peptides and small molecules were carried on Shimadzu LC-20AD reversed-phase HPLC (RP-HPLC) (YMC C18 column, 5 μm, 20×250 mm, Japan) at a flow rate of 8 mL/min. The purifications of proteins were carried on Shimadzu LC-20AD RP-HPLC (Proteonavi C4 column, 5 μm, 10×250 mm, Japan) at a flow rate of 4 mL/min. The analyses of peptides were performed on Shimadzu LC-2010A HPLC (YMC analytic C18 column, Japan, 10 μm, 4.5×150 mm) at a flow rate of 0.7 mL/min and UV detection was set at 215 and 254 nm. The analyses of proteins were performed on Shimadzu LC-2010A HPLC (Proteonavi analytic C4 column, Japan, 5 μm, 4.6×150 mm) at a flow rate of 0.8 mL/min and UV detection was set at 215 and 254 nm. All the solution of HPLC was composed of

solution B (80% acetonitrile/water with 0.06% trifluoroacetic acid) in solution A (water with 0.06% trifluoroacetic acid) with different ratios. All the conversion rates of modified peptides were estimated by UV absorption at 215 nm of the peak in RP-HPLC spectra of the reaction mixture. Conversion was defined as the percentage of pyrazoline-derived peptide or protein peaks versus the sum of all peptide or protein peaks.

Thermo Scientific UltiMate 3000 (ESI-MS) was used to identify peptides and small molecules. Q-TOF MS was used to identify proteins.

NMR spectrum of thiostrepton and its derivatives were recorded on JNM-ECA600 (600MHz). NMR spectrum of peptides **3b**, **4b** and **5b** were recorded on Bruker Avance-HD-800X (800MHz). NMR spectrum of other reactants and products were recorded on JNM-ECZ400S spectrometer (400MHz).

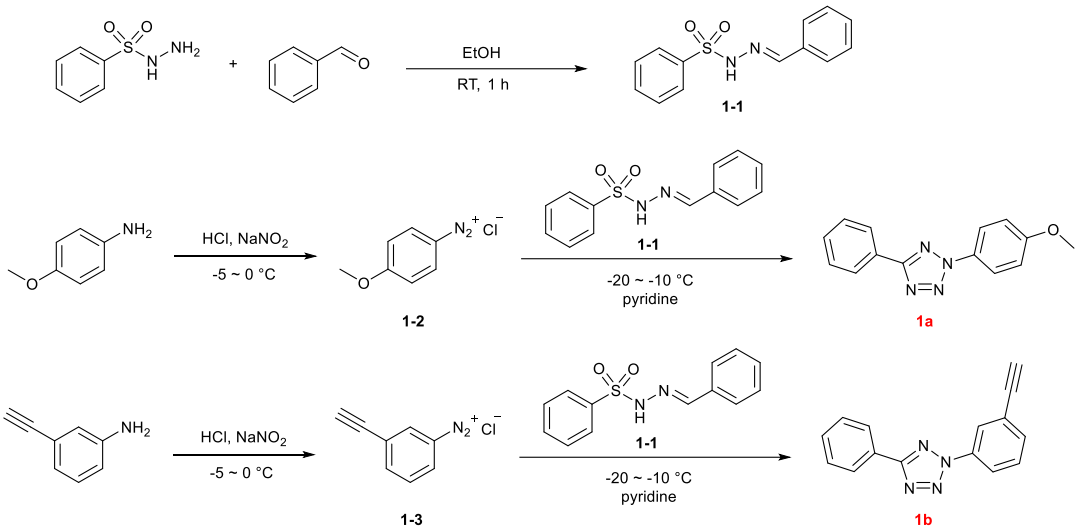
Strategy for dehydroalanine (Dha) modification using 2,5-diaryl tetrazole reagents



Scheme S1. Photoinitiated 1,3-dipolar cycloaddition reaction between a 2,5-diaryl tetrazole and a Dha residue.[1]

General procedure for synthesis and purification of tetrazoles 1a-1d

*Synthesis of tetrazoles **1a** and **1b***



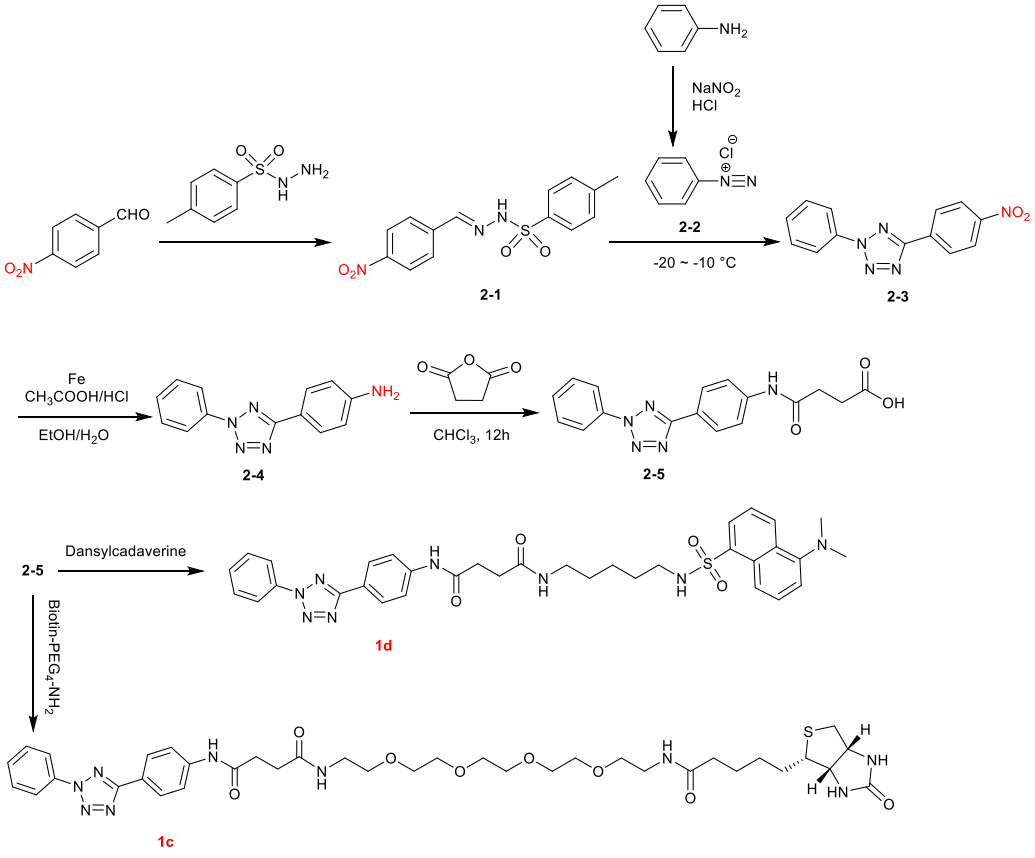
Tetrazoles **1a** and **1b** were synthesized using a previously reported protocol.[2] Benzaldehyde (2.0 mL, 20 mmol) was added to a solution of phenylsulfonylhydrazide (3.4 g, 20 mmol) in absolute ethanol (30 mL). The mixture was stirred for an hour at room temperature. A large amount of white precipitate was generated. The mixture was diluted with water to precipitate the sulfonylhydrazone **1-1**. The precipitate was collected by filtration, washed with aqueous ethanol, and dried under vacuum to obtain the crude product. Recrystallized the crude product with hot EtOH/H_2O, obtaining the white solid product **1-1** with 95.2% yield. Compound **1-1**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 1H), 8.01 (d, *J* = 7.7 Hz, 2H), 7.79 (s, 1H), 7.54 (m, 5H), 7.36 (m, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 148.15, 138.26, 133.39, 133.12, 130.54, 129.13, 128.69, 127.93, 127.43.

p-Anisidine (615.8 mg, 5 mmol) and 2.0 mL of concentrated HCl were added in a mixed solution of EtOH and H_2O (4 mL/ 4 mL). A solution of $NaNO_2$ (345 mg, 5 mmol in 2 mL of H_2O) was added slowly to the above solution to prepare the diazonium salt **1-2**. The reaction temperature was controlled between -5 and 0 °C. Then the **1-2** solution was added carefully to a solution of **1-1** (1031.6 mg, 5 mmol) in pyridine (50 mL) at -20 to -10 °C after 30 minutes. The reactants were stirred at room temperature once the addition was completed. The reaction solution was extracted with chloroform and water. The chloroform layer was combined and washed three times with 2 M HCl. Dry the chloroform layer with anhydrous Na_2SO_4. After filtration to remove Na_2SO_4, the chloroform was removed under vacuum and the residue was purified by silica gel flash column chromatography to give the pure **1a** product using PE/EA (15:1, v/v) as the eluent. The yield of **1a** was 57.3%. Compound **1a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (dd, J = 7.8, 1.7 Hz, 2H), 8.11 (d, J = 9.1 Hz, 2H), 7.51 (m, 3H), 7.06 (d, J = 9.1 Hz, 2H), 3.89 (s, 3H); ^{13}C NMR

(101 MHz, $CDCl_3$): δ 165.10, 160.60, 130.59, 130.53, 129.03, 127.42, 127.10, 121.50, 114.78, 55.77. ESI-IT/TOF MS: m/z calculated for $C_{14}H_{12}N_4O$: 253.1084, $[M+H]^+$. Found: 253.1083, $[M+H]^+$.

m-Ethynylaniline (585.8 mg, 5 mmol) and 2.0 mL concentrated HCl were added to a mixed solution of EtOH and H_2O (4 mL/ 4 mL). A solution of $NaNO_2$ (345 mg, 5 mmol in 2 mL of H_2O) was slowly added to the above solution to prepare the diazonium salt **1-3**. The reaction temperature was controlled between -5 and 0 °C. Then the **1-3** solution was carefully added to a solution of **1-1** (1031.6 mg, 5 mmol) in pyridine (30 mL) at -20 to -10 °C after 30 minutes. The reactants were stirred in room temperature once the addition was completed. The reaction solution was then extracted with chloroform and water. The chloroform layer was combined and washed three times with 2 M HCl. Dry the chloroform layer with anhydrous $MgSO_4$. After filtration to remove $MgSO_4$, the filtrate was concentrated *in vacuo*. The chloroform was removed under vacuum and the residue was purified by silica gel flash column chromatography to give the pure **1b** product using PE/EA (20:1, v/v) as the eluent. The yield of the reaction was 68.5%. Compound **1b**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.34 (t, *J* = 2.0 Hz, 1H), 8.25 (m, 2H), 8.21 (ddd, *J* = 8.1, 2.0, 1.2 Hz, 1H), 7.62 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.53 (m, 4H), 3.21 (s, 1H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 165.42, 136.91, 133.13, 130.75, 129.80, 129.04, 127.15, 124.01, 123.32, 120.08, 82.05, 79.16. ESI-IT/TOF MS: m/z calculated for $C_{15}H_{10}N_4$: 247.0978, $[M+H]^+$. Found: 247.0976, $[M+H]^+$.

*Synthesis of tetrazoles **1c** and **1d***



p-Nitrobenzaldehyde (3.02 g, 20 mmol) was added to a solution of p-toluenesulfonyl hydrazide (3.72 g, 20 mmol) in absolute ethanol (150 mL). The mixture was stirred for an hour at room temperature. A large amount of white precipitate was generated. The mixture was diluted with water to precipitate the sulfonylhydrazone **2-1**. The precipitate was collected by filtration, washed with aqueous ethanol, and dried under vacuum to obtain the crude product. Recrystallized the crude product with hot EtOH/H_2O, obtaining white solid product **2-1** with 94.1% yield. Compound **2-1**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.65 (s, 1H), 8.19 (d, *J* = 8.9 Hz, 2H), 7.89 (d, *J* = 8.4 Hz, 2H), 7.83 (s, 1H), 7.72 (d, *J* = 8.9 Hz, 2H), 7.34 (d, *J* = 8.1 Hz, 2H), 2.42 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 148.62, 144.87, 144.25, 139.15, 134.96, 129.94, 127.97, 127.89, 124.01, 21.68.

Aniline (465.6 μL, 5 mmol) and 2.0 mL of concentrated HCl were added to a mixed solution of EtOH and H_2O (4 mL/ 4 mL). A solution of $NaNO_2$ (345 mg, 5 mmol in 2 mL of H_2O) was slowly added to the above solution to prepare the diazonium salt **2-2**. The reaction temperature was controlled between -5 and 0 ℃. Then the **2-2** solution was carefully added to the solution of **2-1** (1596.7 mg, 5 mmol) in pyridine (50 mL) at -20 to -10 ℃ after 30 minutes. The mixture was allowed to warm at room temperature during an hour once the addition was completed. The reactants were stirred overnight. After adding HCl (10%), the mixture was extracted with chloroform and water. The chloroform layer was combined and washed three times with 2 M HCl. Dry the chloroform layer with anhydrous $MgSO_4$. After filtration to remove $MgSO_4$, the filtrate was concentrated *in vacuo* to give the crude **2-3** product (867.2 mg) as a yellow solid.

Without further purification, the crude **2-3** product and 1.6 g iron powder were directly dissolved in ethanol/water (2:1, v/v) solution. 15 ml of acetic acid/ hydrochloric acid (1/1, v/v) was added into the solution. The reaction was performed at 45 ℃ under ultrasound for 3 hours and monitored by TLC and ESI-MS. After completion of the reaction, the iron powder was removed by filtration, and the remaining solution was extracted three times with ethyl acetate. The organic layer was dried with anhydrous $MgSO_4$. After filtration to remove $MgSO_4$, the filtrate was concentrated under vacuum and purified by silica gel flash column chromatography to give the pure **2-4** product (518.4 mg) as a white solid using DCM/MeOH (8:1, v/v) as the eluent. Compound **2-4**: ^{1}H NMR (400 MHz, CD_3OD): δ 8.09 (d, *J* = 8.1 Hz, 2H), 7.86 (d, *J* = 8.6 Hz, 2H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.47 (m, 1H), 6.74 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (101 MHz, CD_3OD): δ 166.20, 151.30, 137.41, 130.05, 129.91, 128.37, 119.86, 114.92. ESI-MS: m/z calculated for $C_{13}H_{11}N_5$: 238.1, $[M+H]^+$. Found: 238.2, $[M+H]^+$.

A solution of **2-4** (474.5, 2 mmol) in Et_2O (30 mL) was added into a stirred solution of succinic anhydride (200 mg, 2 mmol) in a mixture of benzene and 1,4-dioxane (60 mL, 2:1) in a dropwise fashion at room temperature over a period of 20 min. The mixture was further stirred for 2 hours. The resulting white solid product was filtered, washed with 25 mL of Et_2O, and dried under vacuum to obtain compound **2-5**. The yield was quantitative. Compound **2-5** was used in the subsequent step without further purification.

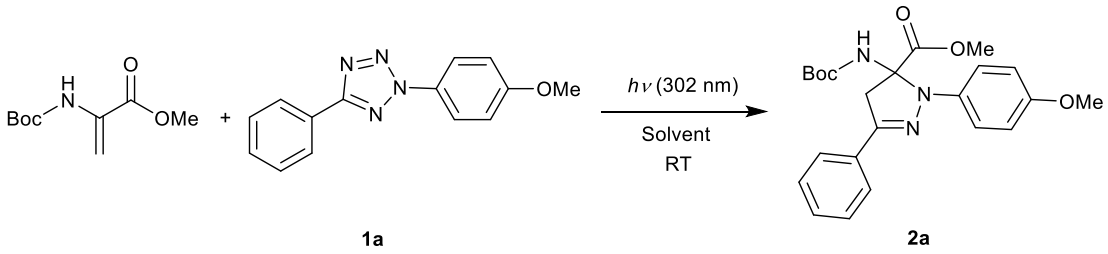
Compound **2-5**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.27 (s, 1H), 8.15 (d, *J* = 8.2 Hz, 2H) , 8.10 (d, *J* = 8.0 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 2H), 7.69 (m, 2H), 7.62 (m,1H), 2.62 (d, *J* = 7.8 Hz, 2H), 2.55 (d, *J* = 7.8 Hz, 2H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 174.35, 171.12, 164.94, 142.19, 136.75, 130.68, 127.94, 121.27, 120.42, 119.74, 31.68, 29.23. ESI-IT/TOF MS: m/z calculated for $C_{17}H_{15}N_5O_3$: 338.1248, $[M+H]^+$. Found: 338.1247, $[M+H]^+$.

Biotin-PEG$_4$-NH$_2$ (231.2 mg, 0.5 mmol) was dissolved in 30 mL DCM/DMF (2:1, v/v) solution. Then compound **2-5** (84.3 mg, 0.25 mmol), HATU (95.1 mg, 0.25 mmol) and DIPEA (87 μL, 0.5 mmol) were added and stirred for 12 hours. After filtration, the solution was washed with 1 M HCl (3 × 50 mL), saturated $NaHCO_3$ solution (3 × 50 mL) and water (3 × 50 mL). The combined organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was recrystallized with CH_2Cl_2/ n-hexane, obtaining white solid product **1c** with 56.3% yield. Compound **1c**: ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.79 (s, 1H), 8.16 (td, *J* = 7.9, 7.2, 1.6 Hz, 4H), 7.77 (d, *J* = 8.7 Hz, 2H), 7.56 (m, 2H), 7.48 (t, *J* = 7.3 Hz, 1H), 4.49 (dd, *J* = 7.7, 4.9 Hz, 1H), 4.30 (dd, *J* = 7.2, 5.3 Hz, 1H), 3.65–3.58 (m, 12H), 3.55 (q, *J* = 5.2 Hz, 4H), 3.43 (ddd, *J* = 16.5, 9.7, 3.8 Hz, 4H), 3.10 (td, *J* = 7.3, 4.8 Hz, 1H), 2.88 (dd, *J* = 12.8, 4.9 Hz, 1H), 2.82–2.63 (br, m, 5H), 2.17–2.09 (br, m, 2H), 1.62 (dt, *J* = 14.9, 8.0 Hz, 4H), 1.39 (tp, *J* = 12.9, 6.2 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$): δ 173.52, 172.80, 171.59, 164.99, 164.05, 140.95, 136.94, 129.67, 129.58, 127.74, 122.23, 119.86, 70.46, 70.14, 69.81, 61.95, 60.19, 55.67, 40.51, 39.46, 35.62, 32.85, 31.36, 29.72, 28.15, 25.51. ESI-IT/TOF MS: m/z calculated for $C_{37}H_{51}N_9O_8S$: 782.3654, $[M+H]^+$; 804.3474, $[M+Na]^+$. Found: 782.3656, $[M+H]^+$; 804.3474, $[M+Na]^+$.

Dansylcadaverine (167.7 mg, 0.5 mmol) was dissolved in 30 mL DCM/DMF (2:1, v/v) solution. Then compound **2-5** (84.3 mg, 0.25 mmol), HATU (95.1 mg, 0.25 mmol) and DIPEA (87 μL, 0.5 mmol) were added and stirred for 12 hours. After filtration, the solution was washed with 1 M HCl (3 × 50 mL), saturated $NaHCO_3$ solution (3 × 50 mL) and water (3 × 50 mL). The combined organic layer was dried with $MgSO_4$ and concentrated *in vacuo*. The crude product was recrystallized with CH_2Cl_2/ n-hexane, obtaining white solid product **1d** with 73.8% yield. Compound **1d**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.24 (s, 1H), 8.44 (d, *J* = 8.5 Hz, 1H), 8.30 (d, *J* = 8.7 Hz, 1H), 8.15 (d, *J* = 7.5 Hz, 2H), 8.12–8.07 (m, 3H), 7.89–7.73 (m, 4H), 7.70 (t, *J* = 7.6 Hz, 2H), 7.64–7.56 (m, 3H), 7.24 (d, *J* = 7.5 Hz, 1H), 2.87 (q, *J* = 6.6 Hz, 2H), 2.81 (s, 6H), 2.75 (q, *J* = 6.6 Hz, 2H), 2.58 (t, *J* = 7.1 Hz, 2H), 2.39 (t, *J* = 7.1 Hz, 2H), 1.32–1.25 (m, 2H), 1.23–1.16 (m, 2H), 1.10 (dt, *J* = 13.3, 6.2 Hz, 2H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 171.53, 171.40, 164.96, 151.83, 142.26, 136.76, 136.70, 130.69, 129.84, 129.64, 129.58, 128.74, 128.30, 127.92, 124.12, 121.22, 120.43, 119.73, 115.65, 45.60, 42.85, 38.84, 32.32, 30.67, 29.32, 29.07, 23.86. ESI-IT/TOF MS: m/z calculated for $C_{34}H_{38}N_8O_4S$: 655.2809, $[M+H]^+$; 677.2629, $[M+Na]^+$. Found: 655.2808, $[M+H]^+$; 677.2817, $[M+Na]^+$.

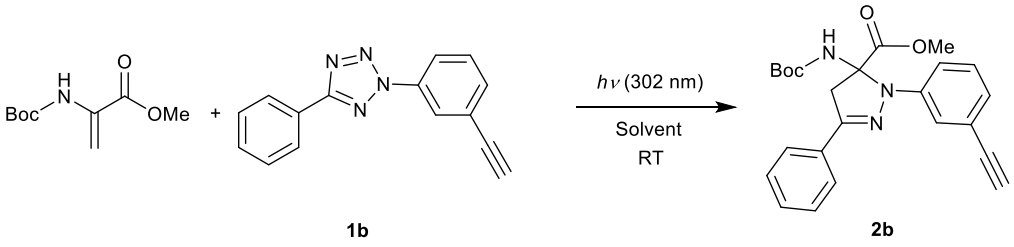
Procedure for photo-initiated 1,3-dipolar cycloaddition between tetrazoles 1a/1b and Boc-Dha-OMe

Boc-Dha-OMe (25 μmol) and excess tetrazole **1a** (30-50 μmol) were dissolved in the solvent in a quartz glass tube equipped with a magnetic stir bar. The reactants were stirred at room temperature in the open air. Next, the solution was irradiated by a hand-held 302-nm UV lamp from one side for 5-10 min. The reaction was monitored using TLC and ESI-MS. After completion of the reaction, the solvent was evaporated, and the product was purified by column chromatography (PE/EA=5/1, v/v), resulting in the isolation of white solid pyrazoline product **2a**. The product was further analyzed by ESI-MS, ^{1}H and ^{13}C NMR. Compound **2a**: ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.68 (d, J = 7.1 Hz, 2H), 7.42 (t, J = 7.3 Hz, 2H), 7.36 (t, J = 7.2 Hz, 1H), 7.18 – 7.08 (m, 2H), 6.84 (d, J = 9.0 Hz, 2H), 3.80 (d, J = 16.0 Hz, 1H), 3.70 (s, 3H), 3.66 (d, J = 16.0 Hz, 1H), 3.65 (s, 3H), 1.20 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6): δ 169.73, 154.63, 145.73, 136.24, 132.07, 128.64, 125.50, 119.43, 113.75, 80.30, 79.22, 55.23, 53.13, 45.98, 27.82. ESI-IT/TOF MS: m/z calculated for $C_{23}H_{27}N_3O_5$: 426.2023, $[M+H]^+$; 448.1843, $[M+Na]^+$. Found: 426.2020, $[M+H]^+$; 448.1845, $[M+Na]^+$.



Scheme S2. Synthesis of small molecule pyrazoline product **2a**.

The synthesis and purification route of pyrazoline **2b** was the same as that of **2a**. Compound **2b**: ^{1}H NMR (400 MHz, CD_3CN): δ 7.75 (dt, J = 6.8, 1.6 Hz, 2H), 7.47–7.40 (m, 3H), 7.24 (t, J = 7.9 Hz, 2H), 7.15 (ddd, J = 8.3, 2.3, 0.8 Hz, 1H), 7.01 (dt, J = 7.5, 1.1 Hz, 1H), 6.63 (s, 1H), 3.82 (m, 2H), 3.76 (s, 3H), 3.37 (s, 1H), 1.22 (s, 9H); ^{13}C NMR (101 MHz, CD_3CN): δ 171.65, 158.24, 153.96, 147.93, 143.33, 132.88, 130.20, 130.17, 129.75, 126.76, 124.87, 123.43, 118.07, 116.16, 86.72, 84.46, 79.43, 78.53, 54.73, 28.26. ESI-IT/TOF MS: m/z calculated for $C_{24}H_{25}N_3O_4$: 420.1918, $[M+H]^+$; 442.1737, $[M+Na]^+$. Found: 420.1926, $[M+H]^+$; 442.1735, $[M+Na]^+$.



Scheme S3. Synthesis of small molecule pyrazoline product **2b**.

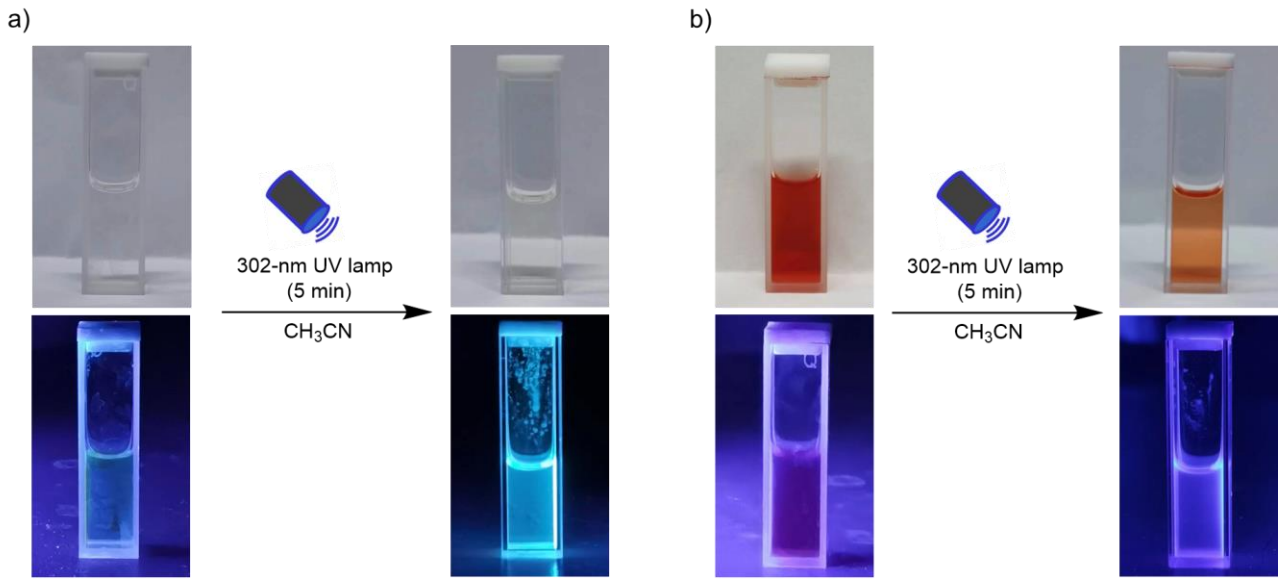


Fig. S1. a) Pictures of the reaction mixture of tetrazole **1a** and Boc-Dha-OMe before and after UV light irradiation. Ex, 365 nm. b) Pictures of the reaction mixture of tetrazole **1b** and Boc-Dha-OMe before and after UV light irradiation. Ex, 365 nm.

Time-course analysis of photo-initiated 1,3-dipolar cycloaddition between tetrazole 1a and Boc-Dha-OMe

The fluorescence spectrum of pyrazoline product **2a** [Fig. S2, a)] was measured on a fluorescence spectrometer. The sample was dissolved in MeCN/H_2O (1/1, v/v). Then the solution was excited at 390 nm, and fluorescence emission was scanned in the region of 400 to 700 nm through a 2 nm slit. And the maximum fluorescence emission wavelength ($\lambda_{em,\,max}$) was determined to be 485 nm [Fig. S2, b)].

Appropriate amounts of tetrazole **1a** (10 mM in MeCN) and Boc-Dha-OMe were diluted into MeCN [Fig. S2, c)] or the acetonitrile-water mixed solvent [Fig. S2, d)] to derive the final Boc-Dha-OMe **1a** concentration of 200 μM, and the final **1a** concentrations of 400 μM,1 mM and 2 mM, respectively. Separate reactions were set up by incubating 200 μL of reaction mixture in a 48-well plate and irradiated with a hand-held 302-nm UV lamp for 30 seconds. The fluorescent cycloaddition product **2a** in the reaction mixtures were monitored by fluorescence assay. The reactions were detected with the excitation wavelength of 390 nm on a multifunctional enzyme marker, and the fluorescence intensity at the emission wavelength of 485 nm was collected. Next, the composition of the reaction solution was irradiated and measured at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18 and 20 minutes from the beginning of the reaction using the same method.

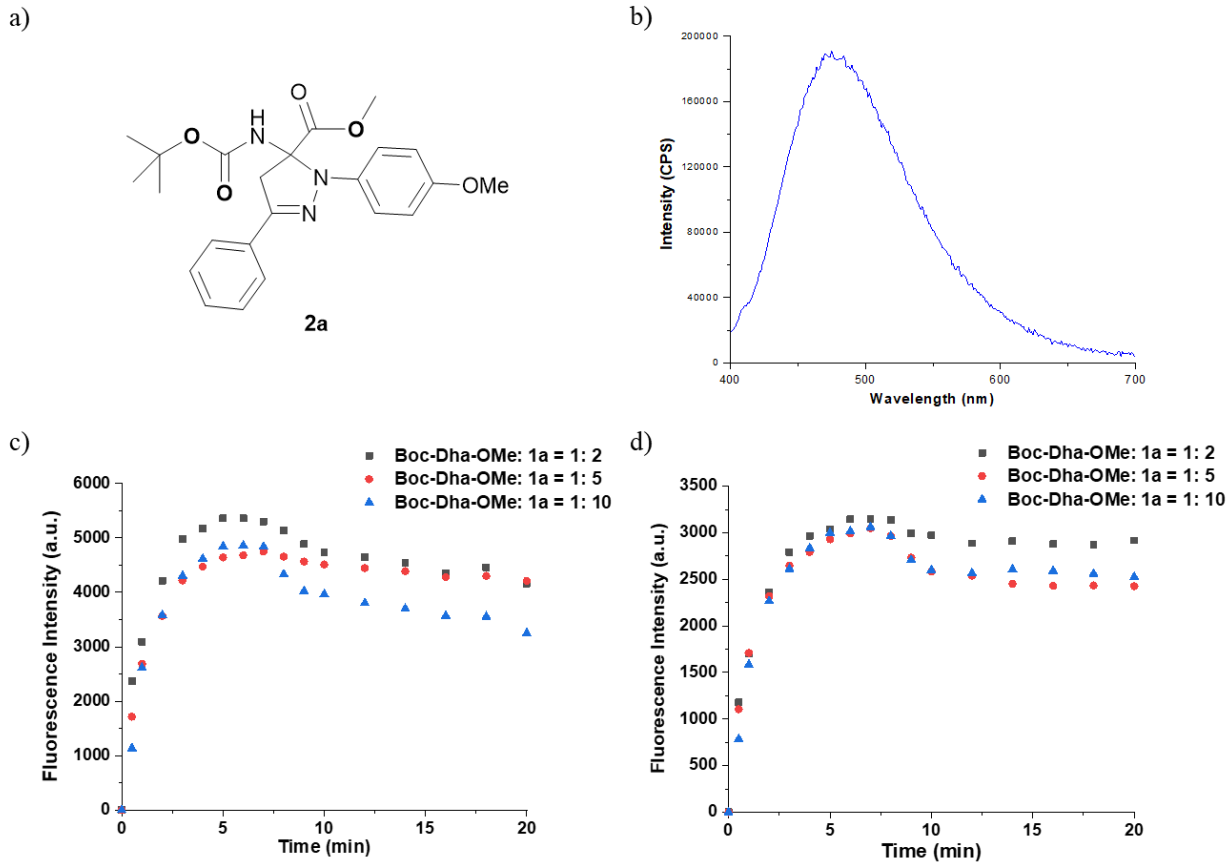


Fig. S2. Time-course studies between Boc-Dha-OMe (200 μM) and tetrazole **1a** (200 μM - 2 mM). a) Chemical structure of **2a**. b) Fluorescence spectrum of **2a** (λ_{ex}= 390 nm, $\lambda_{em,\ max}$= 485 nm). c) Time-course studies in MeCN solvent; d) Time-course studies in the mixed MeCN/H_2O (1/1, v/v) solvent.

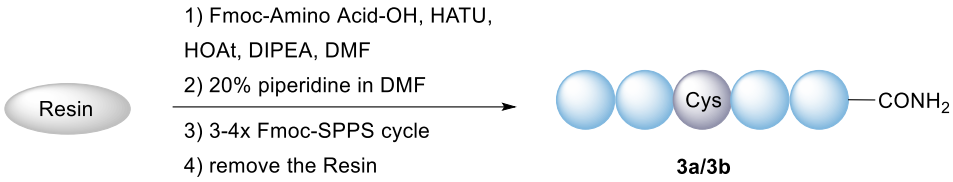
The fluorescence intensity of the product in the reaction mixture reached its maximum at 5 minutes in MeCN [Fig. S2, c)], indicating that the reaction could be completed within 5 minutes in MeCN. Similarly, the reaction could be completed within 5-7 minutes in MeCN/H_2O (1/1, v/v) [Fig. S2, d)].

General procedure for synthesis and purification of Cys-containing peptides 3a-3e

Cysteine-containing peptides **3a-3e** were synthesized through Solid Phase Peptide Synthesis (SPPS) using Fmoc protocol.

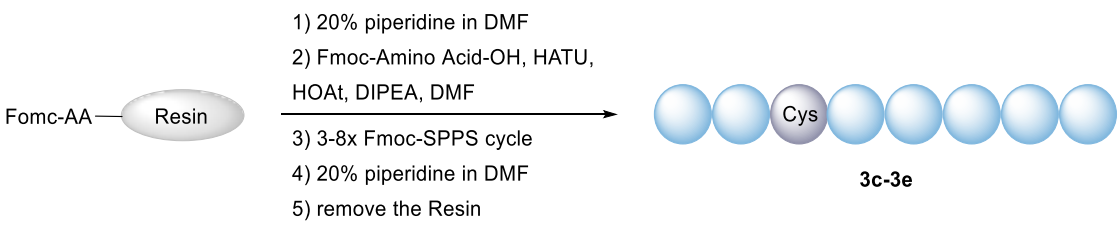
*Synthesis of peptides **3a** and **3b***

Rink Amide MBHA resin (loading 0.272 mmol/g) was swollen in dry DCM (10 mL) for 1 h at 37 °C, and then drained. Fmoc group was deprotected with 20% piperidine in DMF for 15 minutes at 37 °C. Then 4.0 equiv. Fmoc-Leu-OH (**3a**) or Fmoc-Cys-OH (**3b**) was coupled with HATU (4.0 equiv.), HOAt (4.0 equiv.) and DIPEA (8.0 equiv.) in DMF for 1 h. After removing the Fmoc group, other amino acids were sequentially linked using this method. Once the last Fmoc-amino acid was coupled to the resin, the Fmoc group was deprotected, and the peptide was cleaved from the resin with a TFA/TIPS/EDT/H_2O (92.5/2.5/2.5/2.5, v/v/v/v) reagent. After precipitating with diethyl ether, the peptides were purified with preparative RP-HPLC using C18 column and identified with ESI-MS, and then lyophilized to obtain peptide **3a** and **3b**.



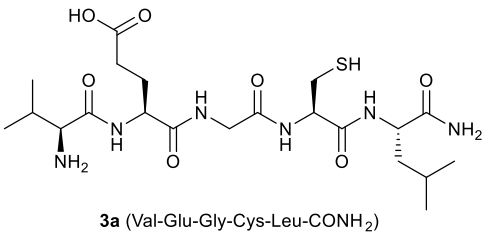
*Synthesis of peptides **3c-3e***

The synthesis of peptides **3c-3e** were performed on the corresponding Fmoc-Amino Acid Wang Resins. First, the Fmoc-Amino Acid Wang resin (0.2 mmol, 1 equiv.) was swollen in dry DCM (10 mL) for 1 h at 37 °C, and then drained. Fmoc group was deprotected with 20% piperidine in DMF for 15 minutes at 37 °C. Then 4.0 equiv. other Fmoc-amino acids were coupled with HATU (4.0 equiv.), HOAt (4.0 equiv.) and DIPEA (8.0 equiv.) in DMF for 1 h after removing Fmoc groups for 4-9 cycles. Once the last Fmoc-amino acid was coupled to the resin, the Fmoc group was deprotected, and the peptide was cleaved from the resin with a TFA/TIPS/EDT/H_2O (92.5/2.5/2.5/2.5, v/v/v/v) reagent. After precipitating with diethyl ether, the peptides were purified with preparative RP-HPLC using C18 column and identified with ESI-MS, and then lyophilized to obtain peptide product.



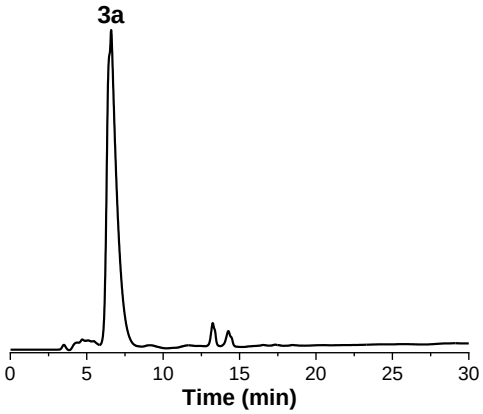
Chemical structures and characterization of Cys-containing peptides 3a-3e

a)



3a (Val-Glu-Gly-Cys-Leu-$CONH_2$)
Chemical Formula: $C_{21}H_{38}N_6O_7S$
Exact Mass: 518.25
Molecular Weight: 518.63

b)



c)

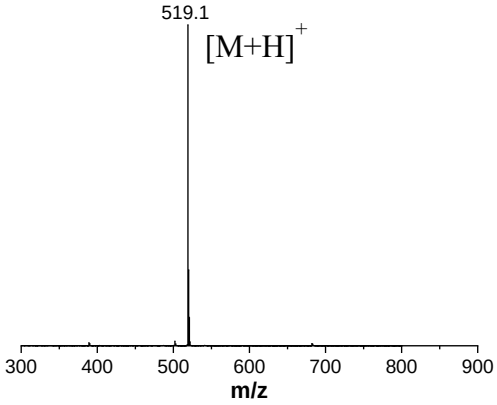
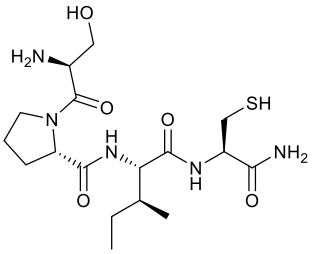


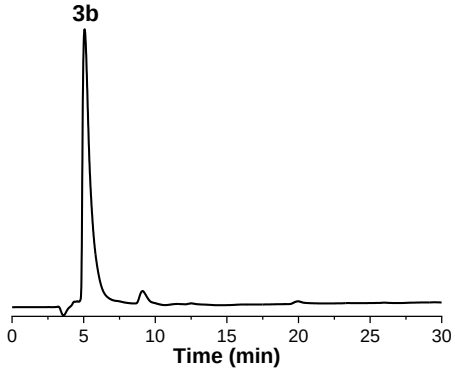
Fig. S3. a) Chemical structure of peptide **3a**; b) RP-HPLC trace of peptide **3a**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{21}H_{38}N_6O_7S$: 519.3, $[M+H]^+$. Found: 519.1, $[M+H]^+$.

a)



3b (Ser-Pro-Ile-Cys-$CONH_2$)
Chemical Formula: $C_{17}H_{31}N_5O_5S$
Exact Mass: 417.20
Molecular Weight: 417.53

b)



c)

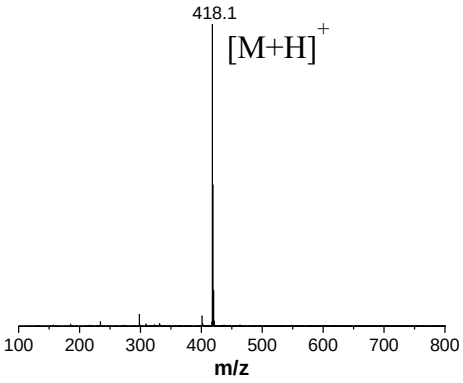
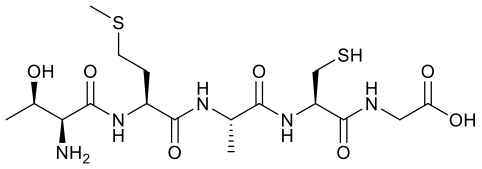


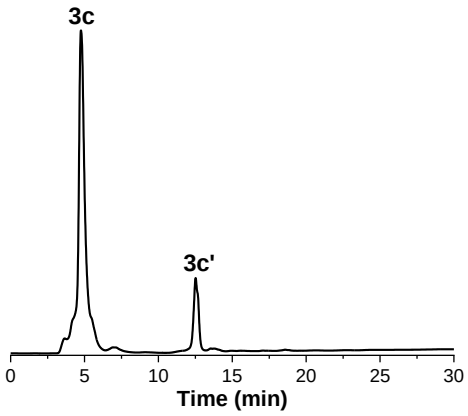
Fig. S4. a) Chemical structure of peptide **3b**; b) RP-HPLC trace of peptide **3b**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{17}H_{31}N_5O_5S$: 418.2, $[M+H]^+$. Found: 418.1, $[M+H]^+$.

a)



3c (Thr-Met-Ala-Cys-Gly)
Chemical Formula: $C_{17}H_{31}N_5O_7S_2$
Exact Mass: 481.17
Molecular Weight: 481.58

b)



c)

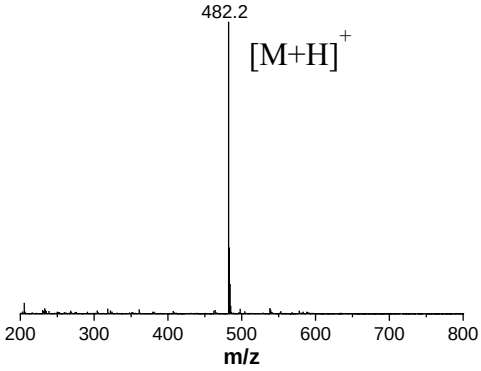
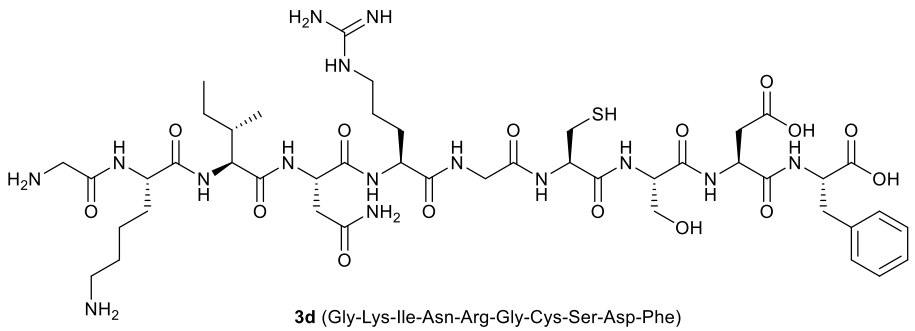


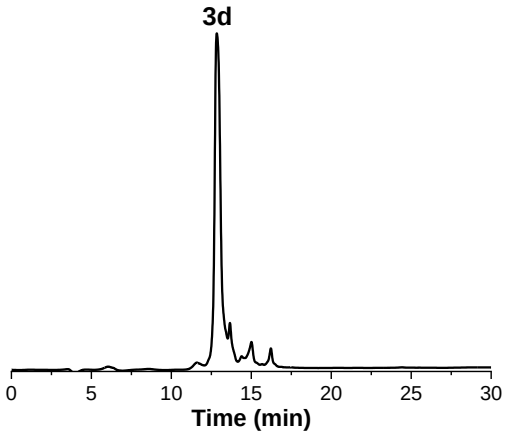
Fig. S5. a) Chemical structure of peptide **3c**; b) RP-HPLC trace of peptide **3c**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{17}H_{31}N_5O_7S_2$: 514.2, $[M+H]^+$. Found: 513.9, $[M+H]^+$. **3c'** is a peptide dimer obtained by forming disulfide bonds between **3c** monomers, which can be reduced to **3c** by adding a small amount of TCEP-HCl.

a)



3d (Gly-Lys-Ile-Asn-Arg-Gly-Cys-Ser-Asp-Phe)
Chemical Formula: $C_{45}H_{73}N_{15}O_{15}S$
Exact Mass: 1095.51
Molecular Weight: 1096.23

b)



c)

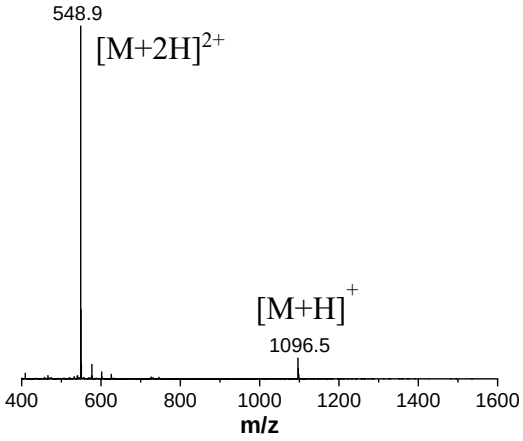
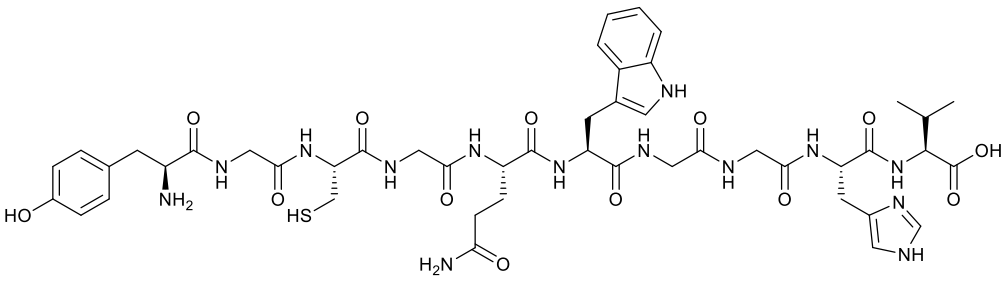


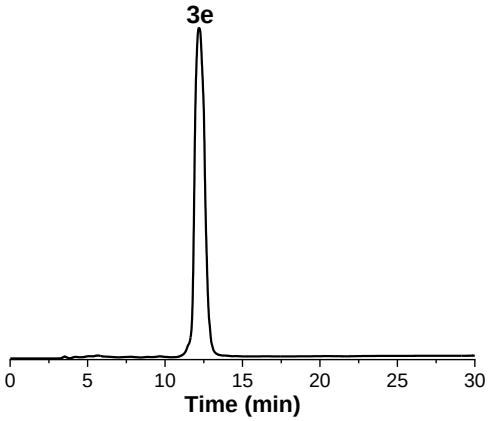
Fig. S6. a) Chemical structure of peptide **3d**; b) RP-HPLC trace of peptide **3d**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{45}H_{73}N_{15}O_{15}S$: 548.8, $[M+2H]^{2+}$; 1596.5, $[M+H]^{+}$. Found: 548.9, $[M+2H]^{2+}$; 1096.5, $[M+H]^{+}$.

a)



3e (Tyr-Gly-Cys-Gly-Gln-Trp-Gly-Gly-His-Val)
Chemical Formula: $C_{47}H_{62}N_{14}O_{13}S$
Exact Mass: 1062.43
Molecular Weight: 1063.16

b)



c)

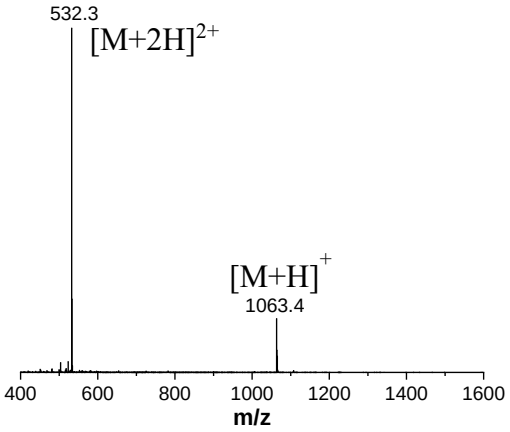
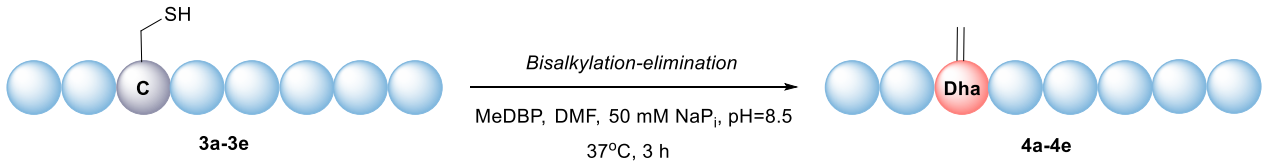


Fig. S7. a) Chemical structure of peptide **3e**; b) RP-HPLC trace of peptide **3e**. HPLC gradient is 15% of solution B in 0-5 min, and 30% to 90% of solution B in 5-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{47}H_{62}N_{14}O_{13}S$: 532.2, $[M+2H]^{2+}$; 1063.4, $[M+H]^{+}$. Found: 532.3, $[M+2H]^{2+}$; 1063.4, $[M+H]^{+}$.

General procedure for synthesis and purification of Dha-containing peptides 4a-4e

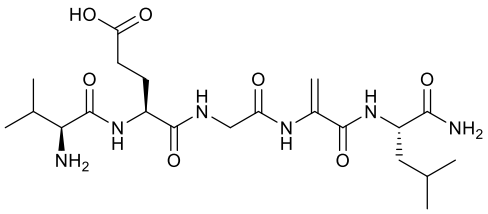
1.0 mg peptide **3** with 0.2 equiv. tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl) was dissolved in 1 mL NaP_i buffer (50 mM, pH 8.5) in a 2 mL Eppendorf tube. Then, 30 equiv. of methyl 2,5-dibromopentanoate (MeDBP) was dissolved in a moderate amount of DMSO until it was completely dissolved. The DMSO solution was added into the NaP_i buffer solution and the mixture was vortexed about 30 s to ensure thorough mixing. The tube was shaken at 37 °C for 3 h in a constant temperature metal bath. Finally, the supernatant was collected after centrifugation of the solution, and the crude peptide was further analyzed by RP-HPLC and ESI-MS. The purified peptide was then lyophilized to obtain the Dha-containing peptide product **4**.



Scheme S4. General procedure for synthesis of Dha-containing peptides **4a-4e**.

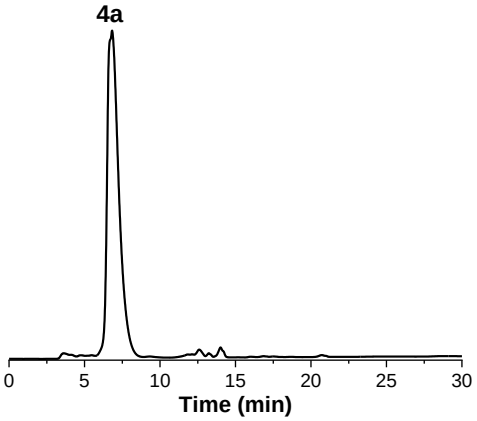
Chemical structures and characterization of Dha-containing peptides 4a-4e

a)



4a (Val-Glu-Gly-Dha-Leu-$CONH_2$)
Chemical Formula: $C_{21}H_{36}N_6O_7$
Exact Mass: 484.26
Molecular Weight: 484.55

b)



c)

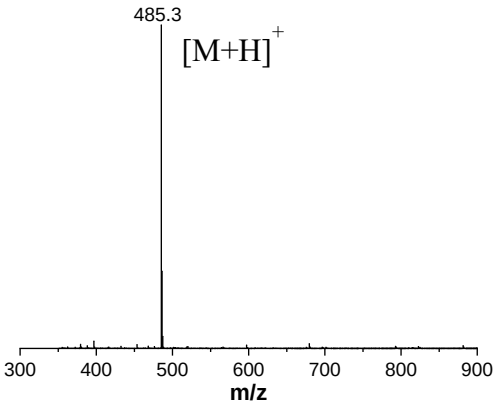
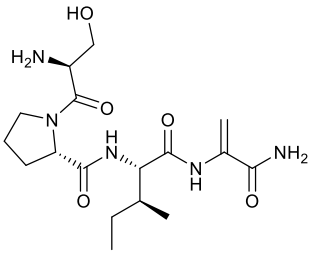


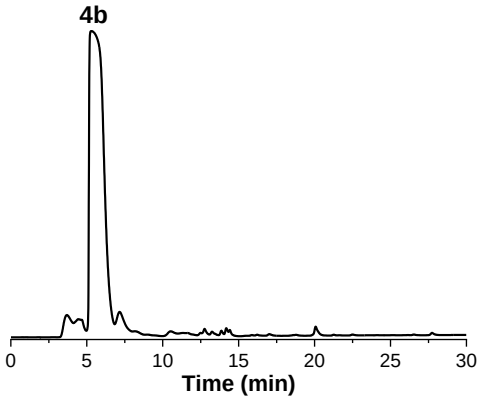
Fig. S8. a) Chemical structure of peptide **4a**; b) RP-HPLC trace of peptide **4a**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{21}H_{36}N_6O_7$: 485.3, $[M+H]^+$. Found: 485.3, $[M+H]^+$.

a)



4b (Ser-Pro-Ile-Dha-$CONH_2$)
Chemical Formula: $C_{17}H_{29}N_5O_5$
Exact Mass: 383.22
Molecular Weight: 383.45

b)



c)

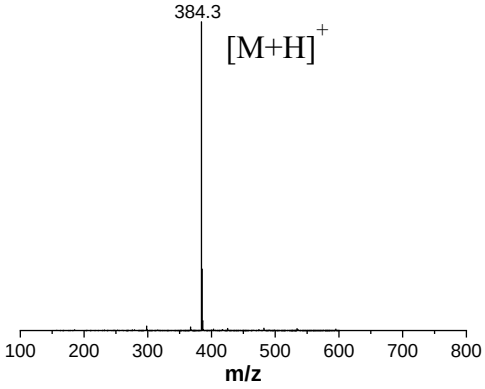
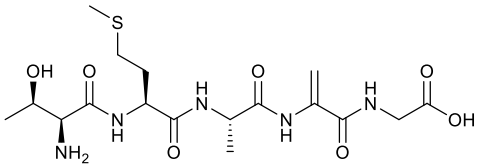


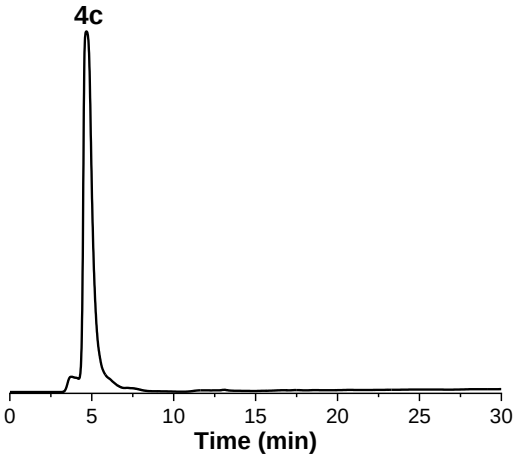
Fig. S9. a) Chemical structure of peptide **4b**; b) RP-HPLC trace of peptide **4b**. HPLC gradient is 5% of solution B in 0-10 min, and 40% to 90% of solution B in 10-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{17}H_{29}N_5O_5$: 384.2, $[M+H]^+$. Found: 384.3, $[M+H]^+$.

a)



4c (Thr-Met-Ala-Dha-Gly)
Chemical Formula: $C_{17}H_{29}N_5O_7S$
Exact Mass: 447.18
Molecular Weight: 447.51

b)



c)

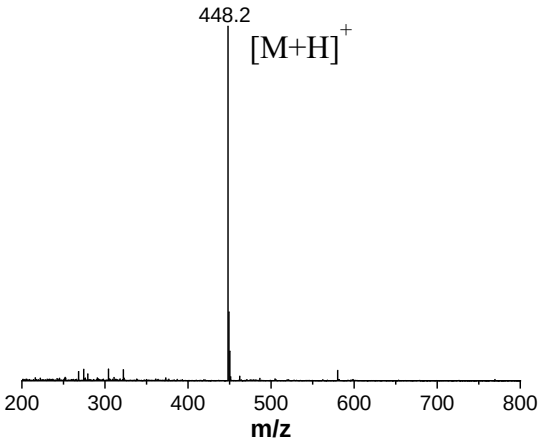
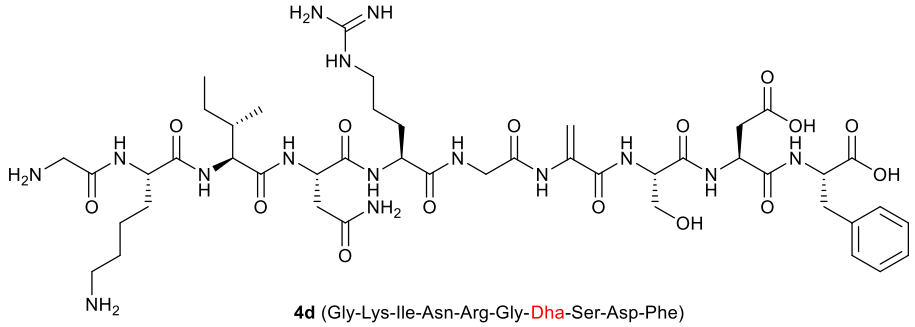


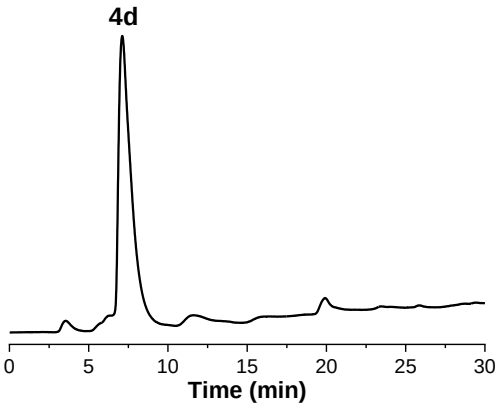
Fig. S10. a) Chemical structure of peptide **4c**; b) RP-HPLC trace of peptide **4c**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{17}H_{29}N_5O_7S$: 448.2, [M+H]. Found: 448.2, $[M+H]^+$.

a)



4d (Gly-Lys-Ile-Asn-Arg-Gly-Dha-Ser-Asp-Phe)
Chemical Formula: $C_{45}H_{71}N_{15}O_{15}$
Exact Mass: 1061.53
Molecular Weight: 1062.15

b)



c)

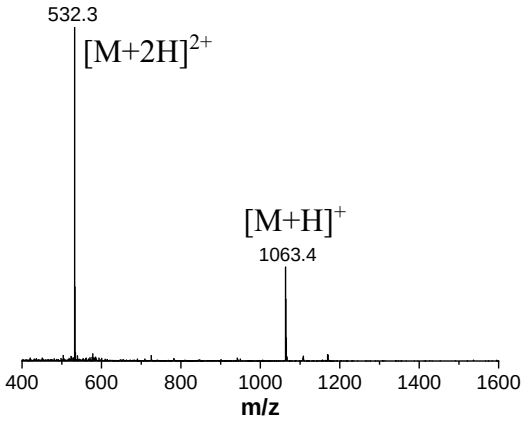
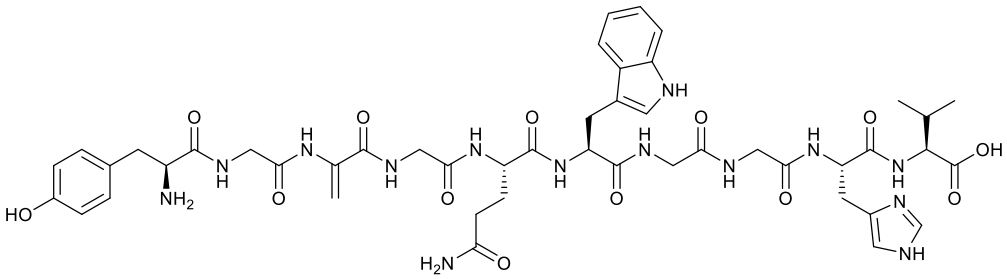


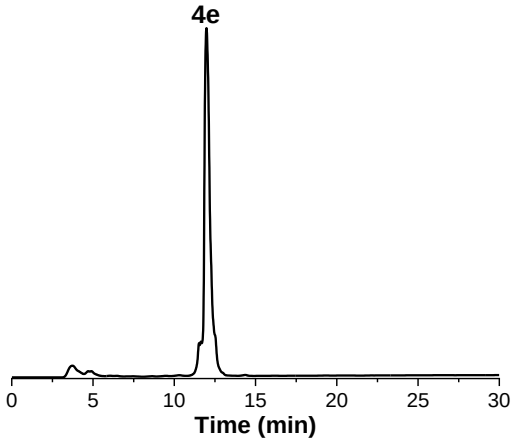
Fig. S11. a) Chemical structure of peptide **4d**; b) RP-HPLC trace of peptide **4d**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{45}H_{71}N_{15}O_{15}$: 531.8, $[M+2H]^{2+}$; 1062.5, $[M+H]^{+}$. Found: 532.3, $[M+2H]^{2+}$; 1063.4, $[M+H]^{+}$.

a)



4e (Tyr-Gly-**Dha**-Gly-Gln-Trp-Gly-Gly-His-Val)
Chemical Formula: $C_{47}H_{60}N_{14}O_{13}$
Exact Mass: 1028.45
Molecular Weight: 1029.08

b)



c)

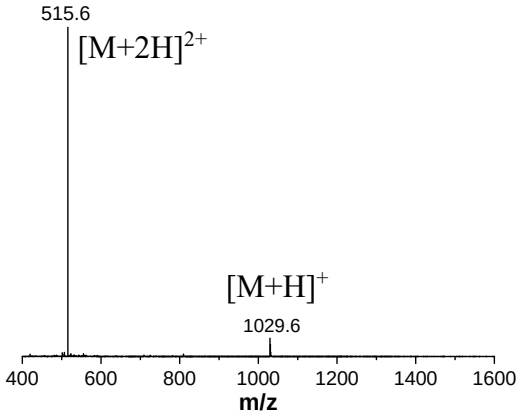
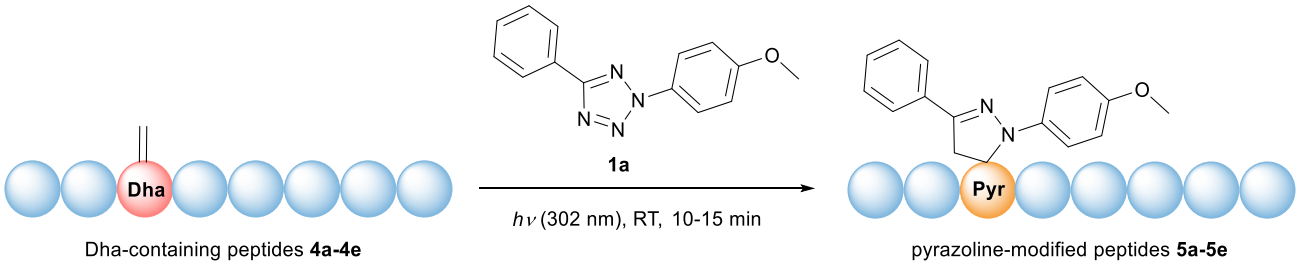


Fig. S12. a) Chemical structure of peptide **4e**; b) RP-HPLC trace of peptide **4e**. HPLC gradient is 15% of solution B in 0-5 min, and 30% to 90% of solution B in 5-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{47}H_{60}N_{14}O_{13}$: 515.8, $[M+2H]^{2+}$; 1029.5, $[M+H]^{+}$. Found: 515.6, $[M+2H]^{2+}$; 1029.6, $[M+H]^{+}$.

General procedure for synthesis of pyrazoline-modified peptides 5a-5e

1 μmol (1.0 equiv.) Dha peptide **4** was fully dissolved in 500 μL H_2O in a quartz glass tube. Then prepare 1 mL of tetrazole **1a** (5 mM in MeCN) solution. 300 μL **1a** solution was added in a quartz glass tube equipped with a magnetic stir bar. The solution was irradiated from one side with a hand-held 302 nm UV lamp for 5 min. Then add 200 μL of the **1a** solution again to derive the final **4** concentration of 1 mM, and the final **1a** concentration of 2.5 mM, respectively. Continue stirring the reaction mixture under UV light for 5-10 minutes. The solution showed a bright green fluorescence. Then the resulting solution was passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and ESI-MS.



Scheme S5. General procedure for synthesis of pyrazoline-modified peptides **5a**-**5e**.

Time-course analysis of photo-initiated 1,3-dipolar cycloaddition on peptide 4c

Prepare solutions of peptide **4c** (4 mM in H_2O) and tetrazole **1a** (5 mM in MeCN), respectively. Next, mix 500 µL of the **4c** solution and 500 µL of the **1a** solution in a quartz glass tube equipped with a magnetic stir bar to obtain a final concentration of 2 mM for **4c** and 2.5 mM for **1a**, respectively. The solution was irradiated from one side with a hand-held 302 nm UV lamp. After stirring for 30 seconds, withdraw 50 µL of the reaction mixture and inject it into the HPLC column. The composition of the reaction mixture was measured at 1, 2, 3, 4, 5, 8, 10, 12, 14, 16, 18, and 20 minutes from the beginning of the reaction using the same method. During this process, the solution concentration and injection volume for each HPLC injection were kept consistent. The relative amounts of peptide substrate **4c** and the pyrazoline-modified product **5c** in the reaction mixtures were monitored by UV absorbance at 215 nm and 254 nm. The integrated areas at 215 nm were used to calculate the conversion rate of the pyrazoline-modified peptide products.

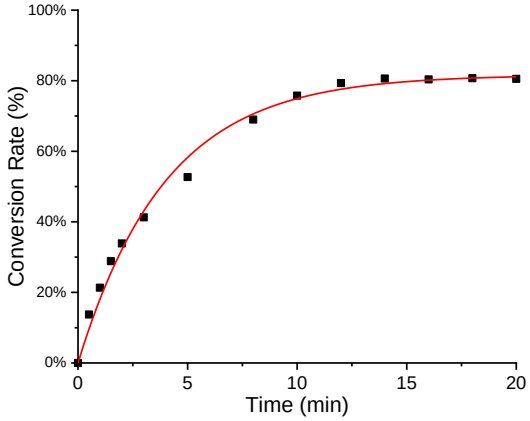
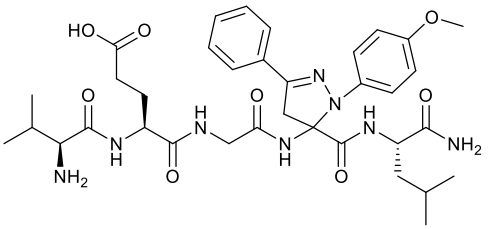


Fig. S13. Time-course study of 1,3-dipolar cycloaddition between tetrazole **1a** and peptide **4c** monitored by RP-HPLC. The reaction approaches completion at around 14 minutes.

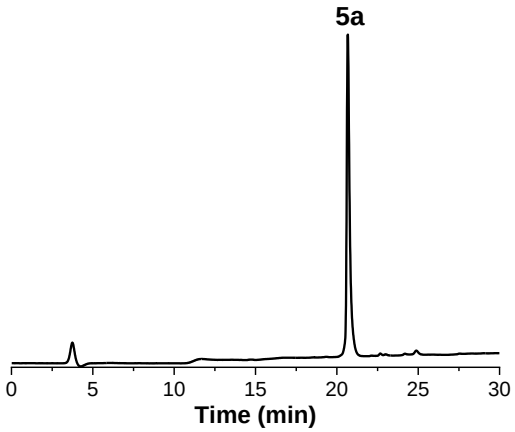
Chemical structures and characterization of pyrazoline-modified peptides 5a-5e

a)



5a
Chemical Formula: $C_{35}H_{48}N_8O_8$
Exact Mass: 708.36
Molecular Weight: 708.82

b)



c)

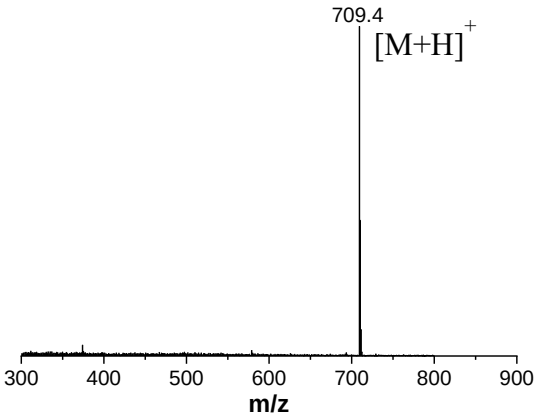
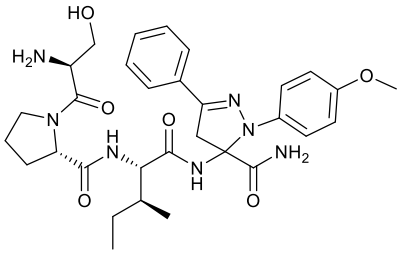


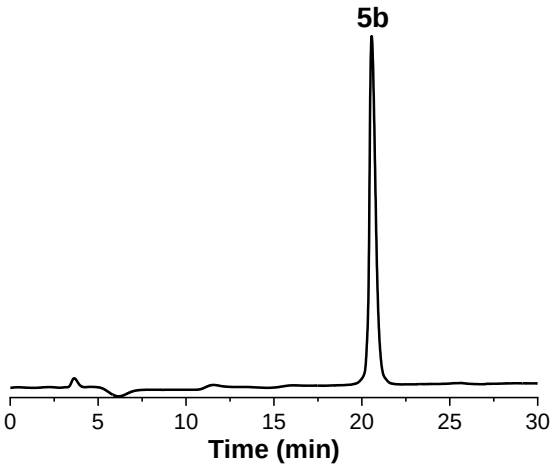
Fig. S14. a) Chemical structure of peptide **5a**; b) Analytic HPLC trace of peptide **5a**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{35}H_{48}N_8O_8$: 709.4, $[M+H]^+$. Found: 709.4, $[M+H]^+$.

a)



5b
Chemical Formula: $C_{31}H_{41}N_7O_6$
Exact Mass: 607.31
Molecular Weight: 607.71

b)



c)

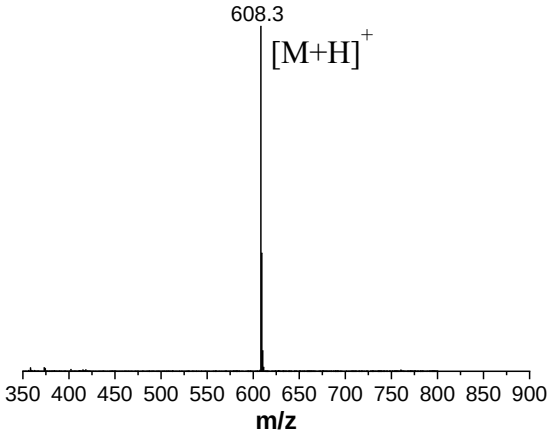
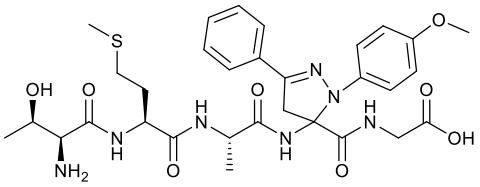


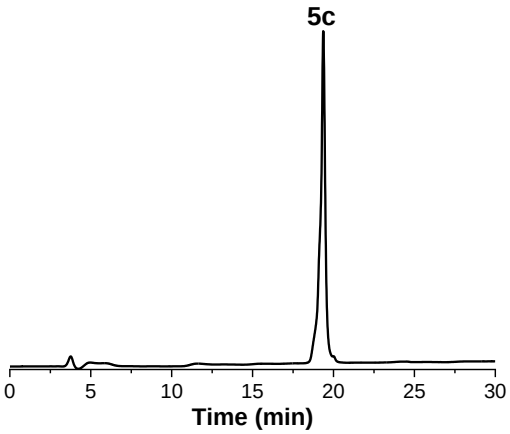
Fig. S15. a) Chemical structure of peptide **5b**; b) Analytic HPLC trace of peptide **5b**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{24}H_{29}N_3O_7S$: 608.3, $[M+H]^+$. Found: 608.3, $[M+H]^+$.

a)



5c
Chemical Formula: $C_{31}H_{41}N_7O_8S$
Exact Mass: 671.27
Molecular Weight: 671.77

b)



c)

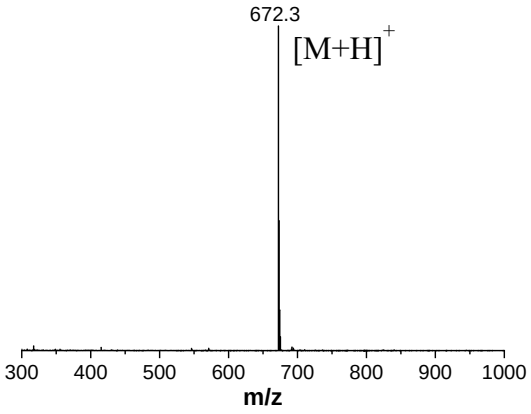
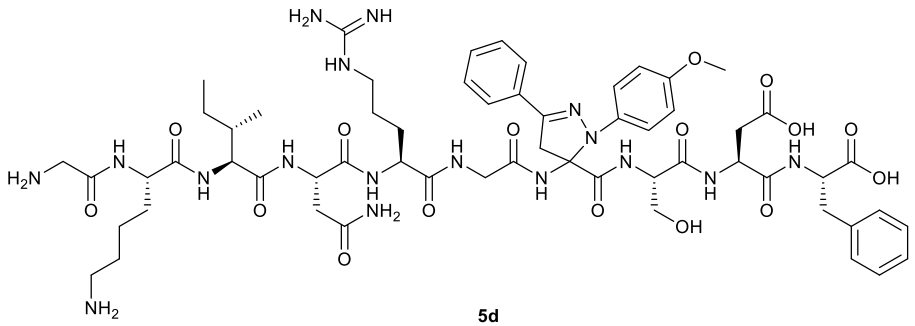


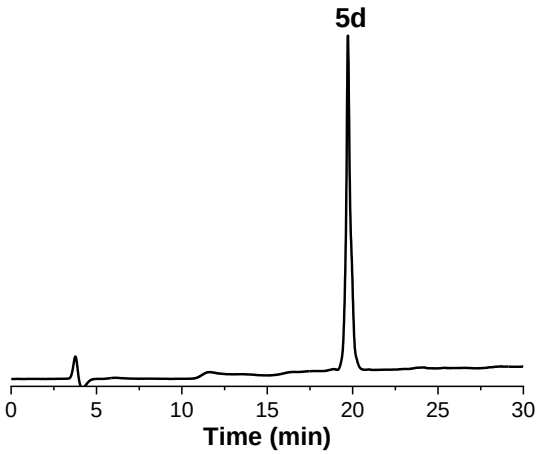
Fig. S16. a) Chemical structure of peptide **5c**; b) Analytic HPLC trace of peptide **5c**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{31}H_{41}N_7O_8S$: 672.3, $[M+H]^+$. Found: 672.3, $[M+H]^+$.

a)



5d
Chemical Formula: $C_{59}H_{83}N_{17}O_{16}$
Exact Mass: 1285.62
Molecular Weight: 1286.42

b)



c)

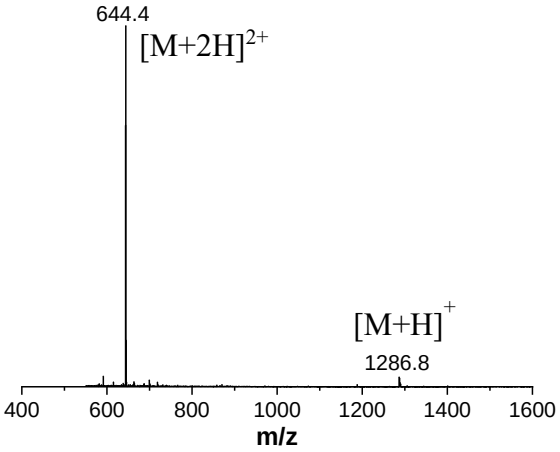
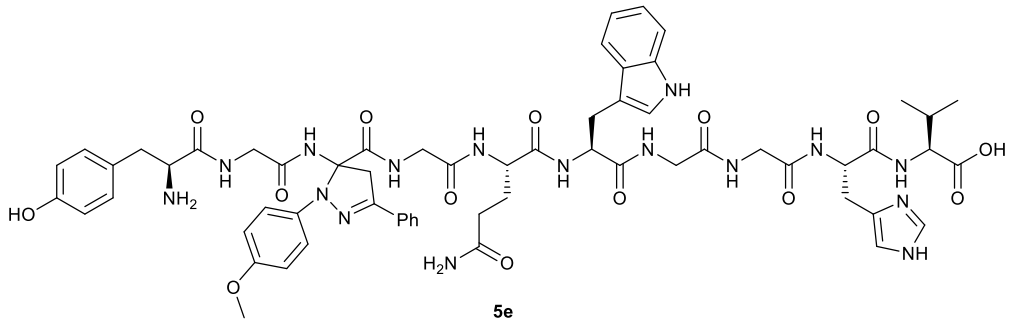


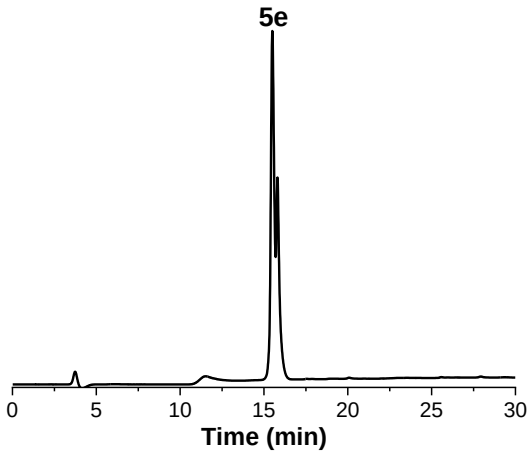
Fig. S17. a) Chemical structure of peptide **5d**; b) RP-HPLC trace of peptide **5d**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{59}H_{83}N_{17}O_{16}$: 643.8, $[M+2H]^{2+}$; 1286.6, $[M+H]^{+}$. Found: 644.4, $[M+2H]^{2+}$; 1286.8, $[M+H]^{+}$.

a)



5e
Chemical Formula: $C_{61}H_{72}N_{16}O_{14}$
Exact Mass: 1252.54
Molecular Weight: 1253.35

b)



c)

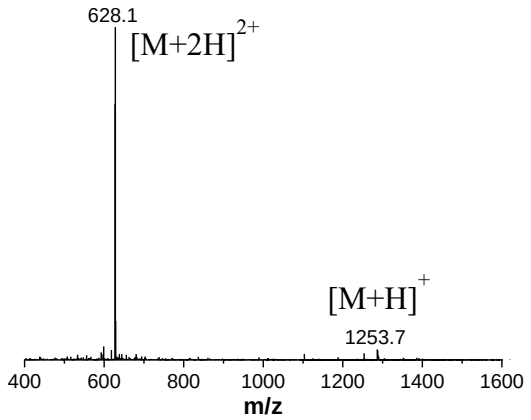
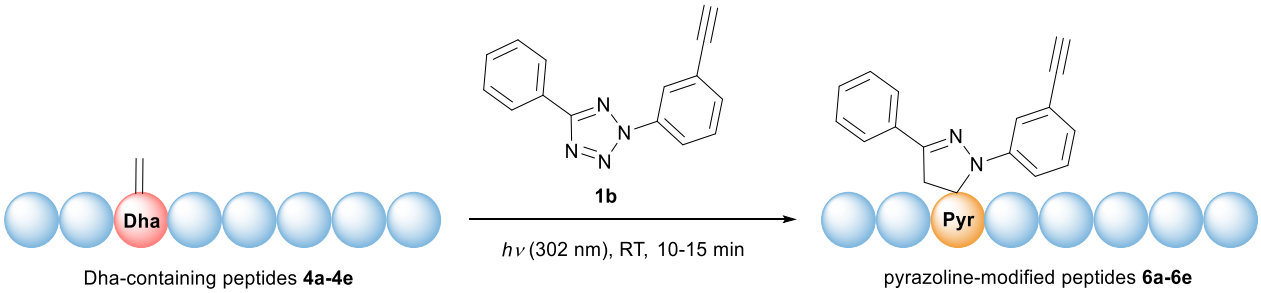


Fig. S18. a) Chemical structure of peptide **5e**; b) RP-HPLC trace of peptide **5e**. HPLC gradient is 15% of solution B in 0-5 min, and 30% to 90% of solution B in 5-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{61}H_{72}N_{16}O_{14}$: 627.3, $[M+2H]^{2+}$; 1253.6, $[M+H]^{+}$. Found: 628.1, $[M+2H]^{2+}$; 1253.7, $[M+H]^{+}$.

General procedure for synthesis of pyrazoline-modified peptides 6a-6e

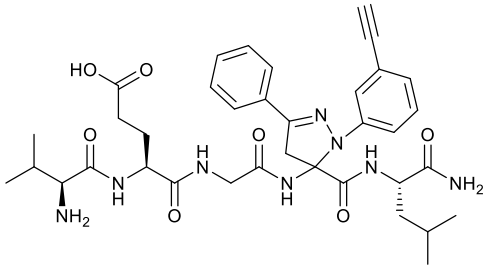
1 μmol (1.0 equiv.) peptide **4** was fully dissolved in 500 μL H_2O in a quartz glass tube. Next, 1 mL of tetrazole **1b** (5 mM in MeCN) solution was prepared. 300 μL **1b** solution was added in the quartz glass tube. The solution was irradiated from one side with a hand-held 302nm UV lamp for 5 min. Then, 200 μL of the **1b** solution was added again to obtain a final concentration of 1 mM for peptide **4** and a final concentration of 2.5 mM for **1b**. Continue stirring the reaction mixture under UV light for 5-10 minutes. The solution showed a bright blue fluorescence. Then the resulting solution was passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and ESI-MS.



Scheme S6. General procedure for synthesis of pyrazoline-modified peptides **6a-6e**.

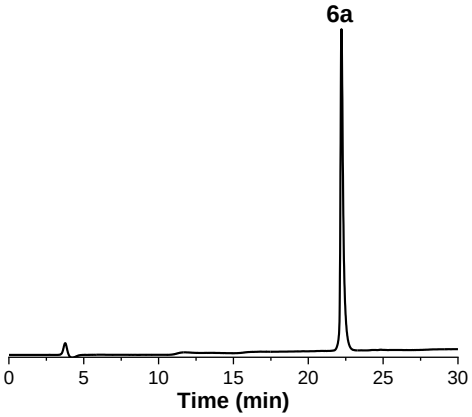
Chemical structures and characterization of pyrazoline-modified peptides 6a-6e

a)



6a
Chemical Formula: $C_{36}H_{46}N_8O_7$
Exact Mass: 702.35
Molecular Weight: 702.81

b)



c)

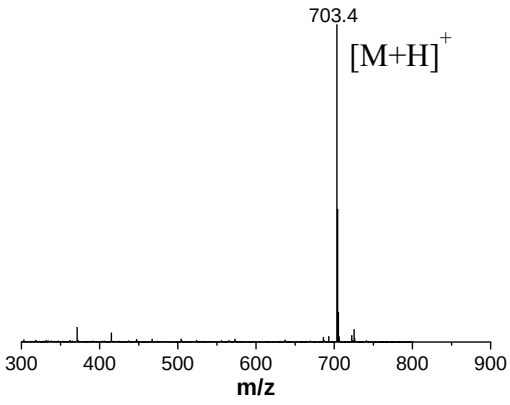
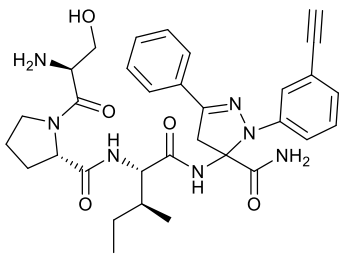


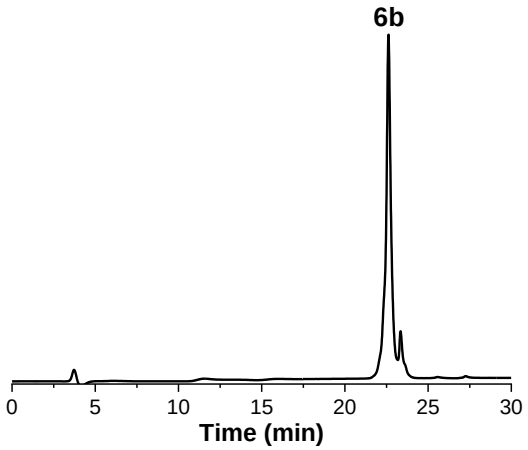
Fig. S19. a) Chemical structure of peptide **6a**; b) Analytic HPLC trace of peptide **6a**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{36}H_{46}N_8O_7$: 703.4, $[M+H]^+$. Found: 703.4, $[M+H]^+$.

a)



6b
Chemical Formula: $C_{32}H_{39}N_7O_5$
Exact Mass: 601.30
Molecular Weight: 601.71

b)



c)

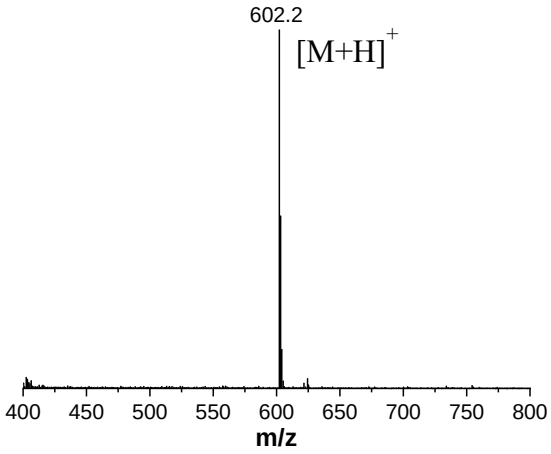
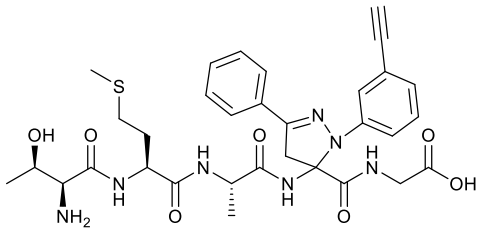


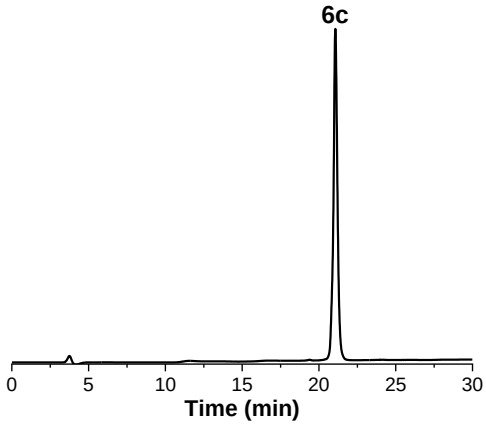
Fig. S20. a) Chemical structure of peptide **6b**; b) Analytic HPLC trace of peptide **6b**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{32}H_{39}N_7O_5$: 602.3, $[M+H]^+$. Found: 602.2, $[M+H]^+$.

a)



6c
Chemical Formula: $C_{32}H_{39}N_7O_7S$
Exact Mass: 665.26
Molecular Weight: 665.77

b)



c)

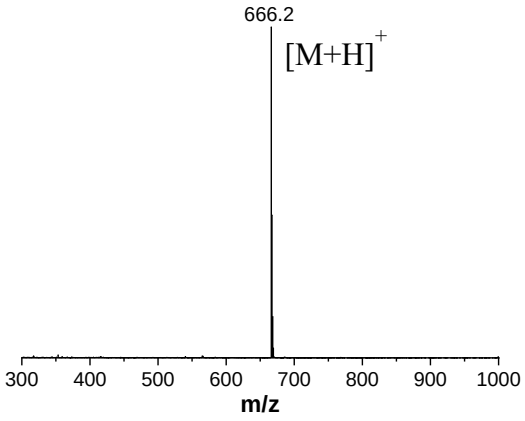
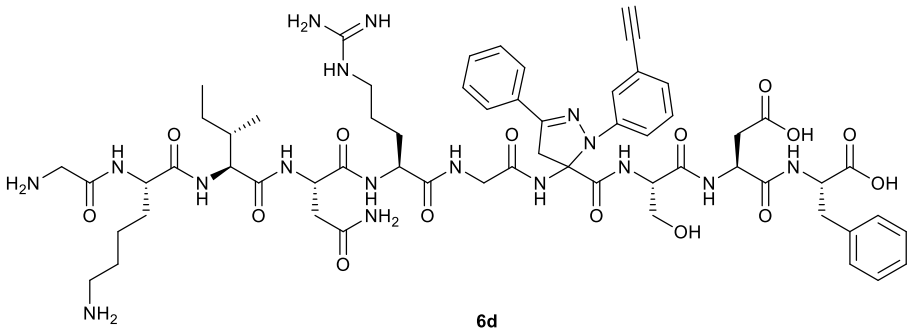


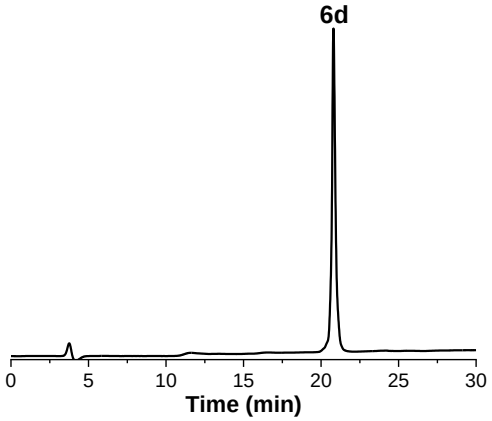
Fig. S21. a) Chemical structure of peptide **6c**; b) Analytic HPLC trace of peptide **6c**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{31}H_{41}N_7O_8S$: 666.3, $[M+H]^+$. Found: 666.2, $[M+H]^+$.

a)



6d
Chemical Formula: $C_{60}H_{81}N_{17}O_{15}$
Exact Mass: 1279.61
Molecular Weight: 1280.41

b)



c)

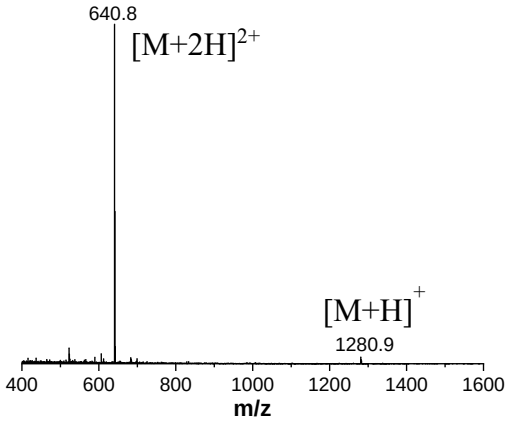
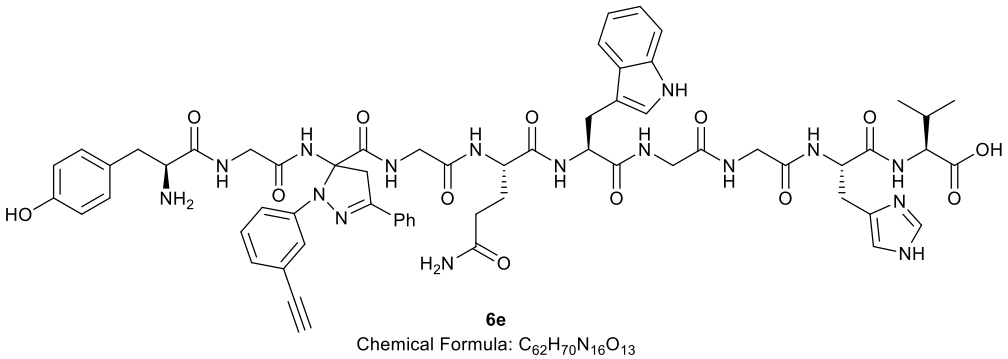


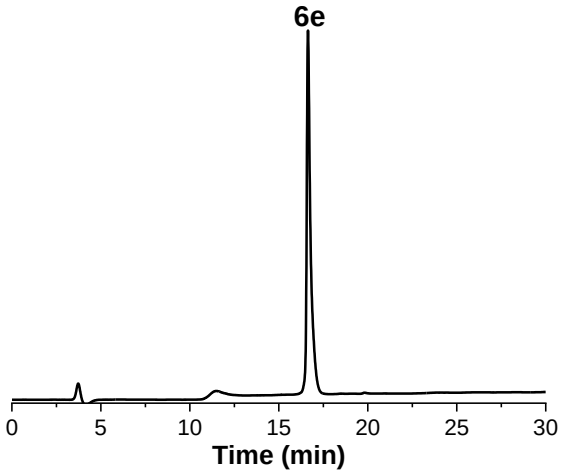
Fig. S22. a) Chemical structure of peptide **6d**; b) RP-HPLC trace of peptide **6d.** HPLC gradient is 15% of solution B in 0-5 min, and 20% to 70% of solution B in 5-30 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{60}H_{81}N_{17}O_{15}$: 640.8, $[M+2H]^{2+}$; 1280.6, $[M+H]^{+}$. Found: 640.8, $[M+2H]^{2+}$; 1280.9, $[M+H]^{+}$.

a)



6e
Chemical Formula: $C_{62}H_{70}N_{16}O_{13}$
Exact Mass: 1246.53
Molecular Weight: 1247.34

b)



c)

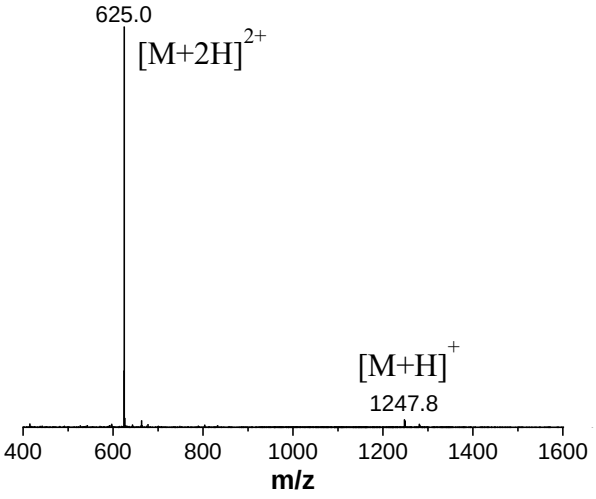
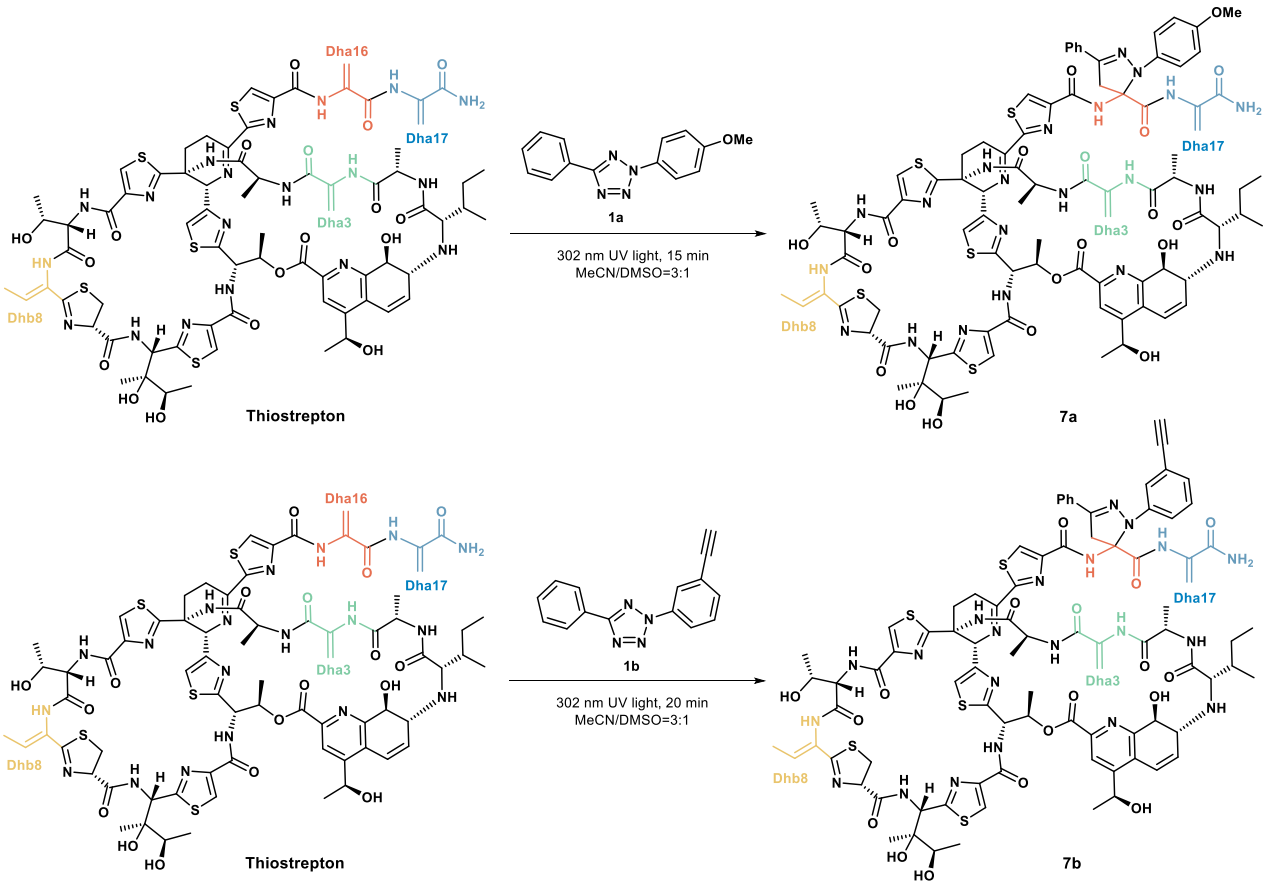


Fig. S23. a) Chemical structure of peptide **6e**; b) RP-HPLC trace of peptide **6e**. HPLC gradient is 15% of solution B in 0-5 min, and 30% to 90% of solution B in 5-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{61}H_{72}N_{16}O_{14}$: 624.3, $[M+2H]^{2+}$; 1247.5, $[M+H]^{+}$. Found: 625.0, $[M+2H]^{2+}$; 1247.8, $[M+H]^{+}$.

Procedure for synthesis of pyrazoline-modified thiostrepton 7a and 7b

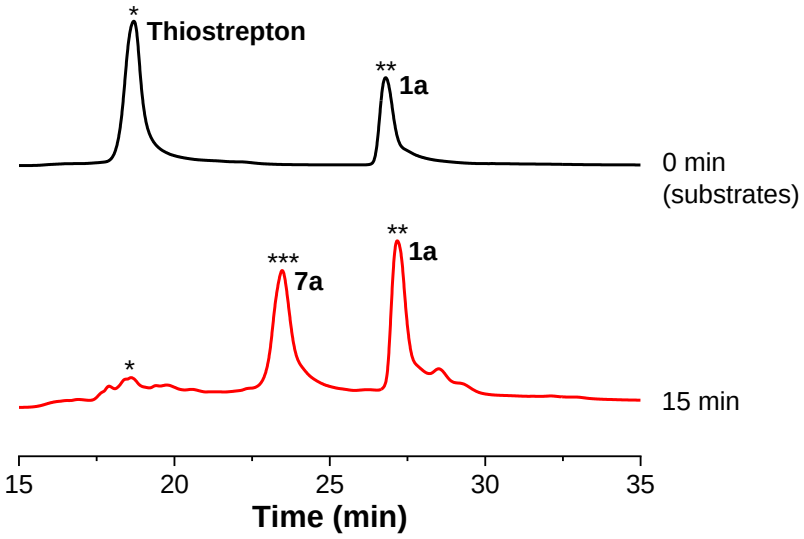
1.66 mg (1 μmol) thiostrepton was fully dissolved in 400 μL of a mixed solvent (MeCN/DMSO 3:1) in a quartz glass tube equipped with a magnetic stir bar. Then prepare 1 mL of tetrazole **1a** (5 mM in MeCN/DMSO 3:1) solution. 300 μL of **1a** solution (1.5 μmol) was added in the quartz glass tube. The solution was stirred and irradiated from one side with a hand-held 302nm UV lamp for 5 min. Then add 300 μL of the **1a** solution again to derive the final thiostrepton concentration of 1 mM, and the final **1a** concentration of 3 mM, respectively. Continue stirring the reaction mixture under UV light for 10 minutes. The solution showed a bright green fluorescence. Then the resulting solution was passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and ESI-MS. 100 μL of the supernatant was diluted with 100 μL H_2O/MeCN 1:1 and analyzed directly by analytical RP-HPLC and ESI-MS. 84.7% conversion of thiostrepton was observed [Fig. S24, a)], as well as the formation of pyrazoline-modified thiostrepton **7a** (m/z = 1889.1, $[M+H]^+$) as the only product. In addition, the isolated yield of **7a** was 73.6%.

Thiostrepton was modified with tetrazole **1b** to obtain **7b**, which was synthesized by the same procedure as **7a**. The conversion rate and isolated yield of **7b** were 83.3% and 64.7%, respectively.



Scheme S7. Procedure for synthesis of pyrazoline-modified thiostrepton **7a** and **7b**.

a)



b)

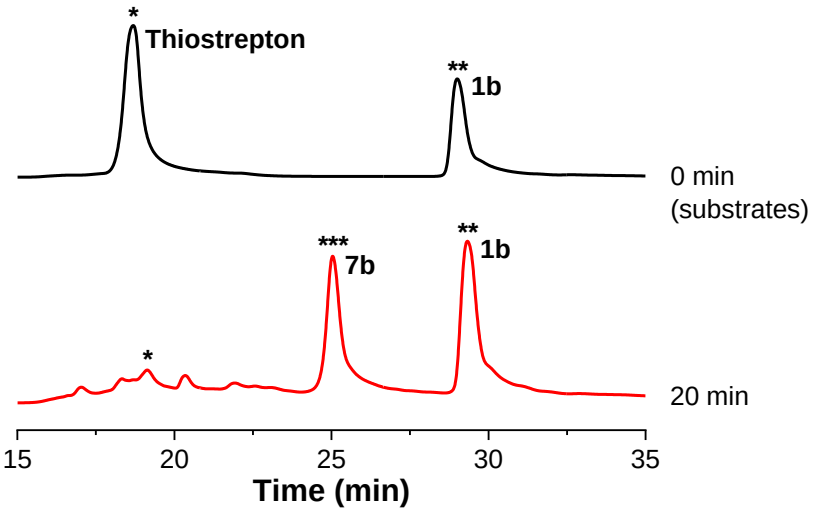
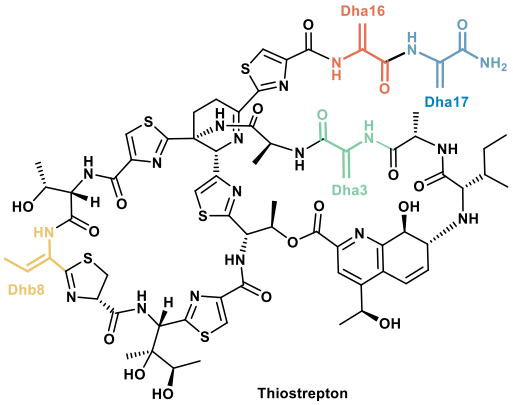


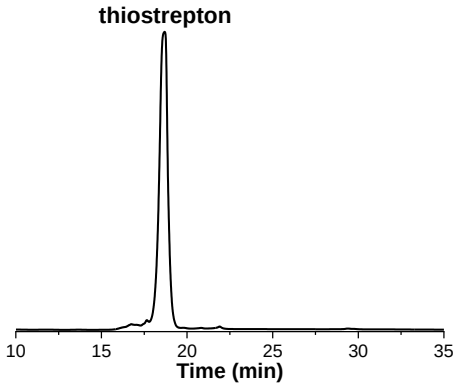
Fig. S24. a) HPLC analysis of the reaction mixture of thiostrepton and tetrazole **1a** at 0 min (top) and 15 min (bottom). b) HPLC analysis of the reaction mixture of thiostrepton and tetrazole **1b** at 0 min (top) and 20 min (bottom).

Chemical structures and characterization of thiostrepton and pyrazoline-modified products 7a and 7b

a)



b)



c)

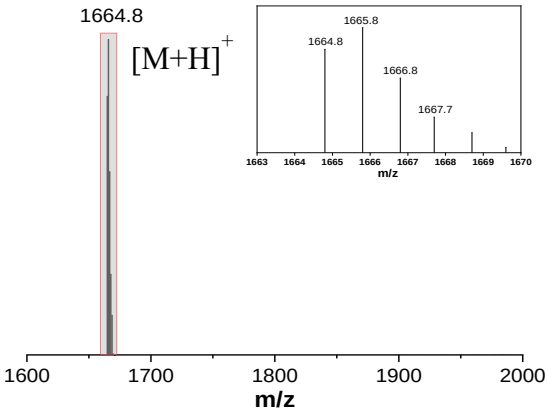


Fig. S25. a) Chemical structure of **thiostrepton**; b) Analytic HPLC trace of **thiostrepton**. HPLC gradient is 30% of solution B in 0-10 min, and 50% to 100% of solution B in 10-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{72}H_{85}N_{19}O_{18}S_5$: 1664.5, $[M+H]^+$. Found:1664.8, $[M+H]^+$.

NMR analysis of thiostrepton: 20 mg purified thiostrepton was dissolved in 600 µL $CDCl_3$/MeOD 3:1. And ^{1}H NMR, ^{1}H-^{1}H COSY and ^{1}H-^{13}C HMBC spectra were recorded. The data of thiostrepton substrate was used to assign the characteristic methylene signals of the different dehydroamino acids.[3-5]

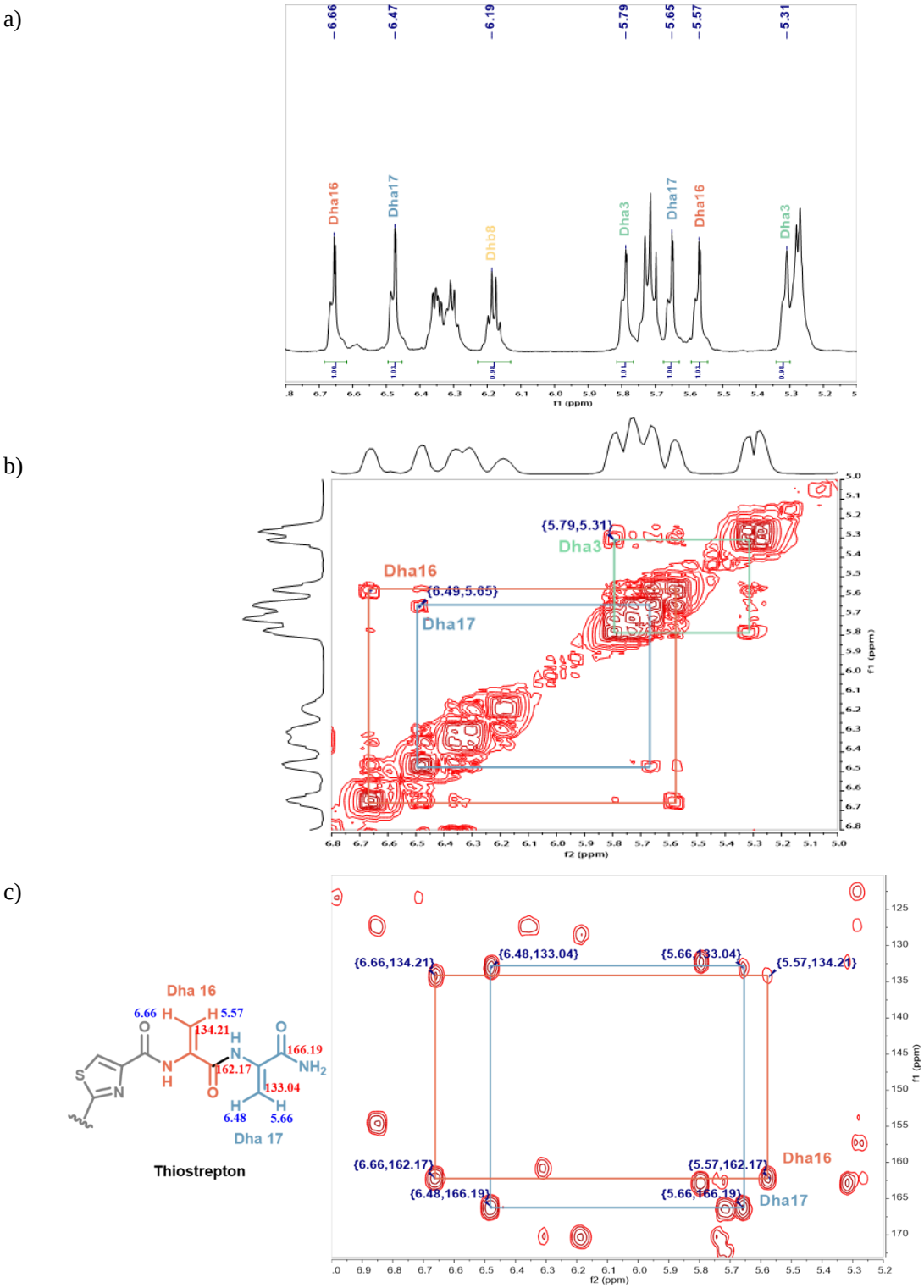
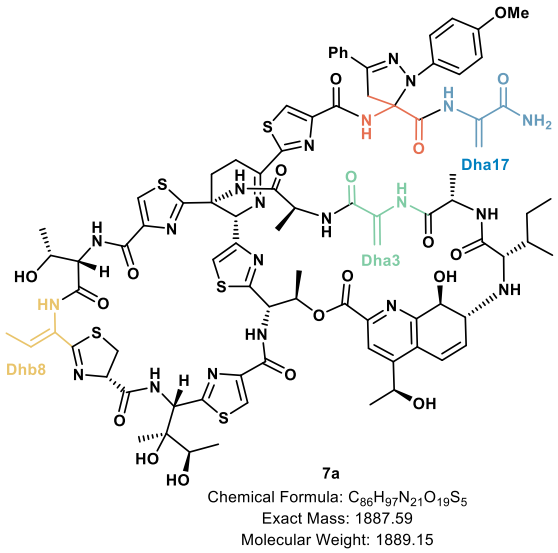
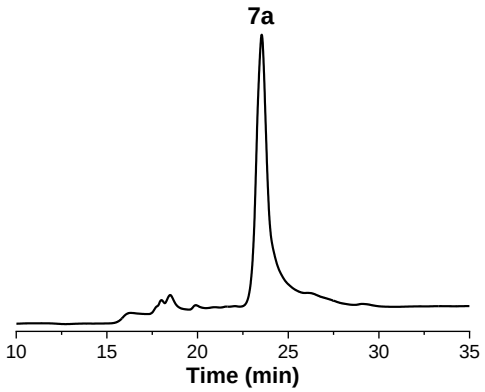


Fig. S26. NMR analysis of **thiostrepton**. a) ^{1}H NMR, b) ^{1}H-^{1}H COSY and c) ^{1}H-^{13}C HMBC spectra of **thiostrepton**.

a)



b)



c)

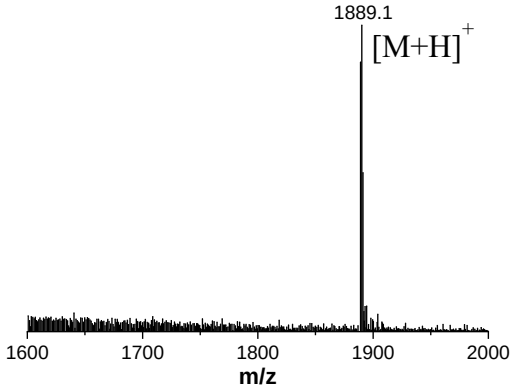


Fig. S27. a) Chemical structure of pyrazoline-modified product **7a**; b) Analytic HPLC trace of **7a**. HPLC gradient is 30% of solution B in 0-10 min, and 50% to 100% of solution B in 10-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{86}H_{97}N_{21}O_{19}S_5$: 1888.6, $[M+H]^+$. Found: 1889.1, $[M+H]^+$.

NMR analysis of 7a: 20 mg purified **7a** was dissolved in 600 µL $CDCl_3$/MeOD 3:1. ^1H NMR, ^1H-^1H COSY and ^1H-^{13}C HMBC spectra were recorded.

a)

b)

c)

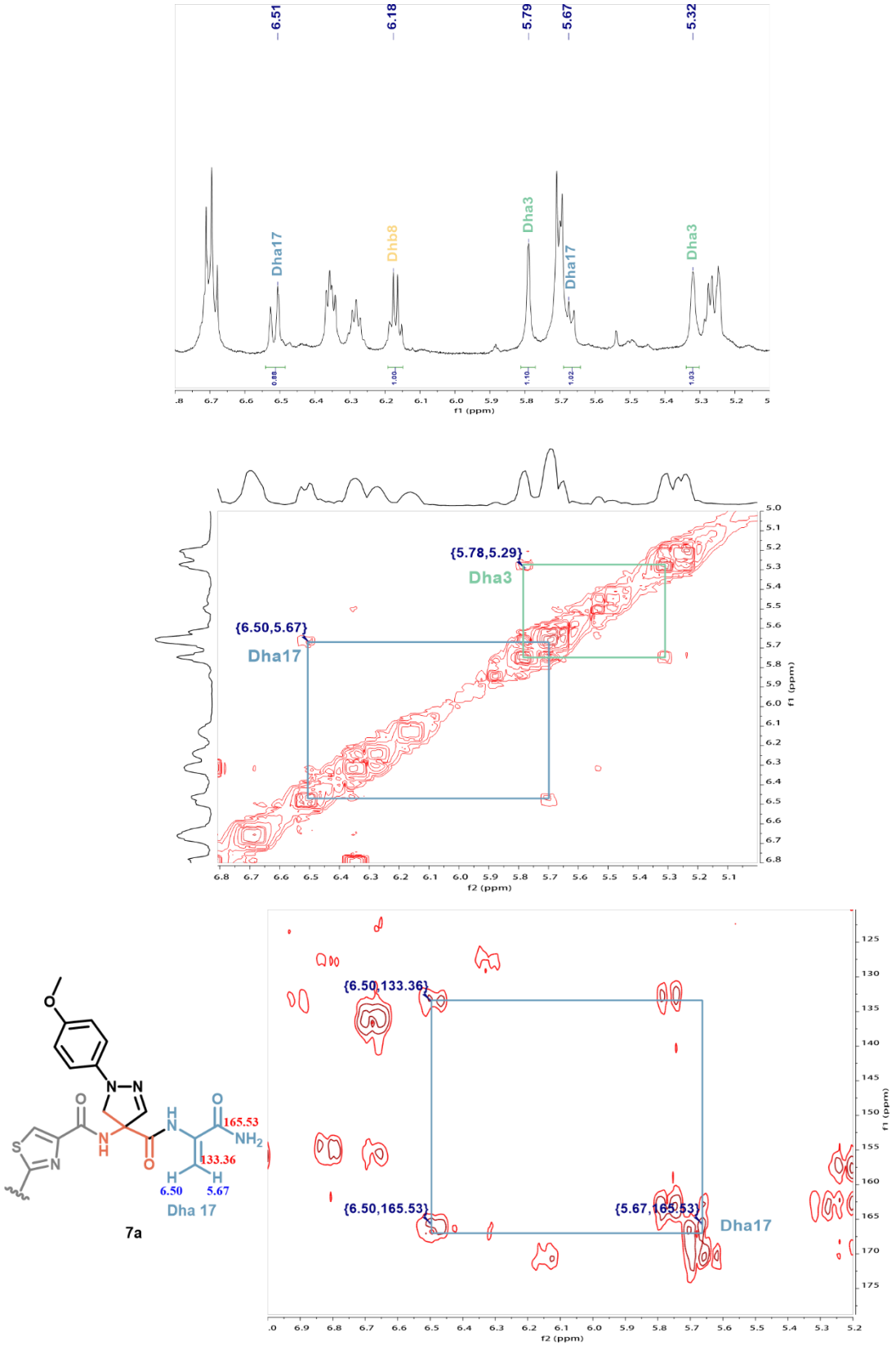
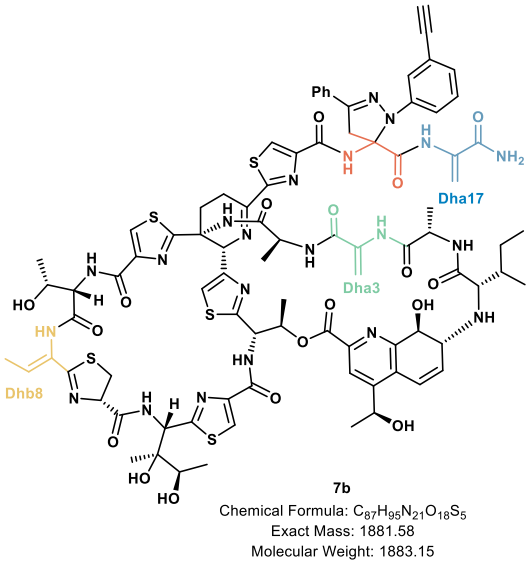


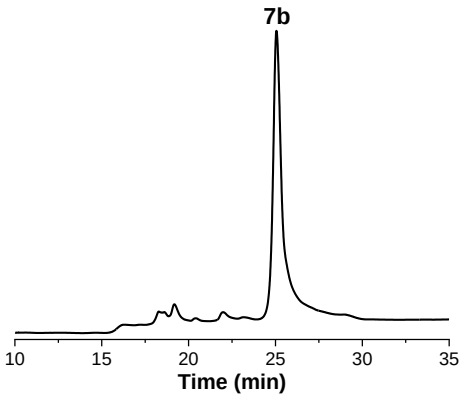
Fig. S28. NMR analysis of **7a**. a) ^1H NMR, b) ^1H-^1H COSY and c) ^1H-^{13}C HMBC spectra of **7a**.

NMR analysis of 7b: The sample **7b** was prepared using the same method as that for thiostrepton and **7a**. ^{1}H NMR, ^{1}H-^{1}H COSY, and ^{1}H-^{13}C HMBC spectra were recorded (see Scheme 4D-4F).

a)



b)



c)

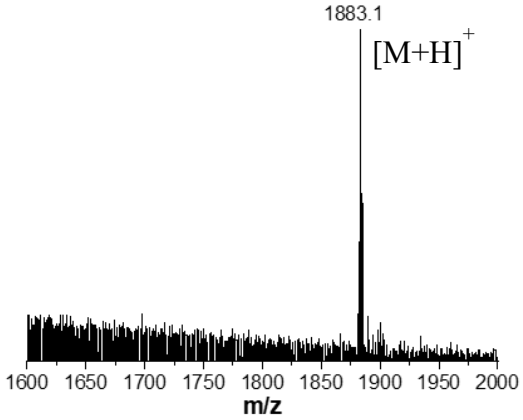
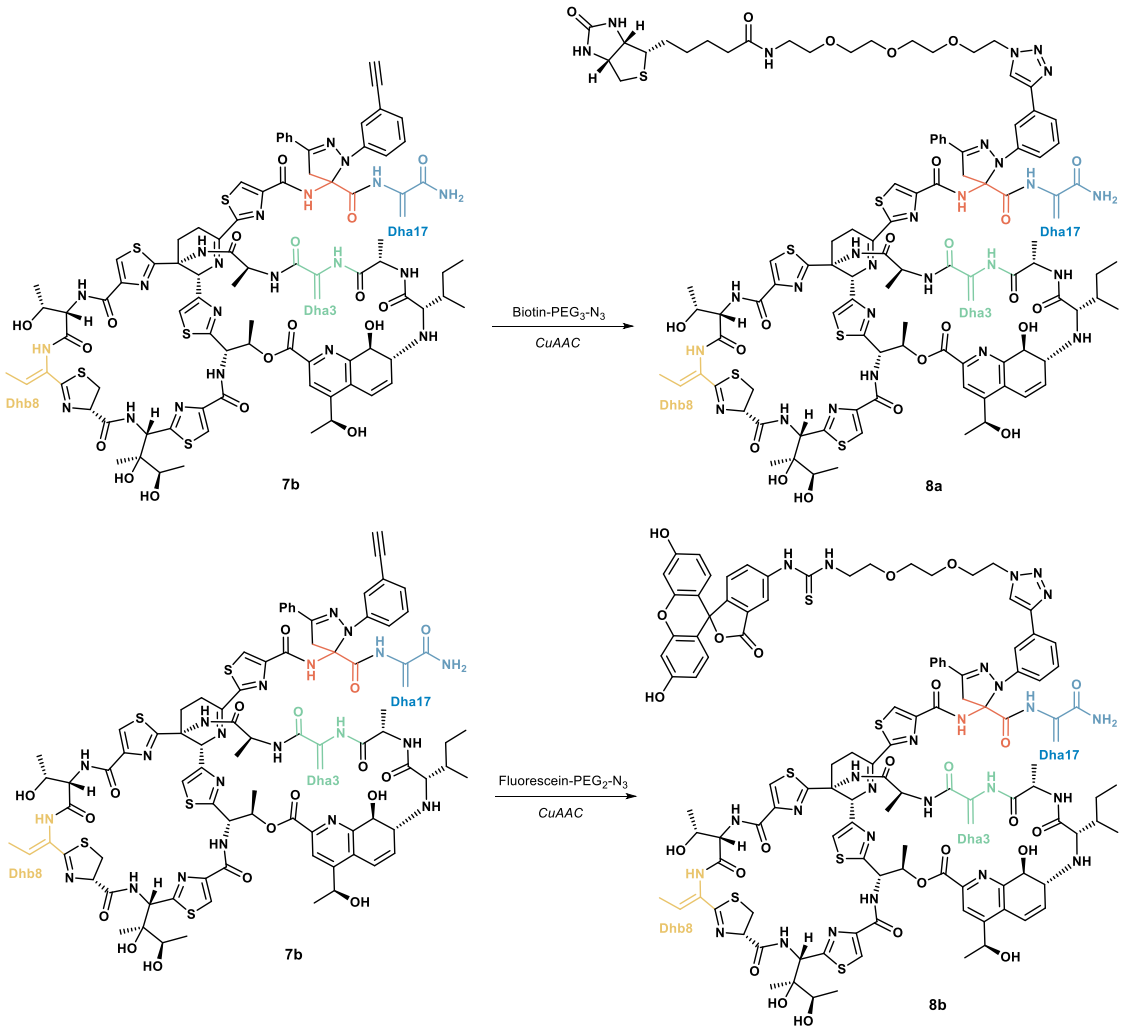


Fig. S29. a) Chemical structure of pyrazoline-modified thiostrepton **7b**; b) Analytic HPLC trace of **7b**. HPLC gradient is 30% of solution B in 0-10 min, and 50% to 100% of solution B in 10-35 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{87}H_{95}N_{21}O_{18}S_5$: 1882.6, $[M+H]^+$. Found: 1883.1, $[M+H]^+$.

General procedure for multi-functionalization of thiostrepton via Click reaction

7b (1.88 mg, 1 μmol), $CuSO_4 \cdot 5H_2O$ (0.15 mg, 0.6 μmol) and sodium ascorbate (0.396 mg, 2 μmol) were fully dissolved by 400 μL of a mixed solvent (MeOH/H_2O=3/1) in a 2 ml Eppendorf tube. Then, biotin-PEG_3-azide (0.45 mg, 1 μmol) was added to the solution. The tube was shaken at 37 °C for 12 hours in a constant temperature metal bath. After reaction, the solution was passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and ESI-MS. After HPLC purification and lyophilization, 1.42 mg of product **8a** was obtained with 61.4% yield.

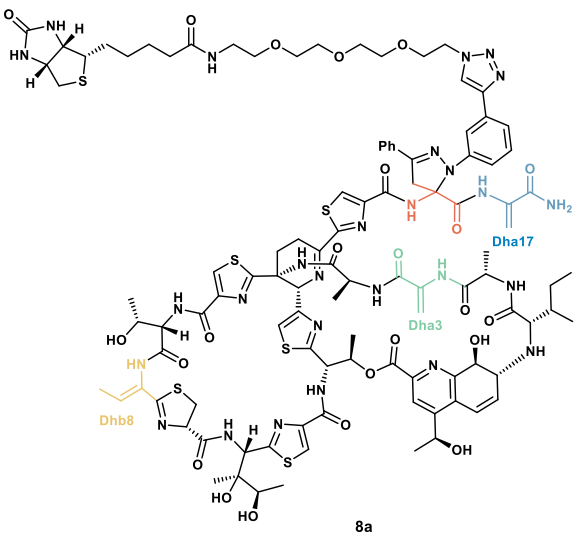
7b (1.88 mg, 1 μmol), $CuSO_4 \cdot 5H_2O$ (0.15 mg, 0.6 μmol) and sodium ascorbate (0.396 mg, 2 μmol) were fully dissolved by 400 μL mixed solvent (MeOH/H_2O=3/1) in a 2 ml Eppendorf tube. Then, fluorescein-PEG_2-azide (0.56 mg, 1 μmol) was added to the solution. The tube was shaken at 37 °C for 12 hours in a constant temperature metal bath. After reaction, the solution was passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and ESI-MS. After HPLC purification and lyophilization, 1.29 mg of product **8b** was obtained with 52.7% yield.



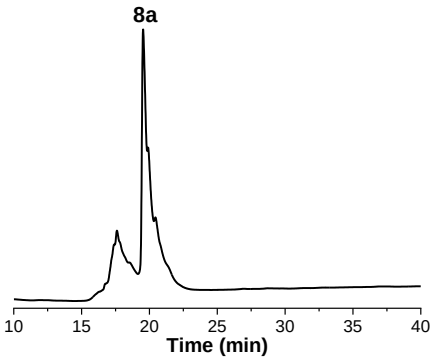
Scheme S8. General procedure for synthesis of thiostrepton derivatives **8a** and **8b**.

Chemical structures and characterization of thiostrepton derivatives 8a and 8b

a)



b)



c)

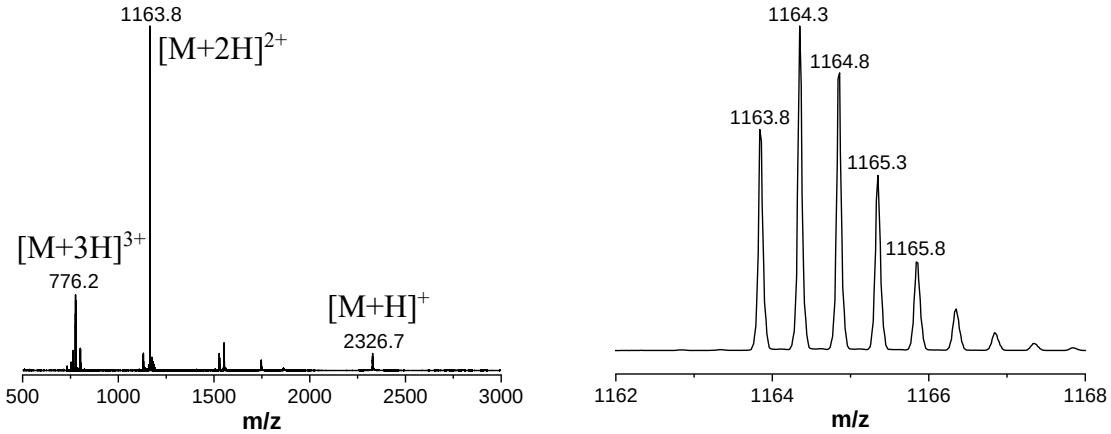
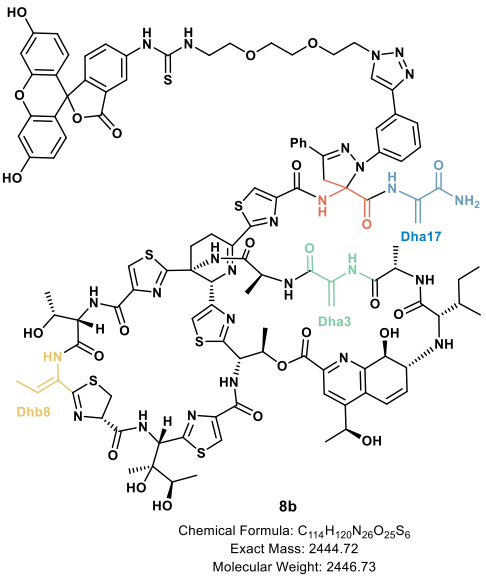
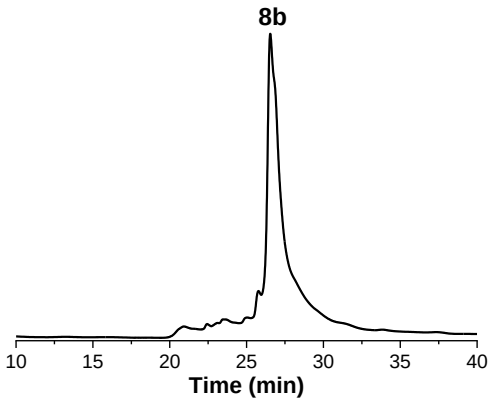


Fig. S30. a) Chemical structure of thiostrepton derivative **8a**; b) Analytic HPLC trace of **8a**. HPLC gradient is 30% of solution B in 0-10 min, 50% to 100% of solution B in 10-35 min, and 100% of solution B in 35-40 min on the YMC C18 column (λ=215 nm). c) Q-TOF MS (ESI): m/z calculated for $C_{105}H_{127}N_{27}O_{23}S_6$: 776.3, $[M+3H]^{3+}$; 1163.9, $[M+2H]^{2+}$; 2326.8, $[M+H]^{+}$. Found: 776.2, $[M+3H]^{3+}$; 1163.8, $[M+2H]^{2+}$; 2326.7, $[M+H]^{+}$.

a)



b)



c)

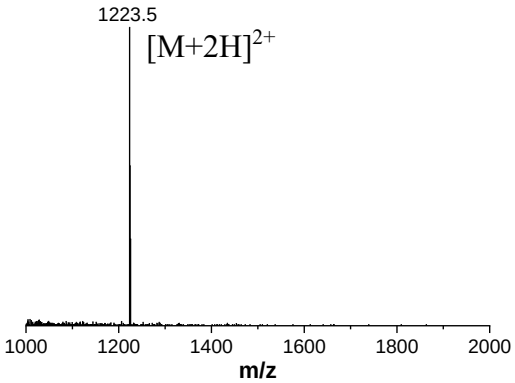


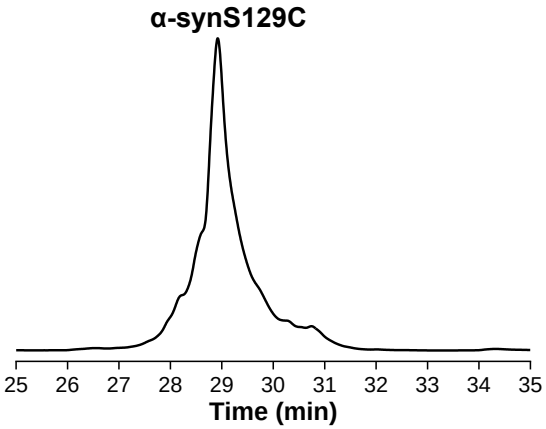
Fig. S31. a) Chemical structure of thiostrepton derivative **8b**; b) Analytic HPLC trace of **8b**. HPLC gradient is 30% of solution B in 0-10 min, 50% to 100% of solution B in 10-35 min, and 100% of solution B in 35-40 min on the YMC C18 column (λ=215 nm). c) ESI-MS: m/z calculated for $C_{114}H_{120}N_{26}O_{25}S_6$: 1223.4, $[M+2H]^{2+}$. Found: 1223.5, $[M+2H]^{2+}$.

Synthesis and characterization of proteins

Expression and purification of recombinant α-synS129C

Recombinant α-SynS129C was expressed and extracted according to existing literature.[6,7] LB medium with 100 μg/mL ampicillin at 37 ℃ was used for all cultures. The overnight culture of transformed BL21(DE3) was induced with 100× of 100 μM IPTG. The supernatant from the osmotic shock treatment containing α-SynS129C was firstly treated with 1 equiv. TCEP-HCl to prevent disulfide bond formation, and then the pH was adjusted to 9.0. Further purification was carried out with preparative HPLC using a Proteonavi column with a linear gradient of 30-70% mobile phase B over 30 min at a flow rate of 4 mL/min. The purified α-SynS129C was then freeze-dried and stored at −80 °C until use. The purity of α-SynS129C was confirmed by analytical HPLC and ESI-MS.

a)



b)

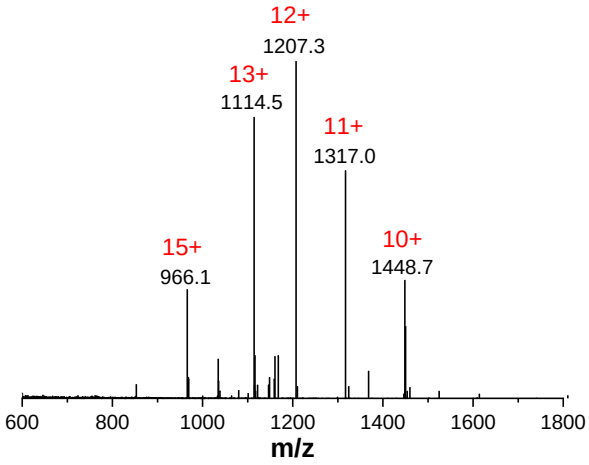
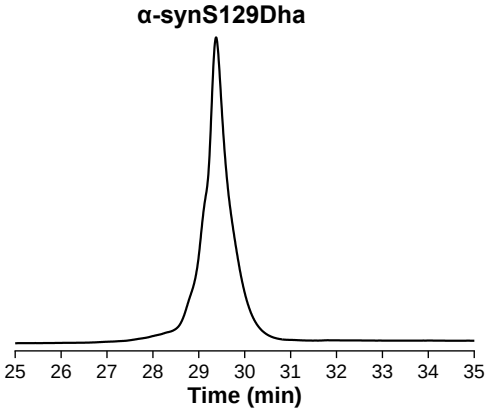


Fig. S32. a) Analytic HPLC trace of **α-synS129C**. HPLC gradient is 10% of solution B in 0-5 min, and 15% to 85% of solution B in 5-40 min on the analytic C4 column (λ=215 nm). b) ESI-MS: Calculated: 14476, $[M+H]^+$. Found: 14476, $[M+H]^+$.

Synthesis and characterization of α-synS129Dha

α-SynS129Dha was synthesized via bisalkylation-elimination from α-SynS129C. 1.0 mg (0.069 μmol) of α-SynS129C and 0.2 equiv. TCEP-HCl were dissolved into 1.0 mL NaPi buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 500 equiv. (5.4 μL, 34.5 μmol) methyl 2,5-dibromopentanoate (MeDBP) was dissolved in 330 μL DMSO. The DMSO solution was added into the NaP_i buffer solution and the mixture was vortexed for 30 s to ensure thorough mixing. The tube was shaken at 37 °C for 5 h in a constant temperature metal bath. After purifying the crude protein with RP-HPLC, the solution was collected and lyophilized to obtain α-synS129Dha. The product was characterized by analytic HPLC and Q-TOF MS.

a)



b)

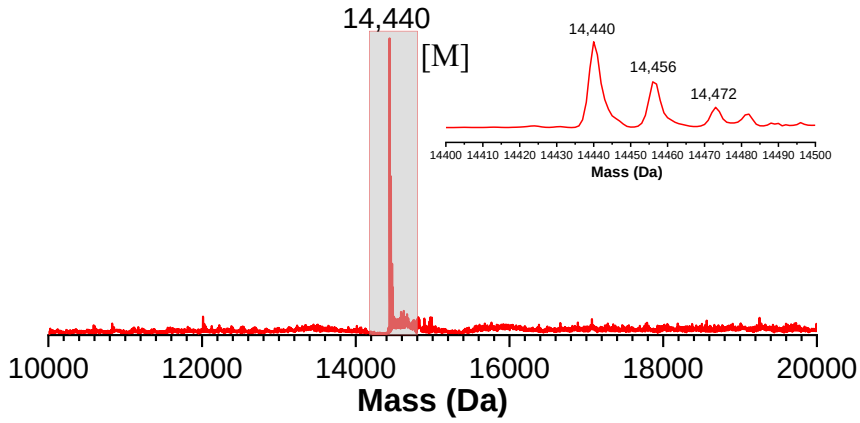


Fig. S33. a) Analytic HPLC trace of **α-SynS129Dha**. HPLC gradient is 10% of solution B in 0-5 min, and 15% to 85% of solution B in 5-40 min on the analytic C4 column (λ=215 nm). b) Deconvoluted mass spectrum of **α-SynS129Dha**. Q-TOF MS: Calculated: 14441, [M]. Found: 14440, [M].

General procedure for synthesis and characterization of pyrazoline-modified proteins 9a-9d

0.5 mg (34.6 µmol) of the protein was dissolved in 200 µL of H_2O. 30 equiv. of **1a** was added into 800 µL of H_2O in an Eppendorf tube. The solution of **1a** was sonicated for 10 min to ensure dissolution. The **1a** solution is then mixed with the protein solution in a quartz glass tube equipped with a magnetic stir bar. The reaction mixture was stirred and irradiated from one side with a hand-held 302-nm UV lamp for 15 minutes.The resulting solution was then passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and Q-TOF MS.

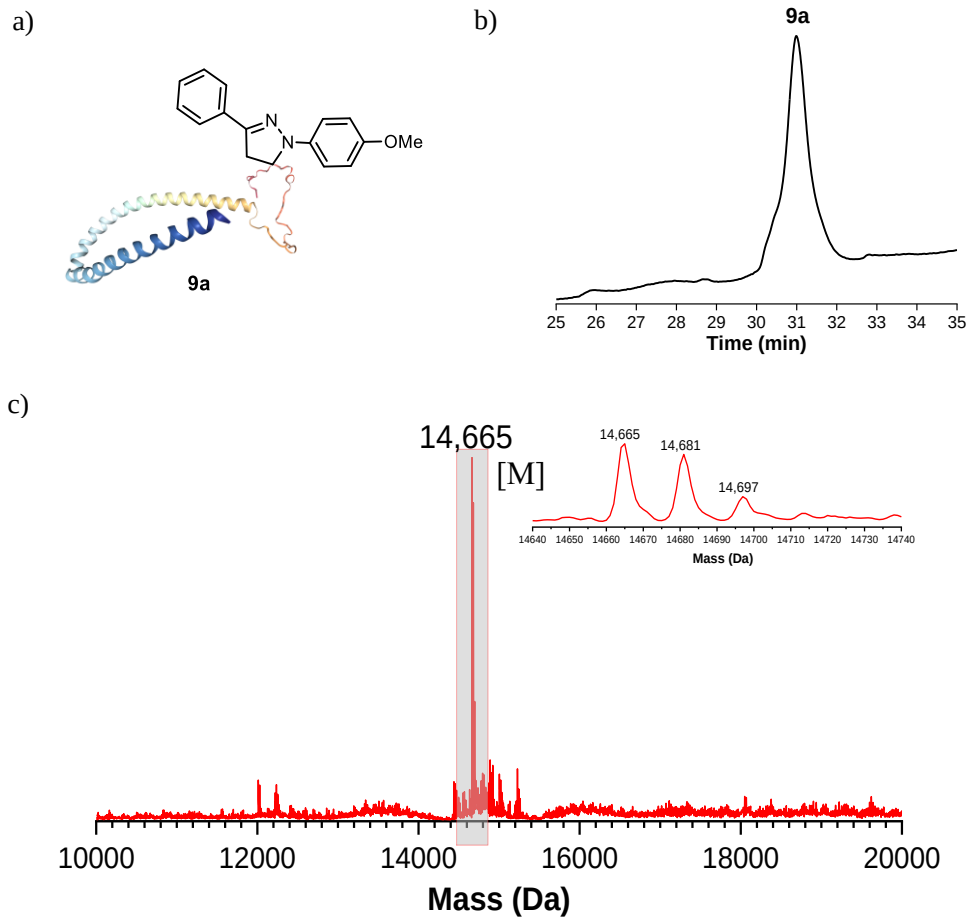


Fig. S34. a) Chemical structure of **9a**. b) Analytic HPLC trace of **9a**. HPLC gradient is 10% of solution B in 0-5 min, and 15% to 85% of solution B in 5-40 min on the analytic C4 column (λ=215 nm). c) Deconvoluted mass spectrum of **9a**. Q-TOF MS: Calculated: 14665, [M]. Found: 14665, [M].

The same protocol was applied for the synthesis of **9b**. 0.5 mg (0.0346 μmol) α-SynS129Dha and 40 equiv. of tetrazole **1b** were dissolved in 1 mL of a mixed solvent (H_2O:MeCN 4:1) in a quartz glass tube equipped with a magnetic stir bar. The reaction mixture was stirred and irradiated from one side with a hand-held 302-nm UV lamp for 20 minutes. The resulting solution was then passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and Q-TOF MS.

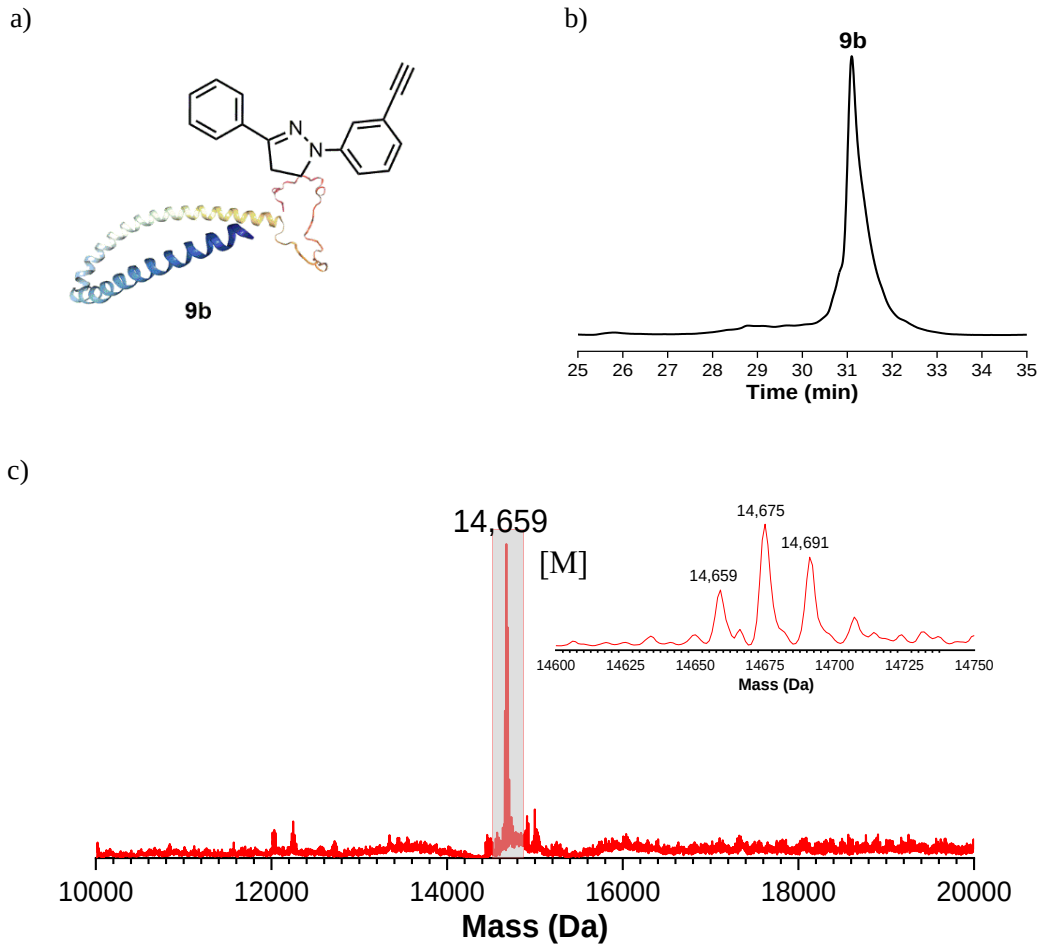


Fig. S35. a) Chemical structure of **9b.** b) Analytic HPLC trace of **9b**. HPLC gradient is 10% of solution B in 0-5 min, and 15% to 85% of solution B in 5-40 min on the analytic C4 column (λ=215 nm). c) Deconvoluted mass spectrum of **9b**. Q-TOF MS: Calculated: 14659, [M]. Found: 14659, [M].

In the synthesis of **9c**, 0.5 mg (0.0346 µmol) α-SynS129Dha and 40 equiv. of tetrazole **1c** were dissolved in 1 mL of a mixed solvent (H_2O:MeCN 4:1) in a quartz glass tube equipped with a magnetic stir bar. The reaction mixture was stirred and irradiated from one side with a hand-held 302-nm UV lamp for 20 minutes. The resulting solution was then passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and Q-TOF MS.

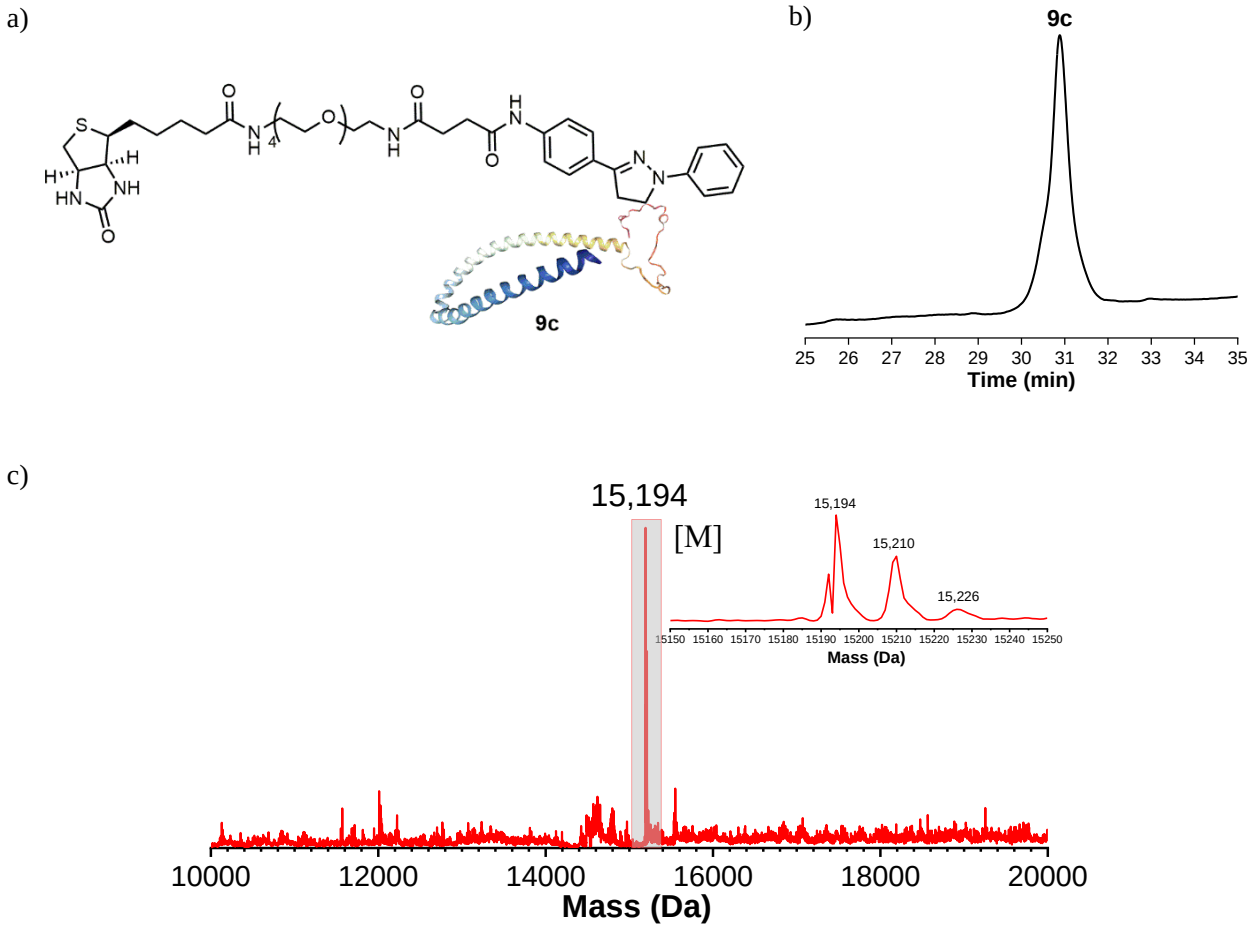


Fig. S36. a) Chemical structure of **9c**. b) Analytic HPLC trace of **9c**. HPLC gradient is 10% of solution B in 0-5 min, and 15% to 85% of solution B in 5-40 min on the analytic C4 column (λ=215 nm). c) Deconvoluted mass spectrum of **9c**. Q-TOF MS: Calculated: 15194, [M]. Found: 15194, [M].

0.5 mg (0.0346 μmol) of α-SynS129Dha and 30 equiv. of tetrazole **1d** were dissolved in 1 mL of a mixed solvent (H_2O:MeCN 4:1) in a quartz glass tube equipped with a magnetic stir bar. The reaction mixture was stirred and irradiated from one side with a hand-held 302-nm UV lamp for 15 minutes. The resulting solution was then passed through a syringe filter, and the filtrate was analyzed by analytical RP-HPLC and Q-TOF MS.

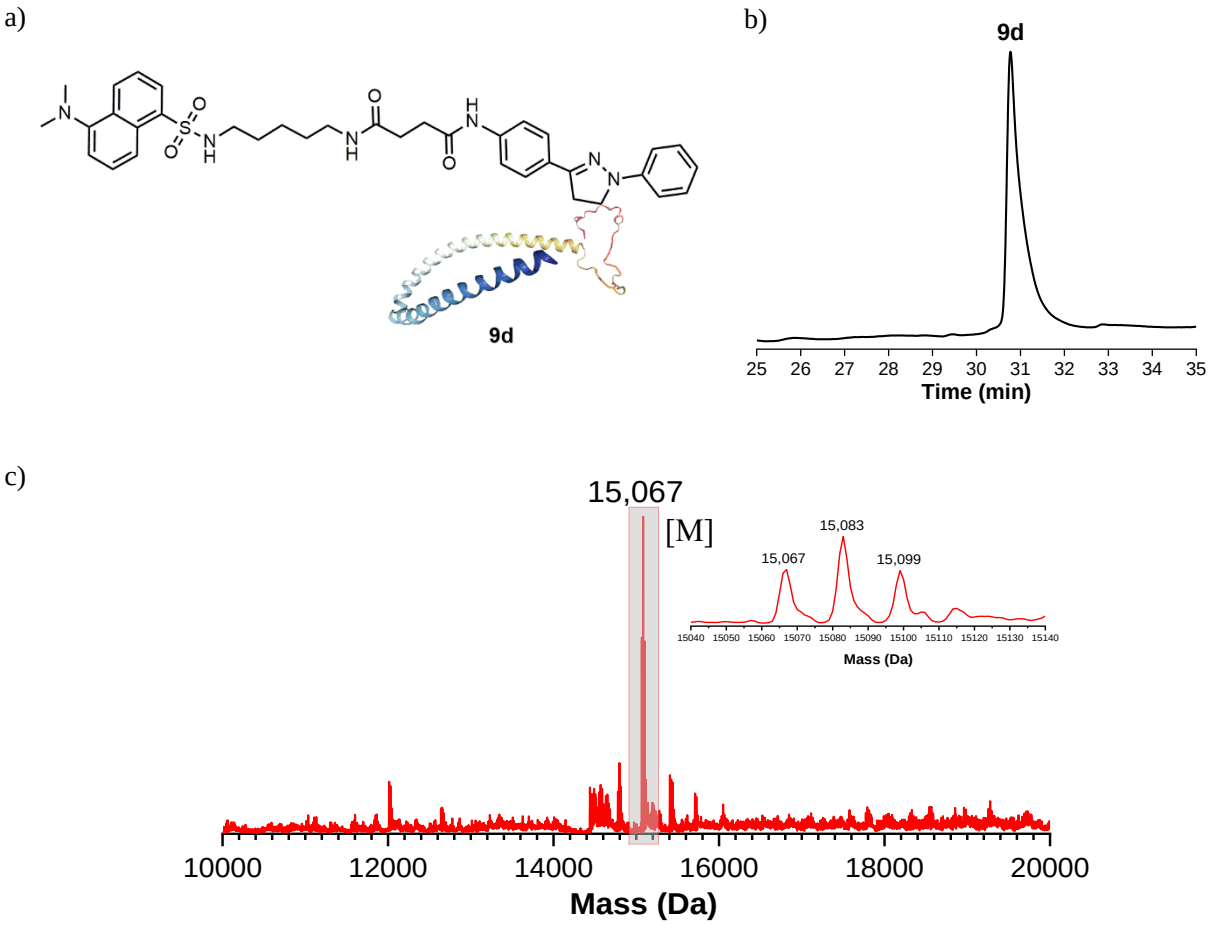
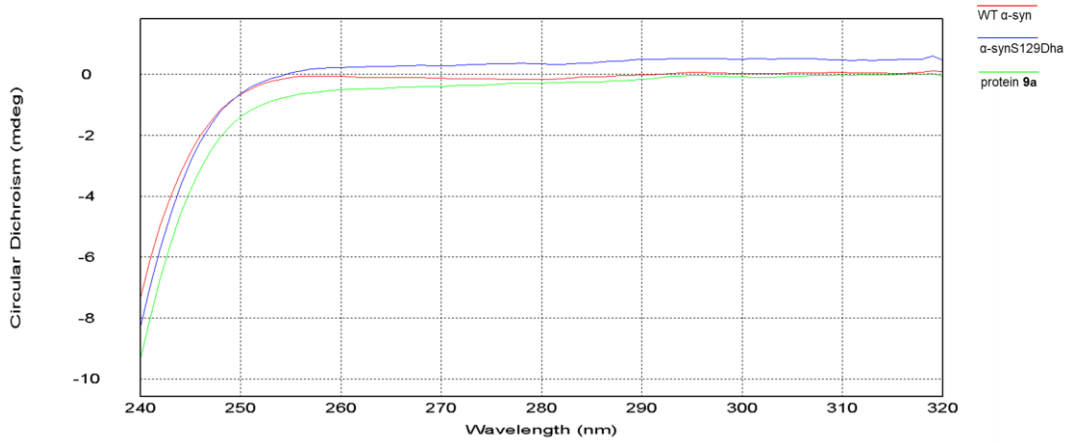


Fig. S37. a) Chemical structure of **9d**. b) Analytic HPLC trace of **9d**. HPLC gradient is 10% of solution B in 0-5 min, and 15% to 85% of solution B in 5-40 min on the analytic C4 column (λ=215 nm). c) Deconvoluted mass spectrum of **9d**. Q-TOF MS: Calculated: 15067, [M]. Found: 15067, [M].

Circular dichroism (CD) spectra measurements of the modified and unmodified protein

Wild-type alpha-synuclein (WT α-syn), the unmodified protein (α-synS129Dha) and the pyrazoline-modified protein **9a** were each dissolved in water to prepare two different solutions: a concentrated solution with a concentration of 2 mg/mL and a diluted solution with a concentration of 0.1 mg/mL. CD spectra of these three proteins were measured using a 1-mm pathlength quartz cuvette. For the determination of the tertiary structure of the proteins, the scanning wavelength range was set from 240 to 320 nm, with a protein sample concentration of 2 mg/mL [Fig. S38, a)]. For the determination of the secondary structure, the scanning wavelength range was set from 185 to 260 nm, with a protein sample concentration of 0.1 mg/mL [Fig. S38, b)]. Each sample was scanned three times, and the average values were taken to improve the signal-to-noise ratio. The obtained CD spectra are shown below:

a)



b)

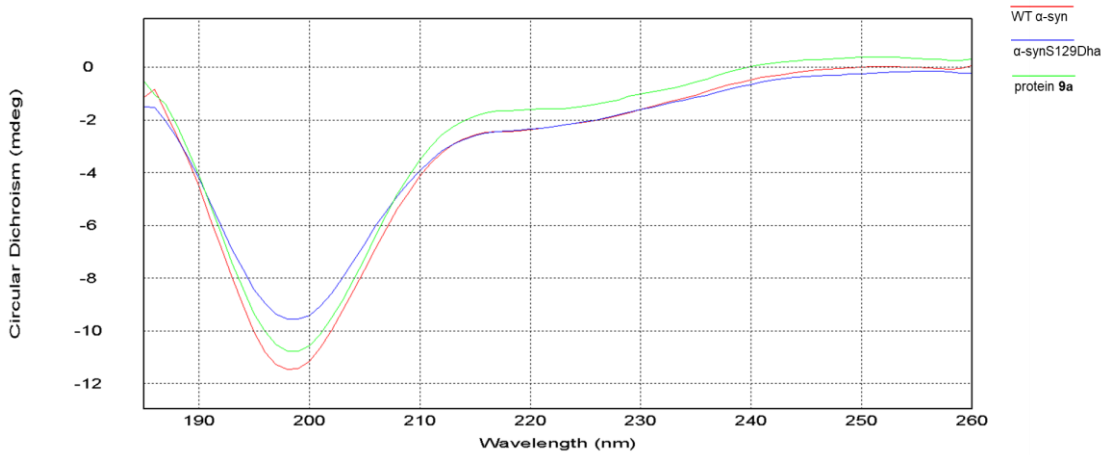


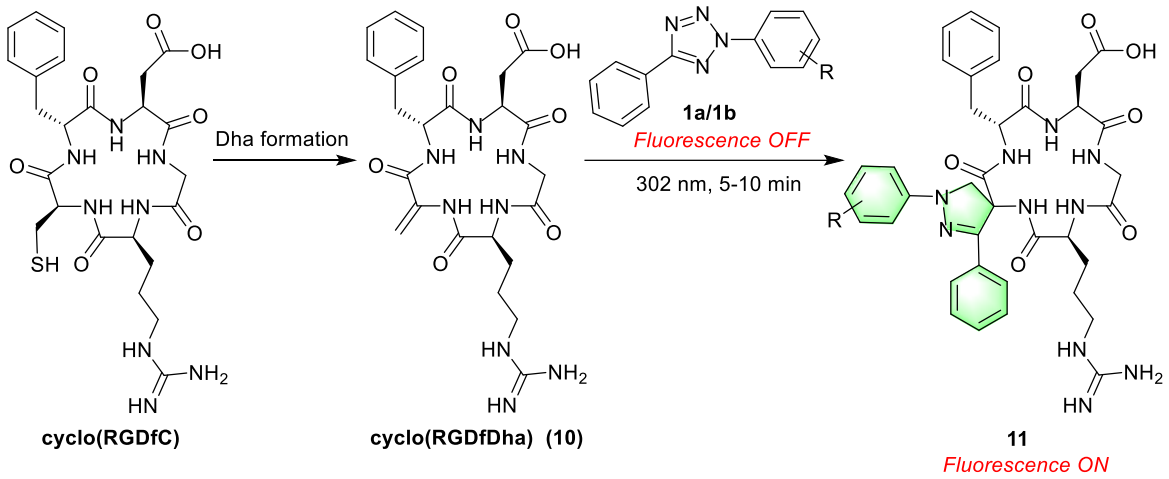
Fig. S38. Circular dichroism (CD) spectra of the wild-type alpha-synuclein (WT α-syn), the unmodified protein (α-synS129Dha) and the pyrazoline-modified protein **9a** for the determination of a) the tertiary structure and b) the secondary structure of the proteins.

Furthermore, CDNN software was used to estimate protein secondary structure from CD spectra. The results are as follows:

	WT α-syn	α-synS129Dha	protein **9a**
Helix	7.65%	7.64%	7.64%
Antiparallel	43.59%	43.92%	43.74%
Parallel	3.50%	3.50%	3.50%
Beta-Turn	17.79%	17.68%	17.77%
Random Coil	27.47%	27.35%	27.44%
Total Sum	100.00%	100.00%	100.00%

Table S1. CDNN analysis of the CD data.

Protocol for fluorescence imaging using cycle RGD peptide and tetrazoles 1a/1b



Scheme S9. Protocol for fluorescence imaging using cycle RGD peptide and tetrazoles **1a/1b**.

Synthesis and characterization of cyclic peptides

Synthesis and characterization of cyclic peptide cyclo(RGDfC)

Firstly, the NH_2-Arg(OtBu)-Gly-Asp(OtBu)-*(D)*-Phe-Cys(Trt)-CTC resin was synthesized using the Fmoc-SPPS protocol. 1.567 g H-Cyc(Trt)-CTC resin (loading 0.319 mmol/g) was swollen in dry DCM (10 mL) for 1 h at 37 °C, and then drained. Then 4.0 equiv. Fmoc-D-Phe-OH was coupled with HATU (4.0 equiv.), HOAt (4.0 equiv.) and DIPEA (8.0 equiv.) in DMF for 1 h. After removing the Fmoc group, Fmoc-Asp(OtBu)-OH, Fmoc-Gly-OH, Fmoc-Arg(OtBu)-OH were sequentially linked by this method. After the Fmoc-Arg(OtBu)-OH was coupled on the resin, the Fmoc group was deprotected and the peptide was cleaved from resin with DCM solution containing 20% trifluoroethanol (TFE) at 37°C for 2 hours, resulting in the formation of NH_2-Arg(OtBu)-Gly-Asp(OtBu)-*(D)*-Phe-Cys(Trt)-COOH. The resin was filtered out, and the filtrate was transferred to a flask. Remove as much solvent as possible through rotary evaporation at 25°C, and then perform lyophilization with the addition of an appropriate amount of H_2O. The freeze-dried peptide was dissolved in 400 mL DCM, and a DMF solution of 1 mmol (135.13 mg) of HOBt was added. Next, 0.6 mL of DIC was slowly added, and the cyclization reaction was carried out at room temperature for 12 hours. After completion of the cyclization reaction, the DCM solvent was removed, and 30 ml of cleavage solution was added for deprotection at 37°C for 3 hours. After precipitating with diethyl ether, the crude product was purified with preparative RP-HPLC using C18 column and identified with ESI-MS, and then lyophilized to obtain cyclic peptide **cyclo(RGDfC)**. Characterization was assessed with analytic RP-HPLC and ESI-MS.

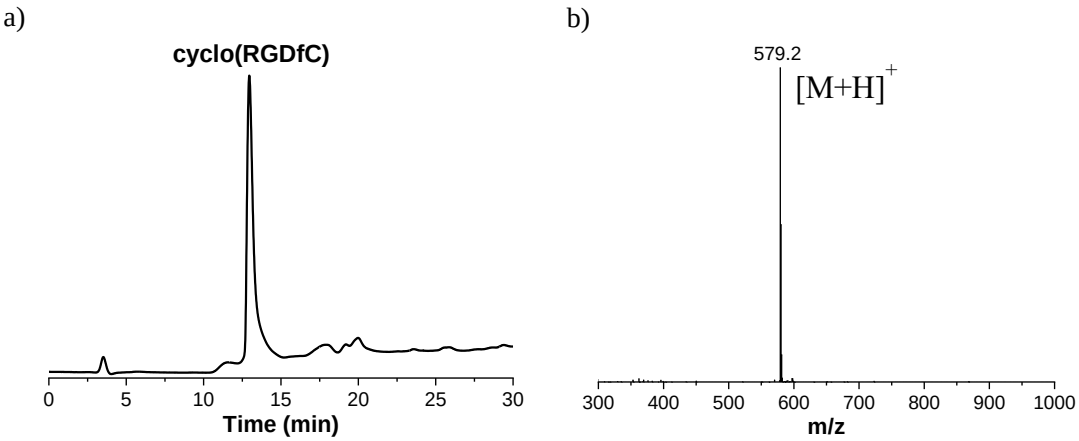
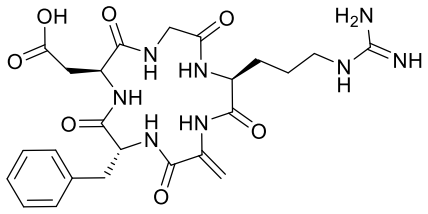


Fig. S39. a) Analytic HPLC trace of cyclic peptide **cyclo(RGDfC)**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 80% of solution B in 5-35 min on the analytic C18 column (λ=215 nm). b) Mass spectrum of **cyclo(RGDfC)**. ESI-MS: Calculated: 579.2, $[M+H]^+$. Found: 579.2, $[M+H]^+$.

Synthesis and characterization of cyclic peptide cyclo(RGDfDha) (10)

Cyclo(RGDfDha) (**10**) was synthesized via bisalkylation-elimination from **cyclo(RGDfC)**. 1.0 mg (1.73 μmol) **cyclo(RGDfC)** and 0.10 mg（0.2 equiv.) TCEP-HCl were dissolved into 1.0 mL NaPi buffer (50 mM, pH 8.0) in a 2 mL Eppendorf tube, then 8.2 μL (30 equiv.) methyl 2,5-dibromopentanoate (MeDBP) was dissolved in 330 μL DMSO. The DMSO solution was added to the NaP_i buffer solution and the mixture was vortexed for 30 s to ensure thorough mixing. The tube was shaken at 37 °C for 3 h in a constant temperature metal bath. Finally, take supernatant after centrifugation and then purify the crude cyclic peptide with preparative RP-HPLC. And the product was obtained by lyophilization. Characterization was assessed with analytic RP-HPLC and ESI-MS.

a)



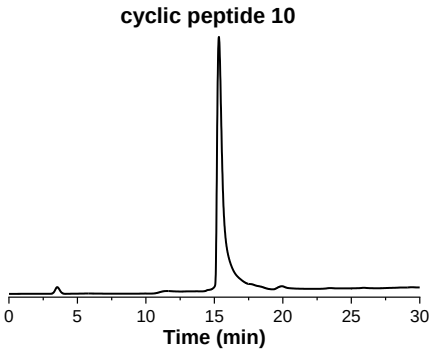
cyclo(RGDfDha) (10)

Chemical Formula: $C_{24}H_{32}N_8O_7$

Exact Mass: 544.24

Molecular Weight: 544.57

b)



c)

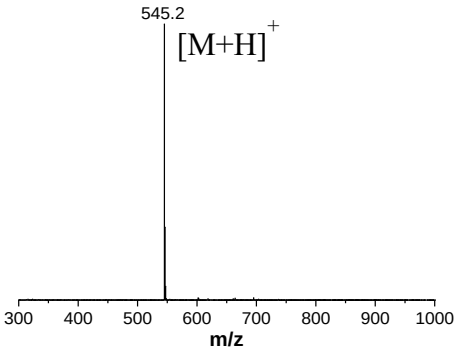


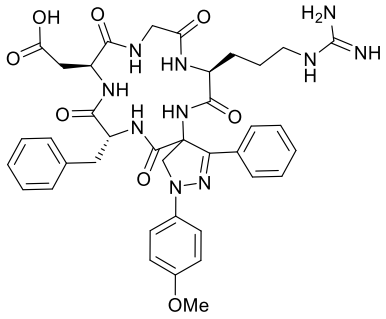
Fig. S40. a) Chemical structure of cyclic peptide **cyclo(RGDfDha) (10)**; b) Analytic HPLC trace of cyclic peptide **10**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 80% of solution B in 5-35 min on the analytic C18 column (λ=215 nm). c) Mass spectrum of cyclic peptide **10**. ESI-MS: Calculated:545.3, $[M+H]^+$. Found: 545.2, $[M+H]^+$.

Synthesis and characterization of pyrazoline-modified cyclic peptides 11a and 11b

Synthesis of 11a

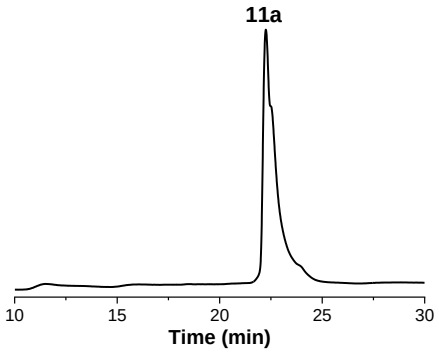
0.58 mg (1 μmol) cyclic peptide **10** was fully dissolved in 400 μL H_2O in a quartz glass tube equipped with a magnetic stir bar. Then prepare 1 mL of tetrazole **1a** (5 mM in MeCN) solution. 600 μL of the **1a** solution was added to the quartz glass tube, resulting in a final concentration of 1 mM for cyclic peptide **10** and 3 mM for **1a**, respectively. The solution was stirred and irradiated from one side with a hand-held 302 nm UV lamp for 15 min. The solution showed a bright green fluorescence after reaction. After passing the solution through a syringe filter, the filtrate was analyzed by analytical RP-HPLC and ESI-MS.

a)



11a
Chemical Formula: $C_{38}H_{44}N_{10}O_8$
Exact Mass: 768.33
Molecular Weight: 768.83

b)



c)

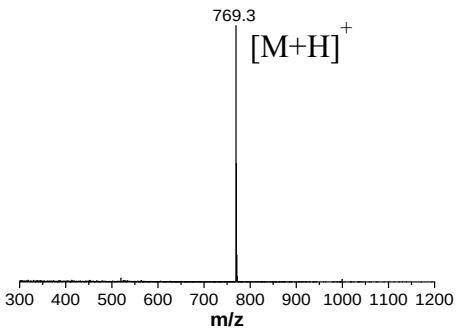
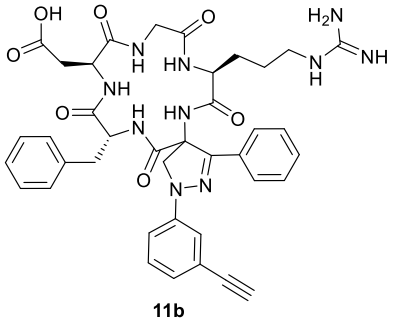


Fig. S41. a) Chemical structure of pyrazoline-modified cyclic peptide **11a**; b) Analytic HPLC trace of **11a**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 80% of solution B in 5-35 min on the analytic C18 column (λ=215 nm). c) Mass spectrum of **11a**. ESI-MS: Calculated: 769.3, $[M+H]^+$. Found: 769.3, $[M+H]^+$.

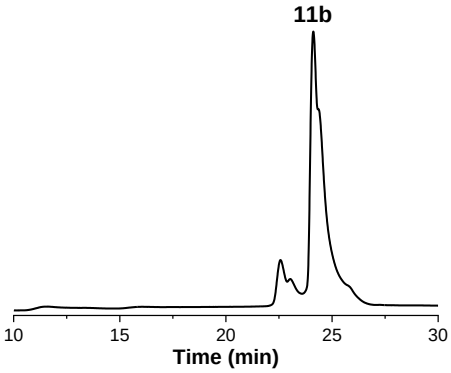
Synthesis of 11b

0.58 mg (1 μmol) cyclic peptide **10** was fully dissolved in 400 μL H_2O in a quartz glass tube equipped with a magnetic stir bar. Then prepare 1 mL of tetrazole **1b** (5 mM in MeCN) solution. 600 μL of the **1b** solution was added to the quartz glass tube, resulting in a final concentration of 1 mM for cyclic peptide **10** and 3 mM for **1b**, respectively. The solution was stirred and irradiated from one side with a hand-held 302 nm UV lamp for 15 min. The solution showed a bright blue fluorescence after reaction. After passing the solution through a syringe filter, the filtrate was analyzed by analytical RP-HPLC and ESI-MS.

a)



b)



c)

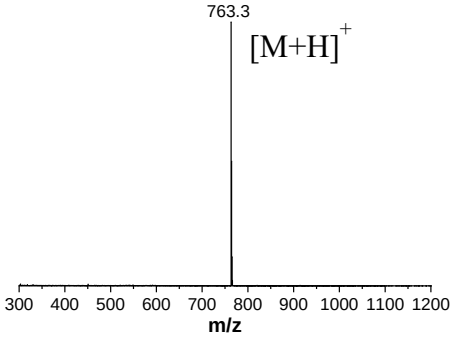


Fig. S42. a) Chemical structure of pyrazoline-modified cyclic peptide **11b**; b) Analytic HPLC trace of **11b**. HPLC gradient is 15% of solution B in 0-5 min, and 20% to 80% of solution B in 5-35 min on the analytic C18 column (λ=215 nm). c) Mass spectrum of **11b**. ESI-MS: Calculated: 763.3, $[M+H]^+$. Found: 763.3, $[M+H]^+$.

Spectroscopic characterization of modified cyclic peptides 11a and 11b

The samples were dissolved in a mixed solvent of MeCN/H_2O (1/1, v/v) solvent, respectively. For sample **11a**, the solution was excited at 340 nm and fluorescence emission was scanned in the region of 390 to 600 nm through a 3 nm slit. For sample **11b**, the solution was excited at 320 nm and fluorescence emission was scanned in the region of 365 to 600 nm through a 3 nm slit.

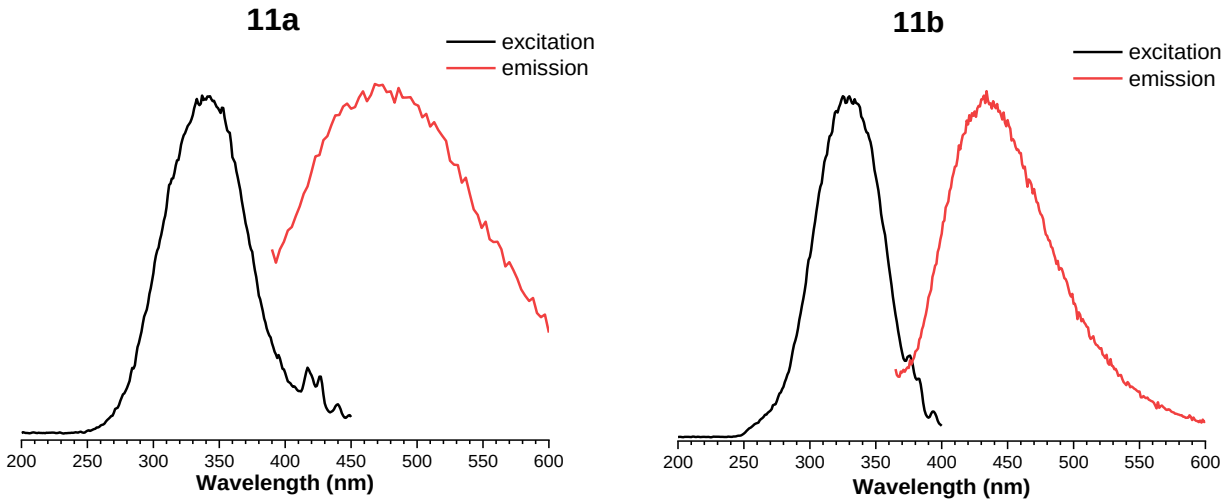


Fig. S43. Fluorescence spectra of modified cyclic peptides **11a** (left) and **11b** (right).

The maximum excitation wavelength and emission wavelength of **11a** are 342 nm and 495 nm, respectively. The maximum excitation wavelength and emission wavelength of **11b** are 325 nm and 450 nm, respectively.

Cytotoxicity assay

B16-OVA cells were cultured in RPMI-1640 medium with 10% FBS and 1% penicillin-streptomycin. A suspension of B16-OVA cells was prepared at a density of 100,000 cells/mL in RPMI-1640 complete medium. Next, 100 μL of B16-OVA cell suspension (10,000 cells per well) was dispensed into each well of a 96-well plate. The plate was incubated at 37 °C in a CO_2 incubator for 12 h to allow for attachment and growth of the cells. Then, remove the medium and add 100 μL of various concentrations of test compounds (tetrazoles **1a/1b**, cyclo(RGDfDha) **10**, modified cyclic peptides **11a/11b**) to each well. The cells were incubated for 18 h in the incubator at 37 °C. To evaluate cell viability, a CCK-8 solution was prepared by adding the reagent to medium at a ratio of 1:9. The medium was then removed from each well, and 100 μL of 10% CCK-8 solution was added to each well. The plate was incubated for 2 h at 37 °C in the CO_2 incubator, and the absorbance of each well was measured at 450 nm using a microplate reader. Cell viability was calculated by comparing the absorbance of the test compound-treated cells to that of the untreated cells (0 μM).

Additionally, the cytotoxicity induced by exposure of cells to 302-nm UV light for different durations of irradiation was evaluated using the same protocol after 12 h incubation.

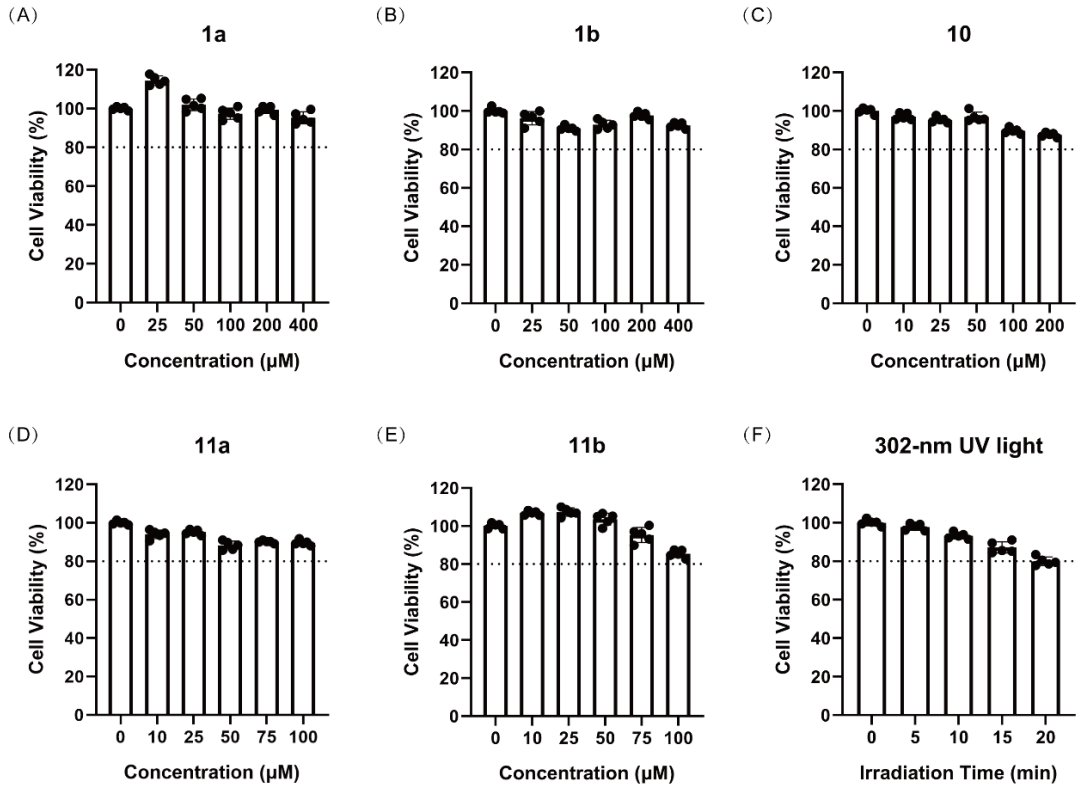


Fig. S44. (A)-(E) The evaluation of the toxicity of compounds **1a**, **1b**, **10**, **11a** and **11b** on B16-OVA cells with different concentrations of reagent treatment after 18 h incubation. (F) The evaluation of the toxicity of 302-nm UV light irradiation on B16-OVA cells for different irradiation time. The data were presented as the mean ± standard deviation (SD). The error bars represent standard deviations derived from five independent experiments.

Confocal experiment of B16-OVA cells

Prepare a suspension of B16-OVA cells in RPMI-1640 complete medium at a density of 150,000 cells/mL. Dispense 200 μL of the cell suspension (30,000 cells per well) into each well of an 8-well glass-bottom cell culture dish. Incubate the cells at 37°C in a CO_2 incubator for 12 hours to allow for attachment and growth. Remove the RPMI-1640 complete medium and treat the cells with cyclic peptide **10** for 12 hours to ensure sufficient binding of the cyclic peptides to the integrin receptors on the cells. Then, remove the medium and wash the cells with PBS. Dissolve tetrazoles **1a** and **1b** separately in DMSO, and dilute the resulting solutions with RPMI medium to obtain corresponding tetrazole solutions containing 1% DMSO. Add 200 μL of each tetrazole solution to the separate wells. After incubating for 30 minutes at 37 °C in the incubator, the cells were exposed to 302 nm UV light for 15 minutes using a hand-held UV lamp. Then remove the medium and wash the cells twice with PBS. The observation of cell imaging was carried out using a confocal microscope through the DAPI channel (excitation wavelength of 405 nm and emission wavelength of 410-585 nm). The cell imaging results were shown in Scheme 6B.

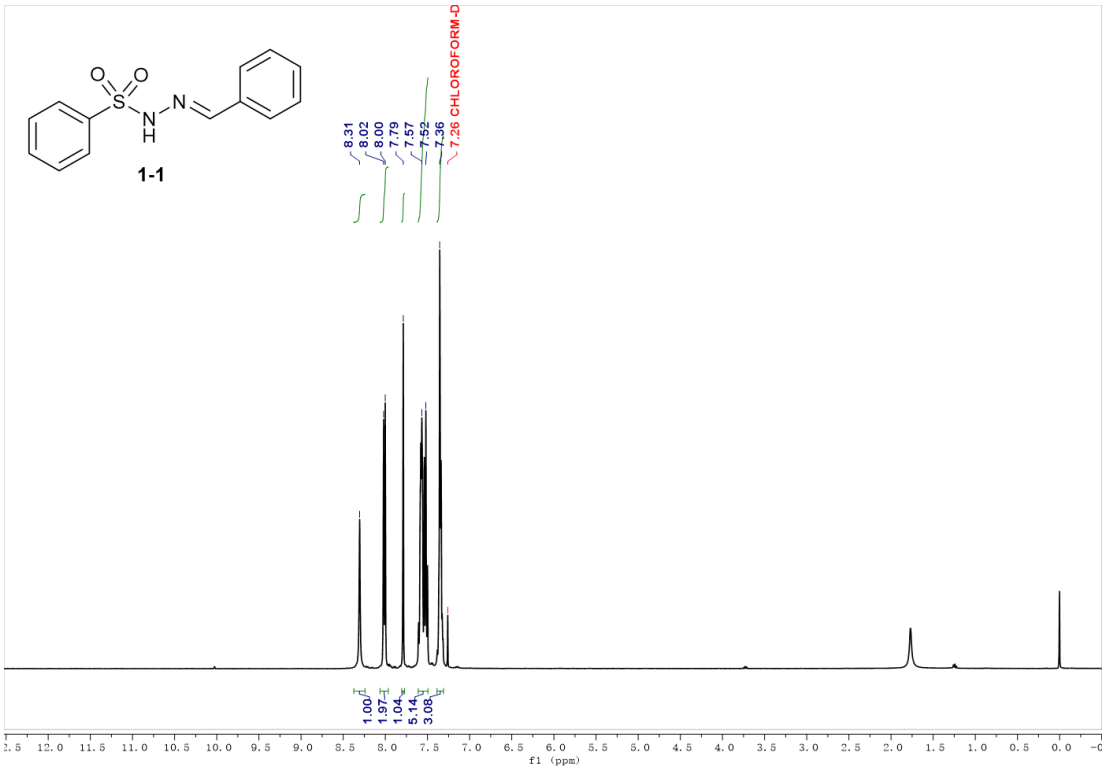
Flow cytometric analysis of B16-OVA cells

Prepare a suspension of B16-OVA cells in RPMI-1640 complete medium at a density of 400,000 cells/mL. Dispense 500 μL of the cell suspension (200,000 cells per well) into each well of a 24-well cell culture plate. Incubate the cells at 37°C in a CO_2 incubator for 12 hours to allow for attachment and growth. Remove the RPMI-1640 complete medium and treat the cells with cyclic peptide **10** for 12 hours to ensure sufficient binding of the cyclic peptides to the integrin receptors on the cells. Then, remove the medium and wash the cells with PBS. Dissolve tetrazoles **1a** and **1b** separately in DMSO, and dilute the resulting solutions with RPMI-1640 medium to obtain corresponding tetrazole solutions containing 1% DMSO. Then add 200 μL of each tetrazole solution to the separate wells. After incubating for 30 minutes at 37 °C in the incubator, the cells were exposed to 302 nm UV light for 15 minutes using a hand-held UV lamp. Then remove the medium and wash the cells with PBS. The single-cell suspensions were prepared using PBS and filtered for subsequent analysis by flow cytometry. The flow cytometric analysis of B16-OVA cells was carried using a 355-nm violet laser for excitation and a 450/50 bandpass filter for fluorescence detection. The cell labeling rates and mean fluorescence intensity of DAPI channel were shown in Scheme 6C.

NMR Spectra

Compound **1-1**

a)



b)

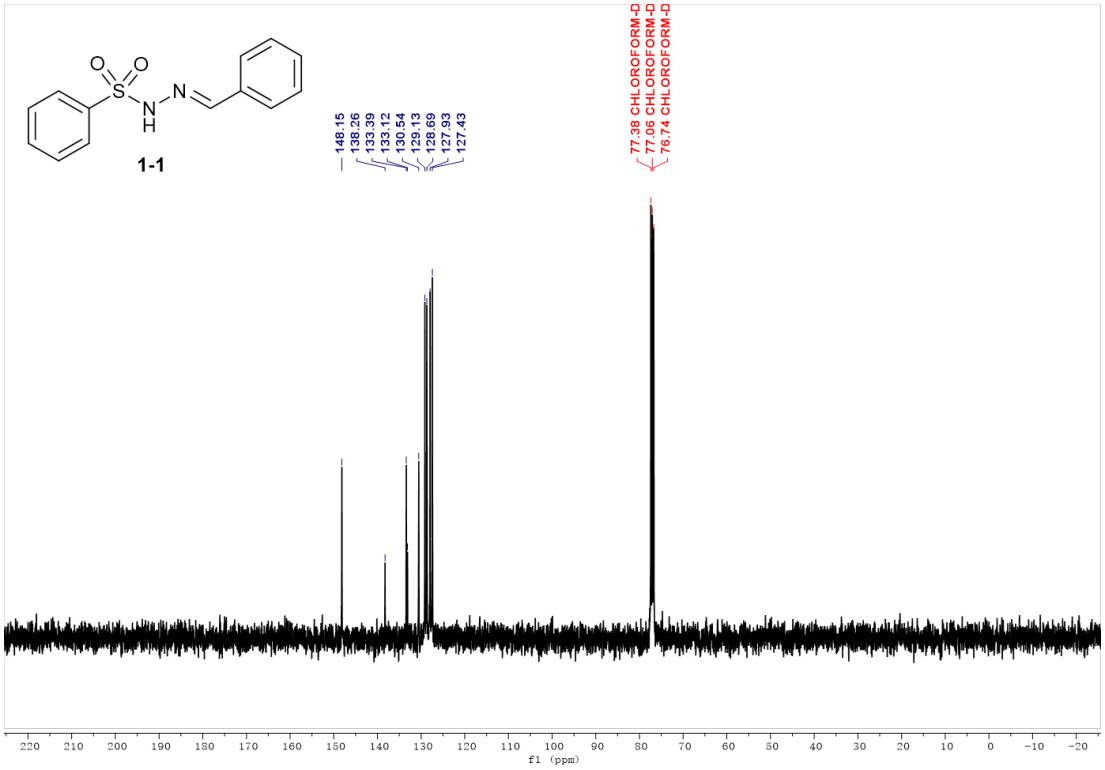


Fig. S45. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **1-1**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of compound **1-1**.

Compound **1a**

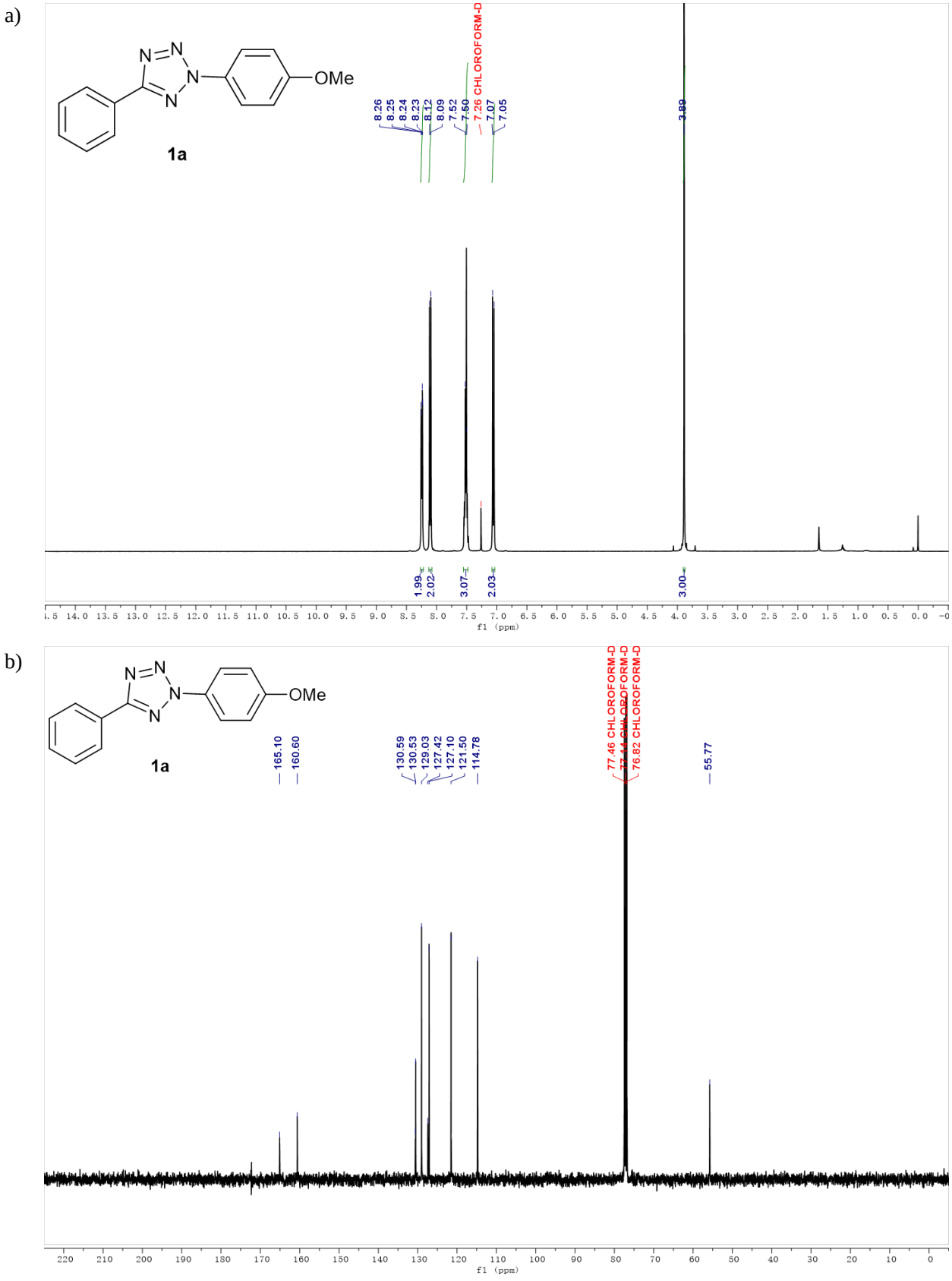


Fig. S46. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **1a**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of compound **1a**.

Compound **1b**

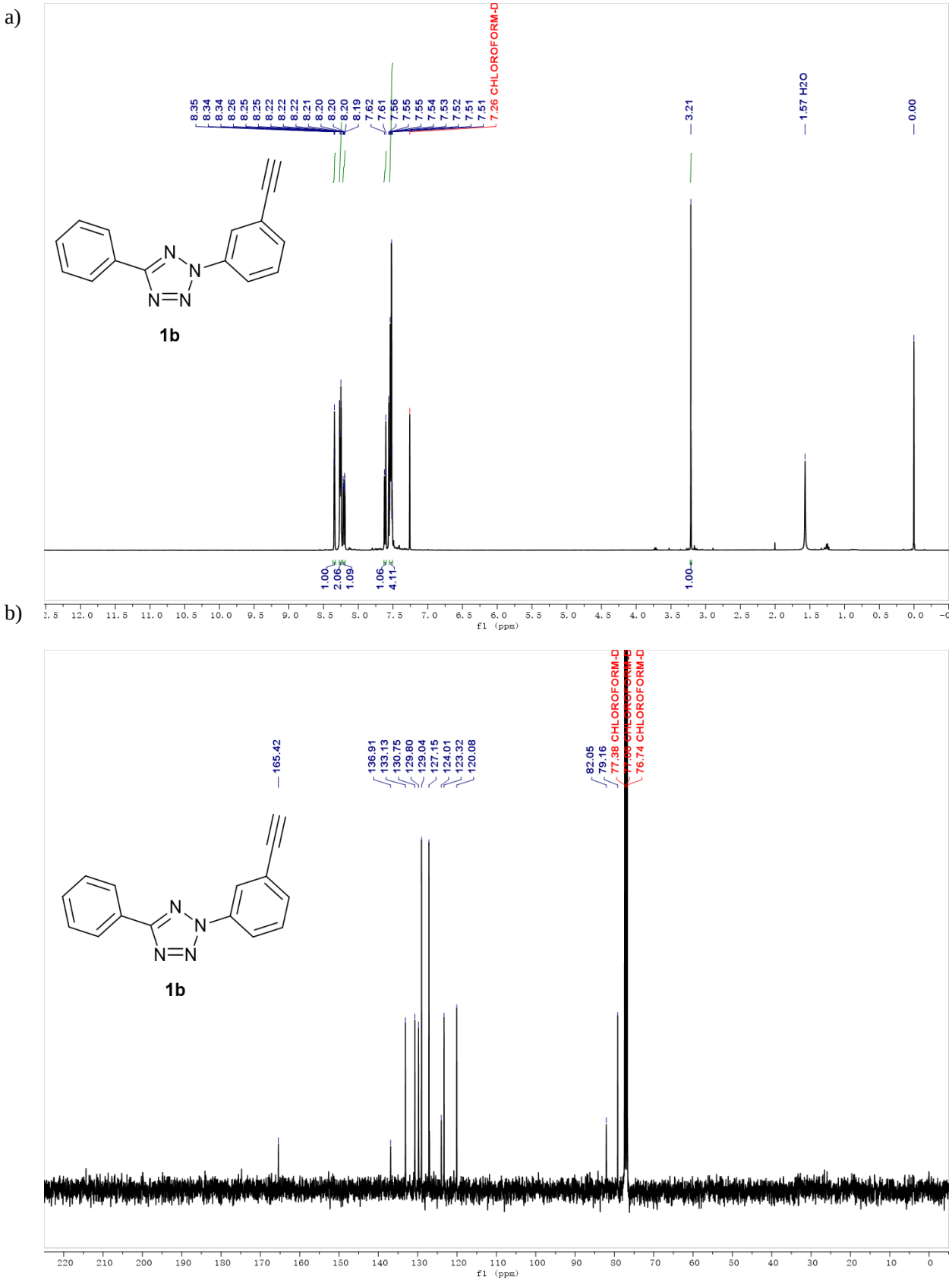


Fig. S47. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **1b**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of compound **1b**.

Compound **2-1**

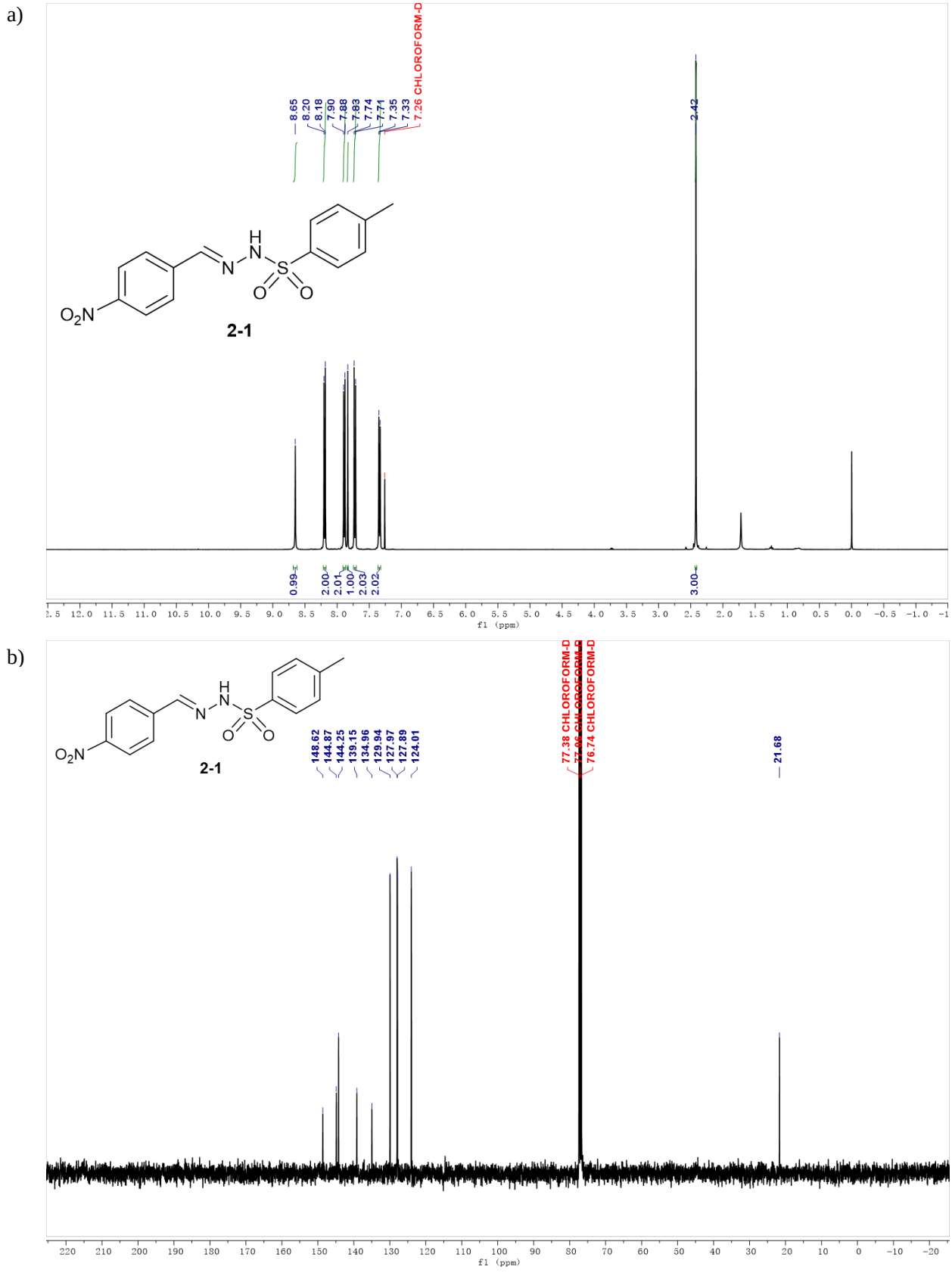


Fig. S48. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **2-1**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of compound **2-1**.

Compound **2-4**

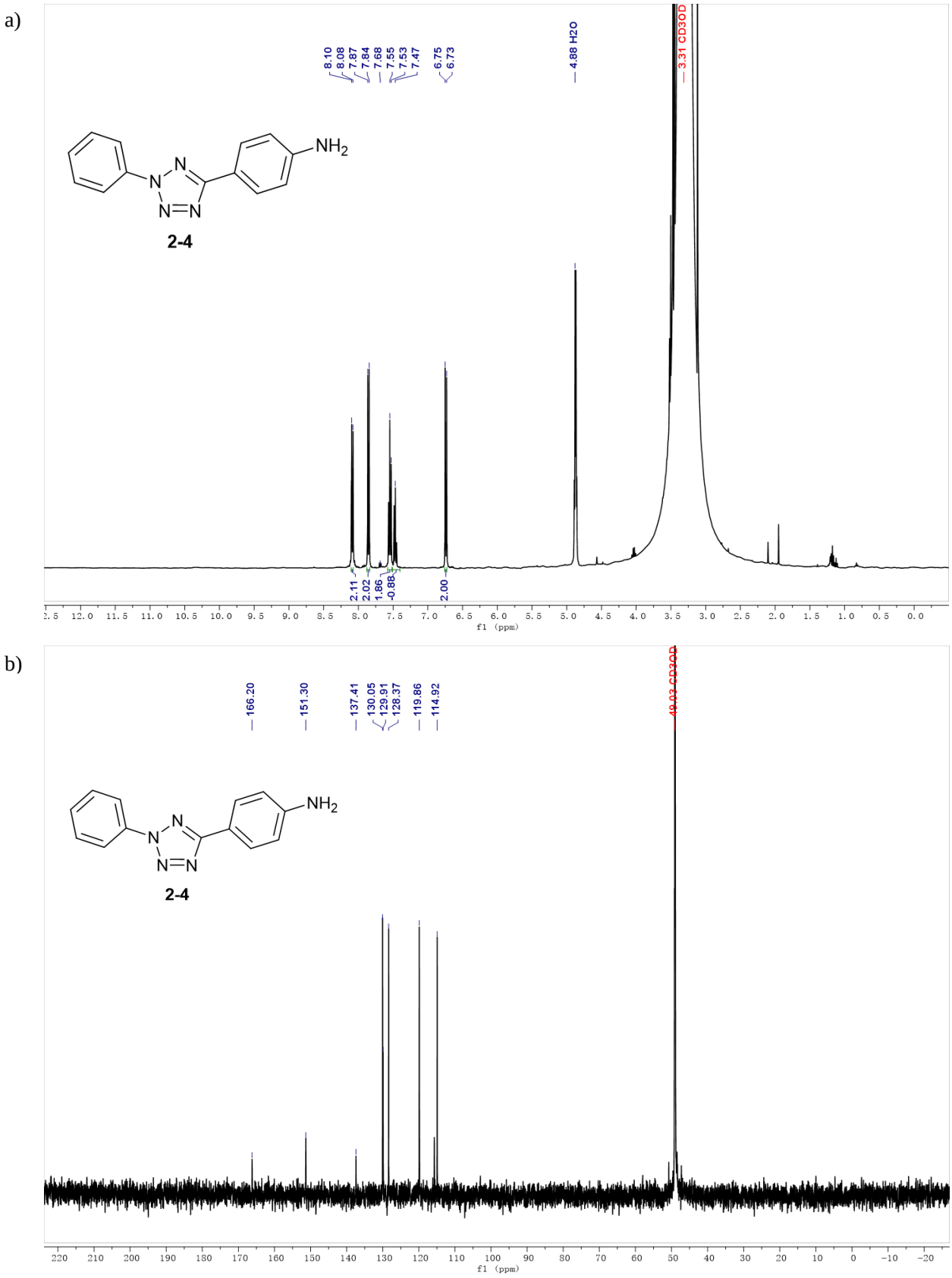


Fig. S49. a) ^{1}H NMR (400 MHz, CD_3OD) of compound **2-4**; b) ^{13}C NMR (101 MHz, CD_3OD) of compound **2-4**.

Compound **2-5**

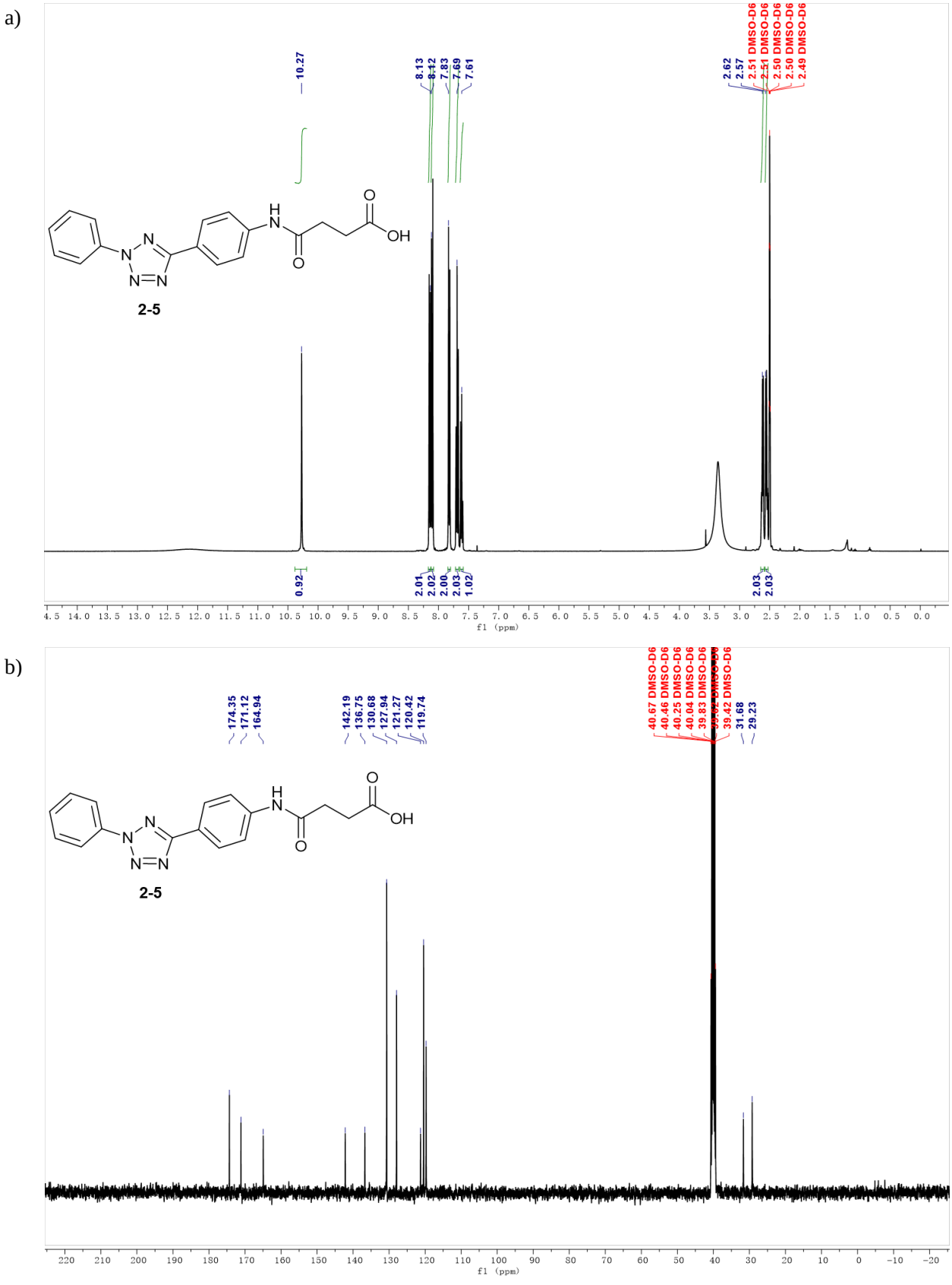


Fig. S50. a) ^{1}H NMR (400 MHz, DMSO-d_6) of compound **2-5**; b) ^{13}C NMR (101 MHz, DMSO-d_6) of compound **2-5**.

Biotin-PEG$_4$-NH$_2$

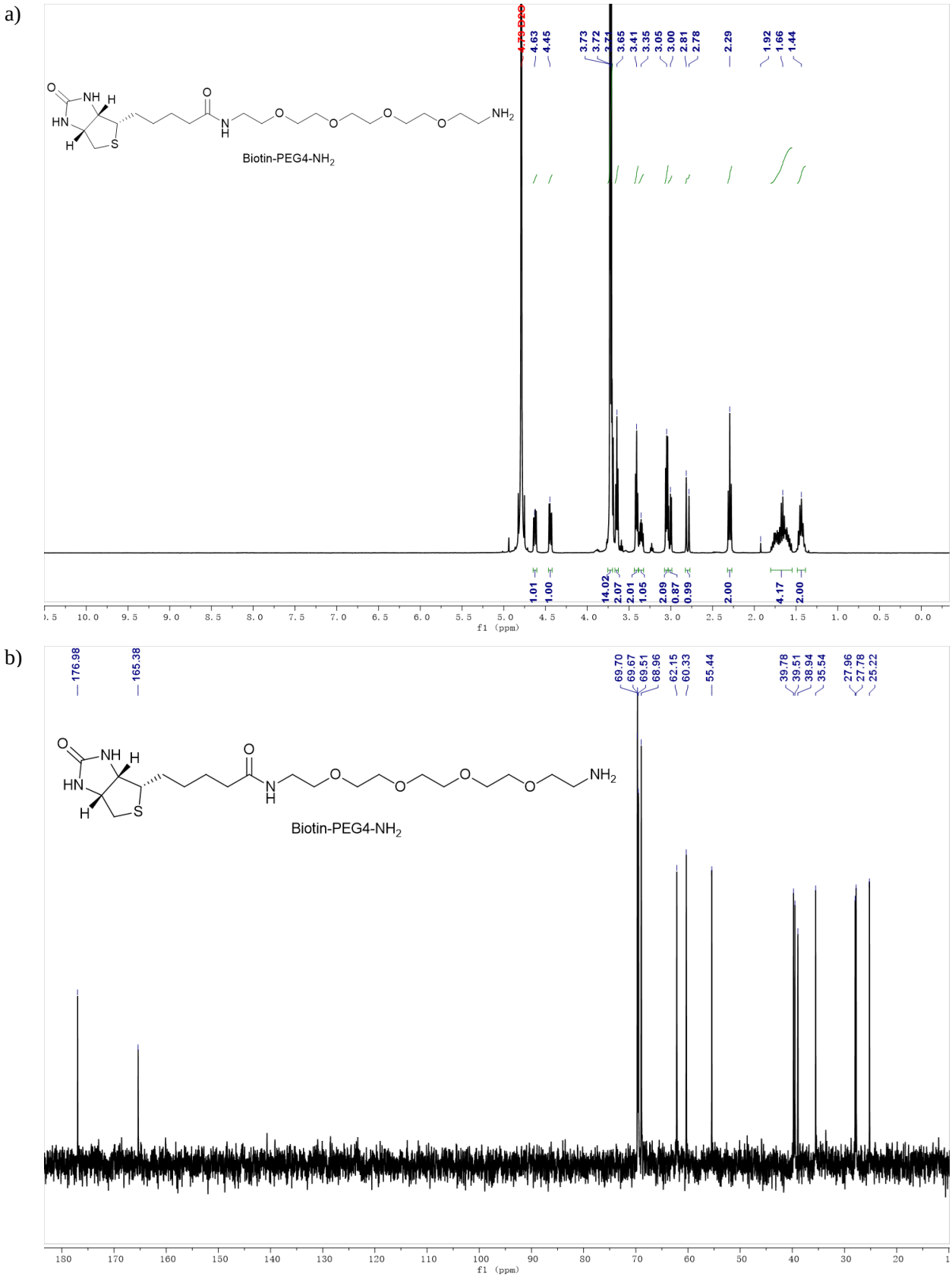


Fig. S51. a) ^{1}H NMR (400 MHz, D_2O) of **biotin-PEG$_4$-NH$_2$**; b) ^{13}C NMR (101 MHz, D_2O) of **biotin-PEG$_4$-NH$_2$**.

Compound **1c**

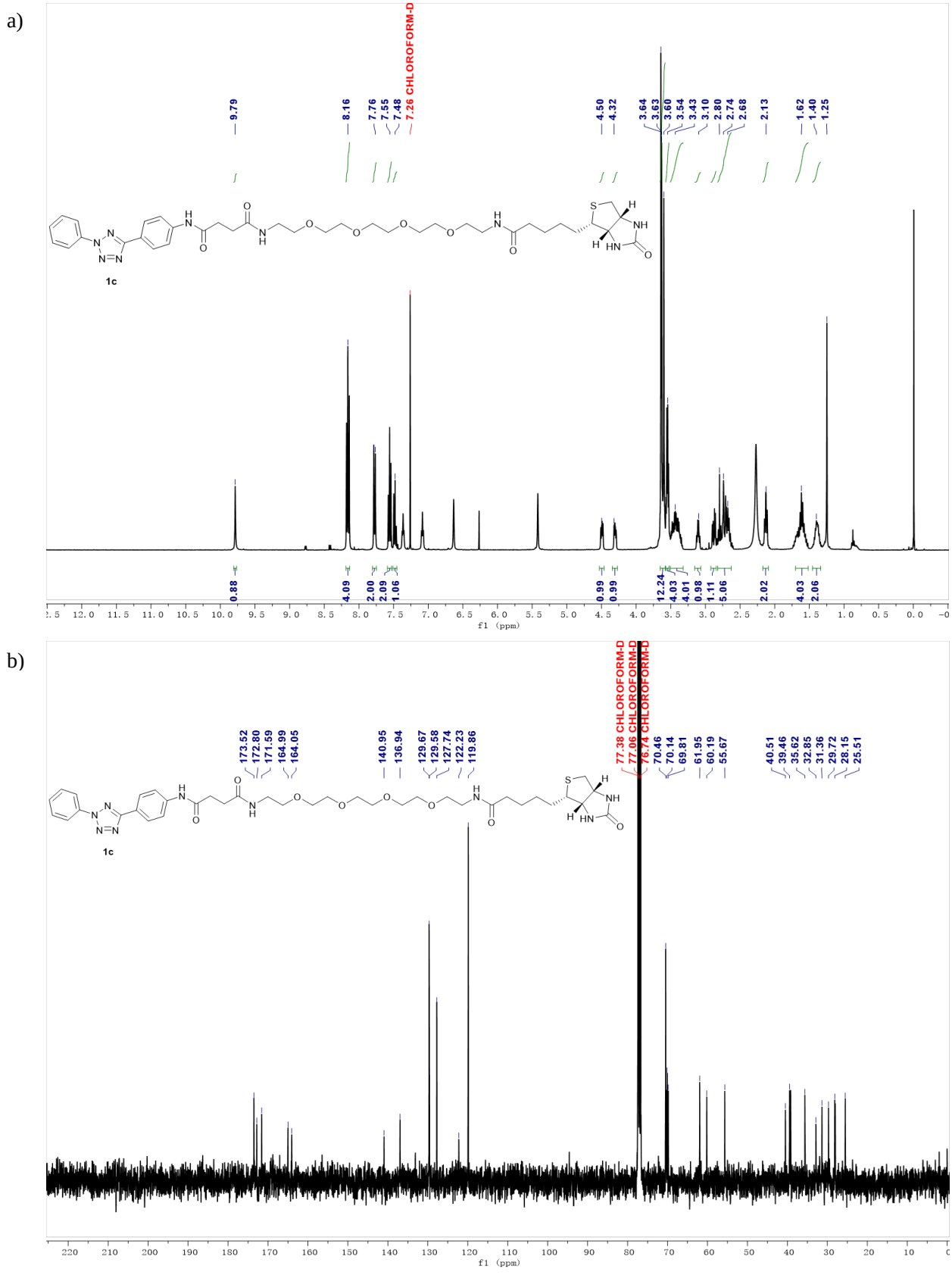


Fig. S52. a) ^{1}H NMR (400 MHz, $CDCl_3$) of compound **1c**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of compound **1c**.

Dansylcadaverine

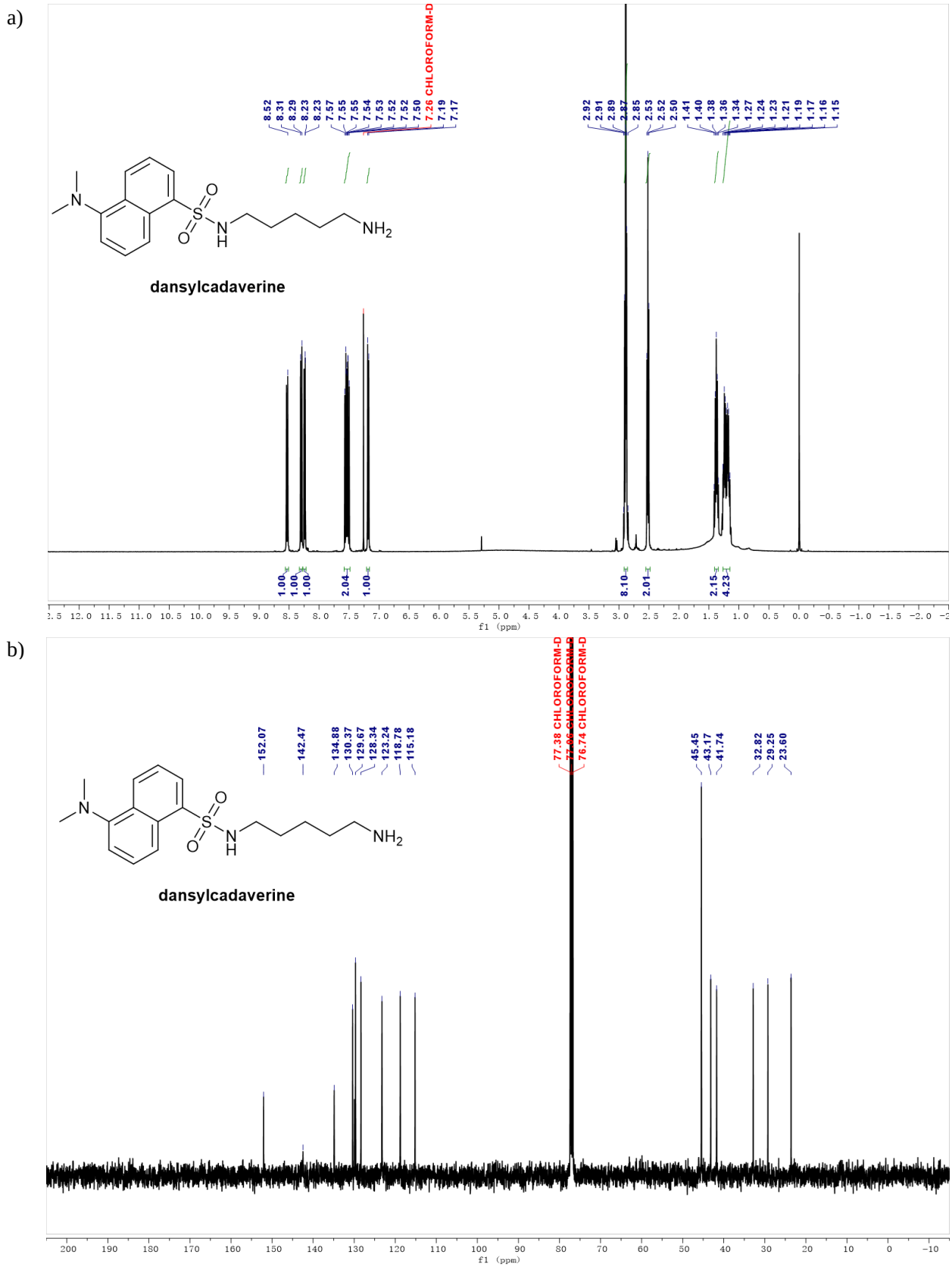


Fig. S53. a) ^{1}H NMR (400 MHz, $CDCl_3$) of **dansylcadaverine**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of **dansylcadaverine**.

Compound **1d**

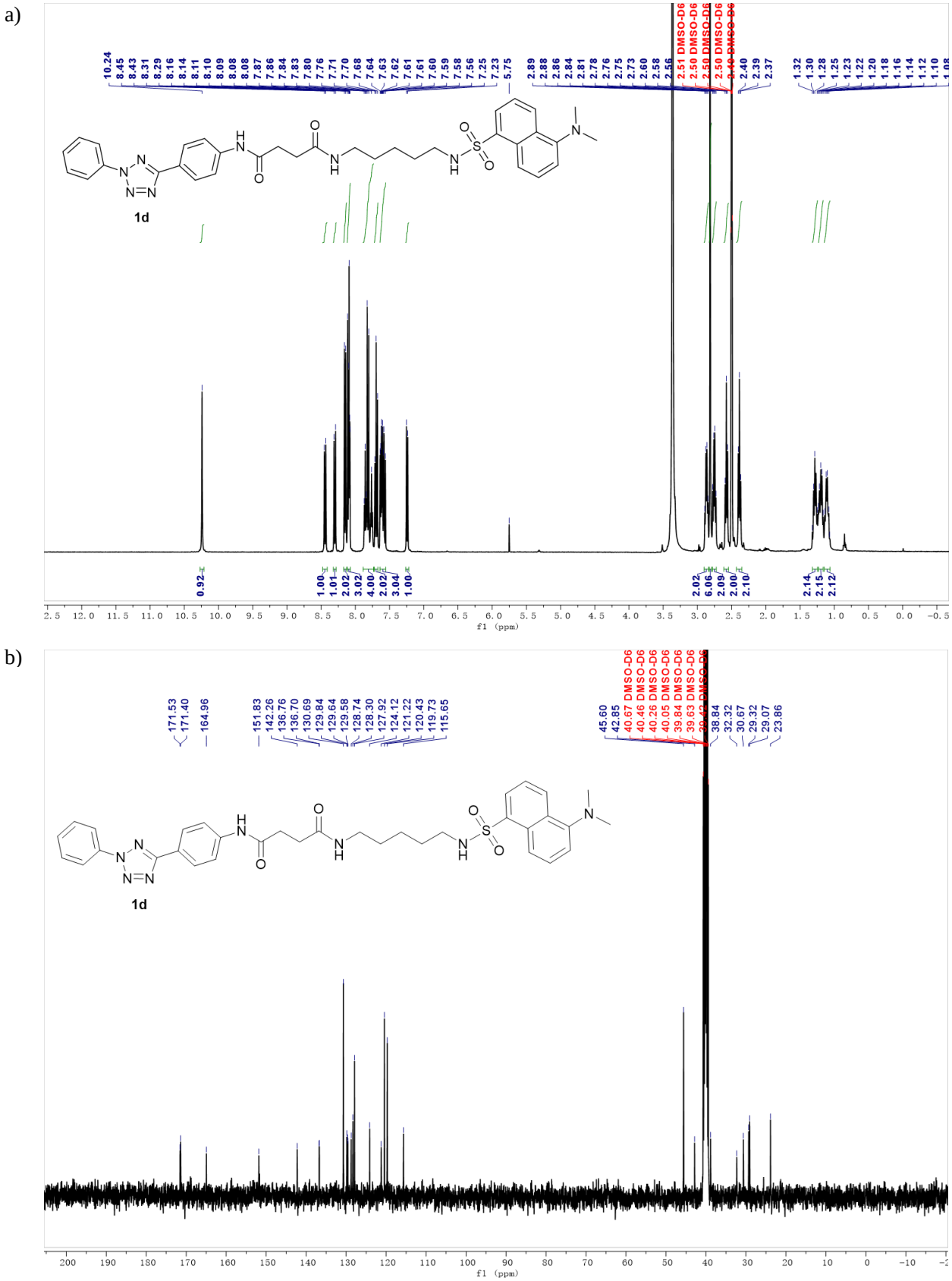


Fig. S54. a) ^{1}H NMR (400 MHz, DMSO-d_6) of compound **1d**; b) ^{13}C NMR (101 MHz, DMSO-d_6) of compound **1d**.

Boc-Dha-OMe

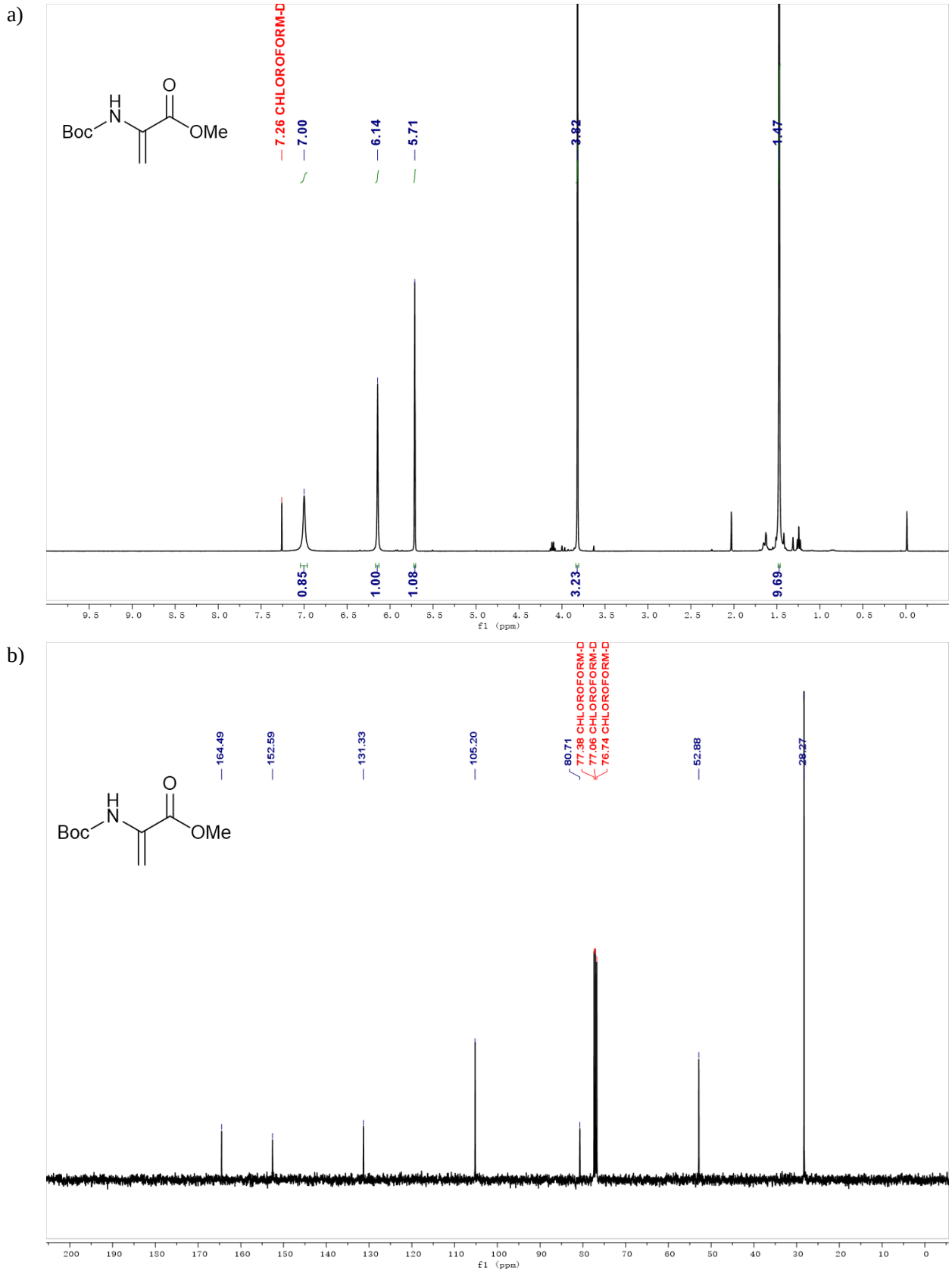


Fig. S55. a) ^{1}H NMR (400 MHz, $CDCl_3$) of **Boc-Dha-OMe**; b) ^{13}C NMR (101 MHz, $CDCl_3$) of **Boc-Dha-OMe**.

Compound **2a**

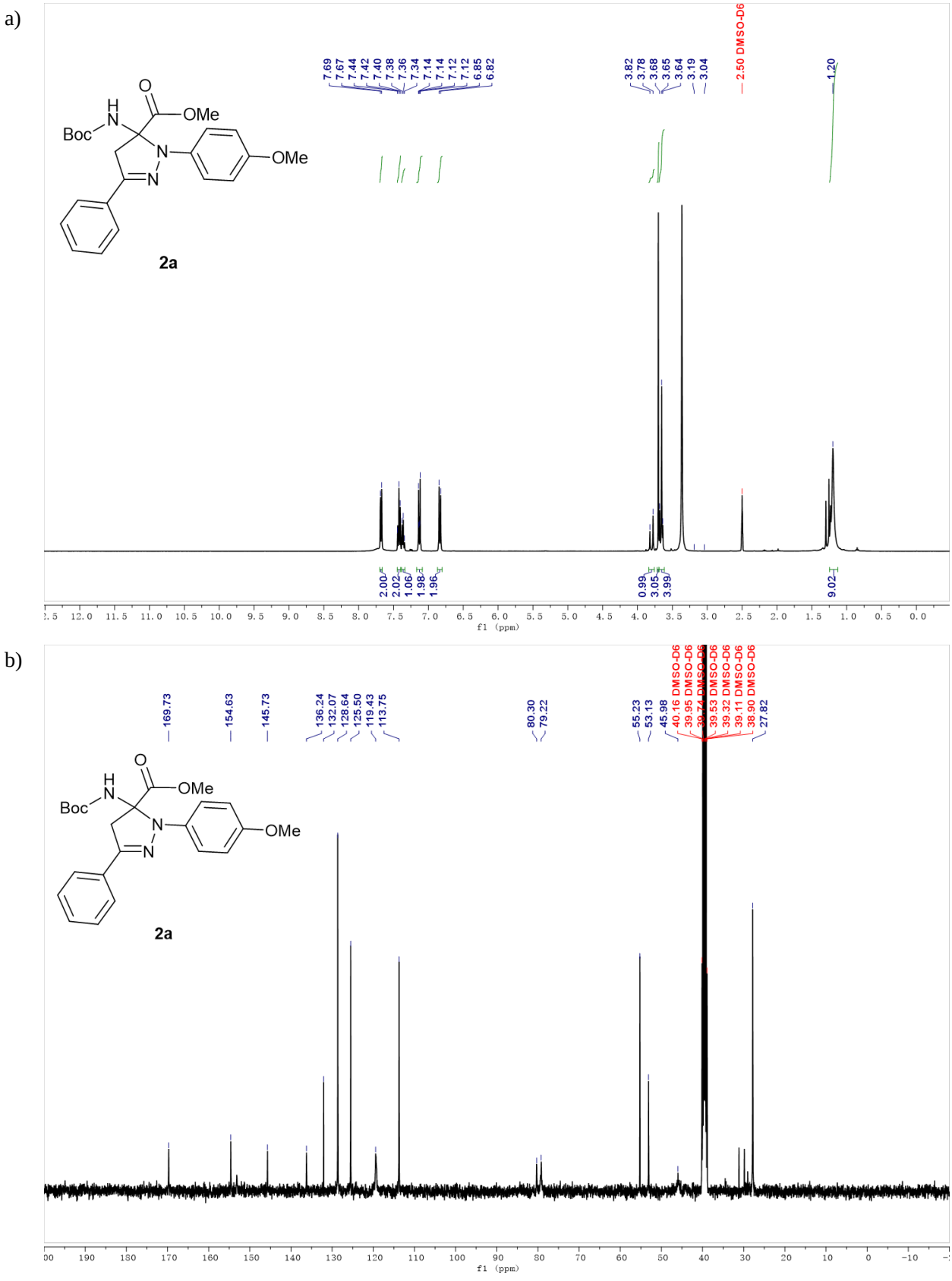


Fig. S56. a) ^{1}H NMR (400 MHz, DMSO-d_6) of compound **2a**; b) ^{13}C NMR (101 MHz, DMSO-d_6) of compound **2a**.

Compound **2b**

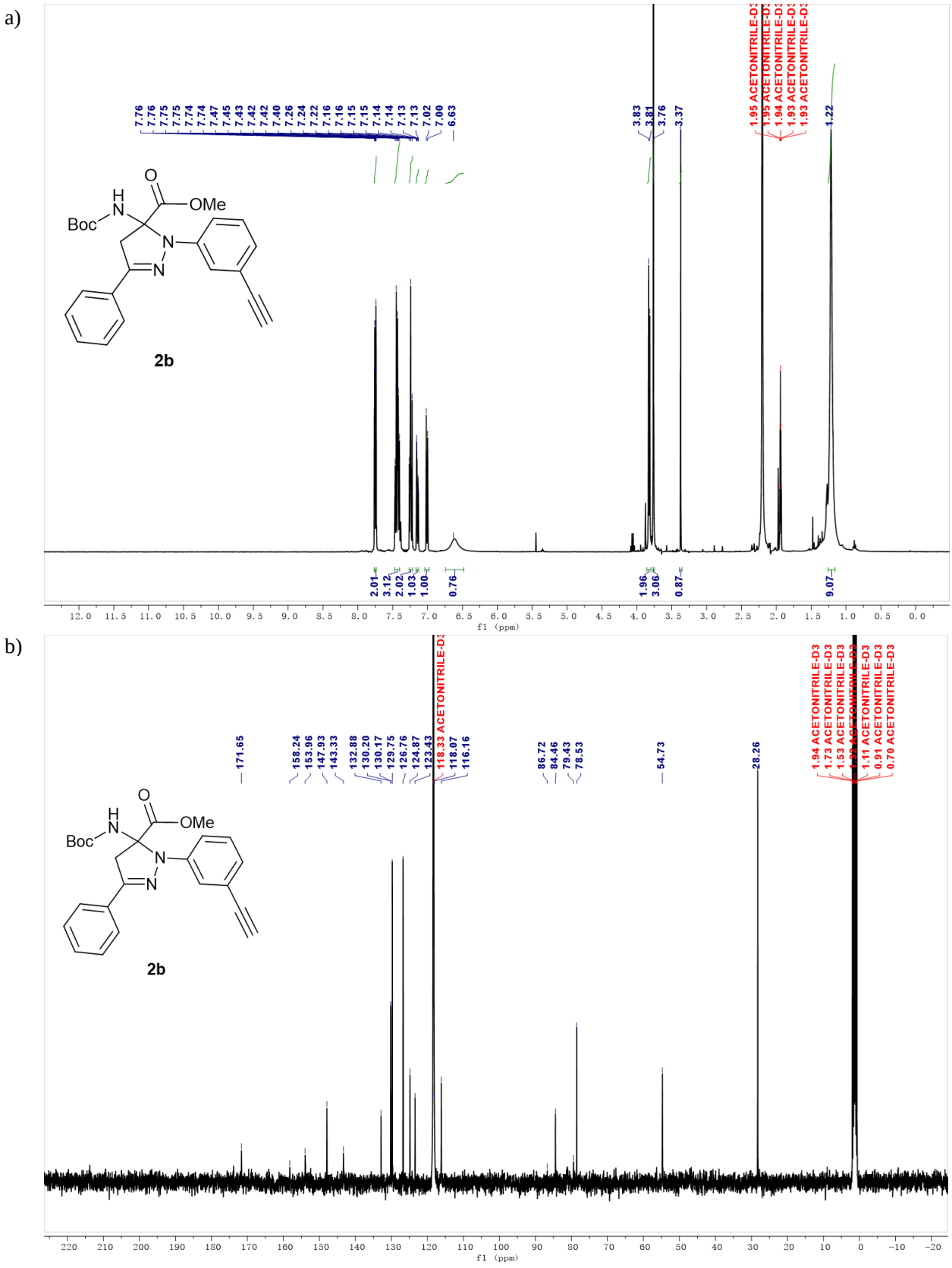


Fig. S57. a) ^{1}H NMR (400 MHz, CD_3CN) of compound **2b**; b) ^{13}C NMR (101 MHz, CD_3CN) of compound **2b**.

Peptide **3b**

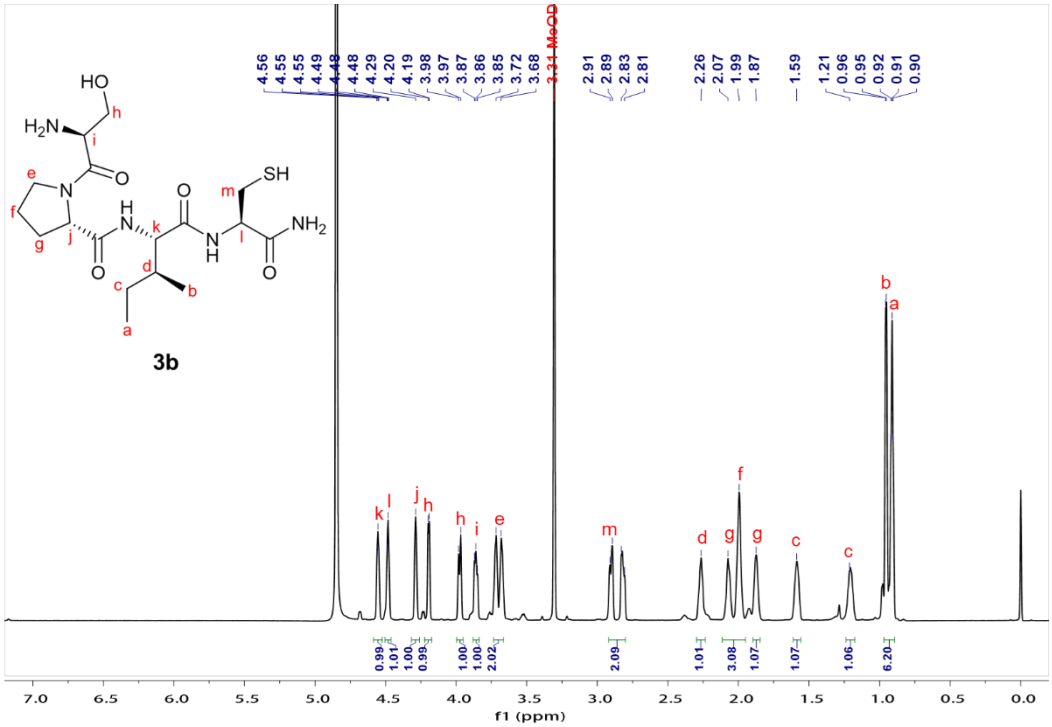


Fig. S58. ^{1}H NMR (800 MHz, CD_3OD) of peptide **3b**.

Peptide **4b**

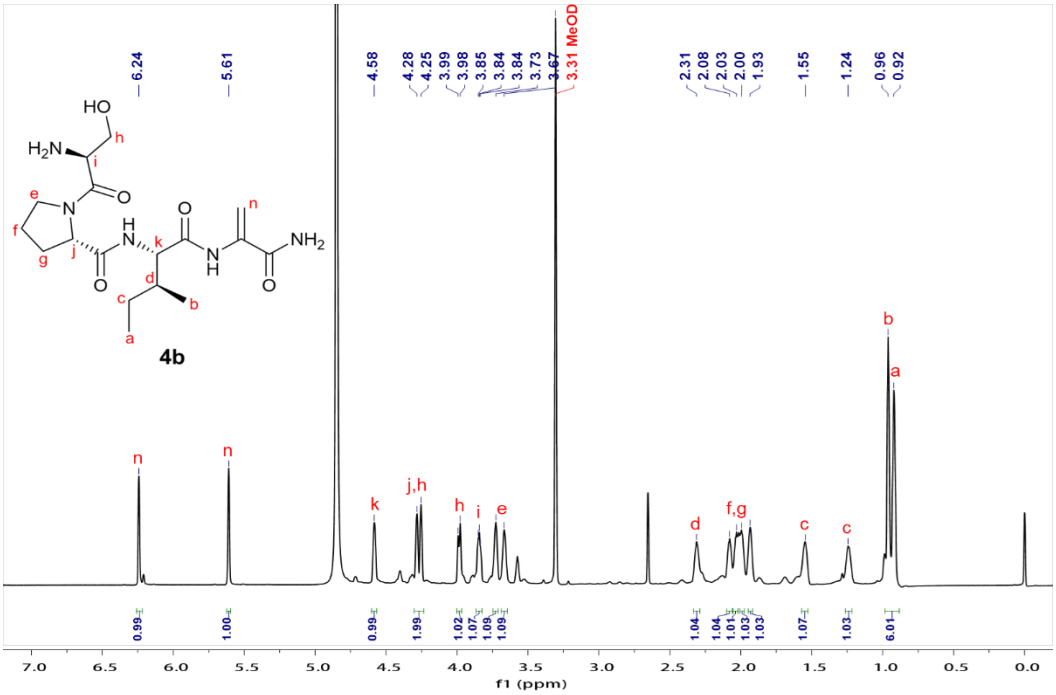


Fig. S59. ^{1}H NMR (800 MHz, CD_3OD) of peptide **4b**.

Peptide **5b**

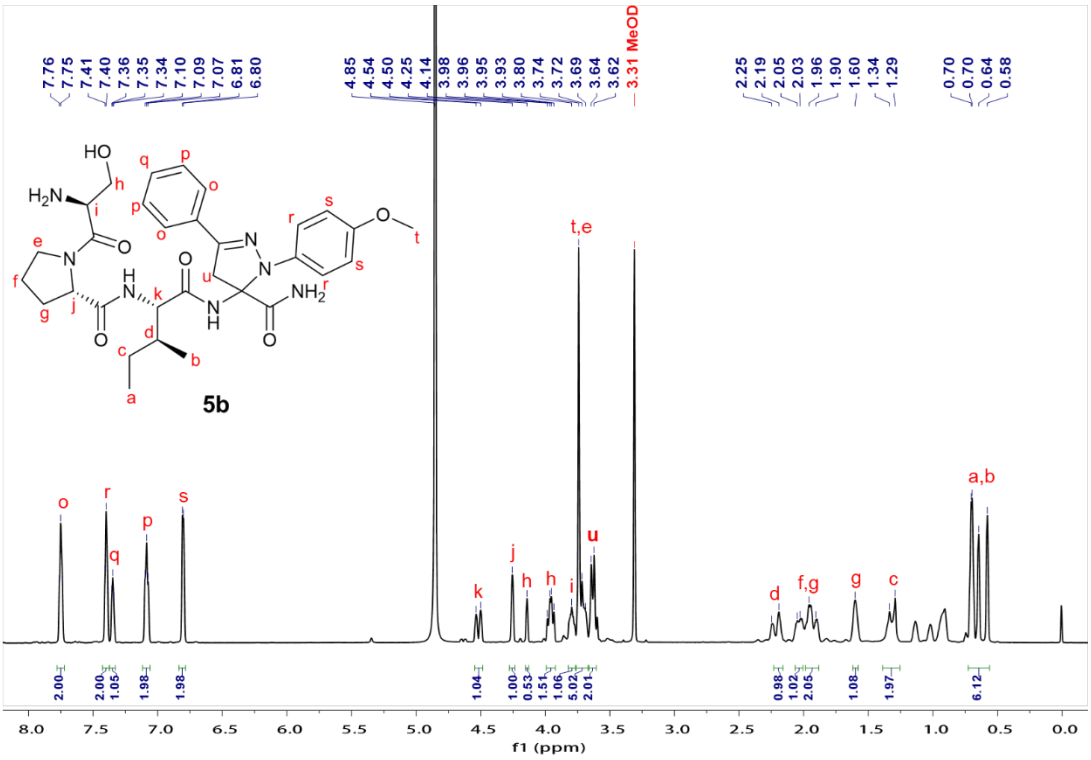
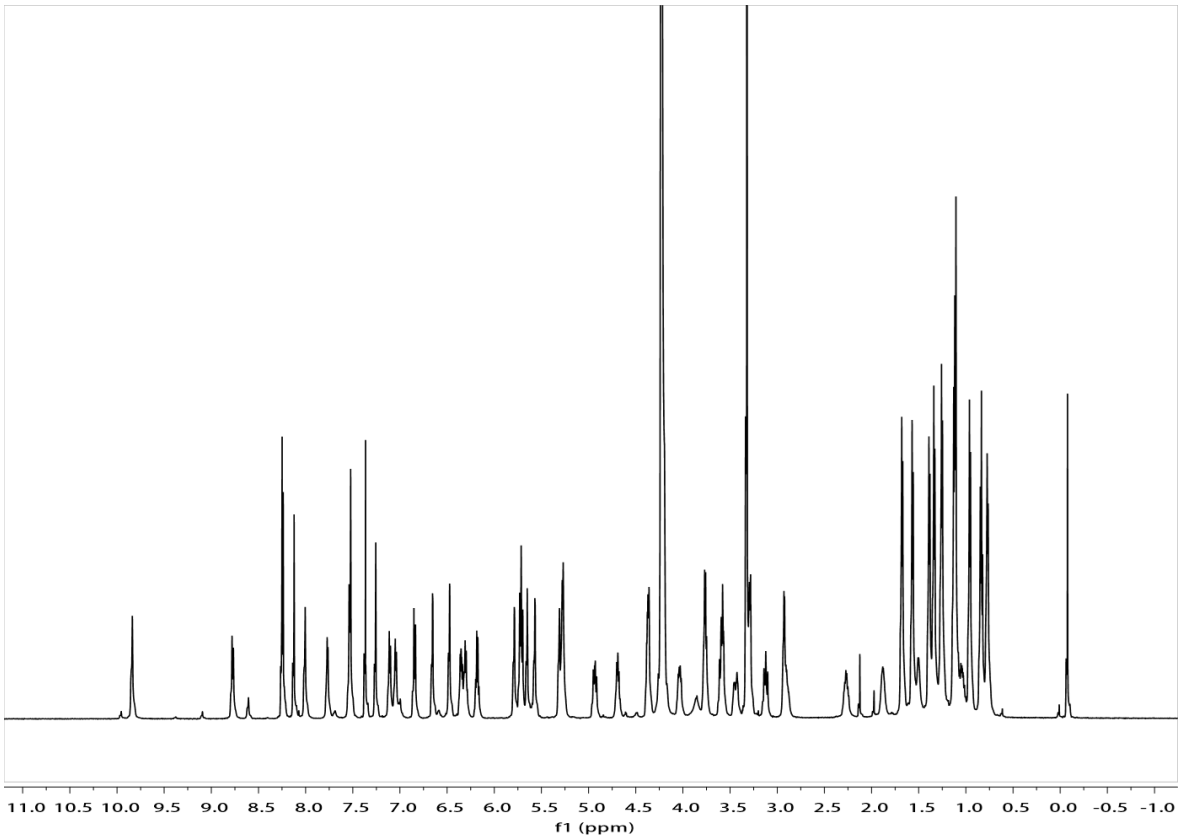


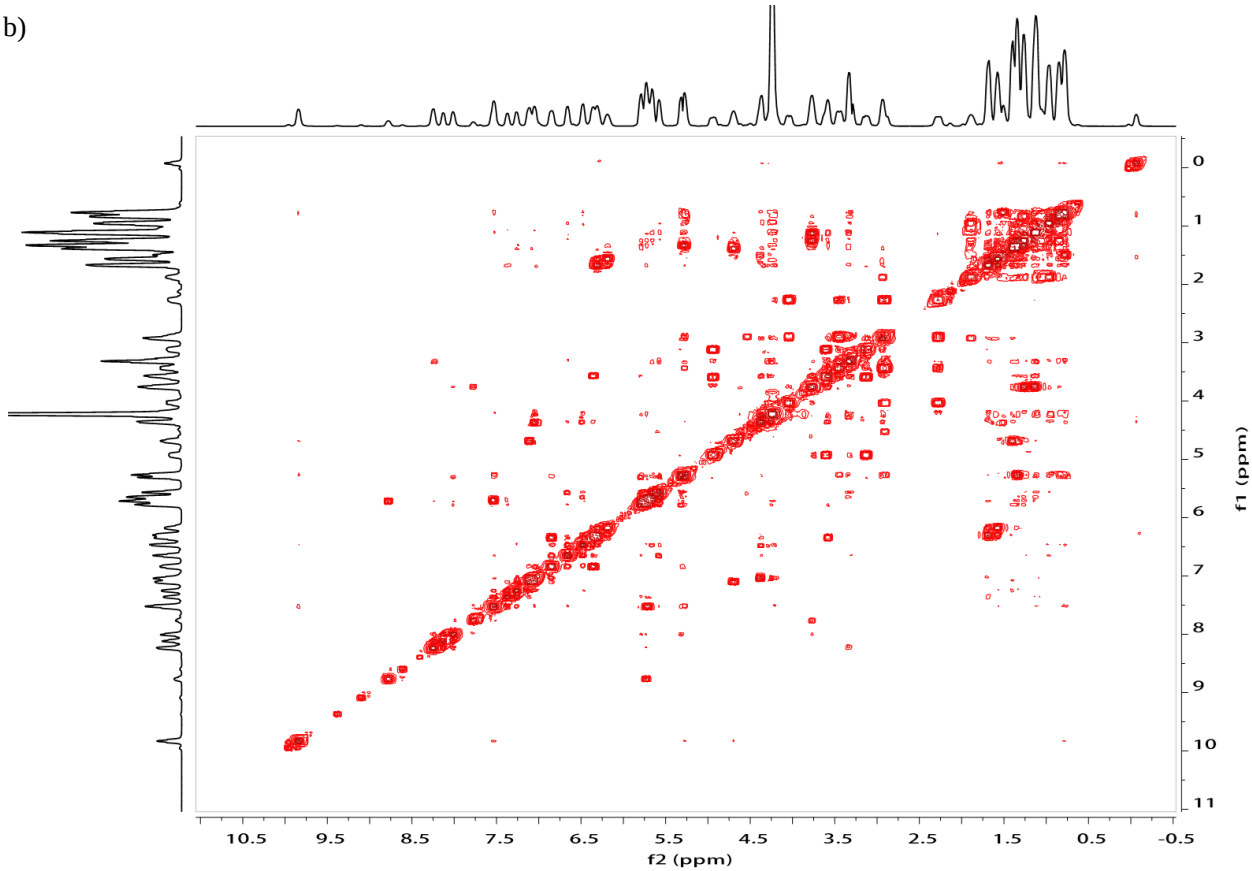
Fig. S60. ^{1}H NMR (800 MHz, CD_3OD) of peptide **5b**.

Thiostrepton

a)



b)



c)

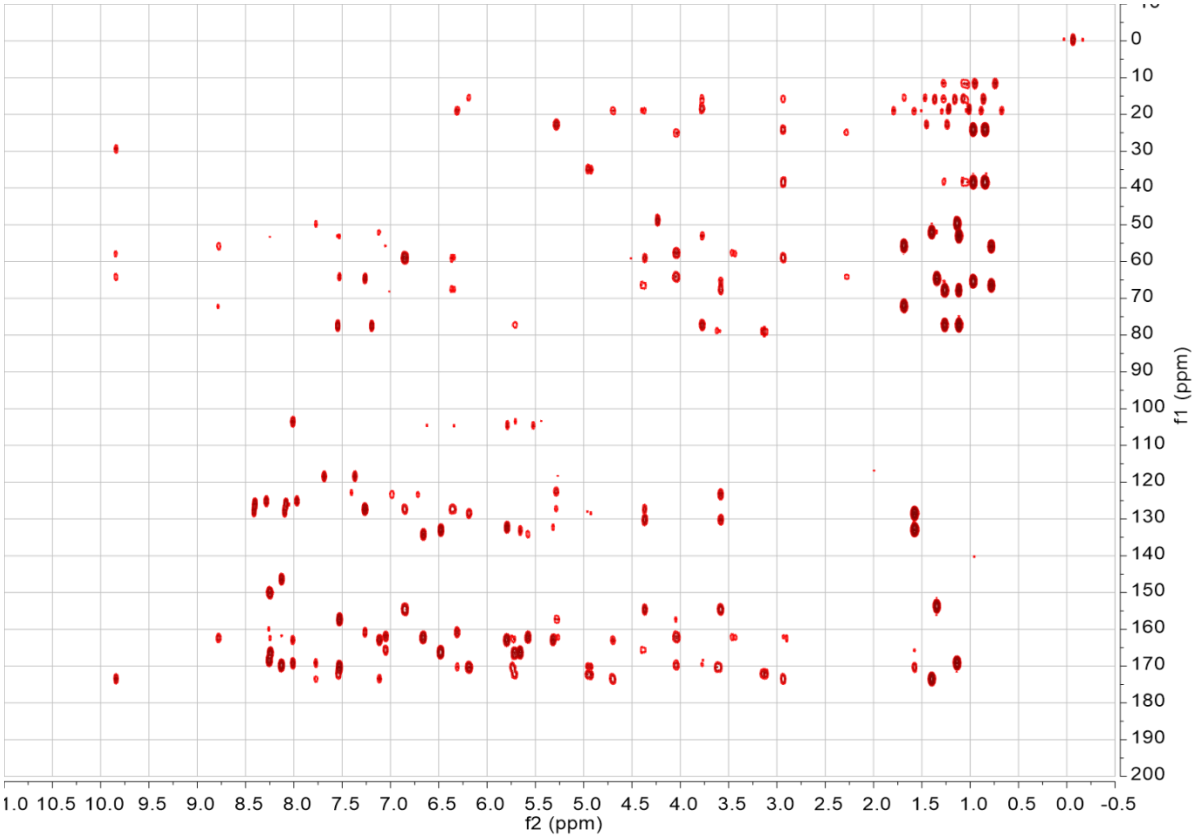
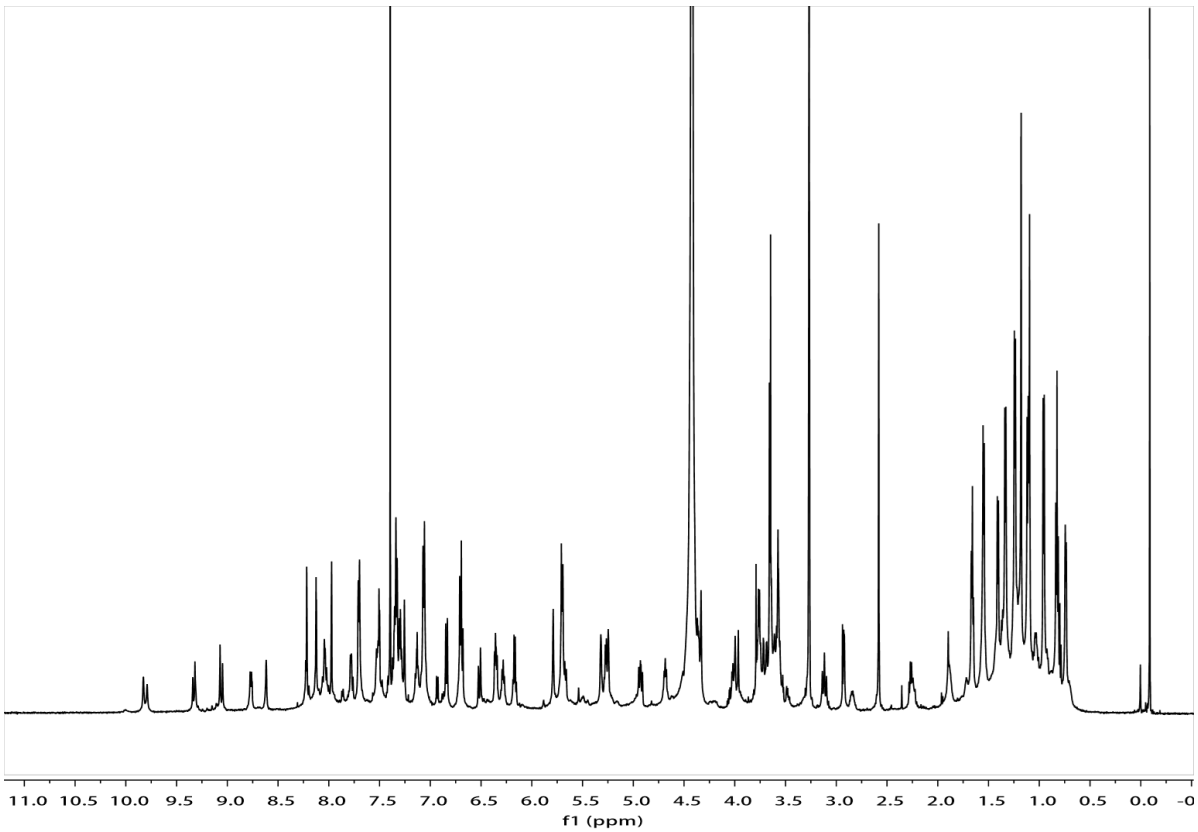
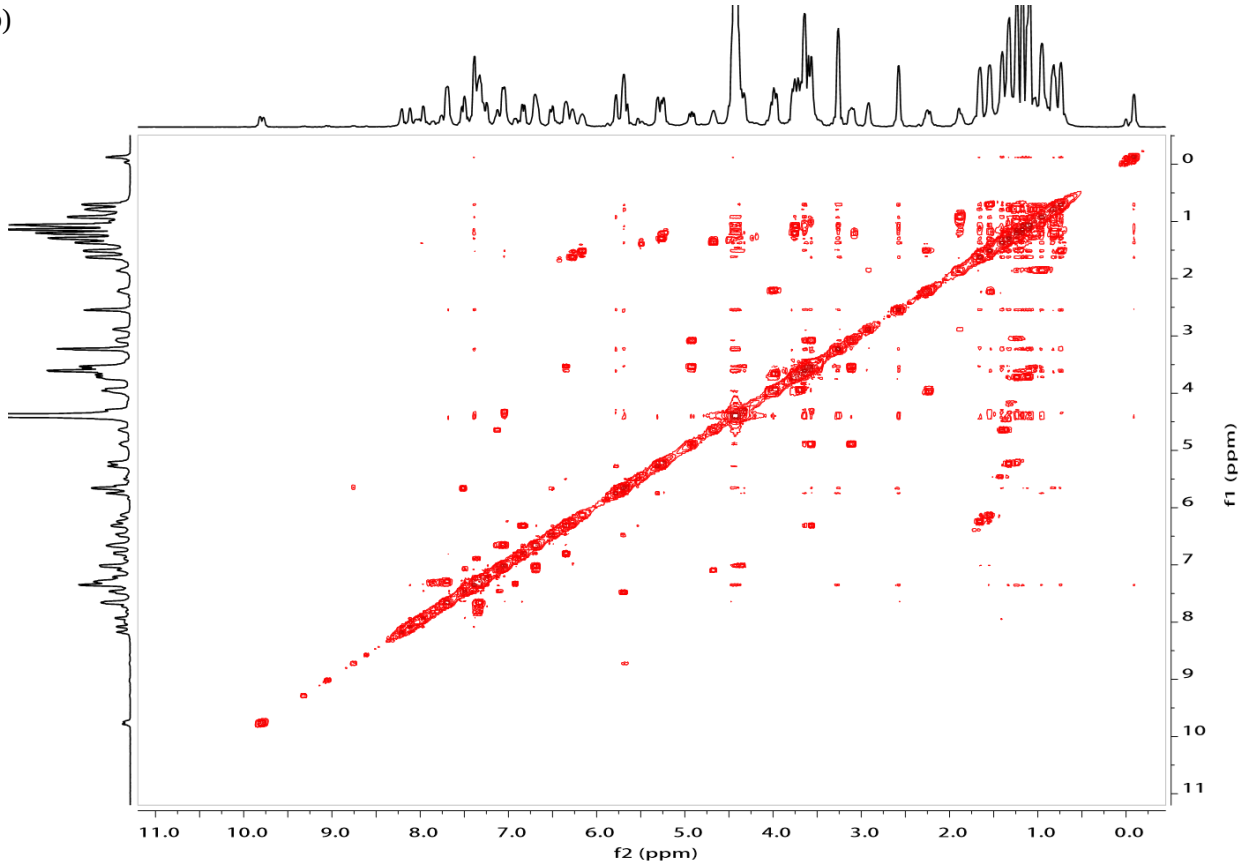


Fig. S61. a) ^{1}H NMR (600 MHz, $CDCl_3$/MeOD 3:1) of thiostrepton; b) ^{1}H-^{1}H COSY NMR of thiostrepton; c) ^{1}H-^{13}C HMBC NMR of thiostrepton.

a)


11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0
f1 (ppm)

b)


11.0 10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0.0
f2 (ppm)
0 1 2 3 4 5 6 7 8 9 10 11
f1 (ppm)

c)

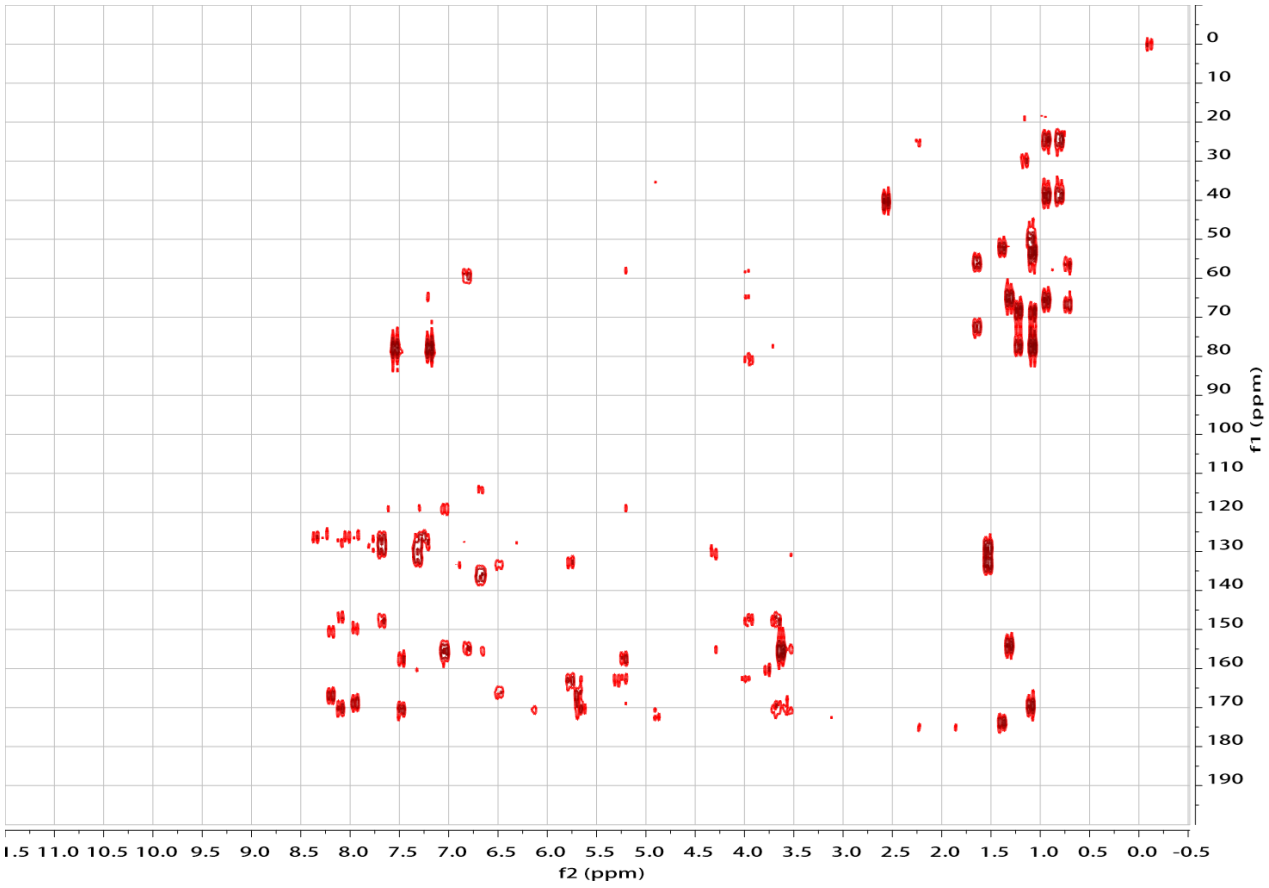
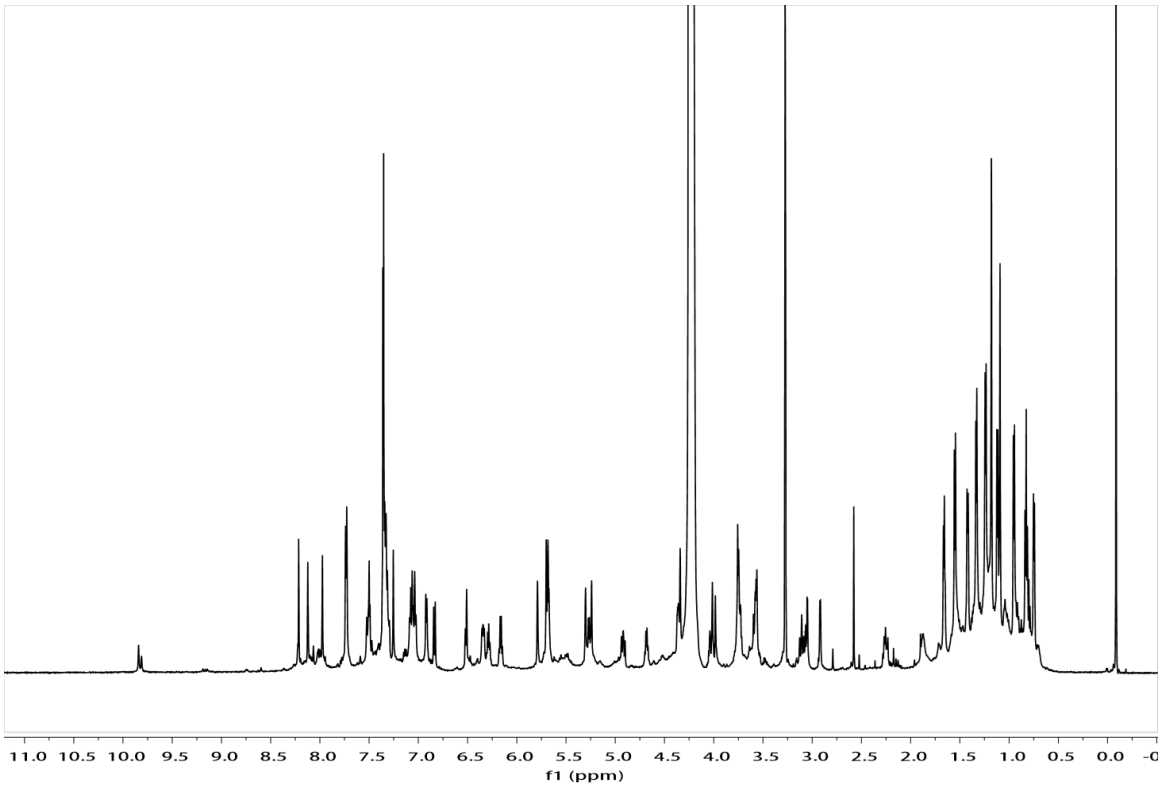
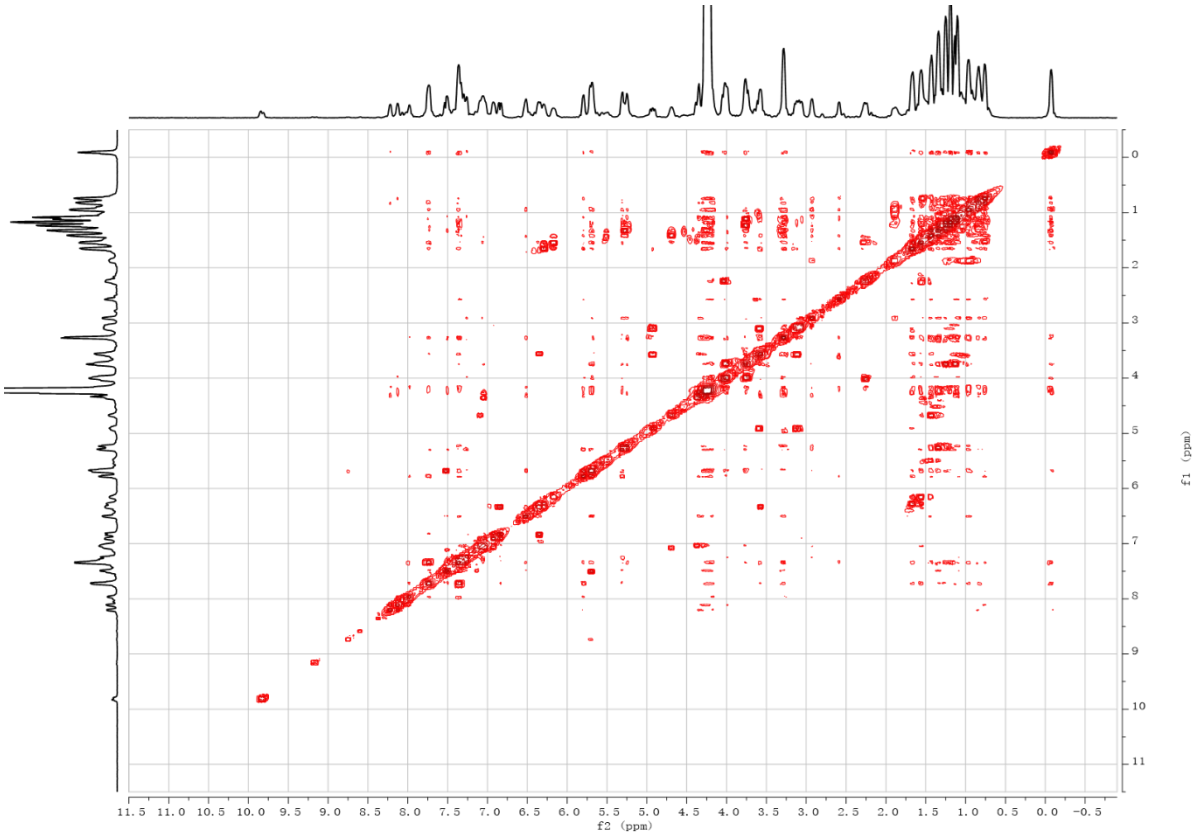


Fig. S62. a) ^{1}H NMR (600 MHz, $CDCl_3$/MeOD 3:1) of **7a**; b) ^{1}H-^{1}H COSY NMR of **7a**; c) ^{1}H-^{13}C HMBC NMR of **7a**.

a)



b)



c)

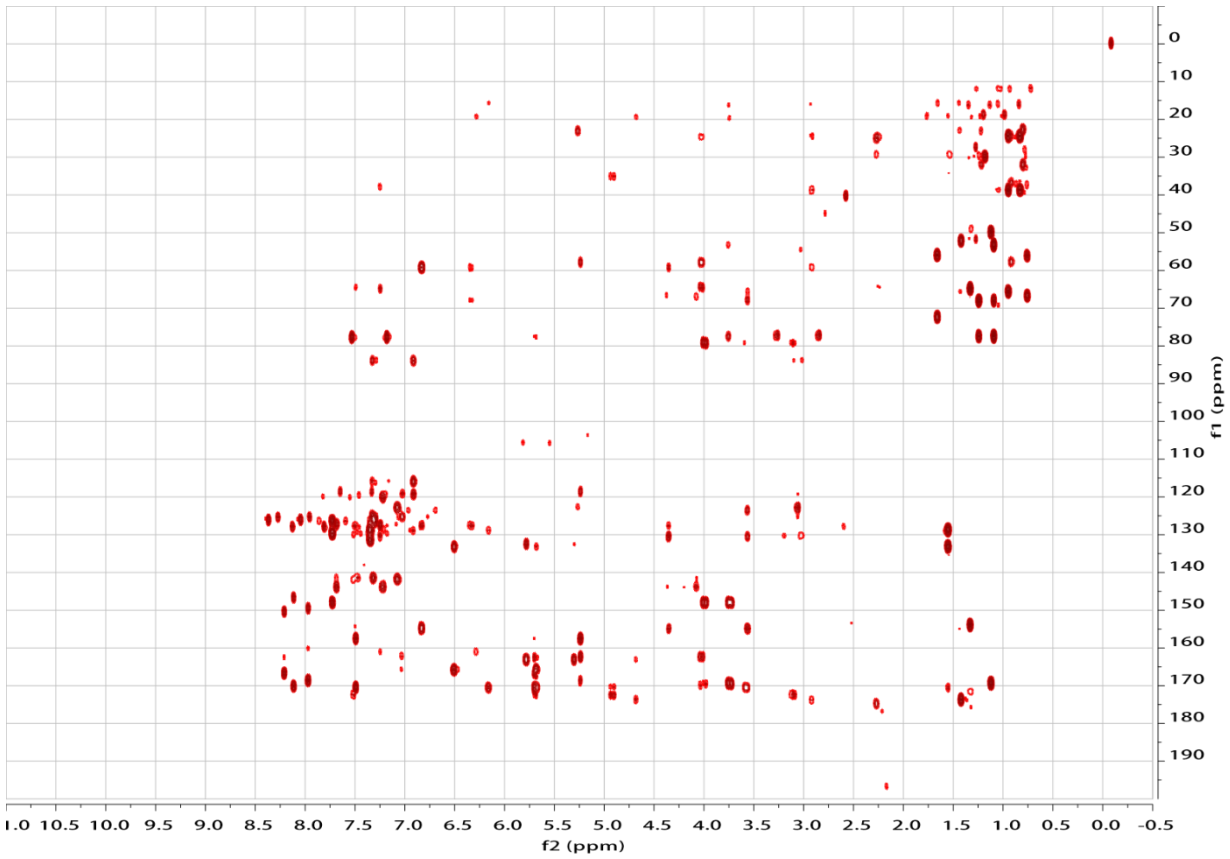


Fig. S63. a) ^{1}H NMR (600 MHz, $CDCl_3$/MeOD 3:1) of **7b**; b) ^{1}H-^{1}H COSY NMR of **7b**; c) ^{1}H-^{13}C HMBC NMR of **7b**.

Mass Spectra of small molecules

Compound **1a**

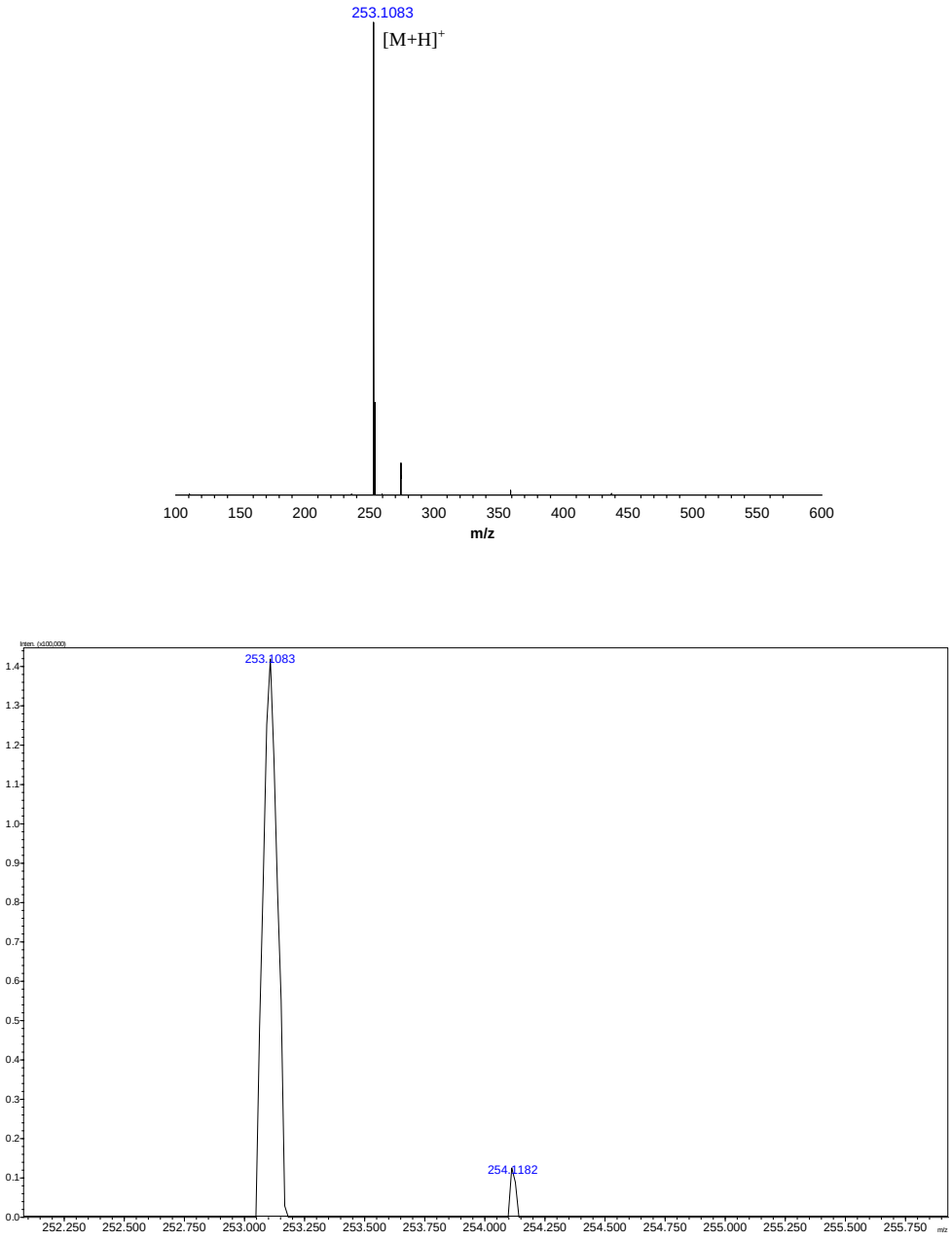


Fig. S64. High resolution mass spectrum (HRMS) of compound **1a.** ESI-IT/TOF MS: m/z calculated for $C_{14}H_{12}N_4O$: 253.1084, $[M+H]^+$. Found: 253.1083, $[M+H]^+$.

Compound **1b**

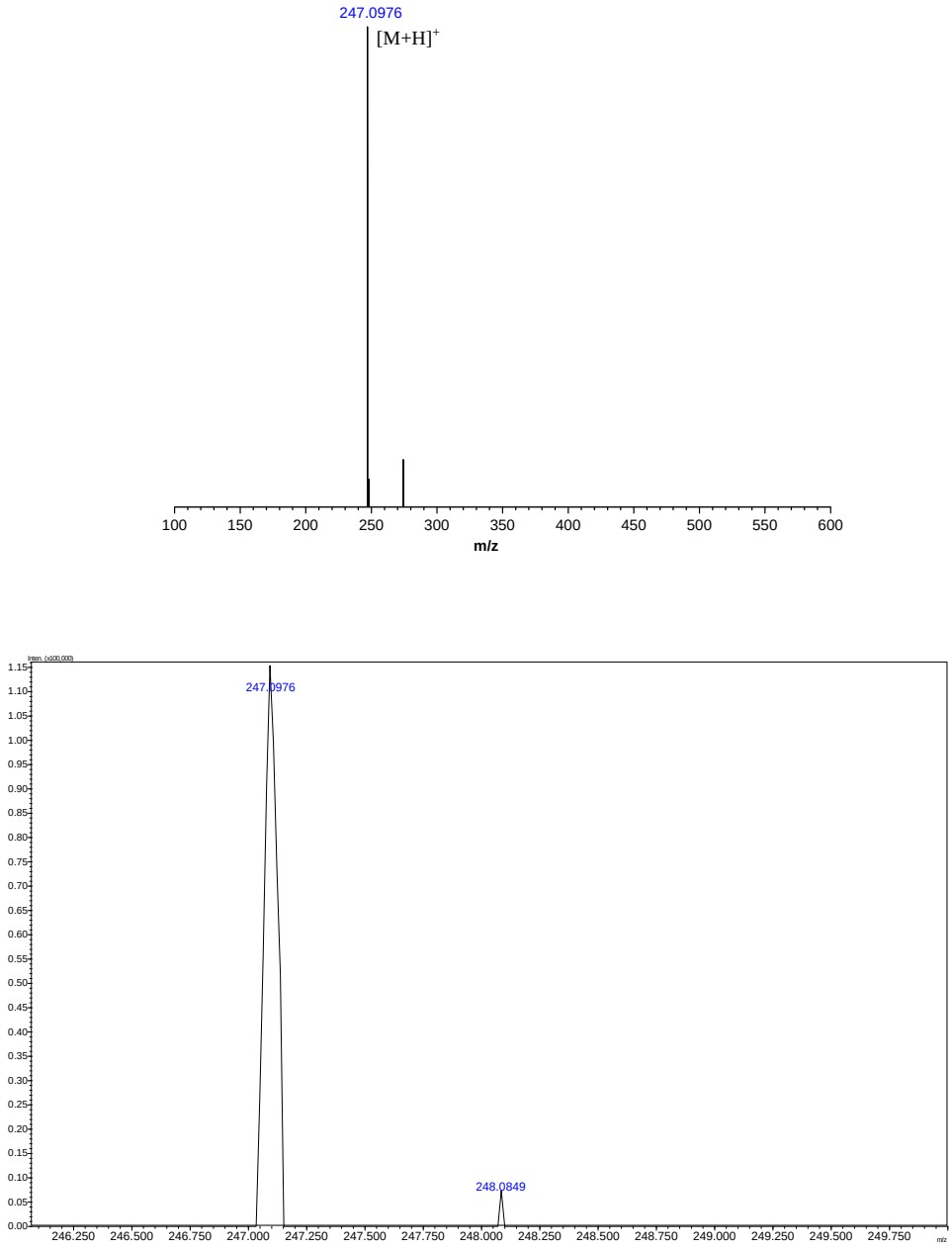


Fig. S65. High resolution mass spectrum (HRMS) of compound **1b.** ESI-IT/TOF MS: m/z calculated for $C_{15}H_{10}N_4$: 247.0978, $[M+H]^+$. Found: 247.0976, $[M+H]^+$.

Compound **2-4**

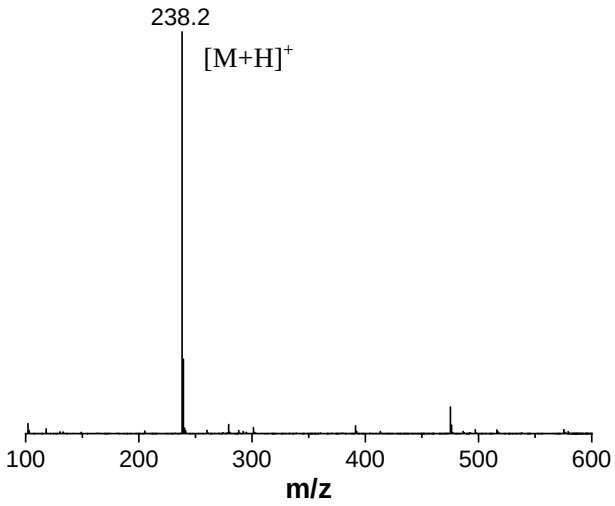
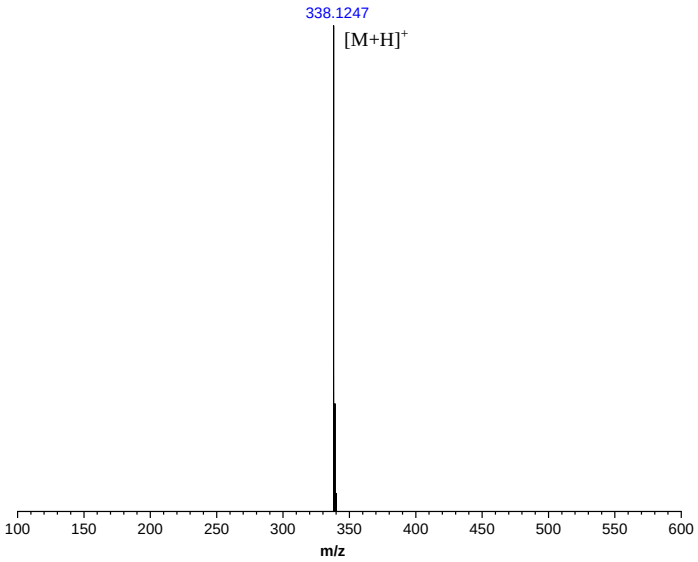


Fig. S66. Mass spectrum of compound **2-4.** ESI-MS: m/z calculated for $C_{13}H_{11}N_5$: 238.1, $[M+H]^+$. Found: 238.2, $[M+H]^+$.

Compound **2-5**



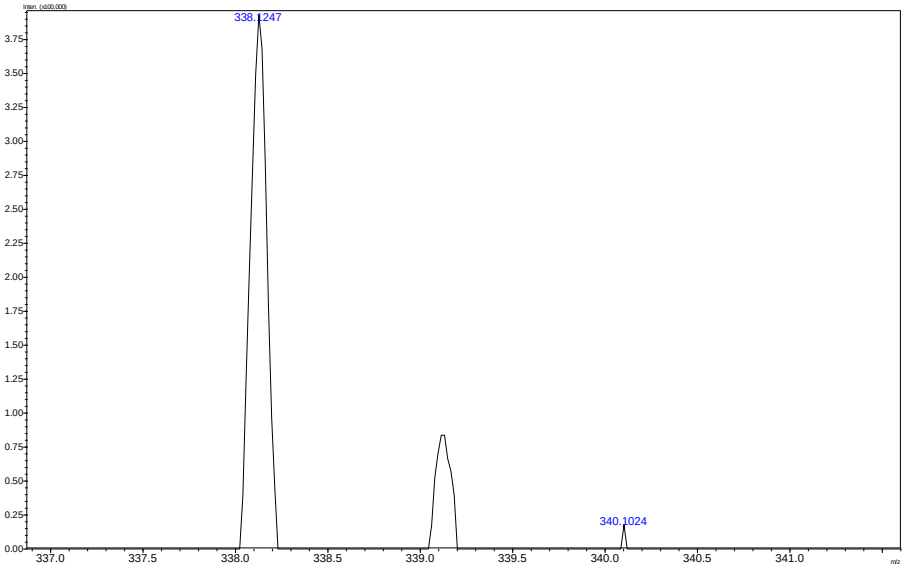
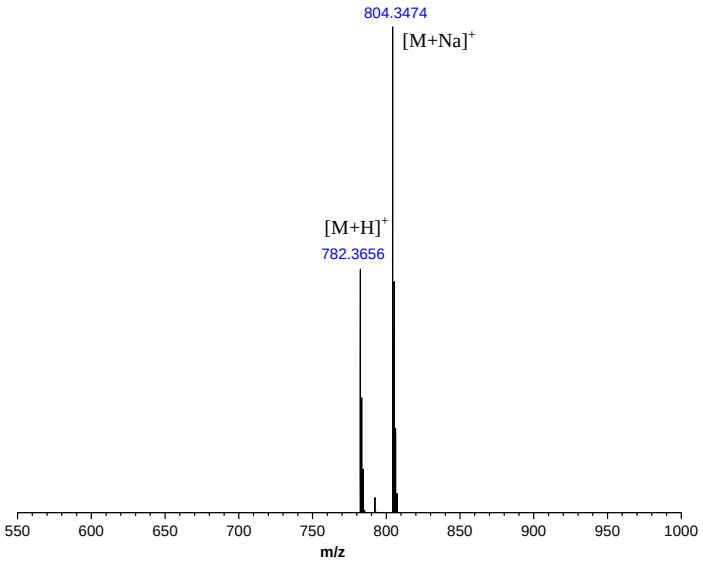


Fig. S67. High resolution mass spectrum (HRMS) of compound **2-5.** ESI-IT/TOF MS: m/z calculated for $C_{17}H_{15}N_5O_3$: 338.1248, $[M+H]^+$. Found: 338.1247, $[M+H]^+$.

Compound **1c**



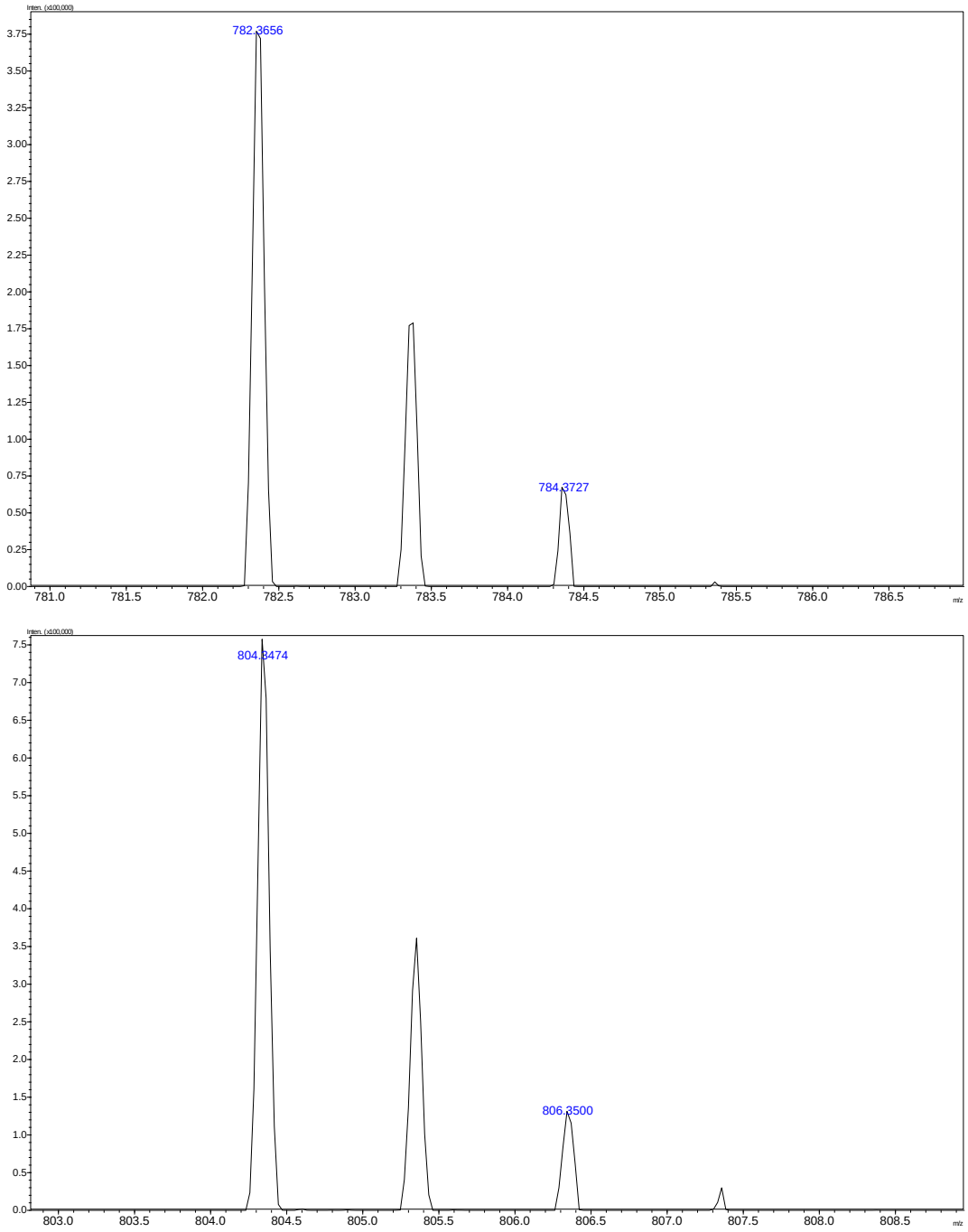


Fig. S68. High resolution mass spectrum (HRMS) of compound **1c.** ESI-IT/TOF MS: m/z calculated for $C_{37}H_{51}N_9O_8S$: 782.3654, $[M+H]^+$; 804.3474, $[M+Na]^+$. Found: 782.3656, $[M+H]^+$; 804.3474, $[M+Na]^+$.

Compound **1d**

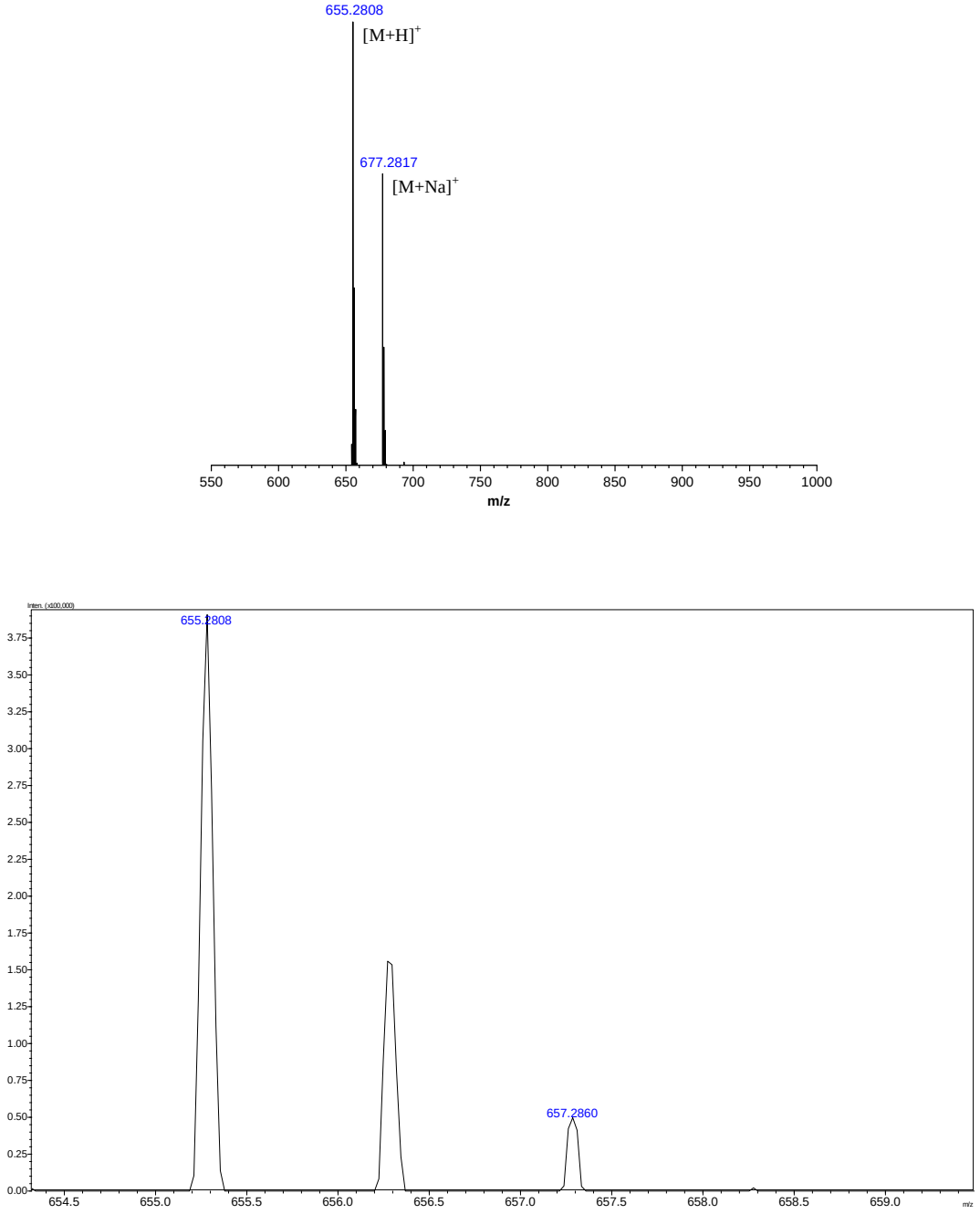


Fig. S69. High resolution mass spectrum (HRMS) of compound **1d.** ESI-IT/TOF MS: m/z calculated for $C_{34}H_{38}N_8O_4S$: 655.2809, $[M+H]^+$; 677.2629, $[M+Na]^+$. Found: 655.2808, $[M+H]^+$; 677.2817, $[M+Na]^+$.

Compound **2a**

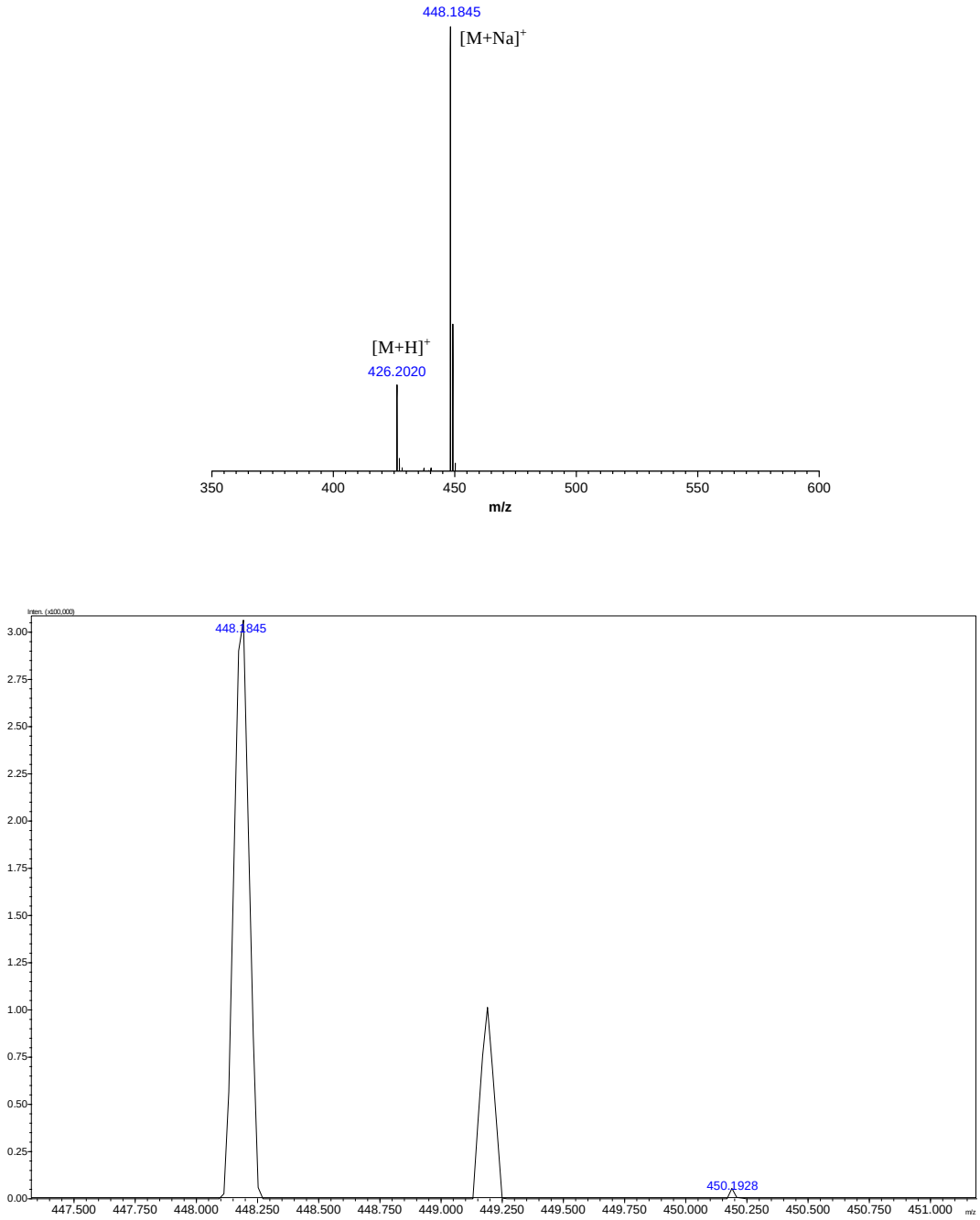


Fig. S70. High resolution mass spectrum (HRMS) of compound **2a.** ESI-IT/TOF MS: m/z calculated for $C_{23}H_{27}N_3O_5$: 426.2023, $[M+H]^+$; 448.1843, $[M+Na]^+$. Found: 426.2020, $[M+H]^+$; 448.1845, $[M+Na]^+$.

Compound **2b**

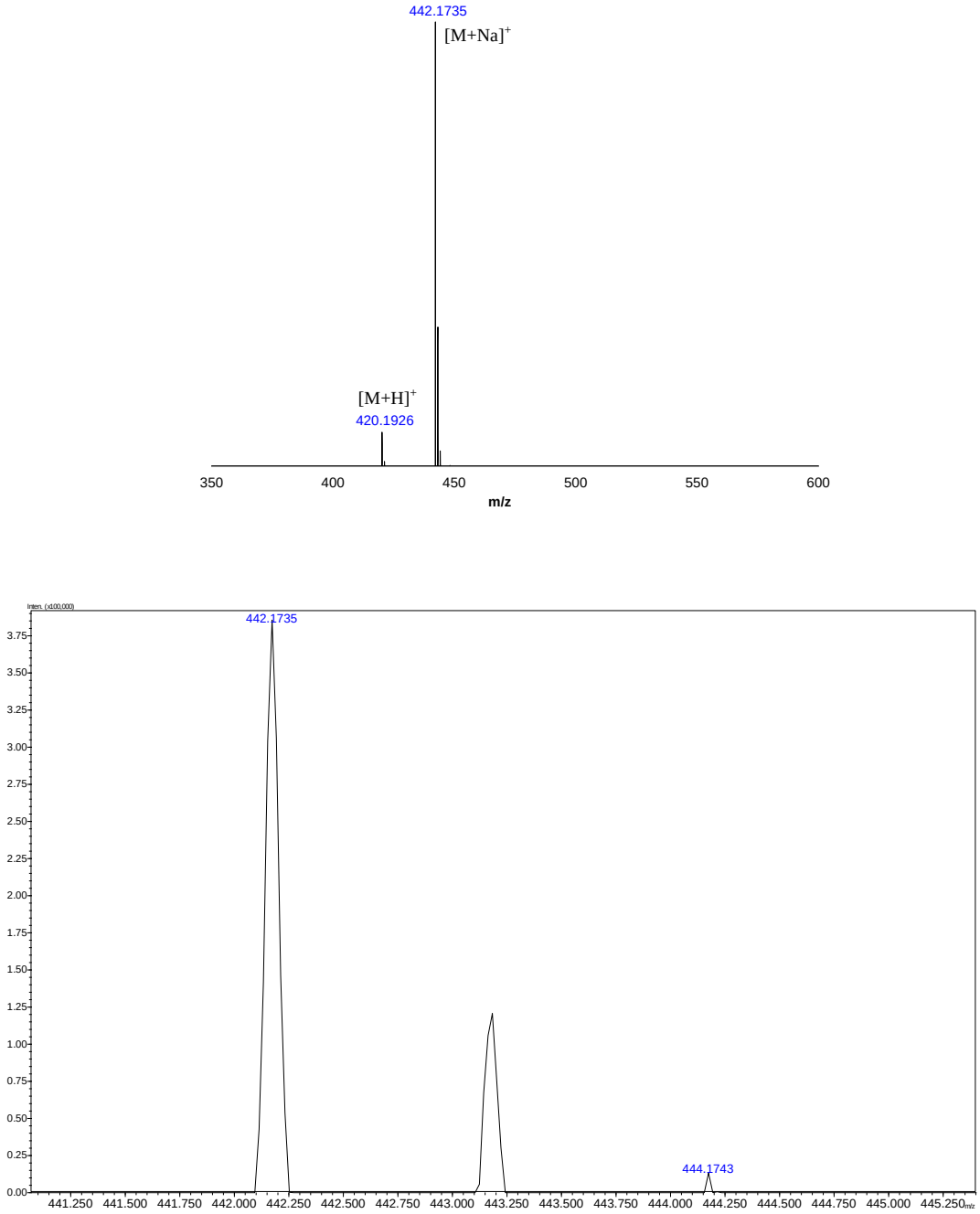


Fig. S71. High resolution mass spectrum (HRMS) of compound **2b.** ESI-IT/TOF MS: m/z calculated for $C_{24}H_{25}N_3O_4$: 420.1918, $[M+H]^+$; 442.1737, $[M+Na]^+$. Found: 420.1926, $[M+H]^+$; 442.1735, $[M+Na]^+$.

Biotin-PEG$_3$-N$_3$

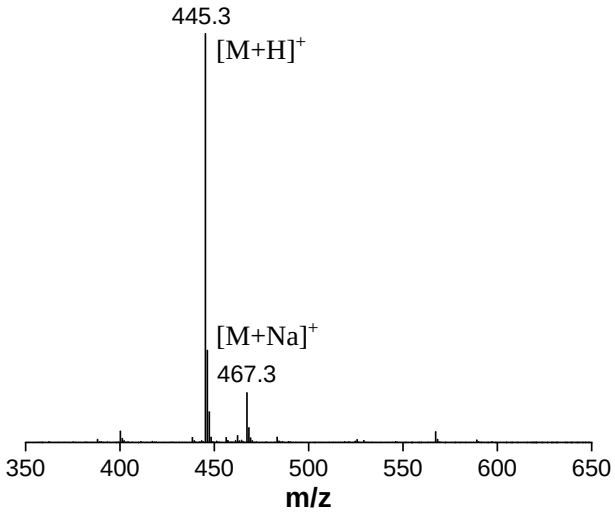


Fig. S72. Mass spectrum of **biotin-PEG$_3$-N$_3$.** ESI-MS: m/z calculated for $C_{18}H_{32}N_6O_5S$: 445.2, $[M+H]^+$; 467.2, $[M+Na]^+$. Found: 445.3, $[M+H]^+$; 467.3, $[M+Na]^+$.

Fluorescein-PEG$_2$-N$_3$

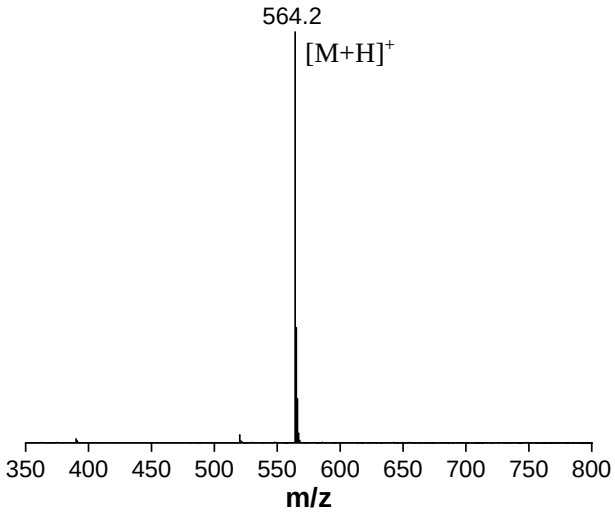


Fig. S73. Mass spectrum of compound **fluorescein-PEG$_2$-N$_3$.** ESI-MS: m/z calculated for $C_{27}H_{25}N_5O_7S$: 564.2, $[M+H]^+$. Found: 564.2, $[M+H]^+$.

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