Supplementary Materials for

Photoredox C-H Functionalization Leads the Site-selective Phenylalanine Bioconjugation

Yue Weng[2]; Chun-Jen Su[3]; Haoyang Jiang[2]; Chien-Wei Chiang[1*]

correspondence to: cwchiang@scu.edu.tw (C.-W. C.)

This PDF file includes:

General Considerations

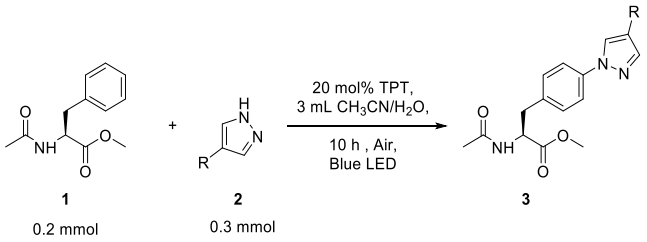
Unless otherwise stated, analytical grade solvents and commercially available reagents were used without further purification. Amino acids were bought at Energy chemistry; biotin, phenylboronic acid, pyrazole and 4-I-1-H-pyrazole was purchased from Bide Pharmatech; porcine insulin was bought at Aladdin. Pyrazoles were generally prepared according to literature procedures.(*1*) (*2*) All manipulations were carried out by using standard Schlenk techniques. Thin layer chromatography (TLC) employed glass 0.25 mm silica gel plates. Flash chromatography columns were packed with 200−300 mesh silica gel in hexane/ ethyl acetate. Gradient flash chromatography was conducted eluting with a continuous gradient from petroleum ether to dichloromethane.

^{1}H-, ^{13}C-, and ^{19}F-NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. For ^{1}H-NMR, chemical shifts (δ) were given in ppm relatives to internal standard (TMS at 0 ppm, $CDCl_3$ at 7.26 ppm, DMSO-d_6 at 49.00 ppm). For ^{13}C-NMR, chemical shifts (δ) were reported in ppm using solvent as internal standard ($CDCl_3$ at 77.16 ppm, MeOH-d_4 at 49.00 ppm, Acetone-d_6 at 206.26 ppm). Data are reported as: s = singlet, d = doublet, t = triplet, q = quartet, p = pentet, m = multiplet, br = broad; coupling constants in Hz; integration.

High resolution mass spectra (HRMS) were obtained by use of a Bruker Compact TOF mass spectrometer in electrospray ionization mode (ESI+). All of the MALDI-TOF-MS and MALDI-TOF-MS/MS spectra were acquired using 5800 MALDI-MS (AB SCIEX, Concord, Canada) equipped with a 355 nm Nd: YAG laser in the reflector positive mode. Samples of 0.6 μ L mixed with 0.6 μ L freshly prepared CHCA matrix were directly loaded onto the stainless steel MALDI plate and allowed to dry in a gentle stream of warm air. Samples were ablated with a power of 3500 while the laser rastered over the target surface. A total of 2000 laser shots were employed in each sample spot. The MS and MS/MS data processing was further performed by DataExplorer 4.0 (AB SCIEX, Concord, Canada).

Supplementary Methods

1. General procedure for bioconjugation of phenylalanine and pyrazoles

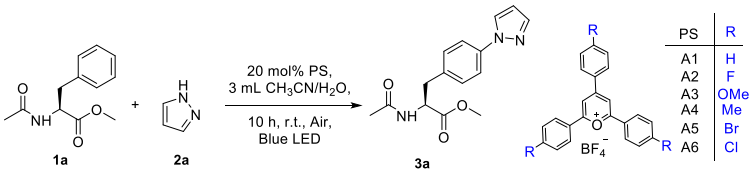


To a solution of methyl acetyl-L-phenylalaninate **1** (0.2 mmol, 1 equiv., 44.2 mg) in CH_3CN/H_2O (1.5 mL/1.5 mL) was added pyrazoles **2** (0.3 mmol, 1.5 equiv.) in presence of photosensitizer $TPT^{+}BF_4^{-}$ (0.04 mmol, 20 mol%, 15.8 mg) under air atmosphere and irradiated by 3W blue LEDs at 25 ˚C for 10 h. After completion of the reaction, the solvents were removed under reduced pressure by rotary evaporation to give a residue, which was then purified by column chromatography on silica gel (hexane: ethyl acetate mixtures to afford the product **3**.

Purification and characterization of methyl (S)-3-(4-(1H-pyrazol-1-yl)phenyl)-2-acetamidopropanoate (3a)

Methyl acetyl-L-phenylalanine **1** was reacted according to the above procedure with pyrazole **2a** (0.3 mmol, 20.4 mg), and $TPT^{+}BF_4^{-}$. The crude reaction mixture was plugged through silica gel and concentrated. Suitable crystals of **3a** for structure determination were obtained by crystallized from CH_2Cl_2/hexane in 28% yield. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.90 (d, J = 2.2 Hz, 1H), 7.72 (d, J = 1.6 Hz, 1H), 7.63 (d, J = 8.5 Hz, 2H), 7.18 (d, J = 8.5 Hz, 2H), 6.47 (dd, J = 2.2, 1.6 Hz, 1H), 4.93 – 4.89 (m, 1H), 3.74 (s, 3H), 3.23 – 3.11 (m, 2H), 2.01 (s, 3H) ppm. ^{13}C-NMR (101 MHz, $CDCl_3$) δ 172.07, 169.82, 141.20, 139.30, 134.25, 130.35, 126.79, 119.37, 107.77, 53.21, 52.55, 37.37, 23.23 ppm. (Figure S15) m/z HRMS(ESI) found $[M+Na]^+$ 310.1170, $C_{15}H_{17}N_3O_3Na^+$ requires 310.1162. (Figure S1A)

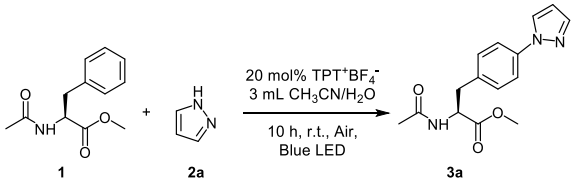
Table S1. Investigation of photosensitizers[a]



Entry	PS	[b]Conv. of **1a**	Yield of **3a**
1	Acr^{+}-$MesClO_4^{-}$	-	n.r.
2	Eosin Y	-	n.r.
3	$Ir(ppy)_3$	-	n.r.
4	$Ru(bpy)_3PF_6$	-	n.r.
5	A1	99 %	28 %
6	A2	42 %	6 %
7	A3	55 %	11 %
8	A4	5 %	n.d.
9	A5	6 %	n.d.
10	A6	23 %	8 %

[a]Conditions: a CH_3CN/H_2O (1.5 mL/1.5 mL) solution of **1**, **2a** and **PS** was irradiated by blue LED for 10 h in the atmosphere. [b]Conversions and yields were determined by FID-GC, using biphenyl as the internal standard.

Table S2. Control experiments

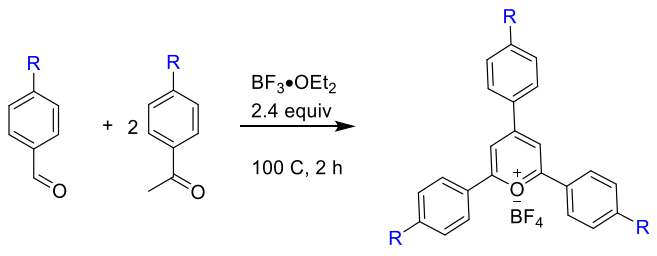


1 2a 3a

Entry	Variations from "standard condition[a]"	Conv. of **1**[b]	Yield of **3a**[b]
1	no light	5 %	2 %
2	no **2a**	60 %	n.d.
3	no phenlyalaine	-	n.d.
4	N_2 instead of Air	5 %	n.d.
5	no $TPT^+BF_4^-$	0 %	n.d.
6	-	99 %	28 %

[a]Standard conditions: a CH_3CN/H_2O (1.5 mL/1.5 mL) solution of **1**, **2a** and **PS** was irradiated by blue LED for 10 h in the atmosphere. [b]Conversions and yields were determined by FID-GC, using biphenyl as the internal standard.

2. Chemical synthesis of photosensitizer $TPT^+BF_4^-$



Catalyst	R
A1	H
A2	F
A3	OMe
A4	Me
A5	Br
A6	Cl

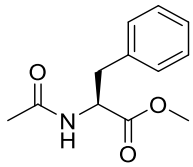
According to the literature procedure,(*3*) BF_3•OEt_2 is slowly added to a mixture of the carbonyl compounds (if starting materials are solids, they are dissolved in a small amount of toluene). After heating for 2 h at 100 ºC the thus formed diethyl ether is evaporated and the oily product dissolved in acetone. Upon the addition of diethyl ether the product precipitates. Purfication is performed by multiple recrystallization from acetone.

3. General procedure of protected amino acid(*4*)

In an oven-dried round-bottom flask (100mL) amino acid (20 mmol) was dissolved in anhydrous MeOH (40 mL). Stirred, cooled to 0 °C. Carefully added (2.8 mL, 40 mmol) $SOCl_2$ to the solution. The reaction mixture was then warmed to room temperature and stirred overnight. The solvent was removed by rotary evaporation to afford amino ester hydrochloride residue. Without further purification, the residue and triethyl amine (5.6 mL, 40 mmol) was added in anhydrous DCM (40 mL) and stirred for 15 min at 0 °C. Acetyl chloride (1.4 mL, 20 mmol) was added to the reaction solution dropwisely. Stirring was continued for 12 h while allowing the mixture to warm up to r.t.. The reaction was washed with saturated $NaHCO_3$ solutions (50 mL×2) and 10% HCl (50 mL×1) to remove any unreacted starting material. The combined organic extracts were dried ($MgSO_4$), filtered,

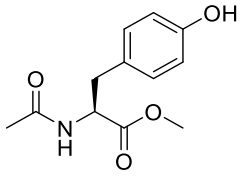
and concentrated in vacuo. Purification of the residue by flash chromatography (SiO_2, hexane:ethyl acetate mixtures) led to the desired protected amino acid.

methyl acetyl-L-phenylalaninate (1)



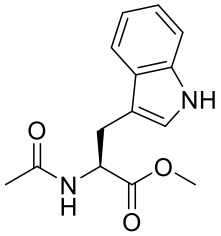
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.38 (d, J = 7.7 Hz, 1H), 7.35 – 7.15 (m, 5H), 4.45 (m, 1H), 3.42 (s, 3H), 3.01 (dd, J = 13.7, 5.6 Hz, 1H), 2.87 (dd, J = 13.7, 9.4 Hz, 1H), 1.79 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 171.28 (d, J = 283.9 Hz), 137.71, 129.47, 128.71, 127.01, 54.11, 52.26, 37.18, 22.68 ppm. (Figure S16)

methyl acetyl-L-tyrosinate



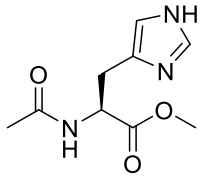
^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.30 (d, J = 7.7 Hz, 1H), 6.99 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.5 Hz, 2H), 4.38 – 4.30 (m, 1H), 3.57 (s, 3H), 2.87 (dd, J = 13.8, 5.8 Hz, 1H), 2.74 (dd, J = 13.8, 9.1 Hz, 1H), 1.79 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.83, 169.84, 156.42, 130.41, 127.67, 115.49, 54.47, 52.20, 36.49, 22.70 ppm. (Figure S17)

methyl acetyl-L-tryptophanate



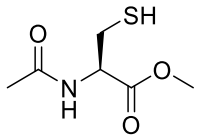
^{1}H NMR (400 MHz, DMSO-d_6) δ 10.88 (s, 1H), 8.35 (d, J = 7.5 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 8.1 Hz, 1H), 7.16 (s, 1H), 7.08 (t, J = 8.0 Hz, 1H), 7.00 (t, J = 7.8 Hz, 1H), 4.50 (td, J = 8.1, 5.8 Hz, 1H), 3.58 (s, 3H), 3.15 (dd, J = 14.6, 5.7 Hz, 1H), 3.03 (dd, J = 14.5, 8.5 Hz, 1H), 1.82 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 173.05, 169.95, 136.55, 127.50, 124.12, 121.47, 118.92, 118.43, 111.93, 109.95, 53.64, 52.26, 27.56, 22.76 ppm. (Figure S18)

methyl acetyl-L-histidinate



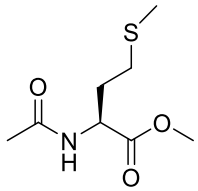
^{1}H NMR (400 MHz, DMSO-d_6) δ 8.31 (s, 1H), 7.55 (s, 1H), 6.82 (s, 1H), 4.46 (m, 1H), 3.60 (s, 3H), 2.88 (m, 2H), 1.83 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.74, 169.78, 135.38, 133.68, 116.89, 52.93, 52.21, 29.50, 22.78 ppm. (Figure S19)

methyl acetyl-L-cysteinate



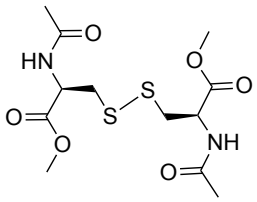
^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.42 (s, 1H), 4.89 (m, 1H), 3.79 (s, 3H), 3.01 (m, 2H), 2.06 (s, 3H), 1.34 (t, J = 9.0 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.59, 169.79, 53.49, 52.82, 26.85, 23.15 ppm. (Figure S20)

methyl acetyl-L-methioninate



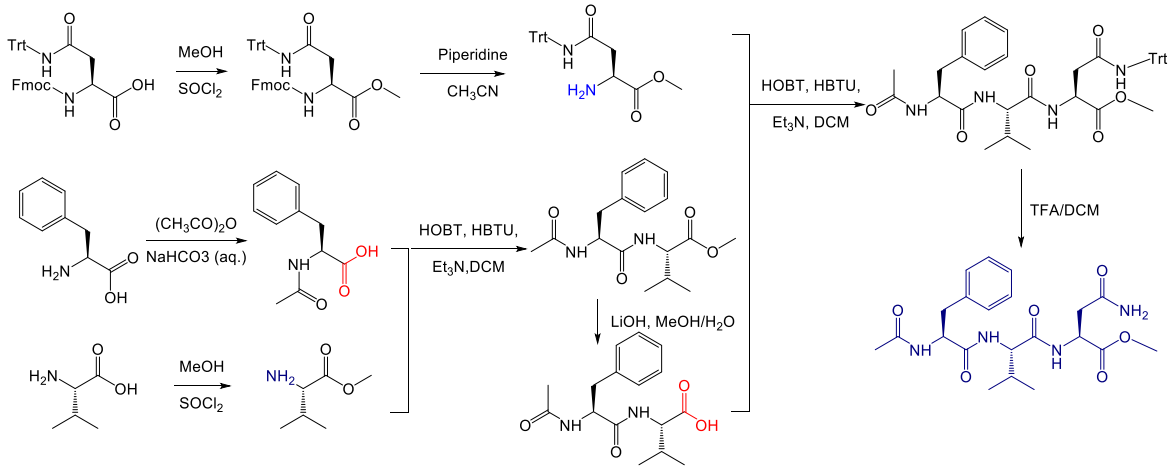
^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.68 (d, J = 8.0 Hz, 1H), 4.62 (m, 1H), 3.67 (s, 3H), 2.44 (t, J = 7.5 Hz, 2H), 2.06 (m, 1H), 2.01 (s, 3H), 1.95 (s, 3H), 1.89 (m, 1H) ppm. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 172.45, 170.03, 52.26, 51.26, 31.31, 29.74, 22.78, 15.18 ppm. (Figure S21)

methyl acetyl-L-cystinate

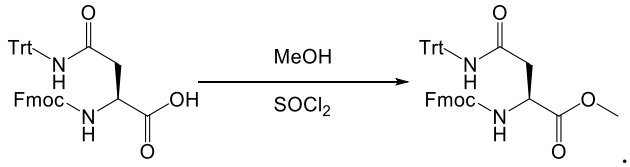


^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.71 (d, J = 7.6 Hz, 1H), 4.84 (m, 1H), 3.74 (s, 3H), 3.16 (m, 2H), 2.03 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.85, 170.11, 52.70, 51.60, 40.51, 22.97. (Figure S22)

4. Chemical synthesis procedure of tripeptide Ac-FVN-OMe(*5*)

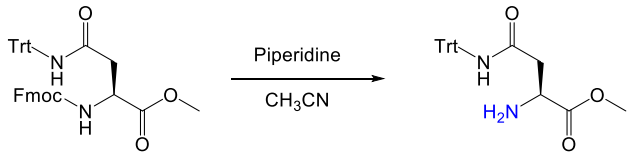


Step1



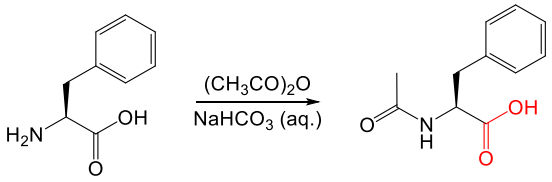
A solution of the N-Fomc-L-asn(N-trityl)-OH (10 mmol) in MeOH was stirred at 0 °C for 10 min and continuously with $SOCl_2$ for 5 min. Stirring was continued for 12 h while allowing the mixture to warm up to RT. The reaction mixture was concentrated in vacuo. Purification of the residue by flash chromatography (SiO_2, hexane:ethyl acetate mixtures) to afford N-Fomc-L-asn(N-trityl)-OMe.

Step2



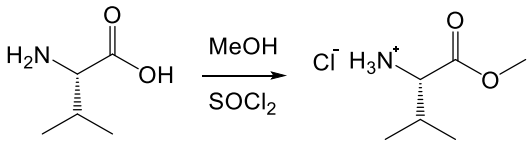
Piperidine (5 mL) was added to a solution of N-Fomc-L-asn(N-trityl)-OMe (9 mmol) in CH_3CN (50 mL), and the resulting mixture was stirred at 25 °C for 2 h to ensure complete removal of the Fmoc protecting group. After concentration in vacuo, the residuewas purified by flash chromatography (SiO_2, hexane:ethyl acetate mixtures).

Step3



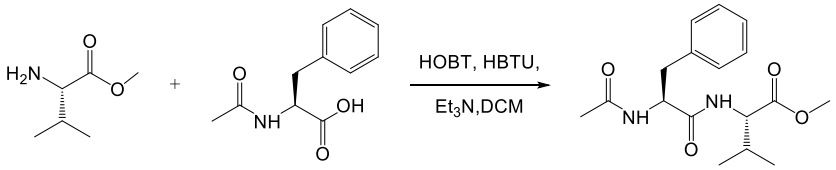
Acetic anhydride (12 mmol) was added continuously in 5 min to a solution of phenylalanine (10 mmol) in saturated $NaHCO_3$ solution. After stirring overnight, the reaction mixture was extracted by CH_2Cl_2, and washed with 10% HCl (50 mL×1) and water (50 mL×1) to remove any unreacted starting material. The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated in vacuo without further purification.

Step4



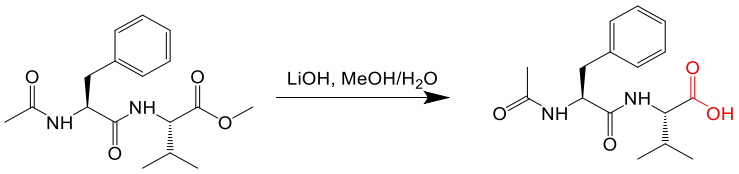
A solution of the valine (10 mmol) in MeOH was stirred at 0 °C for 10 min and continuously with $SOCl_2$ for 5 min. Stirring was continued for 12 h while allowing the mixture to warm up to RT. The reaction mixture was concentrated in vacuo without further purification.

Step5



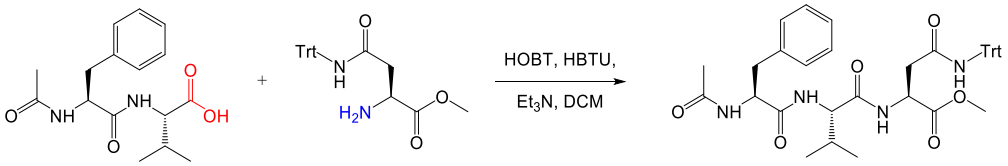
MeO-Val-$NH_3^+Cl^-$ (9 mmol) was dissolved in DMF (40 mL). In another flask, a solution of Ac-Phe-OH (9 mmol) in DMF (40 mL) was treated with HOBt (10 mmol) and HBTU (10 mmol). After 10 min, this mixture and Et_3N (21 mmol) were sequentially added to the above free amino ester. The reaction was stirred at 25 °C for 8 h. After regular workup, the resulting crude product was purified by flash chromatography (EtOAc/ hexanes) to afford linear dipeptide compound.

Step6



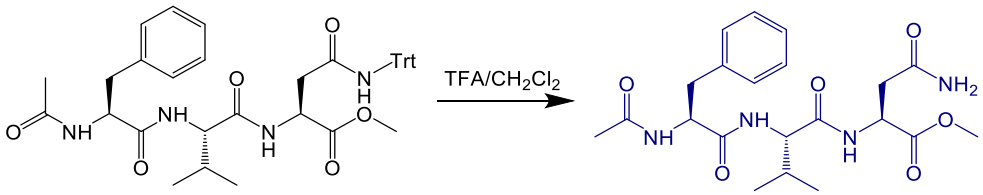
A solution of Ac -protected dipeptide ester（8 mmol） and LiOH（16 mmol） in 2:10:1 MeOH: THF: H_2O（50 mL） was stirred under cooling at room temperature overnight. The reaction solution was cooled to 0°C and acidified with 2 M HCl and then extracted with CH_2Cl_2 (50 mL ×2). The combined extracts were washed with brine (50 mL ×1), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the hydrolyzed product as white solid.

Step7



H_2N-asn(N-trityl)-OMe (5 mmol) was dissolved in DMF (20 mL). In another flask, a solution of Ac-Phe-Val-OH (5 mmol) in DMF (20 mL) was treated with HOBt (6 mmol) and HBTU (6 mmol). After 10 min, this mixture and Et_3N (11 mmol) were sequentially added to the above free amino ester. The reaction was stirred at 25 °C for 8 h. After regular workup, the resulting crude product was purified by flash chromatography (EtOAc/ hexanes) to afford Ac-Phe-Val-Asn(N-trityl)-OMe.

Step8

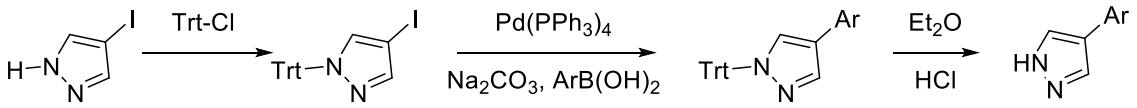


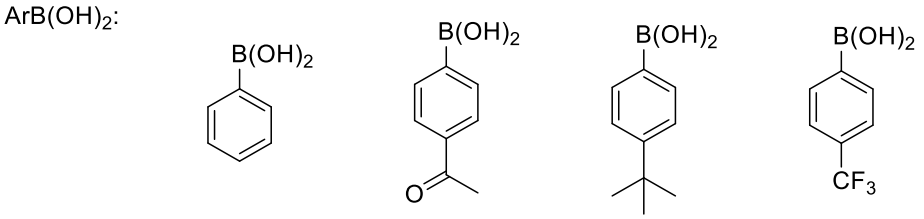
Ac-Phe-Val-Asn(N-trityl)-OMe(2 mmoL) was suspended in anhydrous DCM (5 mL), and TFA(5 ml) was dropwise added. String was continued for 2 h. The reaction mixture was concentrated in vacuo, and purified by flash chromatography (EtOAc/ hexanes) to afford Ac-Phe-Val-Asn-OMe (**Ac-FVN-OMe**).

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (d, J = 7.4 Hz, 1H), 8.10 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 9.0 Hz, 1H), 7.43 (s, 1H), 7.25 (d, J = 4.3 Hz, 4H), 7.21 – 7.13 (m, 1H), 6.97 (s, 1H), 4.57 (q, J = 6.4 Hz, 2H), 4.21 (dd, J = 8.7, 6.6 Hz, 1H), 3.57 (s, 3H), 2.99 (dd, J = 13.9, 3.8 Hz, 1H), 2.71 (dd, J = 13.8, 10.4 Hz, 1H), 2.58 (dd, J = 15.8, 5.9 Hz, 1H), 2.46 (d, J = 6.8 Hz, 1H), 1.97 (tt, J = 13.2, 5.8 Hz, 1H), 1.74 (s, 3H), 0.84 (dd, J = 9.8, 6.9 Hz, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 172.15, 171.80, 171.25, 171.18, 169.67,

138.54, 129.61, 128.46, 126.64, 57.58, 54.25, 52.31, 37.71, 36.88, 31.35, 22.88, 19.50, 18.31 ppm. (Figure S23)

5. General procedure of pyrazoles (*1*)





Step 1

Trityl chloride (1.58g, 5.67mmol) was added to a stirred cold (0-5°C) solution of 4-iodo pyrazole (1 g, 5.15mmol) and triethylamine (1.04g, 10.3mmol) in DCM (12mL). Stirring was continued at room temperature overnight. Cold water was then added and the product was extracted with DCM and the organic layer was washed with saturated sodium bicarbonate solution followed by brine. The organic phase collected was dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (using neutral alumina and 2% EtOAc in hexane as eluent) to afford 1.9g (84.4% yield) of 4-iodo-1-trityl-1H-pyrazole.

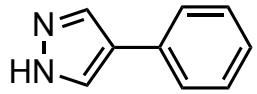
Step 2

Na_2CO_3 (727 mg, 6.86mmol) was added to a stirred solution of 4-iodo-1- trityl-pyrazole (1.5g, 3.43mmol) in THF:H_2O (1:1, 20mL). $Pd(PPh_3)_4$ (790mg, 0.686mmol) and aryl-boronic acid (6.86mmol) were then added and the reaction mixture was heated to reflux for 2 hrs. The reaction mixture was then diluted with water and the product was extracted with ethyl acetate. The organic layer was washed with saturated brine solution, dried over sodium sulfate and concentrated to in vacuo. Purification by column chromatography (using neutral alumina and 5% EtOAc in hexane as eluent).

Step 3

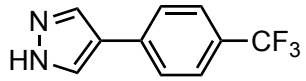
A solution of 4-aryl-1-trityl-pyrazole (2.03mmol) in ether HCl (15 mL) was stirred for 1hr. The reaction mixture was then concentrated under reduced pressure and washed with hexane to afford 4-phenyl-*1H*-pyrazole hydrochloride as white solid. The residue was dissolved in ethyl acetate then washed with saturated $NaHCO_3$, dried over sodium sulfate and concentrated to in vacuo to afford 4-aryl-1-H-pyrazole.

4-phenyl-1H-pyrazole (2b)



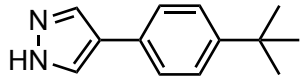
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.01 (br, NH, 1H), 8.22 (s, 1H), 8.00 (s, 1H), 7.66 (d, J = 7.2 Hz, 2H), 7.40 (t, J = 7.7 Hz, 2H), 7.23 (t, J = 7.4 Hz, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 133.35, 129.23, 126.31, 125.56, 121.62, 66.81 ppm. (Figure S24)

4-(4-(trifluoromethyl)phenyl)-1H-pyrazole (2c)



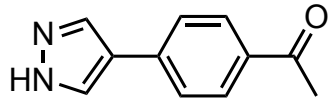
^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (br, NH, 1H), 8.36 (s, 1H), 8.05 (s, 1H), 7.83 (d, J = 8.2 Hz, 2H), 7.69 (d, J = 8.2 Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 137.62, 137.15, 126.16, 126.12, 126.09, 126.05, 125.87, 120.31 ppm. ^{19}F NMR (377 MHz, DMSO-d_6) δ -60.76 ppm. m/z HRMS(ESI) found $[M+H]^+$ 213.0650, $C_{10}H_8F_3N_2^+$ requires 213.0634. (Figure S25)

4-(4-(tert-butyl)phenyl)-1H-pyrazole (2d)



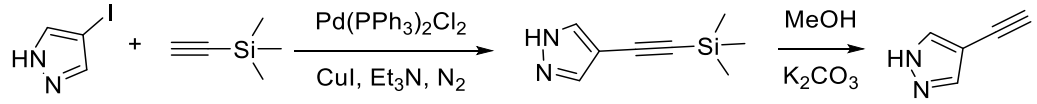
^{1}H NMR (400 MHz, DMSO-d_6) δ 12.86 (s, 1H), 7.99 (s, 2H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.35 (d, *J* = 8.3 Hz, 2H), 1.27 (s, 9H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 148.66, 130.47, 125.91, 125.34, 121.51, 34.58, 31.59 ppm. (Figure S26)

1-(4-(1H-pyrazol-4-yl)phenyl)ethanone (2f)



^{1}H NMR (400 MHz, DMSO-d_6) δ 13.12 (br, NH, 1H), 8.36 (s, 1H), 8.06 (s, 1H), 7.93 (d, J = 8.3 Hz, 2H), 7.76 (d, J = 8.3 Hz, 2H), 2.56 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ 197.71, 138.24, 134.65, 129.46, 125.36, 120.71, 27.04 ppm. (Figure S28)

Synthesis of ethynyl-1H-pyrazole (2e) (*2*)



Step 1

To a solution of 4-iodo-1H-pyrazole (1.93 g, 10 mmol) and ethynyl(trimethyl)silane (4.00 g, 40 mmol) in diethylamine (10 mL) were added bis(triphenylphosphine)- palladium(II) dichloride (350 mg, 0.5 mmol), and copper(I) iodide (44.5 mg, 0.25 mmol), and the reaction mixture was stirred at room temperature for 18 h. The solvent was removed in vacuo and the resulting residue was dissolved in Et_2O (50 mL) and filtered. The filtrate was concentrated and the residue was purified by silica gel chromatography (SiO_2, hexane:ethyl acetate mixtures). 4-((trimethylsilyl)ethynyl)-*1H*-pyrazole (0.88 g, 54 %) as a brown oil.

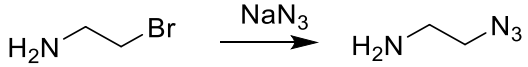
Step 2

To a solution of 4-((trimethylsilyl)ethynyl)-1H-pyrazole (0.82 g, 5 mmol) in MeOH (10 mL) was added a solution of potassium carbonate (1.38 g, 10 mmol) in water (10 mL). After stirring for 18 h at room temperature, the reaction mixture was neutralized with 2 N HCl and concentrated, the resulting residue was dissolved in Et_2O (50 mL). The organic layer was washed with saturated brine solution, dried over sodium sulfate and concentrated to in vacuo. Purification by column chromatography (SiO_2, hexane:ethyl acetate mixtures). ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.76 (s, 2H), 3.05 (s, 1H) ppm. ^{13}C NMR (101 MHz, Chloroform-*d*) δ 137.32, 101.97, 78.59, 74.94 ppm. (Figure S27)

6. Synthesis of biotin-azide (*6*)

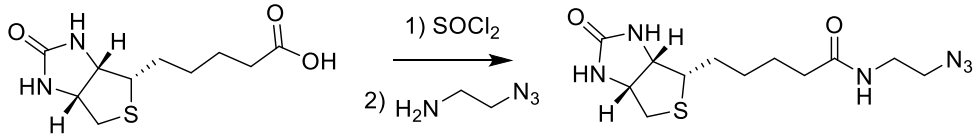
Biotin-azide was generally prepared according to literature procedures with two steps.

Step 1



2-bromoethylamine hydrobromide (500 mg, 2.44 mmol) was add to a solution of sodium azide (475.9 mg, 7.32 mmol, 3 equiv.) in H_2O (2 mL) at 75 °C for 21 h. After the reaction complete, cool the reaction mixture to 0 °C. Et_2O (2 mL) and solid KOH (800 mg) was then added to the reaction mixture. Separate the organic phase and extract the aqueous layer with Et_2O (3X10 mL). The combined organic layer was dried with $MgSO_4$. The resulting solution was filtered and evaporated carefully by rotary evaporation (35 °C, 750 mbar) to afford 2-azidoethylamine.

Step 2



A solution of biotin (0.30 g, 1.23 mmol) in $SOCl_2$ (5 mL) was kept at room temperature for 1 h. The reaction mixture was evaporated under vacuum and co-evaporated with anhydrous toluene (3 × 15 mL) to produce biotin acid chloride. The crude product was then dissolved in anhydrous acetonitrile (15 mL) and drop-wise added to a solution of 2-azidoethanamine (0.22 g, 2.56 mmol) and Et_3N (523 µL, 3.68 mmol) in acetonitrile (15 mL). The reaction mixture was kept at room temperature for 4 h. Evaporation of the solvent yielded a crude product that was purified by column chromatography (EtOAc–MeOH 5:1) producing biotin azide (0.35 g, 92%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.06 (t, J = 5.4 Hz, 1H), 6.45 (s, 1H), 6.38 (s, 1H), 4.30 (m, 1H), 4.15 – 4.04 (m, 1H), 3.33 (t, J = 5.7 Hz, 2H), 3.22 (q, J = 5.6 Hz, 2H), 3.12 – 3.05 (m, 1H), 2.81 (m, 1H), 2.57 (d, J = 12.4 Hz, 1H), 2.07 (t, J = 7.4 Hz, 2H), 1.68 – 1.20 (m, 6H) ppm. ^{13}C NMR (101 MHz, DMSO-d6) δ 172.93, 163.22, 61.49, 59.66, 55.89, 50.44, 38.61, 35.60, 28.67, 28.50, 25.62 ppm. (Figure S29)

7. General procedure for bioconjugation of phenylalanine contained tripeptide Ac-FVN-OMe

To a solution of **Ac-FVN-OMe** (0.2 mmol, 1 equiv., 86.8 mg) in CH_3CN/H_2O (1.5 mL/1.5 mL) was added pyrazole **2a or 2e** (0.3 mmol) in presence of photosensitizer $TPT^+BF_4^-$ (0.04 mmol, 20 mol%, 15.8 mg) under air atmosphere and irradiated by 3W blue LEDs at 25 ˚C for 10 h. After completion of the reaction, the solution was analyzed by ESI-HRMS.

8. Synthesis of biotin tagged tripeptide Ac-F(Pyr-Biotin)VN-OMe by CuAAC reaction.

Pyrazole labeled tripeptide carrying alkyne handles at the concentration 3 mM in CH_3CN/H_2O (1.5 mL/1.5 mL) were labeled with the CuAAC reaction. The reactions were performed by addition of 40 mM $CuSO_4$, 20 mM biotin-azide and 40 mM sodium ascorbate. The reactions were then stirred for 12 hour at room temperature.

9. Procedure for visible-light-induced pyrazole tagging conjugated phenylalanine-targeted biomolecules

a. Polypeptides

To a solution of polypeptide (0.2 mmol, 1 equiv.) in CH_3CN/H_2O (1.5 mL/1.5 mL) was added pyrazole **2a** (0.3 mmol) in presence of photosensitizer $TPT^+BF_4^-$ (0.04 mmol, 20 mol%, 15.8 mg) under air atmosphere and irradiated by 3W blue LEDs at 25 ˚C for 10 h. After completion of the reaction, the solution was analyzed by MS/MS spectroscopy.

b. Insulin

The reaction was performed in co-solvent (CH_3CN/H_2O = 1:1) with 3 mM pig insulin (m/z = 5777 Da) and 10 μM pyrazole in presence of 10 mM $TPT^+BF_4^-$ under the irradiation of blue LED at r.t.. The reaction mixtures with different reaction time were monitored by MALDI-TOF-MS, MS/MS, and HRMS spectroscopies.

10. SAXS measurements

SAXS data for the pyrazole-bound insulin solutions were conducted at the 23A SWAXS endstation of National Synchrotron Radiation Research Center (NSRRC), using a beam of 15.0 keV (wavelength λ= 0.8267 Å) and a sample-to-detector distance 2503 mm. SAXS data were collected on a pixel detector Dectirs-Pilatus 1M detector of an active area of 169 x 179 mm^2 and a detector pixel solution of 172 μm. The scattering wavelength $\mathbf{q} = \mathbf{4}\ ^{-\mathbf{1}}\boldsymbol{sin}$, defined by the scattering θ and , was calibrated with a standard sample of silver behenate. To minimize radiation damages, the 5-mm sample solution with thin (12 μm) kapton windows was gently rocked within an area of 1.5 x 1.5 mm^2 to avoid prolonged spot exposure (ca. 0.5 mm in beam diameter) of the sample solution and measured at room temperature. SAXS data were subtracted with buffer scattering measured under an identical environment as that used for the pyrazole-bound insulin sample solutions; the data were then corrected for incoming flux, sample thickness and electronic noise of the detector, as detailed in a previous report. (*7*)

Supplementary Figures and Data

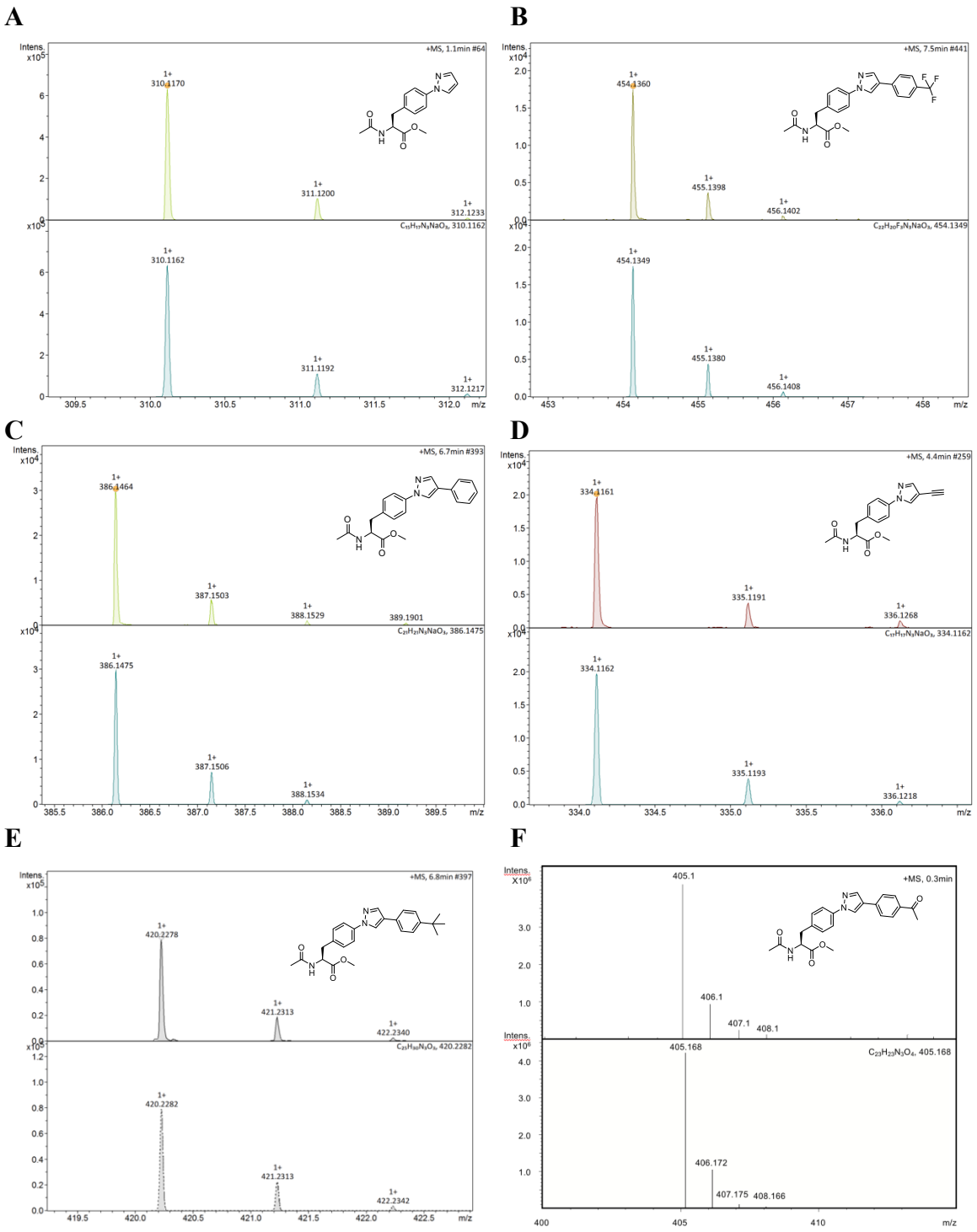


Fig S1. LC-MS analysis of amino acid modifications. The modifications of phenylalanine were analyzed by were analyzed by ESI-HRMS and ESI-MS spectrum. (A) The ESI-HRMS spectrum of pyrazole-labelled Ac-Phe-OMe peaks at m/z = 310.1170, and

the simulated fit for the $C_{15}H_{17}N_3NaO_3$ based on isotopic abundance at m/z = 310.1162. (B) The ESI-HRMS spectrum of trifluorophenylpyrazole-labelled Ac-Phe-OMe peaks at m/z = 454.1360, and the simulated fit for the $C_{22}H_{20}F_3N_3NaO_3$ based on isotopic abundance at m/z = 454.1349. (C) The ESI-HRMS spectrum of phenylpyrazole-labelled Ac-Phe-OMe peaks at m/z = 386.1464, and the simulated fit for the $C_{21}H_{21}N_3NaO_3$ based on isotopic abundance at m/z = 386.1475. (D) The ESI-HRMS spectrum of ethynylpyrazole-labelled Ac-Phe-OMe peaks at m/z = 334.1161, and the simulated fit for the $C_{17}H_{17}N_3NaO_3$ based on isotopic abundance at m/z = 334.1162. (E) The ESI-HRMS spectrum of *tert*-butylphenylpyrazole-labelled Ac-Phe-OMe peaks at m/z = 420.2278, and the simulated fit for the $C_{25}H_{30}N_3O_3$ based on isotopic abundance at m/z = 420.2282. (F) The ESI-MS spectrum of acetylphenylpyrazole-labelled Ac-Phe-OMe peaks at m/z = 405.100, and the simulated fit for the $C_{23}H_{23}N_3O_4$ based on isotopic abundance at m/z = 405.168.

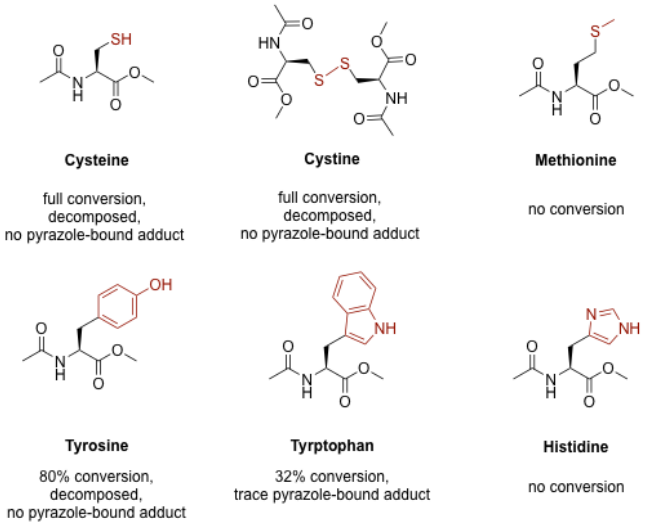
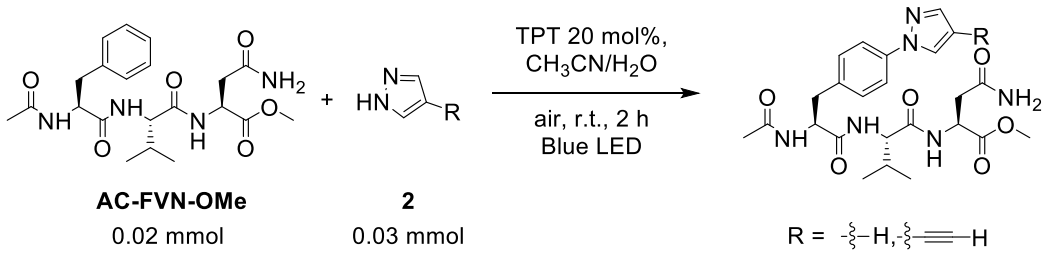


Fig. S2 Visible-light-induced reactivity of pyrazole with other natural amino acids. The reactions were performed in co-solvent (CH_3CN/H_2O = 1:1) with 3 mM amino acids and 10 μM pyrazole in presence of 10 mM $TPT^+BF_4^-$ under the irradiation of blue LED at r.t.. The reaction mixtures were monitored by FID-GC and ESI-MS, using biphenyl as the internal standard.



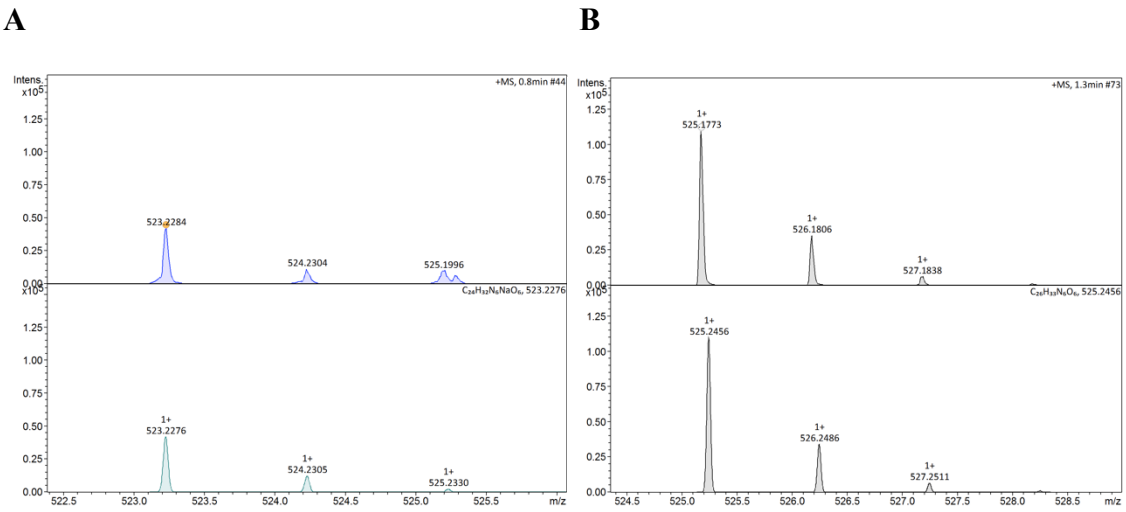


Fig S3. ESI-HRMS analysis of tripeptide modification. The modifications of tripeptide were analyzed by ESI-HRMS spectrum. (A) The ESI-HRMS spectrum of pyrazole-labelled tripeptide peaks at m/z = 523.2284, and the simulated fit for the $C_{24}H_{32}N_6NaO_6$ based on isotopic abundance at m/z = 523.2276. (B) The ESI-HRMS spectrum of 4-ethynyl-1H-pyrazole-labelled tripeptide peaks at m/z = 525.1773, and the simulated fit for the $C_{24}H_{32}N_6NaO_6$ based on isotopic abundance at m/z = 525.2456.

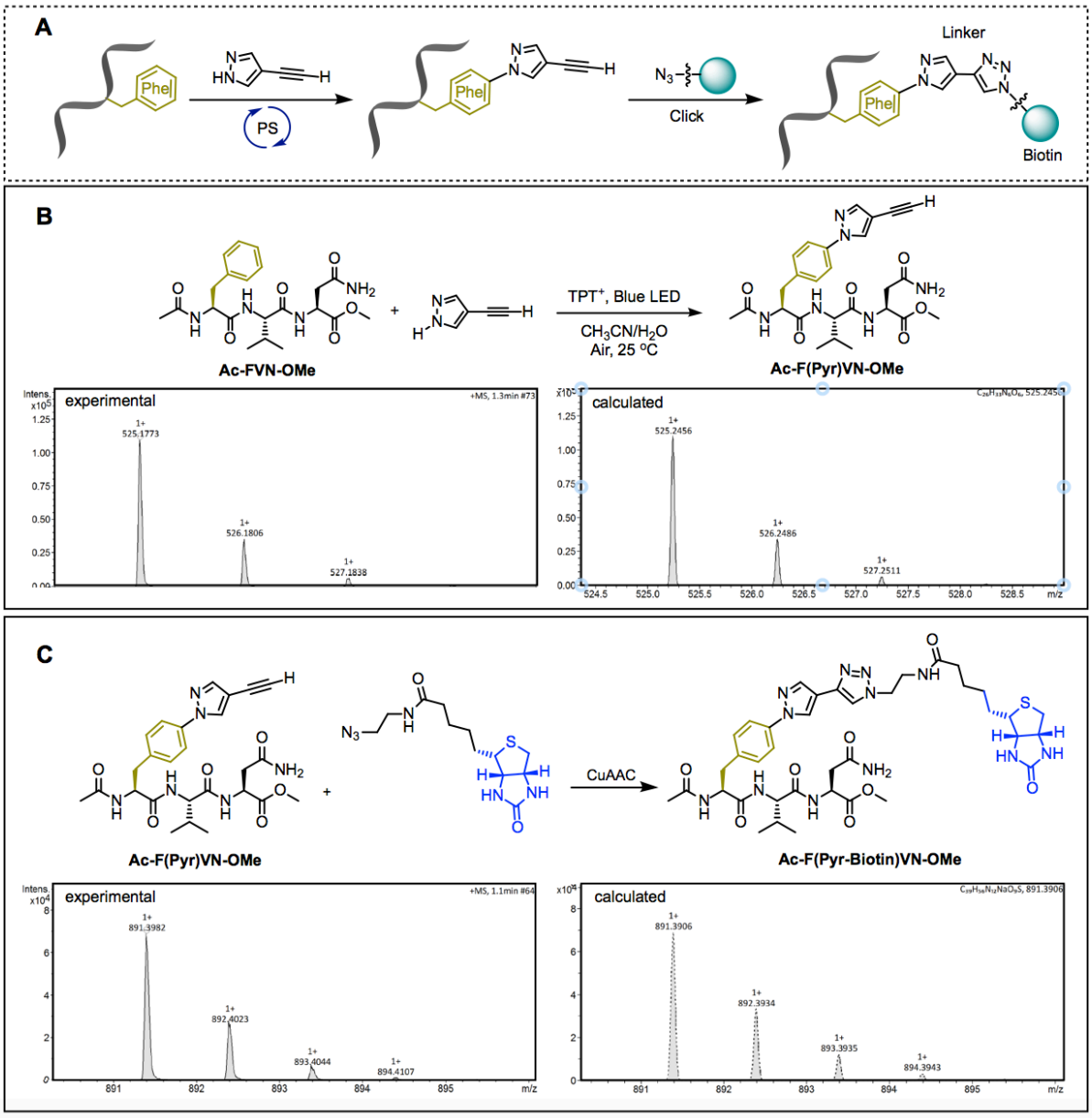


Fig S4. ESI-HRMS analysis of the bio-orthogonal product of the tripeptide Ac-FVN-OMe (**A**) Two-step process for functionalizing the phenylalanine-contained tripeptide (**Ac-FVN-OMe**). (**B**) Visible-light-induced bioconjugation of **Ac-FVN-OMe** (0.02 mmol) with 4-ethynyl-1*H*-pyrazole (0.3 mmol). The reaction conditions are shown with molecular weight listed for the corresponding conjugation. (**C**) Synthesis of the biotin tagged tripeptide **Ac-F(Pyr-Biotin)VN-OMe** by CuAAC reaction. The ESI-HRMS spectrum of ethynylpyrazole-bound tripeptide peaks (**Ac-F(Pyr)VN-OMe**) at *m/z* = 525.1773, and the simulated fit for the **Ac-F(Pyr)VN-OMe** based on isotopic abundance at m/z = 525.2456. For the Click reaction product **Ac-F(Pyr-Biotin)VN-OMe**, ESI-HRMS spectrum exhibiting a base peak at *m/z* = 891.3982, and its simulated peak of (**Ac-F(Pyr-Biotin)VN-OMe·CH$_3$OH·Na$^+$**) at *m/z* = 891.3906.

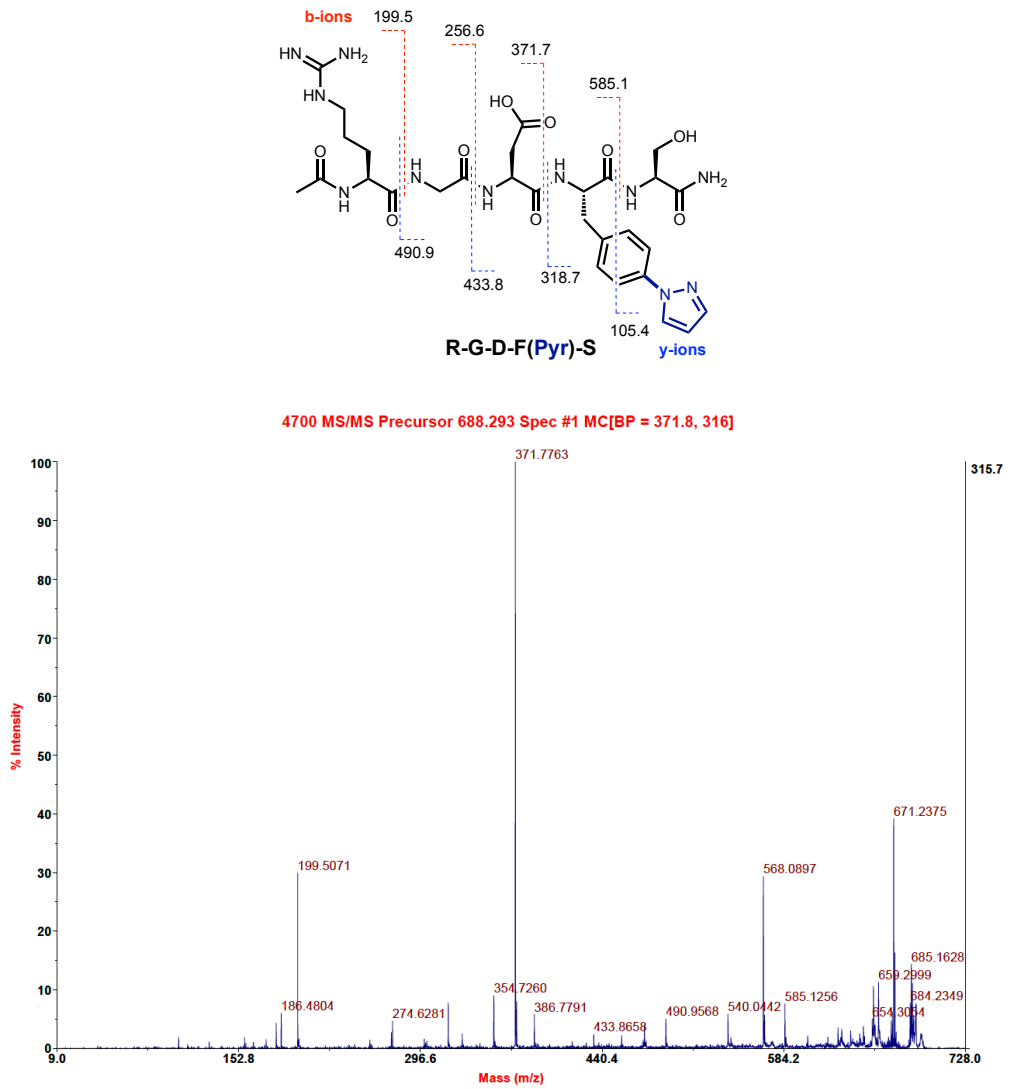


Fig S5. MALDI-TOF-MS/MS analysis of Penta-peptide RGDFS modification. The modification of penta-peptide was analyzed by MS/MS spectrum. MS/MS was key in confirming the site of pyrazolation. Predicted b and y ions are presented in the figure.

OVA Peptide 257-264

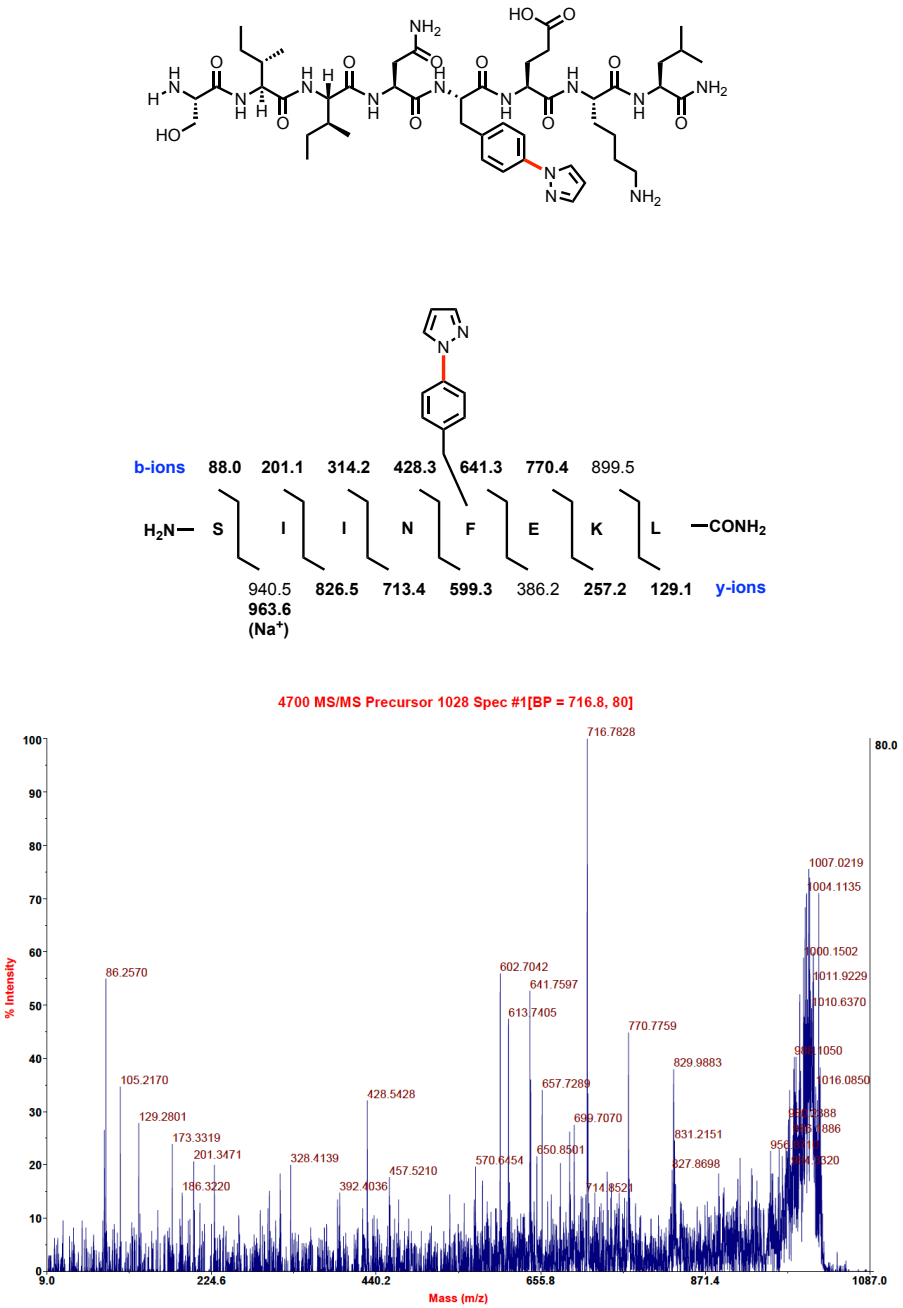
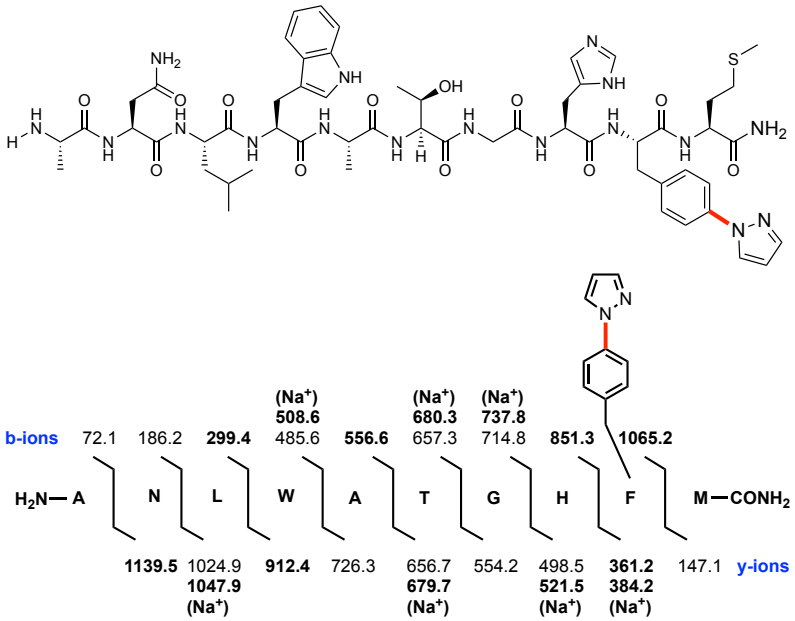


Fig S6. MALDI-TOF-MS/MS analysis of OVA Peptide 257-264 modification. The modification of polypeptide was analyzed by MS/MS spectrum. MS/MS was key in confirming the site of pyrazolation. Predicted b and y ions are presented in the figure. Observed b and y ions are highlighted in bold.

Neuromedin B



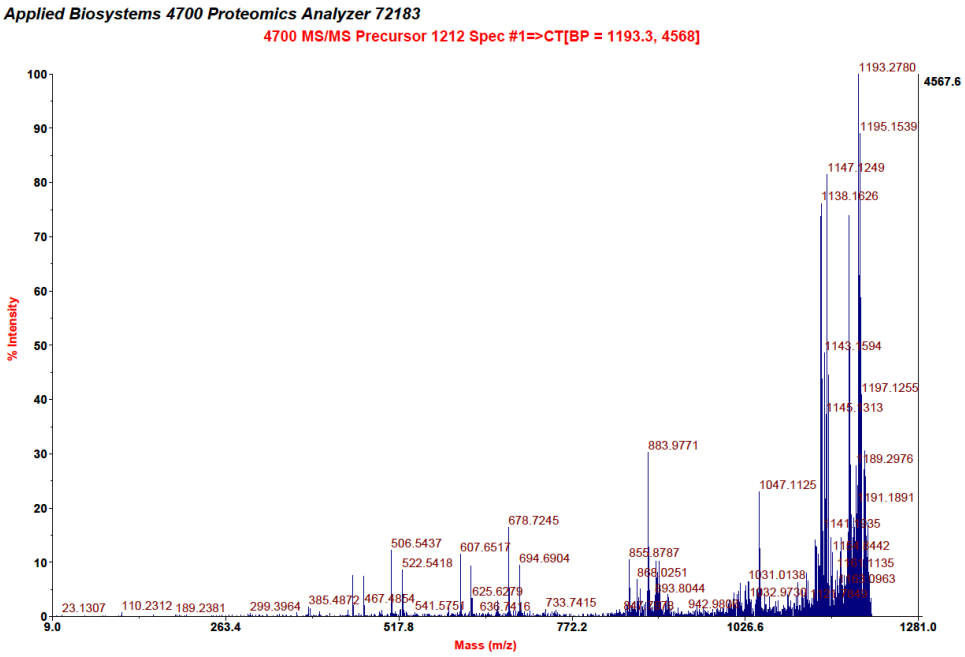
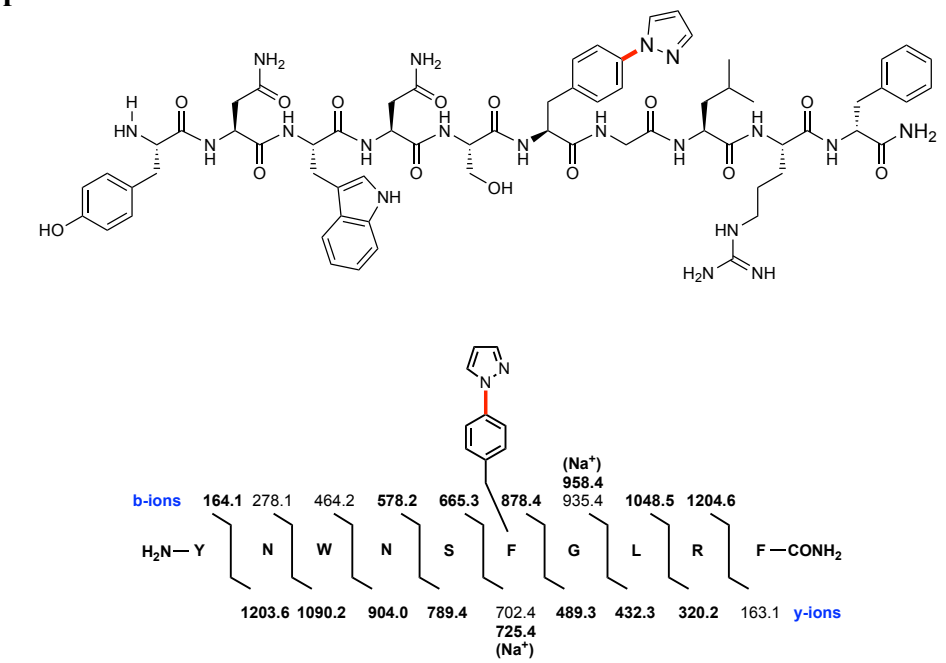


Fig S7. MALDI-TOF-MS/MS analysis of peptide Neuromedin B modification. The modification of polypeptide was analyzed by MS/MS spectrum. MS/MS was key in confirming the site of pyrazolation. Predicted b and y ions are presented in the figure. Observed b and y ions are highlighted in bold.

Kisspeptin-10



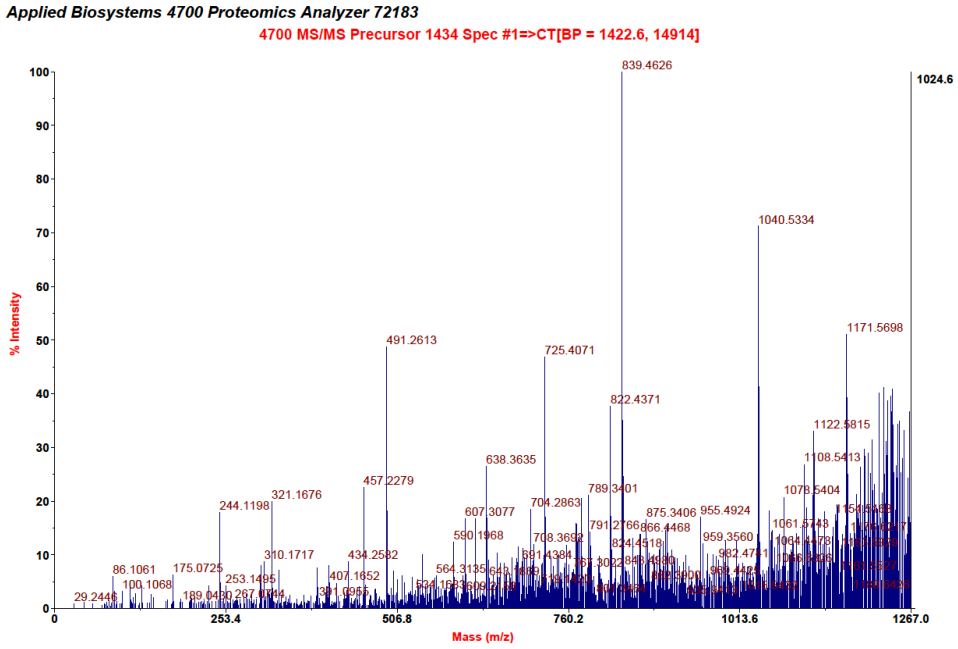
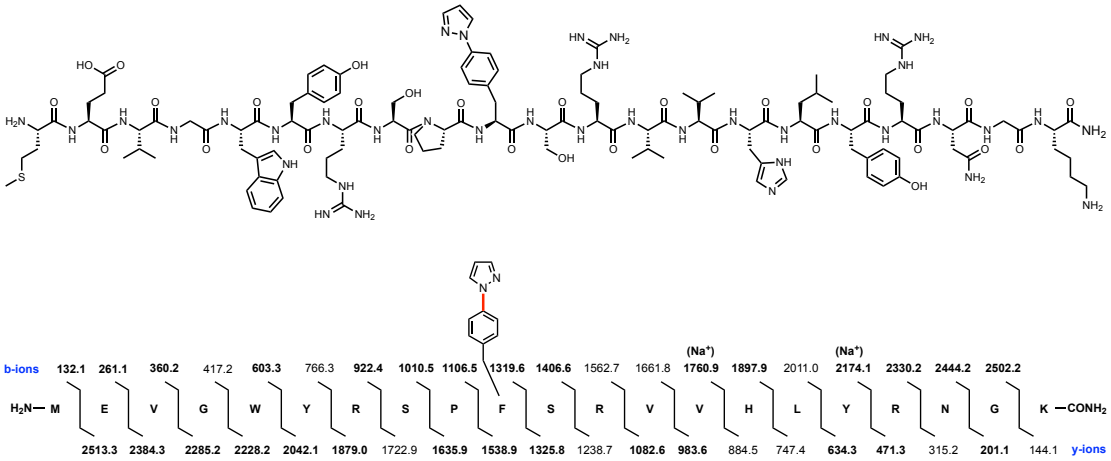


Fig S8. MALDI-TOF-MS/MS analysis of peptide Kisspeptin-10 modification. The modification of polypeptide was analyzed by MS/MS spectrum. MS/MS was key in confirming the site of pyrazolation. Predicted b and y ions are presented in the figure. Observed b and y ions are highlighted in bold.

MOG 35-55



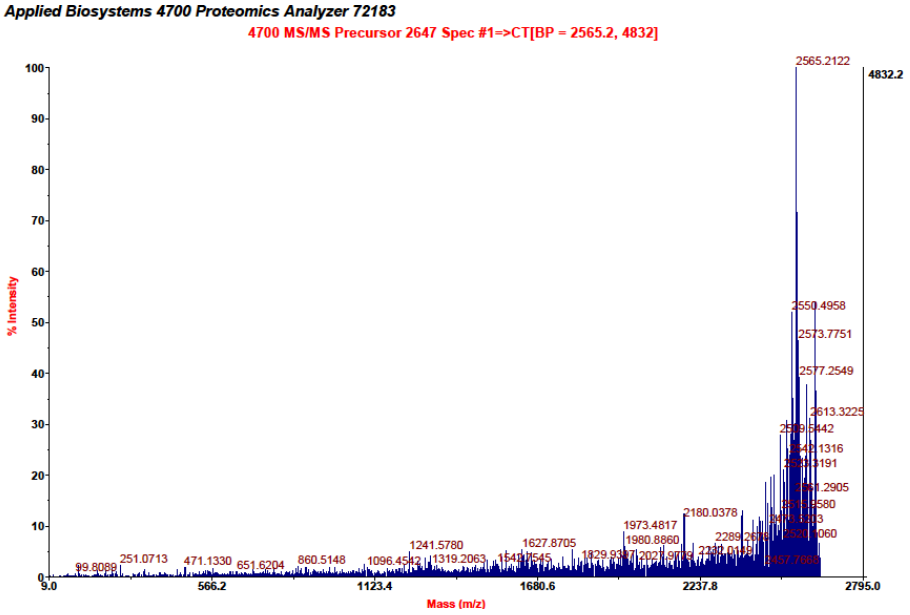
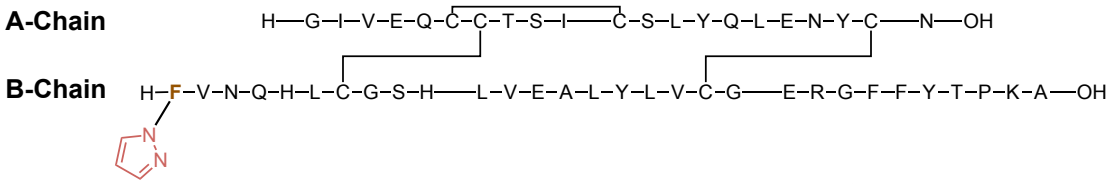


Fig S9. MALDI-TOF-MS/MS analysis of peptide MOG 35-55 modification. The modification of polypeptide was analyzed by MS/MS spectrum. MS/MS was key in confirming the site of pyrazolation. Predicted b and y ions are presented in the figure. Observed b and y ions are highlighted in bold.

Insulin



N-term	y-ion	*C*-term	b-ion
F	**5843.6802**	**A**	**5843.6802**
V	5629.5821	**K**	**5755.6403**
N	**5530.5137**	**P**	**5627.5454**
Q	**5416.4708**	**T**	**5530.4926**
H	5288.4122	**Y**	5429.4449
L	5151.3533	**F**	**5266.3816**
		F	**5119.3132**
		G	4972.2448
		R	4915.2233
		E	**4759.1222**
		G	4630.0796
		C	4573.0581

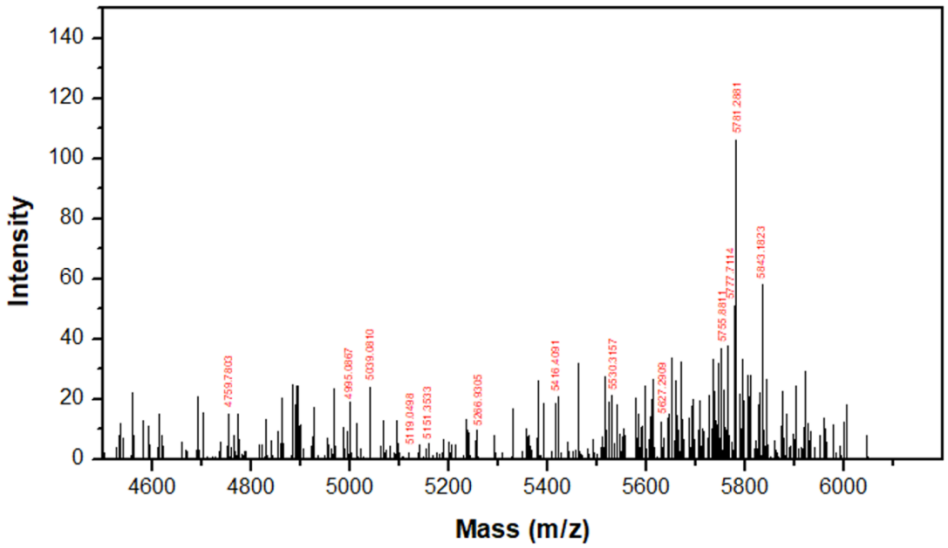


Fig S10. MALDI-TOF-MS/MS analysis of protein insulin modification. The modification of insulin was analyzed by MS/MS spectrum. MS/MS was key in confirming the site of pyrazolation. Predicted b and y ions are tabulated in the table. Observed b and y ions are highlighted in bold.

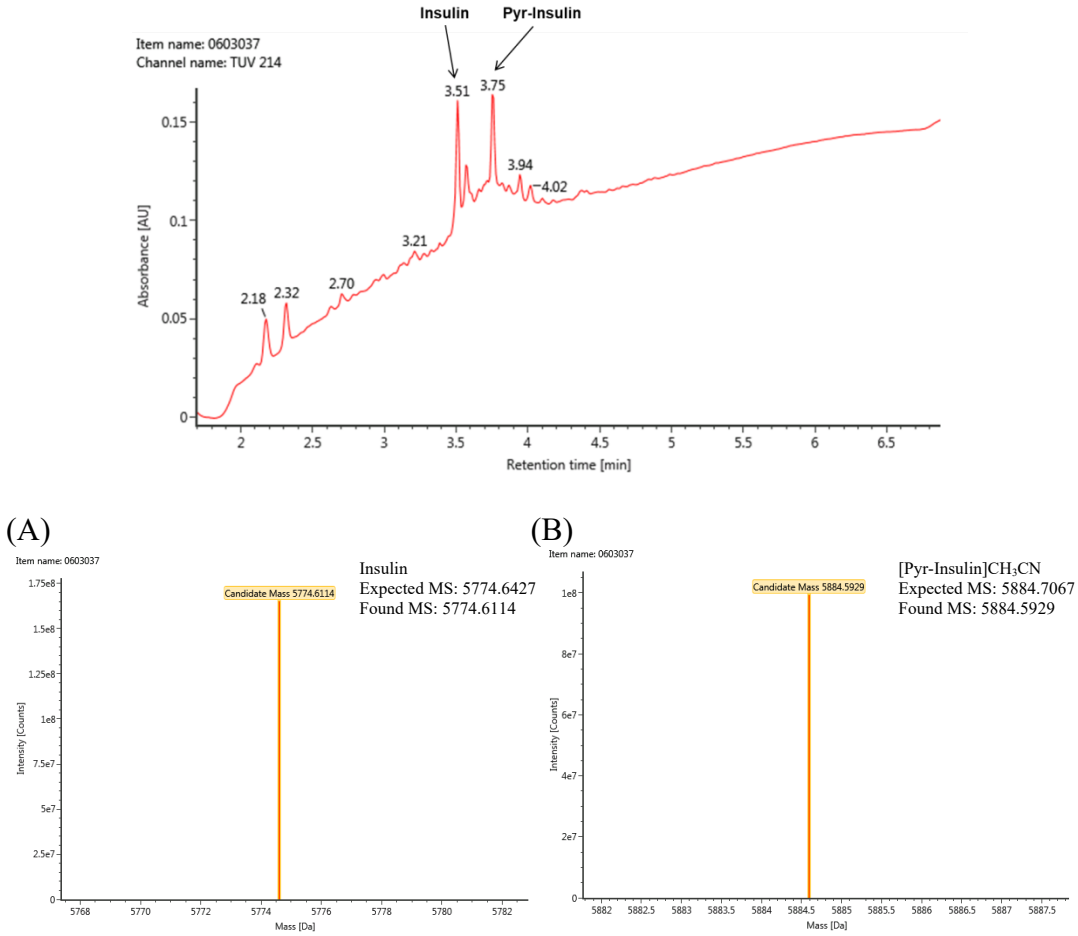


Fig S11. LC-MS analysis of insulin and modified insulin. (Top) LC spectrum of the insulin and pyrazole-bound insulin. (Bottom) LC-MS analysis of insulin without (A) and with (B) a pyrazole labeling. The expected mass and found mass are shown on the top right of deconvoluted peak.

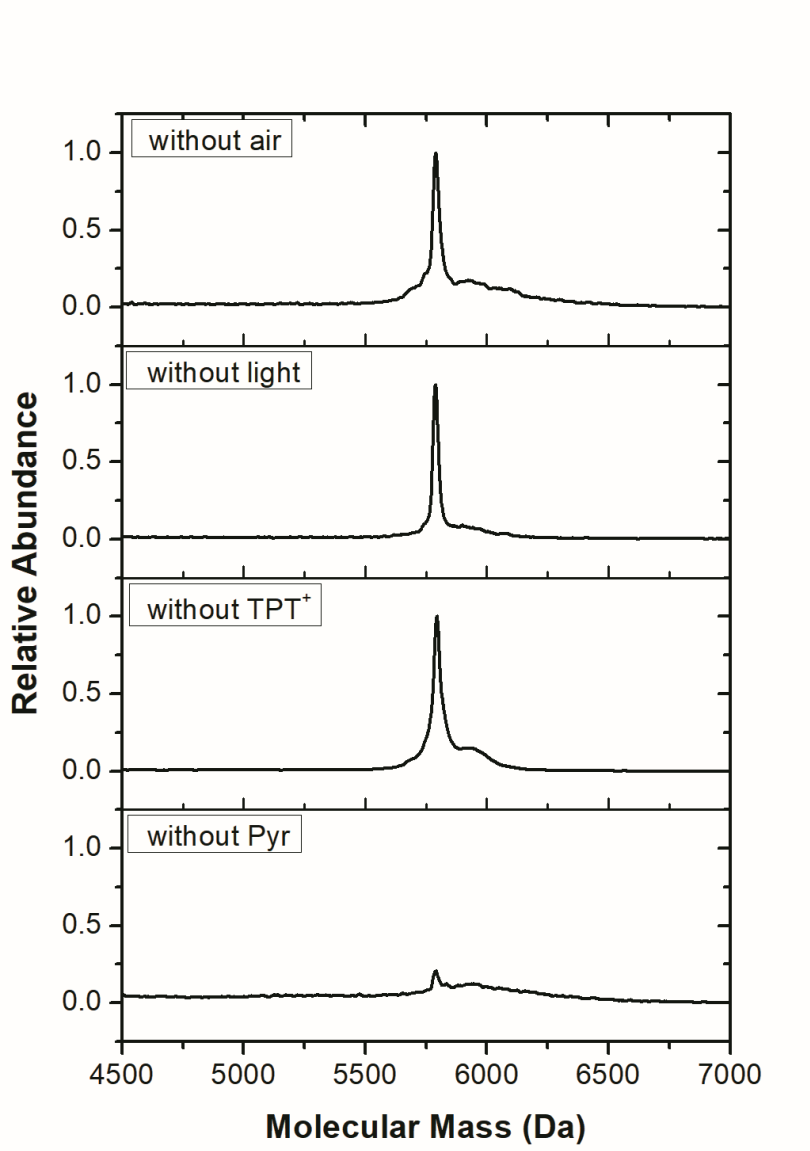
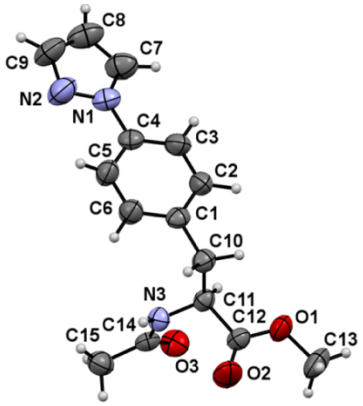


Fig. S12 Control experiments of visible-light-induced pyrazole tagging on insulin. The standard reaction was performed in co-solvent (CH_3CN/H_2O = 1:1) with 3 mM insulin and 10 μM pyrazole in presence of 10 mM $TPT^+BF_4^-$ under the irradiation of blue LED at r.t.. The reaction mixtures were monitored by MALDI-TOF-MS.

Fig. S13 X-Ray crystal data for pyrazole-phenylalanine adduct 3a
CCDC 1912810 contain the supplementary crystallographic data for this paper.



Crystal data and structure refinement for

Identification code	170423
Empirical formula	C15 H17 N3 O3
Formula weight	287.32
Temperature	296 K
Wavelength	1.54178 Å
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a = 5.00990(10) Å α= 90°. b = 8.4438(2) Å β= 90°. c = 34.9640(7) Å γ = 90°.
Volume	1479.07(5) $Å^3$
Z	4
Density (calculated)	1.290 Mg/m3
Absorption coefficient	0.755 mm^{-1}
F(000)	608.0
Crystal size	0.12 x 0.14 x 0.16 mm^3
Theta range for data collection	5.06 to 64.98°.
Index ranges	-5<=h<=5, -9<=k<=9, -27<=l<=41
Reflections collected	11204
Independent reflections	2451 [R_{int} = 0.0495, R_{sigma} = 0.077]
Completeness to theta = 64.97°	99.0 %
Absorption correction	multi-scan
Max. and min. transmission	0.7526 and 0.6179
Refinement method	Full-matrix least-squares on F_2
Data / restraints / parameters	11204/0/228
Goodness-of-fit on F2	1.158
Final R indices [I>2sigma(I)]	R1 = 0.0558, wR2 = 0.1411
R indices (all data)	R1 = 0.0566, wR2 = 0.1419
Largest diff. peak and hole	0.273/-0.300 e.Å-3

No syntax errors found.
Please wait while processing ....

CIF dictionary
Interpreting this report

Datablock: x_a

Bond precision: C-C = 0.0045 A Wavelength=1.54178
Cell: a=5.0099(1) b=8.4438(2) c=34.9640(7)
alpha=90 beta=90 gamma=90
Temperature: 296 K

	Calculated	Reported
Volume	1479.07(5)	1479.07(5)
Space group	P 21 21 21	P 21 21 21
Hall group	P 2ac 2ab	P 2ac 2ab
Moiety formula	C15 H17 N3 O3	?
Sum formula	C15 H17 N3 O3	C15 H17 N3 O3
Mr	287.32	287.32
Dx,g cm-3	1.290	1.290
Z	4	4
Mu (mm-1)	0.755	0.755
F000	608.0	608.0
F000'	609.93	
h,k,lmax	5,9,41	5,9,41
Nref	2511[1516]	2451
Tmin,Tmax	0.886,0.913	0.618,0.753
Tmin'	0.886	

Correction method= # Reported T Limits: Tmin=0.618
Tmax=0.753 AbsCorr = MULTI-SCAN
Data completeness= 1.62/0.98 Theta(max)= 64.970
R(reflections)= 0.0558(2387) wR2(reflections)= 0.1419(2451)
S = 1.158 Npar= 228

The following ALERTS were generated. Each ALERT has the format
test-name_ALERT_alert-type_alert-level.
Click on the hyperlinks for more details of the test.

Alert level C

THETM01_ALERT_3_C The value of sine(theta_max)/wavelength is less than 0.590
Calculated sin(theta_max)/wavelength = 0.5877
PLAT089_ALERT_3_C Poor Data / Parameter Ratio (Zmax < 18) ..... 6.57 Note
PLAT242_ALERT_2_C Low 'MainMol' Ueq as Compared to Neighbors of C12 Check
PLAT340_ALERT_3_C Low Bond Precision on C-C Bonds ..... 0.0045 Ang.
PLAT911_ALERT_3_C Missing FCF Refl Between Thmin & STh/L= 0.588 17 Report
PLAT978_ALERT_2_C Number C-C Bonds with Positive Residual Density. 0 Info

Alert level G

PLAT791_ALERT_4_G Model has Chirality at C11 (Chiral SPGR) S Verify
PLAT909_ALERT_3_G Percentage of I>2sig(I) Data at Theta(Max) Still 88% Note
PLAT910_ALERT_3_G Missing # of FCF Reflection(s) Below Theta(Min). 1 Note
PLAT955_ALERT_1_G Reported (CIF) and Actual (FCF) Lmax Differ by . 1 Units

0 **ALERT level A** = Most likely a serious problem - resolve or explain
0 **ALERT level B** = A potentially serious problem, consider carefully
6 **ALERT level C** = Check. Ensure it is not caused by an omission or oversight
4 **ALERT level G** = General information/check it is not something unexpected

1 ALERT type 1 CIF construction/syntax error, inconsistent or missing data
2 ALERT type 2 Indicator that the structure model may be wrong or deficient
6 ALERT type 3 Indicator that the structure quality may be low
1 ALERT type 4 Improvement, methodology, query or suggestion
0 ALERT type 5 Informative message, check

It is advisable to attempt to resolve as many as possible of the alerts in all categories. Often the minor alerts point to easily fixed oversights, errors and omissions in your CIF or refinement strategy, so attention to these fine details can be worthwhile. In order to resolve some of the more serious problems it may be necessary to carry out additional measurements or structure refinements. However, the purpose of your study may justify

the reported deviations and the more serious of these should normally be commented upon in the discussion or experimental section of a paper or in the "special_details" fields of the CIF. checkCIF was carefully designed to identify outliers and unusual parameters, but every test has its limitations and alerts that are not important in a particular case may appear. Conversely, the absence of alerts does not guarantee there are no aspects of the results needing attention. It is up to the individual to critically assess their own results and, if necessary, seek expert advice.

Publication of your CIF in IUCr journals

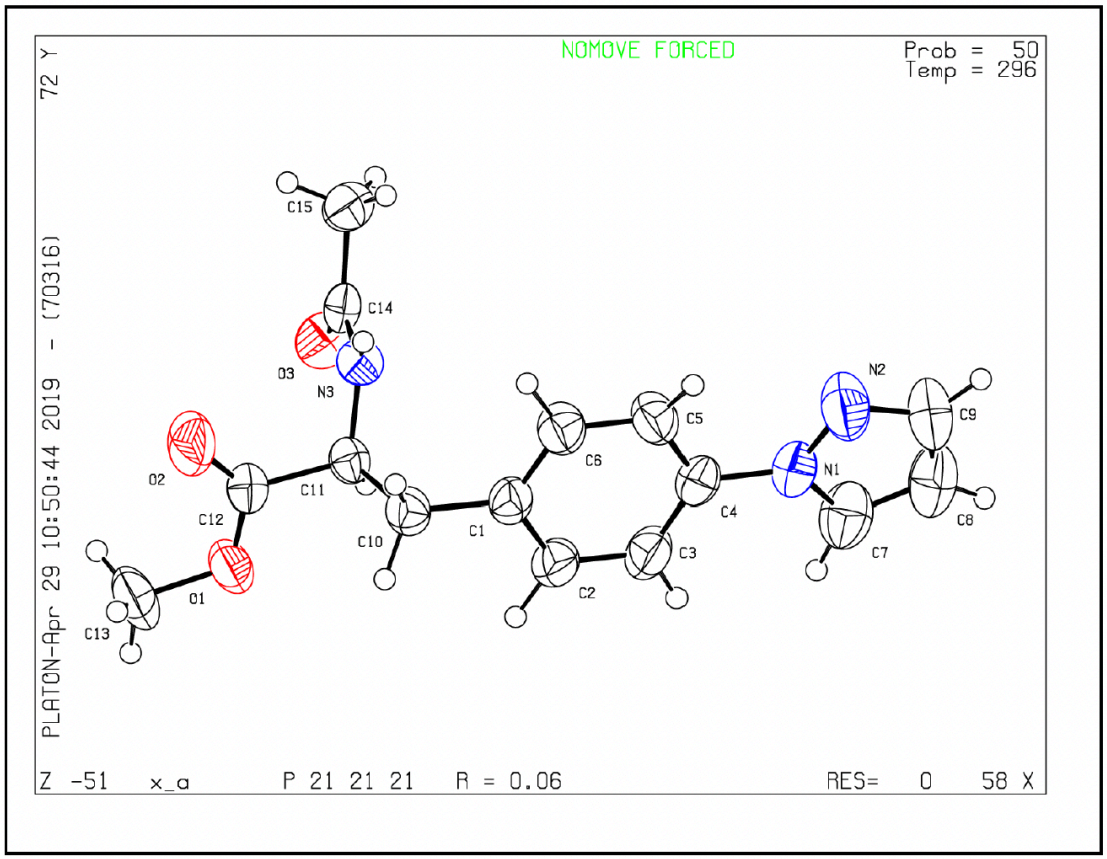
A basic structural check has been run on your CIF. These basic checks will be run on all CIFs submitted for publication in IUCr journals (*Acta Crystallographica*, *Journal of Applied Crystallography*, *Journal of Synchrotron Radiation*); however, if you intend to submit to *Acta Crystallographica Section C* or *E* or *IUCrData*, you should make sure that full publication checks are run on the final version of your CIF prior to submission.

Publication of your CIF in other journals

Please refer to the *Notes for Authors* of the relevant journal for any special instructions relating to CIF submission.

PLATON version of 17/03/2019; check.def file version of 04/03/2019

Datablock x_a – ellipsoid plot



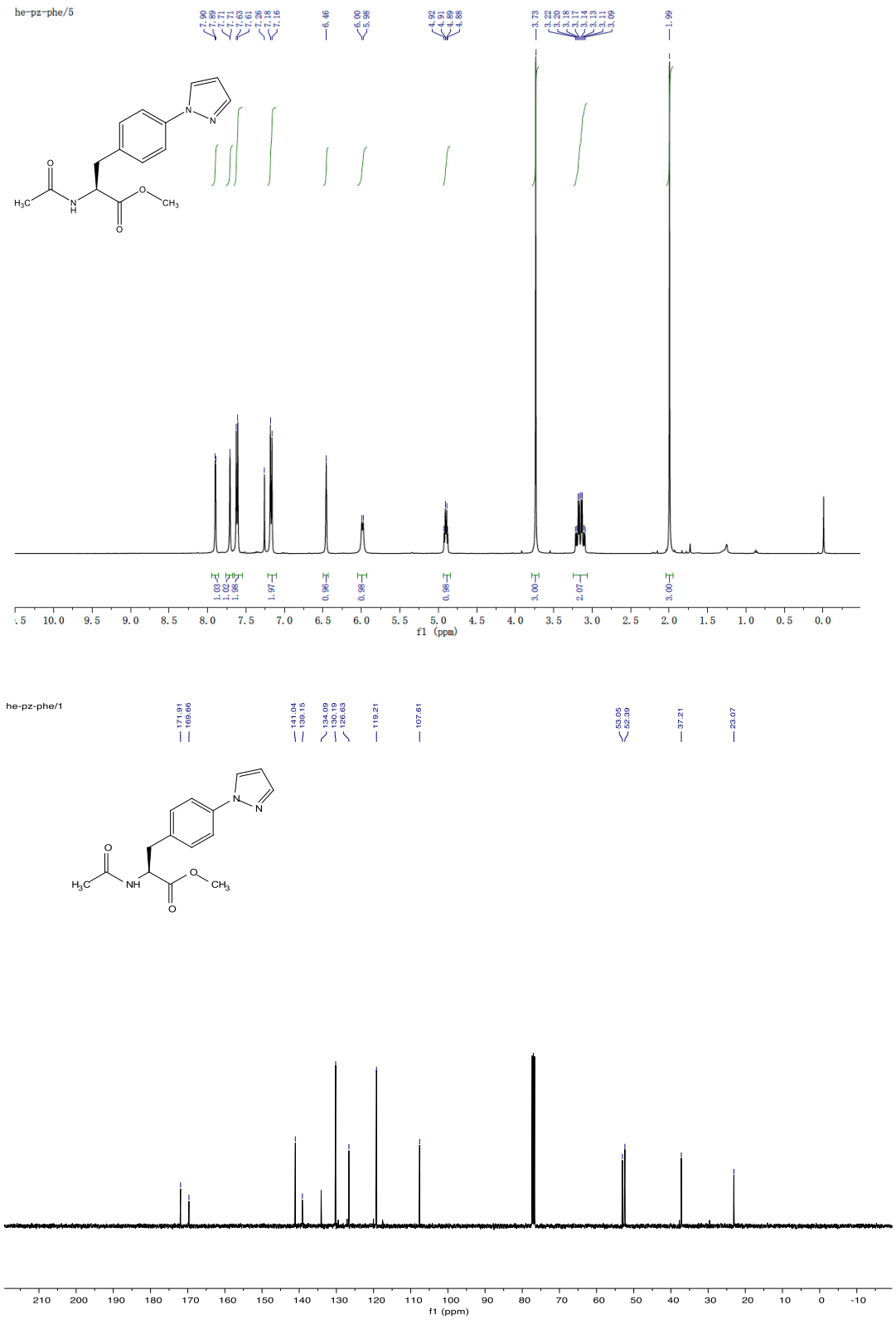


Fig. S14 NMR data of 3a

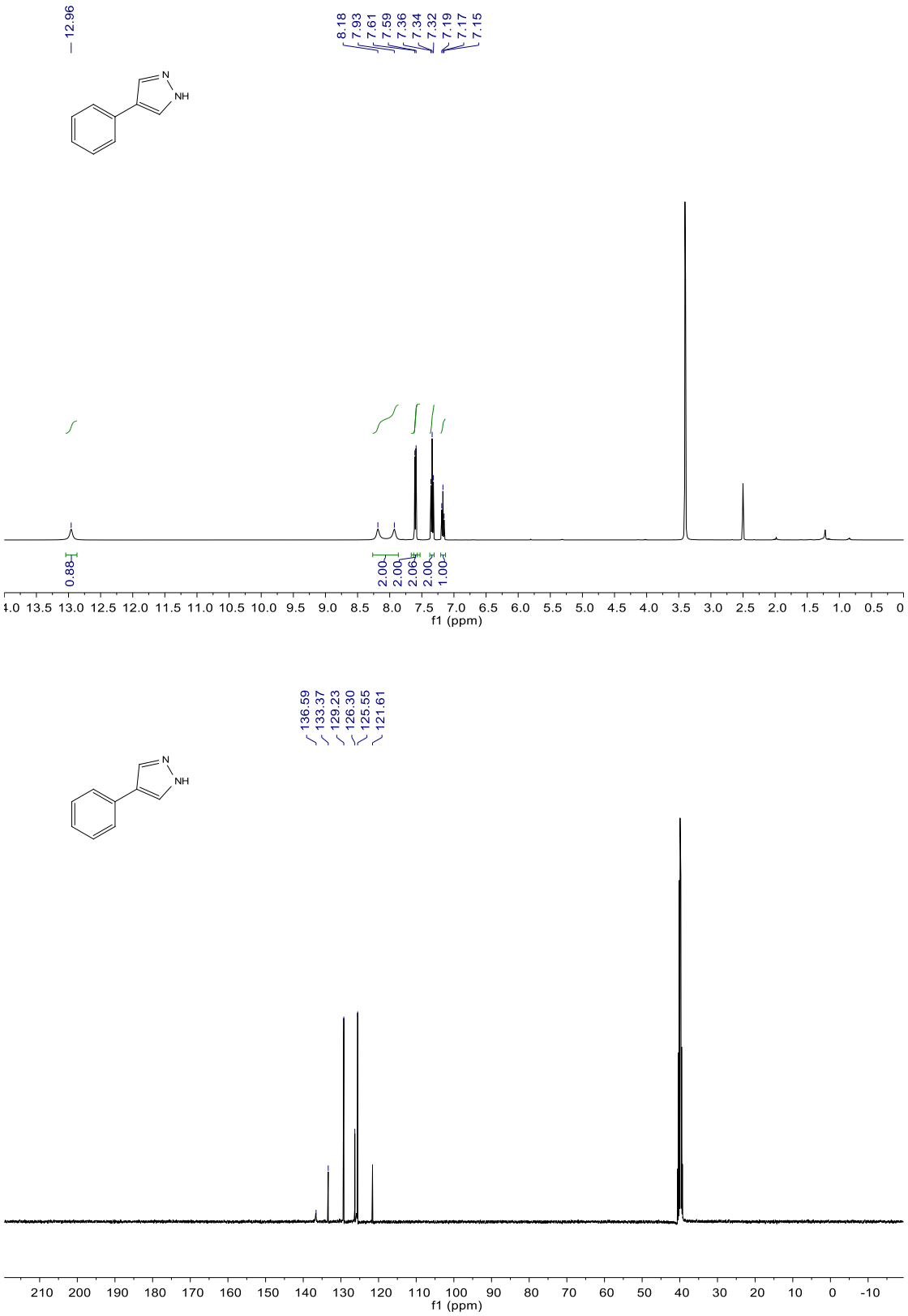
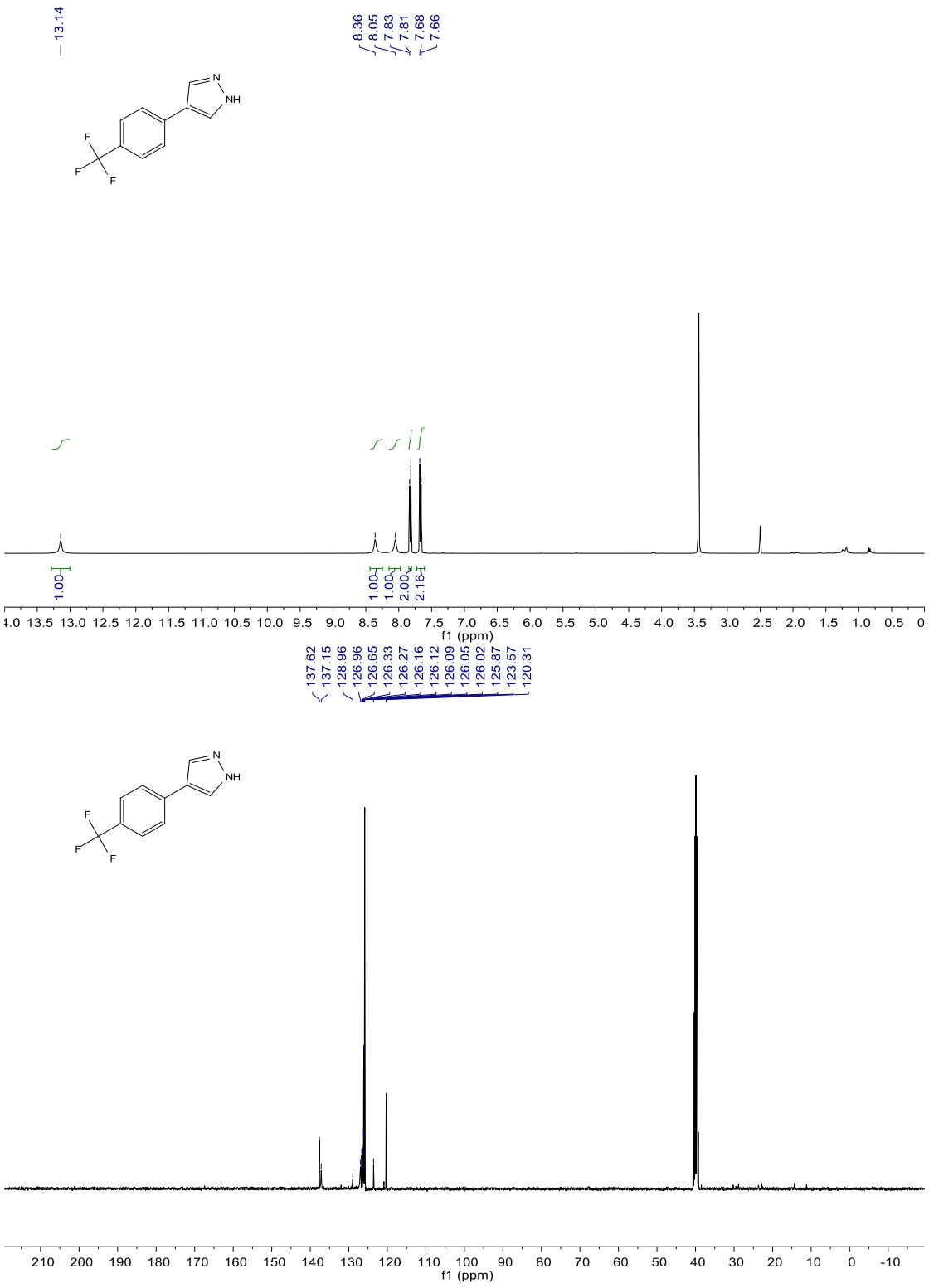


Fig. S15 NMR data of 4-phenyl-1H-pyrazole (2b)


13.14
8.36
8.05
7.83
7.81
7.68
7.66
N
NH
F
F
F
1.00
1.00
1.00
2.00
2.16
f1 (ppm)
137.62
137.15
128.96
126.96
126.65
126.33
126.27
126.16
126.12
126.09
126.05
126.02
125.87
123.57
120.31
f1 (ppm)

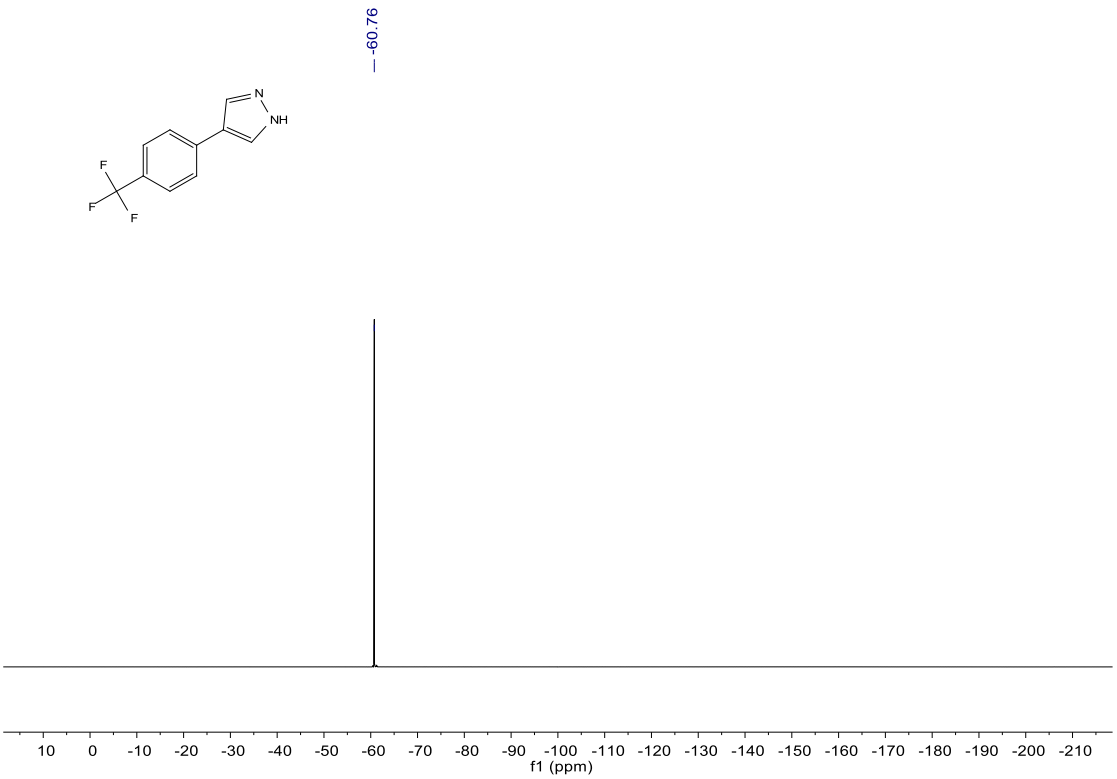


Fig. S16 NMR data of 4-(4-(trifluoromethyl)phenyl)-1H-pyrazole (2c)

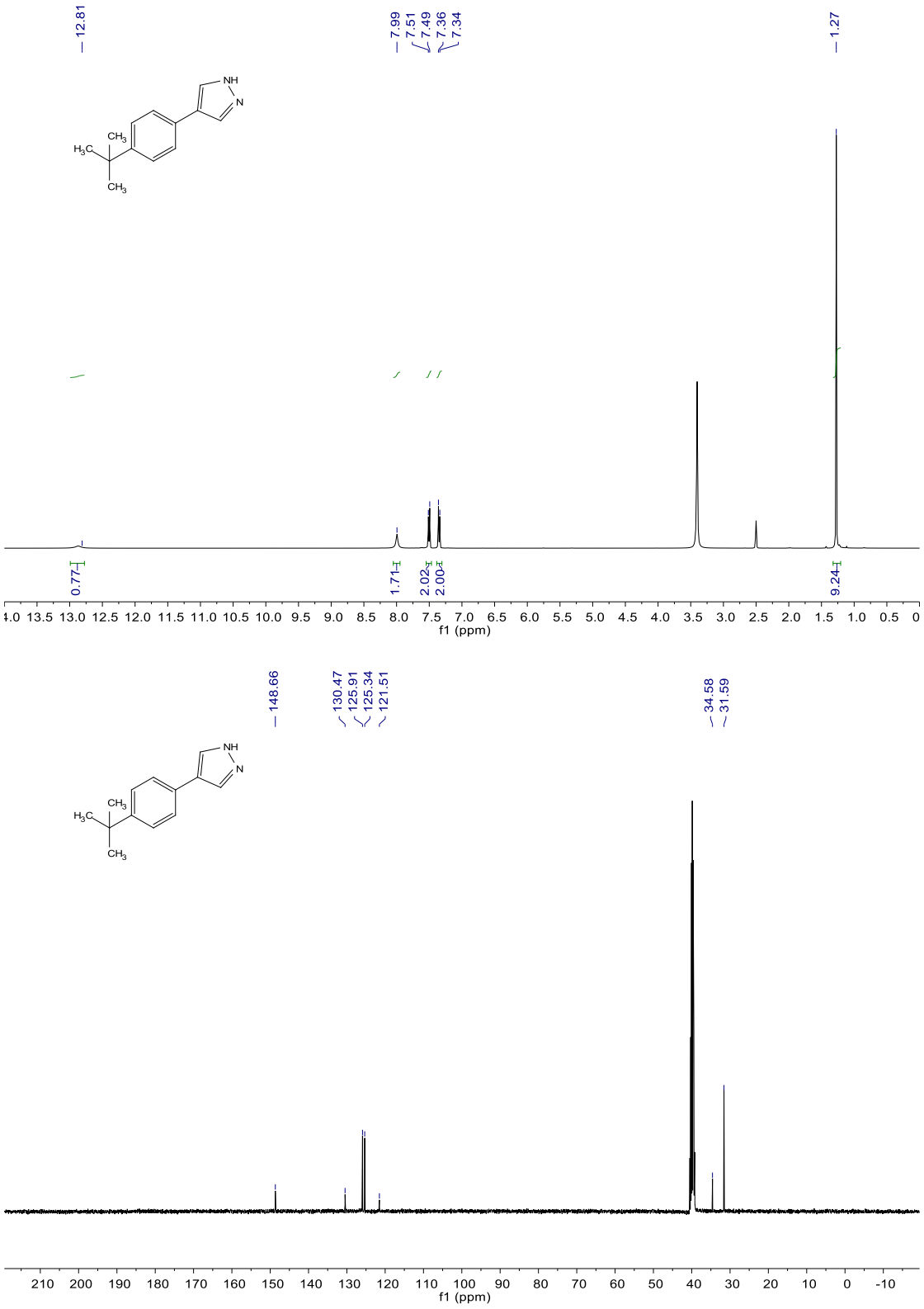


Fig. S17 NMR data of 4-(4-(tert-butyl)phenyl)-1H-pyrazole (2d)

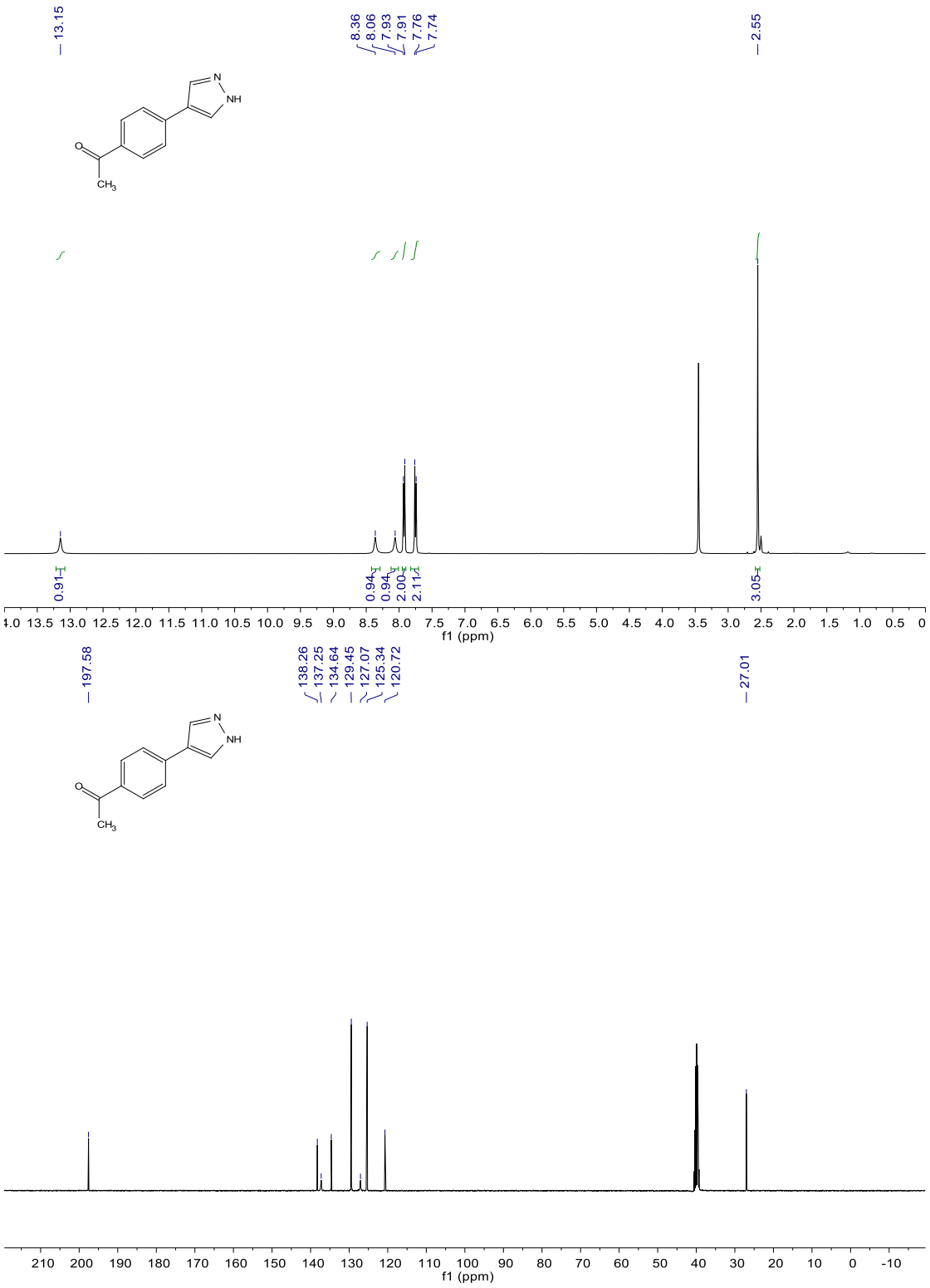


Fig. S18 NMR data of 1-(4-(1H-pyrazol-4-yl)phenyl)ethanone (2f)

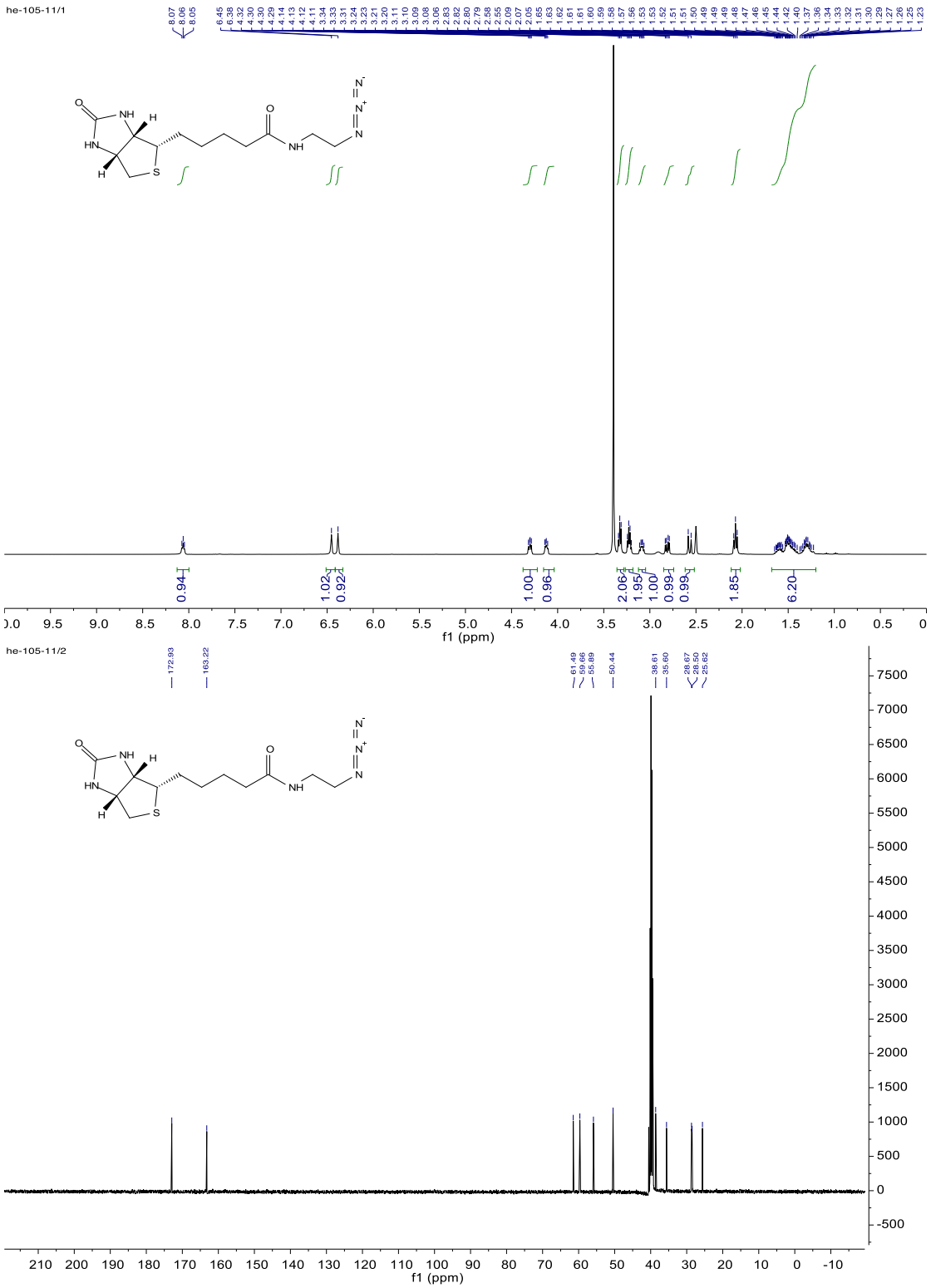


Fig. S19 NMR data of biotin-azide

References

1. E. D. Anderson, D. L. Boger, Inverse Electron Demand Diels–Alder Reactions of 1,2,3-Triazines: Pronounced Substituent Effects on Reactivity and Cycloaddition Scope. *J. Am. Chem. Soc.* **133**, 12285–12292 (2011).

2. E. J. Hanan *et al.*, Discovery of Selective and Noncovalent Diaminopyrimidine-Based Inhibitors of Epidermal Growth Factor Receptor Containing the T790M Resistance Mutation. *J. Med. Chem.* **57**, 10176–10191 (2014).

3. Y. Zhao *et al.*, Photocatalytic Cross-Dehydrogenative Amination Reactions between Phenols and Diarylamines. *ACS Catal.* **7**, 2446–2451 (2017).

4. E. Pedroso, A. Grandas, M. D. Ludevid, E. Giralt, Determination of the preferred tautomeric form of 4-nitrohistidine. *J. Heterocyclic Chem*. **23**, 921–924 (1986).

5. R. A. Serwa, J.-M. Swiecicki, D. Homann, C. P. R. Hackenberger, Phosphoramidate-peptide synthesis by solution- and solid-phase Staudinger-phosphite reactions. *J. Pept. Sci.* **16**, 563–567 (2010).

6. I. A. Inverarity, R. F. H. Viguier, P. Cohen, A. N. Hulme, Biotinylated Anisomycin: A Comparison of Classical and "Click" Chemistry Approaches. *Bioconj. Chem.* **18**, 1593–1603 (2007).

7. U.-S. Jeng *et al.*, A small/wide-angle X-ray scattering instrument for structural characterization of air–liquid interfaces, thin films and bulk specimens. *J. Appl. Cryst*. **43**, 110–121 (2009).