Supplementary Information

Content

1. General reagents and materials.

All commercially available chemical compounds were purchased from Sigma-Aldrich, Alfa Aesar, TCI, J&K, or adamas, and used without further purification. *E. coli* strain DH5α was used for plasmid amplification and BL21 (DE3) was used for protein expression. The antibiotic of kanamycin and ampicillin were purchased from solarbio. The mouse anti-His6, anti-Flag antibodies and HRP-conjugated goat anti-mouse antibodies were purchased from TransGen Biotech. All antibodies diluted with blocking buffer in a ratio of 1:3000 when use. The model peptide was purchased from GL biochem.

2. Instruments.

All NMR spectrums were recorded on a Bruker UltraShield-400 (400 MHz) instrument with chemical shifts recorded relative to tetramethylsilane. Protein mass spectrum was collected by an Ultimate 3000 HPLC coupled with a Q-Exactive Mass Spectrometer (Thermo Fisher Scientific). All protein concentration was detected by using bicinchoninic acid (BCA) protein determination method. Photocrosslinking experiments were performed by a CL-1000L Ultraviolet Crosslinker (UVP) with a fixed wave length of 365 nm. The images of both gel and western blot were all taken by Azure C400 (Azure Biosystems).

3. The HPLC-MS method for peptide and proteins.

Mobile phase used in all HPLC-MS analysis experiments are 0.1% formic acid in H_2O (mobile phase A) and 0.1% formic acid in acetonitrile (mobile phase B).

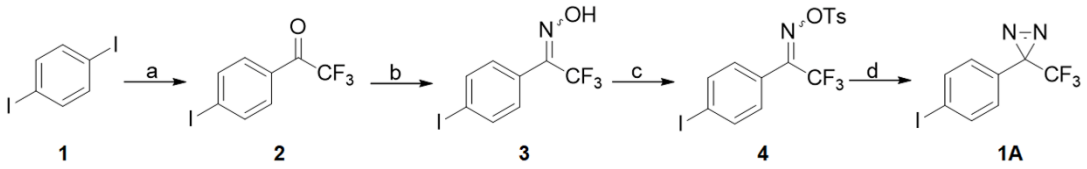
HPLC conditions for peptide: column: inertial® ODS-3, C18, 4.6 × 150 mm, 5 μm; temperature of column: 40°C; gradient: 0-2 min, 5% B; 2-10 min, 5-95% B; 10-11.5 min, 95% B; 11.5-12 min, 95%-5% B; 12-13 min, 5% B. Flow rate: 0.8 mL/min.

HPLC conditions for protein: column: Beijing Yuanbaoshan Chrom-Tech Co. Ltd, China, C4, 4.6 × 10 mm, 5 μm; temperature of column: 60°C; gradient: 0-0.5 min, 5% B; 0.5-2 min, 5-35% B; 2-3.5 min, 35% B; 3.5-5 min, 35-38% B, 5-5.5 min, 38-60% B; 5.5-7.5 min, 60% B;

7.5-8 min, 60-5% B; 8-9 min, 5% B. Flow rate: 0.8 mL/min.

MS conditions: sheath gas flow rate at 35, auxiliary gas flow rate at 10, sweep gas flow rate at 0, spray voltage at 3.5 kV, capillary temperature at 320°C, max injection time at 200 ms. MS full scans were carried out using a mass range of 500–2500 m/z, and the resolution was 70,000.

4. Chemical synthesis.



2,2,2-trifluoro-1-(4-iodophenyl)-ethanone (2)

The compound 2 was synthesized from 1,4-diodobenzene. A solution of butyllithium in hexanes (19.7 mL, 1.6 M, 1.5 equiv) was added into 20 mL dry THF, and the reaction solution was stirred under argon and cooled to -78°C. 1,4-diodobenzene (6.93 g, 21 mmol) was dissolved in 20 mL dry THF and added dropwise. The resulting yellow solution was stirred at -78°C for 1 h, following dropwise added the solution of ethyl trifluoroacetate (7.5 mL, 63 mmol, 3 equiv) in 15 mL dry Et_2O. After warming up to room temperature, 50 mL 1M HCl was added and the reaction mixture was extracted with Et_2O, and the organic layer was washed with brine (50 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue was purified by column chromatography using PE as eluent afforded trifluoroacetophenone as faint yellow solid (3.9 g, 60%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.94 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 180.1 (q, J = 35.1 Hz), 138.6, 131.1, 129.2, 116.5 (q, J = 289.2 Hz), 104.6.

^{19}F NMR (376 MHz, $CDCl_3$): δ −71.5.

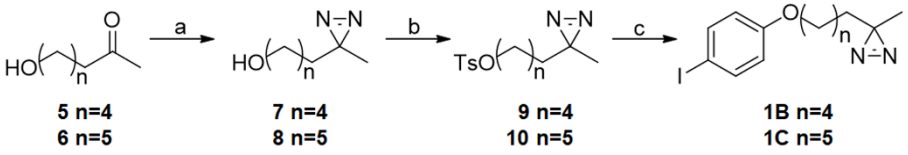
3-(4-iodophenyl)-3-trifluoromethyl-3H-diazirine (1A)

The compound 1A was synthesized from trifluoroacetophenone. Hydroxylamine hydrochloride (0.71 g, 10.2 mmol, 1.7 equiv) was dissolved in 4 mL dry pyridine and stirred, following added 2 mL dry ethanol. The solution of trifluoroacetophenone (1.8 g, 6 mmol, 1 equiv) in 2 mL dry pyridine was added dropwise. The resulting solution was stirred at 60°C for 2 h. The solvent was evaporated and the residue was dissolved in 30 mL Et_2O, and washed with 0.1 M HCl, water and brine, dried over anhydrous Na_2SO_4, and evaporated. The resulted crude oxime and TsCl (1.24 g, 6.58 mmol, 1.5 equiv) were added to 18 mL dry pyridine with stirring, and the reaction mixture was heated to reflux for 2 h. After the completion of this reaction, the pyridine was evaporated and the residue was dissolved in 30 mL Et_2O, and washed with water, dried over anhydrous Na_2SO_4, and evaporated. The crude products were added to 5 mL methanol with stirring at 0°C. A solution of ammonia in methanol (7 M, 6 mL) was added dropwise, and the reaction was allowed to warm up to room temperature and left for additional 10 h. The excess of ammonia was evaporated, and the residue was dissolved in 30 mL Et_2O, and washed with water, dried over anhydrous Na_2SO_4, and evaporated. The crude products were dissolved in 5 mL methanol and stirred at 0°C. Triethylamine (3 equiv) was added dropwise to the reaction system. A solution of iodine (1 equiv) in methanol were added dropwise until the dark brown color of iodine persisted for at least 30 s. The reaction mixture was warm up to room temperature and stirred for additional 30 min. The methanol was evaporated and the residue was dissolved in 30 mL Et_2O, washed with water and brine, dried over anhydrous Na_2SO_4, evaporated, and purified by column chromatography using PE as eluent afforded compound 1A as colorless liquid.

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (d, J = 8.6 Hz, 2H), 6.92 (d, J = 8.4, 2H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 138.0, 128.8, 128.1, 122.0 (q, J = 273.0 Hz, CF_3), 96.0 (C−I), 28.3 (q, J = 40.7 Hz, CN_2).

^{19}F NMR (376 MHz, $CDCl_3$): δ −65.2.



5 n=4
6 n=5

7 n=4
8 n=5

9 n=4
10 n=5

1B n=4
1C n=5

2-(3-methyl-3H-diazirin-3-yl)ethyl 4-methylbenzenesulfonate (9) and 3-(3-methyl-3H-diazirin-3-yl)propyl 4-methylbenzenesulfonate (10)

Compound 9 and 10 were synthesized using 4-hydroxy-2-butanone and 5-hydroxy-2-pentanone as starting reactant, respectively. 4-hydroxy-2-butanone (8.6 g, 98 mmol) or 5-hydroxy-2-pentanone (10 g, 98 mmol) was added to an argon-filled 500 mL three-neck flask with a cold finger condenser containing a 0°C ice bath. A solution of ammonia in methanol (7 M, 98 mL) was added dropwise. The reaction was stirred at 0°C for 5 h under nitrogen. After that, a solution of hydroxylamine-O-sulfonic acid (12.7 g, 112.7 mmol, 1.15 equiv) in 80 mL methanol was added dropwise with a syringe pump, the white suspension liquid was warmed up to room temperature and stirred for another 40 h. The suspension solution was filtered and the filter was evaporated. The crude products were dissolved in 50 mL methanol and stirred at 0°C. Triethylamine (3 equiv) was added dropwise to the reaction system. Solid iodine was added slowly until the dark brown color of iodine persisted for at least 30 s. The reaction mixture was warm up to room temperature and stirred for additional 30 min. The methanol was evaporated and the residue was dissolved in 50 mL ethyl acetate, washed with water, 10% $Na_2S_2O_3$ and brine, dried over anhydrous Na_2SO_4, evaporated. The crude products (2 g) and TsCl (3.34 g, 17.5 mmol, 1 equiv) were dissolved in 50 mL DCM with stirring at 0°C ice bath. The KOH powder (2.95 g, 52.5 mmol, 3 equiv) was added slowly at 0°C. The reaction was stirred at 0°C for 30 min and warmed up to room temperature for additional 3.5 h. The reaction mixture was added 50 mL water and extracted with DCM for three times. The organic layer was combined and washed with brine, dried over anhydrous Na_2SO_4, evaporated. The residue was purified by column chromatograph (5-10% ethyl acetate in PE, linear gradient) affording compound 9 or 10 as faint yellow liquid. (Compound 9, 2.32 g, 10%; Compound 10, 3.75 g, 15% overall yield for four steps).

Compound 9:

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.82 (d, J = 8.3 Hz, 2H), 7.38 (d, J = 8.0 Hz, 2H), 3.96 (t, J = 6.4 Hz, 2H), 2.47 (s, 3H), 1.68 (t, J = 6.4 Hz, 2H), 1.01 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 145.06, 132.72, 129.93, 127.96, 65.14, 34.17, 23.40, 21.67, 19.77.

Compound 10:

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.77 (d, J = 8.0 Hz, 2H), 7.35 (d, J = 8.0 Hz, 2H), 3.98 (t, J = 6.0 Hz, 2H), 2.45 (s, 3H), 1.58-1.46 (m, 2H), 1.40 (dd, J = 8.8, 6.0 Hz, 2H), 0.96 (s, 3H)

^{13}C NMR (101 MHz, $CDCl_3$): δ 132.92, 129.90, 127.84, 69.42, 30.27, 25.02, 23.53, 21.62, 19.67.

3-(2-(4-iodophenoxy)ethyl)-methyl-3H-diazirine (1B) and 3-(2-(4-iodophenoxy)propyl)-methyl-3H-diazirine (1C)

Compound 1B and 1C were synthesized from compound 9 and 10, respectively. Compound 9 (127 mg, 0.5 mmol, 1 equiv) or compound 10 (131 mg, 0.49mmol, 1 equiv), 4-iodophenol (110 mg, 0.5 mmol, 1 equiv), and $CsCO_3$ (244 mg, 0.75 mmol, 1.5 equiv) were added to 5 mL DMF with stirring at room temperature. The reaction mixture was warmed up to 80°C and stirred for another 1 h. After the completion of reaction, 20 mL ethyl acetate was added and the mixture was washed with water and brine, then evaporated. The residue was purified by column chromatograph (5-10% ethyl acetate in PE, linear gradient) affording compound 1B or 1C as colorless liquid. (Compound 1B, 138 mg, 91%; Compound 1C, 146 mg, 94%).

Compound 1B:

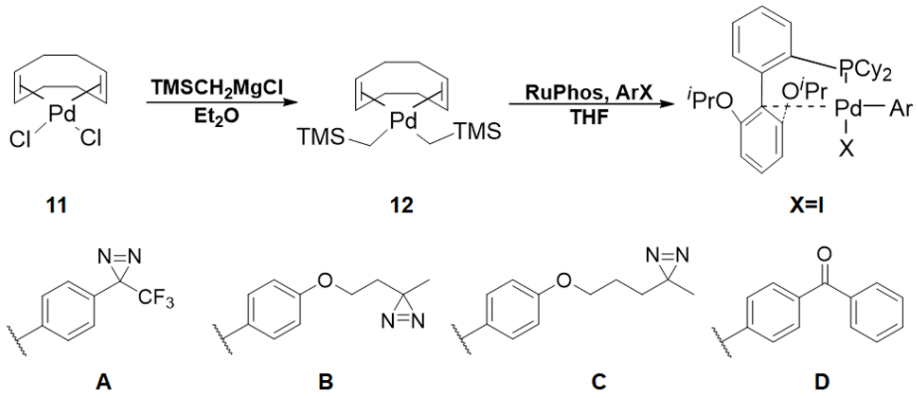
^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58 (d, J = 8.8 Hz, 2H), 6.70 (d, J = 8.8 Hz, 2H), 3.85 (t, J = 6.3 Hz, 2H), 1.83 (t, J = 6.3 Hz, 2H), 1.13 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 158.40, 138.27, 116.96, 83.14, 63.04, 34.23, 24.21, 20.24.

Compound 1C:

^{1}H NMR (400 MHz, $CDCl_3$): δ 7.56 (d, J = 8.8 Hz, 2H), 6.67 (d, J = 8.8 Hz, 2H), 3.90 (t, J = 6.1 Hz, 2H), 1.72-1.63 (m, 2H), 1.56 (dd, J = 8.4, 6.4 Hz, 2H), 1.06 (s, 3H).

^{13}C NMR (101 MHz, $CDCl_3$): δ 158.67, 138.22, 116.87, 82.78, 66.93, 30.86, 25.47, 23.80, 19.89.



$(1,5\text{-COD})PdCl_2$, $(1,5\text{-COD})Pd(CH_2SiMe_3)_2$ and all of the photoaffinity palladium reagents (compound A, B, C and D) were prepared according to literature procedure.[1, 2]

Photoaffinity palladium reagents (A, B, C and D)

General procedure: In an argon-filled glovebox, an oven-dried scintillation vial (10 mL), which was equipped with a magnetic stir bar, was charged with RuPhos (66 mg, 0.14 mmol, 1.1 equiv), Ar–I (0.12 mmol, 1.1 equiv), and THF (1 mL). Solid $(1,5\text{-COD})Pd(CH_2SiMe_3)_2$ (50 mg, 0.13 mmol, 1 equiv) was added rapidly in one portion and the resulting solution was stirred for 16 h at room temperature. After that, 3 mL pentane was added and the resulting mixture was placed into a -20°C freezer for 3 h. The vial was then taken outside of the glovebox, and the resulting precipitate was filtered, washed with pentane (3 × 3 mL), and dried under reduced pressure to afford the oxidative addition complex as faint yellow solid.[2]

Compound A:

^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.65 (m, 2H), 7.47 (t, J = 7.5 Hz, 1H), 7.41 (t, J = 7.5 Hz, 1H), 7.18 (dd, J = 8.5, 1.6 Hz, 2H), 6.87 (d, J = 7.7 Hz, 1H), 6.79 (d, J = 8.2 Hz, 2H), 6.69 (d, J = 8.4 Hz, 2H), 4.62 (hept, J = 6.0 Hz, 2H), 2.14 (m, 2H), 1.77 (s, 6H), 1.68 (d, J = 12.0 Hz, 4H), 1.56 (m, 3H), 1.42 (d, J = 6.0 Hz, 6H), 1.23 (m, 4H), 1.12 (t, J = 13.4 Hz, 2H), 1.05 (d, J = 6.1 Hz, 6H), 0.66 (m, 2H).

^{19}F NMR (376 MHz, CD_2Cl_2): δ -65.99.

^{31}P NMR (121 MHz, CD_2Cl_2): δ 26.93.

Compound B:

^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.64-7.58 (m, 2H), 7.44-7.41 (m, 1H), 7.38-7.35 (m, 1H), 6.89 (d, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 4.2 Hz, 1H), 6.67 (d, *J* = 8.5 Hz, 2H), 6.58 (d, *J* = 8.8 Hz, 2H), 4.59 (hept, J = 6.0 Hz, 2H), 3.80 (t, *J* = 6.4 Hz, 2H), 2.18-2.10 (m, 2H), 1.77-1.72 (m, 6H), 1.65 (br, 4H), 1.59-1.50 (m, 3H), 1.38 (d, *J* = 6.0 Hz, 6H), 1.31-1.24 (m, 4H), 1.22-1.15 (m, 4H), 1.08 (s, 3H), 1.01 (d, *J* = 6.0 Hz, 6H), 0.81-0.72 (m, 2H).

^{31}P NMR (121 MHz, CD_2Cl_2): δ 26.63.

Compound C:

^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.64-7.57 (m, 2H), 7.44-7.41 (m, 1H), 7.38-7.34 (m, 1H), 6.92-6.83 (m, 3H), 6.64 (dd, *J* = 14.4, 8.5 Hz, 2H), 6.57 (dd, *J* = 8.6 Hz, 8.6 Hz, 2H), 4.59 (hept, *J* = 6.0 Hz, 2H), 3.83 (t, *J* = 6.0 Hz, 2H), 2.18-2.10 (m, 2H), 1.77-1.72 (m, 6H), 1.67-1.59 (m, 4H), 1.56-1.48 (m, 3H), 1.38 (d, *J* = 6.0 Hz, 6H), 1.31-1.24 (m, 4H), 1.22-1.15 (m, 4H), 1.12-1.06 (m, 2H), 1.02 (s, 3H), 1.01 (d, *J* = 6.0 Hz, 6H), 0.82-0.74 (m, 2H).

^{31}P NMR (121 MHz, CD_2Cl_2): δ 26.61.

Compound D:

^{1}H NMR (400 MHz, CD_2Cl_2): δ 7.71 (dd, *J* = 8.4,1.6 Hz, 2H), 7.66 (m, 2H), 7.56 (t, *J* = 7.2 Hz, 1H), 7.48-7.43 (m, 3H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.33 (d, *J* = 8.2 Hz, 2H), 7.27 (dd, *J* = 8.6, 1.6 Hz, 2H), 6.85 (ddd, *J* = 7.6, 2.8, 0.8Hz, 1H), 6.68 (d, *J* = 8.5 Hz, 2H), 4.60 (hept, *J* = 6.4 Hz, 2H), 2.19 (m, 2H), 1.79 (m, 6H), 1.67 (m, 4H), 1.61-1.54 (m, 3H), 1.39 (d, *J* = 6.0 Hz, 6H), 1.26-1.10 (m, 6H), 1.02 (d, *J* = 6.0 Hz, 6H), 0.77 (m, 2H).

^{31}P NMR (121 MHz, CD_2Cl_2): δ 26.68.

5. Plasmids construction and site mutation of proteins

pET28a plasmids inserted with ABI1 gene, and pGEX-6p-1 plasmids inserted with PYR1-Flag gene were constructed for corresponding protein expression in *E. coli* cells. Plasmids inserted with PYR1S and PYR1S-H60C/K63C/R116C/T162C/L166C-Flag gene were performed from pGEX-6p-1-PYR1-Flag plasmids by site-directed mutations with a mutation kit.

6. Proteins expression and purification

Plasmids of pET28a-ABI1, pGEX-6p-1-PYR1 and pGEX-6p-1-PYR1S-C were transformed into *E. coli* BL21 (DE3) cells. Cells were grown at 37°C in 800 mL fresh LB medium which containing corresponding antibiotic for about 4 h until OD_{600} reached 0.5, at which point the temperature was lowered to 16°C. Cells were induced by 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) before growing overnight at 16°C. The cells were harvested by centrifugation at 4600 rpm for 15 min, discarding supernatant and the remaining cells lysed by sonication for 30 min in suitable buffer. Buffer 1 (20 mM Tris-HCl, 150 mM NaCl, pH 8.0) was for PYR1. Buffer 2 (50 mM Tris-HCl, 150 mM NaCl, 5 mM $MgCl_2$, 5% glycerol, pH 8.0) was for ABI1. The cell lysates were centrifuged at 12000 rpm for 45 min. After that, recombinational proteins in supernatant were purified by Ni-NTA (Qiagin) affinity column (for His6 tag) or Glutathione-Sepharose beads (for GST tag). GST tagged proteins were eluted by buffer 3 (20 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0). Proteins have no GST tag was cut by prescission protease overnight at 4°C. All purified proteins were concentrated by ultrafiltration and stored at -80°C.

7. ABI1 phosphatase activity assay

The phosphatase activity of ABI1 was measured by a Serine/Threonine phosphatase assay system kit (Promega). In a 50 µL reaction system, 10 µL 5 × reaction buffer (250 mM imidazole, 25 mM $MgCl_2$, 1 mM EGTA, and 0.5 mg/mL BSA, pH 7.2) and certain volume ddH_2O were added firstly, followed by a final concentration of 6 µM PYR1, PYR1 mutants or modified PYR1S-C, 20 µM ABA and 0.3 µM ABI1, and finally, the peptide substrate (supplied in kit). The reaction mixture was incubated at 30°C for 20 min and stopped by adding 50 µL molybdate dye. Another 15 min incubation at room temperature was needed before absorbance measure at 630 nm. The reaction without ABI1 was set as negative control. The data present the means ± standard deviation from three independent experiment results.

8. General procedure for peptide labeling

Peptide (2 μL, 300 μM in water), H_2O (49 μL), and buffer (6 μL, 10 × stock solution of different buffer in Table 1) were combined in a 1.5 mL Eppendorf tube and the solution was mixed by vortexing. A stock solution of each photoaffinity reagents (3 μL, 400 μM; 0.6 μL, 2 mM) in organic solvent was added in one portion, and the reaction tube was vortexed to ensure proper reagent mixing and left for 5 min at room temperature (The reaction time for entry 13 was 30 min.). The reaction was quenched by the addition of 3-mercaptopropionic acid (6.3 μL, 0.05 μL/mL solution in water, 3 equivalents to the palladium complex). After an additional 5 min, a solution of 50% A: 50% B (v/v, 60 μL) was added to the tube and the reaction system was analyzed by HPLC-MS. All yields of aryl conjugate products were calculated from TIC spectra.

9. General procedure for protein labeling

For protein labeling, PYR1S-C was added to 20 mM Tris-HCl buffer (pH 8.0) with a final concentration of 1 μM. Each photoaffinity palladium reagents (125 μL, 200 μM) in organic solvent was added into the protein solution slowly. The solution was pipetted up and down to ensure proper reagent mixing. The reaction mixture was left for 30 min at room temperature, then quenched by the addition of 3-mercaptopropionic acid (25 μL, 10 mM, dissolved in buffer of 20 mM Tris-HCl, pH 8.0). After an additional 5 min at room temperature, the reaction mixture was transferred to a 10K centrifugal filter (Millipore), and concentrated at 3000 g, 4°C, for 30 min. The modified proteins were added 5 mL buffer (20 mM Tris-HCl, 150 mM NaCl, pH 8.0) to remove the residue palladium complex, concentrated to about 50 μL volume and analyzed by HPLC-MS.

10. Photocrosslinking analysis of interactions between modified PYR1 and ABI1.

Following the protein modification, 10 μM the final modified protein with or without 100 μM ABA were incubated with 10 μM ABI1 at buffer (50 mM Tris-HCl, 150 mM NaCl, 5 mM $MgCl_2$, 5% glycerol, pH 8.0) for 10 min. The UV groups were transferred to a 96-well plate and the plate was placed on ice water. After 365 nm UV exposure for 10 min at a distance

of 15 cm, the mixture was cooled down for 2 min. The procedure was repeated two times and total UV exposure time was 20 min. The photocrosslinking products were detected with anti-His6 and anti-Flag antibody after being separated by 12% SDS-PAGE.

11. Protein sequence used in this work

> PYR1-Flag

GPLGSMAPSELTPEERSELKNSIAEFHTYQLDPGS**C**SSLHAQRIHAPPELVWSIVRRFDKPQTYK**H**FI**K**S**C**SVEQNFEMRVG**C**TRDVIVISGLPANTSTERLDILDDERRVTGFSIIGGEH**R**LTNYKSVTTVHRFEKENRIWTVVLESYVVDMPEGNSEDDTRMFAD**T**VVK**L**NLQKLATVAEAMARNSGDGSGSQVT**DYKDDDDK**

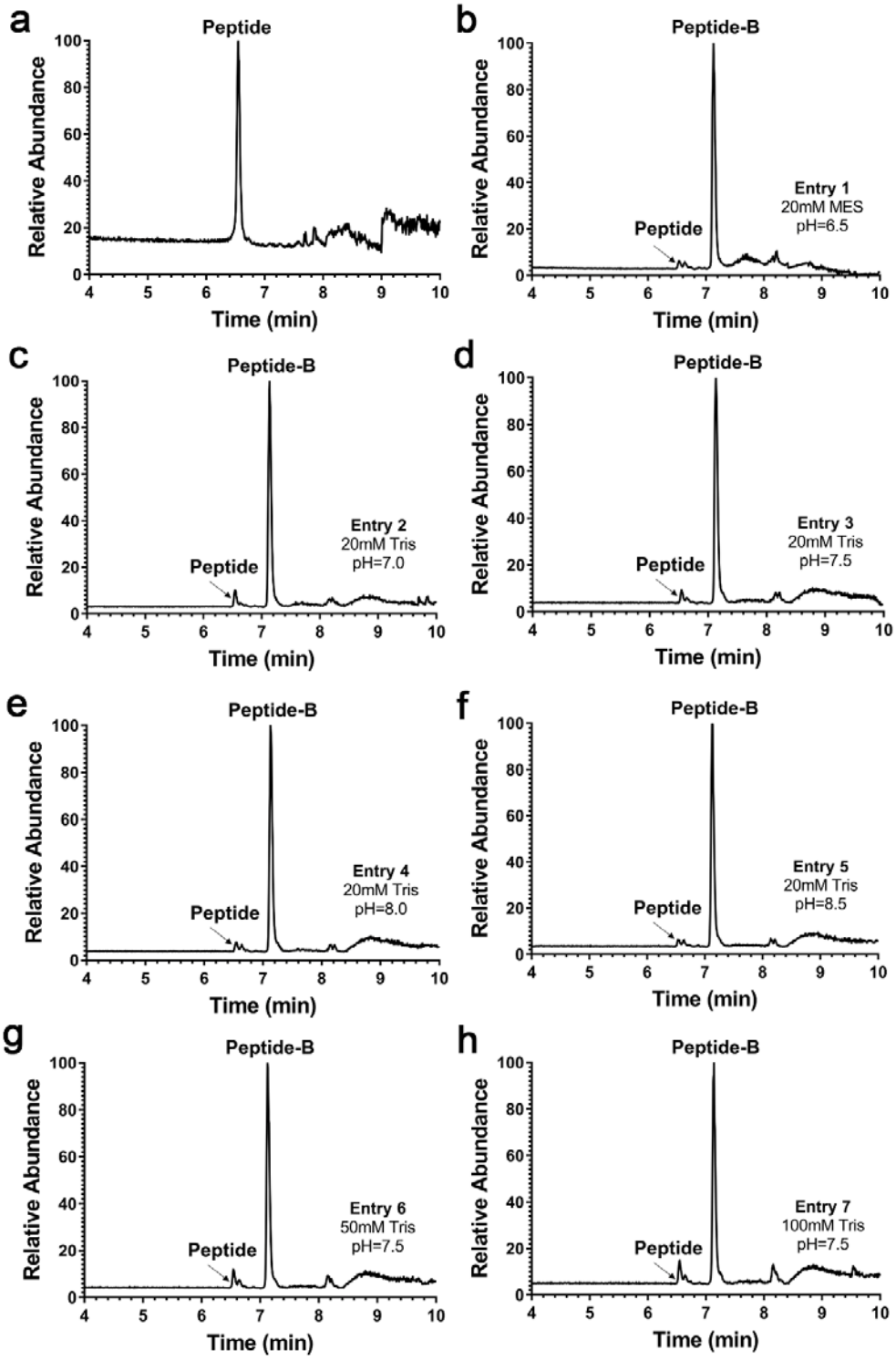
The cysteine in wild type PYR1 were marked as red, and the positions chosen for cysteine mutation were marked as yellow.

> ABI1-His

MGSS**HHHHHH**SSGLVPRGSHMRSLFEFKSVPLYGFTSICGRRPEMEDAVSTIPRFLQSSSGSMLDGRFDPQSAAHFFGVYDGHGGSQVANYCRERMHLALAEEIAKEKPMLCDGDTWLEKWKKALFNSFLRVDSEIESVAPETVGSTSVVAVVFPSHIFVANCGDSRAVLCRGKTALPLSVDHKPDREDEAARIEAAGGKVIQWNGARVFGVLAMSRSIGDRYLKPSIIPDPEVTAVKRVKEDDCLILASDGVWDVMTDEEACEMARKRILLWHKKNAVAGDASLLADERRKEGKDPAAMSAAEYLSKLAIQRGSKDNISVVVVDLKPRRKLKSKPLN&

12. Supplementary Figures.

Figure S1



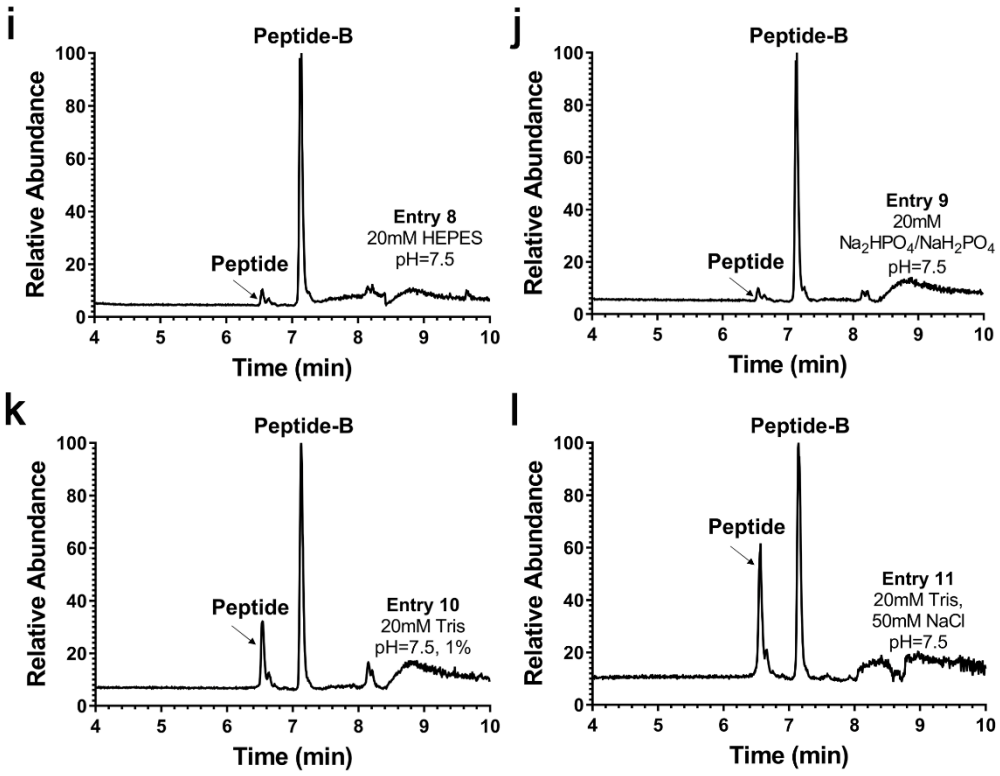


Figure S1. The HPLC analysis of compound B reacting with model peptide in different buffer in Table 1. Above figures showed the TIC data of each sample and the reacting buffer was marked on the bottom right corner.

Figure S2

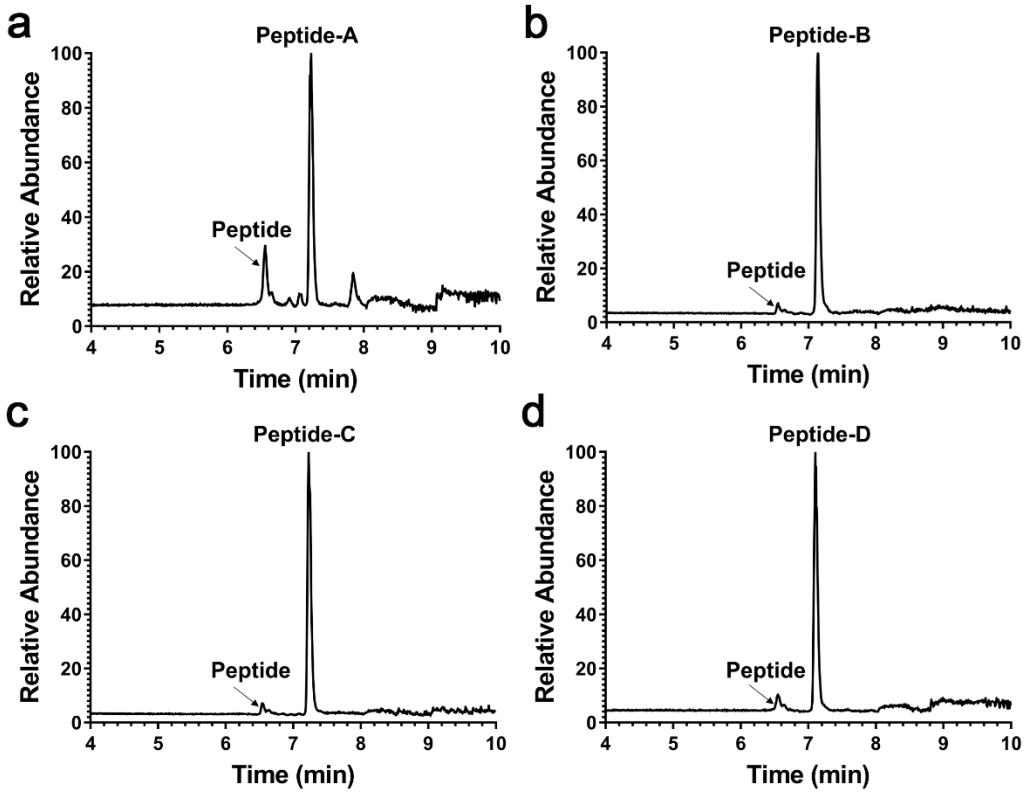
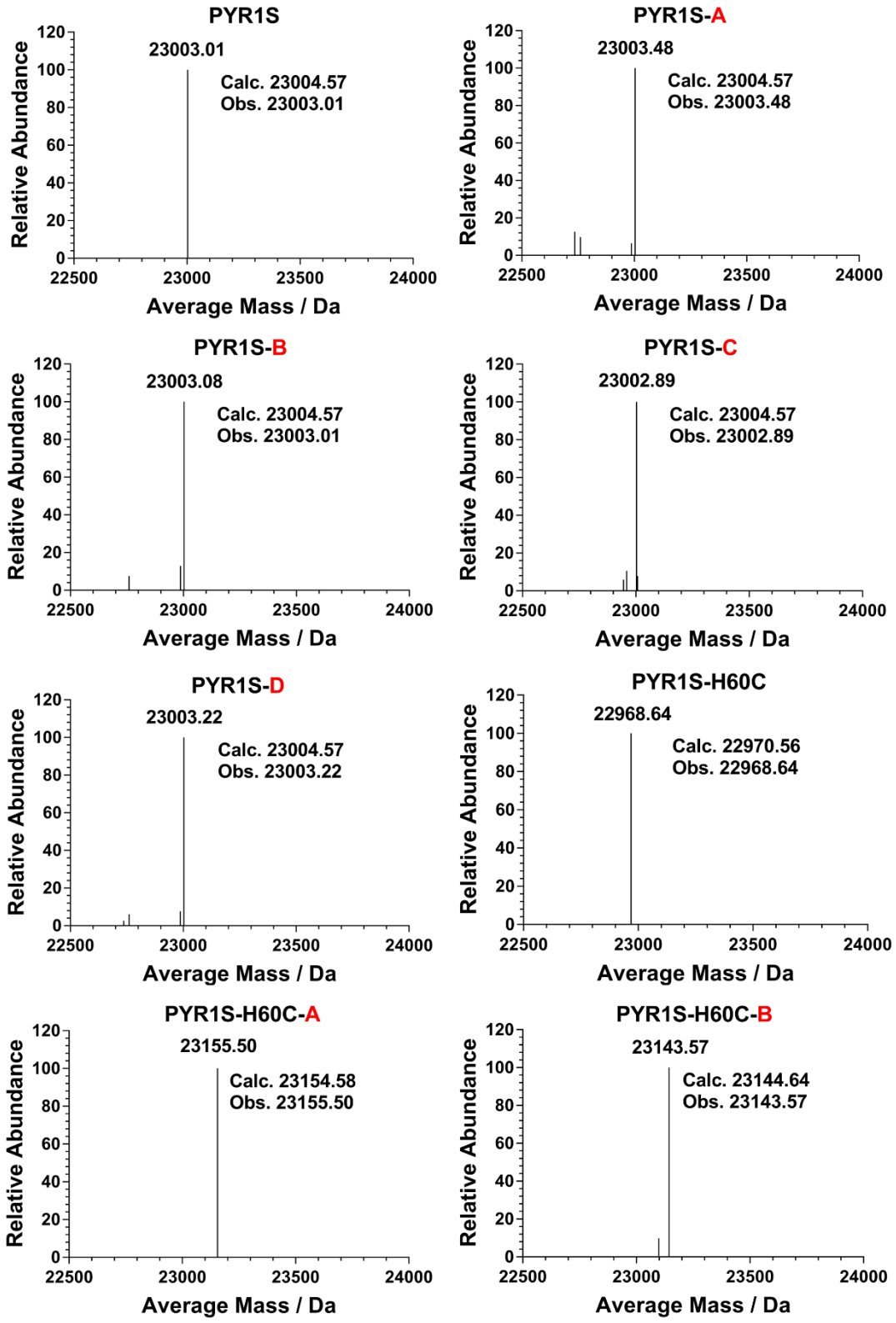


Figure S2. The HPLC analysis of peptides that modified by stored Photoaffinity palladium reagents. The paladium compounds powder, which stored at -80°C for more than one year, was dissolved in organic solvent (50% DMSO and 50% acetonitrile) to a concentration of 2.5 mM. And the stock solution was stored at -80°C for another two month. The TIC data of peptides modified by compound A (a), B (b), C (c) and D (d), demonstrated that stored photoaffinity palladium complex could still react with model peptide generating corresponding aryl-conjugate products.

Figure S3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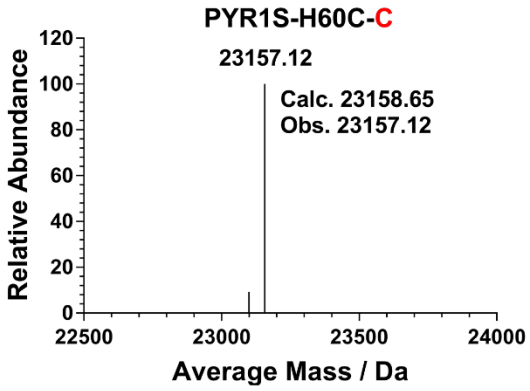
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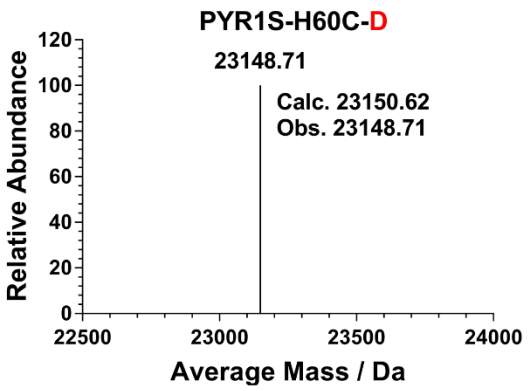
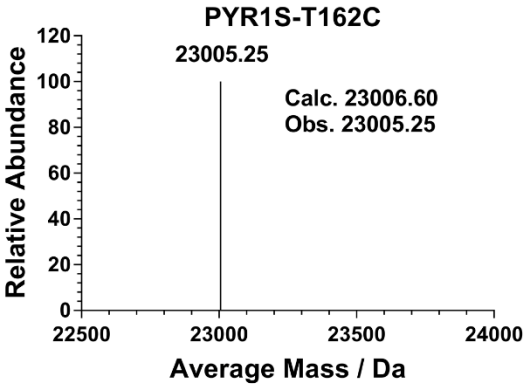
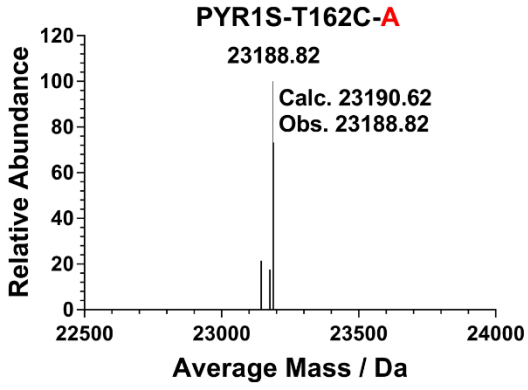
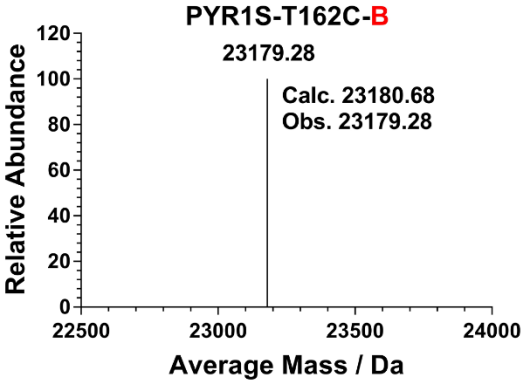
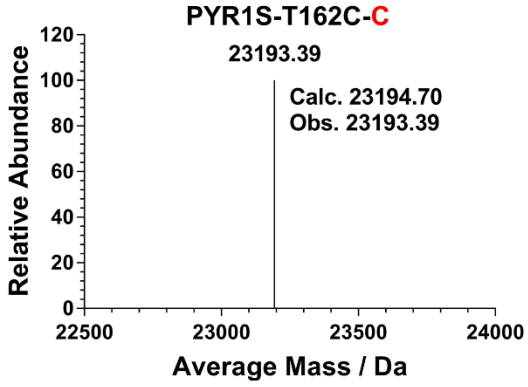
Relative Abundance
Average Mass / Da

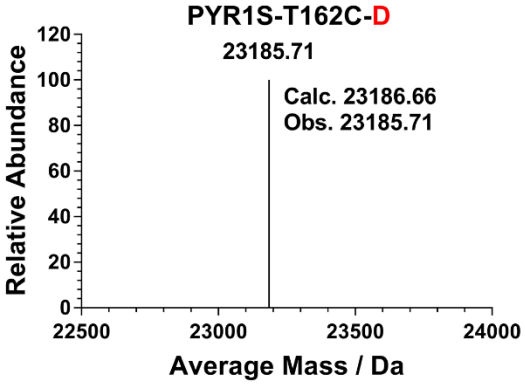

PYR1S-H60C-D
23148.71
Calc. 23150.62
Obs. 23148.71
Relative Abundance
Average Mass / Da

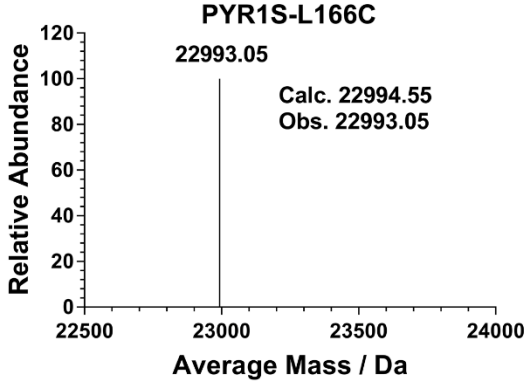

PYR1S-T162C
23005.25
Calc. 23006.60
Obs. 23005.25
Relative Abundance
Average Mass / Da


PYR1S-T162C-A
23188.82
Calc. 23190.62
Obs. 23188.82
Relative Abundance
Average Mass / Da


PYR1S-T162C-B
23179.28
Calc. 23180.68
Obs. 23179.28
Relative Abundance
Average Mass / Da


PYR1S-T162C-C
23193.39
Calc. 23194.70
Obs. 23193.39
Relative Abundance
Average Mass / Da


PYR1S-T162C-D
23185.71
Calc. 23186.66
Obs. 23185.71
Relative Abundance
Average Mass / Da


PYR1S-L166C
22993.05
Calc. 22994.55
Obs. 22993.05
Relative Abundance
Average Mass / Da

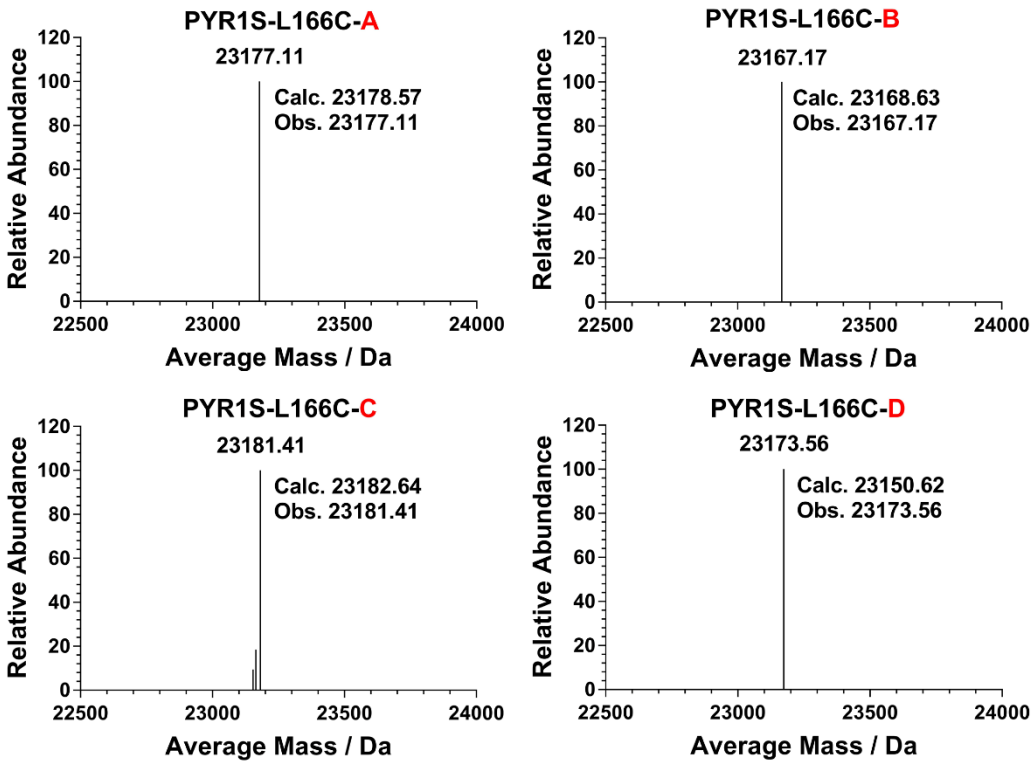


Figure S3. The mass data of wild type PYR1, PYR1 mutants and modified proteins. The deconvoluted mass spectra showed the full-length molecular weight of PYR1S, PYR1S-H60C, PYR1S-T162C, PYR1S-L166C, before and after being modified by compound A, B, C and D. All the cysteine-containing PYR1 mutants could be modified by these four photoaffinity palladium reagents, and generating the corresponding sulfhydryl-aryl conjugate products. The calculated and observed molecular weight were shown in each figure.

Figure S4

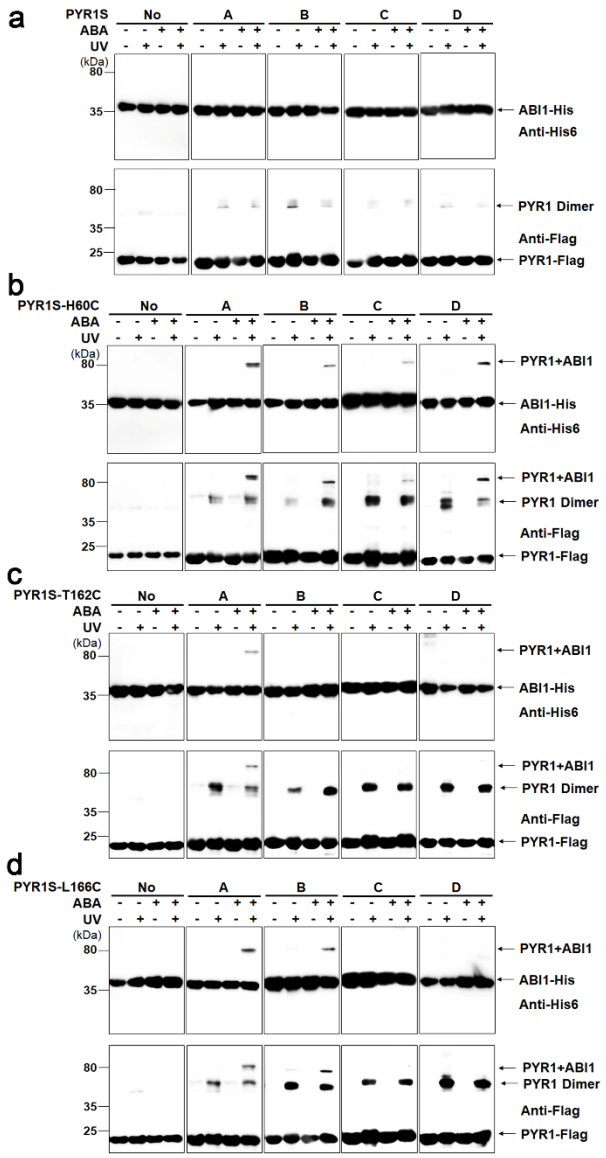
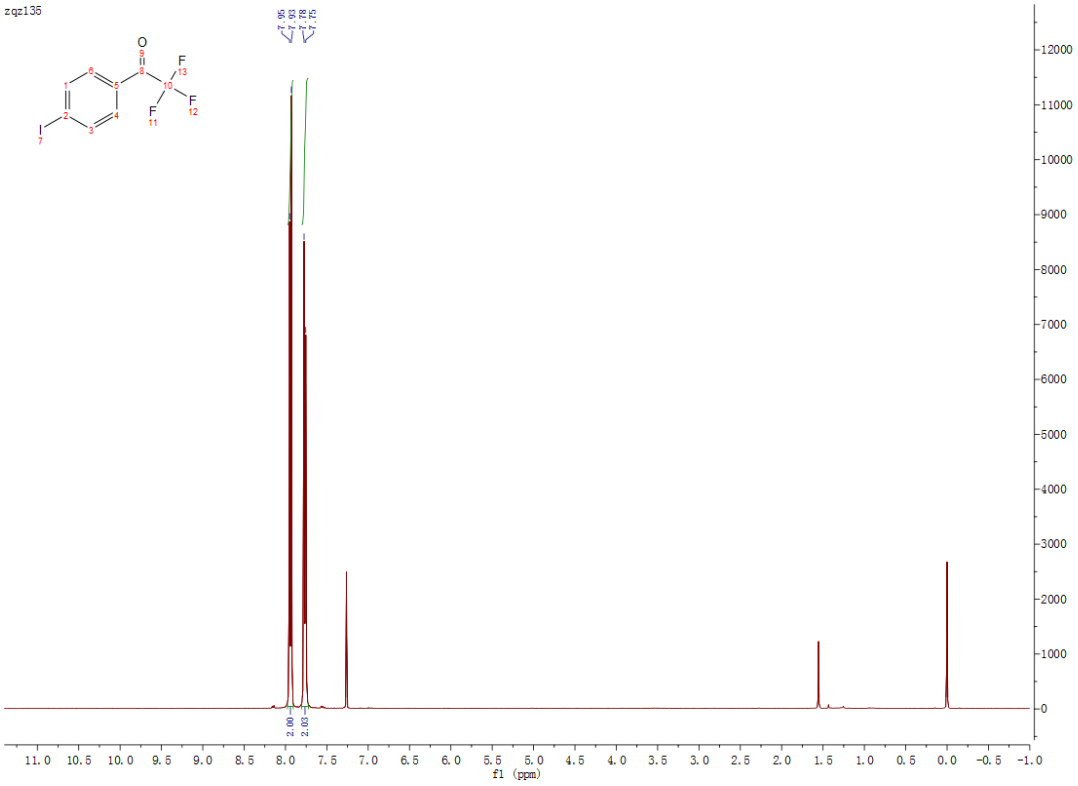
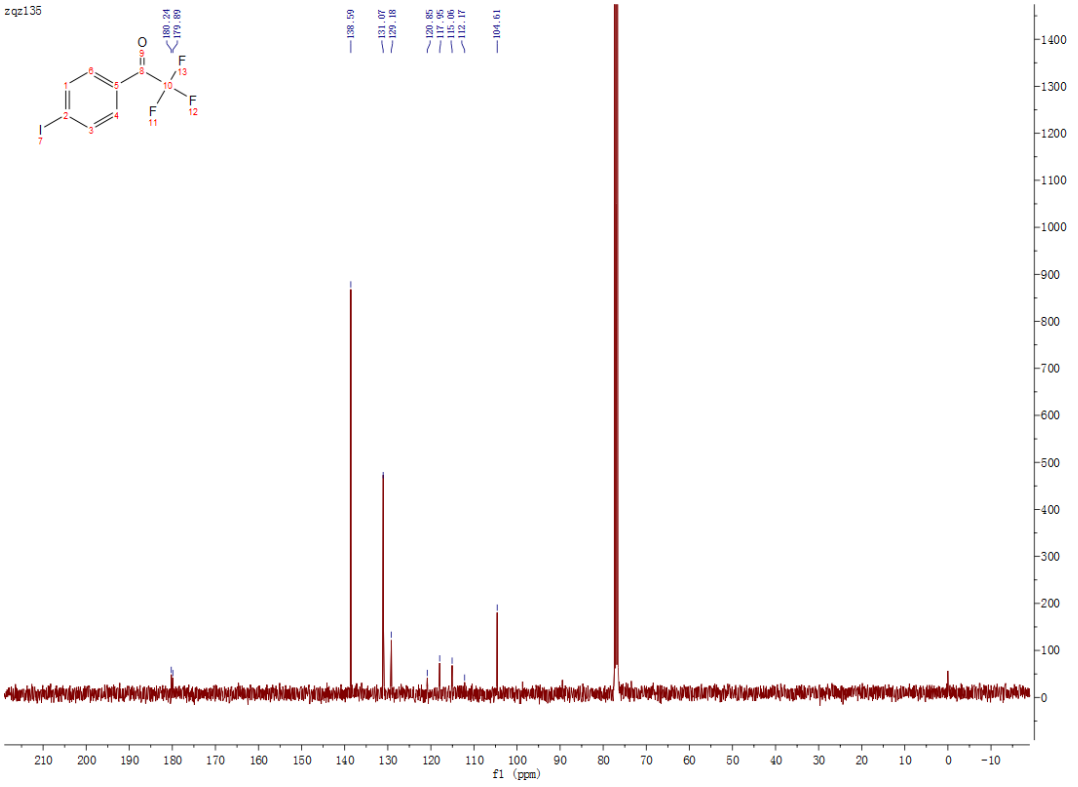
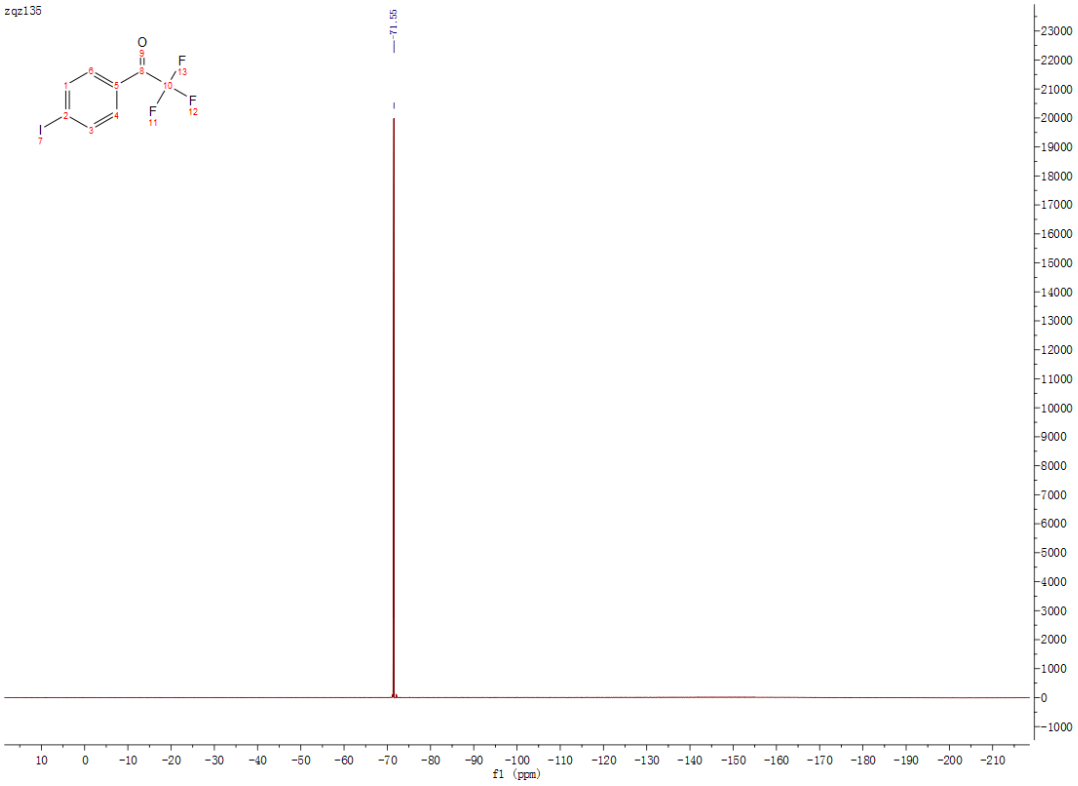


Figure S4. Photocrosslinking analysis of interactions between photoactivatable groups incorporated PYR1S-C and ABI1. 10 μM modified PYR1S-C-Flag and 10 μM purified ABI1-His6 were incubated with 100 μM ABA for 10 min, followed by 365 nm UV irradiation for 20 min at 0°C, non-ABA and non-UV treated samples as control. The photocrosslinking products were separated by 12% SDS-PAGE and detected by anti-His6 and anti-Flag antibody. Above figures were the anti-His6 and anti-Flag western blot results of interactions between ABI1-His and modified PYR1S-C-Flag. (a), (b), (c), and (d) was for protein PYR1S, PYR1S-H60C, PYR1S-T162C, PYR1S-L166C, respectively.

13. NMR Spectrum



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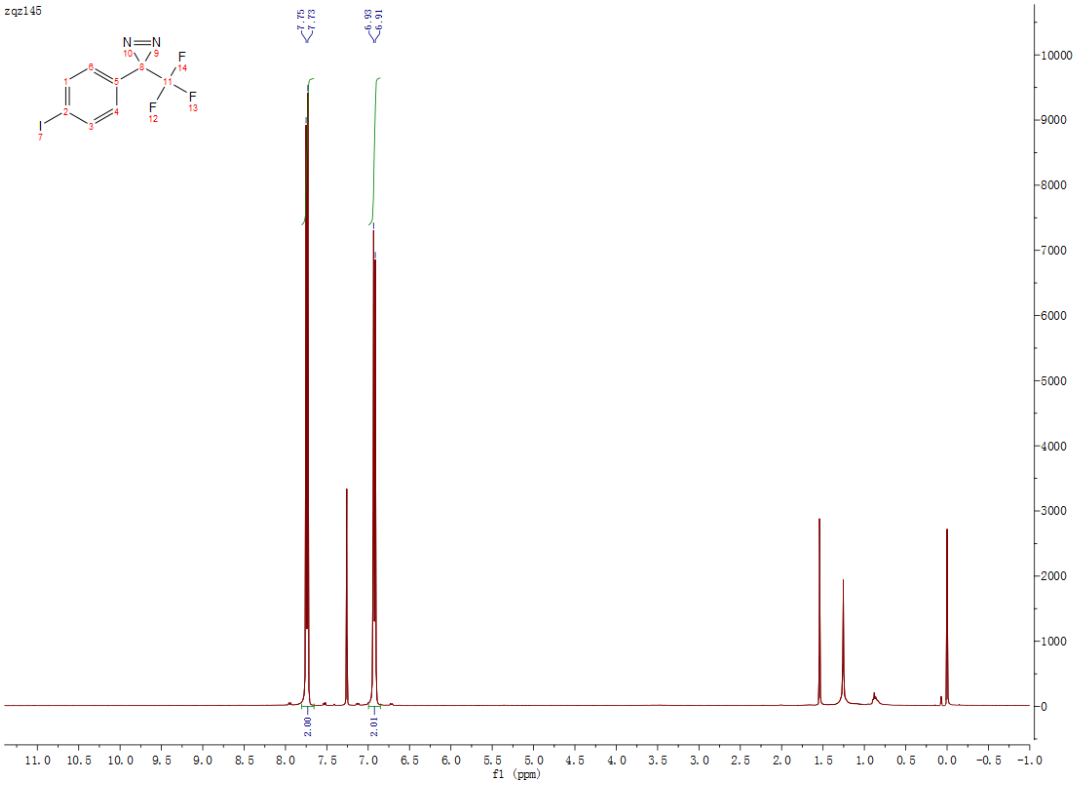
f1 (ppm)


zqz145
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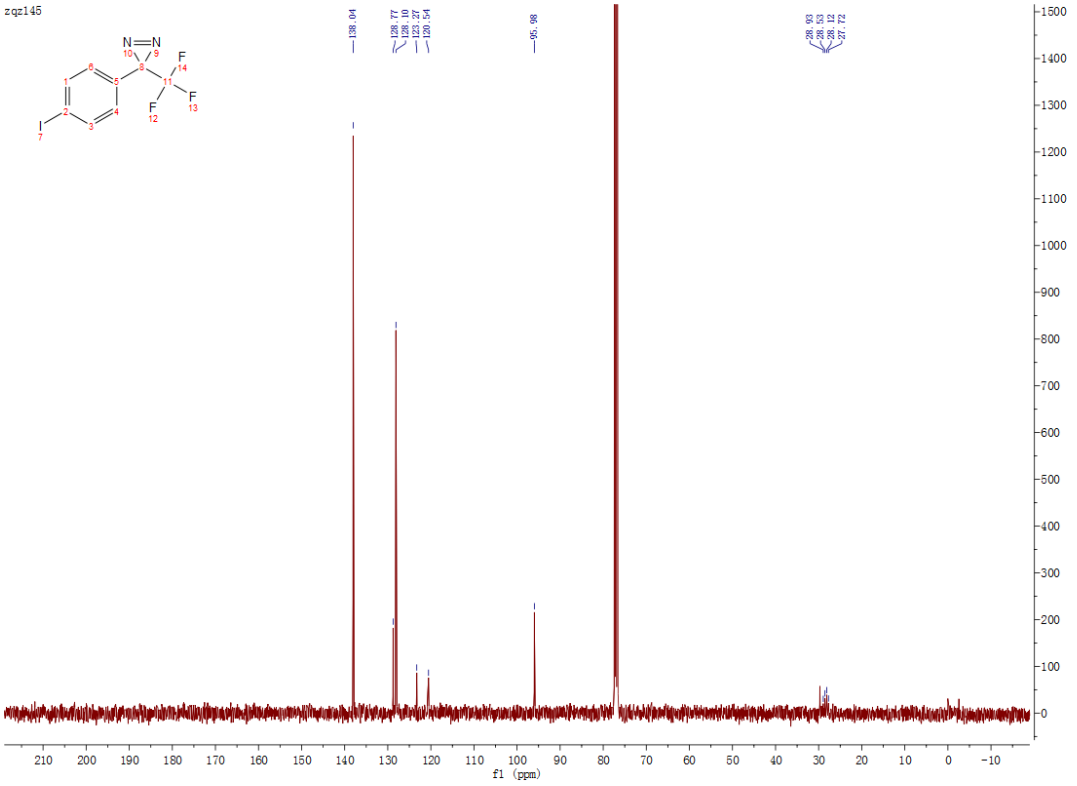
7.73
6.93
6.91
2.00
2.01
f1 (ppm)


zqz145
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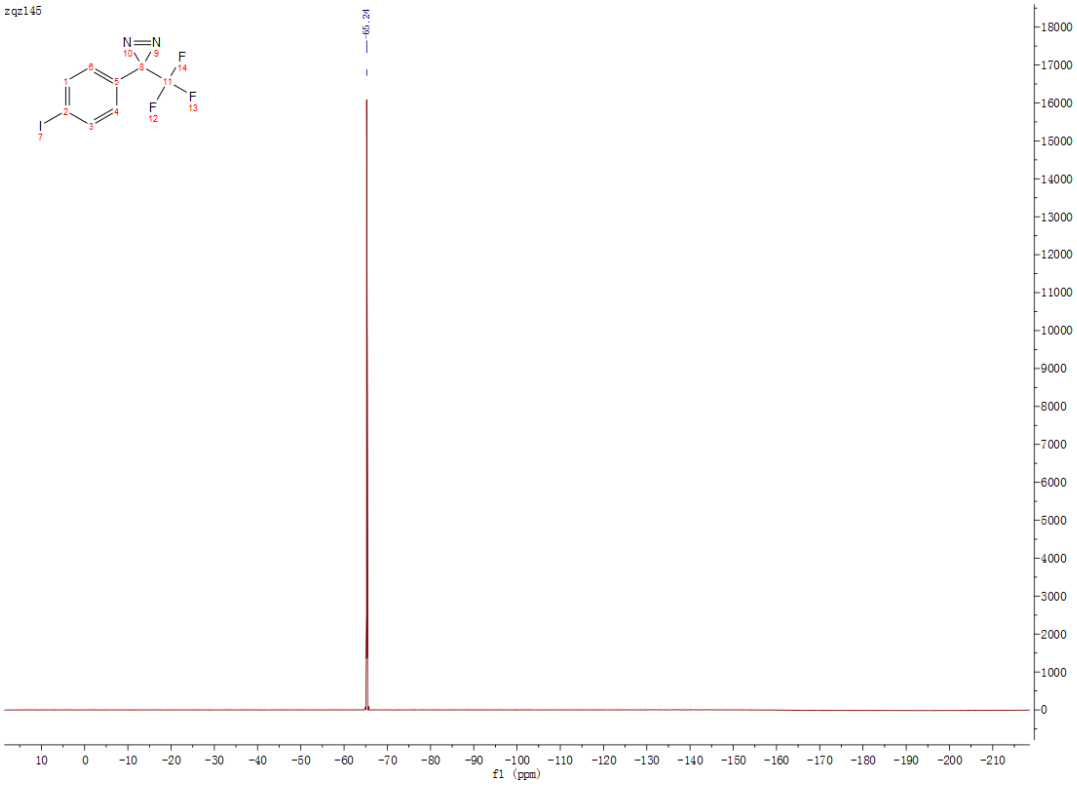
128.77
128.10
123.27
120.54
95.98
28.93
28.53
28.12
27.72
f1 (ppm)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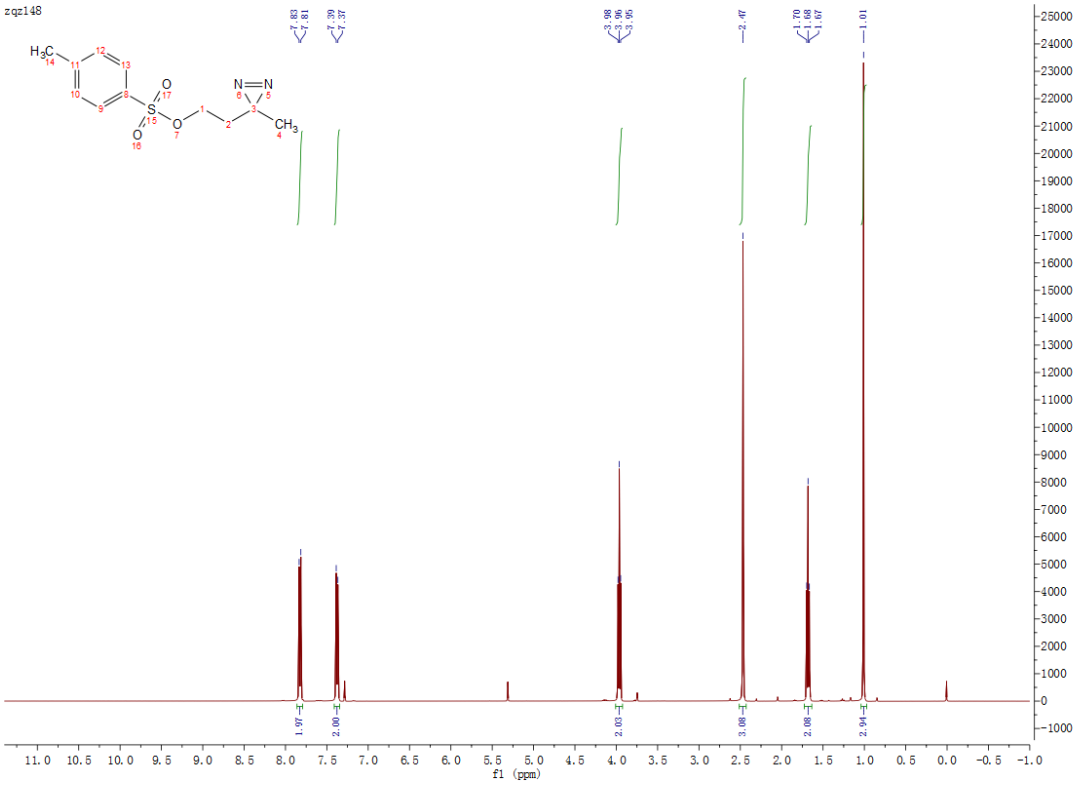
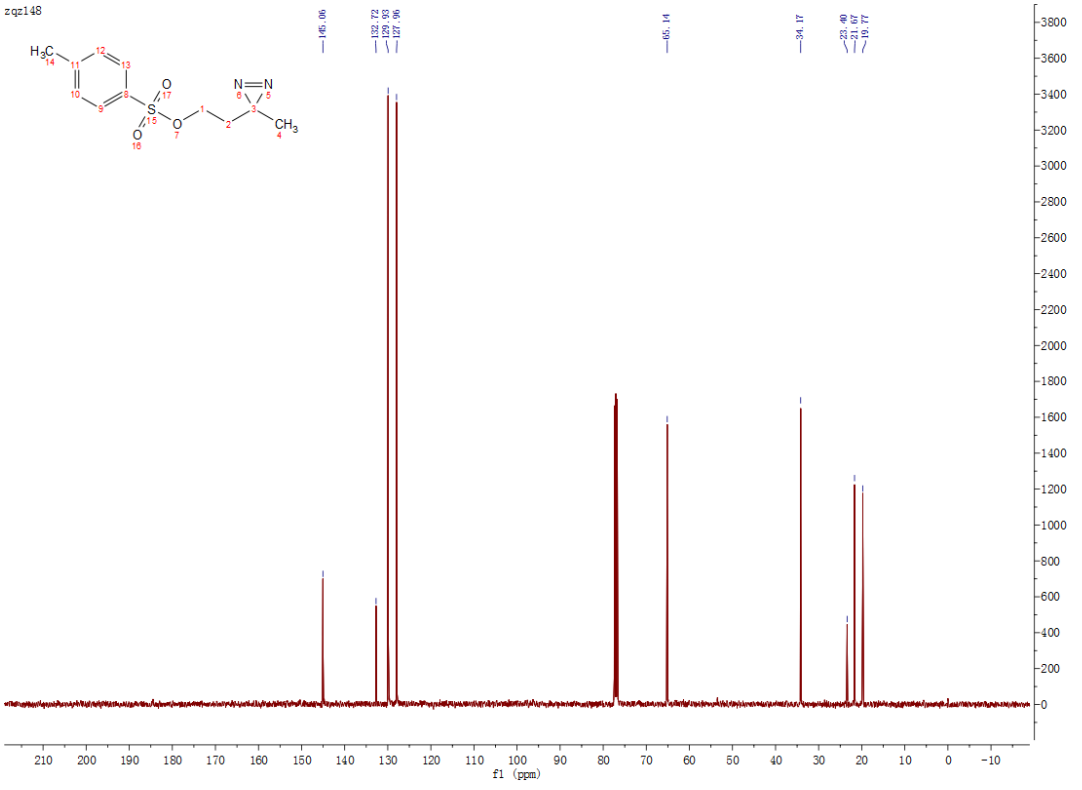

zqz148
7.83
7.81
7.39
7.37
3.98
3.96
3.95
2.47
1.70
1.68
1.67
1.01
1.97
2.00
2.03
3.08
2.08
2.94
f1 (ppm)


zqz148
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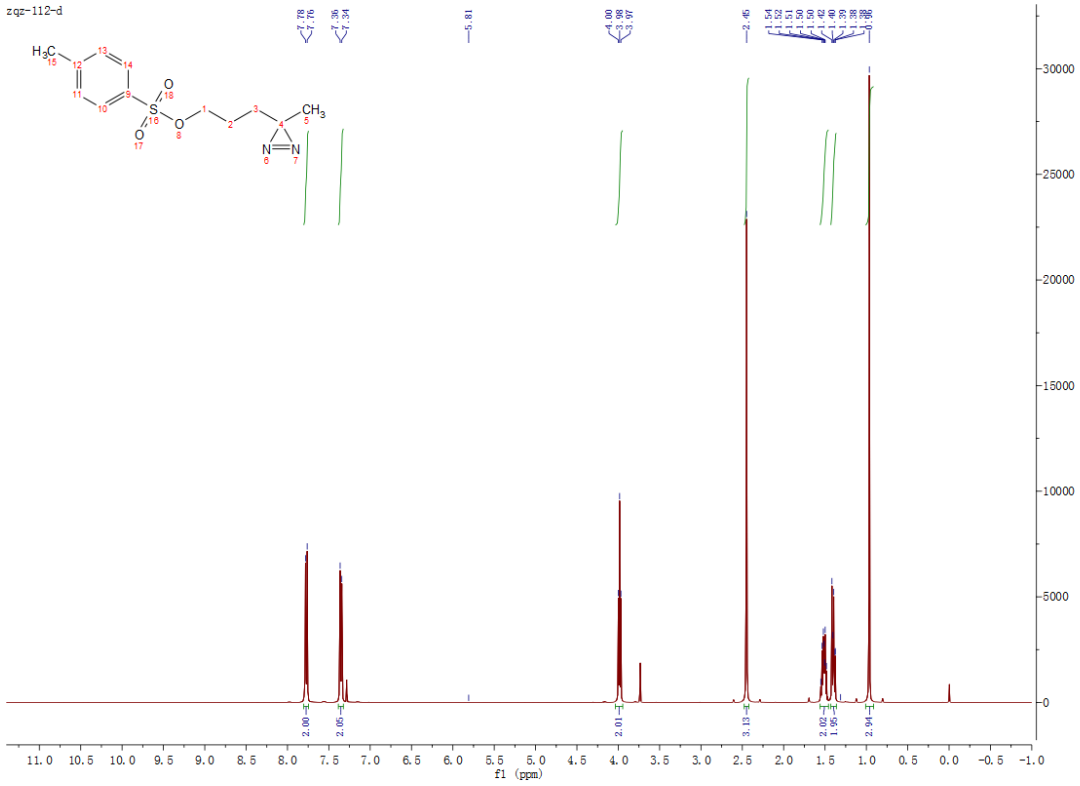
132.72
129.93
127.96
65.14
34.17
23.40
21.67
19.77
f1 (ppm)


zqz-112-d
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7.76
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7.34
5.81
4.00
3.98
3.97
2.45
1.54
1.52
1.51
1.50
1.42
1.40
1.39
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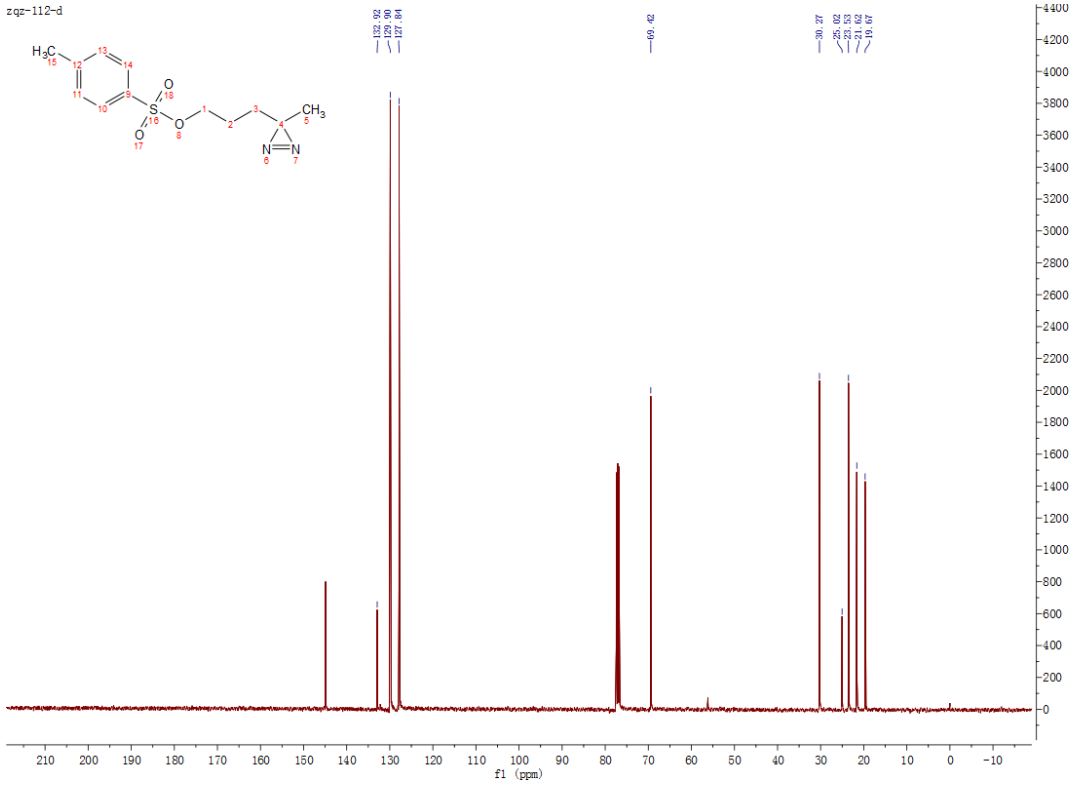
0.96
2.00
2.05
2.01
3.13
2.02
1.95
2.94
f1 (ppm)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25.02
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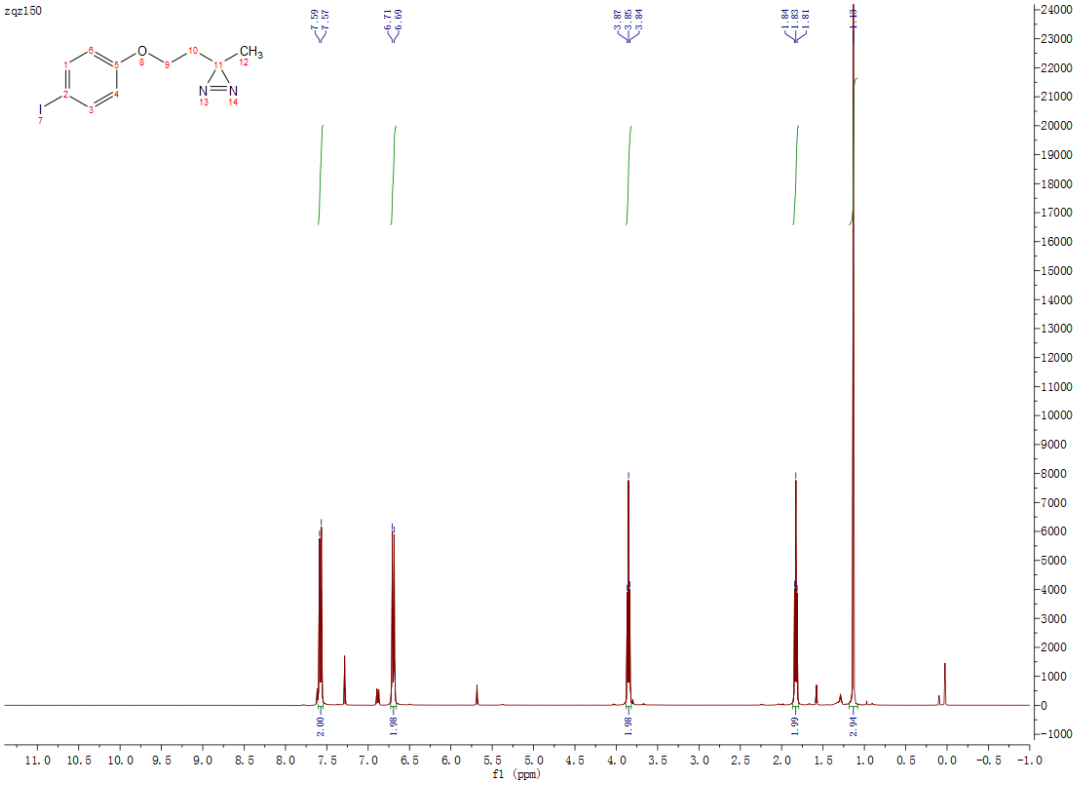
10.67
f1 (ppm)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6.71
6.69
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3.84
1.84
1.83
1.81
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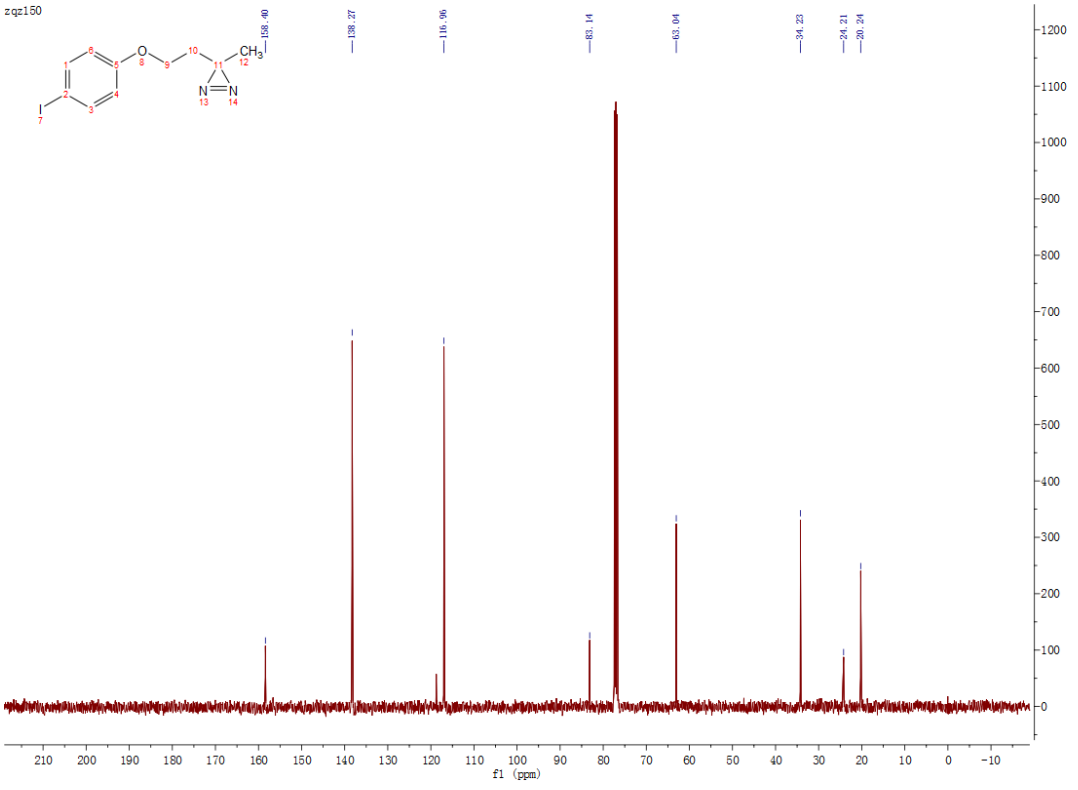
1.98
1.98
1.99
2.94
f1 (ppm)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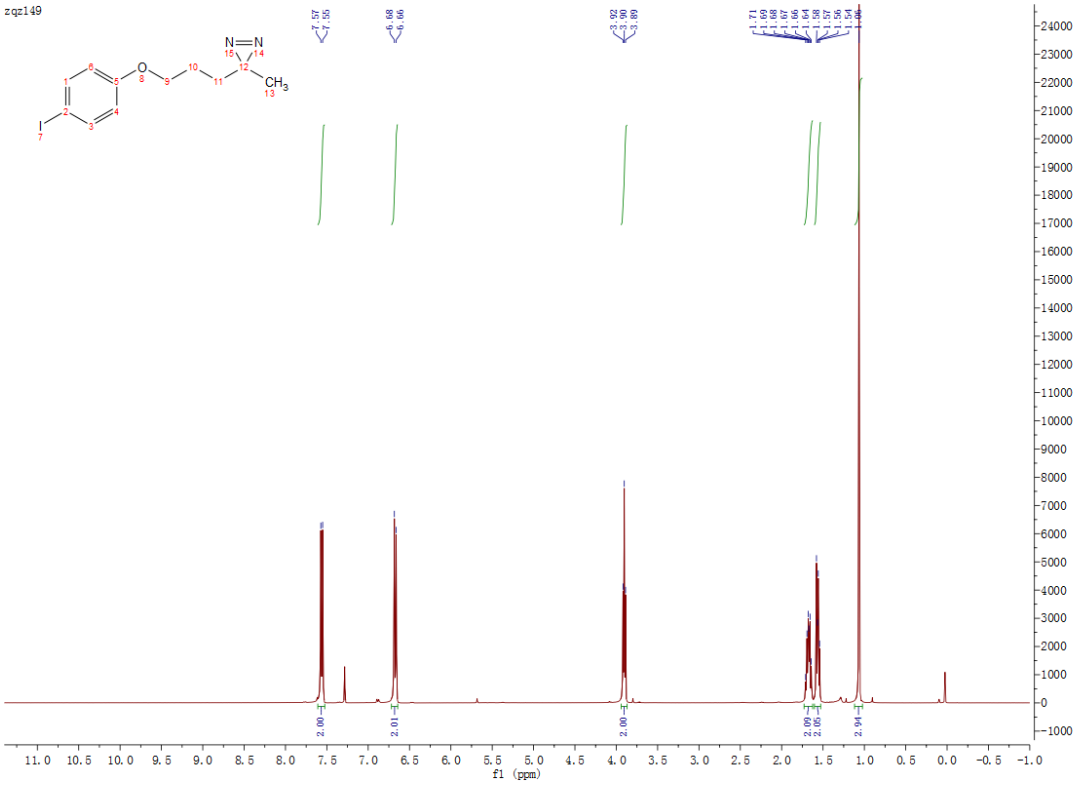
138.27
116.96
83.14
63.04
34.23
24.21
20.24
f1 (ppm)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6.66
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3.89
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1.69
1.68
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2.00
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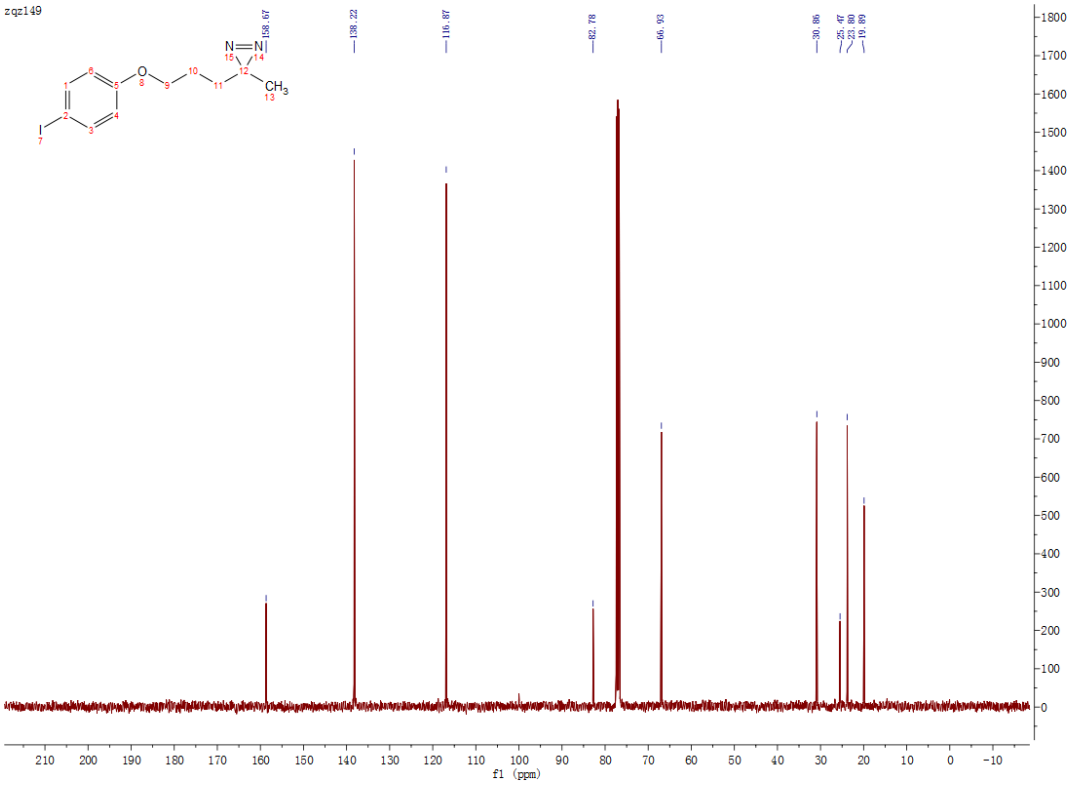
2.94
f1 (ppm)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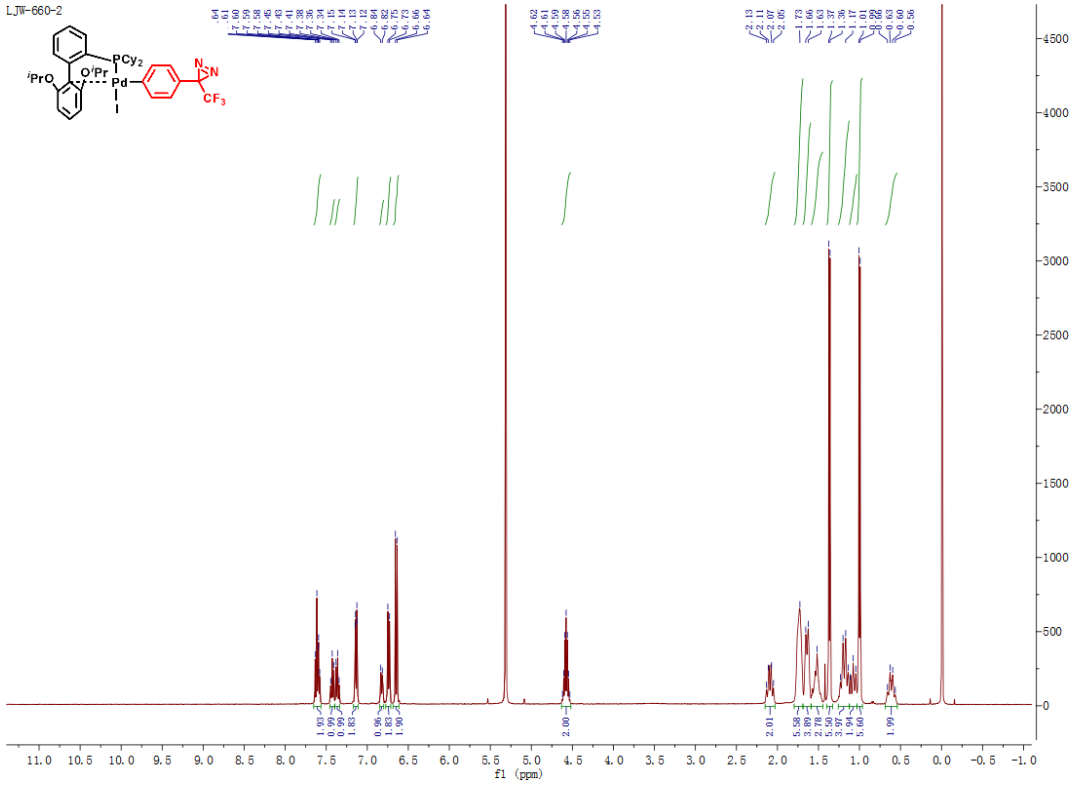
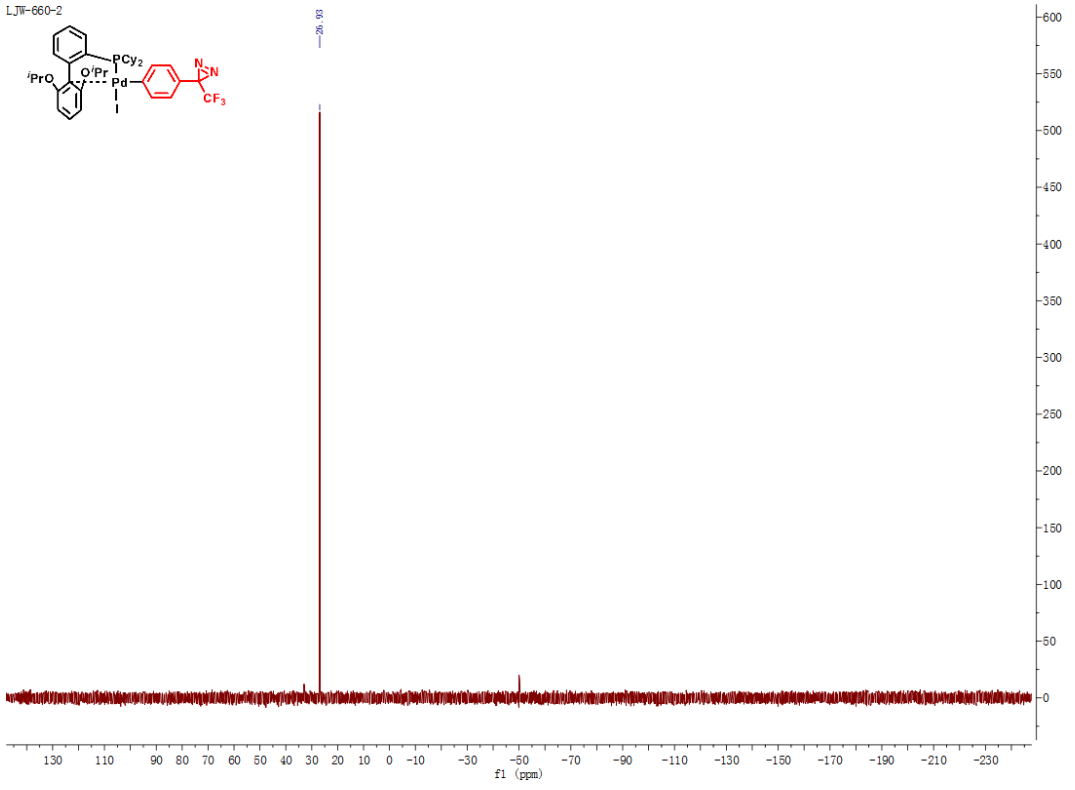
82.78
66.93
30.86
25.47
23.80
19.89
f1 (ppm)


LJW-660-2
PCy2
O'Pr
Pd
iPrO
N
N
CF3
f1 (ppm)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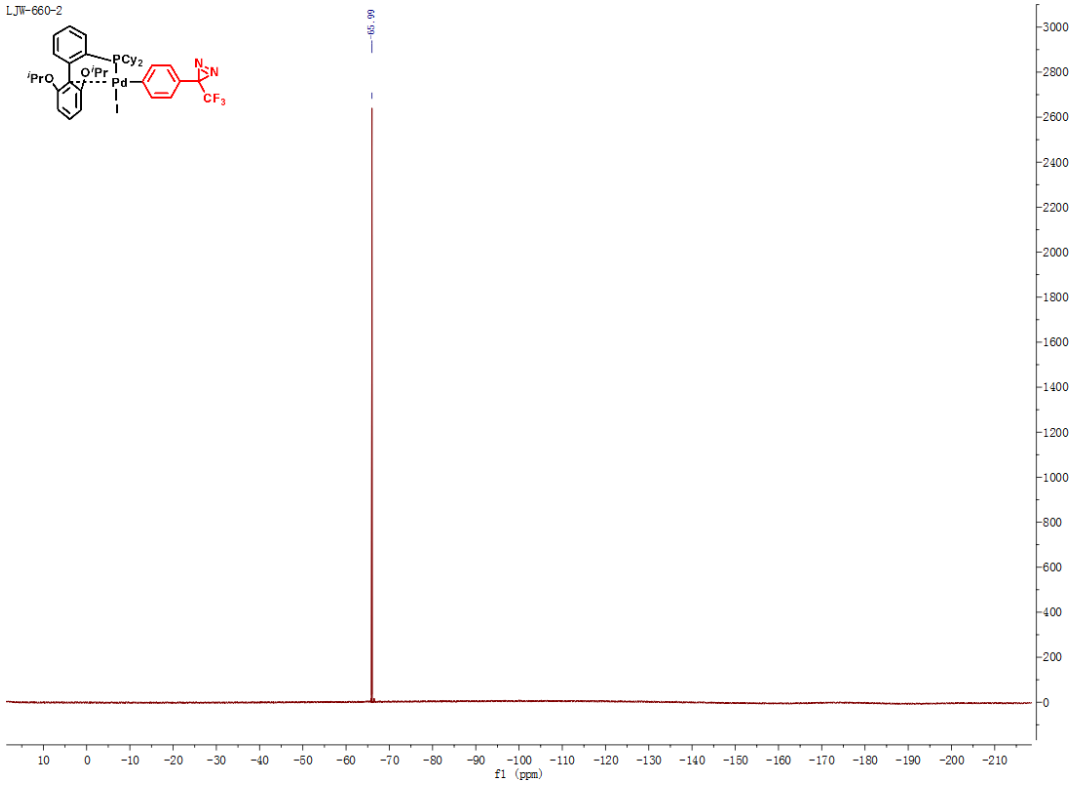
PCy2
O'Pr
Pd
iPrO
N
N
CF3
f1 (ppm)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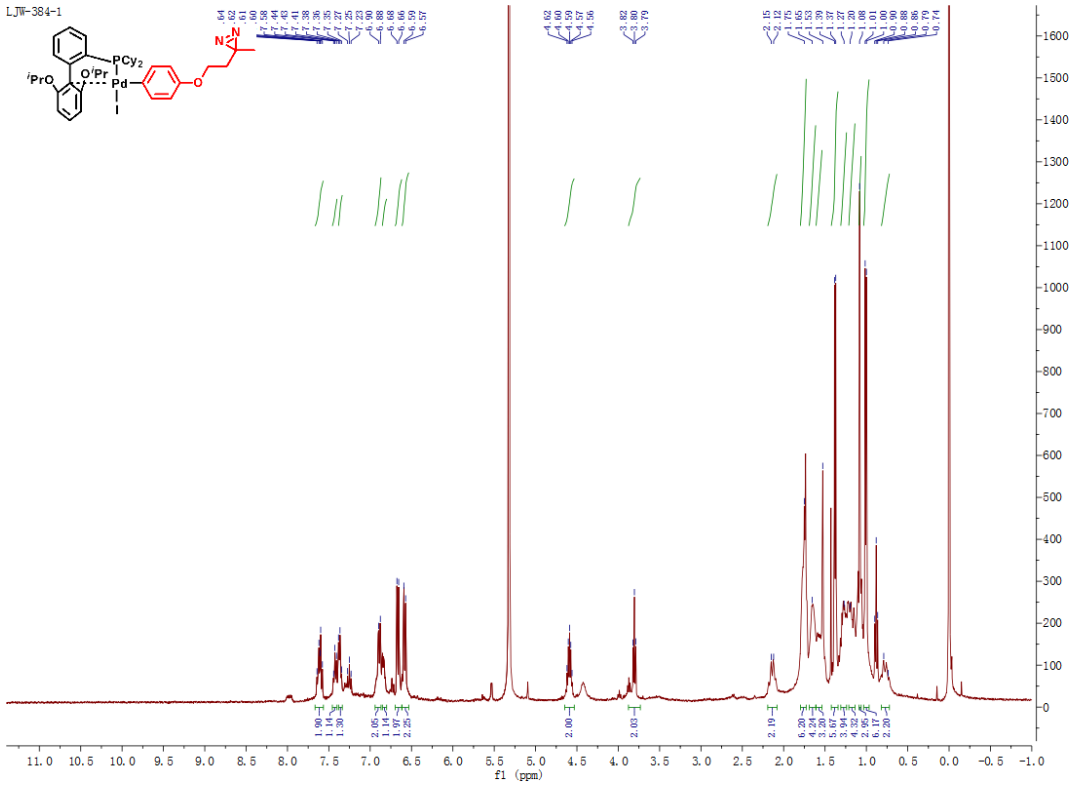
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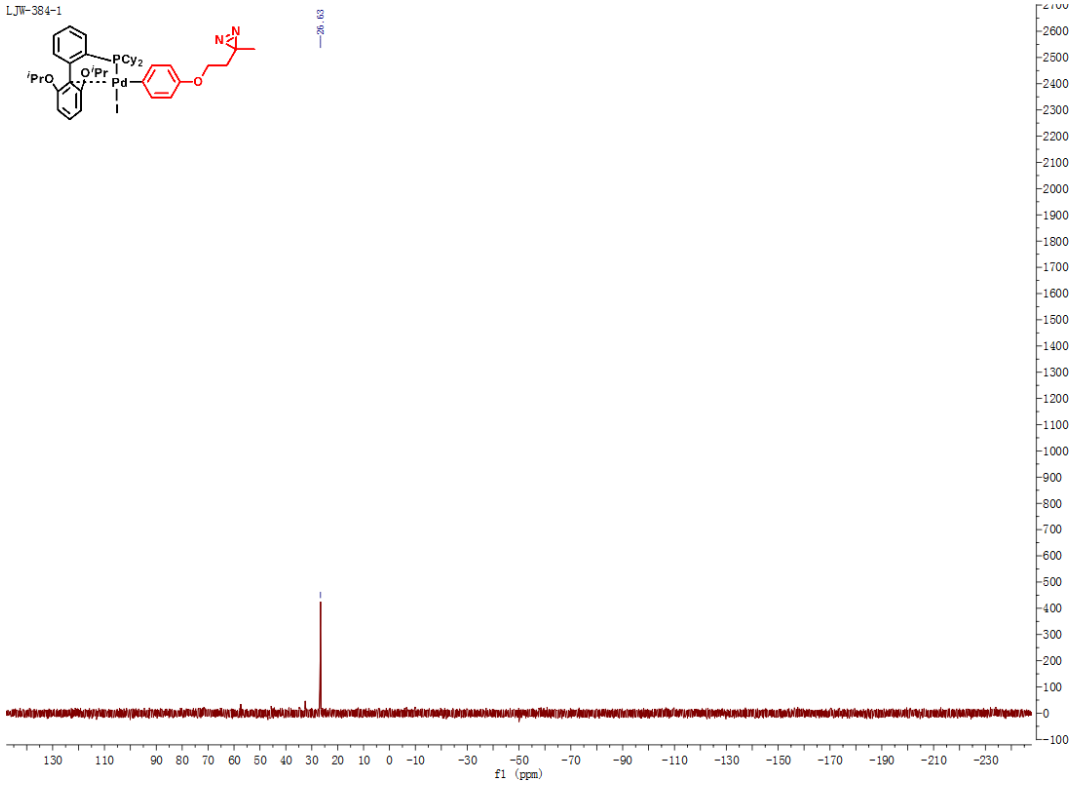
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26.63
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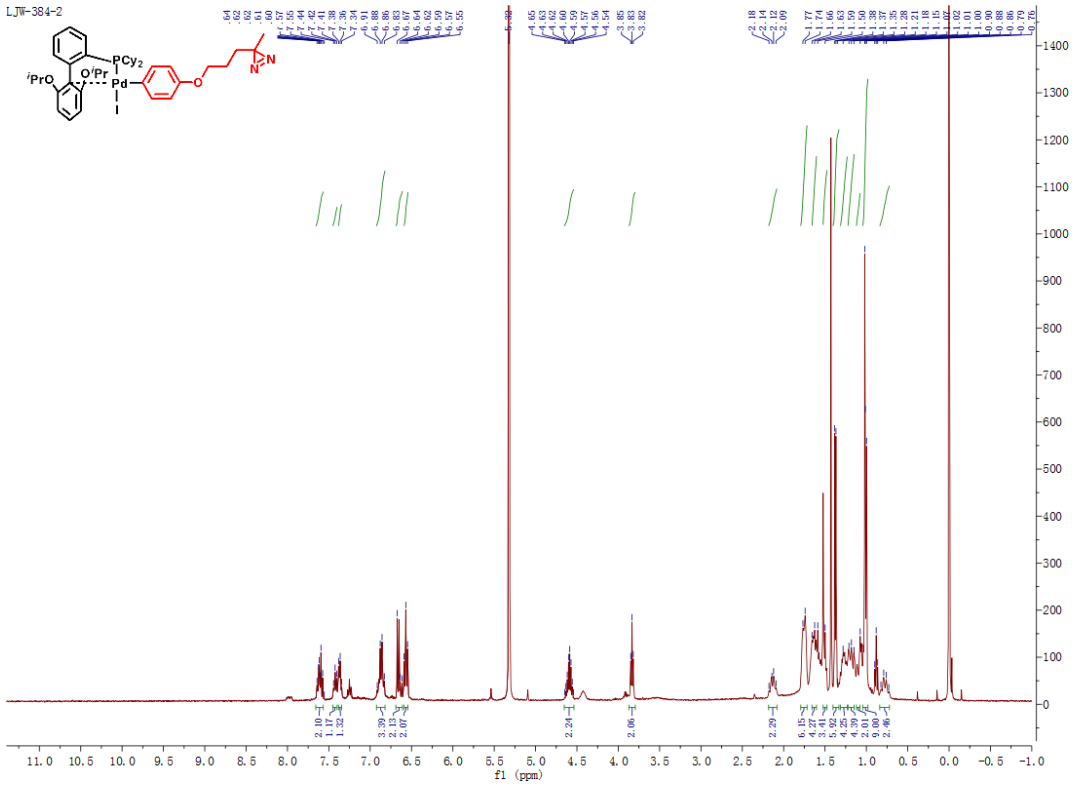
LJW-384-2
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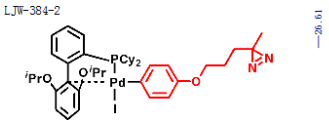
LJW-384-2



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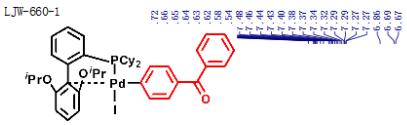

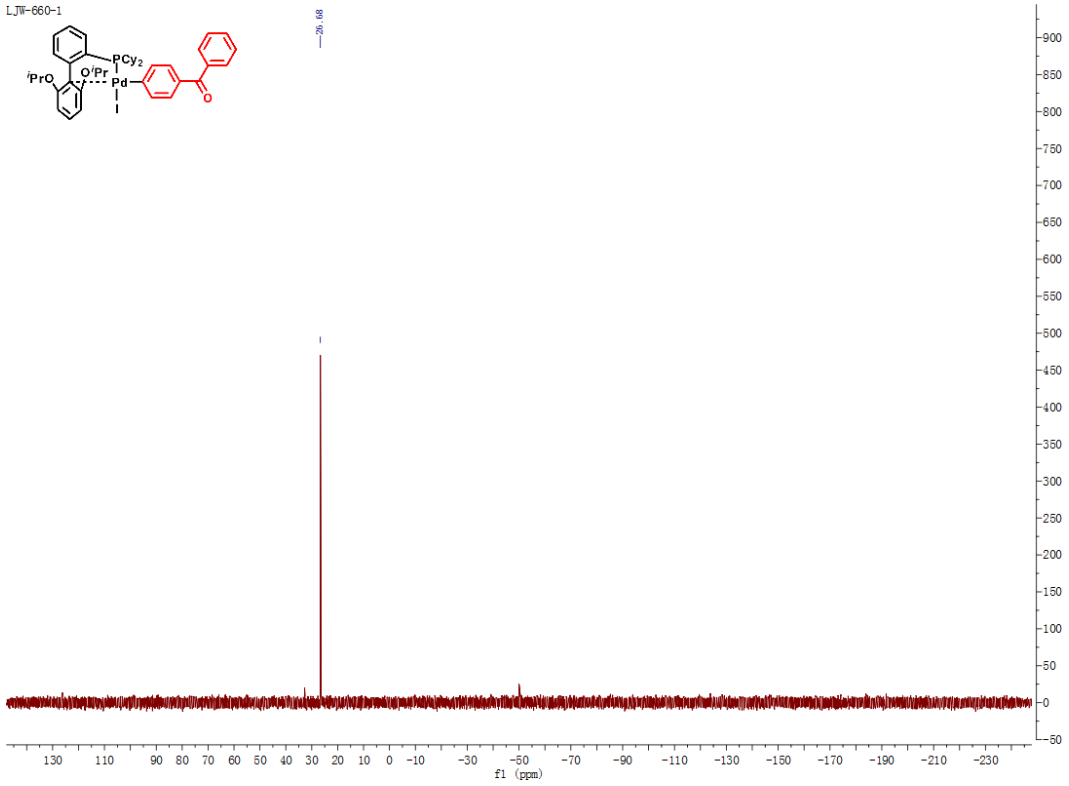

14. References

1. Y. X. Wang, J. W. Liu, L. Cao, W. J. Wang, Y. N. Sun, Z. Yin and Z. Y. Lou, *Chembiochem*, 2018, **19**, 1465-1470.
2. E. V. Vinogradova, C. Zhang, A. M. Spokoyny, B. L. Pentelute and S. L. Buchwald, *Nature*, 2015, **526**, 687-691.