

# Supporting Information

## One-pot nonhydrolysis Staudinger reaction and Staudinger or SPAAC ligation

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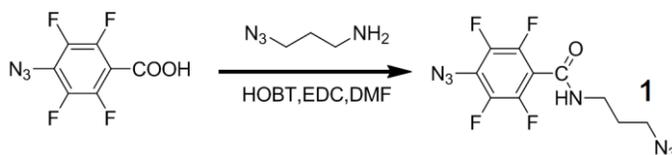
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## 1. Material and Methods

### 1.1 Material and instruments

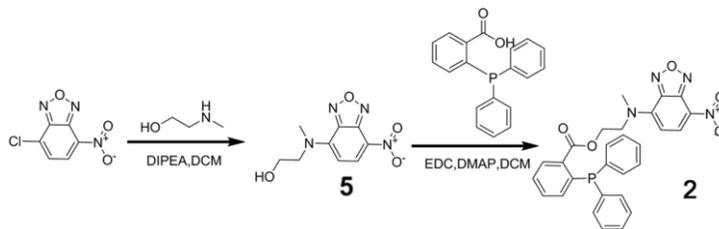
All chemicals and solvents used for synthesis were purchased from commercial suppliers and applied directly in the experiment without further purification. The progress of the reaction was monitored by TLC on pre-coated silica plates (Merck 60F-254, 250  $\mu\text{m}$  in thickness), and spots were visualized by basic  $\text{KMnO}_4$ , UV light or iodine. Merck silica gel 60 (70-200 mesh) was used for general column chromatography purification.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker 400 spectrometer. Chemical shifts are reported in parts per million relative to internal standard tetramethylsilane ( $\text{Si}(\text{CH}_3)_4 = 0.00$  ppm) or residual solvent peaks.  $^1\text{H}$  NMR coupling constants ( $J$ ) are reported in Hertz (Hz), and multiplicity is indicated as the following: *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), *bs* (broad singlet); *dt* (double triplet); *dd* (double doublet). High-resolution mass spectra (HRMS) were obtained on a Varian 7.0 T FTICR-MS or a 6520 Q-TOF LC/MS (Agilent, Santa Clara, CA).

### 1.2 Synthesis

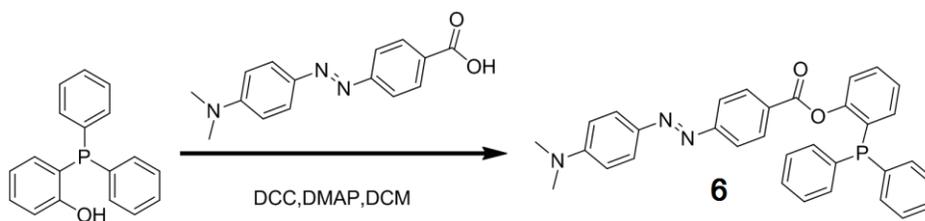


4-Azido-2,3,5,6-tetrafluorobenzoic acid (125 mg, 0.53 mmol) was dissolved in 4 mL DMF, and then EDC (115 mg, 0.6 mmol) and HOBT (81 mg, 0.6 mmol) were added to the solution. After stirring for 10 min, 3-azidopropan-1-amine (50  $\mu\text{l}$ , 0.5 mmol) was added to the DMF solution. The mixture was stirred at room temperature under nitrogen protection overnight. The mixture was extracted with  $\text{CH}_2\text{Cl}_2$  and washed by water and brine. The combined  $\text{CH}_2\text{Cl}_2$  solution was dried by  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The resulted residue was purified by silica gel column chromatography with  $\text{CH}_2\text{Cl}_2/\text{MeOH} = 1000/1$  to obtain a white solid (125 mg, 78%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  6.52 (bs, 1H), 3.52 (q,  $J = 6.4$  Hz, 2H), 3.44 (t,  $J = 6.4$  Hz, 2H), 1.93-1.83 (m,

2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  158.0, 145.4, 142.9, 141.7, 139.3, 122.1, 111.3, 49.3, 38.1, 28.4.  $^{19}\text{F}$  NMR (376 MHz,  $\text{CDCl}_3$ )  $\delta$  -141.2, -150.5. IR spectrum:  $2125.2\text{ cm}^{-1}$ .

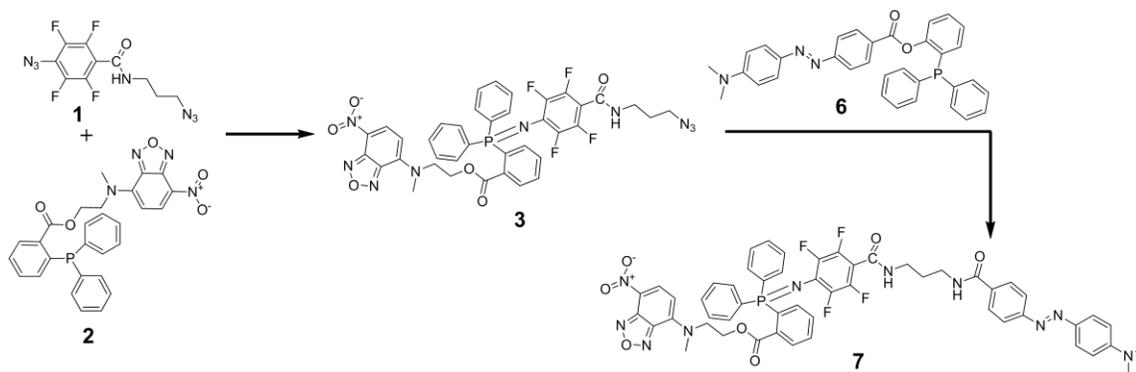


A mixture of NBD-Cl (400 mg, 2 mmol) and 2-(methylamino)ethan-1-ol (200  $\mu\text{L}$ , 2.5 mmol) was dissolved in 20 mL  $\text{CH}_2\text{Cl}_2$ . DIPEA (430  $\mu\text{L}$ , 2.5 mmol) was added to the reaction mixture and stirred at room temperature for 2 h. The mixture was washed by water ( $3 \times 25\text{ mL}$ ) and brine. The  $\text{CH}_2\text{Cl}_2$  solution was dried by  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The resulted residue was purified by silica gel column chromatography to give a red solid **5** (138 mg, 29%).  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  8.54 (d,  $J = 9.1\text{ Hz}$ , 1H), 6.44 (d,  $J = 9.1\text{ Hz}$ , 1H), 4.38-4.28 (m, 2H), 3.95 (t,  $J = 5.5\text{ Hz}$ , 2H), 3.62 (s, 3H). 2-(Diphenylphosphanyl)benzoic acid (123 mg, 0.41 mmol) was dissolved in  $\text{CH}_2\text{Cl}_2$  and then EDC (84 mg, 0.44 mmol) and DMAP (54 mg, 0.44 mmol) were added to the solution. After stirring for 10 min, **5** (80 mg, 0.34 mmol) was added to the solution. The mixture was stirred at room temperature under nitrogen protection overnight. After removing the solvent under reduced pressure, the resulted residue was purified by silica gel column chromatography with  $\text{CH}_2\text{Cl}_2$  to get an orange solid **2** (146 mg, 87%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.36 (d,  $J = 8.9\text{ Hz}$ , 1H), 7.81-7.76 (m, 1H), 7.39-7.29 (m, 8H), 7.25-7.18 (m, 4H), 6.94-6.88 (m, 1H), 6.04 (d,  $J = 9.0\text{ Hz}$ , 1H), 4.52 (t,  $J = 5.3\text{ Hz}$ , 2H), 4.37 (bs, 2H), 3.35 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  166.4, 145.4, 144.7, 140.9, 140.7, 137.5, 135.2, 134.6, 134.0, 133.8, 133.3, 133.1, 132.6, 130.5, 128.9, 128.6, 128.4, 123.2, 102.0, 62.2, 54.2, 41.9. HRMS (ESI):  $m/z$   $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{28}\text{H}_{24}\text{N}_4\text{O}_5\text{P}^+$ : 527.1479; found: 527.1488.



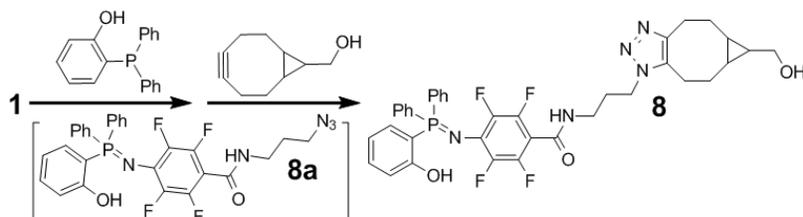
To a stirred solution of 2-(diphenylphosphino)phenol (139 mg, 0.5 mmol) in  $\text{CH}_2\text{Cl}_2$  (9 mL) was added 4-dimethylaminoazobenzene-4'-carboxylic acid (148 mg, 0.55 mmol),

DCC (124 mg, 0.6 mmol), DMAP (31 mg, 0.25 mmol) successively. The resulting mixture was allowed to stir overnight at room temperature. The white solid (1,3-dicyclohexylurea) was removed by filtration, and the filtrate was removed under reduced pressure. The resulted residue was purified by silica gel column chromatography with pentane/EtOAc = 100/3 to obtain a red solid **6** (211 mg, 86%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  7.97-7.90 (m, 4H), 7.79 (d,  $J$  = 8.6 Hz, 2H), 7.48-7.42 (m, 1H), 7.40-7.30 (m, 11H), 7.22-7.16 (m, 1H), 6.92-6.85 (m, 1H), 6.77 (d,  $J$  = 9.2 Hz, 2H), 3.11 (s, 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  164.0, 156.3, 153.1, 152.8, 143.9, 135.5, 134.4, 134.2, 133.6, 131.3, 130.0, 129.2, 128.7, 126.3, 125.7, 122.7, 122.0, 111.6, 40.4.  $^{31}\text{P}$  NMR (162 MHz,  $\text{CDCl}_3$ )  $\delta$  -15.13. HRMS (ESI):  $m/z$   $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{33}\text{H}_{29}\text{N}_3\text{O}_2\text{P}^+$ : 530.1992; found: 530.1989.

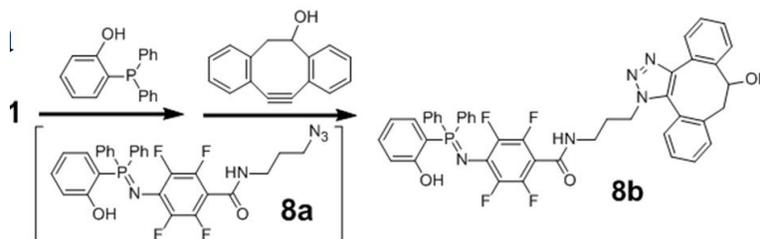


To a stirred solution of **1** (9 mg, 0.029 mmol) in  $\text{H}_2\text{O}$ -DMF (1:4) was added **2** (15 mg, 0.029 mmol). The mixture was stirred at room temperature under nitrogen protection overnight. Then **6** (75.4 mg, 0.143 mmol) was added to the reaction mixture. After 96 h, the mixture was extracted with EtOAc and washed by water and brine. The EtOAc solution was dried by  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The resulted residue was purified by silica gel column chromatography with  $\text{CH}_2\text{Cl}_2/\text{MeOH}$  = 1000/14 to obtain a red solid **7** (10 mg, 32%).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  8.34 (d,  $J$  = 8.9 Hz, 1H), 7.93 (d,  $J$  = 8.5 Hz, 2H), 7.89-7.81 (m, 4H), 7.71-7.61 (m, 6H), 7.59-7.51 (m, 4H), 7.49-7.43 (m, 4H), 6.74 (d,  $J$  = 9.2 Hz, 2H), 6.01 (d,  $J$  = 9.0 Hz, 1H), 4.14-4.09 (m, 4H), 3.75-3.69 (m, 2H), 3.59-3.51 (m, 4H), 3.23 (s, 3H), 3.10 (s, 6H), 2.06-2.01 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  167.4, 166.7, 160.9, 154.9, 152.7, 145.2, 144.5, 144.4, 143.5, 135.3, 135.0, 134.3, 132.6, 132.3, 132.1, 132.0, 131.9, 131.8, 131.5, 131.4, 131.2, 130.6, 130.5, 128.9, 128.8, 128.7, 127.9, 125.7, 125.3, 122.8, 122.2, 122.1, 121.6, 118.5, 111.4,

101.8, 53.3, 40.2, 36.4, 36.1, 31.9, 29.7, 29.3.  $^{19}\text{F}$  NMR (377 MHz,  $\text{CDCl}_3$ )  $\delta$  -145.7, -152.1. HRMS (ESI):  $m/z$   $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{53}\text{H}_{46}\text{F}_4\text{N}_{10}\text{O}_7\text{P}^+$ : 1041.3219; found: 1041.3198.

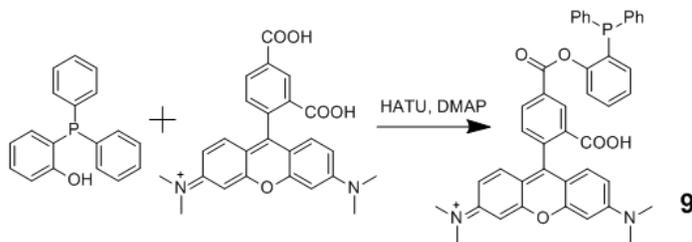


To a solution of **1** (17 mg, 0.054 mmol) in DMSO was added 2-(diphenylphosphino)phenol (11 mg, 0.054 mmol). The mixture was stirred at room temperature under nitrogen protection for 6 h. Then endo-bicyclo[6.1.0]non-4-yn-9-ylmethanol (9 mg, 0.060 mmol) was added to the reaction mixture. After 18 h, the mixture was extracted with EtOAc and washed by water and brine. The EtOAc solution was dried by  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The resulted residue was purified by silica gel column chromatography with  $\text{CH}_2\text{Cl}_2$  to give **8** (17 mg, 59%).  $^1\text{H}$  NMR (400 MHz, MeOD)  $\delta$  7.81-7.76 (m, 4H), 7.67-7.63 (m, 2H), 7.58-7.53 (m, 4H), 7.46-7.42 (m, 1H), 7.25-7.19 (m, 1H), 6.93-6.85 (m, 2H), 4.39-4.32 (m, 2H), 3.66 (dd,  $J = 7.6, 2.7$  Hz, 2H), 3.38-3.35 (m, 4H), 3.09-2.96 (m, 2H), 2.87-2.71 (m, 2H), 2.30-2.16 (m, 2H), 2.14-2.06 (m, 2H), 1.69-1.55 (m, 2H), 1.02-0.97 (m, 2H).  $^{13}\text{C}$  NMR (101 MHz, MeOD)  $\delta$  163.7, 162.1, 145.9, 144.3, 141.9, 135.8, 135.4, 134.4, 134.3, 133.8, 133.5, 133.4, 131.3, 130.4, 130.0, 129.9, 120.5, 120.4, 119.1, 119.0, 112.8, 111.6, 59.6, 46.6, 38.1, 30.5, 26.6, 23.7, 23.4, 22.8, 21.9, 20.7, 20.1.  $^{31}\text{P}$  NMR (162 MHz, MeOD)  $\delta$  23.10.  $^{19}\text{F}$  NMR (377 MHz, MeOD)  $\delta$  -148.03, -153.11. HRMS (ESI):  $m/z$   $[\text{M}+\text{H}]^+$  calcd. for  $\text{C}_{38}\text{H}_{37}\text{F}_4\text{N}_5\text{O}_0\text{P}^+$ : 718.2565; found: 718.2512.



To a solution of **1** (15 mg, 0.047 mmol) in DMSO was added 2-(diphenylphosphino)phenol (13 mg, 0.047 mmol). The mixture was stirred at room temperature under nitrogen protection for 6 h. Then 3-hydroxy-1,2:5,6-dibenzocyclooct-

7-yne (16 mg, 0.073 mmol) was added to the reaction mixture. After 12 h, the mixture was extracted with CH<sub>2</sub>Cl<sub>2</sub> and washed by water and brine. The CH<sub>2</sub>Cl<sub>2</sub> solution was dried by Na<sub>2</sub>SO<sub>4</sub> and concentrated under reduced pressure. The resulted residue was purified by silica gel column chromatography with CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 1000/15 to give **8b** (25 mg, 67%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 7.75-7.65 (m, 4H), 7.61 (dd, *J* = 9.7, 4.7 Hz, 2H), 7.55-7.45 (m, 4H), 7.43-7.27 (m, 4H), 7.25-7.05 (m, 4H), 7.04-6.70 (m, 4H), 5.11-4.90 (m, 1H), 4.64-4.26 (m, 2H), 3.41-2.91 (m, 4H), 2.34-1.77 (m, 2H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 164.1, 160.0, 159.8, 148.6, 146.5, 146.0, 145.0, 140.7, 139.2, 137.9, 136.7, 135.9, 135.7, 134.6, 134.4, 134.0, 133.3, 133.2, 132.9, 132.7, 132.5, 132.3, 132.0, 131.9, 131.6, 131.1, 130.1, 130.0, 129.3, 129.1, 129.0, 128.8, 128.7, 128.6, 127.8, 127.1, 127.0, 126.9, 126.0, 125.7, 123.6, 119.3, 119.2, 119.1, 119.0, 69.9, 68.0, 46.3, 45.8, 45.4, 42.9, 40.6, 40.2, 36.6, 29.7, 29.3, 28.5. HRMS (ESI): *m/z* [M+H]<sup>+</sup> calcd. for C<sub>44</sub>H<sub>35</sub>F<sub>4</sub>N<sub>5</sub>O<sub>3</sub>P<sup>+</sup>: 788.2408; found: 788.2413.



Rhodamine (50 mg, 0.116 mmol) was dissolved in 10 mL DMF. Then HATU (60 mg, 0.139 mmol), DMAP (42.5 mg, 0.174 mmol) and 2-(diphenylphosphino)phenol (39 mg, 0.139 mmol) were added. The mixture was stirred overnight under N<sub>2</sub> gas. After removing the solvent under reduced pressure, the residue was purified by silica gel column chromatography (MeOH:CH<sub>2</sub>Cl<sub>2</sub> = 0.2%) to give a purple solid (40 mg, 50%). <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ 8.87-8.85 (m, 1H), 8.15-8.11 (m, 1H), 7.53-7.48 (m, 1H), 7.34-7.32 (m, 3H), 7.24-7.18 (m, 4H), 7.14-7.08 (m, 6H), 7.07-7.02 (m, 3H), 6.92-6.88 (m, 2H), 6.78-6.76 (m, 2H), 3.24 (s, 12H). <sup>13</sup>C NMR (101 MHz, CDCl<sub>3</sub>) δ 162.9, 162.5, 157.5, 157.3, 139.1, 135.4, 135.3, 135.2, 135.1, 134.8, 134.5, 134.1, 133.9, 133.6, 133.4, 133.2, 131.5, 130.7, 130.5, 130.2, 129.5, 129.3, 129.0, 128.8, 128.7, 128.6, 128.5, 127.0, 126.8, 122.5, 122.0, 114.4, 113.3, 96.8, 40.9. HRMS (ESI): *m/z* [M+H]<sup>+</sup> calcd. for C<sub>43</sub>H<sub>36</sub>N<sub>2</sub>O<sub>5</sub>P<sup>+</sup>: 692.2356; found: 692.2376.

## 2. Procedure of fluorescence measurements

Fluorescence studies were carried out using F-280 spectrophotometer (Tianjin Gangdong Sci & Tech., Development. Co., Ltd) in a 3 mL corvette with 2 mL solution. All spectroscopic measurements were performed in phosphate-buffered saline buffer (PBS, 50 mM, pH 7.4, containing a certain organic solvent for solubility). Compounds **1**, **2**, **9** and **10** were dissolved into DMSO and **6** was dissolved into DMF to prepare their stock solutions of 10-20 mM. Probes were diluted in PBS buffer to afford the final concentration of 1-5  $\mu\text{M}$ . The reaction mixture was shaken uniformly before emission spectra were measured.

**Time-dependent fluorescence kinetics of 1 and 2:** 2  $\mu\text{L}$  **2** (1 mM) in DMSO and 2-10  $\mu\text{L}$  **1** (10 mM) in DMSO were added to 2 mL PBS (50 mM, pH 7.4, containing 30% DMSO) at room temperature. The progress of the reaction was monitored by fluorescence spectra or emission at 540 nm with excitation at 480 nm. The pseudo-first-order rate,  $k_{\text{obs}}$  is determined by fitting the fluorescence intensity data with single exponential function. The linear fitting between  $k_{\text{obs}}$  and concentrations of **1** gives the reaction rate ( $k_2$ ).

**One-pot tandem Staudinger reactions:** One-pot reactions of 800  $\mu\text{L}$  **1** (2 mM) in DMSO, 800  $\mu\text{L}$  **2** (2 mM) in DMSO and 400  $\mu\text{L}$   $\text{H}_2\text{O}$  were mixed for 6 h, and then 10.6 mg **6** was added to the reaction solution for 76 h. For fluorescent tests, 10  $\mu\text{L}$  reaction solution was diluted into 2 mL PBS buffer (50 mM, pH 7.4, containing 40% DMF) at each time point. The fluorescence spectra were recorded with excitation at 480 nm.

**Time-dependent fluorescence kinetics for hydrolysis of 7:** the molecular beacon **7** (2  $\mu\text{M}$ ) was mixed with 0.1-2 M NaOH solution at room temperature. The progress of the reaction was monitored with excitation at 480 nm and fluorescence spectra or emission at 540 nm. The  $k_{\text{obs}}$  is determined by fitting the fluorescence intensity data with single exponential function. The linear fitting between  $k_{\text{obs}}$  and concentrations of NaOH gives the reaction rate ( $k_2$ ).

**Time-dependent fluorescence spectra of 1 and 9:** 10  $\mu\text{L}$  **1** (20 mM) in DMSO and 11  $\mu\text{L}$  **9** (20 mM) in DMSO were added to 180  $\mu\text{L}$  PBS (50 mM, pH 7.4, containing 70% DMSO) at room temperature. For fluorescent tests, 10  $\mu\text{L}$  reaction solution was diluted

into 2 mL PBS buffer (50 mM, pH 7.4, containing 70% DMSO) at each time point. The progress of the reaction was monitored by fluorescence with excitation at 488 nm.

**Time-dependent fluorescence spectra of 1, 9 and 10:** 5  $\mu$ L **1** (20 mM) in DMSO and 5.5  $\mu$ L **9** (20 mM) in DMSO were added to 90  $\mu$ L PBS (50 mM, pH 7.4, containing 70% DMSO) at room temperature for 3 h. The reaction solution was mixed with 3  $\mu$ L **10** (50 mM) in 97  $\mu$ L PBS. For fluorescent tests, 20  $\mu$ L reaction solution was diluted into 2 mL PBS buffer (50 mM, pH 7.4, containing 70% DMSO) at each time point. The progress of the reaction was monitored by fluorescence with excitation at 488 nm.

### 3. Procedure of HPLC measurements

HPLC analysis was performed by ANGELA TECHNOLOGIES HPLC LC-10F using C18 column with 4.6 mm X 250 mm. Buffer A: 0.1% (v/v) trifluoroacetic acid in water; buffer B: methanol; flow: 1 mL/min. For one-pot Staudinger reactions of **1** (0.5 mM) and **2** (2.5 mM), the conditions were: 0-7 min, buffer B: 5-70%; 7-35 min, buffer B: 70-95%. For one-pot chemical reactions of *NSR* and *SPAAC* to produce **8**, the conditions were: 0-3 min, buffer B: 5-70%; 4-20 min, buffer B: 70-95%. The detection wavelength for HPLC is 254 nm.

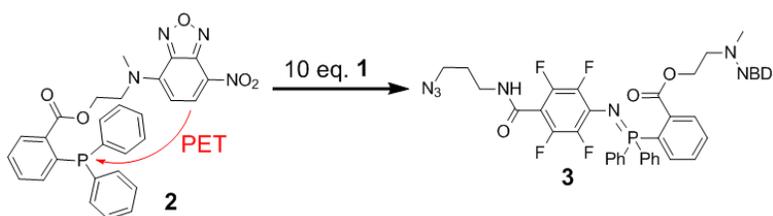
### 4. Cell cultures and bioimaging

**Cell culture:** HEK-293 cells were cultured at 37  $^{\circ}$ C, CO<sub>2</sub> (5%) air environment in high glucose DMEM (GIBICO) supplemented with FBS (10%), penicillin (100  $\mu$ g/ml), streptomycin (100  $\mu$ g/ml) and L-glutamine (4 mM). The cells were maintained in exponential growth phase, and then seeded in a glass-bottom 35 mm plate (~ 2x10<sup>4</sup> cells per well). Cells were passaged every 2–3 days and used between passages 3 and 10.

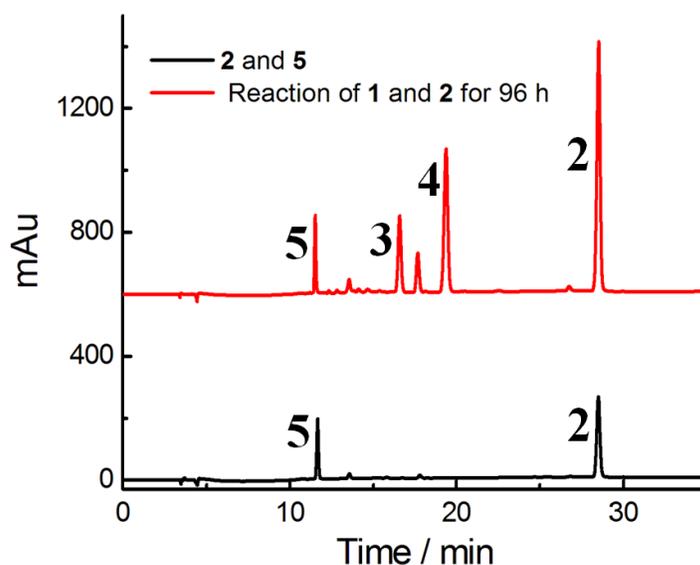
**Confocal imaging experiments:** Cells were imaged on an FV1000 inverted fluorescence confocal microscope (Olympus, Japan) with a UPLSAPO 40 x objective lens. All images were analyzed with Olympus FV1000-ASW software. HEK 293 cells were incubated

with **9** (2  $\mu\text{M}$ ) for 20 min and washed with PBS once. Then the cells were incubated with **1** (5  $\mu\text{M}$ ) for another 20 min and washed with PBS once again. Finally, the cells were incubated with **10** (2  $\mu\text{M}$ ) for 30 min and washed with PBS three times. Control cells were treated with **9** (2  $\mu\text{M}$ ) at 37  $^{\circ}\text{C}$  for 20 min and washed with PBS once, and then **10** (2  $\mu\text{M}$ ) for 30 min and washed with PBS three times. For double-excitation imaging, emission was collected at green channel (500-530 nm) and red channel (555-655 nm) with 488 nm and 543 nm excitation, respectively. For FRET imaging, emission was collected at green channel (500-530 nm) and red channel (555-655 nm) with only 488 nm excitation.

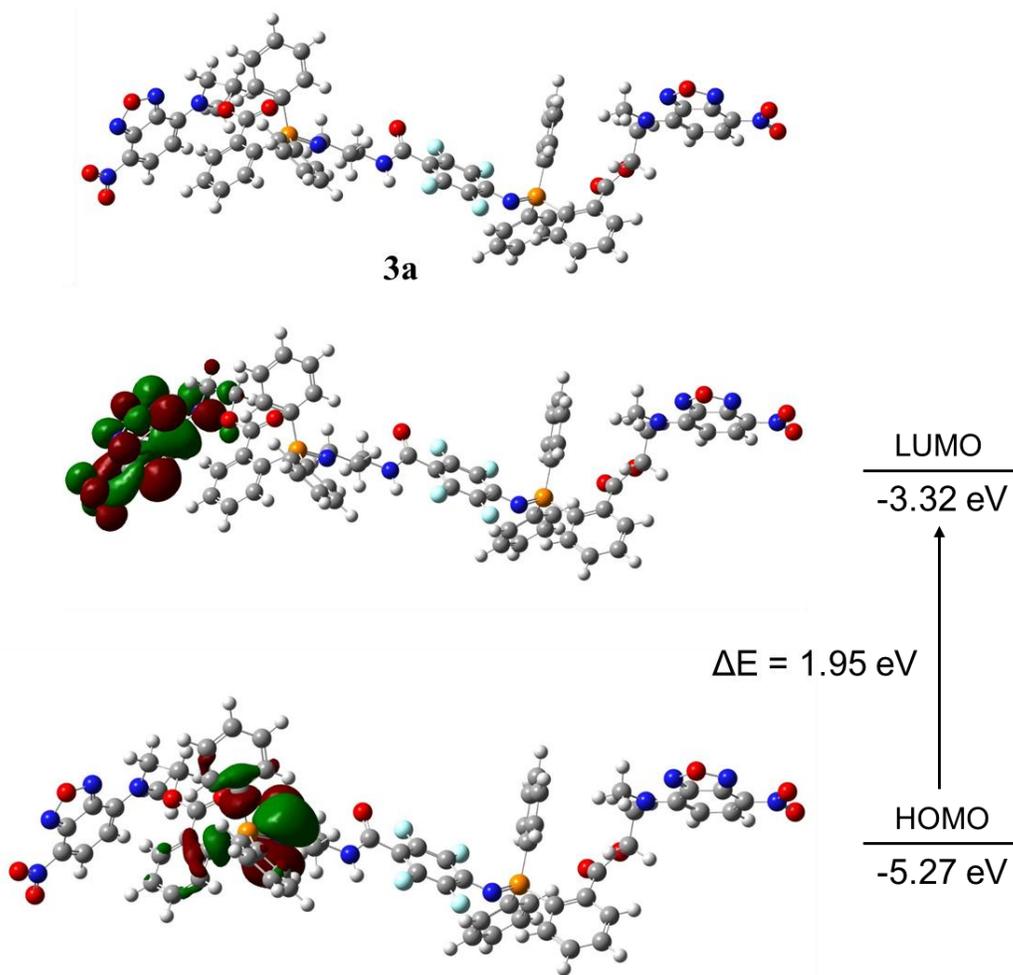
## 5. Supporting figures



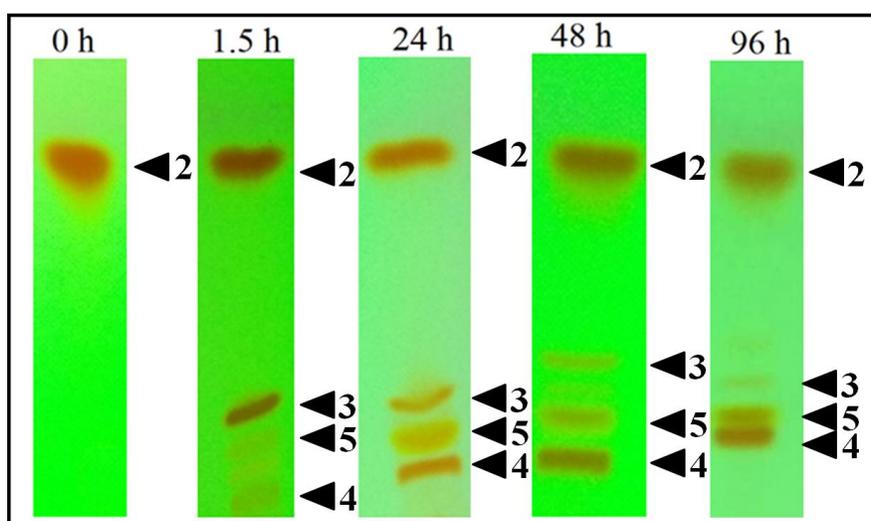
**Scheme S1.** The fluorescent quenching effect of **2** and its reaction with **1** to give **3**.



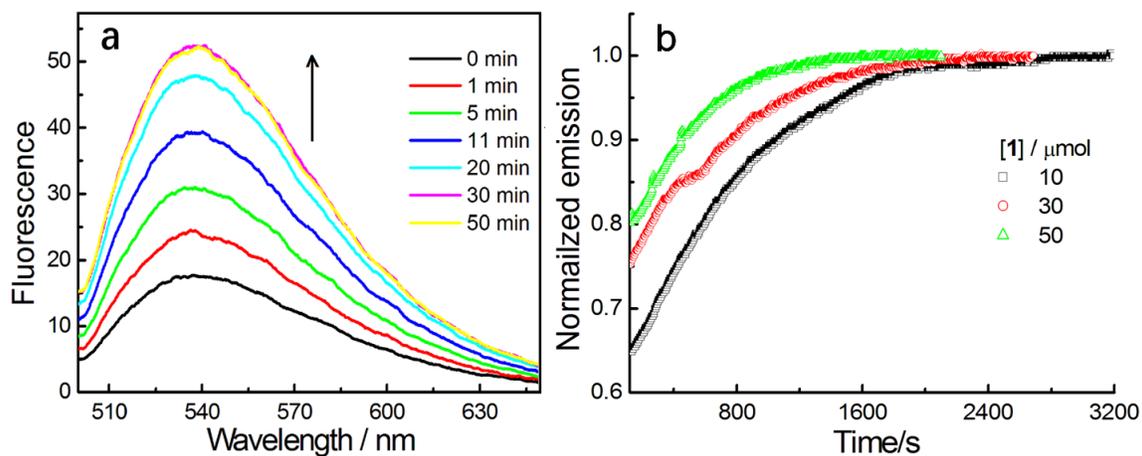
**Figure S1.** The HPLC trace of a mixture of **5** and **2**. The reaction mixture of **1** (0.5 mM) and **2** (2.5 mM) at 96 h was showed in red trace.



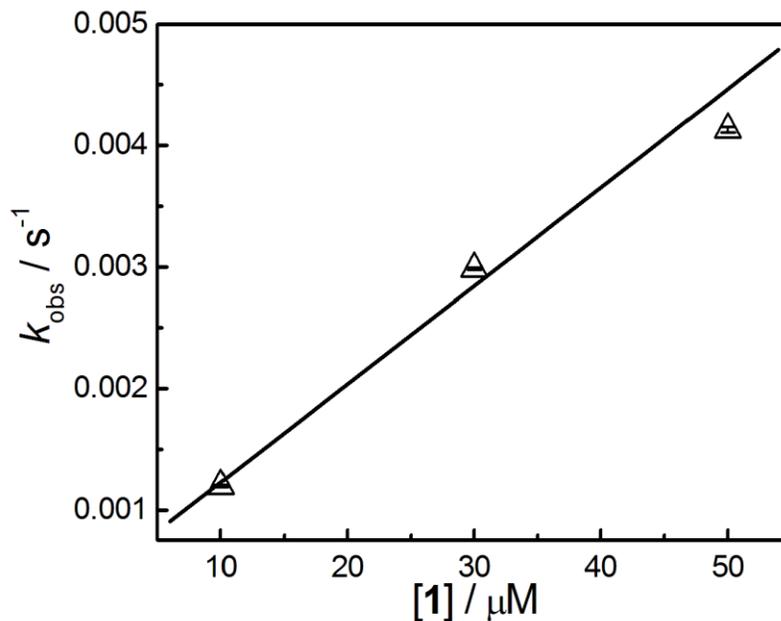
**Figure S2.** Frontier orbital energy of **3a**. Data were calculated by Gaussian 09 with B3LYP/6-31G(d) level (*J. Chem. Theor. Comput.*, 2010, **6**, 370).



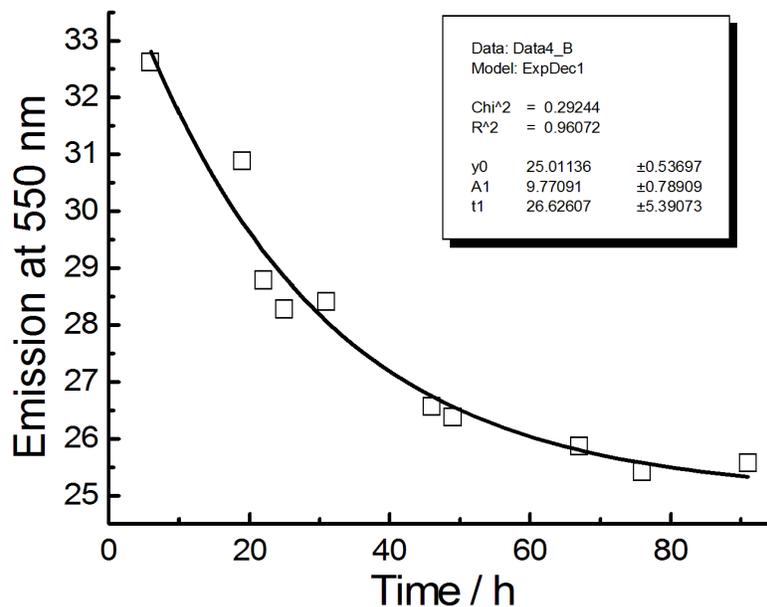
**Figure S3.** Time-course of TLC (5% MeOH in  $\text{CH}_2\text{Cl}_2$ ) for the one-pot reactions of **1** (3 mM) and **2** (15 mM).



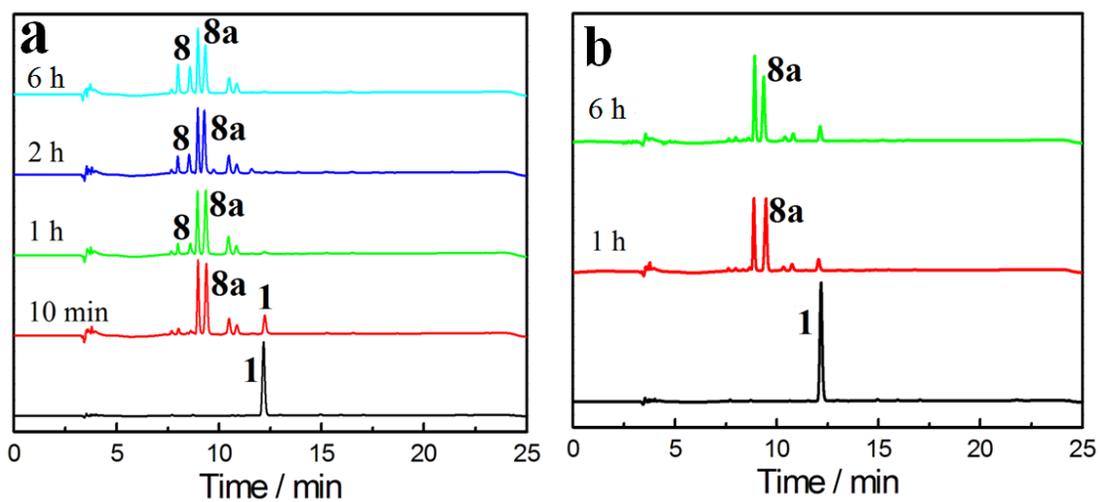
**Figure S4.** (a) Time-dependent fluorescence spectra of **2** (1  $\mu\text{M}$ ) upon treatment with **1** (10  $\mu\text{M}$ ) in PBS (50 mM, pH 7.4, containing 30% DMSO) at room temperature (excitation, 480 nm). The tested time is shown inset. (b) The time-dependent normalized fluorescence intensity at 540 nm of **2** (1  $\mu\text{M}$ ) in the presence of different concentrations of **1** (as indicated inset).



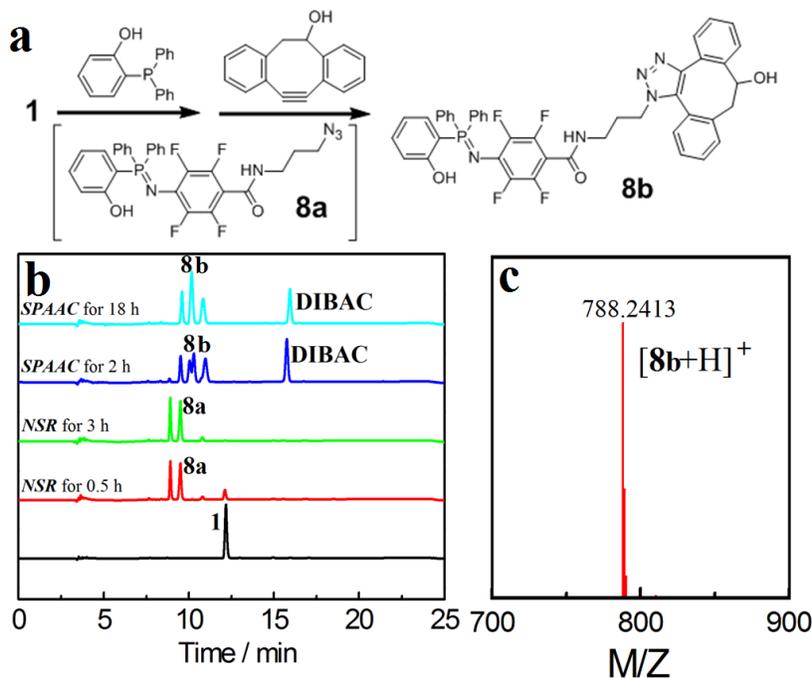
**Figure S5.** The linear relationship between concentrations of **1** and  $k_{\text{obs}}$ . The slope of the best linear fitting gives the reaction rate  $k_2$  ( $73.3 \text{ M}^{-1} \text{ s}^{-1}$ ).



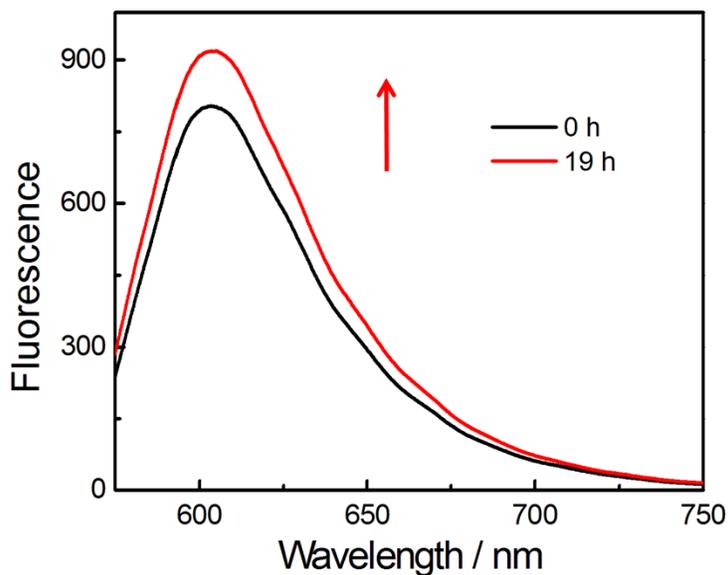
**Figure S6.** The time-dependent fluorescence intensity of the one-pot reactions of **1**, **2** and **6**. **1** (1 mM) and **2** (1 mM) was mixed in DMF/H<sub>2</sub>O (80%) for 6 h, and then **6** (10 mM) was added to start the reaction and tests. The 10  $\mu$ L reaction solution was diluted into 2 mL PBS buffer (50 mM, pH 7.4, containing 40% DMF) at each time point for fluorescent tests. Excitation, 480 nm; emission, 550 nm. The solid line represents the best fitting to give  $t_{1/2}$  = 18.4 h.



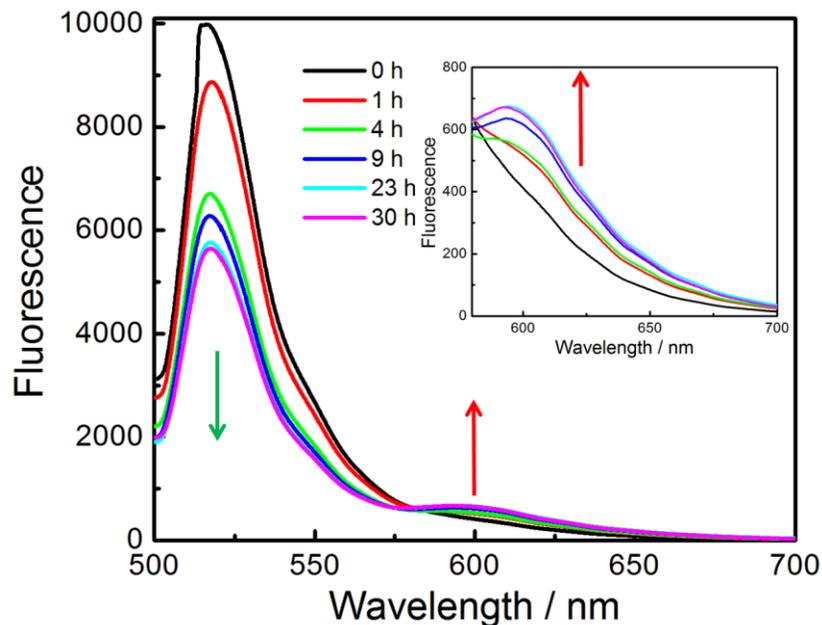
**Figure S7.** Time-dependent HPLC traces for *NSR* and *SPAAC* in one-pot. **1** (1 mM) was added to the mixture of 2-(diphenylphosphino)phenol (1 mM) and BCN (3 mM for (a) or 1 mM for (b)).



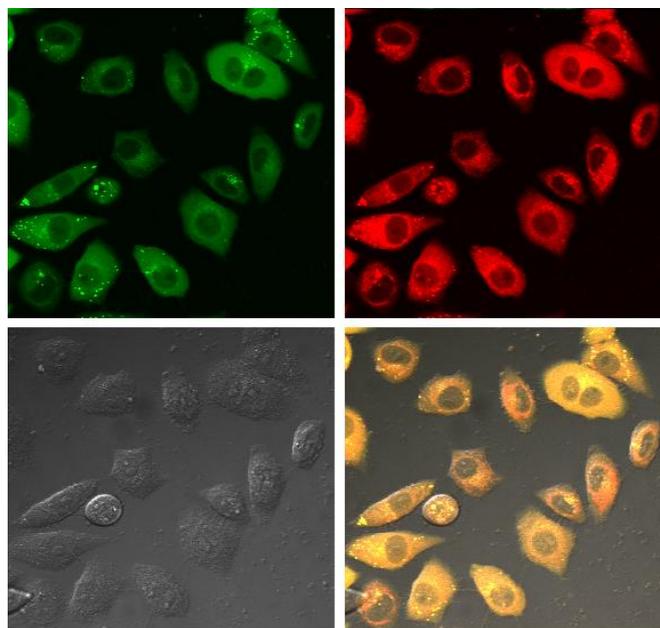
**Figure S8.** (a) One-pot tandem *NSR* and *SPAAC* reactions to produce **8b**. The chemical structure of intermediate **8a** is also shown. (b) Time-dependent HPLC traces for the one-pot reactions. The reactions were performed using **1** (1 mM) and 2-(diphenylphosphino)phenol (1 mM) for 3 h, and then addition of DIBAC (3 mM) for 18 h. (c) HRMS for **8b**. The calculated mass for  $[\mathbf{8b}+\text{H}]^+$  is 788.2413 Da .



**Figure S9.** The one-pot reactions in H<sub>2</sub>O-DMSO (3: 7) of **1** (1 mM) and **9** (1.1mM) for 19 h. The 10  $\mu\text{L}$  reaction solution was diluted into 2 mL PBS buffer (50 mM, pH 7.4, containing 70% DMSO) for fluorescent tests (excitation, 540 nm).

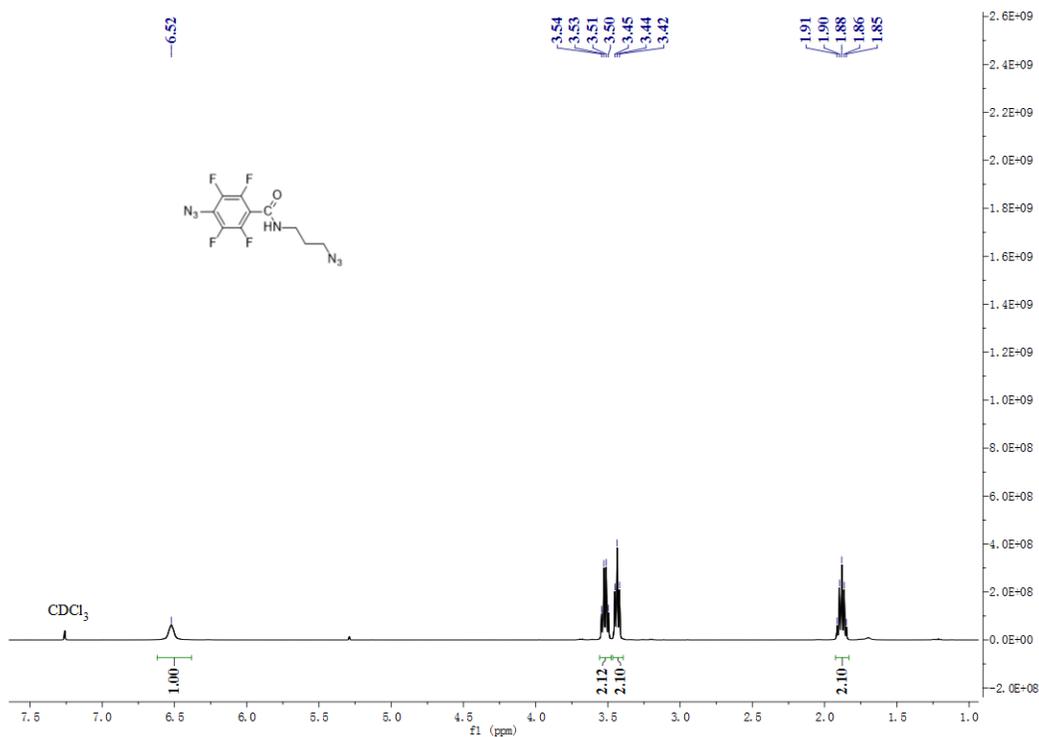


**Figure S10.** The one-pot reactions of **1** (1 mM) and **2** (1.1 mM) for 19 h, and then addition of **5** (1.5 mM) in H<sub>2</sub>O-DMSO (3:7). The 20  $\mu$ L reaction solution was diluted into 2 mL PBS buffer (50 mM, pH 7.4, containing 70% DMSO) at each time point for fluorescent spectra (excitation, 488 nm).

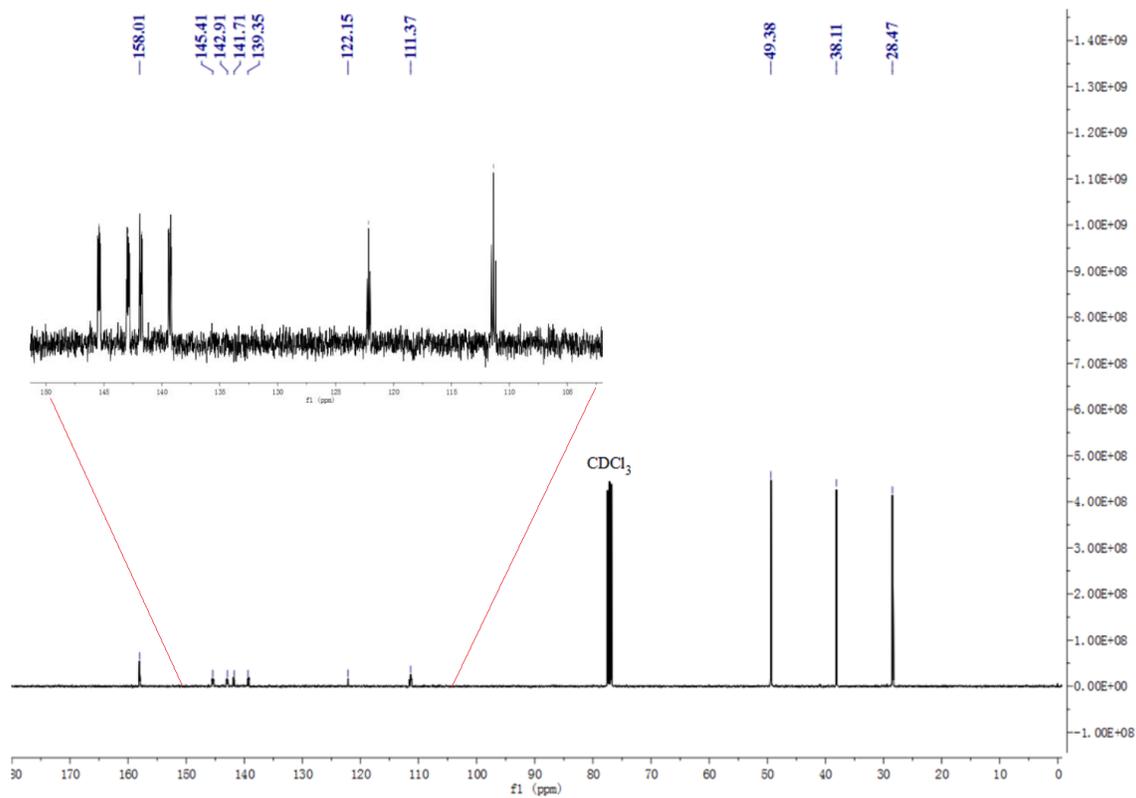


**Figure S11.** Fluorescence labelling of **9** (2  $\mu$ M), **1** (5  $\mu$ M) and **10** (2  $\mu$ M) in HEK-293 cells via *NSR* and *SPAAC*. The emissions were collected at green channel (500-530 nm) and red channel (555-655 nm) upon single excitation at 488 nm. The overlay image (right below) represents the overlap of green channel, red channel, and bright-field image.

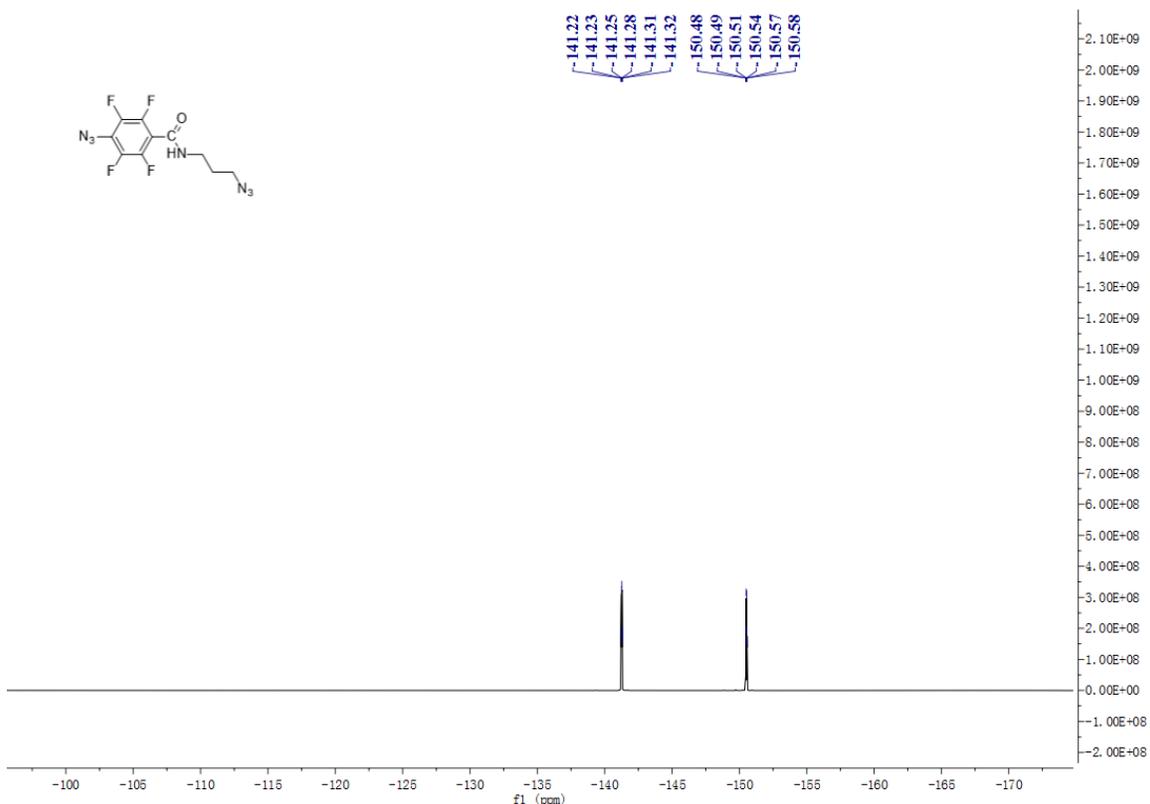
## 6. Supporting NMR and MS figures



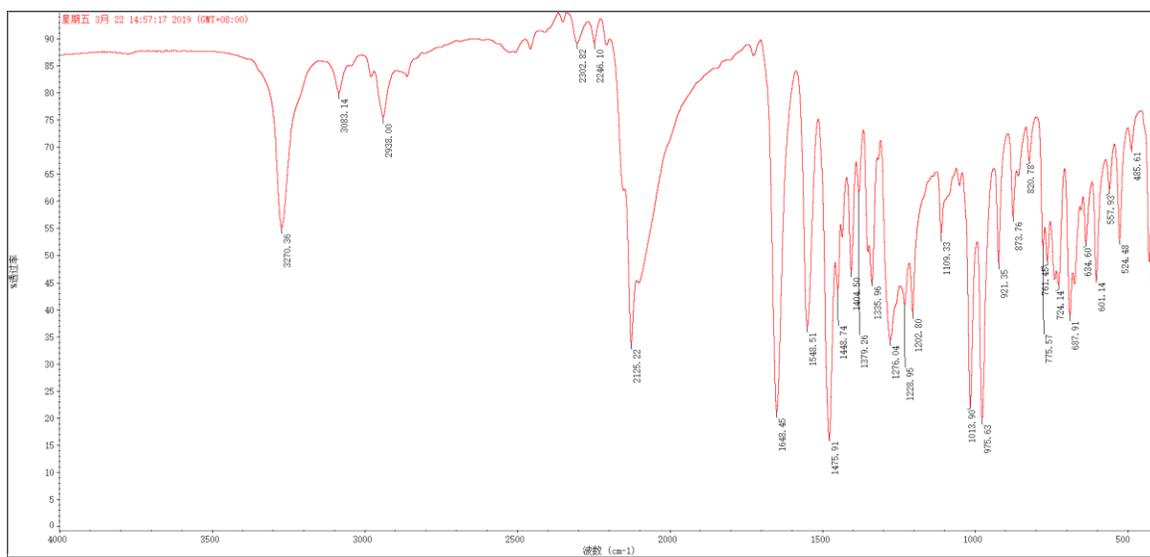
<sup>1</sup>H NMR of compound 1



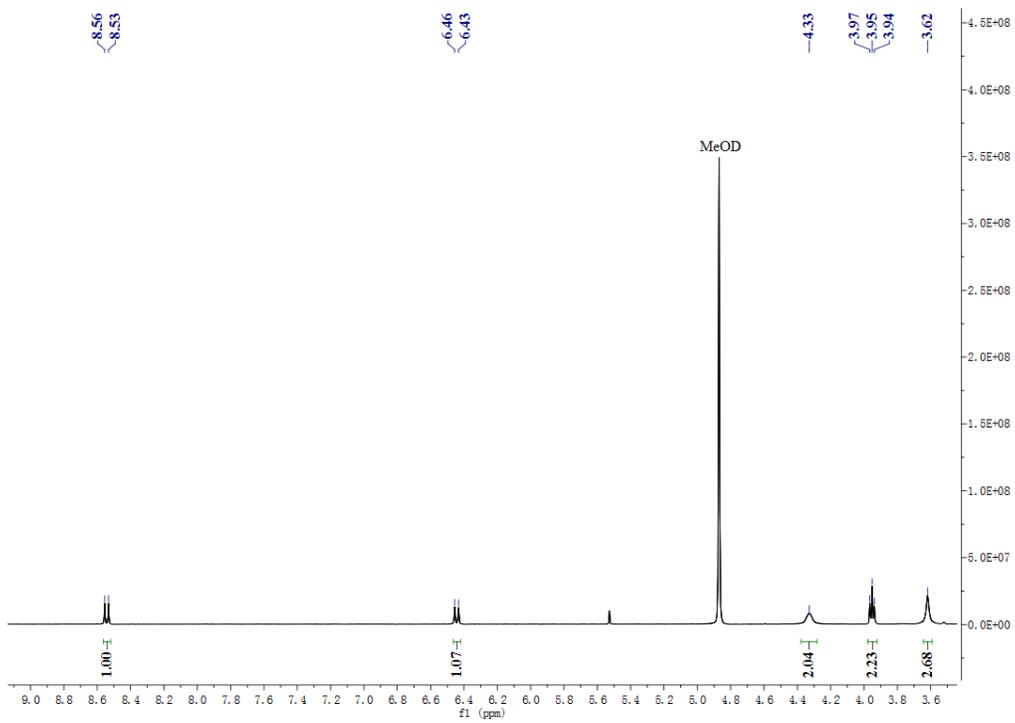
<sup>13</sup>C NMR of compound 1



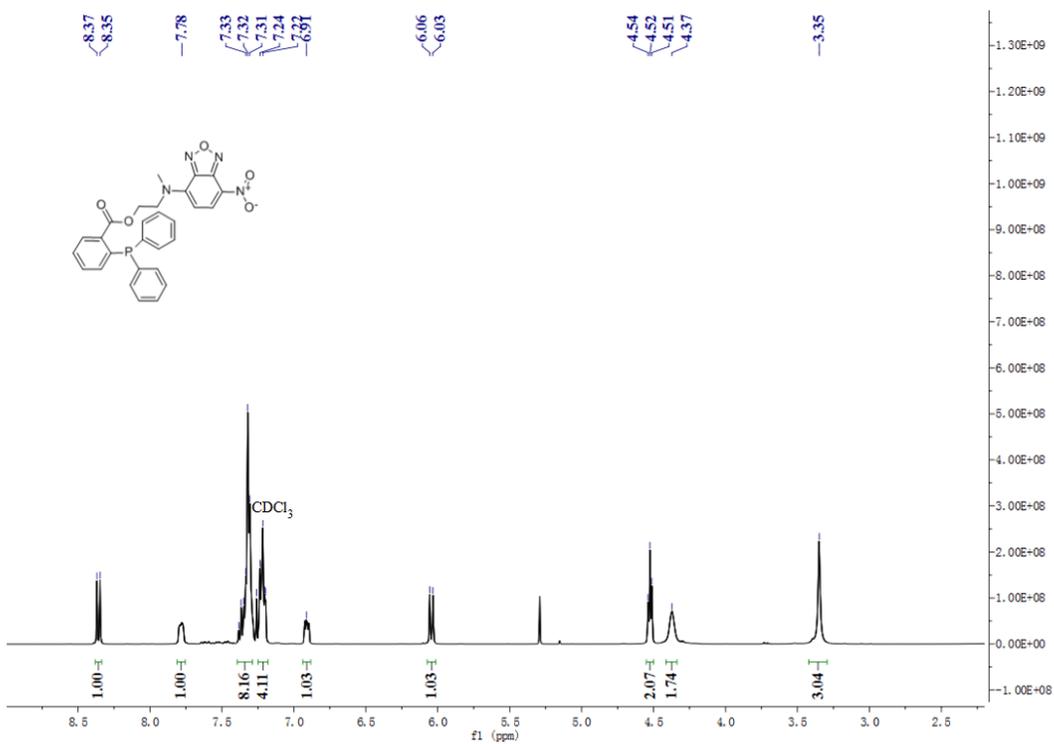
$^{19}\text{F}$  NMR of compound 1



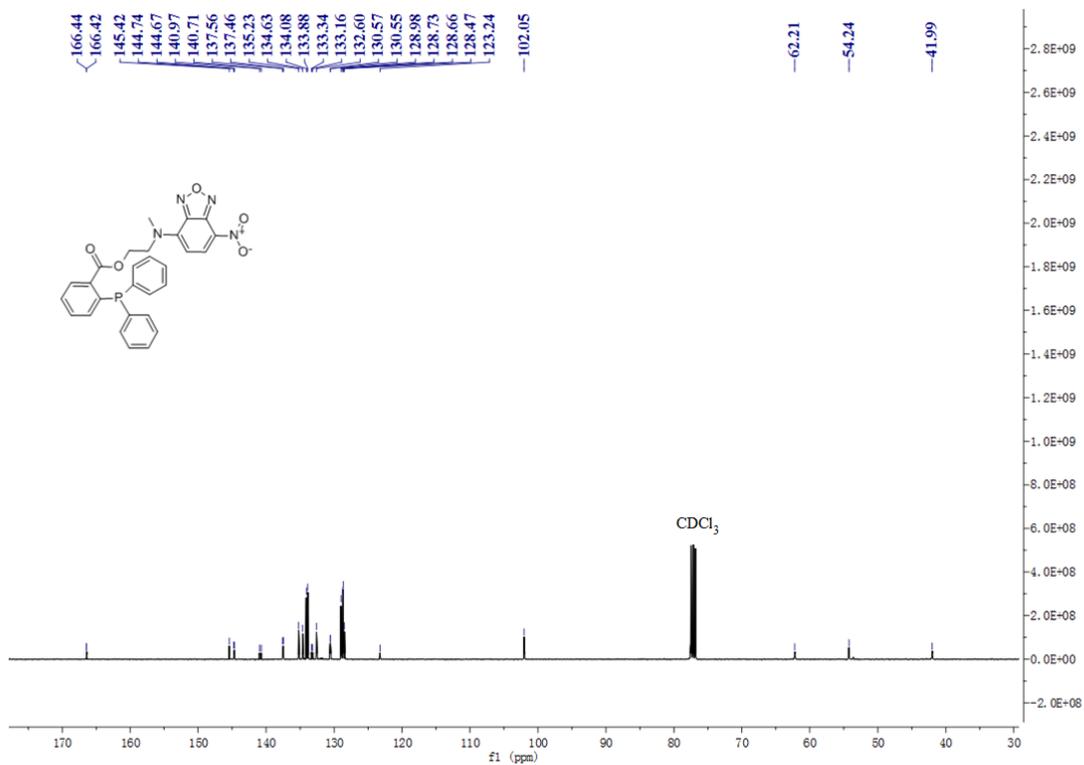
IR of compound 1



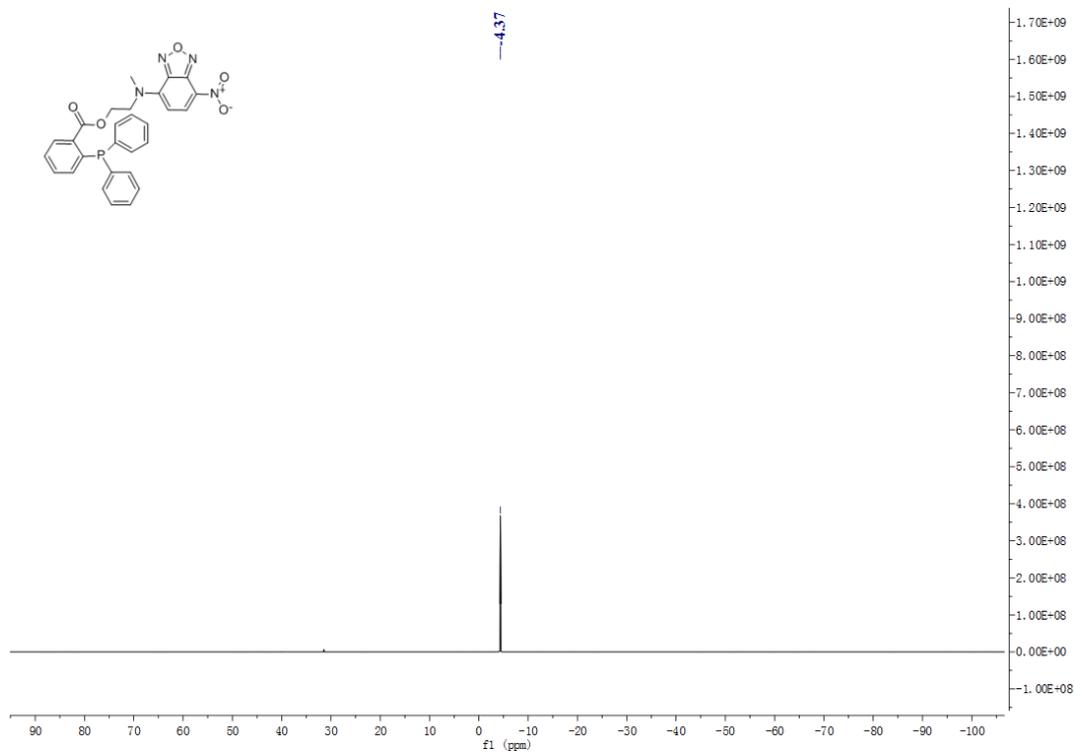
<sup>1</sup>H NMR of compound 5



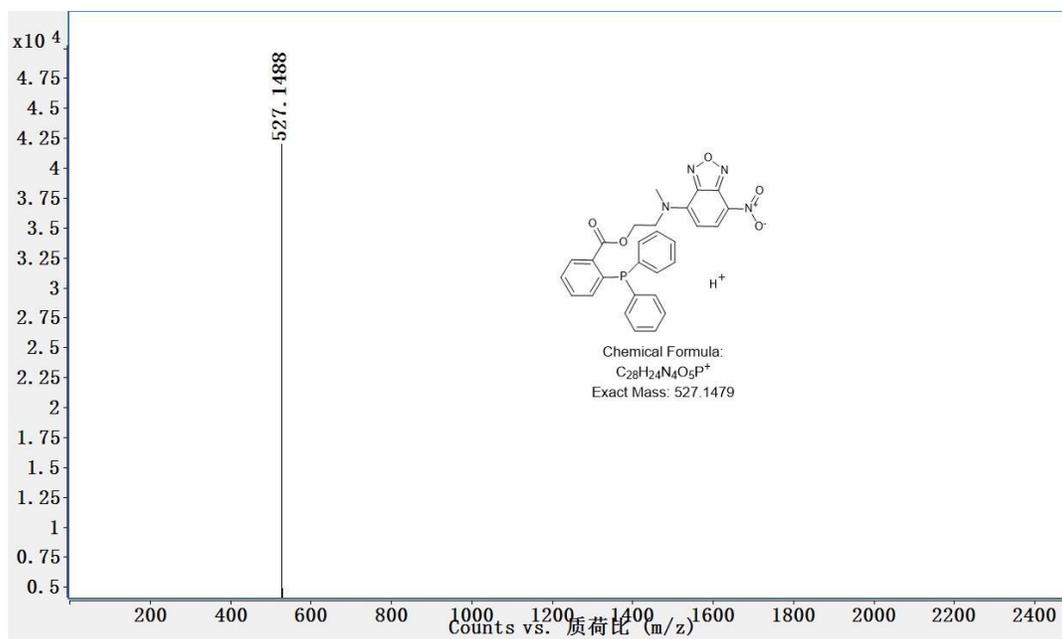
<sup>1</sup>H NMR of compound 2



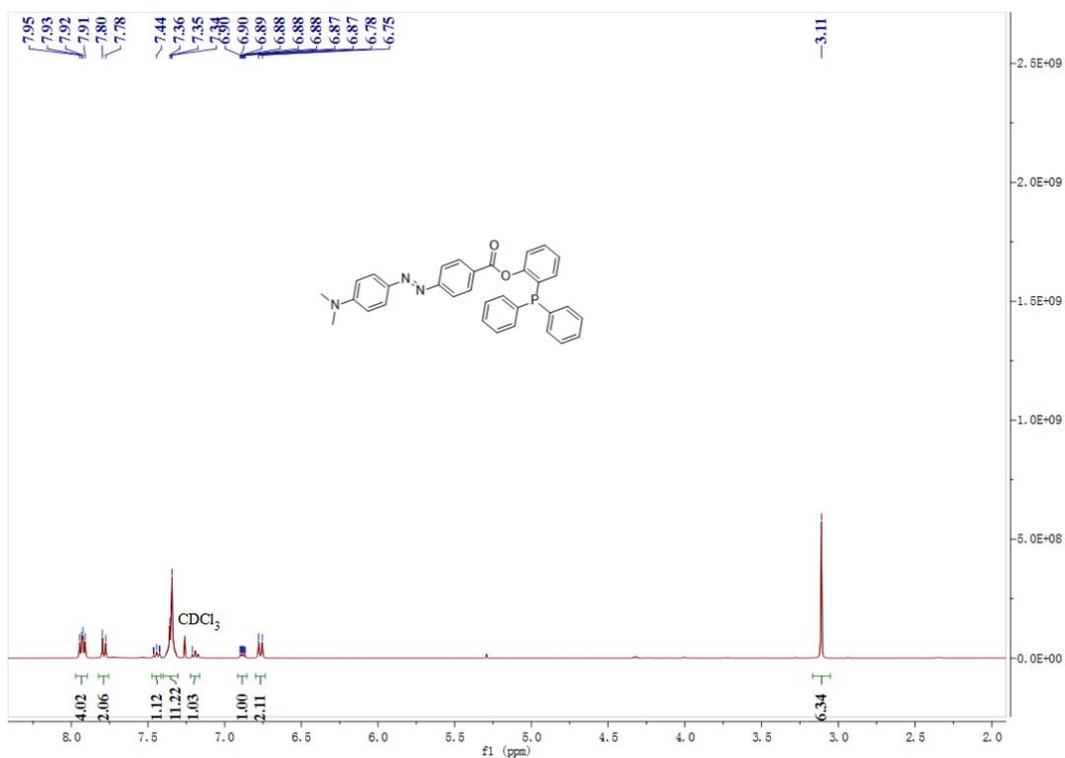
<sup>13</sup>C NMR of compound 2



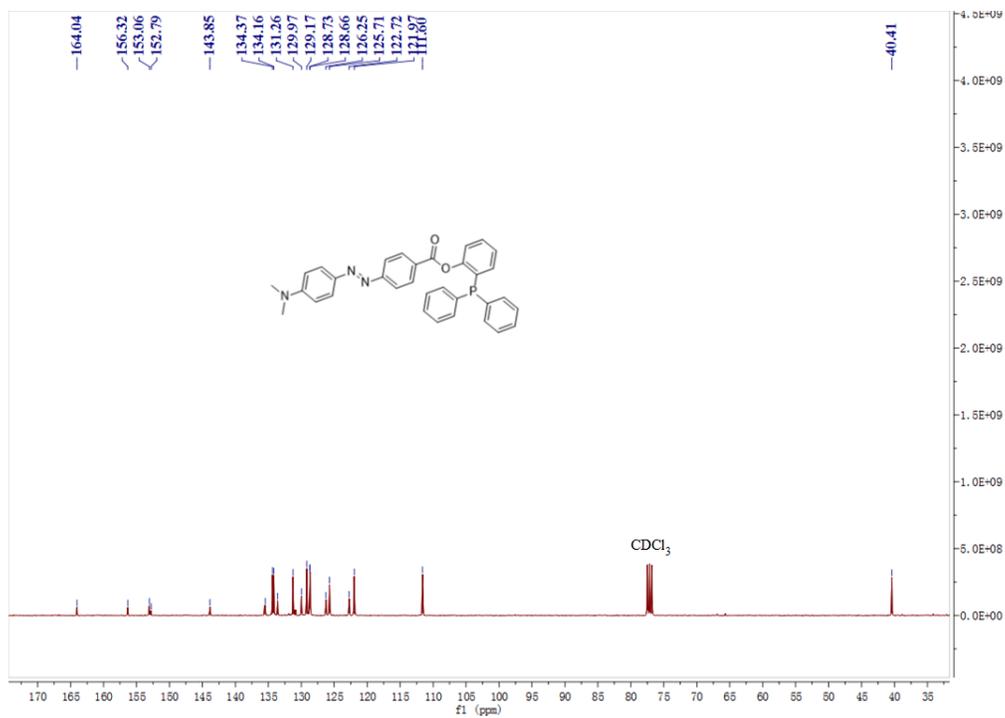
<sup>31</sup>P NMR of compound 2



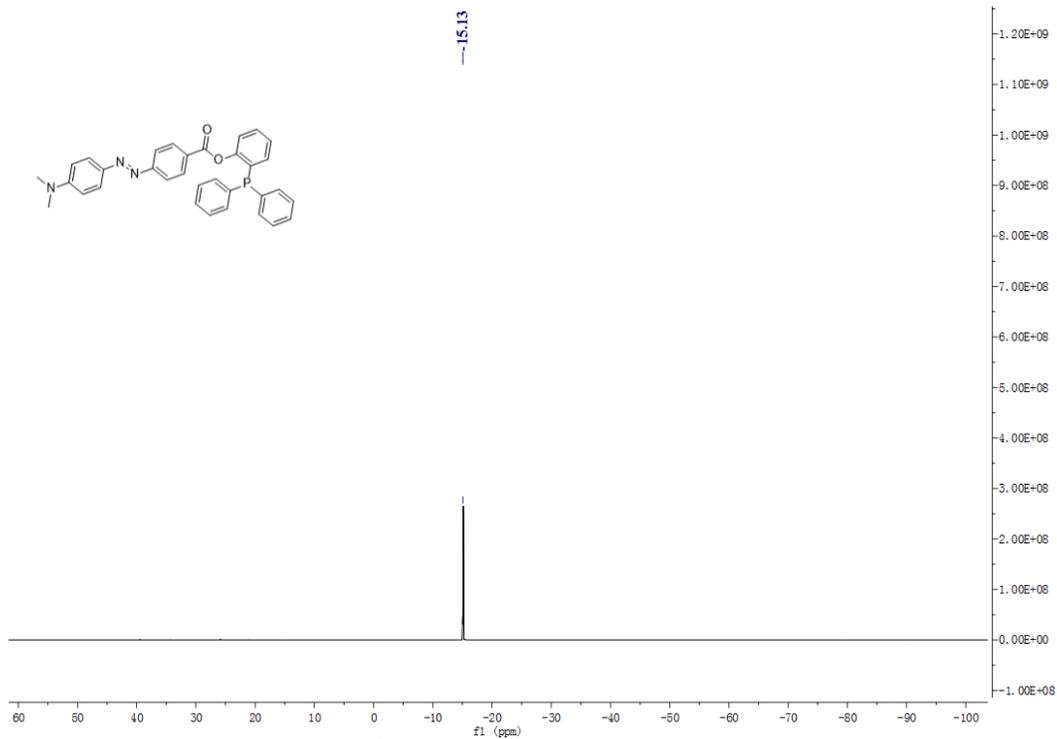
HRMS (ESI) of compound 2



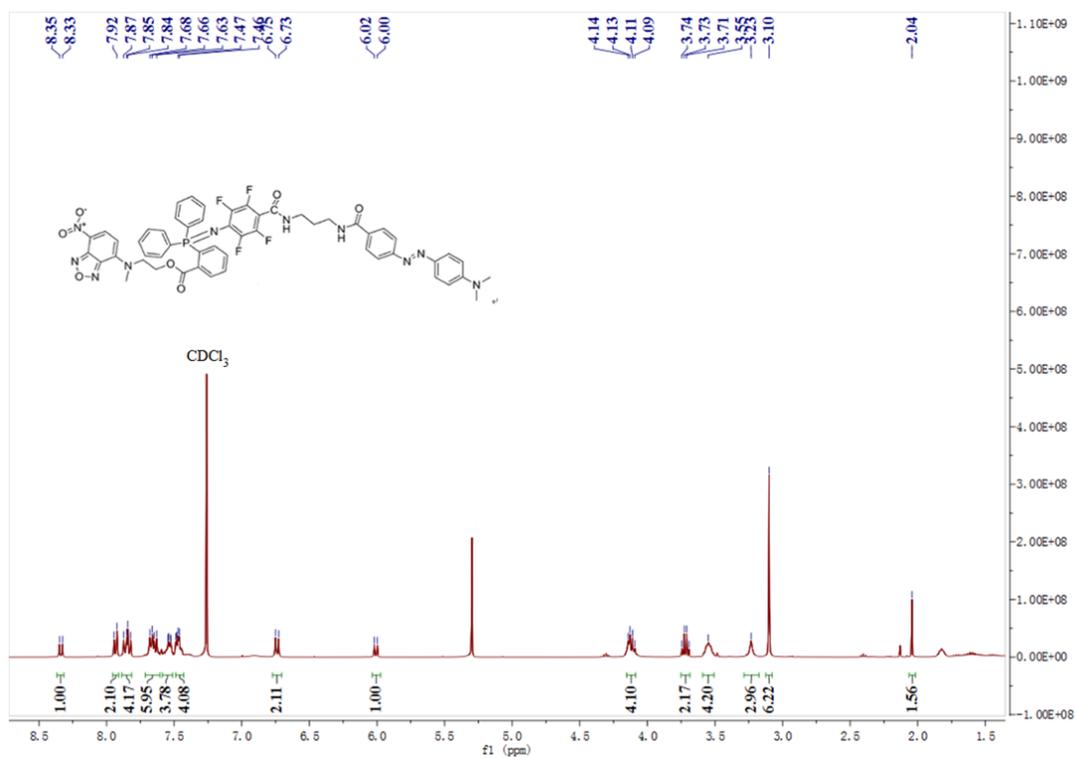
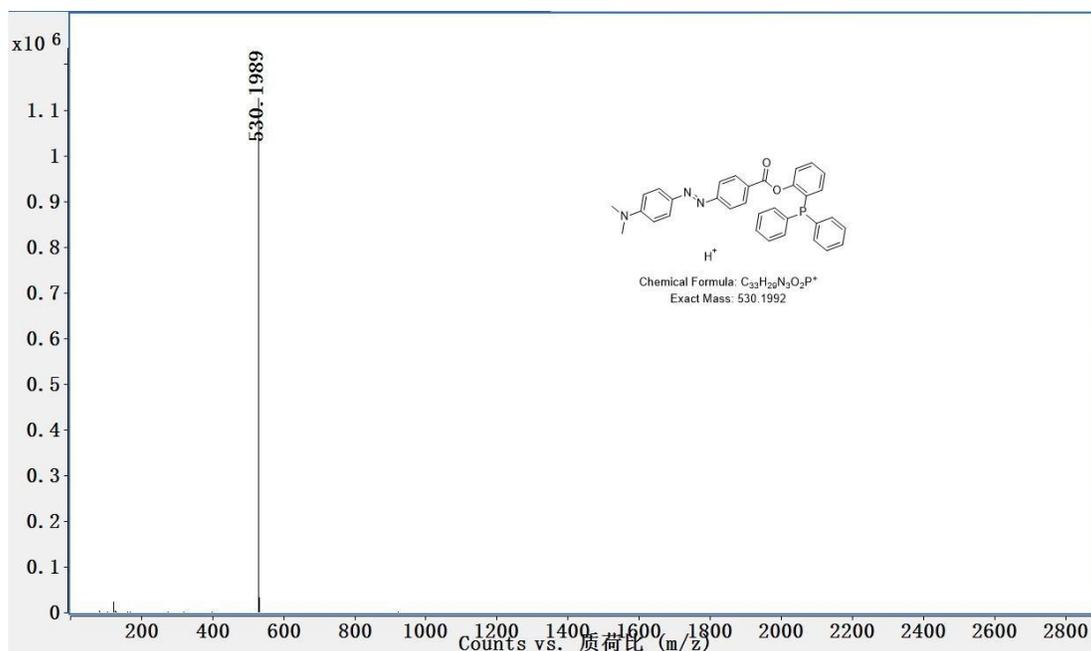
<sup>1</sup>H NMR of compound 6

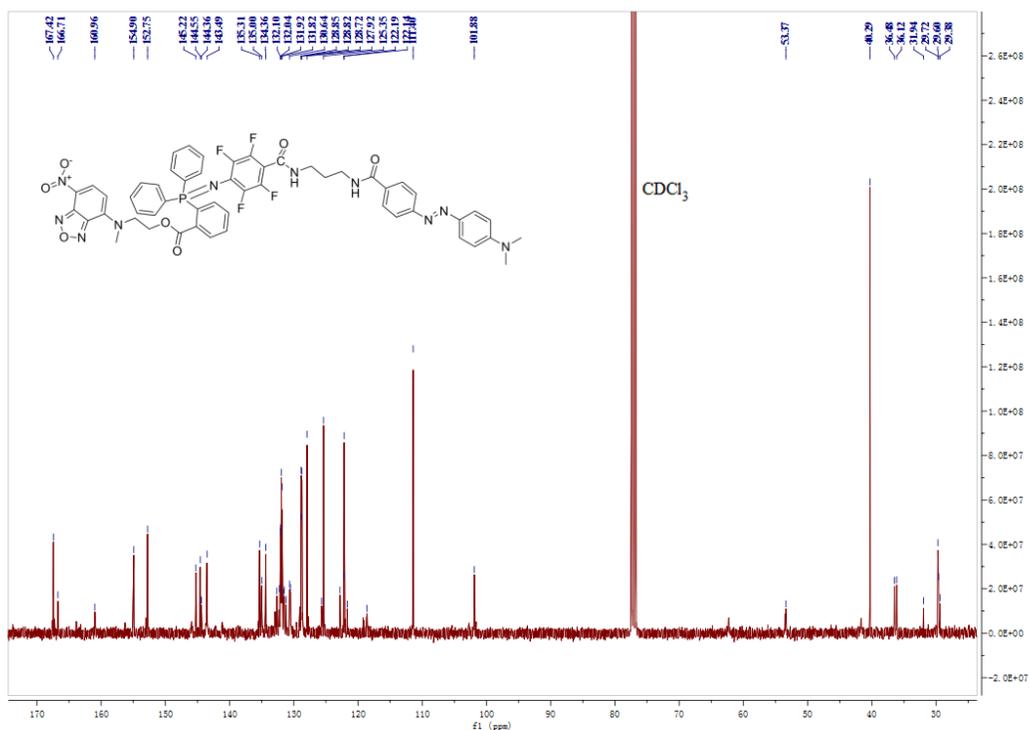


$^{13}\text{C}$  NMR of compound 6

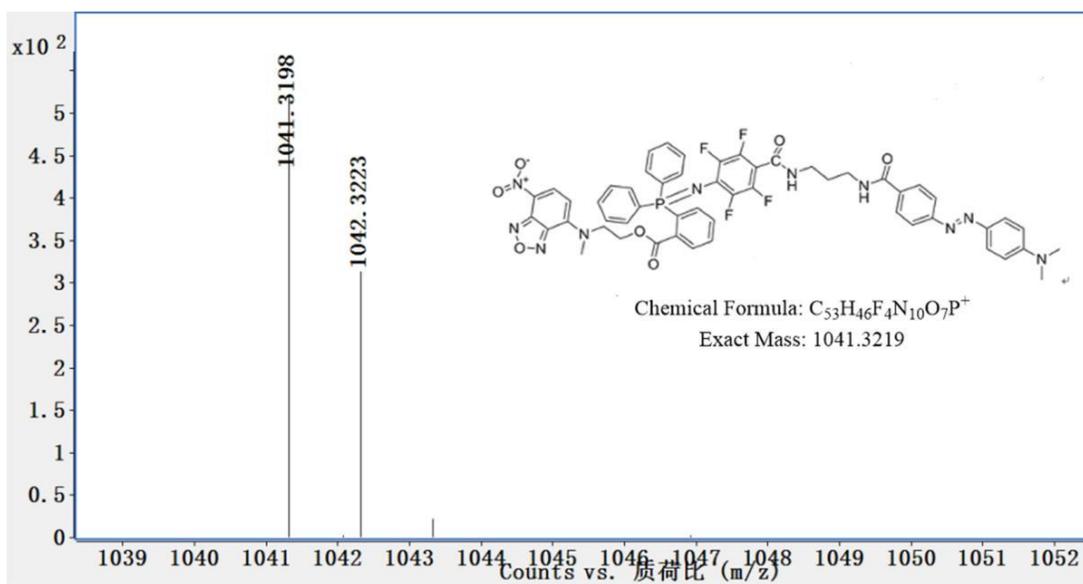


$^{31}\text{P}$  NMR of compound 6

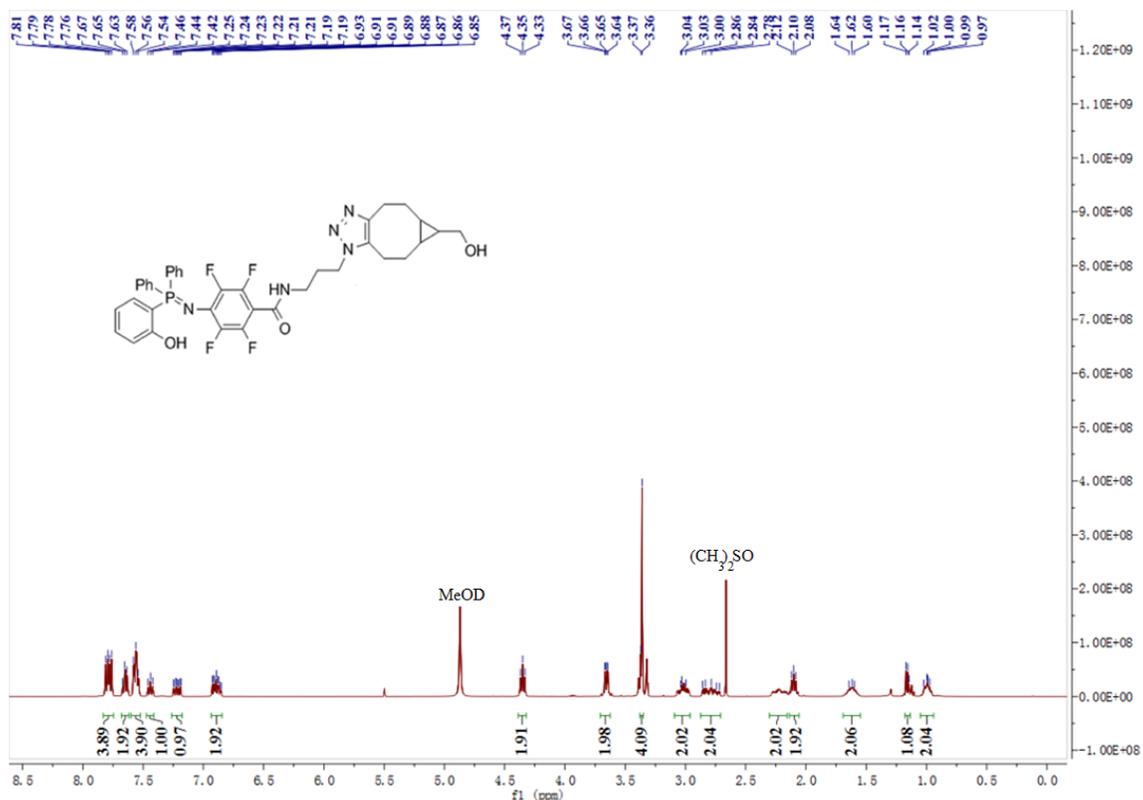




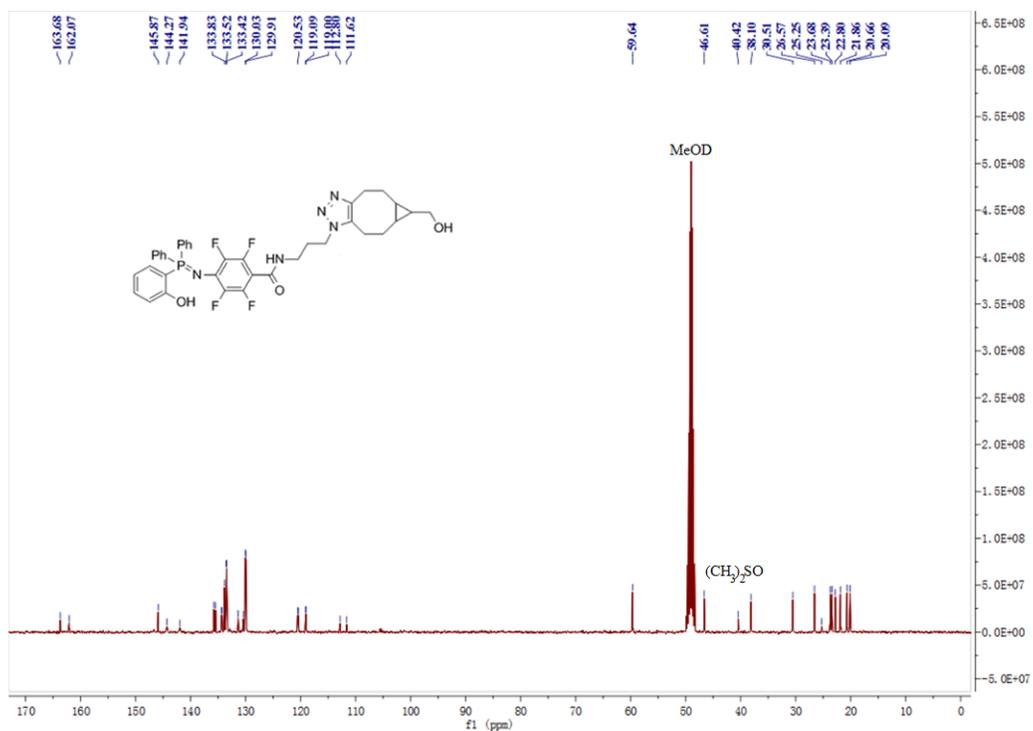
$^{13}\text{C}$  NMR of compound **7**



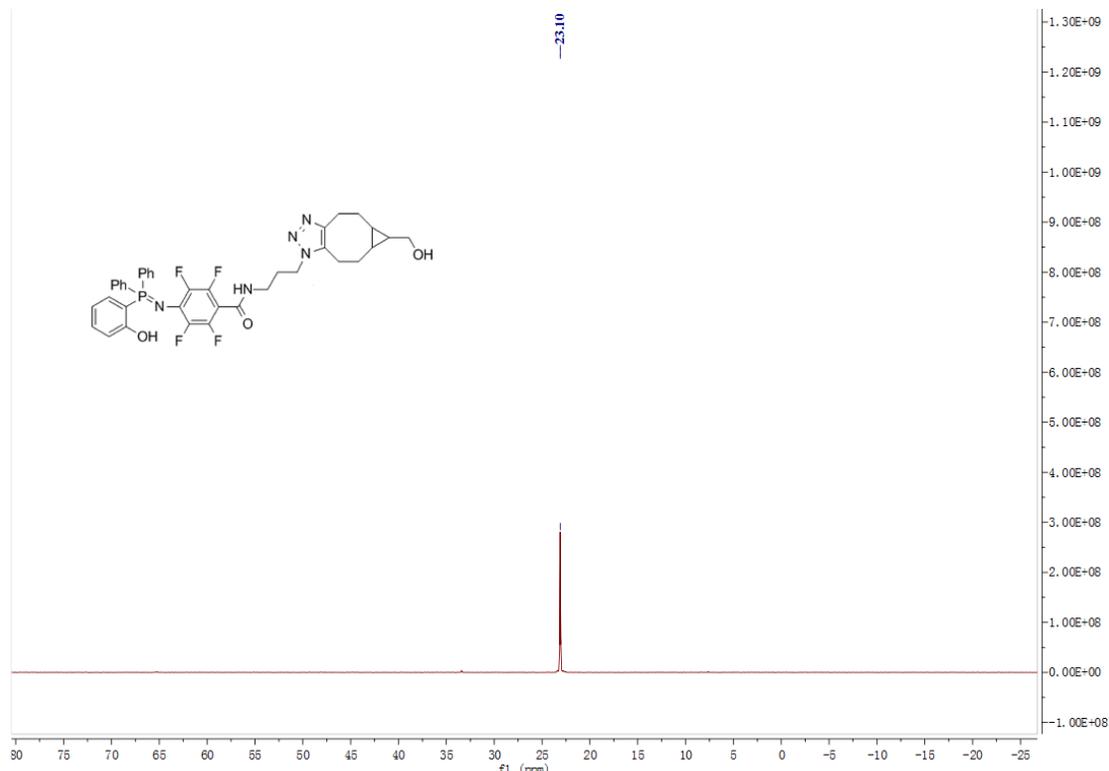
HRMS of compound **7**



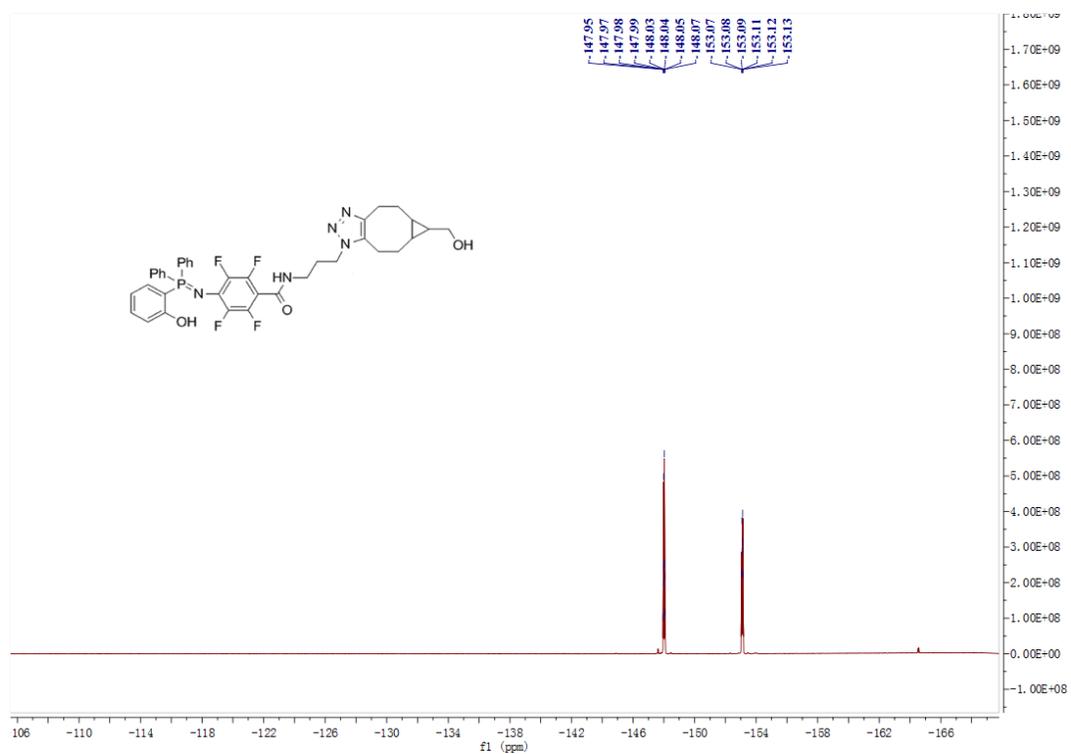
<sup>1</sup>H NMR of compound 8



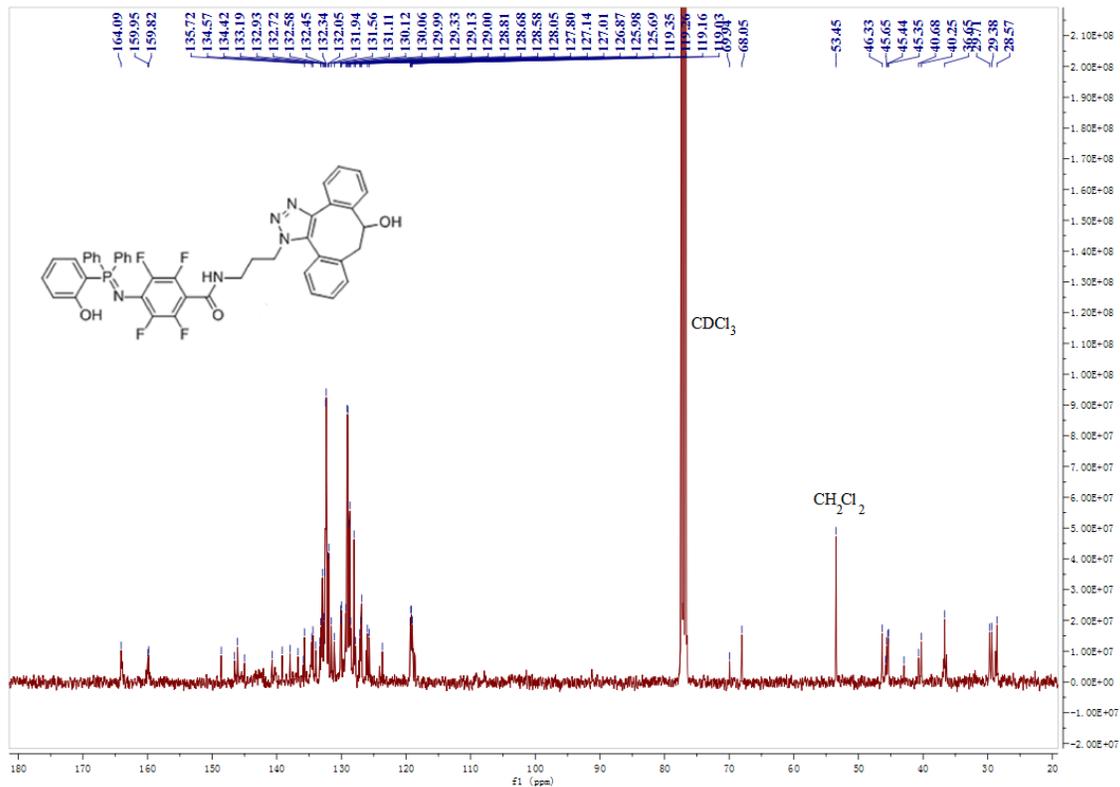
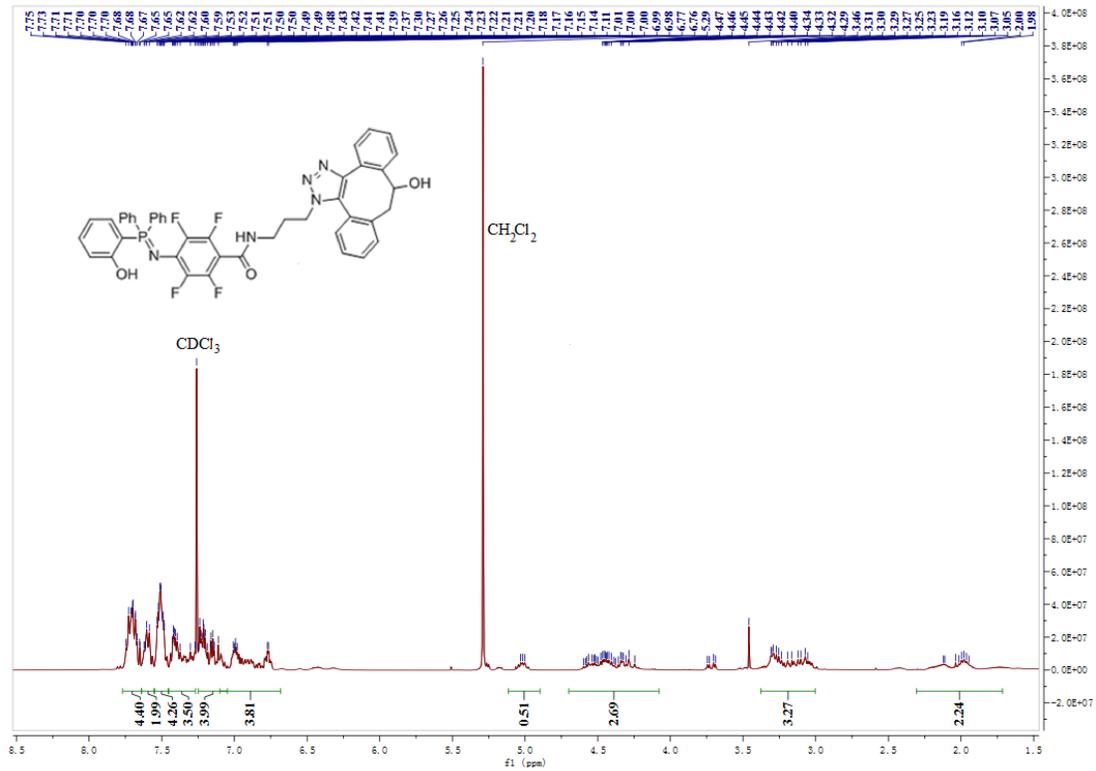
<sup>13</sup>C NMR of compound 8

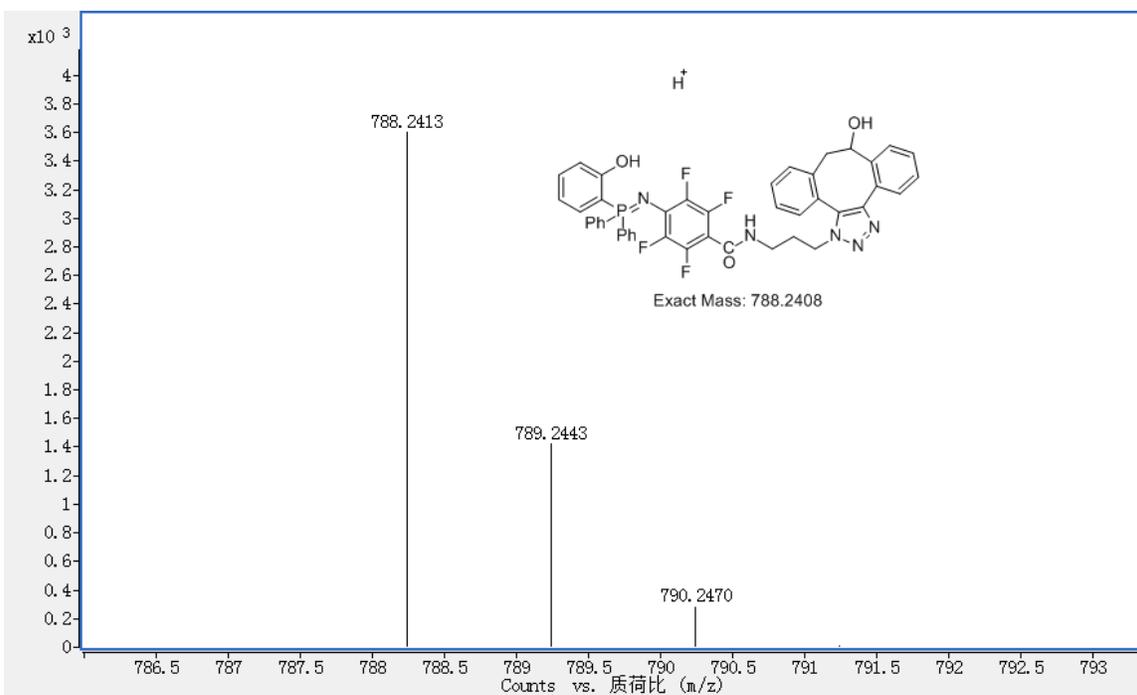


$^{31}\text{P}$  NMR of compound 8

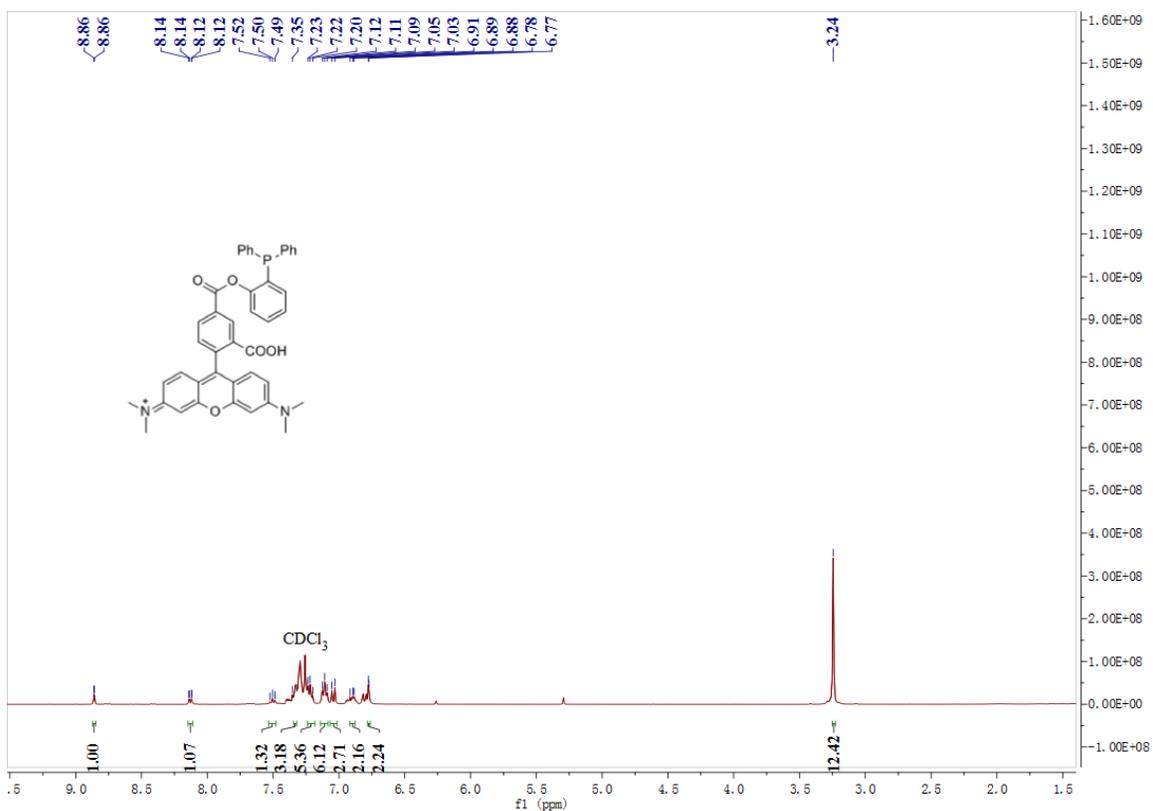


$^{19}\text{F}$  NMR of compound 8

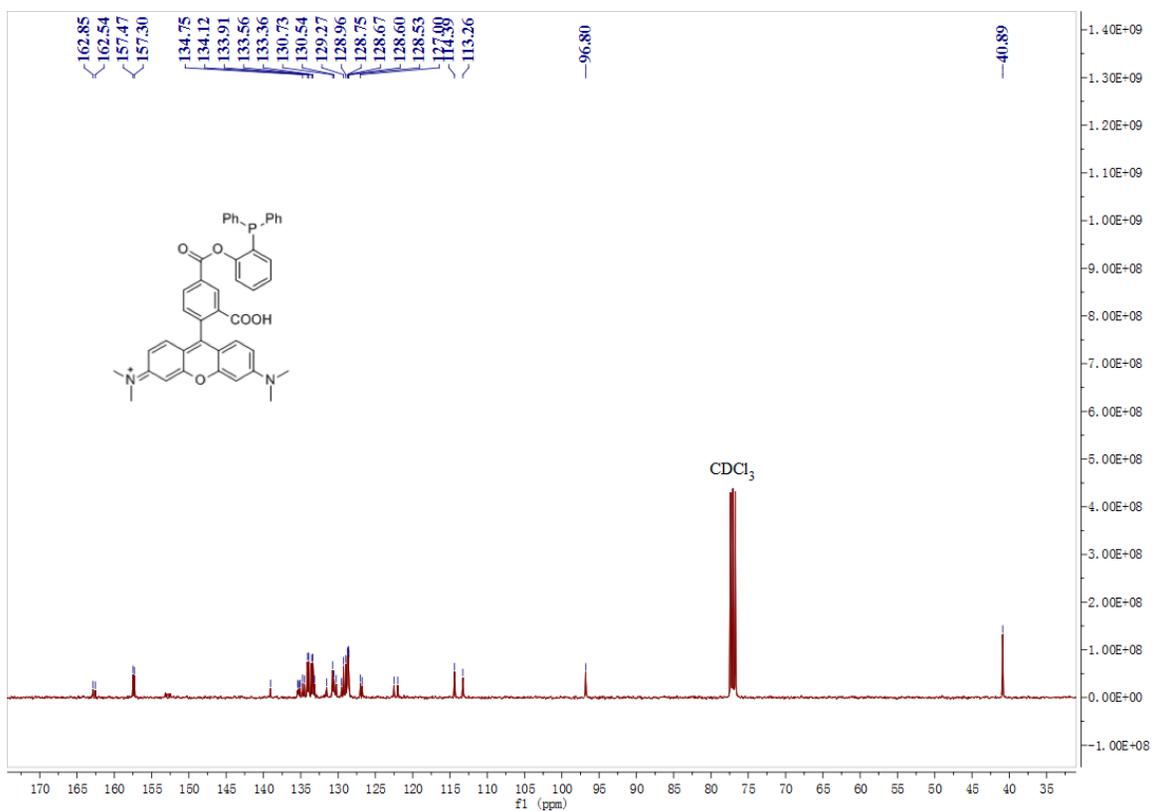




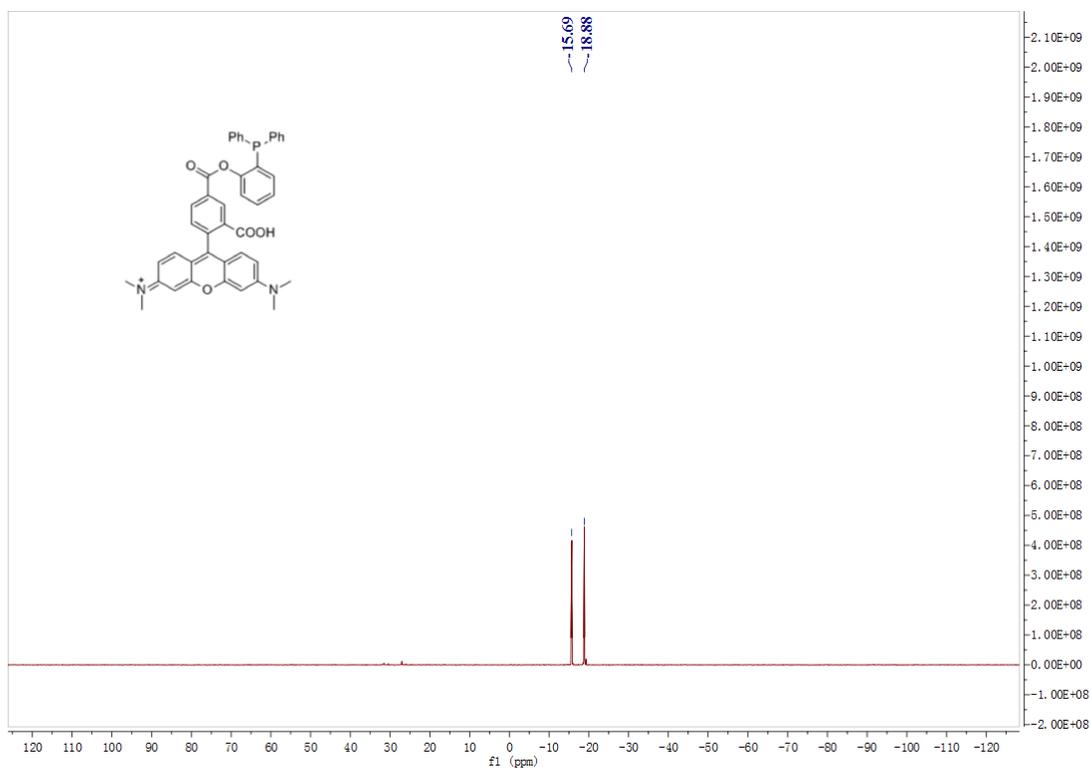
HRMS (ESI) of compound **8b**



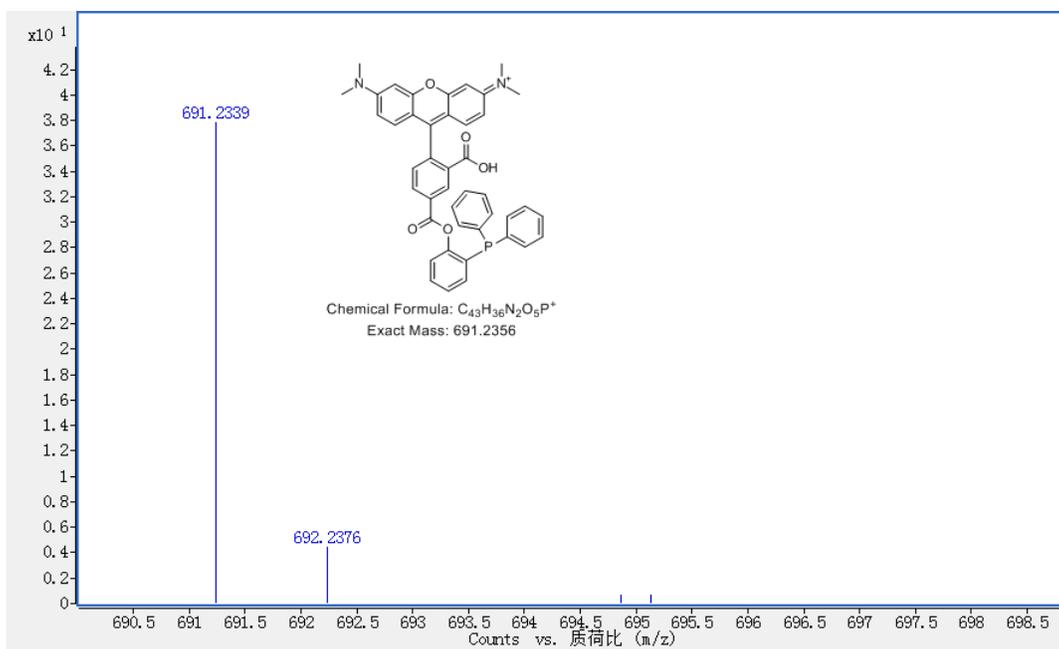
<sup>1</sup>H NMR of compound **9**



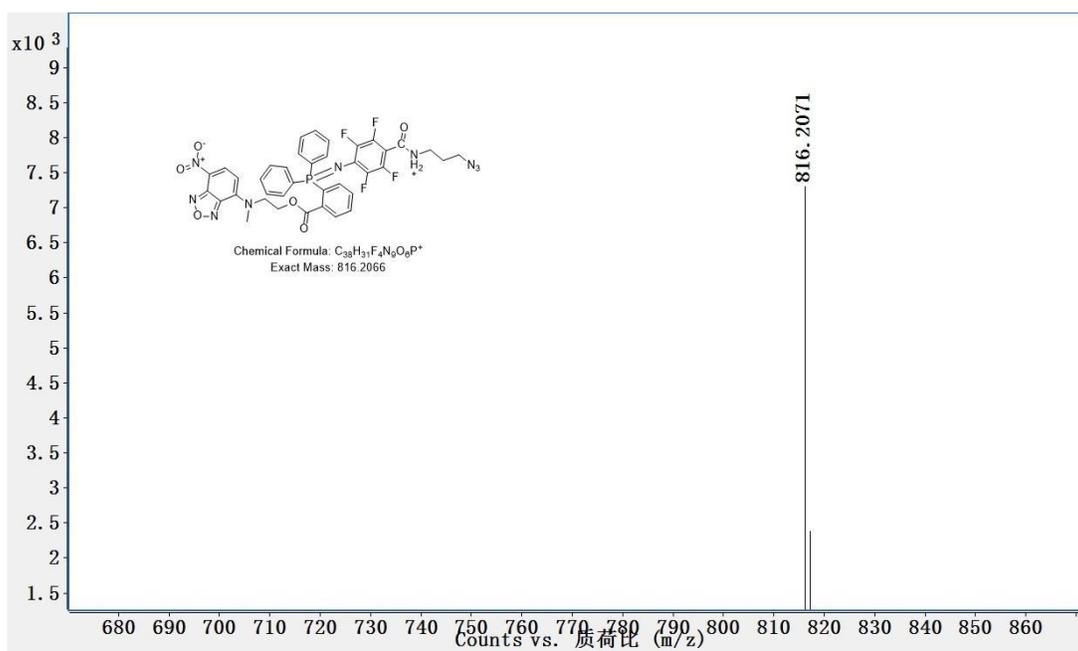
$^{13}\text{C}$  NMR of compound 9



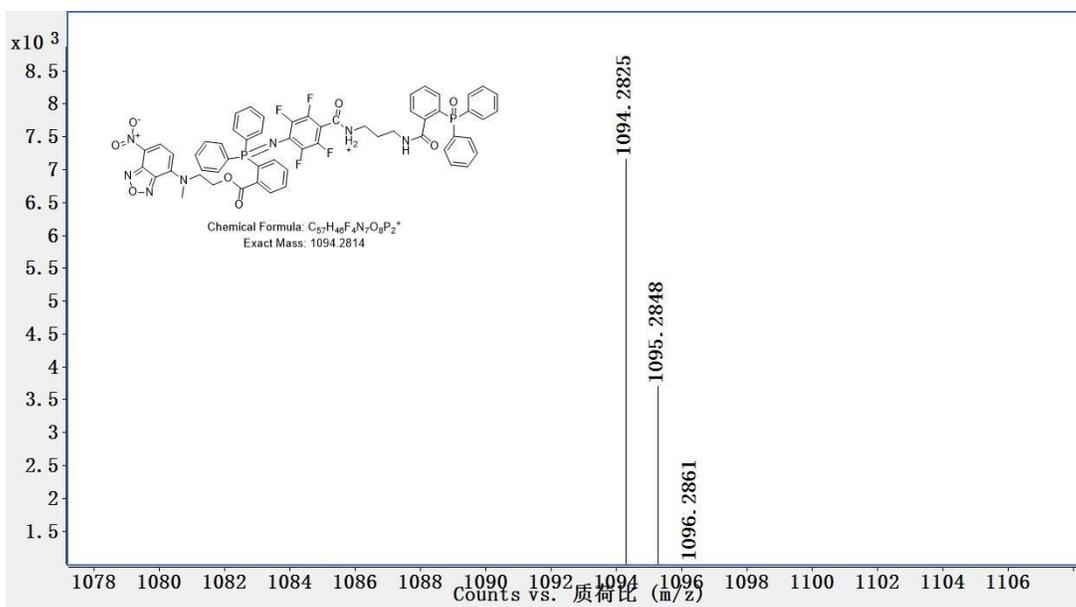
$^{31}\text{P}$  NMR of compound 9



HRMS of compound 9



HRMS of compound 3



HRMS (ESI) of compound 4