Supplementary Information for

Tetrazole-ene Photoactivatable Fluorophore with Improved Brightness and Stability in Protic Solution

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Supplementary Figures and Tables

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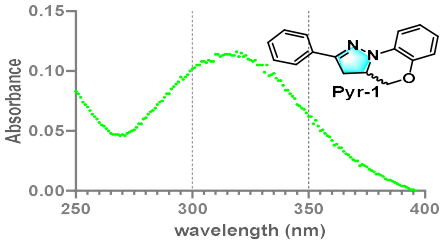

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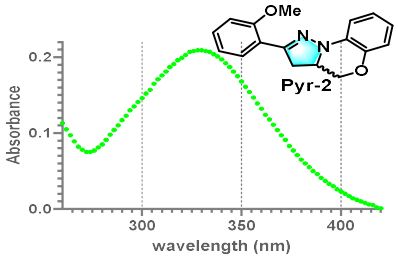

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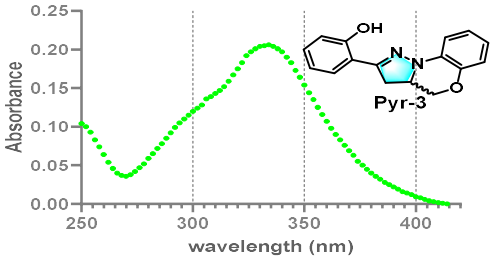

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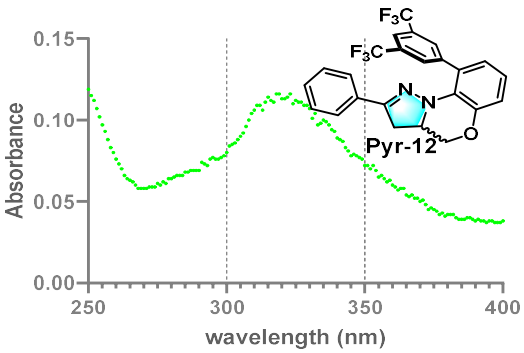


Figure S1 UV-Vis spectra of pyrazolines (a) **pyr-1,** (b) **pyr-2** (c) **pyr-3** and (d) **pyr-12** in acentonitrile with 10 µM concentration.

Table S1. Details of the Crystal Structure of **Tet-9**.

CCDC No.	2165940
empirical formula	$C_{22}H_{17}FN_4O$
formula wt	372.39
crystal system	Monoclinic
space group	P2(1)/c
T (K)	298(2)
a (Å)	5.5665(3)
b (Å)	23.9215(16)
c (Å)	13.9094(9)
α (°)	90
β (°)	91.355(3)
γ (°)	90
V (Å^3)	1851.6(2)
Z	4
ρ_{calcd} (mg/m^3)	1.336
μ (mm^{-1})	0.092
θ range (°)	2.246–25.142
F (000)	776
collected reflcns	17537
unique reflcns no.	3314
R [$I > 2\sigma(I)$]	0.0777
R (ref)	0.1634
wR2 [$I > 2\sigma(I)$]	0.1981
wR2 (ref)	0.2642

Table S2 Photophysical properties of **2-pyr** and **3-pyr** in solution[a].

samples	λ_{max} (nm)	ε_{max} ($M^{-1}cm^{-1}$)	λ_{em} (nm)[b]	Stokes shift (cm^{-1})	Φ_f(%)[c]	Quenching efficiency (%)[d]
3-pyr	328	20,900	484	9980	0.23	-
2-pyr	334	20,600	484	9290	0.01	0.95

[a] Measured in Acetonitrile (10 μM). [b] λ_{ex} = 350 nm. [c] Quinine sulfate (Φ_f = 0.54 in 0.1 M H_2SO_4) as a reference. [d] Quenching efficiency = $1-\frac{Maximum\ Intensity\ of\ Hydroxy-pyrazol}{Maximum\ Intensity\ of\ Methoxy-pyrazoline}$

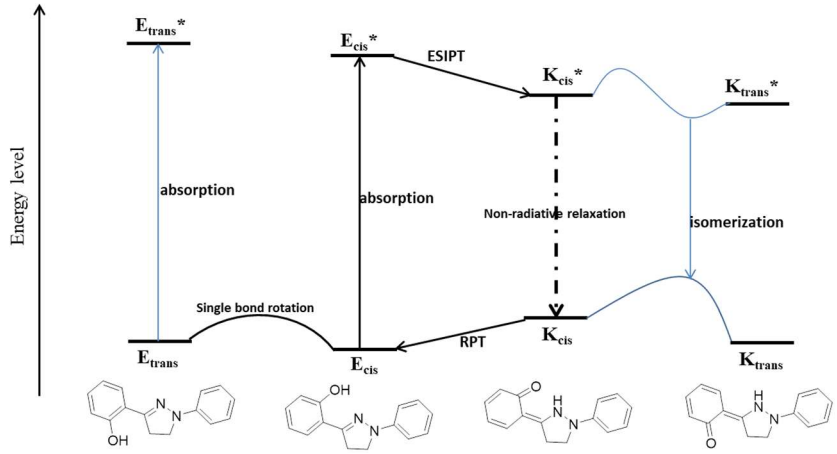
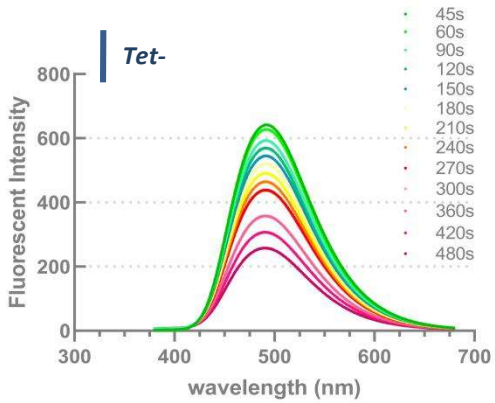
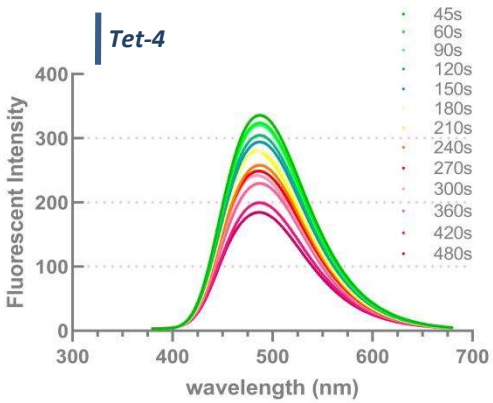
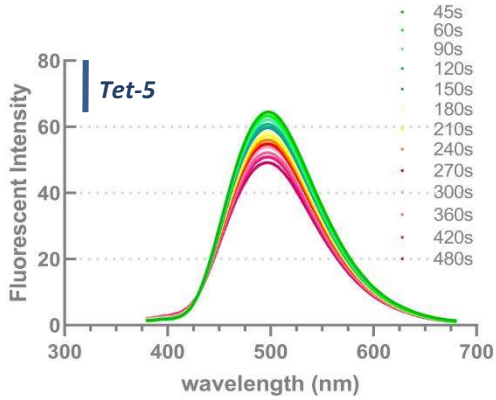


Figure S2. Schematic illustration fluorescence quench mechanism of pyrazoline by excited-state intramolecular proton transfer (ESIPT).


Tet-
45s
60s
90s
120s
150s
180s
210s
240s
270s
300s
360s
420s
480s
Fluorescent Intensity
wavelength (nm)


Tet-4
45s
60s
90s
120s
150s
180s
210s
240s
270s
300s
360s
420s
480s
Fluorescent Intensity
wavelength (nm)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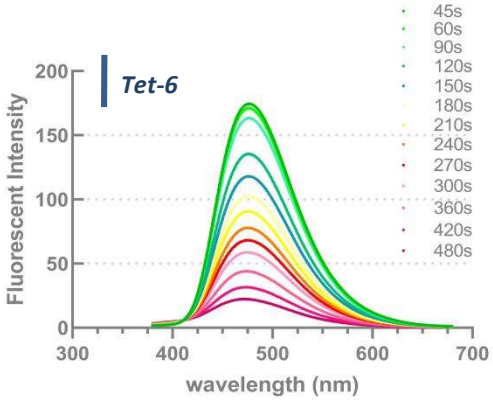
240s
270s
300s
360s
420s
480s
Fluorescent Intensity
wavelength (nm)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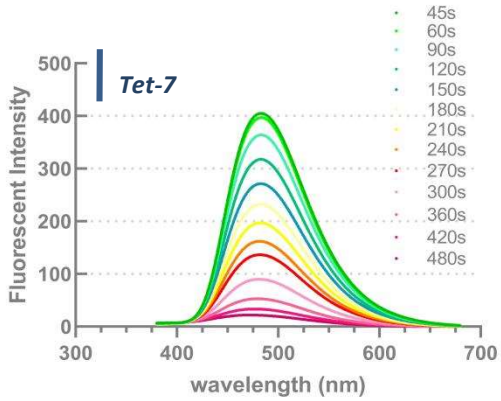
300s
360s
420s
480s
Fluorescent Intensity
wavelength (nm)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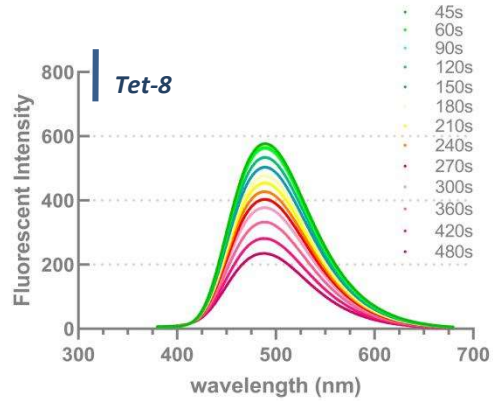
420s
480s
Fluorescent Intensity
wavelength (nm)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300s
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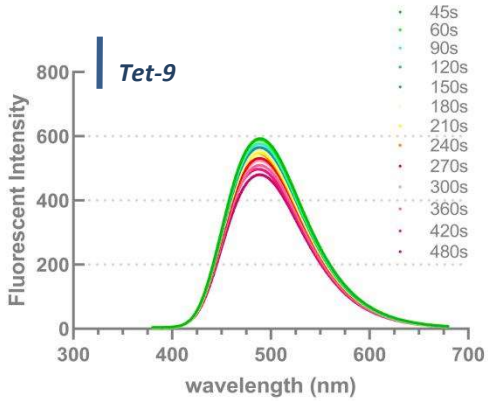
420s
480s
Fluorescent Intensity
wavelength (nm)


Tet-9
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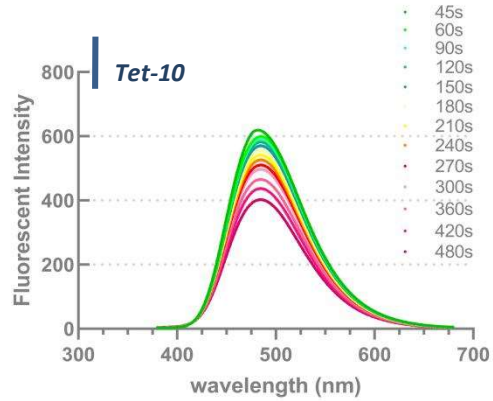
360s
420s
480s
Fluorescent Intensity
wavelength (nm)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wavelength (nm)

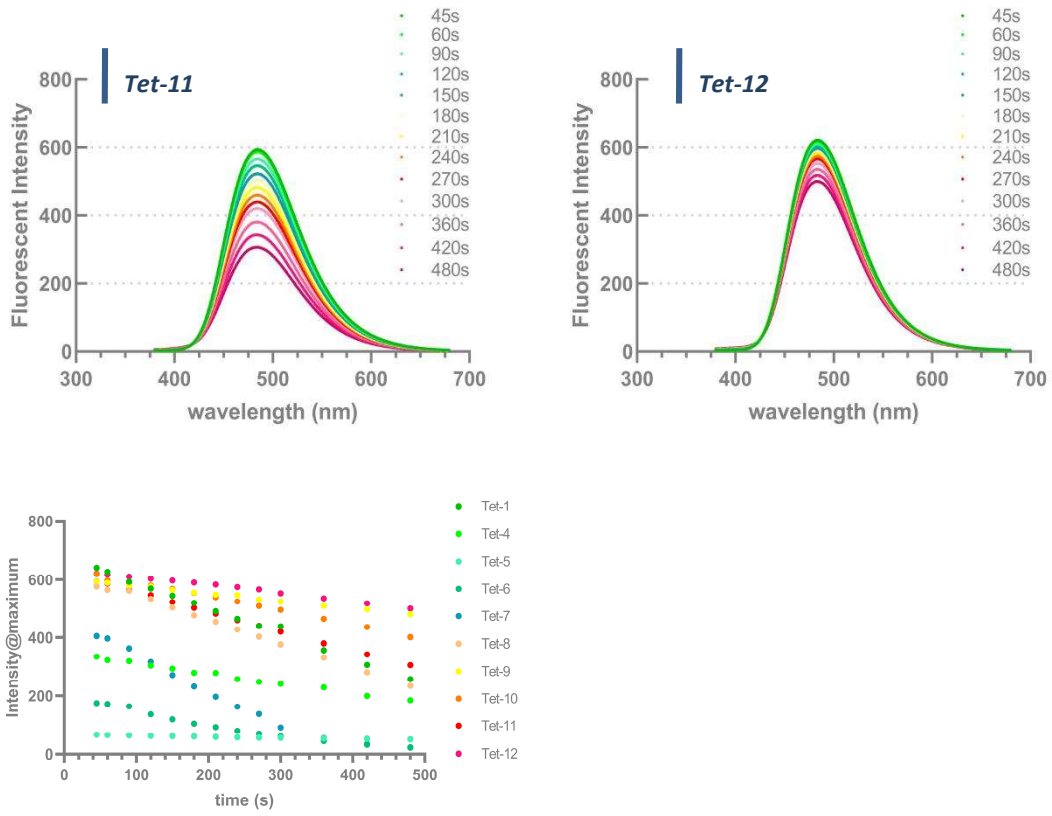


Figure S3 Photostability of pyrazole fluorophores generated from ten tetrazoles: **Tet-1, Tet-4–Tet-12.** The samples were made 10 μM solution in ACN and keep irradiation using 310 nm UV light and monitored by fluorimeter (the pyrazoline fluorophores were generally formed after 45 s irradiation).

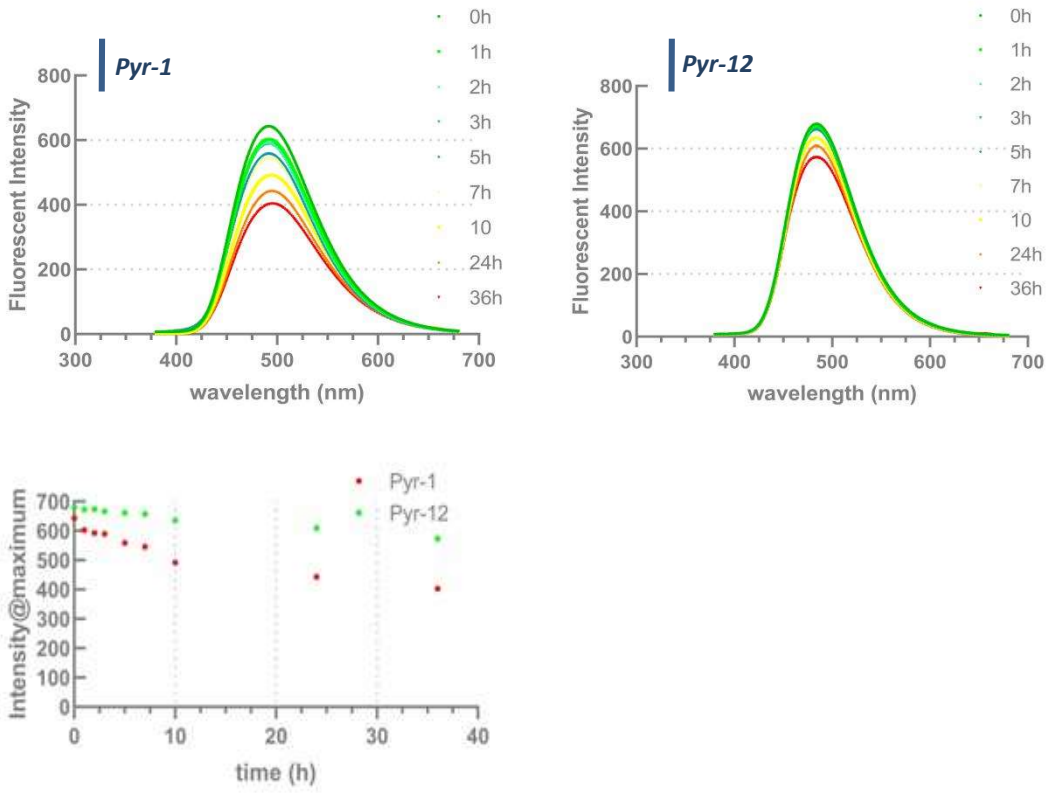
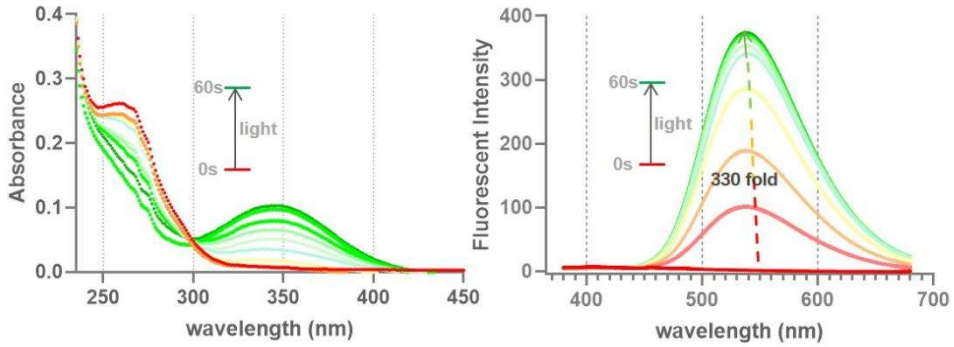


Figure S4 Stability of fluorophores **Pyr-1** and **Pyr-12** at ambient temperature. The samples were made 10 μM solution in ACN at room temperature and monitored by fluorimeter.

(a)



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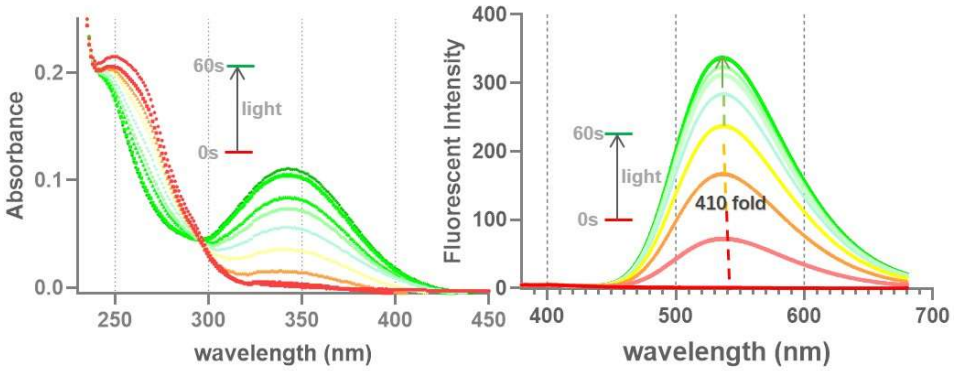

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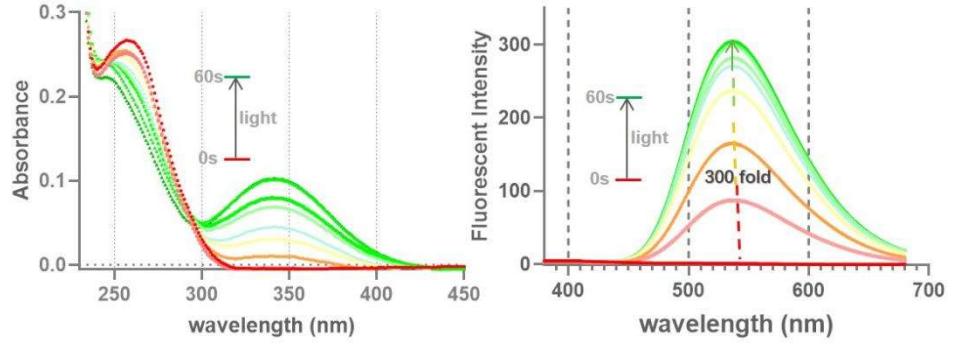


Figure S5 Time course of the photoactivatable fluorophore **TPP-Tet-12** (a), **Cl-Tet-12** (b), and **Mor-Tet-12** (c) as monitored by UV-vis absorption and fluorescence emission during light irradiation using a 310 nm light with 10 μM in PBS/ACN (1:1)

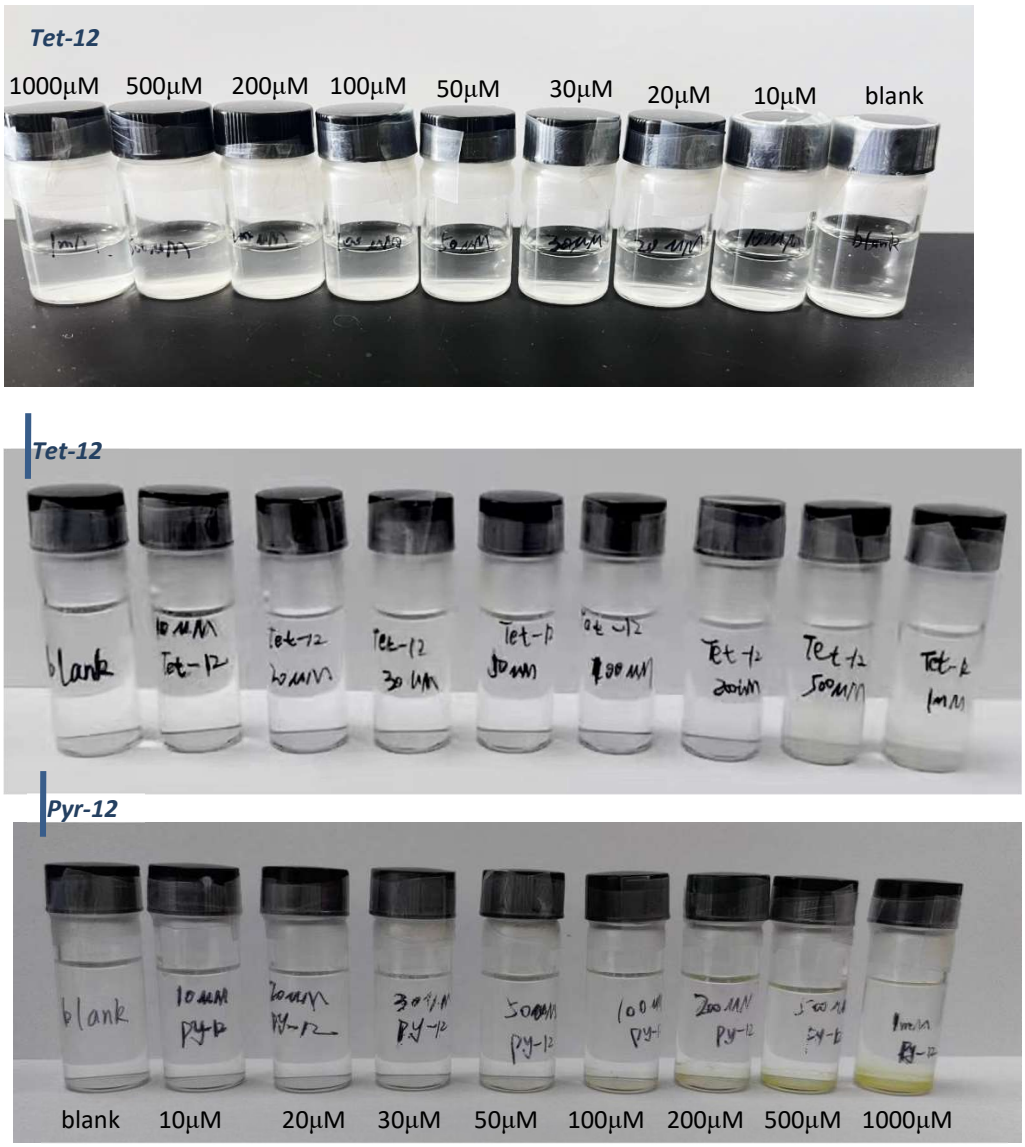


Figure S6 Solubility test of **Tet-12** and **Pyr-12** in pure PBS buffer at different concentration at room temperature.

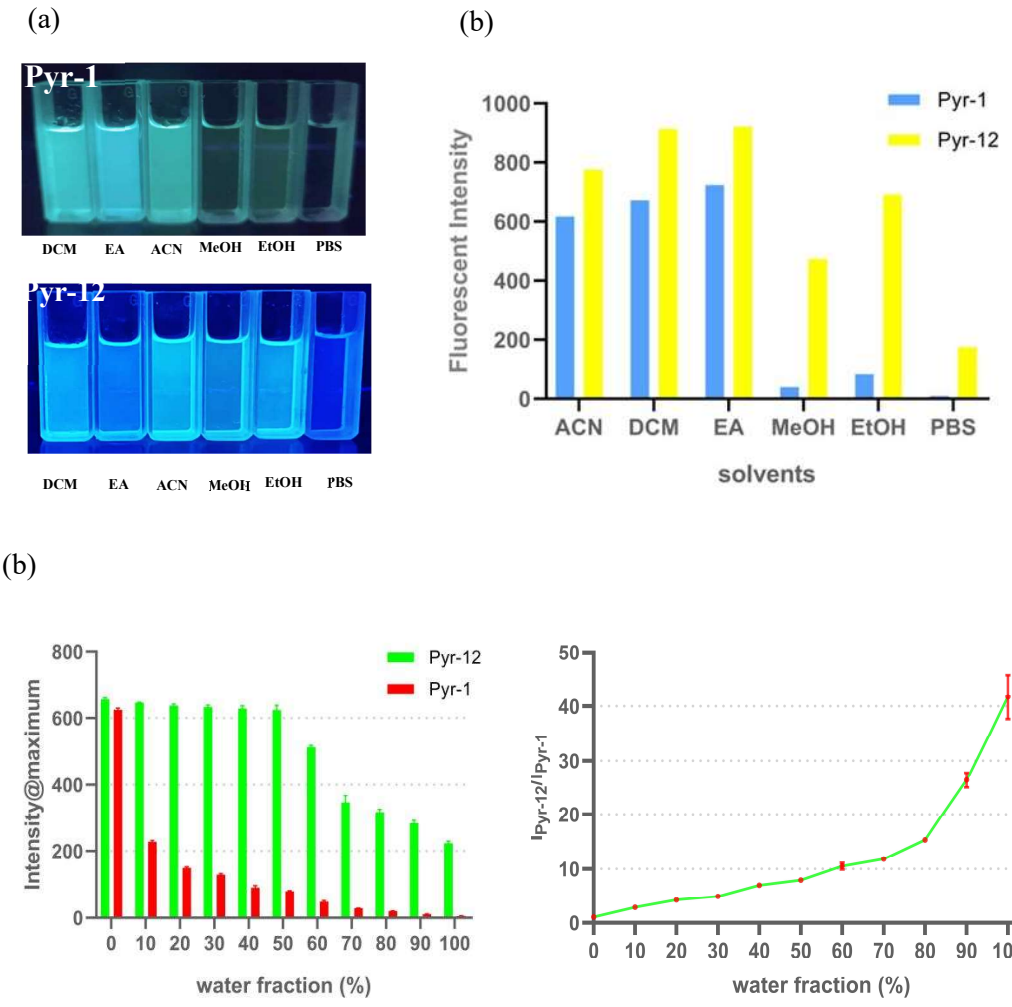


Figure S7 Fluorescence of **Pyr-1** and **Pyr-12** in different solvents at 10 μM concentration. (a) Fluorescence intensity comparison at maximum wavelength of **Pyr-1** and **Pyr-12** in six different solvents. (b) Fluorescence intensity comparison at maximum wavelength of **Pyr-1** and **Pyr-12** in PBS-ACN mixed solvents (the fraction of PBS from 0 to 100%) and the corresponding fluorescent intensity ratio of **Pyr-1** and **Pyr-12** at maximum wavelength.

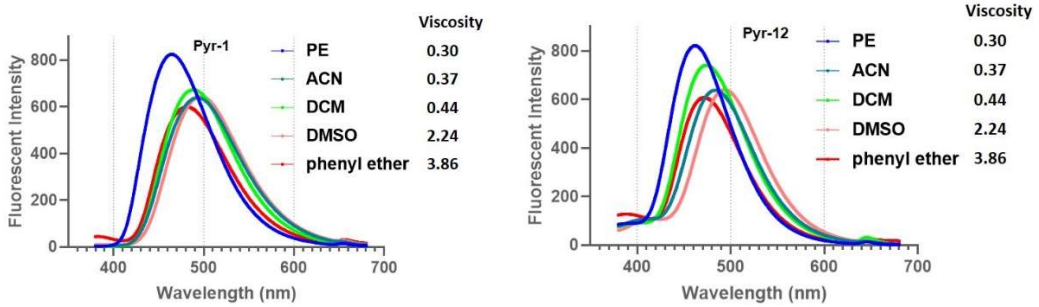


Figure S8 Fluorescence of **Pyr-1** and **Pyr-12** in five solvents with different viscosity. The samples were made 10 μM at room temperature and monitored by fluorimeter.

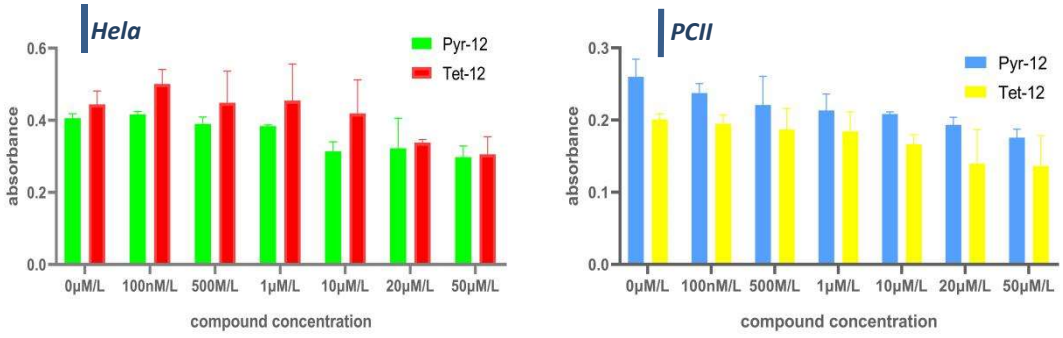


Figure S9 Cell viability of Hela and PCII cells treated with **Pyr-12** and **Tet-12**.

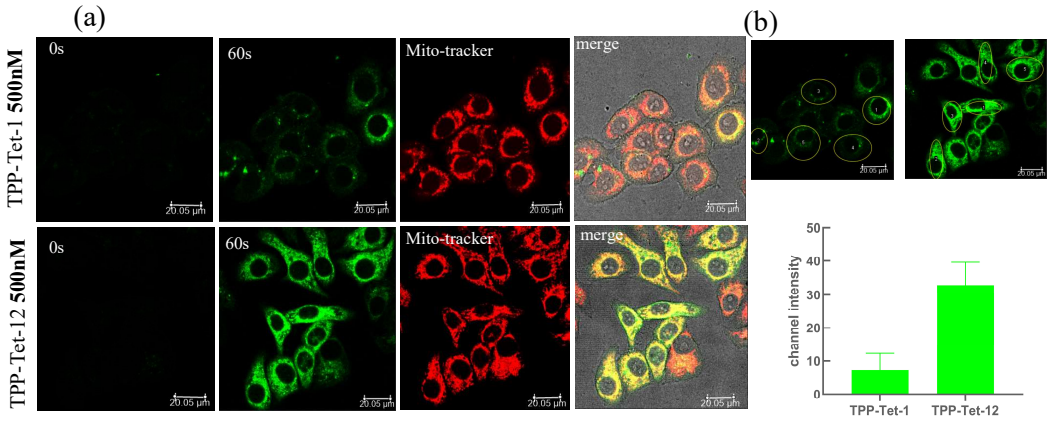


Figure S10 (a) Confocal micrographs of mitochondria-targeting probes **TPP-Tet-1** and **TPP-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. 500 nM photoactivatable fluorophores **TPP-Tet-1** and **TPP-Tet-12** were treated in cell culture. (b) Fluorescence intensity comparison of **TPP-Tet-1** and **TPP-Tet-12.** Fluorescence Histogram represent the mean intensities of 5 cytoskeleton areas (circled on the fluorescent micrographs).

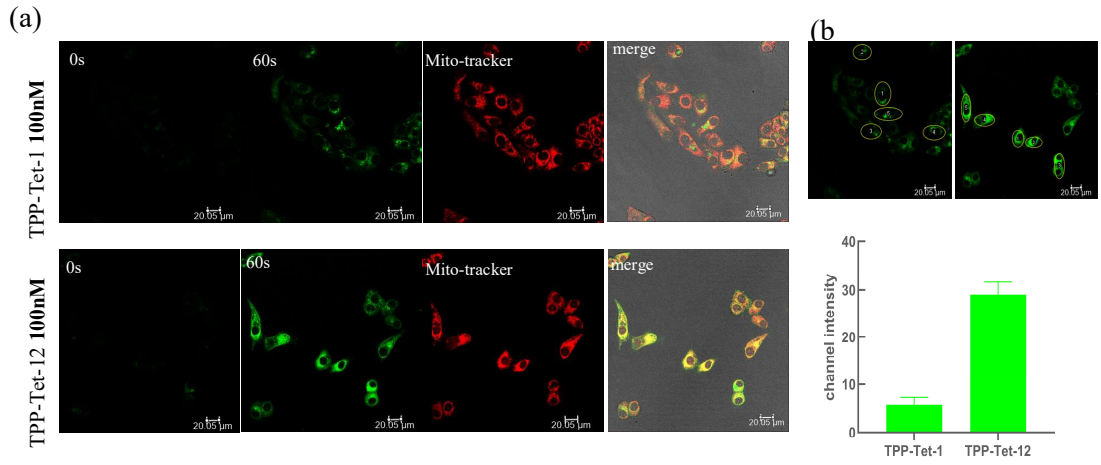


Figure S11 (a) Confocal micrographs of mitochondria-targeting probes **TPP-Tet-1** and **TPP-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. 100 nM photoactivatable fluorophores **TPP-Tet-1** and **TPP-Tet-12** were treated in cell culture. 500 nM photoactivatable fluorophores **TPP-Tet-1** and **TPP-Tet-12** were treated in cell culture. (b) Fluorescence intensity comparison of **TPP-Tet-1** and **TPP-Tet-12.** Fluorescence Histogram represent the mean intensities of 5 cytoskeleton areas (circled on the fluorescent micrographs).

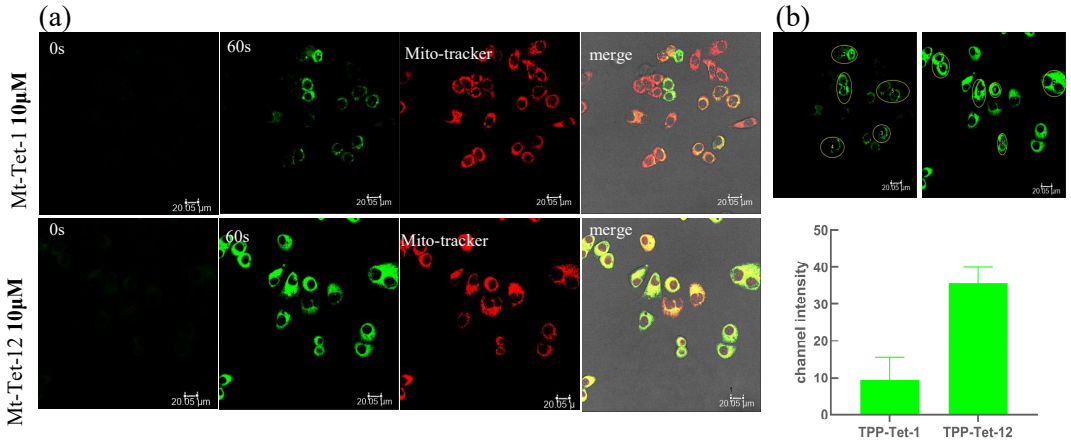


Figure S12 (a) Confocal micrographs of mitochondria-targeting probes **TPP-Tet-1** and **TPP-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. 10 μM photoactivatable fluorophores **TPP-Tet-1** and **TPP-Tet-12** were treated in cell culture. (b) Fluorescence intensity comparison of **TPP-Tet-1** and **TPP-Tet-12.** Fluorescence Histogram represent the mean intensities of 5 cytoskeleton areas (circled on the fluorescent micrographs).

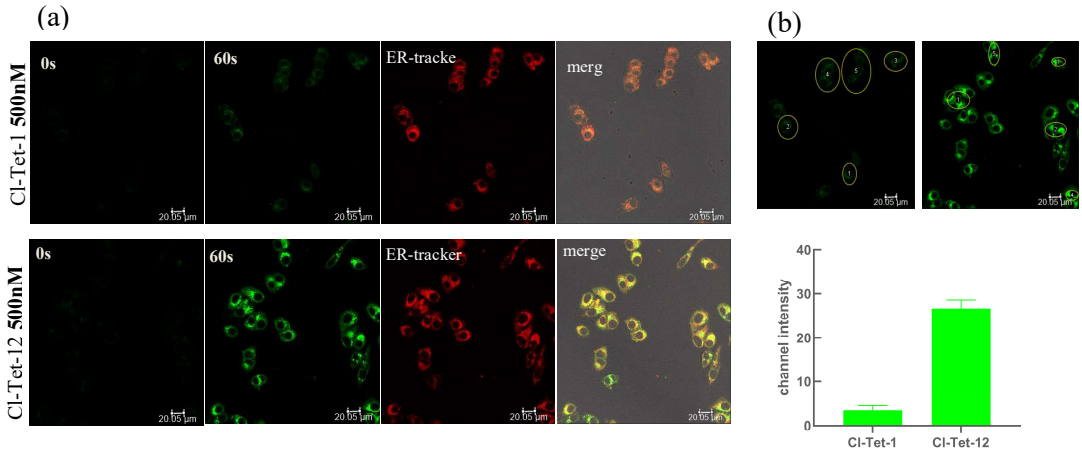


Figure S13 (a) Confocal micrographs of ER-targeting probes **Cl-Tet-1** and **Cl-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. 500 nM photoactivatable fluorophores **Cl-Tet-1** and **Cl-Tet-12** were treated in cell culture. (b)

Fluorescence intensity comparison of **Cl-Tet-1** and **Cl-Tet-12.** Fluorescence Histogram represent the mean intensities of 5 cytoskeleton areas (circled on the fluorescent micrographs).

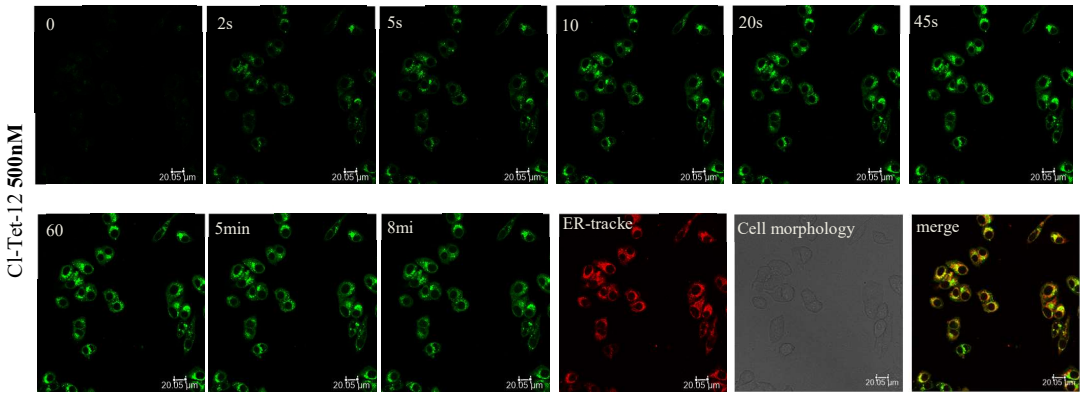


Figure S14 Time-coursed confocal micrographs of ER-targeting probe **Cl-Tet-12** treated Hela cells after photoillumination with 310 nm light. Ex = 405. Scale bar = 20 μM. The cell culture was treated 500 nM concentration of probe. ER-Tracker Red was used as marker for endoplasmic reticulum.

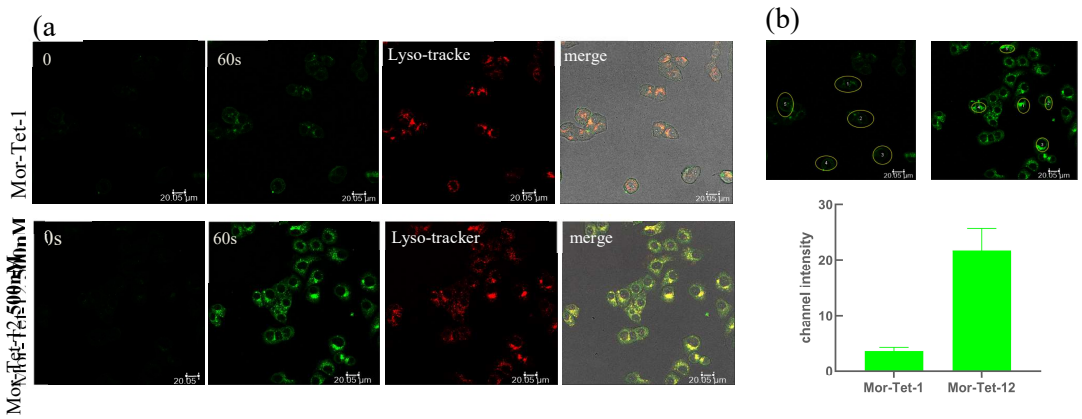


Figure S15 (a) Confocal micrographs of Lysosome-targeting probes **Mor-Tet-1** and **Mor-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. 500 nM photoactivatable fluorophores **Mor-Tet-1** and **Mor-Tet-12** were treated in cell culture. (b) Fluorescence intensity comparison of **Mor-Tet-1** and **Mor-Tet-12.** Fluorescence Histogram represent the mean intensities of 5 cytoskeleton areas (circled on the fluorescent micrographs).

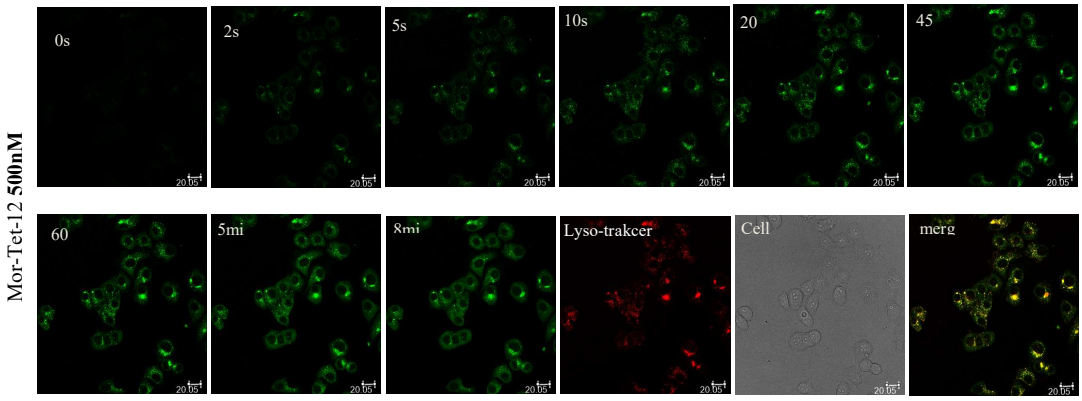


Figure S16 Time-coursed confocal micrographs of Lysosome-targeting probe **Mor-Tet-12** treated Hela cells after photoillumination with 310 nm light. Ex = 405. Scale bar = 20 μM. The cell culture was treated 500 nM concentration of probe. Lyso-Tracker Red was used as marker for lysosome.

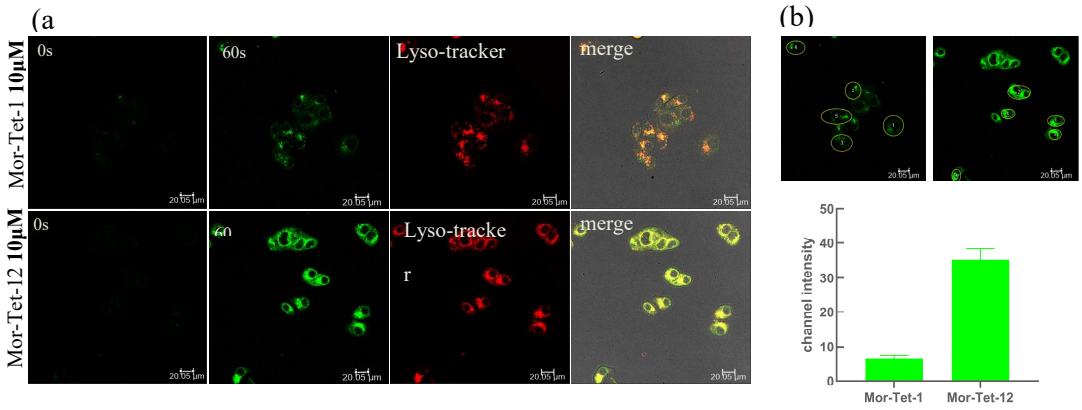


Figure S17 (a) Confocal micrographs of Lysosome-targeting probes **Mor-Tet-1** and **Mor-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. 10 μM photoactivatable fluorophores **Mor-Tet-1** and **Mor-Tet-12** were treated in cell culture. (b) Fluorescence intensity comparison of **Mor-Tet-1** and **Mor-Tet-12.** Fluorescence Histogram represent the mean intensities of 5 cytoskeleton areas (circled on the fluorescent micrographs).

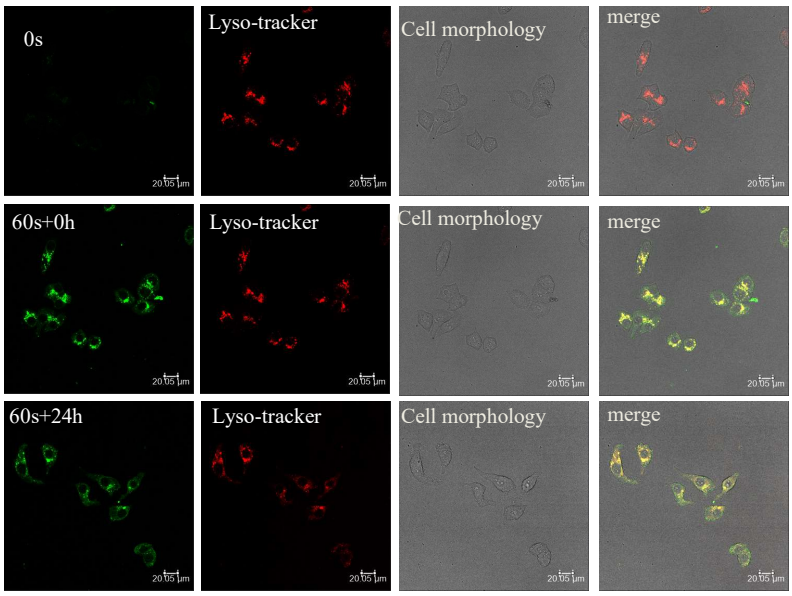


Figure S18 Confocal micrographs of Lysosome-targeting probes **Mor-Tet-12** (500 nmol/L) treated Hela cells before and after 60s photoillumination with 310 nm light lamp. After photoillumination, Hela cells were incubated for 24 hours and live cell images were acquired on a confocal microscope.

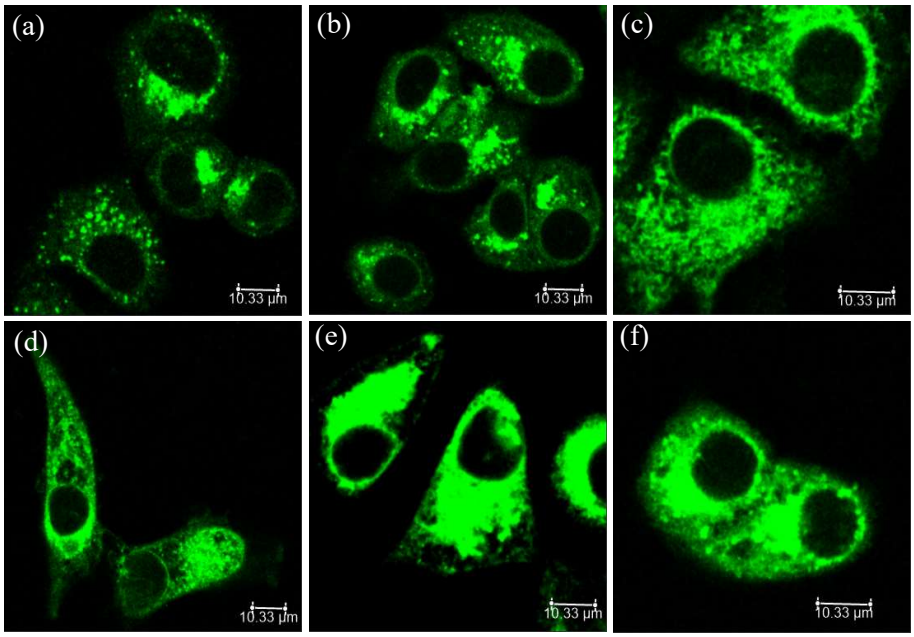


Figure S19 Confocal micrographs of Lysosome-targeting probes **Mor-Tet-12, TPP-Tet-12 and Cl-Tet-12** treated Hela cells before and after 60s photoillumination with 310 nm light lamp. (a) Area of confocal micrograph of **Mor-Tet-12** (500 nmol/L). (b) Area of confocal micrograph of **Cl-Tet-12** (500 nmol/L). (c) Area of confocal micrograph of **TPP-Tet-12** (500 nmol/L). (d) Area of confocal micrograph of **TPP-Tet-12** (100 nmol/L). (e) Area of confocal

micrograph of **TPP-Tet-12** (10 μmol/L). (f) Area of confocal micrograph of **Mor-Tet-12** (10 μmol/L).

Supplemental Methods

Fluorescent quantum yield:

Fluorescent quantum yield determination: Quantum yields measurements were determined using Quinine sulfate(fluorescence quantum yield of 0.54 in H_2SO_4 0.1 M[1]) as a standard. The fluorescence quantum yield, $\Phi_{f,\,sample}$ were calculated according to equation as following:

$\Phi_{f,sample} = (OD_{standard}/ OD_{sample})(I_{sample}/I_{standard})(d^2_{standard}/d^2_{\ sample})\Phi_{f,standard}$

Φf = fluorescent quantum yield;

I: = Integrated emission intensity;

OD: optical density at the excitation wavelength;

d: refractive index of solvents, d_{CH3CN}=1.34; d_{water}=1.33.

Cell Culture:

Hela cells were allowed to grow to about 70-80% confluency in DMEM medium supplemented with 10% FBS and 1% Penicillin-Streptomycin on 35-mm culture dishes pre-installed with a glass cover-slip at the bottom. All probes were prepared as 10 mmol/L stock solutions in DMSO. The stock solutions were diluted with media when used in cell culture. For mitochondria, **TPP-Tet-1** and **TPP-Tet-12** were individually incubated at 100 nM, 500 nM, 10 μM, **MitoTracker® Red CMXRos** was prepared as 200 μM/L stock solution in DMSO. Then it was incubated at 50 nM as Mito-tracker. **Cl-Tet-1** and **Cl-Tet-12** were individually incubated at 500 nM, **ER-Tracker Red** was prepared as 1 μM/L stock solution. Then it was incubated at 50 nM as ER-tracker. **Mor-Tet-1** and **Mor-Tet-12** were individually incubated at 500 nM, 10 μM, **LysoTracker™ Red DND-99** was prepared as 10 μM/L stock solution in DMSO. Then it was incubated at 50 nM as Lyso-tracker. All the tetrazole probes were cultured for three hours and the standard tracker were cultured for 1 hours. Finally, the cells were washed with prewarmed PBS for three times. For the sample was then placed underneath the confocal microscope for photoactivation followed by 310 nm UV lamp. The imaging acquisitions were carried out using a Leica TCS SP8 STED confocal microscope equipped with a continuous laser and fluorescence lifetime (FLIM) detector. Excitation and emission wavelengths are shown below: green channel: λ_{ex} = 405 nm, λ_{em} = 450–570 nm, red channel λ_{ex} = 570 nm, λ_{em} = 583–673 nm. The data quantifications were carried out using the Leica application Suite X, NIH Image J program. **MitoTracker® Red CMXRos** (C1035), **ER-Tracker Red** (C1041)**, LysoTracker™ Red DND-99** (C1046) **were purchased from beyotime.**

Cell viability:

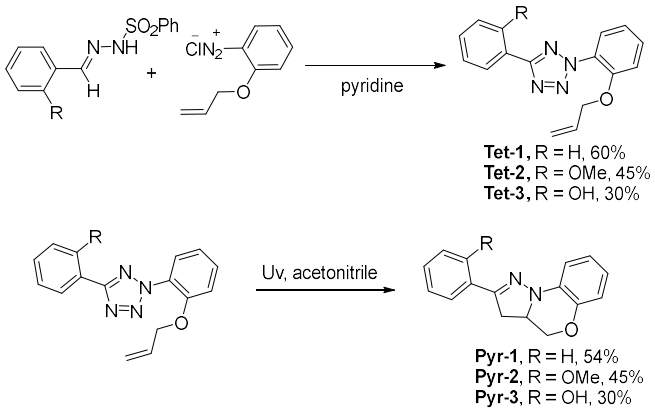
Hela cells were allowed to grow to about 70-80% confluency in DMEM medium supplemented with 10% FBS and 1% Penicillin-Streptomycin on the bottom of a 96-well plate. **Tet-12** and **Pyr-12** were prepared as 10mmol/L stock solution in DMSO. Then they were diluted with media and incubated at varied concentrations: 100 nmol/L, 500 nmol/L, 1 μM/L, 10 μM/L, 20 μM/L, 50 μM/L. Keep about 100uL of medium in each well. All samples were cultured for 24 hours. After 24 hours, 10 uL MTT solution (5mg/ml) was added to sample. After four hours, 100 uL Formazan solution was added to solute formazan crystal. When the formazan crystal was all soluted, the UV absorption of the samples was measured at 570 nm by microplate photometer (Thermo Scientific Multiskan FC). Mtt solution and formazan solution were purchased from beyotime (C009S).

General Information

Solvents and chemicals were purchased from commercial sources and used directly without further purification. Petroleum ether (PE) used had a boiling range of 60−90 °C. Reactions were monitored by TLC on silica gel GF 254 plates. Column chromatography was generally performed through silica gel (200−300 mesh). ^{1}H NMR spectra were recorded with Bruker-600 and Bruker-400 MHz spectrometers. Chemical shifts were reported in ppm using TMS or deuterated solvents as internal standards (TMS, 0.00; $CDCl_3$, 7.26; CD_3OD, DMSO-d_6, 2.50). Multiplicity was reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, brs = broad. ^{13}C NMR spectra were recorded at 150 MHz and 103 MHz, and chemical shifts were reported in ppm using deuterated solvents as internal standards ($CDCl_3$, 77.0; DMSO-d_6, 39.5). UV-Vis absorption spectra were recorded using 1-cm quartz cuvette on a Shimadzu-UV-2600. Fluorescence spectra were recorded using 1-cm quartz cuvette on a Gangdong F-320 spectrofluorometer at 25 ºC. High resolution mass spectrometry was performed on a Thermo LTQ-Orbitrap XL mass spectrometer equipped with collision cells for collision induced dissociation. Single crystal was measured by X-ray single crystal diffractometer (D8 VENTURE).

Experimental Synthesis Procedures and Characterization Data

Scheme S1



2-(2-(allyloxy)phenyl)-5-phenyl-2H-tetrazole (Tet-1): Compound **Tet-1** was synthesizedas a brown solid with 60% yield. The data and procedure was identical as previous reported[2]

2-(2-(Allyloxy)phenyl)-5-(2-methoxyphenyl)-2H-tetrazole (Tet-2)： The allyloxy diazonium salt was prepared in the same synthetic procedure as **Tet-1** using 2-(allyloxy) aniline as starting material. In the same way as **Tet-1**, the **Tet-2** was synthesized as colorless solid in 45% yield: ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.07 (dd, J = 9.0 Hz, 2.0 Hz, 1H), 7.63 (dd, J = 9.0 Hz, 2.0 Hz, 1H), 7.51–7.46 (m, 2H), 7.13–7.10 (m, 3H), 7.08 (d, J = 12.0 Hz, 1H), 5.99–5.92 (m, 1H), 5.36 (dd, J = 18.0 Hz, 3.0 Hz, 1H), 5.22 (dd, J = 12.0 Hz, 3.0 Hz, 1H), 4.63 (d, J = 3.0 Hz, 2H), 3.96 (s, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 162.94, 157.59, 152.56, 132.07, 131.63, 131.51, 130.82, 128.47, 127.05, 120.81, 120.65, 117.53, 114.19, 111.82, 111.79, 69.59, 55.96; HRMS calcd for $C_{17}H_{17}N_4O_2$ 309.1346 [M+H$^+$], found 309.1345.

2-(2-(2-(Allyloxy)phenyl)-2H-tetrazol-5-yl)phenol (Tet-3): **Tet-3** was synthesized as a colorless solid with 30% yield using the same synthetic procedure as **Tet-1**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.74 (s, 1H), 8.17 (d, J = 9.0 Hz, 1H), 7.63 (dd, J = 9.0 Hz, 2.0 Hz, 1H), 7.52 (t, J = 6.0 Hz, 1H), 7.38 (t, J = 6.0 Hz, 1H), 7.15–7.11 (m, 3H), 7.02 (t, J = 6.0 Hz, 1H), 5.97-5.91 (m, 1H), 5.33 (dd, J = 18.0 Hz, 3.0 Hz, 1H), 5.23 (d, J = 6.0 Hz, 1H), 4.63 (d, J = 3.0 Hz, 2H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 163.82, 156.51, 152.44, 132.28, 132.23, 131.92, 127.59, 126.85, 126.29, 121.03, 120.12, 118.07, 117.61, 114.31, 111.26, 69.76; HRMS calcd for $C_{16}H_{15}N_4O_2$ 295.1190 [M+H$^+$], found 295.1189.

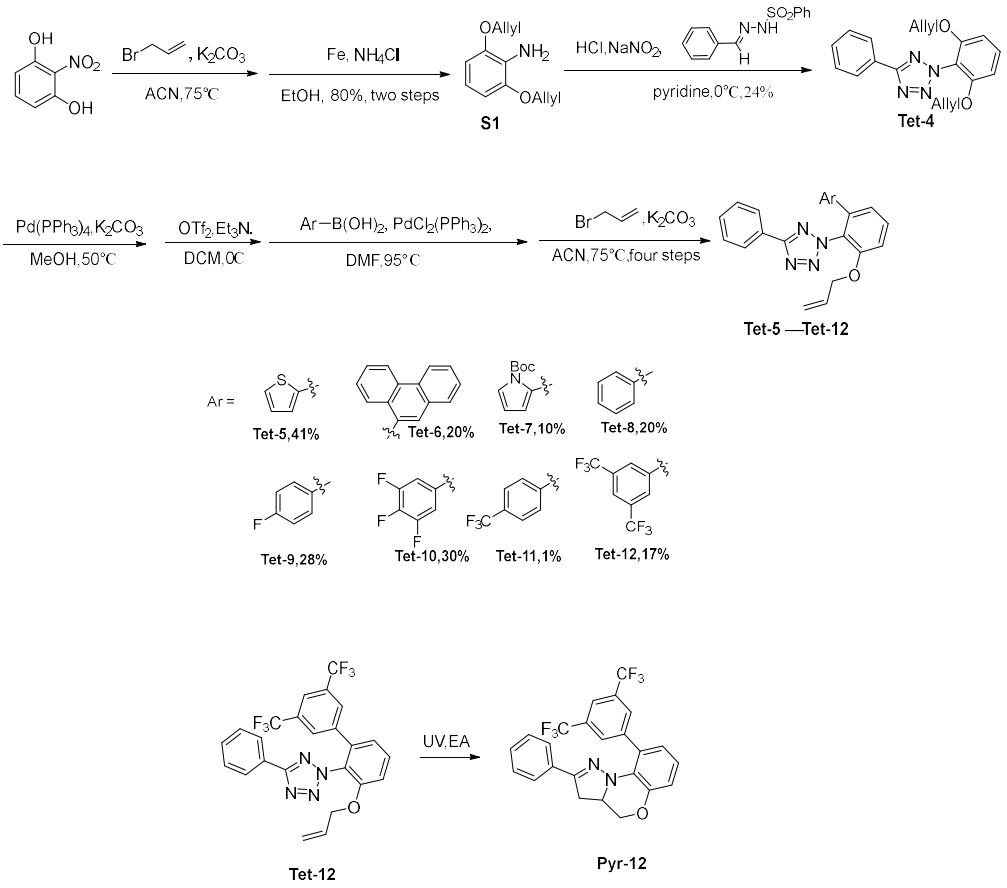
2-phenyl-3a,4-dihydro-3H-benzo[b]pyrazolo[1,5-d][1,4]oxazine (Pyr-1): To a solution of **Tet-1** (150 mg, 0.54 mmol) in acetonitrile (40 mL) in a quartz botter, the reaction mixture was irridated with 310 nm UV light by using the UV-lamp. Upon the tetrazole was completely converted to pyrazoline product (checked by TLC), the mixture was concentrated and purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as eluent to give the title product as a brown solid (74mg, 54%). The data and procedure was identical as previous reported[2]

2-(2-Methoxyphenyl)-3a,4-dihydro-3H-benzo[b]pyrazolo[1,5-d][1,4]oxazine (Pyr-2):
Pyr-2 was synthesized as a light yellow solid with 92% yield using the same synthetic procedure as **Pyr-1**: ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (dd, J = 9.0 Hz, 3.0Hz, 1H), 7.57 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 7.25 (td, J = 9.0 Hz, 3.0 Hz, 1H), 6.96–6.84 (m, 3H), 6.86–6.82 (m, 2H), 4.06–4.02 (m, 1H), 3.75 (s, 3H), 3.48–3.38(m,2H), 3.12 (dd, J = 12.0 Hz, 6.0 Hz, 1H); ^{13}C NMR (150 MHz, CDCl3) δ 157.40, 149.43, 144.74, 131.50, 130.24, 128.82, 121.56, 121.51, 121.19, 120.71, 120.62, 116.87, 111.24, 64.89, 56.23, 55.19, 38.51; HRMS calcd for C17H17N2O2 281.1285 [M+H$^+$], found 281.1284.

2-(3a,4-Dihydro-3H-benzo[b]pyrazolo[1,5-d][1,4]oxazin-2-yl)phenol (Pyr-3): **Pyr-3** was synthesized as a light yellow solid with 90% yield using the same synthetic procedure as **Pyr-1**: ^{1}H NMR (600 MHz, CDCl3) δ 10.65 (s, 1H), 7.44 (d, J = 9.0 Hz, 1H), 7.24 (t, J = 9.0 Hz, 1H), 7.14 (dd, J = 9.0 Hz, 3.0 Hz, 1H), 7.00–6.97 (m, 2H), 6.93–6.92 (m, 2H), 6.88 (td, J

= 9.0 Hz, 3.0 Hz, 1H), 4.14–4.07 (m, 2H), 4.45 (dd, *J* = 12.0 Hz, 9.0 Hz, 1H), 3.37 (t, *J* = 9.0 Hz, 2H), 3.34 (dd, *J* = 12.0 Hz, 3.0 Hz, 1H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 157.31, 151.87, 144.94, 130.79, 130.70, 127.47,122.68, 121.90, 120.80, 119.26, 117.28, 116.65, 115.73, 64.70, 54.80, 35.91; HRMS calcd for $C_{16}H_{15}N_2O_2$ 267.1128 [M+H$^+$], found 267.1125.

Scheme S2



2,6-(diallyloxy)aniline (S1): preparation of 2,6-(diallyloxy)nitrobenzene: to a solution of nitro-1,3-benzenediol (8.01 g, 0.052 mol) in acetonitrile was added 3-bromopropene (10 ml, 0.104 mol) and K_2CO_3 (22.02 g, 0.156 mol), and the mixture was stirred at 75 ℃ for 5 hours. The mixture was filtered and the solvent was removed. The crude nitrobenzene product was used in the next step. Preparation of 2,6-(diallyloxy)aniline: to a solution of above synthesized nitrobenzene in ethanol was added iron powder reduced (10.92 g, 0.195 mol). Ammonium chloride was dissolved in water (20ml) and the NH_4Cl aqueous solution (40 mg/ml) was added to the nitrobenzene suspension. The mixture was stirred at 90 ℃ for 8 hours. Then the mixture was filtered and extracted with ethyl acetate (100 ml × 3) and water. Solvent was evaporated under reduced pressure to obtain product (8.21 g, 80%) as black oil. The crude product was used in the next step. The data was identical as previous reported[3]

5-phenyl-2-(2,6-diallyloxyphenyl)-2H-tetrazole (Tet-4): Preparation of diazonium salt: to a mixed solution of 2,6-(diallyloxy)aniline (8 g, 0.04 mol) in ethanol/water (2:1, 120 ml) cooled with ice-water bath was added sequentially 100mL concentrated HCl and 20 ml sodium nitrite

(3.26 g, 0.048 mol) solution in water were added dropwise and the mixture was stirred for about 10min at ice-water bath. Preparation of tetrazole: to a solution of aboved synthesized sulfohydrazone (in **Tet-1**) (11.44g, 0.04mol) in pyridine (150ml) cooled with an ice-salt bath was slowly added the freshly prepared diazonium salt, and the mixture was stirred while temperature was allowed to gradually increase to room temperature. After about 8 hours, the reaction mixture was washed with 1 N HCl (150 ml) and extracted with ethyl acetate (150 ml × 3) and water. The organic phase was dried over anhydrous Na_2SO_4 and purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as eluent to give the title product (3.125 g, 24%),as a dark yellow solid: ^{1}H NMR (400 MHz, CDCl3) δ 8.26 (dd, J = 8.2 Hz, 1.7 Hz, 2H), 7.53–7.47 (m, 3H), 7.41 (t, J = 8.5 Hz, 1H), 6.67 (d, J = 8.6 Hz, 2H), 5.88–5.79 (m, 2H), 5.19 (dd, J = 17.3 Hz, 1.6Hz, 2H), 5.14 (dd, J = 10.7 Hz, 1.5 Hz, 2H), 4.53 (dt, J = 5.0 Hz, 1.7 Hz, 4H); ^{13}C NMR(101 MHz, $CDCl_3$) δ 164.84, 155.47, 132.31, 132.03, 130.22, 128.87, 127.67, 127.04, 117.65, 116.29, 105.73, 69.08; HRMS calcd for $C_{19}H_{19}N_4O_2$ 335.1508 [M+H$^+$], found 335.1503.

5-phenyl-2-(2-allyloxy-6-(thiophen-2-yl))phenyl-2H-tetrazole (Tet-5): Preparation of 2-(5-phenyl-2H-tetrazol-2-yl)benzene-1,3-diol: to a solution of compound **Tet-4** (400 mg, 1.2 mmol) in methanol was added $Pd(PPh_3)_4$ (14 mg, 0.012 mmol) and K_2CO_3(500 mg, 3.6 mmol). The mixture was done carefully gas exchange to argon and stirred at 50 ℃ for 3 hours. Then the reaction was filtered to obtain the crude product as brown solid. The crude product was used in the next step.

Preparation of 3-hydroxy-2-(5-phenyl-2H-tetrazol-2-yl)phenyl trifluoromethanesulfonate: to a solution of aboved crude product (1.2 mmol) in DCM cooled with ice-water bath was added triethylamine (398 μL, 2.88 mmol).Then trifluoromethanesulfonic anhydride (243 μL,1.44 mmol) was added to the mixture. The reaction was stirred for about 2 hours at ice-water bath. Then the mixture was extracted with DCM (60 ml × 3) and water. After the solvent was removed, the crude product was brown oil, the crude product was used in next step.

Preparation of 2-(5-phenyl-2H-tetrazol-2-yl)-3-(thiophen-2-yl)phenol: to a solution of aboved crude product (1.2 mmol) in DMF was added phenylboronic acid (1.8 mmol, 230mg) and $PdCl_2(PPh_3)_2$ (0.05 mmol, 42 mg). K_2CO_3 (3.6 mmol, 497 mg) was added in the solvent too. The mixture was done carefully gas exchange to argon and stirred at 95 ℃ for 6 hours. After about 6 hours, the mixture was extracted with ethyl acetate (40 ml × 3) and NH_4Cl aqueous solution (40 mg/ml). After the solvent was removed ,the crude product was brown oil and used in the next step.

Preparation of 5-phenyl-2-(2-allyloxyphenyl-6-phenyl)phenyl-2H-tetrazole: to a solution of aboved crude product (1.2 mmol) in acetonitrile was added 3-bromopropene (2.4 mmol, 173 μL) and K_2CO_3 (3.6 mmol, 497 mg). The mixture was stirred at 75℃ for 3 hours. After about 3 hours the mixture was purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as eluent to give the title product (187 mg, 41%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 (dd, J = 7.9 Hz, 2.2 Hz, 2H), 7.55–7.48 (br, 4H), 7.32 (d, J = 7.9 Hz, 1H), 7.20 (d, J = 5.1 Hz, 1H), 7.04 (d, J = 8.4 Hz, 1H), 6.85 (t, J = 3.7 Hz, 1H), 6.73 (dd, J = 3.7 Hz, 1.3 Hz, 1H), 5.87–5.78 (m, 1H), 5.18 (dd, J = 15.7 Hz, 1.6 Hz, 1H), 5.15 (dd, J = 9.1 Hz, 1.5Hz, 1H),

4.56 (d, 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.03, 155.08,137.65, 134.32, 132.15, 131.83, 130.39, 128.91, 127.59, 127.42, 127.09, 126.85, 124.08,122.07, 117.75, 112.47, 69.57; HRMS calcd for $C_{20}H_{16}N_4OSNa$ 383.0943 [M+Na^+], found 383.0940.

5-phenyl-2-(2-allyloxy-6-(-9-Phenanthrene-yl))phenyl-2H-tetrazole(Tet-6): Tetrazole **Tet-6** was synthesized as a brown solid with 20% yield using the same synthetic procedure as tetrazole **Tet-5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.58 (d, J = 8.2 Hz, 1H), 8.54 (d J = 8.2 Hz, 1H), 7.86 (dd, J = 7.4 Hz, 2.3 Hz), 7.78 (d, J = 8.0 Hz, 1H), 7.75 (d, J = 7.5 Hz, 1H), 7.64 (s, 1H), 7.61–7.47 (m, 5H), 7.30 (d, J = 2.6Hz, 2H), 7.29 (s, 1H), 7.17 (t, J = 6.6 Hz, 2H), 5.91–5.81 (m, 1H), 5.22 (d, J = 17.3 Hz, 1H), 5.15 (d, J = 10.6 Hz, 1H), 4.60 (d, J = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.17, 154.47, 140.28, 132.72, 131.97, 131.64, 130.80, 130.67, 130.56, 130.20, 130.02, 128.83, 128.65, 128.38, 127.75, 127.29, 127.00, 126.96, 126.84, 126.81, 126.62,126.52, 126.37, 123.86, 122.67, 122.47, 117.78, 112.79, 69.63; HRMS calcd for $C_{30}H_{23}N_4O$ 455.1872 [M+H^+], found 455.1866.

5-phenyl-2-(2-allyloxy-6-(N-Boc-H-pyrrol-2-yl))phenyl-2H-tetrazole(Tet-7): Tetrazole **Tet-7** was synthesized as a brown solid with 10% yield using the same synthetic procedure as tetrazole **Tet-5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (dd, J = 7.9 Hz, 2.1 Hz, 2H), 7.52–7.44 (m, 4H), 7.18 (q, J = 1.7 Hz, 1H), 7.11(d, J = 1.6 Hz, 1H), 7.09 (d, J = 2.2 Hz, 1H), 6.05 (q, J = 1.7 Hz, 1H), 6.00 (t, J = 3.2 Hz, 1H), 5.90–5.81 (m, 1H), 5.22 (dd, J = 17.3 Hz, 1.6 Hz, 1H), 5.16 (dd, J = 10.7 Hz, 1.5 Hz, 1H), 4.58 (d, J = 5.0 Hz, 2H), 1.36 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.35, 153.79, 148.76, 134.98, 132.05, 130.82, 130.13, 128.80, 127.56, 127.32, 126.97, 126.39, 123.69, 122.30, 117.68, 115.41, 113.10,110.37, 83.69, 69.68, 27.57; HRMS calcd for $C_{25}H_{25}N_5O_3Na$ 466.1855 [M+Na^+], found 466.1846.

5-phenyl-2-(2-allyloxy-6-phenyl)phenyl-2H-tetrazole(Tet-8): Tetrazole **Tet-8** was synthesized as a brown solid with 20% yield using the same synthetic procedure as tetrazole **Tet-5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (dd, J = 7.9 Hz, 2.0 Hz, 2H), 7.52 (t, J = 8.0 Hz), 7.40–17.46 (m, 3H), 7.18 (s, 5H), 7.12 (dd, J = 7.8 Hz, 1.28 Hz, 2H), 7.05 (dd, J = 8.6 Hz, 1.36 Hz, 1H), 5.77–5.86 (m, 1H), 5.18 (dd, J = 17.2 Hz, 1.52 Hz, 2H), 5.13 (dd, J = 12.0 Hz, 1.52 Hz, 2H), 4.54 (d, J = 4.92 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.59, 154.48, 141.59, 136.94, 131.98, 130.29, 128.87, 128.34, 127.99, 127.46, 127.01, 124.94, 122.61, 117.67, 112.44, 69.56; HRMS calcd for $C_{20}H_{16}N_4OSNa$ 377.1378 [M+Na^+], found 377.1370.

5-phenyl-2-(2-allyloxy-6-(4-fluorophenyl))phenyl-2H-tetrazole(Tet-9): Tetrazole **Tet-9** was synthesized as a brown solid with 28% yield using the same synthetic procedure as tetrazole **Tet-5**: ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.16–8.11 (dd , J = 7.9 Hz, 3.4 Hz ,2H), 7.55 (t, J = 8.2 Hz, 1H), 7.48–7.45 (m, 2H), 7.18–7.12 (m, 2H), 7.10 (t, J = 7.8 Hz, 2H), 6.89 (t, J = 8.7 Hz, 2H), 5.89–5.79 (m, 1H), 5.20 (dd, J = 17.3 Hz, 1.6 Hz, 1H), 5.16 (dd, J = 10.7 Hz, 1.5 Hz, 1H), 4.57 (d, J = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.69, 163.74, 161.27, 154.54, 140.58, 132.90, 132.01, 131.88, 130.35, 130.15, 130.06, 128.89, 127.31, 126.99, 124.96, 122.49, 117.75, 115.48, 115.27, 112.57, 69.59; HRMS calcd for $C_{22}H_{18}FN_4O$ 373.1465 [M+H^+], found 373.1460.

5-phenyl-2-(2-allyloxy-6-(3,4,5-trifluorophenyl))phenyl-2H-tetrazole(Tet-10): Tetrazole **Tet-10** was synthesized as a brown solid with 30% yield using the same synthetic procedure

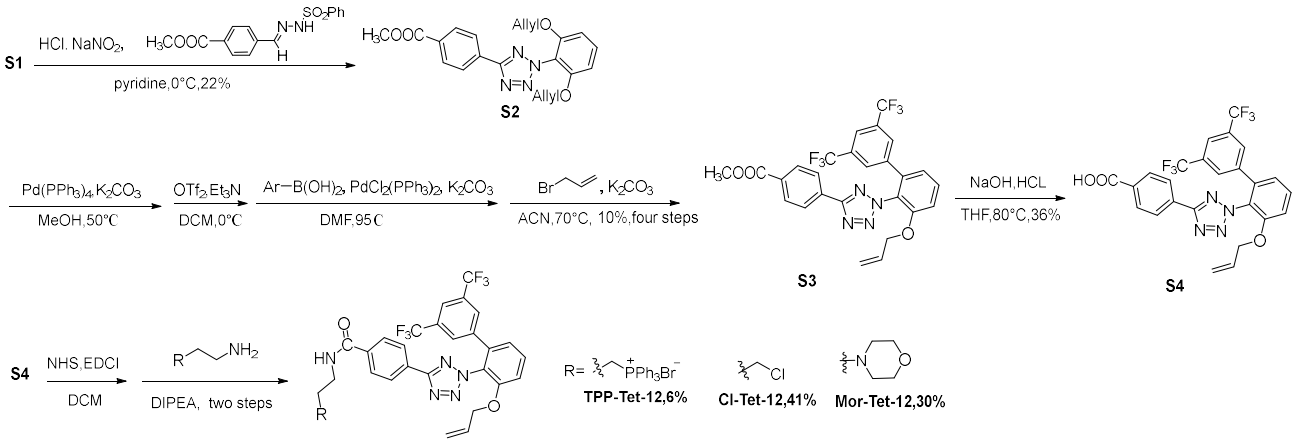
as tetrazole **Tet-5**:1H NMR (400 MHz,$CDCl_3$) δ 8.15 (dd, J = 7.9 Hz, 3.3 Hz, 2H), 7.59 (t, J=8.3 Hz, 1H), 7.51–7.48 (m, 3H), 7.14 (d, J = 8.5 Hz, 1H), 7.07 (dd, J = 7.9 Hz, 1.4 Hz,1H), 6.85 (t, J = 6.4 Hz, 2H), 5.89–5.80 (m, 1H), 5.23–5.16 (m, 2H), 4.59 (d, J = 5.0 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.98, 154.67, 152.15, 152.10, 152.05, 152.01, 149.65, 149.61, 149.55, 149.51, 141.04, 140.89, 140.74, 138.50, 138.37, 138.22, 132.83, 132.78, 132.33, 131.65, 130.53, 128.95, 127.03, 124.72, 122.04, 117.96, 113.51, 113.04, 112.97, 112.88, 112.81, 69.67; HRMS calcd for $C_{22}H_{16}F_3N_4O$ 409.1276 [M+H^+], found 409.1266.

5-phenyl-2-(2-allyloxy-6-(4-(trifluoromethyl)phenyl))phenyl-2H-tetrazole(Tet-11):
Tetrazole **Tet-11** was synthesized as a brown solid with 13% yield using the same synthetic procedure as tetrazole **Tet-5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (dd, J = 7.7 Hz, 3.4 Hz, 2H), 7.61 (t, J = 8.3 Hz, 1H), 7.49–7.46 (m, 5H), 7.33 (s, 1H), 7.31 (s, 1H), 7.14 (dd, J = 6.4 Hz, 1.4 Hz, 1H), 7.12(dd, J = 5.6 Hz, 1.2 Hz, 1H), 5.90–5.81 (m, 1H), 5.24–5.16 (m, 2H), 4.60 (dt, J = 4.9 Hz, 1.7 Hz, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.79, 154.62, 140.55, 140.08, 132.20, 131.77, 130.42, 130.27, 129.95, 128.90, 128.78, 127.19, 127.01, 125.36, 125.32, 125.29, 125.25, 124.85, 122.36, 117.87, 113.21, 69.66; HRMS calcd for $C_{23}H_{18}F_3N_4O$ 423.1433 [M+H^+], found 423.1422.

5-phenyl-2-(2-allyloxy-6-(3,5-bis(trifluoromethyl)phenyl))phenyl-2H-tetrazole(Tet-12):
Tetrazole **Tet-12** was synthesized as a brown solid with 17% yield using the same synthetic procedure as tetrazole **Tet-5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13(dd, J = 8.1 Hz, 2.6 Hz, 2H), 7.74 (s, 1H), 7.70 (s, 2H), 7.62 (t, J = 8.4 Hz, 1H), 7.48–7.45 (m, 3H), 7.20 (dd, J = 8.5 Hz, 1.3 Hz, 1H), 7.16 (dd, J = 7.9 Hz, 1.3 Hz, 1H), 5.23 (dd, J = 17Hz, 1.6 Hz, 1H), 5.18 (dd, J = 10.6 Hz, 1.5 Hz, 1H), 4.60 (d, J = 4.9 Hz, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 165.17, 154.79, 138.97, 138.39, 132.59, 132.20, 131.87, 131.62, 131.54, 131.20, 130.52, 128.90, 128.74, 128.70, 126.96, 124.92, 124.33, 121.99, 121.87, 121.83, 121.80, 121.76, 121.72, 121.61, 117.99, 114.08, 69.73; HRMS calcd for $C_{24}H_{17}F_6N_4O$ 491.1307 [M+H^+], found 491.1295.

9-(3,5-bis(trifluoromethyl)phenyl)-2-phenyl-3a,4-dihydro-3H-benzo[b] pyrazolo [1,5-d][1,4]oxazine (Pyr-12): The compound **Tet-12** (110mg, 0.22mmol) was irradiated with a hand-held 310nm UV lamp in ethyl acetate. The mixture was stirred in a quartz botter and the extent of the reaction was monitored by TLC. After about 2 hours, the mixture was concentrated and purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as eluent to give the title product as a light yellow solid (65mg, 64%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (s, 2H), 7.81 (s, 2H), 7.30–7.24 (br, 2H), 7.09–7.01 (m, 2H), 6.92 (d, J = 7.2 Hz, 1H), 4.08 (d, J = 8.2 Hz, 2H), 3.42 (t, J = 11.7 Hz, 1H), 3.26(q, J = 8.4 Hz, 1H), 2.87 (d, J = 17.4 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 149.54, 146.66, 143.23, 133.59,131.73, 131.21, 130.88, 130.55, 130.23, 129.96, 129.28, 128.96, 128.48, 127.78, 125.89, 125.07, 123.67, 123.60, 122.36,120.34, 120.30, 120.27,119.65, 117.96, 64.67, 56.84, 35.43; HRMS calcd for $C_{24}H_{17}F_6N_2O$ 463.1245 [M+H^+], found 463.1228.

Scheme S3



Methyl 4-(2-(2,6-diallyloxyphenyl)-2H-tetrazole-5-yl)benzoate (S2): Compound **S2** was synthesized as a yellow solid with 22% yield using the same synthetic procedure as **Tet-4** using 2,6-(diallyloxy)aniline and methyl (*E*)-4-((2-(phenylsulfonyl)hydrazono)methyl)benzoate as material: ^{1}H NMR (400 MHz, $CDCl_3$), δ 8.34 (d, J = 8.8 Hz, 2H), 8.18 (d, J = 8.8 Hz, 2H), 7.44 (t, J = 8.0 Hz, 1H), 6.70 (d, J = 8.5 Hz, 2H), 5.90–5.80 (m, 2H), 5.23–5.14 (m, 4H), 4.55 (dt, J = 5.0 Hz, 1.8 Hz, 4H), 3.96 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.64, 164.03, 155.40, 132.45, 131.99, 131.76, 131.52, 130.16, 126.96, 117.74, 116.09, 105.70, 69.58, 52.30, 0.00; HRMS calcd for $C_{21}H_{20}N_4O_4Na$ 415.1382 [M+Na^+], found 415.1379.

4-(2-(3-(allyloxy)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-2-yl)-2H-tetrazol-5-yl) benzoic acid (S4): Compound **S3** was synthesized as a yellow solid with 10% yield using the same synthetic procedure as compound **Tet-4**. To a solution of compound **S3** (70 mg, 0.13 mmol) in 20 mL tetraydrofuran was added 30 mL NaOH aqueous solution (2 mol/L). The mixture was stirred at 80 ℃ for 8 hours. After about 8 hours, the reaction mixture was washed with concentrated HCl (35 ml) and extracted with ethyl acetate (50 ml × 3) and water. The organic phase was dried over anhydrous Na_2SO_4 and purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as eluent to give the title compound (30 mg, 36%) as orange solid: ^{1}H NMR (400 MHz,$CDCl_3$) δ 8.25 (s, 4H), 7.74 (s, 1H), 7.70–7.66 (m, 3H), 7.23 (d, J = 8.6 Hz, 1H), 7.19 (d, J = 7.7 Hz, 1H), 5.93–5.84 (m, 1H), 5.25 (dd, J = 16.2 Hz, 1.4 Hz, 1H), 5.22 (dd, J = 9.3 Hz, 1.44 Hz, 1H), 4.64 (d, J = 4.9Hz, 2H); ^{13}C NMR (101 MHz, CDCl3) δ 171.40, 164.16, 154.72, 138.76, 138.42, 132.72, 132.25, 131.92, 131.77, 131.58, 131.25, 131.03, 130.83, 128.68, 127.00, 124.76, 124.24, 122.03, 121.85, 121.53, 118.18, 114.07, 69.82; HRMS calcd for $C_{25}H_{17}F_6N_4O_3$ 535.1205 [M+H^+], found 535.1191.

4-(2-(3-(allyloxy)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-2-yl)-2H-tetrazol-5-yl)-N-(2-morpholinopropyl)benzamide (Mor-Tet-12): Preparation of

1-(4-(2-(3-(allyloxy)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-2-yl)-2H-tetrazol-5-yl)benzoyl)-1H-pyrrole-2,5-dione: To a solution of aboved benzoic acid (170 mg, 0.32 mmol) in DCM was added NHS (368 mg, 3.2 mmol) and EDCI (246 mg, 1.28 mmol). The reaction was stirred for 5 hours. After about 5 hours, the mixture was extracted with ethyl acetate (60 ml × 3) and water to obtain the brown oil as the crude product. The crude product was used in the next step.

Preparation of 4-(2-(3-(allyloxy)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-2-yl)-2H-tetrazol-5-yl)-N-(3-morpholinopropyl)benzamide: To a solution of aboved crude product in DCM was added DIPEA (262 μL, 1.6 mmol) and N-(3-aminoethyl) morpholine (166 mg, 1.28 mmol). The mixture was stirred for 3 hours. After about 3 hours, the mixture was extracted with ethyl acetate (60ml × 3) and water and purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as eluent to give the title product (60 mg, 30%) as a light yellow solid：^{1}H NMR (400 MHz, CDCl3) δ 8.20 (d, J = 8.4 Hz, 2H), 7.90 (d, J = 8.4 Hz, 2H), 7.68 (t, J = 8.1 Hz, 1H), 7.67 (s, 2H), 7.23 (d, J = 8.6 Hz, 1H), 7.19 (d, J = 7.8 Hz, 1H), 6.88 (s,1H), 5.93–5.83 (m, 1H), 5.25 (dd, J = 17 Hz, 1.5 Hz, 1H), 5.21 (dd, 10.5 Hz, 1.4 Hz, 1H), 4.65 (d, J = 5.0 Hz, 2H), 3.75 (t, J = 4.6 Hz, 4H), 3.60 (q, J = 5.9 Hz, 2H), 2.64 (t, J = 6.0 Hz, 2H), 2.53 (s, 4H). ^{13}C NMR(101 MHz, $CDCl_3$) δ 166.69, 164.28, 154.71, 138.79, 138.39, 136.30, 132.68, 132.21, 131.88, 131.58, 131.21, 129.66, 128.63, 127.56, 127.12, 124.78, 124.24, 122.01, 121.84, 121.53, 118.12, 114.06, 69.79, 66.94, 56.92, 53.35, 36.14; HRMS calcd for $C_{31}H_{29}F_6N_6O_3$ 647.2205 [M+H$^+$], found 647.2191.

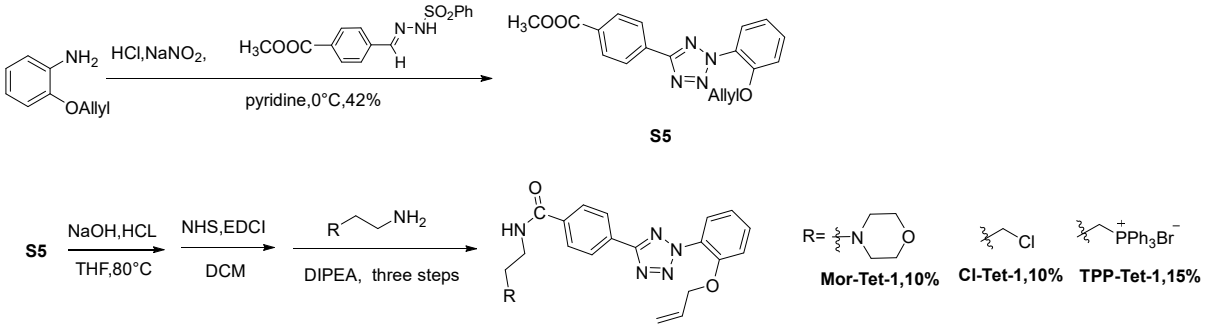
4-(2-(3-(allyloxy)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-2-yl)-2H-tetrazol-5-yl)-N-(3-chloropropyl)benzamide (Cl-Tet-12):

Compound **Cl-Tet-12** was synthesized as a yellow solid with 41% yield using the same synthetic procedure as compound **Mor-Tet-12**: ^{1}H NMR (400MHz, $CDCl_3$) δ 8.18 (d, J =8.6 Hz, 2H), 7.90 (d, J = 8.7 Hz, 2H), 7.73 (s, 1H), 7.68 (t, J = 7.8 Hz, 1H), 7.66 (s, 2H), 7.23 (dd, J = 8.6 Hz, 1.2Hz, 1H), 7.19 (dd, J = 7.8 Hz, 1.2 Hz, 1H), 6.56 (t, J = 3.9 Hz, 1H), 5.24 (dd, J = 17.1 Hz,1.38Hz, 1H), 5.21 (dd, J = 10.6 Hz, 1.44 Hz, 1H), 4.65 (dt, J = 5.0 Hz, 1.7 Hz, 2H), 3.67–3.63 (m, 4H), 2.17–2.11 (m, 2H), ^{13}C NMR (101 MHz, $CDCl_3$) δ ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.04, 164.23, 154.70, 138.78, 138.39, 136.09, 132.69, 132.21, 131.88, 131.58, 131.21, 129.78, 128.64, 127.54, 127.14, 124.76, 124.24, 122.01, 121.84, 121.80, 121.52, 118.14, 114.07, 69.80, 42.67, 37.78, 32.00; HRMS calcd for $C_{25}H_{17}F_6N_4O_3$ 610.1444 [M+H$^+$], found 647.2191.

(3-(4-(2-(3-(allyloxy)-3',5'-bis(trifluoromethyl)-[1,1'-biphenyl]-2-yl)-2H-tetrazol-5-yl) benzamido)propyl)triphenylphosphonium bromide (TPP-Tet-12):

Compound **TPP-Tet-12** was synthesized as a yellow solid with 6% yield using the same synthetic procedure as compound **Mor-Tet-12**: ^{1}H NMR (400 MHz, d_6-DMSO) δ 9.89 (s,1H), 8.05 (s, 3H), 9.98 (s, 1H), 7.86–7.73 (m, 16H), 7.55 (d, J = 8.0 Hz, 1H), 7.49 (s, 1H), 7.43 (d, J = 8 Hz, 1H), 7.36 (s, 1H), 7.23 (s, 1H), 5.90–5.81 (m, 1H), 5.14 (d, J = 8 Hz, 1H), 5.11 (s, 1H), 4.72 (d, J = 8 Hz, 2H), 1.95 (s, 2H). ^{13}C NMR (101 MHz, d_6-DMSO) δ 166.03, 164.19, 154.21, 139.05, 137.40, 136.63, 135.41, 134.06, 133.96, 132.90, 131.03, 130.78, 130.66, 129.19, 128.92, 128.81, 126.65, 124.61, 123.90, 122.82, 122.35, 121.90, 119.25, 118.40, 117.74, 115.52, 69.57, 22.52, 19.23, 18.71. HRMS calcd for $C_{46}H_{37}F_6N_5O_2P$ 836.2589 [M-Br$^-$], found 836.2581.

Scheme S4



Methyl 4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoate(S5):

Compound **S5** was synthesized as a yellow solid with 42%yield using the same synthetic procedure as compound **Tet-4** using 2-(allyloxy)aniline and methyl (*E*)-4-((2-(phenylsulfonyl)hydrazono)methyl)benzoate as material: ^{1}H NMR (400 MHz, $CDCl_3$)δ 8.33 (d, J = 8.0 Hz, 2H), 8.19 (d, J = 8.0 Hz, 2H), 7.63 (dd, J = 8.04 Hz, 2 Hz,1H) 7.52 (m, 1H), 7.14 (m,2H), 6.00–5.90 (m, 1H), 5.35 (m, 1H), 5.23 (m, 1H), 4.64 (dt, J = 4.9 Hz, 1.7 Hz, 2H), 3.36 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.55, 164.01, 152.52, 132.02, 131.69, 131.50, 130.21, 126.93, 126.67, 121.01, 117.83, 114.34, 69.74, 52.30; HRMS calcd for $C_{18}H_{17}N_4O_3$ 337.1301 [M+H$^+$], found 337.1295.

4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)-N-(2morpholinoethyl)benzamide(Mor-Tet-1):

Preparation of 4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoic acid: to a solution of compound **S5** (400 mg, 1.24 mmol) in 30 mL THF was added 30mL NaOH aqueous solution (2 mol/L). The mixture was stirred at 80 ℃ for 8 hours. After about 8 hours, the reaction mixture was washed with concentrated HCl (35 ml) and extracted with ethyl acetate (50 ml × 3) and water. The organic phase was dried over anhydrous Na_2SO_4 and to obtain the crude product as yellow oil. The crude product was used in the next step.

Preparation of 1-(4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzoyl)-1H-pyrrole-2,5-dione: to a solution of aboved crude product (1.24 mmol) in DCM was added NHS (1.42 g, 12.4 mmol) and EDCI (957 mg, 5 mmol). The reaction was stirred for 5 hours. After about 5 hours, the mixture was extracted with ethyl acetate (60 ml × 3) and water to obtain the brown oil as the crude product. The crude product was used in the next step.

Preparation of 4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)-N-(2morpholinoethyl)benzamide: to a solution of aboved crude product (1.24 mmol) in DCM was added DIPEA (1.12 mL, 6.2 mmol) and N-(3-aminoethyl) morpholine (650 mg, 5 mmol). The mixture was stirred for 3 hours. After about 3 hours, the mixture was extracted with ethyl acetate(60 ml × 3) and water and purified by silica gel flash chromatography using ethyl acetate/ Petroleum ether as

Eluent to give the title product (65 mg, 10%) as a white solid：^{1}H NMR (400 MHz, $CDCl_3$) δ 8.32 (d, J = 8.4 Hz, 2H), 7.93 (d, J = 8.4 Hz, 2H), 7.62 (d, J = 8.1 Hz, 1H), 7.53 (t, J = 8.2 Hz, 1H), 7.14 (t, J = 8.2 Hz, 2H), 6.95 (s, 1H), 6.00–5.90 (m, 1H), 5.35 (d, J = 17.3 Hz, 1H), 5.22 (d, J = 10.6 Hz, 1H),4.64 (d, J = 5.0 Hz, 2H), 3.75 (t, J = 4.8 Hz, 4H), 3.60 (q, J = 5.8 Hz, 2H), 2.64 (t, J = 6.1 Hz, 2H), 2.54 (s, 4H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.76, 164.02, 152.50, 136.14, 132.02, 130.17, 127.61, 127.15, 126.91, 126.67, 121.01, 117.81, 114.35, 69.73, 66.93, 56.94, 54.72, 36.17; HRMS calcd for $C_{23}H_{27}N_6O_3$ 435.2145 [M+H$^+$], found 435.2135.

4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)-N-(3chlorineproply)benzamide(Cl-Tet-1):
Compound **Cl-Tet-1** was synthesized as a light yellow solid with 10% yield using the same synthetic procedure as compound **Mor-Tet-1:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, J = 8.6 Hz, 2H), 7.92 (d, J = 8.8 Hz, 2H), 7.62 (dd, J = 8.0 Hz, 1.8 Hz, 1H), 7.55–7.51 (m, 1H), 7.16–7.12 (m, 2H), 6.61 (s, 1H), 6.00–5.90 (m, 1H), 5.35 (m, 1H),5.23 (m, 1H), 4.64 (dt, J = 4.9 Hz, 1.6Hz, 2H), 3.69–3.64 (m, 4H), 2.18–2.12 (m, 2H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 167.12, 163.98,152.50, 135.92, 132.04, 132.01, 130.29, 127.58, 127.19, 126.93, 126.63, 121.03, 117.85, 114.32, 77.25, 69.74, 42.72, 37.80, 32.01; HRMS calcd for $C_{20}H_{20}ClN_5O_2Na$ 420.1203 [M+Na$^+$], found 420.1195.

(3-(4-(2-(2-(allyloxy)phenyl)-2H-tetrazol-5-yl)benzamido)propyl)triphenylphosphonium Bromide (TPP-Tet-1):
compound **TPP-Tet-1** was synthesized as a green solid with 15% yield using the same synthetic procedure as compound **Mor-Tet-1**. the data was idetical as privious reported[4].

Reference:

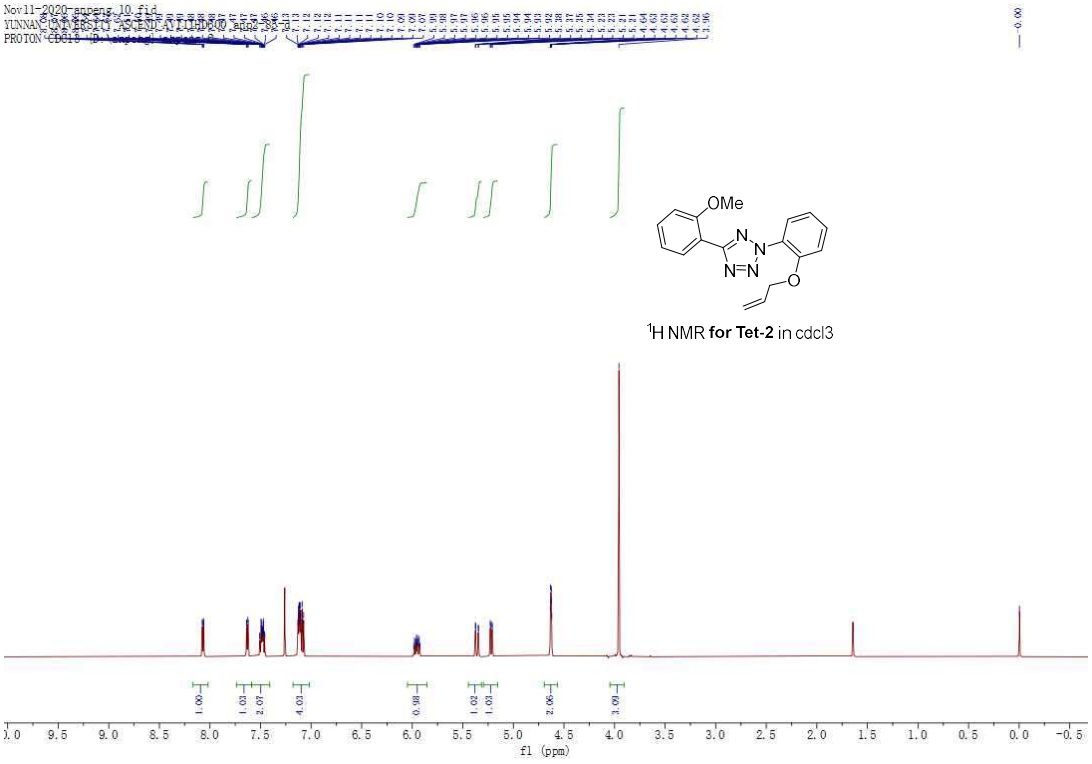
[S1] W. H. Melhuish, *J. Phys. Chem.* **1961***, 65,* 229.

[S2] H. Meier, H. Heimgartner, *Helvetica Chimica Acta.* **1985**, *68*, 1283.

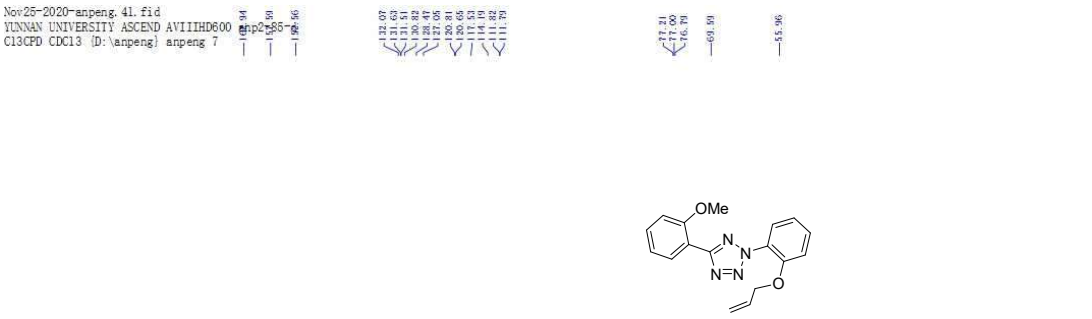
[S3] S. O. Rashid, S. S. Almadhhi, D. J. Berrisford, J. Raftery, I. Vitorica-Yrezabal, G. Whitehead, P. Quayle, *Tetrahedron.* **2019**, *75*, 2413.

[S4] S. Liu, H. Su, L. Bu, J. Yan, G. Li, J. Huang, *Analyst*, **2021**, 146, 1369.

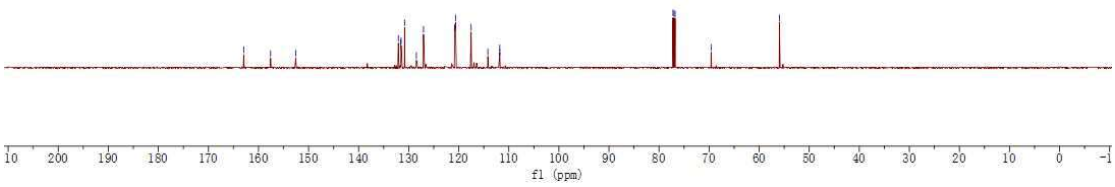
^{1}H and ^{13}C NMR Spectra

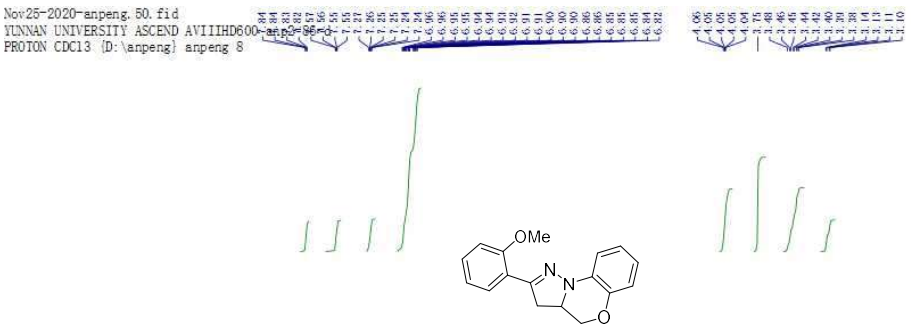


^{1}H NMR **for Tet-2** in cdcl3

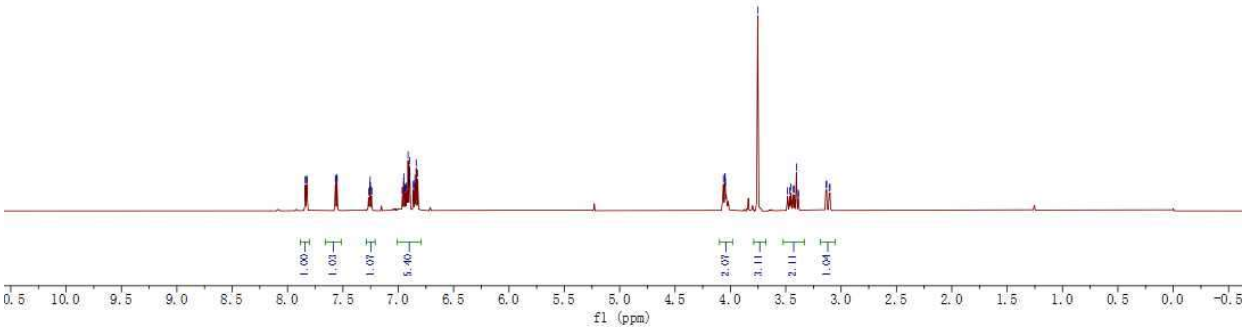


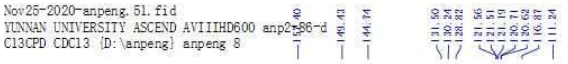
^{13}C NMR **for Tet-2** in cdcl3

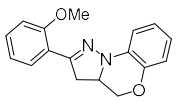




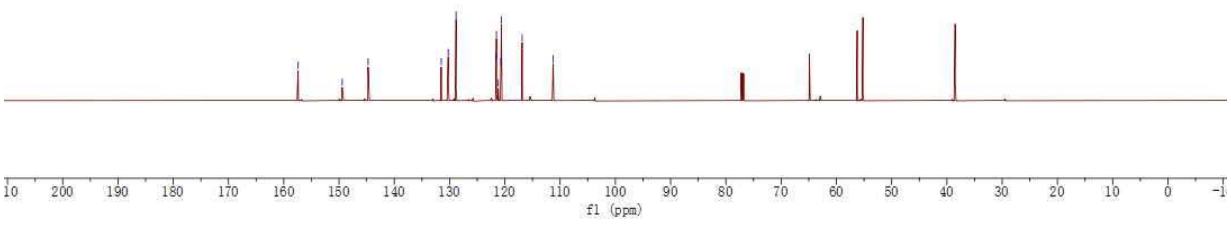
^{1}H NMR **for Pyr-2** in cdcl3

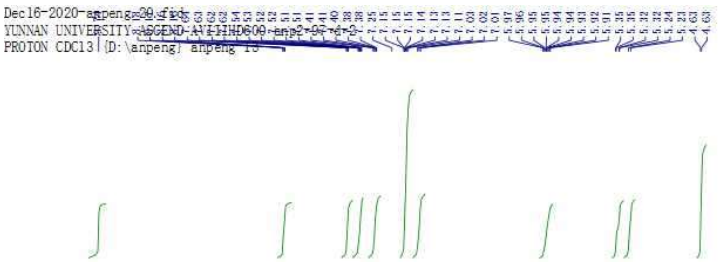


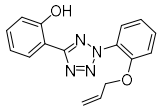




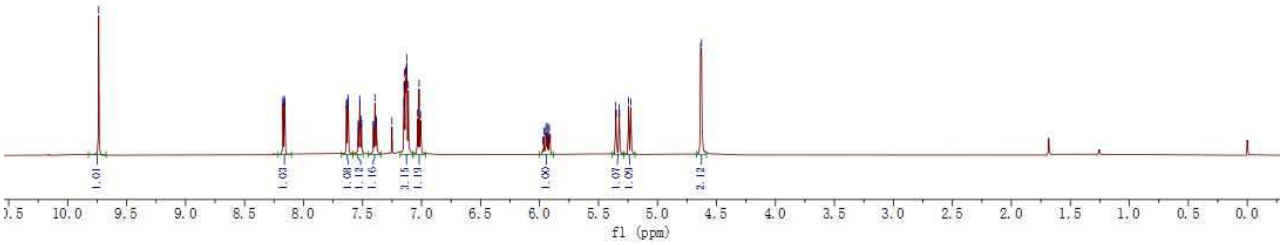
^{13}C NMR **for Pyr-2** in cdcl3

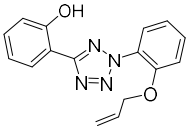




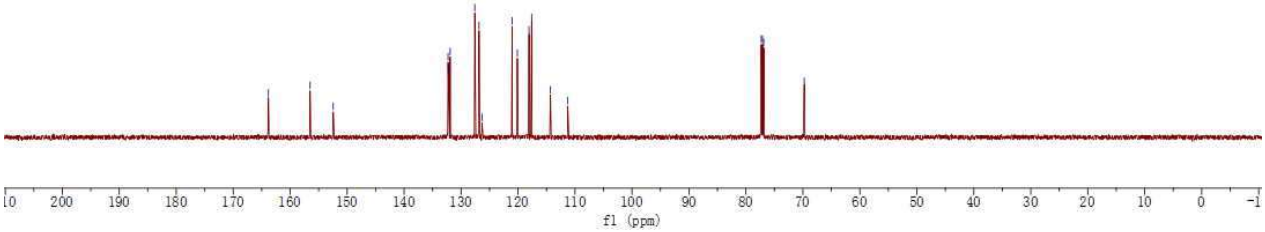


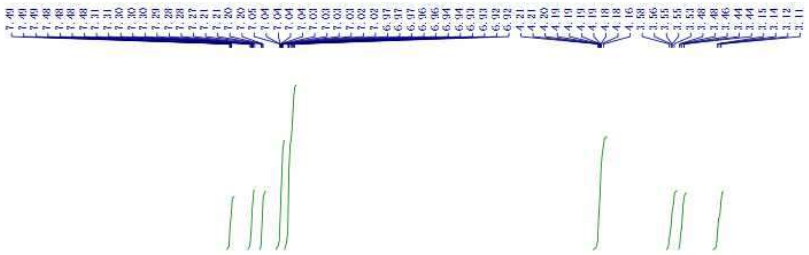
^{1}H NMR **for Tet-3** in cdcl3

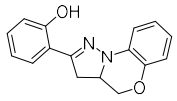




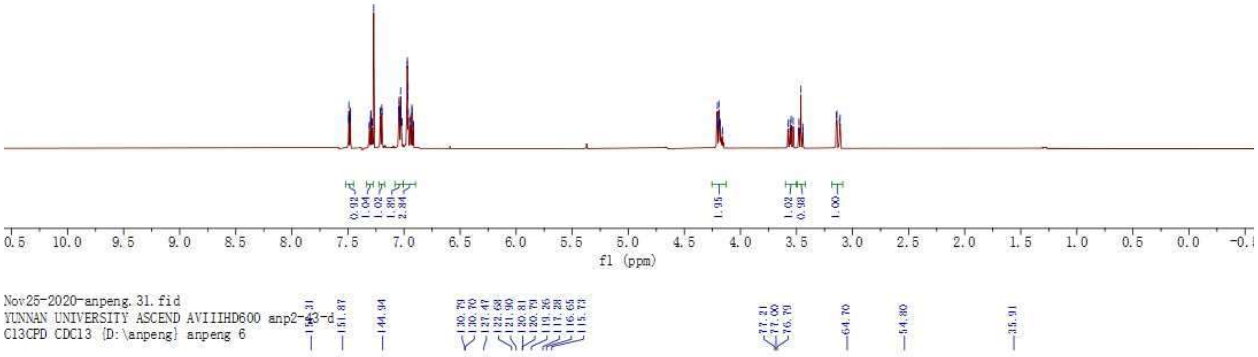
^{13}C NMR **for Tet-3** in cdcl3

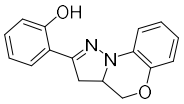




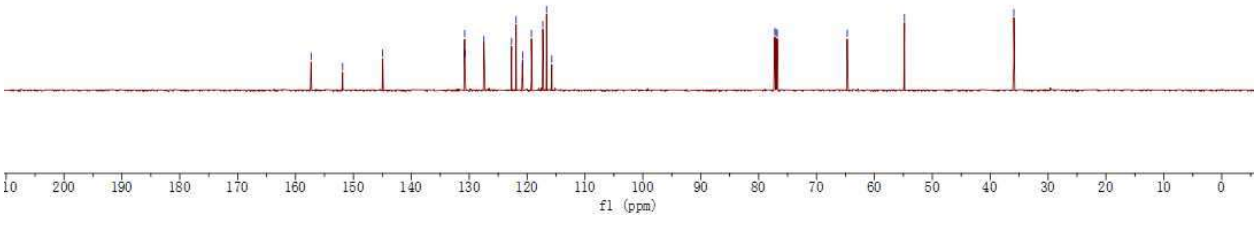


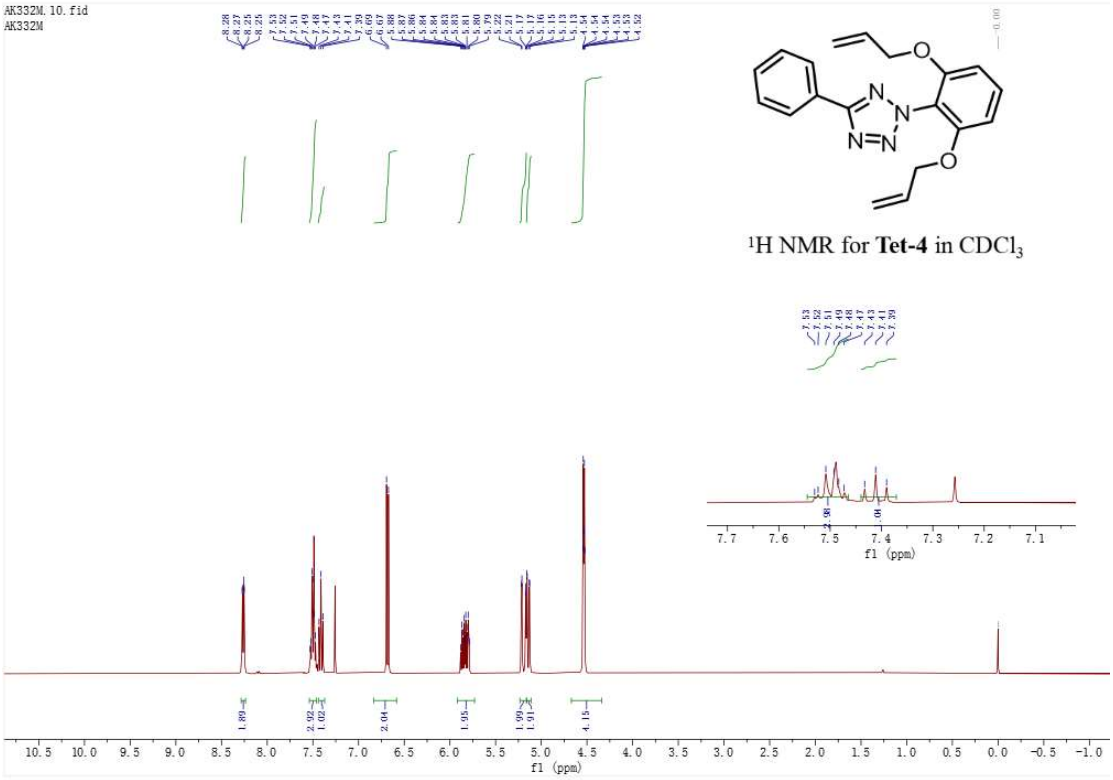
^{1}H NMR **for Pyr-3** in cdcl3



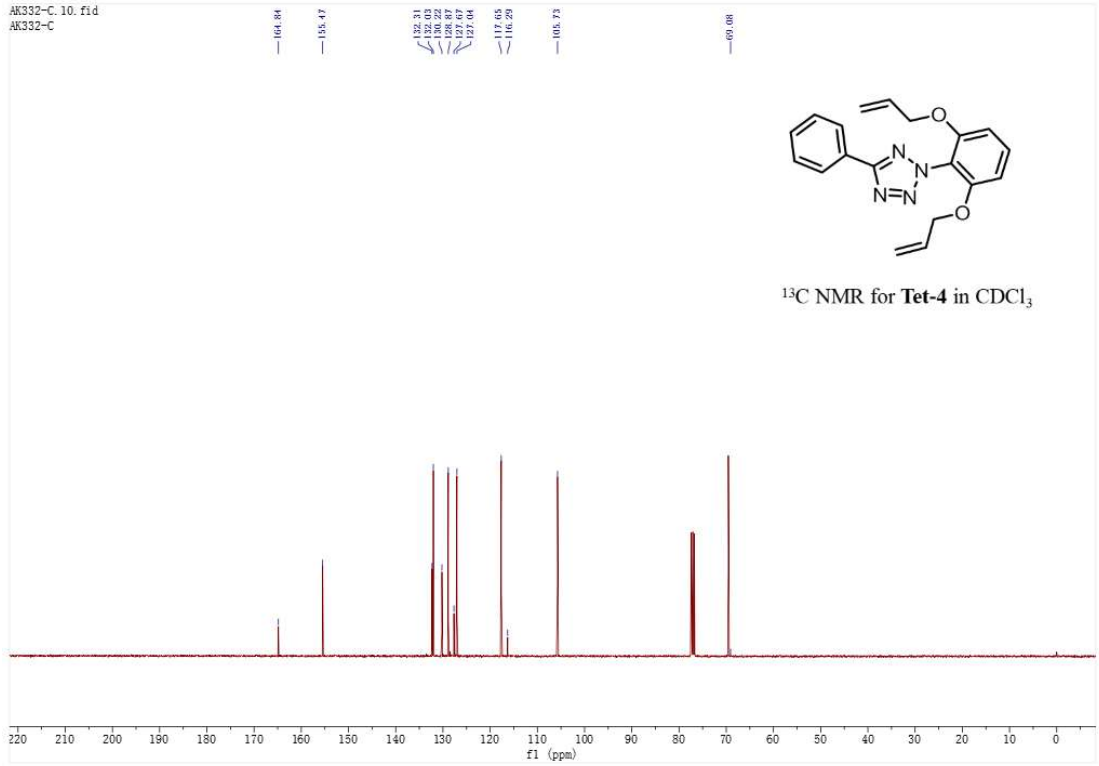


^{1}H NMR **for Pyr-3** in cdcl3

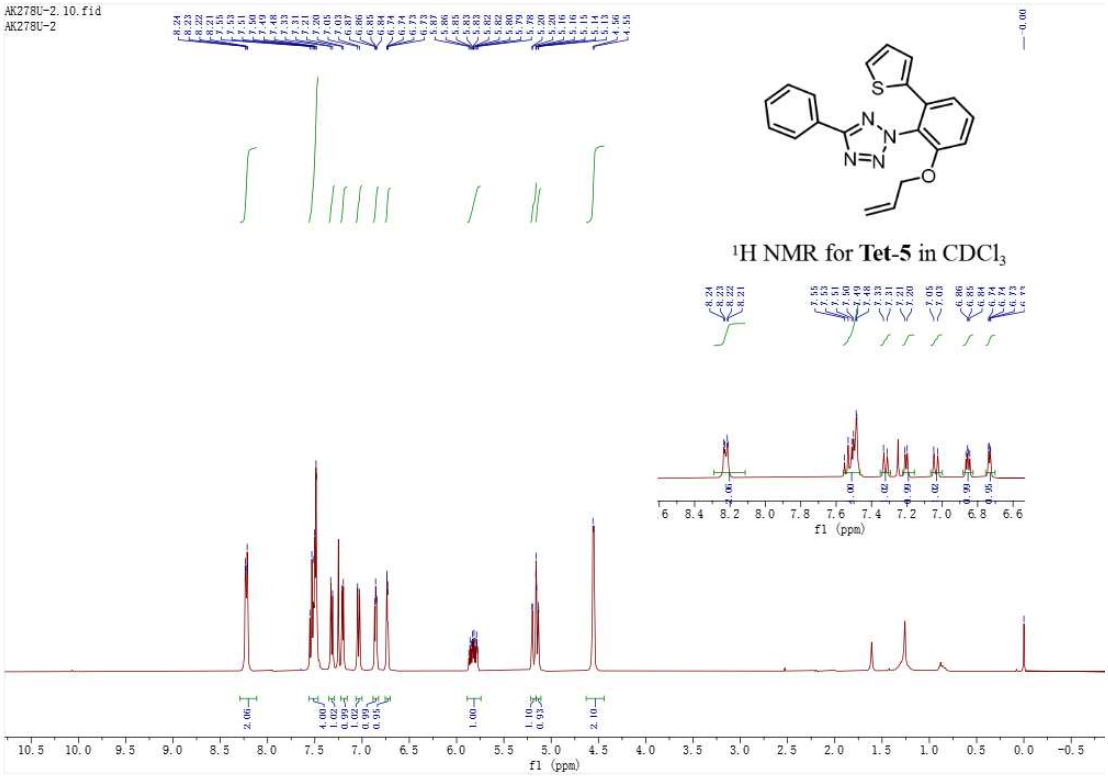




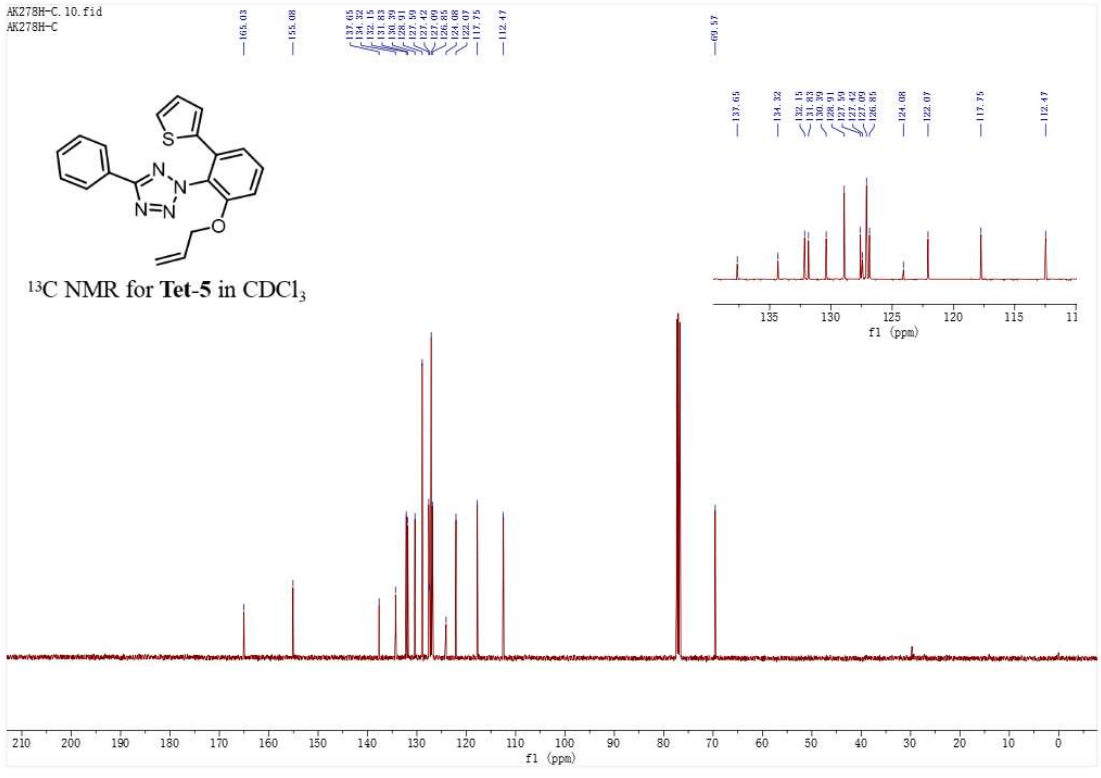
^{1}H NMR for **Tet-4** in $CDCl_3$



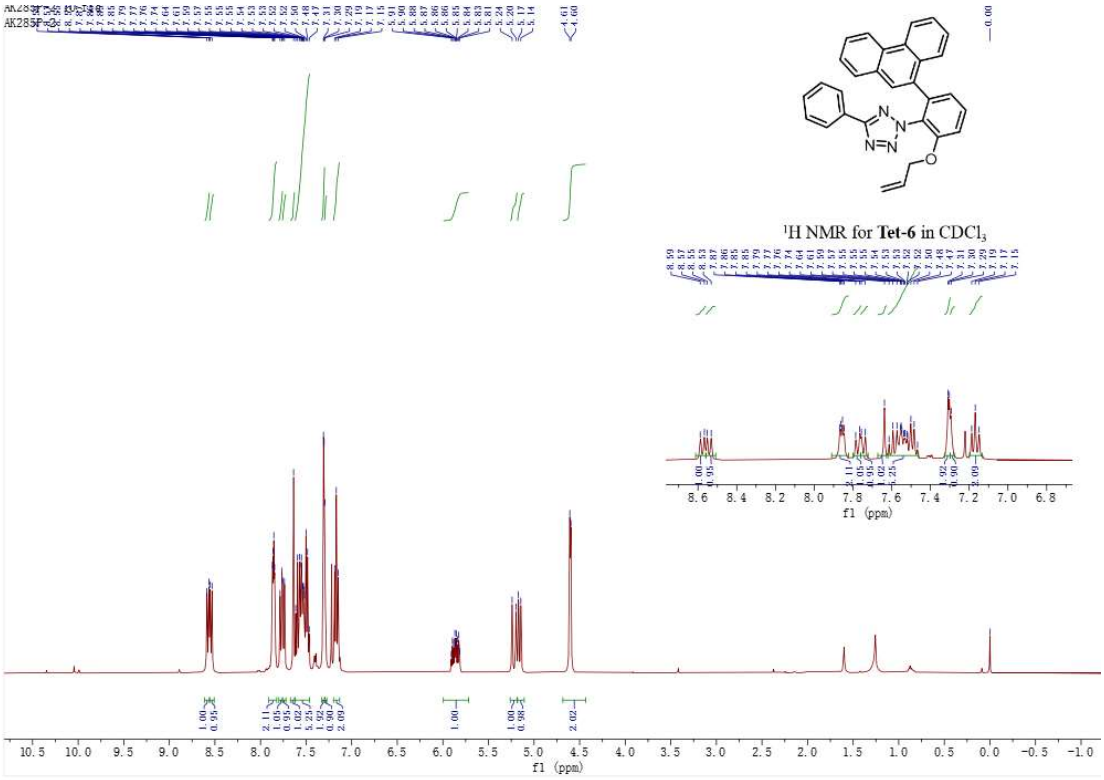
^{13}C NMR for **Tet-4** in $CDCl_3$



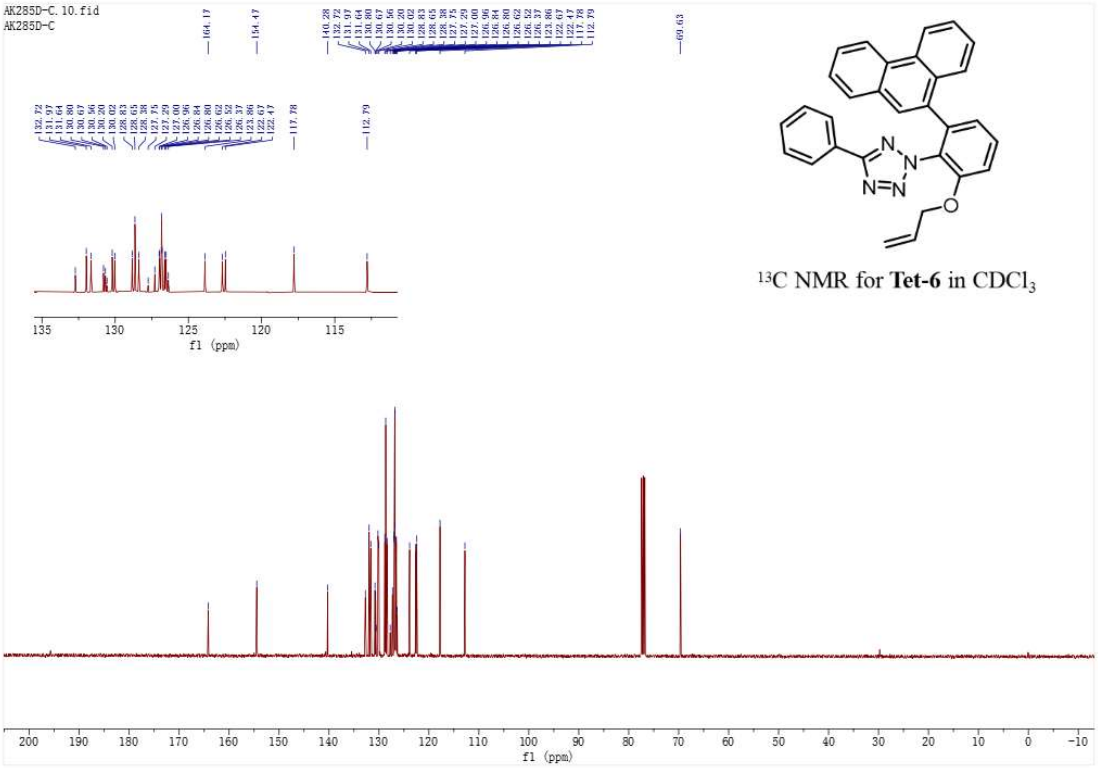
^{1}H NMR for **Tet-5** in $CDCl_3$



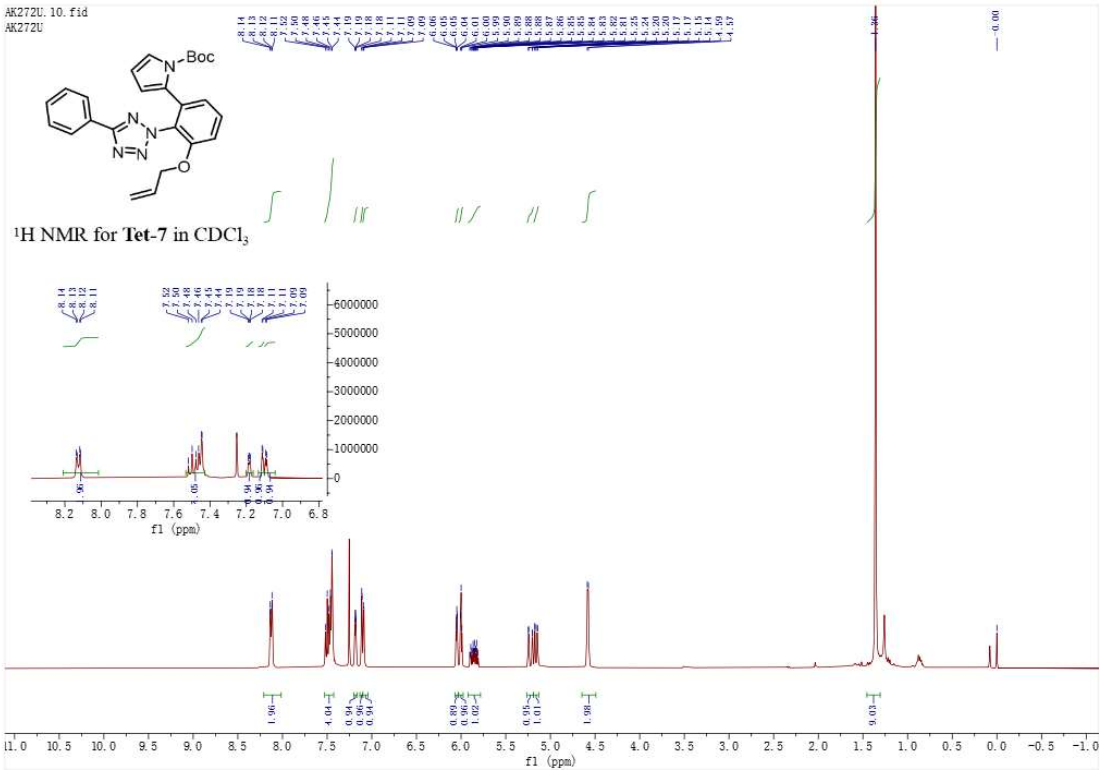
^{13}C NMR for **Tet-5** in $CDCl_3$

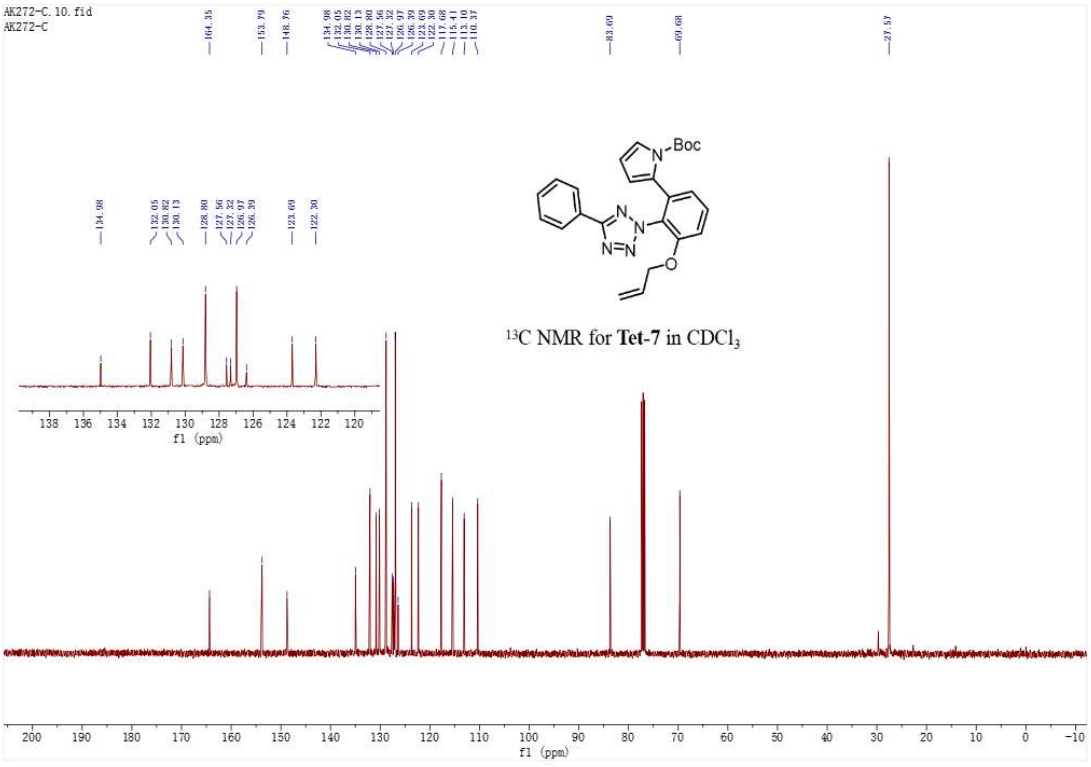


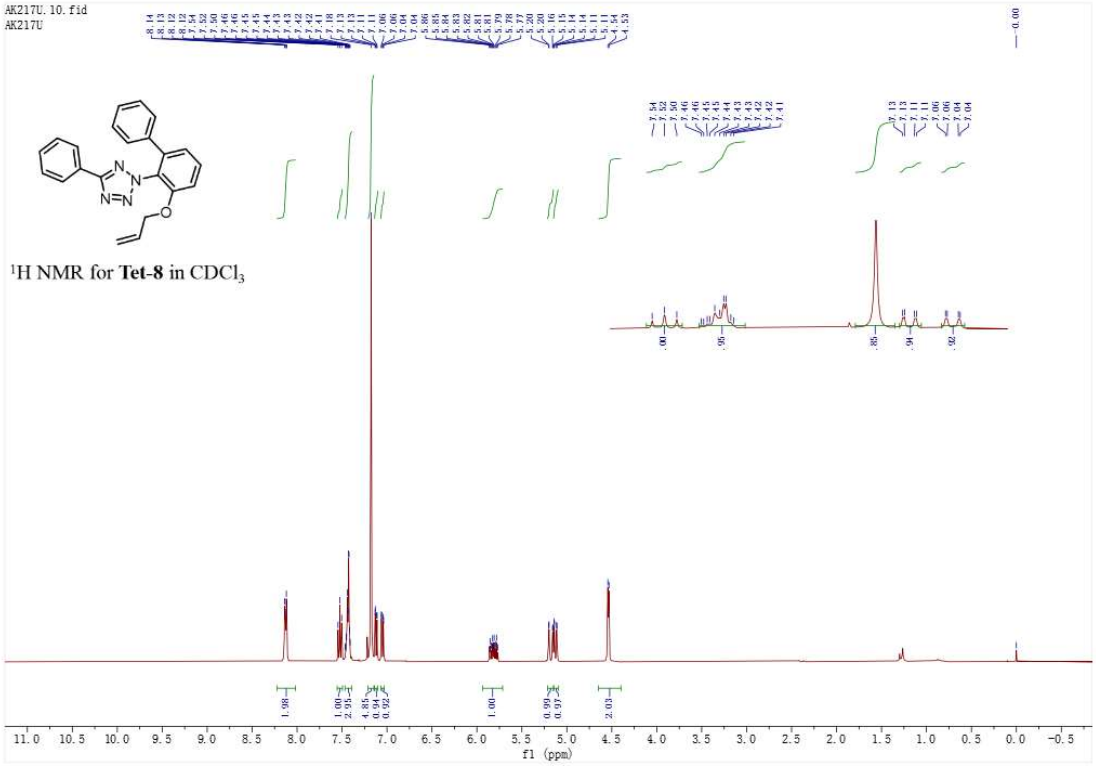
^{1}H NMR for **Tet-6** in $CDCl_3$



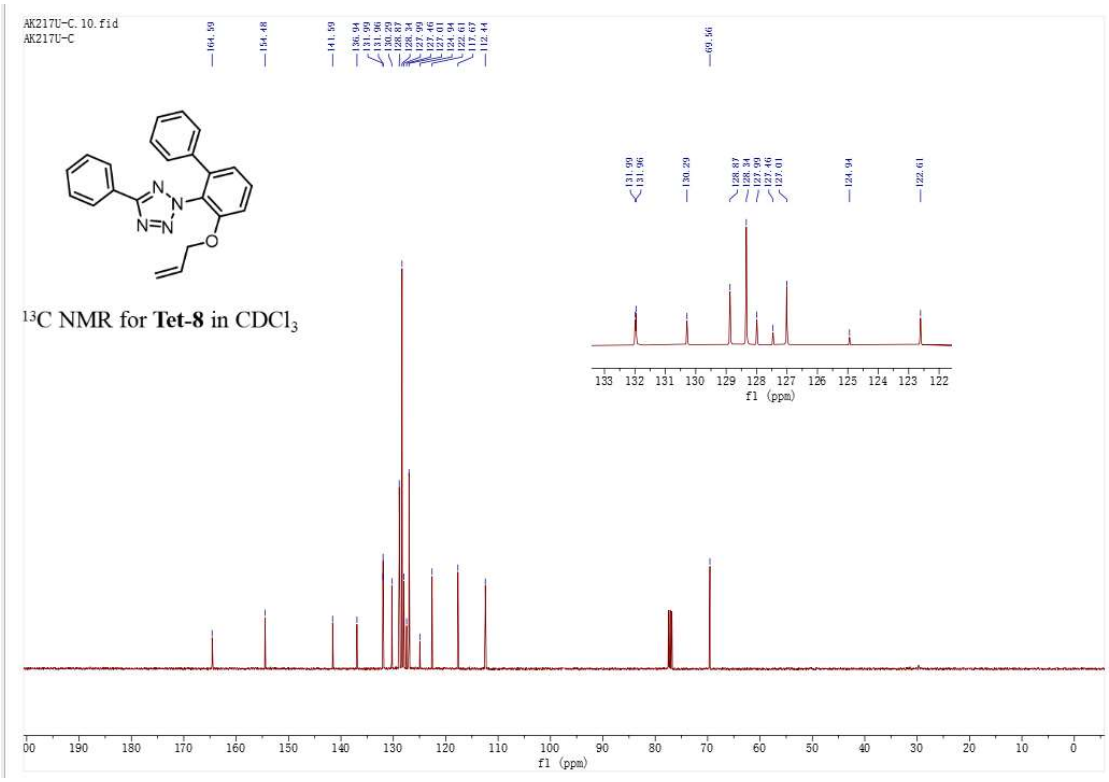
^{13}C NMR for **Tet-6** in $CDCl_3$


AK272U. 10. fid
AK272U
Boc
^{1}H NMR for **Tet-7** in $CDCl_3$
f1 (ppm)

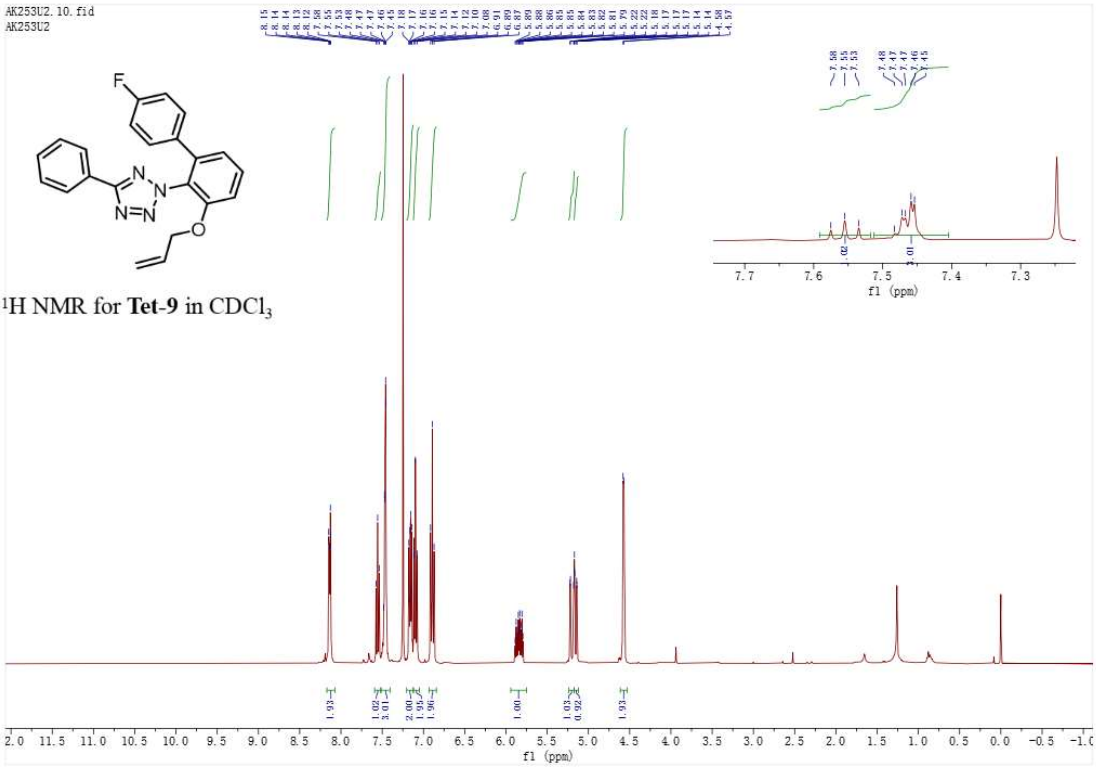

AK272-C. 10. fid
AK272-C
Boc
^{13}C NMR for **Tet-7** in $CDCl_3$
f1 (ppm)



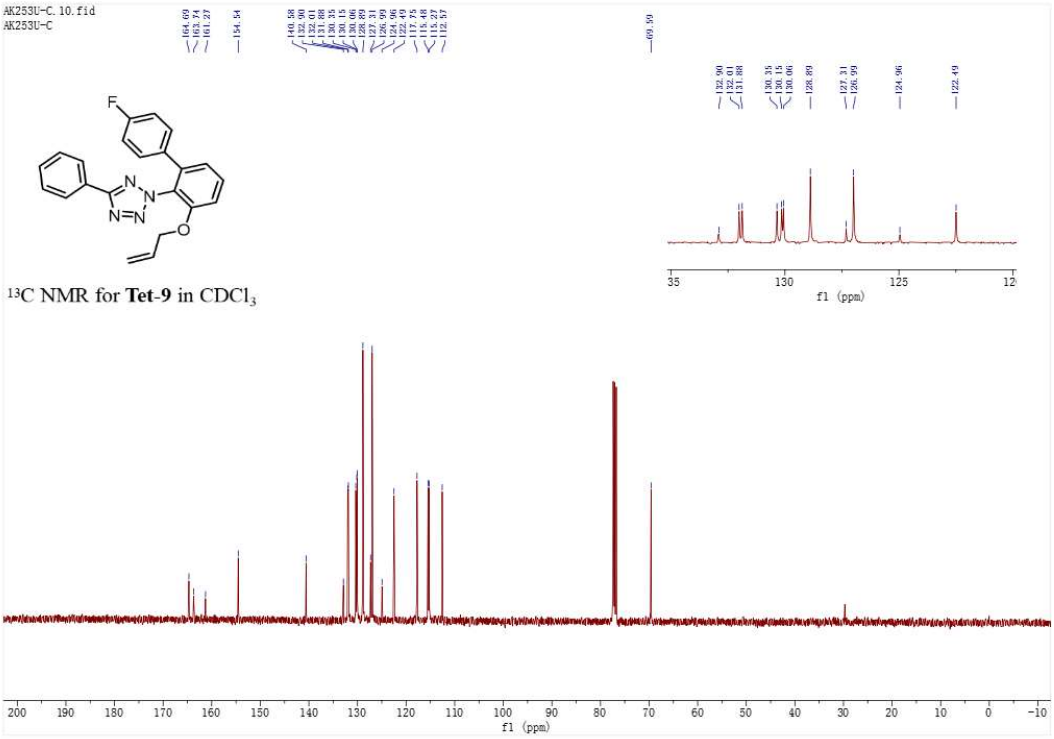
^{1}H NMR for **Tet-8** in $CDCl_3$



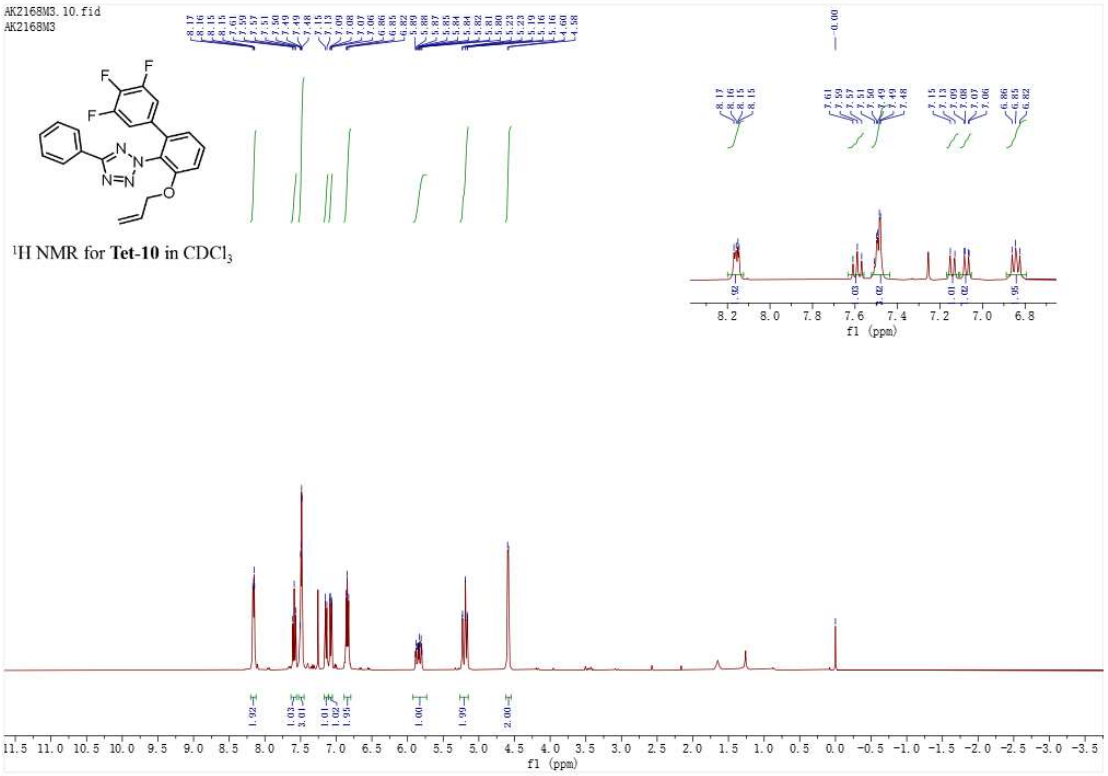
^{13}C NMR for **Tet-8** in $CDCl_3$



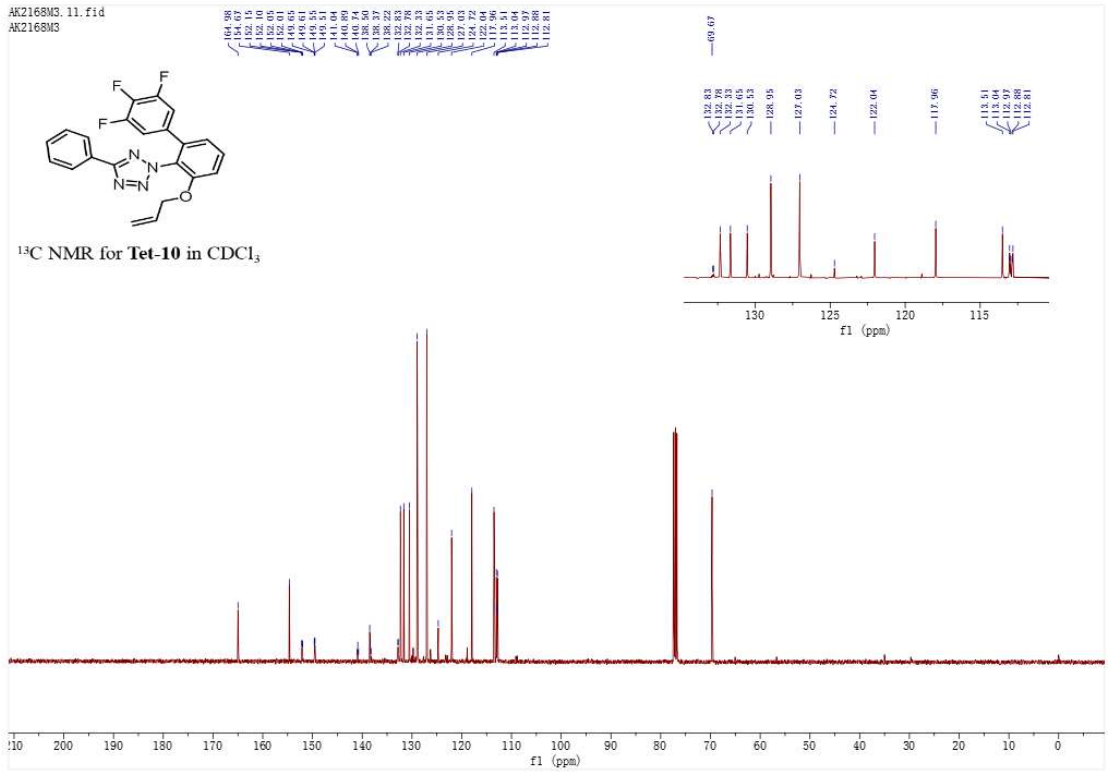
^{1}H NMR for **Tet-9** in $CDCl_3$



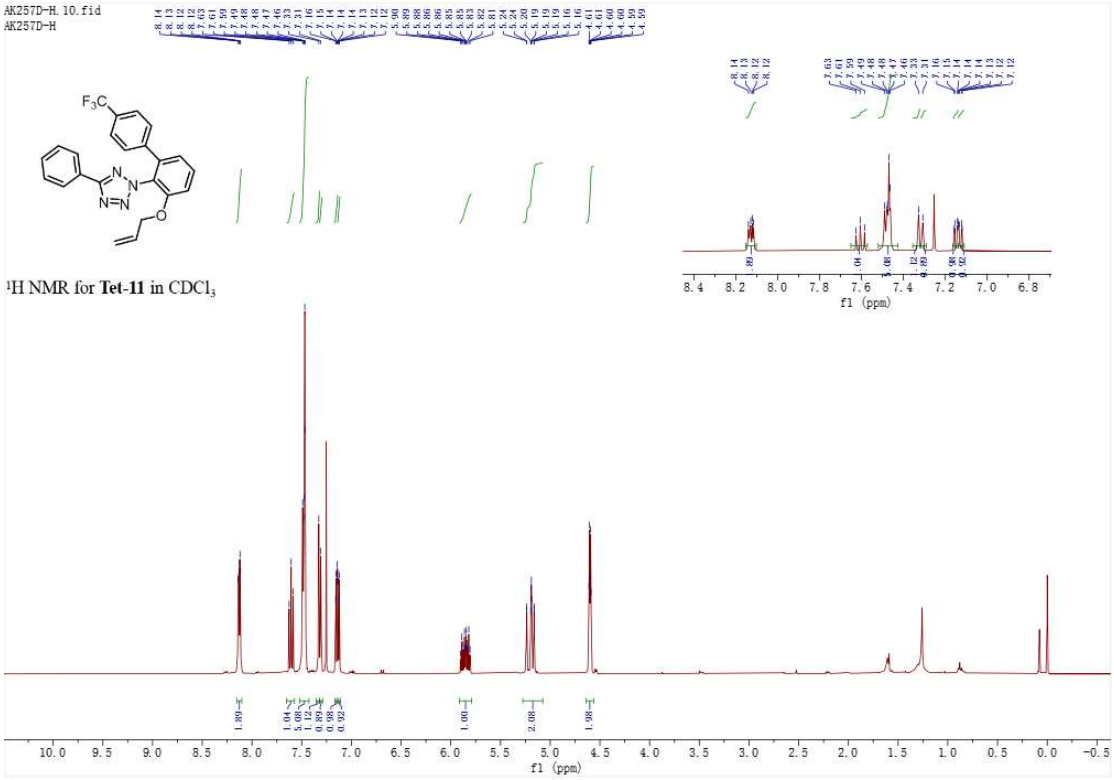
^{13}C NMR for **Tet-9** in $CDCl_3$



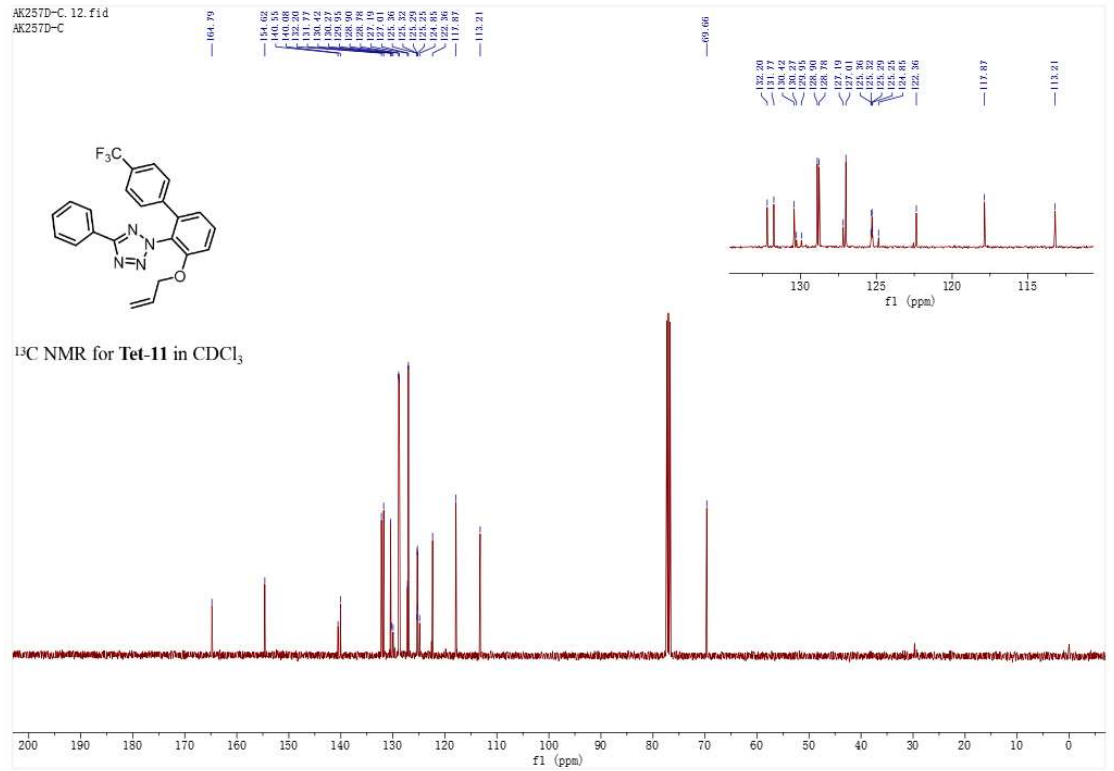
^{1}H NMR for **Tet-10** in $CDCl_3$



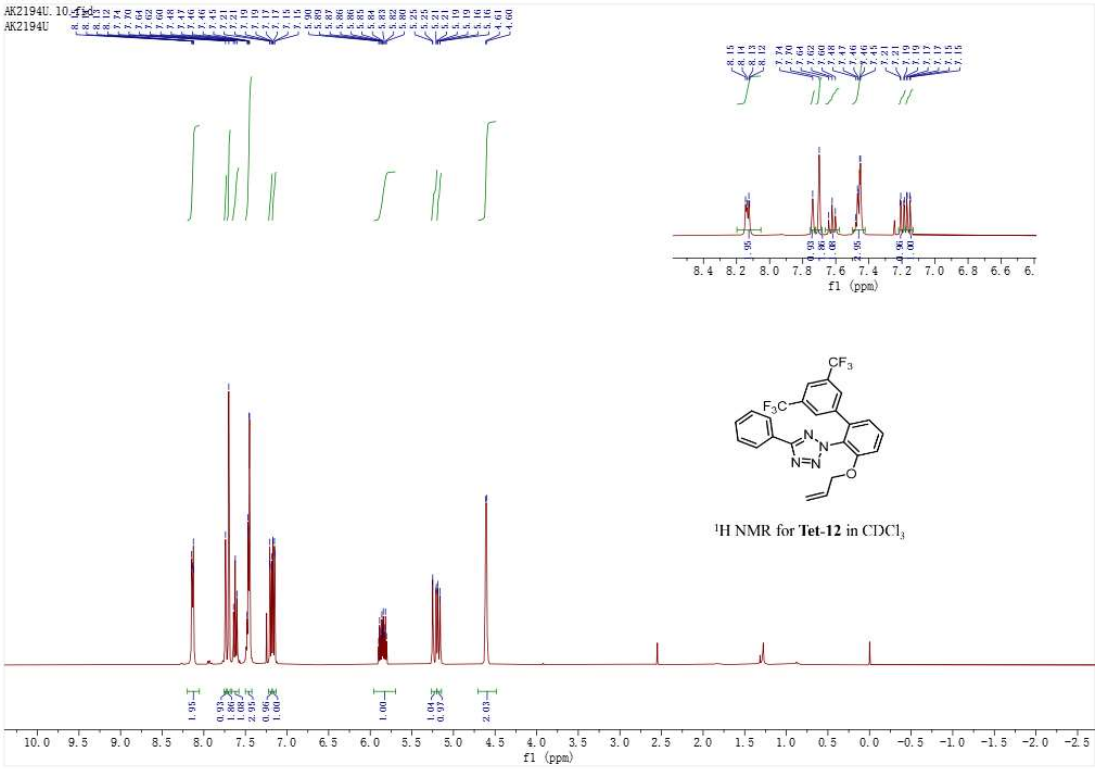
^{13}C NMR for **Tet-10** in $CDCl_3$



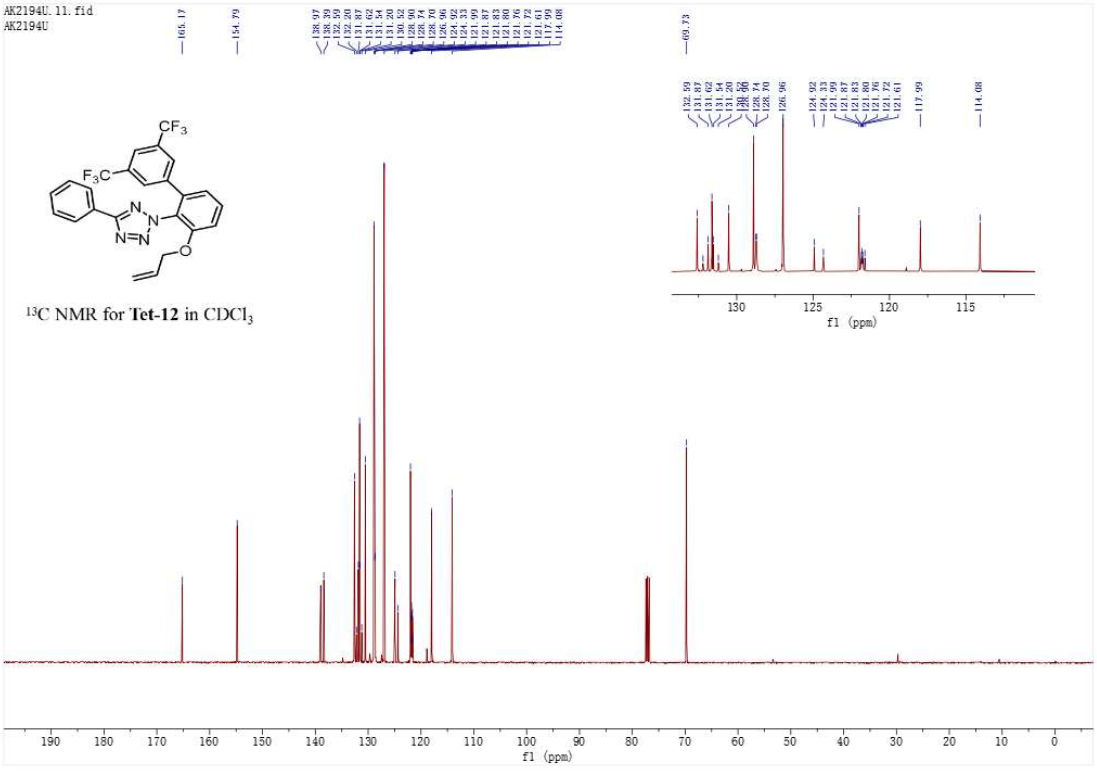
^{1}H NMR for **Tet-11** in $CDCl_3$



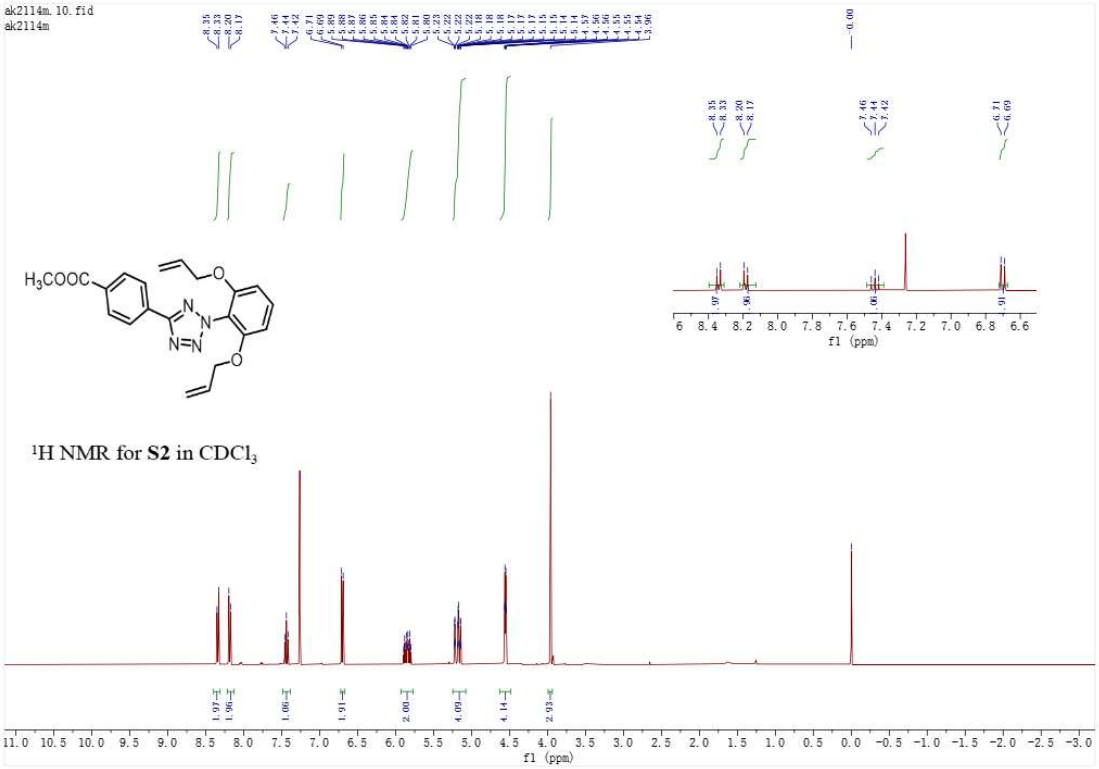
^{13}C NMR for **Tet-11** in $CDCl_3$



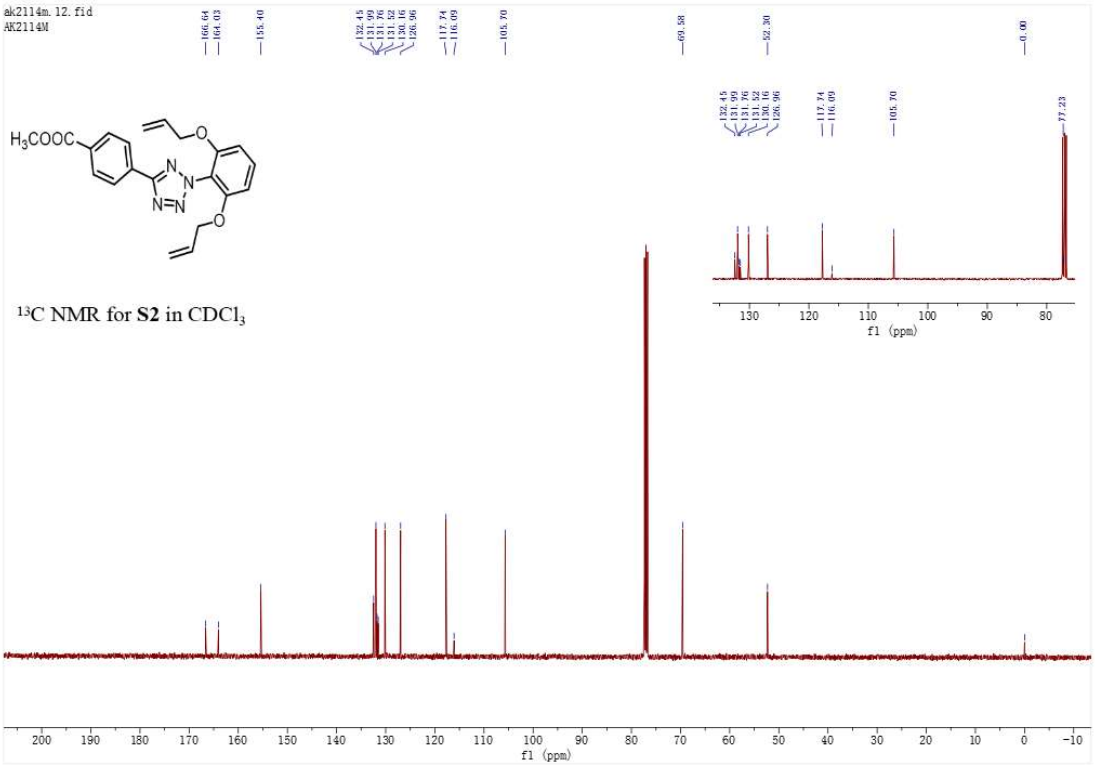
^{1}H NMR for **Tet-12** in $CDCl_3$



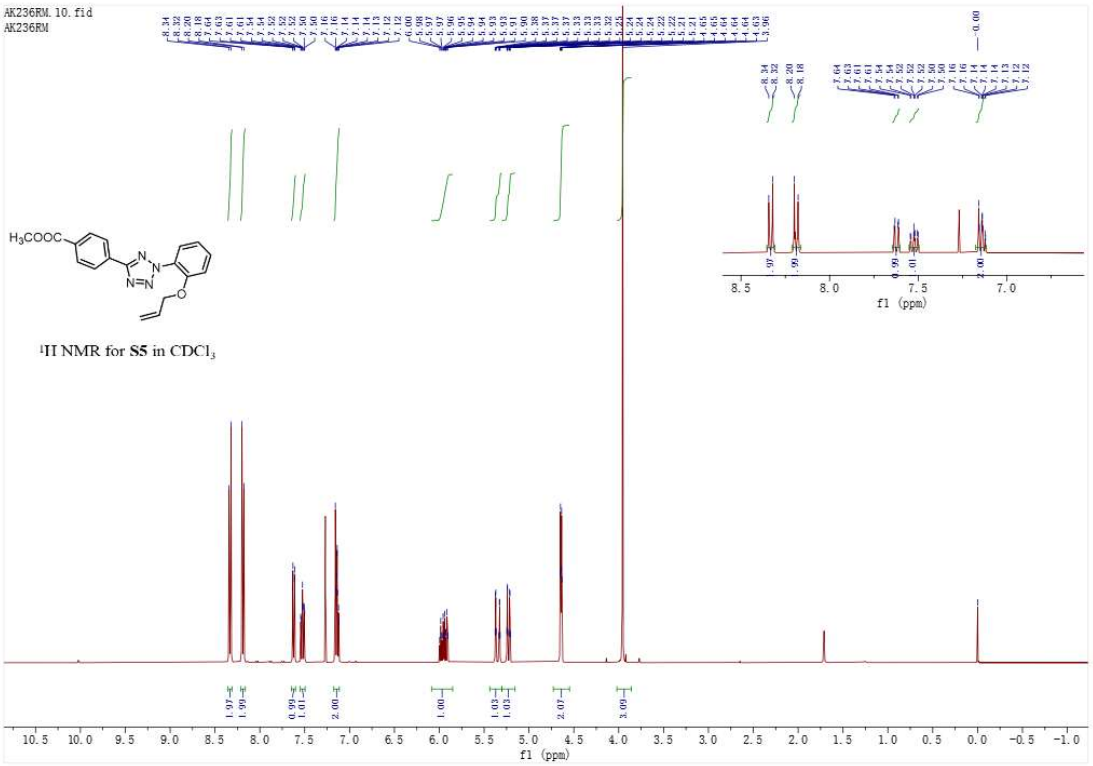
^{13}C NMR for **Tet-12** in $CDCl_3$



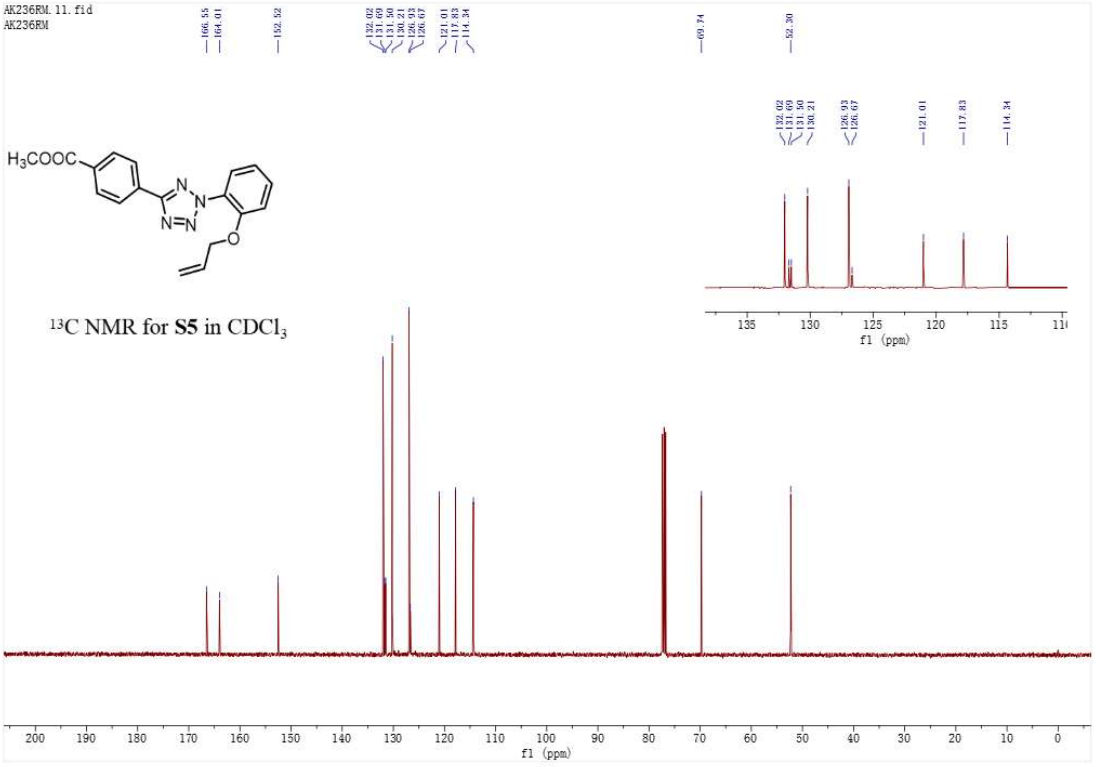
^{1}H NMR for **S2** in $CDCl_3$



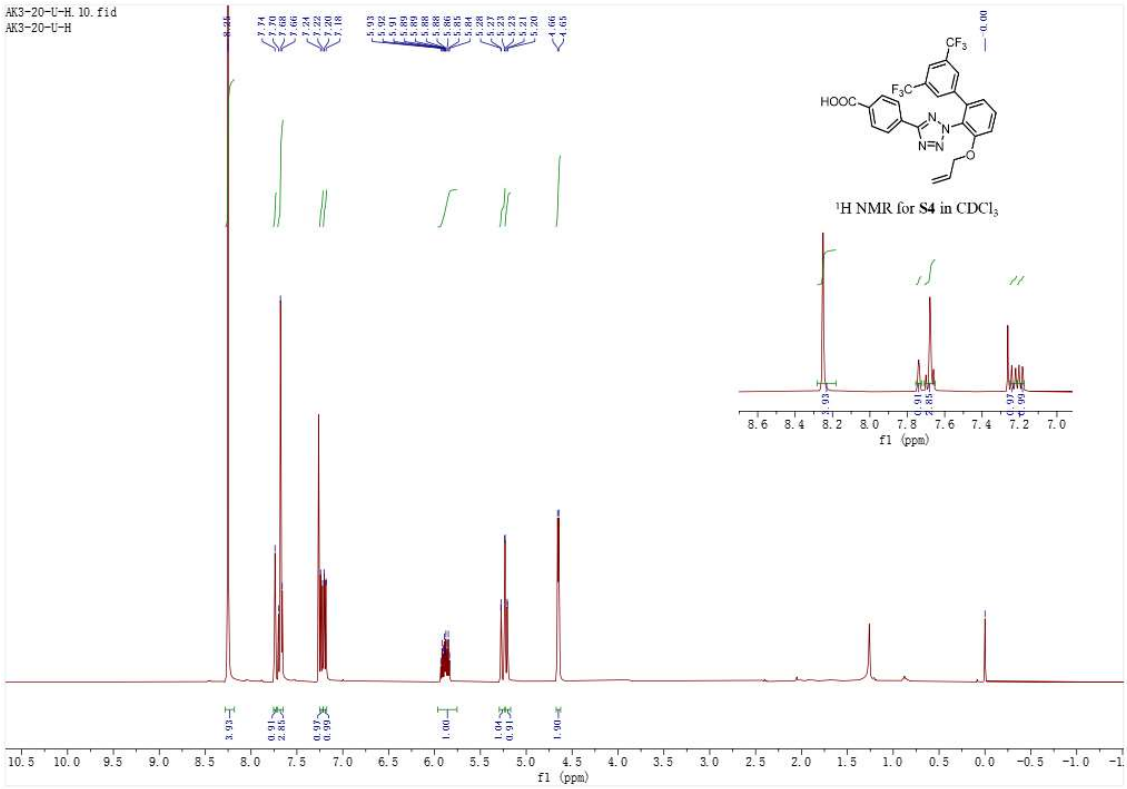
^{13}C NMR for **S2** in $CDCl_3$



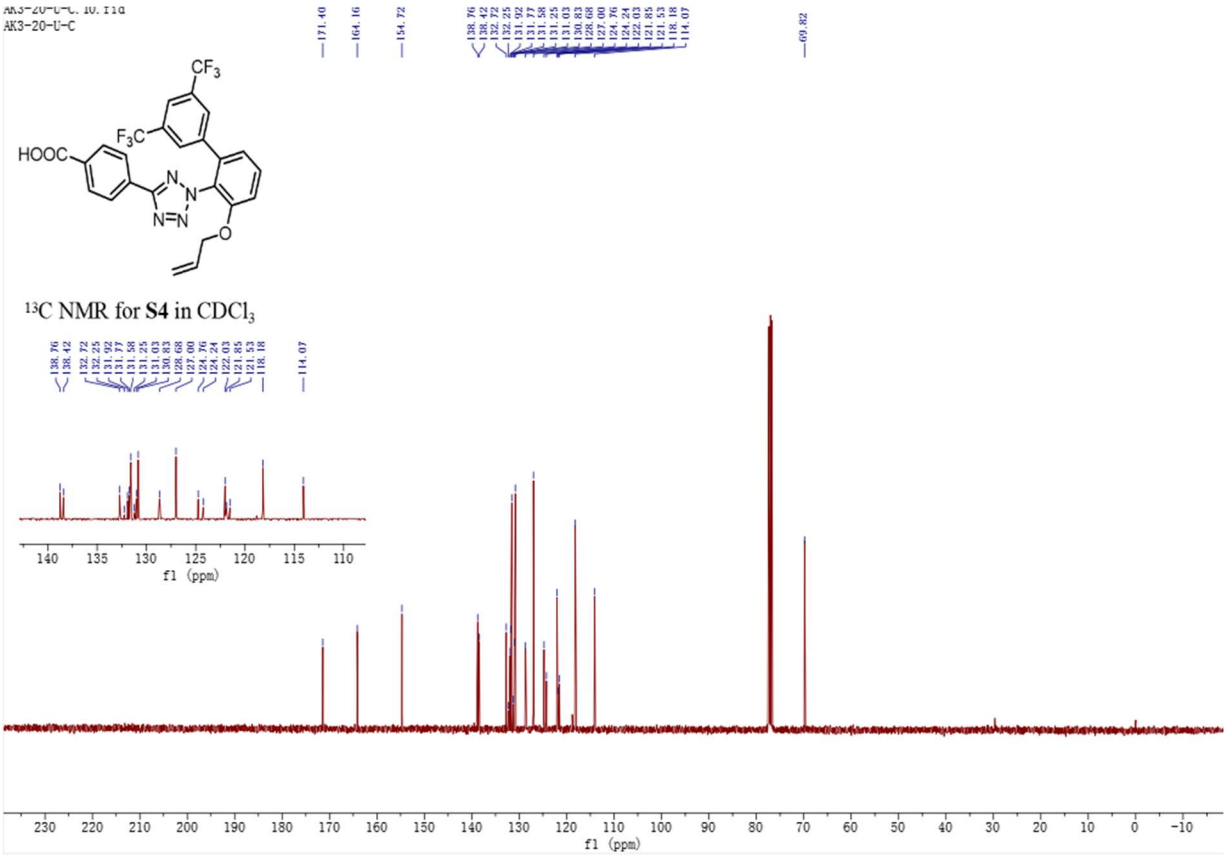
^{1}H NMR for **S5** in $CDCl_3$



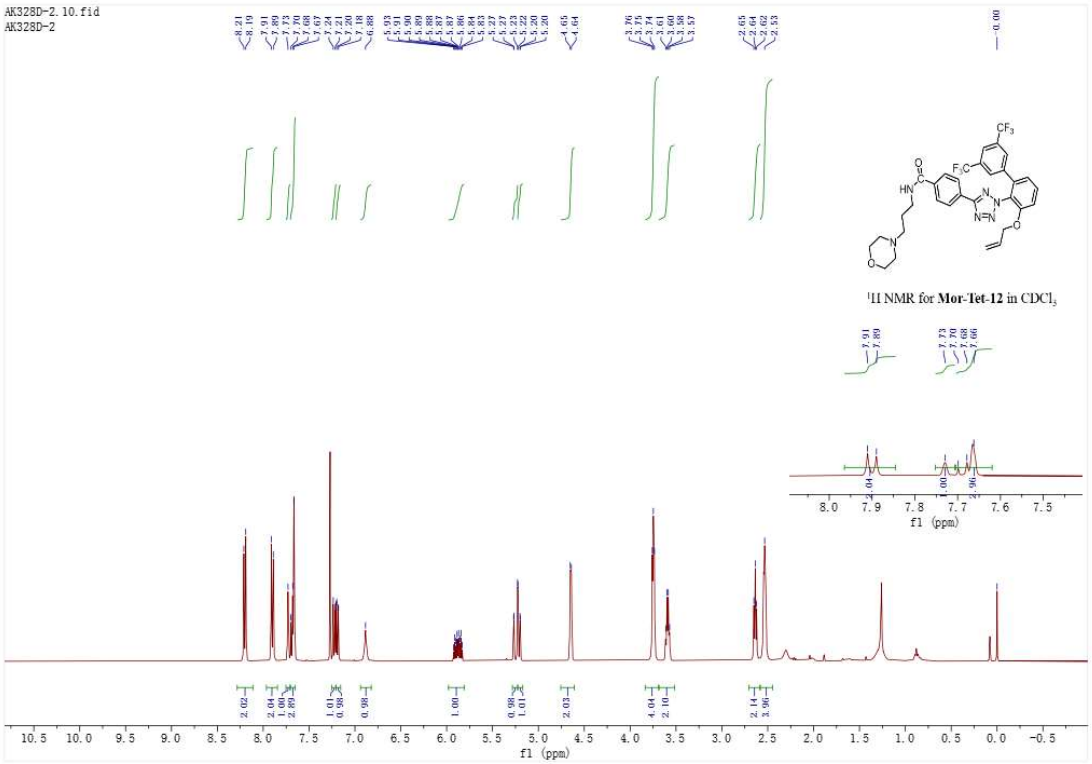
^{13}C NMR for **S5** in $CDCl_3$



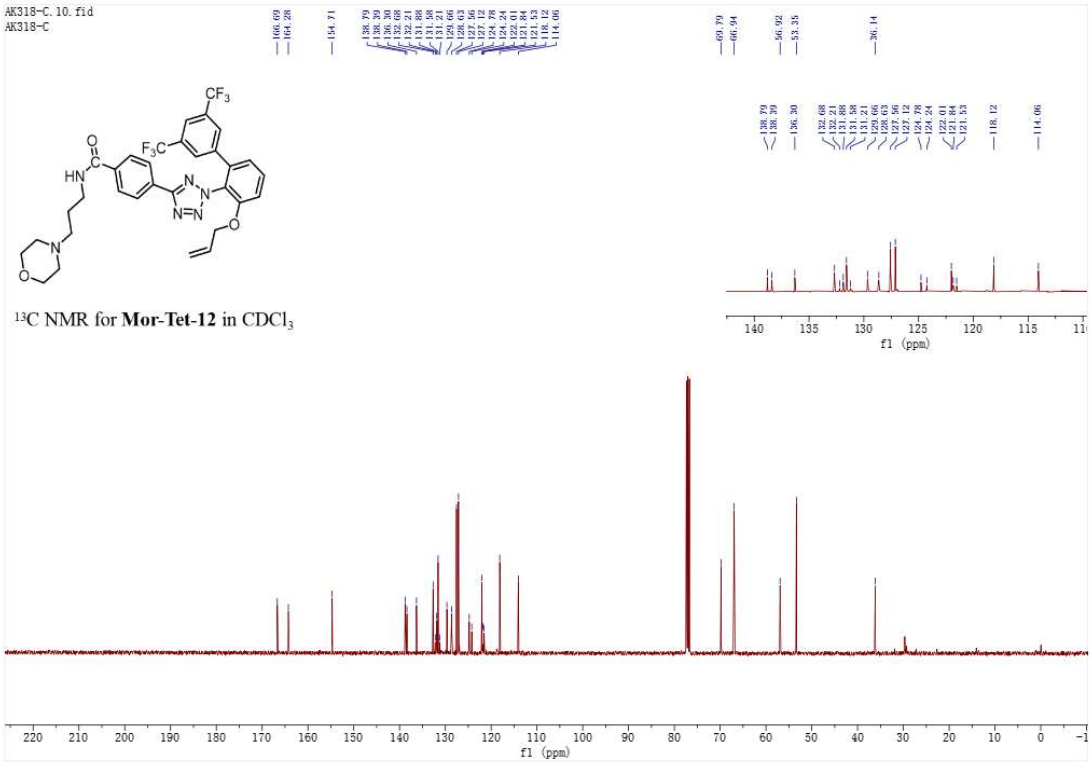
^{1}H NMR for **S4** in $CDCl_3$



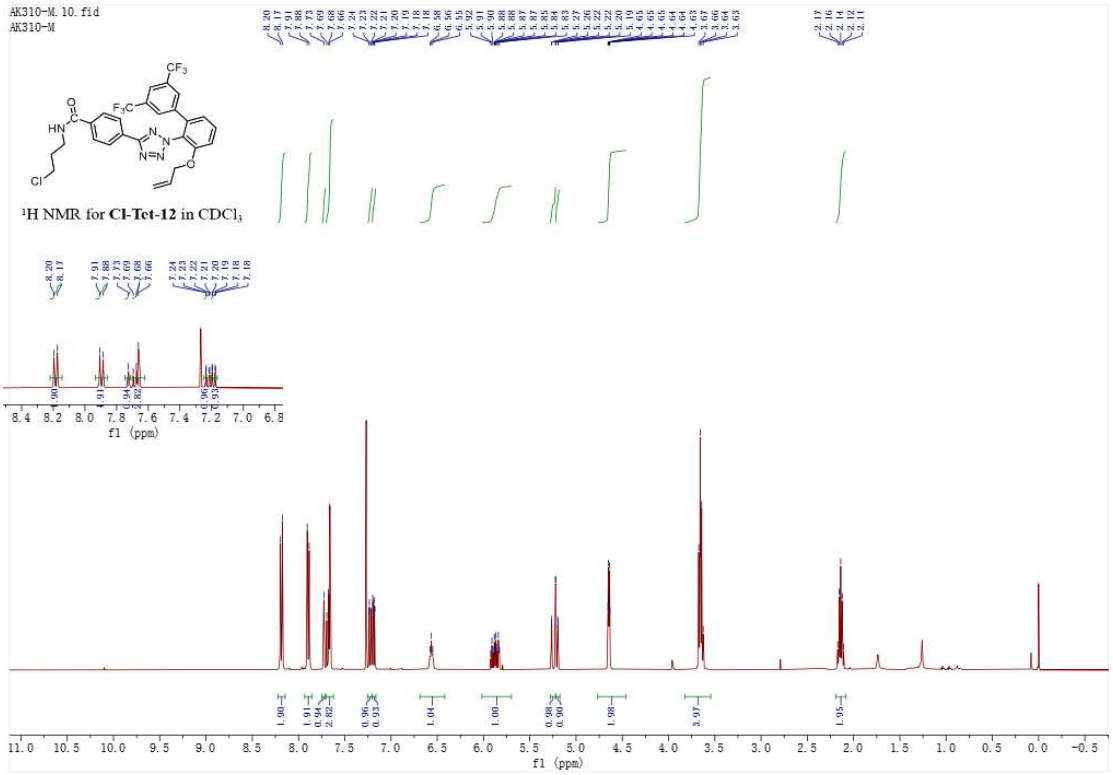
^{13}C NMR for **S4** in $CDCl_3$

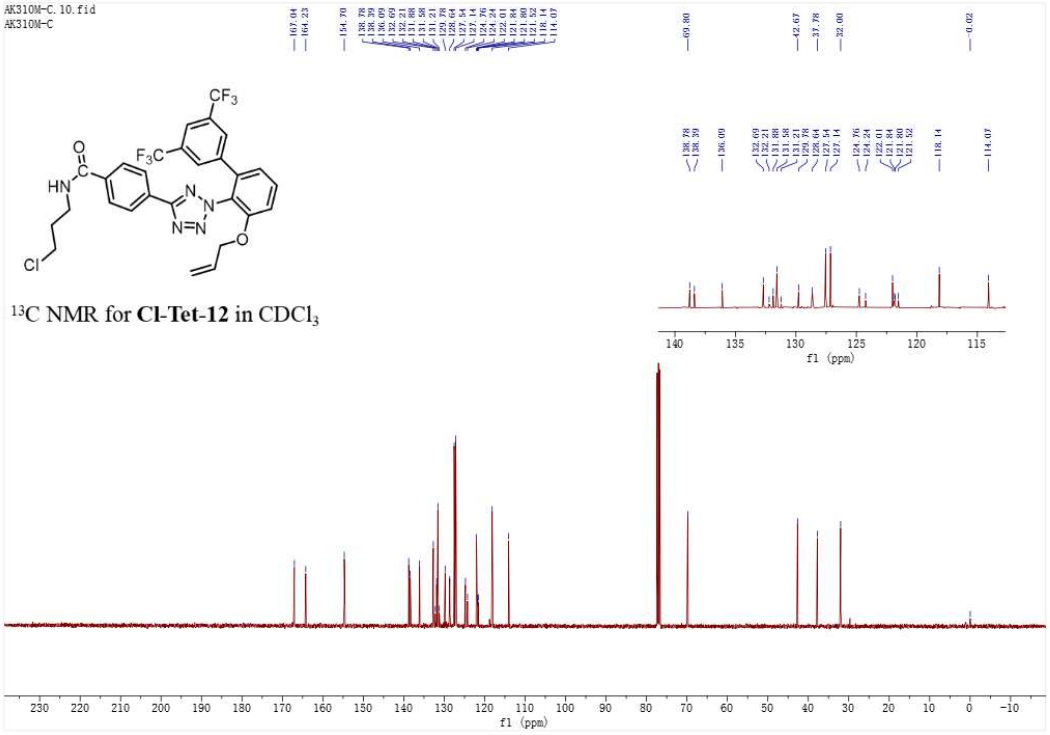


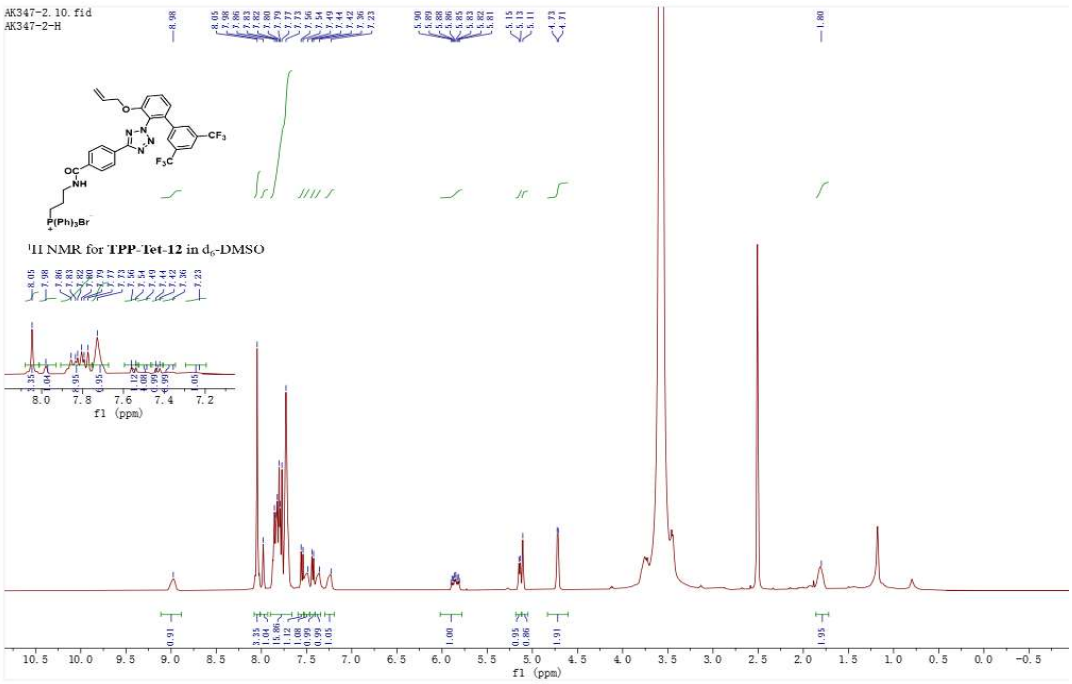
^{1}H NMR for **Mor-Tet-12** in $CDCl_3$



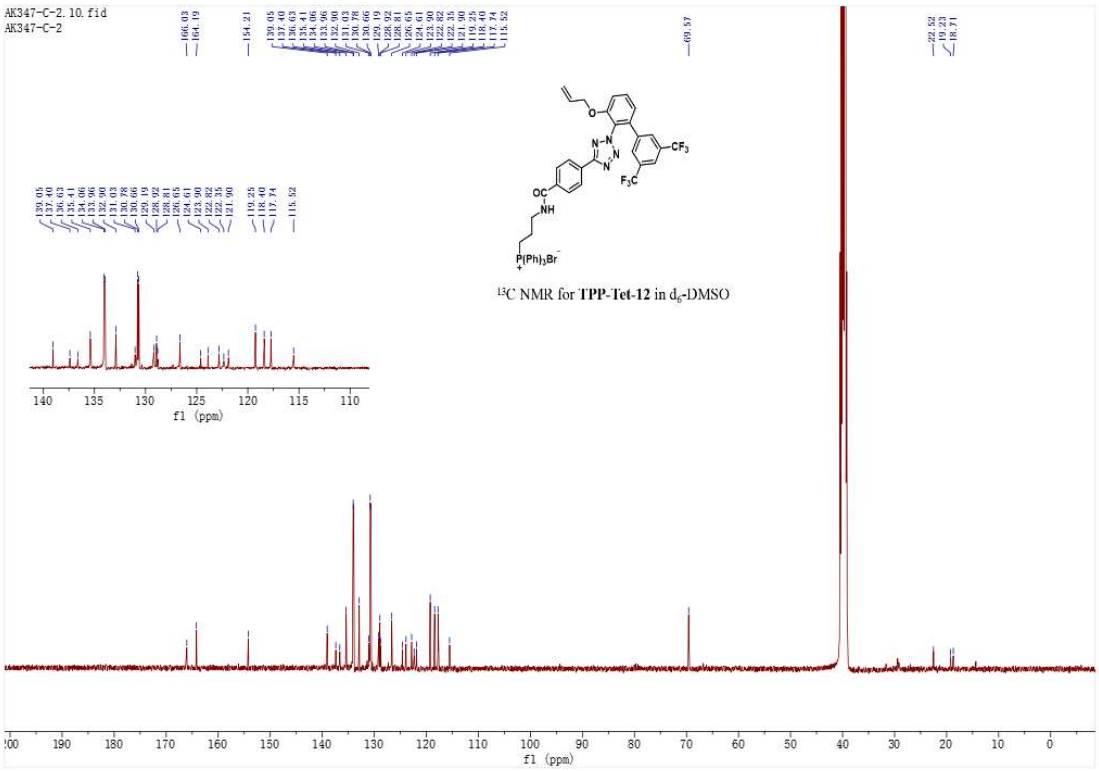
^{13}C NMR for **Mor-Tet-12** in $CDCl_3$


AK310-M.10.fid
AK310-M
^{1}H NMR for **Cl-Tet-12** in $CDCl_3$
f1 (ppm)

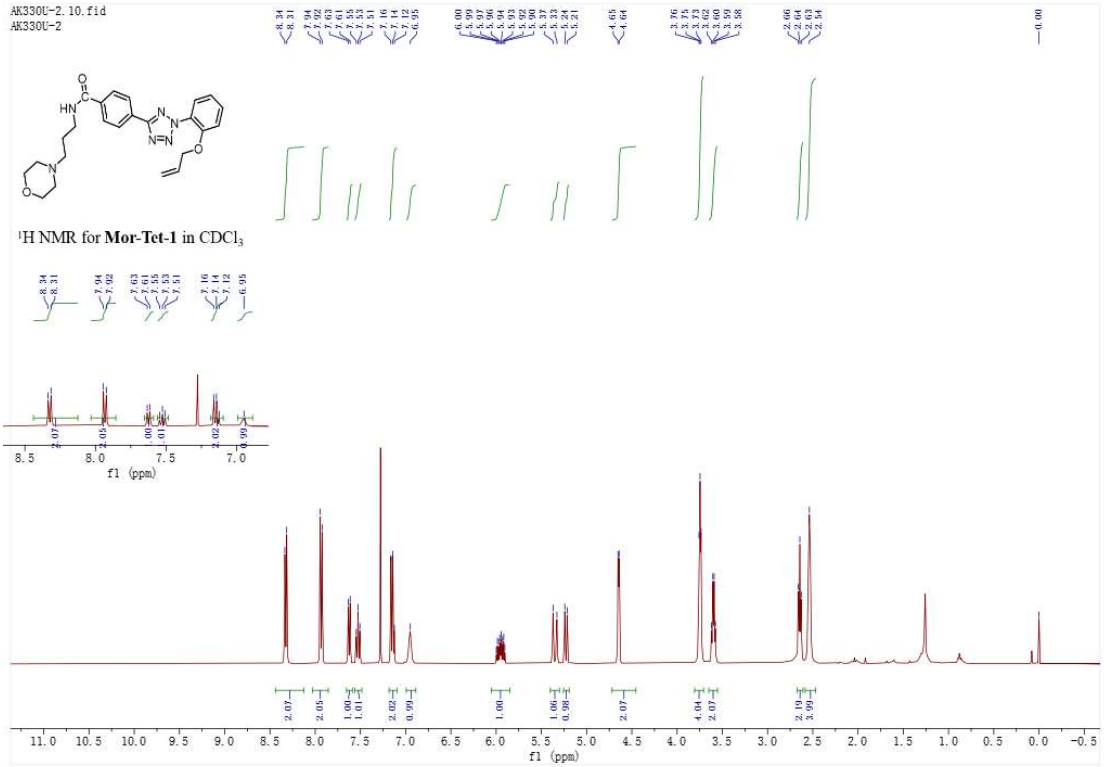

AK310M-C.10.fid
AK310M-C
^{13}C NMR for **Cl-Tet-12** in $CDCl_3$
f1 (ppm)



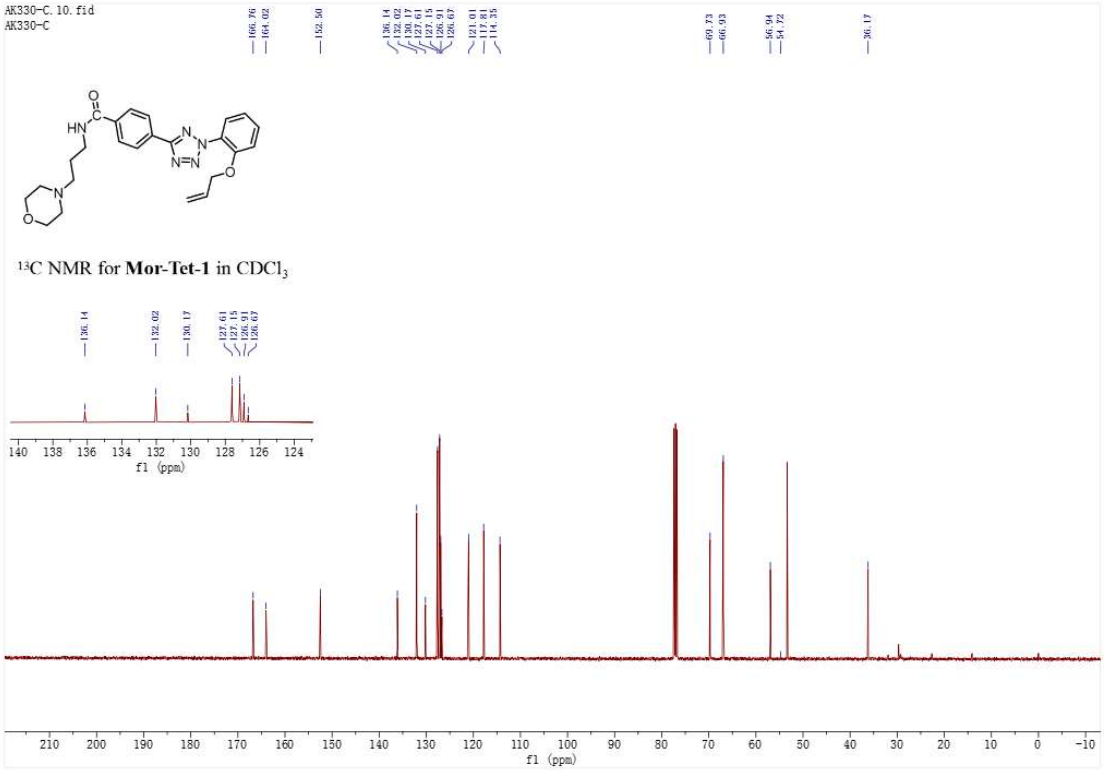
^{1}H NMR for **TPP-Tet-12** in d_6-DMSO



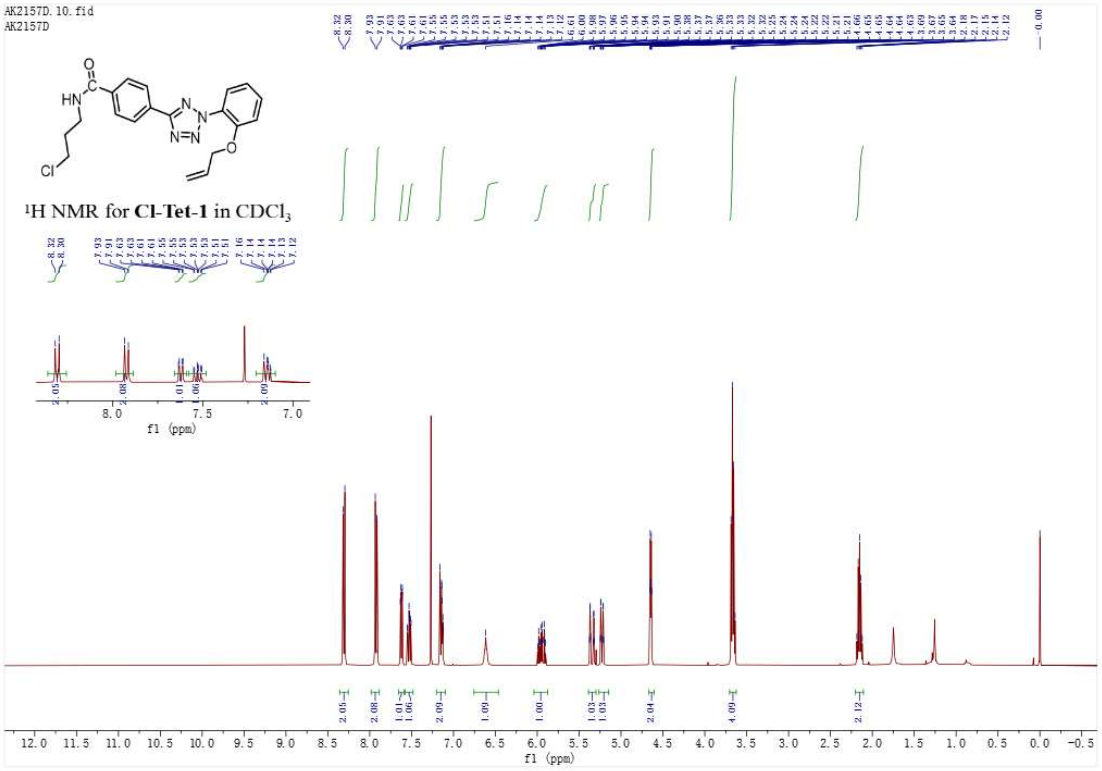
^{13}C NMR for **TPP-Tet-12** in d_6-DMSO



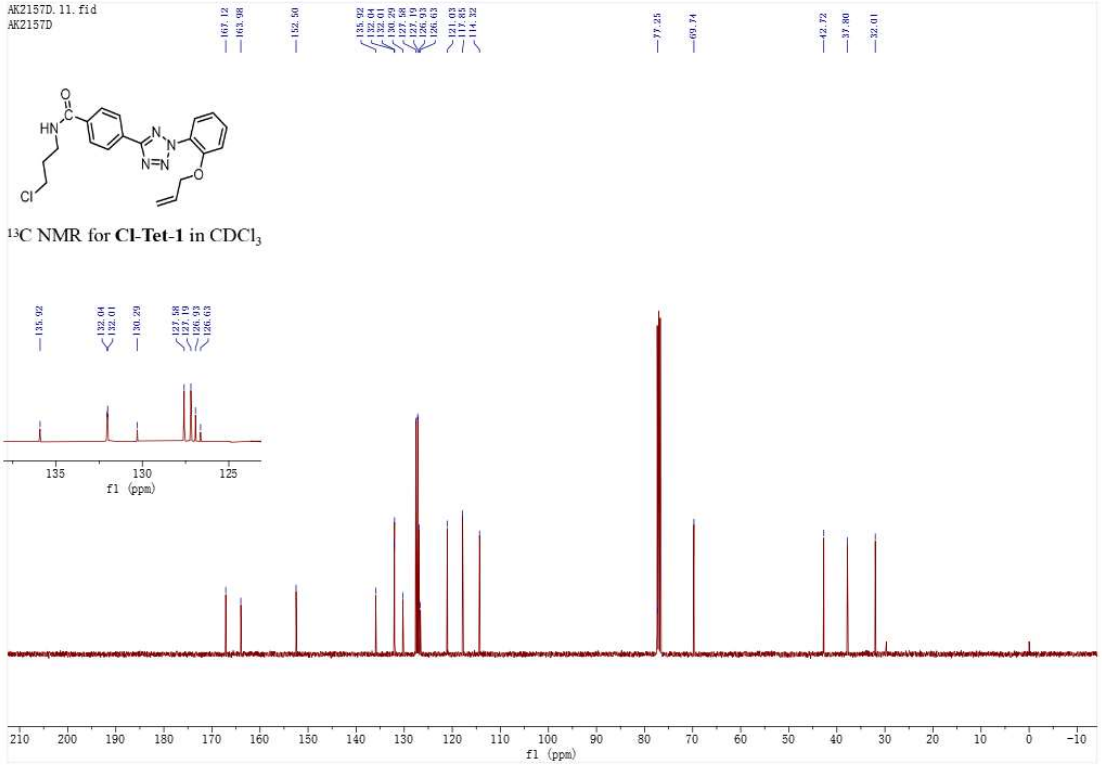
^{1}H NMR for **Mor-Tet-1** in $CDCl_3$



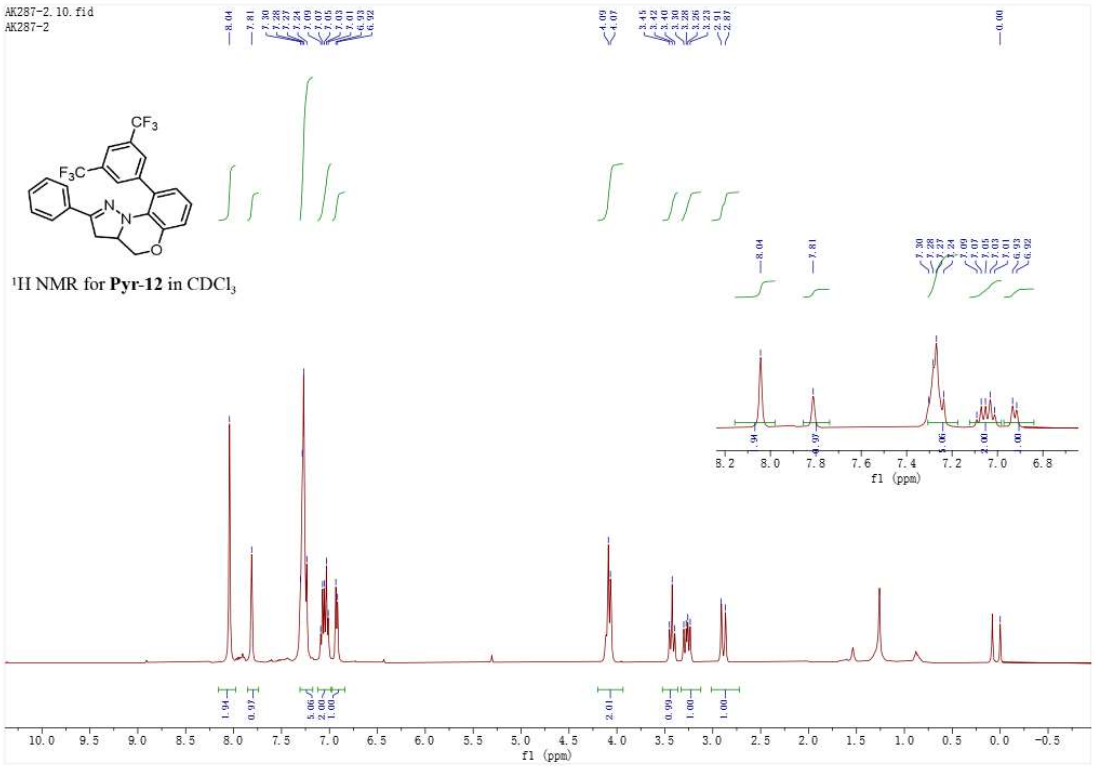
^{13}C NMR for **Mor-Tet-1** in $CDCl_3$



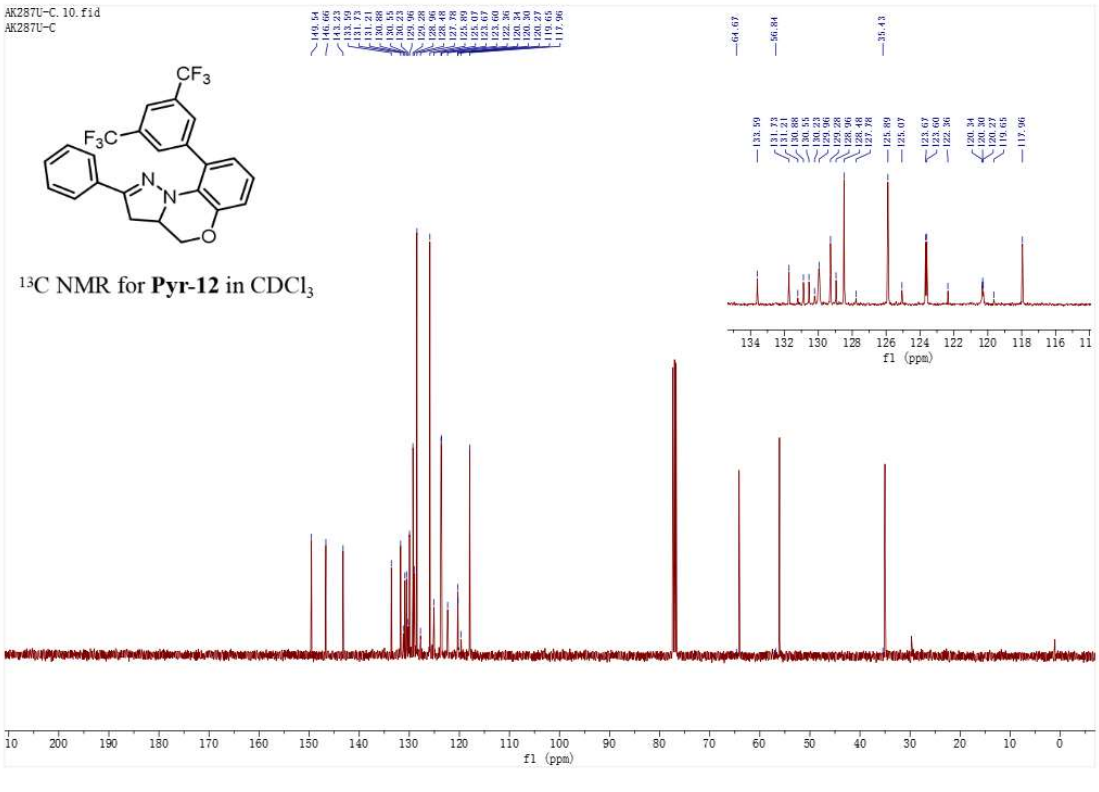
^{1}H NMR for **Cl-Tet-1** in $CDCl_3$



^{13}C NMR for **Cl-Tet-1** in $CDCl_3$



^{1}H NMR for **Pyr-12** in $CDCl_3$



^{13}C NMR for **Pyr-12** in $CDCl_3$