Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

An ultra-sensitive ratiometric fluorescent probe for hypochlorite acid detection by the synergistic effect of AIE and TBET and its application of detecting exogenous/endogenous HOCl in living cells

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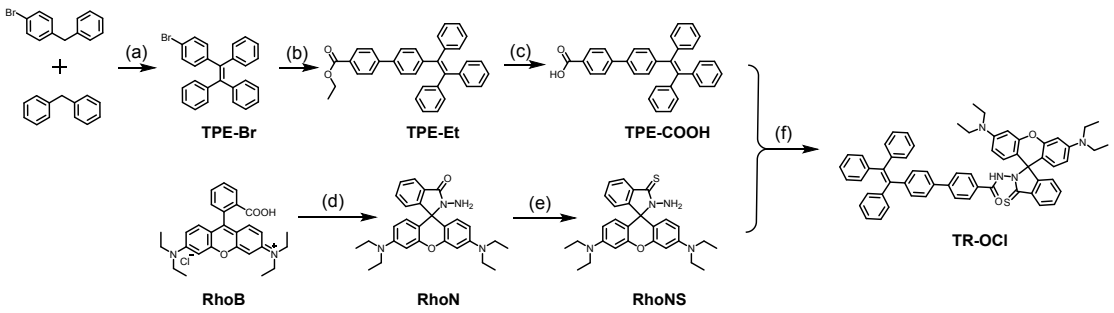
1. Experimental Section

1.1 Materials and chemicals

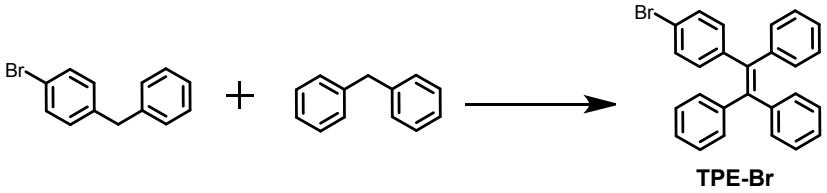
All chemicals used for synthesis were purchased from commercial suppliers and applied directly without purification. Diphenylmethane and 1-benzyl-4-bromobenzene were bought from Meryer. Rhodamine B was purchased from Energy chemical. n-butyllithium, p-toluene-sulphonic acid (p-TSA), 4-ethoxycarbonylphenylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, NaOH, hydrazine hydrate, Lawesson's Reagent, thionyl chloride, Pyridine were obtained from Adamas-beta®. Tetrahydrofuran (THF), toluene, ethanol, and anhydrous dichloromethane (DCM) were purchased from commercial suppliers and used in all reactions as solvent. Dulbecco's modified Eagle's medium (DMEM), PBS, fetal bovine serum (FBS), trypsin-EDTA and penicillin/streptomycin were purchased from Invitrogen. All reactions that utilize air- or moisture sensitive reagents were performed in dried glassware under dry Ar atmosphere. Milli-Q water was used in all experiments. The progress of the reaction was monitored by thin-layer chromatography (TLC; Merck 60F-254). Merck silica gel 60 (70−200 mesh) was used for general column chromatography purification purpose. ^{1}H NMR and ^{13}C NMR spectra were taken on a Bruker 400 MHz NMR spectrometer. For ^{1}H NMR, coupling constants (J) are reported in Hertz (Hz), and multiplicity is indicated as follows: s (singlet), d (doublet), t (triplet), m (multiplet), dd (doublet of doublets), and bs (broad singlet). High-resolution mass spectrometry (HRMS) was performed with Thermo Scientific Q Exactive hybrid quadrupole-orbitrap mass spectrometer. UV absorption spectra were obtained on Shimadzu UV-3600 Plus UV-VIS-NIR Spectrophotometer. Fluorescence spectra were acquired with a FluoroMax-4 fluorescence photometer. Dynamic Light Scattering (DLS) was determined by BI-200SM. Fluorescence images were captured using a ZEISS LSM 800 With Airscan Confocal Laser Scanning Microscope.

1.2 Synthesis and characterization



Scheme S1 The synthetic route for TR-OCl. (a) (i) n-BuLi, THF, (ii) *p*-TSA, toluene; (b) K_2CO_3, $Pd(PPh_3)_4$, Toluene/THF/H_2O, 110 °C, Ar; (c) NaOH, THF/H_2O, 90 °C, Ar; (d)NH_2-$NH_2 \cdot H_2O$, EtOH, 85 °C, reflux; (e) Lawesson's Reagent, Toluene, 110 °C, reflux, Ar; (f) (i) $SOCl_2$, DCM, (ii) Pyridine, 110 °C, Ar.

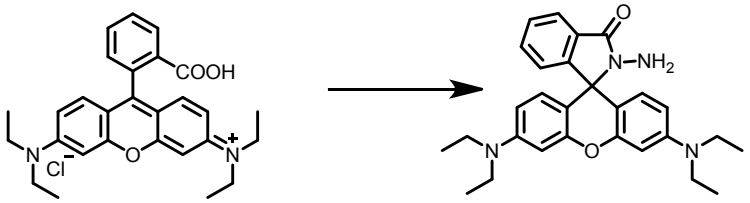
1.2.1 Synthesis of TPE-Br



Diphenylmethane (336 mg, 2.0 mmol) was dissolved in dry THF (10 mL) under Ar atmosphere and cooled at 0 °C. A solution of n-BuLi in hexane (1.2 mL, 3.0 mmol, 2. 5 M) was added by syringe. The mixture was stirred for 30 min. Then, 1-benzyl-4-bromobenzene (390 mg, 1.5 mmol) was added into the reaction solution and stirred for overnight. After that, the aqueous solution of NH_4Cl (10%) was added for quenching the reaction. The solution was extracted by dichloromethane for three times and dried with anhydrous Na_2SO_4. After evaporating the organic solvent, the intermediate product and *p*-toluenesulfonic acid (*p*-TSA) (86 mg, 0.5 mmol) was dissolved in toluene (20 mL) in a reaction flask connected with a Dean-stark trap. The mixture was refluxed for 6 hour and cooled to room temperature. The toluene layer was washed with aqueous $NaHCO_3$ (10%) and dried with anhydrous Na_2SO_4. After removing the organic solvent under vacuum, the crude product was purified by silica gel column chromatography (DCM/hexane=1/3, v/v) to give the compound TPE-Br (345

mg, 42% for two steps). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.17 (m, 4H), 7.15 (m, 5H), 7.07 (m, 8H), and 6.98 (d, J = 8.0 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 143.32, 143.25, 143.15, 142.20, 139.61, 135.21, 132.58, 131.36, 132.31, 129.14, 128.03, 127.97, 127.81, 127.74, 126.95, 126.87, 126.81, 125.19.

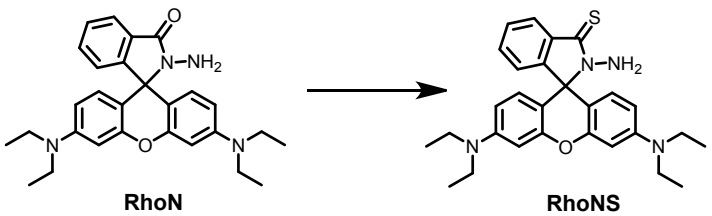
1.2.2 Synthesis of RhoN



RhoB **RhoN**

Rhodamine B (RhoB, 1329 mg, 3.00 mmol) was dissolved in 50 mL ethanol. Hydrazine hydrate (10 mL) was then added at room temperature. The reaction was stirred at 90 °C overnight. The reaction was monitored with TLC till its completion. The mixture was evaporated to remove ethanol. Then, the crude product was purified by column chromatography on silica gel (DCM/hexane= 20/1, v/v) to give the compound RhoN (600 mg, 43%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.93 (m, 1H), 7.45 (m, 2H), 7.10 (m, 1H), 6.46 (d, *J* = 8.8 Hz, 2H), 6.42 (d, *J* = 2.4 Hz, 2H), 6.29 (dd, *J* = 2.5, 8.8 Hz, 2H), 3.61 (s, 2H), 3.34 (q, *J* = 7.0 Hz, 8H), 1.17 (t, *J* = 7.0 Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.16, 153.87, 151.58, 148.91, 132.51, 130.06, 128.11, 123.84, 123.00, 108.06, 104.61, 98.00, 65.92, 44.38, 12.63.

1.2.3 Synthesis of RhoNS

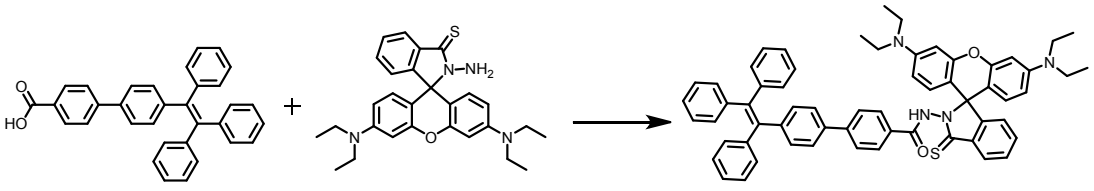


RhoN **RhoNS**

RhoN (456 mg, 1.00 mmol) was dissolved in a degassed toluene (15 mL), Lawesson's Reagent (202 mg, 0.5 mmol) was then placed into the reaction flask under Ar atmosphere. The resulting solution was stirred for 6 hours at 110 ^{o}C under Ar gas. The reaction was monitored with TLC till its completion. The

mixture was evaporated to remove toluene. The crude product was purified by column chromatography on silica gel (pure DCM) to give the compound RhoNS (300 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 6.7 Hz, 1H), 7.47 (m, 2H), 7.11 (d, *J* = 6.5 Hz, 1H), 6.44 (s, 2H), 6.35 (d, *J* = 8.8 Hz, 2H), 6.27 (d, *J* = 8.6 Hz, 2H), 4.82 (s, 2H), 3.34 (q, *J* = 7.0 Hz, 8H), 1.17 (t, *J* = 7.1 Hz, 12H).; ^{13}C NMR (100 MHz, $CDCl_3$) δ: 182.99, 153.50, 149.30, 149.19, 136.39, 132.05, 128.59, 128.10, 124.58, 123.12, 108.11, 103.38, 98.06, 73.31, 53.49, 44.46, 12.60.

1.2.4 Synthesis of TR-OCl



TPE-COOH (226 mg, 0.5 mmol) was added into thionyl chloride (3 mL) in a reaction flask. The reaction was heated at 80 °C and kept stirring for 2 h. After the reaction completed, thionyl chloride was removed under reduced pressure to give a white solid. RhoNS (228 mg, 0.5 mmol) was dissolved in dry pyridine (10 mL), and then the white solid was added to the reaction flask at room temperature under Ar atmosphere. The reaction solution was refluxed for 7 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (DCM/hexane=1/1, v/v) to give a pale-yellow solid (80 mg, 17%). ^{1}H NMR (400 MHz, *d*^{6}-DMSO) δ:10.73 (s, 1H), 8.02 (d, *J* = 6.8 Hz, 1H), 7.62 (m, 6H), 7.50 (d, *J* = 8.3 Hz, 2H), 7.13 (m, 10H), 7.02 (m, 8H), 6.45 (d, *J* = 8.7 Hz, 2H), 6.32 (m, 4H), 3.26 (m, 8H), 1.07 (t, *J* = 6.9 Hz, 12H). ^{13}C NMR (100 MHz, *d*^{6}-DMSO) δ: 170.84, 164.50, 149.09, 143.62, 143.57, 143.55, 142.86, 141.44, 140.44, 137.25, 131.84, 131.74, 131.20, 131.12, 131.10, 130.17, 129.32, 128.94, 128.45, 128.37, 128.28, 127.21, 127.10, 127.05, 126.59, 108.11, 97.47, 60.23, 44.11, 21.24, 14.56, 12.90. ESI-MS m/z calcd. for: $[M+H]^+$: 907.40402 found 907.40363.

1.3 Determination of detection limit

Detection limit or limit of detection was calculated based on the fluorescence titration curve of TR-OCl in the presence of HOCl and mean of the blank. In detail, the fluorescence ratio (I_{589}/I_{477}) of TR-OCl was measured and the standard deviation of blank measurements was achieved and marked as σ using the following equation. σ is the standard deviation of the blank measurements; n is the tested number of blank measure (n= 11); $\bar{x}$ is the mean of the blank measures; X_i is the values of the blank measures.

$$\sigma = \sqrt{\frac{\Sigma(\bar{x} - x_i)^2}{n-1}}$$

In this manuscript, we use the following equation for the calculation of detection limit:

Detection limit = 3σ/s

s is the slope of fluorescence ratio (I_{589}/I_{477}) versus HOCl concentrations.

1.4 Absorption and fluorescence measurement

TR-OCl and other compounds were dissolved in an appropriate amount of DMSO to obtain 1 mM stock solution. NaOCl and other biological analytes were prepared as 1 mM or 10 mM stock solutions in water. All the measurement was taken under room temperature. Firstly, TR-OCl was diluted in CH_3CN/H_2O (4/6, v/v) to afford a final concentration of 5 μM in a 10-mm quartz cuvette. Then, different concentrations of NaOCl or other analytes were added into the quartz cuvette for 1 min incubation respectively. After that, the absorption or fluorescence was measured using Shimadzu 1700 UV/vis Spectrometer and FluoroMax-4 fluorescence photometer respectively. Specially, for the preparation of reactive oxygen species (HO•, $O_2^{\bullet -}$), we followed the protocol from Chang's group. [1] For $ONOO^-$, it was prepared from another literature.[2]

[1] Aaron E. Albers, Voytek S. Okreglak, and Christopher J. Chang, *J. Am. Chem.*

Soc., **2006**, 128, 9640-9641.
[2] Luling Wu, Yang Wang, Maria Weber, Liyuan Liu, Adam C. Sedgwick, Steven D. Bull, Chusen Huang and Tony D. James, *Chem. Commun.*, **2018**, 54, 9953-9956

1.5 MTT assay

B16F10 cells and WI38 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 1% antibiotics (penicillin and streptomycin). Prior to the experiments, the cells were seeded at 1×10^5 cells per well in a 96-well plate, followed by the addition of different concentrations of TR-OCl (0, 5, 10, 25 μM). Subseuquently the medium in each well was removed and the cells were washed with PBS buffer twice. Then, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide solution (MTT, 200 μL, 0.5 mg/mL in medium) was added. The mixture was then incubated for another 4 h. Subsequently, 200 μL of DMSO was added to dissolve the precipitates. Finally, the absorbance of treated cells was measured using the plate reader at 570 nm (A_{570}). All experiments were carried out in quintuplicate. Relative cell viability (%) was determined as a percentage corresponding to the untreated control.

1.6 Cell culture and confocal fluorescence imaging

B16F10 cells and WI38 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics (penicillin and streptomycin). Approximately 1×10^5 cells were seeded in a confocal dish (35 mm). After 24h, the cells were treated with TR-OCl (25 μM) at 37 ^{o}C for 2 hour and then incubated with NaOCl (50 μM) for another 20 min. Cells treated with TR-OCl (25 μM) alone were used as a negative control. Fluorescence images were taken using a ZEISS LSM 800 With Airscan Confocal Laser Scanning Microscope.

2 Supplemental figures and table

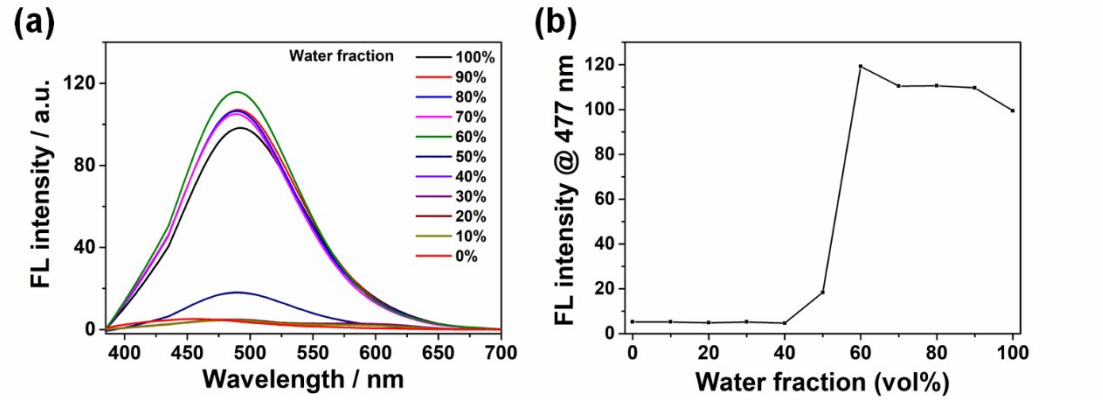


Fig. S1 (A) Fluorescence spectra of TR-OCl (5 μM) at different water fractions; (B) Fluorescence intensity of TR-OCl (5 μM) at 477 nm at different water fraction. Ex: 350 nm.

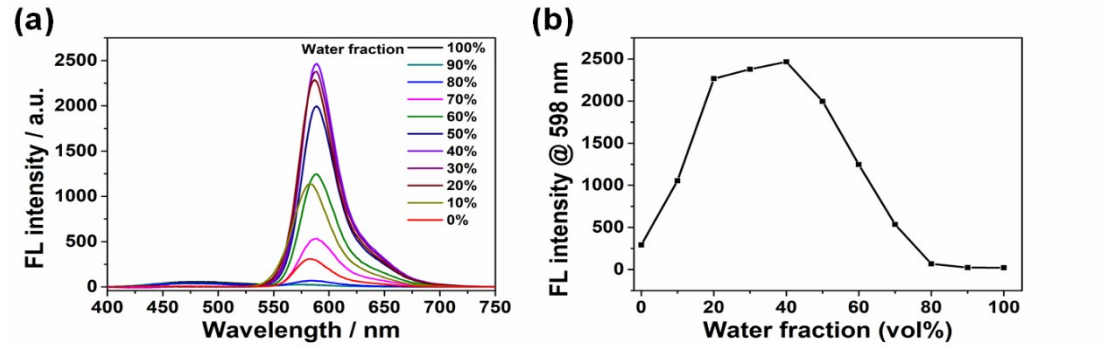


Fig. S2 (A) Fluorescence spectra of TR-OCl (5 μM) and HOCl (100 μM) at different water fractions; (B) Fluorescence intensity of TR--OCl (5 μM) and HOCl (100 μM) at 589 nm at different water fraction. Ex: 350 nm.

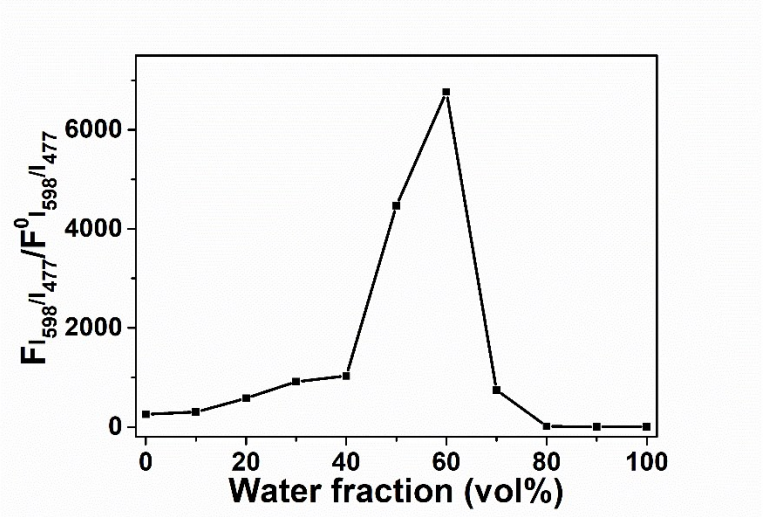


Fig. S3 The fluorescence ratio enhancement of TR-OCl (5 μM) in the presence of HOCl (100 μM) at different water fractions. Ex: 350 nm.

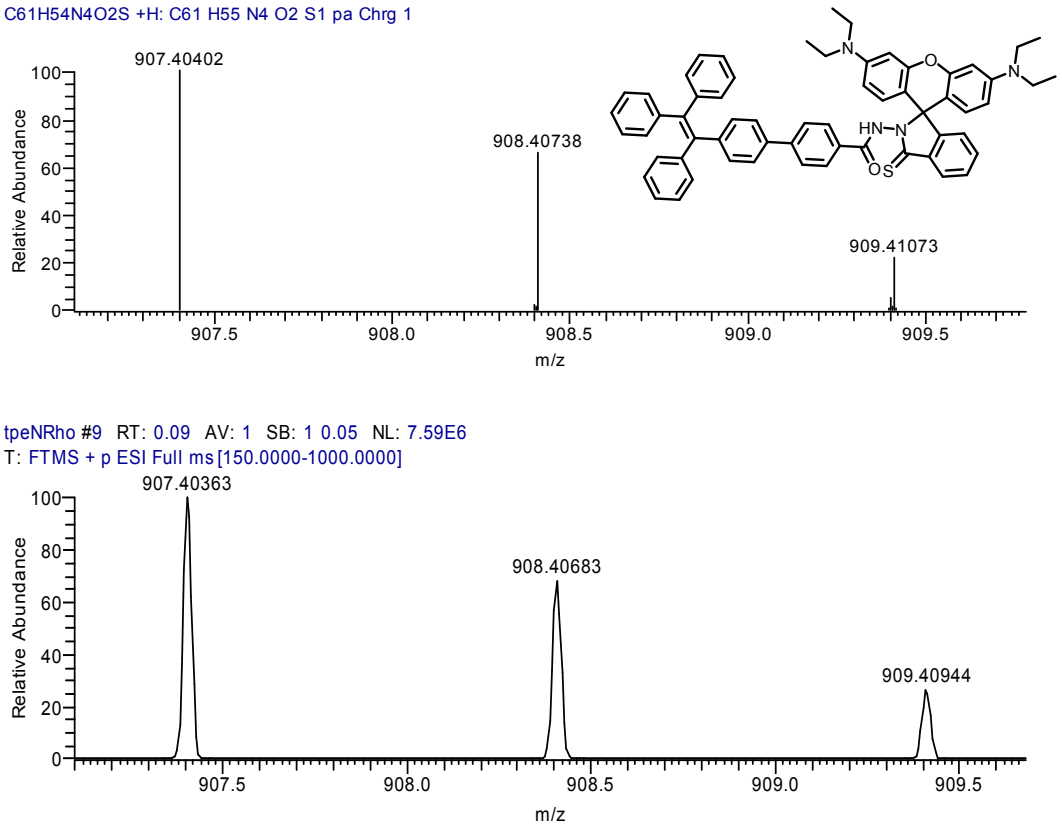


Fig. S4 (A) Calculated mass spectrum of TR-OCl ([M+H]$^+$); (B) HRMS spectrum of TR-OCl ([M+H]$^+$).

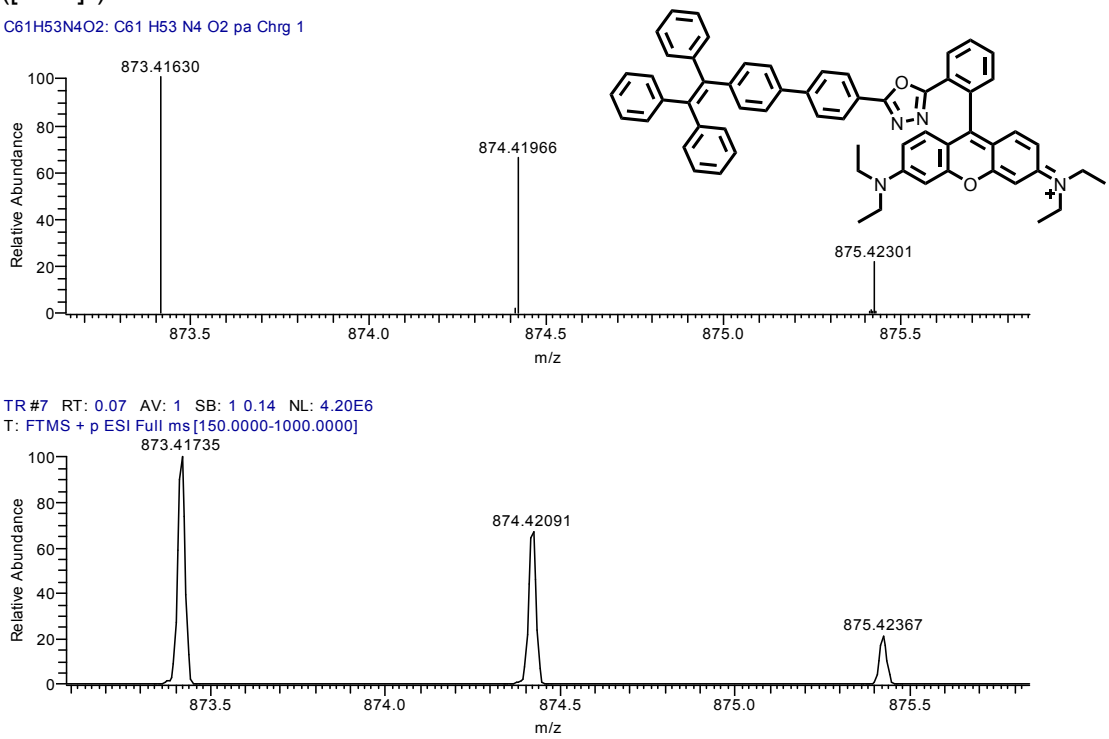


Fig. S5 (A) Calculated mass spectrum of TR-OCl upon addition of HOCl ([M]$^+$); (B) HR-MS spectrum of TR-OCl upon addition of HOCl ([M]$^+$).

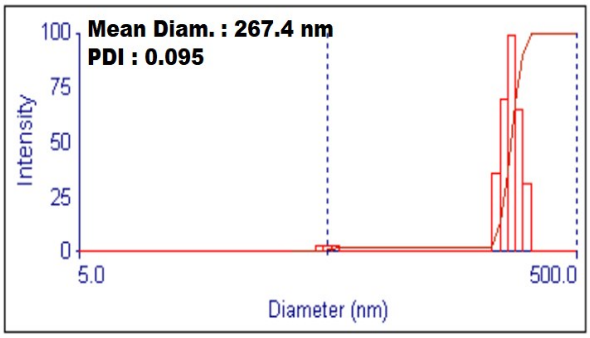


Fig. S6 Particle size of 10 μM TR-OCl measured by DLS in CH_3CN/ H_2O (4/6, v/v).

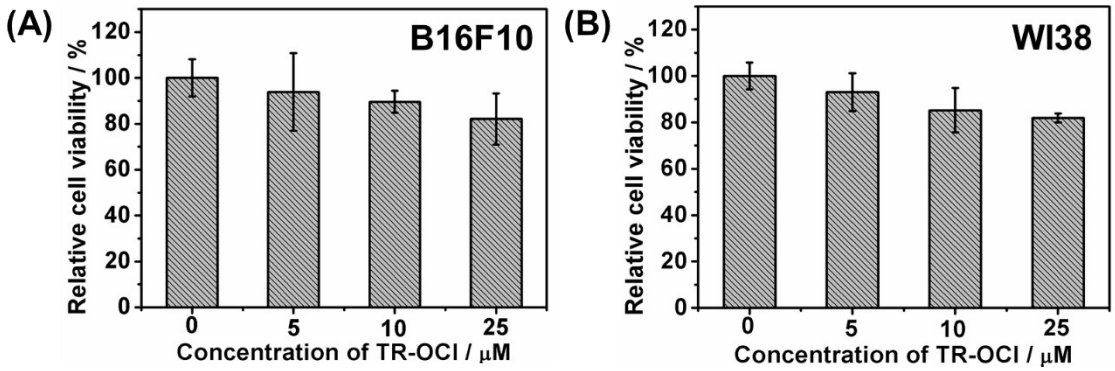


Fig. S7 MTT assay with different concentrations of TR-OCl incubated with B16F10 cells and WI38 cells for 24 h.

Table S1 Comparison of our probe and other fluorescent ratiometric HOCl probes.

Probe	λ_{em}/nm	λ_{ex}/nm	FEF/fold[a]	Detection limit / nM	Reaction time	Imaging application	Type[b]
ThioRB-FITC-MSN Chem. Sci., 2013, 4, 460	490	526↓ 586↑	~	~	~	L929 cells	FRET
Probe 1b Chem. Eur. J. 2012, 18, 2700	414	473↓ 594↑	~	52 (S/N=3)	~	RAW 264.7 Bel 7702 HeLa cells	FRET
Probe 2 Chem. Commun., 2014, 50, 14241	410	470↓ 580↑	25	~	50 s	HeLa cells RAW 264.7 cells	FRET
BRT Anal. Chim. Acta, 2016, 921, 77	525	540↓ 580↑	~	38 (3σ/k)	15 s	RAW 264.7 cells	FRET
DNSRBPH Chin. Sci. Bull, 2011, 56, 3266	370	501↑ 585↑	~	80 (S/N=3)	20 min	HeLa cells	FRET
FL-HA New J. Chem., 2017, 41, 5259	380	455↓ 584↑	~	~	~	HeLa cells RAW 264.7 cells	FRET
RIM Sensor. Actuat. B 2018, 254, 736	370	465↑ 585↑	~	24 (S/N=3)	~	RAW 264.7 cells	FRET
PM Biosens. Bioelectron. 2018, 99, 318	420	444↑ 551↓	~	1.75 (3σ/k)	< 40 s	Zebrafish model	FRET
XWJ Sensor. Actuat. B 2018, 255, 666	370	550↑ 670↓	~	190 (3σ/k)	~	RAW 264.7	FRET
XHZ Talanta 2019, 194, 308	410	470↑ 672↓	91	73 (3σ/k)	~	RAW 264.7 cells	FRET
RIL Sensor. Actuat. B 2018, 263, 252	370	463↓ 588↑	~	27 (S/N=3)	0.2 s	RAW 264.7 cells	FRET
FPD Polym. Chem., 2017, 8, 5795	420	482↑ 576↓	~	5.1 (3σ/k)	30 s	HeLa cells	FRET

Probe	λ_{em}/nm	λ_{ex}/nm	FEF/fold[a]	Detection limit / nM	Reaction time	Imaging application	Type[b]
ZPAC Talanta 2019, 201, 330	410	472↑ 600↓	10	236 (3σ/k)	~	Raw 264.7 cells	FRET
Zcp-Me Sensor. Actuat. B Chem. 2018, 276, 8	420	486↑ 609↓	~	89 (S/N=3)	~	RAW 264.7 cells	FRET
RPM Dyes Pigments 2018, 148, 206	400	462 587↑	~	2080 (3σ/k)	30 s	RAW 264.7 cells	TBET
Naph-Rh Chem. Eur. J. 2015, 21, 19058	350	440↓ 585↑	10	100 (S/N=3)	50 s	RAW 264.7 cells	TBET
RHSNO J. Photoch. Photobio. A 2019, 368, 62	405	467↓ 590↑	260	22.1 (3σ/k)	~	MCF-7 cells	TBET
IRh-Ly Anal. Chim. Acta 2019, 1052, 124	370	462↓ 589↑	~	10.2 Not mentioned	40 s	RAW 264.7 cells	TBET
TR-OCl This work	350	477 ↓ 589 ↑	>7000	1.29	<30 s	B16F10 and WI38 cells	AIE+ TBET

[a] The fluorescence ratio changes before and after incubation with HOCl.

[b] The reported detection limit of corresponding probes. (3σ/k or S/N=3)

~ not mentioned.

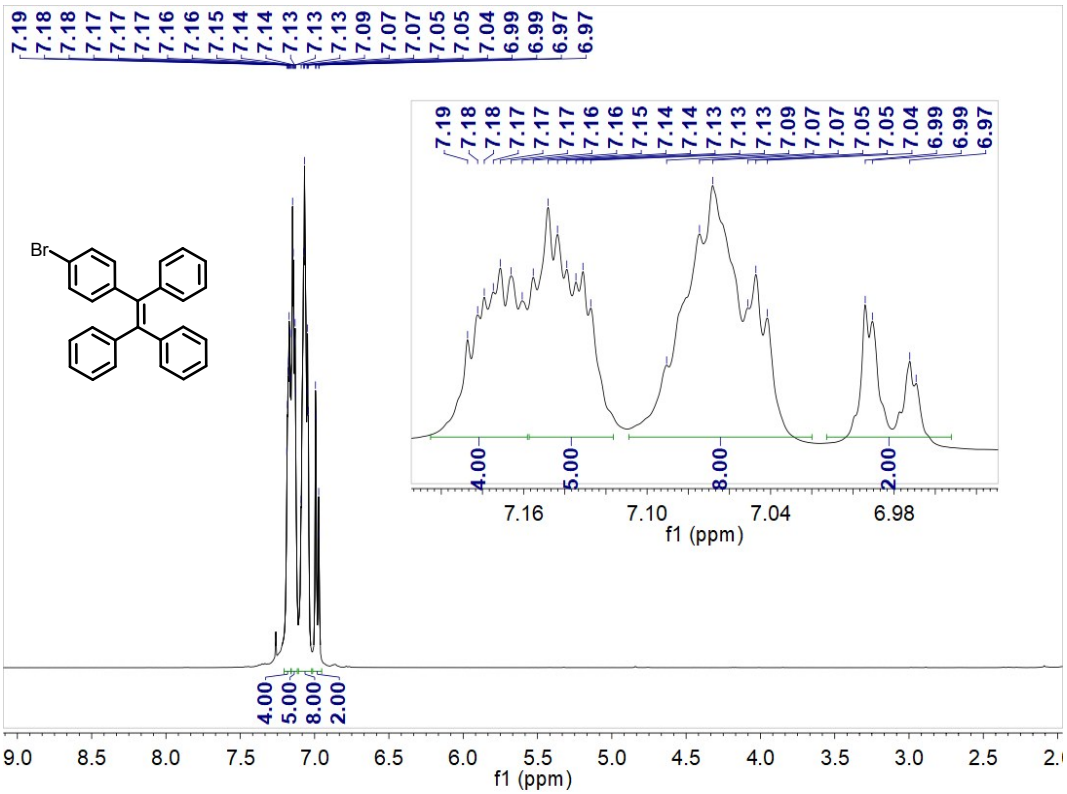


Fig. S8 ^{1}H NMR spectrum of TPE-Br ($CDCl_3$).

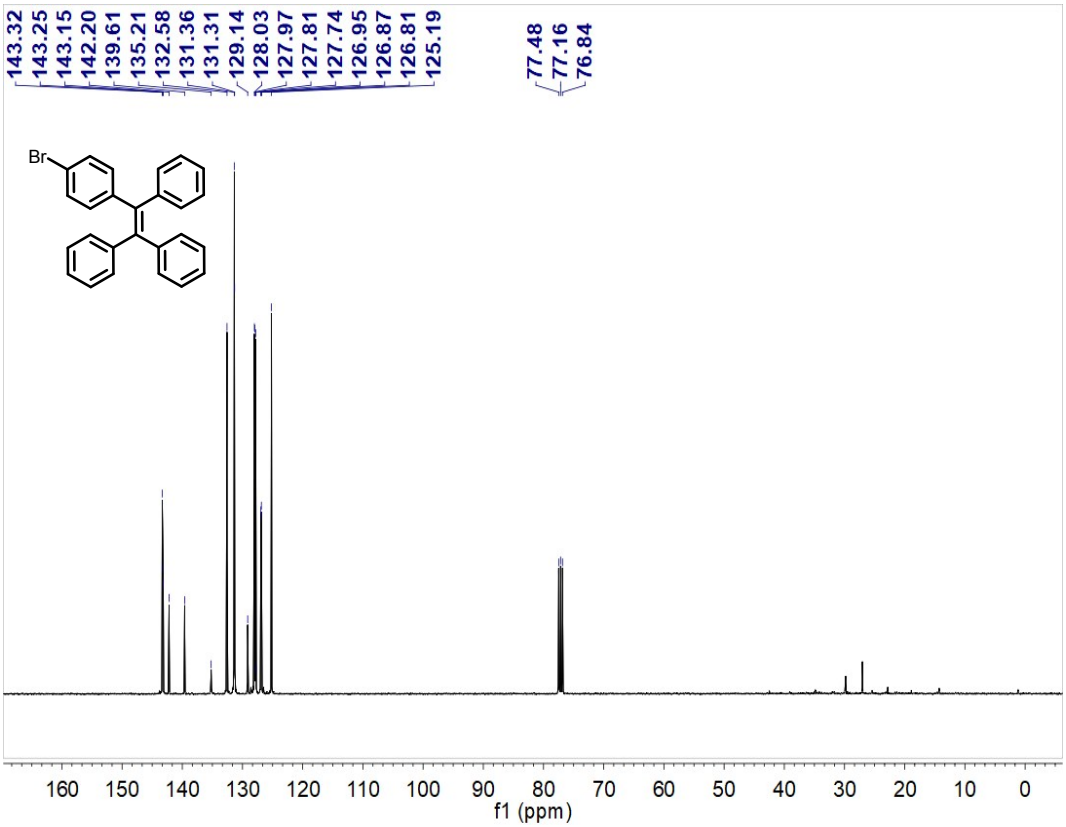


Fig. S9 ^{13}C NMR spectrum of TPE-Br ($CDCl_3$).

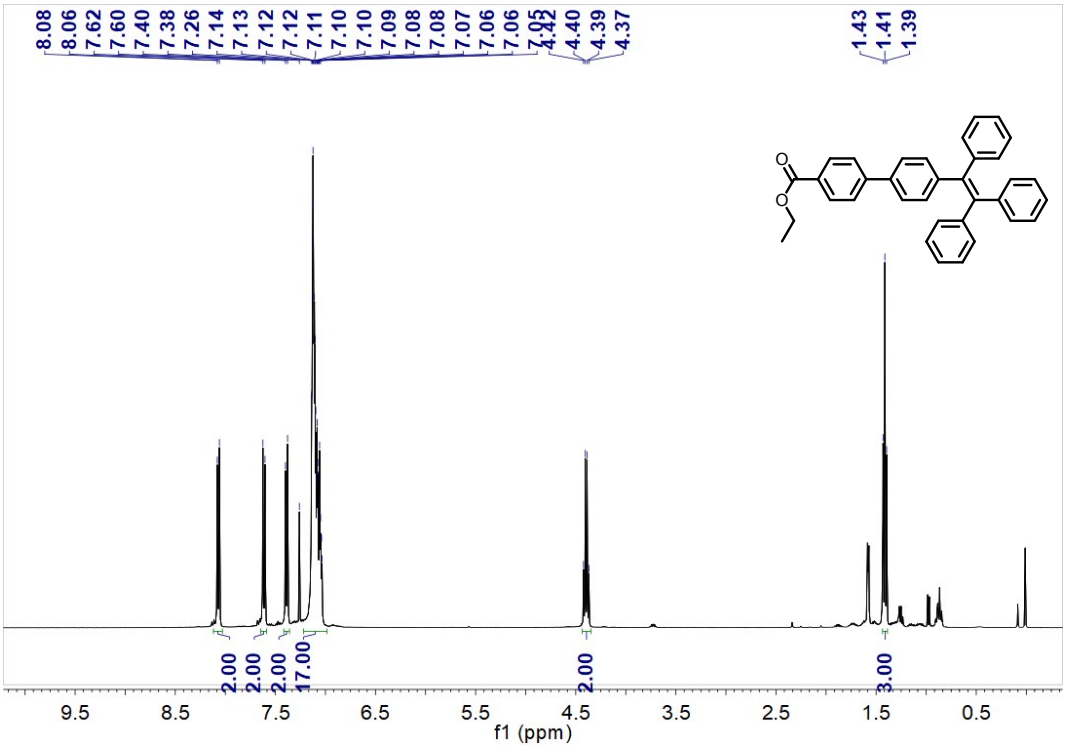


Fig. S10 ^{1}H NMR spectrum of TPE-Et ($CDCl_3$).

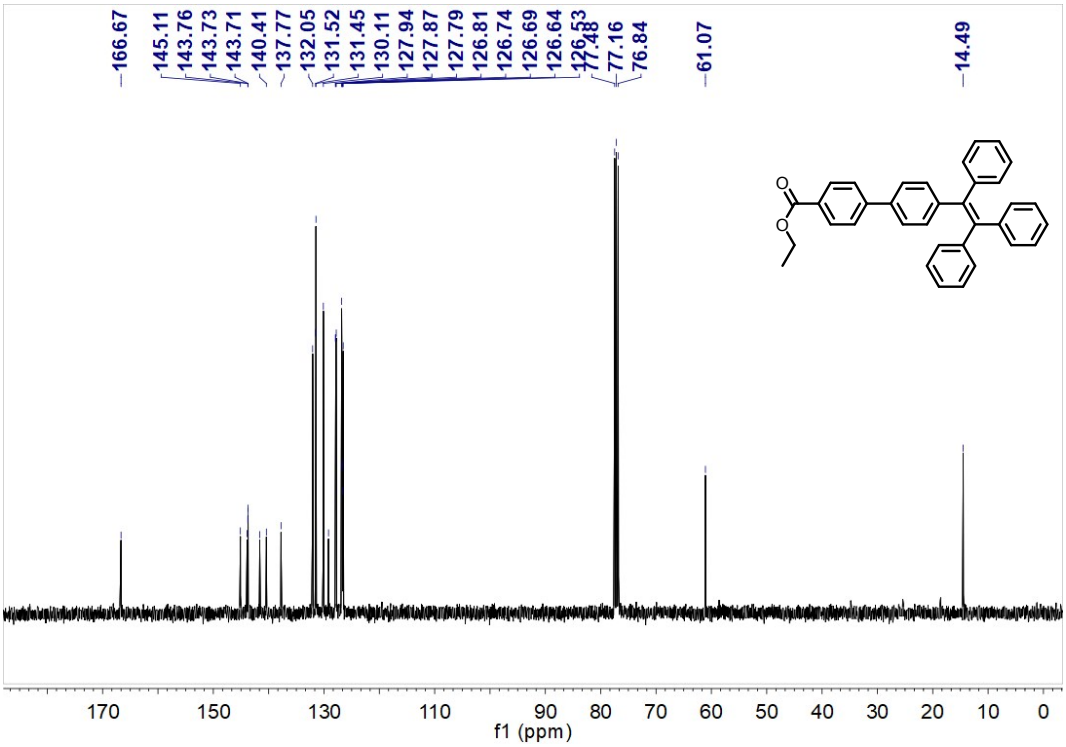


Fig. S11 ^{13}C NMR spectrum of TPE-Et ($CDCl_3$).

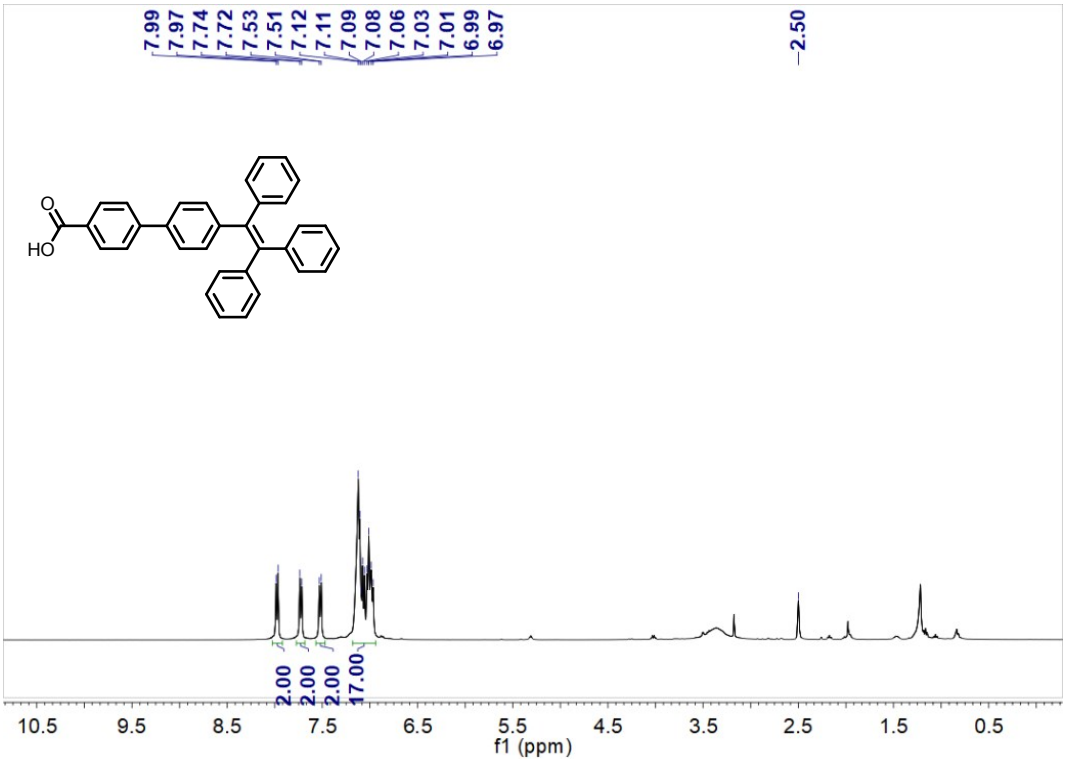


Fig. S12 ^{1}H NMR spectrum of TPE-COOH (d^{6}-DMSO).

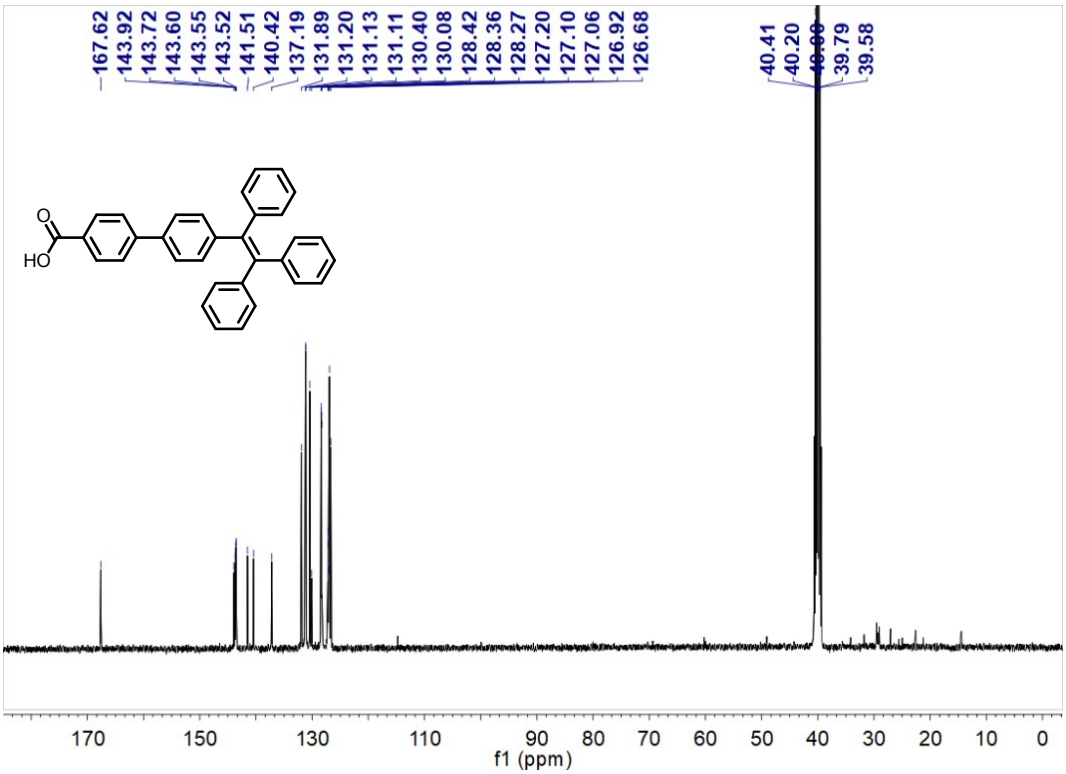


Fig. S13 ^{13}C NMR spectrum of TPE-COOH (d^{6}-DMSO).

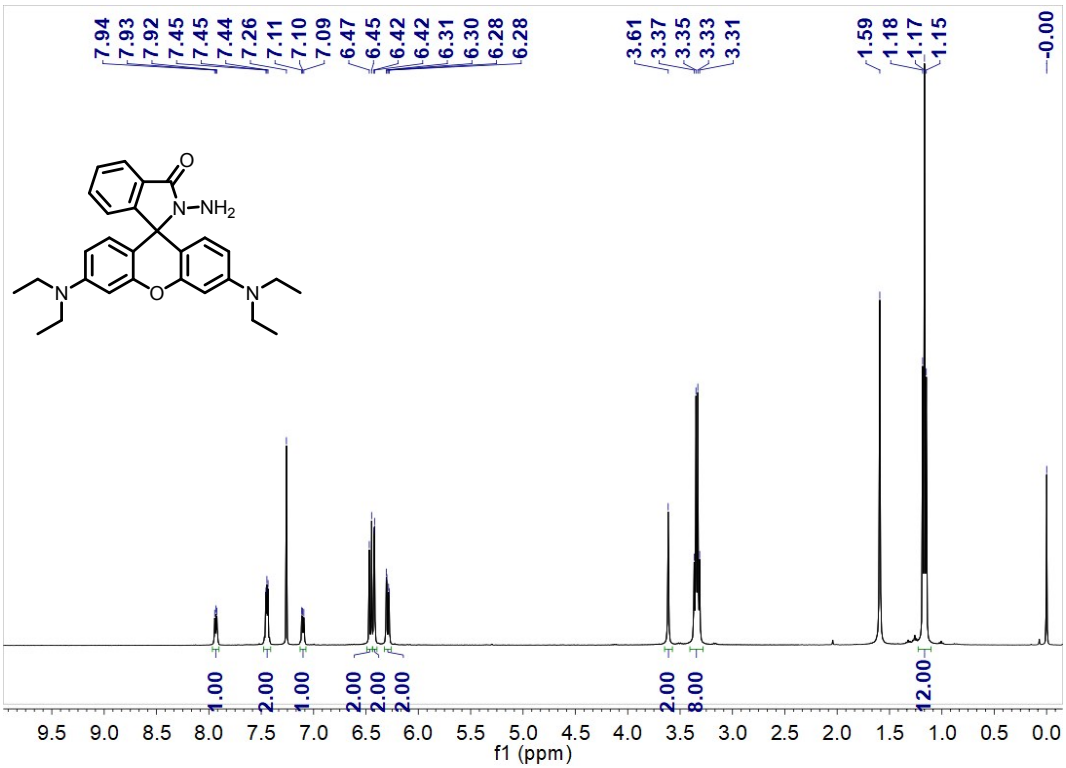


Fig. S14 ^{1}H NMR spectrum of RhoN ($CDCl_3$).

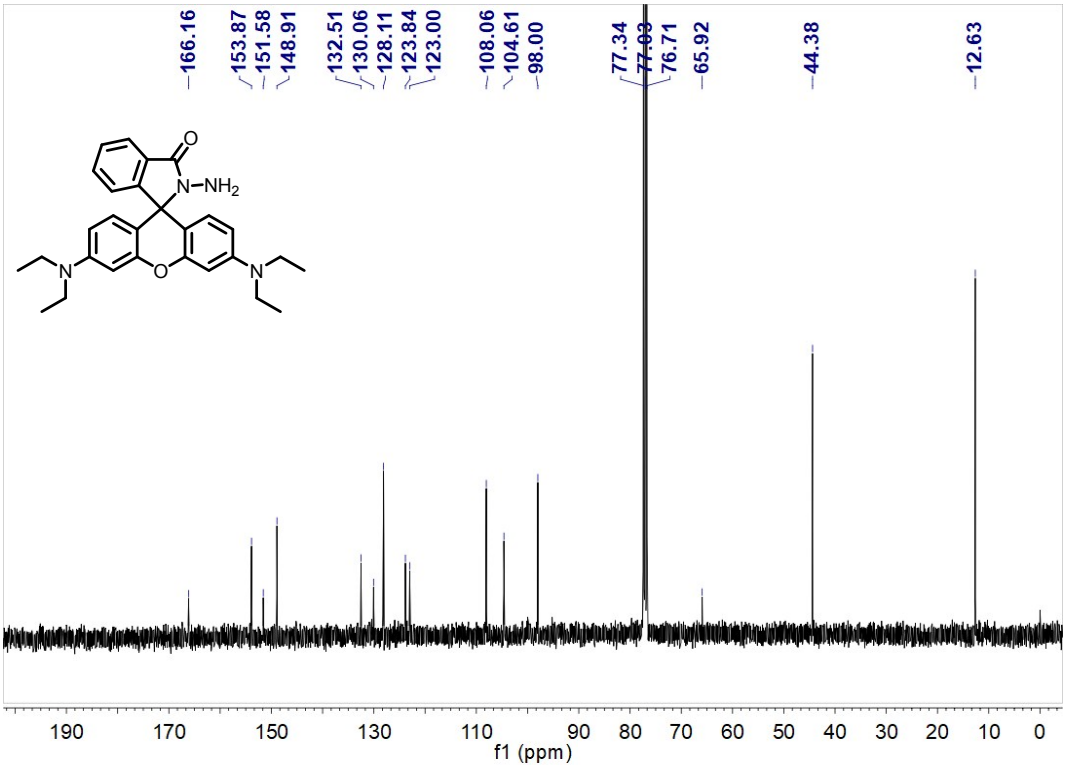


Fig. S15 ^{13}C NMR spectrum of RhoN ($CDCl_3$).

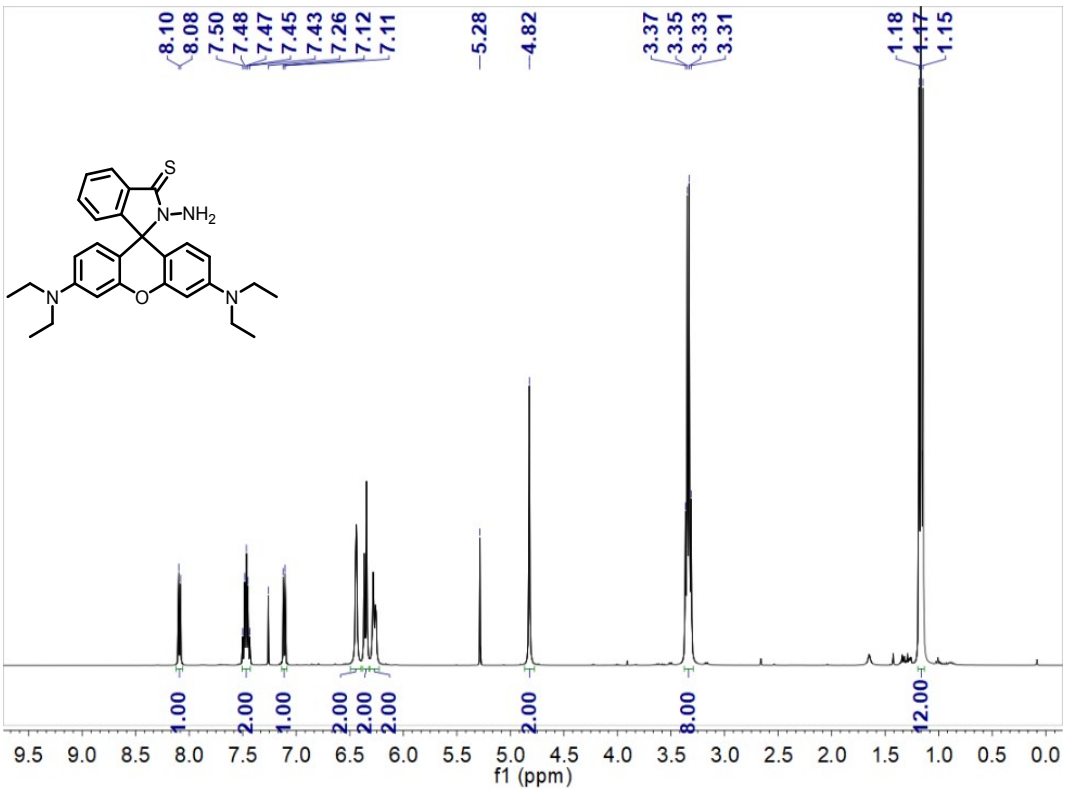


Fig. S16 ^{1}H NMR spectrum of RhoS ($CDCl_3$).

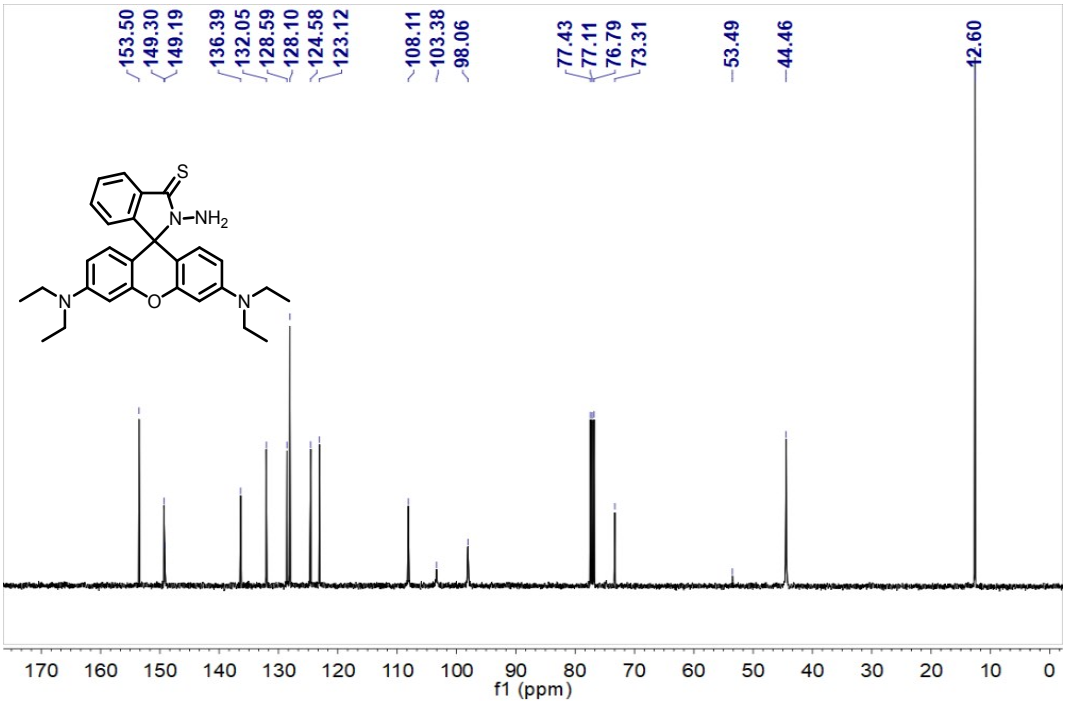


Fig. S17 ^{13}C NMR spectrum of RhoS ($CDCl_3$).

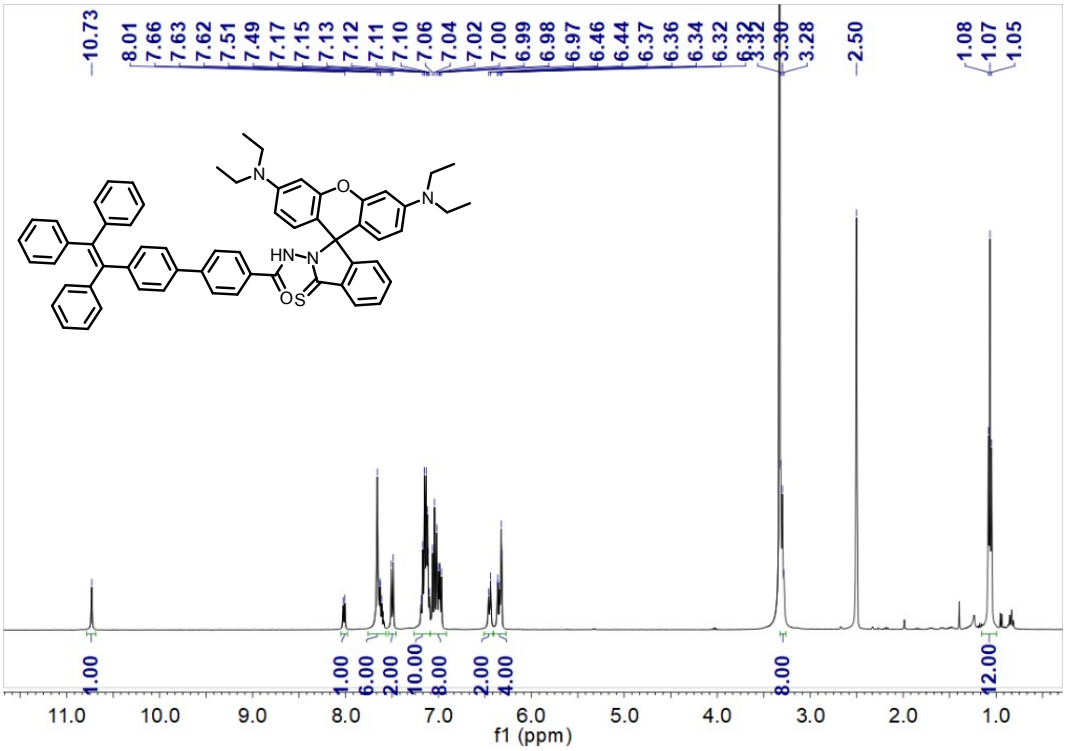


Fig. S18 ^{1}H NMR spectrum of TR-OCl (*d*^{6}-DMSO).

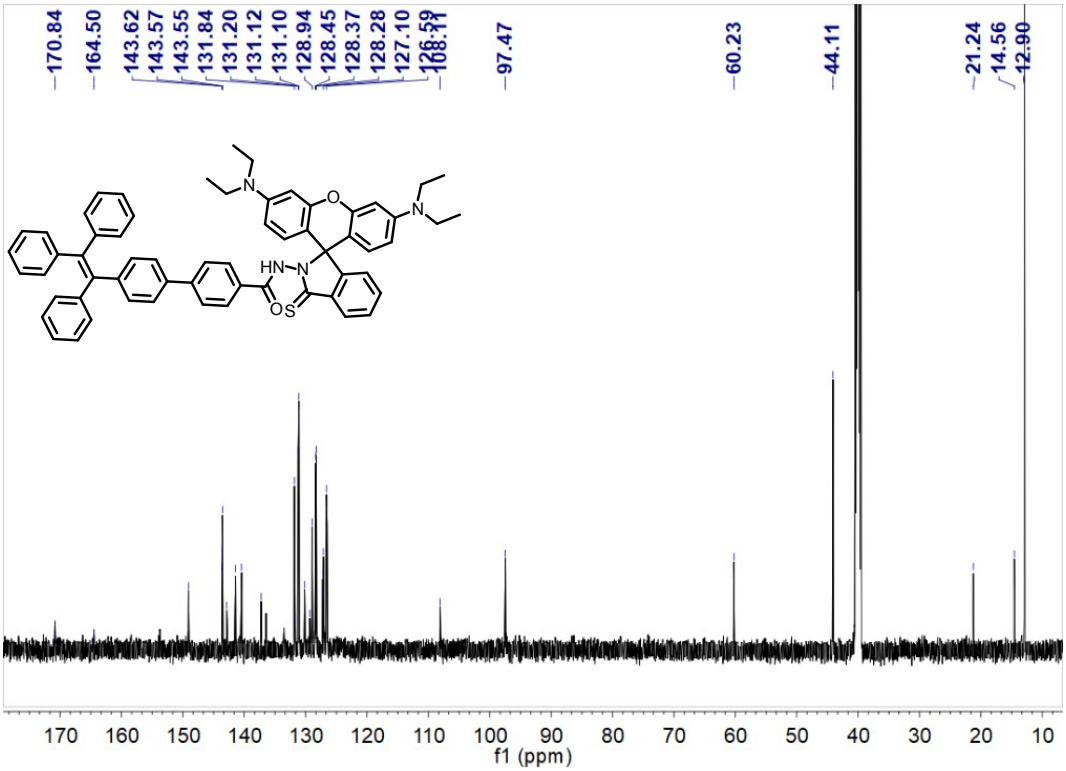


Fig. S19 ^{13}C NMR spectrum of TR-OCl (*d*^{6}-DMSO).