Supporting Information

A Zinc (II)-Tetradentate-Coordinated Probe with Aggregation-Induced Emission Characteristics for Selective Imaging and Photoinactivation of Bacteria

Guangxue Feng,#[a] Chong-jing Zhang,#[a] Xianmao Lu,[a] and Bin Liu*[ab]

a Department of Chemical and Biomolecular Engineering, National University of Singapore, 4 Engineering Drive 4, Singapore, 117585

b Institute of Materials Research and Engineering, Agency for Science, Technology and Research, 3 Research Link, Singapore, 117602

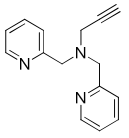
# These authors contribute equally to this work.

* Corresponding author (E-mail: cheliub@nus.edu.sg)

General information

All the chemicals were purchased from commercial vendor and used directly without further purification. Dry dichloromethane (DCM) were distilled over CaH_2. All spectra of ^1H NMR and ^{13}C NMR were recorded on a Bruker ACF-400 MHz NMR spectrometer with $CDCl_3$ or $(CD_3)_2SO$ (DMSO-d_6) as the solvent. Chemical shifts are described in parts per million which is referenced according to residual solvent (7.26 ppm for $CDCl_3$ and 2.50 ppm for DMSO-d_6) for 1 H NMR, (77.0 ppm for CDCl 3 and 40.0 ppm for DMSO-d_6) for 13 C NMR. High resolution mass (HRMS) was recorded in Mass spectra were recorded the AmaZon X LC-MS for ESI. UV-vis absorption spectra were taken on a Shimadzu Model UV-1700 spectrometer. Photoluminescence (PL) spectra were measured on a Perkin-Elmer LS 55 spectrofluorometer.

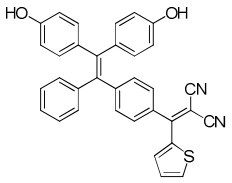
Synthesis of 1



(1)

To the solution of bis(pyridin-2-ylmethyl)amine (750 mg, 3.7 mmol) and 3-bromoprop-1-yne (0.55 mL, 80% (w/w, in toluene), 3.9 mmol) in acetonitrile (10 mL) was added potassium carbonate (1.0 g, 7.5 mmol). The reaction mixture was stirred at room temperature for 12 h. Then the mixture was filtered and the filtrate was concentrated and purified with chromatography to give the product as dark oil (290 mg, 33.0%). ^1H NMR (400 MHz, $CDCl_3$) δ 8.52 (m, 2H), 7.62 (dt, J_1 = 1.2 Hz, J_2 = 7.6 Hz, 2H), 7.47 (d, J = 7.6 Hz, 2H), 7.09-7.13 (m, 2H), 3.87 (s, 4H), 3.36 (d, J = 2.4 Hz, 2H), 2.26 (t, J = 2.4 Hz, 1H); 158.6, 149.1, 136.4, 123.1, 122.0, 78.2, 73.5, 59.3, 42.3; MS (ESI) calcd for $[M+Na]^+$: 260.11, found: 260.12.

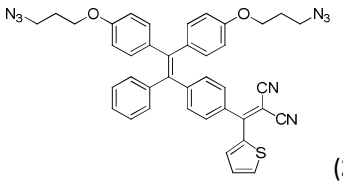
Synthesis of TPETH-2OH



(**TPETH-2OH**)

To the solution of **1** (225 mg, 0.41 mmol) in dry DCM (10 mL) was added boron tribromide (1.0 M in dichloromethane, 1.2 mL) at 0 ^{o}C. Then the reaction mixture was stirred for 3 h at room temperature. The reaction was quenched under ice-water bath by addition of water (10 mL). The organic layer was taken, washed with brine (20 mL), dried by $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography (hexane/ethyl acetate = 10/1—3/1) to give the desired product as red solid (190 mg, 88.8% yield). ^{1}H NMR (400 MHz, $CDCl_3$/MDOD = 10:1) δ 7.79 (dd, J_1 = 1.2 Hz, J_2 = 4.8 Hz, 1H), 7.67 (dd, J_1 = 1.2 Hz, J_2 = 4.0 Hz, 1H), 7.19 (dd, J_1 = 4.0 Hz, J_2 = 4.8 Hz, 1H), 7.07-7.13 (m, 7H), 7.78-7.85 (m, 4H), 6.51-6.54 (m, 4H); ^{13}C NMR (100 MHz, $CDCl_3$/MDOD = 10:1) δ 165.4, 155.7, 155.4, 149.1, 143.4, 143.0, 138.5, 136.9, 136.4, 136.1, 134.6, 134.5, 133.2, 132.7, 132.6, 131.4, 131.2, 129.0, 128.8, 127.8, 126.2, 114.6, 114.4, 113.7, 109.9; HRMS (ESI) calcd for $[M+Na]^+$: 545.1294, found: 545.1299.

Synthesis of 2



(**2**)

To the solution of compound 1 (102 mg, 0.2 mmol) in acetonitrile (8 mL) was added 3-azidopropyl 4-methylbenzenesulfonate (120 mg, 0.47 mmol) and potassium carbonate (138 mg, 1.0 mmol). Then the resulting mixture was stirred at 60 ^{o}C for 12 h. Then the reaction mixture was cooled down to room temperature and filtered to remove the un-dissolved solid. The filtrate was then concentrated and purified with chromatography (Hexane/EA = 50/1-5/1) to give the desired product as red solid (80 mg, 58.0%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.17 (dd, J_1 = 1.2 Hz, J_2 = 4.8 Hz), 7.78 (dd, J_1 = 1.2 Hz, J_2 = 4.0 Hz), 7.35-7.39 (m, 3H), 7.12-7.24 (m, 7H), 6.96-7.03 (m, 4H), 6.74-6.78 (m, 4H), 4.08 (m, 4H), 3.58 (m, 4H), 2.02-2.08 (m, 4H). ^{13}C NMR (100 MHz, Acetone-*d6*) δ 166.1, 159.5, 159.4, 145.1, 138.3, 138.0, 137.3, 134.2, 134.0, 132.9, 132.7 130.8, 130.6, 129.6, 128.1, 116.2, 115.5, 115.4, 115.3, 79.0, 66.2, 66.1, 49.6.

Preparation of bacterial solutions

A single colony of *B. subtilis* (Gram-positive) or *E. coli* (Gram-negative) on a solid Luria Broth (LB) agar plate was transferred to 5 mL of liquid LB culture medium and grown at 37 ^{o}C overnight. The bacteria were then harvested by centrifuging (5000 rpm for 5 min) and washed with phosphate buffer saline (1×PBS, 10 mM, pH = 7.4) for three times. The supernatant was discarded and the remaining bacteria were suspended in 1×PBS or 0.85% NaCl and diluted to desired density based on OD600 1.0 $\approx$ 8×10^8 CFU/mL.

Cell Culture

HeLa cancer cells originated from Human cervix carcinoma were provided by American Type Culture Collection (ATCC). HeLa cells were cultured in DMEM (Invitrogen, Carlsbad, CA) containing 10% heat-inactivated FBS (Invitrogen), 100 U/mL penicillin, and 100 μg/mL streptomycin (Thermo Scientific). The cells were maintained in a humidified incubator at 37 °C with 5% CO_2. Before experiments, the cells were pre-cultured until confluence was reached.

Binding constant determination

A series of 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) buffer solutions (50 mM, 0.1 M KNO_3, pH 7.20) containing diverse amounts of $ZnSO_4$ (0 ~ 9.5 mM) and of EGTA (ethylenebis(oxyethylenenitrilo) tetraacetic acid) (10 mM) were prepared. The free concentration of Zn^{2+} ($[Zn^{2+}]_{free}$) was calculated with $[Zn^{2+}]_{total}$, $[EGTA]_{total}$, and $K_{Zn\text{-}EGTA}$. $K_{Zn\text{-}EGTA}$ is the apparent binding constant and is 3.80×10^8 M^{-1} when pH is 7.20 and ionic strength is 0.1 M. $K_{Zn\text{-}EGTA}$ value was calculated from Equation 1.

$$K_{Zn-EGTA} = \frac{[Zn-EGTA]}{[Zn]_{free}[EGTA]_{free}} \qquad \text{Equation1}$$

When $[Zn^{2+}]_{total}$ and $[EGTA]_{total}$ were used, the Eq.1 can also be as Equation 2

$$K_{Zn-EGTA} = \frac{[Zn]_{toatal}-[Zn]_{free}}{[Zn]_{free}([EGTA]_{total}-[Zn]_{total}+[Zn]_{free})} \qquad \text{Equation 2}$$

In Eq.2, $[EGTA]_{total}$, and $K_{Zn\text{-}EGTA}$ are known value so that the $[Zn^{2+}]_{free}$ could be obtained when different total amounts of $ZnSO_4$ were used. The calculated $[Zn^{2+}]_{free}$ is determined as below:

$[Zn]_{total}$ (mM)	0.5	1	2	3	4	5	6	7	8	9	9.5
$[Zn]_{free}$ (nM)	0.14	0.29	0.66	1.1	1.8	2.6	4.0	6.1	11	24	50

The fluorescent intensities at 630 nm in the emission spectrum of each solution was measured with the excitation wavelength at 440 nm and was fitted to the following equation (Equation 3).

$$R = (R_{min}K_d + R_{max}[Zn^{2+}])/(K_d+[Zn^{2+}]) \qquad \text{Equation 3}$$

$^1O_2$ quantum yield measurement

The $^1O_2$ quantum yield (η) of **TPETH-2Zn** under light illumination was measured using ABDA as the indicator, and Rose Bengal (RB) as the standard reference. To conduct the experiment, ABDA is added into **TPETH-2Zn** or RB aqueous solution to make a working concentration of 10 μM. The mixture is exposed to white light (400-800nm, 100 $mWcm^{-2}$) for designated time. The degradation of ABDA was access by the means of its absorbance changes at 378 nm. And the $^1O_2$ quantum yield is calculated using the following equation:

$^1O_2$ η of **TPETH-2Zn**: $\eta_{AIE} = \eta_{RB}\frac{K_{AIE}A_{RB}}{K_{RB}A_{AIE}}$ Equation 4

where K_{AIE} and K_{RB} represent the decomposition rate constants of the photosensitizing process determined by the plot $\ln(Abs_0/Abs)$ versus irradiation time, where Abs_0 is the initial absorbance of ABDA, Abs is the ABDA absorbance at different irradiation time. A_{RB} and A_{AIE} refer to the light absorbed by RB and **TPETH-2Zn**, respectively, calculated by the integration of their absorption spectra from 400 to 800 nm, η_{RB} is the $^1O_2$ η of RB, which is 75%.

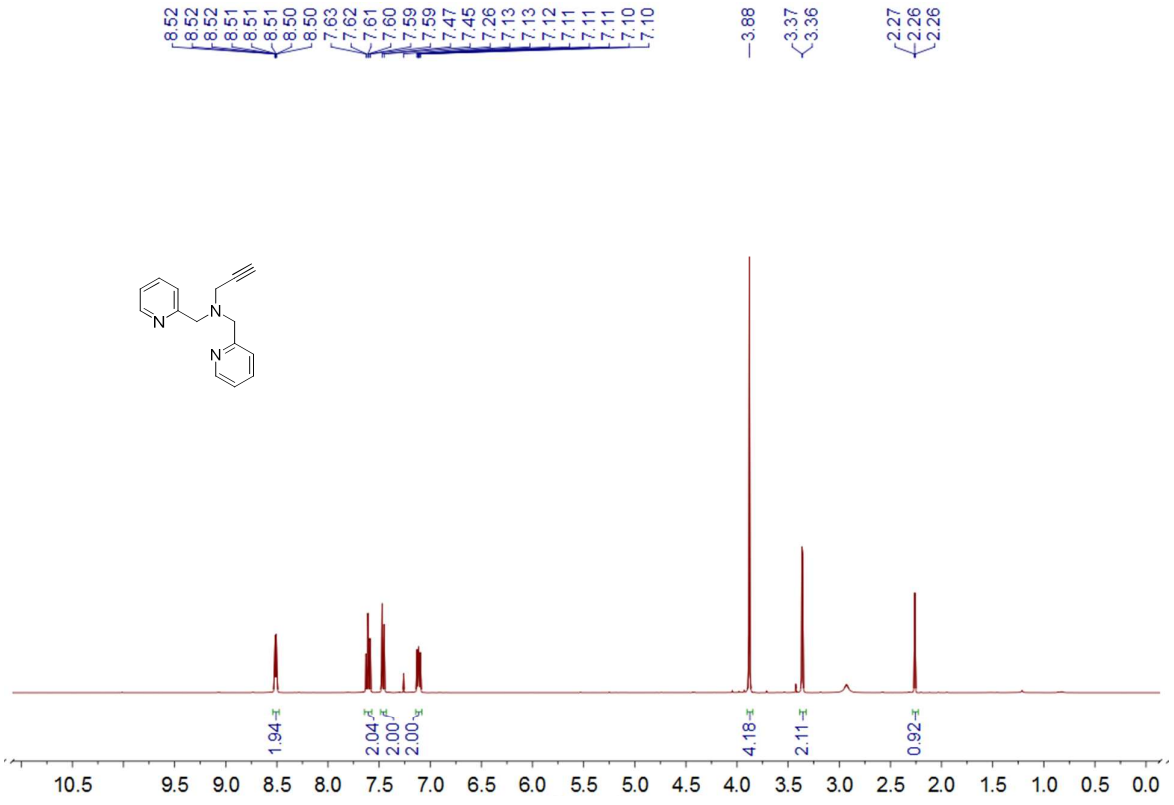


Figure S1 ^{1}H NMR spectra of compound **1** in $CDCl_3$.

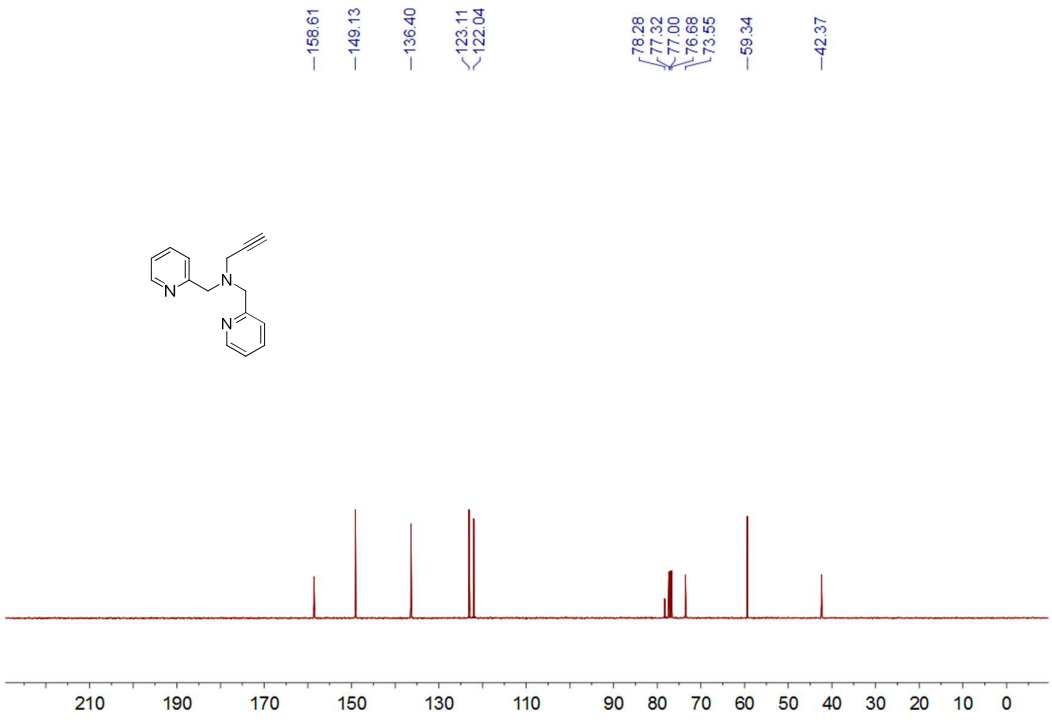
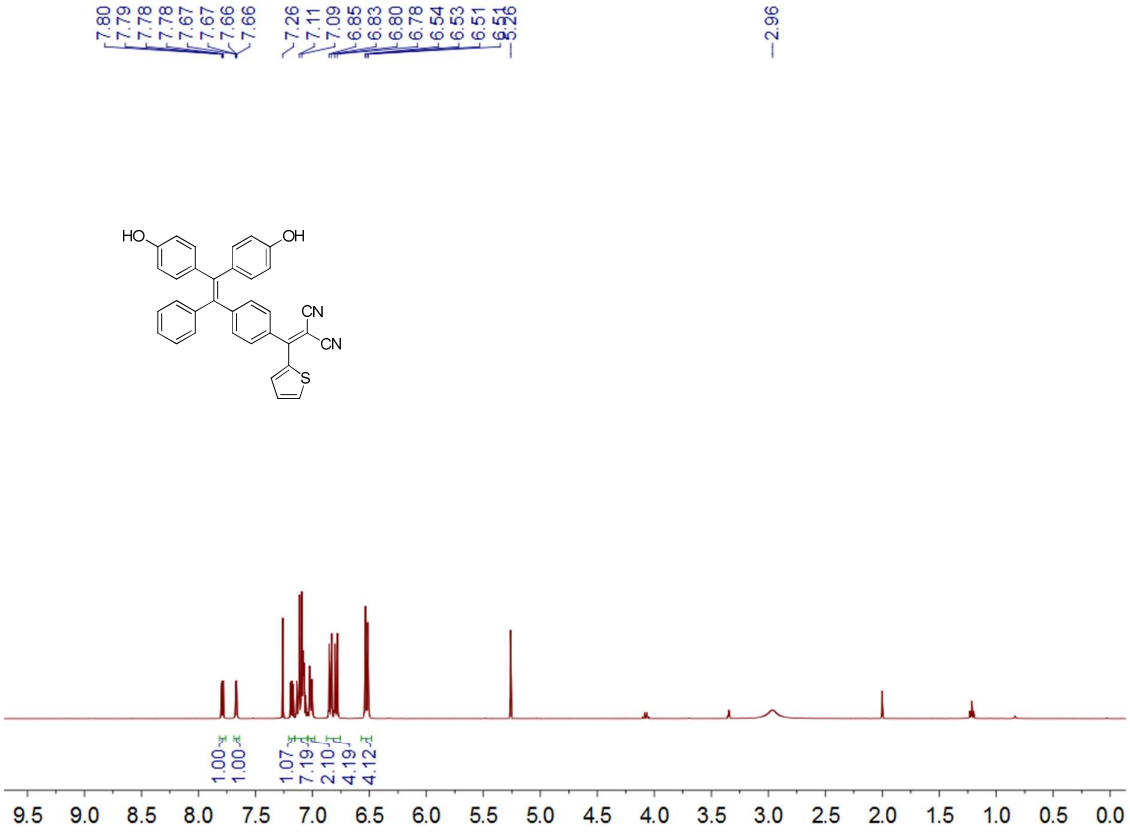
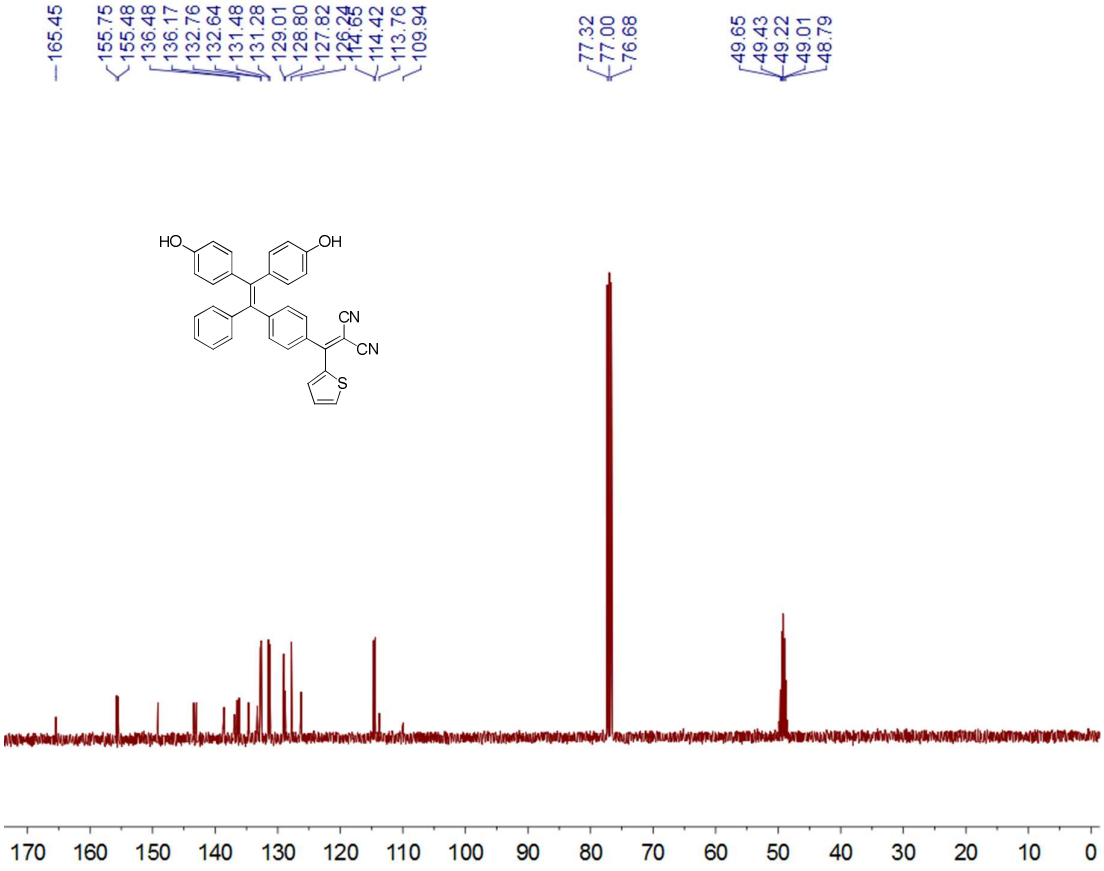


Figure S2 ^{13}C NMR spectra of compound **1** in $CDCl_3$.



Chemical shift (ppm)

Figure S3 ^{1}H NMR spectra of compound **TPETH-2OH** in $CDCl_3$ and MeOD.



Chemical shift (ppm)

Figure S4 ^{13}C NMR spectra of compound **TPETH-2OH** in $CDCl_3$ and MeOD.

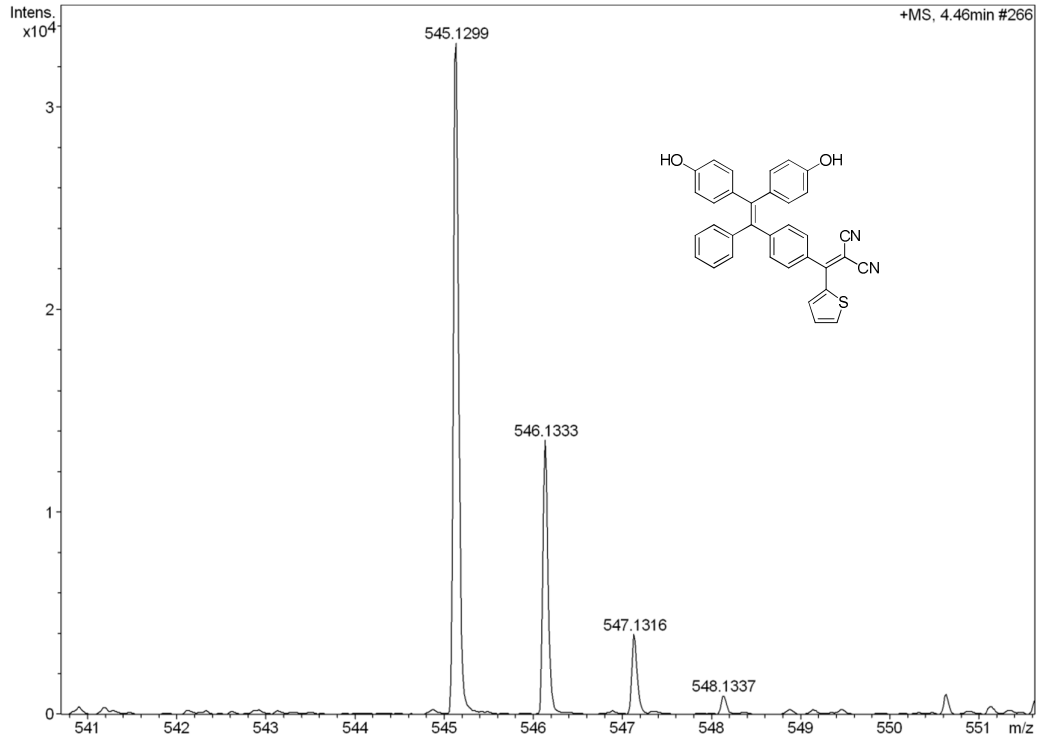


Figure S5 High Resolution mass spectroscopies (ESI) of **TPECM-2OH**

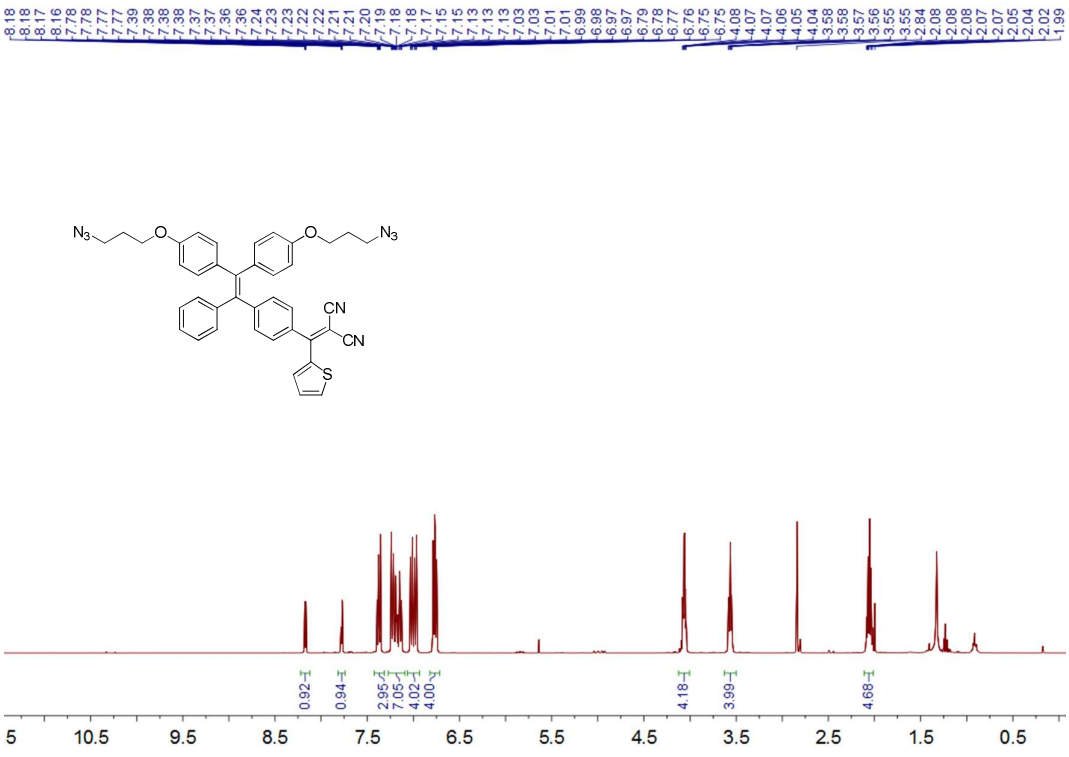


Figure S6 ^{1}H NMR spectra of compound **2** in $CDCl_3$

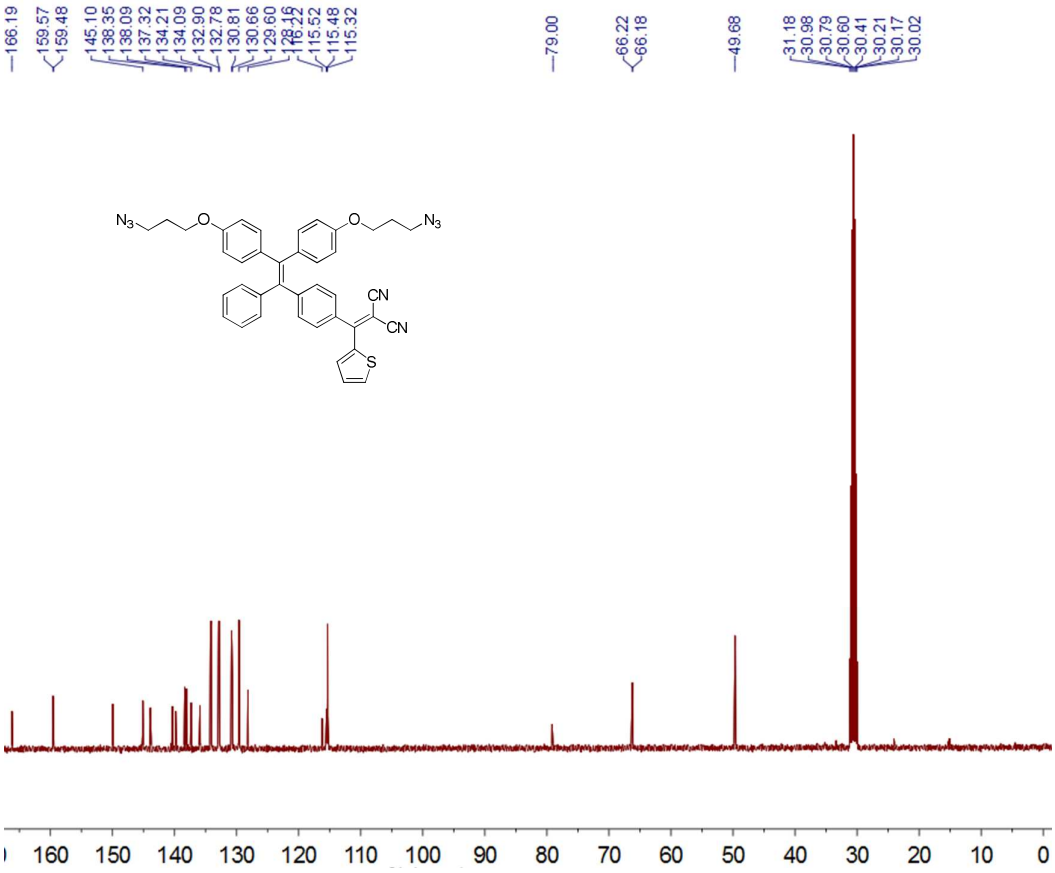


Figure S7 ^{13}C NMR spectra of compound **2** in $CDCl_3$

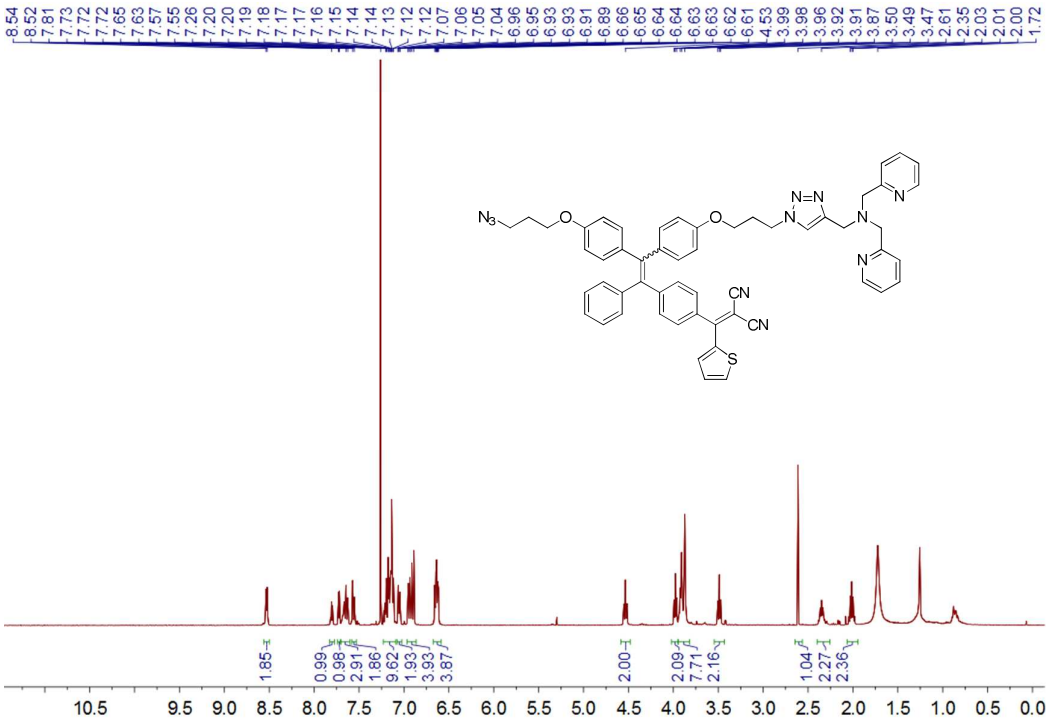


Figure S8 ^{1}H NMR spectra of compound **3** in $CDCl_3$

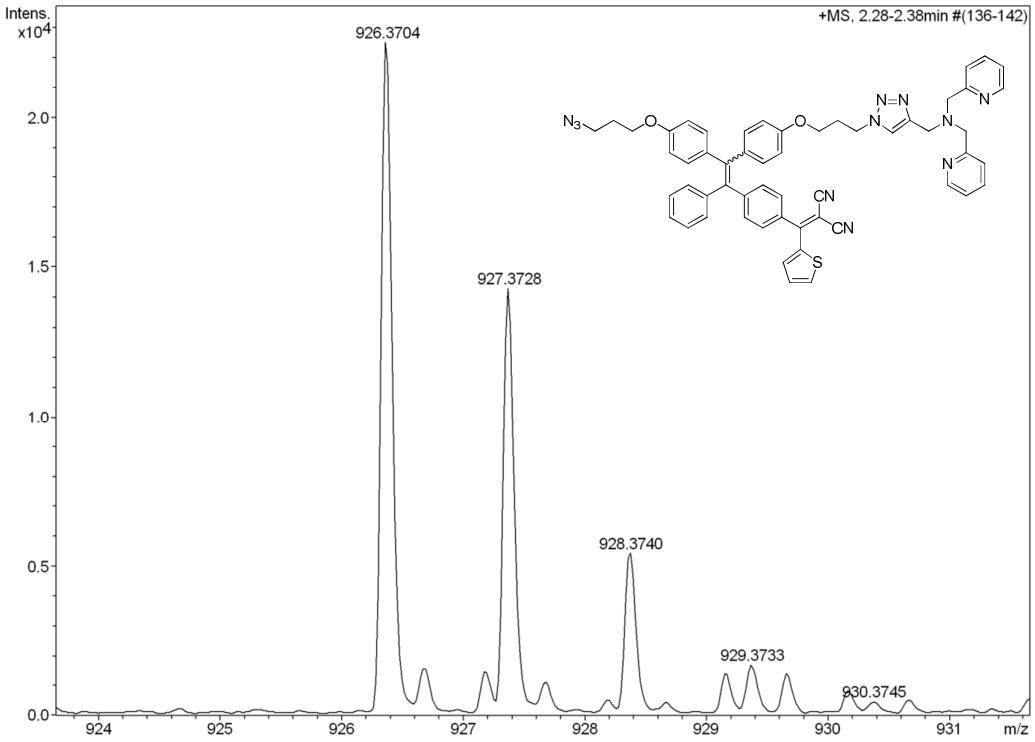


Figure S9 High Resolution mass spectroscopies (ESI) of **3**

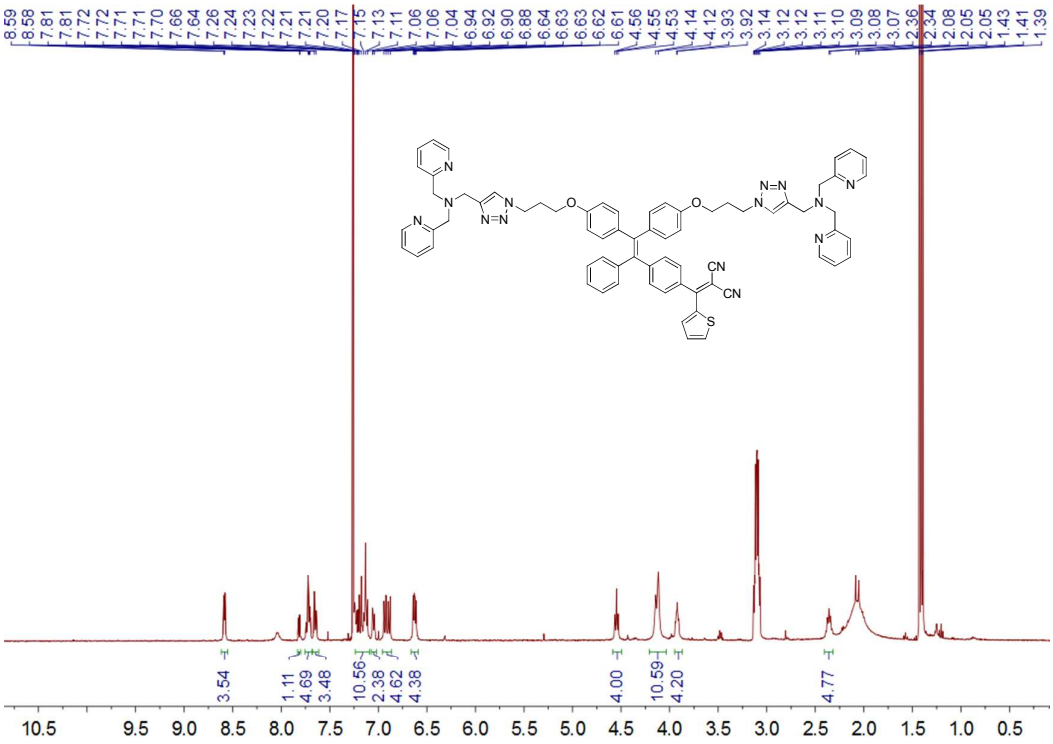


Figure S10 ^{1}H NMR spectra of compound **4** in $CDCl_3$

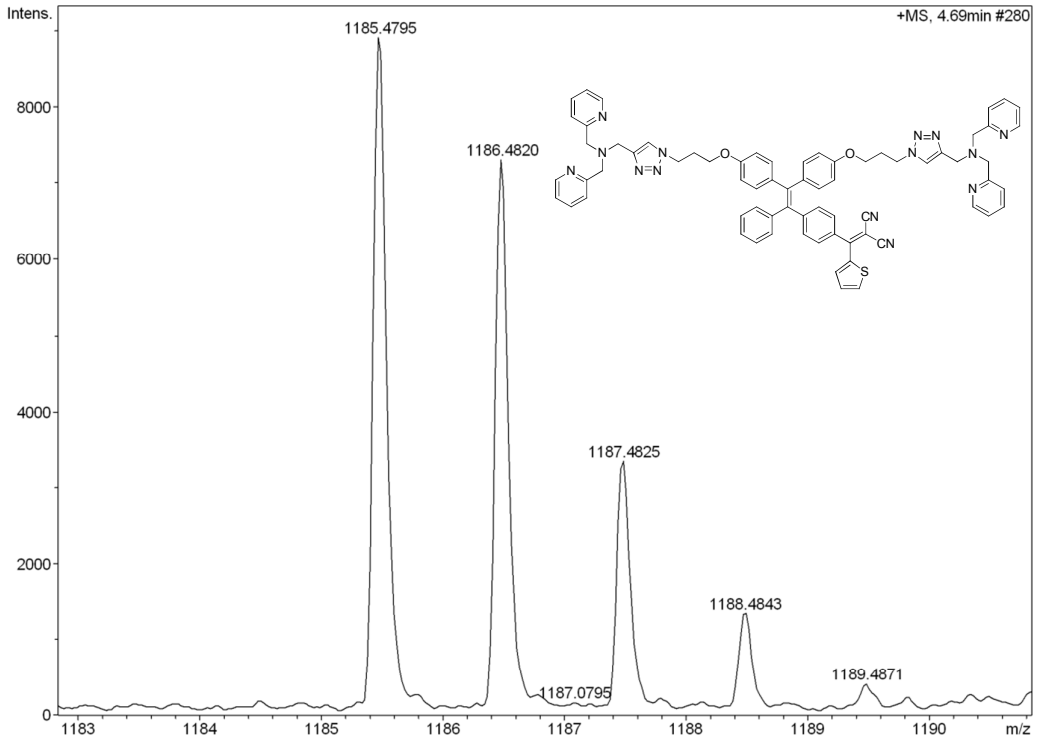


Figure S11 High Resolution mass spectroscopies (ESI) of **4**

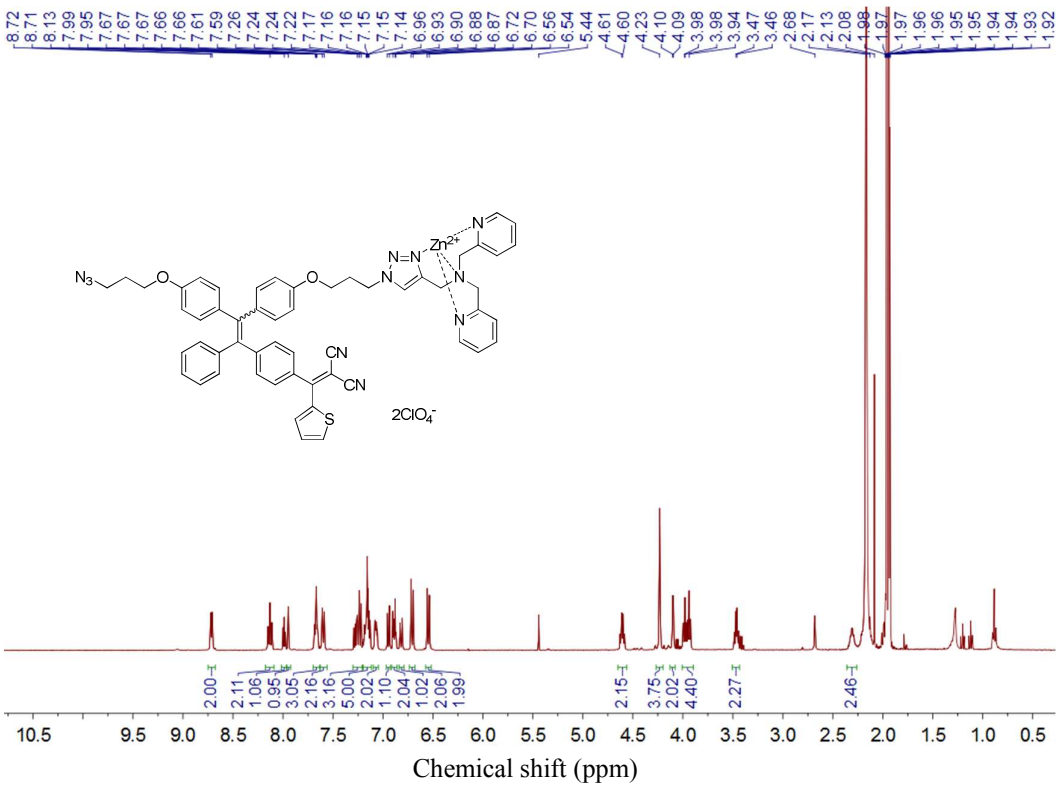


Figure S12 ^{1}H NMR spectra of compound **TPETH-1Zn** in $CDCl_3$

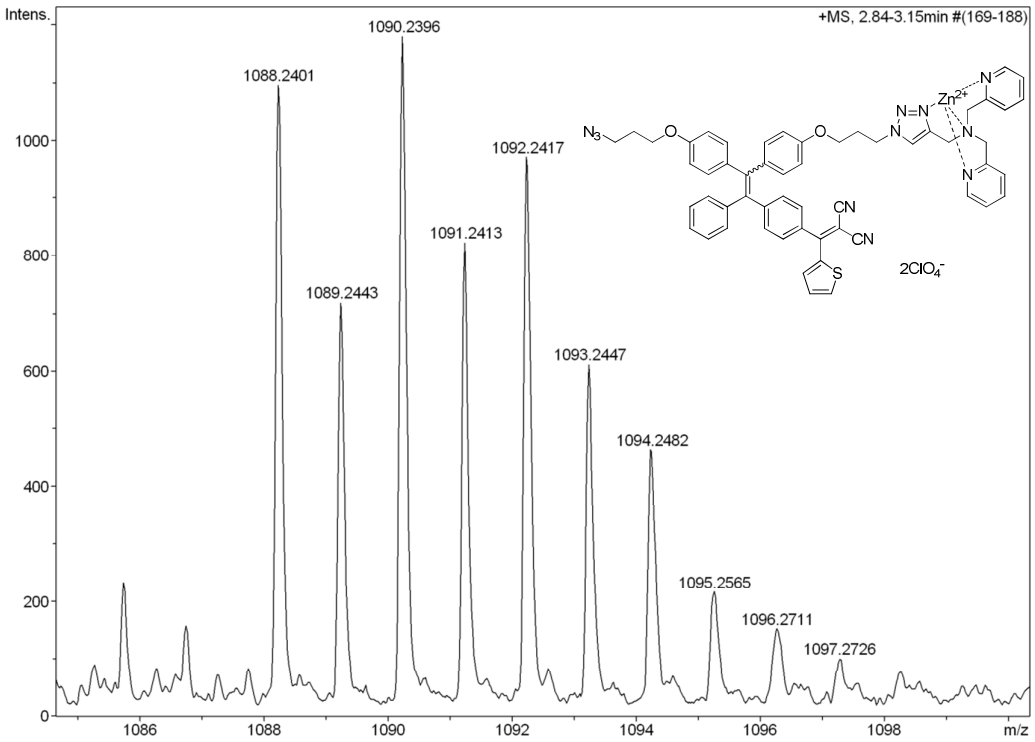


Figure S13 High Resolution mass spectroscopies (ESI) of **TPETH-1Zn**

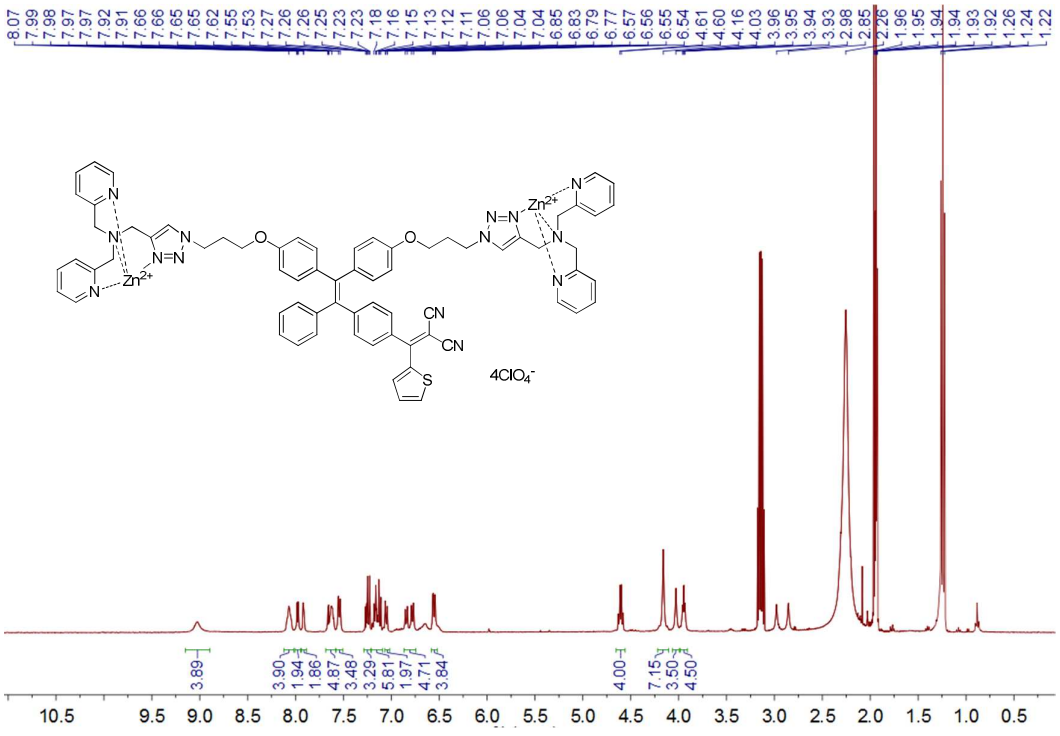


Figure S14 ^{1}H NMR spectra of compound **TPETH-2Zn** in $CDCl_3$

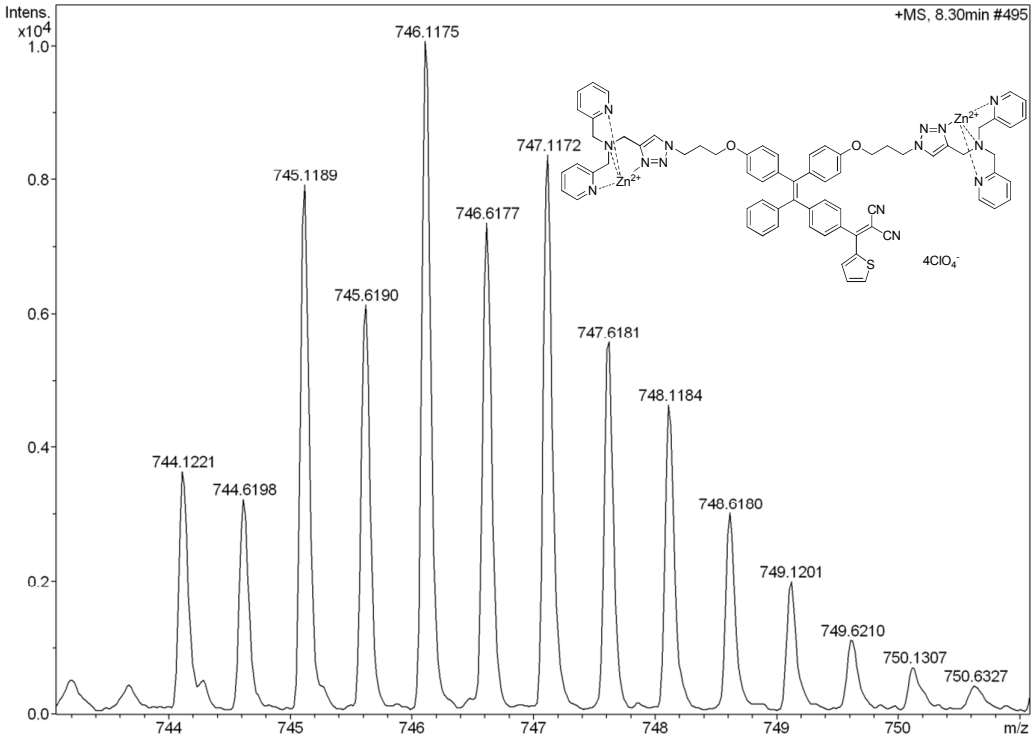


Figure S15 High Resolution mass spectroscopies (ESI) of **TPETH-2Zn**

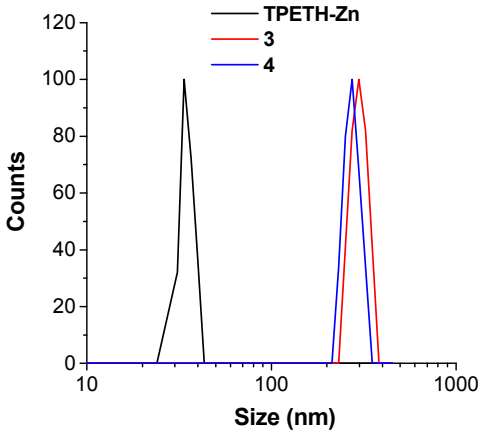


Figure S16. Hydrodynamic diameter measured with laser light scattering (LLS) for 3, 4, **TPETH-1Zn** aqueous solution (DMSO/water: v/v=1:199).

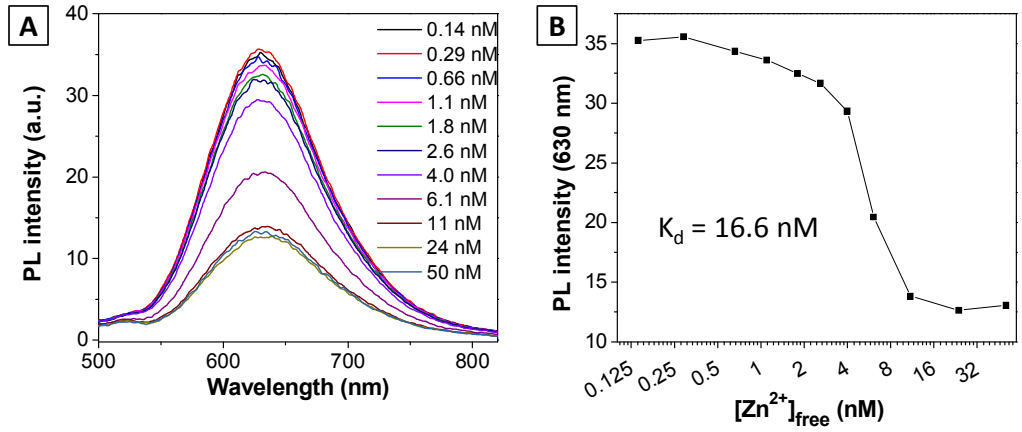


Figure S17 (A) Fluorometric titration of 3 (2.5 μM, λ_{ex} = 440 nm) with $ZnSO_4$ (0–9.5 mM) in HEPES buffer (HEPES: 50 mM, KNO3: 100 mM; EGTA: 10 mM, pH 7.2) at 25 °C. (B) Fluorescence intensity at 630 nm versus free zinc concentration ($[Zn^{2+}]_{free}$). The K_d value is calculated through Graphpad.

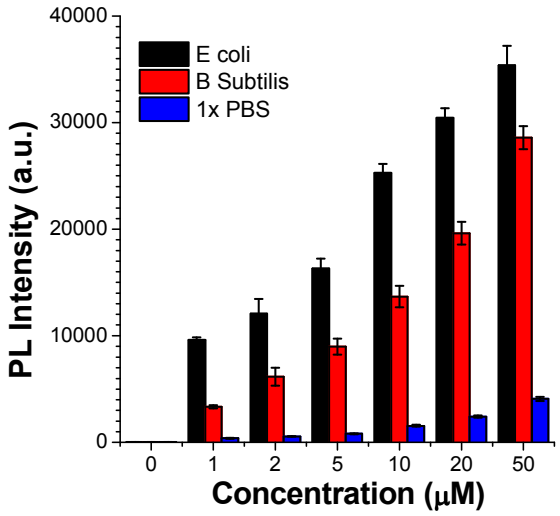


Figure S18. PL intensity at 650 nm of **TPETH-2Zn** in the presence of E coli, B Subtilis, or 1× PBS at different probe concentration.

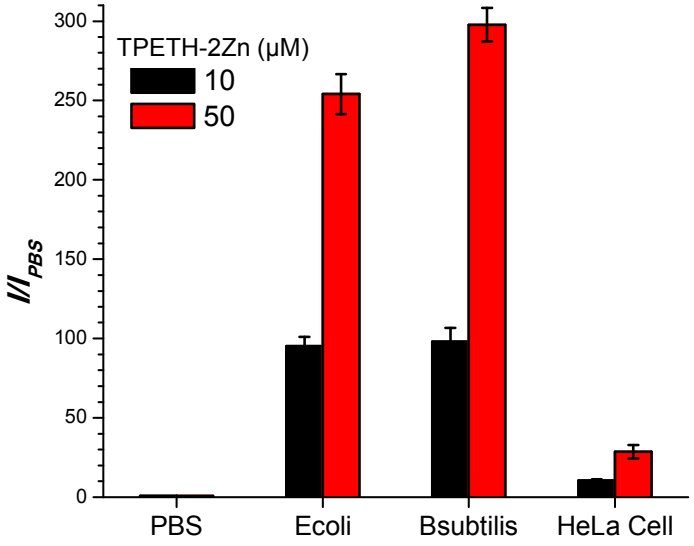


Figure S19. Fluorescence intensity at 650 nm of **TPETH-2Zn** incubated *E coli*, *B subtilis*, HeLa cells and PBS buffer upon varying concentration of **TPETH-2Zn**, respectively.

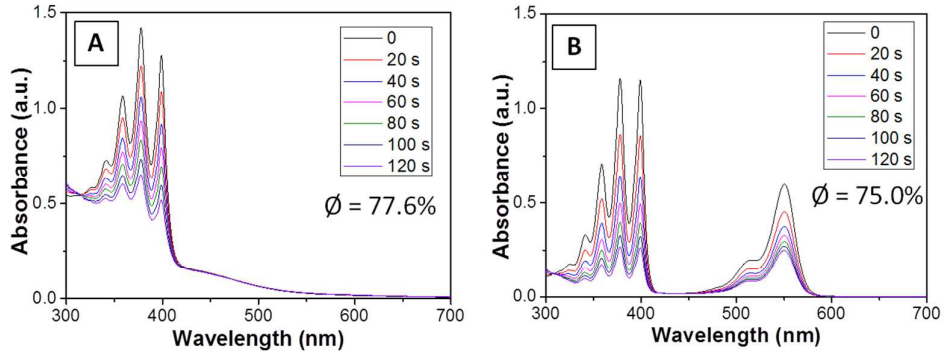


Figure S20. Time-dependent bleaching of ADPA (100 μM) caused by $^1O_2$ generated by **TPETH-2Zn** (10 μM) (A) or Rose bengal (10 μM) (B) irradiated by white light (100 mW cm^{-2}).

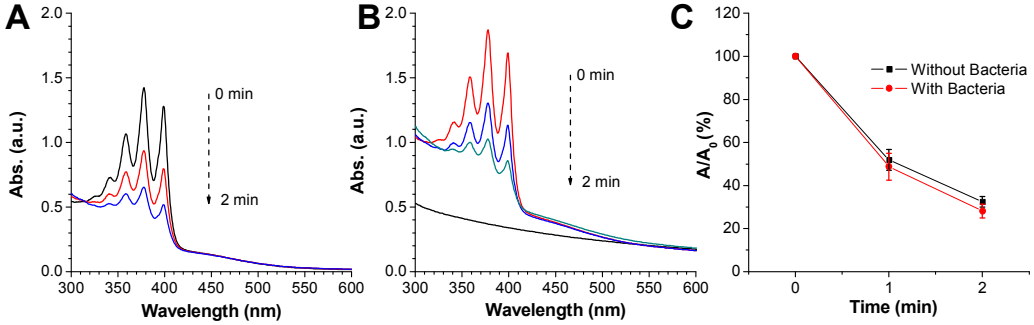


Figure 21. Time-dependent decomposing of ABDA by **TPETH-2Zn** without (A) or with (B) bacteria presence under light irradiation, and C) the corresponding decomposing rate at 399 nm.

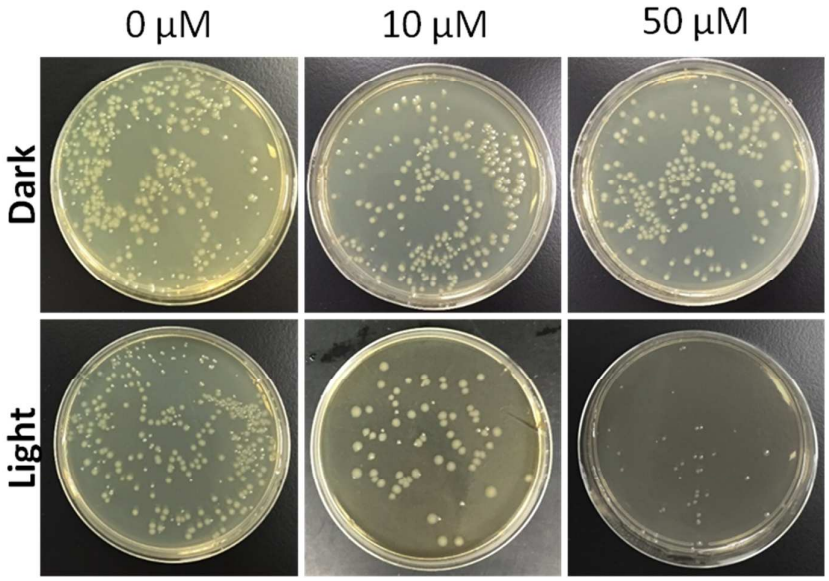


Figure S22. Plate photographs for *E coli* on LB agar plate supplemented with **TPETH-2Zn** with/out white light irradiation (100 mW cm^{-2}) for 6 min then grew overnight.

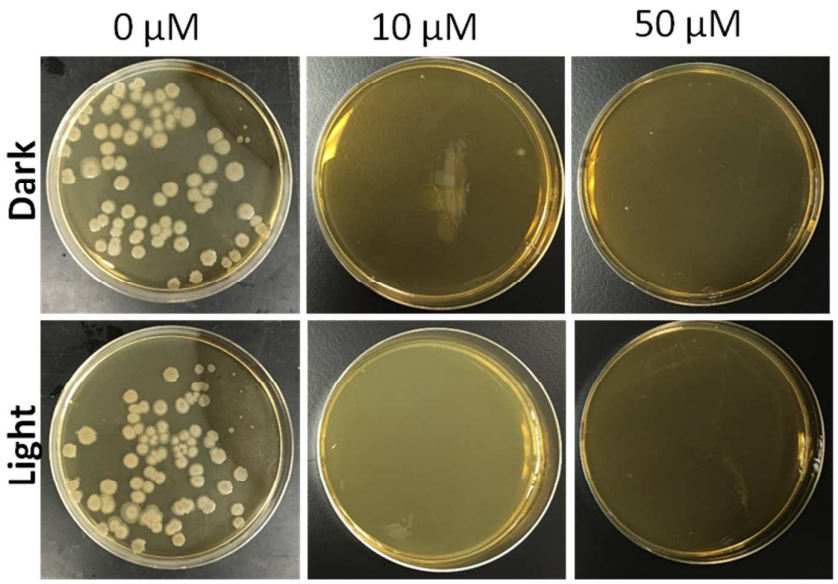


Figure S23. Plate photographs for *B subtilis* on LB agar plate supplemented with **TPETH-2Zn** with/out white light irradiation (100 mW cm^{-2}) for 6 min then grew overnight.

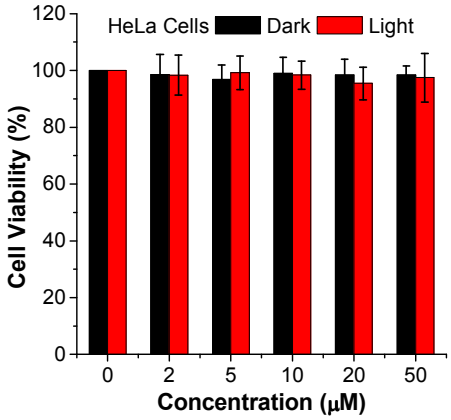


Figure S24. Cell viability of **TPETH-2Zn** treated HeLa cells with or without light irradiation.