

## Supporting Information

# Wavelength-Selective Activation of Photocaged DNazymes for Metal Ion Sensing in Live Cells

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## Experimental Procedures

### 1. Materials and instrument

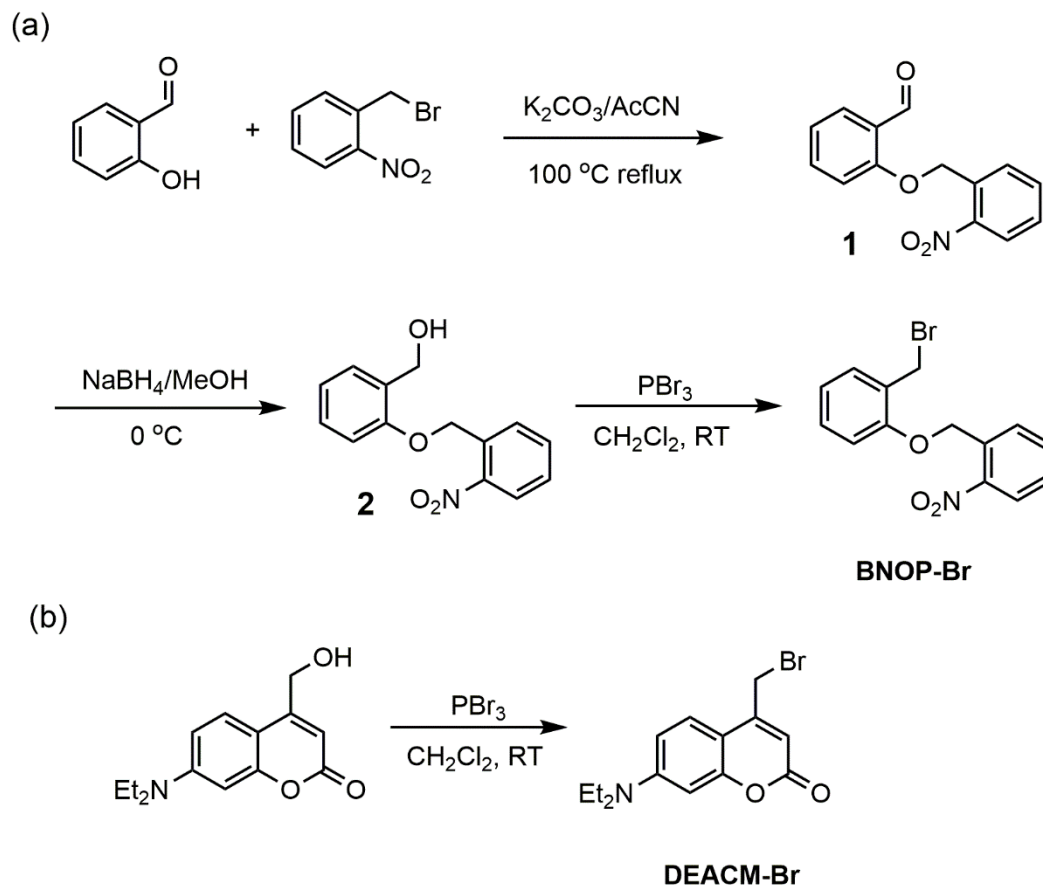
Compound 1-(bromomethyl)-2-nitrobenzene (NP-Br) and potassium diethylthiophosphate were purchased from Sigma Aldrich (Shanghai, China). Compound 7-Diethylamino-4-hydroxymethylcoumarin, salicylaldehyde and other reagents were purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China). Lipofectamine 3000 was from Thermo Fisher Scientific Inc. (MA, USA). Zinc nitrate hexahydrate, 3-morpholinopropanesulfoic acid (MOPS), sucrose, were purchased from Alfa Aesar (Tianjin, China). Dulbecco's modified Eagle's medium (DMEM), Dulbecco's Phosphate Buffered Saline (DPBS), Opti-MEM medium, fetal bovine serum (FBS), 100 IU/mL penicillin-streptomycin and 0.25% Trypsin were purchased from Corning cellgro (NY, USA). HeLa cells were purchased from National Platform of Experimental Cell Resources for Sci-Tech (Beijing, China). Oligonucleotides were synthesized and purified by either Integrated DNA Technologies, Inc. (IA, USA) or Sangon Biotech Co., Ltd. (Shanghai, China).

**Table S1.** List of chemically synthesized RNAs used in this work.

Name	Sequence (left to right: 5' to 3')
PS-T15	TTTTTTTT*TTTTTTT
17Dz	CATCTCTTCTCCGAGCCGGTCGAAATAGTGAGT
PS-17Dz	CATCTCTTCTCCG*A*G*CCGGTCGAAATAGTGAGT
PS-17Dz-DQ	/Spacer9/CATCTCTTCTCCG*A*G*CCGGTCGAAATAGTGAGT/Dabyl/
PS-17Dz-mod-DQ	/Spacer9/GATGAACTTTCCG*A*G*CCGGTCGAAGGGTCAGCT/Dabyl/
17S-FQ	/56-FAM/ACTCACTATr <u>A</u> GGGAAGAGATG/3IABkFQ/
17S-mod-CQ	/5Cy5/AGCTGACCCr <u>A</u> GAAGTTCATC/3IABkFQ/

Note: "N\*" represents a phosphorothioate (PS) linkage at the 3' phosphorodiesther of the nucleotide. "/Spacer9/" represent a commercial 3-mer PEG. "/Dabyl/" is a 4-(4-dimethylaminophenylazo) benzoic acid derivative (Dabyl) quencher. "/3IABkFQ/" is an Iowa Black Quencher. "/56-FAM/" and "/5Cy5/" are fluorescein and Cy5 fluorophores, respectively. Underlined rA is the ribonucleotide for DNAzyme-catalyzed cleavage.

Mass spectra were obtained on a Thermo Scientific LTQ XL Linear Ion Trap mass spectrometer (MA, USA) for ESI-MS, or a SHIMADZU MALDI-TOF mass spectrometer (Kyoto, Japan). Fluorescence spectra were taken on a JASCO FP-6500 fluorometer (Tokyo, Japan). UV-Vis spectra were recorded by a JASCO V-550 UV-VIS spectrophotometer. Fluorescence cell imaging was operated on an OLYMPUS FV1000 confocal laser scanning microscope (Tokyo, Japan). Flow cytometry was operated on a FACSCalibur (Becton Dickinson). Light irradiation was applied using a 45 cm length 36W commercial UV lamp at 365 nm reaching 26 mW/cm<sup>2</sup> to samples, and a 470 nm LED with adjustable DC regulated power supply reaching 13 mW/cm<sup>2</sup> to samples. Powers of light irradiations at samples were measured by a PM100D digital optical power and energy meter purchased from Thorlabs GmbH.



**Scheme S1.** Synthetic routes for NBOP-Br (a) and DEACM-Br (b).

## 2. Synthesis of 1-(bromomethyl)-2-((2-nitrobenzyl)oxy)benzene (NBOP-Br)

$K_2CO_3$  (6.9 g) was added to the solution of 1-(bromomethyl)-2-nitrobenzene (6.8 g, 32 mmol) in 50 mL of acetonitrile, followed by the addition of 2-hydroxybenzaldehyde (4.6 g, 35 mmol) in 50 mL acetonitrile. The mixture was reflux at 100 °C for 2 h. The solvents were removed in the rotary evaporator, the mixture was extracted with ethyl acetate/water. The aqueous layer was acidified by slowly addition of 5% HCl (~10 mL) and extracted with ethyl acetate. The extracts were dried over  $Na_2SO_4$  and concentrated at reduced pressure to give an orange oil as the desired product **1**. Then,  $NaBH_4$  (0.6 g) was added to the solution of product **1** (2 g) in 40 mL of methanol at 0 °C for 1 h. The solvent was evaporated and the residue was purified via extraction with ethyl acetate/water to get product **2**. All the as prepared **2** was then dissolved in dichloromethane, followed by the dropwise addition of 3 mL phosphorus tribromide at room temperature. After stirring for 0.5 h, water was added to quench the reaction and the mixture was extracted with dichloromethane/water. The organic layer was dried over  $Na_2SO_4$ , filtered, and concentrated to obtain the final product NBOP-Br at a 58% total yield.  $^1H$ -NMR (300 MHz,  $CDCl_3$ ),  $\delta$  (ppm): 8.2 (d, 1H), 8.05 (d, 1H), 7.73 (t, 1H), 7.48 (t, 1H), 7.34 (d, 1H), 7.24 (t, 1H), 6.9 (m, 2H), 5.55 (s, 2H), 4.68 (s, 2H).  $^{13}C$ -NMR (300 MHz,  $CDCl_3$ ),  $\delta$  (ppm): 156.04, 146.83, 134.42, 133.79, 131.18, 130.56, 128.57, 128.47, 126.44, 125.12, 121.58, 112.27, 66.94, 29.29. LCMS-IT-TOF spectrometry: calc. for  $C_{14}H_{12}BrNO_3$ ,  $M = 322.0$ , found  $[M-Br]^+ = 242.0$ .

## 3. Synthesis of 4-bromomethyl-7-diethylaminocoumarin (DEACM-Br)

A batch of 0.25 g 4-methoxy-7-diethylaminocoumarin was dissolved in 100 mL dichloromethane, to which 1 mL phosphorus tribromide was dropwise added at room temperature. After stirring for 0.5 h, water was added to the mixture above to quench the reaction. The reaction mixture was extracted with

dichloromethane/water. The organic layer was dried with Na<sub>2</sub>SO<sub>4</sub>, filtered and evaporated to obtain DEACM-Br at almost quantitative yield. <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>), δ (ppm): 7.50 (d, 1H), 6.64 (d, 1H), 6.51 (s, 1H), 4.4 (s, 2H), 3.4 (q, 4H), 1.2 (t, 6H). <sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>), δ (ppm): 161.79, 156.76, 150.91, 150.36, 125.42, 115.41, 109.30, 108.70, 106.20, 97.91, 44.84, 27.17, 12.49. LCMS-IT-TOF spectrometry: calc. for C<sub>14</sub>H<sub>16</sub>BrNO<sub>2</sub>, [M+H]<sup>+</sup> = 310.0, found 310.0.

#### 4. Synthesis of NBOP-PS

To a solution of 0.42 g (2 mmol) potassium diethylthiophosphate dissolved in 50 mL acetonitrile was added 0.48 g (1.5 mmol) NBOP-Br. The mixture was allowed to stand at room temperature with stirring for 1 h. The complete of reaction can be monitored by TLC using ethyl acetate vs. petroleum ether = 1:1 (v/v). After that, the solvent was removed under reduced pressure, and the residue was treated with 50 mL ethyl acetate and 50 mL water. The organic later was collected and washed by 50 mL water for three times, and then dried by anhydrous Na<sub>2</sub>SO<sub>4</sub>. After evaporating the solvent under reduced pressure, PS-NBOPM was obtained as a yellow oil (0.57 g, yield 92%). <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>), δ (ppm): 8.15 (t, 1H), 7.95 (d, 1H), 7.65 (t, 1H), 7.46 (t, 1H), 7.34 (d, 1H), 7.2 (t, 1H), 6.9 (m, 2H), 5.45 (s, 2H), 4.12(m, 6H), 1.2 (t, 6H). LCMS-IT-TOF spectrometry: calc. for C<sub>18</sub>H<sub>22</sub>NO<sub>6</sub>PS, [M+H]<sup>+</sup> = 412.0, found 412.0.

#### 5. Synthesis of DEACM-PS

To a solution of 0.42 g (2 mmol) potassium diethylthiophosphate dissolved in 50 mL acetonitrile was added 0.46 g (1.5 mmol) DEACM-Br. The mixture was allowed to stand at room temperature with stirring for 1 h. The complete of reaction can be monitored by TLC using ethyl acetate vs. petroleum ether = 1:1 (v/v). After that, the solvent was removed under reduced pressure, and the residue was treated with 50 mL ethyl acetate and 50 mL water. The organic later was collected and washed by 50 mL water for three times, and then dried by anhydrous Na<sub>2</sub>SO<sub>4</sub>. After evaporating the solvent under reduced pressure, PS-DEACM was obtained as a yellow oil (0.56 g, yield 94%). <sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>), δ (ppm): 7.45 (d, 1H), 6.62 (q, 1H), 6.5 (d, 1H), 6.13 (s, 1H), 4.15 (m, 4H), 4.09 (s, 1H), 4.02 (s, 1H), 3.41 (q, 4H), 1.32(m, 6H), 1.2 (m, 6H). LCMS-IT-TOF spectrometry: calc. for C<sub>18</sub>H<sub>26</sub>NO<sub>5</sub>PS, [M+H]<sup>+</sup> = 400.1, found 400.1.

#### 6. Synthesis of NBOP- and DEACM-modified DNAzymes

A 20 μL solution of 50 μM DNAzymes containing phosphorothioate modifications in 50 mM sodium phosphate at pH 6.0 was added 20 μL 25 mM arylmethylbromide (NBOP-Br, DEACM-Br or NP-Br) in DMF. Additional 20 μL DMF should be added for NBOPM-Br reaction solutions to make sure a homogeneous solution. The solution was kept on a roller at room temperature for 48 h. The resulting solution was purified by Amicon-3K ultrafilters for 6 times using water to remove excess arylmethylbromide and salts.

#### 7. Polyacrylamide gel electrophoresis (PAGE) analysis of DNAzymes

A solution of 10 μM NBOP- and DEACM-modified DNAzymes in sodium phosphate buffer (pH7.0) was illuminated at 365 nm (26 mW/cm<sup>2</sup>) and 470 nm (13 mW/cm<sup>2</sup>) for desired time, respectively, to serve as irradiated samples. DNAzyme samples without light irradiation were used directly. Each sample was mixed with an equivalent amount of loading buffer containing 8 M urea, 0.03% bromophenol blue and 0.03% xylene cyanol FF, and then electrophoresed at 200 V for 1.5h on a 15% denatured polyacrylamide gels in 1xTBE buffer (pH 7.4). After ethidium bromide (EtBr: 0.5 μg/mL) or Syber Gold (1X) staining, retardation was visualized in a BIO-RAD Universal Hood II.

#### 8. Time-dependent fluorescence measurement of DNAzymes' activity

For each single wavelength (365 nm or 470 nm) activation, the test solution contained 50 nM DNAzyme and 250 nM substrate in 100 mM NaCl, 100 mM MOPS, pH 7.0. Light irradiation was at 365 nm (26 mW/cm<sup>2</sup>)

for 5 min on NBOP-17Dz and at 470 nm (13 mW/cm<sup>2</sup>) for 10 min on DEACM-17Dz, respectively, prior to the experiment if necessary. To start the DNAzyme-catalyzed cleavage reaction, Zn<sup>2+</sup> was added to a final concentration of 0.5 mM with vortexing. The solution was then immediately measured in the fluorometer at excitation/emission = 490/520 nm for fluorescein fluorescence, at 25°C.

For wavelength-selective activation of NBOP-17Dz and DEACM-17Dz, the test solutions contained 50 nM NBOP-17Dz, 50 nM DEACM-17Dz and 250 nM 17S-FQ in 100 mM NaCl, 100 mM MOPS, pH 7.0, added with 0.5 mM Zn<sup>2+</sup>. To make the photolysis reaction slower for a more clear kinetics, the light irradiation was applied at a lower power by doubling the sample-to-lamp distance, which provided 1/4 power as above. The solution was first irradiated at 470 nm at 3.5 mW/cm<sup>2</sup> for desired time, and then at 365 nm at 6.5 mW/cm<sup>2</sup> for desired time. For each time point, the solution was measured in the fluorometer at excitation/emission = 490/520 nm for fluorescein fluorescence, at 25°C.

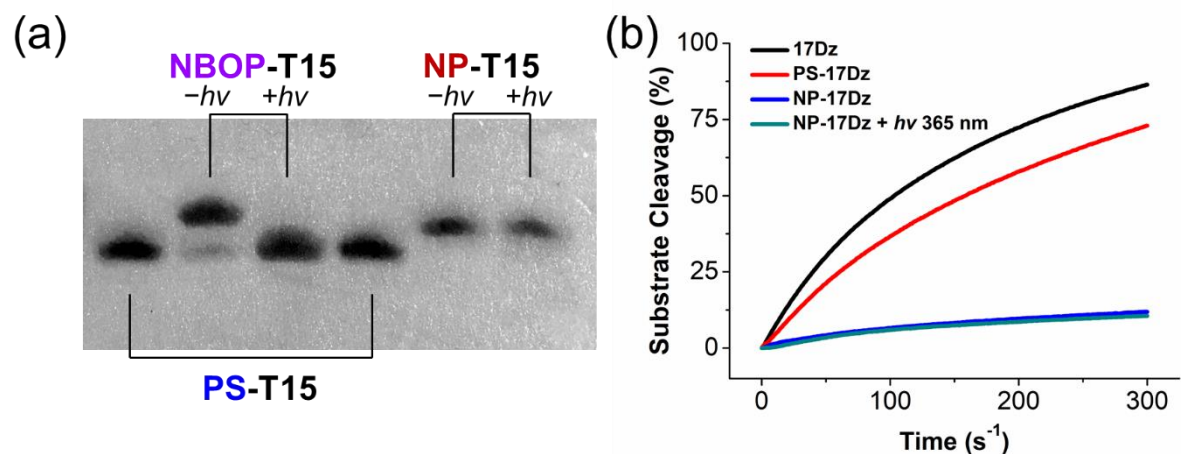
## 9. Cell culture

HeLa cells (a human cervical carcinoma cell line) were cultured in Dulbecco's Modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS) and antibiotics (50 units/mL penicillin and 50 units/mL streptomycin) at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

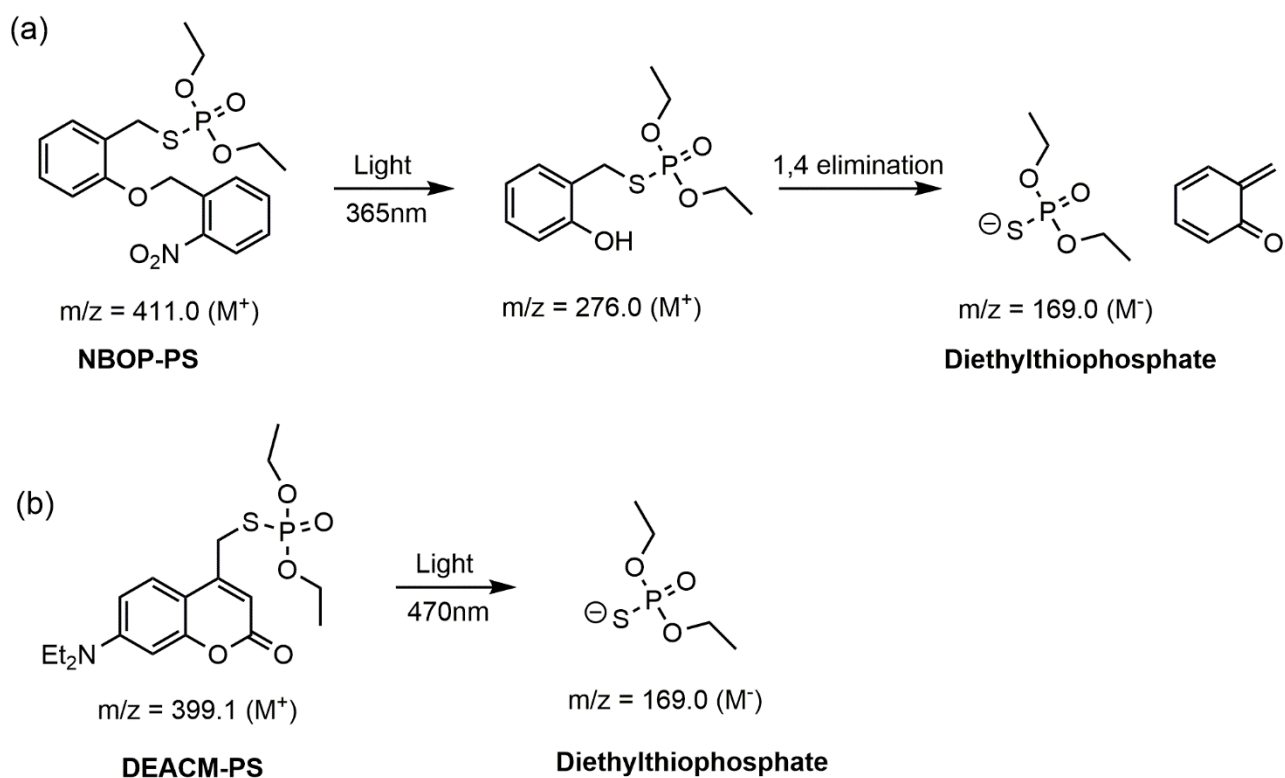
## 10. Confocal fluorescence imaging and flow-cytometry of HeLa cells

HeLa cells (2 × 10<sup>4</sup> cells per well) were plated into dishes covered with 1.5 cm diameter glass or 24-well culture plates and cultured at 37 °C for 24 h before the experiment. Each well was transfected with 100 nM DNAzyme and 100 nM substrate using Lipofectamine 3000 (Invitrogen). After transfection, cells were irradiated at 365 nm (26 mW/cm<sup>2</sup>) for 5 min or 470 nm (13 mW/cm<sup>2</sup>) for 10 min, respectively and then incubate with medium containing 100 μM Zn<sup>2+</sup> for 0.5 h. For wavelength-selective activation, mixtures of DNAzymes and substrates at 100 nM each were transfected together. After transfection, cells were irradiated first at 470 nm (13 mW/cm<sup>2</sup>) for 10 min and then at 365 nm (26 mW/cm<sup>2</sup>) for 5 min, and then incubate with medium containing 100 μM Zn<sup>2+</sup> for 0.5 h. For confocal fluorescence imaging, cells were fixed with 4% paraformaldehyde and images were collected with OLYMPUS FV1000 confocal laser scanning microscope (20X magnification). For flow-cytometry, cells were harvested by trypsin treatment, washing with PBS three times by centrifuging for 1 min at 3000 rpm, and finally resuspended with 500 μL PBS for flow cytometry analysis using FACSCalibur (Becton Dickinson). Fluorescence was determined by counting 10,000 events, and the data were analyzed by FlowJo software.

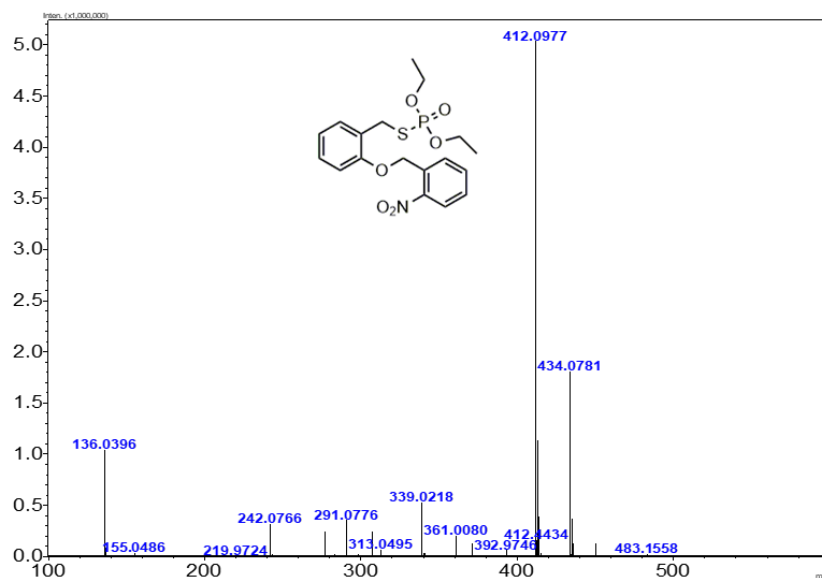
## Additional Figures



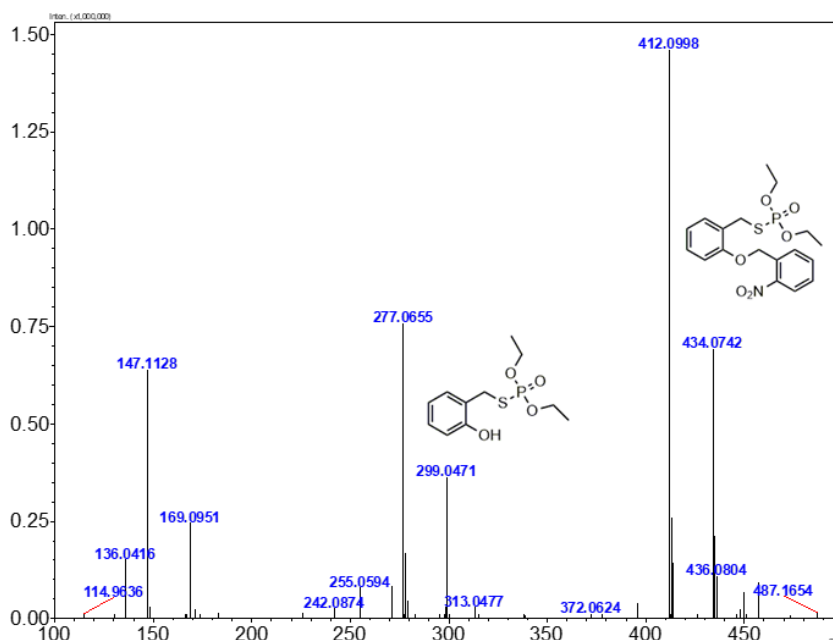
**Figure S1.** (a) PAGE image showing UV light at 365 nm ( $26 \text{ mW/cm}^2$ ) for 30 min was able to degrade NBOP-T15 but not NP-T15.  $10 \mu\text{M}$  DNA in sodium phosphate buffer (pH7.0). (b) NP-17Dz was not activated by UV irradiation at 365 nm ( $26 \text{ mW/cm}^2$ ) for 5 min.  $50 \text{ nM}$  DNAzyme and  $250 \text{ nM}$  17-FQ in  $100 \text{ mM}$  NaCl,  $100 \text{ mM}$  MOPS, pH 7.0 at  $25 \text{ }^\circ\text{C}$ .



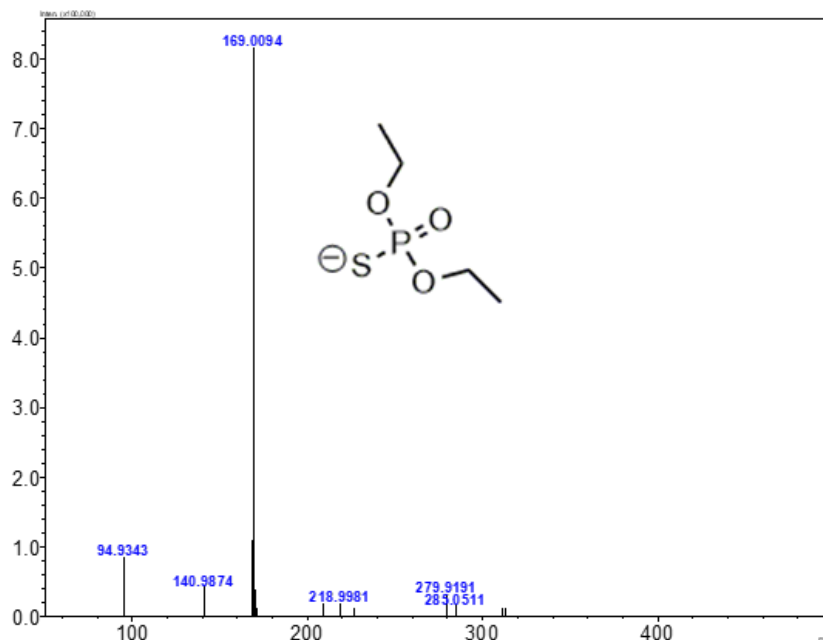
**Figure S2.** The proposed light reactions of NBOP-PS (a) and DEACM-PS (b).



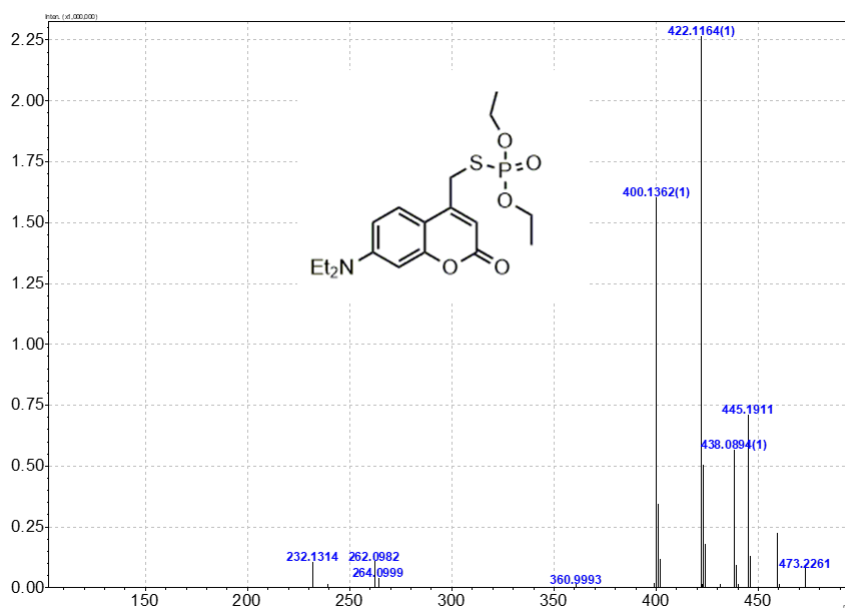
**Figure S3.** The ESI-MS (positive ion) of NBOP-PS ( $[M+H]^+ = 412.0$ ,  $[M+Na]^+ = 434.0$ ).



**Figure S4.** ESI-MS (positive ion) of 1 mM NBOP-PS in CH<sub>3</sub>CN after brief irradiation at 365nm (26 mW/cm<sup>2</sup>). The peak  $m/z = 277.0$  indicates the formation of the intermediate product, S-hydroxybenzyl-diethylphosphorothioate ( $[M+H]^+ = 277.0$ , calc.  $M = 276.0$ ) as shown in Figure S2a.

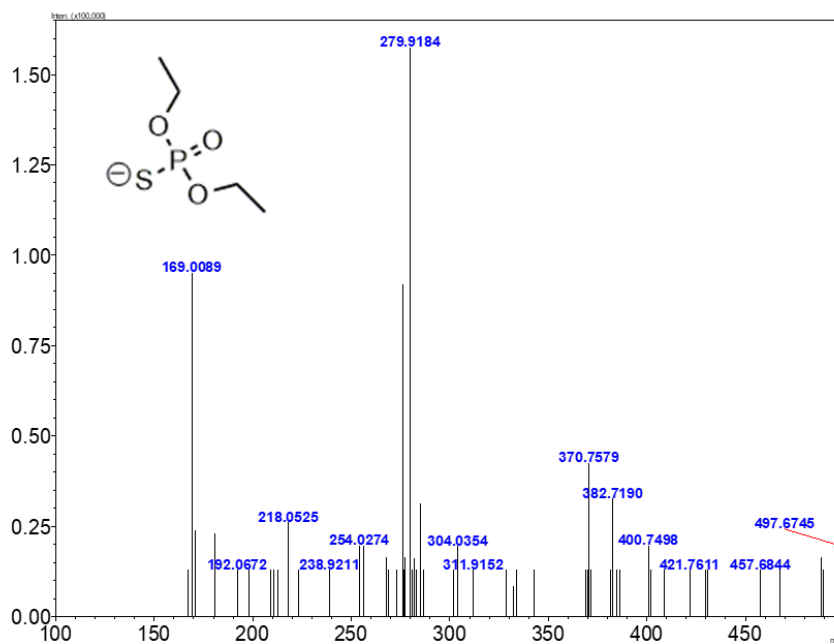


**Figure S5.** ESI-MS (negative ion) of 1 mM NBOP-PS in CH<sub>3</sub>CN after irradiation at 365 nm (26 mW/cm<sup>2</sup>) for 15 min. The peak  $m/z = 169.0$  indicates the formation of diethylthiophosphate as the product.

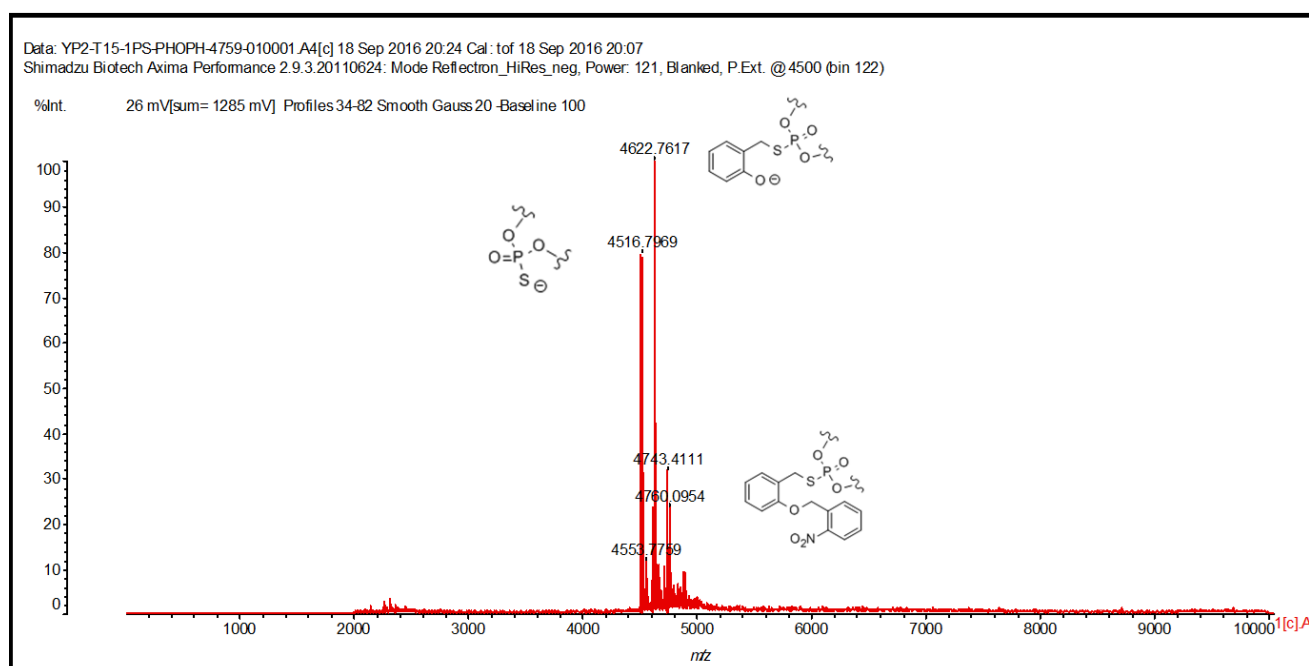


**Figure S6.** ESI-MS (positive ion) of DEACM-PS ( $[M+H]^+ = 400.1$ ,  $[M+Na]^+ = 422.1$ ,  $[M+K]^+ = 438.1$ ).



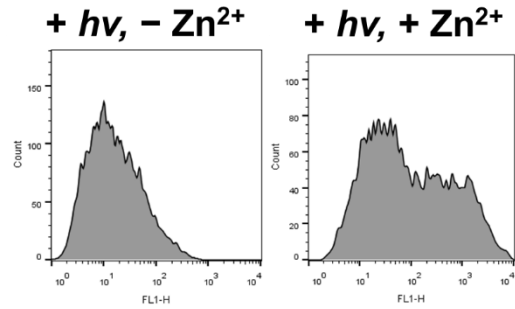


**Figure S7.** ESI-MS (negative ion) of 1 mM DEACM-PS in CH<sub>3</sub>CN after irradiation at 470 nm (13 mW/cm<sup>2</sup>) for 30 min. The peak m/z = 169.0 indicates the formation of diethylthiophosphate as the product.



**Figure S8.** MALDI-TOF MS of NBOP-T15 (m/z = 4760, calc. 4758) after brief UV irradiation at 365 nm (26 mW/cm<sup>2</sup>), showing *o*-hydroxybenzyl-T15 as the intermediate (m/z = 4622, calc. 4623) and PS-T15 as the product (m/z = 4516, calc. 4517).

## PS-17Dz-DQ



## NBOP-17Dz-DQ (365 nm)

## DEACM-17Dz-DQ (470 nm)

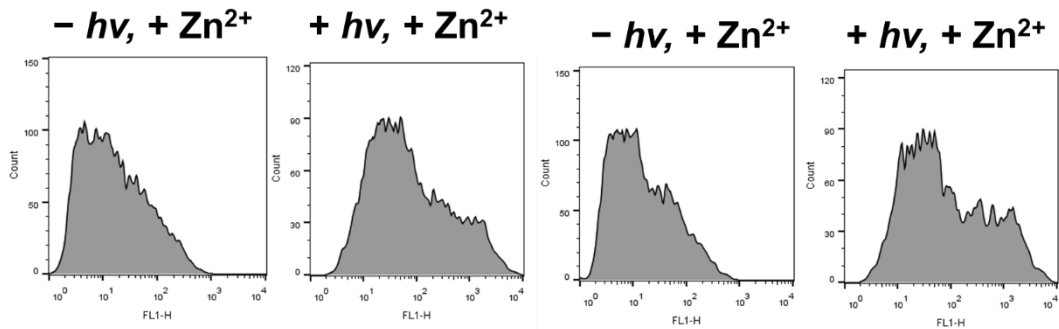


Figure S9. Representative flow cytometry figures for the data in Figure 4c.

## NBOP-17Dz-DQ + DEACM-17Dz-mod-DQ

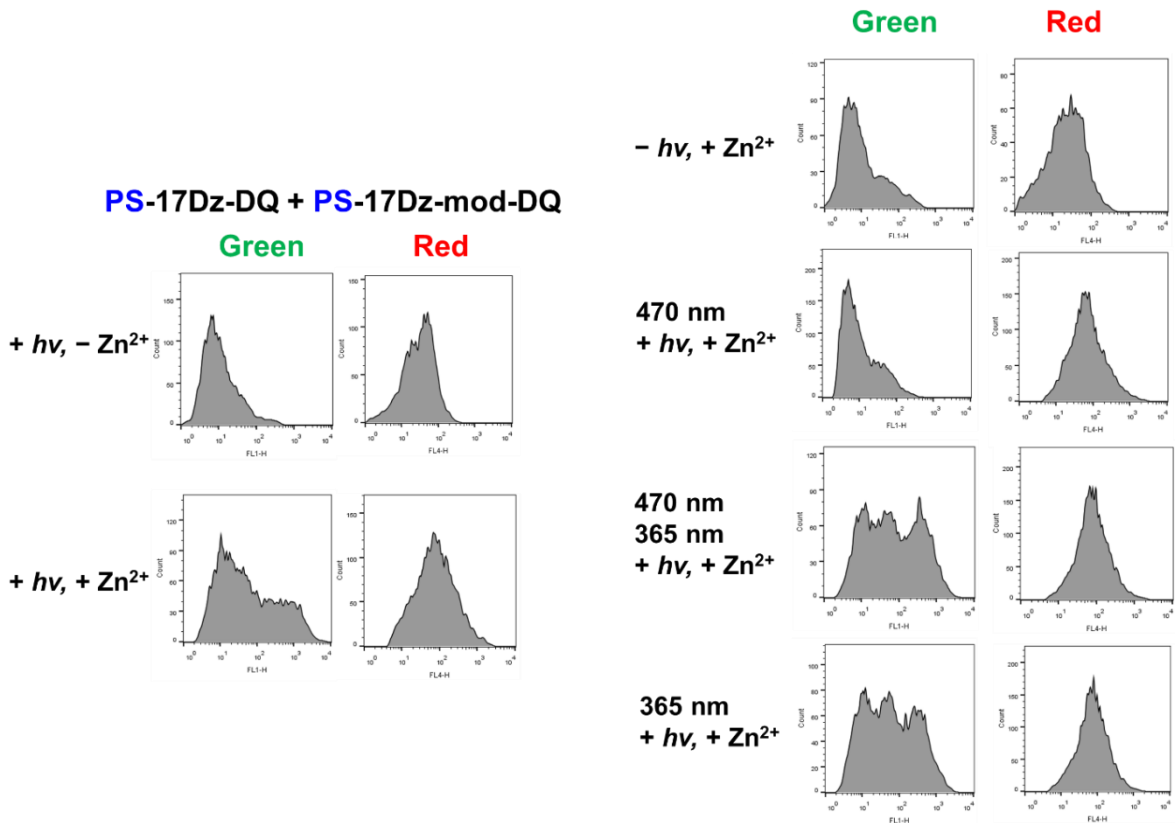


Figure S10. Representative flow cytometry figures for the data in Figure 5d.

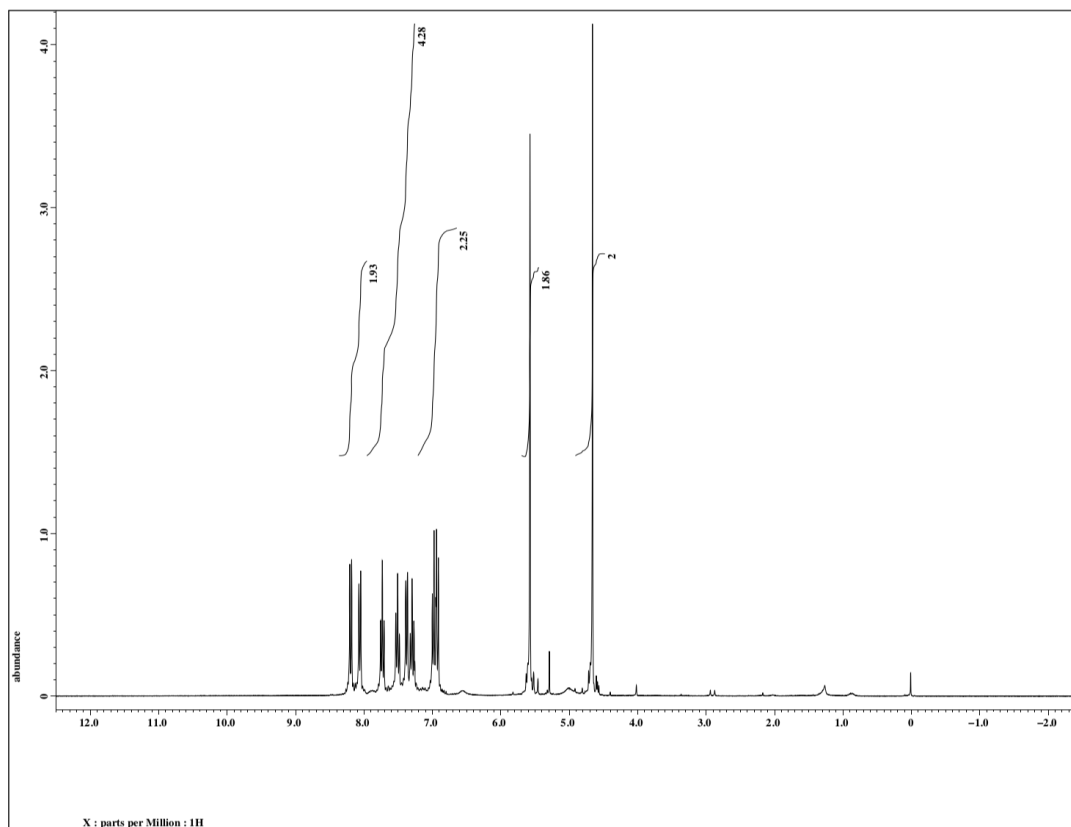


Figure S11.  $^1\text{H}$ -NMR of NBOP-Br

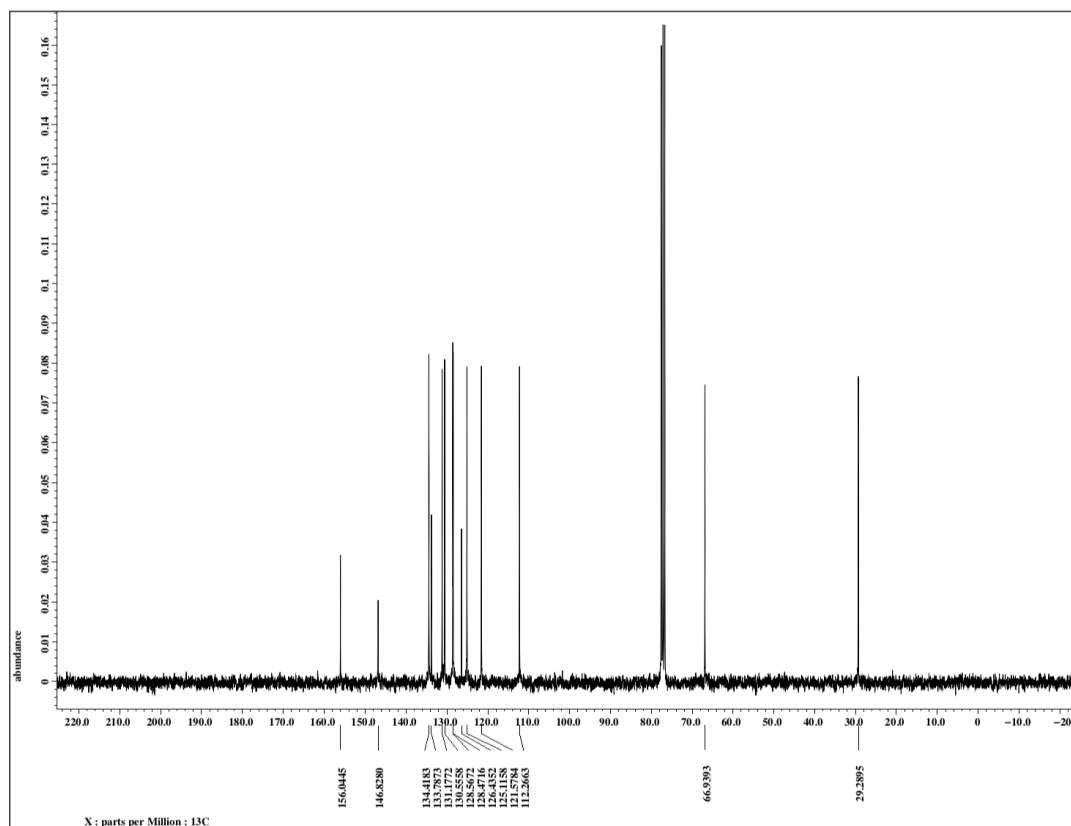


Figure S12.  $^{13}\text{C}$ -NMR of NBOP-Br

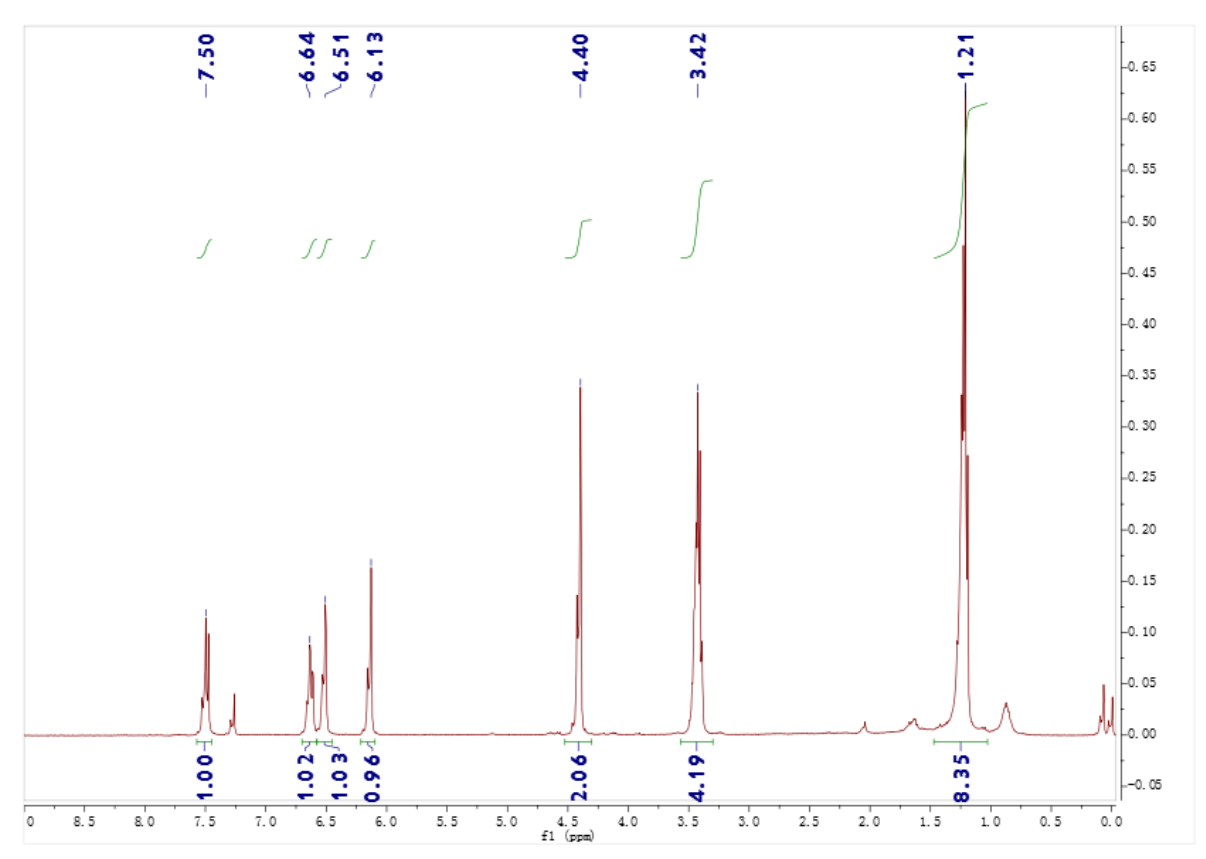


Figure S13.  $^1\text{H-NMR}$  of DEACM-Br

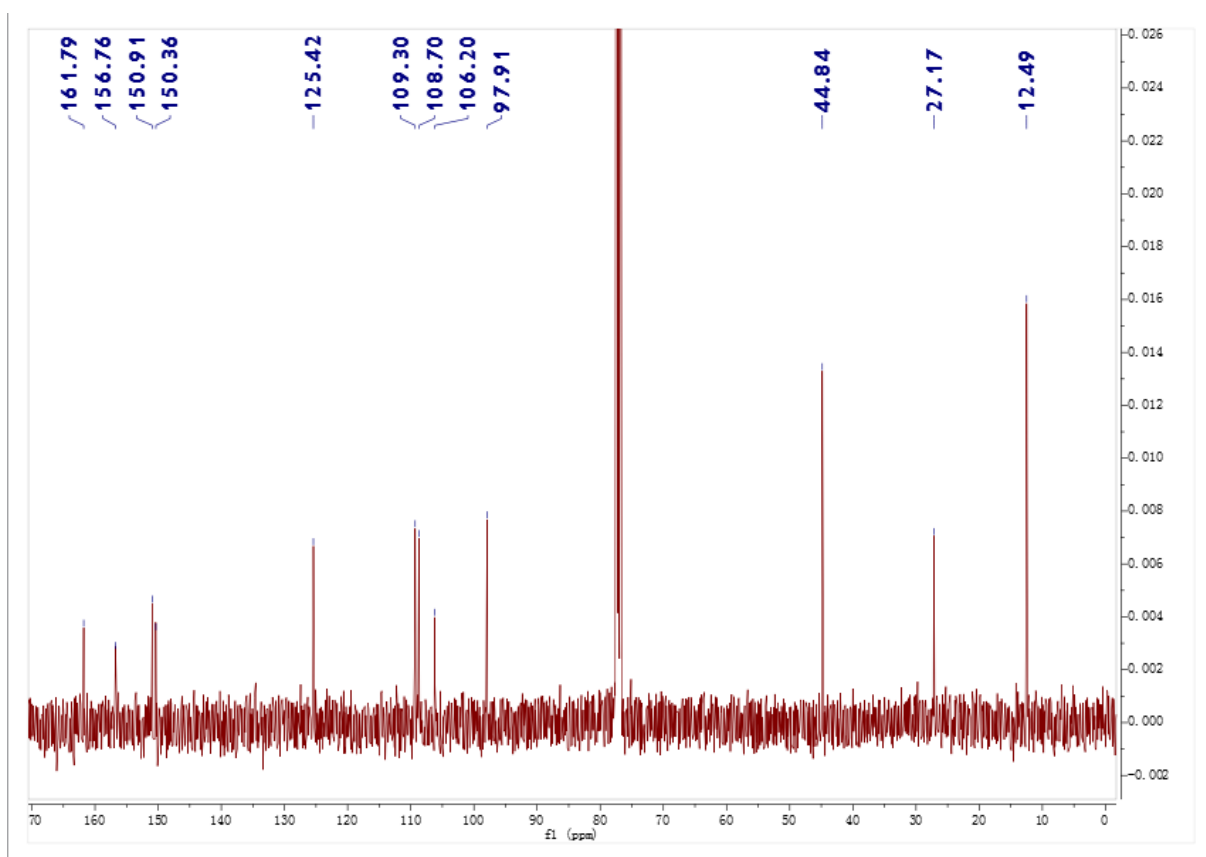


Figure S14.  $^{13}\text{C-NMR}$  of DEACM-Br