

Supporting information for

**A two-photon endoplasmic reticulum-targeting fluorescent
probe for the imaging of pH in living cells and zebrafishes**

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1. Materials and instruments

All reagents were mentioned in this work of analytical grade. ^1H NMR and ^{13}C NMR spectra were collected on an AVANCE III 400 MHz Digital NMR Spectrometer. High resolution mass spectrometric (HRMS) analyses were measured using a Finnigan MAT 95 XP spectrometer. UV-Vis and Fluorescence spectra were acquired on a Shimadzu UV-2700 spectrometer and HITACHI F-4600 fluorescence spectrophotometer, respectively. Fluorescence imaging of living cells and cells was performed on a Nikon A1MP confocal microscopy.

2. Cytotoxicity experiments

Cytotoxicity of **Na-pH** was evaluated using the standard MTT assay. HeLa cells were seeded into the 96 well-plates with the density of around 7000 cells/well. After 24 h, various concentrations of **Na-pH** were added into the wells and the cells were further cultured for 24 h. Then, 10 μL of 5 mg/mL MTT was mixed into cells and the cells were incubated for another 4 h. Subsequently, 100 μL DMSO were used to resolve the formazan and the plate was shaken for 20 min, and finally the absorbance was determined by a Thermo Fisher Scientific microplate reader. Cell viability was expressed as the percentage of the control culture value.

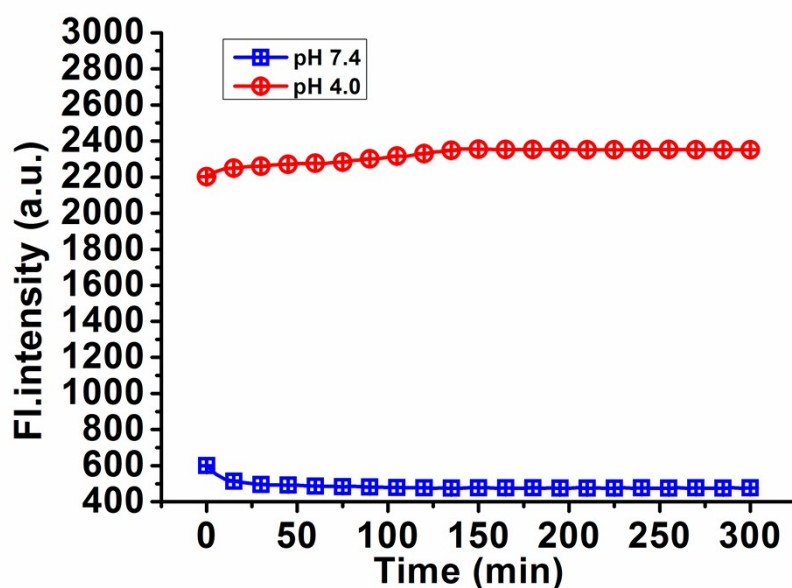
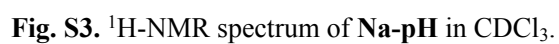
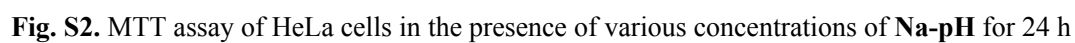


Fig. S1. Photostability of compound **Na-Ph** at different pH values (4.0 and 7.4, at 531nm) .



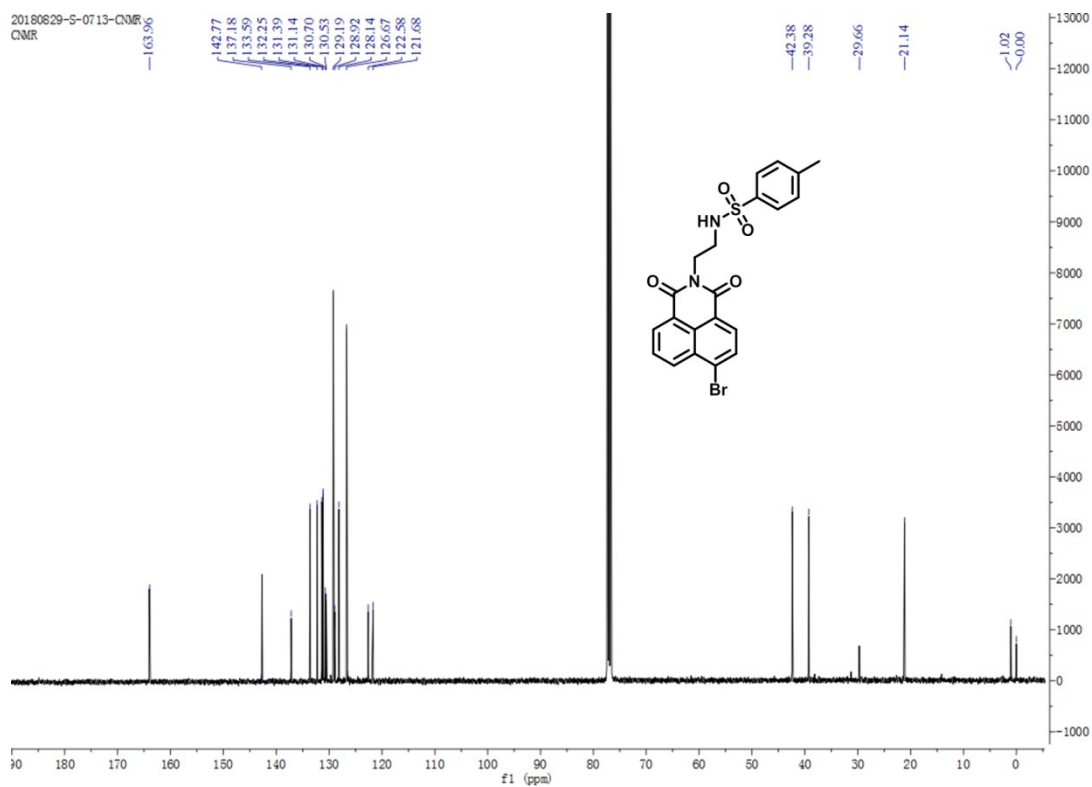


Fig. S6. ^{13}C -NMR spectrum of Compound 1 in CDCl_3 .

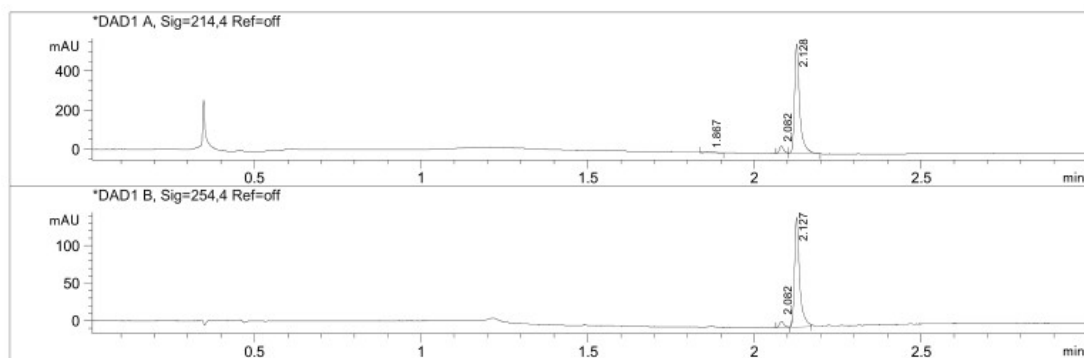


Fig. S7. LC-MS data of compound Na-pH

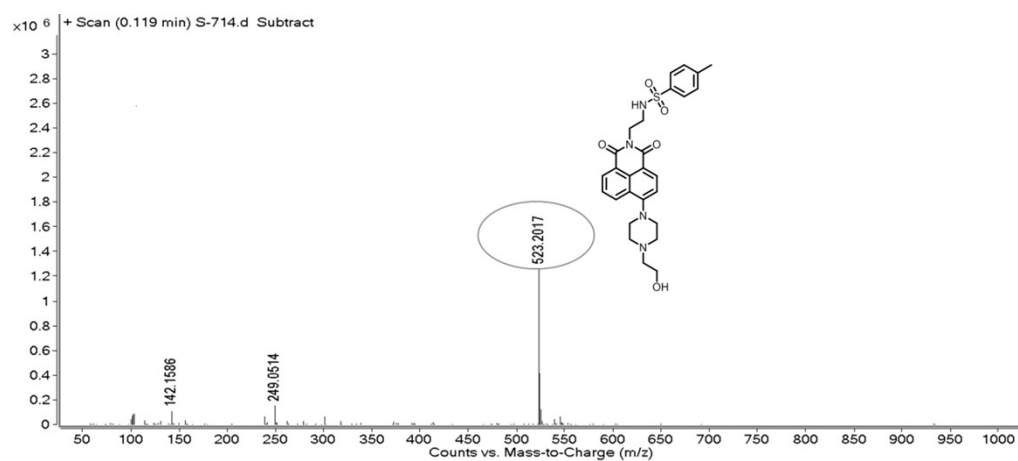


Fig. S8. HR-MS data of **1a-pH**