Electronic Supplementary Information

A ratiometric fluorescent pH probe based on aggregation-induced emission enhancement and its application in live-cell imaging

Panshu Song,[a] Xiaotong Chen, [a] Yu Xiang,[b] Lei Huang,[c] Zhaojuan Zhou,[a] Ruirui Wei,[a] Aijun Tong*[a]

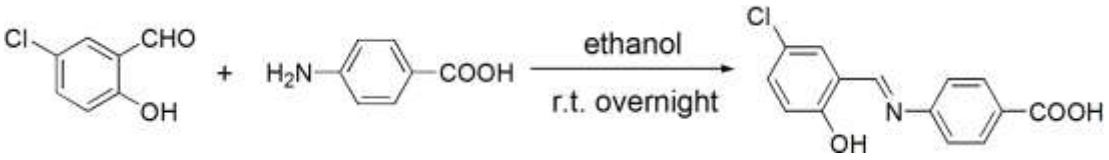
[a] *The Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology (Ministry of Education), Department of Chemistry, Tsinghua University, Beijing 100084, PR China*

[b] *Department of Chemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA*

[c] *Center of Biomedical Analysis, Tsinghua University, Beijing 100084, PR China*

* Corresponding author. Tel & Fax: +86-10-62782485; *E-mail:* tongaj@mail.tsinghua.edu.cn

Synthesis and characterization of compound 1



Scheme S1 Synthesis of compound **1**.

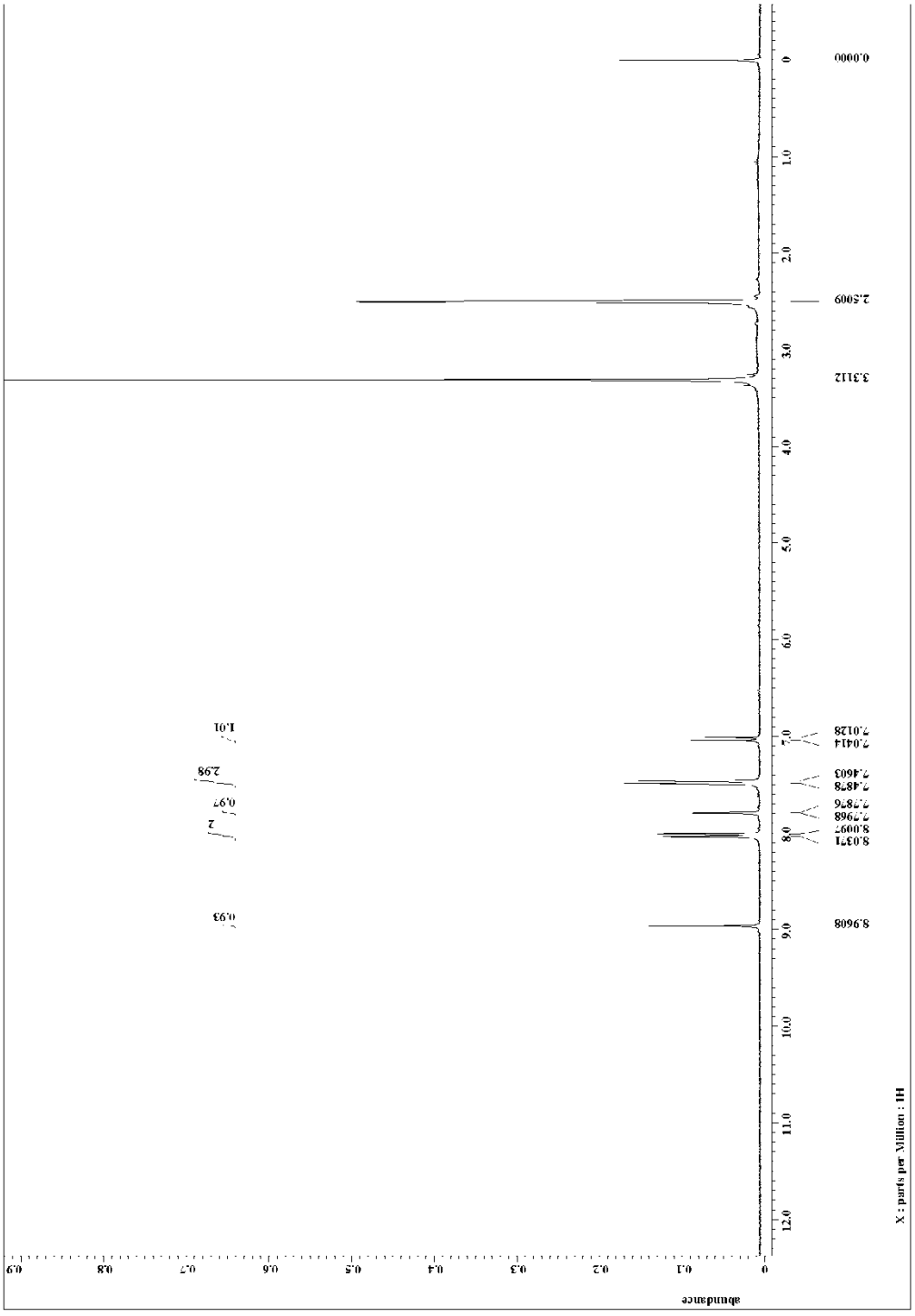


Fig. S1 ^{1}H-NMR spectra of **1** in DMSO - d_6.

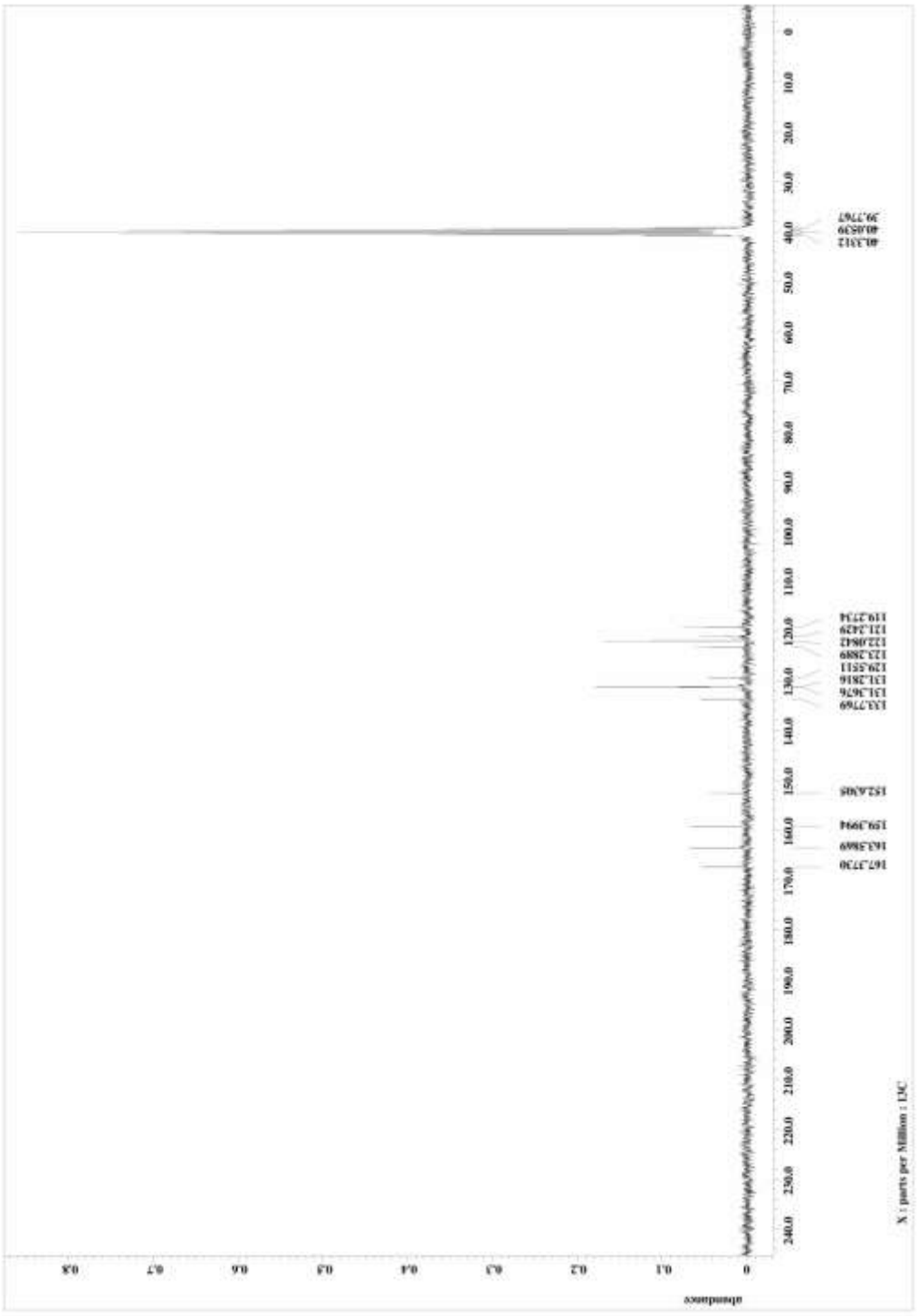


Fig. S2 ^{13}C-NMR spectra of **1** in DMSO - d_6.

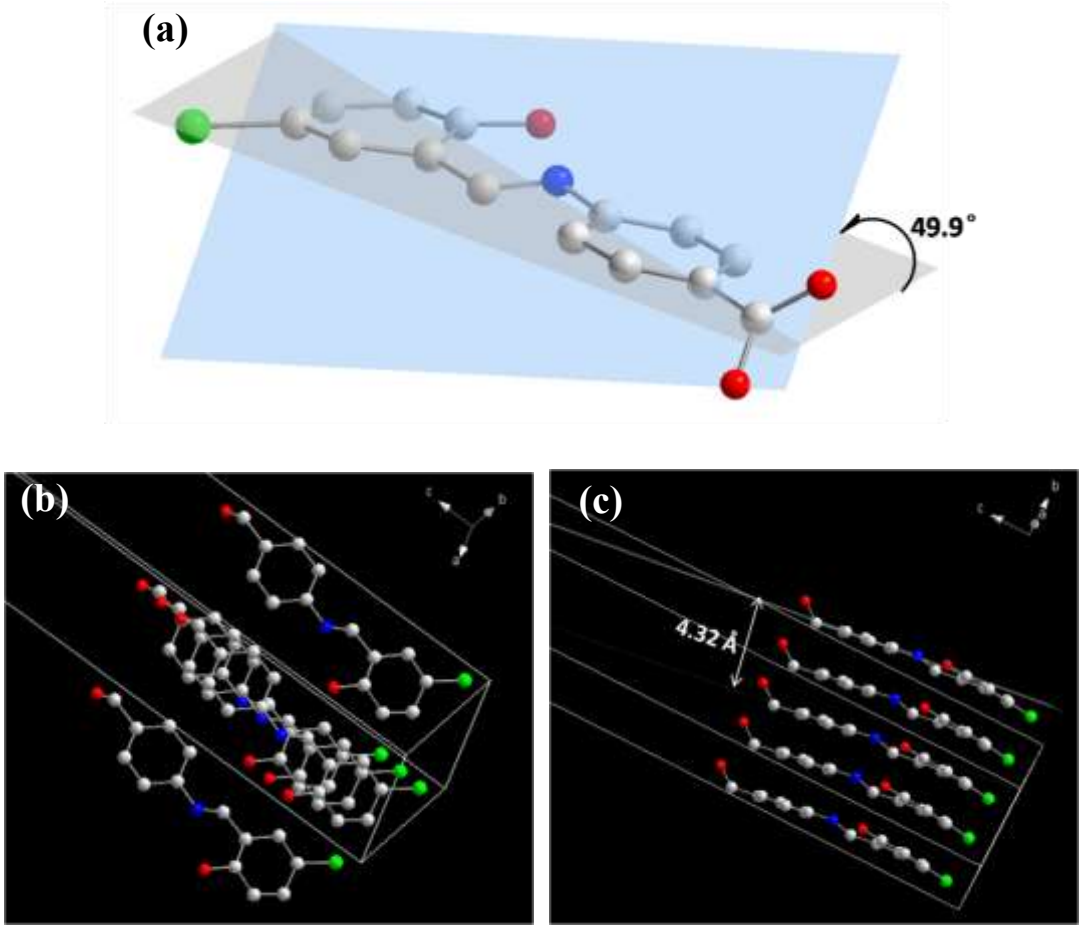


Fig. S3 (a) Single X-ray crystallographic picture of compound **1** with a dihedral angle of 49.9 º for phenyl and carboxyl groups; (b, c) packing views showed the inter-plane distances between adjacent molecular.

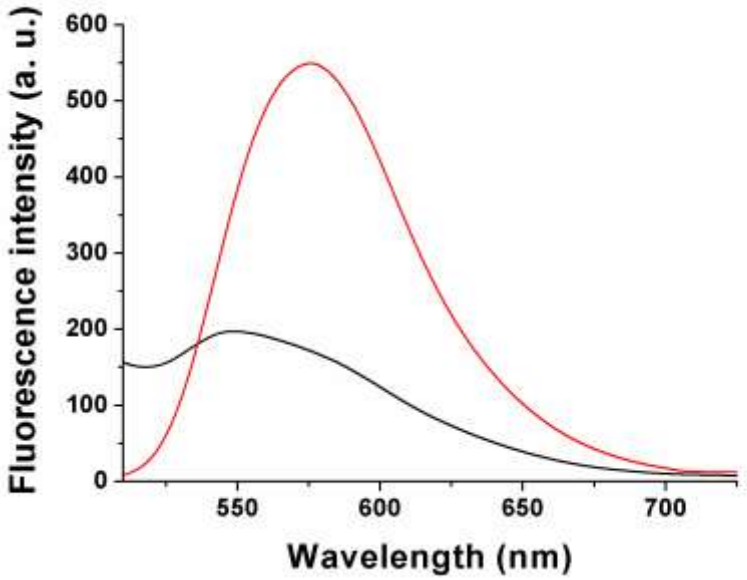
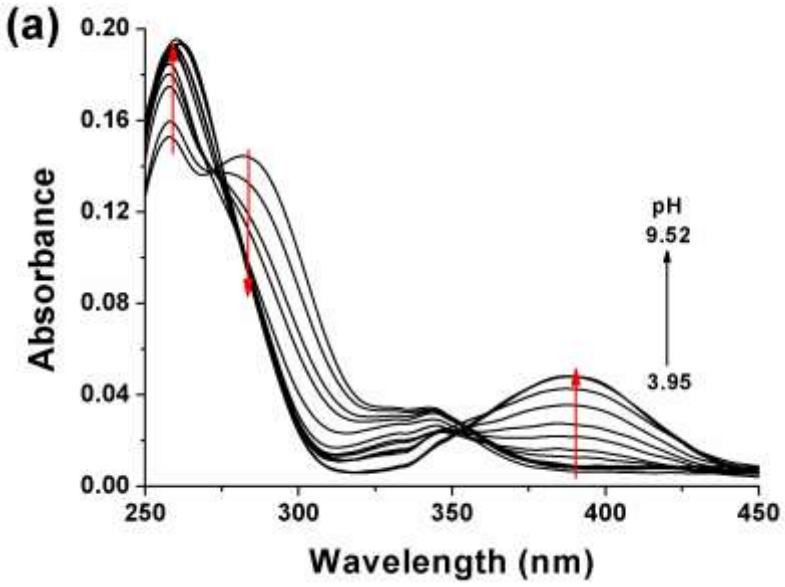


Fig. S4 Fluorescence spectra of compound **1** in powder (red line) and crystal (black line) states. Excitation was performed at 381 nm.



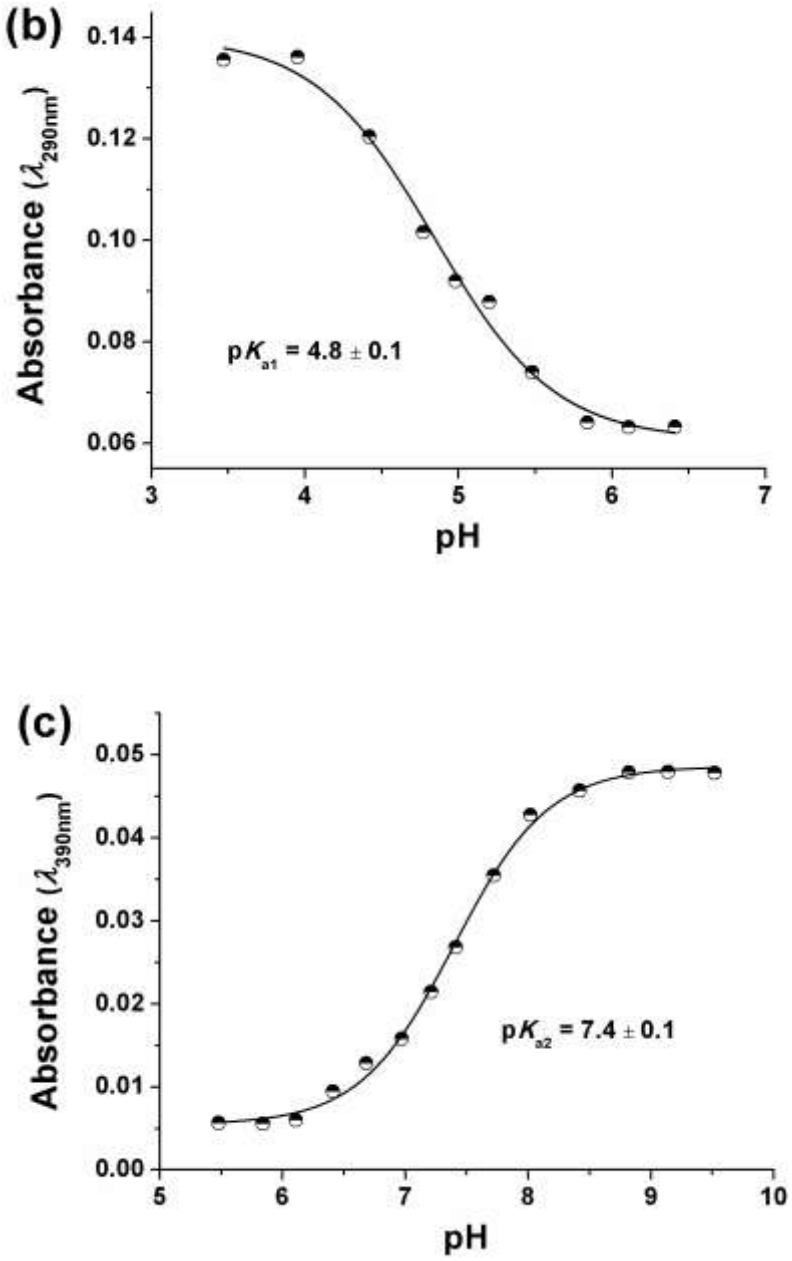


Fig. S5 (a) Absorption spectra of **1** (10 μM) in sodium phosphate buffer at different pH conditions; (b, c) absorption titration against pH at 290 nm and 390 nm. From acidic to basic pH conditions: 3.95, 4.42, 4.77, 4.98, 5.20, 5.48, 5.84, 6.11, 6.41, 6.68, 6.97, 7.21, 7.41, 7.72, 8.02, 8.42, 8.82, 9.14, and 9.52.

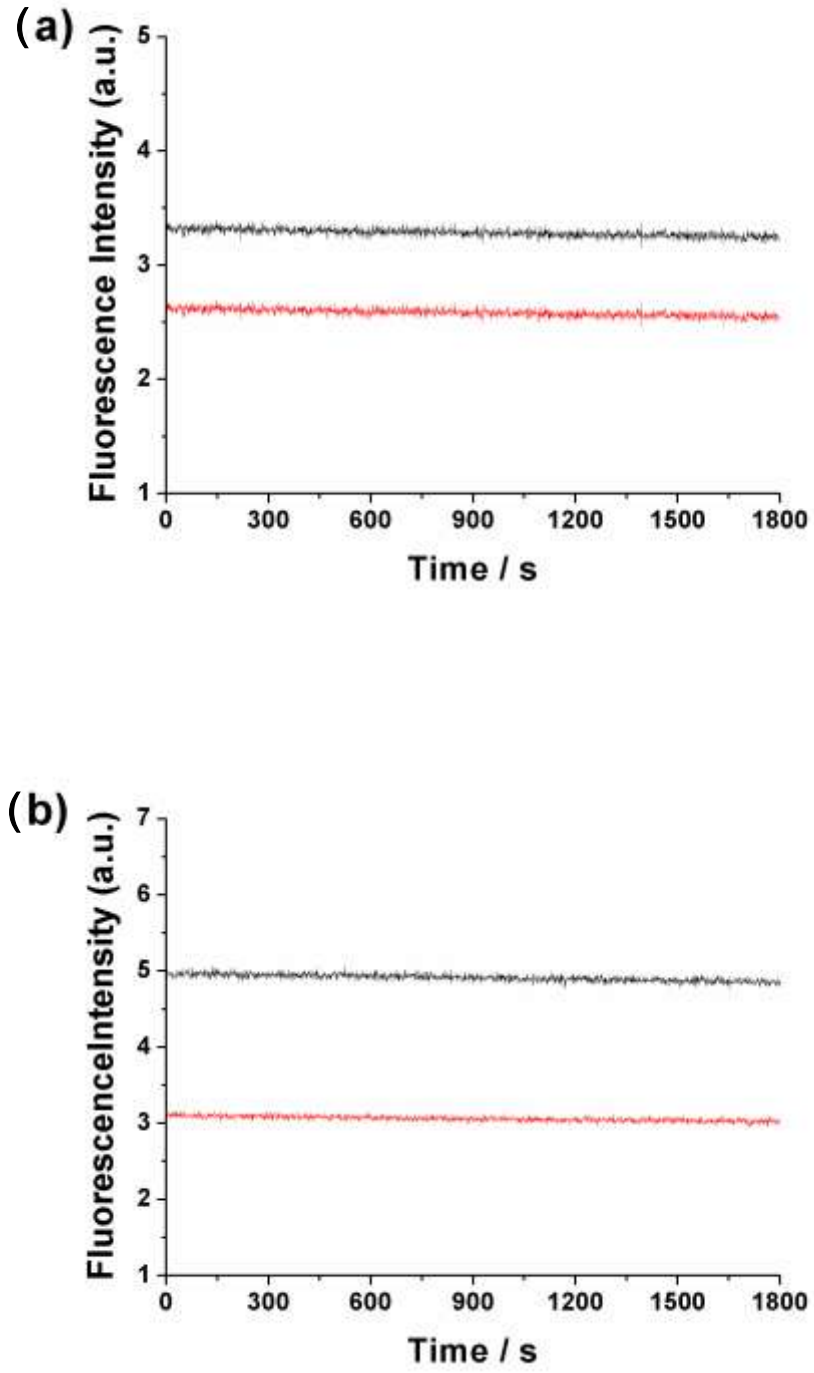


Fig. S6 The time-course fluorescence intensity of **1** (60 μM) in sodium phosphate buffer solution with pH 6.00 (a) and 7.00 (b) at 37 °C. Black line: λ_{ex} = 353 nm and λ_{em} =516 nm; Red line: λ_{ex} = 353 nm and λ_{em} =559 nm.

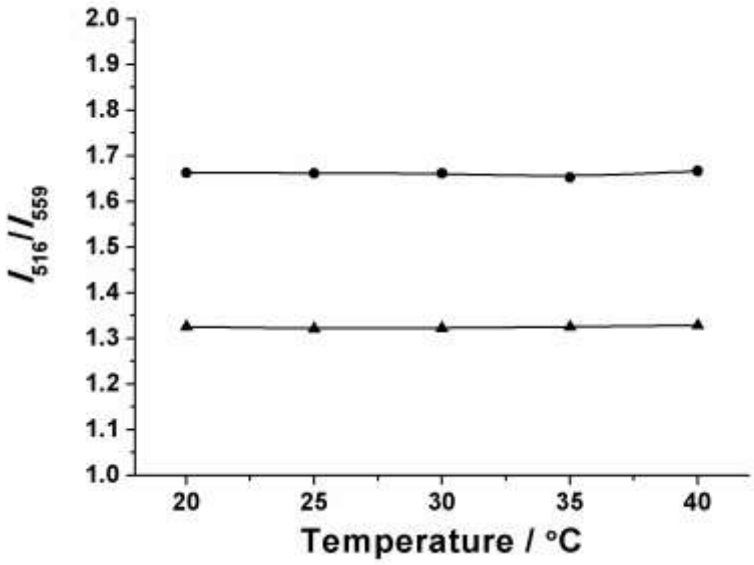


Fig. S7 Ratio of fluorescence intensity at 516 nm and 559 nm of **1** (60 μM) with various temperatures at pH 7.00 (●) and 6.00 (▲). Excitation was performed at 353 nm.

Application of 1 in real water sample analysis

For the measurement of real water samples, 2.94 mL of toning lotion, river water or snow water (River water and snow were collected in Tsinghua University and filtrated before use. Toning lotion was purchased from campus supermarket in Tsinghua University), and 0.06 mL of stock solution of **1** were mixed in a 1 cm quartz cell for 5 min, respectively. Then the fluorescence measurements were performed with excitation wavelength at 353 nm.

Table S1. Measurement of pH in real water samples

Sample	pH known [a]	pH found [b]	R.S.D.(%) [c]
Toning lotion	6.30	6.25	1.35
River water	6.17	6.20	2.53
Snow water	5.98	6.02	2.70

[a] Measured by a METTLER TOLEDO 320 pH meter.

[b] Obtained from the ratiometric calibration curve of $I_{516\ nm}/I_{559\ nm}$.

[c] Relative standard deviations of pH found when n=3.

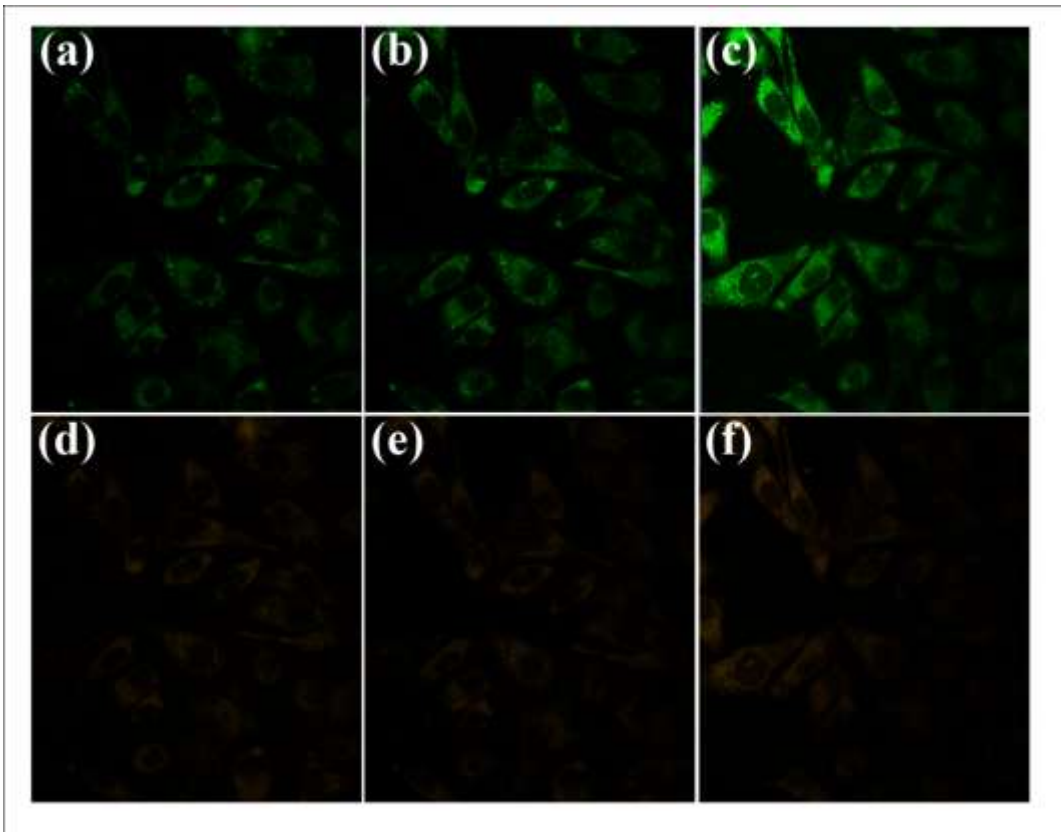


Fig. S8 Confocal fluorescence images of H^+ in HepG2 cells after incubation with **1** (60 μM) and nigericin at 37 ºC (λ_{ex} = 405 nm). Top: collected at channel I (490 - 535 nm) at pH 5.5 (a), 6.0 (b), and 6.6 (c); bottom: collected at channel II (540 - 585 nm) at pH 5.5 (d), 6.0 (e), and 6.6 (f).