Electronic Supplementary Information
for

A biotin-guided formaldehyde sensor selectively detecting endogenous concentrations in cancerous cells and tissues

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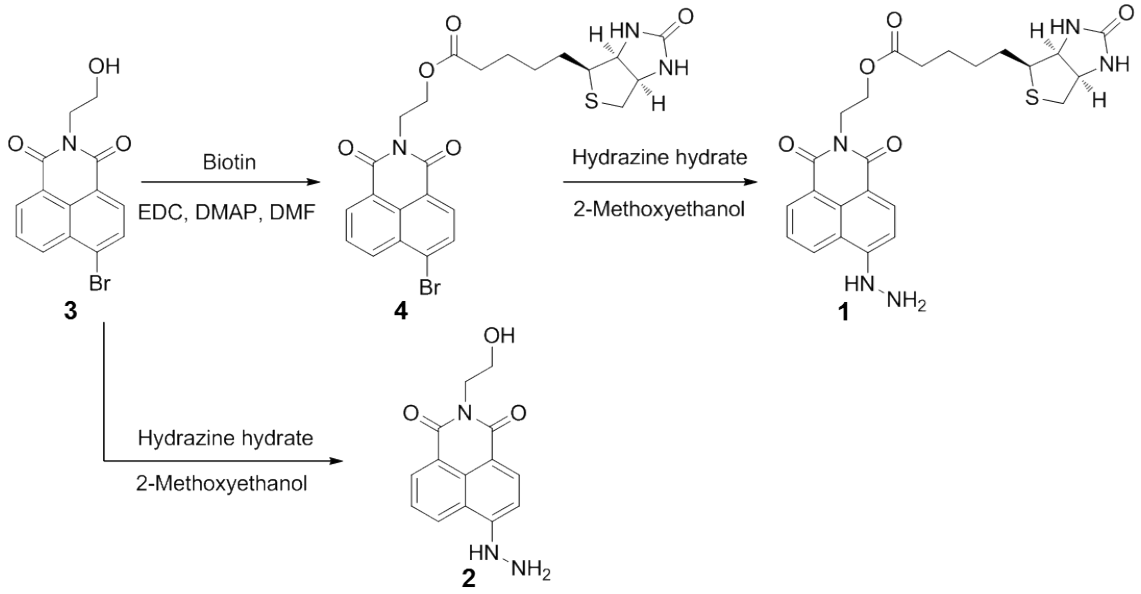
General information and materials. All reactions were carried out under argon atmosphere with flame-dried glassware. Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography. Analytical thin layer chromatography was performed using Merck 60 F254 silica gel (precoated sheets, 0.25 mm thick). All other reagents were purchased from Sigma-Aldrich and were used as received. All fluorescence and UV/Vis absorption spectra were recorded with Shimadzu RF-5301PC and Scinco S-3100 spectrophotometers, respectively. All ^{1}H and ^{13}C NMR spectra were collected in DMSO-d_6 on a Varian 400 MHz spectrometer. All chemical shifts are reported in ppm value using the signals of TMS as an internal reference. ESI mass spectral analyses were carried out using LC/MS-2020 Series (Shimadzu). Reverse-phase HPLC experiments were conducted using an Young Lin HPLC system (YL9100) equipped with a VDSpher 100 C18-E column (5 μm, 250 × 4.6 mm) operated at a flow rates of 1 mL/min using a mobile phase consisting of a binary gradient of solvent A (water with 0.1% *v*/*v* TFA) and solvent B (acetonitrile with 0.1% *v*/*v* TFA).

Spectroscopic data. Stock solutions of **1** (1.0 mM) and **2** (1.0 mM) were prepared in dimethyl sulfoxide (DMSO). Stock solutions (10.0 mM) of 4-methoxybenzaldehyde, 4-nitro-benzaldehyde, acetaldehyde, and trichloroacetaldehyde were prepared in DMSO and glucose, glyoxal, H_2O_2, GSH, $MgCl_2$, $CaCl_2$, Na_2S, tert-butyl hydroperoxide, and $NaHSO_3$ were prepared in twice distilled water. Samples for absorption and emission measurements were contained in quartz cuvettes (4 mL volume). For all measurements of fluorescence spectra, excitation was at 430 nm with all the excitation and emission slit widths at 3.0 nm (10.0 μM of **1**) or 5.0 nm (1.0 μM of **1**). All spectroscopic measurements were performed under physiological conditions (PBS buffer containing 1% of DMSO, pH 7.4, 37 °C).

Synthesis



Scheme S1. Synthetic pathway for probe **1** and **2**.

The compound **3** was prepared according to the previously reported methods with minor modifications.[S1]

Synthesis of **4**

A mixture of biotin (0.31 g, 1.3 mmol), EDC (0.74 g, 3.8 mmol), and DMAP (0.47 g, 3.8 mmol) in anhydrous DMF was stirred under argon atmosphere for 30 min at room temperature. A solution of **3** (1.21 g, 3.8 mmol) in DMF was then added to the mixture. The reaction mixture was stirred overnight. After removal of solvent, the crude product was purified over silica gel using CH_2Cl_2:MeOH (9/1 *v*/*v*) as the eluent to yield **4** as a white solid (0.49 g, 68%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.55-8.50 (m, 2H), 8.30 (d, J = 8.0 Hz, 1H), 8.19 (d, J = 8.0 Hz, 1H), 7.96 (dd, J_1 = 7.3 Hz, J_2 = 1.1 Hz, 1H), 6.37 (s, 1H), 6.34 Hz (s, 1H), 4.30-4.27 (m, 4H), 4.25-4.22 (m, 1H), 4.01-3.97 (m, 1H), 2.87-2.83 (m, 1H), 2.74-2.70 (m, 1H), 2.51 (d, J = 12.6 Hz, 1H), 2.16 (t, J = 7.2 Hz, 2H), 1.40-1.36 (m, 2H), 1.34-1.26 (m, 2H), 1.18-1.12 (m, 2H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 173.5, 163.7, 163.7, 163.3, 133.5, 132.4, 132.1, 131.8, 130.5, 130.0, 129.6, 129.0, 123.3, 122.5, 61.6, 61.4, 59.8, 56.0, 40.7, 39.6, 33.9, 28.6, 28.5, 25.0 ppm. ESI-MS: *m/z* ($M+Na^+$) calcd 568.05, found 568.05.

Synthesis of **2**

A mixture of **3** (165 mg, 0.5 mmol) and 1.5 mL of 98% hydrazine hydrate in 2-methoxyethanol was heated at 70 ^{o}C for 2 h. After cooling to room temperature, the precipitated product was filtered, washed with ether, and dried to afford the desired product as an orange solid (111 mg) with a yield of 80%. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 9.15 (s, 1H), 8.62 (d, J = 8.5 Hz, 1H), 8.42 (d, J = 6.9 Hz, 1H), 8.30 (d, J = 8.5 Hz, 1H), 7.65 (t, J=8.0 Hz, 1H), 7.26 (d, J = 8.5 Hz, 1H), 4.84-4.80 (m, 1H), 4.70 (s, 2H), 4.13 (t, J = 6.5 Hz, 2H), 3.61-3.57 (m, 2H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 164.6, 163.7, 153.8, 134.9,

131.2, 130.0, 128.9, 124.8, 122.5, 119.1, 108.1, 104.6, 58.6, 42.0 ppm. ESI-MS: *m/z* ($M-H^+$) calcd 270.09, found 269.75.

Synthesis of **1**

A mixture of **4** (200 mg, 0.4 mmol) and 1.0 mL of 98% hydrazine hydrate in 2-methoxyethanol was heated at 70 ^oC for 1 h. After cooling to room temperature, the precipitated product was filtered, washed with ether, and dried to afford the desired product as an orange solid (150 mg) with a yield of 82%. ^1H NMR (DMSO-d_6, 400 MHz): δ 8.90 (s, 1H), 8.56 (d, J = 8.5 Hz, 1H), 8.36 (d, J = 8.5 Hz, 1H), 8.24 (d, J = 8.5 Hz, 1H), 7.58 (t, J = 8.0 Hz, 1H), 7.19 (d, J = 8.5 Hz, 1H), 6.41 (s, 1H), 6.34 (s, 1H), 4.77 (t, J = 6.3 Hz, 1H), 4.64 (s, 2H), 4.28-4.25 (m, 1H), 4.10-4.06 (m, 2H), 3.56-3.51 (m, 2H), 3.07-3.02 (m, 1H), 2.80-2.76 (m, 1H), 2.53 (d, J = 12.6 Hz, 1H), 1.96 (t, J = 7.2 Hz, 2H), 1.57-1.53 (m, 2H), 1.47-1.40 (m, 2H), 1.28-1.21 (m, 2H) ppm. ^{13}C NMR (DMSO-d_6, 100 MHz): δ 172.2, 164.6, 163.7, 163.4, 153.8, 134.9, 131.2, 130.0, 128.9, 124.8, 122.5, 119.1, 108.1, 104.6, 61.7, 59.8, 58.6, 56.1, 42.0, 40.5, 33.9, 28.9, 28.7, 25.9 ppm. ESI-MS: *m/z* ($M+K^+$) calcd 536.66, found 536.00.

Cell culture

4T-1(biotin-receptor positive cells), a mouse breast cancer cell line and NIH 3T3 (biotin-receptor negative cells), a mouse embryo fibroblast cell line were cultured with DMEM (Dulbecco's Modified Eagle Medium, Gibico, Grand Island, USA) supplemented with 10% FBS (fetal bovine serum, Tianhang Biotechnology Co.,Ltd., Hangzhou, China) at 37 °C in a 5% CO_2 and 95% air environment.

The day before experiments, both cells were seeded into 20 mm glass dish (Nest, Nest Biotechnology Co., Ltd, Wuxi, China) at a density of 4×10^4 for each dish. After 24 h, cell confluence was up to about 60% for experiments.

Exogenous detection of FA in 4T-1 and NIH 3T3 cells with probes 1 and 2

Before experiments, the cells were washed with PBS twice. 40 μM FA was added into cells and incubated for 20 min, then washed with PBS and 20 μM probes **1** and **2** were put into cells and incubatied for another 20 min. Before detection, the cells were washed with PBS, One-photon and two-photon imaging were achieved with Nikon confocal and multiphoton microscopes (Nikon A1 MP) with a 40× objective. For one-photon imaging, the excitation wavelength was 488 nm, and the detection window was 500-550 nm. For two-photon imaging, the excitation wavelength was 800 nm, the detection window was 500-550 nm.

Endogenous detection of FA in 4T-1 and NIH 3T3 cells with probes 1 and 2

Before the experiments, the cells were washed with PBS twice. 20 μM probes **1** and **2** were put into the cells for incubating another 20 min, then washed with PBS twice for imaging with Nikon confocal and multiphoton microscopes (Nikon A1 MP) with 40× objective. For one-photon imaging, the excitation wavelength was 488 nm, and the detection window was 500-550 nm. For two-photon imaging, the excitation wavelength was 800 nm, and the detection window was 500-550 nm.

Competitive experiments with Biotin for endogenous detection

Before the experiments, the cells were washed with PBS twice. 400 μM biotin (10mM stock solution in 100% DMSO) was pre-incubated into 4T-1 for 30 min, then 20 μM probe **1** or **2** was added to the medium and incubated for another 20 min. For one-photon imaging, the excitation wavelength was 488 nm, and the collected wavelength was 500-550 nm. For two-photon imaging, the excitation wavelength was 800 nm, and the detection window was 500-550 nm.

Inhibition experiments by $NaHSO_3$

Before the experiments, the cells were washed with PBS twice. 50 μM $NaHSO_3$ was pre-incubated with cells for 30 min, then the cells were washed with PBS twice, and 20 μM probes **1** and **2** were added into the medium and incubated for another 20 min. Before detection, the cells were washed once more with PBS. One-photon and two-photon imaging were achieved with Nikon confocal and multiphoton microscopes (Nikon A1 MP) with 40× objective.

Animal tumor models and tissue slice treatment

4 weeks old Balb/c female mice of were purchased from Shandong University Laboratory Animal Centre (Shandong, China) and all animals received, were supplyed with plenty of compound feed and sterile water. For establishment of the tumor models, 2×10^6 of 4T-1 cells were injected subcutaneously into the right flank of the mice. After two more weeks, the volume of the tumor reached about 50 mm^3, and the mice were anesthetized for tumor removal. The tumor slices were cut into 500 μm thickness with a vibrating-blade microtome, and then the slices were incubated with 20 μM probes for 1h at 37 °C. Before imaging, the tissue slices treated with the probes were washed with PBS for three times.

Two-Photon imaging for detecting FA in tumor tissue slices

Two-photon imaging of the probe in tissue slices were obtained with a Nikon confocal and multiphoton microscope (Nikon A1 MP) with 40× objective. For two-photon imaging, the excitation wavelength was 800 nm, and the detection window was 500-550 nm.

Cell cytotoxicity assay by MTT

The toxicity of the probes was tested by an MTT (Solabio Life Sciences, Beijing, China) assay. The day before the experiments, 4×10^3 of 4T-1 cells and 6×10^3 of 3T3 cells in 100 μL culture were seed into 96 well-plates. Next day, 100 μL culture contained various concentrations of probes (0, 1, 5, 10, 20, 30 μM) were added into the cells instead of the old culture. After incubation for 24 h, 10 μL MTT (5 mg/mL in PBS) was added into the each well and incubated for another 4 h. Then, the culture was removed and replaced with 100 μL DMSO, and the plates were put onto a shaker for 20 min at 110 rpm to dissolve the formazan crystals. The absorbance was measured at 570 nm with Microplate Reader (Thermo Fisher Scientific, USA). The cell viability (%) = (OD_{570} (Experiments) − OD_{570} (Blank)) / (OD_{570} (Control) − OD_{570} (Blank)). OD_{570} (Experiments) denotes cells treated with the probes of various concentrations; OD_{570} (Control) denotes cells without the probes; OD_{570} (Blank) denotes plates without the cells treated with the same OD_{570} (Control). Each concentration was conducted with five parallel samples, and the results were expressed as mean ± standard deviation (SD).

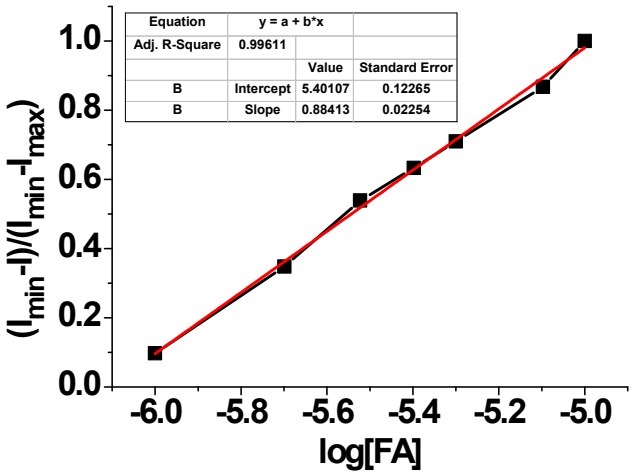


Fig. S1. The detection limit of **1** for FA was calculated from the normalised response of the fluorescence signal with variable concentrations of FA.

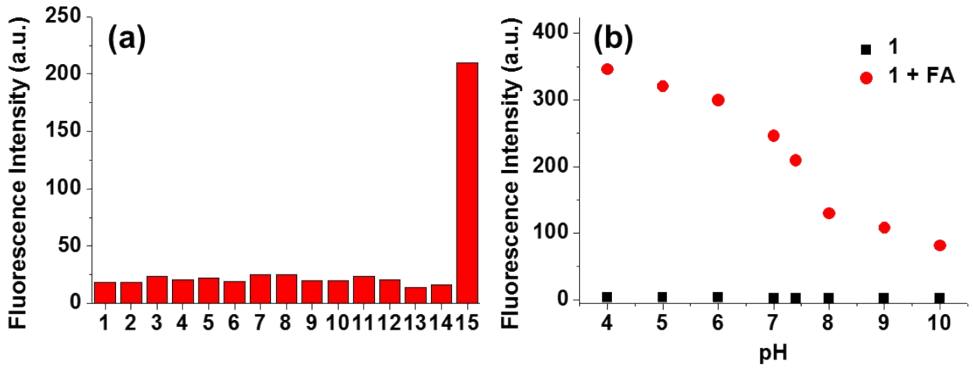


Fig. S2. Fluorescence responses of **1** (10 μM) at 541 nm with (a) various relevant analytes (100 μM) [(1) Only probe **1**; (2) 4-methoxybenzaldehyde; (3) 4-nitro-benzaldehyde; (4) acetaldehyde; (5) trichloroacetaldehyde; (6) glucose; (7) glyoxal; (8) H2O2; (9) GSH (10) MgCl2; (11) CaCl2; (12) Na2S; (13) tert-Butyl hydroperoxide; (14) NaHSO3; (15) FA], and (b) different pH values in the absence (■) or presence (●) of FA (100 μM) after 15 min of incubation at 37 °C in PBS buffer (pH 7.4, 1% DMSO) upon excitation at 430 nm.

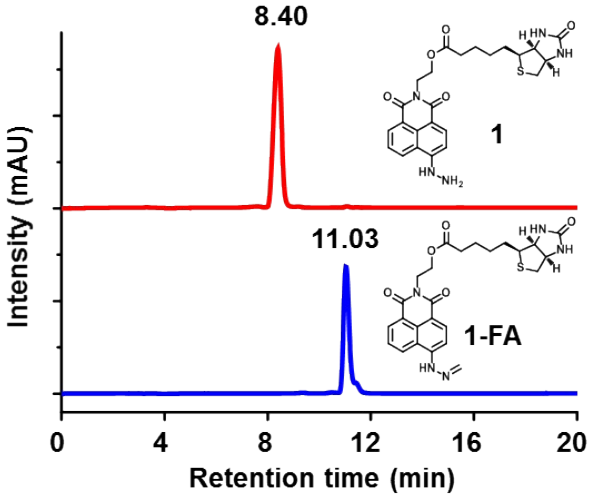


Fig. S3. HPLC analysis of free **1** (red) and **1** with FA (blue) at a single wavelength (430 nm) under gradient conditions (85 to 100 % B for 17 min, then 100% B for 3 min (A = H_2O + 0.5% TFA, B = acetonitrile + 0.5% TFA)).

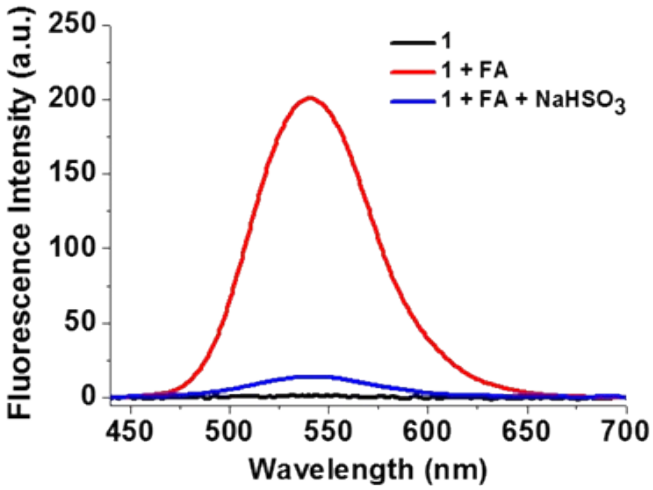


Fig. S4. Fluorescence spectra of the **1** (10 μM) under three different conditions, only probe, 100 μM of FA, and 100 μM of FA pretreated with $NaHSO_3$ (300 μM).

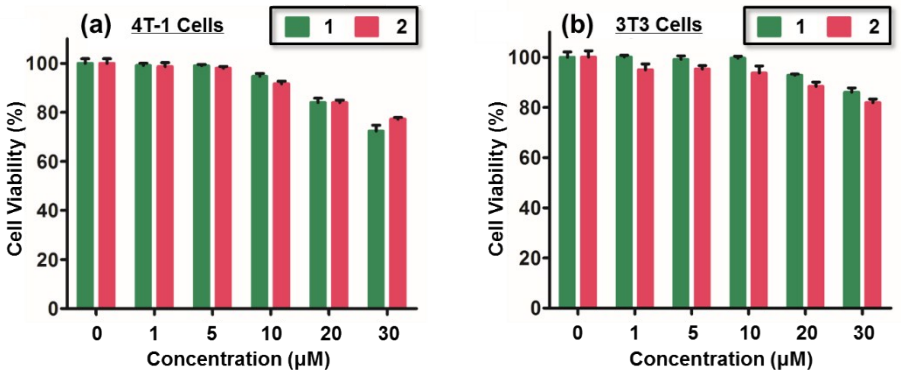


Fig. S5. Cell cytotoxicity of the probes **1** and **2** in (a) 4T-1 cells and (b) 3T3 cells by MTT assay for 24 h. Each concentration was set five parallel trials, and the results were expressed as mean ± standard deviation (SD).

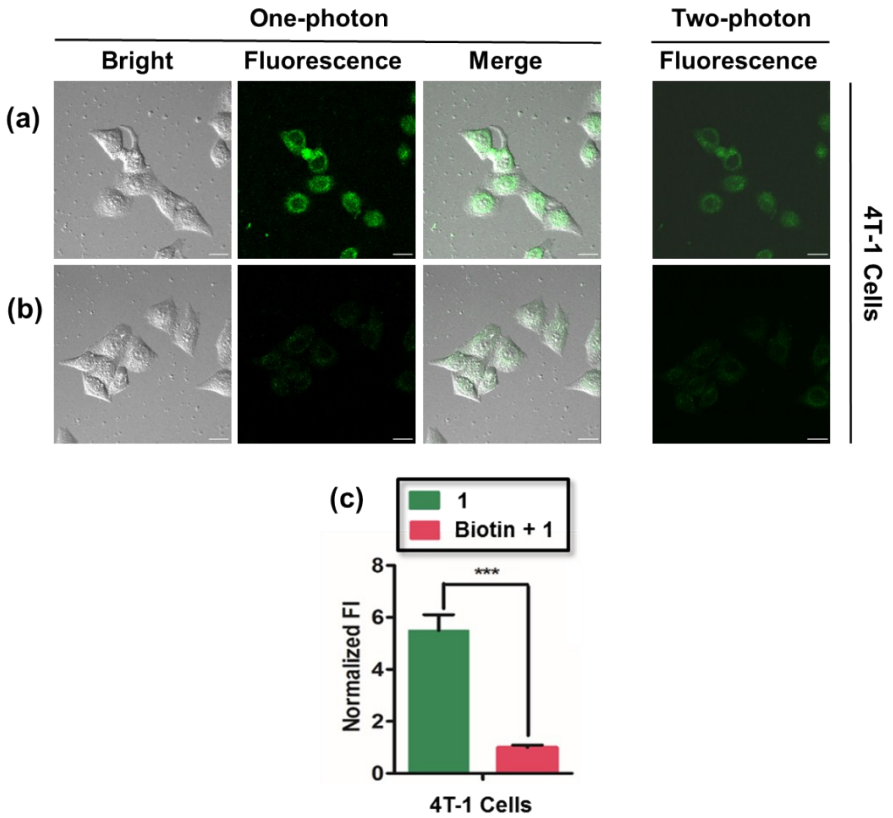


Fig. S6. Imaging of endogenous FA detection with biotin-competitive binding for the probe **1** in 4T-1 cells by one-photon and two-photon modes. (a) 4T-1 cells treated with 20 μM probe **1** for 20 min; (b) 4T-1 cells treated with 400 μM biotin for 30 min and then treated with 20 μM probe **2** for 20 min. Scale bar: 20 μm. (c) Normalised mean fluorescence intensities (F.I.) of 4T-1 cells treated with 20 μM probe **1** for 20 min or 400 μM biotin for 20 min and 20 μM probe **1** for 20 min. The F.I. was normalised *vs.* **1**+ Biotin. Error bars denote SD, n=4. ***P< 0.001.

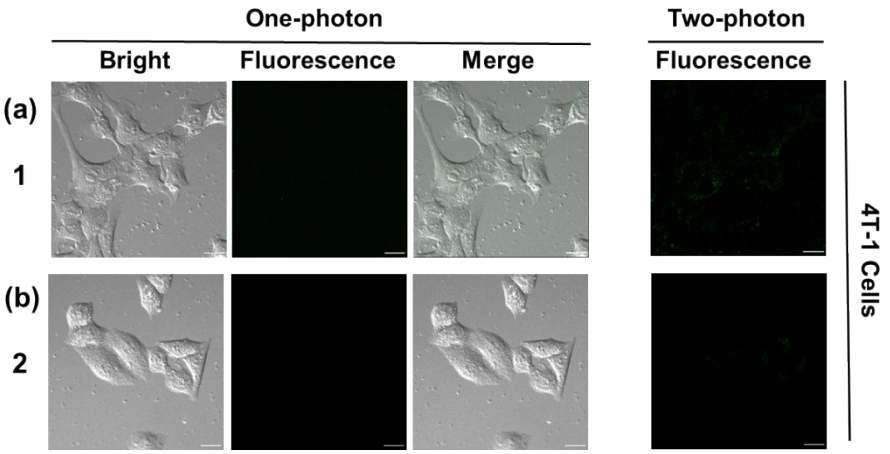


Fig. S7. Imaging of inhibitory experiments with the probes **1** and **2** by the inhibitor of $NaHSO_3$ in 4T-1 by one-photon and two-photon modes. (a) 4T-1 cells treated with 50 μM $NaHSO_3$ for 30 min and then treated with 20 μM probe **1** for 20 min; (b) 4T-1 cells treated with 50 μM $NaHSO_3$ for 30 min and then incubated with 20 μM probe **2** for 20 min.

[1]H-NMR, [13]C-NMR and ESI-MS Analyses

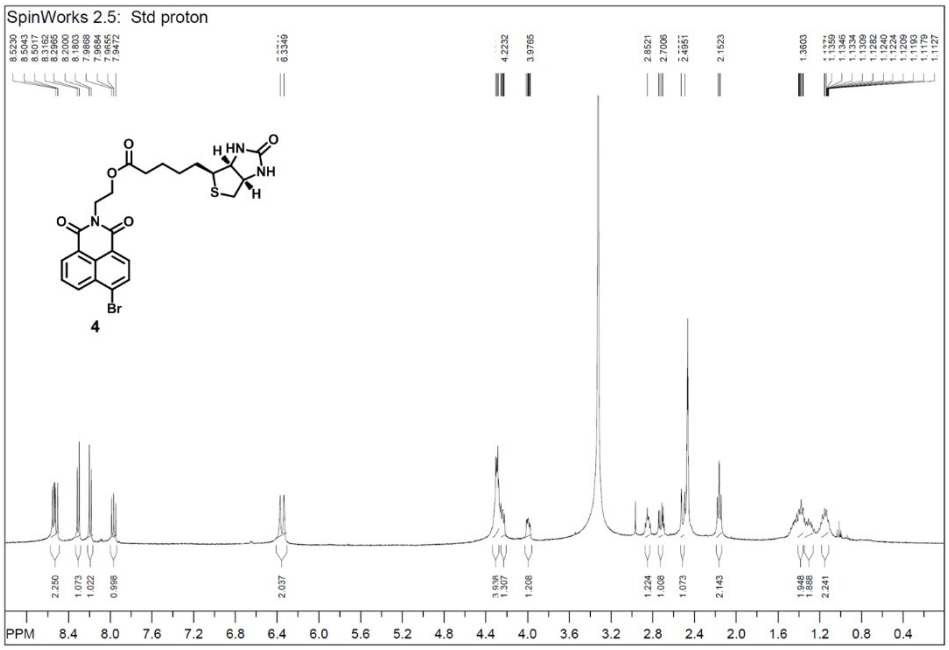


Fig. S8 [1]H NMR spectrum of **4** in DMSO.

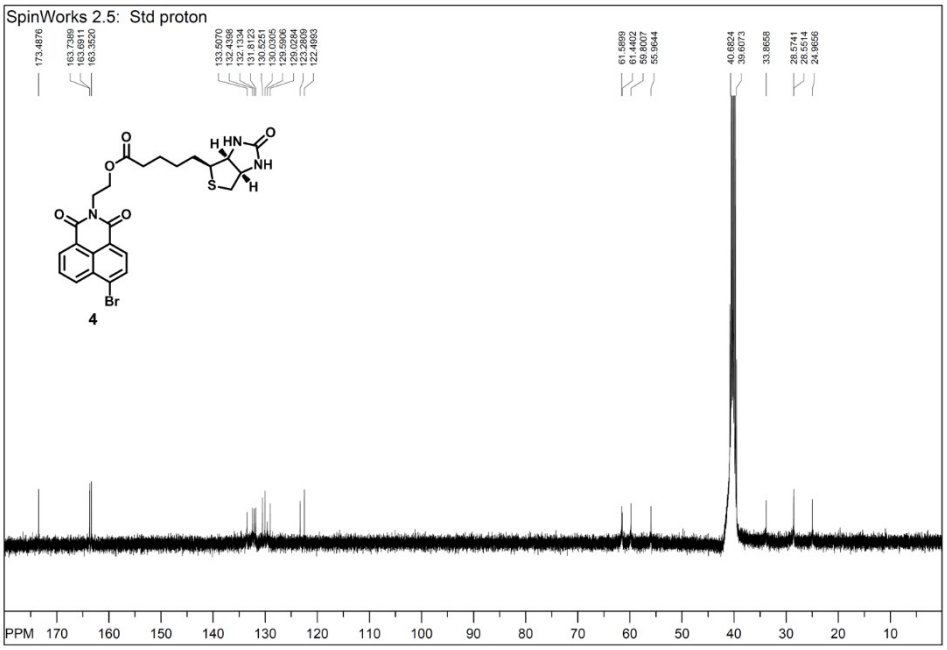


Fig. S9 [13]C NMR spectrum of **4** in DMSO.

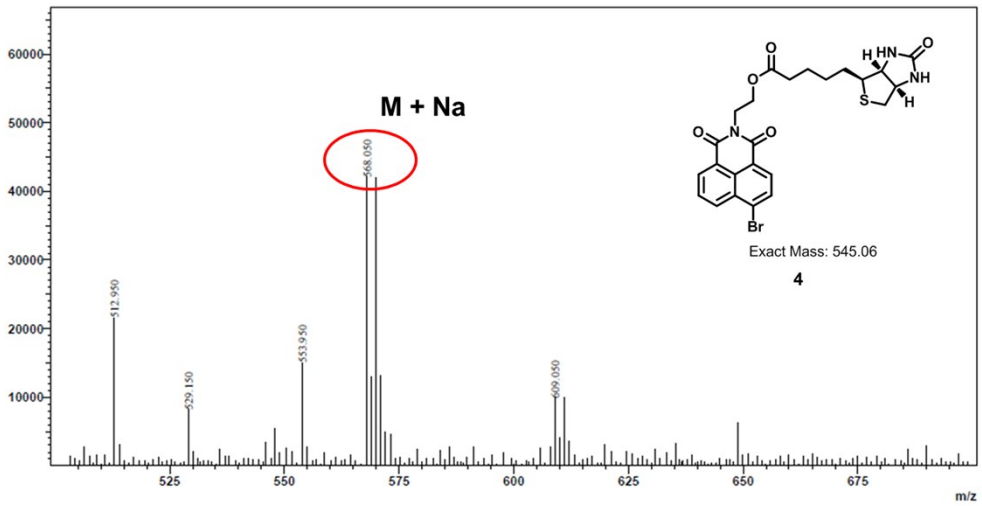


Fig. S10 ESI-MS spectrum of **4**.

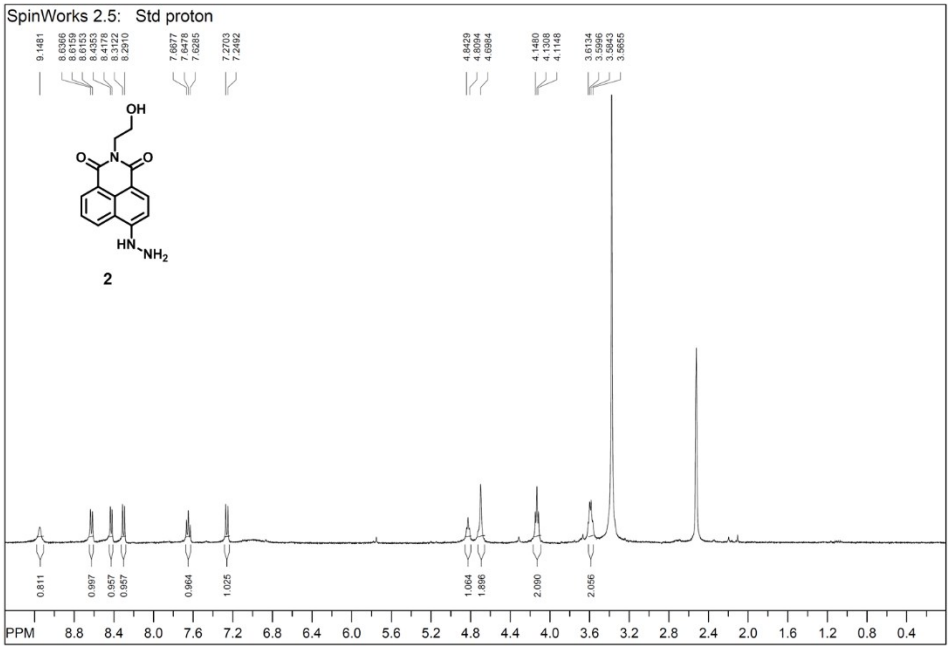


Fig. S11 ^{1}H NMR spectrum of **2** in DMSO.

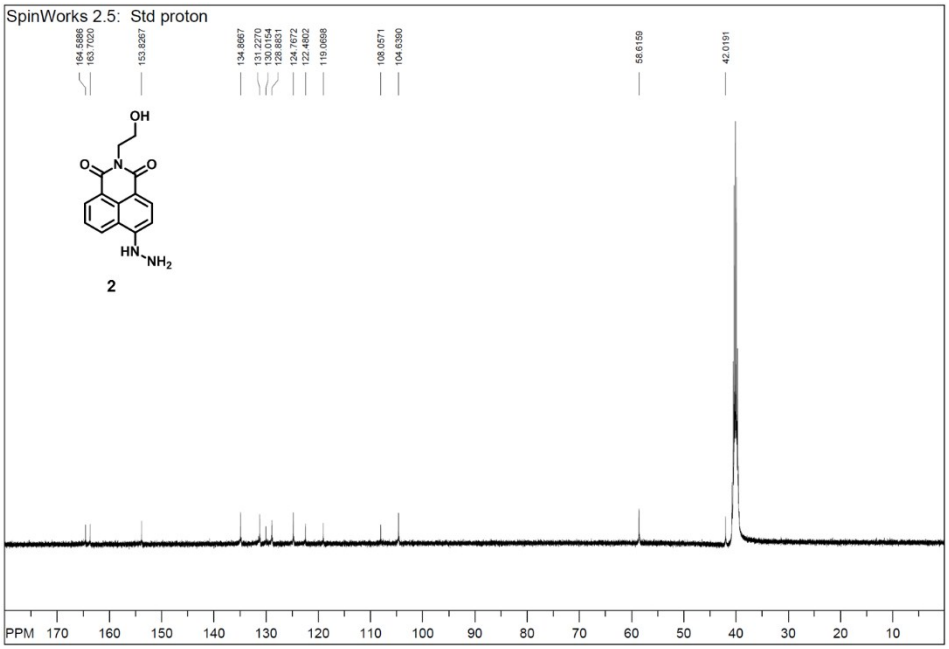


Fig. S12 ^{13}C NMR spectrum of **2** in DMSO.

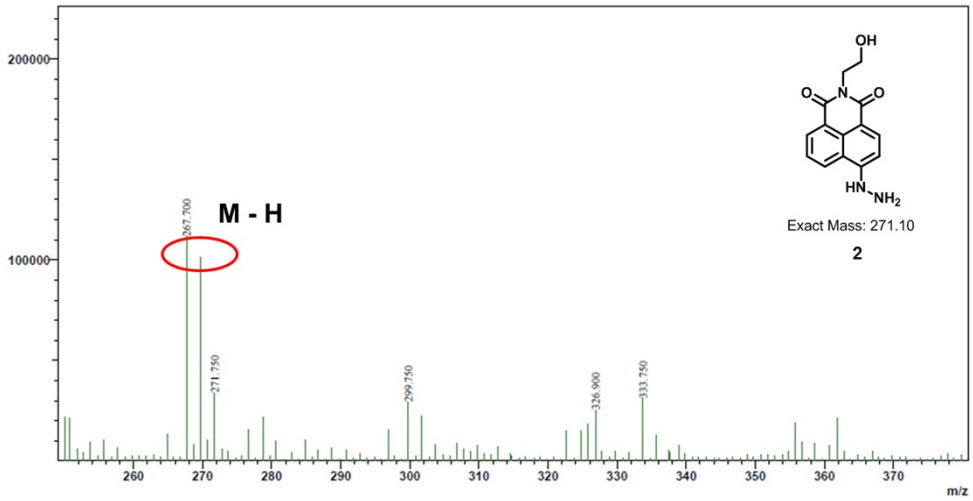


Fig. S13 ESI-MS spectrum of **2**.

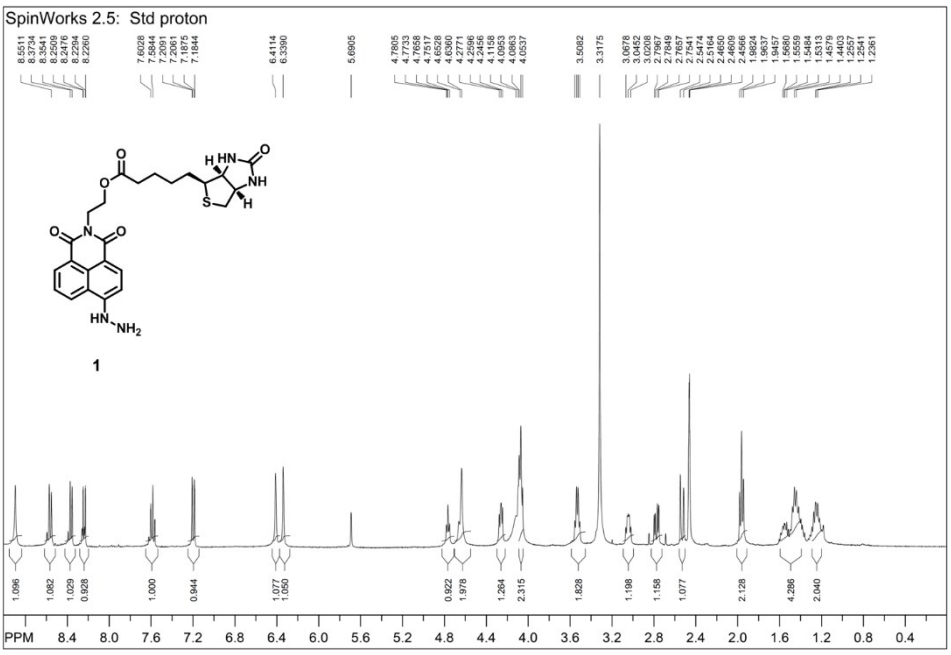


Fig. S14 ^{1}H NMR spectrum of **1** in DMSO.

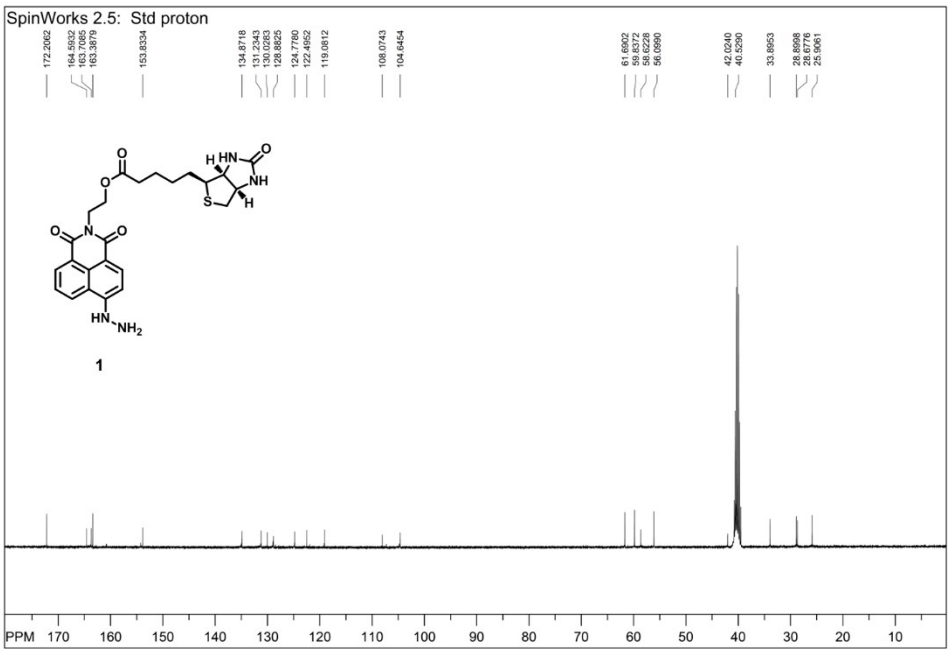


Fig. S15 ^{13}C NMR spectrum of **1** in DMSO.

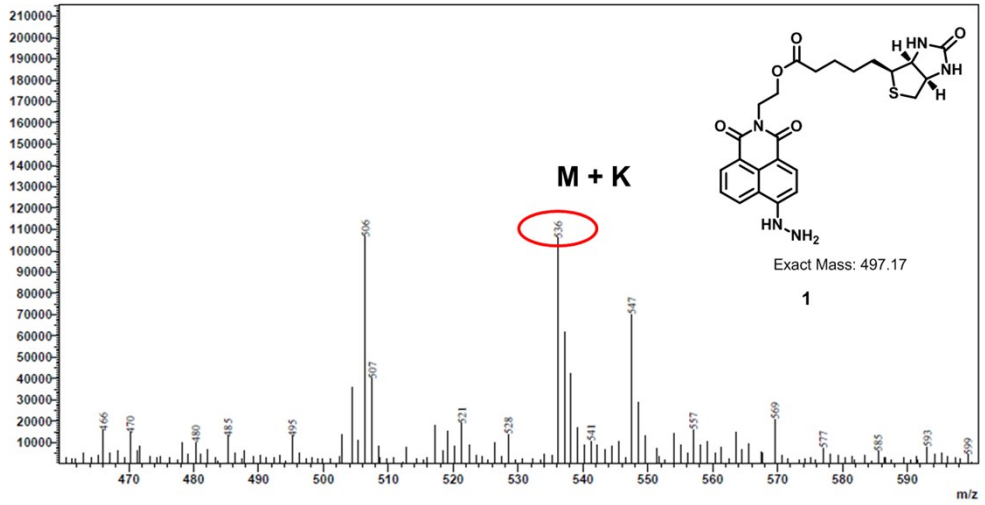


Fig. S16 ESI-MS spectrum of **1**.

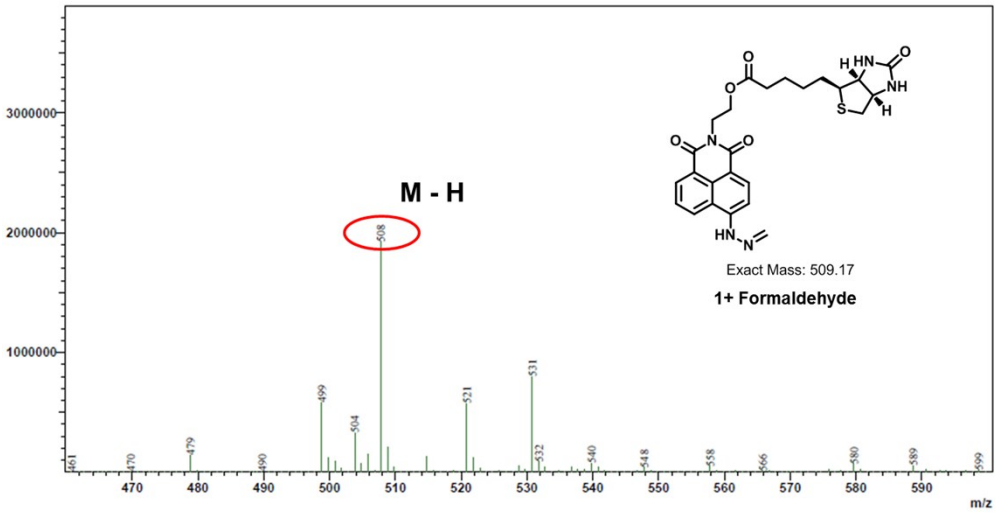


Fig. S17 ESI-MS spectrum of **1** with formaldehyde.

References

S1. X. Wan, T. Liu and S. Liu, *Langmuir*, 2011, **27**, 4082.