A novel colorimetric and fluorescent turn-on pH sensor with notably large Stokes shift for its application

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1. Synthesis route of probe DDTM.

Reagents and Conditions: (a) CH$_3$I, K$_2$CO$_3$, DMF, 50°C, 24 h; (b) POCl$_3$, DMF, 90°C, reflux 5 h; (c) Na$_2$S* 9H$_2$O, Chloroacetaldehyde, K$_2$CO$_3$, DMF; (d) Malononitrile, Et$_3$N, CH$_2$Cl$_2$, R.T. 3 h;

1.1 Synthesis of compound 6- (dimethylamino)-3,4-dihydronaphthalen-1 (2H)-one

To a mixture of compound 6-amino-3,4-dihydronaphthalen-1(2H)-one (1.000 g, 6.2 mmol, 1.0 equiv.) and K$_2$CO$_3$ (1.900 g, 13.6 mmol, 2.2 equiv.) in DMF (40 mL) was added CH$_3$I (2.1 mL, 5.5 equiv.), and the mixture was stirred for 24 h at 50 °C. After being cooled to room temperature, H$_2$O was added and the solution was extracted with EtOAc (3×50 mL). The organic layer was dried with anhydrous Na$_2$SO$_4$ and removed under reduced pressure. The obtained residue was dried and purified by silica gel chromatography using PE: EtOAc = 6:1 as the eluent to obtain compound (Yield: 53.6%, R$_f$ = 0.46, PE: EtOAc = 2:1). $^1$H NMR (600 MHz, CDCl$_3$) $\delta$ 7.92 (d, J=8.9, 1H), 6.56 (dd, J=8.9, 2.5, 1H), 6.37 (d, J=2.0, 1H), 3.02 (s, 6H), 2.89-2.81 (m, 2H), 2.55-2.49 (m, 2H), 2.08 - 2.00 (m, 2H). $^{13}$C NMR (151 MHz, CDCl$_3$) $\delta$ 196.98, 153.59, 146.67, 129.65, 121.96, 110.37, 109.58, 40.23, 38.62, 30.74, 29.87, 23.73. HRMS (C$_{12}$H$_{15}$NO): calcd. for [M+H]$^+$ 190.1154; found: [M+H]$^+$ 190.1137.

1.2. Synthesis of compound 7- (dimethylamino)-4,5- dihydronaphtho[1,2-b] thiophene-2- carbaldehyde (DTC)

POCl$_3$ (0.21 mL, 2.2 mmol, 1.3 equiv.) was added dropwisely to a flask
containing DMF (5 mL) stirred at 0 °C over 30 min. compound 3 (0.3270 g, 1.7 mmol, 1.0 equiv.) in DMF (2 mL) was added slowly with stirring and the mixture was heated for 5 h at 90 °C. Then the mixture was poured into ice water using 20% NaOH solution to adjust the pH to alkalescence. After that the obtained solid was filtered and dried. To a solution of Na₂S•9H₂O (0.1850 g, 0.77 mmol, 1.1 equiv.) and DMF (7 mL) was added the above obtained intermediate (0.1650 g, 0.7 mmol, 1.0 equiv.). The mixture was stirred at 60 °C for 2 h. Then chloroacetaldehyde (0.05 mL, 0.77 mmol, 1.1 equiv.) was added rapidly and the reaction was stirred for 3 h at 60 °C, after that K₂CO₃ (0.106 g, 0.77 mmol, 1.1 equiv.) dissolved in water (1 mL) was added to the reaction. The mixture was stirred for 10 min at 60 °C. Then cooled at room temperature and quenched in water. The aqueous phase was extracted with EtOAc (3×50 mL). The organic layer was dried with Na₂SO₄ and concentrated under reduced pressure. The residue was purified by column chromatography to obtain the desired compound DTC (Yield: 42.2%, Rf = 0.46, PE: EtOAc = 3:1). ¹H NMR (600 MHz, CDCl₃) δ 9.53 (s, 1H), 7.14 (s, 1H), 6.44-6.11 (m, 3H), 2.77 (s, 6H), 2.69 (t, J=7.5, 2H), 2.59 (t, J=7.6, 2H). ¹³C NMR (151 MHz, CDCl₃) δ 182.23, 151.17, 148.46, 138.14, 137.58, 137.17, 135.37, 125.73, 125.30, 119.15, 111.51, 110.78, 40.44, 29.80, 23.96. HRMS (C₁₁H₁₅NOS): calcd. for [M+Na]+ 280.0874; found: [M+Na]+ 280.0771.

2. Equations used for the calculation of fluorescence quantum yield

The quantum yield (Φ) of DDTM denotes the fluorescence quantum yield. It was measured at room temperature referenced to quinine sulphate in aqueous solution of 0.1 M sodium hydroxide, which has a quantum yield of 0.54.

\[ \Phi_{F(X)} = \Phi_{F(S)} \times \left( \frac{A_X \times F_X}{A_S \times F_S} \right) \left( \frac{n_X}{n_S} \right)^2 \]

Φ₉(S) was quantum yield of quinine sulphate; Aₓ and Aₛ indicated the absorption intensity of the sample and the standard at the excitation wavelength, respectively; Fₓ and Fₛ for the sample and the standard fluorescence integral area; nₓ and nₛ was refractive index of the solvent of standard and unknown samples
3. Organic solvents and buffers

![Fluorescence emission spectra of probe DDTM (10 μM) in different solvents with PBS buffer (10 mM, pH 11.0). λ<sub>ex</sub> = 380 nm, slits: 5.0 nm / 5.0 nm, volt:700 v.]

**Fig. S1.** Fluorescence emission spectra of probe DDTM (10 μM) in different solvents with PBS buffer (10 mM, pH 11.0). λ<sub>ex</sub> = 380 nm, slits: 5.0 nm / 5.0 nm, volt:700 v.

![Fluorescence emission spectra of probe DDTM (10 μM) in different buffers (10 mM, pH 11.0). λ<sub>ex</sub> = 380 nm, slits: 5.0 nm / 5.0 nm, volt:700 v.]

**Fig. S2.** Fluorescence emission spectra of probe DDTM (10 μM) in different buffers (10 mM, pH 11.0). λ<sub>ex</sub> = 380 nm, slits: 5.0 nm / 5.0 nm, volt:700 v.

4. Cell culture details

HeLa cells were purchased from the ATCC Cell Bank. Cells were seeded in culture dishes at a concentration of 2 × 10<sup>4</sup> cells/mL and cultured in Dulbecco’s Modified Eagle Medium (DMEM) in an incubator (37°C, 5% CO<sub>2</sub>). The cell were then cultured for 24 h until they were placed on confocal. Probe (10 μM) was added and cells were further incubated for 30 min, then washed three times with phosphate-buffered saline (PBS) and imaged. Meanwhile, further added test substances with pH 10.0, 12.0 and incubated 20 min. The cells fluorescence imaging was obtained by laser scanning confocal fluorescence microscopy Olympus FV1000.

5. Cytotoxicity experiment

The cytotoxicity of probe DDTM towards HeLa cells was determined by CCK-8 assay to evaluate the potential application of probe DDTM in live cell imaging (Fig.
The cells were cultured in DMEM and maintained at 37°C under 5% CO₂. Then a series of probes of different concentrations (0, 5, 10, 15, 20 and 25 μM) were added to the 96-well plates, and the cells were incubated for 24 h. CCK-8 solution was added into each well and then the residual CCK-8 solution was transferred out after 4 h. The absorbance at 450 nm was recorded by a microplate reader (SYNERGY 2). The cell viability (%) was assessed using the following equation:

\[
\text{Cell viability (\%)} = \frac{T}{C} \times 100\%, \text{ where } T \text{ is the } \text{OD}_{450} \text{ value of experience group and } C \text{ is the control group of } \text{OD}_{450} \text{ (optical density) value.}
\]

\(\text{OD}_{450}\) value of each repeated wells is given as mean ± standard deviation (SD)

![Graph showing cell viability against concentration of DFTM (μM)](image)

**Fig. S3.** Cytotoxicity assays of the probe at different concentrations (0 μM; 5 μM; 10 μM; 15 μM; 20 μM; 25 μM) for HeLa cells.

6. **^1H-NMR, ^13C-NMR and HRMS spectra**

![Mass spectrometry mechanism verification spectrum](image)

**Fig. S4.** Mass spectrometry mechanism verification.
Fig. S5. Mass spectrometry analysis of probe DDTM.

Fig. S6. $^1$H-NMR spectrum of compound DTC.
Fig. S7. $^{13}$C-NMR spectrum of compound DTC.

Fig. S8. $^1$H-NMR spectrum of probe DDTM.
Fig. S9. $^{13}$C-NMR spectrum of probe DDTM.

Reference:


