Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting information

A near-infrared probe for real-time detection of lysosome pH value in living cells under "wash free" conditions

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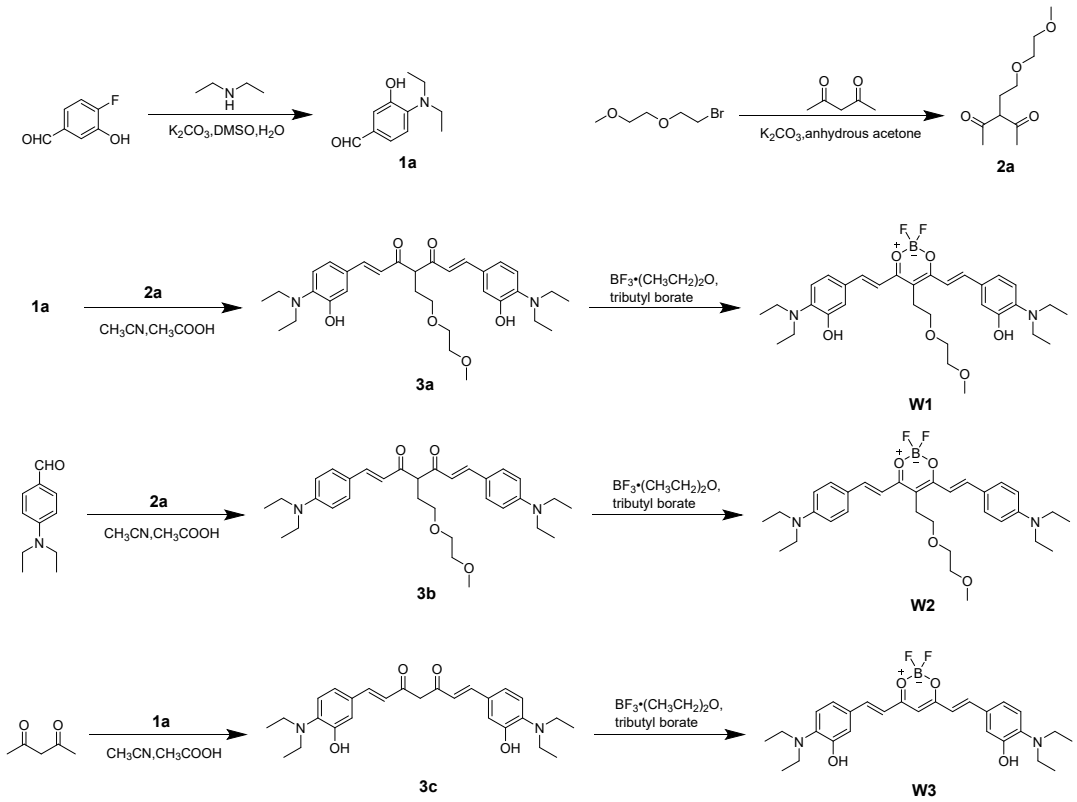
1. Synthesis and characterization



Scheme S1. Synthetic routes of **W1, W2 and W3.**

The synthesis of W1, W2 and W3 was given in detail in Scheme S1.

Synthesis of 1a. 4-Fluoro-3-hydroxybenzaldehyde (280 mg, 2 mmol) and diethylamine (3 mL, 2.2 mmol) were mixed in a flask with 10 mL of DMSO. K_2CO_3 (430 mg, 3.1 mmol) and 3 mL of ddH_2O were added. The mixture was refluxed at 180°C for 15 h. A crude product is obtained by evaporating the solvent. Product **1a** was further purified by silica gel chromatography (hexane/ethyl acetate). Yield is 25.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 9.74 (s, H), 7.51 (d, *J* = 6.9 Hz, H), 7.15 (d, *J* = 14.8 Hz, H), 6.81 (d, *J* = 3.6 Hz, H), 3.42 (d, *J* = 4.1 Hz, 4H), 1.13 (t, *J* = 7.2 Hz, 6H).

Synthesis of 2a. 1-Bromo-2-(2-methoxyethoxy)ethane (800 mg, 4.4 mmol) was dissolved in acetone (8 mL), and then K_2CO_3 (860 mg, 6.2 mmol), KI (catalyst, 10 mg,) and acetylacetone (473 μL, 4.4 mmol) were added to the mixture. The mixture was stirred at 65°C for 20 h. After the reaction, it was poured in water, and then extracted

with ethyl acetate (3×15 mL). The organic layer was washed with saturated NaCl, and dried over Na_2SO_4. Compound **2a** was obtained after flash column chromatography (hexane/ethyl acetate) with a yield of 40.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 3.78 (t, *J* =4.9 Hz, 2H), 3.63 (t, *J* =7.9 Hz, 1H), 3.57-3.52 (m, 2H), 3.44-3.39 (m, 2H), 3.31 (s, 3H), 2.13 (s, 6H), 2.07-1.99 (m, 2H).

Synthesis of 3a. Compound **1a** (400 mg, 2 mmol) was dissolved in acetonitrile (3.0 mL), followed by the additions of acetic acid (6.7 μL, 0.1mmol), tetrahydroisoquinoline (9.3 μL, 0.1 mmol), and **2a** (205 mg, 1 mmol). The resulted solution was stirred at r.t. for 4 h. **3a** was obtained after removing the solvent and subjected to flash column chromatography with methylene chloride. Yield: 42.8%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.63 (d, *J* = 15.7 Hz, 2H), 7.53 (d, *J* = 7.2 Hz, 2H), 7.16 (d, *J* = 15.3 Hz, 2H), 6.72 (d, *J* = 3.8 Hz, 2H), 6.58 (d, *J* = 3.6 Hz, 2H), 6.01 (s, 1H), 3.71 (t, *J* = 10.1, 5.6 Hz, 2H), 3.52-3.49 (m, 2H), 3.42-3.39 (m, 2H), 3.36-3.32 (m, 8H), 3.29 (s, 3H), 2.63 (t, *J* = 6.5 Hz, 2H), 1.13(t, *J* = 7.1 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) = 150.79 (2C), 146.18 (2C), 140.43 (2C), 131.41 (2C), 130.99 (2C), 121.96 (2C), 121.08, 120.16, 119.69, 116.45 (2C), 111.98, 111.57, 71.71, 69.91, 68.34, 59.01, 46.06 (4C), 26.96, 12.41 (4C). HRMS: Calcd. $[M-H]^+$: 551.3199; found value $[M-H]^+$: 551.3181.

Synthesis of W1. $BF_3{\cdot}Et_2O$ (3 mL) was slowly added to **3a** (1.1 g, 2 mmol) in tributyl borate (768 μL, 6.10 mmol) under the protection of nitrogen. The reaction mixture was then stirred at r.t. for 4 h, and the **W1** was obtained after removing the solvent and purified by silica gel chromatography (hexane/ethyl acetate). Yield is 95.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.83 (d, *J* = 15.7 Hz, 2H), 7.52 (d, *J* = 7.8 Hz, 2H), 7.22 (d, *J* = 15.8 Hz, 2H), 6.89 (d, *J* = 3.7 Hz, 2H), 6.75 (d, *J* = 3.8 Hz, 2H), 3.63 (t, *J* = 3.2 Hz, 2H), 3.53-3.51 (m, 2H), 3.43-3.40 (m, 2H), 3.38-3.34 (m, 8H), 3.31 (s, 3H), 2.64 (t, *J* = 6.8 Hz, 2H), 1.14(t, *J* = 7.3 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) = 151.19 (2C), 147.65 (2C), 140.16 (2C), 132.59 (2C), 130.25 (2C), 122.84 (2C), 120.88, 120.26, 119.76, 116.40 (2C), 112.34, 111.77, 71.81, 69.94, 68.74, 59.13, 46.17

(4C), 26.89, 12.35 (4C). HRMS: Calcd. $[M-H]^+$: 599.3492; found value $[M-H]^+$: 599.3529.

Synthesis of 3b. 4-(Diethylamino)benzaldehyde (0.71 g, 2 mmol) was dissolved in acetonitrile (3.0 mL), followed by the additions of acetic acid (6.7 μL, 0.1mmol), tetrahydroisoquinoline (9.3 μL, 0.1 mmol), and **2a** (205 mg, 1 mmol). The resulting solution was stirred at r.t. for 4 h. **3b** was obtained after removing the solvent and subjecting to flash column chromatography with methylene chloride. Yield: 81.4%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.88 (d, *J* = 15.6 Hz, 2H), 7.59 (d, *J* = 4.9 Hz, 4H), 7.23 (d, *J* = 4.2 Hz, 2H), 6.81 (d, *J* = 11.7 Hz, 4H), 5.98 (s, 1H), 3.74 (t, *J* = 3.6 Hz, 2H), 3.54-3.51 (m, 2H), 3.44-3.40 (m, 2H), 3.37-3.34 (m, 8H), 3.31 (s, 3H), 2.65 (t, *J* = 6.2 Hz, 2H), 1.12(t, *J* = 6.9 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) = 145.95 (2C), 142.03 (2C), 134.33 (2C), 130.99 (4C), 126.08 (2C), 120.97, 120.19 (2C), 111.02 (4C), 72.17, 69.43, 68.83, 58.92, 46.18 (4C), 26.71, 12.60 (4C). HRMS: Calcd. $[M+H]^+$: 521.3301; found value $[M+H]^+$: 521.3324.

Synthesis of W2. $BF_3 \cdot Et_2O$ (3 mL) was slowly added to **3b** (1.04 g, 2 mmol) in tributyl borate (768 μL, 6.10 mmol) under the protection of nitrogen. The reaction mixture was then stirred at r.t. for 4 h, and the **W2** was obtained after removing the solvent with a yield of 88.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 8.15 (d, *J* = 16.2 Hz, 2H), 7.63 (d, *J* = 4.3 Hz, 4H), 7.45 (d, *J* = 4.8 Hz, 2H), 6.77 (d, *J* = 11.7 Hz, 4H), 3.72 (t, *J* = 3.6 Hz, 2H), 3.59 (t, *J* = 3.5 Hz, 2H), 3.48 (t, *J* = 4.6 Hz, 2H), 3.42 – 3.39 (m, 8H), 3.35 (s, 3H), 2.74 (t, *J* = 6.2 Hz, 2H), 1.14(t, *J* = 6.9 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) = 145.75 (2C), 141.17 (2C), 133.12 (2C), 132.59 (4C), 126.37 (2C), 121.51, 120.12 (2C), 110.22 (4C), 72.19, 69.50, 68.34, 59.22, 46.26 (4C), 26.75, 12.42 (4C). HRMS: Calcd. $[M+H]^+$: 569.3284; found value $[M+H]^+$: 569.3299.

Synthesis of 3c. **1a** (1.6 g, 8 mmol) was dissolved in acetonitrile (3.0 mL), followed by the additions of acetic acid (6.7 μL, 0.1mmol), tetrahydroisoquinoline (9.3 μL, 0.1 mmol), and acetylacetone (460 μL, 4.0 mmol), the resulting solution was stirred at r.t.

for 8 h. **3c** was obtained after removing the solvent and subjecting to flash column chromatography with methylene chloride. Yield: 75.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.78 (d, *J* = 16.2 Hz, 2H), 7.65 (d, *J* = 7.8 Hz, 2H), 7.27 (d, *J* = 12.1 Hz, 2H), 6.88 (d, *J* = 7.1 Hz, 2H), 6.78 (d, *J* = 15.5 Hz, 2H), 6.16 (m, 2H), 3.83-3.77 (m, 8H), 1.13(t, *J* = 7.1 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) = 182.23 (2C), 152.76 (2C), 139.77 (2C), 135.07 (2C), 130.41 (2C), 124.15, 120.12 (2C), 116.49 (2C), 113.27 (2C), 104.36, 46.12 (4C), 12.37 (4C). HRMS: Calcd. $[M-H]^+$: 451.2519; found value $[M-H]^+$: 451.2534.

Synthesis of W3. $BF_3 \cdot Et_2O$ (3 mL) was slowly added to **4a** (0.89 g, 2 mmol) in tributyl borate (768 μL, 6.10 mmol) under the protection of nitrogen. The reaction mixture was then stirred at r.t. for 4 h. **W3** was obtained after removing the solvent. Yield: 87.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) = 7.95 (d, *J* = 16.4 Hz, 2H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 13.4 Hz, 2H), 6.92 (d, *J* = 7.2 Hz, 2H), 6.81 (d, *J* = 15.9 Hz, 2H), 6.34 (s, 1H), 3.90-3.84 (m, 8H), 1.15(t, *J* = 7.2 Hz, 12H). ^{13}C NMR (101 MHz, DMSO-d_6) δ (ppm) = 181.19 (2C), 151.56 (2C), 140.25 (2C), 135.96 (2C), 129.37 (2C), 123.48, 119.91 (2C), 116.86 (2C), 112.92 (2C), 103.73, 46.28 (4C), 12.31 (4C). HRMS: Calcd. $[M-H]^+$: 497.2531; found value $[M-H]^+$: 497.2559.

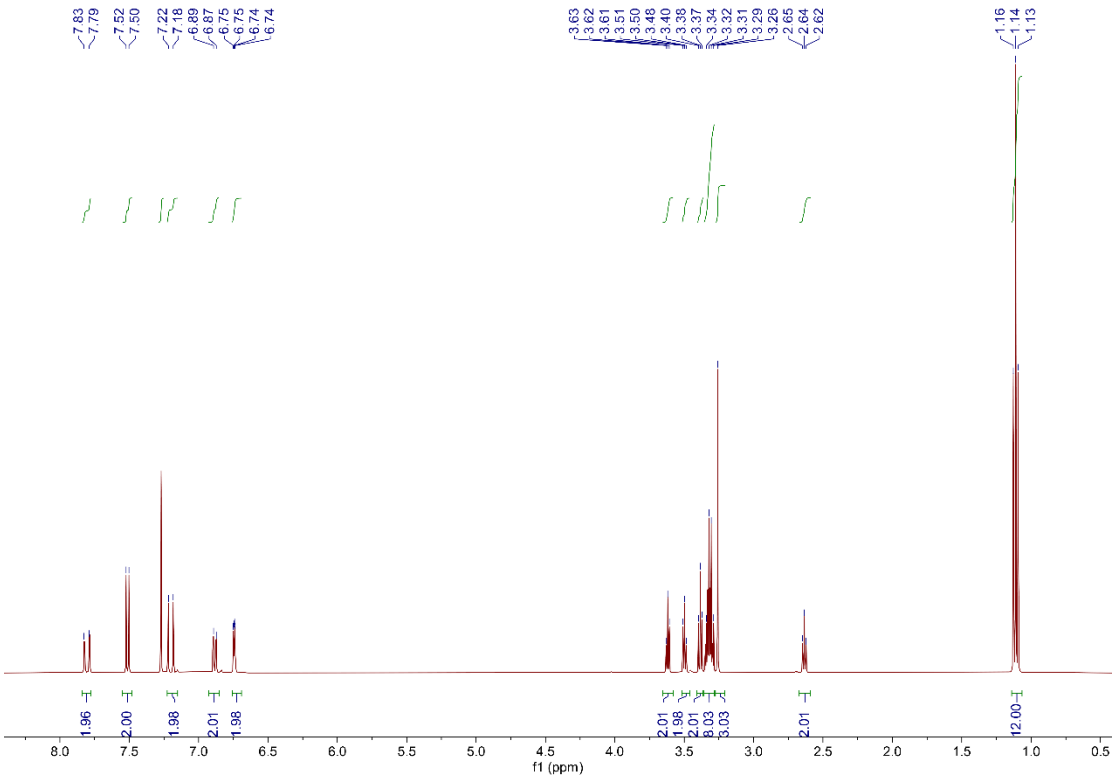


Fig. S1. ^{1}H NMR of **W1.**

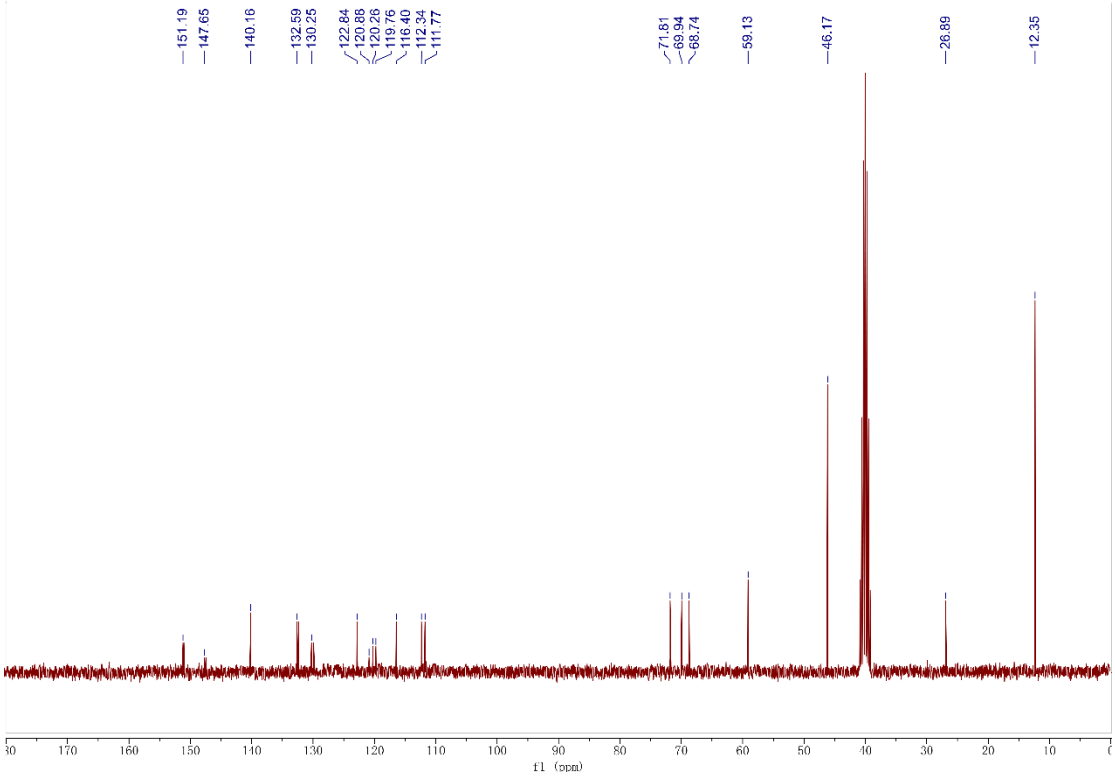


Fig. S2. ^{13}C NMR of **W1.**

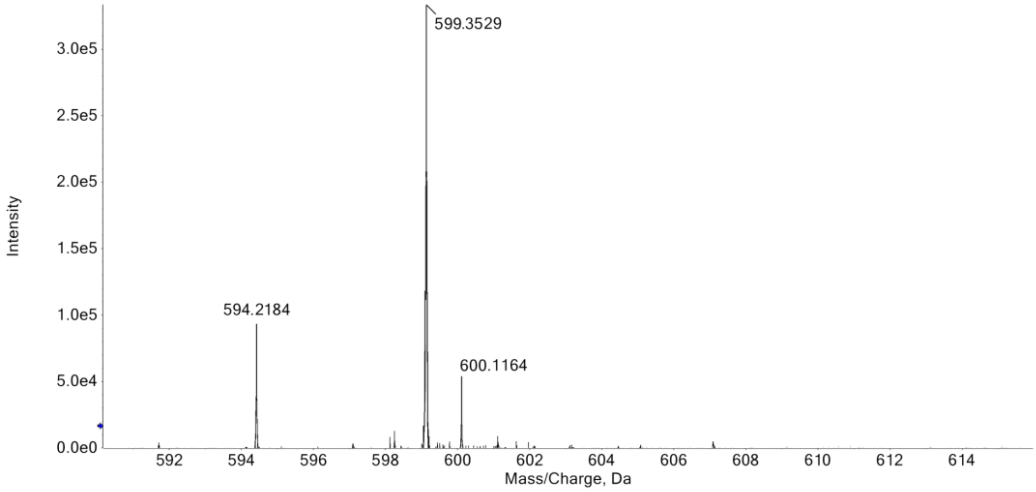


Fig. S3. HRMS of **W1.**

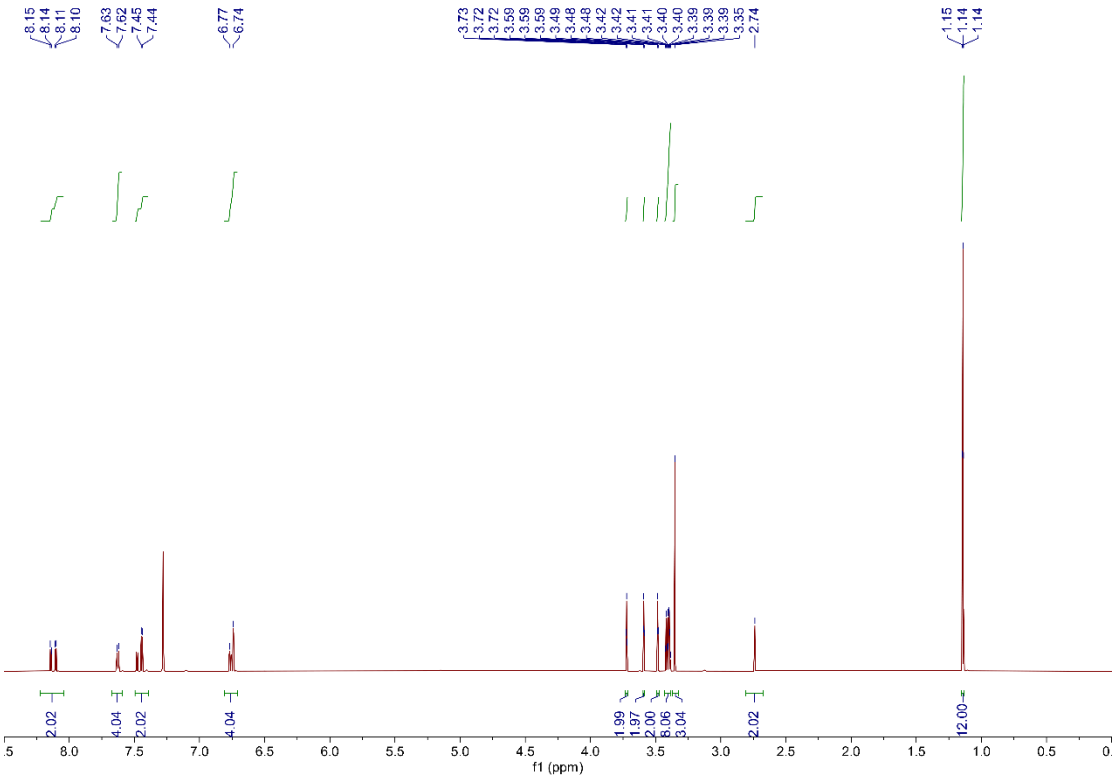


Fig. S4. ^{1}H NMR of **W2.**

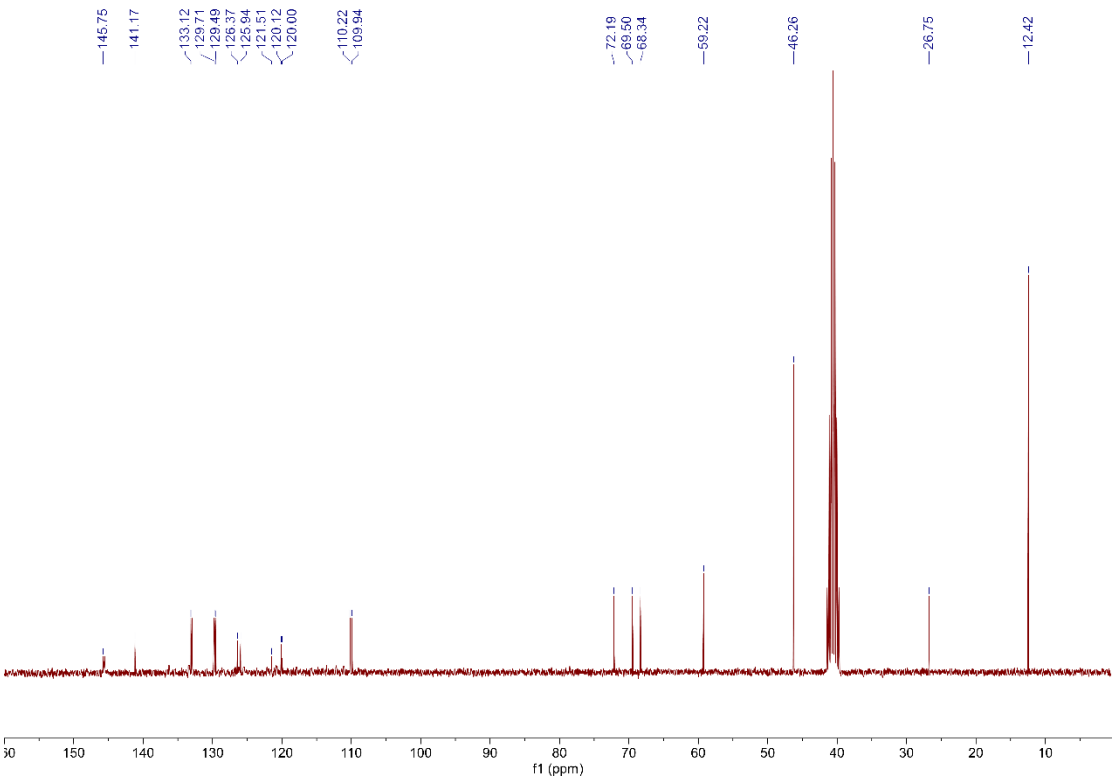


Fig. S5. ^{13}C NMR of **W2.**

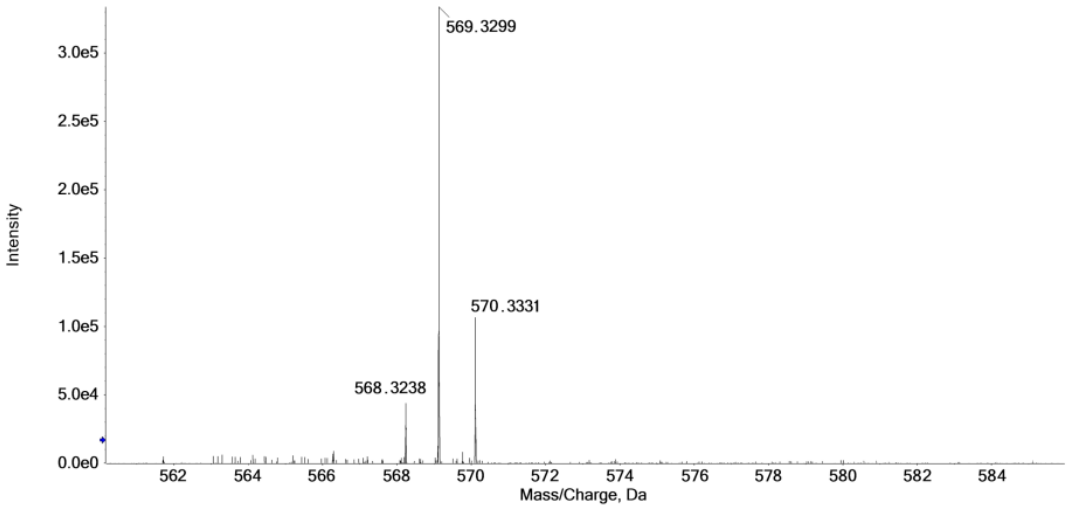


Fig. S6. HRMS of **W2.**

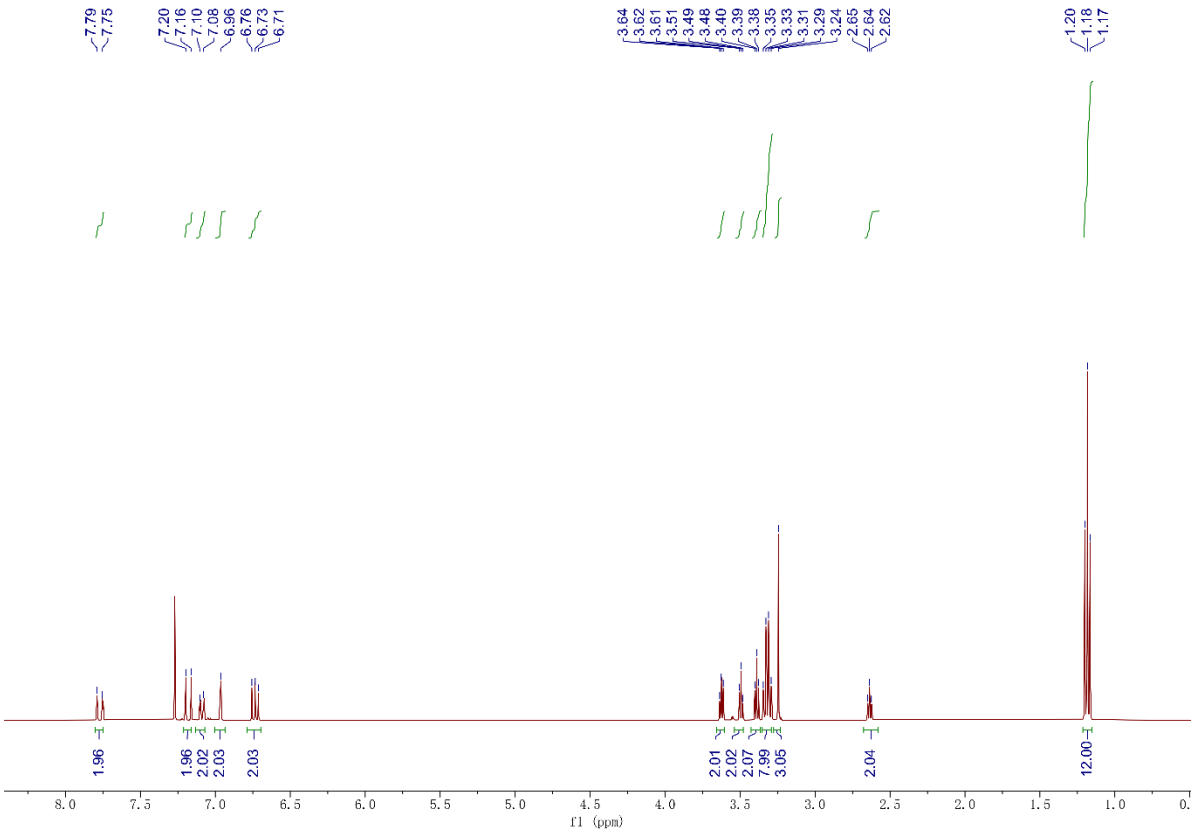


Fig. S7. ^{1}H NMR of **W3.**

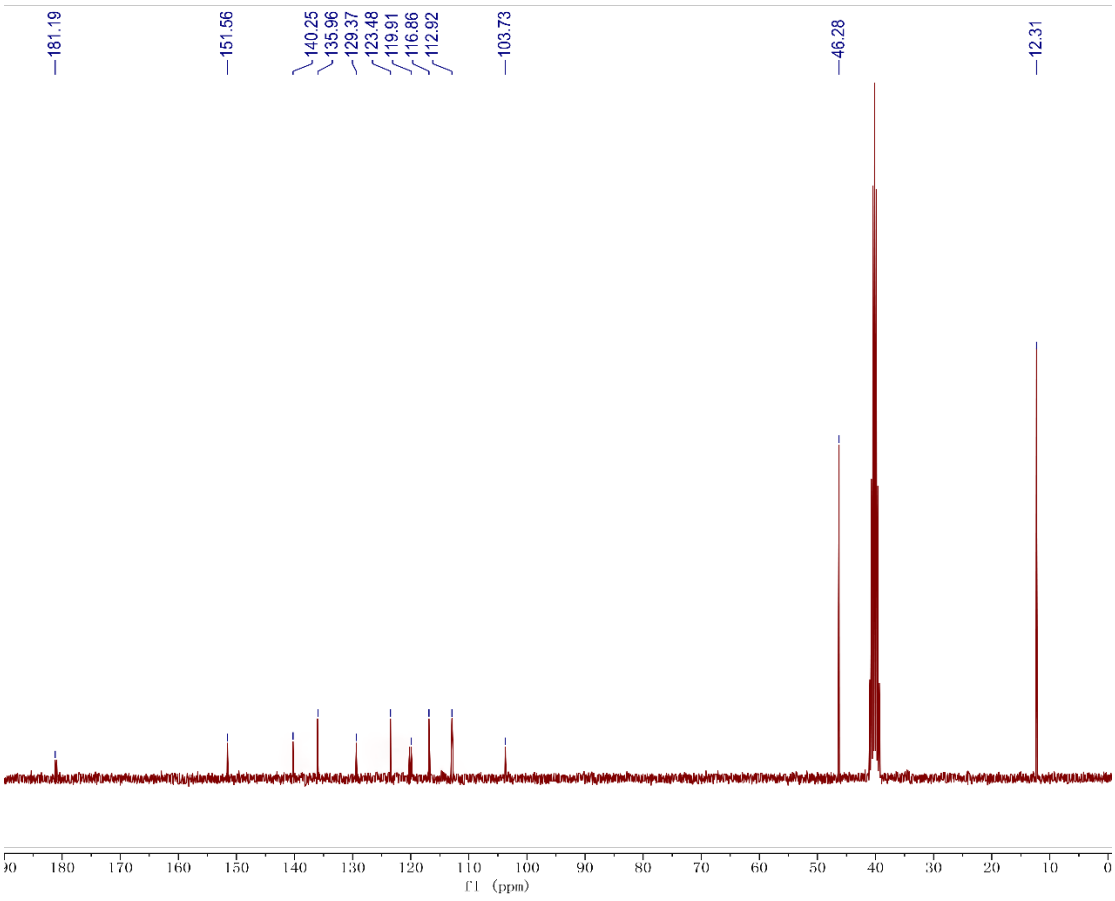


Fig. S8. ^{13}C NMR of **W3.**

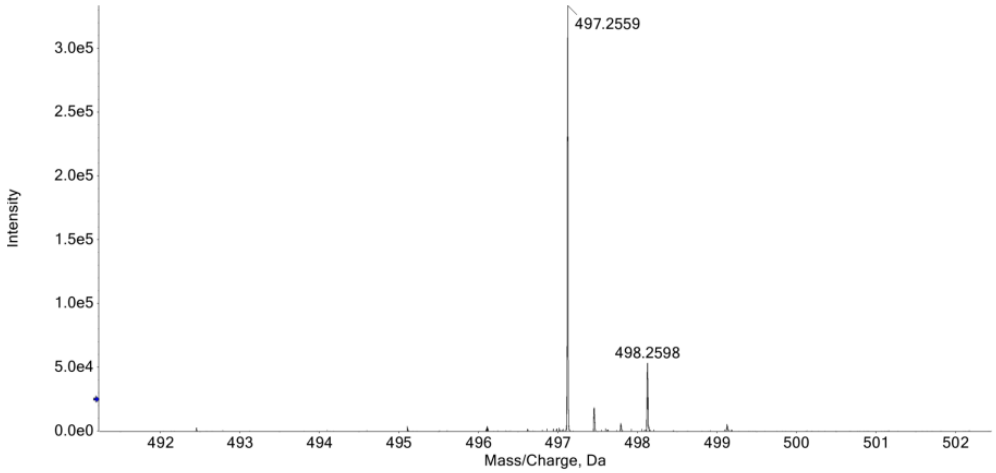


Fig. S9. HRMS of **W3.**

2. Spectral characteristics of W1 molecules

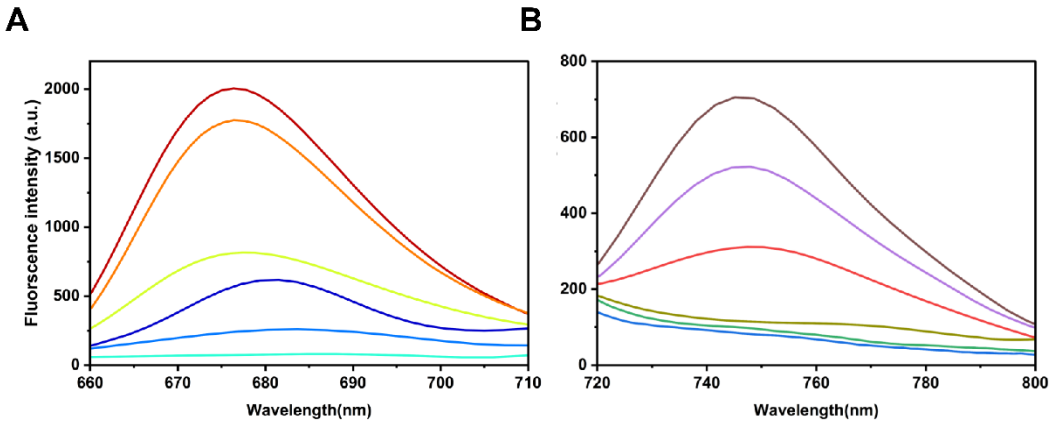


Fig. S10. (A) Fluorescence spectra of **W1** (5 μM) at different pH (4.0-9.0) in the B-R buffer/EtOH (1/1, v/v) solution (λ_{ex} = 580 nm). (B) Fluorescence spectra of **W1** (5 μM) at different pH (4.0-9.0) in the B-R buffer/EtOH (1/1, v/v) solution (λ_{ex} = 660 nm).

3. Spectral characteristics of W2 and W3 molecules

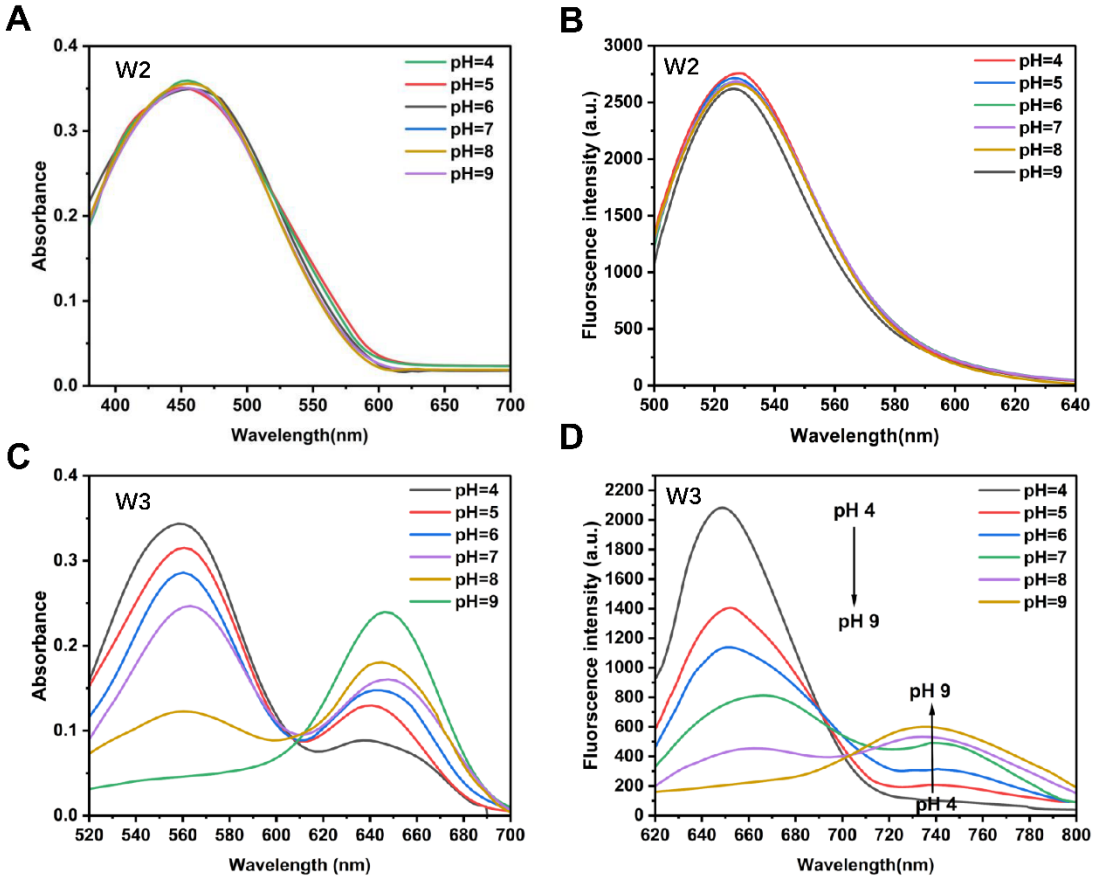


Fig. S11. (A) UV-vis spectra of **W2** (5 μM) at different pH (4.0-9.0) in the B-R buffer/EtOH (1/1, v/v) solution. (B) Fluorescence spectra of **W2** (5 μM) at different pH (4.0-9.0) in the B-R buffer/EtOH (1/1, v/v) solution (λ_{ex} = 450 nm). Thumbnail is the color change of **W2** in different pH solutions. (C) UV-vis spectra of **W3** (5 μM) at different pH (4.0-9.0) in the B-R buffer/EtOH (1/1, v/v) solution. (D) Fluorescence spectra of **W3** (5 μM) at different pH (4.0-9.0) in the B-R buffer/EtOH (1/1, v/v) solution (λ_{ex} = 610 nm).

4. Cytotoxicity evaluation of W1

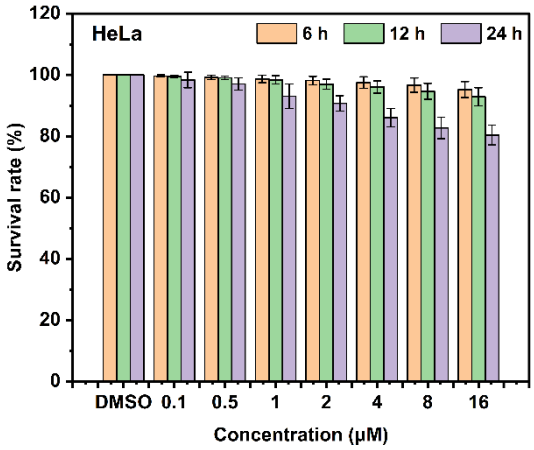


Fig. S12. W1 was incubated with HeLa cells for 6 hours, 12 hours and 24 hours respectively, and the cytotoxicity was evaluated by MTT assay.

5. Images of W1 with different concentrations

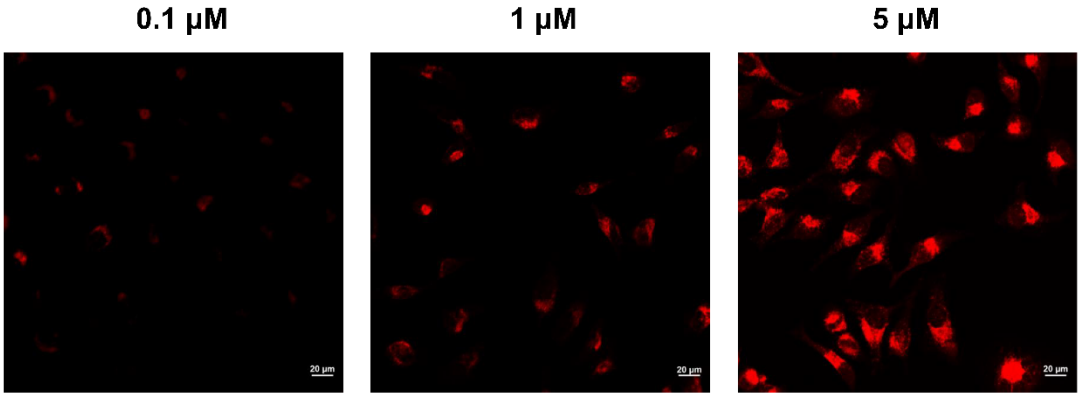


Fig. S13. Images of HeLa cells at different concentrations (0.1, 1, 5 μM) of **W1**. λ_{ex} = 594 nm, emission was collected 660 to 720 nm.

6. Co-location analysis of W3

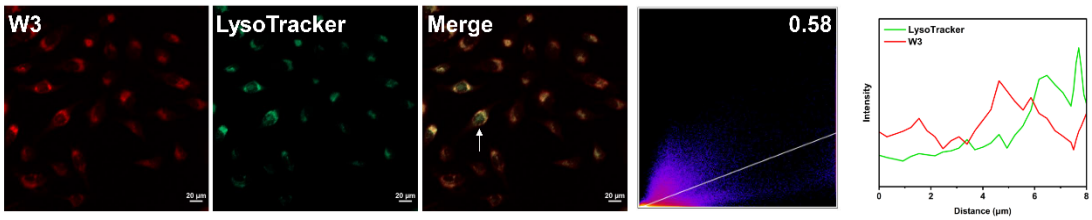


Fig. S14. Intracellular localization of **W3** was performed in HeLa cells. Cells were incubated with **W3** (λ_{ex} = 594 nm, collected 660-720 nm) and Lyso Tracker Green (λ_{ex} = 488 nm, collected 490-530 nm) for 40 min, respectively. The fourth column represents the intensity correlation plot of dyes and probes. The fifth column represents cross-sectional analysis along the white line in the insets. Scale bars: 20 μm.

7. Dielectric constants of common solvents

Solvents	EtOH	MeOH	CH_3CN	DMSO	H_2O
Dielectric constants	24.3	33.6	37.5	47.2	80.4

Table S1. Dielectric constants of common solvents.[1]

References：

1. W. M. Latimer, *Chemical Reviews*, 1949, **44**, 59-67.