Electronic Supplementary Material (ESI) for RSC Advances.

Near-infrared Fluorescent Probes with Higher Quantum Yields and Neutral pKa Values for the Evaluation of Intracellular pH

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Supplementary Information

Experiment section

General methods

The 500 (^{1}H) MHz NMR and 100 (^{13}C) MHz NMR spectra are registered at room temperature on a Bruker 500 MHz spectrometer using perdeuterated solvents as internal standard. Chromatographic purification is conducted with silica gel. All solvent mixtures are given as volume/volume ratios. UV spectra are recorded on TU-1901 UV-Vis Spectrophotometer and fluorescence spectra are recorded on a F-7000 spectrometer. Relative quantum efficiencies of fluorescence of compounds are obtained by comparing the areas under the corrected emission spectrum of the test sample in diluted solvents with a reported aza-BODIPY derivative in toluene which has a quantum efficiency of 0.42 according to the literature.[1] Non-degassed, spectroscopic grade toluene and a 10 mm quartz cuvette are used. Dilute solutions ($0.01 < A < 0.05$) are used to minimize reabsorption effects. Quantum yields are determined using the equation (1):

$$\Phi_F^{(sample)} = \Phi^{(standard)} \times (Abs^{(standard)} \times F^{(sample)}) / (Abs^{(sample)} \times F^{(standard)}) \quad \text{equation (1)}$$

Where $\Phi^{(standard)}$ is the reported quantum yield of the standard, Abs is the absorbance at the excitation wavelength, F is the integrated emission spectra.

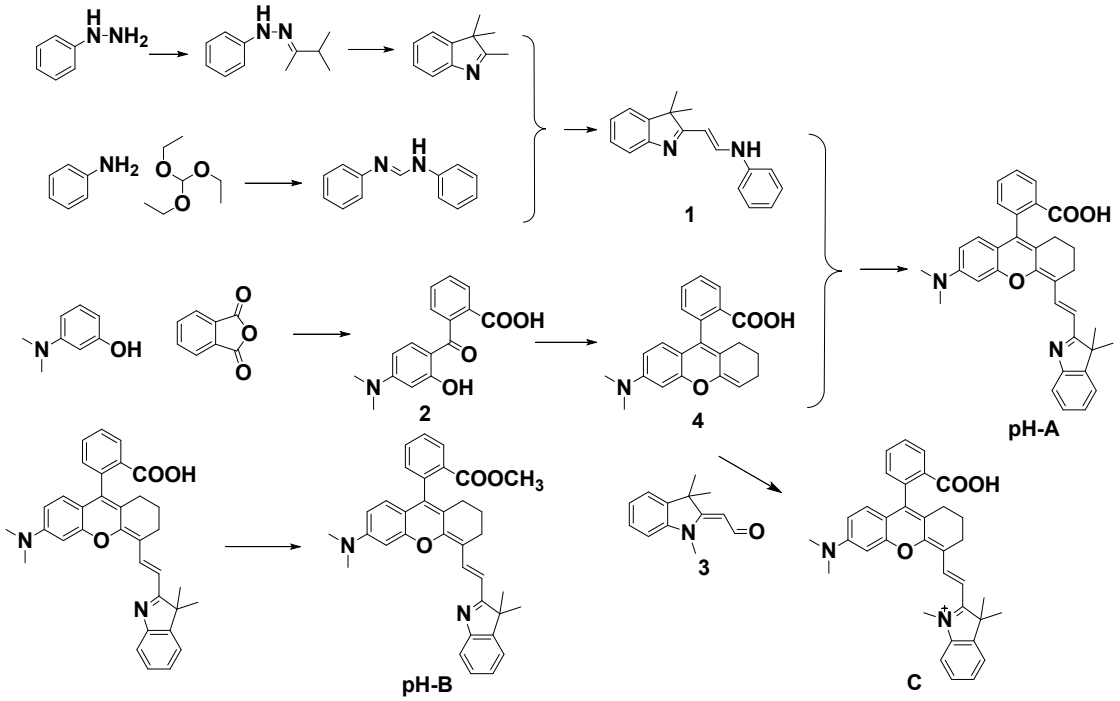
Effects on cell growth/viability

MCF-7 cells are obtained from Institute of Basic Medical Sciences (IBMS) of Chinese Academy of Medical Sciences (CAMS). All cell lines are maintained under

standard culture conditions (atmosphere of 5% CO2 and 95% air at 37 °C) in RPMI 1640 medium or DMEM medium, supplemented with 10% FBS (fetal bovine serum). The cytotoxic effect of **pH-A** and **pH-B** are assessed using the MTT assay. Briefly, the cells in the exponential phase of growth are used in the experimentation. 3×10^3 cells/well are seeded onto 96-well plates and allowed to grow for 24 h prior to treatment with **pH-A** or **pH-B.** The incubation time of **pH-A** or **pH-B** is 24 hours. At the end of this time, MTT is then added to each well (final concentration 0.5 mg/mL) for 4 h at 37 °C and formazan crystals formed through MTT metabolism by viable cells are dissolved in DMSO. Optical densities are measured at 490 nm using a Thermo Scientific Multiskan FC spectrophotometer.

Culture of cells and fluorescent imaging

All cell lines are maintained under standard culture conditions (atmosphere of 5% CO_2 and 95% air at 37 °C) in RPMI 1640 medium or DMEM medium, supplemented with 10% FBS (fetal bovine serum). Cells in the exponential phase of growth on 35 mm glass bottom culture dishes (Φ 20 mm) for 1 day. These cells are used in co-localization experimentation. The cells are washed three times with PBS, and then are incubated with 2 mL containing **pH-A** (6 μM) and Rh-123 (0.5 μM) in an atmosphere of 5% CO_2 and 95% air at 37 °C. Wash cells twice with 1 mL PBS at room temperature, and add 1 mL RPMI 1640 culture medium and then observe under a confocal microscopy (Olympus FV1000).



General procedure for the synthesis of pH-A and pH-B's derivatives

Compound 1, 2, 3 are synthesized according to standard procedures.[2, 3]

Compound 4 Freshly cyclohexanone (6.6 mL, 63.7 mmol) is added to concentrated H_2SO_4 drop-wisely (7.0 mL) and then cooled down to 0 °C. Then, compound 2 (32 mmol) is added in portions with vigorous stirring. The reaction mixture is heated at 90 °C for 1.5 h, cooled down, and poured onto ice (300 g), and $HClO_4$ is added to the solutions and the resulting precipitate is filtered off and washed with cold water (100 mL). Compound 4 obtained as a bluish green is used for the next step without further purification.

pH-A: Compound 1 (100 mg, 0.38 mmol) and compound 4 (200mg, 0.57mmol) are dissolved in a mixture of CH_2Cl_2 (20 mL), CH_3OH (10 mL), and acetic anhydride (1 mL). The mixture is refluxed for 1h. The solvent was removed under reduced pressure to give the crude product, which is purified by silica gel flash chromatography using CH_2Cl_2 to CH_2Cl_2/CH_3OH (200:1 to 20:1) as eluent to afford **pH-A** (80mg, yields 40%). ^{1}H NMR (500 MHz, MeOD) δ 8.47 (d, J = 15.8 Hz, 1H), 7.82 – 7.76 (m, 1H), 7.44 (dt, J = 23.2, 8.2 Hz, 3H), 7.37 (d, J = 7.3 Hz, 1H), 7.30 (t, J = 7.5 Hz, 1H), 7.18

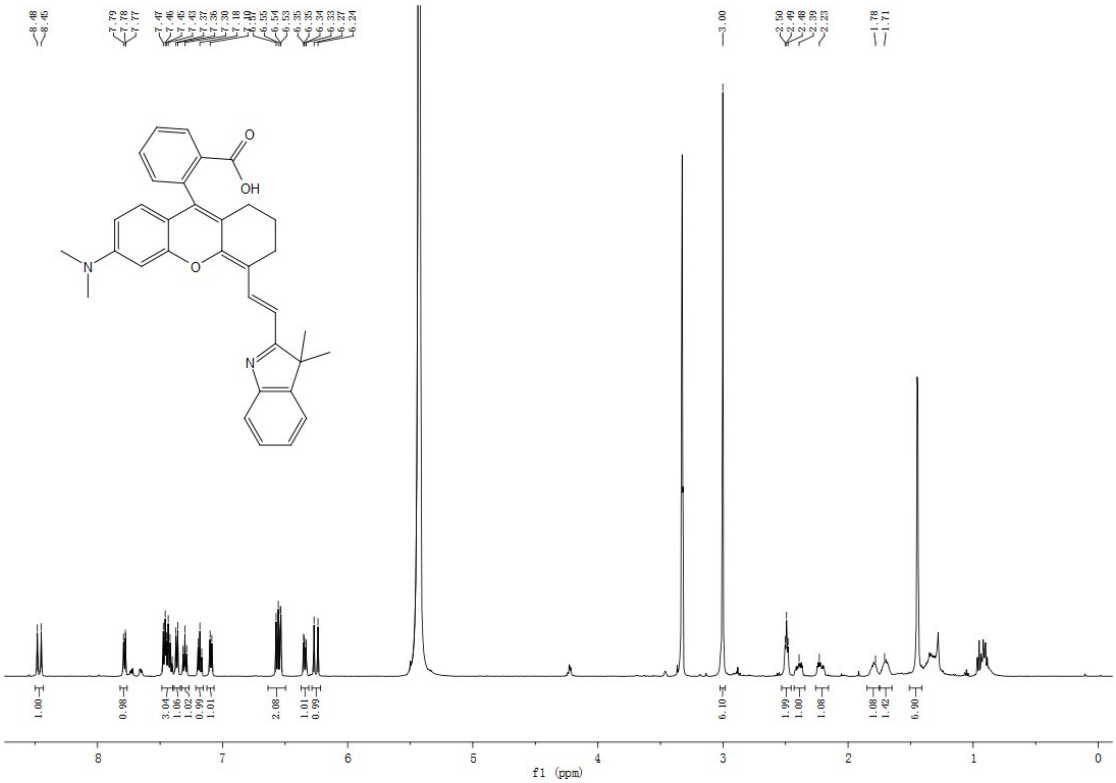
(t, J = 7.4 Hz, 1H), 7.09 (d, J = 7.0 Hz, 1H), 6.34 (dd, J = 8.8, 2.4 Hz, 1H), 6.25 (d, J = 15.8 Hz, 1H), 3.00 (s, 6H), 2.49 (t, J = 5.9 Hz, 2H), 2.39 (s, 1H), 2.23 (s, 1H), 1.78 (s, 1H), 1.71 (s, 1H). ^{13}C NMR (126 MHz, MeOD) δ 186.73 (s), 176.63 (s), 155.19 (s), 154.80 (s), 154.33 (s), 153.06 (s), 147.26 (s), 138.90 (s), 136.29 (s), 135.76 (s), 130.82 (s), 129.89 (s), 129.64 (s), 128.86 (s), 128.69 (s), 128.41 (s), 127.95 (s), 125.90 (s), 122.72 (s), 122.47 (s), 119.73 (s), 114.63 (s), 112.45 (s), 110.69 (s), 108.76 (s), 98.92 (s), 65.39 (s), 53.39 (s), 40.66 (s), 28.50 (s), 25.45 (s), 24.91 (s). m/z (FTMS+p ESI): Calcd $[M+H]^+$ for $C_{34}H_{33}N_2O_3$: 517.2491, found: 517.2479

pH-B: pH-A (0.2 mmol, 100 mg) are added to a 15 mL CH_3OH and $SOCl_2$ (0.6mmol) is dropped. The mixture is heated under reflux and reaction is monitored by TLC. When all the starting material has been consumed, the mixture is cooled to room temperature and solvent is evaporated. The crude product is purified by silica gel flash chromatography using CH_2Cl_2 to CH_2Cl_2/CH_3OH (200:1 to 20:1) as eluent to afford pH-B (56 mg, yields 55%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.87 (s, 1H), 8.15 (d, J = 7.8 Hz, 1H), 7.74 – 7.65 (m, 2H), 7.58 (t, J = 7.6 Hz, 1H), 7.31 (t, J = 7.6 Hz, 2H), 7.17 (d, J = 7.5 Hz, 1H), 7.12 (t, J = 7.4 Hz, 1H), 6.57 (d, J = 8.8 Hz, 1H), 6.51 (d, J = 8.0 Hz, 1H), 5.73 (s, 1H), 3.68 (s, 4H), 3.19 (s, 7H), 2.58 (d, J = 5.8 Hz, 2H), 2.24 (d, J = 3.0 Hz, 2H), 1.79 – 1.74 (m, 2H), 1.48 (s, 7H). ^{13}CNMR (126 MHz, $CDCl_3$) δ 166.17 (s), 136.33 (s), 132.76 (s), 130.93 (s), 129.96 – 129.93 (m), 129.77 (d, J = 21.2 Hz), 128.84 (d, J = 23.8 Hz), 126.85 (s), 123.96 (s), 121.59 (s), 120.47 (s), 113.27 (s), 98.64 (s), 52.39 (s), 40.59 (s), 29.71 (s), 26.86 (s), 26.59 (d, J = 12.3 Hz), 24.70 (s), 20.75 (s), 14.12 (s). m/z (FTMS+p ESI): Calcd $[M+H]^+$ for $C_{35}H_{35}N_2O_3$: 531.2642, found: 531.2640

Compound C: Compound 3 (50 mg, 0.25 mmol) and compound 4 (130mg, 0.37mmol) are dissolved in acetic anhydride (5 mL). The mixture is heated to 50℃ for 1h. The solvent is removed under reduced pressure to give the crude product, which is purified by silica gel flash chromatography using CH_2Cl_2 to CH_2Cl_2/CH_3OH

(200:1 to 20:1) as eluent to afford compound C.^{1}H NMR (400 MHz, CD_3OD) δ 8.70 (d, *J* = 14.4 Hz, 1H), 8.23 (d, *J* = 7.0 Hz, 1H), 7.74 (d, *J* = 4.5 Hz, 1H), 7.67 (d, *J* = 4.4 Hz, 1H), 7.59 – 7.50 (m, 1H), 7.43 (d, *J* = 7.7 Hz, 1H), 7.29 (dd, *J* = 24.4, 6.3 Hz, 3H), 6.77 (d, *J* = 12.9 Hz, 3H), 6.20 (d, *J* = 15.0 Hz, 1H), 3.65 (s, 3H), 3.16 (s, 6H), 2.69 (s, 2H), 2.36 (s, 2H), 2.12 (d, *J* = 65.5 Hz, 2H), 1.83 (s, 6H).^{13}C NMR (126 MHz, MeOD) δ 175.52 (s), 164.64 (s), 156.93 (s), 155.59 (s), 144.34 (s), 143.70 (s), 142.40 (s), 137.30 (s), 133.95 (s), 132.33 (s), 130.86 (s), 130.66 (s), 130.46 (s), 129.85 (s), 129.03 (s), 126.40 (s), 123.38 (s), 122.33 (s), 116.56 (s), 114.95 (s), 113.39 (s), 111.96 (s), 100.74 (s), 97.14 (s), 50.59 (s), 40.40 (s), 31.66 (s), 28.66 (s), 27.88 (s), 21.70 (s). m/z (FTMS+p ESI): Calcd $[M+H]^+$ for $C_{35}H_{35}N_2O_3$: 531.2642, found: 531.2629.

1 Loudet A., Bandichhor R., Wu L., Burgess K. *Tetrahedron* 2008, *64*, 3642 – 3654
2 L. Yuan, W. Lin, Y. Yang and H. Chen, J. Am. Chem. Soc., 2012, 134, 1200-1211.
3 A. M. Kolesnikov and M. I. Povolotskii, Theor. Exp. Chem. 1988, 24, 220-222.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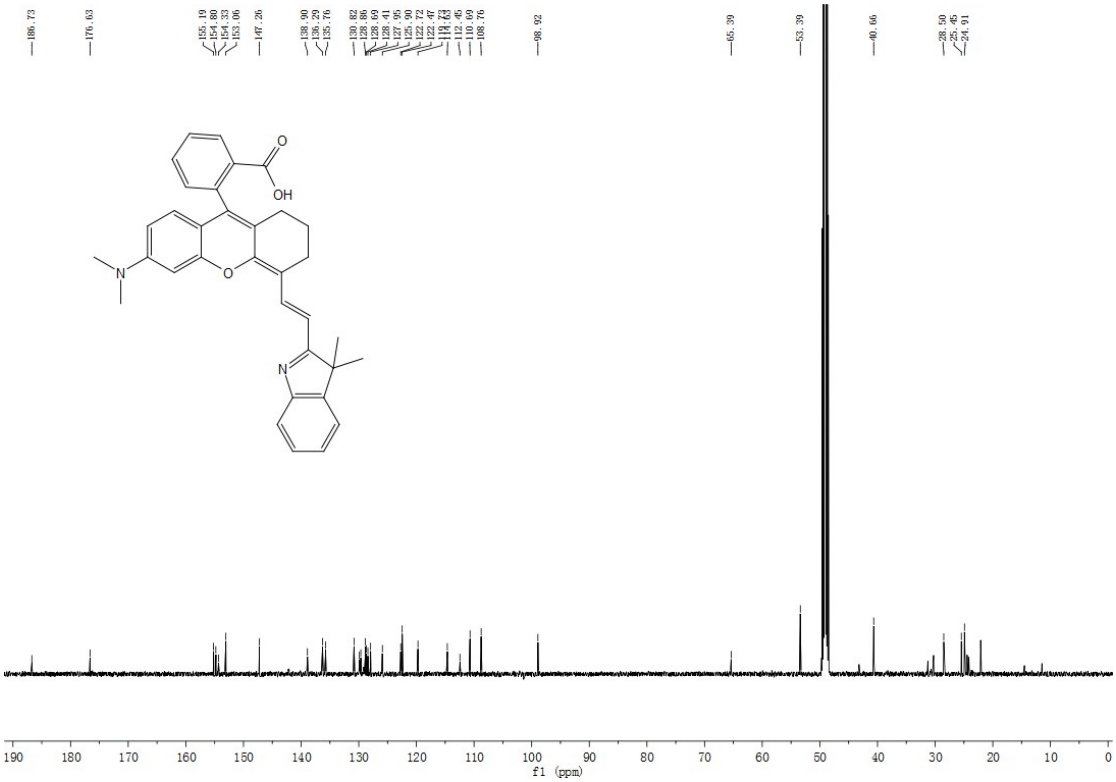
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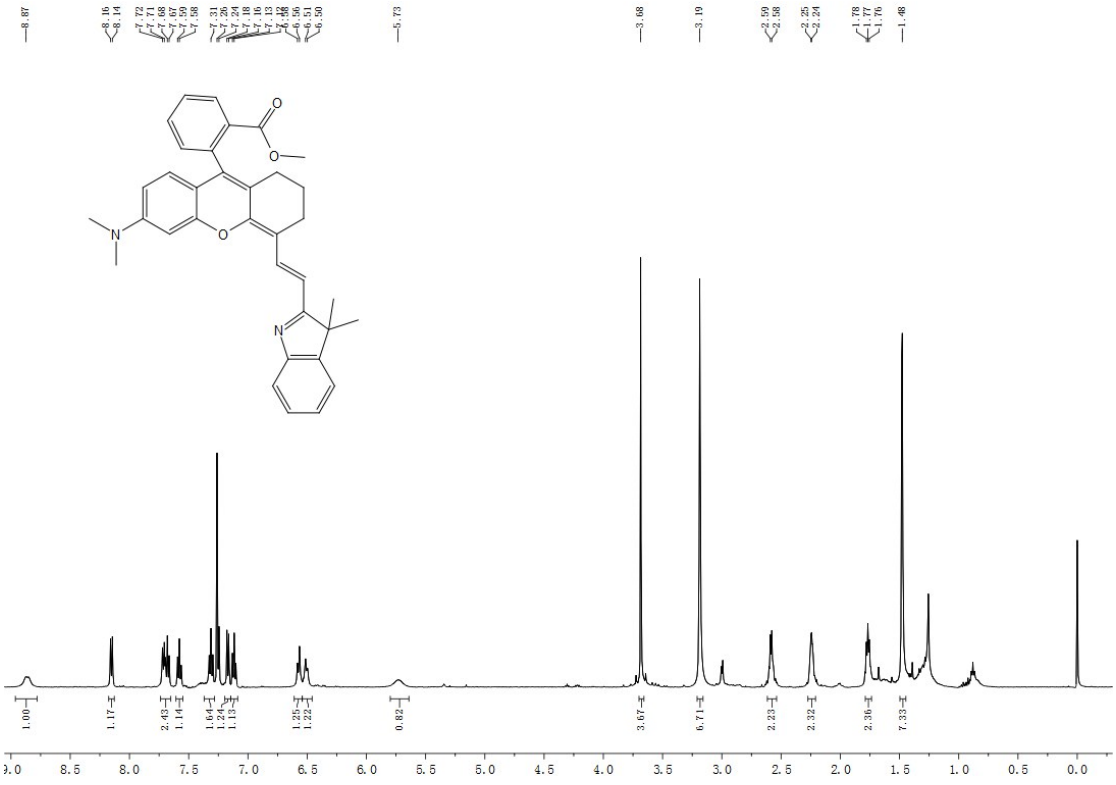
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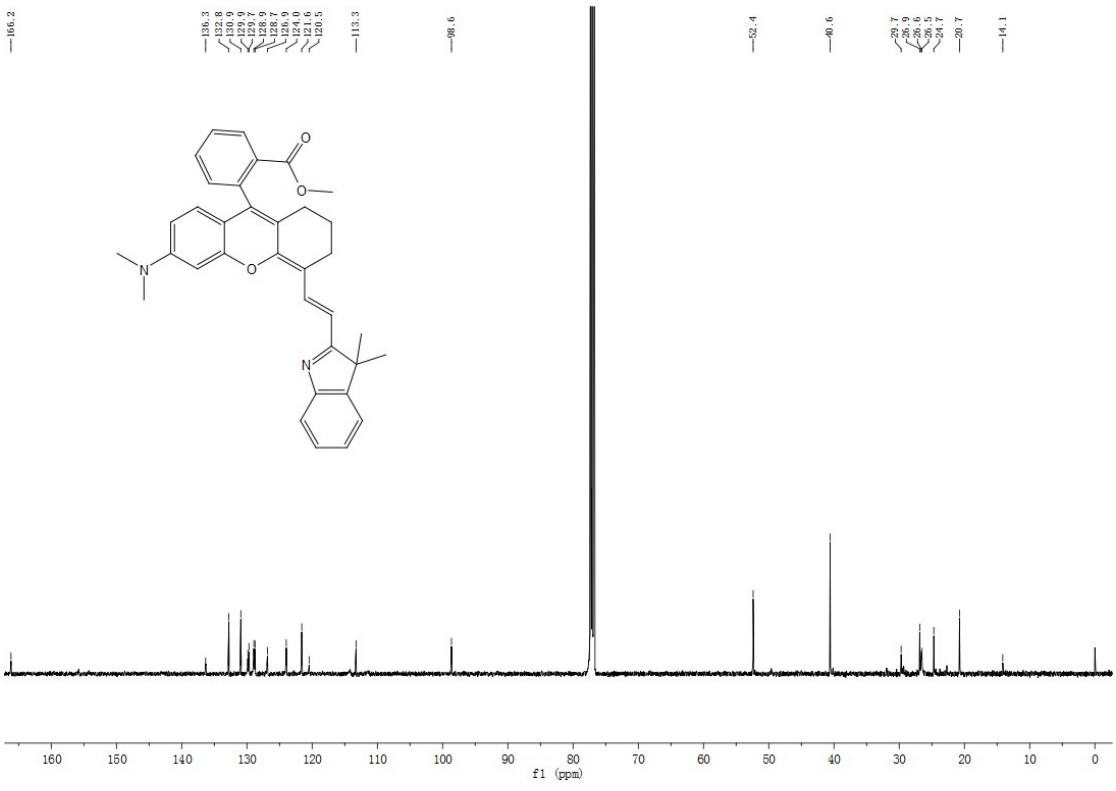
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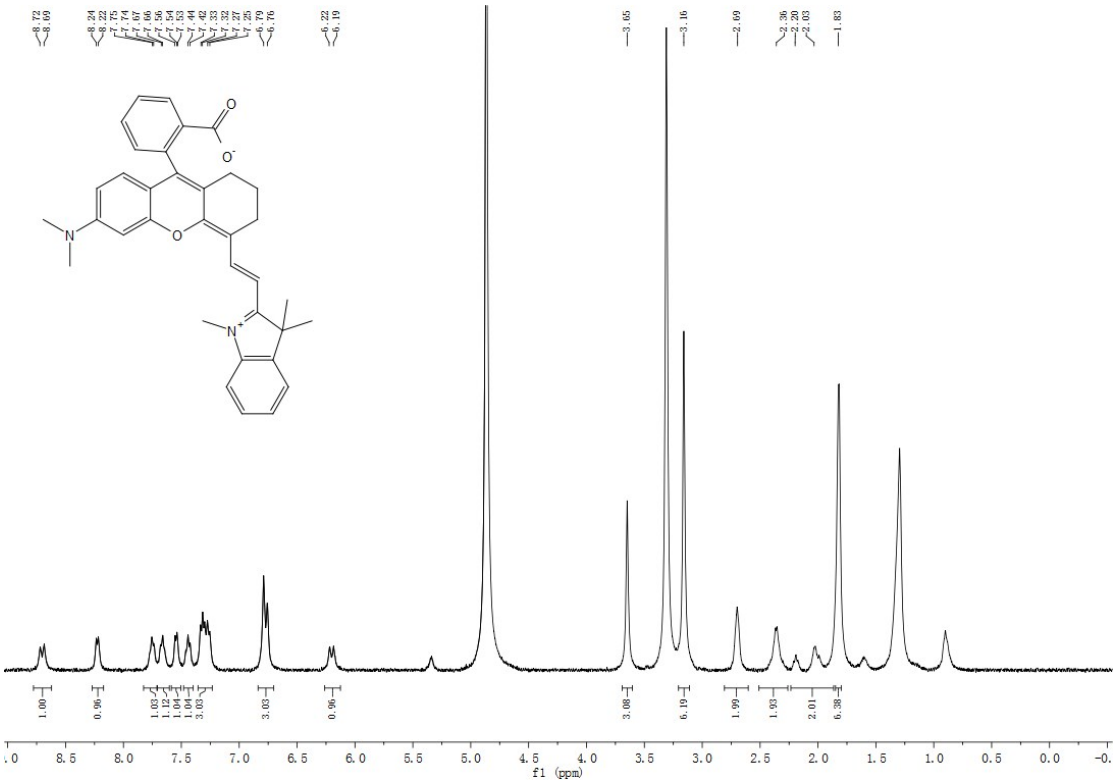
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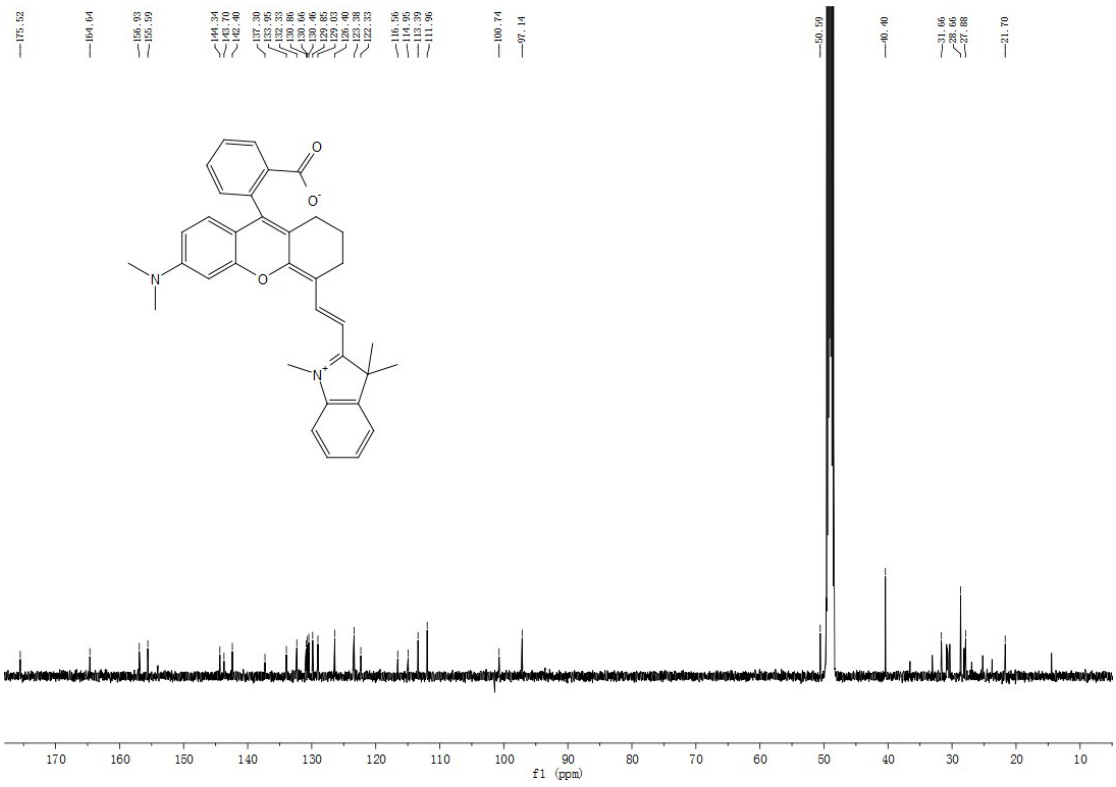
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