Electronic Supplementary Material (ESI) for ChemComm.
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A Ratiometric Fluorescent Probe for Sensing HOCl Based on Rhodamine-coumarin Dyad

Yan-Ru Zhang,[a] Xin-Peng Chen,[b] Jing-Shao,[b] Jia-Yi Zhang,[a] Qiong Yuan,[a] Jun-Ying Miao,*[b] and Bao-Xiang Zhao*[a]

[a] *Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, P.R. China. fax: +86 531 88564464; Tel.: +86 531 88366425; E-mail: bxzhao@sdu.edu.cn (B.X. Zhao)*

[b] *Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, P.R. China. E-mail: miaojy@sdu.edu.cn (J.Y. Miao)*

Apparatus and chemicals

Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Absorption and fluorescence spectroscopy

Preparation of chloride

Cell culture and cell imaging

Synthesis of probes

Fig. S1 HRMS spectrum of the crude product after treatment of probe **1** with 18 equiv. of HOCl, and ^{1}HNMR and HRMS spectra of monochloride

Fig. S2 - S8 Some spectra of probes

Fig. S9 Confocal fluorescence images of living HeLa cells on incubation with probe **2** for different times

^{1}H NMR and ^{13}C NMR spectra of intermediates and probes

Apparatus and chemicals

^{1}HNMR (300 MHz) and ^{13}CNMR (75 MHz) spectra were acquired on a Bruker Avance 300 spectro meter, with $CDCl_3$ or DMSO used as a solvent and tetramethylsilane (TMS) as an internal standard. High-resolution mass spectrometry (HRMS) involved a Q-TOF6510 spectrograph (Agilent). UV–vis spectra were measured by a Hitachi U-4100 spectrophotometer. Fluorescent measurements were performed on a Perkin-Elmer LS-55 luminescence spectrophotometer. Quartz cuvettes with a 1-cm path length and 3-mL volume were used for all measurements. The pH was determined with a model PHS-3C pH meter. Unless otherwise stated, all reagents were purchased from Aladdin, J&K or Sinopharm Chemical Reagent Co. and used without further purification. Twice-distilled water was used throughout all experiments. The salts used in stock aqueous solutions of metal ions were KNO_3, $Ca(NO_3)_2 \cdot 4H_2O$, $NaNO_3$, $Mg(NO_3)_2 \cdot 6H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, $Fe(NO_3)_3 \cdot 9H_2O$.

Preparation of reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Sodium hypochlorite (NaClO), H_2O_2 and *tert*-butylhydroperoxide (TBHP) were diluted from the commercially available solution to 0.1 M in water. Hydroxyl radical (·OH) and *tert*-butoxy radical (*t*-BuO·) were generated by Fenton reactions. Nitric oxide (NO) was generated from potassium nitroprusside dihydrate.[1] Peroxynitrite ($ONOO^-$) solution was prepared as reported.[2] Superoxide ($^{-}O_2$) was prepared from KO_2. Singlet oxygen ($^1O_2$) was synthesized as described.[3]

Absorption and fluorescence spectroscopy

Probe was dissolved in dimethylformamide (DMF) for a stock solution (1 mM). Test solutions were prepared by displacing 100 μL of the stock solution into a 10-mL volumetric flask. The solution was diluted to 10 mL in a mixture of phosphate buffer (0.1 M) and DMF (6 : 4, V/V). Small aliquots of each testing species solution were added. The resulting solutions were shaken well and incubated for 1 h at room temperature before recording spectra.

Preparation of chloride

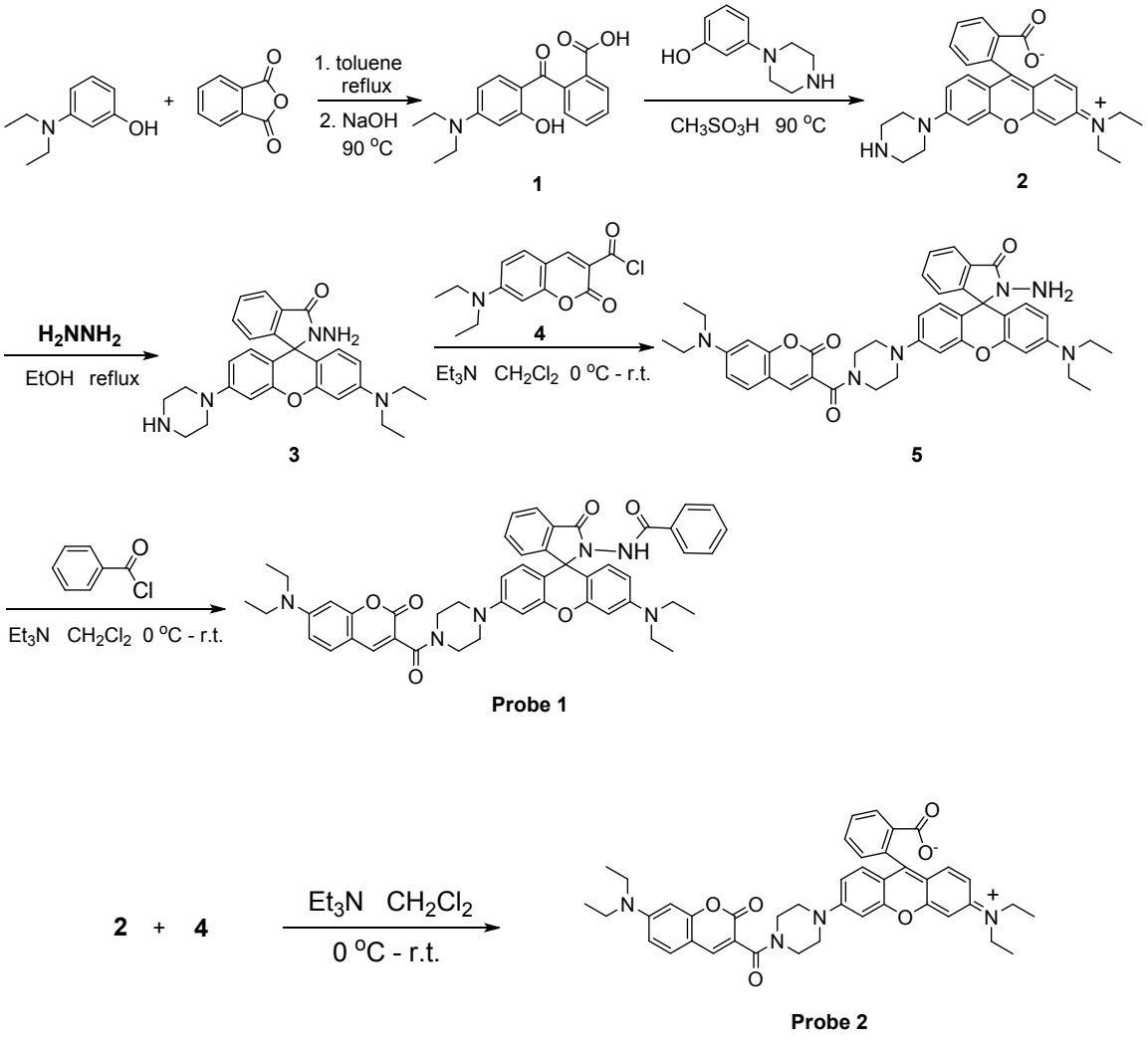
Probe **2** was dispersed into a mixture of NaH_2PO_4 (0.1 M, pH = 5) and EtOH (6 : 4, v/v). Then NaOCl (0.1 M, 18 equiv.) was added to the solution. The mixture was stirred for 0.5 h at room temperature. The EtOH was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate (20 mL × 3). The organic solution was washed with distilled water (20 mL × 3), dried with anhydrous magnesium sulfate and filtered. The solvent was removed under reduced pressure to give the crude product. The crude product was separated into two parts. One part was directly measured for HRMS and the other part was further purified by thin-layer chromatography (petroleum : dichloromethane : methanol = 20 : 20 : 1) to give monochloride.

Cell culture and cell imaging

RAW264.7 cells were cultured in DMEM with 10% fetal bovine serum (FBS). Probe **2** was dissolved in DMSO at a storage concentration of 10 mM and lipopolysaccharide (LPS) dissolved in 1×PBS at a storage concentration of 2 mg mL^{-1}. Cells were adherent-cultured in small glass dish for 24 h, then immersed in LPS (100 ng mL^{-1}) for 12 h. After

washing with phosphate buffered saline (PBS), cells were loaded with probe **2** for 0.5 h, then washed 3 times with PBS and underwent imaging measurement with a confocal microscope (LSM700) at excitation 405 nm. The emission of the blue channel was 405-550 nm and red channel 550-700 nm.

Synthesis of probes



Scheme S1 Synthesis of probes

Compound 1 was synthesized according to the reported method.[4]

Synthesis of compound 2:

To a solution of compound **1** (1.00 g, 3.19 mmol) in methylsulfonic acid (30 mL), 3-(1-piperazino)phenol (0.57 g, 3.19 mmol) was added and the reaction mixture was stirred at 90 ℃ for 24 h under nitrogen atmosphere. After the mixture was cooled to room temperature, EtOH (150 mL) was added, and methylsulfonic acid of the solution was neutralized by excess sodium carbonate. Precipitate was removed by filtration and washed with EtOH. The filtrate was then concentrated under reduced pressure to give brown solid (1.21 g). Yield: 83%. This crude product was used for next step without further purification.

Synthesis of compound 3：

Compound **2** (0.455 g, 1mmol) was dissolved in EtOH (15 mL), and $NH_2NH_2 \cdot H_2O$ (30 mL) was added to the solution. The reaction solution was heated and kept reflux for 4 h. The ethanol was removed under reduced pressure and the aqueous layer was extracted with dichloromethane (100 mL × 3). The organic solution was washed with distilled water (50 mL× 3), dried with anhydrous magnesium sulfate and then filtered. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (dichloromethane : methanol = 3:1) to give white solid (0.288 g). Yield: 61%. ^{1}H NMR (300 MHz, $(CD_3)_2SO$) δ 7.77 (dd, *J* = 5.6, 2.8 Hz, 1H), 7.53–7.42 (m, 2H), 6.97 (dd, *J* = 5.6, 2.6 Hz, 1H), 6.68 (d, *J* = 2.3 Hz, 1H), 6.62 (dd, *J* = 8.8, 2.4 Hz, 1H), 6.41–6.36 (m, 4H), 4.31 (s, 2H), 3.32 (q, *J* = 6.9 Hz, 4H), 3.15–3.11 (m, 4H), 2.94–2.79 (m, 4H), 1.08 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (75 MHz, $(CD_3)_2SO$) δ 165.35, 152.88, 152.63, 151.88, 151.65, 148.18, 132.42, 129.50, 128.21, 127.66, 127.37, 123.43, 122.20,

111.30, 109.32, 107.98, 105.27, 101.56, 97.37, 64.63, 48.28, 47.95, 44.91, 43.65, 12.40. HRMS: m/z $[M+H]^+$ calcd for $C_{28}H_{31}N_5O_2$: 470.2556, found: 470.2550.

Compound 4 was synthesized according to the reported method.[5]

Synthesis of compound 5:

Compound **3** (0.117 g, 0.25 mmol) was dissolved in dry dichloromethane (5 mL) and Et_3N (0.5 mL). The solution was cooled with stirring under nitrogen atmosphere to 0 ℃. Subsequently, Compound **4** (0.070 g, 0.25 mmol) was added into the solution in batches. After addition, the reaction was stirred at 0 ℃ for 30 min and then at room temperature for 3 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (ethyl acetate : dichloromethane : methanol = 20:20:1) to give red solid (0.103 g). Yield: 58%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.95–7.89 (m, 2H), 7.46 (dd, J = 5.1, 3.3 Hz, 2H), 7.36–7.21 (m, 2H), 7.13–7.03 (m, 1H), 6.68 (s, 1H), 6.60 (dd, J = 8.9, 2.1 Hz, 1H), 6.54 (s, 2H), 6.51–6.37 (m, 3H), 6.32 (s, 1H), 3.89 (s, 2H), 3.65 (s, 2H), 3.56 (s, 2H), 3.47–3.40 (m, 4H), 3.38–3.31 (m, 8H), 1.25–1.15 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.25, 165.10, 159.13, 157.39, 153.64, 153.52, 151.82, 151.30, 145.39, 132.65, 129.91, 128.35, 128.10, 123.82, 123.10, 116.14, 112.16, 109.64, 109.42, 108.19, 107.84, 103.04, 98.10, 97.05, 65.64, 48.31, 47.03, 44.98, 44.42, 42.07, 12.58, 12.44, HRMS: m/z $[M+H]^+$ calcd for $C_{42}H_{44}N_6O_5$: 713.3451, found: 713.3454.

Synthesis of probe 1:

Compound **5** (0.178 g, 0.25 mmol) was dissolved in dry dichloromethane (5 mL) and

Et_3N (0.5 mL). The solution was cooled with stirring under nitrogen atmosphere to 0 ℃. Subsequently, benzoyl chloride (0.084 g, 0.6 mmol) in dry dichloromethane (2 mL) was dropped into the solution. After addition, the reaction was stirred at 0 ℃ for 30 min and then at room temperature for 6 h. The reaction process was detected by TCL. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel with eluent (ethyl acetate : dichloromethane : methanol = 20:20:1) to give yellow solid (0.049 g). Yield: 24%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (dd, *J* = 6.0, 2.1 Hz, 1H), 7.88 (s, 1H), 7.62–7.45 (m, 5H), 7.40 (s, 1H), 7.31 (d, *J* = 8.9 Hz, 2H), 7.13 (dd, *J* = 6.0, 1.8 Hz, 1H), 6.82 (s, 1H), 6.72–6.50 (m, 4H), 6.48 (d, *J* = 2.2 Hz, 1H), 6.38–6.33 (m, 2H), 3.86 (s, 2H), 3.54 (s, 2H), 3.43 (q, *J* = 7.1 Hz, 4H), 3.37–3.29 (m, 8H), 1.25–1.14 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.70, 165.07, 159.13, 157.36, 153.68, 153.49, 151.79, 149.11, 145.35, 133.34, 132.38, 131.69, 130.91, 129.93, 129.69, 129.23, 129.12, 128.84, 128.52, 128.32, 127.49, 124.17, 123.67, 116.07, 111.77, 109.41, 108.31, 107.82, 102.35, 97.71, 97.01, 65.56, 48.12, 46.99, 44.97, 44.35, 42.06, 12.60, 12.43. HRMS: m/z $[M+H]^+$ calcd for $C_{49}H_{48}N_6O_6$: 817.3713, found: 817.3746.

Synthesis of probe 2:

To a solution of compound **2** (1.00 g, 2.20 mmol) in dry CH_2Cl_2 (28 mL) and Et_3N (3 mL), compound **4** (0.71 g 2.52 mmol) was added at 0℃ under nitrogen atmosphere. The mixture was stirred for 30 min at 0℃ and for further 3 h at room temperature. The solvent was removed under reduced pressure and the crude product was purified by column chromatography on silica gel (dichloromethane : methanol = 15:1) to give red

solid (0.84 g). Yield: 55%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, *J* = 6.6 Hz, 1H), 7.92 (s, 1H), 7.60–7.54 (m, 2H), 7.32 (d, *J* = 8.8 Hz, 1H), 7.17 (d, *J* = 7.1 Hz, 1H), 6.71–6.59 (m, 5H), 6.48 (s, 2H), 6.41 (s, 1H), 3.87 (s, 2H), 3.57 (s, 2H), 3.48–3.30 (m, 12H), 1.25–1.15 (m, 12H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.69, 165.12, 159.16, 157.40, 153.17, 152.95, 152.43, 151.82, 149.67, 145.44, 134.59, 129.93, 129.34, 128.95, 127.54, 124.89, 124.16, 116.08, 111.94, 110.50, 109.43, 108.42, 107.84, 102.51, 97.68, 97.04, 48.17, 46.97, 44.98, 44.53, 42.04, 12.54, 12.44. HRMS: m/z $[M+H]^+$ calcd for $C_{42}H_{42}N_4O_6$: 699.3182, found: 699.3164.

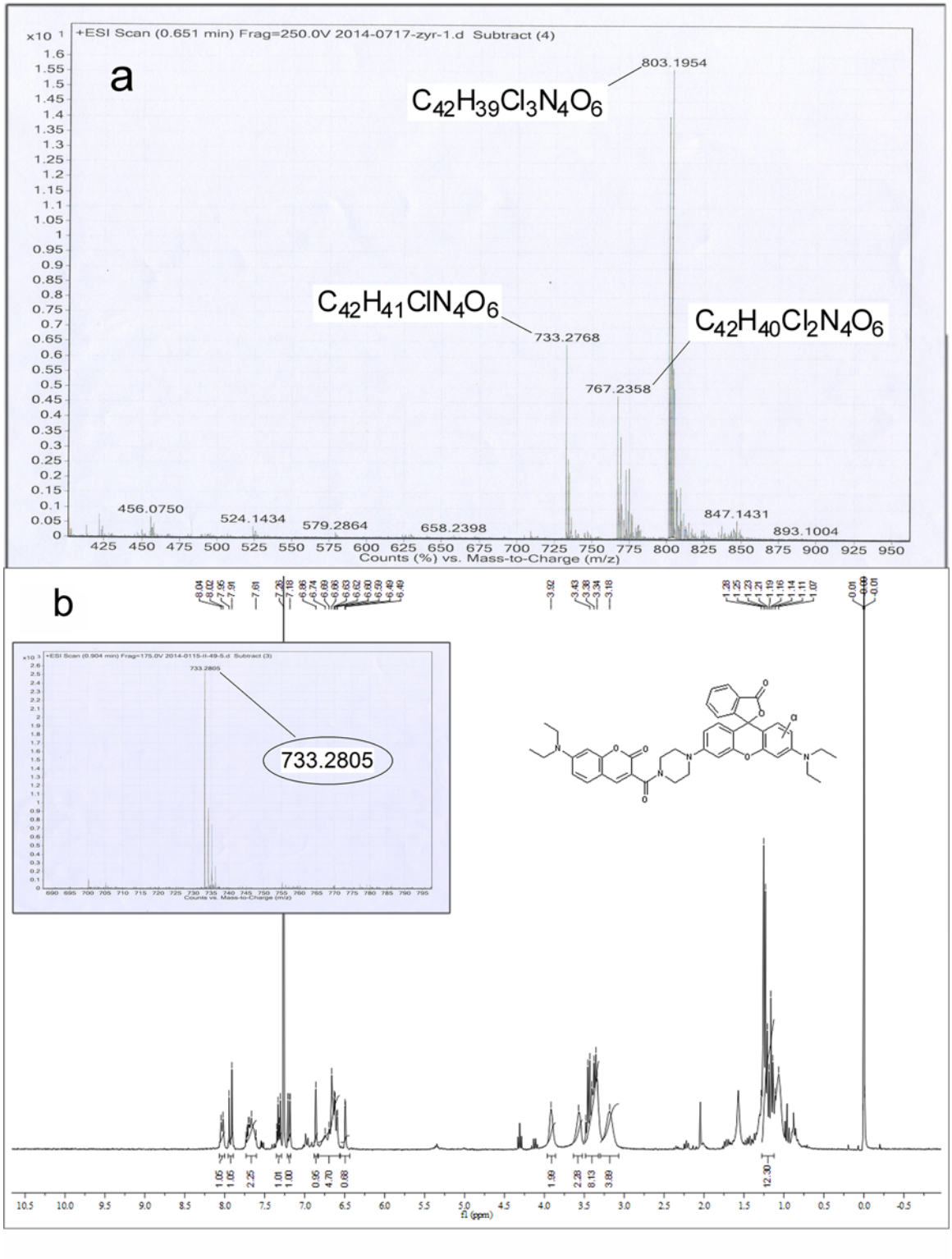


Fig. S1 (a) HRMS spectrum of the crude product after treatment of probe **1** with 18 equiv. of HOCl. (b) ^{1}HNMR and HRMS (insert) spectra of monochloride separated from the

crude product.

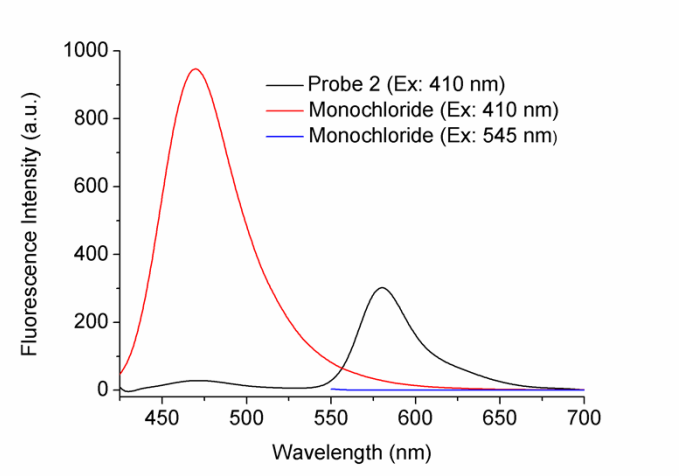


Figure S2 Fluorescence spectra of probe **2** and monochloride with difference in excitation at 410 and 545 nm (slit widths: 12 nm/5 nm). Condition: [Probe **2**] = 10 μM, [Monochloride] = 10 μM, NaH_2PO_4 (0.1 M, pH = 5) : DMF (6: 4, v/v).

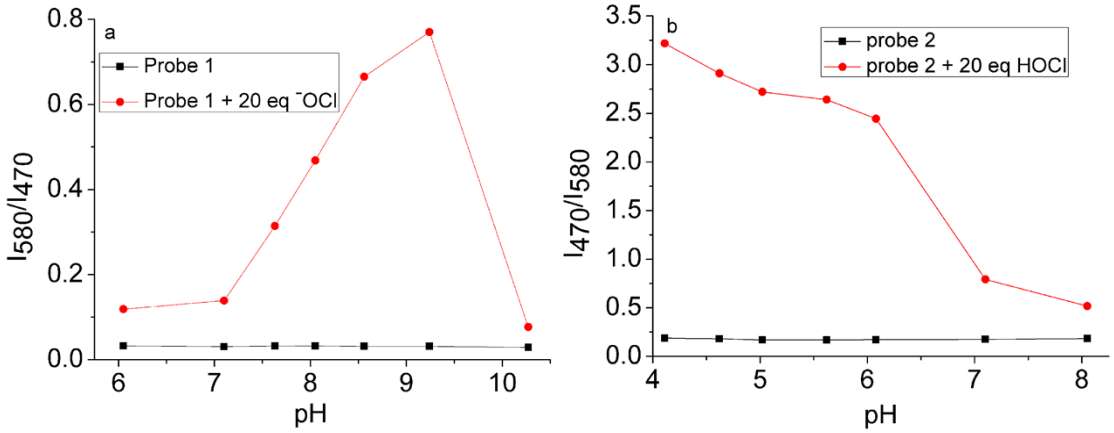


Fig. S3 pH-dependent fluorescence intensity ratio changes of (a) probe **1** and (b) probe **2**. Condition: [Probe **1**] = 10 μM, Na_2HPO_4 (0.1 M, pH = 8) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm). [Probe **2**] = 10 μM, NaH_2PO_4 (0.1 M, pH = 5) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm)

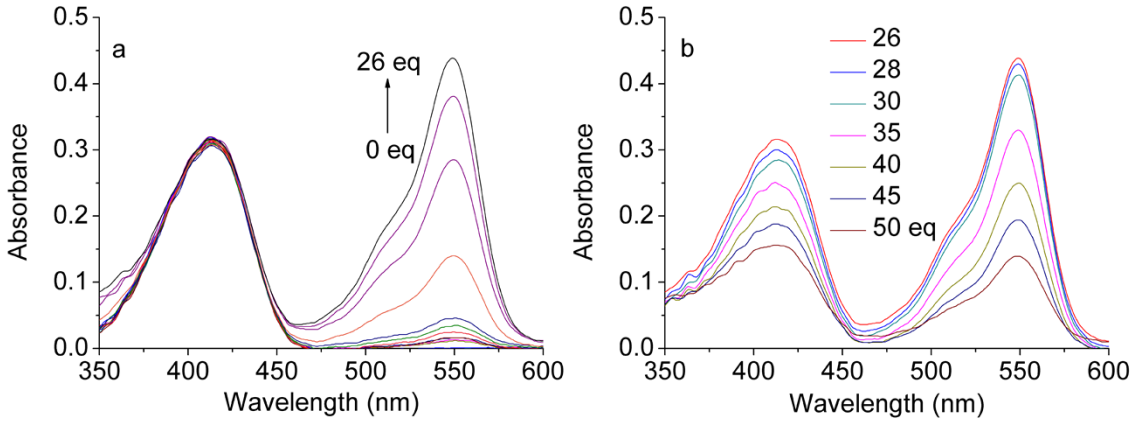


Fig. S4 (a) Absorption spectra of probe **1** up addition of $^{-}$OCl (0–26 equiv.). (b) Absorption spectra of probe **1** up addition of $^{-}$OCl (26–50 equiv.). Condition: [Probe **1**] = 10 μM, Na_2HPO_4 (0.1 M, pH = 8) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm).

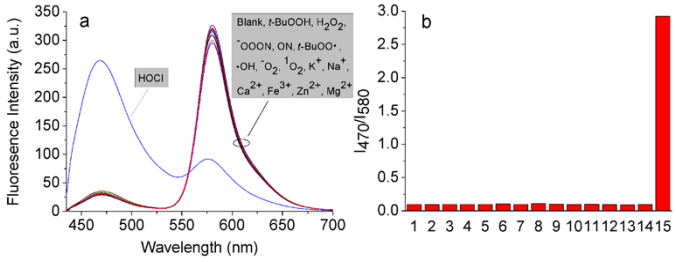


Figure S5 (a) Fluorescence spectra and (b) fluorescence intensity ratio (I_{470}/I_{580}) changes of probe **2** to HOCl (20 equiv.), other ROS/RNS and metal ions (20 equiv. for (1) HO·, (2) $ONOO^-$, (3) NO, (4) H_2O_2, (5) *t*-BuOOH, (6) *t*-BuOO·, (7) $^1O_2$, (8) $^-O_2$, (9) Ca^{2+}, (10) Fe^{3+}, (11) Mg^{2+}, (12) Zn^{2+}, (13) K^+, (14) Na^+ and (15) HOCl). Condition: [Probe **2**] = 10 μM, NaH_2PO_4 (0.1 M, pH = 5) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm).

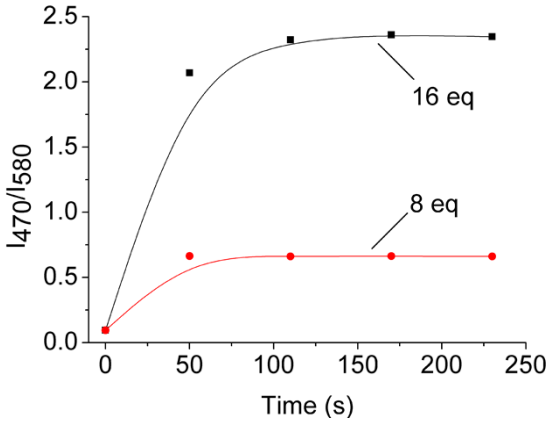


Fig. S6 Time-dependent fluorescence intensity ratio (I_{470}/I_{580}) changes of probe **2**. Condition: [Probe **2**] = 10 μM, NaH_2PO_4 (0.1 M, pH = 5) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm).

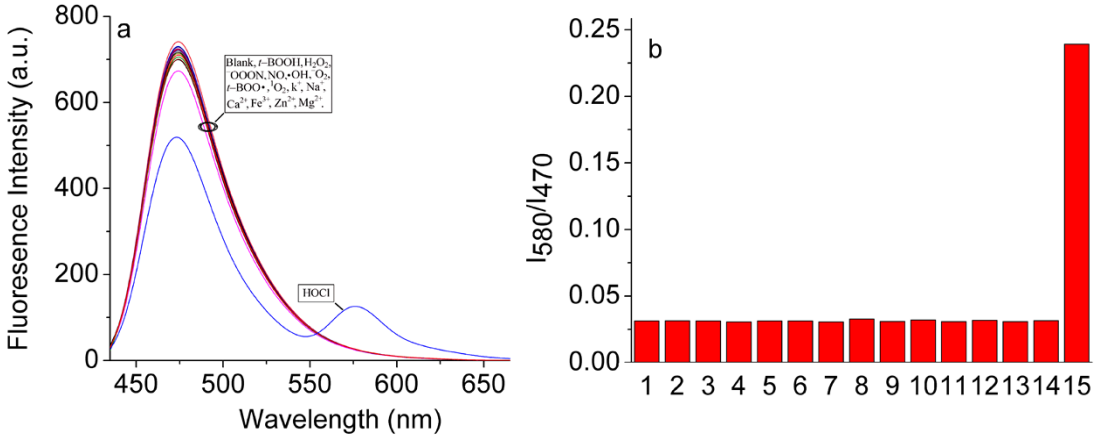


Fig. S7 (a) Fluorescence spectra and (b) fluorescence intensity ratio (I_{580}/I_{470}) changes of the probe **1** to $^{-}$OCl, other ROS/RNS and metal ions (20 equiv. for (1) $HO^{\cdot}$, (2) $ONOO^{-}$, (3) NO, (4) H_2O_2, (5) *t*-BuOOH, (6) *t*-$BuOO^{\cdot}$, (7) $^1O_2$, (8) $^{-}O_2$, (9) Ca^{2+}, (10) Fe^{3+}, (11) Mg^{2+}, (12) Zn^{2+}, (13) K^+, (14) Na^+ and (15) HOCl). Condition: [Probe **1**] = 10 μM, Na_2HPO_4 (0.1 M, pH = 8) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm).

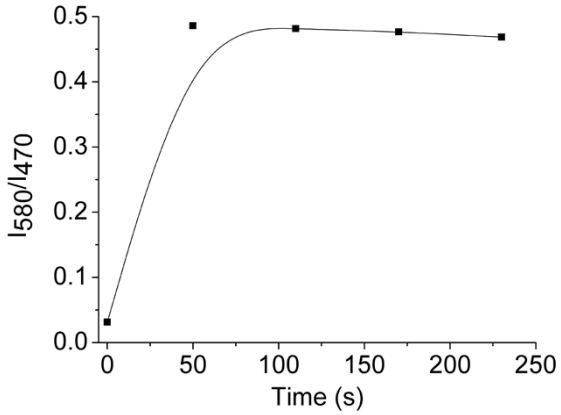


Fig. S8 Time-dependent fluorescence intensity ratio (I_{580}/I_{470}) changes of the probe **1** upon addition of HOCl (26 equiv.). Condition: [Probe **1**] = 10 μM, NaH_2PO_4 (0.1 M, pH = 8) : DMF (6: 4, v/v), λ_{ex}: 410 nm (slit widths: 12 nm/5 nm).

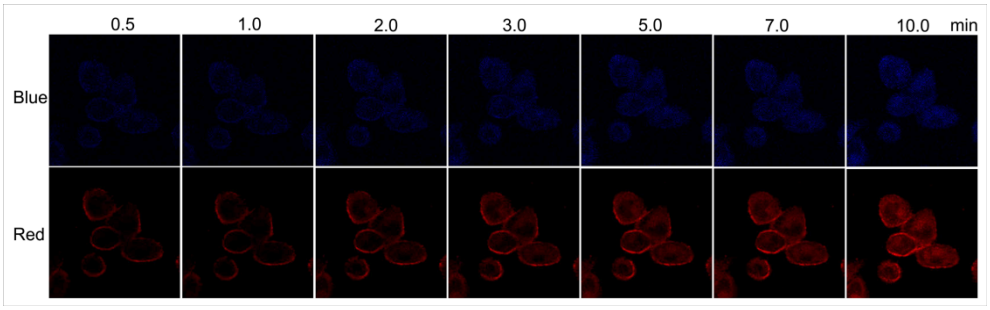
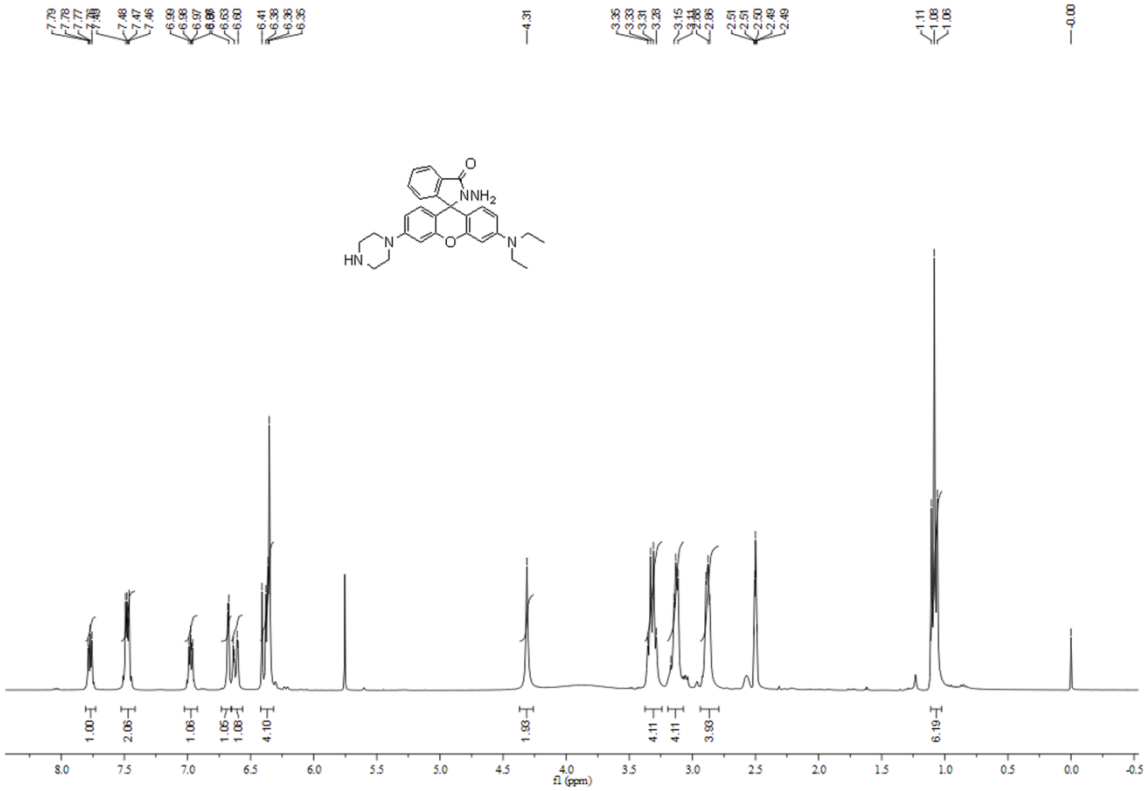


Fig. S9 Confocal fluorescence images of living HeLa cells on incubation with probe **2** (1.0 μM) for different times (0.5, 1.0, 2.0, 3.0, 5.0, 7.0, 10.0 min. excitation: 405 nm, emission: blue 405–550 nm, red 550–700 nm).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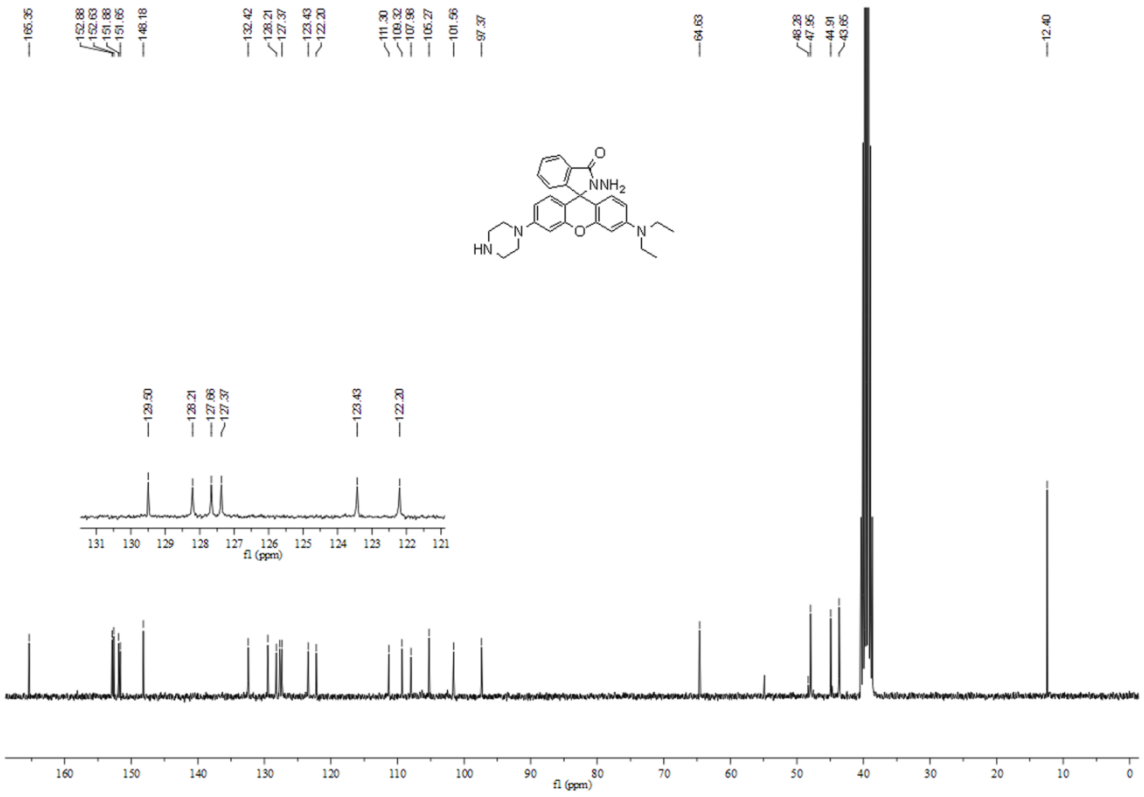
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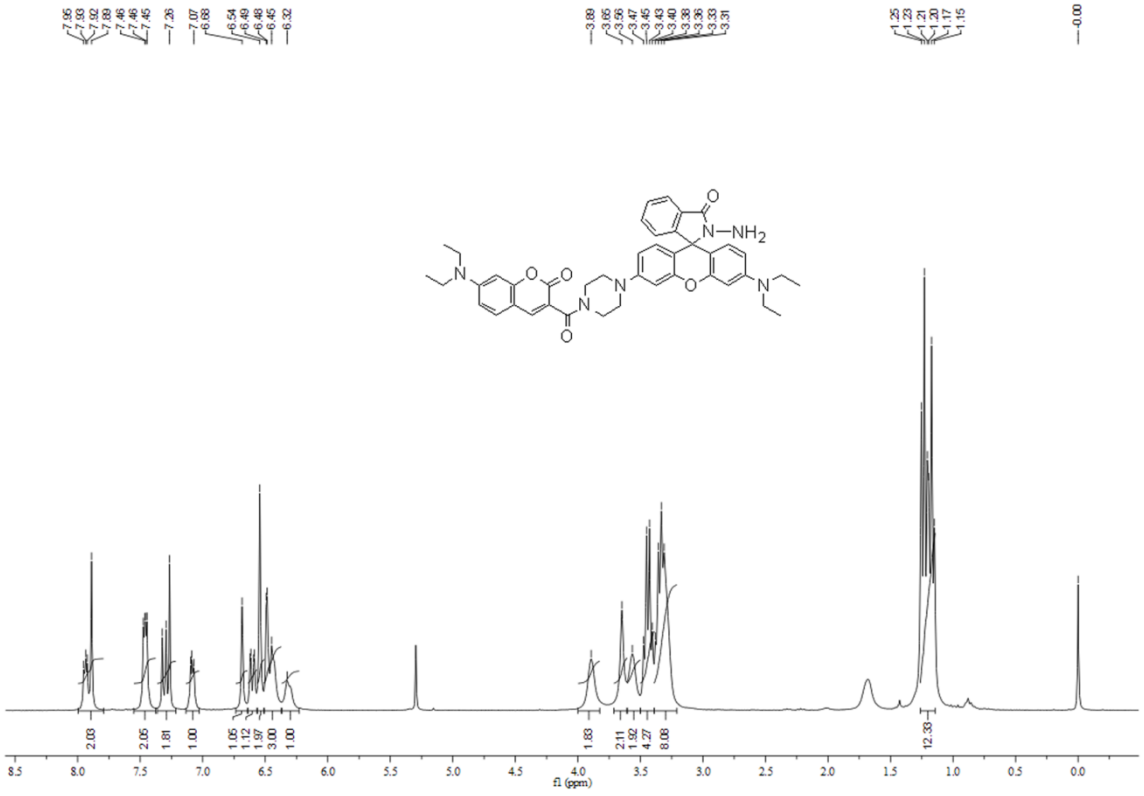
f1 (ppm)
f1 (ppm)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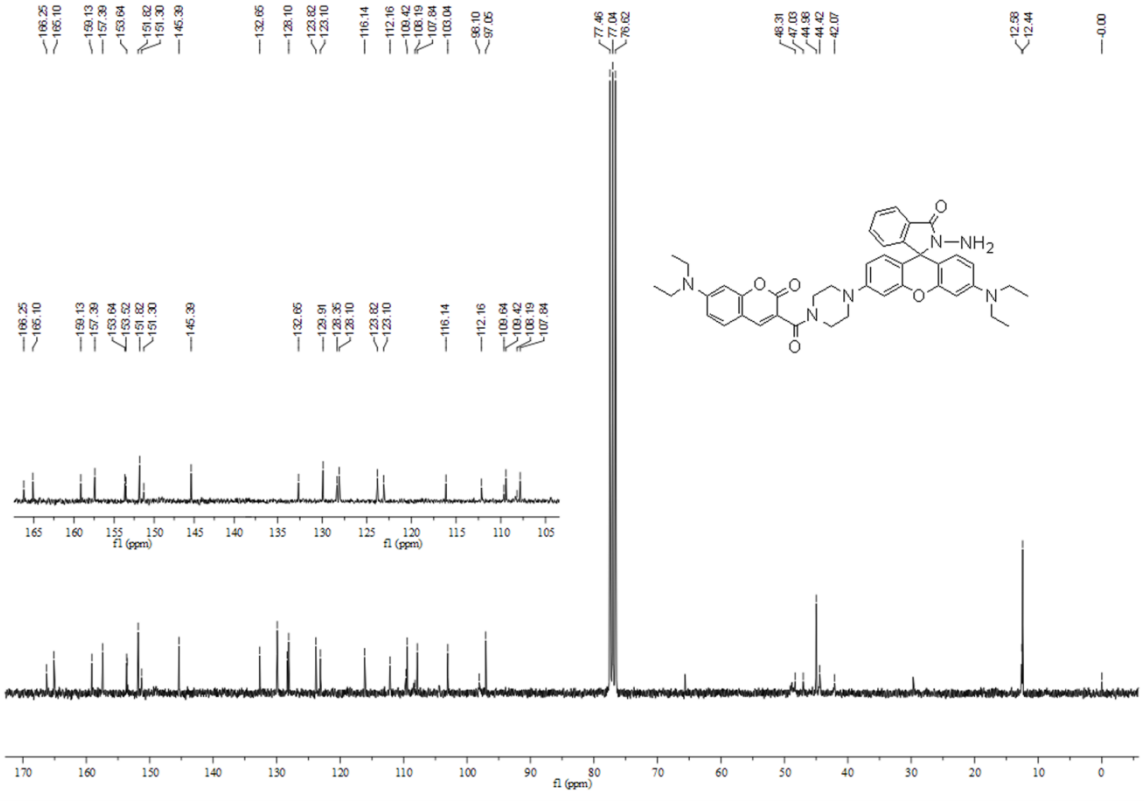
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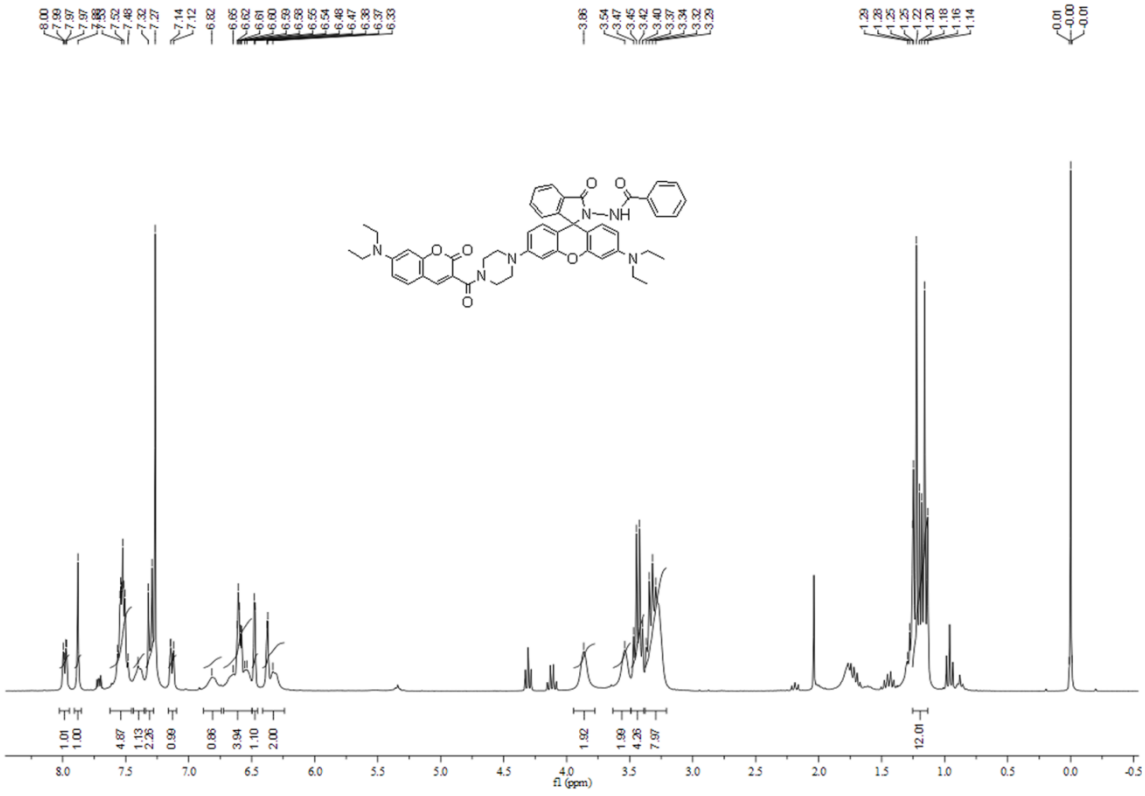

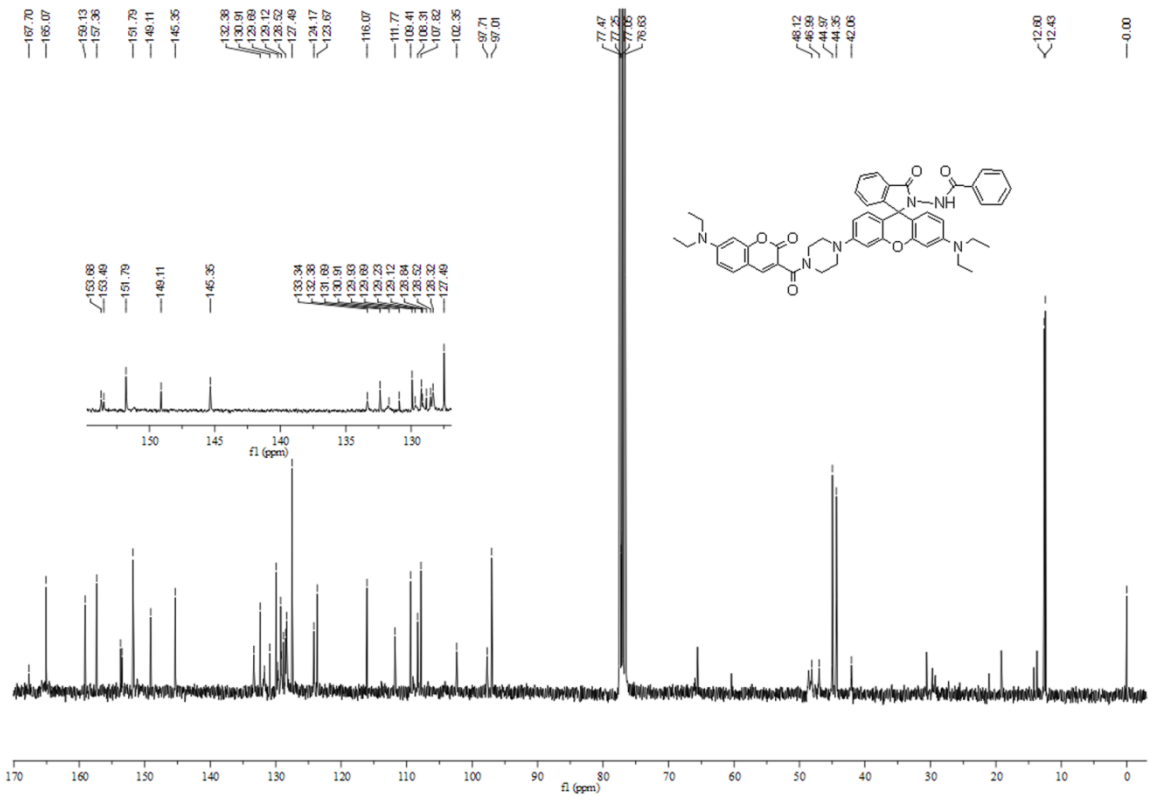

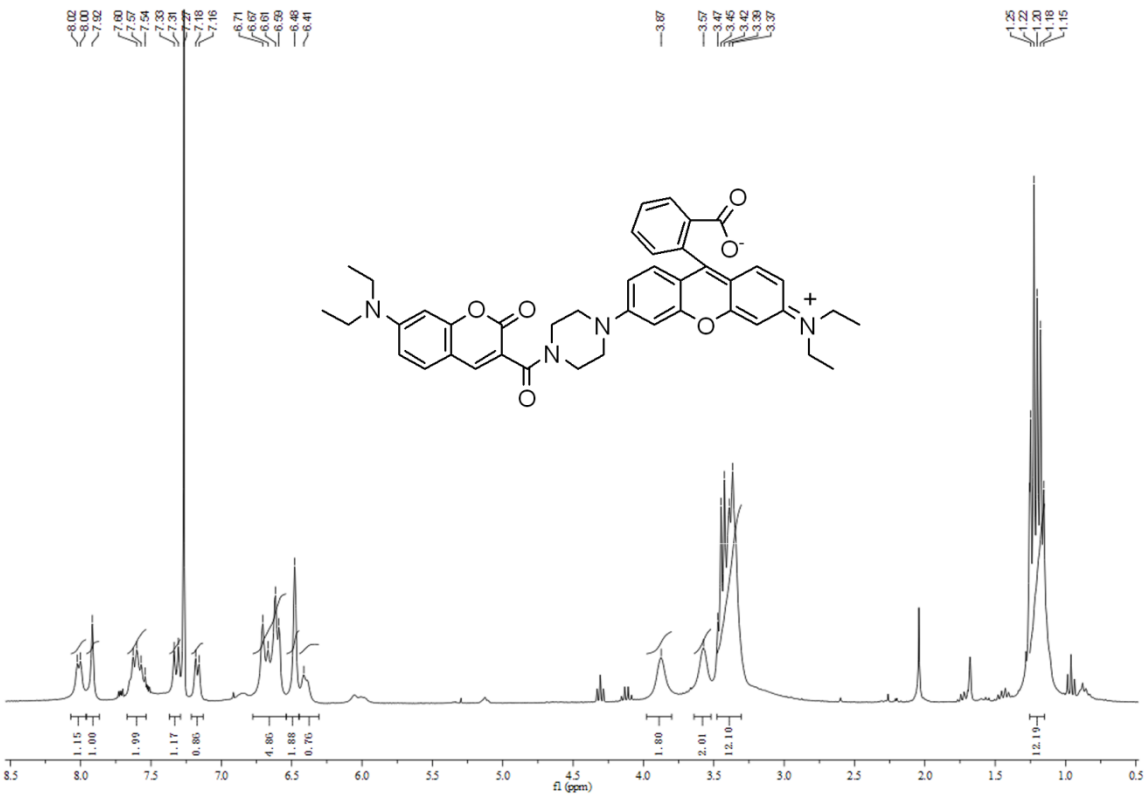
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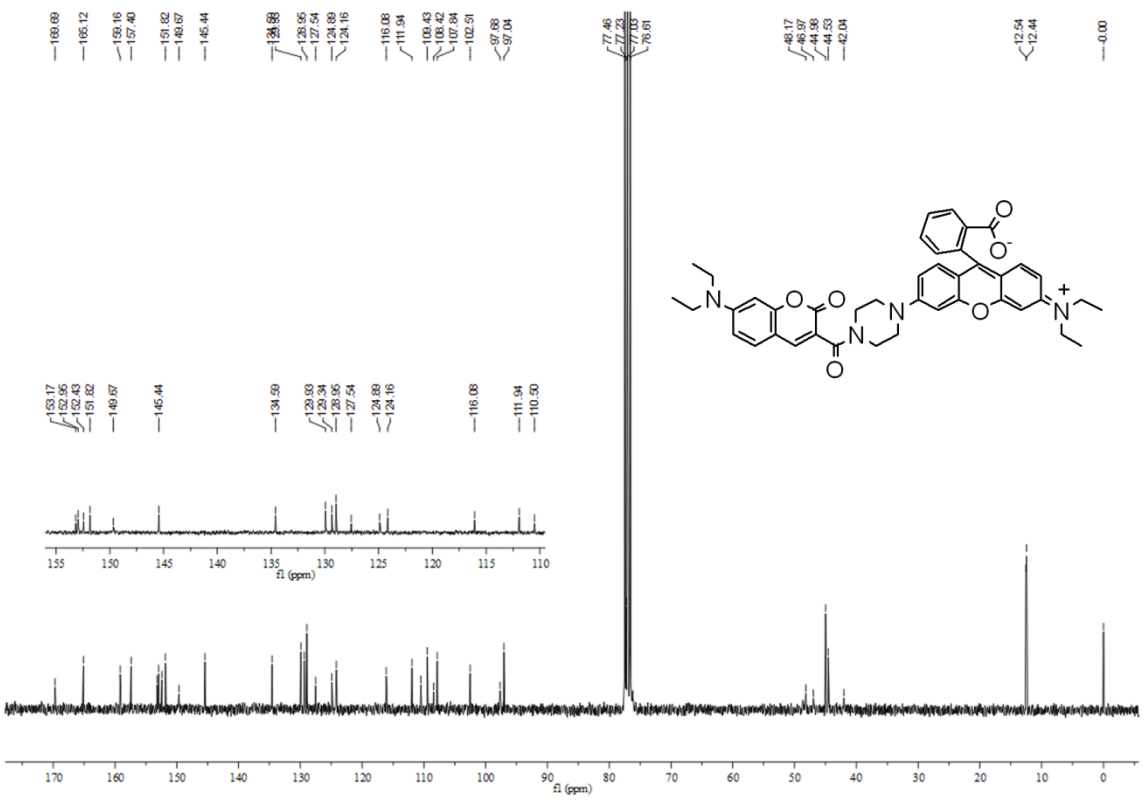
12.19
f1 (ppm)


160.69
155.12
159.16
157.40
151.82
149.67
145.44
134.59
129.93
129.34
128.95
127.54
124.89
124.16
116.08
111.94
110.43
108.42
107.84
102.51
97.88
97.04
77.46
77.23
77.03
76.61
48.17
46.97
44.98
44.53
42.04
12.54
12.44
0.00
153.17
152.95
152.43
151.82
149.67
145.44
134.59
129.93
129.34
128.95
127.54
124.89
124.16
116.08
111.94
110.50
f1 (ppm)
f1 (ppm)

Reference

(1) I. Ioannidis, M. Bätz, T. Paul, H. Korth, R. Sustmann, H. De Groot, *Biochem. J.*, 1996, **318**, 789-795.

(2) J. W. Reed, H. H. Ho, W. L. Jolly, *J. Am. Chem. Soc.*, 1974, **96**, 1248-1249. Ma, Z.; W. Sun, L. Z. Chen, J. Li, Z. Z. Liu, H. X. Bai, M. L. Zhu, P. Du, X. D. Shi, M. Y. Li, *Chem. Commun.,* 2013, **49**, 6295-6297.

(3) X. H. Li, G. X. Zhang, H. M. Ma, D. Q. Zhang, J. Li, D. B. Zhu, *J. Am. Chem. Soc.* 2004, **126**, 11543-11548.

(4) Q. H. Liu, X. L. Yan, J. C. Guo, D. H. Wang, L. Li, F. Y. Yan, L. G. Chen, *Spectrochimica Acta Part A* 2009, **73,** 789–793.

(5) G. J. He, D. Guo, C. He, X. L. Zhang, X. W. Zhao, C. Y. Duan, *Angew. Chem. Int. Ed.* 2009, **48,** 6132–6135.