Electronic Supplementary Material (ESI) for Analyst

Supplementary Information

Ratiometric Fluorescence Imaging of Cellular Hypochloric Acid Based on Heptamethine Cyanine Dye

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Contents

1. General experimental section

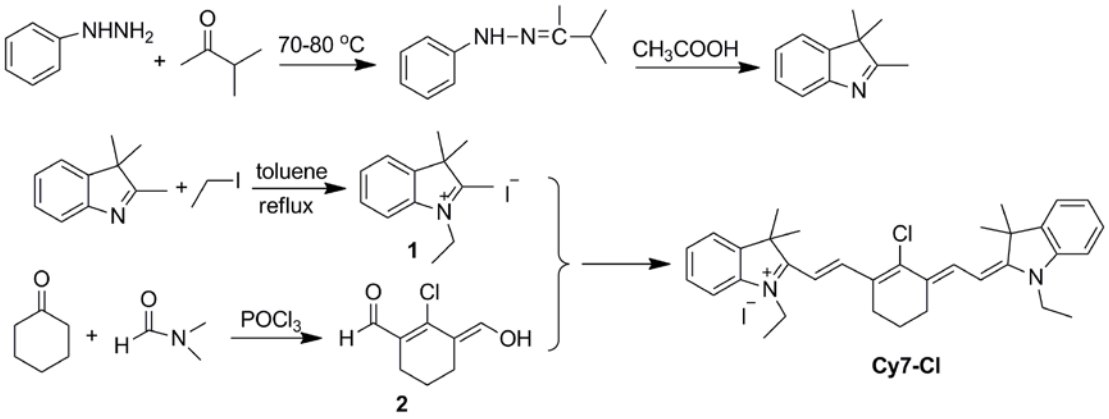
Materials and characterization: The $ONOO^-$ source was the donor 3-Morpholinosydnonimine hydrochloride (SIN-1, 10.0 mmol/ml)[1]. NO is generated in form of sodium nitroprusside (SNP)[2]. $^1O_2$ was generated by the reaction of H_2O_2 with NaOCl[3] and $O_2^{-}\cdot$ was created by KO_2[4]. ·OH was generated by Fenton reaction between Fe^{II}(EDTA) and H_2O_2 quantitively, and Fe^{II}(EDTA) concentrations represented ·OH concentrations[5]. Tert-butylhydroperoxide (*t*-BuOOH) and cumene hydroperoxide (CuOOH) could also use to induce ROS in biological systems[6]. Hypochlorous acid (HOCl) was standardized ($\varepsilon_{292\ nm}$= 350 $M^{-1}cm^{-1}$)[7]. Common reagents or materials were obtained from commercial source of analytical reagent grade, and used without further purification except as otherwise noted. Ultrapure water was used throughout the analytical experiments. ^{1}H NMR, ^{13}C NMR were obtained on a Bruker DRX-400 spectrometer, and the chemical shifts (δ) are reported in ppm relative to TMS (Me_4Si) as internal reference.

Absorption and fluorescence analysis: Steady-state UV/Vis were measured at room temperature on a Lambda 35 UV-visible Spectrophotometer (Perkin-Elmer) with 1.0-cm quartz cells. Fluorescence emission spectra were obtained at room temperature on a Fluoromax-4 Spectrofluorometer (Horiba-Jobin Yvon), with a Xenon lamp and 1.0-cm quartz cells. The probe Cy7-NR (N, N-dimethylformamide, 50 μL, 1.0 mM) was added to a 10.0-mL color comparison tube. After dilution to 5.0 μM with 20 mM PBS buffers (pH=7.40), NaClO was added. The mixture was equilibrated for 6 min before measurement. The fluorescence intensity was measured at λ_{ex} = 540 nm. The excitation and emission slits were set to 5 and 5 nm, respectively.

Cell culture and confocal imaging: HeLa cells were seeded at a density of 1×10^6 cells mL^{-1} for confocal imaging in RPMI 1640 Medium supplemented with 15% fetal bovine serum (FBS), $NaHCO_3$ (2 g/L), and 1% antibiotics (penicillin /streptomycin, 100 U/ml). Cultures were maintained at 37 °C under a humidified atmosphere containing 5% CO_2. The cells were subcultured by scraping and seeding on 35 mm × 12 mm glass bottom cell culture dishes according to the instructions from the manufacturer. Florescent images were acquired on a FV1000 confocal laser-scanning microscope (Olympus) with an objective lens (×40, ×60). Prior to imaging, the medium was removed. Cell imaging was carried out after washing cells with physiological saline (0.9 %) for three times.

2. Synthesis and characterization of compounds

The general synthetic route of the compound Cy7-Cl was described in Scheme S1.



Synthesis of 1,2,3,3-tetramethyl-3H-indolium iodide (1). Compounds 1 was synthesized according to a slight modification of the literature procedure[8]. To a 250 ml three-neck flask containing phenylhydrazine (54.10 g ,0.5 mol), 3-Methylbutanone (43.42 g, 0.5 mol) was dropped. After the mixture was stirred and heated to 80 ^{o}C for 4 h, the colourless lower layer was removed and the orange upper layer was collected. Then acetic acid (160 mL) was added to the collected orange solution and refluxed for 3.5 hours. After the mixture was cooled down, saturated sodium bicarbonate solution (50 mL) was added. The resulting solution was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and the solvent was evaporated on a rotary evaporator, yellow liquid (2,3,3-trimethyl-3H-indole) was obtained by reduced pressure distillation. The obtained 2,3,3-trimethyl-3H-indole (24.03 g, 0.15 mol) and iodoethane (40.03 g, 0.25 mol) in 60 ml toluene were stirred for 22 hours at 100 ^{o}C. After of which, the mixture was filtered and the precipitate was washed with ethyl acetate, a pink solid was gained (37.57 g, 79.5 %).

Synthesis of 2-Chloro-1-formyl-3-hydroxymethylenecyclohexen (2). Compounds 2 was synthesized according to a slight modification of the literature procedure[9]. A solution of 40 ml of N,N'-dimethylformamide in 40 ml dichloromethane was cooled to -6 ^{o}C and phosphorus oxychloride (37 ml) was added dropwise with stirring, followed by cyclohexanone (10.00g, 0.10 mol). The mixture was refluxed for 3 h. After cooled, the solution was poured onto 200 g of ice and allowed to stand overnight. A yellow solid was collected and dried (8.04 g, 46.5 %).

Synthesis of 2-[4-Chloro-7-(1-ethyl-3,3-dimethyl(indolin-2-ylidene)]-3,5-(propane -1,3-diyl)-1,3,5-heptatrien-1-yl)-1-ethyl-3,3-dimethyl-3H-indolium (Cy7-Cl). Cy7-Cl was synthesized according to a slight modification of the literature procedure[10]. Under the nitrogen atmosphere, to a three-neck flask containing compound **1** (11.23 g, 36 mmol) in 70 ml acetic anhydride, sodium acetate (2.95 g, 36 mmol) was added. The solution was heated to 70 ^{o}C for 1 h. After of which, the reaction solution was poured to potassium iodide aqueous solution, stirred. The generated mixture was filtered, and the precipitate was washed thoroughly with diethyl ether. A green solid was obtained and dried (7.88 g, 68.6 %). ^{1}H NMR (400 MHz, $CDCl_3$) δ(ppm): 8.36 (d, J=16.0 Hz, 2H), 7.43-7.41 (m, 4H), 7.29-7.24 (m, 2H), 7.19 (d, J=8.0 Hz, 2H), 6.23 (d, J=12.0 Hz, 2H), 4.27 (q, J=6.7 Hz, 4H), 2.76 (t, J=6.0 Hz, 4H), 1.99-1.98 (m, 2H), 1.73 (s, 12H), 1.47 (t, J=8.0 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ(ppm): 171.96, 171.89, 154.29, 150.62, 144.49, 141.77, 141.33, 141.17, 133.49, 128.88, 127.50, 125.40, 125.34, 122.37, 122.31, 110.74, 101.77, 101.05, 49.43, 49.36, 40.13, 28.51, 28.08, 27.95, 26.83, 20.71, 12.50. HRMS: m/z

$C_{34}H_{40}N_2Cl^+$ Calcd 511.2880, found 511.2885.

Synthesis of Cy7-NR(1-4). Under the nitrogen atmosphere, a mixture containing **Cy7-Cl** and 10 equiv of NRH(**1**: morpholine; **2**: piperidine; **3**: piperazine; **4**: thiomorpholine) in anhydrous N, N'-dimethylformamide (DMF) was stirred at 90 ^oC. After there was no **Cy7-Cl**, the reaction was stopped. Then, the DMF was removed under reduced pressure. The resulting residue was purified on a short-column chromatography (silica gel, ethyl acetate : methanol = 5:1).

Synthesis of Cy7-NR5. Under the nitrogen atmosphere, a mixture containing **Cy7-Cl**, 10 equiv of **NRH** (**5**: dimethylamine hydrochloride) and Et_3N was stirred at 90 ^oC. The next procedure was same to the synthesis of **Cy7-NR(1-4)**.

Cy7-NR1: 1H NMR (400 MHz, $CDCl_3$) δ(ppm): 7.77 (d, J=12.0 Hz, 2H), 7.34 (t, J=6.0 Hz, 4H), 7.16 (t, J=8.0 Hz, 2H), 7.03 (d, J=8.0 Hz, 2H), 5.93 (d, J=16.0 Hz, 2H), 4.09 (q, J=6.7 Hz, 4H), 3.98 (t, J=4.0 Hz, 4H), 3.72 (t, J=4.0 Hz, 4H), 2.56 (t, J=6.0 Hz, 4H), 1.88 (t, J=6.0 Hz, 2H), 1.69 (s, 12H), 1.44 (t, J=6.0 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ(ppm): 171.78, 168.91, 142.16, 141.59, 140.39, 128.65, 125.31, 123.97, 122.10, 109.57, 96.91, 68.31, 55.01, 48.30, 39.05, 28.84, 25.32, 21.69, 12.0. HRMS: m/z $C_{38}H_{48}N_3O^+$ Calcd 562.3797, found 562.2762.

Cy7-NR2: 1H NMR (400 MHz, $CDCl_3$) δ(ppm): 7.55 (d, J=16.0 Hz, 2H), 7.34-7.27 (m, 4H), 7.11 (t, J=8.0 Hz, 2H), 6.95 (d, J=8.0 Hz, 2H), 5.75 (d, J=12.0 Hz, 2H), 3.98-3.95 (m, 4H), 3.86 (br, 4H), 2.50 (t, J=6 Hz, 4H), 1.92-1.84 (m, 6H), 1.74 (br, 2H), 1.66 (s, 12H), 1.38 (t, J=8.0 Hz, 6H). HRMS: m/z $C_{39}H_{50}N_3^+$ Calcd 560.4005, found 560.3985.

Cy7-NR3: 1H NMR (400 MHz, $CDCl_3$) δ(ppm): 7.85 (d, J=12.0 Hz, 2H), 7.42 (d, J=8.0 Hz, 2H), 7.36 (t, J=6.0 Hz, 2H), 7.19 (t, J=8.0 Hz, 2H), 7.05 (d, J=8.0 Hz, 2H), 5.90 (d, J=16.0 Hz, 2H), 4.07-4.03 (m, 8H), 3.53 (s, 4H), 2.52 (t, J=6.0 Hz, 4H), 1.88-1.85 (m, 2H), 1.80 (s, 12H), 1.44 (t, J=8.0 Hz, 6H). HRMS: m/z $C_{38}H_{49}N_4^+$ Calcd 561.3957, found 561.3971.

Cy7-NR4: 1H NMR (400 MHz, $CDCl_3$) δ(ppm): 7.89 (d, J=12.0 Hz, 2H), 7.36 (t, J=6.0 Hz, 4H), 7.20 (t, J=6.0 Hz, 2H), 7.09 (d, J=8.0 Hz, 2H), 6.00 (d, J=16.0 Hz, 2H), 4.14 (q, J=6.7 Hz, 4H), 3.73 (t, J=4.0 Hz, 4H), 2.90 (t, J=6.0 Hz, 4H), 2.56 (t, J=6.0 Hz, 4H), 1.88 (t, J=6.0 Hz, 2H), 1.74 (s, 12H), 1.44 (t, J=6.0 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ(ppm): 171.32, 169.75, 142.19, 142.07, 140.57, 130.90, 128.83, 128.73, 127.76, 124.38, 122.12, 109.93, 98.36, 65.55, 56.38, 48.60, 39.39, 30.57, 29.35, 28.58, 25.50, 21.58, 19.17, 13.70, 12.13. HRMS: m/z $C_{38}H_{48}N_3S^+$ Calcd 578.3569, found 578.5587.

Cy7-NR5: 1H NMR (400 MHz, $CDCl_3$) δ(ppm): 7.47 (d, J=12.0 Hz, 2H), 7.32-7.28 (m, 4H), 7.08 (t, J=8.0 Hz, 2H), 6.93 (d, J=8.0 Hz, 2H), 5.67 (d, J=12.0 Hz, 2H), 3.94 (q, J=8.0 Hz, 4H), 3.65 (s, 6H), 2.51 (t, J=6.0 Hz, 4H), 1.86 (t, J=6.0 Hz, 2H), 1.66 (s, 12H), 1.37 (t, J=8.0 Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$) δ(ppm): 175.32, 166.96, 162.55, 142.55, 140.62, 140.19, 128.28, 122.87, 122.15, 121.95, 108.45, 93.74, 47.88, 47.80, 38.28, 36.53, 34.85, 31.45, 29.51, 25.39, 21.62, 11.64. HRMS: m/z $C_{36}H_{46}N_3^+$ Calcd 520.3692, found 520.3690.

3. Photographs of Cy7-NR solutions before and after the addition of NaOCl

Figure S1. The absorption photographs of Cy7-NR in the presence (Above) and absence (Below) of NaOCl.

4. The fluorescence spectra of Cy7-NR responding to NaOCl

The emission spectra of Cy7-NR in the absence and presence of NaOCl were shown in Figure S2. It can be noted from Figure S2 that the initial peaks of Cy7-NR disappeared and new peaks centrated around 560 nm emerged.

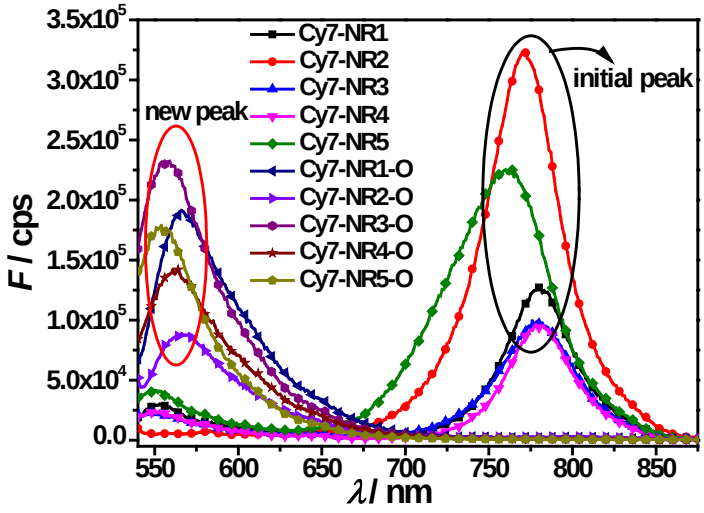


Figure S2. Fluorescence spectra of Cy7-NR (5.0 μM) in the absence (a) and presence (b) of 3 equiv NaOCl.

5. Fluorescence titration experiment of Cy7-NR1

The fluorescence titration experiment of Cy7-NR1 upon the addition of NaClO with different concentrations were performed. The relationship of ratio (I_{566}/I_{780}) and the concentrations of NaOCl was shown in Figure S3a, and the linearity curve between the ratio and concentrations (0-20 μM) was shown in Figure S3b.

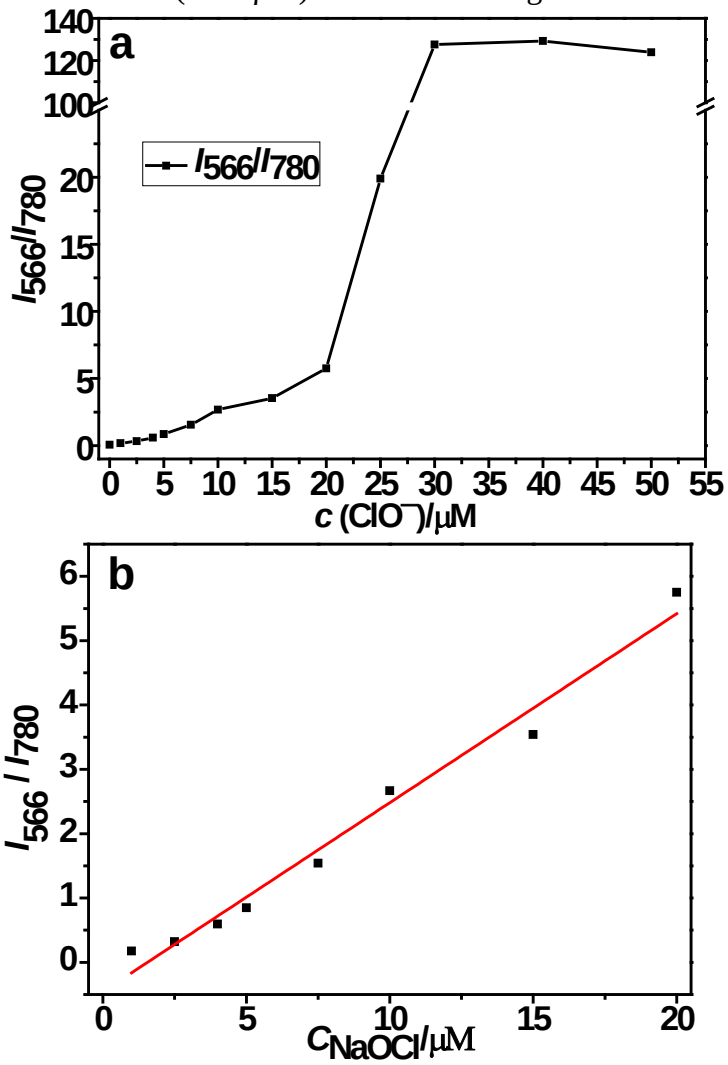


Figure S3. (a) The changes in fluorescence ratio (I_{566}/I_{780}) of Cy7-NR1 (5.0 μM) upon the addition of various concentrations of NaOCl: (0, 1.0, 2.5, 4.0, 5.0, 7.5, 10.0, 15.0, 20.0, 25.0, 30.0, 40.0, 50.0 μM). (b) The linearity curve between the fluorescence ratio (I_{566}/I_{780}) and the concentrations of NaOCl (0-20 μM).

6. Determination of the limit of detection

The limit of detection was calculated based on the method used in the previous literature[11]. The fluorescence ratio (I_{566}/I_{780}) of Cy7-NR1 was measured by twelve times and the standard deviation of blank measurement was obtained. The fluorescence ratio (I_{566}/I_{780}) was plotted as a concentration of NaClO. The limit of detection was calculated with the following equation:

Limit of detection (LOD) = 3σ/k (1)

Where σ is the standard deviation of blank measurement, k is the slope between the fluorescence ratio (I_{566}/I_{780}) versus the NaOCl concentration.

7. The quantum yields of Cy7-NR

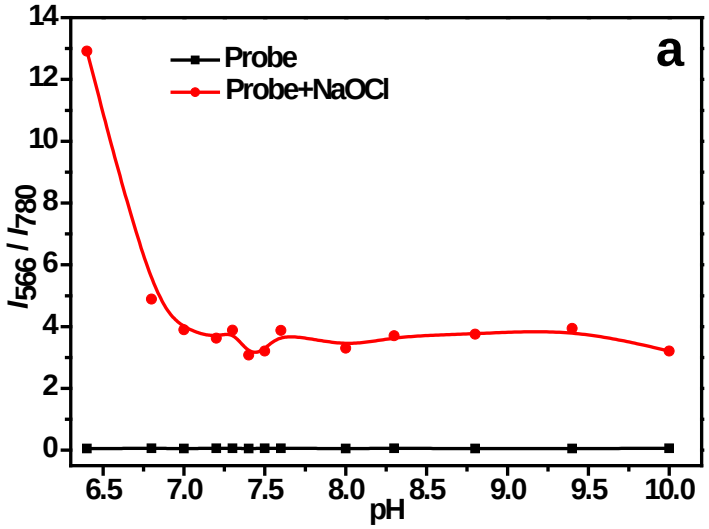
The quantum yields of Cy7-NR were obtained with a solution of cresyl violet in methanol (φ = 0.54) as a fluorescence standard[12].

Table S1. The quantum yields of Cy7-NR.

Cy7-NR	$Cy7\text{-}NR_1$	$Cy7\text{-}NR_2$	$Cy7\text{-}NR_3$	$Cy7\text{-}NR_4$	$Cy7\text{-}NR_5$
Quantum Yield	0.0011	0.0020	0.0007	0.0007	0.0031
Cy7-NR + NaOCl	$Cy7\text{-}NR_1$	$Cy7\text{-}NR_2$	$Cy7\text{-}NR_3$	$Cy7\text{-}NR_4$	$Cy7\text{-}NR_5$
Quantum Yield	0.0035	0.0368	0.0023	0.0018	0.0072

8. The effects of pH values

The pH effects on the ratio (I_{566}/I_{780}) of Cy7-NR1 in the absecnce and presence of NaOCl (Figure S4a), the fluorescence intensities at 566 nm and 780 nm of Cy7-NR1 in the presence of NaClO (Figure S4b), the fluorescence intensity at 780 nm (Figure S4c) and the absorbance at 682 nm (Figure S4d) of free Cy7-NR1 were investigated.



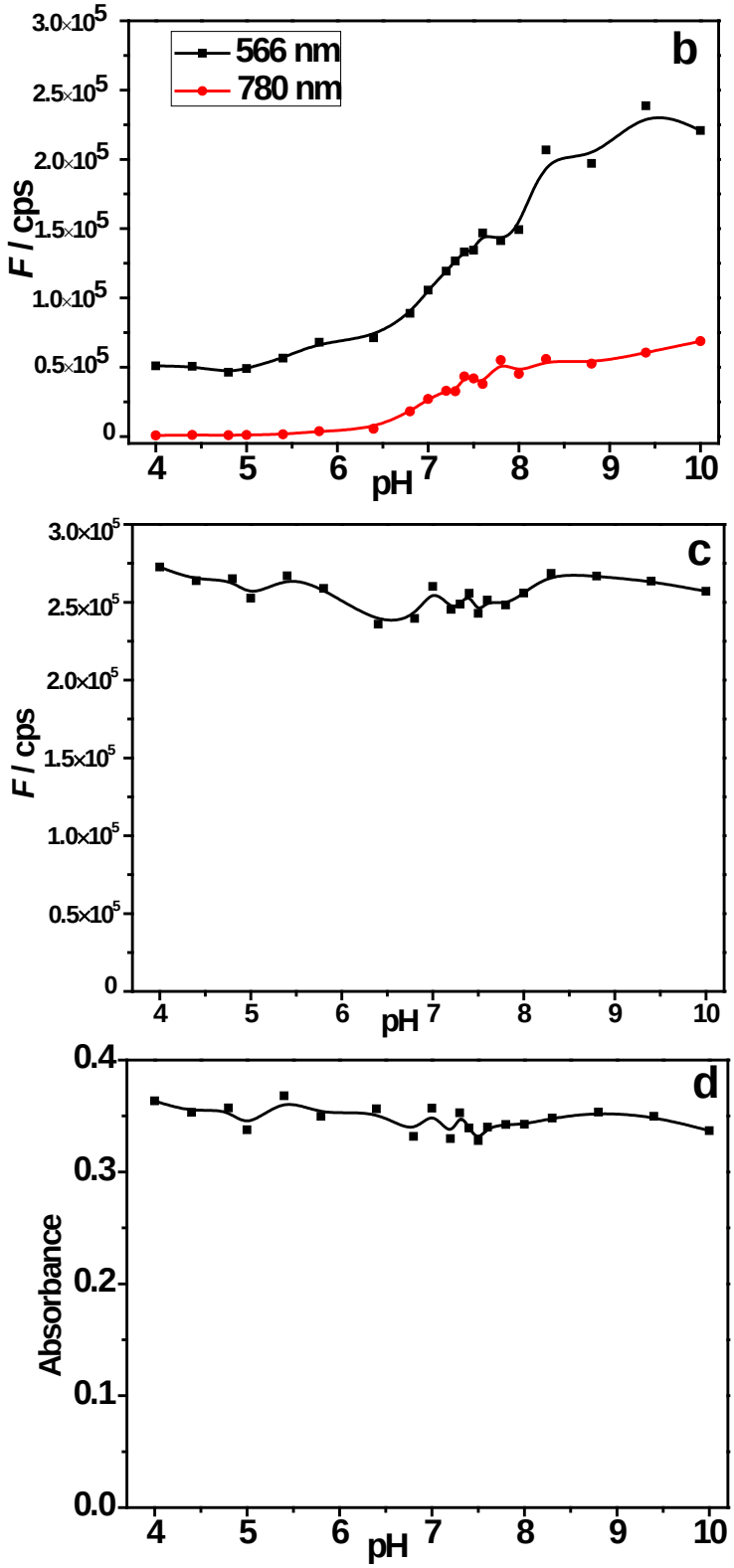
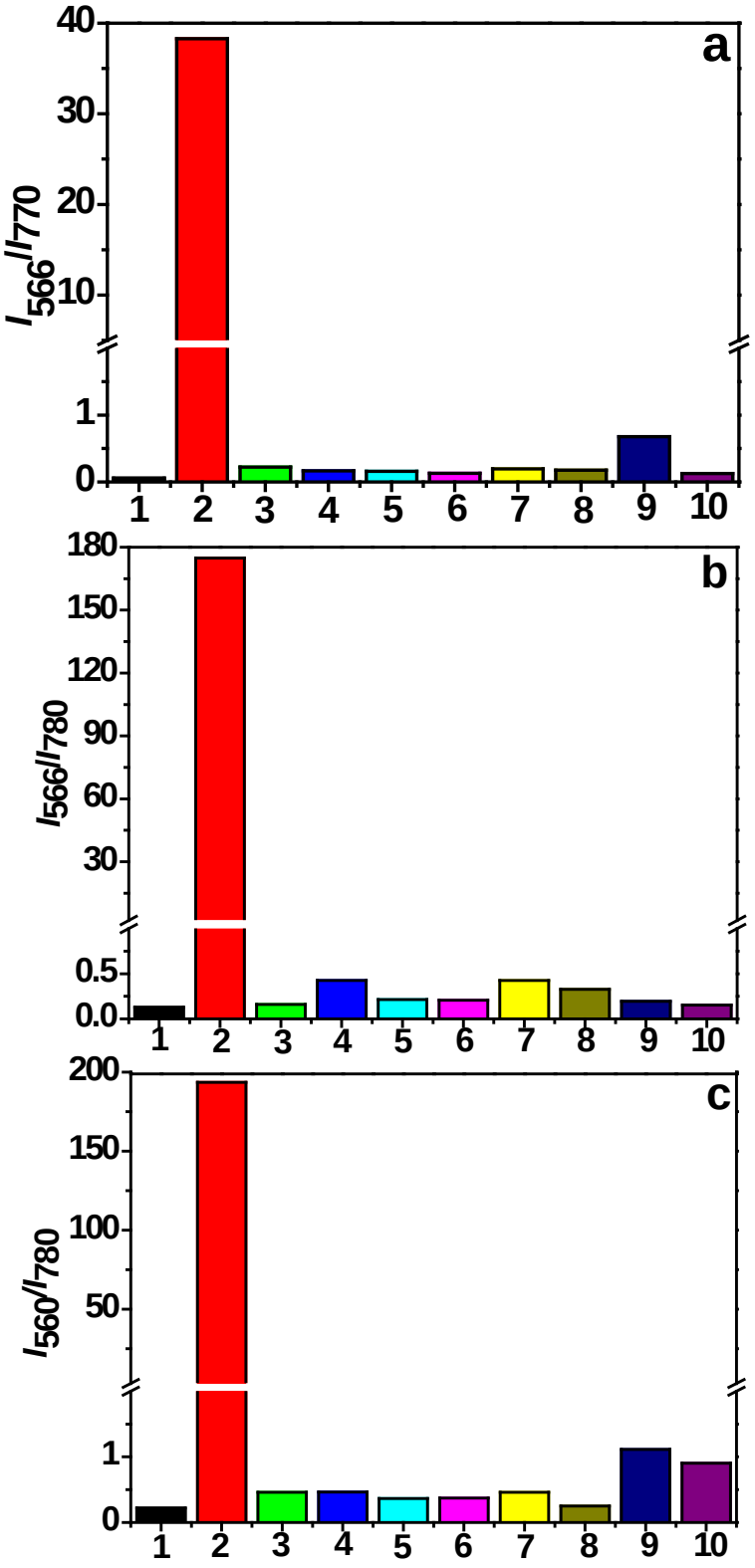


Figure S4. The effects of pH on the spectral properties of Cy7-NR1(5.0 μM). (a) the ratio (I_{566}/I_{780}) in the absecnce (black, square) and presence (red, circle) of 3 equivalent of NaOCl. pH= 6.4, 6.8, 7.0, 7.2, 7.3, 7.4, 7.5, 7.6, 8.0, 8.3, 8.8, 9.4, 10.0 (b) The fluorescent emission intensities at 566 nm (black, square) and 780 nm (red, circle) in the presence of NaClO (3 equivalent) at various pH values. (c) The fluorescent emission intensity at 780 nm. (d) The absorbance at 682 nm at various pH values. pH= 4.0, 4.4, 4.8, 5.0, 5.4, 5.8, 6.4, 6.8, 7.0, 7.2, 7.3, 7.4, 7.5, 7.6, 7.8, 8.0, 8.3,

8.8, 9.4, 10.0 (20 mM PBS buffer solution).The mixture was equilibrated for 6 min before measurement. Excitation wavelength was λ= 540 nm, excitation and emission slits were set to 5 and 5 nm, respectively.

9. The selectivity of Cy7-NR for NaOCl



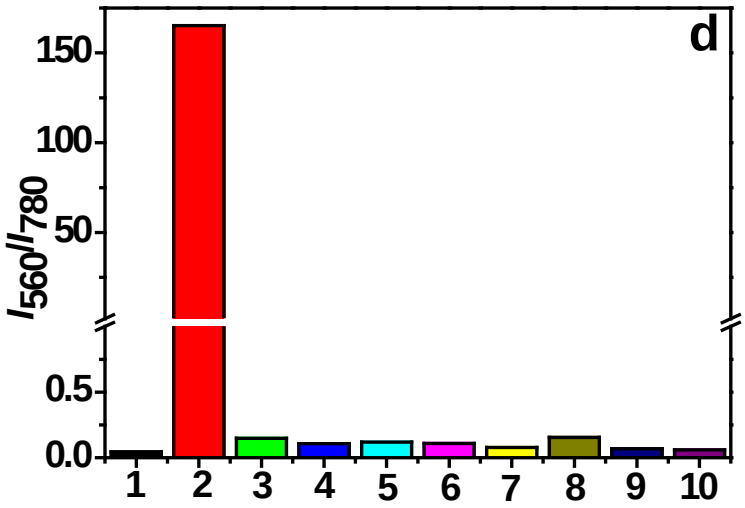


Figure S5. Fluorescence responses of Cy7-NR (5.0 μM) to various analytes. (a) Cy7-NR2; (b) Cy7-NR3; (c) Cy7-NR4; (d) Cy7-NR5. Bars represent the ratio between the intensities of short-wavelength and those of long-wavelength, responding to various ROS: 1. Cy7-NR (control); 2. NaClO (15.0 μM); 3. H_2O_2 (250.0 μM); 4. *t*-BuOOH (250.0 μM); 5. CuOOH (250.0 μM); 6. O^-_2 (250.0 μM); 7. ·OH (250.0 μM); 8. $^1O_2$ (250.0 μM); 9. NO (15.0 μM); 10. $ONOO^-$ (15.0 μM). The mixture was equilibrated for 25 min before measurement.

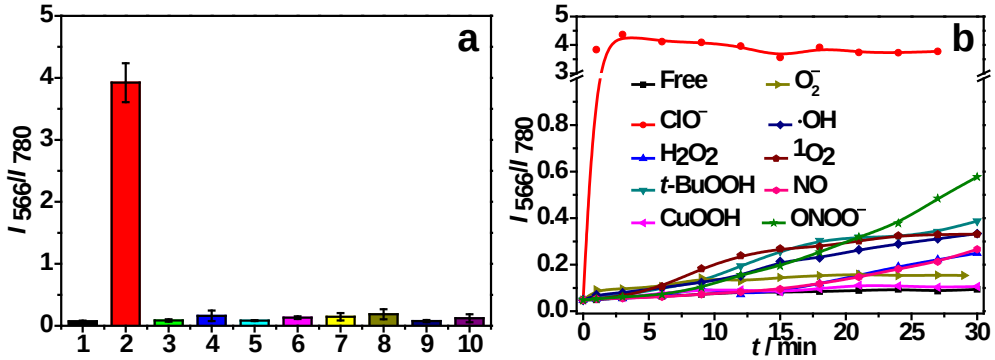


Figure S6. (a) Fluorescence responses of Cy7-NR1 to various analytes. Bars represent the ratio of I_{566}/I_{780}, responding to various ROS: 1. Cy7-NR1 (control); 2. NaOCl (15.0 μM); 3. H_2O_2 (250.0 μM); 4. *t*-BuOOH (250.0 μM); 5. CuOOH (250.0 μM); 6. O^-_2 (250.0 μM); 7. ·OH (250.0 μM); 8. $^1O_2$ (250.0 μM); 9. NO (15.0 μM); 10. $ONOO^-$ (25.0 μM). The mixture was equilibrated for 6 min before measurement. (b) Kinetic profiles of Cy7-NR1 (5.0 μM) and the reaction with various ROS for 30 min.

10. The kinetic assay

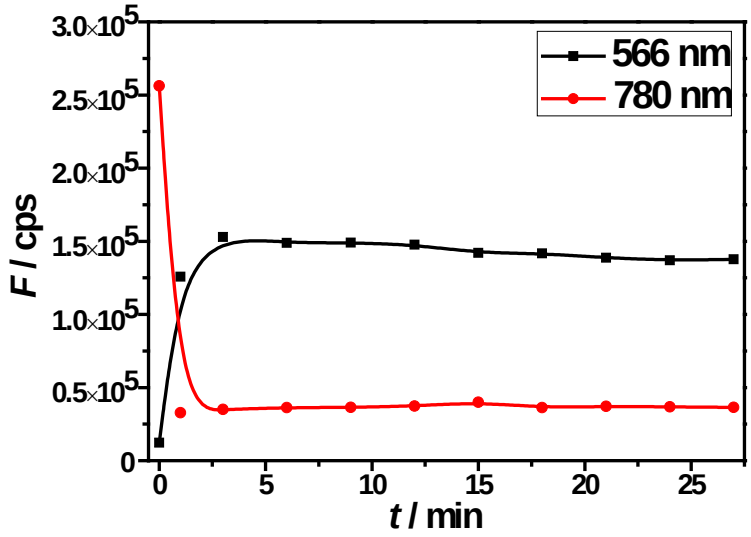


Figure S7. The time-relevant curves of fluorescent emission intensities at 566 nm (black, square) and 780 nm (red, circle) of Cy7-NR1 (5.0 μM) treated with NaClO (15.0 μM, 3 equivalent). The mixture was equilibrated for 6 min before measurement. Excitation wavelength was λ= 540 nm, excitation and emission slits were set to 5 and 5 nm, respectively.

11. Additional fluorescent confocal images

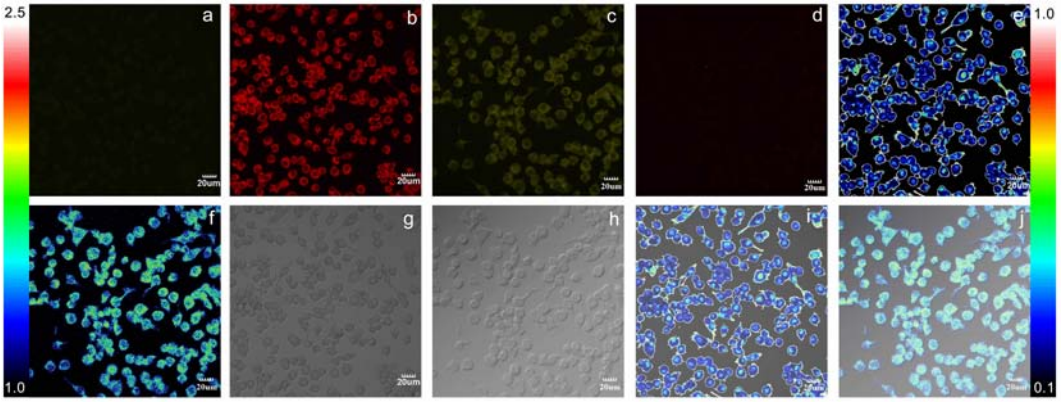


Figure S8. Confocal fluorescence images of HOCl in living Raw264.7 cells. (a) Raw264.7 cells incubated with 10 μM Cy7-NR1 for 5 min, the image was collected from 550 to 650 nm; (b) The image was collected from 700 to 800 nm of cells in (a); (c) Raw264.7 cells incubated with 10 μM Cy7-NR1 for 5 min and then coincubated with 1.0 mM NaClO for 5 min, the image was collected from 550 to 650 nm; (d) The image was collected from 700 to 800 nm of cells in (c); (e) The ratiometric image of (a) and (b); (f) The ratiometric image of (c) and (d); (g) The bright-field image of (a); (h) The bright-field image of (c); (i) The overlay of (e) and (g); (j) The overlay of (f) and (h). Scale bar represents 20 μm. The ratio scale of image (e) is in the left and (f) is in the right. Florescent images were acquired with an objective len of ×40.

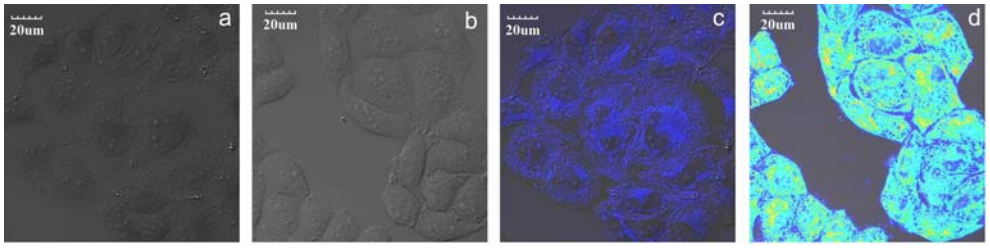


Figure S9. (a) The bright-field image of Figure 4a; (h) The bright-field image of Figure 4c; (i) The overlay of (a) and Figure 4e; (j) The overlay of (b) and Figure 4f.

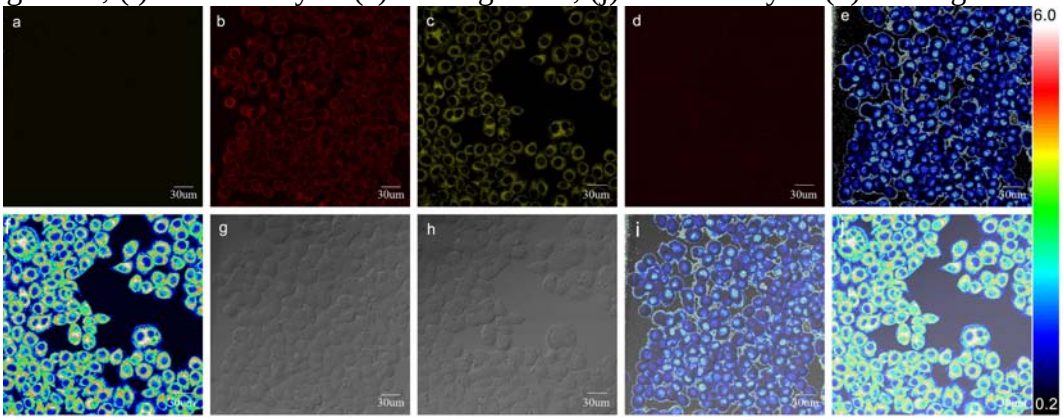


Figure S10. Confocal fluorescence images of HOCl in living HeLa cells. (a) HeLa cells incubated with 10 μM Cy7-NR1 for 5 min, the image was collected from 545 to 645 nm; (b) The image was collected from 700 to 800 nm of cells in (a); (c) HeLa cells incubated with 10 μM Cy7-NR1 for 5 min and then coincubated with 1.0 mM NaClO for 5 min, the image was collected from 550 to 650 nm; (d) The image was collected from 700 to 800 nm of cells in (c); (e) The ratiometric image of (a) and (b); (f) The ratiometric image of (c) and (d); (g) The bright-field image of (a); (h) The bright-field image of (c); (i) The overlay of (e) and (g); (j) The overlay of (f) and (h). Scale bar represents 30 μm. Florescent images were acquired with an objective len of ×40.

12. The influence of endogenous HOCl

The influence of endogenous HOCl in HeLa cells have been investigated. As shown in Figure S10 and 11, there is no distinguish between the control and taurine (a HOCl scavenger[13]) treated cells in the ratiometric images ($F_{545\text{-}645}/F_{700\text{-}800}$). The average ratio of $F_{545\text{-}645}/F_{700\text{-}800}$ in images e and f are both mainly in the range from 0.5 to 0.8. Hence, the influence of endogenous HOCl in HeLa cells can be eliminated.

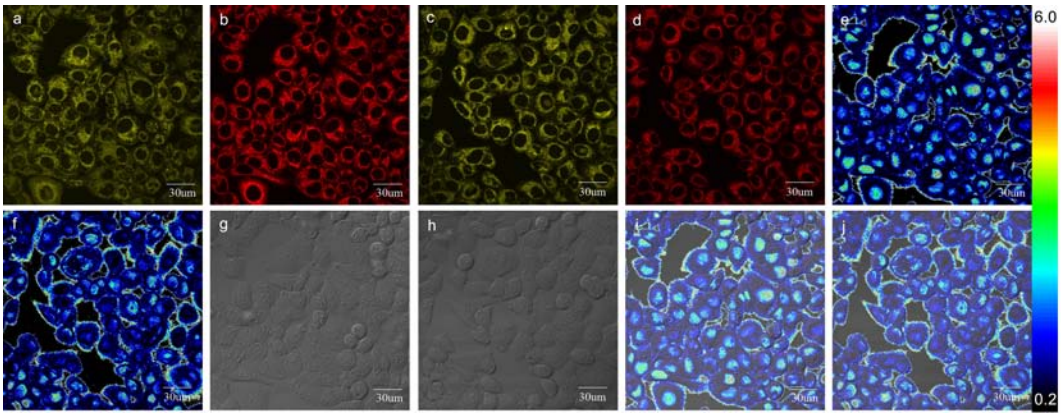


Figure S11. Confocal fluorescence images in living HeLa cells. (a) HeLa cells incubated with 10 μM Cy7-NR1 for 5 min, the image was collected from 545 to 645 nm; (b) The image was collected from 700 to 800 nm of cells in (a); (c) HeLa cells incubated with 5 μg / ml taurine for 10min and then 10 μM Cy7-NR1 for 5 min, the image was collected from 545 to 645 nm; (d) The image was collected from 700 to 800 nm of cells in (c); (e) The ratiometric image of (a) and (b); (f) The ratiometric image of (c) and (d); (g) The bright-field image of (a); (h) The bright-field image of (c); (i) The overlay of (e) and (g); (j) The overlay of (f) and (h). Scale bar represents 30 μm. Florescent images were acquired with an objective len of ×60.

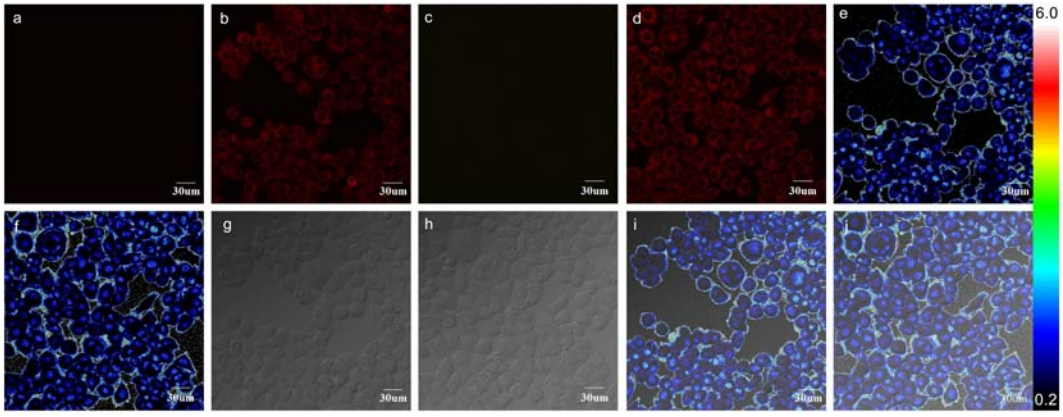
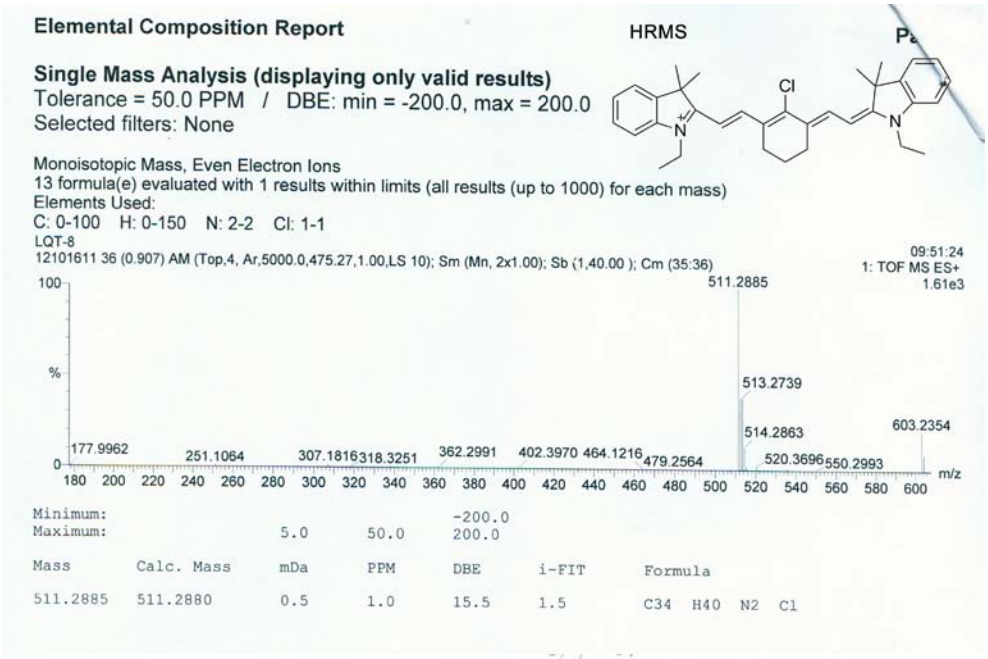
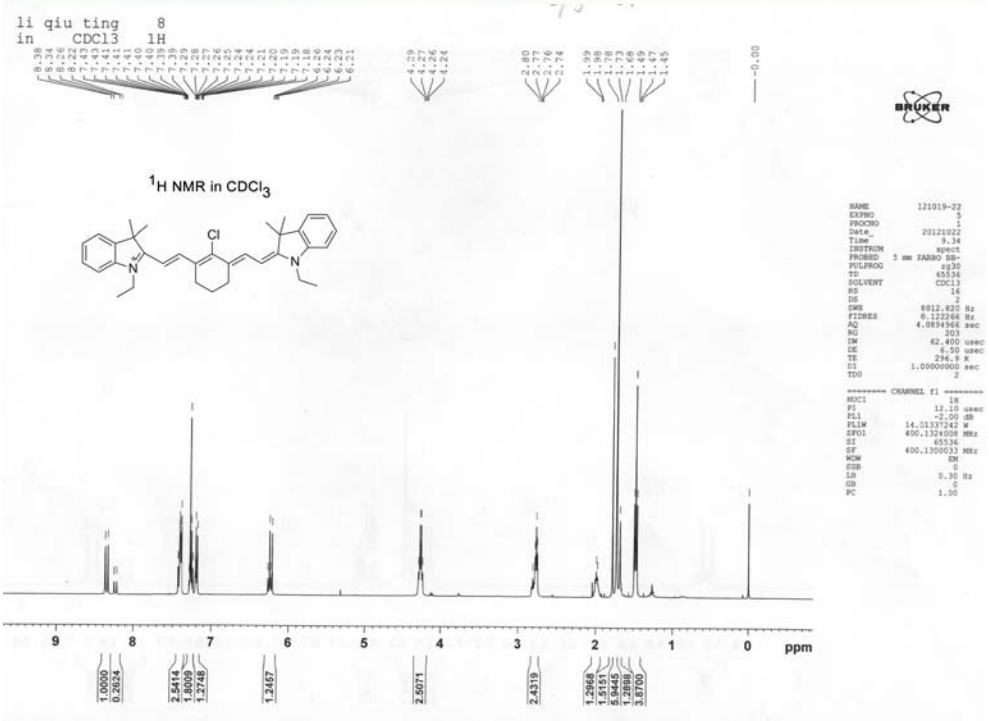
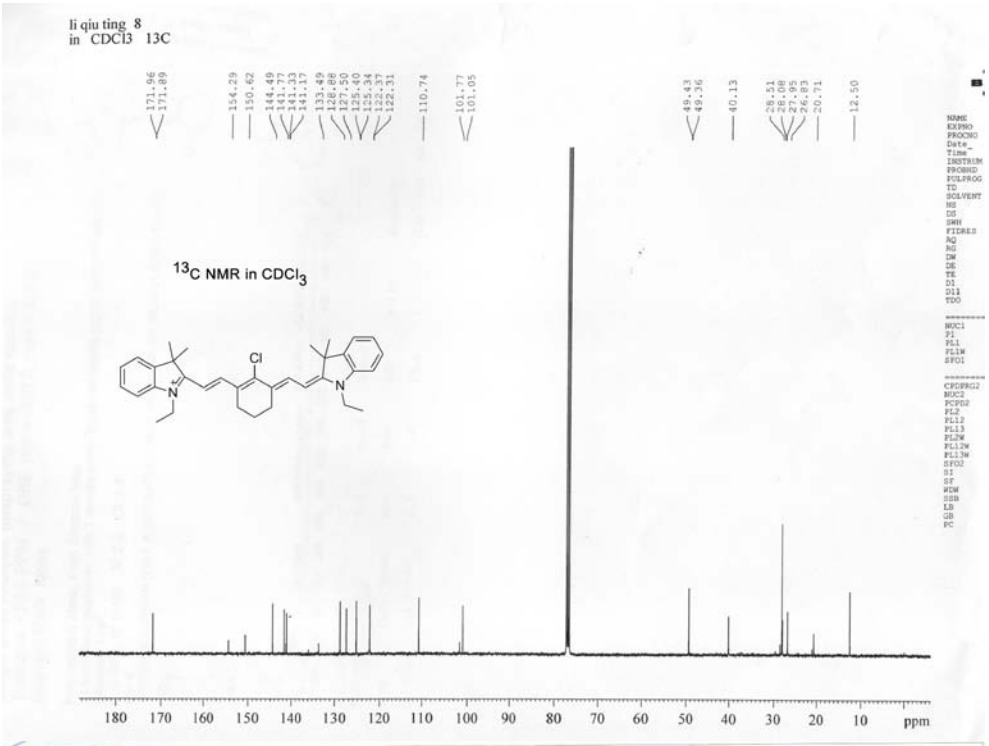


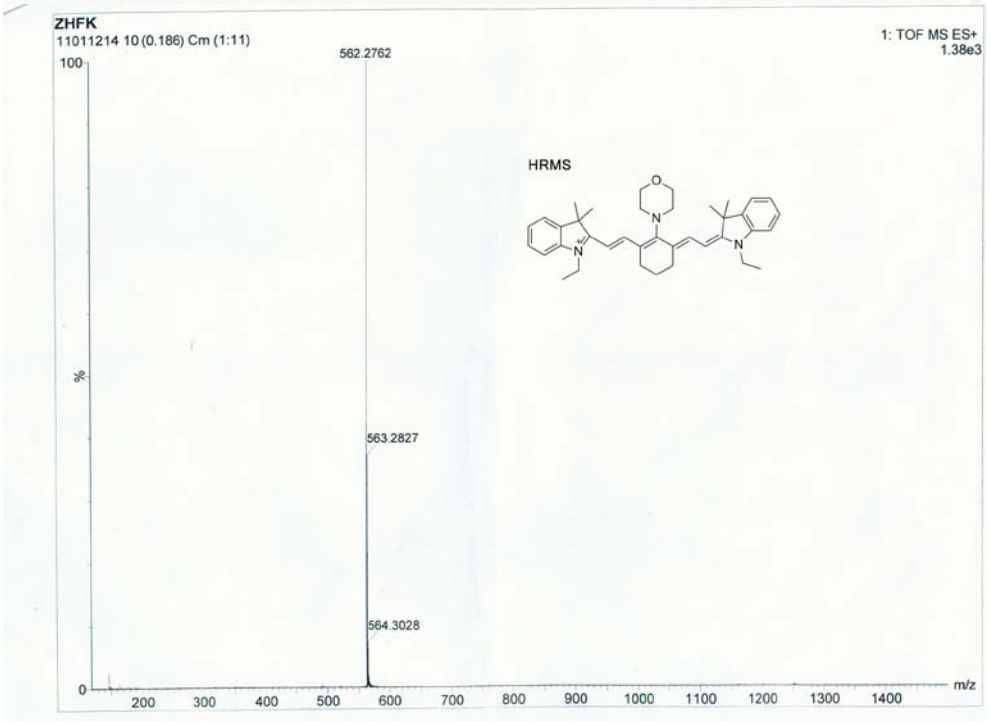
Figure S12. Confocal fluorescence images in living HeLa cells. (a) HeLa cells incubated with 10 μM Cy7-NR1 for 5 min, the image was collected from 545 to 645 nm; (b) The image was collected from 700 to 800 nm of cells in (a); (c) HeLa cells incubated with 5 μg / ml taurine for 10min and then 10 μM Cy7-NR1 for 5 min, the image was collected from 545 to 645 nm; (d) The image was collected from 700 to 800 nm of cells in (c); (e) The ratiometric image of (a) and (b); (f) The ratiometric image of (c) and (d); (g) The bright-field image of (a); (h) The bright-field image of (c); (i) The overlay of (e) and (g); (j) The overlay of (f) and (h). Scale bar represents 30 μm. Florescent images were acquired with an objective len of ×40.

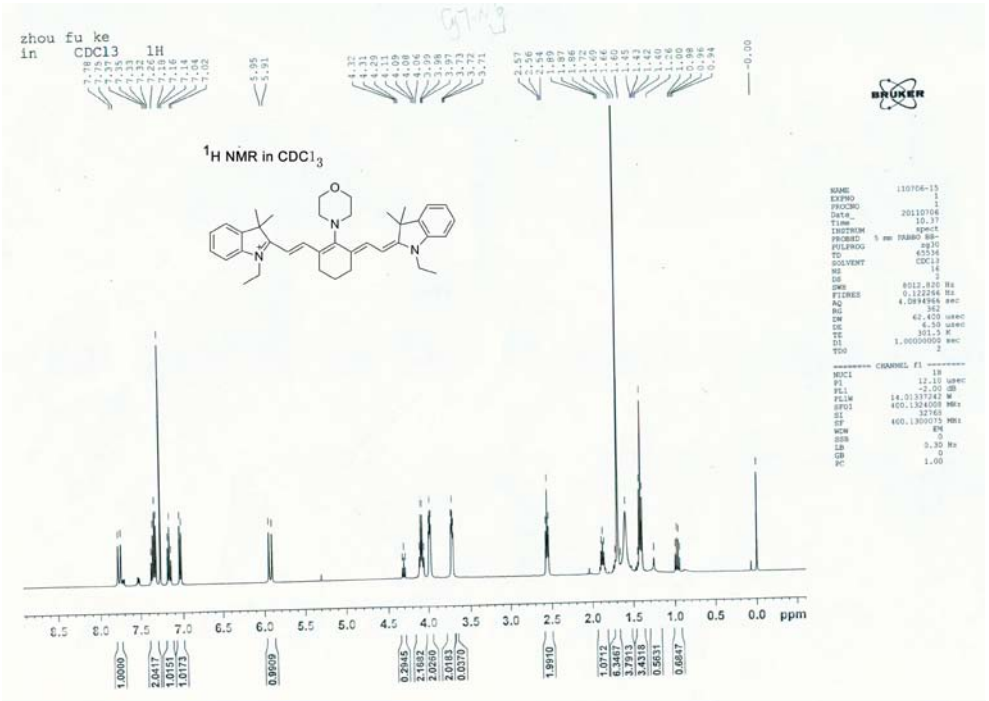
13. Additional Spectroscopic Data

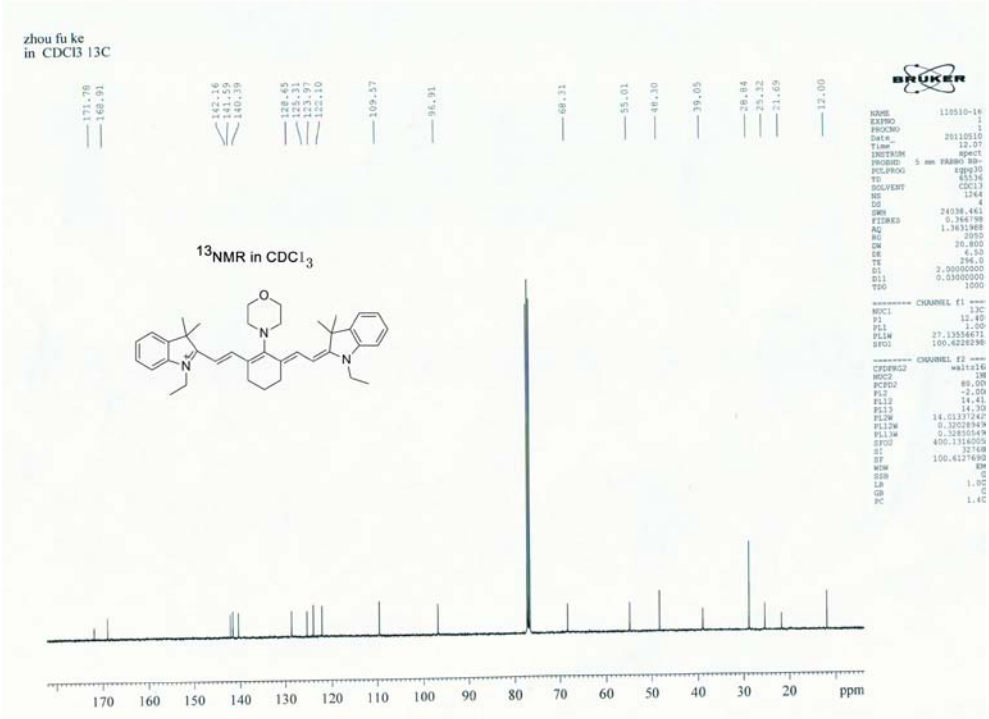






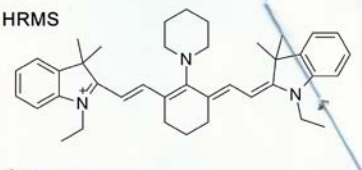






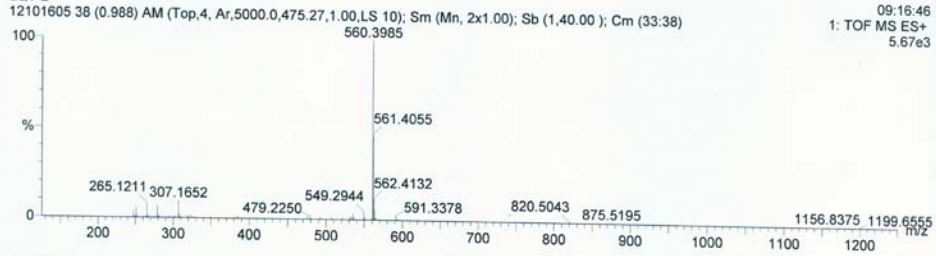
Elemental Composition Report

HRMS



Single Mass Analysis (displaying only valid results)
Tolerance = 50.0 PPM / DBE: min = -200.0, max = 200.0
Selected filters: None

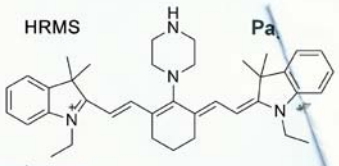
Monoisotopic Mass, Even Electron Ions
13 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 N: 3-3
LQT-2
12101605 38 (0.988) AM (Top,4, Ar,5000.0,475.27,1.00,LS 10); Sm (Mn, 2x1.00); Sb (1,40.00); Cm (33:38)
09:16:46
1: TOF MS ES+
5.67e3



Minimum:		5.0	50.0	-200.0		
Maximum:				200.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
560.3985	560.4005	-2.0	-3.6	16.5	8.3	C39 H50 N3

Elemental Composition Report

HRMS Pa



Single Mass Analysis (displaying only valid results)
Tolerance = 50.0 PPM / DBE: min = -200.0, max = 200.0
Selected filters: None

Monoisotopic Mass, Even Electron Ions
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Elements Used:
C: 0-100 H: 0-150 N: 4-4
LQT-1
12101604 25 (0.653) AM (Top,4, Ar,5000.0,475.27,1.00,LS 10); Sm (Mn, 2x1.00); Sb (1,40.00); Cm (14:25)
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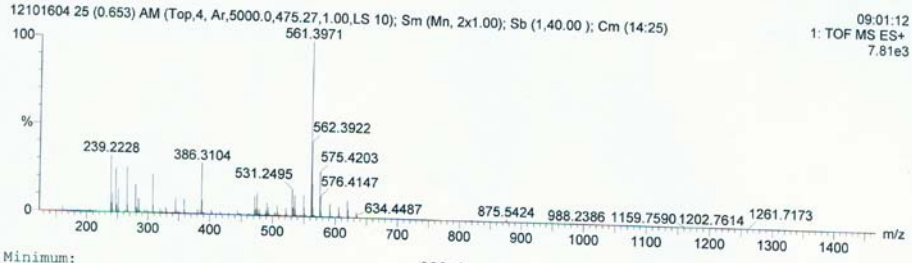
1: TOF MS ES+
7.81e3



Minimum:		5.0	50.0	-200.0		
Maximum:				200.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
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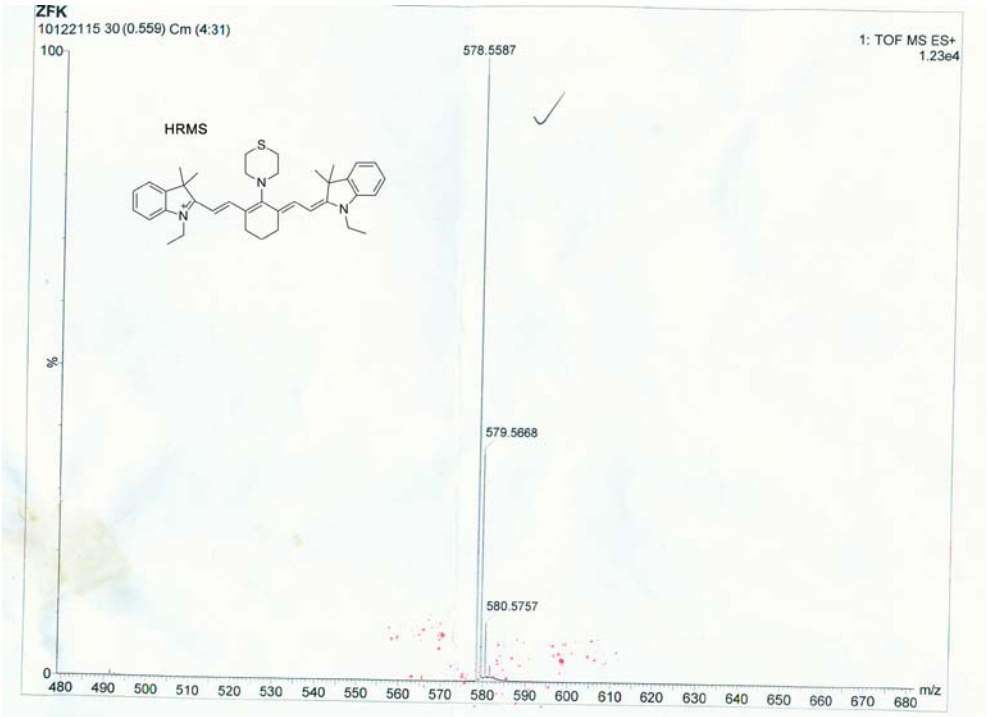
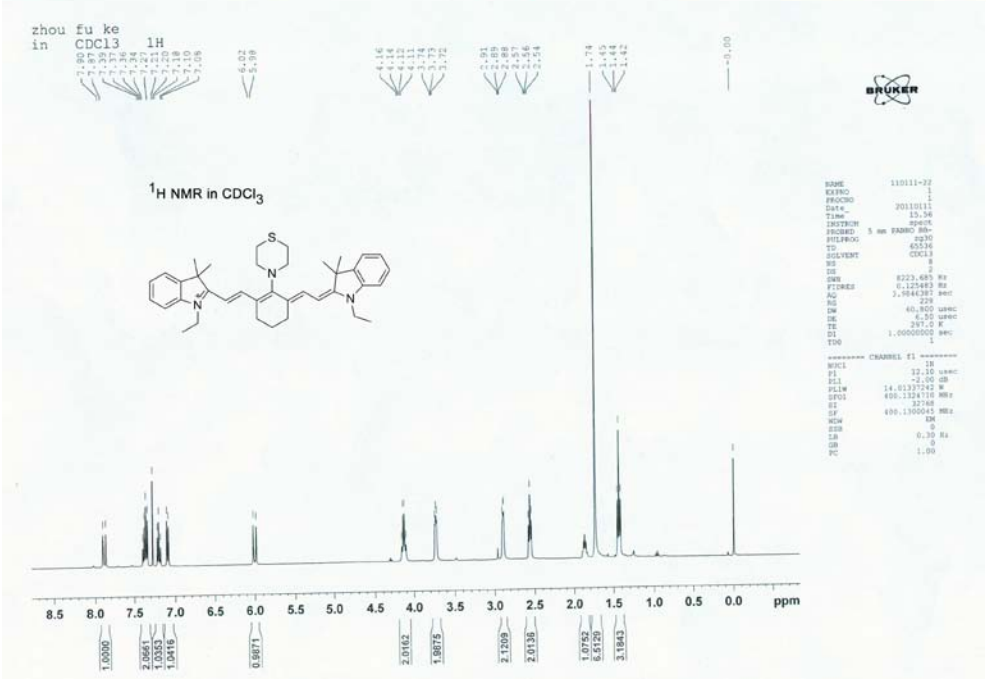
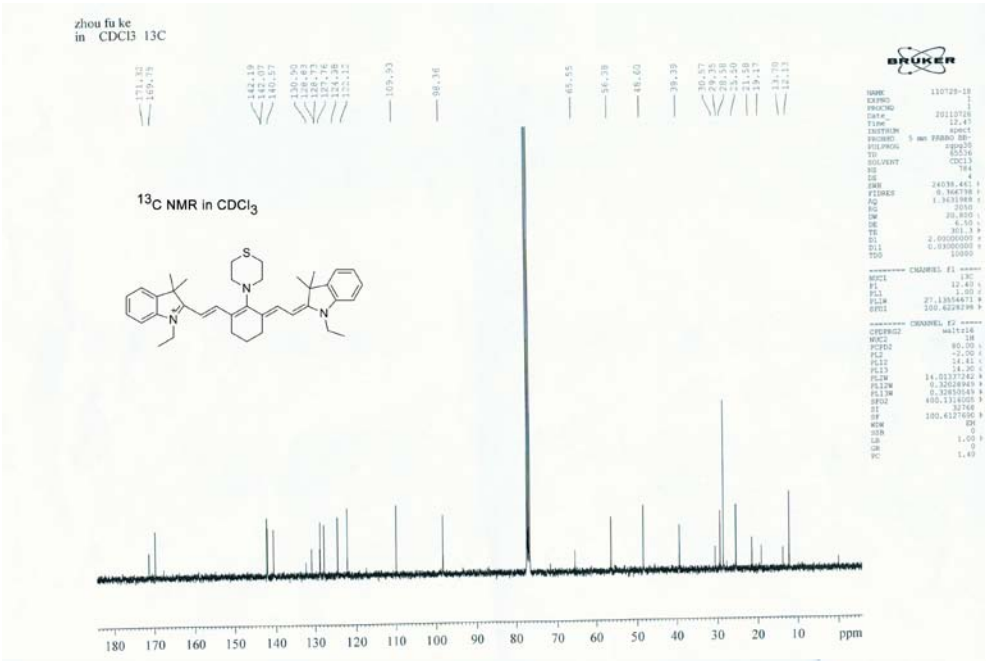
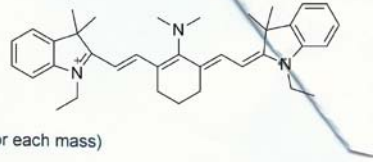






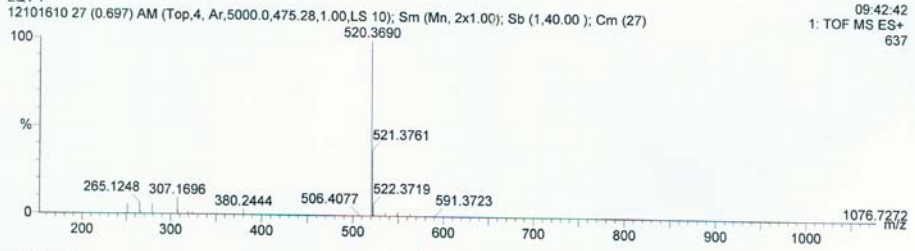
Elemental Composition Report

HRMS

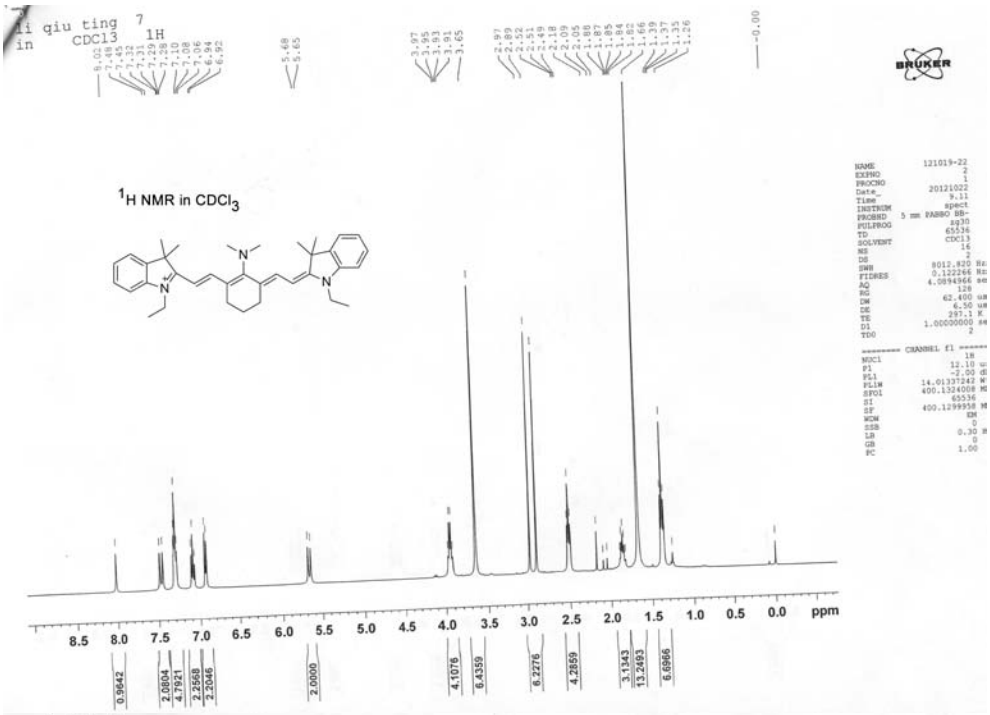


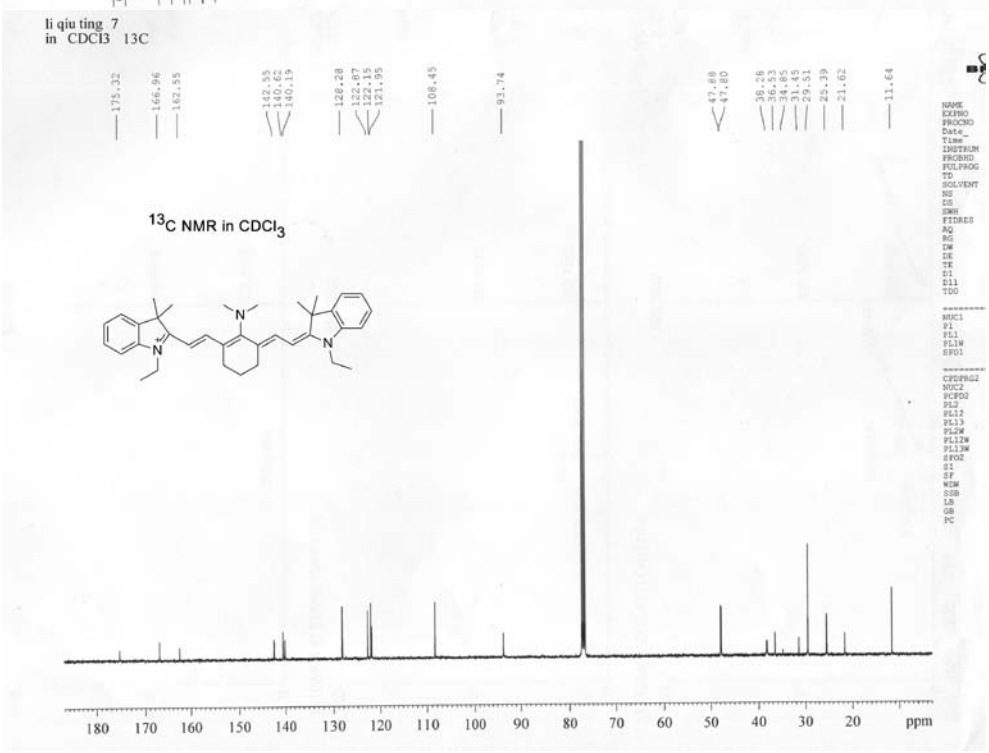
Single Mass Analysis (displaying only valid results)
Tolerance = 50.0 PPM / DBE: min = -200.0, max = 200.0
Selected filters: None

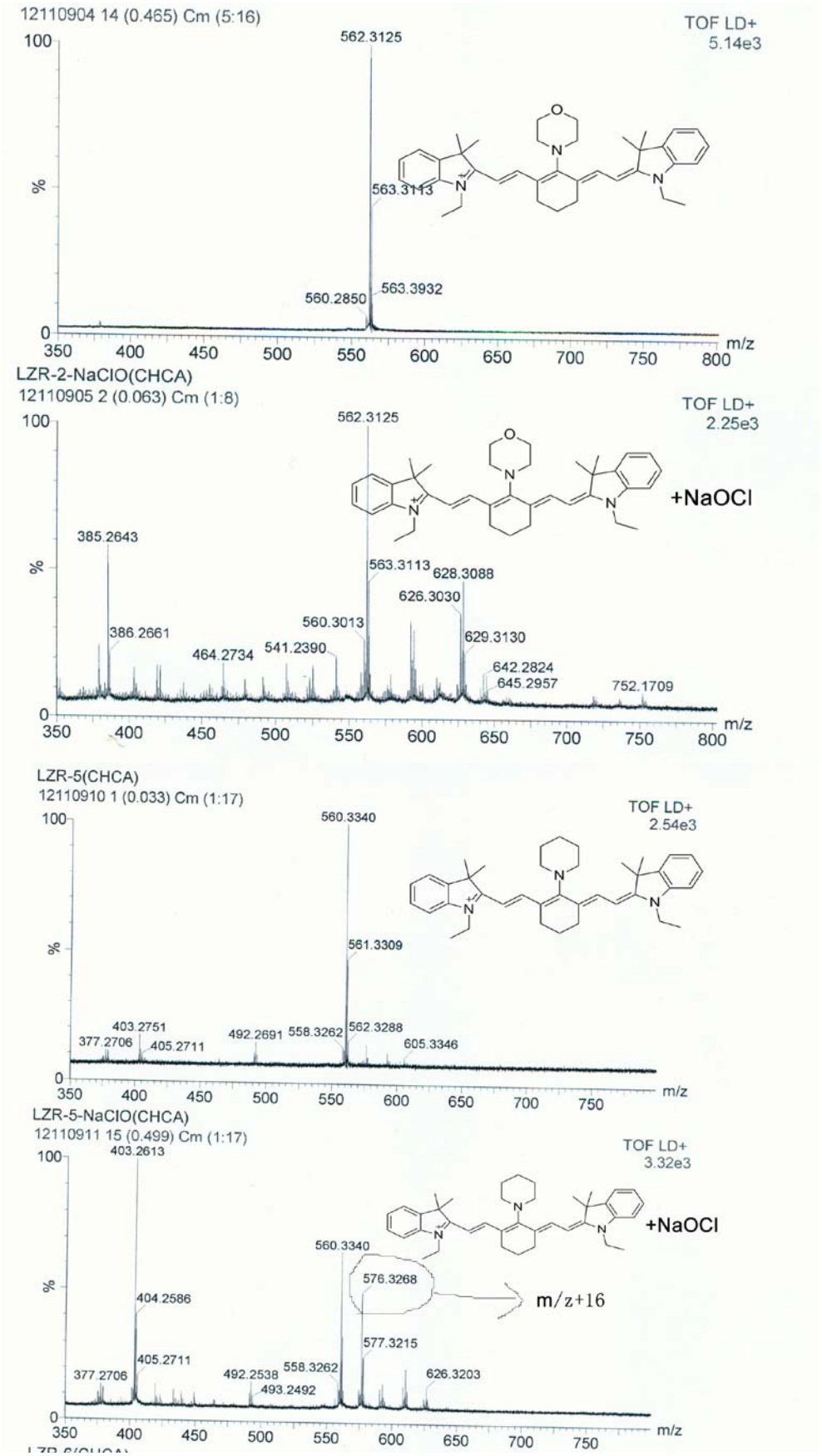
Monoisotopic Mass, Even Electron Ions
12 formula(e) evaluated with 1 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-100 H: 0-150 N: 3-3
LQT-7
12101610 27 (0.697) AM (Top,4, Ar,5000.0,475.28,1.00,LS 10); Sm (Mn, 2x1.00); Sb (1,40.00); Cm (27)
09:42:42
1: TOF MS ES+
637

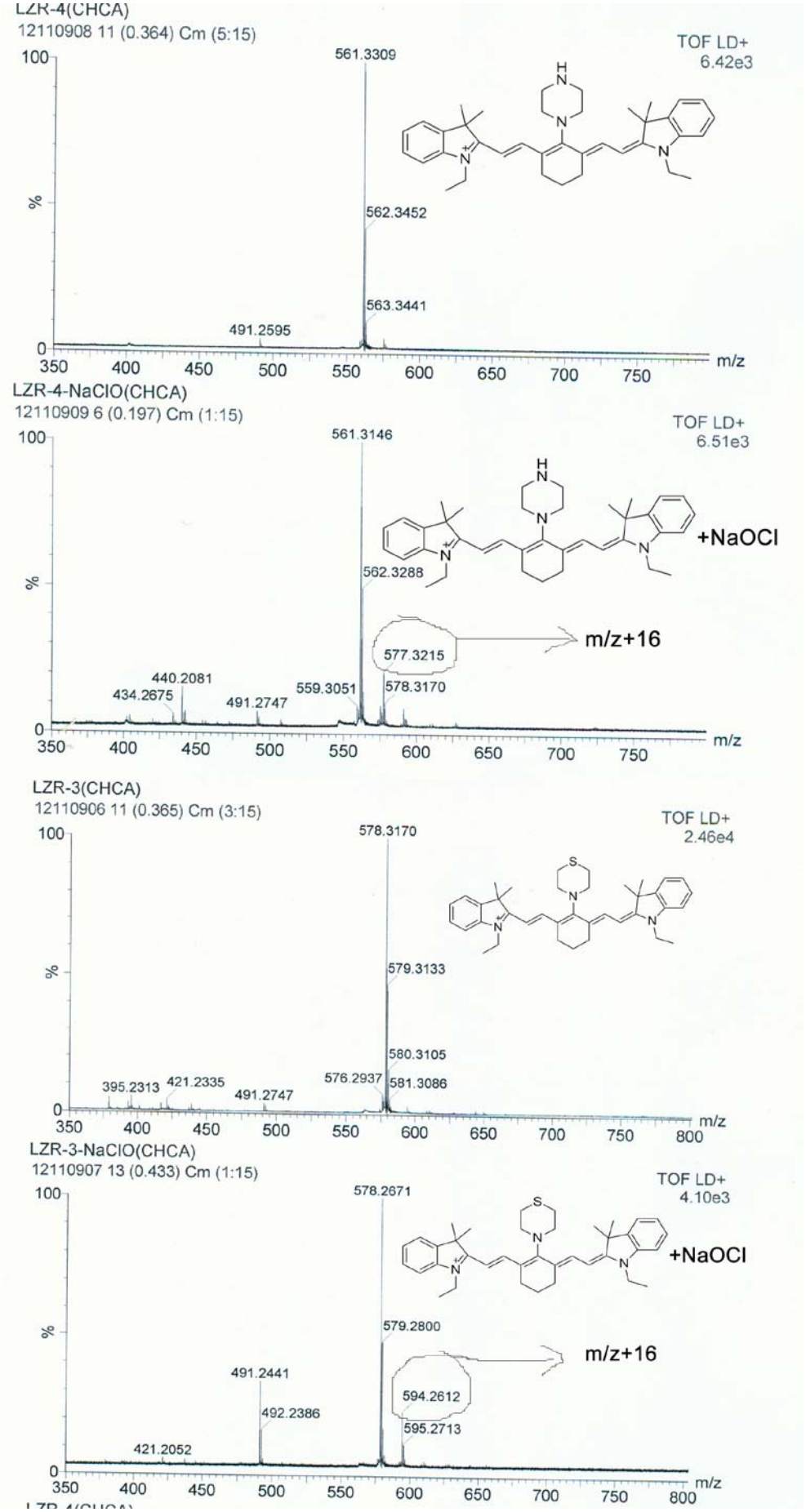


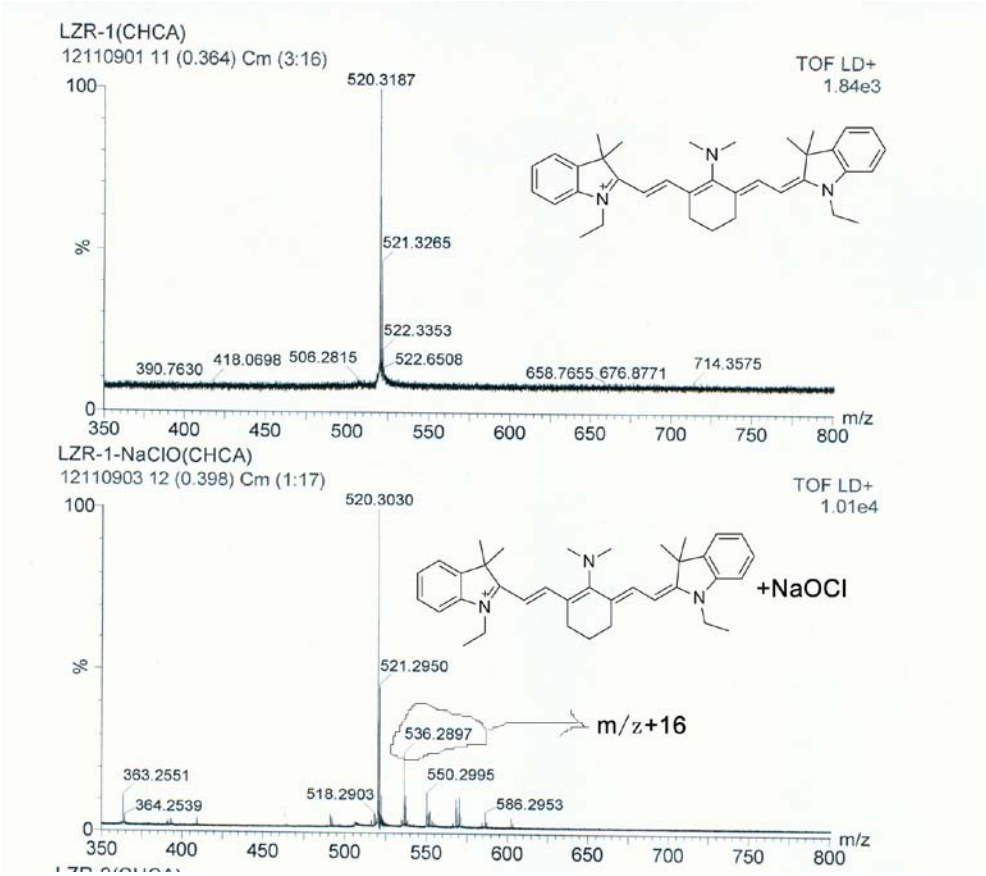
Minimum:				-200.0		
Maximum:		5.0	50.0	200.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
520.3690	520.3692	-0.2	-0.4	15.5	2.6	C36 H46 N3











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