Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information

Spiroboronate Si-rhodamine as a near-infrared probe for imaging lysosomes based on reversible ring-opening process

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General Methods.

Materials. General chemicals were of the best grade available, supplied by Adams, J&K chemical LTD. and Acros Organics were used without further purification. All solvents were used after appropriate distillation or purification.

Apparatus. All reactions were monitored by thin-layer chromatography (TLC) on gel F254 plates. Flash chromatography was carried out on silica gel (200-300 mesh; Qingdao Ocean Chemicals). NMR spectra were recorded on a Bruker AC-300P spectrometer at 300 MHz for ^{1}H NMR and at 75 MHz for ^{13}C NMR or on a Bruker AC-600P spectrometer at 600 MHz for ^{1}H NMR and at 150 MHz for ^{13}C NMR. Spectral data are reported in ppm relative to tetramethylsilane (TMS) as internal standard. Mass spectra (MS) were measured with an API-3000 MS spectrometer using electrospray ionization (ESI). High-resolution mass spectra (HRMS) were recorded on an Agilent Technologies 6538 UHD Accurate-Mass Q-TOF MS spectrometer using ESI. All pH measurements were performed with a pH-3c digital pH-meter (Shanghai Lei Ci Device Works, Shanghai, China) with a combined glass-calomel electrode. UV-visible spectra were obtained on a Agilent Cary 100 UV-vis spectrophotometer. Fluorescence spectroscopic studies were performed on a Hitachi F-7000. Fluorescence images were obtained on a Leica TCS SP5 confocal laser scanning microscope.

Spectroscopic Measurements. Absorption spectra and fluorescence spectra were obtained with a 1 cm standard quartz cell. The fluorescence quantum yields were determined by using rhodamine B (Φ = 0.31 in water) and Cy5 (Φ = 0.27 in PBS) for respective **R-B** and **SiR-B** as the reference. pK_{cycl} values were calculated by fluorescence intensities

Cell incubation.

HepG2 and PC12 cells were cultured in DMEM supplemented with 10% FBS, 50 unit/mL of penicillin and 50 *u*g/mL of streptomycin at 37 ℃ in a 5% CO_2 humidified incubator, and culture media were replaced with fresh media every day. For fluorescence imaging, HepG2 and PC12 cells were seeded in 35-mm glass-bottom culture dishes at a density of 5×10^4 cells in culture media for 24h.

Cytotoxicity Test.

Cell viability was evaluated using the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazoilum bromide (MTT) reduction assay. HepG2 cells with a density of 5×10^4 cells per mL were grown in 96-well plates to a total volume of 100 mL per well at 37 ℃ under an atmosphere of 5% CO_2 for 24 h. Subsequently, different concentrations probe **SiR-B** of 1 μM, 5 μM, 10 μM and 20 μM in fresh medium containing 1‰ DMSO were incubated with HepG2 cells for 24 h, respectively, while cells in medium without probes were used as control. After incubation, MTT (20 μL, 5 mg/mL) in PBS was added to each well for 4 h. Then DMSO (150 μL) was added to each well to dissolve the purple products after removing media. After shaking for 10 min, the absorbance was measured at 490 nm on a plate reader. Cell viability was expressed as a percentage of the control culture value.

Imaging of probe SiR-B and LysoTracker Green DND-26.

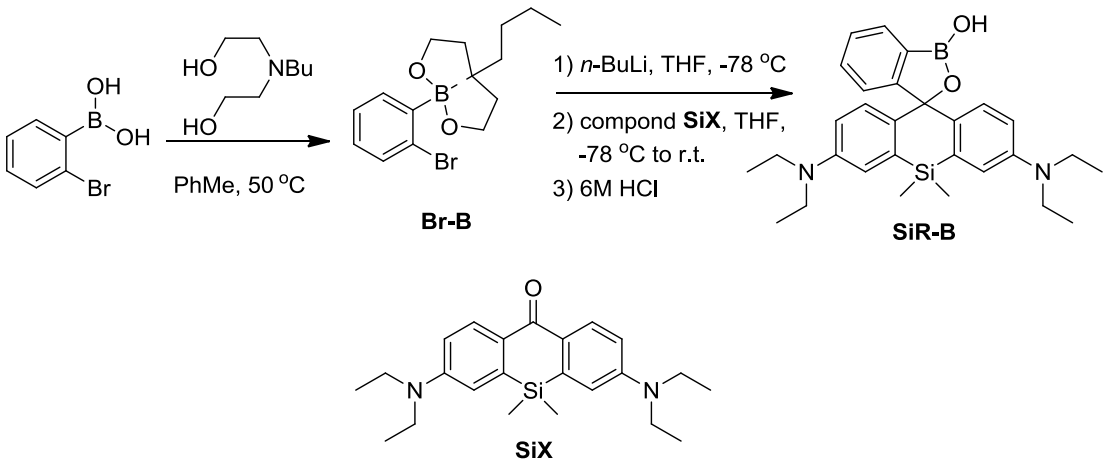
HepG2 and PC12 cells were incubated with **SiR-B** (5 μM, 1% DMSO) in culture media at 37 ℃. After 30 min, the cells were washed three times with PBS, LysoTracker Green DND-26 (1 μM) was used to co-stain the cells for another 20 min.

Then the cells were washed three times with PBS again. Confocal microscopy and image analysis was acquired with a laser scanning confocal microscopy. Probe **SiR-B** was excited at 633 nm and LysoTracker Green DND-26 was excited at 488 nm.

Chloroquine-induced lysosomal pH change.

HepG2 cells were incubated with probe **SiR-B** for 30 min and washed with PBS for three times. Then, the cells were exposed to 100 μM chloroquine for 2 min .

Synthesis of Compounds SiR-B and R-B.

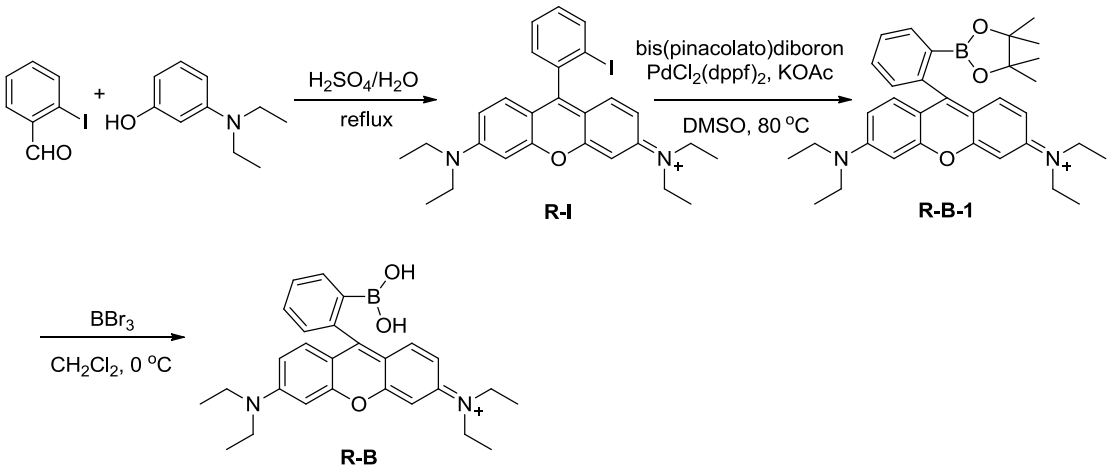


Scheme S1 Synthesis of compound **SiR-B**.

2-(2'-Bromophenyl)-6-butyl[1,3,6,2]dioxazaborocan (Br-B). To a suspension of 2-bromophenylboronic acid (4.0 g, 20 mmol) in dry toluene (30 mL) was added N-butyldiethanolamine (3.2 g, 20 mmol). The mixture was heated at 50°C for 2 h. After cooling to room temperature, the toluene was evaporated under reduced pressure. The remaining clear colorless crude oil was treated with heptane (50 ml ×3) to remove the residual toluene. The resulting suspension was allowed to stand at room temperature overnight. The solid that precipitated was collected by filtration, washed with heptane, and dried overnight to give the title compound **Br-B** as a white solid (6.4 g, 98% yield). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.05-7.81 (m, 4H), 4.09-4.21 (m, 4H), 3.24-3.33 (m, 2H), 3.00-3.07 (m, 2H), 2.59-2.65 (m, 2H), 1.48-1.59 (m, 2H), 1.11-1.23 (m, 2H), 0.83 (t, 3H, *J* = 7.3 Hz).

SiR-B. To a dried flask flushed with argon, compound **Br-B** (326 mg, 1.0 mmol) and anhydrous THF (10 mL) were added. The solution was cooled to –78 ℃, 1.6 M *n*-BuLi (0.75 mL, 1.2 mmol) was added over a period of 10 min and the mixture was stirred for further 20 min. At the same temperature, a solution of **SiX**[1] (380 mg, 1.0 mmol) in anhydrous THF (10 mL) was slowly added. The mixture was allowed to stir for 20 min at –78 ℃, then allowed to warm to room temperature gradually. After stirring for further 1 h, 6M HCl solution (10 mL) was added and the mixture was

stirred for an additional 30 min. The resulting blue solution was neutralized with $NaHCO_3$, and extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel (CH_2Cl_2 : MeOH = 10 : 1) to give **SiR-B** as a light blue solid (165 mg, 34% yield). HRMS (ESI): m/z calcd for $C_{29}H_{37}BN_2O_2Si$ $[M+H]^+$: 485.2790; found:485.2839. ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.26 (s, 1H), 6.52-7.76 (m, 10H), 3.57 (q, 8H, J = 6.9 Hz), 1.06 (t, 12H, J = 6.9 Hz), 0.58 (s, 3H), 0.49 (s, 3H); ^{13}C NMR (75 MHz, DMSO-d_6): δ 163.27, 145.76, 135.16, 134.80, 131.12, 130.92, 128.19, 126.88, 123.75, 115.55, 113.29, 88.34, 43.96, 12.89, 0.61, -0.1.



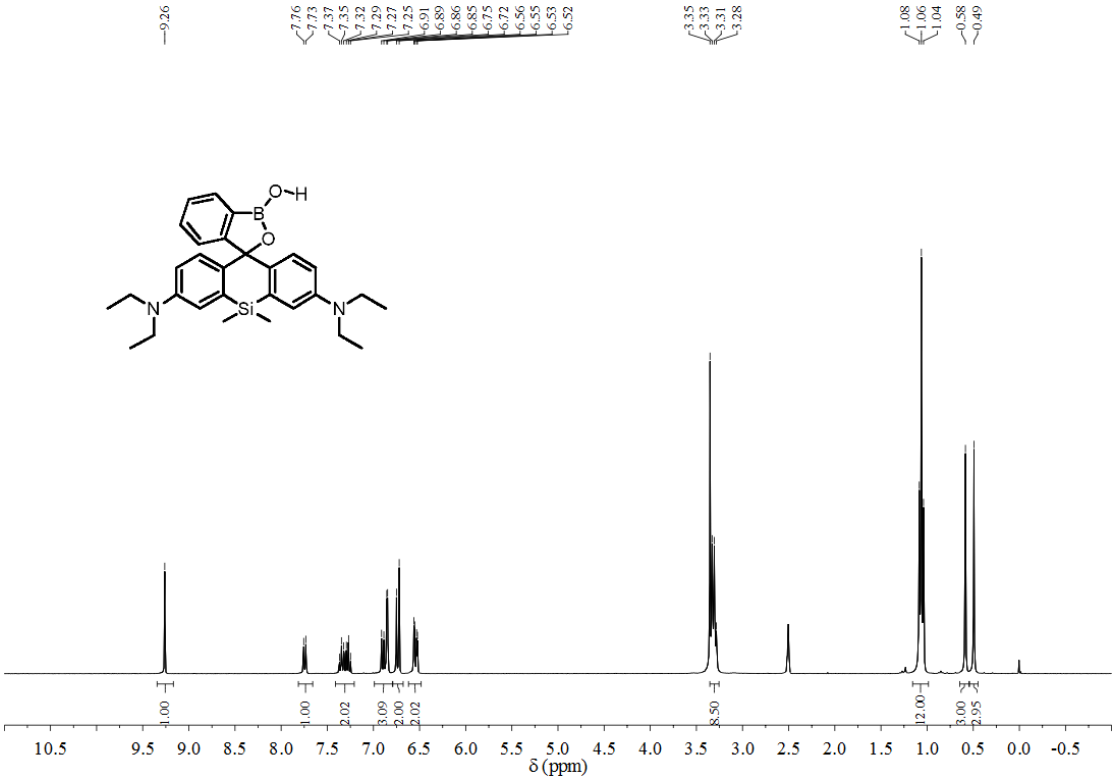
Scheme S2 Synthesis of compound **R-B**.

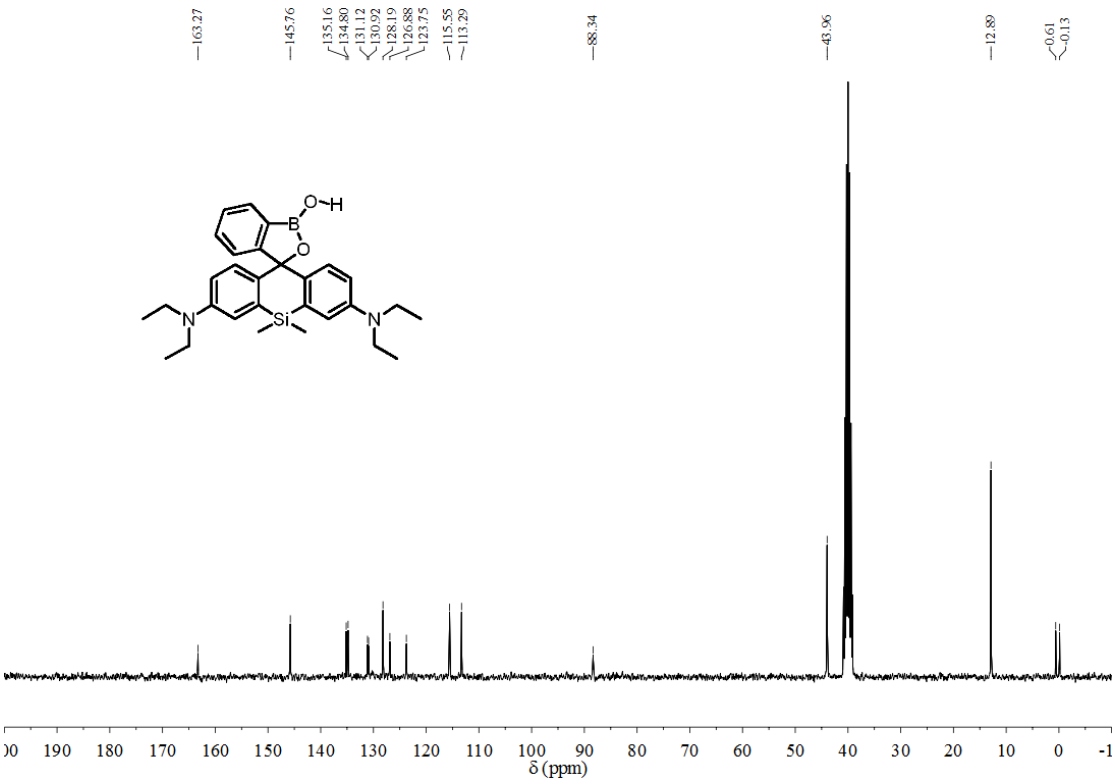
R-I[2]. To a solution of concentrated H_2SO_4 (12 ml) and water (8 ml) was added 2-iodobenzaldehyde (1.16 g, 5 mmol) and the solution was stirred and gradually heated to reflux. Then 3-dimethylaminophenol (1.37 g, 10 mmol) was slowly added over 1 h. The resulting solution was further stirred under reflux for 12 h. After cooling to room temperature, the mixture the carefully neutralized with 20% NaOH to pH = 7.0. The red mixture was extracted with CH_2Cl_2 (containing small amount of MeOH). The organic layer was washed with brine and dried over Na_2SO_4. After removal of the solvent under reduced pressure, the residue was purified by column chromatography on silica gel (CH_2Cl_2 : MeOH = 20 : 1) to give **R-I** as a dark red solid (0.92 g, 35%

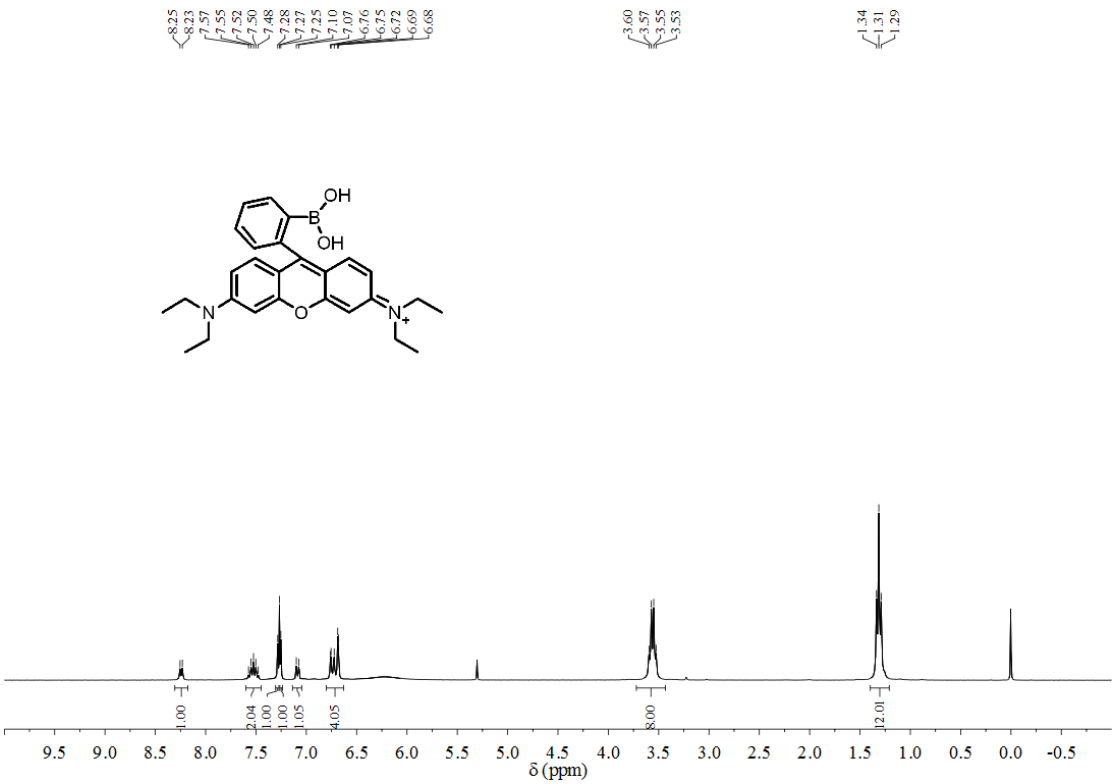
yield). ESI-MS m/z 525.4. ^{1}H NMR (300 MHz, $CDCl_3$): 8.07-6.85 (m, 10H), 3.64 (q, 8H, *J* = 7.1 Hz), 1.32 (t, 12H, *J* = 7.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.0, 157.8, 155.7, 139.8, 137.3, 131.5, 129.9, 128.7, 128.1, 126.3, 114.5, 113.1, 96.7, 46.3, 12.7.

R-B. Compound **R-I** (530 mg, 1 mmol), Bis(pinacolato)diboron (280 mg, 1.1 mmol), copper(I) iodide (2 mg, 0.01 mmol), $PdCl_2(dppf)_2$ (53 mg) and KOAc (275 mg, 2.8 mmol) were dissolved in dry DMSO (10 mL) in a Schlenk tube. The solution was freeze-pump-thawed three times, then stirred at 80^{o}C for 12 h under argon. The resulting red solution was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under reduced pressure and the crude **R-B-1** was used without further purification. The preceding crude **R-B-1** was dissolved in dry CH_2Cl_2 (12 mL) and stirred at 0 ℃ under argon. Boron tribromide (1 M in CH_2Cl_2, 5 mL, 5 mmol) was slowly added. The reaction mixture was stirred at 0 ℃ for 1 h and slowly warmed to room temperature, then stirred for another 1 h. Methanol (10 mL) was added dropwise (CAUTION) at 0 ℃ to quench the reaction. The resulting red solution was extracted with CH_2Cl_2. The organic layer was washed with brine and dried over Na_2SO_4. The solution was concentrated in vacuo then purified by flash chromatography on silica gel (dichloromethane : methanol = 20 : 1) to give pure **R-B** as deep red solid (80 mg, 18% yield). HRMS (ESI): m/z calcd for $C_{27}H_{32}BN_2O_3$ $[M]^+$: 443.2500; found:443.2554. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.25-6.68 (m, 10H), 3.56 (q, 8H, *J* = 7.0 Hz) , 1.31(t, 12H, *J* = 7.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.4, 158.0, 155.2, 137.3, 136.2, 132.9, 129.0, 128.8, 127.6, 114.9, 113.2, 95.8, 45.8, 12.6.

^{1}H NMR, ^{13}C NMR and HRMS Spectra.



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1.29
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OH
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O
N+
1.00
2.04
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1.05
4.05
8.00
12.01
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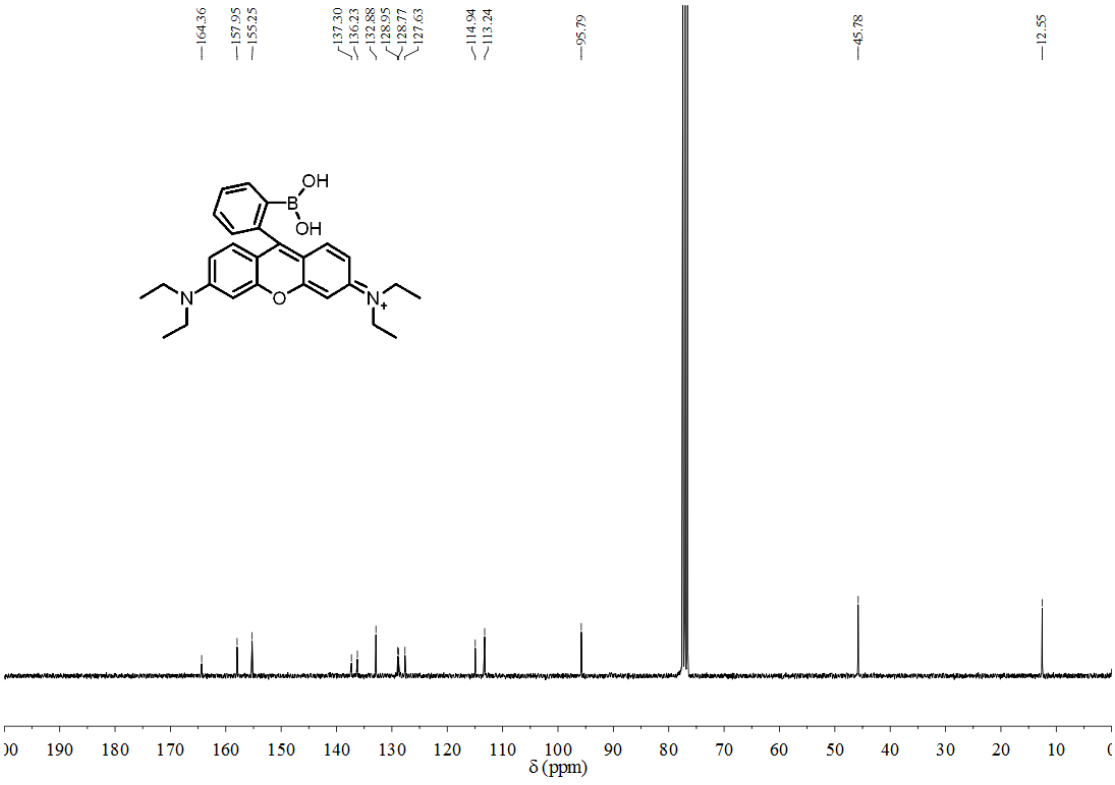
δ (ppm)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OH
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O
N+
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δ (ppm)

Qualitative Analysis Report

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Comment			

User Spectra

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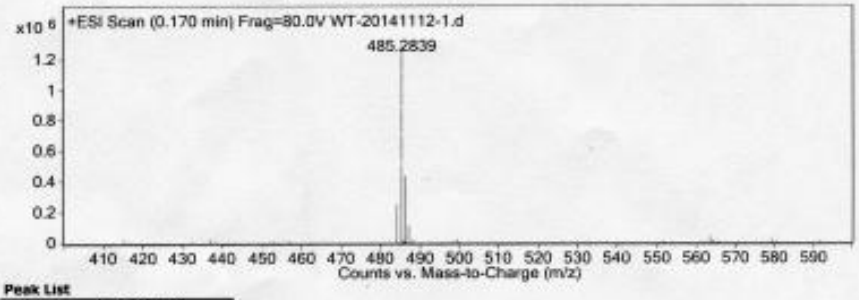

Peak List

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274.2746		116946
280.2639		109832
282.282	1	1116723
283.2834	1	214348
484.2864		257064
485.2839	1	1278989
486.2863	1	437237
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Formula Calculator Element Limits

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O	0	20
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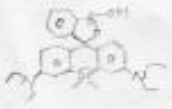

Qualitative Analysis Report

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Comment			

User Spectra

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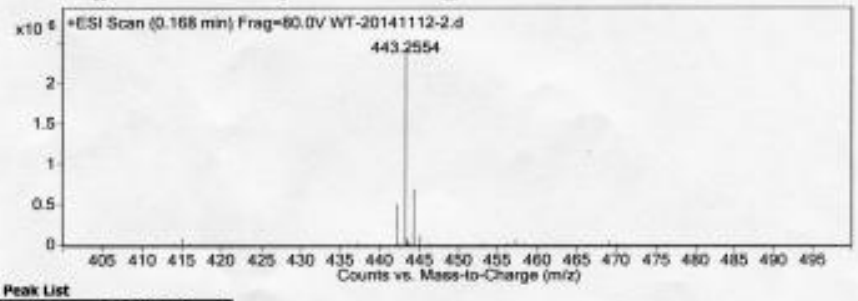

Peak List

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443.2554	1	2437740
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Formula Calculator Element Limits

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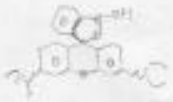

$C_{21}H_{41}BN_2O_4$

442.24

Optical properties

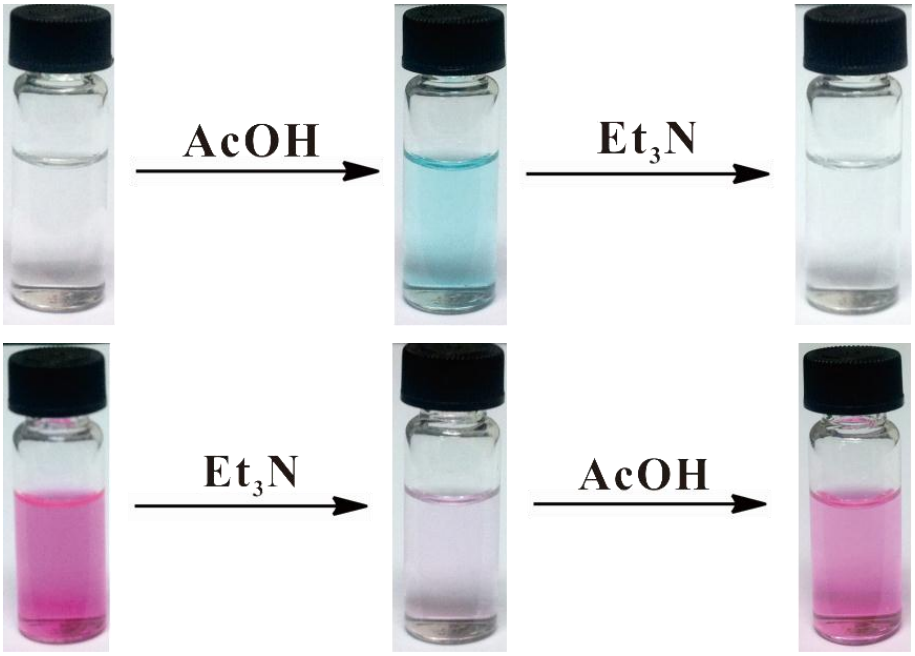

Fig. S1 A reversible color changes of **SiR-B** (blue color) and **R-B** (red color) solution (10 *u*M, MeCN) after adding AcOH or Et_3N.

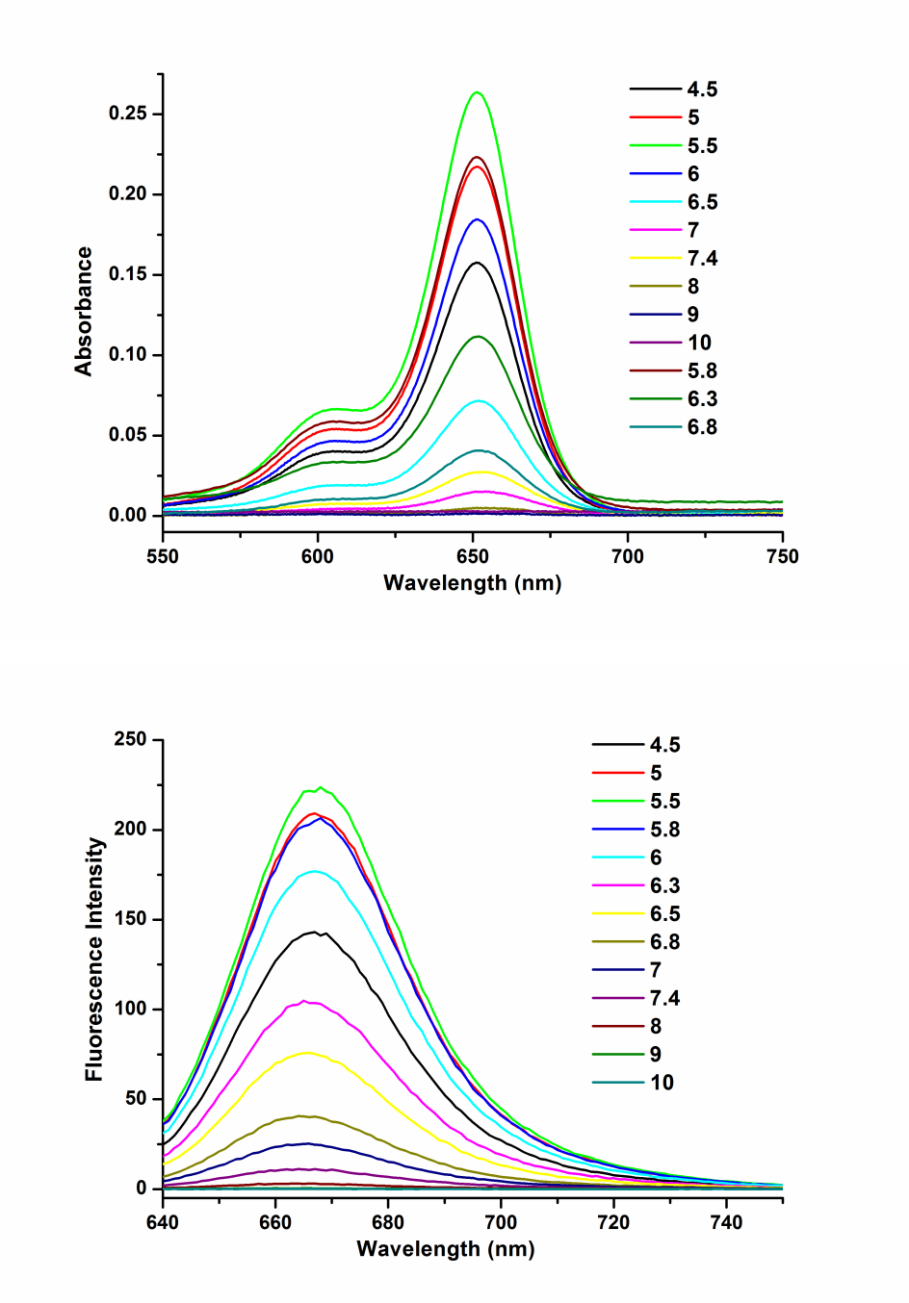

Fig. S2 Changes of the absorption and fluorescence spectra of probe **SiR-B** towards various pH.

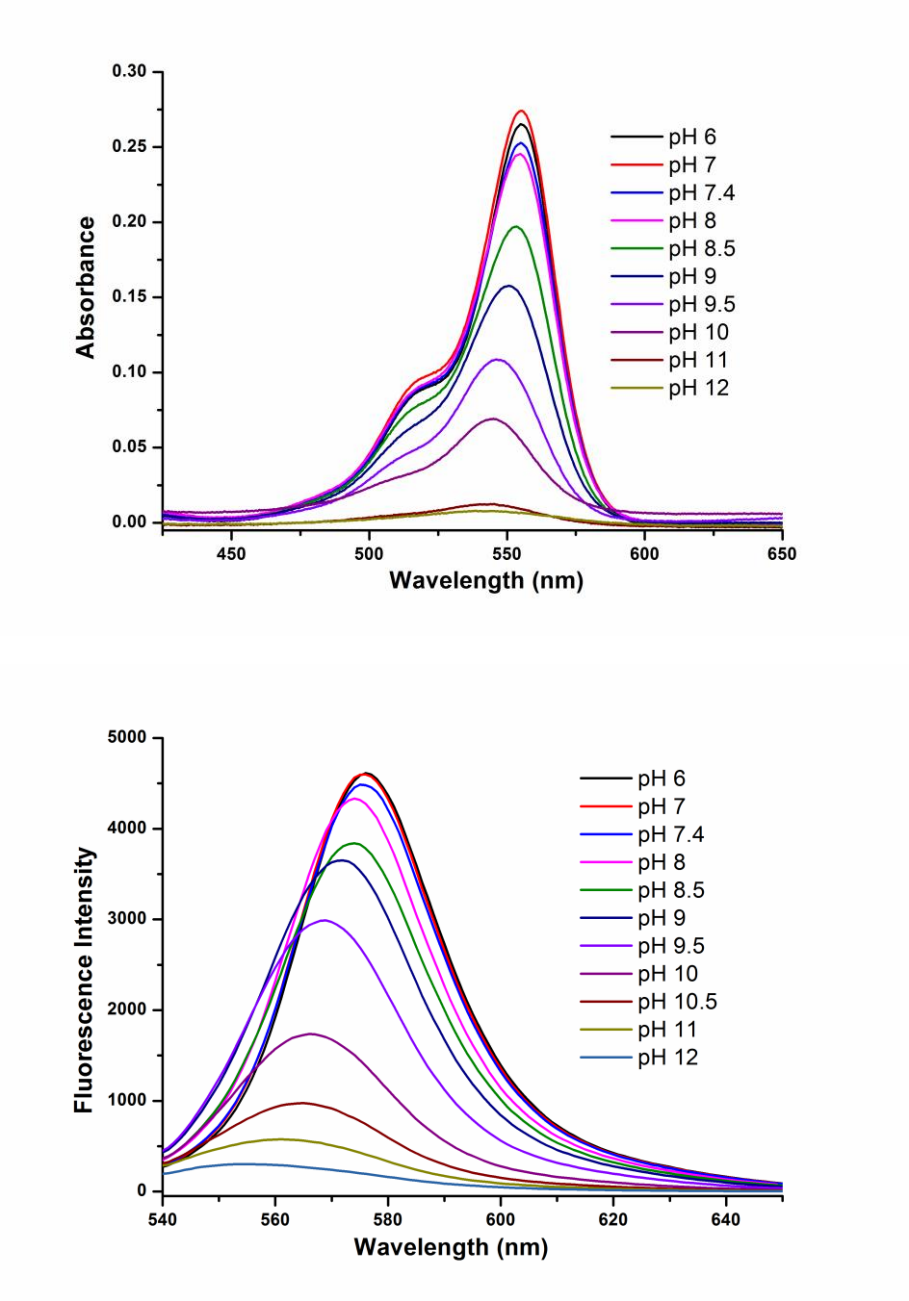

Fig. S3 Changes of the absorption and fluorescence spectra of probe **R-B** towards various pH.

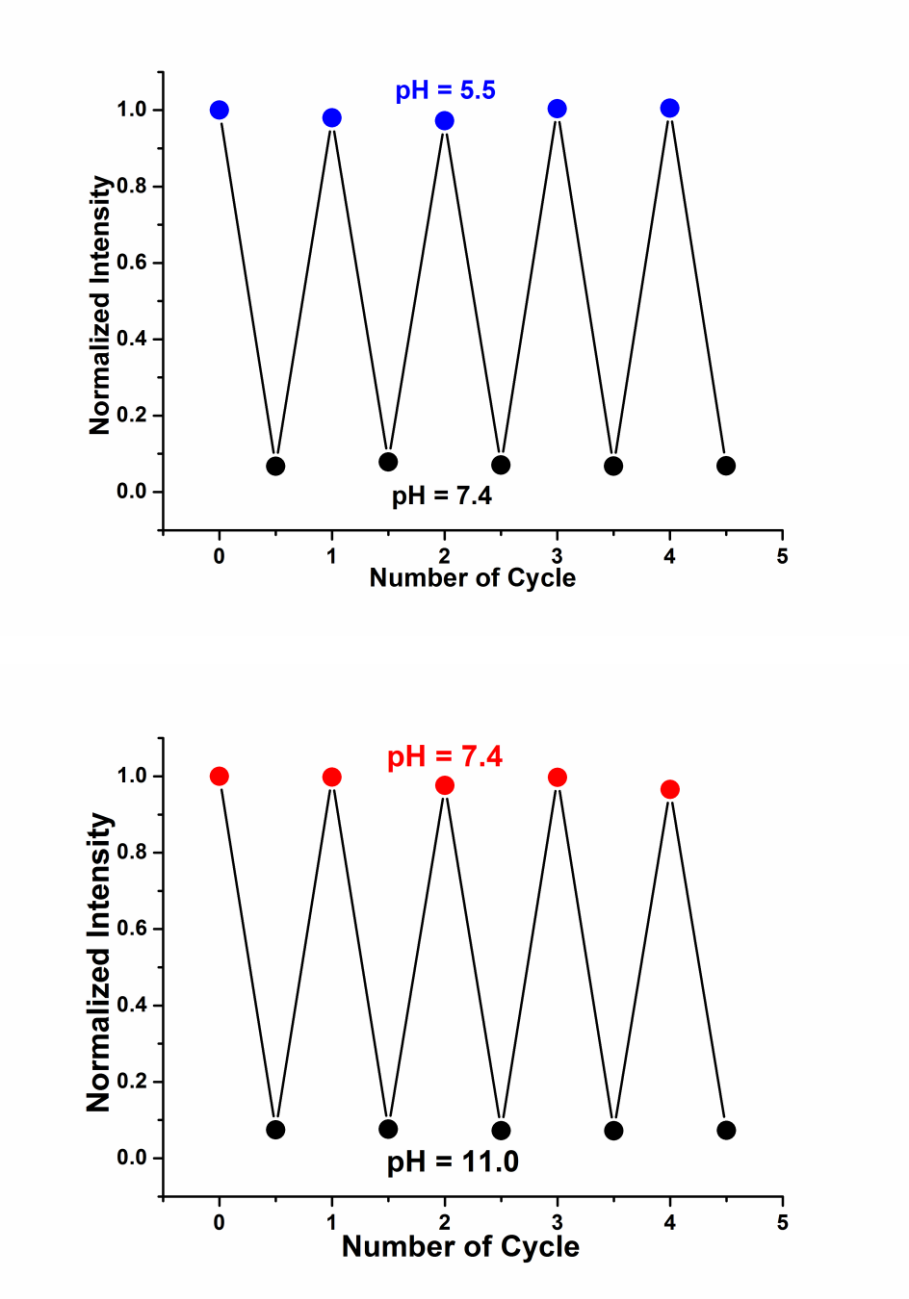

Fig. S4 The reversible fluorescence responses of probes **SiR-B** (top, changes in the fluorescence at 668 nm between pH 7.4 and 5.5) and **R-B** (bottom, changes in the fluorescence at 576 nm between pH 11.0 and 7.4).

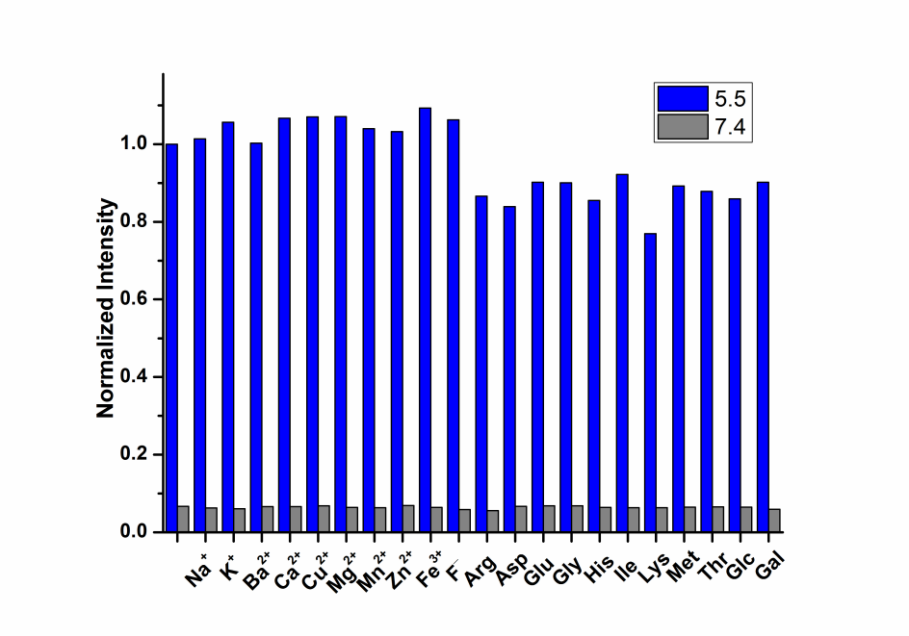

Fig. S5 Fluorescence responses of probes **SiR-B** (blue bars: pH 5.5; gray bas: pH 7.8) upon the addition of various species, respectively.

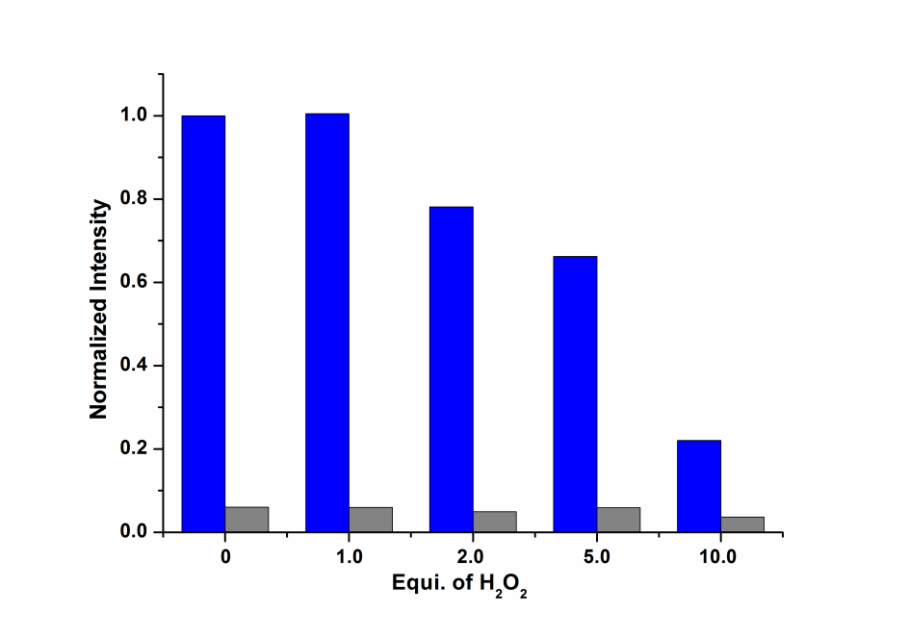

Fig. S6 Fluorescence responses of probes **SiR-B** (blue bars: pH 5.5; gray bas: pH 7.8) after treatment of H_2O_2 for 10 min.

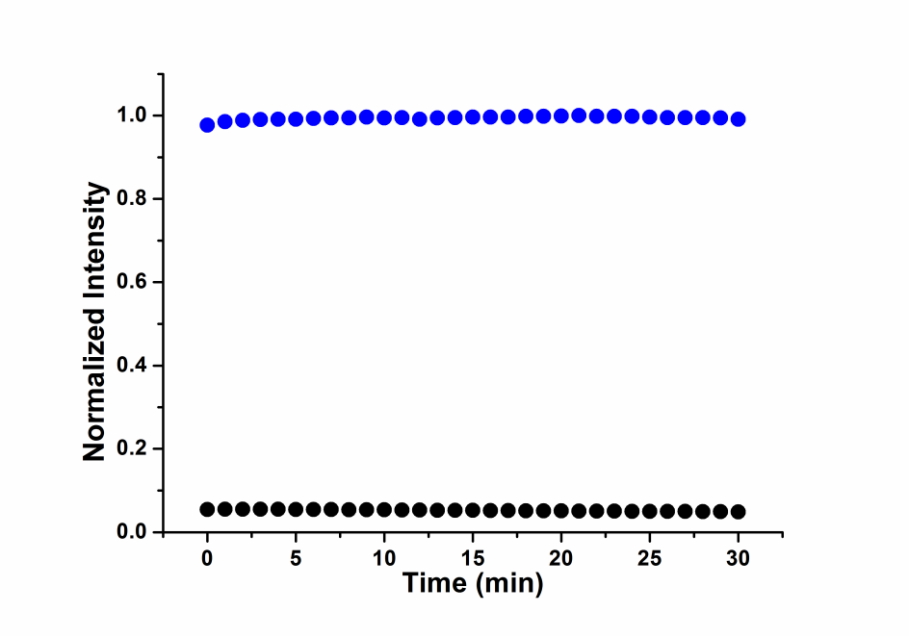

Fig. S7 Photostability of probe **SiR-B** (2.5 μM) in PBS buffer solution at pH 7.4 (black) and pH 5.5 (blue), respectively. The sample was continuously irradiated by a xenon lamp (150 W) at 10 nm slit width at 633 nm.

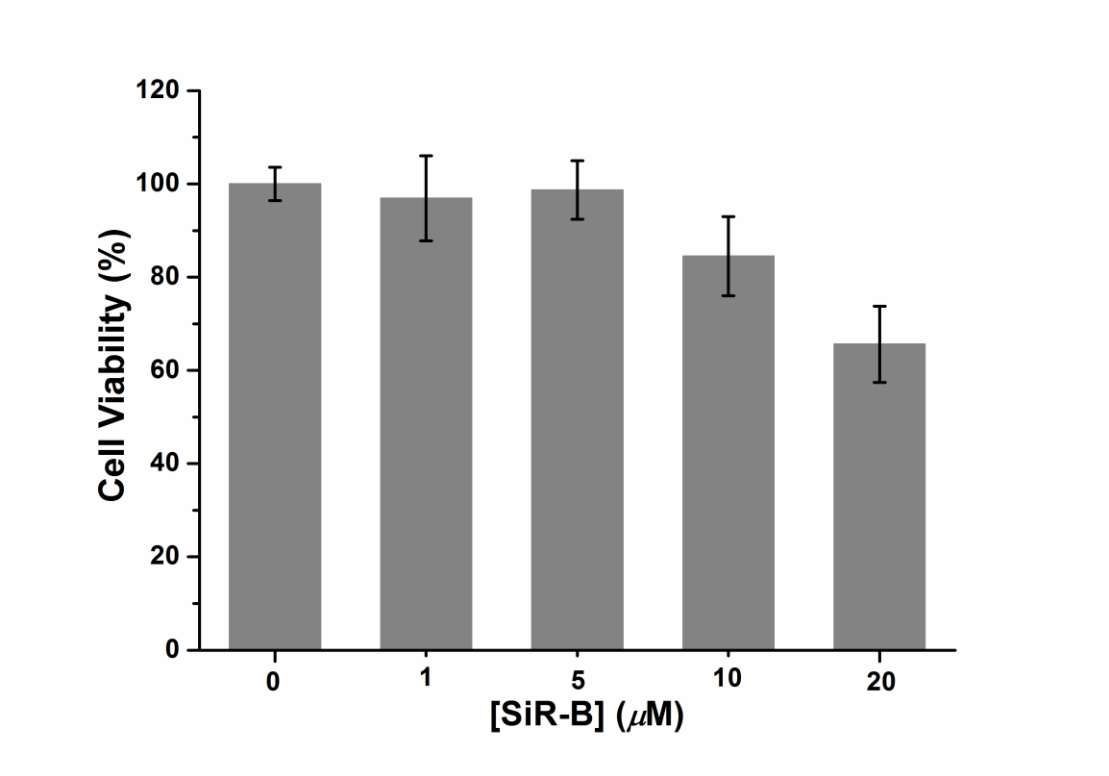

Fig. S8 Cytotoxic effects of probe **SiR-B** on HepG2 cells. Date are expressed as mean values ± standard error of the means of five independent experiments.

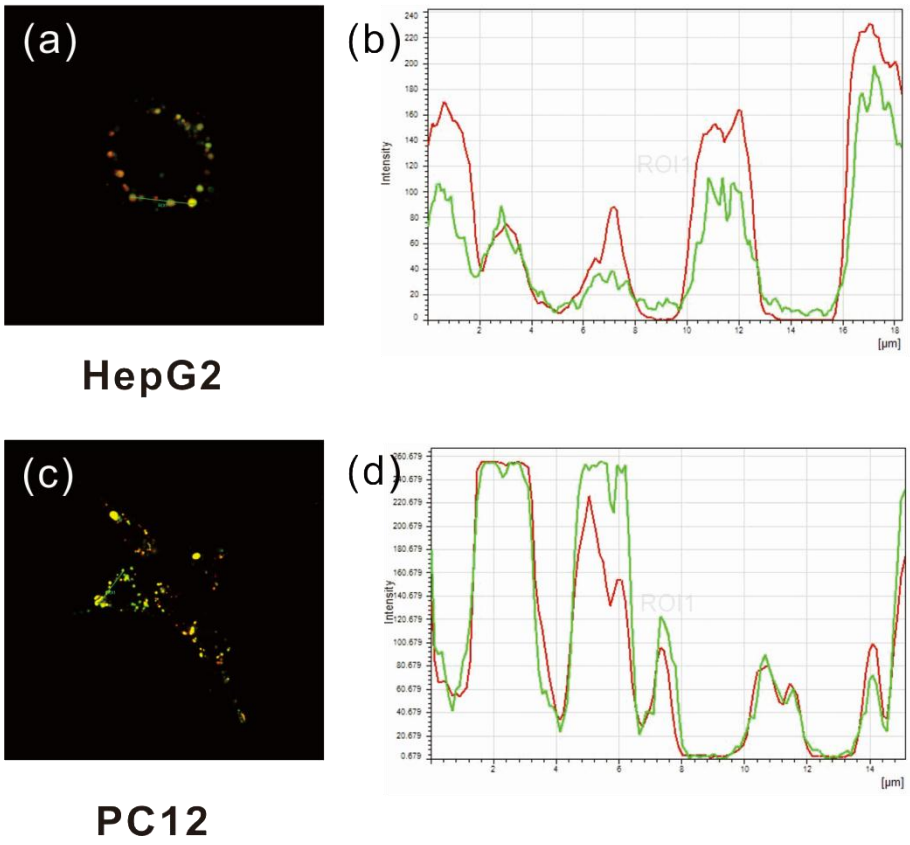

Fig. S9 Fluorescence images of HepG2 (top) and PC12 (bottom) cells co-stained with 5.0 μM probe **SiR-B** and 1 μM LysoTracker Green DND-26. (a) and (c) Merged fluorescence microscopic images. (b) and (d) Intensity profiles of region of interest cross the cells.

References

S1. Wang, T.; Zhao, Q.-J.; Hu, H.-G.; Yu, S.-C.; Liu, X.; Liu, L.; Wu, Q.-Y., *Chem. Commun.* **2012**, *48*, 8781-8783.

S2. Yuan, L.; Lin, W.; Cao, Z.; Wang, J.; Chen, B., *Chem. Eur. J.* **2012,** *18*, 1247-1255.