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Supporting Information

Molecular engineering of a dual emission near-infrared ratiometric fluorophore for detection of pH at the organism level

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Contents:

Figure S1 Absorption and fluorescence spectra of **NIR-HBT** S2

Figure S2 Spectra of **NIR-HBT** with different pH with excitation at 638 nm S2

Figure S3 Plots of I_{764}/I_{713} and I_{713}/I_{764} versus pH values S2

Figure S4 Effect of various substances on the fluorescence response of **NIR-HBT** S3

Figure S5 Time-dependent fluorescence intensity change of **NIR-HBT** S3

Figure S6 Cytotoxicity of **NIR-HBT** evaluated on HepG-2 cells by CCK-8 assay S3

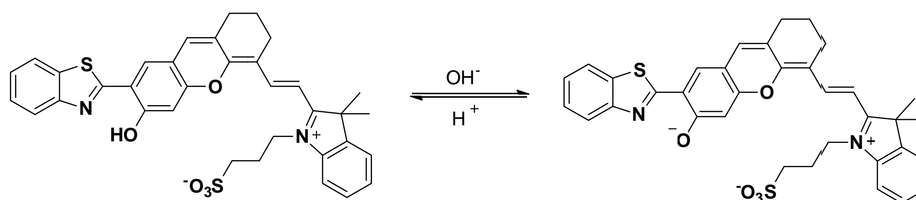
Figure S7. Confocal images of HepG-2 cells stained with **NIR-HBT** S4

Figure S8. Photophysical properties of **NIR-HBT** by the scanning lambda (xyλ) mode S4

Figure S9. Flow cytomerty analysis of HepG-2 cells stained with **NIR-HBT** S5

Figure S10. Evaluation of dye leakage in **NIR-HBT** stained HepG-2 cells S5

Figure S11-S16. ^{1}H NMR, ^{13}C NMR, HRMS. S6-S8



Scheme S1. Working principle of **NIR-HBT**.

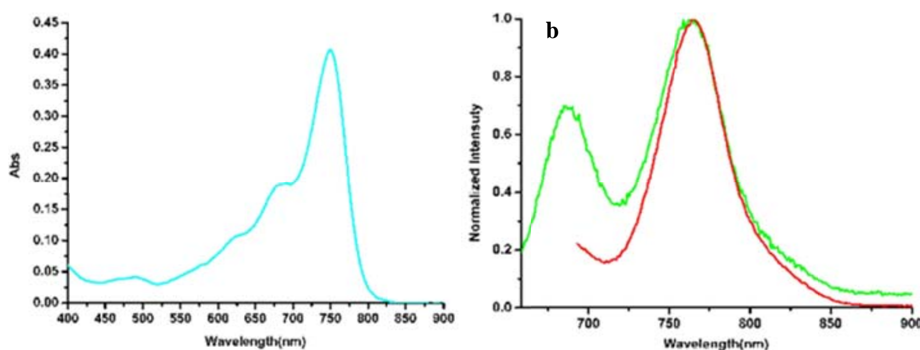
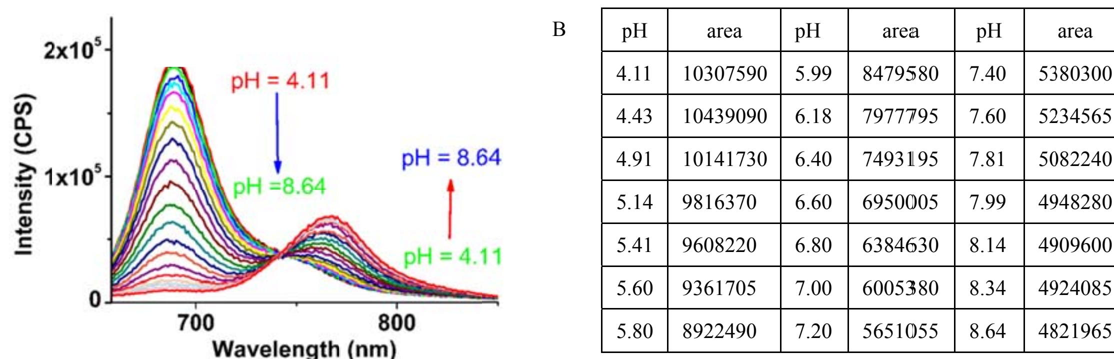


Figure S1 (a) Absorption spectra of **NIR-HBT** (5 μM) in the PBS buffer (b) Fluorescence emission spectra of **NIR-HBT** (5 μM) with an excitation at 683 nm (red line) and 638 nm (green line).



pH	area	pH	area	pH	area
4.11	10307590	5.99	8479580	7.40	5380300
4.43	10439090	6.18	7977795	7.60	5234565
4.91	10141730	6.40	7493195	7.81	5082240
5.14	9816370	6.60	6950005	7.99	4948280
5.41	9608220	6.80	6384630	8.14	4909600
5.60	9361705	7.00	6005380	8.34	4924085
5.80	8922490	7.20	5651055	8.64	4821965

Figure S2 (A) Fluorescence emission spectra of 5 μM **NIR-HBT** at different pH value (4.11, 4.43, 4.91, 5.14, 5.41, 5.60, 5.80, 5.99, 6.18, 6.40, 6.60, 6.80, 7.00, 7.20, 7.40, 7.60, 7.81, 7.99, 8.14, 8.34, 8.64). All samples were measured in 10 mM sodium phosphate buffer (0.01% Triton x 100) and 1% DMSO as a cosolvent, Ex = 638 nm. (B) The integral areas at different pH values.

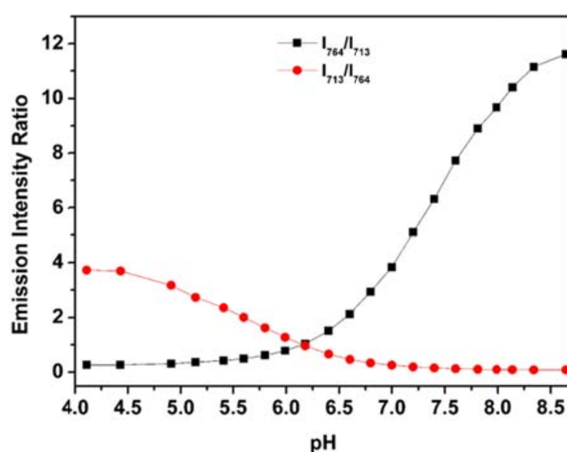


Figure S3 Plots of I_{764}/I_{713} (dark line) and I_{713}/I_{764} (red line) versus pH values in the range pH 4.11– 8.64.

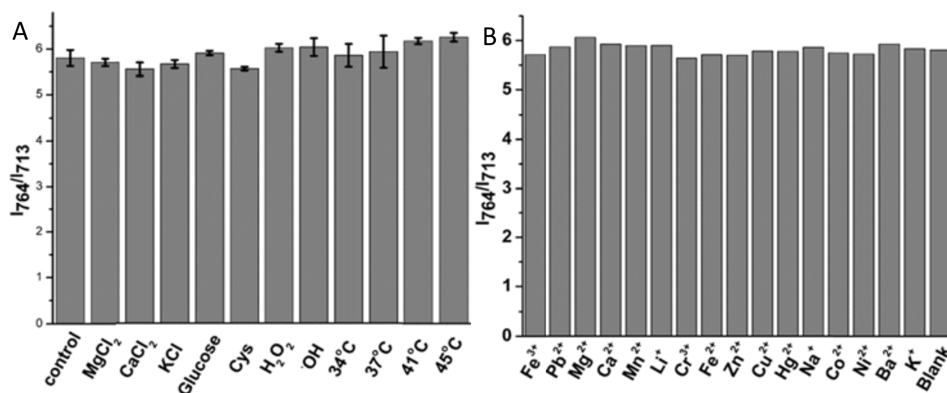


Figure S4 (A) Fluorescence responses of **NIR-HBT** (5 μM) to various substances and temperature: $MgCl_2$ (1 mM), $CaCl_2$ (1 mM), KCl (150 mM), glucose (10 mM), cysteine (1 mM), H_2O_2(100 μM) and ·OH (100 μM). (B) Fluorescence responses of **NIR-HBT** (5 μM) to various cationic ions. The concentration of each ion is 50μM. All samples were measured in 10 mM sodium phosphate buffer (pH 7.4, 0.01% Triton x 100) and 1% DMSO as a cosolvent, Ex = 683 nm.

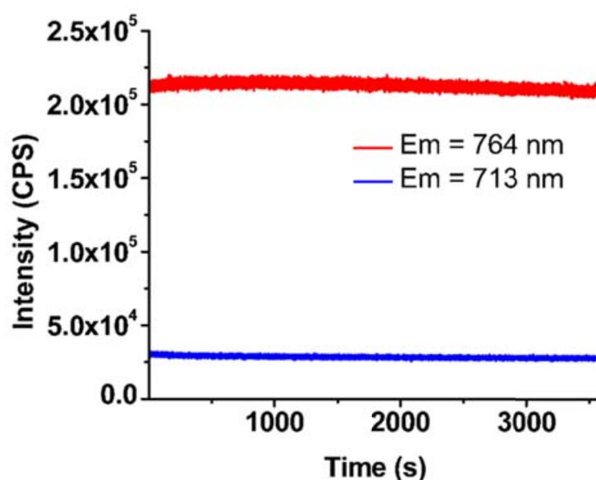


Figure S5 Time-dependent fluorescence intensity change of **NIR-HBT** (5 μM) in 10 mM sodium phosphate buffer (pH 7.4, 0.01% Triton x 100) and 1% DMSO as a cosolvent. Ex = 683 nm

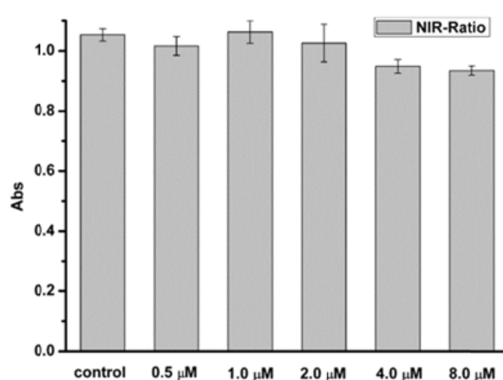


Figure S6 Viability of HEPG 2 cells in the presence of **NIR-HBT** as measured by using CCK-8 assay. The cells were incubated with probe for 24 h

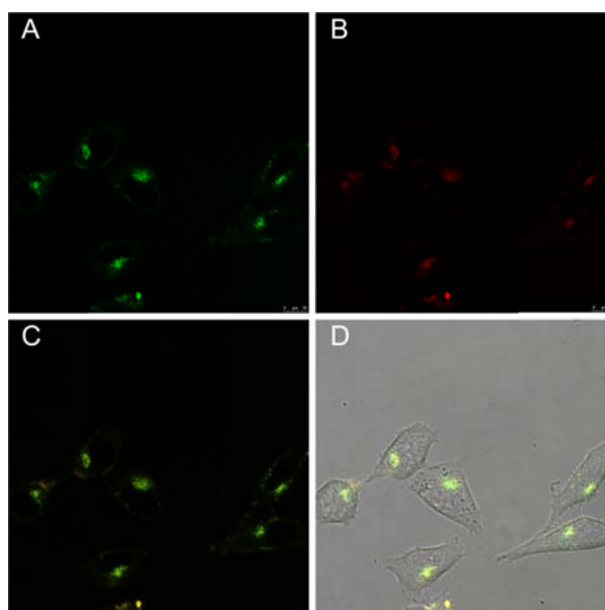


Figure S7.Confocal images of HEPG-2 cells incubated with **NIR-HBT** for 30 min under excitation at 638 nm (A) Green channel 680 ± 20 nm and (B) 750 ± 30 nm. (C) Image merged from those in panels A and B. (D) Image merged from that in panel C and the bright-field image. Scale bar: 10 μm. [**NIR-HBT**] = 5×10^{-6} M.

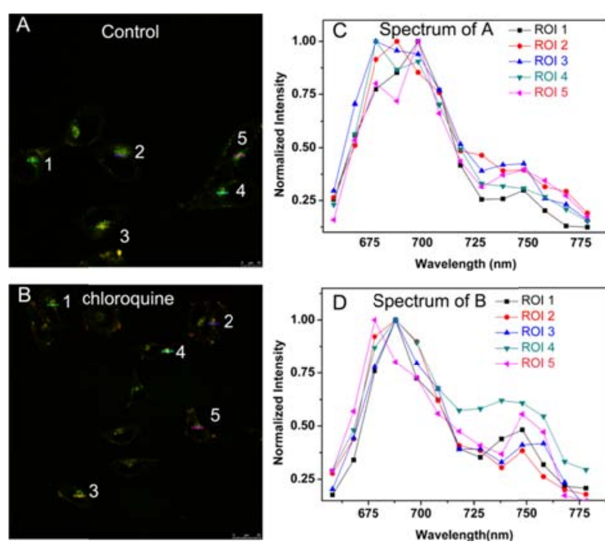


Figure S8 (A) Fluorescence ratiometric images with **NIR-HBT** stained HepG-2 cells. (B) Fluorescence ratiometric images with 100 μM chloroquine treated **NIR-HBT** stained HepG-2 cells. (C) and (D) The ROIs spectrum of **NIR-HBT** in A and B by scanning lambda (xyλ) mode. Ex = 638 nm; Scanning range: 658 nm ~ 778 nm.

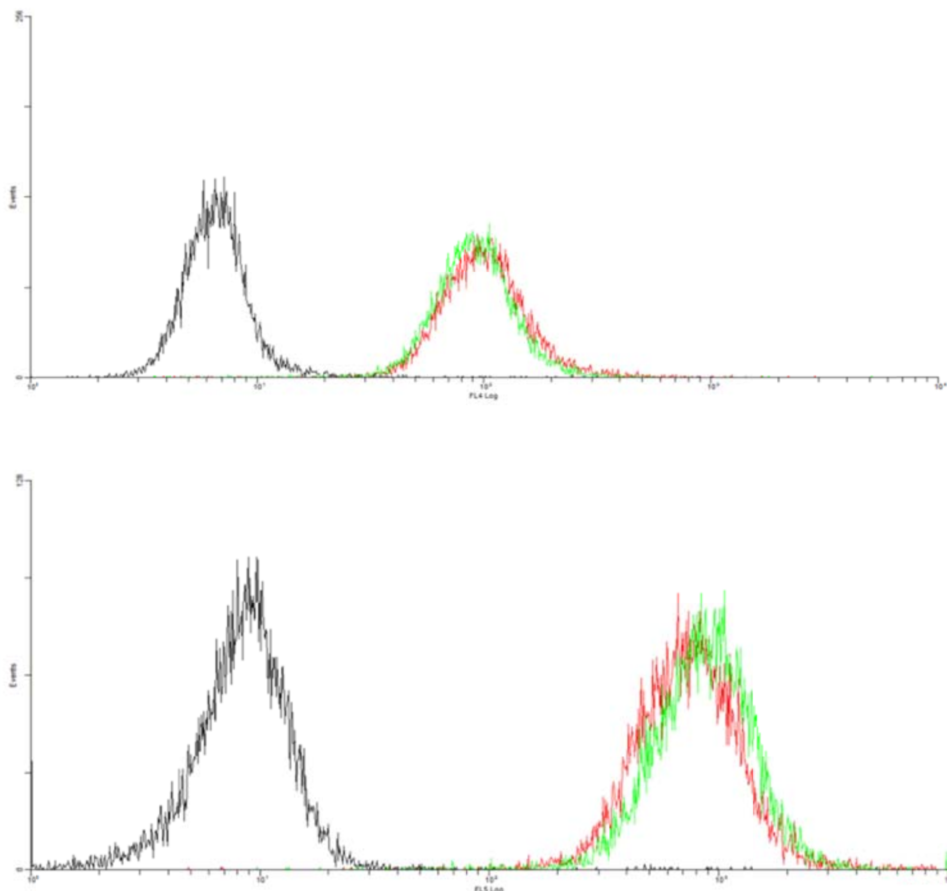


Figure S9 Flow cytomerty analysis of HepG-2 cells stained with **NIR-HBT**. The cells were in PBS (red line), treated with NH_4Cl (green line). The cells without staining are shown as control (black line). Up: Signal from the F4 channel (Em: 670 nm). Down: Signal from the F5 channel (Em: 770 nm).

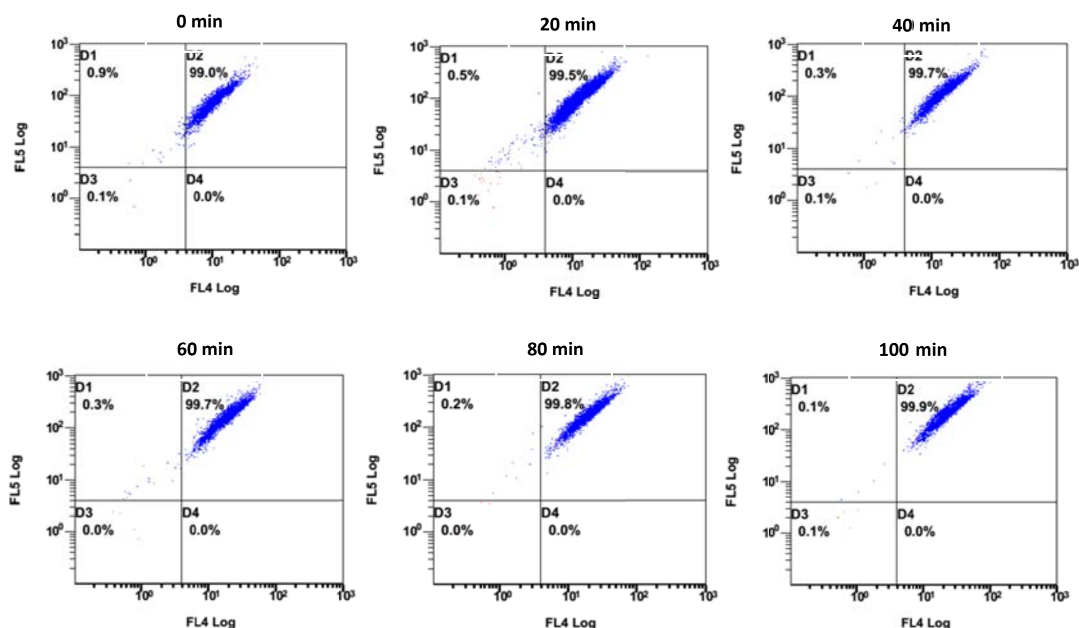


Figure S10 Evaluation of dye leakage in **NIR-HBT** stained HepG2 cells at room temperate in 10 mM PBS buffer (pH 7.4). Data are collected by flow cytometer with the sampling of 10,000 cells in each event

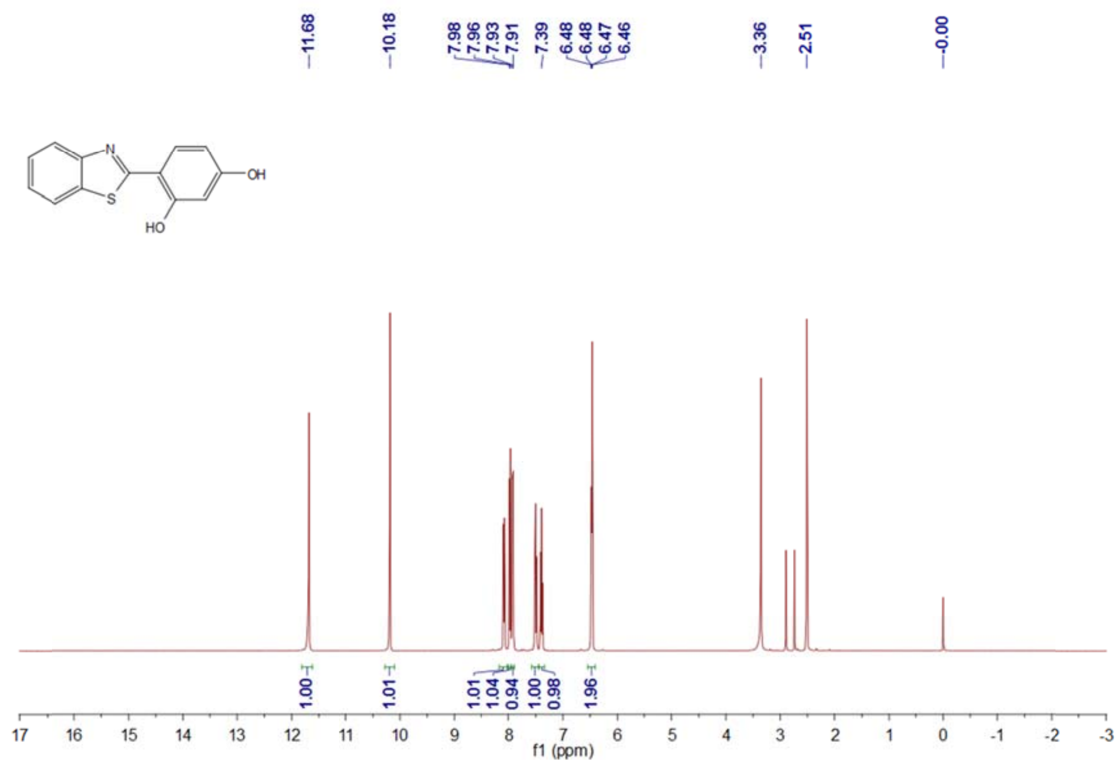


Figure S11 ^{1}HNMR spectrum of compound **8** in d^{6}-DMSO

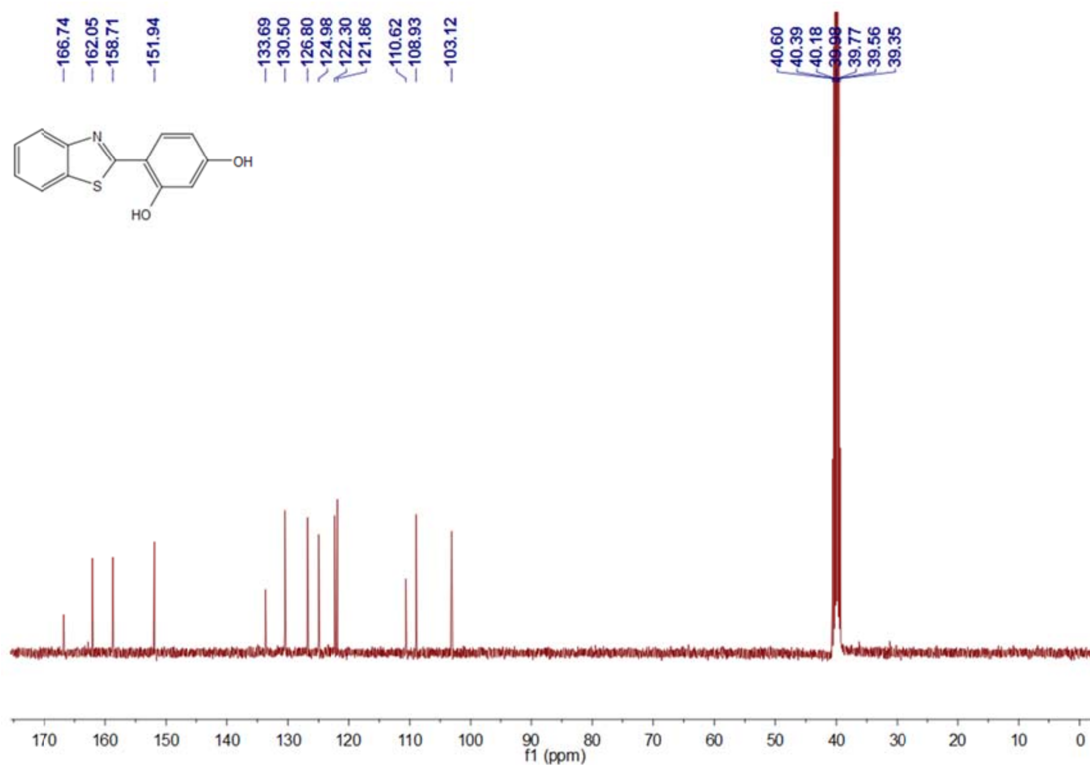


Figure S12 ^{13}CNMR spectrum of compound **8** in d^{6}-DMSO

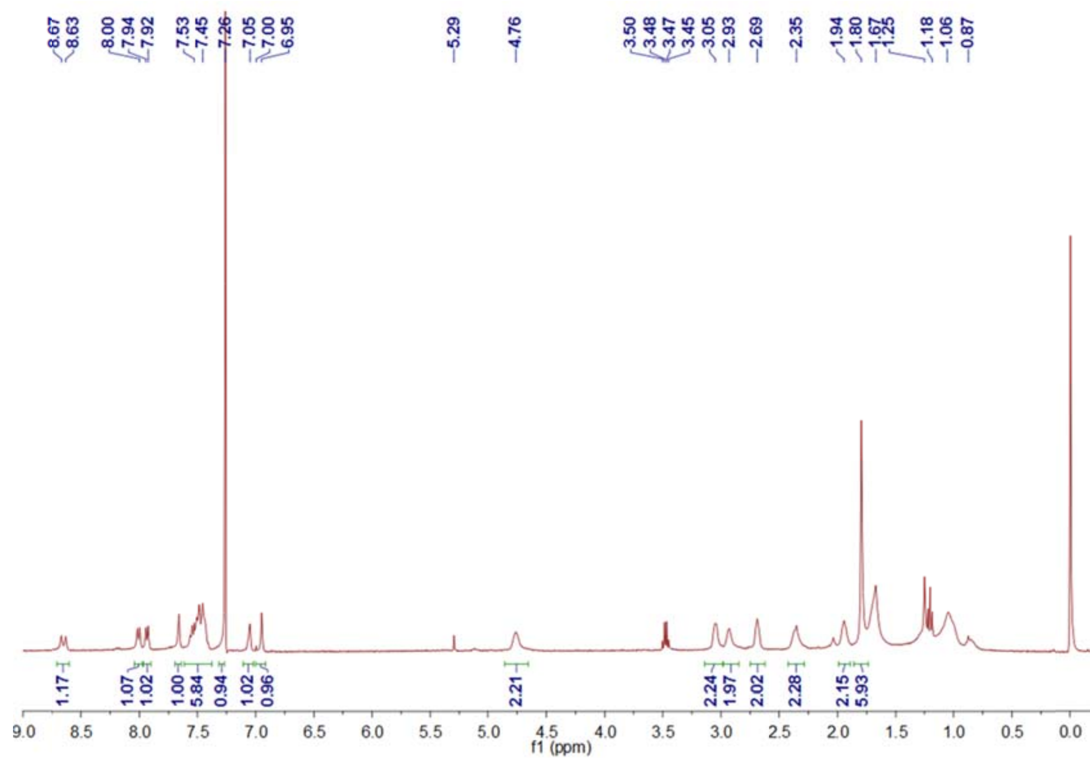


Figure S13 ^{1}HNMR spectrum of compound **NIR-HBT** in $CDCl_3$

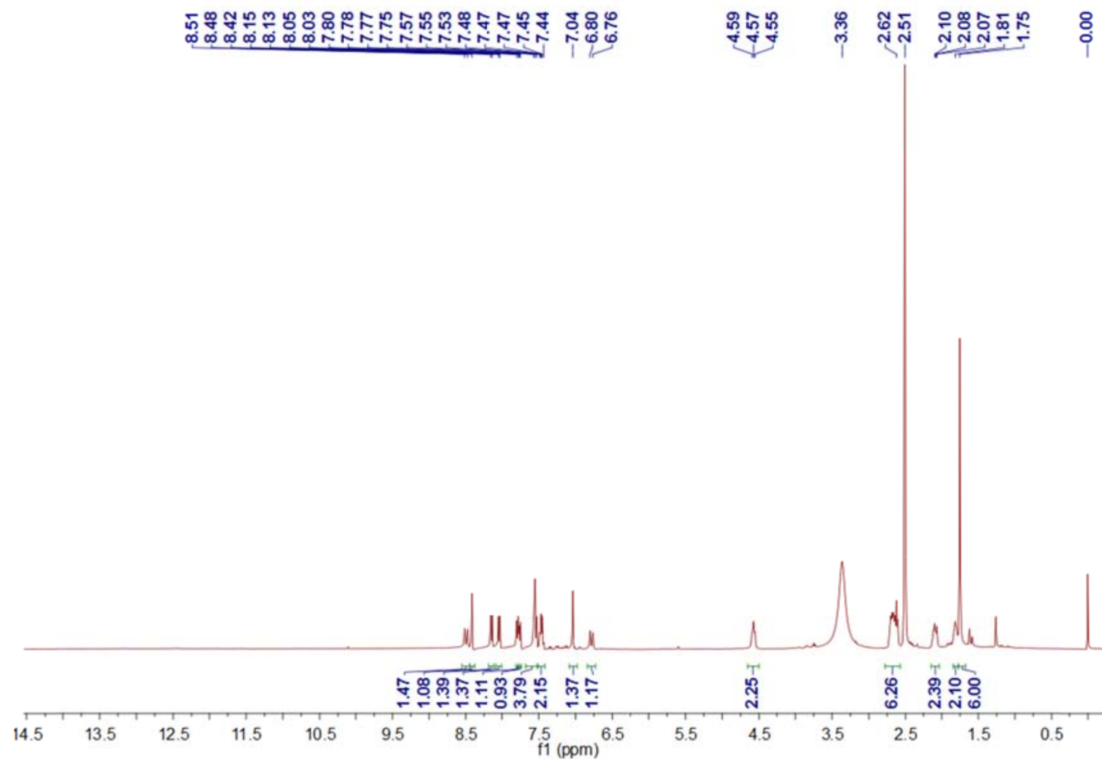


Figure S14 ^{1}HNMR spectrum of compound **NIR-HBT** in d^6-DMSO

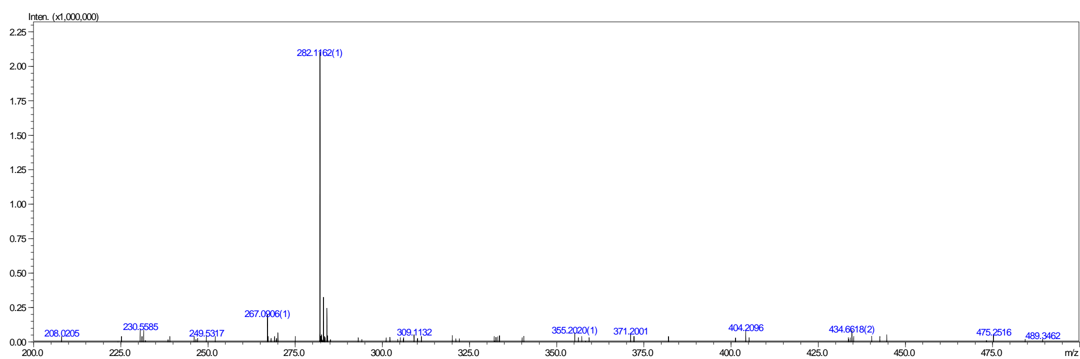


Figure S15 HRMS spectrum of compound **5**

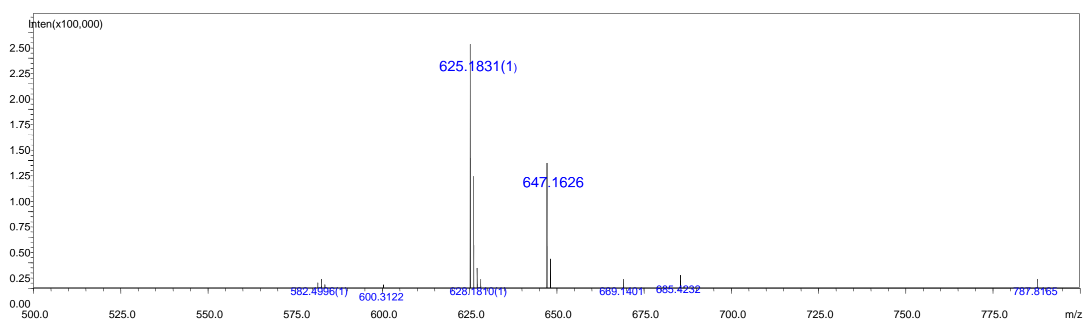


Figure S16 HRMS spectrum of **NIR-HBT**