Electronic Supplementary Material (ESI) for Chemical Communications.

Design, Synthesis and Applications of NIR-emissive Scaffolds of Diketopyrrolopyrrole-aza-BODIPY Hybrids

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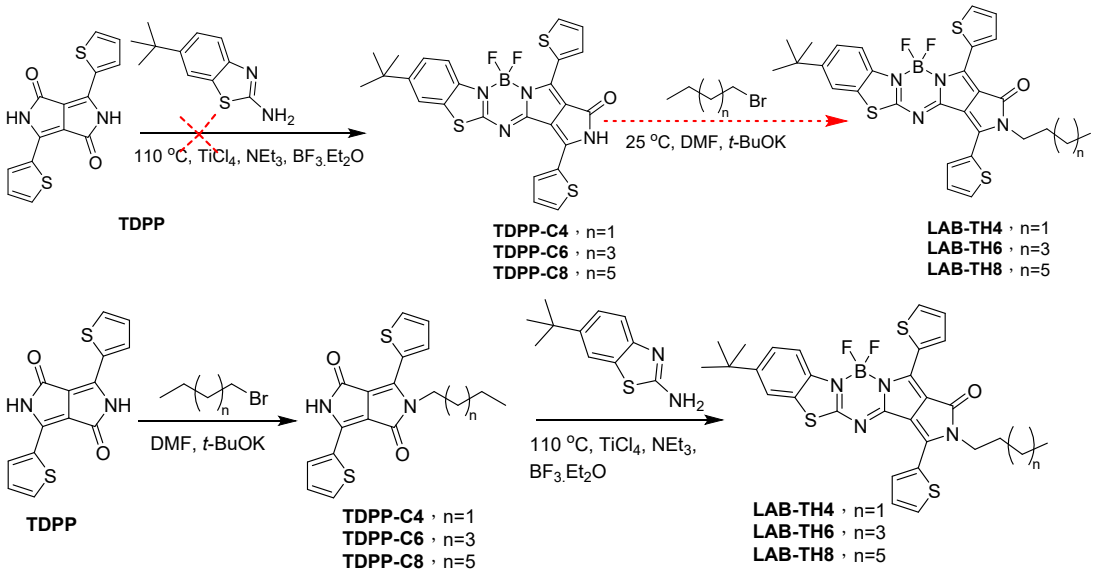
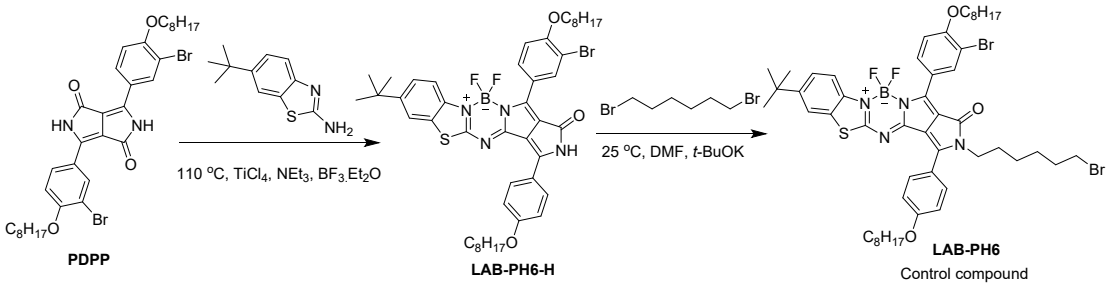
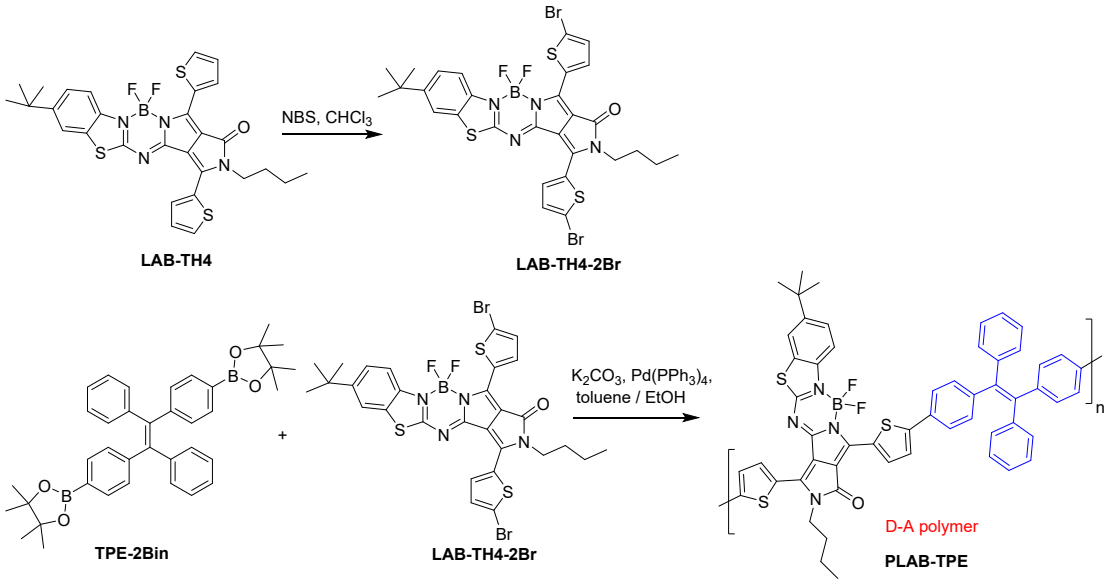


Scheme S1 The synthetic routes of **LAB-TH4 ~ LAB-TH8**.



Scheme S2 The synthetic routes of **LAB-PH6**.



Scheme S3 The synthetic routes of **PLAB-TPE**.

1. Experimental section

1.1. Instrumentation and materials

All chemicals used in this study were analytical reagent grade. The UV-vis absorption spectra were recorded using a Helios Alpha UV-Vis scanning spectrophotometer with a 1 cm quartz cell. Fluorescence spectra were quantitatively measured by FluoroMax-4 spectrofluorometer with a xenon lamp and 0.5 cm quartz cells. High-resolution mass spectra were carried on LCQ Fleet LC-MS System (Thermo Fisher Scientific). Mass spectra were recorded on Waters ACQUITY TQD liquid chromatograph-mass spectrometer using APCI ionization. ^{1}H NMR and ^{13}C NMR spectra were carried on a Bruker spectrometer.

Size distribution was analyzed on a dynamic light scattering (DLS) using a Malvern Zetasizer Nano ZSP. The cell viability was detected by CCK-8 kit, and the absorbance of each sample was measured at 450 nm using a microplate reader (BioTek). The cellular fluorescence images were taken by confocal laser scanning microscope (CLSM, ZEISS-LSM880).

2 Synthesis

2.1 Synthetic procedures. 3,6-di(thiophen-2-yl)-2,5-dihydropyrrolo[3,4-c] (**TDPP**), **TPDPP-C4**, **TPDPP-C6** and **TPDPP-C8** were synthesized according previous methods [S1]. **LAB-PH6** was synthesized according our previous method [S2].

Synthesis of LAB-TH4

A mixture of **TDPP-C4** (100 mg, 0.28 mmol) and 6-(*tert*-butyl)benzo[d]thiazol-2-amine (57.88 mg, 0.28 mmol) was dissolved in 40 mL of dry toluene. This mixture was stirred for 30 min at 45 ^{o}C. Then, trimethylamine (4.26 mmol) and $TiCl_4$ (1.52 g, 1.68 mmol) were added. The resulting solution was refluxed at 110 ^{0}C. Upon **TDPP-C4** was consumed by TLC, $BF_3.Et_2O$ (3.93 mmol) was added and the solution was refluxed at 110 ^{0}C for 90 min. The reaction mixture was poured into 200 mL of NaCl solution and extracted with ethyl acetate. The crude product was purified by column chromatography on silica (CH_2Cl_2 / petroleum ether = 2/3, v/v) to give 30 mg dark blue solid **LAB-TH4** in 18% yield.

LAB-TH4. m.p. 210-214 ^{o}C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 9.52 (d, J = 3.9 Hz,

1H), 8.76 (d, *J* = 3.9 Hz, 1H), 7.96 (d, *J* = 8.8 Hz, 1H), 7.87 (d, *J* = 5.0 Hz, 1H), 7.67 (dd, *J* = 10.7, 3.2 Hz, 2H), 7.55 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.44 (t, *J* = 4.5 Hz, 1H), 7.26 (t, *J* = 4.5 Hz, 1H), 4.25 – 4.10 (m, 2H), 1.78 (dt, *J* = 15.5, 7.6 Hz, 2H), 1.49 (dd, *J* = 15.0, 7.5 Hz, 2H), 1.41 (s, 9H), 1.00 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 161.54, 151.19, 148.51, 146.43, 140.62, 140.40, 138.84, 134.35, 131.63, 131.60, 129.15, 129.13, 129.10, 127.84, 126.99, 125.35, 125.30, 120.25, 118.14, 117.14, 112.07, 42.44, 35.03, 31.50, 29.75, 22.74, 20.16, 14.17, 13.69. HRMS(ESI): *m/z* [M+H] calcd. for $C_{29}H_{28}BF_2N_4OS_3$: 593.1408, found: 593.1481.

LAB-TH6 and **LAB-TH8** were synthesized by the same method as **LAB-TH4**.

LAB-TH6. Yield: 18%. m.p. 219-223 °C. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.52 (d, *J* = 3.0 Hz, 1H), 8.76 (d, *J* = 3.1 Hz, 1H), 7.97 (d, *J* = 8.5 Hz, 1H), 7.89 (d, *J* = 5.0 Hz, 1H), 7.68 (dd, *J* = 9.4, 2.9 Hz, 2H), 7.56 (dd, *J* = 8.7, 1.8 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.26 (d, *J* = 4.8 Hz, 1H), 4.22 – 4.11 (m, 2H), 1.86 – 1.73 (m, 2H), 1.41 (s, 9H), 1.36 (dd, *J* = 8.8, 5.3 Hz, 6H), 0.92 (t, *J* = 6.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.46, 161.62, 148.57, 146.42, 140.46, 138.84, 134.41, 134.37, 134.31, 131.92, 131.69, 131.61, 129.18, 129.15, 127.90, 125.35, 123.93, 120.23, 118.16, 117.15, 112.85, 42.70, 35.05, 31.67, 31.49, 31.43, 31.34, 29.65, 26.53, 22.57, 14.03. HRMS(ESI): *m/z* $[M+Na]^+$ *calcd*. for $C_{31}H_{31}BF_2N_4NaOS_3$: 643.1621, found: 643.1613.

LAB-TH8. Yield: 18%. m.p. 213-215℃. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.52 (d, *J* = 3.9 Hz, 1H), 8.76 (d, *J* = 3.1 Hz, 1H), 7.97 (d, *J* = 8.7 Hz, 1H), 7.89 (d, *J* = 5.0 Hz, 1H), 7.68 (dd, *J* = 9.7, 3.3 Hz, 2H), 7.55 (dd, *J* = 8.8, 1.8 Hz, 1H), 7.50 – 7.44 (m, 1H), 7.26 (d, *J* = 4.8 Hz, 1H), 4.22 – 4.11 (m, 2H), 1.88 – 1.69 (m, 3H), 1.41 (s, 9H), 0.96 – 0.81 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.35, 161.53, 151.17, 148.52, 146.46, 140.60, 140.36, 138.84, 134.41, 134.33, 131.63, 129.12, 127.91, 127.85, 126.98, 125.36, 125.30, 118.15, 117.14, 112.73, 112.08, 42.70, 35.03, 31.76, 31.49, 29.65, 29.21, 29.13, 26.86, 22.65, 14.13. HRMS (ESI):*m/z* $[M+H]^+$ *calcd*. for $C_{33}H_{36}BF_2N_4OS_3$: 649.2034, found: 649.2107.

Synthesis of LAB-TH-2Br

Compound **LAB-TH4** (296 mg, 0.5 mmol) was taken in a 150 mL round bottom flask in 1:1 solvent mixture of chloroform: acetic acid (v/v) (30.0 mL), and then N-

bromosuccinimide (208 mg, 1.2 mmol) was added at room temperature, which was dissolved in 5 mL of chloroform. The resulting solution was stirred at room temperature overnight in the absence of light. The mixture was extracted with 40 mL of CH_2Cl_2. After concentration under reduced pressure, the crude product was purified by chromatography on a silica gel column with a mixture of petroleum: ethyl acetate (15:1). Compound **LAB-TH-2Br** was obtained as a dark violet solid (150 mg, 40%). m.p. > 300 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.31 (d, J = 4 Hz, 1H), 8.54 (d, J = 4 Hz, 1H), 7.94 (d, J = 8 Hz, 1H), 7.65 (s, 1H), 7.55 (d, J = 4 Hz, 1H), 7.40 (d, J = 4 Hz, 1H), 7.19 (d, J = 4 Hz, 1H), 4.07 (t, J = 8 Hz, 2H), 1.78-1.70 (m, 2H), 1.50-1.44 (m, 2H), 1.39 (s, 9H), 1.01- 0.98 (t, J = 4 Hz, J = 8 Hz, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.45, 161.38, 151.20, 148.85, 144.85, 140.70, 139.53, 138.69, 134.86, 133.07, 132.38, 131.01, 130.48, 126.96, 125.53, 123.30, 120.51, 118.19, 117.22, 112.93, 112.11, 42.54, 35.08, 31.80, 31.47, 20.17, 13.70. HRMS (ESI): *m/z* $[M+H]^+$ *calcd*. for $[C_{29}H_{25}BBr_2F_2N_4OS_3+Na]^+$: 770.9512, found: 770.9516.

Synthesis of PLAB-TPE

Compound **LAB-TH4** (372 mg, 0.5 mmol) and (*E*)-1,2-diphenyl-1,2-bis(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)ethene (**TPE-2Bin**, 287 mg, 0.5 mmol) $Pd(PPh_3)_4$ (2.75 mg, 0.05 mmol) and K_2CO_3 aqueous solution were added to toluene/EtOH mixture (1/1, v/v, 10 mL). After degassing three times by freeze–pump–thaw cycles, the mixture was stirred for 12 h at 90 °C. After cooling to room temperature, the reaction mixture was poured into methanol and stirred for 0.5 h. After filtration, the product was subjected to Soxhlet extraction using methanol, acetone to afford black solids (461 mg, 50%). GPC: M_n = 7259, M_w = 12340, PDI = 1.7. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.60 (b, 1H), 8.73 (b, 1H), 7.94 (b, 1H), 7.63-7.38 (b, 7H), 7.19-7.05 (b, 17H), 4.18 (b, 2H), 1.38-0.83 (m, 16H).

2.2 Preparation of LAB-TH4 NPs

A modified co-precipitation method was used to prepare **LAB-TH4** NPs. In a typical experiment, 2 mg of **LAB-TH4** dissolved in 1 mL THF solution, and 5 mg of pluronic

F-127 was dissolved in 5 mL deionized water. Then **LAB-TH4** solution was injected into pluronic F-127 solution. Then the mixture was evaporated to remove THF completely on a rotary evaporator at 40 °C. The resulting mixture was cooled to room temperature. The obtained solution of **LAB-TH4** NPs was filtered by a 0.22 μm filter and then stored at 4 °C for further use.

3 ROS detection

2,7-Dichlorodi-hydrofluorescein diacetate (H2DCF-DA, 20 μM) is used as the ROS indicator. **LAB-TH4**, **LAB-TH6**, **LAB-TH8** and **LAB-PH6** was prepared as 80 μM in THF, which was mixed with H2DCF-DA solution. Then the cuvette was exposed to white light (300-700 nm) for different amounts of time, and the fluorescence spectra were observed immediately after each irradiation. In the control group, only H2DCF-DA solution was subjected to irradiation. The fluorescence was excited at 488 nm.

4.1 Cell culture

Cells were cultured in high-glucose Dulbecco's Modified Eagle's Medium (H-DMEM) containing 10% fetal bovine serum (FBS) and 1% penicillin streptomycin at 37 °C in a humidified environment containing 5% CO_2. Before the experiment, the cells were pre-cultured until confluence was reached.

4.2 Cell imaging

HeLa cells were seeded in the 12-well plate and cultured in H-DMEM with 10%FBS at 37 °C in a humidified environment containing 5% CO_2. After an 80% confluence, the medium was removed and the adhered cells were rinsed twice with 1 × PBS. Cells were incubated with different concentrations of **LAB-TH4** NPs for different times. After washing, the culture dishes three times with PBS, fluorescence imaging experiments were carried out on a confocal laser scanning microscope (CLSM, ZEISS-LSM880).

4.3 Detection of Intracellular ROS

HeLa cells were incubated with **LAB-TH4** NPs (10 μM) for 24 h. Then, cells were washed with 1 × PBS for three times, and fresh medium containing 10 μM of DCFH-DA (Sigma-Aldrich) was added. After further incubation for 30 min, the cells were

washed three times with 1 × PBS and then irradiated by white light (35 mW/cm^2, 5 min). Finally, the fluorescence images of the cells were taken by a confocal laser scanning microscopy (CLSM). The fluorescence of DCF was excited at 488 nm and collected within 500-560 nm.

4.4 Cytotoxicity assay by CCK-8

HeLa cells were grown in 96-well plates with a confluence of about 5.0 × 10^3 cells/well for 24 h incubation. Subsequently, the medium was replaced with the fresh medium containing different concentrations of **LAB-TH4** NPs. After further incubation for 24 h, the medium was removed and washed with PBS for three times. Cells were then incubated with fresh serum-free medium containing 10% CCK-8 for 2 h in the dark. Finally, the absorbance of the products was measured at a wavelength of 450 nm by a microplate reader.

The negative control referred to the same procedure was carried out but no **LAB-TH4** NPs was used. The positive controls meant that **LAB-TH4** NPs was replaced by equal concentration of DMSO.

4.5 Cytotoxicity of photosensitizers to different cells under light irradiation.

The cells were seeded in 96-well plates at a density of 5000 cells per well. After 24 h at 37 °C, the medium was replaced with fresh medium (100 μL) containing different concentrations of **LAB-TH4** NPs. After incubation for 24 h, the cells were exposed to white light (35 mW/cm^2) for 5 min, and another array of plates with the cells containing different concentrations of **LAB-TH4** NPs were kept in the dark as a control. After that, the plates were subjected to the same treatment as the cytotoxicity study.

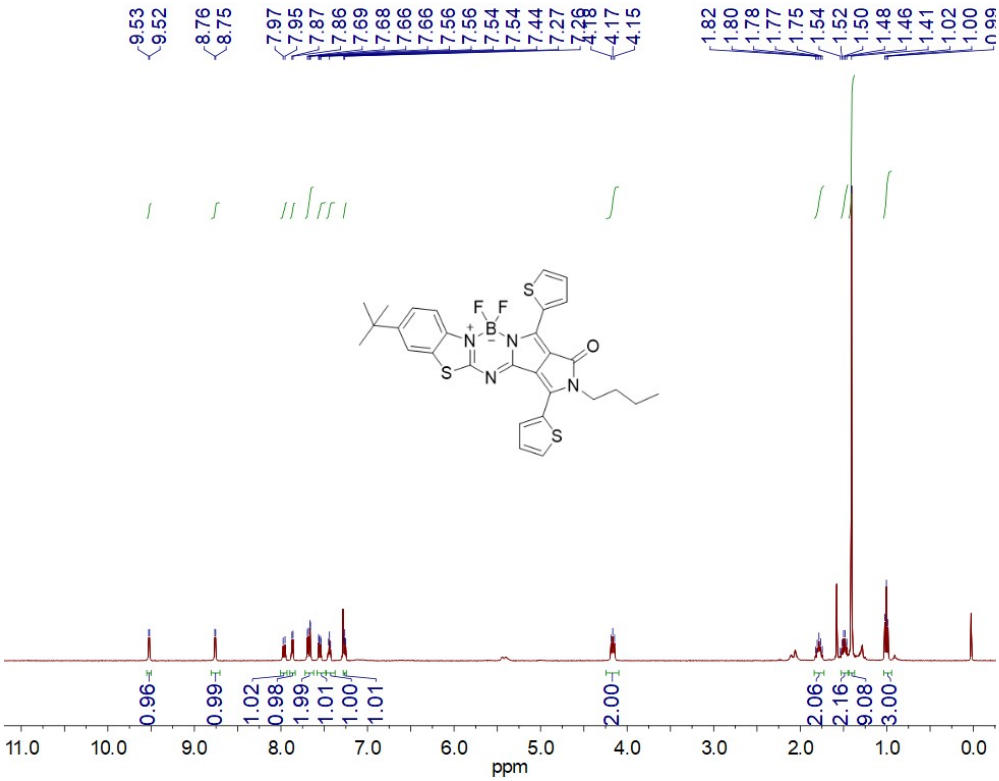


Figure S1 ^{1}H NMR spectrum of **LAB-TH4** in $CDCl_3$.

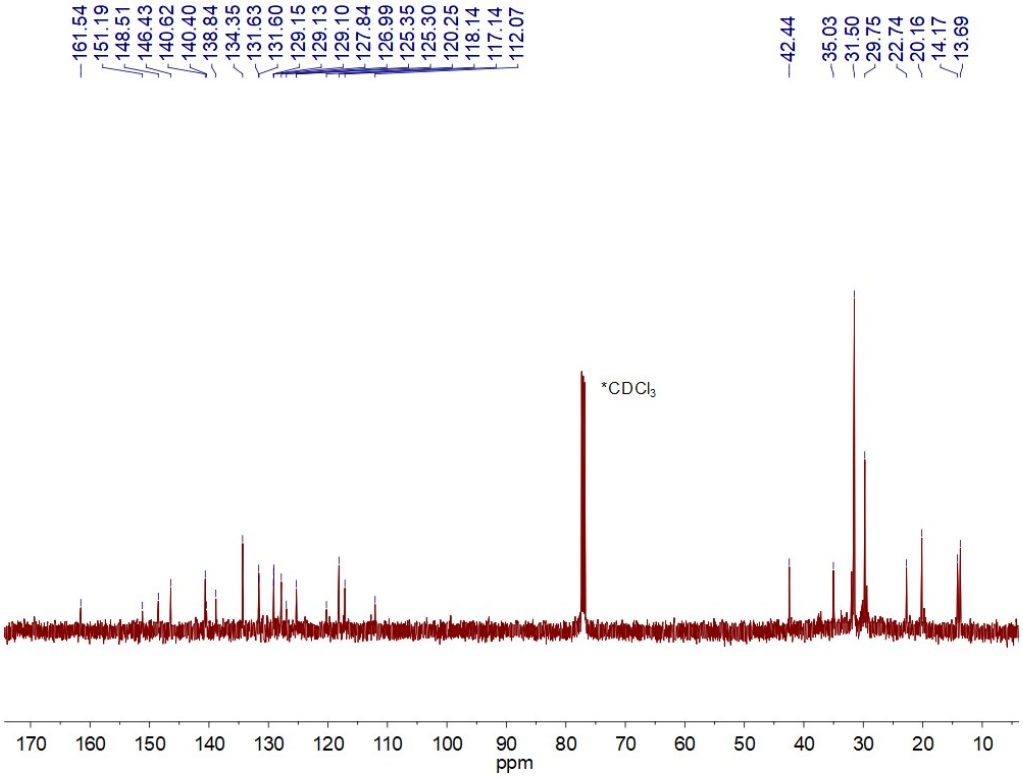


Figure S2 ^{13}C NMR spectrum of **LAB-TH4** in $CDCl_3$.

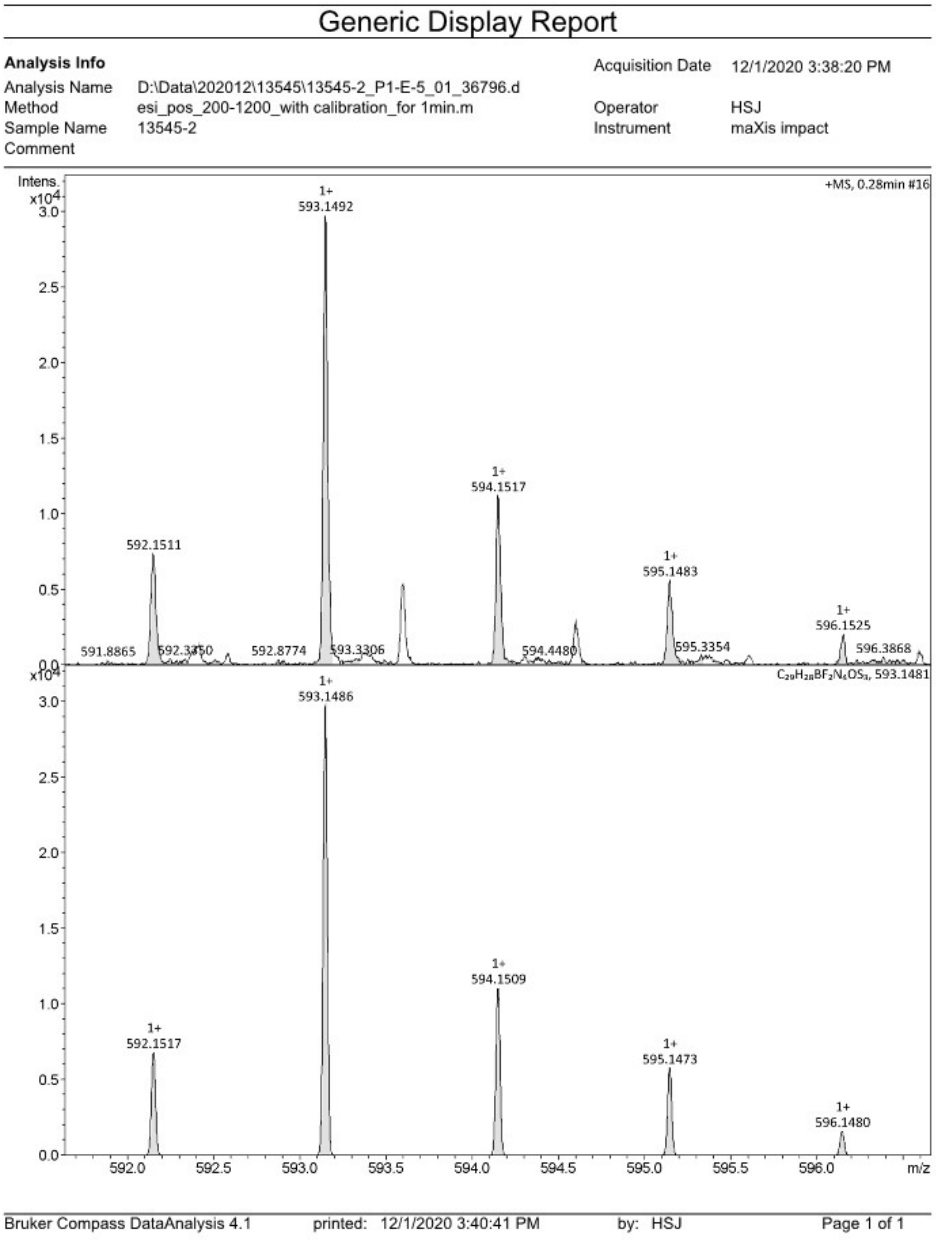


Figure S3 HRMS spectrum of **LAB-TH4**.

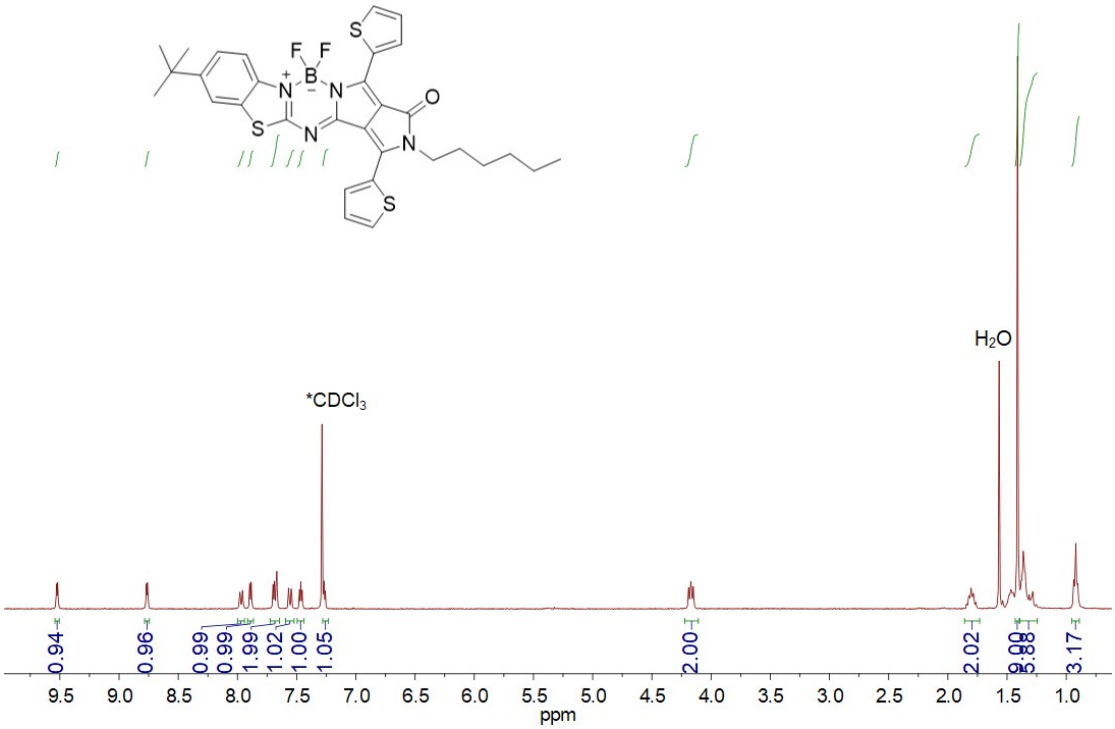


Figure S4 ^{1}H NMR spectrum of **LAB-TH6** in $CDCl_3$.

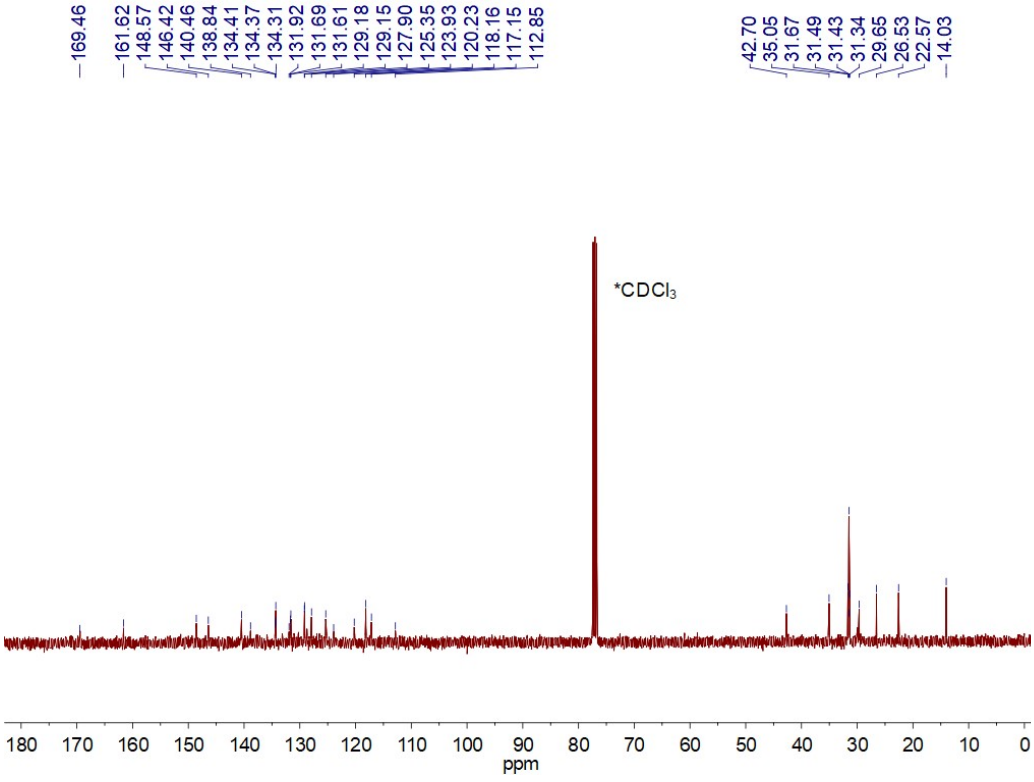


Figure S5 ^{13}C NMR spectrum of **LAB-TH6** in $CDCl_3$.

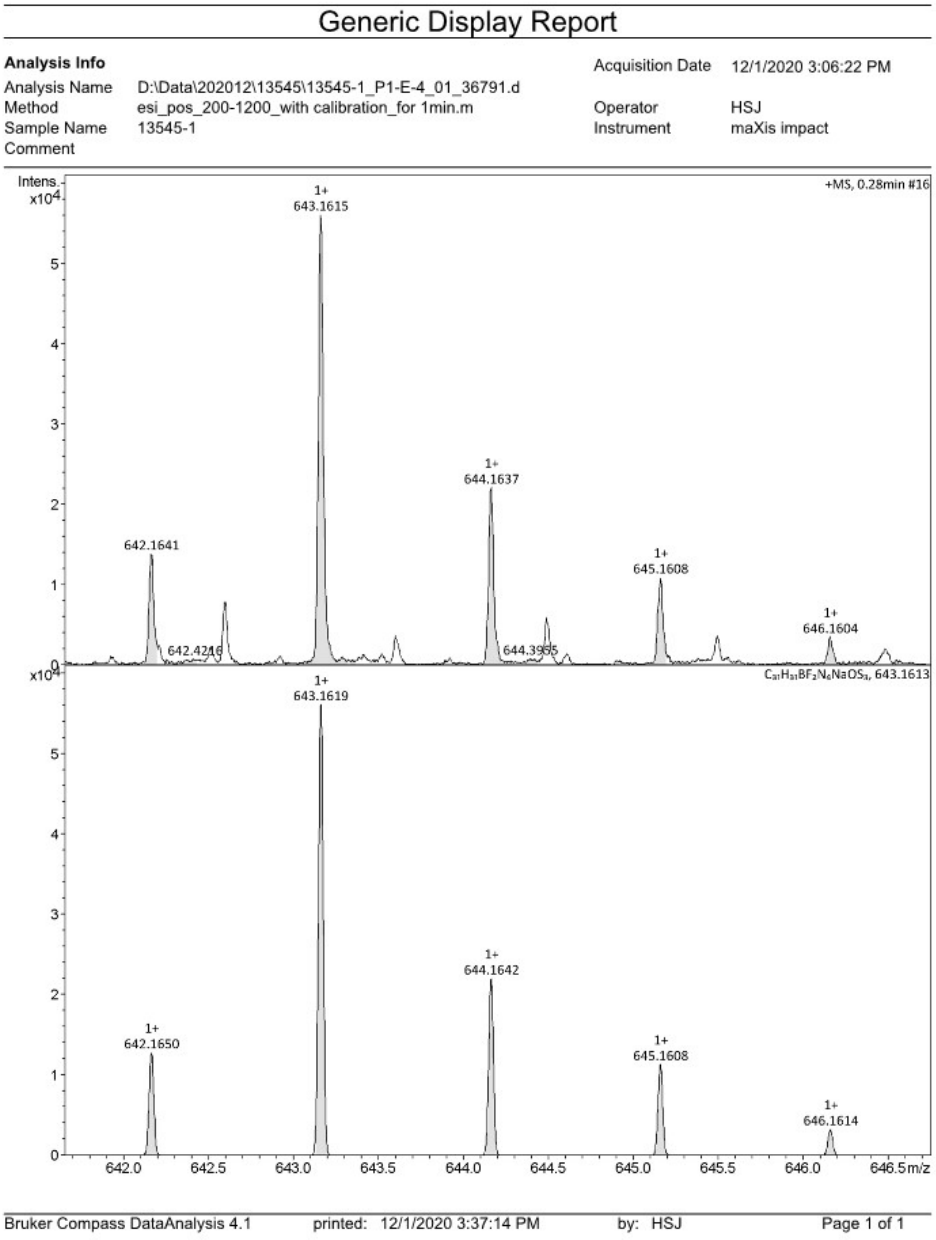


Figure S6 HRMS spectrum of **LAB-TH6**.

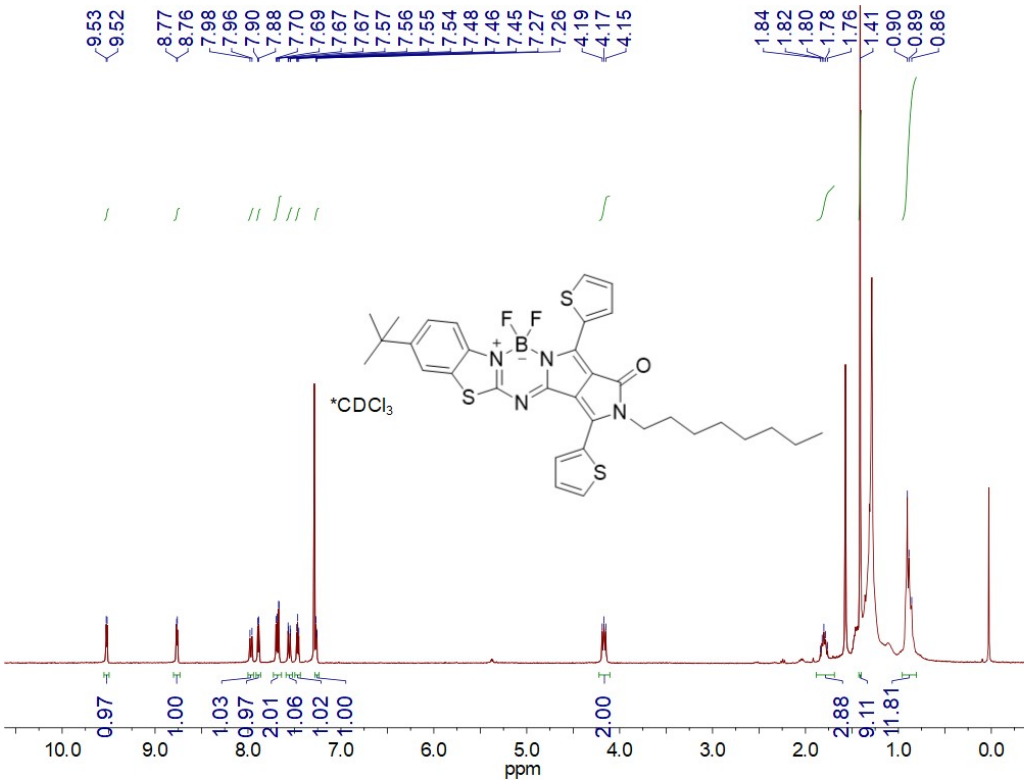


Figure S7 ^{1}H NMR spectrum of **LAB-TH8** in $CDCl_3$.

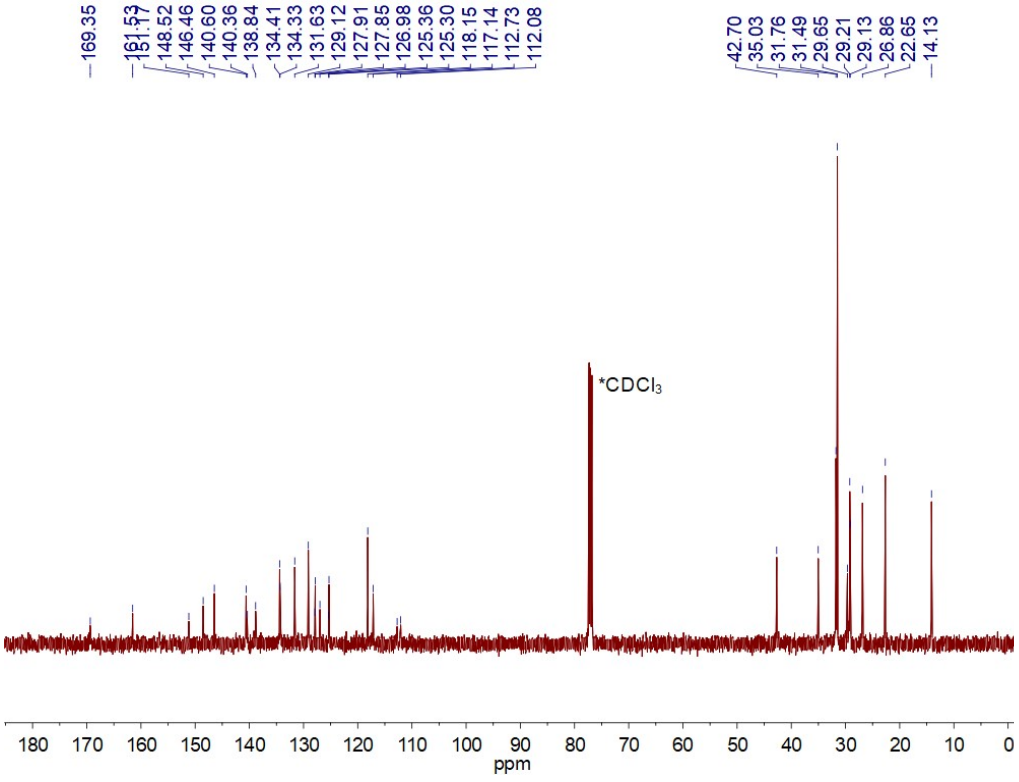


Figure S8 ^{13}C NMR spectrum of **LAB-TH8** in $CDCl_3$.

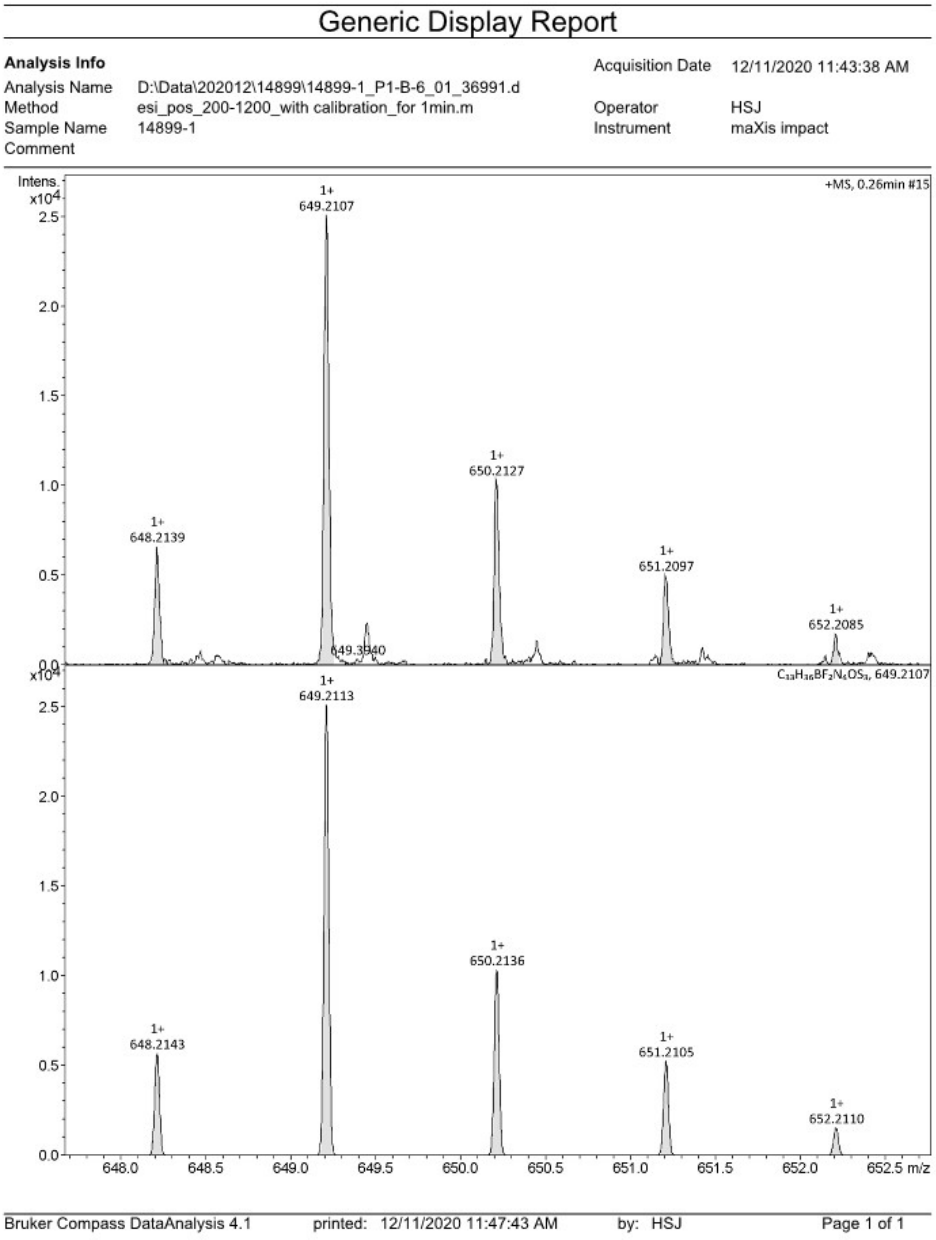


Figure S9 HRMS spectrum of **LAB-TH8**.

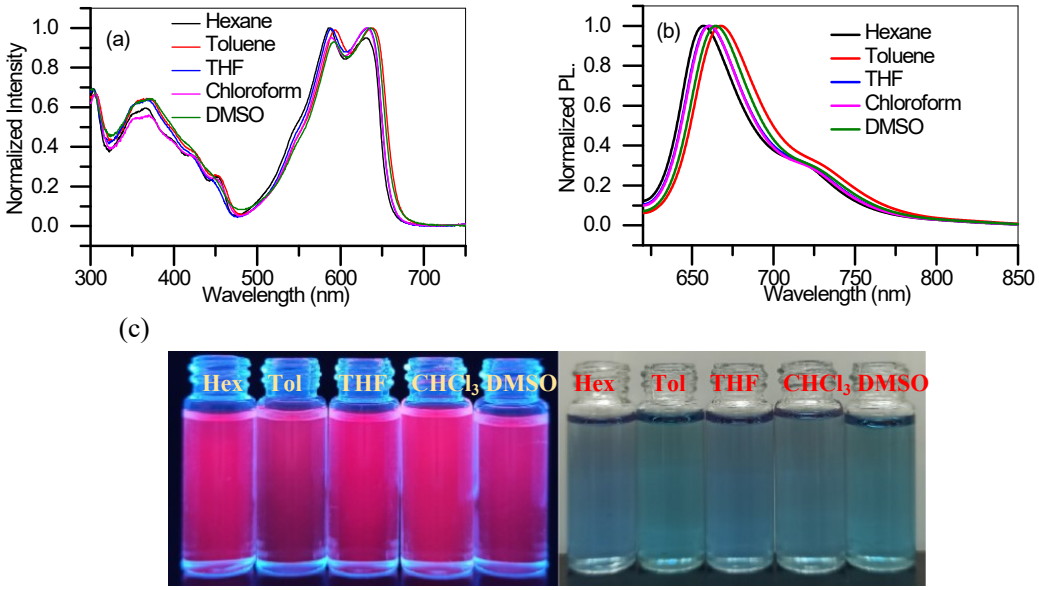


Figure S10 The normalized (a) UV-vis and (b) emission spectra of **LAB-TH4** (10 μM) in different solvents. (c) The photos of **LAB-TH4** (10 μM) in different solvents under daylight and 365 nm irradiation (hexane: Hex; toluene: Tol).

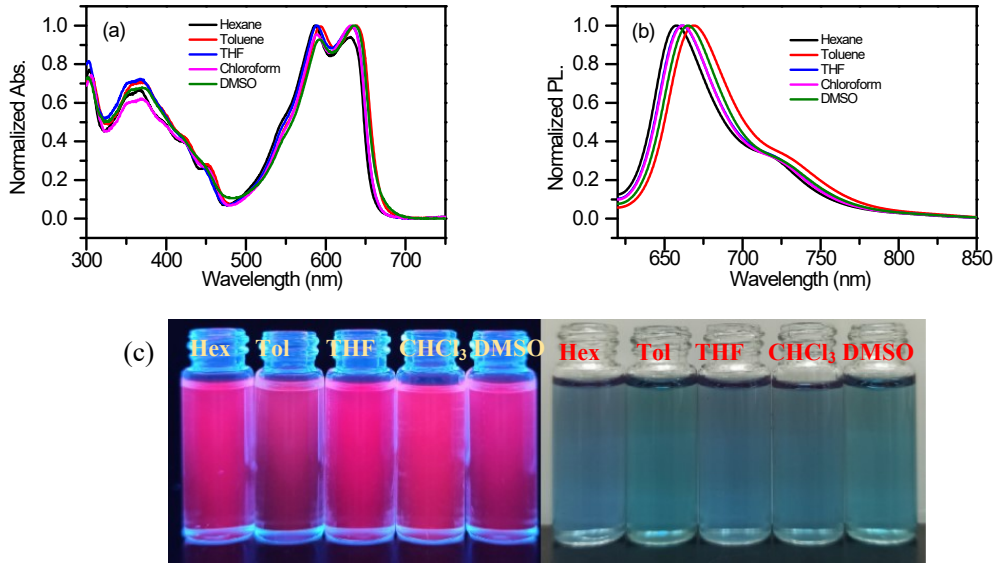


Figure S11 The normalized (a) UV-vis and (b) spectra of **LAB-TH6** (10 μM) in different solvents. (c) The photos of **LAB-TH6** (10 μM) in different solvents under daylight and 365 nm irradiation (hexane: Hex; toluene: Tol).

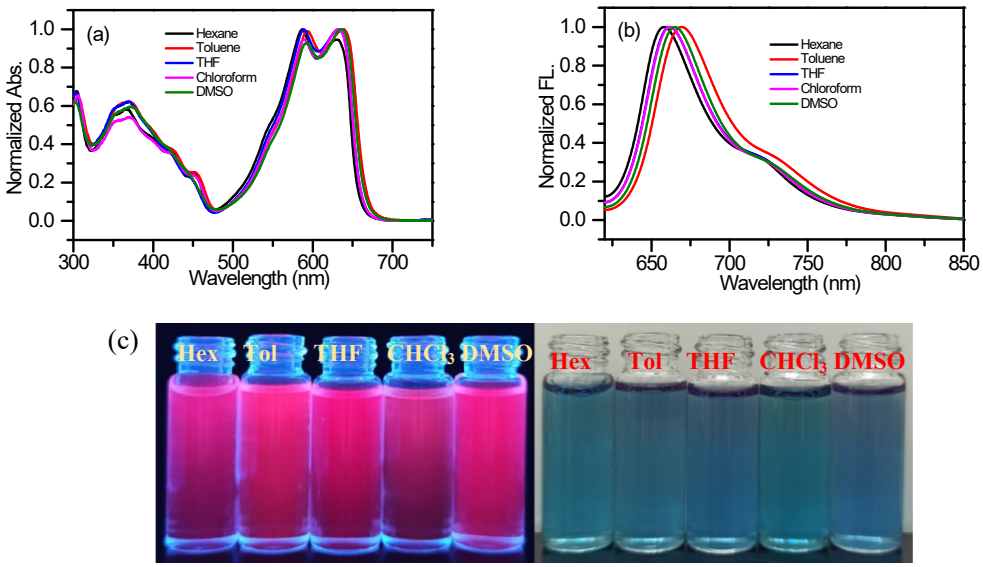


Figure S12 The normalized (a) UV-vis and (b) spectra of **LAB-TH8** (10 μM) in different solvents. (c) The photos of **LAB-TH8** (10 μM) in different solvents under daylight and 365 nm irradiation (hexane: Hex; toluene: Tol).

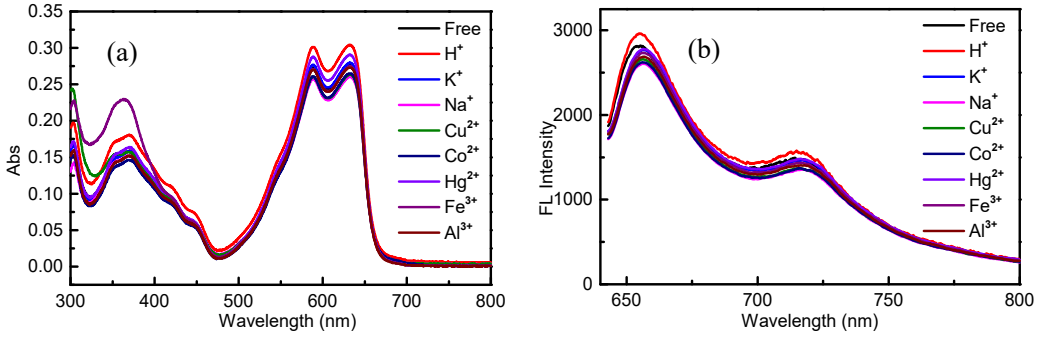


Figure S13 The (a) UV-vis and (b) emission spectra of **LAB-TH4** (10 μM) in THF in presence of different metal ions and HCl (20 μM).

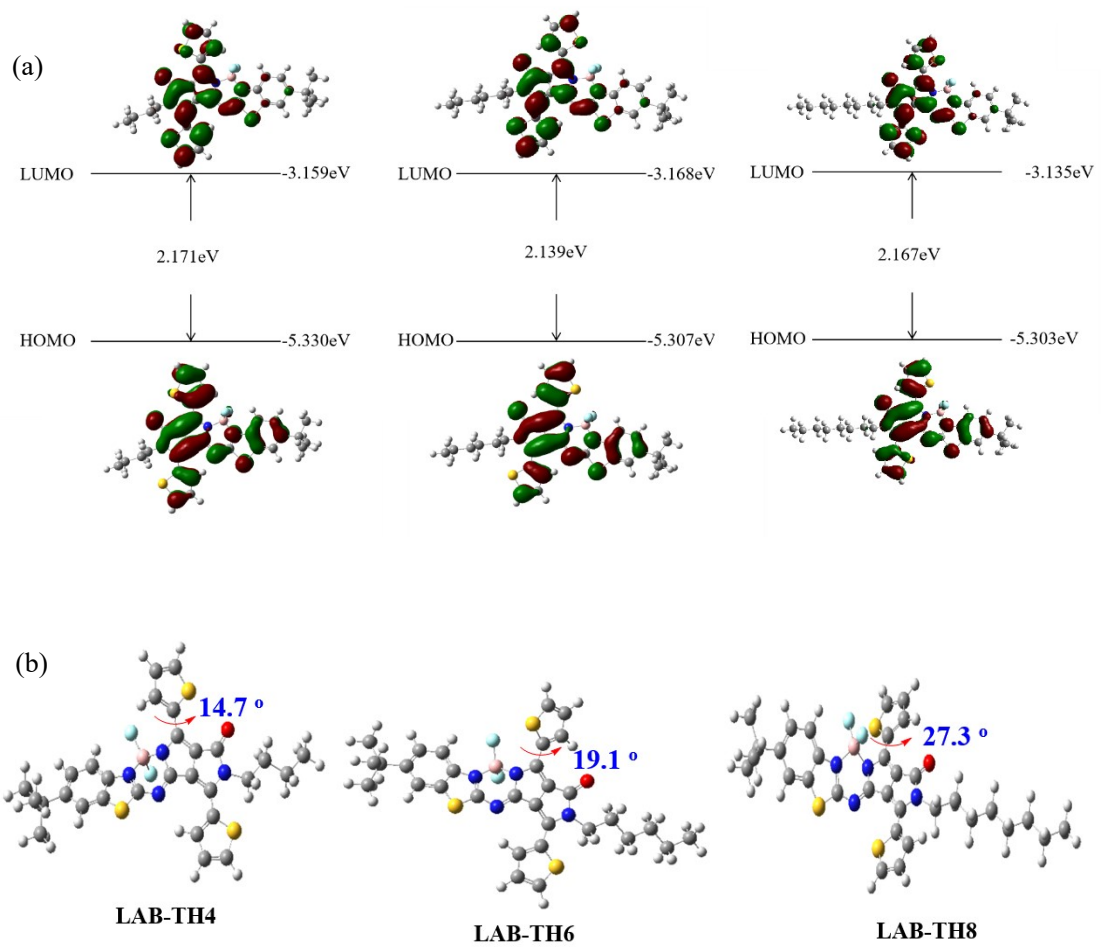


Figure S14 (a) The DFT diagram and (b) optimal geometry of **LAB-TH4** ~ **LAB-TH6**.

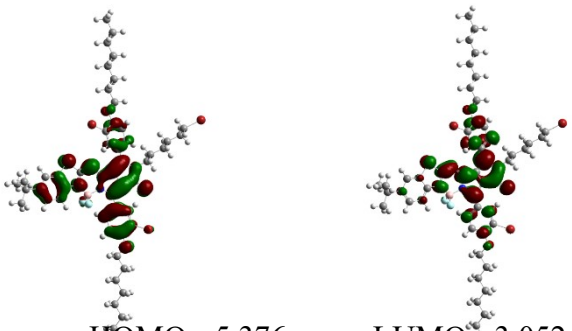


Figure S15 The DFT data of **LAB-PH6**.

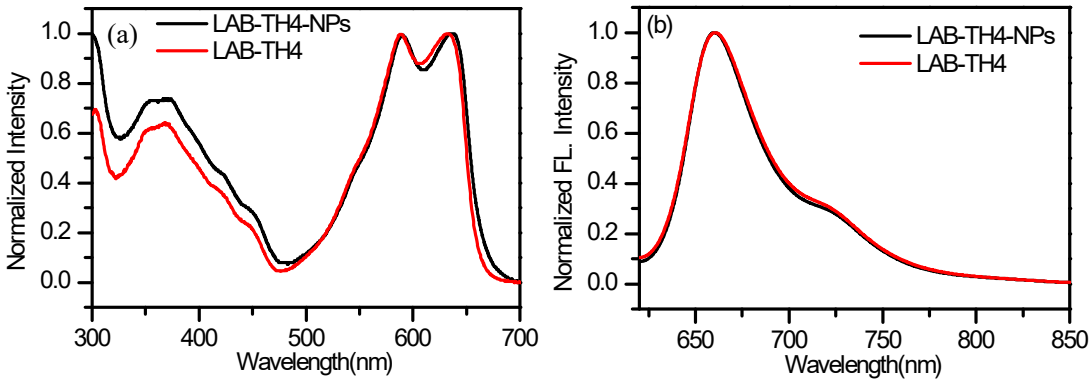


Figure S16 (a) The UV-vis and (b) emission spectra of **LAB-TH4** and **LAB-TH4** NPs.

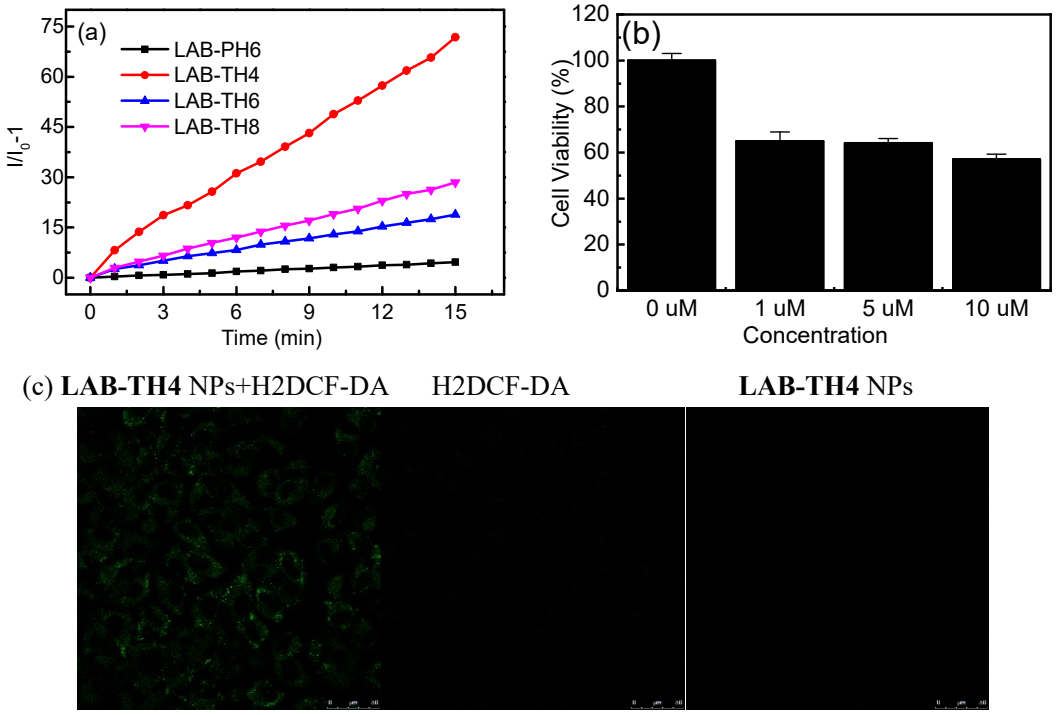


Figure S17 (a) ROS generation upon light irradiation of **LAB-TH4, LAB-TH6** and **LAB-TH8** (1 μM). Relative change in fluorescent intensity (I/I_0-1) at 525 nm of H2DCF-DA (10 μM) upon white light irradiation for different time. (b) The cells viability after incubation with different concentrations of **LAB-TH4** NPs (1, 5 and 10 μM) in the presence of white light irradiation for 5 min. (c) Detection of intracellular ROS production by H2DCF-DA in HeLa cells after incubation with **LAB-TH4** NPs and control groups under white light irradiation for 5 min.

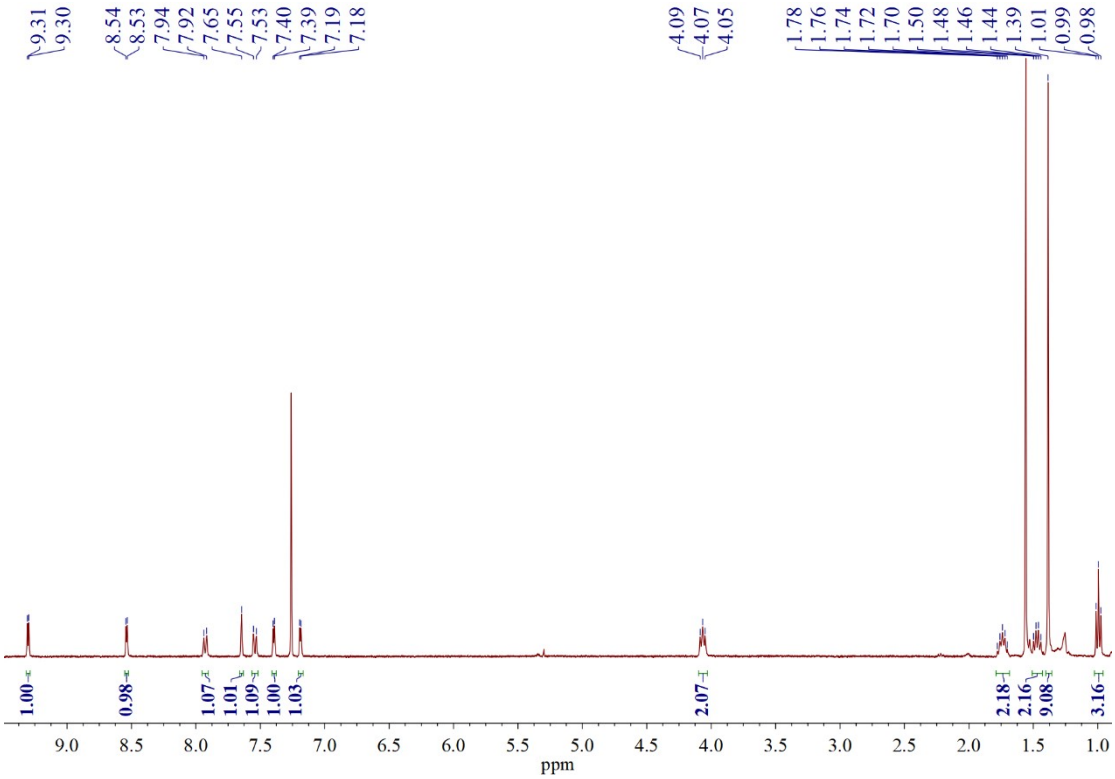


Figure S18 The ^{1}H NMR spectrum of **LAB-TH4-2Br**.

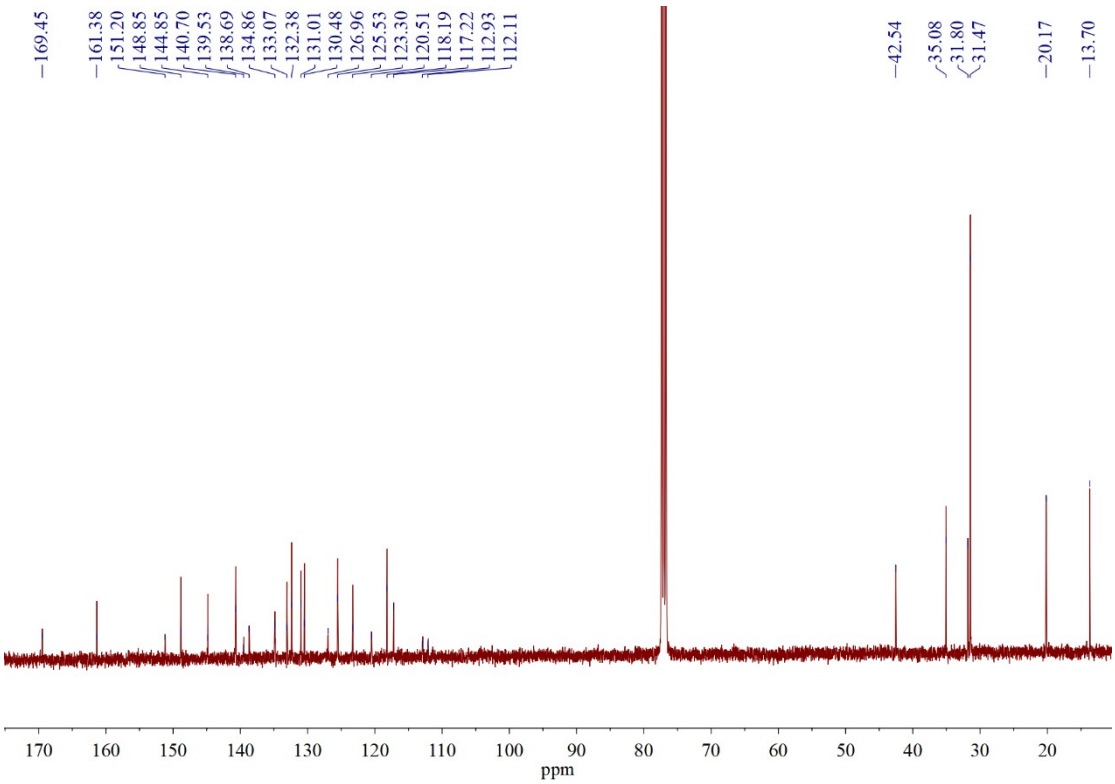


Figure S19 The ^{13}C NMR spectrum of **LAB-TH4-2Br**.

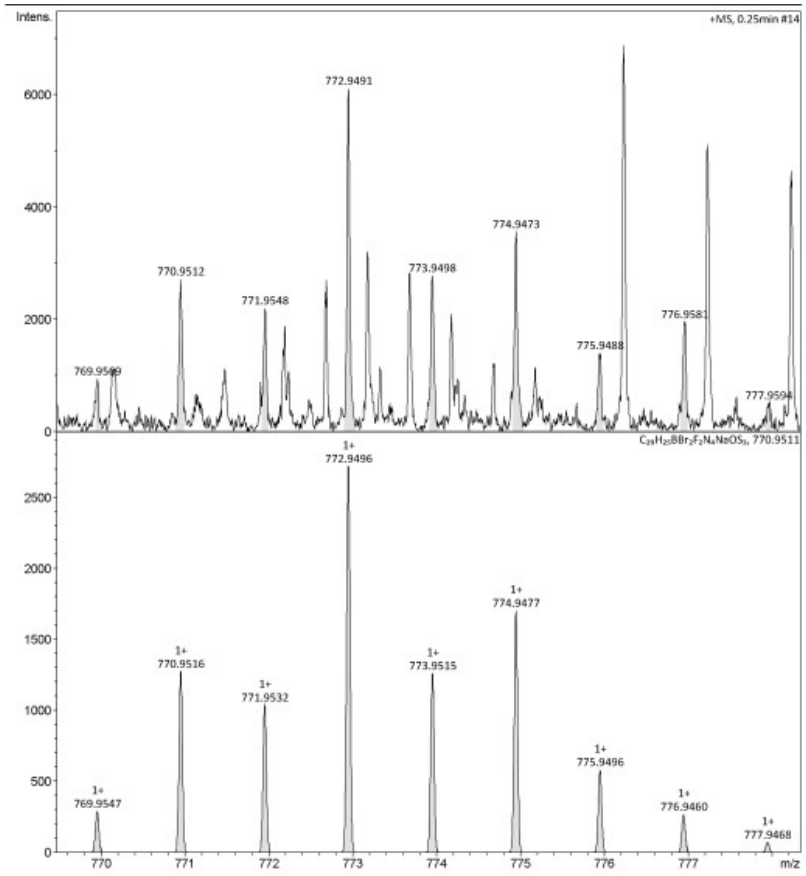


Figure S20 The HRMS spectrum of **LAB-TH4-2Br**.

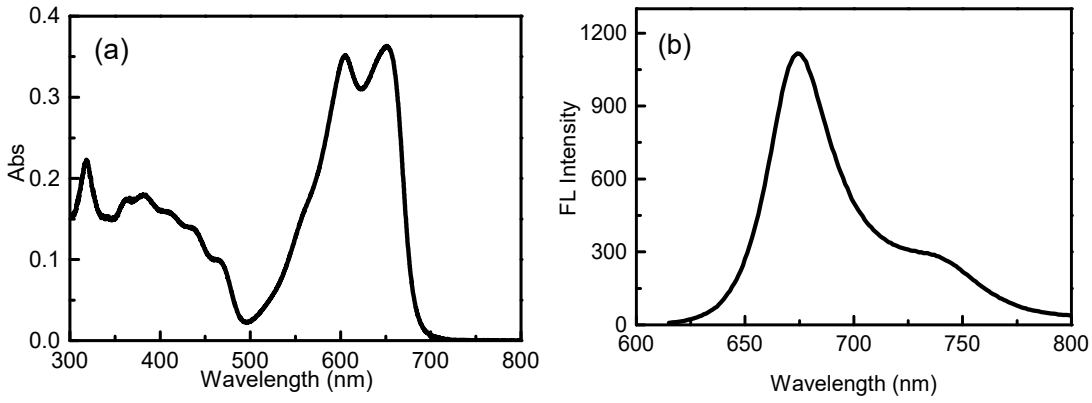


Figure S21 The (a) UV-vis and (b) emission spectra of **LAB-TH4-2Br** in THF.

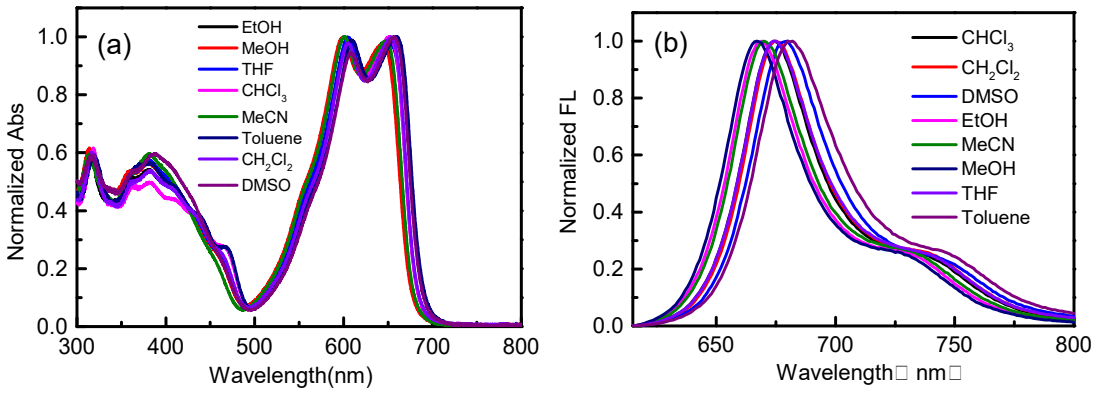


Figure S22 The normalized UV-vis and emission spectra of **LAB-TH4-2Br**.

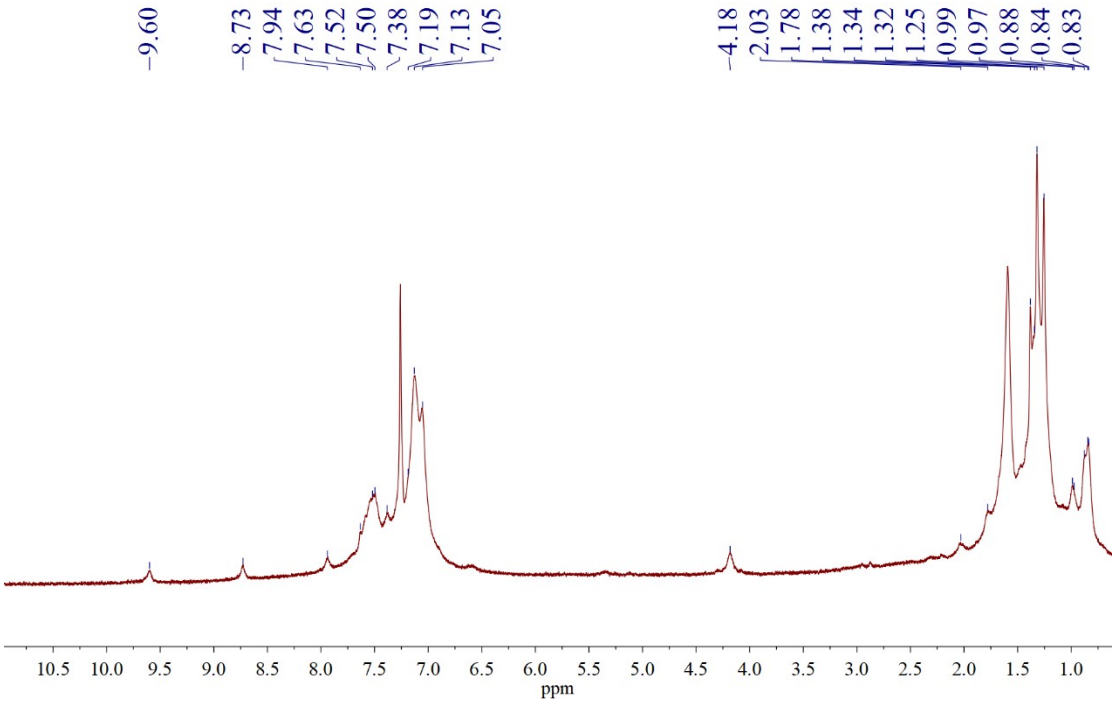


Figure S23 The ^{1}H NMR spectrum of **PLAB-TPE**.

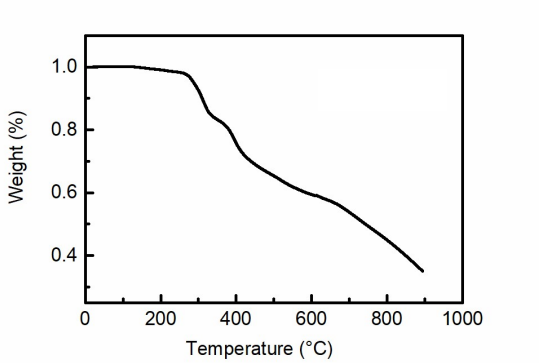


Figure S24 The TG curve of **PLAB-TPE**.

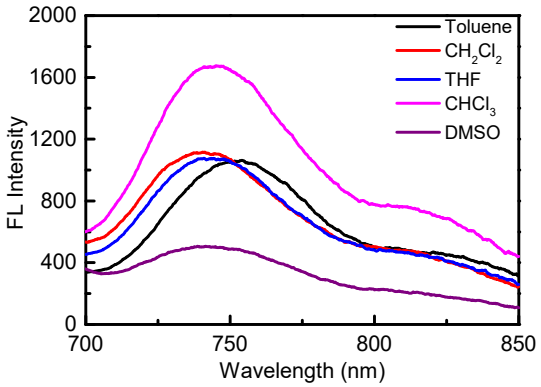


Figure S25 The emission spectra of **PLAB-TPE** in different solvents.

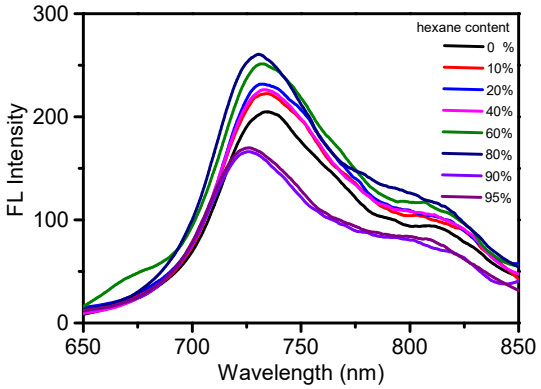


Figure S26 The emission spectra of **PLAB-TPE** in THF-n-hexane mixture.

Table S1 The photophysical properties of **LAB-TH4**, **LAB-TH6**, **LAB-TH8** (10 μM) in different solvents.

Compound	Solvent	$\lambda_{max,\ abs}$ (nm)	$\lambda_{max,\ em}$ (nm)	Stokes shift (nm)	ε ($M^{-1}.cm^{-1}$)
LAB-TH4	Hexane	638	656	18	$1.96*10^4$
	Toluene	639	667	28	$2.06*10^4$
	THF	633	660	27	$2.14*10^4$
	Chloroform	633	660	27	$1.94*10^4$
	DMSO	637	664	27	$2.12*10^4$
LAB-TH6	Hexane	637	657	20	$2.40*10^4$
	Toluene	638	669	31	$2.53*10^4$
	THF	633	661	28	$2.48*10^4$
	Chloroform	633	661	28	$2.39*10^4$
	DMSO	637	665	28	$2.67*10^4$
LAB-TH8	Hexane	636	658	22	$2.95*10^4$
	Toluene	638	669	31	$2.99*10^4$
	THF	634	661	27	$2.92*10^4$
	Chloroform	633	662	29	$2.94*10^4$
	DMSO	637	665	28	$3.23*10^4$

Table S2 The photophysical properties of **LAB-TH4-2Br** (5 μM) in different solvents.

Solvent	$\lambda_{max\ ab}$ (nm)	λ_{em}(nm)	Stokes shift (nm)	ε ($M^{-1}.cm^{-1}$)
Toluene	658	681	23	$6.76*10^4$
DCM	654	675	21	$7.78*10^4$
THF	652	674	22	$7.06*10^4$
$CHCl_3$	652	674	22	$7.26*10^4$
EtOH	647	669	22	$8.02*10^4$
MeOH	644	667	23	$6.94*10^4$
MeCN	648	670	22	$7.20*10^4$
DMSO	656	679	23	$6.66*10^4$

Table S3 The photophysical properties of **PLAB-TPE** (5 μM) in different solvents.

Solvent	$\lambda_{max\ ab}$ (nm)	λ_{em}(nm)	Stokes shift (nm)	ε ($M^{-1}.cm^{-1}$)
Toluene	655	753	98	$3.12*10^4$
DCM	650	740	90	$3.42*10^4$
THF	651	743	92	$3.38*10^4$
$CHCl_3$	653	745	92	$3.38*10^4$
DMSO	657	743	86	$3.20*10^4$

[S1] (a) Xiaoxu Xie, Jian Wang, Yongchao Yan, Xiao Zhang, Chenchen Liu, Ji Yang and Jianli Hua, A new mitochondria-targeted ratiometric fluorescent probe based on diketopyrrolopyrrole for imaging endogenous HOCl in living cells, Analyst, 2018, 143, 5736–5743. (b) Ye Liu, Zhuo Qu, Hongyan Cao, Hongyan Sun, Yuan Gao, Xingyu Jiang, pH Switchable Nanoassembly for Imaging a Broad Range of Malignant Tumors, ACS Nano 2017, 11, 12446−12452.

[S2] Lingyun Wang, Hui Ding, Hao Tang, Derong Cao, Xueguang Ran, A novel and efficient chromophore reaction based on a lactam-fused *aza*-BODIPY for polyamine detection, Analytica Chimica Acta, 2020, 1135, 38-46