Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Synthesis, crystal structures, one/two-photon optical properties and application for bioimaging of two organic molecules with D-A and D-π-A models containing 6-phenyl-2,2'-bipyridine

*Zhaodi Liu,[a,b] Fuying Hao,[a] Jie Liu,[a] Yingzhong Zhu,[b] Wei Du,[b] Qiong Zhang,[b] Jieying Wu *[b]*

[a] *Department of chemistry and materials engineering, Fuyang Normal College, Fuyang, 230039, P. R. China.*

[b] *Department of Chemistry, Anhui University and Key Laboratory of Functional Inorganic Materials of Chemistry of Anhui Province, Hefei 230039, P. R. China.*

E-mail: *jywu1957@163.com*

Content

Experimental Section

1 General procedure

^{1}HNMR spectra were performed on a Bruker 400 Hz Ultrashield spectrometer and were reported as parts per million (ppm) from TMS (δ) and ^{13}C NMR spectra were obtained on a Bruker Avance 100 MHz NMR spectrometer. IR spectra were recorded on NEXUS 870 (Nicolet) spectrophotometer in the 400-4000 cm^{-1} region using a powder sample on a KBr plate. Mass spectrometry analyses were performed by a Bruker Biflex III matrix assisted laser desorption/ionization time of flight (MALDI-TOF and ESI-source) mass spectrometer.

2 Optical measurements and structure solution

All the solvents used for absorption and fluorescence measurements were HPLC grade. For dilute solutions of $c = 5.0\times10^{-6}$ M, in quartz cuvettes of 1 cm path length, one-photon absorption (OPA) spectra were recorded on a UV-265 spectrometer. One-photon excited fluorescence (OPEF) spectra were recorded on a PerkinElmer LS55 fluorescence spectrometer. The fluorescence quantum yields and lifetime fluorescence were performed using Horiba Fluoro Max-4P fluorescence spectrophotometer. The two-photon fluorescence spectra of all compounds were measured in DMF with $c = 5.0\times10^{-4}$ mol/L using femtosecond laser pulse and Ti: sapphire system (680-900 nm, 80 MHz, 140 fs, Chameleon II) as the light source. All measurements were carried out in air at room temperature. Thus, the two-photon absorption cross sections (δ) of samples were obtained according to literature methods reported[8].

Single crystals were determined by single crystal X-ray diffraction analyses. Data collections were performed using a Siemens SMART CCD area detector diffractometer with Mo/Ka radiation with an ω-scan mode (λ = 0.71073 Å). The structures were solved with direct methods using the SHELXTL program and refined anisotropically with SHELXTL using full-matrix least squares procedure. In compound **L1**, the terminated-propyl and phenyl moieties are disordered over two positions. During refinement, the sum of the occupancy factors for the two orientations was kept fixed at 1.0, and the occupancy factors for the primed orientations refined to 0.494 (1) and 0.497 (7), respectively. All non-hydrogen atoms were refined anisotropically. Additional crystallographic details and complete listings of the compounds have been deposited with the Cambridge Crystallographic Data Center as supplementary publications with reference number CCDC 951012 for **L1**, 951011 for **L2**, (Copies of the data can be obtained free of charge CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44-1223-336033; e-mail: deposit@ccdc.cam.ac.uk).

3 Theoretical calculations

The orbital energy of the compounds was calculated by using the Gaussian 03 program at the B3LYP Time-Dependent Density Functional Theory (TD-DFT). The 6-31G* was used to optimize their ground-state geometries. The TDB3LYP/6-31G* level of theory has been used to compute the absorption spectra in gas phase. All the calculations were performed with Gaussian03 package[9].

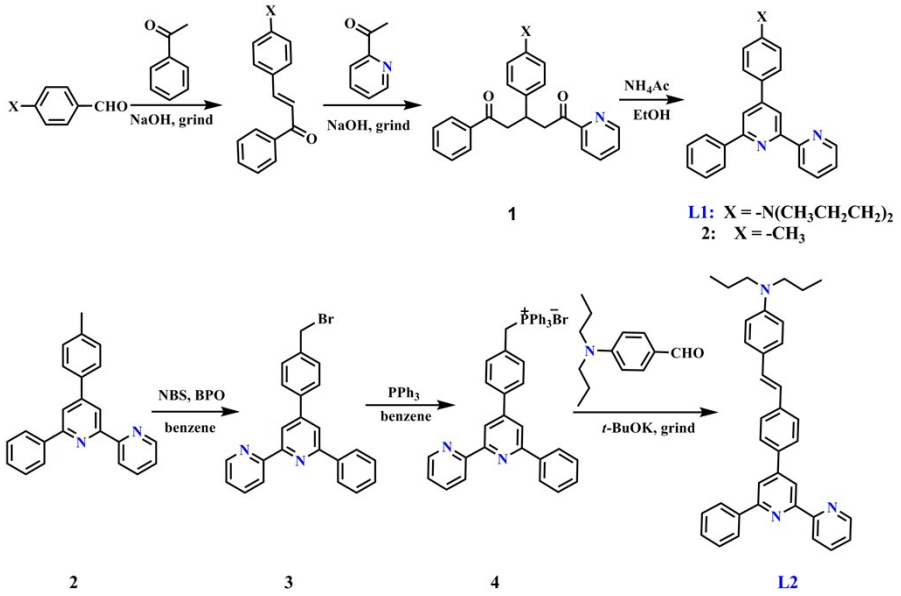
GAUSSIAN package to optimize the molecule geometries in gas phase was calculated at the HF/6-31G* level and then the calculation 2PA is performed by the response theory method at the hybrid density functional theory (DFT/B3LYP) level with 6-31G* basis set using the DALTON 2011 program.

4 Cytotoxicity and cell image

Cytotoxicity tests on HepG2 cell line of the 6-phenyl-2,2'-bipyridine derivatives were studied by the MTT (3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyltetrazolium bromide) assay. The culture cancer cells and the cytotoxicity test/calculation were similar to the literature procedures except that the compound stock solutions (1 mM in DMSO) were added to obtain the final concentrations of 10, 20, 40, 60 and 80 μM[10].

The cells imaging were carried out by using confocal laser scanning microscopy (Zeiss LSM 710 META) and water immersion lenses. Excitation energy of 720 nm for L1 (830 nm for L2) was used and the fluorescence emission measured at 495-582 nm.

5 Synthesis



Scheme S1. Synthetic route for compound **L1**, **L2**.

***4'-[4-(N,N-dipropylamino)phenyl]-6-phenyl-2,2'-bipyridine* (L1)**

4-(N,N-dipropylamino)benzaldehyde (5.60 g, 30 mmol) and
acetophenone (3.60 g, 30 mmol) were mixed together as liquids with a pestle and mortar in the presence of NaOH for *ca*. 30 min to form the α, β-unsaturated ketone, which was then ground with 2-acetylpyridine (3.63 g, 60 mmol) to give the required 1,5-dicarbonyl compound (**1**). Then **1** was consequently treated with ammonium acetate (13.86 g, 180 mmol) in glacial acetic acid (50 mL) and was refluxed with stirring for 10 h. On cooling, the solution was poured into water (200 mL) and extracted with dichloromethane three times. The organic extract was dried over $MgSO_4$. Chromatography on silica gel with dichloromethane /methanol (95:5) gave a yellow solid. Yield: 6.9 g, 56.5 %. ^{1}H NMR (400 MHz, d_6-DMSO): δ (ppm) 8.75 (d, J = 4.4 Hz, 1H), 8.61 (d, J = 8 Hz, 1H), 8.56 (s, 1H), 8.33 (d, J = 7.6 Hz, 2H)), 8.18 (s, 1H), 7.99-8.03 (m, 1H), 7.82 (d, J = 8.8 Hz, 2H), 7.47-7.58 (m, 4H), 6.79 (d, J = 8.8 Hz, 2H), 3.32 (t, J = 8.8 Hz, 4H), 1.54-1.63 (m, 4H), 0.92 (t, J = 7.6 Hz, 6H).^{13}C NMR (100 MHz, d_6-DMSO）: δ (ppm) 156.14, 155.57, 155.36, 149.34, 149.19, 148.78, 138.89, 137.27, 129.06, 128.67, 127.86, 126.84, 124.16, 122.99, 120.75, 116.21, 114.73, 111.61, 51.79, 20.01, 11.16. FT-IR (KBr, cm^{-1}): 3473 (m), 3411 (s), 2956 (m), 2871(w), 1600 (s), 1581 (s),1523 (s), 1504 (w), 1450 (w), 1367 (s), 1234 (m), 1203 (m), 1157 (m), 795 (w), 735 (w), 696 (w). MALDI-TOF MS (*Cal*. 407.23, *Found*: M+1 = 408.23). Melting point: 108.7-109.2 °C. Anal. calcd for $C_{28}H_{29}N_3$: C, 82.52; H,7.17, N, 10.31. Found: C, 82.55; H, 7.18, N, 10.30.

***4'-[(4-bromomethyl)phenyl]-6-phenyl-2,2'-bipyridine* (3)**

4-(*p*-Tolyl)-6-phenyl-2,2'-bipyridine (**2**) (3.2 g, 10 mmol), NBS (2.2 g,12 mmol) and a catalytic amount of 0.15 g benzoyl peroxide (BPO) were mixed in tetrachloromethane (200 mL) and

refluxed for 12 h. After the reaction was completed, the solvent was removed under reduced pressure. A precipitate was produced with addition of ethanol, a white solid was prepared after filtered, washed with ethanol and drying. Yield: 2.5 g, 62.5 %. FTIR (KBr, cm^{-1}): 3035, 2969, 2889, 1584, 1540, 1471, 1376, 835, 775, 702.^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.76 (d, J = 3.94 Hz, 1H), 8. 63 (t, 2H), 8.36 (dd, J = 5.25, 3.27 Hz, 2H), 8.30 (t, 1H), 8.06~8.01 (m, 1H), 7.99 (d, J = 8.29 Hz, 2H), 7. 66 (d, J = 8.31 Hz, 2H), 7.60~7.56(m, 2H), 7.54~7.51 (m, 2H), 4.81 (s, 2H).

4-(6-phenyl-2,2'-bipyridine-4')-benzyl triphenyl phosphonium bromide **(4)**

Compound **3** (2.0 g, 5 mmol) and trimethyl phosphate (1.3 g, 5 mmol) were mixed in dried benzene (50 mL). The mixture was refluxed for 10 h and filtered at a high temperature. The liquid phase was cooled to room temperature. A khaki solid precipitate was filtered and air-dried. Yield: 2.4 g, 73.3 %. The product obtained was used in the next step without characterization.

4'-{4-[4-(N,N-dipropylamino)styryl]phenyl}6-phenyl-2,2'-bipyridine (**L2**)

Compound **4** (1.96 g, 3 mmol), 4-(1H-imidazol-1-yl) benzaldehyde (0.8 g, 3.9 mmol) and *t*-BuOK (0.8 g, 7 mmol) were placed in a dry mortar. The mixture milled vigorously for about 20 min. It became sticky, and then *t*-BuOK (0.4 g, 3.5 mmol) added. It was continuously milled for 10 min and monitored by Thin Layer Chromatography (TLC). After completion of the reaction, the mixture was poured into distilled water (100 mL). The product was extracted with dichloromethane three times. The organic extracts were dried over $MgSO_4$. Chromatography on silica gel with dichloromethane/methanol (10:1) gave a yellow solid. Yield: 0.98 g, 65 %. ^{1}H NMR(400 MHz, d_6-CD_3COCD_3)δ: 8.72-8.77 (m, 3H), 8.38 (d, J = 7.6 Hz, 2H), 8.28 (s, 1H), 7.95-8.02 (m, 3H), 7.73 (d, J = 8 Hz, 2H), 7.57 (d, J = 7.6 Hz, 2H), 7.45-7.51 (m, 4H), 7.27 (d, J = 16 Hz, 1H), 7.05 (d, J = 16 Hz, 1H), 6.71 (d, J = 8.4 Hz, 2H), 3.34 (d, J = 7.6 Hz, 4H), 1.55-1.68 (m, 4H), 0.95 (t, J = 7.4 Hz, 6H). ^{13}C NMR (100 MHz, d_6-CD_3COCD_3): δ (ppm) 157.92, 157.23, 156.97,150.61, 150.14, 149.13, 140.61, 140.25, 137.87, 136.99, 131.08, 129.99, 129.57, 128.91, 128.23, 127.94, 127.44, 125.20, 124.97, 123.16, 121.85, 118.50, 117.30, 112.56, 53.27, 21.19, 11.56. FT-IR (KBr, cm^{-1}): 3473 (m), 3411 (s), 2958 (m), 1597 (s), 1520 (s), 1392 (w), 1352 (w), 1242 (m), 1186 (m), 964 (w), 823 (w), 773 (w), 692 (w). MS(ESI) (Cal. 509.28, Found: M+1 =510.29). Melting point: 156.5-157.5 °C. Anal. calcd for $C_{36}H_{35}N_3$: C, 84.83; H,6.92, N, 8.24. Found: C, 84.82; H, 6.90, N, 8.23.

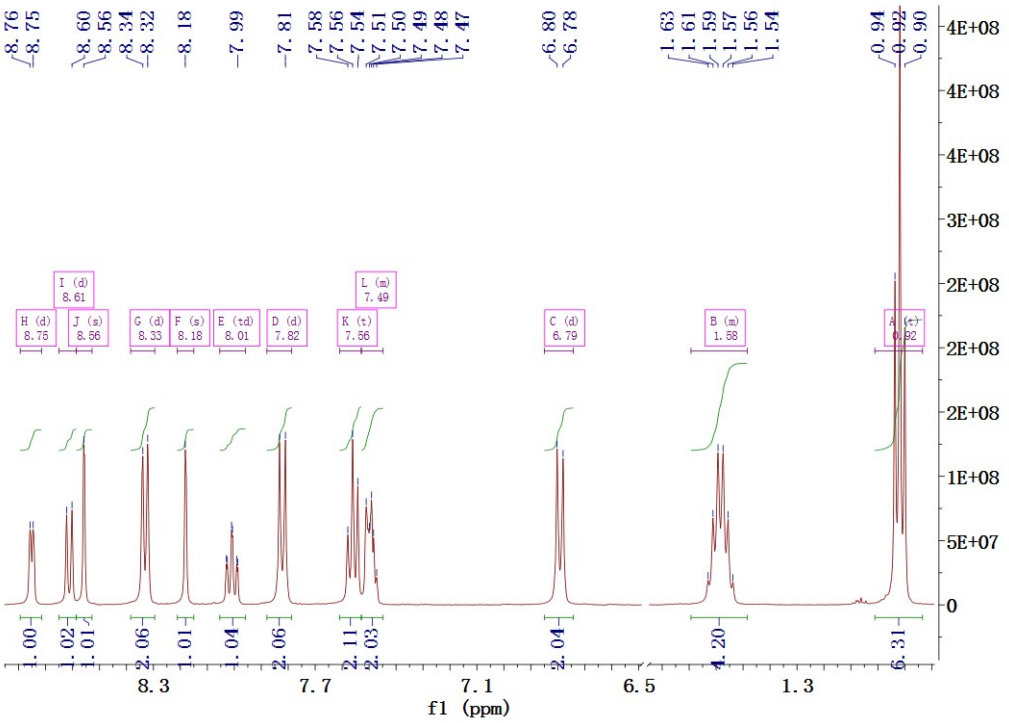


Fig. S1 The ^{1}H NMR spectrum of L1

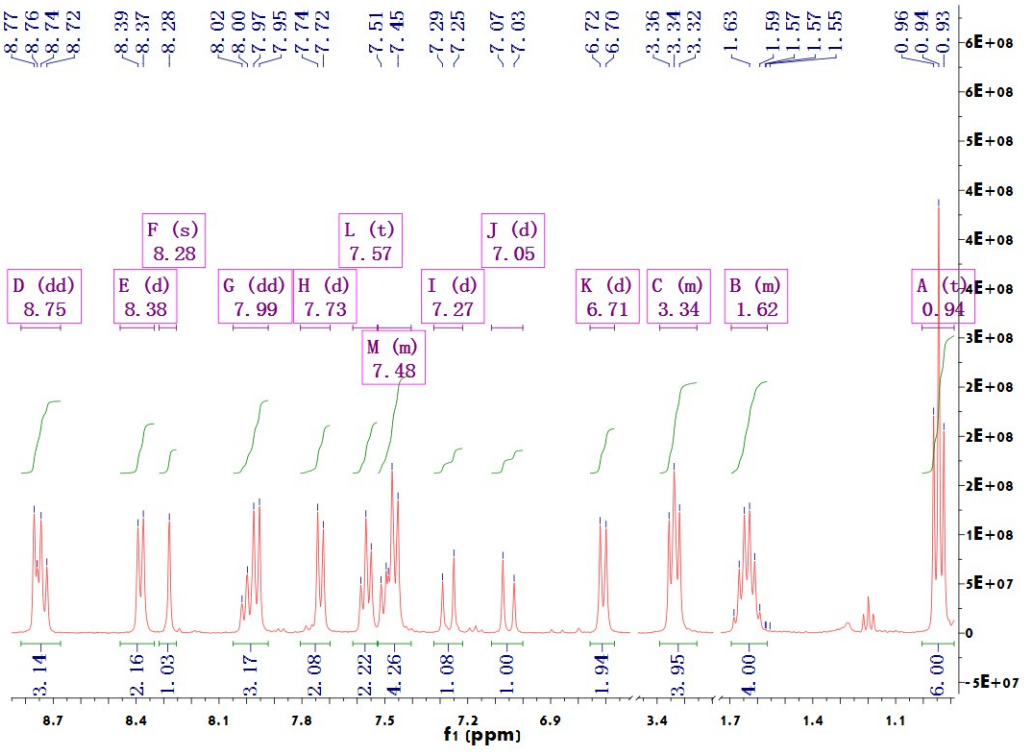


Fig. S2 The ^{1}H NMR spectrum of L2

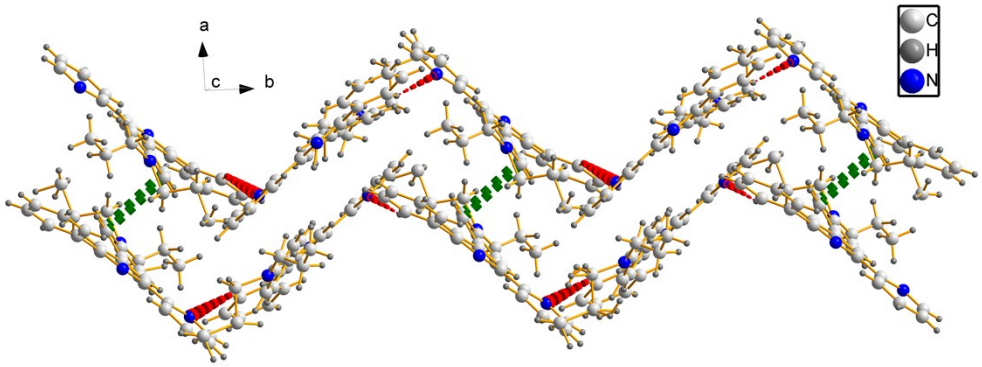


Fig. S3 The crystal stracking photograph of L1 is generating by π···π intermolecular interaction and N-H bond.

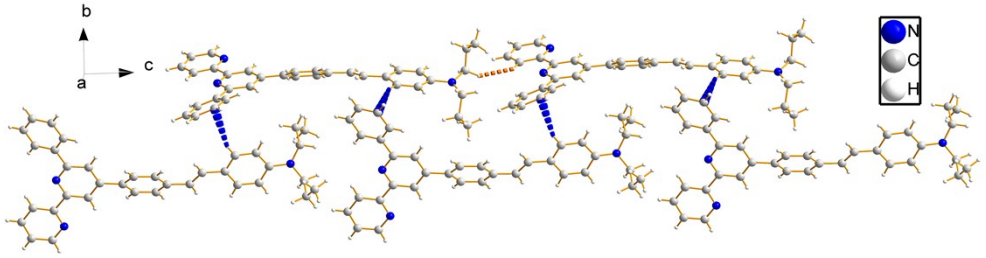


Fig. S4 The crystal stracking photograph of L2 is generating by C-H···π intermolecular and H···H interaction.

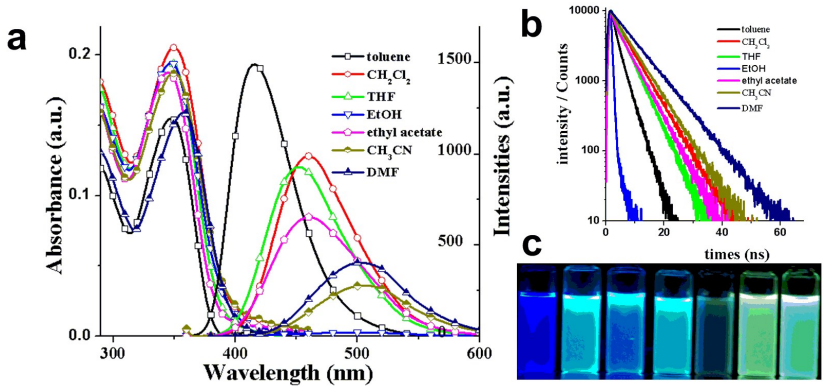


Fig. S5 (a) Linear absorption and emission spectra of **L1** in different solvents; (b) Time-resolved fluorescence curves of **L1** in different solvents; (c) Change in fluorescence color of **L1** in different solvents under 365 nm UV light.

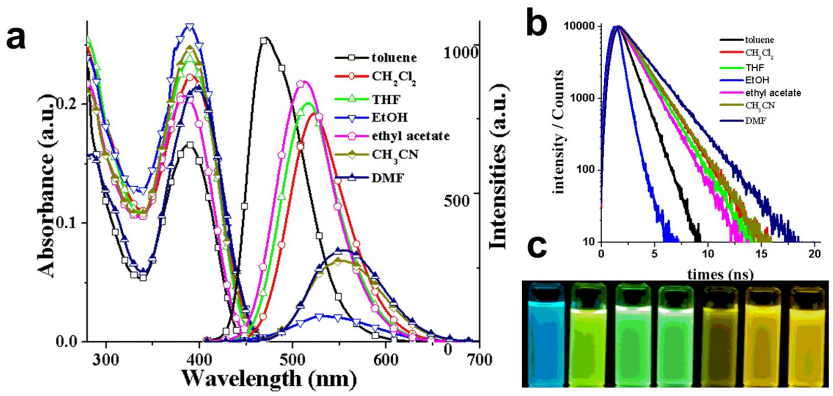
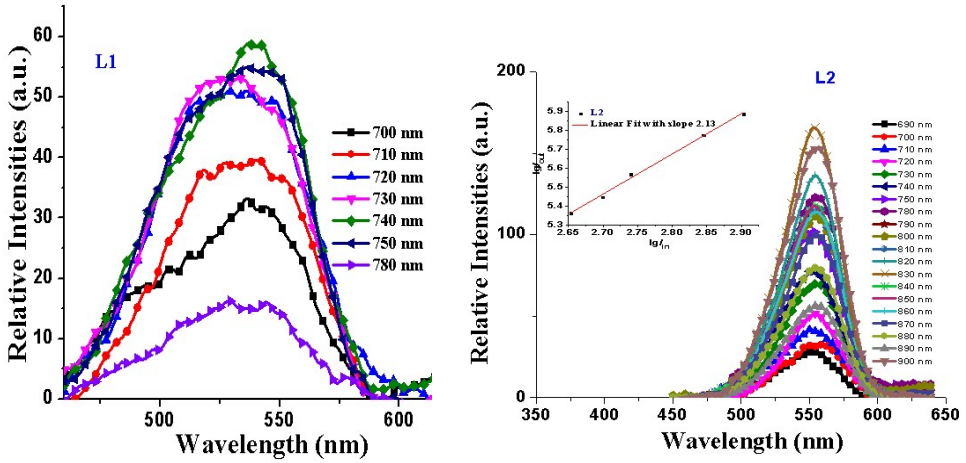


Fig. S6 (a) Linear absorption and emission spectra of **L2** in different solvents; (b) Time-resolved fluorescence curves of **L2** in different solvents; (c) Change in fluorescence color of **L2** in different solvents under 365 nm UV light.

Table. S1 The calculated fluorescent data of **L1** and **L2**.

Compound	Fluorescent energy	Fluorescent wavelength	oscillator strength
L1	3.63	341	0.24
L2	2.75	451	1.38



(a) (b)

Fig. S7 (a) The 2PEF spectra of 5 **L1**; (b) The TPEF spectra of 5 **L2**, Insert figure: Outputfluorescence (Iout) versus the square of input laser power (Iin) under the optimal excitation wavelength in DMF at 820 nm.

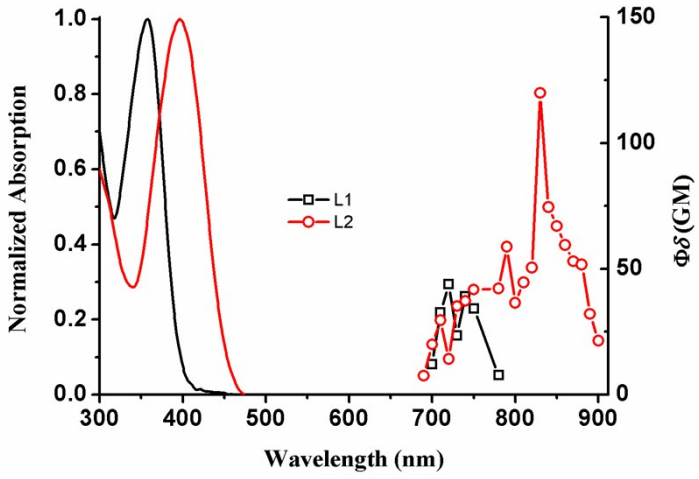


Fig. S8 The normalized absorption spectra and two-photon absorption cross-section of L1 and L2 in DMF.

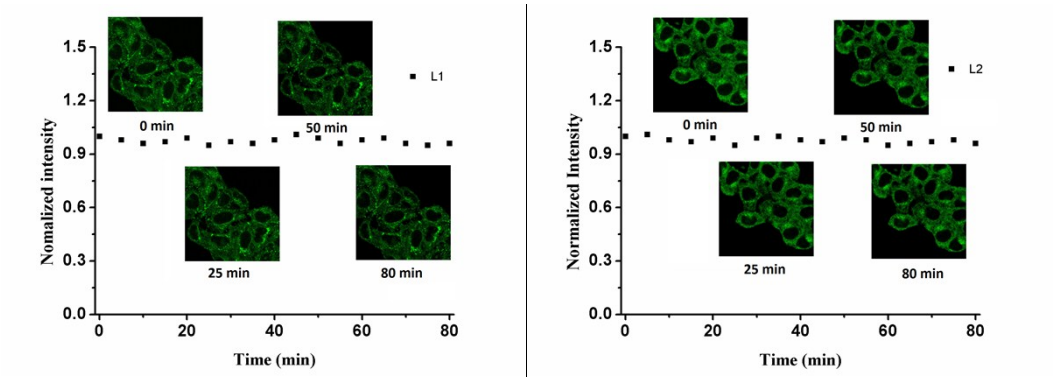


Fig. S9 Time series fluorescence intensity of L1 (left) and L2 (right) in HepG2 cells (20 μM, 30 min) under laser exposure over 80 min, inset imaging from times point 0, 25, 50, and 80 min (excited at 720 nm).

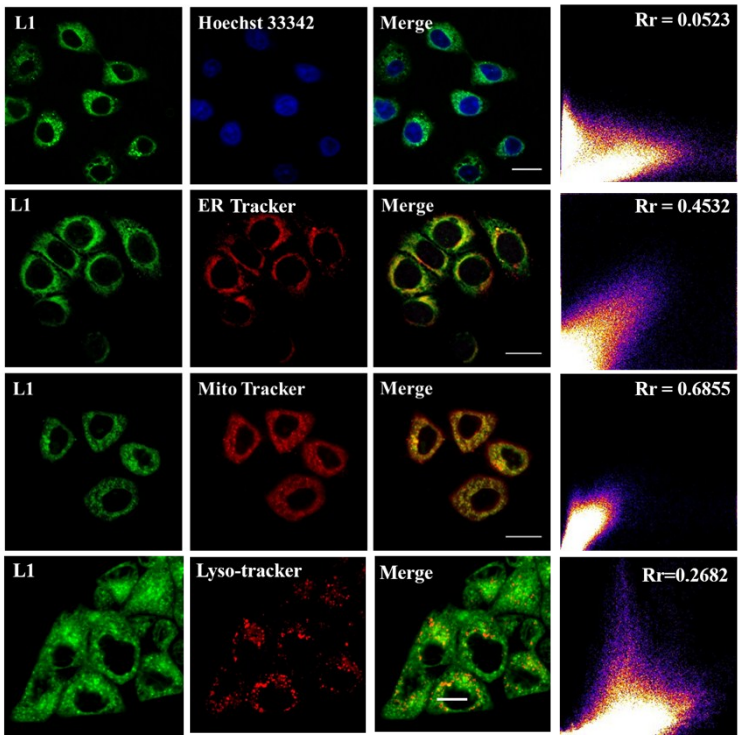


Fig. S10 The co-localization of **L1** with commercial dyes Hoechst 33342, ER Tracker, Mito Tracker Red, and Lyso-Tracker Red.

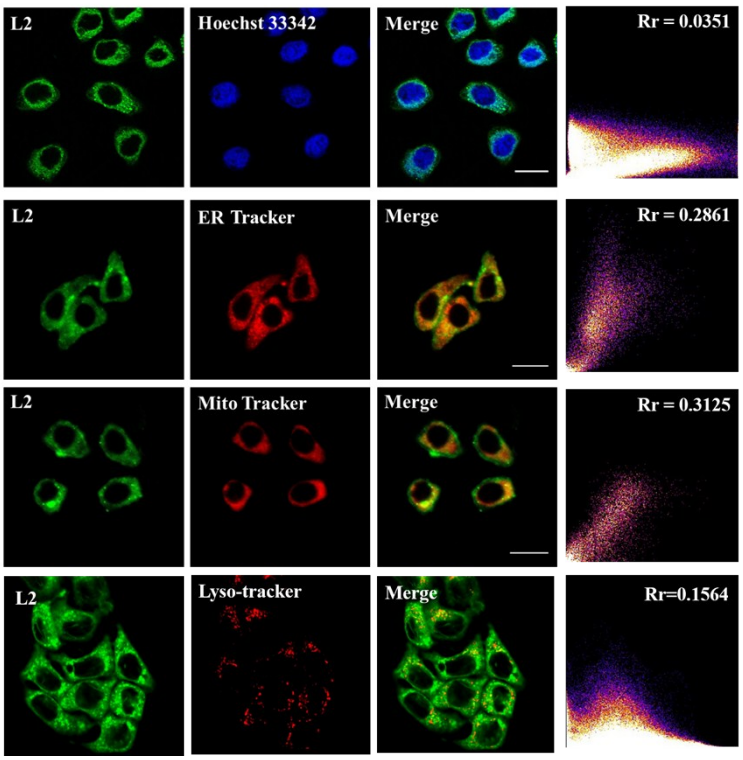


Fig. S11 The co-localization of **L2** with commercial dyes Hoechst 33342, ER Tracker, Mito Tracker Red and Lyso-Tracker Red.

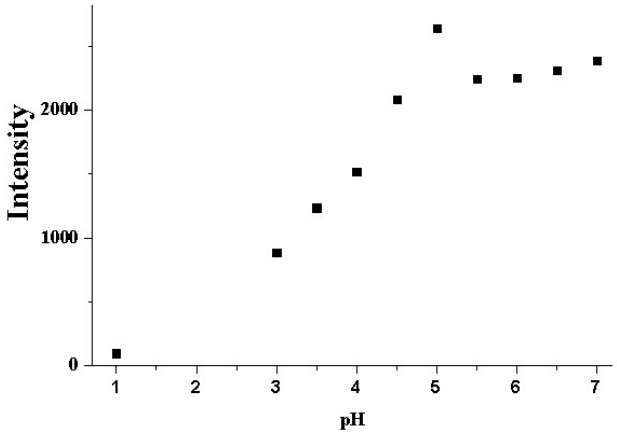


Fig. S12 Single-photon fluorescence intensity change of L1 (5.0×10-6 M) in different pHs water.