Supporting Information for

Tetraphenylethylene-Induced Cross-Linked Vesicles with Tunable Luminescence and Controllable Stability

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General Method

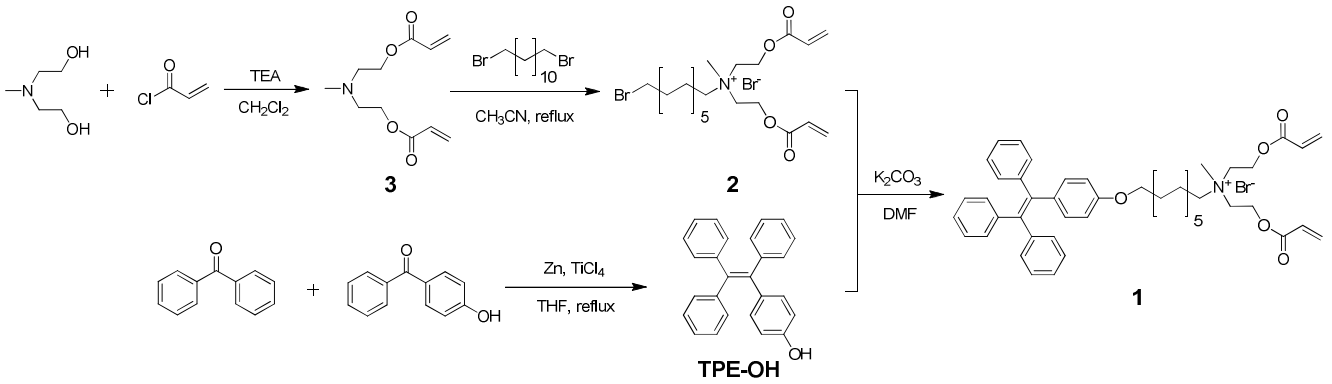
Routine NMR spectra were obtained on a Bruker AV II-400. The ^{1}H NMR chemical shifts were measured relative to D_2O or $CDCl_3$ as the internal reference (D_2O: δ 4.79 ppm; $CDCl_3$: δ 7.26 ppm). The ^{13}C NMR chemical shifts were given using $CDCl_3$ as the internal standard ($CDCl_3$: δ 77.16 ppm). Mass spectrometry was performed on a Waters Q-Tof premier instrument. The fluorescence emission was measured by using a Hitachi F-7000 fluorescence spectrometer. The absorbance curve was measured with UV-5301PC (Shimadzu, Japan). The particle size was measured with a Dynamic Light Scattering (DLS) Analyzer (Malvern ZetasizerNano ZS90). TEM studies were carried out using a TecnaiG2F20S-TWIN instrument, operating at 120 kV. The TEM specimens were prepared by gently placing a carbon-coated copper grid on the surface of the sample. The TEM grid was then removed, stained with an aqueous solution of 2% phosphotungstic acid, dried for 0.5 h at room temperature, and then subjected to TEM observation. Human liver cancer (HepG2) was obtained from Chinese Academy of Science Cell Bank for Type Culture Collection (Shanghai, China) and used for all of cell experiments and animal experiments. The cell line was grown in Dulbecco's modification of Eagle's medium Dulbecco supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin in an incubator under 5% CO_2 at 37 ^{o}C. Cell toxicity was evaluated by measuring the percentage of cell viability via the Cell Counting Kit-8 assay (CCK-8). The absorbance was then measured using a microplate reader Varioscan Flash (ThermoFisher SCIENTIFIC). The cell viability (%) was obtained according to the manufacturer's instructions. The cellular uptake of HepG2 cells incubated with TPE-CVs@RhB was observed under confocal laser scanning microscopy (CLSM, LSM780).

Chemicals: Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Compounds (4-hydroxyphenyl)(phenyl)methanone, benzophenone and

Rhodamine B were purchased from Adamas-beta Ltd, and 2,2'-(methylazanediyl)bis(ethan-1-ol) was bought from Aladdin Ltd. All solvents for reactions were freshly distilled prior to use. Deionized water was used in all aqueous experiments.

Synthesis

Scheme 1S. Synthesis of compound **1**.



Synthesis of Compound 3. Acryloyl chloride (6.84 g, 75.5 mmol) was added dropwise to a solution of *N*-methyldiethanolamine (3.0 g, 25.2 mmol) and triethylamine (TEA, 10.2 g, 100.7 mmol) in 70 mL of dry dichloromethane (CH_2Cl_2) at 0 ^oC. The reaction mixture was stirred at room temperature for 24 h. Then the reaction mixture was concentrated and the residue was purified by column chromatography over silica gel (petroleum ether : ethyl acetate = 2:1) to give **3** (3.0 g, 53%) as a clear oil. ^1H NMR (400 MHz, $CDCl_3$) δ 2.38 (s, 3 H), 2.77 (t, *J* = 5.8 Hz, 4 H), 4.26 (t, *J* = 5.8 Hz, 4 H), 5.82. (dd, *J* = 1.2, 1.6 Hz, 2 H).

Synthesis of Compound 2. Compound **3** (1.023 g, 4.5 mmol) and 1,12-dibromododecane (984 mg, 3.0 mmol) were refluxed in dry acetonitrile (CH_3CN) under N_2 atmosphere for 36 h. After cooling to room temperature, the reaction mixture was concentrated, diluted with water (50 mL), and extracted with CH_2Cl_2 (50 mL × 3). Te combine organic layer was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography over silical gel (dichloromethane :

CH_3OH = 10:1) to give the product as yellow liquid (752 mg, 45%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24-1.42 (m, 16 H), 1.80-1.97(m, 4 H), 3.40 (t, *J* = 6.8 Hz, 2 H), 3.54 (s, 3 H), 3.58-3.62 (m, 2 H), 4.14-4.24 (m, 4 H), 4.18-4.20 (m, 4 H), 4.72 (s, 4 H), 5.93-5.96 (m, 2 H), 6.08-6.14 (m, 2 H), 6.45-6.94 (m, 2 H).

Synthesis of TPE-OH.[1] Under N_2 atmosphere, a three-necked flask equipped with a magnetic stirrer was charged with zinc powder (1.6 g, 24 mmol) and dry tetrahydrofuran (THF, 40 mL). The mixture was cooled to -5 to 0 ^{o}C, and dry $TiCl_4$ (1.3 mL, 12 mmol) was slowly added by a syringe with the temperature kept under 10 ^{o}C. The suspending mixture was warmed to room temperature and stirred for 0.5 h, then heated over refluxing for 2.5 h. The mixture was again cooled to -5 to 0 ^{o}C, charged with pyridine (0.5 mL, 6 mmol) and stirred for 10 min. The solution of diphenyl ketone and 4-hydroxy diphenyl ketone (2.4 mmol, in 1:1.2 mole ratio) in 15 mL THF was added slowly. After addition, the reaction mixture was heated over refluxing until the carbonyl compounds were consumed (monitored by TLC). The reaction was quenched with 10% K_2CO_3 aqueous solution and taken up with CH_2Cl_2. The organic layer was collected. After solvent evaporation, the crude product was purified on a silica gel column (petroleum ether : ethyl acetate from 20:1 to 7:1) to give the desired product as a white powder (600 mg, 76%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.68 (s, 1 H), 6.56 (d, *J* = 8 Hz, 2 H), 6.89 (d, *J* = 8 Hz, 2 H), 7.00-7.12 (m, 15 H).

Synthesis of Compound 1. Potassium carbonate (103.7 mg, 0.75 mmol) was added to a solution of TPE-OH (174 mg, 0.5 mmol) in DMF (4 mL) at room temperature. After the mixture was stirred at 60 ^{o}C for 2 h, compound **2** (276.6 mg, 0.5 mmol) was added slowly. The resulting reaction mixture was further stirred overnight at 60 ^{o}C under N_2, cooled to room temperature, and poured over 500 mL of icy water. The mixture was then extracted with CH_2Cl_2 (100 mL × 3). The combined organic phase was

washed with brine (50 mL), dried over anhydrous $MgSO_4$, and concentrated *in vacuo*. The crude product was purified on a silica gel column with CH_2Cl_2/CH_3OH (10/1) as the eluent to give the product as yellow liquid (300 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24-1.40 (m, 18 H), 1.69 -1.73 (m , 2 H), 3.53 (s, 3 H), 3.58-3.62 (m, 2 H), 3.83-3.86 (m, 2 H), 4.13-4.20 (m, 4 H), 4.72 (s, 4 H), 5.93-6.08 (m, 2 H), 6.11-6.14 (m, 2 H), 6.45-6.50 (m, 2 H), 6.59-6.61 (m, 2 H), 6.89-6.91 (m, 2 H), δ 7.00-7.08 (m, 2 H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 22.9, 23.1, 26.2, 26.4, 29.3, 29.4, 29.5, 29.6, 50.0, 57.9, 61.1, 63.9, 66.6, 67.9, 76.8, 77.2, 77.5, 113.7, 114.6, 126.3, 126.4, 127.1, 127.2, 127.7, 127.8, 128.9, 131.0, 131.4, 131.5, 132.6, 133.0, 133.2, 136.0, 140.1, 140.7, 144.1, 157.8, 165.2. High resolution ESI-MS: $[M+Na]^+$ calcd for $C49H60NO5^+$ 742.4466, found 742.4488.

Typical preparation of tetraphenylethylene-induced vesicles (TPE-Vs). Compound **1** (50 μL, 0.12 M in propanol) was added dropwise into 5.0 mL of deionized water under vortex at room temperature. The resulting solution was then left to stand and the TPE-Vs formed spontaneously within minutes as a pale blue emulsion.

Carboxyfluorescein Leakage Assay.[2,3] Compound **1** (50 μL, 0.12 M in propanol) was added dropwise into 5.0 mL of aqueous solution of 5(6)-Carboxyfluorescein (CF, 3 mM) under vortex at room temperature. A portion (1.0 mL) of the resulting vesicle solution was passed through a column of Shepadex G-50 using millipore water as the eluent to remove the extravesicular CF. The vesicle fractions were combined and diluted to 5.0 mL with the same deionized water. The concentration of compound **1** in the solution was 0.24 mM. At this moment, the fluorescence emission at 523 nm (λ_{ex} = 492 nm) was recorded. After that, 20 μL of Triton X-100 (1%) was added to lyse the vesicles, and the fluorescence emission (λ_{ex} = 492 nm) was measured again. The sharp increase of fluorescence at 523 nm suggests the formation of vesicular structure.

Typical procedure for measurement of the critical vesicle concentration (CVC) of compound 1. The critical vesicle concentration (CVC) of **1** was measured by the fluorescence emission of TPE buried in compound **1**. Briefly, the stock solution of compound **1** was diluted to various desired concentrations (from 1.0 μM to 512.0 μM). The fluorescence spectra of all solutions were measured with the excitation wavelength at 373 nm, and the intensity values of all solutions at 475 nm were recorded to determine the CVC. Based on triplicate experiments with separately prepared solutions, the CVC was measured to be ~59 μg/mL.

Typical preparation of tetraphenylethylene-induced cross-linked vesicles (TPE-CVs). To 5.0 mL aqueous solution of TPE-Vs (1.2 mM), dithiothreitol (DTT, 1.1 mg, 7.2 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at 40 ^{o}C for 16 h and dialyzed against deionized water for 2 days (Spectra/Pore, MWCO 1000) to get the TPE-CVs as a pale blue solution.

Typical preparation of RhB entrapped tetraphenylethylene-induced cross-linked vesicles (TPE-CVs@RhB) and non-acid labile TPE-CVs@RhB. Compound **1** (50 μL, 0.12 M in propanol) was added dropwise into 5.0 mL of aqueous solution of RhB (5.4 μM) under vortex at room temperature. To above aqueous solution of TPE-Vs@RhB, dithiothreitol (DTT, 1.1 mg, 7.2 mmol) was added under nitrogen atmosphere. The reaction mixture was stirred at 40 ^{o}C for 16 h and dialyzed against deionized water for 2 days (Spectra/Pore, MWCO 1000) to get the TPE-CVs@RhB as a pale red solution.

For non-acid-labile TPE-CVs@RhB, the TPE-Vs@RhB in millipore water (1.2 mM, 5 mL) was treated with $K_2S_2O_8$ (0.032 mg, 2%). The reaction mixture was stirred at 60 ^{o}C for 24 h and dialyzed against deionized water for 48 h (Spectra/Pore, MWCO 1000) to get the non-acid-labile TPE-CVs@RhB as a pale red solution.

For calculation of the loading content (LC) of RhB. The TPE-Vs@RhB was destroyed by DMSO/H_2O (V/V, 9/1) to release RhB. The released RhB was calculated by the fluorescence spectra using a pre-established calibration curve with various RhB concentrations. The LC was calculated as follows:

$$\text{LC (wt \%)} = (\text{weight of loaded RhB/total weight of TPE-Vs@RhB}) \times 100\%$$

Calculations of energy-transfer efficiency (Φ_{ET}). Energy-transfer efficiency, Φ_{ET}, the fraction of the absorbed energy that is transferred to the acceptor is experimentally measured as a ratio of the fluorescence intensities of the donor in the absence and presence of the acceptor (I_D and I_{DA}).[4]

$$\Phi_{ET} = 1 - \frac{I_{DA}}{I_D}$$

The energy-transfer efficiency (Φ_{ET}) was calculated as 47.8% and 56.5% in water (pH = 7.4), measured in the condition of TPE-Vs@RhB ([RhB] = 0.36 mmol%) and TPE-CVs@RhB ([RhB] = 0.36 mmol%, λ_{em} = 475 nm.

Stability test of TPE-CVs. The dilution stability of TPE-CVs was evaluated by diluting the concentrations of **1** below its CMC (59 μg/mL). Briefly, the TPE-CVs ([**1**] = 1.0 mg/mL) was diluted to the concentrations of 500 μg/mL, 100 μg/mL, 50 μg/mL, 20 μg/mL, respectively. Afterwards, the particle sizes of above solutions were recorded by DLS to evaluate its stability.

The FBS stability of TPE-CVs and TPE-Vs was investigated by incubation with 10% (V/V) FBS. Briefly, 9 mL of TPE-CVs and TPE-Vs ([**1**] = 1.2 mM, in deionized water) were mixed with 1 mL FBS, respectively. The particle sizes at 0 h and over 12 h of incubation at 37 °C were recorded to evaluate their stability.

Environmental durability of TPE-CVs@RhB and TPE-Vs@RhB. (1) *Heating*. The TPE-CVs@RhB and TPE-Vs@RhB ([**1**] = 1.2 mM, [RhB] = 4.3 μM) were elevated to 30 ^{o}C, 40 ^{o}C, 50 ^{o}C, 60 ^{o}C and 70 ^{o}C, respectively. Afterwards, the emission intensity ratio of I_{578}/I_{475} at every elevated temperature was recorded to evaluate their optical stability over heating.

(2) *Dilution.* The TPE-CVs@RhB and TPE-Vs@RhB ([**1**] = 1.2 mM, [RhB] = 4.3 μM) were diluted to the concentrations of 500 μg/mL, 100 μg/mL, 50 μg/mL, 20 μg/mL, 10 μg/mL, respectively. Afterwards, the emission intensity ratios of I_{578}/I_{475} were recorded to evaluate their optical stability over dilution.

(3) *Doping of organic solvent*. The different volumes of ethanol were added in the aqueous solution of TPE-CVs@RhB and TPE-Vs@RhB (([**1**] = 1.2 mM, [RhB] = 4.3 μM), respectively. After vibrated for 2 min, the emission intensity ratios of I_{578}/I_{475} were recorded to evaluate their optical stability over doping of organic solvent.

(4) *Storage in serum.* The TPE-CVs@RhB and TPE-Vs@RhB ([**1**] = 1.2 mM, [RhB] = 4.3 μM) were incubation with 10% (V/V) FBS, respectively. Afterwards, the emission intensity ratios of I_{578}/I_{475} at 0 h, 3 h, 6 h, 9 h, and 12 h were recorded to evaluate their optical stability over storage in serum.

Optical stability of acid-labile TPE-CVs@RhB and non-acid-labile TPE-CVs@RhB at pH 5.0 and pH 7.4. The acid-labile TPE-CVs@RhB and non-acid-labile TPE-CVs@RhB were incubated in different buffers [PBS buffer (pH = 7.4), and acetate buffer (pH = 5.5)], respectivley. Afterwards, the emission intensity ratios of I_{578}/I_{475} at 0 h, 3 h, 6 h, 9 h, and 12 h were recorded to evaluate their optical stability in different pH conditions.

Cellular uptake evaluation by confocal laser scanning microscopy (CLSM). HepG2 cells (5×10^4 cells/mL) were seeded in a Φ = 35 mm glass Petri dish and incubated at 37 ^{o}C/5% CO_2 for 24 h.

Subsequently, the cells were cultured with equal concentration of TPE-CVs@RhB and non-acid labile TPE-CVs@RhB ([RhB] = 0.2 μg/mL, λ_{ex} = 480 nm, λ_{em} = 578 nm), at 37 ^{o}C for 3 h, 6 h, 12 h respectively. The culture medium was removed and the cells were washed three times and another 200 μL PBS was added. The cellular uptake was then observed under confocal laser-scanning microscopy.

Cytotoxicity assay. *In vitro* cytotoxicity was assessed by the Cell Counting Kit-8 assay (CCK-8). Briefly, HepG2 cells (5000/well) were seeded in 96-well culture plates and incubated at 37 ^{o}C/5% CO_2. After 24 h, culture media was removed and fresh media (200 μL) containing acid labile TPE-CVs@RhB ([RhB] = 0.36 mol%) which ranging from 0.05 μg/mL to 200 μg/mL were added to each well, separately. Cells without any treatment were set as control. After 24 h, culture media was removed and fresh media (100 μL) containing CCK-8 (10 μL) was added to each well and the plates were incubated at 37 ^{o}C for another 2 h. Then, the absorbance at 450 nm of each sample was measured using a microplate reader Varioscan Flash.

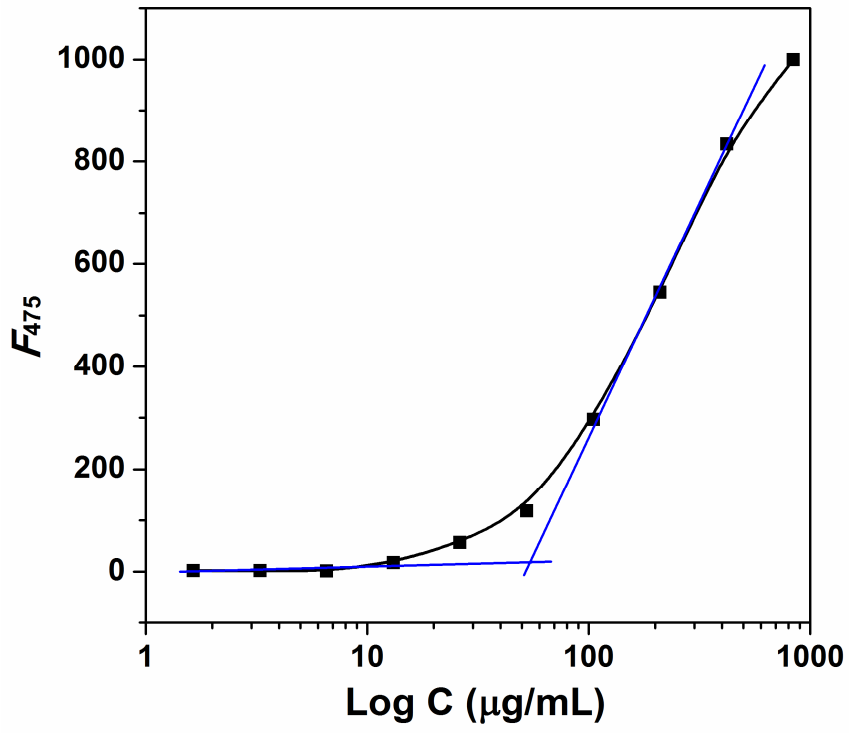


Figure 1S. Critical vesicle concentration (CVC) of compound **1** dertermined by the emission intensity at 475 nm (F_{475}) as a function of [**1**] in water. λ_{ex} = 373 nm.

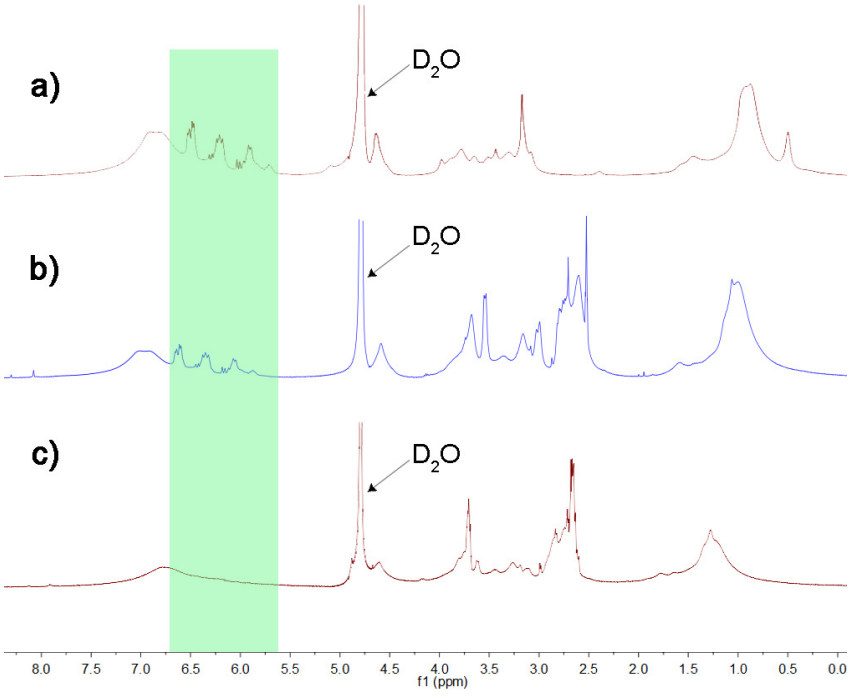


Figure 2S. ^{1}H NMR spectra of a 6 mM aqueous solution of TPE-Vs (a), the mixture of TPE-Vs and DTT in D_2O before (b) and after (c) cross-linking in D_2O.

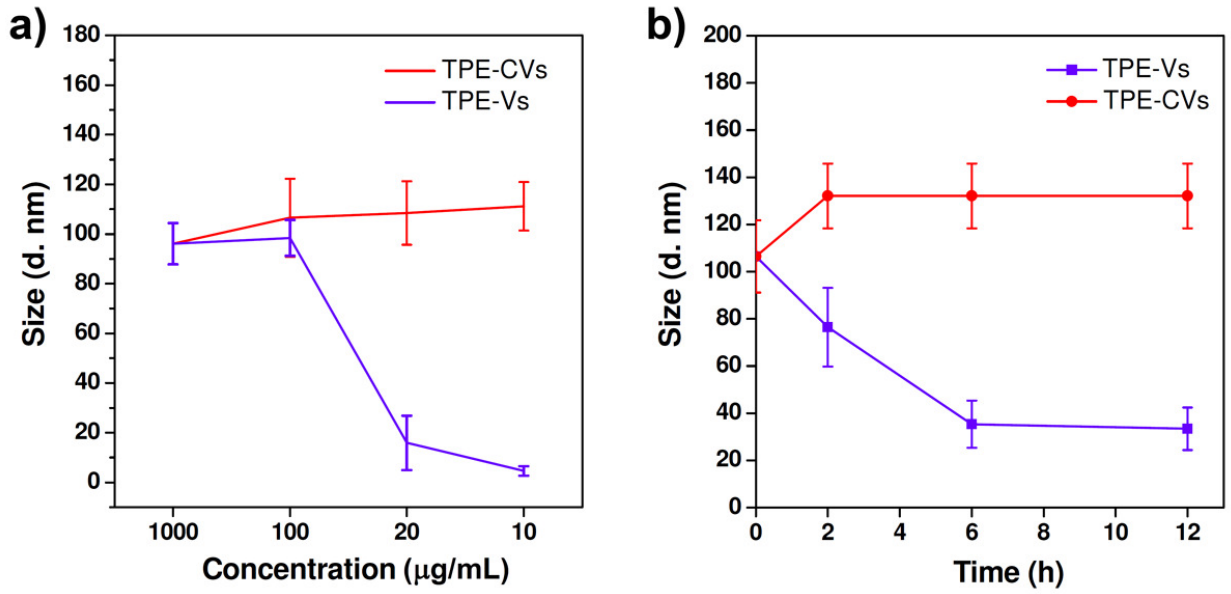


Figure 3S. Stability tests of TPE-Vs and TPE-CVs. (a) Size of TPE-Vs and TPE-CVs as a function of the concentration of **1** in water. (b) Particle sizes of TPE-Vs and TPE-CVs after incubation with 10% FBS at 37 °C over time ([**1**] = 0.25 mM).

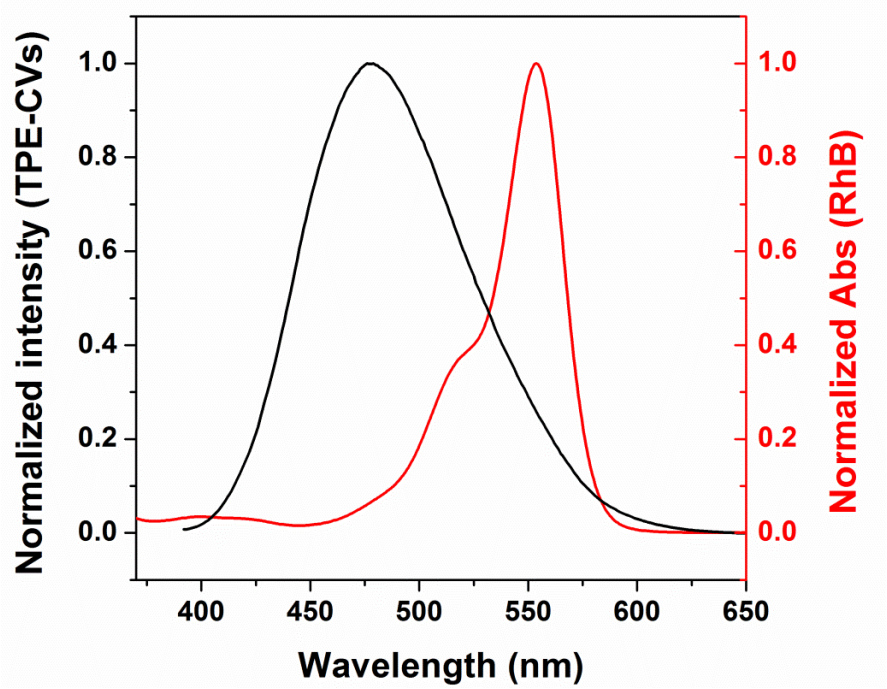


Figure 4S. Normalized fluorescence spectrum of TPE-CVs (black) and UV-Vis absorption spectrum of RhB (red) in water. λ_{ex} = 373 nm.

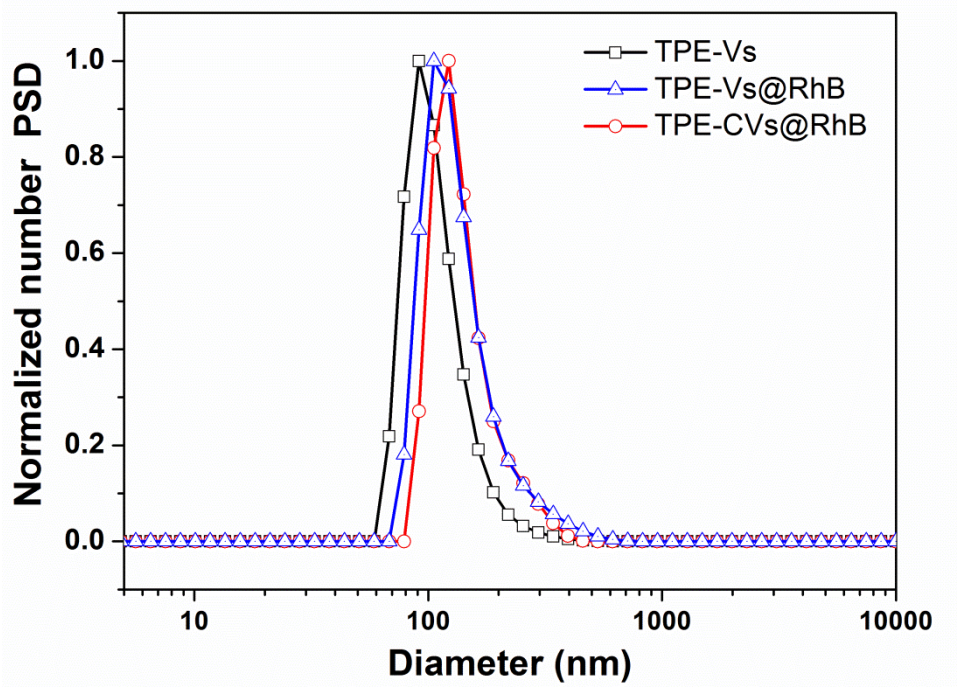


Figure 5S. Distribution of the hydrodynamic diameters of TPE-Vs, TPE-Vs@RhB, and TPE-CVs@RhB in aqueous solution determined by DLS. [**1**] = 1.2 mM, [RhB] = 4.3 μM.

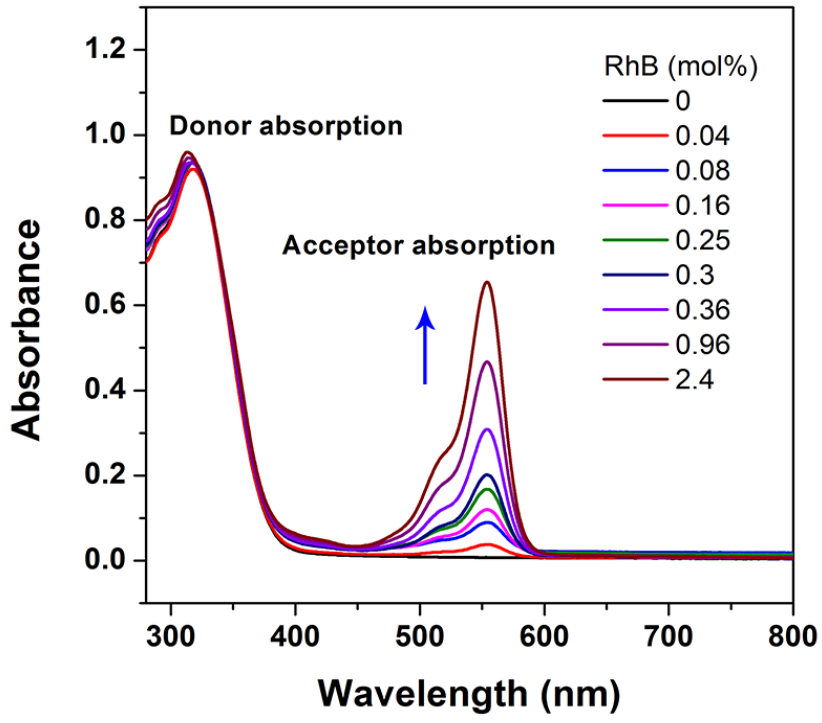


Figure 6S. UV-Vis spectra of TPE-CVs@RhB with different amounts of RhB in water.

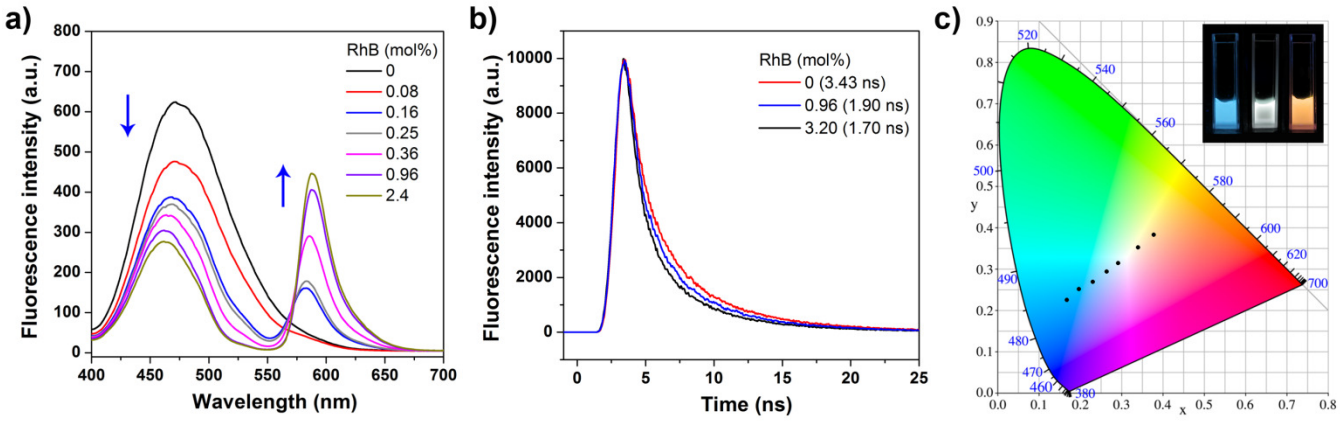


Figure 7S. (a) Fluorescence spectra of TPE-Vs@RhB with different amounts of RhB in water. λ_{ex} = 373 nm. (b) Fluorescence decay profiles of TPE-Vs@RhB in water at λ_{ex} = 373 nm. (c) The CIE chromaticity diagram that shows the luminescent color changes of TPE-Vs@RhB. The right upper insert shows the water solution of TPE-Vs@RhB with different concentrations of RhB under 365 nm UV light).

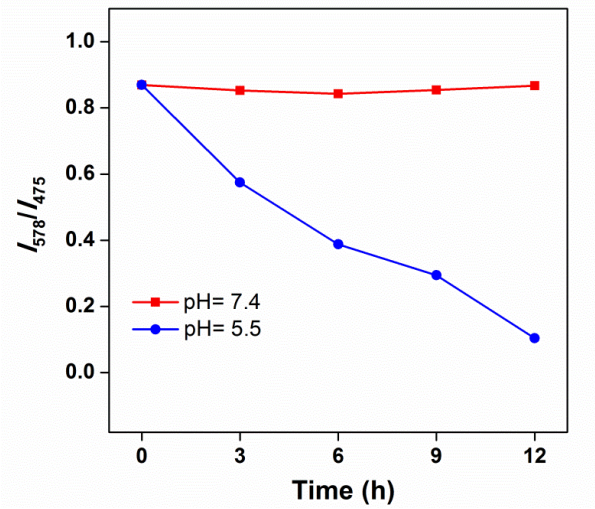


Figure 8S. Optical stability of acid-labile TPE-CVs@RhB in the media of pH = 7.4 (PBS buffer) and pH = 5.5 (acetate buffer) at 37 °C over time.

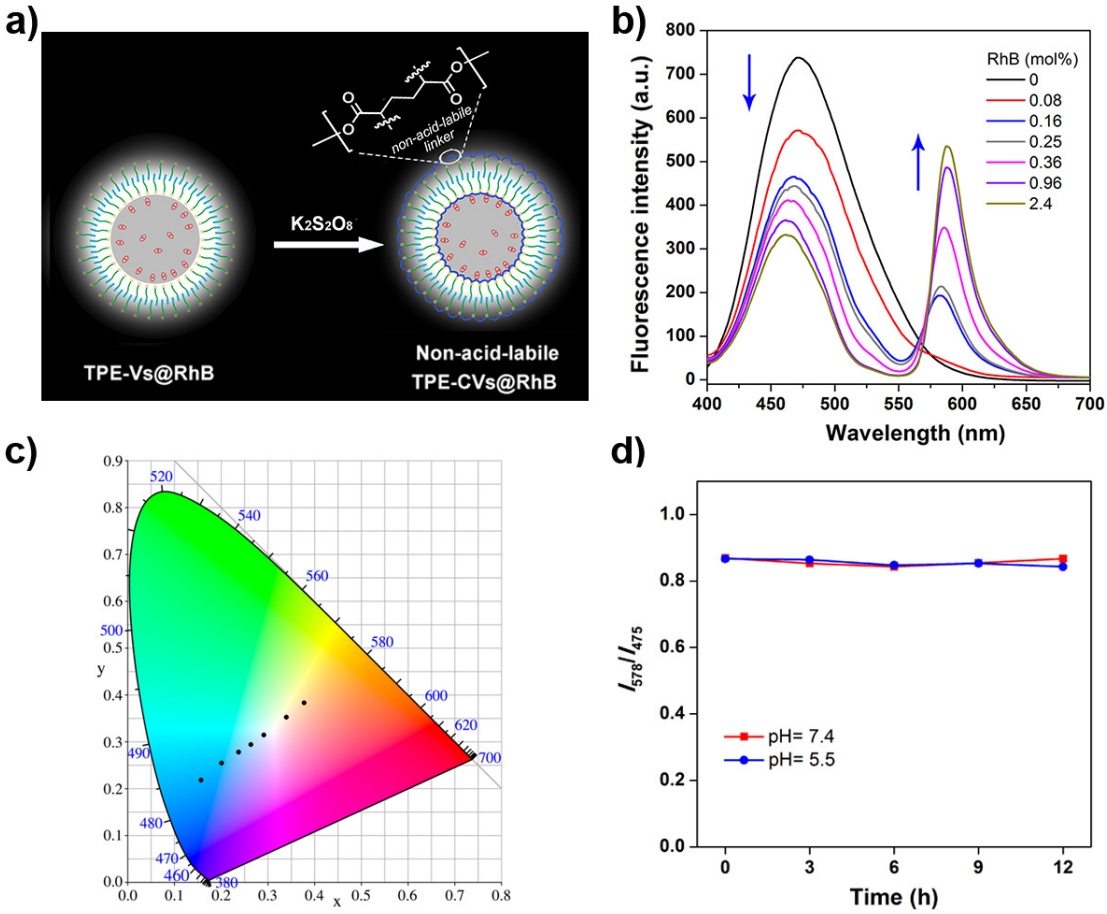


Figure 9S. Synthesis and luminescence properties of the non-acid-labile TPE-CVs@RhB. (a) Scheme for the preparation of non-acid-labile TPE-CVs@RhB. (b) Fluorescence spectra of non-acid-labile TPE-CVs@RhB with different amounts of RhB in water. λ_{ex} = 373 nm. (c) The CIE chromaticity diagram that shows the luminescent color changes of non-acid-labile TPE-CVs@RhB. (d) Optical stability of non-acid-labile TPE-CVs@RhB in the media of pH = 7.4 (PBS buffer) and pH = 5.0 (acetate buffer) at 37 °C over time.

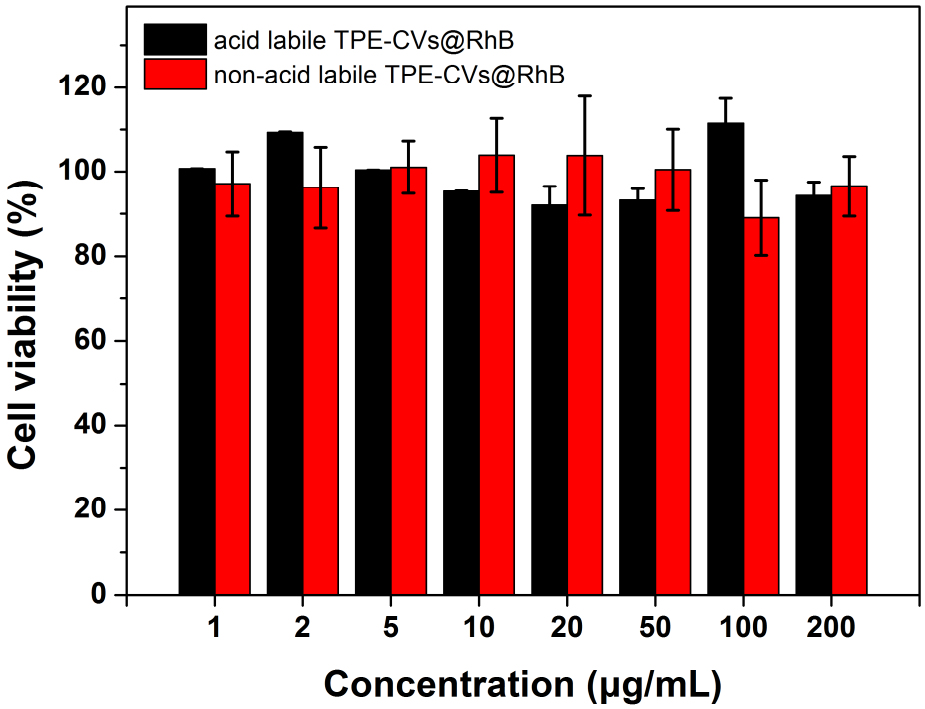


Figure 10S. Cell viability of acid labile TPE-CVs@RhB and non-acid labile TPE-CVs@RhB against HepG2 cells after incubation for 24 h at 37 °C with a series of concentrations. HepG2 cells incubated without any materials were used as the control (mean ± SD, n = 5).

Table 1S. Comparsion of luminescence property of TPE-Vs@RhB and TPE-CVs@RhB in water.

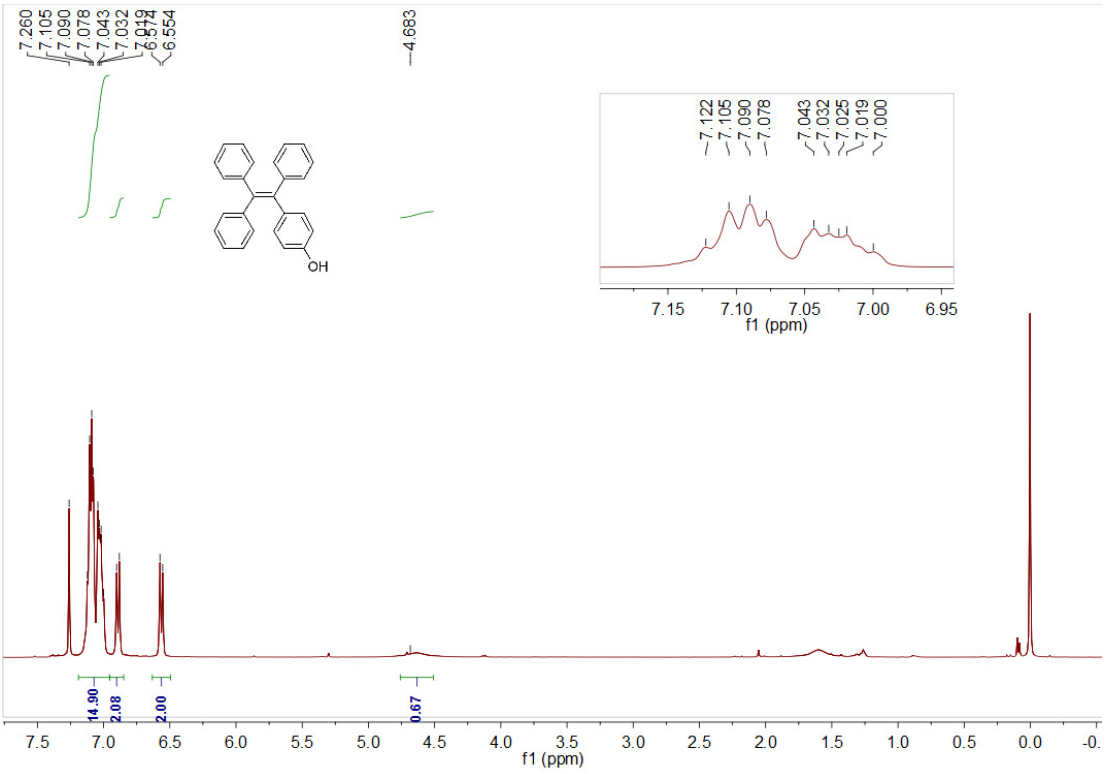
Entry	**RhB (mol%)**	**Materials**[a]	τ_1 **(ns)**[b]	Φ_F[c]
1	0	TPE-Vs	3.43	13.2
		TPE-CVs	3.53	17.7
2	0.36	TPE-Vs@RhB	1.90	18.4
		TPE-CVs@RhB	2.05	20.8
3	2.4	TPE-Vs@RhB	1.70	22.3
		TPE-CVs@RhB	1.79	28.3

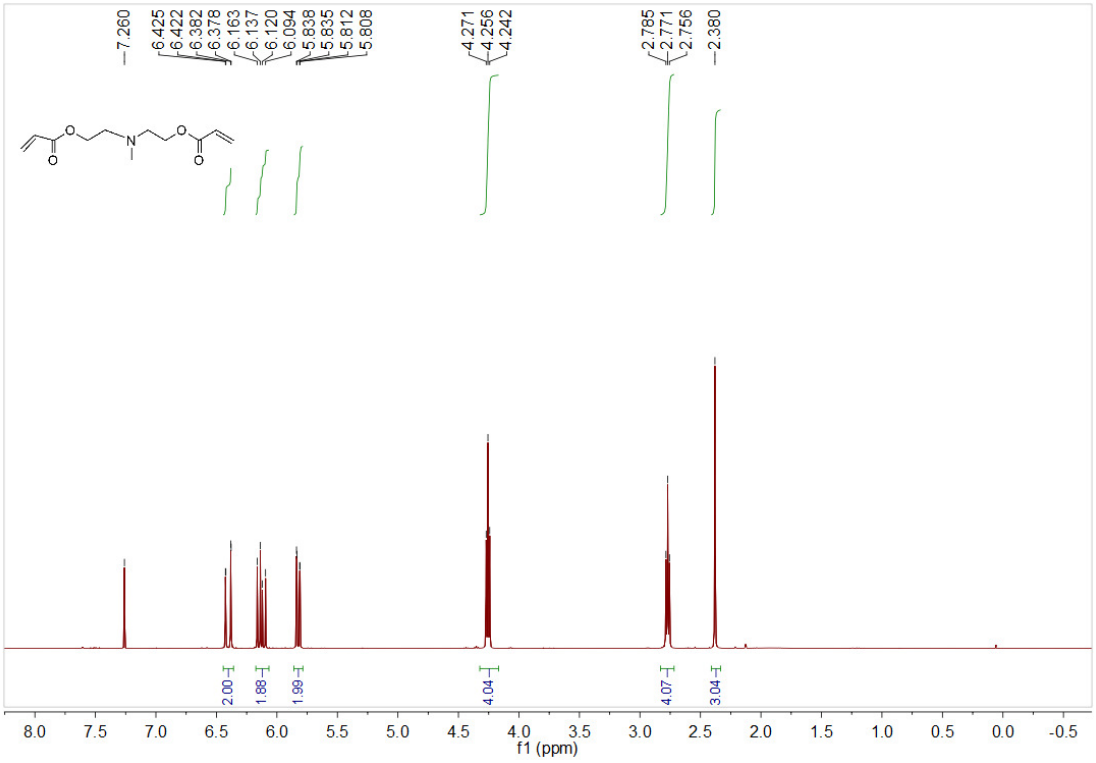
[a] For each material, the concentration of [**1**] is 1.2 mM.

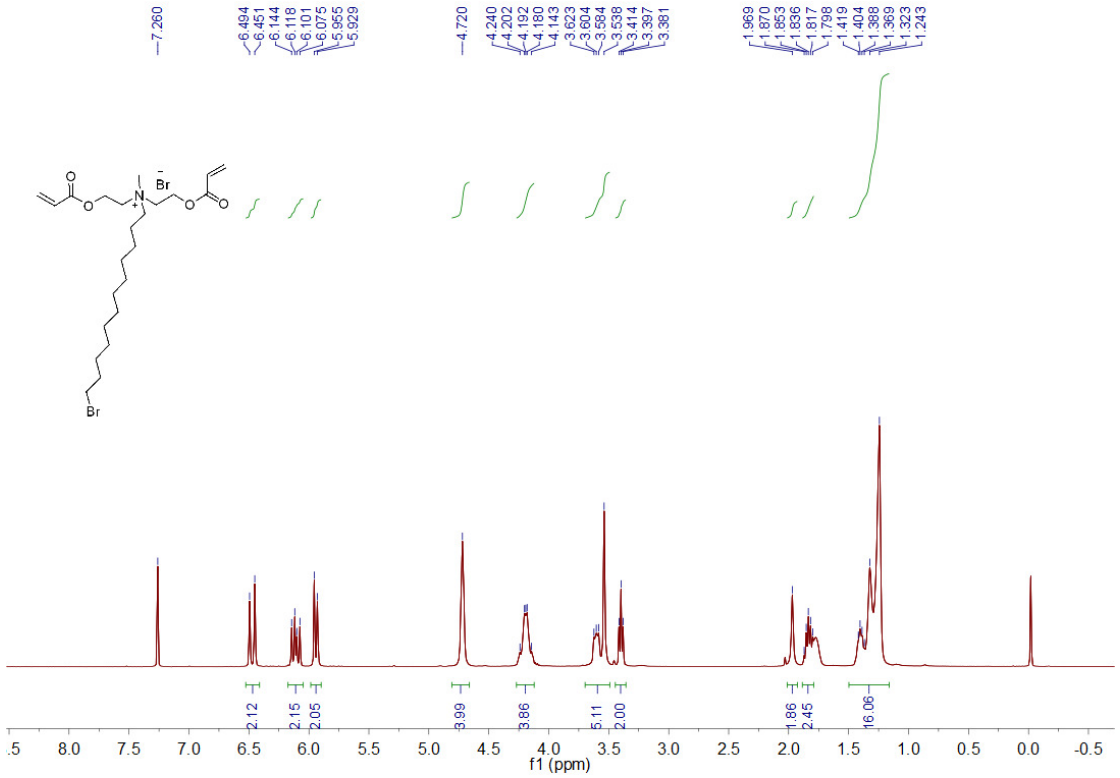
[b] Luminescence lifetimes (τ_i, ns) at 475 nm.

[c] Absolute quantum yield (determined with an integrating sphere system).

[1]H, [13]C NMR, and MS Spectra of Key Compounds



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4.180
4.143
3.623
3.604
3.584
3.538
3.414
3.397
3.381
1.969
1.870
1.853
1.836
1.817
1.798
1.419
1.404
1.388
1.369
1.323
1.243
Br
Br
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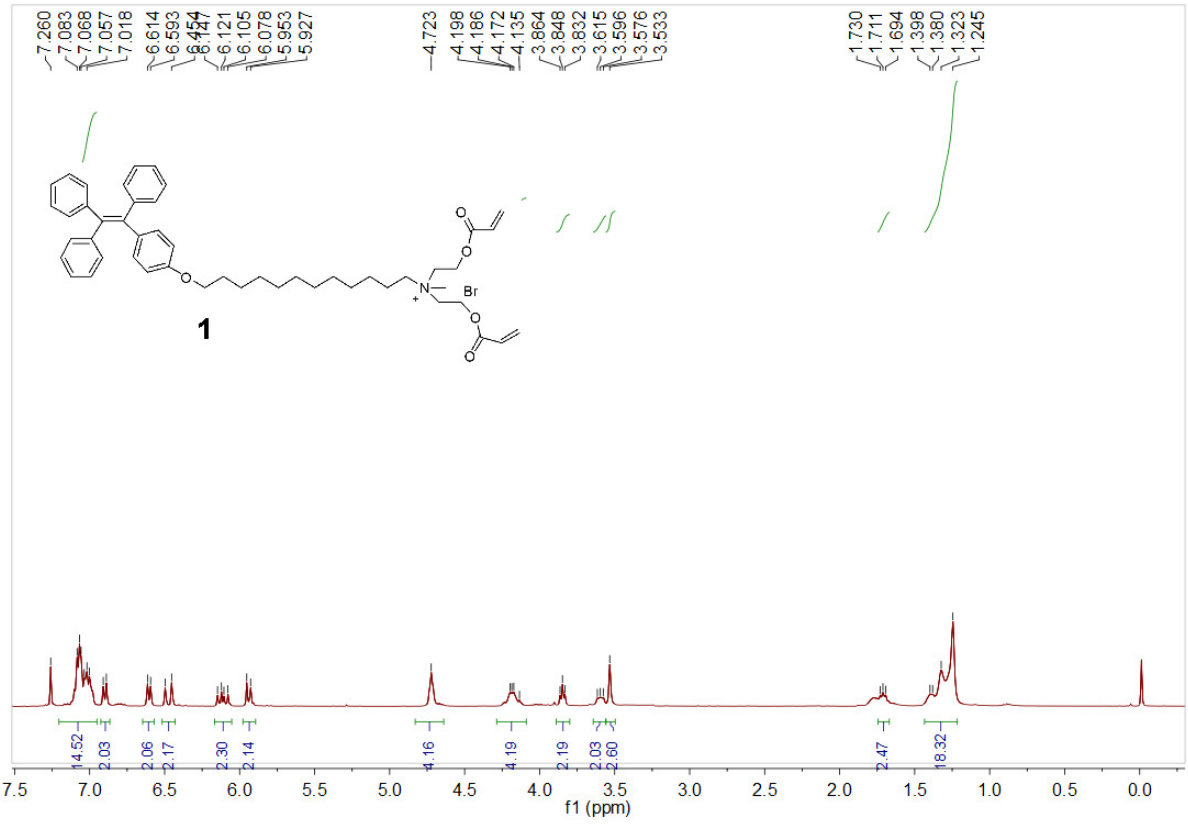
2.05
3.99
3.86
5.11
2.00
1.86
2.45
16.06
f1 (ppm)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7.057
7.018
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6.105
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3.832
3.615
3.596
3.576
3.533
1.730
1.711
1.694
1.398
1.380
1.323
1.245
1
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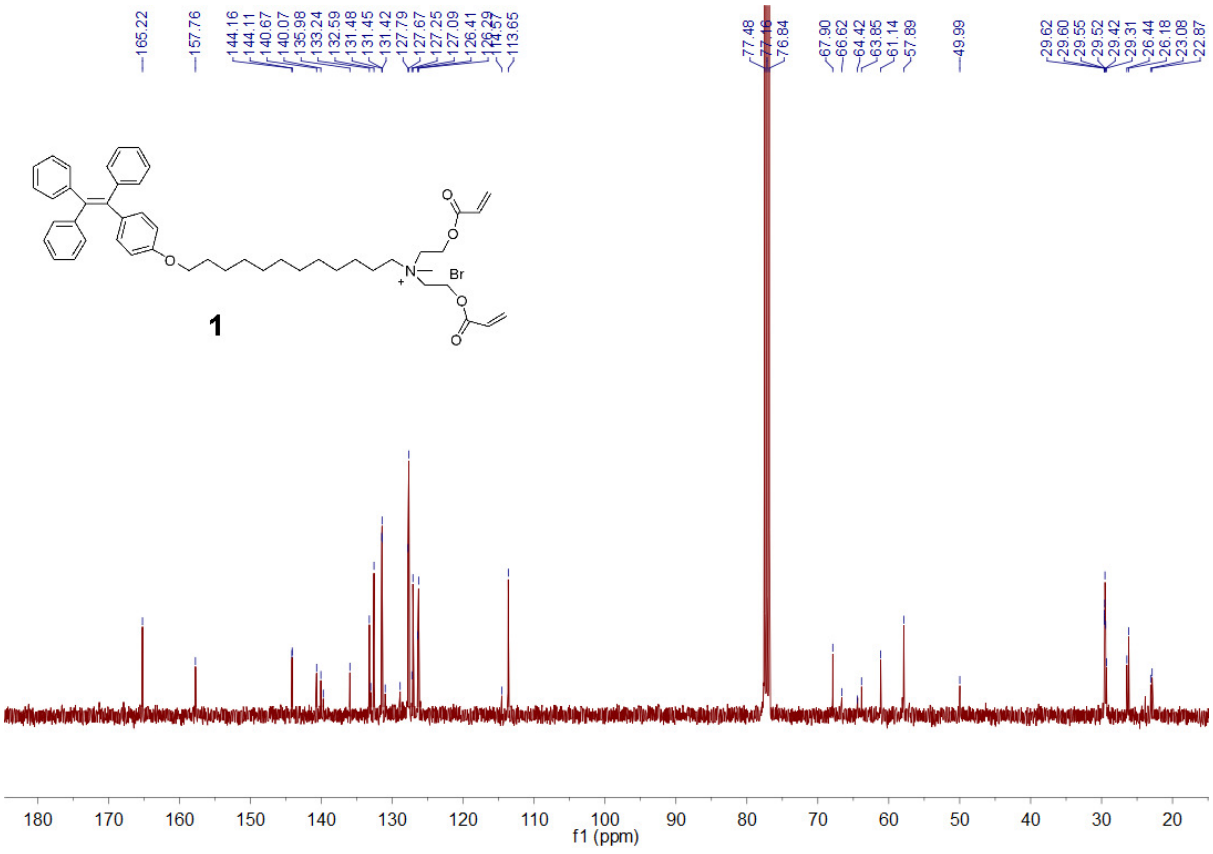
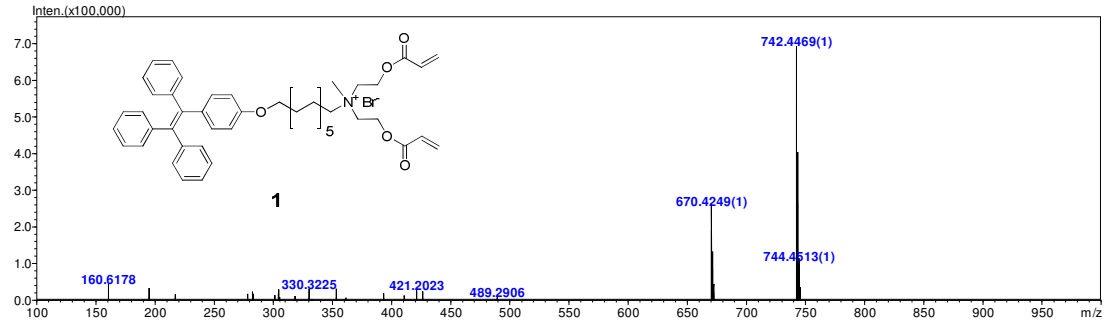
2.03
2.06
2.17
2.30
2.14
4.16
4.19
2.19
2.03
2.60
2.47
18.32
f1 (ppm)





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