Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supporting Information for:

A BODIPY-Based fluorescent dye for mitochondria in living cells, with low cytotoxicity and high photostability

Si Zhang,[a] Tong Wu,[a] Jiangli Fan,[a] Zhiyong Li,[a] Na Jiang,[a] Jingyun Wang,*[b] Bairui Dou,[b] Shiguo Sun,[a] Fengling Song,[a] Xiaojun Peng*[a]

State Key Laboratory of Fine Chemicals, Dalian University of Technology, 2 Linggong Road, Dalian 116024, P.R. China

School of Life Science & Biotechnology, Dalian University of Technology, 2 Linggong Road, Dalian 116024, P.R. China

pengxj@dlut.edu.cn； wangjingyun@dl.cn

Materials and methods

All solvents and other chemicals were of reagent grade, and were used without further purification unless otherwise stated. CCCP was purchased from Acros Organics (USA). Tris base was from Promega Co. (USA). MitoTracker Green FM, MitoTracker Deep Red FM, and MitoTracker Red CXMRos were purchased from Invitrogen (USA). DRAQ5 was purchased from eBioscience (USA). Silica gel (200-300 mesh) and aluminum oxide (neutral. 100-200 mesh) used for flash column chromatography.

Partition Coefficient

The partition coefficient（P）is defined as the ratio of the equilibrium concentions of a dissolved substance in a two-phase system consisting of two largely immiscible solvents. In the case of n-octanol and water:

$$P_{ow} = c_{n\text{-}octanol}/c_{water}$$

P_{ow} values in the range log P_{ow} between -2 and 4 (occasionally up to 5) can be experimentally determined by the shake flask method, as described in the literature[1].

Spectroscopic measurements

Absorption spectra were recorded on an Agilent HP-8453 (Agilent, USA) absorption spectrometer. The steady-state fluorescence emission and excitation spectra were obtained by using a Cray Edipse fluorescence spectrophotometer (Varian, Australia). The fluorescence quantum yields of **OBEP** and **OBP** were determined according to the literature[2]:

$$\Phi_x=\Phi_s(F_x/F_s)(A_s/A_x)(\lambda ex_s/\lambda ex_x)(n_x/n_s)^2$$

Where Φ is quantum yield; F is the integrated area under the corrected emission spectrum; A is the absorbance at the excitation wavelength; λex is the excitation wavelength; n is the refractive index of the solution; the subscripts x and s refer to the unknown and the standard, respectively. Fluorescein (Φ_F = 0.90) in 0.1mol/L NaOH was used as the standard[2]. Mass spectral studies were carried out using LC/Q-Tof mass spectra (Micromass, England). NMR spectra were obtained with a Jasco FP-6500 spectrophotometer (Jasco, Japan).

Photodegradation experiments

The photodegradation test was carried out in square cross-section quartz cells (l × 1 cm) and solutions of the samples were irradiated with a 500W iodine-tungsten lamp at room temperature. To eliminate the heat and absorb short wavelength light, a cold trap (5 L solution of 60 g/L $NaNO_2$ in 10 cm (width) × 30 cm (length) ×20 cm (height) was set up between the cells and the lamp. The distance between the cells and the lamp was 25 cm. The irreversible bleaching of the dyes at the absorption peak was monitored as a function of time. Samples were tightly sealed, but not deoxygenated with nitrogen, before the test.

Cell incubation and imaging

HeLa cells (human cervical carcinoma cells) were cultured in DEME medium supplemented with 10% fetal bovine serum at 37 ℃ in an atmosphere containing 5% CO_2. For live cell imaging, compounds were added to cells grown in a confocal microscope dish for 1 h and washed with PBS (phosphate-buffered saline) three times. After replacement of the medium, cells were imaged using a Leica TCS-SP2 confocal laser scanning microscope with a 200×objective lens (excited with the FITC channel). For subcellular localization analysis of **OBEP** staining, organelle specific fluorescent dyes were used. HeLa cells were first stained with 5 μM of **OBEP** at 37 ℃ in an atmosphere of 5% CO_2 for 1 h, and then washed with PBS three times. Cells were then incubated with MitoTracker Deep Red FM (0.4 μM) at 37 ℃ in an atmosphere of 5% CO_2 for 15 minutes or with DRAQ5 (10 μM) for 40 minutes, and then washed with PBS three times. After replacement of medium, cells were imaged using a Leica TCS-SP2 confocal laser scanning microscope and OLYMPUS FV1000 confocal fluorescence microscope with a 200×objective lens.

Effects of CCCP on uptake of dyes

HeLa cells were treated with 10 μM DMSO, or 10 μM carbonyl cyanide m-chlorophenylhydrazone (CCCP). Thirty minutes after the treatment the cells were stained with 0.4 μM MitoTracker Green FM, 0.2 μM MitoTracker Red CXMRos or 5 μM **OBEP**. MitoTracker Green FM, whose mitochondrial uptake is not dependent dye on mitochondrial membrane potential, stained in the same manner in the absence or presence of CCCP. However the staining given by MitoTracker Red CXMRos, whose mitochondrial uptake is dependent on mitochondrial membrane potential, was decreased in the presence of CCCP, to a level lower than that in the DMSO control. The staining pattern of **OBEP** remained the same in the absence or presence of CCCP, suggesting that the staining properties of

OBEP are independent of the membrane potential.

Cell Cytotoxicity Assay

Toxicities of **OBEP** and MitoTracker Red CXMRos towards HeLa cells were assessed by the MTT cytotoxicity assay. This involves the reduction of MTT tetrazolium to MTT formazan pigment by the metabolic activity of living cells. HeLa cells were seeded at a density of 1×10^5 cells/ml in a 96-well plate. After 24 h of cell attachment, HeLa cells were treated with the two dyes at serial concentrations (0, 0.5, 2, 5, 10 μM) for 12 h. Six replicate wells were used for each control and tested concentrations. After incubation for 12 h, the medium was removed and cells were washed with PBS twice. MTT tetrazolium solution (100 μL of 0.5 mg/ml in PBS) was added to each well, and the cells further incubated at 37 ℃ for 4 h in a 5% CO_2 humidified atmosphere. Excess MTT tetrazolium solution was then carefully removed and the colored formazan was dissolved in 100 μL dimethyl sulfoxide (DMSO). The plate was shaken for 10 minutes and the absorbance was measured at 570 and 630 nm using a microplate reader.

Protocols for dye synthesis

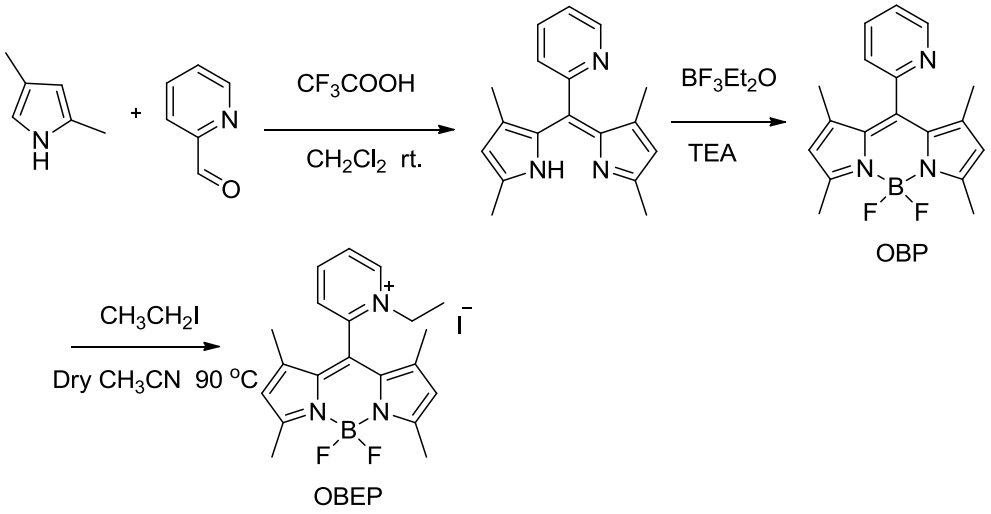


Fig. S1. Synthesis of **OBP** and **OBEP**

To a solution of 2, 4-dimethylpyrrole (1.0 mL, 0.92 g, 8.8 mmol, 2 eq) in dry dichloromethane (DCM, 250 mL), the 2-pyridinecarboxaldehyde (4.4 mmol, 1 eq) was added, followed by a catalytic amount of trifluoroacetic acid (TFA). After overnight stirring of the resulting reddish material under a nitrogen atmosphere, the mixture was concentrated to 50.0 mL under vacuum, and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ, 1.5 eq) was added and stirred for 2 h. Triethylamine (6.0 mL) and $BF_3{\cdot}OEt_2$ (7.0 mL) were then added. After 2 h the mixture was concentrated under vacuum, redissolved in DCM and washed with water. The water layer was extracted with DCM and the combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The mixture was purified by silica flash column chromatography (DCM/methanol=50:1) to yield the product **OBP** (0.47 g, 35% yield). The HPLC purity of **OBP** was determined to be 98.17%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (d, J = 6.1 Hz, 1H), 7.90 (t, J = 7.8 Hz, 1H), 7.50 (m, 2H), 5.99 (s, 2H), 2.56 (s, 6H), 1.32 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ_C 156.34, 153.99, 150.20, 142.55, 138.58, 136.94, 131.41, 124.39, 123.85, 121.24, 14.65, 13.72. HR-TOF-MS Exact mass calculated for $C_{18}H_{18}BN_3F_2$ + H requires 326.1640. Found m/z 326.1650.

To a solution of **OBP** (0.65 g, 2 mmol) in dry acetonitrile, iodoethane (3.12 g, 20 mmol) was added. The mixture was then stirred at 70 ℃ for 5 h under a nitrogen atmosphere. The mixture was cooled to room temperature and concentrated under vacuum. The residue was purified by neutral aluminum oxide flash column chromatography (DCM/ethanol = 50 : 1) to yield **OBEP**(0.56 g, 58% yield). The HPLC purity of **OBEP** was determined to be 99.93%. ^{1}H NMR (400 MHz, D_2O) δ 9.23 (d, J = 5.9 Hz, 1H), 8.73 (t, J = 8.0 Hz, 1H), 8.32 (t, J = 6.9 Hz, 2H), 6.29 (s, 2H), 4.68 (q, J = 7.5 Hz, 2H), 2.51 (s, 6H), 1.50 (t, J = 7.3 Hz, 3H), 1.35 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ_C 159.15, 147.63, 147.25, 146.59, 142.02, 130.79, 129.94, 129.71, 125.87, 123.18, 54.91, 54.17, 15.28, 14.59, 13.12. HR-TOF-MS Exact mass calculated for $C_{20}H_{23}BN_3F_2$ requires 354.1953. Found m/z 354.1945.

All reactions were carried out under nitrogen, and dry molecular sieves were used to remove the water produced during the synthesis of the BODIPY derivatives. CH_2Cl_2 and CH_3CN were dried by distillation from CaH_2 under a nitrogen atmosphere.

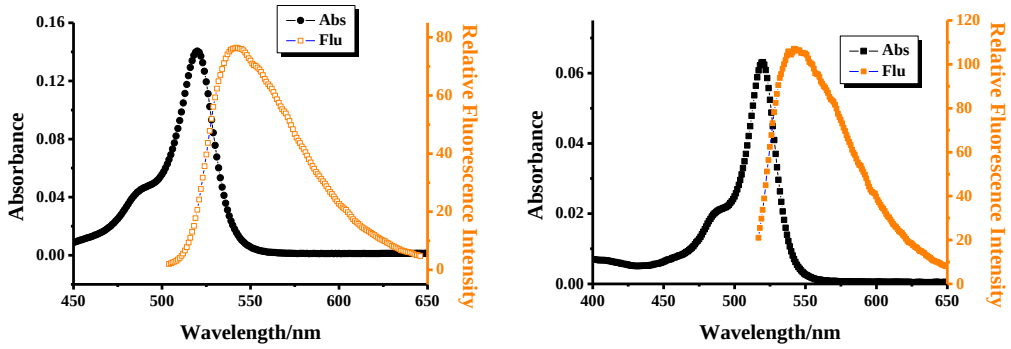


Fig. S2. Absorbance and emission spectra of **OBEP** in Tris-HCl buffer (left) and acetonitrile (right).

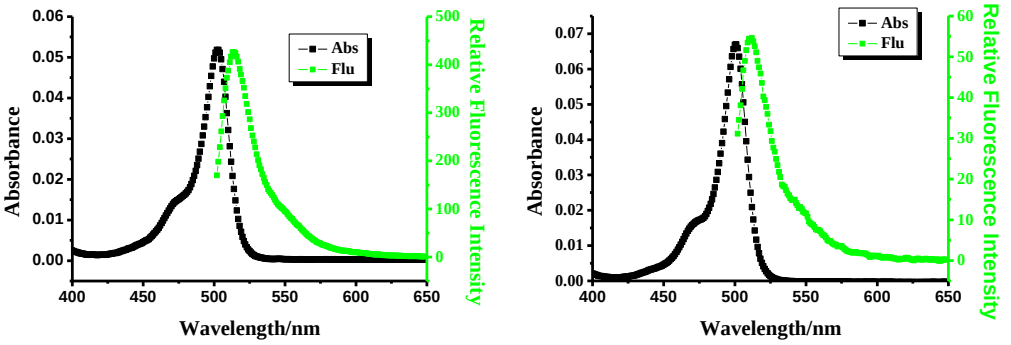


Fig. S3. Absorbance and emission spectra of **OBP** in Tris-HCl buffer (left) and acetonitrile (right).

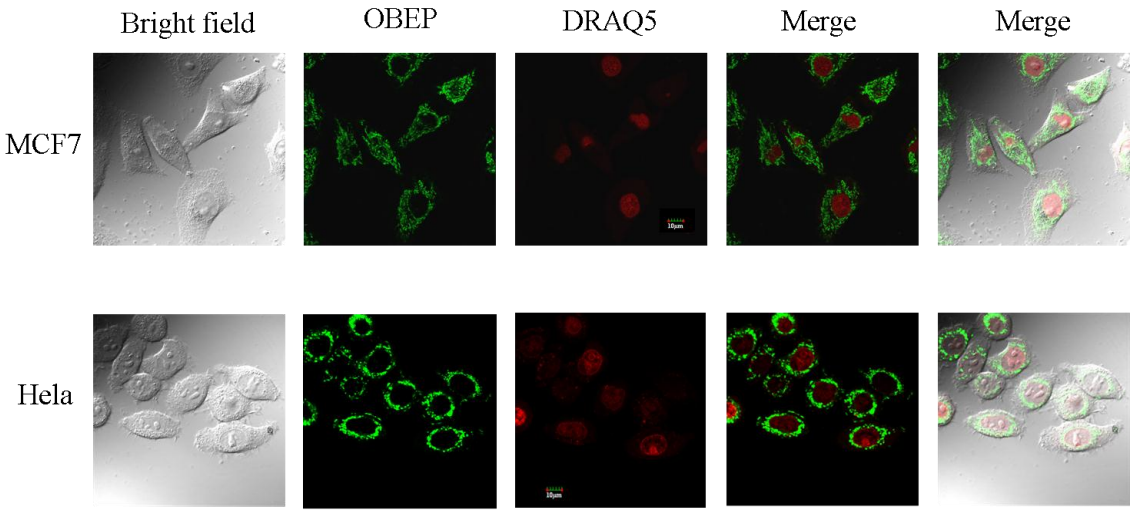


Fig. S4. Fluorescence confocal images of **OBEP** and **DRAQ5** in MCF7 and HeLa cells. The cells were cultured with 5 μM **OBEP** for 1 h and 10 μM **DRAQ5** for 40 min. The images were obtained using the FITC and Cy5 channels. (Scale bar = 10 μm).

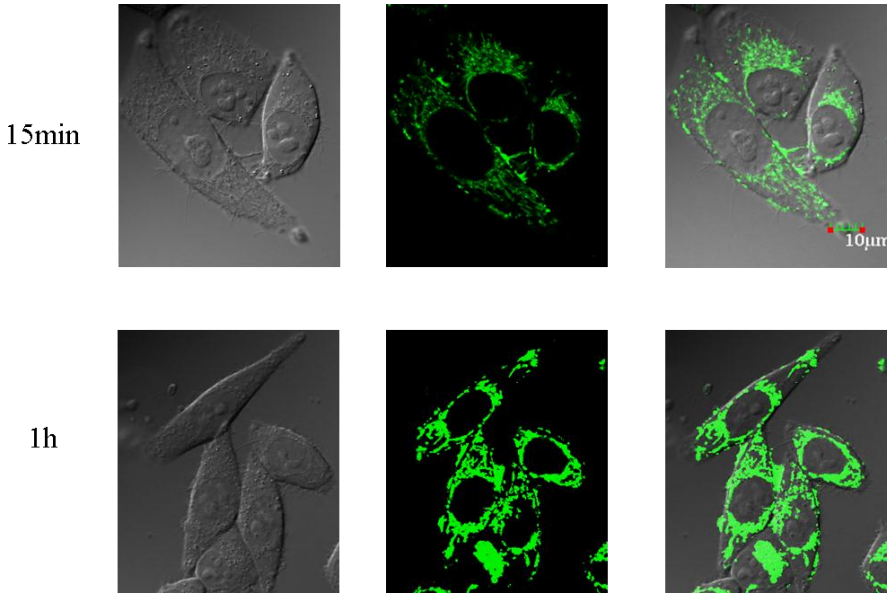


Fig. S5. Fluorescence confocal images of **OBEP,** after different incubation times, in HeLa cells. The cells were cultured with 5 μM OBEP for 15 min (up) or 1 h (down). The images were obtained using the FITC channel. (Scale bar = 10 μ m).

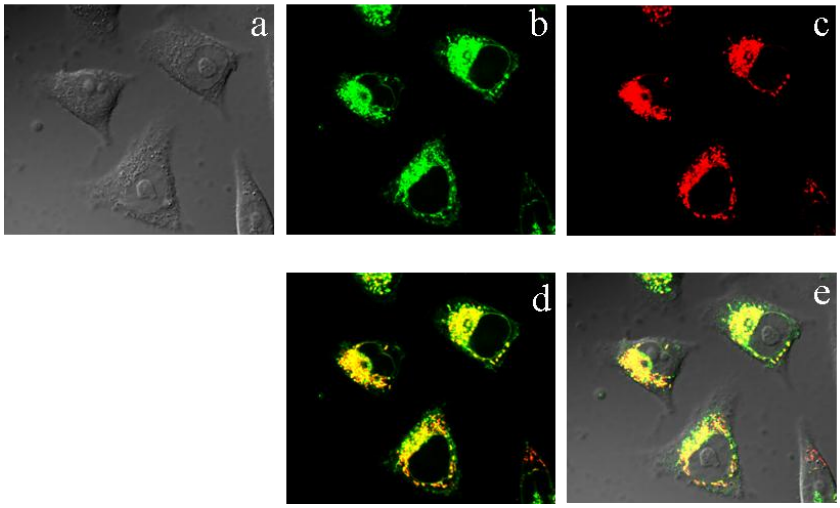


Fig. S6. Fluorescence confocal images of **OBEP** and of the mitochondrially specific dye MitoTracker Deep Red FM in MCF7 cells. (a) Bright field; (b) Fluorescence image of MCF7 cells stained with **OBEP**; (c) Fluorescence image of MCF7 cells stained with MitoTracker Deep Red FM; (d) Merged image of (b) and (c); (e) Merged image of (a), (b) and (c).

^{1}H and ^{13}C NMR spectra

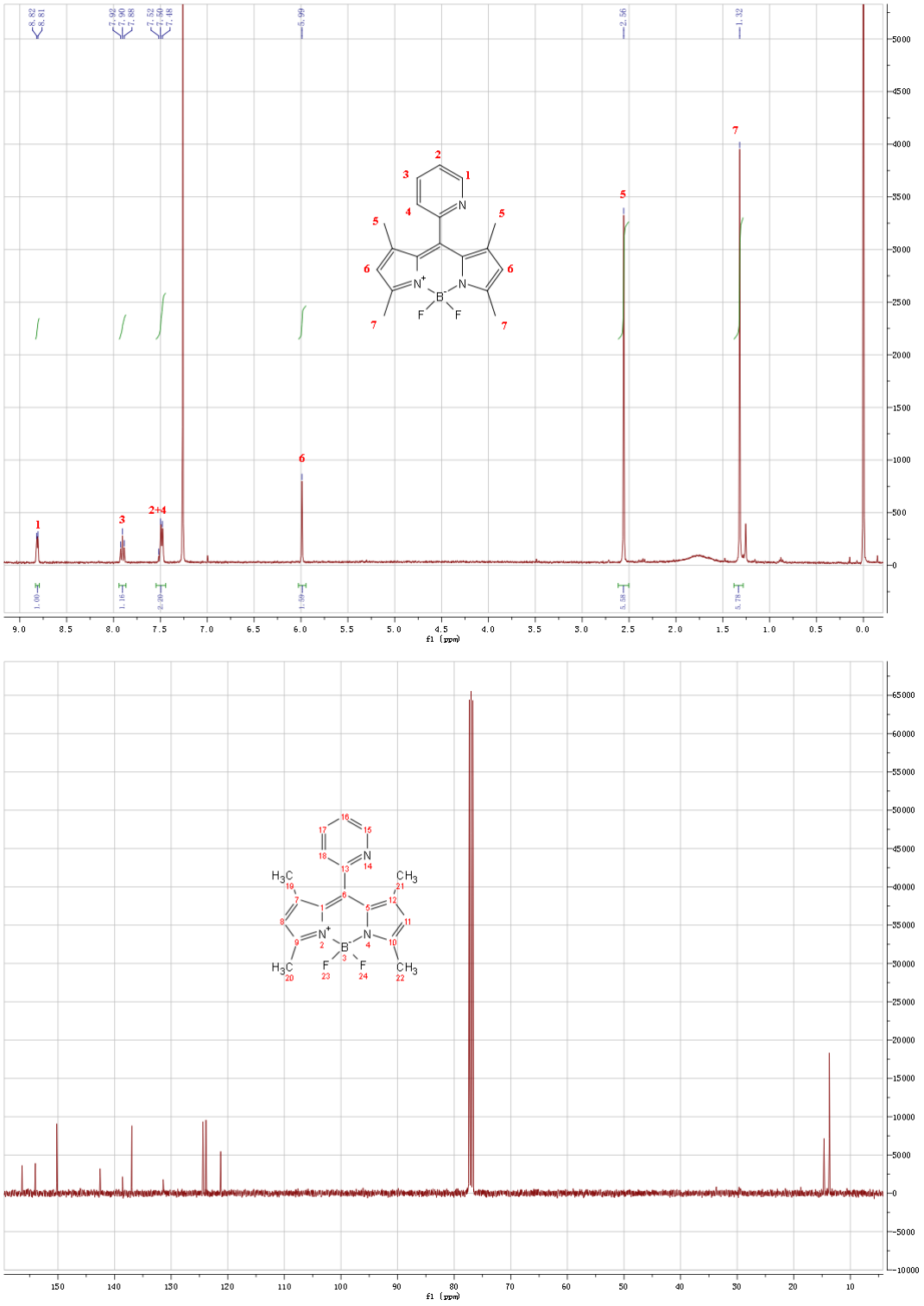


Fig. S7. ^{1}H and ^{13}C NMR spectra of **OBP**

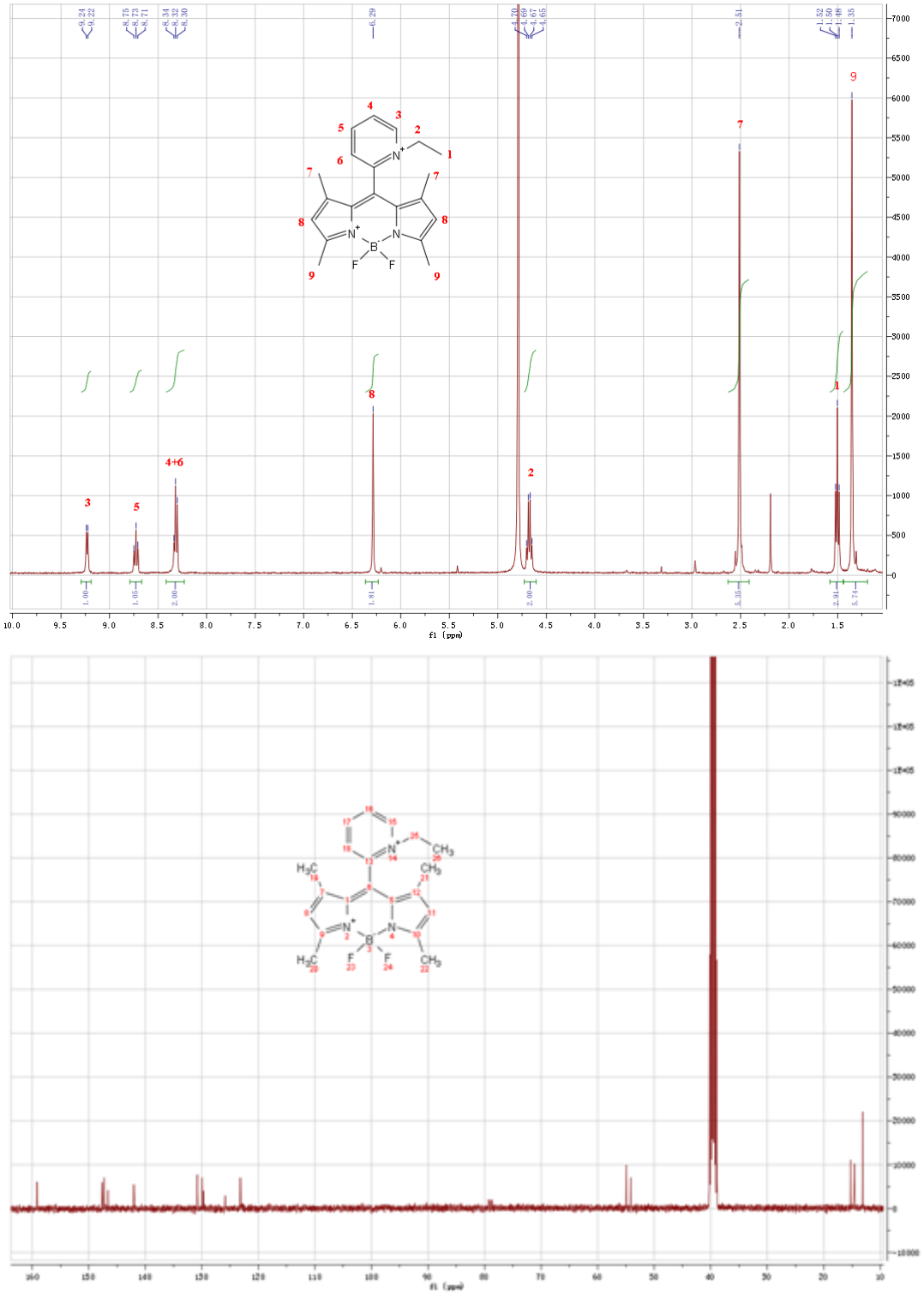
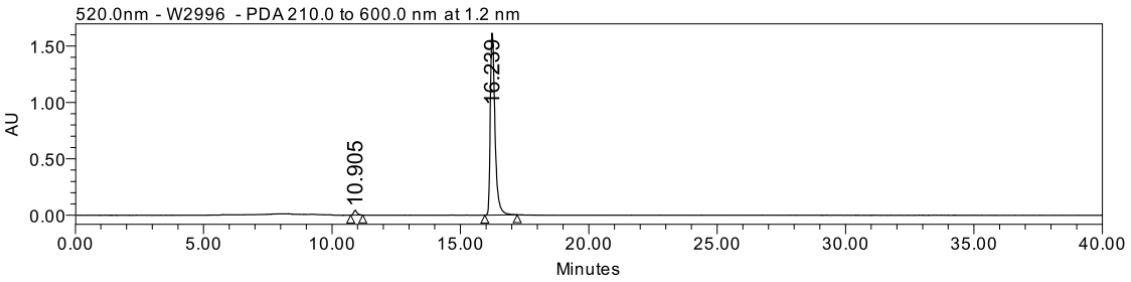


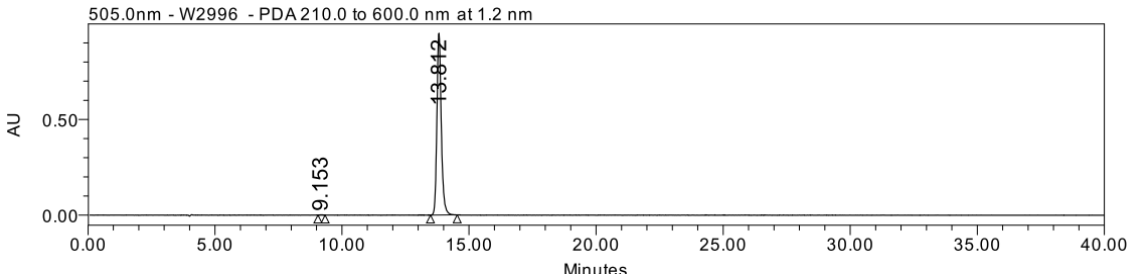
Fig. S8. ^{1}H and ^{13}C NMR spectra of **OBEP**



Processed Channel Descr.: PDA 520.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 520.0 nm	10.905	383770	1.83	41488
2	PDA 520.0 nm	16.239	20566671	98.17	1614742

Fig. S9 The HPLC purity of **OBP**.



Processed Channel Descr.: PDA 505.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 505.0 nm	9.153	7443	0.07	947
2	PDA 505.0 nm	13.812	11348917	99.93	952747

Fig. S10 The HPLC purity of **OBEP**.

1. M. R. Gill, J. Garcia-Lara, S. J. Foster, C. Smythe, G. Battaglia and J. A. Thomas, *Nature Chemistry*, 2009, **1**, 662-667.
2. R. A. Velapoldi and H. H. Tonnesen, *J. Fluoresc.*, 2004, **14**, 465-472.