Electronic Supplementary Material (ESI) for Chemical Communications
This journal is © The Royal Society of Chemistry 2013

Electronic Supporting Information

A near-infrared dye based on BODIPY for tracking morphology changes in mitochondria

5 Na Jiang,[a] Jiangli Fan,[*a] Tao Liu,[a] Jianfang Cao,[a] Bo Qiao,[a] Jingyun Wang,[b] Pan Gao,[b] and Xiaojun Peng[*a]

[a] State Key Laboratory of Fine Chemicals, Dalian University of Technology, Dalian 116024, China [b] School of Life Science and Biotechnology, Dalian University of Technology, Dalian 116024, China

10 fanjl@dlut.edu.cn; pengxj@dlut.edu.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). MitoTracker Green FM, MitoTracker Deep Red FM, and LysoTracker 15 Green DND were purchased from Invitrogen (USA). Silica gel (200-300 mesh) and aluminum oxide (neutral. 100-200 mesh) used for flash column chromatography. **DPT** and **M-DPT** were dissolved in dimethyl sulphoxide (DMSO) to produce 1 mM stock solutions. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a VARIAN INOVA-400 spectrometer. Chemical shifts (δ) were reported as ppm (in $CDCl_3$ or CD_3SOCD_3, with TMS as the internal standard). Mass spectrometric (MS) data were obtained with HP1100LC/MSD MS and an LC/Q-TOF-MS instruments. Fluorescence measurements were 20 performed on a VAEIAN CARY Eclipse fluorescence spectrophotometer (Serial No. FL0812-M018). Excitation and emission slit widths were modified to adjust the fluorescence intensity to a suitable range. Absorption spectra were measured on a Perkin Elmer Lambda 35 UV/VIS spectrophotometer. All pH measurements were performed using a Model PHS-3C meter.

25 **Absorption and Fluorescence Quantum Yields Measurements.**

Mass spectral studies were carried out using LC/Q-Tof mass spectrometer. NMR spectra were obtained using a Varian INOVA 400 MHz spectrometer. Visible absorption spectra were measured on a Lamda LS35 spectrophotometer. Fluorescence spectra were obtained with a FP-6500 spectrophotometer (Jasco, Japan). The relative fluorescence quantum yields were determined with Rhodamine B (Φ_F = 0.97) in ethanol as a standard and calculated using the following 30 equation[s1]:

$$\Phi_x = \Phi_s (F_x / F_s) (A_s / A_x) (\lambda_{exs}/\lambda_{exx}) (n_x/n_s)^2$$

Where Φ represents quantum yield; F stands for integrated area under the corrected emission spectrum; A is absorbance at the excitation wavelength; λ_{ex} is the excitation wavelength; n is the refractive index of the solution (because of the low concentrations of the solutions (10^{-7}-10^{-8} mol/L), the refractive indices of the solutions were replaced with those of the 35 solvents); and the subscripts x and s refer to the unknown and the standard, respectively.

Partition Coefficient.

The partition coefficient (P) is defined as the ratio of the equilibrium concentrations 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} = cn-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[s2].

5

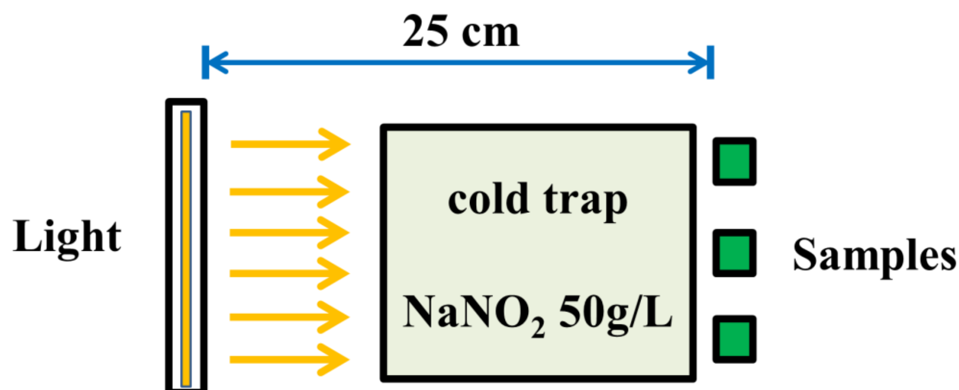
Polarity interference experiment.

The solvents were obtained by mixing deionized water-1, 4-dioxane system in different proportions. The stock solution of **M-DPT** (1.0 mM, 2.0 μL) was added into the system above. The solution obtained was left to stand to eliminate air bubbles, and measured in the fluorescence spectrophotometer.

10

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 500 W 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 15 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.



20

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, 25 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 **M-DPT** staining, organelle specific fluorescent dyes were used. HeLa cells were first stained with 0.5 μM of **M-DPT** 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 Green FM (0.5 μM) at 37 ℃ in an atmosphere of 5% CO_2 for 15 minutes or with Lyso Tracker Green DND (1.0 μM) for 10 minute, and then washed with PBS three times. After 30 replacement of medium, cells were imaged using OLYMPUS FV1000 confocal fluorescence microscope with a

200×objective lens.

Cytotoxicity determined by MTT method.

HeLa cells were prepared for cell viability studies in 96-well plates (1 × 105 cellsper well that were incubated in 100 μL). 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.

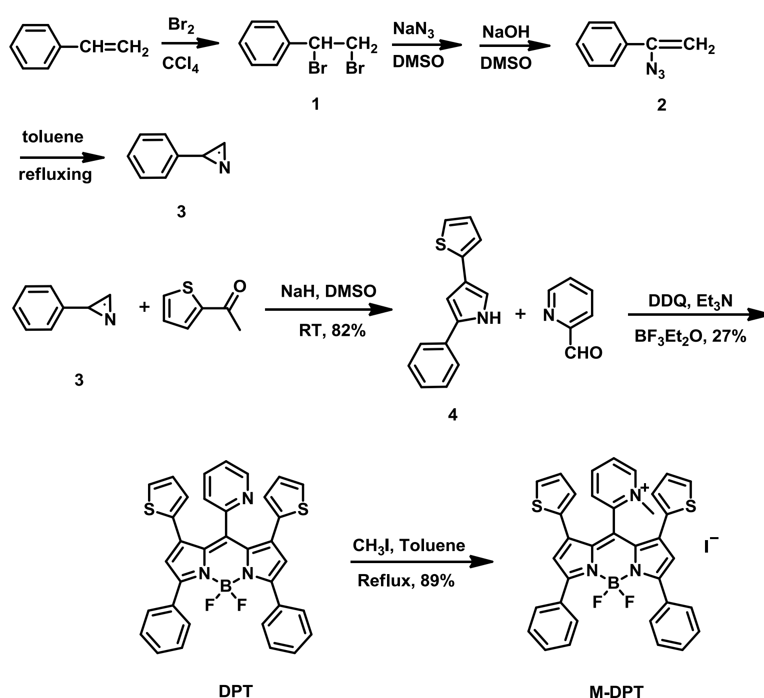
Effects of CCCP on uptake of dyes.

MCF-7 cells were treated with 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 0.5 μM **M-DPT**. Mito Tracker 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. The staining pattern of **M-DPT** remained the same in the absence or presence of CCCP, suggesting that the staining properties of **M-DPT** are independent of the membrane potential.

Quantum calculation.

All of the quantum-chemical calculations were done with the Gaussian 09 suite. The geometry of the molecules at the ground state (S_0) and the S_1 excited state was optimized by density functional theory (DFT) and time-dependent DFT (TDDFT) methods with B3-LYP functional and 6-31G ** basis set. No constraints to bonds/ angles/dihedral angles were applied in the calculations and all atoms were free to optimize.

Synthetic procedures of dye and intermediates.



Scheme S1. The synthetic routes of the dyes.

Synthetic routes of **3** [S3]

Bromine (4.0 g, 0.05 mol) in 15 ml of CCl_4, was added slowly to a cooled (15-20°) solution of styrene (2.6 g, 25 mmol) in 20 ml of CCl_4. After stirred for 2 h, the CCl_4 was removed in vacuo and got the crystalline **1** (1,2-dibromostyrene). Then **1** was dissolved in 35 ml of DMSO. Next 2.45 g (37.5 mol) of sodium azide was added at 15-20° under N_2. The mixture became thick with precipitated azido bromide and was stirred for a further 13 h at RT (room temperature). After cooling to 12° the reaction mixture was treated with a solution of 1.0 g NaOH in 1.0 ml of H_2O. Stirring was continued at RT for another 24 h. The mixture was poured into 100 mL 2% $NaHCO_3$ solution and extracted with CH_2Cl_2. Then evaporated to yield crude 1-azidostyrene as a red oil. The oil was passed through a column of alumina using petroleum ether as an eluent. The eluate was evaporated and got the residual pale yellow oil **2**. Next 2 was dissolved in 50 mL toluene and the solution was refluxed (4h) until the evolution of nitrogen ceased. Removal of the solvent and distillation of the crude product **3** (1.75g, yield 61%).

Synthetic routes of **4**

2-Acetylthiophene (1.27 g, 10 mmol) and NaH (purity: 60%, 0.4 g, 10 mmol) were dissolved in 10 ml DMSO in the ice bath. 2-phenyl-1-azirine (1.13 g, 10 mmol) was then added into the mixture with the colour becomes purple rapidly. After stirred for 6 h at RT, the resulting mixture was poured into 150 ml ice water. The crude brown solid was obtained by filtration and dried under vacuum. Then **2** was purified by silica column chromatography using CH_2Cl_2 /Petroleum ether (1:1) mixture as eluent, yielding white powder (1.84 g, 82%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 8.32(s, 1H), 7.56 (d, 2H, J = 8.0 Hz), 7.35 (t, 2H, J = 8.0 Hz), 7.22-7.19 (m, 2H), 7.10-7.08 (m, 2H), 7.05-7.03 (m, 1H), 6.72-6.70 (m, 1H). m/z (TOF-LD): Calcd. M^- For $C_{14}H_{11}NS$: 224.0534, found: 224.0535.

Synthetic routes of **DPT**.

2-Pyridinecarboxaldehyde (107 mg, 1 mmol) and **2** (450 mg, 2 mmol) in deoxygenated CH_2Cl_2 (50 ml), several drops of TFA was added and the solution was stirred overnight under N_2 at RT. Then the mixture was treated with DDQ (228 mg, 1 mmol) for 1 h followed by the addition of Et_3N (15 ml). $BF_3 \cdot Et_2O$ (15 ml) was added slowly 30 min later and the stirring was continued for further 24 h. After the mixture washed with water, the organic layer was dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product. Purification by column chromatography on silica gel eluting with CH_2Cl_2/EtOAc (4:1) provided a dark blue solid (157.4 mg, 27%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS): δ 8.28 (d, 2H, J = 4.0 Hz), 7.75 (d, 1H, J = 8.0 Hz), 7.52 (d, 2H, J = 4.0 Hz), 7.23 (t, 2H, J = 4.0 Hz), 6.92-6.83 (m, 7H), 7.77-6.74 (m, 7H), 6.52-6.49 (m, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 150.2, 148.8, 148.2, 146.7, 135.3, 134.04, 134.00, 131.9, 131.8, 131.7, 130.0, 129.1, 128.5, 127.9, 127.5, 126.4, 123.4, 123.1.

m/z (HR-MALDI-MS): Calcd. M^+ For $C_{34}H_{22}BF_2N_3S_2$: 586.1395, found: m/z = 586.1441

.

Synthesis of **M-DPT**.

M-DPT was synthesized by mixing **DPT** (59 mg, 0.1mmol) and iodomethane (140 g, 1mmol) in toluene (5 ml) and heating to reflux for 5 h. After the solution cooled down, the crude product was evaporated and purified by silica column chromatography using CH_2Cl_2/CH_3OH (10:1) mixture as eluent, yielding green solid (69 mg, 89%). ^{1}H NMR (400 MHz, $CDCl_3$, TMS) : δ 8.36 (d, 2H, J = 12.0 Hz), 8.24 (d, 1H, J = 4.0 Hz), 7.95 (d, 2H, J = 8.0 Hz), 7.88 (d, 2H, J = 8.0 Hz), 7.83-7.79 (m, 1H), 7.42-7.38 (m, 1H), 7.32 (t, 2H, J = 4.0 Hz), 7.08-6.94 (m, 12H) . ^{13}C NMR (100 MHz, $CDCl_3$, TMS): δ 151.5, 147.9, 146.6, 141.7, 139.4, 133.8, 132.6, 132.2, 129.8, 128.7, 128.3, 124.5.

m/z (TOF-LD): Calcd. M^+ For $C_{35}H_{26}BF_2N_3S_2$: m/z = 600.1551, found: 600.1557.

Table S1. Spectral Data of the Dye **DPT** and **M-DPT** in Different Solvents

	DPT					**M-DPT**				
solvents	λ_{abs}[a]	λ_{em}[b]	$\Delta\lambda$[c]	ε[d]	Φ_f[e]	λ_{abs}[a]	λ_{em}[b]	$\Delta\lambda$[c]	ε[d]	Φ_f[e]
DCM	630	655	25	3.92	0.57	670	718	48	0.70	0.18
CH_3CN	621	650	29	3.67	0.54	655	697	42	0.67	0.31
DMSO	635	661	26	4.40	0.52	665	706	41	0.72	0.26
EtOH	629	653	24	5.05	0.47	661	701	40	0.71	0.23
H_2O	---	---	---	---	---	656	698	32	0.61	0.17

[a] The absorption peaks of dyes (nm). [b] The fluorescence emission peaks of dyes (nm). **DPT** and **M-DPT** were excited at 570 nm and 630 nm respectively. [c] Stocks shift (nm) [d] $\times 10^5 mol^{-1} cm^{-1}$ L. [e] Rhodamine B was used as a standard reference with a fluorescence quantum yield of 0.97 in ethanol.

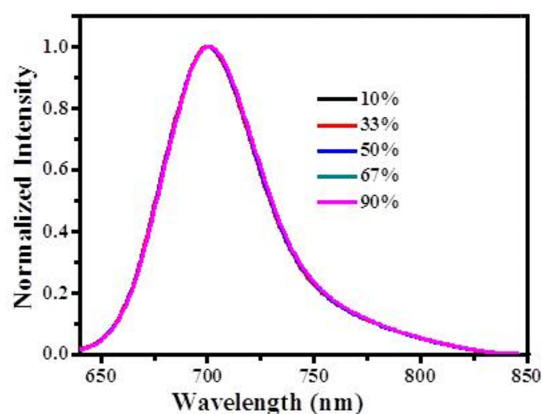


Figure S1. Fluorescence spectra of **M-DPT** (2 μM) in different proportional water-1, 4-dioxane system, excited at 630 nm. Volume fractions of 1, 4-dioxane were 10%, 33%, 50%, 67%, 90% respectively.

5

Table S2. The theoretical calculation results of **DPT** and **M-DPT**

Molecular		**DPT**	**M-DPT**
	HOMO	-5.53	-6.02
Ground State (eV)	LUMO	-2.99	-3.66
	Energy gap	2.54	2.36
	HOMO	-5.38	-5.88
Excited State(eV)	LUMO	-3.09	-3.88
	Energy gap	2.29	2.00

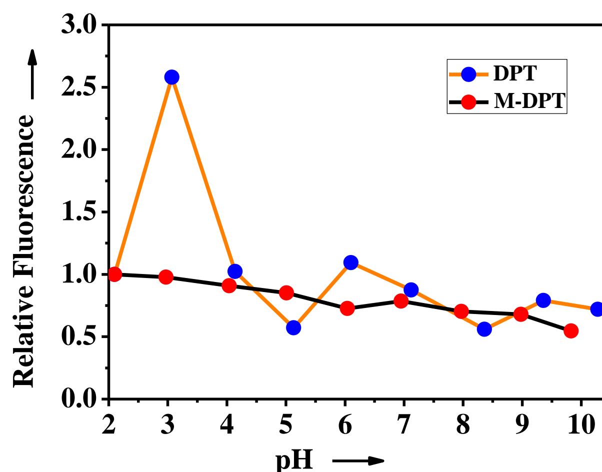


Figure S2. The fluorescence intensity of **M-DPT** (2 μM) or **DPT** (2 μM) with different pHs. **DPT** was in H_2O/
10 C_2H_5OH=1:1, **M-DPT** in H_2O.

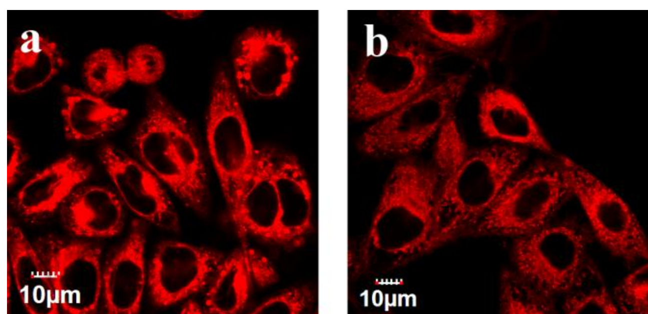
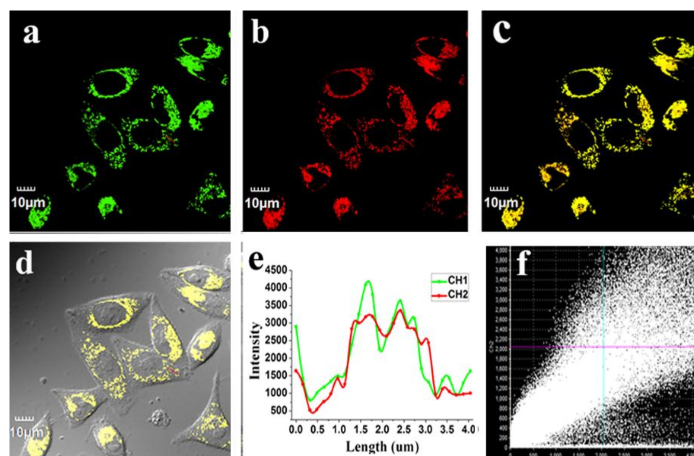
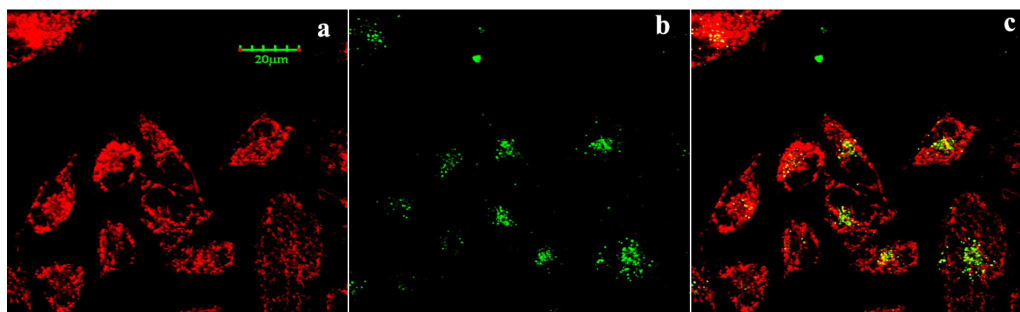


Figure S3. Confocal fluorescence images of **DPT** (a) or **M-DPT** (b) in MCF-7 cells. The cells were incubated with **DPT** (1.0 μM) or **M-DPT** (1.0 μM) for 15 min.



5 **Figure S4.** Confocal fluorescence images of MCF-7 cells stained with (a) Mito Tracker Green FM (2.0 μM) for 15 min and (b) **M-DPT** (0.8 μM) for 15 min. (c) Merged image of a and b. (d) Merged image of c and bright-field image. (e) Intensity profile of ROIs across MCF-7 cells. (f) Correlation plot of Mito Tracker Green FM and **M-DPT** intensities.



10

Figure S5. Fluorescence confocal images of **M-DPT** and LysoTracker Green DND in MCF-7 cells. (a) Fluorescence image of MCF-7 cells stained with **M-DPT**; (b) fluorescence image of MCF-7 cells stained with LysoTracker Green DND; (c) merged image of (a) and (b).

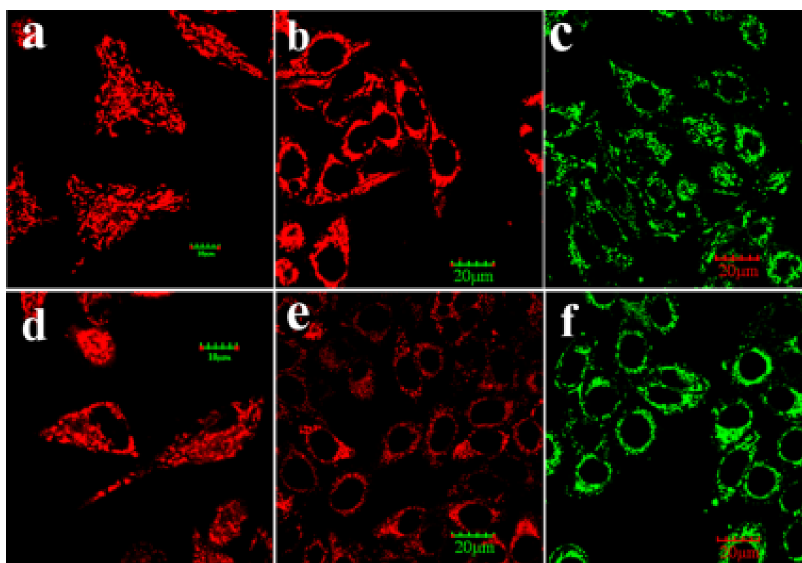


Figure S6. Staining patterns of **M-DPT** and mitochondrially specific dyes in MCF-7 cells. The cells were incubated in the absence (a, b, c) or presence (d, e, f) of 10 μM CCCP for 30 min, and then stained with 0.4 μM MitoTracker Green FM for 15 min, 0.2 μM MitoTracker Red CXMRos for 15 min, and 1.0 μM **M-DPT** for 15 min.

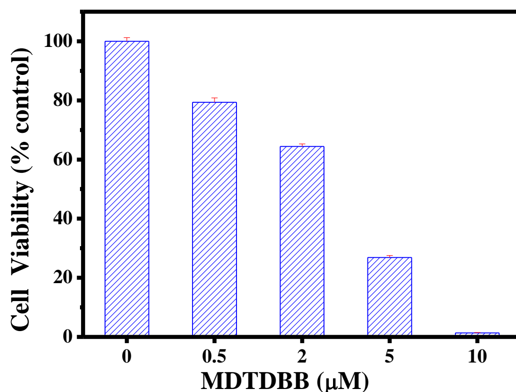


Figure S7. Cytotoxicity of **M-DPT** at various concentrations (0.5 μM, 2 μM, 5 μM, 10 μM) in living HeLa cells for 12 h.

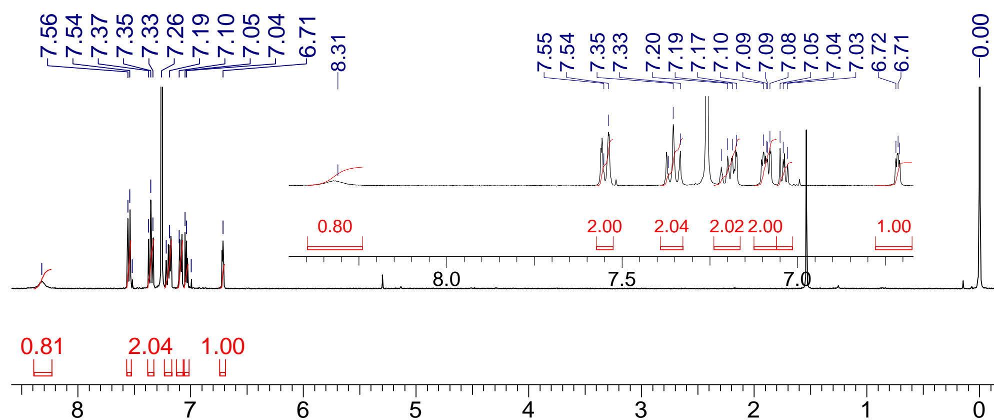
References:

S1. Velapoldi R, Tønnese H., *Journal of Fluorescence*, 2004, **14**, 465-472.

S2. M. R. Gill, J. Garacia-Lara, S. J. Foster, C. Smythe, G. Battaglia and J. A. Thomas, *Nature Chemistry*, 2009, **1**,662-667.

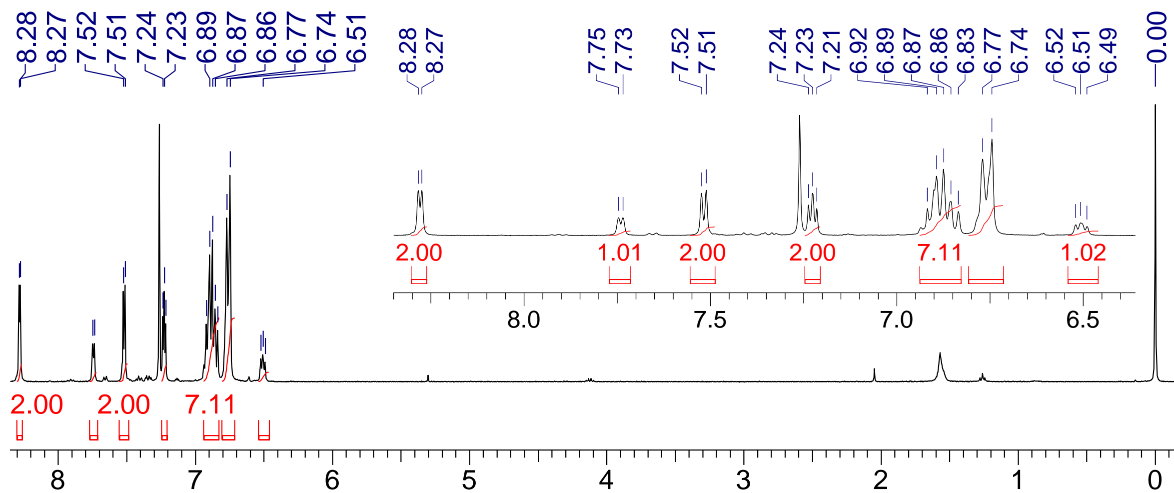
S3. Hortmann A, Robertson D, Gillard B., *J. Org. Chem.*, 1972, **37**, 322-324.

^{1}H-NMR spectra of **4**



5

^{1}H-NMR spectra of **DPT**



10

^{13}C-NMR spectra of **DPT**