Supporting Information for

Copper-mediated phospha-annulation to attain water-soluble polycyclic luminophores

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Experimental

General comments

9,10-bis(2-bromophenyl)anthracene was synthesized according to the published procedure.[1] Tetrahydrofuran (THF) was distilled over Na-benzophenoneketyl under a nitrogen atmosphere prior to use. Other reagents and solvents were used as received. The solution ^{1}H, ^{31}P$\{^1$H$\}$ and ^{13}C NMR spectra were recorded on Bruker Avance 400 and AMX-400 spectrometers. Mass spectra were measured on a Bruker MaXis instrument in the ESI$^+$ mode. Microanalyses were carried out in the analytical laboratory of the University of Eastern Finland.

9,10-Bis(2-diphenylphosphinophenyl)anthracene (dpa***P²***). The synthesis was carried out under a nitrogen atmosphere. A solution of 9,10-bis(2-bromophenyl)anthracene (600 mg, 1.23 mmol) in THF (100 ml) was cooled to −78 °C, and a 1.7 M pentane solution of *t*-BuLi (1.7 ml, 2.90 mmol) was added dropwise within 10 min. to give a light yellow solution. It was stirred for 30 min. at −60 °C and then treated dropwise with neat PPh$_2$Cl (540 mg, 2.46 mmol). The reaction mixture was allowed to reach room temperature and was stirred for 1 hour, quenched with methanol (5 ml) and evaporated. The resulting pale solid residue was washed with methanol (3 x 20 ml), diethyl ether (2 x 10 ml) and dried *in vacuo* to afford dpa***P²*** as a colorless microcrystalline material (640 mg, 74 %). ^{1}H NMR (CDCl$_3$, 298 K; δ): 7.56–7.60 (m, 2H, -C$_6$H$_4$-), 7.44–7.54 (m, 6H, -C$_6$H$_4$-), 7.30–7.33 (m, 4H, -anthracene-), 7.15–7.22 (m, 12H, *para+ortho* H -PPh$_2$), 7.05–7.09 (m, 8H, *meta* H -PPh$_2$), 7.01–7.04 (m, 4H, -anthracene-). ^{13}C$\{^1$H$\}$ NMR (CDCl$_3$; 298 K; δ): 146.1 (d, *J*=33.1 Hz), 140.2 (d, *J*=12.8 Hz), 138.0, 137.9, 137.1, 134.9, 134.2 (d, *J*=20.2 Hz), 132.6 (d, *J*=5.9 Hz), 130.6 (d, *J*=4.9 Hz), 129.6, 128.6,

127.4, 125.1. $^{31}P\{^1H\}$ NMR (CDCl$_3$; 298 K; δ): -14.3 (s, 2P, PPh$_2$). Anal. Calcd for C$_{50}$H$_{36}$P$_2$: C, 85.94; H, 5.19. Found: C, 86.10; H, 5.01.

9,10-Bis(2-diphenylphosphinophenyl)anthracene dioxide (1). A solution of dpa*P^2* (150 mg, 0.21 mmol) in dichloromethane (20 ml) was treated with an excess of hydrogen peroxide (30% aqueous solution, 1 ml) at room temperature for 2 h under vigorous stirring. The reaction mixture was washed with water (10 ml), dried over anhydrous sodium sulfate and dried *in vacuo*. Chromatography purification (Silica gel 70-230 mesh, eluent dichloromethane-methanol, 9:1 v/v mixture) afforded colourless solid of **1** (140 mg, 91 %). ^1H NMR (CD$_2$Cl$_2$, 298 K; δ): 7.79–7.85 (m, 4H, -C$_6$H$_4$-), 7.63–7.67 (m, 2H, -C$_6$H$_4$-), 7.38–7.41 (m, 2H, -C$_6$H$_4$-), 7.24–7.28 (m, 4H, -anthracene-), 7.14–7.23 (m, 16H, -anthracene-, *para+meta* H -PPh$_2$), 6.98–7.03 (m, 8H, *ortho* H -PPh$_2$). $^{13}C\{^1H\}$ NMR (CD$_2$Cl$_2$; 298 K; δ): 143.6 (d, *J*=9.1 Hz), 136.5, 134.0, 132.9, 131.6 (d, *J*=9.4 Hz), 131.2 (d, *J*=4.8 Hz), 130.4, 127.6, 128.0, 127.4, 125.0. $^{31}P\{^1H\}$ NMR (CD$_2$Cl$_2$; 298 K; δ): 25.7 (s, 2P, PPh$_2$O). Anal. Calcd for C$_{50}$H$_{36}$P$_2$O$_2$: C, 82.18; H, 4.97. Found: C, 81.98; H, 4.81.

9,10-Bis(2-diphenylmethylphosphoniumphenyl)anthracene diodide salt (2). A solution of dpa*P^2* (160 mg, 0.23 mmol) in dichloromethane (3 ml) was stirred with an excess of methyl iodide (570 mg, 4.00 mmol) at room temperature for 3 h resulting in a colourless suspension. The reaction mixture was cooled to 0 ℃, the precipitate was collected, washed with diethyl ether (2 x 10 ml) and dried *in vacuo* to afford colourless microcrystalline material (198 mg, 88 %). Recrystallization from hot chloroform produced the crystals suitable for X-ray diffraction analysis. ESI-MS (*m/z*): [M]$^{2+}$ 364.1372 (calcd 364.1375). ^1H NMR (CD$_3$CN, 298 K; δ): 8.13–8.20 (m, 2H, -Ph-), 7.90–7.96 (m, 4H, -C$_6$H$_4$-), 7.65–7.68 (m, 2H, -C$_6$H$_4$-), 7.58 (t, J_{HH} = 8.6 Hz, 4H, *para* H -PPh$_2$), 7.14–7.32 (m, 24H, -anthracene-, *ortho+meta* H -PPh$_2$), 1.87 (d, J_{HP} = 13.6 Hz, 6H, -Me). $^{13}C\{^1H\}$ NMR (CD$_3$CN; 298 K; δ): 144.5, 136.9 (d, *J*=11.9 Hz), 136.0, 134.9, 133.9, 132.93 (d, *J*=10.0 Hz), 129.7, 127.4, 126.6, 120.2, 119.3. $^{31}P\{^1H\}$ NMR (CD$_3$CN; 298 K; δ): 20.8 (s, 2P, PPh$_2$Me$^+$). Anal. Calcd for C$_{52}$H$_{42}$P$_2$I$_2$: C, 63.56; H, 4.31. Found: C, 63.48; H, 4.42.

Cyclophosphonium-dpa phosphine oxide chloride (3). CuCl$_2$·2H$_2$O (68 mg, 0.40 mmol) was added to the degassed solution of dpa*P^2* (250 mg, 0.36 mmol) in dichloromethane-methanol (2:1 v/v mixture, 15 ml). The resulting orange reaction mixture was stirred for 30 min. at room temperature under a nitrogen atmosphere, filtered through a pad of Silica gel (70-230 mesh, 2×2 cm, eluent dichloromethane-methanol, 8:2 v/v mixture) and evaporated. The orange residue was dissolved in dichloromethane (10 ml) and an excess of hydrogen peroxide (30% aqueous solution, 1 ml) was added. After stirring at room temperature for 2 h the solvents were removed. The residue was purified by two-step column chromatography (Silica gel 70-230 mesh, 20×3 cm,

eluent dichloromethane-methanol, 98:2 → 93:7 v/v mixture; then aluminium oxide neutral 150 mesh, 15×3 cm, eluent dichloromethane-methanol, 98:2 v/v mixture) to afford salt **3** as an orange solid (124 mg, 46 %). ESI-MS (m/z): $[M]^{1+}$ 713.2208 (calcd 713.2158). ^{1}H NMR (CD$_2$Cl$_2$, 298 K; δ): 8.47 (d, J_{HH} = 8.9 Hz, 1H), 8.29 (dd, J_{HH} = 8.1, 5.6 Hz, 1H), 7.15–8.09 (br unresolved m, 31H), 7.09 (br s, 2H). ^{1}H NMR (D$_2$O, 298 K; δ): 6.50–7.88 (br unresolved m, 34H), 6.14 (br s, 1H). ^{13}C{^{1}H} NMR (CD$_2$Cl$_2$; 298 K; δ): 141.4, 140.4, 137.5, 136.2, 135.9, 134.5, 133.4, 132.7. 132.2, 130.7, 130.0 (d, J=8.5 Hz), 129.6, 129.2, 128.8 (d, J=10.4 Hz), 127.4 (d, J=8.3 Hz), 127.0, 126.6. ^{31}P{^{1}H} NMR (CD$_2$Cl$_2$; 298 K; δ): 24.0 (s, 1P, PPh$_2$O), 4.1 (s, 1P, P$_{cyclo}$Ph$_2$$^+$), ^{31}P{^{1}H} NMR (D$_2$O; 298 K; δ): 31.5 (s, 1P, PPh$_2$O), 4.6 (s, 1P, P$_{cyclo}$Ph$_2$$^+$). Anal. Calcd for C$_{50}H_{35}P_2$ClO: C, 80.16; H, 4.71. Found: C, 80.02; H, 4.92.

Cyclophosphonium-dpa phosphonium dichloride (4). CuCl$_2$·2H$_2$O (68 mg, 0.40 mmol) was added to the degassed solution of dpa*P^2* (250 mg, 0.36 mmol) in dichloromethane-methanol (2:1 v/v mixture, 15 ml). The resulting orange reaction mixture was stirred for 30 min. at room temperature under a nitrogen atmosphere, filtered through a pad of Silica gel (70-230 mesh, 2×2 cm, eluent dichloromethane-methanol, 8:2 v/v mixture) and evaporated. The orange residue was dissolved in dichloromethane (10 ml) and an excess of methyl iodide (710 mg, 5.00 mmol) was added. The mixture was stirred under a nitrogen atmosphere for 12 h, and then treated with a solution of sodium chloride (580 mg, 10 mmol) in methanol (5 ml). The solvents were removed and the residue was purified by two-step column chromatography (Silica gel 70-230 mesh, 20×3 cm, eluent dichloromethane-methanol, 98:2 → 93:7 v/v mixture; then aluminium oxide neutral 150 mesh, 15×3 cm, eluent dichloromethane-methanol, 98:2 v/v mixture) to afford salt **4** as a brown amorphous solid (90 mg, 32 %). ESI-MS (m/z): $[M]^{2+}$ 356.1250 (calcd 356.1219). ^{1}H NMR (CD$_2$Cl$_2$, 298 K; δ): 8.56 (d, J_{HH} = 8.9 Hz, 1H), 8.39 (br s, 1H), 7.38-8.19 (br unresolved m, 33H), 2.33 (d, J_{HP} = 13.2 Hz, 3H, -Me). ^{1}H NMR (D$_2$O, 298 K; δ): 6.64–8.04 (br unresolved m, 34H), 6.47 (br s, 1H), 2.07 (d, J_{HP} = 13.3 Hz, 3H, -Me). ^{13}C{^{1}H} NMR (CD$_2$Cl$_2$; 298 K; δ): 143.0, 139.5, 136.8, 137.3, 136.0, 135.6, 133.6 (d, J=10.6 Hz), 133.1, 133.4, 131.5 (d, J=13.5 Hz), 131.0 (d, J=12.9 Hz), 130.5 (t, J=12.1 Hz), 129.2, 128.9, 127.4, 125.0 (d, J=5.9 Hz). ^{31}P{^{1}H} NMR (CD$_2$Cl$_2$; 298 K; δ): 19.8 (s, 1P, PPh$_2$Me$^+$), 4.1 (s, 1P, P$_{cyclo}$Ph$_2$$^+$), ^{31}P{^{1}H} NMR (D$_2$O; 298 K; δ): 19.4 (s, 1P, PPh$_2$Me$^+$), 4.8 (s, 1P, P$_{cyclo}$Ph$_2$$^+$). Anal. Calcd for C$_{51}H_{38}P_2Cl_2$: C, 78.16; H, 4.89. Found: C, 77.98; H, 4.96.

X-ray structure determination. The crystal of **2** was immersed in cryo oil, mounted in a Nylon loop, and measured at a temperature of 150 K. The diffraction data were collected with Bruker Kappa Apex II Duo diffractometer using Mo Kα radiation (λ = 0.71073 Å). The *APEX2*[2] program package was used for cell refinements and data reductions. The structures were solved by direct methods using the *SHELXS*-2014[3] programs with the *WinGX*[4] graphical user interface.

A semiempirical absorption correction (*SADABS*[5]) was applied to all data. Structural refinements were carried out using *SHELXL*-2014.[3] All H atoms in **2** were positioned geometrically and constrained to ride on their parent atoms, with C–H = 0.95−0.98 Å, and U_{iso} = 1.2–1.5U_{eq} (parent atom). The crystallographic details are summarized in Table S1.

Table S1. Crystal data and structure refinement for **2**.

Identification code	2
Empirical formula	$C_{52}H_{42}I_2P_2$
Formula weight	982.59
T (K)	150(2)
λ (Å)	0.71073
Crystal system	Orthorhombic
Space group	*Pccn*
a (Å)	25.6523(9)
b (Å)	12.2403(5)
c (Å)	13.5209(5)
α (deg)	90
β (deg)	90
γ (deg)	90
Volume (Å^3)	4245.5(3)
Z	4
ρ_{calc} (Mg/m^3)	1.537
μ (mm^{-1})	1.593
F(000)	1960
Crystal size (mm^3)	0.169 x 0.082 x 0.082
θ range for data collection (deg.)	1.588 to 34.380
Index ranges	-32<=h<=40, -19<=k<=16, -21<=l<=21
Reflections collected	48631
Unique reflections	8910
R_{int}	0.0256
Completeness to θ= 25.242°	100.0 %
Absorption correction	Semi-empirical from equivalents
Max. and min. transmission	0.878 and 0.764
Refinement method	Full-matrix least-squares on F2
Data / restraints / parameters	8910 / 0 / 253
GOOF on F^2	1.112
Final R indices [I>2sigma(I)][a]	R1 = 0.0340, wR2 = 0.0734
R indices (all data)	R1 = 0.0463, wR2 = 0.0772
Largest diff. peak and hole (e.Å^{-3})	1.219 and -1.222

[a] $R1 = \Sigma ||F_o| - |F_c||/\Sigma|F_o|$; $wR2 = [\Sigma[w(F_o^2 - F_c^2)^2]/ \Sigma[w(F_o^2)^2]]^{1/2}$

Photophysical Measurements. Steady-state absorption and emission measurements were recorded on a Hitachi (U-3310) spectrophotometer and an Edinburgh (FS920) fluorimeter, respectively. Both the wavelength- dependent excitation and emission response of the fluorimeter have been calibrated. To determine the relative photoluminescence quantum yields in solution, Coumarin 480 (C480, Exciton) in methanol with a quantum yield of 0.87 and Rhodamine 6G (R6G, Exciton) in methanol with a Q.Y of 0.95, served as the standards. Absolute quantum yields were measured using an integrating sphere with luminescence spectrometer (HORIBA FluroMax-4P) as the optical detector. Lifetime studies were performed with an Edinburgh FL 900 photon-counting system using a hydrogen-filled lamp as the excitation source. The emission decays were fitted by the sum of exponential functions with a temporal resolution of 300 ps by the deconvolution of instrument response function.

Two-photon absorption (TPA) property. The open aperture Z-scan experiments[6] were conducted to measure TPA of compounds **2–4**. In this study, a mode-locked Ti sapphire laser (Tsunami, Spectra Physics) produced a single Gaussian pulse, which was then coupled to a regenerative amplifier that generated a ~ 180 fs, 1 mJ pulse (800 nm, 1 kHz). The pulse energy, after proper attenuation, was reduced to 1.00-2.00 μJ. The laser beam was focused and passed through a 1.00 mm cell and the beam radius at the focal position was ~ 5.09×10^{-3} cm. When the sample cell was translated along the beam direction (z-axis), the transmitted laser intensity was detected by a photodiode. The TPA induced decrease in transmittance, T(z), can be fitted with eq. S1 incorporating the TPA coefficient (β):

$$T(z) = \sum_{n=0}^{\infty} \frac{(-q)^n}{(n+1)^{3/2}} ; q = \frac{\beta I_0 L}{1 + \dfrac{z^2}{z_0^2}} \quad (S1)$$

where n is an integer number from 0 to ∞ and has been truncated at $n = 1000$, L is the sample length, I_0 is the input intensity, z represents the sample position with respect to the focal plane, and z_0 denotes the diffraction length of the incident beam (Rayleigh range). After obtaining the TPA coefficient (β), TPA cross section (σ_2) can be deduced with eq. S2:

$$\beta = \frac{\sigma_2 N_A d \times 10^{-3}}{h\upsilon} \quad (S2)$$

where N_A is the Avogadro constant, d is the sample concentration, and $h\upsilon$ is the incident photon energy.

In-Vitro Cytotoxicity. To access the cytotoxicity of the compounds **1–4**, the Hela cell viability was analyzed by using a colorimetric assay agent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Roche). The Hela cells were seeded in a 96-well plate with 5×10^3 cells per well in a 90% Dulbecco's Modified Eagle Medium supplemented (HyClone) with 10% heat-inactivated fetal bovine serum (HyClone), penicillin and streptomycin (HyClone). To contrast with the control, 6 different concentrations of 1-4 were added to each well (highest to 1.2 µM). After 15 hours of incubation, wells were washed twice with PBS buffer and then incubated with 200 µL of the culture medium with 10% MTT agent per well. After 4 hours of reaction time, the culture medium was removed and refilled per well with 200 µL of dimethyl-sulfoxide (Sigma-Aldrich) per well to dissolve the purple MTT formazan crystal. The optical density of these samples was measured at 595 nm. All measurements were done with three replicates using an ELISA reader (VersaMax™ Microplate Spectrophotometers; Molecular-Devices).

Imaging Experiments. For cell imaging experiments, 5×10^4 cell per well of HeLa or CCD-986Sk (ATCC® CRL-1947™) cells were seeded in a 35 mm glass bottom dish with 2 mL of DMEM in each well, and were incubated at 37 °C supplied with 5 % CO_2/95% air. After 24 h of incubation, each well was washed twice with PBS buffer and 1 µM solution of a compound **2–4** then added. After further incubated for 1 hour, cells were washed three times with PBS buffer and then added phenol red-free culture medium in each dish. Then, live cells were observed by a Zeiss LSM710 NLO confocal spectral microscope equipped with 63X objective for Hela cell (P-APO, 1.40 oil immersion) and 40X objective (C-APO, 1.2 water immersion) for CCD fibroblast. For co-staining experiments, the final concentration of MitoTracker® Deep Red (Invitrogen) and test compound were 500 nM and 1 µM, respectively. Diode laser 405 nm or 458 nm (Argon ion laser) were used as single photon excitation sources for compound **2-4** and 633 nm (He-Ne laser) for MitoTracker. Femtosecond NIR Laser (80MHz/100fs, Mai Tai DeepSee, Spectra-Physics) was used as a two–photon excitation source. All microscopic experiments for live cell image were carried out at a temperature of 37 °C, maintained by a circulation chamber.

Photostability test. Photostability of compounds **1–4** was compared with that of widely used chromophores (Coumarin 480 and Fluorescein). All experiments were performed in PBS (10 mM, pH = 7.4 with 1% MeOH for 1). Samples were kept at the same absorbance (A = 0.1) at their excitation wavelength (λ_{ex} = 405 nm diode laser for 1, 2 and coumarin 480; λ_{ex} = 458 nm Argon ion laser for 3 ,4 and fluorescein). Fluorescence intensity at the respective emission maxima was recorded with continuous exposure to excitation light and collected by Zeiss

LSM710 NLO confocal spectral microscope equipped with 40X objective (C-APO, 1.2 water immersion).

Computational Details. Compounds **1**–**4** were studied using the hybrid PBE0 density functional method (DFT-PBE0).[7] Non-hydrogen atoms were described by a triple-zeta-valence quality basis set with polarization functions (def-TZVP) and a split-valence basis set without polarization functions was used for hydrogen atoms (def2-SV(P)).[8] Multipole-accelerated resolution-of-the-identity technique was used to speed up the calculations.[9] To facilitate comparisons with the experiments, C_i point group symmetry was applied for compounds **1** and **2**. The geometries of all complexes were first fully optimized using the DFT-PBE0 method. The optimized geometry of the compound **2** is in good agreement with the available X-ray structure (the coordinates of the optimized structures are included as Supporting Information). The excited states were investigated using the Time-Dependent DFT formalism.[10] The singlet excitations were determined at the optimized ground state S_0 geometries, while the lowest energy singlet emissions were determined at the optimized S_1 geometry. All electronic structure calculations were carried out with the TURBOMOLE program package (version 7.0).[11]
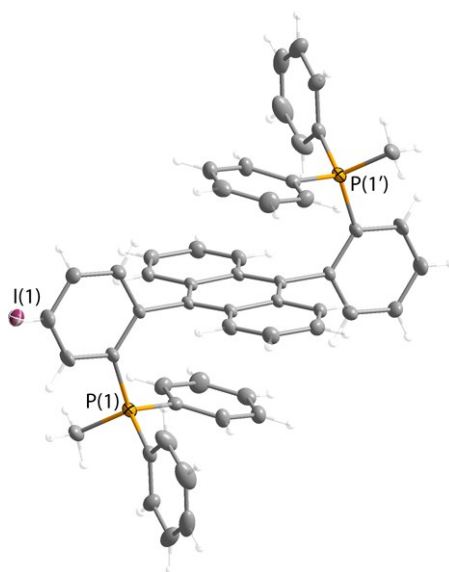
Fig. S1. Molecular view of the diphosphonium salt [**2**]I$_2$ (thermal ellipsoids are shown at the 50% probability level). One iodide counterion is depicted.
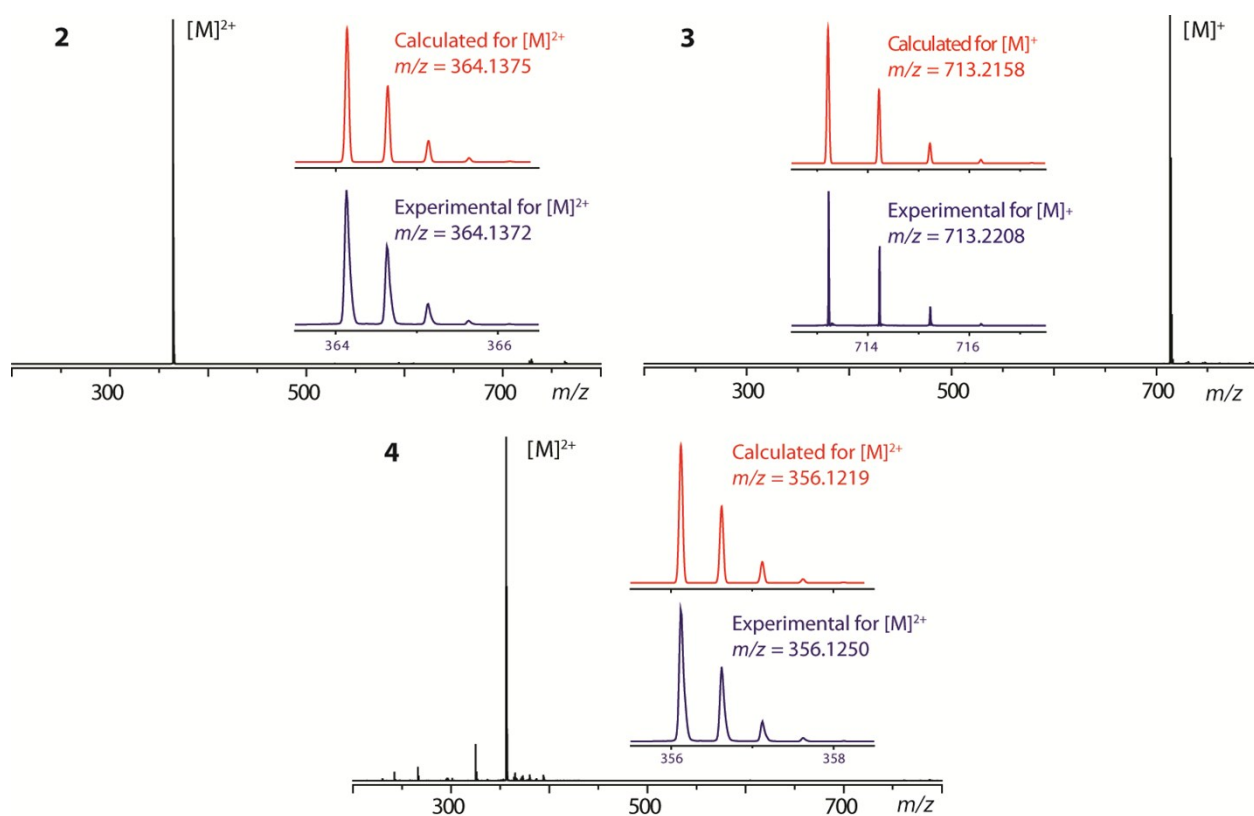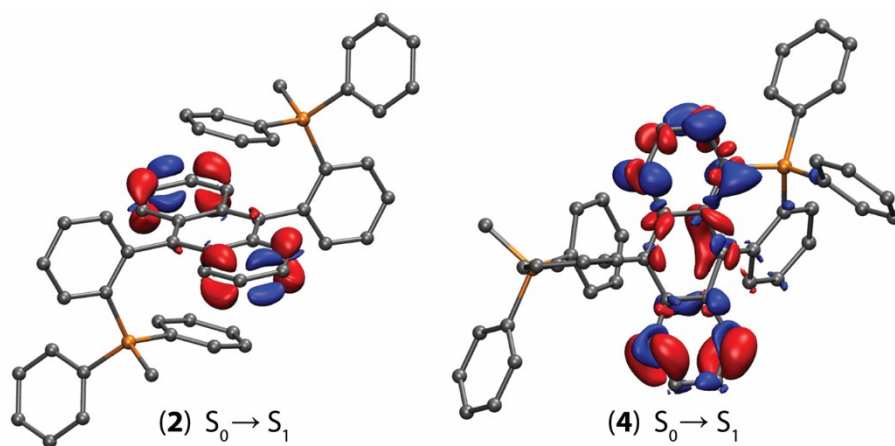
Fig. S2. ESI-MS of compounds **2–4**.
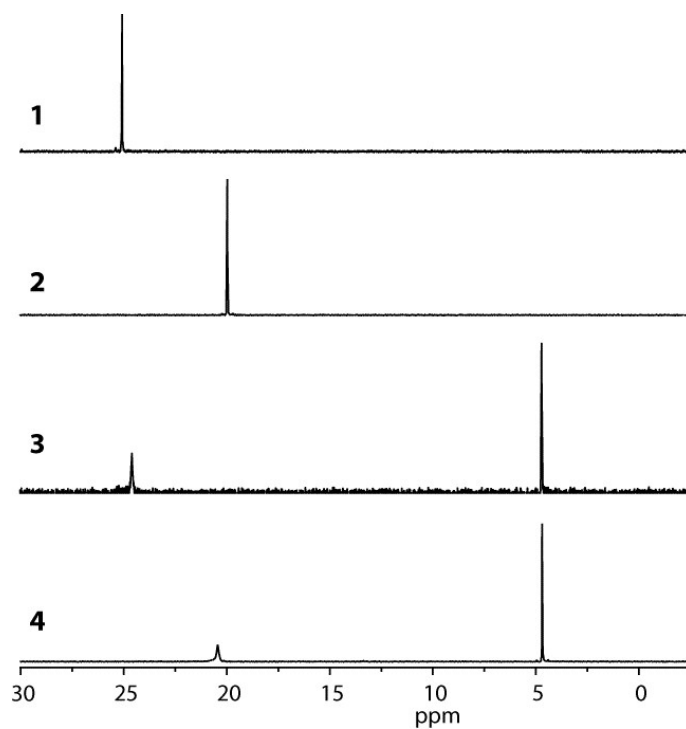
Fig. S3. 162 MHz ^{31}P{^{1}H} NMR spectra of compounds **1-4** (**1**, **3**, **4** in CD$_2$Cl$_2$, **2** in CD$_3$CN, 298 K).

**(2) $S_0 \rightarrow S_1$**   **(4) $S_0 \rightarrow S_1$**

Fig. S4. Electron density difference plots for the lowest energy singlet excitation ($S_0 \rightarrow S_1$) of the compounds **2** and **4** (isovalue 0.002 a.u.). During the electronic transition, the electron density increases in the blue areas and decreases in the red areas. Hydrogen atoms omitted for clarity.
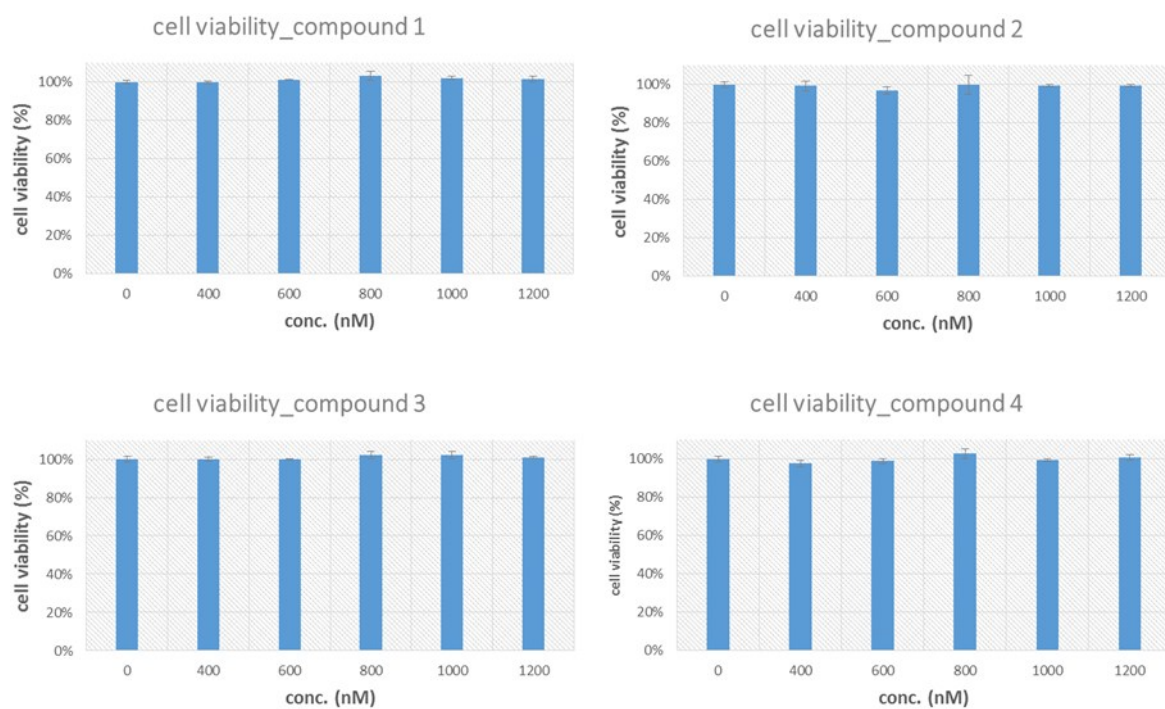

Fig. S5. MTT assay for the viability of Hela cells treated with various concentrations of compounds **1**–**4**. Error bars represent the standard deviations of three measurements.
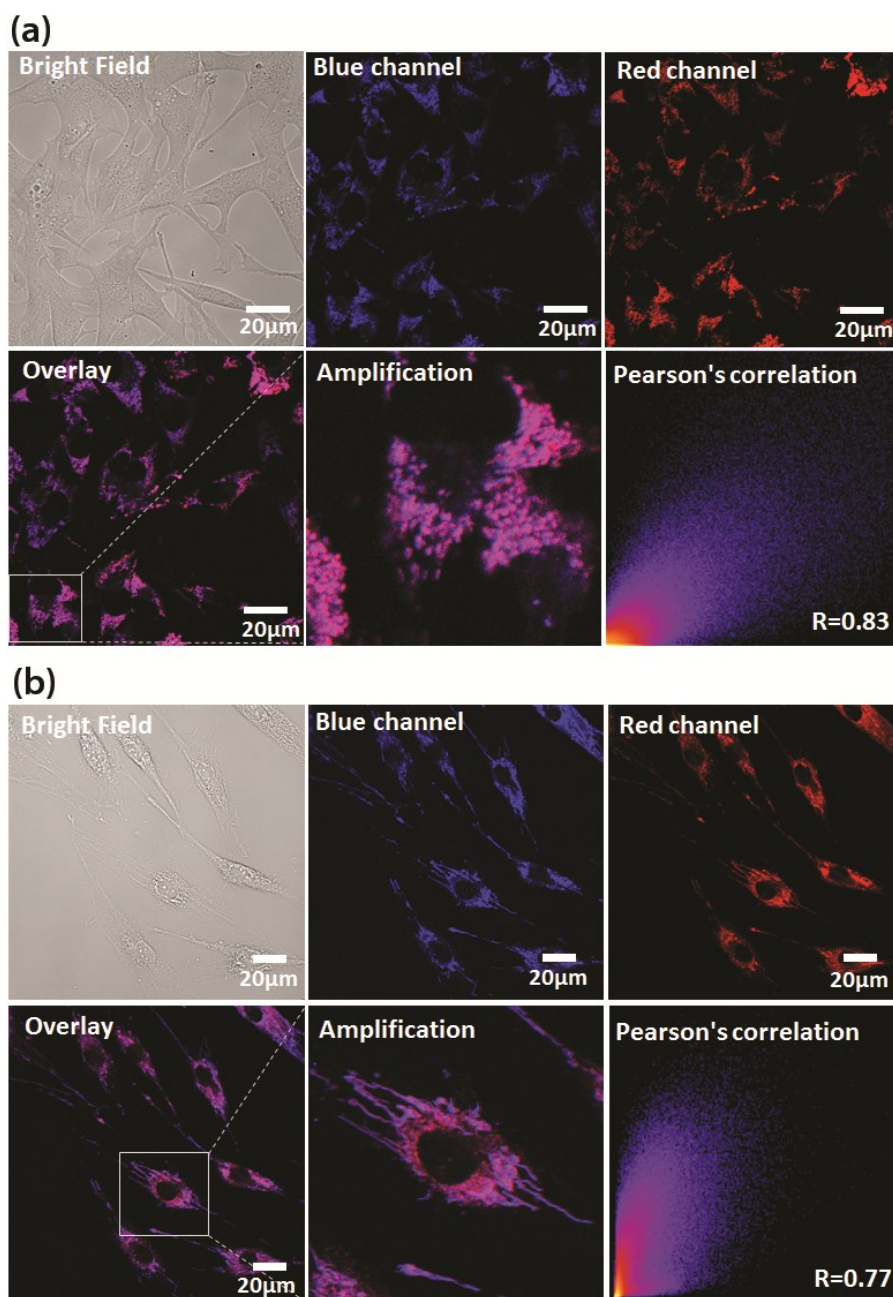
Figure S6. Confocal images of living (a) Hela cells and (b) CCD cells incubated with [**2**]I_2 (1μM) for 1 hour and mitotracker (500 nM) for another 30 mins, 37 °C and under 5% CO_2. Blue channel: acquisition wavelength from 410 to 550 nm, λ_{ex}= 405 nm for [**2**]I_2; Red channel: acquisition wavelength from 640-740 nm, λ_{ex}= 633 nm for MitoTracker deep red. The image of overlay area is shown in magenta. The amplification images are zoomed from the white box, and co-localization analysis from the overlay images are quantified by Pearson's correlation coefficient (R).
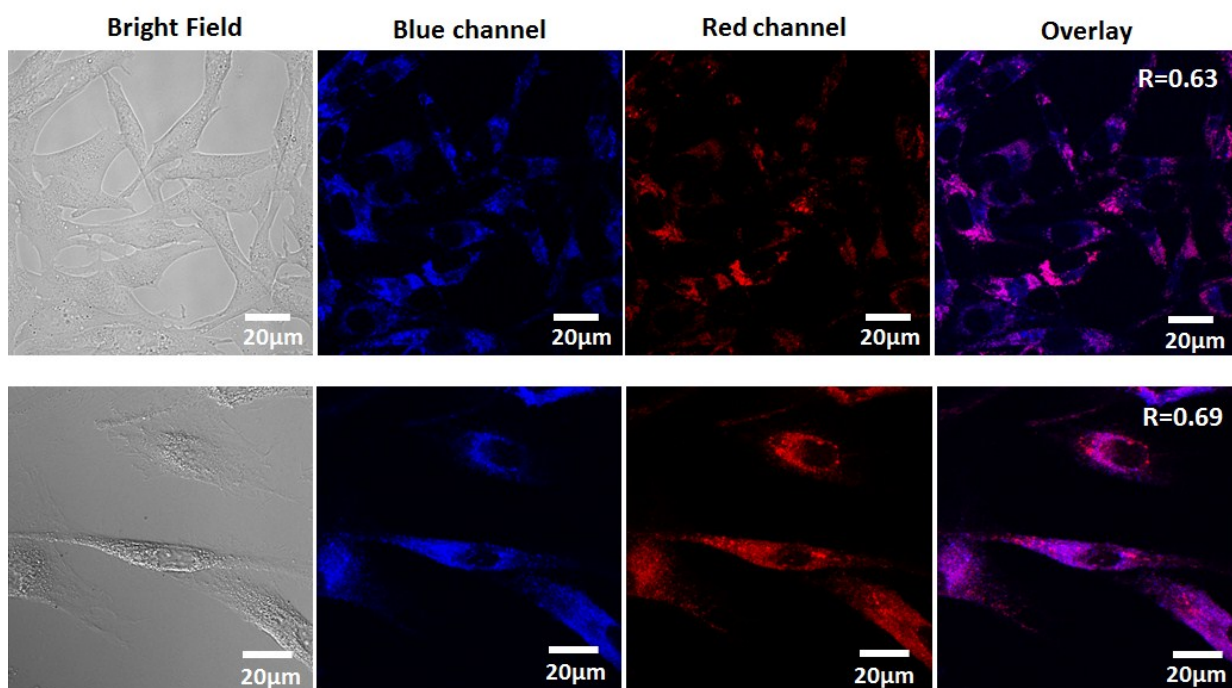
Fig. S7. Confocal images of living Hela cells (upper) and CCD cells (lower) incubated with **3** (1μM) for 1 hour and mitotracker (500 nM) for another 30 min., 37 °C and under 5% CO_2. Images from left to right are Bright field, one photon luminescence images of **3** (blue channel, 460-620 nm, λ_{ex}= 458 nm) and of MitoTracker deep red (red channel, 640-740 nm, λ_{ex}= 633 nm), and the image of overlay area shown in magenta. The co-localization analysis from the overlay images are quantified by Pearson's correlation coefficient (R).
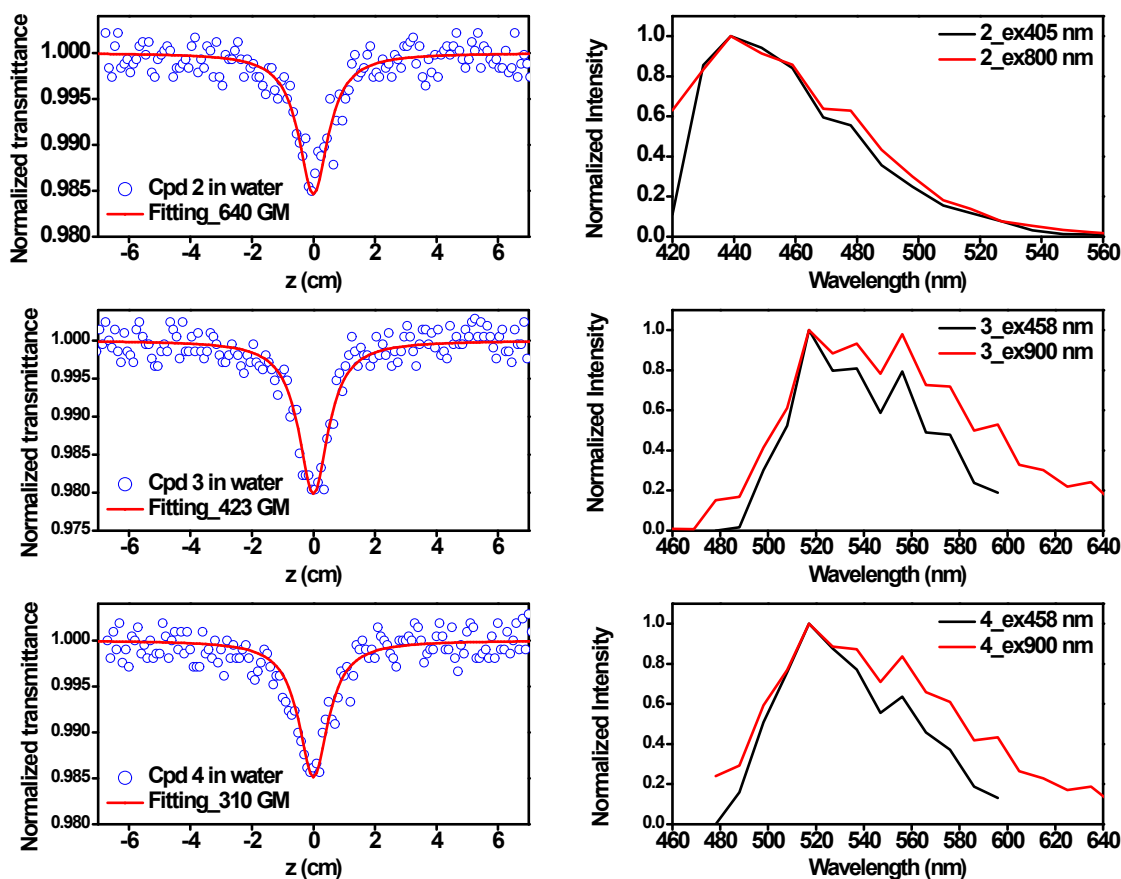
Fig. S8. Left: Z-scan experimental data for compounds **2**–**4** in water (1×10^{-4} M for **2** and 2×10^{-4} M for **3** and **4**) in a 1 mm cell, λ_{ex} = 800 nm. Solid lines are the best fit for the data points by eqs. S1 and S2. Right: one photon induced emission (black line) and two photon induced fluorescence (red line) spectra of compounds **2**–**4** in water.
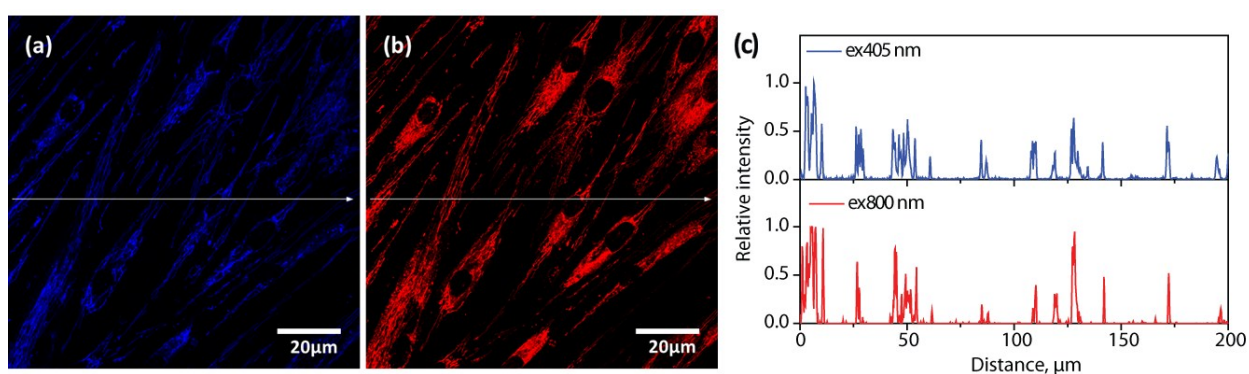

Fig. S9. Confocal image of compound **2** in CCD cell line (a) excitation at 405 nm diode laser; (b) excited by femtosecond Ti:Sapphire laser, λ_{ex} = 800 nm; (c) the profile drawn from the white line appearing in (a) and (b), which shows high selectivity staining of mitochondria.
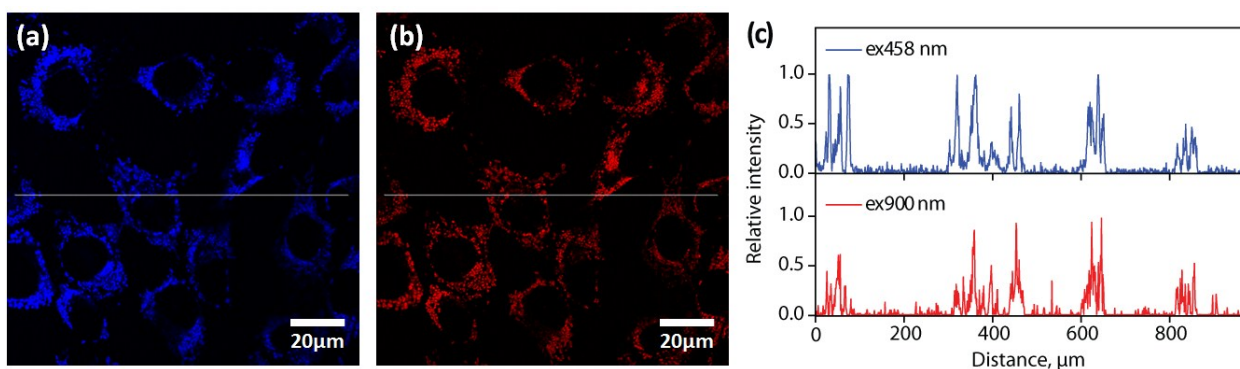
Fig. S10. Confocal image of compound **4** in Hela cell line (a) excitation at 458 nm Argon ion laser; (b) excited by femtosecond Ti:Sapphire laser, λ_{ex}=900 nm; (c) the profile drawn from the white line appearing in (a) and (b), which shows high selectivity stained on mitochondria.
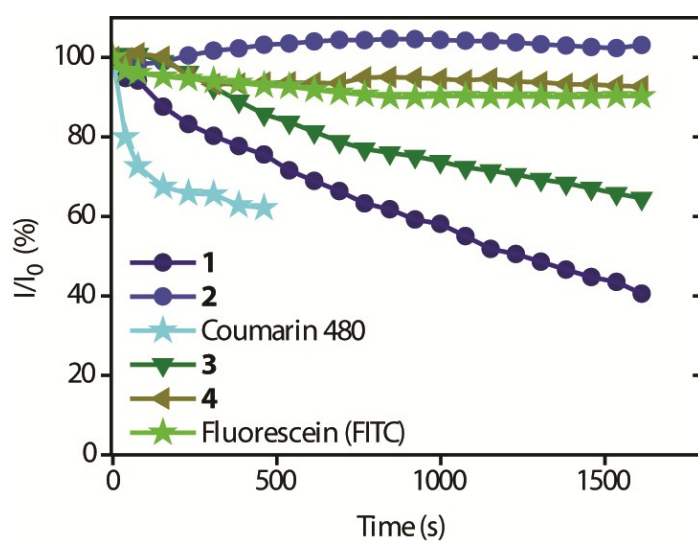
Fig. S11. Photostability test of the compounds **1**–**4** compared with widely used chromophores (Coumarin 480 and Fluorescein). All experiments were performed in PBS (10 mM, pH = 7.4 with 1% MeOH for **1**). Samples were kept at the same absorbance (A = 0.1) at their excitation wavelength (λ_{ex} = 405 nm diode laser for **1**, **2** and coumarin 480; λ_{ex} = 458 nm Argon ion laser for **3**, **4** and fluorescein). Fluorescence intensity at the respective emission maxima was recorded with continuous exposure to excitation light and collected by Zeiss LSM710 confocal spectral microscope equipped with 40X objective (C-APO, 1.2 water immersion).
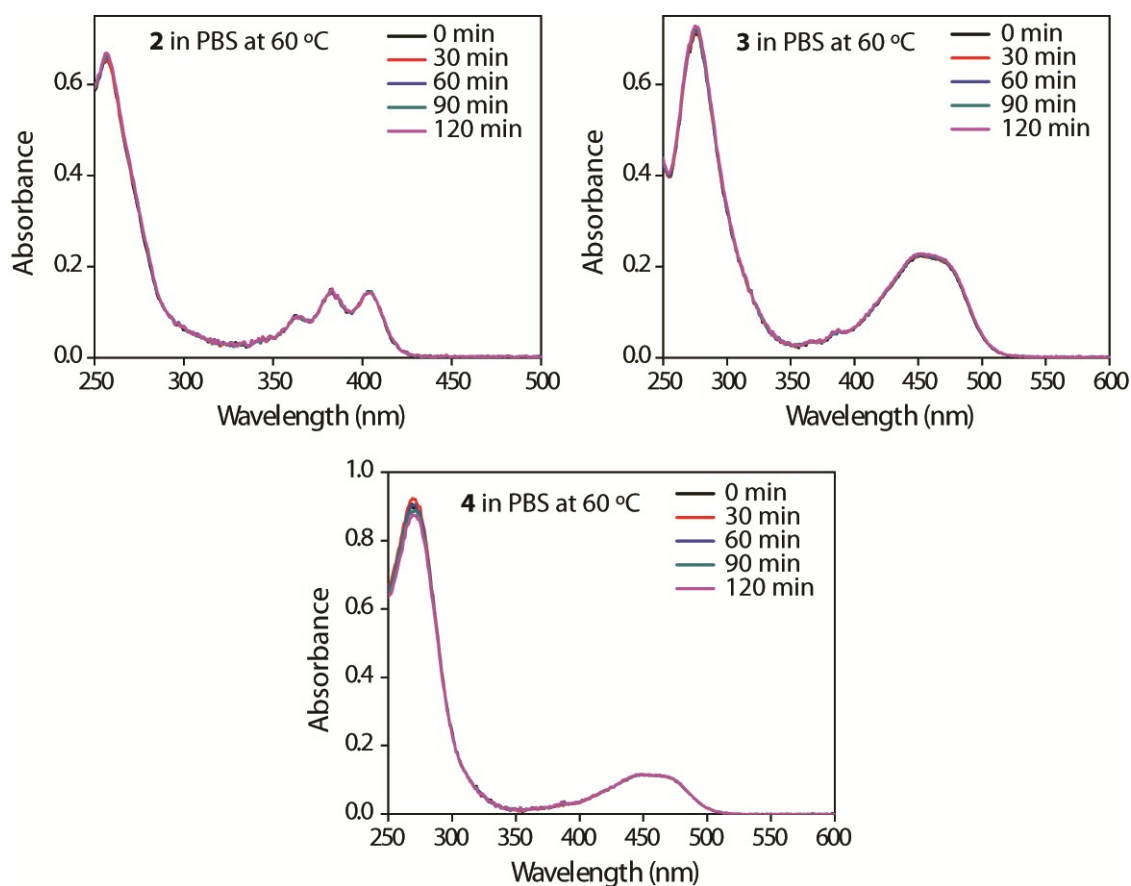
Fig. S12. Thermal stability test of the compounds **2–4.** Samples were prepared in PBS buffer solution and continuous heated under 60°C up to 2 hours. The absorption spectra of these compounds showed negligible changes.

References

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