Supporting Information for

Photoswitchable fluorescent diarylethene in a *turn-on* mode for live cell imaging

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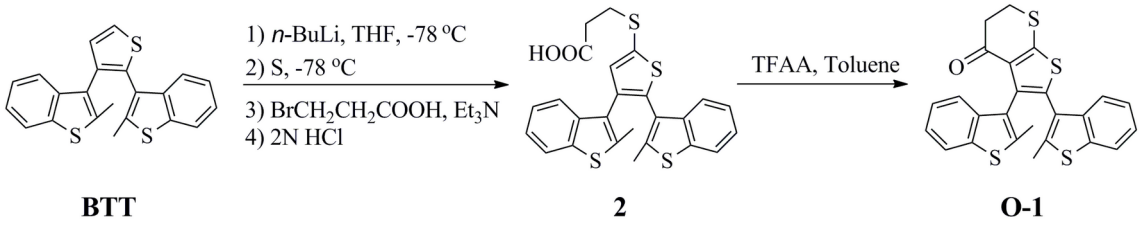
Methods

General

All reagents and spectrograde solvents were purchased from Sigma-Aldrich. Melting points were determined with a Laboratory Devices Mel-Temp 3.0 melting point apparatus. The ^{1}H and ^{13}C NMR spectra were obtained using a JEOL JNM-AL300 spectrometer at 300 and 75 MHz in $CDCl_3$, respectively, with tetramethylsilane as the internal reference. High resolution mass spectrometry (HRMS) spectra were obtained with JEOL JMS-700 spectrometer. Fourier transform infrared (FTIR) spectroscopy measurements were performed using a JASCO FTIR-4200 instrument. HPLC was performed using a Young Lin SP-930D liquid chromatography coupled with a Young Lin UV-730D spectrophotometric detector. UV absorption spectra were recorded on a Shimadzu UV-3100 spectrophotometer in spectroscopy grade ethyl acetate. Fluorescence spectra were collected in spectroscopy grade ethyl acetate on a Fluoro Max-2 spectrophotometer equipped with a 150 W ozone-free xenon lamp. UV and visible irradiations were performed with standard lamps used for visualizing TLC plates (VL6L; 312

nm, 8 mW/cm^2) and a 400 W tungsten lamp and the samples were placed in a glass chamber maintained at room temperature. Photochromic changes as a function of time were monitored using a 500 W xenon lamp (Newport-74000) equipped with a monochromator (Newport-66921). Flash column chromatography was performed with Merck silica gel 60 (70-230 mesh). BTT was synthesized according to the literature.[1]

Synthesis of O-1



BTT **2** **O-1**

Scheme S1. Synthesis of **O-1**

Synthesis of compound 2

Synthesis of compound **2** was performed as in literature[2] with several modifications. Briefly, a solution of *n*-butyllithium (2 mL of 2.5 M in *n*-hexane, 5.0 mmol) was added to a solution of 2,3-bis(2-methylbenzo[b]thiophene-3-yl)thiophene (**BTT**) (753 mg, 2.0 mmol) in dry THF (20 mL) at -78^{o}C for a period of 10 min and stirred at -78^{o}C for 30 min under a nitrogen atmosphere. Granular sulfur (128 mg, 4.0 mmol) was added in one portion and then the reaction mixture was stirred at -78^{o}C for 1 h. A cold solution of 3-bromopropanoic acid (612 mg, 4.0 mmol) dissolved in a solution of triethylamine (1.0 mL, excess) in dry THF (5 mL) is then added slowly while maintaining the temperature at -78^{o}C . After the addition is complete the temperature is gradually raised to room temperature over a period of 16 h. Then the reaction was quenched with cold 2 N HCl solution. The mixture was extracted with ethyl acetate (2 × 30 mL) and the combined organic layers were washed with water (2 × 30 mL), followed by brine solution (30 mL) and dried over magnesium sulfate. This organic layer was concentrated to give the crude product, which was purified by column chromatography (stationary phase: silica gel 60-120, mobile phase: 25% ethyl acetate in hexane) to give **2** (0.85 g, 85%) as a white powder; mp 151.6-152.3^{o}C;

^{1}H NMR ($CDCl_3$, 300 MHz) δ; 7.86 (d, 0.6 H, *J* = 7.7 Hz, Ar-H), 7.72 (d, 1.4 H, *J* = 7.7 Hz, Ar-H), 7.63 (d, 1.4 H, *J* = 7.1 Hz, Ar-H), 7.25-7.37 (m, 4.2 H, Ar-H), 7.15-6.99 (m, 1.4 H, Ar-H), 3.20-3.15 (t, 2.0 H, *J* = 6.7 Hz, CH_2-H), 2.87-2.83 (t, 2.0 H, *J* = 6.7 Hz, CH_2-H), 2.36 (s, 1.1H, p-CH_3), 2.24 (s, 1.1H, p-CH_3), 1.77 (s, 3.8H, ap-CH_3). Ratio of p: ap = 36:64. ^{13}C NMR ($CDCl_3$, 75 MHz) δ; 177.55, 139.81, 138.06, 137.68, 137.19, 136.69, 134.91, 133.22, 127.28, 124.86, 124.48, 124.31, 123.96, 123.76, 122.25, 122.05, 121.92, 34.44, 32.91, 14.89, 14.76.

HRMS (FAB+): *m/z* calcd for $C_{25}H_{20}O_2S_4$: 480.0346; found: 480.0342.

Anal. Calcd for $C_{25}H_{20}O_2S_4$: C, 62.47; H, 4.19. Found: C, 62.52; H, 4.11

Synthesis of compound O-1[3]

To the solution of **2** (0.85 g, 1.77 mmol) in toluene (5 mL) at 0°C was added trifluoroacetic anhydride (0.5 mL, 3.54 mmol) at 0-5°C. The mixture was warmed to 25 °C and monitored by TLC until ring closure was complete (ca. 12 h). The reaction mixture was cooled to 0-5°C and quenched into cold H_2O (10 mL, 5°C) keeping the temperature at < 25°C. The mixture was stirred for 30 min and the layers were separated. The organic layer was washed with brine (2 × 10 mL) and dried over magnesium sulfate. This organic layer was concentrated to give the crude product, which was purified by column chromatography (stationary phase: silica gel 60–120, mobile phase: 10% ethyl acetate in hexane) to give **O-1** (0.43 g, 52%) as a yellow powder; mp 233.7-234.7°C;

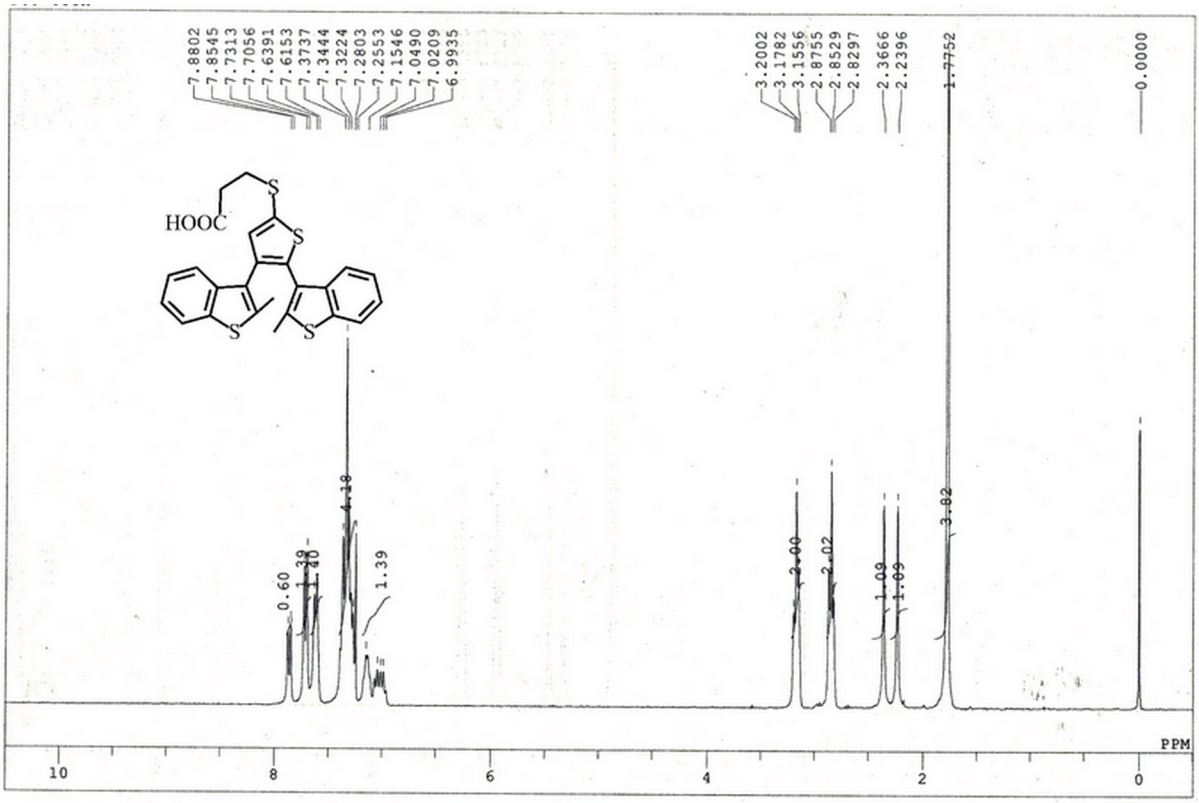
^{1}H NMR ($CDCl_3$, 300 MHz) δ; 7.66-7.70 (m, 2.0H, Ar-H), 7.54 (d, 0.8H, *J* = 7.7 Hz, Ar-H), 7.20-7.31 (m, 3.8H, Ar-H), 7.08-6.90 (m, 1.4 H, Ar-H), 3.39 (m, 2.0 H, CH_2-H), 2.83 (t, 2.0 H, CH_2-H), 2.39 (s, 1.1H, p-CH_3), 2.21 (s, 1.0H, p-CH_3), 1.85-1.80 (d, 3.9H, *J* = 14.8 Hz, ap-CH_3). Ratio of p: ap = 35:65. ^{13}C NMR ($CDCl_3$, 75 MHz) δ; 187.52, 152.45, 141.35, 140.21, 139.99, 137.83, 137.54, 137.32, 134.47, 133.40, 131.09, 131.08, 127.33, 124.29, 123.93, 123.65, 123.44, 123.24, 122.04, 121.96, 121.84, 121.62, 39.31, 29.79, 15.11, 14.96.

HRMS (FAB+): *m/z* calcd for $C_{25}H_{19}OS_4$ $[M+H]^+$: 463.0319; found: 463.0332.

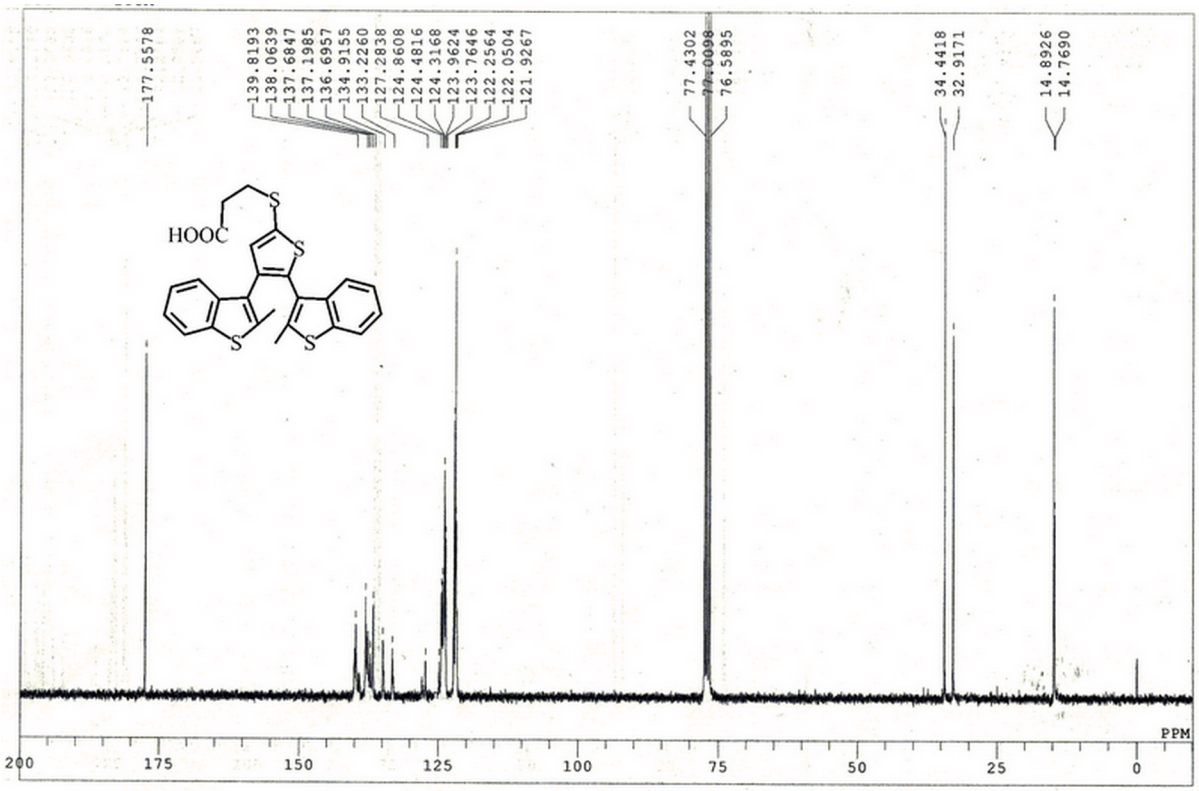
Anal. Calcd for $C_{25}H_{18}OS_4 \cdot H_2O$: C, 64.90; H, 3.92. Found: C, 64.89; H, 3.92

NMR Spectra of Compound 2 and O-1

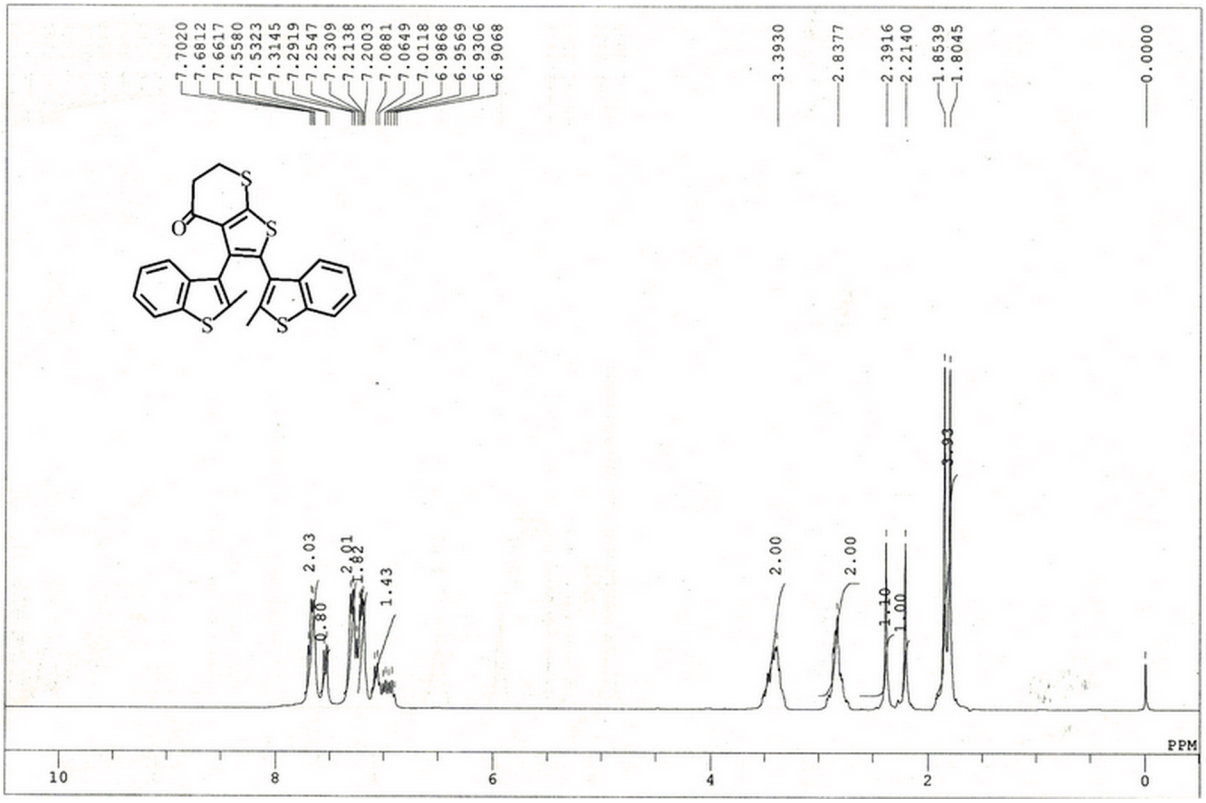
^{1}H NMR of compound **2**



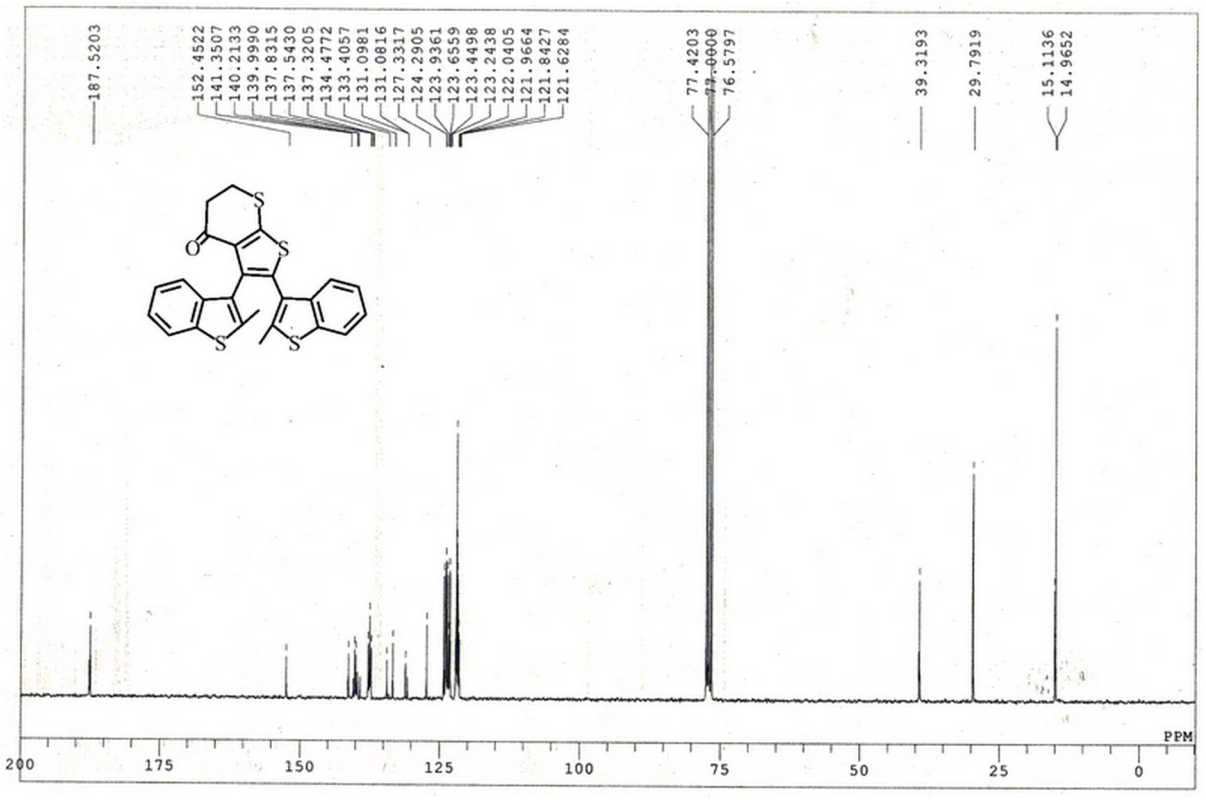
^{13}C NMR of compound **2**



^{1}H NMR of compound **O-1**



^{13}C NMR of compound **O-1**



Preparation of liposome

To prepare liposomes, 25 μmol of lipid(s) (*see* main text) and 0.5 μmol of compound **1** were first dissolved in 2 mL chloroform. The lipid(s)/compound **1** molar ratio was maintained at 50:1. When DC-Chol was mixed with DPPC or DOPE, the molar ratio was maintained at 1:1. After complete mixing, the solvent was removed completely by successive vacuuming to form a dry film. The film was hydrated with 2 mL of 10 mM potassium phosphate buffer (pH 7.5) to a final concentration of 10 mg/mL at room temperature overnight. Then the suspended film was vortexed vigorously and agitated in an ultrasonic bath. This preparation yielded single lamellar liposomes with a narrow size distribution. The liposomes were diluted in distilled water to a concentration of 0.5 mg/mL concentration and then used for chemophysical measurements and for cell treatment. Average particle size and zeta potential were measured by dynamic light scattering (DLS) analysis using a Zetasizer NanoS (Malvern Instruments, UK).

Atomic force microscopy (AFM) analysis

The formation of liposomes and their sizes were confirmed by atomic force microscopy. The samples were prepared on mica and air-dried for 18 h. Non-contact mode images were obtained on a Park System XE-70 AFM. The scanning speed was 1 Hz and the resolution was 256 × 256. For each sample, scanning was performed with a scan size of 3 μm × 3 μm. Integrated silicon tips (MikroMasch, NSC15/AlBS) were used on the cantilevers. The resonance frequency was 348 kHz and the force constant was 46 N/m. The AFM images revealed the presence of uniform, well-dispersed liposomes with sizes comparable to those obtained from DLS analysis.

Cell culture and image acquisition

HeLa cells (ATCC, Rockville, MD, USA) were grown in DMEM (pH 7.4, Dulbecco's modified Eagles medium, GIBCO, Gaithersburg, MD, USA) containing 10% FBS (fetal

bovine serum, GIBCO) and 1× antibiotic-antimycotic agent (GIBCO). The cells were maintained in plastic tissue culture dishes (Falcon) at 37°C in an incubator with a humidified atmosphere consisting of 5% CO_2 / 95% air. On the day prior to treatment of the compound **1**, the cells were seed onto a round cover glass (ϕ 25 mm, No. 1, Deckglaser, Germany). Compound **1** was added to adherent cells on the cover glass before the experiment, followed by 4 h incubation. The medium was then removed, and adherent cells were rinsed three times with phosphate buffered saline (PBS) to remove the unbound compounds. Cover glasses with adherent cells were placed under the objective lens (APO 100×/1.65 Oil HR, Olympus) on a stage equipped with a microcell incubator (Live Cell Instrument, Seoul, Korea). The samples were alternatively irradiated by visible light (7724 EVA, 375 nm-5000 nm, halogen lamp, Philips, Germany) for 10 min and a UV lamp (ENF-240C/FE, 254 nm, Spectroline, Westbury, NY, USA) for 10 min. For liposome treatment, cells were incubated with various liposomes (0.5 mM) for 3 h. Each of the liposome mixtures was irradiated with UV light (VL-6LC; 254 nm, 6 W) or visible light (overhead projector, 400 W) for 30 min before application to the cells. For direct treatment, compound **1** in DMSO was added to cells in a microincubator to achieve a final concentration of 12 μM. After 4 h, the cells were extensively washed with PBS, irradiated with UV light or visible light for the indicated time periods, and monitored using our integrated TIRFM-DIC system. The fluorescence signal was obtained under a fluorescence microscope (Axiovert; Carl Zeiss, Oberko, Germany) with a rhodamine filter.

Assessment of the cytotoxicity of compound 1 using a modified MTT assay

Cell viability was assessed using a modified MTT assay.[4] Compound **1** in DMSO was added at various concentrations (1.0 μM to 500 μM) to cells. After 4 h incubation, 25 μL of a 5 mg/mL MTT solution was added to the samples and the plates were incubated for 2 h at 37°C. Thiazolyl blue tetrazolium bromide (MTT, Sigma Aldrich) was added to each well (final concentration 0.5 mg/mL) and incubated for 2 h at 37°C in a humidified atmosphere of 95% air / 5% CO_2. 0.08 N HCl in isopropanol was added to solubilize the blue MTT-formazan product and the cells were incubated for a further 30 min at room temperature. The absorbance of the solution was read at a test wavelength of 570 nm against a reference wavelength of 630 nm.

Integrated TIRFM-DIC detection system with a microcell incubator

Total internal reflection fluorescence microscopy (TIRFM), Nomarski differential interference contrast (DIC), and a microcell incubator (Live Cell Instrument, Seoul, Korea) were combined with a conventional inverted epifluorescence microscope to create an integrated detection system (Figure S4). An Olympus IX71 inverted microscope (Olympus Optical, Tokyo, Japan) equipped with two DIC sliders (U-DICTS and IX2-AN, Olympus) was used to detect distinct cellular morphological features using transmitted light. The designed TIRFM objective lens had the high numerical apertures critical for proper laser alignment and specimen illumination. The 532 nm laser (GL532T-050, Shanghai Laser Century Technology Co., Ltd., China) was used as a light source for TIRFM. The filter cube was composed of 590/20 nm bandpass filters (Olympus). A cooled charge-coupled device (CCD) camera (Cascade 512B, Photometrics, Tucson, AZ, USA) was mounted on the microscope. The CCD exposure time was 100 ms. To reduce photobleaching, a Uniblitz mechanical shutter (model LS3Z2, Vincent Associates, Rochester, NY, USA) was used to block the laser beam when the camera was off. All images were collected using MetaMorph 7.1 software (Universal Imaging Co., Downing Town, PA, USA).

***In vitro* photochromism assay**

Live HeLa cells were prepared on 6-well plates (SPL, Korea) and grown in DMEM supplemented with FBS, penicillin-streptomycin, and L-glutamine (Cellgro) at 37°C in a 5% CO_2 / 95% air in a humidified incubator. After 4 h post-incubation with open-form compound **1** (15 μM), cells were washed three times with an excess amount of DPBS, detached enzymatically using trypsin-EDTA, and initially collected by a gentle spin-down (500 × g). Cells were further rinsed and transferred to a quartz cuvette containing cell culture media. Steady-state photoluminescence (PL) spectra were measured and recorded using a Hitachi F-4500 spectrometer. The PL quantum yields of compound **1** were determined at λ_{ex} = 520 nm. Cells were irradiated with UV light from standard UV lamps (VL-6LC, 6 W) for 3 min and

visible light from a halogen lamp (overhead projector, 400 W) for 5 min. The optimal irradiation time was determined from the results shown in Fig. S8.

References

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3 T. J. Blacklock, P. Sohar, J. W. Butcher, T. Lamanec and E. J. J. Grabowski, *J. Org. Chem*. 1993, **58**, 1672-1679.

4 T. Mosmann. *J. Immunol. Methods* 1983, **65**, 55-63.

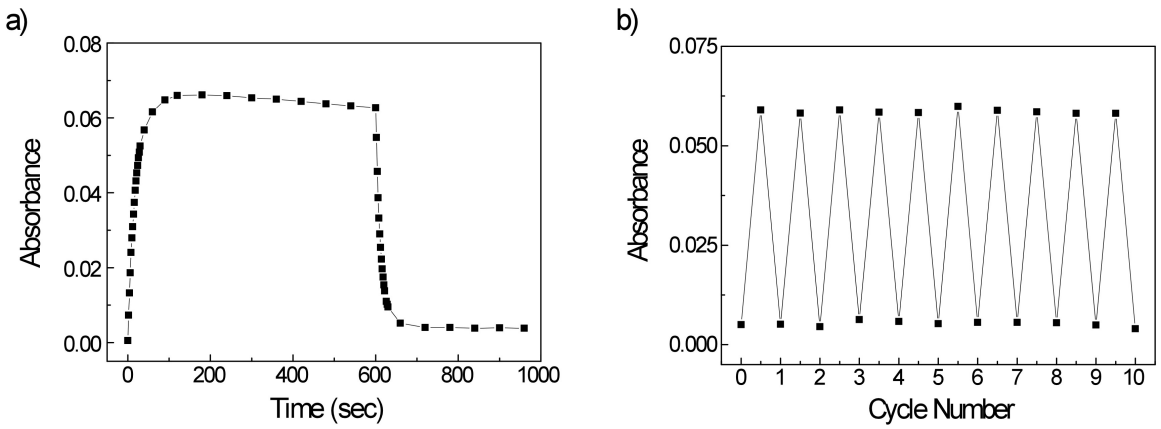

Fig. S1. (a) Time-course measurement of the absorption spectrum of compound **1** (1.0×10^{-5} M in ethyl acetate at room temperature) upon UV irradiation (312 nm, 1-600 seconds), followed by visible light illumination (520 nm, 600-1000 sec). (b) UV absorption switching of **1** (1.0×10^{-5} M in ethyl acetate at room temperature) between ring-open and ring-closed states upon alternative illumination with UV and visible light as monitored at 520 nm.

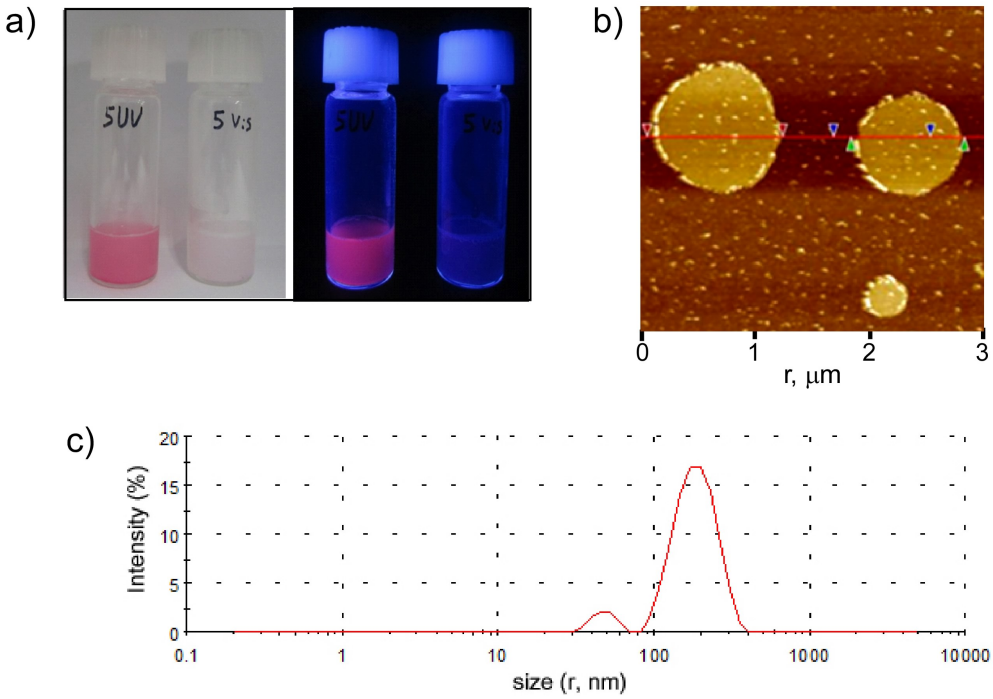

Fig. S2 (a) Photographs of DC-Chol/DPPC solution containing compound **1**. Solutions in the left and right vials were UV- and visible-light irradiated, respectively. (b) Atomic force microscopy (AFM) analysis was performed to confirm liposome formation and the size distribution of liposomes. (c) Dynamic light scattering (DLS) analysis of compound **1** when integrated into a DC-Chol/DPPC liposome complex.

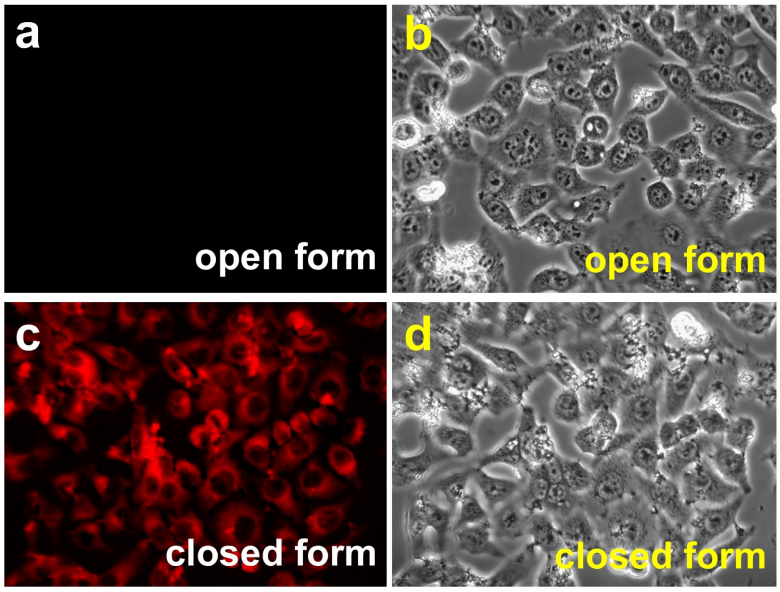

Fig. S3 Liposome mediated internalization of compound **1**. DC-Chol/DOPC liposomes (1:1 molar ratio) with compound 1 were either irradiated with visible- (a, b) or UV-light (c, d) and then incubated with cultured HeLa cells for 3 h. Representative fluorescence (a, c) and DIC images (b, d) after cells were fixed are shown.

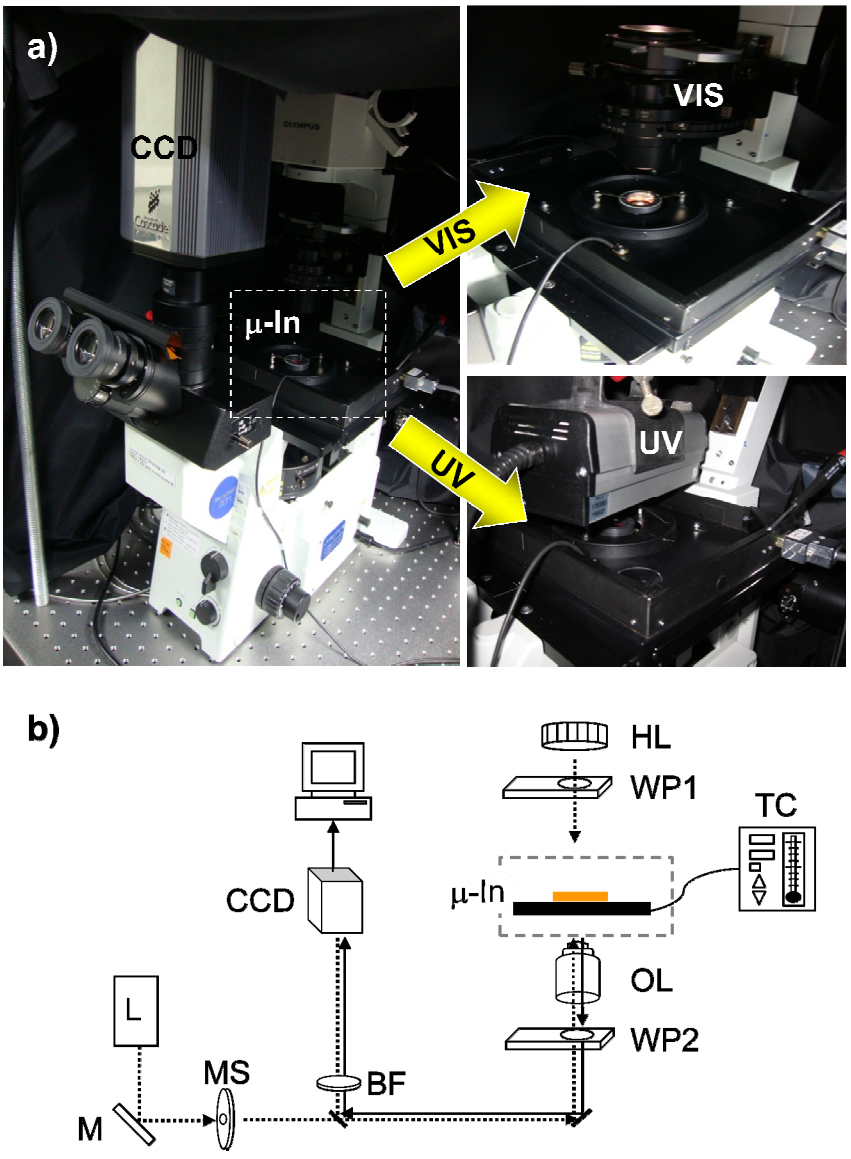

Fig. S4 (a) A photograph of the integrated TIRFM and Nomarski DIC system. (b) Schematic diagram of the integrated system. Indicators: L, laser; M, mirror; MS, mechanical shutter; BF, bandpass filter; HL, halogen light lamp; WP, Wollaston prism; TC, temperature controller; OL, objective lens; CCD, charge-coupled device; μ-In, microcell incubator.

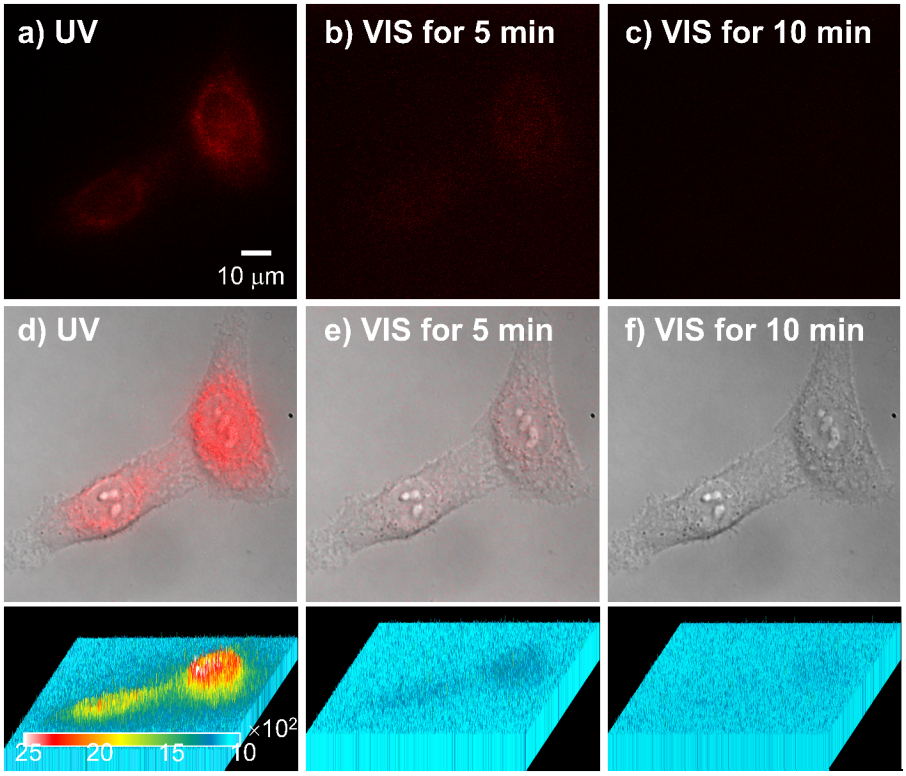

Fig. S5 TIRFM images (a-c), merged images of TIRFM and DIC (d-f) and profile images (bottom) of living HeLa cells incubated with UV-irradiated compound **1** (ring-closed form) for 4 h. Images: after a 30 min exposure to a UV lamp (254 nm) (a, d); after a 5 min (b, e) or 10 min(c, f) exposure to a halogen light lamp (VIS).

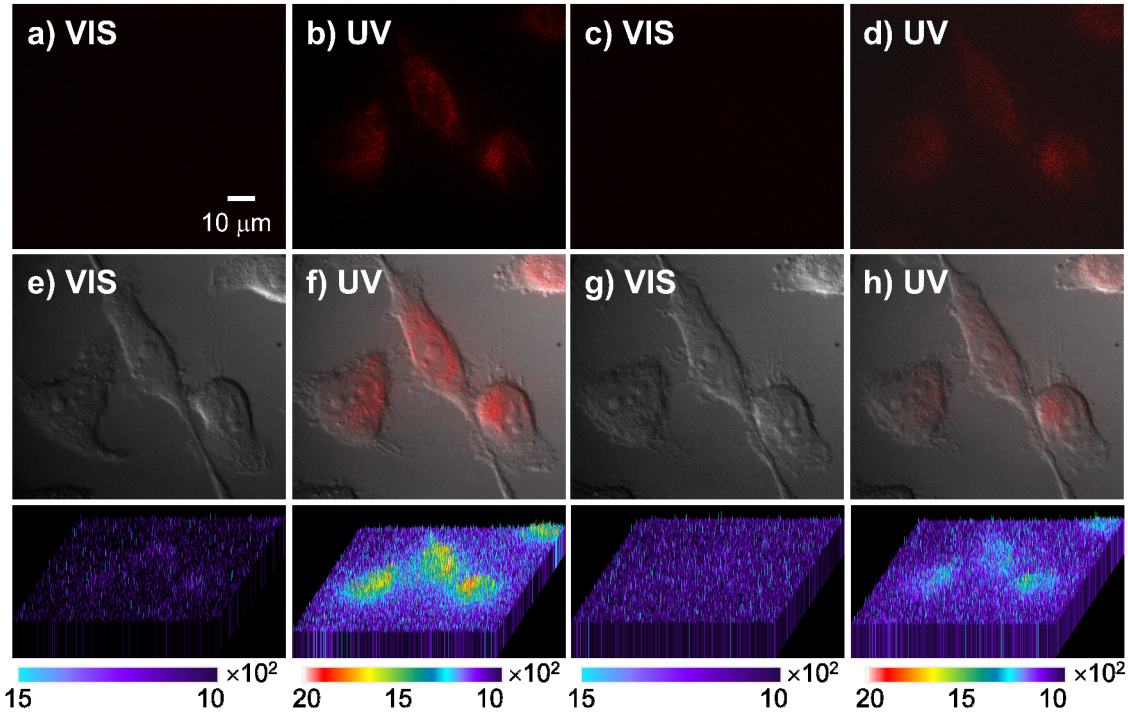

Fig. S6 Same as the top panel of Fig. 2, except HeLa cells were initially treated with visible-light irradiated compound **1** (ring-opened), followed by UV and visible-light irradiations.

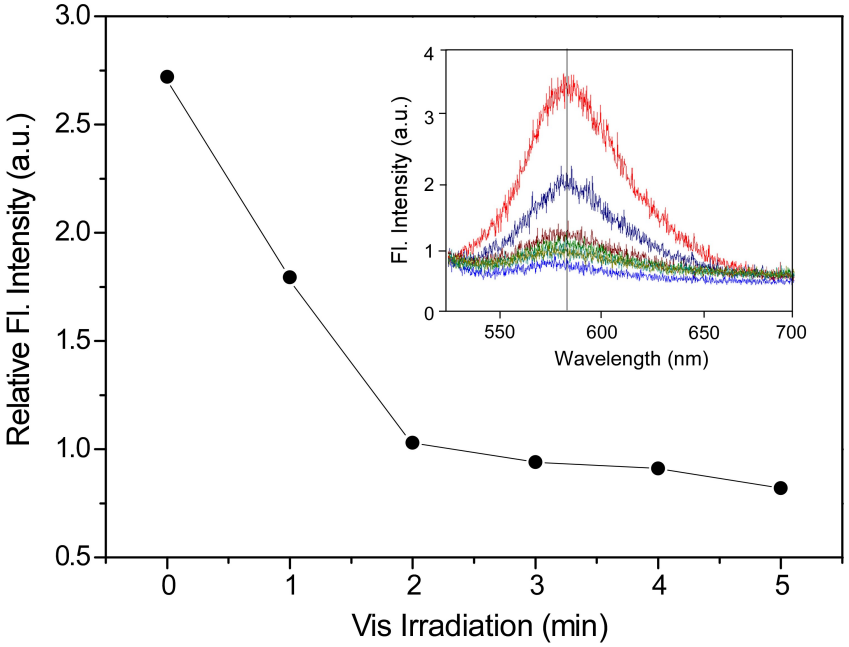

Fig. S7 Time-course determination of ring-opening of compound **1** upon visible-light irradiation. The inset is the time-course emission spectra of **1** in its closed form after excitation of 520 nm. The light blue line is the spectrum prior to irradiation with UV light; the red line is the spectrum after irradiation with UV for 3 min; the dark blue is the spectrum after exposure to natural visible light for 3 min; the brown line is the spectrum after exposure to natural visible light for 3 min and additional irradiation with a halogen lamp for 2 min; the green line is the spectrum after exposure to natural visible light for 3 min and additional irradiation with a halogen lamp for 3 min; the skyblue line is the spectrum after exposure to natural visible light for 3 min and additional irradiation with a halogen lamp for 4 min; the olive line is the spectrum after exposure to natural visible light for 3 min and additional irradiation with a halogen lamp for 5 min.