Supporting information

Synthesis

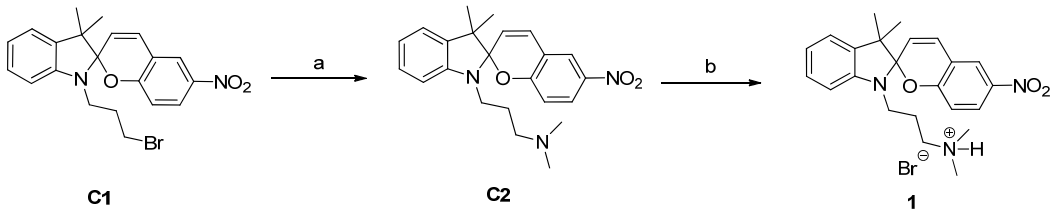
General Methods and Materials

^{1}H NMR (400 MHz) and ^{13}C NMR (100.6 MHz) spectra were recorded on Varian Unity 400 or JEOL Eclipse 400 spectrometer at ca. 20 °C. In the ^{1}H NMR spectra, chemical shifts (δ/ppm) are referenced to internal reference $(CH_3)_4Si$ (0.00 ppm in $CDCl_3$) and to DMSO-d_6 (2.50 ppm). In the ^{13}C NMR spectra, chemical shifts ((δ/ppm) are referenced to the carbon signal of the deuterated solvents; 77.2 ppm in $CDCl_3$, 39.5 ppm in DMSO-d_6).

Thin-layer chromatography was performed on silica gel plates (Merck kieselgel 60, F_{254}) to monitor the reactions. Spots were made visible with UV-light.

2M Me_2NH solution in THF was purchased from Aldrich Company. Solvents, 99.7% ethanol (ab EtOH) and dichloromethane (DCM) were used as received.

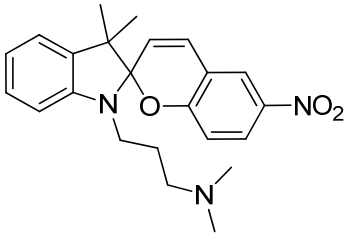
Experimental procedures for the synthesis of compounds C2 and 1.



Scheme S1[a] Synthetic scheme for preparing compounds **C2** and **1**.

[a]Reagents and conditions: (a) 2M Me_2NH solution in THF, rt, 6 h; (b) 0.2M HBr in EtOH, rt.

1′-(3″-Bromopropyl)-3′,3′-dimethy-6-nitrospiro[(2*H*)-1-benzopyran-2,2′-indoline] (**C1**) was synthesized according to published procedures.[1]



C2

N,*N*-Dimethyl-*N*-[3-[3′,3′-dimethy-6-nitrospiro[(2*H*)-1-benzopyran-2,2′-indoline]-1′-yl]propyl]amine (**C2**)

To 1′-(3″-bromopropyl)-3′,3′-dimethy-6-nitrospiro[(2*H*)-1-benzopyran-2,2′-indoline] (4.29 g, 10 mmol) in a flask was added 2M Me_2NH in THF solution (50 mL, 100 mmol). The flask was sealed with a rubber septum and the mixture solution was stirred magnetically at rt under dark for 6 h. The white needle crystals formed in the reaction mixture (Me_2NH_2Br) were filtered off. THF was removed from the filtrate giving a solid. The solid was dissolved in DCM (100 mL) and 0.5 M $NaHCO_3$ (30 mL) was added. The mixture solution was stirred for 20 min. The DCM layer was separated and the aq layer was extracted with DCM (2 x 25 mL). The combined DCM layer was washed with brine and water, dried over anhydrous Na_2SO_4. The DCM was removed under reduced pressure. The crude product was recrystallized from ab EtOH to afford the pure compound **C2** (2.95 g, 75%).

^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.02-7.97 (m, 2H; Ar-H), 7.19 (td, *J* = 8.0, 1.2 Hz, 1H; Ar-H),
7.08 (dd, *J* = 7.2, 1.2 Hz, 1H; Ar-H), 6.90 (d, *J* = 10.4 Hz, 1H; vinyl-H), 6.87 (td, *J* = 7.6, 1.2 Hz, 1H; Ar-H), 6.74 (d, *J* = 8.8 Hz, 1H; Ar-H), 6.61 (d, *J* = 7.6 Hz, 1H; Ar-H), 5.86 (d, *J* = 10.4 Hz, 1H; vinyl-H), 3.33-3.13 (m, 2H; CH_2), 2.31 (t, *J* = 6.8 Hz, 2H; CH_2), 2.21 (s,

6H; $N(CH_3)_2$), 1.90-1.68 (m, 2H; CH_2), 1.28 (s, 3H; CH_3), 1.18 (s, 3H; CH_3) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$) δ = 159.8, 147.2, 141.0, 136.0, 128.3, 127.9, 126.0, 122.9, 122.2, 121.8, 119.5, 118.6, 115.7, 106.9, 106.8, 57.5, 52.9, 45.7, 41.9, 27.3, 26.2, 20.0 ppm.

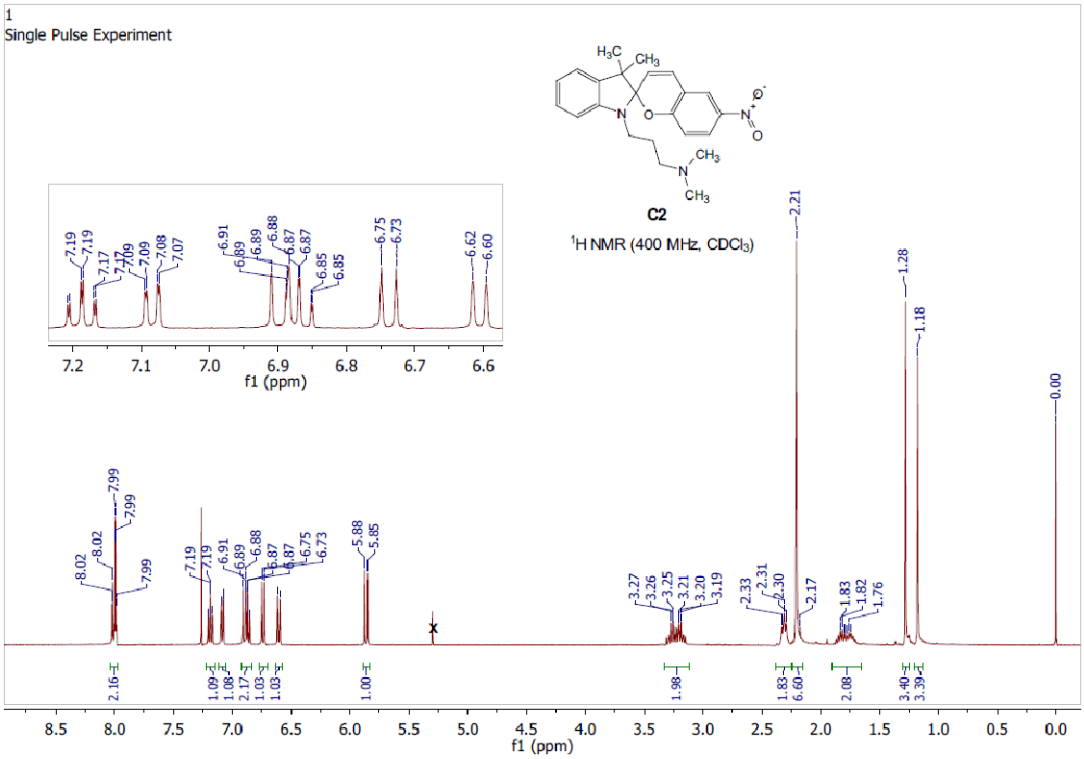


Fig. S1 ^{1}H NMR spectrum of compound **C2**.

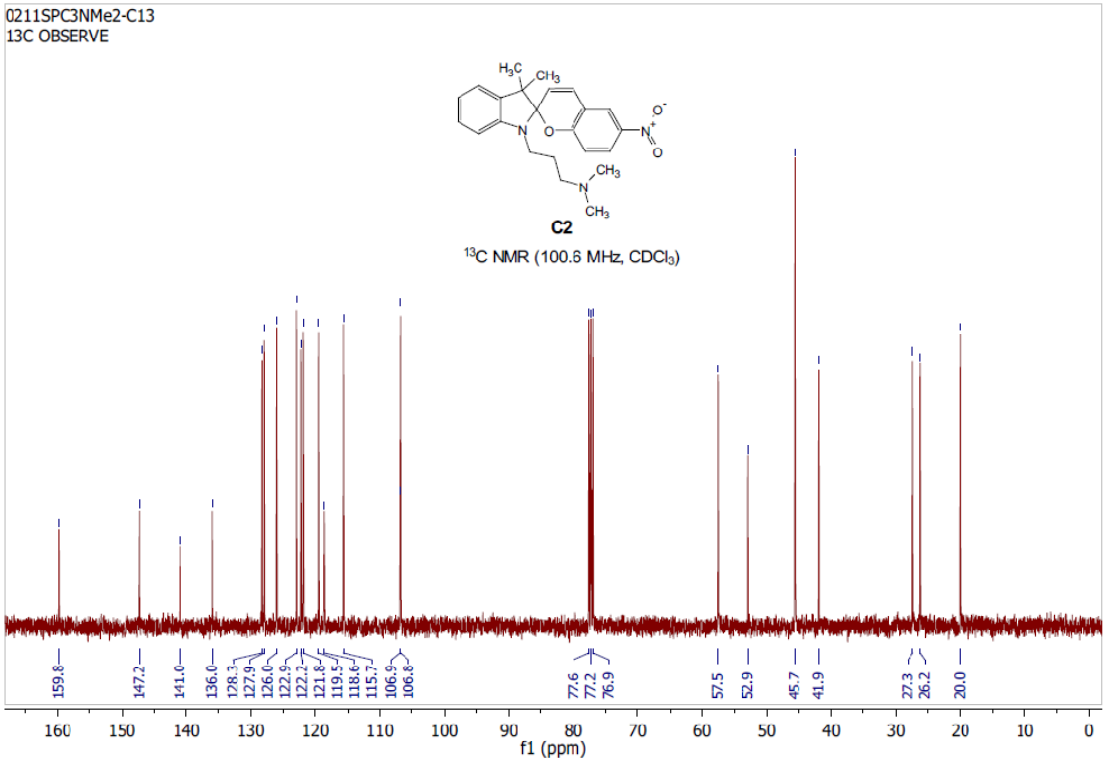
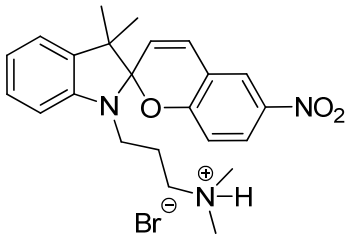


Fig. S2 ^{13}C NMR spectrum of compound **C2**.



1

N,*N*-Dimethyl-*N*-[3-[3′,3′-dimethyl-6-nitrospiro[(2*H*)-1-benzopyran-2,2′-indoline]-1′-yl]propyl]amine hydrobromide (**1**)

N,*N*-Dimethyl-*N*-[3-[3′,3′-dimethy-6-nitrospiro[(2*H*)-1-benzopyran-2,2′-indoline]-1′-yl]propyl]amine (**C2**) (1.18 g, 3 mmol) was dissolved in ab EtOH (60 mL). The solution was cooled on an ice-bath and 0.2 M HBr in EtOH (15 mL, 3 mmol) was added dropwise. The light yellow precipitate formed was filtered off and washed with cooled ab EtOH (0.85 g). The filtrate and washings were combined and concentrated under reduced pressure. The residue was extracted with $CHCl_3$. Removal of $CHCl_3$ by evaporation under reduced pressure gave a light yellow solid (0.55 g). ^{1}H NMR examinations indicated that the light yellow precipitate and the light yellow solid are pure compound **1** (total yield, 98%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.30 (br s, 1H; NH), 8.25 (d, J = 2.8 Hz, 1 H; Ar-H), 8.02 (dd, J = 8.8, 2.8 Hz, 1H; Ar-H), 7.24 (d, J = 10.4 Hz, 1 H; vinyl-H), 7.18-7.12 (m, 2 H; Ar-H), 6.90 (d, J = 9.2 Hz, 1H; Ar-H), 6.83 (t, J = 7.2 Hz, 1H; Ar-H), 6.70 (d, J = 7.6 Hz, 1H; Ar-H), 6.06 (d, J = 10.4 Hz, 1H; vinyl H), 3.27-2.99 (m, 4H; CH_2), 2.73 (s, 6H; $N(CH_3)_2$), 2.05-1.75 (m, 2H; CH_2), 1.21 (s, 3H; CH_3), 1.13 (s, 3H; CH_3) ppm.

^{13}C NMR (101 MHz, DMSO-d_6) δ = 159.1, 146.5, 140.5, 135.6, 128.3, 127.6, 125.8, 122.9, 121.8, 121.7, 119.4, 118.8, 115.5, 106.7, 106.4, 54.6, 52.3, 42.1, 40.3, 25.8, 23.6, 19.6 ppm.

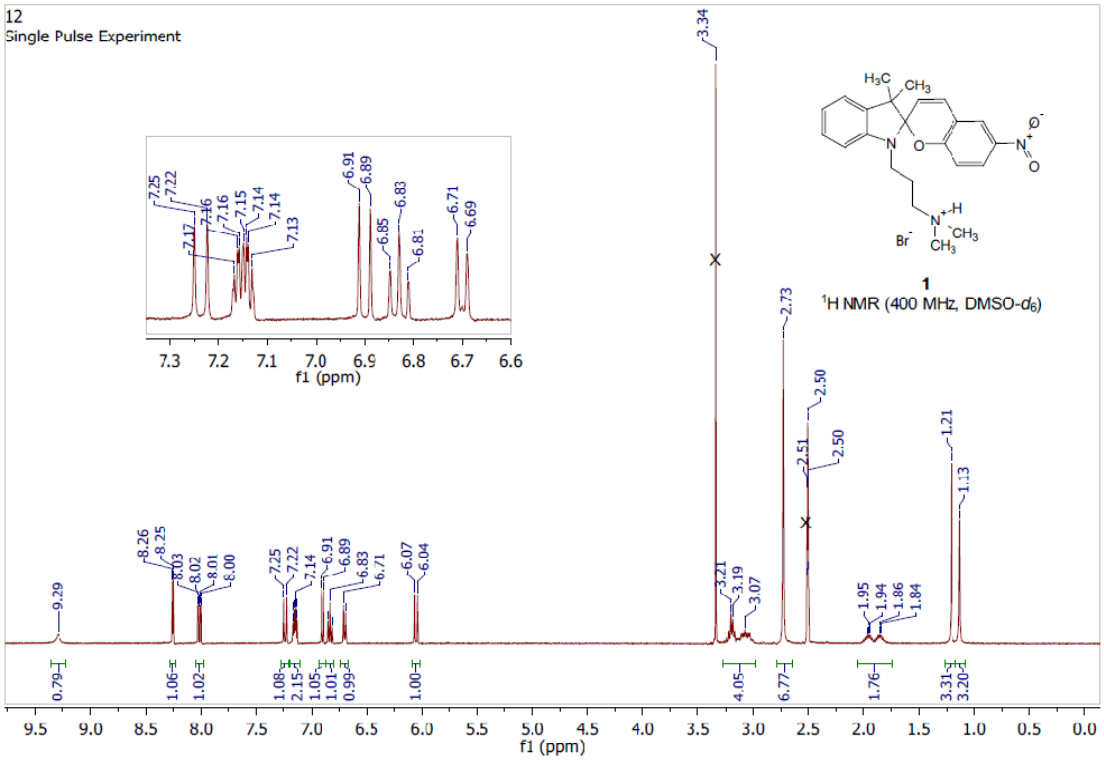


Fig. S3 ^{1}H NMR spectrum of compound **1**.

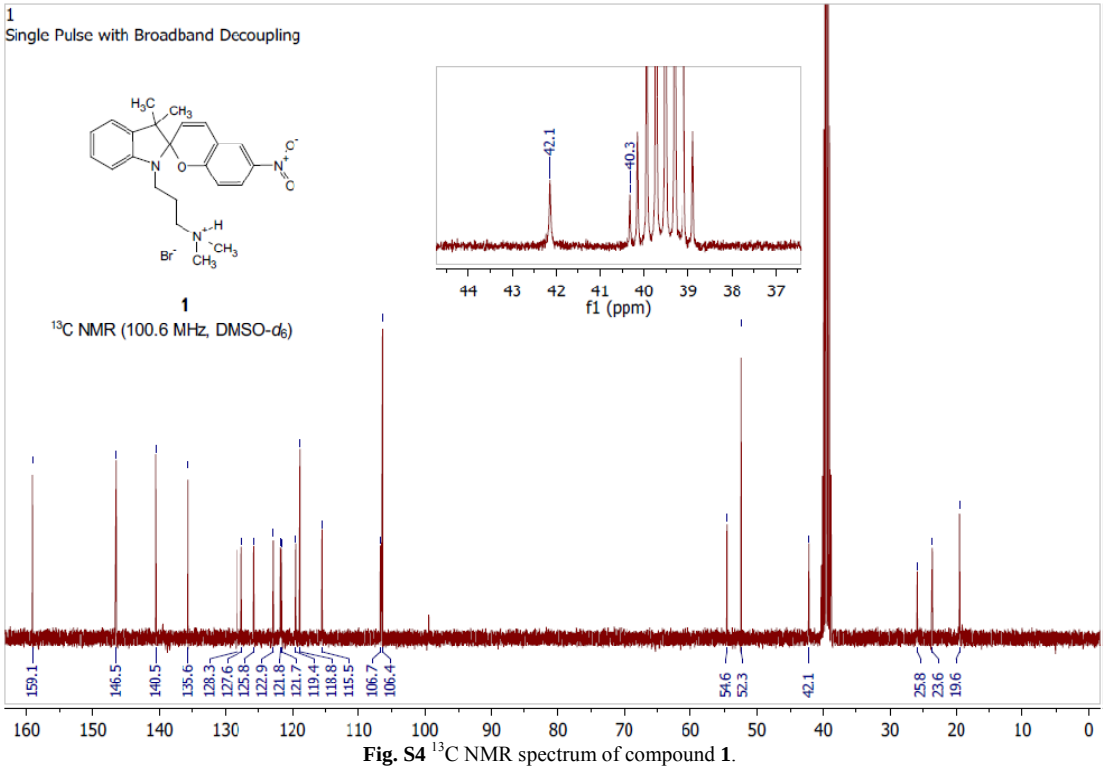


Fig. S4 ^{13}C NMR spectrum of compound **1**.

Spectroscopic characterization

All spectroscopic measurements were performed with mQ water as solvent. The absorption measurements were carried out using a Cary 5000 UV/Vis/NIR spectrometer (for absorption spectra) or a Cary Bio 50 UV/Vis spectrometer (for kinetic measurements). A SPEX Fluorolog *τ*2 was used for the emission measurements. The visible light used for isomerization was generated by a 500 W Xe lamp equipped with a hot mirror (A=1.8 at 900 nm) to reduce the IR intensity and a 455 nm long-pass filter, giving a light power density of ~ 35 mW/cm^2. The 254 nm UV-light was generated by a UVP hand-held UV-lamp model UVGL-25 with a power density of 700 µW/cm^2 at the sample. Total sample volume was ca. 2.2 mL. When applying the visible light, only ~ 1/2 of the sample volume was exposed to the light at any given time, whereas the whole sample volume was exposed to the 254 nm light. The absorption spectra shown in Fig. S5 were recorded with samples in which virtually 100% had been converted to the appropriate forms. The experimental protocol has been described in the literature previously.[2]

UV-VIS Absorption spectra and emission spectrum of SP 1.

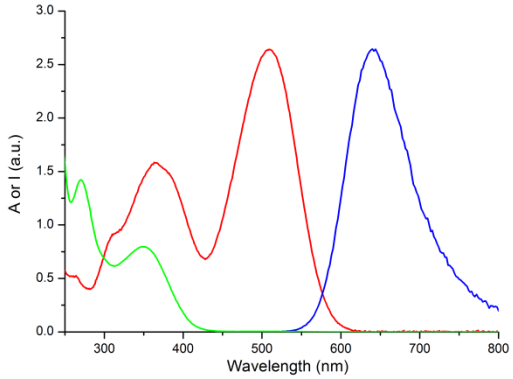


Fig. S5 Absorption spectra of **1c** (green) and **1o** (red) and normalized emission spectrum of **1o** (blue) in mQ water.

Cell culture and imaging

Human embryonic kidney (HEK) 293T or 293A cells were cultured in RPMI-1640 media supplemented with 10% FCS, 2 mM L-glutamine, 1 × non-essential amino acids, 1 mM sodium pyruvate, 50 U/ml penicillin-streptomycin, 50 µM β-mercaptoethanol at 37°C, 5% CO_2. Before the imaging experiments, cells were seeded in Lab-Tek^{TM} chambers and left to adhere for approximately 24 h. SP stock solutions were prepared by dissolving **1** in water and irradiating the solution with 254 nm UV-light until 50/50 **1c**/**1o** was reached. The stock solution was added directly to the chambers, yielding a total SP concentration of 6 µM. The resulting dilution of the cell medium with water was at most 10%. The dye was left in the medium during the measurements. Live cells were imaged at 10× (for the large cell population experiments summarized in Table 1), 20× (for uptake experiments, Figs. S6 and S7) and 63× (all other images) magnification using a LSM 5 Pascal confocal microscope (Carl Zeiss, Oberkochen, Germany) equipped with an environmental chamber kept at 37°C, 5% CO_2. Isomerization of the spiropyran was achieved using 10 s illumination with UV-light (300 nm < λ < 400 nm) from the microscope mercury arc lamp. The open merocyanine form **1o** was probed with argon ion laser excitation at 488 nm and fluorescence collection through an LP505 filter. The size of the pinhole was varied to balance the desired fluorescence intensity at the detector and the Z-resolution. Post experimental image processing was performed in ImageJ 1.43U. The contrast and brightness of the wide field channel was adjusted equally across the entire image for each image. A region of interest was selected to reduce image size.

Cellular uptake experiments

The cellular uptake experiments were performed at 37 °C and at 4 °C (on ice). The 37 °C incubation was carried out as previously described. For the 4 °C incubation the cells were first cultured as above, and then transferred to an ice-cold environment. Next, ice-cold 50/50 **1c**/**1o** stock solution was added to the incubation mixture, yielding a total concentration of 6 µM. After incubation (30 min) images were acquired before and after a 10 s UV pulse for both temperatures. For the higher temperature the microscope stage were kept at 37 °C and for the lower temperature the stage was kept cool with ice packages ensuring that the sample was not significantly warmed up during imaging. Fig. S6 shows one of the eight image pairs acquired at both temperatures. In the quantification of mean intracellular fluorescence (represented in Fig. S7), the pre-UV mean intracellular fluorescence was subtracted from the post-UV ditto for each before/after image pair. The data illustrated in Fig. S7 is the mean value (±SD) of the 8 before/after image pairs for each temperature.

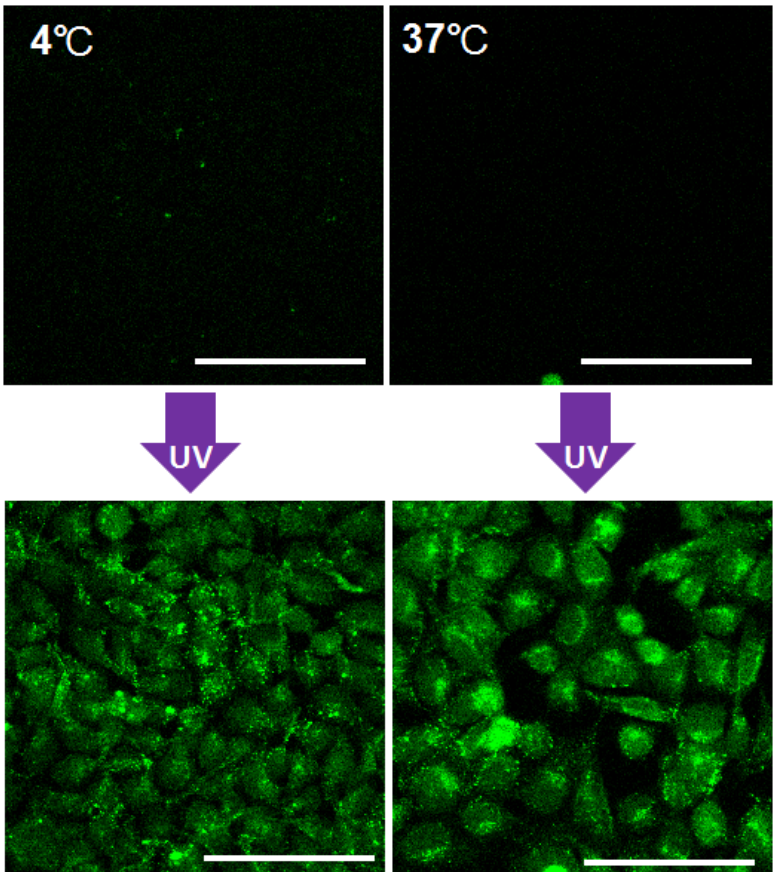


Fig. S6 Images of HEK 293 cells incubated with 50/50 **1c**/**1o** at 4 °C and 37 °C acquired before and after a 10 s UV pulse at both 4 °C and 37 °C. Scale bar = 100 μm.

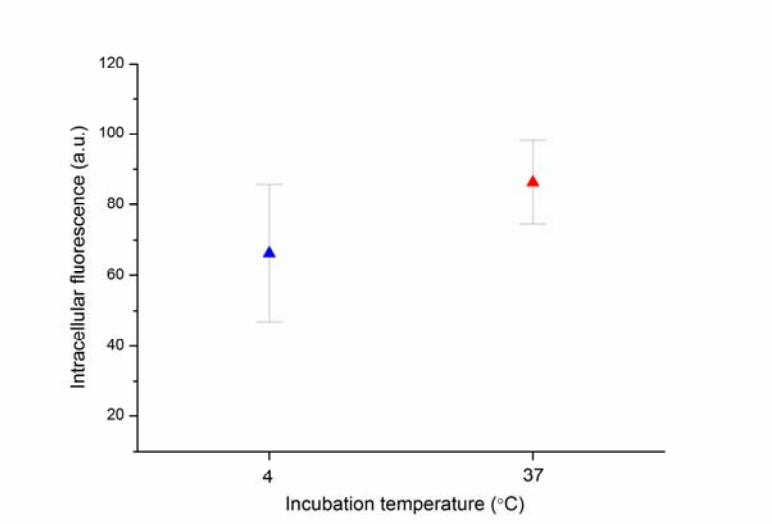


Fig. S7 Net intracellular fluorescence from HEK 293 cells incubated with 50/50 **1c**/**1o** at 4 °C and 37 °C. Mean (±SD) of 8 before/after image pairs.

Video 1

This video shows a confocal time lapse taken immediately after 50/50 **1c**/**1o** (total concentration 6 µM) was added to the cells. Images were acquired every 3 min for the first 516 min, thereafter 10 s UV exposure was applied (at t = 516 min) to the entire field of view and the imaging interval was changed to 20 s. The video displays images at 9 frames per second. Scale bar = 30 µm.

Intracellular vs. non-cellular thermal isomerization.

The cells in Video 1 showed no signs of reduced viability during the course of the time lapse (8.6 h). A very slow increase in fluorescence intensity was noted during the entire time lapse, reflecting the thermal intracellular isomerization **1c** → **1o**. When exposing the entire field of view to 10 s UV-light following this intracellular thermal isomerization, a pronounced increase in the fluorescence intensity was observed (compare time points t = 516 min to t > 516 min).
To contrast the intracellular isomerization to a non-cellular isomerization, a complementary study was made where **1** was dissolved in mQ water at 37 °C and left in the dark. The absorbance changes were monitored over time. As seen in Fig. S8, the non-cellular thermal isomerization reached equilibrium after 90 min. When further **1c** → **1o** isomerization was attempted using 254 nm UV-light, the photoinduced increase ceased after 3 min (purple region in Fig. S8), yielding a negligible absorbance increase. This shows that the thermal isomerization is much slower in an intracellular environment compared to a non-cellular environment. It also suggests that the intracellular thermal process yields a very low concentration of **1o** in the cells (even after 9 h), compared to the corresponding UV-induced isomerization process.

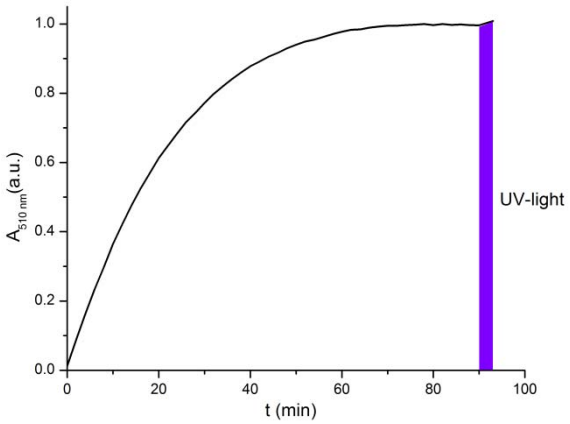


Fig. S8 Absorbance changes at 510 nm upon dissolving **1c** in water at 37 °C, reflecting the thermal isomerization **1c** → **1o**. The absorbance was normalized to 1 at t = 82 min. UV-light (254 nm) was applied once thermal equilibrium (t= 90 min) was reached, isomerising the sample to the photostationary distribution.

Video 2

Prior to this confocal time lapse video the cells were first incubated with 50/50 **1c**/**1o** (total concentration 6 µM) for 15 min, then exposed to a 10 s UV pulse directed exclusively to the circular area marked in the video. Images were acquired every minute during the entire time lapse. The video displays all acquired images at 9 frames per second. Scale bar = 30 µm.

Video 3

This confocal time lapse video shows cells that were first incubated *in the absence* of **1** for 15 min and then exposed to a 10 s UV pulse directed exclusively to the circular area marked in the video. Images were acquired every minute during the entire time lapse. The video displays all acquired images at 9 frames per second. Scale bar = 30 µm.

References

1 J. Andersson, S. M. Li, P. Lincoln, J. Andréasson, *J. Am. Chem. Soc.* 2008, **130**, 11836.
2 T. Stafforst, D. Hilvert, *Chem. Commun.* 2009, 287.