

# Chemistry–A European Journal

Supporting Information

## Excited-State Dynamics in Borylated Arylisoquinoline Complexes in Solution and *in cellulo*

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## Supporting Information

## Experimental Procedures

### 1. Materials and methods

#### General information and materials

$^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in  $\text{CDCl}_3$  at 400 MHz or 100 MHz, respectively, using the residual solvent signal as reference.  $^{11}\text{B}$  NMR spectra were recorded with complete proton decoupling at 160 MHz, using  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  (0.00 ppm for  $^{11}\text{B}$  NMR) as standard. The solvents *n*-hexane, EtOAc, methanol,  $\text{CH}_2\text{Cl}_2$ , and DMF were used as received. The starting materials **3-6**,<sup>[1]</sup> 2-azidoethyl methanesulfonate,<sup>[2]</sup> and the PEG-linkers<sup>[3]</sup> were synthesized according to procedures described in the literature.

#### Synthesis of azide **7**

To a round-bottom flask, containing **5** (0.050 mmol, 26 mg) and  $\text{K}_2\text{CO}_3$  (0.10 mmol, 14 mg), a solution of 2-azidoethyl methanesulfonate (0.20 mmol, 33 mg) in 0.5 mL of DMF was added. The resulting reaction mixture was stirred for 3 hours at 60 °C, and then it was diluted with EtOAc (20 mL) and washed with a saturated aqueous solution of  $\text{NH}_4\text{Cl}$  ( $2 \times 5$  mL). The organic phase was dried over anhydrous  $\text{MgSO}_4$ , filtered, concentrated to dryness, and the crude product was purified by column chromatography on silica gel (*n*-hexane/EtOAc 10:1) to afford **7** (20 mg, 68 % yield) as a viscous yellow oil.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 343 K):  $\delta$  8.68 (d, 1H,  $J$  = 8.6 Hz, Ar-H), 8.41 (d, 1H,  $J$  = 6.7 Hz, Ar-H), 8.42 (m, 1H, Ar-H), 8.18 (m, 1H, Ar-H), 7.84 (t, 1H,  $J$  = 8.2 Hz, Ar-H), 7.80 (t, 1H,  $J$  = 7.6 Hz, Ar-H), 7.60 (ddd, 1H,  $J$  = 8.5, 6.6, and 1.5 Hz, Ar-H), 7.47-7.42 (m, 3H, Ar-H), 7.41 (s, 1H, Ar-H), 6.62 (br s, H, Ar- $\text{H}_{\text{Mes}}$ ), 4.33 (t, 2H,  $J$  = 5.0 Hz,  $\text{OCH}_2$ ), 3.69 (t, 2H,  $J$  = 5.0 Hz,  $\text{CH}_2\text{N}_3$ ), 2.16 (s, 6H,  $2 \times \text{CH}_3\text{Mes}$ ), 1.94 (br s, 9H,  $3 \times \text{CH}_3\text{Mes}$ ), 1.42 (s, 3H,  $\text{CH}_3\text{Mes}$ ) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  179.4 (C), 161.2 (C), 156.6 (C), 155.6 (C), 144.3 (br s, C), 140.9 (br s, C), 138.1 (C), 137.3 (CH), 134.1 (CH), 132.5 (CH), 131.0 (CH), 129.9 ( $2 \times \text{CH}$ ), 127.0 (CH), 126.8 (CH), 126.2 (CH), 125.7 (CH), 124.8 (C), 124.7 (CH), 124.4 (CH), 124.1 (C), 123.2 (CH), 118.1 (CH), 107.9 (CH), 67.1 ( $\text{OCH}_2$ ), 50.6 ( $\text{CH}_2\text{N}_3$ ), 25.0 (br s,  $2 \times \text{CH}_3\text{Mes}$ ), 20.7 ( $4 \times \text{CH}_3\text{Mes}$ ) ppm; (C-B not observed).  $^{11}\text{B}$  NMR (128 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  4.9 (br s) ppm. HRMS (ESI) calcd. for  $\text{C}_{39}\text{H}_{38}\text{BN}_4\text{O}^+$  ( $\text{M} + \text{H}^+$ ) 589.3133. Found 589.3125.

#### Dye **1**

To a round-bottom flask containing **6** (0.079 mmol, 44 mg) and  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  (10 mol%, 2.0 mg), a solution of the PEG-azide (0.12 mmol, 29 mg) in 0.5 mL of wet DMF and a solution of sodium ascorbate (20 mol%, 3.1 mg) in 0.5 mL of wet DMF were consecutively added. The resulting reaction mixture was heated overnight at 60 °C. Then, the solution was cooled down to room temperature, diluted with EtOAc (15 mL), and washed with  $\text{H}_2\text{O}$  ( $3 \times 10$  mL). The organic phase was dried over anhydrous  $\text{MgSO}_4$ , filtered, concentrated to dryness, and the crude product was purified by column chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ; gradient 10:1  $\rightarrow$  5:1) to afford **1** (47 mg, 74 % yield) as a yellow foam.

$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  8.65 (d, 1H,  $J$  = 8.6 Hz, Ar-H), 8.39 (d, 1H,  $J$  = 6.6 Hz, Ar-H), 8.27 (d, 1H,  $J$  = 8.1 Hz, Ar-H), 8.17 (d, 1H,  $J$  = 8.3 Hz, Ar-H), 7.86-7.79 (m, 2H, Ar-H), 7.62-7.58 (m, 2H, Ar-H and  $\text{H}_{\text{triazole}}$ ), 7.51 (s, 1H, Ar-H), 7.47-7.38 (m, 3H, Ar-H), 6.78 (br s, 2H, Ar- $\text{H}_{\text{Mes}}$ ), 6.52 (br s, 1H, Ar- $\text{H}_{\text{Mes}}$ ), 6.39 (br s, 1H, Ar- $\text{H}_{\text{Mes}}$ ), 5.40 (br s, 2H,  $\text{OCH}_2$ ), 4.49 (t, 2H,  $J$  = 5.3 Hz,  $\text{NCH}_2$ ), 3.84 (br s, 2H,  $\text{OCH}_2$ ), 3.66-3.51 (br s, 10H,  $\text{OCH}_2$ ), 2.54 (br s, 2H,  $J$  = 6.1 Hz,  $\text{CH}_2\text{CO}_2\text{H}$ ), 2.24-2.16 (br s, 12H,  $4 \times \text{CH}_3\text{Mes}$ ), 1.66-1.55 (br s, 6H,  $2 \times \text{CH}_3\text{Mes}$ ) ppm.  $^{13}\text{C}$  NMR (100 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  173.9 ( $\text{CO}_2\text{H}$ ), 160.8 (C), 156.1 (C), 150.4 (C), 143.5 (C), 137.8 (C), 136.9 (CH), 132.5 (CH), 130.7 (C), 130.3 (br s,  $\text{C}_{\text{Mes}}$ ), 130.1 (br s,  $\text{C}_{\text{Mes}}$ ), 129.7 (CH), 126.9 (CH), 126.8 (CH), 126.1 (CH), 125.5 (C), 124.5 (CH), 124.4 (C), 124.1 (CH), 123.7 (C), 123.1 (CH), 118.2 (CH), 107.7 (CH), 70.5 ( $2 \times \text{OCH}_2$ ), 70.3 ( $\text{OCH}_2$ ), 70.1 ( $\text{OCH}_2$ ), 69.4 ( $\text{OCH}_2$ ), 66.3 ( $\text{OCH}_2$ ), 61.8 ( $\text{OCH}_2$ ), 50.3 ( $\text{NCH}_2$ ), 34.6 ( $\text{CH}_2\text{CO}_2\text{H}$ ), 25.4 ( $2 \times \text{CH}_3\text{Mes}$ ), 20.7 ( $4 \times \text{CH}_3\text{Mes}$ ) ppm; (C-B not observed).  $^{11}\text{B}$  NMR (128 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  5.7 (br s) ppm. HRMS (ESI) calcd. for  $\text{C}_{49}\text{H}_{53}\text{BN}_4\text{NaO}_6^+$  ( $\text{M} + \text{Na}^+$ ) 827.3950. Found 827.3949.

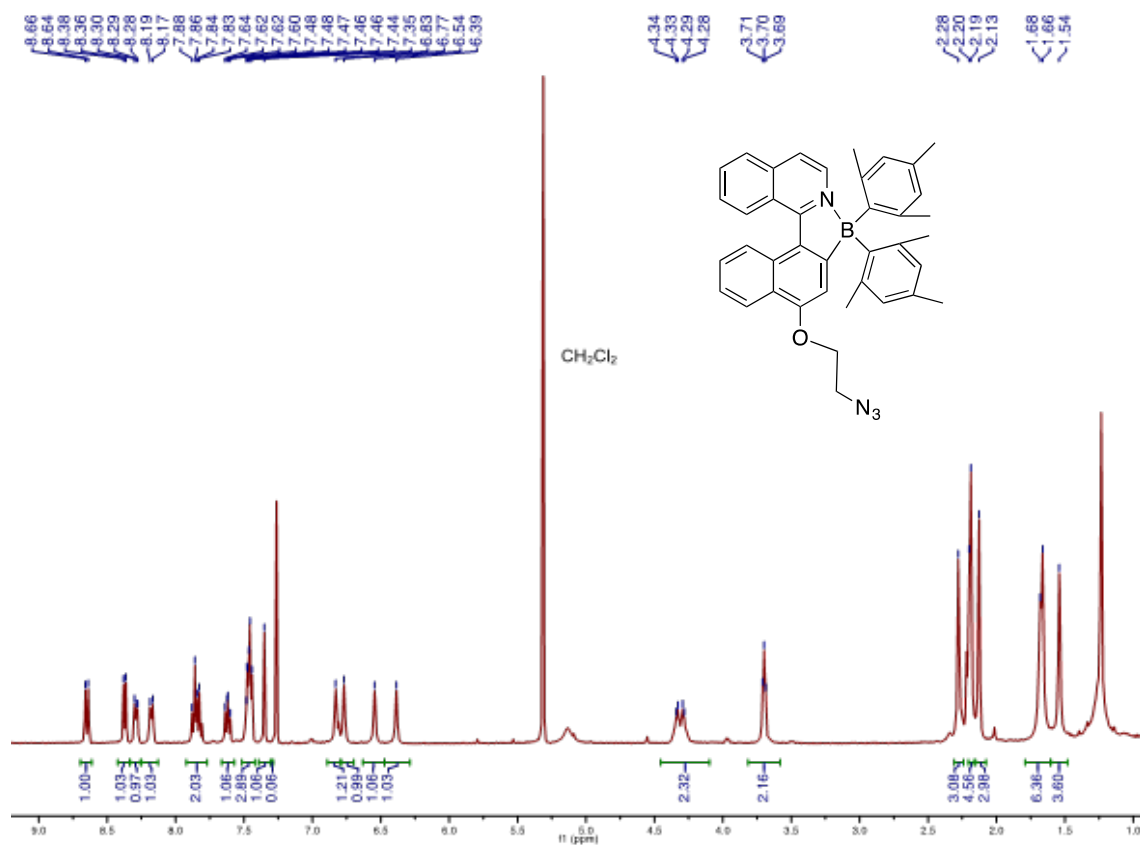
#### Dye **2**

To a round-bottom flask containing **7** (0.075 mmol, 44 mg) and  $\text{CuSO}_4 \cdot 5 \text{H}_2\text{O}$  (10 mol%, 1.9 mg), a solution of the PEG-alkyne (0.11 mmol, 33 mg) in 0.5 mL of wet DMF and a solution of sodium ascorbate (20 mol%, 3.0 mg) in 0.5 mL of wet DMF were consecutively added. The resulting reaction mixture was heated overnight at 60 °C. Then, the solution was cooled down to room temperature, diluted with EtOAc (15 mL), and washed with  $\text{H}_2\text{O}$  ( $3 \times 10$  mL). The organic phase was dried over anhydrous  $\text{MgSO}_4$ , filtered, concentrated to dryness, and the crude product (**8**) was dissolved in 0.5 mL of  $\text{CH}_2\text{Cl}_2$  and treated with trifluoroacetic acid (1.3 mmol, 100  $\mu\text{L}$ ). After 1 h at room temperature, the reaction mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (10 mL) and washed with an aqueous saturated solution of  $\text{NaHCO}_3$  ( $3 \times 5$  mL). The organic phase was dried over anhydrous  $\text{MgSO}_4$ , filtered, concentrated to dryness, and the crude product was purified by chromatography on silica gel ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$ ; gradient 10:1  $\rightarrow$  5:1) to give **2** (30 mg, 52 % yield) as a viscous yellow oil.

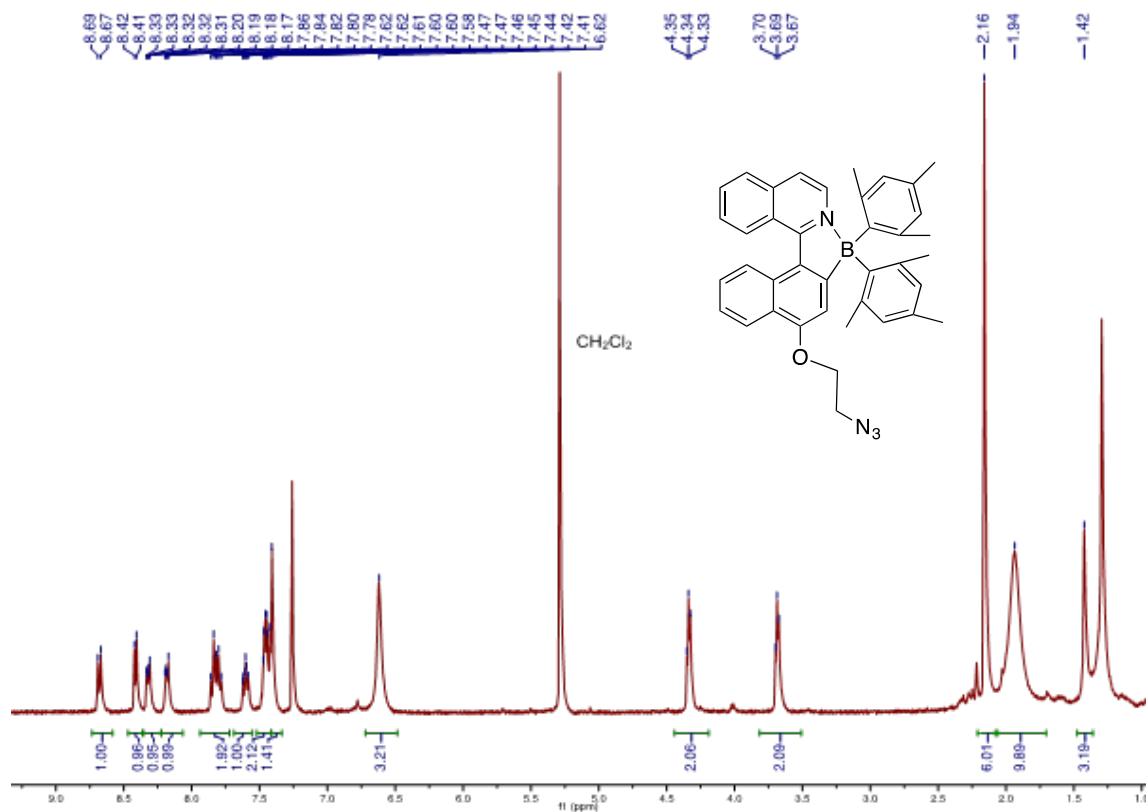
$^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ , 298 K):  $\delta$  8.61 (d, 1H,  $J$  = 8.4 Hz, Ar-H), 8.39 (d, 1H,  $J$  = 6.3 Hz, Ar-H), 8.18-8.13 (m, 2H, Ar-H), 7.87-7.77 (m, 3H, Ar-H), 7.61 (t, 1H,  $J$  = 7.5 Hz, Ar-H), 7.45-7.44 (m, 3H, Ar-H), 7.36 (s, 1H,  $\text{H}_{\text{triazole}}$ ), 6.77 (br s, 2H, Ar- $\text{H}_{\text{Mes}}$ ), 6.53 (br s, 1H, Ar-

$H_{Mes}$ , 6.39 (br s, 1H, Ar- $H_{Mes}$ ), 4.84 (br s, 2H,  $OCH_2$ ), 4.65 (br s, 2H,  $OCH_2$ ), 4.51 (br s, 2H,  $NCH_2$ ), 3.82-3.58 (br s, 10H,  $OCH_2$ ), 3.13 (br s, 4H,  $CH_2NH_2$ ), 2.21-2.14 (br s, 12H,  $4 \times CH_{3Mes}$ ), 1.67-1.60 (br s, 6H,  $2 \times CH_{3Mes}$ ) ppm.  $^{13}C$  NMR (100 MHz,  $CDCl_3$ , 298 K):  $\delta$  176.4 (C), 169.6 (C), 160.7 (C), 155.6 (C), 144.5 (br s, C), 137.8 (C), 137.0 (CH), 132.6 (CH), 130.7 (br s, C), 130.2 (br s, C), 129.6 (CH), 127.0 (CH), 126.3 (CH), 125.1 (C), 124.8 (CH), 124.7 (CH), 124.5 (CH), 123.8 (CH), 122.4 (CH), 118.5 (CH), 107.4 (CH), 70.4 ( $OCH_2$ ), 70.1 ( $OCH_2$ ), 69.9 ( $OCH_2$ ), 69.4 ( $OCH_2$ ), 67.2 ( $OCH_2$ ), 66.0 ( $OCH_2$ ), 63.6 ( $OCH_2$ ), 49.9 ( $NCH_2$ ), 40.2 ( $CH_2NH_2$ ), 25.5-22.7 ( $2 \times CH_{3Mes}$ ), 20.7 ( $4 \times CH_{3Mes}$ ) ppm, (C-B not observed).  $^{11}B$  NMR (128 MHz,  $CDCl_3$ , 298 K):  $\delta$  5.7 (br s) ppm. HRMS (ESI) calcd. for  $C_{48}H_{55}BN_5O_4^+$  ( $M + H^+$ ) 776.4342. Found 776.4338.

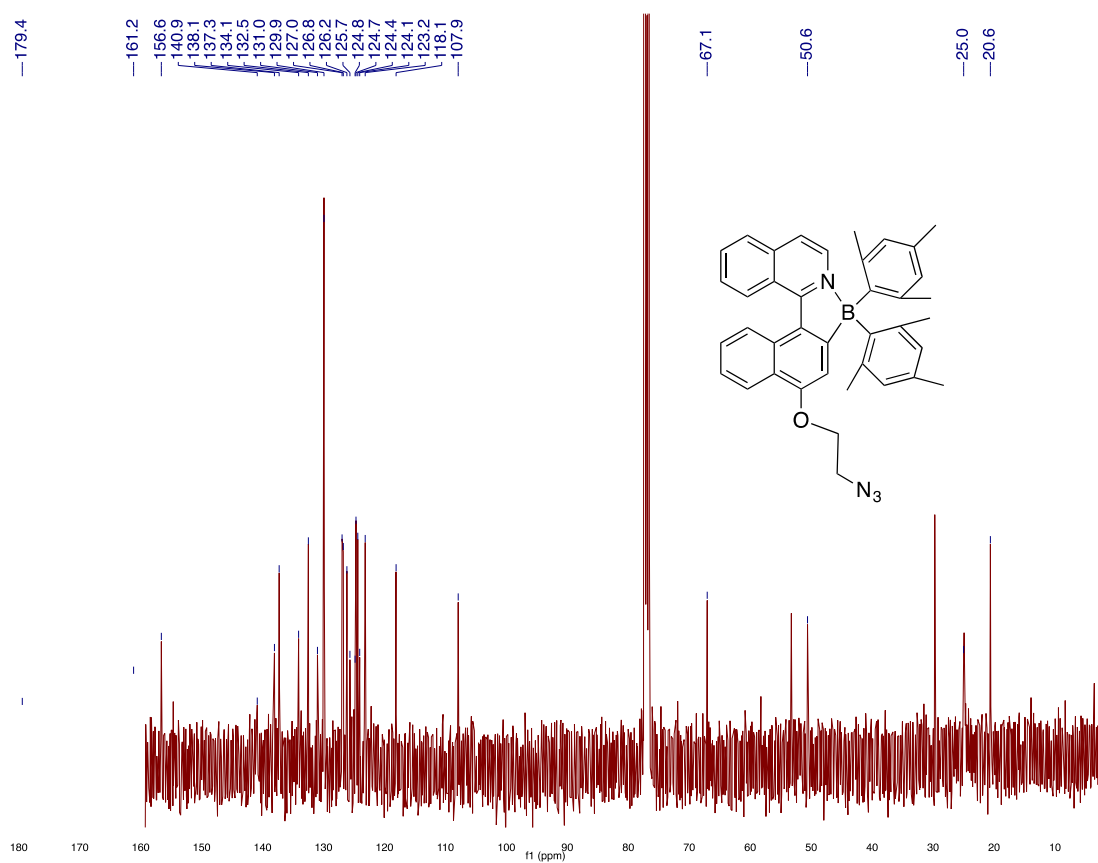
<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz, 263K) for **7**:



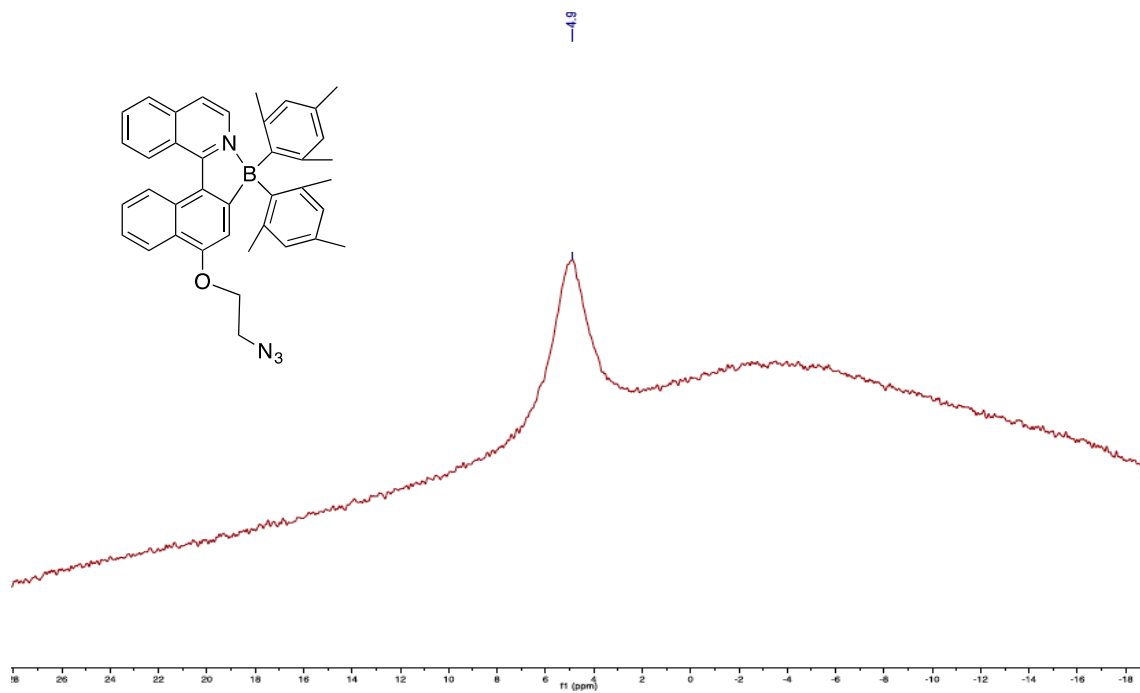
<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz, 343K) for **7**:



$^{13}\text{C}$ -NMR ( $\text{CDCl}_3$ , 100 MHz, 343K) for **7**:



$^{11}\text{B}$ -NMR ( $\text{CDCl}_3$ , 128 MHz, 298K) for **7**:



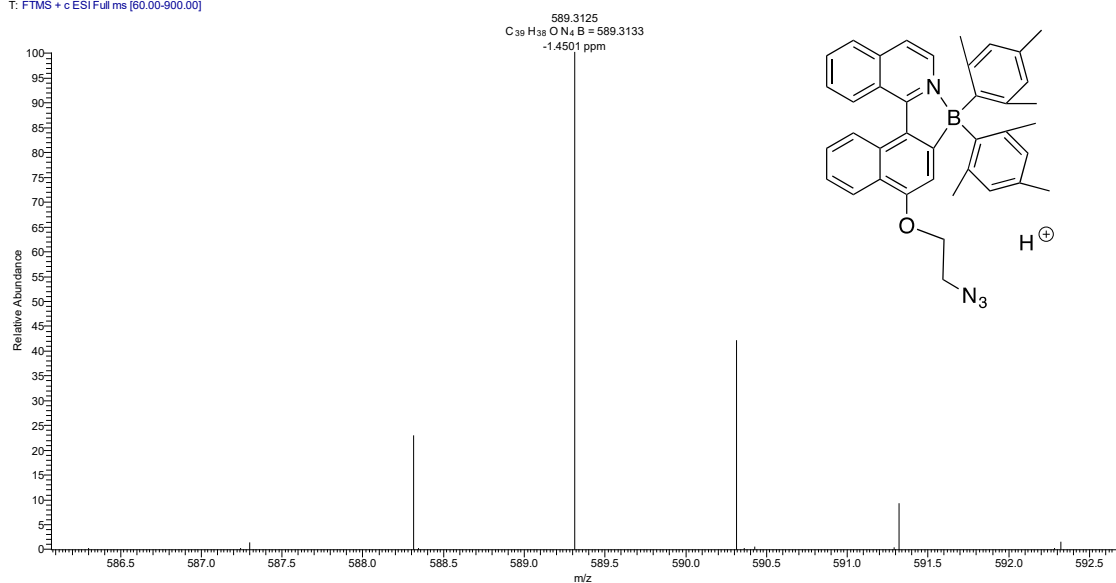
# HRMS (ESI) for 7:

171122\_ROS358

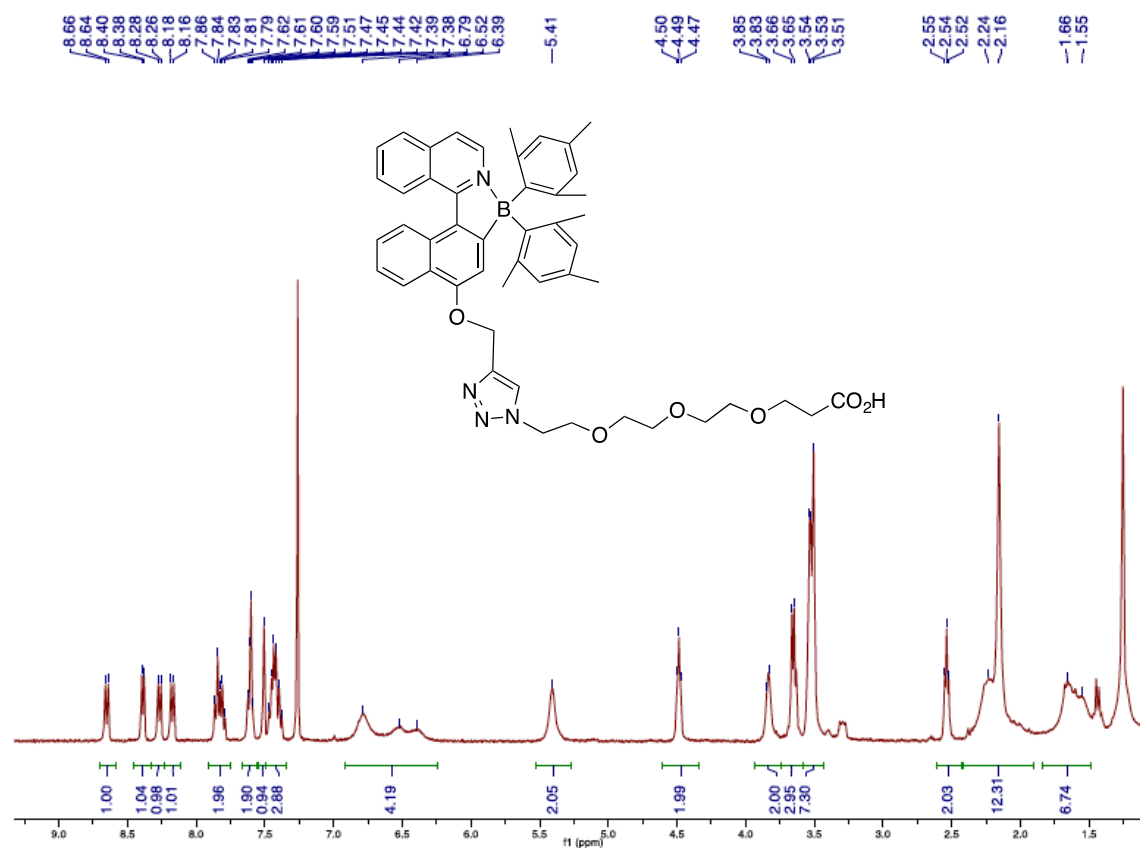
11/22/17 14:04:37

ROS-358 PM=588 C39H37BN4O

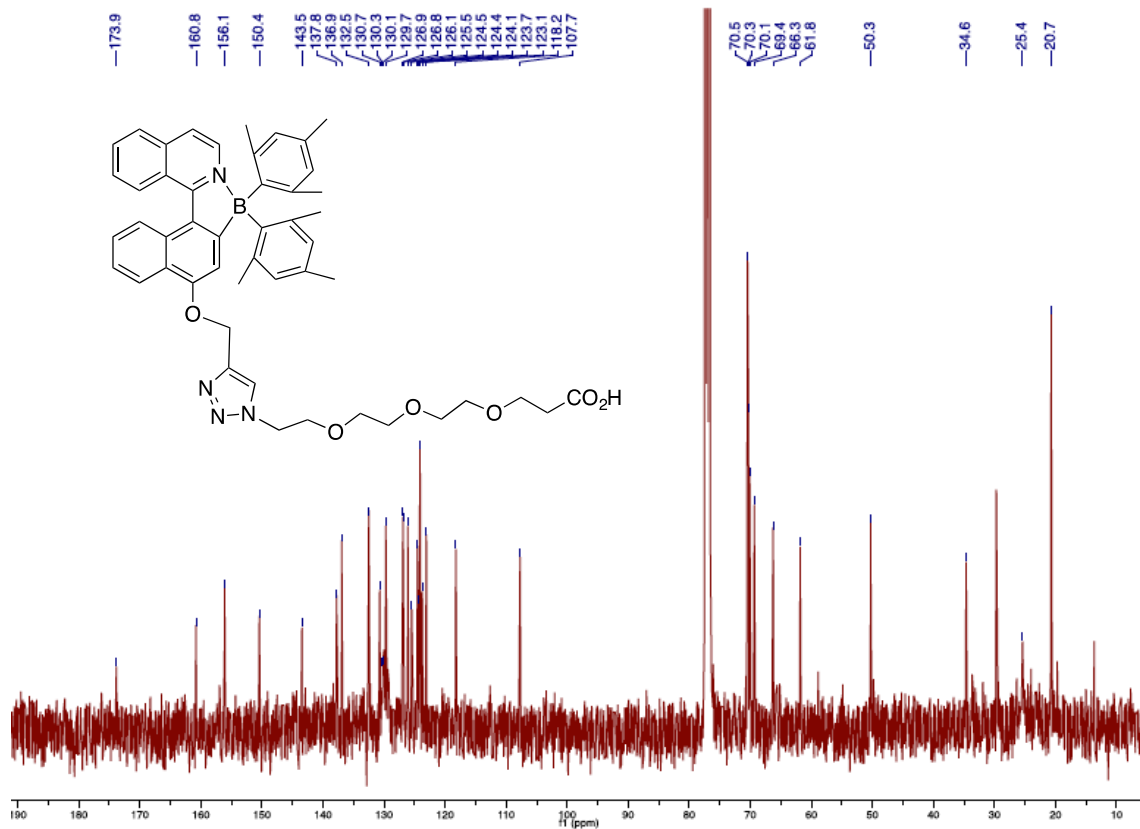
171122\_ROS358 #81-99 RT: 0.32-0.39 AV: 19 NL: 4.50E6  
T: FTMS + c ESI Full ms [60.00-900.00]



<sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz, 298K) for 1:

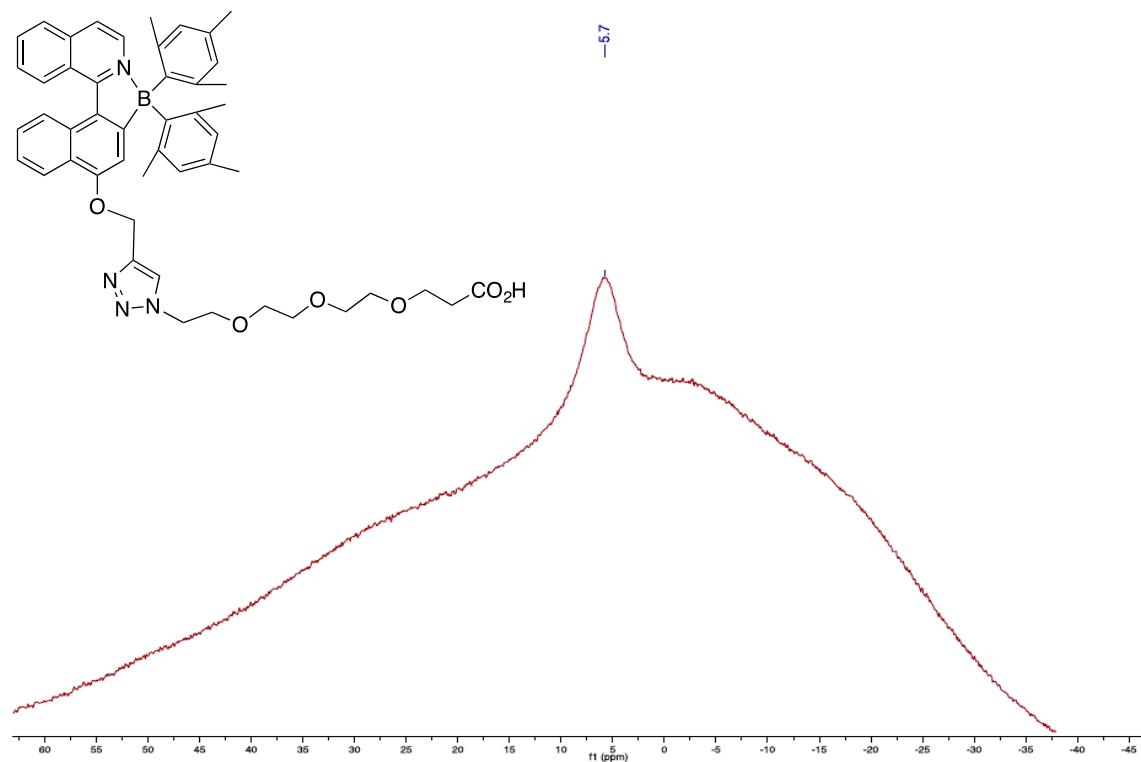


<sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz, 298K) for 1:

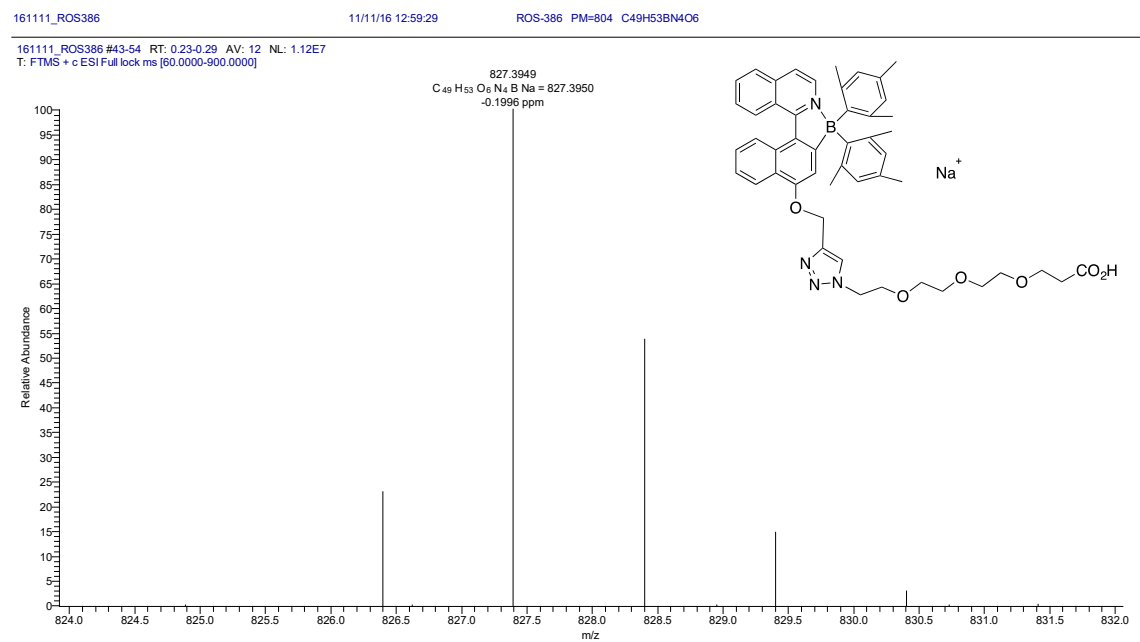




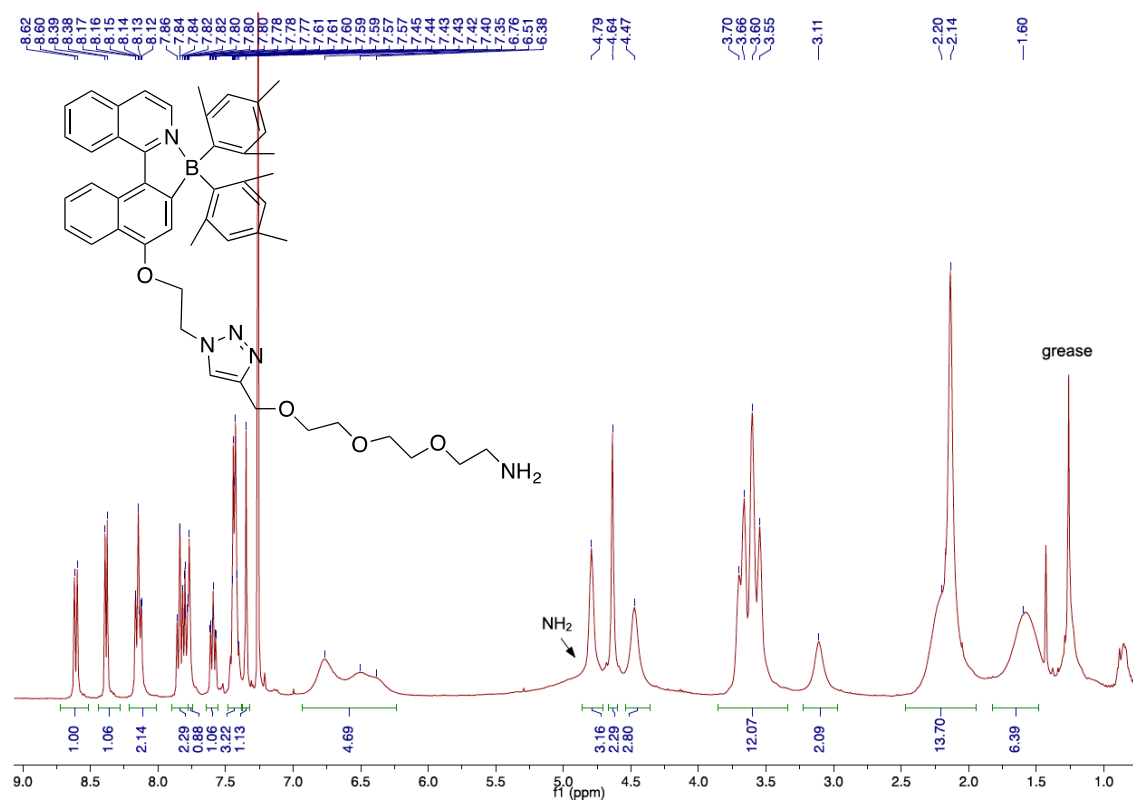
$^{11}\text{B}$ -NMR ( $\text{CDCl}_3$ , 128 MHz, 298K) for **1**:



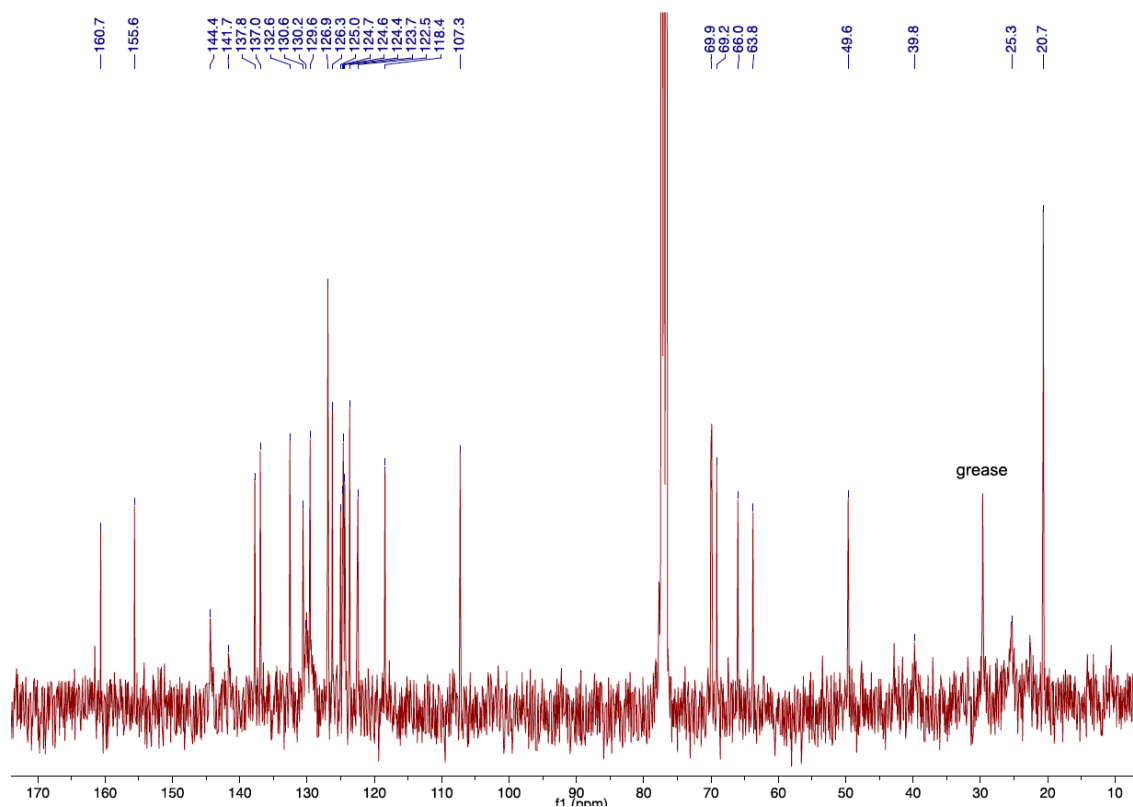
HRMS (ESI) for **1**:



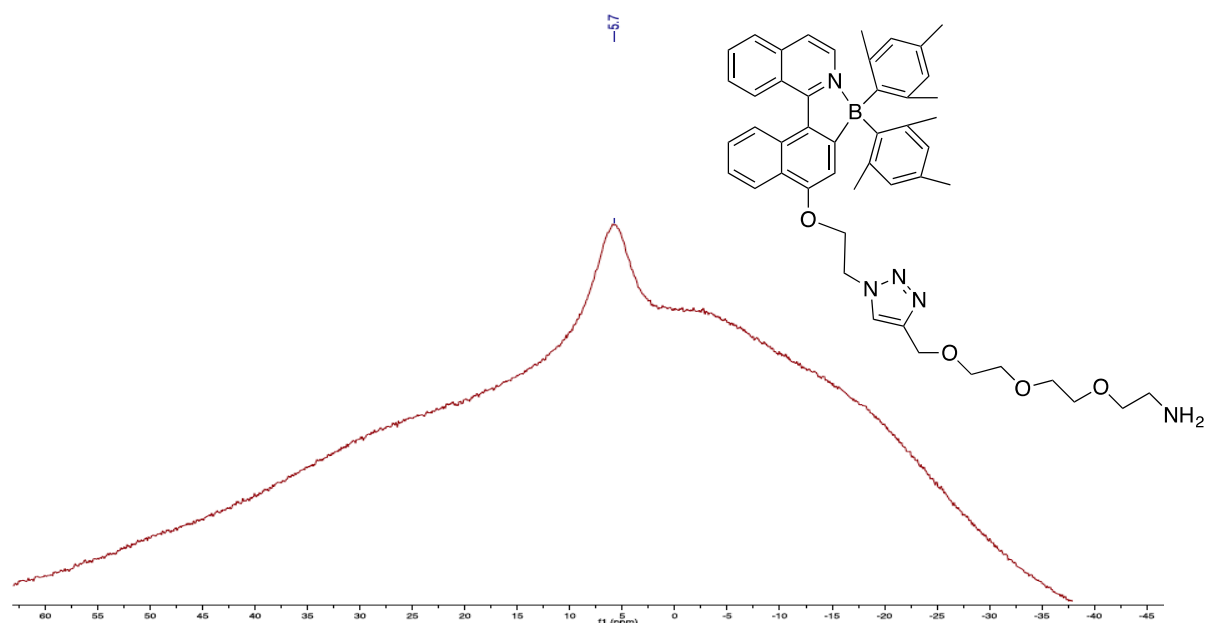
$^1\text{H-NMR}$  ( $\text{CDCl}_3$ , 400 MHz, 298K) for **2**:



$^{13}\text{C-NMR}$  ( $\text{CDCl}_3$ , 100 MHz, 298K) for **2**:



<sup>11</sup>B-NMR (CDCl<sub>3</sub>, 128 MHz, 298K) for **2**:



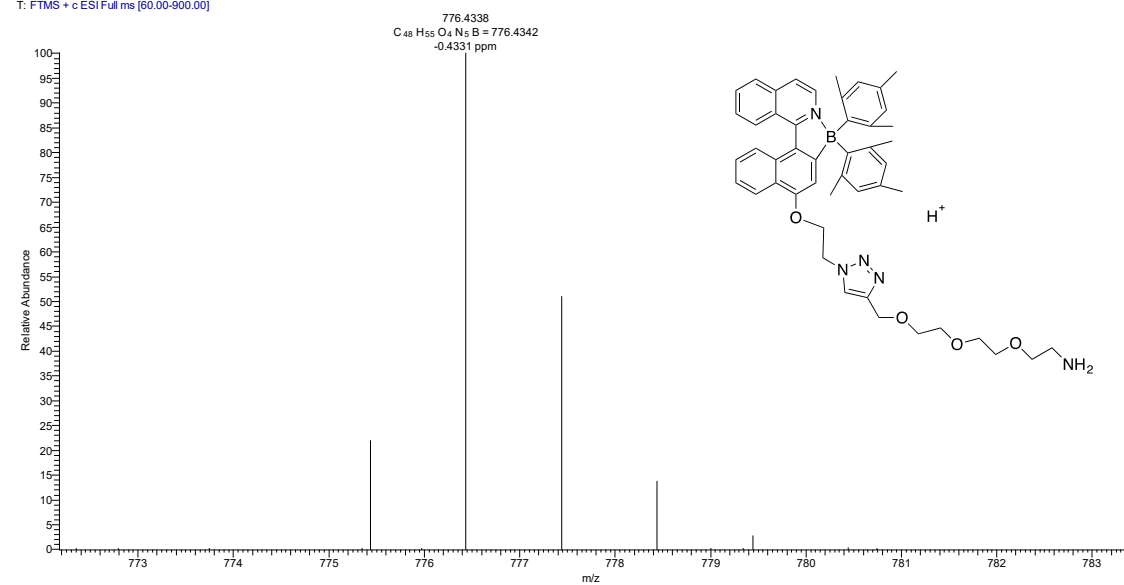
HRMS (ESI) for **2**:

161130\_ROS390F2

11/30/16 11:50:49

Ros-390F2 PM=775 C48H54BN5O4

161130\_ROS390F2 #50-65 RT: 0.20-0.26 AV: 16 NL: 2.79E6  
T: FTMS + c ESI Full ms [60.00-900.00]



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## Absorption and emission measurements

UV/vis absorption spectra of **1** and **2** in DMSO and water were recorded with a JASCO V-760 spectrophotometer. The steady-state emission spectra were recorded with a FLS980 spectrometer (Edinburgh Instruments Ltd., Livingston, UK). All samples were measured in 1cm quartz cuvettes under air-equilibrated conditions at room temperature (25°C).

Absolute fluorescence quantum yields were determined using an FLS980 spectrometer equipped with an integrating sphere. The optical densities of the solutions were set to  $\sim 0.1$  to avoid inner filter effects and re-absorption of the photoluminescence. An excitation wavelength of 420 nm was used.

## Time-resolved emission spectroscopy measurements

Fluorescence decay curves were determined by employing a Hamamatsu HPDTA streak camera (C4334, Hamamatsu Photonics K.K., Hamamatsu, Germany). Samples were excited by a pulse centered at 400 nm created by frequency-doubling the output of a Ti:Sapphire laser (Tsunami, Newport Spectra-Physics GmbH, Berlin, Germany). The repetition rate of the fundamental is produced at 400 kHz by a pulse selector (model 3980, Newport Spectra-Physics GmbH, Berlin, Germany). Fluorescence spectra were collected for DMSO and water in air equilibrated conditions in a 1cm quartz cuvette at a 90° angle and spectrally dispersed on the detector using a CHROMEX spectrograph (Chromex, Albuquerque, NM, USA). A glass plate was used to record the instrumental response function (IRF) by reflecting parts of the attenuated excitation beam directly into the detector. IRF is typically in the order of 250 ps. The emission kinetics were spectrally integrated and the resulting decay traces fitted with Origin software.

## Femtosecond transient absorption spectroscopy measurements

The femtosecond (fs) transient absorption spectra were obtained with a home-built setup, which has been described in detail elsewhere.<sup>[4]</sup> A Ti:sapphire amplifier (Libra, Coherent, USA) with a 1 kHz repetition rate was used for pulse regeneration. The output is split into two fractions by a 50/50 beam splitter. One fraction is used to generate the 530 nm pump beam with a time duration of  $\sim 110$  fs with two optical parametric amplifiers (TOPAs, LightConversion Ltd).<sup>[5]</sup> The other one is focused on a rotating CaF<sub>2</sub> plate to generate a supercontinuum light used to probe the absorbance of the sample between 350 to 800 nm. A mechanical chopper is used to reduce the repetition rate of the pump pulses to 0.5 kHz and the polarization between the probe and pump beam set at the magic angle ( $\sim 54.7^\circ$ ) by adjusting a Berek compensator and a polarizer. Throughout the measurement, the pump power was set to  $\sim 250$   $\mu$ W at the sample position. The probe beam was delayed in time with respect to the pump beam by passing through an optical delay line. The temporal resolution of the experiment is limited to 300 fs due to the strong contributions from coherent artifact signals to the data.<sup>[6]</sup> The data was chirp-corrected after exclusion of a temporal window of 300 fs around time-zero and subsequently globally fitted with a sum of exponential functions by using custom software (Pascher Instruments AB, Lund, Sweden). All samples were measured in 1cm quartz cuvettes.

## Nanosecond transient absorption spectroscopy measurements

Nanosecond (ns) transient absorption spectroscopy was employed to study the spectra at long decay time, which are invisible in the fs-transient absorption data. The pump pulses centred at 430 nm were produced by a continuum Surelite Nd:YAG laser system with a pulse duration of 5 ns and a repetition rate of 10 Hz. The probe light is provided by a xenon arc lamp. The probe beams are focused into the sample by the spherical concave mirrors and then sent into the monochromator (Acton, Princeton Instruments). The spectrally selected probe light is detected by a Hamamatsu R928 photomultiplier. The signal is amplified and processed by a commercially available detection system (Pascher Instruments AB, Lund, Sweden). The power of the pump beam was kept at  $\sim 0.27$  mJ and  $\sim 0.38$  mJ for Figure 2f and Figure S7, respectively. All samples were measured in 1cm quartz cuvettes. Oxygen-free solutions were prepared in Glove box and measured in 1cm inert quartz cuvettes.

## Cell culture procedure and sample preparation

MCF-7 cells were chosen as a cell line sample and incubated in a 25 cm<sup>2</sup> cell culture flask (Thermo Fisher) with RPMI 1640 medium with L-glutamine and sodium bicarbonate (Sigma-Aldrich), which was supplemented with 10% fetal bovine serum (Sigma-Aldrich), together with 1% streptomycin-penicillin (Sigma-Aldrich). Cells were incubated at 37°C with 5% CO<sub>2</sub> incubator. At approximately 90% confluency, the cells were detached by a 0.25% Trypsin/0.53 mM EDTA solution (ATCC) and then split and seeded in 35 mm glass-bottom  $\mu$ -dishes (Cellvis), then placed in the incubator for overnight. The cells in dishes were rinsed with phosphate-buffered saline (PBS) (Sigma-Aldrich) and stained with 20  $\mu$ M of **1** and **2** for  $\sim 3$  h (the stock solution was prepared in dimethyl sulfoxide (DMSO) solvent), respectively. Finally, the cells are rinsed twice with PBS to remove any unwanted residue of complexes on the cells. Prior to the measurements, the irradiated areas were marked and imaged (Figure S15). During the experiments, the cells were covered with Hanks' balanced salt solution (HBSS) (Sigma-Aldrich). After the measurements, the cells were treated with Trypan Blue Solution (0.4%) (gibco, USA) to check if the cells were damaged by laser pulses (see Figure S15). The irradiated area of the cell was imaged by a combination of a Raspberry Pi camera and Carl Zeiss Axiovert 25 microscope with the objective EC Plan-Neofluar 10X/0.30 M27.

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## Time-resolved pump-probe setup on the bulk of live cells measurements.

A home-built time-resolved pump-probe setup was used to perform the measurements in the bulk of cells. The 400 nm pump beam was generated from a non-collinear optical-parametric amplifier (TOPASwhite, LightConversion Ltd.), and the generation of the probe beam was also obtained via a non-collinear optical-parametric amplifier (TOPASwhite, LightConversion Ltd.). The probe beam was split into two sets after a beam splitter. One of the beams was sent to a reference photodiode while the other one was allowed to pass through the sample then recorded by photodiode and read out by the detections system purchased from Pascher Instruments AB. The pump and probe beam spatial and temporal overlap on the sample. The 400 nm pump beam is adjusted to an average pulse energy of 25 nJ, while the probe power is typically an order of magnitude lower. Sample concentrations are set as 20  $\mu$ M.

## Fluorescence lifetime imaging

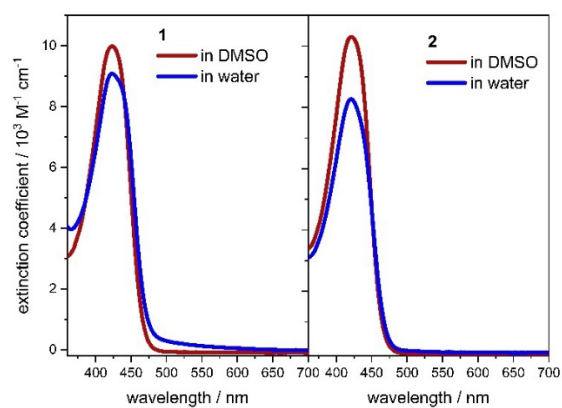
FLIM experiments were performed on a custom Abberior Expert Line laser scanning STED microscope with 100 $\times$ /1.4 oil immersion objective lens from Olympus. Pulsed 440 nm excitation laser repetition rate 40 MHz was used for the lifetime imaging with very low excitation power since the dye was extremely bright. Fluorescence was collected with a 1.0 airy unit pinhole, and finally collected by single photon counting SPCM-AQRH-14-TR avalanche photodiodes (Excelitas Technologies) equipped with appropriate filters. For the cellular lifetime measurements, the pixel size was 200 nm in all dimensions and the pixel dwell time was 10  $\mu$ s with 1-line accumulation. MCF-7 cells were carefully seeded on a glass coverslip (#1.5) and each coverslip was separately incubated for 3h with a 20  $\mu$ M concentration of each of the dyes. Thereafter the coverslip was mounted on an Attolfluor chamber (Thermo Fisher Scientific) with HBSS as a buffer. Then it was mounted on the microscope for imaging. For generation of fluorescence lifetime images of single molecules, the signal of the avalanche photodiode was fed into a time-correlated single-photon-counting device Time Tagger from Swabian Instruments, Germany. SPCImage software (Becker & Hickl, Berlin, Germany) was used for fitting the lifetime data.

## Lattice-SIM Microscopy

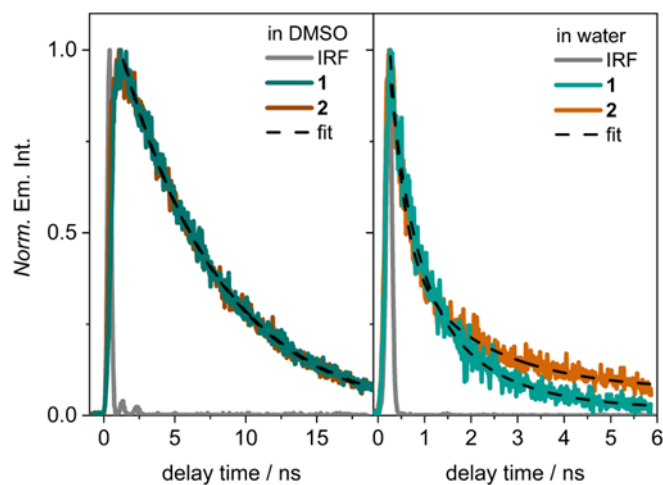
Lattice-SIM images were acquired on a Zeiss Elyra 7 microscope equipped with a Plan-Apochromat 63X/1.4 Oil DIC M27 objective and a pco.edge sCMOS (version 4.2 CL HS) camera. The system is equipped with fiber-coupled 405 nm and 642 nm diode laser, as well as 488 nm and 561 nm OPAL lasers.

For the Lattice-SIM images, z-stacks consisting of 90 nm step-width have been acquired. The 405 nm laser line (50 mW total power before fiber) was used for excitation at a power level of 2.5 % with the camera set to an acquisition time of 20 ms. For each layer in the z-stack, 13 phase individual steps have been acquired, which have been used to reconstruct the final SIM image. SIM reconstruction has been performed in ZEN black 3.0 using standard parameters on auto settings, resulting in a final pixel size of 30 nm x 30 nm (xy).

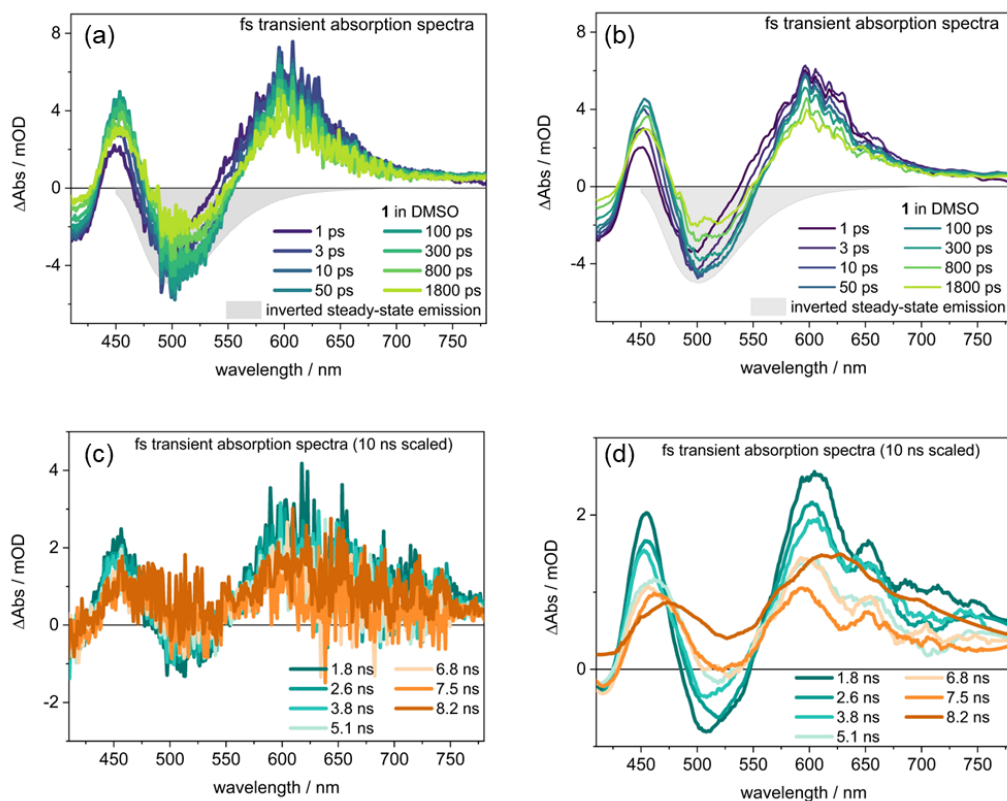
## Results and Discussion



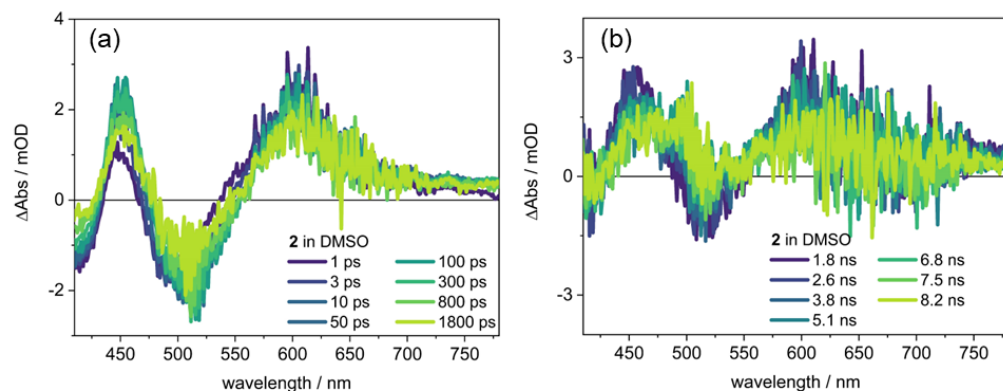
**Figure S1.** UV/vis absorption spectra of **1** and **2** in DMSO and water, respectively.



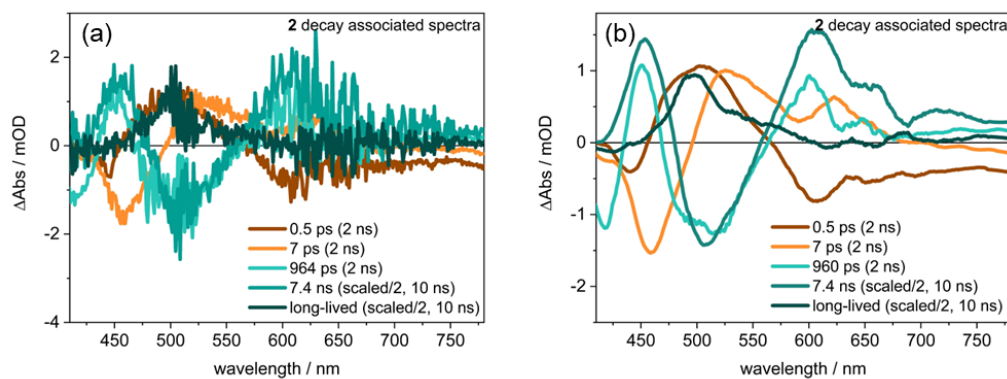
**Figure S2.** Normalized fluorescence kinetics of **1** and **2** in DMSO and water, respectively. Recorded with streak camera.



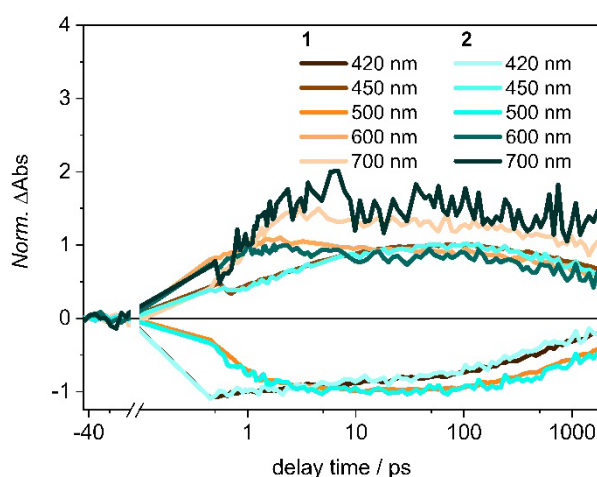
**Figure S3.** The original data of fs TA spectra of **1** in DMSO at selected delay time (a) and its smoothed data (b). Transient absorption spectra of **1** in DMSO in 10-ns time window (c) and its smooth data (d).



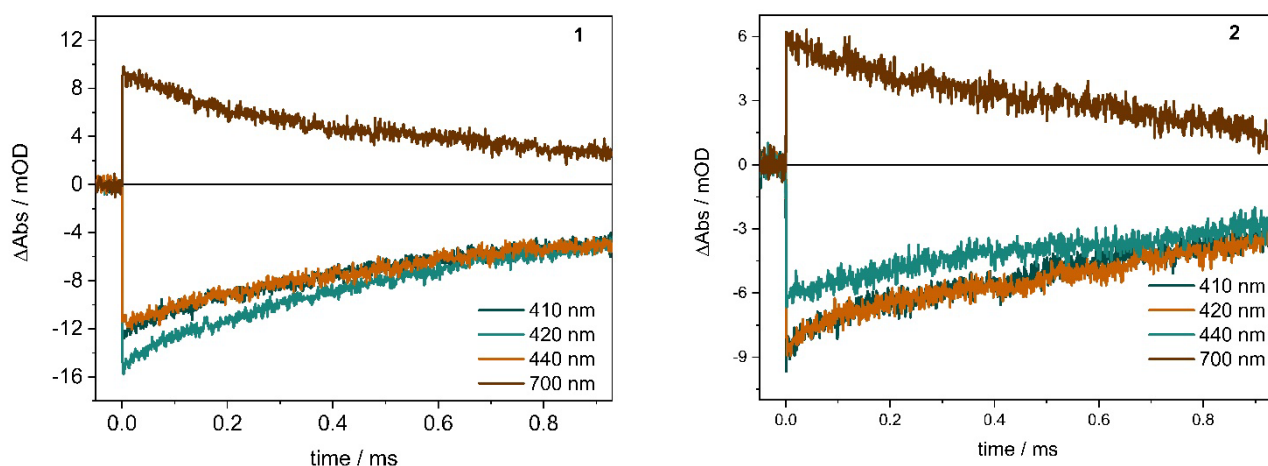
**Figure S4.** The original fs TA spectra of **2** in DMSO in 2ns time window (a) and 10 ns time window (b).



**Figure S5.** Decay-associated spectra (DAS) resulted from the global fit of the fs TA data (a) recorded from 2ns and 10 ns time windows (b) for **2**.



**Figure S6.** Kinetic traces of selected wavelengths of **1** and **2** recorded in DMSO.



**Figure S7.** Kinetic traces of selected wavelengths of **1** and **2** recorded in DMSO at 1ms time window.

## Estimation of triplet-state contribution to the ns and $\mu$ s transient absorption signal

To quantify the contribution of the triplet-excited state absorption to the transient absorption signal recorded at long delay times, we consider the number of molecules initially excited by each pump pulse.

Given the concentration of **1** and **2** in DMSO ( $\text{mol/L}$ ):

$$c_1 = 3.6 \times 10^{-5} \text{ mol/L} = 3.6 \times 10^{-8} \text{ mol} \cdot \text{cm}^{-3}$$

$$c_2 = 3.4 \times 10^{-5} \text{ mol/L} = 3.4 \times 10^{-8} \text{ mol} \cdot \text{cm}^{-3}$$

and the sample volume excited by the pump laser in our experimental configuration:

$$v = \pi \times 0.2 \text{ cm} \times 0.3 \text{ cm} \times 1 \text{ cm} \approx 0.19 \text{ cm}^3$$

The number of molecules (**1** and **2**) participating a pump-probe experiment in DMSO is determined to:

$$n_1 = c_1 \times v = 3.6 \times 10^{-8} \text{ mol} \cdot \text{cm}^{-3} \times 0.19 \text{ cm}^3 = 6.84 \times 10^{-9} \text{ mol}$$

$$n_2 = c_2 \times v = 3.4 \times 10^{-8} \text{ mol} \cdot \text{cm}^{-3} \times 0.19 \text{ cm}^3 = 6.46 \times 10^{-9} \text{ mol}$$

Considering the absorption cross-section  $\sigma$  of a given molecules,  $\sigma$ , expressed as a function of the molar extinction coefficient  $\epsilon$ , the number density of molecules and the Avogadro number  $N_A$ :<sup>[7]</sup>



$$\sigma = \frac{2303(cm^3 L^{-1})}{N_A} \varepsilon(L \cdot mol^{-1} \cdot cm^{-1})$$

as well as the value for the extinction coefficient  $\varepsilon$  as determined from steady-state absorption spectroscopy (Figure S1):<sup>[8]</sup>

$$\varepsilon_{\lambda_1=430} = 9.8 \times 10^3 L \cdot mol^{-1} \cdot cm^{-1}$$

$$\varepsilon_{\lambda_2=430} = 10^4 L \cdot mol^{-1} \cdot cm^{-1}$$

we yield:

$$\sigma_1 = 3.7 \times 10^{-17} cm^2$$

$$\sigma_2 = 3.8 \times 10^{-17} cm^2$$

The number of photons per square cm and excitation pulse yields:

$$n' = \frac{E_{laser}}{h \frac{c}{\lambda}} = \frac{\frac{0.38 mJ}{\pi \times 0.2 cm \times 0.3 cm}}{(6.63 \times 10^{-34} \times 10^3 mJ \cdot s) \times \frac{3 \times 10^8 \times 10^2 cm/s}{430 \times 10^{-7} cm}} = 4.1 \times 10^{15} cm^{-2}$$

Thus, combining the number of photons per square centimeter and excitation pulse, we calculate the excitation efficiency,  $\phi$ , i.e., the fraction of molecules excited by the pump pulse:

$$\phi_1 = n' \times \sigma_1 = 4.1 \times 10^{15} cm^{-2} \times 3.7 \times 10^{-17} cm^2 = 15\%$$

$$\phi_2 = n' \times \sigma_2 = 4.1 \times 10^{15} cm^{-2} \times 3.8 \times 10^{-17} cm^2 = 16\%$$

Multiplying the excitation efficiency with the number of molecules with the volume of the laser beam in the sample, the number of actually excited molecules can be estimated to

$$n_1'' = \phi_1 n = 15\% \times 6.84 \times 10^{-9} mol = 1.03 \times 10^{-9} mol$$

$$n_2'' = \phi_2 n = 16\% \times 6.46 \times 10^{-9} mol = 1.03 \times 10^{-9} mol$$

Based on the number of excited molecules, the ground-state absorption cross section as well as the laser volume and the optical path length, we can estimate  $\Delta OD^{theory}$ , i.e. the ground state bleach to be expected if at a given delay time all initially excited molecules still did not return to the ground-state and no excited-state absorption would overlap with ground-state bleach in the spectral window considered

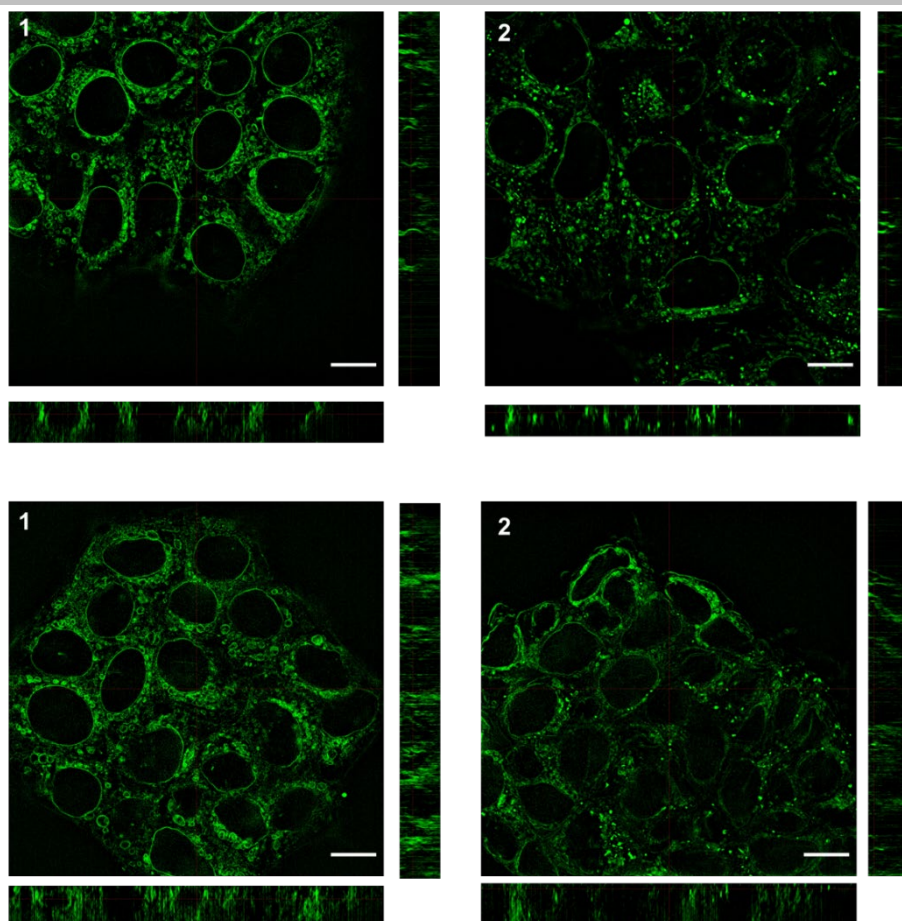
$$\Delta OD^{theory} = \varepsilon \times n'' / \nu \times l$$

$l$ , the length of the cuvette, is 1 cm.

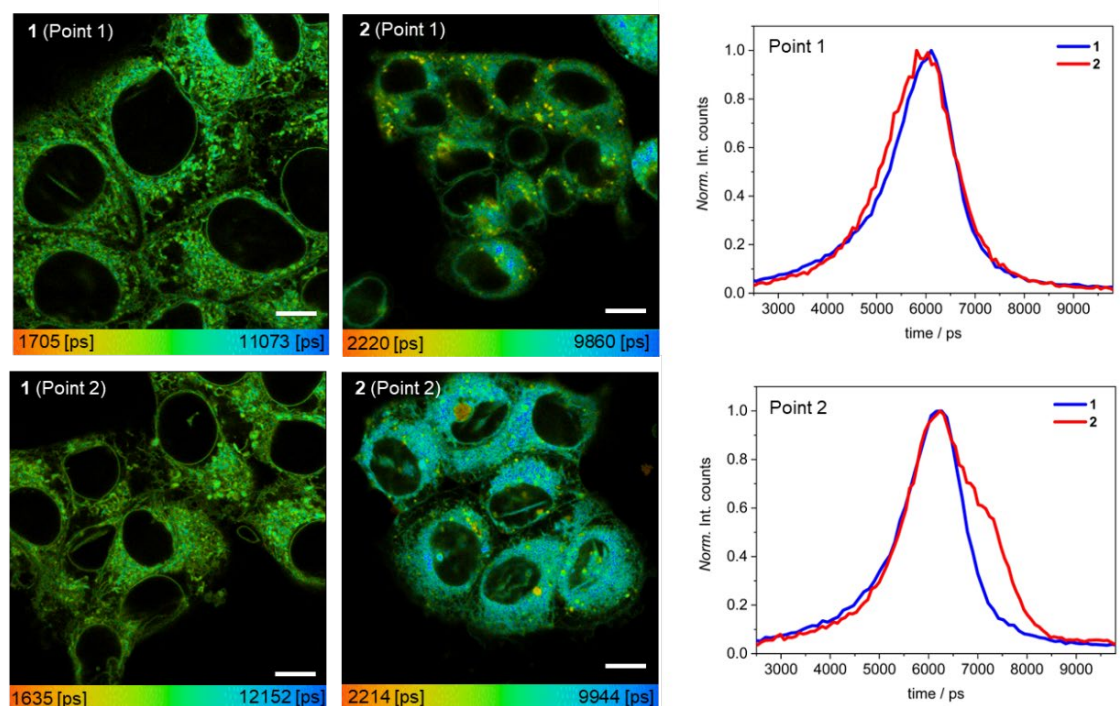
Multiplying  $1/\Delta OD^{theory}$ , with  $\Delta OD^{real}$ , the experimentally observed ground state bleach at a given delay time, we obtain  $\Phi_{eff}$ , an expression of the fraction of molecules, which did not return to the ground state bleach at a given point in time.

$$\Phi_{eff} = \Delta OD^{real} / \Delta OD^{theory}$$

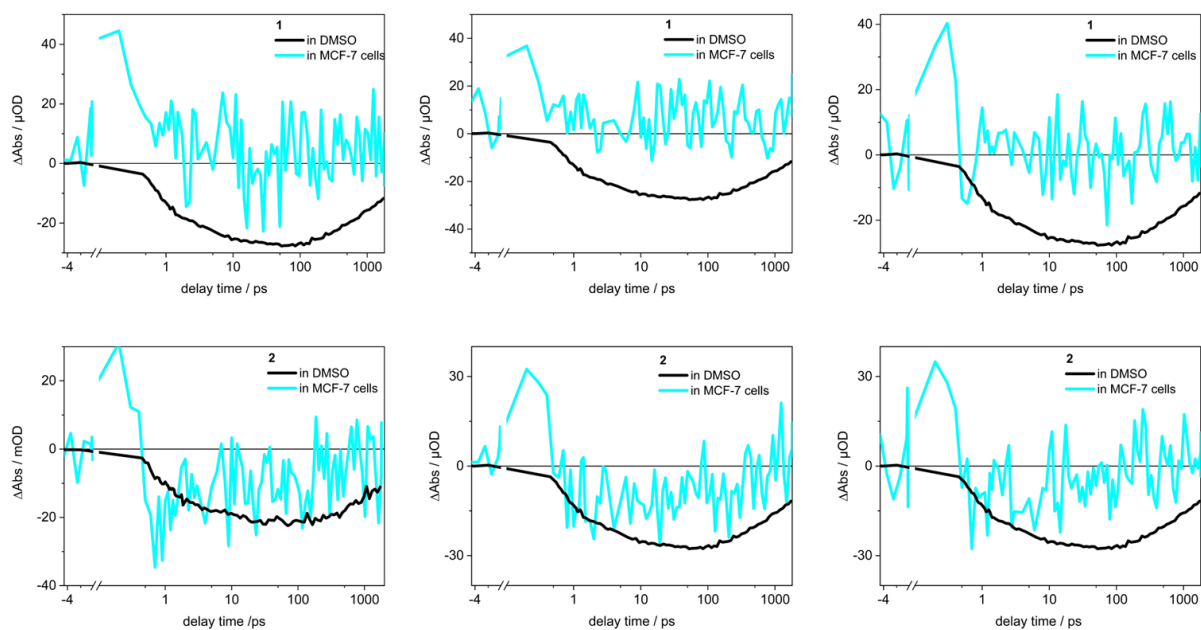
To consider the triplet yield of the compounds under investigation, we calculate  $\Phi_{eff}$  at a pump-probe delay of 7  $\mu s$ ; given the about three-orders of magnitude faster fluorescence lifetime at 7  $\mu s$  molecules are either in the (non-emissive) triplet state or in the electronic ground state.



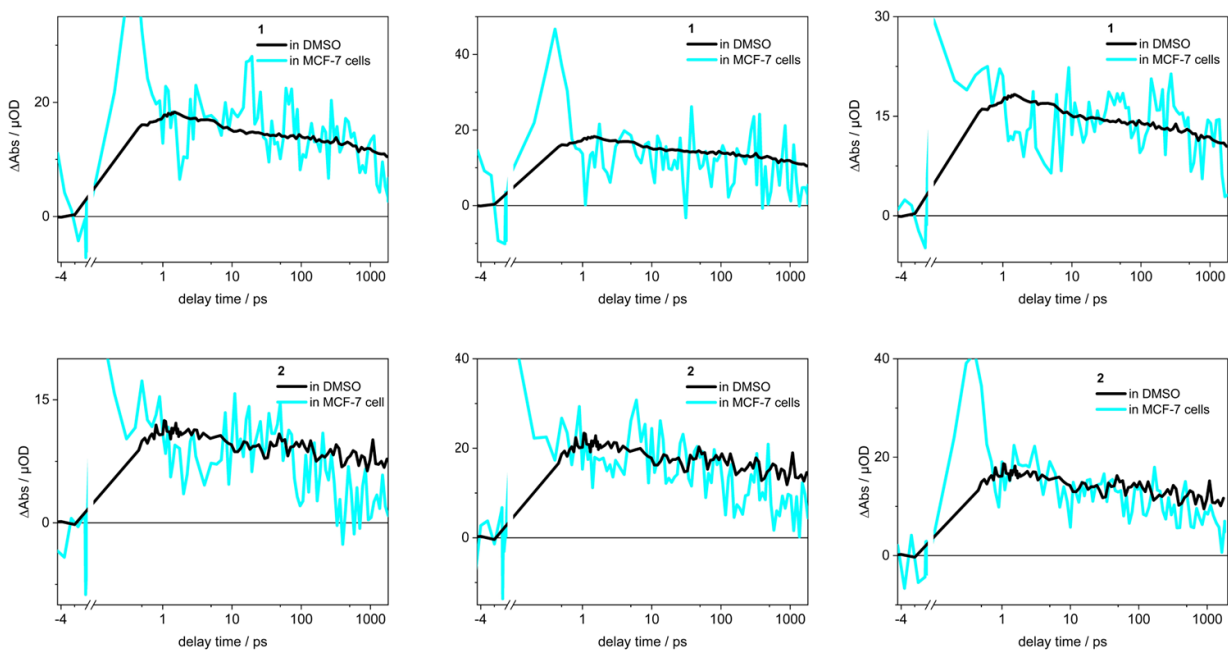
**Figure S8.** Fluorescence images of **1** and **2** taken by Lattice-SIM microscopy. Scale bar: 10  $\mu\text{m}$ .



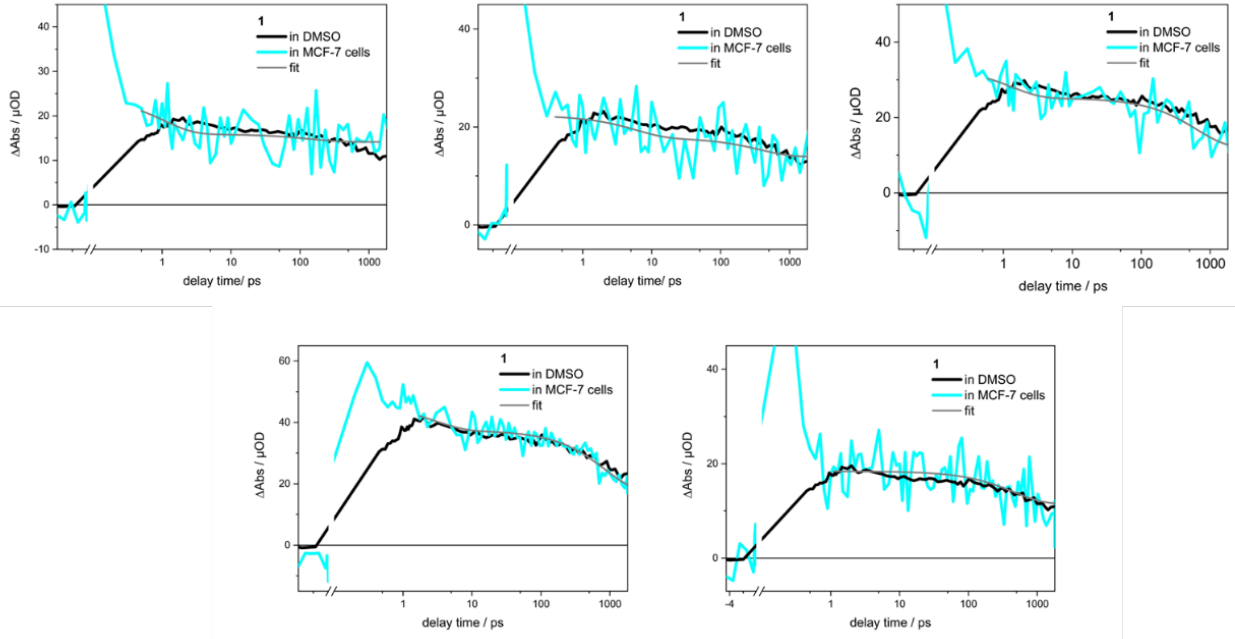
**Figure S9.** FLIM data of **1** and **2** in different measured points. Scale bar: 5  $\mu\text{m}$ .



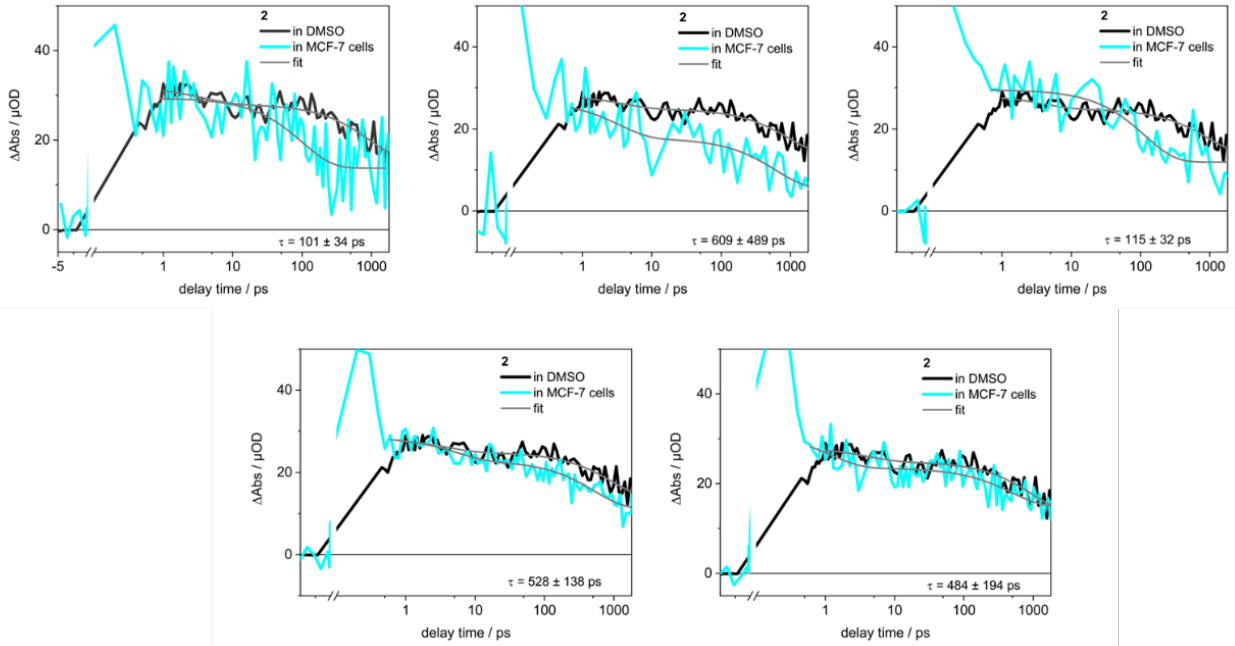
**Figure S10.** Kinetic traces of **1** and **2** in different Petri dishes of live MCF-7 cells, respectively. Probed at 515 nm.



**Figure S11.** Kinetic traces of **1** and **2** in different Petri dishes of live MCF-7 cells, respectively. Probed at 580 nm.



**Figure S12.** Kinetic traces of **1** in live MCF-7 cells of different Petri dishes, respectively. Probed at 600 nm.



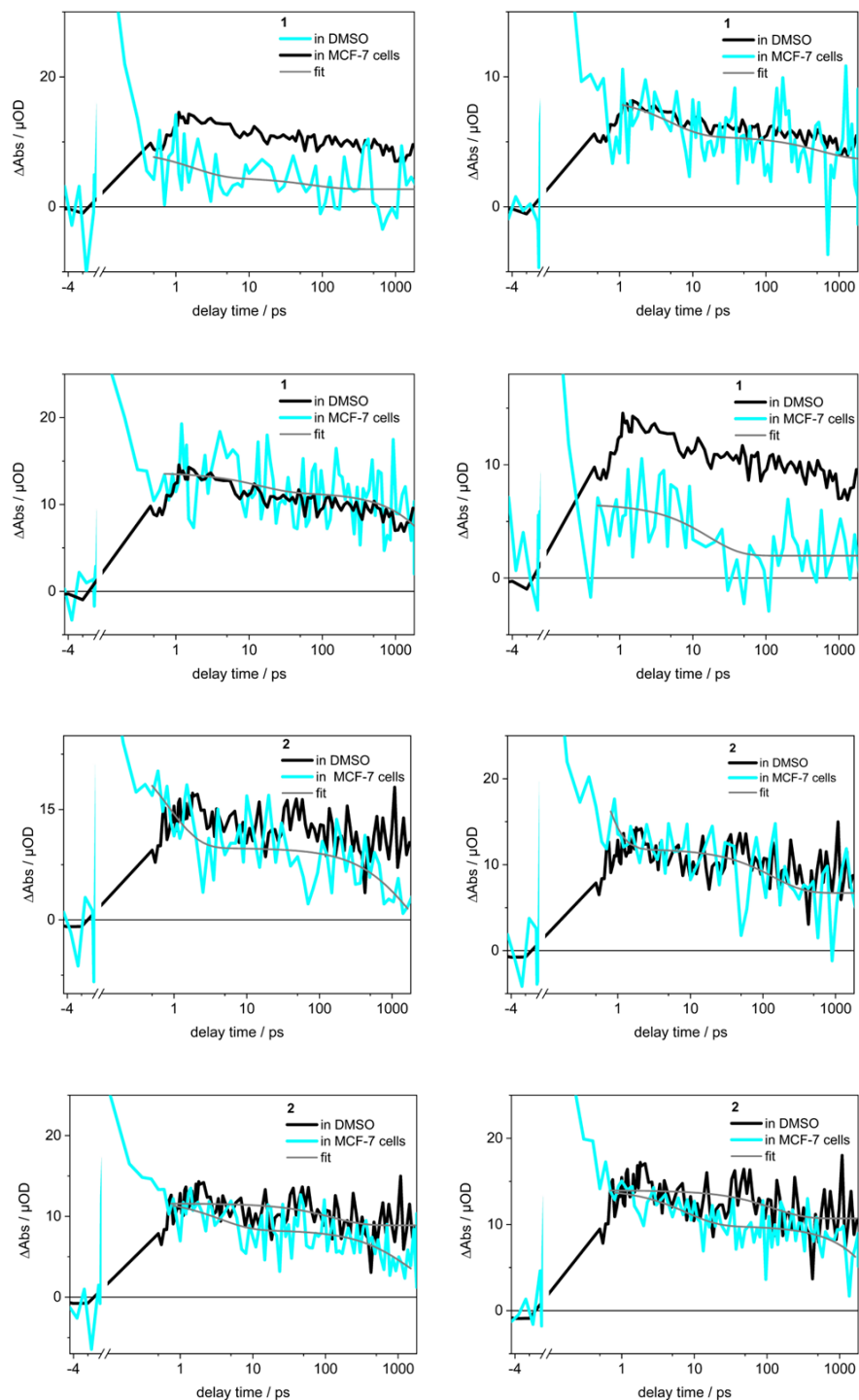
**Figure S13.** Kinetic traces of **2** in live MCF-7 cells of different Petri dishes, respectively. Probed at 600 nm.

$$\tau = \frac{101 + 609 + 115 + 528 + 484}{5} (ps) = 360 (ps)$$

$$x = \frac{34 + 489 + 32 + 138 + 194}{5} = 177$$

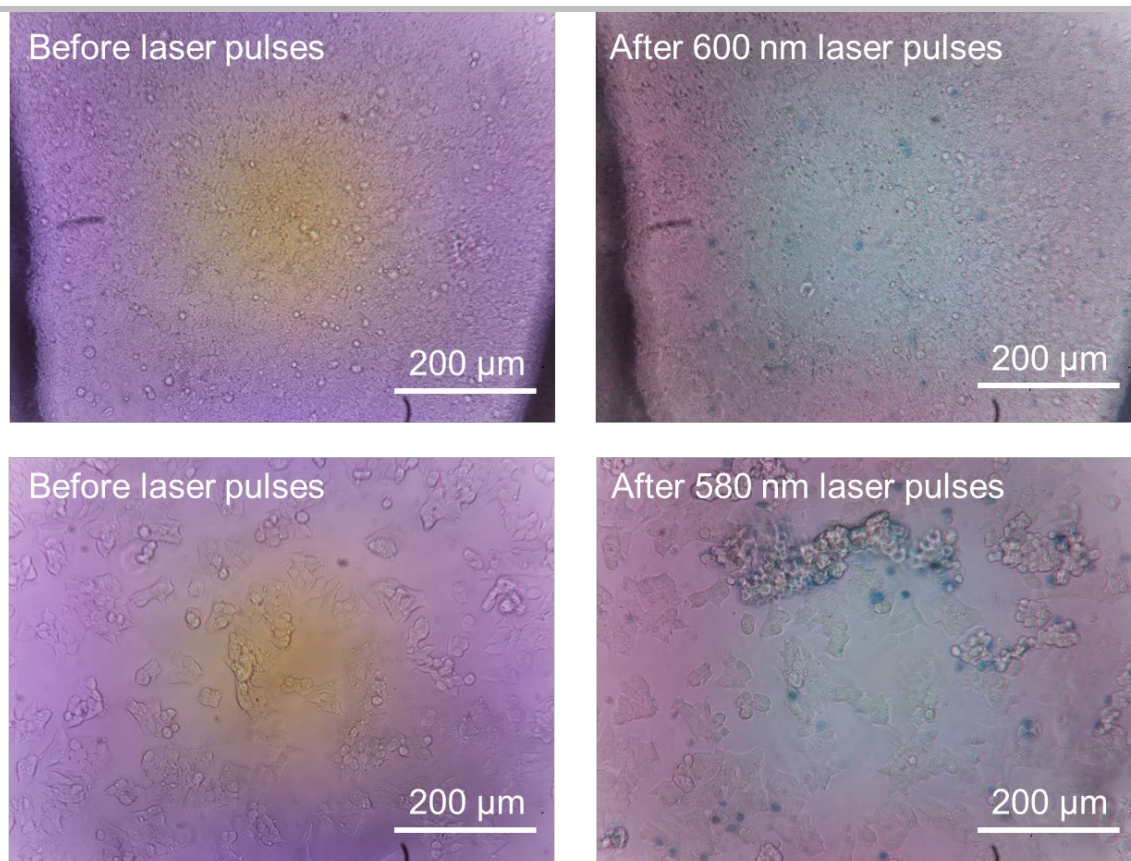
$$D = \sqrt{\frac{(34 - 177)^2 + (489 - 177)^2 + (32 - 177)^2 + (138 - 177)^2 + (194 - 177)^2}{5}} = 170$$

$$\tau = 360 \pm 170 ps$$



**Figure S14.** Kinetic traces of **1** and **2** in live MCF-7 cells of different Petri dishes, respectively. Probed at 650 nm.





**Figure S15.** Cytotoxicity and laser damage effect checked by live/dead cells staining agent, trypan blue. Left column: the cells before measurements without trypan blue. Right column: the cells after measurements with trypan blue. The colouration points mean the dead cells, but not a clan of cells stained by trypan blue, which indicates that the cells are not damaged by laser pulses.

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