**Electronic Supplementary Information (ESI)**

# Pegylated metal-free and zinc(II) phthalocyanines: Synthesis, photophysicochemical properties and *in vitro* photodynamic activities against head, neck and colon cancer cell lines

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## Experimental

### Chemical and Materials

3-Nitrophthalonitrile, 4-nitrophthalonitrile and tetraethyleneglycol monomethyl ether were purchased from Sigma-Aldrich, Germany. Silica gel Kieselgel 60, 200–400 mesh) was used in the separation and purification of compounds by column chromatography. All reagents and solvents were of reagent grade quality obtained from commercial suppliers. All solvents were dried and purified, as described by Perrin and Armarego [1]. The homogeneity of the products was tested in each step by TLC (silica gel).

### Instruments

$^{1}$H NMR spectra were recorded on an Agilent 500 MHz spectrometer using TMS as the internal reference. $^{13}$C NMR spectrum was recorded on an Agilent 126 MHz NMR. IR spectra were recorded on a Perkin-Elmer Spectrum One FT-IR (ATR sampling accessory) spectrophotometer. Electronic spectra were recorded on a Scinco LabProPlus UV/vis spectrophotometer. Mass spectra were performed on Bruker Microflex LT MALDI-TOF MS mass spectrometers. Fluorescence spectra were measured using a Varian Eclipse spectrofluorometer using 1 cm path length cuvettes at room temperature. Photo-irradiations were measured using a General Electric quartz line lamp (300W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations respectively. An interference filter (Intor, 700 nm with a bandwidth of 40 nm) was additionally placed in the light path before the sample. Light intensities were measured with a POWER MAX5100 (Mol electron detector incorporated) power meter.

### Culture condition of cell lines

Cells were purchased from ATCC. A253 (ATCC, HTB-41), human submaxillary salivary gland epidermoid carcinoma cells were cultured in McCoy's 5a medium modified (Gibco, Cat No 30-2007) supplemented with final concentration 10% heat-inactivated fetal bovine serum (Gibco, Cat No 16140071) and 1% penicillin (10,000 Units/mL) - streptomycin (10,000 Units/mL) (Gibco, Cat No 15140122). FaDu (ATCC, HTB-43), human pharynx squamous cell carcinoma were cultured in Minimum Essential Medium Eagle with jocklic modification (Sigma Aldrich, Cat No M0518) supplemented with final concentration 2.0 g/L sodium bicarbonate (Gibco, Cat No 25080094), 10% heatinactivated fetal bovine serum (Gibco, Cat No 16140071) and 1% penicillin (10,000 Units/mL) - streptomycin (10,000 Units/mL) (Gibco, Cat No 15140122). HT29 (ATCC, HTB-38), human colon colorectal adenocarcinoma cells cultured in McCoy's 5A Modified Medium (Gibco, Cat No 30-2007)

supplemented with final concentration 10% heat-inactivated fetal bovine serum (Gibco, Cat No 16140071) and 1% penicillin (10,000 Units/ml) - streptomycin (10,000 Units/ml) (Gibco, Cat No 15140122).

**Continuous cell culture**

Cell passage was carried out two to three times per week, considering their population doubling time. Firstly, the serum-containing medium was removed by washing with DPBS (Gibco, Cat No 14190250). Cells were detached from the flask through incubation with 0.25% trypsin-EDTA (Gibco, Cat No 25200056) for around 5 minutes at 37°C. Then, trypsin was neutralized with three times more serum-containing "complete medium" and centrifuged at 1300 rpm for 5 minutes to eliminate waste components. After discarding the supernatant, the cell pellet was suspended with complete medium and was seeded with appropriate aliquots in new cell culture flasks. During cell culture studies, cell counting was carried by trypan blue dye, which do not cell-permeable and could not pass from the membrane of healthy cells while can stain dead cells. Therefore, living cells appear white, and dead cells take the color of the dye, blue. Counting was performed using an automated cell counting system, Luna II, that also demonstrates percent viability.

**Cytotoxicity (Dark and PDT)**

The cells were detached from the cell culture when cell confluence was around 70-80%. Cells were suspended in their complete media with cell viability higher than 90% as final concentration $5\times10^4$ cells/mL. The cells were seeded into 96 well plates ($5\times10^3$ cells/well) and incubated in a 37°C, 5% $CO_2$ humidified incubator for 24 hours. After 24 hours of incubation, Pcs in different concentrations (75 µM, 7.5 µM, 0.75 µM, 75 nM, 7.5 nM, 0.75 nM) that were prepared in complete cell culture media were applied onto the cells, then the cells were incubated in a 37°C, 5% $CO_2$ humidified incubator for an hour in order to cellular uptake of Pc molecules. After cellular uptake of Pcs, the PDT group was irradiated at 685 nm with 2 joules/$cm^2$ using Cetoni-LED, the dark group was kept at dark and incubated in a 37°C, 5% $CO_2$ humidified incubator as covered with aluminum foil for 24 hours. Post-24 hours of the treatment, cell viability evaluation was carried out by colorimetric WST-1 assay based on the cleavage of tetrazolium salts into formazan dye by 8 mitochondrial dehydrogenases in viable cells. WST-1 solution is diluted 1:10 in complete media, and the media in each well was replaced with a 1:10 WST-1 solution. The cells were incubated to avoid light in a 37°C, 5% $CO_2$ humidified incubator for 3 hours. After incubation, endpoint absorbance of the wells was taken at 450 nm; references 650 nm through Cytation 5 multi-plate reader. References values were distracted from an actual value. As a control, non-treated cells and cells treated with 10% DMSO were used. Percentage values were calculated respected to non-treated cells.

**Oxidative stress evaluation (ROS-DCFDA)**

In PDT, one of the photo damage processes is triggered through reactive oxygen species formation. In order to carry out ROS formation, the cells were detached from the cell culture flask when cell confluence was around 70-80%. Cells were suspended in their complete media with cell viability higher than 90% as the final concentration of $10\times10^4$ cells/mL. The cells were seeded into 96 well plates ($10\times10^3$ cells/well) and incubated in a 37°C, 5% $CO_2$ humidified incubator for 24 hours. After 24 hours of incubation, Pcs in different concentrations (75 µM, 7.5 µM, 0.75 µM, 75 nM, 7.5 nM, 0.75 nM) prepared in complete cell culture media administrated into the cells. Following treatment with Pc molecules, the photo-irradiated group was irradiated at 685 nm with 2 joules/$cm^2$ using CETONI-LED equipment after 1-hour incubation with Pc molecules. Following irradiation, dark and photo-irradiated groups were incubated in a 37°C, 5% $CO_2$ humidified incubator covered with aluminum foil for 24 hours. At the end of the incubation, reactive oxygen species intensity was evaluated using 2',7'-Dichlorofluorescin diacetate, DCFDA (Sigma, D6883). DCFDA is a non-fluorescent cell-permeable probe that undergoes esterase reaction in the presence of reactive oxygen species and yields highly fluorescent 2',7'-dichlorofluorescein. All treated and control groups media were removed and replaced with 10 µM DCFDA in HBSS (Gibco, Cat No 14175129) and

incubated by avoiding light in a 37°C, 5% $CO_2$ humidified incubator for 30 minutes. After that, endpoint fluorescent intensity was read where the excitation wavelength is 504 nm, and the emission wavelength is 529 nm. The results were normalized in percentage relative to the number of cells compared to the control in non-Pc-treated samples.

**Cellular death mechanism (Apoptosis/Necrosis)**

The effects of PDT with Pcs on the cellular death mechanism on cancer cells were determined using the Apoptosis/Necrosis Detection Kit (Abcam, Ab176749). During apoptosis, changes occur in the membranes of apoptotic cells. The most noticeable change is that negatively charged phosphaotidylserine units, generally located on the cytoplasmic surface of the cell membrane, protrude to the cell membrane's outer surface. Apopxin Green fluorescent dye included in the kit binds to these negatively charged phosphatidylserine units and provides the detection of the presence of apoptotic cells by giving a green fluorescent image. While CytoCalcein Violet 450 fluorescent dye stains living cells in blue color, 7-AAD stains necrotic cells in red and enables them to be determined under fluorescent images.

Firstly, the cells were detached from the cell culture flask by 0.25% trypsin-EDTA when cell confluence was around 70-80%. Cells were suspended in their complete media with cell viability higher than 90% as the final concentration of $10\times10^4$ cells/mL. The cells were seeded into 96 well plates ($10\times10^3$ cells/well) and incubated in a 37°C, 5% $CO_2$ humidified incubator for 24 hours. After 24 hours of incubation, Pcs in different concentrations (7.5 µM and 0.75 µM) that were prepared in complete cell culture media were applied onto the cells, then the cells were incubated in a 37°C, 5% $CO_2$ humidified incubator for an hour in order to cellular uptake of Pcs. After cellular uptake of Pcs, the PDT group was irradiated at 685 nm with 2 joules/$cm^2$ using Cetoni-LED; the dark group was kept at dark and incubated in a 37°C, 5% $CO_2$ humidified incubator as covered with aluminum foil for 24 hours. At the end of 24 hours of incubation, the cellular death mechanism of cells was determined using the Apoptosis/Necrosis Detection Kit. First, the wells were washed with 200 µL of Apopxin buffer solution. Afterward, 2 µL of Apopxin Green, 1 µL of Cyt0 Calcein Violet 450, and 1 µL of 7-AAD in 200 µL of Apopxin buffer solution were given to the wells and incubated in the dark for 1 hour at room temperature. After the incubation, the wells were washed with 200 µL of Apopxin buffer solution, then 200 µL of buffer was added, cell images were taken and analyzed on the Bright Field, DAPI (CytoCalcein Violet 450), GFP (Apopxin Green) and Texas Red (7-AAD) filters of the Cytation 5 device.

**References**

1. D.D. Perrin, W.L.F. Armarego, Purification of Laboratory Chemicals (2nd ed.), Pergamon Press: Oxford, 1989.

**SUPPLEMENTARY FIGURES**

**NMR spectra of 1, 2, 1a,b and 2a,b**

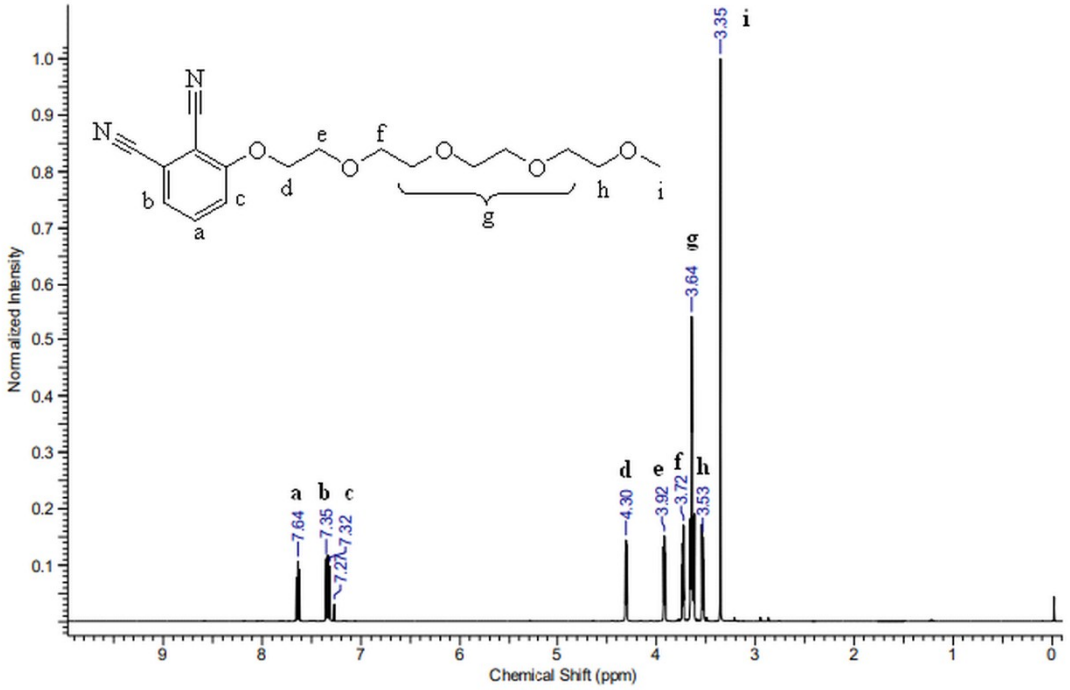


**Figure S1:** $^{1}H$ NMR spectrum of **1** in $CDCl_3$.

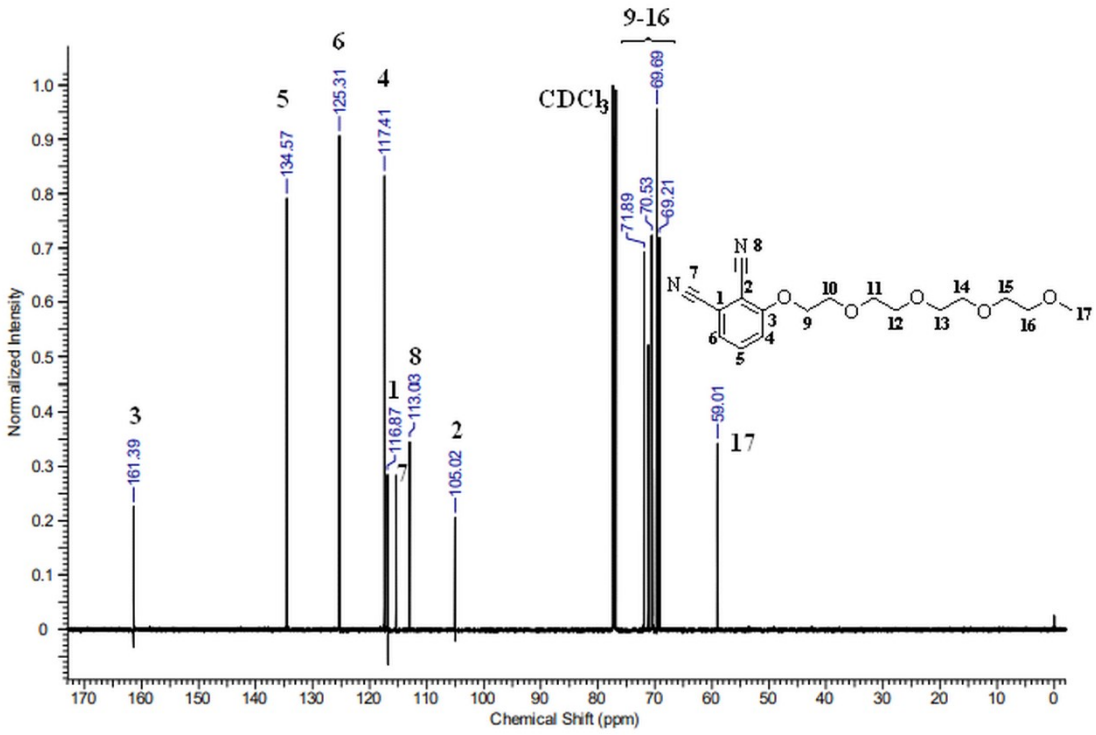


**Figure S2:** $^{13}C$ NMR spectrum of **1** in $CDCl_3$.

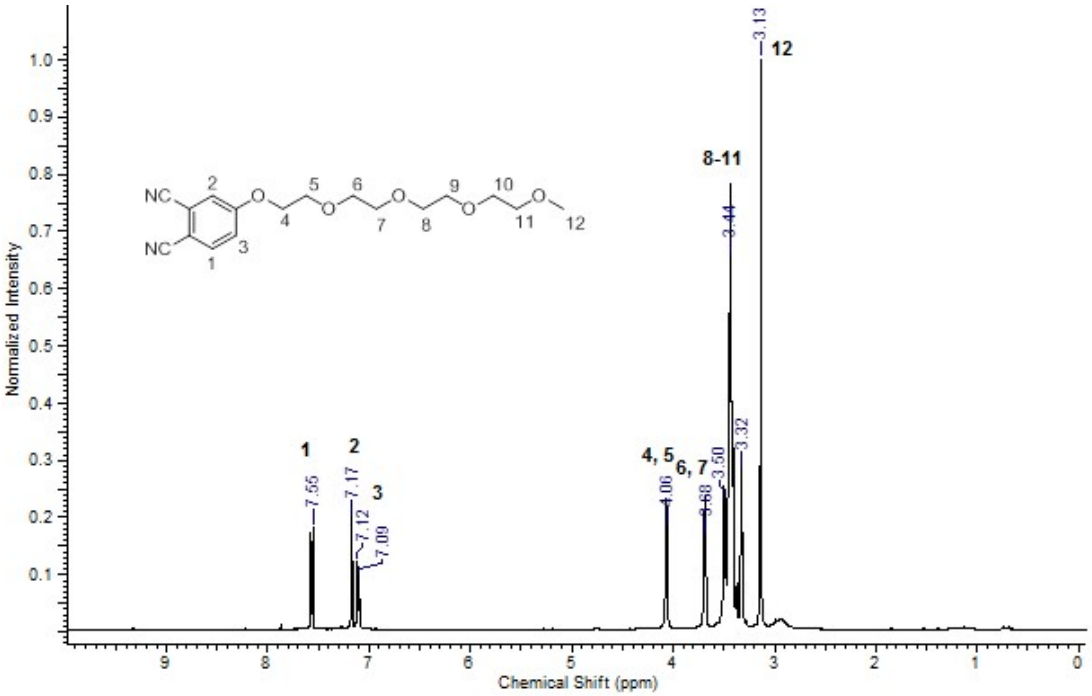


**Figure S3:** $^{1}H$ NMR spectrum of **2** in $CDCl_3$.

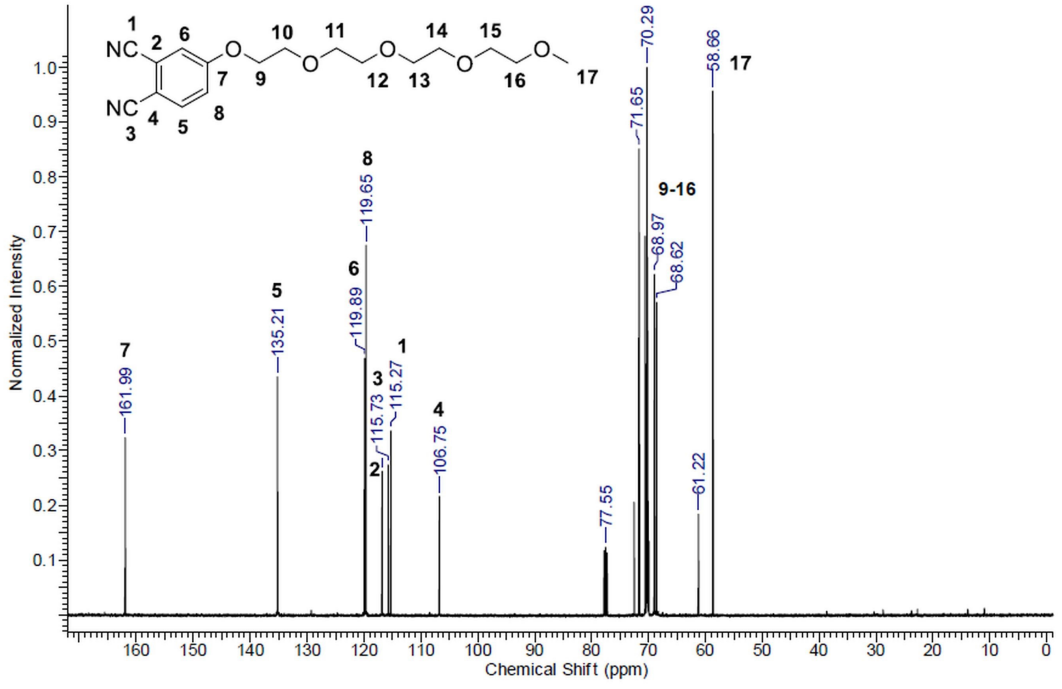


**Figure S4:** $^{13}C$ NMR spectrum of **2** in $CDCl_3$.

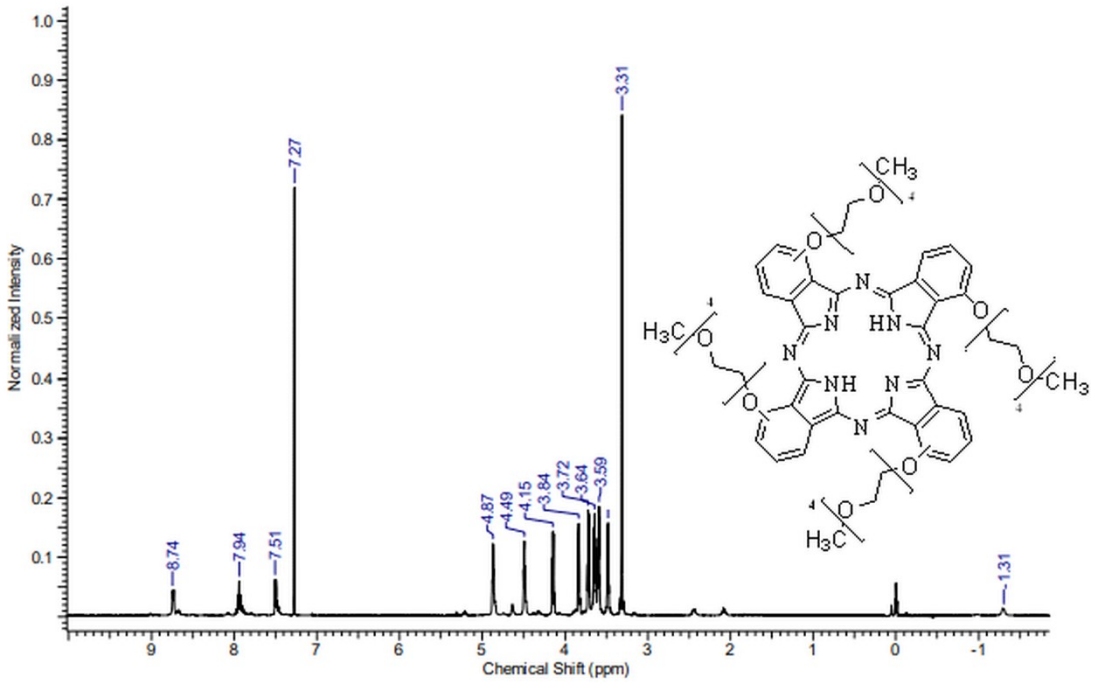


**Figure S5:** $^{1}$H NMR spectrum of **1a** in $CDCl_3$.

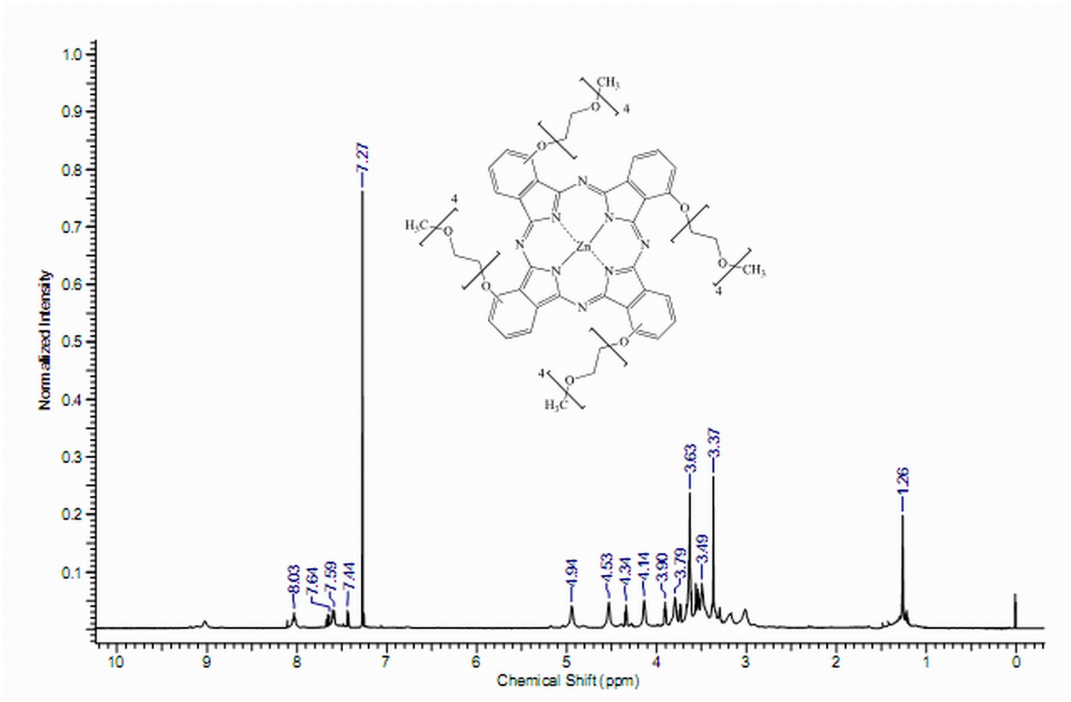


**Figure S6:** $^{1}$H NMR spectrum of **1b** in $CDCl_3$.

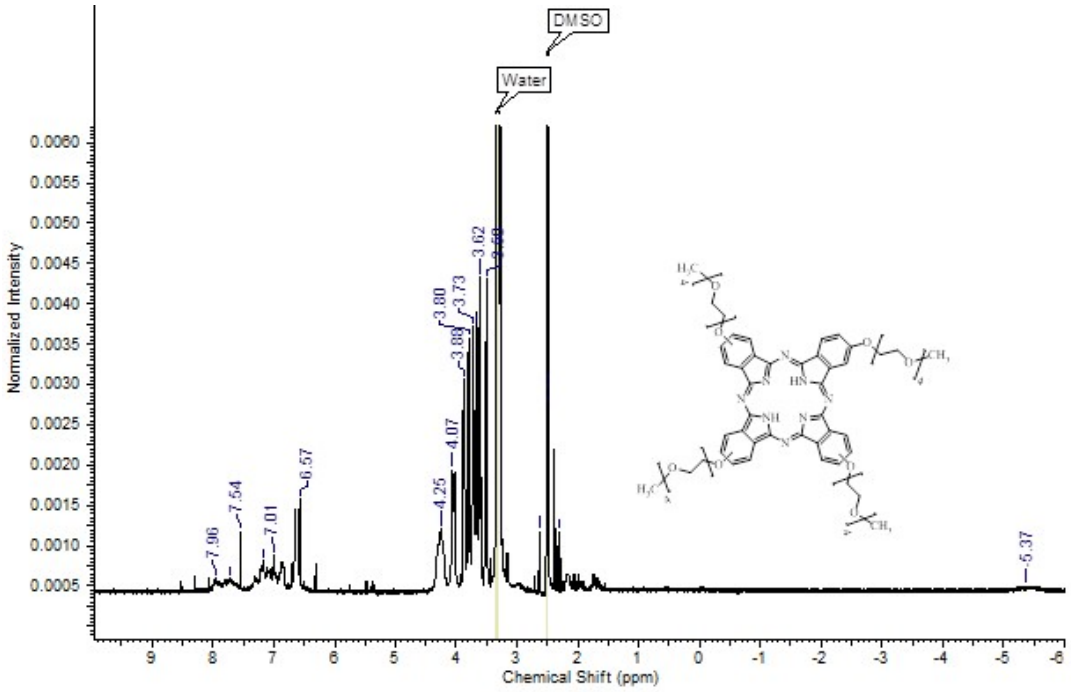


**Figure S7:** $^{1}$H NMR spectrum of **2a** in $d_6$-DMSO.

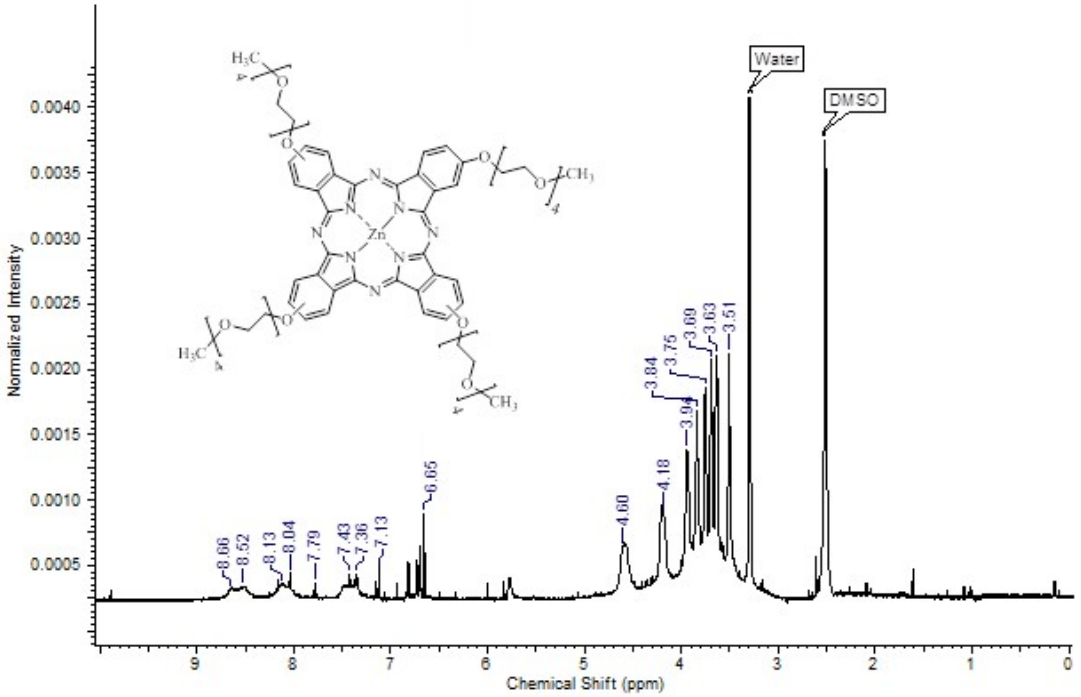


**Figure S8:** $^{1}$H NMR spectrum of **2b** in $d_6$-DMSO.

**MALDI-TOF MS spectra of 1, 2, 1a,b and 2a,b**

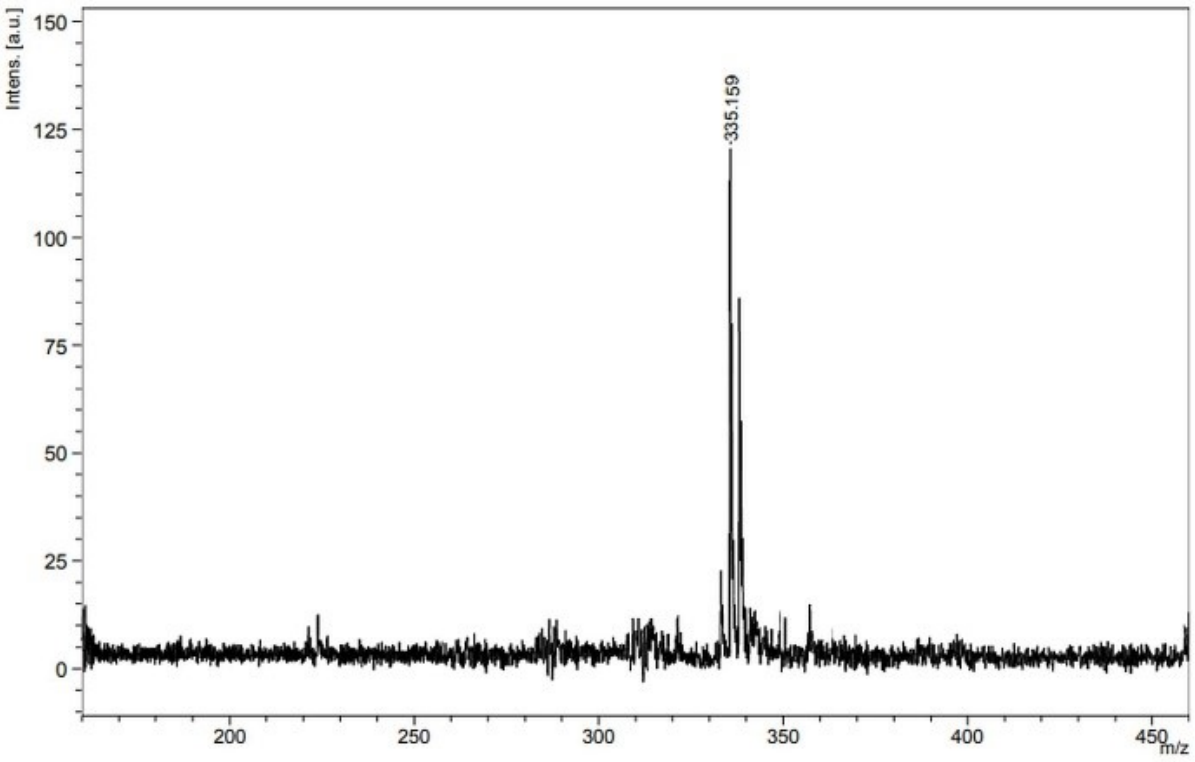


**Figure S9:** MALDI-TOF MS spectrum of **1**.

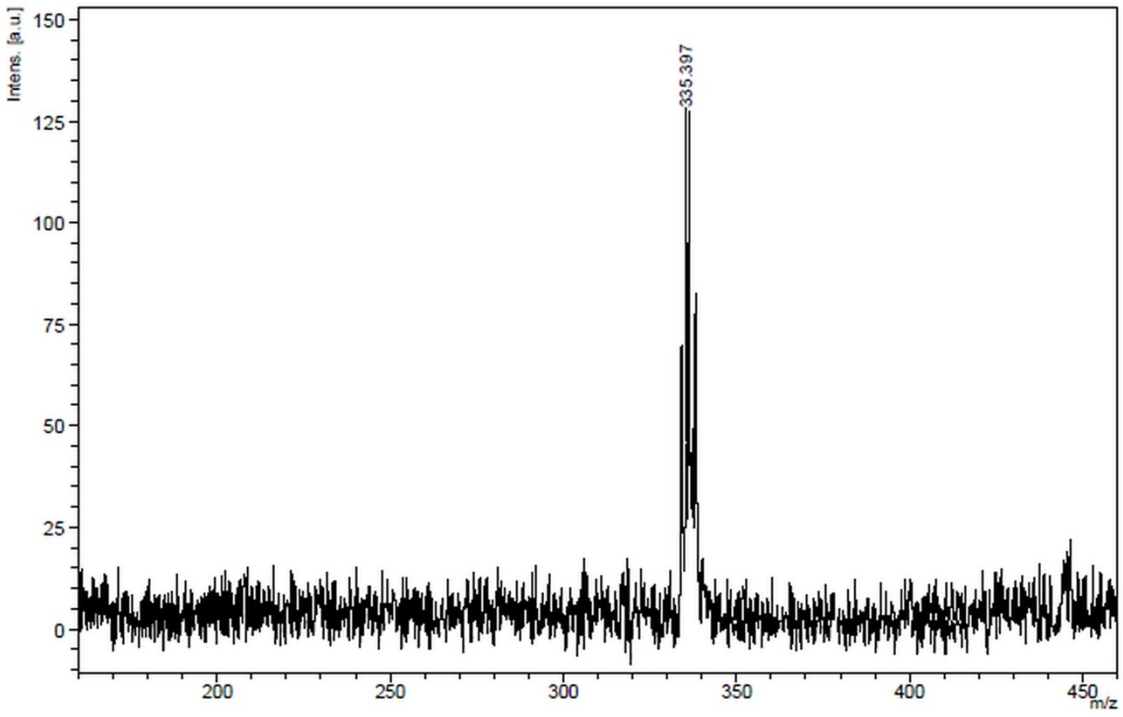


**Figure S10:** MALDI-TOF MS spectrum of **2**.

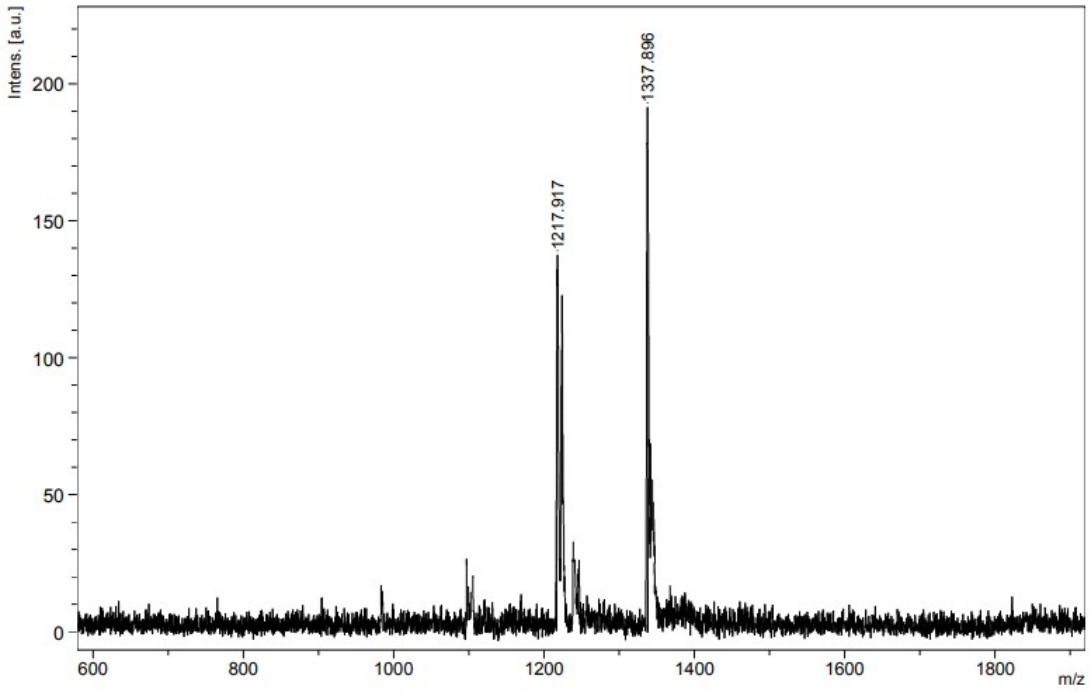


**Figure S11:** MALDI-TOF MS spectrum of **1a**.

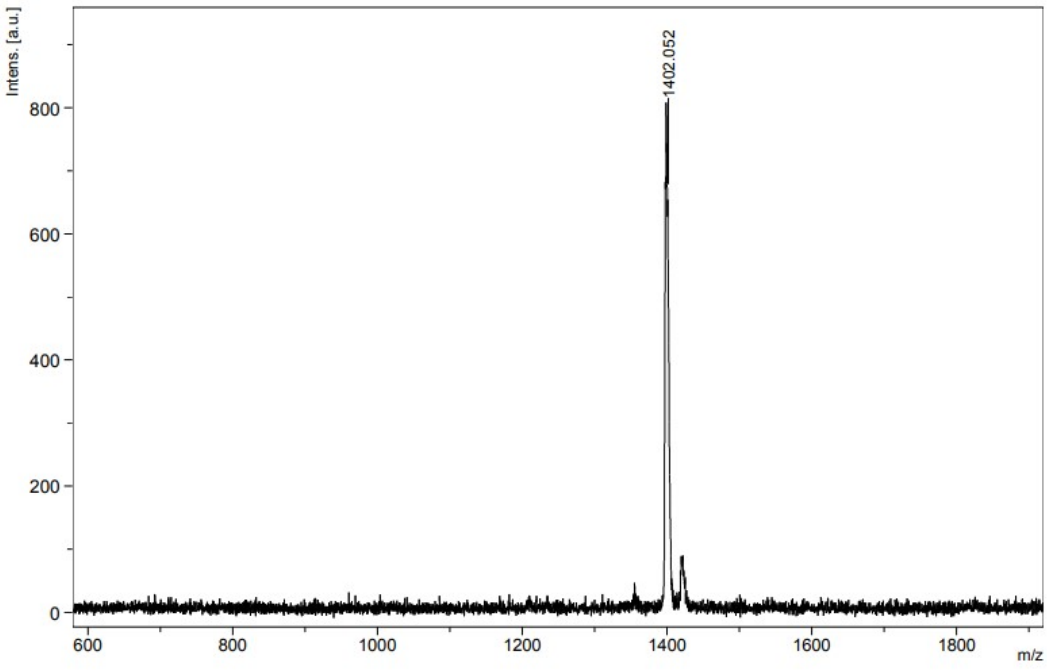


**Figure S12:** MALDI-TOF MS spectrum of **1b**.

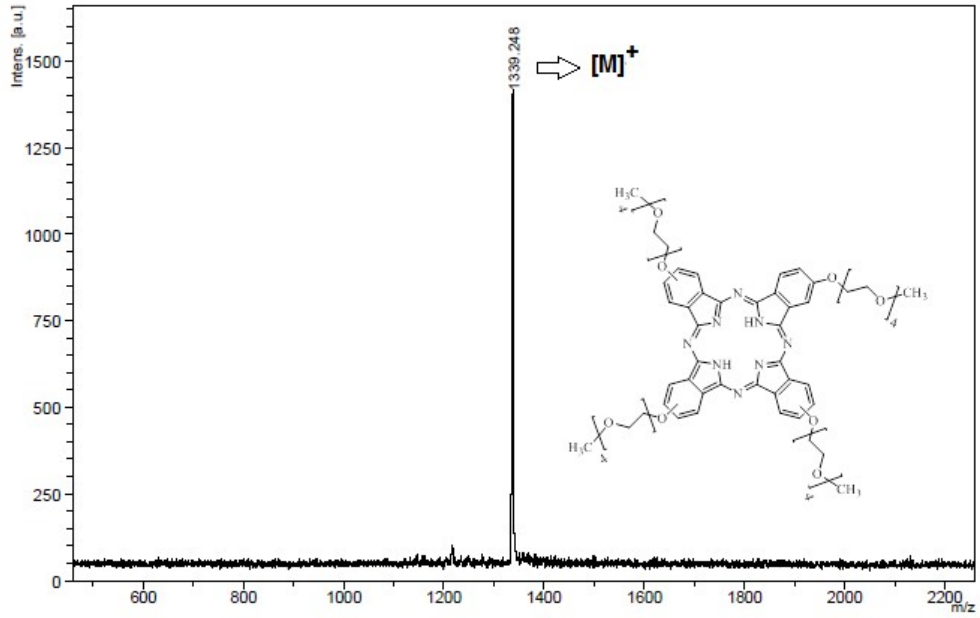


**Figure S13:** MALDI-TOF MS spectrum of **2a**.

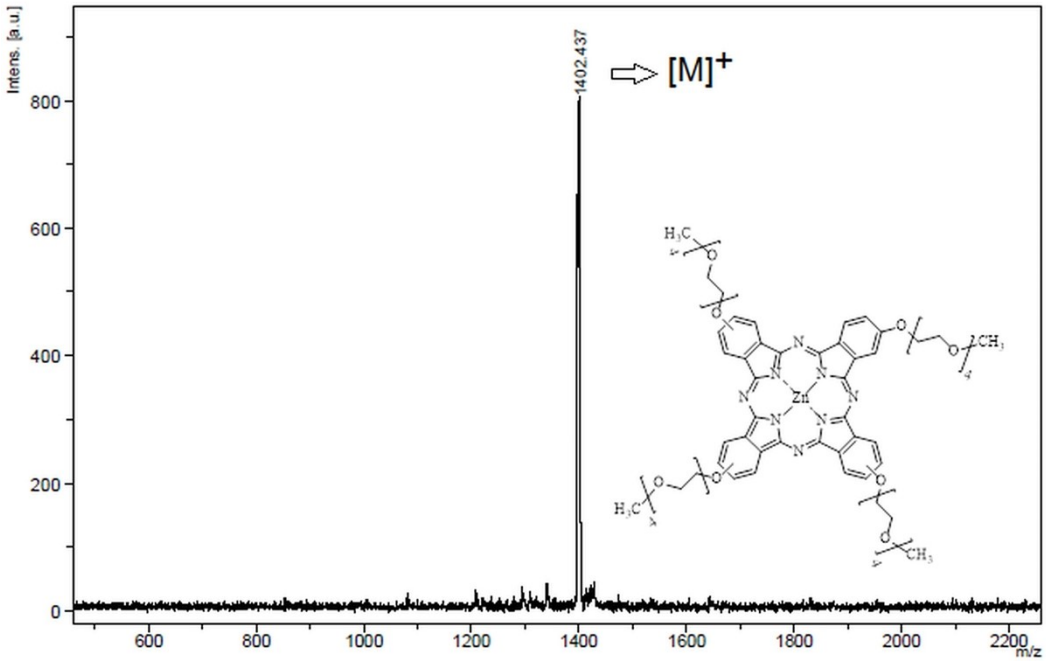


**Figure S14:** MALDI-TOF MS spectrum of **2b**.

**Photophysical and photochemical studies**

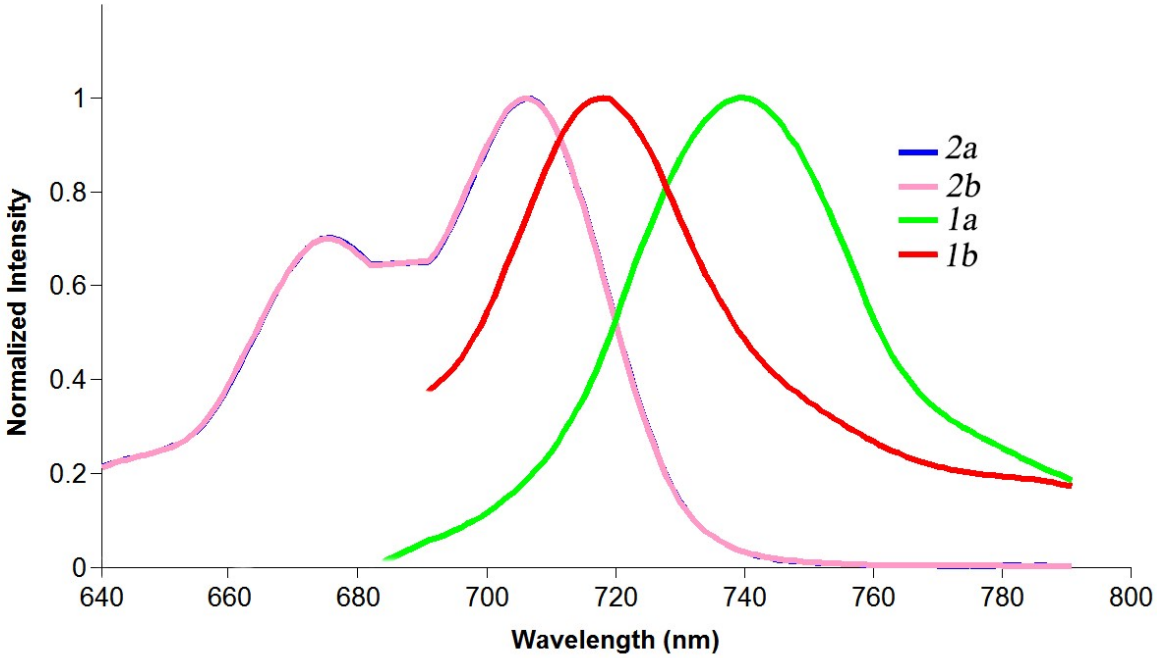


**Figure S15**: Normalized emission spectra of **1a** (739 nm), **1b** (718 nm), **2a** (706 nm) and **2b** (706 nm) in DMSO.

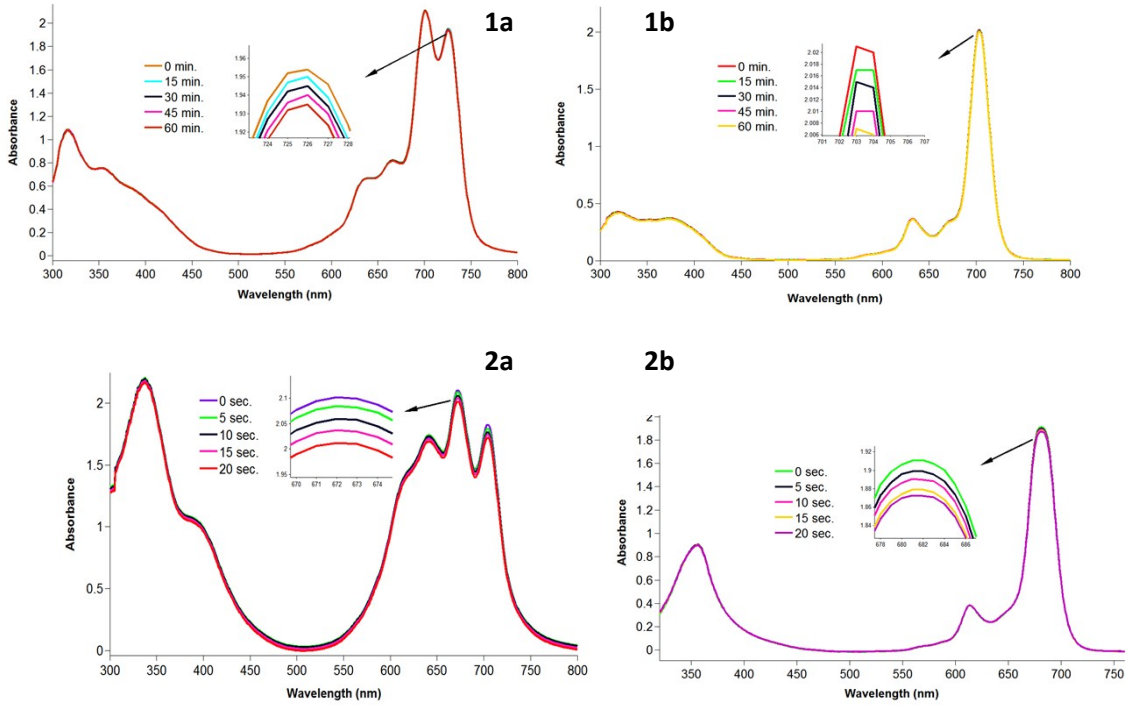


**Figure S16:** A typical spectrum for the determination of photodegradation of the **1a**,**b** and **2a**,**b** in DMSO.