SUPPLEMENTARY INFORMATION

In vitro photodynamic inactivation of conidia of the phytopathogenic fungus *Colletotrichum graminicola* with cationic porphyrins

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1. General methods, materials and chemicals

All reagents and solvents were of reagent grade and they were used as obtained. Pyrrole, 4-pyridinecarboxaldehyde, *N,N*-dimethylformamide (DMF) and 3-diphenylisobenzofuran (DPBF) were purchased from Sigma-Aldrich®. Benzaldehyde, propionic acid and ethyl ether were purchased from VETEC®. Dimethyl sulfoxide (DMSO) and methyl iodide were from Merck®. Thin-layer chromatography (TLC) was performed on silica gel coated aluminum sheets 60 F_{254} (Merck®) using solvent mixtures measured on a v/v basis. Column chromatography was carried out using silica gel 60 35-70 mesh (Fluka®). ^{1}H spectra were recorded in 5 mm NMR tubes on a Bruker AVANCE III 400 NMR spectrometer at 400.13 MHz in DMSO-d6 (Sigma-Aldrich®). NMR spectra calibration was performed using signal at 2,50 ppm (DMSO-d6) as reference. Assignments and magnitudes of coupling constants were obtained from ^{1}H NMR spectra by first-order analyses. The appearance of signals is indicated using the abbreviations b, s, d, t, q, p, and m for broad, singlet, doublet, triplet, quartet, pentet, and multiplet, respectively. MALDI mass spectra were recorded on a MALDI-TOF/MS model Autoflex II Bruker Daltonics using HCCA (α-cyano-4-hydroxycinnamic acid) as matrix. The ultraviolet-visible spectra were obtained with a UV-1800 Shimadzu spectrophotometer.
Materials used for the culture media preparation were oatmeal (Nestlé®), bacteriologic Agar (VETEC®) and Sabouraud Dextrose Agar (ASD, Kasvi®). Sterile 6-well plates (well volume: 15.53 mL) for photodynamic inactivation were purchased from TPP®.
The light source used for the photodynamic and photophysical assays was a Lumacare LC 122A with a halogen/quartz 250 W lamp. Fluorescence measurements for the binding assay were peformed with a RF-5301PC Shimadzu spectrofluorometer. Centrifugation for photosensitizer binding and microscopic analysis (11000 rpm, 5 min) were performed in centrifuge Sigma, model 1-14. Microscopic images were recorded using Fluorescence Microscope (Olympus BX51TF) with FITC filter and microscopic magnification of eighty. Images were evaluated with Cell^F software, 5.0 version (Olympus Europe Software Information).

2. Synthesis of the cationic porphyrins

Synthesis of the neutral *meso*-pyridyl porphyrin were typically performed by adding 10 mL of propionic acid, 280 μL (268 mg, 4 mmol) of pyrrole, 100 μL (106 mg, 1 mmol) of benzaldehyde and 320 μL (321 mg, 3 mmol) of 4-pyridine-carboxaldheyde in a 50 mL round-bottomed flask. The mixture was kept at 145°C for 1 hour, under reflux. After reaction time, reaction mixture volume was reduced by heating (down to ~2 mL) and then cooled to room temperature. Acetone (5 mL) was added to the cooled mixture to give a precipitate containing a mixture of porphyrins, which was collected by filtration and washed with acetone. The purple solid was suspended in chloroform and loaded onto a silica gel column (20 × 4 cm), using chloroform:methanol (99:1) as mobile phase. The separation process was evaluated by performing thin layer chromatography analysis of the column fractions and the retention factors of the porphyrins containing one, two-*trans*, two-*cis*, three and four pyridine-*meso*-rings were 0.48, 0.24, 0.16, 0.12 and 0.08, respectively. Each porphyrin was dried and maintained in dark and percentage yields of the five compounds were 0.9% (5.5 mg), 1.1% (6.8 mg), 1.0% (6.1 mg), 3.1% (19.1 mg) and 2.5% (15.4 mg), respectively. This procedure was repeated as much as necessary until enough material for the next step was obtained.

For porphyrin cationization, each of the neutral *meso*-pyridyl porphyrins (30 mg) was solubilized in DMF (3 mL) in a 5 mL screw cap flask and a large excess of methyl iodide (300 μL) was then added to the solution. Reaction was stirred under room temperature for 18 hours in the dark. The volume of the reaction mixture was then reduced to approximately 1.5 mL and methanol was added (1.5 mL). The resulting mixture was added of 10 mL of diethyl ether to precipitate the cationic porphyrin. The purple solids were collected by filtration, washed with cold diethyl ether and dried. Porphyrins **1**, **2**, **3**, **4** and **5** were obtained with yields of 90% (iodide salt, 33.2 mg), 38% (diiodide salt, 16.6 mg), 27% (diiodide salt, 11.8 mg), 60% (triiodide salt, 30.4 mg) and 64% (tetraiodide salt, 36.8 mg), respectively.

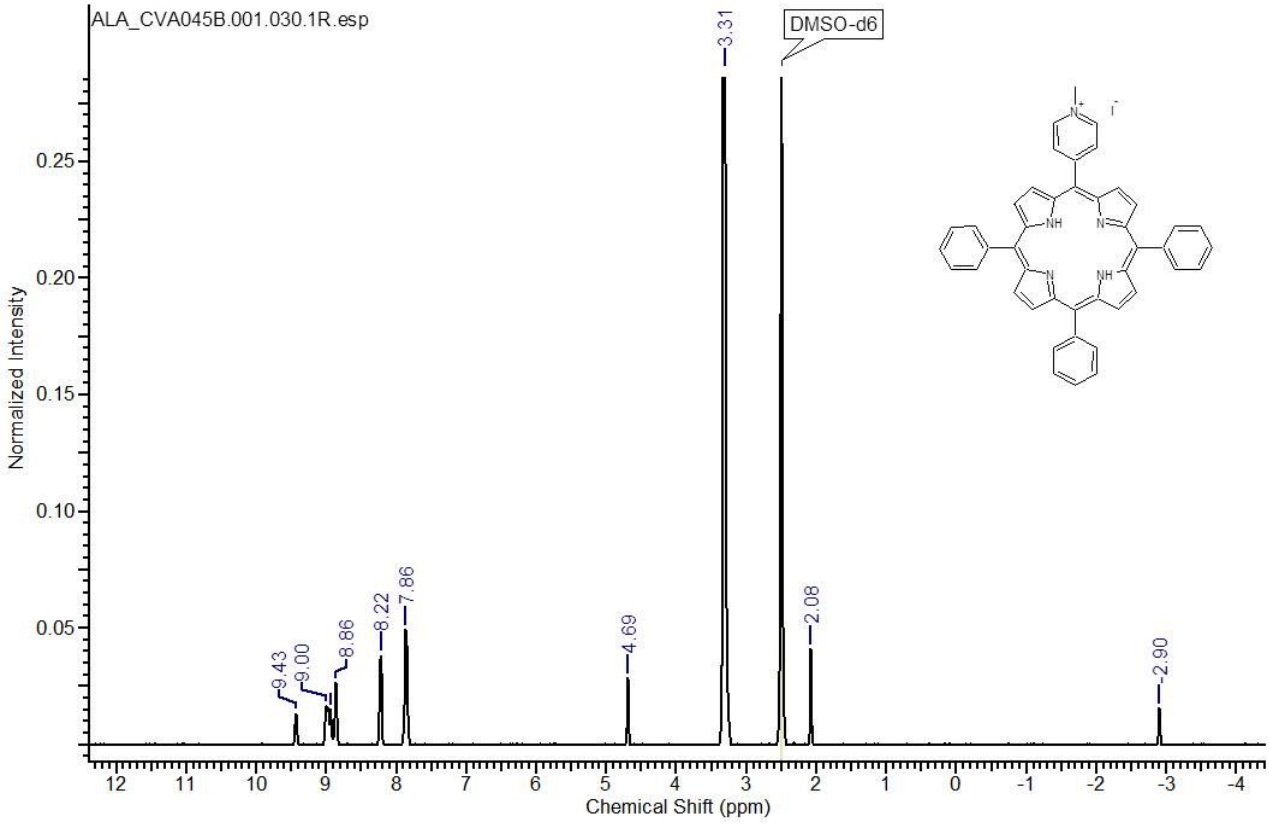
3. NMR, MALDI-TOF and UV spectra of compounds 1-5

^{1}H NMR data for all compounds here in studied are consistent with literature spectral data[43].

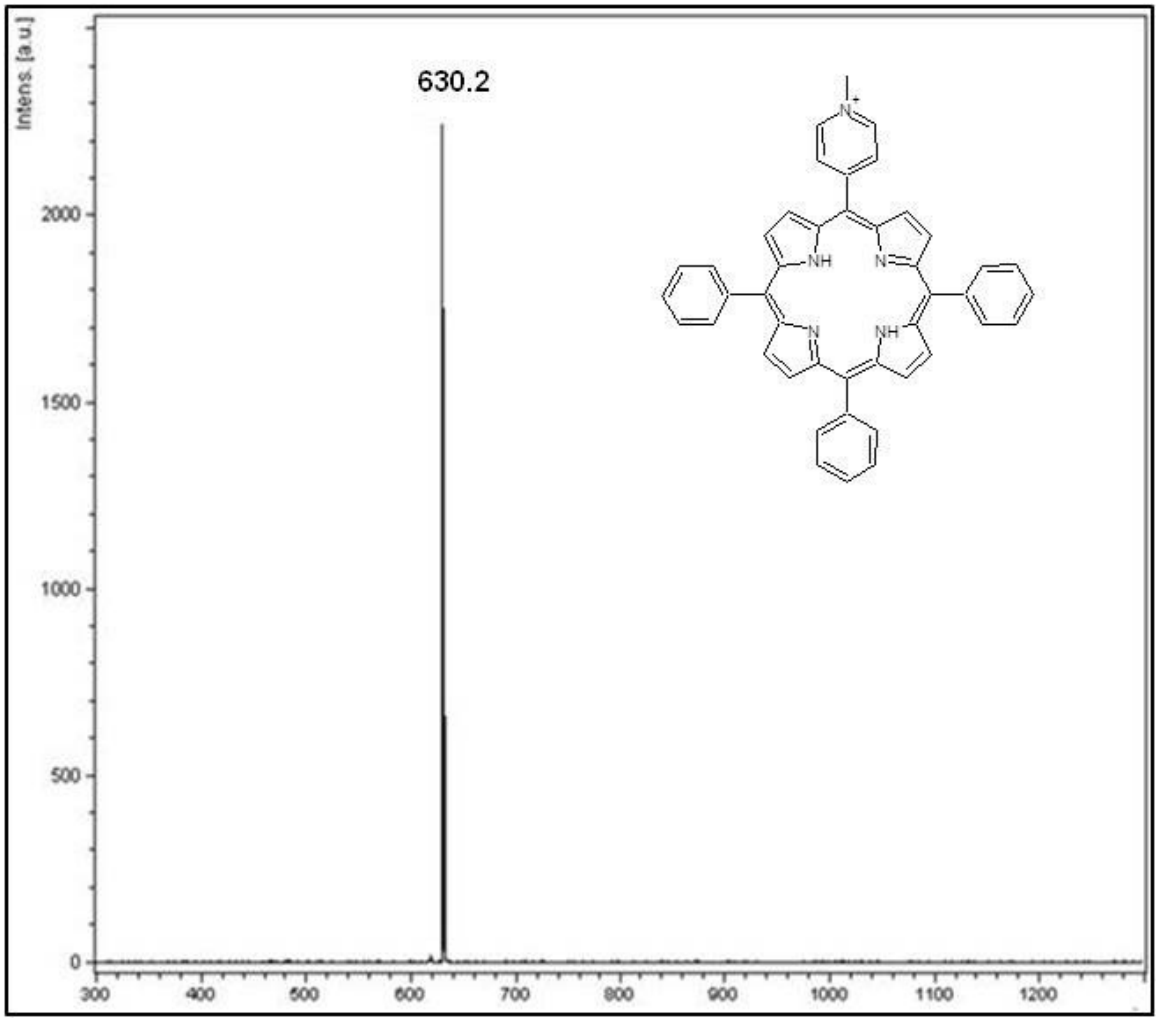
Compound **1** – 5,10,15-triphenyl-20-(1-methyl-4-pyridinio)porphyrin iodide

^{1}H NMR(400 MHz, $DMSO_{d6}$): 9.43 (d, 2H, *m*-Pyr), 9.00 (d, 4H, pyrr-H), 9.00 (d, 2H, *o*-Pyr), 8.86 (m, 4H, pyrr-H), 8.22 (m, 6H, *o*-Ph), 7.86 (m, 9H, *m & p*-Ph), 4.69 (s, 3H, NCH), -2,90 (s, 2H, NH). MALDI-TOF *m/z* $[M-I]^+$: 630. UV-Vis (in H_2O) λ_{max} (nm): 445 (Soret band), 528, 563, 600 and 655 (Q-bands).

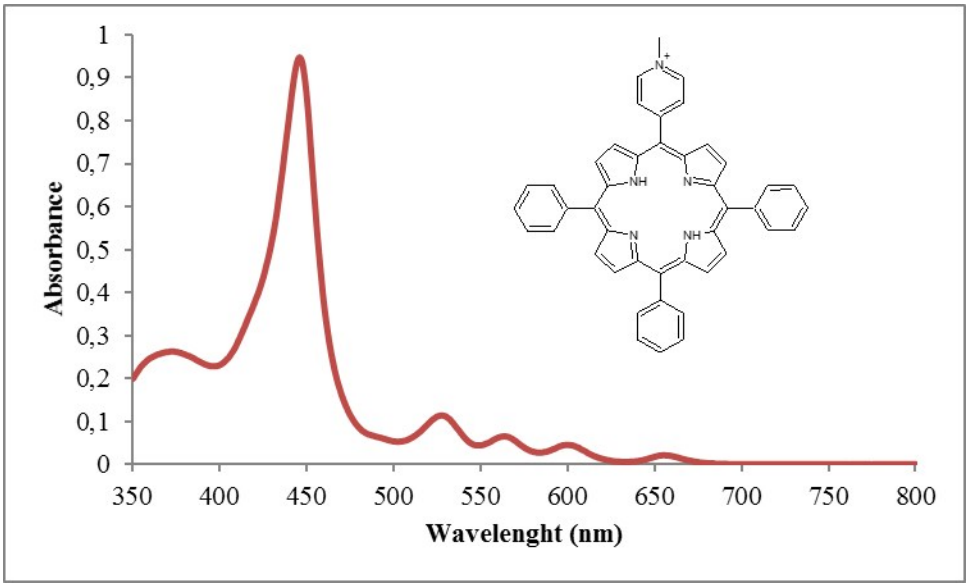
^{1}H NMR spectrum of compound **1**



MALDI-TOF spectrum of compound **1**



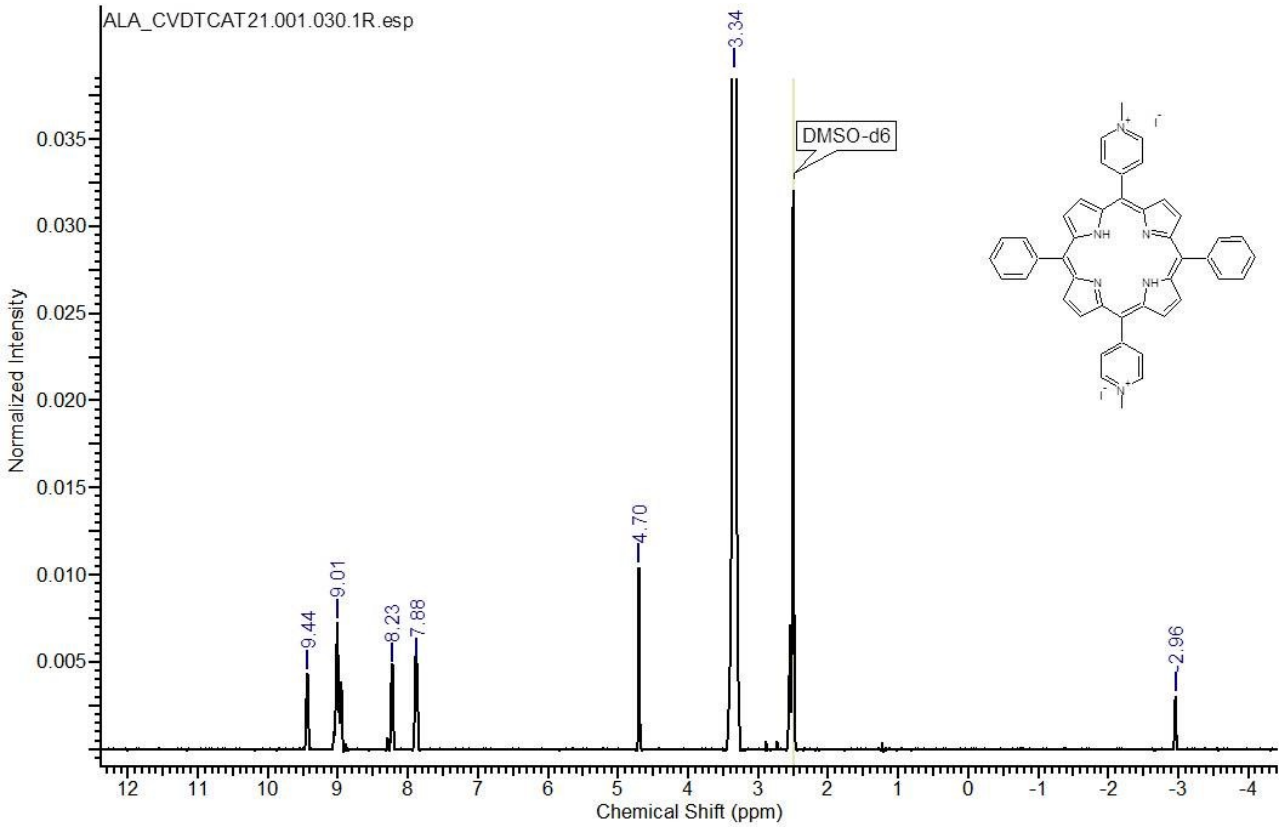
UV-VIS spectrum of compound 1



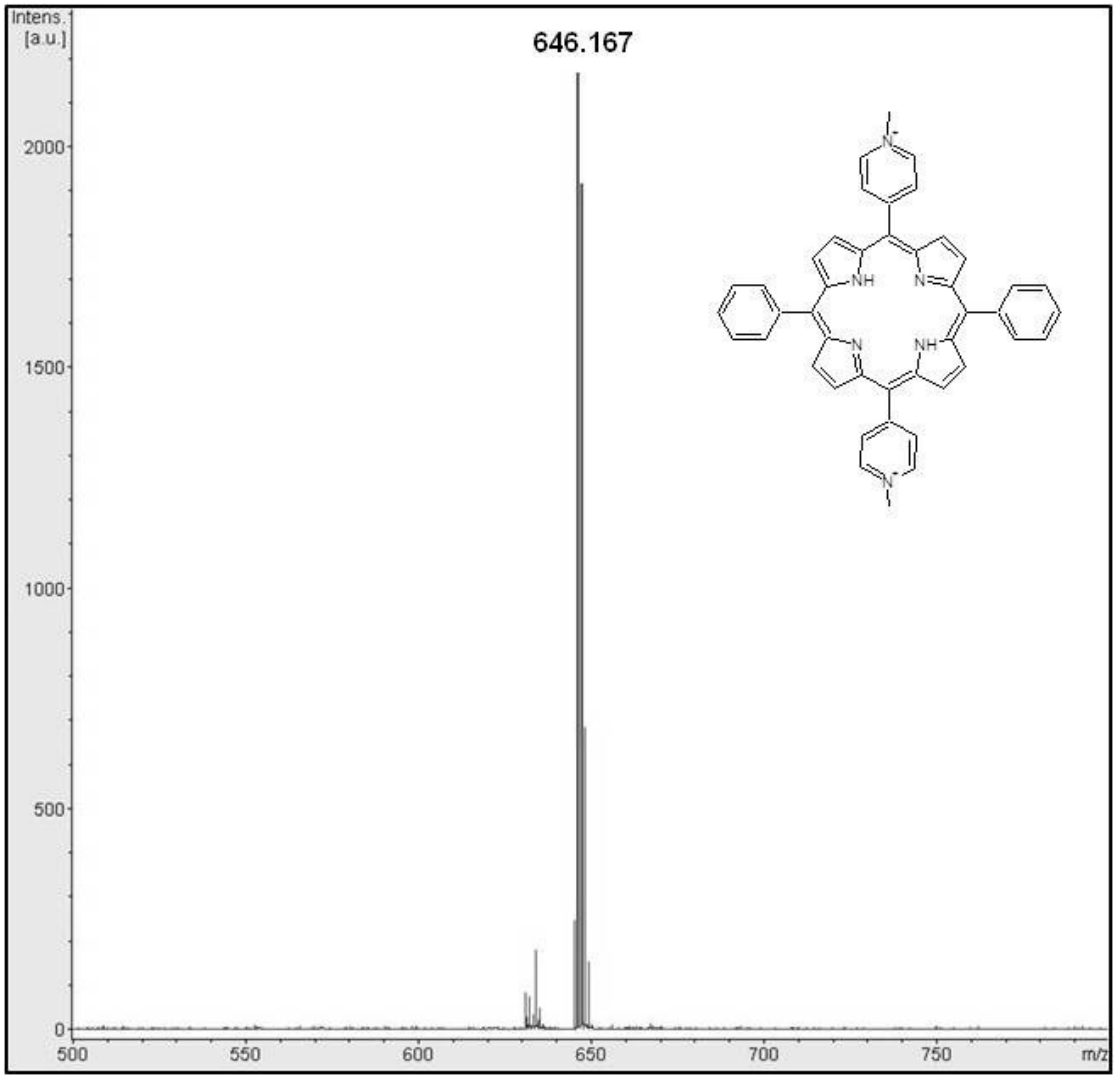
Compound **2 -** 5, 15-diphenyl-10,20-di(1-methyl-4-pyridinio)porphyrin diiodide

^{1}H NMR(400 MHz, $DMSO_{d6}$): 9.44 (d, 4H, *m*-Pyr), 9.01 (m, 8H, pyrr-H), 9.01 (d, 4H, *o*-Pyr), 8.23 (m, 4H, *o*-Ph), 7.88 (m, 6H, *m* & *p*-Ph), 4.70 (s, 6H, NCH), -2,96 (s, 2H, NH). MALDI-TOF m/z $[M-2I]^+$: 646. UV-Vis (in H_2O) λ_{max} (nm): 419 (Soret band), 520, 558, 583 and 643 (Q-bands).

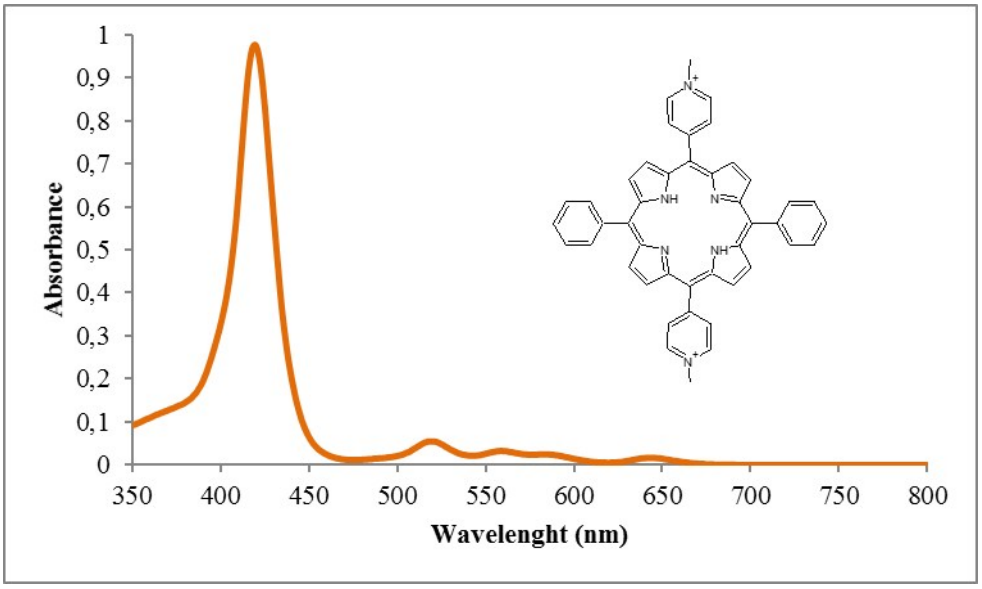
^{1}H NMR spectrum of compound **2**



MALDI-TOF spectrum of compound **2**



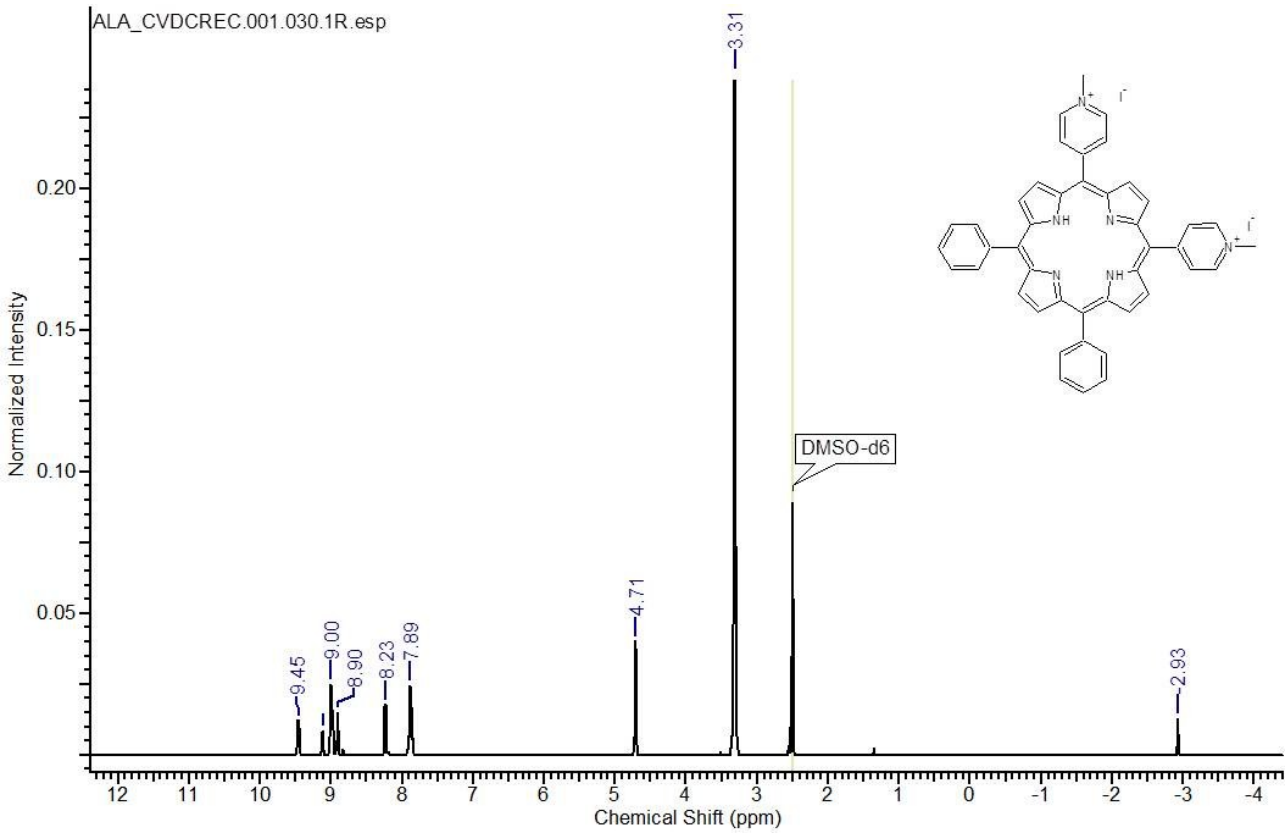
UV-VIS spectrum of compound **2**



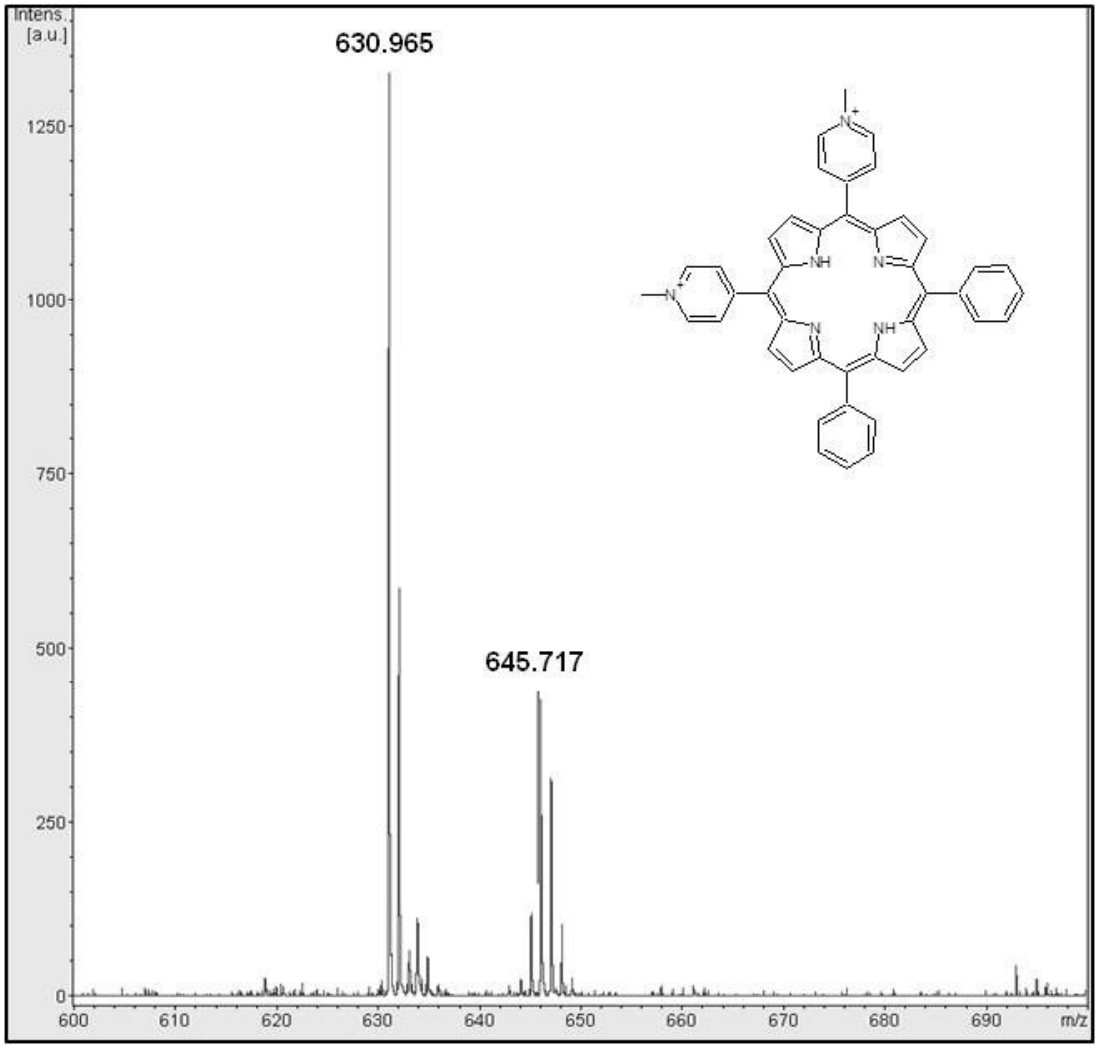
Compound **3 -** 5,10-diphenyl-15,20-di(1-methyl-4-pyridinio)porphyrin diiodide

^{1}H NMR(400 MHz, $DMSO_{d6}$): 9.45 (d, 4H, *m*-Pyr), 9.11 (d, 2H, pyrr-H), 9.00 (m, 4H, pyrr-H), 9.01 (d, 4H, *o*-Pyr), 8.90 (s, 2H, pyrr-H), 8.23 (d, 4H, *o*-Ph), 7.89 (m, 6H, *m* & *p*-Ph), 4.71 (s, 6H, NCH), -2,93 (s, 2H, NH). MALDI-TOF m/z $[M-2I]^+$: 646. UV-Vis (in H_2O) λ_{max} (nm): 421 (Soret band), 520, 559, 581 and 640 (Q-bands).

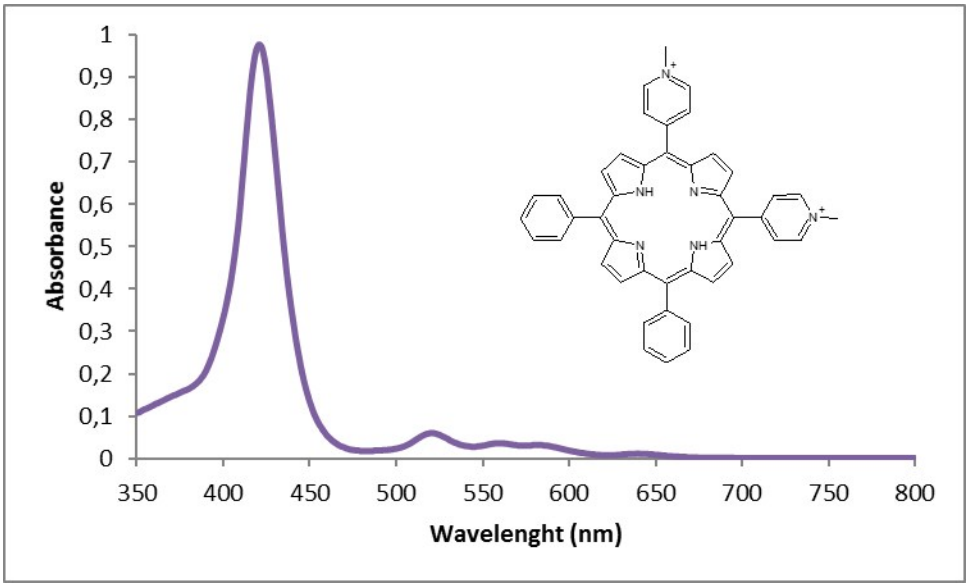
^{1}H NMR spectrum of compound **3**



MALDI-TOF spectrum of compound **3**



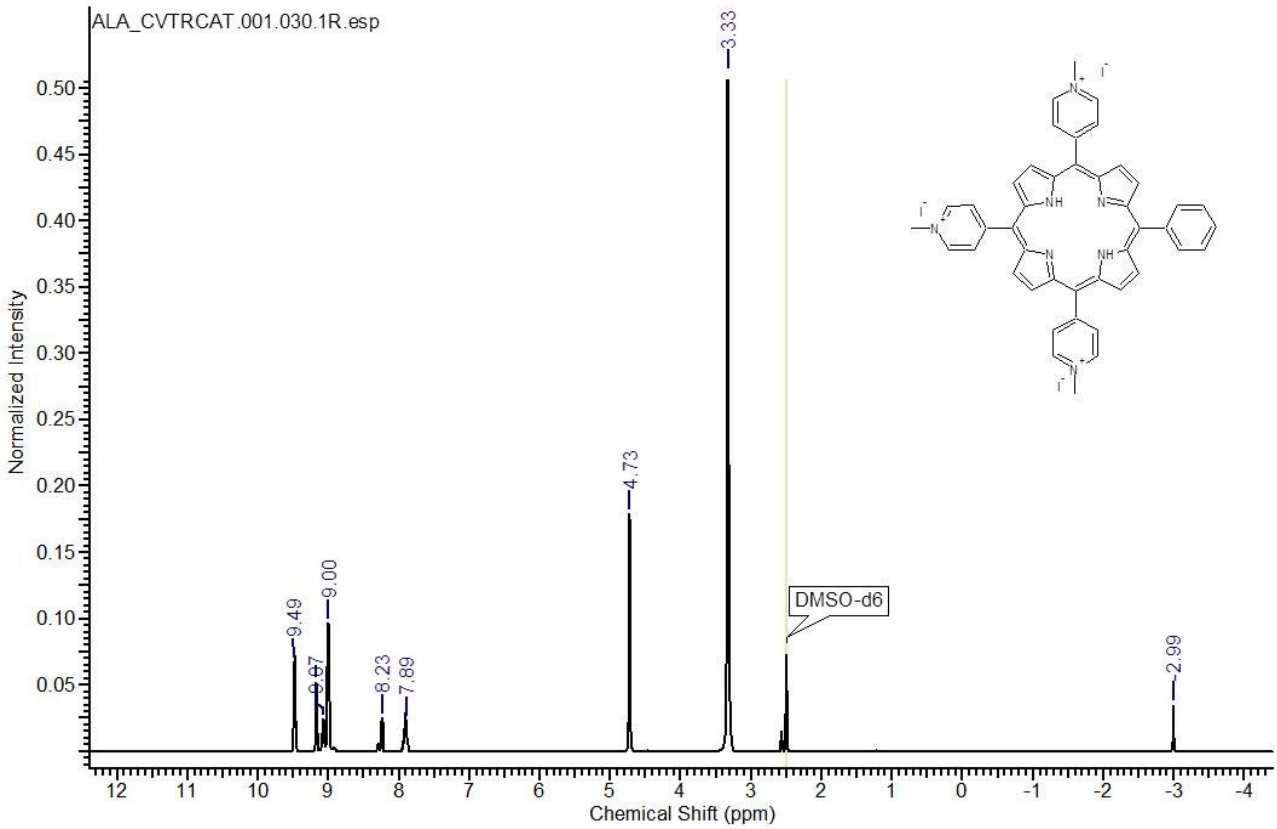
UV-VIS spectrum of compound **3**



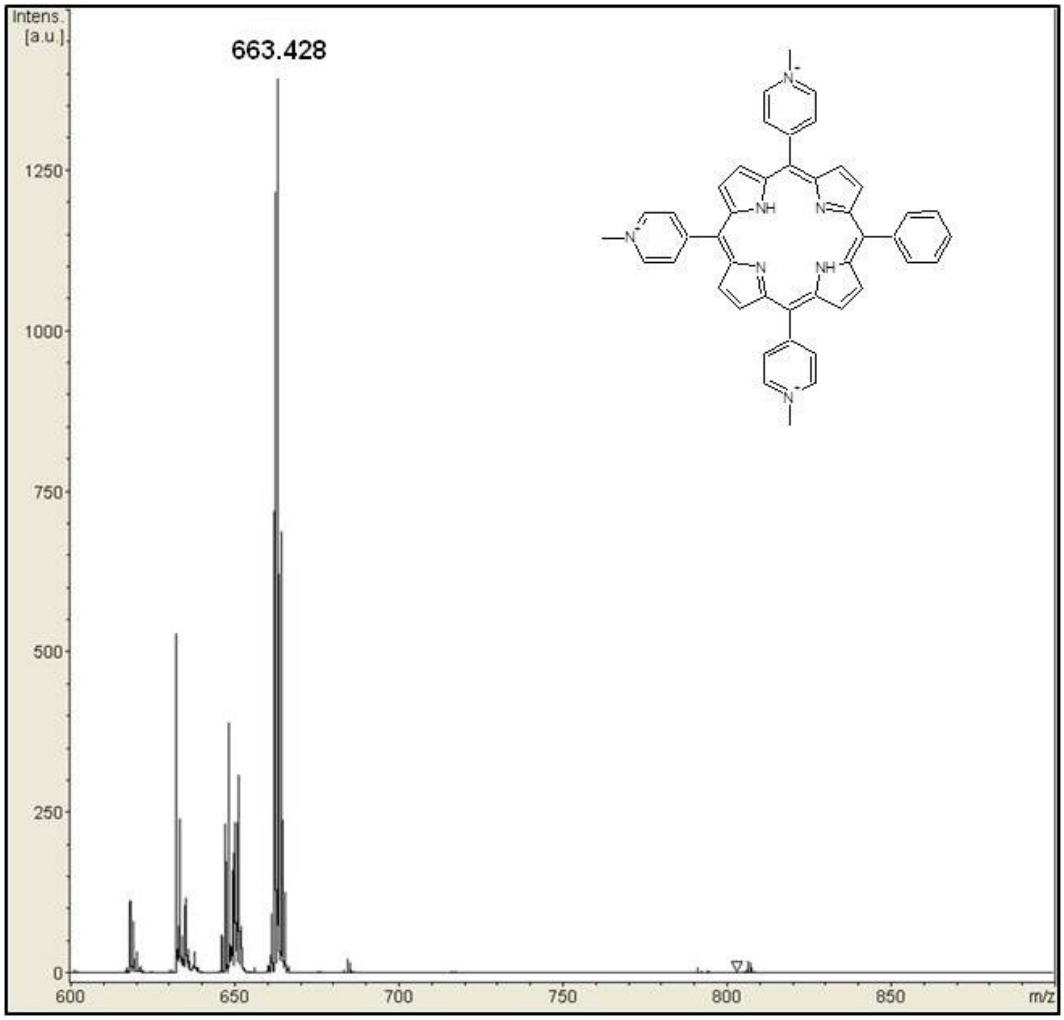
Compound **4 -** 5-phenyl-10,15,20-tri(1-methyl-4-pyridinio)porphyrin triiodide

^{1}H NMR(400 MHz, $DMSO_{d6}$): 9.49 (m, 6H, *m*-Pyr), 9.17 (d, 4H, pyrr-H), 9.07 (d, 6H, *o*-Pyr), 9.00 (d, 4H, pyrr-H), 8.23 (d, 2H, *o*-Ph), 7.91 (m, 3H, *m* & *p*-Ph), 4.73 (s, 9H, NCH), -2,99 (s, 2H, NH). MALDI-TOF m/z $[M-3I+H]^+$: 663. UV-Vis (in H_2O) λ_{max} (nm): 422 (Soret band), 519, 558, 582 and 642 (Q-bands).

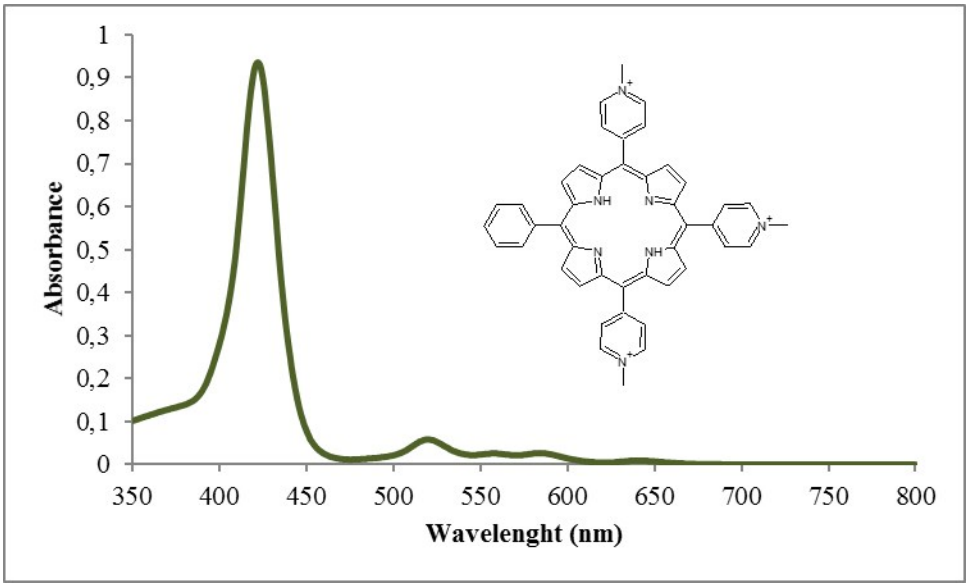
^{1}H NMR spectrum of compound **4**



MALDI-TOF spectrum of compound **4**



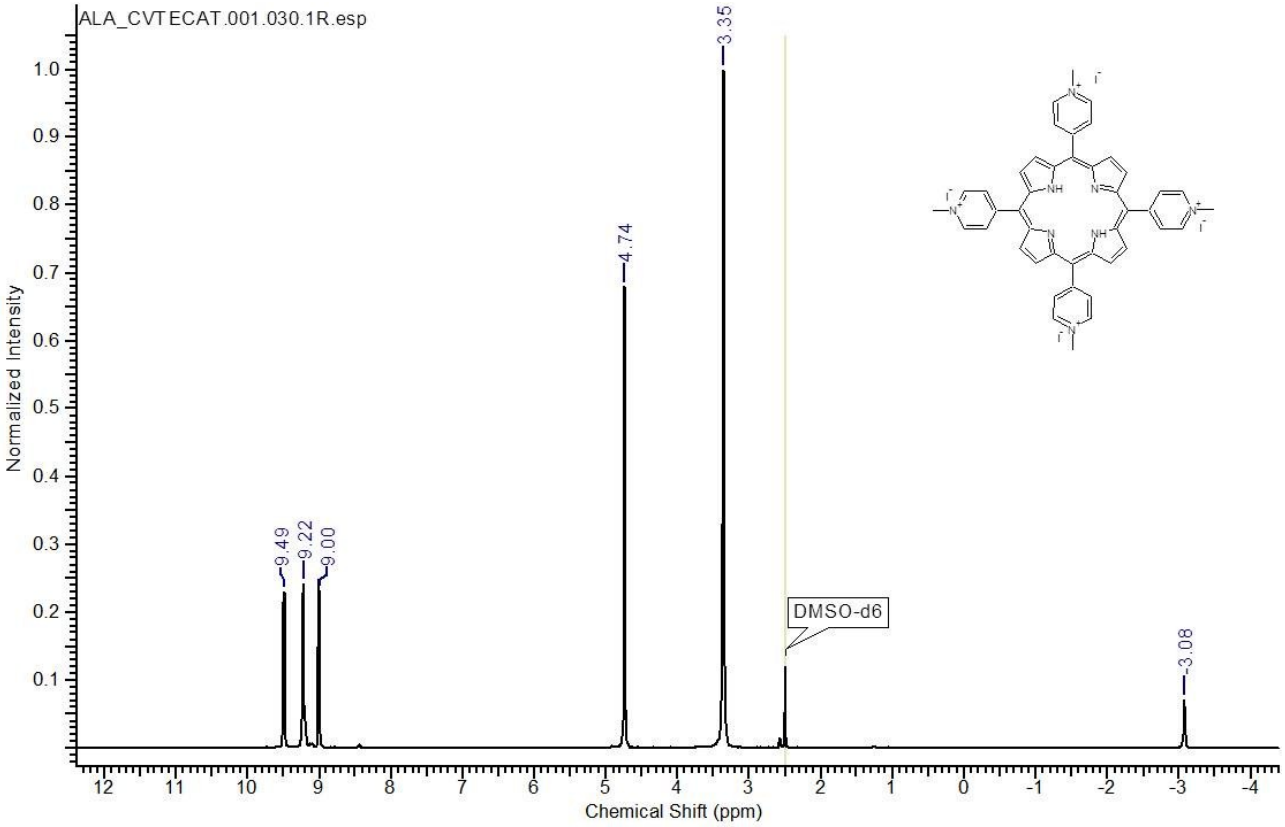
UV-VIS spectrum of compound **4**



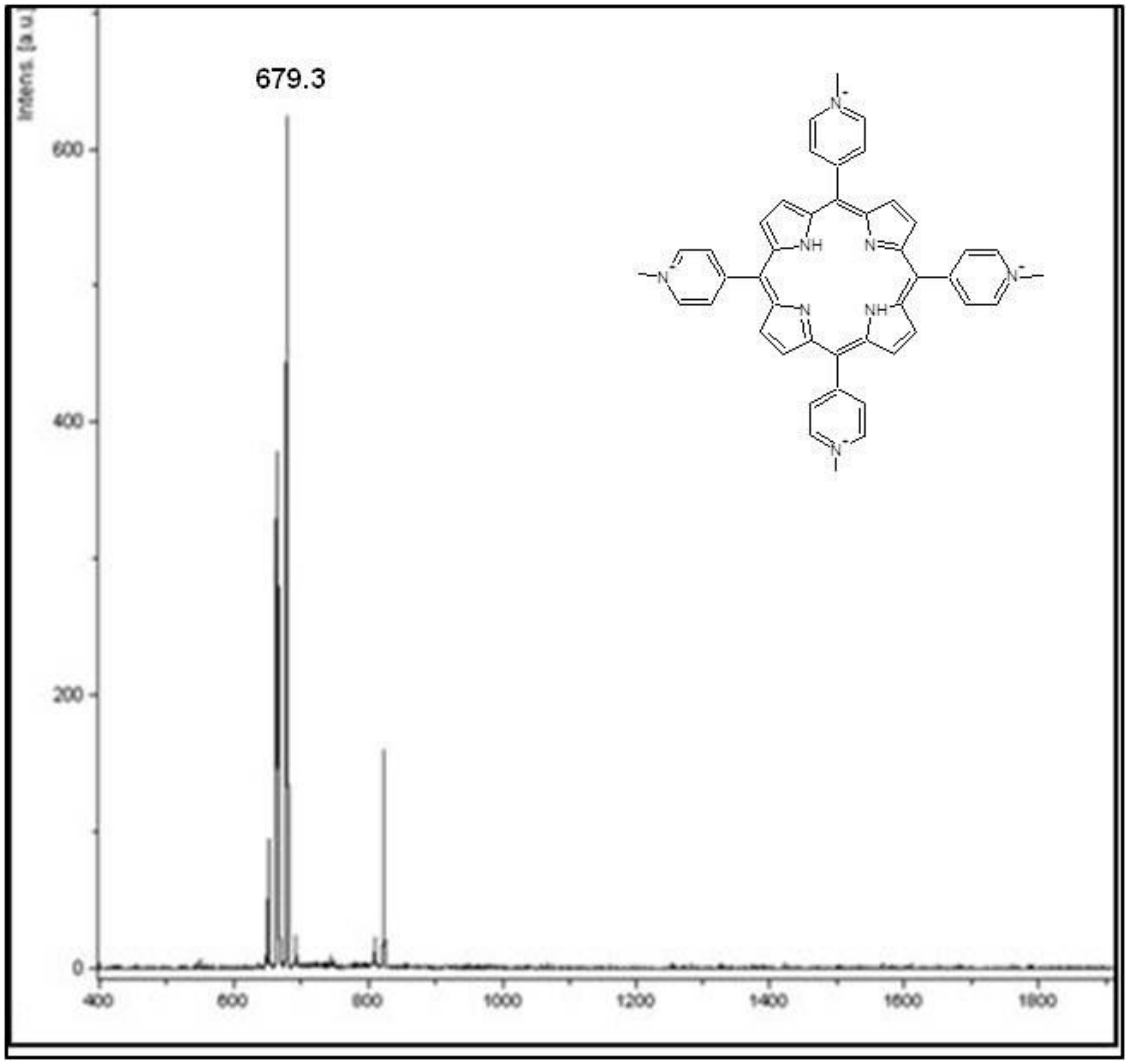
Compound **5 -** 5,10,15,20-*tetrakis*(1-methyl-4-pyridineo)porphyrin tetraiodide

^{1}H NMR(400 MHz, $DMSO_{d6}$): 9.49 (d, 8H, *m*-Pyr), 9.22 (s, 8H, pyrr-H), 9.00 (d, 8H, *o*-Pyr), 4.74 (s, 12H, NCH), -3,08 (s, 2H, NH). MALDI-TOF m/z $[M-4I+H]^+$: 679. UV-Vis (in H_2O) λ_{max} (nm): 422 (Soret band), 519, 554, 585 and 640 (Q-bands).

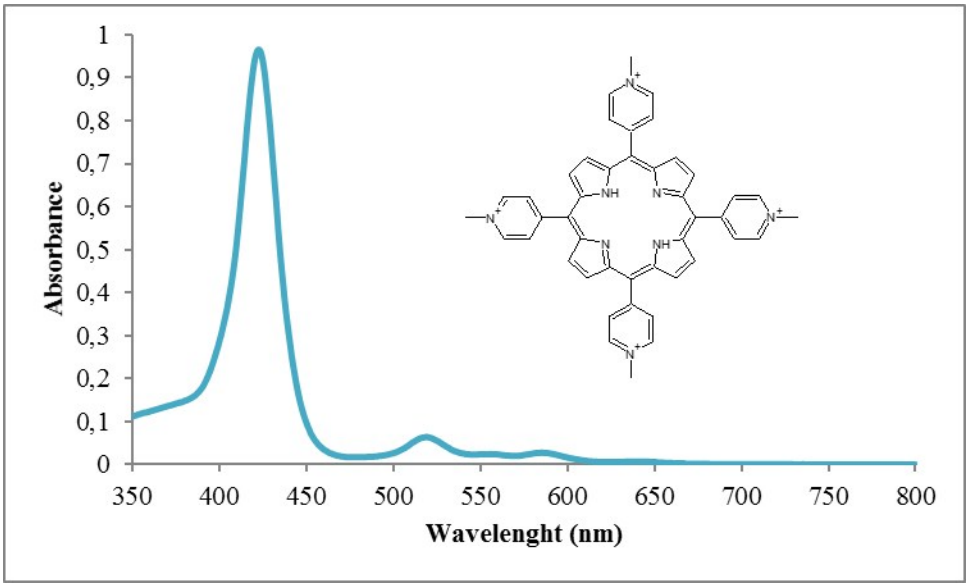
^{1}H NMR spectrum of compound **5**



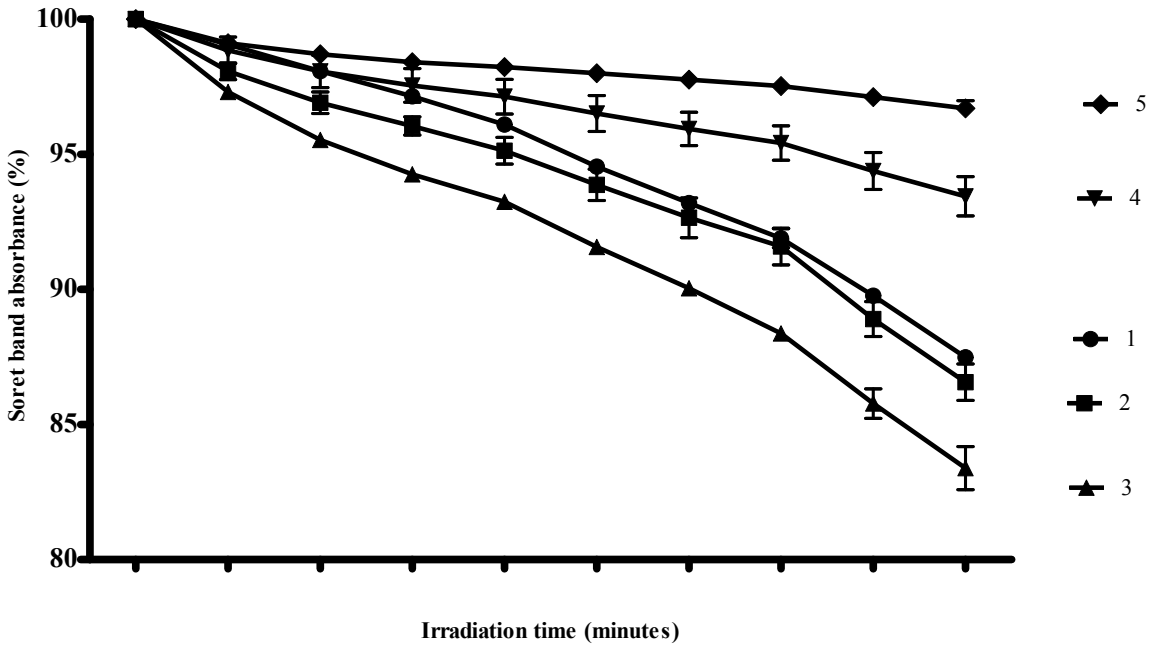
MALDI-TOF spectrum of compound **5**



UV-VIS spectrum of compound **5**

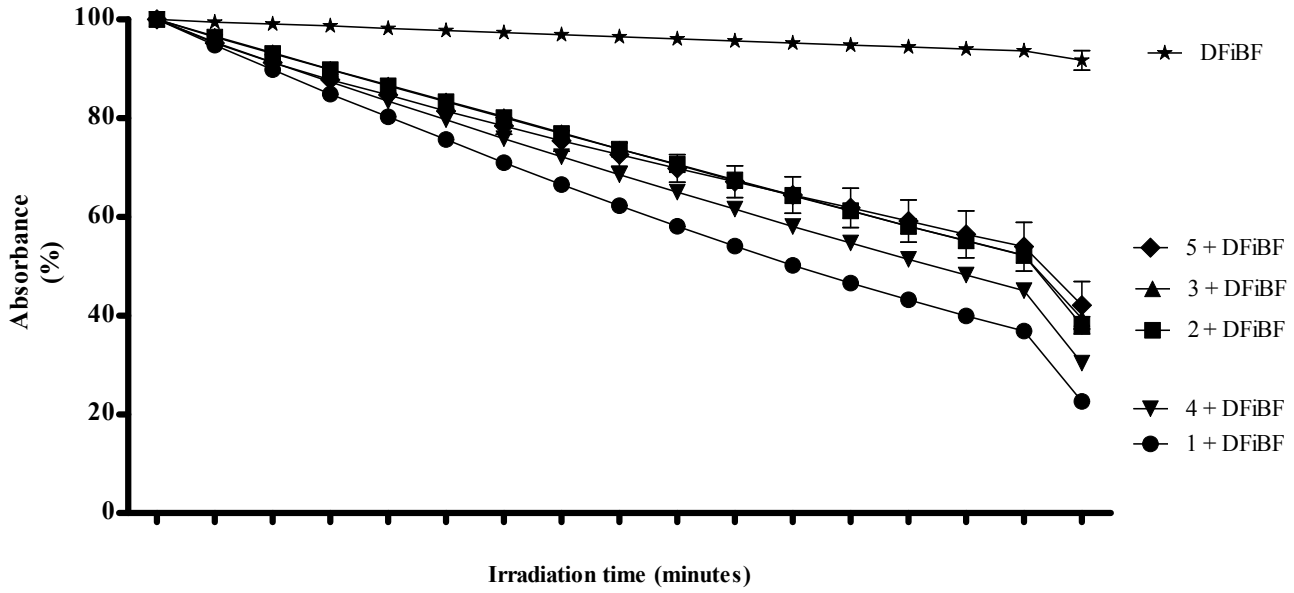


4. Photostability Assay of porphyrins 1–5



Photostability results are expressed as percentage from the initial absorbance of porphyrins Soret band, during white light irradiance (100.0 mW cm^{-2}). Complete monitoring (1–20 min) is described.

5. Singlet Oxygen generation by compounds 1–5



Singlet oxygen generation was assessed by the DPBF photooxidation (50 mmol L^{-1}; in DMF/H_2O 9:1) upon white light irradiance filtered through a cut-off filter for wavelengths <540 nm (9.0 mW cm^{-2}), with or without photosensitizer (0.5 mmol L^{-1}).