Supporting Information

Temocene: the porphycene analogue of temoporfin (Foscan®)

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1. Chemical synthesis

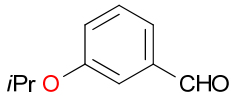
General.

All reactions were carried out with standard Schlenk techniques under an Ar atmosphere. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400-MR (^{1}H at 400 MHz and ^{13}C at 100.6 MHz) spectrometer. All NMR data were obtained in $CDCl_3$ and DMSO-d_6. Chemical shifts are reported in parts per million (ppm, δ) and are referenced to the residual proton signal of the solvent. Coupling constants are reported in Hertz (Hz). Spectral splitting patterns are designated as s: singlet, d: doublet, t: triplet, q: quartet, m: complex multiplet (chemically non-equivalent H's), brs: broad signal. Infrared spectra were recorded in a Nicolet Magna 560 FTIR spectrophotometer. All MS were registered at the Unidade de Espectrometria de Masas (Universidade de Santiago de Compostela) using a Micromass Autospec spectrometer. Flash chromatography was performed using silica gel 60 A C.C 35-70 μm (SDS ref. 2000027).

Materials.

Solvents and reagents were reagent-grade and were used without further purification (Aldrich). THF was distilled from K / benzophenone prior to use.

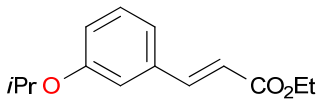
_m_-isopropoxybenzaldehyde (4)



70 mL of *n*BuLi (1.6 M in hexanes, 112 mmol) were added dropwise *via* syringe to a solution of 20 g of 1-bromo-3-isopropoxybenzene (93 mmol) in 100 mL anhydrous THF at -78 ºC under an Ar atmosphere. The reaction mixture was stirred at -78 ºC for 15 min; then 16 mL of DMF was added in one portion. The temperature of the mixture was allowed to rise to room temperature and the stirring was maintained for 30 minutes. The clear reaction mixture was poured into H_2O (150 mL) and extracted with pentane (3×50 mL). The combined organic layers were washed with H_2O (50 mL), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to afford 15 g of *m*-isopropoxybenzaldehyde as a yellowish liquid (η = 98%, 91 mmol).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.96 (s, 1H), 7.46 – 7.10 (m, 4H), 4.63 (dt, *J* = 6.0, 12.1, 1H), 1.36 (d, *J* = 6.1, 6H).
^{13}C NMR (100 MHz, $CDCl_3$): δ = 192.18, 158.47, 137.79, 130.06, 123.10, 123.03, 114.14, 70.18, 21.88.

(*E*)-ethyl 3-(*m*-isopropoxyphenyl)acrylate (5)



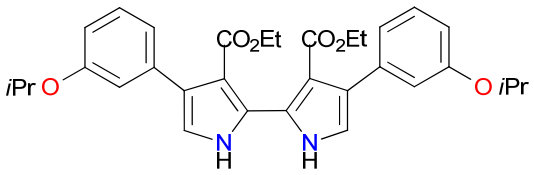
To a 150 mL solution of anhydrous THF 29.2 mL of triethyl phosphonoacetate (130 mmol) were poured under a N_2 atmosphere keeping the temperature at -78ºC by using a dry ice / acetone bath. Next, 85.3 mL of an *n*BuLi solution (1.6 M in hexanes; 136 mmol) were added in a drop wise fashion. After 5 minutes of mixing, a solution of 20.4 g of *m*-isopropoxybenzaldehyde (124 mmol) in THF was added drop by drop *via* syringe. Temperature was allowed to rise to room temperature and stirring was maintained for an extra 2 hours. The reaction mixture was washed with a 10% NH_4Cl solution and the organic layer extracted with ethyl acetate. The yellow oil obtained after removing the solvent was purified by means of a silica pad (ethyl acetate / cyclohexane; 1:1). The filtration afforded 22.3 g of (*E*)-ethyl 3-(*m*-isopropoxyphenyl)acrylate as a yellow liquid (η = 77%, 95 mmol).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.63 (d, J = 16.0, 1H), 7.25 (t, J = 7.9, 1H), 7.07 (d, J = 7.6, 1H), 7.02 (s, 1H), 6.89 (d, J = 8.2, 1H), 6.40 (d, J = 16.0, 1H), 4.55 (m, 1H), 4.25 (q, J = 7.1, 2H), 1.33 (t, 3H), 1.31 (d, J = 3.5, 6H);

^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.95, 158.18, 144.59, 135.80, 129.84, 120.51, 118.36, 117.87, 114.97, 69.99, 60.47, 21.98, 14.30;

EA (%) Calc for $C_{14}H_{18}O_3$: C, 71.77; H, 7.74. Found: C, 71.50; H, 7.62;

Diethyl 4, 4'-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-[2,2'-bipyrrole]-3,3'-dicarboxylate (6)



n-BuLi (1.6 M in hexanes, 39.8 mL, 63.7 mmol) was added to a solution of TosMIC (6.59 g, 31.9 mmol) in THF (50 mL) at -78 °C. After 5 min of stirring at -78 °C, 65 mL of Me_3SnCl (1 M in THF, 63.7 mmol) was added dropwise. After another 5 min of stirring at -78 °C, a solution of (*E*)-ethyl 3-(*m*-isopropoxyphenyl)acrylate (7.45g, 31.8 mmol) in THF (100 mL) was added dropwise. The temperature of the reaction mixture was allowed to rise to room temperature in 30 min, and stirring was continued for 2 h. To this solution 1.9 g of $Cu(NO_3)_2 \cdot 3H_2O$ (8 mmol) were added in a single portion. The mixture was stirred at room temperature for 40 minutes. The solvent was removed under reduced pressure and the residue was dissolved in ethyl acetate. The organic layer was washed with 10% ammonia (3×50 mL), water (3×50 mL) and brine. The solvent was dried over $MgSO_4$ and the volume of the solution was reduced to 1/3 of the total. The resulting slurry was placed in the fridge overnight and the precipitate was collected by filtration and washed with cold ethyl acetate (η = 44%, 3.8 g).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 13.36 (s, 2H), 7.22 (t, J = 7.9, 2H), 6.92 – 6.78 (m, 8H), 4.57 (dt, J = 6.0, 12.1, 2H), 4.10 (q, J = 7.1, 4H), 1.35 (d, J = 6.0, 12H), 0.96 (t, J = 7.1, 6H);

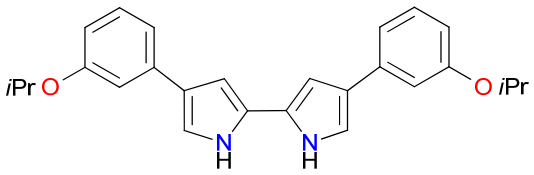
^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.64, 157.21, 138.09, 129.50, 128.80, 128.35, 121.98, 117.80, 117.33, 113.65, 109.65, 69.76, 60.71, 22.14, 13.38;

EA (%) Calc. for $C_{32}H_{36}N_2O_6$: C, 70.57; H, 6.66; N, 5.14. Found: C, 70.51; H, 6.76; N, 5.02;

IR (KBr) ν_{max}/cm^{-1}: 3442, 2980, 1649, 14945, 1421, 1194, 1027, 776;

HRMS (FAB+) m/z Calc. for $C_{32}H_{36}N_2NaO_6$ 567.2471. Found 567.2466.

4,4´-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-2,2´-bipyrrole (7)



Diethyl 4, 4'-bis(m-isopropoxyphenyl)-1*H*, 1'*H*-[2,2'-bipyrrole]-3,3'-dicarboxylate (7.6 g, 14 mmol), ethylene glycol (120 mL) and sodium hydroxide (3.4 g, 85 mmol) were combined in a 100 mL round bottomed flask and degassed for 1h with a stream of N_2. The system was turned to an Ar atmosphere and the flask was heated to 180 ºC for 90 minutes. The mixture was allowed to cool to 100 ºC and then 50 mL of water (previously degassed) was added. The precipitate was collected by filtration under an Ar sweep and dried *in vacuo* to afford 4.85 g (η = 87%, 12 mmol) of 4,4´-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-2,2´-bipyrrole as a green powder.

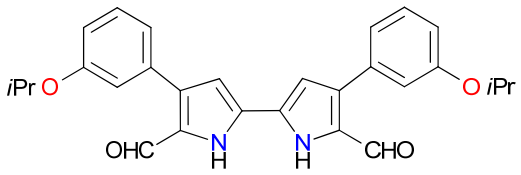
^{1}H NMR (400 MHz, DMSO-d_6): δ = 11.13 (brs, 2H), 7.52 (d, *J* = 7.9 Hz, 4H), 7.27 (d, *J* = 7.9 Hz, 4H), 7.22 (brs, 2H), 6.67 (brs, 2H), 4.38 (s, 4H), 3.29 (s, 6H);

^{13}C NMR (100 MHz, DMSO-d_6): δ = 135.6, 135.1, 128.6, 127.3, 124.5, 124.1, 115.2, 101.0, 74.0, 57.8;

IR (KBr) ν_{max}/cm^{-1}: 3316, 2924, 1610, 1510, 1079, 1059, 927, 845, 790;

HRMS (FAB$^+$) *m/z* Calc. for $C_{24}H_{25}N_2O_2$: 373.1916. Found: 373.1917

4, 4'-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-[2,2'-bipyrrole]-5,5'-dicarbaldehyde (8)



4.8 g (12 mmol) of 4,4´-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-2,2´-bipyrrole were dissolved in dry DMF (180 mL) under Ar at 0 °C. Then, freshly distilled $POCl_3$ (5.7 mL, 61 mmol) was added drop wise with stirring *via* syringe. Once the addition was completed, the mixture that resulted was heated to 60 °C for 1h, and then cooled at room temperature. The solution was chilled in an ice/water bath while adding 40 mL of half-saturated aqueous sodium acetate. This biphasic mixture was then heated at 85 ºC for 1h. The slurry was allowed to cool to room temperature and then the precipitate was collected by filtration and washed with water. The product was dried *in vacuo* to yield 4, 4'-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-[2,2'-bipyrrole]-5,5'-dicarbaldehyde (η = 89%, 4.9 g) as a yellow powder.

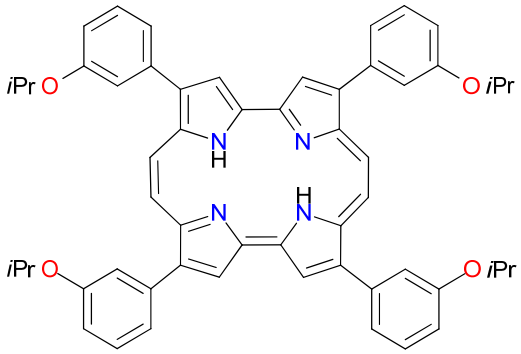
^{1}H NMR (400 MHz, DMSO-d_6): δ = 12.43 (brs, 2H), 9.61 (s, 2H), 7.58 (d, *J* = 7.9 Hz, 4H), 7.42 (d, *J* = 7.9 Hz, 4H), 7.19 (s, 2H), 4.47 (s, 4H), 3.33 (s, 6H);

^{13}C NMR (100 MHz, DMSO-d_6): δ = 178.5, 137.8, 135.9, 132.2, 129.9, 128.9, 128.6, 127.8, 110.1, 73.1, 57.5;

IR (KBr) ν_{max}/cm^{-1}: 3426, 3259, 2925, 1619, 1446, 1345, 1241, 1100, 823, 783;

HRMS (FAB$^+$) *m/z* Calc. for $C_{26}H_{24}N_2O_4$: 428.1736. Found: 428.1734

2,7,12,17-tetrakis(*m*-isopropoxyphenyl)porphycene (9)



Activated Zn (14.3, 65 mmol) and Cu_2Cl_2 (2.2 g, 218.6 mmol) were suspended in THF (800 mL), $TiCl_4$ (12.1 mL, 111 mmol) was added dropwise and the resulting mixture was heated at reflux for 3 h. A solution of 4, 4'-bis(*m*-isopropoxyphenyl)-1*H*, 1'*H*-[2,2'-bipyrrole]-5,5'-dicarbaldehyde (2 g, 4.38 mmol) in THF (200 mL) was added drop wise, and the slurry was stirred at reflux for 3 min. Finally, the resulting mixture is cooled to room temperature and filtered through a silica pad. The filtrate was hydrolyzed at 0 ºC with 200 mL of 10% K_2CO_3 solution and stirred under oxygen atmosphere for 30 min. The aqueous solution was extracted with dichloromethane (3×50 mL) and dried over $MgSO_4$. The solvent was removed under reduced pressure and the residue was purified using silica gel column chromatography (hexanes/CH_2Cl_2 1:9). The title compound was obtained (η = 20%, 380 mg) as dark blue a powder.

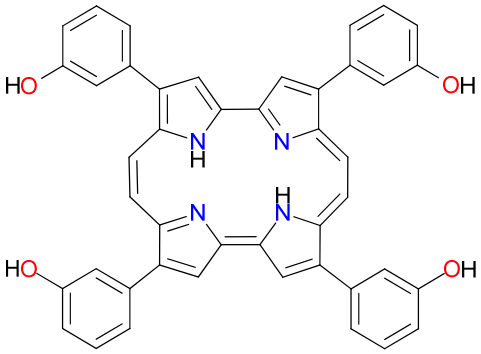
^{1}H-NMR (400 MHz, $CDCl_3$): δ = 9.92 (s, 4H), 9.69 (s, 4H), 8.32 (d, *J* = 7.9 Hz, 8H), 7.79 (d, *J* = 7.9 Hz, 8H), 4.75 (s, 8H), 3.82 (brs, 2H), 3.59 (s, 12H);

^{13}C-NMR (100 MHz, $CDCl_3$): δ = 158.5, 144.9, 142.5, 137.3, 134.4, 130.6, 125.2, 122.7, 118.5, 115.6, 114.9;

UV/vis λ_{max}(benzene)/nm 659 (ε/dm^3 mol^{-1} cm^{-1} 57 576), 629 (53 472), 587 (39 516), 378 (13 0783)

HRMS (ESI-TOF) *m/z* Calc. for $C_{56}H_{55}N_4O_4$: 847.4218. Found: 847.4210

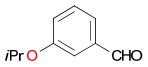
2,7,12,17-tetrakis(*m*-hydroxyphenyl)porphycene (THPPo) (1)

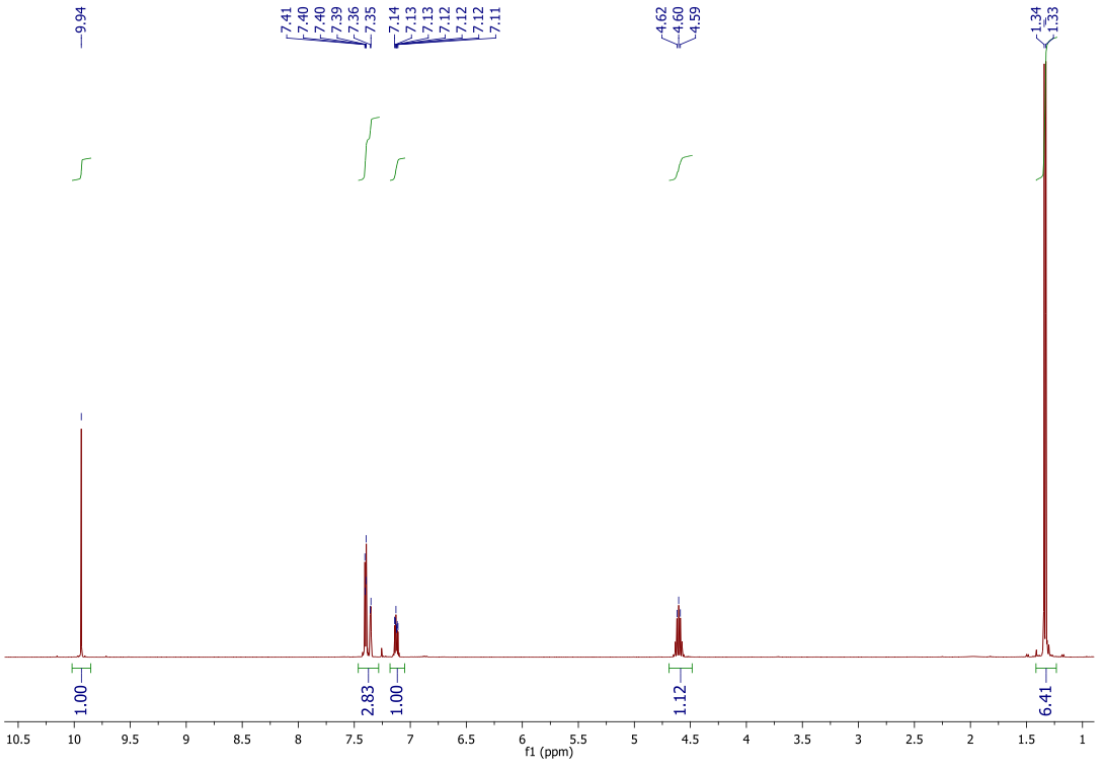


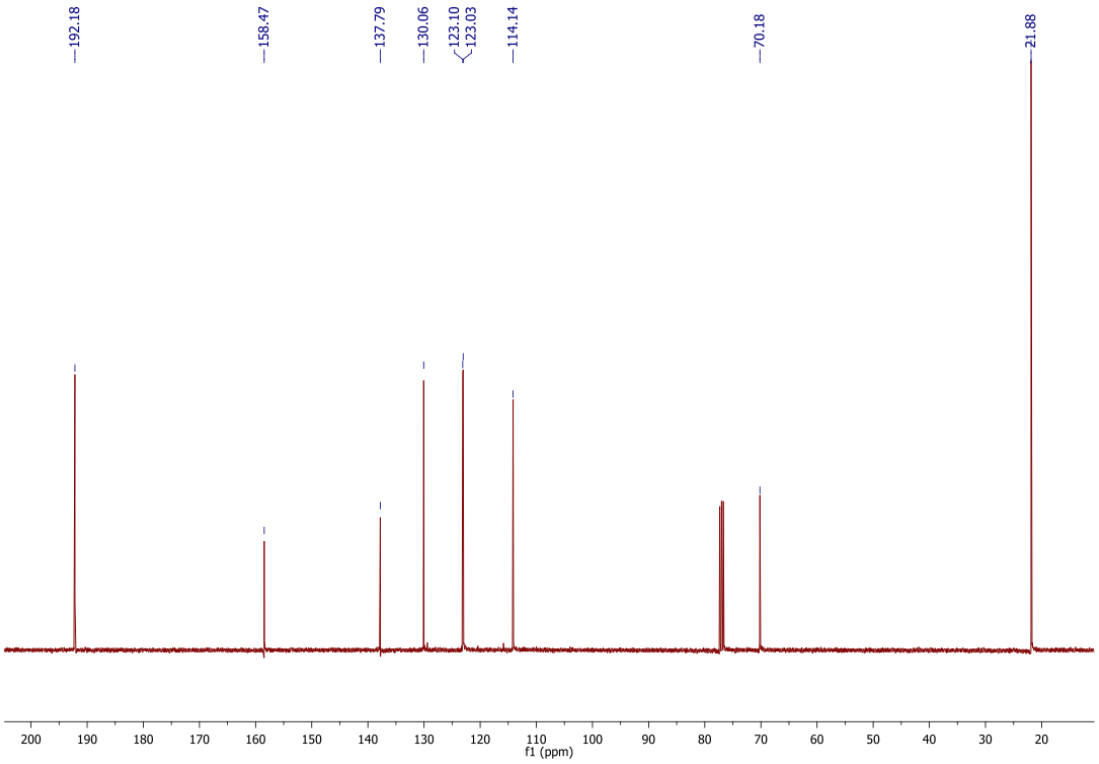
105 mg (0.177 mmol) of 2,7,12,17-tetrakis(*m*-isopropoxyphenyl)porphycene were dissolved in 50 mL of anhydrous dichloromethane. Then, 117 mg (0.89 mmol) of anhydrous aluminum trichloride were added in one portion while standing the system in a water / ice bath for 3 hours. Then the reaction mixture is quench with the careful addition of later 10 mL of chilled water. The organic layer was washed with a $NaHCO_3$ saturated solution. The organic layer was dried over $MgSO_4$ and the solvent removed under reduced pressure. The crude was purified by silica gel column chromatography (ethyl acetate/cyclohexane 1:9) yielding 95 mg of 2,7,12,17-tetrakis(*m*-hydroxyphenyl)porphycene (η = 79%) as deep blue powder.

^{1}H-NMR (400 MHz, $CDCl_3$): δ = 10.14 (s, 4H), 10.06 (s, 4H), 9.93 (brs, 4H), 7.80 (m, 8H), 7.68 (t, *J* = 8 Hz, 4H), 7.13 (d, *J* = 4 Hz, 4H), 3.76 (brs, 2H);
^{13}C-NMR (100 MHz, $CDCl_3$): δ = 144.6, 142.7, 137.9, 135.8, 134.4, 131.5, 128.4, 123.9, 114.5, 74.7, 58.4;
UV/vis λ_{max}(THF)/nm 656 (ε/dm^3 mol^{-1} cm^{-1} 69 085), 624 (65 250), 583 (48 888), 373 (156 428)
HRMS (ESI-TOF) *m/z* Calc. for $C_{44}H_{31}N_4O_4$: 679.2340. Found: 679.2336

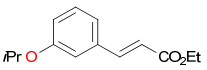
[^{1}H and ^{13}C NMR spectra of **4**]

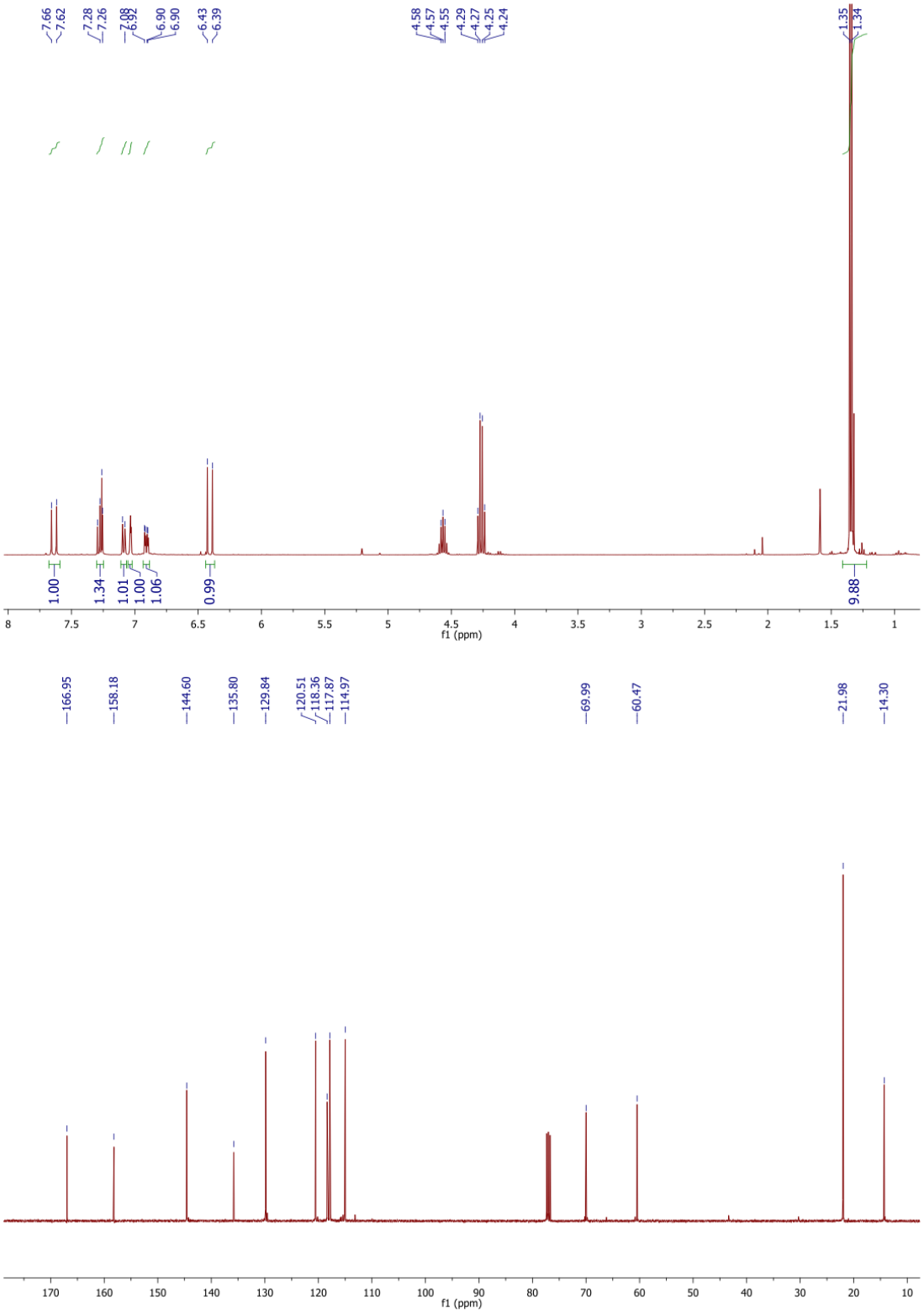




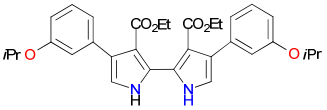


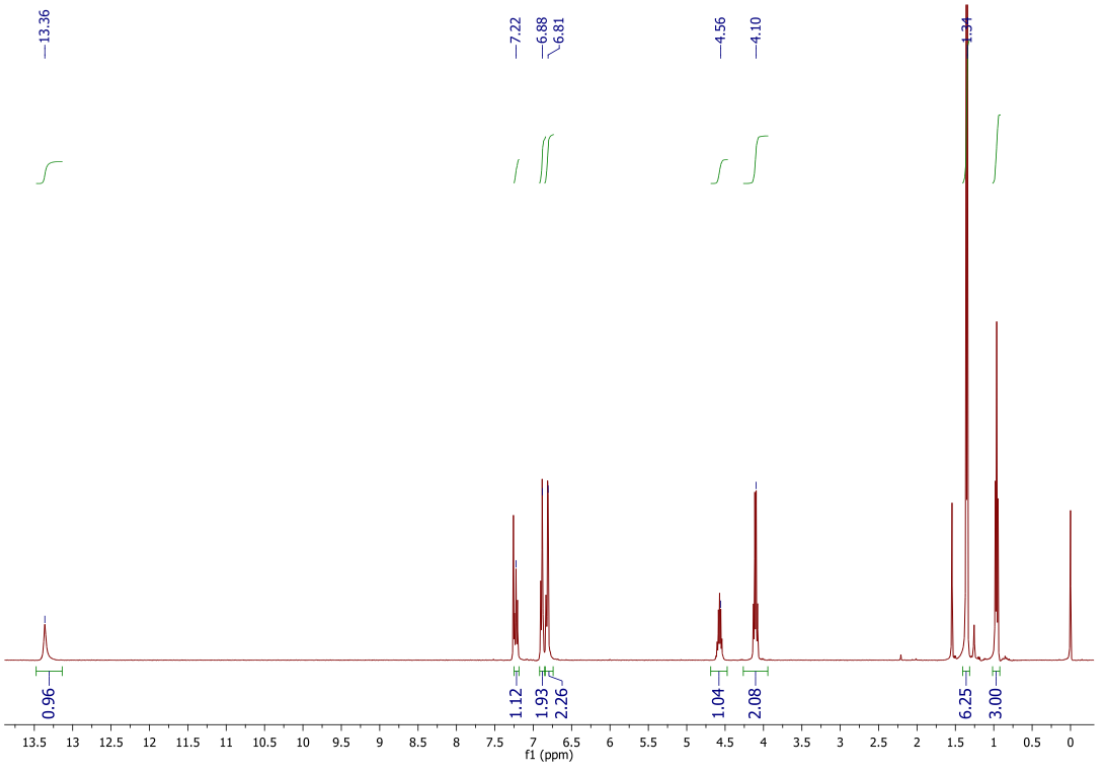
[^{1}H and ^{13}C NMR spectra of **5**]

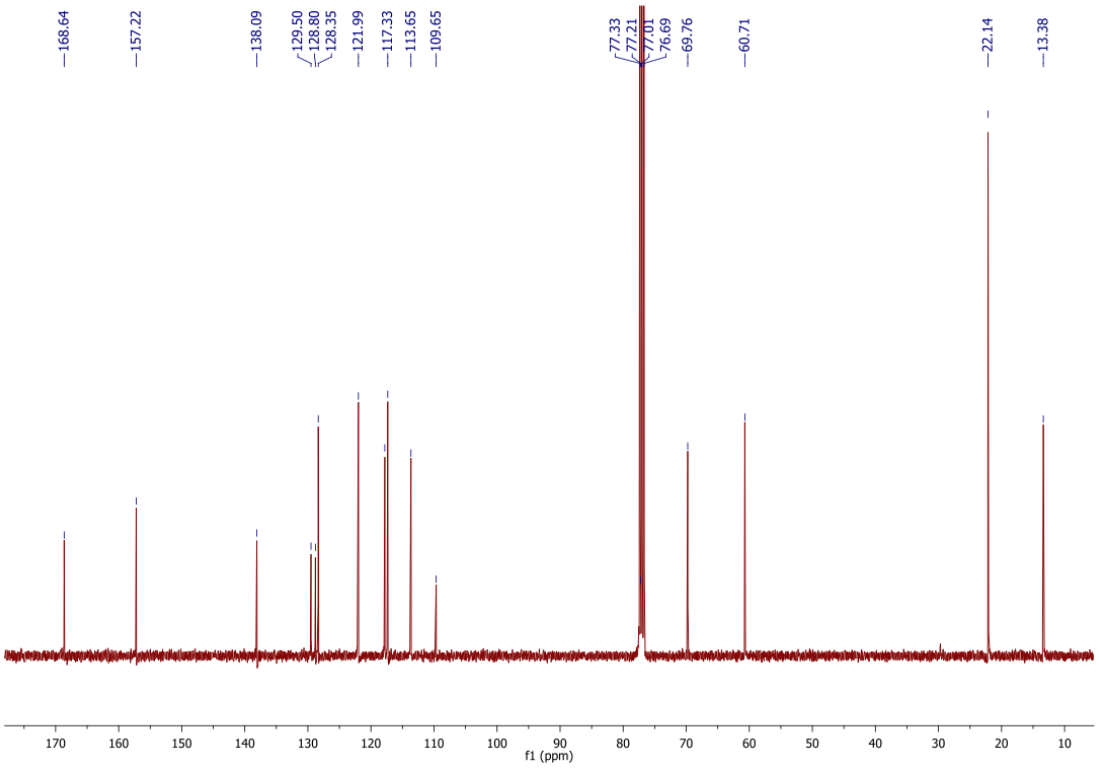




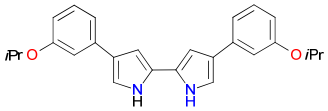
[^{1}H and ^{13}C NMR spectra of **6**]

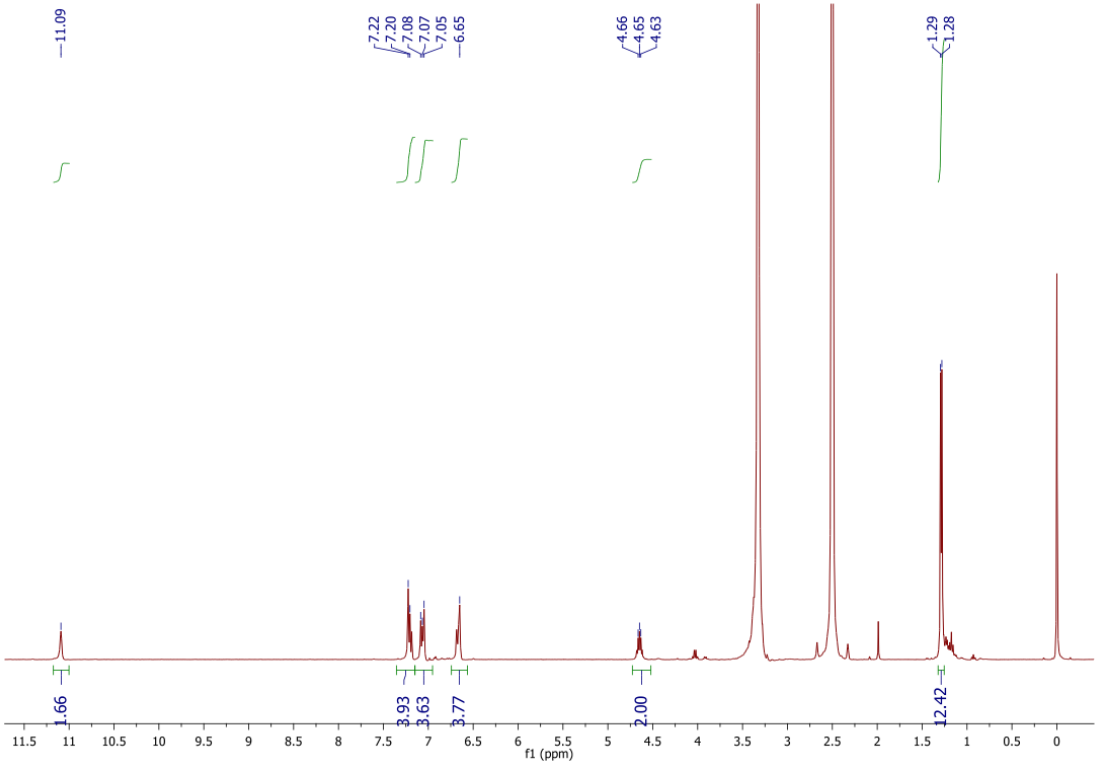


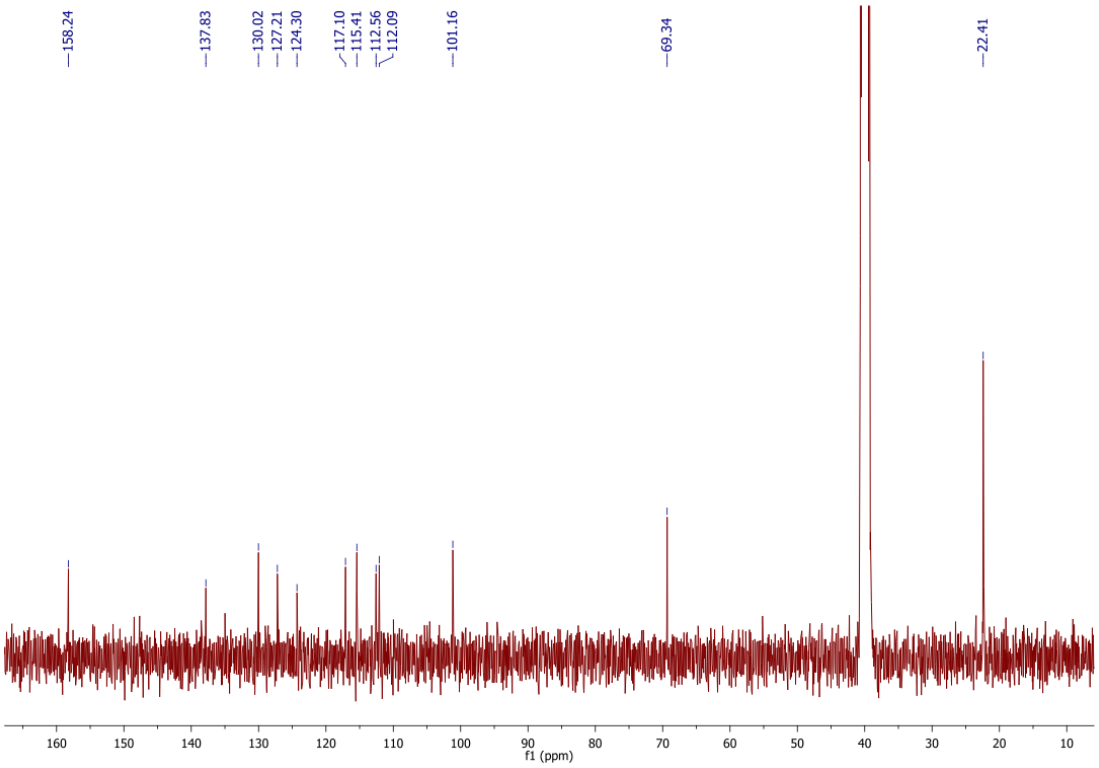




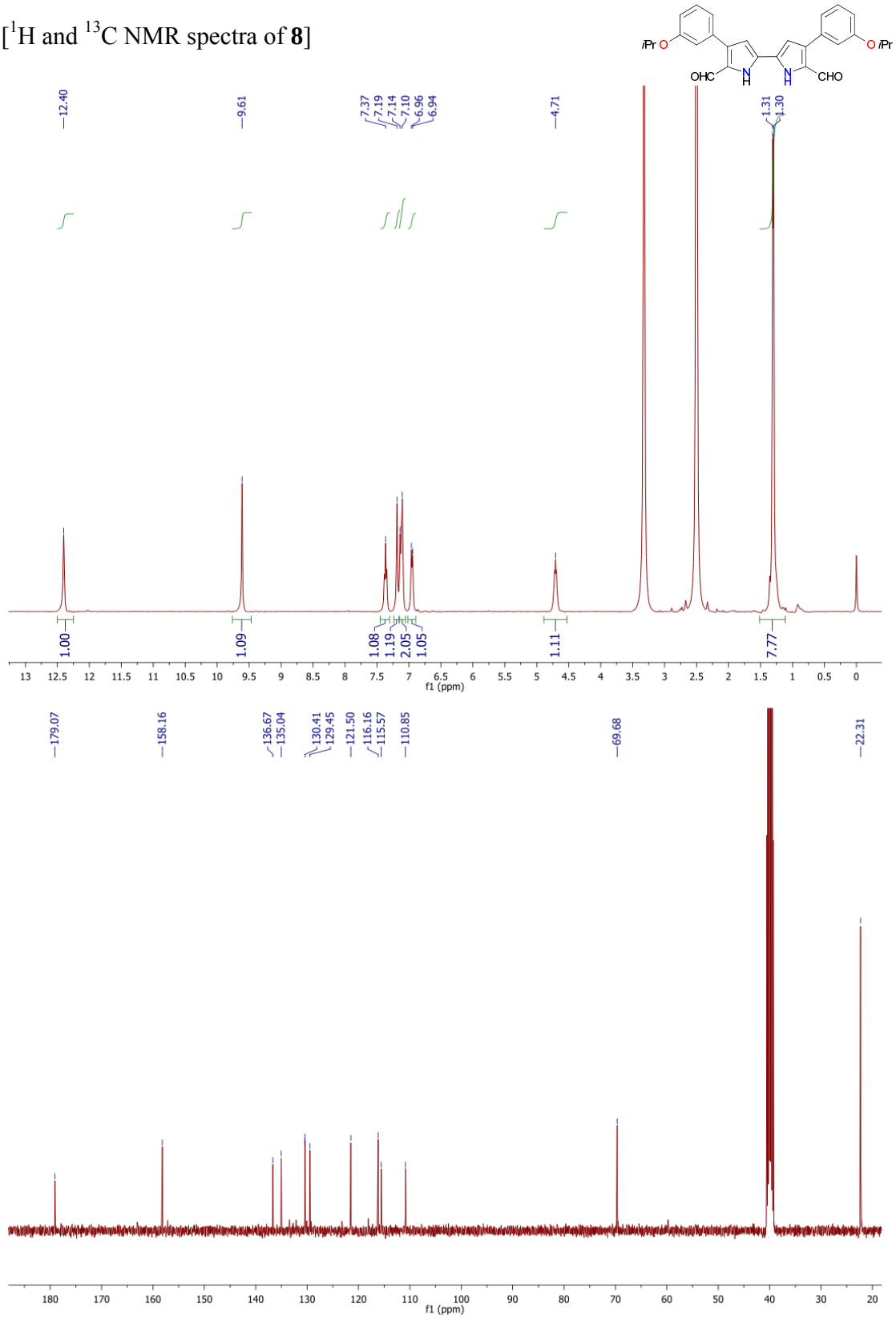
[^{1}H and ^{13}C NMR spectra of **7**]



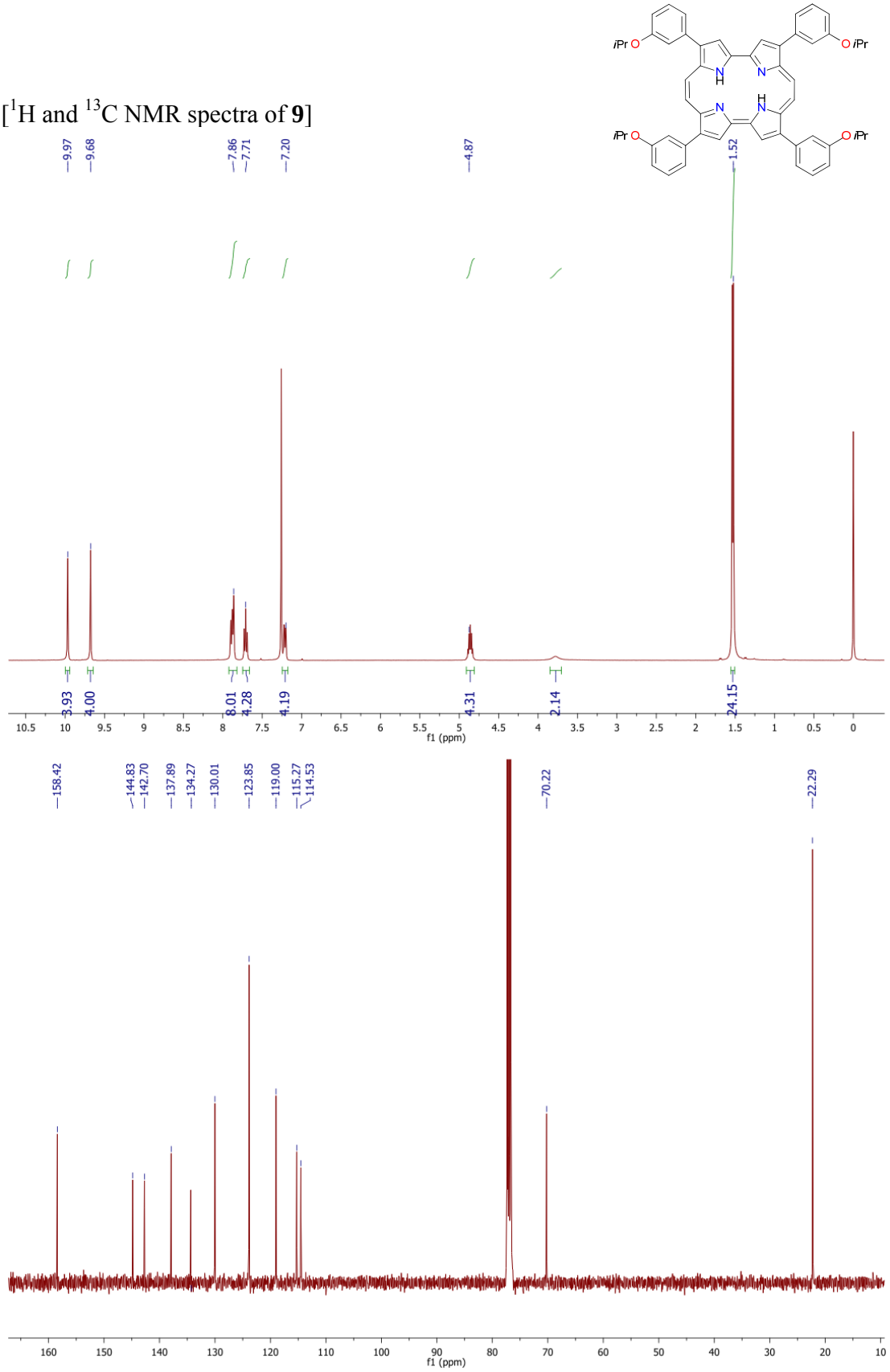




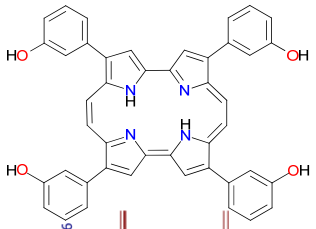
[^{1}H and ^{13}C NMR spectra of **8**]

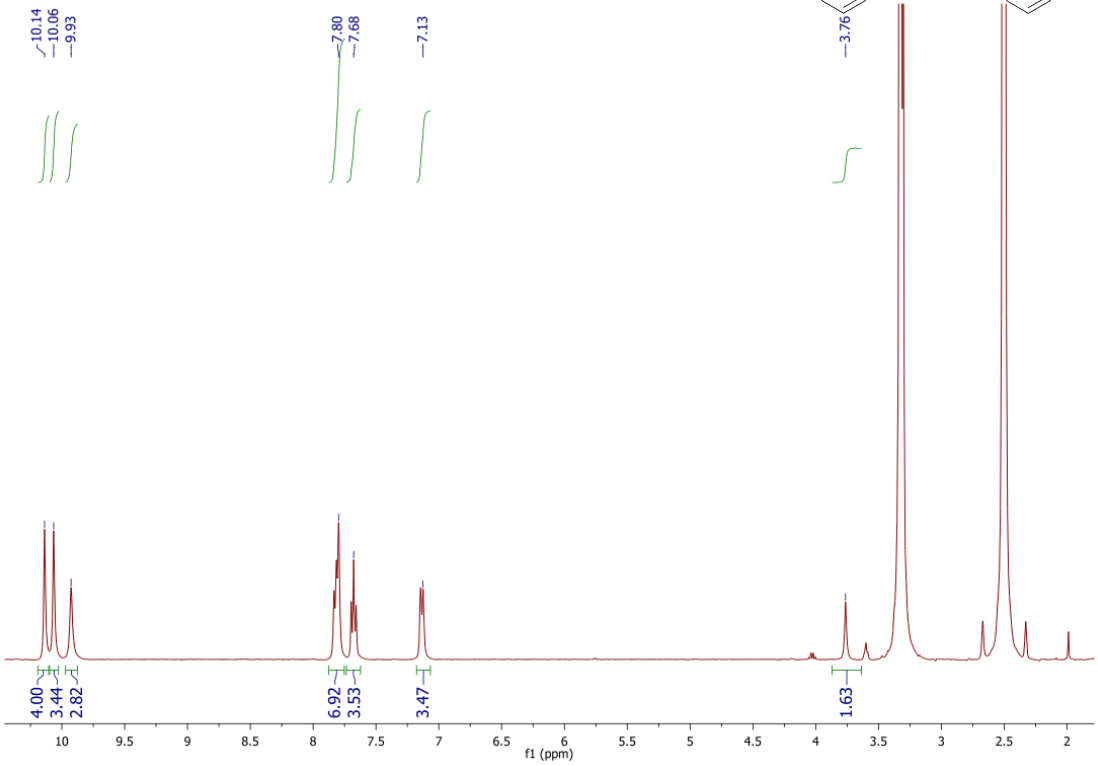


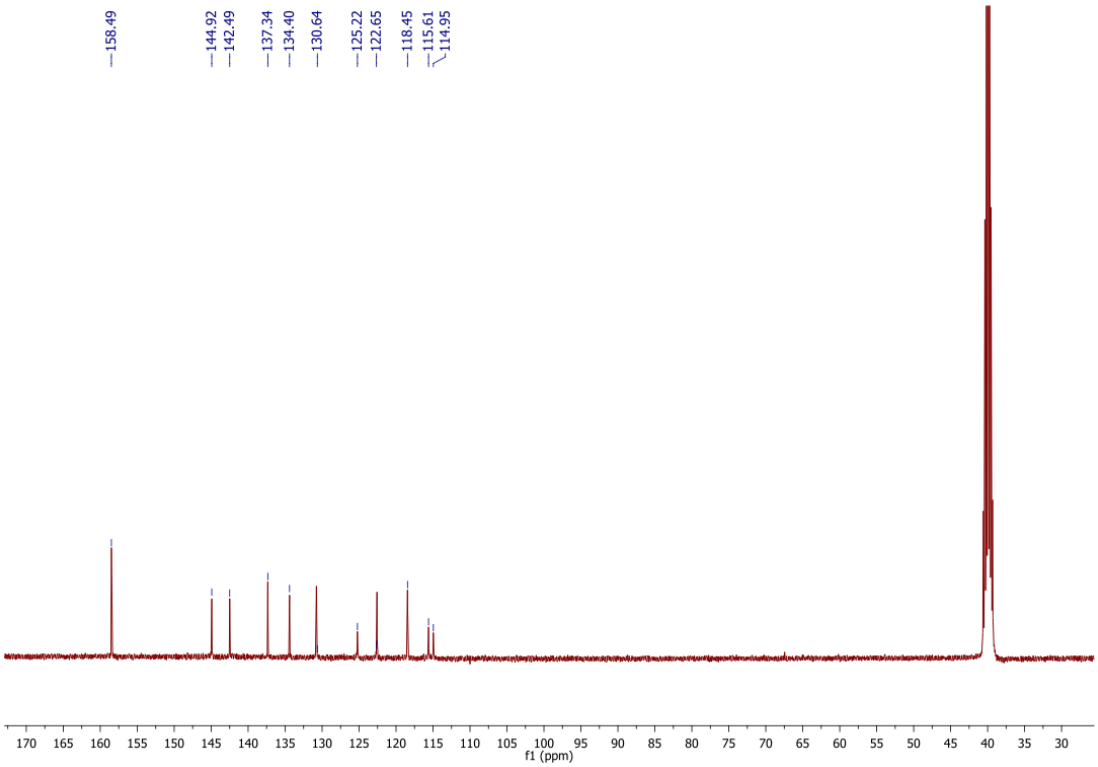
[^{1}H and ^{13}C NMR spectra of **9**]



[^{1}H and ^{13}C NMR spectra of **1**]







3. HPLC analysis of *m*-THPPo 1

Liquid Chromatography (HPLC) was performed with a HP 1090 series liquid chromatograph equipped with a diode array detector. *m*-THPPo was analyzed on a 30 mm x 4 mm, 3 μm particle, Lichrocart Purospher STAR RP-18E column. Detection was achieved at 375 nm. All chromatography runs were performed at room temperature with a mobile phase flow rate of 1.0 mL min^{-1}. Isocratic elution was performed with 77:23 ACN/H_2O.

As shown in Fig. S1, a majority peak can be observed at 5.4 min, with a relative integral intensity higher than 97%. The minority peaks observed at 10.0 and 14.3 min have a porphycene-like UV-Vis spectrum and they can be attributed to porphycene aggregates.

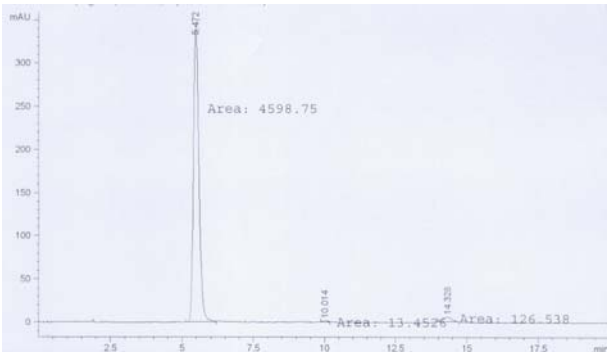


Fig. S1 Liquid chromatography of *m*-THPPo detected at 375 nm.

UV-Vis spectra were recorded every 1 s and found to be identical throughout the peak (Fig. S2).

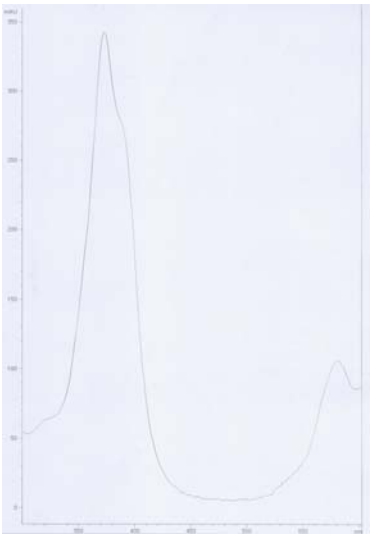


Fig. S2 Absorption spectrum of *m*-THPPo peak at 4.3 min obtained by liquid chromatography and diode array detection.

4. Physical and photophysical properties

General spectroscopic measurements.

Absorption spectra were recorded on a Cary 4E spectrophotometer (Varian, Palo Alto, CA). Fluorescence emission spectra were recorded in a Spex Fluoromax-2 spectrofluorometer (Horiba Jobin-Ybon, Edison, NJ). Fluorescence decays were recorded with a time-correlated single photon counting system (Fluotime 200, PicoQuant GmbH, Berlin, Germany) equipped with a red sensitive photomultiplier. Excitation was achieved by means of a 375 nm picosecond diode laser working at 10 MHz repetition rate. The counting frequency was always below 1%. Fluorescence lifetimes were analyzed using PicoQuant FluoFit 4.0 data analysis software. Transient absorption spectra were monitored by nanosecond laser flash photolysis using a Q-switched Nd-YAG laser (Surelite I-10, Continuum) with right-angle geometry and an analysing beam produced by a Xe lamp (PTI, 75 W) in combination with a dual-grating monochromator (mod. 101, PTI) coupled to a photomultiplier (Hamamatsu R928). Kinetic analysis of the individual transients was performed with software developed in our laboratory.

$^1O_2$ phosphorescence was detected by means of a customized PicoQuant Fluotime 200 system. $^1O_2$ signal amplitudes were determined by analysis of the data using the FluoFit 4.0 software. All spectroscopic measurements were carried out in 1-cm quartz cuvettes (Hellma, Germany) at room temperature.

Time-resolved fluorescence.

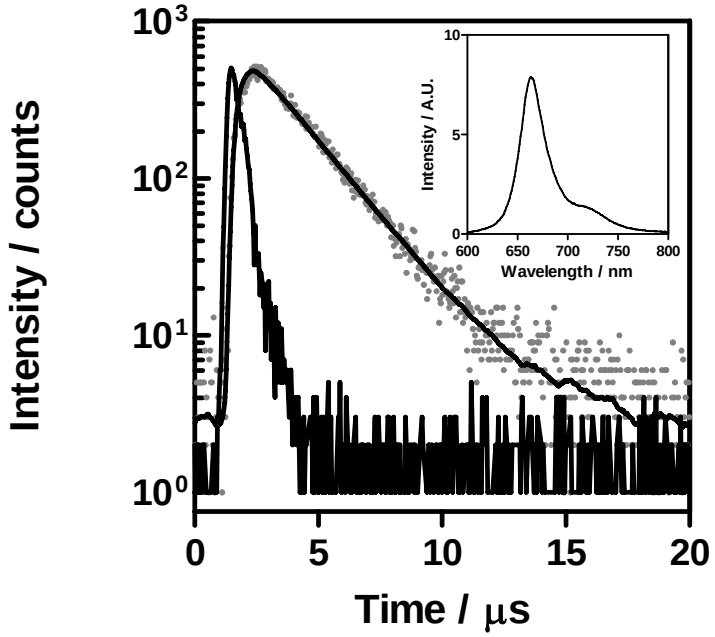


Fig. S3 Time-resolved fluorescence of *m*-THPPo in THF, λ_{exc} = 375 nm, λ_{obs} = 660 nm. Inset: fluorescence spectrum.

The fluorescence decay of *m*-THPPo could be fitted with a single exponential decay model. The singlet lifetime is 2.2 ± 0.05 ns.

Fluorescence quantum yield.

The fluorescence quantum yield, Φ_F, was determined by means of equation 1:

$$\phi_F(\text{sample}) = \frac{F_{\text{sample}} \cdot n^2_{\text{sample}}}{F_{\text{ref}} \cdot n^2_{\text{ref}}} \cdot \phi_F(\text{ref}) \qquad \text{Equation 1}$$

where F_i is the fluorescence intensity integrated over the entire emission spectrum corrected by the absorption factor ($1\text{-}10^{-A}$) and n_i is the refractive index of the solvent used in each case.

The absorption and emission spectra of optically-matched solutions at 532 nm of cresyl violet in MeOH as reference, and *m*-THPPo in THF are shown in Fig. S4.

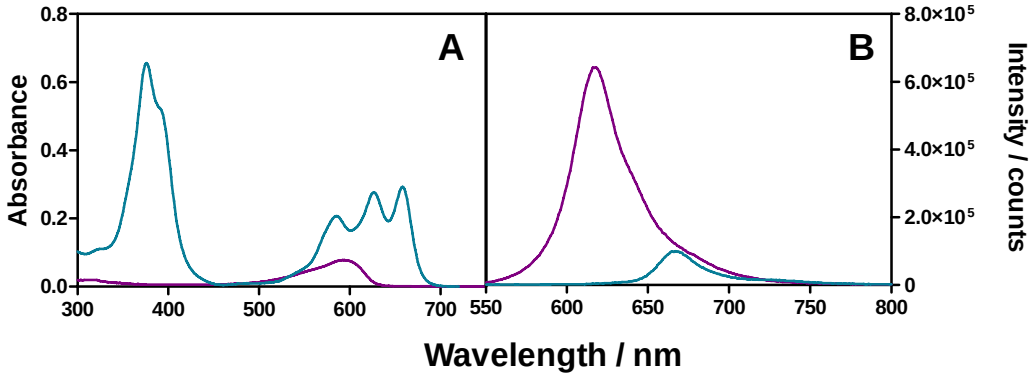


Fig. S4 (A) Absorption and (B) fluorescence spectra of cresyl violet in MeOH (violet) and *m*-THPPo in THF (blue).

Singlet oxygen quantum yield.

The quantum yield of singlet oxygen photosensitizations is defined as the number of photosensitized $^1O_2$ molecules per absorbed photon. The pre-exponential factor *S(0)*, which is proportional to Φ_Δ, was determined by fitting the equation 2 to the time-resolved phosphorescence intensity at 1270 nm.

$$S(t) = S(0) \cdot \frac{\tau_\Delta}{\tau_T - \tau_\Delta} \cdot \left(e^{-t/\tau_T} - e^{-t/\tau_\Delta} \right) \qquad \text{Equation 2}$$

The quantum yield of $^1O_2$ production was determined by comparison of *S(0)* to that produced by an optically matched reference in the same solvent and at the same excitation wavelength and intensity (Equation 3)

$$\phi_\Delta(\text{sample}) = \frac{S(0)_{\text{sample}}}{S(0)_{\text{ref}}} \cdot \phi_\Delta(\text{ref}) \qquad \text{Equation 3}$$

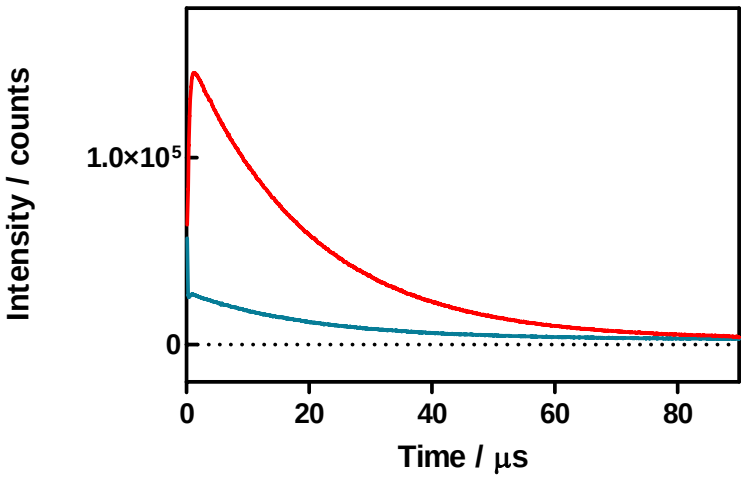


Fig. S5 Singlet oxygen phosphorescence kinetics at 1270 nm of TPP (red) and *m*-THPPo (blue) in THF.

Transient absorption spectra.

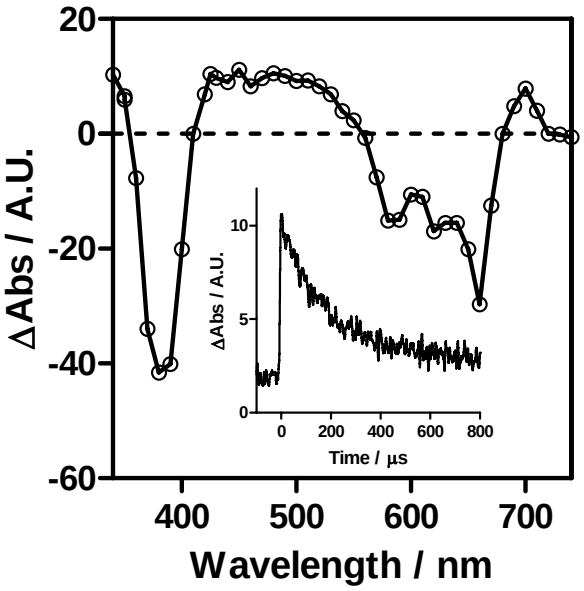


Fig. S6 Triplet-minus-singlet absorption spectrum of *m*-THPPo in argon-saturated acetone. Inset: Transient decay at 490 nm.

The triplet state of *m*-THPPo has a lifetime of 181 μs in acetone. The triplet-minus-singlet absorption spectrum shows its maximum at 490 nm. Monoexponential decay kinetics with the same lifetime were observed throughout the spectrum.

Oxygen quenching rate constant (k_{qT}^{O2})

The method is based on the Stern-Volmer relationship where τ_T is measured as a function of the quencher's concentration. The plot of τ_T^{-1} *vs* oxygen concentration yielded the quenching rate constant.

$$1/\tau_T = 1/\tau_T^0 + k_{qT}^{O_2} \cdot [O_2] \qquad \text{Equation 4}$$

As shown in Fig. S7, the rate constant for triplet decay ($1/\tau_T$) increases linearly with the concentration of oxygen, yielding a quenching rate constant of 2.1 x 10^9 M^{-1} s^{-1}.

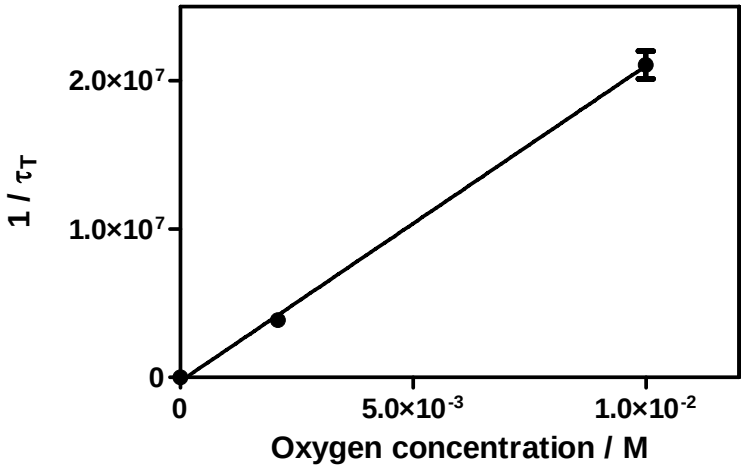


Fig. S7 Stern-Volmer plot of $1/\tau_T$ at different oxygen concentrations in THF.

5. Photobleaching study

Optically-matched solutions of *m*-THPC or *m*-THPPo in acetone were irradiated with a Q-switched Nd-YAG laser (Surelite I-10, Continuum) tuned to 532 nm. At intervals the cuvette was removed and the spectrum in the range of 450-800 was recorded in order to follow the course of photobleaching.

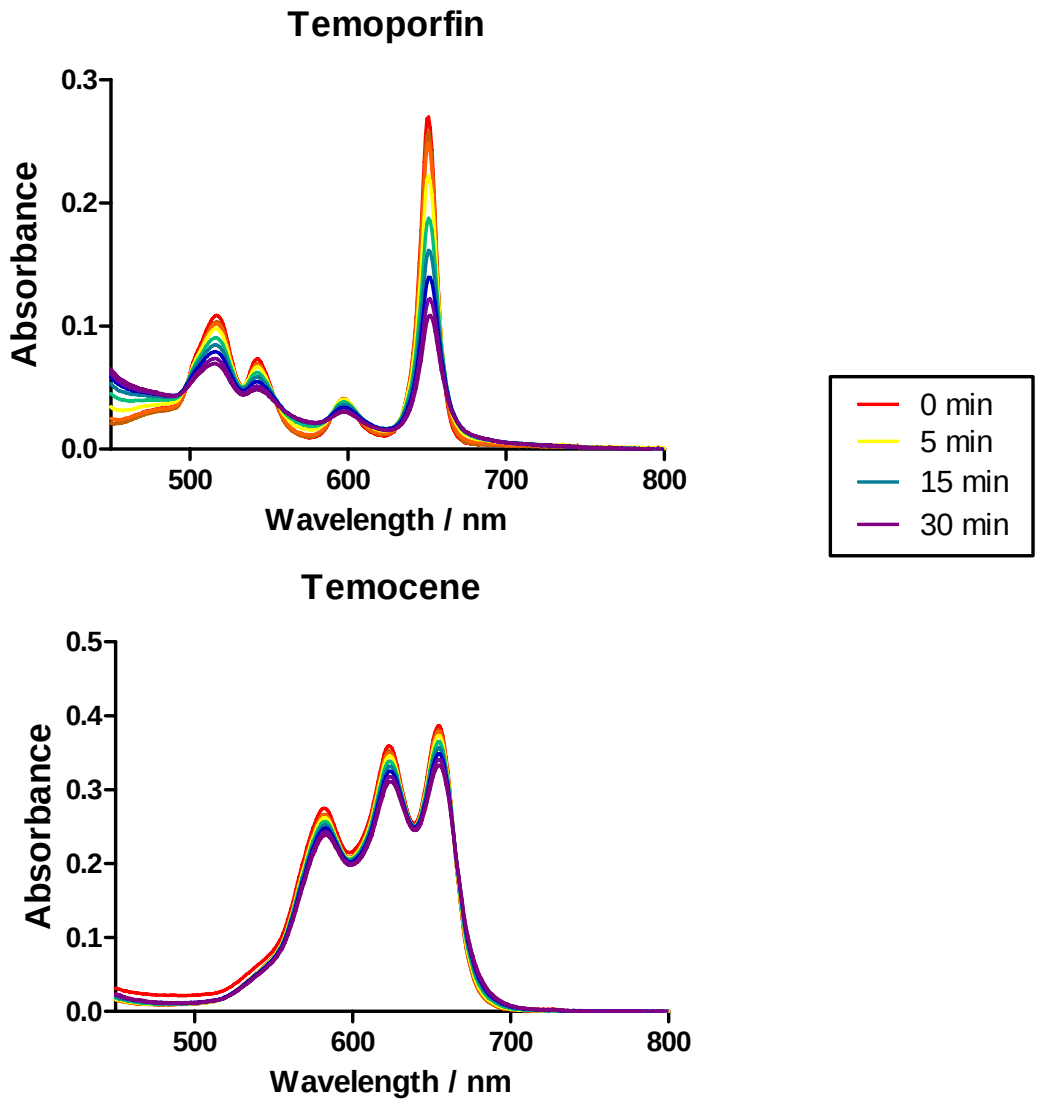


Fig. S8 Changes in the visible spectrum of *m*-THPC, temoporfin and *m*-THPPo, temocene in acetone (aerated solution) on irradiation at 532 nm.

6. Light dose and concentration dependence phototoxicity

HeLa cells were seeded in 24-well plates and cultured towards 80-85% confluence. They were then incubated in the dark at 37 ºC with serum-free DMEM containing 1-10 μM *m*-THPPo in DMSO. After 18 h incubation, cells were washed three times with PBS and replenish with fresh DMEM. Irradiation was carried out with Sorisa Photocare LED source with wavelength range of 620-645nm. The light intensity at the irradiation site was 24 mW·cm^{-2}, measured with a LaserStar Ophir power meter. Cells were irradiated for different light doses and then incubated for 24 h before the MTT assay for cell viability. Experiments were performed in quadruplicate.

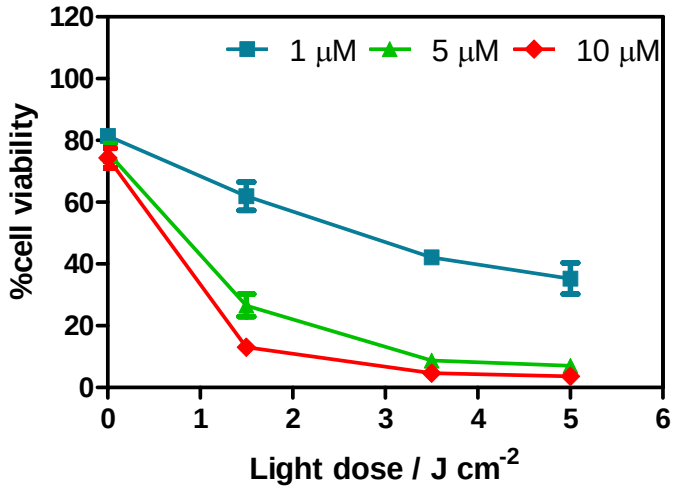


Fig. S9 Light dose and concentration dependence photodynamic induced cytotoxicity of *m*-THPPo at different concentrations. Mean ± SD of at least four independent experiments are shown.

7. Mitochondrial localization

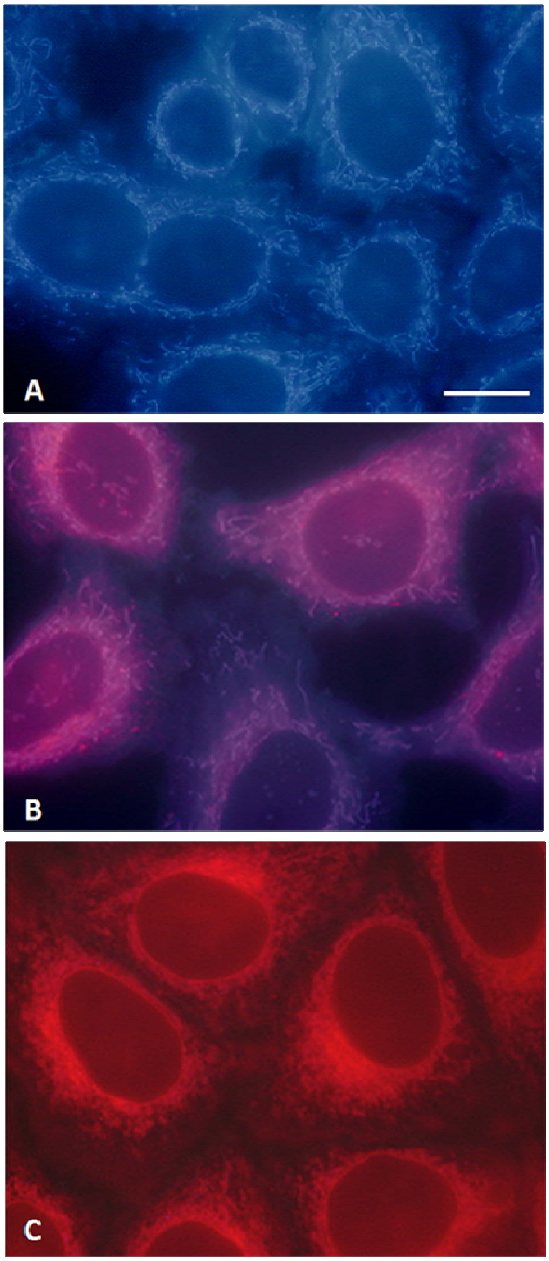


Fig. S9 Fluorescence microscopy images of living HeLa cells incubated 18 h with different concentrations of DMSO-loaded *m*-THPPo and observed under ultraviolet (365 nm) excitation. (A) Control cells. (B) and (C) 1 μM and 10 μM *m*-THPPo, respectively. Scale bar: 20 μm.