Platinum(II) ring-fused Chlorins as Near-infrared Emitting Oxygen Sensors and Photodynamic Agents

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Supporting Information

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1. Experimental Section

1.1 General

^{1}H NMR spectra were recorded on a Bruker Avance III spectrometer operating at 400 MHz and ^{13}C NMR spectra were recorded on same instrument operating at 100 MHz. Tetramethylsilane (TMS) was used as internal standard. Chemical shifts are expressed in parts per million related to TMS, and coupling constants (J) are in Hertz. HRMS spectra were obtained on a VG Autospect M spectrometer (TOF MS ESI). TLC analyses were carried out on Merck Silica gel 60 F254 plates and flash column chromatography was performed with silica gel 60 as the stationary phase.

Absorption and emission spectra (fluorescence and phosphorescence) were recorded at room temperature with a Shimadzu UV-2100 spectrophotometer and a Horiba-Jobin–Yvon-SPEX Fluorolog spectrofluorometer (equipped with a pulsed lamp for phosphorescence measurements), respectively. Fluorescence excitation spectra of the chlorins were in good agreement with the corresponding absorption spectra. The time-resolved phosphorescence spectra were obtained in an adapted nanosecond transient absorption spectrometer setup (ns-TA) operating in emission mode. This consists of an Applied Photophysics laser flash photolysis equipment pumped by a Nd:YAG (Spectra Physics) laser with excitation wavelength at 532 nm and coupled to a Hamamatsu R5509-42 photomultiplier cooled to 193 K in a liquid nitrogen chamber (Products for Research model PC176TSCE-005) as detector.

Room temperature fluorescence and phosphorescence quantum yields were obtained using TPP in toluene (ϕ_F=0.11)[1] as standard. In this case the room temperature emission spectra of the Pt(II)-chlorin derivatives display an unstructured long wavelength phosphorescence emission band in addition to the blue-shifted characteristic fluorescence emission band of the non-substituted chlorin (see Figures S4-5 and S7). Thus, due to the sensitivity of the phosphorescence emission band to oxygen, which results in a strong overlap between the phosphorescence and the fluorescence emission bands when the concentration of O_2 in solution is reduced, the fluorescence quantum yields for chlorins 5 and 6 were measured in oxygen saturated toluene solutions, [O_2]= 8.63 mmol L^{-1} at 20ºC [2]. Although it was not possible to fully quench the phosphorescence emission (due to the solubility limit of O_2 in the solvent) the overlap between the phosphorescence emission and the fluorescence emission spectra was greatly reduced. Moreover in this case the vibrational progression of the fluorescence emission spectra closely resembles that observed for the metal free chlorins derivatives (namely the ratio between the first and second vibronic bands). Thus due to the reduced overlap between the fluorescence and phosphorescence emissions bands it was possible to obtain the fluorescence and phosphorescence quantum yields in the O_2 saturated solutions (by integrating each spectroscopic feature and comparing to that of the optically matched reference compound, TTP). Additionally, the phosphorescence quantum yields were also obtained using the time-resolved phosphorescence data obtained in the ns-TA setup. In this case the integrated area of the decays at the maxima emission collected in the presence and absence of O_2 were compared to those of the reference compound tris(2,2'-bipyridyl)ruthenium (II) in water (ϕ_F = 0.042), see Figures S7C and S7D.[3]

The room-temperature singlet oxygen phosphorescence was detected at 1270 nm using an Horiba-Jobin-Ivon SPEX Fluorog 3-22 equipped with a Hamamatsu R5509-42 photomultiplier cooled to 193 K in a liquid nitrogen chamber (Products for Research model PC176TSCE-005). The use of a Schott RG1000 filter was essential to eliminate from the infrared signal all of the first harmonic contribution of the sensitizer emission in the region below 850 nm. The sensitized phosphorescence emission spectra of singlet oxygen from optically matched solutions of the samples and that of reference were obtained in identical experimental conditions (see Figure S9). The singlet oxygen formation quantum yield was then determined by comparing the integrated area under the sensitized emission spectra of singlet oxygen of the sample solutions ($\int I(\lambda)^{cp} d\lambda$) and that of the reference solution ($\int I(\lambda)^{ref} d\lambda$) and applying equation 1,

$$\phi_{\Delta}^{cp} = \frac{\int I(\lambda)^{cp} d\lambda}{\int I(\lambda)^{ref} d\lambda} \cdot \phi_{\Delta}^{ref} \qquad (1)$$

with ϕ_{Δ}^{ref} the singlet oxygen formation quantum yield of the reference compound. Phenazine in benzene solution, ϕ_{Δ}= 0.83, was used as standard [4].

1.2 Synthesis

1.2.1. Platinum (II) 2^2,2^3-bis(methoxycarbonyl)-5,10,15,20-tetraphenyl-2,2^1,2^6,3-tetrahydropyrazolo[1',5':1,6]pyrido[3,4-*b*]chlorin (**5**)

A solution of 2,2-dioxo-1*H*,3*H*-pyrazolo[1,5-*c*][1,3]thiazole-6,7-dicarboxylate [29] (**3** 19 mg, 0.07 mmol) and Pt(II) porphyrin **1** [18] (113 mg, 0.14 mmol, 2 equiv.) in 1,2,4-trichlorobenzene (2 mL) was flushed with argon, and irradiated in a microwave reactor (CEM Focused Synthesis System, Discover S-Class) with the temperature set to 250 °C for 20 min. After cooling to room temperature, some drops of triethylamine were added, and the mixture was purified by flash chromatography (CH_2Cl_2/ethyl acetate, 95:5, v/v) to afford unreacted Pt(II) porphyrin **1** (56 mg, 50%) and Pt(II) chlorin **5** (14 mg, 0.014 mmol, 20%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.41 (s, 2H, β-H pyrrolic), 8.38-8.36 (m, 2H, β-H pyrrolic), 8.16-8.13 (m, 2H, β-H pyrrolic), 8.11-7.94 (m, 7H, Ar), 7.82-7.76 (m, 3H, Ar), 7.71-7.60 (m, 10H, Ar), 5.78-5.71 (m, 1H, reduced β-H pyrrolic), 5.39-5.32 (m, 1H, reduced β-H pyrrolic), 4.33 (dd, J = 13.5, 7.7 Hz, 1H, CH_2 ring-fused), 3.93 (dd, J = 13.5, 9.8 Hz, 1H, CH_2 ring-fused), 3.88 (s, 3H, CO_2Me), 3.74 (s, 3H, CO_2Me), 3.58 (dd, J = 15.8, 6.4 Hz, 1H, CH_2 ring-fused), 2.64 (dd, J = 15.8, 10.4 Hz, 1H, CH_2 ring-fused) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.6, 162.2, 150.7, 148.4, 146.4, 146.2, 143.3, 143.0, 140.8, 140.6, 140.3, 138.4, 138.2, 135.9, 135.7, 134.5, 134.1, 133.3, 133.2, 132.3, 132.3, 131.6, 131.5, 128.9, 128.7, 128.6, 128.3, 128.1, 128.0, 127.7, 127.6, 127.5, 127.0, 126.9, 126.6, 126.1, 126.0, 113.5, 113.3, 110.7, 52.5, 51.6, 49.2, 45.6, 43.4, 26.2 ppm. ESI-HRMS found 1017.2585, calcd. 1017.2601 ($C_{53}H_{38}N_6O_4Pt$ M^+).

1.2.2. Platinum (II) 2^2,2^3-bis(hydroxymethyl)-5,10,15,20-tetraphenyl-2,2^1,2^6,3-tetrahydropyrazolo[1',5':1,6]pyrido[3,4-*b*]chlorin (**6**)

A solution of Pt(II) chlorin **5** (36 mg, 0.035 mmol) in dry dichloromethane (3 mL) was added dropwise to a stirred suspension of $LiAlH_4$ (16 mg, 0.421 mmol, 12 equiv.) in dry diethyl ether (3 mL), at 0 °C. After addition was complete, the mixture was heated to 50 °C and was left stirring at this temperature for 4 hours. The reaction mixture was cooled to 0 °C (ice bath) and quenched over a 30 min period with ethyl acetate (30 mL), water (10 mL) and aqueous 10% sodium hydroxide (0.2 mL). Then the reaction mixture was warmed to room temperature and left stirring for 16 hours. The aqueous phase was extracted with ethyl acetate (30 mL). Combined organic extracts were dried (Na_2SO_4), reduced in volume, and the product was purified by flash chromatography (ethyl acetate) to afford Pt(II) chlorin **6** (20 mg, 0.021 mmol, 59%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.40 (s, 2H, β-H pyrrolic), 8.38-8.35 (m, 2H, β-H pyrrolic), 8.17-8.16 (m, 2H, β-H pyrrolic), 8.10-8.08 (m, 1H, Ar), 8.06-7.93 (m, 6H, Ar), 7.82-7.80 (m, 1H, Ar), 7.76-7.58 (m, 12H, Ar), 5.72-5.65 (m, 1H, reduced β-H pyrrolic), 5.35-5.29 (m, 1H, reduced β-H pyrrolic), 4.52 (s, 2H, CH_2OH), 4.22 (d, J = 12.6 Hz, 1H, CH_2OH), 4.15 (d, J = 12.6 Hz, 1H, CH_2OH), 4.10 (dd, J = 13.3, 7.8 Hz, 1H, CH_2 ring-fused), 3.89 (dd, J = 13.3, 9.5 Hz, 1H, CH_2 ring-fused), 2.91 (dd, J = 15.2, 6.3 Hz, 1H, CH_2 ring-fused), 2.47 (dd, J = 15.2, 10.2 Hz, 1H, CH_2 ring-fused) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 151.4, 149.5, 149.3, 146.2, 146.1, 140.8, 140.5, 138.2, 138.1, 137.8, 135.8, 135.6, 134.7, 134.3, 133.2, 132.2, 132.1, 131.6, 131.5, 128.7, 128.6, 128.5, 128.1, 127.9, 127.6, 127.4, 127.3, 127.0, 126.6, 126.4, 126.0, 115.8, 113.5, 57.9, 54.2, 53.4, 48.5, 46.1, 44.3, 25.0 ppm. ESI-HRMS found 961.2691, calcd. 961.2702 ($C_{51}H_{38}N_6O_2Pt$ M^+).

1.3 Biology

1.3.1. Cell culture conditions

The A375 (CRL1619) human melanoma cell line was purchased from American Type Culture Collection and cultured according to standard procedures using the Dulbecco's Modified Eagle medium (Sigma–Aldrich, Inc; Sigma D-5648) supplemented with 10% heat-inactivated fetal bovine serum (Gibco Invitrogen Life Technologies; Gibco 2010-04), 1% Penicillin–Streptomycin (Gibco Invitrogen Life Technologies; 100 U/mL penicillin and 10 µg/mL streptomycin – Gibco 15140-122) and 100 µM sodium pyruvate (Gibco Invitrogen Life Technologies; Gibco 1360) at 37 ºC, in a humidified incubator with 95% air and 5% CO_2. For all studies, cells were detached using a solution of 0.25% trypsin-EDTA (Gibco).

1.3.2. Photodynamic activity

For each experiment, A375 cells were plated and kept in the incubator overnight, in order to allow the attachment of the cells. The initial formulation of the sensitizers consisted of a 1 mg/mL solution in DMSO and the desired concentrations were achieved by successive dilutions, being always administered to the cells at 1% DMSO. After sensitizer administration (from 1 nM to 10 µM), cells were incubated for 24 h. Controls were performed on every test. Cells were washed with PBS and new drug-free medium was added. Each plate was irradiated with a fluence rate of 7.5 mW/cm^2 until a total of 10 J was reached, using a light source equipped with a red filter (cut off < 560 nm).

For the evaluation of photocytotoxicity a MTT assay was performed. Cell culture plates were washed and incubated with a solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg/mL, Sigma) in PBS, pH 7.4, in the dark at 37 °C for 4

hours. To solubilize formazan crystals, a 0.04 M solution of hydrochloric acid in isopropanol was added. Absorbance was measured using a Biotek Synergy HT Plate Reader. Cytotoxicity was expressed as the inhibition percentage of cultures subjected to PDT, correlated with cultures only treated with the vehicle administration of sensitizers. This procedure allowed us to establish dose-response curves, obtained using Origin 8.0, and to calculate the concentration of sensitizers that inhibits the proliferation of cultures in 50% (IC_{50}). Each experiment was performed in duplicate and repeated in three sets of tests.

1.3.3. Cytotoxicity/ Viability

The determination of cellular adenosine triphosphate (ATP) was performed using A375 cells grown in black 96-well plates. Cell viability was assessed with ATPlite one-step assay kit (Perkin Elmer, Inc) according to the manufacturer's instructions using an EnSpire Multimode Plate Reader.

1.3.4. Luminescence response to O_2 Concentration

Luminescence response to O_2 of micellar solution of chlorin **6** were performed using an IVIS Lumina XR optical imaging system equipped with a 150 W Tungsten/Halogen lamp and appropriate filters (Caliper LifeSciences, Hopkinton, MA, USA) excitation/emission pairs as follows: λexc = 405 nm λem = 575−650 nm; λem = 695−770 nm. The solutions were saturated with O_2/N_2 mixtures with $\%O_2$ = 21, 12, 8, 5.5, 3 and 0.

2. ^{1}H and ^{13}C NMR spectra of chlorins 5 and 6

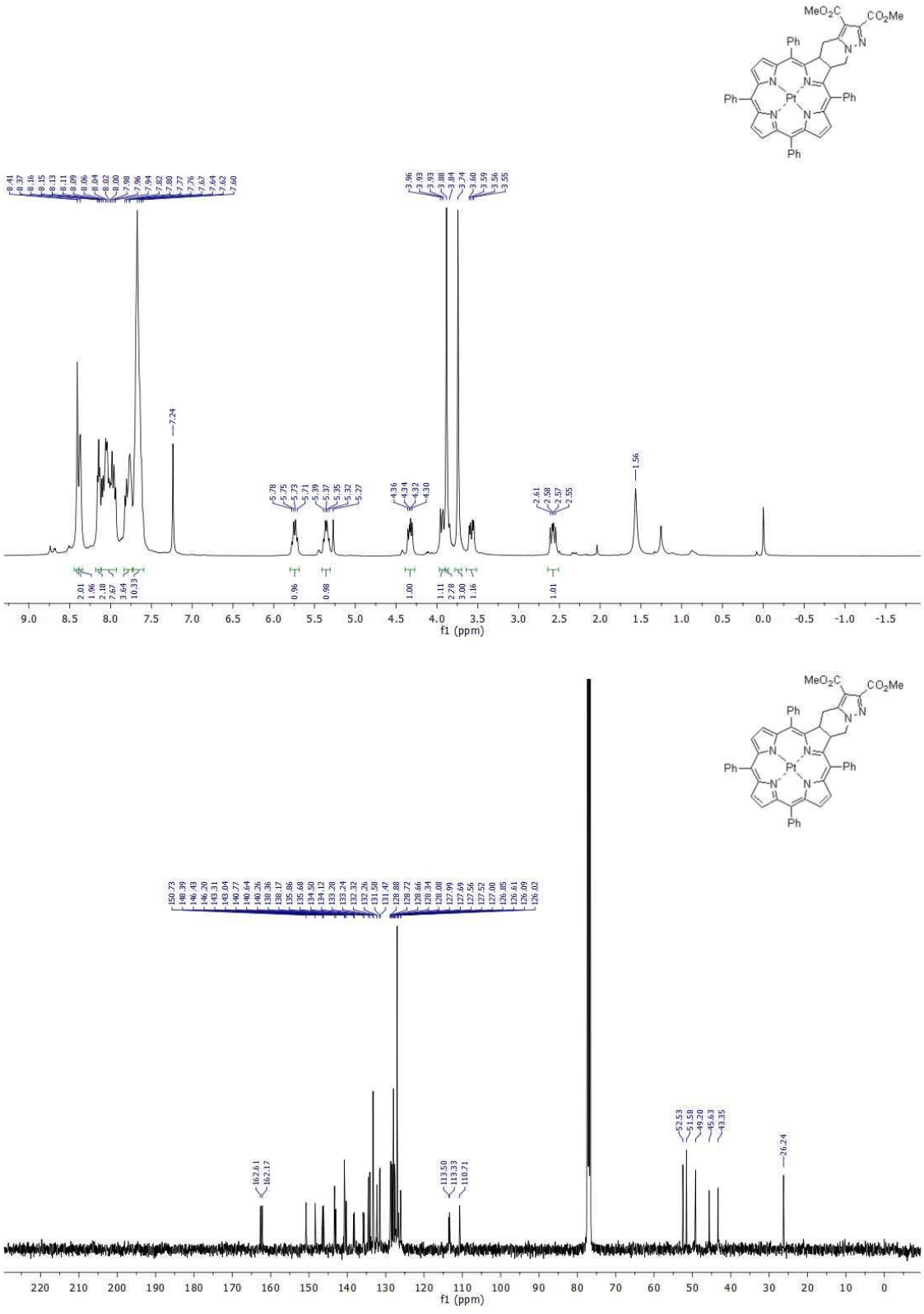

Fig. S1. ^{1}H NMR and ^{13}C NMR spectra of chlorin **5** in $CDCl_3$.

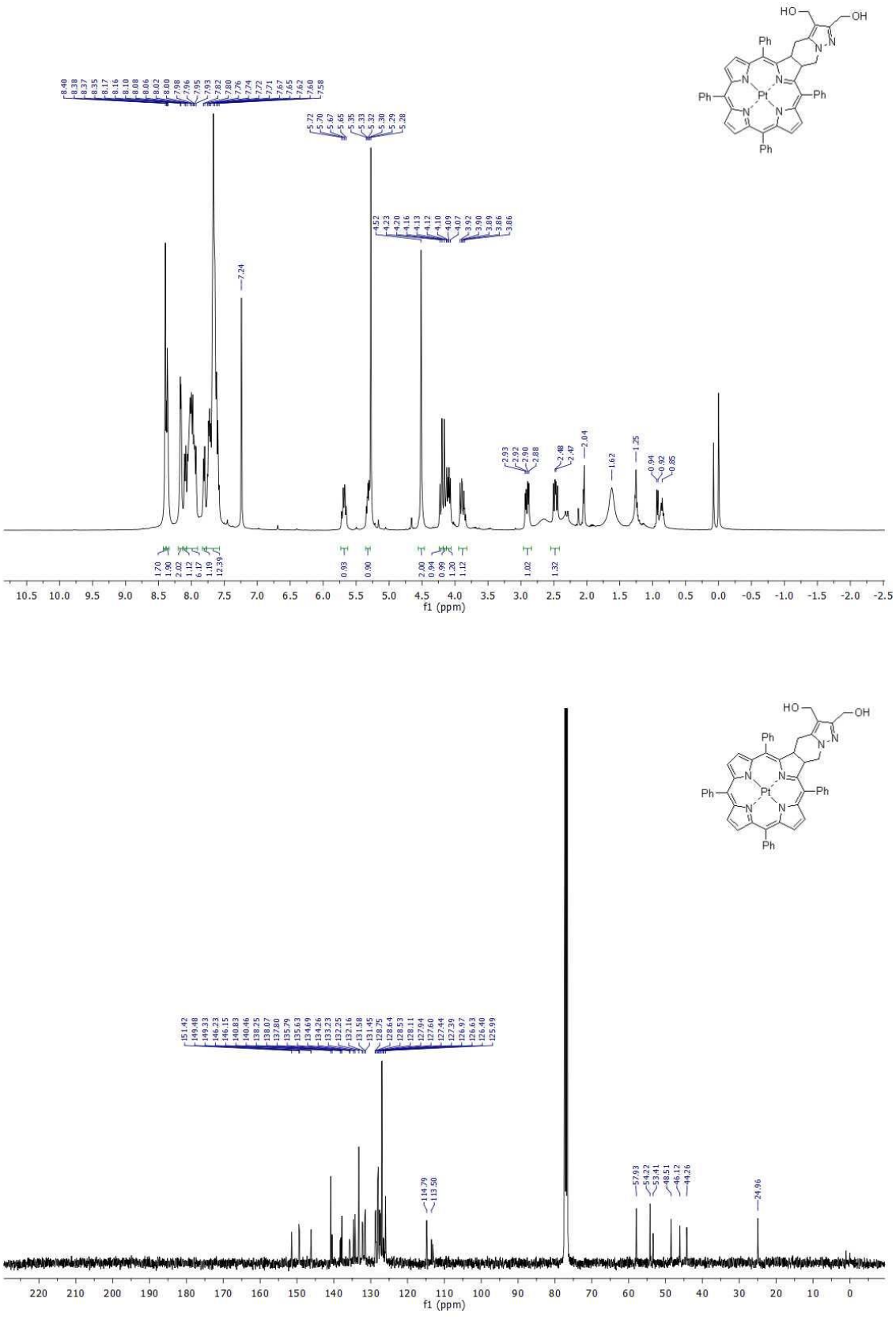

Fig. S2. ^{1}H NMR and ^{13}C NMR spectra of chlorin **6** in $CDCl_3$.

3. Absorption and fluorescence emission spectra for 5,10,15,20-tetraphenylporphyrin (TPP) and Pt(II) complex

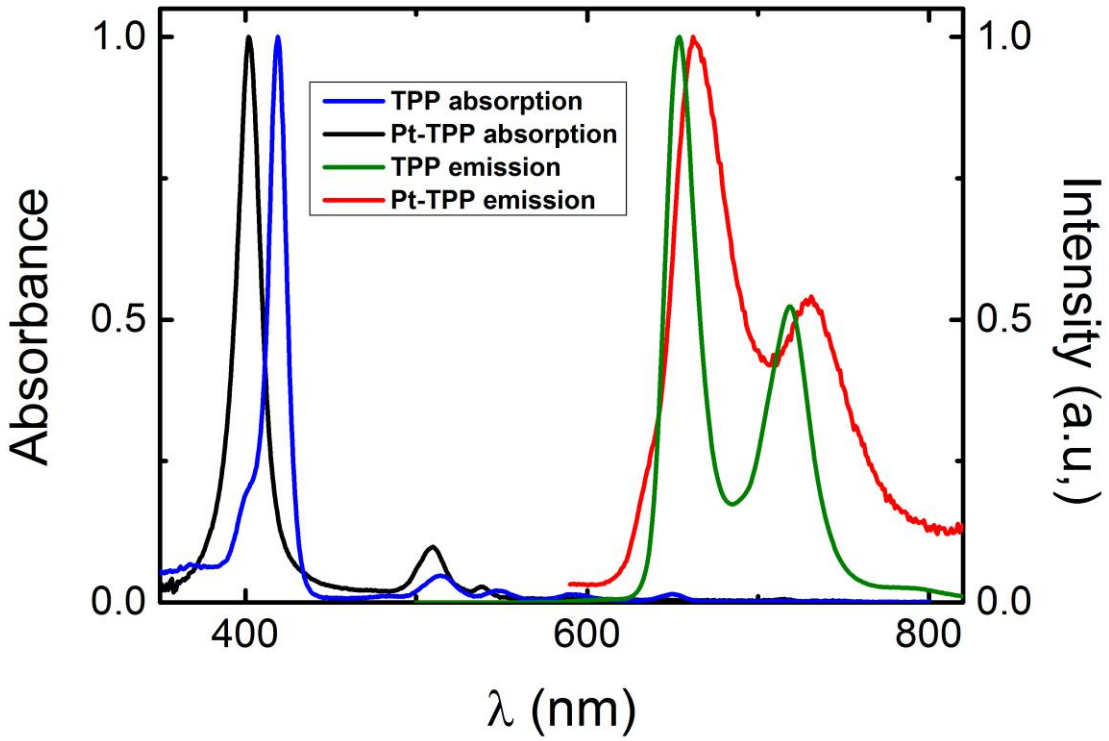

Fig. S3. Room temperature normalized absorption and fluorescence emission spectra for 5,10,15,20-tetraphenylporphyrin (TPP) and their Pt(II) complex (Pt-TPP) in aerated toluene solutions.

4. Luminescence spectra of chlorin 5 and 6 (in the absence and presence of oxygen)

The following luminescence spectra were recorded on a Horiba-Jobin-Ivon Fluorog 3-22 spectrometer (Fig. S4). Phosphorescence measurements (phosphorescence emission and decays) were made at room temperature using the Horiba-Jobin-Ivon Fluorog 3-22 spectrometer in phosphorescence mode (using a pulsed excitation source), see Fig. S5 and S6 respectively.

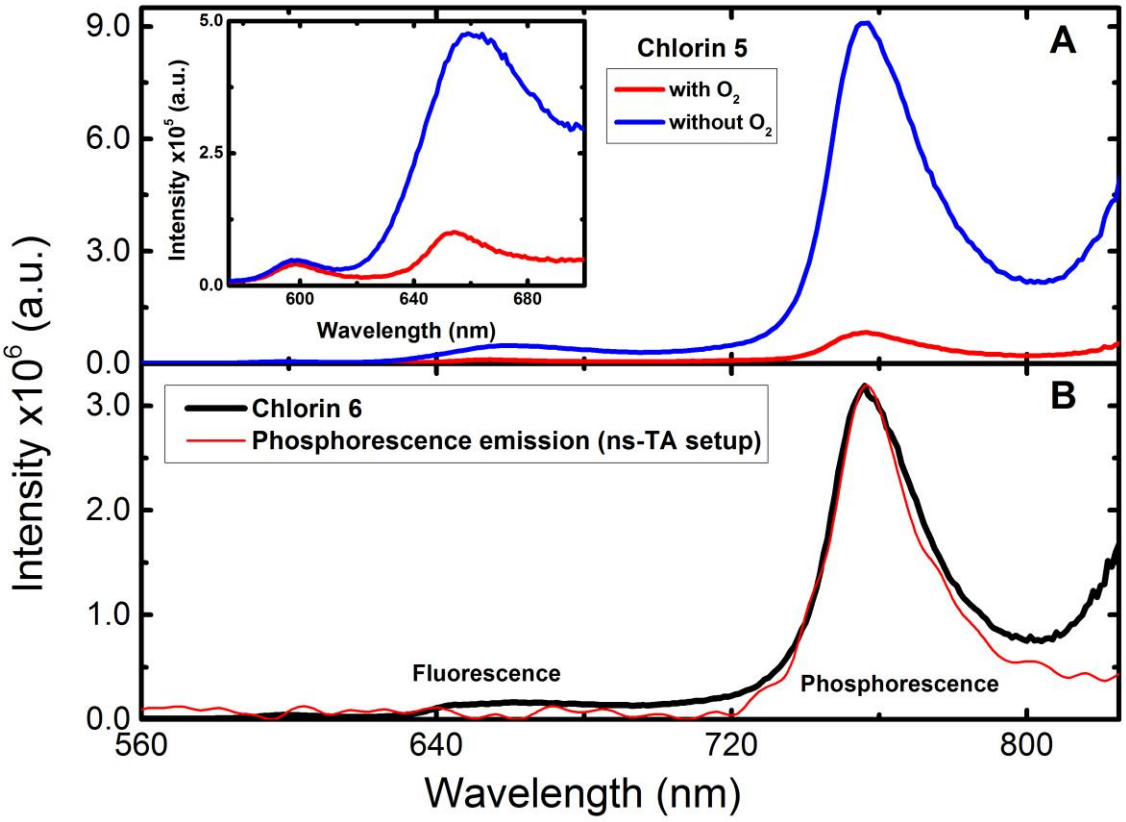

Fig. S4. (A) Room temperature luminescence spectra (λ_{exc} = 410 nm) for Pt(II)-chlorin **5** in toluene solution in the presence (air saturated solution) and absence of O2 (after deoxygenation by bubbling N_2 for 20 min). Also shown as inset is a magnified view of the fluorescence emission bands present in the 575-700 nm range; (B) Room temperature luminescence spectra for Pt(II)-chlorin **6** collected with λ_{exc}= 413 nm in nitrogen saturated toluene solution, together with the normalized phosphorescence band obtained in the ns-TA setup for comparison purposes.

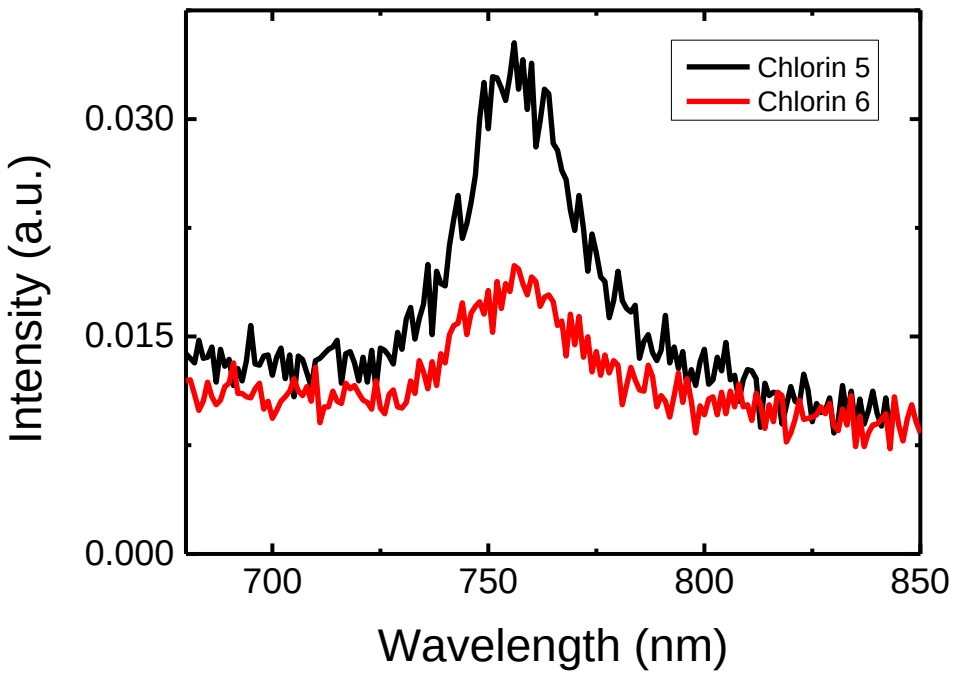

Fig. S5. Room temperature phosphorescence emission spectra for Pt(II)-chlorin 5 and 6 in nitrogen saturated toluene solution collected with λ_{exc}= 415 nm and delay after flash of 5 μs.

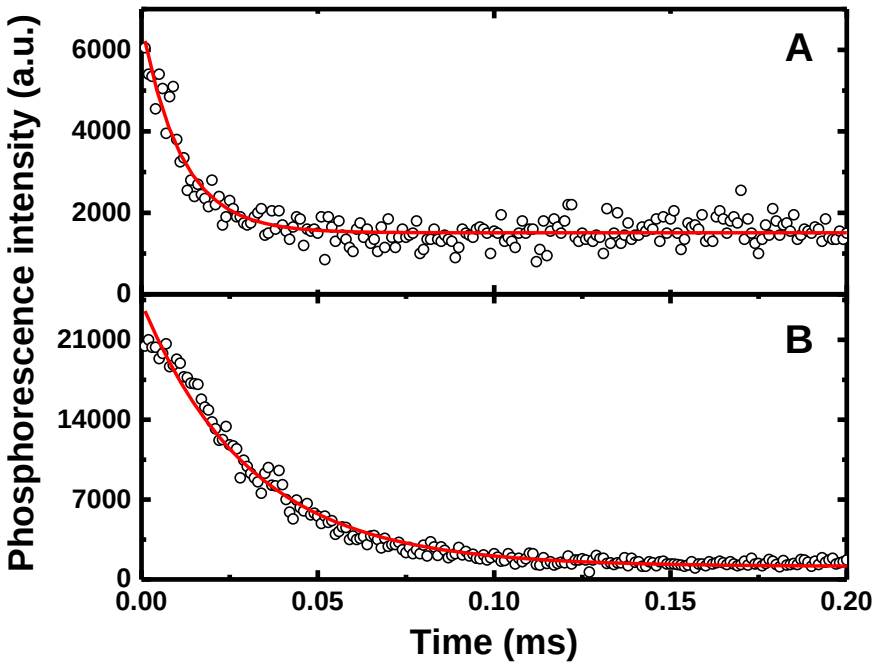

Fig. S6. Phosphorescence decays of the Pt(II)-chlorin **6** in DMSO:Tween80:H2O (2:2:96, v/v/v) solution in the presence (A) and in the absence of oxygen (B). The decay data analysis was performed with a single exponential fit, giving a phosphorescence lifetime of 11.3 and 30.9 μs respectively.

The time resolved phosphorescence spectra for chlorins **5** and **6** were also obtained in a nanosecond transient absorption setup (ns-TA, operating in emission mode) equipped with an Hamamatsu R5509-42 photomultiplier cooled to 193 K in a liquid nitrogen chamber (Products for Research model PC176TSCE-005).

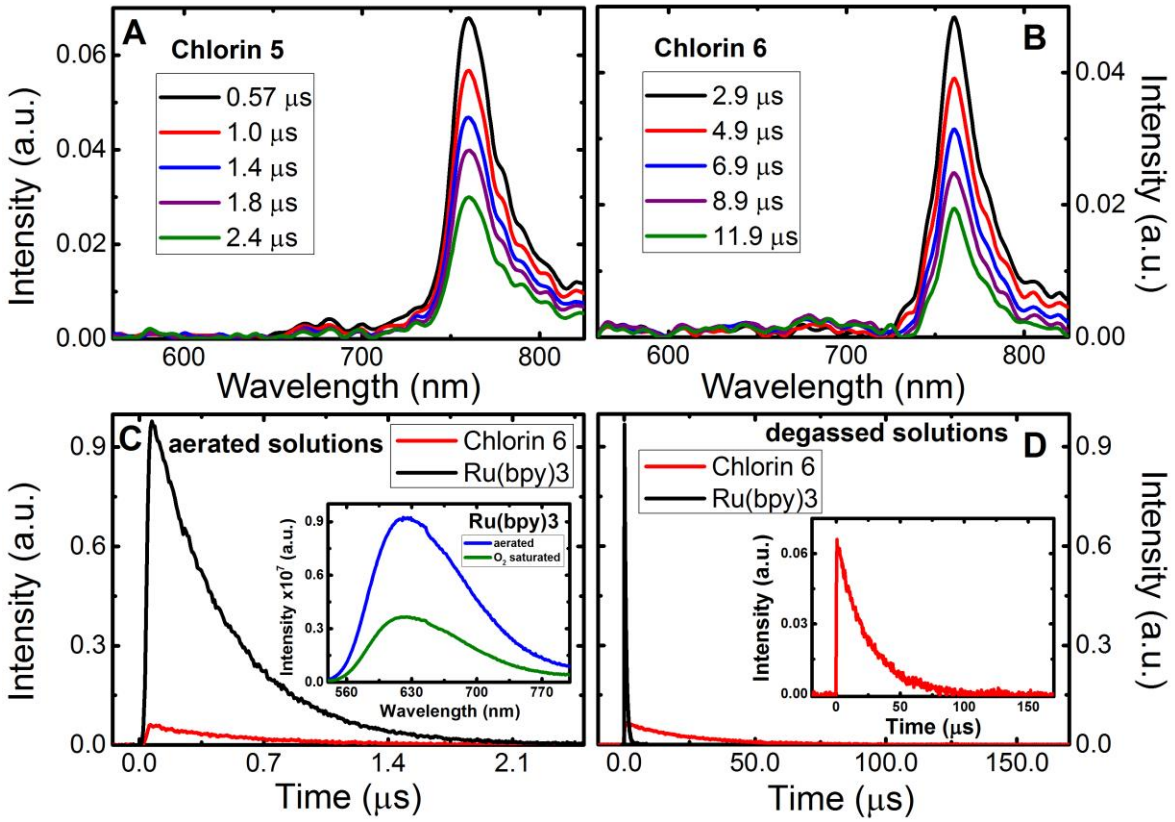

Fig. S7. (A, B) Room temperature time-resolved phosphorescence spectrum for chlorins **5** and **6** in degassed toluene solutions obtained in the ns-TA setup; together with the (C, D) phosphorescence decays for chlorin 6 in toluene and tris(2,2'-bipyridyl)ruthenium (II), Ru(by)3, in water, collected at λ_{em}= 755 nm and 620 nm, respectively, in air equilibrated and degassed solutions (after bubbling with N_2 for 30 min); Also shown as inset in (C) are the room temperature luminescence spectra for Ru(bpy)3 in water collect in aerated and O_2 saturated solution (by bubbling O_2 for 3 hours).

5. Thermal stability of chlorins 5 and 6

The thermal stability of chlorins **5** and **6** was evaluated by isotermal thermogravimetric analysis of the samples at 60 ^{o}C during 3 hours. Negligible mass loss (less than 1%) was observed for the two studied compounds.

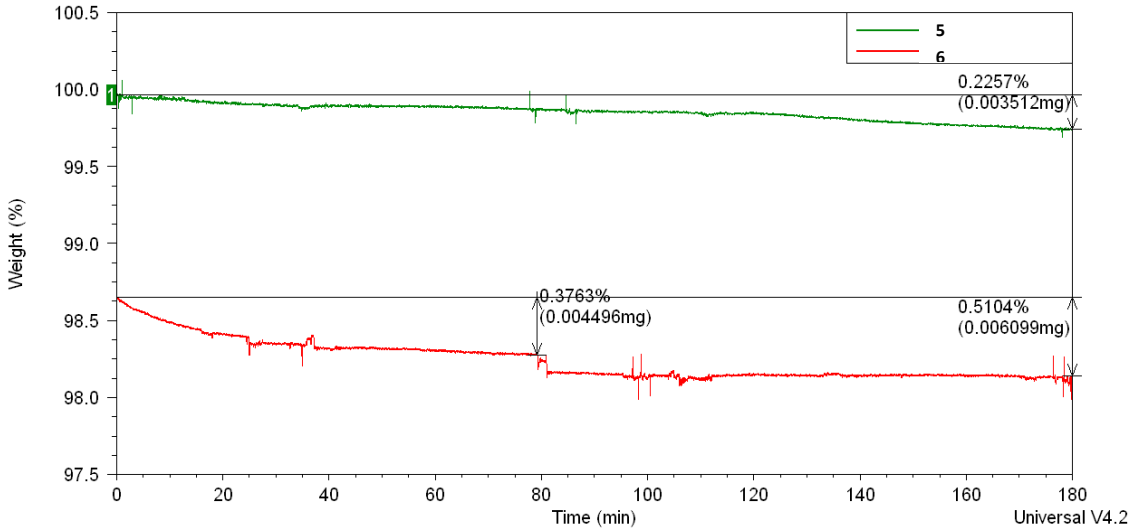

Fig. S8. Isotermal thermogravimetric analysis of chlorin **5** (green) and chlorin **6** (red).

6. Singlet oxygen formation upon photolysis of chlorin 6

Singlet oxygen formation on photolysis of aerated solution of chlorin **6** was demonstrated by its near infrared phosphorescence around 1270 nm. Phenazine was used as the standard for the determination of singlet oxygen formation quantum yields (ϕ_Δ), Figure S8 [27].

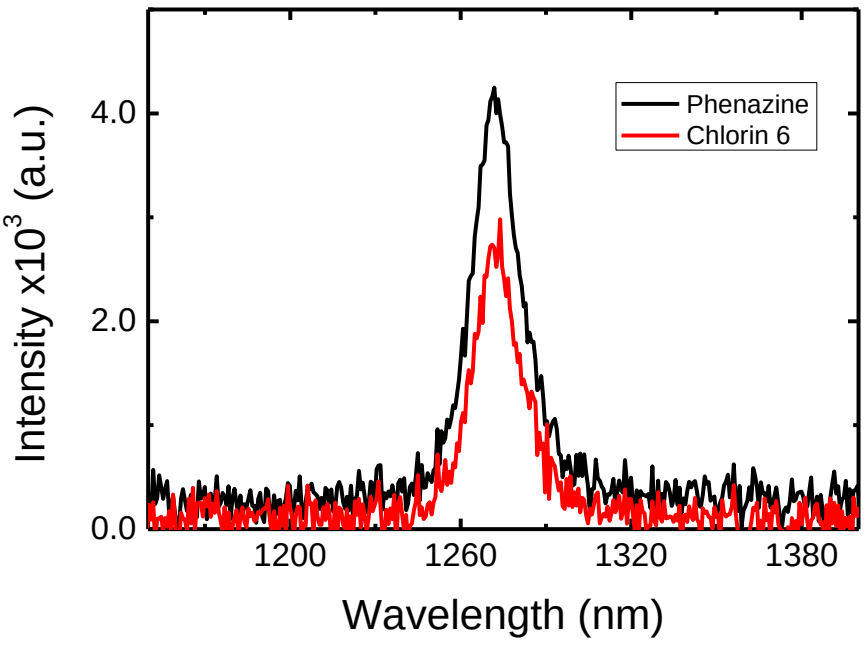

Fig. S9. Room temperature singlet oxygen phosphorescence emission spectra for chlorin **6** together with the reference compound phenazine in air saturated ethanol solutions.

7. References

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