SUPPORTING INFORMATION

Functionalization of a Ru(II) Polypyridine Complex with an Aldehyde Group as a Synthetic Precursor for Photodynamic Therapy

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EXPERIMENTAL SECTION

Instruments & Materials

All starting materials were obtained from commercial sources and used without further purification. Utilized solvents were dried with molecular sieves before use and experiments conducted under inert atmosphere. When protection from light was necessary, tinfoil wrapped glassware was used. The compounds (2,2'-bipyridine)-4-carbaldehyde (C. Dolan, A. Byrne, C. Long, K. Czamara, A. Kaczor, M.Baranska, M. Keyes, *RSC Adv.* **2017**, *7* (69), 43743–43754), [Ru(dimethyl sulfoxide)$_4$Cl$_2$] (I. Brastos, E. Alessio, M. E. Ringenberg, T. B. Rauchfuss, *Inorg. Synth.*, **2010**, *35*, 148–163), and [Ru(1,10-phenanthroline)$_2$(Cl)$_2$] (A. Notaro, M. Jakubaszek, N. Rotthowe, F. Maschietto, R. Vinck, P. S. Felder, B. Goud, M. Tharaud, I. Ciofini, F. Bedioui, R. F. Winter and G. Gasser, *J. Am. Chem. Soc.*, **2020**, *142*, 6066–6084) were prepared as described in literature. ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker 400 MHz NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm). Coupling constants (J) are reported in Hertz (Hz). Following abbreviations are used for multiplicity description: s -(singlet), d (doublet), dd (doublet of doublet), m (multiplet). ESI mass spectra were recorded on an Advion expression compact mass spectrometer.

Synthesis

Synthesis of [Ru(dimethyl sulfoxide)$_4$Cl$_2$] (1)

[Ru(dimethyl sulfoxide)$_4$Cl$_2$] was synthesized by adapting a previously reported procedure (I. Brastos, E. Alessio, M. E. Ringenberg, T. B. Rauchfuss, *Inorg. Synth.*, **2010**, *35*, 148–163). Ruthenium-trichloride-hydrate (1.97 g, 5.5 mmol) was suspended in ethanol and the mixture heated at reflux for 3 hours. After this time, the reaction mixture was filtered and the solvent removed under reduced pressure. The residue was suspended in a minimum quantity of dimethyl sulfoxide (~2 mL) and the solution heated at reflux for 2 hours. After cooling down to room temperature, dry acetone cooled to 0 °C (200 mL) was added and the mixture was placed in a freezer at -24 °C overnight. The formed precipitate was filtered off and washed with acetone to form the product. The product was dried under vacuum. A yellow powder was isolated (77%,

2.81 g). ^{1}H-NMR (400 MHz, D_2O) δ = 3.52-3.33 (m, 18H), 2.73 (s, 6H) ppm. The analytic data was found to be in agreement with the previous literature.

Synthesis of [Ru(1,10-phenanthroline)$_2$(Cl)$_2$] (2)

[Ru(1,10-phenanthroline)$_2$(Cl)$_2$] was synthesized by adapting a previously reported (A. Notaro, M. Jakubaszek, N. Rotthowe, F. Maschietto, R. Vinck, P. S. Felder, B. Goud, M. Tharaud, I. Ciofini, F. Bedioui, R. F. Winter and G. Gasser, *J. Am. Chem. Soc.*, **2020**, *142*, 6066–6084) . [Ru(dimethyl sulfoxide)$_4$Cl$_2$] (1.74 g, 3.6 mmol, 1.0 equiv.), 1,10-phenanthroline (2.50 g, 7.5 mmol, 2.0 equiv.), and lithium chloride (1.07 g, 25.2 mmol, 8.5 equiv.) were dissolved in *N*,*N*-dimethylformamide (150 mL) and heated at reflux for 4 hours. The solvent was reduced to roughly 5 mL under reduced pressure, acetone (500 mL) was added, and the mixture was placed in a freezer at -24 °C overnight. The formed precipitate was isolated by filtration, the solids thoroughly washed with water (30 mL), acetone (30 mL), and diethyl ether (50 mL). The product was dried under vacuum. A purple powder was isolated (54 %, 1.04 g). ^{1}H-NMR (400 MHz, CD_2Cl_2) δ = 10.05 (d, *J* = 5.5 Hz, 2H), 8.48 (s, 2H), 8.35 (d, *J* = 4.9 Hz, 2H), 8.09 (d, *J* = 4.8 Hz, 2H), 7.85 (s, 2H), 7.63-7.51 (m, 2H), 7.39 (d, *J* = 4.7 Hz, 2H), 6.74 (m, 2H) ppm. The analytic data was found to be in agreement with the previous literature.

Synthesis of 4-(2,2'-bipyridyl)benzaldehyde (3)

4-(2,2'-bipyridyl)benzaldehyde was synthesized by adapting a previously reported procedure (C. Dolan, A. Byrne, C. Long, K. Czamara, A. Kaczor, M.Baranska, M. Keyes, *RSC Adv.* **2017**, *7* (69), 43743–43754). 4-Bromo-2,2'-bipyridine (200 mg, 0.85 mmol, 1.0 equiv.) was added to a solution of 4-formylphenyl boronic acid (153 mg, 1.02 mmol, 1.2 equiv.) and [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) dichloride (65 mg, 0.08 mmol, 10 mol%) in dioxane (2 mL). Following, a solution of potassium carbonate (218 mg,1.58 mmol, 1.6 equiv.) in water (0.5 mL) was added and the mixture heated at reflux for 6 hours. After cooling to room temperature, dichloromethane (3 x 30 mL) was added to the mixture and the organic phase extracted and washed with brine (50 mL). The organic phase was dried over magnesium sulphate. The solvent was removed under reduced pressure. The product was isolated by recrystallization from over layering of a chloroform solution with cold

pentane. The generated precipitate was obtained by filtration and washed with pentane (25 mL). The product was dried under vacuum. A brown powder was isolated (79 %, 170 mg). ^{1}H-NMR (400 MHz; $CDCl_3$) δ = 10.11 (s, 1H), 9.03 (s, 1H), 8.81 (dd, *J* = 1.2, 5.0 Hz, 2H), 8.72 (d, *J* = 2.0 Hz, 1H), 8.05 (m, 4H), 7.73 (d, *J* = 1.1 Hz, 1H), 7.55 (m, 2H) ppm.

Synthesis of [Ru(1,10-phenantroline)$_2$-4-(2,2'-bipyridyl)benzaldehyde] (4)

[Ru(1,10-phenanthroline)$_2$(Cl)$_2$] (69 mg, 129 µmol, 1.0 equiv.) and 4-(2,2'-bipyridyl)benzaldehyde (37 mg, 129 µM, 1.0 equiv.) were suspended in water/methanol (1:1, 10 mL) and the mixture heated at reflux overnight. After this time, the solution was cooled down to room temperature and a saturated aqueous ammonia hexafluorophosphate solution was added dropwise. The formation of a red precipitate was immediately observed. The solid was isolated by filtration and thoroughly washed with water (30 mL) and diethyl ether (50 mL). The product was dried under vacuum. A red powder was isolated (69 %, 90 mg). ^{1}H-NMR (400 MHz, MeOD) δ = 10.10 (s, 1H), 9.11 (s, 1H), 8.98 (m, 2H), 8.77 (d, *J* = 5.1 Hz, 2H), 8.69 (d, *J* = 4.8 Hz, 2H), 8.45 (t, *J* = 3.8 Hz, 1H), 8.34 (m, 6H), 8.12 (m, 3H), 8.02 (m, 2H), 7.93 (m, 4H), 7.69 (m, 3H), 7.42 (m, 1H) ppm; ^{13}C-NMR (100 MHz, MeOD) δ = 193.2, 158.7, 158.0, 153.6, 153.2, 152.9, 148.8, 148.6, 148.3, 142.1, 138.5, 138.1, 137.6, 131.8, 131.0, 129.0, 128.8, 128.3, 126.7, 125.7, 125.2, 122.8 ppm; ESI (pos. detection mode): calcd. for $C_{41}H_{28}N_6ORuPF_6$ $[M\text{-}PF_6]^+$ m/z 867.2; found: 867.4. HPLC: tR = 6.0 min; elemental analysis calcd for $C_{41}H_{28}N_6ORuP_2F_{12}\times H_2O$ (%): C 47.82, H 2.94, N 8.16; found: C 47.84, H 3.48, N 7.89.

Lipophilicity

The lipophilicity of the sample was determined using an octanol/PBS system. Equal volumes of pre-saturated 1-octanol and PBS solutions were prepared by mixing for 24 hours. The complex was dissolved in PBS, and an equal volume of 1-octanol was added. The mixture was shaken overnight at room temperature and the phases separated. The concentrations of the compound in both the octanol and PBS phases were quantified using UV/Vis spectrometry. The partition coefficient (logP) was

calculated as the logarithm of the ratio of the compound's concentration in the octanol phase to its concentration in the PBS phase.

$$LogP\left(\frac{octanol}{water}\right) = Log\left(\frac{[D]octanol}{[D]water}\right)$$

Water solubility

Stock solutions of **4** were prepared in DMSO and diluted with PBS in a 1:9 ratio. Prepared concentrations were: 0.01 mM, 0.1 mM, 1 mM, 5 mM, 10 mM, 20 mM, 25 mM.

Water stability

The stability of the compound in DMSO/water was determined by UV/Vis spectroscopy. The compound was dissolved and stored at room temperature in the dark. The absorption spectrum from 250 to 650 nm was recorded after 0, 12, 24 and 48 h and compared.

Photostability

The sample was prepared in an air saturated acetonitrile solution and irradiated for 10 minutes with 100 μW/cm² using a 420 nm long-pass filter. After, the UV/Vis spectrum was recorded and the irradiation process repeated multiple times. As qualitative comparison, the same measurement was performed with Photofrin.

Absorption

For the absorption measurement, a 1 mM stock solution of **4** in acetonitrile was prepared. The measurement was conducted in water with a concentration of 30 μM of **4**, to keep the absorption intensity in the lambert-beer region. The used instrument was a Jasco V-670 Spectrometer.

Emission

For the emission measurement, a 1 mM stock solution of **4** in acetonitrile was prepared. The measurement was conducted in water with a concentration of 10 μM of **4**. The used slit width was 5 nm for both emission and excitation. The used instrument was a Jasco Spectrofluorometer FP-8300.

Computational Calculations

All quantum mechanical calculations were performed using Gaussian 16 M. (J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, G. A. Petersson, H. Nakatsuji, X. Li, M. Caricato, A. V. Marenich, J. Bloino, B. G. Janesko, R. Gomperts, B. Mennucci, H. P. Hratchian, J. V. Ortiz, A. F. Izmaylov, J. L. Sonnenberg, Williams, F. Ding, F. Lipparini, F. Egidi, J. Goings, B. Peng, A. Petrone, T. Henderson, D. Ranasinghe, V. G. Zakrzewski, J. Gao, N. Rega, G. Zheng, W. Liang, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, K. Throssell, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. J. Bearpark, J. J. Heyd, E. N. Brothers, K. N. Kudin, V. N. Staroverov, T. A. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. P. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, J. M. Millam, M. Klene, C. Adamo, R. Cammi, J. W. Ochterski, R. L. Martin, K. Morokuma, O. Farkas, J. B. Foresman, D. J. Fox, *Gaussian 16* Rev. C.01, Wallingford, CT, **2016**). The molecular structure was optimized via B3LYP functional with a customized basis set. All organic atoms were modelled with 6-31G(d) and ruthenium with LANL2DZ basis set. To ensure that each stationary point is in fact a minimum, vibrational frequency calculation was performed under the same conditions. As solvent model CPCM method with acetonitrile was employed. DFT method was used to optimize the structure and to calculate HOMO-LUMO gap as well as triplet excited energy. The excited singlet state energy was obtained using TD-DFT. Additionally, the electrostatic potential map was attained through Hirshfeld method.

Measurement of Singlet Oxygen Generation Quantum Yield

For the measurements in acetonitrile, samples were prepared in an air-saturated solution containing the complex (absorbance of 0.1 at the irradiation wavelength), N,N-dimethyl-4-nitrosoaniline (24 μM), and imidazole (12 mM).

For the measurements in PBS buffer, samples were prepared in an air-saturated solution containing the complex (absorbance of 0.1 at the irradiation wavelength), N,N-dimethyl-4-nitrosoaniline (20 μM), and histidine (10 mM).

All samples were irradiated with 450 nm wavelength for one minute 10 times, with absorbance measurements in between. The absorbance was recorded using a Shimadzu UV-1900 spectrophotometer. The absorbance change (A_0 - A) was

determined at 420 nm for acetonitrile and 440 nm for PBS, then plotted against irradiation time. The used reference values are: Φ_{MeCN} = 0.57 (A. Abdel-Shafi, P. Beer, R. Mortimer, F. Wilkinson, Photosensitized Generation of Singlet Oxygen from (Substituted Bipyridine)ruthenium(II) Complexes, *Helv. Chim. Acta*, **2001**, *84*, 2784-2795.) and Φ_{PBS} = 0.22 (D. Garcìa-Fresnadillo, Y. Georgiadou, G. Orellana, A. M. Braun, E. Oliveros, Singlet-Oxygen Production by Ruthenium(II) complexes containing polyazaheterocyclic ligands in methanol and in water, *Helv. Chim. Acta*, **1996**, *79*, 1222-1238.). The slope of the linear regression was calculated and the absorbance correction factor determined. Quantum yields were calculated using the following formula

$$\Phi_{sample} = \Phi_{reference} \times \frac{S_{sample}}{S_{reference}} \times \frac{I_{reference}}{I_{sample}}$$

$$I = I_0 \times (1 - 10^{-A})$$

Φ = singlet oxygen quantum yield, S = slope of the linear regression, I = absorbance correction factor, I_0 = light intensity of the irradiation source, A = absorbance of the sample at the irradiation wavelength.

SUPPORTING FIGURES

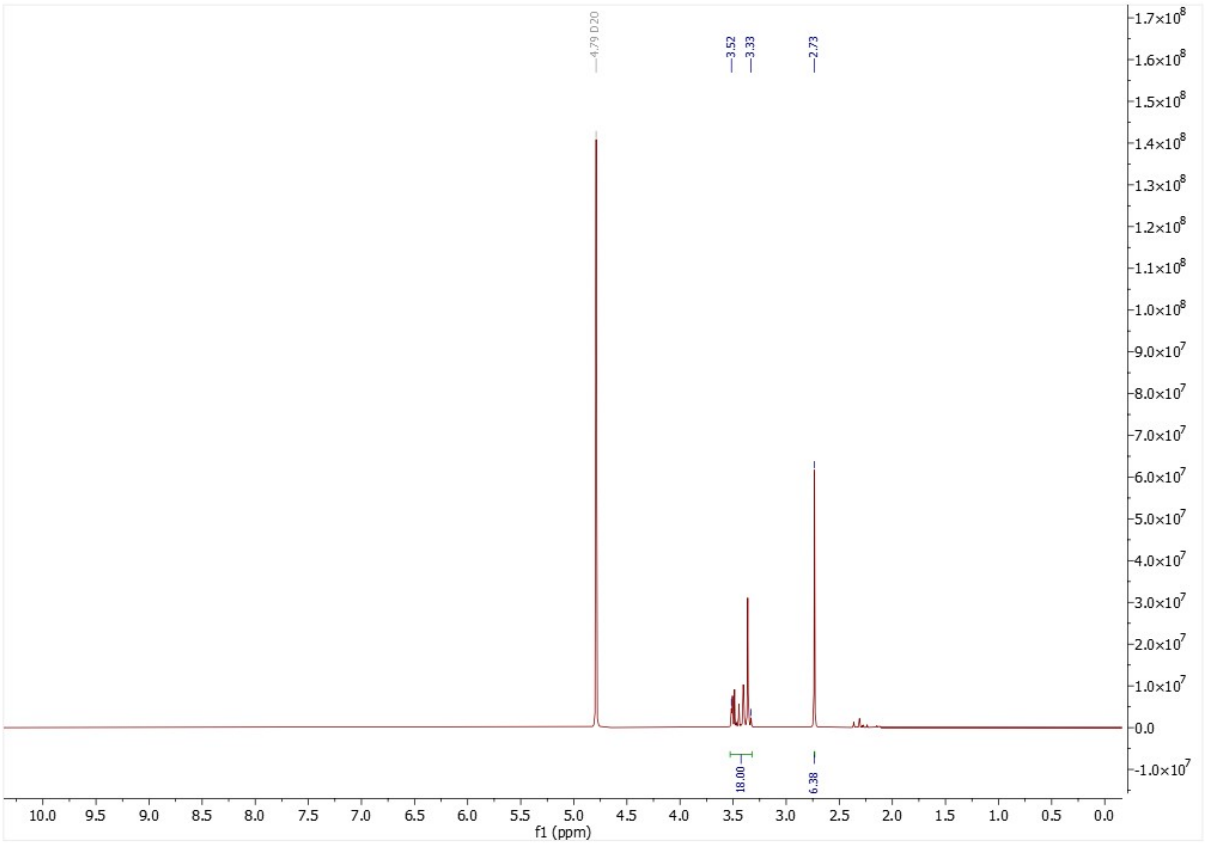


Figure S1. ^{1}H-NMR spectrum (400 MHz) of **1** in D_2O.

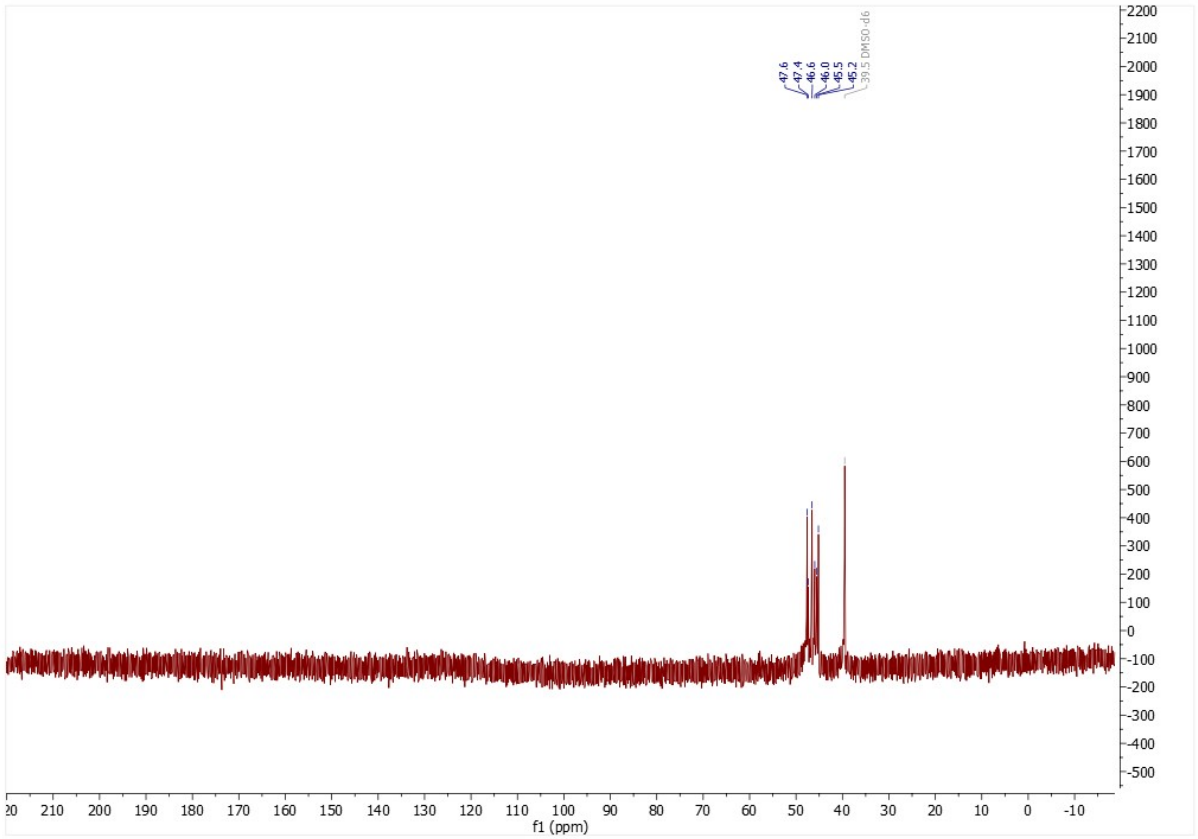


Figure S2. ^{13}C-NMR spectrum (100 MHz) of **1** in DMSO-d_6.

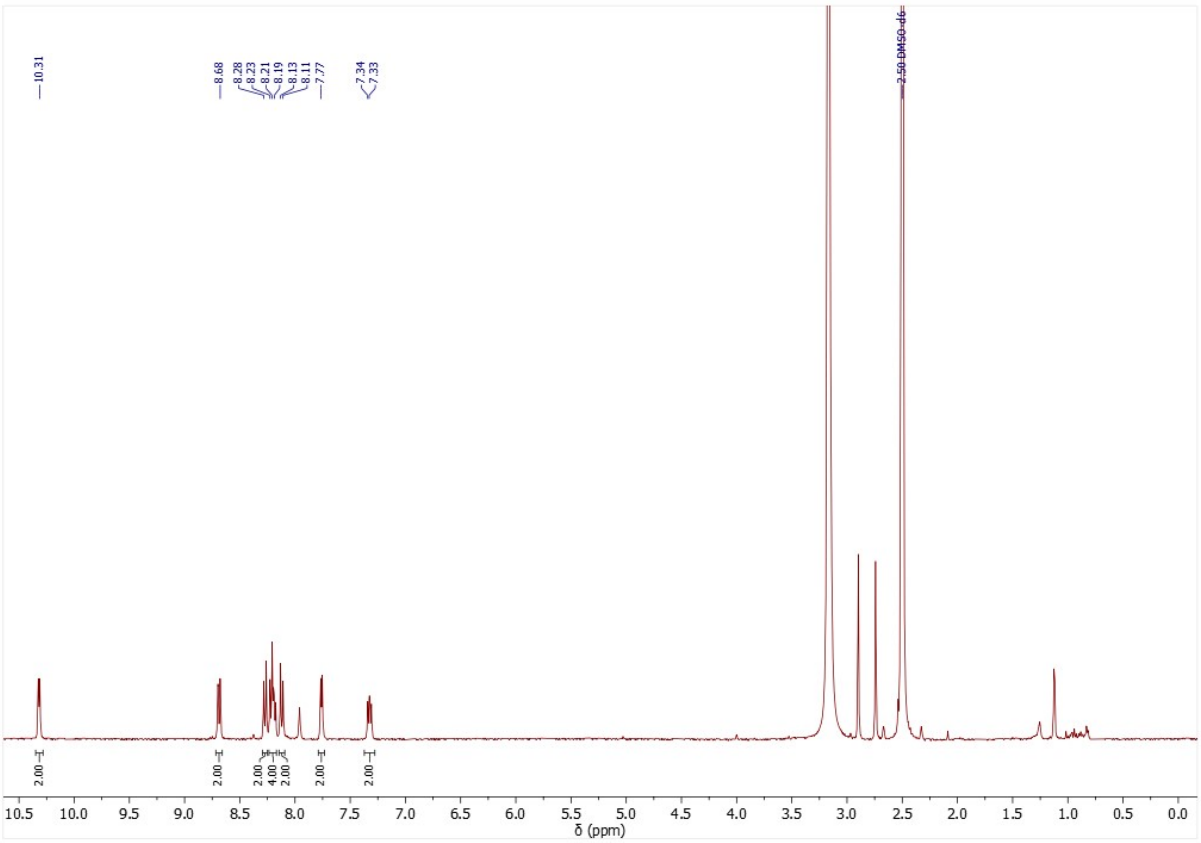


Figure S3. ^{1}H-NMR spectrum (400 MHz) **2** in DMSO-d_6.

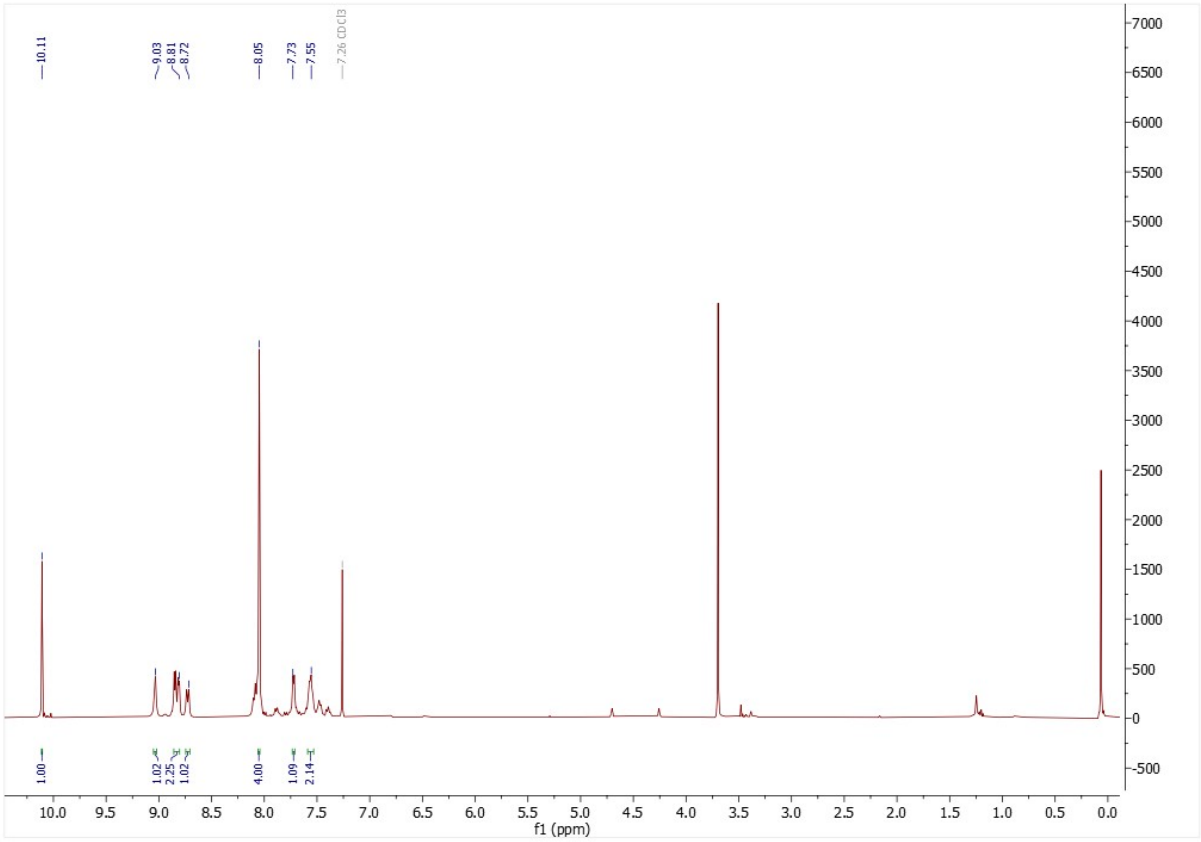


Figure S4. ^{1}H-NMR spectrum (400 MHz) of **3** in CD_3Cl.

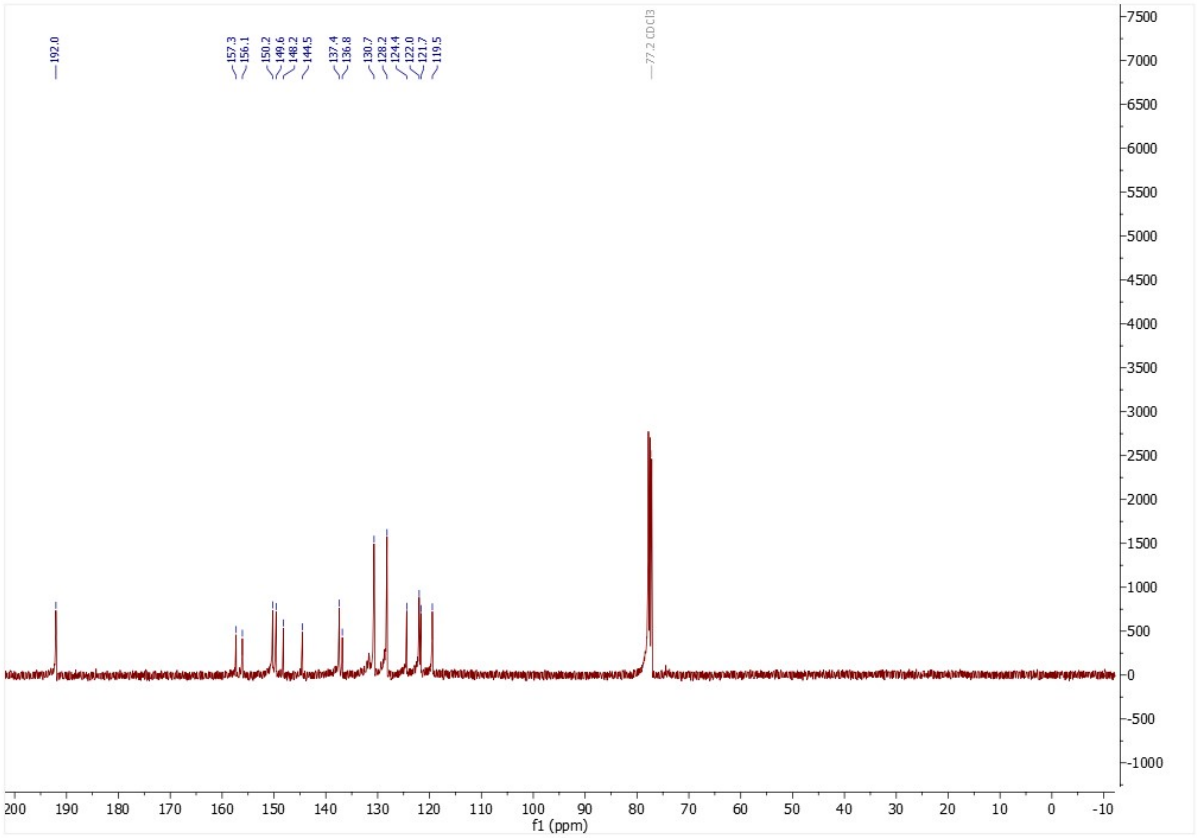


Figure S5. ^{13}C-NMR spectrum (100 MHz) of **3** in CD_3Cl.

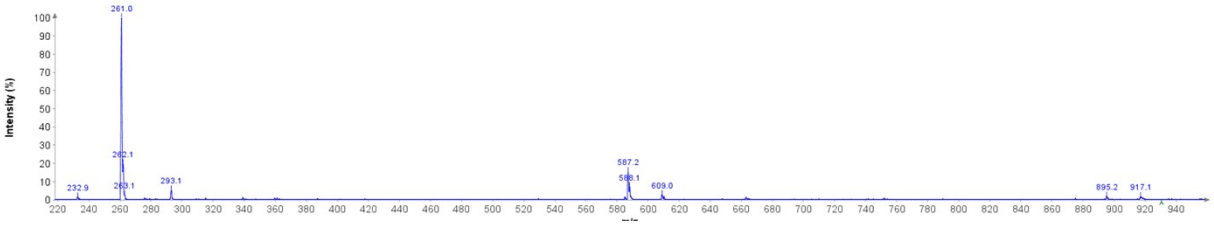


Figure S6. ESI/MS of **3**.

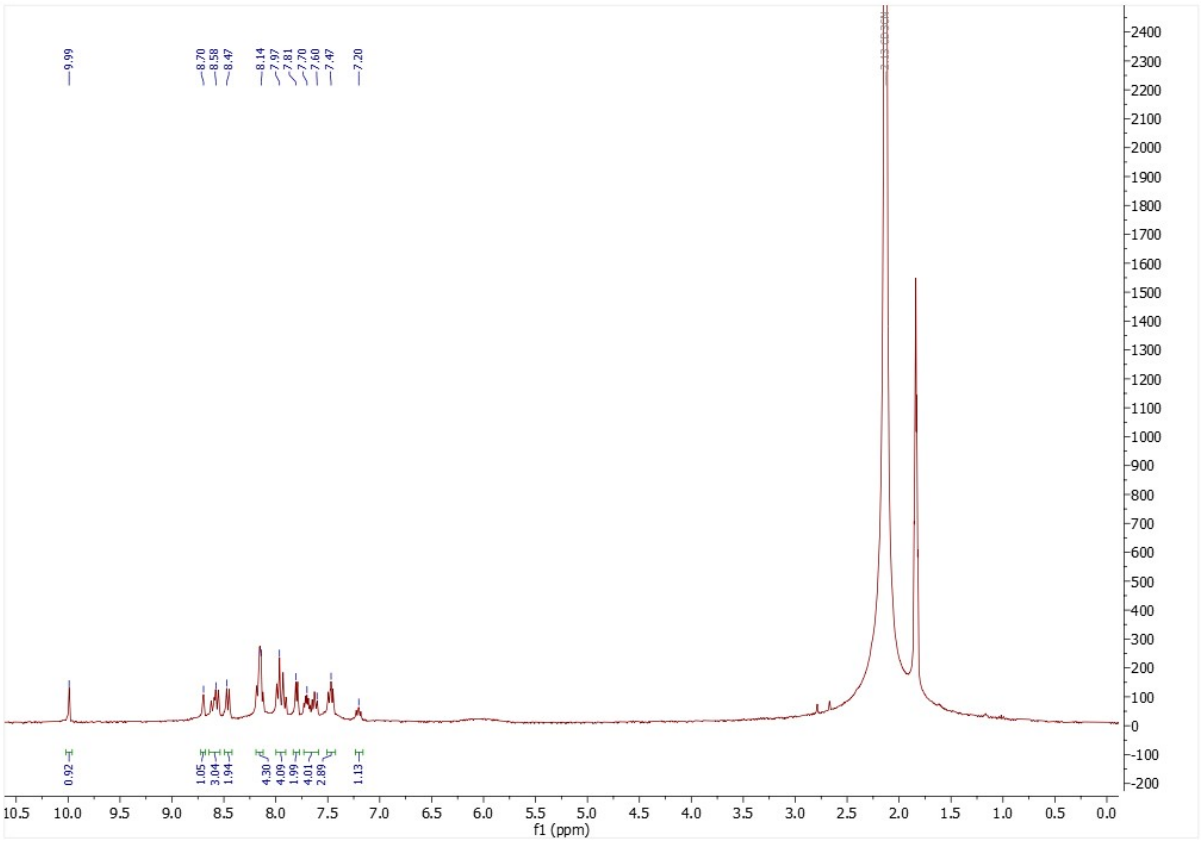


Figure S7. ^{1}H-NMR spectrum (400 MHz) of **4** in acetonitrile.

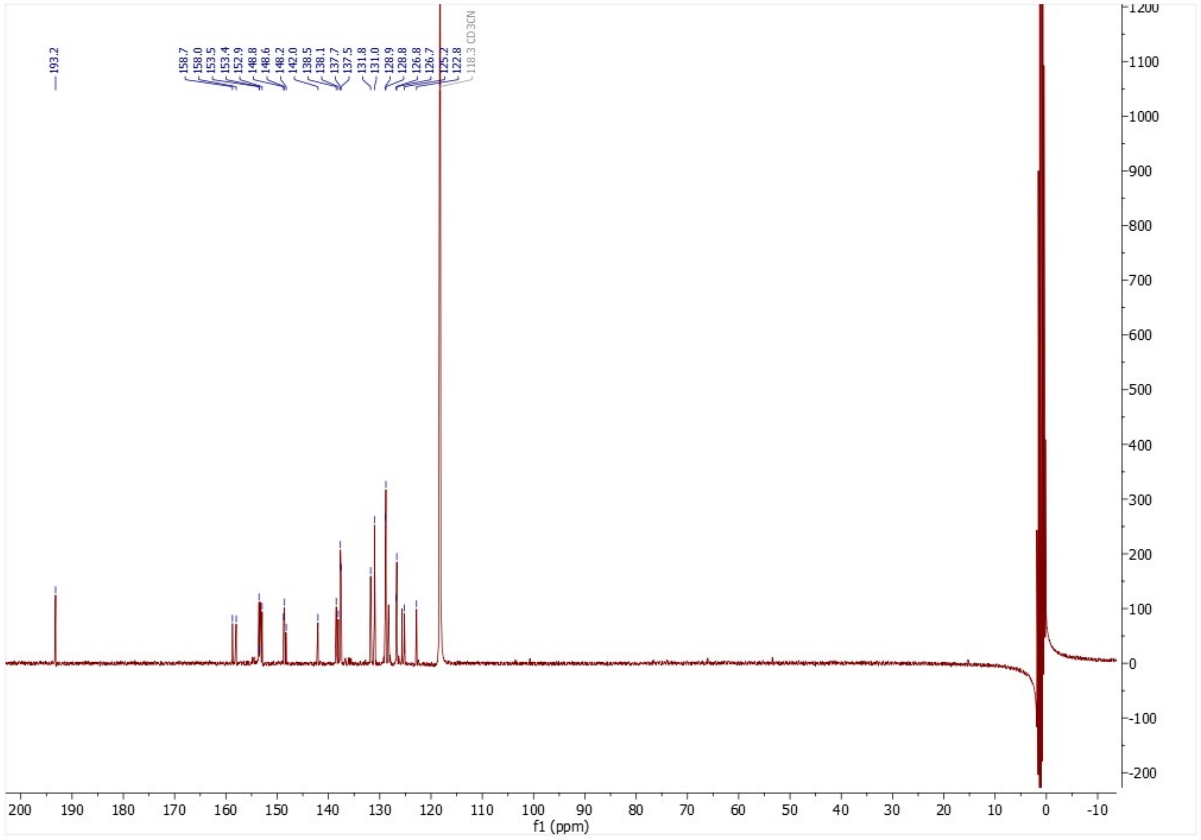


Figure S8. ^{13}C-NMR spectrum (100 MHz) of **4** in acetonitrile.

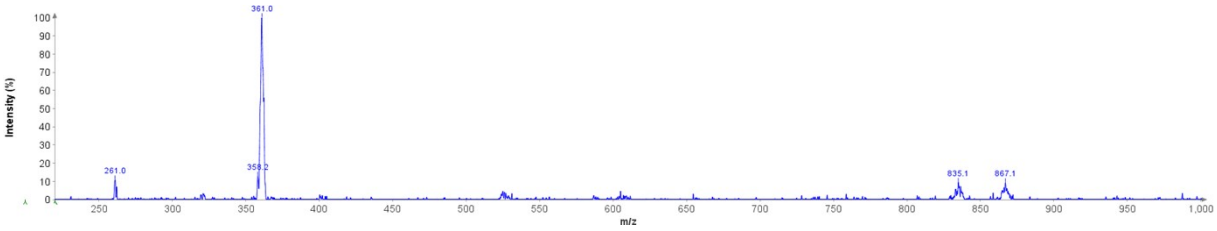


Figure S9. ESI/MS of **4**.

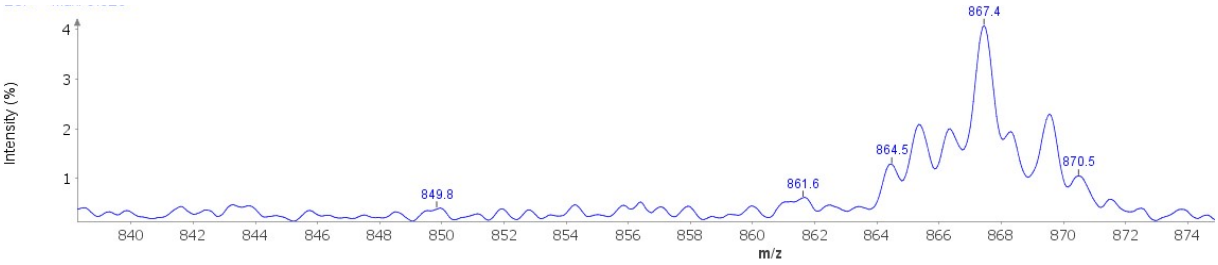


Figure S10. Zoomed ESI/MS signal of the **4** cation $[RuC_{41}H_{28}N_6O\text{-}PF_6]^+$.

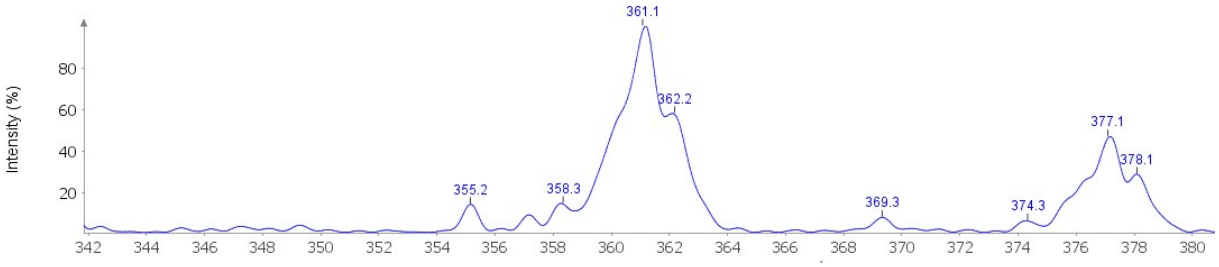


Figure S11. Zoomed ESI/MS signal of the **4** cation $[RuC_{41}H_{28}N_6O]^{2+}$.

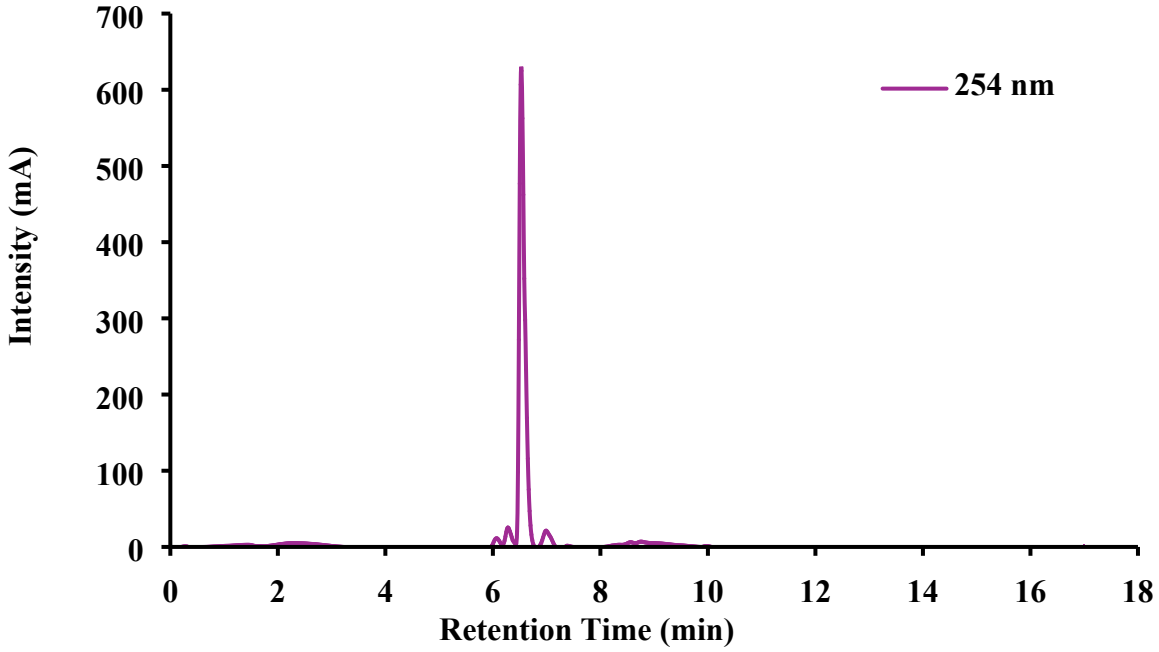


Figure S12. HPLC of **4** with a 60:40 H_2O/acetonitrile to 100% acetonitrile gradient and a flow rate of 1 mL/min.

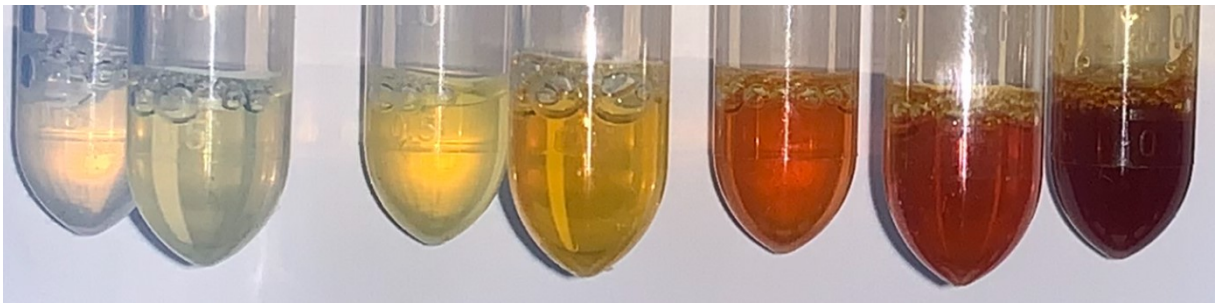
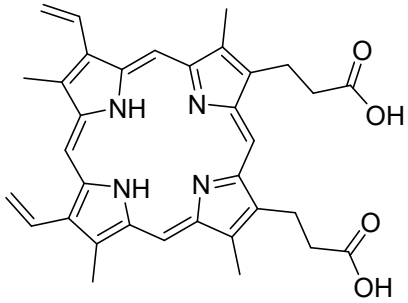
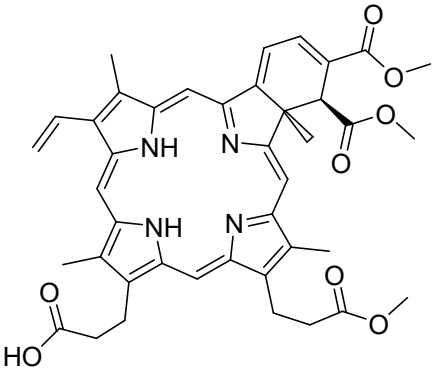
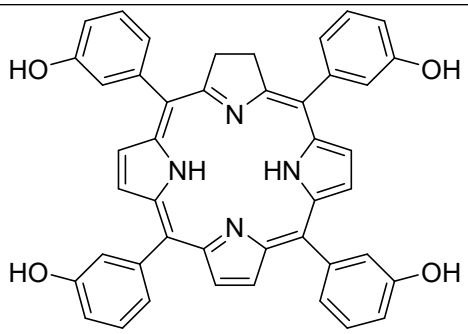


Figure S13. Water solubility of **4** in a 9:1 PBS/DMSO mixture. Concentrations from left to right: 0.01 mM, 0.1 mM, 1 mM, 5 mM, 10 mM, 20 mM, and 25 mM.

Table S1: Clinically approved photosensitizers Protoporphyrin IX, Verteporfin Temporfin and their aqueous solubility.

Compound	Structure	Solubility
Protoporphyrin IX		0.17 mg/ml in water[a]
Verteporfin		0.1 mg/mL in 1:7 DMF:PBS[b]

Temoporfin		Practically insoluble in water (10 mg/mL in DMSO)[c]

[a] National Center for Biotechnology Information (2025). PubChem Compound Summary for CID 4971, protoporphyrin IX.

[b] Cayman Chemical, Product Information, Verteporfin, Item No. 17334

[c] Cayman Chemical, Product Information, Temoporfin, Item No. 17333

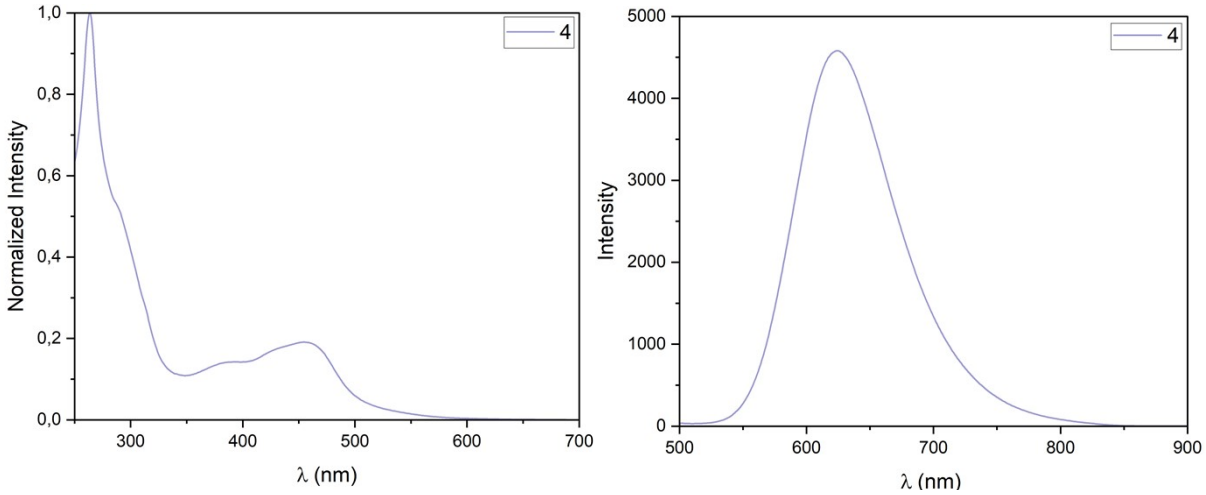


Figure S14. Left) Normalized absorption spectrum of **4** in PBS. Right) Emission spectrum of **4** in PBS upon excitation at 280 nm.

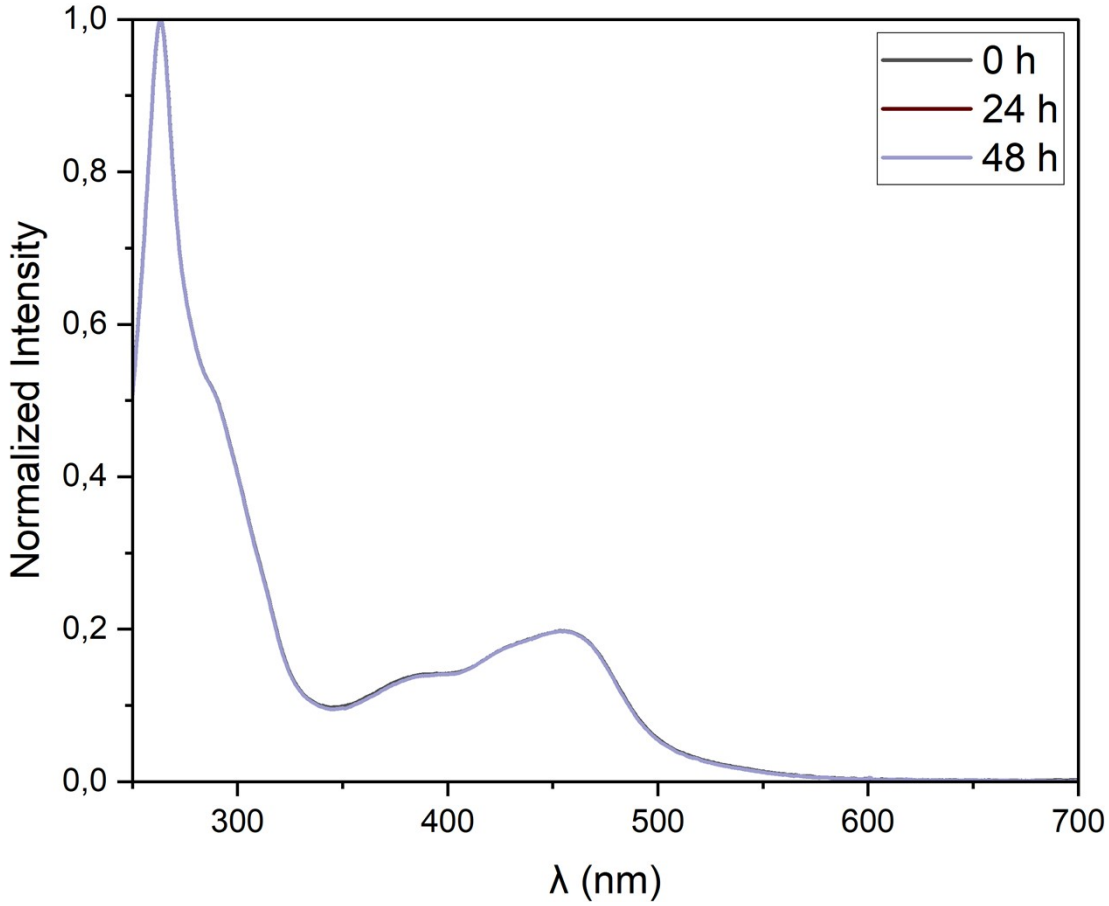


Figure S15. UV/Vis water stability measurement of **4** in dimethyl sulfoxide performed in the dark after 0, 24 and 48 h.

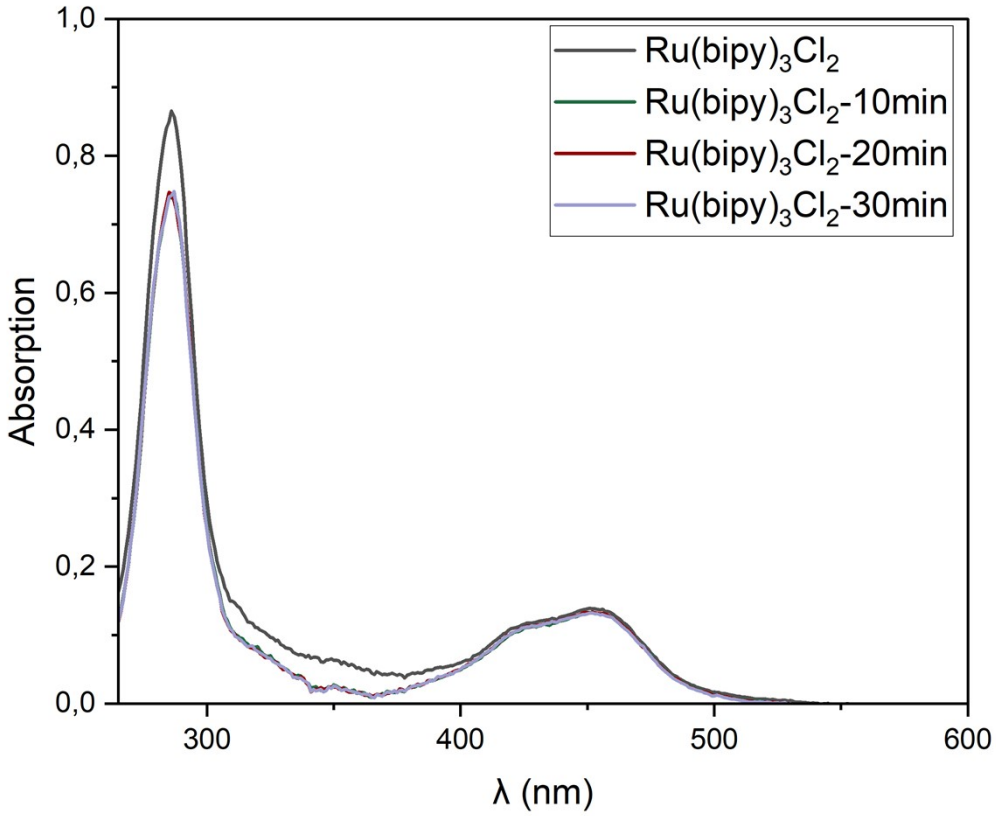


Figure S16. UV/Vis photostability measurement of [$Ru(bipy)_3Cl_2$] without, after 10 minutes, 20 minutes and 30 minutes of irradiation using a 420 nm long-pass filter, as reference for a photostable compound.

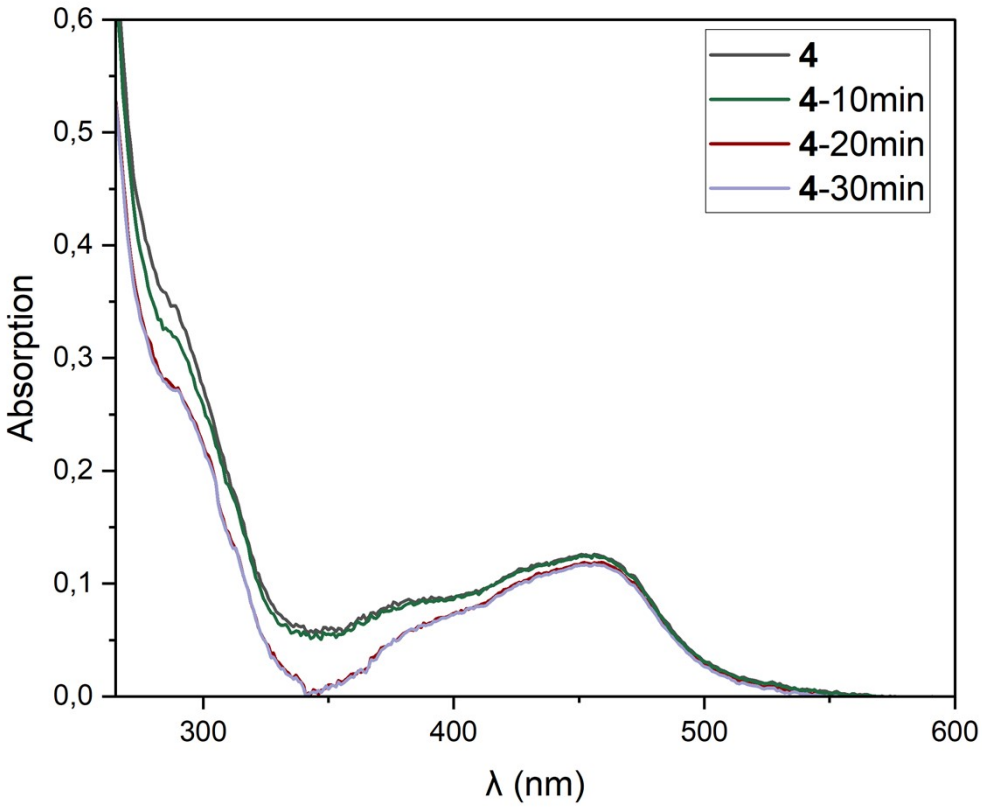


Figure S17. UV/Vis photostability measurement of **4** without, after 10 minutes, 20 minutes and 30 minutes of irradiation using a 420 nm long-pass filter.

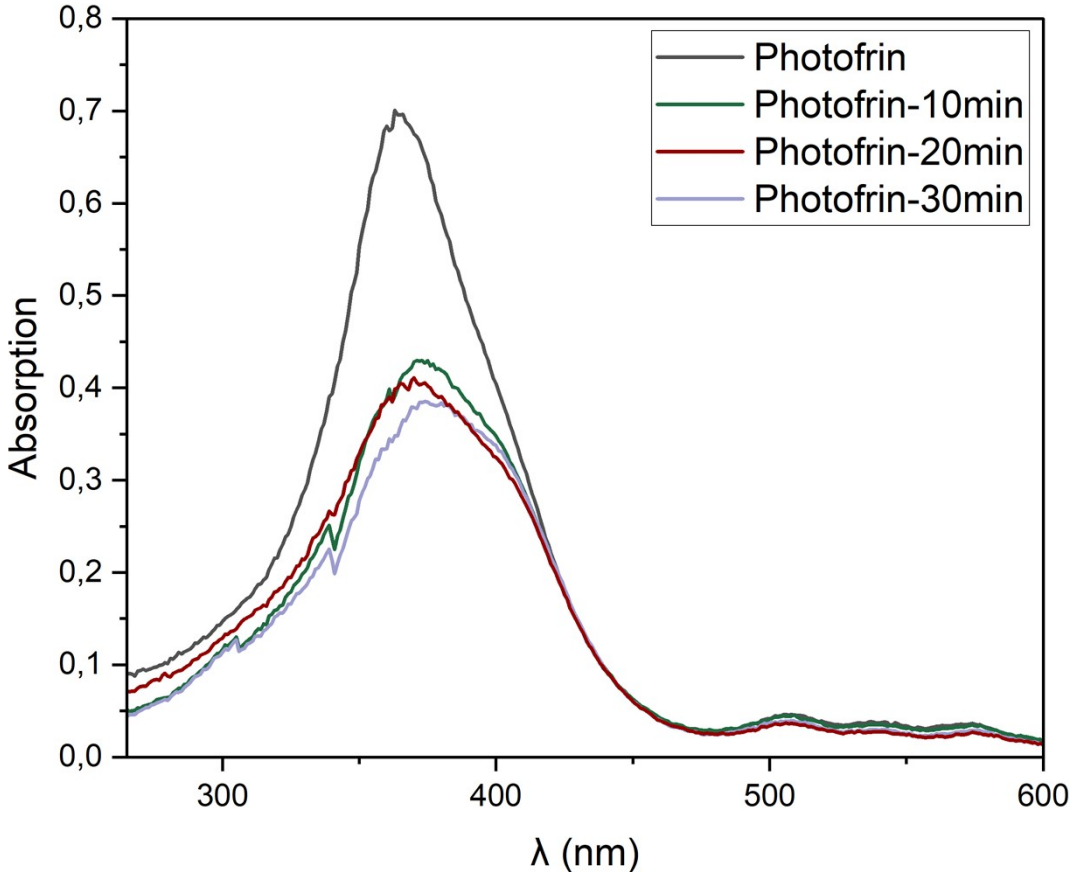


Figure S18. UV/Vis photostability measurement of Photofrin without, after 10 minutes, 20 minutes and 30 minutes of irradiation using a 420 nm long-pass filter.

Table S2. All barycenter cartesian coordinates of the ES1, ES3 and TE1.

	Positive barycenter			**Negative barycenter**		
Coordinates	x	y	z	x	y	z
Excited singlet	-1.08197	0.21218	0.10852	1.40985	-0.42174	0.55549
Excited vertical triplet	-0.92263	0.21119	0.16407	1.51833	-0.38393	0.50687
Third Excited vertical singlet	-0.52768	-0.06058	0.35796	-2.18384	-0.85552	-0.22718

Table S3. Charge-transfer distances between ground state and ES1/ES3/TE1.

Excited state	Charge-Transfer distances
Excited singlet	2.6097590575780125
Excited vertical triplet	2.535745732602914
Third excited vertical singlet	1.9280055512868728

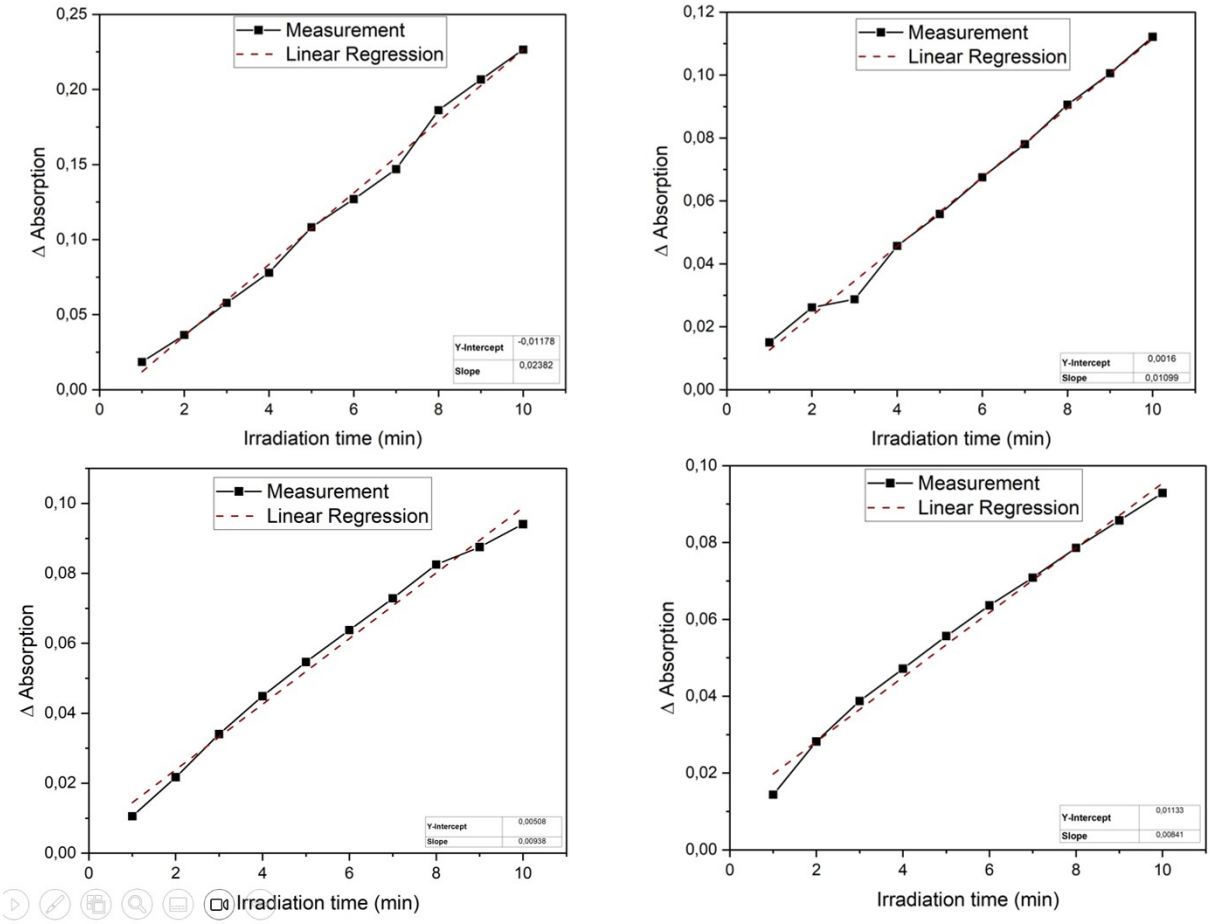


Figure S19. Linear regression of the singlet oxygen quantum yield. Top Left: Complex **4** in acetonitrile. Top Right: Reference in acetonitrile. Bottom Left: Complex **4** in PBS. Bottom Right: Reference in PBS.