Supporting Information

Synthesis, characterization and biomolecule-binding properties of novel tetra-platinum(II)-thiopyridylporphyrins

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Contents

1 Experimental section

1.1 Synthesis and characterization of zinc(II) thiopyridylporphyrins and platinum(II)-thiopyridylporphyrins

5,10,15,20-Tetrakis[2,3,5,6-tetrafluoro-4-(4-pyridylsulfanyl)phenyl]porphyrinato zinc(II), $ZnTPPF_{16}(SPy)_4$ ***2***:

Compound 5,10,15,20-Tetrakis[2,3,5,6-tetrafluoro-4-(4-pyridylsulfanyl)phenyl]porphyrin $H_2TPPF_{16}(SPy)_4$ **1** was synthesized as previously described by Tomé and co-workers.[1] $H_2TPPF_{16}(SPy)_4$ **1** (31.6 mg, 0.024 mmol) and zinc acetate (23.7 mg, 0.13 mmol, 5 equiv.) were carried out in a mixture of $CHCl_3$/MeOH (9:1; 5 mL) at reflux. The reaction was stirred for 3 hours under N_2 atmosphere and ended by precipitation from a mixture of $CHCl_3$/MeOH (98:2)/hexane. The solid was them washed with water in order to remove the excess of zinc acetate. Compound $ZnTPPF_{16}(SPy)_4$ **2** was obtained in 96% yield. **^{1}H NMR (300 MHz, $CDCl_3$):** δ 7.23 (d, J = 5.7 Hz, 8H, Py-*o*-H), 8.07 (d, J = 5.7 Hz, 8H, Py-*m*-H), 8.95 (s, 8H, 8 β-H); **^{19}F NMR (282 MHz, $CDCl_3$):** δ -154.93 (dd, J = 24.5, 11.8 Hz, 8F, 8 *m*-F), -151.46 (dd, J = 24.5, 11.8 Hz, 8F, 8 *o*-F). **MALDI-MS:** *m/z* 1401.2 $[M+H]^+$.

Tetra-platinum(II)-thiopyridylporphyrin $H_2TPPF_{16}(SPyPt)_4$ ***3***:

In a 25 mL round-bottom flask, $H_2TPPF_{16}(SPy)_4$ **1** (25.6 mg, 0.019 mmol) and dichloro(2,2′-bipyridine)platinum(II) (33.1 mg, 0.078 mmol, 4.1 equiv.) were dissolved in dry DMF (2 mL) under N_2 atmosphere. The reaction mixture was stirred for 24 h at 50 ºC. After evaporation of part of the solvent, the product was precipitated with an excess of NH_4PF_6 solution, filtrated and washed with abundant amount of cold water. Compound $H_2TPPF_{16}(SPyPt)_4$ **3** was obtained in 85% yield (56.5 mg, 0.016 mmol) after crystallization in acetonitrile. **^{1}H NMR (500 MHz, DMSO-d_6):** δ -3.11 (s, 2H, NH), 7.82 – 7.85 (m, 8H, BiPy), 8.07 – 8.12 (m, 4H, Py-*o*-H), 8.40 – 8.43 (m, 8H, BiPy), 8.50 – 8.55 (m, 4H, Py-*m*-H), 8.58 (dd, J = 8.2, 0.7 Hz, 8H, BiPy), 8.60 – 8.66 (m, 4H, Py-*o*-H), 8.92 – 9.01 (m, 4H, Py-*m*-H), 9.48 – 9.50 (m, 8H, BiPy), 9.61 (s, 8H, β-H). **^{19}F NMR (282 MHz, DMSO-d_6):** δ -160.74 (dd, J = 174.8, 23.9 Hz, 8F), -155.72 (dd, J = 174.8, 23.9 Hz, 8F). **Anal. Calcd for** $C_{104}H_{58}Cl_6F_{28}N_{16}P_2Pt_4S_4$: C, 38.47; H, 1.80; Cl, 6.55; F, 16.38; N, 6.90; P, 1.91; Pt, 24.03; S, 3.95; **Found:** C, 38.24; H, 2.15; N, 6.82; S, 3.59. **UV-Vis (CH_3CN), λ_{max}(log ε):** 307 (4.68), 320 (4.71), 360 (4.51), 410 (5.32), 504 (4.10), 536 (3.14), 580 (3.60), 633 (2.59).

Tetra-platinum(II)-thiopyridylporphyrinato zinc(II) ZnTPPF$_{16}$(SPyPt)$_4$ ***4***:

In a 25 mL round-bottom flask, $H_2TPPF_{16}(SPyPt)_4$ **1** (26.7 mg, 0.019 mmol) and zinc acetate (17.4 mg, 0.095 mmol, 5 equiv.) were carried out in a mixture of $CHCl_3$/MeOH (9:1, 5 mL) at reflux. The reaction was stirred for 3 hours under N_2 atmosphere and ended by precipitation from a mixture of $CHCl_3$/MeOH (98:2)/hexane. The solid was then washed with water in order to remove the excess of zinc acetate. Compound $ZnTPPF_{16}(SPyPt)_4$ **4** was obtained in 99% yield (66.4 mg, 0.019 mmol) after crystallization in acetonitrile. **^{1}H NMR (500 MHz, DMSO-d_6):** δ 7.83 – 7.86 (m, 8H, BiPy), 8.09 – 8.10 (m, 4H, Py-*o*-H), 8.42 (ddd, *J* = 8.0, 7.6, 1.5 Hz, 8H, BiPy), 8.53 – 8.54 (m, 4H, Py-*m*-H), 8.58 (ddd, *J* = 8.3, 1.4, 0.7 Hz, 8H, BiPy), 8.61 – 8.63 (m, 4H, Py-*o*-H), 8.95 – 8.96 (m, 4H, Py-*m*-H), 9.43 – 9.45 (m, 8H, β-H), 9.50 (ddd, *J* = 5.9, 1.5, 0.6 Hz, 8H, BiPy). **^{19}F NMR (282 MHz, DMSO-d_6):** δ -160.51 (dd, *J* = 153.7, 23.8 Hz, 8F), -156.33 (dd, *J* = 170.0, 18.4 Hz, 8F). **UV-Vis (CH_3CN), λ_{max}(log ε):** 308 (4.62), 321 (4.67), 396 (4.42), 419 (5.27), 552 (4.03), 625 (2.58). **ESI-HRMS *m/z*: calcd for** $C_{104}H_{56}Cl_4F_{16}N_{16}Pt_4S_4Zn$ (M^{4+}) 736.0019; **found:** *m/z* 737.2538.

1.2 Fluorescence assays

The emission spectra of the porphyrin derivatives $H_2TPPF_{16}(SPyPt)_4$ **3** and $ZnTPPF_{16}(SPyPt)_4$ **4** were measured in acetonitrile in 1 x 1 cm quartz optical cells under normal air conditions on a computer controlled Horiba JobinYvon FluoroMax-3 spectrofluorimeter. The fluorescence quantum yields (Φ_f) of **3** and **4** were calculated in acetonitrile by comparison of the area below the corrected emission spectra (between 600 to 800 nm) using **TPP** as standard ($\lambda_{excitation}$ at 420 nm, Φ_f = 0.15 in acetonitrile)[2] for that, the following equation was used:

$$\Phi_f^{sample} = \Phi_f^{standard} \frac{AUC^{sample}(1 - 10^{-Abs_{standard}})}{AUC^{standard}(1 - 10^{-Abs_{sample}})}$$

where *AUC* is the integrated area under the fluorescence curves for each sample and standard, and *Abs* is the absorbance of the samples and the standard at the excitation wavelength ($\lambda_{excitation}$ at 420 nm).

1.3 Photostability assays

Phosphate buffered saline (PBS) solutions of porphyrin derivatives **1-4** at 1.5 μM were freshly prepared in PBS with 0.5% v/v DMSO and kept in the dark at room temperature. The irradiation experiments were performed in magnetically stirred cuvette solutions with 2 mL of sample, over a period of 30 min with white light (400-800 nm) delivered by an illumination system (LC-122 LumaCare, London) equipped with a halogen/quartz 250 W lamp coupled to an interchangeable optic fiber probe (400-800 nm). Light was delivered at a fluence rate of 50 mW.cm^{-2}. The fluence rate was measured with an energy power meter Coherent FieldMaxII-Top combined with a Coherent PowerSens PS19Q energy sensor. The absorbance at 415 nm (compounds **1** and **3**) or 425 nm (compound **2** and **4**) was determined before and after irradiation at 0, 5, 10, 15, 20, 25 and 30 min periods of time irradiation. The results were expressed as follows:

$$Photostability\ (\%) = \frac{Abs\ at\ a\ given\ time\ of\ irradiation}{Abs\ before\ irradiation}$$

Table SI 1 – Photostability of PBS solutions of porphyrin derivatives **1-4** at 1.5 μM, after irradiation with white light (400-800 nm) at a fluence rate of 50 mW.cm^{-2} for different periods of time (0-30 min). The results are presented in percentage calculated by the ratio of residual absorbance at Soret-bands at different periods of time and absorbance before irradiation.

Compounds	**Irradiation time (min)**						
	0	**5**	**10**	**15**	**20**	**25**	**30**
$H_2TPPF_{16}(SPy)_4$ 1	100	96	95	95	95	95	95
$ZnTPPF_{16}(SPy)_4$ 2	100	100	100	99	99	93	93
$H_2TPPF_{16}(SPyPt)_4$ 3	100	100	95	89	86	84	83
$ZnTPPF_{16}(SPyPt)_4$ 4	100	98	98	97	97	97	96

1.4 Singlet oxygen production

For the determination of singlet oxygen production, solutions containing DPBF (25 μM) with or without porphyrin derivatives 0.25 μM were prepared in DMF/H_2O (9:1 v/v) in a quartz cuvette. The

solutions were irradiated at room temperature and under gentle magnetic stirring, with a LEDs array system emitting red light ($\lambda > 600$ nm) at a fluence rate of 12 mW.cm^{-2}. The breakdown of DPBF was monitored by measuring the decrease in absorbance at 415 nm at pre-established irradiation intervals. The results were expressed by plotting the DPBF depletion against the irradiation time. The depletion of DPBF was calculated as follows:

$$DPBF\ depletion = \frac{Abs_t}{Abs_0},$$

where Abs_0 and Abs_t are the absorbance values at 415 nm before and after irradiation, respectively.

1.5 Human serum albumin (HSA) interaction assays

Stock solutions of derivatives **1**-**4** were prepared at concentrations of 20 mM in DMSO and stored in the dark at room temperature. The working solutions were freshly prepared prior to use, by diluting the stock solutions in PBS (10 mM NaH_2PO_4, 70 mM Na_2HPO_4 and 145 mM NaCl at pH 7.4) with the concentration of DMSO being always inferior of 1% (v/v). Human serum albumin (HSA, Sigma) was freshly prepared at concentration of 2 μM in PBS.

For the determination of porphyrin derivatives **1**-**4** interaction with HSA, 2 mL of HSA solution (2 μM in PBS) was titrated with increasing concentrations of porphyrin derivatives (ranging from 0 to 8 μM). The emission spectra of the HSA´s tryptophan residues were acquired for the wavelength range of 300-450 nm upon photoexcitation at 280 nm. The emission quenching curves were obtained by plotting the tryptophan residues quenching (in percentage) against porphyrins concentration. The tryptophan residues quenching (in percentage) were calculated, as follows:

$$Quenching\ (\%) = \frac{(F_0 - F)}{F_0} \times 100,$$

where F_0 and F are the HSA emission intensities in the absence and presence of a quencher ($H_2TPPF_{16}(SPyPt)_4$ **3** or $ZnTPPF_{16}(SPyPt)_4$ **4**.

K_a and n values were determined by plotting the $log((F_0-F)/F)$ against $log(Por\ concentration)$, giving a linear plot, where $log(K_a)$ and n are the ordinate at the origin and slope, respectively.

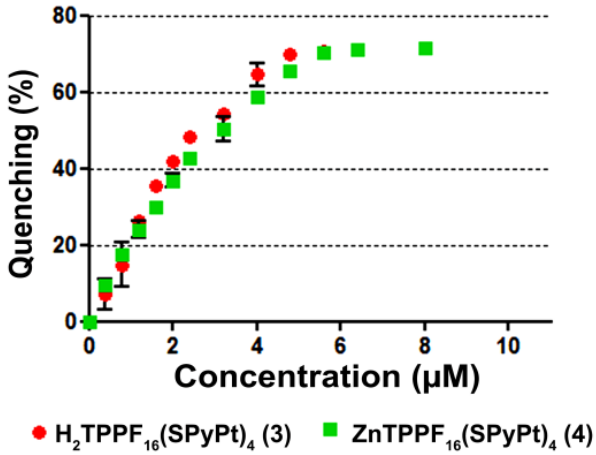


Figure SI 1 – Emission quenching curves of 2 µM of HSA, after addition of $H_2TPPF_{16}(SPyPt)_4$ **3** and $ZnTPPF_{16}(SPyPt)_4$ **4** at several concentrations (between 0 to 8 µM). Quenching = $(F_0-F)/(F_0)$, where F_0 and F are the HSA emission intensities in the absence and presence of the supramolecules **3** and **4** ($\lambda_{excitation}$ at 280 nm and wavelength emission at 335 nm).

1.6 Deoxyribonucleic acid (DNA) interaction assays

Stock solutions and working solutions of porphyrin derivatives **1-4** were prepared as previously described. For the determination of porphyrin derivatives **3** and **4** for interaction with DNA, spectral measurements were performed at room temperature in PBS at pH 7.4. The DNA base pair concentrations of low molecular weight from salmon sperm (ssDNA) and DNA from calf thymus (ctDNA) were determined by a spectroscopic method, using the molar extinction coefficients 6,600 $M^{-1}.cm^{-1}$ and 13,100 $M^{-1}.cm^{-1}$ (per base pair) at 260 nm, respectively.

PBS solutions of porphyrins **3** and **4** at 2 µM were titrated with increasing concentrations of ssDNA and ctDNA (ranging from 0 to 8 µM). The absorption spectra of porphyrin complexes **3** and **4** were acquired for the wavelength range between 370-900 nm. The emission spectra of compounds **3** and **4** were acquired for the wavelength range of 600-800 nm upon photoexcitation at 420 nm.

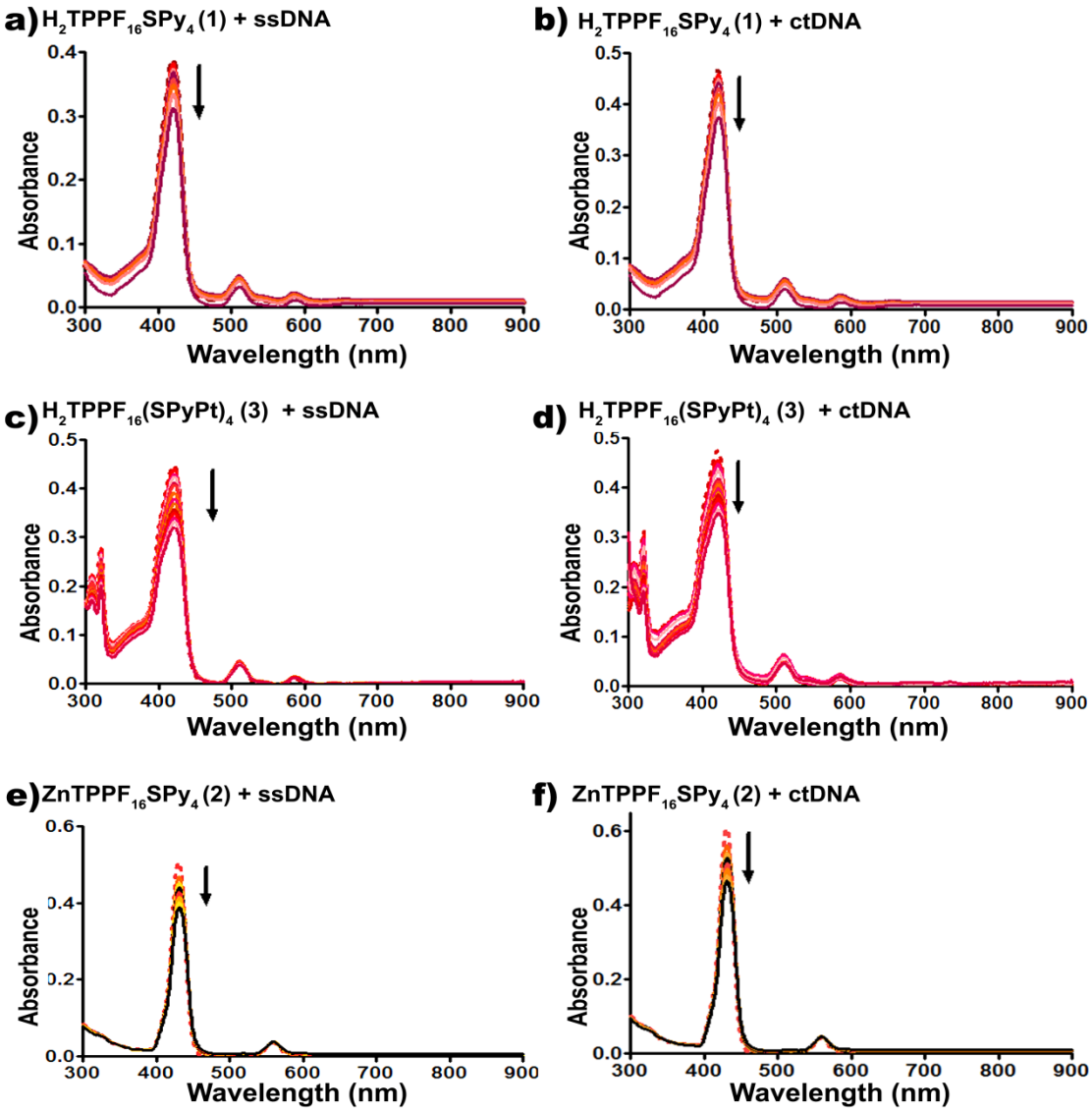


Figure SI 2 – UV-Vis absorption spectra of $H_2TPPF_{16}(SPy)_4$ **1** (**a**, **b**), $ZnTPPF_{16}(SPy)_4$ **2** (**e**, **f**) and $H_2TPPF_{16}(SPyPt)_4$ **3** (**c**, **d**) with increasing ssDNA and ctDNA concentrations (ranging from 0 to 8 μM) in PBS.

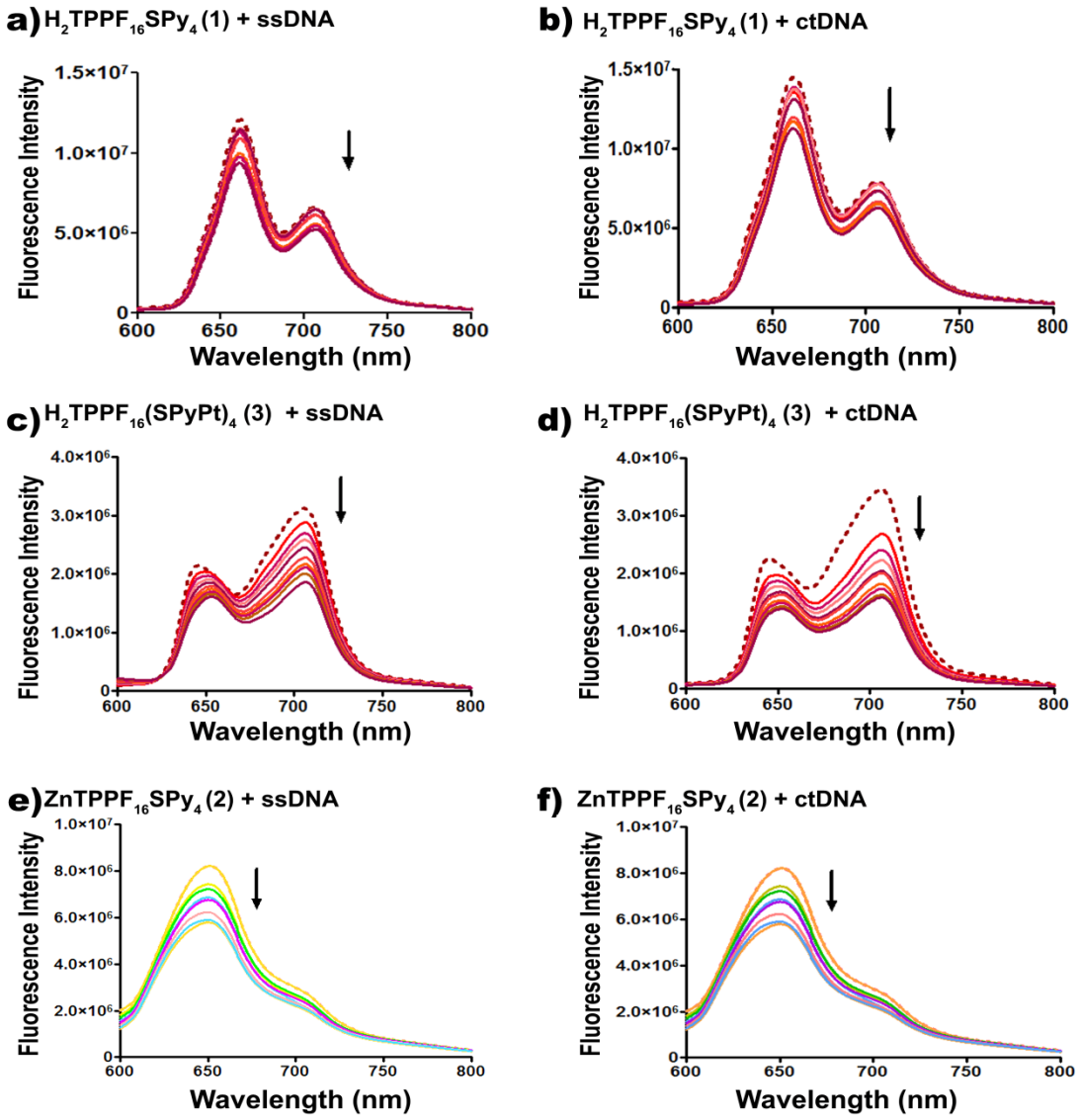


Figure SI 3 – Emission spectra of $H_2TPPF_{16}(SPy)_4$ **1** (**a**, **b**), $ZnTPPF_{16}(SPy)_4$ **2** (**e**, **f**) and $H_2TPPF_{16}(SPyPt)_4$ **3** (**c**, **d**) (2 μM) with increasing ssDNA and ctDNA concentrations (ranging from 0 to 8 μM) in PBS ($\lambda_{excitation}$ 420 nm).

1.7 DNA cleavage assays

Stock solutions of porphyrin derivatives **1-4** were prepared at concentrations of 2 mM in DMSO and stored in the dark at room temperature. The working solutions were freshly prepared prior to use, by diluting the stock solutions in buffer (3 mM Tris–HCl, 0.3 mM EDTA, pH 8.0) keeping the concentration of DMSO always lower than 2% (v/v).

DNA cleavage studies were performed by the exposure of supercoiled pMT123 DNA (Form I) to porphyrins **1**-**4** and irradiation. Typically, solutions of the porphyrin derivatives (0, 1, 10 or 40 μM) were incubated in darkness during 1 h with 1 μg of plasmid DNA at room temperature. The mixtures were then irradiated for 1 h with a LED array system composed of a matrix 24 × 16 LEDs, totalizing 384 light sources overall and emitting light with two emission peaks at $\lambda = 450 \pm 20$ nm and $\lambda = 550 \pm 50$ nm (white light).[3] Immediately after the treatments, the resultant mixtures underwent electrophoresis on 1% (w/v) agarose gel (containing ethidium bromide stain) at 50 V for 90 min. The agarose gel was prepared in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0). Gels were visualized on a UV transiluminator (GelDoc 2000, Bio-Rad). DNA cleavage was determined by the formation of relaxed circular DNA (Form II).

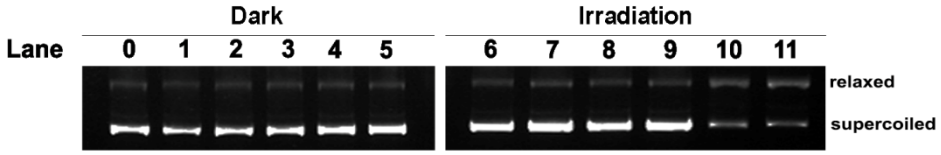


Figure SI 4 – Left part: Agarose gel electrophoresis (1%) of pMT123 plasmid DNA incubated with porphyrin derivatives **1**-**4** in darkness. Lane 0: DNA; lane 1: DNA+DMSO (2%); lane 2: DNA+$H_2TPPF_{16}(SPy)_4$ **1** (40 μM); lane 3: DNA+ $ZnTPPF_{16}(SPy)_4$ **2** (40 μM); lane 4: DNA+$H_2TPPF_{16}(SPyPt)_4$ **3** (40 μM); lane 5: DNA+$ZnTPPF_{16}(SPyPt)_4$ **4** (40 μM). Right part: Agarose gel electrophoresis (1%) of supercoiled pMT123 plasmid DNA photosensitized with porphyrin derivatives **1**-**4**. Lane 6: DNA+irradiation; lane 7: DNA+DMSO (2%)+irradiation; lane 8: DNA+$H_2TPPF_{16}(SPy)_4$ **1** (40 μM)+irradiation; lane 9: DNA+ $ZnTPPF_{16}(SPy)_4$ **2** (40 μM)+irradiation; lane 10: DNA+$H_2TPPF_{16}(SPyPt)_4$ **3** (40 μM)+irradiation; lane 11: DNA+$ZnTPPF_{16}(SPyPt)_4$ **4** (40 μM)+irradiation.

1.8 NMR spectra of porphyrin derivatives

1.8.1 ^{1}H NMR spectra

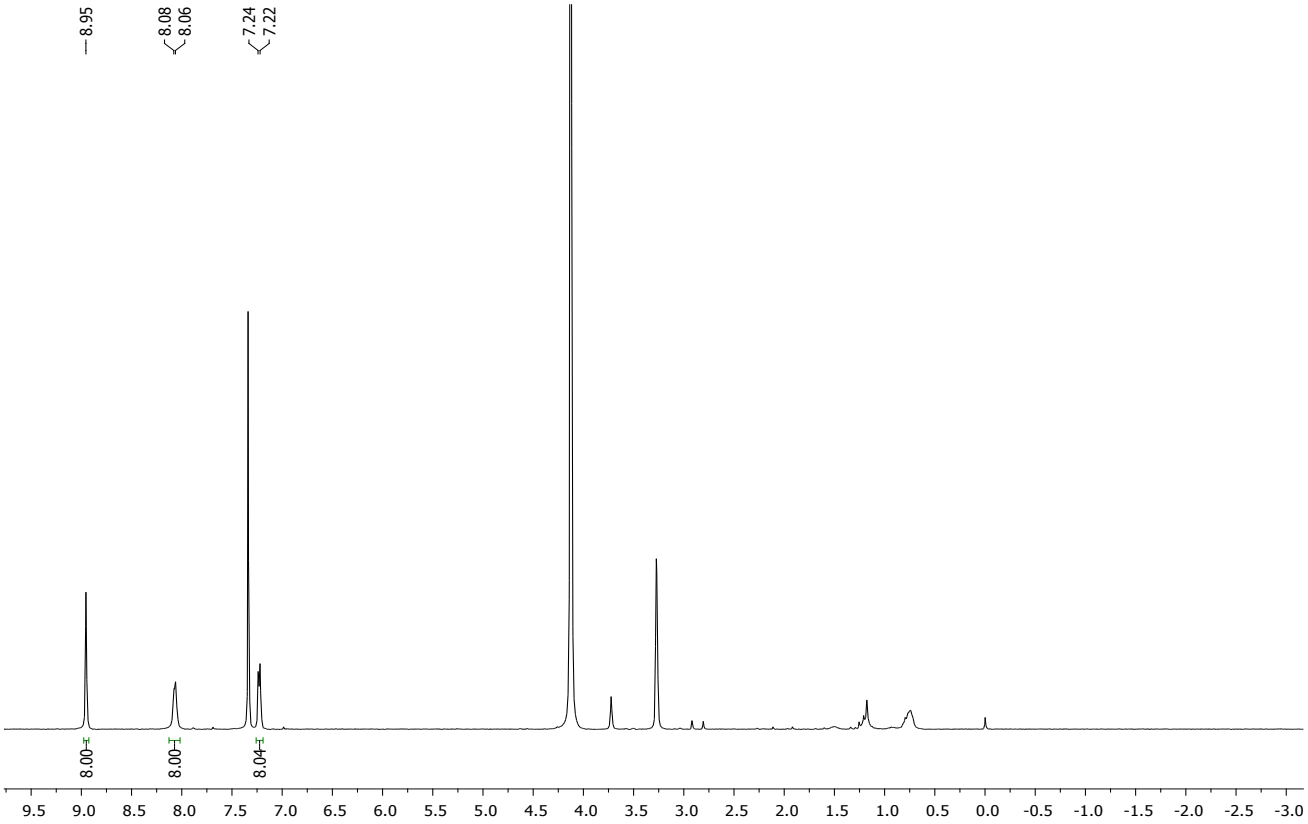


Figure SI 5 – ^{1}H NMR spectrum of $ZnTPPF_{16}(SPy)_4$ **2** in $CDCl_3$.

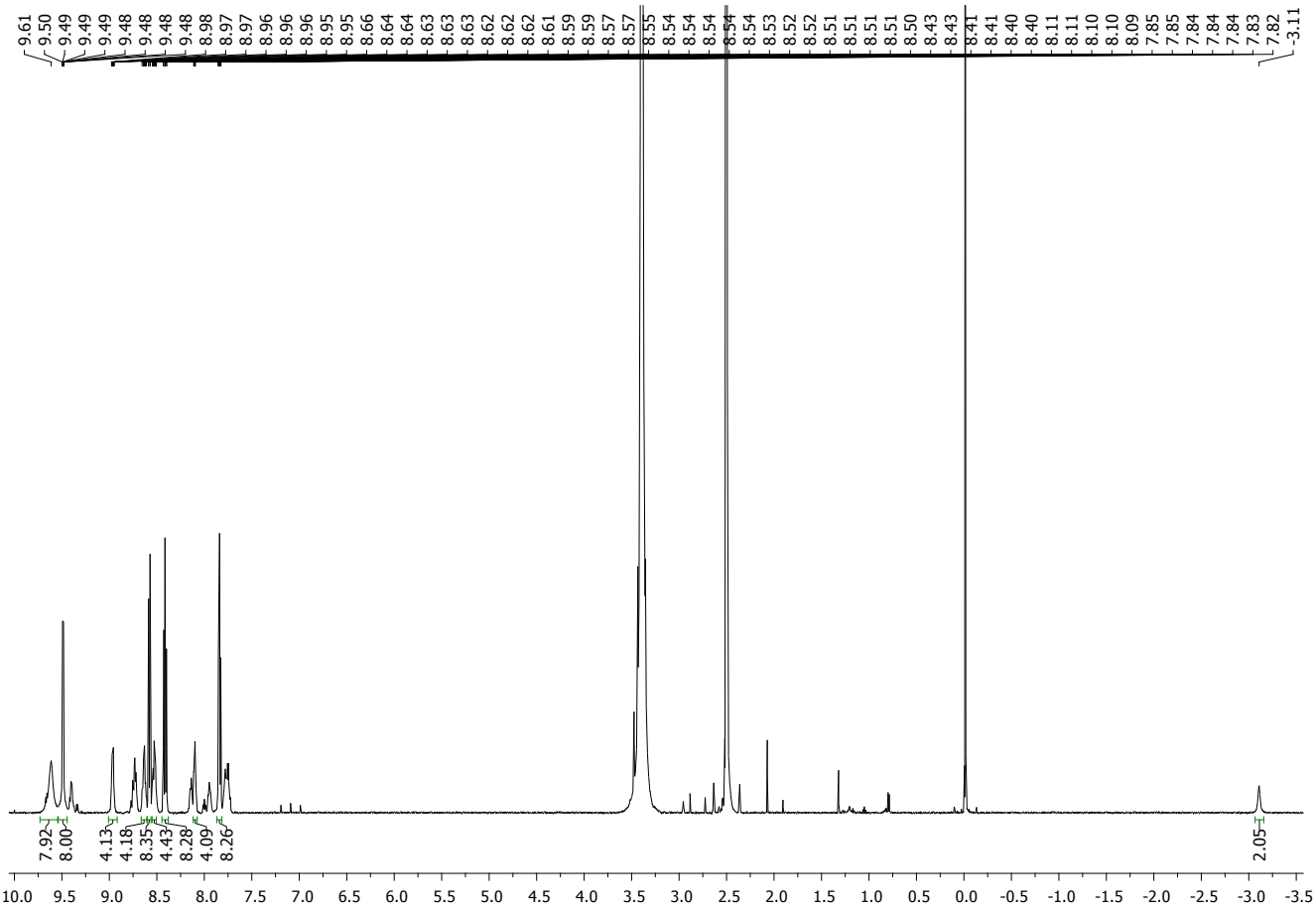


Figure SI 6 – ^{1}H NMR spectrum of $H_2TPPF_{16}(SPyPt)_4$ **3** in DMSO-d_6.

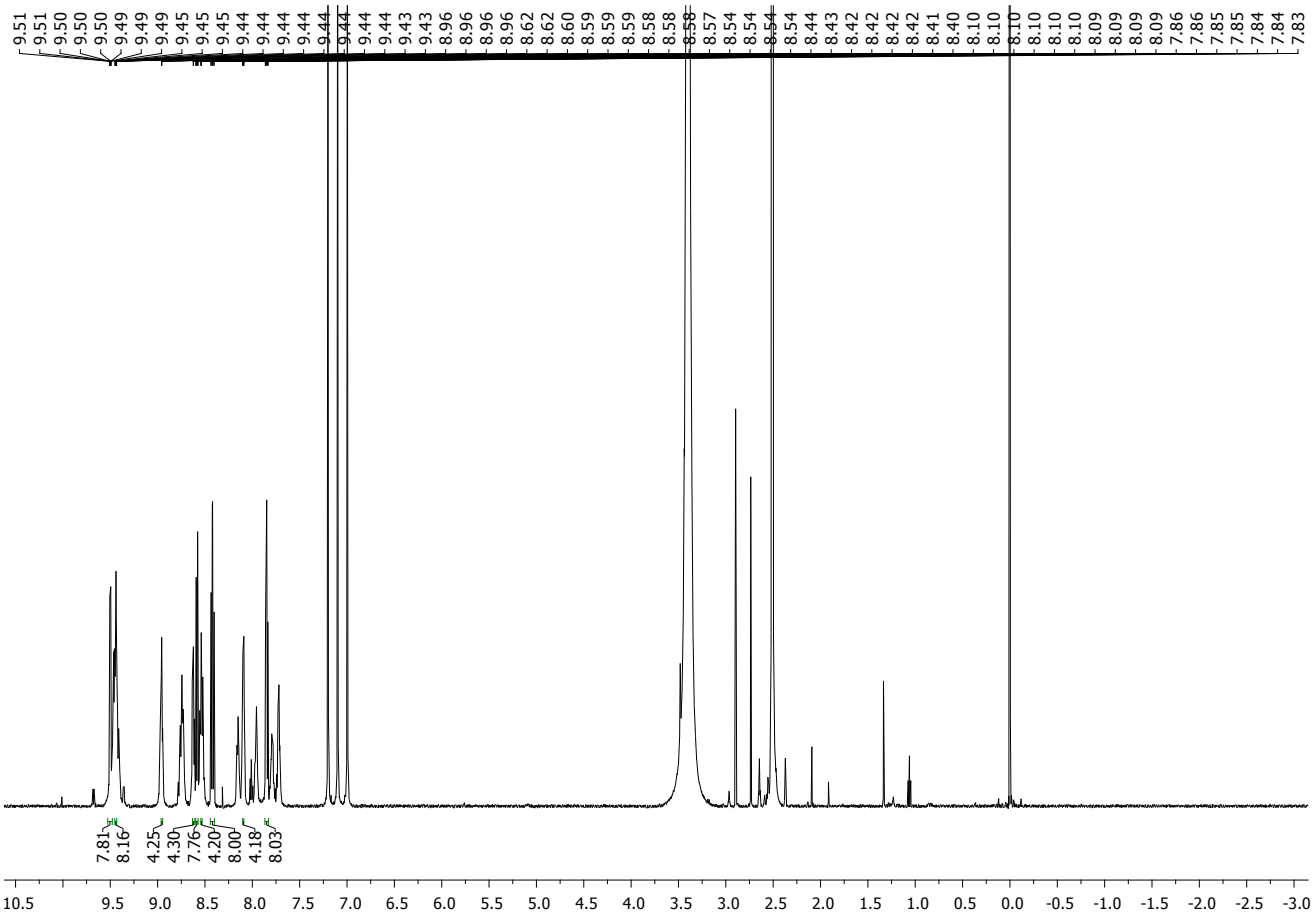


Figure SI 7 – ^{1}H NMR spectrum of $ZnTPPF_{16}(SPyPt)_4$ **4** in DMSO-d_6.

1.8.2 COSY 2D ^{1}H-^{1}H NMR spectra

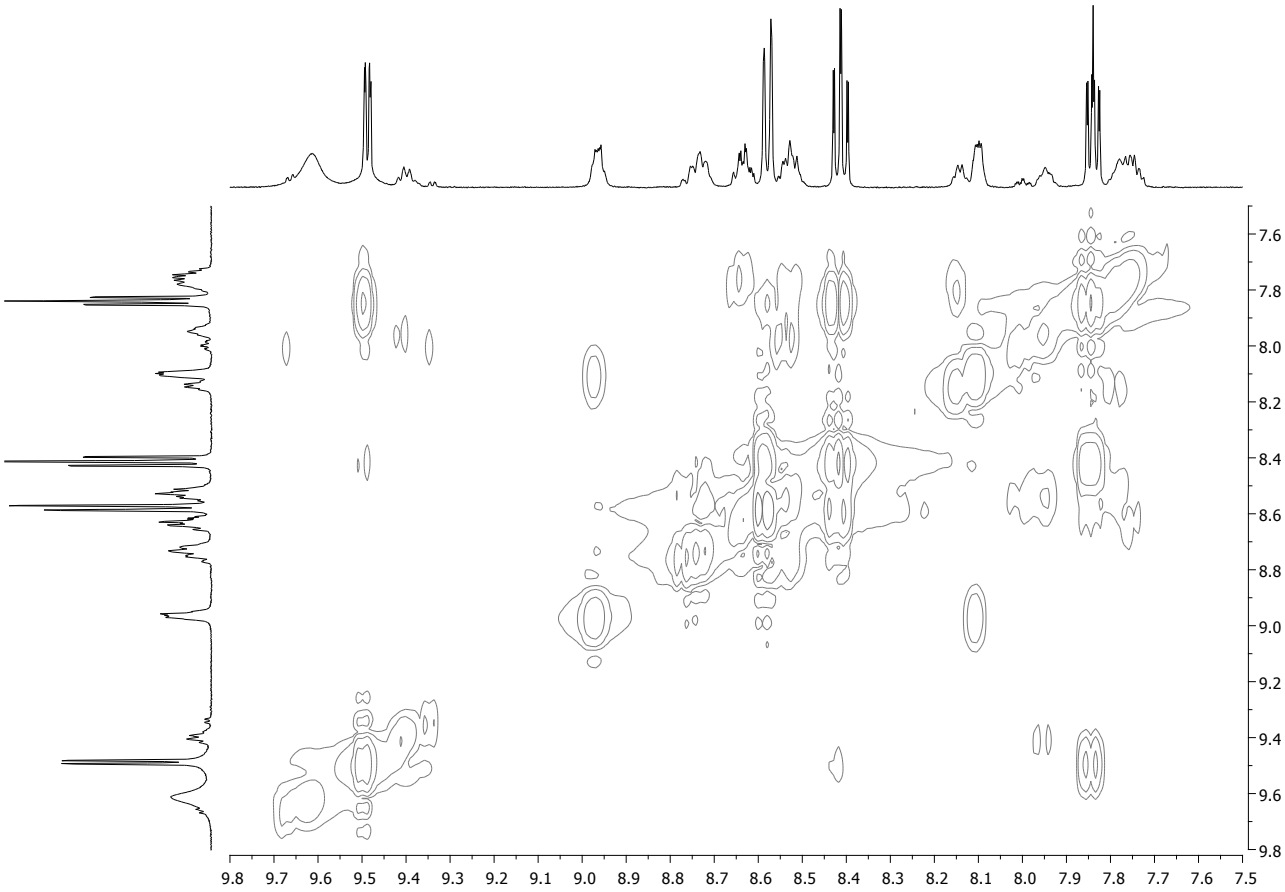


Figure SI 8 – COSY 2D ^{1}H-^{1}H NMR spectrum of $H_2TPPF_{16}(SPyPt)_4$ **3** in DMSO-d_6.

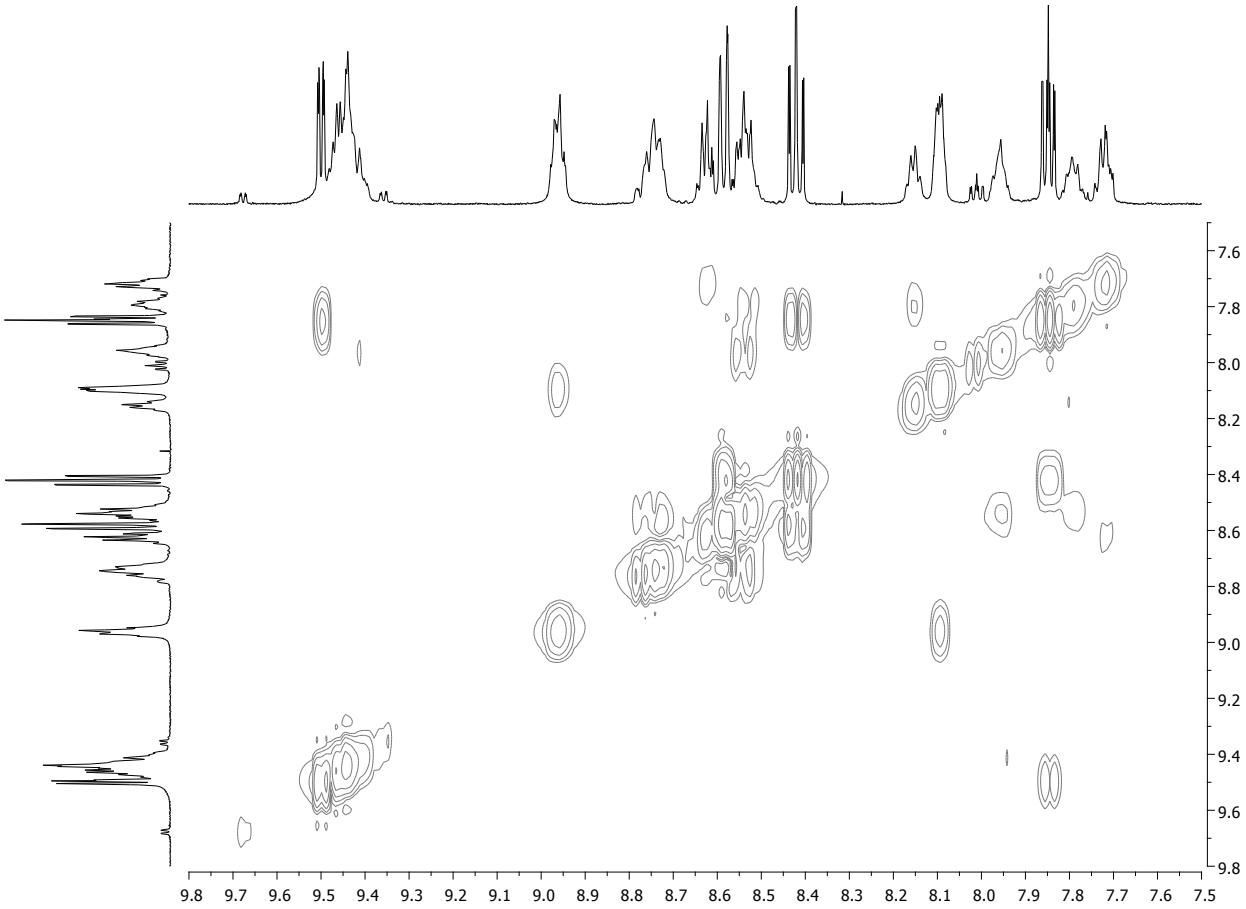


Figure SI 9 – COSY 2D ^{1}H-^{1}H NMR spectrum of $ZnTPPF_{16}(SPyPt)_4$ **4** in DMSO-d_6.

1.8.3 ^{19}F NMR spectra

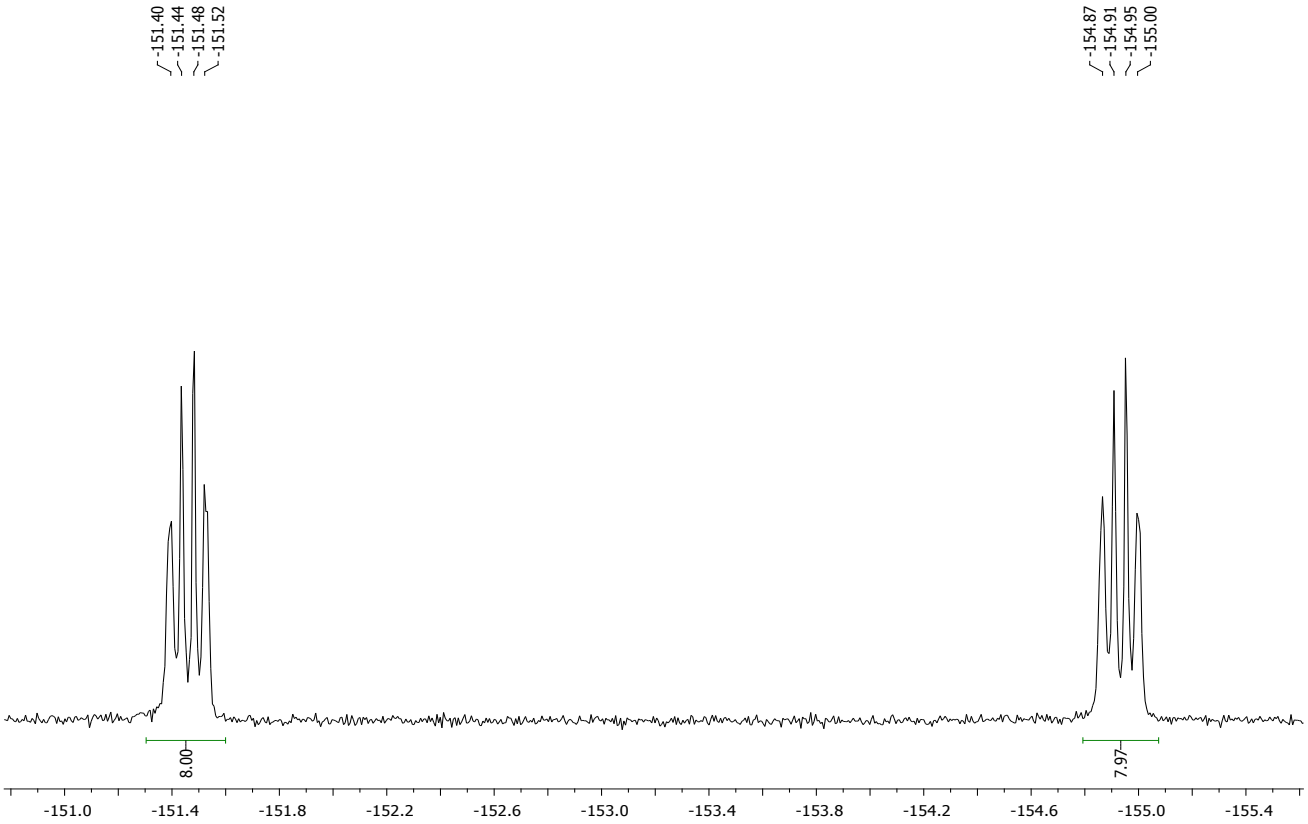


Figure SI 10 – ^{19}F NMR spectrum of $ZnTPPF_{16}(SPy)_4$ **2** in $CDCl_3$.

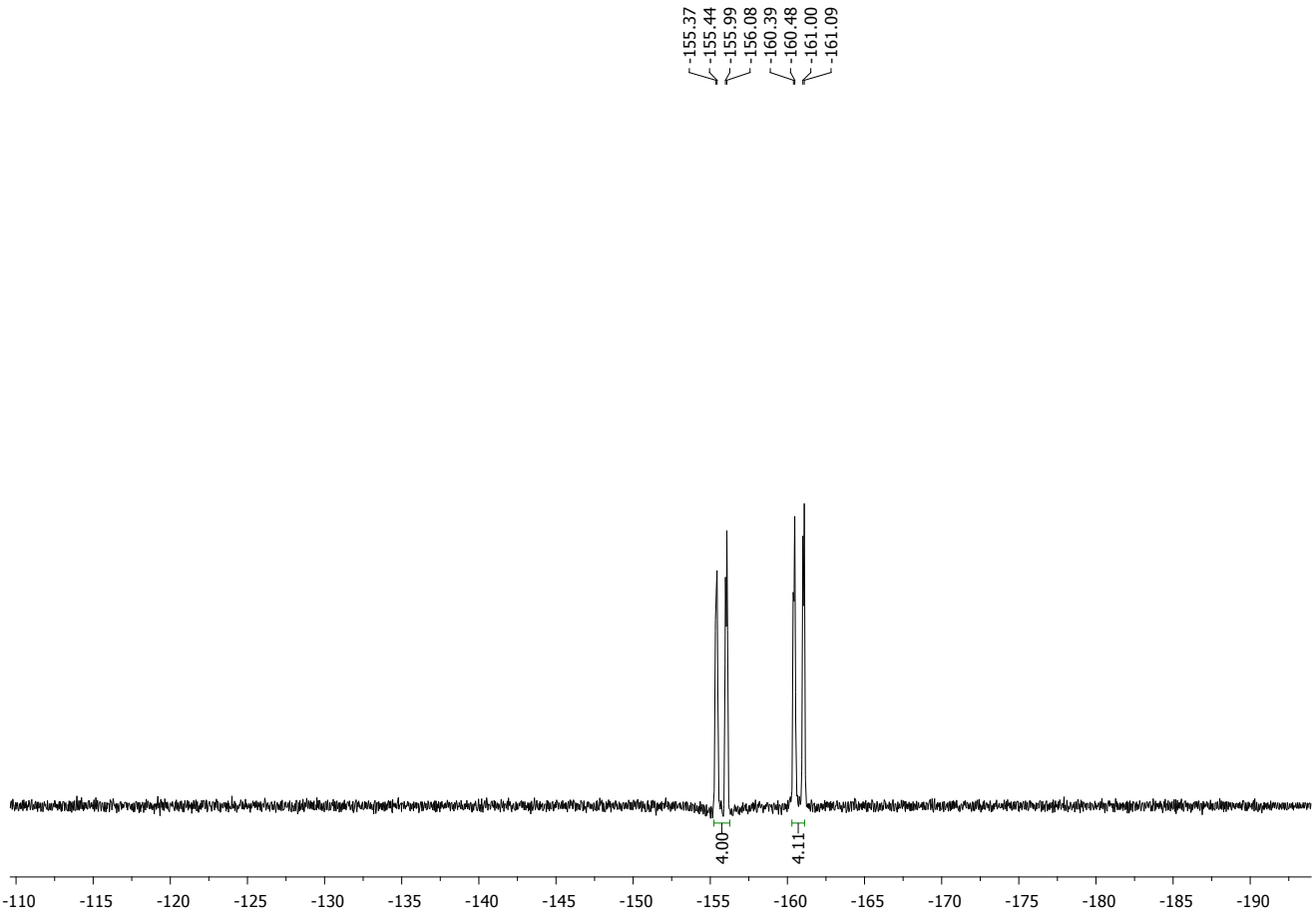


Figure SI 11 – ^{19}F NMR spectrum of $H_2TPPF_{16}(SPyPt)_4$ **3** in DMSO-d_6.

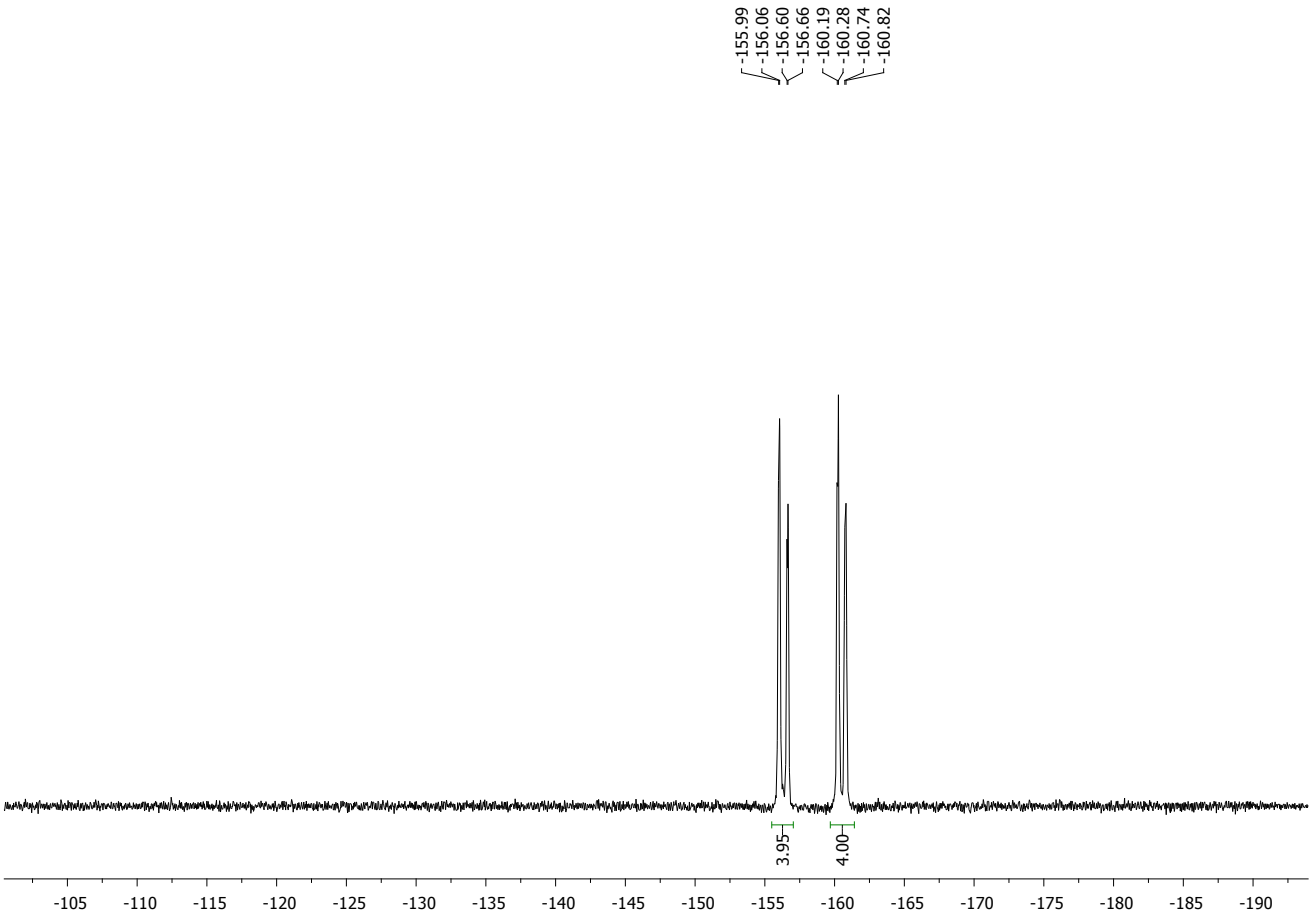
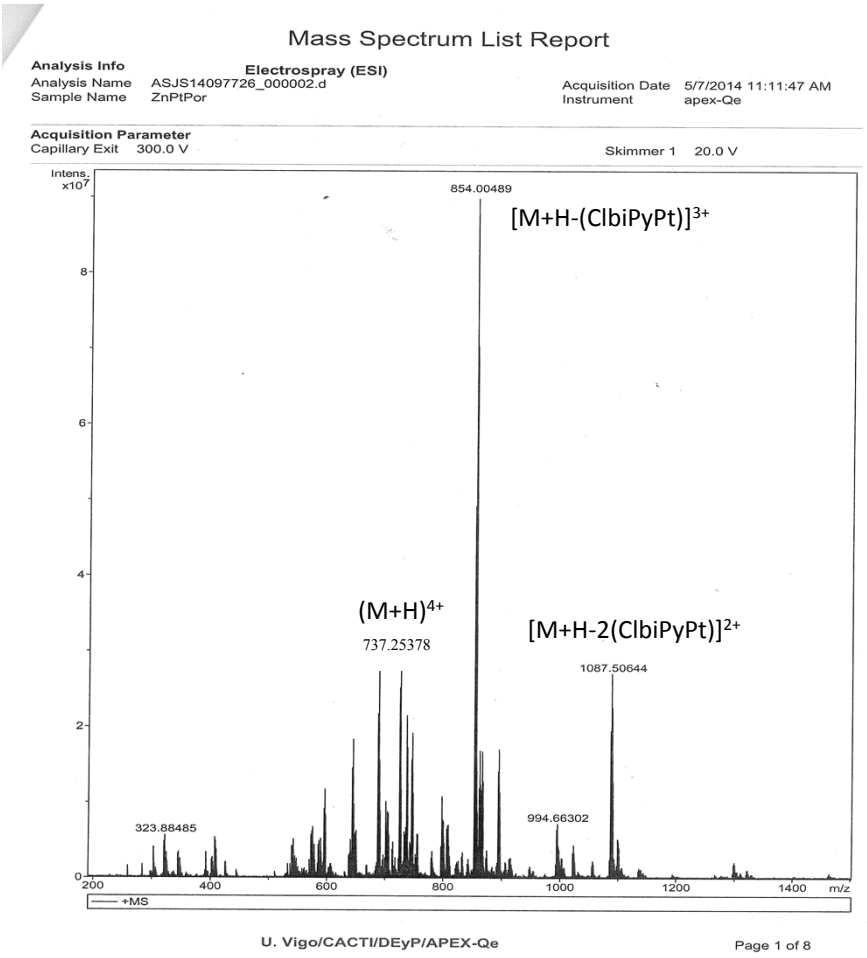


Figure SI 12 – ^{19}F NMR spectrum of $ZnTPPF_{16}(SPyPt)_4$ **4** in DMSO-d_6.

1.9 Mass spectra of porphyrin derivatives



Mass Spectrum Molecular Formula Report

Meas. m/z	#	Formula	Score	m/z	err [mDa]	err [ppm]	mSigma	rdb	e⁻ Conf	N-Rule
737.25378	1	C 105 H 57 Cl 4 F 16 N 15 Pt 4 S 4 Zn	100.00	735.75306	-0.59	-0.79	204.0	75.0	even	ok
	2	C 104 H 56 Cl 4 F 16 N 16 Pt 4 S 4 Zn	24.38	736.00186	-1.79	-2.43	209.6	75.0	even	ok
	3	C 104 H 54 Cl 4 F 16 N 16 Pt 4 S 4 Zn	4.22	735.49795	-5.69	-7.71	242.0	76.0	even	ok
	4	C 104 H 58 Cl 4 F 16 N 16 Pt 4 S 4 Zn	0.00	736.50578	2.11	2.86	510.2	74.0	even	ok
	5	C 105 H 52 Cl 4 F 16 N 16 Pt 4 S 4 Zn	0.00	737.99404	-10.29	-13.95	615.5	78.0	even	ok
854.00489	1	C 94 H 48 Cl 3 F 16 N 14 Pt 3 S 4 Zn	100.00	852.66902	-2.55	-2.98	66.0	68.5	even	-
	2	C 95 H 49 Cl 3 F 16 N 13 Pt 3 S 4 Zn	20.43	852.33728	-0.98	-1.15	115.3	68.5	even	-
	3	C 93 H 47 Cl 3 F 16 N 15 Pt 3 S 4 Zn	0.36	853.00076	-4.15	-4.86	150.1	68.5	even	-
	4	C 95 H 51 Cl 3 F 16 N 13 Pt 3 S 4 Zn	0.01	853.00916	4.26	4.99	159.5	67.5	even	-
	5	C 92 H 46 Cl 3 F 16 N 16 Pt 3 S 4 Zn	0.00	853.33250	-5.72	-6.69	255.8	68.5	even	-
	6	C 94 H 50 Cl 3 F 16 N 14 Pt 3 S 4 Zn	0.00	853.34090	2.69	3.15	257.4	67.5	even	-
	7	C 93 H 49 Cl 3 F 16 N 15 Pt 3 S 4 Zn	0.00	853.67265	1.06	1.24	357.8	67.5	even	-
	8	C 90 H 40 Cl 2 F 16 N 11 Pt 4 S 4	0.00	852.00067	-2.92	-3.42	424.7	67.5	even	ok
	9	C 92 H 48 Cl 3 F 16 N 16 Pt 3 S 4 Zn	0.00	854.00439	-0.50	-0.59	446.8	67.5	even	-
	10	C 91 H 41 Cl 2 F 16 N 10 Pt 4 S 4	0.00	851.66893	-1.10	-1.29	507.5	67.5	even	ok
	11	C 94 H 43 Cl 2 F 16 N 8 Pt 4 S 4	0.00	855.00546	0.54	0.63	555.5	68.5	even	ok
	12	C 93 H 42 Cl 2 F 16 N 9 Pt 4 S 4	0.00	855.33720	-2.64	-3.09	599.2	68.5	even	ok

Figure SI 13 – ESI-HRMS spectrum and report of $ZnTPPF_{16}(SPyPt)_4$ **4**.

1.10 References

1. M. C. Gomes, S. M. Woranovicz-Barreira, M. A. F. Faustino, R. Fernandes, M. G. P. M. S. Neves, A. C. Tomé, N. C. M. Gomes, A. Almeida, J. A. S. Cavaleiro, Â. Cunha and J. P. C. Tomé, *Photochem. Photobiol. Sci*., 2011, **10**, 1735.
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3. P. M. R. Pereira, J. J. Carvalho, S. Silva, J. A. S. Cavaleiro, R. Schneider, R. Fernandes and J. P. C. Tomé, *Org. Biomol. Chem.*, 2014, **12**, 1804.