A combined experimental and computational study of the substituent effect on photodynamic efficacy of amphiphilic Zn(II)phthalocyanines

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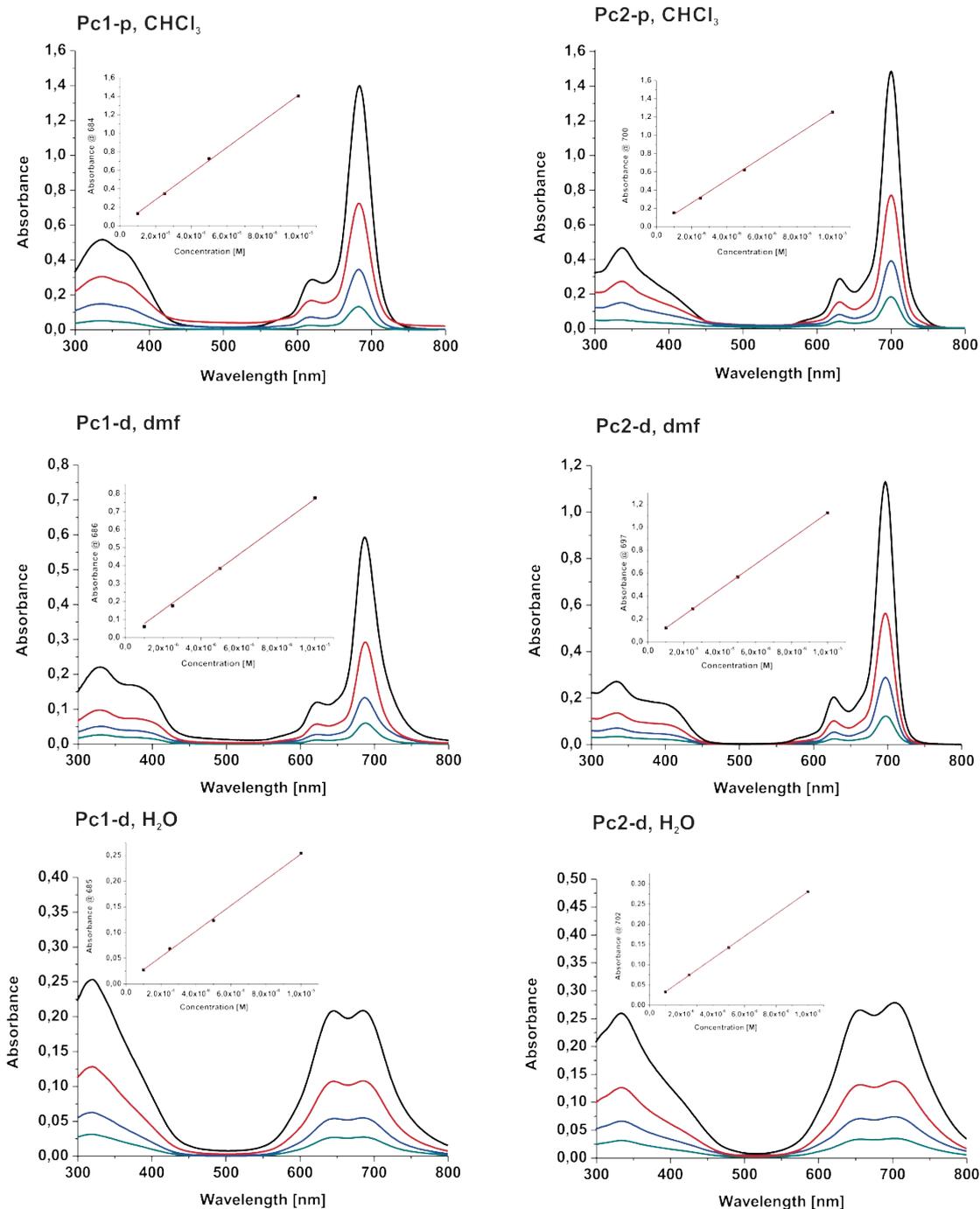[d] DFG EXC 1003 'Cells in Motion' Cluster of Excellence, University of Münster, Germany

Supporting Information

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1. Photophysical Data

1.1. Absorption spectra

Figure S1. UV-Visible spectra of **Pc1-p, Pc2-p, Pc1-d** and **Pc2-d** in different solvents ($CHCl_3$, DMF and H_2O) at different concentrations (1 to 10 μM). The inset of each spectrum plots the Q-band absorbance *vs* the concentration of the Zn(II)phthalocyanine and the line represents the best-fitted straight line.

1.2. Excitation and emission spectra

Pc1-p, CHCl$_3$

Pc2-p, CHCl$_3$

Pc1-d, dmf

Pc2-d, dmf
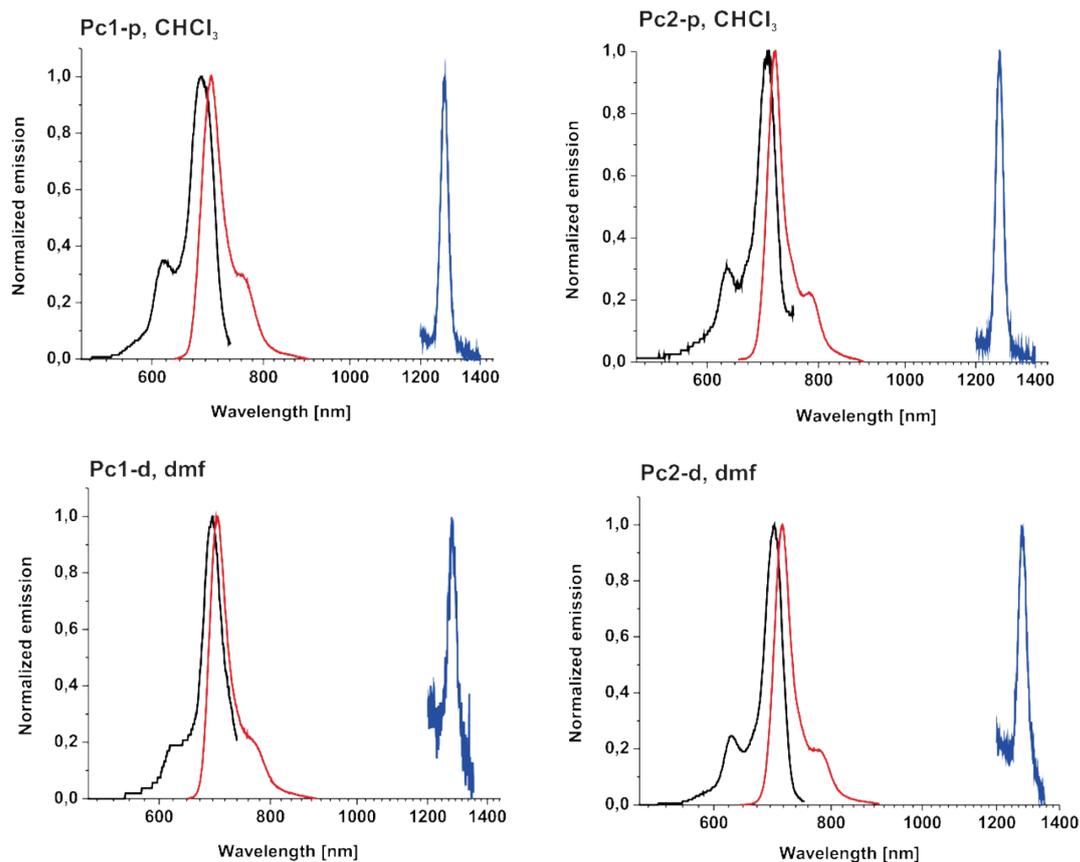
Figure S2. Excitation, fluorescence and singlet oxygen emission spectra of **Pc1-p** (λ_{exc}=610nm, λ_{em}=760nm) and **Pc2-p** (λ_{exc}=625nm, λ_{em}=784nm) in CHCl$_3$ and **Pc1-d** (λ_{exc}=610nm, λ_{em}=760nm) and **Pc2-d** (λ_{exc}=625nm, λ_{em}=785nm) in DMF.

1.3. Time resolved luminescence dacays

X^2 (red) = 0.958
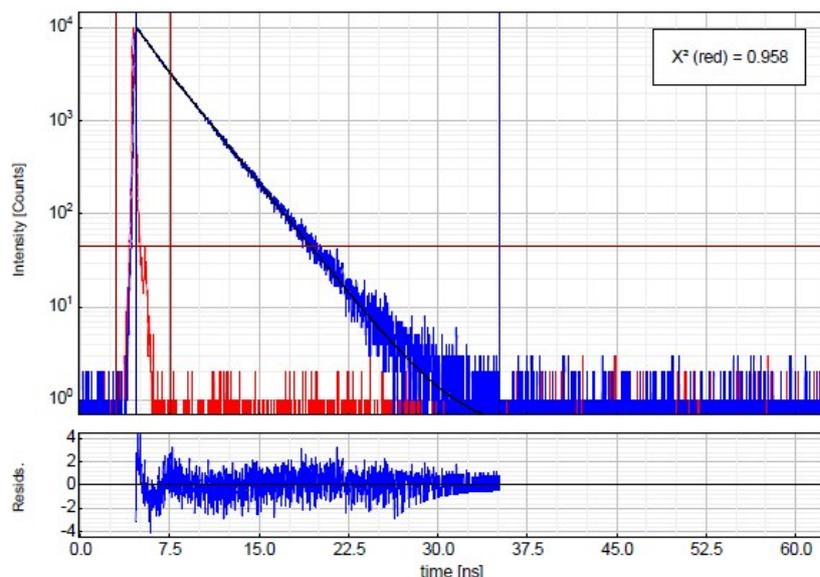
Parameter	Value	Conf. Lower	Conf. Upper	Conf. Estimation
A_1 [Cnts]	11470.9	-59.9	+59.9	Fitting
τ_1 [ns]	2.75108	-0.00987	+0.00987	Fitting
Bkgr. Dec [Cnts]	0.477	-0.431	+0.431	Fitting
Bkgr. IRF [Cnts]	45.49	-2.10	+2.10	Fitting
Shift IRF [ns]	-0.1187	-0.0134	+0.0134	Fitting

Figure S3. Time-resolved luminescence decay of **ZnPc1-p** in $CHCl_3$ including the instrument response function and the residuals (λ_{exc}=635 nm) and fitting parameters including pre-exponential factors and confidence limits.

X^2 (red) = 1.000
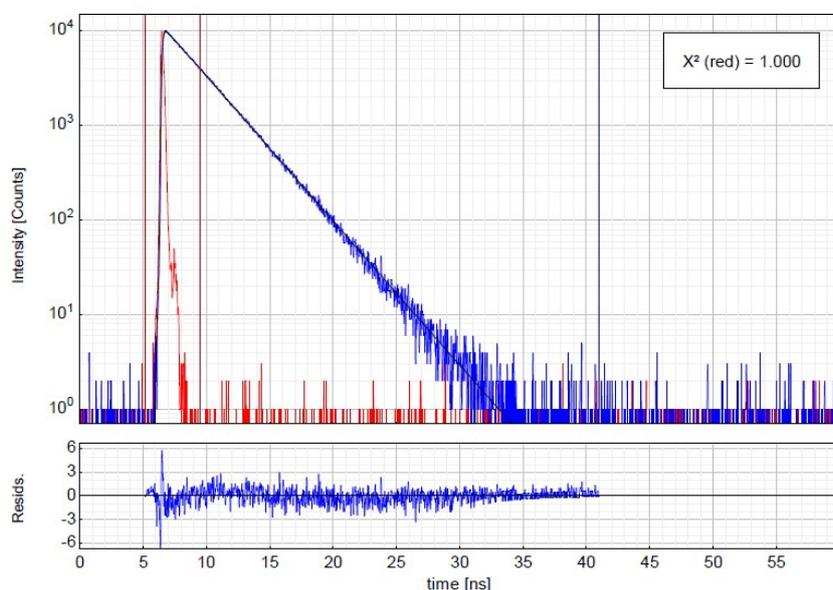
Parameter	Value	Conf. Lower	Conf. Upper	Conf. Estimation
A_1 [Cnts]	11435.2	-60.3	+60.3	Fitting
τ_1 [ns]	2.8378	-0.0106	+0.0106	Fitting
Bkgr. Dec [Cnts]	0.0357	-0.381	+0.381	Fitting
Bkgr. IRF [Cnts]	0.496	-0.662	+0.662	Fitting
Shift IRF [ns]	0.01966	-0.00265	+0.00265	Fitting

Figure S4. Time-resolved luminescence decay of **ZnPc2-p** in $CHCl_3$ including the instrument response function and the residuals (λ_{exc}=635 nm) and fitting parameters including pre-exponential factors and confidence limits.

X^2 (red) = 1.004
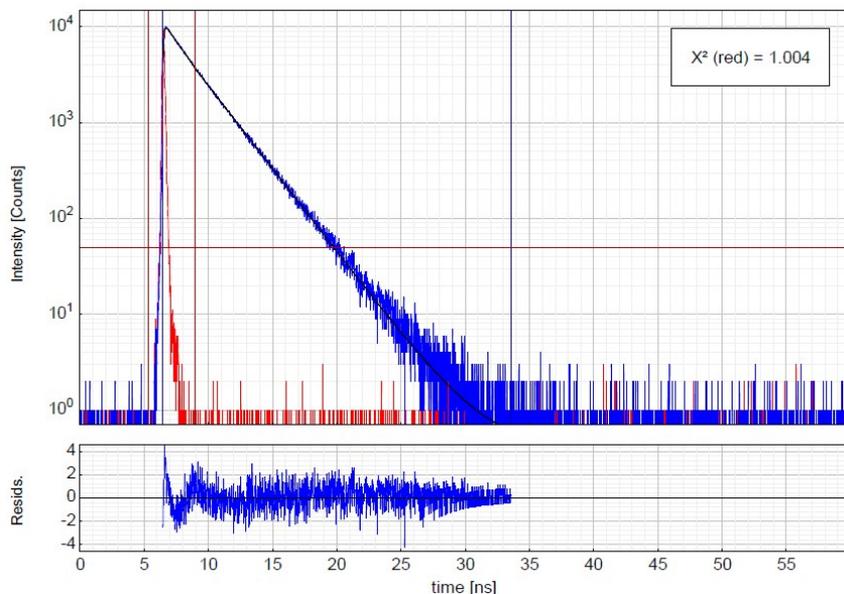
Parameter	Value	Conf. Lower	Conf. Upper	Conf. Estimation
A_1 [Cnts]	11342.5	-58.4	+58.4	Fitting
τ_1 [ns]	2.50436	-0.00919	+0.00919	Fitting
Bkgr. Dec [Cnts]	0.415	-0.459	+0.459	Fitting
Bkgr. IRF [Cnts]	49.09	-2.36	+2.36	Fitting
Shift IRF [ns]	-0.02066	-0.00300	+0.00300	Fitting

Figure S5. Time-resolved luminescence decay of **ZnPc1-d** in DMF including the instrument response function and the residuals (λ_{exc}=635 nm) and fitting parameters including pre-exponential factors and confidence limits.

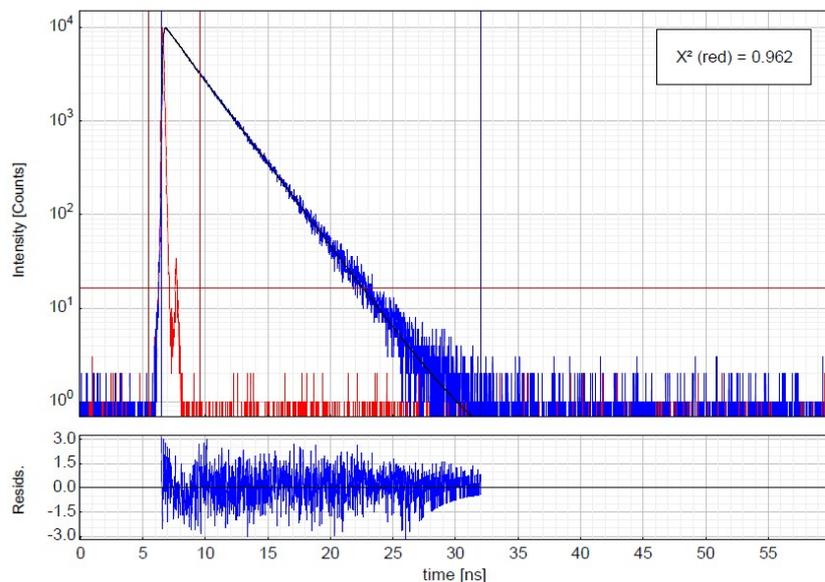X^2 (red) = 0.951
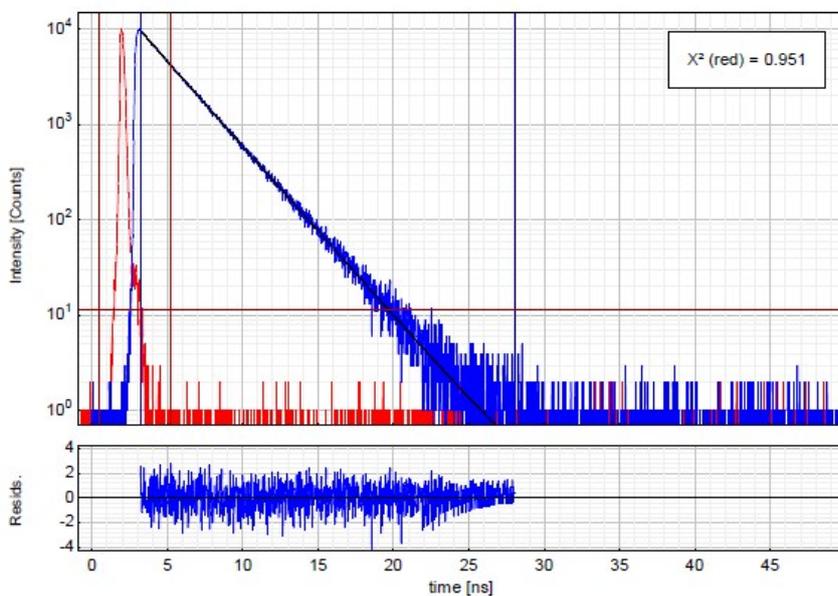
Parameter	Value	Conf. Lower	Conf. Upper	Conf. Estimation
A_1 [Cnts]	16067.1	-80.6	+80.6	Fitting
τ_1 [ns]	2.44662	-0.00687	+0.00687	Fitting
Bkgr. Dec [Cnts]	0.087	-0.479	+0.479	Fitting
Bkgr. IRF [Cnts]	11.44	-2.15	+2.15	Fitting
Shift IRF [ns]	-0.9254	-0.0120	+0.0120	Fitting

Figure S6. Time-resolved luminescence decay of **ZnPc2-d** in DMF including the instrument response function and the residuals (λ_{exc}=635 nm) and fitting parameters including pre-exponential factors and confidence limits.

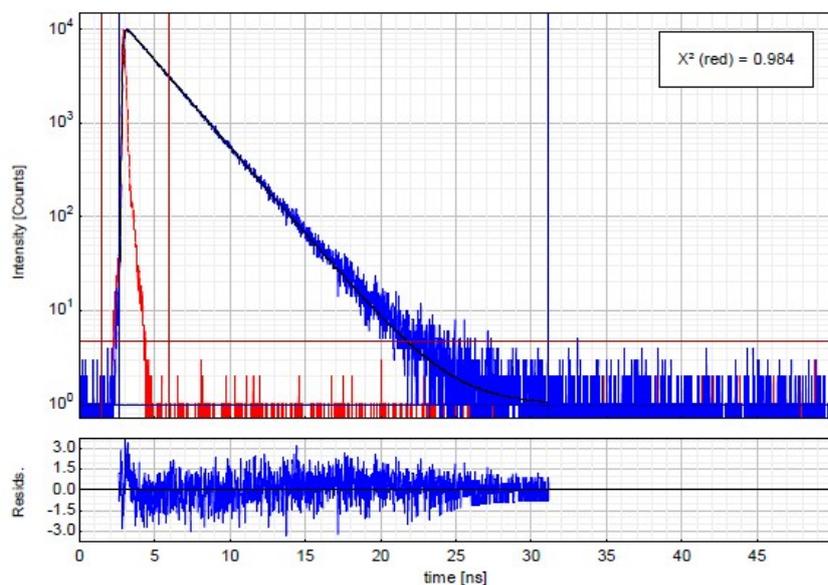X² (red) = 0.962

| Parameter	Value	Conf. Lower	Conf. Upper	Conf. Estimation
A_1 [Cnts]	11208.7	-53.9	+53.9	Fitting
τ_1 [ns]	2.46851	-0.00846	+0.00846	Fitting
Bkgr. Dec [Cnts]	0.230	-0.484	+0.484	Fitting
Bkgr. IRF [Cnts]	16.31	-1.95	+1.95	Fitting
Shift IRF [ns]	-0.05793	-0.00221	+0.00221	Fitting
A Scat [Cnts]	-4080	-1520	+1520	Fitting

Figure S7. Time-resolved luminescence decay of **ZnPc1-d** in H_2O including the instrument response function and the residuals (λ_{exc}=635 nm) and fitting parameters including pre-exponential factors and confidence limits.


X² (red) = 0.984

Parameter	Value	Conf. Lower	Conf. Upper	Conf. Estimation
A_1 [Cnts]	11325.3	-59.1	+59.1	Fitting
τ_1 [ns]	2.34455	-0.00873	+0.00873	Fitting
Bkgr. Dec [Cnts]	0.985	-0.435	+0.435	Fitting
Bkgr. IRF [Cnts]	4.73	-1.64	+1.64	Fitting
Shift IRF [ns]	0.13918	-0.00219	+0.00219	Fitting
A Scat [Cnts]	25250	-2210	+2210	Fitting

Figure S8. Time-resolved luminescence decay of **ZnPc2-d** in H_2O including the instrument response function and the residuals (λ_{exc}=635 nm) and fitting parameters including pre-exponential factors and confidence limits.

## 1.4. Determination of singlet molecular oxygen quantum yields
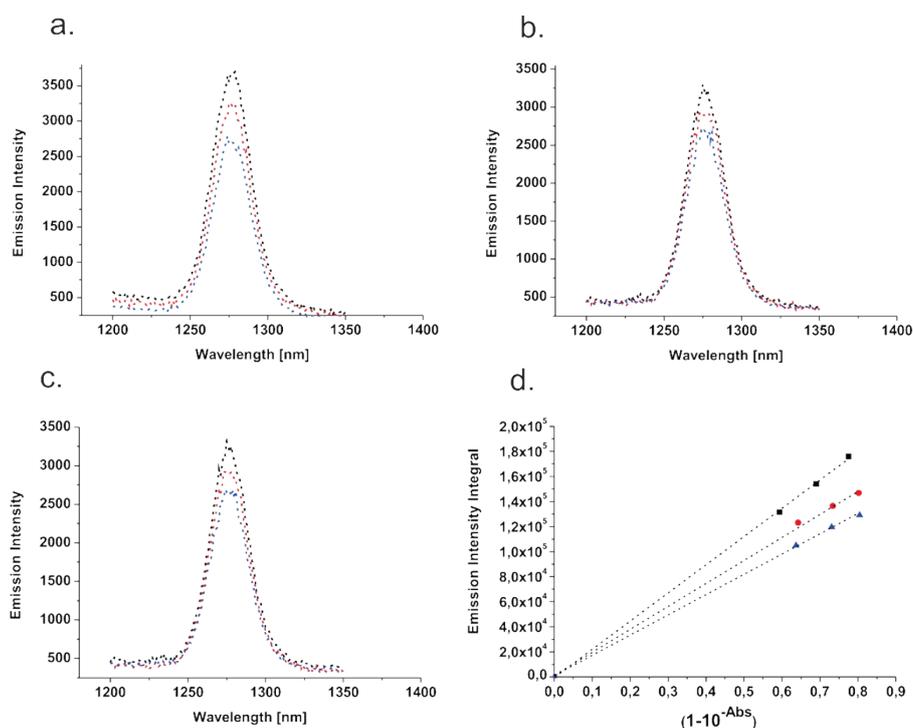
Figure S9. Phosphorescence spectrum of the photogenerated singlet oxygen for tetra-*t*-butylphthalocyaninato zinc(II) (a), **Pc1-p** (b) and **Pc2-p** (c) in CHCl₃ for three concentrations. Plots of the emission intensity against the fraction of absorbed light tetra-*t*-butylphthalocyaninato zinc(II) (balck), **Pc1-p** (blue) and **Pc2-p** (red). The line represents the best-fitted straight line.
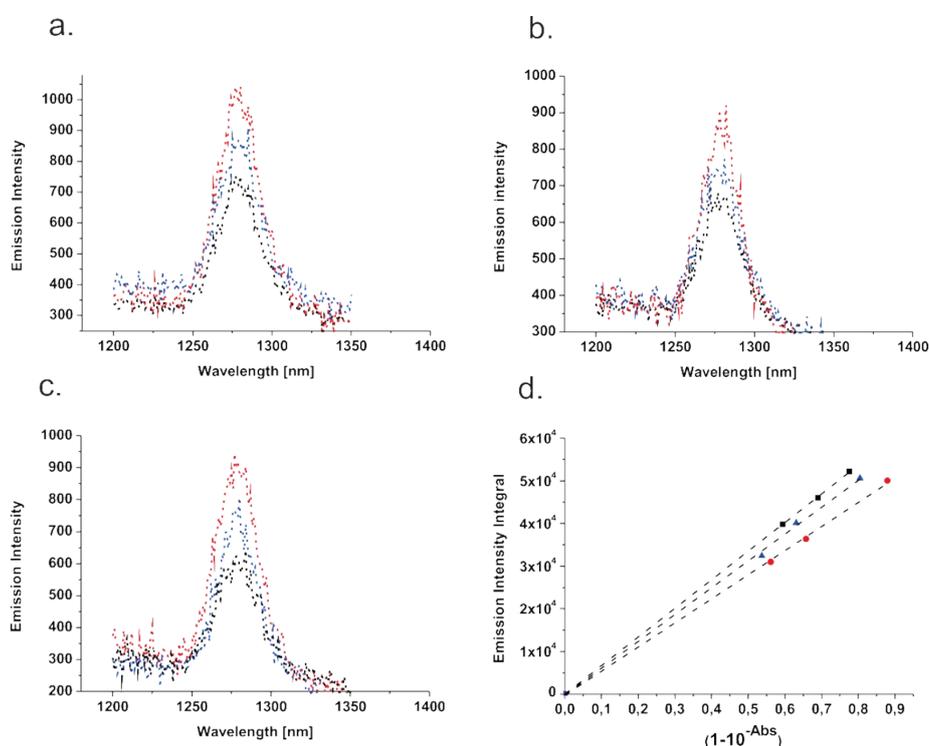

Figure S10. Phosphorescence spectrum of the photogenerated singlet oxygen for tetra-*t*-butylphthalocyaninato zinc(II) (a), **Pc1-d** (b) and **Pc2-d** (c) in DMF for three concentrations. Plots of the emission intensity against the fraction of absorbed light tetra-*t*-butylphthalocyaninato zinc(II) (balck), **Pc1-d** (red) and **Pc2-d** (blue). The line represents the best-fitted straight line.

2. Partition coefficients
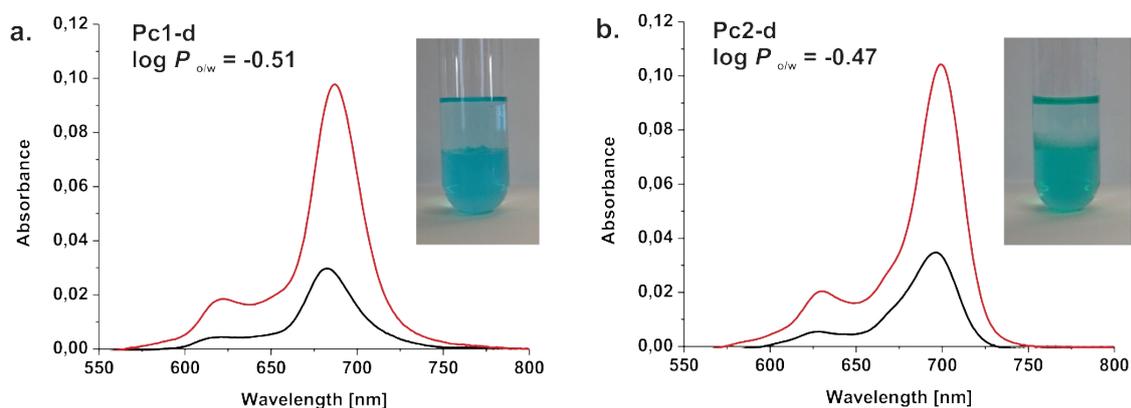
Figure S11. Absorption spectra of **Pc1-d** (a) and **Pc2-d** (b) from water phase – red line and 1-octanol phase – black line.

## 3. Binding to Human Serum Albumin
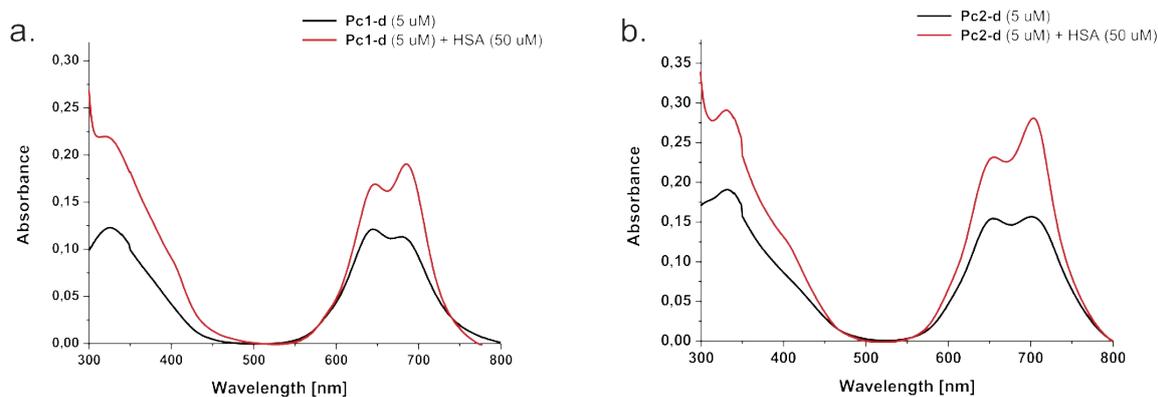
Figure S12. Absorption spectra of **Pc1-d** (a) and **Pc2-d** (b) in the absence (black line) and presence of HSA (red line).

4. Theoretical calculations

Table S1. List of selected molecular orbital energies and HOMO–LUMO energy gaps [eV] for the model system **Pc1-m** and **Pc2-m**.

Orbital	Pc1-m	Pc2-m
LUMO + 5	-0.7878	-1.0928
LUMO + 4	-0.8014	-1.2267
LUMO + 3	-0.8931	-1.2278
LUMO + 2	-1.1886	-1.2498
LUMO + 1	-2.8107	-2.7239

LUMO	-2.8216	-2.7451
HOMO	-4.9498	-4.7971
HOMO – 1	-6.3645	-5.8145
HOMO – 2	-6.4959	-5.8456
HOMO – 3	-6.5095	-6.1650
HOMO – 4	-6.5490	-6.4252
HOMO – 5	-6,8113	-6.7204
HOMO-LUMO gap	2.1282	2.0520

0.0011	0.0037	0.000
0.014	0.022	0.000
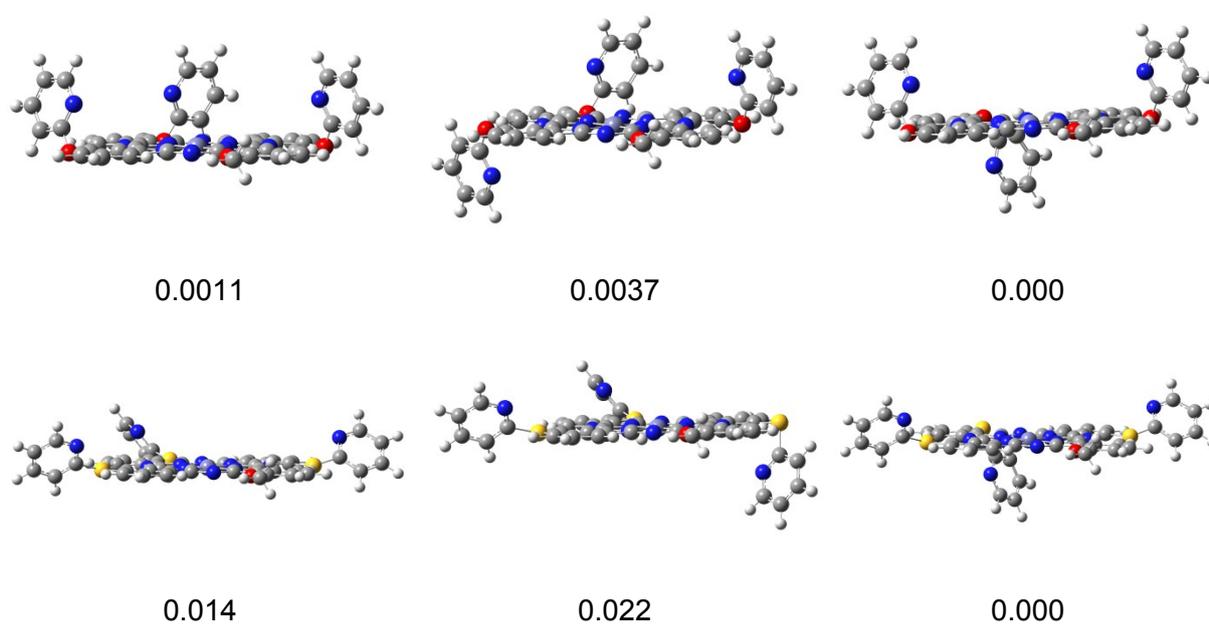
Figure S13. Optimized structures of different conformers of the model system **Pc1-m** and **Pc2-m** and the calculated relative energies (kcal mol^{-1}) using B3LYP/6-311G(d,p).

5. *In vitro* studies

Table S2. Summary of the irradiation experiments.

| Compound		Pos. Control	H₂O Control	Neg. Control	10µg/ml	5µg/ml	1µg/ml
Pc1-d	irradiated	0.00 (±0.16)	99.31 (±2.88)	100.00 (±1.86)	17.28 (±3.60)	57.92 (±12.74)	93.97 (±4.06)
	dark	0.00 (±0.04)	106.15 (±9.11)	100.00 (±7.65)	97.92 (±8.63)	104.87 (±9.38)	108.98 (±14.78)
Pc2-d	irradiated	0.00 (±0.24)	104.56(±6.28)	100.00(±8.92)	2.43 (±1.95)	16.38 (±5.21)	77.98 (±7.39)
	dark	0.00 (±0.15)	111.61(±15.73)	100.00(±9.16)	88.86 (±7.89)	95.80(±11.20)	100.68(±3.90)

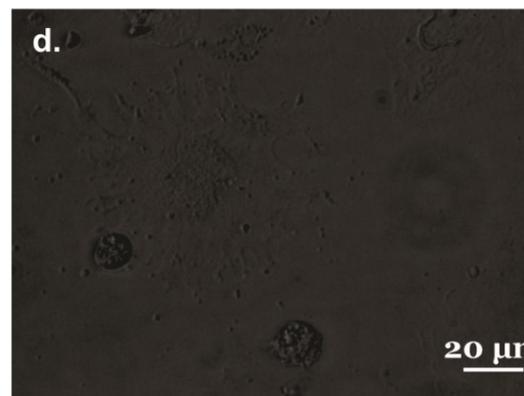**Pc1-d** 0mg/ml 24h after irradiation
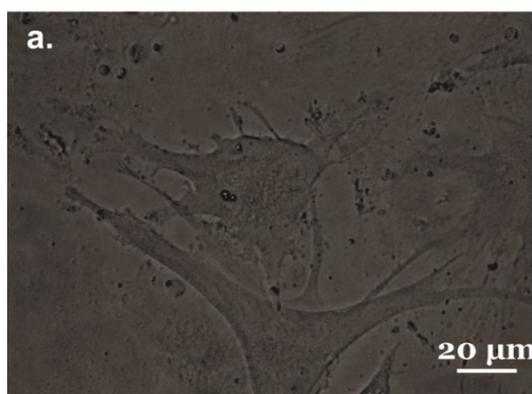
Pc1-d 10mg/ml 24h after irradiation
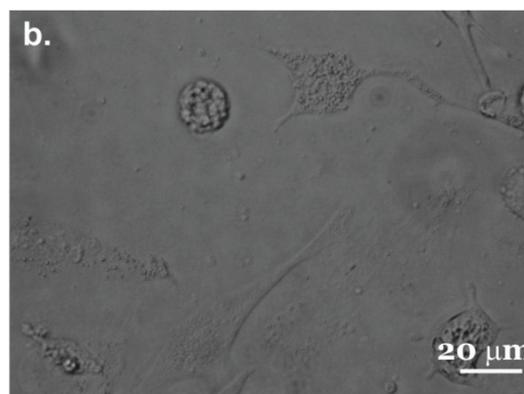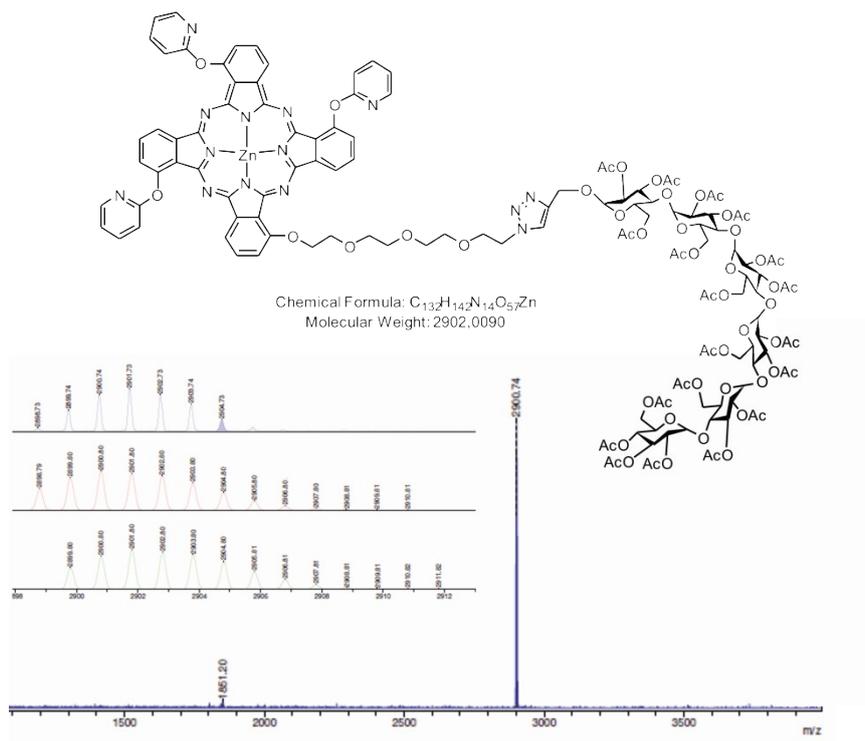
Pc2-d 0mg/ml 24h after irradiation
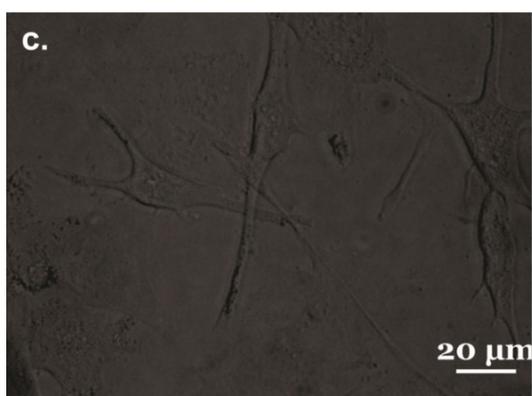
Pc2-d 10mg/ml 24h after irradiation

Figure S14. Morphological changes after PDT for COLO-818 cell line incubated with 10 mg/ml **Pc1-d** (b) and **Pc2-d** (d) 24h after irradiation. Negative controls for **Pc1-d** (a) and **Pc2-d** (c).

6. Analytical Data

Chemical Formula: $C_{132}H_{142}N_{14}O_{57}Zn$
Molecular Weight: 2902,0090

Figure S15. MALDI-MS spectra of **Pc1-p** measured in trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile DCTB matrix (CHCl$_3$). Insert: measured and calculated isotope pattern.

Chemical Formula: $C_{132}H_{142}N_{14}O_{54}S_3Zn$
Molecular Weight: 2950,1920
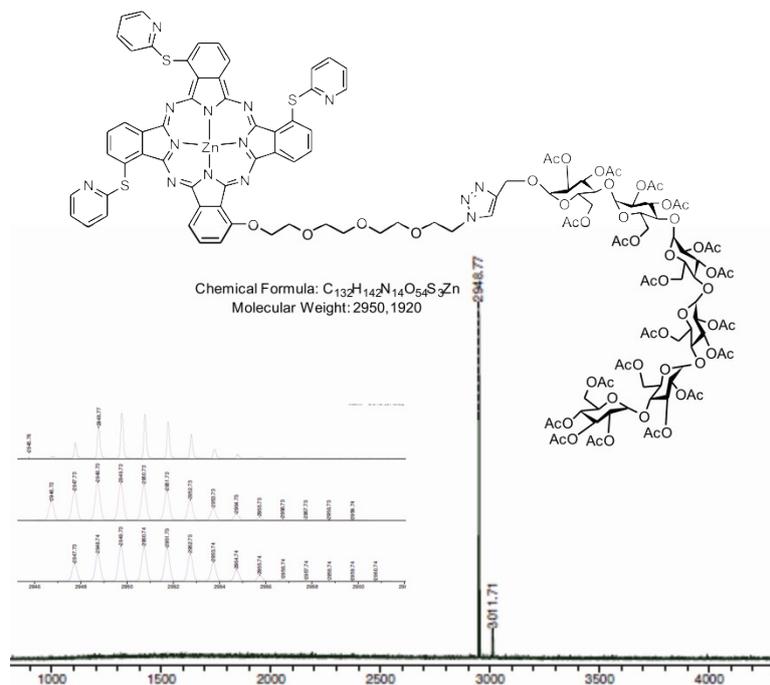
Figure S16. MALDI-MS spectra of **Pc2-p** measured in trans-2-[3-(4-tert-Butylphenyl)-2-methyl-2-propenylidene]malononitrile DCTB matrix (CHCl$_3$). Insert: measured and calculated isotope pattern.
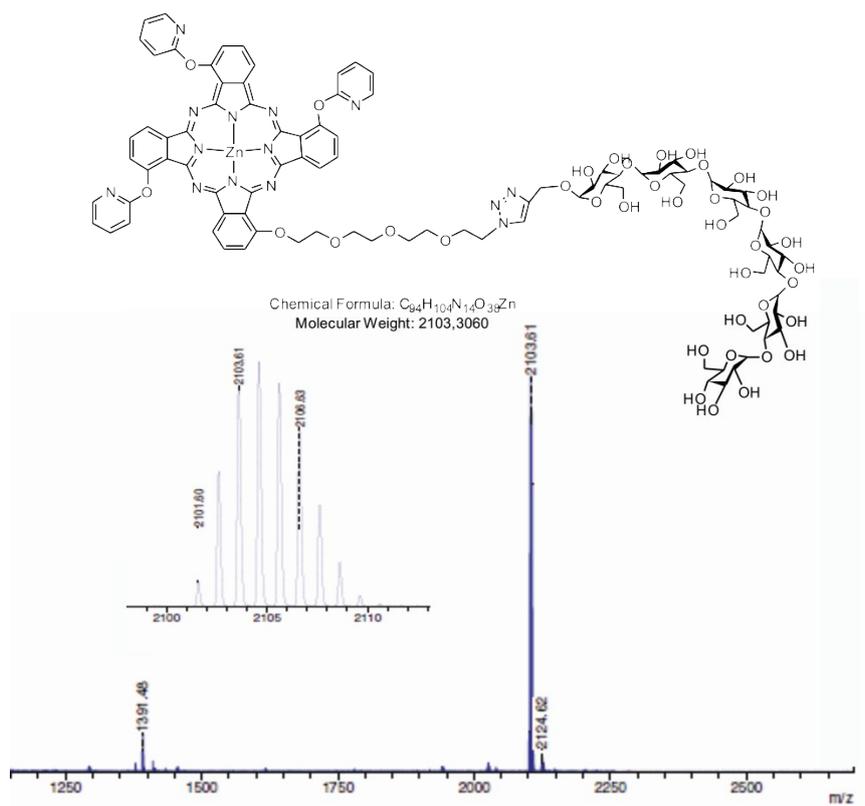
Figure S17. MALDI-MS spectra of **Pc1-d** measured in 2,5-Dihydroxybenzoic acid DHB matrix (H_2O/ACN). Insert: measured isotope pattern.
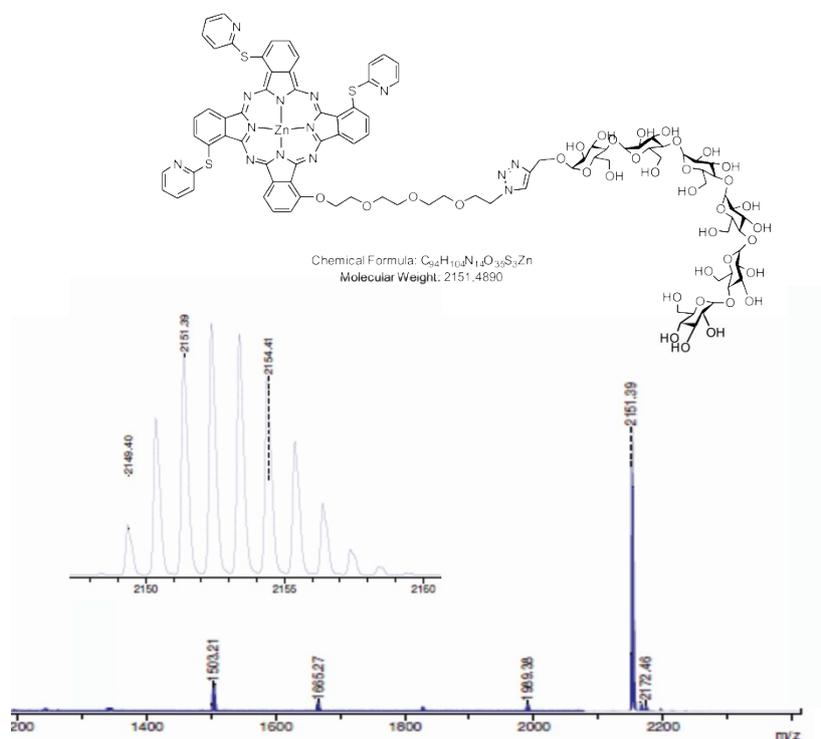
Figure S18. MALDI-MS spectra of **Pc2-d** measured in 2,5-Dihydroxybenzoic acid DHB matrix (H_2O/ACN). Insert: measured isotope pattern.
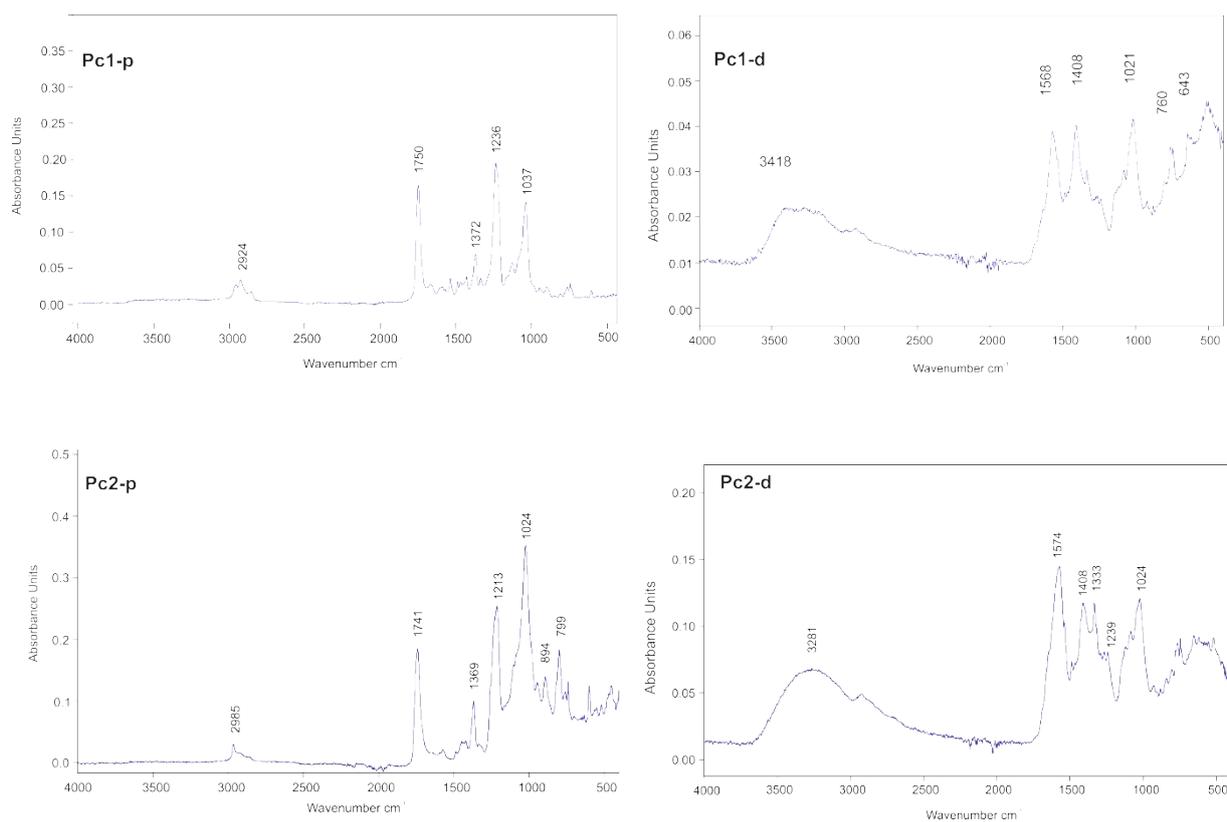
Figure S19. FT-IR spectra of **Pc1-p, Pc1-d, Pc2-p** and **Pc2-d**.

1H NMR spectra show broad chemical shifts likely due to the self-aggregation at NMR concentration as well as presence of four positional isomers.