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Synthesis and photophysical properties of a new glycoconjugate based on *meso*-arylporphyrin

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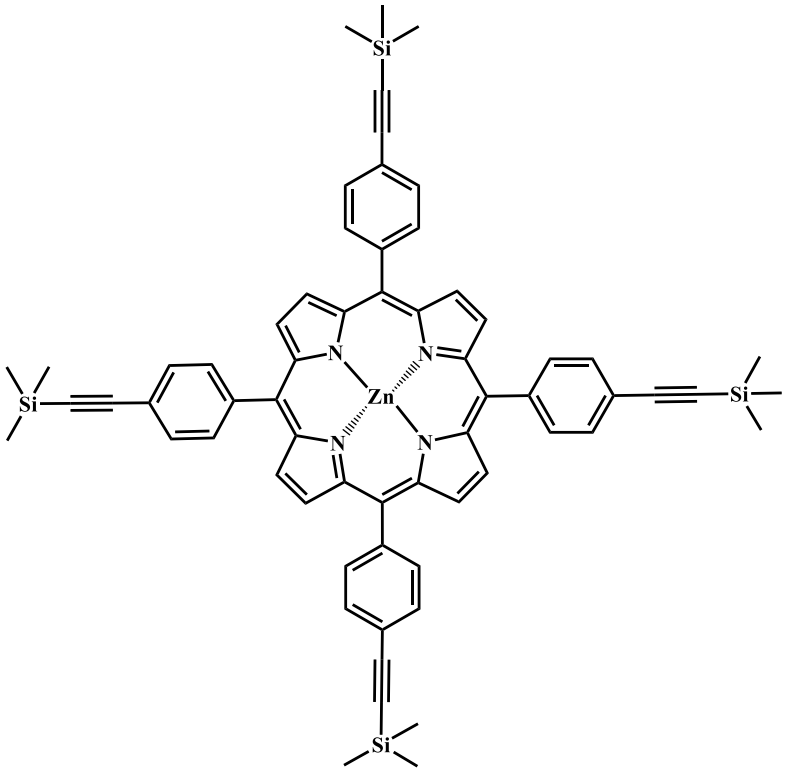
1. General information

All commercial chemicals and reagents were of analytical purity and purchased from Sigma-Aldrich. All solvents were dried according to standard procedures. Silica gel for column chromatography was purchased from Macherey-Nagel (Kieselgel 60, 200-400 mesh) and thin layer chromatography (TLC) was performed on aluminum sheets coated with silica gel 60 F254 (Macherey-Nagel). NMR spectra (^{1}H, ^{13}C and ^{1}H-^{1}H-COSY) of the studied solutions in $CDCl_3$ or DMSO were recorded on a Bruker MSL-300 pulsed FTIR spectrometer or q-ONE AS400 Quatntum I Plus on the frequency 399.879 MHz with the deuterium inner stabilization with the TMS as an internal standard. Mass spectra were registered using a Bruker Daltonics Ultraf lex time-of-flight (TOF) mass spectrometer (Bruker Daltonics Inc., Germany) and 3,5-dihydroxybenzoic acid as a matrix. HRMS spectra were recorded on a high-resolution chromatography-mass spectrometer Maxis Impact.

2. Preparation of porphyrin derivatives

5,10,15,20-Tetrakis(4-(2-(trimethylsilyl)ethynyl)phenyl)porphyrin (2)



100 mg (1.495 mmol) of pyrrole and 302 mg (1.495 mmol) of 4-((trimethylsilyl)ethynyl)benzaldehyde were dissolved in 100 mL CH_2Cl_2. The reaction mixture was saturated with argon for 15 min, then 20 μl of $BF_3 \cdot OEt_2$ and 200 μl of C_2H_5OH. The mixture

was stirred for 40 min at room temperature in the dark. Then 340 mg (1.495 mmol) DDQ was added and the reaction was carried out under stirring for 2 hours. The reaction mixture was concentrated in vacuum. Then extraction in CH_2Cl_2/H_2O system with addition of aqueous ammonia solution was carried out. The substance was purified by column chromatography in the system CH_2Cl_2 : *n*-C_6H_{14} = 1:1. The yield amounted to 55%.

Electron absorption spectrum (λmax, nm, logε): 422 (5.00), 517 (3.55), 553 (3.31), 592 (2.94), 649 (2.59). ^{1}H NMR (300MHz, $CDCl_3$) δ, ppm: 8.82 (m, 8H, CH-pyrrole), 8.19 – 8.07 (m, 8H, ArH), 7.91 – 7.82 (m, 8H, ArH), 0.38 (m, 36H, TMS). -2.83 (s, 2H, NH- pyrrole). ^{13}C NMR (75 MHz, $CDCl_3$) δ, ppm: 142.43, 134.52, 130.53, 122.96, 119.78, 105.13, 95.82, 33.69, 32.69, 32.09, 29.86, 29.83, 29.52, 27.47, 22.85, 14.25. MALDI-TOF m/z: Calcd for $C_{64}H_{62}N_4Si_4$ 998.55 $[M]^+$ Found 999.193.

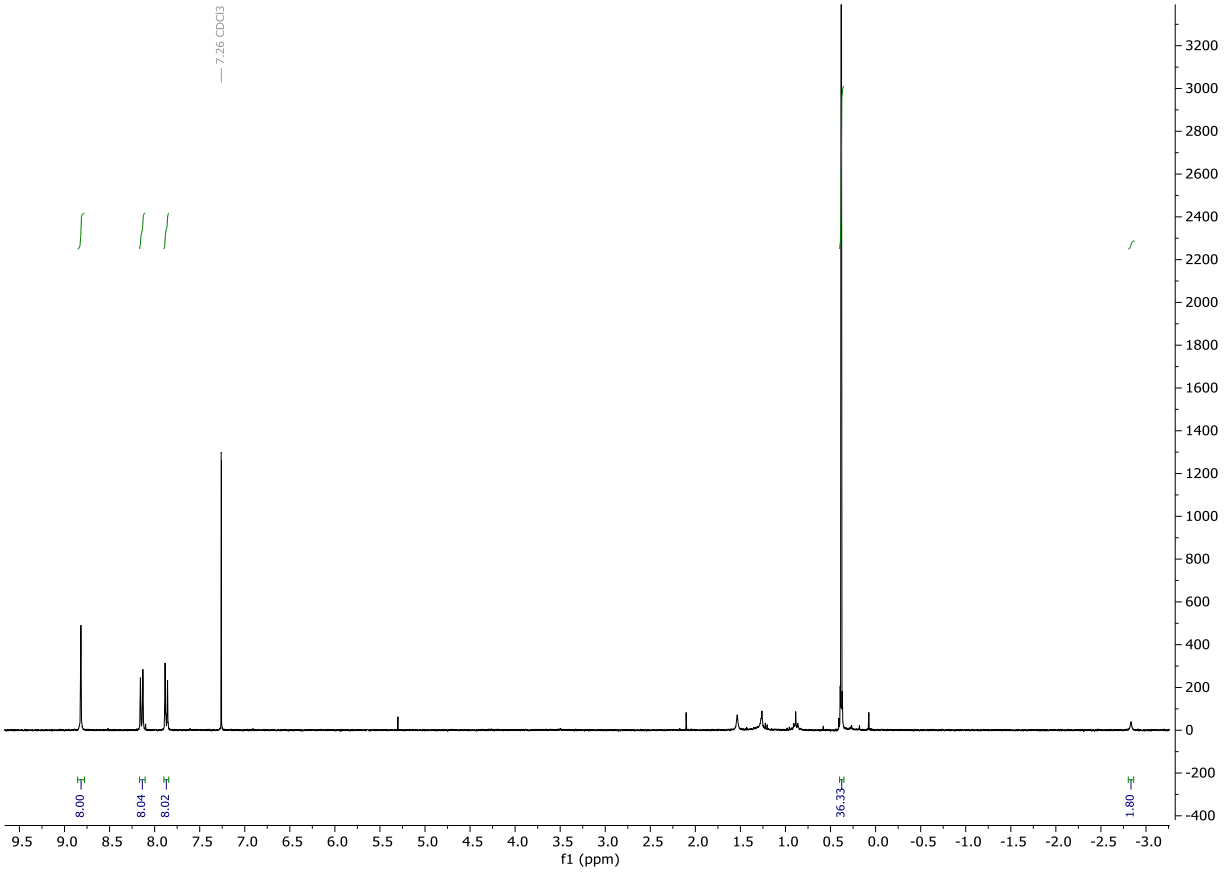


Figure S1 ^{1}H NMR spectrum of compound **2** in $CDCl_3$ at 300 MHz.

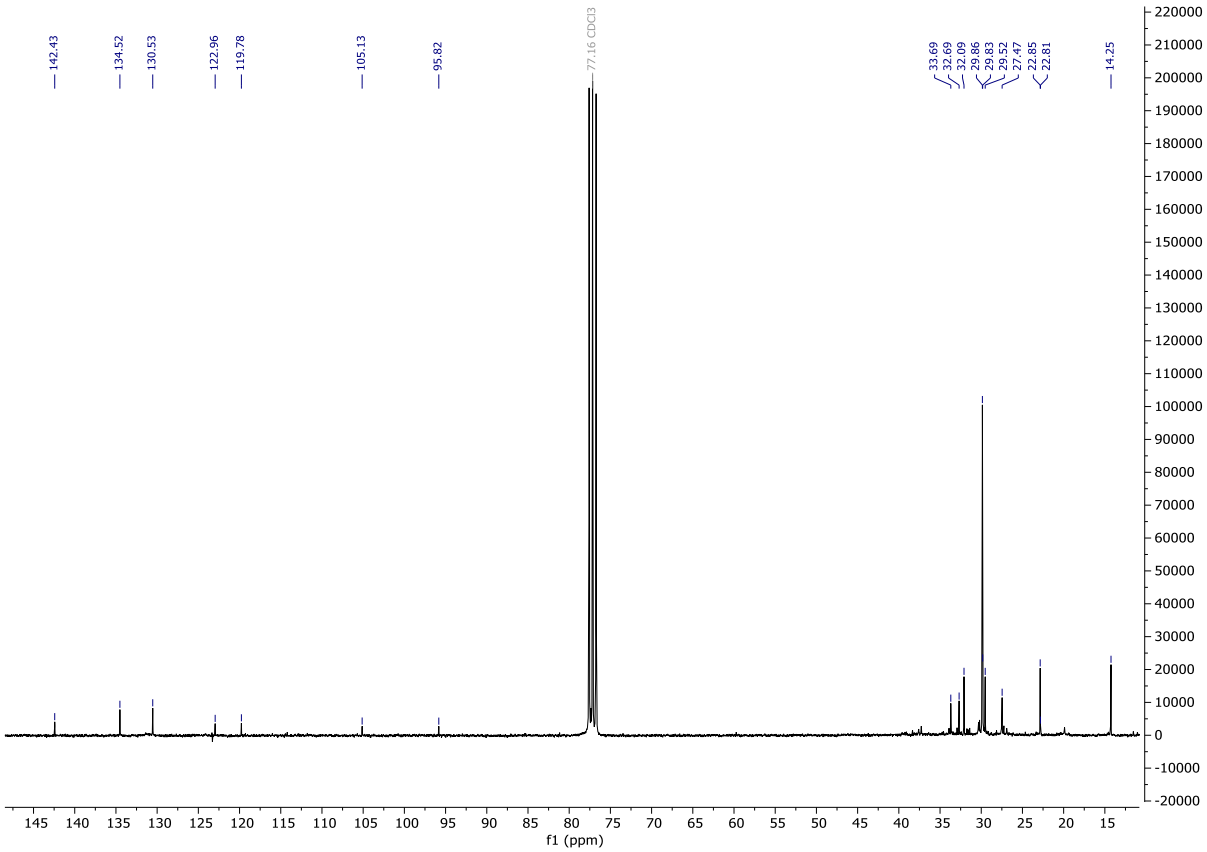


Figure S2 ^{13}C NMR spectrum of compound **2** in $CDCl_3$ at 75 MHz.

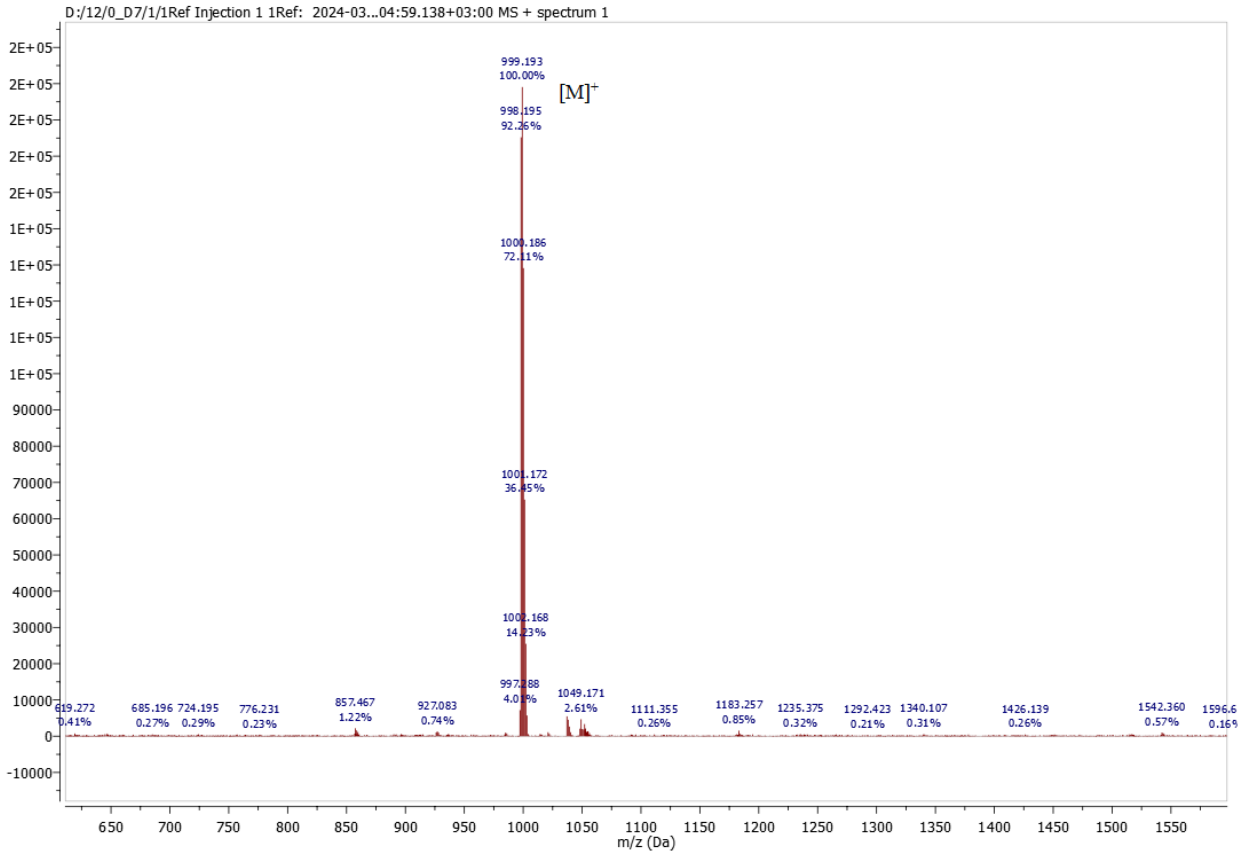
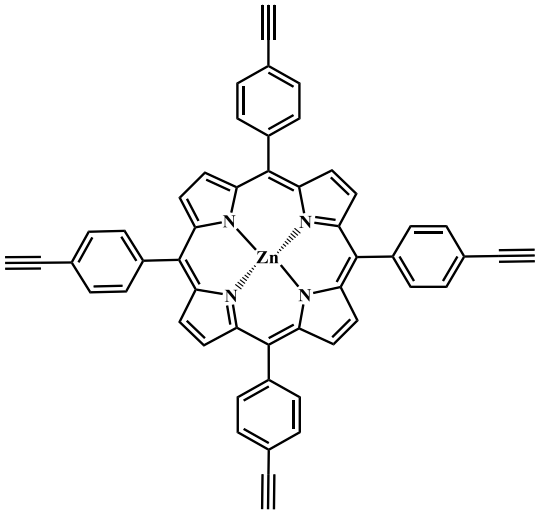


Figure S3 MALDI-TOF spectrum of compound **2**.

Zinc(II) complex with 5,10,15,20-tetrakis(4-ethynylphenyl)porphyrin (3)



Zinc acetate 110 mg (0.5 mmol, 10 eq.) was dissolved in 5 mL of methanol and added to 50 mg (0.05 mmol, 1 eq.) of porphyrin **2** dissolved in CH_2Cl_2. The reaction was carried out for 3 hours. The completion of the reaction was judged by spectrophotometric analysis. Porphyrin was extracted into the system CH_2Cl_2 - H_2O. The obtained metal complex was concentrated in a vacuum. The substance was then dissolved in 10 mL THF, and 50 μL of 80% aqueous Bu_4NF solution was added. The reaction was carried out for 30 min. The product obtained was purified by column chromatography in the system CH_2Cl_2 : *n*-C_6H_{14} = 1:1. The yield was 95%.

Electron absorption spectrum (λmax, nm, logε): 422 (5.45), 549 (4.06), 588 (3.44). ^{1}H NMR (300 MHz, CDCl3) δ 8.91 (s, 8H, CH-pyrrole), 8.17 (m, 8H, 2,6-ArH), 7.93 – 7.84 (m, 8H, 3,5-ArH), 3.31 (s, 4H ≡CH). ^{13}C NMR (75 MHz, $CDCl_3$) δ 59.25, 29.84, 24.37, 19.93, 13.84. MALDI-TOF m/z: Calcd for $C_{52}H_{28}N_4Zn$ 772.16 $[M]^+$ Found 772.064.

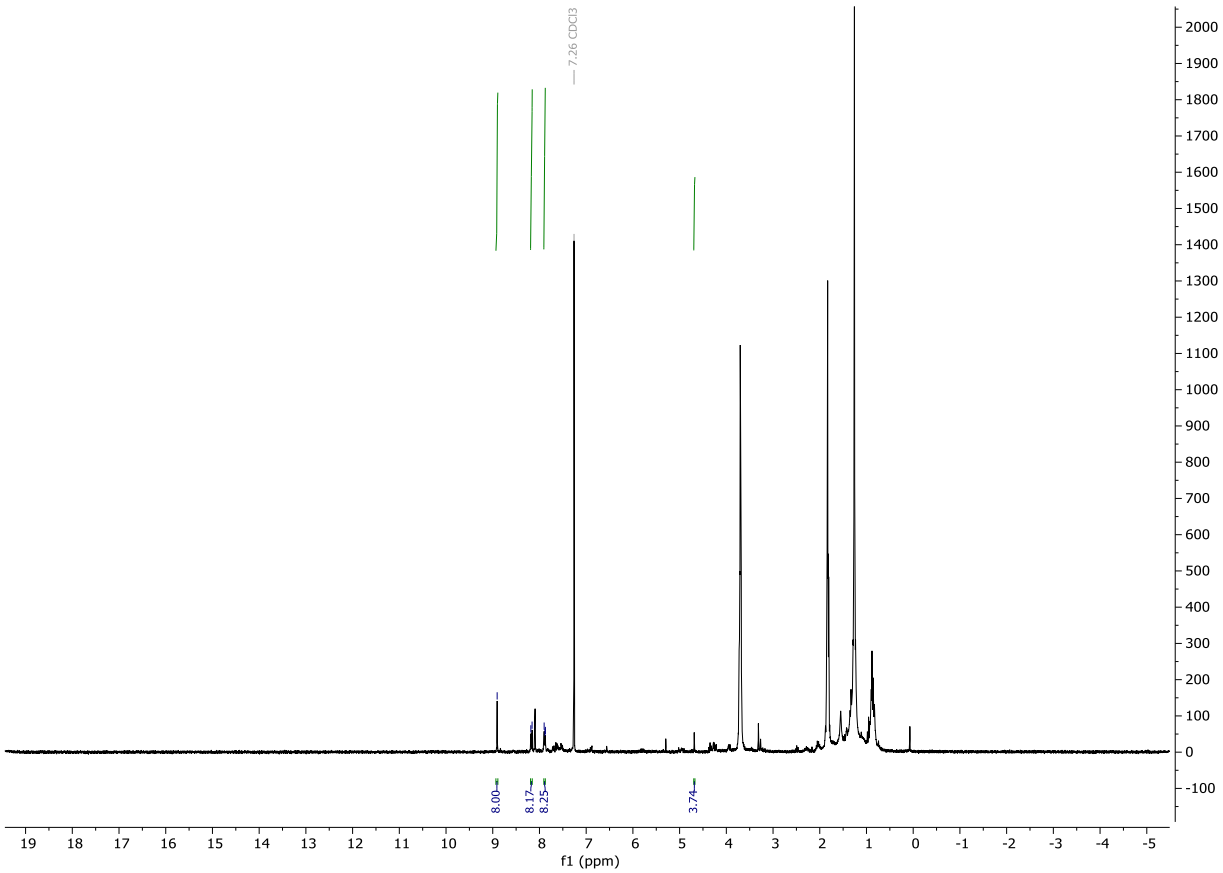


Figure S4 ^{1}H NMR spectrum of compound **3** in $CDCl_3$ at 300 MHz.

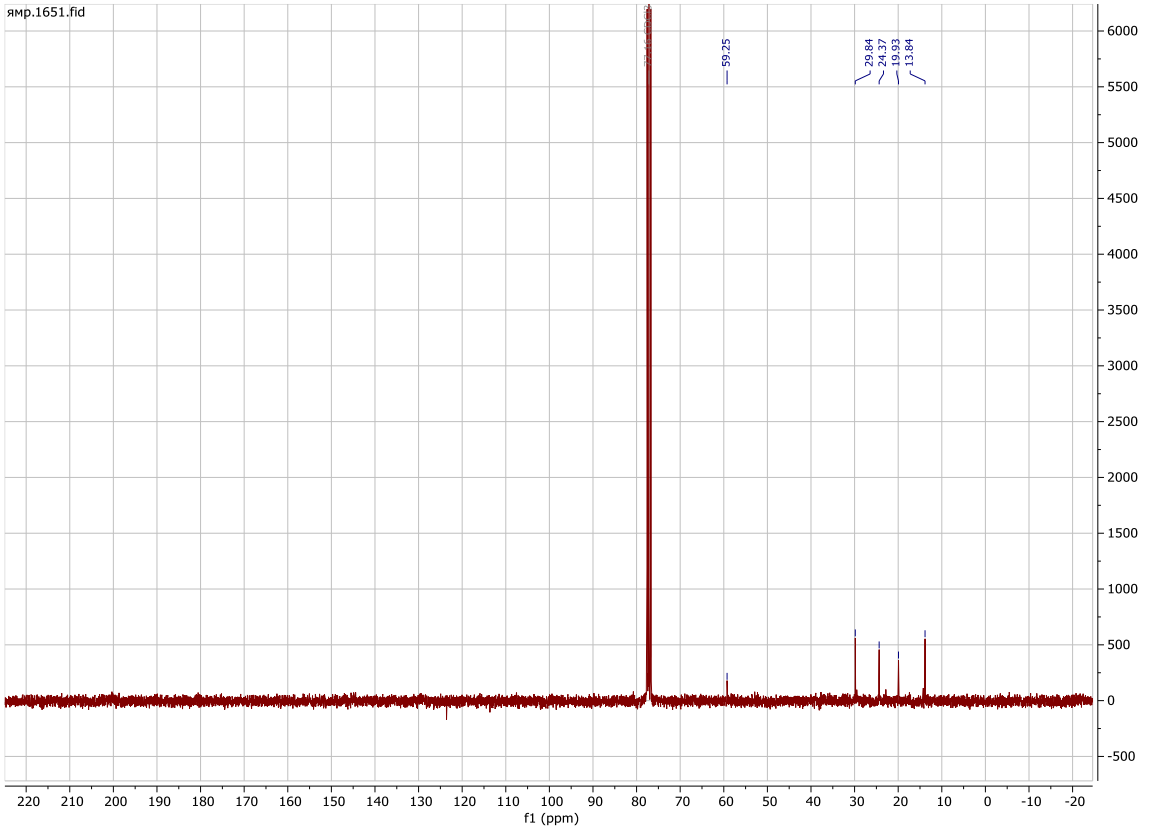


Figure S5 ^{13}C NMR spectrum of compound **3** in $CDCl_3$ at 75 MHz.

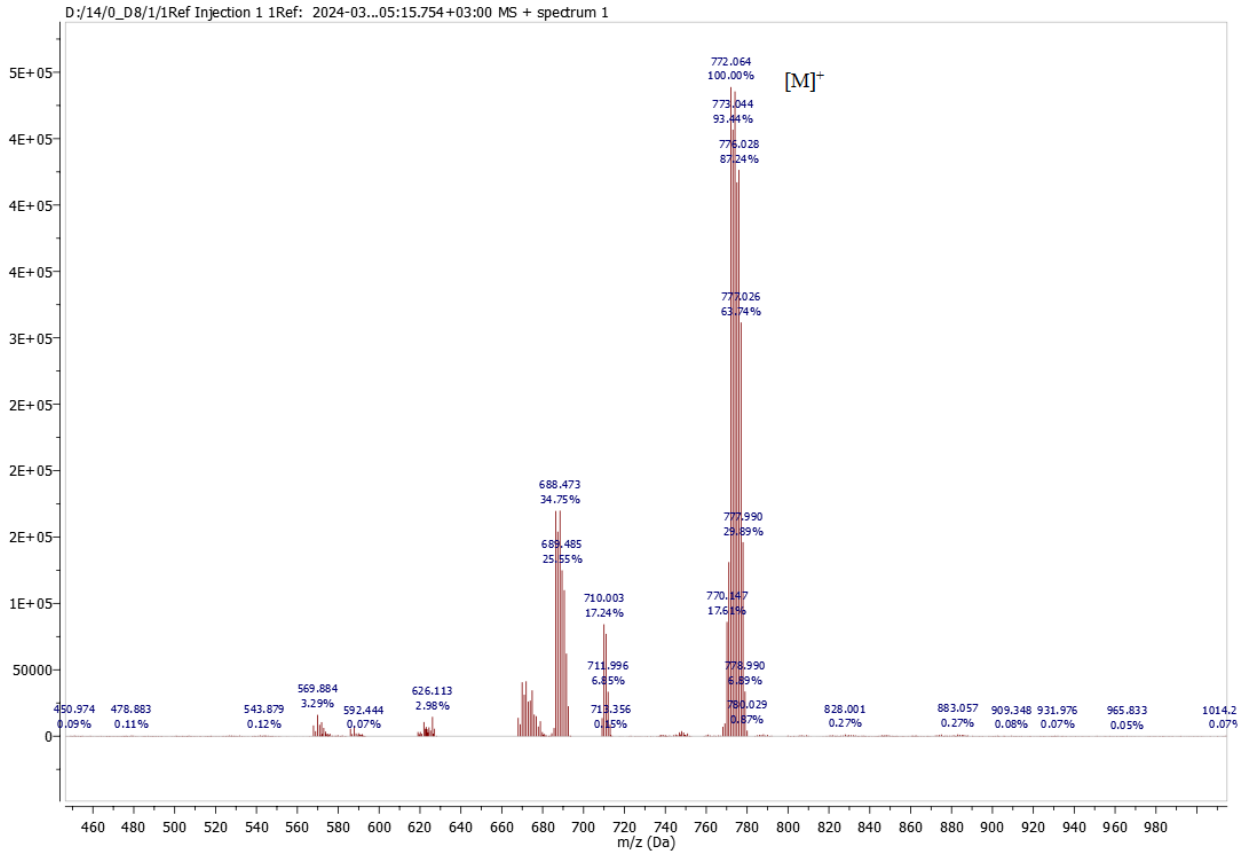
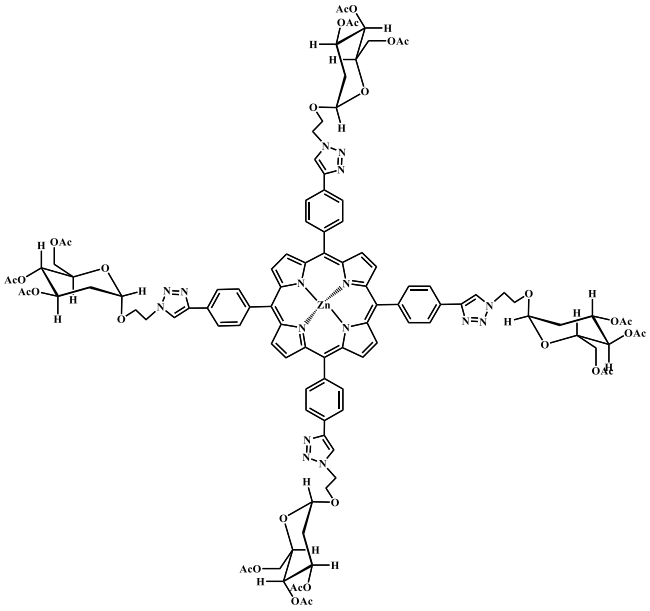


Figure S6 MALDI-TOF spectrum of compound **3**.

Conjugate with tri-O-acetoxy-2-deoxy-D-glucose (4)



To a solution of porphyrin **3** in THF was added 1.2 eq. for each ethynyl group of glucose derivative **1**. Then 6 ml of a solution of 1.6 eq. $CuSO_4*5H_2O$ and 3.3 eq. sodium ascorbate per

each ethynyl group was added. The reaction was allowed to proceed for 12 hours. The conjugate was extracted in THF - saturated aqueous NaCl solution system. The obtained products were purified by column chromatography in the system CH_2Cl_2:MeOH = 50:1. The yield was 97%.

Electron absorption spectrum (λmax, nm, logε): 423 (5.71), 549 (4.30), 588 (3.67).^{1}H NMR (300 MHz, $CDCl_3$) δ, ppm: 8.97 (s, 8H, CH- pyrrole), 8.22 (d, J = 7.9 Hz, 8H, 2,6-(ArH)), 8.11 (d, *J* = 7.6 Hz, 8H, 3,5-(ArH)), 8.01 (s, 4H, CH-triazole), 5.30 – 5.13 (m, 4H, 1-Glc), 4.98 – 4.84 (m, 8H, 3,5-Glc), 4.67 (s, 8H, -CH_2-triazole), 4.14 (td, *J* = 13.9, 4.9 Hz, 8H, 4,6-Glc), 4.03 – 3.93 (m, 4H, 6-Glc), 3.91 – 3.81 (m, 4H, -OCH_2), 3.55 – 3.43 (m, 4H, -OCH_2), 2.16 (dd, *J* = 12.7, 5.1 Hz, 4H, 2-Glc), 2.06 (s, 12H, Ac-Glc), 1.93 (d, *J* = 1.4 Hz, 12H, Ac-Glc), 1.84 (s, 12H, Ac-Glc), 1.77 (s, 4H, 2-Glc). ^{13}C NMR (75 MHz, $CDCl_3$) δ, ppm: 170.69, 170.31, 169.76, 150.09, 147.76, 142.86, 135.05, 132.00, 129.64, 124.03, 121.43, 120.62, 97.32, 96.88, 68.79, 68.35, 66.26, 65.60, 62.17, 50.12, 34.66, 32.42, 22.70, 20.78, 20.71. MALDI-TOF m/z: Calcd for $C_{108}H_{112}N_{16}O_{32}Zn$ $[M]^+$ 2009.79 Found 2210.953.

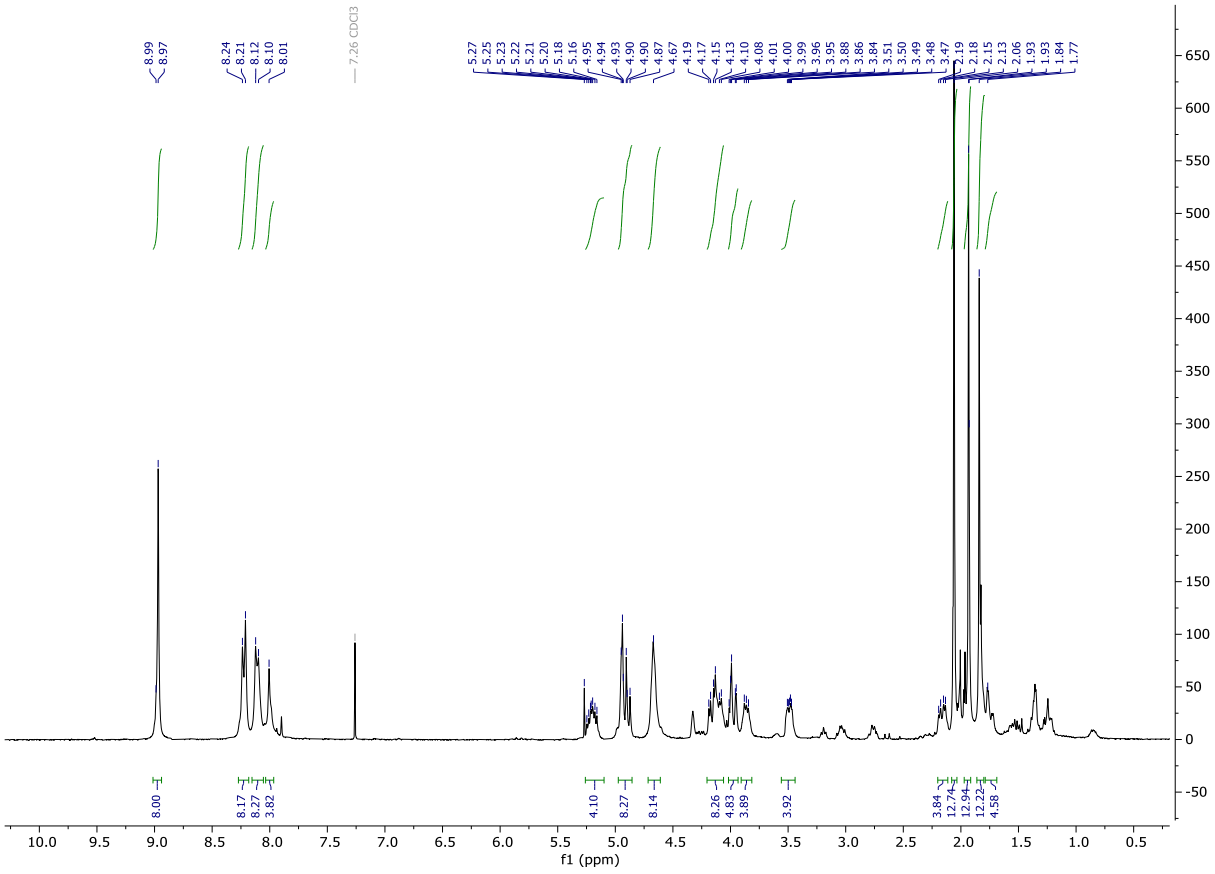


Figure S7 ^{1}H NMR spectrum of compound **4** in $CDCl_3$ at 300 MHz.

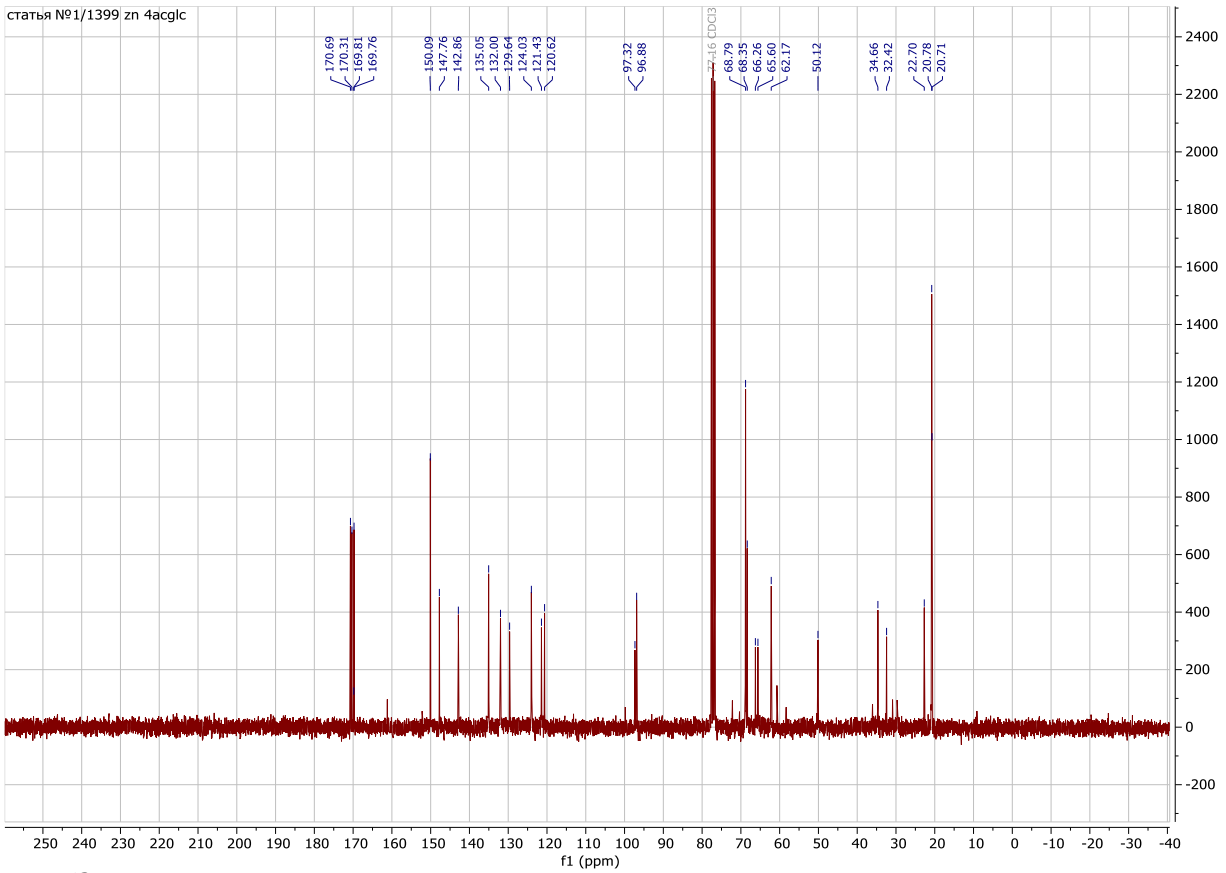


Figure S8 ^{13}C NMR spectrum of compound **4** in $CDCl_3$ at 75 MHz.

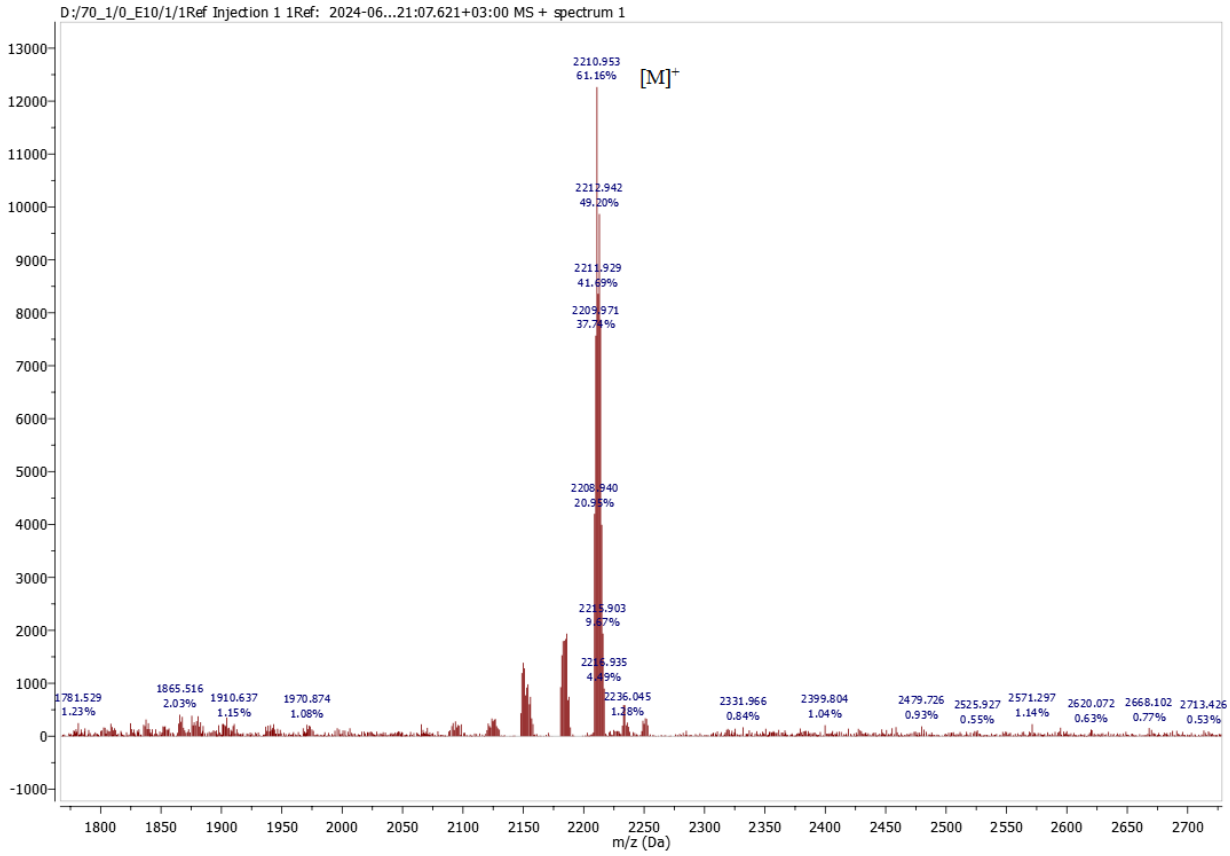
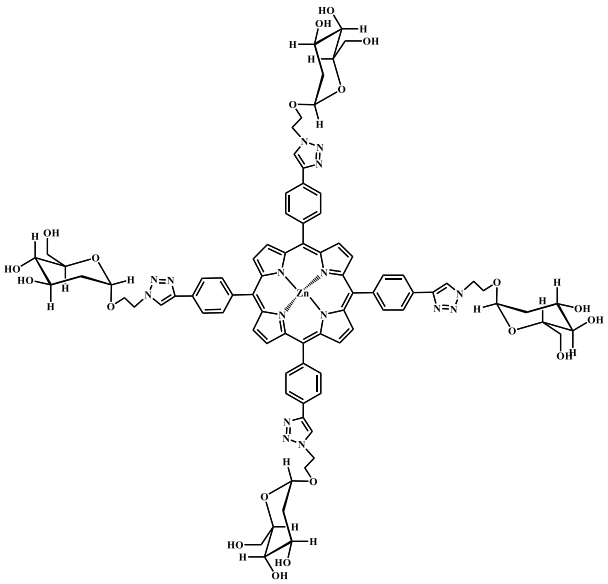


Figure S9 MALDI-TOF spectrum of compound **4**.

Conjugate with 2-deoxy-D-glucose (5)



Acetyl protecting groups were removed from the 2-deoxy-D-glucose residues of 50 mg of conjugate **4** using 10 mL of 0.1 M CH_3ONa solution in methanol. The reaction mixture was then adjusted to neutral pH using Dowex 50WX8 (H+ form). The resulting conjugate was purified by recrystallisation from methanol in diethyl ether. The yield was 90%.

Electron absorption spectrum (λmax, nm, logε): 432 (5.73), 563 (4.24), 604 (4.04). ^{1}H NMR (400 MHz, DMSO) δ, ppm: 8.90 (s, 8H, CH- pyrrole), 8.82 (s, 4H, CH-triazole), 8.28 (s, 16H, ArH), 4.94 (dd, *J* = 9.4, 4.2 Hz, 8H, 3,5-Glc), 4.83 (d, *J* = 4.8 Hz, 4H, 1-Glc), 4.73 (s, 8H, -CH2-triazole), 4.63 – 4.51 (m, 4H, OH), 4.12 – 4.05 (m, 4H, OH), 3.95 – 3.88 (m, 4H, OH), 3.64 (ddd, J = 31.6, 11.4, 5.7 Hz, 8H, 4,6-Glc), 3.50 (dt, *J* = 11.7, 5.9 Hz, 4H, 6-Glc), 3.29 (dd, *J* = 11.0, 5.3 Hz, 4H, -OCH_2), 3.09 – 3.05 (m, 4H, -OCH_2), 1.94 (dd, *J* = 12.8, 5.0 Hz, 4H, 2-Glc), 1.48 (td, J = 12.6, 3.7 Hz, 4H, 2-Glc). ^{13}C NMR (101 MHz, DMSO) δ, ppm: 149.39, 146.37, 142.27, 134.90, 131.86, 130.06, 123.59, 122.23, 120.15, 96.85, 73.53, 71.58, 67.97, 64.97, 61.04, 49.86, 37.82. MALDI-TOF m/z: Calcd for $C_{84}H_{88}N_{16}O_{20}Zn$ $[M]^+$ 1704.57 Found 1707.431.

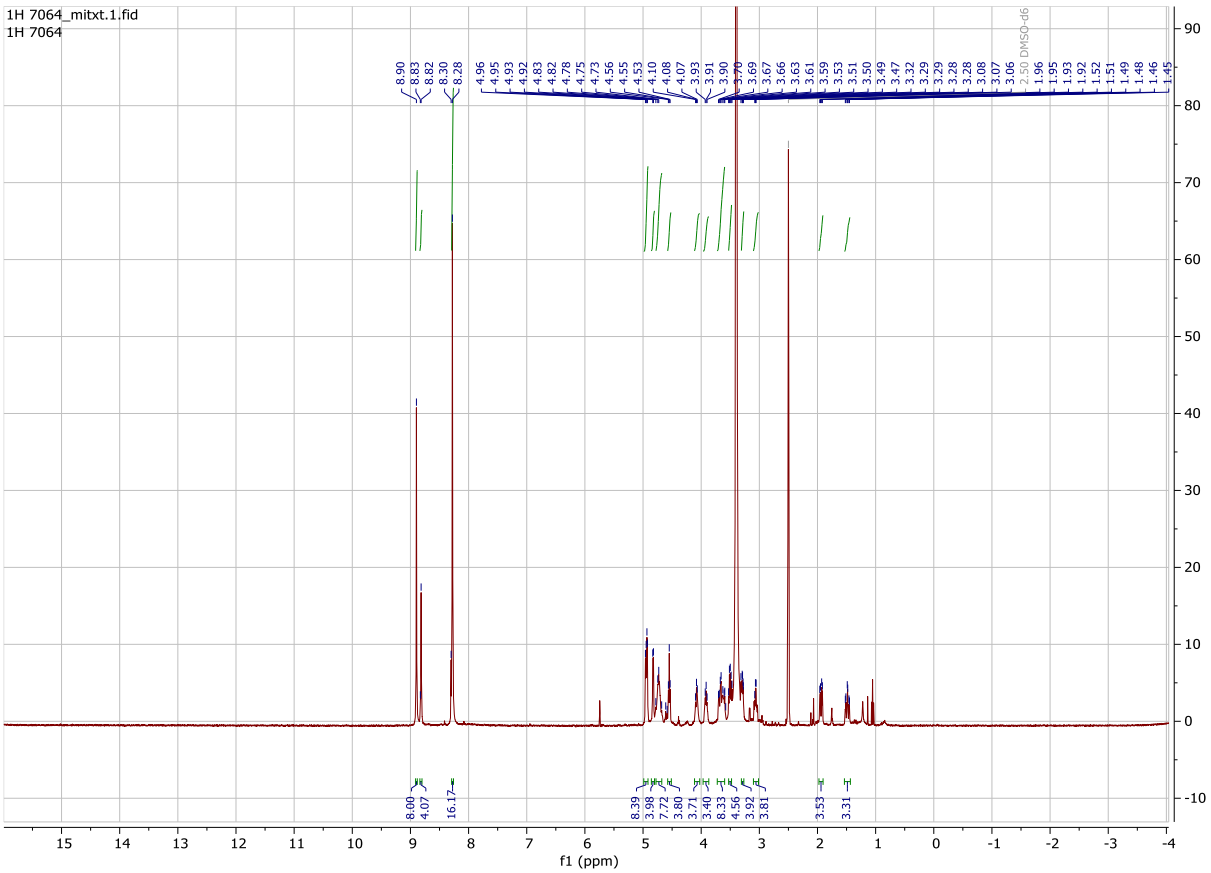


Figure S10 ^{1}H NMR spectrum of compound **5** in DMSO at 400 MHz.

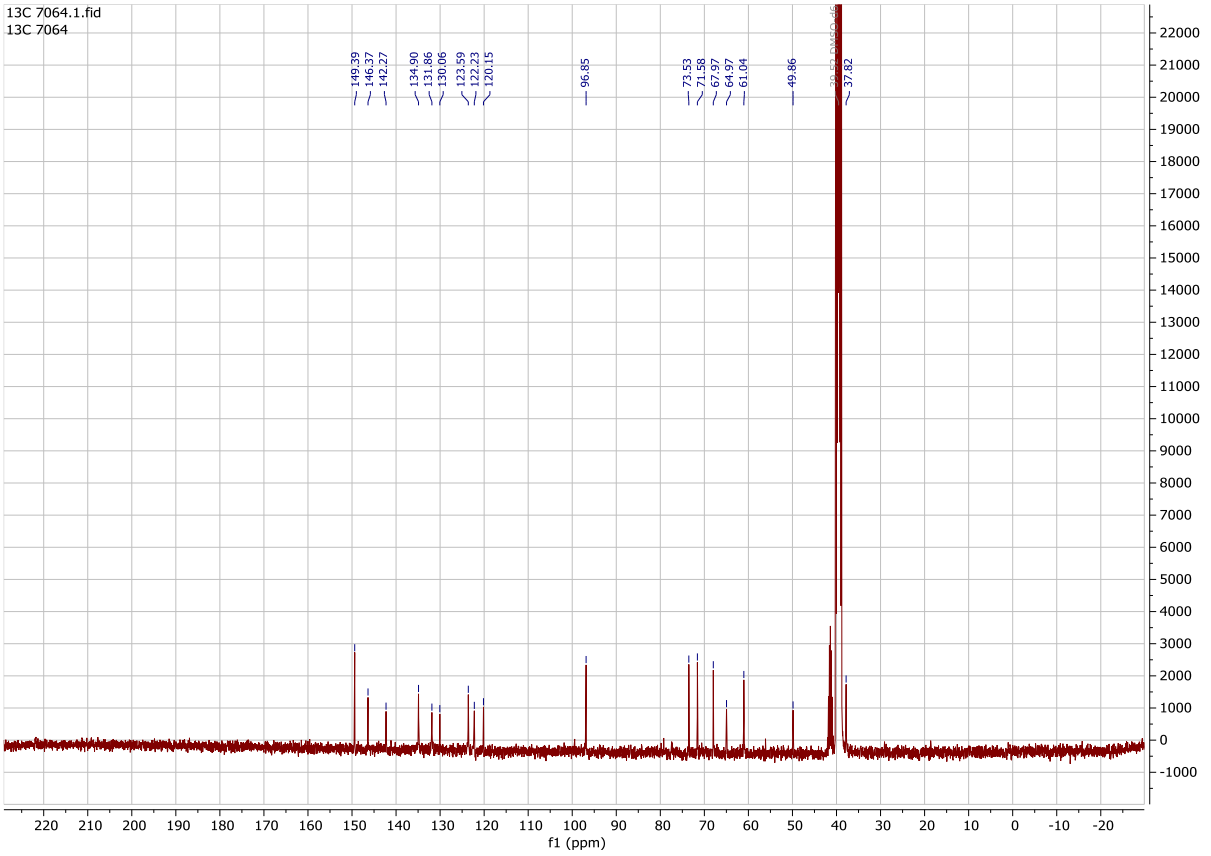


Figure S11 ^{13}C NMR spectrum of compound **5** in DMSO at 101 MHz.

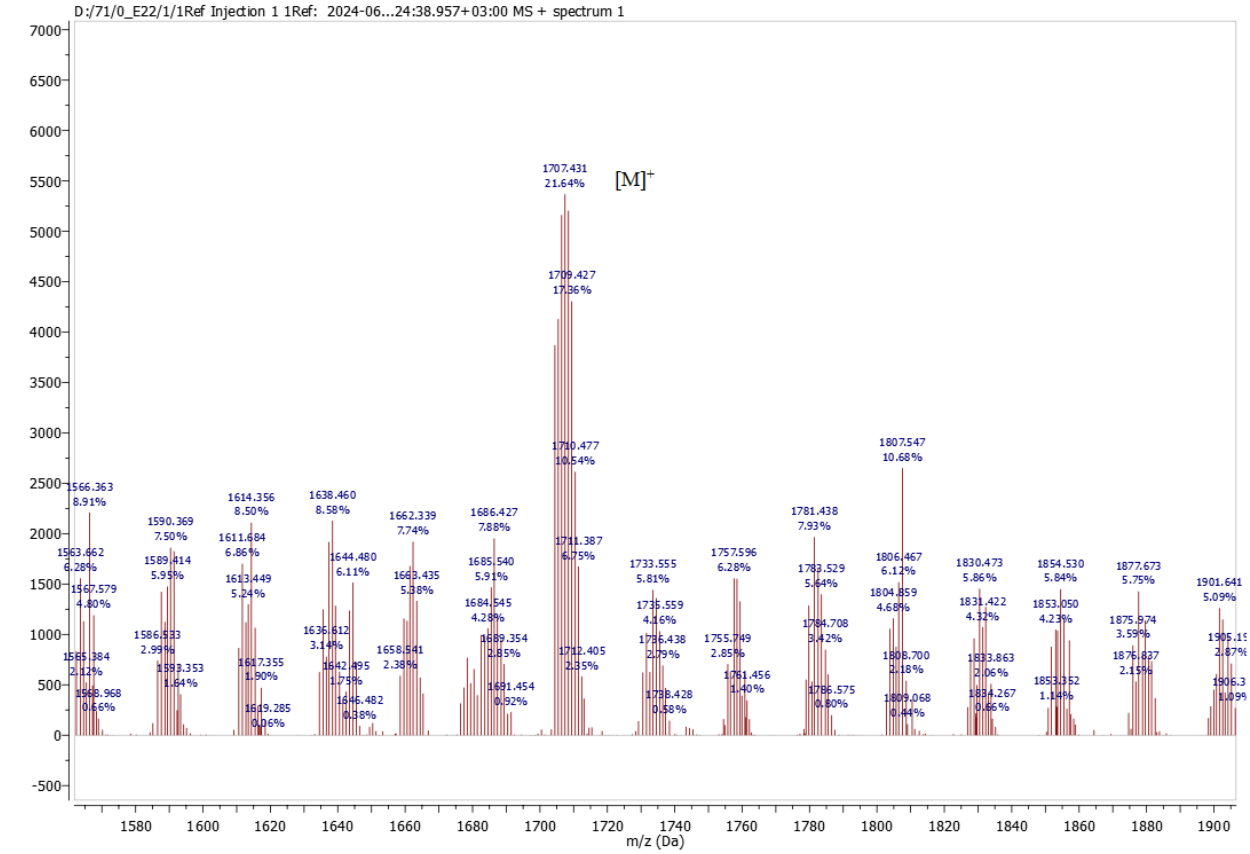


Figure S12 MALDI-TOF spectrum of compound **5**.

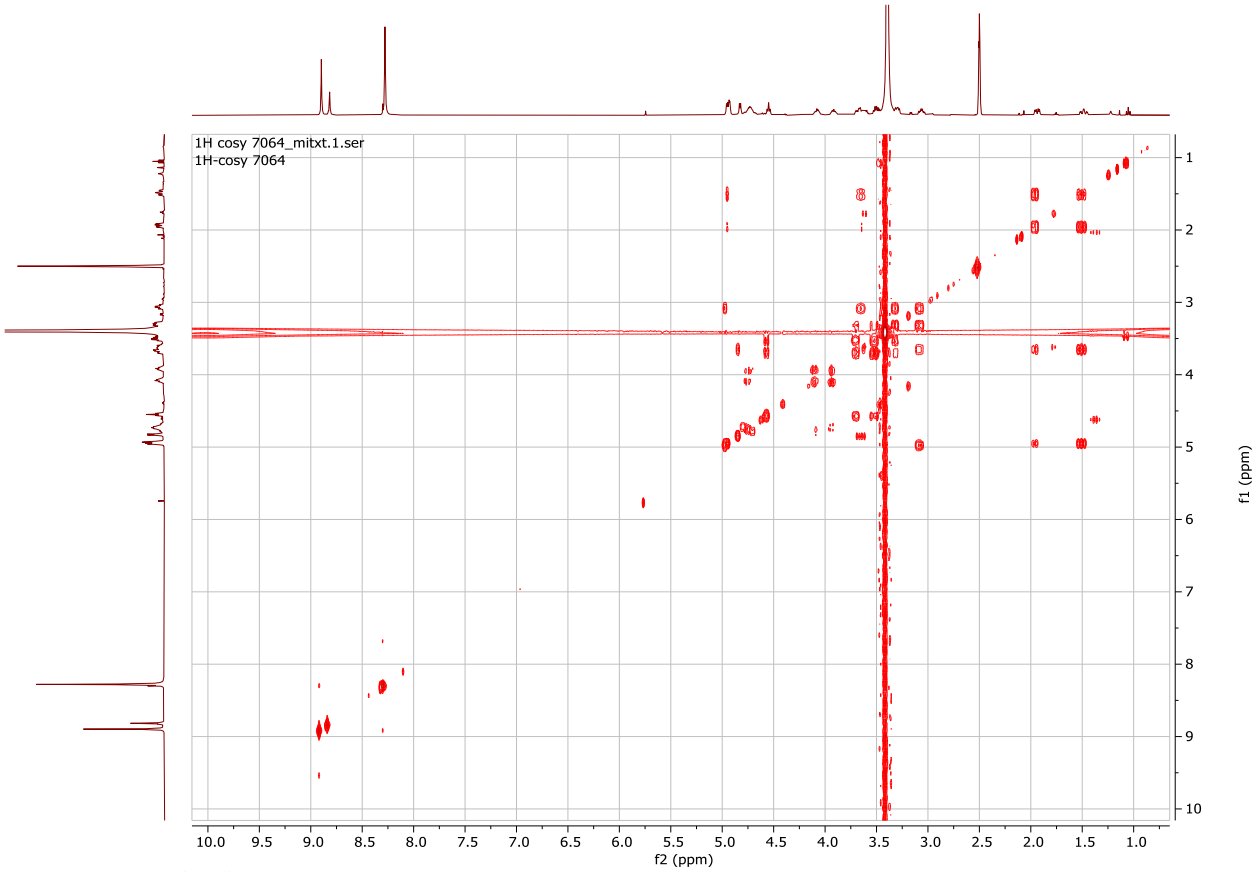


Figure S13 COSY ^{1}H-^{1}H NMR spectrum of compound **5** in DMSO at 400 MHz.

3. Photophysical properties

Electronic absorption spectra of porphyrins were obtained using a HACH DR-4000V (Hach-Lange, Ames, IA, USA) spectrophotometer operating in 320–800 nm spectral range with 10 mm quartz cells at room temperature. Steady-state fluorescence spectra were recorded using a Perkin Elmer LS-50 luminescence spectrometer (Perkin Elmer, USA) under similar conditions at a monochromator slit width of 10 nm and excitation wavelength corresponding to the maximum of the Sore band. Concentrations of porphyrin solutions in DMSO were 1.15 μM.

Fluorescence quantum yield values Φ_F for the studied compounds in DMSO were calculated according to the standard procedure[S1,S2] using ZnTPP ($\Phi_{F\,=}$ 0.033) as a standard.[S3-S5] The error in determining the fluorescence quantum yield was 10%.

For the determination of the singlet oxygen quantum yield, the samples were irradiated in 10 mm quartz cells at room temperature. The light sources included a 150 W halogen lamp, a three-lens spherical condenser with a reflector, and a 500 nm cut-off filters. The photosensitized singlet oxygen generation efficiency of the porphyrin solutions was estimated from the absorbance decrease at 418 nm, corresponding to the concentration of the selective $^1O_2$ acceptor – 1,3-diphenylisobenzofuran (DPBF), which was added to the porphyrin solution in DMSO immediately before the start of irradiation (C_{DPBF} = 0.1 mM). The porphyrin concentration was maintained at 1–2 μM to minimize the internal filter effect in all the photochemical experiments. Calculation of the singlet oxygen quantum yield (Φ_Δ) was carried out according to a procedure described elsewhere,[S6,S7] for which ZnTPP was used as a standard (Φ_Δ = 0.74).[S8] The error of the determination of the singlet oxygen quantum yield was 10%.

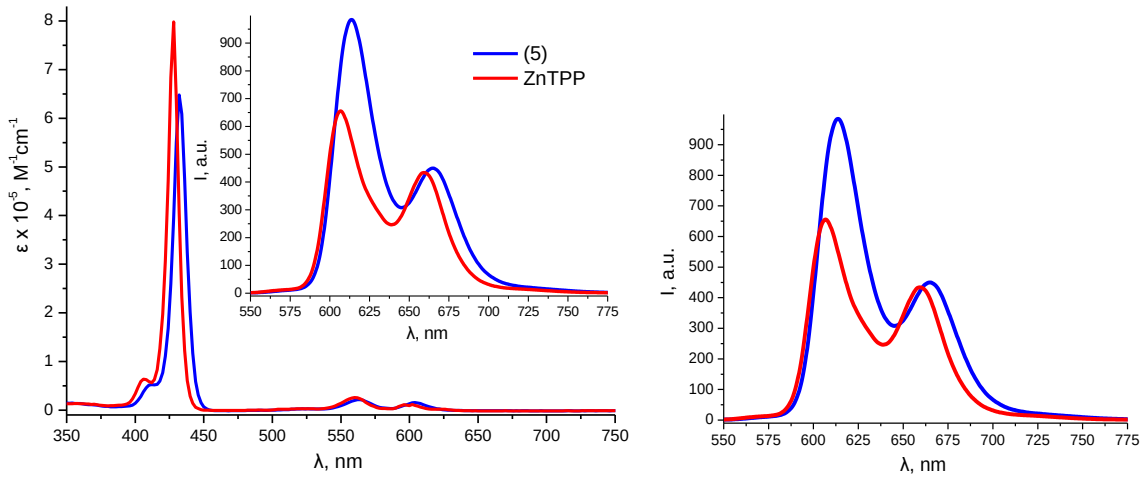


Figure S14

Table S1 Photophysical properties of compound **5** in DMSO

Compound	λ_B, nm	lgε	$\Delta\lambda_{1/2}$, nm	λ_{Q1} / λ_{Q2}	λ_{em}, nm	I_1 / I_2	logP	Φ_F	Φ_Δ
5	432	5.81	12	563 / 604	614 / 665	2.19	0.31	0.055	0.78

4. Partition coefficient

The partition coefficients were measured using 1-octanol and aqueous phosphate buffered saline (PBS), each solvent presaturated with the other. Porphyrin **5** (1 mg) was added to 3 mL of 1-octanol and 3 mL of PBS solution, sonicated for 30 min and centrifuged for 10 min to accelerate the separation of the two layers. The absorption of each layer was measured spectrophotometrically at the photosensitizer Soret band. Due to low concentration of porphyrin in the aqueous layer, relatively large volume of the aqueous layer was required (2 mL) and this sample was combined with 0.5 mL of DMF. The absorbance measurements were used to calculate partition coefficients using the formula shown below

$$P = \frac{A(org) * d(org)}{A(aq) * d(aq)}$$

where A(org) and A(aq) are absorbance values for the organic and aqueous layers at the Soret band, and d(org) and d(aq) are the dilution factors for the organic and aqueous layers. The dilution factor is the ratio of the final volume of the sample to the aliquot, as indicated below:

$$d(org) = \frac{Vf(org)}{V(org)}$$

$$d(aq) = \frac{Vf(aq)}{V(aq)}$$

where Vf(org) and Vf(aq) are the final volumes of the sample from the organic and aqueous layers, while V(org) and V(aq) are the volumes of the aliquots of the organic and aqueous layers.[S9]

5. Biological activity

The dark and light-induced toxicity of the photosensitizer **5** was determined on two cell lines - MCF7 (breast ductal adenocarcinoma) and NKE (normal kidney epithelial). During the experiments, compounds were added to the cell culture and irradiated using a Medical Therapy Philips TL 20W/52 lamp, wavelength 420 nm. The irradiation dose was 8.073 J/cm^2. After the end of irradiation time, the cell culture was incubated for another 24 hours at +37˚C in 5% CO_2 in the dark. In parallel with the same serial dilutions, photosensitizers were added to the cell cultures and, without irradiation, left in the dark for 24 hours at +37°C in 5% CO_2.

Table S2. Dark and light-induced toxicity of compound **5**.

Cell line	IC_{50} dark, μM	IC_{50} light, μM	IC_{50} dark/IC_{50} light
NKE	>3	0.067±0.010	>45
MCF7	>3	0.042±0.007	>71

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