Tetrahedron Letters
journal homepage: www.elsevier.com

Synthesis and photophysics of an octathioglycosylated zinc(II) phthalocyanine

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Contributions: A. Aggarwal, photophysical studies, partition coefficient, cell uptake; S. Singh, synthesis and characterization of the Pc; Y Zhang and R. Gao singlet oxygen quantum yields; M. Anthes and D. Samaroo, cell uptake studies; C.M. Drain data analysis and manuscript preparation.

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Note that at concentrations used in the NMR spectra, there is significant aggregation, causing some shifts and broadening of the peaks (see UV-visible and fluorescence studies).

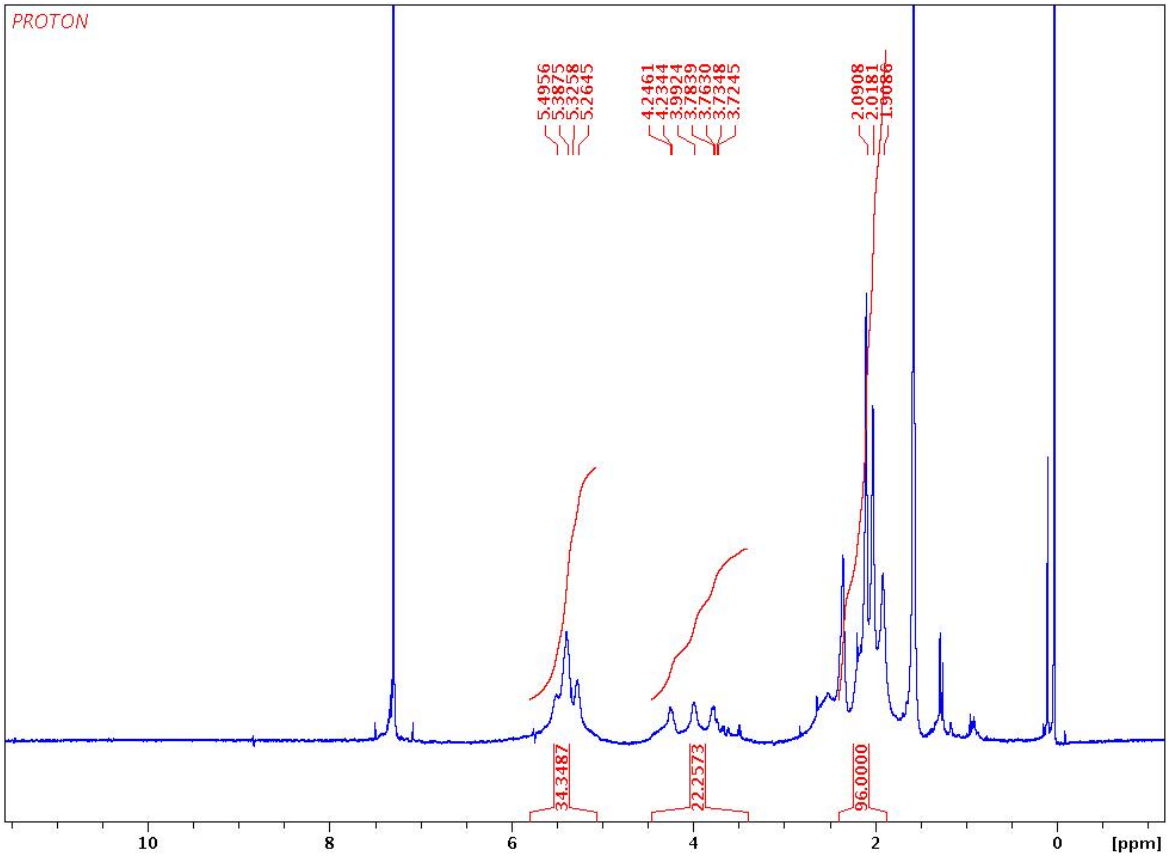


Figure SM-1. ^{1}H NMR of compound **1a** ZnPcGlcAc$_8$ ($CDCl_3$ 7.28 ppm, water 1.6 ppm).

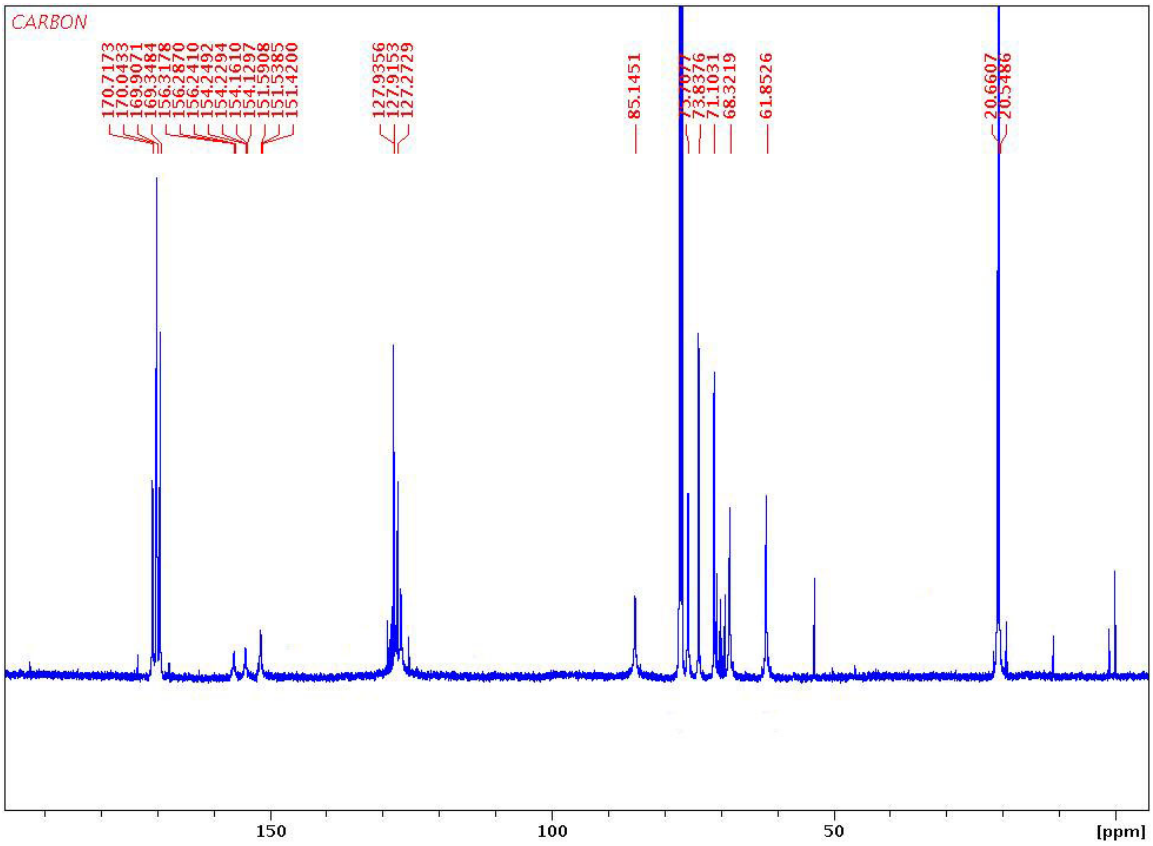


Figure SM-2. ^{13}C NMR of compound **1a** $ZnPcGlcAc_8$ ($CDCl_3$ 77 ppm).

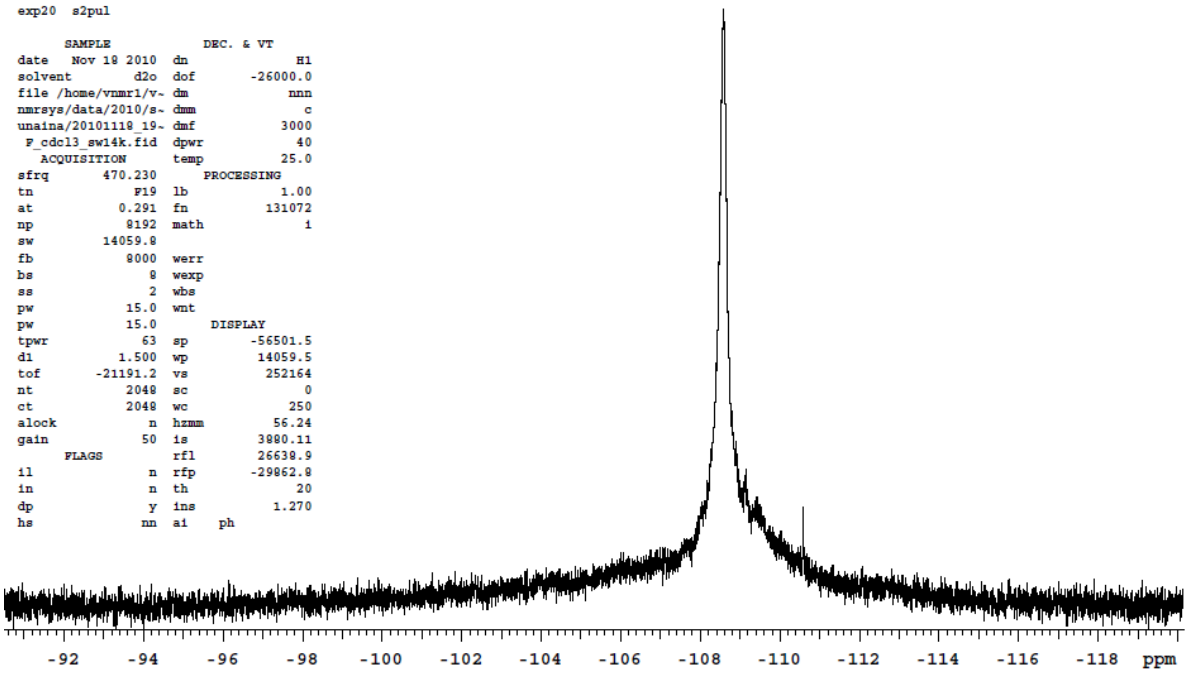
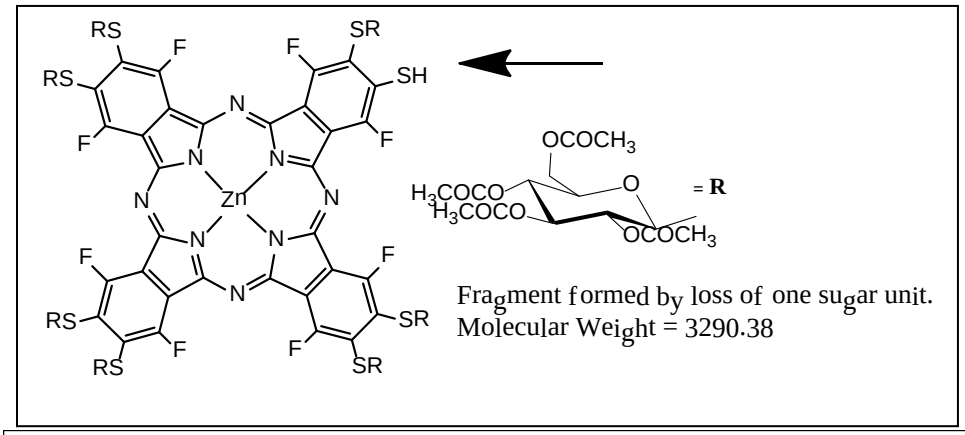


Figure SM-3. ^{19}F NMR of compound **1a** $ZnPcGlcAc_8$ ($CDCl_3$). The broadened peak is due to aggregation and clearly shows the absence of the α F.



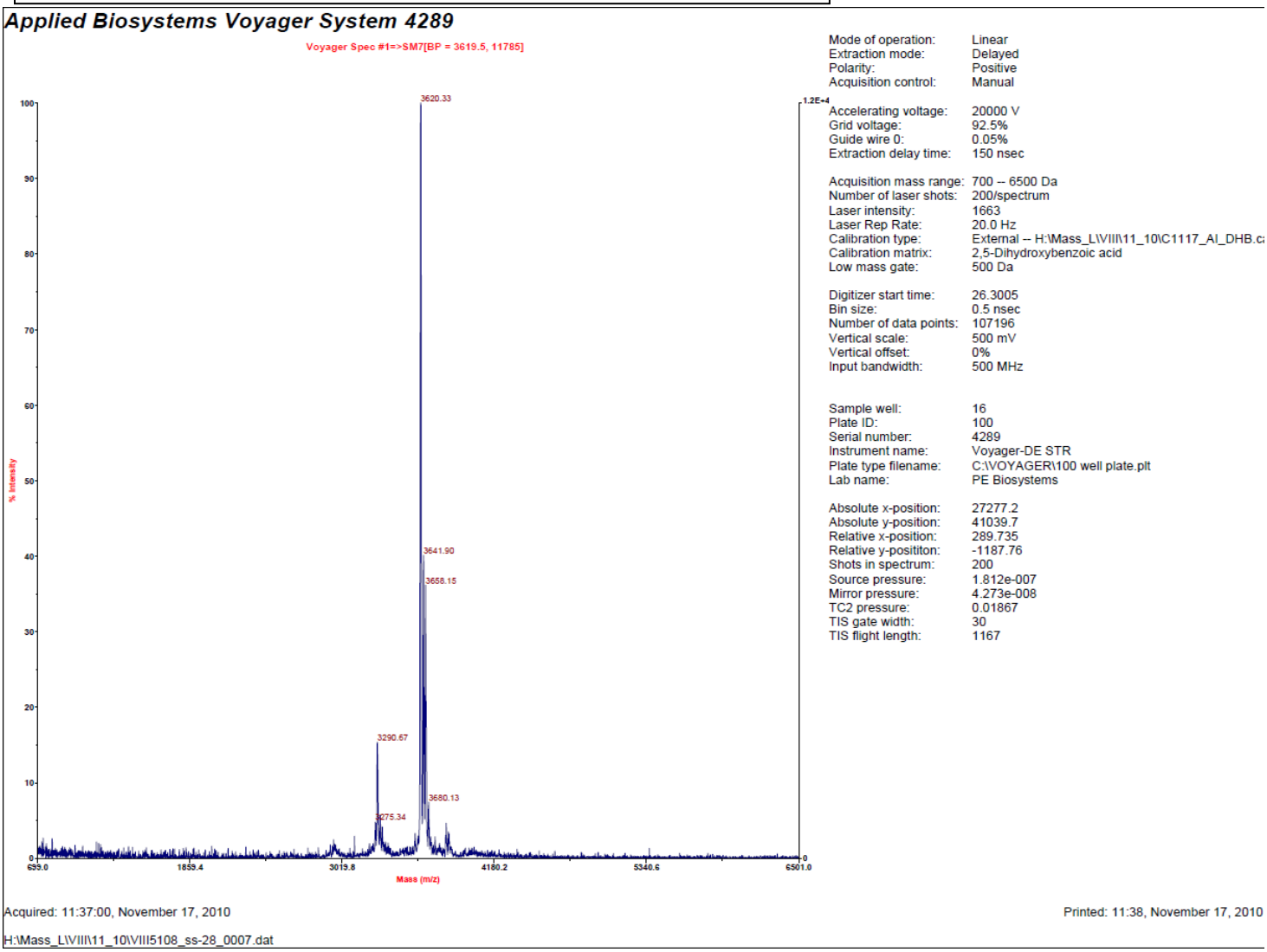


Figure SM-4. MALDI of compound **1a**. The small peak at m/e 3290.67 is the loss of a sugar, leaving the thiol on the Pc, see structure above.

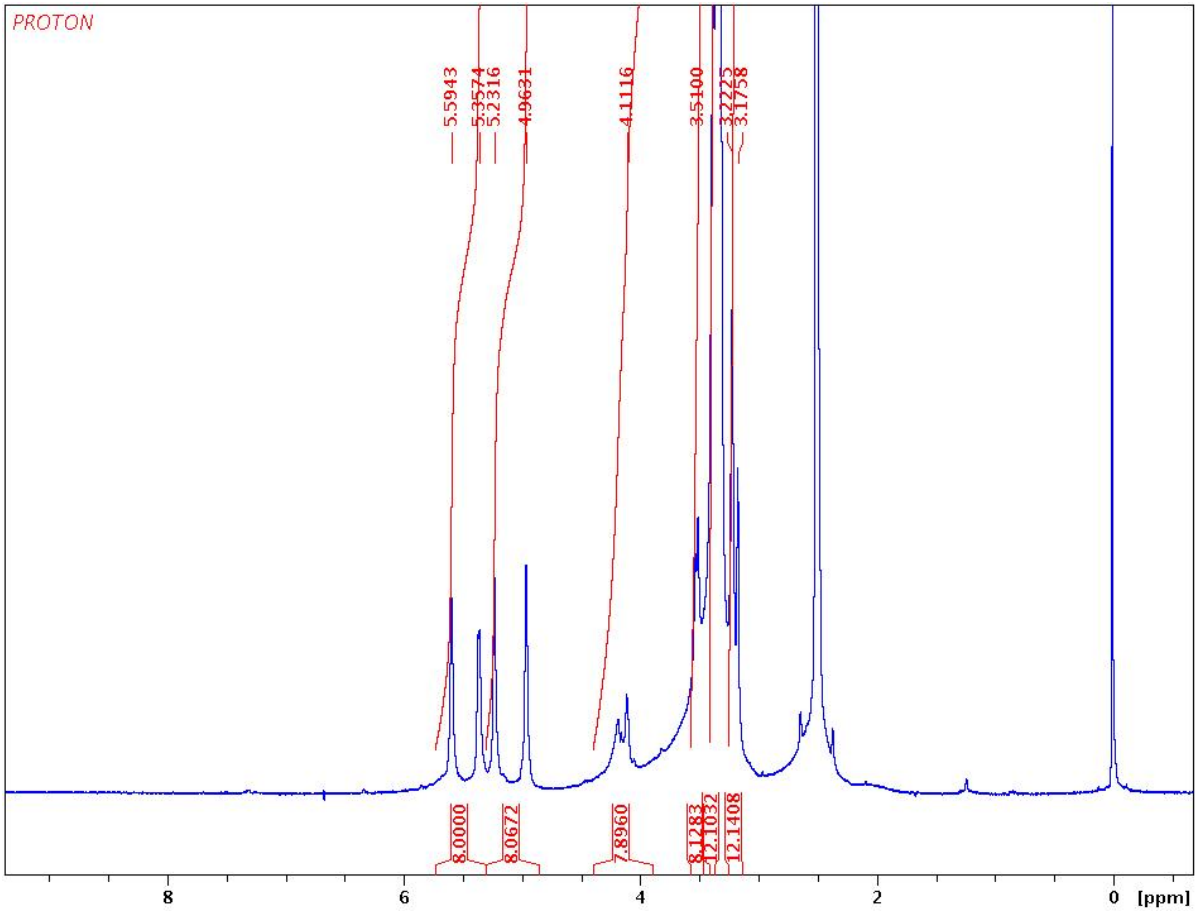


Figure SM-5. ^{1}H NMR of compound **1b** $ZnPcGlc_8$ (DMSO-d_6 2.5 ppm, water 3.3 ppm).

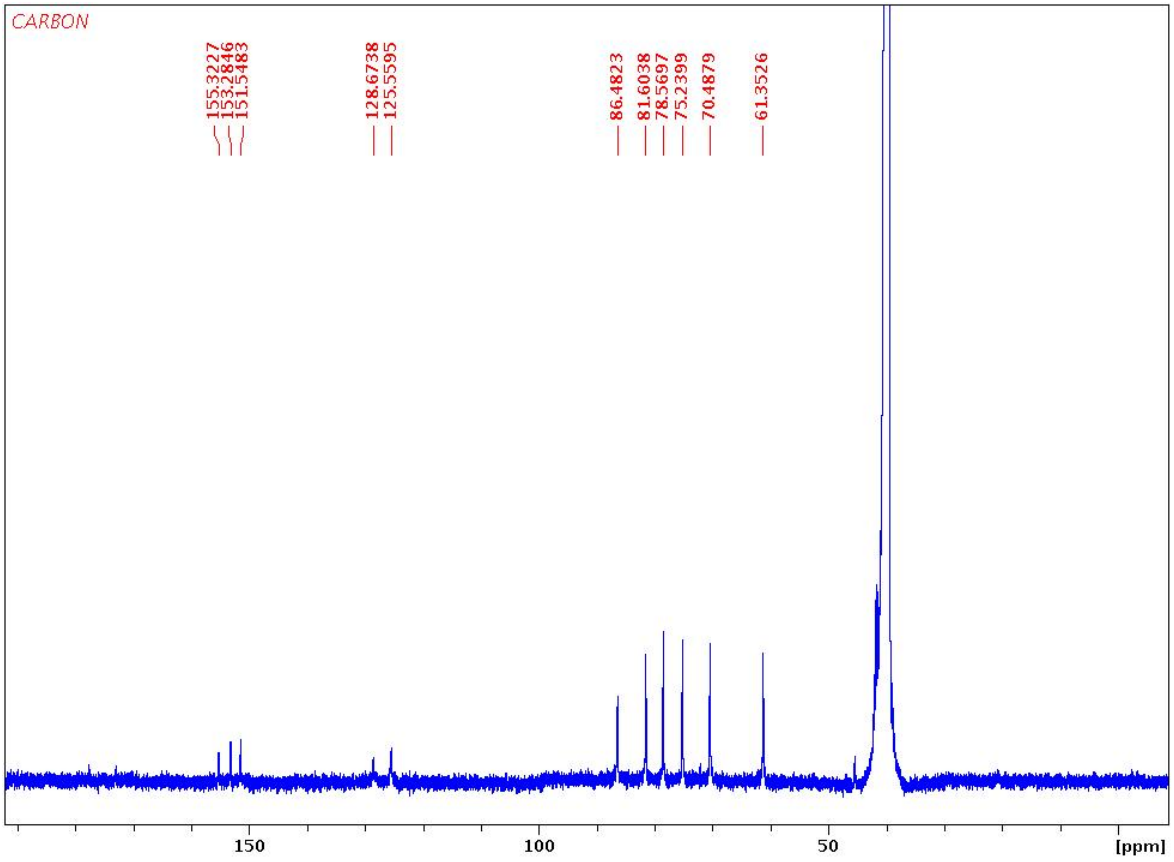


Figure SM-6. ^{13}C NMR of compound **1b** $ZnPcGlc_8$ (DMSO 40 ppm).

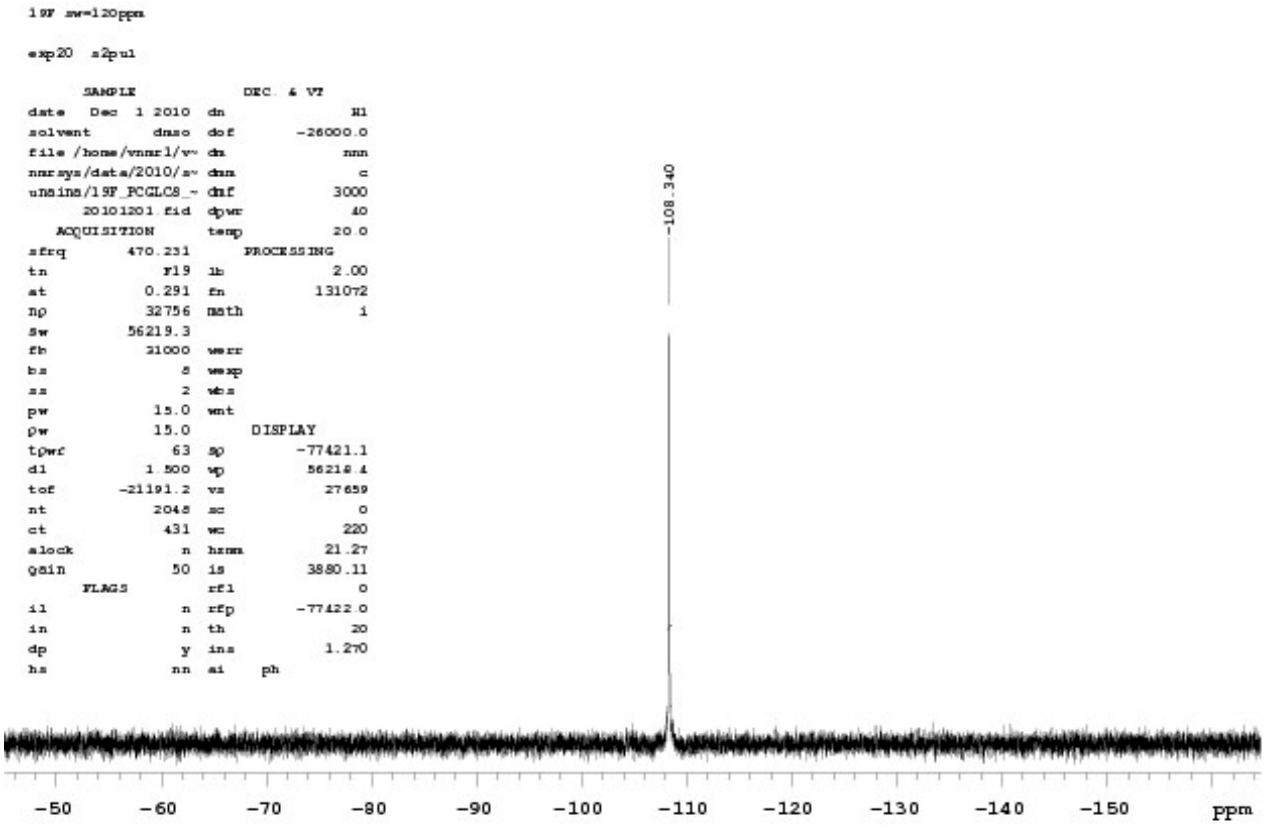
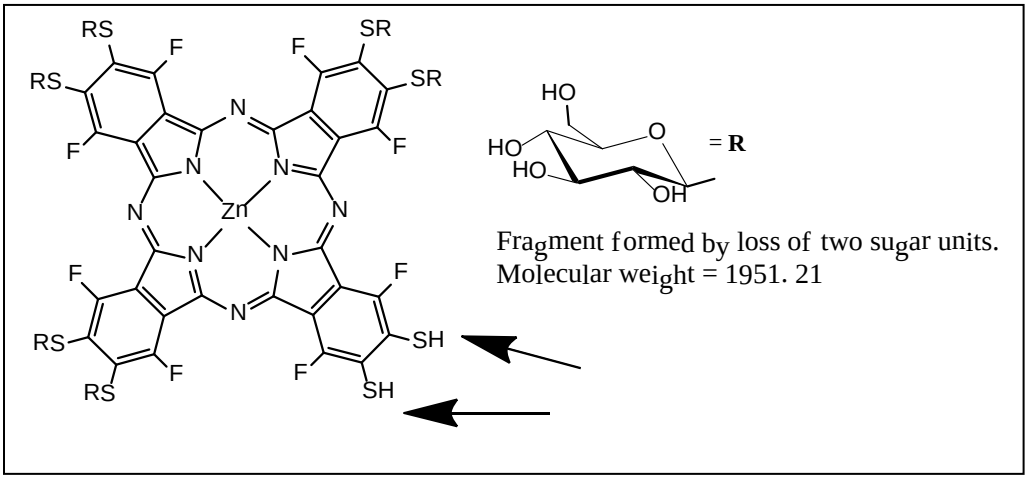


Figure SM-7. ^{19}F NMR of compound **1b** $ZnPcGlc_8$ (DMSO-d_6).



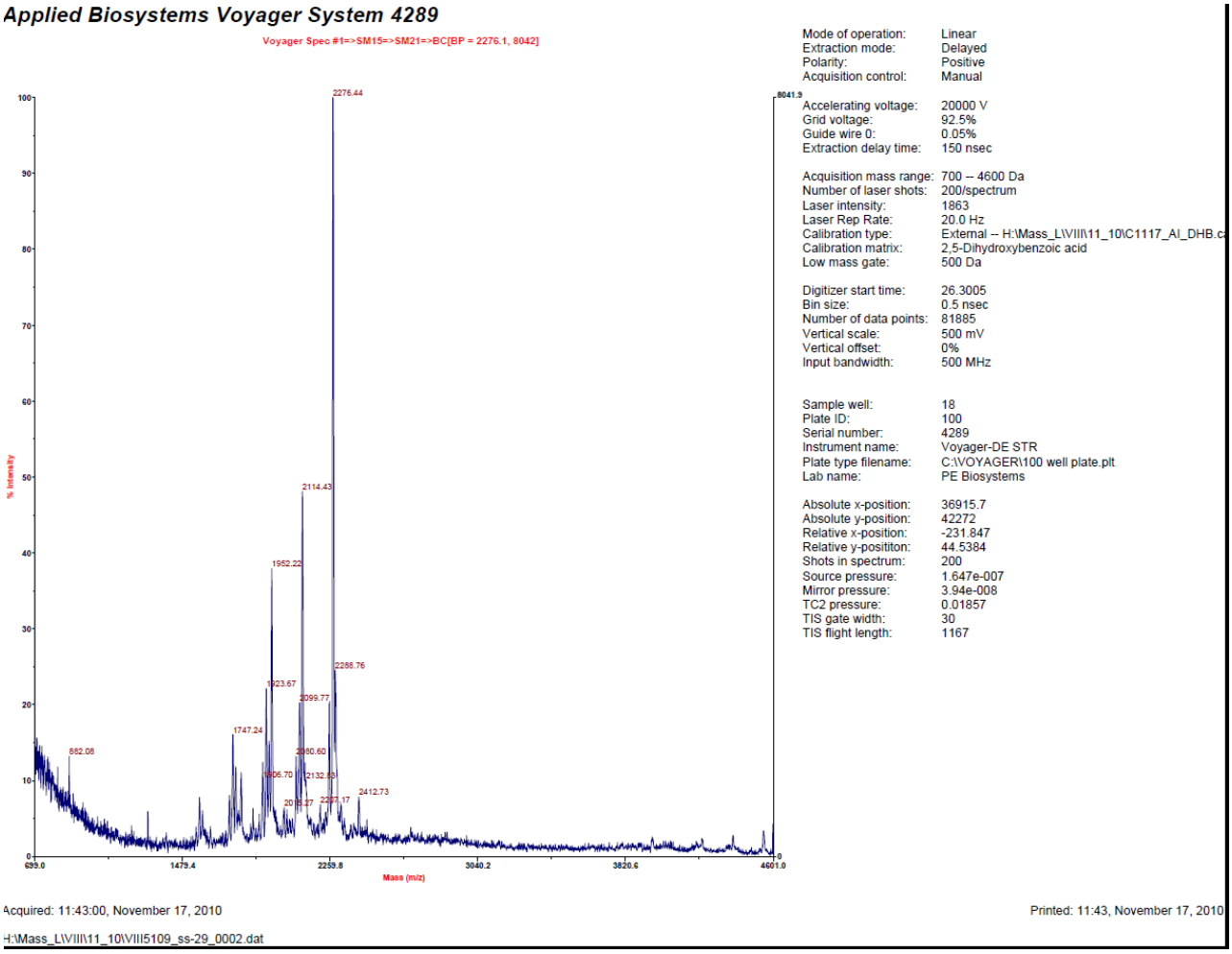


Figure SM-8. MALDI of compound **1b** $ZnPcGlc_8$ show that parent ion and the loss of 1, 2, 3, and 4 Glc units, that leaves a thiol on the macrocycle, e.g. the structure above shows the loss of two sugars.[1]

UV-visible spectroscopy:

UV-visible spectra were recorded on a Varian Bio3 spectrophotometer using dilute solutions, typically ~ 2 µM, of compound $ZnPcGlc_8$ in DMSO, toluene, ethyl acetate, ethanol, phosphate buffer saline (PBS) and 1:1 DMSO: H_2O mixture solvent. The spectra were obtained from 350 to 850 nm using 1 cm quartz cuvettes. The UV-visible spectra of ZnPc and $ZnPcF_{16}$, ~ 2 µM in DMSO were also recorded to show the difference in the spectra with the change in the nature of substituent from electron withdrawing F atoms to electron donating S atoms in thioglucose group.

Emission spectroscopy, fluorescence quantum yield, and fluorescence lifetime measurements:

Steady-state fluorescence (emission) spectra and fluorescence lifetime were measured with a Fluorolog τ3, Jobin-SPEX Instrument S.A., Inc. For ZnPc and $ZnPcF_{16}$ emission spectra were recorded in DMSO, whereas for $ZnPcGlc_8$ spectra were recorded in DMSO, toluene, ethyl acetate, ethanol, phosphate buffer saline (PBS) and 1:1 DMSO: H_2O mixture solvent. The concentrations of each compound in these solutions were typically ~2 µM. In each case the compounds were excited at 646 nm where the O.D for each compound was 0.027. Both, the excitation and emission monochromators had a band pass of 2 nm. The corrected emission (for instrument response) and absorption (UV-visible) spectra were used to calculate the quantum yield. The quantum yields were calculated relative to ZnPc in DMSO, which has a fluorescence quantum yield of 0.20.[2] The quantum yields were measured indirectly using ZnPc; thus these values may have some systematic error. All experiments were carried out on the same day, using identical concentrations and similar experimental conditions to minimize any experimental errors. To measure the fluorescence decay lifetime, 401 nm laser was used to excite the molecule, average power = 13.6 pJ/pulse, and emission decay was recorded at 740 nm. The band pass for both excitation and emission monochromators were 3 nm. The decays were obtained with 1000 counts or more in the peak channel. The data was fitted with first exponential fit curve and the goodness of the fit was judged by reduced χ^2 values, the random distribution of residuals and the autocorrelation of the residuals.

Dynamic Light Scattering (DLS) for particle size measurement:

A solution of $ZnPcGlc_8$ was found to aggregates in various studied solvents. A Precision Detector PD2000DLS Cool-Batch dynamic light scattering (DLS) instrument was used in batch mode at 25 ^oC to determine particle size. These solutions were also sonicated in Fisher SF15 sonicator to break the large aggregates into the small ones. To measure the size of $ZnPcGlc_8$ in 2% solution of DMSO in PBS, the solution was sonicated for ca. 30 minutes and left to rest for another 30 min and then the size was measured. Without sonication large sized particle were observed mainly but after sonication the large sized particles, 265 nm broke down to smaller sized particles, 65 nm.

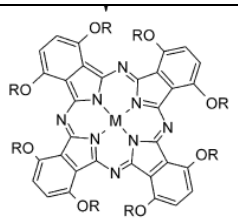
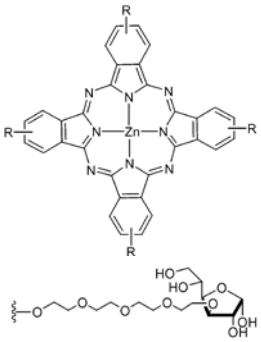
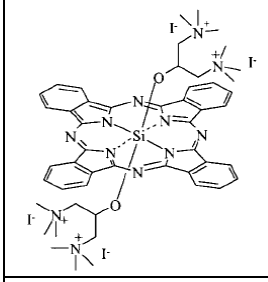
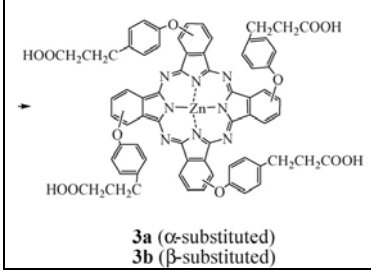
Octanol/Water partition coefficient:

For octanol/water partition coefficient, a saturated solution of $ZnPcGlc_8$ was prepared in 1:1 (V/V) mixture of these two solvents. The mixture solution was vigorously shaken, followed by letting the mixture stand for 8-10 hours to separate the two layers. UV-visible spectra were recorded for the two layers. The partition coefficient for this compound was calculated by measuring the Soret band and Q-Band intensities in two solvents. Since the compound aggregates in water, to ensure accuracy a 50 µL of each layer of the above saturated solution of $ZnPcGlc_8$ in mixture solvent (1:1=octanol:water) were withdrawn and were mixed again and diluted with 1 mL of DMSO, to break any aggregates formed (if there), and then the spectra were recorded to calculate the partition coefficient.

Photo bleaching of $ZnPcGlc_8$ in sunlight:

5 μM solution of $ZnPcGlc_8$ in DMSO was exposed to sunlight (on a full sunny day) over a time period of 2 h and 30 min and absorption and emission spectra were recorded at different interval of times. The power of sunlight was measured to be 60-95 W/m^2.

Table SM-1. Photophysical properties of selected Pcs from the literature for comparison.

Compound	**solvent**	**Φ_F**	**τ_F, ns**	**Φ_Δ**	**reference**
ZnPc	Acetone DMSO	0.17 0.20	 1.2	0.17 0.67	[3] [4]
$ZnPcF_{16}$	Acetone Liposome	0.04 0.08-0.15		0.13 0.23-0.61	[3] [5]
$ZnPcF_{12}(SR)_4$	DMSO	0.08	0.78	0.65	[4]
$ZnPcF_8(SR)_8$ $SCH[(CH_2O(CH_2CH_2O)_2C_2H_5)_2]$	DMSO	0.15	1.57	0.60	[6]
	DMSO	0.07		0.5	[7]
	DMF	0.23		0.58	[8]
	DMF	0.64		0.26	[9] Si(IV)Pc
 **3a** (α-substituted) **3b** (β-substituted)	DMF	0.16-0.23	3.4-3.5	0.58-0.70	[10]

There is a strong solvent dependence, especially on the $^1O_2$ quantum yield.[4] There are a variety of bioconjugates to Pcs reported.[11,12]

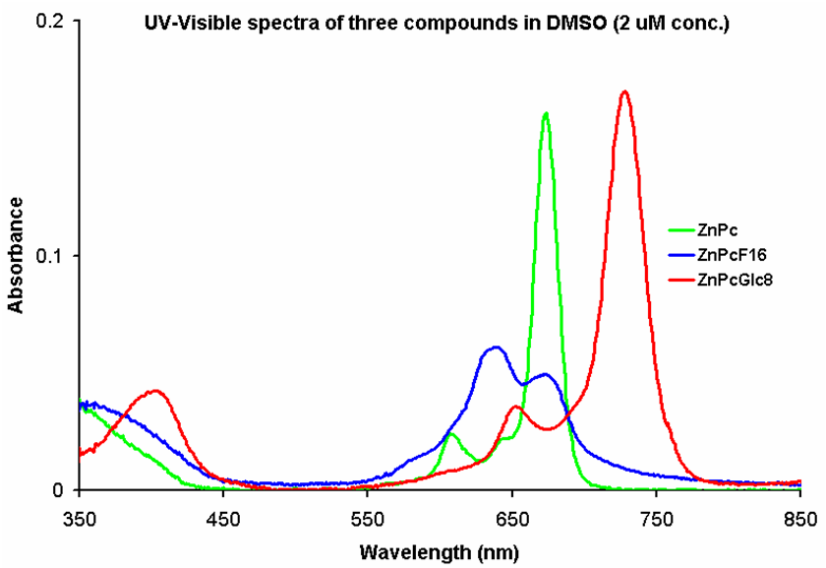


Figure SM-9. UV-visible spectra of three compounds ZnPc, $ZnPcF_{16}$ and $ZnPcGlc_8$ in DMSO. The concentration of each solution was 2 µM. The blue shift in the Q-band for $ZnPcF_{16}$ is because of the electron withdrawing effect of the F atoms whereas in case of $ZnPcGlc_8$ a strong red shift was observed because of the electron donating effect of S-atoms in thioglucose moieties.

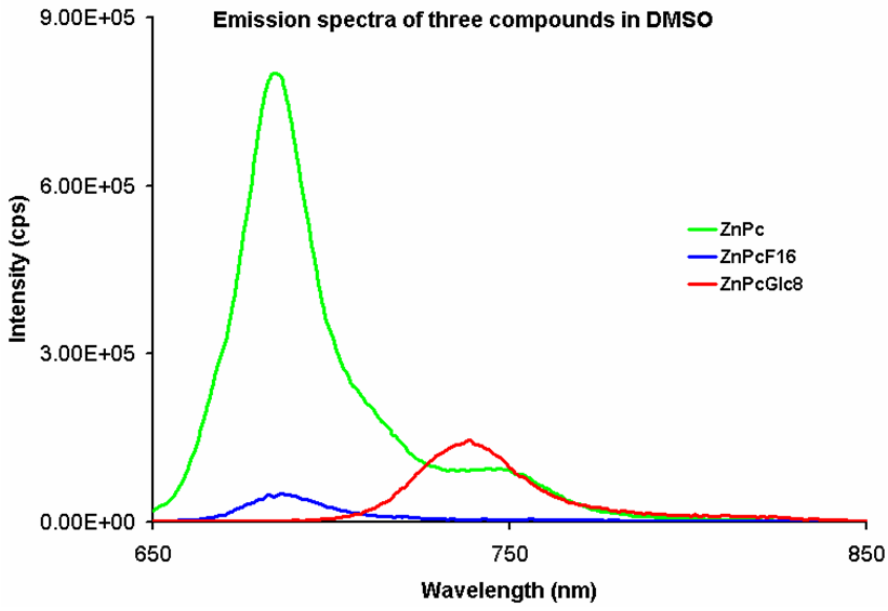


Figure SM-10. Emission spectra of three compounds ZnPc, $ZnPcF_{16}$, and $ZnPcGlc_8$ in DMSO. The three compounds were excited at 646 nm where absorbance of each compound was 0.027. The band pass for both emission and excitation monochromators were 2 nm.

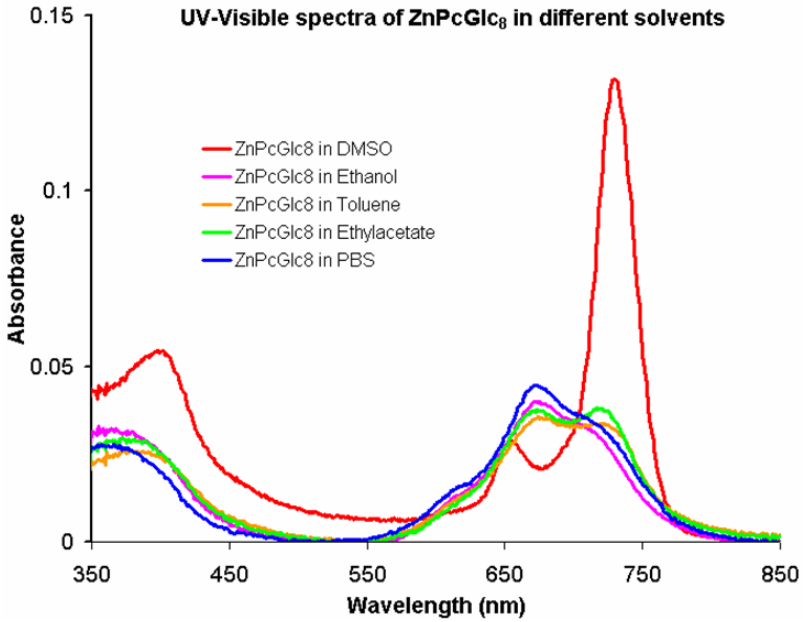


Figure SM-11. UV-visible spectra of $ZnPcGlc_8$ compound in different solvents. 1 mM $ZnPcGlc_8$ in DMSO was used as stock solution to prepare these solutions. The final concentration of the compound in each solution was 2 μM.

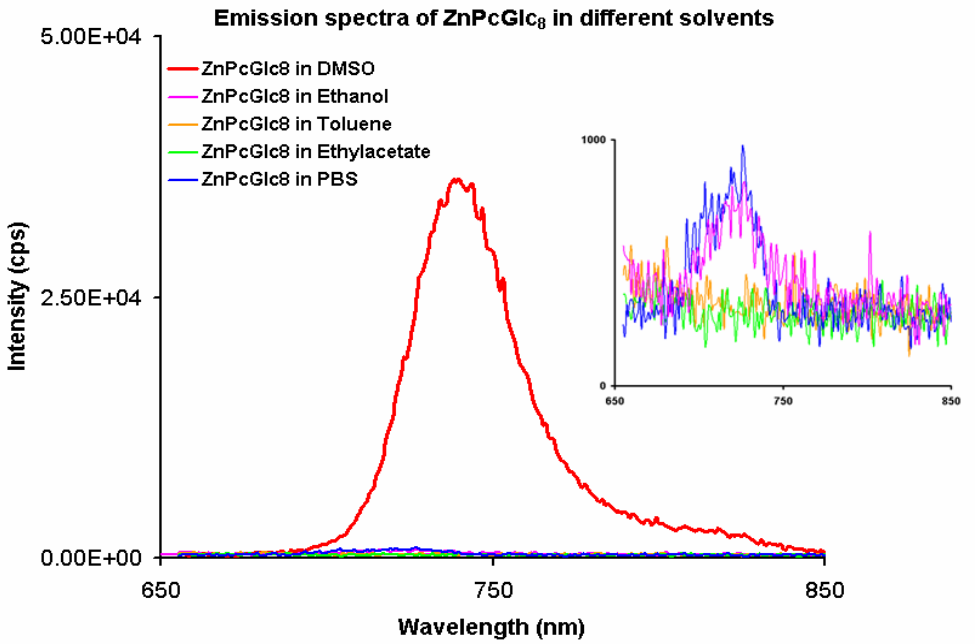


Figure SM-12. Emission spectra of $ZnPcGlc_8$ in different solvents. The compounds were excited at 647 nm where the absorbance of each compound was 0.026 in the indicated solvents; the bandpass for both excitation and emission monochromators were 2 nm. The area under these curves was used to find the quantum yield of $ZnPcGlc_8$ in different solvents, using ZnPc as standard (Φ_F = 0.20 in DMSO).

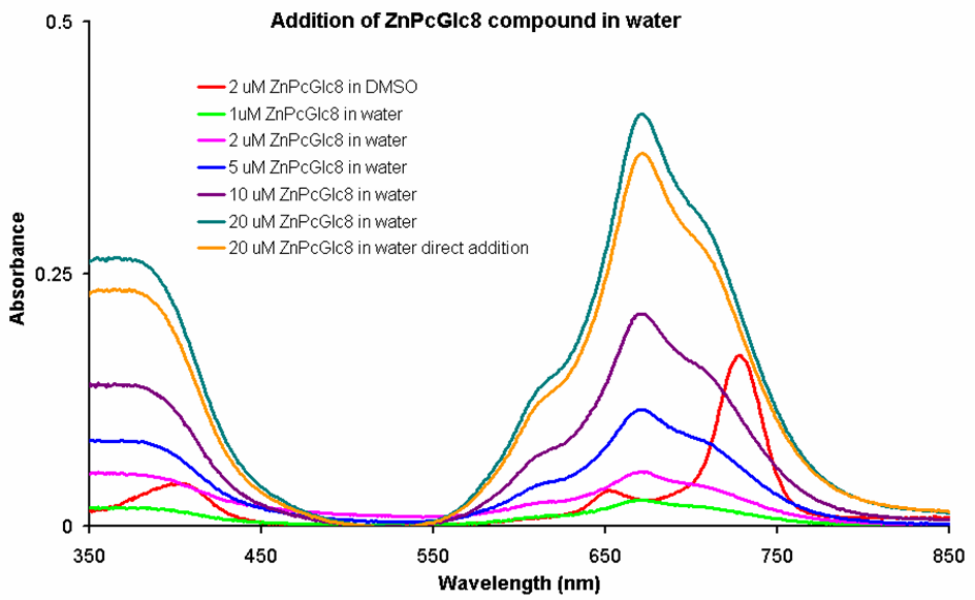


Figure SM-13. UV-visible spectra of $ZnPcGlc_8$ compound in water. Solutions of different concentration ranges from 1 µM to 20 µM of $ZnPcGlc_8$ in water were prepared from a stock solution, 1 mM $ZnPcGlc_8$ in DMSO. In water the Q-band of $ZnPcGlc_8$ is blue shifted by 59 nm. Broadening of the Q-band is because of the aggregation of the thioglycosylated phthalocyanine in water. The shoulder on the blue end of the Q-band is indication of H-aggregation.

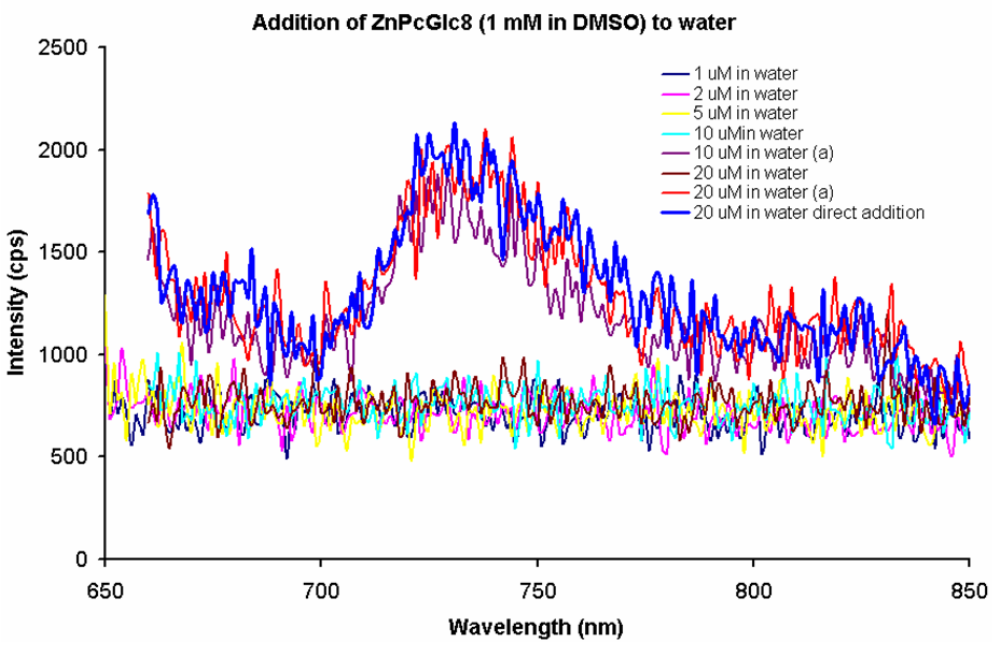


Figure SM-14. Emisson spectra of $ZnPcGlc_8$ in water. Ex = 647 nm, Bandpass =2 nm. The graphs with (a) have band pass = 5 nm.

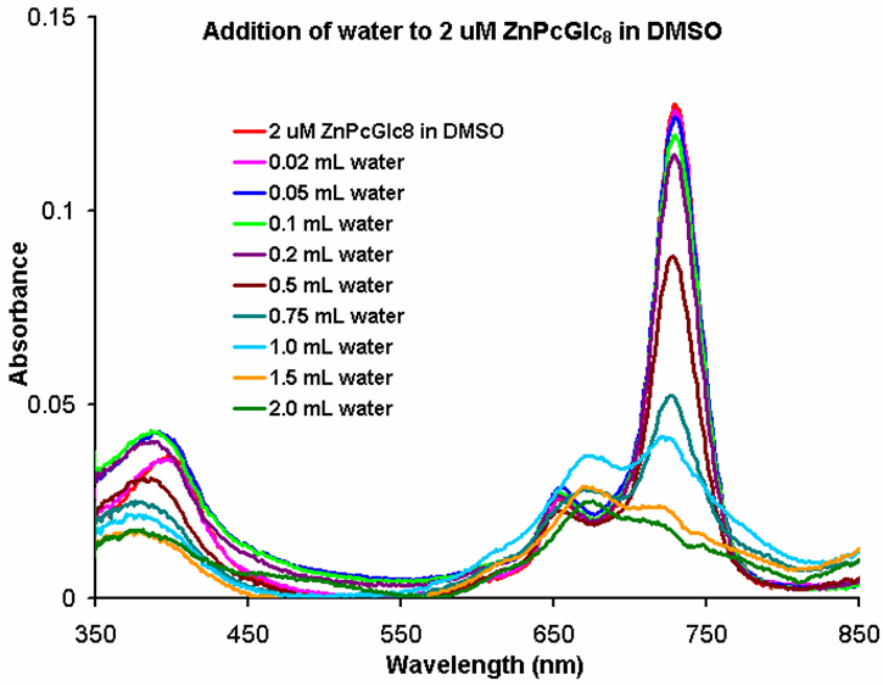


Figure SM-15. UV-visible spectra of solutions after addition of different volumes of water to the 2 µM stock solution of $ZnPcGlc_8$ in DMSO. After addition, the mixture was mixed thoroughly and left to stand to establish the equilibria.

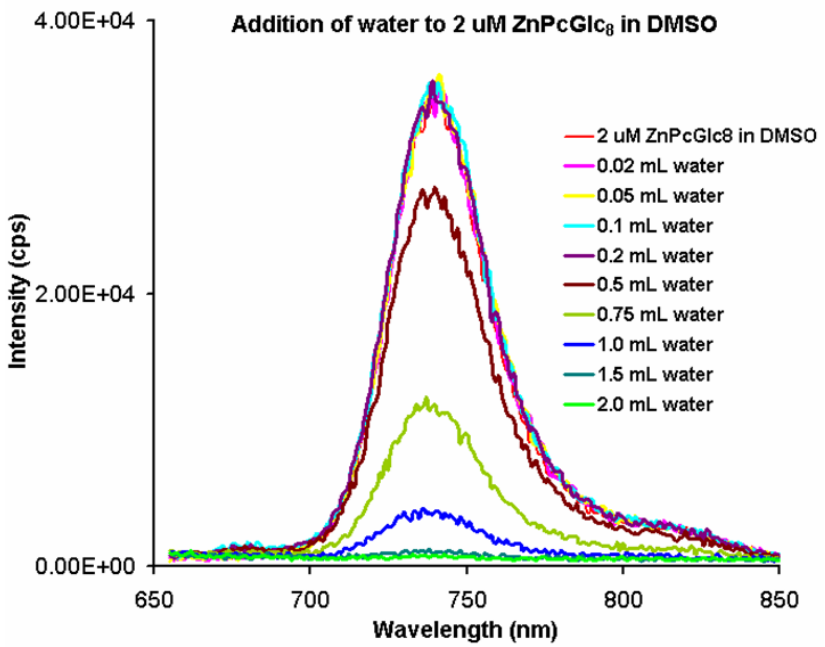


Figure SM-16. Emission spectra of the solutions after addition of different volumes of water to 2 µM stock solution of $ZnPcGlc_8$ in DMSO. The emission was fully quenched in 1:1 mixture solution of DMSO:water.

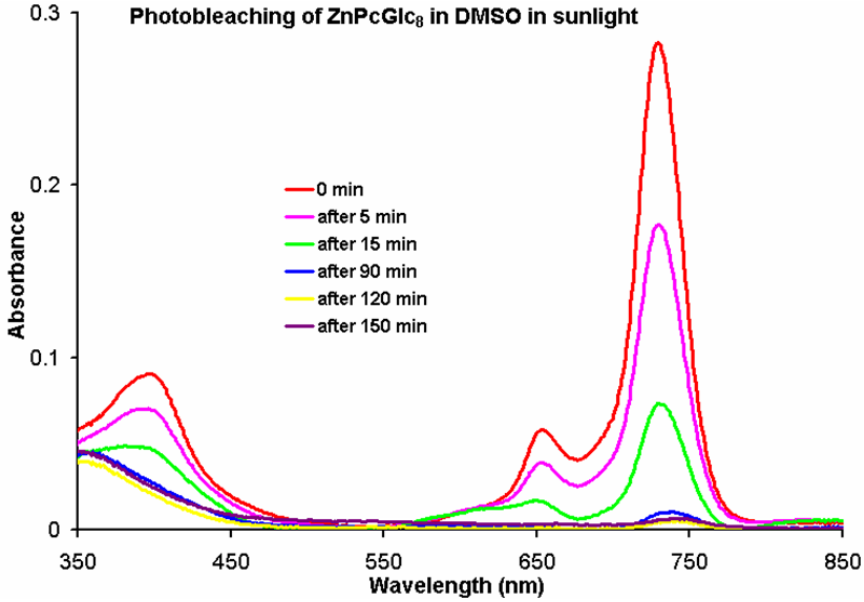


Figure SM-17. Photobleaching of 5 μM $ZnPcGlc_8$ in dry DMSO. The sample was exposed to sunlight over a time period of 2 h 30 min and the UV-visible spectra was recorded. The power of sunlight was measured to be 60-95 W/m^2. A continuous decrease in the absorption intensity indicates the compound photobleaches under these conditions.

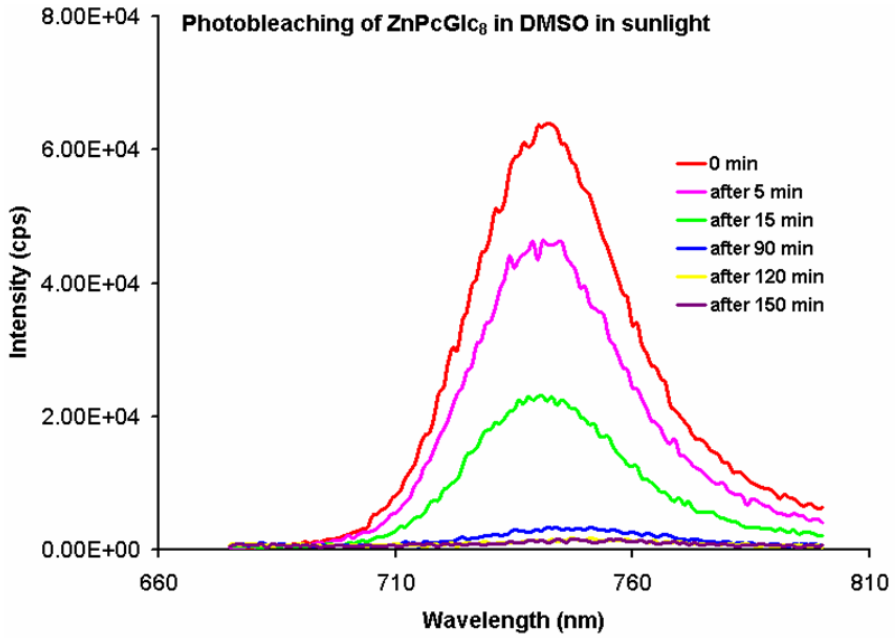


Figure SM-18. A continuous decrease in the emission intensity is in agreement with the decrease in the absorption intensity for this solution. Ex = 654 nm, Band pass for both emission and excitation monochromators were 2 nm.

Table SM-2. Photophysical Data of $ZnPcGlc_8$ in other solvents:

Compound	Solvent	Absorption Wavelength	Emission wavelength	Φ_F		τ_F ,ns (%) (χ^2)	
				Air	N_2	Air	N_2
$ZnPcGlc_8$	Ethanol	361, 671, 704	726	0.0022	0.0021	T_1= 0.18(18%) (1.19) T_2= 2.19(82%)	T_1=0.19(17%) (1.20) T_2=2.21(83%)
	Ethyl acetate	370, 672, 716	-----	------	------	----------	----------
	Toluene	379, 673, 719	----	------	------	----------	----------

Table: Φ_F was measured by exciting the molecule at 647 nm where all the compounds have same absorbance 0.027. Both quantum yield and singlet state life time was measured in air and under N_2 by purging N_2 gas through the solution for 10 min. To measure the singlet state life time 401 nm laser was used, emission = 740 nm, and band pass = 5nm.

Cell Uptake studies*:*

Materials: Human breast cancer cells (MDA-MB-231) were obtained from David A. Foster's laboratory, Department of Biological Sciences, Hunter College. Hanks' Balanced Salt solution, DMEM, Antibiotic-Antimycotic Solution and Trypsin-EDTA solution 10×, were all purchased from Sigma-Aldrich. Fetal bovine serum was obtained from Biowest. Fluorescent Mounting Medium was obtained from Dako. PBS (4.3 mM Na_2HPO_4, 137 mM NaCl, 2.7 mM KCl, 1.4 mM KH_2PO_4 pH 7.4) was made using chemicals all purchased from Sigma-Aldrich.

Instrumentation. Image Core's Nikon Optiphot 2 fluorescence microscope with mercury lamp was used to capture cell images. Fluorescence filters used: position 2 single filter FITC excitation: 465-495nm, band absorption: 515-555 nm; position 3 single filter TxRed excitation: 540-580, band absorption: 600-660; position 4 triple filter DAPI, FITC and TxRed excitation: 340-380, 465-495, 540-580, band absorption: 435-485, 515-555 and 600-660. Images were all captured as JPEG files.

Cell Culture. MDA-MB-231 cells were sustained in DMEM medium containing 10% fetal bovine serum and 1% antimycotic. Cells were kept in an incubator set at 37 °C with a 5% CO_2 atmosphere. Cells were seeded at 2 x 10^5 cells/mL at 2mL per well. Incubation of $ZnPcGlc_8$ was done at approximately 80% cell confluency 24 hours prior to fixing and mounting cells onto glass slides.

Fluorescence Imaging of Cells. MDA-MB-231 breast cancer cells were seeded in culture dishes containing cover slips. Cells were grown to 80% confluency then incubated for 24 hours with $ZnPcGlc_8$, dissolved in DMSO, at final concentrations of 50 nM. Cells were washed 5-6 times with PBS buffer to remove unbound $ZnPcGlc_8$ compound and then fixed with 4% paraformaldehyde in PBS and washed again 5-6 times with PBS. Cover slips were then mounted on slides with Dako fluorescent mounting medium and left to air-dry for 1 hour. Corners were sealed with clear nail polish. Slides were visualized with Image Core's Nikon Optiphot 2 fluorescence microscope. Microscope set at 20x magnification, quick mode, exposure 2, interval time 30 sec at positions 2, 3 and 4 of microscope. Four days later, fixed slides were again visualized under same conditions of original images.

$ZnPcGlc_8$ as a sensitizer for singlet oxygen ($^1O_2$) production

Experimental Section

Materials and instrumentation. Reagents and solvents were obtained commercially and used without further purification. *meso*-Tetra(4-carboxylphenyl) porphine (TCPP) was purchased from Frontier Scientific, Inc., [2-(dicyclohexyl phosphino) ethyl]trimethyl ammonium chloride (> 98%) from Strem Chemicals, Inc., Hydrochloric acid (37.5%) was from Fisher Scientific. Dimethyl sulfoxide-d_6 (DMSO, 99.9% of D) was from Cambridge Isotope Laboratories. Inc. Dimethylanthracene (DMA), Zinc phthalocyanine (ZnPc), Deuterium oxide (D_2O, 99% of D), Tris(hydroxymethyl)aminomethine (> 99.8%), Ethylenedinitrilotetraacetic acid and disodium salt dihydrate (EDTA, > 99%) were from Sigma-Aldrich. Deionized water was obtained from Nanopure Water (Barnsted System, USA). Steady-state photooxidation was conducted in oxygen-saturated solution using a 150 W Xenon lamp (6255 Xenon lamp housed in 66907 Arc Lamp Source, Newport Oriel Instruments) equipped with an IR blocking filter (59042, Newport Oriel Instruments) and a monochromator with primary wavelength region 450-2000 nm (77250 1/8 m Monochromator and 77305 Grating, Newport Oriel Instruments). Other instruments employed in this research include a BioMate 3 UV-visible spectrophotometer (Thermo Scientific) and a Cary 300 UV-visible spectrophotometer (Varian, Inc.) for taking absorbances and spectra. The determination of photooxidation products was done on a 300 MHz Bruker Spectrospin FT-NMR. All of the measurements were carried out at ambient temperature. Samples were protected from light when not being irradiated.

Direct observation of $^1O_2$ after irradiation of $ZnPcGlc_8$ at 532 nm. The kinetics of $^1O_2$ luminescence at 1270 nm was monitored in pH 7.4 D_2O Tris buffer solution by time-resolved Nd:YAG laser equipped with a low temperature cooled Ge detector, as previously described.[13-16] An absorption spectrum is shown in Figure SM-19. $^1O_2$ luminescence at 1270 nm was observed upon irradiation of air-saturated $ZnPcGlc_8$ solution at excitation wavelength of 532 nm. The control was carried out under identical conditions but in the presence of 20 mM NaN_3. NaN_3 is a well-known efficient $^1O_2$ quencher that reacts with $^1O_2$ at a rate constant of $5.0x10^8$ M^{-1} s^{-1} in water.[17]

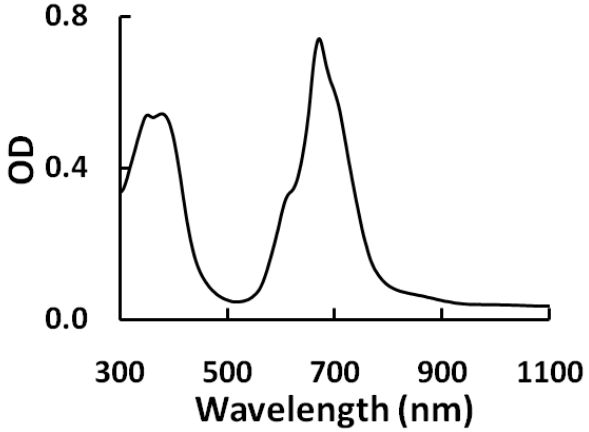
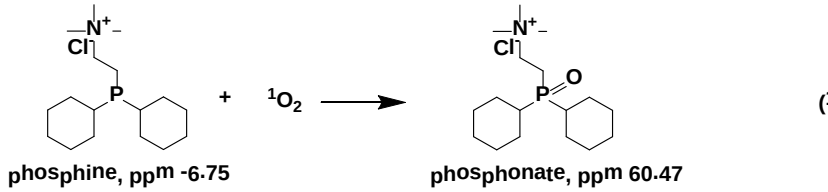


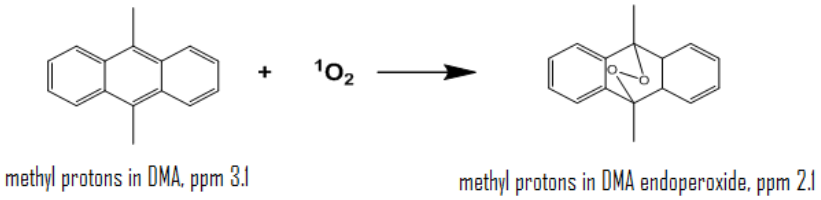
Figure SM-19. Absorption spectrum of $5.4x10^{-6}$ M $ZnPcGlc_8$ in 50 mM pH7.4 H_2O Tris buffer with an estimated extinction coefficient of $1.37x10^5$ M^{-1} cm^{-1} at a maximum absorption wavelength of 671 nm.

Quantum yield of $^1O_2$ production (Φ_Δ). Φ_Δ was determined according to previously reported method[18] in air-saturated pH 7.4 D_2O Tris buffer on a relative basis by steady-state photolysis. 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin (TCPP) in pH 7.4 D_2O Tris buffer and zinc(II) phthalocyanine (ZnPc) in DMSO

were used as references for excitation at 532 nm and 700 nm, respectively. Φ_Δ values of 0.53 for TCPP in weak base solutions[14] and 0.67 for ZnPc in DMSO[2] have been reported. A water-soluble phosphine, [2-(dicyclohexylphosphino)ethyl]trimethylammonium chloride, and 9,10-dimethylanthracene (DMA) were used as $^1O_2$ traps in aqueous solution and in DMSO, respectively. A 1.50 mL mixture of $ZnPcGlc_8$ ($OD_{532\ nm}$ = 0.2-0.3 and $OD_{700\ nm}$ = 0.4-0.5) and phosphine (5.0-10.0 mM) was added into a 1-cm quartz cuvette and irradiated at 532 nm or 700 nm for 20 minutes, followed by an immediate measurement of phosphine oxidation by ^{31}P NMR. $^1O_2$ photooxidation of phosphine trap leads to the formation of a sole product phosphonate (eq. 1). The peaks at δ -6.45 (s, 1P) and δ 60.50 (s, 1P) represent phosphine and phosphonate, respectively. The percent yields of phosphonates were controlled below 20% and calculated by comparison of the integrated ^{31}P NMR peaks of phosphine with those of phosphonate. Trapping experiments under identical conditions were conducted for the reference sensitizer TCPP at excitation wavelength of 532 nm. The absorbance of $ZnPcGlc_8$ and TCPP at 532 nm was adjusted the same. For 700 nm excitation, trapping experiments for the reference sensitizer of ZnPc were conducted in air-saturated DMSO solution using DMA as an $^1O_2$ acceptor. A 1.50 mL mixture of ZnPc ($OD_{700\ nm}$ = 0.4-0.5) and DMA (5.0-10.0 mM) was added into a 1-cm quartz cuvette and irradiated at 700 nm for 20 minutes, then followed by an immediate measurement of ^1H NMR with methyl proton peak in DMA at δ 3.1 ppm and in DMA endoperoxides at δ 2.1 ppm (eq. 2). Control experiments in dark as well as in the absence of sensitizers were carried out in order to correct phosphine or DMA oxidation by heat or by ground-state oxygen molecules if there was any. Φ_Δ values were calculated according to eq. 3. Where $\Phi_{\Delta,\ sample}$ and $\Phi_{\Delta,\ reference}$ are the quantum yields of sample (e.g., $ZnPcGlc_8$) and reference sensitizers, respectively; $\%_{trap\ oxidation\ by\ sample}$ and $\%_{trap\ oxidation\ by\ reference}$ represent the percent yields of trap oxides formed after irradiation of sample and reference sensitizers, respectively. Examples of ^{31}P NMR and ^1H NMR spectra for Φ_Δ measurements are given in Figure SM-20 and Figure SM-21.



(1)



(2)

$$\frac{\Phi_{\Delta,\ sample}}{\Phi_{\Delta,\ reference}} = \frac{\%_{trap\ oxidation\ by\ sample}}{\%_{trap\ oxidation\ by\ reference}} \qquad (3)$$

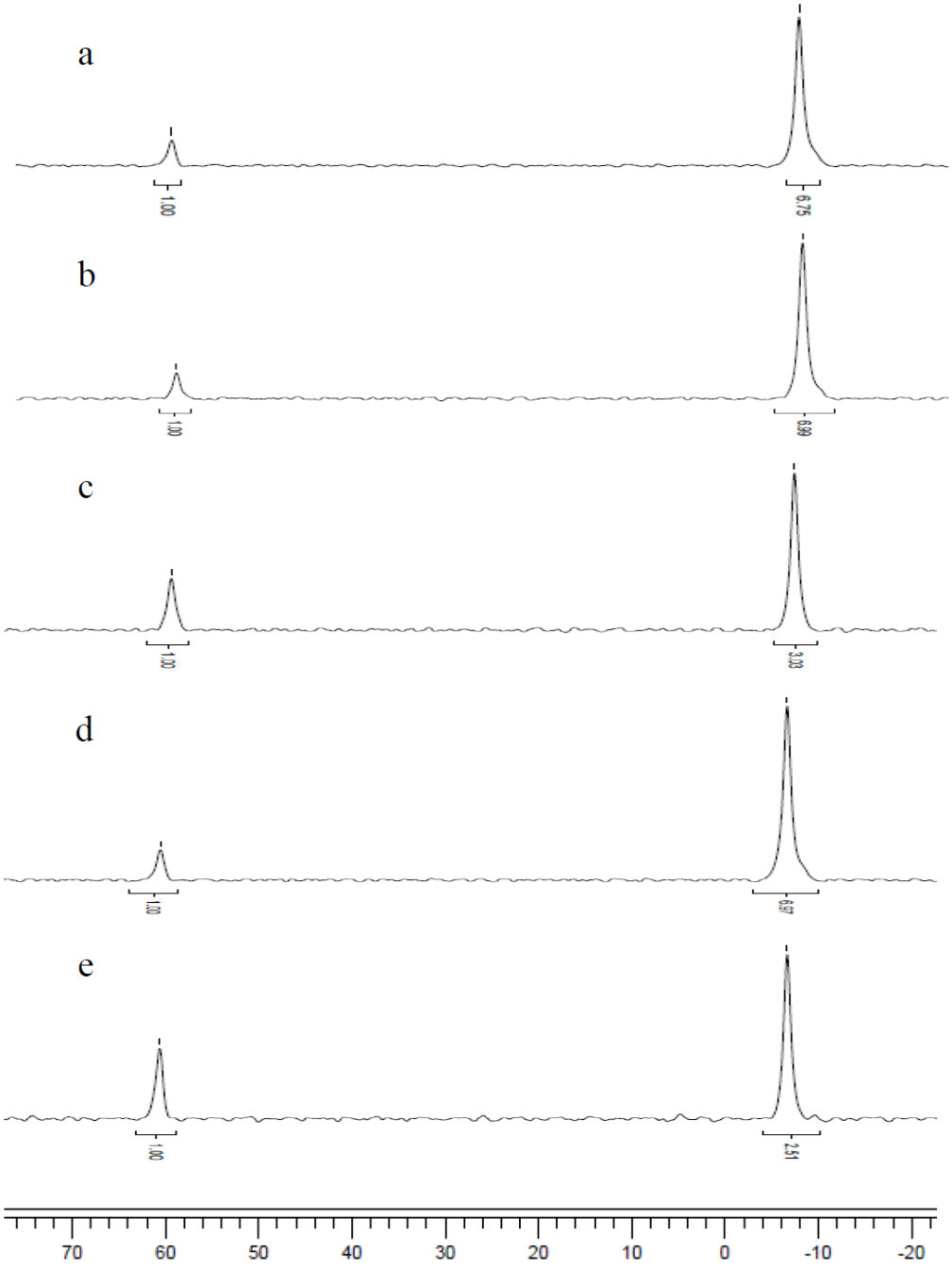


Figure SM-20. ^{31}P NMR spectra for Φ_{Δ} measurements in pH 7.4 D_2O Tris buffer at excitation wavelength of 532 nm using a water-soluble phosphine, [2-dicyclohexylphosphino)ethyl]trimethylammonium chloride as an $^1O_2$ trap. (a) irradiation of phosphine (6.0 mM) in the absence of a sensitizer for 20 minutes; (b) a mixture of phosphine (6.0 mM) and $ZnPcGlc_8$ (OD = 0.28 at 532 nm) before irradiation, (c) irradiation of (b) for 20 minutes, (d) a mixture of phosphine (6.0 mM) and TCPP (OD = 0.28 at 532 nm) before irradiation, (e) irradiation of (d) for 20 minutes.

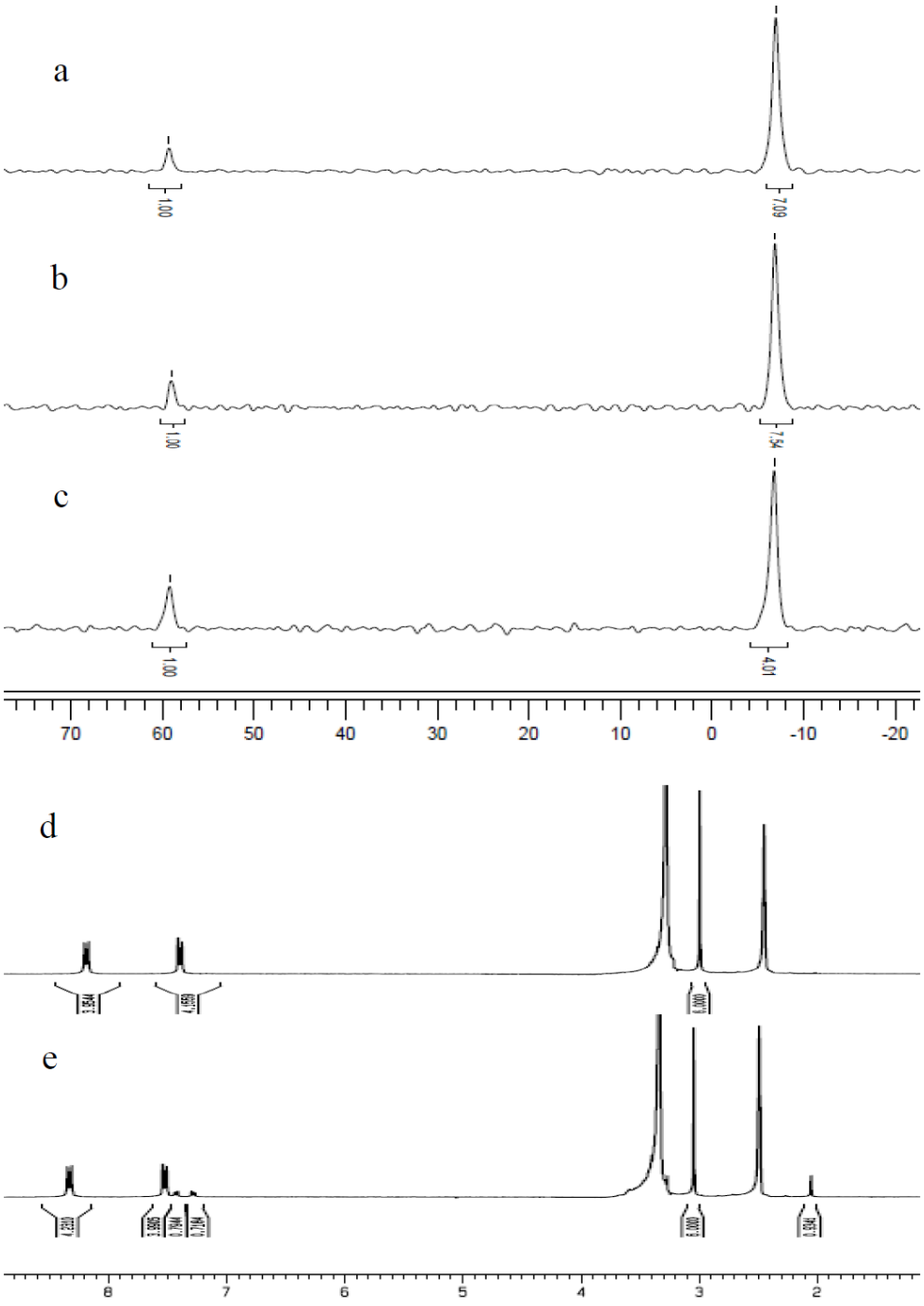


Figure SM-21. ^{31}P NMR (a-c) and ^{1}H NMR (d-e) spectra for Φ_{Δ} measurements in pH 7.4 D_2O Tris buffer at excitation wavelength of 700 nm using ZnPc as a reference and DMA as an $^{1}O_2$ trap in DMSO. (a) irradiation of phosphine (6.0 mM) in pH 7.4 D_2O Tris buffer in the absence of $ZnPcGlc_8$ for 20 minutes; (b) a mixture of phosphine (6.0 mM) and $ZnPcGlc_8$ (OD = 0.47 at 700 nm) before irradiation, (c) irradiation of (b) for 20 minutes, (d) a mixture of DMA (10 mM) and ZnPc (OD = 0.47 at 700 nm) before irradiation, (e) irradiation of (d) for 20 minutes.

Results and brief discussion

Direct observation of $^1O_2$ luminescence at 1270 nm. Kinetic decay of $^1O_2$ luminescence was observed directly at 1270 nm in air-saturated pH 7.4 D_2O Tris buffer upon irradiation of $ZnPcGlc_8$ at 532 nm (Fig. SM-22). The decay trace was assigned to $^1O_2$ luminescence because the kinetics of $^1O_2$ was sensitive to the presence of NaN_3. The total decay rate constant (k_d) of $^1O_2$ removal in pH 7.4 D_2O Tris buffer was measured to be $1.1x10^4$ s^{-1} that is comparable with literature value of $1.5x10^4$ s^{-1} in D_2O.[19]

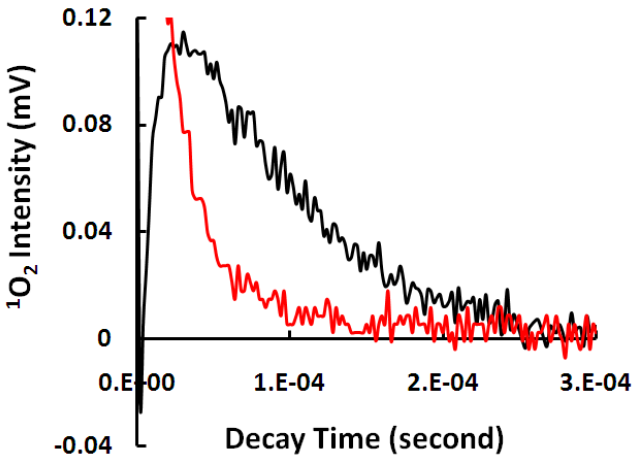


Figure SM-22. Time-resolved $^1O_2$ luminescence upon pulsed laser irradiation of $ZnPcGlc_8$ at 532 nm (OD $_{532\ nm}$ = 0.12) in pH7.4 Tris D_2O buffer in the absence (black line) and presence (red line) of 20 mM NaN_3.

Determination of Φ_Δ. Φ_Δ values were determined on a relative basis that requires a reference, such as TCPP and ZnPc used in this work. The reactions of traps (phosphine and DMA) with $^1O_2$ are shown in eq. 1 and eq. 2. The conversion yields of trap oxidation were controlled below 20% to assure an efficient trapping condition. The Φ_Δ values were calculated according to the eq. 3 and summarized in Table SM-3. Control experiments in the absence of $ZnPcGlc_8$ as well as in dark did not show any increase in the conversion yield of trap oxidation although the initial phosphonate peaks were observed due to the slow phosphine oxidation by triplet oxygen in air.

Table SM-3. Φ_Δ for $ZnPcGlc_8$ in pH7.4 D_2O Tris buffer

$\lambda_{excitation}$, nm	Φ_Δ
532	0.42 ± 0.01
700	0.41 ± 0.01

The fact that the Φ_Δ is independent of excitation wavelength affirms that Kasha's rule indeed holds for $ZnPcGlc_8$. Kasha's rule states that molecules excited on their higher energy levels relax within 0.1-1 ps down to the first excited singlet (S_1) or triplet (T_1) states prior to emission or lose of energy.[20,21] Following the dictates of Kasha's rule, $^1O_2$ production originated from a common state. The immediate precursor to $^1O_2$ in $ZnPcGlc_8$ is the same state regardless of excitation wavelengths at 532 nm or 700 nm.

All of the measurements for $ZnPcGlc_8$ were conducted in physiological pH 7.4 Tris buffer, in which TCPP is fairly soluble. Phthalocyanines (Pc) are ideal $^1O_2$ references for longer wavelength excitation. However, water-soluble Pc are either commercially unavailable or have not been well developed as $^1O_2$ references. We therefore used ZnPc as a reference and DMA as a trap in DMSO for Φ_Δ measurements at 700 nm.

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