Supporting Information for

Assessment of the relevance of GaPc substituted with azidopolyethylene glycol chains for photodynamic therapy. Design, synthetic strategy, fluorescence, singlet oxygen generation and pH-dependent spectroscopic behaviour

Veysel Koç[†], Sevinc Zehra Topal^{†,*}, Özge Dilara Ateş, Duygu Aydın Tekdaş, Emel Önal, Fabienne Dumoulin, Ayşe Gül Gürek^{*} and Vefa Ahsen

Gebze Technical University, Department of Chemistry, Gebze, Kocaeli, 41400, Turkey

* Correspondance: <u>gurek@gtu.edu.tr</u>, <u>sztopal@gtu.edu.tr</u>; Tel.: +902626053017; Fax: +902626053005

[†] These authors contributed equally to this work.

1. Materials, Methods and Equipments

Synthetic procedures were carried out under a dry argon atmosphere, unless otherwise specified. All reagents and solvents were purchased at the highest commercial quality available and used without further purification. Column chromatography was carried out on silica gel Merck-60 (230–400 mesh, 60 A), and TLC on aluminum sheets pre-coated with silica gel 60 F254 (E. Merck). FT-IR spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrophotometer. Elemental analyses were obtained from Carlo Erba 1106 Instrument. Normal resolution mass spectra were recorded on a LCQ-ion trap (Thermo Finnigan, San Jose, CA), equipped with an ES (Electrospray) ionization source. MALDI-TOF MS mass spectrometry was carried out on Bruker microflex. Positive ion and linear mode MALDI-TOF-MS spectrum of phthalocyanine derivatives were obtained in 2,5-dihydroxybenzoic acid (DHB) or α -cyano-4-hydroxycinnamic acid (CHCA) MALDI matrix using nitrogen laser accumulating 50 laser shots.

¹H and ¹³C NMR spectra were recorded on a Bruker and Varian 500MHz spectrometers using TMS ($\delta = 0$) as an internal reference.

Synthesis of 3-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-ethoxy)phthalonitrile (1) was achieved according to related literatures [1,2].

4,5-bis(4,7,10-trioxaundecan-1-sulfanyl) phthalonitrile (**4**) was synthesized and purified according to the published methods [3,4].

2. Photophysical and photochemical measurements

2.1. Absorption and Fluorescence Measurements

Electronic absorption spectra were recorded with a Shimadzu 2001 UV spectrophotometer, and steady-state fluorescence excitation and emission spectra were recorded by using a Varian Cary Eclipse spectrofluorometer using 10 mm path length cuvettes at room temperature. If **5-GaCl** and **6-GaCl** readily dissolved in DMSO, we observed that it was necessary to prepare the **ClGaPc** solution one day before measurement to have complete dissolution in the solvent used (spectroscopic grade DMSO).

Fluorescence rate constants (k_F) were determined with the ϕ_F and τ_F values (k_F= ϕ_F/τ_F). Fluorescence lifetimes (τ_F) were measured by using Horiba FL3-2IHR spectrofluorometer with a Time Correlated Single Photon Counting (TCSPC), equipped with a NanoLED 670-LH and a standard air cooled R928 PMT detector. In the course of measurements, the Instrument Response Function (IRF) was obtained from a non-fluorescence suspension of a colloidal silica (LUDOX 30%, Sigma Aldrich) in water, held in 10 mm path length quartz cell and was considered to be wavelength independent.

2.2. Fluorescence quantum yield determination

Fluorescence quantum yield values (ϕ_F) were determined by using William's method [5]. Accordingly, the UV-Vis absorbance and fluorescence emission spectra at different concentrations of the reference standard (**ZnPc**) and samples were measured under identical conditions. Integrated fluorescence intensities versus absorbance for **ZnPc** (ϕ_F = 0.18 in DMSO [6]) and **5-GaCl**, **6-GaCl** and **ClGaPc** were plotted. Slopes of the plots were proportional to the quantity of the quantum yield. Equation 1 was used to calculate quantum yield (ϕ_F) values. In the equation, Grad is the gradient of the plot and *n* is the refractive index of the solvent used for samples and standards.

$$\Phi_{F} = \Phi_{F}^{Sid} \begin{pmatrix} Grad \\ Grad \\ Grad \\ Sid \end{pmatrix} \begin{pmatrix} \underline{n}^{2} \\ n^{2} \\ sid \end{pmatrix}$$
(1)

2.3. Determination of Partition Coefficients

Determination of partition coefficients of **5-GaCl** and **6-GaCl** were studied as described by Fukushima et al. [7]. Partition coefficient values of **5-GaCl** and **6-GaCl** was carried out between octanol and water. Both solvents were equilibrated with each other. After shaking at the room temperature, the phases were separated by using centrifugation during 2 minutes. The concentration of **5-GaCl** and **6-GaCl** in both phases were determined by their absorbance values.

2.4. Singlet oxygen quantum yield determination

Singlet oxygen quantum yield (Φ_{Δ}) determinations were carried out a 1 mL portion of the solutions containing the singlet oxygen quencher irradiated in the Q band region with the photo-irradiation set-up described in the reference [8]. For singlet oxygen measurements, DPBF was used as chemical quencher ($\Phi_{\Delta} = 0.67$ in DMSO [9]). DPBF degradation at 417 nm was monitored by UV-Vis spectroscopy. The solutions of photosensitizers containing DPBF ($\sim 1 \times 10^{-5}$ M) were prepared in the dark and irradiated in the Q band region. The light intensity of 7.05×10^{15} photons s⁻¹ cm⁻² was used for Φ_{Δ} determinations.

Singlet oxygen quantum yields (Φ_{Δ}) of **5-GaCl** and **6-GaCl** were calculated using the equation 2. In the equation, *R* and *R*_{Std} were DPBF photobleaching rates in the presence of the respective samples and standard, respectively, while I_{abs} and I_{abs} were the rates of light absorption by the samples and standard, respectively.

$$\Phi_{\Delta} = \Phi_{\Delta}^{Std} \frac{R \cdot I_{Abs}^{Std}}{R \cdot I_{Abs}}$$
(2)

Photo-irradiations were studied using a General Electric Quartz line lamp (300W). A 600 nm glass cut off filter (Schott) and a water filter were used to filter off ultraviolet and infrared radiations, respectively. An interference filter (Intor, 700 nm with a band width of 40 nm) was additionally placed in the light path before the sample. Light intensities were measured with a POWER MAX5100 (Molelectron detector incorporated) power meter.

2.5. Photodegradation quantum yields

Photodegradation quantum yield (Φ_d) determinations were carried out with the experimental set-up as described in the literature [10]. Photodegradation quantum yields were calculated using the equation 3.

$$\Phi_{d} = \frac{(C_0 - C_t) \cdot V \cdot N_A}{I_{abs.}S. t}$$
(3)

where C_0 and C_t are the concentration of the samples before and after irradiation, respectively. V is the reaction volume. N_A is the Avogadro's constant. S is the irradiated cell area. t is their radiation time. I_{abs} is the overlap integral of the radiation source light intensity and the absorption of **5-GaCl** and **6-GaCl**. A light intensity of 2.35×10^{16} photons s⁻¹.cm⁻² was employed for Φ_d determinations.

2.6. Determination of acidity constant (pKa)

Titrations of the molecules were performed in acetonitrile by adding small aliquots of perchloric acid (HClO₄) or tetrabutyl ammonium hydroxide (TBAOH). pH values were recorded with a digital pH meter (WTW). All of the measurements were performed at room temperature (25 °C) and under atmospheric pressure. Before the starting usage, the pH readings had to be calibrated against buffer solutions with potential readings of pH of 7.0 and pH 4.0 in order to obtain reliable and reproducible titration curves. Absorbance of the solution as a function of pH was recorded and absorbance changes *vs* pH were plotted.

3. Spectroscopic Studies

Table S1. Q band absorption maxima of **5-GaCl** and **6-GaCl** in chloroform, ethanol, DMSOand THF.

	Q band max, nm			
Solvent	Chloroform	Ethanol	DMSO	THF
5-GaCl	724	719	721	716
6-GaCl	729	720	726	722



Fig. S1 FT-IR spectrum of 3-(2-{2-[2-(2-methylsulfanyl-ethoxy)ethoxy]ethoxy}ethoxy)phthalonitrile (**2**)



Fig. S2 ¹H NMR spectrum of $3-(2-\{2-[2-(2-methylsulfanyl-ethoxy)ethoxy]ethoxy\}-ethoxy)phthalonitrile (2) in CDCl₃$



ethoxy)ethoxy]ethoxy}-ethoxy)phthalonitrile (2) in CDCl₃



Fig. S4 ESI-MS spectrum of 3-(2-{2-[2-(2-methylsulfanyl-ethoxy)ethoxy]ethoxy}ethoxy)phthalonitrile (**2**)



Fig. S5 FT-IR spectrum of 3-(2-{2-[2-(2-azidoethoxy-ethoxy]ethoxy}-ethoxy)phthalonitrile (3)



ethoxy)phthalonitrile (3) in CDCl₃



Fig. S8 MALDI-MS spectrum of 3-(2-{2-[2-(2-azidoethoxy-ethoxy]ethoxy}-ethoxy)phthalonitrile (**3**) (no matrix).

Fig. S9 FT-IR spectrum of 5-Zn.

Fig. S10 ¹H NMR spectrum of **5-Zn** in DMSO- d_6

Fig. S11 MALDI-MS spectrum of 5-Zn (Matrix: DHB)

Fig. S12 FT-IR spectrum of 6-Zn

Fig. S13 ¹H NMR spectrum of **6-Zn** in DMF- d_7

Fig. S14 MALDI-MS spectrum of 6-Zn (Matrix: DHB)

Fig. S15 FT-IR spectrum of 5-2H

Fig. S16 ¹H NMR spectrum of **5-2H** in DMSO- d_6

Fig. S17 MALDI-MS spectrum of 5-2H (Matrix: DHB)

Fig. S19 ¹H NMR spectrum of **6-2H** in DMSO- d_6

Fig. S20 MALDI-MS spectrum of 6-2H (Matrix: CHCA).

Fig. S21 Absorption spectra of **5-Zn** and **5-2H** in dichloromethane $(6 \times 10^{-6} \text{ M})$.

Fig. S22 Absorption spectra of **6-Zn** and **6-2H** in dichloromethane (6×10^{-6} M).

Fig. S23 FT-IR spectrum of 5-GaCl.

Fig. S24 ¹H NMR spectrum of 5-GaCl in DMSO-d₆

Fig. S25 MALDI-MS spectrum of 5-GaCl (Matrix: DHB)

Fig. S26 FT-IR spectrum of 6-GaCl.

Fig. S27 ¹H NMR spectrum of **6-GaCl** in DMSO- d_6

Fig. S28 MALDI-MS spectrum of 6-GaCl. (Matrix: CHCA)

Fig. S29 Electronic absorption, excitation and emission spectra of compound 5-Zn in DMSO.

Fig. S30 Electronic absorption, excitation and emission spectra of compound 6-Zn in DMSO.

Fig. S31 Electronic absorption, excitation and emission spectra of compound **5-GaCl** in DMSO.

Fig. S32 Electronic absorption, excitation and emission spectra of compound **6-GaCl** in DMSO.

Fig. S33 3D fluorescence emission spectra of 5-GaCl in DMSO.

Fig. S34 3D fluorescence emission spectra of 6-GaCl in DMSO.

Fig. S35 Fluorescence decay profiles of **5-GaCl** and **6-GaCl** (absorbance at excitation wavelength: ~0.5).

Fig. S36 Determination of singlet oxygen quantum yield of **5-Zn** in DMSO. Inset: Plot of DPBF absorbance at 417 nm *vs* time.

Fig. S37 Determination of singlet oxygen quantum yield of **6-Zn** in DMSO. Inset: Plot of DPBF absorbance at 417 nm *vs* time.

Fig. S38 Determination of singlet oxygen quantum yield of **ZnPc** in DMSO. Inset: Plot of DPBF absorbance at 417 nm *vs* time.

Fig. S39 Photodegradation profiles of 5-Zn in DMSO (10 μ M). Inset: Plot of absorbance at 704 nm vs time.

Fig. S40 Photodegradation profiles of 6-Zn in DMSO (10 μ M). Inset: Plot of absorbance at 705 nm vs time.

Fig. S41 Photodegradation profiles of 5-GaCl in DMSO (10 μ M). Inset: Plot of absorbance at 721 nm vs time.

Fig. S42 Photodegradation profiles of 6-GaCl in DMSO (10 μ M). Inset: Plot of absorbance at 726 nm vs time.

Fig. S43 Absorption spectra of **5-GaCl** in acetonitrile (5×10^{-6} M, 25 ml) at varying amount of TBAOH (0.1 M).

Fig. S44 Absorption spectra of **6-GaCl** in acetonitrile (5×10^{-6} M, 25 ml) at varying amount of TBAOH (0.1 M).

References

[1] A. Galstyan, K. Riehemann, M. Schaefers, A. Faust, J. Mater. Chem. B, 2016, 4, 5683.

[2] S. Tuncel, F. Dumoulin, J. Gailer, M. Sooriyaarachchi, D. Atilla, M. Durmuş, D. Bouchu,

H. Savoie, R. W. Boyle and V. Ahsen, Dalton Trans., 2011, 40, 4067.

[3] S. Dabak, F. Heinemann, V. Ahsen, P. Zugenmaier, *Mol. Cryst. Liq. Cryst.*, 2000, 348, 111.

[4] D. Atilla, N. Saydan, M. Durmuş, A. G. Gürek, T. Khan, A. Rück, H. Walt, T. Nyokong and V. Ahsen *J. Photochem. Photobiol. A: Chem.* 2007, **186**, 298.

[5] A.T.R. Williams, S.A.Winfield and J.N. Miller, Analyst, 1983, 108, 1067.

[6] P. Jacques and A. M. Braun, Helv. Chim. Acta, 1981, 64, 1800.

[7] K. Fukushima, K. Tabata and I. Okura, J. Porphyrins Phthalocyanines 1998, 2, 219.

[8] A. Ogunsipe and T. Nyokong, J. Photochem. Photobiol. A: Chem. 2005, 173, 211.

[9] N. Kuznetsova, N. Gretsova, E. Kalmkova, E. Makarova, S. Dashkevich, V.

Negrimovskii, O. Kaliya and E. Luk'yanets, Russ. J. Gen. Chem. 2000, 70, 133.

[10] I. Seotsanyana-Mokhosi, N. Kuznetsova and T. Nyokong, *J. Photochem. Photobiol. A: Chem.* 2001, **140**, 215.