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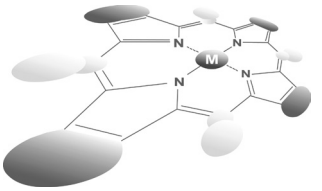

# Dendrimeric-like hexadecahydroxylated zinc phthalocyanine. Synthesis and evaluation of photodynamic efficiency

**Serkan Alpugan[a], Guillaume Garcia[b,c,d,e], Florent Poyer[b,d,e,f], Mahmut Durmuş[a], Philippe Maillard[b,c,d,e◊], Vefa Ahsen[a◊] and Fabienne Dumoulin*[a◊]**

[a] *Gebze Institute of Technology, Department of Chemistry, P.O. Box 141, Gebze, 41400 Kocaeli, Turkey*
[b] *Institut Curie, Section de Recherche, Bât 110-112, Centre Universitaire, F-91405 Orsay, France*
[c] *UMR 176 CNRS, Bât 110, Centre Universitaire, F-91405 Orsay, France*
[d] *Université Paris-Sud, Centre Universitaire, F-91405 Orsay, France*
[e] *CNRS GDR 3049 PHOTOMED, UMR 5623 Université Paul Sabatier, F-31062 Toulouse Cedex 9, France*
[f] *U759 INSERM, Bât 112, Centre Universitaire, F-91405 Orsay, France*

*Dedicated to Professor Özer Bekaroğlu on the occasion of his 80th birthday*

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**ABSTRACT:** The design of a dendrimeric-like diglycerol-tetrasubstituted Zn(II) phthalocyanine resulted in a remarkably water-soluble compound due to the presence of 16 hydroxyls. Several parameters relevant to evaluate the photodynamic efficiency of a potentiel photosensitizer such as: aggregation behavior, fluorescence properties, singlet oxygen generation, binding to a carrier protein model (Bovine Serum Albumin) and partition coefficient have been measured. Biocompatibility was demonstrated by dark cytotoxicity in *in vitro* experiments. The absence of phototoxicity can be explained by an elevated hydrophilicity. All the collected data have confirmed that this new substitution pattern is promising to be used on phthalocyanines aiming at being photodynamic therapy agents.

**KEYWORDS:** glycerol, phthalocyanine, photosensitizer, photodynamic therapy.



## INTRODUCTION

Photodynamic therapy is a cancer treatment alternative or complementary to surgery, radiotherapy and chemotherapy. It is based on the interaction of three factors: light at appropriate wavelength, molecular oxygen and photosensitizer. The photosensitizer excited by light returns to its ground state by transferring the energy of its excited triplet to molecular oxygen, subsequently converted into its toxic singlet species. The resulting oxidative effect damages cell component, leading to cell death. In the last four decades, much effort led to significantly optimized photosensitizers. In order to gather the ideal properties of photosensitizers [1] on single molecule, second-generation photosensitizers were elaborated to overcome the first drawbacks of the clinically used Photofrin® and Visudyne®, which are badly characterized and exists under mixture forms. Most of current research efforts are directed towards the elaboration of third-generation photosensitizing systems, such as theranostics combining imaging and photodynamic agents. The development of optimized second-generation photosensitizers is likely to be useful for further conception of these advanced systems.

Due to their photophysical and photochemical properties, phthalocyanines are among the most attractive tetrapyrrolic photosensitizing derivatives. Their maximum absorption is largely red-shifted compared to porphyrins, and phthalocyanines are much more stable than bacteriochlorins. Liposomal formulation of Zn phthalocyanine (CGP55847) [2], Pc4 [3] and Photosens [4] (a mixture of sulfonated aluminum phthalocyanines developed in Russia) are the best examples of phthalocyanines clinically used or on advanced trial stages.

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*Correspondence to: Fabienne Dumoulin, email: fdumoulin@gyte.edu.tr, fax: +90 262-605-3101, tel: +90 262-605-3106

Nevertheless, except these latest examples, phthalocyanines remain the poor relation of tetrapyrrolic photosensitizers, probably mainly due to their unjustified reputation of poor water solubility. There are actually a broad range of pathways to confer them water-solubility [5]. Besides, phthalocyanines exhibit most of the properties desired on photosensitizers for PDT: a near-IR absorption in the therapeutic window allowing deeper tissue penetration, which is sought for most of the treatments (except those of oesophagus and ORL cases), no dark phototoxicity, suitable body clearance, excellent stability especially compared to bacteriochlorins absorbing in the same range of wavelength, reproducible syntheses in satisfying yields. Among water-solubilizing substitution patterns, non-ionic ones were developed after ionic ones but are expected to allow better cell uptake and different biodistributions. Non-ionic substitution patterns are based on polyoxo and polyhydroxylated moieties. Among them, symmetrical glycerol substitution gave promising results, especially in non-peripheral position [6]. Since then, many of our efforts tended to the optimization of the photodynamic efficiency of glycerol-substituted phthalocyanines, either by the introduction of carbohydrates, grafting onto nanoparticles, or tailored variations of the amphiphilicity of the phthalocyanines. In the present work, we focused on the development of a new glycerol-based substituent: diglycerol moieties leading to the dendrimeric-like water-soluble hexadecahydroxylated Zn phthalocyanine **1** (Fig. 1), the aim to increase the biocompatibility of this potential new photosensitizer by conferring suitable hydrophilicity and amphiphilicity.

The photodynamic potential of **1** was investigated here, through the determination of its photophysical and photochemical parameters, as well as its *in vitro* dark toxicity and phototoxicity.

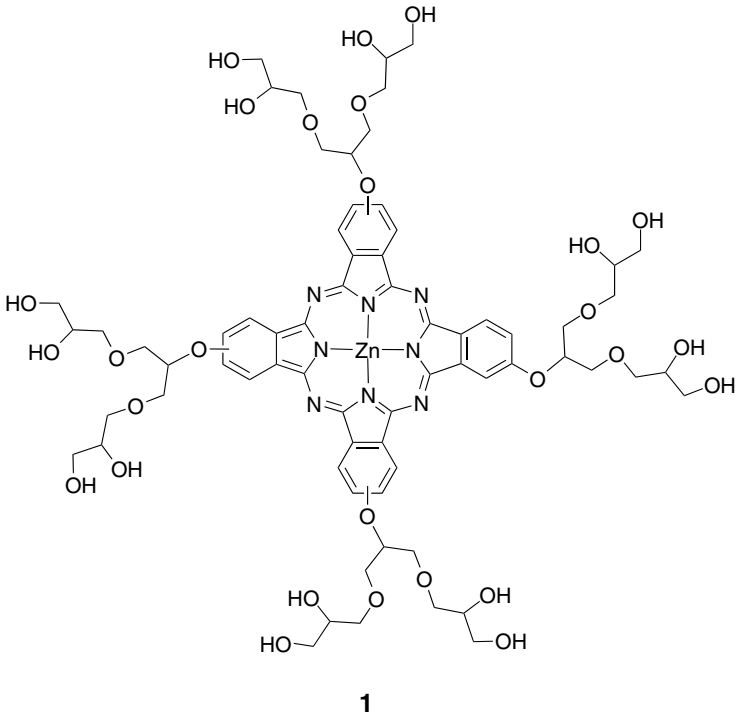
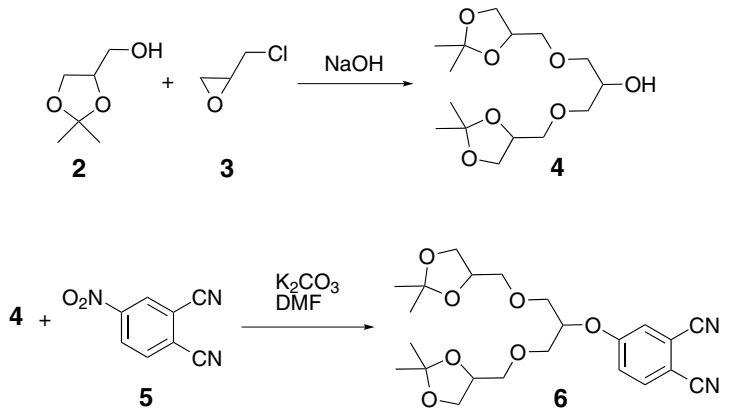



**Fig. 1.** Structure of the dendrimeric-like diglycerol-substituted phthalocyanine **1**




**Scheme 1.** Preparation of phthalonitrile **6**

## RESULTS AND DISCUSSION

### Syntheses and characterizations

Phthalocyanine **1** was obtained in four steps. Starting from solketal **2** and epichlorhydrin **3**, disolketal **4** was obtained and grafted on 4-nitrophthalonitrile **5** to yield phthalonitrile **6**. Subsequent cyclotetramerization of **6** and final acidic hydrolysis yielded **1** (Scheme 1).

The opening of epichlorhydrin by two molecules of alcohols is a well-known reaction providing access to symmetrically substituted secondary alcohols [7], and already applied to solketal [8]. We slightly modified the conditions, using solketal itself as the reaction bulk solvent and sodium hydroxide as the base. Disolketal **4** was obtained in high yield (79%) and in large scale: up to 120 g could be obtained in a single reaction. Nucleophilic displacement of the nitro group on **5** was achieved in relatively moderate yield (44%), can be considered normal as this is a secondary alcoholate, quite sterically hindered.

The cyclotetramerization of **6** was achieved equally in dimethylaminoethanol or pentanol/DBU systems in the presence of zinc acetate to obtain the corresponding protected phthalocyanine **7** in high yield (46%). The acidic hydrolysis of the isopropylidene protecting groups

**Table 1.** Q-band electronic absorption data for **1**

| Solvent | $\lambda_{max}$ Q-band, nm | Log ε |
|---|---|---|
| DMSO | 684 | 5.18 |
| DMF | 682 | 4.67 |
| water | 637, 675 | 4.47, 4.22 |
| PBS | 638, 679.5 | 4.64, 4.39 |
| MeOH | 678 | 4.60 |

was performed using trifluoroacetic acid, and the final unprotected phthalocyanine was obtained pure in 80% yield after reprecipitation from ethanol in dichloromethane and ethylacetate, followed by thorough washing with acetone.

## Photophysics and photochemistry

Parameters which allow to estimate the potential of a molecule as a photosensitizer for photodynamic therapy have been determined: preliminary ground state absorption measurements and aggregation state in water permitted to select the best solvent for further investigation, such as the fluorescence and singlet oxygen generation behavior. In addition, study of the photodegradation of **1**, as well as its binding to a carrier protein model were performed, to provide a full picture of the potential use of **1** as a photodynamic therapy agent on a photophysical and photochemical point of view.

***Ground state electronic absorption and aggregation behavior in water.*** The shape of the electronic absorption spectrum of phthalocyanine gives precious indication of its aggregation state. This information is crucial for further measurements that must be conducted in non-aggregated states to avoid misleading interpretations. The electronic absorption spectra of **1** were recorded in polar solvents: DMSO, DMF, methanol, water and phosphate buffer solution. Extinction coefficient values and Q-band maximum absorption wavelengths in these solvents are summarized in Table 1. Corresponding spectra at 10 µM concentrations are presented in Fig. 2 (top). These results prompted us to select DMSO as the solvent in which to perform further photophysical and photochemical measurements, as it appeared to be a solvent in which **1** is not aggregated at all (see Fig. 2 (bottom) for the linearity of the absorption towards the concentration), thus avoiding artefact due to aggregation likely to interfere with the interpretation of the measurements.

In water, phthalocyanine is quite aggregated, despite observations with naked eye showing that **1** readily dissolves in water. To confirm that the shape of the Q-band in water is due to aggregation, Triton X100, a detergent inhibiting the aggregation of molecules in aqueous media, was added. The Q-band significantly became sharp, indicating the monomerization of the phthalocyanine (Fig. 3). The water/octanol partition coefficient was determined to be 0.04 (log *P* -1.4), reflecting its elevated

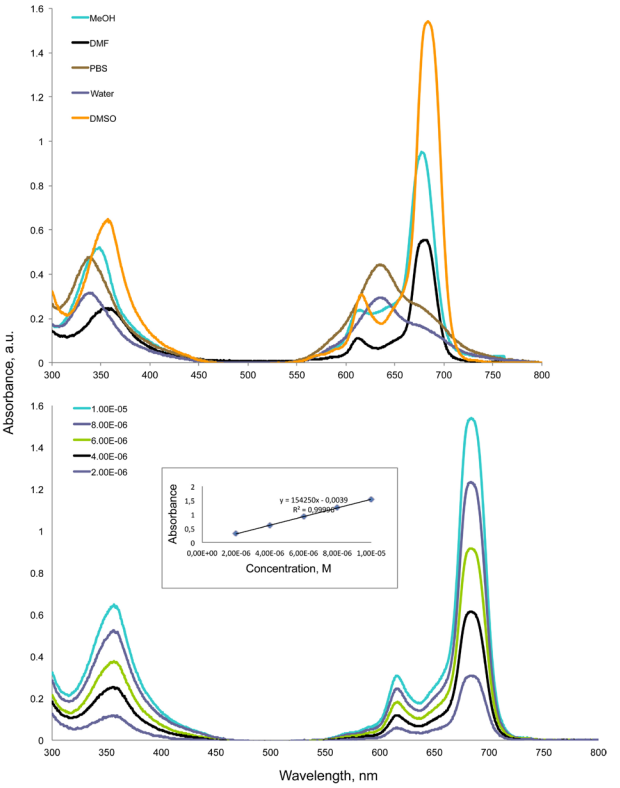



**Fig. 2.** (Top) electronic absorption spectra of **1** in DMSO, DMF, methanol, water and phosphate buffer, at concentration: 10 µM, and (bottom) linearity of the absorption in DMSO at different concentrations

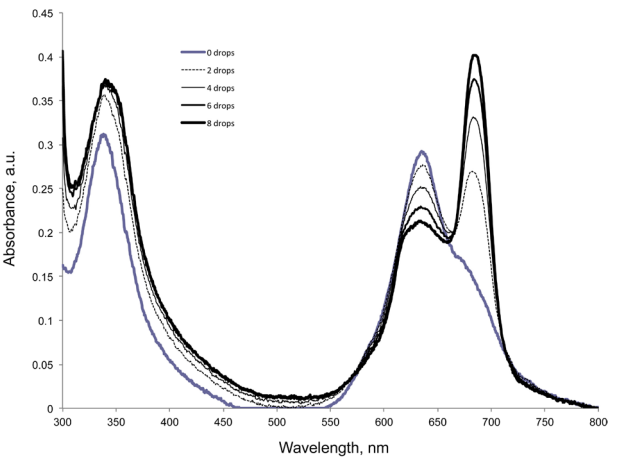



**Fig. 3.** Electronic absorption spectra of **1** in water and upon addition of increasing quantities of Triton X100

hydrophilicity compared to Lipinski rule [9]. The high solubility in aqueous media, despite the aggregation, is promising for further biological experiments. The behavior in PBS, more similar to biological media than pure water and in which phthalocyanine **1** is even less aggregated than in water, is a good indication that further biological works are relevant.

***Fluorescence spectroscopy, quantum yields and lifetimes.*** A photosensitizer exhibiting some fluorescence properties allows to follow its *in vitro* repartition, and Zn(II) phthalocyanines are even now used as fluorescence probes [10]. As the emission of fluorescence is concurrent with the singlet oxygen generation, an ideal photosensitizer for PDT must have a rather low fluorescence, as most of Zn(II) phthalocyanines. The two important parameters to measure are the fluorescence quantum yield and lifetime. These values for **1**, as well as those of the reference compound used for this study (unsubstituted ZnPc) are summarized in Table 2. The emission of **1** proved to be of a satisfactory level ($\Phi_F$ 0.17) for further biological investigations, such as quantification of cell uptake and subcellular localization.

***Singlet oxygen generation and photodegradation.*** A satisfying generation of singlet oxygen is a key property of a good photosensitizer, and reflected by the singlet oxygen generation quantum yield $\Phi_\Delta$. The disappearance of a singlet oxygen quencher upon irradiation allows to calculate this yield, using irradiation in conditions close to those of biological experiments. On the other hand, the generation of singlet oxygen is likely to damage the photosensitizer itself. This phenomenon called photodegradation must be measured as well to estimate the photostability of the photosensitizer. An aered solution of the photosensitizer is irradiated with stronger light and without singlet oxygen quencher, and subsequent modification of the UV spectrum reflects the photodegradation of the photosentizer. Relevant data for the singlet oxygen generation and photodegradation are summarized in Table 3. 1,3-Diphenylisobenzofuran (DPBF) is used as the organosoluble singlet oxygen quencher, and its quenching upon singlet oxygen generation by **1**, reflected by the diminution of its proper maximum absorption at 417 nm is illustrated in Fig. 4. The diminution of the absorption is time-linear. The corresponding singlet oxygen generation yield is 77%, higher than the reference unsubstituted phthalocyanine. This high value perfectly fits the expectations of a good photosensitizer.

**Table 2.** Fluorescence data for **1** in DMSO

| Compound | Excitation | Emission | Stokes shift | $\Phi_F$ | $\tau_F$, ns |
|---|---|---|---|---|---|
| 1 | 687 | 694 | 10 | 0.17 | 1.41 |
| ZnPc [11] | 672 | 682 | 10 | 0.20 | 1.22 |

**Table 3.** Singlet oxygen generation and photodegradation data for **1** in DMSO

| Compound | $\Phi_\Delta$ | $\Phi_d(10^{-5})$ |
|---|---|---|
| 1 | 0.77 | 5.30 |
| ZnPc [11a, 12] | 0.67 | 2.61 |

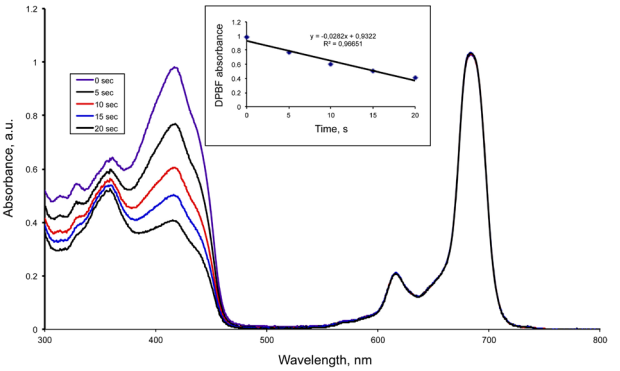



**Fig. 4.** Decrease of DPBF absorption upon singlet oxygen generation by **1**. Concentration 10 μM. Inset: plots of DPBF absorbance *vs.* time

**Table 4.** Binding and fluorescence quenching data for interaction of BSA with **1** in PBS

| Compound | $K_{SV}^{BSA}/10^5$, $M^{-1}$ | $k_q/10^{13}$, $M^{-1}.s^{-1}$ | $K_b/10^{-6}$, $M^{-1}$ | n |
|---|---|---|---|---|
| 1 | 1.12 | 1.12 | 2.76 | 1.07 |

The photodegradation of **1** is reflected by the absorption changes upon irradiation (Table 3). The photodegradation is time-linear and has a quantum yield $\Phi_d$ of $5.3 \times 10^{-5}$. **1** can be considered as moderately stable, which is a good point for PDT photosensitizer as it helps to avoid any accumulation in organisms.

***Interactions with a carrier protein model.*** Bovine Serum Albumin (BSA) is a carrier protein model frequently used to estimate the potential efficacy of new medicines, as their blood circulation is directly related to their ability to bind and unbind to carrier proteins.

Fluorescence of the BSA at 348 nm due to its tryptophane moieties is quenched when the protein is bound to quencher, such as for example phthalocyanine. Following the fluorescence of BSA upon the addition of phthalocyanine allows the quantification of the binding, as it decreases linearly upon the addition of phthalocyanine, until the saturation of the BSA (Fig. 5) by **1**. Calculations of relevant data are summarized in Table 4.

The slope of the plots (Fig. 5, top inset) gave Stern–Volmer quenching constants ($K_{SV}$) value of **1**. Using the reported fluorescence lifetime of BSA (10 ns) [13], the bimolecular quenching constant ($k_q$) was determined (see relevant equation in Supporting information). This value is of the order of $10^{13}$ $M^{-1}.s^{-1}$, which exceed the proposed value of $10^{10}$ $M^{-1}.s^{-1}$ for diffusion-controlled (dynamic) quenching (according to the Einstein–Smoluchowski approximation) at room temperature [14]. This confirms that the mechanism of BSA quenching by **1** is not diffusion-controlled and that the quenching is static, not dynamic. The slope of the plot of Fig. 5 (bottom), gave n values and their intercepts of these plots gave $K_b$ values. The values of $K_b$ and n are typical of MPc-BSA

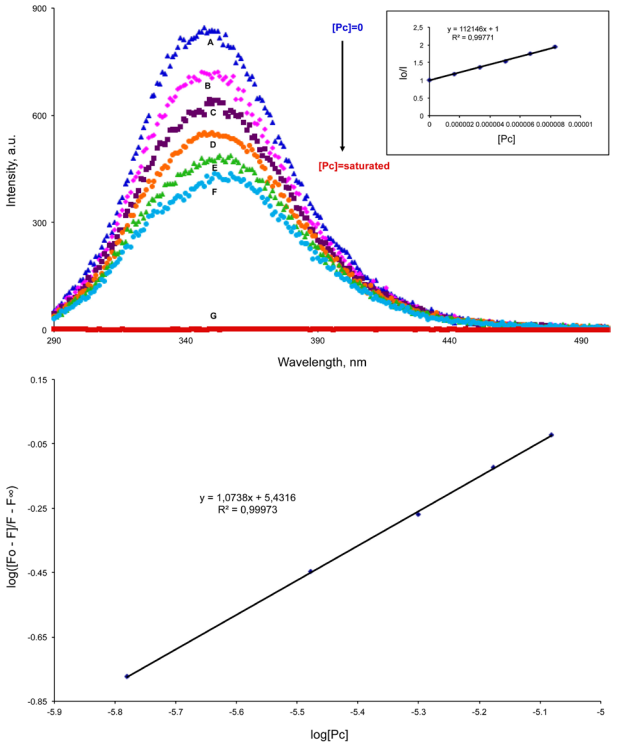



**Fig. 5.** Top: fluorescence emission spectral changes of BSA (C = 30 μM) on addition of varying concentrations of **1** in PBS. [**1**]: A = 0, B = 1.66 μM, C = 3.33 μM, D = 5 μM, E = 6.66 μM, F = 8.3 μM, G = saturated with **1**. Bottom: determination of **1**-BSA binding constant (and number of binding sites on BSA). [BSA] = $3.00 \times 10^{-5}$ M and [**1**] = 0, $1.66 \times 10^{-6}$, $3.33 \times 10^{-6}$, $5.00 \times 10^{-6}$, $6.66 \times 10^{-6}$, $8.33 \times 10^{-6}$ M in PBS

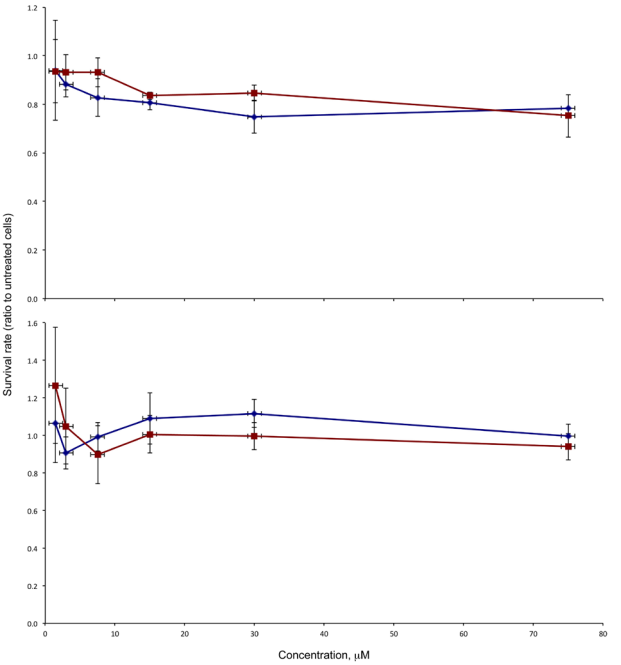



**Fig. 6.** *In vitro* dark toxicity (blue) and phototoxicity (red) of **1** against Y79 (top) and HT-29 (bottom) cell lines: survival rate (ratio to untreated cells

interactions in aqueous solutions [15]. n values of near unity suggest that complex **1** forms 1:1 adducts with BSA. The decrease in the intrinsic fluorescence intensity of tryptophan with **1** concentration indicates that this complex readily bind to BSA, which implies that the phthalocyanine reaches the subdomains where tryptophan residues are located in BSA.

### *In vitro* dark toxicity and phototoxicity

A mandatory property of a good photosensitizer is its dark toxicity, as it must exhibit toxicity only upon irradiation. It was evaluated against two cell lines, Y79 and HT-29, after 24 h of incubation (Fig. 6). The survival rate, compared to the untreated cells, remains complete even at elevated concentrations, up to 80 μM, which is a good indication of the suitability of phthalocyanine **1** as a photodynamic therapy agent. Further tests are being carried out to determine its cell uptake before evaluating its phototoxicity.

Upon irradiation, no cell death could be observed, reflecting an absence of phototoxicity (Fig. 6). This is not correlated with the singlet oxygen generation ability. Several hypotheses may be emitted: **1** may be too hydrophilic to croos the amphiphilic cell membrane and be internalized. Another possibility is the release of the photosensitizer during the incubation time. A third assumption could be that even if **1** is internalized, it remains aggregated inside the cell, the aggregation preventing the electronic events required for the singlet oxygen generation leading to the cell death, or that it does not go to the appropriate organelles. Different incubation times and internalization and/or subcellular localization attemps are being performed using confocal microscopy.

## EXPERIMENTAL

### Material and methods

4-Nitrophthalonitrile (**5**) was prepared following the literature [16]. All reaction solvents were dried and purified as described by Perrin and Armarego [17]. Optical spectra in the UV-visible region were recorded with a Shimadzu 2101 UV spectrophotometer using a 1 cm path length cuvette at room temperature. The mass spectra were recorded on a LCQ-ion trap (Thermo Finnigan, San Jose, CA, USA), equipped with an ES (Electrospray) source and MALDI (matrix assisted laser desorption ionization) BRUKER Microflex LT using 2,5-dihydroxybenzoic acid as matrix. $^{1}$H and $^{13}$C NMR spectra were recorded in deuterated solvents solutions on a Varian 500 MHz spectrometer.

## Syntheses

**1,3-bis((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)-propan-2-ol (4).** Sodium hydroxide (38 g, 0.95 mol) was added portion wise to solketal **2** (235 mL, 250 g, 1.9 mol). The slurry was stirred at 80 °C for 2 h and allowed to cool down. Epichlorhydrine (44 g, 38 mL) was then added drop by drop at room temperature. The resulting reaction mixture was stirred at 110 °C overnight. After cooling to room temperature, the reaction mixture was diluted with water and extracted three times by dichloromethane. Combined organic phase were dried on sodium sulphate, filtered, concentrated, and the excess of solketal was distilled under vacuum. The expected product was purified by silica gel column chromatography, eluent hexane/ethyl acetate (1/2, v/v), yielding 120 g (79%) of a colorless transparent oil. $C_{15}H_{28}O_7$, MW 320.38. $^1$H NMR ($CDCl_3$): δ, ppm 4.26–4.33 (m, 2H), 4.06–4.09 (m, 2H), 3.99–4.02 (m, 1H, CHOH), 3.73–3.77 (m, 2H), 3.53–3.64 (m, 8H, 2 $CH_2OCH_2$), 1.45 (bs, 6H, 2 $CH_3$), 1.39 (bs, 6H, 2 $CH_3$). $^{13}$C NMR: δ, ppm 109.09, 74.38, 74.36, 72.55, 72.52, 72.51, 72.15, 69.03, 66.24, 66.22, 26.42, 25.07. MS (ESI): isotopic cluster peaking at *m/z* 343.17 found 343.25 $[M + Na]^+$. Anal. calcd. for $C_{15}H_{28}O_7$: C, 56.23; H, 8.81%. Found C, 56.28; H, 8.74%.

**4-{1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl)-methoxy]propan-2-yloxy}phthalonitrile (6).** Disolketal **4** (4.2 g, 13 mmol), 4-nitrophthalonitrile **5** (16 mmol, 2.72 g, 1.2 eq) and potassium carbonate (100 mmol, 15 g) were stirred overnight at 80 °C in dry DMF. After cooling to room temperature, the reaction mixture was filtered, diluted with water and extracted by ethyl acetate. The organic phase was then washed with water, dried on sodium sulphate, filtered and concentrated. The expected product was purified by silica gel column chromatography, eluent hexane/ethyl acetate (1/2, v/v), yielding 2.56 g (44%) of a pale yellow wax. $C_{23}H_{30}N_2O_7$, MW 446.49. $^1$H NMR ($CDCl_3$): δ, ppm 7.67 (d, 1H, aromatic CH), 7.40 (dd, 1H, aromatic CH), 7.30 (dd, 1H, aromatic CH), 4.49 (m, 1H, CH–O–Ar), 4.21, 4.00 (2m, 4H, 2 $CH_2$-Osolketal), 3.47–3.78 (2 m, 10H, 2 $CH_2$–CH–$CH_2$), 1.39 (bs, 6H, 2 $CH_3$), 1.34 (bs, 6H, 2 $CH_3$). $^{13}$C NMR ($CDCl_3$): δ, ppm 162.08, 135.32, 121.11, 120.69, 117.29, 115.60, 109.57, 107.55, 78.21, 74.57, 74.23, 72.66, 72.61, 71.06, 71.02, 70.84, 70.81, 66.32, 26.71, 25.25. MS (ESI): isotopic cluster peaking at *m/z* 469.19 found 469.32 $[M + Na]^+$. Anal. calcd. for $C_{23}H_{30}N_2O_7$: C, 61.87; H, 6.77; N, 6.27%. Found C, 61.31; H, 6.72; N, 6.81%.

**2(3),9(10),16(17),23(24)-tetrakis-{1,3-bis[(2,2-dimethyl-1,3-dioxolan-4-yl)methoxy]propan-2-yloxy} phthalocyaninato Zn(II) (7).** Phthalonitrile **6** (250 mg, 0.56 mmol) and zinc acetate (0.28 mmol) were stirred in refluxing *N,N′*-dimethylaminoethanol (5 mL) overnight. The reaction mixture was cooled down and poured into water. The blue solid was filtered and purified on a silica gel column chromatography, eluent hexane/ethyl acetate (1/10, v/v), yielding 120 mg (46%) of a dark blue wax. $C_{92}H_{120}N_8O_{28}Zn$, MW 1851.38. $^1$H NMR (DMSO-$d_6$): δ, ppm 9.23 (m, 4H, aromatics), 8.95 (m, 4H, aromatics), 7.83 (m, 4H, aromatics), 5.29 (m, 4H, CH-OPc), 4.31 (m, 8H, CH), 4.05, 3.69 (2 m, 48H, 24 $CH_2$), 1.34, 1.24 (2 m, 48H, 16 $CH_3$). MS (MALDI-TOF): *m/z* 1849.75 found 1850.87 $[M + H]^+$. Anal. calcd. for $C_{92}H_{120}N_8O_{28}Zn$, $2H_2O$: C 58.54, H 6.62, N 5.94%. Found C, 58.72; H, 6.50; N, 5.98%.

**2(3),9(10),16(17),23(24)-tetrakis-[1,3-bis(2,3-dihydroxypropoxy)propan-2-yloxy]phthalocyaninato Zn(II) (1).** Phthalocyanine **7** (100 mg, 54 mmol) was stirred at room temperature in aqueous 90% trifluoro-acetic acid (5 mL) for 5 h, then the reaction mixture was concentrated under vacuum. The resulting solid was thoroughly washed with dichloromethane, ethyl acetate, acetone, and finally dissolved in methanol and reprecipitated in ethyl acetate, yielding 66 mg (80%) of a dark blue solid. $C_{68}H_{88}N_8O_{28}Zn$, MW 1530.87. $^1$H NMR (DMSO-$d_6$): δ, ppm 9.31 (m, 4H, aromatics), 9.25 (m, 4H, aromatics), 7.83 (m, 4H, aromatics), 5.27, 5.21 (m, 4H, CH–OPc), 4.69 (m, 8H, CH), 3.93 (m, 48H, 24 $CH_2$). MS (MALDI-TOF): *m/z* 1528.50 found 1529.873 $[M + H]^+$, 1552.51 $[M + H + Na]^+$. Anal. calcd. for $C_{68}H_{88}N_8O_{28}Zn$: C 53.35, H 5.79, N 7.32%. Found C, 53.72; H, 5.63; N, 7.11%.

## Water/octanol partition coefficient calculation

An excess of **1** was stirred in a solution of octanol and water in order to obtain saturated phases. The solution was filtered. An aliquot of each phase was 20-fold diluted by DMSO. The electronic absorption of each solution (in which the phthalocyanine is monomeric) was measured. The coefficient partition value was obtained by dividing the DMSO-diluted octanol phase absorbance by the DMSO-diluted water phase absorbance.

## Photophysics and photochemistry

Details regarding the photophysical and photochemical experiments are provided in the supplementary material (see Supporting information).

## Cell culture conditions and *in vitro* photocytotoxicity

The suspension cell line Y79 (human retinoblastoma cells) and adherent cell line HT-29 (human colorectal adenocarcinoma cells) were obtained from the American Type Culture Collection (Rockville, MD, USA, HTB-18 and HTB-38, respectively). Y79 and HT-29 cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Eurobio, les Ulis, France) Glutamax™ supplemented with 20% and 10% fetal calf serum (FCS, Eurobio) respectively and antibiotics in humidified atmosphere under 5% $CO_2$ in air at 37 °C. For Y79 cells and HT-29 cells, $5.10^5$ and $5.10^4$ cells/well in 1 mL respectively, were seeded into 24-microwell plates with the appropriate culture medium. After 2–3 h of incubation at 37 °C for Y79 and 24 h for

HT-29, tested compound, in DMSO solution, were added in the dark at a final concentration ranging from 1.5 to 75 μM in duplicate (1.5, 3, 7.5, 15, 30, 75 μM). Two plates of each cell line were realized, one for dark cytotoxicity and the other for photocytotoxicity. Illuminated control cells received 7.5 μL of DMSO free of dye. After 24 h of incubation with PS at 37 °C in the dark, the cells were washed with phosphate buffered saline (PBS, Eurobio) and fresh medium free of drug was added. Illumination was performed for 27 min and 20 s (5 J/cm$^2$) through the bottom of the 24-microwell plates using a "light box" made of six Phillips TL 13W tubes covered by a diffusing glass fitted with an orange filter (emission wavelength $\lambda > 540$ nm), leading to a final irradiance of 3 mW/cm$^2$. Plates were left to incubate in the dark for three days before evaluation of the cell viability by determination of mitochondrial activity using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT, Sigma, Saint-Quentin Fallavier, France) assay. At the time of counting, 50 μL of a MTT (5 mg/mL) solution was added to each well. After 30 min of incubation and removal of the medium, formazan crystals were taken up with 600 μL of DMSO and absorbance at 562 nm was measured with a microplate reader (Bio-Rad, Marnes-la-Coquette, France). Survival rate was expressed as ratio of the absorbance of treated and illuminated cells to untreated and illuminated controls.

## CONCLUSION

Phthalocyanine **1** reported here possesses numerous features desired for photodynamic therapy agents. The molecular design lead to an elevated water-solubility. Relevant fluorescence investigations showed sufficient emission to later on investigate the cell uptake and subcellular localization. Singlet oxygen generation was particularly efficient. Binding to BSA, a carrier model protein, evidenced the aptitude of **1** to circulate in biological media. Its photophysical and photochemical behavior fit the expectations of a photodynamic therapy agent. Besides, the absence of *in vitro* dark toxicity determined on two cell lines confirmed the potential of phthalocyanine **1** as a photosensitizer. Further works are being conducted to interpret the absence of phototoxicity in the conditions used. Anyway, the present works confirm the potential of **1** as a photodynamic therapy agent. More amphiphilic derivatives are being prepared.

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### Supporting information

Experimental details on the photophysical and photochemical measurements are provided in the supplementary information. Supplementary material is available free of charge *via* the Internet at http://www.worldscinet.com/jpp/jpp.shtml.

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