Journal of Inorganic Biochemistry xxx (2014) xxx-xxx



Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry



journal homepage: www.elsevier.com/locate/jinorgbio

Synthesis, photophysical studies and ¹O₂ generation of carboxylate-terminated zinc phthalocyanine dendrimers

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ARTICLE INFO

Article history: Received 2 October 2013 Received in revised form 16 February 2014 Accepted 18 February 2014 Available online xxxx

Keywords: Phthalocyanines Dendrimers Singlet oxygen generation

ABSTRACT

Highly water-soluble dendrimers have been prepared consisting of a central zinc phthalocyanine moiety and dendritic wedges with terminal carboxylate groups. The biggest polyelectrolyte comprises 32 negative charges at the dendrimer surface. The photophysical studies reveal a strong correlation between the degree of dendritic environment, the extent of aggregation, and the ability to generate singlet oxygen in aqueous media. Compared to dendrimers having an axial derivatization the functionalization on the outer rim also significantly improves the phthalocyanine's ability to photosensitize singlet oxygen.

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1. Introduction

The structural motif of phthalocyanines has attracted significant research efforts throughout the last couple of decades [1–6]. Whereas this class of molecules has often been used as dyes and pigments in the early days, this interest has shifted in recent decades towards their utilization as building blocks for the construction of new molecular materials for electronics and optoelectronics [1–6], and dye-sensitized solar cells [7–14].

This notwithstanding, phthalocyanines have also demonstrated their potential in the production of singlet molecular oxygen (${}^{1}O_{2}$) [15–17]. This reactive oxygen species is well known to play a crucial role in applications such as photocatalysis [18–22] or photodynamic therapy (PDT) [23–26]. Both applications rely on the interaction between light in either the UV or visible region of the spectrum and a photosensitizer (PS), but only the latter takes advantage of this effect to selectively kill cancer cells [27,28]. The growing popularity of this therapeutic method can be attributed to the high selectivity of destructing diseased tissues and tumors through the localized generation of cytotoxic ${}^{1}O_{2}$ while surrounding healthy cells remain mostly

http://dx.doi.org/10.1016/j.jinorgbio.2014.02.007 0162-0134/© 2014 Elsevier Inc. All rights reserved. unaffected. Several metallophthalocyanines disclosed their capability to act as photosensitizing agents like e.g., zinc [29-36], silicon [37-41], or ruthenium as metal in the central cavity [42-45]. Some of these specimens are dendritic structures in which the phthalocyanine macrocycle is embedded inside the fractal scaffold [46,47]. One of the latest approaches has been described by Kataoka et al. [29,30]. It confers a promising example with a 100-fold photochemical enhancement of transgene expression in vitro while at the same time showing reduced photocytotoxicity. This ternary system consists of a core containing DNA that is packaged with cationic peptides and surrounded at the surface by a given number of anionic phthalocyanine dendrimers. This sophisticated system was then also applied in animal experiments and showed transgene expression only in the laser-irradiated region, thus presenting the first example of photochemical-internalizationmediated gene delivery in vivo. On the other hand, dendritic porphyrins are not likely to be internalized by cancer cells, yet they can inflict lethal photodynamic damage and kill them from the outside [48].

Recently, we described a series of ruthenium phthalocyanine dendrimers with oligoethylene end groups which provided a straightforward means to switch their ability to produce ${}^{1}O_{2}$ on and off between non-polar and polar environments [42] (Chart 1). In continuation of our research on phthalocyanine-derived PSs for photodynamic applications, we report herewith on the photophysical characterization of a series of zero to second generation zinc phthalocyanine dendrimers appended at the surface through carboxylate moieties, whose potential to generate ${}^{1}O_{2}$ was evaluated for pre-screening purposes. In contrast to the work

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Chart 1. Structures of amphiphilic zinc phthalocyanine dendrimers ZnPc1-3 with up to 32 terminal carboxylate groups.

of Kataoka et al. [29,30,49], the primary photophysical properties and the ability to generate ${}^{1}O_{2}$ for the complete series of dendrimers demonstrate a positive effect of the dendrimer shell.

2. Materials and methods

2.1. Chemicals and materials

All reagents were used as purchased from commercial sources without further purification. Solvents were dried using standard techniques prior to use. Dendritic alcohols 2-4 have been prepared according to a procedure as previously described in the literature [50]. All reactions were performed in standard glassware under an inert argon atmosphere. Reactions were monitored by thin-layer chromatography (TLC) using TLC plates precoated with silica gel 60F₂₅₄ (Merck). Column chromatography was carried out on a Merck silica gel 60, 40-63 µm (230-400 mesh). Gel permeation chromatography (GPC) was performed using Biorad, Biobeads SX-1 (200-400 mesh). ¹H and ¹³C NMR spectra were recorded using Bruker AC 300 (300 MHz) instruments (H_{pc}: phthalocyanine protons, H_{ar}: aromatic protons); the solvent signal was used for internal calibration. UV-visible (UV-vis) spectra were recorded with a JASCO V-660 spectrophotometer. MALDI-TOF high resolution mass spectra (HRMS) were recorded with a Bruker Reflex III spectrometer.

2.2. Chemistry

2.2.1. Synthesis of generation zero phthalonitrile (5)

To a solution of **1** (305 mg, 1.76 mmol) and dendritic benzyl alcohol **2** (400 mg, 1.59 mmol) in dry DMF (16 mL) was added anhydrous K_2CO_3 (975 mg, 7.05 mmol) in three portions. After addition was complete, the reaction mixture was heated at 60 °C for 24 h under argon atmosphere. After cooling to room temperature, the crude mixture was poured into water and the aqueous phase extracted several times with CH₂Cl₂. The combined organic phase was dried with Na₂SO₄, filtered and the solvent evaporated to dryness. The resulting viscous oil was purified by gradient column chromatography (CH₂Cl₂ to CH₂Cl₂/EtOAc

2:1) to yield **5** (474 mg, 79%) as a white solid. ¹H NMR (300 MHz, CDCl₃): $\delta = 8.68$ (s, 1H, H_{ar}), 8.27 (s, 2H, H_{ar}), 7.74 (d, J = 9 Hz, 1H, H_{ar}), 7.36 (d, J = 3 Hz, 1H, H_{ar}), 7.27 (dd, J = 9 Hz, J = 3 Hz, 1H, H_{ar}), 5.23 (s, 2H, CH₂), 4.43 (q, J = 7 Hz, 4H, CH₂), 1.43 (t, J = 7 Hz, 6H, CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 165.2$, 161.2, 135.4, 135.1, 132.4, 131.6, 130.7, 119.8, 119.5, 117.5, 115.3, 114.8, 108.1, 69.8, 61.6, 14.1 ppm. MALDI-TOF HRMS (matrix DCTB + PEGNa300 + PEGNa400 + NaI): calc. for: C₂₁H₁₈N₂NaO₅ [M + Na⁺]: m/z = 401.1108, found 401.1118.

2.2.2. Synthesis of first generation phthalonitrile (6)

To a solution of 1 (54 mg, 312 μ mol) and dendritic benzyl alcohol 3(170 mg, 279 µmol) in dry DMF (7 mL) was added anhydrous K₂CO₃ (188 mg, 1.36 mmol) in three portions. After addition was complete, the reaction mixture was heated at 60 °C for 24 h under argon atmosphere. After cooling to room temperature, the crude mixture was poured into water and the aqueous phase extracted several times with CH₂Cl₂. The combined organic phase was dried with Na₂SO₄, filtered and the solvent evaporated to dryness. The resulting viscous oil was purified by column chromatography (CH₂Cl₂/EtOAc 5:1) to yield 6 (140 mg, 68%) as a colorless viscous oil. ¹H NMR (300 MHz, CDCl₃): $\delta = 8.63$ (s, 2H, H_{ar}), 8.28 (s, 4H, H_{ar}), 7.69 (d, J = 9 Hz, 1H, H_{ar}), 7.29 (d, J = 3 Hz, 1H, H_{ar}), 7.21 (dd, J = 9 Hz, J = 3 Hz, 1H, H_{ar}), 6.63 (m, 3H, H_{ar}), 5.13 (s, 4H, CH₂), 5.11 (s, 2H, CH₂), 4.42 (q, J = 7 Hz, 8H, CH₂), 1.42 (t, *J* = 7 Hz, 12H, CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃): $\delta = 165.5, 161.5, 160.0, 137.4, 137.2, 131.4, 130.2, 119.6, 117.4, 115.5,$ 115.1, 107.7, 106.4, 102.0, 70.6, 69.1, 61.5, 14.3 ppm. MALDI-TOF HRMS (matrix ditranol + PEG600 + NaI): calc. for: C₄₁H₃₈N₂NaO₁₁ $[M + Na^+]$: m/z = 757.2368, found 757.2404.

2.2.3. Synthesis of second generation phthalonitrile (7)

To a solution of **1** (14.2 mg, 82.0 μ mol) and dendritic benzyl alcohol **3** (120 mg, 90.8 μ mol) in dry DMF (7 mL) was added anhydrous K₂CO₃ (63 mg, 456 μ mol) in three portions. After addition was complete, the reaction mixture was heated at 60 °C for 24 h under argon atmosphere. After cooling to room temperature, the crude mixture was poured into water and the aqueous phase extracted several times with CH₂Cl₂. The

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Scheme 1. Preparation of dendritic phthalonitriles 5–7 with terminal ethyl ester functions. Reagents and conditions: (i) dendritic alcohol 2–4, K₂CO₃, DMF, reflux, 1d (5: 79%, 6: 68%, 7: 86%).

combined organic phase was dried with Na₂SO₄, filtered and the solvent evaporated to dryness. The resulting viscous oil was purified by gradient column chromatography (CH₂Cl₂ to CH₂Cl₂/EtOAc 8:1) to yield **7** (102 mg, 86%) as a pale yellow viscous oil. ¹H NMR (300 MHz, CDCl₃): $\delta = 8.63$ (s, 4H, H_{ar}), 8.29 (s, 8H, H_{ar}), 7.68 (d, J = 9 Hz, 1H, H_{ar}), 7.30 (d, J = 3 Hz, 1H, H_{ar}), 7.22 (dd, J = 9 Hz, J = 3 Hz, 1H, H_{ar}), 6.69 (m, 3H, H_{ar}), 6.59 (m, 6H, H_{ar}), 5.13 (s, 8H, CH₂), 5.11 (s, 4H, CH₂), 5.00 (s, 2H, CH₂), 4.42 (q, 16H, CH₂), 1.39 (t, 24H, CH₃) ppm. ¹³C NMR (75 MHz, CDCl₃): δ 165.5, 165.2, 160.2, 159.8, 139.3, 137.6, 137.4, 135.4, 132.5, 131.4, 130.2, 119.9, 119.6, 117.6, 117.5, 106.6, 106.4, 106.3, 101.6, 101.6, 70.6, 69.1, 69.9, 61.4, 14.1. MALDI-TOF HRMS (matrix ditranol + Nal): calc. for: C₈₁H₇₈N₂NaO₂₃ [M + Na⁺]: m/z = 1469.4888, found 1469.4846.

2.2.4. General procedure for the preparation of ester-appended zinc phthalocyanines (8–10)

A mixture of the corresponding dendritic phthalonitrile (4 eq) and $Zn(OAc)_2$ (1.2 eq) in *n*-pentanol was heated at 90 °C. At this temperature a few drops of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) were added and the mixture heated at reflux overnight under argon atmosphere. After cooling to room temperature, the solvent was removed under reduced pressure to give a greenish oil, which was purified by column chromatography and size exclusion chromatography.

2.2.5. Synthesis of generation zero ester-appended zinc phthalocyanine (8)

Ester-appended zinc phthalocyanine: Prepared from dendritic phthalonitrile **5** (250 mg, 661 µmol), Zn(OAc)₂ (48 mg, 262 µmol) and a few drops of DBU in *n*-pentanol (8 mL). Purification by column chromatography (SiO₂; EtOAc/Hexane 10:1) followed by GPC (eluent: THF) yielded dendritic zinc phthalocyanine **8** (167 mg, 53%) as dark green solid. NMR data indicate that transesterification of ethyl to pentyl end groups occurred during the synthesis. ¹H NMR (300 MHz, THF-d₈): $\delta = 8.76-8.98$ (m, 4H, H_{pc}), 8.62 (m, 12H, H_{ar}), 8.30–8.56 (m, 4H, H_{pc}), 7.50–7.73 (m, 4H, H_{pc}), 5.66 (m, 8H, CH₂), 4.44 (q, *J* = 7 Hz, 16H, CH₂), 1.88 (m, 16H, CH₂), 1.50 (m, 32H, CH₂), 0.98 (m, 24H, CH₃) ppm. UV-vis (THF): λ_{max} (log ε) = 675 (5.3), 610 (4.6), 350 (4.97), 284 (4.6). MALDI-TOF (matrix DHB): calcd. for C₁₀₈H₁₂₀N₈O₂₀Zn [M]⁺: m/z = 1914.8, found 1914.8.

2.2.6. Synthesis of first generation ester-appended zinc phthalocyanine (9)

Ester-appended zinc phthalocyanine: Prepared from dendritic phthalonitrile **6** (197 mg, 268 µmol), $Zn(OAc)_2$ (20 mg, 109 µmol) and a few drops of DBU in *n*-pentanol (4 mL). Purification by column chromatography (SiO₂; EtOAc/Hexane 10:1) followed by GPC (eluent: THF) yielded dendritic zinc phthalocyanine **9** (97 mg, 39%) as dark green viscous oil. NMR data indicate that transesterification of ethyl to pentyl end groups occurred during the synthesis. ¹H NMR (300 MHz, THF-d₈ + 1% (CD₃)₂SO): δ = 8.55 (s, 8H, H_{ar}), 8.41 (br m, 4H, H_{pc}),



Scheme 2. Preparation of zinc phthalocyanine dendrimers 8–10 with terminal ethyl ester functions and subsequent saponification under the formation of polyelectrolyte dendrimers ZnPc1–3. Reagents and conditions: (i) Zn(AcO)₂, DBU, *n*-pentanol, 90 °C to reflux, 16–20h (8: 53%, 9: 39%, 10: 31%); (ii) NaOH, H₂O/MeOH (v/v: 1:4), 40 °C, 4 h (ZnPc1: 81%, ZnPc2: 83%, ZnPc3: 83%).

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 $\begin{array}{l} 8.33 \; (s, 16H, H_{ar}), 7.71 \; (m, 4H, H_{pc}), 7.38 \; (m, 4H, H_{pc}), 6.82 \; (s, 8H, H_{ar}), \\ 6.76 \; (s, 4H, H_{ar}), 5.25 \; (s, 8H, CH_2), 5.23 \; (s, 4H, CH_2), 4.33 \; (t, 32H, J = 7 \; Hz, CH_2), 1.78 \; (m, 32H, CH_2), 1.42 \; (m, 64 \; H, CH_2), 0.93 \; (m, 48H, CH_3) \; ppm. UV-vis (THF): \\ \lambda_{max} \; (log \; \epsilon) = 677 \; (4.4), 611 \; (3.7), 350 \; (4.2). \\ \mbox{MALDI-TOF HRMS (matrix ditranol): calcd. for: $C_{212}H_{248}N_8O_{44}Zn \; [M^+]: $m/z = 3677.6764, found 3677.6900. \\ \end{array}$

2.2.7. Synthesis of second generation ester-appended zinc phthalocyanine (10)

Ester-appended zinc phthalocyanine: Prepared from dendritic phthalonitrile **7** (119 mg, 82.2 µmol), Zn(AcO)₂ (6 mg, 32.7 µmol) and a few drops of DBU in *n*-pentanol (2 mL). Purification by column chromatography (SiO₂; EtOAc/CH₂Cl₂ 10:1) followed by GPC (eluent: THF) yielded dendritic zinc phthalocyanine **10** (45 mg, 31%) as dark green viscous oil. NMR data indicate that transesterification of ethyl to pentyl end groups occurred during the synthesis. ¹H NMR (300 MHz, THF-d₈ + 1% (CD₃)₂SO): δ = 9.26 (m, 4H, H_{pc}), 9.03 (m, 4H, H_{pc}), 8.51 (br s, 16H, H_{ar}), 8.31 (br s, 32H, H_{ar}), 7.83 (m, 4H, H_{pc}), 7.09 (m, 8H, H_{ar}), 6.73–6.93 (br m, 28H, H_{ar}), 5.63 (br s, 8H, CH₂), 1.36 (br s, 128H, CH₂), 0.88 (s, 96H, CH₃) ppm. UV–vis (THF): λ_{max} (log ε) = 678 (4.94), 612 (4.12), 350 (4.7), 275 (4.92). MALDI-TOF (matrix DCTB): calcd. for: C₃₂₄H₃₁₂N₈O₉₂Zn [M⁺]: m/z = 7201.4, found 7201.4.

2.2.8. General procedure for the hydrolysis of ester-appended zinc phthalocyanine dendrimers

The corresponding ester-terminated zinc phthalocyanine **8–10** was dissolved in a small amount of THF and the solution added slowly to a saturated solution NaOH in a mixture of water/methanol (v/v: 1:4). The solution was then heated at 40 °C until total consumption of the starting material (ZnPc1–3: 4 h). The organic solvents were evaporated and the remaining aqueous solution was subjected to dialysis. Finally, the solid was treated with CH₂Cl₂, acetone, and ethyl acetate and dried to give the final product.

2.2.9. Synthesis of generation zero zinc phthalocyanine polyelectrolyte dendrimer (ZnPc1)

Prepared from dendritic zinc phthalocyanine **8** (60 mg, 38.0 µmol) in THF (3 mL) and saturated solution of NaOH in water/ methanol (v/v 1:4; 25 mL). Carboxylate-terminated zinc phthalocyanine dendrimer ZnPc1 was obtained after dialysis as a dark green powder (47 mg, 81%). ¹H NMR (300 MHz, D₂O + 1% (CD₃)₂SO): δ = 9.26 (br m, 4H, H_{pc}), 8.84 (m, 4H, H_{pc}), 8.61 (br, 12H, H_{ar}), 7.97 (m, 4H, H_{pc}), 5.45 (s, 8H, CH₂) ppm. UV-vis (H₂O): λ_{max} (log ε) = 684 (4.4), 639 (4.2), 344 (4.33). MALDI-TOF (matrix DHB): calc. for: C₆₈H₄₀N₈O₂₀Zn [M⁺]: m/z 1354.2, found 1354.1.

2.2.10. Synthesis of first generation zinc phthalocyanine polyelectrolyte dendrimer (ZnPc2)

Prepared from dendritic zinc phthalocyanine **9** (20 mg, 6.66 µmol) in THF (1 mL) and saturated solution of NaOH in water/methanol (v/v 1:4; 15 mL). Carboxylate-terminated zinc phthalocyanine dendrimer ZnPc2 was obtained after dialysis as a dark green powder (16 mg, 83%). ¹H NMR (300 MHz, D₂O + (CD₃)₂SO): δ = 8.17 (s, 8H, H_{ar}), 7.90 (s, 16H, H_{ar}), 6.68 (s, 16H, CH₂), 6.63 (s, 8H, CH₂) ppm. UV-vis (H₂O): λ_{max} (log ϵ) = 680 (3.9), 638 (3.97), 336 (4.1).

2.2.11. Synthesis of second generation zinc phthalocyanine polyelectrolyte dendrimer (ZnPc3)

Prepared from dendritic zinc phthalocyanine **10** (25 mg, 4.27 µmol) in THF (1.5 mL) and saturated solution of NaOH in water/methanol (v/v 1:4; 20 mL). Carboxylate-terminated zinc phthalocyanine dendrimer ZnPc3 was obtained after dialysis as a dark green powder (20 mg, 83%). ¹H NMR (300 MHz, D₂O + (CD₃)₂SO): δ = 8.15 (s, 16H, H_{ar}), 7.90 (s, 32H, H_{ar}), 6.62 (s, 24H, H_{ar}), 6.63 (br, 48H,

CH_2) ppm. UV-vis (H_2O): λ_{max} (log $\epsilon) = 686$ (4.0), 638 (4.0), 642 (4.4), 275 (4.6).

2.3. Photophysical measurements

Absorption spectra were recorded on a double beam Cary 6000i spectrophotometer (Varian, Palo Alto, CA). Time-resolved near-infrared phosphorescence measurements were carried out using a customized setup composed by PicoQuant Fluotime 200 system and its software for the data analysis. Direct ¹O₂ phosphorescence detection was achieved by means of a diode-pumped pulsed Nd:YAG laser (FTSS355-Q, Crystal Laser, Berlin, Germany) working at a 10 kHz repetition rate for sample excitation at 355 nm (5 mW, 0.5 µJ per pulse). A 1064 nm rugate notch filter (Edmund Optics, U.K.) was placed at the exit port of the laser to remove any residual component of its fundamental emission in the near-IR region. The luminescence exiting from the 90° side of the sample was filtered by two long-pass filters of 355 and 532 nm (Edmund Optics, York, U.K.) and two narrow bandpass filters at 1275 nm (NB-1270-010, Spectrogon, Sweden; bk-1270-70-B, bk Interferenzoptik, Germany) to remove any scattered laser radiation. A near-IR sensitive photomultiplier tube assembly (H9170-45, Hamamatsu Photonics, Hamamatsu City, Japan) was used as the detector. Photon counting was achieved with a multichannel scaler (PicoQuant's Nanoharp 250).

Transient absorption experiments in the UV–visible (UV–vis) region were carried out using a home-built nanosecond laser flash photolysis system. In this instrument, the 3rd harmonic (355 nm) of a Continuum Surelite I-10 Nd:YAG laser (10 Hz, 5 ns pulse width, 1–10 mJ per pulse) was directed onto the sample. Changes in the sample absorbance were detected by a Hamamatsu R928 photomultiplier in order to monitor the intensity variations of an analyzing beam produced by a 75 W short arc Xe lamp (USHIO) and spectral discrimination was provided by a PTI 101 monochromator. The signal was fed to a Lecroy Wavesurfer 454 oscilloscope for digitizing and averaging (3–10 shots, typically) and finally transferred through a GPIB interface (National Instruments) to a PC computer for data storage and analysis. The TTL sync output of the



Fig. 1. Normalized absorption spectra of anionic phthalocyanines ZnPc1 (red solid line), ZnPc2 (green dotted line) and ZnPc3 (blue dashed line) in DMSO (a) or D_2O (b). The bulk concentration is ca. 10 μ M for all compounds. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 1

Summary of the photophysical characterization of the anionic zinc dendrimer phthalocyanines in D₂O.

Compound	Q-band $\lambda_{max}\!/nm^a$	$\Phi_{\!\Delta}{}^{\mathbf{b}}$	$\tau_{\Delta}\!/\!\mu s^{c}$	$k_q^{O_2}/M^{-1} s^{-1d}$	$\tau_T/\mu s^e$	$F_T^{O_2 f}$
ZnPc1	675 (682)	0.02	55.1	$5.8 imes10^8$	38	0.82
ZnPc2	682 (685)	0.06	59.8	6.2×10^{8}	59	0.88
ZnPc3	679 (684)	0.15	62.8	$6.1 imes 0^8$	171	0.95

DMSO as solvent in parentheses.

b Singlet oxygen quantum yield in air-saturated solutions.

Singlet oxygen lifetime in air-saturated solutions.

d Rate constant for triplet quenching by oxygen.

Triplet lifetime in argon-saturated solutions.

f Fraction of phthalocyanine triplet excited states deactivated by oxygen in airsaturated solutions ($F_T^{O_2}$).

laser was used to trigger the oscilloscope. The energy of the laser pulse was varied by controlling the Q-switch delay and measured with a pyroelectric energy meter (RJP 735 and RJ 7610) from Laser Precision Corp. The system was controlled by the in house-developed LKS software (LabView, National Instruments).

The singlet oxygen quantum yields (Φ_{Δ}) were determined by comparing the intensity of the 1270 nm signals to those of optically-matched solutions of a reference PS. 5,10,15,20-Tetrakis(4-sulfonatophenvl) porphine (TPPS, Φ_{Δ} , $_{D20} = 0.64$) was used as reference [51]. Measurements were carried out in 1×1 cm quartz fluorescence cuvettes at room temperature. The concentration of the phthalocyanines was in the range 1–10 μ M. Time-resolved phosphorescence signals (S_t) were then fitted by Eq. (1) using the PicoQuant FluoFit 4.0 data analysis software, thereby extracting amplitude (A) and lifetime values (τ_{Δ} and τ_{T}). Since A is an instrumental quantity proportional to the quantum yield, Φ_{Λ} values were calculated from the amplitudes according to Eq. (2):

$$S_{t} = \mathbf{A} \times \frac{\tau_{\Delta}}{\tau_{\Delta} - \tau_{T}} \times \left(e^{-t/\tau_{\Delta}} - e^{-t/\tau_{T}} \right)$$
(1)

$$\Phi_{\Delta}(\text{sample}) = \Phi_{\Delta}(\text{ref}) \times \frac{A(\text{sample})}{A(\text{ref})}.$$
(2)

The rate constant for oxygen quenching of the triplet state of ZnPcs 1–3 $(k_q^{O_2})$ was determined by measuring the lifetime of the triplet– triplet transient-absorption signals as a function of oxygen concentration in solution. The samples were excited at 355 nm and the transients were observed at 635 nm. A plot of the reciprocal lifetime vs



Fig. 2. Time-resolved near-IR signals of ¹O₂ photosensitized by ZnPc1 (red), ZnPc2 (green), ZnPc3 (blue) and reference TPPS (gray) in D₂O upon excitation at 355 nm. The changes in signal intensity are greater in this order. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

oxygen concentration afforded $k_q^{O_2}$ as the slope of the linear fit. The concentration of oxygen in the solutions was changed by gentle bubbling with solvent-saturated argon or oxygen for at least 30 min. The fraction of triplets quenched by oxygen, $F_T^{O_2}$, was calculated from the $k_a^{O_2}$ and k_T^0 values following Eq. (3):

$$k_T^{O_2} = \frac{k_q^{O_2} \times [O_2]}{k_T^{O_1} + k_q^{O_2} \times [O_2]}.$$
 (3)

3. Results and discussion

3.1. Synthesis and characterization

Dendritic molecules have been an inspiration to chemists since its discovery in the late 1970s. The structural characteristics brought along with such macromolecular architectures remain appealing not least due to the possibility to tune and control the overall properties by pure chemical means. Likewise, the generation-dependant behavior offers the incentive to study the often peculiar properties of a given core moiety that is surrounded by an increasing branched shell. In this nexus, hydrophilic dendrimers can be easily obtained through the implementation of polar groups, which assist to overcome the intrinsically strong lipophilic character of phthalocyanine macrocycles [52]. This can be accomplished upon decoration of the dendritic scaffolds by multiple charges at the surface with for instance modified Fréchet-type polyarylether dendrons, or aliphatic Newkome-type dendrons. Similarly, introducing polar entities such as oligoethylene glycol chains confers a certain hydrophilicity [42,53-56]. Whereas the former approach relying on multiple charges allows to obtain highly water-soluble scaffolds, the latter comprising neutral polar end groups offers the possibility to examine the material properties in both common organic solvents as well as aqueous media [42,53-56].

The synthesis of the zinc phthalocyanine dendrimers presented herein started with the preparation of dendritic wedges 2-4, which have been obtained following the procedure of Fréchet et al. [50]. The different alcohols of generation zero to two have then been subjected to *ipso*-substitution reactions with 4-nitrophthalonitrile (1) in the presence of potassium carbonate as base to provide the branched ortho-dinitriles in good to high yields of 68 to 86%. With these dendritically modified phthalonitriles 5-7 in hand, the corresponding cyclotetramerizations have been conducted in the presence of zinc acetate as metal template (Scheme 1). The target dendrimers 8-10 have been obtained as intense green products in reasonably good yields of 31 to 53% after purification by gradient column chromatography and size exclusion chromatography. However, it has to be noted that the often observed transesterification occurred during the synthesis giving rise to the formation of the pentylester-terminated dendrimers rather than the ethyl-appended derivatives. The transesterification products have been obtained quantitatively as can be deduced from the ¹H NMR integrals. In addition, no signals have been observed in the MALDI-TOF mass spectra for partially transesterified products. As the last step of the synthetic route, the terminal ester groups were saponified under the use of sodium hydroxide in a mixture of water and methanol (v/v: 1:4) and furnished the final dendrimers ZnPc1-3 after dialysis in high yields (ZnPc1: 81%, ZnPc2: 83%, ZnPc3: 83%) (Scheme 2).

All phthalonitriles (5-7) and phthalocyanines with ester end groups (8-10) have been characterized by common techniques including NMR, MALDI-TOF mass spectrometry and UV-vis spectroscopy. Due to the multiple charges prevailing in ZnPc1-3, the characterization of these polyelectrolytes was more difficult. Using the NMR technique, the corresponding resonances in the proton spectra have been obtained as broad signals even after accumulation over an elongated period of time. MALDI-TOF mass spectrometry was attempted under the use of the respective uncharged acid species. However, these attempts proved only

Please cite this article as: F. Setaro, et al., Synthesis, photophysical studies and ¹O₂ generation of carboxylate-terminated zinc phthalocyanine dendrimers, J. Inorg. Biochem. (2014), http://dx.doi.org/10.1016/j.jinorgbio.2014.02.007

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Fig. 3. Transient absorption decays of ZnPc1 (red, top line in panels a and b), ZnPc2 (green, middle line in panels a and b) and ZnPc3 (blue, bottom line in panels a and b) excited at 355 nm and observed at 635 nm in argon-saturated solutions (a), air-saturated solutions (b) or oxygen-saturated solutions (c) in D₂O. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

successful for the generation zero derivative and no suitable signal has been observed neither for the first nor for the second generation dendrimer.

3.2. Spectroscopic and photophysical characterization

Fig. 1 shows the normalized absorption spectra of all three phthalocyanines either in deuterated water (D₂O) or in DMSO. In this latter media, compounds present a narrow shape and the maxima are found in the less energetic but more intense band of the Q-region. When changing to D₂O, a remarkable change in the relative intensities of the two bands of the Q-region appears, mainly due to different extents of inter- or intramolecular aggregation effects. Comparison between both solvents reveals that ZnPc3 is the only compound that maintains the ratio between the two less energetic bands of the Q-region >1 in aqueous media (least energetic band as dividend of the ratio). On the contrary, for ZnPc1 and ZnPc2 this ratio is below 1, indicating that these latter compounds are still largely aggregated. Moreover, for all the cases a small but clear hypsochromic shift occurs and bands broaden, another archetypal aggregation feature. Altogether, the degree of complexity of the dendrimer seems to be responsible for the extent of aggregation, the more branched the easier to disaggregate in aqueous media.

A detailed compilation of the spectroscopic and photophysical properties of the family of anionic zinc phthalocyanines ZnPc1–3 is summarized in Table 1. Apart from the aforementioned spectral changes, further differences are observed regarding their ability to sensitize the production of ${}^{1}O_{2}$. The photosensitizing properties of the phthalocyanines hinged on the degree of ramification, the more efficient the higher the degree of dendrimerization.

As it can be seen in Table 1 and Fig. 2, the Φ_{Δ} values increased 3-fold and over 7-fold for ZnPc2 and ZnPc3, respectively, as compared to parent zero generation ZnPc1 in D₂O. A remarkable Φ_{Δ} value of 0.15 is achieved for ZnPc3, one order of magnitude higher than that of other reported dendritic phthalocyanines [42]. On the other hand, the ester form of ZnPc3 shows a Φ_{Δ} value of 0.42 in tetrahydrofuran, where it is predominantly in monomeric form.

Regarding the τ_{Δ} values, the compounds exhibited a small but not negligible ${}^{1}O_{2}$ quenching ability that correlated, once again, with the level of complexity and degree of aggregation. Thus, ZnPc1 exhibited a lifetime value of ca. 55 µs in solution, shorter than the reported value of 65 µs in D₂O [57], which is progressively more attained by ZnPc2 and ZnPc3.

In contrast with apically-substituted dendritic phthalocyanines, the $k_q^{O_2}$ values remained essentially constant among all compounds (Table 1). It has been previously published that apical dendritic wedges are able to partially shield the photoactive core from molecular oxygen, resulting in an increase of the triplet lifetime upon increasing the dendrimer complexity [54,58,59]. Our series of phthalocyanines behave in a similar fashion, as observed in Fig. 3 and Table 1, but the effect must originate from the higher degree of disaggregation in the more branched derivatives. Such effect is expected to also lead to an increase in the Φ_{Δ} value — as a result of the lower non-radiative deactivation of its precursors, consistent with our observations. In addition, the fraction of triplets quenched by oxygen, $F_T^{O_2}$, is very similar in all compounds. This suggests that functionalization at the outer rim of the phthalocyanine macrocycle is a valuable strategy for improving the solubility of the phthalocyanines without shielding of the chromophore from oxygen.

4. Conclusion

A new series of monodisperse generation zero to two zinc phthalocyanine-centered dendrimers has been synthesized. Within the last step of the synthetic route the terminal ethyl esters have been saponified giving rise to a dramatic change in polarity under the formation of multiply charged polyelectrolyte species that are well-soluble in aqueous media. Along with increasing dendritic shell surrounding the photoactive zinc phthalocyanine centerpiece, a significant increase in the generation of singlet oxygen has been observed even in airsaturated solutions. Hence, such highly hydrophilic phthalocyanine dendrimers may thus be advantageous for the purpose of producing ${}^{1}O_{2}$ in biological media by photosensitization.

Abbreviations

- ¹O₂ singlet oxygen
- A amplitude
- DBU 1,8-diazabicyclo[5.4.0]undec-7-ene
- DCTB *trans*-2-[3-(4*-tert*-butylphenyl)-2-methyl-2-propenylidene] malononitrile
- DHB 2,5-dihydroxybenzoic acid
- DMF dimethylformamide
- $F_T^{O_2}$ fraction of triplet states quenched by oxygen
- GPC gel permeation chromatography
- HRMS high resolution mass spectrometry
- $k_a^{O_2}$ rate constant for oxygen quenching
- MALDI-TOF matrix-assisted laser desorption ionization
- Nd:YAG neodymium-doped yttrium aluminum garnet
- PDT photodynamic therapy
- PEG polyethylene glycol
- PS photosensitizer
- *S_t* time-resolved phosphorescence signals
- THF tetrahydrofuran
- TLC thin-layer chromatography
- TPPS 5,10,15,20-tetrakis(4-sulfonatophenyl)porphine
- TTL transistor-transistor logic

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- ZnPc zinc phthalocyanine
- Φ_{Δ} singlet oxygen quantum yields
- τ lifetime values

Acknowledgments

F.S. thanks the Universidad Autónoma de Madrid, Spain, for a PhD fellowship FPI-UAM. R.R.-G. thanks the Generalitat de Catalunya (DURSI) and Fons Social Europeu for a predoctoral fellowship. Financial support for this research was obtained from the Spanish Ministry of Economy and Competitivity (MEC) and the MICINN (CTQ2010-20870-C03-01, CTQ-2011-24187/BQU, Consolider-Ingenio, Nanociencia Molecular CSD2007-00010) and the Comunidad de Madrid (MADRISOLAR-2, S2009/PPQ/1533).

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