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Fluorine-substituted tetracationic ABAB-phthalocyanines for efficient photodynamic inactivation of Gram+ and Gram- bacteria

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ABSTRACT

Herein, we report the synthesis and characterization of new amphiphilic phthalocyanines (Pcs), the study of their singlet oxygen generation capabilities, and biological assays to determine their potential as photosensitizers for photodynamic inactivation of bacteria. In particular, Pcs with an ABAB geometry (where A and B refer to differently substituted isoindole constituents) have been synthesized. These molecules are endowed with bulky bis(trifluoromethylphenyl) groups in two facing isoindoles, which hinder aggregation and favour singlet oxygen generation, and pyridinium or alkylammonium moieties in the other two isoindoles. In particular, two water-soluble Pc derivatives (**PS-1** and **PS-2**) have proved to be efficient in the photoinactivation of *S. aureus* and *E. coli*, selected as models of Gram-positive and Gram-negative bacteria.

KEYWORDS: Phthalocyanine; Cationic; Amphiphile; Photodynamic inactivation; Bacteria

1. Introduction

The therapeutic properties of light have been extensively applied as a medical practice,^[1] yet photodynamic therapy (PDT) was principally developed in the last century. Among the therapies based on the management of light through the use of chemical substances called photosensitizers (PS), the photodynamic inactivation (PDI) of microbial cells is nowadays taking a leading position,^[2,3] owing to the emergence of microbial resistance to antibiotics.^[4] Within this therapy, the combination of a PS, light of an appropriate wavelength, and molecular oxygen enables the generation of reactive oxygen species, such as singlet oxygen (¹O₂), that trigger a phototoxic cascade leading to cell death. In this context, a large variety of cationic PS have been developed and successfully tested for bacterial photo-killing.^[5–8] The presence of outer positively charged terminal groups in the PS permits electrostatic interactions with the

negatively charged membrane of bacteria, and therefore, facilitates their uptake by them. Phthalocyanines (Pcs), and in particular ZnPcs, are very interesting PS as they present high singlet oxygen quantum yields (Φ_{Δ}).^[8–10] To sort out their inherent insolubility in aqueous media, these chromophores can be endowed with hydrophilic groups;^[11–17] in particular, functionalization with positively charged moieties imparts water-solubility to the Pcs and makes them potential PS in PDI.^[18–20] However, owing to their extended π -conjugation, Pcs exhibit a high aggregation tendency in aqueous media, forming oligomers in solution that can be either photoactive (i.e. J-type aggregates),^[21] or present cancelled ¹O₂ generation abilities. Aggregation in aqueous media can be reduced by the introduction of bulky substituents in axial or peripheral positions of the Pc. Recently, our research group has described a new strategy to prepare ZnPcs presenting both water-solubility and hindered aggregation. The approach consists in introducing

two types of peripheral substituents in the Pc in a crosswise, ABAB architecture (A and B coding for differently functionalized isoindole constituents), by using a phthalonitrile precursor (**B**) functionalized with bulky groups in the *ortho*-positions which is not able to self-condensate due to steric constrictions.^[22,23] Therefore, these molecules hold two facing isoindoles endowed with bulky bis(trifluoromethylphenyl) moieties that avoid the aggregation in solution, and other two that can be functionalized with substituents that render water-solubility to the molecule. Furthermore, we have recently demonstrated that the functionalization with trifluoromethylphenyl units at the B units of ABAB ZnPcs gives rise to high Φ_A .^[24, 25]

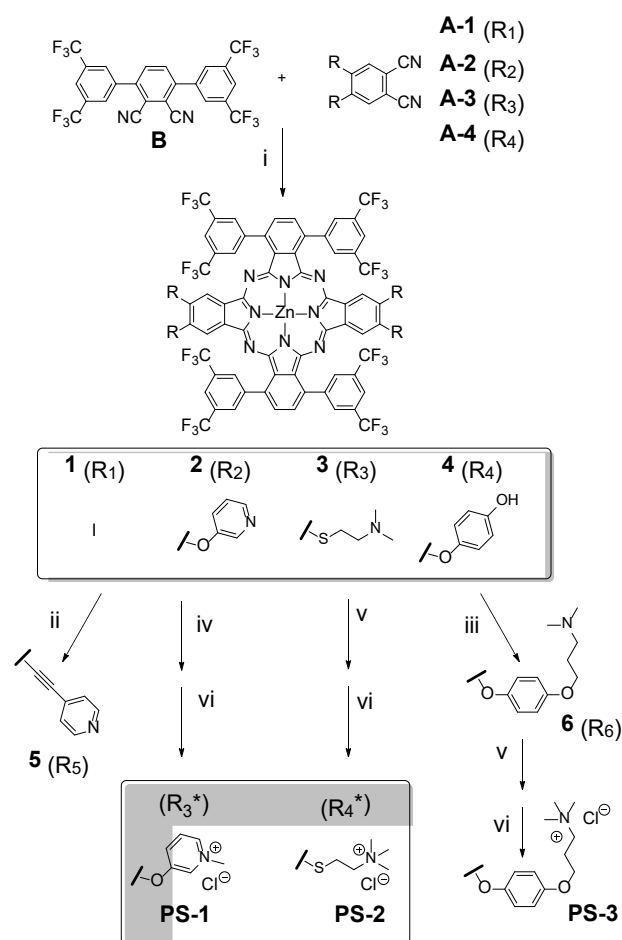
Taking advantage of this successful design, the objective of the present work is to synthesize and characterize new ZnPcs with potential use as PS for PDI, by incorporating hydrophilic cationic-terminated substituents in the A isoindole that provide these ABAB ZnPcs with solubility in aqueous media. *In vitro* assays with two selected ZnPcs (**PS-1** and **PS-2**) have been performed to examine their toxicity in different bacterial strains and their potential and efficiency as PS for PDI.

2. Results and Discussion

2.1. Synthesis

Two different functionalization approaches have been undertaken to perform the preparation of amphiphilic tetracationic ABAB ZnPcs, namely the functionalization with either pyridines or tertiary amines, which could be quaternized in a final step (Scheme 1). The aim is to compare the effect of the nature of the positively charged groups and/or the bridge between the positive charge and the aromatic core on their activity as PS. The synthesis of phthalonitrile **B** was carried out following the methodology described in our group.^[22] The preparation of ZnPcs functionalized with pyridine units was undertaken by two different synthetic approaches. First, we prepared the already described ZnPcs **1**^[22] and subjected it to a four-fold Sonogashira coupling with 4-ethynylpyridine, yielding **5**. Although the product was isolated and fully characterized, the low yield of this reaction (11%), preceded by the 10% yield of the mixed cyclotetramerization between **A-1** and **B**, which makes an overall yield of 1%, made us discard this route as an operative method to obtain pyridine

functionalized PS for *in vitro* assays. Therefore, we prepared **2**, resulting from the straightforward cross-condensation between bulky phthalonitrile **B** and **A-2** (Scheme 1). Although the cross-condensation yield was low (8%), **2** was easily purified, and the pyridyl moieties could be easily quaternized by reaction with MeI. In a last step, the molecules were exposed to Dowex® resin in order to exchange the initial counterions for chloride anions, rendering a compound that was partially soluble in water, from now coded as **PS-1** for the photophysical characterization and further *in vitro* experiments.



Scheme 1. Synthesis of pyridil or alkylamino -substituted ZnPcs and their respective pyridinium and ammonium salts. i) Zn(OAc)₂, *o*-DCB/DMF (2:1), 150 °C, overnight; ii) 4-ethynylpyridine, PdCl₂(PPh₃)₂, CuI, diisopropylamine, 70 °C, overnight; iii) 3-(dimethylamino)propyl methyl carbonate, CH₃CN, 180 °C, MW, 90 min; iv) MeI, DMF, 4h; v) MeI, EtOH, reflux, overnight; vi) Dowex® (1x8 200-400), MilliQ water, DMSO.

Moving to the preparation of ABAB ZnPcs functionalized with alkyl ammonium moieties, we intended to explore the

preparation of a series of compounds with different separation between the aromatic core and the positive charges. The first tailored ABAB ZnPc derivative was compound **3**, in which the tertiary amines were separated from the ZnPc core by a $(\text{CH}_2)_2\text{-S-}$ bridge. Since alkyl thioether chains can be easily introduced in the starting phthalonitriles by aromatic nucleophilic substitution over 4,5-dichlorophthalonitrile,^[26] phthalonitrile **A-3** was synthesized and reacted with phthalonitrile **B** under the typical conditions applied for the preparation of ABAB ZnPc derivatives. Worth mentioning, the reaction proceeded in 20% yield, proving this conversion as one of the most efficient cross-condensation reactions performed between bulky phthalonitrile **B** and another differently substituted phthalonitrile performed to date. **3** was then methylated with MeI in dry EtOH, in 36% yield. Noteworthy, after this transformation, the solubility of the resulting product changed drastically, becoming soluble only in polar solvents such as DMSO or DMF. In a last step, the product was exposed to Dowex® resin for ionic exchange, thus yielding a compound soluble in water. From now this product will be coded as **PS-2** for the photophysical characterization and *in vitro* tests. Our second approach towards an alkyl-ammonium functionalized ZnPc was to increase the distance between the ZnPc and the positive charge by preparing compound **4**, which was consecutively subjected to a four-fold O-alkylation to introduce *N*-dimethylamino-*N*-propyl residues. In particular, we performed the O-alkylation by heating at 180 °C a mixture of **4** and excess of 3-(dimethylamino)propyl methyl carbonate^[27] in acetonitrile. The formation of **6** was efficient, namely, 11% yield starting from the mixture of **A-4** and **B** phthalonitriles). **6** was then subjected to a methylation reaction, and further exposed to Dowex® resin to exchange the initial iodides for chloride anions. Unfortunately, this compound, coded as **PS-3** in Scheme 1, proved unstable in solution, undergoing Hoffman-type eliminations of the alkylammonium moieties that lead to molecules with terminal double bonds in the peripheral alkyl chains, as detected by mass spectrometry. This chemical instability made us discard **PS-3** for *in vitro* assays.

Fig. 1 shows the ¹H-NMR spectra of **PS-1** and **PS-2**. In both cases, the signals are well resolved due to their non-aggregated state in DMSO-*d*₆ solutions. The reduced number of signals derives from the high molecular symmetry these compounds possess (i.e. *D*_{2h}). In the case

of **PS-1**, the spectrum presents four singlets for the aromatic protons of the isoindole units and the CF₃-substituted phenyl rings, a group of signals corresponding to the *ortho* substituted pyridines (two doublets, a doublet of doublets and one singlet), and one singlet for the methyl groups. In the case of **PS-2**, the distinctive signals of this product (i.e. the two triplets for the methylenes) appear overlapped with the DMSO signal, but the singlet corresponding to the methyl groups of the tertiary amines could be clearly observed. Moreover, HR-MS characterization confirmed the structure of the compound.

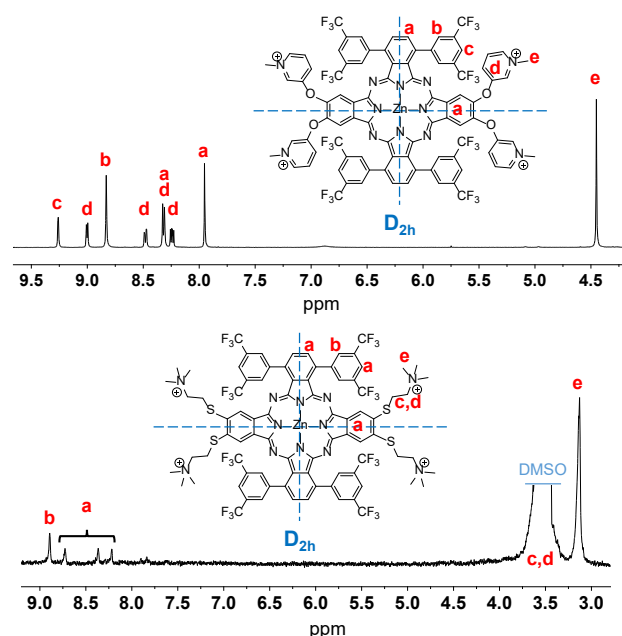


Fig 1. ¹H-NMR in DMSO-*d*₆ of **PS-1** (up); and **PS-2** (down).

2.2. Photophysical characterization

Table 1 presents a summary of the photophysical properties of **PS-1** and **PS-2** in methanol, including molar absorption coefficients (ϵ), wavelength, lifetime (τ_s) and quantum yield (Φ_F) of fluorescence emission, and singlet oxygen quantum yield (Φ_Δ), while the photophysical data for ZnPcs **1-6** are included in the Supporting Information (Fig. S2). The absorption spectra of **PS-1** and **PS-2** (see Fig. 2 for the UV-vis spectra in MeOH) show symmetric, non-split Q bands, which is not usual for MPcs with *D*_{2h}-symmetry. The thioether-derivatized Pc (**PS-2**) shows a Q-band more shifted to the red than the ether-derivatized one (**PS-1**), and with a higher absorption coefficient. Indeed, the absorption spectra of these compounds in MeOH do not show any evidence of aggregation, as otherwise expected for these compounds

due to the presence of rather bulky substituents at the non-peripheral positions of the Pc core. Absorption spectra were registered in a range of concentrations (between 8.7×10^{-7} M and 4.1×10^{-6} M for **PS-1**, and 1.6×10^{-6} M and 4.9×10^{-6} M for **PS-2**) (Fig. S3). For the verification of the Lambert-Beer law, an analysis of linear regression between the intensity of the Q-band and the concentration was performed, with R^2 values of ~ 0.999 . Fluorescence studies (Table 1 and Fig. S4) that were also performed in MeOH are in line with absorption assays; the monoexponential fluorescence decay kinetics confirm that **PS-1** and **PS-2** are in monomeric form in solution. Also, the quantification of the Φ_{Δ} was performed for these compounds, by direct observation of the $^1\text{O}_2$ phosphorescence at 1275 nm after excitation at 355 nm (Fig. S5). The values of Φ_F and τ_S are similar for the two compounds, however **PS-2** shows a lower Φ_{Δ} than **PS-1**. These results point to an enhanced internal conversion in **PS-2**, which is consistent with the higher flexibility of its structure. Comparing the photophysical parameters of **PS-1** with those of a neutral ZnPc described previously with a similar functionalization (i.e. 4-methoxyphenoxy moieties)^[25], τ_S of **PS-1** is 50% longer, its Φ_F has almost doubled and its Φ_{Δ} has decreased by 42%, which indicate a decrease in the intersystem-crossing rate constant, probably due to the presence of the positive charges. Still, the values are adequate for phototherapeutic applications. Importantly, we tried to perform measurements of $^1\text{O}_2$ generation in water, but only residual signals were detected, which can be rationalized by the formation of aggregates, which is not fully hindered for these PS in aqueous media (see below).

Table 1. Photophysical properties of **PS-1** and **PS-2** in MeOH.

Sample	Solvent	$\log \varepsilon (\lambda)^*:_{\text{max}}$	λ_f / nm	Φ_F	τ_S / ns	Φ_{Δ}
PS-1	MeOH	4.59 (350), 5.13 (678)*	683	0.13	2.7	0.49
PS-2	MeOH	4.74 (359), 5.20 (694)*	699	0.11	2.4	0.35

2.3. Aggregation studies and determination of the $\log P_{OW}$

In addition to the photophysical and photochemical characterization, some aggregation studies are also

needed to evaluate the possible application of our ABAB ZnPcs as potential PS. Due to the hydrophobic nature of the Pc ring, these chromophores have tendency to form aggregates in solution to minimize the solvation energy, particularly in the case of polar solvents as water. Aggregation is a complex process that depends on the van der Waals interaction, π - π stacking, concentration and the environment characteristics. Although a certain organization of the PS in vesicles or micelles can facilitate their transport through the blood system, it is important that once the PSs are delivered to the target cell they stay as non-aggregated species, at least to some extent, leading to a more efficient $^1\text{O}_2$ generation after light excitation. For that reason, aggregation studies in aqueous media are fundamental for the former evaluation of the potential PS. These studies are usually performed by means of UV-vis studies. As mentioned above, **PS-2** is soluble in pure water, but not **PS-1**, although it happens to be soluble upon addition of a stock of polar organic solvent solution, such as MeOH or DMSO, to water. In fact, **PS-1** remained soluble until a 99:1 water/organic solvent ratio was reached. Therefore, we performed aggregation studies by progressively adding water to MeOH solutions of **PS-1** and **PS-2**, keeping the concentration constant (Fig. 2). Upon the addition of water, the two compounds showed a similar behaviour that is a decrease in the intensity of the Q band and a slight red shift and broadening. While **PS-2** proved only slightly aggregated in MeOH/water 99:1 solution, a much more pronounced aggregation was observed for **PS-1**, due to the larger hydrophobic nature of the pyridine rings compared to the alkyl amines of **PS-2**.

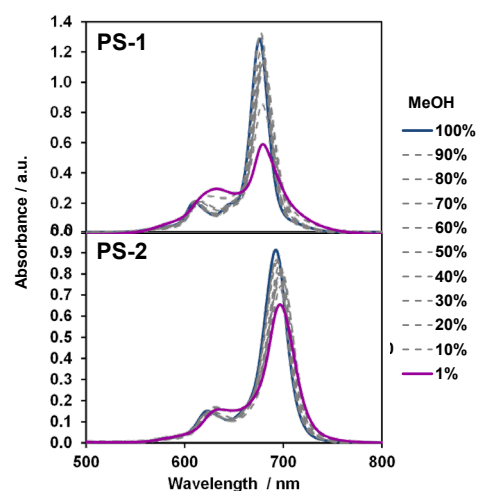


Fig. 2. Aggregation tendency upon increasing water percentage over **PS-1** and **PS-2** solutions in MeOH at constant concentration of the PS in the range: $6\text{--}9 \cdot 10^{-6}$ M.

In order to compare the aggregation behaviour of both PS in physiologically relevant medium, that is phosphate buffered saline (PBS), concentration-dependent UV-vis studies were performed (Fig. 3). The solutions were prepared starting from stock solutions in DMSO, following the same protocol than will be further used for dosing the PS to bacteria.

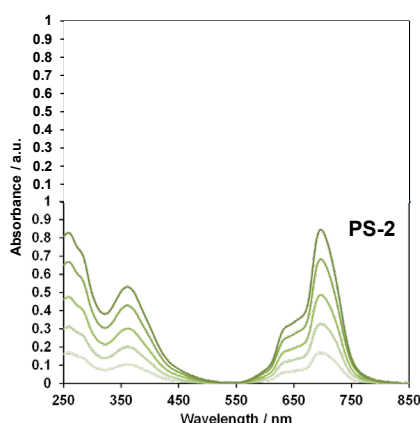


Fig. 3. Aggregation study in PBS (from stock solution in DMSO <2%), for **PS-1** and **PS-2** (concentration range from $\approx 3 \cdot 10^{-6}$ to $2 \cdot 10^{-5}$ M).

Although the recorded spectra resemble those typical of non-aggregated Pcs, the relative intensity of the Q band for **PS-1** (at 681 nm), and for **PS-2** (at 695 nm) with respect to their respective shoulder at 650 nm is very far from that of molecularly dissolved Pcs. Moreover, while a Beer-Lambert plot is apparently linear (Figure S6), the slope yields values of $\log(\epsilon / \text{cm}^{-1} \cdot \text{M}^{-1}) = 4.7$ for both PS, which are one order of magnitude lower than in methanol. Also the intercept is negative for both PS, which suggests a curve with upward-deviation from linearity. Indeed, a much better fit can be obtained by using the monomer-dimer equilibrium model equation,^[28] indicating that the dimer absorbs more than two monomers. Data reduction yields the absorption coefficients for the monomer and the dimer, and the equilibrium constant for dimerization (K) (Table 2). Formation of aggregates is consistent with the almost complete inhibition of $^1\text{O}_2$ production in water.

Table 2. Photophysical properties and $\log P_{OW}$ of **PS-1** and **PS-2** in PBS.

Sample	$\epsilon(m^*)^{[a]} / \text{M}^{-1} \cdot \text{cm}^{-1}$	$\epsilon(m^*)^{[b]} / \text{M}^{-1} \cdot \text{cm}^{-1}$	$\epsilon(d^{**})^{[b]} / \text{M}^{-1} \cdot \text{cm}^{-1}$	K	$\log P_{OW}$
PS-1	50110	24181	106290	$1.1 \cdot 10^6$	2.33

PS-2	53818	48422	107351	$7.5 \cdot 10^5$	0.73
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* m: monomer; ** d: dimer; [a] Beer Lambert; [b] Lavenberg-Marquardt;

Photodynamic efficiency depends also on lipophilicity, which has been recognized as a factor that can determine cellular uptake, and consequently, the phototoxicity of PSs.^[29,30] The affinity of a compound for cell membranes may be numerically represented by its membrane-water partition coefficient, that is, the membrane-water concentration ratio. As this value is usually difficult to measure, the *n*-octanol/PBS partition coefficient (P_{OW}) is a useful quantitative parameter for evaluating the lipophilic/hydrophilic balance, and has been extensively utilized to predict the relative tendency of drugs to interact or incorporate in biological membranes.^[31-33] To evaluate the affinity of **PS-1** and **PS-2** for cell membranes, and the relative lipophilicity of the compounds, the *n*-octanol/PBS partition coefficient for each Pc was determined using the shake-flask method (see Supporting Information), which does not require standard compounds and is based on the direct determination of equilibrium partition concentrations of a compound in a biphasic system. Although both compounds have a common structural core, they exhibited different amphiphilic character. **PS-1** renders a higher octanol/water partition coefficient than **PS-2**, (see Table 2) indicating that the former has a more lipophilic nature. In fact, the Pc concentration reached for **PS-1** in *n*-octanol was approximately 200 times higher than in PBS, while the **PS-2** concentration was only 5-6 times higher in *n*-octanol than in PBS. Therefore, the $\log P_{OW}$ values suggest that **PS-1** is mostly hydrophobic and have very high affinity for membranes. Nevertheless, **PS-2** is more amphiphilic and its presence in the aqueous phase is bigger, which is concomitant with its higher solubility in this medium.

2. 4. Photodynamic inactivation studies

The photodynamic studies were performed testing **PS-1** and **PS-2** in *S. aureus* and *E. coli* bacteria, chosen as models for Gram-positive and Gram-negative bacteria, respectively (Fig. 4). The inactivation studies present a typical light- and concentration-dependent profile. **PS-1** and **PS-2** were able to induce 99.9% (3 log decrease in colony-forming units, CFUs) of *S. aureus* inactivation at 0.1 and 0.5 μM , respectively, using a red light fluence of $33 \text{ J} \cdot \text{cm}^{-2}$. This observation indicates that the ZnPcs must be in monomeric, i.e., photochemically active, form, which in turn indicates that they are bound to the bacterial cell wall, where they de-

aggregate.^[34] Reducing the light fluence to 11 J·cm⁻² only resulted in a cell survival increase of 1 log CFU. Full inactivation (7 log CFUs) could be achieved at 10 μM for both **PS-1** and **PS-2**, even though 4-logs can be attributed to dark cytotoxicity at such high concentration. Regarding *E. coli*, larger concentrations were required to induce a comparable cell death. Up to 50 μM of either photosensitizer was needed to completely inactivate the bacterial strain, whilst 10 μM and 33 J·cm⁻² were enough to achieve a disinfection (99.9%) status. In this case, no dark toxicity could be observed even at the highest concentration, which likely indicates that the compounds are not taken up by the bacteria but stick to the outer membrane.^[35] This confirms that Gram-negative bacteria are harder to photo-inactivate by PDT than Gram-positive ones, an observation that has been thoroughly described in the literature.^[36] The reason behind this difference is found in the composition of the bacterial wall. Importantly, there are no dramatic differences in activity between **PS-1** and **PS-2** against the microbial strains tested, indicating a minor effect of the nature of the cationic moieties, as observed previously by Ruiz-Gonzalez and co-workers for two related porphycene macrocycles.^[37] Compared to other symmetrically-substituted cationic Pcs, the bulky bis(trifluoromethylphenyl) groups attached to **PS-1** and **PS-2** provide them with much stronger photo-antimicrobial activity. Indeed, related Pcs lacking these groups achieved only a modest 1-2-log CFUs cell death, owing to their strong aggregation.^[38,39] This issue has now been solved by constructing asymmetric compounds with large steric hindrance that alleviate the aggregation problems.

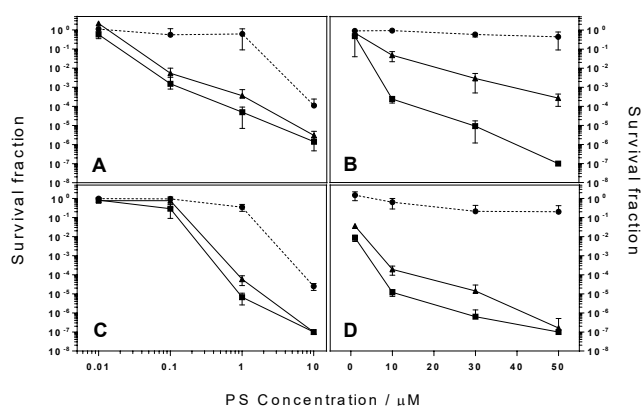


Fig. 4. Survival curves of *S. aureus* (A, C) and *E. coli* (B, D) with **PS-1** (A, B) and **PS-2** (C, D) after PDT treatment. Circles, triangles and squares represent 0 (dark toxicity), 11 and 33 J·cm⁻², respectively.

3. Conclusions

Two different cationic ABAB-ZnPcs (**PS-1** and **PS-2**) have been synthesized as PS for PDT. The peculiar functionalization of these Pcs with facing bulky substituents that provide the molecules with hampered aggregation and lipophilicity, and facing hydrophilic pyridinium (**PS-1**) or alkylammonium (**PS-2**) moieties that impart water-solubility, makes these molecules suitable candidates for the inactivation of bacteria. In order to probe the validity of the design, photophysical evaluation of their ¹O₂ generation capabilities, as well as aggregation assays, have been carried out with **PS-1** and **PS-2**. Non-aggregated MeOH solutions of both PS efficiently generate ¹O₂, but only residual signals were detected in water, which can be rationalized by a certain degree of aggregation. In fact, UV-vis experiments in aqueous media indicate that both PS form aggregates at some extent. Nevertheless, *in vitro* photodynamic assays carried out over *E. coli* and *S. aureus* show that they are photochemically active, inducing bacteria inactivation under red light irradiation, which in turn indicates that they are bound to the bacterial cell wall, where they de-aggregate. It has been proved that **PS-1** and **PS-2** are not toxic for bacteria in darkness in the selected range of concentrations, but when the colonies are irradiated a high fatality turns out. In this regard, there is a minor effect of the nature of the cationic moieties on the activity of these PS. Nevertheless, an important conclusion is that these cationic PS have proved much stronger activity than other related cationic Pcs as a result of the rational design that has rendered improved oxygen generation abilities,^[25] well-balanced lipophilicity/hydrophilicity and diminished aggregation issues.

4. Experimental Section

4.1. General methods and characterization techniques

Chemical reagents were purchased from Merck-Sigma Aldrich, Alfa Aesar, Acros Organics, TCI or Fluorochem, as main commercial suppliers, and were used without further purification unless it is indicated. Solvents were purchased from Carlo Erba Reagents, and anhydrous solvents were dried with 4Å molecular sieves (Panreac). The monitoring of the reactions has been carried out by thin layer chromatography (TLC), employing aluminium sheets coated with silica gel type 60 F254 (0.2 mm thick, E. Merck). Purification and separation of the synthesized products was

performed by column chromatography, using silica gel (230-400 mesh, 0.040-0.063 mm, Merck). Gel permeation chromatography (GPC) was performed using Bio-Beads S-X1 (200-400 mesh, Bio-Rad). Microwave (MW) reactions were carried out in a Biotage Initiator +4.1.2, in closed glass vials (Biotage 2-5 mL, 0.2-0.5 mL) under argon atmosphere. Aggregation studies were performed using aqueous milliQ-water or phosphate-buffered saline solution (PBS), and organic solvents. Infrared (IR) spectra were recorded on a Cry 630 FTIR spectrophotometer from Agilent Technologies, using solid samples (diamond ATR). Mass spectrometry (MS) and high resolution MS (HR-MS) were recorded using positive ESI Positive TOF_MS or Matrix-Assisted Laser Desorption/Ionization (MALDI). MALDI-TOF MS and high-resolution (HR-MS) mass spectra were recorded with a Bruker Ultrareflex III spectrometer. Electrospray Ionization (ESI) mass spectra were recorded with an API Q-Star Pulsar i from Sciex. Matrix used are indicated in each spectrum. MS data are expressed in m/z units. All MS experiments were carried out at the Servicio Interdepartamental de Investigación (SIdI) of the Universidad Autónoma de Madrid. Nuclear magnetic resonance spectra (^1H -NMR and ^{13}C -NMR) were recorded on Bruker AC-300 (300 MHz) or Bruker XRD-500 (500 MHz) instruments. Deuterated solvents employed are indicated in each spectrum. UV-vis spectra were recorded with a UV-vis JASCO-V660 spectrophotometer using spectroscopy grade solvents and 10x10mm quartz cuvettes for aggregation experiments.

4.2. Synthesis

Compounds 3,3'',5,5''-tetrakis(trifluoromethyl)-[1,1':4',1''-terphenyl]-2',3'-dicarbonitrile (**B**),^[22] 4,5-diiodophthalonitrile (**A-1**),^[40] 4,5-bis(pyridin-3-yloxy)phthalonitrile (**A-2**),^[41] 4,5-bis(4-hidroxiphenoxy)phthalonitrile (**A-3**),^[42] 4,5-bis[(2-(dimethylamino)ethyl)thio]phthalonitrile (**A-4**),^[26] ZnPc **1**,^[22] 4-ethinylpyridine,^[43] and 3-(dimethylamino)propyl methyl carbonate (**8**),^[44] were prepared according to previously reported procedures.

ZnPc 2: Phthalonitrile **B** (1 eq), phthalonitrile **A-2** (1 eq) and anhydrous $\text{Zn}(\text{OAc})_2$ (1 eq) were placed in a 5 mL high pressure resistant flask equipped with a magnetic stirrer, and then 2.7 mL ($[\text{B}]=0.1\text{ M}$) of dry *o*-DCB/DMF 2:1 were added. The mixture was heated to 150-160°C overnight under an argon atmosphere. After cooling the solvent was removed under vacuum. The product was purified by

column chromatography on SiO_2 (heptane/EtOAc 1:3 with 1% pyridine) where the second blue-green fraction to elute containing the desired product **2**. A second batch of the product that was strongly retained can be eluted from the column using THF:pyridine (7:1). After evaporation of the solvent a blue-green solid was obtained, which was precipitated from MeOH with water. Yield: 18.8 mg, (8%). ^1H -NMR (300 MHz, THF-d_8): δ 8.75 (s, 8H, Ar), 8.41 (dd, $J = 11.15\text{ Hz}$, $J = 2.76\text{ Hz}$, 8H, Ar-py), 8.23 (s, 4H, Ar), 8.10 (s, 4H, Ar), 8.03 (s, 4H, Ar), 7.46-7.31 (m, 8H, Ar-py); ^{13}C -NMR (125 MHz, DMSO-d_6): δ 120.1, 123.4, 124.5, 124.7, 130.5, 131.2, 134.8, 136.1, 139.7, 142.4, 144.1, 144.8, 147.9, 148.7, 149.8, 151.7, 152.5, 153.7; IR(ATR) ν^{-1} (cm^{-1}): 2920, 2851, 1592, 1458, 1377, 1275, 1132. HR-MS (MALDI, matrix DCTB + PMMANa 2100 + NaI) for $\text{C}_{84}\text{H}_{36}\text{F}_{24}\text{N}_{12}\text{O}_4\text{Zn}$: m/z 1796.1857 [M^+] (calculated: 1796.1885).

PS-1: 2 (10 mg, $5.56 \cdot 10^{-3}$ mmol) was placed in a 10 mL round bottom flask equipped with a magnetic stirrer and 1 mL of dry DMF (dried over 4Å molecular sieves) were added. Then, an excess of MeI (150 μL) was added and the mixture was stirred at room temperature for 4h under argon atmosphere. Then 10 mL of diethylether were added and the product was collected by filtration and washed several times with diethylether as a blue solid. Yield: 8.0 mg (61%). 250 mg of Dowex® (1x8 200-400) were suspended in 10 mL of MilliQ water, the product was previously dissolved in 0.2 mL of DMSO was added and stirred for 2 h. Then, the mixture was filtered, washed with diethylether, and evaporated under vacuum. ^1H -NMR (500 MHz, DMSO-d_6): δ 9.26 (s, 4H, Ar), 9.00 (d, $J^1 = 6.00\text{ Hz}$, 4H, Ar-py), 8.83 (s, 8H, Ar), 8.48 (d, $J^2 = 8.87\text{ Hz}$, 4H, Ar-py), 8.33 (s, 4H, Ar), 8.31 (s, 4H, Ar-py), 8.24 (dd, $J^2 = 8.87\text{ Hz}$, $J^1 = 6.00\text{ Hz}$, 4H, Ar-py), 7.95 (s, 4H, Ar), 4.45 (s, 12H, Me); IR (ATR) ν^{-1} (cm^{-1}): 3016, 2931, 1584, 1498, 1409, 1276, 1171, 1125. HR-MS (ESI Positive TOF_MS-100-3500.m) for $\text{C}_{88}\text{H}_{48}\text{F}_{24}\text{N}_{12}\text{O}_4\text{Zn}$: m/z 630.4153 [MCl^+] (calculated: 630.4167); 963.1093 [MCl_2^{2+}] (calculated: 963.1098).

ZnPc 3: Phthalonitrile **B** (1 eq), phthalonitrile **A-3** (1 eq) and anhydrous $\text{Zn}(\text{AcO})_2$ (1 eq) were placed in a 5 mL high pressure resistant flask equipped with a magnetic stirrer, and then 2.7 mL ($[\text{B}]=0.1\text{ M}$) of dry *o*-DCB/DMF 2:1 were added. The mixture was heated to 150-160°C overnight under an argon atmosphere. After cooling the solvent was removed under vacuum. The product was purified by column chromatography on Bio-Beads using CHCl_3 as

eluent. After evaporation of the solvent a blue solid was obtained, which was washed with heptane. Yield: 50 mg, (20%). ¹H-NMR (300 MHz, THF-d₈): δ 2.35 (s, 24H, NMe₂); 2.81 (t, *J* = 7.09 Hz, 8H, CH₂); 3.23 (t, *J* = 7.09 Hz, 8H, CH₂); 8.12 (s, 4H, Ar); 8.24 (s, 4H, Ar); 8.58 (s, 4H, Ar); 8.84 (s, 8H, Ar). ¹³C-NMR (75 MHz, THF-d₈): δ 33.4 (CH₂), 45.6 (NMe₂), 58.7 (CH₂), 122.4, 122.8 (brs, C*CF₃), 125.0 (q, *J* = 272.42 Hz, CF₃), 132.1, 132.4, 132.8, 136.4, 136.9, 137.9, 142.0, 144.3, 153.4, 154.4 (C=N). IR (ATR) ν⁻¹ (cm⁻¹): 2930 (ar, C-H st), 1729, 1668, 1380 (pyrrole ring), 1279, 1179 (C-F st), 1134 (C-F st). HR-MS (MALDI, matrix: DCTB+PMMA 2100+Nal) for C₈₀H₆₀F₂₄N₁₂S₄Zn: *m/z* 1837.2917 [M+H]⁺ (calculated: 1837.2928).

PS-2: 3 (1 eq) was placed in a 5 mL high pressure resistant flask equipped with a magnetic stirrer and 0.9 mL of dry ethanol (EtOH) (dried over 4 Å molecular sieves) were added. Then, an excess of IMe (20 eq) was added and the mixture was refluxed overnight. After cooling the solvent was removed under vacuum. The residue was washed with CHCl₃, heptane, diethylether and CHCl₃ again, and a green solid was obtained. Yield: 13 mg (36%). 250 mg of Dowex® (1x8 200-400) were suspended in 10 mL of MilliQ water, the product was dissolved in 0.2 mL of DMSO was added and stirred for 2h. Then, the mixture was filtered, washed with diethylether, and evaporated under vacuum. ¹H-NMR (300 MHz, DMSO-d₆): δ 3.13 (s, 36H, NMe₃); 8.22 (s, 4H, CHAr); 8.36 (s, 4H, CHAr); 8.73 (s, 4H, CHAr); 8.89 (s, 8H, CHAr); CH₂ signals are overlapped by the solvent. IR (ATR) ν⁻¹ (cm⁻¹): 2929 (ar, C-H st), 1682, 1482, 1388 (pyrrole ring), 1278, 1179 (C-F st), 1132 (C-F st). HR-MS (ESI Positive TOF_MS-50-3000.m) for C₈₄H₇₂F₂₄N₁₂S₄Zn: *m/z* 474.0942 [M]⁴⁺ (calculated: 474.0943); 643.7829 [MCl]³⁺ (calculated: 643.7822); 983.1589 [MCl₂]²⁺ (calculated: 983.1580).

ZnPc 4: Phthalonitrile **B** (1 eq), phthalonitrile **A-4** (1 eq) and anhydrous Zn(AcO)₂ (1 eq) were placed in a 5 mL high pressure resistant flask equipped with a magnetic stirrer, and then 2.7 mL ([**B**]=0.1 M) of dry *o*-DCB/DMF 2:1 were added. The mixture was heated to 150-160°C overnight under an argon atmosphere. After cooling the solvent was removed under vacuum. The product was purified by column chromatography on SiO₂ (dioxane/heptane in gradient from 1:1 to 2:1) where the first fraction to elute containing the desired ABAB-ZnPc **4**. The product was further purified by an additional column chromatography on Bio-Beads using CHCl₃ as eluent. After evaporation of the solvent, a blue solid was obtained, which were recrystallized

from DCM/heptane. Yield: 17%. ¹H-NMR (300 MHz, DMSO-d₆): δ 6.84 (d, *J* = 8.72 Hz, 8H, O-Ph-O); 6.98 (d, *J* = 8.72 Hz, 8H, O-Ph-O); 7.72 (s, 4H, CHAr); 8.03 (s, 4H, CHAr); 8.23 (s, 4H, CHAr); 8.77 (s, 8H, CHAr); 9.33 (s, 4H, OH). ¹³C-NMR (75 MHz, DMSO-d₆): δ 116.1, 118.7, 125.2, 129.8, 130.3, 131.1, 132.1, 133.6, 134.6, 136.0, 136.9, 142.3, 147.1, 149.7, 150.8, 152.1, 153.6; IR (ATR) ν⁻¹ (cm⁻¹): 3392 (O-H st), 2924 (ar, C-H st), 1505, 1411, 1378 (pyrrole ring), 1277 (C-O-C st as), 1196 (C-F st), 1181 (O-H δ ip), 1134 (C-F st); HR-MS (MALDI, matrix: DCTB) for C₈₈H₄₀F₂₄N₈O₈Zn: *m/z* 1856.1870 (calculated: 1856.1872).

ZnPc 5: 1 (15 mg, 1 eq), **7** (6.67 eq.), PdCl₂(PPh₃)₂ (0.040 eq.) and CuI (0.040 eq.), were dissolved in 1 mL of dry diisopropylamine (distilled with CaH₂ and collected over 4 Å activated molecular sieves) and stirred at 70°C overnight under argon atmosphere. The mixture was diluted with chloroform (20 mL) and washed with water (3x20 mL), dried over MgSO₄, filtrated and evaporated under vacuum. The product was purified by column in Bio-Beads using chloroform as eluent, and then by column chromatography on SiO₂ (EtOAc/pyridine 99:1 and then THF/pyridine) being the product the last eluted fraction. After evaporation the green solid was washed with heptane. Yield: 1.5 mg (11%). ¹H-NMR (300 MHz, THF-d₈): δ 7.67 (d, *J* = 5.76 Hz, 8H, py), 8.34 (s, 4H, Ar), 8.45 (s, 4H, Ar), 8.63 (s, 4H, Ar), 8.72 (d, *J* = 5.76 Hz, 8H, py), 8.85 (s, 8H, Ar). ¹³C-NMR: The low amount obtained due to the low yield prevented obtaining the spectrum. IR (ATR) ν⁻¹ (cm⁻¹): 2959, 2927, 2359, 1591, 1377, 1275, 1165, 1132. HR-MS (MALDI (ULTRAFLEX III) DCTB + PEGNa 2000) for C₉₂H₃₆F₂₄N₁₂Zn: *m/z* 1828.2112 [M]⁺ (calculated: 1828.2089).

ZnPc 6: 4 (15 mg, 0.008 mmol) and **8** (15.6 mg, 0.09 mmol) were placed in a 5 mL high pressure resistant flask equipped with a magnetic stirrer and 0.85 mL of dry acetonitrile were added. Solution was heated for 90 minutes at 180 °C under MW irradiation. After cooling, the mixture was transferred into a flask and the solvent evaporated to dryness. Finally, product was poured onto heptane and the resulting green precipitate was filtered off and dried over vacuum. Yield: 11 mg (62%). ¹H-NMR (500 MHz, DMSO-d₆): δ 1.91 (t, *J* = 6.52 Hz, 8H, CH₂), 2.19 (s, 24H, CH₃), 2.45 (m, 8H, CH₂), 4.03 (t, *J* = 6.45 Hz, 8H, CH₂), 7.02 (d, *J* = 8.34 Hz, 8H, Ar), 7.06 (d, *J* = 8.34 Hz, 8H, Ar), 7.75 (s, 4H, Ar), 7.97 (s, 4H, Ar), 8.23 (s, 4H, Ar), 8.77 (s, 8H, Ar). ¹³C-NMR (125 MHz, DMSO-d₆): δ 26.9, 45.1, 55.8, 66.3, 115.5, 115.7, 116.1, 118.5, 122.4, 129.9, 130.2, 131.2, 132.1,

134.6, 136.0, 152.2. IR (ATR) ν^{-1} (cm⁻¹): 2948 (Ar, C-H st), 1711, 1593 (pyrrole ring), 1500, 1276, 1199 (C-F st), 1132 (C-F st). MS (MALDI, matrix: DCTB) for C₁₀₈H₈₄F₂₄N₁₂O₈Zn: m/z [M] + [M+H]⁺ mixture 2196.5 (calculated: 2196.5), 2151.4 (calculated: 2151.5) [M-(HNMe₂)], 2106.3 (calculated: 2016.4) [M-2(HNMe₂)]. Due to the large number of ions in the region of the molecular ion it is not possible to measure the exact mass reliably by possible interference with the internal standard.

PS-3: ZnPc **6** (1 eq.) was placed in a 5 mL high pressure resistant flask equipped with a magnetic stirrer and 0.9 mL of dry ethanol (EtOH) (dried over 4Å molecular sieves) were added. Then, an excess of Mel (20 eq.) was added and the mixture was refluxed overnight. After cooling the solvent was removed under vacuum. The residue was washed with EtOAc and heptane and it was filtered off to obtain a green solid. Yield: 2 mg (19%). 250 mg of Dowex® (1x8 200-400) were suspended in 10 mL of MilliQ water, the product was previously dissolved in 0.2 mL of DMSO was added and stirred for 2h. Then, the mixture was filtered, washed with diethylether, and evaporated under vacuum. ¹H-NMR (500 MHz, DMSO-d₆): δ 2.25 (br s, 8H, CH₂), 3.14 (s, 36H, CH₃), 3.54 (m, 8H, CH₂), 4.10 (br s, 8H, CH₂), 6.85-7.17 (m, 16H, Ar), 7.76 (s, 4H, Ar), 7.98 (s, 4H, Ar), 8.24 (s, 4H, Ar), 8.77 (s, 8H, Ar). IR (ATR) ν^{-1} (cm⁻¹): 2929 (ar, C-H st), 1616 (pyrrole ring), 1501, 1277, 1196 (C-F st), 1133 (C-F st). HR-MS (ESI Positive TOF_MS-100-3500.m) for C₁₁₂H₉₆F₂₄N₁₂O₈Zn: m/z 763.8681 [MCl]³⁺ (calculated: 763.8685); 1163.2876 [MCl₂]²⁺ (calculated: 1163.2874).

Declaration of competing interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article (photophysical measurements, partition experiments, and biological assays) can be found online at...

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