A series of symmetrical cationic phthalocyanines (Pcs) with either Zn(II) or Si(IV) metal ions and two bulky axial ligands on the silicon complex was synthesized in high yields. The photophysical (absorption, emission, fluorescence, and singlet oxygen quantum yields) and cellular (uptake, toxicity, and subcellular localization) properties of this series of Pcs were investigated. The Si(IV)–Pcs exist mainly as monomers in aqueous media and have higher fluorescent quantum yields in protic solvents (methanol and water) than the Zn(II)–Pcs. The presence of eight short PEG groups at the periphery of a Zn(II)–Pc significantly increases its solubility in protic solvents, but a centrally chelated silicon ion and associated bulky axial ligands were more efficient in preventing aggregation of the Pc macrocycles. The singlet oxygen quantum yields for this series of Pcs in DMSO are in the range 0.09–0.15. All Pcs were readily taken up by human HEP2 cells, and the extent of their accumulation within cells depends on their hydrophobic character. Intracellularly, all Pcs localized preferentially within the cell lysosomes. The Zn(II)–Pc 11 and Si(IV)–Pcs 12 and 14 were found to be the most phototoxic (IC50 = 2.2 µM at a 1 J cm−2 light dose) of this series of compounds.

Introduction

Photodynamic therapy (PDT) is a binary modality for cancer treatment that uses a photosensitizer (PS) and visible light to produce reactive oxygen species which selectively destroy malignant cells.1 PDT is currently used in several countries for the treatment of several types of cancer, including skin, mouth, esophageal, lung, and bladder tumors.2–7 The selectivity of the PDT treatment of several types of cancer, including skin, mouth, esophageal, lung, and bladder tumors.8–11 Since the first approval of hematoporphyrin derivative (HpD) as a PS for PDT clinical applications, a large number of porphyrin derivatives have been synthesized and investigated for application in PDT; these include meso-tetraarylporphyrins, porphyrin dendrimers, core modified porphyrins, chlorins, bacteriochlorins, and Pcs.12–14 From these studies, a number of second generation Pcs have emerged. For example, tetra(meta-hydroxyphenyl)chlorin, 2-(1-hexyloxyethyl)-2-devinyl-pyropheophorbide a, and mono-1-aspartyl chlorin ε are currently being evaluated in PDT clinical trials. Pcs are particularly promising Pcs for PDT because of their intense absorptions around 680 nm (about 2 orders of magnitude larger in intensity than porphyrin long wavelength absorptions) and their ability for generating singlet oxygen.1–4,8–14 However, Pcs are notorious for their strong tendency to aggregate in aqueous solutions,14,15 which can significantly decrease their photosensitizing ability through self-quenching.16 In order to decrease Pc aggregation and to increase their photodynamic activity, several hydrophobic and amphiphilic groups (e.g., carboxylates, sulfonates, phosphonates, PEGs)17–20 and bulky axial ligands (e.g., OSiMe2(CH2)3NMe2 and OCH(CH2NMe2)2)21–24 have been introduced at the macrocycle periphery and core, respectively.

Of particular interest are positively charged Pcs, since such molecules could potentially target highly vulnerable intracellular sites and cause effective DNA photodamage.25,26 For example, it was reported that a series of cationic zinc pyridyloxy Pcs have higher photodynamic activity than purified HpD, the first FDA-approved PS for PDT, and that their biological efficacy could be modulated upon the introduction of alkyl chains of different length on the pyridyloxy groups.27–29 Furthermore, positively charged Pcs have been successfully used for the photoinactivation of both Gram-negative and -positive bacteria.30,31 On the other hand, Si(IV)–Pcs containing one or two bulky axial ligands usually show reduced aggregation, enhanced water-solubility, and high photodynamic efficacy. Recently, two glucosylated Si(IV)–Pcs were shown to have high phototoxicity toward human carcinoma HT29 and HepG2 cells and a Si(IV)–Pc bearing two solketal axial substituents was found to be highly phototoxic to both 14C and B16F10 cell lines.33 In our continuing investigation of new water-soluble and effective PDT sensitizers, we decided to combine, in a single macrocycle, peripheral cationic pyridyloxy groups and two bulky axial ligands on centrally chelated Si(IV) ions. Herein, we report the synthesis, photophysical evaluation, and in vitro investigations of a series of cationic 3-pyridyloxy Pcs bearing either a methyl or a short PEG chain on the pyridyl groups and Zn(II) or Si(IV) coordinating metals, with two large axial ligands on the silicon complexes. The properties of this new series of water-soluble Pcs are compared and discussed.

Experimental Section

1. Chemistry. All air and moisture sensitive reactions were performed under an argon atmosphere. All solvents and reagents were purchased from commercial sources, unless otherwise noted. Dry solvents excluding methanol were purified using a Braun solvent purification system. Dry methanol was obtained by redistillation after refluxing over calcium hydride for 5 h. Silica gel 60 (230 × 400 mesh, Sorbent Technologies) was used for column chromatography. Analytical thin-layer chromatography (TLC) was carried out using polyester backed TLC plates 254 (precoated, 200 µm) from Sorbent Technologies. NMR spectra were recorded on DPX-250 or ARX-300 Bruker or Varian Inova-500 spectrometers (250, 300, or 500 MHz for 1H; 63, 75, or 125 MHz for 13C). The chemical shifts are reported in δ ppm using the following deuterated solvents as internal references: CDCl3 5.32 (1H), 53.8 ppm (13C);
of 15 mL of dry DMF at 80 °C under argon. Potassium carbonate (4.5 g, 32.6 mmol) was added to the reaction solution in 8 portions every 5 min. The reaction solution was heated for 3 h, then cooled to room temperature, and poured into 100 mL of ice-water. After filtration under vacuum, the crude product was purified by column chromatography on neutral alumina using methanol/dichloromethane 1:10 for elution. The title compound (1.37 g) was obtained as a pale white solid in 86% yield. 1H NMR (CDCl3): δ 8.53–8.51 (m, 2H, Py-H), 8.42 (s, 2H, Py-H), 7.42–7.41 (m, 4H, Py-H), 7.31 (s, 2H, Ar-H), 1.3C NMR (CDCl3): δ 151.53, 147.46, 142.19, 127.18, 125.24, 123.67 (Ar-C, Py-C), 50.00 (CH3). 13C NMR (CDCl3): δ 151.53, 147.46, 142.19, 127.18, 125.24, 123.67 (Ar-C, Py-C), 50.00 (CH3). 1H NMR (CDCl3): δ 9.60 (s, 8H, Ar-H), 9.00 (s, 8H, Py-H), 8.56 (d, J = 6.1 Hz, 8H, Py-H), 8.48 (d, J = 8.4 Hz, 8H, Py-H), 8.00–8.04 (m, 8H, Py-H), 4.32 (s, 2H, CH3). 13C NMR (D2O): δ 157.24, 151.70, 147.57, 142.07, 137.20, 136.06, 134.63, 130.38, 117.26 (Ar-C, Py-C), 50.00 (CH3). MS (MALDI-TOF) m/z 1320.48 [M – 81 – 8CH3]+, 1335.43 [M – 81 – 7CH3]+, calcd. for C72H40N16O8Zn 1320.25, C72H41N16O7Zn 1335.27.

ZnPc (11). Pe 4 (0.02 g, 0.015 mmol) and CH3(OCH2CH2)2I (1.5g, 5.5 mmol) were heated to 70 °C in a sealed 10 mL thick-walled tube for 6 days. The product was isolated from the reaction solution by centrifugation and washed twice with acetone and twice with dichloromethane. The crude product was purified on a Sephadex LH-20 column using methanol for elution. The product was vacuum-dried at 30 °C for 2 days to afford the title compound as a dark green solid (56 mg) in 92% yield; mp 173–175 °C. UV–vis (DMSO): δmax (log ε) 675 (4.74), 609 (4.10), 354 (4.36) nm. UV–vis (H2O): δmax (log ε) 674 (4.36), 611 (3.94), 351 (4.27). 1H NMR (D2O): δ 9.69 (s, 8H, Ar-H), 8.75 (d, J = 5.6 Hz, 8H, Py-H), 8.68 (s, J = 8.4 Hz, 8H, Py-H), 8.69 (s, J = 8.3 Hz, 8H, Py-H), 3.70 (s, 2H, CH3). 1H NMR (CD3OD): δ 9.69 (s, 8H, Ar-H), 8.75 (d, J = 5.6 Hz, 8H, Py-H), 8.68 (s, J = 8.4 Hz, 8H, Py-H), 8.69 (s, J = 8.3 Hz, 8H, Py-H), 3.70 (s, 2H, CH3). 1H NMR (DMSO): δ 9.69 (s, 8H, Ar-H), 8.75 (d, J = 5.6 Hz, 8H, Py-H), 8.68 (s, J = 8.4 Hz, 8H, Py-H), 8.69 (s, J = 8.3 Hz, 8H, Py-H), 3.70 (s, 2H, CH3). 13C NMR (D2O): δ 157.24, 151.70, 147.57, 142.07, 137.20, 136.06, 134.63, 130.38, 117.26 (Ar-C, Py-C), 50.00 (CH3). MS (MALDI-TOF) m/z 1320.48 [M – 81 – 8CH3]+, 1335.43 [M – 81 – 7CH3]+, calcd. for C72H40N16O8Zn 1320.25, C72H41N16O7Zn 1335.27.

SiPc (5). Dry dimino-sindoinolide 3 (0.5 g, 1.5 mmol) was added to 5 mL of freshly redistilled quinoline. The reaction solution was stirred under argon for 10 min. and, then, silicon tetrachloride (0.5 mL, 2.2 mmol) was added dropwise to the solution. The temperature was raised to 220 °C and maintained for 1 h. The solution was cooled to room temperature, and the solid was filtered under vacuum and washed successively with water, methanol, and acetone. The title compound was obtained as a dark green solid (0.5 g) in 98% yield; mp > 250 °C. UV–vis (DMSO): δmax (log ε) 677 (3.83) nm.
SiPc (13). Pc 8 (35 mg, 0.02 mmol) and Cl-I (8 mL) reacted as described above for Pc 10, and the title compound was obtained as a dark green solid (28 mg, 96%); mp > 250 °C. UV–vis (H2O): λ: 677 (4.84), 648 (4.05), 610 (4.07), 356 (4.54) nm. 1H NMR (d-THF): δ: 9.99 (s, 8H, Ar-H), 9.70 (s, 8H, Py-H), 9.22 (d, 8H, J = 5.5 Hz, Py-H), 8.88 (d, 8H, J = 7.4 Hz, Py-H), 8.48–8.46 (m, 8H, 8H, Py-H), 4.73 (s, 24H, N-CH3), −1.07 (d, 36H, J = 3.5 Hz, CH3), −1.99 (s, 8H, CH3), −2.06 (m, 6H, CH3). 13C NMR (d-THF): δ: 154.36, 151.52, 148.90, 146.18, 141.22, 132.32, 126.53, 125.68, 116.01 (Ar-C, Py-C), 16.11, 11.42 (CH(CH3)2). MS (MALDI-TOF) m/z: 1631.23 [M + H]+, 1457.42 [M – (OSi(CH3)3)]+, calculated for C105H82N16O10Si3 1810.57. HRMS-ESI m/z: 1539.26 [M – (OSi(PbBu)3)]+, calculated for C104H78N16O10Si3 1794.54, C104H78N16O10Si3 1794.54, C105H82N16O10Si3 1810.57.

SiPc (14). Pc 9 (20 mg, 0.01 mmol) and Cl-I (4 mL) reacted as described above for Pc 10, and the title compound was obtained as a dark green solid (29 mg, 91%); mp 217–219 °C. UV–vis (H2O): λ: 679 (4.81), 649 (3.96), 612 (4.01), 352 (4.29) nm. 1H NMR (d-THF): δ: 9.97 (s, 8H, Ar-H), 9.71 (s, 8H, Py-H), 9.25 (d, 8H, J = 5.3 Hz, 8H, Py-H), 8.88 (d, 8H, J = 7.8 Hz, 8H, Py-H), 8.57–8.54 (m, 8H, 8H, Py-H), 6.90 (t, 4H, J = 7.3 Hz, Ar-H), 6.50 (t, 4H, J = 7.4 Hz, Ar-H), 6.88 (d, 8H, J = 7.0 Hz, 8H, Ar-H), 4.89 (d, 8H, J = 7.0 Hz, 8H, Ar-H), 4.77 (s, 24H, N-CH3), −1.24 (s, 8H, CH3(CH3)2). 13C NMR (d-THF): δ: 154.45, 154.17, 146.12, 146.13, 141.19, 133.79, 130.33, 130.21, 129.94, 126.94, 126.39, 125.78, 119.55 (Ar-C, Py-C), 24.99, 17.00 (CH3(CH3)2). MS (MALDI-TOF) m/z: 1594.37 [M+], 1539.26 [M – (OSi(PbBu)3)]+.
were then prepared to 3.125 μM, and the cells were incubated overnight. Cell toxicity was measured using Promega’s CellTiter Blue Viability Assay kit as per manufacturer’s instructions, with untreated cells considered 100% viable and cells treated with 0.2% saponin as 0% viable.

4.3. Phototoxicity. The cells were prepared as described above with Pc concentration range 0.3125 to 10 μM. After loading overnight, the medium was replaced with medium containing 50 nM HEPES pH 7.2. The cells were exposed to a 100 W halogen lamp filtered through a 610 nm long pass filter to provide approximately 1 J cm⁻² light dose. The cells were kept cool by filtering the IR radiation through 10 mm of water and placing the culture plate on an aluminum block in an ice–water bath, which acted as a heat sink. After exposure to light, the plate was incubated overnight. Cell toxicity was then measured as described above.

4.4. Microscopy. The cells were plated on Laboratory Tek II chamber slides (Nunc) and allowed to grow for 48 h. The cells were then exposed to 10 μM Pc overnight. Organelle tracers were obtained from Invitrogen and used at the following concentrations: LysoSensor Green (to visualize lysosomes and acidic compartments) 50 nM, Mitotracker Green (to visualize mitochondria) 250 nM, ER Tracker (to visualize the ER) 100 nM, and BODIPY FL Ceramide (to visualize the Golgi complex) 1 μM. Images were acquired using a Zeiss Axiovert 200 M inverted fluorescent microscope equipped with an AxioCam MRm CCD camera. Pc fluorescence was imaged using standard Texas Red filters, and organelle tracers were imaged with standard FITC filters (Chroma Technologies).

Results and Discussion

1. Synthesis and Characterization. The water-soluble octapyridyloxy-Pcs 10–15 were synthesized as shown in Scheme 1, via quaternization of the corresponding Zn(II) or Si(IV) complexes. The dipipyridylo-phthalonitrile 2 was prepared in 86% yield from commercially available 4,5-dichlorophthalonitrile 1 and 3-hydroxy pyridine, as it has been previously reported by Wöhrlle and co-workers. The X-ray structure of this key intermediate is shown in Figure 1. One of the pyridine rings is disordered by 2-fold rotation, such that N4 and C17 superimpose. The other pyridine appears to be ordered. The two OPy groups are oriented differently with respect to the phthalonitrile, with torsion angles −59.8(2)° about C1−O1 and −7.0(2)° about C2−O2.

The macrocyclization of phthalonitrile 2 was accomplished under mild conditions, upon heating in dry 1-pentanol in the presence of zinc acetate and a catalytic amount of DBU, to give Zn(I)−Pc 4 in an improved 88% yield from that previously reported.28 The neutral octapyridylo-Pc 4 is poorly soluble in most common organic solvents, but it is soluble in TFA. Alkylation of the pyridyl groups using either methyl iodide or CH₂(COH₂CH₂)₂I afforded the octa-cationic Zn(II)−Pcs 10 and 11 in 96 and 92% yields, respectively. Although highly soluble in DMSO and partially soluble in water, Pcs 10 and 11 aggregate in aqueous solutions, as indicated by the broadening of their Q bands and fluorescence quenching (vide infra).

Since eight alkylated pyridyl groups at the periphery of the Zn−Pc macrocycle still do not prevent its aggregation in aqueous solutions, we prepared a series of octapyridyloxy Si(IV)−Pcs bearing two bulky axial ligands. Phthalonitrile 2 was converted to the diminoisoindoline 3 in 91% yield by reaction with ammonia gas (Scheme 1). Diminoisoindoline 3 and an excess of silicon tetrachloride were heated to 220 °C in freshly distilled quinoline for 1 h giving Si(IV)−Pcs 5 in 98% yield. The dihydroxylation of Pc 5 to give Pc 6 was accomplished using sodium methoxide in ethanol/water. The presence of the OH groups in Pc 6 was confirmed by FT-IR, which shows a broad peak at 3382.3 cm⁻¹ (O−H) and a sharp

peak at 844.6 cm\(^{-1}\) (Si=O). Pcs 7–9 were obtained by reacting Pc 6 with a large excess of the corresponding alkylchlorosilane in dry pyridine, at 115 °C under an argon atmosphere. The excess silane reagent was washed out with pentane, and Pcs 7–9 were purified by column chromatography on silica gel, using THF for elution. The octapyridyloxy Si(IV)–Pcs 7–9 show high solubility in polar organic solvents, such as in DMF, THF, DMSO, methanol, and acetonitrile. The \(^1\)H NMR spectra of 7–9 in either deuterated THF or DMF characteristically show the Pc macrocycle protons in the downfield region at > 9 ppm, the pyridyl protons between 8 and 9 ppm, the aliphatic protons on the axial ligands significantly upfield shifted below 0 ppm, and the axial ligand aromatic protons below 7 ppm. The MALDI-TOF MS spectra of Pcs 5–9 all show the base peak corresponding to the cleavage of one axial ligand, as it is often observed for this type of compound (e.g., see ref 41). Alkylation of Si(IV)–Pcs 7–9 gave the corresponding octa-cationic derivatives 10–15 in yields higher than 90%. All cationic Si(IV)–Pcs were also characterized by NMR, MS, UV–vis, and fluorescence spectroscopy (vide infra) and are highly soluble in protic solvents such as methanol and water. All Pcs were >99% pure, as determined by HPLC (see Table S1 and Figures S1–S5 in the Supporting Information).

2. Photophysical Studies. The spectral properties of Pcs 10–15 in DMSO, methanol, and water at different pH values are summarized in Table 1. Additional concentration- and time-dependent absorption and emission spectra in phosphate buffer at pH 7.4 and spectral data obtained in DMF and acetonitrile are shown in Tables S3 and S4 and in Figures S6–S21 in the Supporting Information. The absorption and emission properties of this series of phthalocyanines depend significantly on the solvent used and the solution pH. Both the nature of the N-pyridyl group (methyl vs triethylene glycol) and the centrally chelated metal and associated axial ligands affect the photophysical properties of the Pc macrocycles. The Si(IV)–Pcs bearing two bulky axial ligands show significantly higher...
fluorescence quantum yields than the Zn(II)–Pcs, in all solvents studied. Among the Si(IV)–Pcs, Pc 12 bearing two flexible tri(n-propyl)siloxy axial groups has the highest fluorescence quantum yield in aqueous media at all pH values investigated (5.0, 6.0, 7.0, 7.4, and 8.0), whereas Pc 13 bearing two tri(isopropyl)siloxy axial groups, the most hydrophobic of the Si(IV)–Pcs synthesized (vide infra), has the highest quantum yield in organic solvents (DMSO and methanol). The neutral Si(IV)–Pcs 7–9 also showed high fluorescence quantum yields in organic solutions (see Table S4 of the Supporting Information). The fluorescence quantum yields of Pcs 10–15 in aqueous media were found to generally decrease with the pH, being the largest at pH 5.0 as we have previously observed. 32 The cationic Pcs bearing triethylene glycol chains on the pyridyloxy groups showed higher fluorescence quantum yields in organic solvents than the corresponding methylated Pcs, although in aqueous media similar values were observed (Table 1).

In DMSO, all Pcs show strong absorption and emission bands at ~677 and 681 nm, respectively, and Stokes shifts of 3 and 5 nm for the Si(IV) and Zn(II) complexes, respectively, as it is typical for this type of compound (Figure 2). 42–45 Although the Zn(II)–Pcs show aggregation in protic solvents (methanol and water), as evidenced by the splitting and/or broadening of their Q absorption bands and significant decrease in the intensity of their emission bands and quantum yields, the Si(IV)–Pcs show intense and sharp Q bands and the highest fluorescence quantum yields in methanol solution. Pc aggregation in solution often results in broadening of the Q band absorptions and bathochromic and hypsochromic shifts. 46,47 Whereas only a few examples of J-type aggregates have been documented, for example protonation of a tetrasulfonated Zn(II)–Pc in aqueous acetonitrile caused a bathochromic shift of the Q band, 48 most of the aggregates are believed to be H-type, causing blue shifts and fluorescence quenching. 46,49–51 Although the octa-cationic Zn(II)–Pcs 10 and 11 exist in their monomeric forms in DMSO, showing sharp Q absorption bands at 677 nm (Figure 2a and b) and emission bands at 682 nm (Figure 2g and h), in aqueous media, they both form H-type aggregates as seen by the broadening and pronounced reduction in the intensity of their absorption and emission bands, along with reduction of their fluorescence quantum yields. Among all the Pcs evaluated in this study, Zn(II)–Pc 10 has the strongest tendency for aggregation in aqueous media, and as a result, it shows fluorescence quantum yields in water about one order of magnitude lower than all other cationic Pcs 11–15. The presence of the eight tri(ethylene glycol) chains on Zn(II)–Pc 11 significantly decreases its tendency for aggregation in aqueous media compared with Zn(II)–Pc 10 bearing eight methyl groups, and as a result, Pc 11’s Q absorption band follows the Lambert–Beer Law (see Figure S6 in the Supporting Information). However, the most efficient structural feature for minimizing aggregation of these cationic macrocycles is the presence of a centrally chelated silicon ion and associated bulky axial ligands. All Si(IV)–phthalocyanines 12–15 were found to exist mainly as monomers in both DMSO and aqueous solutions, showing similar intense and sharp Q band absorptions (Figure 2c–f) that strictly follow the Lambert–Beer Law (see Figures S7–S10 in the Supporting Information) and similarly intense emission bands (Figure 2i–l and Figures S7–S10 in the Supporting Information) in both media.

The singlet oxygen quantum yields for Pcs 11–15 were determined in DMSO as previously reported, 38 in the range 0.09–0.15, as shown in Table S5 of the Supporting Information. These values are characteristic for this type of Pc. 32,38 Si(IV)–Pc 13 was found to have the highest singlet oxygen quantum yield whereas Si(IV)–Pc 15 bearing eight short PEG chains had the lowest. It is possible that the singlet oxygen is inactivated by the PEG chains, as it has been previously observed. 52,53 Pc 13, Table 1. Spectral Properties of Phthalocyanines 10–15 in Different Mediaa

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a abs, absorption maxima (nm); em, emission maxima (nm); SS, Stokes shift (nm); QY, quantum yield.
the most hydrophobic of all the Si(IV)-Pcs synthesized, shows the highest quantum yields in DMSO.

3. Cell Culture Studies. 3.1. Time-Dependent Cellular Uptake. The results obtained for the time-dependent uptake of Pcs 11–15 at a concentration of 10 µM in human HEp2 cells are shown in Figure 3. The cellular properties of Zn(II)-Pc 10 were not investigated due to its poor solubility in aqueous solution. All Pcs showed similar uptake kinetics with rapid accumulation at short time points and reaching a plateau after about 2 h. Zn(II)-Pc 11 bearing eight tri(ethylene glycol) groups was found to have the highest uptake at all time points investigated of this series of cationic Pcs, whereas Si(IV)-Pc 15 had the lowest. This result indicates that Zn(II)-Pc 11 probably has the most favorable amphiphilicity for crossing the HEp2 cells plasma membranes. Although PEG-containing molecules generally have enhanced water solubility, serum life, and tumor accumulation, we have recently shown that the accumulation of porphyrins within HEp2 cells depends on the number of PEG-substituents at the macrocycle periphery. meso-Tetraphenylporphyrins containing three or four low molecular weight PEG chains accumulated to a lower extent within HEp2 cells than those containing one or two PEGs.

The cellular uptake of phthalocyanines and related macrocycles is known to depend on their hydrophobic character, overall charge, and charge distribution. On the basis of the octanol/aqueous buffer (pH 7.4) distribution coefficients of this series of Pcs (see Table S6 of the Supporting Information), the hydrophobic character of this series of Pcs, and the extent of their cellular uptake, increases in the order 15 < 12 < 14 < 13 < 11. Si(IV)-Pc 13 bearing two triisopropylsiloxylaxial groups is the most hydrophobic among the silicon Pcs whereas Si(IV)-Pc 15 bearing eight short PEG chains is the most hydrophilic of this series of Pcs and consequently the least taken up by cells.

3.2. Cytotoxicity. The dark-toxicity of Pcs 11–15 was evaluated in human HEp2 cells exposed to increasing concentrations of each Pc up to 100 µM, and the results are shown in Figure 4. Only Zn(II)-Pc 11, the most accumulated within cells, showed measurable dark toxicity with an estimated IC 50 ∼ 85 µM. All Si(IV)-Pcs were found to be nontoxic to HEp2 cells in the dark up to 100 µM concentrations. However, upon exposure to a low light dose (1 J cm −2), all Pcs were highly toxic to HEp2 cells, as shown in Figure 5. Pcs 11, 12, and 14 were the most phototoxic (IC 50 ∼ 2.2 µM), followed by Si(IV)-Pc 15 (IC 50 ∼ 3.7 µM) and Si(IV)-Pc 13 (IC 50 ∼ 7.2 µM). These results show, as previously observed, that the phototoxicity of Pcs depends not only on the extent of their uptake into cells but also on their intracellular distribution and ability for generating reactive oxygen species (ROS). Si(IV)-Pcs 12 and 14 showed the highest quantum yields in aqueous media at pH 7.4 (vide supra) and might have higher ability for producing ROS. Although Pc 13 exhibited the highest singlet oxygen quantum yield in DMSO of this series of Pcs, its phototoxicity was lower than that of the other Si(IV)-Pcs, possibly due to its higher hydrophylicity compared with the other Si-Pcs and consequently its tendency for aggregation in aqueous solution. Pc 13 was also found to have the highest fluorescence quantum yield in DMSO but the lowest of all
Si(IV)-Pcs in water at pH 7.0 (Table 1). It has been previously reported that although a dichlorinated Pc exhibited a higher singlet oxygen quantum yield in DMSO than the corresponding nonchlorinated Pc, its ability for generating ROS was lower probably as a consequence of its higher hydrophobicity and tendency for aggregation. On the other hand, the high phototoxicity of Zn(II)-Pc might result from both its high cellular uptake and ability for generating ROS, including singlet oxygen. Our results show that the nature of the axial ligands on Si(IV)-Pcs determines not only their photophysical properties but also their aggregation behavior and phototoxicity.

3.3. Intracellular Localization. The preferential sites of subcellular localization of this series of cationic Pcs were evaluated by fluorescence microscopy, upon exposure of HEp2 cells to 10 µM Pc concentrations overnight. Figure 6 shows the fluorescent pattern observed for Si(IV)-Pc and its overlay with the organelle specific fluorescent probes BODIPY Ceramide (Golgi), LysoSensor Green (lysosomes), MitoTracker Green (mitochondria), and DiOC₆ (ER). Additional subcellular images for Pcs 11–14 are provided in the Supporting Information, Figures S27–S30. All Pcs were found to localize mainly within vesicles, which correlated well with the cell lysosomes (Figure 6f and Supporting Information). We did not observe any Pc relocalization during irradiation, as previously detected for carboxylate-substituted Pcs. In agreement with our results, a series of porphyrin- and Pc-peptide conjugates bearing multiple cationic residues, and of PEG-substituted porphyrins, were found to localize preferentially within the cell lysosomes. Preferential lysosome localization has also been reported for several cationic porphyrins (e.g., see refs 61 and 62) probably as a result of an endocytic mechanism of cellular uptake. The lysosomes are considered important targets for the PDT-induced initiation of apoptosis.

Conclusions
A series of cationic pyridyloxoy Pcs (11–15) bearing either Zn(II) or Si(IV) coordinated metal ions and methyl or short PEG groups on the pyridyl, was synthesized. The Si(IV)-Pcs bearing two bulky axial ligands exist mainly as monomers in both organic and aqueous solutions and show higher fluor-
cence quantum yields in solution than the Zn(II)–Pcs. Both Zn(II)–Pcs form aggregates in aqueous medium, in particular Pc 10 bearing eight N-methyl groups; the presence of eight short PEGs increases the solubility of the Pc macrocycle in protic solvents, but a centrally chelated silicon ion and associated bulky axial ligands are more efficient in minimizing Pc aggregation. This series of Pcs exhibited singlet oxygen quantum yields in DMSO in the range 0.09–0.15, and their in vitro properties depended on their hydrophobic character. The most hydrophobic Pc tested (11) accumulated the most within HEp2 cells and was highly phototoxic (IC50 = 2.2 μM at a 1 J cm−2 light dose). Among the Si(IV)–Pcs, the amphiphilic Pcs 12 and 14 were also highly phototoxic (IC50 = 2.2 μM at a 1 J cm−2 light dose). Despite their octa-cationic nature, all Pcs localized subcellularly preferentially within the lysosomes.

Acknowledgment. The authors thank Dr. Irina Nesterova for assistance with the photophysical studies. This work was supported by the National Science Foundation, grant number CHE-304833.

Supporting Information Available: Additional spectral data, octanol/aqueous buffer distribution coefficients, 1H NMR, and fluorescence microscopy images for Pcs 11–14. This material is available free of charge via Internet at http://pubs.acs.org.

References


Cationic Water-Soluble Phthalocyanines


JM070781F