Highly Photocytotoxic Glucosylated Silicon(IV) Phthalocyanines. Effects of Peripheral Chloro Substitution on the Photophysical and Photodynamic Properties

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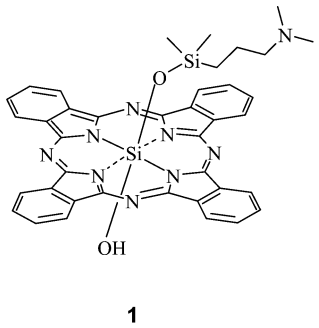
Two novel glucoconjugated silicon(IV) phthalocyanines (compounds **3** and **4**) have been prepared and examined for their photophysical and biological properties. With two axial 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose substituents linked to the silicon center through the tetraethylene glycol chain, both compounds are highly soluble and remain nonaggregated in *N*,*N*-dimethylformamide. The dichloro-substituted phthalocyanine **4** exhibits a weaker fluorescence emission and higher efficiency to generate singlet oxygen compared with the nonchlorinated counterpart **3** as a result of the heavy atom effect. Both compounds are highly photocytotoxic against HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells, particularly the nonchlorinated phthalocyanine **3**, of which the IC_{50} values are as low as 6 nM. The lower photodynamic activity of the chlorinated derivative (IC_{50} = 17−21 nM) can be attributed to its higher aggregation tendency in the biological media, leading to a lower efficiency to generate reactive oxygen species inside the cells. Fluorescence microscopic studies have also revealed that compound **3** has a high and selective affinity to the lysosomes, but not the mitochondria, of HT29 cells.

Introduction

Photodynamic therapy (PDT[a]) has emerged as a promising therapeutic modality for some localized and superficial cancers and wet age-related macular degeneration.[1−3] It employs nontoxic photosensitizers and harmless visible light in combination with oxygen to produce cytotoxic reactive oxygen species (ROS) that destroy malignant cells and tissues by multifactorial mechanisms, including a direct cell-killing by necrosis and/or apoptosis, destruction of vasculature to deprive the malignant tissues of oxygen and nutrients, and induction of acute inflammation that attracts leukocytes.[4] For cancer treatment, PDT has several potential advantages including its minimally invasive nature, tolerance of repeated doses, and high specificity that can be achieved through precise application of the light with modern fiber-optic systems and various types of endoscopy.[3] As a result, PDT has received considerable attention, not only at the fundamental level, but also in the clinical avenue.

Porfimer sodium and temoporfin are the two systemic photosensitizing drugs approved for oncological indications.[3] Although their therapeutic efficacy toward a range of cancers has been confirmed, they still have some deficiencies such as the low absorption in tissue-penetrating red light, sustained skin photosensitivity, low initial selectivity, and long drug-to-light intervals (48−96 h). As a result, various kinds of new-generation photosensitizers with improved properties are being developed.[5] Owing to the desirable absorption and photophysical properties, phthalocyanines are one of the most promising classes of candidates for this application.[6] The silicon(IV) phthalocyanine Pc4 (**1**) developed by Kenney et al. is perhaps the most representative example.[7] Its good in vitro and in vivo performance has stimulated the search for even better candidates and studies of their PDT actions.

Over the past few years, we have been interested in rational modification of phthalocyanines for this application. Several new series of silicon(IV)[8−14] and zinc(II)[15−16] phthalocyanines have been synthesized and evaluated for the photophysical and biological properties. The amphiphilic analogue **2** is by far the most potent compound that we have synthesized.[11,14] It exhibits a high photocytotoxicity against a range of cancer cell lines with IC_{50} values (defined as the dye concentration required to kill 50% of the cells) down to 17 nM. This compound also shows a high and selective affinity to the mitochondria of cells causing cell death mainly through apoptosis.[13] We report herein two novel glucoconjugated silicon(IV) phthalocyanines which have an even higher photocytotoxicity, including their molecular design, synthesis, photophysical properties, and in vitro photodynamic activities.



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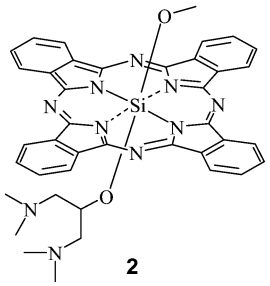

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Results and Discussion

Molecular Design and Chemical Synthesis. Silicon(IV) phthalocyanines are good candidates for PDT application. The closed-shell silicon center not only imparts desirable photophysical characteristics to the macrocycles,[17] but also allows

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[a] Abbreviations: DCFDA, 2′,7′-dichlorodihydrofluorescein diacetate; DMEM, Dulbecco's modified Eagle's medium; DMF, *N*,*N*-dimethylformamide; DPBF, 1,3-diphenylisobenzofuran; ESI, electrospray ionization; $IC_{50/90}$, dye concentration required to kill 50% (or 90%) of the cells; PBS, phosphate buffered saline; PDT, photodynamic therapy; ROS, reactive oxygen species; THF, tetrahydrofuran; ZnPc, unsubstituted zinc(II) phthalocyanine; Φ_F, fluorescence quantum yield; Φ_Δ, singlet oxygen quantum yield.

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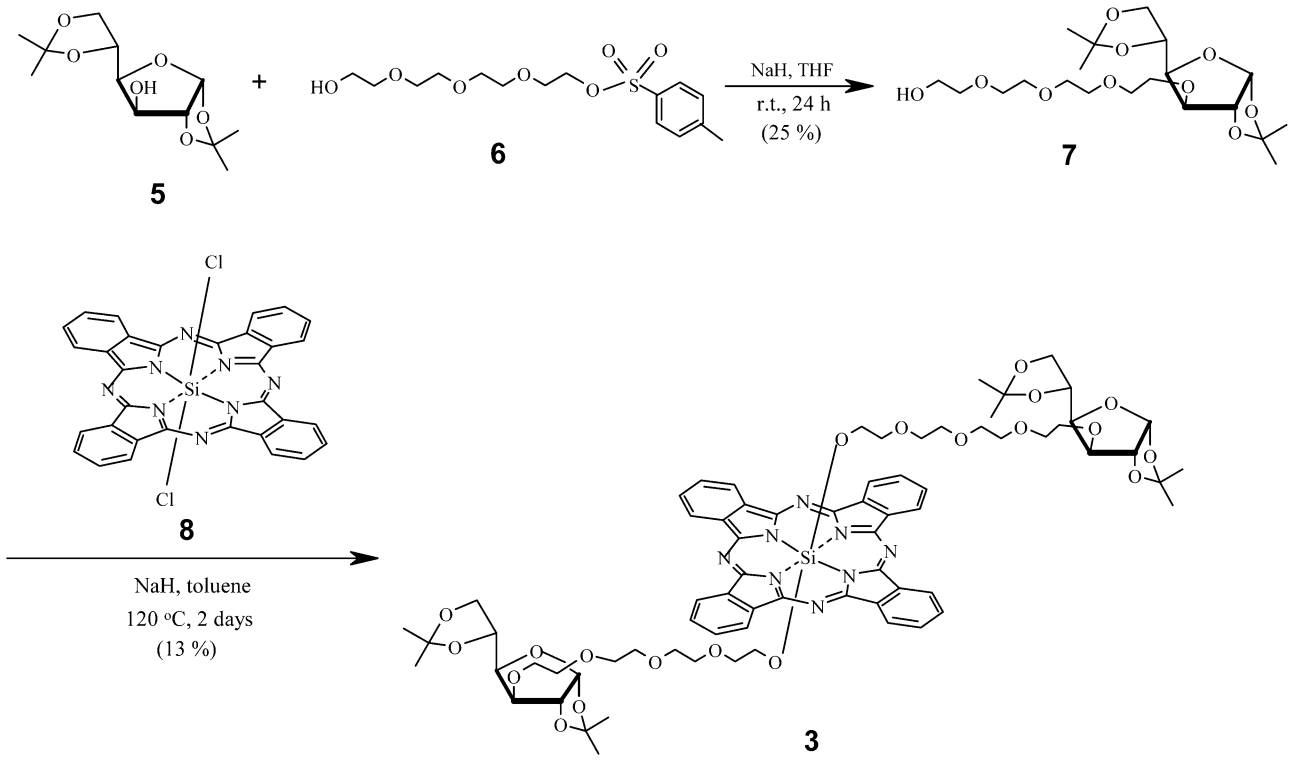
Scheme 1. Synthesis of Glucoconjugated Phthalocyanine **3**



the introduction of appropriate axial ligands to tailor the properties of the macrocycles such as their solubility in biological media, aggregation behavior, and targeting properties. Recently, we employed the isopropylidene-protected galactose moiety as the axial ligand.[12,14] It was hoped that this biocompatible unit can increase the water solubility of the macrocyclic core, reduce its $\pi-\pi$ stacking tendency, and more importantly, enhance the uptake through the glucose transporters overexpressed in cancer cells.[18−21] The resulting glycosylated silicon(IV) phthalocyanines were found to be highly potent in vitro giving IC_{50} values as low as 0.10 μM. On this basis, we extended the study to two novel glucosylated silicon(IV) phthalocyanines (compounds **3** and **4**). Both compounds contain two axial 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose substituents, which are linked to the silicon center through the tetraethylene glycol chain. The latter was introduced to further enhance the water solubility of the macrocycles.

In an attempt to promote the singlet oxygen formation indirectly through the heavy atom effect,[22] two chloro groups were also added on the periphery of the macrocycle in compound **4**. Such approach has been used for other photosensitizers such as chalcogenapyrylium dyes,[23] carbocyanines,[24] hypericins,[25] bacteriochlorins,[26] squaraines,[27] and BF_2-chelated tetraarylazadipyrromethenes[28] and found to be able to improve their photophysical and/or biological properties. We have also examined the effects of peripheral halo-substitution of silicon(IV) phthalocyanines with two axial polyethylene glycol chains.[10,29] Although the octa-chloro and bromo analogues give higher intersystem crossing and singlet oxygen quantum yields (Φ_Δ) than the nonhalogenated counterpart in organic solvents, their in vitro photodynamic activity is reduced as a result of their lower solubility and higher aggregation tendency in the culture media. Hence, only two instead of eight chloro groups were introduced in compound **4**.

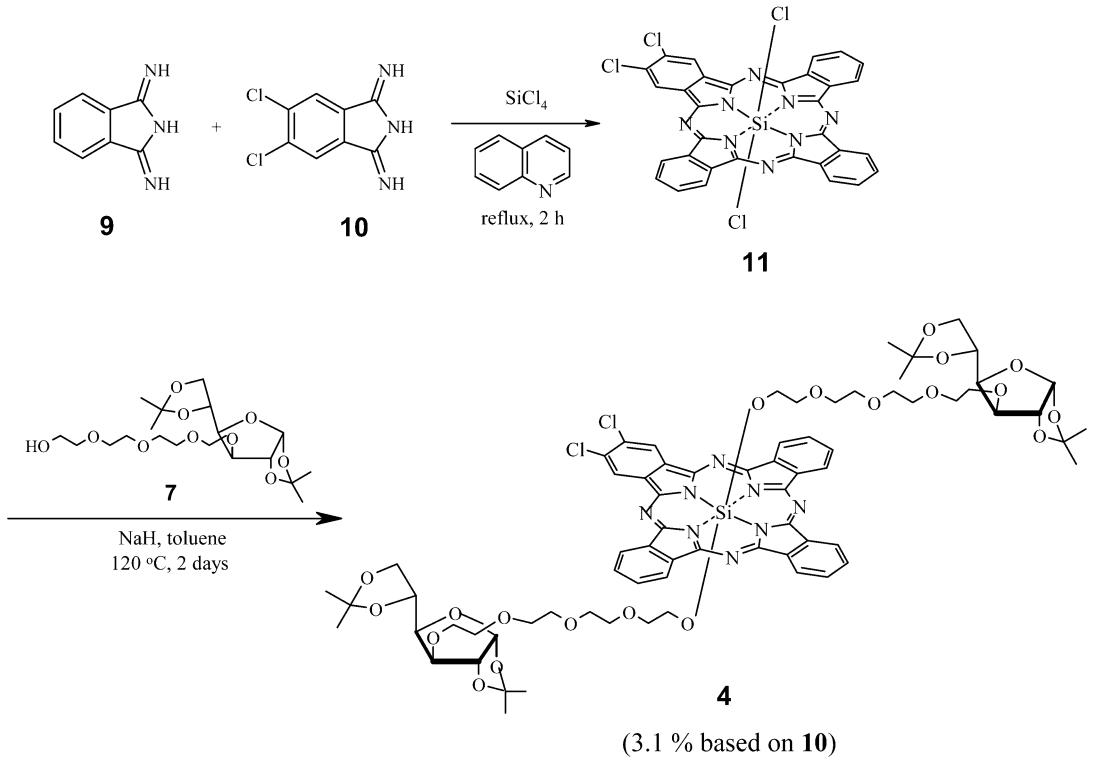
Scheme 1 shows the synthetic route for compound **3**. Reaction of 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (**5**) with tetraethylene glycol mono(*p*-toluenesulfonate) (**6**) and NaH gives the substituted product **7**. Treatment of this compound with the readily available silicon(IV) phthalocyanine dichloride (**8**) and NaH in toluene affords the diglucosylated phthalocyanine **3** in 13% yield. Attempts to purify this compound by silica gel column chromatography were not successful. The compound decomposed slowly in the column, which might be due to its susceptibility toward the slightly acidic nature of the silica gel. The compound, however, could be purified by column chromatography using neutral alumina followed by gel permeation chromatography.

To prepare compound **4**, a "3+1" mixed cyclization of 1,3-diiminoisoindoline (**9**) and the dichloro derivative **10** was first performed in the presence of $SiCl_4$ in refluxing quinoline (Scheme 2). Due to the poor solubility of the desired product **11**, it was not separated from the other side products, and the crude product was treated directly with the protected glucose derivative **7** and NaH. From the reaction mixture, the desired glucosylated dichlorophthalocyanine **4** could be isolated by the same chromatographic procedures. This compound represents a very rare example of A_3B-type silicon(IV) phthalocyanines.

Both compounds **3** and **4** are highly soluble in common organic solvents. They also exhibit substantial solubility in water (at least 50 μM, see the UV−vis study below). Considering the fact that phthalocyanine **8** is completely insoluble in water and even in many other organic solvents, it is clear that the tetraethylene glycol linked glucose moiety can greatly enhance the hydrophilicity of the phthalocyanine core. Due to the sensitivity of these compounds toward acid, deprotection of the glucose moieties was not performed.

Spectroscopic Characterization and Photophysical Properties. Both glucosylated phthalocyanines **3** and **4** were fully characterized with various spectroscopic methods. The ^{1}H NMR spectrum of **3** in $CDCl_3$ showed two AA'BB' multiplets for the phthalocyanine α and β ring protons, while that of **4** also showed a singlet at δ 9.68 assignable to the α ring protons

Scheme 2. Synthesis of Glucoconjugated Dichlorophthalocyanine **4**



(3.1 % based on **10**)

adjacent to the chloro groups. Due to the ring current effect, the signals for the tetraethylene glycol were spread from δ −1.9 to 3.6 as eight well-separated triplets or multiplets. These protons as well as those of the protected glucose unit could be unambiguously assigned by 2D COSY experiment (these spectra are given in Supporting Information). The $^{13}C\{^1H\}$ spectra also showed the expected number of signals and a partial assignment could be made (see the Experimental Section).

Satisfactory elemental analysis data were obtained for compound **3**, but not for compound **4**. This could be attributed to the presence of a trace amount of impurities in the latter sample and/or the fact that phthalocyanines are notoriously difficult to combust.[30] However, as revealed by HPLC (see the chromatograph in Figure S4 in Supporting Information), compound **4** was deemed to be pure. In fact, its ^1H NMR spectrum showed well-resolved doublets for H1, H2, and H3 protons of the glucofuranose ring (Figure S3 in Supporting Information). The $^{13}C\{^1H\}$ NMR spectrum also showed 16 signals for the phthalocyanine ring (Figure S5 in Supporting Information). These results clearly indicated that the other self- and mix-cyclized products are not present.

The electronic absorption and basic photophysical data of **3** and **4** in *N*,*N*-dimethylformamide (DMF) are summarized in Table 1. Both compounds give very similar UV−vis spectra, which are typical for nonaggregated phthalocyanines. The spectra show the B-band at 355 nm, a vibronic band at 606 nm, and an intense and sharp Q-band at 673 nm, which strictly follows the Lambert Beer's law (see Figure S6 for compound **3** in Supporting Information). Peripheral substitution with two chloro groups slightly shifts the fluorescence emission from 676 (for **3**) to 679 (for **4**) nm and reduces the fluorescence quantum yield (Φ_F) from 0.34 ± 0.02 to 0.24 ± 0.02. The Φ_Δ, as determined by a steady-state method using 1,3-diphenylisobenzofuran (DPBF) as the scavenger,[31] increases from 0.32 ± 0.01 to 0.41 ± 0.01 upon dichloro substitution (Supporting Information). The results can be attributed to the heavy atom effect, which promotes the intersystem crossing leading to a lower Φ_F and a higher efficiency to generate singlet oxygen. Similar results were observed for the halogenated analogues with two axial polyethylene glycol chains.[10,29]

Both compounds **3** and **4** are also soluble in water. Hence, the absorption spectra can also be recorded in this medium. Figure S8 (Supporting Information) shows the spectra of **3** in water with <0.5% tetrahydrofuran (THF) with concentrations ranging from 9.4 to 47.2 μM. All the absorptions [λ_{max} (nm) (log ϵ): 361 (4.62), 628 (4.35), and 684 (4.82)] are significantly red-shifted with a lower molar absorptivity compared with those in DMF. The Q-band is also significantly broadened, but still follows the Lambert Beer's law, showing that aggregation of this compound under these conditions is not so significant. However, the spectra of **4** (from 9.0 to 53.8 μM; Figure S9 in Supporting Information) show a much broader Q-band (at 691 nm), which indicates that this compound has a higher aggregation tendency in water.

In Vitro Photodynamic Activities. The in vitro photodynamic activities of compounds **3** and **4** in Cremophor EL emulsions were investigated against two different cell lines, namely, HT29 human colorectal carcinoma and HepG2 human hepatocarcinoma cells. Figure 1 shows the effects on the former cells. It can be seen that both compounds are essentially noncytotoxic in the absence of light, but exhibit a very high photocytotoxicity. The photodynamic action against HepG2 is similar, and the corresponding IC_{50} and IC_{90} values are summarized in Table 2. It can be seen that both compounds are highly potent and the effects on HT29 are greater than those on HepG2. The nonchlorinated phthalocyanine **3** is particularly potent with the IC_{50} values down to 6 nM, which are even lower than those of **2** (ca. 20 nM).[11,14] The in vitro photocytotoxicity of these compounds is much higher than that of the classical photosensitizer porfimer sodium (IC_{50} = 7.5 μg mL^{-1} vs 7.8 ng mL^{-1} for **3** against HT29),[32] pheophorbide *a* (IC_{50} = 0.3 μg mL^{-1} or 0.5 μM for HT29),[32] and several other glucoconjugated chlorins.[33]

Table 1. Electronic Absorption and Photophysical Data for **3** and **4** in DMF

cmpd	λ_{max} (nm) (log ϵ)	λ_{em}[a] (nm)	Φ_F[b]	Φ_Δ[c]
3	355 (4.87), 606 (4.56), 673 (5.38)	676	0.34 ± 0.02	0.32 ± 0.01
4	355 (4.91), 606 (4.58), 673 (5.28)	679	0.24 ± 0.02	0.41 ± 0.01

[a] Excited at 610 nm. [b] Using ZnPc in 1-chloronaphthalene as the reference ($\Phi_F = 0.30$). [c] Using ZnPc as the reference ($\Phi_\Delta = 0.56$ in DMF).

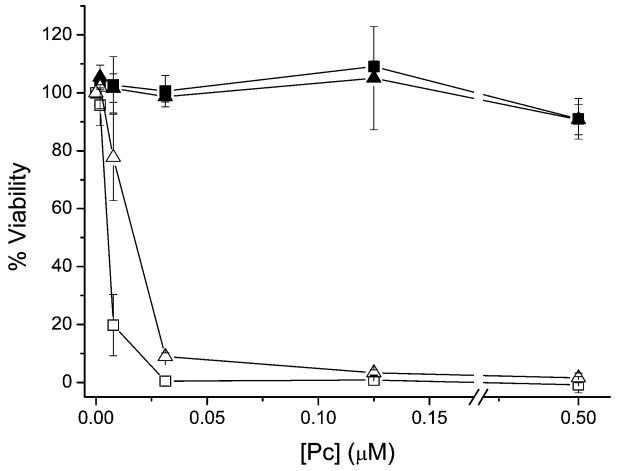


Figure 1. Effects of **3** (squares) and **4** (triangles) on HT29 in the absence (closed symbols) and presence (open symbols) of light. For the latter, the cells were irradiated with a red light (λ > 610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± S.E.M. of three independent experiments, each performed in quadruplicate.

Table 2. Comparison of the IC_{50} and IC_{90} Values of Phthalocyanines **3** and **4** against HT29 and HepG2

	HT29		HepG2	
cmpd	IC_{50}[a] (nM)	IC_{90}[b] (nM)	IC_{50}[a] (nM)	IC_{90}[b] (nM)
3	6	20	6	ca. 200
4	17	31	21	>500

[a] Defined as the dye concentration required to kill 50% of the cells. [b] Defined as the dye concentration required to kill 90% of the cells.

For the light source that we used (halogen lamp, λ > 610 nm), only a narrow region of the light (at ca. 610−700 nm) is absorbed by the compounds to initiate the photodynamic action. Hence, the actual total fluence required should be much lower than 48 J cm^{-2}. To study the effects of light dose, the cytotoxicity of **3** against HT29, upon illumination with a diode laser at 675 nm, was also evaluated. It was found that a total fluence of 8 J cm^{-2} (power = 0.2 W) is sufficient to attain a similar effect (IC_{50} = 7 nM). By reducing the total fluence to 2 J cm^{-2}, the photoactivity was lower giving an IC_{50} value of 46 nM (Supporting Information).

It is worth noting that although the dichlorophthalocyanine **4** exhibits a higher Φ_Δ in DMF (Table 1), its photocytotoxicity is lower than that of the unsubstituted counterpart **3** (Table 2). To account for the results, the absorption spectra of these compounds in the culture medium were recorded. As shown in Figure 2, the Q-band for compound **3** remains very sharp and intense, while that for **4** is significantly weaker and broadened. This is a strong indication that compound **4**, having two hydrophobic and large chloro groups, is significantly more aggregated in the medium. This is corroborated with its weaker fluorescence emission compared with **3** (Supporting Information).

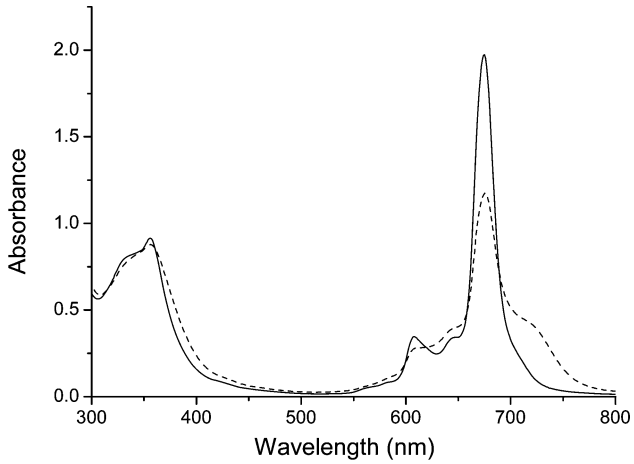


Figure 2. Electronic absorption spectra of **3** (—) and **4** (---), formulated with Cremophor EL, in the DMEM culture medium (both at 8 μM).

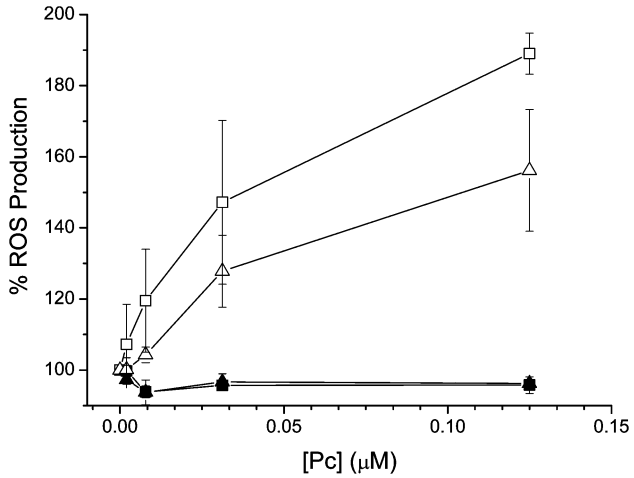


Figure 3. ROS production induced by **3** (squares) and **4** (triangles) in HT29 cells. The cells were incubated with different concentrations of these compounds and illuminated with red light (open symbols) or kept in dark (closed symbols). Immediately after illumination, the cells were washed with PBS and incubated in 100 μM DCFDA at 37 °C for 60 min. Fluorescence measurements were made in a plate reader with a 485 nm excitation filter and a 535 nm emission filter. Each data point represents the mean value ± S.E.M. of three independent experiments, each performed in quadruplicate.

The intracellular production of ROS by these compounds was also studied using 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) as the quencher.[34] As shown in Figure 3, both **3** and **4** can generate ROS upon illumination, and the efficiency is lower for the dichlorophthalocyanine **4**, which again may be due to its higher aggregation tendency. These results are in contrast to those for the halogenated squaraines[27] and BF_2-chelated tetraarylazadipyrromethenes,[28] which show higher photocytotoxicities than the nonhalogenated counterparts as a result of the heavy atom effect. The present study indicates that heavy-atom substitution is not a general approach to improve the efficiency of photosensitizers. In fact, these substituents, usually hydrophobic in nature, may induce aggregation of the photosensitizers in biological media, which counteracts the heavy atom effect.

To have a better understanding on the role of the glucose moieties, we performed a competitive experiment. During the incubation with **3** (from 0 to 0.5 μM), a solution of glucose in phosphate buffered saline (PBS; 10 or 20 μM after dilution with the medium) was added to the HT29 or HepG2 cells. For both of these cell lines, the photodynamic response was found to be

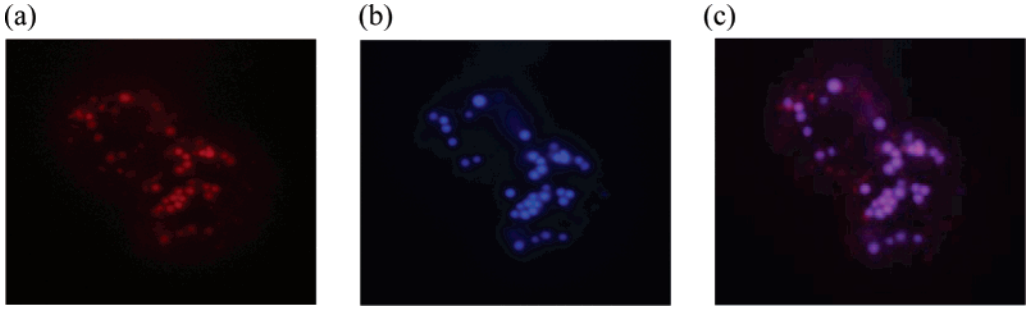


Figure 4. Visualization of intracellular fluorescence of HT29 using filter sets specific for (a) the LysoTracker (in red) and (b) phthalocyanine **3** (in blue). Figure c shows the corresponding superimposed image.

virtually identical with that in the absence of glucose. The results suggested that the cellular uptake of **3** may not involve the glucose transporters, so the protected glucose moieties of **3** do not play the related functional role.

In addition to the cell viability studies, we also employed fluorescence microscopy to investigate the cellular uptake and subcellular localization of compound **3**. After incubation with **3** (formulated with Cremophor EL) for 2 h and upon excitation at 630 nm, the HT29 cells showed a strong intracellular fluorescence as bright and granular spots throughout the cytoplasm (Supporting Information), indicating that there were substantial uptakes of the dye. As lysosomes and mitochondria are important targets for the initiation of apoptosis by PDT,[35−37] it would be important to reveal whether the dye has a selective affinity to these subcellular components. We stained the HT29 cells with LysoTracker HCK-123 or MitoTracker Green FM, which are specific fluorescence dyes for lysosomes and mitochondria, respectively, together with **3**. As shown in Figure 4, the fluorescence caused by the LysoTracker (excited at 465 nm, monitored at 500−575 nm) can superimpose with the fluorescence caused by **3** (excited at 630 nm, monitored at >660 nm). This observation indicates that compound **3** can target lysosomes. By contrast, the fluorescence images of **3** and the MitoTracker (excited at 490 nm, monitored at 500−575 nm) cannot be superimposed (Supporting Information), indicating that **3** is not localized in the mitochondria. The subcellular localization property of this compound in HT29 cells is very different from that of **2**, which has a high and selective affinity to the mitochondria.[14]

In conclusion, we have prepared and characterized two novel glucoconjugated silicon(IV) phthalocyanines. The peripherally unsubstituted analogue **3** is a highly promising photosensitizer, which shows an extremely high in vitro photocytotoxicity and a highly selective subcellular localization property targeting the lysosomes. Attempts to optimize its photodynamic activity by the heavy atom effect were not successful due to the adverse effect of aggregation. The detailed mechanism of the PDT action of this compound and its in vivo PDT efficacy are under investigation.

Experimental Section

Materials and Methods. All the reactions were performed under an atmosphere of nitrogen. THF and toluene were distilled from sodium benzophenone ketyl and sodium, respectively. Chromatographic purifications were performed on silica gel (Macherey-Nagel, 70−230 mesh) or neutral alumina (Merck, 70−230 mesh) columns with the indicated eluents. Gel permeation chromatography was carried out on Bio-Rad Bio-Beads S-X1 beads (200−400 mesh). All other solvents and reagents were of reagent grade and used as received. The protected glucose **5**,[38] tosylate **6**,[39] phthalocyanine **8**,[40] and 1,3-diiminoisoindolines **9**[40] and **10**[10] were prepared as described.

^{1}H and ^{13}C{^{1}H} NMR spectra were recorded on a Bruker DPX 300 spectrometer (^{1}H, 300; ^{13}C, 75.4 MHz) in $CDCl_3$. Spectra were referenced internally using the residual solvent (^{1}H: δ 7.26) or solvent (^{13}C: δ 77.0) resonances relative to $SiMe_4$. Electrospray ionization (ESI) mass spectra were measured on a Thermo Finnigan MAT 95 XL mass spectrometer. Elemental analyses were performed by Medac Ltd., Brunel Science Centre, U.K.

UV−vis and steady-state fluorescence spectra were taken on a Cary 5G UV−vis-NIR spectrophotometer and a Hitachi F-4500 spectrofluorometer, respectively. The Φ_F values were determined by the equation: $\Phi_{F(sample)} = (F_{sample}/F_{ref})(A_{ref}/A_{sample})(n_{sample}^2/n_{ref}^2)\Phi_{F(ref)}$,[41] where F, A, and n are the measured fluorescence (area under the emission peak), the absorbance at the excitation position (610 nm), and the refractive index of the solvent, respectively. The unsubstituted zinc(II) phthalocyanine (ZnPc) in 1-chloronaphthalene was used as the reference [$\Phi_{F(ref)} = 0.30$].[42] To minimize reabsorption of radiation by the ground-state species, the emission spectra were obtained in very dilute solutions where the absorbance at 610 nm was less than 0.03. The Φ_Δ values were measured in DMF by the method of chemical quenching of DPBF using ZnPc as reference ($\Phi_\Delta = 0.56$).[31]

3-*O*-(11-Hydroxy-3,6,9-trioxaundecyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (7). The protected glucose **5** (6.0 g, 23.1 mmol) was added to a suspension of NaH (60% in mineral oil, 0.92 g, 23.0 mmol) in THF (50 mL). After the evolution of gas bubbles had ceased, a solution of the monotosylate **6** (4.0 g, 11.5 mmol) in THF (30 mL) was added slowly. The mixture was stirred vigorously at room temperature for 24 h. A few drops of water were then added to quench the reaction, then the volatiles were removed under reduced pressure. The residue was mixed with water (120 mL) and the mixture was extracted with chloroform (100 mL × 3). The combined organic extracts were dried over anhydrous $MgSO_4$ and evaporated under reduced pressure. The residue was subjected to silica-gel column chromatography using ethyl acetate as eluent. The product was obtained as a colorless transparent liquid (1.25 g, 25% yield). ^{1}H NMR: δ 5.88 (d, J = 3.6 Hz, 1 H, H1), 4.58 (d, J = 3.6 Hz, 1 H, H2), 4.28−4.36 (m, 1 H, H5), 4.07−4.15 (m, 2 H, H6), 3.98−4.01 (m, 1 H, H4), 3.91 (d, J = 3.0 Hz, 1 H, H3), 3.58−3.77 (m, 16 H, CH_2), 2.88 (t, J = 7.5 Hz, 1 H, OH), 1.48 (s, 3 H, Me), 1.41 (s, 3 H, Me), 1.34 (s, 3 H, Me), 1.30 (s, 3 H, Me). ^{13}C{^{1}H} NMR: δ 111.7 (CMe_2), 108.9 (CMe_2), 105.2 (C1), 82.6, 81.1, 72.5, 70.6 (several overlapping signals), 70.4, 70.3, 70.1, 67.1, 61.7, 26.8 (two overlapping signals) (Me), 26.2 (Me), 25.4 (Me). MS (ESI): an isotopic cluster peaking at m/z 459 {100%, $[M + Na]^+$}. HRMS (ESI): m/z calcd for $C_{20}H_{36}NaO_{10}$ $[M + Na]^+$, 459.2201; found, 459.2204.

Phthalocyanine 3. A mixture of silicon phthalocyanine dichloride (**8**; 1.02 g, 1.7 mmol), protected glucose **7** (3.73 g, 8.6 mmol), and NaH (60% in mineral oil, 0.83 g, 20.8 mmol) in toluene (80 mL) was refluxed for 2 days. After evaporating the solvent in vacuo, the residue was subjected to column chromatography on neutral alumina using $CHCl_3$ as eluent, followed by gel permeation chromatography using THF as eluent. The crude product was then chromatographed again on neutral alumina using CH_2Cl_2, and then $CHCl_3/CH_2Cl_2$ (1:4) as eluent. The product was collected as a blue solid (0.30 g, 13%). ^{1}H NMR: δ 9.62−9.67 (m, 8 H, Pc-H$_\alpha$), 8.32−

8.38 (m, 8 H, Pc-H$_\beta$), 5.78 (d, J = 3.6 Hz, 2 H, H1), 4.44 (d, J = 3.6 Hz, 2 H, H2), 4.14−4.23 (m, 2 H, H5), 4.06 (dd, J = 3.0, 7.5 Hz, 2 H, H4), 3.91−4.15 (m, 4 H, H6), 3.81 (d, J = 3.0 Hz, 2 H, H3), 3.54−3.58 (m, 4 H, CH_2), 3.34−3.37 (m, 4 H, CH_2), 3.20−3.23 (m, 4 H, CH_2), 2.95−2.98 (m, 4 H, CH_2), 2.45−2.49 (m, 4 H, CH_2), 1.68−1.71 (m, 4 H, CH_2), 1.45 (s, 6 H, Me), 1.38 (s, 6 H, Me), 1.26 (s, 6 H, Me), 1.22 (s, 6 H, Me), 0.41 (t, J = 5.7 Hz, 4 H, CH_2), −1.89 (t, J = 5.7 Hz, 4 H, CH_2). $^{13}C\{^1H\}$ NMR: δ 149.2 (Pc), 136.0 (Pc), 130.8 (Pc), 123.6 (Pc), 111.6 (CMe_2), 108.8 (CMe_2), 105.1 (C1), 82.5, 82.4, 81.0, 72.4, 70.1 (two overlapping signals), 69.9, 69.8, 69.3, 68.5, 67.0, 54.7, 30.3, 26.8 (Me), 26.7 (Me), 26.1 (Me), 25.3 (Me). MS (ESI): isotopic clusters peaking at m/z 976 {100%, $[M - OR]^+$} and 1434 {62%, $[M + Na]^+$}. HRMS (ESI): m/z calcd for $C_{72}H_{86}N_8NaO_{20}Si$ $[M + Na]^+$, 1433.5620; found, 1433.5632. Anal. Calcd for $C_{72}H_{86}N_8O_{20}Si$: C, 61.26; H, 6.14; N, 7.94. Found: C, 61.41; H, 6.23; N, 7.44.

Silicon 2,3-Dichlorophthalocyanine Dichloride (11). A mixture of 1,3-diiminoisoindoline (**9**; 3.88 g, 26.7 mmol), dichloro-1,3-diiminoisoindoline (**10**; 1.46 g, 6.8 mmol), and $SiCl_4$ (5.0 mL, 43.6 mmol) in quinoline (60 mL) was heated at reflux for 2 h. The mixture was then poured into toluene (400 mL) to give a blue paste, which was filtered and washed thoroughly with toluene, methanol, and acetone. The resulting blue solid was then Soxhlet extracted with a mixture of these solvents plus chloroform for 2 days to give a blue solid (3.98 g), which contained the desired product. The crude product was used for the following reaction without further purification.

Phthalocyanine 4. According to the procedure for **3**, treatment of crude **11** (0.58 g) with protected glucose **7** (1.66 g, 3.8 mmol) and NaH (60% in mineral oil, 0.44 g, 11.0 mmol) in toluene (50 mL) gave the product as a greenish blue solid (45 mg, 3.1% based on **10**). ^1H NMR: δ 9.68 (s, 2 H, Pc-H$_\alpha$), 9.58−9.65 (m, 6 H, Pc-H$_\alpha$), 8.32−8.38 (m, 6 H, Pc-H$_\beta$), 5.76 (d, J = 3.7 Hz, 2 H, H1), 4.43 (d, J = 3.7 Hz, 2 H, H2), 4.16−4.23 (m, 2 H, H5), 4.02−4.06 (m, 2 H, H4), 3.90−4.00 (m, 4 H, H6), 3.79 (d, J = 3.0 Hz, 2 H, H3), 3.54−3.58 (m, 4 H, CH_2), 3.34−3.38 (m, 4 H, CH_2), 3.21−3.25 (m, 4 H, CH_2), 2.97−3.01 (m, 4 H, CH_2), 2.47−2.50 (m, 4 H, CH_2), 1.70−1.73 (m, 4 H, CH_2), 1.44 (s, 6 H, Me), 1.36 (s, 6 H, Me), 1.24 (s, 6 H, Me), 1.21 (s, 6 H, Me), 0.40 (t, J = 5.7 Hz, 4 H, CH_2), −1.91 (t, J = 5.7 Hz, 4 H, CH_2). $^{13}C\{^1H\}$ NMR: δ 150.3 (Pc), 150.1 (Pc), 149.1 (Pc), 146.5 (Pc), 136.1 (Pc), 136.0 (Pc), 135.9 (Pc), 135.0 (Pc), 134.6 (Pc), 131.3 (Pc), 131.2 (Pc), 131.0 (Pc), 125.1 (Pc), 123.9 (Pc), 123.8 (Pc), 123.6 (Pc), 111.6 (CMe_2), 108.8 (CMe_2), 105.1 (C1), 82.6, 82.5, 81.0, 72.4, 70.2 (two overlapping signals), 69.9, 69.4, 69.3, 68.6, 67.1, 54.7, 29.7, 26.8 (Me), 26.7 (Me), 26.1 (Me), 25.3 (Me). MS (ESI): isotopic clusters peaking at m/z 975 {100%, $[M - OR - 2\,Cl + 2\,H]^+$}, 1043 {51%, $[M - OR]^+$}, 1434 {97%, $[M - 2\,Cl + 2\,H + Na]^+$}, 1504 {42%, $[M + Na]^+$}. HRMS (ESI): m/z calcd for $C_{72}H_{84}Cl_2N_8NaO_{20}Si$ $[M + Na]^+$, 1501.4840; found, 1501.4835.

Cell Lines and Culture Conditions. The HT29 human colorectal carcinoma cells (from ATCC, no. HTB-38) were maintained in Dulbecco's modified Eagle's medium (DMEM; Invitrogen, cat no. 10313-021) supplemented with fetal calf serum (10%), penicillin-streptomycin (100 units mL^{-1} and 100 mg mL^{-1}, respectively), L-glutamine (2 mM), and transferrin (10 mg mL^{-1}). The HepG2 human hepatocarcinoma cells (from ATCC, no. HB-8065) were maintained in RPMI medium 1640 (Invitrogen, cat no. 23400−021) supplemented with fetal calf serum (10%) and penicillin-streptomycin (100 units mL^{-1} and 100 mg mL^{-1}, respectively). Approximately 3 × 10^4 (for HT29) or 4 × 10^4 (for HepG2) cells per well in these media were inoculated in 96-multiwell plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere.

Photocytotoxicity Assay. Phthalocyanines **3** and **4** were first dissolved in DMF to give 1.5 mM solutions, which were diluted to 80 μM with an aqueous solution of Cremophor EL (Sigma, 4.7 g in 100 mL of water). The solutions were filtered with a 0.2 μm filter, then diluted with the culture medium to appropriate concentrations (4-fold dilutions from 0.5 μM). The cells, after being rinsed with PBS, were incubated with 100 μL of these phthalocyanine solutions for 2 h at 37 °C under 5% CO_2. The cells were then rinsed again with PBS and refed with 100 μL of the culture medium before being illuminated at ambient temperature. The light source consisted of a 300 W halogen lamp, a water tank for cooling, and a color glass filter (Newport) cut-on 610 nm. The fluence rate (λ > 610 nm) was 40 mW cm^{-2}. An illumination of 20 min led to a total fluence of 48 J cm^{-2}. Alternatively, the cells were irradiated with a diode laser (Biolitec Ceralas) at 675 nm operated at 0.2 W. An illumination on a spot size of 1.13 cm^2 for 11.3 or 45.0 s led to a total fluence of 2 or 8 J cm^{-2}.

Cell viability was determined by means of the colorimetric MTT assay.[43] After illumination, the cells were incubated at 37 °C under 5% CO_2 overnight. An MTT (Sigma) solution in PBS (3 mg mL^{-1}, 50 μL) was added to each well followed by incubation for 2 h under the same environment. A solution of sodium dodecyl sulfate (SDS, Sigma; 10% by weight, 50 μL) was then added to each well. The plate was incubated in an oven at 60 °C for 30 min, then 80 μL of *iso*-propanol was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 10 s before the absorbance at 540 nm at each well was taken. The average absorbance of the blank wells, which did not contain the cells, was subtracted from the readings of the other wells. The cell viability was then determined by the following equation: % viability = $[\sum(A_i/A_{control} \times 100)]/n$, where A_i is the absorbance of the ith data (i = 1, 2, ..., n), $A_{control}$ is the average absorbance of the control wells in which the phthalocyanine was absent, and n (= 4) is the number of the data points.

ROS Measurements. ROS production was determined by using DCFDA (Molecular Probes). Approximately 3 × 10^4 HT29 cells were placed in a 96-well plate and incubated for 24 h before photodynamic treatment. After being washed with PBS, the cells were incubated with 100 μL of a 100 μM DCFDA solution in PBS at 37 °C for 60 min. Fluorescence measurements were made in a fluorescence plate reader (TECAN Polarion) with a 485 nm excitation filter and a 535 nm emission filter set at a gain of 60.

Subcellular Localization Studies. About 6.0 × 10^4 HT29 cells in the culture medium (2 mL) were seeded on a coverslip and incubated overnight at 37 °C under 5% CO_2. The medium was then removed. For the study using LysoTracker, the cells were incubated with a solution of **3** (formulated with Cremophor EL) in the medium (8 μM, 2 mL) for 100 min under the same conditions. LysoTracker HCK-123 (Molecular Probes; 1 mM in DMSO, 4 μL) was then added, and the cells were incubated under these conditions for a further 20 min. For the study using MitoTracker, the cells were incubated with 2 μL of MitoTracker Green FM (Molecular Probes) and 2 μL of pluronic acid (both in DMSO) in the medium (2 mL) for 30 min. Then the cells were rinsed with PBS and incubated again with a solution of **3** in the medium (8 μM, 2 mL) for 2 h under the same conditions. For both cases, the cells were then rinsed with PBS and viewed with an Olympus IX 70 inverted microscope. The excitation light source (at 465, 490, and 630 nm for LysoTracker, MitoTracker, and **3**, respectively) was provided by a multiwavelength illuminator (Polychrome IV, TILL Photonics). The emitted fluorescence (at 500−575 nm for LysoTracker and MitoTracker, and >660 nm for **3**) was collected using a digital cooled CCD camera (Quantix, Photometrics). Images were digitized and analyzed using MetaFluor V.4.6 (Universal Imaging). The subcellular localization of **3** was revealed by comparing the intracellular fluorescence images caused by the LysoTracker or MitoTracker and this dye.

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Supporting Information Available: ^{1}H NMR spectra of **3** and **4** in $CDCl_3$; $^{13}C\{^{1}H\}$ NMR spectrum of **4** in $CDCl_3$ showing the aromatic region; COSY spectrum of **3** in $CDCl_3$; HPLC chromatograph of **4**; UV−vis spectra of **3** in DMF; UV−vis spectra of **3** and **4** in water; comparison of the rates of decay of DBPF sensitized by **3**, **4**, and ZnPc in DMF; comparison of the photocytotoxicities of **3** on HT29 upon illumination with a halogen lamp or a diode laser; fluorescence emission spectra of **3** and **4** in the DMEM culture medium; intracellular fluorescence image of HT29 after incubation with **3**; and visualization of intracellular fluorescence of HT29 using filter sets specific for the MitoTracker and **3** and their superimposed image. This material is available free of charge via the Internet at http://pubs.acs.org.

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