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Effects of Peripheral Chloro Substitution on the Photophysical Properties and in vitro Photodynamic Activities of Galactose-Conjugated Silicon(IV) Phthalocyanines

Pui-Chi Lo,[a] Wing-Ping Fong,[b] and Dennis K. P. Ng*[a]

A series of silicon(IV) phthalocyanines with two axial isopropylidene-protected galactose moieties and one, two, or eight chloro group(s) on the periphery of the macrocycle have been synthesised and spectroscopically characterised. The photophysical properties and in vitro photodynamic activities of these compounds have been studied and compared with those of the nonchlorinated analogue. All the compounds, with the exception of the octachlorinated counterpart which has a limited solubility, are essentially nonaggregated in N,N-dimethylformamide. The fluorescence quantum yield decreases and the singlet oxygen quantum yield increases as the number of chloro substituent increases, which is in accord with the heavy-atom effect. The non-, mono-, and dichlorinated phthalocyanines formulated with Cremophor EL are all photodynamically active against HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells with IC_{50} values ranging from 0.03 to 1.05 μM. The photocytotoxicity as well as the efficiency to generate intracellular reactive oxygen species decrease along this series because of the increase in aggregation tendency upon chloro substitution. The nonchlorinated analogue exhibits the highest potency and can target the lysosomes of HT29 cells, whereas the monochlorinated counterpart is not localised in the lysosomes.

Introduction

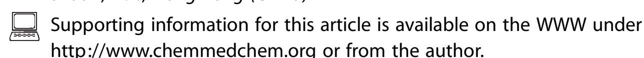
Photodynamic therapy (PDT) is a promising therapeutic modality for the treatment of a variety of premalignant and malignant diseases.[1] It utilises the combined action of three individually nontoxic components, namely a photosensitiser, light, and molecular oxygen to cause cellular and tissue damage. Singlet oxygen generated through the photosensitisation process is believed to be the major cytotoxic reactive oxygen species (ROS) responsible for the damage. As the photosensitisers play a crucial role in the overall efficacy of the treatment, optimisation of their photophysical and photobiological characteristics has become one of the major research focuses. Over the last decade, significant progress has been made in this avenue.[2]

From the perspective of molecular design, one of the logical approaches to improve the photosensitising efficiency of photosensitisers is to introduce heavy atoms to the photosensitisers. Generally, this chemical modification can promote intersystem crossing through spin-orbit coupling, leading to a higher triplet state population and singlet oxygen quantum yield. These expected effects have been demonstrated in several classes of photosensitisers including chalcogenapyrylium dyes,[3] carbocyanines,[4] porphyrins,[5] hypericins,[6] bacteriochlorins,[7] phthalocyanines,[8] and squaraines.[9] However, a shortening of the triplet lifetime has also been observed in some cases when a heavy metal centre is incorporated into some macrocyclic photosensitisers.[10] Whereas most of these studies focus on the changes in photophysical properties in organic media, relatively little is known about the actual photobiological effects of such heavy-atom substitution.[11–15] O'Shea and co-workers have recently prepared a series of BF_2-chelated tetraarylazadipyrromethenes and evaluated their in vitro photodynamic activities.[11] It has been found that introduction of two bromo substituents to the azadipyrromethene skeleton can enhance the photocytotoxicity by more than 1000 times, showing that the heavy-atom effect is also viable in cellular systems. However, such enhancement has not been observed for some tetrapyrrole-based photosensitisers including porphyrins,[12] corroles,[13] and phthalocyanines.[14] The heavy-atom substituted analogues exhibit a significantly lower in vitro photocytotoxicity. Further investigation is therefore warranted to examine the general applicability of this heavy-atom approach.

We have recently prepared a novel silicon(IV) phthalocyanine with two axial isopropylidene-protected galactose moieties (compound **1**).[16] This compound is highly potent against HepG2 human hepatocarcinoma, and HT29 and T84 human colon adenocarcinoma cells. The IC_{50} values, defined as the dye concentration required to kill 50% of the cells, are as low

[a] Dr. P.-C. Lo, Prof. D. K. P. Ng
Department of Chemistry and Centre of Novel Functional Molecules
The Chinese University of Hong Kong
Shatin, N.T., Hong Kong (China)
Fax: (+852) 2603-5057
E-mail: dkpn@cuhk.edu.hk

[b] Prof. W.-P. Fong
Department of Biochemistry and Centre of Novel Functional Molecules
The Chinese University of Hong Kong
Shatin, N.T., Hong Kong (China)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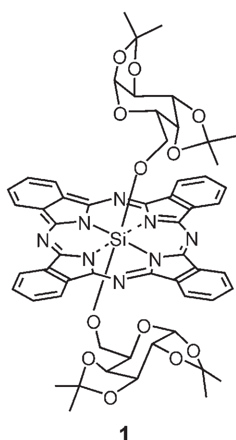


as 0.03 μM. We have therefore chosen **1** as the parent compound and introduced one, two, or eight chloro substituent(s) on the peripheral position(s) of the macrocycle. The synthesis of this novel series of chlorinated phthalocyanines as well as the effects of chloro substitution on their photophysical properties and in vitro photodynamic activities are reported herein.

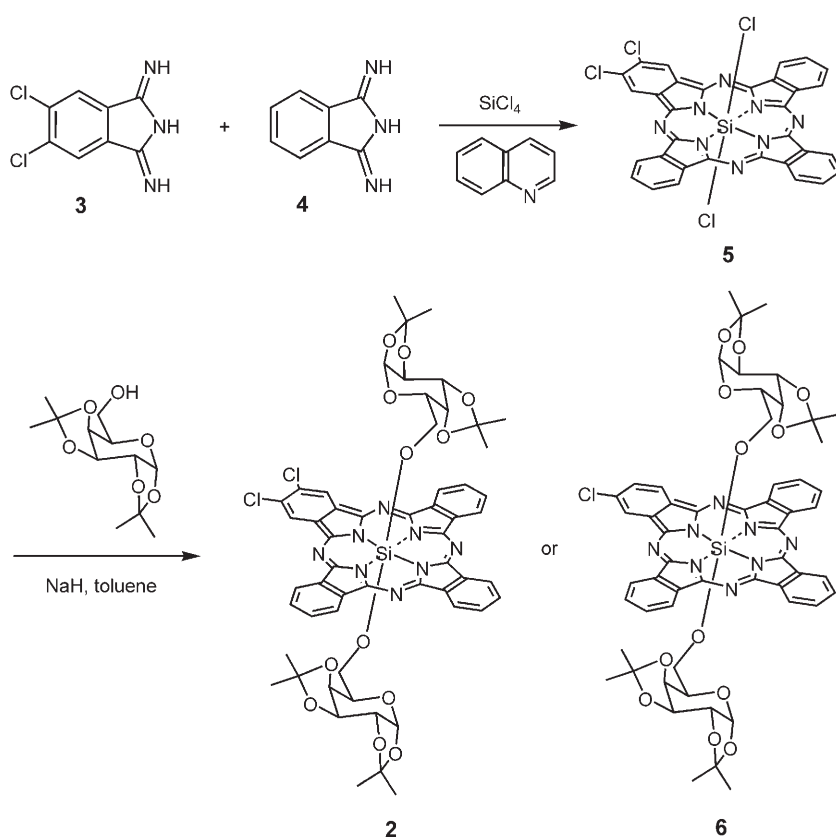
Results and Discussion

Synthesis and characterisation

We first prepared the dichloro analogue **2** according to Scheme 1. Mixed condensation of the 1,3-diiminoisoindolines **3** and **4** in the presence of $SiCl_4$ resulted in the formation of a mixture of silicon(IV) phthalocyanines which contained the desired dichloro derivative $[Si(Cl_2Pc)Cl_2]$ (**5**).[14b] After Soxhlet extraction, the crude product was treated with the protected galactose and NaH in toluene at 120 °C for two days. The galactosylated dichlorophthalocyanine $[Si(Cl_2Pc)(PGal)_2]$ (**2**) could be isolated by repeated column chromatography in 3.5 % yield based on **3**. Several mole ratios of **3** to **4** were attempted and it was found that a mole ratio of 1:4 led to a better separation of this product. Interestingly, by increasing the reaction temperature to 170 °C and extending the reaction time to four days, the monochlorinated phthalocyanine $[Si(ClPc)(PGal)_2]$ (**6**) was isolated instead in 4.3 % yield based on **3** (Scheme 1), showing that one of the chloro substituents was cleaved under a harsher condition. To the best of our knowledge, this way of preparing monochlorinated phthalocyanines is unprecedented. These two compounds represent very rare examples of A_3B-type silicon(IV) phthalocyanines, which in principle can be further functionalised through manipulation of the chloro substituent(s).

For comparison, the octachlorinated analogue **7** was also prepared by treating the silicon(IV) octachlorophthalocyanine dichloride (**8**)[14a] with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose in the presence of NaH in toluene (Scheme 2). This compound was stable under ambient conditions and could be purified by silica-gel column chromatography and recrystallisation from $EtOH/CHCl_3$.

The galactose-conjugated chlorinated phthalocyanines **2**, **6**, and **7** were fully characterised with various spectroscopic methods and elemental analysis. The ^1H NMR signals for the phthalocyanine ring protons of these compounds are very distinct and can be assigned unambiguously. As shown in Figure 1, the ^1H NMR spectrum of $[Si(Cl_8Pc)(PGal)_2]$ (**7**) in $CDCl_3$ shows a singlet at δ 9.65 for the eight phthalocyanine α ring protons. The spectrum of $[Si(Cl_2Pc)(PGal)_2]$ (**2**) shows a singlet at δ 9.68 (2 H) for the α ring protons adjacent to the chloro substituents, and two multiplets at δ 9.58–9.63 and 8.32–8.35 (6 H each) for the remaining α and β ring protons, respectively. The spectrum of $[Si(ClPc)(PGal)_2]$ (**6**) shows a virtual doublet at δ 9.58 (1 H), a doublet at δ 9.53 (1 H), and a doublet of doublet at δ 8.25 (1 H) for the ring protons near the chloro group, together with two multiplets at δ 9.59–9.65 and 8.31–8.36 (6 H each) for the remaining α and β ring protons,



Scheme 1. Preparation of di- and monochlorinated phthalocyanines **2** and **6**.

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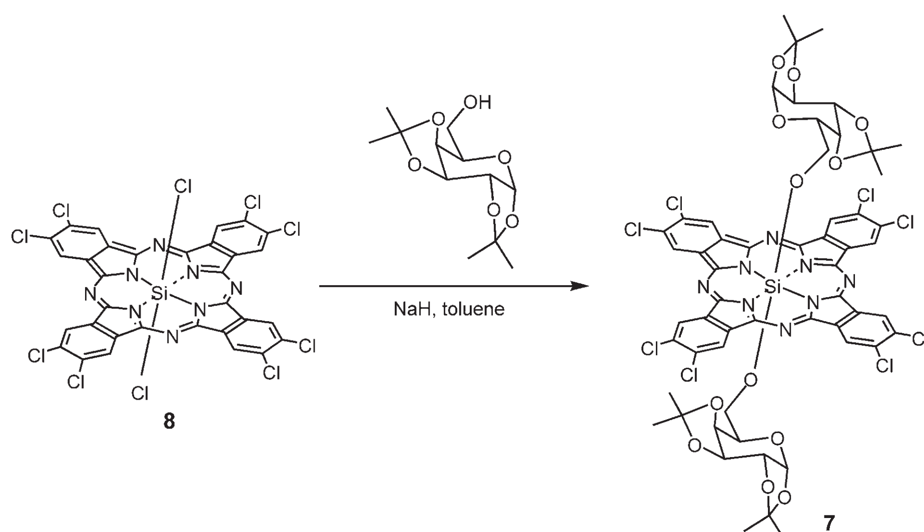

Scheme 2. Preparation of octachlorinated phthalocyanine **7**.

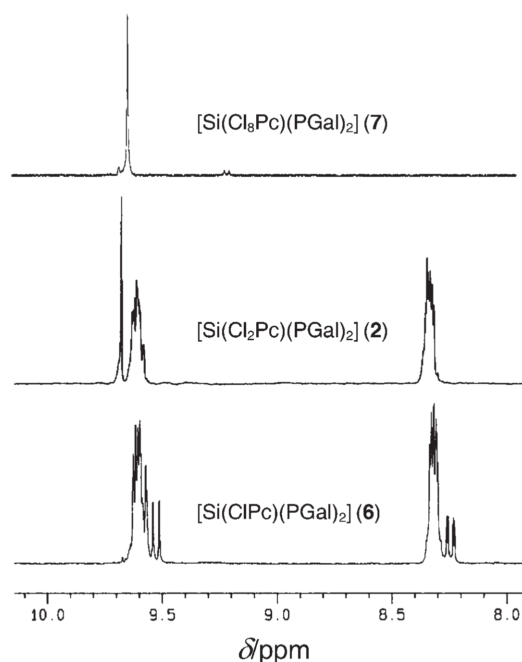

Figure 1. The aromatic region of the ^{1}H NMR spectra of the chlorinated phthalocyanines **2**, **6**, and **7** in $CDCl_3$.

respectively. As in the case of the nonchlorinated analogue **1**,[16a] the signals for the isopropylidene-protected galactose moieties are all shifted upfield by the phthalocyanine ring current, particularly those for the two C6 methylene protons at approximately δ −(1.7–1.9) and −(2.1–2.4). The assignment of these sugar signals could similarly be made with the aid of the ^{1}H-^{1}H COSY spectra.

The ^{13}C{^{1}H} NMR spectra of these compounds were also in good agreement with the proposed structures. Whereas four signals were observed in the spectrum of **7** for the phthalocyanine ring, a total of 16 peaks (two of them were overlapped) were observed in the spectrum of **2**, which is in accord with the lowering in symmetry from D_{4h} to C_{2v}. For the monochlorinated analogue **6**, many of the phthalocyanine carbon signals were overlapped. Nevertheless, the most downfield signal (at δ 148.3) in the spectrum of **7** was split into eight signals. In addition to these aromatic signals, a total of 12 signals were observed for the sugar moieties of these compounds. The assignment (see Experimental Section) was supported by the HMQC with BIRD spectra (see the spectrum of **2** given in the Supporting Information, Figure S1 as an example).

The mass spectra of phthalocyanines **2**, **6**, and **7** were also recorded. The molecular ion signals were detected in all cases with expected isotopic pattern.

Electronic absorption and photophysical properties

The electronic absorption and basic photophysical data of the mono-, di-, and octachlorinated phthalocyanines **6**, **2**, and **7** are summarised in Table 1. The data for the nonchlorinated counterpart **1** are also included for comparison. Most of the data were measured in *N*,*N*-dimethylformamide (DMF). However, because of the limited solubility of **7** in DMF, the UV-Vis spectrum was recorded in $CHCl_3$. All the compounds give typical absorption spectra of nonaggregated phthalocyanines, showing the B band at 356–357 nm, an intense and sharp Q band at 673–679 nm, together with one to two vibronic bands. For the dichlorophthalocyanine [Si(Cl_2Pc)(PGal)$_2$] (**2**), a shoulder appears at the higher energy side of the Q band as a result of its lower symmetry (Figure 2). The Q band, however, still fol-

Table 1. Electronic absorption and photophysical data for phthalocyanines **1**, **2**, **6**, and **7**.[a]

Compound	λ_{max} [nm] (log ε)	λ_{em}[nm][b]	Φ_F[c]	Φ_Δ[d]
[SiPc(PGal)$_2$] (**1**)	356 (4.88), 606 (4.59), 644 (4.54), 673 (5.40)	676	0.33	0.35
[Si(ClPc)(PGal)$_2$] (**6**)	356 (4.89), 606 (4.58), 675 (5.34)	679	0.28	0.37
[Si(Cl_2Pc)(PGal)$_2$] (**2**)	356 (4.89), 607 (4.51), 678 (5.25)	682	0.27	0.41
[Si(Cl_8Pc)(PGal)$_2$] (**7**)	357 (4.86), 611 (4.72), 649 (4.65), 679 (5.53)[e]	681	0.04	0.08

[a] Recorded in DMF unless otherwise stated. [b] Excited at 610 nm. [c] Using unsubstituted zinc(II) phthalocyanine (ZnPc) in 1-chloronaphthalene as the reference (Φ_F = 0.30). [d] Using ZnPc in DMF as the reference (Φ_Δ = 0.56). [e] Recorded in $CHCl_3$.

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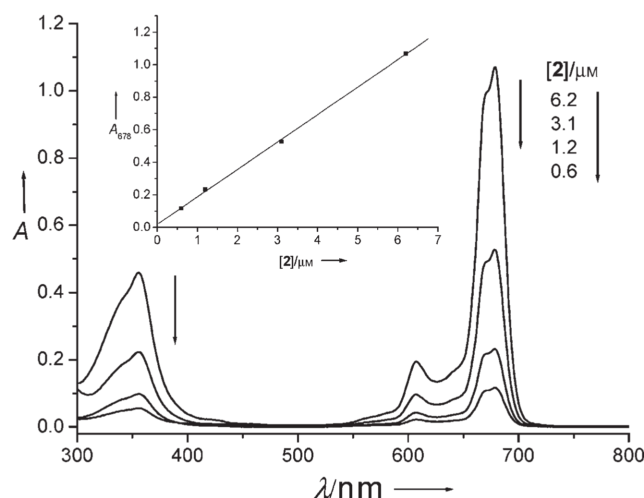

Figure 2. UV-Vis spectra of [Si(Cl_2Pc)(PGal)$_2$] (**2**) in DMF at various concentrations. The inset plots the absorbance at 678 nm versus the concentration of **2**.

lows the Lambert Beer's law showing that aggregation of this compound is not significant.

As shown in Table 1, introduction of chloro substituents slightly shifts the Q band from 673 (for **1**) to 679 (for **7**) nm, and the fluorescence emission from 676 (for **1**) to 681 (for **7**) nm. The fluorescence quantum yield (Φ_F) decreases as the number of chloro substituent increases (that is, **1** > **6** > **2** ≫ **7**) as a result of the heavy-atom effect. The very low Φ_F value of [Si(Cl_8Pc)(PGal)$_2$] (**7**) may be also due to the incomplete solvation of this compound in DMF.

To evaluate the photosensitising efficiency of these compounds, their singlet oxygen quantum yields (Φ_Δ) were determined by a steady-state method using 1,3-diphenylisobenzofuran (DPBF) as the scavenger. The change in concentration of the quencher was monitored spectroscopically at 411 nm with time, from which the values of Φ_Δ could be determined by the method described previously.[17] As shown in Table 1, the value of Φ_Δ follows the order **2** > **6** > **1** ≫ **7**. The trend (excluding **7**) is also consistent with the heavy-atom effect, which promotes the intersystem crossing leading to a higher efficiency to generate singlet oxygen. The unexpectedly low Φ_Δ value of **7** is again attributed to its poor solubility in DMF, leading to an incomplete solvation.

In vitro photodynamic activities

The photodynamic activities of the mono-, di-, and octachlorinated phthalocyanines **6**, **2**, and **7** in Cremophor EL emulsions were investigated against two different cell lines, namely HT29 human colon adenocarcinoma and HepG2 human hepatocarcinoma cells. Figure 3 shows the dose-dependent curves for compounds **2** and **6** both in the absence and presence of light. The corresponding curves for the nonchlorinated analogue **1** are also included for comparison.[16] The octachlorinated counterpart **7** has virtually no effect on these cells. It can be seen in Figure 3 that all the three compounds are essentially noncytotoxic in the absence of light, but they exhibit substantial photocytotoxicity. The corresponding IC_{50} values are summarised in Table 2. Generally, these compounds are more photocytotoxic toward HT29 than HepG2 cells. Chloro substitution has an adverse effect on the photocytotoxicity of this series of compounds. The IC_{50} value increases along the series **1** < **6** < **2**. These results are in contrast to those for the halogenated squaraines[15b] and BF_2-chelated tetraarylazadipyrromethenes,[11b] which show a higher photocytotoxicity than the nonhalogenated counterparts as a result of the heavy-atom effect.

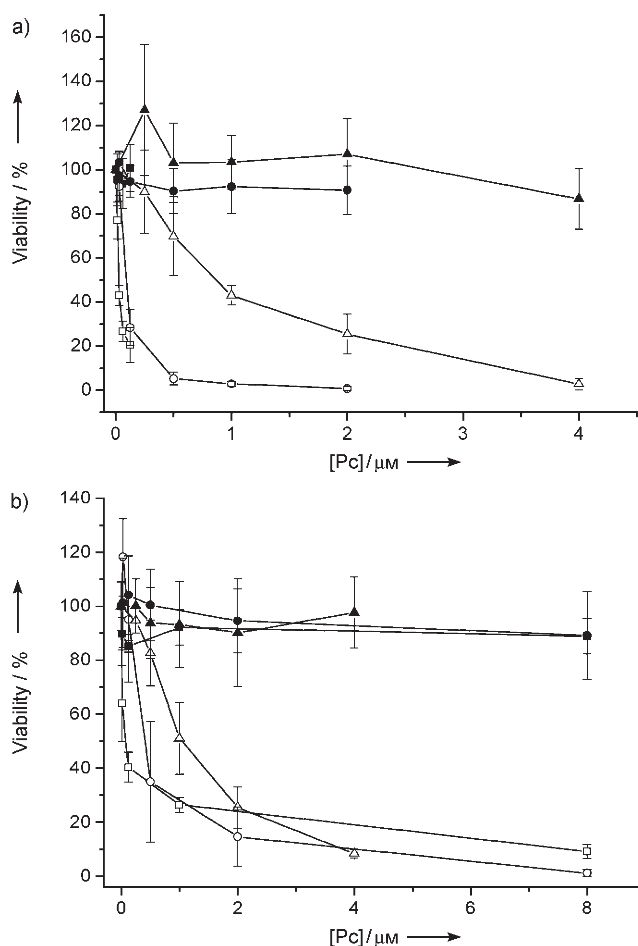

Figure 3. Effects of **1** (squares), **2** (triangles), and **6** (circles) on a) HT29 and b) HepG2 in the absence (closed symbols) and presence (open symbols) of light (λ > 610 nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean values ± SEM of three independent experiments, each performed in quadruplicate.

Table 2. Comparison of the IC_{50} values of phthalocyanines **1**, 2, and **6** against HT29 and HepG2.

Compound	For HT29 [μM]	For HepG2 [μM]
[SiPc(PGal)$_2$] (**1**)	0.03[16b]	0.10[16a]
[Si(ClPc)(PGal)$_2$] (**6**)	0.09	0.40
[Si(Cl_2Pc)(PGal)$_2$] (**2**)	0.87	1.05

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To account for the lower photodynamic activity of the chlorinated phthalocyanines, we examined their aggregation behaviour in the culture media using absorption and fluorescence spectroscopic methods. Figure 4 shows the absorption spectra

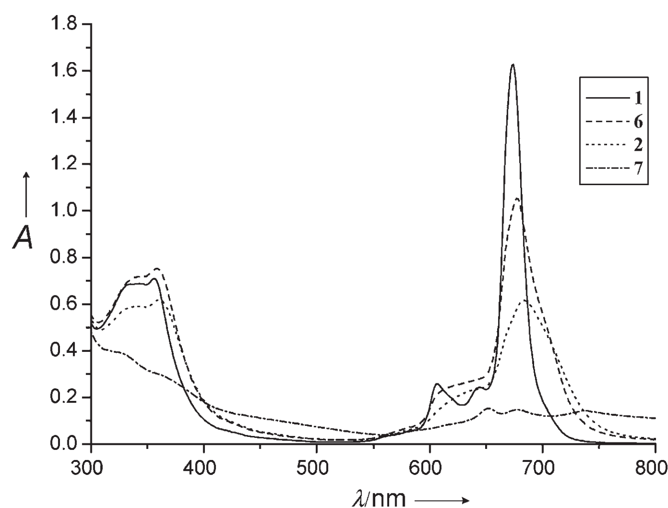

Figure 4. Electronic absorption spectra of phthalocyanines **1**, **2**, **6**, and **7**, formulated with Cremophor EL, in culture medium (DMEM, all at 8 μM).

of these compounds in DMEM (for HT29 cells). Whereas an intense and sharp Q band is retained for the nonchlorinated phthalocyanine **1** in this medium, it becomes broadened and weaker as the number of chloro substituents increases. The Q band is almost invisible for the octachlorinated analogue **7**. The intensity of the fluorescence emission also follows the order **1** > **6** > **2** > **7** (see Figure S2 in the Supporting Information). Very similar results were obtained when the spectra were recorded in the RPMI medium 1640 (for HepG2 cells). These observations strongly indicate that upon peripheral chlorination, the compounds tend to be more aggregated in the culture media, and the degree of aggregation increases with the number of chloro substituents. It is interesting to note that the introduction of even one chloro substituent will have these undesirable effects.

The intracellular production of ROS by phthalocyanines **1**, **2**, **6**, and **7** was also studied using 2′,7′-dichlorodihydrofluorescein diacetate (DCFDA) as the quencher.[18] In the absence of light, all these compounds could not generate ROS. Upon irradiation, the non-, mono-, and dichlorinated analogues **1**, **6**, and **2** could sensitise the production of ROS, and the efficiency followed the order **1** > **6** > **2** (Figure 5). The octachlorinated counterpart **7** could not generate ROS inside the cells even in the presence of light. These results are consistent with the trend in in vitro photocytotoxicity of these compounds and can again be explained by their different aggregation tendency.

The uptake of phthalocyanines **1**, **2**, and **6** by HT29 cells was also examined by fluorescence microscopy. After incubation with these compounds for 2 h and upon excitation at 630 nm, the HT29 cells showed an intracellular fluorescence as granular

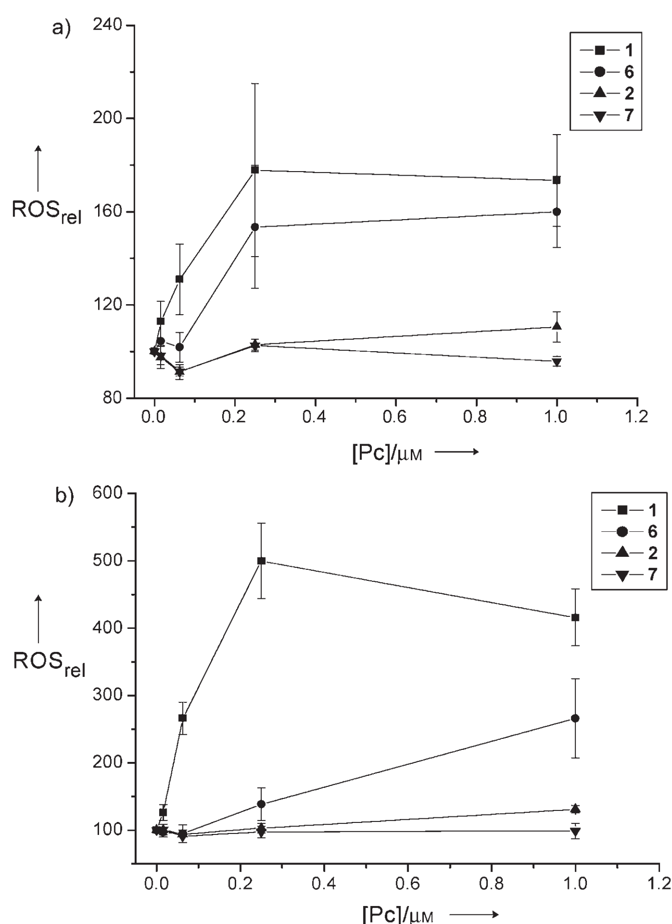

Figure 5. Relative ROS production (ROS_{rel}) sensitised by phthalocyanines **1**, **2**, **6**, and **7** in a) HT29 and b) HepG2 cells. Each data point represents the mean value ± SEM of three independent experiments, each performed in quadruplicate.

spots throughout the cytoplasm, indicating that there was a substantial uptake of the dyes. The intensity for the dichloro analogue **2** was relatively weak. To further investigate the subcellular localisation of the non- and monochlorinated analogues **1** and **6**, we stained the HT29 cells with LysoTracker HCK-123, which is a specific fluorescence dye for lysosomes, together with these dyes. As shown in Figure 6, the fluorescence caused by the LysoTracker (excited at 465 nm, monitored at 500–575 nm) can superimpose with the fluorescence caused by **1** (excited at 630 nm, monitored at > 660 nm). This observation indicates that this compound has a high and selective affinity to lysosomes, which are an important target for the initiation of apoptosis by PDT.[19] By contrast, the nonsuperimposed image for the monochlorinated analogue **6** indicates that it is not localised in the lysosomes. These results show that the peripheral chloro substituent changes not only the aggregation tendency, photocytotoxicity, and uptake of these compounds, but also their subcellular localisation property.

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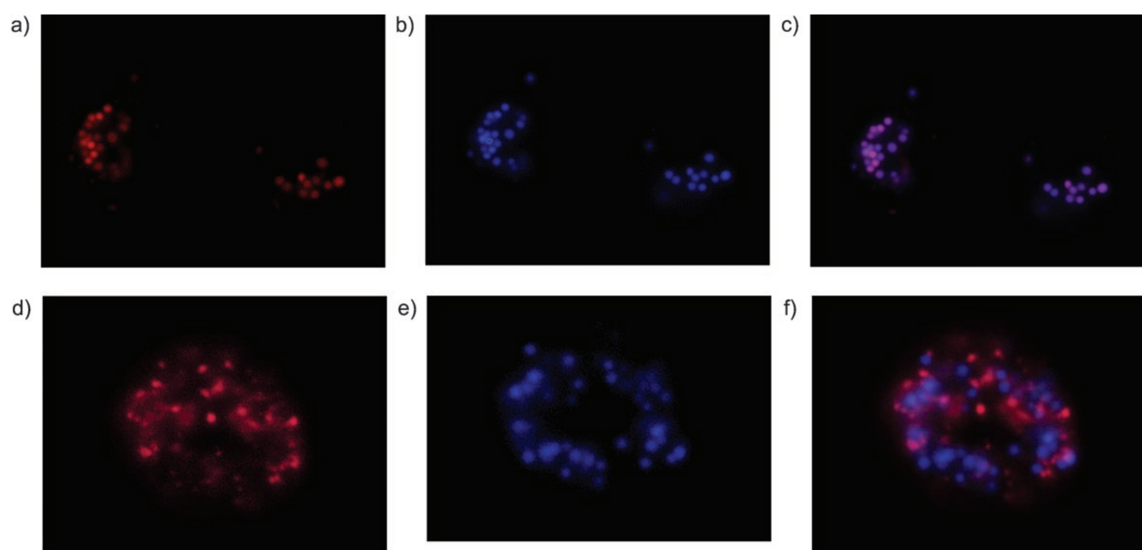

Figure 6. Visualisation of intracellular fluorescence of HT29 using filter sets specific for a) the LysoTracker (in red) and b) phthalocyanine **1** (in blue). The corresponding superimposed image is shown in (c) (in violet). The corresponding microscopic images for phthalocyanine **6** are shown in (d), (e), and (f), respectively.

Conclusions

We have prepared and characterised a series of galactose-conjugated silicon(IV) phthalocyanines substituted with one, two, or eight chloro group(s) on the periphery of the macrocycle. Their photophysical properties and in vitro photodynamic activities have been studied and compared with those of the nonchlorinated counterpart. The results show that the mono- and dichlorinated analogues **6** and **2** are also photodynamically active, but the potency is lower than that of the nonchlorinated counterpart **1** as a result of their higher aggregation tendency in the culture media. The introduction of even one chloro substituent will lead to this undesirable effect. Hence heavy-atom substitution is not a general approach to improve the efficiency of photosensitisers. For photosensitisers having a large hydrophobic core such as phthalocyanines, these substituents may exaggerate their aggregation, which outweighs the heavy-atom effect.

Experimental Section

General

All the reactions were performed under an atmosphere of nitrogen. Toluene was distilled from sodium. Quinoline was predried over anhydrous sodium sulfate and fractionally distilled from zinc dust in vacuo. Chromatographic purifications were performed on silica gel (Macherey–Nagel, 70–230 mesh) columns with the indicated eluents. All other solvents and reagents were of reagent grade and used as received. The 1,3-diiminoisoindolines **3** and **4**,[14a] and phthalocyanines **1**[16a] and **8**[14a] 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 $SiMe_4$. MALDI-TOF mass spectra were recorded on a Bruker bench TOF mass spectrometer equipped with a standard UV-laser desorption source, using α-cyano-4-hydroxycinnamic acid as the matrix. ESI mass spectra were measured on a Thermo Finnigan MAT 95 XL mass spectrometer. Elemental analyses were performed by Medac Ltd., Brunel Science Centre, UK.

UV-Vis and steady-state fluorescence spectra were taken on a Cary 5G UV-Vis-NIR spectrophotometer and a Hitachi F-4500 spectrofluorometer, respectively. Fluorescence quantum yields (Φ_F) were determined by the equation: $\Phi_{F(sample)} = (F_{sample}/F_{ref})(A_{ref}/A_{sample})(n_{sample}^2/n_{ref}^2)\Phi_{F(ref)}$,[20] for which 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 [$\Phi_{F(ref)} = 0.30$].[21] To minimise reabsorption of radiation by the ground-state species, the emission spectra were obtained in very dilute solutions of which the absorbance at 610 nm was less than 0.03. Singlet oxygen quantum yields (Φ_Δ) were measured in DMF by the method of chemical quenching of DPBF using ZnPc as the reference ($\Phi_\Delta = 0.56$).[22]

[Si(Cl$_2$Pc)Cl$_2$] (5). A mixture of dichloro 1,3-diiminoisoindoline **3** (1.46 g, 6.8 mmol), nonchlorinated 1,3-diiminoisoindoline **4** (3.88 g, 26.7 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, MeOH, and acetone. The resulting blue solid was Soxhlet extracted with a mixture of these solvents plus $CHCl_3$ for two days to give a blue solid (3.98 g), which contained the desired product **5**. The crude product was used for the following reactions without further purification.

[Si(Cl$_2$Pc)(PGal)$_2$] (2). A mixture of crude **5** prepared above (2.08 g), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (4.60 g, 17.7 mmol), and NaH (0.69 g, 28.8 mmol) in toluene (80 mL) was held at

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reflux at 120 °C for two days. After evaporating the solvent in vacuo, the residue was chromatographed using ethyl acetate/hexane (3:7) as eluent. The crude product was further purified by column chromatography using ethyl acetate/hexane (1:4) as eluent. The product was collected as a blue solid (0.14 g, 3.5% based on **3**). R_f [ethyl acetate/hexane (3:7)] = 0.70; ^{1}H NMR: δ = 9.68 (s, 2H, Pc-H$_\alpha$), 9.58–9.63 (m, 6H, Pc-H$_\alpha$), 8.32–8.35 (m, 6H, Pc-H$_\beta$), 4.39 (d, J = 4.8 Hz, 2H, H1), 3.35 (dd, J = 2.1, 4.8 Hz, 2H, H2), 3.26 (dd, J = 2.1, 8.4 Hz, 2H, H3), 0.81–0.84 (m, 2H, H4), 0.79 (s, 6H, Me), 0.71 (s, 6H, Me), 0.67–0.70 (m, 2H, H5), 0.51 (s, 6H, Me), 0.33 (s, 6H, Me), −1.70 (dd, J = 5.4, 8.4 Hz, 2H, OCH), −2.33 ppm (vt, J = 9.0 Hz, 2H, OCH); ^{13}C{^{1}H} NMR: δ = 150.6, 150.3, 149.2, 146.5, 136.3, 136.2, 136.1, 134.8, 134.7, 130.9 (two overlapping signals, Pc-C$_\beta$), 130.7 (Pc-C$_\beta$), 125.0 (Pc-C$_\alpha$), 123.8 (Pc-C$_\alpha$), 123.7 (Pc-C$_\alpha$), 123.5 (Pc-C$_\alpha$), 107.2 (Me_2C), 107.1 (Me_2C), 94.8 (C1), 69.6 (C2), 68.7 (C3), 67.2 (C4), 63.9 (C5), 52.9 (OCH_2), 25.2 (Me), 24.4 (Me), 24.2 (Me), 22.8 ppm (Me); HRMS (ESI): m/z calcd for $C_{56}H_{52}Cl_2N_8NaO_{12}Si$ $[M+Na]^+$: 1149.2743; found: 1149.2739; Anal. Calcd for $C_{56}H_{52}Cl_2N_8O_{12}Si$: C 59.63, H 4.65, N 9.93; found: C 59.92, H 4.86, N 9.52.

[Si(ClPc)(PGal)$_2$] (6). A mixture of crude **5** prepared above (1.49 g), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (4.86 g, 18.7 mmol), and NaH (1.35 g, 56.3 mmol) in toluene (80 mL) was held at reflux at 170 °C for four days. After evaporating the solvent in vacuo, the residue was chromatographed using ethyl acetate/hexane (3:7) as eluent. The crude product was further purified by column chromatography using ethyl acetate/hexane (1:4) as eluent. The product was collected as a blue solid (0.12 g, 4.3% based on **3**). R_f [ethyl acetate/hexane (3:7)] = 0.67; ^{1}H NMR: δ = 9.59–9.65 (m, 6H, Pc-H$_\alpha$), 9.58 (vd, J = 1.8 Hz, 1H, Pc-H$_\alpha$), 9.53 (d, J = 8.1 Hz, 1H, Pc-H$_\alpha$), 8.31–8.36 (m, 6H, Pc-H$_\beta$), 8.25 (dd, J = 1.8, 8.1 Hz, 1H, Pc-H$_\beta$), 4.39 (d, J = 5.1 Hz, 2H, H1), 3.34 (dd, J = 2.1, 4.8 Hz, 2H, H2), 3.24 (dd, J = 2.1, 8.1 Hz, 2H, H3), 0.79–0.82 (m, 2H, H4), 0.80 (s, 6H, Me), 0.70 (s, 6H, Me), 0.67–0.71 (m, 2H, H5), 0.51 (s, 6H, Me), 0.33 (s, 6H, Me), −1.72 to −1.67 (m, 2H, OCH), −2.35 ppm (vt, J = 9.0 Hz, 2H, OCH); ^{13}C{^{1}H} NMR: δ = 150.1, 150.0, 149.9, 149.8, 149.3, 149.2, 148.0, 147.4, 137.2, 136.6, 136.2, 134.1, 130.7, 130.6, 124.7, 123.7, 123.5, 123.3, 107.2 (Me_2C), 107.1 (Me_2C), 94.8 (C1), 69.7 (C2), 68.7 (C3), 67.2 (C4), 63.9 (C5), 52.9 (OCH_2), 25.2 (Me), 24.3 (Me), 24.2 (Me), 22.7 ppm (Me) (some of the phthalocyanine carbon signals are overlapped); HRMS (ESI): m/z calcd for $C_{56}H_{53}ClN_8NaO_{12}Si$ $[M+Na]^+$: 1115.3133; found: 1115.3147; Anal. Calcd for $C_{56}H_{53}ClN_8O_{12}Si$: C 61.50, H 4.88, N 10.24; found: C 61.44, H 5.00, N 9.65.

[Si(Cl$_8$Pc)(PGal)$_2$] (7). A mixture of [Si(Cl$_8$Pc)Cl$_2$] (**8**) (0.53 g, 0.6 mmol), 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (1.53 g, 5.9 mmol), and NaH (0.72 g, 30.0 mmol) in toluene (50 mL) was held at reflux for three days. After evaporating the solvent in vacuo, the residue was chromatographed using $CHCl_3$/EtOH (9:1) as eluent. The crude product was recrystallised by layering EtOH onto a $CHCl_3$ solution. The greenish-blue solid formed was collected by filtration and dried in vacuo (0.21 g, 26%). R_f [$CHCl_3$/EtOH (9:1)] = 0.84; ^{1}H NMR: δ = 9.65 (s, 8H, Pc-H$_\alpha$), 4.41 (d, J = 4.8 Hz, 2H, H1), 3.39–3.42 (m, 2H, H2), 3.32–3.36 (m, 2H, H3), 0.99–1.02 (m, 2H, H4), 0.76 (s, 6H, Me), 0.74 (s, 6H, Me), 0.60–0.66 (m, 2H, H5), 0.45 (s, 6H, Me), 0.42 (s, 6H, Me), −1.88 to −1.83 (m, 2H, OCH), −2.14 ppm (vt, J = 9.0 Hz, 2H, OCH); ^{13}C{^{1}H} NMR: δ = 148.3, 135.8, 135.0, 125.3 (Pc-C$_\alpha$), 107.3 (two overlapping signals, Me_2C), 94.8 (C1), 69.5 (C2), 68.9 (C3), 67.8 (C4), 64.2 (C5), 53.4 (OCH_2), 25.0 (Me), 24.6 (Me), 24.2 (Me), 23.3 ppm (Me); MS (MALDI-TOF): an isotopic cluster peaking at m/z 1357.0 $[M+Na]^+$; Anal. Calcd for $C_{56}H_{46}Cl_8N_8O_{12}Si$: C 50.39, H 3.47, N 8.39; found: C 50.95, H 3.54, N 8.16.

Cell lines and culture conditions

The HT29 human colon adenocarcinoma 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-well plates and incubated overnight at 37 °C in a humidified 5% CO_2 atmosphere.

Photocytotoxicity assays

All the phthalocyanines 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, 0.47 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. The cells, after being rinsed with phosphate buffered saline (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 re-fed 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 colour glass filter (Newport) cut-on 610 nm. The fluence rate ($\lambda >$ 610 nm) was 40 mW cm^{-2}. An illumination of 20 min led to a total fluence of 48 J cm^{-2}.

Cell viability was determined by means of the colorimetric MTT assay.[23] 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 (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 isopropanol was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 10 sec 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 equation: $\%\,\text{Viability} = [\Sigma(A_i/A_{control}\times100)]/n$, for which 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) as the quencher. Approximately 3×10^4 (for HT29) or 4×10^4 (for HepG2) cells were placed in a 96-well plate and incubated with the phthalocyanine solutions 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.

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Subcellular localisation studies

About 6×10^4 HT29 cells in the culture medium (2 mL) were seeded on a cover slip and incubated overnight at 37 °C under 5% CO_2. The medium was then removed. The cells were incubated with a solution of **1** or **6** (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. Then the cells were rinsed with PBS and viewed with an Olympus IX 70 inverted microscope. The excitation light source (at 465 and 630 nm for LysoTracker and phthalocyanine **1** or **6**, respectively) was provided by a multiwavelength illuminator (Polychrome IV, TILL Photonics). The emitted fluorescence (at 500–575 nm for LysoTracker and $>$ 660 nm for **1** or **6**) was collected using a digital cooled CCD camera (Quantix, Photometrics). Images were digitised and analysed using MetaFluor V.4.6 (Universal Imaging). The subcellular localisation of **1** and **6** was revealed by comparing the intracellular fluorescence images caused by the LysoTracker and these dyes.

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