VIP

New Amphiphilic Silicon(IV) Phthalocyanines as Efficient Photosensitizers for Photodynamic Therapy: Synthesis, Photophysical Properties, and in vitro Photodynamic Activities

Pui-Chi Lo,[a] Jian-Dong Huang,[a, b] Diana Y. Y. Cheng,[a] Elaine Y. M. Chan,[c] Wing-Ping Fong,[c] Wing-Hung Ko,[d] and Dennis K. P. Ng*[a]

Abstract: A novel series of silicon(IV) phthalocyanines substituted axially with one or two 1,3-bis(dimethylamino)-2-propoxy group(s) have been prepared by ligand substitution and alkoxy exchange reactions. Two dicationic and tetracationic phthalocyanines have also been prepared by methylation of two of these compounds. The nonionic phthalocyanines are essentially nonaggregated in common organic solvents and show a weak fluorescence emission, while the methylated derivatives are also nonaggregated, even in aqueous media, and exhibit a strong fluorescence emission. These new phthalocyanines, in particular the unsymmetrical and amphiphilic analogues, are highly potent against HepG2 human hepatocarcinoma cells and J774 mouse macrophage cells with IC_{50} values down to 0.02 μM. The photodynamic activities are related to the cellular uptake and the efficiency to generate singlet oxygen. A higher positive charge at the phthalocyanine hinders the uptake, reflected by the lower intracellular fluorescence intensity. Fluorescence microscopic studies have also revealed that the unsymmetrical phthalocyanine $SiPc[C_3H_5(NMe_2)_2O](OMe)$ (**4**) has a high and selective affinity to the mitochondria of HepG2 cells.

Keywords: fluorescence · photodynamic therapy · photophysics · photosensitizers · phthalocyanines · silicon

Introduction

Being a versatile class of functional dyes, phthalocyanines have been studied extensively over the last few decades.[1] Owing to the strong absorption in the red visible region, high efficiency to generate reactive oxygen species, such as singlet oxygen, and ease of chemical modification and formulation, this class of compounds has found to be highly promising as second-generation photosensitizers for photodynamic therapy (PDT).[2,3] A substantial number of phthalocyanines with different functionalities have been synthesized and examined for their photophysical and biological properties, among which sulfonated derivatives of zinc(II) and aluminum(III) phthalocyanines have received the most attention, because of their water solubility and ease of preparation.[3] To our knowledge, only a few phthalocyanine-based photosensitizers have been used in clinical trials so far; these include the silicon(IV) phthalocyanine Pc4, a liposomal preparation of zinc(II) phthalocyanine (CGP55847), and a mixture of sulfonated aluminum(III) phthalocyanines (Photosens).[3c]

Pc4 with the structure shown below is one of the most efficient phthalocyanine-based photosensitizers. Apart from its nonaggregated nature and the fact that the compound does not exist as structural isomers, this compound exhibits high photodynamic activities, both in vitro and in vivo, against a range of model cell lines and tumors.[4] The preparation of this compound, however, is rather tedious. It involves more than five steps from commercially available precursors, including a photolysis reaction to replace the axial methyl group with a hydroxy group.[5] The lengthy procedure somewhat hampers the chemical modification of this

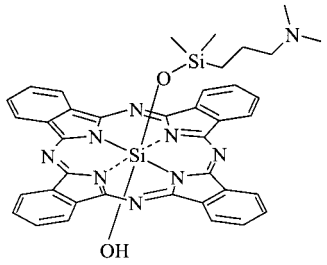
[a] P.-C. Lo, Dr. J.-D. Huang, D. Y. Y. Cheng, Prof. D. K. P. Ng
Department of Chemistry
The Chinese University of Hong Kong
Shatin, N.T., Hong Kong (China)
Fax: (+852) 2603-5057
E-mail: dkpn@cuhk.edu.hk

[b] Dr. J.-D. Huang
Present address:
Institute of Research on Functional Materials
Department of Chemistry, Fuzhou University
Fuzhou 350002 (China)

[c] E. Y. M. Chan, Prof. W.-P. Fong
Department of Biochemistry
The Chinese University of Hong Kong
Shatin, N.T., Hong Kong (China)

[d] Prof. W.-H. Ko
Department of Physiology
The Chinese University of Hong Kong
Shatin, N.T., Hong Kong (China)

 DOI: 10.1002/chem.200400462



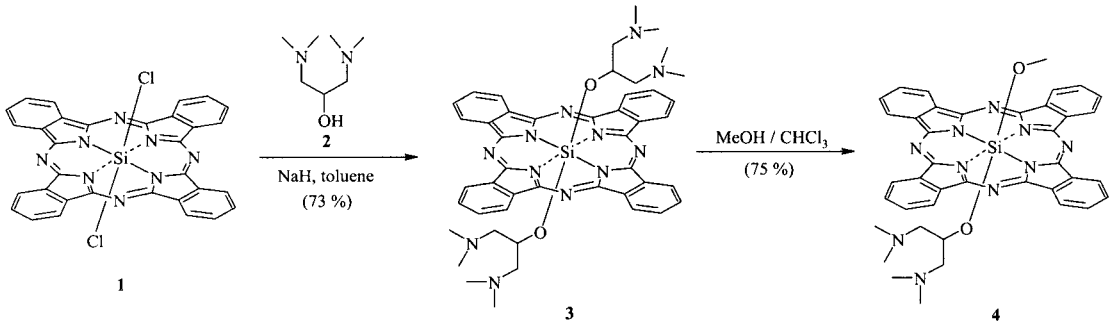
Pc4

compound which is important to reveal the structure-activity relationships.[6] We describe herein an unexpected yet facile synthesis of a new series of amphiphilic silicon(IV) phthalocyanines. These compounds are highly photoactive in in vitro assays with a high cellular uptake. The results suggest that these readily accessible compounds may form a new series of efficient phthalocyanine-based photosensitizers.

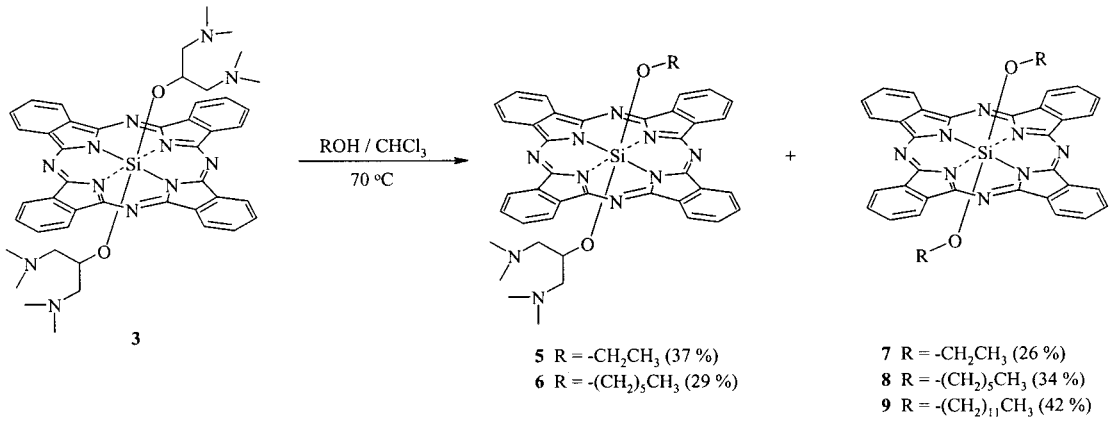
Results and Discussion

Synthesis and characterization: Treatment of the commercially available silicon phthalocyanine dichloride (**1**) with 1,3-bis(dimethylamino)-2-propanol (**2**) in the presence of NaH in toluene gave the disubstituted product **3** in 73% yield (Scheme 1). Purification of this compound on silica gel columns led to partial decomposition, probably due to the slightly acidic nature of the silica gel. The compound, however, could be purified by column chromatography by using basic alumina. Interestingly, upon further purification by recrystallization in a mixture of MeOH and $CHCl_3$, one of the 1,3-bis(dimethylamino)-2-propoxy groups was replaced by a methoxy group giving the unsymmetrical phthalocyanine **4** in high yield. It is likely that the bulky 1,3-bis(dimethylamino)-2-propoxy group makes the compound susceptible to ligand-exchange reactions. It is worth mentioning that axially substituted silicon(IV) phthalocyanines are usually prepared by substitution reactions of **1** or the dihydroxy analogue $SiPc(OH)_2$.[7] Such ligand-exchange reactions remain extremely rare.[8]

To study the generality of this reaction, we replaced methanol with ethanol and 1-hexanol during the recrystallization of **3**. It was found that compound **3** remained intact in both cases. However, by stirring a solution of **3** in $CHCl_3$ in the presence of ethanol, 1-hexanol, or 1-dodecanol at 70 °C for two days, one or both of the 1,3-bis(dimethylamino)-2-propoxy group(s) could be replaced by the corresponding alkoxy groups, giving the monosubstituted products **5** and **6** and the disubstituted products **7**–**9** (Scheme 2), which could be separated by column chromatography. Apparently, a longer alcohol promoted disubstitution, with the monosubstituted product only as a minor product. The monosubstituted 1-dodecoxy analogues of **5** and **6** could not be isolated from the reaction mixture. The reasons accounting for the different reactivities of these alcohols remain elusive at this stage.

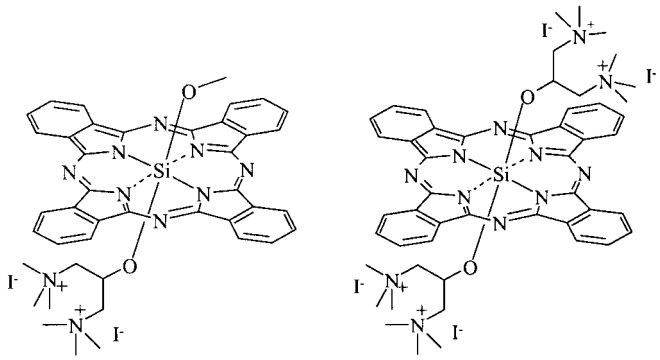


Scheme 1. Preparation of phthalocyanines **3** and **4**.



Scheme 2. Preparation of phthalocyanines **5**–**9**.

To increase the hydrophilicity and to examine the effects of charge on the photodynamic activities, methylation was performed on compounds **3** and **4**. Treatment of **4** with a large excess of iodomethane in $CHCl_3$ at room temperature for three days gave the dicationic phthalocyanine **10** (in 68% yield), which could be separated readily from the reaction mixture by filtration. Reaction of **3** with iodomethane under the same conditions led to incomplete methylation as shown by ^{1}H NMR spectroscopy. The desired tetracationic phthalocyanine **11**, however, could be prepared in 45% yield by increasing the temperature (at reflux) and shortening the reaction time (to 1 h).[9]



10 **11**

All the new compounds were characterized with various spectroscopic methods and elemental analysis (or accurate mass measurement for **6** and **8**). The ^{1}H NMR spectra of all these compounds showed two typical downfield ($\delta = 8$–10 ppm) AA′BB′ multiplets for the α and β protons of the phthalocyanine ring. The 1,3-bis(dimethylamino)-2-propoxy group resonated as a singlet (for the methyl protons), two doublets of doublets (for the two sets of diastereotopic CH_2 protons), and a quintet (for the OCH proton). These signals appeared at very upfield positions (down to $\delta = -3$ ppm) due to the shielding effect of the phthalocyanine ring. Similarly, the alkoxy protons of compounds **4**–**10** were also shifted upfield.

Compounds **3** and **8** were also characterized structurally by X-ray diffraction analysis. Single crystals of **3** were obtained by slow diffusion of diethyl ether into a CH_2Cl_2 solution of **3**, while those of **8** were obtained by slow evaporation of a $CDCl_3$ solution of **8**. Figure 1a shows a perspective view of the molecular structure of **3**, which contains an inversion center (at the silicon atom) relating the two halves of the molecule. The silicon atom is hexacoordinate with a Si–O bond length of 1.703(2) Å and an average Si–N bond length of 1.921(2) Å. The structural parameters of this molecule are not unusual. Compound **8** crystallizes in the triclinic system with one molecule per unit cell. The molecular structure (Figure 1b) also contains an inversion center. The Si–O (1.703(2) Å) and average Si–N (1.931(2) Å) bond lengths together with other structural features closely resemble those of **3**.

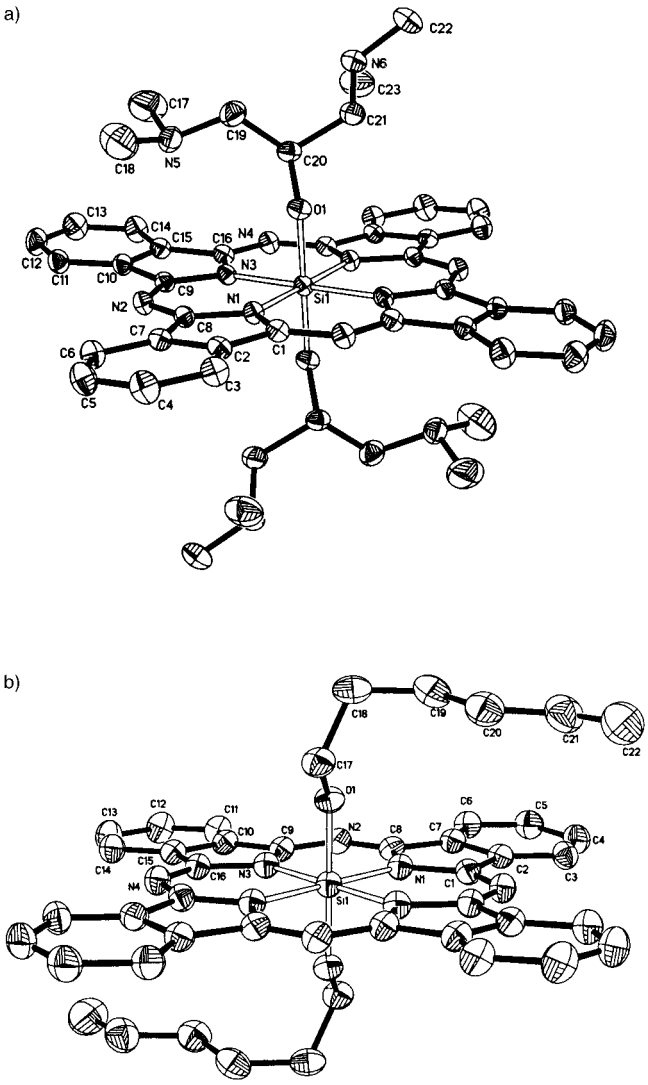


Figure 1. Molecular structure of a) **3** and b) **8** showing the 30% probability thermal ellipsoids for all non-hydrogen atoms.

Electronic absorption and photophysical properties: The data for the new phthalocyanines **3**–**6**, **10**, and **11** measured in DMF are summarized in Table 1. The absorption spectra

Table 1. Electronic absorption and photophysical data for **3**–**6**, **10**, and **11** in DMF.

Compound	λ_{max} [nm] (log ε)	λ_{em} [nm][a]	Φ_F[b]	Φ_Δ[c]
3	353 (4.68), 608 (4.39), 645 (4.33), 677 (5.19)	677	0.02	0.25
4	354 (4.98), 607 (4.68), 644 (4.61), 676 (5.48)	677	0.08	0.26
5	355 (5.37), 607 (5.08), 643 (5.00), 675 (5.85)	676	0.06	0.35
6	354 (4.82), 607 (4.54), 643 (4.47), 675 (5.32)	678	0.09	0.39
10	356 (5.02), 612 (4.75), 682 (5.36)	690	0.48	0.81
11	329 (4.72), 604 (4.18), 670 (4.94)	683	0.64	0.26

[a] Excited at 610 nm. [b] Using unsubstituted zinc(II) phthalocyanine (ZnPc) in 1-chloronaphthalene as the reference (fluorescence quantum yield (Φ_F) = 0.30. [c] Using ZnPc as the reference (singlet oxygen quantum yield (Φ_Δ) = 0.56 in DMF).

of **3–6** in DMF are typical for nonaggregated phthalocyanines, with a B-band (or Soret band) at 353–355 nm, an intense and sharp Q-band at 675–677 nm, together with two vibronic bands at 607–608 and 643–645 nm. The absorption positions of all these compounds are very similar, indicating that the phthalocyanine π system is not perturbed by the axial substituents. Upon excitation at 610 nm, these compounds have a weak fluorescence emission at 676–678 nm. It is likely that the amino groups in these compounds reductively quench the singlet excited state,[10] leading to a low fluorescence quantum yield ($\Phi_F = 0.02$–0.09). The excitation spectra of these compounds were also recorded (monitored at the corresponding emission maximum). The close similarity of these spectra with the absorption spectra confirms that the emission bands are due to the phthalocyanines. Methylation slightly shifts the Q band and the fluorescence emission to the red and greatly increases the fluorescence quantum yield as shown in Table 1.

Figure 2 shows the absorption spectra of the tetracationic phthalocyanine **11** in water in different concentrations. A B-band at 353 nm, a strong and sharp Q-band at 692 nm, together with a vibronic band at 624 nm are also observed. The inset shows a plot of the Q-band absorbance versus the concentration of **11**. The linear relationship shows that this absorption obeys the Lambert–Beer law, and the compound is essentially free from aggregation in aqueous media. This is corroborated with the strong fluorescence emission at 697 nm in water ($\Phi_F = 0.30$). The dicationic analogue **10** has similar spectral features in water, with absorption maxima at 353, 619, and 690 nm, and an emission band at 696 nm with a fluorescence quantum yield of 0.31. This nonaggregated property is extremely important for photosensitization, as aggregation provides an efficient nonradiative energy relaxation pathway, greatly shortening the excited state lifetimes.[11] Due to the very high aggregation tendency of the hydrophobic phthalocyanine π systems, fluorescence is rarely observed for these macrocycles in aqueous media.[12]

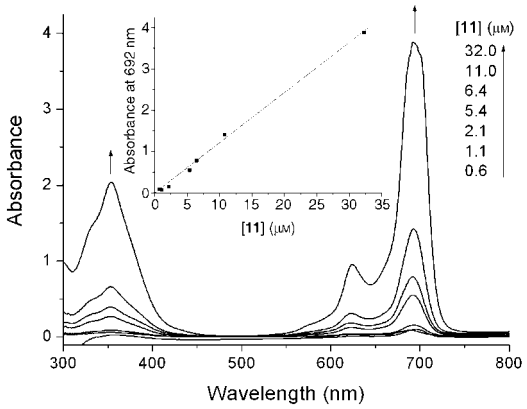


Figure 2. Electronic absorption spectra of **11** in water in different concentrations. The inset plots the absorbance at 692 nm versus the concentration of **11**.

To evaluate the photosensitizing efficiency of these compounds, their singlet oxygen quantum yields (Φ_Δ) were determined by a steady-state method with 1,3-diphenylisobenzofuran (DPBF) as the scavenger. The concentration of the quencher was monitored spectroscopically at 411 nm against time, from which the values of Φ_Δ could be determined by the method described by Wöhrle et al.[13] As shown in Table 1, all the phthalocyanines are efficient singlet oxygen generators and the value of Φ_Δ follows the order $\mathbf{3} \approx \mathbf{4} \approx \mathbf{11} < \mathbf{5} < \mathbf{6} \ll \mathbf{10}$. The singlet oxygen quantum yield of the dicationic derivative **10** is significantly higher than those of the others.

In vitro photodynamic activities: The photodynamic activities of compounds **3–6**, **10**, and **11** in Cremophor EL emulsions were investigated against two different cell lines, namely human hepatocellular carcinoma HepG2 and murine macrophage J774. The IC_{50} values defined as the dye concentration required to kill 50% of the cells are summarized in Table 2. Figure 3, which shows the effects of **5** on HepG2, is a typical dose-dependent survival curve for all these phthalocyanines. While these compounds at concentrations lower than 1 μM are essentially noncytotoxic in the absence of light (except **6** on J774, for which the viability drops to ca. 80% at 1 μM of **6**), they exhibit a very high photocytotoxicity, particularly the unsymmetrical and amphiphilic phthalocyanines **4–6** and **10**, of which the IC_{50} values are down to 0.02 μM (Table 2). The length of the alkoxy groups for compounds **4–6** does not have significant effects on the photo-activities toward both HepG2 and J774. Figure 4 compares the cytotoxicities of the amphiphilic dicationic phthalocyanine **10** and the symmetrical tetracationic phthalocyanine **11** against HepG2. It is clear that the

Table 2. Comparison of the IC_{50} values of phthalocyanines **3–6**, **10**, and **11** against HepG2 and J774.

Compound	IC_{50} (μM)[a]	
	for HepG2	for J774
3	0.70	0.09
4	0.06	0.02
5	0.05	0.02
6	0.06	0.04
10	0.09	0.36
11	2.31	1.56

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

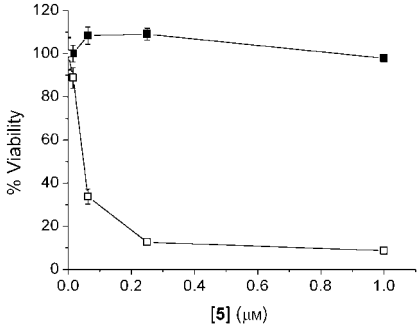


Figure 3. Effects of **5** on HepG2 in the absence (■) and presence (□) of light. For the latter, the cells were illuminated with a red light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean ± SD ($n = 4$).

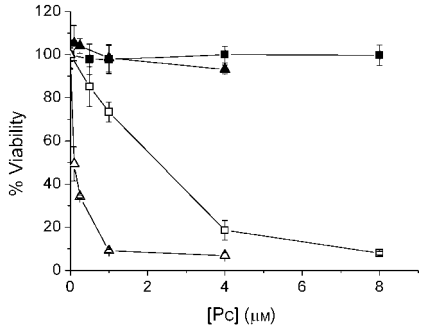


Figure 4. Effects of **10** (triangles) and **11** (squares) on HepG2 in the absence (closed symbols) and presence (open symbols) of light. For the latter, the cells were illuminated with a red light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean ± SD ($n = 4$).

amphiphilic analogue exhibits a much higher photocytotoxicity, which is as good as those of the unsymmetrical neutral counterparts **4**–**6**. A higher charge at the photosensitizer does not enhance the photocytotoxicity. These results are in accord with the previous finding that amphiphilic photosensitizers are usually more photodynamically active.[2b] It is believed that amphiphilicity can facilitate the localization of photosensitizers in the hydrophobic–hydrophilic interfaces in membranes and on the surface of proteins, and change their aggregation state and eventually their photosensitizing efficiency.

To reveal whether the different photoactivities of these compounds are related to the cellular uptake, we employed fluorescence microscopy to quantify the cellular uptake of compounds **3**, **4**, **10**, and **11**. The HepG2 cells were incubated with 8 μM of these compounds in Cremophor EL emulsions respectively on coverslips at 37 °C and 5 % CO_2 for 2 h. The cells were rinsed with phosphate buffered saline (PBS) before being viewed with an inverted microscope. Figure 5a shows a typical fluorescence microscopic image of HepG2 cells after incubation with these compounds. It is clear that the dyes enter into the cells causing a substantial fluorescence in the cytoplasm. The average relative fluorescence intensity per cell (from six independent experiments) of these compounds was determined and is depicted in Figure 5b. It can be seen that compound **4** has the highest uptake and a higher positive charge reduces the cellular uptake. The observed trend for the photocytotoxicity of compounds **3**, **4**, and **11** (**4** > **3** > **11** as shown in Table 2),

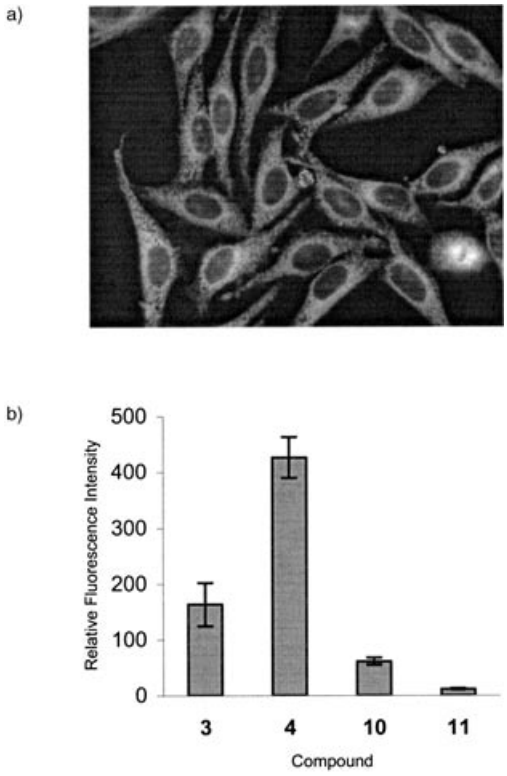


Figure 5. a) Visualization of intracellular fluorescence of HepG2 after incubation with **4** for 2 h. b) Comparison of the relative intracellular fluorescence intensity for **3**, **4**, **10**, and **11**. Data are expressed as mean ± SD ($n = 6$).

which have almost the same singlet oxygen quantum yield (0.25–0.26), is in good agreement with the trend in uptake. The very high photocytotoxicity of compound **10** can be attributed to the high singlet oxygen quantum yield (0.81), even though the uptake of this compound is not particularly high. Thus the different photocytotoxicities of these compounds may be rationalized by the differences in cellular uptake and the efficiency to generate singlet oxygen, which are two important factors determining the overall PDT efficacy.

To further reveal the subcellular localization, we stained the HepG2 cells with MitoTracker Green FM, which is a specific fluorescence dye for mitochondria, prior to the treatment with **4**. This phthalocyanine was selected for the study because it has the highest uptake. Figure 6 clearly

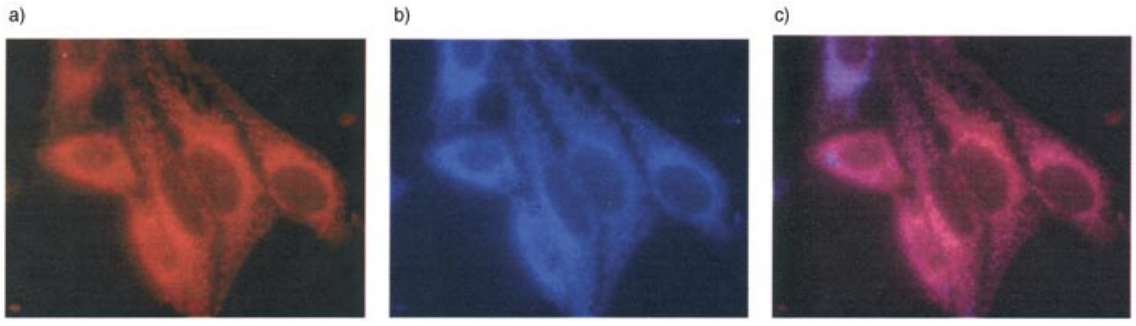


Figure 6. Visualization of intracellular fluorescence of HepG2 using filter sets specific for a) the MitoTracker (in red) and b) phthalocyanine **4** (in blue). c) The corresponding superimposed image in violet.

shows that the fluorescence caused by the MitoTracker (excited at 490 nm, monitored at 500–575 nm) can superimpose with the fluorescence caused by **4** (excited at 630 nm, monitored at >660 nm). This observation indicates that compound **4** can target mitochondria. This property is of particular importance as mitochondria are commonly believed to be the targets for the initiation of apoptosis by PDT.[2c] The detailed mechanism of the PDT action of these compounds is under investigation.

Conclusion

We have prepared and characterized a novel series of silicon(IV) phthalocyanines substituted axially with one or two 1,3-bis(dimethylamino)-2-propoxy group(s) as well as the methylated derivatives of two of these compounds. The unsymmetrical amphiphilic analogues exhibit an extremely high photocytotoxicity against human hepatocellular carcinoma HepG2 and murine macrophage J774, which can be attributed to the high cellular uptake and/or efficiency to generate singlet oxygen. Compound **4** has a high and selective affinity to mitochondria of HepG2 as revealed by fluorescence microscopy. The results show that these compounds, which have a well-defined structure, good photophysical properties, and high photodynamic activity, are potentially useful as a new generation of phthalocyanine-based photosensitizers.

Experimental Section

General: All the reactions were performed under an atmosphere of nitrogen. Toluene was distilled from sodium. Chromatographic purifications were performed on basic alumina columns (Merck, 70–230 mesh ASTM) with the indicated eluents. All other solvents and reagents were of reagent grade and used as received.

^{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 $CDCl_3$ or [D_6]DMSO. Spectra were referenced internally by using the residual solvent resonances ($\delta=$ 7.26 ppm for $CDCl_3$, $\delta=2.49$ ppm for [D_6]DMSO) relative to $SiMe_4$. Fast atom bombardment (FAB) mass spectra were measured on a Thermo Finnigan MAT 95 XL mass spectrometer. Elemental analyses were performed by Medac, Brunel Science Centre (UK) and the Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences (China).

UV-visible and steady-state fluorescence spectra were taken on a Cary 5G UV-Vis-NIR spectrophotometer and a Hitachi F-4500 spectrofluorometer, respectively. The fluorescence quantum yields were determined by the equation: $\Phi_{F(sample)}=(F_{sample}/F_{ref})(A_{ref}/A_{sample})(n_{sample}^2/n_{ref}^2)\Phi_{F(ref)}$,[14] in 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. ZnPc in 1-chloronaphthalene was used as the reference [$\Phi_{F(ref)}=0.30$].[15] To minimize re-absorption 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. Singlet oxygen quantum yields (Φ_Δ) were measured by the method of chemical quenching of DPBF described by Wöhrle et al.,[13] except that the light intensity of our system was not determined. All measurements were performed in DMF and referenced to ZnPc ($\Phi_\Delta=0.56$).

Preparation of SiPc[$C_3H_5(NMe_2)_2O$]$_2$ (3): A mixture of $SiPcCl_2$ (**1**) (2.0 g, 3.3 mmol), 1,3-bis(dimethylamino)-2-propanol (**2**) (1.61 g, 11.0 mmol), and NaH (0.53 g, 22.1 mmol) in toluene (50 mL) was refluxed for 2 days. After evaporating the solvent in vacuo, the residue was subjected to column chromatography using $CHCl_3$/EtOH (9:1) as eluent. The product was further purified by recrystallization from $CHCl_3$/EtOH (1:10) to afford shiny purple microcrystals (2.0 g, 73%). ^{1}H NMR ($CDCl_3$): $\delta=$ 9.59–9.65 (m, 8H; Pc-H$_\alpha$), 8.27–8.32 (m, 8H; Pc-H$_\beta$), 0.48 (s, 24H; NMe), -0.80 (dd, $J=5.1$, 12.6 Hz, 4H; CH_2), -1.56 (dd, $J=5.1$, 12.6 Hz, 4H; CH_2), -2.72 ppm (quintet, $J=5.1$ Hz, 2H; CH); ^{13}C{^{1}H} NMR ($CDCl_3$): $\delta=149.5$, 136.2, 130.5 (Pc-C$_\beta$), 123.3 (Pc-C$_\beta$), 61.4 (two overlapping signals; CH and CH_2), 44.9 ppm (NMe; as supported by HMQC with BIRD); HRMS (FAB): m/z calcd for $C_{46}H_{51}N_{12}O_2Si$ [M+H]$^+$: 831.4027; found: 831.4073; elemental analysis calcd (%) for $C_{46}H_{50}N_{12}O_2Si$: C 66.48, H 6.06, N 20.22; found: C 65.89, H 5.99, N 19.80.

Preparation of SiPc[$C_3H_5(NMe_2)_2O$](OMe) (4): MeOH was slowly added to the top of a solution of **3** (1.42 g, 1.71 mmol) in $CHCl_3$. The bilayer system was left under ambient conditions for 3 days. The precipitate formed was filtered off and subjected to chromatography with $CHCl_3$/hexane (1:2) as eluent to give a greenish blue solid (0.92 g, 75%). R_f ($CHCl_3$/hexane 1:2) = 0.86; ^{1}H NMR ($CDCl_3$): $\delta=$ 9.60–9.66 (m, 8H; Pc-H$_\alpha$), 8.29–8.36 (m, 8H; Pc-H$_\beta$), 0.47 (s, 12H; NMe), -0.78 (dd, $J=5.4$, 12.6 Hz, 2H; CH_2), -1.57 (dd, $J=5.4$, 12.6 Hz, 2H; CH_2), -1.78 (s, 3H; OMe), -2.71 ppm (quintet, $J=5.4$ Hz, 1H; CH); ^{13}C{^{1}H} NMR ($CDCl_3$): $\delta=149.4$, 136.2, 130.6, 123.5, 61.7 (two overlapping signals), 45.0, 43.0 ppm; HRMS (FAB): m/z calcd for $C_{40}H_{37}N_{10}O_2Si$ [M+H]$^+$: 717.2870; found: 717.2846; elemental analysis calcd (%) for $C_{40}H_{36}N_{10}O_2Si$: C 67.02, H 5.06, N 19.54; found: C 66.03, H 4.99, N 19.06.

Preparation of SiPc[$C_3H_5(NMe_2)_2O$](OEt) (5) and SiPc(OEt)$_2$ (7): A mixture of **3** (0.40 g, 0.48 mmol) and EtOH (1 mL) in $CHCl_3$ (20 mL) was heated at 70°C for 2 days. The volatiles were rotary evaporated; then the residue was subjected to column chromatography with CH_2Cl_2/hexane (3:1) as eluent. Two green bands were developed and collected to give compounds **7** (0.08 g, 26%) and **5** (0.13 g, 37%), respectively. The latter was further purified by recrystallization from $CHCl_3$/EtOH (1:10) to afford shiny greenish blue microcrystals.

Data for compound 5: R_f (CH_2Cl_2/hexane 3:1) = 0.79; ^{1}H NMR ($CDCl_3$): $\delta=$ 9.60–9.65 (m, 8H; Pc-H$_\alpha$), 8.29–8.34 (m, 8H; Pc-H$_\beta$), 0.47 (s, 12H; NMe), -0.80 (dd, $J=5.4$, 12.6 Hz, 2H; NCH_2), -1.59 (dd, $J=5.4$, 12.6 Hz, 2H; NCH_2), -2.06 to -1.92 (m, 5H; OEt), -2.74 ppm (quintet, $J=5.4$ Hz, 1H; CH); ^{13}C{^{1}H} NMR ($CDCl_3$): $\delta=149.4$, 136.2, 130.5, 123.5, 61.6, 61.5, 50.2, 45.0, 15.4 ppm; HRMS (FAB): m/z calcd for $C_{41}H_{39}N_{10}O_2Si$ [M+H]$^+$: 731.3026; found: 731.3030; elemental analysis calcd (%) for $C_{41}H_{38}N_{10}O_2Si$: C 67.38, H 5.24, N 19.16; found: C 66.84, H 5.46, N 18.73.

Data for compound 7:[16] ^{1}H NMR ($CDCl_3$): $\delta=$ 9.61–9.65 (m, 8H; Pc-H$_\alpha$), 8.32–8.36 (m, 8H; Pc-H$_\beta$), -2.05 to -1.99 ppm (m, 10H; OEt); HRMS (FAB): m/z calcd for $C_{36}H_{26}N_8O_2Si$ [M]$^+$: 630.1948; found: 630.1940.

Preparation of SiPc[$C_3H_5(NMe_2)_2O$](OC_6H_{13}) (6) and SiPc(OC_6H_{13})$_2$ (8): A mixture of **3** (0.40 g, 0.48 mmol) and 1-hexanol (3 mL) in $CHCl_3$ (20 mL) was heated at 70°C for 2 days. The volatiles were removed in vacuo; then the residue was subjected to chromatography with CH_2Cl_2 as eluent. Two green bands were developed and collected separately to give compounds **8** (0.12 g, 34%) and **6** (0.11 g, 29%), respectively. The latter was further purified by recrystallization from $CHCl_3$/EtOH (1:10) to afford a green solid.

Data for compound 6: R_f (CH_2Cl_2) = 0.80; ^{1}H NMR ($CDCl_3$): $\delta=$ 9.60–9.65 (m, 8H; Pc-H$_\alpha$), 8.28–8.34 (m, 8H; Pc-H$_\beta$), 0.47 (s, 12H; NMe), 0.18–0.23 (m, 5H; CH_2CH_3), -0.52 to -0.42 (m, 2H; CH_2), -0.80 (dd, $J=5.4$, 12.6 Hz, 2H; NCH_2), -1.48 to -1.38 (m, 2H; CH_2), -1.58 (dd, $J=5.4$, 12.6 Hz, 2H; NCH_2), -1.70 (quintet, $J=7.2$ Hz, 2H; CH_2), -2.09 (t, $J=6.3$ Hz, 2H; OCH_2), -2.73 ppm (quintet, $J=5.4$ Hz, 1H; CH); ^{13}C{^{1}H} NMR ($CDCl_3$): $\delta=149.4$, 136.3, 130.5, 123.4, 61.6 (two overlapping signals), 54.6, 45.0, 29.9, 29.1, 23.3, 21.8, 13.3 ppm; HRMS (FAB): m/z calcd for $C_{45}H_{47}N_{10}O_2Si$ [M+H]$^+$: 787.3652; found: 787.3661.

Data for compound 8: ^{1}H NMR ($CDCl_3$): $\delta=$ 9.61–9.66 (m, 8H; Pc-H$_\alpha$), 8.30–8.35 (m, 8H; Pc-H$_\beta$), 0.16–0.22 (m, 10H; CH_2CH_3), -0.48 (quintet, $J=7.5$ Hz, 4H; CH_2), -1.43 (quintet, $J=7.5$ Hz, 4H; CH_2), -1.69 (quintet, $J=6.6$ Hz, 4H; CH_2), -2.11 ppm (t, $J=6.3$ Hz, 4H; OCH_2); HRMS (FAB): m/z calcd for $C_{44}H_{42}N_8O_2Si$ [M]$^+$: 742.3200; found: 742.3192.

Preparation of SiPc($OC_{12}H_{25}$)$_2$ (9):[17] A mixture of **3** (0.40 g, 0.48 mmol) and 1-dodecanol (3 mL) in $CHCl_3$ (20 mL) was heated at 70°C for 2 days. The mixture was then poured into MeOH (150 mL) and filtered. The blue solid obtained was further purified by recrystallization from

$CHCl_3$/EtOH (1:10) (0.18 g, 42%). ^1H NMR ($CDCl_3$): $\delta = 9.60–9.65$ (m, 8H; Pc-H_α), 8.30–8.35 (m, 8H; Pc-H_β), 1.11–1.32 (m, 12H; CH_2), 0.99–1.09 (m, 4H; CH_2), 0.90 (t, $J = 7.2$ Hz, 6H; CH_3), 0.79–0.89 (m, 4H; CH_2), 0.51 (quintet, $J = 7.5$ Hz, 4H; CH_2), 0.16 (quintet, $J = 7.5$ Hz, 4H; CH_2), −0.48 (quintet, $J = 7.5$ Hz, 4H; CH_2), −1.45 (quintet, $J = 7.5$ Hz, 4H; CH_2), −1.69 (quintet, $J = 6.6$ Hz, 4H; CH_2), −2.11 ppm (t, $J = 6.3$ Hz, 4H; OCH_2); HRMS (FAB): m/z calcd for $C_{56}H_{66}N_8O_2Si$ $[M]^+$: 910.5078; found: 910.5095.

Preparation of {SiPc[$C_3H_5(NMe_3)_2O$](OMe)}I_2 (10): A mixture of **4** (0.17 g, 0.24 mmol) and iodomethane (15 mL) in $CHCl_3$ (25 mL) was stirred at room temperature for 3 days. The resulting green precipitate was collected by filtration, washed with $CHCl_3$, and dried in vacuo (0.17 g, 72%). ^1H NMR ([D_6]DMSO): $\delta = 9.72–9.78$ (m, 8H; Pc-H_α), 8.58–8.64 (m, 8H; Pc-H_β), 1.31 (s, 18H; NMe), 0.72 (dd, $J = 5.4$, 13.8 Hz, 2H; CH_2), −0.61 (quintet, $J = 5.4$ Hz, 1H; CH), −1.01 (dd, $J = 5.4$, 13.8 Hz, 2H; CH_2), −2.01 ppm (s, 3H; OMe); $^{13}C\{^1H\}$ NMR ([D_6]DMSO): $\delta = 149.4$, 134.9, 133.2, 124.3, 64.7 (two overlapping signals), 52.2, 42.8 ppm; HRMS (FAB): m/z calcd for $C_{42}H_{42}IN_{10}O_2Si$ $[M-I]^+$: 873.2308; found: 873.2309; elemental analysis calcd (%) for $C_{42}H_{42}I_2N_{10}O_2Si$: C 50.41, H 4.23, N 14.00; found: C 50.32, H 4.31, N 13.76.

Preparation of {SiPc[$C_3H_5(NMe_3)_2O]_2$}I_4 (11): A mixture of **3** (0.20 g, 0.24 mmol), iodomethane (20 mL), and $CHCl_3$ (20 mL) was refluxed for 1 h. After cooling to room temperature, the mixture was filtered and the resulting green precipitate was washed with $CHCl_3$ and dried in vacuo (0.15 g, 43%). ^1H NMR ([D_6]DMSO): $\delta = 9.80–9.84$ (m, 8H; Pc-H_α), 8.67–8.72 (m, 8H; Pc-H_β), 1.27 (s, 36H; NMe), 0.63–0.71 (m, 4H; CH_2), −0.67 to −0.56 (m, 2H; CH), −1.18 to −1.09 ppm (m, 4H; CH_2); $^{13}C\{^1H\}$ NMR ([D_6]DMSO): $\delta = 149.4$, 134.7, 133.9, 124.6, 64.5 (two overlapping signals), 52.1 ppm; elemental analysis calcd (%) for $C_{50}H_{62}I_4N_{12}O_2Si$: C 42.93, H 4.47, N 12.02; found: C 43.84, H 4.82, N 11.36.

In vitro studies: Phthalocyanines were first dissolved in DMF (1.5 mM) and the solutions were diluted to 80 μM with a about 0.01 M aqueous solution of Cremophor EL (Sigma, 0.47 g in 100 mL of water). The solutions were clarified with a 0.45 μm filter, then diluted with the medium to appropriate concentrations.

The HepG2 human hepatocarcinoma cells and J774 mouse macrophage cells (both from ATCC) were maintained in RPMI medium 1640 (Invitrogen) supplemented with 10% fetal calf serum (Invitrogen). About 2×10^4 (for HepG2) or 3×10^4 (for J774) cells per well in this medium were inoculated in 96-multiwell plates and incubated overnight at 37°C under 5% CO_2. The cells were rinsed with PBS and incubated with the above phthalocyanine solutions (100 μL) for 2 h under the same conditions. The cells were then rinsed again with PBS and re-fed with the growth medium (100 μL) 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 ($\lambda >$ 610 nm) was 40 mWcm^{-2}. An illumination of 20 min led to a total fluence of 48 Jcm^{-2}.

Cell viability was determined by means of the colorimetric MTT assay.[18] After illumination, the cells were incubated at 37°C under 5% CO_2 overnight. An MTT (Sigma) solution in PBS (50 μL, 3 mgmL^{-1}) was added to each well followed by incubation for 2 h (for HepG2) or 5 h (for J774) under the same environment. A solution of sodium dodecyl sulfate (Sigma) in 0.04 M HCl(aq) (50 μL, 10% by weight) was then added to each well. The plate was incubated in a 60°C oven for 30 min, then *iso*-propanol (80 μL) was added to each well. The plate was agitated on a Bio-Rad microplate reader at ambient temperature for 20 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 equation: Cell Viability (%) $= [\Sigma(A_i/A_{control}\times100)]/n$, where A_i is the absorbance of the ith data ($i = 1, 2, \ldots n$), $A_{control}$ is the average absorbance of the control wells, in which the phthalocyanine was absent, and n (=4) is the number of the data points.

Cellular uptake: About 2.5×10^4 HepG2 cells in the growth medium (2 mL) were seeded on a coverslip and incubated overnight at 37°C under 5% CO_2. The medium was removed, then the cells were incubated with an 8 μM phthalocyanine dilution (2 mL) in the medium for 2 h under the same conditions. The cells were then rinsed with PBS and viewed with an Olympus IX 70 inverted microscope. The excition light source (at 490 and 630 nm) was provided by a multiwavelength illuminator (Polychrome IV, TILL Photonics). The emitted fluorescence (500–575 and >660 nm) was collected using a digital cooled CCD camera (Quantix, Photometrics). Images were digitized and analyzed using MetaFluor V.6.0 (Universal Imaging).

Subcellular localization studies: About 2.5×10^4 HepG2 cells in the growth medium (2 mL) were seeded on a coverslip and incubated overnight at 37°C under 5% CO_2. After removing the medium, the cells were incubated with MitoTracker Green FM (Molecular Probes; 2 μL) and pluronic acid (2 μL) (both in DMSO) in the medium (2 mL) for 30 min under the same conditions. Then the cells were rinsed with PBS and incubated again with a solution of **4** in the medium (8 μM, 2 mL) for 2 h under the same conditions. The cells were then rinsed with PBS, and the subcellular localization of **4** was revealed by comparing the intracellular fluorescence images caused by the MitoTracker and **4**, using the above microscopic setup.

X-ray crystallographic analysis of 3 and 8: Crystal data and details of data collection and structure refinement are given in Table 3. Data were collected on a Bruker SMART CCD diffractometer with an $Mo_{K\alpha}$ sealed tube ($\lambda = 0.71073$ Å) at 293 K, using a ω scan mode with an increment of 0.3°. Preliminary unit cell parameters were obtained from 45 frames. Final unit cell parameters were obtained by global refinements of reflections obtained from integration of all the frame data. The collected frames were integrated using the preliminary cell-orientation matrix. SMART software was used for collecting frames of data, indexing reflections, and determination of lattice constants; SAINT-PLUS for integration of intensity of reflections and scaling;[19] SADABS for absorption correction;[20] and SHELXL for space group and structure determination, refinements, graphics, and structure reporting.[21] CCDC-238400 and CCDC-238401 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk).

Table 3. Crystallographic data for compounds **3** and **8**.

	3	**8**
formula	$C_{46}H_{50}N_{12}O_2Si$	$C_{44}H_{42}N_8O_2Si$
M_r	831.07	742.95
crystal size [mm^3]	0.50×0.40×0.20	0.40×0.35×0.30
crystal system	orthorhombic	triclinic
space group	*Pbca*	$P\bar{1}$
a [Å]	21.920(7)	8.7499(17)
b [Å]	8.825(3)	9.4952(19)
c [Å]	22.374(7)	11.920(2)
α [°]	90	80.97(3)
β [°]	90	79.93(3)
γ [°]	90	78.49(3)
V [$Å^3$]	4328(2)	947.8(3)
Z	4	1
F (000)	1760	392
ρ_{calcd} [$Mg\,m^{-3}$]	1.275	1.302
μ [mm^{-1}]	0.108	0.112
θ range [°]	1.82 to 25.00	2.64 to 25.57
reflections collected	21839	2944
independent reflections	3821 ($R_{int} = 0.0674$)	2944 ($R_{int} = 0.0000$)
parameters	277	252
$R1$ [$I > 2\sigma(I)$]	0.0548	0.0642
w$R2$ [$I > 2\sigma(I)$]	0.1318	0.1810
goodness of fit	1.046	1.068

Acknowledgement

This work was supported by the Research Grants Council of the Hong Kong Special Administrative Region, China (RGC Ref. No. 402103). We

also thank the Croucher Foundation for a Chinese Visitorship to Dr. J.-D. Huang.

[1] a) *Phthalocyanines—Properties and Applications*, *Vols. 1–4* (Eds.: C. C. Leznoff, A. B. P. Lever), VCH, New York, **1989–1996**; b) N. B. McKeown, *Phthalocyanine Materials: Synthesis, Structure and Function*, Cambridge University Press, Cambridge, **1998**.

[2] For reviews on PDT, see: a) R. Bonnett, *Chemical Aspects of Photodynamic Therapy*, Gordon and Breach, Amsterdam, **2000**; b) I. J. MacDonald, T. J. Dougherty, *J. Porphyrins Phthalocyanines* **2001**, *5*, 105–129; c) N. L. Oleinick, R. L. Morris, I. Belichenko, *Photochem. Photobiol. Sci.* **2002**, *1*, 1–21; d) M. B. Vrouenraets, G. W. M. Visser, G. B. Snow, G. A. M. S. van Dongen, *Anticancer Res.* **2003**, *23*, 505–522; e) D. E. J. G. J. Dolmans, D. Fukumura, R. K. Jain, *Nat. Rev. Cancer* **2003**, *3*, 380–387.

[3] For reviews on phthalocyanine-based photosensitizers, see: a) H. Ali, J. E. van Lier, *Chem. Rev.* **1999**, *99*, 2379–2450; b) E. A. Lukyanets, *J. Porphyrins Phthalocyanines* **1999**, *3*, 424–432; c) C. M. Allen, W. M. Sharman, J. E. van Lier, *J. Porphyrins Phthalocyanines* **2001**, *5*, 161–169; d) A. C. Tedesco, J. C. G. Rotta, C. N. Lunardi, *Curr. Org. Chem.* **2003**, *7*, 187–196.

[4] See for example: a) N. Ahmad, D. K. Feyes, R. Agarwal, H. Mukhtar, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 6977–6982; b) K. Kalka, N. Ahmad, T. Criswell, D. Boothman, H. Mukhtar, *Cancer Res.* **2000**, *60*, 5984–5987; c) C. M. Whitacre, D. K. Feyes, T. Satoh, J. Grossmann, J. W. Mulvihill, H. Mukhtar, N. L. Oleinick, *Clin. Cancer Res.* **2000**, *6*, 2021–2027; d) M. Srivastava, N. Ahmad, S. Gupta, H. Mukhtar, *J. Biol. Chem.* **2001**, *276*, 15481–15488; e) M. Lam, N. L. Oleinick, A.-L. Nieminen, *J. Biol. Chem.* **2001**, *276*, 47379–47386; f) R. L. Morris, M. E. Varnes, M. E. Kenney, Y.-S. Li, K. Azizuddin, M. W. McEnery, N. L. Oleinick, *Photochem. Photobiol.* **2002**, *75*, 652–661; g) J. Usuda, S.-M. Chiu, E. S. Murphy, M. Lam, A.-L. Nieminen, N. L. Oleinick, *J. Biol. Chem.* **2003**, *278*, 2021–2029.

[5] N. L. Oleinick, A. R. Antunez, M. E. Clay, B. D. Rihter, M. E. Kenney, *Photochem. Photobiol.* **1993**, *57*, 242–247.

[6] Several Pc4 analogues have been synthesized and compared for their photodynamic activities. See: a) X.-J. Zhao, S. Lustigman, M. E. Kenney, E. Ben-Hur, *Photochem. Photobiol.* **1997**, *66*, 282–287; b) J. He, H. E. Larkin, Y.-S. Li, B. D. Rihter, S. I. A. Zaidi, M. A. J. Rodgers, H. Mukhtar, M. E. Kenney, N. L. Oleinick, *Photochem. Photobiol.* **1997**, *65*, 581–586; c) C. Y. Anderson, K. Freye, K. A. Tubesing, Y.-S. Li, M. E. Kenney, H. Mukhtar, C. A. Elmets, *Photochem. Photobiol.* **1998**, *67*, 332–336.

[7] See for example: a) B. L. Wheeler, G. Nagasubramanian, A. J. Bard, L. A. Schechtman, D. R. Dininny, M. E. Kenney, *J. Am. Chem. Soc.* **1984**, *106*, 7407–7410; b) C. Farren, C. A. Christensen, S. FitzGerald, M. R. Bryce, A. Beeby, *J. Org. Chem.* **2002**, *67*, 9130–9139; c) J.-D. Huang, S. Wang, P.-C. Lo, W.-P. Fong, W.-H. Ko, D. K. P. Ng, *New J. Chem.* **2004**, *28*, 348–354.

[8] Z. Li, M. Lieberman, *Inorg. Chem.* **2001**, *40*, 932–939.

[9] S. Gürsoy, A. Cihan, M. B. Koçak, Ö. Bekaroğlu, *Monatsh. Chem.* **2001**, *132*, 813–819.

[10] M. E. Daraio, A. Völker, P. F. Aramendía, E. San Román, *Langmuir* **1996**, *12*, 2932–2938.

[11] L. Howe, J. Z. Zhang, *J. Phys. Chem. A* **1997**, *101*, 3207–3213.

[12] a) A. C. H. Ng, X.-Y. Li, D. K. P. Ng, *Macromolecules* **1999**, *32*, 5292–5298; b) X.-Y. Li, X. He, A. C. H. Ng, C. Wu, D. K. P. Ng, *Macromolecules* **2000**, *33*, 2119–2123; c) C. Farren, S. FitzGerald, A. Beeby, M. R. Bryce, *Chem. Commun.* **2002**, 572–573; d) N. M. Speirs, W. J. Ebenezer, A. C. Jones, *Photochem. Photobiol.* **2002**, *76*, 247–251.

[13] W. Spiller, H. Kliesch, D. Wöhrle, S. Hackbarth, B. Röder, G. Schnurpfeil, *J. Porphyrins Phthalocyanines* **1998**, *2*, 145–158.

[14] D. F. Eaton, *Pure Appl. Chem.* **1988**, *60*, 1107–1114.

[15] G. Ferraudi in *Phthalocyanines—Properties and Applications*, *Vol. 1* (Eds.: C. C. Leznoff, A. B. P. Lever), VCH, New York, **1989**, p. 301.

[16] P. C. Krueger, M. E. Kenney, *J. Org. Chem.* **1963**, *28*, 3379–3381.

[17] T. Koyama, T. Suzuki, K. Hanabusa, H. Shirai, N. Kobayashi, *Inorg. Chim. Acta* **1994**, *218*, 41–45.

[18] MTT = 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide. H. Tada, O. Shiho, K. Kuroshima, M. Koyama, K. Tsukamoto, *J. Immunol. Methods* **1986**, *93*, 157–165.

[19] SMART and SAINT for Windows NT Software Reference Manuals, Version 5.0, Bruker Analytical X-Ray Systems, Madison, WI, **1997**.

[20] G. M. Sheldrick, SADABS - A Software for Empirical Absorption Correction, University of Göttingen (Germany), **1997**.

[21] SHELXL Reference Manual, Version 5.1, Bruker Analytical X-Ray Systems, Madison, WI, **1997**.

Received: May 12, 2004
Published online: August 17, 2004

