Synthesis and Photodynamic Activities of Silicon 2,3-Naphthalocyanine Derivatives

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Bis(*tert*-butyldimethylsiloxy)- (**7**), bis(dimethylthexylsiloxy)- (**8**), bis(tri-*n*-hexylsiloxy)- (**9**), and bis(dimethyloctadecylsiloxy)silicon 2,3-naphthalocyanines (**10**) were prepared via substitution of the bis(hydroxy) precursor with the corresponding chlorosilane ligands and characterized by spectroscopic and combustion analyses. They show strong absorption around 780 nm where tissues exhibit optimal transparency. Compounds **7–10** are capable of producing singlet oxygen. They are relatively photostable although less stable than the analogous phthalocyanine, i.e., the bis(dimethylthexylsiloxy)silicon phthalocyanine (**12**). They were evaluated as potential photosensitizers for the photodynamic therapy (PDT) of cancer *in vitro* against V-79 cells and *in vivo* against the EMT-6 tumor in Balb/c mice. *In vitro* all four dyes showed limited phototoxicity combined with substantial dark toxicity. Surprisingly, *in vivo* (iv, 0.1 μmol/kg, 24 h prior to the photoirradiation of the tumor with 780-nm light, 190 mW/cm^2, 400 J/cm^2) all dyes induced tumor regression in at least 50% of mice whereas compound **8** gave a complete tumor response in 80% of mice without apparent systemic toxicity at doses as high as 10 μmol/kg. At 24 h postinjection, compound **8** showed a favorable tumor to muscle ratio of 7, assuring minimal damage to the healthy tissue surrounding the tumor during PDT. Our data confirm the potential of silicon naphthalocyanines as far-red-shifted photosensitizers for the PDT of cancer and indicate the importance of the selection of the two axial silicon ligands for optimal photodynamic efficacy.

Introduction

Clinical trials for the treatment of a variety of cancers with red light after systemic administration of a mixture of hematoporphyrin derivatives (Photofrin), known as photodynamic therapy (PDT), are in progress in medical centers throughout the world, and in Canada approval of this protocol has been secured for the treatment of bladder cancer. It is believed that PDT has the potential to evolve as a valid alternative or adjuvant therapy for a variety of solid neoplasms.[1] Over the past years, several alternative classes of dyes with physicochemical properties selected for improved efficiency of PDT have been proposed as new sensitizers for PDT.[2] Among them, the phthalocyanines (Pc) and naphthalocyanines (Nc) have received increasing attention.[3] Compared to Photofrin ($\epsilon \simeq 10^3$ M^{-1} cm^{-1}), these dyes offer high molar extinction coefficients ($\epsilon \simeq 10^5$ M^{-1} cm^{-1}) and red-shifted absorption maximum at 680 (Pc) and 780 nm (Nc) resulting from the benzene rings condensed to the periphery of the porphyrin like macrocycle. Like Photofrin, Nc and Pc are capable of generating singlet oxygen ($^1O_2$), the reactive species generally believed to be responsible for the cytotoxic effect. The use of 780-nm red light in the case of Nc allows for light penetration into tissues to twice the depth of that possible at 630 nm, i.e., the wavelength currently used for porphyrin-mediated PDT.[4] Convenient light sources are available in the far-red–near IR region of the spectrum, including the solid-state diode and tunable Ti-Sapphire lasers. The singlet oxygen yields obtained with Nc are comparable to those reported for Pc and porphyrins;[5] however, compared to the analogous Pc, Nc are more prone to photobleaching.[6]

Phthalocyanines and naphthalocyanines form stable chelates with metal cations, and the photosensitizing properties of aluminum, zinc, and silicon naphthalocyanines have been documented.[7] We now report on the potential of four silicon naphthalocyanine derivatives (SiNc) as photosensitizers for PDT. The choice of silicon as the central metal ion was based on its tetravalence which allows for the substitution with two axial ligands, whereby variations in the nature of the latter provide a base for the evaluation of structure–activity relationships. Furthermore, addition of bulky substituents to these molecules should reduce their tendency to aggregate, favoring the monomeric and photoactive form of the dye. Four axial substituents with increasing lengths of aliphatic chains (C_4–C_{18}) were selected, and the syntheses of the following derivatives are reported: the bis(*tert*-butyldimethylsiloxy)- (**7**), bis(dimethylthexylsiloxy)- (**8**), bis(tri-*n*-hexylsiloxy)- (**9**), and bis(dimethyloctadecylsiloxy)silicon 2,3-naphthalocyanines (**10**) (Scheme 1). Dyes were formulated as Cremophor EL emulsions, and their photodynamic activity was evaluated *in vitro* against V-79 cells and *in vivo* against the EMT-6 tumor implanted intradermally in BALB/c mice. Pharmacokinetics of the most active dye **8** were studied in the same animal model.

Results

Chemistry. The synthesis of the naphthalocyanine (Scheme 1) and phthalocyanine (Scheme 2) derivatives was adapted from established procedures.[8] The dihydroxysilicon naphthalocyanine and phthalocyanine were selected as precursor for the introduction of substituents of different hydrophobicity and bulkiness.

Briefly, α,α,α′,α′-tetrabromo-*o*-xylene (**1**) was reacted with fumaronitrile (**2**) at 75 °C, using sodium iodide as scavenger for bromine, to yield 2,3-dicyanonaphthalene (**3**). This product was condensed with NH_3 in refluxing methanol in the presence of sodium methoxide as catalyst to give the 1,3-diiminobenz[*f*]isoindoline (**4**).[8b] Compound **4** was then heated with tetrachlorosilane, tri-*n*-butylamine, and tetrahydronaphthalene at 200 °C for 4 h. The resulting

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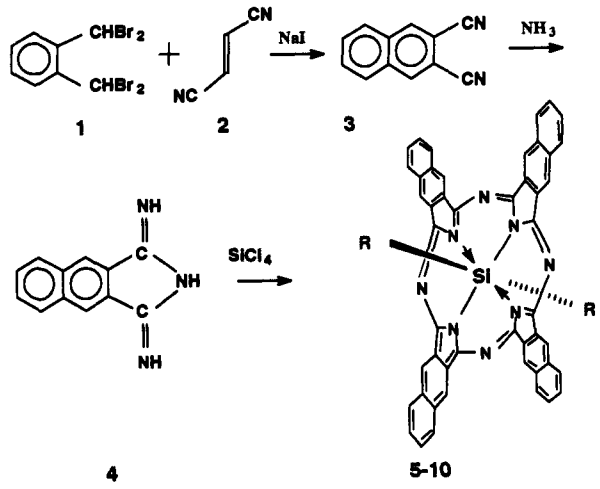
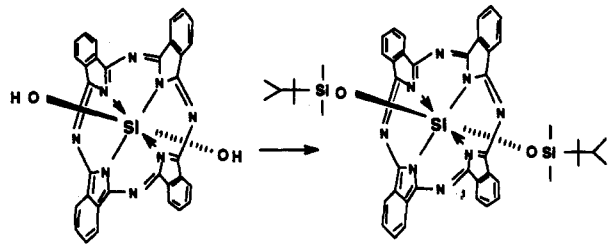
Scheme 1



	R
5	Cl
6	OH
7	$OSi(CH_3)_2C(CH_3)_3$
8	$OSi(CH_3)_2C(CH_3)_2CH(CH_3)_2$
9	$OSi(C_6H_{13})_3$
10	$OSi(CH_3)_2C_{18}H_{37}$

Scheme 2



dichlorosilicon naphthalocyanine (5) was removed from the reaction mixture by filtration and converted to the corresponding dihydroxy derivative 6 by treatment with sulfuric acid and subsequent reflux in concentrated ammonium hydroxide.

The dihydroxysilicon naphthalocyanine 6 was reacted with *tert*-butyldimethylchlorosilane in 2,4,6-collidine and tri-*n*-butylamine at reflux for 15 h to give 7 as a dark green product. Compounds 8–10 were prepared in a similar manner using the appropriate chlorosilane reactants. All four compounds were purified by aluminum oxide column chromatography and characterized by their spectral properties.

For comparative studies, a phthalocyanine derivative containing the thexyl substituents was also synthesized. The condensation of 1,3-diiminoisoindoline with tetrachlorosilane in quinoline at 200 °C afforded the dichlorosilicon phthalocyanine,[8c] which was converted to the corresponding dihydroxysilicon phthalocyanine 11 with aqueous sodium hydroxide in pyridine.[8d] The compound 11 was reacted with dimethylthexylchlorosilane in 2,4,6-collidine and tri-*n*-butylamine, as described for the analogous Nc derivative 8, to give 12.

Figure 1 shows the absorption spectra of compound 9 in dimethylformamide (DMF) and in culture medium containing 1% serum with and without 0.4% Cremophor,

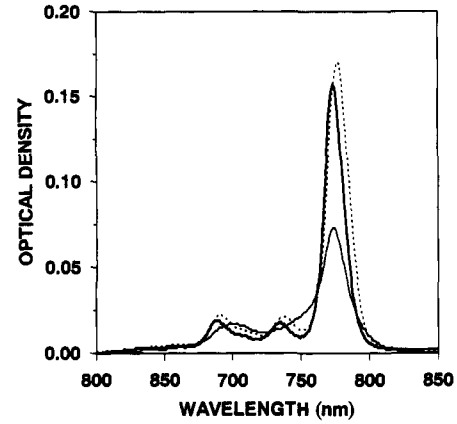


Figure 1. Absorption spectra of a 0.4 μM solution of compound 9 in DMF (dotted line) and culture medium containing 1% serum in the presence (bold line) or absence (fine line) of 0.04% Cremophor.

Table 1. Rates of Photobleaching and Quantum Yields of Singlet Oxygen

dye	solvent	rate of photobleaching,[a] mol s^{-1}	Φ ($^1O_2$)[b]
7	DMF	4.1×10^{-5}	0.18
8	DMF	3.6×10^{-5}	0.33
9	DMF	2.4×10^{-5}	0.32
	PBS–H_2O	4.3×10^{-6}	
	PBS–D_2O	1.8×10^{-5}	
10	DMF	3.6×10^{-5}	0.33
12	DMF	8.0×10^{-6}	

[a] First-order rate of decay extrapolated to $t = 0$ during exposure of various SiNc and SiPc (10 μM) to 20–400 J/cm^2 of red light ($\lambda >$ 600 nm). [b] Quantum yields for the production of singlet oxygen, Φ ($^1O_2$), were measured in oxygen-saturated DMF at 2 μM dye concentration. The luminescence intensity of $^1O_2$ at 1276 nm was quantified using 9,10-diphenylanthracene as a scavenger.[9]

which served as injection vehicle for the dye. The sharp absorption maximum around 775 nm suggests that the dye is in a monomeric state, even in aqueous solutions. In the absence of Cremophor, the position of the maximum wavelength was not significantly shifted although its intensity was diminished.

No important differences in photostability were found among the four SiNc derivatives diluted in DMF and exposed to light doses up to 100 J/cm^2 (Table 1). The bis(dimethylthexylsiloxy)silicon phthalocyanine 12 was about 7 times more photostable than the corresponding naphthalocyanine 8. The photostability (up to 400 J/cm^2) of compound 9 was also studied in phosphate-buffered saline (PBS) containing 0.5% Cremophor. When the same experiment was performed in PBS formulated in D_2O instead of H_2O, the rate of photobleaching of 9 increased about 3-fold (Table 1). Quantum yields for the production of singlet oxygen were taken from Marengo *et al.*[9] and are listed in Table 1.

No difference in hydrophobicity among the four SiNc derivatives was observed when partition in the system octanol/tris buffer (1:1) was used as a criterion; all dyes were completely recovered in the organic phase (data not shown). However, when the hydrophobicity was related to the migration distance on TLC (R_f) or the retention time on HPLC (t_R), a good correlation was observed with the length of the alkyl chain of the axial substituents (Table 2).

Biological Properties. The effect of SiNc derivatives and light on the survival of V-79 cells is expressed as the extracellular dye concentration (μM) required to achieve

Table 2. Light and Dark Toxicity toward V-79 Cells and Chromatographic Mobilities of Differently Substituted SiNc

dye (axial substituents)	LD_{50}[a] (- light), μM	LD_{50}[a] (+ light), μM	R_f[b] (TLC)	t_R[b] (HPLC), min
7 (*tert*-butyldimethylsiloxy)	36	12	0.16	9.2
8 (dimethylthexylsiloxy)	6.5	5.5	0.18	9.0
9 (tri-*n*-hexylsiloxy)	46	29	0.53	5.0
10 (dimethyloctadecylsiloxy)	4.2	1.8	0.34	5.1

[a] Extracellular drug concentration (LD_{50}) required to kill 50% of V-79 cells after a 1-h incubation period (- light) and after subsequent exposure to 2.4 J/cm² red light (+ light). [b] Dye mobility relative to the solvent front (R_f) upon silica gel TLC in petroleum ether/chloroform (3:1) and retention time (t_R) on normal-phase HPLC in petroleum ether/chloroform (19:1).

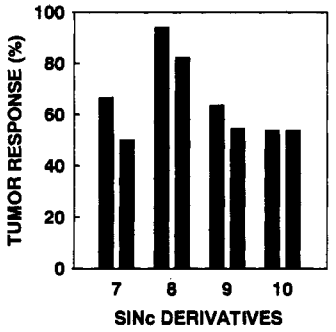


Figure 2. EMT-6 tumor response in BALB/c mice ($n \geq 8$) treated with 780-nm laser light (190 mW/cm², 400 J/cm²) 24 h after iv administration of 0.1 μmol/kg SiNc derivatives 7–10. Shaded area, flat and necrotic tumor within a few days after phototreatment; dark area, absence of a palpable tumor within 2 weeks after phototreatment.

50% cell kill (LD_{50}, Table 2). All four SiNc derivatives 7–10 exhibited substantial dark toxicity. Exposure to light further reduced the cell survival to varying degrees with the exception of compound 8 which induced similar toxicity with or without light. A correlation between increasing hydrophobicity of the dye and efficiency to inactivate cells (LD_{50}) is noted, with the exception of compound 9 (Table 2). The latter is the most hydrophobic dye of the series but also the least active in terms of potential to induce photosensitized cell killing.

The concentrations of dimethylthexyl derivative 8 in plasma, tumor, skin, and muscle at different time intervals after iv administration (1 μmol/kg) to tumor-bearing mice are presented in Table 3. Plasma clearance follows first-order kinetics, with a half-time for elimination of around 7 h. The tumor accumulated significant amounts of photosensitizer, reaching a maximum at 12 h after administration, while dye elimination was slow. Dye uptake by the muscle was very low, providing excellent tumor to muscle ratios reaching a maximum of 10 at 12 h postinjection. However, at this time point, plasma levels are still high, suggesting that the best time for the photodynamic treatment of the tumor is between 24 and 72 h after dye administration. Tumor dye levels also exceeded those of the skin throughout the study (tumor to skin ratio: 1.5-1.8). High levels of dye were retained by the liver and the spleen even at 1 week after injection (data not shown).

The tumor response upon exposure to red light (780 nm, 190 mW/cm², 400 J/cm²) 24 h after iv injection of compounds 7–10 is summarized in Figure 2. Control animals exposed to light alone showed no apparent tumor effect. Also, nonirradiated control tumors of animals injected with dye showed no regression. At a drug dose of 0.1 μmol/kg (≃0.1 mg/kg), compound 8 was the most active and induced a complete tumor regression in 80% of mice compared to 50% response for compounds, 7, 9, and 10. In addition, no apparent systemic toxicity was observed with compound 8 at doses as high as 10 μmol/kg.

Discussion

The preparation of all four SiNc 7–10 bearing different axial substituents followed straightforward synthetic procedures to yield single isomeric products. The latter is a major advantage over synthetic routes involving substitutions of various positions on the macrocycle which often results in difficult to resolve, complex isomeric mixtures. The final products were purified by conventional aluminum oxide column chromatography. The purity of the compounds was assessed by normal-phase HPLC and silica gel TLC, which revealed the various SiNc as single green spots. Fast atom bombardment mass spectrometry or combustion analyses were used to confirm the molecular composition. The SiNC derivatives 9 and 10 gave adequate combustion analyses and definite melting points <300 °C whereas compounds 7 and 8 had inadequate combustion data, probably due to their high melting point (>300 °C). Instead, the latter dyes were characterized by their spectral properties and appropriate molecular ions.

The sharp absorption peak around 775 nm of compound 9 suggests that this dye is in a monomeric state both in DMF and aqueous solutions containing Cremophor (0.04%) as emulsifier (Figure 1). In biological media void of Cremophor, the solubility of the dye is maintained by its association with serum proteins. The intensity of the absorption maximum under the latter condition is diminished; however, the absence of a significant blue-shift suggests that the dye is in a monomeric state. These observations support the prediction that the addition of two axial substituents onto the centrally coordinated Si(IV) ion prevents stacking of the ring structures. However, a water-soluble derivative of SiNc obtained by the addition of two poly(ethylene glycol) ($n = 44$) ligands on the central metal [SiNc (OPEGM-1900)$_2$] showed extensive aggregation in PBS,[10] suggesting that for extremely large substituents other intermolecular interactions will occur.

All SiNc 7–10 were shown to generate singlet oxygen in DMF solution with quantum yields varying from 0.18 to 0.35 (Table 1).[5,9] The relative potential for photobleaching of our four SiNc derivatives was studied in DMF. The photostability of the SiNc was not significantly affected by the different substituents (Table 1). For comparison, compound 12, the phthalocyanine (Pc) analog of 8, was included and shown to exhibit a 5-fold higher photostability (Table 1). These differences in photostability are far less important than those observed for the aluminum derivative, since it has been reported that AlNc is about 1000 times less photostable than AlPc.[6] The photostability of 9 was further improved upon formulation in PBS containing 0.5% Cremophor. The higher rate of photobleaching in DMF could reflect the 2-fold longer lifetime of singlet oxygen ($^1O_2$) as well as the higher oxygen solubility in DMF as compared to H_2O.[11] The involvement of $^1O_2$ in the photobleaching process is also suggested by the higher rate of photobleaching of 9 in D_2O compared to normal water, i.e., under conditions whereby the lifetime of $^1O_2$ is increased 10-fold.[12] Since compound 9, even in an aqueous environment, is mainly in its photoactive monomeric form and relatively photostable, photobleach-

Table 3. Blood Clearance and Tissue Distribution of Bis(dimethylthexylsiloxy)silicon 2,3-Naphthalocyanine (8) in Tumor-Bearing Mice

	μg/g or μg/mL (SE) or ratio[a]							
tissue	1 h	3 h	6 h	12 h	1 d	2 d	3 d	7 d
plasma	12.94	11.28	9.12	5.51	2.06	0.21	0.02	0.00
	(0.99)	(0.36)	(0.13)	(0.71)	(0.30)	(0.04)		
tumor	0.43	0.46	0.65	0.90	0.71	0.73	0.77	0.51
	(0.05)	(0.03)	(0.02)	(0.28)	(0.09)	(0.14)	(0.09)	(0.04)
muscle	0.12	0.32	0.98	0.09	0.11	0.08	0.13	0.08
	(0.02)	(0.05)	(0.02)	(0.01)	(0.02)	(0.01)	(0.06)	(0.01)
skin	0.13	0.51	0.36	0.56	0.46	0.43	0.44	0.35
	(0.01)	(0.06)	(0.03)	(0.08)	(0.04)	(0.05)	(0.01)	(0.02)
tumor/plasma	0.03	0.04	0.07	0.16	0.34	3.5	38	
tumor/muscle	3.6	1.4	0.7	10.0	6.4	9.1	5.9	6.4

[a] Mean tissue uptake (μg/g), plasma concentration (μg/mL), or uptake ratio and standard error (SE) for BALB/c mice bearing the EMT-6 tumor (n = 3), after iv injection of 1 μmol/kg (1.058 mg/kg) of compound 8.

ing is unlikely a limiting factor during SiNc-mediated tumor PDT.

Our *in vitro* results indicate that the cellular inactivation induced by SiNc derivatives increases with increasing hydrophobicity of the dye, albeit the differences in polarity between the analogs are very small (Table 2). The hydrophobicity relates to the chain length of the two axial substituents of the central Si metal, but steric hindrance, due to the spatial configuration of these chains, may perturb the correlation. Thus, in the case of 9, the lower than expected *in vitro* activity may reflect reduced cellular uptake due to the presence of the bulky tri-*n*-hexylalkyl chains, rather than changes in hydrophobicity. As already reported for other Nc,[7d,e] our silicon derivatives also induce significant cellular dark toxicity. This may be attributed to the larger size of the Nc macrocycle as compared to the closely related, nontoxic Pc, causing disruption of the cell physiology.[7e]

Dye concentrations in plasma, tumor, muscle, and skin were obtained at different time intervals after iv injection of 1 μmol/kg of compound 8 (Table 3). Significant amounts of dye accumulated in the tumor between 12 and 72 h postinjection while only low concentrations were detected in the muscle, providing favorable tumor/muscle ratios of 6–10. At these ratios, damage to healthy tissue surrounding the tumor is minimal during PDT. The slow rate of dye elimination from the tumor also allows for repeated treatments after a single dye administration. Similar pharmacokinetic data were reported for a related bis(alkylsiloxy) SiNc derivative, the bis(diisobutyloctadecylsiloxy)silicon 2,3-naphthalocyanine (iso-BOSiNc).[7a,13] Although the concentration of compound 8 in the tumor remains stable between 12 and 72 h, plasma dye levels decrease at a first-order rate during the same period, resulting in varying tumor to plasma ratios. Dye plasma levels could be a major determinant in PDT efficiency, since it has been shown that responsiveness to another photosensitizer, the mono-L-aspartyl chlorine e_6, correlated with plasma dye levels rather than tumor concentrations of the dye.[14] Studies on the role of tumor to plasma ratios of 8 on PDT response are in progress in our laboratory.

In contrast to the *in vitro* results, comparison of the *in vivo* PDT activities of our four SiNc 7–10 showed no correlation between dye hydrophobicity and biological activity. It is unlikely that discrepancies between *in vitro* and *in vivo* activities relate to the use of different cell lines since similar results were obtained with other Pc-based photosensitizers using the same EMT-6 cell line for both *in vitro* and *in vivo* studies (unpublished results). In the case of metallophthalocyanines substituted on the macrocycle ring system with different numbers of sulfonate groups[15] or alkylhydroxy groups of varying chain lengths,[16] good correlations between *in vitro* and *in vivo* results were observed, albeit differences in activities among the dyes were more accentuated *in vitro*. Among our SiNc, the best therapeutic effect was obtained with compound 8 which induced a complete tumor regression in 80% of the animals as compared to 50% for the three other derivatives, using the same treatment protocol (0.1 μmol/kg, 400 J/cm^2) (Figure 2). These results are in good agreement with those reported for iso-BOSiNc which induced complete tumor destruction at 0.2 mg/kg and 135 J/cm^2 of 774-nm light.[7b] With compound 8, no apparent systemic toxicity was observed in mice after PDT, even at 10 μmol/kg, i.e., the maximum concentration which could be administered in Cremophor emulsion. This provides for a very large phototherapeutic window, with a ratio of the phototoxic dose to the phototherapeutic dose of over 100. For comparison, in the case of Photofrin, using the same animal model, the minimum drug dose required to achieve tumor regression was 10 mg/kg, while PDT after a dye dose of 20 mg/kg induced mortality.[17]

In conclusion, the axial substituents of SiNc affect the *in vitro* activity on V-79 cells in a manner which correlates to hydrophobicity and steric hindrance. No correlation between *in vitro* photoactivity and *in vivo* PDT potential was observed. Furthermore, in spite of the limited *in vitro* phototoxicity, *in vivo* these dyes exhibited excellent photodynamic activity. The bis(alkylsiloxy)silicon naphthalocyanines, in particular the bis(dimethylthexylsiloxy) derivative 8, offer a good potential as sensitizers for PDT at far-red wavelengths with a large phototherapeutic window. Compound 8 formulated as a Cremophor emulsion induces tumor regression at a low dye dose, assuring minimal risk for long-term skin phototoxicity. Finally, the pharmacokinetic behavior of 8 suggests the possibility of repeated photodynamic treatments after a single dye injection.

Experimental Section

Solvents and reagents were purchased from Aldrich, Sigma, or Fisher and used as such. Crude reaction mixtures were purified by column chromatography on 60–200-mesh aluminum oxide. Ultraviolet and visible (UV–Vis) absorption spectra were recorded with a Varian 2200 spectrophotometer. ^{1}H NMR spectra were obtained with a Bruker AC 300 instrument (300 MHz). Chemical shifts are reported on the δ scale relative to TMS. A Hewlett-Packard Model 5988A quadrupole instrument was used to obtain fast atom bombardment mass spectra (FABMS). Combustion analyses were carried out by Guelph Chemical Laboratories Ltd, Guelph, Ontario, Canada.

To evaluate photostability, stock solutions of SiNc derivatives in dimethylformamide (DMF) or formulated in 10% Cremophor

emulsion (200 μM) were diluted to 10 μM in the following solvents: DMF, PBS, or PBS formulated in deuterium oxide (D_2O, Aldrich) instead of deionized water. Two milliliters of dye solution were irradiated in 60-mm-diameter glass Petri dishes with the same light source as described below for cell illumination (10 mW/cm², 20–400 J/cm²). After various light exposures, 200-μL aliquots were taken in triplicate and diluted 10-fold in DMF, and absorption spectra were recorded. Photobleaching rates were determined from the decrease in optical density (λ_{max} 785–780 nm, ϵ 5 × 10^5 M^{-1} cm^{-1}). No significant shifts around the λ_{max} were observed as a function of illumination time.

The relative hydrophobicity of SiNc derivatives **7–10** was determined both by thin-layer chromatography (TLC) and by high-performance liquid chromatography (HPLC). TLC was performed on 0.25-mm-thick Brinkmann analytical silica gel plates coated with fluorescent indicator (UV 254 nm) in petroleum ether (50–110 °C)/chloroform (3:1). The mobilities are given in terms of migration distance relative to the solvent front (R_f). HPLC (Shimadzu, Japan) was performed on a normal-phase column (8- × 100-mm Novapak silica cartridge, 4-μm particle size, Waters) eluted at 1.5 mL/min isocratically with petroleum ether (50–110 °C)/chloroform (19:1). Dyes were detected at 760 nm, and their retention times (t_R) were recorded.

The four SiNc derivatives **7–10** were formulated in Cremophor emulsion for biological studies. The dyes were first dissolved in a minimal volume of DMF. Cremophor EL (10% final) and 1,2-propanediol (3% final) were added under sonication, whereafter DMF was evaporated under vacuum. The solution was diluted with phosphate-buffered saline (PBS), pH 7.4 and filtered (0.2 μm). The final SiNc concentration was estimated spectroscopically after dissolution of the emulsion in DMF (λ_{max} 775–780 nm, ϵ 5 × 10^5 M^{-1} cm^{-1}).

General Synthetic Methods for Derivatives 7–10. A mixture of dihydroxysilicon naphthalocyanine (0.5 mmol), the appropriate alkyl chlorosilane (1.3 mmol), tri-*n*-butylamine (1 mL), and 2,4,6-collidine (10 mL) was refluxed for 16 h, cooled, and filtered, and the solid was washed with pyridine. The filtrate was mixed with ethanol/water (1:1) (50 mL) and filtered to give a green solid. The crude product was dissolved in the specified solvent and purified over an aluminum oxide column eluted as indicated. Yields varied from 40 to 70%.

Bis(*tert*-butyldimethylsiloxy)silicon 2,3-Naphthalocyanine (7). The crude product was dissolved in DMF, applied to the column, and eluted with hexane/ethyl acetate (90:10) to yield **7** as a green powder: mp >300 °C; Vis (DMF) λ_{max} 780 nm (ϵ 5 × 10^5 M^{-1} cm^{-1}); ^{1}H NMR (DMSO-d_6 + $CDCl_3$) (δ) −2.59 (m, 12H, 4 × $SiCH_3$), −1.18 (m, 18H, 2 × *t*-Bu), 7.96 (m, 8H, ArH), 8.69 (m, 8H, ArH), 10.15 (m, 8H, ArH); FABMS (3-nitrobenzyl alcohol) *m/z* (rel intensity) 1002 (M^+, 100), 871 (85).

Bis(dimethylthexylsiloxy)silicon 2,3-Naphthalocyanine (8). The crude product was washed with 2-propanol, dissolved in tetrahydrofuran, applied to the column, and eluted with hexane/ethyl acetate (80:20) to yield **8** as a green powder: mp >300 °C; Vis (DMF) λ_{max} 780 nm (ϵ 5 × 10^5 M^{-1} cm^{-1}); ^{1}H NMR (THF-d_8) (δ) −2.88 (m, 12H, 4 × $SiCH_3$), −1.52, −1.51 (m, 12H, 2 × $SiC(CH_3)_2$), −1.16, −1.14 (m, 12H, 2 × Si-C-$C(CH_3)_2$), −0.95 (m, 2H, 2 × CH), 7.59–7.63 (m, 8H, ArH), 8.35–8.38 (m, 8H, ArH), 9.81–9.82 (s, 8H, ArH); FABMS (*o*-nitrophenyl octyl ether) *m/z* (rel intensity) 1058 (M^+, 80), 924 (100).

Bis(tri-*n*-hexylsiloxy)silicon 2,3-Naphthalocyanine (9). The crude product was dissolved in ethyl acetate, applied to the column, and eluted with hexane and subsequently with hexane/ethyl acetate (99:1) to give, after trituration from acetonitrile, **9** as a dark green powder: mp 275–276 °C (lit.[8b] mp 278 °C); Vis (acetone) λ_{max} 770 nm (ϵ 5 × 10^5 M^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$) (δ) −2.08 (m, 12H, 6 × $SiCH_2$), −0.97 (m, 12H, 6 × CH_2), 0.07 (m, 12H, 6 × CH_2), 0.22 (m, 12H, 6 × CH_2), 0.41 (m, 18H, 6 × CH_3), 0.61 (m, 12H, 6 × CH_2), 7.92 (m, 8H, ArH), 8.67 (m, 8H, ArH), 10.11 (m, 8H, ArH); FABMS (ethyl acetate + *o*-nitrophenyl octyl ether) *m/z* (rel intensity) 1340 (M^+, 66), 1197 (100). Anal. ($C_{84}H_{102}N_8O_2Si_3$) C, H, N.

Bis(dimethyloctadecylsiloxy)silicon 2,3-Naphthalocyanine (10). The product was purified as described for compound **9**: mp 214–215 °C; Vis (hexane) λ_{max} 763 nm (ϵ 5.2 × 10^5 M^{-1} cm^{-1}); ^{1}H NMR ($CDCl_3$) (δ) −2.52 (s, 12H, 4 × $SiCH_3$), −1.89 (m, 4H, 2 × $SiCH_2$), −1.05 (m, 4H, 2 × CH_2), −0.15 (m, 4H, 2 × CH_2), 0.15 (m, 4H, 2 × CH_2), 0.51 (m, 4H, 2 × CH_2), 0.68 (m, 4H, 2 × CH_2), 0.95 (m, 4H, 2 × CH_2), 1.11 (m, 4H, 2 × CH_2), 1.25 (m, 42H, 18 × CH_2 + 2 × CH_3), 7.94 (m, 8H, ArH), 8.67 (m, 8H, ArH), 10.11 (bm, 8H, ArH). Anal. ($C_{88}H_{110}N_8O_2Si_3$) C, H, N.

Bis(dimethylthexylsiloxy)silicon Phthalocyanine (12). A mixture of dihydroxysilicon phthalocyanine (11) (0.5 g), thexyldimethyl silicon chloride (0.5 mL), and tri-*n*-butylamine (3 mL) in 2,4,6-collidine (30 mL) was refluxed for 16 h. The solvent was evaporated under vacuum, and the residue was dissolved in ethyl acetate, washed with water, dried over anhydrous sodium sulfate, and taken to dryness. The crude product was triturated with hexane, applied on an aluminum oxide column, and eluted with hexane/ethyl acetate (90:10). The fraction containing the first blue band was evaporated to dryness, and the solid was triturated with 2-propanol to give the blue compound **12**, 0.44 g (59%); mp > 300 °C, Vis (ethyl acetate) λ_{max} 666 nm (ϵ 2 × 10^5 M^{-1} cm^{-1}); ^{1}H NMR (THF-d_8) (δ) −3.39 (s, 12H, 4 × $SiCH_3$), −1.87 (s, 12H, 2 × $SiC(CH_3)_2$), −1.43 (m, 14H, 2 × $CH(CH_3)_2$), 7.89–7.94 (m, 8H, ArH), 9.18–9.24 (m, 8H, ArH); FABMS (*o*-nitrophenyl octyl ether) *m/z* (rel intensity) 858 (M^+, 12), 773 (22), 699 (100), 615 (19). Anal. ($C_{48}H_{54}N_8O_2Si_3$) C, H, N.

Cellular Photoinactivation. The survival of V-79 Chinese Hamster lung fibroblasts after incubation with SiNc derivatives **7–10**, with or without light treatment, was estimated by a colony formation assay as previously described.[18] Briefly, 200 cells in exponential phase were inoculated in 60-mm-diameter Petri dishes and incubated for 3 h at 37 °C in 5% CO_2 to allow cell attachment. The cells were then rinsed with PBS and incubated in the dark for 1 h at 37 °C with 1 mL of MEM medium (Gibco) containing 1% fetal bovine serum (MEM-1% serum) and photosensitizer (1–30 μM). After the medium was removed, cells were exposed to red light, refed with growth medium, and incubated at 37°C in 50% CO_2 for 6 or 7 days. The light source consisted of two 500-W tungsten/halogen lamps (GTE Sylvania, Canada) fitted with a circulating, refrigerated, aqueous Rhodamine filter (OD_{580nm} 1.25). Cells were irradiated for 4 min with a fluence rate, calculated over the absorbance peak of the dyes (760–800 nm), of 10 mW/cm² providing a fluence of 2.4 J/cm². The plating efficiency of control cells incubated with Cremophor-medium only and exposed to red light was taken as 100% survival. No cytotoxicity was observed due to the added Cremophor (<1%). The mean value of triplicate plates was plotted for three different experiments and the extracellular dye concentration required to kill 50% of cells incubated in the dark or exposed to light (LD_{50}) was calculated.

Experimental Tumors. Male Balb/c AnN mice (18–20 g) were supplied by Charles River Breeding Laboratories (Montréal, Canada). Experiments were conducted in accordance with the recommendations of the Canadian Council on Animal Care and an in-house ethics committee. The animals were allowed free access to water and food throughout the course of the experiments. EMT-6 mouse mammary tumor cells were obtained from Dr. C.-W. Lin (Massachusetts General Hospital, Boston) and maintained according to an established protocol[19] as previously described.[20] Before tumor implantation, hair in the tumor area was removed with a chemical depilatory (Nair, Whitehall Lab., Mississauga, Canada). A tumor was implanted on each hind thigh by intradermal injection of 2 × 10^5 EMT-6 cells suspended in 0.05 mL of Waymouth growth medium (Gibco, Canada). Mice were used 6–8 days after cell inoculation when the tumor diameter and thickness reached 4–8 mm and 2–4 mm, respectively. At this stage of development of the tumor no spontaneous necrosis was observed.

Pharmacokinetic Studies. Tumor-bearing mice were injected iv via the caudal vein with 1 μmol/kg of compound **8** in 10% Cremophor emulsion (0.2 mL/20 g). At different time intervals after dye administration, blood was collected from the orbital sinus by means of an heparinized capillary, whereafter the animals (n = 3) were sacrificed by cervical dislocation. The blood was centrifuged in eppendorf tubes for 5 min at 2000*g*, and the plasma was collected. Tissues of interest were then removed, washed with saline 0.9%, blotted dry, and weighed. Aliquots of 0.1–0.2 g of minced tissue or 0.1 mL of plasma were treated with 1.9 mL of DMF using a polytron (PT 10/35, Beckmann, Canada). The homogenate was incubated overnight at 37 °C under mechanical agitation and then centrifuged at 900*g* for 10 min.

The supernatant was further centrifuged under the same conditions, and the dye concentration in the clear supernatant was measured by fluorescence using a SLM-Aminco SPF-500C spectrofluorometer (λ_{ex} 766 nm, λ_{em} 780 nm, 5 nm bandpass). Calibration curves were established for each tissue by adding known amounts of dye diluted in DMF to 0.1–0.2 g of tissue from control mice, whereafter the tissue was treated as described above. No fluorescence was found in control tissue samples to which no dye had been added.

Photodynamic Therapy. Tumor-bearing mice were given an intravenous injection of the various dye preparations in 10% Cremophor emulsion, via the lateral tail vein (0.2 mL/20 g). The right tumor was irradiated at 24 h after dye administration while the left was used as a control. The light system consisted of a CW Ti-Sapphire laser (Spectra Physics, Model 3900, Mountain View, CA) tuned to 780 nm by a birefringent filter and pumped by a 6-W argon laser system (Coherent Inc., Model innova 90–6, Palo Alto, CA). The output beam was split using a 50–50 beam splitter (Melles Griot, Model 03BTF029, Irvine, CA). The fluence rate delivered at the level of the tumor surface over a 8-mm-diameter spot was 190 mW/cm^2 for a total fluence of 400 J/cm^2. No hyperthermia was observed under these conditions as monitored by means of a microthermocouple inserted in the tumor and coupled with a digital readout device. Two end-points were selected to assess tumor response: (a) tumor necrosis, i.e., development of a flat and necrotic tumor within a few days after phototreatment, and (b) tumor regression, i.e., absence of a palpable tumor within 2 weeks after phototreatment.

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