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Synthesis and in vitro photodynamic activity of novel galactose-containing phthalocyanines

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Abstract—A novel series of silicon(IV) phthalocyanines with one or two axial acetal-protected galactose substituent(s) have been prepared by typical substitution reactions. The compounds exhibit a high photodynamic activity against HepG2 human hepatocarcinoma cells with IC_{50} values down to 0.10 μM.

Phthalocyanines have been known for more than seven decades. Apart from their traditional use as industrial dyes and pigments, these compounds have been extensively studied as advanced materials for various applications.[1] Owing to the strong absorption in the red visible region (the Q band appears at ca. 680 nm with a molar absorptivity of ca. $10^5\ M^{-1}cm^{-1}$), high efficiency to generate reactive oxygen species, and apparently low dark toxicity, these compounds have emerged as promising second-generation photosensitizers for photodynamic therapy (PDT).[2] Most of the studies so far have focused on the sulfonated zinc(II) and aluminium(III) phthalocyanines, and the well-known silicon(IV) phthalocyanine Pc4. Photodynamic activities of other functionalized phthalocyanines remain relatively little studied.[3] We describe herein the preparation and in vitro photodynamic activity of a novel series of galactose-containing silicon(IV) phthalocyanines. By introducing one or two bulky acetal-protected galactose substituent(s) at the axial position(s), we aimed to increase the hydrophilicity and inhibit the self-aggregation of these compounds. More importantly, on the basis that various types of glucose transporters which are specific for different monosaccharides are over-expressed in cancer cells,[4] we hoped to enhance the cellular uptake of the dyes through glycoconjugation, which in turn may increase the photodynamic activity. Such an approach has been employed for other photosensitizers such as porphyrins,[5] chlorins[6] and pheophorbides.[7] By contrast, covalently linked sugar-containing phthalocyanines are extremely rare. To our knowledge, only the synthesis and preliminary characterization data of a zinc(II) phthalocyanine substituted with four glucose moieties have been reported so far.[8]

The preparation of the galactose-substituted phthalocyanines is shown in Scheme 1. Treatment of the readily available silicon(IV) phthalocyanine dichloride (**1**) with 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose (**2**) in the presence of NaH in toluene gave the disubstituted product **3** in 38% yield. This compound was highly soluble in a wide range of organic solvents and could be purified readily by column chromatography. To increase the amphiphilicity of the compounds which is believed to be one of the desirable characteristics for efficient photosensitisers,[9] the unsymmetrical analogues **4**–**6** were also prepared by mixed substitution with **2** and the corresponding alcohols in the presence of NaH. As expected, these reactions also afforded the symmetrical products **3** and the dialkoxy silicon(IV) phthalocyanines $SiPc(OR)_2$, which were separated from the desired products by column chromatography. To maximize the yield of the unsymmetrical products, compound **1** was treated with 0.8 equiv of deprotonated **2** in refluxing toluene overnight prior to the addition of another 0.8 equiv of NaOR. Nevertheless, these products could only be isolated in 11–18%.

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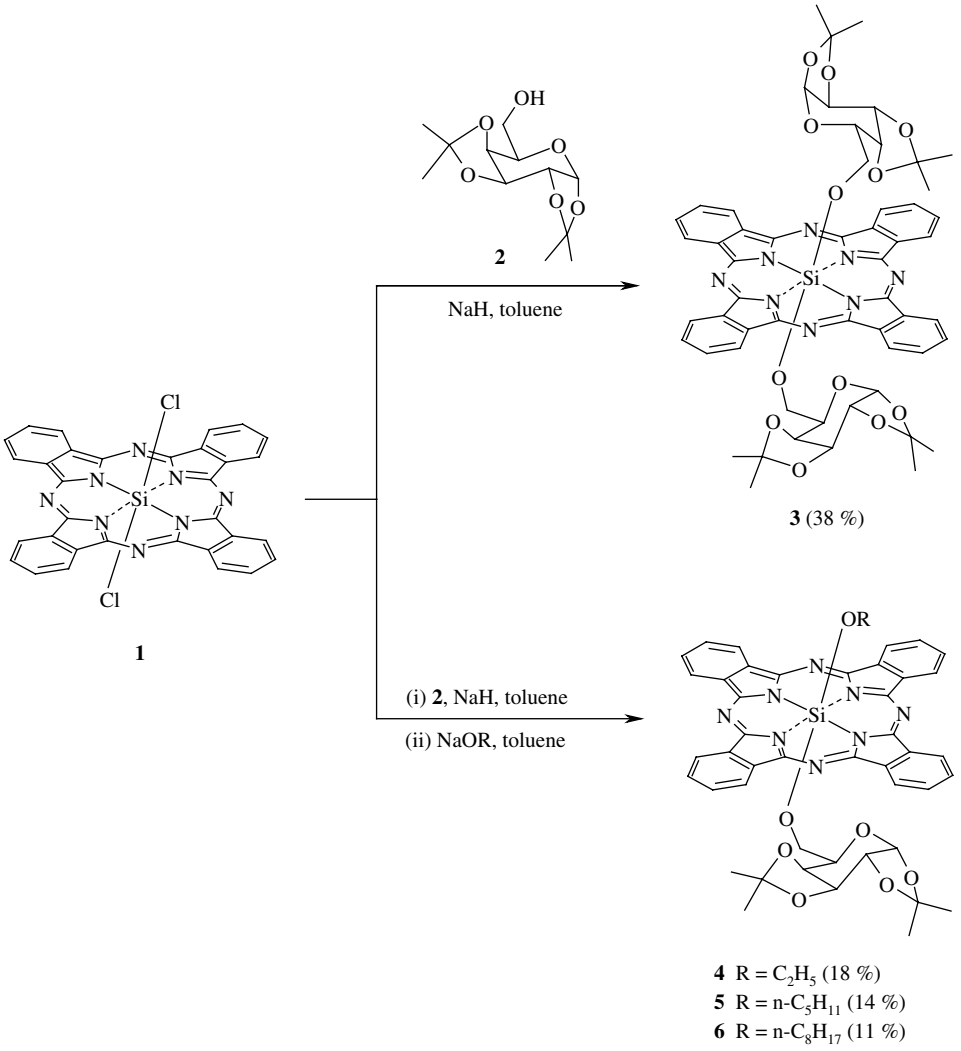

Scheme 1. Preparation of galactose-containing silicon(IV) phthalocyanines.

All the new compounds (**3**–**6**) were characterized with ^{1}H NMR and UV–vis spectroscopic methods together with accurate mass measurements.[10] The ^{1}H NMR spectrum of **3** in $CDCl_3$ showed two typical AA′BB′ multiplets at δ 9.60–9.67 and 8.29–8.35 for the phthalocyanine α- and β-ring protons, respectively. All the signals for the acetal-protected galactose group were significantly shifted upfield by the phthalocyanine ring current, particularly those for the two hydroxymethyl protons at C5, which appeared at δ −1.68 and −2.36 both as a doublet of doublet. The H5 signal was embedded by one of the methyl singlets. Unambiguous assignments could be made with the aid of its ^{1}H–^{1}H COSY spectrum (see Fig. S1 in the supporting information). The replacement of one of the acetal-protected galactose groups with an alkoxy chain in **4**–**6** did not significantly shift the signals for the phthalocyanine and sugar units, while additional signals due to the alkoxy protons were spread in the upfield positions.

The UV–vis spectra of **3**–**6** in DMF were typical for non-aggregated phthalocyanines. As summarized in Table 1, all the compounds show similar absorption positions, including the B band at 352–356 nm, the Q band at 671–673 nm together with two vibronic bands at 604–606 and 641–644 nm. Upon excitation at 610 nm, these compounds showed a strong fluorescence emission at 675–679 nm with a stoke shift of 4–6 nm and a fluorescence quantum yield (Φ_f) of 0.39–0.47.[11] To evaluate the photosensitizing efficiency of these phthalocyanines, their singlet oxygen quantum yields (Φ_Δ) were also

Table 1. Electronic absorption and photophysical data for compounds **3**–**6** in DMF

Compound	λ_{max} (nm) (log ε)	λ_{em} (nm)[a]	Φ_f[b]	Φ_Δ[c]
3	356 (5.28), 606 (4.99), 644 (4.92), 673 (5.76)	679	0.39	0.94
4	356 (4.90), 604 (4.61), 641 (4.53), 672 (5.40)	677	0.45	0.79
5	354 (5.00), 604 (4.70), 641 (4.63), 671 (5.48)	675	0.47	0.82
6	352 (5.13), 604 (4.75), 641 (4.69), 671 (5.55)	676	0.42	0.88

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

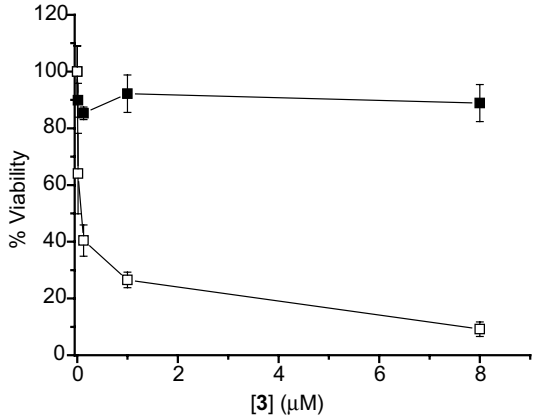

Figure 1. Effects of **3** on HepG2 in the absence (■) and presence (□) of light. For the latter, the cells were illuminated with a red light ($\lambda > 610$ nm, 40 mW cm^{-2}, 48 J cm^{-2}). Data are expressed as mean ±SD ($n = 4$).

determined by a steady-state method using 1,3-diphenylisobenzofuran as the scavenger.[12] It was found that all these compounds are excellent singlet-oxygen generators with a Φ_Δ value ranging from 0.79 to 0.94.[11]

The photodynamic activities of the galactose-containing phthalocyanines **3**–**6** in Cremophor EL emulsions were evaluated against human hepatocellular carcinoma HepG2 using the procedure described earlier.[3d,e] Figure 1 shows a typical dose-dependent survival curve for **3**. While this compound at concentrations lower than 8 μM is essentially non-cytotoxic in the absence of light, it exhibits a high photocytotoxicity with an IC_{50} value, defined as the dye concentration required to kill 50% of the cells, of 0.10 μM. The absorption spectrum of **3** in Cremophor EL emulsion showed a very sharp and intense Q band at 673 nm (see Fig. S2 in the supporting information), indicating that the compound remains essentially non-aggregated in this medium. This property is particularly important for photosensitizing applications.[13] The unsymmetrical analogues **4**–**6** behaved similarly with an IC_{50} value of 0.10, 0.48 and 0.79 μM, respectively. Apparently, the photodynamic activity decreases as the length of the alkoxy chain increases. Under the same conditions, the bis(hexyloxy) analogue $SiPc(OC_6H_{13})_2$ was not cytotoxic and the bis-[poly(ethylene glycol)] analogue $SiPc[(OCH_2CH_2)_nOMe]_2$ showed an IC_{50} value of ca. 1 μM.[3d] The higher photodynamic activity of **3**–**6** should be related to the galactose moieties although their role is not clear at this stage.

To study the cellular uptake, we employed fluorescence microscopy to monitor the intracellular fluorescence caused by the phthalocyanines. The HepG2 cells were incubated with 8 μM of these compounds in Cremophor EL emulsions on coverslips for 2 h. The cells were rinsed with phosphate buffered saline before being viewed with an inverted microscope. Upon excitation at 630 nm, a strong fluorescence was observed in the cytoplasm of the cells showing that there were substantial uptakes of the dyes. As mitochondria are commonly believed to be the targets for the initiation of apoptosis by PDT,[14] it would be important to reveal whether the dyes have selective affinities in these subcellular components. We stained the HepG2 cells with MitoTracker Green FM, which is a specific fluorescence dye for mitochondria, prior to the treatment with **3**. As shown in Figure 2a, the MitoTracker reveals the mitochondria distribution in the cytoplasm (excited at 490 nm, monitored at 500–575 nm). Exciting the cells at 630 nm (monitored at >660 nm) also shows the intracellular fluorescence of **3** (Fig. 2b). The fluorescence appears as bright and granular spots throughout the cytoplasm. However, when the two images are superimposed (Fig. 2c), only certain mitochondria exhibit a co-localization with compound **3**. This indicates that **3** is not exclusively localized in the mitochondria.

In summary, we have prepared and characterized a novel series of galactose-containing silicon(IV) phthalocyanines. The compounds exhibit a high photodynamic activity against HepG2 human hepatocellular carcinoma, which can be attributed to the high cellular uptake and efficiency to generate singlet oxygen. Further studies are in progress to reveal the role of the galactose moieties in cellular uptake and to optimize the PDT efficacy of these glycoconjugated phthalocyanine systems.

Acknowledgements

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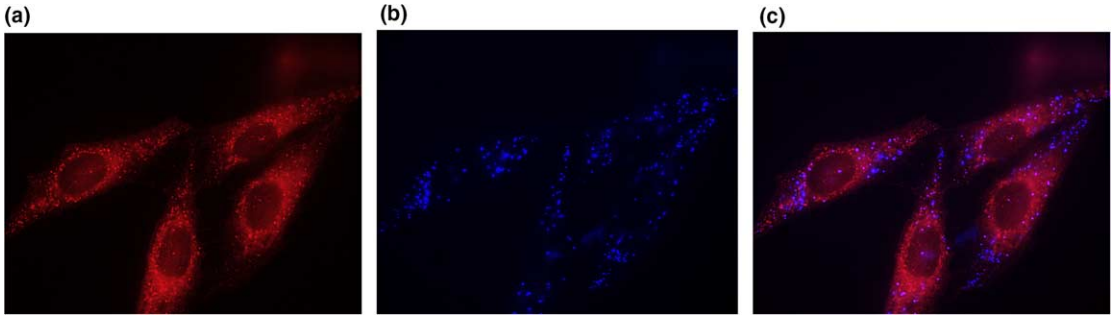

Figure 2. Visualization of intracellular fluorescence of HepG2 using filter sets specific for (a) the MitoTracker and (b) phthalocyanine **3**. Figure c shows the corresponding superimposed image.

Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2004.12.137.

References and notes

1. See for example (a) Perry, J. W.; Mansour, K.; Lee, I.-Y. S.; Wu, X.-L.; Bedworth, P. V.; Chen, C.-T.; Ng, D.; Marder, S. R.; Miles, P.; Wada, T.; Tian, M.; Sasabe, H. *Science* **1996**, *273*, 1533–1536; (b) van Nostrum, C. F. *Adv. Mater.* **1996**, *8*, 1027–1030; (c) Bao, Z.; Lovinger, A. J.; Dodabalapur, A. *Adv. Mater.* **1997**, *9*, 42–44; (d) Bao, Z.; Lovinger, A. J.; Brown, J. J. *Am. Chem. Soc.* **1998**, *120*, 207–208; (e) de la Torre, G.; Vázquez, P.; Agulló-López, F.; Torres, T. *J. Mater. Chem.* **1998**, *8*, 1671–1683; (f) Schön, J. H.; Kloc, C.; Bao, Z.; Batlogg, B. *Adv. Mater.* **2000**, *12*, 1539–1542.
2. (a) Ali, H.; van Lier, J. E. *Chem. Rev.* **1999**, *99*, 2379–2450; (b) Lukyanets, E. A. *J. Porphyrins Phthalocyanines* **1999**, *3*, 424–432; (c) Allen, C. M.; Sharman, W. M.; van Lier, J. E. *J. Porphyrins Phthalocyanines* **2001**, *5*, 161–169.
3. See for example (a) Hu, M.; Brasseur, N.; Yildiz, S. Z.; van Lier, J. E.; Leznoff, C. C. *J. Med. Chem.* **1998**, *41*, 1789–1802; (b) Brasseur, N.; Ouellet, R.; La Madeleine, C.; van Lier, J. E. *Br. J. Cancer* **1999**, *80*, 1533–1541; (c) Fabris, C.; Jori, G.; Giuntini, F.; Roncucci, G. *J. Photochem. Photobiol. B: Biol.* **2001**, *64*, 1–7; (d) Huang, J.-D.; Wang, S.; Lo, P.-C.; Fong, W.-P.; Ko, W.-H.; Ng, D. K. P. *New J. Chem.* **2004**, *28*, 348–354; (e) Lo, P.-C.; Huang, J.-D.; Cheng, D. Y. Y.; Chan, E. Y. M.; Fong, W.-P.; Ko, W.-H.; Ng, D. K. P. *Chem. Eur. J.* **2004**, *10*, 4831–4838; (f) Choi, C.-F.; Tsang, P.-T.; Huang, J.-D.; Chan, E. Y. M.; Ko, W.-H.; Fong, W.-P.; Ng, D. K. P. *Chem. Commun.* **2004**, 2236–2237.
4. (a) ZamoraLeon, S. P.; Golde, D. W.; Concha, I. I.; Rivas, C. I.; DelgadoLopez, F.; Baselga, J.; Nualart, F.; Vera, J. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 1847–1852; (b) Kumamoto, K.; Goto, Y.; Sekikawa, K.; Takenoshita, S.; Ishida, N.; Kawakita, M.; Kannagi, R. *Cancer Res.* **2001**, *61*, 4620–4627; (c) Chandler, J. D.; Williams, E. D.; Slavin, J. L.; Best, J. D.; Rogers, S. *Cancer* **2003**, *97*, 2035–2042.
5. Chen, X.; Hui, L.; Foster, D. A.; Drain, C. M. *Biochemistry* **2004**, *43*, 10918–10929, and references cited therein.
6. (a) Laville, I.; Figueiredo, T.; Loock, B.; Pigaglio, S.; Maillard, Ph.; Grierson, D. S.; Carrez, D.; Croisy, A.; Blais, J. *Bioorg. Med. Chem.* **2003**, *11*, 1643–1652; (b) Li, G.; Pandey, S. K.; Graham, A.; Dobhal, M. P.; Mehta, R.; Chen, Y.; Gryshuk, A.; Rittenhouse-Olson, K.; Oseroff, A.; Pandey, R. K. *J. Org. Chem.* **2004**, *69*, 158–172.
7. Zhang, M.; Zhang, Z.; Blessington, D.; Li, H.; Busch, T. M.; Madrak, V.; Miles, J.; Chance, B.; Glickson, J. D.; Zheng, G. *Bioconjugate Chem.* **2003**, *14*, 709–714.
8. Maillard, P.; Guerquin-Kern, J.-L.; Momenteau, M.; Gaspard, S. *J. Am. Chem. Soc.* **1989**, *111*, 9125–9127.
9. MacDonald, I. J.; Dougherty, T. J. *J. Porphyrins Phthalocyanines* **2001**, *5*, 105–129.
10. Characterizing data for **3**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.60–9.67 (m, 8H, Pc–H_α), 8.29–8.35 (m, 8H, Pc–H_β), 4.38 (d, J = 5.1 Hz, 2H, H1), 3.33 (dd, J = 2.1, 5.1 Hz, 2H, H2), 3.23 (dd, J = 2.1, 8.1 Hz, 2H, H3), 0.81 (s, 6H, Me), 0.79 (dd, J = 1.5, 8.1 Hz, 2H, H4), 0.70 (virtual s, 8H, Me and H5), 0.51 (s, 6H, Me), 0.32 (s, 6H, Me), −1.68 (dd, J = 5.4, 8.4 Hz, 2H, OCH), −2.36 (dd, J = 8.4, 9.6 Hz, 2H, OCH). HRMS (FAB) calcd for $C_{56}H_{54}N_8O_{12}Si$ (M^+): 1058.3630; found: 1058.3634. **4**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.61–9.66 (m, 8H, Pc–H_α), 8.29–8.36 (m, 8H, Pc–H_β), 4.38 (d, J = 4.8 Hz, 1H, H1), 3.33 (dd, J = 2.1, 4.8 Hz, 1H, H2), 3.23 (dd, J = 2.1, 8.4 Hz, 1H, H3), 0.81 (s, 3H, Me), 0.78 (dd, J = 1.8, 8.4 Hz, 1H, H4), 0.70 (virtual s, 4H, Me and H5), 0.51 (s, 3H, Me), 0.32 (s, 3H, Me), −1.72 (dd, J = 5.1, 8.4 Hz, 1H, OCH), −2.05 to −1.96 (m, 5H, OEt), −2.37 (dd, J = 8.4, 9.6 Hz, 1H, OCH). HRMS (FAB) calcd for $C_{46}H_{40}N_8O_7Si$ (M^+): 844.2789; found: 844.2763. **5**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.59–9.65 (m, 8H, Pc–H_α), 8.29–8.35 (m, 8H, Pc–H_β), 4.38 (d, J = 5.1 Hz, 1H, H1), 3.33 (dd, J = 2.1, 5.1 Hz, 1H, H2), 3.23 (dd, J = 2.1, 8.1 Hz, 1H, H3), 0.81 (virtual s, 4H, Me and H4), 0.70 (virtual s, 4H, Me and H5), 0.51 (s, 3H, Me), 0.32 (s, 3H, Me), −0.14 (t, J = 7.5 Hz, 3H, Me), −0.42 to −0.32 (m, 2H, CH_2), −1.48 to −1.38 (m, 2H, CH_2), −1.72 to −1.62 (m, 3H, CH_2 and OCH), −2.07 (t, J = 6.3 Hz, 2H, OCH_2), −2.37 (dd, J = 8.4, 9.6 Hz, 1H, OCH). HRMS (FAB) calcd for $C_{49}H_{46}N_8O_7Si$ (M^+): 886.3258; found: 886.3229. **6**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.59–9.66 (m, 8 H, Pc–H_α), 8.30–8.33 (m, 8H, Pc–H_β), 4.38 (d, J = 5.1 Hz, 1H, H1), 3.33 (dd, J = 2.1, 5.1 Hz, 1H, H2), 3.23 (dd, J = 2.1, 8.1 Hz, 1H, H3), 0.78–0.90 (m, 6H, Me, H4, and CH_2), 0.64–0.72 (m, 7H, Me, H5, and CH_2CH_3), 0.48–0.56 (m, 5H, Me and CH_2), 0.32 (s, 3H, Me), 0.10–0.22 (m, 2H, CH_2), −0.52 to −0.42(m, 2H, CH_2), −1.50 to −1.40 (m, 2H, CH_2), −1.72 to −1.62 (m, 3H, CH_2 and OCH), −2.09 (t, J = 6.6 Hz, 2H, OCH_2), −2.37 (dd, J = 8.4, 9.6 Hz, 1H, OCH). HRMS (FAB) calcd for $C_{52}H_{52}N_8O_7Si$ (M^+): 928.3728; found: 928.3731.
11. The unsubstituted zinc(II) phthalocyanine was used as reference (Φ_f = 0.30 in 1-chloronaphthalene, Φ_Δ = 0.56 in DMF). See Ng, A. C. H.; Li, X.-y.; Ng, D. K. P. *Macromolecules* **1999**, *32*, 5292–5298.
12. Spiller, W.; Kliesch, H.; Wöhrle, D.; Hackbarth, S.; Röder, B.; Schnurpfeil, G. *J. Porphyrins Phthalocyanines* **1998**, *2*, 145–158.
13. Li, X.-y.; He, X.; Ng, A. C. H.; Wu, C.; Ng, D. K. P. *Macromolecules* **2000**, *33*, 2119–2123, and references cited therein.
14. Oleinick, N. L.; Morris, R. L.; Belichenko, I. *Photochem. Photobiol. Sci.* **2002**, *1*, 1–21.