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Synthesis and Phototoxic Property of Tetra- and Octa-Glycoconjugated Tetraphenylchlorins

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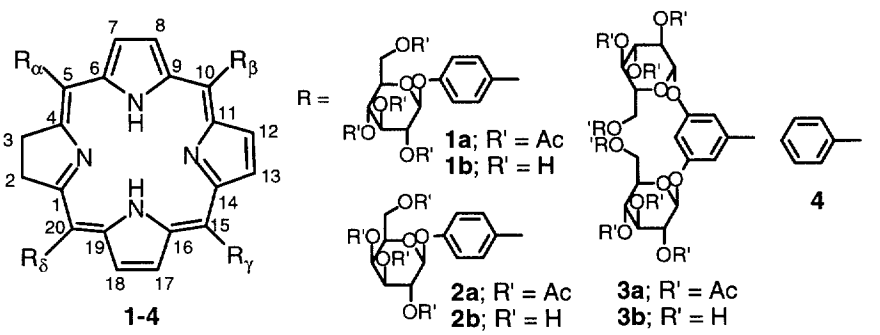
Abstract
New tetraphenylchlorin derivatives having four or eight glucose molecules were synthesized. Phototoxicity against the HeLa cell, singlet oxygen producing ability, and cell permeability were examined to evaluate the activity on photodynamic therapy of the compounds.

Keywords: photodynamic therapy; porphyrin and analogues; glycosides; oxygen, singlet

Photodynamic therapy (PDT) is one of the most clean and effective treatments for cancer.[1,2] To obtain the water-solubility and penetration activity into cells, several porphyrin derivatives linked to sugar moieties have been synthesized as a photosensitizer for PDT.[3-6] Recently, we reported the necessity of screening of the sugar moiety and protective group of the glycoconjugated tetraphenylporphyrins as sugar-linked photosensitizers.[7] Here we report the synthesis of sugar-linked chlorins which have markedly large molar extinction coefficient on long-wavelength region compared with corresponding porphyrins.[8] In addition to spectroscopic character, their activity for singlet oxygen production, phototoxicity against the HeLa cell, and cell permeability using microscopic analysis were studied.

Chart I.



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Compounds **1-3a** were prepared by the diimide reduction[9,10] of corresponding porphyrins.[4,7] Overreduction was occurred, however, chloranil oxidation was effective to obtain the chlorin (57-73 %). Deprotection of these compounds by NaOMe in MeOH/$CHCl_3$ gave **1-3b** quantitatively.[11] It was difficult to obtain the N-acetylglucosamine derivative from corresponding porphyrin by similar procedure due to the problem of solubility of the product.

The electronic absorption spectra of these chlorins were recorded in DMSO. The λ_{max} and ε of the compounds are summarized in Table 1. All compounds have larger molar extinction coefficient on their Q_I band compared with corresponding porphyrin derivative **5** having four OH-protected glucoses (*i. e.* porphyrin analog of compound **1a**).

Table 1. Spectral Data of Sugar-linked Chlorins in DMSO

Compound	λ_{max} /nm (ε /10^4 $M^{-1}cm^{-1}$)				
	Soret	Band IV	Band III	Band II	Band I
1a	423 (20.1)	523 (1.48)	549 (1.19)	598 (0.63)	652 (3.34)
1b	423 (16.5)	523 (1.22)	550 (1.03)	598 (0.53)	652 (2.80)
2a	423 (12.6)	523 (1.02)	550 (0.80)	599 (0.44)	652 (2.15)
2b	423 (12.9)	523 (0.90)	551 (0.77)	599 (0.38)	652 (2.01)
3a	420 (17.8)	517 (1.31)	543 (0.76)	599 (0.52)	652 (3.15)
3b	422 (13.3)	519 (0.91)	546 (0.54)	599 (0.33)	653 (2.32)
5[a]	421 (45.4)	517 (1.75)	554 (1.04)	593 (0.52)	648 (0.56)

[a] From ref. 7.

The photosensitizing ability of these compounds yielding singlet oxygen ($^1O_2$) was evaluated by degradation of diphenylisobenzofuran (DPBF) in DMSO at long wavelength region ($\lambda > 600$ nm).[7] The observed rate constants (k_{obs}) are listed in Table 2. Under the same experimental conditions, these sugar-linked chlorins were more effective than tetraphenylporphyrin tetrasulfonic acid (TPPS) which is known as photosensitizers that produce singlet oxygen efficiently.[12,13] Sugar-linked porphyrin derivative **5** was also proved to have lesser activity. The tetragalactosylated compounds **2** exhibited rather small k_{obs} values, presumably due to their small molar extinction coefficients at Q bands.

Table 2. Observed Rate Constants of DPBF Degradation by Singlet Oxygen in DMSO Solution[a]

Compound	**1a**	**1b**	**2a**	**2b**	**3a**	**3b**	TPPS	**5**
k_{obs} /10^{-2}·s^{-1}	1.2	0.94	0.68	0.62	1.1	1.1	0.24	0.34

[a] At 27 °C. Errors are within ± 5 %. Initial concentrations: [sensitizer] = 6.5×10^{-7} M, [DPBF] = 4.4×10^{-5} M. Light source: 250 W halogen lamp ($\lambda > 600$ nm).

The photocytotoxic properties of these glycosylated sensitizers were evidenced against the HeLa cell line. The cell survival is plotted as a function of concentration of sensitizers in Figure 1, in which the results of tetraphenylchlorin **4** and porphyrin derivative **5** are included.

Since almost no remarkable difference in the photodynamic efficiency for generating singlet oxygen among all the chlorin compounds was observed, the *in vitro* photocytotoxic results reflects the extent of the incorporation of the drug into the cell. In constrast to the results from porphyrin derivatives,[7] the higher activity was observed in OH-free compounds for tetraglycoconjugated chlorins **1** and **2**. This is in good agreement to the tumor localizing property of chlorin derivatives prepared from pheophorbide.[14] The opposite effect of OH-protection was observed in **3**. Octa-glucosylated derivative **3b** exhibited small phototoxicity probably arising from its poor transmembrane activity owing to excessive hydrophilicity. Compound **5**, in spite of the lesser activity of singlet oxygen production in the present experimental condition ($\lambda > 600$ nm), was the most effective sensitizer indicating the superior membrane permeability of this compound.[15]

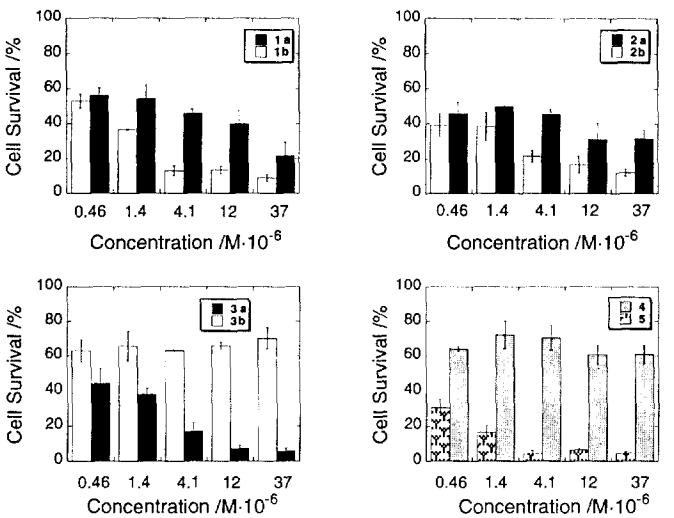

Figure 1. Photocytotoxicity of compounds **1-5** against the HeLa cell. Irradiation time was 8 min; the percentage of cell survival was determined by MTT assay after 24 h incubation based on the value for no drug treatment. Light source: 500 W halogen lamp ($\lambda > 600$ nm), fluence 50 mW/cm^2.

The higher reactivity of compounds **1-3** than **4** demonstrates the benefit of sugar moiety in glycoconjugated photosensitizers. For the sake of strong fluorescent ability of chlorins, the effect of sugar moiety in drug incorporation into cells were investigated by fluorescent mycroscopic analysis. After the exposure of HeLa cell to photosensitizers (**1a**, **3a** and **4**) for 2 hours, the cells were washed with buffer and fluorescence from cells were monitored. Figure 2 clearly shows the advantage of introduction of sugar moiety in membrane permeability of

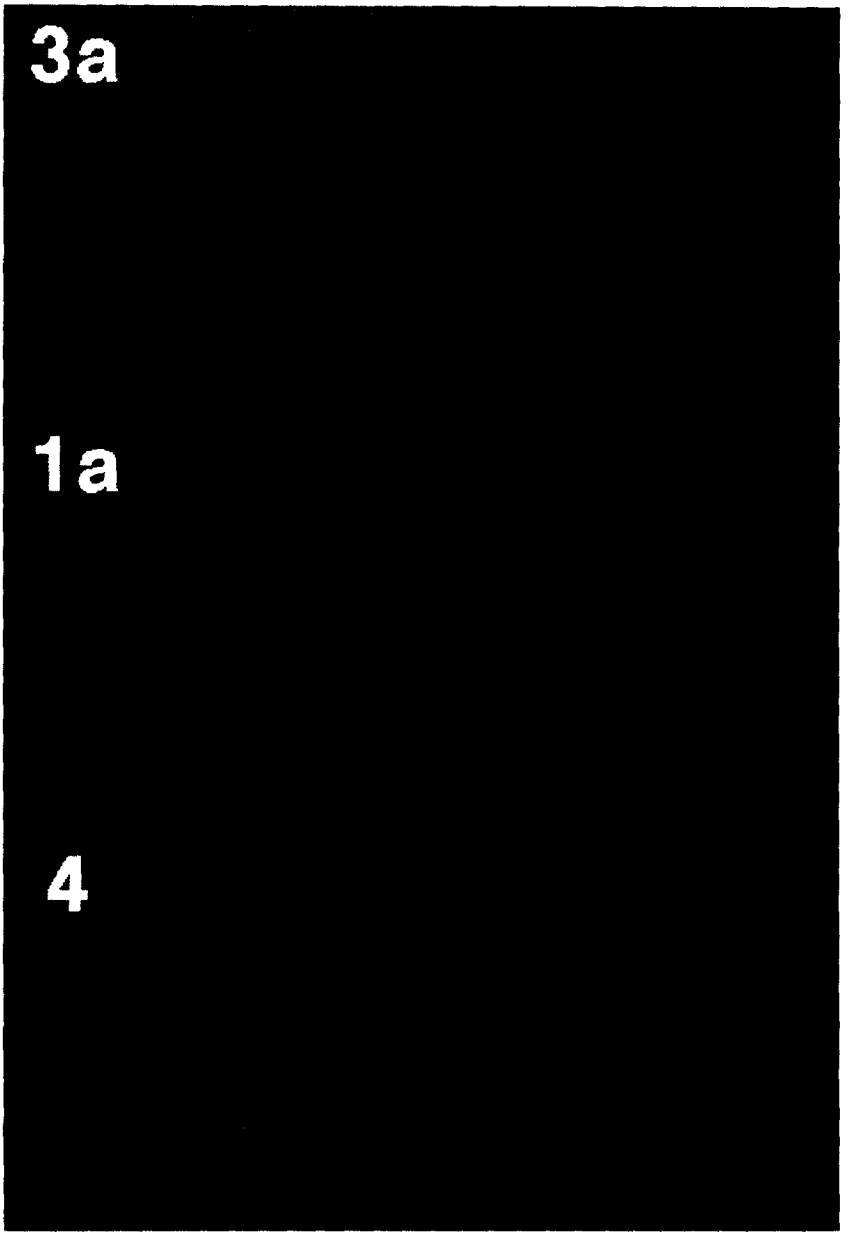

Figure 2. Fluorescent mycroscopic analysis of compounds **1a**, **3a**, and **4** incorporated into the HeLa cell. Left: under white light. Right: under excitaion light (400-440 nm).

octaglycosilated porphyrin **3a**. These observations are very important for the exploration of new glycoconjugated photosensitizers.

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11. Selected data: For **1a**, ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ -1.479 (2H, brs, $W_{1/2}$ = 5.4 Hz. NH), 2.091 (6H, s, Ac × 2), 2.093 (12H, s, Ac × 4), 2.097 (6H, s, Ac × 2), 2.105 (6H, s, Ac × 2), 2.112 (6H, s, Ac × 2), 2.185 (6H, s, Ac × 2), 2.194 (6H, s, Ac × 2), 4.018 (2H, ddd, J = 10.0, 5.4, 2.5 Hz, α,δGlc-H5), 4.032 (2H, ddd, J = 10.0, 5.4, 2.4 Hz, β,γGlc-H5), 4.141 (4H, brs, $W_{1/2}$ = 3.5 Hz, H2,3), 4.282 (2H, dd, J = 12.3, 2.5 Hz, α,δGlc-H6a), 4.285 (2H, dd, J = 12.3, 2.4 Hz, β,γGlc-H6a), 4.395 (2H, dd, J = 12.3, 5.4 Hz, α,δGlc-H6b), 4.401 (2H, dd, J = 12.3, 5.4 Hz, β,γGlc-H6b), 5.265-5.302 (4H, m, Glc-H4), 5.375 (2H, d, J = 7.8 Hz, α,δGlc-H1), 5.410 (2H, d, J = 7.8 Hz, β,γGlc-H1), 5.404-5.471 (8H, m, Glc-H2,3), 7.296-7.317 (8H, m, Ar-H3,5), 7.779 (4H, d, J = 8.7 Hz, α,δAr-H2,6), 8.009 (4H, d, J = 8.7 Hz, β,γAr-H2,6), 8.178 (2H, d, J = 4.9 Hz, H7,18), 8.408 (2H, s, H12,13), 8.563 (2H, d, J = 4.9 Hz, H2,17). HRMS (FAB) calcd for $C_{100}H_{104}N_4O_{40}$ 2000.6227, found 2000.6146.

For **1b**, ^{1}H NMR (CD_3OD, 600.13 MHz) δ 3.492-3.533 (4H, m, Glc-H4), 3.563-3.660 (12H, m, Glc-H2,3,5), 3.806 (2H, dd, J = 12.0, 5.5 Hz, α,δGlc-H6a), 3.815 (2H, dd, J = 12.0, 5.5 Hz, β,γGlc-H6a), 3.989 (2H, dd, J = 12.0, 2.4 Hz, α,δGlc-H6b), 4.003 (2H, dd, J = 12.0, 2.5 Hz, β,γGlc-H6b), 4.112 (4H, brs, $W_{1/2}$ = 3.7 Hz, H2,3), 5.176 (2H, d, J = 7.4 Hz, α,δGlc-H1), 5.228 (2H, d, J = 7.5 Hz, β,γGlc-H1), 7.417 (4H, d, J = 8.5 Hz, α,δAr-H3,5), 7.453 (4H, d, J = 8.5 Hz, β,γAr-H3,5), 7.742 (4H, d, J = 8.5 Hz, α,δAr-H2,6), 7.975 (4H, d, J = 8.5 Hz, β,γAr-H2,6), 8.190 (2H, d, J = 4.9 Hz, H7,18), 8.367 (2H, s, H12,13), 8.574 (2H, d, J = 4.9 Hz, H8,17). HRMS (FAB) calcd for $C_{68}H_{73}N_4O_{24}$ (M + H) 1329.4615, found 1329.4633.

For **2a**, ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ -1.473 (2H, brs, $W_{1/2}$ = 5.7 Hz, NH), 2.063 (6H, s, Ac × 2), 2.071 (12H, s, Ac × 4), 2.074 (6H, s, Ac × 2), 2.199 (6H, s, Ac × 2), 2.208 (6H, s, Ac × 2), 2.248 (6H, s, Ac × 2), 2.252 (6H, s, Ac × 2), 4.146 (4H, brs, $W_{1/2}$ = 5.4 Hz, H2,3), 4.197-4.282 (8H, m, Gal-H5,6a), 4.359 (4H, dd, J = 11.2, 6.8 Hz, Gal-H6b), 5.237 (2H, dd, J = 10.5, 3.4 Hz, α,δGal-H3), 5.248 (2H, dd, J = 10.5, 3.4 Hz, β,γGal-H3), 5.339 (2H, d, J = 8.0 Hz, α,δGal-H1), 5.375 (2H, d, J = 8.0 Hz, β,γGal-H1), 5.546 (2H, brd, J = 3.4 Hz, α,δGal-H4), 5.552 (2H, brd, J = 3.4 Hz, β,γGal-H4), 5.650 (2H, dd, J = 10.5, 8.0 Hz, α,δGal-H2),

5.669 (2H, dd, J = 10.5, 8.0 Hz, β,γGal-H2), 7.318 (4H, brd, J = 8.6 Hz, α,δAr-H3,5), 7.325 (4H, brd, J = 8.7 Hz, β,γAr-H3,5), 7.783 (4H, d, J = 8.6 Hz, α,δAr-H2,6), 8.013 (4H, d, J = 8.7 Hz, β,γAr-H2,6), 8.179 (2H, s, J = 4.7 Hz, H7,18), 8.411 (2H, s, H12,13), 8.565 (2H, d, J = 4.7 Hz, H8,17). HRMS (FAB) calcd for $C_{100}H_{104}N_4O_{40}$ 2000.6227, found 2000.6213.

For **2b**, ^{1}H NMR (CD_3OD, 600.13 MHz) δ 3.695 (2H, dd, J = 9.7, 3.4 Hz, α,δGal-H3), 3.717 (2H, dd, J = 9.8, 3.4 Hz, β,γGal-H3), 3.802-3.862 (8H, m, Gal-H5,6a), 3.878-3.928 (4H, m, Gal-H6b), 3.952 (2H, dd, J = 9.7, 7.8 Hz, α,δGal-H2), 3.974 (2H, dd, J = 9.8, 7.7 Hz, β,γGal-H2), 3.985 (2H, brd, J = 4.1 Hz, α,δGal-H4), 3.992 (2H, brd, J = 4.4 Hz, β,γGal-H5), 4.118 (4H, brs, $W_{1/2}$ = 3.6 Hz, H2,3), 5.126 (2H, d, J = 7.8 Hz, α,δGal-H1), 5.180 (2H, d, = 7.7 Hz, β,γGal-H1), 7.430 (4H, d, J = 8.6 Hz, α,δAr-H3,5), 7.467 (4H, d, J = 8.3 Hz, β,γAr-H3,5), 7.744 (4H, d, J = 8.6 Hz, α,δAr-H2,6), 7.977 (4H, d, J = 8.3 Hz, β,γAr-H2,6), 8.200 (2H, d, J = 4.8 Hz, H7,18), 8.373 (2H, s, H12,13), 8.589 (2H, d, J = 4.8 Hz, H8,17). HRMS (FAB) calcd for $C_{68}H_{73}N_4O_{24}$ (M + H) 1329.4615, found 1329.4614.

For **3a**, ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ -1.675 (2H, brs, $W_{1/2}$ = 5.5 Hz, NH), 1.381 (6H, s, Ac × 2), 1.468 (6H, s, Ac × 2), 1.476 (6H, s, Ac × 2), 1.584 (6H, s, Ac × 2), 1.969 (6H, s, Ac × 2), 1.983 (6H, s, Ac × 2), 1.984 (6H, s, Ac × 2), 1.991 (6H, s, Ac × 2), 2.015 (12H, s, Ac × 4), 2.018 (6H, s, Ac × 2), 2.021 (6H, s, Ac × 2), 2.077 (6H, s, Ac × 2), 2.090 (6H, s, Ac × 2), 2.101 (6H, s, Ac × 2), 2.107 (6H, s, Ac × 2), 3.783 (2H, ddd, J = 10.0, 5.6, 2.3 Hz, β,γ-3-Glc-H5), 3.808-3.837 (4H, m, α,β,γ,δ-5-Glc-H5), 3.859 (2H, ddd, J = 10.0, 5.6, 2.4 Hz, α,δ-3-Glc-H5), 3.967 (2H, dd, J = 12.0, 2.3 Hz, β,γ-3-Glc-H6a), 4.020-4.043 (4H, m, α,δ-5-Glc-H6a, β,γ-5-Glc-H6a), 4.074 (2H, dd, J = 12.2, 2.4 Hz, α,δ-3-Glc-H6a), 4.099 (2H, dd, J = 12.0, 5.6 Hz, β,γ-3-Glc-H6b), 4.133-4.170 (6H, m, β,γ-5-Glc-H6b, α,δ-3-Glc-H6b, α,δ-5-Glc-H6b), 4.205 (4H, brs, $W_{1/2}$ = 3.0 Hz, H2,3), 5.117-5.173 (8H, m, Glc-H4), 5.282-5.349 (24H, m, Glc-H1,2,3), 6.940 (2H, dd, J = 2.2, 2.2 Hz, α,δAr-4), 7.005 (2H, dd, J = 2.2, 2.2 Hz, β,γAr-4), 7.190 (2H, dd, J = 2.2, 1.3 Hz, α,δAr-2), 7.212 (2H, dd, J = 2.2, 1.3 Hz, α,δAr-6), 7.436 (2H, dd, J = 2.2, 1.3 Hz, β,γAr-2), 7.447 (2H, dd, J = 2.2, 1.3 Hz, β,γAr-6), 8.310 (2H, d, J = 4.9 Hz, H7,18), 8.481 (2H, s, H12,13), 8.661 (2H, d, J = 4.9 Hz, H8,17). HRMS (FAB) calcd for $C_{156}H_{176}N_4O_{80}$ 3384.9827, found 3384.9766.

For **3b**, ^{1}H NMR (CD_3OD, 600.13 MHz) δ 3.344-3.426 (8H, m, Glc-H4), 3.463-3.552 (24H, m, Glc-H2,3,5), 3.654-3.710 (8H, m, Glc-H6a), 3.844-3.894 (8H, m, Glc-H6b), 4.196-4.255 and 4.282-4.341 (4H, A_2B_2-type, H2,3), 5.143 (2H, d, J = 7.6 Hz, α,δ-3-Glc-H1), 5.163 (2H, d, J = 7.5 Hz, α,δ-5-Glc-H1), 5.195 (4H, d, J = 7.5 Hz, β,γGlc-H1), 7.200 (2H, brt, J = 2.2 Hz, α,δAr-H4), 7.274-7.293 (8H, m, α,δAr-H2,6, β,γAr-H4), 7.487-7.500 (4H, m, β,γAr-H2,6), 8.342 (2H, d, J = 4.9 Hz, H7,18), 8.482 (2H, s, H12,13), 8.713 (2H, d J = 4.9 Hz, H8,17). HRMS (FAB) calcd for $C_{92}H_{112}O_{48}N_4Na$ (M + Na) 2063.6344, found 2063.6292

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15. The reason of this surprising result was preliminary investigated. Octanol/water partition coefficients of these compounds showed the sugar dependent lipophilicity change, however, no meaningful difference between compounds **1a** and **5** was detected. Further details are now under investigation in our laboratory.