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SUGAR-DEPENDENT PHOTOCYTOTOXIC PROPERTY OF TETRA- AND OCTA-GLYCOCONJUGATED TETRAPHENYLPORPHYRINS

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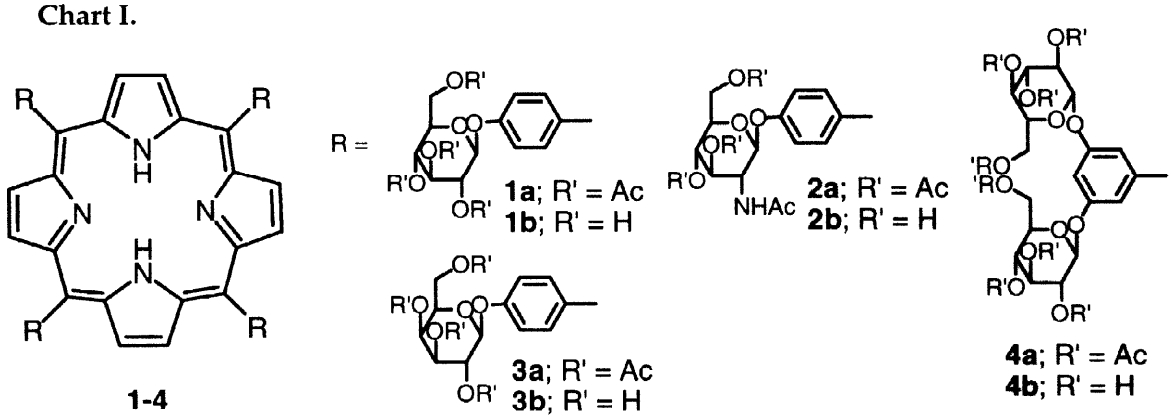
Abstract: New tetraphenylporphyrin derivatives having eight glucose molecules were synthesized. Phototoxicity of these compounds against the HeLa cell line were compared to tetraglycosylated tetraphenylporphyrins. The highest activity was observed for a derivative having four OH-protected glucose moieties. Singlet oxygen producing ability was also examined to explain the difference in photocytotoxicities.

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

New photosensitizers in the photodynamic therapy (PDT) have been extensively explored in this decade.[1,2] Since the antitumor activity is believed to be the result of activation of molecular oxygen by the sensitizer incorporated into the cancer cell, many efforts have been made to increase the singlet oxygen quantum efficiency and cellular recognition property of the chromophore.[3-5] Recently, many porphyrins linked to sugar moieties have been synthesized and some derivatives have been proved to possess an activity in PDT.[6-13] However, no systematic study focused on the phototherapeutic effect of the hydrophilic nature of sugar-linked porphyrin derivatives has been reported. It is therefore, important to check out the correlation between the hydrophilic property of the compound and the phototherapeutic

Chart I.



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activity. Here we report the *in vitro* photocytotoxicity of four families of OH-protected and unprotected sugar-linked tetraphenylporphyrins, in order to clarify the effect of amphiphilic character and carbohydrate recognition on photodynamic activity.

Compounds **1-3** were prepared by the literature methods.[8,9] Octaglucosylated derivative **4a** was synthesized by modified Lindsey's method[9] in 32 % yield from bis(tetraacetylglucopyranosyloxy)benzaldehyde (**5**), which was obtained by condensation of 3,5-dihydroxybenzaldehyde with acetobromoglucose[14] in the presence of Ag_2O[15] (90 %). Deprotection of **4a** by NaOMe in MeOH gave **4b** quantitatively. All new compounds gave expected spectrum properties and sufficient purity.[16]

The electronic absorption spectra of these porphyrins were recorded in DMSO. The λ_{max} and ε of the compounds are summarized in Table 1. Compounds **4a** and **4b** have rather smaller molar extinction coefficients at bands I and III, which might indicate the development of asymmetry in the π-electron cloud.[17]

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

	λ_{max} /nm (ε /10^4 $M^{-1}cm^{-1}$)				
Compound	Soret	Band IV	Band III	Band II	Band I
1a	421 (45.4)	517 (1.75)	554 (1.04)	593 (0.52)	648 (0.56)
1b	423 (46.2)	518 (1.74)	555 (1.17)	593 (0.54)	648 (0.63)
2a	422 (48.7)	517 (1.87)	554 (1.16)	594 (0.57)	648 (0.62)
2b	422 (43.5)	518 (1.59)	554 (1.05)	593 (0.49)	648 (0.56)
3a	421 (40.7)	517 (1.59)	553 (1.16)	587 (0.67)	648 (0.52)
3b	423 (46.7)	518 (1.76)	553 (1.18)	593 (0.56)	649 (0.65)
4a	419 (41.0)	453 (1.86) 513 (1.96)	547 (0.59)	587 (0.67)	642 (0.37)
4b	422 (36.8)	452 (1.66) 515 (1.68)	550 (0.56)	588 (0.57)	642 (0.35)

The photosensitizing ability of these compounds, yielding singlet oxygen ($^1O_2$), was evaluated by degradation of diphenylisobenzofuran (DPBF) in DMSO.[18] The observed rate constants (k_{obs}) are listed in Table 2. Retardation by sodium azide, which is a well-known singlet oxygen quencher, confirms that this reaction is accompanied with generation of singlet oxygen. Under the same experimental conditions, these sugar-linked porphyrins were almost as effective as hematoporphyrin (HP) and tetraphenylporphyrin tetrasulfonic acid (TPPS) which are known as photosensitizers that produce singlet oxygen efficiently.[19,20] No remarkable difference in photodynamic activity among the sugar moieties of the sensitizer was found. The octaglucosylated compounds **4** exhibited slightly 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**	**1a**[b]	**1b**	**2a**	**2b**	**3a**	**3b**	**4a**	**4b**	HP	TPPS
k_{obs} /10^{-2}·s^{-1}	1.6	0.013	1.5	1.7	1.5	1.6	1.9	1.2	1.0	1.3	1.5

[a] Errors are within ± 5 %. Initial concentrations: [sensitizer] = 6.5×10^{-7} M, [DPBF] = 4.4×10^{-5} M. Light source: 250 W halogen lamp ($\lambda > 500$ nm). [b] In the presence of 9.5×10^{-2} M of NaN_3.

The photocytotoxic properties of these glycosylated sensitizers were evidenced against the HeLa cell line. 10^3 cancer cell incubated in the growth media (100 µl of MEM (Minimum Essential Medium) contains 10 % of Fetus Bovine Serum) at 37 °C overnight was incubated for 2 more hours in the presence of photosensitizer . The cell was washed with cold MEM, 200 µl of MEM was added, and irradiated for 8 minutes (light source: 500 W halogen lamp ($\lambda > 500$ nm), fluence 65 mW/cm^2). Incubation was continued for 24 more hours and the number of surviving cells was analyzed by MTT assay. The cytotoxicity of these compounds in the dark was found to be small at low drug concentrations (*e.g.* ~15 % at 10^{-5} M). The cell survival is plotted as a function of concentration of sensitizers in Figure 1, in which the results of TPPS and α,β,γ,δ-terakis(1-methylpyridinium-4-yl)porphyrin *p*-toluenesulfonate (TMPyP) are included for comparison. Most reactive derivative **1a** was also examined at lower concentrations and was revealed to be at least 10 times more reactive than TPPS and TMPyP (Data not shown).

The effect of protection of the hydroxyl group in the carbohydrate moiety was drastic in tetraglucose-linked porphyrin **1**. Similar but smaller effect was seen in **2**. Almost no effect of OH protection was observed in **3**. Comparing the data of porphyrins that have four protected sugar parts (*i.e.*, **1a**, **2a** and **3a**), tetraacetylglucose was found to bring about the most effective results in a series of glycopyranosyl substituents examined here.

Since the photodynamic efficiency in generating singlet oxygen is almost identical in all the compounds, the *in vitro* photocytotoxic results of tetracarbohydrated porphyrins **1-3** indicate that the glucose moiety protected with acetyl groups specifically increases the incorporation of the drug into the cell. Octa-glucosylated derivative **4a**, which was synthesized in order to increase the site of recognition toward the membrane permeability, did not exhibit any marked effect, probably because of its bulky spherical structure. A globular structure with the highly water-soluble character of **4b** was inferior to a flat hydrophobic form like compound **1a** with respect to the incorporation of photosensitizers into the cell.

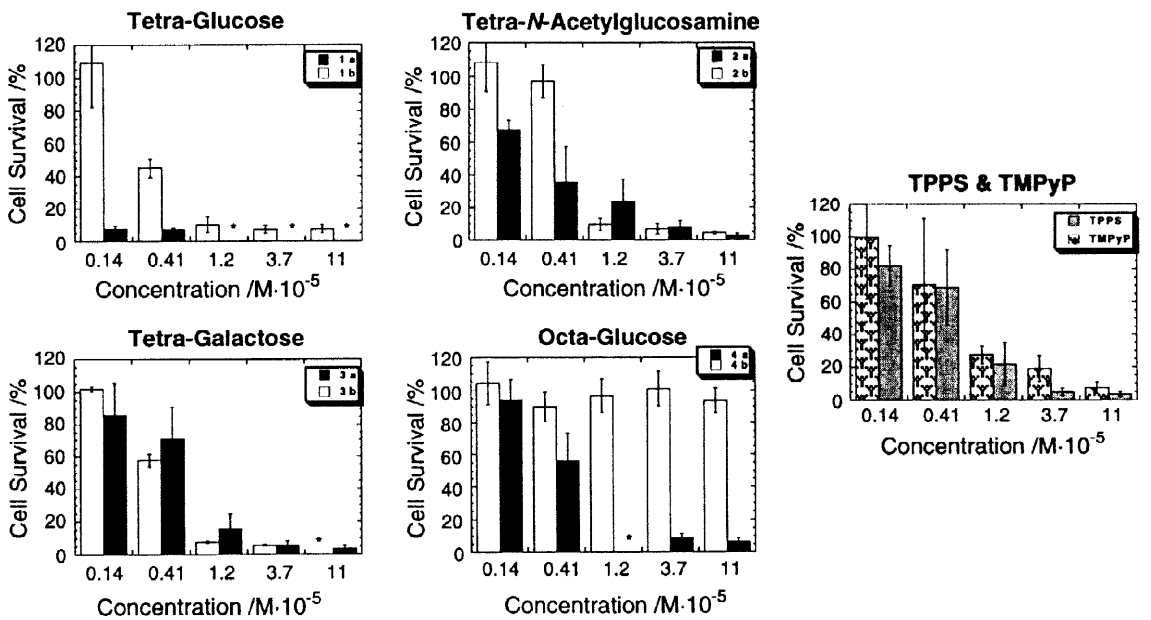

Figure 1. Photocytotoxicity of compounds **1-4** against the HeLa cell. Irradiation time was 8 min; the percentage of cell survival was determined by MTT assay after 24 h incubation. Light source: 500 W halogen lamp ($\lambda > 500$ nm), fluence 65 mW/cm^2. * = Not determined.

The high reactivity of compound **1a** demonstrates the necessity of screening of the sugar moiety and protective group of the sugar-linked photosensitizers. These observations could provide important information for the exploration of new glycoconjugated photosensitizers.

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16. Selected data: For **4a**, ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ -2.99 (2H, s, NH), 1.37 (24H, s, -OAc), 1.97 (24H, s, -OAc), 2.02 (24H, s, -OAc), 2.10 (24H, s, -OAc), 3.81 (8H, ddd, J = 10.0, 5.6, 2.3 Hz, Glc-H-5), 4.00 (8H, dd, J = 12.2, 2.3 Hz, Glc-H-6), 4.13 (8H, dd, J = 12.2, 5.6 Hz, Glc-H-6), 5.15 (8H, dd, J = 10.0, 9.1 Hz, Glc-H-4), 5.30-5.35 (16H, m, Glc-H-2, Glc-H-3), 5.39 (8H, d, J = 7.8 Hz, Glc-H-1), 7.07 (4H, t, J = 2.2 Hz, Ar-H-4), 7.55 (8H, d, J = 2.2 Hz, Ar-H-2), 8.93 (8H, s, pyrrole-H). Anal. calcd for $C_{156}H_{174}N_4O_{80}\cdot 0.5CHCl_3$: C, 54.57; H, 5.11; N, 1.63. Found: C, 54.54; H, 5.12; N, 1.68. HRMS (FAB) calcd for $C_{156}H_{174}N_4O_{80}Na$ (M + Na) 3405.9568, found 3405.9556.
For **4b**, ^{1}H NMR (CD_3OD, 600.13 MHz) δ 3.41 (8H, dd, J = 9.9, 8.6 Hz, Glc-H-4), 3.51 (8H, dd, J = 9.2, 8.6 Hz, Glc-H-3), 3.53 (8H, ddd, J = 9.9, 5.8, 2.0 Hz, Glc-H-5), 3.55 (8H, dd, J = 9.2, 7.6 Hz, Glc-H-2), 3.70 (8H, dd, J = 12.2, 5.8 Hz, Glc-H-6), 3.89 (8H, dd, J = 12.2, 2.0 Hz, Glc-H-6), 5.24 (8H, d, J = 7.6 Hz, Glc-H-1), 7.35 (4H, t, J = 2.1, Ar-H-4), 7.61 (8H, d, J = 2.1, Ar-H-2), 8.97 (8H, brs, $W_{1/2}$ = 75 Hz, pyrrole-H). Anal. calcd for $C_{92}H_{110}N_4O_{48}\cdot 17H_2O$: C, 47.10; H, 6.19; N, 2.39. Found: C, 47.06; H, 5.63; N, 2.39. HRMS (FAB) calcd for $C_{92}H_{111}N_4O_{48}$ (M + H) 2039.6368, found 2039.6372.
For **5**, ^{1}H NMR ($CDCl_3$, 600.13 MHz) δ 2.04 (6H, s, -OAc), 2.06 (6H, s, -OAc), 2.07 (6H, s, -OAc), 2.10 (6H, s, -OAc), 3.94 (2H, ddd, J = 10.1, 5.9, 2.4 Hz, Glc-H-5), 4.19 (2H, dd, J = 12.3, 2.4 Hz, Glc-H-6), 4.24 (2H, dd, J = 12.3, 5.9 Hz, Glc-H-6), 5.14 (2H, dd, J = 10.1, 9.2 Hz, Glc-H-4), 5.19 (2H, d, J = 7.7 Hz, Glc-H-1), 5.26 (2H, dd, J = 9.4, 7.7 Hz, Glc-H-2), 5.32 (2H, dd, J = 9.4, 9.2 Hz, Glc-H-3), 6.86 (1H, t, J = 2.3 Hz, Ar-H-4), 7.21 (2H, d, J = 2.3 Hz, Ar-H-2), 9.90 (1H, s, -CHO). Anal. calcd for $C_{35}H_{42}O_{21}\cdot CHCl_3$: C, 47.09; H, 4.72. Found: C, 47.08; H, 4.72. HRMS (FAB) calcd for $C_{35}H_{41}O_{21}$ (M – H) 797.2140, found 797.2150.
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