meso-Tetraphenylporphyrin Having Hexa-maltosyl and Decyl Chain as an Amphiphilic Photosensitizer toward Photodynamic Therapy

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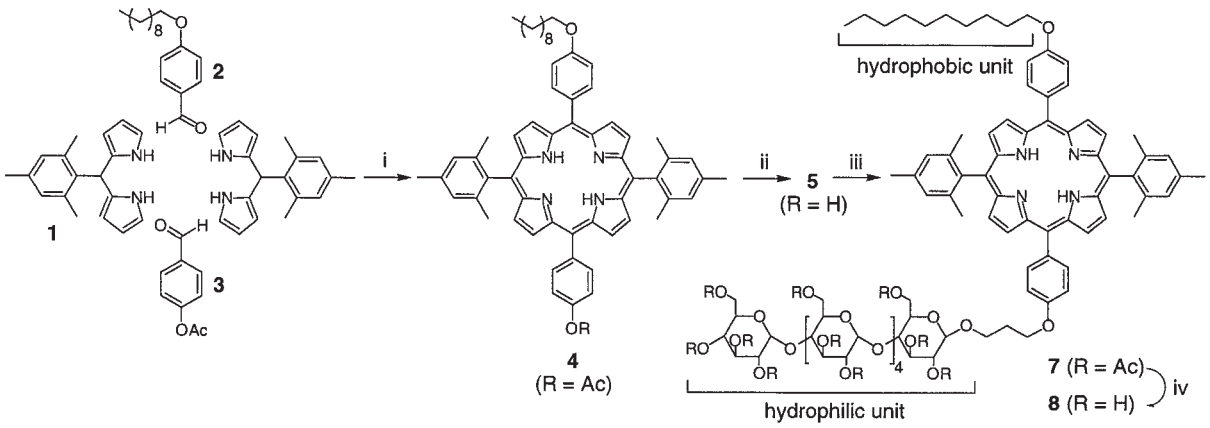
meso-Tetraphenylporphyrin having hexa-maltosyl and decyl chain was synthesized *via* dipyrromethane coupling. Its singlet oxygen producing ability and phototoxicity against the HeLa cell were estimated.

meso-Tetraphenylporphyrins (TPP) and chlorins comprise a new class of photosensitizers (PSs), which are promising molecules in the treatment of cancer, i.e., photodynamic therapy (PDT).[1] We have prepared a series of new TPP and chlorin derivatives having mono- and disaccharides and examined their singlet oxygen producing ability and photocytotoxicity against the HeLa cell.[2,3] The amphiphilic property of PS is one of the important characteristics for PDT, so that it is necessary to suitably design porphyrin possessing hydrophilic and hydrophobic substituents. Recently, the importance of oligosaccharide has been recognized for its specific interaction with the cell membrane, indicating that incorporating oligosaccharide, highly hydrophilic molecule, into porphyrin is expected to increase the selective accumulation of porphyrin on the desired tissue.[4] In addition, lipophilicity of the functional group attached to the porphyrin increases the affinity to the cell membrane. In this study, we designed a novel amphiphilic porphyrin, i.e., phenylporphyrin substituted with oligosaccharide and alkyl long chain. Scheme 1 shows our synthetic strategy. For the preparation of the decanoxy- and hydroxy-substituted porphyrin as a starting material, we used an effective method for producing phenylporphyrin with two different substituted in 5,15-positions as the key reaction, which has been developed by Lindsey's group.[5,6] In addition, PDT activity of the porphyrin having hexa-maltosyl and decyl groups was evaluated using HeLa cells.

A mixture of three porphyrins, which was obtained from the acid-catalyzed condensation of 5-mesityldipyrromethane (**1**) with 4-decanoxybenzaldehyde (**2**) and 4-acetoxybenzaldehyde (**3**), was easily separated by column chromatography to give an asymmetrical porphyrin, 5-(4-acetoxyphenyl)-15-(4-decanoxyphenyl)-10,20-dimesitylporphyrin (**4**) (yield, 17.5%). The deacetylated compound of **4**, 5-(4-decanoxyphenyl)-10,20-dimesityl-15-(4-hydroxyphenyl)porphyrin (**5**) was coupled with 3-iodopropyl nonadeca-*O*-acetyl-1-hexa-β-D-maltoside (**6**)[7] using K_2CO_3 in DMF to afford the acetyl hexa-maltose-linked porphyrin (**7**). Deacetylation of **7** was carried out using NaOMe to give a desired compound, 5-(4-decanoxyphenyl)-10,20-dimesityl-15-[4-{3-(hexa-β-D-maltosyl)propoxy}phenyl]porphyrin (**8**).[8] (Total yield based on **1**, 9.6%)

The relative quantum yield of singlet oxygen using 1,3-diphenylisobenzofuran as a scavenger for **8** was examined in order to qualify the photosensitivity, and turned out to be almost the same as for the value of 5,10,15,20-tetrakis(*m*-hydroxyphenyl)porphyrin (*m*-THPP). Figure 1(a) shows cell-survival ratio (%) in the presence of **8** (5 and 10 μM) without irradiation, indicating that **8** had low cytotoxicity under the dark condition.[9] The photocytotoxicity of **8** (5 and 10 μM) toward HeLa cells was evaluated after visible light irradiation. Although the ratios of cell-survival for **8** and *m*-THPP (5 μM) were 43.2 and 3.7%, respectively, the cell-survival at 10 μM for **8** was remarkably reduced to 2.3%, as shown in Figure 1(b). For the practical use in PDT, low cytotoxicity of PS under the dark condition is required. The cytotoxicity of PSs in the dark was also estimated by the same condition and turned out to have only low cytotoxicity.



i) **1** (2 eq.), **2** (1 eq.), **3** (1 eq.), TFA, CH_2Cl_2, 15 min, then DDQ; ii) KOH-EtOH, reflux, 1 h; iii) **6**, K_2CO_3, DMF, r.t., 72 h; iv) MeONa, MeOH, reflux, 1 h.

Scheme 1.

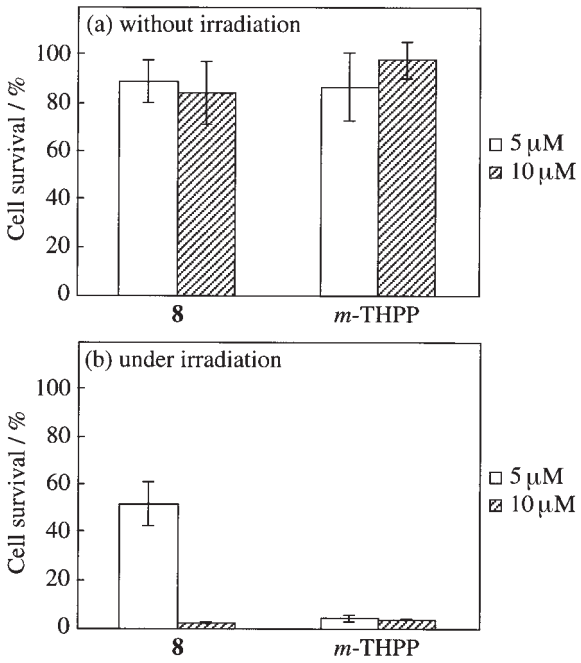


Figure 1. (a) Survival of HeLa cells incubated with **8** and *m*-THPP in the dark for 2 h at 30 °C and (b) survival of HeLa cells incubated with **8** and *m*-THPP for 2 h at 37 °C and then irradiated using 500 W halogen lamp attenuated by a 500 nm cut-off filter for 8 min. The results are expressed as nmol sensitizer per gram cell protein. Bars represent standard errors of the means for four experiments.

In summary, the novel amphiphilic phenylporphyrin having hexa-maltosyl and decyl groups, **8**, have been synthesized through successive reactions based on Lindsey's method. We confirmed that the photosensitizer **8** exhibited the photocytotoxicity with low cytotoxicity under dark conditions, which is a suitable characteristic for PDT reagents. Thus the present molecular design, i.e., photosensitizer consisting of oligosaccharide and lipid, should be one of promising concept for developing "second generation" photosensitizers toward PDT.

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8 "Carbohydrate-Protein Interactions" ed. by I. A. Wilson, A. E. Clarke, Springer-Verag, New York (1998). Spectral data of **8**: ^{1}H NMR(300 MHz, DMSO-d$_6$): δ(ppm) = 8.83 (d, ^3J = 5.4 Hz, 2H, β-pyr*H*), 8.82 (d, ^3J = 4.8 Hz, 2H, β-pyr*H*), 8.62 (m, 4H, β-pyr*H*), 8.25 (d, ^3J = 8.4 Hz, 2H, 3,5-Ar*H*), 8.07 (d, ^3J = 8.4 Hz, 2H, 2,6-Ar*H*), 7.36 (d, ^3J = 8.7 Hz, 2H, 3′,5′-Ar*H*), 7.34 (s, 4H, 2,6-Mes*H*), 7.30 (d, ^3J = 8.8 Hz, 2H, 2′,6′-Ar*H*), 3.77 ~ 3.75 (m, 4H, Ar-O-C*H*$_2$-), 2.58 (s, 6H, 4-MesC*H*$_3$), 2.27 (m, 2H, saccharide-O-C*H*$_2$-), 2.20 (m, 2H, OCH$_2$C*H*$_2$CH$_2$O), 1.85 ~ 1.76 (m, 12H, 2,6-MesC*H*$_3$), 1.76 ~ 1.27 (m, 16H, decyl-C*H*$_2$), 0.86 (t, ^3J = 6.4 Hz, 3H, decyl-C*H*$_3$). ESI-MASS (m/z) = $[M+H]^+$ calcd for $C_{99}H_{128}N_4O_{33}$, 1901.8461; found, 1902.5458.

9 Procedure for photocitotoxity evaluation: *cells and cultures*; HeLa cells were obtained from Cell Resource Center for Biomedical Research, Tohoku University. DMEM medium, FBS, PSM were purchased from GIBCO. MTT was purchased from SIGMA. *Procedure*; HeLa cells (1.0×10^4 cells/well) were incubated in 100 μL of DMEM containing 10% FBS and 1% PSM at 37 °C for 24 h. After removal of media, cells were washed once with PBS, and incubated with serum-free DMEM solution of porphyrin derivatives (5 and 10 μM) for 2 h. After the cells were washed twice with PBS, 100 μL of DMEM containing 10% FBS and 1% PSM was added. The cell was irradiated for 8 min with a 500 W halogen lamp equipped with cut-off filter Y-50 (HOYA) which cut off the light shorter than 500 nm. After incubating for 20 h, 10 μL of PBS solution of MTT (5 mg·mL^{-1}) was added, and incubated again for 4 h. After the supernant was removed, 100 μL of DMSO was added to dissolve the formazan crystals. The cell-survival ratio was determined by measurement of the optical density at 570 nm using microplate reader (Model 550, BIORAD). As control experiment, cells were treated in the same manner in the absence of the photosensitizer.