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Silylation enhancement of photodynamic activity of tetraphenylporphyrin derivative

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ABSTRACT

Singlet oxygen sensitization of the water-soluble silylated tetraphenylporphyrin derivative has been studied. Quantum yield of singlet oxygen sensitization of the silylated compound was improved compared with that of the non-silylated one. To clarify the mechanism of the improvement, photophysical processes have been studied. The silylation increased the fraction of the triplet state quenched responsible for the formation of singlet oxygen, and resultantly, this effect improved the quantum yield of singlet oxygen sensitization. To demonstrate the silylated compound suitable for a photosensitizer in photodynamic therapy for cancer, a cell culture study was carried out with a human cancer cell line. We found that the silylated compound displayed much higher photodynamic activity than the non-silylated one. We conclude that this high activity was caused by the improvements of both quantum yield of singlet oxygen sensitization and cellular uptake efficiency. We emphasize that improved lipophilicity by silylation contributes much to the high cellar uptake efficiency of porphyrin derivatives.

1. Introduction

Photodynamic therapy (PDT) is accepted as one of the promising treatments of a variety of cancers including bladder cancer, cervical cancer, lung cancer, esophageal cancer, and gastric cancer [1,2]. PDT is based on photosensitizers, which are preferentially taken up and/or retained by tumors. Upon excitation of a photosensitizer with visible light, a singlet oxygen ($^1\Delta_g$) is sensitized by the triplet state of the photosensitizer. Although other reactive intermediates such as superoxide and hydroxyl radicals may also be photogenerated [3], the singlet oxygen is nowadays accepted as the foremost cytotoxic mediator to the tumor cells in PDT [4]. As a photosensitizer, the following five qualities are required:

(1) Soluble in water.
(2) Selective accumulating quality to tumors; photosensitizer targeting has been studied for establishing a proper drug delivery system (DDS) [5–15].
(3) High cellular uptake efficiency; numerous photosensitizers have been extensively studied to improve cellular uptake efficiency [5,6,16]. The cellular uptake efficiency reportedly shows rough correlation with a partition coefficient of photosensitizers [17–20].
(4) Efficient light absorption quality (high molar absorption coefficient) from red to near-IR wavelength region; chlorins [5,16,21,22], phthalocyanines [23–25] and porphycenes [26,27] have been studied to improve the molar absorption coefficient in this region.
(5) High sensitization efficiency of the singlet oxygen (high quantum yield of singlet oxygen sensitization). Various compounds have been synthesized [5,28], to obtain the high sensitization efficiency.

Recently, we synthesized 5,10,15,20-tetrakis(4-trimethylsilylphenyl)porphyrin (SiTPP) to reach the five qualities, and found that the silylation of tetraphenylporphyrin (TPP) improves the quantum yield of singlet oxygen sensitization [29]. On the other hand, it has been reported that the introduction of a silicon atom into some previously known drug molecules leads to a significant change in biological activities [30–32].

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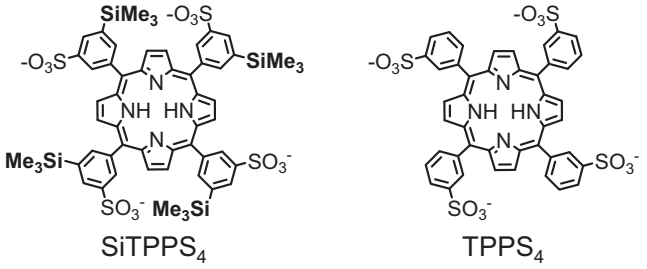

Fig. 1. Molecular structures of photosensitizers.

Considering these studies, we examined the effect of silylation on the photodynamic activity of porphyrin derivatives. Although SiTPP displayed a higher quantum yield of singlet oxygen sensitization than TPP, this compound was not soluble in water, suggesting that it is not qualified as a five quality-fulfilling photosensitizer. In this paper, we have introduced silyl groups to a water-soluble porphyrin derivative and studied its photophysical properties and photodynamic activity using U251 human glioma cells.

2. Results and discussion

2.1. Photophysical and photochemical processes

We synthesized 5,10,15,20-tetrakis(3-sulfonatophenyl)porphyrin sodium salt ($TPPS_4$) and its silylated derivative ($SiTPPS_4$) whose chemical structures are shown in Fig. 1. UV–vis absorption spectra of $TPPS_4$ and $SiTPPS_4$ in ethanol are shown in Fig. 2. $TPPS_4$ displayed absorption peaks at 415.0, 512.5, 547.0, 590.0, and 645.5 nm. This spectrum corresponds well to that of tetraphenylporphyrin (TPP) [29], indicating that sulfonato groups introduced at the 3-position of the phenyl moieties do not cause any significant change in the electronic structure of TPP. Likewise, $SiTPPS_4$ displayed absorption peaks at 417.5, 515.0, 549.5, 591.0, and 647.0 nm, which are very similar to those of $TPPS_4$, indicating that the silylation at the 5-position of the phenyl moieties also do not cause any significant change in the electronic structure of $TPPS_4$. Fluorescence spectra of $SiTPPS_4$ and $TPPS_4$ are also shown in Fig. 2, and two fluorescence peaks were similarly observed at 651 and 717 nm, and 649 and 715 nm for $SiTPPS_4$ and $TPPS_4$, respectively. These spectra are similar to that of TPP [29], indicating that both sulfonato and silyl groups did not have a notable effect on the fluorescence spectrum of TPP as in the case on the absorption spectrum. Fluorescence excitation spectra of $SiTPPS_4$ and $TPPS_4$ were essentially the same as their absorption

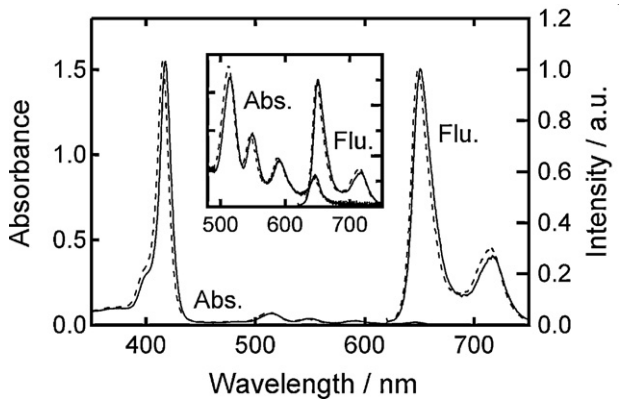

Fig. 2. Absorption and fluorescence spectra of $SiTPPS_4$ (full line) and $TPPS_4$ (broken line) in ethanol at room temperature.

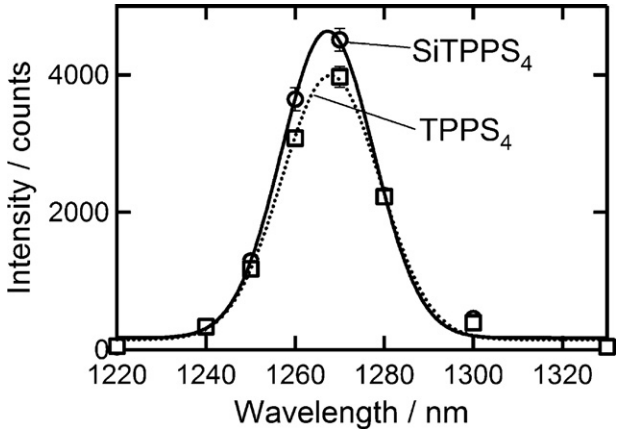

Fig. 3. Phosphorescence spectra of the singlet oxygen sensitized by $SiTPPS_4$ and $TPPS_4$ in air-saturated ethanol at room temperature.

spectra (data not shown), indicating that relaxation processes of the excited singlet state are independent of the excitation wavelength.

To determine the quantum yield of the singlet oxygen sensitization Φ_Δ, a phosphorescence spectrum of the singlet oxygen was measured in air-saturated ethanol. Fig. 3 shows phosphorescence spectra of the singlet oxygen produced upon excitation of $SiTPPS_4$ and $TPPS_4$ with the 355-nm light. Absolute Φ_Δ value was determined using perinaphthenone as a reference compound ($\Phi_\Delta = 0.98$). [33] Φ_Δ of $TPPS_4$ and $SiTPPS_4$ was determined to be 0.57 and 0.66, respectively. Since Φ_Δ of $TPPS_4$ was similar to that of TPP in tetrahydrofuran (0.58), the sulfonato groups appeared to induce virtually no significant change in Φ_Δ. Φ_Δ of $SiTPPS_4$ turned out to be 1.15 times larger than that of $TPPS_4$, indicating that the silylation at the 5-position also improves Φ_Δ as in the case of that at the 4-position in SiTPP ($\Phi_\Delta = 0.77$[29]). Φ_Δ of $SiTPPS_4$ was smaller than that of SiTPP. Because the silylation at the 2-position increases steric hindrance significantly and this derivative is hard to be synthesized, we did not determine the Φ_Δ of 2-silylated derivative. Instead, we introduced the silyl groups at both 3- and 5-positions (5,10,15,20-tetrakis(3,5-bis(trimethylsilyl)phenyl)porphyrin) and we found that its Φ_Δ is higher (0.76) than that of TPP. Every silylated compound studied exhibited higher Φ_Δ than the corresponding non-silylated compound. Thus, the silyl group position on the phenyl moieties of TPP appears to be unimportant for the enhancement of Φ_Δ.

To clarify the mechanism of the improvement, photophysical processes were studied. Fluorescence lifetime and quantum yield in air-saturated ethanol were determined to be 12.3 ns and 0.059 for $SiTPPS_4$, and 12.6 ns and 0.057 for $TPPS_4$, respectively (Table 1). The fluorescence rate constant k_f and non-radiative rate constant k_{nr} were estimated using equations $k_f = \Phi_f/\tau_f$ and $k_{nr} = \tau_f^{-1} - k_f$ to be $4.8 \times 10^6\ s^{-1}$ and $7.4 \times 10^7\ s^{-1}$ for $SiTPPS_4$, and $4.5 \times 10^6\ s^{-1}$ and $7.0 \times 10^7\ s^{-1}$ for $TPPS_4$, respectively, indicating that the deactivation processes of the first excited singlet state (S_1) seem to be independent of the silyl group. The quantum yield of singlet oxygen sensitization Φ_Δ can be described as follows:

$$\Phi_\Delta = \Phi_T P_T^{O_2} f_\Delta^T$$

where Φ_T is the quantum yield of intersystem crossing, $P_T^{O_2}$ is the quenching efficiency of the triplet state (T_1) by molecular oxygen and f_Δ^T is the fraction of T_1 quenched responsible for the formation of singlet oxygen (sensitization efficiency). We first determined Φ_T by use of the nanosecond transient absorption technique. Fig. 4a shows transient absorption spectra of $SiTPPS_4$ and $TPPS_4$ in Ar-saturated ethanol. Both spectra are very similar to each other,

Table 1
Photophysical parameters of $SiTPPS_4$ and $TPPS_4$.

	ε_{Soret} (M^{-1} cm^{-1})	Φ_f	Φ_T	τ_f (ns)	Φ_{ic}	k_f (10^6 s^{-1})	k_{ic} (10^6 s^{-1})	k_{TS} (10^7 s^{-1})	k_{TS}^{Ar} (10^4 s^{-1})	Φ_Δ	f_Δ^T
$TPPS_4$	3.91×10^5	0.057	0.89	12.6	0.06	4.5	5	7.0	5.0	0.57	0.66
$SiTPPS_4$	4.33×10^5	0.059	0.89	12.3	0.04	4.8	3	7.4	5.0	0.66	0.76

and are also similar to that of TPP [29]. Thus, these transient absorption spectra are considered to consist of the T–T absorption and the bleaching of the ground state. Because the T–T absorption spectra of $SiTPPS_4$ and $TPPS_4$ are similar to each other, they are assumed to have the same molar absorption coefficient. Based on this assumption, we estimated the relative Φ_T from the initial absorbance of T_1 at 665 nm. Fig. 4b depicts similar decay time profiles of the transient absorption at 665 nm of $SiTPPS_4$ and $TPPS_4$ in Ar-saturated ethanol. They were best-fitted by a single exponential function with the initial absorbance of 0.013 and the decay rate constant of T_1 (k_{TS}^{Ar}) of 5.0×10^4 s^{-1} for both $SiTPPS_4$ and $TPPS_4$. From the initial absorbance, Φ_T of $SiTPPS_4$ and $TPPS_4$ were estimated to be equivalent to each other. The absolute value was determined to be 0.89 relative to the triplet–triplet absorption of TPP [34]. Based on the values of Φ_f, Φ_T, and τ_f, rate constants of intersystem crossing from S_1 to T_1 (k_{ST}) and internal conversion (k_{ic}) were also determined to be 7.4×10^7 s^{-1} and 3×10^6 s^{-1} for $SiTPPS_4$, and 7.0×10^7 s^{-1} and 5×10^6 s^{-1} for $TPPS_4$, respectively. These k_{ST} and k_{ic} values of $SiTPPS_4$ are similar to those of $TPPS_4$, respectively. Because Φ_T of $SiTPPS_4$ is similar to that of $TPPS_4$, the increase in $P_T^{O_2}$ and/or f_Δ^T appears to be responsible for the improvement of Φ_Δ. $P_T^{O_2}$ is described by the following equation:

$$P_T^{O_2} = \frac{k_{TS}^{O_2} - k_{TS}^{Ar}}{k_{TS}^{O_2}}$$

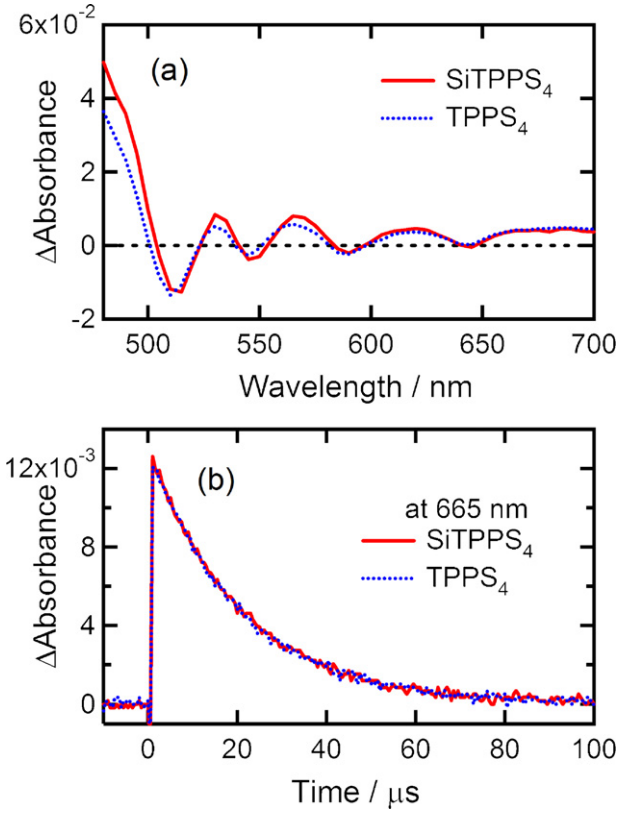

Fig. 4. Transient absorption spectra (a) of $SiTPPS_4$ and $TPPS_4$ in Ar-saturated ethanol observed immediately after the excitation, and decay time profiles monitored at 665 nm (b).

where $k_{TS}^{O_2}$ is a decay rate constant of T_1 in the presence of oxygen. To determine $P_T^{O_2}$, the transient absorption spectrum was also measured in air-saturated ethanol. The decay rate constant of T_1 under air ($k_{TS}^{O_2}$) was estimated to be 1.9×10^6 s^{-1} for both $SiTPPS_4$ and $TPPS_4$. From k_{TS}^{Ar} and $k_{TS}^{O_2}$, $P_T^{O_2}$ under air was estimated to be 0.97 for both $SiTPPS_4$ and $TPPS_4$. Then, the sensitization efficiency f_Δ^T was estimated to be 0.76 and 0.66 for $SiTPPS_4$ and $TPPS_4$, respectively. Based on these results, it is concluded that the increase in the sensitization efficiency f_Δ^T is responsible for the improvement of the quantum yield of singlet oxygen sensitization Φ_Δ. This result is also similar to that of TPP [29]. f_Δ^T of $TPPS_4$ (0.66) is slightly smaller than that of TPP (0.70 [29]). This may be due to the effect of sulfonato groups and/or solvent. f_Δ^T of $SiTPPS_4$ (0.76 [29]) is smaller than that of SiTPP (0.89), indicating that the effect of the silylation at the 5-position may be smaller than that at the 4-position.

The singlet oxygen is considered to be produced by the quenching of the triplet sensitizer $^3M^*$ by molecular oxygen according to Scheme 1 [35]. From the singlet encounter complex $^1(^3M^{*}\cdots O_2, {}^3\Sigma_g^-)$, the singlet oxygen and the photosensitizer in the ground state are produced (1), but the singlet oxygen is not sensitized from the triplet encounter complex $^3(^3M^{*}\cdots O_2, {}^3\Sigma_g^-)$ (2). This quenching process from the triplet encounter complex $^3(^3M^{*}\cdots O_2, {}^3\Sigma_g^-)$ (2) was reported to take place via the charge transfer complex $^3(M^{\delta+}\cdots O_2{}^{\delta-})$ [35], and thus the sensitization efficiency f_Δ^T is expected to increase with suppression of the charge transfer. In TPP, the silylation induced an increase in the free energy change of the charge transfer ΔG^{CT}, resulting in improvement of the sensitization efficiency f_Δ^T [29]. Therefore, a similar increase in ΔG^{CT} is also expected for $TPPS_4$ and $SiTPPS_4$. ΔG^{CT} can be roughly described by the equation $\Delta G^{CT} = F(E_{ox}^S - E_{red}^{O_2} - E_T)$ [35], where F is the Faraday constant, E_{ox}^S is the half-wave oxidation potential of the sensitizer in the ground state, $E_{red}^{O_2}$ is the half-wave reduction potential of oxygen (−0.78 V vs. SCE [36]), and E_T is the triplet state energy. To evaluate ΔG^{CT}, we tried to estimate E_T by near-IR phosphorescence measurements in ethanol at 77 K. However, the phosphorescence signal was too weak to analyze the spectrum. We also tried to estimate E_{ox}^S by cyclic voltammetry in water using sodium sulfate as an electrolyte, but the oxidation signal from the sensitizers was hidden by the oxidation signal of water. Thus, we failed to estimate the ΔG^{CT} value experimentally. However, we presume that the increase in ΔG^{CT} may induce the improvement of f_Δ^T based on the similarity of photophysical and quenching processes of $SiTPPS_4$, $TPPS_4$, SiTPP and TPP. Although the mechanism is only partially clear, the silylation of water-soluble porphyrin improves Φ_Δ, and thus this new silylated compound is promising for photodynamic therapy.

2.2. Photodynamic activity

To evaluate cytotoxicity of $SiTPPS_4$ and $TPPS_4$ in vitro, we cultured U251 human glioma cells in media with various concentrations of photosensitizers for 12 h in the dark. After the culture, cell survival was measured in a no light-irradiating condition by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT) assay. Below 50 μM, $SiTPPS_4$ and $TPPS_4$ did not show any cytotoxicity to U251 cells, and $TPPS_4$ also did not have any effect on their survival up to 100 μM (Fig. 5). However, over 50 μM $SiTPPS_4$, the cell survival became shorter. This higher cytotoxicity of $SiTPPS_4$ appears to have resulted from higher cellular uptake effi-

$$\overset{1/9\,k_d}{\rightleftharpoons}\ {}^1({}^3M^* \cdots O_2, {}^3\Sigma_g^-)^* \xrightarrow{k_{et}} M + O_2({}^1\Delta_g) \quad (1)$$

$${}^3M^* + O_2({}^3\Sigma_g^-) \overset{3/9\,k_d}{\rightleftharpoons}\ {}^3({}^3M^* \cdots O_2, {}^3\Sigma_g^-)^* \xrightarrow{k_{isc}} M + O_2({}^3\Sigma_g^-) \quad (2)$$

$$\overset{5/9\,k_d}{\rightleftharpoons}\ {}^5({}^3M^* \cdots O_2, {}^3\Sigma_g^-)^*$$

Scheme 1. Quenching processes of the triplet state by molecular oxygen.

ciency which is described below. Next we compared photodynamic activity of $SiTPPS_4$ and $TPPS_4$. We cultured U251 cells with 25 μM $SiTPPS_4$ or $TPPS_4$ for 12 h in the dark. After changing the medium to the fresh photosensitizer-free medium, we then irradiated visible light to the cells. Since the absorption spectral shape of $SiTPPS_4$ is almost the same as that of $TPPS_4$, use of a monochromatic light was not necessary. After irradiation, cell survival decreased strikingly in both cases (Fig. 6), and notably $SiTPPS_4$ displayed much higher phototoxic activity than $TPPS_4$ did, indicating that this activity is significantly enhanced by the silylation. Since the light dose yielding a 50% lethal effect (LD_{50}) is known as an important index for evaluating photodynamic activity, [5] we calculated LD_{50} to be 5.5 and 15.1 J cm^{-2} for $SiTPPS_4$ and $TPPS_4$, respectively. As a result, the photodynamic activity of $SiTPPS_4$ is three times higher than that of $TPPS_4$. As one of the important factors for this enhancement, the higher quantum yield of singlet oxygen sensitization Φ_Δ has been pointed out for $SiTPPS_4$ as discussed above. However, actual enhancement of the photodynamic activity was much higher than that of Φ_Δ, suggesting that the silylation may have improved other qualities than Φ_Δ. Considering the five qualities proposed for effective photosensitizers in Introduction, we found it difficult to evaluate the second quality "selective accumulating quality to tumors." Since the fourth quality, "molar absorption coefficient" of $SiTPPS_4$ and $TPPS_4$, was similar to each other, we presumed that the silylation may have improved one of the remaining two qualities. We tested the third quality, "cellular uptake efficiency," and found that fluorescence from U251 cells was much more intense with $SiTPPS_4$ than with $TPPS_4$, when the cells were cultured with 25 μM photosensitizer for 12 h (Fig. 7a and b). Because the fluorescence quantum yield of $SiTPPS_4$ (0.059) is almost the same value for $TPPS_4$ (0.057), the intense fluorescence appears to reflect the higher cellular uptake of $SiTPPS_4$ compared to that of $TPPS_4$, suggesting the enhancing effect of silylation depends on the cellular uptake. To evaluate the cellular uptake in a quantitative manner, photosensitizer molecules were extracted from the cells and evaluated the fluorescence intensity of the extracted solution (Fig. 7c). Although the fluorescence intensity was still going up even at 12 h, the intracellular concentration of $SiTPPS_4$ displayed a high plateau level. The cellular uptake rate was similar to the value reported by Di Stasio et al. [5] and Schneider et al. [6] Fluorescence intensity of $SiTPPS_4$ was approximately two times higher than that of $TPPS_4$ at the 12-h incubation point (Fig. 7c), indicating that the silylation improves the cellular uptake. Thus, the enhancement of the PDT activity is explained by the increase in the quantum yield of singlet oxygen sensitization as well as by the cellular uptake efficiency. Since the trialkylsilyl group is known to be lipophilic, $SiTPPS_4$ is presumably more lipophilic than $TPPS_4$, and this highly lipophilic feature may lead to improve cell membrane penetration. [31] Indeed, Bom et al. reported that the introduction of a silyl group into camptothecin increases its incorporation to small unilamellar vesicles [37]. To evaluate the lipophilic property, we measured the octanol–water partition coefficient ($\log P$), although the relationship between $\log P$ and cellular uptake efficiency was approximate, and an inverted relationship was sometimes found for individual compounds [17,18]. The $\log P$ was determined to be 3.7×10^{-3} and -1.22 for $SiTPPS_4$ and $TPPS_4$, respectively. Since the $\log P$ value of $TPPS_4$ (-1.22) suggests the low affinity to the biological membrane, it is presumably hard for $TPPS_4$ to penetrate through the cell membrane. Considering that the $\log P$ value is negative, $TPPS_4$ may be taken up by endocytosis. On the other hand, the $\log P$ value of $SiTPPS_4$ is almost 0, thus $SiTPPS_4$ is thought to have high affinity to the biological membrane, and results inefficient penetration through the plasma membrane, perhaps with endocytosis. As seen in Fig. 7, subcellular localization of $SiTPPS_4$ appears to be similar to that of $TPPS_4$, and both photosensitizers were not localized to the nucleus, instead localized to organelles surrounding the nucleus. In terms of organelle candidates, lysosomes are mostly suggested (see Fig. 7).

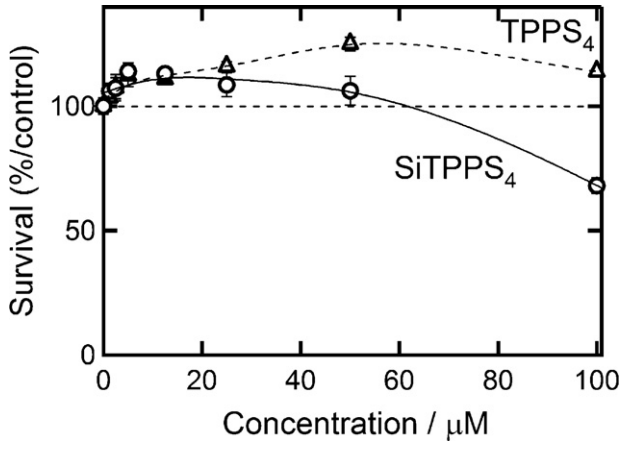

Fig. 5. Survival of U251 cells cultured with either $SiTPPS_4$ or $TPPS_4$ at various concentrations for 12 h in the dark.

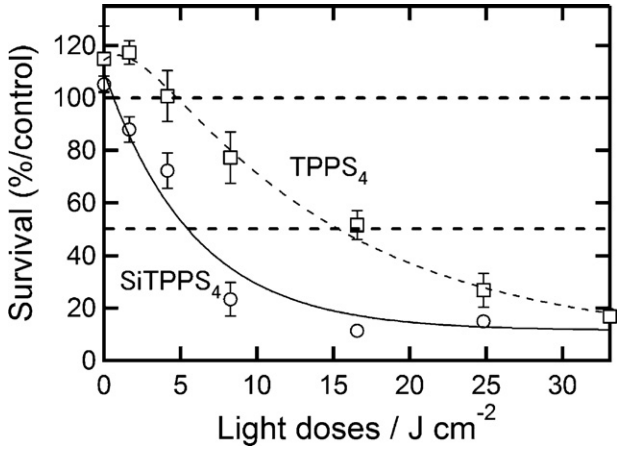

Fig. 6. Survival of U251 cells cultured with either 25 μM of $SiTPPS_4$ or $TPPS_4$ for 12 h as a function of the irradiation light dose.

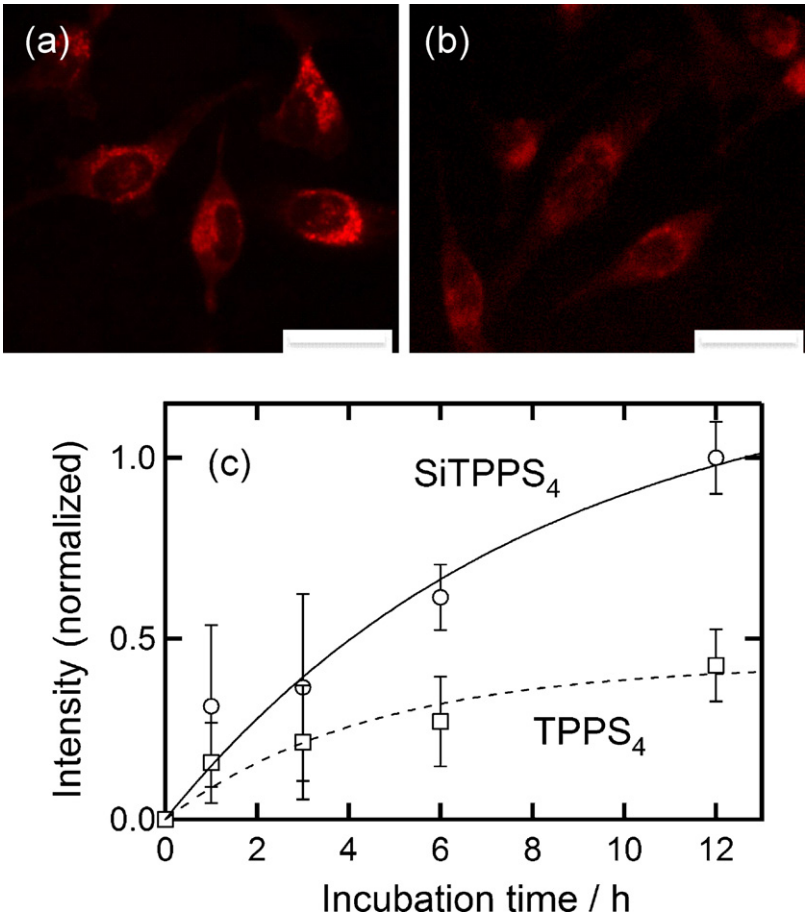

Fig. 7. U251 cells were cultured with either 25 μM $SiTPPS_4$ (a) or 25 μM $TPPS_4$ (b) for 12 h. Fluorescence image were observed by U-MWIG2 filter (535 nm excitation; 580 nm emission filter) under olympas microscopy. Bar 20 μm. Fluorescence intensity of the extracted solution of photosensitizers from U251 cells cultured with either 25 μM of $SiTPPS_4$ or $TPPS_4$ as a function of the incubation time (c).

3. Summary

The silylation of 5,10,15,20-tetrakis(3-sulfonatophenyl)porphyrin sodium salt ($TPPS_4$) improves the quantum yield of singlet oxygen sensitization Φ_Δ as observed with tetraphenylporphyrin. The silylation does not change the photophysical parameters including the quantum yield of intersystem crossing Φ_T, but it increases the fraction of the triplet sensitizer quenched responsible for the formation of singlet oxygen f_Δ^T. Although the detailed mechanism to increase f_Δ^T has not yet been clarified experimentally, the suppression of the charge transfer from the triplet photosensitizer to molecular oxygen in the triplet encounter complex is suggested to improve f_Δ^T according to the results of the silylation of tetraphenylporphyrin. The silylation also improves cellular uptake efficiency. The increase in lipophilicity by the silylation is considered to be an essential factor for enhancing the cellular uptake efficiency. The silylation improved both quantum yield of singlet oxygen sensitization Φ_Δ and cellular uptake efficiency to improve the photodynamic activity significantly. Thus, we propose that the silylation is a promising strategy to improve photosensitizers effectively for photodynamic therapy.

4. Materials and methods

4.1. Synthesis

The reaction was carried out under an argon atmosphere. Carbon tetrachloride was distilled from calcium hydride. Trimethylsilyl chlorosulfonate (Aldrich) and a cellulose membrane (Viskase, tubing, diameter: 18/32 in., molecular weight cut-off (MWCO): 12,000–16,000) were purchased. Tetrasodium 5,10,15,20-tetrakis(3′-sulfonatophenyl)porphyrin ($TPPS_4$) [38] and 5,10,15,20-tetrakis[3′,5′-bis(trimethylsilyl)phenyl]porphyrin [39] were prepared by published procedures. A phosphate buffer was prepared by dissolving sodium dihydrogenphosphate dihydrate and disodium hydrogenphosphate dodecahydrate in water.

A ^{1}H NMR spectrum was obtained with a JEOL JNM-LA500 spectrometer. A mass spectrum was recorded on an Applied Biosystems/MDS Sciex API-100 mass spectrometer.

4.1.1. Synthesis of tetrasodium 5,10,15,20-tetrakis(3′-sulfonato-5′-trimethylsilylphenyl)porphyrin ($SiTPPS_4$)

Trimethylsilyl chlorosulfonate (0.223 g, 1.18 mmol) was added dropwise to a solution of 5,10,15,20-tetrakis[3′,5′-bis(trimethylsilyl)phenyl]porphyrin (0.101 g, 0.085 mmol) in carbon tetrachloride (17 mL) at room temperature. The reaction mixture was stirred for 1.5 h at room temperature. Aqueous sodium hydroxide (1 mol/L, 7 mL) was added to the reaction mixture, and the mixture was stirred for 30 min at room temperature. The aqueous layer was washed with chloroform three times and dialyzed with a cellulose membrane for 4 days. The resulting solution was concentrated under reduced pressure, and the residue was separated by medium-pressure liquid chromatography (ODS, methanol–0.01 mol/L phosphate buffer (8:2)) to give an $SiTPPS_4$ fraction. The $SiTPPS_4$ fraction was concentrated under reduced

pressure and dialyzed with a cellulose membrane for 4 days. The solvents were removed under reduced pressure to give $SiTPPS_4$ (0.029 g, 26%) as a purple solid.

^1H NMR (DMSO-d_6, 80 °C) δ −2.73 (s, 2H), 0.42 (s, 36H), 8.26 (s, 8H), 8.47 (s, 4H), 8.81 (s, 8H); ESI-MS (negative, methanol–water (1:1)) m/z 1243.0 ($[M-3Na^+ + 2H^+]^-$).

Purity of the $SiTPPS_4$ synthesized is verified using thin layer chromatography (TLC), ^1H NMR, UV–Vis spectroscopy, fluorescence spectroscopy, and HPLC. No signal due to impurity was observed in all measurements, indicating that the purity is more than 95%.

4.2. Measurements for photophysical and photochemical processes

Absorption spectra were recorded on a Hitachi U3310 spectrophotometer. Fluorescence emission and excitation spectra were measured using a Hitachi F4500 fluorescence spectrometer. Fluorescence quantum yields were determined using an Absolute PL Quantum Yield Measurement System (Hamamatsu C9920-02). Fluorescence lifetimes were measured using an Edinburgh Analytical Instruments FL900CDT Spectrometer system (H_2 pulser, pulse width 0.8 ns, 10^8 photons/pulse, repetition rate 40 kHz).

Nanosecond transient absorption spectra were measured using a Unisoku TSP601H nanosecond laser photolysis system with an Nd^{3+}:YAG laser (Tokyo Instruments Lotis II, 355 nm, 2.0 mJ/pulse, pulse width: 8 ns, 10 Hz).

For the phosphorescence measurements of the singlet oxygen, the 355-nm light from an Nd^{3+}:YAG laser (Tokyo Instruments Lotis II, 1.0 mJ/pulse, pulse width: 8 ns, 10 Hz) was used as the excitation light source. Phosphorescence was detected with a photomultiplier tube for NIR region (Hamamatsu R5509-42) cooled at −80 °C after dispersion with a monochromator (Ritsu MC-10N, blaze wavelength: 1250 nm and slit width: 0.7 mm). Signals from the photomultiplier tube were amplified by five times with a DC-300 MHz amplifier (Stanford Research Systems SR445) and processed with a gated photon counter (Stanford Research Systems SR400). Gate width and delay of the photon counter was set at 0.5 μs and 0.5 μs, respectively, and data acquisition was 300 times.

4.3. Procedure for in vitro experiments

4.3.1. Cell culture conditions

Human glioma cells (U251) were grown in 64 cm^2 plastic tissue culture dish in Dulbecco' modified Eagle medium (DMEM) supplemented with 10% of foetal calf serum (FCS) solution and 1% of solution of 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin in a 5% CO_2 atmosphere at 37 °C. Cells were subcultured by dispersal with 0.25% trypsin and seeded 5×10^4 cells/mL.

4.3.2. Cell survival measurements

Cell survival was measured using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) assay. Before performing growth inhibition assays, we examined the linearity of the MTT assay with increasing number of U251 cells plated between 0 and 2×10^5 cells/mL and a good linear relationship was obtained. U251 cells were seeded at the initial density of 1×10^6 cells/mL in 96-well microtitration plates. Forty-eight hours after plating, cells were exposed to photosensitizers in RPMI supplemented with 9% of bovine serum albumin (BSA). After 12-h incubation at 37 °C, the medium was removed, cells were washed with phosphate buffered saline (PBS) and the fresh supplemented DMEM was added. In the case of photocytotoxicity experiment, visible light was irradiated. After 24-h incubation at 37 °C, the MTT assay was carried out. Absorbance was measured using a microplate reader (Corona Electric Co., Ltd. MTP500). As a light source, output from a 500 W Xe short-arc lamp was used after passing through long-pass filter (390 nm; Sigma Koki Co., Ltd., SCF-50S-39L). The light intensity was estimated to be 28.1 mW cm^{-2} in the wavelength region from 250 nm to 800 nm by a Ushio Spectro-Reflectance Meter (USR-45 V/D).

4.3.3. Fluorescence microscopy

U251 cells (3×10^4 cells/mL) were cultured in plastic chamber slide (0.6 cm^2/chamber)and cultured for 48 h for proper attachment to the substratum. The U251 cells were then exposed to photosensitizers (25 μM) in RPMI supplemented with 9% of BSA from 1 to 12 h. In the case of dual staining experiments, specific organelle markers in RPMI were added at 11.5 h of the exposed time and cultured further 0.5 h. After incubation with the photosensitizers, the cells were washed with PBS. Fluorescence images were observed by a fluorescence microscope (Olympus BX50). Excitation wavelengths were selected at ca. 535 nm and 475 nm to excite photosensitizers and specific organelle marker, respectively.

4.3.4. Cellular uptake efficiency

U251 cells (3×10^4 cells/mL) were inoculated in 6 well plate and cultured for 48 h for proper attachment to the substratum. The U251 cells were then exposed to photosensitizers (25 μM) in RPMI supplemented with 9% of BSA from 1 to 12 h. After the exposure, cells were washed by PBS, and 1 mL of PBS and 20 μL of 5 M KOH were added to extract photosensitizer from cells. Fluorescence spectrum of the extracted solution was measured by use of a Hitachi F-2500 fluorescence spectrophotometer and relative cellular uptake efficiency was estimated by the fluorescence intensity.

4.3.5. Partition coefficient

An aqueous solution of the photosensitizer (10^{-5} M) was prepared and its absorption spectrum was measured. The maximum absorbance around 415 nm (Soret band) of this solution was defined as A_0. An equal amount of 1-octanol was added to this aqueous solution and mixed. After the phase separation between water and 1-octanol layers, absorption spectrum of the water layer was measured. The maximum absorbance around 415 nm (Soret band) of this solution was defined as A_w. The partition coefficient log P was estimated using the equation $\log P = \log c_o/c_w = \log\{(A_0 - A_w)/A_w\}$, where c_o and c_w are the concentration of the photosensitizer in 1-octanol and water, respectively.

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References

[1] T.J. Dougherty, Photodynamic therapy, Photochem. Photobiol. 58 (1993) 895–900.
[2] D. Dolmans, D. Fukumura, R.K. Jain, Photodynamic therapy for cancer, Nat. Rev. Cancer 3 (2003) 380–387.
[3] C. Schweitzer, R. Schmidt, Physical mechanisms of generation and deactivation of singlet oxygen, Chem. Rev. 103 (2003) 1685–1757.
[4] G. Jori, Tumour photosensitizers: approaches to enhance the selectivity and efficiency of photodynamic therapy, J. Photochem. Photobiol. B 36 (1996) 87–93.
[5] B. Di Stasio, C. Frochot, D. Dumas, P. Even, J. Zwier, A. Müller, J. Didelon, F. Guillemin, M.L. Viriot, M. Barberi-Heyob, The 2-aminoglucosamide motif improves cellular uptake and photodynamic activity of tetraphenylporphyrin, Eur. J. Med. Chem. 40 (2005) 1111–1122.
[6] R.L. Schneider, F. Schmitt, C. Frochot, Y. Fort, N. Lourette, F. Guillemin, J.F. Muller, M. Barberi-Heyob, Design, synthesis, and biological evaluation of folic acid targeted tetraphenylporphyrin as novel photosensitizers for selective photodynamic therapy, Bioorg. Med. Chem. 13 (2005) 2799–2808.

[7] M. Zeisser-Labouebe, N. Lange, R. Gurny, F. Delie, Hypericin-loaded nanoparticles for the photodynamic treatment of ovarian cancer, Int. J. Pharm. 326 (2006) 174–181.
[8] S. Sortino, A. Mazzaglia, L.M. Scolaro, F.M. Merlo, V. Valveri, M.T. Sciortino, Nanoparticles of cationic amphiphilic cyclodextrins entangling anionic porphyrins as carrier–sensitizer system in photodynamic cancer therapy, Biomaterials 27 (2006) 4256–4265.
[9] M. Kongshaug, J. Moan, L.S. Cheng, G.M. Garbo, S. Kolboe, A.R. Morgan, C. Rimington, Binding of drugs to human plasma-proteins, exemplified by Sn(IV)-etiopurpurin dichloride delivered in Cremophor and DMSO, Int. J. Biochem. 25 (1993) 739–760.
[10] K. Woodburn, D. Kessel, The alteration of plasma-lipoproteins by Cremophor EL, J. Photochem. Photobiol. B 22 (1994) 197–201.
[11] X. Damoiseau, H.J. Schuitmaker, J.W.M. Lagerberg, M. Hoebeke, Increase of the photosensitizing efficiency of the bacteriochlorin a by liposome-incorporation, J. Photochem. Photobiol. B 60 (2001) 50–60.
[12] U. Isele, K. Schieweck, R. Kessler, P. Vanhoogevest, H.G. Capraro, Pharmacokinetics and body distribution of liposomal zinc phthalocyanine in tumor-bearing mice – influence of aggregation state, particle-size, and composition, J. Pharm. Sci. 84 (1995) 166–173.
[13] J. Taillefer, M.C. Jones, N. Brasseur, J.E. Van Lier, J.C. Leroux, Preparation and characterization of pH-responsive polymeric micelles for the delivery of photosensitizing anticancer drugs, J. Pharm. Sci. 89 (2000) 52–62.
[14] J. Taillefer, N. Brasseur, J.E. van Lier, V. Lenaerts, D. Le Garrec, J.C. Leroux, In-vitro and in-vivo evaluation of pH-responsive polymeric micelles in a photodynamic cancer therapy model, J. Pharm. Pharmacol. 53 (2001) 155–166.
[15] N. Nishiyama, H.R. Stapert, G.D. Zhang, D. Takasu, D.L. Jiang, T. Nagano, T. Aida, K. Kataoka, Light-harvesting ionic dendrimerporphyrins as new photosensitizers for photodynamic therapy, Bioconjugate Chem. 14 (2003) 58–66.
[16] T. Bastogne, L. Tirand, D. Bechet, M. Barberi-Heyob, A. Richard, System identification of photosensitiser uptake kinetics in photodynamic therapy, Biomed. Signal Process. Control 2 (2007) 217–225.
[17] J. Moan, Q. Peng, J.F. Evensen, K. Berg, A. Western, C. Rimington, Photosensitizing efficiencies, tumor-uptake and cellular-uptake of different photosensitizing drugs relevant for photodynamic therapy of cancer, Photochem. Photobiol. 46 (1987) 713–721.
[18] G. Oenbrink, P. Jurgenlimke, D. Gabel, Accumulation of porphyrins in cells – influence of hydrophobicity aggregation and protein-binding, Photochem. Photobiol. 48 (1988) 451–456.
[19] S. Bonneau, P. Morliere, D. Brault, Dynamics of interactions of photosensitizers with lipoproteins and membrane-models: correlation with cellular incorporation and subcellular distribution, Biochem. Pharmacol. 68 (2004) 1443–1452.
[20] A.C. Serra, M. Pineiro, A. Gonsalves, M. Abrantes, M. Laranjo, A.C. Santos, M.F. Botelho, Halogen atom effect on photophysical and photodynamic characteristics of derivatives of 5,10,15,20-tetrakis(3-hydroxyphenyl)porphyrin, J. Photochem. Photobiol. B 92 (2008) 59–65.
[21] G. Schermann, A. Volcker, K. Seikel, R. Schmidt, H.D. Brauer, F.P. Montforts, Potential photosensitizers for photodynamic tumor-therapy. 1. Photophysical properties of 2 chlorin derivatives, Photochem. Photobiol. 51 (1990) 45–51.
[22] R. Schneider, L. Tirand, C. Frochot, R. Vanderesse, N. Thomas, J. Gravier, F. Guillemin, M. Barberi-Heyob, Recent improvements in the use of synthetic peptides for a selective photodynamic therapy, Anti-Cancer Agents Med. Chem. 6 (2006) 469–488.
[23] E.I. Yslas, V. Rivarola, E.N. Durantini, Synthesis and photodynamic activity of zinc(II) phthalocyanine derivatives bearing methoxy and trifluoromethylbenzyloxy substituents in homogeneous and biological media, Bioorg. Med. Chem. 13 (2005) 39–46.
[24] E.I. Yslas, E.N. Durantini, V.A. Rivarola, Zinc-(II) 2,9,16,23-tetrakis (methoxy) phthalocyanine: potential photosensitizer for use in photodynamic therapy in vitro, Bioorg. Med. Chem. 15 (2007) 4651–4660.
[25] J.W. Hofman, F. van Zeeland, S. Turker, H. Talsma, S.A.G. Lambrechts, D.V. Sakharov, W.E. Hennink, C.F. van Nostrum, Peripheral and axial substitution of phthalocyanines with solketal groups: synthesis and in vitro evaluation for photodynamic therapy, J. Med. Chem. 50 (2007) 1485–1494.
[26] N. Rubio, F. Prat, N. Bou, J.I. Borrell, J. Teixido, A. Villanueva, A. Juarranz, M. Canete, J.C. Stockert, S. Nonell, A comparison between the photophysical and photosensitising properties of tetraphenylporphycenes and porphyrins, New J. Chem. 29 (2005) 378–384.
[27] N. Rubio, D. Sanchez-Garcia, A. Jimenez-Banzo, O. Rey, J.I. Borrell, J. Teixido, S. Nonell, Effect of aza substitution on the photophysical and electrochemical properties of porphycenes: characterization of the near-IR-absorbing photosensitizers 2,7,12,17-tetrakis(p-substituted phenyl)-3,6,13,16-tetraazaporphycenes, J. Phys. Chem. A 110 (2006) 3480–3487.
[28] A. Dudkowiak, E. Teslak, J. Habdas, Photophysical studies of tetratolylporphyrin photo sensitizers for potential medical applications, J. Mol. Struct. 792 (2006) 93–98.
[29] H. Horiuchi, T. Tanaka, K. Yoshimura, K. Sato, S. Kyushin, H. Matsumoto, H. Hiratsuka, Enhancement of singlet oxygen sensitization of tetraphenylporphyrin by silylation, Chem. Lett. 35 (2006) 662–663.
[30] G. Bikzhanova, I. Toulokhonova, S. Gately, R. West, Novel silicon-containing drugs derived from the indomethacin scaffold: synthesis, characterization and evaluation of biological activity, Silicon Chem. 3 (2007) 209–217.
[31] W. Bains, R. Tacke, Silicon chemistry as a novel source of chemical diversity in drug design, Curr. Opin. Drug Discov. Dev. 6 (2003) 526–543.
[32] S. Gately, R. West, Novel therapeutics with enhanced biological activity generated by the strategic introduction of silicon isosteres into known drug scaffolds, Drug Dev. Res. 68 (2007) 156–163.
[33] R. Schmidt, C. Tanielian, R. Dunsbach, C. Wolff, Phenalenone, a universal reference compound for the determination of quantum yields of singlet oxygen $O_2(^1\Delta_g)$ sensitization, J. Photochem. Photobiol. A 79 (1994) 11–17.
[34] L. Pekkarinen, H. Linschitz, Studies on metastable states of porphyrins. II. Spectra and decay kinetics of tetraphenylporphine, zinc tetraphenylporphine and bacteriochlorophyll, J. Am. Chem. Soc. 82 (1960) 2407–2660.
[35] F. Wilkinson, D.J. McGarvey, A.F. Olea, Excited triplet-state interactions with molecular-oxygen – influence of charge-transfer on the bimolecular quenching rate constants and the yields of singlet oxygen (O_2^*, $^1\Delta_g$) for substituted naphthalenes in various solvents, J. Phys. Chem. 98 (1994) 3762–3769.
[36] G.J. Kavarnos, N.J. Turro, Photosensitization by reversible electron-transfer – theories, experimental-evidence, and examples, Chem. Rev. 86 (1986) 401–449.
[37] D. Bom, D.P. Curran, S. Kruszewski, S.G. Zimmer, J.T. Strode, G. Kohlhagen, W. Du, A.J. Chavan, K.A. Fraley, A.L. Bingcang, L.J. Latus, Y. Pommier, T.G. Burke, The novel silatecan 7-tert-butyldimethylsilyl-10-hydroxycamptothecin displays high lipophilicity, improved human blood stability, and potent anticancer activity, J. Med. Chem. 43 (2000) 3970–3980.
[38] B.-H. Ye, Y. Naruta, A novel method for the synthesis of regiospecifically sulfonated porphyrin monomers and dimers, Tetrahedron 59 (2003) 3593–3601.
[39] S. Kyushin, K. Yoshimura, K. Sato, H. Matsumoto, Hyperchromic effect of silyl groups on the UV–visible spectrum of 5,10,15,20-tetraphenylporphyrin, Chem. Lett. 38 (2009) 324–325.