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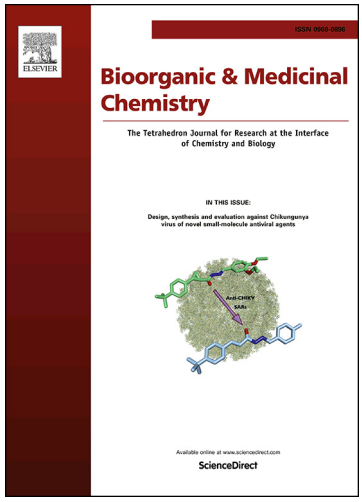

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Article

Synthesis, Photophysical Properties, and Photodynamic Activity of Positional Isomers of TFPP-Glucose Conjugates

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ABSTRACT

The synthesis and characterization of a 'complete set' of positional isomers of tetrakis(perfluorophenyl)porphyrins (TFPP)-glucose conjugates (**1OH**, **2OH**, **3OH**, **4OH**, and **6OH**) are reported herein. The cellular uptake and photocytotoxicity of these conjugates were examined in order to investigate the influence of location of the TFPP moiety on the D-glucose molecule on the biological activity of the conjugates. An *In vitro* biological evaluation revealed that the certain of these isomers have a greater effect on cellular uptake and cytotoxicity than others. The TFPP-glucose conjugates **1OH**, **3OH**, and **4OH** were found to exert exceptional photocytotoxicity in several types of cancer cells compared to **2OH** and **6OH** substituted isomers.

1. Introduction

Human life expectancy is increasing year by year thanks to the dramatic developments in medical technology and the availability of advanced medical treatment.[1] With such a social situation, numerous people are seeking cancer treatment that has few side effects and is non-invasive. Photodynamic therapy (PDT) represents such a treatment that satisfies these requirements.[2] PDT is a therapeutic method based on generating cytotoxic reactive oxygen species (ROS) by the visible light irradiation of a tumor-containing photosensitizer (PS) for selectively killing tumor tissue by apoptosis or necrosis. Porphyrin derivatives are the most useful PS in PDT applications, because of their inherent ability to selectively accumulate in tumors and for their ability to efficiently generate ROS.[2,3] Until now, considerably efforts have been devoted to developing high performance PS in terms of light absorbing ability in a longer wavelength region (frequently referred to as the PDT-window), efficient ROS generation, and high tumor selectivity.

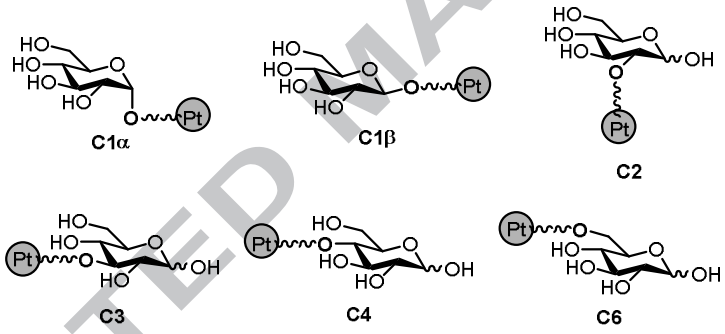
In the development of drugs with improved tumor accumulation, one approach is to conjugate the PS to an inactive site and transform it into an activated PDT-PSs by light or enzymes, namely prodrug activation.[3] Another simple approach involves the conjugation of a PS with a biologically active element such as a peptide,[4] an anti-tumor monoclonal antibody,[5] folic acid,[6] or a sugar.[7] Among these approaches, glycoconjugation, a chemical modification using sugar, is a promising strategy and has attracted considerable attention.[8] In addition to providing polar hydroxy groups and to specifically bind to receptors on the cell surface,[9] sugars are also known to have a key role in cancer,[8a,10] immune dysfunction,[11] congenital disorders,[12] and infectious diseases.[8b] The potential of sugar is also supported by

that a radiolabeled D-glucose derivative, namely 2-[^{18}F]fluoro-2-deoxy-D-glucose (^{18}F-FDG), is frequently used in cancer diagnosis by positron emission tomography (PET).[8a,13] Many sugar-conjugated PSs have been synthesized and their photophysical properties, cellular uptake behavior, and *in vitro* photocytotoxicity examined.[7a,14] A few sugar-conjugated PSs have also been tested in *in vivo* conditions.[7a,14b,15] The findings indicate sugar-conjugated PSs exhibit a high cell uptake and photocytotoxic effect by virtue of the fact that the molecule contains a sugar moiety. Kessel and co-workers reported that not only the type of substituent (namely, the biologically active element) conjugated to the tetraphenylporphyrin sulfonic acid (TPPS), but also the substitution number and pattern (i.e. mono-, bis *cis*-, bis *trans*-, tri-, and tetra-sulfonic acids) greatly influence the extent of cellular uptake and the overall effect of the PS.[16] In a previous study, we reported on the synthesis of tetrakispentafluorophenyl porphyrin (TFPP) derivatives with glucose oligomers with varying chain lengths and binding positions (namely, mono-, bis *cis*-, bis *trans*-, tri-, and tetra-glucoses) and examined the PDT effect. The findings indicated that the TFPP bis *trans*-glucose derivatives exhibited an almost 20-fold higher photocytotoxicity compared with the other TFPPs.[17]

Lippard and co-workers recently investigated the effect of the position of substitution on D-glucose on the biological activity of Pt complex-glucose conjugates (Glc-Pts) for an anticancer agent.[8c] Although the synthesis of a complete set of positional isomers of a glycoconjugate and a study of the influence the substitution position on its biological activity has not been reported over the past two decades, they prepared all possible positional isomers (**C1*α***, **C1*β***, **C2**, **C3**, **C4**, and **C6**) of a Glc-Pt complex studied their

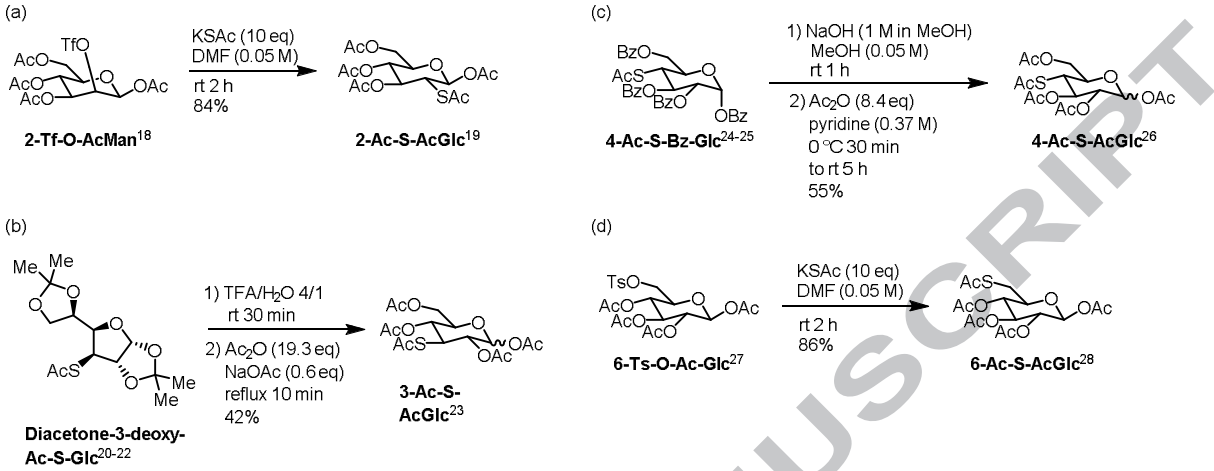
effects, both *in vitro* and *in vivo* (Chart 1). Their findings indicate that the recognition and efficiencies varied for different positional isomers of a glycoconjugate and that different positions of substitution resulted in a different response for cellular uptake, cytotoxicity profile, and the inhibition of the facilitative membrane-bound glucose transporter (GLUT1) which is usually specifically overexpressed in various cancers. While the **C3** isomer showed the lowest efficiency in cancer cells, the **C1*α*** and **C2** isomers accumulation the most efficiently, compared to the others. Furthermore, the **C2** isomer exhibited the highest GLUT1-specific internalization in cancer cells.

Chart 1 Structure of Pt complex-glucose conjugates[8c]



In order to study the effect of D-glucose positional isomers on their chemical and biological properties, we synthesized a complete set of positional isomers of D-glucose by attaching TFPP to the 2-, 3-, 4-, and 6-positions by sulfide bonding, i.e., at the **2OH**, **3OH**, **4OH**, and **6OH** positions examined their chemical and biological properties, and compared their activities with those for the **1OH** isomer The findings indicate that the position of substitution is an important factor in ROS generation, cellular uptake, GLUT-1 inhibition, and cytotoxicity.

Scheme 1. Synthesis of **2-**, **3-**, **4-**, and **6-Ac-S-AcGlc**



2. Results and discussion

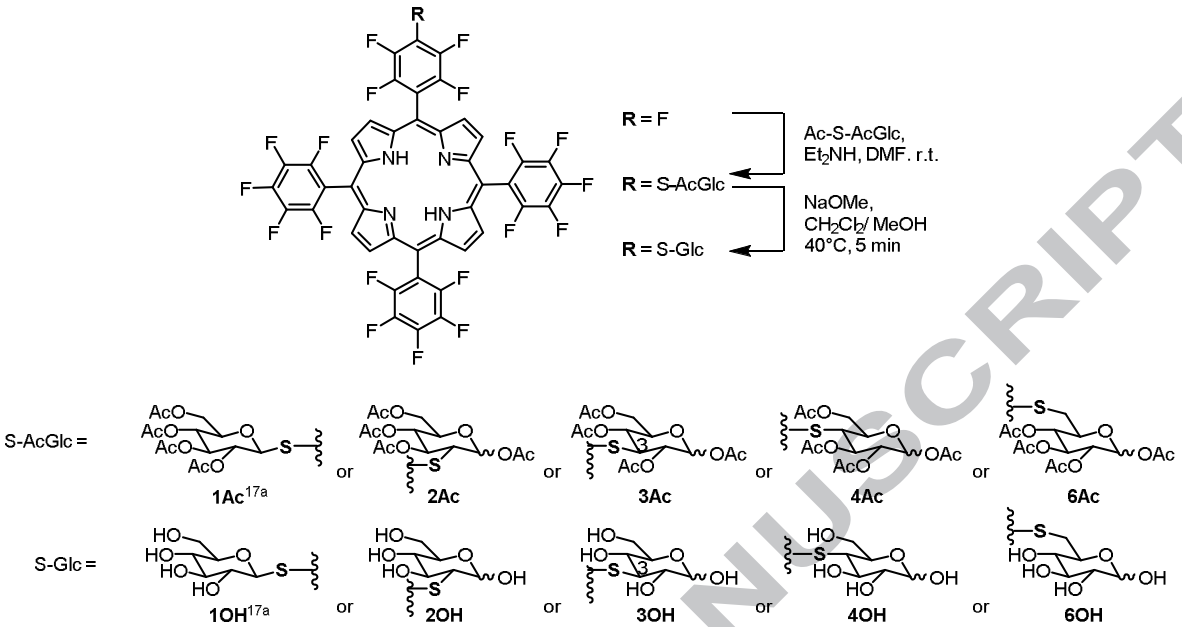
2.1. Chemistry

To systematic study the effect of D-glucose positional isomers, D-glucose derivatives that were deoxythiolated at the 2-, 3-, 4-, and 6-positions were synthesized following previously reported procedures, with some modifications. Starting with D-mannose, tetraacetyl mannose 2-triflate (**2-Tf-O-AcMan**) was prepared through peracetylation followed by rearrangement of the acetyl group through orthoester formation (details of their precursor preparations are shown in Supporting Information).[18] An acethylthio group was introduced by an S_N2 reaction to afford tetraacetyl 2-thio glucose (**2-Ac-S-AcGlc**) as the β-anomer.[19]

From commercially available diacetone D-glucose, the free 3-hydroxy group was epimerized through oxidation and reduction[20] followed by triflation[21] and acetyl thiolation[22] to give diacetone 3-deoxy-3-acetylthio D-glucose (**Diacetone-3-deoxy-Ac-S-Glc**), which was transformed into the pyranose form to yield the tetraacetyl 3-thio glucose (**3-Ac-S-**

AcGlc) as an α/β-anomeric mixture.[23] For the tetraacetyl 4-thio glucose (**4-Ac-S-AcGlc**), the synthesis was started from D-galactose, the most reliable and reproducible route. Global benzoylation except for the axial 4-hydroxy group was successful, giving 4-hydroxyl tetrabenzoyl glucose in moderate yield,[24] which was transformed to the triflate derivative.[25] Reaction with potassium thioacetate gave **4-Ac-S-Bz-Glc** in moderate yield. Finally, the fully **4-Ac-S-AcGlc** was synthesized as an α/β-anomeric mixture, which was previously prepared via a different route.[26] The benzoyl groups of **4-Ac-S-Bz-Glc** were replaced with acetyl groups because the removal of benzoyl group was difficult at the stage of the last step after the conjugation with TFPP. 6-Acetyl 6-thio (**6-Ac-S-AcGlc**)[27] was prepared from D-glucose by a one-pot regioselective tosylation/tetraacetylation[28] and acetylthiolation was accomplished similar to the procedure used for the synthesis of **2-Ac-S-AcGlc**.[19]

Scheme 2. Synthesis of TFPP-glucose conjugates



S_NAr reactions of TFPP with peracetylated thioglucose were carried out using a method similar to that used to prepare the TFPP-glucose conjugate **1Ac** (Scheme 2).[17a] All other thioglucopyranoses, **2-Ac-S-AcGlc**, **3-Ac-S-AcGlc**, **4-Ac-S-AcGlc**, and **6-Ac-S-AcGlc**, were less reactive than **1-Ac-S-AcGlc**. The S_NAr reaction of TFPP with **6-Ac-S-AcGlc** required an unusually high temperature of 40 ~ 50°C or a longer reaction time of 3 days or more. This is because the other positions (C2, C3, C4, and C6) react more slowly than the anomeric position,[29] that is, it appears to be difficult to generate thiolate ions in these compounds, and therefore, to bind with porphyrin. This was confirmed by the fact that the thioglucopyranose derivatives (**2-Ac-S-AcGlc**, **3-Ac-S-AcGlc**, **4-Ac-S-AcGlc**, and **6-Ac-S-AcGlc**) were recovered from the reaction mixture after column purification. The reaction mixtures were separated by conventional silica gel column chromatography to give the peracetylated TFPP-glucose conjugates **2Ac**, **3Ac**, **4Ac**, and **6Ac** as deep purplish red

solids. These compounds were fully characterized by 1D (^{1}H, ^{13}C, and ^{19}F) and 2D (COSY and HSQC) NMR, UV-vis spectroscopy, MALDI-TOF high resolution mass spectrometry. ^{1}H NMR spectra of **2Ac**, **3Ac**, **4Ac**, and **6Ac** were recorded in $CDCl_3$. The β-pyrroles afforded two peaks centered at 8.9 ppm due to asymmetric conjugation, which is similar to that for **1Ac**.[17a] The protons of acetyl groups and inner pyrrole protons were found around 2.3 ~ 2.1 ppm and −2.9 ppm, respectively. Glucose protons afforded heavily coupled peaks in the range of 7 ~ 3 ppm. The peracetylated TFPP-glucose conjugates **2Ac** and **6Ac** were obtained only β-anomeric form. In contrast, **3Ac** and **4Ac** were obtained as anomeric mixtures. These compounds showed two doublet peaks that were assigned to the α-anometic proton (^{3}J = 4.0 Hz) at 6.4 ppm and the β-anomeric one (^{3}J = 8.0 Hz) at 5.8 ppm.[17b,30] The α- and β-isomeric ratios for these derivatives were determined to be 5:5 and 3:7 for **3Ac** and **4Ac**, respectively. Interestingly, this stereochemical difference resulted in the chemical shift of the 2,6- and 3,5-F of glucose-substituted perfluorophenyl group, which are separated from the anomeric position. Stereochemically pure **2Ac** and **6Ac** showed two peaks corresponding to the 2,6- and 3,5 positions at −136.54 ~ −138.02 and −131.85 ~ −133.77 ppm. In contrast, **3Ac** and **4Ac** afforded two set of two peaks whose area ratios approximately coincided with that determined by ^{1}H NMR spectroscopy.

Further separation of the anomeric mixture was not feasible or very inefficient at least. Fortunately, this did not impact our goal because all acetyl groups were removed to give anomeric mixtures of all these glucose conjugates, which existed in an equilibrium between α- and β-anomers in solution and were subjected to further physiological and biological investigations (*vide infra*). The part of crude acetylated compounds **2Ac**, **3Ac**, **4Ac** and **6Ac**

were used for the synthesis of deacetylated compounds. After chromatographic purification, four TFPP-glucose conjugates **2OH**, **3OH**, **4OH**, and **6OH** were obtained in moderate yields and acceptable purities. The structures of TFPP-glucose conjugates **2OH**, **3OH**, **4OH**, and **6OH** were confirmed by ^{1}H, ^{19}F, and ^{13}C NMR spectroscopies with a 2D technique such as COSY, HSQC and ^{1}H-^{19}F HOESY, UV-vis spectroscopy, Steady-state luminescence spectroscopy, and MALDI-TOF high resolution mass spectrometry. All of the TFPP-glucose conjugates were detected as $[M]^{+}$ species in MALDI-TOF high resolution mass spectrometry.

After hydrolysis by treatment with sodium methoxide and chromatographic purification, all peaks assigned to the acetyl groups disappeared. ^{19}F NMR spectra of **2OH**, **3OH**, **4OH**, and **6OH** clearly indicated that remaining 4-F nucleus of the pentafluorophenyl groups were conserved. Unlike peracetylated TFPP-glucose conjugates, all of the TFPP-glucose conjugates showed two sets of two peaks conforming the 2,6- and 3,5-F nucleus of glucose-conjugated perfluorophenyl group. This is reasonably explained by the fact that the α- and β-pyranose forms are in equlibrium. The peak area ratio between the two sets was approximately 4:6 which is in reasonable agreement with the value for D-glucose in water (37.3:62.7).

The stock solution of all compounds **2OH**, **3OH**, **4OH**, and **6OH** were prepared using qNMR data, and the physical properties (ROS-generation test and *In vitro* assay) were evaluated using these stock solutions.

Table 1 UV-Vis and Photoluminescence Spectral Data of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in DMSO at 25°C.[a]

	UV-vis					Luminescence
	λ_{max} / nm ($\varepsilon \times 10^{-4}$ / M^{-1} cm^{-1})				$f_{>500\ nm}$[b]	λ_{max} / nm[c]
	Soret	Q bands				
1OH	412.5 (29.3)	505.5 (2.09)	537.5 (0.07)	582.0 (0.61)	0.03	637.5, 702.5
2OH	412.5 (28.9)	506.0 (1.93)	537.5 (0.03)	582.0 (0.55)	0.03	637.5, 702.5
3OH	413.0 (30.4)	505.5 (2.13)	537.5 (0.06)	581.0 (0.63)	0.03	637.5, 703.0
4OH	413.0 (30.2)	506.0 (2.01)	537.5 (0.02)	582.0 (0.57)	0.03	637.5, 703.0
6OH	412.5 (35.1)	505.5 (2.52)	536.5 (0.06)	581.0 (0.71)	0.04	637.5, 702.5

[a] [**1OH**] = 5.00 μM, [**2OH**] = 4.44 μM, [**3OH**] = 4.48 μM, [**4OH**] = 4.05 μM, [**6OH**] = 4.19 μM. [b] Oscillator strength in the range above 500 nm estimated as $4.32 \times 10^{-9} \int \varepsilon(\nu) d\nu$. [c] The excitation wavelength was adjusted to the maximum absorption wavelength of the Soret band.

All TFPP-glucose conjugates **2OH**, **3OH**, **4OH**, and **6OH** exerted typical phyllo-type UV-vis spectra with one intense peak (Soret band) at approximately 413 nm and three vibronic peaks (Q bands) around 505 to 648 nm in DMSO (Table 1 and *vide infra*). The TFPP-glucose conjugates **2OH**, **3OH**, **4OH**, and **6OH** afforded broad peaks in UV-vis spectra recorded in PBS containing 1 vol% DMSO (Figure 1 and Table 2), and resemble to the

spectrum of **1OH** that was reported previously.[17a] These results suggested that the TFPP-glucose conjugates form aggregates similar to **1OH** in PBS. In addition, the oscillator strength in the region over 500 nm ($f_{>500nm}$), which is an important measure for evaluating the probability of photoexicitation, was nearly the same for of the TFPP-glucose conjugates.

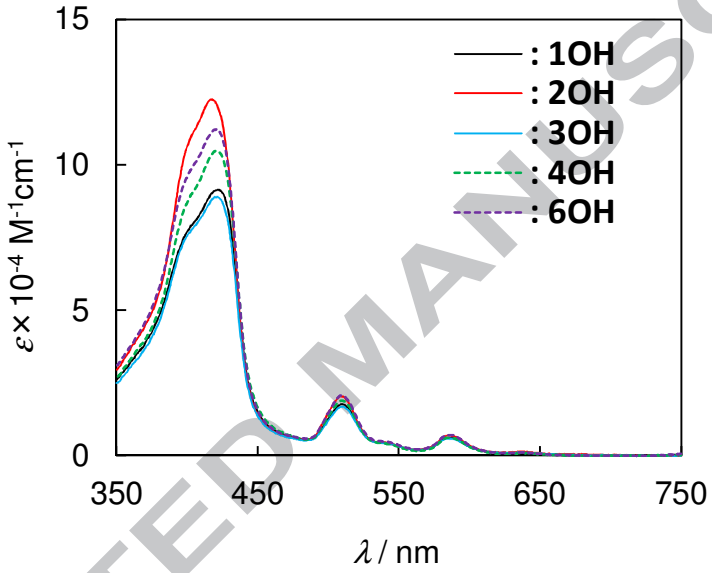


Figure 1. UV-vis spectra of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in PBS Solution containing 1% DMSO at 25°C.

Table 2 UV-Vis and Photoluminescence Spectral Data of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in PBS containing 1% DMSO at 25°C.[a]

	UV-vis					Luminescence
	λ_{max} / nm ($\varepsilon \times 10^{-4}$ / M^{-1} cm^{-1})				$f_{>500\ nm}$[b]	λ_{max} / nm[c]
	Soret	Q bands				
1OH	421.5 (9.1)	509.5 (1.77)	557.0 (0.23)	586.5 (0.60)	0.06	645.0, 709.0
2OH	417.5 (12.2)	509.0 (2.04)	556.5 (0.23)	586.0 (0.69)	0.06	646.5, 662.0, 702.5
3OH	421.5 (8.90)	509.0 (1.67)	557.0 (0.23)	586.0 (0.58)	0.05	645.5, 663.0, 703.0
4OH	421.5 (10.5)	510.0 (1.90)	557.0 (0.21)	586.5 (0.61)	0.06	646.0, 662.5, 703.0
6OH	421.0 (11.2)	510.0 (2.06)	557.0 (0.25)	586.0 (0.70)	0.06	645.5, 702.5

[a] [**1OH**] = 5.00 μM, [**2OH**] = 4.44 μM, [**3OH**] = 4.48 μM, [**4OH**] = 4.05 μM, [**6OH**] = 4.19 μM. [b] Oscillator strength in the range above 500 nm estimated as $4.32 \times 10^{-9} \int \varepsilon(v) dv$. [c] The excitation wavelength was adjusted to the maximum absorption wavelength of the Soret band.

PDT efficacy crucially depends on the generation of reactive oxygen species (ROS) by the photoirradiation of photosensitizers located in tumor tissue.[2,3a] There are two types of photochemical reactions, namely hydrogen or electron transfer generating superoxide anions, hydroxyl radicals (•OH) and other (types I reaction) and energy transfer producing singlet oxygen ($^1O_2$) (type II reaction). In general, $^1O_2$ is known to be the major cytotoxic

species in PDT applications because of its relatively long lifetime. In recent years, it was reported that hematoporphyrin (HP) and imidazole conjugated porphyrin not only generate singlet oxygen, but also hydroxyl radicals.[17] Therefore, both the type and efficiency of ROS generation are important issues for successful photosensitizers in PDT applications. The relative quantum yield for $^1O_2$ generation (Φ_Δ) was determined by means $^1O_2$ luminescence measurements at 1270 nm in O_2-saturated MeOH and normalized to the values for **1OH**. On the other hand, the relative quantum yields ($\Phi_{\bullet OH}$) of •OH were evaluated by the rate constant for the increment of the initial rate of HPF-fluorescence intensity[31] and normalized to the value for hematoporphyrin (HP). The relative Φ_Δ and $\Phi_{\bullet OH}$ values for the TFPP-glucose conjugates are listed in Table 3. No differences in the Φ_Δ values was found in methanol. However, similar to **1OH**,[17b] TFPP-glucose conjugates **2OH**, **3OH**, **4OH**, and **6OH** showed higher •OH generation than HP. The $\Phi_{\bullet OH}$ values for the TFPP-glucose conjugates increased in the order of **2OH < 1OH < 4OH < 3OH << 6OH**. Hence, the $\Phi_{\bullet OH}$ values for TFPP-glucose conjugates were dependent, not only on the *S*-glycosylation pattern, but also the specific of D-glucose.

Table 3 Relative Quantum Yield of Singlet Oxygen (Φ_{Δ}) and Hydroxyl Radical ($\Phi_{\bullet OH}$) of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH**.

	Φ_{Δ} [a]	$\Phi_{\bullet OH}$ [b]
1OH	1.0	25.0
2OH	1.0	20.5
3OH	1.0	36.9
4OH	1.0	28.4
6OH	1.0	61.2
HP	–	1.0

[a] In O_2-saturated methanol and normalized to the values for **1OH**. The excitation wavelength was 399.4 nm (**1OH**), 397.6 nm (**2OH**), 395.4 nm (**3OH**), 397.6 nm (**4OH**), and 389.8 nm (**6OH**) (Abs = 0.2). [b] The $\Phi_{\bullet OH}$ value in O_2-saturated PBS containing 1 vol% DMSO and normalized to the values for HP.

The hydrophobicity parameter (Log *P*), the logarithmic scale of the partition coefficient between *n*-octanol and water, is closely correlated to the penetration and uptake behavior of drugs through the cellular membrane.[32] The Log *P* values of the TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** were determined by reverse phase thin layer chromatography (RP-TLC) (Table 4). Their hydrophobicity increased in the order of **1OH < 3OH < 4OH < 6OH < 2OH**.

Table 4 R_f values in RP-TLC and Hydrophobicity Parameter (Log *P*) of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH**.

	R_f	Log P^a
1OH	0.19	6.05
2OH	0.11	6.87
3OH	0.17	6.21
4OH	0.16	6.30
6OH	0.14	6.50

[a] Log $P = 3.46 \times \text{Log}\,(1/R_f) + 3.55$.

2.2. Biology

The cellular uptake of these compounds was examined in stomach cancer, namely an RGK gastric carcinoma mucosal cell line (RGK cells). Figure 2 shows the relative cellular uptake of the TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** by RGK cells after incubation with each compound (c = 2.5 μM) for 8 and 24h. The value was normalized to that of **1OH** for an 8h incubation. The amounts of these compounds that were taken up increased with increasing incubation time from 8 to 24h. TFPP-glucose conjugate **4OH** showed a slightly higher uptake than the other conjugates.

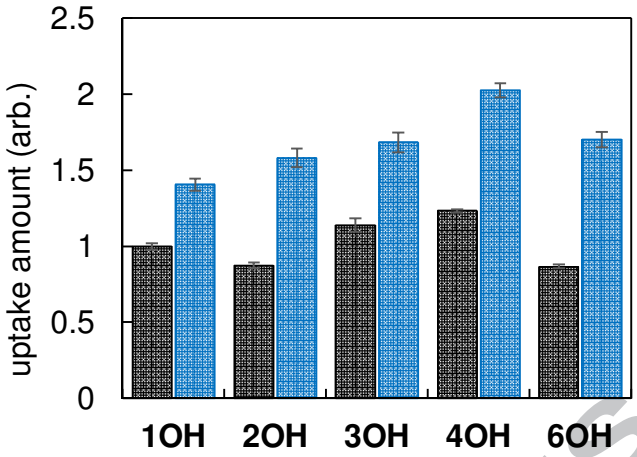


Figure 2. Relative uptake amount of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in RGK-1 cells for 8h (gray stick bars) and 24h (blue stick bars) co-incubation. The concentration of the conjugates was 2.5 μM. Values are the mean ± standard deviation of three replicate experiments.

Lippard and co-workers recently reported cellular uptake studies of platinum complexes that were ligated to different positions on D-glucose (**C1*α***, **C1*β***, **C2**, **C3**, **C4**, and **C6**; Chart 1) using A2780, A549, and DU 145 cells after 7.5 ~ 72h co-incubation.[8c] They reported that Glc-Pt **C2** showed a high uptake in cancer cells that overexpress the membrane-bound glucose transporter (GLUT1), resulting in a high anticancer activity. It is known that GLUT1 is overexpressed in brain tumors and stomach cancer.[35] Therefore, the cellular uptake of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in the presence of cytochalasin B, an inhibitor of GLUT1, was examined in RGK cells after a 8 and 24 h co-incubation (Figure 3). The uptake of **2OH**, **3OH**, and **4OH** by RGK cells appeared to be slightly inhibited in the presence of cytochalasin B. This result suggests that the cellular

uptake of these conjugates is partly driven by GLUT1. However the effect of cytochalasin B was too small, probably due to the strong hydrophobic nature of these conjugates, which would result in enhanced non-specific interactions with cellular membrane.

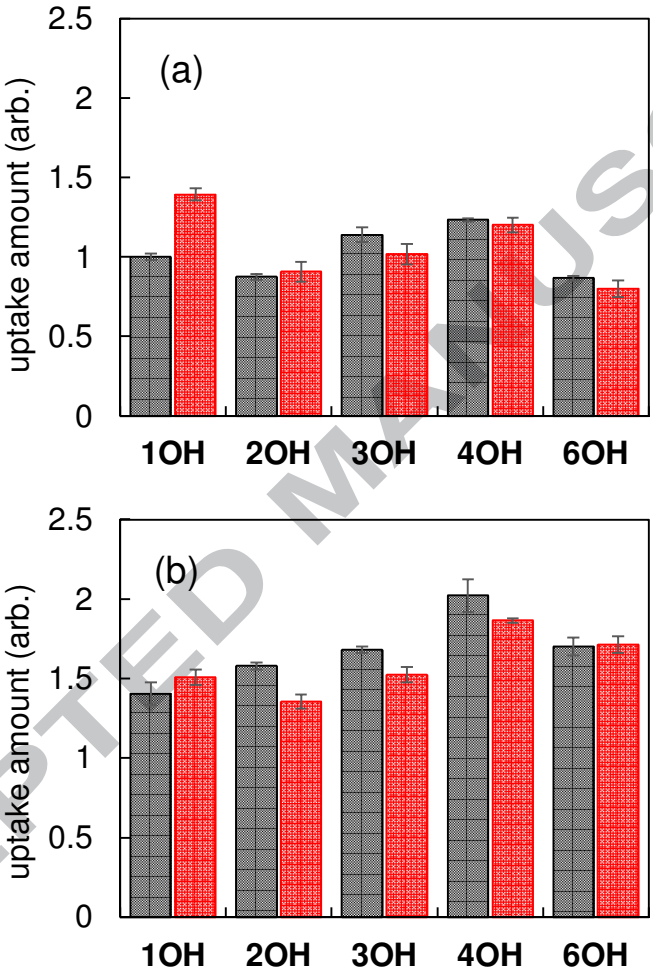


Figure 3. Relative uptake amount of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in RGK cells in the absence (red stick bars) and presence (gray stick bars) of cytochalasin B (c = 50 μM), which is a GLUT1 inhibitor, for 8h (a) and 24h (b) co-incubation. The concentration of the conjugates was 2.5 μM. Values are the mean ± standard deviation of three replicate experiments.

The photocytotoxicity of the TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** were evaluated in Human cervical cell line (HeLa cells) at a concentration of 1.0 μM in the dark and by irradiation. The cells were incubated with photosensitizers for 24h, and then photoirradiated using a 100 W halogen lamp (λ> 500 nm) at a light dose of 0 J cm^{-2} (dark) or 16 J cm^{-2}. cell survival (%) at 24h post-photoirradiation was evaluated in terms of the mitochondrial activity of NADH dehydrogenase using the WST-8® reagent from Cell Counting Kit-8 and the value was normalized to the survival rate in the absence of photosensitizer, namely vehicle. None of the conjugates showed any cytotoxicity in the dark (*vide infra*). In contrast, these conjugates, except for **2OH,** showed potent photocytotoxicity under photoirradiation (Figure 4).

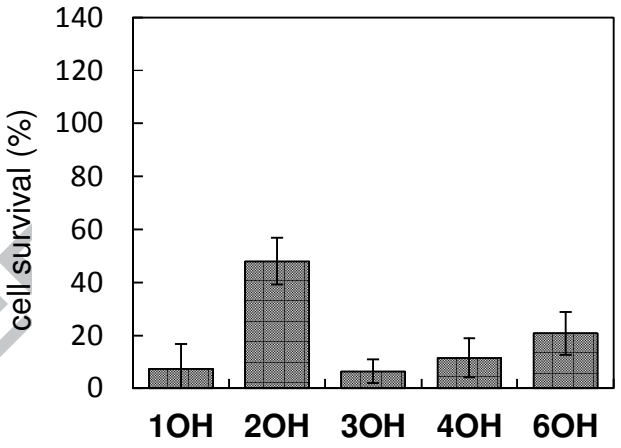


Figure 4. Photocytotoxicity of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in HeLa cells. The concentration of the conjugate was 1.0 μM. The light dose was 16 J cm^{-2} from a 100 W halogen lamp (λ > 500 nm). Values are the mean ± standard deviation of six replicate experiments.

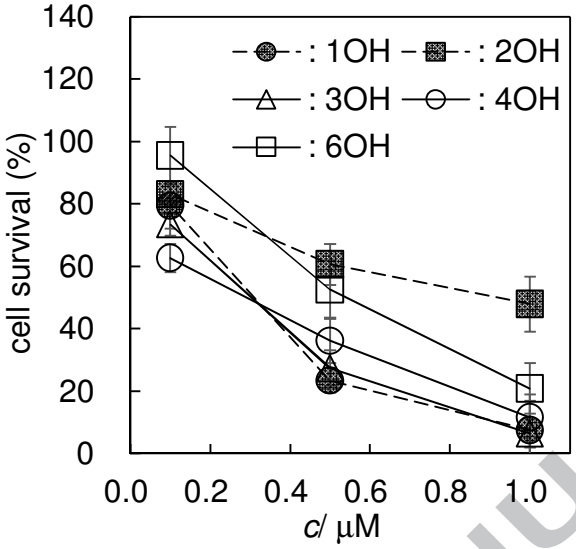


Figure 5. Plot of the cell survival rate (%) of HeLa cells treated with TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** as a function of the concentration. The light dose was 16 J cm^{-2} from a 100 W halogen lamp (λ > 500 nm). Incubation time with the conjugates was 24 h. Values are the mean ± standard deviation of six replicate experiments.

The photocytotoxicity of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in HeLa cells was examined in the concentration range from 0.1 to 1.0 μM (Figure 5). The drug concentration needed to induce 50% cell death (EC_{50}) values for the TFPP-glucose conjugates **1OH**, **3OH**, and **4OH** were somewhat lower than the values for **2OH** and **6OH**, indicating a slightly higher photocytotoxic effect. In previous studies of TFPPs having *S*-glucopyranosyl groups with possible patterns (mono-, *cis*-bis-, *trans*-bis-, tris-, and tetrakis-substituted), we found that the photocytotoxic effect of these compounds is correlated with the amount taken up by cells.[17] In contrast, no correlation between cellular uptake and

photocytotoxicity was found for these mono-glucose conjugates. This can be attributed to the high hydrophobicity of TFPP-glucose conjugates. PDT has been applied to the treatment of early lung cancer, early esophageal cancer, uterine cancer and breast cancer, and more recently it was approved as a treatment for early gastric cancer and brain cancer.[36] Therefore, the photocytotoxicity of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH** and **6OH** was examined in brain tumors, namely the human glioblastoma cell line (U251), and RGK cells under the same conditions as for HeLa cells. Figure 6 shows cell survival (%) as a function of concentration. The effect of positional isomers in both cells was found to be similar to that in HeLa cells.

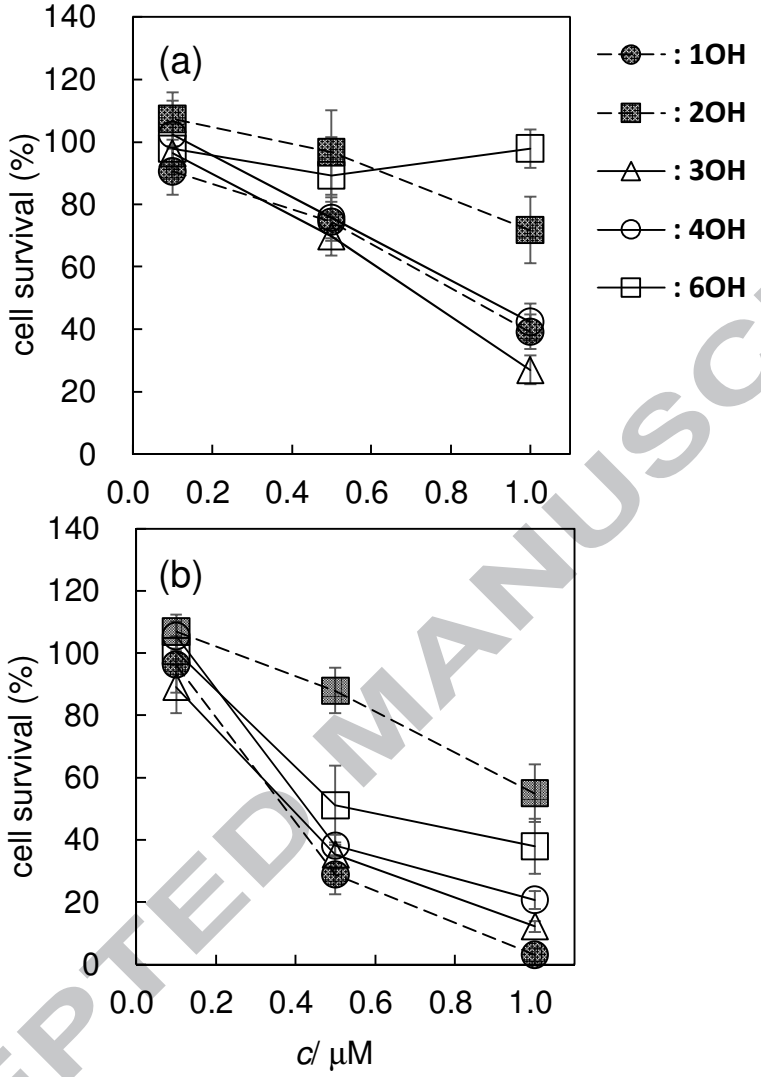


Figure 6. Plot of the cell survival rate (%) of U251 cells (a) and RGK cells (b) treated with TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** as a function of the concentration. The light dose was 16 J cm^{-2} from a 100 W halogen lamp (λ > 500 nm). Incubation time with the conjugates was 24 h. Values are the mean ± standard deviation of six replicate experiments.

3. Conclusion

We successfully synthesized five possible positional isomers of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH**. The TFPP-glucose conjugates **1OH**, **3OH**, and **4OH** exerted a higher photocytotoxicity in several cancer cells, such as HeLa cells, RGK cells and U251 cells, compared to the **2OH** and **6OH** isomers. Hence, the location of substitution on the D-glucose molecule influence the photocytotoxicity of the TFPP-glucose conjugates. In addition, the cellular uptake of these conjugates in RGK cells suggested that the cellular internalization of **2OH**, **3OH**, and **4OH** was partly facilitated by the GLUT1 pathway. Accordingly, the specificity of these TFPP-glucose conjugate isomers would be expected to be reduced by reducing their hydrophobicity.

4. Experimental

4.1. Materials

All chemicals were of analytical grade. Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui pharamaceutical Co. Ltd. (Tokyo, Japan). Fetal calf serum (FCS) was purchased from Hyclone Laboratories, Inc. (Logan, UT, USA). Antibiotic-Antimyconic was purchased from Life Technologies Japan Ltd. (Tokyo, Japan). DMEM nutrient mixture F-12 HAM (F-12 Ham/DMEM, 1/1) and tetraphenylporphyrin tetrasulfonic acid (TPPS) and 1,3-diphenylisobenzofuran (DPBF) were purchased from Sigma-Aldrich Japan. Cytochalasin B, hematoporphyrin (HP), 3,5-bis(trifluoromethyl)benzoic acid, Dulbecco's phosphate buffered saline (PBS) and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka,

Japan). Hydroxyphenyl fluorescein (HPF) was purchased from Goryo Chemical, Inc. (Tokyo, Japan). 5,10,15,20-Tetrakispentfluorophenyl porphyrin (TFPP) was prepared according to the previous literature. [36] 5-[4-(2,3,4,6-Tetra-*O*-acetyl-1-thio- -D-glucopyranos-1-*S*-yl)-2,3,5,6-tetrafluoro-phenyl)]-10,15,20-tris(2,3,4,5,6-pentafluorophenyl)porphyrin (**1Ac**) and 5-[4-(1-thio-D-glucospyranos-1-*S*-yl)-2,3,5,6-tetrafluorophenyl)]-10,15,20-tris(pentafluorophenyl)porphyrin (**1OH**) were prepared according to our previous papers [17a] Stock solutions of photosensitizers were prepared by weighing the dried photosensitizers and dissolving them in DMSO, and kept in freezer (–30°C) until use.

4.2. General Methods

Reverse-phase thin layer chromatography (RP-TLC) was carried out using R-18F_{254s} (Merck Japan Ltd., Tokyo, Japan). The purities of peracetyrated TFPP-Glucose conjugates **2Ac**, **3Ac**, **4Ac** and **6Ac** were determined to be >99% on an HPLC system (Jasco PU-2086 Plus Intelligent pump, Jasco Co., Ltd., Tokyo, Japan) equipped with a UV-vis detector (UV-2075 Plus, Jasco Co., Ltd.) and a silica gel column (COSMOSIL 5SL-II packed column, 4.6 mmϕ × 150 mm, Nacalai Tesque, Inc., Kyoto, Japan) using a mixture of CH_2Cl_2 and ethyl acetate (9/1, v/v) at 30°C. The purities of TFPP-Glucose conjugates **2OH**, **3OH**, **4OH** and **6OH** were determined to be >97% on an HPLC system (Jasco PU-2086 Plus Intelligent pump) equipped with a UV-vis detector (UV-2075 Plus) and a silica gel column (COSMOSIL 5SL-II packed column, 4.6 mmϕ × 150 mm, Nacalai Tesque, Inc.) using a mixture of CH_2Cl_2 and CH_3OH (9/1, v/v) at 30°C. Mass spectra were recorded

using JEOL spiral TOF JMS-S3000 (MALDI-TOF) (JEOL Ltd., Tokyo, Japan). UV-vis spectra were recorded on UV-2550 spectrophotometer (Shimadzu Co., Kyoto, Japan) and V-570 spectrophotometer (JASCO Co., Ltd., Tokyo, Japan). Steady-state fluorescence (FL) spectra were recorded on FP-6300 spectrofluorometer (JASCO Co., Ltd., Tokyo, Japan). NMR spectra were recorded using an AVANCE III HD (500 MHz; Bruker Biospin K.K., Yokohama, Japan) and a NMR-DD2 500PS (500 MHz; Agilent Technologies, CA, USA). Luminescence spectra of singlet oxygen sensitized by each compound's solution was recorded using a spectrometer (Jobin Yvon SPEX fluorolog3, HORIBA, Ltd., Kyoto, Japan) equipped with a photomultiplier (NIR-PMT R5509-72, Hamamatsu Photonics K.K., Shizuoka, Japan) cooled to 193 K. The absorbance and the fluorescence intensity of each well were determined using plate readers (Multiscan JX and Fluoroskan Ascent, Thermo Fisher Scientific Co., Yokohama, Japan).

4.3.1. 1,3,4,6-Tetra-O-acetyl-2-S-acetyl-2-thio-β-D-glucopyranose (2-Ac-S-AcGlc, CCDC 1581347)[18-19]

A 0.05 M solution of **2-Tf-O-AcMan** (501 mg, 1 mmol) in dimethylformamide (DMF, 21 mL) was treated with potassium thioacetate (KSAc, 1.2 g, 10 mmol) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 2h and was partitioned between diethyl ether (Et_2O) and water. The organic phase was dried, filtered, and concentrated in vacuo. Purification by column chromatography on silica with hexane : ethyl acetate (EtOAc) = 3 : 1 as the eluant followed by recrystallization from Et_2O gave **2-Ac-S-AcGlc** as colorless solids (354 mg, 84%). R_f value 0.46 (hexane : EtOAc = 1 : 1). m.p.

117–119°C. HRMS (ESI) : *m/ z* for $C_{16}H_{22}NaO_{10}S$ $[M+Na]^+$ calcd 429.08314, found 429.08357 (error 0.43 mmu, 1.00 ppm). IR (NaCl, neat) ν_{max} 1755, 1707, 1369, 1221, 1103, 1049 cm^{-1}. ^1H NMR ($CDCl_3$, 500.16 MHz, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 5.83 (1H, d, $^3J_2$ = 9.0 Hz, 1-Glc*H*), 5.31 (1H, dd, $^3J_2$ = 11.5 Hz, $^3J_4$ = 9.0 Hz, 3-Glc*H*), 5.10 (1H, dd, $^3J_5$ = 10.0 Hz, $^3J_3$ = 9.0 Hz, 4-Glc*H*), 4.32 (1H, dd, $^3J_{6'}$ = 12.5 Hz, $^3J_5$ = 4.5 Hz, 6-Glc*H*), 4.10 (1H, dd, $^3J_6$ = 12.5 Hz, $^3J_5$ = 1.5 Hz, 6'-Glc*H*), 3.85 (1H, ddd, $^3J_4$ = 10.0 Hz, $^3J_6$ = 4.5 Hz, $^3J_{6'}$ = 1.5 Hz, 5-Glc*H*), 3.73 (1H, dd, $^3J_3$ = 11.0 Hz, $^3J_1$ = 9.5 Hz, 2-Glc*H*), 2.34 (3H, s, $SCOCH_3$), 2.09 (6H, s, $2COCH_3$), 2.017 (3H, s, $COCH_3$), 2.015 (3H, s, $COCH_3$). ^{13}C NMR ($CDCl_3$, 125.77 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 192.3 ($SCOCH_3$), 170.7 ($OCOCH_3$), 170.0 ($OCOCH_3$), 169.5 ($OCOCH_3$), 169.0 ($OCOCH_3$), 92.0 (1-Glc*C*), 72.6 (3-Glc*C*), 71.0 (4-Glc*C*), 68.8 (5-Glc*C*), 61.5 (6-Glc*C*), 47.1 (2-Glc*C*), 30.7 ($SCOCH_3$), 20.7 ($OCOCH_3$), 20.6 ($OCOCH_3$), 20.5 ($OCOCH_3$). $[\alpha]_D^{30}$ +15.9 (*c* 1.00 in $CHCl_3$).

4.3.2. 1,2,4,6-Tetra-O-acetyl-3-S-acetyl-3-thio-D-glucopyranose (3-Ac-S-AcGlc)[20-23]

A solution of **Diacetone-3-deoxy-Ac-S-Glc** (330 mg, 1 mmol) in CF_3COOH/H_2O (4:1, 1.4 mL) was stirred at room temperature under nitrogen atmosphere for 30 min. Concentration of the mixture gave a colorless residue, which was dissolved in acetic anhydride (Ac_2O, 1.9 mL) containing sodium acetate (NaOAc, 48 mg, 0.6 mmol). This mixture was heated at reflux (10 min) before being poured into ice/water. Standard work-up (EtOAc) and column chromatography on silica with hexane : EtOAc = 3 : 1 as eluant gave **3-Ac-S-AcGlc** as a yellowish oil (180 mg, 42%). R_f value 0.40 (hexane : EtOAc = 1 : 1). HRMS (ESI): *m/ z* for $C_{16}H_{22}NaO_{10}S$ $[M+Na]^+$ calcd 429.08314, found 429.08323 (error 0.09 mmu, 0.22 ppm).

IR (NaCl, neat) ν_{max} 1753, 1702, 1373, 1221, 1068 cm^{-1}. ^1H NMR ($CDCl_3$, 500.16 MHz, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 5.72 (1H, d, $^3J_2$ = 8.0 Hz, 1-Glc*H*), 5.12 (2H, m, 2-,6-Glc*H*), 4.26 (1H, m, $^3J_{6'}$ = 12.5 Hz, $^3J_5$ = 5.0 Hz, 4-Glc*H*), 4.11 (1H, m, 5-Glc*H*), 3.86 (2H, m, 3-,6'-Glc*H*), 2.34 (3H, s, SCO*C*H_3), 2.10 (3H, s, CO*C*H_3), 2.08 (3H, s, CO*C*H_3), 2.03 (6H, s, 2CO*C*H_3). ^{13}C NMR ($CDCl_3$, 125.77 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 193.1 (S*C*OCH$_3$), 170.7 (O*C*OCH$_3$), 169.3 (O*C*OCH$_3$), 169.2 (O*C*OCH$_3$), 169.0 (O*C*OCH$_3$), 93.1 (1-Glc*C*), 75.3 (2-Glc*C*), 69.1 (4-Glc*C*), 66.7 (5-Glc*C*), 61.7 (6-Glc*C*), 47.7 (3-Glc*C*), 30.6 (SCO*C*H_3), 20.8 (OCO*C*H_3), 20.7 (OCO*C*H_3), 20.52 (OCO*C*H_3), 20.51 (OCO*C*H_3). $[\alpha]_D^{28}$ +17.8 (*c* 1.00 in $CHCl_3$).

4.3.3. 1,2,3,6-Tetra-O-acetyl-4-S-acetyl-4-thio-D-glucopyranose (4-Ac-S-AcGlc, CCDC 1581348 for β- form)[24-25]

4-Ac-S-BzGlc (20 mg, 30 μmol) was debenzoylated in methanol (MeOH, 0.6 mL) with sodium methoxide (MeONa, 0.1 M, 31 μL). After stirring at room temperature for 60 min, the mixture was neutralized with acetic acid (AcOH) and concentrated. The resulted residue was dissolved in anhydrous pyridine (83 μL), cooled to 0°C and Ac_2O (24 μL, 26 μmol) was added. After 30 min the reaction mixture was allowed to reach room temperature, stirred for additional 5h, solvent was removed under reduced pressure and the residue was dissolved in dichloromethane (CH_2Cl_2, 10 mL). The solution was washed with saturated sodium hydrogen carbonate ($NaHCO_3$) solution followed by 0.2 M hydrochloric acid (HCl) aq. and water, dried, concentrated under reduced pressure, and purified by column chromatography on silica with hexane : EtOAc = 5 : 1 to give the product **4-Ac-S-AcGlc** as

white solids (6.8 mg, 55%). R_f value 0.38 (hexane : EtOAc = 1 : 1). m.p. 30–32°C. HRMS (ESI): *m/ z* for $C_{16}H_{22}NaO_{10}S$ $[M+Na]^+$ calcd 429.08314, found 429.08333 (error 0.19 mmu, 0.45 ppm). IR (NaCl, neat) ν_{max} 1754, 1707, 1369, 1223, 1073, 1046, 918, 732 cm^{-1}. ^1H NMR ($CDCl_3$, 500.16 MHz, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 6.36 (0.3H, d, $^3J_{2\alpha}$ = 4.0 Hz, 1α-Glc*H*), 5.71 (1H, d, $^3J_{2\beta}$ = 8.5 Hz, 1β-Glc*H*), 5.34 (0.3H, dd, $^3J_{2\alpha}$ = 10.5 Hz, $^3J_{4\alpha}$ = 7.5 Hz, 3α-Glc*H*), 5.27 (1H, dd, $^3J_{2\beta}$ = 11.0 Hz, $^3J_{4\beta}$ = 9.5 Hz, 3β-Glc*H*), 5.08-5.12 (1.3H, m, 2α-,2β-Glc*H*), 4.38 (1H, dd, $^3J_{6'\beta}$ = 13.0 Hz, $^3J_{5\beta}$ = 5.0 Hz, 6β-Glc*H*), 4.20 (1H, dd, $^3J_{6\beta}$ = 12.0 Hz, $^3J_{5\beta}$ = 1.5 Hz, 6'β-Glc*H*), 4.10-4.15 (0.9H, m, 6α-,6'α-,5α-Glc*H*), 3.96 (1H, ddd, $^3J_{4\beta}$ = 11.0 Hz, $^3J_{6\beta}$ = 4.5 Hz, $^3J_{6'\beta}$ = 2.5 Hz, 5β-Glc*H*), 3.81 (0.3H, dd, $^3J_{5\alpha}$ = 11.0 Hz, $^3J_{3\alpha}$ = 11.0 Hz, 4α-Glc*H*), 3.74 (1H, dd, $^3J_{5\beta}$ = 11.0 Hz, $^3J_{3\beta}$ = 11.0 Hz, 4β-Glc*H*), 2.33 (3H, s, SCO*C*H_3), 2.11 (3H, s, CO*C*H_3), 2.09 (3H, s, CO*C*H_3), 2.02 (6H, s, CO*C*H_3). ^{13}C NMR ($CDCl_3$, 125.77 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 192.4 (S*C*OCH$_3$), 170.7 (O*C*OCH$_3$), 169.9 (O*C*OCH$_3$), 169.4 (O*C*OCH$_3$), 168.9 (O*C*OCH$_3$), 91.5 (1-Glc*C*), 73.6 (2-Glc*C*), 71.4 (3-Glc*C*), 71.1 (5-Glc*C*), 62.6 (6-Glc*C*), 43.3 (4-Glc*C*), 30.7 (SCO*C*H_3), 20.8 (OCO*C*H_3), 20.7 (OCO*C*H_3), 20.54 (OCO*C*H_3), 20.50 (OCO*C*H_3). $[\alpha]_D^{20}$ +22.8 (*c* 1.00 in $CHCl_3$).

4.3.4. 1,2,3,4-Tetra-O-acetyl-6-S-acetyl-6-thio-β-D-glucopyranose (6-Ac-S-AcGlc, CCDC 1581349)[27-28]

A 0.05 M solution of **6-Ts-OAcGlc** (450 mg, 890 μmol) in DMF (18 mL) was treated with KSAc (1.0 g, 8.9 mmol) at room temperature under nitrogen atmosphere. The reaction mixture was stirred for 2h and was partitioned between Et_2O and water. The organic phase was dried, filtered, and concentrated in vacuo. The product **6-Ac-SAcGlc** was isolated by

column chromatography on silica with hexane : EtOAc = 2 : 1 as the eluant as white solids (312 mg, 86%). R_f value 0.55 (hexane : EtOAc = 1 : 1). m.p. 126–127°C. HRMS (ESI): *m/z* for $C_{16}H_{22}NaO_{10}S$ $[M+Na]^+$ cacld 429.08314, found 429.08333 (error 0.19 mmu, 0.45 ppm). IR (NaCl, neat) ν_{max} 1757, 1695, 1369, 1215, 1074, 1037 cm^{-1}. ^1H NMR ($CDCl_3$, 500.16 MHz, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 5.66 (1H, d, $^3J_2$ = 8.0 Hz, 1-Glc*H*), 5.21 (1H, dd, $^3J_2$ = 9.5 Hz, $^3J_4$ = 9.5 Hz, 3-Glc*H*), 5.10 (1H, dd, $^3J_1$ = 8.0 Hz, $^3J_3$ = 9.5 Hz, 2-Glc*H*), 5.02 (1H, dd, $^3J_3$ = 9.5 Hz, $^3J_5$ = 9.5 Hz, 4-Glc*H*), 3.80 (1H, ddd, $^3J_4$ = 9.5 Hz, $^3J_6$ = 6.0 Hz, $^3J_{6'}$ = 3.0 Hz, 5-Glc*H*), 3.21 (1H, dd, $^3J_{6'}$ = 14.5 Hz, $^3J_5$ = 3.0 Hz, 6-Glc*H*), 3.14 (1H, dd, $^3J_6$ = 14.5 Hz, $^3J_5$ = 6.0 Hz, 6'-Glc*H*), 2.34 (3H, s, SCO*C*H_3), 2.11 (3H, s, CO*C*H_3), 2.08 (3H, s, CO*C*H_3), 2.02 (3H, s, CO*C*H_3), 2.00 (3H, s, CO*C*H_3). ^{13}C NMR ($CDCl_3$, 125.77 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 194.6 (S*C*OCH$_3$), 170.1 (O*C*OCH$_3$), 169.7 (O*C*OCH$_3$), 169.2 (O*C*OCH$_3$), 168.9 (O*C*OCH$_3$), 91.5 (1-Glc*C*), 73.7 (2-Glc*C*), 72.7 (3-Glc*C*), 70.2 (4-Glc*C*), 69.7 (5-Glc*C*), 30.4 (SCO*C*H_3), 29.6 (6-Glc*C*), 20.8 (OCO*C*H_3), 20.7 (OCO*C*H_3), 20.6 (OCO*C*H_3), 20.5 (OCO*C*H_3). $[\alpha]_D^{25}$ -11.4 (*c* 1.00 in $CHCl_3$).

4.4.1. 5-[4-(2-Thio-D-glucospyranos-2-S-yl)-2,3,5,6-tetrafluorophenyl)]-10,15,20-tris-(pentafluorophenyl)porphyrin (2OH)

TFPP (192.2 mg, 197 μmol), **2-Ac-S-AcGlc** (117.8 mg, 290 μmol), and diethylamine (DEA, 60 μL, 580 μmol) were dissolved in DMF (40 mL). The reaction mixture was stirred at room temperature for 24h, diluted with CH_2Cl_2 (15 mL) and washed with distilled water (15 mL × 5). The extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was separated by column chromatography (silica gel,

CH_2Cl_2 to CH_2Cl_2/EtOAc = 100–90:10) to give acetylated TFPP-glucose conjugate **2Ac** (41.2 mg).

The crude acetylated compounds **2Ac** was used for the synthesis of deacetylated compounds. The deprotection was conducted by dissolving **2Ac** (48.7mg, 37 μmol) in CH_2Cl_2 (20 mL) and MeOH (20 mL). NaOMe was added to adjust the pH to 9 and this mixture was heated at 40°C. After 5 min acetic acid was added to the mixture. The solvent was removed and the crude product was washed with destilled water (15 mL × 5). The crude product was purified by column chromatography (silica gel, CH_2Cl_2/MeOH = 9/1) and washed with distilled water to give the deacetylated TFPP-glucose conjugate **2OH** (28.3 mg, total yield 10.5%) as a deep brownish purple solids.

*Characterization for acetylated TFPP-glucose conjugate **2Ac***

MALDI-TOF high resolution mass spectrometry (HRMS): *m/ z* for $C_{58}H_{29}N_4O_9F_{19}S$ ($[M]^+$) cacld 1318.13560, found 1318.13464 (error 0.97 mmu, 0.73 ppm). ^{1}H NMR ($CDCl_3$, 499.91 MHz, Si(CH_3)$_4$ = 0 ppm): δ (ppm) = 8.93 (8H, br s, 2,3,7,8,12,13,17,18-β-pyrrole*H*), 6.02 (1H, d, ^3J = 9.0 Hz, 1'-Glc*H*), 5.36 (1H, dd, ^3J = 10.0 Hz and 9.0 Hz, 3'-Glc*H*), 5.24 (1H, dd, ^3J = 10.0 Hz and 10.0, 4'-Glc*H*), 4.44 (1H, dd, ^3J = 12.0 Hz and 4.0 Hz, 6'-Glc*H*), 4.17 (1H, dd, ^3J = 12.0 Hz and 2.00 Hz, 6-Glc*H*), 3.99 (1H, ddd, ^3J = 12.0 Hz, 4.0 Hz and 2.0 Hz, 5'-Glc*H*), 3.64 (1H, dd, ^3J = 9.0 Hz and 9.0 Hz, 2'-Glc*H*), 2.26 (3H, s, CH_3), 2.25 (3H, s, CH_3), 2.14 (3H, s, CH_3), 2.12 (3H, s, CH_3), −2.92 (2H, br s, N*H*). ^{13}C NMR ($CDCl_3$, 125.72 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 170.64 (*C*=O), 170.26 (*C*=O), 169.64 (*C*=O), 169.55 (*C*=O), 147.48−145.48 (2,6-Ph*C*, 3,5-Ph*C*, α-pyrrole*C*), 130.98 (β-pyrrole*C*), 122.35 (4-Ph*C*), 115.47 (1-Ph*C*), 103.84 (meso*C*), 94.17 (1'-Glc*C*), 72.72 (5'-

Glc*C*), 71.58 (3'-Glc*C*), 68.94 (4'-Glc*C*), 61.45 (6'-Glc*C*), 52.82 (2'-Glc*C*), 20.78 (*C*H_3), 20.69 (*C*H_3), 20.68 (*C*H_3), 20.61 (*C*H_3). ^{19}F NMR ($CDCl_3$, 470.34 MHz, C*F*$_3$CO_2H = −76.05 ppm): δ (ppm) = −131.85 (2F, dd, $^3J_{F\text{-}F}$ = 25.9 Hz, $^2J_{F\text{-}F}$ = 13.3 Hz, 3,5-Ph*F*Glc), −136.54 (2F, dd, $^3J_{F\text{-}F}$ = 25.8 Hz, $^5J_{F\text{-}F}$ = 12.4 Hz, 2,6-Ph*F*Glc), −137.18 (6F, dd, $^3J_{F\text{-}F}$ = 15.2 Hz, $^5J_{F\text{-}F}$ = 6.7 Hz, 3,5-Ph*F*), −151.78 (3F, dd, $^3J_{F\text{-}F}$ = 20.0 Hz, $^5J_{F\text{-}F}$ = 20.0 Hz, 4-Ph*F*), −161.92 (6F, dd, $^3J_{F\text{-}F}$ = 14.3 Hz, $^5J_{F\text{-}F}$ = 6.7 Hz, 2,6-Ph*F*). UV-Vis (c = 5.00 μM, DMSO, path length = 1 cm, 25°C): λ /nm= 412, 505, 532, 580, 631.

Characterization for deacetylated TFPP-glucose conjugate ***2OH***

Purity (HPLC): >99%. MALDI-TOF high resolution mass spectrometry (HRMS): *m*/ *z* for $C_{50}H_{19}N_4O_4F_{19}S$ ([M – H_2O]$^+$) cacld 1132.08085, found 1132.08181 (error -0.97 mmu, -0.85 ppm). Purity (^{19}F qNMR, 3,5-bis(trifluoromethyl)benzoic Acid): 84.1 wt%. ^{1}H NMR (CD_3OD, 499.91 MHz, C*H*D_2OD = 3.30 ppm): δ (ppm) = 9.32, 9.06 (8H, s,β-pyrrole*H*), 5.70 (1H, d, ^3J = 2.0 Hz, 1'α-Glc*H*), 5.60 (1H, d, ^3J = 4.0 Hz, 1'β-Glc*H*), 4.82 (1H, m, 3'α-Glc*H*), 4.72 (1H, m, 4'α-Glc*H*), 4.68 (1H, m, 6'α-Glc*H*), 4.48 (1H, m, 6''α-Glc*H*), 4.16 (3H, m, 3'β,5'αβ-Glc*H*), 3.54 (4H, m, 2'αβ-,6'β-,6''β-Glc*H*), 3.34 (1H, m, 4'β-Glc*H*). ^{13}C NMR (CD_3OD, 125.72 MHz,CD_3OD = 49.0 ppm): δ (ppm) = 149.06-129.57 (α,β-pyrrole*C*, 2,6-Ph*C* and 3,5-Ph*C*), 122.15 (4-Ph*C*), 116.65 (1-Ph*C*), 105.90, 105.50 (meso*C*), 104.97 (1'β-Glc*C*), 101.23 (1'α-Glc*C*), 90.95 (3'α-Glc*C*), 83.89 (6',6''α-Glc*C*), 81.66 (4'α-Glc*C*), 72.96 (3'β-Glc*C*), 72.49 (5'αβ-Glc*C*), 72.31 (2'α-Glc*C*), 72.30 (4'β-Glc*C*), 59.10 (2'β-Glc*C*), 58.90 (6',6''β-Glc*C*). ^{19}F NMR (CD_3OD, 470.34 MHz, C*F*$_3$CO_2H = −76.05 ppm): δ (ppm) = −134.41 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^5J_{F\text{-}F}$ = 12.4 Hz, 3,5-Ph*F*Glc), −139.23 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^5J_{F\text{-}F}$ = 11.4 Hz, 2,6-Ph*F*Glc), −139.67 (6F, m,

$^{3}J_{F\text{-}F}$ = 21.9 Hz, 3,5-Ph*F*), −154.86 (3F, t, $^{3}J_{F\text{-}F}$ = 35.3 Hz, $^{5}J_{F\text{-}F}$ = 18.1 Hz, 4-Ph*F*), −164.47 (6F, dd, $^{3}J_{F\text{-}F}$ = 36.2 Hz, $^{5}J_{F\text{-}F}$ = 21.0 Hz, 2,6-Ph*F*). UV-Vis (*c* = 4.44 μM, DMSO, path length = 1 cm, 25°C): λ /nm ($\varepsilon \times 10^{-4}$/M^{-1} cm^{-1}) = 412.5 (28.94), 506.0 (1.93), 537.5 (0.03), 582 (0.55). FL (*c* = 4.44 μM in DMSO, path length = 1 cm, λ_{ex} = 412.5 nm, 25°C): λ/ nm = 637.5, 702.5.

4.4.2. 5-[4-(3-Thio-D-glucospyranos-3-S-yl)-2,3,5,6-tetrafluorophenyl)]-10,15,20-tris-(pentafluorophenyl)porphyrin (3OH)

A similar procedure described for acetylated TFPP-glucose conjugate **2Ac** was applied to TFPP (112.8.mg, 116 μmol), **3-Ac-S-AcGlc** (91.7 mg, 226 μmol), and DEA (40 μL, 387 μmol) to yield the acetylated TFPP-glucose conjugate **3Ac** (83.0 mg) as a deep purplish red solids.

The deacetylation was conducted as described for glucosylated TFPP **2OH** by using crude **3Ac** (30.9 mg, 23 μmol) yielding deacetylated TFPP-glucose conjugate **3OH** (10.0 mg, total yield 20.0%) as a deep brownish purple solids.

*Characterization for acetylated TFPP-glucose conjugate **3Ac***

MALDI-TOF high resolution mass spectrometry (HRMS): *m/ z* for $C_{58}H_{29}N_4O_9F_{19}S$ ([M]$^{+}$) cacld 1318.13569, found 1318.13464 (error 1.05 mmu, 0.80 ppm). ^{1}H NMR ($CDCl_3$, 499.91 MHz, Si(CH_3)$_4$ = 0 ppm): δ (ppm) = 8.99 (2H, br s, 3,7-β-pyrrole*H*), 8.93 (6H, br s, 2,8,12,13,17,18-β-pyrrole*H*), 6.44 (0.5H, d, ^{3}J = 4.0 Hz, 1'α-Glc*H*), 5.83 (1H, dd, ^{3}J = 8.0 Hz, 1'β-Glc*H*), 5.42-5.37 (2.5H, m, 2'α-,2'β-,6''β-Glc*H*), 5.32 (0.5H, m, 6''α-Glc*H*), 4.36-4.31 (1.5H, m, 4'αβ-Glc*H*), 4.23-4.18 (2H, m, 3'α-,5'αβ-Glc*H*), 3.97-3.90 (1.5H, m,

6'α-,3'β-Glc*H*), 3.71 (1H, dd, ^{3}J = 10.0 Hz, 6'β-Glc*H*), 2.29 (3H, s, CH_3), 2.28 (3H, s, CH_3), 2.27 (3H, s, CH_3), 2.23 (3H, s, CH_3), 2.21 (3H, s, CH_3), 2.20 (3H, s, CH_3), 2.159 (3H, s, CH_3), 2.156 (3H, s, CH_3), −2.91 (2H, br s, N*H*). ^{13}C NMR ($CDCl_3$, 125.72 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 170.74 (*C*=O), 169.45 (*C*=O), 169.16 (*C*=O), 169.12 (*C*=O), 147.45−145.45 (2,6-Ph*C*, 3,5-Ph*C*, α-pyrrole*C*), 131.26 (β-pyrrole*C*), 122.03 (4-Ph*C*), 115.48 (1-Ph*C*), 103.87 (meso*C*), 93.13 (1'α-Glc*C*), 88.88 (1'β-Glc*C*), 75.40 (6'α-Glc*C*), 71.28 (5'αβ-Glc*C*), 70.73 (2'αβ-Glc*C*), 70.33 (6''α-Glc*C*), 68.20 (6''β-Glc*C*), 62.00 (4'αβ-Glc*C*), 61.91 (3'α-Glc*C*), 54.18 (6'β-Glc*C*), 50.33 (3'β-Glc*C*), 20.92 (*C*H_3), 20.80 (*C*H_3), 20.63 (*C*H_3), 20.58 (*C*H_3). ^{19}F NMR ($CDCl_3$, 470.34 MHz, CF_3CO_2H = −76.05 ppm): δ (ppm) = −131.74 (2F, dd, $^{3}J_{F\text{-}F}$ = 25.8 Hz, $^{2}J_{F\text{-}F}$ = 12.4 Hz, 3,5-Ph*F*Glc), −136.59 (2F, dd, $^{3}J_{F\text{-}F}$ = 25.8 Hz, $^{5}J_{F\text{-}F}$ = 12.4 Hz, 2,6-Ph*F*Glc), −137.18 (6F, dd, $^{3}J_{F\text{-}F}$ = 22.0 Hz, $^{5}J_{F\text{-}F}$ = 22.0 Hz, 3,5-Ph*F*), −151.84 (3F, dd, $^{3}J_{F\text{-}F}$ = 32.4 Hz, $^{5}J_{F\text{-}F}$ = 19.0 Hz, 4-Ph*F*), −161.94 (6F, dd, $^{3}J_{F\text{-}F}$ = 12.4 Hz, $^{5}J_{F\text{-}F}$ = 6.9 Hz, 2,6-Ph*F*). UV-Vis (*c* = 5.00 μM, DMSO, path length = 1 cm, 25°C): λ /nm = 412, 505, 532, 580, 631.

Characterization for deacetylated TFPP-glucose conjugate ***3OH***

Purity (HPLC): >99%. MALDI-TOF high resolution mass spectrometry (HRMS): *m*/ *z* for $C_{50}H_{21}N_4O_5F_{19}S$ ([M]$^{+}$) cacld 1150.09196, found 1150.09238 (error -0.42 mmu, -0.36 ppm). Purity (^{19}F qNMR, 3,5-bis(trifluoromethyl)benzoic Acid): 88.1 wt%. ^{1}H NMR (CD_3OD, 499.91 MHz, C*H*D_2OD = 3.30 ppm): δ (ppm) = 9.32, 9.06 (8H, s,β-pyrrole*H*), 5.20 (1H, d, ^{3}J = 4.0 Hz, 1'α-Glc*H*), 4.63 (1H, d, ^{3}J = 8.0 Hz, 1'β-Glc*H*), 4.00-3.93 (2H, m, 4'β-,6'α-Glc*H*), 3.86-3.70 (5H, m, 2'α-,3'α-,4'α-,5'αβ-Glc*H*), 3.60 (1H, dd, ^{3}J = 10.0 Hz, 3'β-Glc*H*), 3.50-3.46 (2H, m, 2'β-,6'β-Glc*H*). ^{13}C NMR (CD_3OD, 125.72 MHz, CD_3OD =

49.0 ppm): δ (ppm) = 148.95-129.60 (α,β-pyrrole*C*, 2,6-Ph*C* and 3,5-Ph*C*), 121.02 (4-Ph*C*), 116.70 (1-Ph*C*), 106.34, 104.87 (meso*C*), 99.45 (1'α-Glc*C*), 93.72 (1'β-Glc*C*), 80.51 (2'β-Glc*C*), 76.26 (6'β-Glc*C*), 73.94 (3'α-Glc*C*), 73.87 (4'β-Glc*C*), 71.62 (2'α-Glc*C*), 62.85 (4'α-Glc*C*), 62.70 (5'αβ-Glc*C*), 59.37 (3'β-Glc*C*), 56.79 (6'α-Glc*C*). ^{19}F NMR (CD_3OD, 470.34 MHz, CF_3CO_2H = −76.05 ppm): δ (ppm) = −133.72 (2F, dd, $^3J_{F\text{-}F}$ = 25.8 Hz, $^5J_{F\text{-}F}$ = 12.4 Hz, 3,5-Ph*F*Glc), −139.67 (6F, m, $^3J_{F\text{-}F}$ = 15.2 Hz, $^5J_{F\text{-}F}$ = 6.6 Hz, 3,5-Ph*F*), −140.65 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^5J_{F\text{-}F}$ = 11.4 Hz, 2,6-Ph*F*Glc), −154.88 (3F, t, $^3J_{F\text{-}F}$ = 20.0 Hz, $^5J_{F\text{-}F}$ = 8.6 Hz, 4-Ph*F*), −164.49 (6F, dd, $^3J_{F\text{-}F}$ = 22.9 Hz, $^5J_{F\text{-}F}$ = 6.7 Hz, 2,6-Ph*F*). UV-Vis (c = 4.48 μM, DMSO, path length = 1 cm, 25°C): λ /nm ($\varepsilon \times 10^{-4}$/M^{-1} cm^{-1}) = 413.0 (30.39), 505.5 (2.13), 537.5 (0.06), 581.0 (0.63). FL (c = 4.48 μM in DMSO, path length = 1 cm, λ_{ex} = 413.0 nm, 25°C): λ/ nm = 637.5, *703.0.*

4.4.3. 5-[4-(4-Thio-D-glucospyranos-4-S-yl)-2,3,5,6-tetrafluorophenyl)]-10,15,20-tris-(pentafluorophenyl)porphyrin (4OH)

A similar procedure described for acetylated TFPP-glucose conjugate **2Ac** was applied to TFPP (111.9 mg, 115 μmol), **4-Ac-S-AcGlc** (20.9 mg, 52 μmol), and DEA (37 μL, 358 μmol) to yield the acetylated TFPP-glucose conjugate **4Ac** (14.7 mg) as a deep brownish purple solids.

The deacetylation was conducted as described for glucosylated TFPP **2OH** by using crude **4Ac** (21.4 mg, 16 μmol) yielding deacetylated TFPP-glucose conjugate **4OH** (14.7 mg, total yield 30.8%) as a deep brownish purple solids.

Characterization for acetylated TFPP-glucose conjugate ***4Ac***

MALDI-TOF high resolution mass spectrometry (HRMS): *m/ z* for $C_{58}H_{29}N_4O_9F_{19}S$ ($[M]^+$) cacld 1318.13565, found 1318.13464 (error 1.01 mmu, 0.77 ppm). ^{1}H NMR ($CDCl_3$, 499.91 MHz, Si(CH_3)$_4$ = 0 ppm): δ (ppm) = 9.05 (2H, br s, 3,7-β-pyrrole*H*), 8.92 (6H, br s, 2,8,12,13,17,18-β-pyrrole*H*), 6.43 (0.3H, d, ^3J = 4.0 Hz, 1'α-Glc*H*), 5.85 (1H, dd, ^3J = 8.0 Hz, 1'β-Glc*H*), 5.62 (0.3H, dd, ^3J = 10.0 Hz, 3'α-Glc*H*), 5.35 (1H, dd, ^3J = 11.0 Hz, 9.0 Hz, 3' β-Glc*H*), 5.20-5.27 (1.3H, m, 2'αβ-Glc*H*), 4.70-4.76 (1.3H, m, 6''αβ-Glc*H*), 4.61 (1H, dd, ^3J = 12.0 Hz, 2.0 Hz, 6'α-Glc*H*), 4.47-4.52 (0.6H, m, 6'α-,5'α-Glc*H*), 4.17-4.20 (1H, m, 5'β-Glc*H*), 3.89-3.97 (1.3H, m, 4' αβ-Glc*H*), 2.26 (3H, s, CH_3), 2.25 (3H, s, CH_3), 2.24 (3H, s, CH_3), 2.17 (3H, s, CH_3), 2.13 (3H, s, CH_3), 2.11 (3H, s, CH_3), −2.92 (2H, br s, N*H*). ^{13}C NMR ($CDCl_3$, 125.72 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 170.53 (*C*=O), 170.05 (*C*=O), 169.62 (*C*=O), 168.93 (*C*=O), 147.46−145.48 (2,6-Ph*C*, 3,5-Ph*C*, α-pyrrole*C*), 131.25 (β-pyrrole*C*), 122.12 (4-Ph*C*), 115.51 (1-Ph*C*), 103.81 (meso*C*), 91.49 (1'β-Glc*C*), 75.15 (5'β-Glc*C*), 72.08 (3'β-Glc*C*), 71.77 (2'β-Glc*C*), 63.11 (6'β-Glc*C*), 48.00 (4'β-Glc*C*), 20.86 (*C*H$_3$), 20.74 (*C*H$_3$), 20.68 (*C*H$_3$), 20.63 (*C*H$_3$). ^{19}F NMR ($CDCl_3$, 470.34 MHz, CF_3CO_2H = −76.05 ppm): δ (ppm) = −132.23 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^2J_{F\text{-}F}$ = 11.5 Hz, 3,5-Ph*F*Glc), −136.29 (2F, dd, $^3J_{F\text{-}F}$ = 23.8 Hz, $^5J_{F\text{-}F}$ = 11.5 Hz, 2,6-Ph*F*Glc), −136.54 (6F, dd, $^3J_{F\text{-}F}$ = 24.5 Hz, $^5J_{F\text{-}F}$ = 10.5 Hz, 3,5-Ph*F*), −151.96 (3F, dd, $^3J_{F\text{-}F}$ = 21.9 Hz, $^5J_{F\text{-}F}$ = 21.9 Hz, 4-Ph*F*), −162.04 (6F, dd, $^3J_{F\text{-}F}$ = 17.2 Hz, $^5J_{F\text{-}F}$ = 6.9 Hz, 2,6-Ph*F*). UV-Vis (*c* = 5.00 μM, DMSO, path length = 1 cm, 25°C): λ /nm = 412, 505, 532, 580, 631.

Characterization for deacetylated TFPP-glucose conjugate ***4OH***

Purity (HPLC): >99%. MALDI-TOF high resolution mass spectrometry (HRMS): *m/ z* for $C_{50}H_{21}N_4O_5F_{19}S$ ($[M]^+$) cacld 1150.09283, found 1150.09238 (error 0.45 mmu, 0.39 ppm). Purity (^{19}F qNMR, 3,5-bis(trifluoromethyl)benzoic Acid): 80.1 wt%. ^{1}H NMR (CD_3OD, 499.91 MHz, C*H*D_2OD = 3.30 ppm): δ (ppm) = 9.32, 9.07 (8H, s,β-pyrrole*H*), 5.27 (1H, d, ^3J = 4.0 Hz, 1'α-Glc*H*), 4.62 (1H, d, ^3J = 8.0 Hz, 1'β-Glc*H*), 4.22-4.16 (2H, m, 6''β-,5'α-Glc*H*), 4.13-4.10 (2H, m, 6',6''α-Glc*H*), 3.98-4.07 (2H, m, 6'β-,4'α-Glc*H*), 3.73-3.69 (2H, m, 3'β-,3'α-Glc*H*), 3.52-3.60 (3H, m, 4'β-,5'β-,2'α-Glc*H*), 3.33 (1H, d, 2'β-Glc*H*). ^{13}C NMR (CD_3OD, 125.72 MHz, CD_3OD = 49.0 ppm): δ (ppm) = 149.07-129.79 (α,β-pyrrole*C*, 2,6-Ph*C* and 3,5-Ph*C*), 121.954-Ph*C*), 116.71(1-Ph*C*), 106.03, 104.93 (meso*C*), 98.12 (1'α-Glc*C*), 94.17 (1'β-Glc*C*), 77.96 (3'α-Glc*C*), 77.88 (3'β-Glc*C*), 77.50 (2'β-Glc*C*), 75.34 (2'α-Glc*C*), 73.51 (4'α-Glc*C*), 72.71 (6''β-Glc*C*), 63.50 (5'α-Glc*C*), 63.496 (6''α-Glc*C*), 63.40 (6'α-Glc*C*), 63.399 (6'β-Glc*C*), 52.76 (4'β-Glc*C*), 52.70 (5'β-Glc*C*). ^{19}F NMR (CD_3OD, 470.34 MHz, CF_3CO_2H = −76.05 ppm): δ (ppm) = −133.03 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^5J_{F\text{-}F}$ = 11.4 Hz, 3,5-Ph*F*Glc), −139.68 (6F, m, $^3J_{F\text{-}F}$ = 24.8 Hz, 3,5-Ph*F*), −139.90 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^5J_{F\text{-}F}$ = 11.5 Hz, 2,6-Ph*F*Glc), −154.87 (3F, t, $^3J_{F\text{-}F}$ = 21.0 Hz, 4-Ph*F*), −164.48 (6F, dd, $^3J_{F\text{-}F}$ = 20.0 Hz, 2,6-Ph*F*). UV-Vis (*c* = 4.05 μM, DMSO, path length = 1 cm, 25 °C): λ /nm ($\varepsilon \times 10^{-4}$/$M^{-1}$ cm^{-1}) = 413.0 (30.15), 506.0 (2.01), 537.5 (0.02), 582.0 (0.57). FL (*c* = 4.05 μM in DMSO, path length = 1 cm, λ_{ex} = 413.0 nm, 25°C): λ/ nm = 637.5, 703.0.

4.4.4. 5-[4-(6-Thio-D-glucospyranos-6-S-yl)-2,3,5,6-tetrafluorophenyl)]-10,15,20-tris-(pentafluorophenyl)porphyrin (6OH)

TFPP (100.0 mg, 103 μmol), **6-Ac-S-AcGlc** (127.6 mg, 314 μmol), and DEA (45 μL, 435 μmol) were dissolved in DMF (25 mL). The reaction mixture was stirred at 60°C for 24h, diluted with CH_2Cl_2 (15 mL) and washed with distilled water (15 mL × 5). The extract was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The reaction mixture was separated by column chromatography (silica gel, CH_2Cl_2 to CH_2Cl_2/EtOAc = 100-90:10) to give acetylated TFPP-glucose conjugate **6Ac** (8.5 mg, 6 μmol).

The deacetylation was conducted as described for glucosylated TFPP **2OH** by using crude **6Ac** (8.5 mg, 6 μmol) yielding deacetylated TFPP-glucose conjugate **6OH** (6.0 mg, total yield 5.1%) as a deep brownish purple solids.

*Characterization for acetylated TFPP-glucose conjugate **6Ac***

MALDI-TOF high resolution mass spectrometry (HRMS): *m/ z* for $C_{58}H_{29}N_4O_9F_{19}S$ ($[M]^+$) cacld 1318.13593, found 1318.13464 (error 1.29 mmu, 0.98 ppm). ^{1}H NMR ($CDCl_3$, 499.91 MHz, $Si(CH_3)_4$ = 0 ppm): δ (ppm) = 8.99 (2H, br s, 3,7-β-pyrrole*H*), 8.92 (6H, br s, 2,8,12,13,17,18-β-pyrrole*H*), 5.87 (1H, d, ^3J = 8.0 Hz, 1'-Glc*H*), 5.37 (1H, dd, ^3J = 9.0 Hz, 9.0 Hz, 4'-Glc*H*), 5.30-5.23 (2H, m, 2',3'-Glc*H*), 4.14-4.11 (1H, m, 5'-Glc*H*), 3.45 (1H, dd, ^3J = 9.0 Hz, 9.0 Hz, 6'α-Glc*H*), 3.35 (1H, m, 6''-Glc*H*), 2.16 (3H, s, CH_3), 2.09 (3H, s, CH_3), 2.07 (3H, s, CH_3), 1.87 (3H, s, CH_3), −2.92 (2H, br s, N*H*). ^{13}C NMR ($CDCl_3$, 125.72 MHz, $CDCl_3$ = 77.0 ppm): δ (ppm) = 170.17 (*C*=O), 169.68 (*C*=O), 169.33 (*C*=O), 168.83 (*C*=O), 147.52−145.46 (2,6-Ph*C*, 3,5-Ph*C*, α-pyrrole*C*), 131.27 (β-pyrrole*C*), 120.43 (4-Ph*C*), 115.51 (1-Ph*C*), 103.57 (meso*C*), 91.72 (1'-Glc*C*), 74.98 (5' -Glc*C*), 72.61

(4'-Glc*C*), 70.68 (3'-Glc*C*), 70.35 (2'-Glc*C*), 35.56 (6'β-Glc*C*), 20.74 (*C*H_3), 20.65 (*C*H_3), 20.58 (*C*H_3), 20.53 (*C*H_3). ^{19}F NMR ($CDCl_3$, 470.34 MHz, C*F*$_3$CO_2H = −76.05 ppm): δ (ppm) = −133.77 (2F, dd, $^3J_{F\text{-}F}$ = 24.8 Hz, $^2J_{F\text{-}F}$ = 12.4 Hz, 3,5-Ph*F*Glc), −137.29 (6F, dd, $^3J_{F\text{-}F}$ = 23.8 Hz, $^5J_{F\text{-}F}$ = 11.5 Hz, 3,5-Ph*F*), −138.02 (2F, dd, $^3J_{F\text{-}F}$ = 25.8 Hz, $^5J_{F\text{-}F}$ = 12.5 Hz, 2,6-Ph*F*Glc), −152.00 (3F, dd, $^3J_{F\text{-}F}$ = 33.3 Hz, $^5J_{F\text{-}F}$ = 21.0 Hz, 4-Ph*F*), −162.05 (6F, dd, $^3J_{F\text{-}F}$ = 17.2 Hz, $^5J_{F\text{-}F}$ = 6.9 Hz, 2,6-Ph*F*). UV-Vis (*c* = 5.00 μM, DMSO, path length = 1 cm, 25°C): λ /nm = 412, 505, 532, 580, 631.

Characterization for deacetylated TFPP-glucose conjugate ***6OH***

Purity (HPLC): >99%. MALDI-TOF high resolution mass spectrometry (HRMS): *m*/ *z* for $C_{50}H_{21}N_4O_5F_{19}S$ ($[M]^+$) cacld 1150.09181, found 1150.09238 (error -0.57 mmu, -0.49 ppm). Purity (^{19}F qNMR, 3,5-bis(trifluoromethyl)benzoic Acid): 83.7 wt%. ^{1}H NMR (CD_3OD, 499.91 MHz, C*H*D_2OD = 3.30 ppm): δ (ppm) = 9.32, 9.07 (8H, s,β-pyrrole*H*), 5.27 (1H, d, ^3J = 4.0 Hz, 1'α-Glc*H*), 4.62 (1H, d, ^3J = 8.0 Hz, 1'β-Glc*H*), 4.22-4.16 (2H, m, 6''β-,5'α-Glc*H*), 4.13-4.10 (2H, m, 6',6''α-Glc*H*), 3.98-4.07 (2H, m, 6'β-,4'α-Glc*H*), 3.73-3.69 (2H, m, 3'β-,3'α-Glc*H*), 3.52-3.60 (3H, m, 4'β-,5'β-,2'α-Glc*H*), 3.33 (1H, d, 2'β-Glc*H*). ^{13}C NMR (CD_3OD, 125.72 MHz, CD_3OD = 49.0 ppm): δ (ppm) = 149.07-129.79 (α,β-pyrrole*C*, 2,6-Ph*C* and 3,5-Ph*C*), 121.954-Ph*C*), 116.71(1-Ph*C*), 106.03, 104.93 (meso*C*), 98.12 (1'α-Glc*C*), 94.17 (1'β-Glc*C*), 77.96 (3'α-Glc*C*), 77.88 (3'β-Glc*C*), 77.50 (2'β-Glc*C*), 75.34 (2'α-Glc*C*), 73.51 (4'α-Glc*C*), 72.71 (6''β-Glc*C*), 63.50 (5'α-Glc*C*), 63.496 (6''α-Glc*C*), 63.40 (6'α-Glc*C*), 63.399 (6'β-Glc*C*), 52.76 (4'β-Glc*C*), 52.70 (5'β-Glc*C*). ^{19}F NMR (CD_3OD, 470.34 MHz, C*F*$_3$CO_2H = −76.05 ppm): δ (ppm) =

−133.03 (2F, dd, $^{3}J_{F\text{-}F}$ = 24.8 Hz, $^{5}J_{F\text{-}F}$ = 11.4 Hz, 3,5-Ph*F*Glc), −139.68 (6F, m, $^{3}J_{F\text{-}F}$ = 24.8 Hz, 3,5-Ph*F*), −139.90 (2F, dd, $^{3}J_{F\text{-}F}$ = 24.8 Hz, $^{5}J_{F\text{-}F}$ = 11.5 Hz, 2,6-Ph*F*Glc), −154.87 (3F, t, $^{3}J_{F\text{-}F}$ = 21.0 Hz, 4-Ph*F*), −164.48 (6F, dd, $^{3}J_{F\text{-}F}$ = 20.0 Hz, 2,6-Ph*F*). UV-Vis (c = 4.19 μM, DMSO, path length = 1 cm, 25°C): λ /nm ($\varepsilon \times 10^{-4}$/M^{-1} cm^{-1}) = 412.5 (35.11), 505.5 (2.52), 536.5 (0.06), 581 (0.71).FL (c = 4.19 μM in DMSO, path length = 1 cm, λ_{ex} = 412.5 nm, 25°C): λ/ nm = 637.5, 702.5.

4.5. Hydrophobicity Parameters Determined by RP-TLC

The hydrophobicity parameter (logarithm of the partition coefficient between *n*-octanol and water; Log *P*) is well-correlated with the capacity factor *k′* in reverse-phase (i.e., partition) chromatography as follows:

$$\text{Log } P = a \text{ Log } k' + b \qquad (1)$$

Where *a* and *b* are constants for a given chromatographic system. The capacity factor *k′* was determined by the R_f value in RP-TLC and a mixture of MeOH and H_2O (9/1, v/v) as the eluent, and calculated as follows:

$$k' = 1 / R_f \qquad (2)$$

m-Hydroxybenzaldehyde (Log *P* = 1.70),[33] *p*-fluorobenzaldehyde (Log *P* = 1.39),[33] *m*-fluorobenzaldehyde (Log *P* = 1.89),[33] 5,15-bis(4-(2-hydroxyethoxy)-2,3,5,6-tetrafluorophenyl)-10,20-bis(pentafluorophenyl)porphyrin (Log *P* = 6.5),[34] 5,10-bis(4-(2-hydroxyethoxy)-2,3,5,6-tetrafluorophenyl)-15,20-bis(pentafluorophenyl)porphyrin (Log *P* = 6.5),[34] and 5,10,15-tris(4-(2-hydroxyethoxy)-2,3,5,6-tetrafluorophenyl)-20-pentafluorophenylporphyrin (Log *P* = 5.4)[34] were used as standards to calibrate our RP-TLC system.

4.6. ROS Measurements

4.6.1. Relative quantum yield of singlet oxygen generation (Φ_{1O2})

TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** under study was dissolved in MeOH. The absorbance of the solution was adjusted to be 0.2 at an appropriate excitation wavelength (*vide infra*) and purged with oxygen gas for 1 min. Luminescence spectra of singlet oxygen generated by the photoirradiation of the solution were recorded on a spectrophotometer equipped with a photomultiplier cooled at 193 K.

4.6.2. Relative quantum yield of Hydroxyl radical ($\Phi_{\bullet OH}$)

TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** (c = 1.00 μM), HP (c = 1.00 μM), and HPF (c = 225 nM) were dissolved in PBS containing 0.2% DMSO and placed into a cell. Oxygen gas was introduced to the solution for approximately 1 min prior to photoirradiation. Then, the solution was exposed to light from a 100 W halogen lamp (KBEX-102A, USHIO Inc., Tokyo, Japan) through a Y-50 cutoff filter (λ > 500 nm, Toshiba Co., Tokyo, Japan) at 25°C. The initial rate of fluorescence intensity increments at 510 nm was monitored under the excitation at 490 nm. The rate constant was estimated from the first-order plot of fluorescence intensity increments against photoirradiation time. The relative quantum yield ($\Phi_{\bullet OH}$) was evaluated by the rate constant and was normalized to the value of HP.

4.7. In vitro studies

4.7.1. Cell culture

Human cervical cell line, HeLa (ATCC CCL-2), was obtained from Riken cell bank. Cells were grown in DMEM supplemented with 10% FCS. Human glioblastoma cell lines, U251, were obtained from the American Type Culture Collection. U251 cells were grown in DMEM supplemented with 10% FCS. An *N*-methyl-*N'*-nitro-*N*-nitroso guanidine (MNNG)-induced mutant of a rat murine RGM gastric carcinoma mucosal cell line, RGK, was kindly provided by Dr. Matsui (Faculty of Medicine, University of Tsukuba). Cells were grown in a 1:1 mixture of DMEM and F12 supplemented with 10% FCS and Antibiotic-Antimycotic.

4.7.2. Cellular uptake inhibition by cytochalasin B

The cellular uptake of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** by RGK cells was examined as follows: Cells (2.5×10^6 cells/well) in 1.5 mL of culture medium were plated in a 6-well plate (Sigma-Aldrich Co.) and incubated for 24h (37°C, 5% CO_2). The medium was aspirated, 1.5 mL fresh culture medium with or without cytochalasin B (50 μM final concentration) was added and the cells were incubated for 2h.[8c] The medium was aspirated, 1.5 mL of 2.5 μM compound in culture medium containing 1% DMSO was added to each well and the plate was incubated for 8 or 24h. Then, the cells were washed twice with PBS. The cells were lysed in 150 μL of DMSO. The fluorescence intensity of each extract was measured with a plate reader using

excitation and an emission wavelengths of 430 and 650 nm, respectively. The concentration of compounds was calculated on the basis of the calibration obtained for each compounds in DMSO. The cellular uptake is given as the means of three replicate experiments.

4.7.3. Photocytotoxicity Test

The photocytotoxicity of TFPP-glucose conjugates **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in these cell lines (HeLa, U251, and RGK cells) was examined as follows: Cells (5×10^3 cells/well) in 100 μL of culture medium were plated in a 96-well plate (Thermo Fisher Scientific Co.) and incubated for 24h (37°C, 5% CO_2). One hundred microliters of a TFPP-glucose conjugates in culture medium containing 2% DMSO was added to each well. The plate was then incubated at 24h in the presence of these compounds. The concentration of these compounds was varied from 0.1 μM to 1 μM in culture medium (final DMSO content was 1% in all cases). The cells were washed twice with PBS and 100 μL of the fresh culture medium was added. The cells were exposed to light from a 100 W halogen lamp equipped with a water jacket and Y-50 cutoff filter (λ>500 nm). The light intensity was measured by using a UV-vis power meter (ORION/TH, Ophir Optronics Ltd., Jerusalem, Israel). The irradiation time was adjusted to obtain the desired light dose of 16 $J \cdot cm^{-2}$. The mitochondrial activity of NADH dehydrogenase of the cells in each well was measured at 24h after photoirradiation using WST-8 reagent (10 μL) from Cell Counting Kit-8 according to the manufacturer's instructions. The absorbance at 450 nm was measured using a plate reader. The percentage cell survival was calculated by normalization with respect to the value for no drug treatment.

4.7.4. Statistical analysis

All statistical evaluations were performed using Welch's t test. All values for cellular uptake and cytotoxicity are expressed as mean ± standard deviation.

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A. Supplementary data

HPLC chart, ^1H, ^{13}C and ^{19}F NMR, ^{19}F qNMR, ^1H-^{19}F HOESY, UV-vis, photoluminescence spectra of TFPP-glucose conjugates, luminescence spectra and HPF fluorescence spectra of $^1O_2$ and •OH generated by the photosensitization of TFPP-glucose conjugates of **1OH**,

2OH, **3OH**, **4OH**, and **6OH** and *in vitro* dark and photocytotoxicity of TFPP-glucose conjugates of **1OH**, **2OH**, **3OH**, **4OH**, and **6OH** in U251 and RGK cell lines. This material is available free of charge via the Internet at http://www.sciencedirect.com/science/journal/09680896.

References

(1) Statistics and Information Department. Vital Statistics 2014. Tokyo: Ministry of Health, Labor and Welfare 2015.

(2) (a) Kessel D. *Photodiagn. Photodyn. Ther.* 2004; 1: 3. (b) Zhang Y, Lovell JF. *Theranostics* 2012; 2: 905. (c) Bonnett R. *Chemical Aspects of Photodynamic Therapy*; Gordon and Breach Science Publishers, CRC Press: Boca Raton, FL, 2000.

(3) (a) Yano S, Hirohara S, Obata M, Hagiya Y, Ogura S, Ikeda A, Kataoka H, Tanaka M, Joh T. *J. Photochem. Photobiol. C: Photohem. Rev.* 2011; 12: 46. (b) Moser JG. Definition and General Properties of 2nd and 3rd Generation Photosensitizers. In *Photodynamic Tumor Therapy, 2nd and 3rd Generation Photosensitizers*; Moser, J. G., Ed.; Harwood Academic Publishers: The Netherlands, 1998. (c) Wu J, Han H, Jin Q, Li Z, Li H, Ji J. *ACS Appl. Mater. Interfaces* 2017; 9: 14596. (d) Dai LL, Cai R, Li M, Luo Z, Yu Y, Chen W, Shen X, Pei Y, Zhao X, Cai K. *Chem. Mater.* 2017; 29: 6976. (e) Chow SYS, Zhao S, Lo P-C, Ng D. K. P. *Dalton Trans.* 2017; 46: 11223.

(4) (a) Sehgal I, Sibrian-Vazquez M, Vicente MGH. *J. Med. Chem.* 2008; 51: 6014. (b) Tirand L, Frochot C, Vanderesse R, Thomas N, Trinquet E, Pinel S, Viriot M-L, Guillemin F, Barberi-Heyob M. *J. Controlled Release* 2006; 111: 153. (c) Sibrian-Vazquez M, Jensen

TJ, Vicente MGH. *J. Med. Chem.* 2008; 51: 2915. (d) Kamarulzaman EE, Gazzali AM, Acherar S, Frochot C, Barberi-Heyob M, Boura C, Chaimbault P, Sibille E, Wahab HA, Vanderesse R. *Int. J. Mol. Sci.* 2015; 16: 24059. (e) Li S-Y, Cheng H, Qiu W-X, Liu, L-H, Chen S, Hu Y, Xie B-R, Li B, Zhang X-Z. *ACS Appl. Mater. Interfaces* 2015; 7: 28319.

(5) (a) Ogura S-I, Tabata K, Fukushima K, Kamachi T, Okura I. *J. Porphyr. Phthalocyanines* 2006; 10: 1116. (b) Vrouenraets MB, Visser GW, Stigter M, Oppelaar H, Snow GB, van Dongen G. A. *Int. J. Cancer* 2002; 98: 793. (c) Vrouenraets MB, Visser GW, Stigter M, Oppelaar H, Snow GB, van Dongen G. A. *Cancer Res.* 2001; 61: 1970.

(6) (a) Gravier J, Schneider R, Frochot C, Bastogne T, Schmitt F, Didelon J, Guillemin F, Barberi-HeyobM. *J. Med. Chem.* 2008; 51: 3867. (b) Schneider R, Schmitt F, Frochot C, Fort Y, Lourette N, Guillemin F, Müller JF, Barberi-Heyob M. *Bioorg. Med. Chem.* 2005; 13: 2799. (c) Ke M-R, Yeung S-L, Ng DK, Fong W-P, Lo P-C P. *J. Med. Chem.* 2013; 56: 8475. (d) Stallivieri A, Colombeau L, Jetpisbayeva G, Moussaron A, Myrzakhmetov B, Arnoux P, Acherar S, Vanderesse R, Frochot C. *Bioorg. Med. Chem.* 2017; 25: 1.

(7) (a) Hirohara S, Oka C, Totani M, Obata M, Yuasa J, Ito H, Tamura M, Matsui H, Kakiuchi K, Kawai T, Kawaichi M, Tanihara M. *J. Med. Chem.* 2015; 58: 8658. (b) Moylan C, Scanlan EM, Senge MO. *Curr. Med. Chem.* 2015; 22: 2238. (c) Zhang KY, Tso KK-S, Louie M-W, Liu H-W, Lo KK-W. *Organometallics* 2013; 32: 5098. (d) Singh S, Aggarwal A, Bhupathiraju NVSDK, Arianna G, Tiwari K, Drain CM. *Chem. Rev.* 2015; 115: 10261. (e) Shivran N, Tyagi M, Mula S, Gupta P, Saha B, Patro BS, Chattopadhyay S. *Eur. J. Med. Chem.* 2016; 122: 352.

(8) (a) Waki A, Fujibayashi Y, Yokoyama A. *Nucl. Med. Biol.* 1998; 25: 589. (b)

Calvaresi EC, Hergenrother PJ. *Chem. Sci.* 2013; 4: 2319. (c) Patra M, Awuah SG, Lippard SJ. *J. Am. Chem. Soc.* 2016; 138: 12541. (d) Zheng G, Graham A, Shibata M, Missert JR, Oseroff OR, Dougherty TJ, Pandey RK. *J. Org. Chem.* 2001; 66*:* 8709.

(9) (a) Ballut S, Makky A, Chauvin B, Michel J-P, Kasselouri A, Maillard P, Rosilio V. *Org. Biomol. Chem.* 2012; 10: 4485. (b) Lafonta D, Zorlub Y, Savoiec H, Albrieuxd F, Ahsenb V, Boylec RW, Dumoulin F. *Photodiagnosis Photodyn. Ther.* 2013; 10: 252. (c) MacDonald IJ, Dougherty TJ. *J. Porphyrins Phthalocyanines* 2001; 5: 105. (d) Liu J-Y, Lo, P-C, Fong W-P, Ng DKP. *Org. Biomol. Chem.* 2009; 7: 1583. (e) Daly R, Vaz G, Davies AM, Senge MO, Scanlan EM. *Chem. Eur. J.* 2012; 18: 14671. (f) Sears P, Wong CH. *Angew. Chem., Int. Ed.* 1999; 38: 2300. (g) Lee YC, Lee RT. C. *Acc. Chem. Res.* 1995; 28: 321.

(10) Warburg O. *Science* 1956; 123: 309.

(11) Yin Z, Comellas-Aragones M, Chowdhury S, Bentley P, Kaczanowska K, BenMohamed L, Gildersleeve JC, Finn MG, Huang X. *ACS Chem. Biol.* 2013; 8: 1253.

(12) Orlean P. *J. Clin. Invest.* 2000; 105: 131.

(13) (a) Ido T, Wan C-N, Casella V, Fowler JS, Wolf AP. *J. Label. Compounds Radiopharm.* 1978; 14: 171. (b) Gallagher BM, Ansari A, Atkins H, Casella V, Christman DR, Fowler JS, Ido T, MacGregor RR, Som P, Wan CN, Wolf AP, Kuhl DE, Reivich M. *J. Nucl. Med.* 1977; 18: 990. (c) Som P, Atkins HL, Bandoypadhyay D, Fowler JS, MacGregor RR, Matsui K, Oster ZH, Sacker DF, Shiue CY, Turner H, Wan C-N, Wolf AP Zabinski SV. *J. Nucl. Med.* 1980; 21: 670. (d) Fowler JS, Wolf AP. *Appl. Radiat. Isot.* 1986; 37: 663.

(14) (a) Hirohara S, Kawasaki Y, Funasako R, Yasui N, Totani M, Alitomo H, Yuasa J, Kawai T, Oka C, Kawaichi M, Obata M, Tanihara M. *Bioconjugate Chem.* 2012; 23: 1881. (b) Zheng X, Morgan J, Pandey SK, Chen Y, Tracy E, Baumann H, Missert JR, Batt C, Jackson J, Bellnier DA, Henderson BW, Pandey RK. *J. Med. Chem.* 2009; 52: 4306.

(15) Tanaka M, Kataoka H, Mabuchi M, Sakuma S, Takahashi S, Tujii R, Akashi H, Ohi H, Yano S, Morita A, Joh T. *Anticancer Res.* 2011; 31: 763.

(16) Kessel D, Thompson P, Saatio K, Nantwi KD. *Photochem. Photobiol.* 1987; 45: 787.

(17) (a) Hirohara S, Nishida M, Sharyo K, Obata M, Ando T, Tanihara M. *Bioorg. Med. Chem.* 2010; 18: 1526. (b) Hirohara S, Sharyo K, Kawasaki Y, Totani M, Tomotsuka A, Funasako R, Yasui N, Hasegawa Y, Yuasa J, Nakashima T, Kawai T, Oka C, Kawaichi M, Obata M, Tanihara M. *Bull. Chem. Soc. Jpn.* 2013; 86: 1295.

(18) Toyokuni T, Kumar JSD, Gunawan P, Basarah ES, Liu J, Barrio JR, Satyamurthy N. *Mol. Imaging. Biol.* 2004; 6: 324.

(19) Knapp S, Kirk BA. *Tetrahedron Lett.* 2003; 44: 7601.

(20) Lee J-C, Chang S-W, Liao C-C, Chi F-C, Chen C-S, Wen Y-S, Wang C-C, Kulkarni SS, Puranik R, Liu Y-H, Hung S-C. *Chem. Eur. J.* 2004; 10: 399.

(21) Hall LD, Miller DC. *Carbohydr. Res.* 1976; 47: 299.

(22) Contour-Galcera M-O, Guillot J-M, Ortiz-Mellet C, Pflieger-Carrara F, Defaye J, Gelas. J. *Carbohydr. Res.* 1996; 81: 99.

(23) Stick RV, Stubbs KA. *Tetrahedron: Asymmetry* 2005; 16: 321.

(24) Garegg PJ, Hultberg H. *Carbohydr. Res.* 1982; 110: 261.

(25) Leon B, Liemann S, Klaffke W. *J. Carbohydr. Chem.* 1993; 12: 597.

(26) Vegh L, Hardegger E. *Helv. Chim. Acta* 1973; 56: 2020.

(27) Maschauer S, Haubner R, Kuwert T, Prante O. *Mol. Pharmaceutics* 2014; 11: 505.

(28) Akagi M, Tejima S, Haga M. *Chem. Pharm. Bull*. 1962; 10: 562.

(29) Miljković, M. *Carbohydrates Synthesis, Mechanisms, and Stereoelectronic Effects*; Springer, NY, 2010.

(30) Roslund MU, Tähtinen P, Niemitz M, Sjöholm R. *Carbohydr. Res.* 2008; 343: 101.

(31) Selectivity of hydroxyl radical species by HPF-detection was reported in the following article; Setsukinai K-i, Urano Y, Kakinuma K, Majima HJ, Nagano T. *J. Biol. Chem.* 2003; 278: 3170.

(32) (a) Yee KKL, Soo KC, Bay BH, Olivo M. *Photochem.Photobiol.* 2002; 76: 678. (b) Zheng G, Potter WR, Camacho SH, Missert JR, Wang G, Bellnier DA, Henderson BW, Rodgers MAJ, Dougherty TJ, Pandey RK. *J. Med. Chem.* 2001; 44: 1540.

(33) Calculated values using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (©1994-2009 acd/Labs).

(34) (a) Obata M, Hirohara S. *J. Photochem. Photobiol. B: Biol.* 2016; 162: 324. (b) Hirohara S, Kubota Y, Akiyama N, Obata M, Matsui H, Kakiuchi K. Synthesis and Photocytotoxicity of Fluorophenylporphyrin Derivatives having Two Different Functional Groups. In *Spectroscopic Tools for the Diagnosis and Treatment of Cancer*; Pacifichem Symposium; Chapter 10, 2017; pp 94-103.

(35) (a) Calvo MB, Figueroa A, Pulido EG, Campelo RG, Aparicio LA. *Int. J. Endocrinol.* 2010; 2010: 205357. (b) Szablewski L. *Biochim. Biophys. Acta, Rev. Cancer*

2013; 1835: 164.

(36) (a) Ethirajan M, Chen Y, Joshi P, Pandey RK. *Chem. Soc. Rev.* 2011; 40: 340. (b) MacDonald IJ, Dougherty TJ. *J. Porphyrins Phthalocyanines* 2001; 5: 105.

(37) Kashiwagi Y, Imahori H, Araki Y, Ito O, Yamada K, Sakata Y, Fukuzumi S. *J. Phys. Chem. A* 2003; 107: 5515.

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