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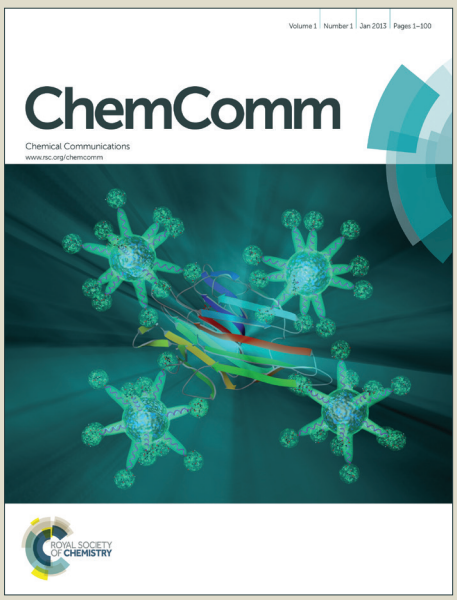

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Efficient singlet oxygen generation from sugar pendant C_{60} derivatives for photodynamic therapy†

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The amidation reaction between C_{60} with activated ester group (1) and acetylated Glc (AcGlc) with amino group (2) was performed to yield target AcGlc-pendant C_{60} compound (3). The water soluble deacethylated compound, Glc-pendant C_{60} compound (4) exhibited high photocytotoxicity against HeLa cells due to the more efficient singlet oxygen generation as compared with that of Glc-pendant azafulleroids.

The photodynamic action is initiated by the absorption of a photon followed by many competing radiative and nonradiative reactions, which ultimately result in the oxidation and degradation of vital biomolecules. Molecular oxygen plays a key role in propagation of the initial molecular damage, resulting in vascular collapse, tissue destruction, cell death. Photodynamic therapy (PDT) has attracted much attention as a less invasive method for treating cancer, because PDT induces tumour cell necrosis and/or apoptosis by producing reactive oxygen species (ROS) through activated photosensitiser (PS) that accumulates specifically to the tumor.[1,2] The potential applications of fullerenes and their derivatives have increased in recent years, particularly in the fields of biology and medicine, where they can be used as DNA photo-cleaving agents, anti-HIV protease inhibitors, antibacterial agents and PS for PDT.[3-10] Although carbohydrates play essential roles in biological systems, their usage in fullerene-based PDT has yet to be fully explored. We have previously reported that sugar-pendant [60]fullerene (C_{60}) derivatives prepared from carbohydrate linked azides exhibited singlet oxygen producing ability in DMSO to demonstrate the carbohydrate-dependent photocytotoxicity against the HeLa cell.[11,12] However, the singlet oxygen yields of these D-glucose (Glc) pendant azafulleroids were significantly smaller than that of pristine C_{60}.[11] It is highly desired to develop water soluble sugar-pendant C_{60} compounds without decreasing the singlet oxygen yield upon photoirradiation.

We report herein the synthesis of a new family of Glc-pendant C_{60} compounds, in which sugar and photosensitive units are connected via the cyclopropane bridged carbon-linkage to retain the conjugated property of pristine C_{60}, such as 2'-(2',3',4',6'-O-tetraacetyl-β-D-glucopyranosyl)ethyl 3'*H*-cyclopropa[1,9](C_{60}-I_h)[5,6]-fullereno-3'-carboxylic amide (**3**) and 2'-(β-D-glucopyranosyl)ethyl 3'*H*-cyclopropa[1,9](C_{60}-I_h)[5,6]fullereno-3'-carboxylic amide (**4**) (Chart 1). The photocytotoxicities against HeLa cells and photophysical processes of **3** and **4** were compared with previously prepared Glc-pendant azafulleroids, such as 1a-aza-1a-[2'-(2',3',4',6'-*O*-tetraacetyl-β-D-glucopyranosyl)ethyl]-1(2)a-homo(C_{60}-I_h)[5,6]fullerene (**5**) and 1a-aza-1a-[2'-(β-D-glucopyranosyl)-ethyl]-1(2)a-homo(C_{60}-I_h)[5,6]-fullerene (**6**) (Chart 1).[11]

Scheme 1 shows the synthetic procedure of **3** and **4**. 3'*H*-Cyclopropa[1,9][5,6]fullereno-C_{60}-I_h-3'-carboxylic acid *N*-hydroxysuccinimide ester (**1**) was prepared from the corresponding C_{60} acid derivative, 3'*H*-cyclopropa[1,9][5,6]fullereno-C_{60}-I_h-3'-carboxylic acid,[13] and *N*-hydroxysuccinimide (NHS). 2-Aminoethyl 2,3,4,6-*O*-tetraacetyl-β-D-glucopyranoside (**2**) was prepared by the similar procedure as that described in the literature,[14] in which Pd/C was used in place of Lindlar catalyst. We performed the amidation reaction between **1** and **2** in dry $CHCl_3$, followed by the purification using

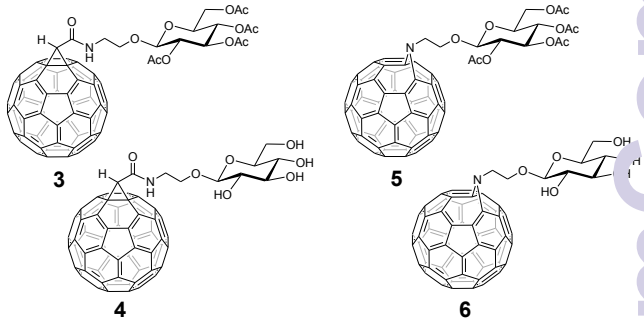
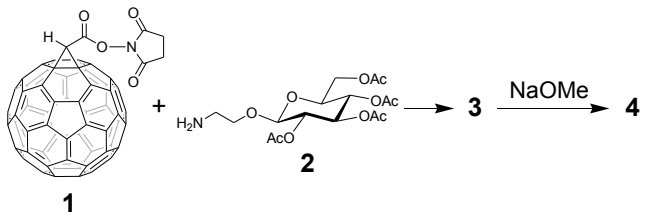


Chart 1 Structures of Glc-pendant C_{60} compounds 3 and 4 in this study and previously prepared Glc-pendant azafulleroids 5 and 6.

Scheme 1 Synthesis of sugar-pendant C_{60} compounds



silica gel column chromatography (eluent: $CHCl_3$/MeOH = 50/1, R_f = 0.20) to afford **3** as a brown solid. Subsequently, **3** was treated by sodium methoxide in dry THF to quantitatively afforded final target compound **4**. The detailed procedures are described in Electronic Supporting Information (ESI).

The photodynamic activities were evaluated using human cervical cancer HeLa cells, to compare between two kinds of Glc-pendant C_{60} derivatives (**4** and **6**) (Fig. 1). Following incubation with the respective C_{60} derivatives, the cells were exposed to light with wavelengths between 350-550 nm. The results showed that no samples had dark toxicity, even at the highest concentrations used. On the other hand, the viabilities of HeLa cells were reduced dependent on photoirradiation. These photodynamic activities of Glc-pendant C_{60} derivatives were drug dose-dependent and the medium inhibitory concentrations (IC_{50} values) were estimated to be ca. 0.4 μM for **4** and 1.6 μM for **6**. Furthermore, similar tendencies as for the photodynamic activities were observed even when the light wavelengths were changed to 400-550 nm at same light dose (Fig. 2). The IC_{50} values were estimated to be ca. 0.4 μM for **4** and 1.5 μM for **6** (Table S1 in ESI). These data indicate that the photodynamic activity of **4** is about 4 times higher than that of **6.**

In order to rationalize such drastic differences in the photodynamic activity, the singlet oxygen generation properties were characterized for **3** and **5**. These samples produced singlet oxygen in the oxygen-saturated C_6D_6 solutions by photoirradiation, which could be quantified from the peak area due to the singlet oxygen phosphorescence at 1270 nm (Fig. S1 in ESI).[15,16] The quantum yields of singlet oxygen generation from **3** was determined to be 0.61, which is much larger than that from **5** (0.22). The larger singlet oxygen yield from **3** results from the efficient formation of the triplet excited state of the C_{60} moiety, which has the much longer lifetime as compared with that derived from **5**. This was also supported by femtosecond laser-induced transient absorption measurements of **4** and **6**. Femtosecond laser excitation of a deaerated DMSO solution of **4** at 355 nm resulted in instant observation of an transient absorption band at 700 nm due to the triplet excited state of C_{60}, which increased at 3000 ps as shown in Fig. 3a. In contrast to the case of **4**, femtosecond laser excitation of a deaerated DMSO solution of **6** at 355 nm resulted in formation of $C_{60}^{\bullet-}$ as revealed by the transient absorption at 1000 nm,[17] together with the triplet excited state of C_{60} at 700 nm, which decayed significantly at 3000 ps as shown in Fig. 3b. Similar results were obtained for the acetylated compounds of **3** and **5** (Figs. S2 and S3 in ESI).

The formation of the triplet excited state of C_{60} without forming $C_{60}^{\bullet-}$ of **4** was confirmed by nanosecond laser transient absorption spectra, whereas the transient absorption spectra of $C_{60}^{\bullet-}$ were observed for **6** as shown in Fig. S4 (ESI), where the formation of the triplet excited state of C_{60} of **3** and $C_{60}^{\bullet-}$ of **5** are also observed. The formation of $C_{60}^{\bullet-}$ of **5** and **6** was also confirmed by EPR spectra measured after photoirradiation and measured at 143 K as shown in Fig. S5 (ESI),[18] where only weak EPR signal due to $C_{60}^{\bullet-}$ was observed for **3** and **4**.

No observation of $C_{60}^{\bullet-}$ (Fig. 3a) and slow formation of the triplet excited state of C_{60} (Fig. 3a) indicate that no

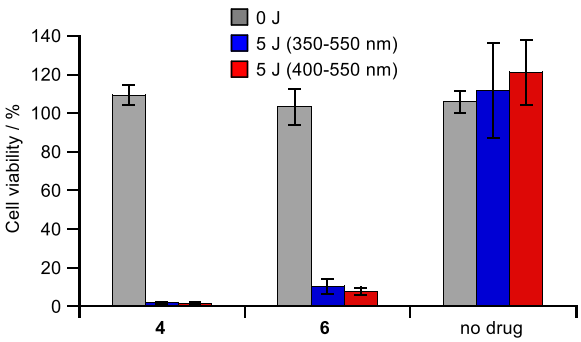


Fig. 1 Cytotoxicity in the dark (gray bar) and photocytotoxicity (green and red bar) of **4** and **6** in HeLa cells ([**4** or **6**] = 5 μM). The cells (1.7×10^4 cells/well) were irradiated with xenon lamp (total 5 J/cm^2) after incubation with photosensitiser for 24 h. The cell survival was determined by WST-8® assay. Each experiment was performed three times.

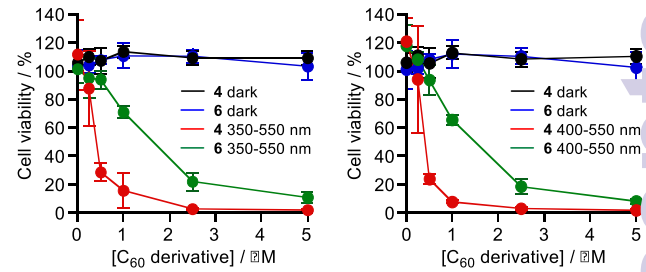


Fig. 2 Cell viability with **4** and **6** at various concentrations under dark and photoirradiation. Data represent the mean values from three independent experiments. Error bars are standard deviations.

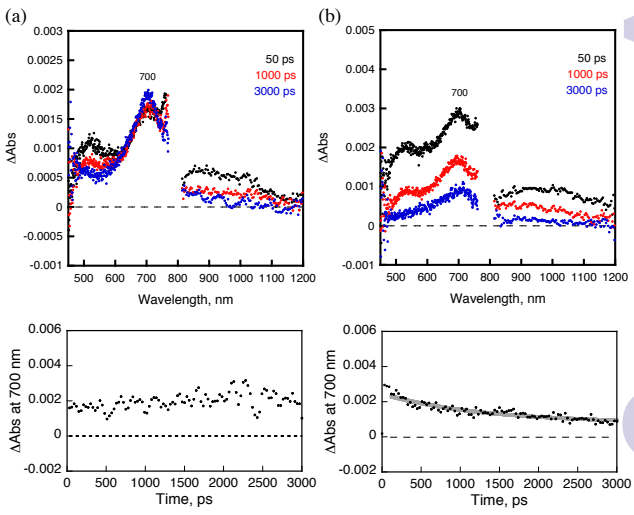
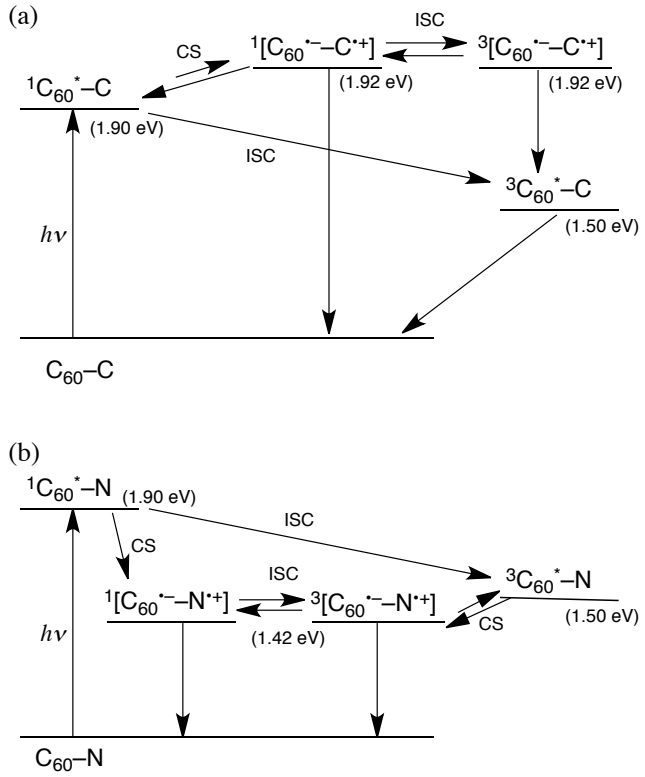


Fig. 3 Transient absorption spectra (upper panels) and time profiles (lower panels) at 700 nm of (a) **4** and (b) **6** in deaerated DMSO taken 50, 1000, and 3000 ps after laser excitation at 355 nm.

Scheme 2 Energy diagrams of (a) **4** and (b) **6**



photoinduced electron transfer occurs from the Glc moiety to the singlet excited state and the triplet excited state of the C_{60} moiety. This is verified by the determination of the redox potentials of **3-6** by cyclic votammetry and second harmonic ac voltammetry (SHACV)[19] as shown in Figs. S6-S9 in ESI). The one-electron oxidation potential of the Glc moiety of **4** was determined to be 1.28 V *vs*. SCE by SHACV, whereas the one electron reduction potential of the C_{60} moiety of **4** was determined to be –0.64 V *vs*. SCE by CV. Because the singlet excited state energy of the C_{60} moiety of **4** was determined to be 1.92 eV, the free energy change of electron transfer from the Glc moiety of **4** to the singlet excited state of C_{60} is evaluated to be 0.02 eV, which means that the electron transfer is slightly endergonic as shown in Scheme 2a. In such a case, electron transfer from the Glc moiety of **4** to the singlet excited state of C_{60} may be followed by faster back electron transfer to the singlet excited state of C_{60} or the triplet excited state of C_{60} without observation of $C_{60}^{\bullet-}$.

The occurrence of electron transfer from the Glc moiety of **6** to the singlet excited state of C_{60} (Fig. 3b) is verified by the lower oxidation potential of **6** as compared with that of **4** because of the *N*-linkage in **6** (Fig. S9 in ESI). In this case, the free energy change of electron transfer from the Glc moiety of **6** to the singlet excited state of C_{60} is evaluated to be -0.40 eV, which means the electron transfer is highly exergonic (Scheme 2b). Because the energy of the charge-separated state (1.50 eV) is the same as the energy of the triplet excited state of C_{60}, the triplet excited state of C_{60} is produced via the intersystem crossing of the charge-separated state (Scheme 2b). However, the triplet excited state of C_{60} of **6** decays via the charge-separated state with the faster rate than the case of **4**.

In conclusion, D-glucose (Glc) pendant C_{60} compound (**4**) exhibited significantly higher photocytotoxicity against HeLa cells than the corresponding azafulleroid, because of no involvement of the charge-separated state in the decay of the triplet excited sate of the C_{60} moiety of **4**, which resulted in the higher yield of singlet oxygen. The present study provides rational design of water soluble sugar-pendant C_{60} derivatives for more efficient PDT.

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Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental procedures and Figs. S1-S9, Table S1. See DOI: 10.1039/c000000x/

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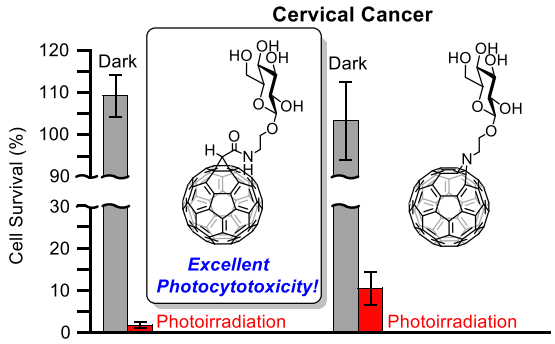

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