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# Detection of 1270 nm Emission from Singlet Oxygen and Photocytotoxic Property of Sugar-Pendant [60] Fullerenes

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**Abstract**—Sugar-pendant [60] fullerene derivatives have been prepared from carbohydrate-linked azides **1a**–**e**. Both monosugar (**4a**–**e**) and bissugar derivatives (**5a**–**e**) produce singlet oxygen ($^1O_2$) under laser irradiation (355 nm) proved by the direct observation of $^1O_2$ emission at 1270 nm. Monosugar derivatives exhibit photocytotoxicity varying by the attached sugar molecule.


The fullerene derivatives show many interesting properties including their superconductivity, organic soft ferromagnetism, electron-transfer behavior such as formation of relatively stable hexaanion in cyclic voltammetry, and non-linear optical property, derived from spherical aromatic structure with carbon-only skeleton.[1] The application of [60] fullerene as medicinal devices[2–4] is also an important approach especially because [60] fullerene generates singlet oxygen upon photoirradiation,[5,6] in addition to the tumor accumulation property.[7] Fullerene biology has been studied extensively by the groups of Nakamura and Sugiura,[8,9] Friedman and Wudl,[10,11] and Miyata.[12,13]

Photodynamic therapy (PDT) is an effective cancer treatment where porphyrins and related macrocycles are used as photosensitizers.[14] Many approaches have been reported to solubilize the photosensitizers that are usually hydrophobic aromatic compounds. Attachment of sugar moiety to chromophores is very attractive not only because of improvement in water solubility but also carbohydrates play important role in cell–cell interaction.[15–18] Thus, several sugar-attached [60] fullerenes have been prepared.[19–26] Here we describe the first sugar-dependent phototoxicity of carbohydrate-pendant [60] fullerene derivatives.

Sugar-pendant [60] fullerenes are synthesized by the procedure shown in Scheme 1.[27] Azides **1a**–**e**[27–29] were reacted with one equivalent of [60] fullerene in chlorobenzene under reflux for 12 h. After evaporation of the solvent the residue was separated by silica gel column chromatography (eluent:toluene/ethyl acetate = 11/1) giving rise to 1/1 adducts **2a**–**e** ($R_f = \sim 0.7$ in toluene/ethyl acetate = 2/1) in 15–30%. Furthermore, 2:1 adducts **3a**–**e** ($R_f = \sim 0.2$ in toluene/ethyl acetate = 2/1) were isolated in 30–50% (eluent:toluene/ethyl acetate = 3/1). Unreacted [60] fullerene was recovered in 30–50% at the first stage of the chromatography (eluent: toluene). The products were characterized by $^1H$ and $^{13}C$ NMR, ESI-MS, and elemental analyses.[30] D-Mannose derivatives **2b** and **3b** has been prepared and characterized by Kato

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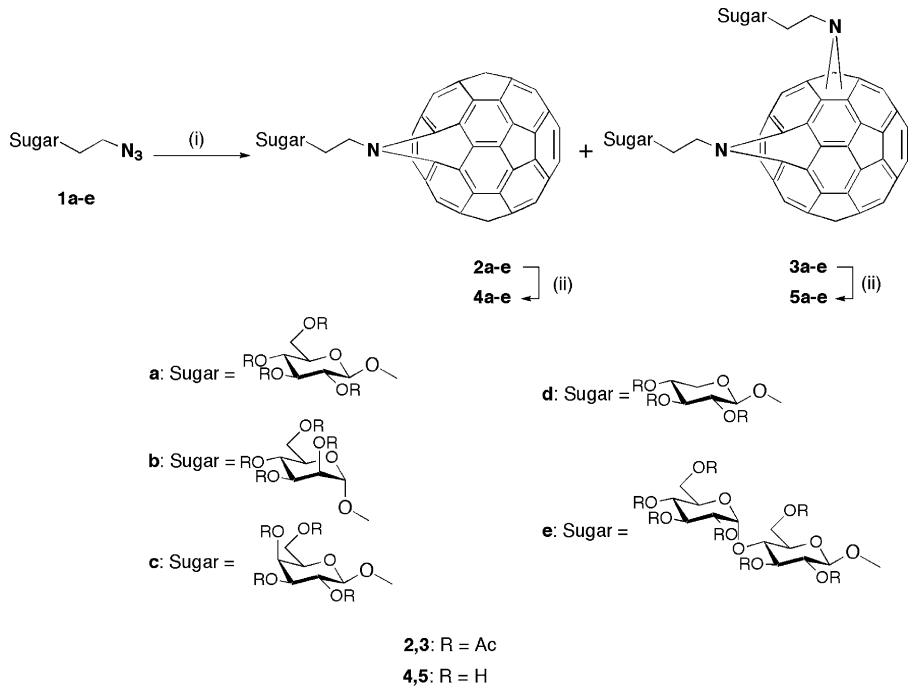


**Scheme 1.** Synthesis of sugar-pendant fullerene derivatives. Conditions: (i) $C_{60}$ in chlorobenzene, reflux (12 h). (ii) NaOMe in $CHCl_3$/MeOH (1/1).

et al., demonstrating that **2b** can be separated to [5,6]-azafulleroid and [6,6]-aziridino fullerene.[26] However, we could not separate [5,6]-azafulleroid and [6,6]-aziridino fullerene for monoadducts **2**. We consider that the compounds obtained could be a mixture of two types of fullerene adducts composed mainly [5,6]-azafulleroid. These materials were used without further separation. Bisadducts **3** could be a 1,1-bis-glycopyranosyl [5,6]-azafulleroid from the reaction mechanism.[26,31]

The removal of acetyl groups of **2a–e** and **3a–e** by sodium methoxide in $CHCl_3$/MeOH (1/1) quantitatively afforded sugar-pendant fullerenes **4a–e** and **5a–e**, respectively (Scheme 1). The structure of the compounds was confirmed by IR, ESI-MS, and elemental analyses.[30] The compounds **4** are not completely soluble in water, however, soluble in 1% aqueous dimethylsulfoxide (DMSO). The $^1H$ NMR spectra of **4** in DMSO-$d_6$ gave very broad signals probably due to high aggregation nature of these compounds even at 70 °C in DMSO solution.

The photosensitizing ability of the sugar-pendant fullerenes **4a–e** and **5a–e** to produce singlet oxygen ($^1O_2$) in DMSO solution was demonstrated by the most reliable direct observation of $^1O_2$ system. The amount of $^1O_2$ generated was directly measured by near IR emission around 1270 nm from $^1O_2$ deactivated, which corresponds to the $O_2(^1\Delta_g)$–$O_2(^3\Sigma_g^-)$ transition. We have built a direct detection system which consists of a Nd:YAG laser (THG 355 nm, 20 Hz, Surelite SL1-20 Continuum) as an excitation light source, a quartz cuvette as an irradiation cell, spectrograph, and a near IR multi-channel detector. Since the IR emission was very feeble, the near IR multi-channel detector was a newly developed ICCD using a near IR gated image intensifier (NIR-II, HAMAMATSU). The experimental conditions are described in the legend for Figure 1. The $^1O_2$ emission spectra obtained are shown in Figure 1, clearly indicating that 1/1 adducts **4a–e** produced more singlet oxygen than 2/1 adducts **5a–e**. Sequential functionalization of the fullerene core reduces the efficiency of singlet oxygen production.[32] The normalized emission intensities of compounds **4a–e** divided by the absorption at 355 nm are, however, ~5% of the parent [60] fullerene and approximately half of the OH-protected derivatives **2a–e** (data not shown). This is probably due to the disruption of fullerene aromaticity and high aggregation property of OH-free sugar-pendant [60] fullerenes. The singlet oxygen yields are hardly dependent on the nature of the pendant sugar moiety.

Photocytotoxicity of these sugar-pendant fullerenes was evaluated against the HeLa cell. The cells ($1\times10^5$ cells/well) were incubated for 12 h in PBS in the 24-well plate (diameter: 16 mm), then the photosensitizers were added to the medium to make [sensitizer] = 25 μM in 1% DMSO, and further incubated for 6 h. After washing, the cells were irradiated with Nd/YAG laser (355 nm, 50 mW/cm$^2$) for 200 s (total 10 J/cm$^2$), and incubated for 24 h, then, the number of living cells were counted by MTT assay. The results are shown in Figure 2. Dark cytotoxicity was found to be small.[9] Significant photocytotoxicity is observed for **4a–c**, which are D-glucose, D-mannose, and D-galactose derivatives with one carbohydrate unit. D-Xylose (**4d**) and maltose (**5d**) derivatives are less phototoxic under these conditions in spite of the fact that they produce similar amount of singlet oxygen in vitro. The difference in the amount of the photosensitizers incorporated might explain these differences. Compounds **5a–e**, which have two sugar units, do not exhibit photocytotoxicity probably because they produce very little of singlet oxygen under the present experimental condition. The alternative explanation is

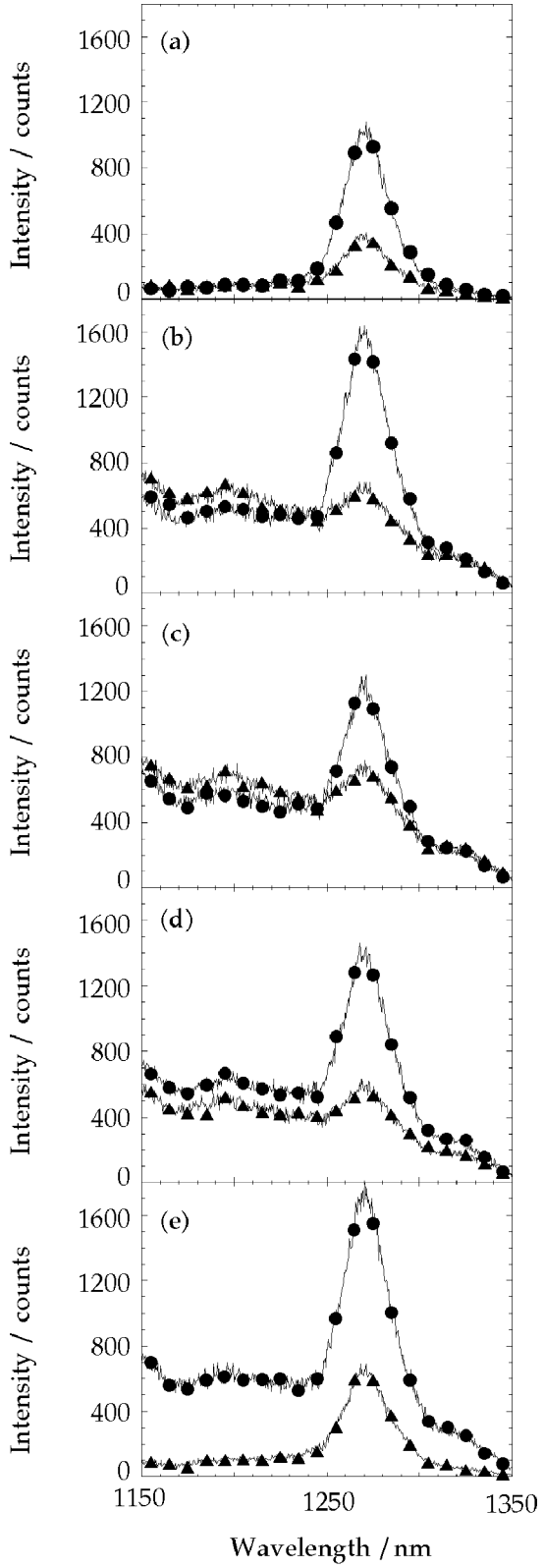


**Figure 1.** Emission spectra from singlet oxygen generated by laser irradiation (355 nm) of sugar-pendant [60] fullerene derivatives ($1\times10^{-5}$ M in: DMSO solution). Excitation laser intensity: 280 mW/cm$^2$. Gate time: 0.5–5.5 μs after laser pulse. Collection times: 100 s (total 28 J/cm$^2$). Lines with circle marks represent spectra for monosugar-pendant derivatives **4a–e**; triangles represent bissugar derivatives **5a–e**. (a) D-Glucose; (b) D-Mannose; (c) D-Galactose; (d) D-Xylose; (e) Maltose derivatives.

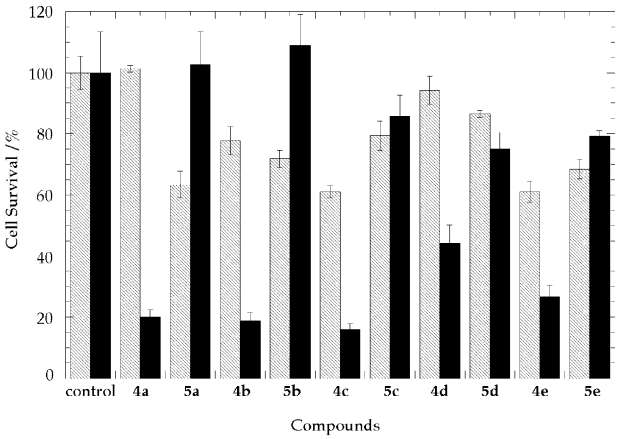


**Figure 2.** Cytotoxicity of sugar-pendant [60] fullerene derivatives against the HeLa cell. See text for experimental details. Striped bars represent dark toxicity and solid bars represent phototoxicity. The error bars represent ±1 estimated standard deviation from at least three independent experiments.

the inferior incorporation property of the bis-sugar derivatives into the cell. The estimation of the amount of the photosensitizers in cell could solve this matter. In addition, study of cellular localization of the photosensitizers is important for investigation of the photodynamic property of the water-soluble fullerene derivatives.[33] Such studies are now in progress in our laboratory.

In summary, carbohydrate-pendant [60] fullerene derivatives generate singlet oxygen upon photoirradiation and exhibit carbohydrate-dependent photocytotoxicity against the HeLa cell.

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30. All the new compounds gave satisfactory spectral data and elemental analyses consistent with their proposed structures. Selected data for **4a**: ESI-MS $m/z = 972.1104$ $(M + CH_3OH\text{-}H)^-$ ($C_{69}H_{18}NO_7$ requires 972.1083), Anal. calcd (Found) for **4a**·$7H_2O$ ($C_{68}H_{15}NO_6{\cdot}7H_2O$): C, 76.48(76.91); H, 2.74 (2.70); N, 1.31 (1.79)%. for **4b**: ESI-MS $m/z = 972.1075$ $(M + CH_3OH\text{-}H)^-$ ($C_{69}H_{18}NO_7$ requires 972.1083), Anal. calcd (Found) for **4b**·$5H_2O$ ($C_{68}H_{15}NO_6{\cdot}5H_2O$): C, 79.15(78.98); H, 2.44(2.58); N, 1.36(1.49)%. for **4c**: ESI-MS $m/z = 972.1065$ $(M + CH_3OH\text{-}H)^-$ ($C_{69}H_{18}NO_7$ requires 972.1083), Anal. calcd (Found) for **4c**·$8H_2O$ $C_{68}H_{15}NO_6{\cdot}8H_2O$: C, 75.21(75.17); H, 2.88(2.70); N, 1.29(1.55)%. for **4d**: ESI-MS $m/z = 942.0986$ $(M + CH_3OH\text{-}H)^-$ ($C_{68}H_{16}NO_6$ requires 942.0978), Anal. calcd (Found) for **4d**·$5H_2O$ $C_{67}H_{13}NO_5{\cdot}5H_2O$: C, 80.32(80.13); H, 2.31(2.48); N, 1.46(1.79)%. for **4e**: ESI-MS $m/z = 1134.1627$ $(M + CH_3OH\text{-}H)^-$ ($C_{75}H_{28}NO_{12}$ requires 1134.1612), Anal. calcd (Found) for **4e**·$7H_2O$ $C_{74}H_{25}NO_{11}{\cdot}7H_2O$: C, 72.25(72.53); H, 3.20(3.30); N, 1.14(1.38)%. for **5a**: ESI-MS $m/z = 1193.1992$ $(M + CH_3OH\text{-}H)^-$ ($C_{77}H_{33}N_2O_{13}$ requires 1193.1983), Anal. calcd (Found) for **5a**·$5H_2O$ $C_{76}H_{30}N_2O_{12}{\cdot}5H_2O$: C, 72.84(73.28); H, 3.22(3.45); N, 2.24(2.76)%. for **5b**: ESI-MS $m/z = 1193.1960$ $(M + CH_3OH\text{-}H)^-$ ($C_{77}H_{33}N_2O_{13}$ requires 1193.1983), Anal. calcd (Found) for **5b**·$6H_2O$ $C_{76}H_{30}N_2O_{12}{\cdot}6H_2O$: C, 71.81(71.82); H, 3.33(3.54); N, 2.20(2.40)%. for **5c**: ESI-MS $m/z = 1193.1980$ $(M + CH_3OH\text{-}H)^-$ ($C_{77}H_{33}N_2O_{13}$ requires 1193.1983), Anal. calcd (Found) for **5c**·$5H_2O$ $C_{76}H_{30}N_2O_{12}{\cdot}5H_2O$: C, 72.84(72.82); H, 3.22(3.35); N, 2.24(2.44)%. for **5d**: ESI-MS $m/z = 1133.1776$ $(M + CH_3OH\text{-}H)^-$ ($C_{75}H_{29}N_2O_{11}$ requires 1133.1771), Anal. calcd (Found) for **5d**·$6H_2O$ $C_{74}H_{26}N_2O_{10}{\cdot}6H_2O$: C, 73.39(73.24); H, 3.16(3.12); N, 2.31(2.47)%. for **5e**: ESI-MS $m/z = 1517.3033$ $(M + CH_3OH\text{-}H)^-$ ($C_{89}H_{53}N_2O_{23}$ requires 1517.3039), Anal. calcd (Found) for **5e**·$9H_2O$ $C_{88}H_{50}N_2O_{22}{\cdot}9H_2O$: C, 64.08(63.90); H, 4.16(4.53); N, 1.70(1.93)%.
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