Electronic Supporting Information

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

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ESI. 1: Synthesis.

Scheme S1 shows the synthetic procedure of 2'-(2',3',4',6'-*O*-tetraacetyl- β -D-glucopyranosyl)ethyl 3'*H*-cyclopropa[1,9](C₆₀-*I*_h)[5,6]fullereno-3'-carboxylic amide (**3**) and 2'-(β -D-glucopyranosyl)ethyl 3'*H*-cyclopropa[1,9](C₆₀-*I*_h)[5,6]fullereno-3'-carboxylic amide (**4**). 3'*H*-Cyclopropa[1,9][5,6]fullereno-C₆₀-*I*_h-3'-carboxylic acid *N*-hydroxysuccinimide ester (**1**) was prepared by the condensation reaction between 3'*H*-cyclopropa[1,9][5,6]fullereno-C₆₀-*I*_h-3'-carboxylic acid¹³ 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 CHCl₃, followed by the purification using silica gel column chromatography (eluent: CHCl₃/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**.



Scheme S1 Synthesis of sugar-pendant C₆₀ compounds

Materials. Dry toluene was prepared by refluxing toluene (Junsei Chemical Co., Japan, 99.0 %) over sodium and benzophenone, followed by distillation. Dry THF was prepared by refluxing THF (Kanto Chemical Co., Japan, >99.5 %) over sodium and benzophenone, followed by distillation. Dry chloroform (CHCl₃) was prepared by refluxing CHCl₃ (Junsei Chemical Co., Japan, 99.0 %) over calcium hydride, followed by distillation, respectively. Dry N,N-dimethylformamide (DMF) was prepared by DMF (Junsei Chemical Co., Japan, 99 %) over calcium hydride, followed by distillation. Palladium (10 % on carbon), N-hydroxysuccinimide (NHS, >98.0%), 1-ethyl-3-(3dimethylaminopropyl)carbodiimide (EDCl, >98.0%), and 4-dimethylaminopyridine (DMAP, >99.0%) were purchased from Tokyo Chemical Industry Co., Ltd., Japan, and used as received. Dry THF containing 2.0 wt% sodium methoxide (NaOMe) was prepared from NaOMe (ca. 5 mol/L in methanol, Wako Pure Chemical Industries, Japan) and dry THF. Deionized water (electrical conductivity = $18.3 \text{ M}\Omega$ cm) was prepared by Advantec Aquarius GSH-5000 and Millipore Milli-Q Jr. All other chemicals were obtained from commercial sources and used as received unless otherwise stated.

3'H-Cyclopropa[1,9][**5,6**]**fullereno-C**₆₀-*I*_h-**3'-carboxylic acid** *N*-hydroxysuccinimide ester (1). The mixture of 3'*H*-cyclopropa[1,9][5,6]fullereno-C₆₀-*I*_h-3'-carboxylic acid¹³ (557 mg, 0.717 mmol), *N*-hydroxysuccinimide (NHS, 166 mg, 1.44 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCl, 276 mg, 1.44 mmol), and 4-dimethylaminopyridine (DMAP, 8.80 mg, 0.072 mmol) in dry toluene (300 mL) was stirred at 40 °C for under nitrogen atmosphere. After 48 h, the mixture was evaporated to dryness. The residue was purified by flash column chromatography (silica gel, toluene) to give **1** as a brown solid (418 mg, 66.7 %). *R*_f = 0.29 (toluene). ¹H NMR (400 MHz, CDCl₃, δ): 4.99 (s, 1H), 3.00 (s, 4H, -CH₂CH₂-).

2-Aminoethyl 2,3,4,6-*O***-tetraacetyl-\beta-D-glucopyranoside (2).** Compound **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. To the mixture of 2-azidoethyl 2,3,4,6-*O*-tetraacetyl- β -D-glucopyranoside (600 mg, 1.45 mmol) in the 4:3 EtOH/THF (v/v) mixed solvent (7 mL), palladium (10 % on carbon, 33.6 mg) was added in a glove box. After stirred for 21 h under a hydrogen atmosphere at room temperature, the mixture was filtered by a 0.45 µm membrane filter. The filtrate was evaporated to dryness to give **2**

as a pale yellow viscous liquid (556 mg, 99.0 %). $R_f = 0$ (hexane/ethyl acetate = 1:1). ¹H NMR (D₂O 400 MHz) δ (ppm): 5.24-5.19 (dd, $J_1 = J_2 = 9.3$ Hz, 1H, H3), 5.11-5.09 (dd, $J_1 = J_2 = 9.8$ Hz, 1H, H4), 5.03-4.98 (dd, $J_1 = 7.8$ Hz, $J_2 = 9.3$ Hz, 1H, H2), 4.55-4.54 (d, J = 7.8 Hz, 1H, H1), 4.28-4.24 (dd, $J_1 = 4.9$ Hz, $J_2 = 12.2$ Hz, 1H, H6), 4.17-1.14 (dd, $J_1 = 2.4$ Hz, $J_2 = 12.2$ Hz, 1H, H6), 3.91-3.86 (m, 1H, OCH₂CH₂N), 3.73-3.69 (ddd, $J_1 = 2.4$ Hz, $J_2 = 4.9$ Hz, $J_3 = 9.8$ Hz, 1H, H5), 3.61-3.56 (m, 1H, OCH₂CH₂N), 2.94-2.80 (m, 2H, OCH₂CH₂N).

2'-(2',3',4',6'-*O*-Tetraacetyl-β-D-glucopyranosyl)ethyl 3'H-cyclopropa[1,9](C₆₀- $I_{\rm h}$)[5,6]fullereno-3'-carboxylic amide (3). The mixture of 1 (125 mg, 145 μ mol), 2 (112 mg, 290 µmol), and catalytic amounts of DMAP in dry CHCl₃ (10.0 mL) was stirred at 40 °C under nitrogen atmosphere. After 48 h, the mixture was evaporated to dryness. The residue was purified by flash column chromatography (silica gel, CHCl₃/MeOH = 50:1) to give **3** as a brown solid (56.1 mg, 36.3 %). $R_f = 0.20$ (CHCl₃/MeOH = 50:1). ¹H NMR (CDCl₃, 400 MHz) δ (ppm): 7.08-7.05 (dd, $J_1 = J_2 =$ 5.4 Hz, 1H, NHCO), 5.30-5.25 (dd, $J_1 = J_2 = 9.8$ Hz, 1H, H3), 5.16-5.11 (dd, $J_1 = J_2 =$ 9.8 Hz, 1H, H4), 5.10-5.06 (dd, $J_1 = 7.8$ Hz, $J_2 = 9.8$ Hz, 1H, H2), 4.77 (s, 1H, $C_{60}CHCO$, 4.65-4.63 (d, J = 7.8 Hz, 1H, H1), 4.36-4.33 (dd, $J_1 = 2.8$ Hz, $J_2 = 12.4$ Hz, 1H, H6), 4.32-4.29 (dd, $J_1 = 4.7$ Hz, $J_2 = 12.4$ Hz, 1H, H6), 4.05-3.96 (m, 2H, OCH₂CH₂N), 3.82-3.76 (m, 3H, OCH₂CH₂N, H5), 2.15 (s, 3H, acetyl), 2.14 (s, 3H, acetyl), 2.05 (s, 3H, acetyl), 2.04 (s, 3H, acetyl). ¹³C NMR (CDCl₃, 400 MHz) δ (ppm): 170.82 (acetyl C=O), 170.17 (acetyl C=O), 169.73 (acetyl C=O), 169.42 (acetyl C=O), 165.21 (CONH), 148.66, 146.36, 145.70, 145.23, 145.23, 145.16, 145.12, 145.03, 145.02, 144.80, 144.67, 144.67, 144.64, 144.57, 144.57, 144.50, 144.29, 143.96, 143.68, 143.32, 143.10, 142.99, 142.94, 142.77, 142.44, 142.23, 142.16, 142.16, 142.07, 141.10, 140.89, 140.42, 136,14 (C₆₀), 101.15 (C1), 72.50 (C3), 72.28 (C5), 71.55 (C2), 71.41, 69.26 (OCH₂CH₂N), 68.20 (C4), 61.29 (C6), 41.32 (C₆₀CHCO), 39.94 (OCH₂CH₂N), 20.92, 20.60 (cyclopropane bridgehead carbon). ESI-TOF MS (m/z): $[M+Na]^+$ calcd for $C_{78}H_{25}O_{11}NNa$, 1175.023; found, 1175.543. Anal: calcd for C₇₈H₂₅O₁₁N·0.5H₂O; C, 80.69

H, 2.26; N, 1.21; found: C, 80.58; H, 2.38; N, 1.22.

2'-(β-D-Glucopyranosyl)ethyl 3'H-cyclopropa[1,9](C₆₀- I_h)[5,6]fullereno-3'carboxylic amide (4). Compound 3 (50.0 mg, 46.9 μmol) was added to Teflon® bottle with a magnetic stir bar. To the bottle, dry THF containing 2.0 wt% CH₃ONa (19 mL) was added. The mixture was stirred at room temperature under nitrogen atmosphere with blocking the light. After 12 h, DMSO (8 mL) was added and the resultant homogeneous mixture was evaporated to remove THF. The residue was dialyzed against water using Spectra/Por[®] dialysis tubing (MWCO 1,000) for 2 days and then freeze-dried to give **4** as a brown solid (46 mg, 100 %). ¹H NMR (DMSO-*d*₆, 400 MHz) δ (ppm): 9.11-9.08 (dd, $J_1 = J_2 = 5.3$ Hz, 1H, NHCO), 5.38 (s, 1H, C₆₀CHCO), 5.07-5.06 (d, $J_1 = 3.4$ Hz, 1H), 5.00-4.99 (d, $J_1 = 3.9$ Hz, 1H), 4.95-4.94 (d, $J_1 = 4.9$ Hz, 1H), 4.66-4.63 (dd, $J_1 = J_2 = 5.4$ Hz, 1H), 4.25-4.22 (d, $J_1 = 7.8$ Hz, 1H, H1), 4.25-3.92 (m, 1H), 3.91-3.71 (m, 2H), 3.70-3.68 (d, $J_1 = 7.8$ Hz, 1H), 3.17-3.15 (m, 1H), 3.08-3.02 (m, 2H). ESI-TOF MS (m/z): [M+Na]⁺ calcd for C₇₀H₁₇O₇NNa, 1006.318; found, 1006.88. Anal: calcd for C₇₀H₁₇O₇N·3H₂O; C, 81.05; H, 2.20; N, 1.43; found: C, 81.00; H, 2.23; N, 1.35.

ESI.2: Photodynamic Activity Experiments

HeLa cells were maintained in CO₂ Independent Medium (Gibco BRL) supplemented with 10% fetal calf serum at 37 °C in 5% CO₂. For experiments conducted to determine the photodynamic activities of D-glucose pendant C₆₀ derivatives, the cells were seeded in 48-well culture plates at a density of 1.7×10^4 cells per well. After growing overnight, the cells were treated with D-glucose pendant C₆₀ derivatives for 24 h in the dark. For the treatment of cells, D-glucose pendant C₆₀ derivatives were dissolved in DMSO and injected to cell medium at a 0.50% (v/v) DMSO concentration. After treatments with D-glucose pendant C₆₀ derivatives (4, 6), the cells were washed with PBS and exposed to light at room temperature. The light irradiation was performed using a xenon lamp (MAX-301, 300 W; Asahi Spectra Co. Ltd.) equipped with a purpose-built UV-VIS mirror module (300-550 nm) and a long-pass filter with a cut-off at 350 or 400 nm. The light dose was 7 J/cm² at the cell level. To measure the viability of cells as a ratio (%) compared with cells which were not treated, a WST-8 assay was carried out 24 h after light irradiation using the Cell Counting Kit-8 (Dojindo Laboratories) according to the manufacturer's instructions.

	IC ₅₀ (μM)	
	350-550 nm	400-550 nm
4	1.64	1.42
6	0.41	0.40

Table S1 50% Inhibitory concentration (IC₅₀)

ESI.3: Photochemical Experiments.



Fig. S1 Emission spectra of singlet oxygen at 1280 nm generated from C_{60} (gray), 3 (blue), and 5 (red) in C_6D_6 .



Fig. S2 Transient absorption spectra of (a) **3** and (b) **5** in deaerated DMSO taken 50, 1000, and, 3000 ps after laser excitation at 355 nm (17 mJ/pulse).



Fig. S3 Time profiles of absorbance at 700 nm of (a) **3** and (b) **5** in deaerated DMSO after laser excitation at 355 nm (17 mJ/pulse).



Fig. S4 Transient absorption spectra of (a) **3**, (b) **4**, (c) **5** and (d) **6** in deaerated DMSO taken 2 μ s after laser excitation at 355 nm (17 mJ/pulse).



Fig. S5 ESR spectra of (a) **3**, (b) **4**, (c) **5**, and (d) **6** after photo irradiation with highpressure mercury lump and measured at 143 K.



Fig. S6 (a) CV and (b) SHACV of **3** in deaerated DMSO containing TBAPF_6 (0.10 M) at 298 K.



Fig. S7 (a) CV and (b) SHACV of 4 in deaerated DMSO containing TBAPF_6 (0.10 M) at 298 K.



Fig. S8 (a) CV and (b) SHACV of 5 in deaerated DMSO containing TBAPF_6 (0.10 M) at 298 K.



Fig. S9 (a) CV and (b) SHACV of 6 in deaerated DMSO containing TBAPF_6 (0.10 M) at 298 K.

Experimental details

Electrochemical measurements. Cyclic voltammetry (CV) measurements of fullerene derivatives were performed with an ALS630B electrochemical analyzer in deaerated DMSO containing 0.1 M Bu₄N⁺ClO₄⁻ (TBAP) as a supporting electrolyte at 298 K. The platinum working electrode (BAS, surface i.d. 1.6 mm) was polished with BAS polishing alumina suspension and rinsed with acetone before use. The counter electrode was a platinum wire (0.5 mm dia.). The measured potentials were recorded with respect to an Ag/AgNO₃ (0.01 M) reference electrode. The values of redox potentials (vs Ag/AgNO₃) are converted into those vs SCE by addition of 0.29 V.^{S1}

Transient absorption measurements. Femtosecond transient absorption spectroscopy experiments were conducted using an ultrafast source: Integra-C (Quantronix Corp.), an optical parametric amplifier: TOPAS (Light Conversion Ltd.) and a commercially available optical detection system: Helios provided by Ultrafast Systems LLC. The source for the pump and probe pulses were derived from the fundamental output of Integra-C (780 nm, 2 mJ/pulse and fwhm = 130 fs) at a repetition rate of 1 kHz. 75% of the fundamental output of the laser was introduced into TOPAS which has optical frequency mixers resulting in tunable range from 285 nm to 1660 nm, while the rest of the output was used for white light generation. Prior to generating the probe continuum, a variable neutral density filter was inserted in the path in order to generate stable continuum, then the laser pulse was fed to a delay line that provides an experimental time window of 3.2 ns with a maximum step resolution of 7 fs. In our experiments, a wavelength at 355 nm of TOPAS output, which is fourth harmonic of signal or idler pulses, was chosen as the pump beam. As this TOPAS output consists of not only desirable wavelength but also unnecessary wavelengths, the latter was deviated using a wedge prism with wedge angle of 18°. The desirable beam was irradiated at the sample cell with a spot size of 1 mm diameter where it was merged with the white probe pulse in a close angle ($< 10^{\circ}$). The probe beam after passing through the 2 mm sample cell was focused on a fiber optic cable that was connected to a CCD spectrograph for recording the time-resolved spectra (410-800 nm). Typically, 2500 excitation pulses were averaged for 5 seconds to obtain the transient spectrum at a set delay time. Kinetic traces at appropriate wavelengths were assembled from the time-resolved spectral data. All measurements were conducted at room temperature, 295 K.

Nanosecond time-resolved transient absorption measurements were carried out using the laser system provided by UNISOKU Co., Ltd. Measurements of nanosecond transient absorption spectrum were performed according to the following procedure. A deaerated solution containing a sample in a quartz cell $(1 \text{ cm} \times 1 \text{ cm})$ was excited by a Nd:YAG laser (Continuum SLII-10, 4-6 ns fwhm, $\lambda_{ex} = 355 \text{ nm}$, 5mJ/pulese). The photodynamics were monitored by continuous exposure to a xenon lamp (150 W) as a probe light and a photomultiplier tube (Hamamatsu 2949) as a detector. The solution was oxygenated by nitrogen purging for 15 min prior to measurements.

Near-IR emission measurements: Singlet oxygen generation was detected by its phosphorescence emission at 1270 nm. An O_2 -saturated benzene- d_6 solution containing fullerene derivative in a quartz cell (optical path length 10 mm) was excited by an LED laser at 532 nm using an Otsuka Electronics Co. Ltd. Vis-NIR emission spectrophotometer.

(S1) C. K. Mann and K. K. Barnes, In *Electrochemical Reactions in Non-aqueous Systems*, Mercel Dekker, New York, 1970.