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Cyclodextrin Complexed [60]Fullerene Derivatives with High Levels of Photodynamic Activity by Long Wavelength Excitation

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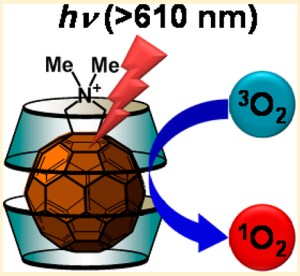
Supporting Information

ABSTRACT: We have evaluated the photodynamic activities of C_{60} derivative·γ-cyclodextrin (γ-CDx) complexes and demonstrated that they were significantly higher than those of the pristine C_{60} and C_{70}·γ-CDx complexes under photoirradiation at long wavelengths (610−720 nm), which represent the optimal wavelengths for photodynamic therapy (PDT). In particular, the cationic C_{60} derivative·γ-CDx complex had the highest photodynamic ability because the complex possessed the ability to generate high levels of $^1O_2$ and provided a higher level of intracellular uptake. The photodynamic activity of this complex was greater than that of photofrin, which is the most widely used of the known clinical photosensitizers. These findings therefore provide a significant level of information toward the optimization of molecular design strategies for the synthesis of fullerene derivatives for PDT.



KEYWORDS: *Fullerenes, cyclodextrin, photodynamic therapy, photosensitizers*

Water-solubilized fullerenes (C_{60} and C_{70}) have recently been reported for their use as potential photosensitizers because C_{60} and C_{70} are efficient visible-light triplet-sensitizers with pronounced photoproduction abilities for the generation of reactive oxygen species (ROS).[1] γ-Cyclodextrin (CDx)-complexed C_{60} or C_{70} can be isolated as independent units through γ-CDx inclusion in water, allowing for the self-quenching of photoexcited states to be avoided.[2−4] To improve the photoactivity of these fullerene·γ-CDx complexes, it is important that the fullerene or γ-CDx units are effectively functionalized. Furthermore, for pharmaceutical applications such as photodynamic therapy (PDT), improvements are required in a number of areas, including (i) the generation activity of ROS during the photoirradiation at longer wavelength with high biological tissue permeability; (ii) intracellular uptake; and (iii) the stability and solubility of the complexes. Unfortunately, however, the functionalization of fullerene or γ-CDx often results in a reduction in the overall stability of the complex.[3,5] In a recent publication, we reported that several functionalized C_{60} derivatives, such as the *N*-methylpyrrolidine, *N*,*N*-dimethylpyrrolidinium, and *N*-acetylpyrrolidine derivatives (**1**−**3**, Figure 1) of C_{60}, could form stable complexes with γ-CDx using a mechanochemical high-speed vibration milling apparatus according to Komatsu's method.[3,6,7] The γ-CDx-complexed C_{60} derivatives of **1**−**3** were found to be soluble in water at high concentrations (i.e., >1.0 mM), with solubility levels comparable to that of unmodified C_{60} (2.2 mM).[6,7] These complexes possessed a 1:2 stoichiometry of the γ-CDx unit to the C_{60} derivative and existed in a [2]pseudorotaxane conformation.[7]

Herein, we report a comparison of the photodynamic activities of γ-CDx-complexed C_{60} derivatives containing three different nitrogen atom-containing groups, including amino, ammonium, and amide groups. The photodynamic activities of the γ-CDx-complexed C_{60} derivatives toward human cervical cancer HeLa cells were evaluated under visible-light irradiation at long wavelengths (610−740 nm). This wavelength range has been reported to be optimal for PDT, in that no cytotoxicity issues were observed in this wavelength range when pristine C_{60} and C_{70} were incorporated into liposomes.[8−17]

HeLa cells were incubated for 24 h in the presence of 0.1−2.0 μM of the fullerenes complexes before being exposed to light with a wavelength greater than 610 nm (610−740 nm) for 30 min. The power of the light at the cellular level was 9 mW cm^{-2}. To measure the viability of the cells as a percentage ratio relative to the cells that had not been treated with the fullerenes, a WST-8 assay was conducted using the Cell Counting Kit-8 24 h after the irradiation of the cells with light. The results are shown in Figure 2. No cytotoxicity was observed when the fullerene·γ-CDx complexes were added to the cells in the absence of any exposure to light (Figure 2a). Furthermore, no photodynamic activity was observed in the

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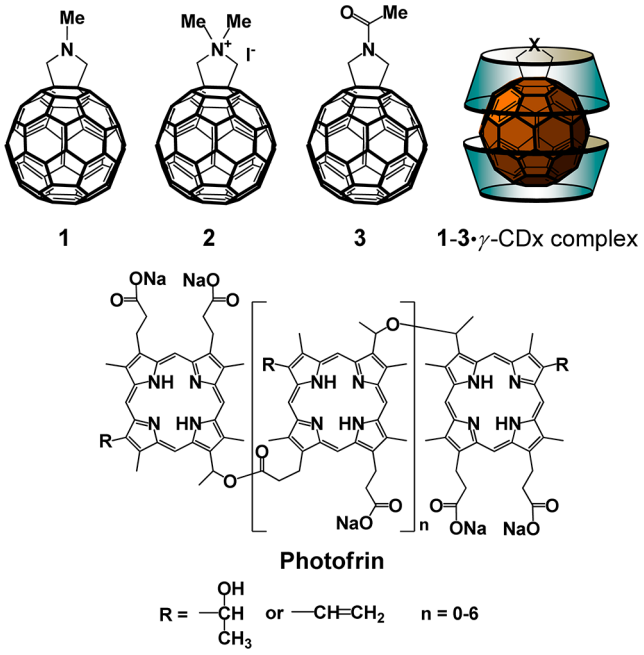

Figure 1. Structures of the C_{60} derivatives (**1**–**3**) and photofrin and schematic illustration of those C_{60} derivative·γ-CDx complexes.

C_{60}, C_{70}, and **1**·γ-CDx complexes. In contrast, the photodynamic activities of the γ-CDx complexes of **2** and **3** were drug dose-dependent, and their half maximal inhibitory concentration (IC_{50}) values in combination with 30 min of light irradiation (610–740 nm) were estimated to be approximately 0.47 and 0.96 μM, respectively (Figure 2b). On the basis of these results, the order of the photodynamic activity of these complexes was **2** > **3** ≫ **1** ≈ C_{60} ≈ C_{70}. The IC_{50} values of the γ-CDx complexes of **2** and **3** were lower than that of photofrin, which is the most widely used clinical photosensitizer. Photofrin provided an IC_{50} value of 2 μM under the same conditions, when the number of moles was converted to the number of porphyrin units because photofrin consists of porphyrin oligomers containing two to eight units (Figure 2c). These results revealed that the photodynamic activities of the γ-CDx complexes of **2** and **3** were approximately four and two times higher than that of photofrin, respectively.

At this stage, it was not clear why the γ-CDx complexes of **2** and **3** possessed higher levels of photodynamic activity than the γ-CDx complexes of C_{60}, C_{70}, and **1**. Two possible explanations were suggested to explain these differences, including (i) an increase in the levels of cytotoxic ROS in the presence of the γ-CDx complexes of **2** and **3** and (ii) an improvement in the intracellular uptake of the γ-CDx complexes of **2** and **3**. Photoexcited C_{60} has been reported to generate superoxide anions ($O_2^{\bullet-}$) through an electron transfer Type I reaction, as well as singlet oxygen molecules ($^1O_2$) through an energy transfer Type II reaction as ROS.[18] To assess the plausibility of explanation (i), the identities of the major ROS generated from the γ-CDx complexes of **2** and **3** were determined. To identify the species of reactive oxygen, the effect of D-mannitol and L-histidine, which are scavengers of hydroxyl radicals and $^1O_2$, respectively, were analyzed.[19,20] As shown in Figure 3, L-histidine effectively inhibited the photocytotoxicity of the γ-CDx complexes of **2** and **3**, whereas D-mannitol was provided no inhibition.[21] These results suggested that the $^1O_2$ generated via the energy transfer Type II reaction played a major role in

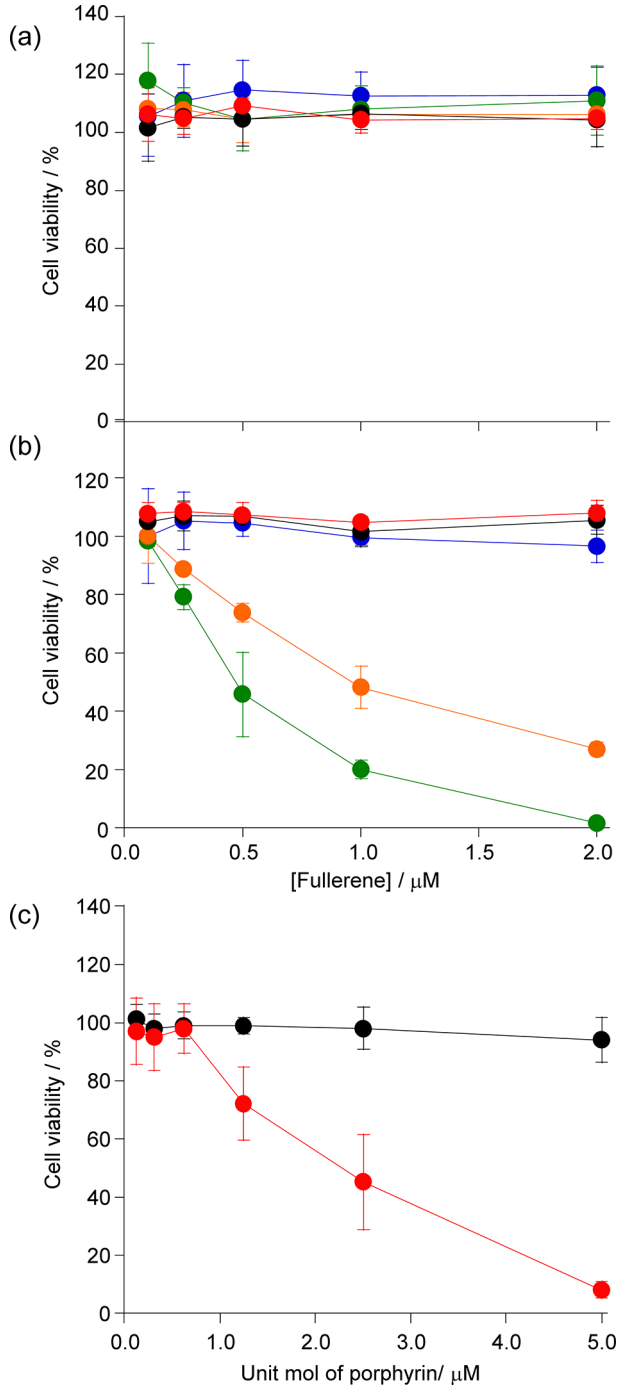

Figure 2. Concentration dependency of the cytotoxicities of the γ-CDx complexes of C_{60} (black line), C_{70} (red line), **1** (blue line), **2** (green line), and **3** (orange line) in (a) the absence and (b) the presence of light irradiation (610–740 nm, 30 min) and (c) the cytotoxicity of photofrin in the absence (black line) and the presence of light irradiation (red line) (610–740 nm, 30 min). Cell viability was evaluated according to the WST-8 method. Each value represents the mean ± standard deviation (SD) of three experiments.

the photodynamic activity of the γ-CDx complexes of **2** and **3**. The amount of $^1O_2$ generated during the processes was therefore measured according to a chemical method using 9,10-anthracenedipropionic acid disodium salt (ADPA)[22] as a detector to clarify the reason for the differences in biological activities of the γ-CDx complexes of C_{60}, C_{70}, **1**, **2**, and **3**. The level of ADPA absorption at 400 nm (absorption maximum for

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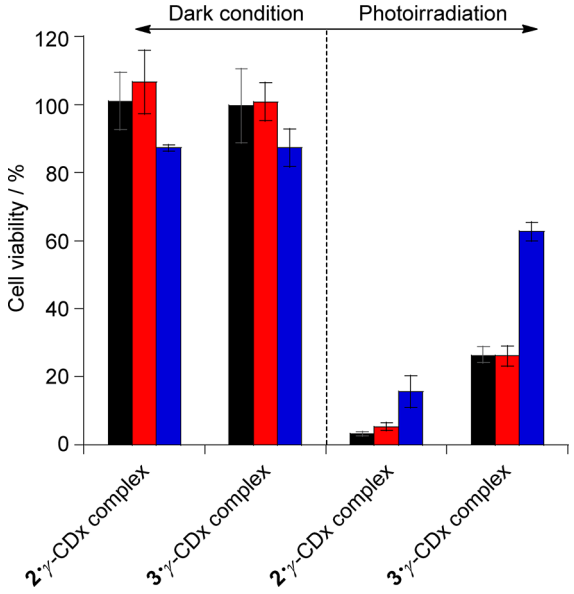

Figure 3. Effect of ROS scavengers on the photodynamic activities of the γ-CDx complexes of **2** and **3**. The photodynamic activities of the γ-CDx complexes of **2** and **3** (2.0 μM) were measured in the absence (black bar) and presence of 50 mM D-mannitol (red bar) or 50 mM L-histidine (blue bar). Each value represents the mean ± SD of three experiments.

ADPA) was monitored as a function of time following the irradiation of 15 μM samples of the complexes (Figure 4). The

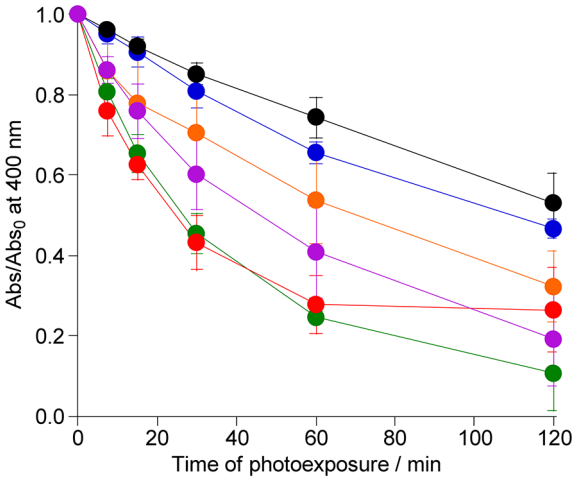

Figure 4. Time-dependent bleaching of 9,10-anthracenedipropionic acid disodium salt (ADPA) caused by the $^1O_2$ generated from γ-CDx complexes of C_{60} (black line), C_{70} (red line), **1** (blue line), **1** (purple line, pH 2.0), **2** (green line), and **3** (orange line). Changes in the ADPA absorption at 400 nm upon photoirradiation (620 nm, 0.6 mW cm^{-2}) were monitored as a function of time (Abs_0, initial absorbance). [complex] = 15 μM, and [ADPA] = 25 μM; under an oxygen atmosphere at 25 °C.

results indicated that the different γ-CDx complexes generated $^1O_2$ in the order of **2** ≈ C_{70} > **3** > **1** > C_{60}. With the exception of C_{70}, this order was consistent with that of the photodynamic activities of the γ-CDx complexes. The results for the amounts of $^1O_2$ generated by the C_{60} derivatives were compatible with those previously reported for the monoadduct C_{60} derivatives, such as **1**–**3**, which were approximately 20–60% greater than that of pristine C_{60}.[1,23,24] These results can be understood in the sense that although the monoadduct C_{60} derivative gave a reduced quantum yield of $^1O_2$ production compared with pristine C_{60} (532 nm was used in ref 23), the molar extinction coefficients of the monoadduct C_{60} derivatives were greater at longer wavelengths (>500 nm).[1] Consequently, the low levels of photodynamic activity and $^1O_2$ generation observed for the C_{60}·γ-CDx complex originated from the poor absorption of C_{60} at wavelengths greater than 610 nm (Figure S1b, Supporting Information).

The resulting low level of $^1O_2$ generation from the **1**·γ-CDx complex relative to the γ-CDx complexes of **2** and **3** can be explained in the following way. The results can be attributed predominantly to the lower $^1O_2$ generation ability of the **1**·γ-CDx complex relative to those of the γ-CDx complexes of **2** and **3** from the fact that the γ-CDx complexes of **1**, **2**, and **3** have similar molar extinction coefficients at longer wavelengths (>600 nm) (Figure S1b and Table S1, Supporting Information). It is well-known that the presence of the lone pair of electrons on an amine can lead to electron-transfer quenching.[25−27] The triplet-excited state of **1** ($^3C_{60}$*−**1**) could therefore be quenched by the lone pair of electrons on its amine moiety prior to the occurrence of any energy transfer toward $^3O_2$, whereas the absence of such an amino group in **2** and **3** would prevent the occurrence of any such quenching event. To confirm this explanation of the experimental results, the level of $^1O_2$ generated by the **1**·γ-CDx complex was measured under low pH conditions (pH = 2.0) because **1** would be converted to the corresponding ammonium salt by protonation. As shown in Figure 4 (purple line), the validity of this explanation was confirmed experimentally because the level of $^1O_2$ generation significantly increased following the protonation of **1**.

In spite of its low level of photodynamic activity, the C_{70}·γ-CDx complex showed a high level of ADPA bleaching. Although this was a reasonable result, in that C_{70} has a higher level of absorbance over 610 nm than the other C_{60} derivatives, the result was in conflict with the photodynamic activity of the C_{70}·γ-CDx complex. We have already reported the incorporation of C_{70} into a cell membrane, with the material successfully acting as a photodynamic sensitizer for a cancer cell.[28] The experiments, however, were carried out in physiological saline (0.9 w/v% NaCl solution). Given that the C_{70}·γ-CDx complex was much more labile than the other fullerene·γ-CDx complexes, it was envisaged that the C_{70}·γ-CDx complex could decompose and release C_{70}, which could interfere with the level of uptake in the HeLa cells via its incorporation into several proteins, low density lipoprotein (LDL) and high density lipoprotein (HDL), in the fetal calf serum. In fact, only the C_{70}·γ-CDx complex exhibited a broadened absorption in the cell culture medium supplemented with 10% fetal calf serum, indicating that the C_{70} self-aggregated to be incorporated in the proteins and the LDL and HDL (Figure S2, Supporting Information).

To evaluate explanation (ii) mentioned previously, the intracellular uptakes of the γ-CDx complexes of **1**–**3** ([complex] = 4.0 μM) were quantified by flow cytometric analysis (FACS). As shown in Figure S3, Supporting Information, the γ-CDx complexes of **1**–**3** possessed similar fluorescence intensities when they were excited at the wavelength (λ_{ex} = 488 nm) used in the FACS. HeLa cells were incubated with 25 μM of γ-CDx complexes of **1**–**3** at 37 °C for 24 h. Following the incubation, the cellular uptakes of

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the γ-CDx complexes of **1**−**3** were analyzed on a FACScan flow cytometer. The histograms of fluorescence distribution were plotted as the number of cells versus fluorescence intensity on a logarithmic scale (Figure S4, Supporting Information). As shown in Figure S4, the cellular uptake of the cationic **2**·γ-CDx complex was greater than those of the neutral γ-CDx complexes of **1** and **3**, indicating that the intracellular uptake of the **2**·γ-CDx complex increased as a consequence of the electrostatic interactions between the anionic surface of the HeLa cells and the cationic **2**·γ-CDx complex.[29] This result was consistent with the higher levels of photodynamic activity observed for the **2**·γ-CDx complex. In contrast, there was no correlation between the cellular uptake efficiencies and the photodynamic activities of the γ-CDx-complexes of **1** and **3**. Instead, the difference in the level of photodynamic activity between the γ-CDx-complexes of **1** and **3** was related to their ability to generate $^1O_2$ because the ability of the **1**·γ-CDx complex to generate $^1O_2$ was significantly lower.

In summary, we have demonstrated that the γ-CDx complexes of **2** and **3** showed significantly higher levels of photodynamic activity than the γ-CDx complexes of C_{60}, C_{70}, and **1** under photoirradiation at long wavelengths (610−740 nm). In particular, the photodynamic activity of the **2**·γ-CDx complex was found to be the highest of the fullerene derivatives tested in the current letter and four times greater than that of photofrin. The high level of photodynamic activity observed for the **2**·γ-CDx complex was attributed both to its higher level of $^1O_2$ generation and its higher level of intracellular uptake. In contrast, the **1**·γ-CDx complex showed a low level of cytotoxicity, similar to the γ-CDx complexes of C_{60} and C_{70}, because of the quenching effect of the lone pair of electrons on the amino group. These findings therefore provide a significant level of information toward the optimization of the molecular design of fullerene derivatives for PDT. We aim to pursue the development of γ-CDx-complexed C_{60} derivatives with higher photodynamic activities in a continuation of this study.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimantal procedures, Table S1, and Figures S1−S5. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS

ROS, reactive oxygen species; CDx, cyclodextrin; PDT, photodynamic therapy; IC_{50}, half maximal inhibitory concentration; ADPA, 9,10-anthracenedipropionic acid disodium salt; FACS, flow cytometric analysis

■ REFERENCES

(1) Hamano, T.; Okuda, K.; Mashino, T.; Hirobe, M.; Arakane, K.; Ryu, A.; Mashikoc, S.; Nagano, T. Singlet oxygen production from fullerene derivatives: effect of sequential functionalization of the fullerene core. *Chem. Commun.* **1997**, 21−22.

(2) Andersson, T.; Nilsson, K.; Sundahl, M.; Westman, G.; Wennerström, O. C_{60} embedded in γ-cyclodextrin: a water-solublefullerene. *J. Chem. Soc., Chem. Commun.* **1992**, 604−606.

(3) Komatsu, K.; Fujiwara, K.; Murata, Y.; Braun, T. Aqueous solubilization of crystalline fullerenes by supramolecular complexation with γ-cyclodextrin and sulfocalix[8] arene under mechanochemical high-speed vibration milling. *J. Chem. Soc., Perkin Trans. 1* **1999**, 2963−2966.

(4) Yoshida, Z.; Takekuma, H.; Takekuma, S.; Matsubara, Y. Molecular recognition of C_{60} with γ-cyclodextrin. *Angew. Chem., Int. Ed.* **1994**, *33*, 1597−1599.

(5) Kuroda, Y.; Nozawa, H.; Ogoshi, H. Kinetic behaviors of solubilization of C_{60} into water by complexation with γ-cyclodextrin. *Chem. Lett.* **1995**, *24*, 47−48.

(6) Ikeda, A.; Genmoto, T.; Maekubo, N.; Kikuchi, J.; Akiyama, M.; Mochizuki, T.; Kotani, S.; Konishi, T. Water-soluble inclusion complexes of [60]fullerene derivatives using gamma-cyclodextrin. *Chem. Lett.* **2010**, *39*, 1256−1257.

(7) Ikeda, A.; Aono, R.; Maekubo, N.; Katao, S.; Kikuchi, J.; Akiyama, M. Pseudorotaxane structure of a fullerene derivative-cyclodextrin 1:2 complex. *Chem. Commun.* **2011**, *47*, 12795−12797.

(8) Ikeda, A.; Sato, T.; Kitamura, K.; Nishiguchi, K.; Sasaki, Y.; Kikuchi, J.; Ogawa, T.; Yogo, K.; Takeya, T. Efficient photocleavage of DNA utilising water-soluble lipid membrane-incorporated [60]-fullerenes prepared using a [60]fullerene exchange method. *Org. Biomol. Chem.* **2006**, *3*, 2907−2909.

(9) Ikeda, A.; Doi, Y.; Nishiguchi, K.; Kitamura, K.; Hashizume, M.; Kikuchi, J.; Yogo, K.; Ogawa, T.; Takeya, T. Induction of cell death by photodynamic therapy with water-soluble lipid-membrane-incorporated [60]fullerene. *Org. Biomol. Chem.* **2007**, *5*, 1158−1160.

(10) Doi, Y.; Ikeda, A.; Akiyama, M.; Nagano, M.; Shigematsu, T.; Ogawa, T.; Takeya, T.; Nagasaki, T. Intracellular uptake and photodynamic activity of water-soluble [60]- and [70]fullerenes incorporated in liposomes. *Chem.—Eur. J.* **2007**, *14*, 8892−8897.

(11) Ikeda, A.; Sue, T.; Akiyama, M.; Fujioka, K.; Shigematsu, T.; Doi, Y.; Kikuchi, J.; Konishi, T.; Nakajima, R. Preparation of highly photosensitizing liposomes with fullerene-doped lipid bilayer using dispersion-controllable molecular exchange reactions. *Org. Lett.* **2008**, *10*, 4077−4080.

(12) Ikeda, A.; Kawai, Y.; Kikuchi, J.; Akiyama, M. Effect of phase transition temperature of liposomes on preparation of fullerene-encapsulated liposomes by the fullerene-exchange reaction. *Chem. Commun.* **2010**, *46*, 2847−2849.

(13) Ikeda, A.; Akiyama, M.; Ogawa, T.; Takeya, T. Photodynamic activity of liposomal photosensitizers via energy transfer from antenna molecules to [60]fullerene. *ACS Med. Chem. Lett.* **2010**, *1*, 115−119.

(14) Ikeda, A.; Kawai, Y.; Kikuchi, J.; Akiyama, M.; Nakata, E.; Uto, Y.; Hori, H. Formation and regulation of fullerene-incorporation in liposomes under the phase transition temperature. *Org. Biomol. Chem.* **2011**, *9*, 2622−2627.

(15) Ikeda, A.; Kiguchi, K.; Shigematsu, T.; Nobusawa, K.; Kikuchi, J.; Akiyama, M. Location of [60]fullerene incorporation in lipid membranes. *Chem. Commun.* **2011**, *47*, 12095−12097.

(16) Ikeda, A.; Mori, M.; Kiguchi, K.; Yasuhara, K.; Kikuchi, J.; Nobusawa, K.; Akiyama, M.; Hashizume, M.; Ogawa, T.; Takeya, T. Advantages and potential of lipid-membrane-incorporating fullerenes

prepared by the fullerene-exchange method. *Chem.—Asian J.* **2012**, *7*, 605−613.
(17) Kato, S.; Kikuchi, R.; Aoshima, H.; Saitoh, Y.; Miwa, N. Defensive effects of fullerene-C_{60}/liposome complex against UVA-induced intracellular reactive oxygen species generation and cell death in human skin keratinocytes HaCaT, associated with intracellular uptake and extracellular excretion of fullerene-C_{60}. *J. Photochem. Photobiol., B* **2010**, *98*, 144−151.
(18) Yamakoshi, Y.; Umezawa, N.; Ryu, A.; Arakane, K.; Miyata, N.; Goda, Y.; Masumizu, T.; Nagano, T. Active oxygen species generated from photoexcited fullerene (C_{60}) as potential medicines: $O_2^{-\bullet}$ versus $^1O_2$. *J. Am. Chem. Soc.* **2003**, *125*, 12803−12809.
(19) Goldstein, S.; Czapski, G. Mannitol as an OH• scavenger in aqueous solutions and in biological systems. *Int. J. Radiol.* **1984**, *46*, 725−729.
(20) Lindig, B. A.; Rodgers, M. A. J. Rate parameters for the quenching of singlet oxygen by water-soluble and lipid-soluble substrates in aqueous and micellar systems. *Photochem. Photobiol.* **1981**, *33*, 627−634.
(21) To further confirm these results, the generation of $O_2^{\bullet-}$ was evaluated by chemical methods using nitroblue tetrazolium (NBT) as a detector.[18] The reduction of NBT with $O_2^{\bullet-}$ was not detected following the photoirradiation of the γ-CDx complexes of C_{60}, **1**, **2**, and **3**, although formazan was readily detected in the C_{60}·γ-CDx complex positive control samples in the presence of NADH (Figure S5, Supporting Information).[30] These results indicate that $O_2^{\bullet-}$ is not generated by these complexes under the photoirradiation conditions.
(22) Lindig, B. A.; Rodgers, M. A.; Schaap, A. P. Determination of the lifetime of singlet oxygen in D_2O using 9,10-anthracenedipropionic acid, a water-soluble probe. *J. Am. Chem. Soc.* **1980**, *102*, 5590−5593.
(23) Anderson, J. L.; An, Y.-Z.; Rubin, Y.; Foote, C. S. Photophysical characterization and singlet oxygen yield of a dihydrofullerene. *J. Am. Chem. Soc.* **1994**, *116*, 9763−9764.
(24) Prat, F.; Marti, C.; Nonell, S.; Zhang, X. J.; Foote, C. S.; Moreno, R. G.; Bourdelande, J. L.; Font, J. C_{60} fullerene-based materials as singlet oxygen $O_2(^1\Delta_g)$ photosensitizers: a time-resolved near-IR luminescence and optoacoustic study. *Phys. Chem. Chem. Phys.* **2001**, *3*, 1638−1643.
(25) Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; Sandanayake, K. R. A. S. Molecular fluorescent signaling with "fluor-spacer-receptor" systems: approaches to sensing and switching devices via supramolecular photophysics. *Chem. Soc. Rev.* **1992**, *21*, 187−195.
(26) Bissell, R. A.; de Silva, A. P.; Gunaratne, H. Q. N.; Lynch, P. L. M.; Maguire, G. E. M.; McCoy, C. P.; Sandanayake, K. R. A. S. Fluorescent PET (photoinduced electron transfer) sensors. *Top. Curr. Chem.* **1993**, *168*, 223−264.
(27) James, T. D.; Sandanayake, K. R. A. S.; Iguchi, R.; Shinkai, S. Novel saccharide-photoinduced electron-transfer sensors based on the interaction of boronic acid and amine. *J. Am. Chem. Soc.* **1995**, *117*, 8982−8987.
(28) Ikeda, A.; Matsumoto, M.; Akiyama, M.; Kikuchi, J.; Ogawa, T.; Takeya, T. Direct and short-time uptake of [70]fullerene into the cell membrane using an exchange reaction from a [70]fullerene-gamma-cyclodextrin complex and the resulting photodynamic activity. *Chem. Commun.* **2009**, 1547−1549.
(29) Miller, C. R.; Bondurant, B.; McLean, S. D.; McGovern, K. A.; O'Brien, D. F. Liposome-cell interactions in vitro: Effect of liposome surface charge on the binding and endocytosis of conventional and sterically stabilized liposomes. *Biochemistry* **1998**, *37*, 12875−12883.
(30) Nakanishi, I.; Fukuzumi, S.; Konishi, T.; Ohkubo, K.; Fujitsuka, M.; Ito, O.; Miyata, N. DNA cleavage via superoxide anion formed in photoinduced electron transfer from NADH to γ-cyclodextrin-bicapped C_{60} in an oxygen-saturated aqueous solution. *J. Phys. Chem. B* **2002**, *106*, 2372−2380.