Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

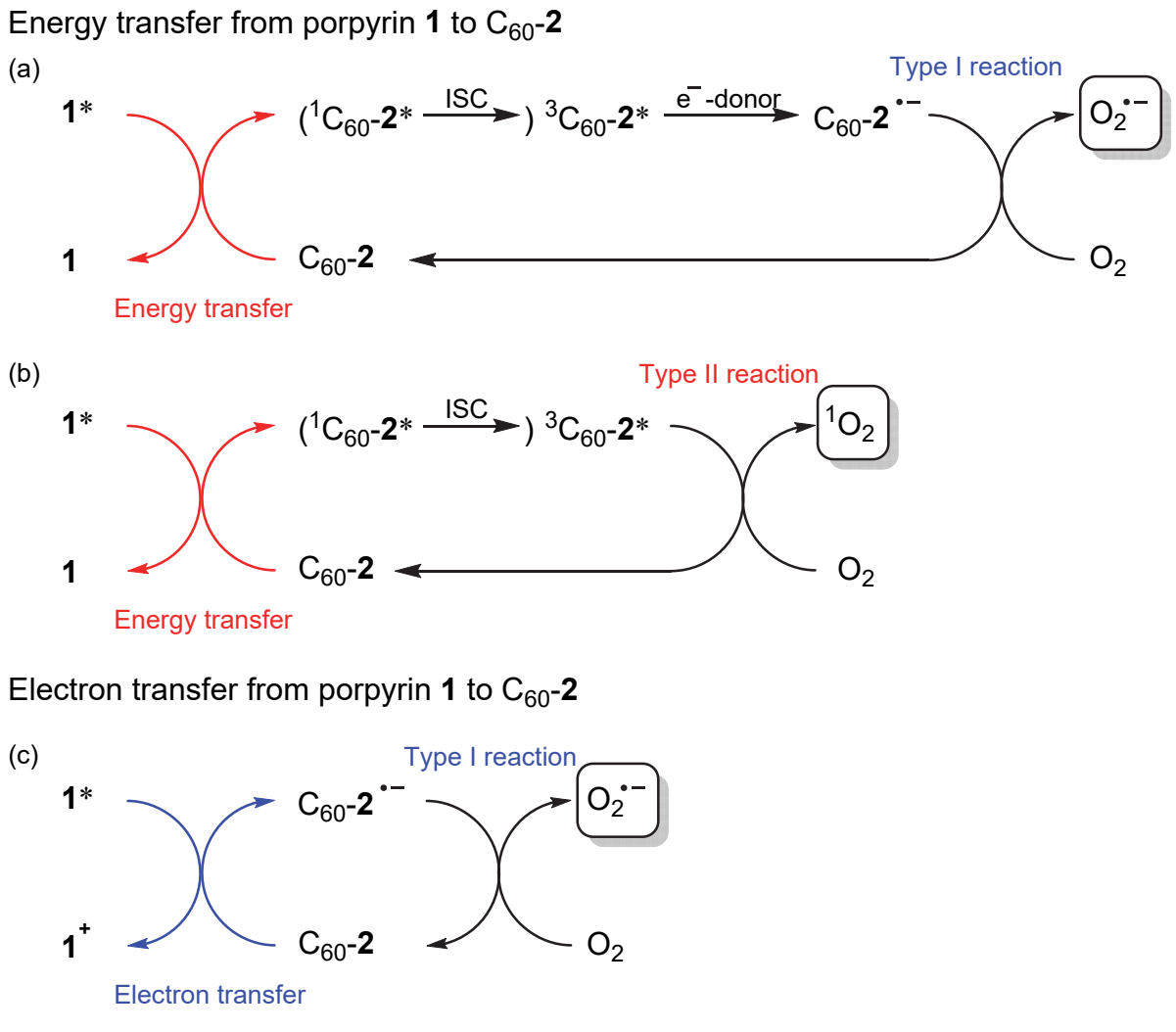
Supporting Information

Improvement in photodynamic activity by porphyrin–fullerene composite system in lipid membrane

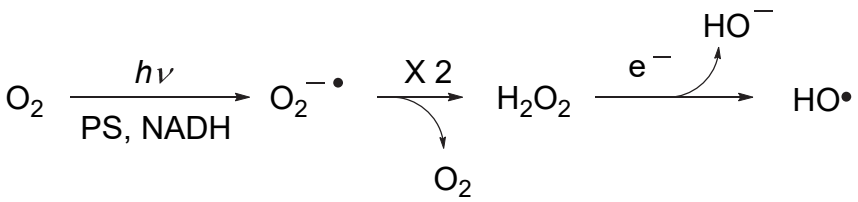
Kotaro Nishimura, Keita Yamana, Riku Kawasaki and Atsushi Ikeda *

Applied Chemistry Program, Graduate School of Advanced Science and Engineering, Hiroshima University, 1-4-1 Kagamiyama, Higashi-Hiroshima 739-8527, Japan

Scheme S1. Energy or electron transfer mechanism from photoactivated **1** to C_{60}-**2**. If the triplet excited state of C_{60}-**2** ($^3C_{60}$-**2***) is generated via energy transfer from photoactivated **1** to C_{60}-**2**, either $O_2^{\bullet-}$ or $^1O_2$ can be produced by a Type I or Type II reaction between C_{60}-**2** and dissolved oxygen. Conversely, if the radical anion of C_{60}-**2** (C_{60}-**2**$^{\bullet-}$) is generated via electron transfer from photoactivated **1** to C_{60}-**2**, only $O_2^{\bullet-}$ can be produced by a Type I reaction between C_{60}-**2**$^{\bullet-}$ and dissolved oxygen.

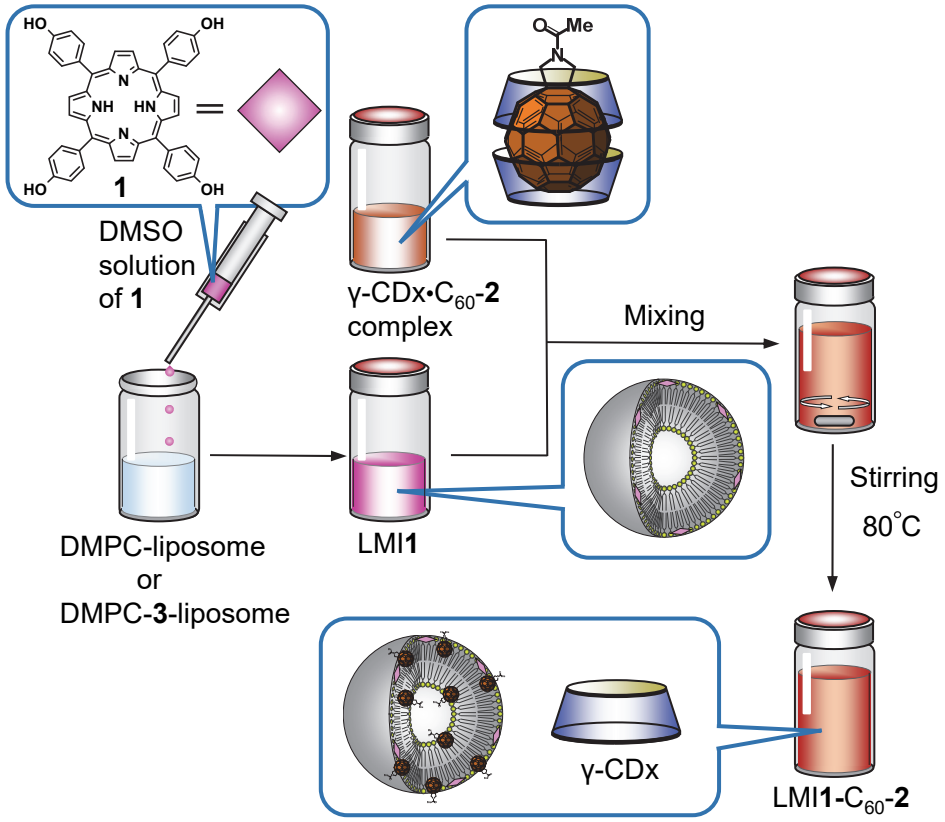


Scheme S2. Generation $O_2^{\bullet-}$ and then OH• from dissolved oxygen by photosensitizer[23]

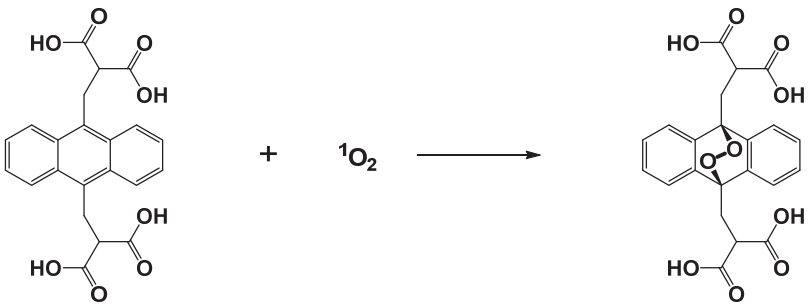


PS: Photosensitizer (C_{60} or porphyrin)

Scheme S3. Representation of the exchange method implemented for the preparation of LMI**1**–C_{60}-**2** (γ-CDx = γ-cyclodextrin; LMI = lipid membrane–incorporated).

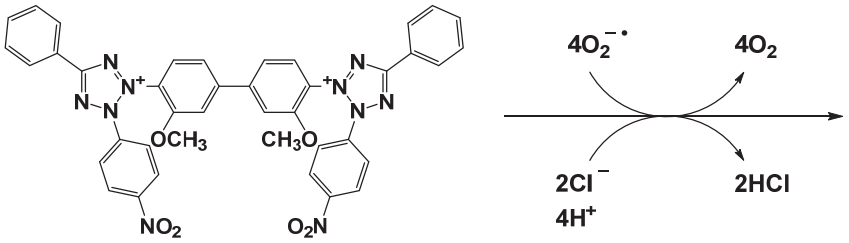


Scheme S4. Conversion of 9,10-anthracenediyl-bis(methylene)dimalonic acid to the corresponding endoperoxide as a result of reaction with singlet oxygen ($^1O_2$).



9,10-Anthracenediylbis(methylene)dimalonic acid

Scheme S5. Conversion of nitroblue tetrazolium to formazan as a result of reaction with superoxygen ($O_2^{\bullet -}$).



Nitroblue tetrazolium

Formazan

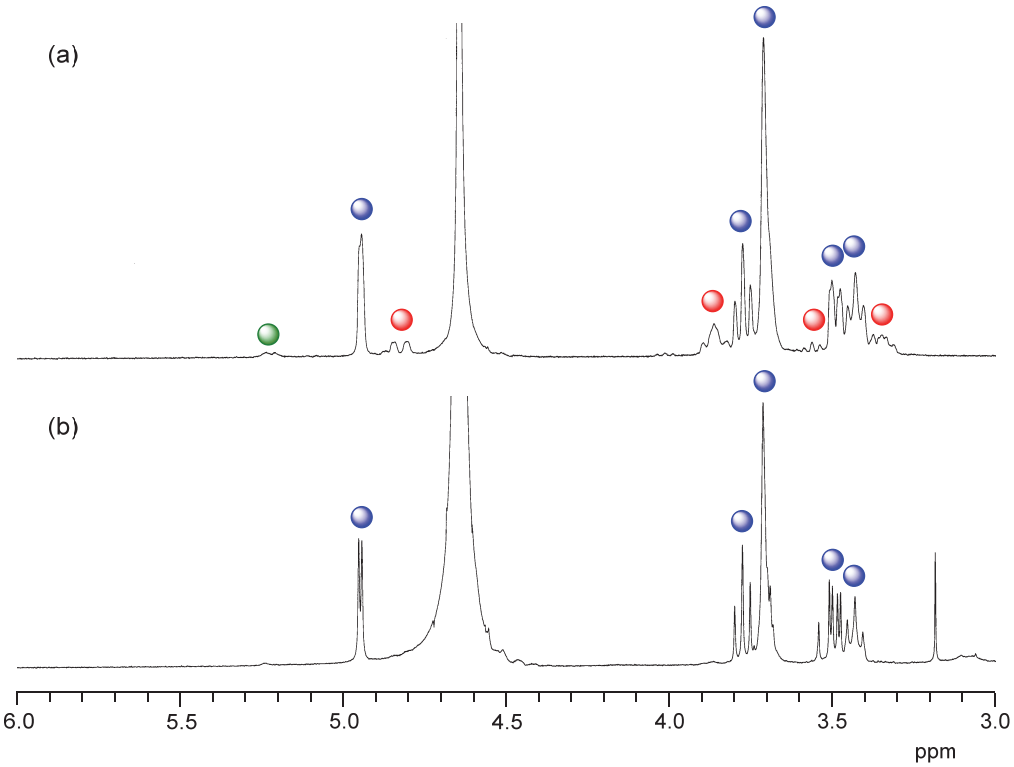


Fig. S1 Sections of the ^{1}H NMR spectra of the γ-cyclodextrin–C_{60}-**2** complex (a) before and (b) after the addition of the DMPC liposomes in D_2O. The blue circles indicate peaks due to free γ-cyclodextrin; the red circles indicate peaks due to γ-cyclodextrin in the γ-cyclodextrin–C_{60}-**2** complex; the green circle indicates the peak due to C_{60}-**2** in the γ-cyclodextrin–C_{60}-**2** complex. (a) [C_{60}-**2**] = 0.05 mM; [γ-cyclodextrin] = 0.7 mM; (b) [DMPC] = 1.0 mM; [C_{60}-**2**]/[DMPC] = 5.0 mol%; [DiD]/[DMPC] = 2.5 mol%.

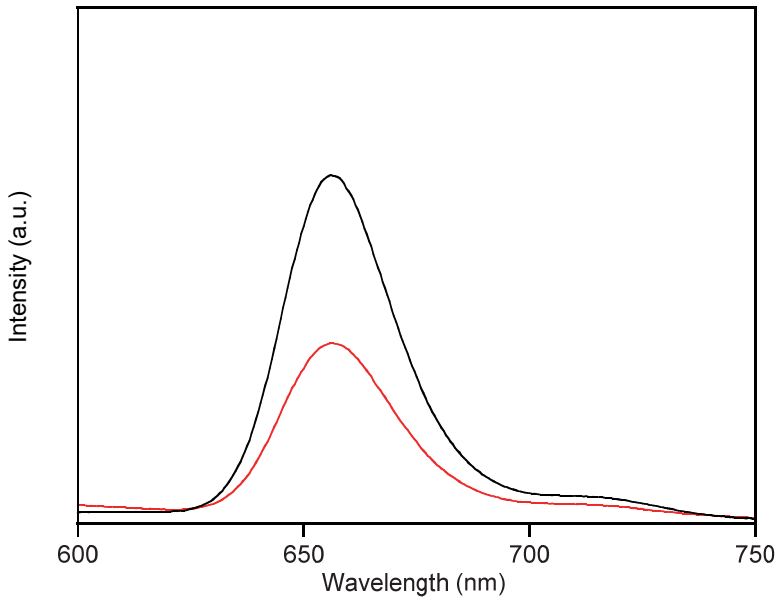


Fig. S2 Fluorescence spectra (λ_{ex}: 570 nm) of LMI**1** (black line) and LMI**1**–C_{60}-**2** (red line) ([DMPC] = 0.1 mM; [**1**]/[DMPC] = 2.5 mol%; [C_{60}-**2**]/[DMPC] = 5.0 mol%) in water.

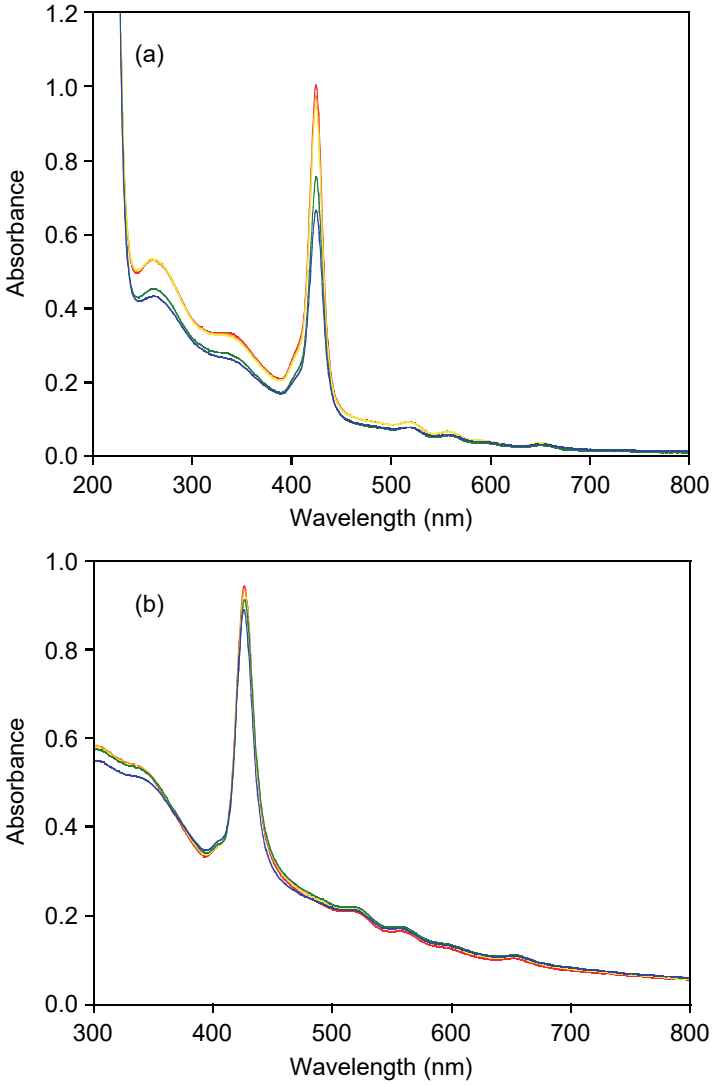


Fig. S3 UV–Vis absorption spectra of LMI**1**–C_{60}-**2** (a) in water after incubation times of 0 (red), 1 (orange), 3 (yellow), 5 (green), and 7 (blue) days and (b) in blood serum after incubation times of 0 (red), 2 (orange), 4 (yellow), 6 (green), and 24 (blue) hours at 37 °C using a 1-cm cell ([DMPC] = 0.1 mM; [**1**] = 2.5 μM; [C_{60}-**2**] = 5.0 μM).

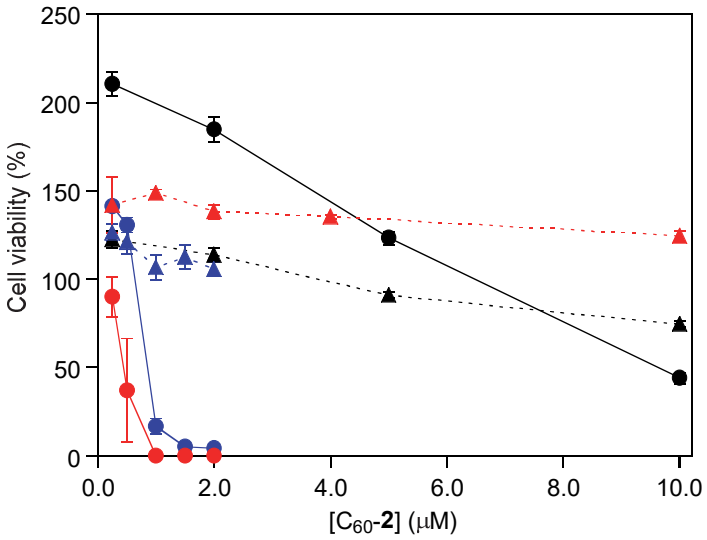


Fig. S4 Cell viability of Colon26 cells treated with LMIC$_{60}$-**2** (black), LMI**1**–C$_{60}$-**2** (red), and LMI**1**+LMIC$_{60}$-**2** mixture (blue) in the dark (triangles and dashed lines) and after photoirradiation at 610–740 nm for 30 min (circles and solid lines) at concentrations of C$_{60}$-**2** ranging from 0.25 to 10.0 μM. The cell viability was evaluated 24 h after treatment using the WST-8 method. The error bars represent the mean ± standard deviation for n = 3.

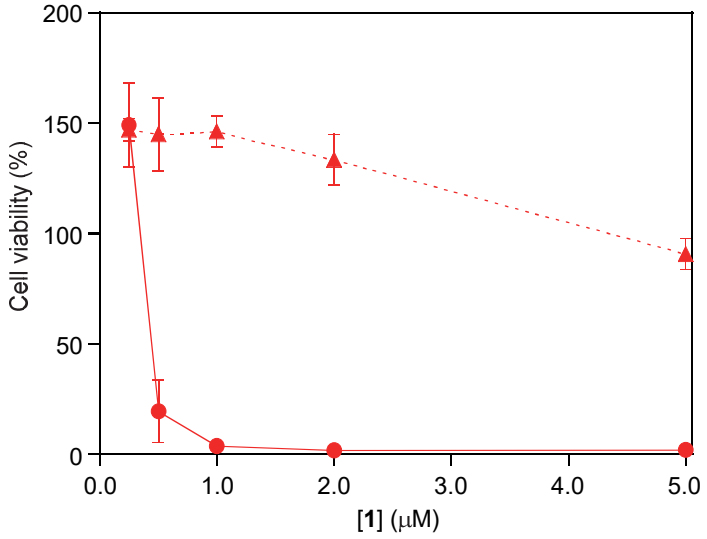


Fig. S5 Cell viability of L929 cells treated with LMI**1**–C$_{60}$-**2** in the dark (triangles and dashed lines) and after photoirradiation at 610–740 nm for 30 min (circles and solid lines) at concentrations of C$_{60}$-**2** ranging from 0.25 to 5.0 μM. The cell viability was evaluated 24 h after treatment using the WST-8 method. The error bars represent the mean ± standard deviation for n = 3.

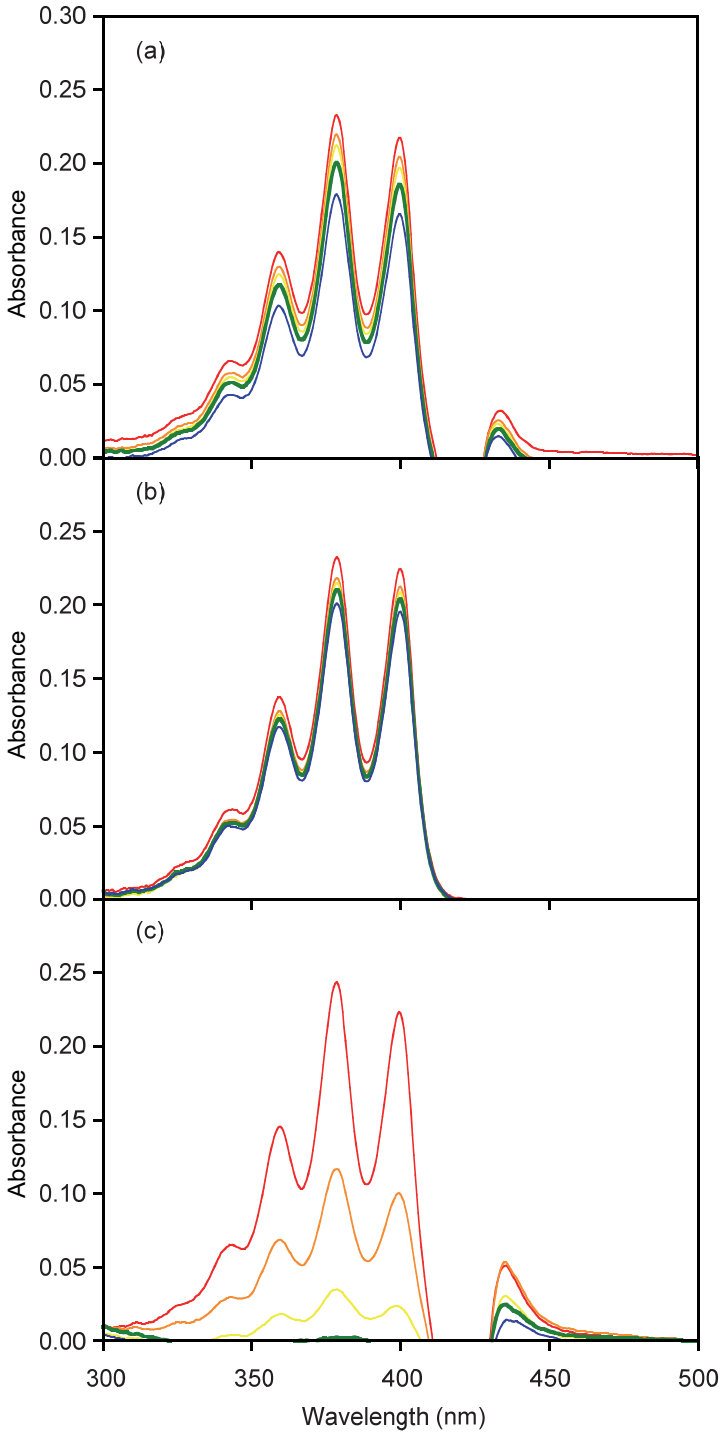


Fig. S6 Time-dependent bleaching of 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) caused by the generation of singlet oxygen by (a) LMI**1**, (b) LMIC_{60}-**2**, and (c) LMI**1**–C_{60}-**2**, upon photoirradiation (λ > 620 nm, 15 mW cm^{-2}) for 0 (red line), 7.5 (orange line), 15 (yellow line), 30 (green line), and 60 (blue line) min. A dimethyl sulfoxide solution of ABDA was injected into aqueous solutions of the liposomes. The absorption spectra were obtained by subtracting the absorption of LMI**1**, LMIC_{60}-**2**, and LMI**1**–C_{60}-**2**, respectively. [DMPC] = 0.1 mM, [**1**] = 0 or 2.5 μM, [C_{60}-**2**] = 0 or 5.0 μM, and [ABDA] = 25 μM; 1-cm cell. The experiments were conducted under an oxygen atmosphere at 25 °C.

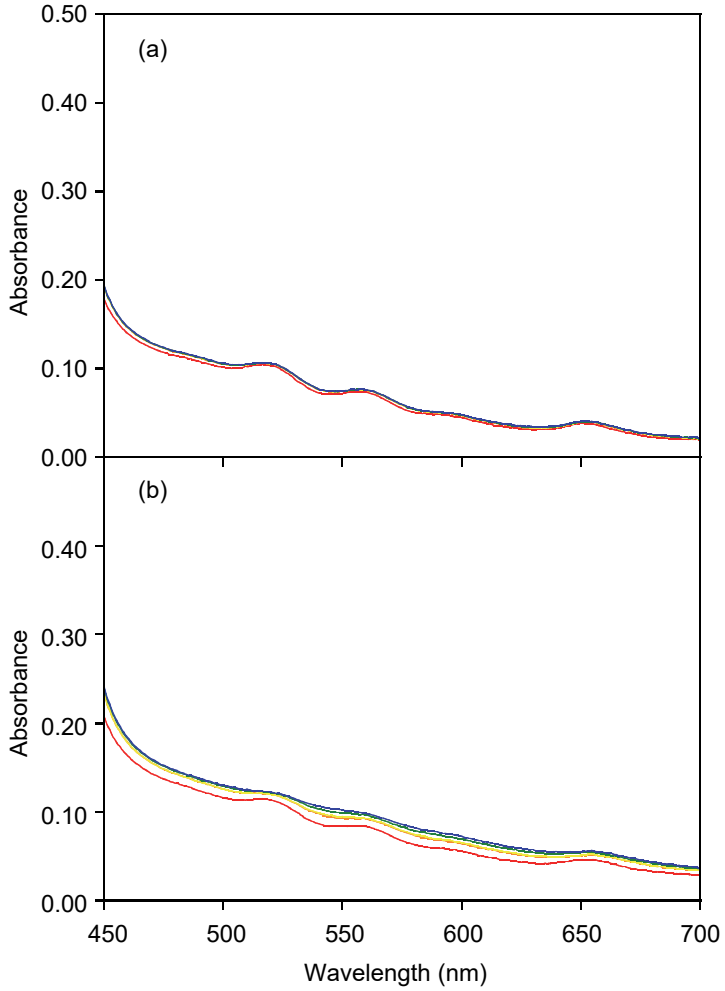


Fig. S7 Changes in the UV–Vis absorption of formazan generated by the reduction of NBT in the presence of LMI**1**–C_{60}-**2** in (a) the absence and (b) the presence of NADH, after photoirradiation at a wavelength greater than 620 nm for 0 (red line), 7.5 (orange line), 15 (yellow line), 30 (green line), and 60 (blue line) min. [DMPC] = 0.1 mM, [**1**] = 0 or 2.5 μM, [C_{60}-**2**] = 0 or 5.0 μM, [NBT] = 0.20 mM, and [NADH] = 0 or 0.50 mM; 1-cm cell. The experiments were conducted under an oxygen atmosphere at 25 °C.

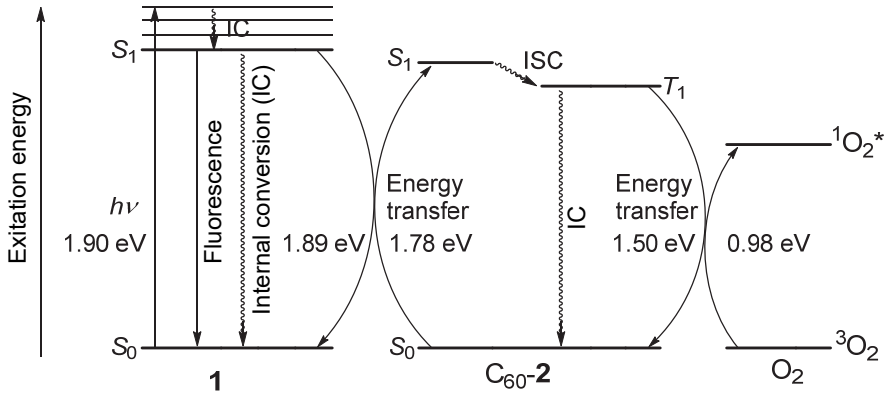


Fig. S8 Schematic illustrations of Jablonski diagrams in LMI**1**–C_{60}-**2** (The energy potential ($S_0 \rightarrow S_1$) of C_{60}-**2** was determined using the wavelength of maximum fluorescent emission of γ-cyclodextrin–C_{60}-**2** complex.[28] The energy potential ($T_1 \rightarrow S_0$) of C_{60}-**2** was determined from wavelength of maximum phosphorescence emission of *N*-methyl fulleropyrrolidine in methylcyclohexane, which was reported in Ref. S1 and S2, because the phosphorescence of C_{60}-**2** has not reported.).

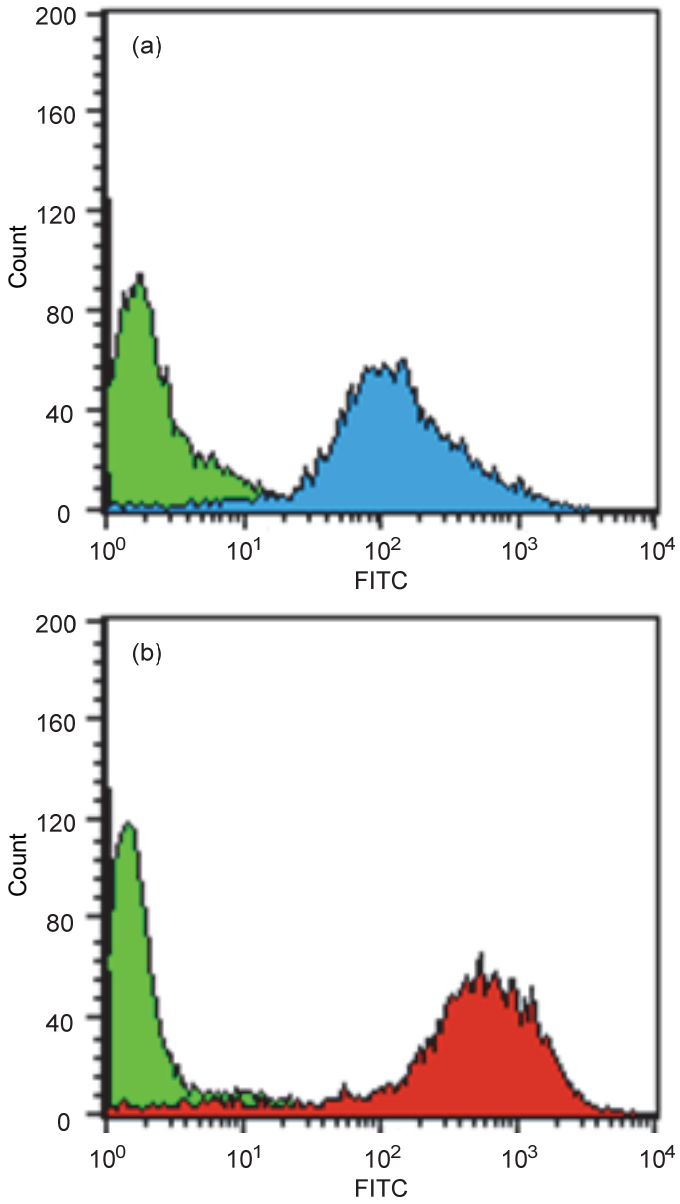


Fig. S9 Flow cytometry analysis for the detection of LMI**1**–C_{60}-**2** in (a) Colon26 (blue) and (b) HeLa (red) cells treated with LMI**1**–C_{60}-**2** ([DMPC] = 0.04 mM, [**1**] = 1.0 μM, [C_{60}-**2**] = 2.0 μM) and control (green; [DMPC] = [**1**] = [C_{60}-**2**] =0 mM).

References

S1 R. M. Williams, J. M. Zwier and J. W. Verhoeven, *J. Am. Chem. Soc.*, 1995, **117**, 4093–4099.

S2 C. Luo, M. Fujitsuka, A. Watanabe, O. Ito, L. Gan, Y. Huang and C.-H. Huang, *J. Chem. Soc., Faraday Trans.*, 1998, **94**, 527–532.