Supporting Information

Photodynamic Activities of Porphyrin Derivative–Cyclodextrin Complexes by Photoirradiation

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Experimental Section

Materials: Photofrin and 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA) were purchased from Takeda Pharmaceutical Co. Ltd. (Osaka, Japan) and Sigma-Aldrich (Milwaukee WI, USA), respectively. Compounds **1**, **3**, **4**, **6**, nitroblue tetrazolium (NBT), D-mannitol, and NADH were purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). TMe-β-CDx and L-histidine were purchased from Wako Pure Chemical Industries Ltd. (Tokyo, Japan). Compounds **2** and **5** were synthesised by methods described in previous literature.[S1,S2]

Preparation of the TMe-β-CyD-complexed with 1–6: Compounds **1**–**6** (5.00 mg) and TMe-β-CyD (23.3 mg, 1.63×10^{-5} mol) were placed in an agate capsule with two agate-mixing balls. The resulting mixture was vigorously agitated at 30 Hz for 20 min using a high-speed vibration mill (MM 200; Retsch Co., Ltd., Haan, Germany). The solid mixture was suspended in either pure water or D_2O (1.5 mL) to produce a dark purple emulsion. Subsequent centrifugation (18,000 × *g*, 25 °C, 20 min) allowed for the removal of non-dispersed **1**–**6** from the solution. The concentration of **1**–**6** in the TMe-β-CyD-complexed with **1**–**6** were determined to be 2.90, 4.40, 4.80, 3.72, 4.23, and 2.80 mM, from measurement of the absorbance of the solution at λ_{max} in water. The molar absorption coefficient for the water-soluble TMe-β-CyD-complexed with **1**–**6** were $\varepsilon_{416} = 3.30 \times 10^5$, $\varepsilon_{419} = 3.25 \times 10^5$, $\varepsilon_{425} = 2.70 \times 10^5$, $\varepsilon_{418} = 3.77 \times 10^5$, $\varepsilon_{418} = 3.49 \times 10^5$, and $\varepsilon_{415} = 2.43 \times 10^5$ $dm^3\ mol^{-1}\ cm^{-1}$, respectively.

Photodynamic Activity Experiments: HeLa cells were maintained in a CO_2-independent medium (Gibco BRL, Eggenstein, Germany), supplemented with 10% fetal calf serum at 37 °C in 5% CO_2. To determine the photodynamic activities of the TMe-β-CyD-complexed with **2**–**4**, the cells were seeded in 48-well culture plates at a density of 8.55×10^4 cells per well. The cells were left overnight, after which they were incubated with the TMe-β-CyD-complexed with **2**–**4** for 24 h in the absence of light. The cells then were washed with PBS and were exposed to light for 30 min at 25 °C. Light irradiation was performed using a xenon lamp (MAX-301, 300 W; Asahi Spectra Co., Ltd., Osaka, Japan) equipped with a VIS mirror module (385−740 nm) and a long-pass filter with a cut-off of 610 nm. The power of the light at the cellular level was 9 mW cm^{-2} (610–740 nm). To measure the viability of cells as a

percentage ratio relative to the cells that were not treated, a WST-8 assay was conducted 24 h after the photoirradiation process using a Cell Counting Kit-8 (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions.

Identification of Reactive Oxygen: Following their treatment with the TMe-β-CyD-complexed with **2**–**4** (0.4 μM), the cells were incubated with 50 mM L-histidine or D-mannitol for 2 h. After the incubation, the cells were exposed to light in the presence of the scavengers, and the effects of the scavengers on cell viability were determined. The detection of ROS generation using ABDA and NBT was conducted as previously described in the literature.[7,8] For the ABDA bleaching method, all of the samples were prepared using a pure water solution. ABDA was added to DMSO, to a concentration of 2.5 mM. The end concentrations of the TMe-β-CyD-complexed with **2**–**4** and ABDA in the mixed solutions were 15 μM and 25 μM, respectively. The NBT method was carried out at a concentration of 50 μM of the TMe-β-CyD-complexed with **2**–**4** and 0.2 mM of the NBT. For the positive control experiment, 0.5 mM NADH was added to the TMe-β-CyD-complexed with **2** solution. Oxygen was bubbled through all of the samples for 30 min, prior to photoirradiation to generate the necessary aerobic conditions. Photoirradiation was performed using a xenon lamp (SX-UID500X, 500 W; Ushio Inc., Tokyo, Japan) equipped with a long-pass filter with a cut-off at 620 nm. The light was cooled by passing it through a water filter. The power of the light at the sample was 16 mW cm^{-2} (over 620 nm).

Fluorescence Spectroscopy: Fluorescence spectra of the TMe-β-CyD-complexed with **2**, **3**, and **4** were obtained using an FluoroMax-4 spectrofluorometer (Horiba, Kyoto, Japan). An excitation wavelength of 540 nm was used with emission wavelengths in the range of 550–800 nm. Fluorescence quantum yields were determined by using a calibrated integrating sphere system.

Fluorescence Microscopy of HeLa Cells: After growing HeLa cells overnight, the cells were incubated with 0.4 μM TMe-β-CyD-complexed with **2**–**4**·at 37°C for 24 h. After the incubation, the cells were washed with PBS and the medium was replaced (DMEM). The cells and the introduction of the TMe-β-CyD-complexed with **2**–**4** was monitored by fluorescence

microscopy. The cells were observed using an Olympus IX71 epifluorescence microscope equipped with a 20 × objective lens. Fluorescent images were recorded using a Hamamatsu ImagEM EM-CCD camera (C9100-13) under irradiation of an excitation light beam from a mercury lamp through an optical filter (U-MNIBA2, Olympus).

References

S1 Smykalla, L.; Shukrynau, P.; Mende, C.; Ruffer, T.; Lang, H.; Hietschold, M. *Surf. Sci.* **2014**, 628, 92–97.

S2 Li, D.; Huang, K. K.; Hu, B.; Shi, Z.; Wang, G.; Feng, S. H. *J. Mol. Struct.* **2009**, 938, 82–88.

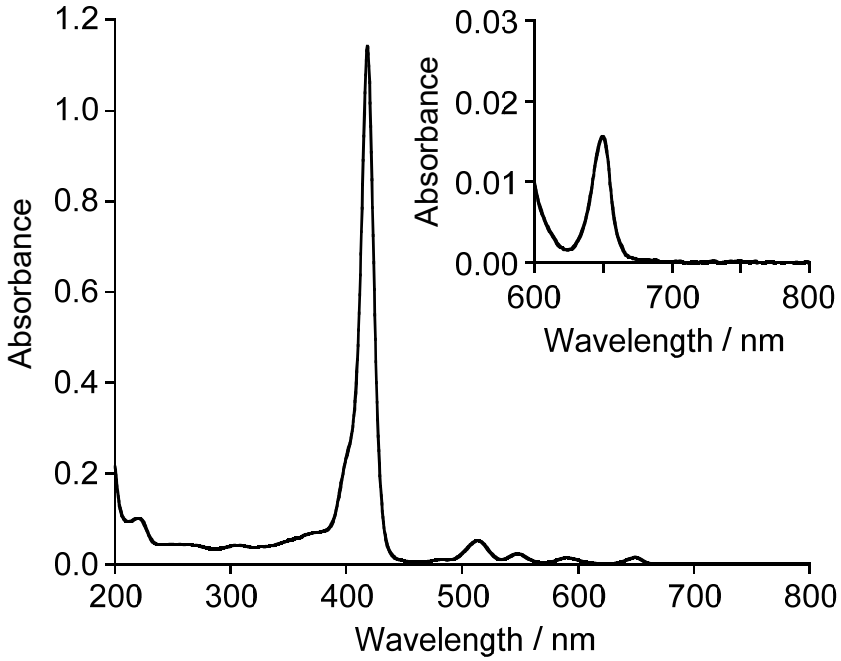

Figure S1. UV-vis absorption spectra of the TMe-β-CyD-complexed with **5** ([TMe-β-CyD-complexed with **5**] = 30 μM). All of absorption spectra were measured in H_2O at 25 °C (1 mm cell).

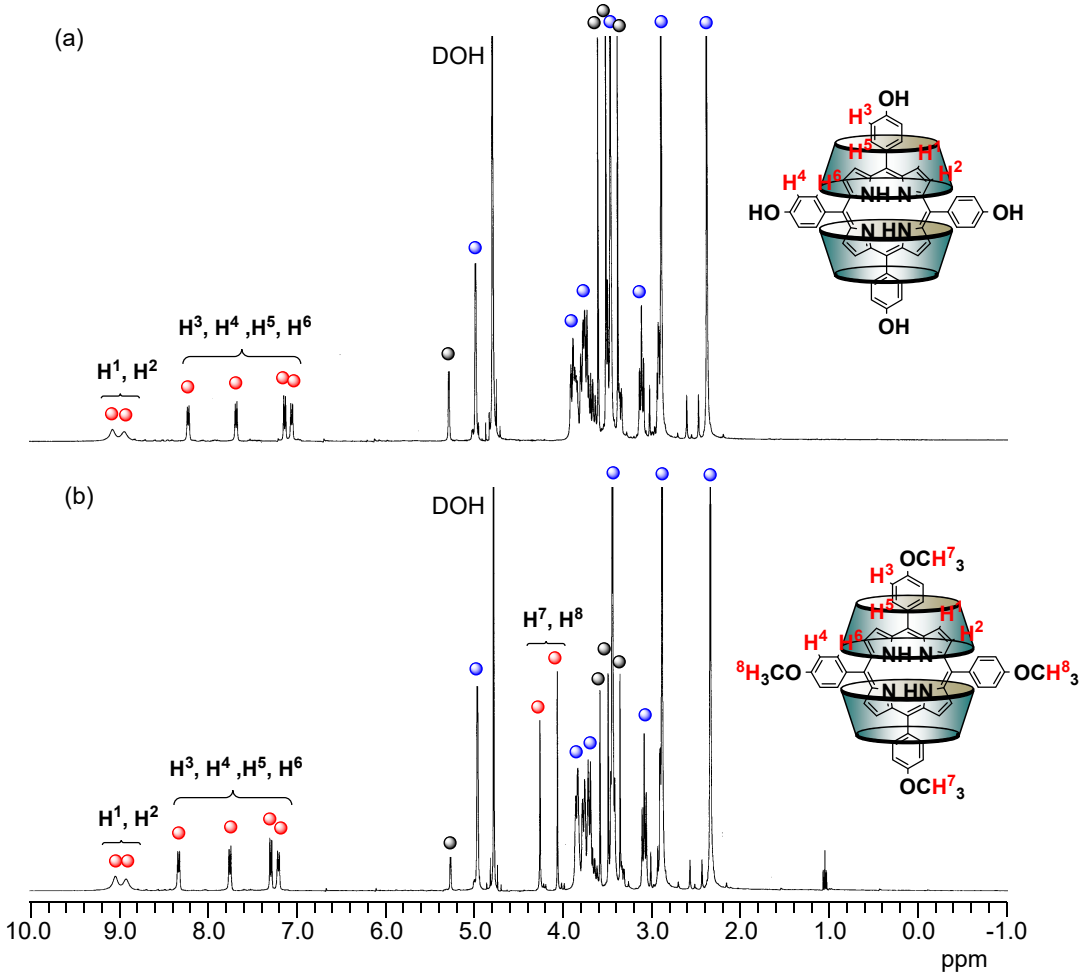

Figure S2. Complete ^{1}H NMR spectra of the TMe-β-CyD-complexed with (a) **2** and (b) **5** in D_2O at 25 °C (●: free TMe-β-CyD, ●: **2** or **5**, and ●: TMe-β-CyD in the TMe-β-CyD-complexed with **2** or **5**).

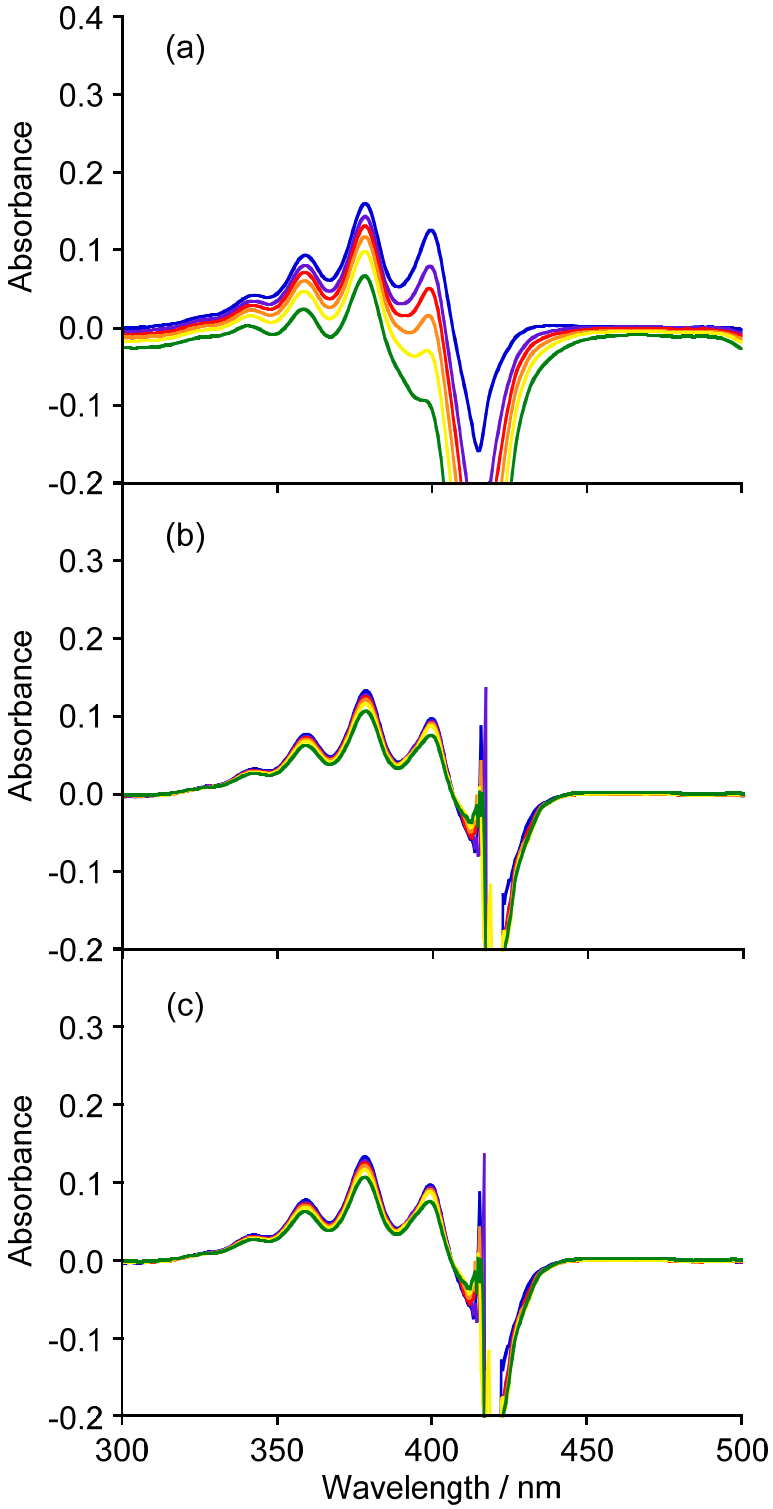

Figure S3. UV-vis absorption spectral change of ABDA in the presence of the TMe-β-CyD-complexed with (a) **1**, (b) **2**, and (c) **3** after photoirradiation at a wavelength greater than 620 nm for 0 (blue line), 7.5 (purple line), 15 (red line), 30 (orange line), 60 (yellow line), and 120 (green line) min. Absorption spectra were obtained by subtracting the absorption spectra of the TMe-β-CyD-complexed with **1**, **2**, or **3** and were measured at 25 °C (1 cm cell).

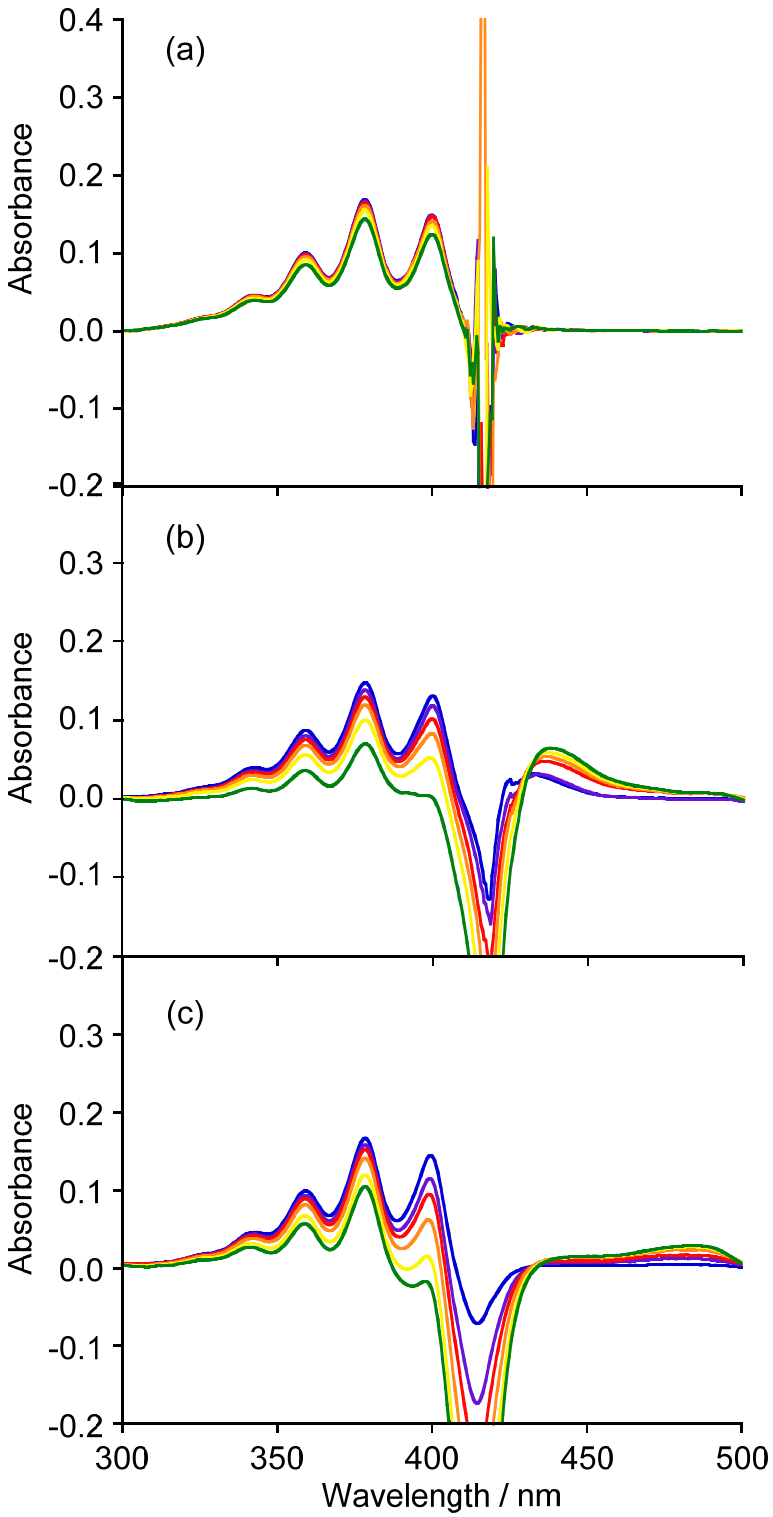

Figure S4. UV-vis absorption spectral change of ABDA in the presence of the the TMe-β-CyD-complexed with (a) **4**, (b) **5**, and (c) **6** after photoirradiation at a wavelength greater than 620 nm for 0 (blue line), 7.5 (purple line), 15 (red line), 30 (orange line), 60 (yellow line), and 120 (green line) min. These absorption spectra were obtained by subtracting the absorption spectra of the TMe-β-CyD-complexed with **4**, **5**, or **6** and were measured at 25 °C (1 cm cell).

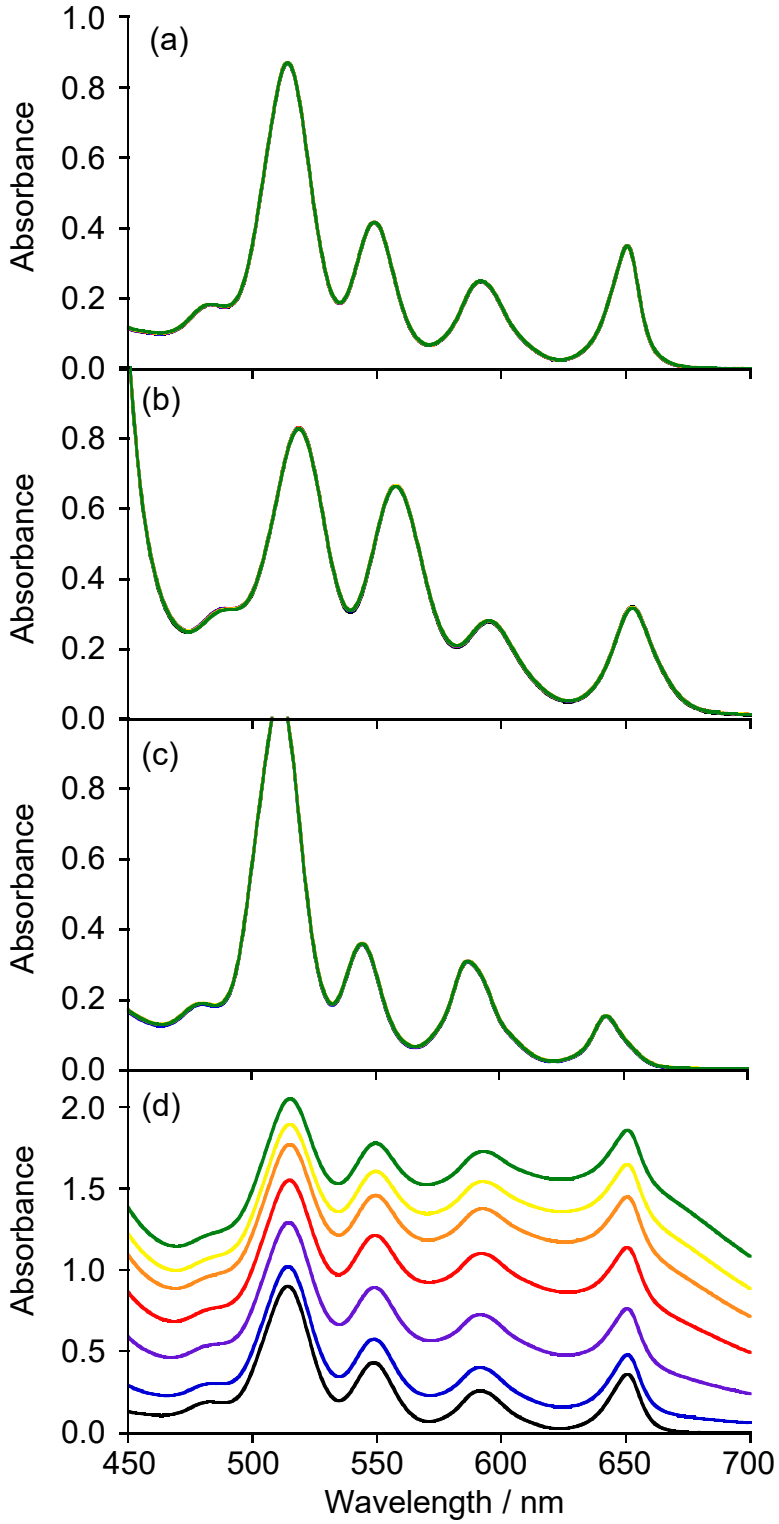

Figure S5. UV-vis absorption change of formazan generated by the reduction of NBT in the presence of the TMe-β-CyD-complexed with (a) **2**, (b) **3**, and (c) **4** and (d) the the TMe-β-CyD-complexed with **3** with NADH, after photoirradiation at a wavelength greater than 620 nm for 0 (black line), 10 (blue line), 20 (purple line), 30 (red line), 40 (orange line), 50 (yellow line), and 60 (green line) min. ([TMe-β-CyD-complexed with **2**–**4**] = 50 μM, [NBT] = 0.20 mM, [NADH] = 0 or 0.50 mM; 1 cm cell).

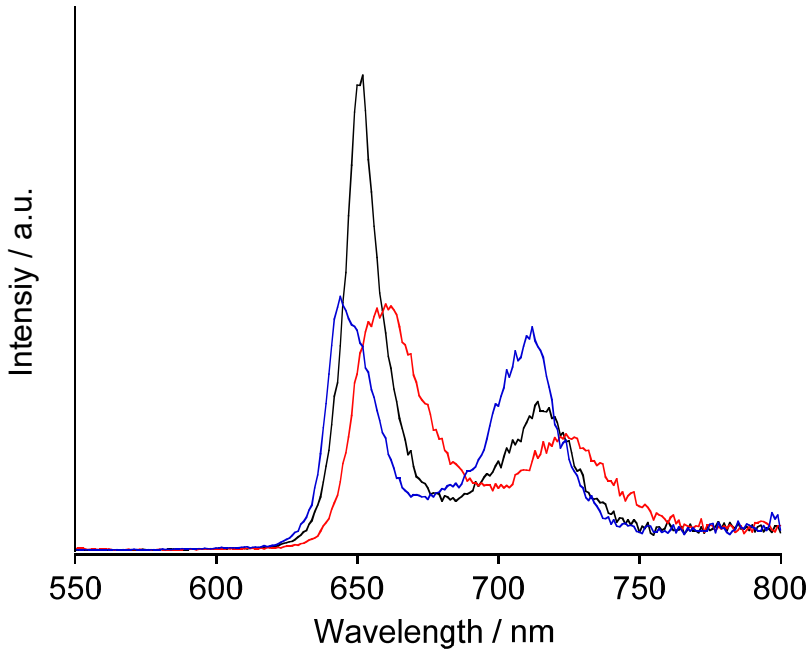

Figure S6. Fluorescence spectra (λ_{ex} = 540 nm) of the TMe-β-CyD-complexed with **2** (black line), **3** (red line), and **4** (blue line) (30 μM).

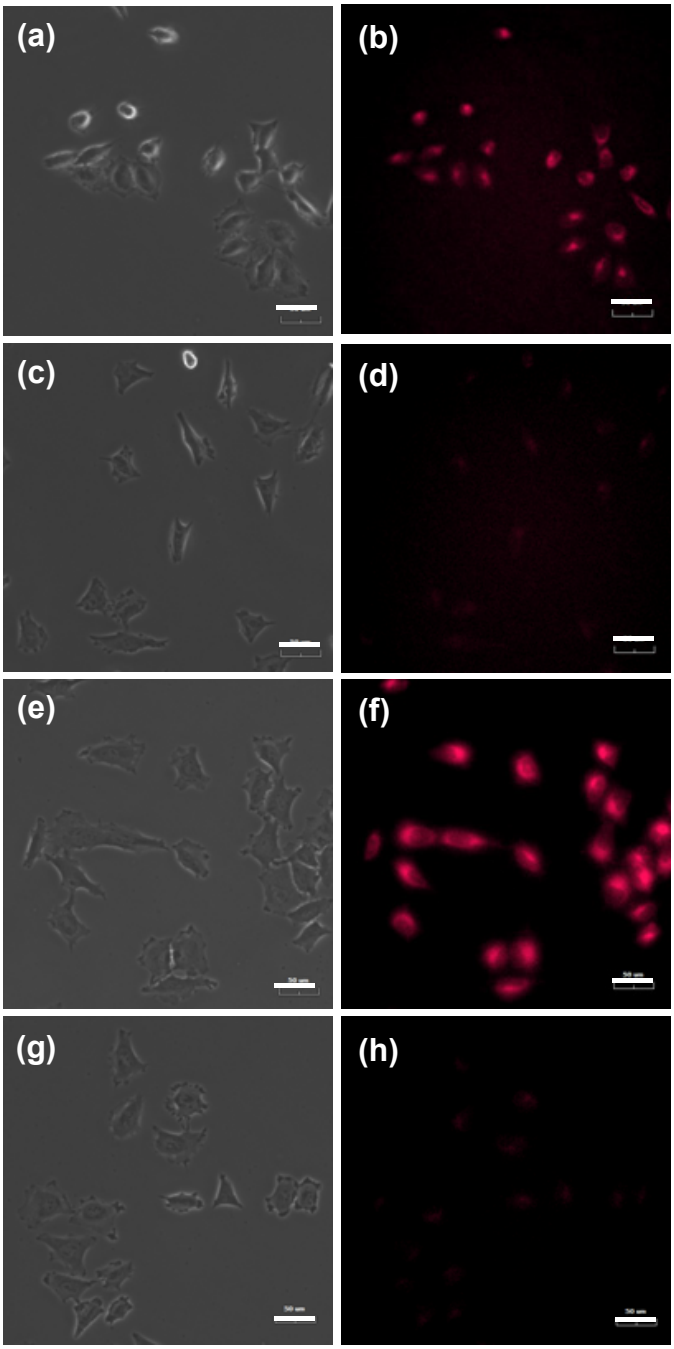

Figure S7. Inhibition of liposome uptake by low temperature. HeLa cells were incubated with the TMe-β-CyD-complexed with (a–d) **2**, or (e–h) **3**, for 30 min at 37 °C (a, b, e, and f) or 4 °C (c, d, g, and h). Fluorescence images were obtained by using laser scanning microscopy. Phase contrast (a, c, e, and g) and fluorescence (b, d, f, and h) images. The scale bar represents 50 μm.