

Improved photodynamic activities of liposome-incorporated [60]fullerene derivatives bearing a polar group†

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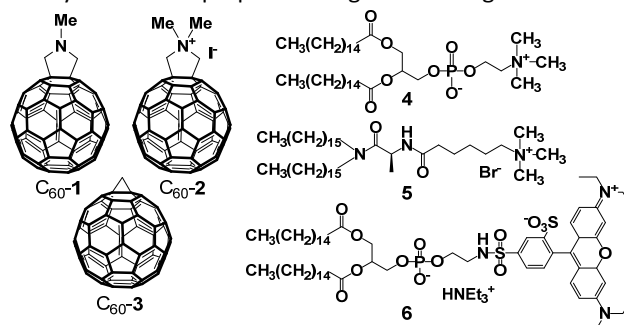
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[60]Fullerene (C₆₀) derivatives were incorporated into liposomes using a fullerene exchange method involving the transfer of the fullerene from the cavity of two γ -cyclodextrin molecules to a liposome. A lipid-membrane-incorporated C₆₀ derivative bearing a polar group showed much higher photodynamic activity than the analogous system incorporating pristine C₆₀.

Photodynamic therapy (PDT) is a promising approach for the treatment of malignant tumours and macular degeneration.¹ The photosensitisers (PSs) used in PDT are activated by light irradiation and transfer their energy to nearby oxygen molecules (³O₂) to form singlet oxygen (¹O₂), which results in irreversible damage to the tumour cells.^{1,2} Fullerenes³ and their derivatives^{4,5} are efficient visible light triplet-sensitisers and exhibit high photoproduction ability for ¹O₂.⁶ In particular, the *N,N*-dimethylpyrrolidinium derivatives of [60]fullerenes (C₆₀) complexed with γ -cyclodextrins (γ -CDxs) have higher photodynamic activities than pristine C₆₀, as well as several other C₆₀ derivatives and photofrin, which is commonly used in clinical practice for PDT.⁷ The incorporation of the C₆₀ derivatives into the lipid membranes (LMIC₆₀ derivatives) would lead to the formation of LMIC₆₀ derivatives with high photodynamic activities. However, lipid-membrane-incorporated guest molecules can be difficult to prepare using guest molecules with a high dipole moment, because some of the guest molecules precipitate from solution or leak into the bulk water.⁸ In an attempt to solve these problems, it was envisaged that lipid membrane-incorporated C₆₀ derivatives (LMIC₆₀-derivatives) could be prepared based on the exchange

reaction of a functionalised C₆₀ from a γ -CDx•C₆₀ derivative complex⁹ to a liposome, which we defined as the 'exchange method' (Scheme S1).^{3a,c} LMIC₆₀-derivatives would be expected to have high photodynamic activities because of the high photodynamic activities of the γ -CDx•C₆₀ derivative complexes. Furthermore, the results of molecular dynamics simulations of fullerenes inside the lipid bilayer have shown that the C₆₀ molecules exist between the two membranes of the liposomes.¹⁰ We have confirmed these results experimentally.¹¹ These reports suggest that ³O₂ molecules have to migrate into the membranes to come into contact with photoexcited fullerenes before being converted to ¹O₂ through an energy transfer process. The introduction of a C₆₀ derivative in close proximity to the surface of the liposome should therefore lead to an increase in the photodynamic activity by enhancing the likelihood of there being contact between ³O₂ and the photoactivated C₆₀ derivative. In this paper, we describe the preparation of three lipid membrane-incorporated C₆₀ systems, including pyrrolidine, *N,N*-dimethylpyrrolidinium iodide and methylene derivatives of C₆₀ (LMIC₆₀-1–C₆₀-3).¹² All three of these systems were prepared using the exchange method and



their biological activities were evaluated under visible light irradiation. The results revealed that LMIC₆₀-2 showed a dramatic improvement in photodynamic activity compared with pristine C₆₀.

LMIC₆₀-1–C₆₀-3 were prepared using a fullerene exchange reaction between the liposomes and the γ -CDx•C₆₀-1, C₆₀-2 and C₆₀-3 complexes⁹ by heating at 80°C for 1 h, as described in our

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previous report (Scheme S1a).^{3c} The size distributions of the liposomes were studied using dynamic light scattering (DLS). Table S1 shows that the hydrodynamic diameters (D_{hy}) changed from 110–145 nm before the exchange reactions to 105, 138 and 112 nm for LMIC₆₀-1, C₆₀-2 and C₆₀-3, respectively, indicating that the incorporation of the fullerene derivatives had very little impact on the size of the liposomes. The ¹H NMR peaks assigned to the γ -CDx•C₆₀-1, C₆₀-2, and C₆₀-3 complexes disappeared completely after the C₆₀ derivative-exchange reactions (Figs. 1, S1 and S2). These results indicated that all of the C₆₀-1, C₆₀-2 and C₆₀-3 molecules had been released from the γ -CDx cavities in the presence of the liposomes. Furthermore, it is well known that all of the peaks belonging to the guest molecules and the lipids disappear completely in these systems as a consequence of peak broadening following the formation of the liposomes. The disappearance of the peaks belonging to the C₆₀-1 and C₆₀-2 molecules in the current study therefore suggests that all of these molecules were completely incorporated into the liposomes (Fig. 1, blue circles). In contrast, the rapid mixing of an aqueous solution of the γ -CDx•C₆₀-2 complex with a solution of liposomes solution resulted in a new peak (5.4 ppm). This peak was attributed to a self-aggregate of C₆₀-2 coated with γ -CDx, which was similar to that of the self-aggregated γ -CDx•C₆₀ complex obtained by heating (5.41 ppm) (Fig. S2d).^{3a} This result indicated that part of the C₆₀-2 molecule self-aggregated outside the liposome. We therefore investigated the dropwise addition of an aqueous solution of γ -CDx•C₆₀-2 complex to an aqueous solution of liposomes (Scheme S1b). ¹H NMR showed that the peak at 5.4 ppm associated with the self-aggregation of C₆₀-2 did not appear (Fig. S2e), indicating that almost all of the C₆₀-2 molecules had been successfully incorporated into the lipid membranes, resulting in the formation of LMIC₆₀-2.

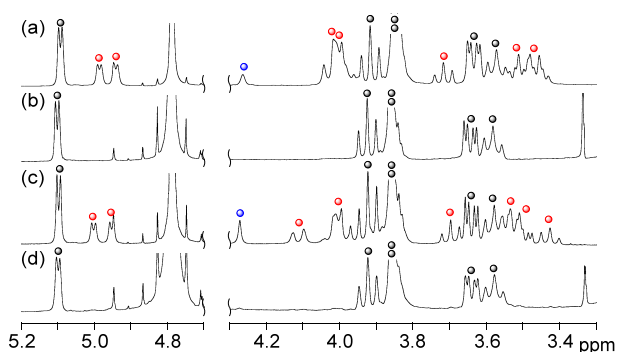


Fig. 1 Partial ¹H NMR spectra of the γ -CDx•C₆₀-1 complex (a) before and (b) after the addition of liposome-4-5 and γ -CDx•C₆₀-2 complex (c) before and (d) after the addition of liposome-4-5 (●: free γ -CDx, ●: γ -CDx in the γ -CDx•C₆₀ derivative complex, ●: C₆₀ derivative in the γ -CDx•C₆₀ derivative complex, [C₆₀-1 or C₆₀-2]/[4 + 5] = 5 mol%, [4 + 5] = 1.0 mM).

The UV-vis absorption spectra of LMIC₆₀-1–C₆₀-3 exhibited broad absorption bands in the range of 200–600 nm (Figs. 2a and S3), which were absent from their cyclodextrin complexes, indicating that C₆₀-1, C₆₀-2 and C₆₀-3 formed self-aggregates in the lipid membranes. These results were similar to that of the LMIC₆₀ material prepared by the exchange method at 80 °C.

However, because the spectrum of LMIC₆₀-2 contained sharper peaks than those of LMIC₆₀-1 and LMIC₆₀-3, this result suggested that C₆₀-2 self-aggregated to a much lesser extent than C₆₀-1 or C₆₀-3. This difference was mainly attributed to electrostatic repulsion between the cationic C₆₀-2 molecules.

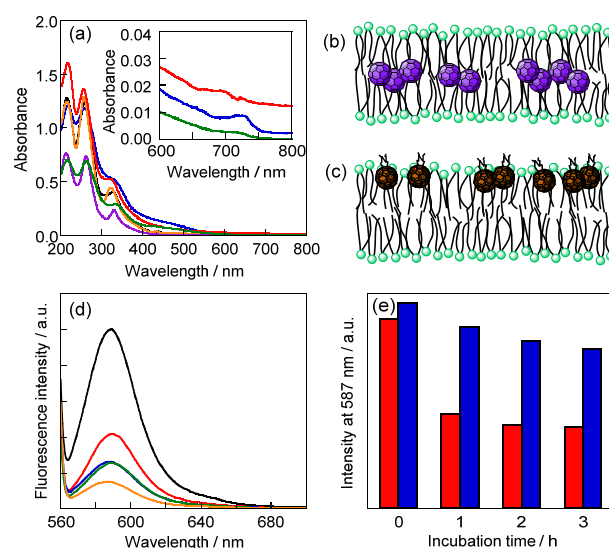


Fig. 2 (a) UV-vis absorption spectra of the γ -CDx•C₆₀-1 (black line), γ -CDx•C₆₀-2 (orange line) and γ -CDx•C₆₀-3 (purple line) complexes and LMIC₆₀-1 (blue line), LMIC₆₀-2 (red line) and LMIC₆₀-3 (green line) after the exchange reaction. The inset shows the 600–800 nm regions of LMIC₆₀-1 (blue line), LMIC₆₀-2 (red line) and LMIC₆₀-3 (green line) ([C₆₀-1, C₆₀-2 or C₆₀-3]/[4 + 5] = 5 mol%, [4 + 5] = 2.0 mM). All of these spectra were recorded at 25 °C with a 1 mm cell. Schematic illustrations of (b) C₆₀ and (c) C₆₀-2 in the lipid membranes. (d) Fluorescence spectra (λ_{ex} 550 nm) of liposome-4-5-6 (black line) and RhB-LMIC₆₀ (red line), RhB-LMIC₆₀-1 (blue line), RhB-LMIC₆₀-2 (orange line) and RhB-LMIC₆₀-3 (green line) consisting of liposome-4-5-6. ([6]/[4 + 5] = 0.25 mol%, [5]/[4 + 5] = 10 mol%, [C₆₀, C₆₀-1, C₆₀-2 or C₆₀-3]/[4 + 5] = 5 mol%). (e) Incubation time-dependent fluorescence intensities at 587 nm (λ_{ex} 550 nm) of liposome-4-6 and an RhB-LMIC₆₀-2 mixture consisting of liposome-4 (red bars) and liposome-4-5-6 and RhB-LMIC₆₀-2 (blue bars) mixture consisting of liposome-4-5, respectively. [6]/[4 or 4 + 5] = 0.25 mol%, [C₆₀-2]/[4 or 4 + 5] = 5 mol%, [5]/[4 + 5] = 10 mol%.

We have shown experimentally that C₆₀ exists between the two membranes of liposomes (Fig. 2b).¹¹ Furthermore, these findings were consistent with the results predicted by molecular dynamics simulations for the fullerenes inside the lipid bilayer (Fig. 2b).¹⁰ In contrast, molecular dynamics simulations for C₆₀ derivative bearing hydrophilic moieties on a half sphere revealed that this material most likely exists on the hydrophilic surface of the liposome (Fig. 2c).^{10b} It was therefore envisaged that a large number of ¹O₂ would be produced by an effective energy transfer process from the photo-excited C₆₀-2 molecules to the dissolved oxygen molecules because of the increasing number of collisions. To directly confirm the location of these fullerenes, we measured fluorescence quenching effects of rhodamine B-dipalmitoyl phosphatidylethanolamine (6) on the fullerenes in the liposome of 4 containing 0.25 mol% 6 (RhB-LMIC₆₀, RhB-LMIC₆₀-1, RhB-LMIC₆₀-2 and RhB-LMIC₆₀-3) (Fig. 2d). RhB-LMIC₆₀, RhB-LMIC₆₀-1, RhB-LMIC₆₀-2 and RhB-LMIC₆₀-3 led to fluorescence quenching activities of 58, 74, 85 and 75%, respectively, indicating that C₆₀-2 with a higher hydrophilicity was positioned much closer to the rhodamine moiety than C₆₀,

C₆₀-1 or C₆₀-3 (i.e., C₆₀-2 exists in close proximity to the head groups of the lipids).

We wanted to confirm whether C₆₀-2 was released from the lipid membranes because of the hydrophilicity of C₆₀-2 (Scheme S2a). C₆₀-2 was found to be insoluble in water in the absence of a polar organic solvent such as DMSO.¹³ As shown in Fig. 2d, the fluorescence of RhB-LMIC₆₀-2 was considerably quenched. Therefore, if C₆₀-2 was released from the lipid membranes of LMIC₆₀-2 to move in amongst liposome-4-6, the fluorescence characteristics of liposome-4-6 would be quenched after mixing with the LMIC₆₀-2 solution because C₆₀-2 molecules released during this process would move into the lipid membranes of liposome-4-6. The fluorescence of 6 decreased slowly after the mixing of the LMIC₆₀-2 with solutions of liposome-4-6 solutions (Fig. 2e, red bars). However, this decrease implied that (i) the C₆₀-2 molecules were migrating into the lipid membrane of liposome-4-6 (Scheme S2a); and (ii) the membrane fusion and fission processes were being repeated between LMIC₆₀-2 and liposome-4-6 (Scheme S2b). In an attempt to suppress the fusion and fission processes using electrostatic repulsion, we added the cationic lipid 5 ([5]/[4 + 5] = 10 mol%) to the lipid membrane of LMIC₆₀-2 and liposome-4-6. This process inhibited the quenching (Fig. 2e, blue bars), which suggested that most of the C₆₀-2 molecules had not been transferred from LMIC₆₀-2 to liposome-4-5-6 (i.e., very little C₆₀-2 had been released from the lipid membranes) (Scheme S2c).

The photodynamic activity of LMIC₆₀-1-C₆₀-3 was evaluated in HeLa cells using a cationic lipid 5 ([5]/[4 + 5] = 10 mol%).¹⁴ A cationic lipid was selected for this experiment because LMIC₆₀ bearing a cationic liposome surface efficiently induced cell death under photoirradiation, whereas LMIC₆₀ systems bearing a neutral or anionic liposome surface had no discernible effect.³ LMIC₆₀ and photofrin were used as control samples. After incubation with each LMIfullerene ([fullerene]/[lipids] = 5 mol%), the cells were exposed to light at a wavelength in the range of 610–740 nm. No cytotoxicity was observed when all of the LMIfullerenes were added to the cells without light exposure (Fig. 3a). Furthermore, no photodynamic activity was observed when LMIC₆₀, LMIC₆₀-1 or LMIC₆₀-3 was used in combination with light irradiation for wavelengths in the range of 610–740 nm (Fig. 3b, black, blue and green lines). In contrast, the photodynamic activity of LMIC₆₀-2 was much higher than those of LMIC₆₀, LMIC₆₀-1 and LMIC₆₀-3 (Fig. 3b, red line). We determined that the medium inhibitory concentration (IC₅₀ value) was 1.1 μ M for LMIC₆₀-2 ([C₆₀-2]/[lipids] = 5 mol%, [lipids] = 0.25–5.00 μ M), as shown in Fig. 3b (red line). The IC₅₀ value of LMIC₆₀-2 was lower than that of photofrin, which gave an IC₅₀ value of 2.1 μ M under the same conditions (Fig. S4b) when the number of moles was converted to the number of porphyrin units because photofrin consists of porphyrin oligomers containing two to eight units (Fig. S4a). These results therefore revealed that the photodynamic activity of LMIC₆₀-2 was approximately two times higher than that of photofrin.

We wanted to determine why the photodynamic activity of LMIC₆₀-2 was higher than that of LMIC₆₀. In this regard, there are two possible explanations, including (i) LMIC₆₀-2 could improve intracellular uptake by the formation of electrostatic

interactions with the anionic surface of the cells, with cationic C₆₀-2 leading to an increase in the cationic density of the

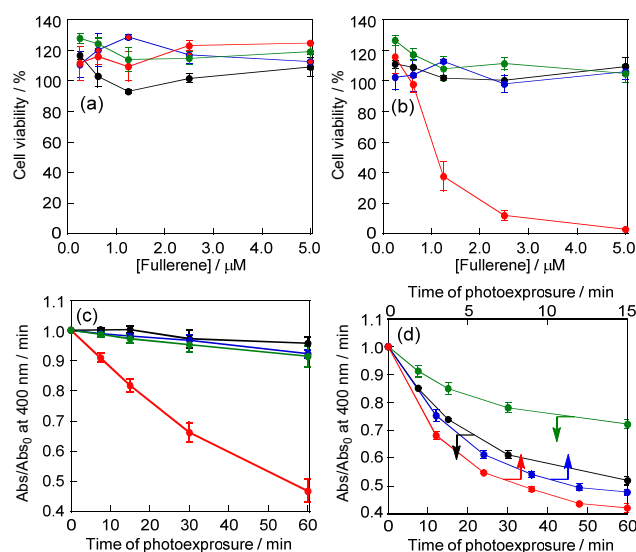


Fig. 3 Concentration-dependent cytotoxicities of LMIC₆₀ (black line), C₆₀-1 (blue line), C₆₀-2 (red line) and C₆₀-3 (green line) (a) without and (b) with light irradiation (610–740 nm, 30 min). Cell viability was assayed by the WST-8 method. (c) Detection of ¹O₂ generation for LMIC₆₀ (black circles), LMIC₆₀-1 (blue circles), LMIC₆₀-2 (red circles) and LMIC₆₀-3 (green circles) with light irradiation at wavelengths greater than 620 nm. (d) Detection of ¹O₂ generation for γ-CDx•C₆₀ complex (black circles), γ-CDx•C₆₀-2 complex (blue circles), LMIC₆₀ (green circles) and LMIC₆₀-2 (red circles) with light irradiation at wavelengths greater than 350 nm by the ABDA bleaching method. A DMSO solution of ABDA was injected into an aqueous solution of the liposomes or γ-CDx complexes. The bleaching of ABDA was monitored as a reduction in the absorbance of ABDA at 400 nm. All data represent the mean values of three independent experiments. Error bars represent the standard deviations.

liposomal surfaces;^{3b,14} and (ii) the formation of cytotoxic reactive oxygen species (ROS) could increase as a consequence of the effective energy or electron transfer between C₆₀-2 and the dissolved oxygen molecules. To investigate the validity of explanation (i), we determined the surface potentials of liposomes 3-4 in the absence and presence of C₆₀, C₆₀-1, C₆₀-2 and C₆₀-3 using zeta potential measurements. The results of these experiments are summarised in Table S2. The zeta-potential of liposomes 4-5 changed from +41 to +24–35 mV, respectively, following the addition of the fullerenes ([fullerenes]/[lipids] = 5 mol%), indicating that the surface densities of LMIC₆₀-2 were largely unaffected by the addition of cationic C₆₀-2 compared with the other fullerenes. To test the validity of explanation (ii), we measured the level of ¹O₂ generation according to a chemical method using 9,10-anthracenediyl-bis(methylene)dimalonic acid (ABDA)¹⁵ as a detector. This experiment was conducted to clarify the reason for the differences observed in the biological activities of LMIC₆₀, LMIC₆₀-1, LMIC₆₀-2 and LMIC₆₀-3. The absorption of ABDA at 400 nm (absorption maximum for ABDA) was monitored as a function of time after the irradiation of the samples with light at a lipid concentration of 150 μ M (Fig. 3c). The results revealed severe time-dependent bleaching of ABDA for LMIC₆₀-2 compared with LMIC₆₀, LMIC₆₀-1 and LMIC₆₀-3, indicating that much higher levels of ¹O₂ were generated in LMIC₆₀-2 compared with LMIC₆₀, LMIC₆₀-1 and LMIC₆₀-3. We previously reported

that the photodynamic activity of the γ -CDx•C₆₀-2 complex is much higher than that of γ -CDx•C₆₀-1 complex for wavelengths in the range of 610–740 nm because of the quenching effect of the lone pair of electrons on the amino group.⁷ For comparison, we measured the generation of ¹O₂ in LMIC₆₀-3, which does not have any amino groups. However, the amount of ¹O₂ generated by LMIC₆₀-3 was much lower than that of LMIC₆₀-2, but similar to those of the LMIC₆₀ and LMIC₆₀-1 complexes (Fig. 3c). Furthermore, the difference in the ¹O₂-generation abilities of LMIC₆₀-2 and LMIC₆₀-3 could be attributed to differences in the amount of light absorbed by these materials at wavelengths greater than 620 nm (inset of Fig. 2a, red and green lines). These results suggested that the high photoactivity of C₆₀-2 could be attributed to the introduction of a polar substituent rather than simply being associated with the derivatization of C₆₀. It is therefore very difficult to prepare non-polar C₆₀ derivatives, as reference compounds with high photodynamic activities (e.g., C₆₀-2).

In the absence of a good reference compound, we compared the ¹O₂ generating activity of γ -CDx with those the liposome incorporated C₆₀ and its derivatives. Furthermore, given that C₆₀ generates very little ¹O₂ under photoirradiation at wavelengths greater than 620 nm, we employed light with a wavelength greater than 350 nm. As shown in Fig. 3d, the γ -CDx•C₆₀ complex generated higher levels of ¹O₂ than LMIC₆₀. In contrast, LMIC₆₀-2 generated higher levels of ¹O₂ than the γ -CDx•C₆₀-2 complex. The contrasting results between C₆₀ and C₆₀-2 were attributed to (i) LMIC₆₀ quenching itself in the lipid membrane to a much greater extent than LMIC₆₀-2 through self-aggregation (as indicated above in Fig. 2a); and (ii) LMIC₆₀ inhibiting energy transfer toward dissolved oxygen in the lipid membranes to a much greater extent than LMIC₆₀-2. Furthermore, the higher ¹O₂ generating ability of LMIC₆₀-2 compared with the γ -CDx•C₆₀-2 complex suggests that C₆₀-2 exists in close proximity to the surface of the lipid membrane. This result also indicates that photoexcited C₆₀-2 can readily interact with dissolved oxygen rather than self-aggregating in the same way as LMIC₆₀-2. In other words, although γ -CDx isolates C₆₀, it simultaneously protects photoexcited C₆₀ from the dissolved oxygen.¹⁶ The location of the C₆₀ derivatives in the lipid membrane is therefore one of most important factors for determining the efficiency of ¹O₂ generation.

In summary, we have successfully prepared a series of LMIC₆₀ derivatives using an exchange reaction. The photodynamic activity of LMIC₆₀-2 was much higher than that of LMIC₆₀, LMIC₆₀-1 and LMIC₆₀-3 and approximately two times higher than that of the porphyrin units of photofrin for the same photon flux (> 610 nm). The main reason for the high photodynamic activity of this material was attributed to the high ¹O₂ generating ability of C₆₀-2. The location and isolation of C₆₀-2 on the lipid membrane surface also played important roles in the photodynamic activity of this material compared with other fullerene derivatives. The latter of these two factors enhanced the generation of ¹O₂ by LMIC₆₀-2. These findings could have important implications in biological, medicinal and materials chemistry applications involving the use of fullerene-based materials.

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Notes and references

- 1 T. J. Dougherty, C. J. Gomer, B. W. Henderson, G. Jori, D. Kessel, M. Korbelik, J. Moan and Q. Peng, *J. Natl. Cancer Inst.*, 1998, **90**, 889–905.
- 2 Y. N. Konan, R. Grunz and E. Allemann, *J. Photochem. Photobiol., B*, 2002, **66**, 89–106.
- 3 (a) A. Ikeda, T. Sato, K. Kitamura, K. Nishiguchi, Y. Sasaki, J. Kikuchi, T. Ogawa, K. Yogo and T. Takeya, *Org. Biomol. Chem.*, 2005, **3**, 2907–2909; (b) A. Ikeda, Y. Doi, K. Nishiguchi, K. Kitamura, M. Hashizume, J. Kikuchi, K. Yogo, T. Ogawa and T. Takeya, *Org. Biomol. Chem.*, 2007, **5**, 1158–1160; (c) A. Ikeda, T. Sue, M. Akiyama, K. Fujioka, T. Shigematsu, Y. Doi, J. Kikuchi, T. Konishi and R. Nakajima, *Org. Lett.*, 2008, **10**, 4077–4080.
- 4 For reviews, see: (a) Y. Tabata and Y. Ikada, *Pure Appl. Chem.*, 1999, **71**, 2047–2053; (b) S. Bosi, T. Da Ros, G. Spalluto and M. Prato, *Eur. J. Med. Chem.*, 2003, **38**, 913–923; (c) E. Nakamura and H. Isobe, *Acc. Chem. Res.*, 2003, **36**, 807–815.
- 5 (a) N. Nitta, A. Seko, A. Sonoda, T. Tanaka, M. Takahashi, K. Murata, S. Takemura, T. Sakamoto and Y. Tabata, *Cardiovasc. Intervent. Radiol.*, 2008, **31**, 359–366; (b) M. Hurtgen, A. Debuigne, A. Mouithys-Mickalad, R. Jérôme, C. Jérôme and C. Detrembleur, *Chem.-Asian J.*, 2010, **5**, 859–868.
- 6 (a) J. L. Anderson, Y.-Z. An, Y. Rubin and C. S. Foote, *J. Am. Chem. Soc.*, 1994, **116**, 9763–9764; (b) T. Hamano, K. Okuda, T. Mashino, M. Hirobe, K. Arakane, A. Ryu, S. Mashiko and T. Nagano, *Chem. Commun.*, 1997, 21–22.
- 7 A. Ikeda, T. Iizuka, N. Maekubo, R. Aono, J. Kikuchi, M. Akiyama, T. Konishi, T. Ogawa, N. Ishida-Kitagawa, H. Tatebe and K. Shiozaki, *ACS Med. Chem. Lett.*, 2013, **4**, 752–756.
- 8 A. Ikeda, K. Ashizawa, Y. Tsuchiya, M. Ueda and K. Sugikawa, *RSC Adv.*, 2016, **6**, 78505–78513.
- 9 (a) A. Ikeda, R. Aono, N. Maekubo, S. Katao, J. Kikuchi and M. Akiyama, *Chem. Commun.*, 2011, **47**, 12795–12797; (b) A. Ikeda, A. Hirata, M. Ishikawa, J. Kikuchi, S. Mieda and W. Shinoda, *Org. Biomol. Chem.*, 2013, **11**, 7843–7851.
- 10 (a) R. Qiao, A. P. Roberts, A. S. Mount, S. J. Klaine and P. C. Ke, *Nano Lett.*, 2007, **7**, 614–619; (b) R. S. G. D'Rozario, C. L. Wee, E. J. Wallace and M. S. P. Sansom, *Nanotechnology*, 2009, **20**, 115102–115108.
- 11 A. Ikeda, K. Kiguchi, T. Shigematsu, K. Nobusawa, J. Kikuchi and M. Akiyama, *Chem. Commun.*, 2011, **47**, 12095–12097.
- 12 D. He, X. Du, Z. Xiao and L. Ding, *Org. Lett.*, 2014, **16**, 612–615.
- 13 A. M. Cassell, W. A. Scrivens and J. M. Tour, *Angew. Chem., Int. Ed.*, 1998, **37**, 1528–1531.
- 14 We prepared LMIC₆₀, C₆₀-1, C₆₀-2 and C₆₀-3 with a cationic liposome surface using by a cationic lipid **5** ([**5**]/[**4** + **5**] = 10 mol%) since it has been known that LMIC₆₀ with the cationic liposome surface efficiently engenders cell death by photoirradiation, as compared with LMIC₆₀ with a neutral or an anionic liposome surface.^{3a}
- 15 C. R. Miller, B. Bondurant, S. D. McLean, K. A. McGovern and D. F. O'Brien, *Biochemistry*, 1998, **37**, 12875–12883.
- 16 A. Masuhara, M. Fujitsuka and O. Ito, *Bull. Chem. Soc. Jpn.*, 2000, **73**, 2199–2206.