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Syntheses of Phospholipids Containing 2-Nitrobenzyl Ester Moieties at the Terminals of Alkyl Chains and Properties of Photodegradable Liposomes from the Lipids

Kazuo Yamaguchi,* Yoshihiro Tsuda, Taka-aki Shimakage, and Akihiro Kusumi†

Department of Materials Science, Faculty of Science, Kanagawa University, Hiratsuka, Kanagawa 259-1293

†Department of Biological Science, Graduate School of Science, Nagoya University, Chikusa-ku, Nagoya 464-01

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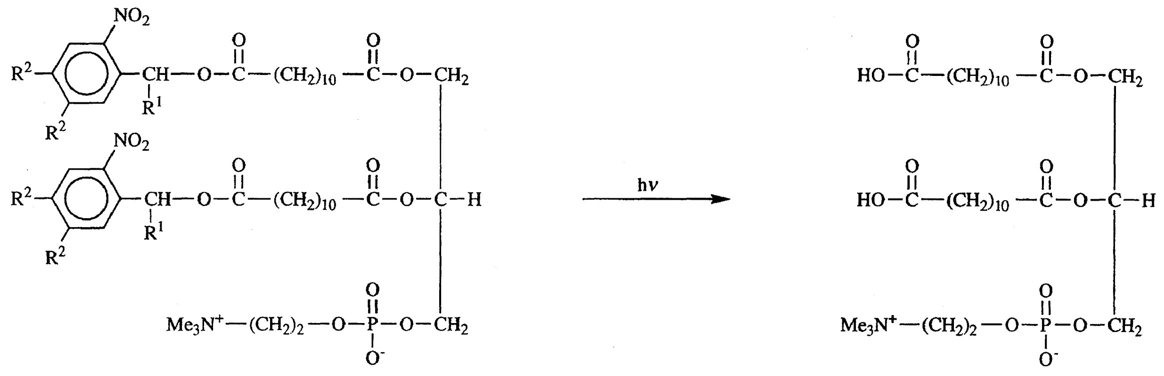
Phospholipid **1a** bearing 2-nitrobenzyl ester moieties as a photocleavable group at the terminal of alkyl chains was synthesized from the corresponding terminal carboxy-bearing phospholipid **2** by the reaction with 2-nitrophenyl-substituted diazomethane. Phospholipid, **1b** bearing α-methyl-2-nitrobenzyl group, **1c** bearing 4,5-dimethoxy-2-nitrobenzyl group, and **1d** bearing α-methyl-4,5-dimethoxy-2-nitrobenzyl group were similarly synthesized by the use of the respective diazo compounds. The order of photolysis rates of 2-nitrobenzyl ester linkage of phospholipids by ultrahigh-pressure mercury lamp is **1b**$\geq$**1d** > **1a** > **1c**. Liposomes of **1a**—**1d** containing calcein in the inner aqueous layer were prepared by vortexing, sonication, and gel-filtration. UV irradiation resulted in fast release of the entrapped fluorescence dye. The order of release rates : **1b**$\geq$**1d** > **1c** > **1a** is consistent with that of photolysis rates except for **1c**, which has poor retention of the dye.

Concentration-jump experiments in a cell with biologically active compounds such as substrate or inhibitor for enzymes are very useful for the study of biological processes. For these experiments, compounds are developed which, while inactive themselves, are rapidly transformed into biologically active ones. These photosensitive precursor molecules have been termed ‘caged’ compounds.[1)] The basic idea for caged compounds is to protect carboxyl, hydroxyl, and amino groups, and phosphoric acid as photocleavable 2-nitrobenzyl derivatives.[2)] The functional groups are generated upon photoirradiation. For example, caged ATP, which is γ-1-(2-nitrophenyl)ethyl ester of ATP is extensively applied for studies on muscle contraction.[3)] 2-Nitrobenzyl ester of cyclic AMP as caged cyclic AMP is a lipophilic phosphoric triester and easily crosses membranes.[4)] When the concentration of Ca^{2+} in a controlled cell is changed, the cellular physiology is largely affected. Upon photoirradiation, calcium-chelating agent is transformed into a ketone which has a lower binding constant of Ca^{2+}, resulting in release of chelated Ca^{2+}.[5)] More recently, novel caged compounds[6)] as well as improvements of photocleavable groups[7)] have been reported.

Liposomes have been widely employed as models of biological membranes since their discovery in 1965.[8)] This membrane model is also studied for application to microcapsules entrapping various compounds in the inner aqueous phase, especially for drug delivery systems.[9)] Development of methods to improve their stability and retention ability of drugs and to control release of drugs (targeting) is essential for this purpose. Polymeric liposomes are devised for improvement of the stability and retention ability.[10)] This system is based on polymerization of reactive moieties such as a styryl or methacryl group introduced into normal phospholipids in liposomes. On the other hand, temperature-, pH-, and target-sensitive liposomes have been reported as a liposome to release the entrapped molecules in response to outer stimuli.[11)]

Photosensitive liposomes have also been reported.[12)] The ideas are based on photochemical transformation of the head group of amphiphiles,[12b,12d,12e)] photoisomerization of the azo group or conjugated polyene compound,[12a,12c,12f,12g,12h)] photopolymerization of phospholipids,[12j,12l)] and photoinduced pH-decrease.[12k)] We have already reported a preliminary study of photodegradable liposomes from phospholipid **1a** containing a 2-nitrobenzyl ester,[12i)] which is different from the other reported methods described above. As shown in Scheme 1, our system is conceptually designed to cleave the 2-nitrobenzyl ester of phosphatidylcholine derivatives **1** upon photoirradiation and to generate a carboxyl group at the terminal of the alkyl chains located in the middle, hydrophobic region of the bilayer membrane, leading to the membrane disintegration and the subsequent release of the entrapped molecules. If ‘photodegradable liposomes’ are developed which disintegrate upon photoirradiation, releasing the entrapped compounds rapidly as caged compounds, extensive use of their systems as cages for a variety of water-soluble compounds can be expected. Compared with the response rate for release of the entrapped solutes and the retention ability,[12c,12f,12h,12i,12j,12l)] our system appears to be most promising for such purposes, although comparison may be difficult due to the different experimental conditions.

In this study, we report syntheses of phospholipids **1a**—**1d** containing substituted derivatives of the 2-nitrobenzyl group and the retention ability and photoinduced release of an en-



1a: $R^1 = R^2 = H$
1b: $R^1 = Me$, $R^2 = H$
1c: $R^1 = H$, $R^2 = MeO$
1d: $R^1 = Me$, $R^2 = MeO$

2

Scheme 1.

trapped fluorescent dye of the liposomes. If this liposome is delivered into a cell by the endocytosis method or pipette-injection method, and then photoirradiated, the entrapped compounds are released along with degradation of the liposomal structure. This liposome should be applicable as a cage for a variety of water-soluble molecules different from each caged compound described above and should be useful for biophysical and biochemical studies of cells as a phototriggered rapid-mixing microcapsules.

Results and Discussion

Phospholipid **1a** has already been synthesized from *sn*-glycero-3-phosphocholine and 11-(2-nitrobenzyloxycarbonyl)undecanoic acid (**5**).[12i] In this paper, as shown in Scheme 2, phospholipids **1a**—**1d** were synthesized by the reaction of phospholipid **2** containing a carboxy group at the terminal of both alkyl chains as a common intermediate with the corresponding diazo compounds (**10a**—**10d**) similar to syntheses of caged cyclic AMP and GMP.[4]

The intermediate **2** was synthesized by Pd/C-hydrogenolysis of phospholipids containing benzyl esters (**7**) prepared by a method similar to that for **1a**.[12i] Although a large number of synthetic methods of diazoalkanes are reported,[13] only two of them have been applied to caged compounds. Relatively stable diazomethane derivatives such as **10a** and **10c** are prepared and isolated by a basic treatment of tosylhydrazone.[4a,4c,14] Less stable diazoethane derivatives such as **10b** and **10d**, which are prepared by oxidation of hydrazones with manganese(IV) oxide, are usually used in the next step without isolation.[3c,6a] However, synthesis of **10c** by the latter method has been reported.[4d] In this study, synthesis of the desired diazoalkanes (**10a**—**10d**) was attempted by the latter method and empolyed for further reaction with **2** without isolation.

Reaction of **2** in methanol with a chloroform solution of diazo compounds (**10a**—**10d**) prepared from a large excess amount of hydrazones (**9a**—**9d**) at room temperature for 5—7 d gave the desired products (**1a**—**1d**) in high yields, although the reaction time was longer than those for syntheses of caged cyclic AMP and GMP.[4c,4d] The products were identified by ^{1}H NMR spectra and elemental analyses. This result shows that a method using manganese(IV) oxide is applicable for syntheses of diazomethane derivatives as well as diazoethanes, consistent with the literature.[4d]

UV spectra of **1a**—**1d** in ethanol are shown in Fig. 1. The presence of the methyl group at the benzylic α-position has almost no effect on the spectra, but introduction of methoxy groups at the aromatic rings gives more absorption at longer wavelengths. The effect of these substituents on the absorption spectra is consistent with data for caged compounds already reported, including the value of molar extinction coefficient.[1a,3b,4c] Spectral changes of **1c** and **1d** in ethanol upon UV irradiation from an ultrahigh-pressure mercury lamp are shown in Fig. 2. Isosbestic points are observed in **1d**, but not in **1c**. Similar photoinduced changes of absorption spectra in **1a** and **1b** were observed (data not shown). These results are explained by the difference in the photoreactions: **1b** yields 2′-nitrosoacetophenone in one

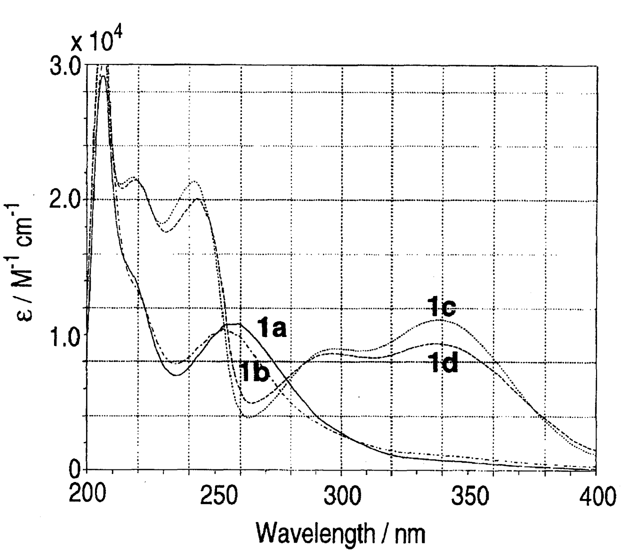
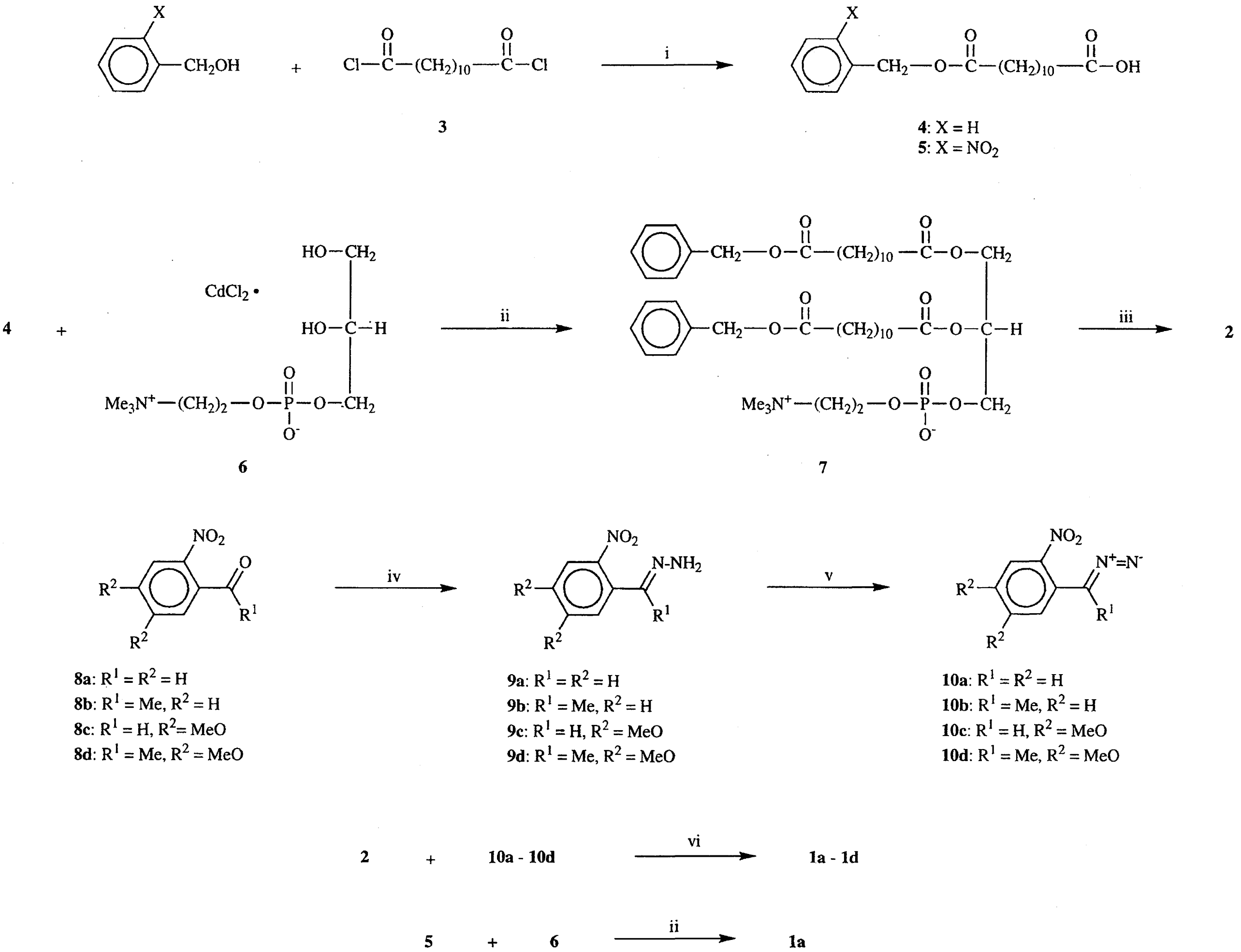


Fig. 1. UV spectra of **1a**—**1d** in EtOH.



Scheme 2. (i) (1) Et_3N/THF, r.t., overnight; (2) Et_3N/H_2O, r.t., 1 h, 38—50%; (ii) DCC and DMAP/$CHCl_3$, r.t., 5—6 d, 50—73%; (iii) Pd/C and H_2/EtOH, r.t., 3 d, 95%; (iv) hydrazine monohydrate and AcOH/EtOH, reflux, 3 h, 85—89%; (v) $MnO_2/CHCl_3$, r.t., 5 min; (vi) r.t., 5—7 d, 50—91%.

step, while the resulting 2-nitrosobenzaldehyde from **1a** reacts bimolecularly to form an azo compound.[15]

Decomposition of phospholipids was monitored by HPLC when an ethanolic solution of the lipids was irradiated by white light and by 364-nm light through an interference filter from an ultrahigh-pressure mercury lamp. The results for white-light irradiation are shown in Fig. 3. Times required for decomposition of 50% of lipids (**1a**—**1d**) upon irradiation by white light as well as 364-nm light are summarized in Table 1. The order of decomposition rates of the lipids irradiated by white light is **1b**$\geq$**1d** > **1a** > **1c**. Although there was almost no change in UV spectra by introduction of a methyl group at the benzylic α-position, **1b** and **1d** bearing methyl groups decomposed faster than **1a** and **1c**, respectively. This shows that quantum yields of photodecomposition of **1b** and **1d** are higher than those of **1a** and **1c**. Irradiation at 364 nm resulted in the order of photolysis rates of **1d**$\geq$**1b** > **1c** > **1a** different from the result obtained by white-light irradiation. Probably, this is due to the fact that molar extinction coefficients at 364 nm of **1c** and **1d** bearing methoxy groups at the aromatic rings are much larger than those of **1a** and **1b**. However, the effect of methoxy groups on photolysis rates is much smaller than the difference in ε-values between **1c** and **1a** (**1d** and **1b**). A methyl group introduced at the benzyl position is more effective than a methoxy group at the aromatic ring in terms of photodecomposition rate.

The spectral changes and decomposition rates of phospholipids in liposomes obtained were similar to those in an ethanolic solution, although scattering of irradiated light by particles of liposomes resulted in larger variation of the measured values.

Retention of calcein entrapped in liposomes of **1a**—**1d** was measured.[16] Time for retention of 75% of calcein is shown in Table 1. Retention abilities are in the order of **1b** > **1d** > **1a** > **1c**. Liposomal membranes of **1b** and **1d** containing methyl groups at the benzylic α-position exhibited relatively high retention ability. However, introduction of methoxy groups to the aromatic rings tended to lower the retention ability.

White and 364-nm lights were employed for measurement of photoinduced release rate. The results of release of calcein induced by white-light irradiation are shown in Fig. 4 and the time required for release of 50% of calcein from liposomes irradiated by white and 364-nm lights is summarized in Table 1. The release rates by white-light irradiation

Table 1. Photodecomposition of Phospholipid **1a**—**1d** in Ethanol and Retention and Photoinduced Release of Calcein Entrapped in Liposomes of **1a**—**1d**

Lipid	Photodecomposition[a)]		Retention[b)]	Photoinduced release[c)]		
	White light	364 nm		White light	259 nm	364 nm
1a	1.7	26.6	3.8	11	1500	433
1b	0.6	7.1	9.4	1.9	462	68
1c	2.6	13.1	2.1	6.1	840	56
1d	0.7	6.6	4.4	2.3	600	26

a) Time (min) for photodecomposition of 50% of phospholipid by UV irradiation in EtOH. b) Time (h) for retention of 75% of calcein at 37 °C. c) Time (s) for photoinduced release of 50% of calcein.

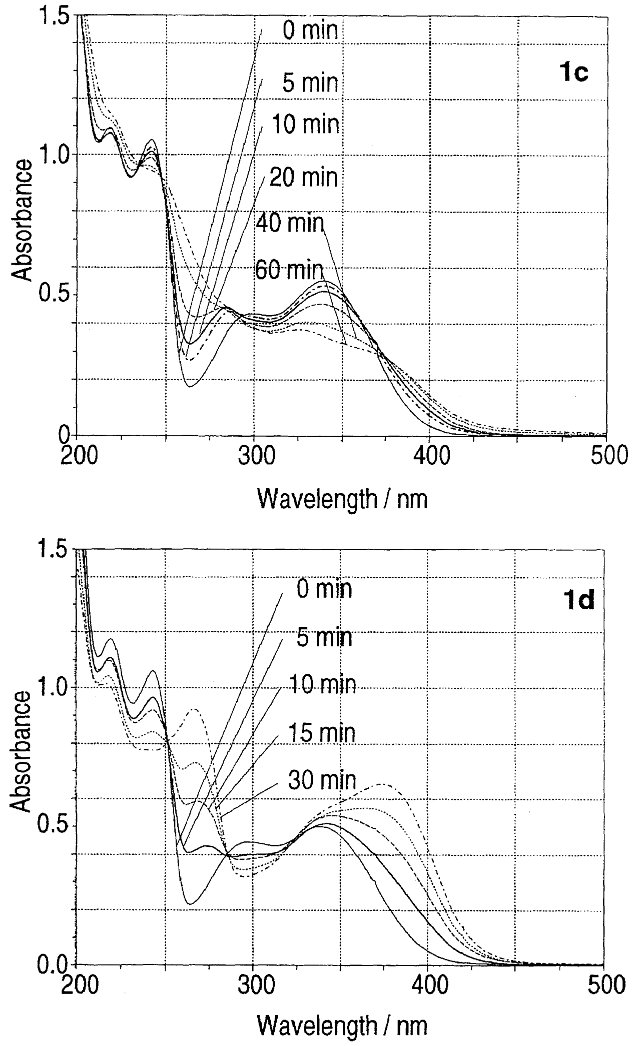


Fig. 2. Changes in UV spectra of **1c** (top) and **1d** (bottom) by photorradiation at 364 nm.

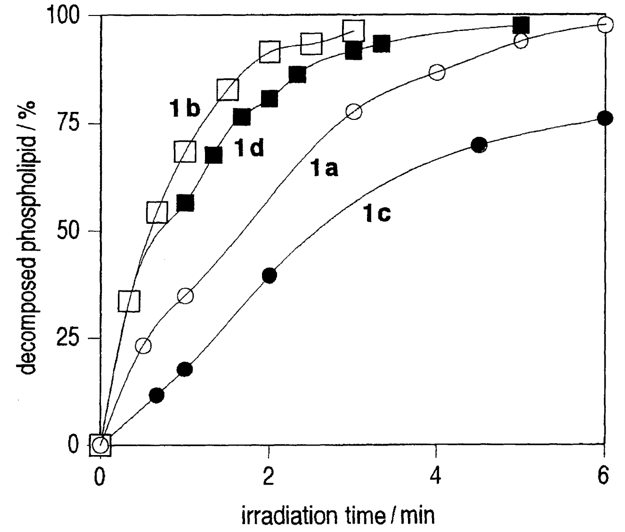


Fig. 3. Decomposition of **1a**—**1d** in EtOH Irradiated by white light.

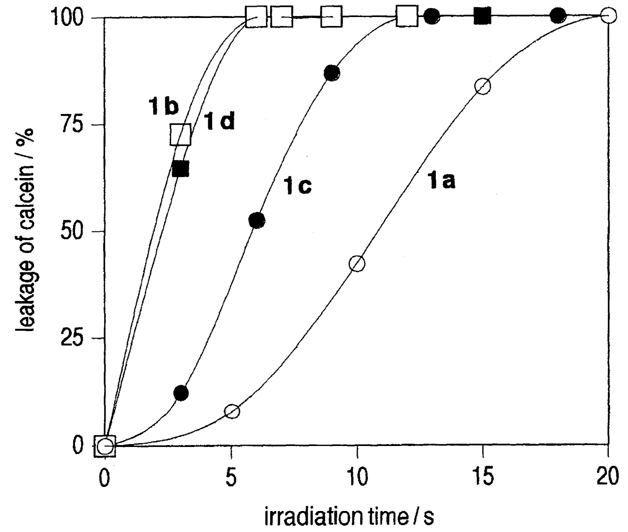


Fig. 4. Leakage of calcein from liposomes of **1a**—**1d** induced by white-light irradiation.

are in the order of **1b**≒**1d** > **1c** > **1a**. Irradiation by white light releases calcein rapidly from membranes of **1b** and **1d**, which is consistent with the results of photolysis rates described above. The membrane of **1c** released slowly upon irradiation, and the release from **1a** was much slower. This is in contrast to the photolysis rates of **1a** and **1c**, and may result from poor retention ability of membranes of **1c**. In the case of irradiation at 364 nm, **1d** was most sensitive to irradiation; however, **1b** was less sensitive than **1c**. This could be explained by poor retention ability of liposomes of **1c** and by relative enhancement of the decomposed rate of **1c** to that of **1b** compared to the case of white-light irradiation. The decomposed rate of **1a** was too small to show a photoinduced release rate comparable to the other membranes.

The release rate of calcein from liposomes by white-light irradiation is much larger than the photodecomposition rate of the respective phospholids in EtOH, as shown in Figs. 3 and 4 and in Table 1. Twenty-seconds irradiation by white light is required for complete release of calcein from liposome of **1a** (Fig. 4), while only 23% of **1a** is decomposed by 30-s irradiation in an ethanol solution (Fig. 3). Although it is difficult to determine exactly the amount of phospholipid decomposed at the end point of release, photodecomposition of 10—20% of **1a** may be estimated after the irradiation time for complete release. In the case of the other liposomes which release the fluorescence dye more rapidly when induced by irradiation, about 10-% or less amount of the lipids is likely to decompose at the end point of release. Similar results are obtained when 364-nm light is used (the original data are not shown). It is found that decomposition of small amounts of lipids is necessary for complete release of the entrapped dye.

In conclusion, phospholipids were synthesized which have photocleavable 2-nitrobenzyl esters at the terminal of the alkyl chains. It was found that release of a water-soluble fluorescent dye entrapped in the inner aqueous phase of liposomes of these lipids as well as decomposition of the lipids was triggered by UV irradiation. In order to apply this system to biochemical and biophysical studies, we are currently investigating structural changes in liposomal membranes and release of different water-soluble materials induced by photoirradiation.

Experimental

Materials. Dry $CHCl_3$ was prepared by distillation from P_2O_5. 2-Nitrobenzyl alcohol (Tokyo Chemical Industry Co.) was recrystallized from H_2O. Cadmium chloride complex of *sn*-glycero-3-phosphocholine **6** was purchased from Sigma Chamical Co. 4,5-Dimethoxy-2-nitrobenzaldehyde **8c** (Tokyo Chemical Industry Co.) was recrystallized from EtOH. All other chemicals were used without further purification. Calcein and 1,4-piperazinediethanesulfonic acid (PIPES) were purchased from Dojindo Laboratories. MnO_2 was purchased from Merck & Co. Sephadex® G-50 Medium was purchased from Pharmacia LKB Biotechnology. DCC, 3,4-dimethoxyacetophenone, DMAP, dodecanedioic acid, and 2′-nitroacetophenone **8b** were purchased from Tokyo Chemical Industry Co. Benzyl alcohol, Et_3N, glacial acetic acid, hydrazine monohydrate, 2-nitrobenzaldehyde **8a**, 5% Pd/C, thionyl chloride, and Triton® X-100 (so-called "polyoxyethylene(10) octylphenyl ether") were purchased from Wako Pure Chemical Industries. Amberlite IR-120 (plus) (Aldrich Chemical Co.) was used as cation exchange resin. Hydrazone of **8b**, **9b**, was prepared from **8b** by a reported method.[3c] 3′,4′-Dimethoxyacetophenone was nitrated to obtain **8d**,[17] which was converted to **9d**.[6a]

Methods. TLC was performed on precoated TLC plates silica gel 60 F_{254} (0.2 mm) (Merck & Co.) with UV or iodine visualization. Column chromatography was performed using Wakogel® C-300 (200—300 mesh) (Wako Pure Chemical Industries). HPLC was performed using a Shim-pack CLC-ODS column (0.15×6.0 mm) (Shimadzu Co.) with a tunable UV detector at 30 °C. MeOH–H_2O (47 : 3, v/v) was used as the mobile phase at a flow rate of 0.5 mL min^{-1}. ^1H NMR spectra were recorded on JEOL JNM-EX 90 (90 MHz) or 400 (400 MHz) for solutions in $CDCl_3$ unless otherwise noted, and the chemical shifts are reported in ppm relative to TMS as an internal standard. IR spectra were recorded on a Nicolet 510 as liquid film between NaCl plates or KBr pellet. UV spectra were recorded on JASCO V560 or V570. Microanalyses were performed on a Perkin–Elmer 2400 CHN. Fluorescence intensities were measured on Hitachi F-4010. Photoirradiation was performed using a 500-W ultrahigh-pressure mercury lamp (Ushio USH-500D) with interference filter (259 or 364 nm). Sonication was performed using a 200-W probe-type sonicator (Otake Works).

Dodecanedioyl Dichloride 3. According to a reported procedure,[18] a mixture of dodecanedioic acid (25.0 g, 109 mmol) and thionyl chloride (40.0 mL, 558 mmol) was refluxed for 5 h. The excess of thionyl chloride was distilled off, and the crude product was distilled under reduced pressure to give **3** (28.0 g, 95%, 120 °C/0.2 mmHg, 1 mmHg = 133.322 Pa) as a colorless liquid: ^1H NMR (90 MHz) $\delta = 1.3$ (12H, s), 1.6—1.8 (4H, m), 2.9 (4H, t, $J = 7.1$ Hz); IR (neat) 1799 cm^{-1}.

11-(Benzyloxycarbonyl)undecanoic Acid 4. To an ice-cooled, stirred solution of **3** (9.1 g, 34 mmol) in dry THF (100 mL) was added dropwise a solution of benzyl alcohol (3.6 mL, 35 mmol) and Et_3N (4.8 mL, 34 mmol) in dry THF (75 mL) over 2.5 h. After the addition was complete, the mixture was stirred at room temperature overnight. To the mixture was added dropwise a solution of H_2O (25 mL) and Et_3N (4.8 mL, 34 mmol) in THF (25 mL) over 1 h, and the mixture was stirred for 2 h and concentrated by a rotary evaporator. After the addition of H_2O (50 mL) to the residue, the mixture was extracted with Et_2O (50 mL×3). The combined Et_2O layers were washed with H_2O (50 mL×3), dried over $MgSO_4$, and concentrated by rotary evaporator. To the residue was added EtOAc (50 mL), and the suspension was filtered to remove dodecanedioic acid as by-product from unreacted **3**. To the filtrate was added silica gel (50 g), and the mixture was concentrated by a rotary evaporator. The mixture was chromatographed on 350 g of silica gel with hexane–EtOAc (4 : 1 and 2 : 1, v/v) to give **4** (5.5 g, 50%) as a white powder: $R_f = 0.4$ (hexane–EtOAc 2 : 1, v/v); ^1H NMR (90 MHz) $\delta = 1.3$ (12H, s), 1.6—1.8 (4H, m), 2.4 (4H, t, $J = 7.3$ Hz), 5.1 (2H, s), 7.4 (5H, s); IR (KBr) 1737 and 1702 cm^{-1}. Found: C, 71.16; H, 8.91%. Calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81%.

11-(2-Nitrobenzyloxycarbonyl)undecanoic Acid 5. The procedure for preparation of **4** gave **5** (4.6 g, 38%) as a white powder from **3** (9.0 g, 34 mmol) and 2-nitrobenzyl alcohol (5.2 g, 34 mmol): $R_f = 0.3$ (hexane–EtOAc 2 : 1, v/v); ^1H NMR (90 MHz) $\delta = 1.3$—1.6 (16H, m), 2.3—2.5 (4H, m, $J = 7.3$ Hz), 5.5 (2H, s), 7.4—8.1 (4H, m); IR (KBr) 1740 and 1700 cm^{-1}. Found: C, 62.38; H, 7.59; N, 3.77%. Calcd for $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83%.

1, 2- Bis[11- (benzyloxycarbonyl)undecanoyl]- *sn*- glycero- 3-phosphocholine 7. A suspension of **6** (0.20 g, 0.46 mmol), **4** (0.60 g, 1.9 mmol), DMAP (0.12 g, 1.0 mmol), and DCC (0.48 g, 2.4 mmol) in dry $CHCl_3$ (5.0 mL) was stirred for 5 d at room temperature. The reaction mixture was passed through a 100-mL cation-exchange resin column with $CHCl_3$–MeOH–H_2O (4 : 5 : 1, v/v) to remove Cd^{2+} and DMAP. The resulting solution was concentrated by a rotary evaporator, and the residue was chromatographed on 200 g of silica gel with $CHCl_3$–MeOH (1 : 1, v/v) and then $CHCl_3$–MeOH–H_2O (65 : 25 : 4, v/v) to give **7** (0.28 g, 73%) as a white solid: ^1H NMR (400 MHz) $\delta = 1.3$ (24H, s), 1.57—1.64 (8H, m), 2.2—2.4 (8H, m), 3.4 (9H, s), 3.8 (2H, br s), 3.9—4.4 (6H, m), 5.1 (4H, s), 5.2 (1H, br s), 7.31—7.35 (10H, m). Found: C, 62.75; H, 8.83; N, 1.86%. Calcd for $C_{46}H_{72}NO_{12}P\cdot H_2O$: C, 62.78; H, 8.48; N, 1.59%.

1,2-Bis(11-carboxyundecanoyl)-*sn*-glycero-3-phosphocholine 2. A mixture of **7** (0.26 g, 0.30 mmol) and 5% Pd/C (0.061 g) in EtOH (3.3 mL) was stirred for 3 d at room temperature under hydrogen atmosphere. The reaction mixture was filtered to remove Pd/C. The filtrate was concentrated by a rotary evaporator and freeze-dried from benzene to give **2** (0.19 g, 95%) as a white solid, which was used without further purification: ^{1}H NMR (90 MHz; CD_3OD) $\delta = 1.3$—1.6 (32H, m), 2.2—2.4 (8H, m), 3.2 (9H, s), 3.6—3.7 (2H, m), 3.9—4.4 (6H, m), 5.2 (1H, br s).

Hydrazone of 2-Nitrobenzaldehyde 9a. In a modification of the synthetic procedures for **9b**[3c)] and **9d**,[6a)] synthesis was carried out without glacial acetic acid. A solution of 2-nitrobenzaldehyde **8a** (5.0 g, 33 mmol) and hydrazine monohydrate (3.3 g, 66 mmol) in EtOH (60 mL) was refluxed for 3 h. The reaction mixture was concentrated by rotary evaporator, water (20 mL) was added, and the mixture was extracted with $CHCl_3$ (50 mL×3). The combined $CHCl_3$ layers were washed with H_2O (50 mL×3) to remove hydrazine. Then they were dried over $MgSO_4$ and concentrated by a rotary evaporator. The residue was recrystallized from $CHCl_3$–hexane to give **9a** (4.9 g, 89%) as yellowish crystals: ^{1}H NMR (90 MHz) $\delta = 5.9$ (2H, s), 7.3—8.1 (4H, m), 8.3 (1H, s). Found: C, 51.01; H, 4.20; N, 25.12%. Calcd for $C_7H_7N_3O_2$: C, 50.91; H, 4.27; N, 25.44%.

Hydrazone of 4,5-Dimethoxy-2-nitrobenzaldehyde 9c. Following the procedure for preparation of **9a**, we treated the mixture of 4,5-dimethoxy-2-nitrobenzaldehyde **8c** (0.93 g, 4.4 mmol) and hydrazine monohydrate (0.52 g, 10 mmol) in EtOH (12 mL). The raction mixture was cooled to room temperature and filtered. The precipitate was recrystallized from EtOH to give **9c** (0.85 g, 85%) as yellowish crystals: ^{1}H NMR (90 MHz) $\delta = 3.96$ (3H, s), 4.00 (3H, s), 5.8 (2H, br s), 7.5 (1H, s), 7.6 (1H, s), 8.4 (1H, s). Found: C, 48.06; H, 4.90; N, 18.66%. Calcd for $C_9H_{11}N_3O_4$: C, 48.00; H, 4.92; N, 18.66%.

Preparation of Diazo Compounds 10a—10d. According to a reported method for preparation of **10b**,[3c)] diazo compounds **10a**—**10d** were prepared from **9a**—**9d**, respectively. Typically, a mixture of **9a** (0.5 g, 3.0 mmol) and MnO_2 (2.1 g, 24 mmol) in $CHCl_3$ (30 mL) was stirred for 5 min at room temperature. The reaction mixture was filtered to remove MnO_2; then the filtrate was washed with 0.1 M $NaHCO_3$ (100 mL, 1 M = 1 mol dm^{-3}) and dried over $MgSO_4$ to give a solution of **10a** in $CHCl_3$ (30 mL). The concentration was estimated to be 0.1 M on the assumption that the reaction was complete. The solution of **10a** was used for the next step without isolation.

1,2-Bis(11-(2-nitrobenzyloxycarbonyl)undecanoyl)-*sn*-glycero-3-phosphocholine 1a. To a solution of **2** (0.15 g, 0.23 mmol) in MeOH (30 mL) was added 0.1 M of **10a** in $CHCl_3$ (30 mL, 3.0 mmol) and this mixture was stirred for 6 d at room temperature. After the addition of 0.20 mL of glacial acetic acid, the reaction mixture was stirred for 1 h and then concentrated by rotary evaporator. The residue was chromatographed on 200 g of silica gel with $CHCl_3$–MeOH (1 : 1, v/v) and then $CHCl_3$–MeOH–H_2O (65 : 25 : 4, v/v) to give **1a** (0.11 g, 50%) as a white solid: $R_f = 0.3$ ($CHCl_3$–MeOH–H_2O 65 : 25 : 4, v/v); ^{1}H NMR (90 MHz) $\delta = 1.3$—1.6 (32H, m), 2.2—2.5 (8H, m), 3.4 (9H, s), 3.7—4.3 (8H, m), 5.2 (1H, s), 5.5 (4H, s), 7.5—8.1 (8H, m); UV (EtOH) λ_{max} 259 nm (ε 10800). Found: C, 56.51; H, 7.85; N, 4.70%. Calcd for $C_{46}H_{70}N_3O_{16}P\cdot H_2O$: C, 56.96; H, 7.48; N, 4.33%.

Compound **1a** (0.26 g, 0.27 mmol) was also prepared from **6** (0.17 g, 0.38 mmol) and **5** (0.55 g, 1.50 mmol) according to the procedure for preparation of **7** from **6** and **4**.[12i)]

1,2-Bis{11-[1-(2-nitrophenyl)ethoxycarbonyl]undecanoyl}-*sn*-glycero-3-phosphocholine 1b. Following the procedure for preparation of **1a** from **10a**, to a solution of **2** (0.15 g, 0.23 mmol) in MeOH (30 mL) was added 0.08 M **10b** in $CHCl_3$ (30 mL, 2.4 mmol) and this mixture was stirred for 5 d at room temperature to give **1b** (0.15 g, 64%) as a white solid: $R_f = 0.3$ ($CHCl_3$–MeOH–H_2O 65 : 25 : 4, v/v); ^{1}H NMR (90 MHz) $\delta = 1.2$ (24H, s), 1.4—1.8 (8H, m), 1.6 (6H, d, $J = 6.6$ Hz), 2.2—2.3 (8H, m), 3.4 (9H, s), 3.8—4.3 (8H, m), 5.2 (1H, s), 6.3 (2H, q, $J = 6.6$ Hz), 7.4—8.0 (8H, m); UV (EtOH) λ_{max} 253 nm (ε 10400). Found: C, 57.60; H, 7.85; N, 4.07%. Calcd for $C_{48}H_{74}N_3O_{16}P\cdot H_2O$: C, 57.76; H, 7.67; N, 4.21%.

1,2-Bis[11-(4,5-dimethoxy-2-nitrobenzyloxycarbonyl)undecanoyl]-*sn*-glycero-3-phosphocholine 1c. Following the procedure for preparation of **1a** from **10a**, to a solution of **2** (0.15 g, 0.22 mmol) in 30 mL of MeOH was added 0.1 M **10c** in $CHCl_3$ (30 mL, 3.0 mmol) and this mixture was stirred for 5 d at room temperature to give **1c** (0.19 g, 78%) as a white solid: $R_f = 0.3$ ($CHCl_3$–MeOH–H_2O 65 : 25 : 4, v/v); ^{1}H NMR (90 MHz) $\delta = 1.3$ (24H, s), 1.6—1.7 (8H, m), 2.3—2.4 (8H, m), 3.4 (9H, s), 3.8—4.4 (8H, m), 3.95 (6H, s), 3.98 (6H, s), 5.2 (1H, s), 5.5 (4H, s), 7.0 (2H, s), 7.7 (2H, s); UV (EtOH) λ_{max} 242, 298, and 338 nm (ε 21400, 9000, and 11200). Found: C, 55.22; H, 7.82; N, 3.79%. Calcd for $C_{50}H_{78}N_3O_{20}P\cdot H_2O$: C, 55.09; H, 7.40; N, 3.85%.

1,2-Bis{11-[1-(4,5-dimethoxy-2-nitrophenyl)ethoxycarbonyl]-undecanoyl}-*sn*-glycero-3-phosphocholine 1d. Following the procedure for preparation of **1a** from **10a**, to a solution of **2** (0.15 g, 0.22 mmol) in MeOH (30 mL) was added 0.1 M solution of **10d** in $CHCl_3$ (30 mL, 3.0 mmol) and the mixture was stirred for 7 d at room temperature to give **1d** (0.22 g, 91%) as a white solid: $R_f = 0.3$ ($CHCl_3$–MeOH–H_2O 65 : 25 : 4, v/v); ^{1}H NMR (400 MHz) $\delta = 1.2$ (24H, s), 1.57—1.61 (8H, m), 1.61 (6H, d, $J = 6.4$ Hz), 2.3—2.4 (8H, m), 3.4 (9H, s), 3.8—4.4 (8H, m), 3.9 (6H, s), 4.0 (6H, s), 5.2 (1H, s), 6.5 (2H, q, $J = 6.4$ Hz), 7.0 (2H, s), 7.6 (2H, s); UV (EtOH) λ_{max} (EtOH) 243, 297, and 336 nm (ε 20100, 8600, and 9400). Found: C, 56.38; H, 7.84; N, 3.75%. Calcd for $C_{52}H_{82}N_3O_{20}P$: C, 56.77; H, 7.51; N, 3.82%.

Photolysis of Lipid in EtOH. In a quartz cell was placed 5×10^{-5} M lipid in EtOH (2.4 mL) and the solution was photoirradiated. The UV spectra of the solution were measured after given irradiation times. An aliquot of the resulting solution was analyzed by HPLC to determine the amount of decomposed lipid.

Preparation of Liposome. In a test tube (12 mm×75 mm) was placed 10 mM lipid in $CHCl_3$ (254 μL, 2.54 mmol); lipid film was obtained by N_2 gas and dried under reduced pressure. To the film was added 100 mM calcein/10 mM PIPES buffer (pH 7.2) (254 μL), vortexed and sonicated (20 kHz, 20 W) for 90 s to give unilamellar vesicles (ULVs). The vesicle solution was passed through Sephadex® G-50 column (1.5 cm×30 cm) with 189 mM NaCl/10 mM PIPES buffer (pH 7.2) to separate the vesicles and entrapped calcein from the free calcein. The eluted vesicular fractions were diluted to 5×10^{-5} M lipid on the basis of absorbance measured by UV spectra.

Measurement of Retention and Photoinduced Release of Calcein.[16)] In a quartz cell was placed the solution containing ULVs (2.4 mL), then the fluorescence intensity (F_0) at 520 nm (excited at 490 nm) was measured. The sample was kept at 37 °C or photoirradiated, and the value of F_t was measured for a given time. After the addition of 10% Triton® X-100 (12 mL), the values of F_{100} was measured. The amount of calcein entrapped in liposome (A) and released from liposome (B) was calculated from the following equation:

$$A = [1 - (F_t - F_0)/(F_{100} - F_0)] \times 100,$$

$$B = [(F_t - F_0)/(F_{100} - F_0)] \times 100.$$

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