Dithiane-Based Photolabile Amphiphiles: Toward Photolabile Liposomes[1,2]

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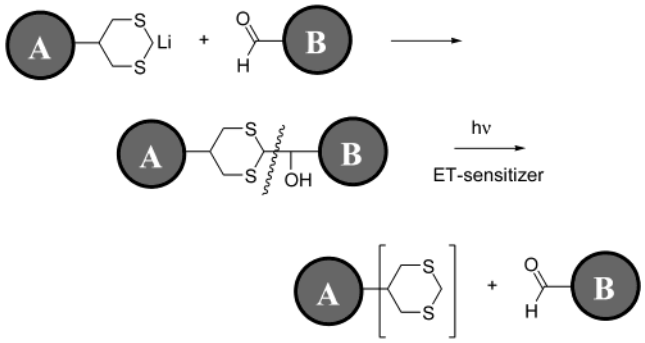
A series of externally and internally sensitized photolabile amphiphiles having their hydrophilic headgroups and hydrophobic tails tethered via the dithiane-based photocleavable "latch" is prepared. These lipids are tested in various formulations with naturally occurring phospholipids and/or cholesterol to prepare light-sensitive liposomes. Irradiation of such liposomes in phosphate-buffered saline solution (medium-pressure mercury lamp, Pyrex filter, $\lambda > 300$ nm) increases the bilayer permeability and accelerates the release of entrapped small organic molecules by up to an order of magnitude. A novel assay, based on ^{1}H or ^{19}F pulsed field gradient NMR measurements of diffusion coefficients, is developed to monitor the lifetimes of dark leakage and light-induced unloading of the probe molecules.

Introduction

Modulation of membrane permeability triggered by light offers an attractive alternative to temperature, pH, or chemically induced changes in bilayer properties. There are several general approaches developed for disrupting the integrity of the lipid bilayer photochemically: (i) photoinduced polymerization of polymerizable lipids diluted with polymorphic, nonpolymerizable co-lipid, causing phase separation in the bilayer and enhancing leakage at the boundaries of polymerized domains;[3] (ii) perturbation caused by significant conformational changes occurring as a result of photoisomerization in an azobenzene or alkene fragment incorporated into the lipid molecule;[4] (iii) photoinduced release of a masked hydrophilic group in the hydrophobic region of the bilayer membrane;[5] (iv) phototransformation of lipid headgroups, causing a considerable decrease in hydrophilicity, e.g., severing a hydrophilic headgroup via oxidative scission of a double bond.[6]

The photolabile lipids are then used to prepare vesicles capable of entrapping and releasing molecular objects upon irradiation. The driving force behind the extensive studies toward photolabile liposomes is their potential applications in controlled drug delivery. These research efforts have not yet produced an efficient and fully biocompatible prototype. However, some progress was achieved, especially in O'Brien's lab, where polymerization of sorbate-bearing amphiphiles was shown to significantly destabilize the two-component vesicles after rather short irradiation times.[3]

We have been developing a general approach to assembly and photoinduced disassembly of photolabile molecular systems which utilizes Corey−Seebach dithiane-aldehyde adducts as photolabile "molecular latches".



This methodology allows us to link various molecular blocks with latches that can be unfastened on demand via photoinduced electron transfer.[7] In this paper we report the synthesis of novel photolipids mimicking natural phosphatidylcholines, in which the hydrophilic phosphocholine headgroups are connected to the hydrophobic tails

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(1) This paper is dedicated to Professor David O'Brien in tribute of all his contributions to the field.

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via a photolabile, dithiane-based tether. The photocleavable unit can also be outfitted with the dual-purpose nitropyridinamino group, which serves as an internal ET-sensitizer and as a model element of molecular recognition.[7d] We show that such photolabile lipids can be used "as is" or in formulations with egg palmitoyl−oleoyl phosphatidylcholine (POPC) and cholesterol to form vesicles capable of unloading their contents upon irradiation. To monitor the release of small organic molecules entrapped in the photolabile liposomes, we have developed a simple PFG NMR assay, which is also discussed in this paper.

Experimental Section

Common reagents were purchased from the Sigma-Aldrich Chemical Co. and used without further purification. THF was refluxed over and distilled from potassium benzophenone ketyl prior to use. Egg palmitoyl−oleoyl phosphatidylcholine (POPC) and cholesterol were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). ^{1}H and ^{13}C NMR spectra were recorded at 25 °C on a Varian Mercury 400 MHz instrument. In $CDCl_3$, TMS was used as an internal standard; in D_2O, DOH was used as an internal standard. Column chromatography was performed on silica gel, 70−230 mesh ASTM. The irradiations were carried out in a carousel Rayonet photoreactor, with medium-pressure mercury UV lamps.

Synthetic Procedures. [1,3]Dithian-5-yl-methanol (4). *(a) Synthesis of Diethyl 2,2-Bis-(acetylthiomethyl)malonate.* Ten grams (18.9 mmol) of diethyl 2,2-bis-(tosyloxymethyl)malonate[8] and 4.75 g (41.6 mmol) potassium thioacetate in DMF (150 mL) were stirred at 80 °C for 6 h. DMF was removed under reduced pressure, and water (100 mL) and CH_2Cl_2 (100 mL) were added. The water layer was extracted with 100 mL of CH_2Cl_2, and the combined organic layers were washed two times with 100 mL of water and dried over Na_2SO_4. The solvent was removed under reduced pressure to give 5.85 g of oil. This was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.01 (q, J = 6.8 Hz, 4H), 3.33 (s, 4H), 2.15 (s, 6H), 1.09 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.7, 168.5, 62.2, 58.0, 31.1, 30.3, 14.0.

(b) Synthesis of [1,3]Dithiane-5-carboxylic Acid. A 5.0 g (14.9 mmol) portion of diethyl 2,2-bis-(acetylthio-methyl)malonate and 1.21 g of 37% formaldehyde (14.9 mmol) were refluxed in 70 mL of 2 N HCl for 1 day. The solvent was removed, and brine (10 mL) was added. This was extracted with 5 × 10 mL of CH_2Cl_2, and the combined extracts were dried over Na_2SO_4. The solvent volume was reduced under reduced pressure, causing crystallization. The crystals were collected by filtration to give 1.4 g of [1,3]dithiane-5-carboxylic acid (57.2%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.89 (d, J = 13.6 Hz, 1H), 3.56 (dt, J_1 = 13.6 Hz, J_2 = 1.2 Hz, 1H), 3.10−2.94 (m, 5H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 178.5, 41.5, 31.6, 31.0. Calcd for $C_5H_8O_2S_2$: C, 36.56%; H, 4.91%. Found: C, 36.67%; H, 4.99%.

(c) Synthesis of ***4****.* To a solution of 1 g (6.1 mmol) of [1,3]dithiane-5-carboxylic acid in 10 mL of THF, 6.1 mL (9.1 mmol) of BH_3−THF (1.5 M) was added dropwise at 0 °C. The mixture was stirred at room temperature overnight and then added slowly to 20 mL of saturated $NaHCO_3$ aqueous solution. The solution was extracted with 3 × 20 mL of CH_2Cl_2, and the combined CH_2Cl_2 was washed with 20 mL of water and then dried over Na_2SO_4. Removal of solvent gave 0.86 g of crude product. Chromatographic purification (silica gel, ethyl acetate−hexane, 1:1) gave 0.70 g of white solid (76.7%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.85 (d, J = 13.6 Hz, 1H), 3.69 (d, J = 6.8 Hz, 2H), 3.61 (d, J = 13.6 Hz, 1H), 2.91 (dt, J_1 = 13.2 Hz, J_2 = 1.2 Hz, 2H), 2.65 (dd, J_1 = 13.6 Hz, J_2 = 8.8 Hz, 2H), 2.15−2.07 (m, 1H), 1.83 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 65.9, 37.8, 32.1, 32.0. Calcd for $C_5H_{10}OS_2$: C, 39.97%; H, 6.71%. Found: C, 40.06%; H, 6.75%.

3,4-Bis(dodecyloxy)benzaldehyde (5b). To a solution of 3,4-dihydroxybenzaldehyde (2.76 g, 20 mmol) in 100 mL of anhydrous EtOH was added KOH (2.33 g, 41.6 mmol). The solution was stirred until KOH dissolved and 1-bromododecane (10.97 g, 44.0 mmol) was added. The reaction mixture was refluxed overnight. After that, it was cooled to room temperature and the solvent was removed under reduced pressure. Water was added, and the residue was extracted with diethyl ether (60 mL × 3). The organic extracts were combined, washed with 5% KOH (50 mL × 2), water (50 mL), and brine (50 mL), and dried with $MgSO_4$, and the solvent was removed. The product was recrystallized from EtOH (activated charcoal) to yield **5b** (2.0 g, 21.1%) as white flakes. ^{1}H NMR ($CDCl_3$): δ, 9.83 (s, 1H), 7.43−7.38 (m, 2H), 6.95 (d, J = 8 Hz, 1H), 4.08 (t, J = 7 Hz, 2H), 4.05 (t, J = 7 Hz, 2H), 1.90−1.80 (m, 4H), 1.52−1.18 (m, 36H), 0.88 (t, J = 7 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ, 191.7, 155.4, 150.2, 130.6, 127.3, 112.5, 111.7, 69.8, 69.8, 32.6, 30.4, 30.3, 30.1, 30.0, 29.8, 29.7, 26.7, 26.6, 23.4, 14.8.

4-{2-[(3,4-Bis(dodecyloxy)phenyl)hydroxymethyl]-[1,3]-dithian-5-yl}phenol (6b). To a solution of 4-[1,3]dithian-5-yl-phenol (**2**) (0.312 g, 1.47 mmol) in freshly distilled THF (15 mL) was added 2.3 mL of 1.6 M *n*-butyllithium (3.68 mmol) at −20 to −25 °C. The solution was stirred at this temperature for 2 h. The solution of lithiodithiane derivative was added slowly to a solution of 3,4-bis(dodecyloxy)benzaldehyde (**5b**) in 10 mL of THF at −78 °C, and the reaction mixture was stirred overnight at −20 °C. It was quenched with 20 mL of saturated NH_4Cl and extracted with diethyl ether (20 mL × 3). The organic extracts were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and the product was recrystallized from MeOH to give 0.910 g (90.4%) of off-white solid. ^{1}H NMR ($CDCl_3$): δ, 7.05 (d, J = 9 Hz, 2H), 7.00−6.84 (m, 3H), 6.78 (d, J = 9 Hz, 2H), 4.84 (d, J = 6 Hz, 1H), 4.47 (d, J = 6 Hz, 1H), 4.05−3.98 (m, 4H), 3.08−2.81 (m, 5H), 2.53 (s, broad, 1H), 1.88−1.76 (m, 4H), 1.52−1.24 (m, 36H), 0.89 (t, J = 7 Hz, 6H). ^{13}C NMR ($CDCl_3$): δ, 154.7, 149.4, 149.0, 136.8, 132.5, 127.8, 119.0, 115.6, 113.1, 112.0, 75.8, 69.3, 69.1, 54.9, 42.5, 36.6, 36.2, 3.9, 26.7, 29.6, 29.5, 29.4, 29.3, 26.0, 22.7, 14.1.

Photolabile Lipid (7b). To a cooled solution of phenol **6b** (473 mg, 0.690 mmol) and triethylamine (139 mg, 1.38 mmol) in dry benzene (10 mL) was added dropwise 2-chloro-2-oxo-1,3,2-dioxaphospholane (164 mg, 1.03 mmol). The procedure for preparing compound **35** was followed (see below) to give the product (0.112 g, 19.1%) as lightly yellow oil. ^{1}H NMR (CD_3OD): δ, 7.35−6.78 (m, 7H), 4.71 (d, J = 6.4 Hz, 1H), 4.52 (d, J = 6.4 Hz, 1H), 4.36−4.27 (m, 2H), 4.04−3.93 (m, 4H), 3.62−3.58 (m, 2H), 3.15 (s, 9H), 3.20−2.70 (m, 5H), 1.82−1.70 (m, 4H), 1.52−1.24 (m, 36H), 0.89 (t, J = 6.4 Hz, 6H). HRMS Calcd for $C_{46}H_{78}NO_7PS_2$ (MH^+): 852.5035. Found: 852.5015.

4-(Hexadecyloxy)-3-hydroxybenzaldehyde (12). A mixture of 3,4-dihydroxybenzaldehyde (4.14 g, 30 mmol), 1-bromohexadodecane (9.16 g, 36 mmol), and potassium carbonate (4.98 g, 36 mmol) in DMF (100 mL) was stirred at room temperature for 24 h. The solvent was removed under reduced pressure, and water (60 mL) and CH_2Cl_2 (60 mL) were added. The aqueous layer was extracted twice with CH_2Cl_2 (50 mL), and the combined organic layers were washed with water (100 mL). After the mixture was dried over Na_2SO_4, the solvent was removed under reduced pressure. The residue was recrystallized from $CHCl_3$/CH_3OH to give 4.53 g of the product (41.7%). ^{1}H (400 MHz, $CDCl_3$): δ 9.84 (s, 1H), 7.44 (d, J = 2.2 Hz, 1H), 7.41 (dd, J_1 = 8.1 Hz, J_2 = 2.2 Hz, 1H), 6.95 (d, J = 8.1 Hz, 1H), 5.71 (s, 1H), 4.13 (t, J = 6.6 Hz, 2H), 1.89−1.82 (m, 2H), 1.51−1.43 (m, 2H), 1.40−1.21 (m, 24H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 191.0, 151.3, 146.2, 130.4, 124.5, 114.0, 110.8, 69.3, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.0, 25.9, 22.7, 14.1. HRMS Calcd for $C_{23}H_{38}O_3Na^+$: 385.2713. Found: 385.2712.

4-(Hexadecyloxy)-3-(octadec-9-enyloxy)benzaldehyde (13). Oleyl bromide (1 g, 3.0 mmol) was added to a mixture of **12** (0.95 g, 2.62 mmol) and potassium carbonate (0.43 g, 3.6 mmol) in acetonitrile (20 mL). The mixture was refluxed for 24 h, and then the solvent was removed under reduced pressure. The residue was vigorously stirred with 20 mL of water and 20 mL of CH_2Cl_2, the layers were separated, and the organic layer was washed with water (20 mL) and dried over Na_2SO_4. The solvent was evaporated under reduced pressure, and the residue was recrystallized from $CHCl_3$/CH_3OH to give 1.2 g of **13** as a white solid (74.5%). ^{1}H (400 MHz, $CDCl_3$): δ 9.83 (s, 1H), 7.41 (dd, J_1 = 8.1 Hz, J_2 = 2.2 Hz, 1H), 7.39 (d, J = 2.2 Hz, 1H), 6.95 (d, J

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= 8.1 Hz, 1H), 5.35 (t, J = 5.1 Hz, 2H), 4.08 (t, J = 7.3 Hz, 2H), 4.05 (t, J = 6.6 Hz, 2H), 2.06−1.96 (m, 4H), 1.89−1.80 (m, 4H), 1.51−1.44 (m, 4H), 1.41−1.21 (m, 44H), 0.92−0.84 (m, 6H). ^{13}C (100 MHz, $CDCl_3$): δ 191.0, 154.7, 149.4, 129.9, 129.9, 129.8, 126.6, 111.8, 111.0, 69.1, 32.6, 31.9, 31.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.4, 29.3, 29.3, 29.1, 29.0, 27.2, 26.0, 25.9, 22.7, 14.1. HRMS Calcd for $C_{41}H_{72}O_3Na^+$: 635.5374. Found: 635.5372.

(4-(Hexadecyloxy)-3-(octadec-9-enyloxy)phenyl)(5-hydroxymethyl-[1,3]dithian-2-yl)methanol (14). To a solution of **4** (0.15 g, 1 mmol) in freshly distilled THF (10 mL) was added 1.4 mL of 1.6 M *n*-butyllithium (2.2 mmol) at −20 to −25 °C. The solution was stirred at this temperature for 2.5 h. Then a solution of **13** (0.613 g, 1 mmol) in THF (10 mL) was added at −78 °C and stirred for 2 h. The reaction mixture was allowed to warm overnight to room temperature. It was quenched with 15 mL of saturated NH_4Cl and extracted with CH_2Cl_2 (3 × 20 mL). The organic extracts were washed with water (30 mL) and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by chromatography (silica gel, ethyl acetate−hexane, 2:1) to give 0.74 g of **14** (94.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.94 (d, J = 2.0 Hz, 1H), 6.90 (dd, J_1 = 8.0 Hz, J_2 = 1.6 Hz, 1H), 6.84 (d, J = 8.4 Hz, 1H), 5.35 (t, J = 4.8 Hz, 2H), 4.81 (dd, J_1 = 7.2 Hz, J_2 = 2.8 Hz, 1H), 4.18 (d, J = 6.8 Hz, 1H), 4.01−3.96 (m, 4H), 3.60 (t, J = 5.6 Hz, 2H), 2.99−2.52 (m, 4H), 2.04−1.99 (m, 4H), 1.83−1.72 (m, 5H), 1.46−1.26 (m, 48H), 0.88 (t, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.6, 149.2, 132.9, 130.2, 130.0, 119.5, 113.4, 112.4, 75.3, 69.6, 69.4, 65.8, 54.4, 37.6, 32.1, 32.1, 31.5, 30.9, 30.0, 30.0, 29.9, 29.9, 29.9, 29.8, 29.8, 29.7, 29.6, 29.5, 27.4, 26.3, 22.9, 14.3. HRMS Calcd for $C_{46}H_{82}O_4S_2Na^+$: 785.5547. Found: 785.5572.

Photolabile Lipid (15). To a solution of **14** (0.52 g, 0.68 mmol) and triethylamine (96.0 mg, 0.95 mmol) in 20 mL of dry benzene was added dropwise 2-chloro-2-oxo-1,3,2-dioxaphospholane (132 mg, 0.75 mmol). The mixture was stirred at room temperature for 20 h. The hydrochloride was removed by filtration. The solvent was evaporated under reduced pressure to give a viscous oil. The oil was transferred to a pressure tube, and 15 mL of acetonitrile was added. Trimethylamine was added at −78 °C, and the tube was sealed. The mixture was heated at 60−65 °C for 2 days with stirring. The tube was cooled to −78 °C and opened. The solvent was removed under reduced pressure, and the residue was chromatographed on silica gel ($CHCl_3$−CH_3OH−H_2O = 65:25:2) to give 0.227 g of **14** and 0.24 g of **15** as a white solid (67.3% based on reacted **14**). ^{1}H NMR (400 MHz, CD_3OD): δ 7.01 (s, 1H), 6.89 (s, 2H), 5.35 (t, J = 4.4 Hz, 2H), 4.72 (d, J = 6.4 Hz, 1H), 4.30 (d, J = 6.4 Hz, 1H), 4.25 (m, 2H), 3.99 (m, 4H), 3.81 (t, J = 6.4 Hz, 2H), 3.63 (m, 2H), 3.21 (s, 9H), 2.96−2.60 (m, 4H), 2.07−2.00 (m, 5H), 1.81−1.74 (m, 4H), 1.51−1.46 (m, 4H), 1.43−1.22 (m, 44H), 0.90 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 150.4, 150.2, 135.9, 130.9, 130.9, 121.0, 114.9, 114.3, 76.7, 70.6, 69.8, 60.4, 55.8, 54.8, 38.5, 38.4, 33.1, 32.6, 32.3, 31.0, 30.9, 30.8, 30.8, 30.7, 30.6, 30.5, 30.5, 30.4, 28.2, 28.2, 27.4, 23.8, 14.5, 14.5. HRMS (MH^+) Calcd for $C_{51}H_{95}NO_7PS_2$: 928.62876, Found: 928.63547.

4-(Hexadecyloxy)-3-(octadec-9-enyloxy)benzophenone (17). *Octadec-9-enyl tosylate* was prepared as described[9] from oleyl alcohol and tosyl chloride in the presence of dry pyridine. ^{1}H (400 MHz, $CDCl_3$): δ 7.78 (d, J = 8.1 Hz, 2H), 7.34 (d, J = 8.1 Hz, 2H), 5.38−5.31 (m, 2H), 4.02 (t, J = 6.6 Hz, 2H), 2.45 (s, 3H), 2.05−1.93 (m, 4H), 1.66−1.59 (m, 2H), 1.37−1.15 (m, 22H), 0.88 (t, J = 6.6 Hz, 3H).

To a stirred mixture of 3,4-dihydroxybenzophenone (4.28 g, 20 mmol) and potassium carbonate (3.32 g, 24 mmol) in 50 mL of DMF, a solution of 1-bromohexadecane (6.1 g, 20 mmol) in 100 mL of DMF was added dropwise at room temperature. The reaction mixture was stirred at room temperature for 48 h and then poured into 150 mL of water. The precipitate was filtered, washed with water (2 × 50 mL), and dried. It was purified by chromatography on silica gel (hexane/ethyl acetate = 5:1) to give 4.12 g of 4-(hexadecyloxy)-3-hydroxybenzophenone (47.0%) and 3.13 g of 3,4-bis(hexadecyloxy)benzophenone (47.2%).

4-(Hexadecyloxy)-3-hydroxybenzophenone. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (dd, J_1 = 8.1 Hz, J_2 = 1.5 Hz, 2H), 7.56 (t, J = 8.1 Hz, 1H), 7.50−7.43 (m, 3H), 7.39 (dd, J_1 = 8.8 Hz, J_2 = 2.2 Hz, 1H), 6.89 (d, J = 8.8 Hz, 1H), 5.71 (s, 1H), 4.13 (t, J = 6.6 Hz, 2H), 1.86 (m, 2H), 1.51−1.22 (m, 26H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.3, 149.5, 145.1, 137.9, 131.6, 130.5, 129.5, 127.9, 123.6, 116.0, 110.2, 68.9, 31.7, 29.4, 29.4, 29.4, 29.3, 29.3, 29.1, 29.1, 28.8, 25.7, 22.5, 13.9. HRMS Calcd for $C_{29}H_{42}O_3Na^+$: 461.3026. Found: 461.3040.

3,4-Bis(hexadecyloxy)benzophenone. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.79−6.86 (m, 8H), 4.07 (t, J = 6.8 Hz, 2H), 4.05 (t, J = 6.8 Hz, 2H), 1.90−1.80 (m, 4H), 1.52−1.43 (m, 4H), 1.40−1.21 (m, 48H), 0.88 (t, J = 6.8 Hz, 6H). HRMS Calcd for $C_{45}H_{74}O_3Na^+$: 685.5530. Found: 685.5502.

A mixture of 4-hexadecyloxy-3-hydroxybenzophenone (1.41 g, 3.2 mmol), octadec-9-enyl tosylate (1.36 g, 3.2 mmol), and potassium carbonate (0.66 g, 4.8 mmol) in 100 mL of DMF was stirred at room temperature for 24 h. Water (100 mL) was added, and the precipitate was filtered, washed with water (50 mL), and dried to give 1.74 g (78.5%) of **17**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.76 (d, J = 6.6 Hz, 2H), 7.57 (t, J = 8.1 Hz, 1H), 7.50−7.44 (m, 3H), 7.35 (dd, J_1 = 8.1 Hz, J_2 = 2.2 Hz, 1H), 6.87 (d, J = 8.1 Hz, 1H), 5.40−5.33 (m, 2H), 4.07 (t, J = 6.6 Hz, 2H), 4.05 (t, J = 6.6 Hz, 2H), 2.05−1.93 (m, 4H), 1.90−1.80 (m, 4H), 1.52−1.43 (m, 4H), 1.42−1.20 (m, 44H), 0.91−0.85 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 195.6, 153.3, 148.8, 138.4, 131.7, 129.9, 129.9, 129.8, 129.7, 128.1, 125.4, 114.4, 111.4, 69.2, 69.0, 32.6, 31.9, 31.9, 29.8, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 29.3, 29.3, 29.2, 29.0, 27.2, 26.0, 26.0, 22.7, 14.1. HRMS Calcd for $C_{47}H_{76}O_3Na^+$: 711.5687. Found: 711.5641.

(4-(Hexadecyloxy)-3-(octadec-9-enyloxy)phenyl)(5-hydroxymethyl-[1,3]dithian-2-yl)phenylmethanol (18). Compound **18** was synthesized from **17** (0.69 g, 1.0 mmol) and **4** (0.15 g, 1.0 mmol) as described above for **14**, yield 0.54 g (64.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, J = 7.3 Hz, 2H), 7.29 (t, J = 7.3 Hz, 2H), 7.23−7.19 (m, 1H), 7.12 (d, J = 2.2 Hz, 1H), 7.05 (dd, J_1 = 8.6 Hz, J_2 = 2.2 Hz, 1H), 6.78 (d, J = 8.6 Hz, 1H), 5.40−5.31 (m, 2H), 5.04 (s, 1H), 3.98−3.89 (m, 4H), 3.43 (s, 1H), 3.40 (d, J = 6.2 Hz, 2H), 2.88−2.79 (m, 2H), 2.60−2.50 (m, 2H), 2.16 (br, 1H), 2.05−1.91 (m, 5H), 1.81−1.69 (m, 4H), 1.48−1.19 (m, 48H), 0.88 (t, J = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.4, 148.3, 143.7, 136.0, 129.8, 129.7, 127.8, 127.2, 126.1, 118.9, 112.9, 112.4, 79.7, 69.2, 68.8, 65.9, 59.2, 38.2, 32.5, 32.5, 31.8, 31.8, 29.7, 29.6, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.3, 29.2, 29.2, 29.2, 27.1, 27.1, 25.9, 25.9, 22.6, 14.0. HRMS Calcd for $C_{52}H_{86}O_4S_2Na^+$: 861.5860. Found: 861.5880.

Photolabile Lipid (19). Compound **19** was synthesized from **18** (0.45 g, 0.54 mmol) and 2-chloro-2-oxo-1,3,2-dioxaphospholane (0.15 g, 1.08 mmol) as described above for **15**. Yield 0.42 g (80.0%). ^{1}H NMR (400 MHz, CD_3OD): δ 7.54−6.75 (m, 8H), 5.39−5.28 (m, 2H), 5.16 (s, 1H), 4.28−4.14 (m, 3H), 3.93−3.84 (t, J = 6.0 Hz, 4H), 3.74 (t, J = 5.8 Hz, 2H), 3.59 (d, J = 7.3 Hz, 2H), 3.18 (s, 9H), 2.92−2.67 (m, 4H), 2.10−1.93 (m, 5H), 1.75−1.65 (m, 4H), 1.48−1.20 (m, 48H), 0.89−0.85 (m, 6H). ^{13}C NMR (100 MHz, CD_3OD): δ 149.7, 149.6, 146.3, 139.0, 131.6, 130.9, 130.9, 128.7, 128.0, 121.2, 115.3, 114.4, 81.3, 70.6, 70.4, 66.1, 60.9, 60.5, 60.4, 54.8, 45.44, 38.9, 33.6, 33.2, 33.2, 31.0, 31.0, 30.9, 30.8, 30.8, 30.7, 30.7, 30.6, 30.6, 30.5, 28.3, 28.3, 27.5, 27.4, 23.9, 14.7. HRMS Calcd for $C_{57}H_{98}NO_7PS_2Na^+$: 1026.6414. Found: 1026.6474.

4-(Hexadecyloxy)-5-hydroxy-2-nitrobenzaldehyde (21). To a solution of aldehyde **12** (5.22 g, 14.4 mmol) and a catalytic amount of $NaNO_2$ in 150 mL of CH_2Cl_2 and 10 mL of acetic acid, 70% nitric acid (1.1 mL) was added dropwise at 0 °C. The reaction was stirred at 0 °C for 5 h and then was poured into 200 mL of ice water. The organic layer was separated, washed with 3 × 50 mL of water, and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was separated by silica gel chromatography (hexanes−ethyl acetate, 15:1) to give 2.12 g of **21** (36.1%) and 1.92 g of **22** (32.7%)

*1,2,4,5-Isomer **21***. ^{1}H (400 MHz, $CDCl_3$): δ 10.38 (s, 1H), 7.63 (s, 1H), 7.43 (s, 1H), 6.66 (br, 1H), 4.22 (t, J = 6.6 Hz, 2H), 1.94−1.87 (m, 2H), 1.54−1.45 (m, 2H), 1.40−1.18 (m, 24H), 0.88 (t, J = 6.6 Hz, 3H). ^{13}C (100 MHz, $CDCl_3$): δ 187.8, 150.5, 149.0, 143.0, 126.4, 114.2, 107.7, 70.2, 31.8, 29.6, 29.6, 29.5, 29.5, 29.4, 29.3, 29.2, 28.7, 25.7, 22.6, 14.0. HRMS Calcd for $C_{23}H_{37}NO_5Na^+$: 430.2564. Found: 430.2551.

*1,2,3,4-Isomer **22***. ^{1}H (400 MHz, $CDCl_3$): δ 10.05 (s, 1H), 8.37 (br, 1H), 7.46 (d, J = 8.4 Hz, 1H), 7.09 (d, J = 8.4 Hz, 1H), 4.16 (t, J = 6.6 Hz, 2H), 1.93−1.85 (m, 4H), 1.49−1.46 (m, 4H), 1.37−

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1.23 (m, 20H), 0.88 (t, $J = 7.0$ Hz, 3H). HRMS Calcd for $C_{23}H_{37}NO_5Na^+$: 430.2564. Found: 430.2576.

4-(Hexadecyloxy)-2-nitro(5-octadec-9-enyloxy)benzaldehyde (23). To a solution of **21** (0.37 g, 0.91 mmol) and octadec-9-enyl tosylate (0.46 g, 1.1 mmol) in 15 mL of DMF, potassium carbonate (0.304 g, 2.2 mmol) was added. The mixture was stirred under N_2 at 110−120 °C for 36 h. The solvent was removed under reduced pressure, then 20 mL of water and 20 mL of CH_2Cl_2 was added. The water layer was separated and extracted with 2 × 20 mL of CH_2Cl_2. The combined CH_2Cl_2 solutions were washed with 20 mL water and dried over Na_2SO_4. The solvent was removed under reduced pressure, and the residue was purified by chromatography (silica gel, hexanes−ethyl acetate, 20:1) to give 0.17 g of **23** (28.3%). ^{1}H (400 MHz, $CDCl_3$): δ 10.43 (s, 1H), 7.58 (s, 1H), 7.38 (s, 1H), 5.41−5.31 (m, 2H), 4.13 (t, $J = 6.6$ Hz, 4H), 2.06−1.95 (m, 4H), 1.92−1.83 (m, 4H), 1.53−1.44 (m, 4H), 1.42−1.19 (m, 44H), 0.88 (t, $J = 6.6$ Hz, 6H). ^{13}C (100 MHz, $CDCl_3$): δ 187.9, 153.2, 152.3, 143.6, 130.0, 129.7, 125.3, 110.6, 108.1, 69.8, 69.7, 31.9, 29.8, 29.7, 29.7, 29.6, 29.5, 29.5, 29.4, 29.4, 29.3, 29.2, 29.2, 29.2, 28.8, 28.8, 27.2, 27.2, 25.8, 22.7, 14.1. HRMS Calcd for $C_{41}H_{71}NO_5Na^+$: 680.5224. Found: 680.5249.

(4-(Hexadexyloxy)-2-nitro-5-(octadec-9-enyloxy)phenyl)-(5-hydroxymethyl-[1,3]-dithian-2-yl)methanol (24). Compound **24** was prepared analogously to compound **14** using **23** (0.11 g, 0.17 mmol) and **4** (50.1 mg, 0.334 mmol) in THF, yield 56 mg (41.4%). ^{1}H (400 MHz, $CDCl_3$): δ 7.61 (s, 1H), 7.28 (s, 1H), 5.81 (d, $J = 5.1$ Hz, 1H), 5.35 (m, 2H), 4.36 (d, $J = 5.1$ Hz, 1H), 4.16−3.99 (m, 4H), 3.65 (d, $J = 6.6$ Hz, 2H), 3.14 (br, 1H), 3.03−2.49 (m, 4H), 2.10−1.92 (m, 6H), 1.88−1.80 (m, 4H), 1.51−1.20 (m, 48H), 0.88 (t, $J = 6.6$ Hz, 6H). ^{13}C (100 MHz, $CDCl_3$): δ 153.4, 148.1, 139.8, 130.7, 129.9, 129.7, 111.5, 109.4, 77.2, 70.6, 69.4, 65.4, 53.1, 37.0, 31.9, 31.5, 30.6, 29.7, 29.7, 29.6, 29.6, 29.5, 29.5, 29.3, 29.3, 29.2, 28.9, 28.9, 27.2, 25.9, 25.9, 22.6, 14.1. HRMS Calcd for $C_{46}H_{81}NO_6S_2Na^+$: 830.5397. Found: 830.5333.

Photolabile Lipid (25). Compound **25** was synthesized analogously to compound **15** from **24** (110 mg, 0.14 mmol) and 2-chloro-2-oxo-1,3,2-dioxaphospholane (39 mg, 0.28 mmol), yield 63.4 mg (47.9%). ^{1}H (400 MHz, THF-d8/D_2O): δ 7.52 (s, 1H), 7.40 (s, 1H), 5.65 (d, $J = 4.2$ Hz, 1H), 5.36−5.28 (m, 2H), 4.32 (d, $J = 4.2$ Hz, 1H), 4.27 (br, 2H), 4.08 (t, $J = 6.0$ Hz, 2H), 3.98 (t, $J = 6.2$ Hz, 2H), 3.76 (br, 2H), 3.68 (br, 2H), 3,25 (s, 9H), 3.02−2.88 (m, 2H), 2.69−2.45 (m, 2H), 2.18−1.92 (m, 5H), 1.84−1.73 (m, 4H), 1.57−1.18 (m, 48H), 0.85 (t, $J = 6.2$ Hz, 6H). ^{13}C (100 MHz, CD_3OD): δ 154.1, 148.6, 140.5, 134.0, 130.5, 130.4, 113.0, 109.7, 71.3, 70.0, 69.9, 69.0, 60.2, 60.1, 55.2, 54.9, 37.6, 32.7, 30.7, 30.6, 30.5, 30.5, 30.5, 30.3, 30.3, 30.2, 30.1, 30.1, 30.0, 30.0, 28.0, 27.9, 27.0, 26.9, 23.4, 14.4. HRMS Calcd for $C_{51}H_{93}N_2O_9PS_2Na^+$: 995.5952. Found: 995.6004.

5,5-Bis(dodecyloxymethyl)-[1,3]dithiane (27b). To a solution of (5-hydroxymethyl-[1,3]dithian-5-yl)methanol (**3**) (0.682 g, 3.78 mmol) in DMF was added NaH (0.273 g, 60% dispersion in mineral oil, 11.4 mmol, washed twice with hexanes before use). After the gas evolution ceased, 1-bromododecane (2.36 g, 9.48 mmol) was added. The reaction mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure, 20 mL of water was added, and the product was extracted with CH_2Cl_2 (20 mL × 3). The organic phase was dried with $MgSO_4$ and concentrated. Crude product was obtained as a colorless oil (1.83 g, 93.8%), which solidified after several days. It was used without further purification. ^{1}H NMR ($CDCl_3$): δ 3.65 (s, 2H), 3.54 (s, 4H), 3.42 (t, $J = 7$ Hz, 4H), 2.75 (s, 4H), 1.63−1.50 (m, 4H), 1.38−1.16 (m, 36H), 0.88 (t, $J = 7$ Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 72.6, 71.6, 34.9, 33.8, 32.0, 31.8, 29.8, 29.7, 29.6, 29.5, 26.3, 22.8, 14.3.

***C*-(5,5-Bis(dodecyloxymethyl)-[1,3]dithian-2-yl)-*C*-[4-(tetrahydropyran-2-yloxy)phenyl]methylamine (32).** A solution of 0.431 g (2.67 mmol) of 1,1,1,3,3,3-hexamethyldisilazane in 15 mL of freshly distilled THF was cooled to 0 °C and 1.8 mL of *n*-butyllithium (1.6 M solution in hexanes, 2.88 mmol) was added while stirring under N_2 atmosphere. The reaction mixture was stirred at this temperature for 1 h. Then, 0.551 g (2.67 mmol) of 4-(tetrahydropyran-2-yloxy)benzaldehyde was added slowly, and the resulting mixture was stirred at 0 °C for 1 h. The lithiated 5,5-bis(dodecyloxymethyl)-[1,3]dithiane (**27b-Li**) was prepared by adding 1.8 mL of 1.6 M *n*-butyllithium (2.88 mmol) to a solution of 1.38 g (2.67 mmol) of **27b** in 15 mL of freshly distilled THF at −20 to −25 °C and stirring at this temperature for 2 h. The solution of lithiodithiane derivative was added slowly to the solution of silylated 4-(tetrahydropyran-2-yloxy)benzaldimine (**31**), and the reaction mixture was stirred overnight at −20 °C. It was quenched with 20 mL of saturated NH_4Cl and extracted with diethyl ether (20 mL × 3). The organic extracts were combined and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, furnishing **32** (1.73 g, 90%) as a yellow oil. It was used without further purification.

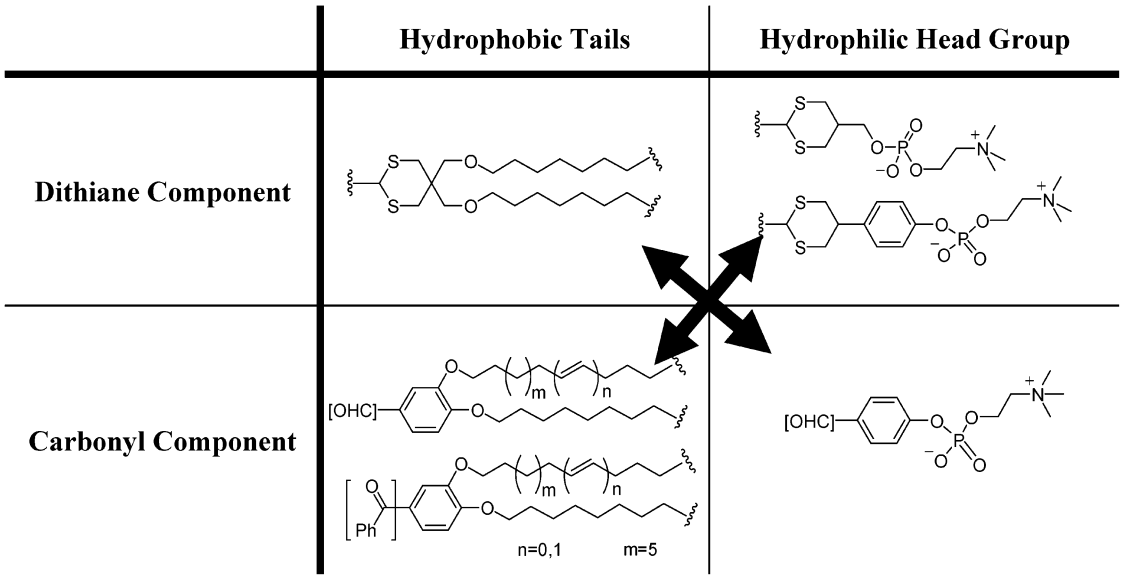
{(5,5-Bis-(dodecyloxy)methyl-[1,3]dithian-2-yl)[4-(tetrahydropyran-2-yloxy)phenyl]methyl}(5-nitropyridin-2-yl)amine (34-OTHP). The aminopyridine derivative was obtained by boiling **32** (1.27 g, 1.76 mmol) and 2-fluoro-5-nitropyridine (0.375, 2.64 mmol) in 2-propanol for 18 h in the presence of triethylamine (0.267 g, 2.64 mmol). The solvent was then removed under reduced pressure, and the reaction mixture was chromatographed (silica gel, hexanes−ethyl acetate, 5:1) to give a yellow solid, 0.964 g, 64.8%. ^{1}H NMR ($CDCl_3$): δ 9.00 (d, $J = 2.8$ Hz, 1H), 8.11 (dd, $J_1 = 2.8$ Hz, $J_2 = 8.8$ Hz, 1H), 7.27 (d, $J = 8.4$ Hz, 2H), 7.03 (d, $J = 8.4$ Hz, 2H), 6.26−6.20 (m, 2H), 5.42−5.37 (m, 1H), 5.15 (s, broad, 1H), 4.80 (dd, $J_1 = 1$ Hz, $J_2 = 6$ Hz, 1H), 3.92−3.84 (m, 1H), 3.78−3.55 (m, 1H), 3.66 (s, 2H), 3.43 (t, $J = 6$ Hz, 2H), 3.37 (t, $J = 6$ Hz, 2H), 3.30 (s, 2H), 2.86−2.65 (m, 4H), 2.02−1.92 (m, 1H), 1.87−1.79 (m, 2H), 1.72−1.45 (m, 7H), 1.36−1.19 (m, 36H), 0.88 (t, $J = 6.4$ Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 160.3, 157.2, 146.7, 136.4, 133.0, 130.8, 127.9, 116.4, 96.3, 74.3, 71.6, 71.5, 69.5, 62.0, 53.0, 34.7, 33.8, 33.5, 31.9, 30.3, 29.6, 29.4, 29.3, 26.2, 26.1, 22.7, 18.7, 14.1.

4-[(5,5-Bis(dodecyloxy)methyl-[1,3]dithian-2-yl)(5-nitropyridin-2-ylamino)methyl]phenol (34-OH). A solution of **34-OTHP** (0.877 g, 1.04 mmol) and pyridinium *p*-toluenesulfonate (78 mg, 0.3 mmol) in 20 mL of ethanol was stirred at 55 °C for 3 h. The solvent was evaporated under reduced pressure, and the residue was chromatographed on silica gel column (hexanes−ethyl acetate, 4:1) to afford a yellow solid, 0.580 g, 73.3%. ^{1}H NMR ($CDCl_3$): δ 8.99 (d, $J = 3.0$ Hz, 1H), 8.12 (dd, $J_1 = 3.0$ Hz, $J_2 = 9.6$ Hz, 1H), 7.21 (d, $J = 8.8$ Hz, 2H), 6.78 (d, $J = 8.8$ Hz, 2H), 6.30 (d, $J = 9.6$ Hz, 1H), 6.27−6.21 (m, 1H), 5.51 (s, 1 Hz), 5.15 (s, broad, 1H), 4.37 (d, $J = 6$ Hz, 1H), 3.67−3.62 (m, 2H), 3.43 (t, $J = 7$ Hz, 2H), 3.37 (t, $J = 7$ Hz, 2H), 3.32−3.27 (m, 2H), 2.85−2.68 (m, 4H), 1.59−1.46 (m, 4H), 1.37−1.20 (m, 36H), 0.88 (t, $J = 6.4$ Hz, 6H). ^{13}C NMR ($CDCl_3$): δ 160.3, 155.8, 146.6, 132.4, 133.1, 130.0, 128.2, 115.6, 74.4, 71.7, 71.6, 69.5, 53.0, 34.8, 33.8, 33.5, 31.9, 29.7, 29.7, 29.6, 26.5, 29.4, 29.3, 26.2, 26.1, 22.7, 14.1.

Photolabile Lipid (35). To a cooled solution of the phenol **34-OH** (499 mg, 0.659 mmol) and triethylamine (93 mg, 0.922 mmol) in dry benzene (10 mL) was added dropwise 2-chloro-2-oxo-1,3,2-dioxaphospholane (128 mg, 0.725 mmol). The mixture was stirred at room temperature for 10 h. The crystalline $Et_3N\cdot$HCl was removed by filtration. The solvent was evaporated under reduced pressure to give an oil. The oil was transferred to a pressure tube, and 10 mL of CH_3CN was added. Trimethylamine (1.5 mL) was added at −78 °C, and the tube was sealed. It was heated at 60−65 °C for 2 days with stirring. The tube was cooled to −78 °C and opened. The solvent was removed under reduced pressure. The residue was chromatographed on silica gel ($CHCl_3$−MeOH−H_2O = 65:25:4), and the product (0.24 g, 39.4%) was obtained as a slightly brown oil. ^{1}H NMR (CD_3OD): δ 8.87 (d, $J = 3.0$ Hz, 1H), 8.06 (dd, $J_1 = 3.0$ Hz, $J_2 = 9.6$ Hz, 1H), 7.40 (d, $J = 8.8$ Hz, 2H), 7.20 (d, $J = 8.8$ Hz, 2H), 6.57 (d, $J = 9.6$ Hz, 1H), 5.58 (s, broad, 1H), 4.35−4.26 (m, 2H), 3.64−3.55 (m, 4H), 3.45−3.34 (m, 8H), 3.12 (s, 9H), 2.81−2.60 (m, 4H), 1.60−1.47 (m, 4H), 1.40−1.20 (m, 36H), 0.88 (t, $J = 6.4$ Hz, 6H). HRMS Calcd for $C_{47}H_{81}N_4O_8PS_2$ (MH^+): 925.5312. Found: 925.5294.

1-Methyl-4-(trifluoromethyl)pyridinium Chloride (36). To a solution of 4-(trifluoromethyl)pyridine (0.200 g, 1.36 mmol) in 10 mL of CH_3CN was added MeI (1.00 g, 7.04 mmol). The reaction mixture was stirred at room temperature overnight. The solvent and excess amount of MeI were removed under reduced pressure. The yellow solid obtained was eluted through an ion-exchange column (AG 21K anion-exchange resin chloride form 50−100 mesh). Water was removed to give a hygroscopic colorless solid (0.250 g, 93%). ^{1}H NMR (D_2O): δ 9.17 (d, $J = 7$ Hz, 2H), 8.48 (d, $J = 7$ Hz, 2H), 4.57 (s, 3H). ^{19}F NMR gives a sharp singlet 54.0 ppm downfield of KF in PBS buffer. (Aqueous

Chart 1



F^- is −125.3 from $CFCl_3$, i.e., **36** has a resonance at −71.3 ppm with respect to $CFCl_3$).

Bispyridinium Bromide (37). To a solution of 4-(trifluoromethyl)pyridine (0.390 g, 2.65 mmol) in 10 mL of CH_3CN was added 1,3-dibromopropane (0.267 g, 1.33 mmol). The reaction mixture was refluxed for 2 days. The white precipitate thus formed was collected by filtration and used without further purification (0.125 g, 19.0%). ^1H NMR (D_2O): δ 9.32 (d, $J = 7$ Hz, 2H), 8.56 (d, $J = 7$ Hz, 2H), 5.06−4.98 (m, 4H), 3.00−2.88 (m, 2H).

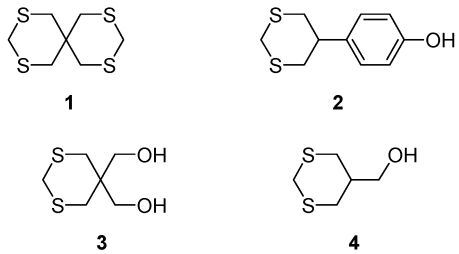
Liposomes Leakage Studies. Liposomes were prepared by the extrusion technique developed by MacDonald.[10] A representative example (photolabile amphiphile **35**) is as follows: **35**, POPC (egg), and cholesterol (20:50:30) in chloroform were dried under reduced pressure overnight. The lipid was hydrated in a 0.15 M PBS solution (pH = 7.0, 10% D_2O for fluorine containing probes, or 50% D_2O for TMSNa probe) containing 0.25 M probe compound at 55 °C for 2 h and subjected to four freeze−thaw cycles in a dry ice/ethanol bath. The multilamellar vesicles were extruded through a 100 nm pore size polycarbanate membrane mounted in the miniextruder (Avanti Polar Lipids) at 55 °C 21 times. The bulk probe molecules were removed by elution through a gel-filtration column (Sephadex g-75 40−120 μm) with 0.40 M PBS (pH = 7.0, 10% D_2O for fluorine containing probes or 50% D_2O for TMSNa probe).

TEM of Stained Liposomes. Lipid vesicles prepared as described above were diluted 1:100 in the same PBS buffer. Four microliters of the solution was applied to 400 mesh Formvar carbon coated copper grids (Electron Microscopy Sciences) and allowed to settle for 2 min before wicking away excess buffer. The sample was then rinsed and stained with 1% uranyl acetate for 30 s. The samples were viewed and photographed at 20000× magnification with a Hitachi 7000 transmission electron microscope.

The diffusion measurements (PFG-LED technique) were carried out with a Varian Mercury 400 MHz spectrometer equipped with a Performa I PFG module and a PFG-capable, 4 nuclei autoswitchable probe. The PFG module is capable of forming PFG pulses up to 21 G/cm strength, which was sufficient to drive the signal amplitude of free probe molecules to zero. We used a modified watersLED sequence (with water-suppression turned off completely).

Results and Discussion

Synthesis of Photolabile Amphiphiles. Earlier we synthesized several substituted dithianes including compounds **1**−**3**,[7e] which are capable of forming Corey−Seebach adducts with carbonyl compounds and at the same time are suitable for tethering the necessary molecular blocks through position 5 of the dithiane ring. In this study we used compounds **2** and **3** and also synthesized 5-hydroxymethyldithiane (**4**) to tether the hydrophilic headgroup and the hydrophobic tails.



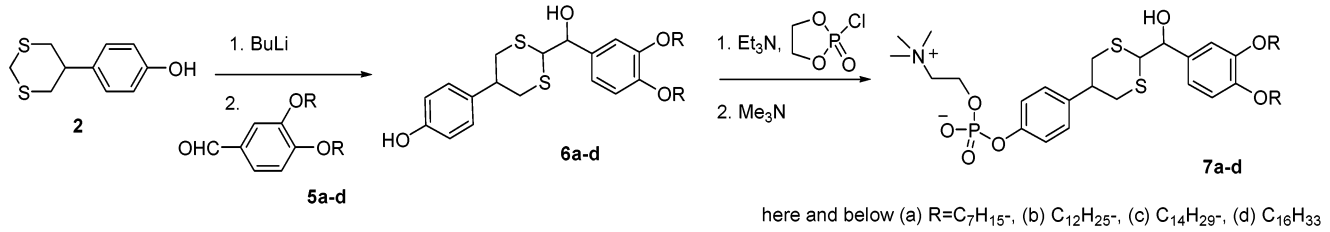
In general, dithiane-based photolabile amphiphiles can be synthesized either by having the dithiane unit carry the hydrophilic headgroup, while the hydrophobic tails are attached to the carbonyl component (for example, 3,4-dihydroxybenzaldehyde), or, conversely, with the aldehyde carrying the hydrophilic phosphatidyl choline moiety and the dithianyl component being outfitted with hydrophobic alkyl chains (Chart 1). For a hydrophobic dithiane, we used alkylated bis(hydroxymethyl)dithiane (**3**),[7e] whereas (hydroxyphenyl)dithiane (**2**) and (hydroxymethyl)dithiane (**4**) were employed to carry the phosphocholine hydrophilic headgroup. For the carbonyl part, hydrophilic modules were built based on 4-hydroxybenzaldehyde, while 3,4-dihydroxybenzaldehyde and 3,4-dihydroxybenzophenone were used to synthesize the hydrophobic carbonyl components.

The first series of amphiphiles with hydroxyphenyl dithiane carrying the phosphocholine group was synthesized according to Scheme 1. 3,4-Dihydroxybenzaldehyde was alkylated with heptyl, dodecyl, tetradecyl, and hexadecyl hydrophobic tails and reacted with bis-lithiated **2**. Phenol **6** was outfitted with phosphocholine via a standard procedure[11] involving reaction with 1-chlorophosphadioxolane and ring opening of the phosphadioxolane cycle with trimethylamine in a sealed tube at 60−65 °C.

(10) MacDonald, R. C. *Biochim. Biophys. Acta* **1991**, *1061*, 297.

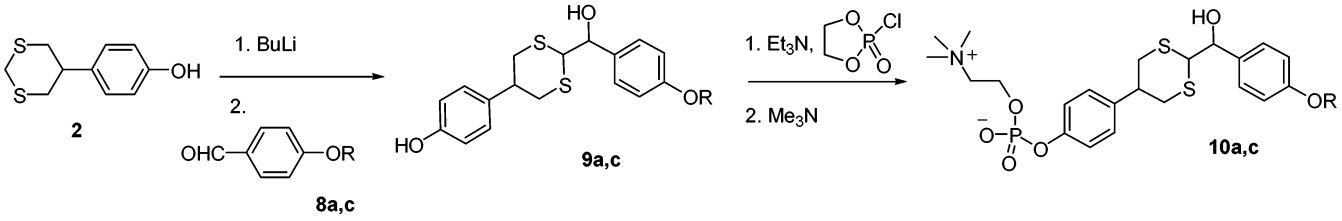
(11) (a) Roodsari, F. S.; Wu, D.; Pum, G. S.; Hajdu, J. *J. Org. Chem.* **1999**, *69*, 7727. (b) Menger, F. M.; Chen, X. Y.; Brocchini, S.; Hopkins, H. P., Hamilton, D. *J. Am. Chem. Soc.* **1993**, *115*, 6600.

Scheme 1. Synthesis of Photolabile Amphiphiles Based on Hydroxyphenyl Dithiane

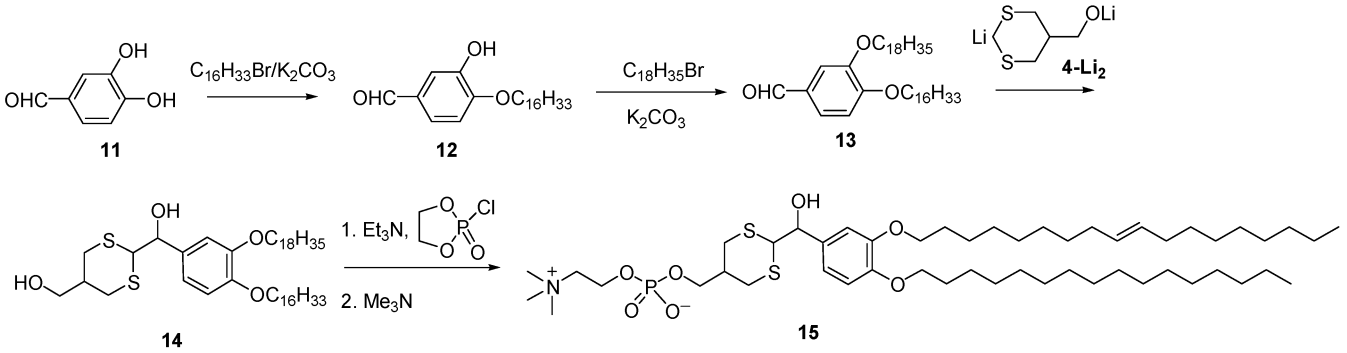


here and below (a) R=C_7H_{15}-, (b) $C_{12}H_{25}$-, (c) $C_{14}H_{29}$-, (d) $C_{16}H_{33}$

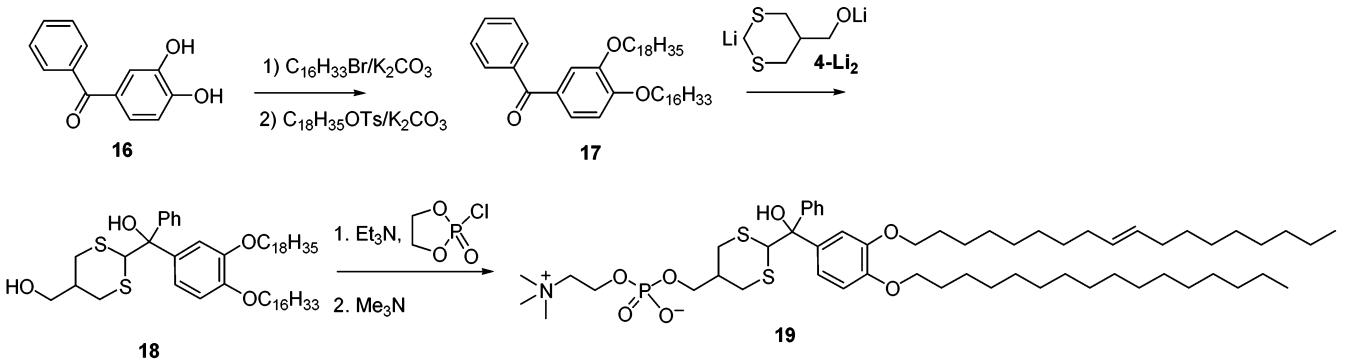
Scheme 2. Single-tailed Amphiphiles



Scheme 3. Synthesis of Photolabile Amphiphiles Based on Hydroxymethyl Dithiane



Scheme 4. Synthesis of Dihydroxybenzophenone-Based Amphiphile 19



Amphiphiles bearing one hydrophobic alkyl chain were similarly synthesized (Scheme 2).

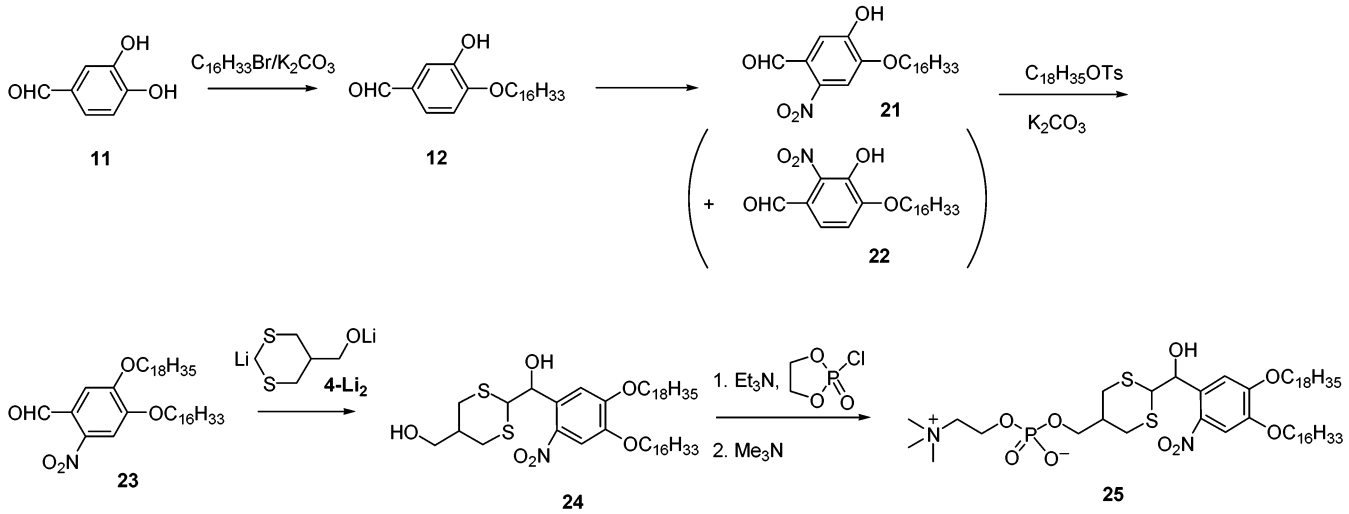
Due to a significant difference in pK_a, alkylation of the two hydroxy groups of 3,4-dihydroxybenzaldehyde can be carried out in a stepwise manner. This provided ready access to amphiphiles possessing two different hydrophobic alkyl/alkenyl chains, e.g., hexadecane/octadecene in benzaldehyde **13**. Thus, compound **15** was synthesized via addition of bis-lithiated 5-hydroxymethyldithiane to **13** with subsequent phosphorylation (Scheme 3).

Amphiphile **19** was synthesized from 3,4-dihydroxybenzophenone (**16**) in a similar fashion (Scheme 4).

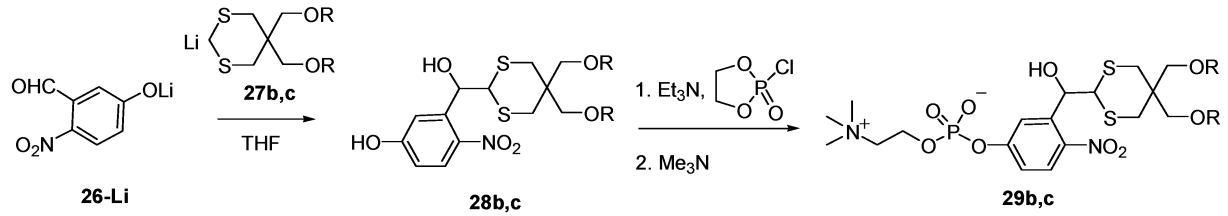
Two types of nitro-substituted self-sensitized systems were also obtained. Schemes 5 and 6 show syntheses of amphiphiles possessing the *o*-nitrobenzaldehyde moiety in their structure, bearing either lipophilic alkyl chains or hydrophilic phosphocholine. In the synthesis of **25** (Scheme 5), to improve the regioselectivity of nitration, the 4-hydroxy group of 3,4-dihydroxybenzaldehyde (**11**) was alkylated, leaving the 3-hydroxy group free. Nitration of **12** with $HNO_3/NaNO_2$ in CH_2Cl_2/AcOH produced the desired 1,2,4,5-isomer **21**, although the 1,2,3,4-isomer **22** was also formed along with traces of a dinitro compound. After chromatographic separation, the residual hydroxy group in **21** was alkylated and the aldehyde was reacted with bis-lithiated 5-hydroxymethyldithiane (**4**). The phosphocholine moiety was introduced as outlined above.

Another nitro-group-containing amphiphile, **29**, was obtained from precursor **28**, which was synthesized by coupling lithium phenolate of 3-hydroxy-6-nitrobenzaldehyde (**26**) with bis-alkylated 5,5-bis(hydroxymethyl)-dithiane (**27**). Lithiation of nitrobenzaldehyde (**26**) prior to dithiane addition not only prevented wasting an extra mole of dithiane but also reduced the undesired electron-

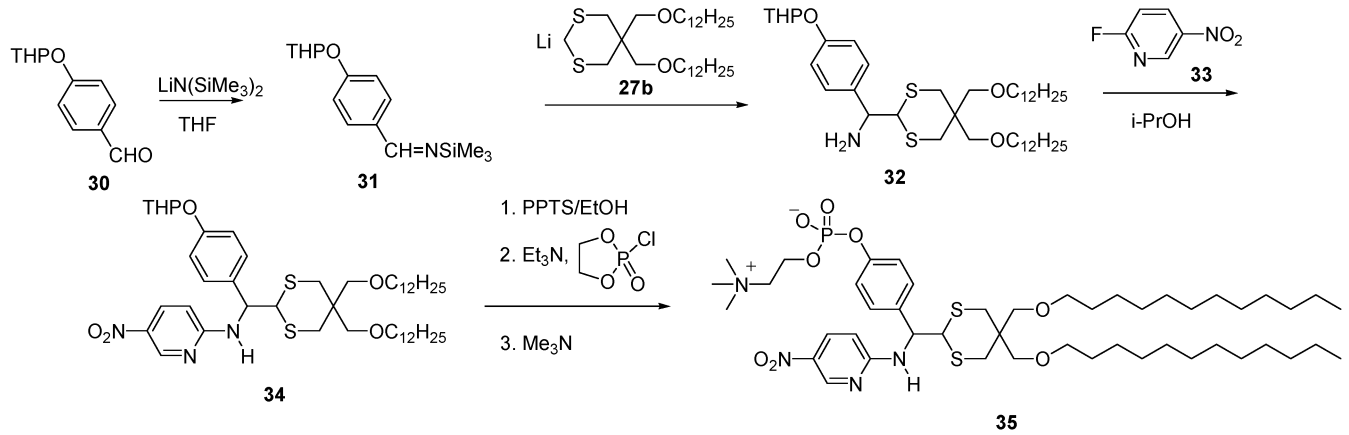
Scheme 5



Scheme 6



Scheme 7. Synthesis of Nitropyridine-Based Self-Sensitized Photolabile Amphiphile 35



transfer reduction of the nitrobenzaldehyde by the dithianyl carbanion, while not affecting the efficiency of addition to the cross-conjugated carbonyl group (Scheme 6).

Finally, we synthesized the nitropyridine-based amphiphile **35** (Scheme 7), which not only is self-sensitized but also contains a model molecular recognition motif, aminopyridine. Lithiated bis(alkoxymethyl)dithiane was first reacted with N-sylilated imine **31** to furnish amine **32**. Imine **31** was generated in situ by treating THP-protected 4-hydroxybenzaldehyde (**30**) with LHMDS in THF. Amine **32** was then reacted with 2-fluoro-5-nitropyridine in an aromatic nucleophilic substitution to give aminopyridine **34**, which was deprotected with PPTS/ethanol and outfitted with the phosphocholine headgroup.

Liposomes carrying entrapped small probe molecules were then prepared from a two- or three-component mixture of photolipids with cholesterol, sensitizer (if not self-sensitized) and natural egg-POPC. First, a thoroughly dried lipid mixture film was hydrated with 0.15 M phosphate buffered saline solution (PBS, pH = 7.0) containing the probe molecule. The suspension was subjected to four freeze−thaw cycles to disrupt large multilamellar vesicles (LMV) and extruded 21 times through a polycarbonate filter with 100 nm pores to form large unilamellar vesicles (LUV). Subsequent gel filtration on Sephadex was carried out to remove untrapped probes (see below).

Monitoring the Leakage by PFG NMR. Conventional assays of liposome leakage are based on fluorescence recovery derived from reduced efficiency of collisional quenching in the bulk solution as opposed to nearly total quenching inside the vesicle due to the higher internal concentration of fluorophore quencher.[12,3b,c,g] Most commonly the ANTS (8-aminonaphthalene-1,3,6-trisulfonic acid)−DPX (*p*-xylenebis(pyridinium bromide)) fluorescence recovery assay, first introduced by Ellens and Bentz, is used. While this is a useful assay for studying liposome

(12) Ellens, H.; Bentz, J.; Szoka, F. C. *Biochemistry* **1984**, *23*, 1532.

leakage induced by changes in pH, temperature, and other nonradiative chemical processes, we detected considerable photobleaching of several fluorophores during the photochemical experiments, rendering fluorescent assays less suitable for quantitative monitoring of photoinitiated release. Also, light absorption by the probe molecule may interfere with the desired photochemistry, especially when accurate quantum yield measurements are required. We suggest that these shortcomings in photochemical experiments can be overcome by utilizing the pulsed field gradients (PFG) NMR technique[13] for determining the self-diffusion coefficients of small probe molecules which do not have any interfering UV absorption. Conceptually, the PFG assay is very straightforward. When the probe is confined inside the vesicle, its apparent diffusion coefficient is equal to that of the carrier liposome. It changes by orders of magnitude, depending on the hydrodynamic size ratio, when the probe is released into the bulk solution. We further suggest using probes that carry fluorine-containing groups, e.g., trifluoromethyl, for easy detection with ^{19}F PFG NMR.[14] In such a case, the monitoring is reduced to observing one signal with no interference from other peaks and no need for water suppression.

The PFG diffusion measurements were carried out with Varian Mercury 400 MHz NMR spectrometer equipped with Performa I PFG module and a PFG-enabled probe, which is capable of forming PFG pulses of up to 21 G/cm strength. This was sufficient to drive the signal amplitude of free probes to zero. In these studies we employed a modified watersLED sequence, which is a stimulated spin−echo technique and is more sophisticated than the original spin−echo experiment by Stejskal and Tanner.[13b]

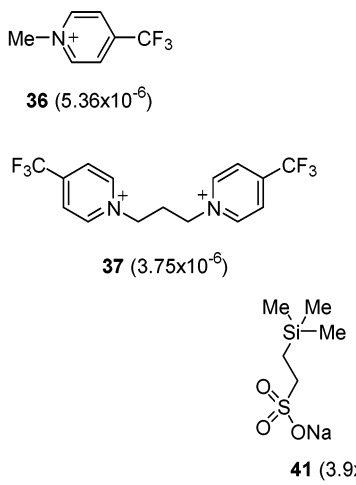
The echo amplitude in a PFG NMR experiment is a Gaussian function of the applied field strength and is related to the diffusion coefficient by the expression

$$A = A_o \exp[-(\gamma\delta G)^2(\Delta - \delta/3)D_s] \qquad (1)$$

where γ is the magnetogyric ratio, δ is the length of pulsed field gradient, g is the gradient strength, Δ is the diffusion time, and D_s is the diffusion coefficient.[13]

Chart 2 shows several cationic and anionic probes that we tested for ^{19}F (**36**−**40**) and ^{1}H (**41**) PFG NMR monitoring of membrane permeability. Small anions (**38**−**40**) showed unacceptably high rates of dark leakage,[15] even when control experiments were run with liposomes made of well-documented stable POPC−cholesterol formulations. At room temperature the lifetimes of leakage for salts (**38**−**40**) were less than 0.5 h, making them impractical for monitoring. Larger cations (**36**, **37**) escaped much slower, with lifetimes of dark leakage exceeding 18 h for **36** and no detectable leakage for **37**.

Chart 2. Ionic Probes Tested for ^{19}F (36−40) and ^{1}H (41) PFG NMR Study[a]



[a] Measured self-diffusion coefficients ($cm^2\ s^{-1}$) of 10 mM solutions in PBS are shown in parentheses.

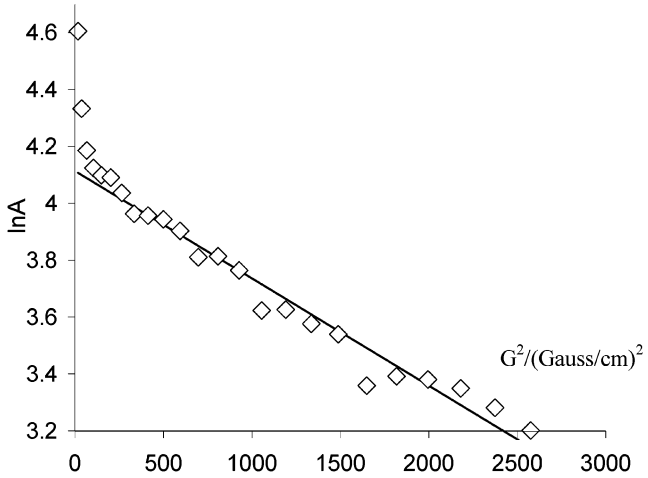


Figure 1. Entrapped probe allows measuring the liposome's mobility.

While methylpyridinium **36** was our probe of choice for this study, we note that sodium 2-(trimethylsilyl)ethanesulfonate (**41**), commonly used as an ^{1}H NMR reference for aqueous solutions, also is an adequate PFG probe molecule, with an easily identifiable rightmost peak in the proton spectrum.

The gradient strength of the Performa I PFG module was insufficient for accurate determination of the diffusion coefficient of 100 nm liposomes. For this task we used a Varian Inova 500 MHz NMR spectrometer equipped with a Performa II PFG module capable of forming PFG pulses up to 51 G/cm. Figure 1 shows the graph ln A vs G^2 giving a liposome diffusion coefficient of 4.3×10^{-8} $cm^2\ s^{-1}$. The initial (steeper) slope is due to a small amount of free probe.

This value is in remarkably good agreement with the 100 nm average size of the vesicles, provided the self-diffusion of liposomes is subject to the Stokes−Einstein equation,[16] $D_S = kT/6\pi\eta R$. The 2 orders of magnitude difference in diffusion coefficients is sufficient to accurately monitor the probe release. The bar graph (Figure 2) helps

(13) (a) Altieri, A. S.; Hinton, D. P.; Byrd, R. A. *J. Am. Chem. Soc.* **1995**, *117*, 7566. (b) For the original paper see: Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288. (c) For an in depth review on NMR diffusion measurements to characterize membrane transport and solute binding see: Waldeck, A. R.; Kuchel, P. W.; Lennon, A. J.; Chapman, B. E. *Prog. Nucl. Magn. Reson. Spectrosc.* **1997**, *30*, 39.

(14) ^{19}F PFG NMR was utilized to monitor diffusion of anionic species, e.g., BF_4^- or $CF_3SO_2O^-$, in polymers and ionic liquids: (a) Ferry, A.; Orädd, G.; Jacobsson, P. *Macromolecules* **1997**, *30*, 7329. (b) Kataoka, H.; Saito, Y.; Sakai, T.; Deki, S.; Ikeda, T. *J. Phys. Chem. B* **2001**, *105*, 2546. (c) Noda, A.; Hayamizu, K.; Watanabe M. *J. Phys. Chem. B* **2001**, *105*, 4603.

(15) Here and below "dark" implies "not irradiated". The liposome solutions were kept in NMR tubes wrapped with aluminum foil. No special effort was made to prevent accidental ambient light exposure of the liposome solutions in the lab. There was no detectable difference in the lifetimes of leakage for liposome solutions kept wrapped in aluminum foil and that of the solutions which were rigorously kept in the dark. However, samples exposed to direct sunlight did show accelerated leakage.

(16) Tyrrell, H. J. V.; Harris, K. R. *Diffusion in Liquids*; Butterworths: London, 1984.

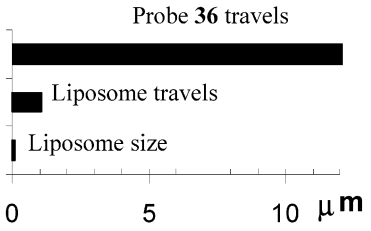


Figure 2.

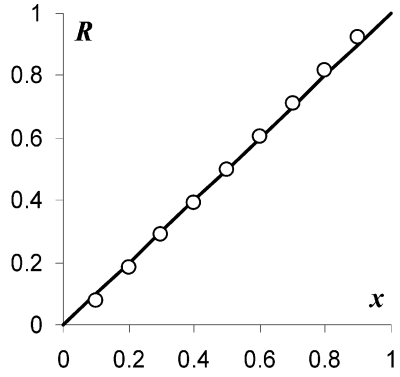


Figure 3.

visualize the distance traveled during the pulse delay between the two gradient pulses, 150 ms in our experiments, by the unconstrained probe **36**, the liposome, and also the size of the liposome for comparison.

To monitor the probe release, we alternated the PFG strength between the two values, 100 and 1600 DAC (digital to analog converter) units, which corresponds to 0.99 and 15.78 G/cm. It follows from eq 1 that, at 15.78 G/cm, the signal from the free probe should nearly disappear (about 2.5% of its initial value), while the signal of the probe trapped inside the liposome is hardly affected (97.1%). At 0.99 G/cm, the signal intensities are only slightly reduced for both free (98.6%) and trapped (99.99%) probe. If x is a fraction of trapped material, the ratio, R, of signal intensities at 15.78 and 0.99 G/cm is then

$$R = \frac{A_{15.78}}{A_{0.99}} = \frac{97.1x + 2.5(1 - x)}{99.9x + 98.6(1 - x)} = \frac{94.6x + 2.5}{1.3x + 98.6} \quad (2)$$

Conversely, x can be expressed in terms of R as

$$x = \frac{98.6R - 2.5}{94.6 - 1.3R} \quad (3)$$

For $0 \le R \le 1$, x can be approximated with high accuracy as

$$x = 1.0564R - 0.0288 \quad (4)$$

As follows from eq 4, for all practical purposes the ratio of the signal intensities at the high and the low PFG values is itself a reasonable measure for the percentage of trapped material. The graph (Figure 3) shows excellent correlation between x and R; the difference never exceeds 3% of the overall probe content.

Dark Stability of the Vesicles. A series of liposomes was prepared using formulations of natural egg−POPC, cholesterol, and photolipids **7a**−**d** or **10a**,**c**. The dark stability of liposomes was studied using the PFG NMR assay. Liposomes prepared with amphiphiles **7a** and **10a**,**c** showed high membrane permeability. It is known that the overall dynamic molecular shape of a lipid determines its phase preference.[17] The effective headgroup area as well as the length, shape, and the degree of saturation of the hydrocarbon chains of the lipid influence its phase behavior. Liposomes made with amphiphiles **10a**,**c**, which have only one hydrophobic tail, or **7a**, having two short tails, showed unacceptably high rates of leakage. In this series, only lipids **7b**−**c** pack well with POPC and cholesterol to form stable liposomes with relatively low membrane permeability. For the three-component formulation (the ratio POPC/cholesterol/**7b** = 5:3:2), dark permeability of the vesicles was similar to that of the all natural POPC−cholesterol (7:3) vesicles, with a lifetime of about 30 h. A higher molar fraction of the photolabile amphiphiles destabilized liposomes. Vesicles with the photolipid content exceeding 35% had dark leakage lifetimes less than 5−10 h.

Vesicles formulated with self-sensitized, nitropyridine-containing amphiphile **35** showed moderate to good dark stability: for example, for 5:3:2 (POPC/cholesterol/photolipid) ratio, the lifetime of leakage was found to be 19 h.

We have found that benzaldehyde-based amphiphiles bearing two *saturated* alkyl chains produced liposomes that were on average less stable and easier to aggregate. This prompted us to synthesize compounds **15**, **19,** and **25** having both alkyl *and* alkenyl tails. Also, these amphiphiles are based on 5-hydroxymethyldithiane and thus have their phosphocholine part connected via a shorter tether. Stable liposomes can be prepared with much higher molar fractions of these photolipids, and even without POPC. For example, in formulations with 22% cholesterol (2:7 ratio, no POPC), **15** forms stable liposomes with the dark leakage lifetime exceeding 30 h. The presence of cholesterol is necessary for stability of the vesicles. Mixing **15** and benzophenone in a 7:2 ratio without cholesterol produced less stable liposomes with a dark leakage lifetime of 12.8 h. Various combinations of **19** or **25** and 20−30% cholesterol produced vesicles with 27−30 h lifetimes for dark leakage.

Solution Photochemistry. Photochemistry of externally sensitized amphiphiles was studied in 10% aqueous acetonitrile to evaluate the efficiency of *solution* photofragmentation. Aqueous acetonitrile was used because these compounds are not soluble in dry acetonitrile and aggregate in water. The quantum yields of benzophenone-sensitized fragmentation in aqueous acetonitrile were found to be in the range of 0.09−0.14, which is in keeping with our previous observations.

The mass balance for the cleavage of hydroxyalkyl dithianes was previously determined to be nearly quantitative.[7a] Although C−C bond cleavage in the self-sensitized reaction of aminopyridine **35** was expected by analogy with the previously observed photofragmentations in the externally sensitized aminoalkyldithianes, we carried out a product study and investigated the mass balance of the model reaction of **42**, i.e., the adduct of a DHP-protected *p*-hydroxybenzaldehyde. Direct irradiation of **42** in aqueous acetonitrile produced only the corresponding imine and products of its hydrolysis (Scheme 8). After chromatographic separation, 17% of imine **45** was isolated, along with 35% of aldehyde **44**, 39% of 2-amino-5-nitropyridine **43**, and 21% of unreacted **42**, accounting for at least 71% of the photoreaction resulting in C−C bond cleavage.

Liposome Photochemistry. Several external sensitizers were tested in liposomes formulated with photolipids **7**, **15**, and **19**. Small water-soluble sensitizers, such as

(17) Litzinger, D. C.; Huang, L. *Biochim. Biophys. Acta* **1992**, *1113*, 201.

Scheme 8. Mass Balance in Fragmentation of Self-Sensitized 42

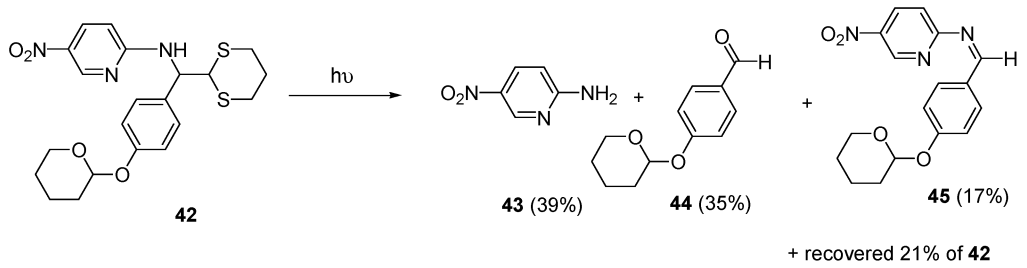


Table 1. Liposomes Based on Externally Sensitized Amphiphile 15

entry	components	molar ratio	dark lifetime, h (r^2)	lifetime for irradiated samples, h (r^2)
1	**15**/cholesterol	7:2	30.6 (0.579)	
2	**15**/cholesterol/benzophenone	7:2:1	27.5 (0.845)	2.73 (0.999)
3	**15**/cholesterol/benzophenone	7:2:2	26.6 (0.854)	10.55 (0.592)
4	**15**/benzophenone	7:2	12.8 (0.828)	2.3 (0.938)
5	**15**/cholesterol/1,4-dinitrobenzene	7:2:1	58.5 (0.670)	
6	**15**/anthraquinone	8:2	8.93 (0.923)	
7	**15**/cholesterol/anthraquinone	7:2:1	13.5 (0.964)	7.38 (0.598)
8	**15**/cholesterol/benzophenone[a]	7:2:1	16.2 (0.736)	5.2 (0.743)

[a] *N*-Trifluoroacetyl glucosamine as the probe.

anthraquinone-1,5-disulfonic acid and benzophenone carboxylic and tetracarboxylic acids, added to the bulk solution did not initiate the fragmentation. With the dithiane moiety buried in the membrane, the initial oxidative electron transfer was apparently not efficient.

Results with sensitization by lipophilic electron-transfer sensitizers dissolved in the membrane of **15** are shown in Table 1. A 7:2 formulation with cholesterol had satisfactory stability, with the dark leakage lifetime exceeding 30 h. There was a very small destabilizing effect upon addition of benzophenone to the formulation (entries 2 and 3, $\tau_{1/2}$ = 27.5 and 26.6 h, respectively). Entry 2 shows a rate acceleration of 1 order of magnitude as a result of sample irradiation. Mixing amphiphile **15** with benzophenone does not much improve the irradiated leakage, while the dark stability significantly deteriorates (entry 4, $\tau_{1/2}$ = 12.8 h).

Other electron-transfer sensitizers were tested. Anthraquinone did not pack well with photolipid **15**, and their 8:2 formulation produced unstable liposomes (entry 6). Addition of cholesterol did not produce considerable improvement (entry 7). On the contrary, 1,4-dinitrobenzene formulation with **15** and cholesterol gave very stable vesicles with dark lifetime of leakage exceeding 2 days. Irradiation of the vesicles containing 1,4-dinitrobenzene produced inconclusive results due to aggregation and precipitation during photolyses. Liposomes produced with self-sensitized nitro-group-containing systems **25** also immediately aggregated and precipitated upon irradiation. While the increased fusability of photolabile liposomes was also confirmed by transmission electron microscopy (TEM) for vesicles made with nitropyridines **35** (see below), the aggregation of vesicles having the nitrobenzaldehyde-based photolipids **25** was massive and fast. At this point we are unable to provide a mechanistic rationale for this behavior, because the aggregated reaction mixtures were intractable and we were unable to isolate individual products of these photolyses.

A neutral ^{19}F PFG probe molecule, *N*-trifluoroacetylglucosamine, was also tested (entry 8). Comparison of entries 2 and 8 indicate that, other conditions being equal, the two probes leak out with somewhat different rates. With certain reservations this can be interpreted in terms of irradiation uniformly affecting the permeability of the bilayer as opposed to the membrane catastrophic rupture.

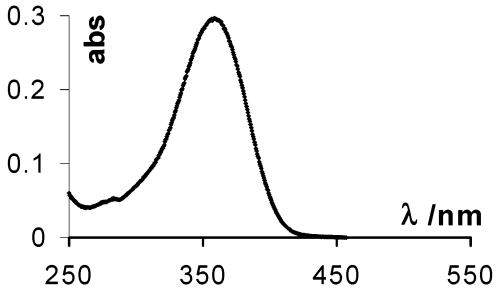


Figure 4. UV absorption of liposomes prepared with nitropyridine **35**.

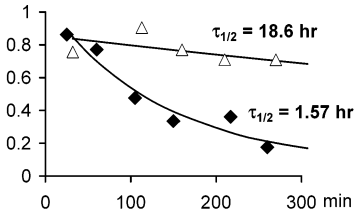


Figure 5. Release of probe **36** from POPC−cholesterol−**35** in the dark (△) and irradiated (◆).

Another representative of the self-sensitized compounds, the nitropyridine-based photolipid **35**, produced moderately stable liposomes only in formulations with POPC and cholesterol. Liposomes prepared with less than 20% of photolipid **35** had a lifetime of dark leakage on the order of 20 h or better. UV spectrum of a liposome solution, Figure 4, shows a strong absorption band with λ_{max} = 350 nm. The tailing absorption allows for sample illumination up to 380−400 nm.

Irradiation of liposomes prepared from a mixture of **35**/POPC/cholesterol in 2:5:3 ratio with a medium-pressure mercury UV lamp and a Pyrex filter (λ > 300 nm) for 75 min decreased the lifetime of leakage by a factor of 12 to 1.57 h (Figure 5). The reaction mixture was extracted with chloroform, and NMR analysis showed that 55−60% of the photolabile lipid was cleaved. This means that an order of magnitude acceleration of leakage can be achieved via photoinduced fragmentation in only 11−12 mol % of the total bilayer material.

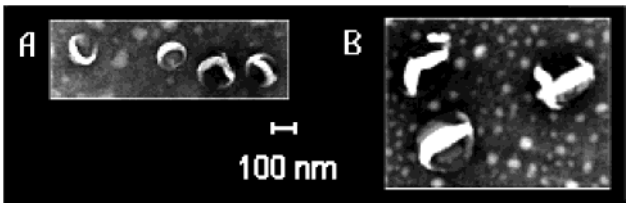


Figure 6. TEM images of photolabile liposomes before (A) and after irradiation (B), same scale.

TEM images of the negatively stained vesicles suggested that some fusion occurred during irradiation (Figure 6). Judging by the size, two to three 100 nm liposomes fuse into larger unilamellar vesicles. The resulting vesicles have almost "normal" spherical shapes, although the staining pattern was slightly different (conceivably due to accumulation of the fragmented lipid, which increased positive staining by stain exclusion). We hypothesize that irradiation uniformly affected the permeability of photolabile liposomes, although we cannot rule out that the increase in liposome fusability is also related to photorelease of the entrapped material.

In conclusion, we have developed a dithiane-based modular approach for synthesis of photolabile lipids capable of forming liposomes in formulations with POPC and/or cholesterol. The lipids can be equipped with hydrogen-bond-based elements of molecular recognition offering a possibility to rationally modify the surface of vesicles. Obviously, before this chemistry could be used to solve "real" problems of drug delivery, the efficiency of the photofragmentation and subsequent release will have to be further improved.

We also have developed a simple assay to monitor the release of small organic molecules based on PFG NMR. The potential advantage of ^{19}F monitoring is that many biomedically relevant compounds of interest can be labeled via, for example, trifluoroacetylation, and their unloading from liposomes or other delivery vehicles can then be followed easily.

Work is in progress in our laboratories to improve the dark stability of photosensitive liposomes and the efficiency of photorelease and to model recognition events based on vesicle surface modification.

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