Supporting information for

Influence of Hydrophobic Structures on the Plasma Membrane Permeability of Lipid-like Molecules

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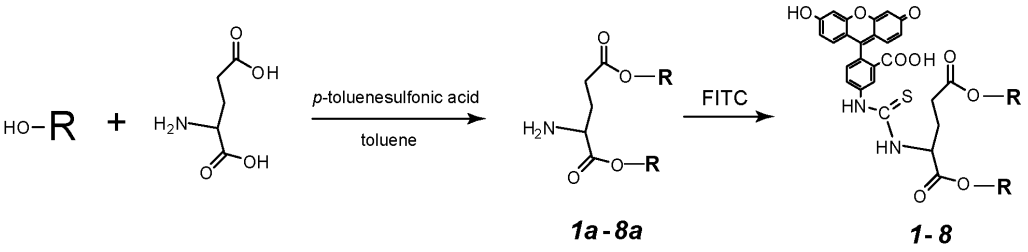
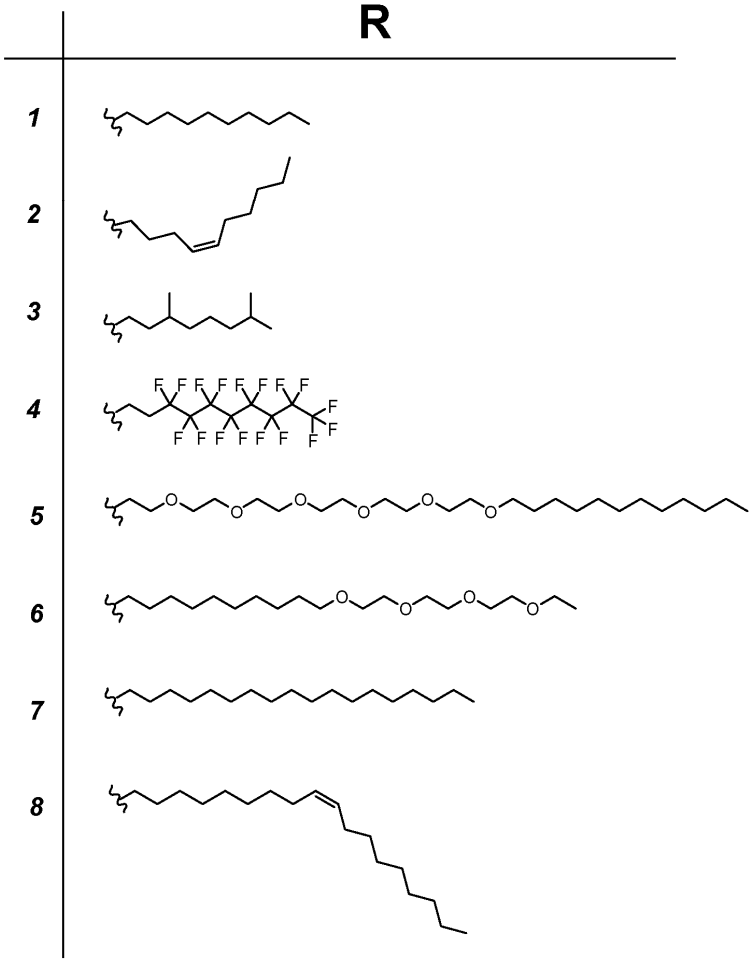
Materials and methods

All reagents were used without further purification. Thin-layer chromatography (TLC) and Preparative Layer Chomatography (PLC) were performed on glass-backed, precoated silica gel plate (E. Merck silica gel 60F254). Molecules were visualized by Cerium molybdate (10% Cerium (IV) Sulfate, 15% aqueous sulfuric acid solution). Products were isolated by column chromatography on silica-gel (Kanto Chemical, 60 N, spherical, neutral, 40-50 μm). Products were characterized by ^{1}H-NMR, MALDI-TOF MS, and if appropriate High-resolution mass spectra (HR-MS). ^{1}H-NMR spectra were recorded on JEOL-ECX 400 (400 MHz) spectrometer (JEOL, Japan). MALDI-TOF-MS spectra were measured with a Voyager-DE STR-H spectrometer (Applied Bio Systems) using 2,5 –dihydroxybenzoicacid (Bluker, Germany) as the matrix. HR-MS were recorded at Center for Instrumental Analysis, Hokkaido University using electrospray ionization (ESI) mass spectrometry on a JEOL JMS-T100LC mass spectrometer (JEOL).





Supporting Scheme 1. Synthesis of lipid-like molecule **1**-**8**.

Didecyl 2-aminopentanedioate (1a). Decyl alcohol (5.0 g, 31.9 mmol), L-glutamic acid (2.0 g, 13.6 mmol) and *p*-TsOH (3.2 g, 16.8 mmol) were dissolved in toluene (100 ml) and refluxed with Dean-Stark trap for 5.5 h at 130 ºC. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$-EtOAc (1:1) to give **1a** as white solid; R_f = 0.51 (silica, $CHCl_3$/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.29-3.91 (m, 4H), 3.63 (t, 1H, *J*= 6.66Hz), 2.59-2.44 (m, 1H), 2.43-2.29 (m, 2H), 2.26-2.15 (m, 1H), 1.64 (qn, 2H, *J*= 7.16 Hz), 1.56 (qn, 2H, *J*= 7.40), 1.41-1.17 (m, 28H), 0.88 (t, 6H, *J*= 6.66Hz); MALDI-TOF MS (m/z) calcd. for $C_{25}H_{50}NO_4$ $[M+H]^+$: 428.66; found 428.16.

FITC-didecyl 2-aminopentanedioate (1). The molecule **1a** (0.3 g, 0.8 mmol) in DMF (5 ml) was added FITC-I (0.4 g, 0.9 mmol) and triethylamine. The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was purified by PLC with $CHCl_3$-MeOH (9:1) to give **1** as yellow solid; R_f = 0.56 (silica, $CHCl_3$/CH_3OH 9:1); MALDI-TOF MS (m/z) calcd. for $C_{46}H_{61}N_2O_9S$ $[M+H]^+$: 818.04; found 816.93.

Di(Z)-dec-4-enyl 2-aminopentanedioate (2a). *cis*-4-decen-1-ol (3.9 g, 25.0 mmol), L-glutamic acid (1.6 g, 10.6 mmol) and *p*-TsOH (2.5 g, 13.2 mmol) were dissolved in toluene (200 ml) and refluxed with Dean-Stark trap for 8 h at 130 ºC. The reaction mixture was concentrated under reduced pressure then the residue was purified by column chromatography on silica gel with $CHCl_3$-EtOAc (3:8) to give **2a** (0.7 g, 15.6 %) as yellow oil; R_f = 0.50 (silica, $CHCl_3$/EtOAc = 3/8); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.47-5.25 (m, 4H), 4.12 (t, 2H, *J*= 6.68 Hz), 4.08 (t, 2H, *J*= 6.66 Hz), 3.47 (dd, 1H, *J*= 6.40, 8.28 Hz), 2.47 (t, 2H, *J*= 7.54 Hz), 2.11 (q, 5H, *J*= 7.50 Hz), 2.01 (q, 4H, *J*= 7.02 Hz), 1.90-1.77 (m, 8H), 1.69 (m, 4H), 1.40-1.20 (m, 12H), 0.89 (t, 6H, *J*= 6.90 Hz); MALDI-TOF MS (m/z) calcd. for $C_{25}H_{46}NO_4$ $[M+H]^+$: 424.63; found 424.25.

FITC-di(Z)-dec-4-enyl 2-aminopentanedioate (2). The molecule **2a** (0.1g, 0.3 mmol) in DMF (5 ml) was added FITC-I (0.1 g, 0.2 mmol) and a few ml of triethylamine. The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was purified by PLC with EtOAc to give **2** as yellow oil; R_f = 0.78 (silica, EtOAc); MALDI-TOF MS (m/z) calcd. for $C_{46}H_{57}N_2O_9S$ $[M+H]^+$: 814.01; found 813.23.

Bis(3,7-dimethyloctyl) 2-aminopentanedioate (3a). 3,7-Dimethyl-1-octanol (1.6 g, 10.4 mmol), L-glutamic acid (0.7 g, 4.4 mmol) and *p*-TsOH (1.0 g, 5.5 mmol) were dissolved in toluene (30 ml) and refluxed with Dean-Stark trap for 3 h at 130 ºC then for overnight at room temperature . The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$-MeOH (9:1) to give **3a** (1.44 g, 76.23 %) as yellow oil; R_f = 0.69 (silica, $CHCl_3$/MeOH = 9/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.23-4.10 (m, 4H), 3.46 (dd, 1H, *J*= 5.32, 8.28 Hz), 2.45 (t, 2H, *J*= 7.56 Hz), 2.14-2.02 (m, 1H), 1.89-1.77 (m, 1H), 1.59-1.46 (m, 8H), 1.25-1.05 (m, 12H), 0.87 (d, 18H, *J*= 6.52 Hz); MALDI-TOF MS (m/z) calcd. for $C_{25}H_{50}NO_4$ $[M+H]^+$: 428.66; found 428.61.

FITC-bis(3,7-dimethyloctyl) 2-aminopentanedioate (3). The molecule **3a** (0.4 g, 1.0 mmol) in toluene (3 ml) was added triethylamine. After stirring for 1 min, the reaction mixture was concentrated under reduced pressure. The residue was added FITC-I (0.4 g, 1.1 mmol) in DMF (5 ml). The reaction mixture was stirred for 19 h at room temperature and then concentrated under reduced pressure. The crude was purified by PLC with $CHCl_3$-MeOH (19:1) to give **3** (0.3 g, 34.5 %) as yellow solid; R_f= 0.45 (silica, $CHCl_3$/MeOH = 9/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.31-7.39 (m, 3H), 7.12-577 (m, 5H), 4.63-3.62 (m, 5H), 2.85-0.64 (m, 40H); MALDI-TOF MS (m/z) calcd. for $C_{46}H_{61}N_2O_9S$ $[M+H]^+$: 818.04; found 816.93.

Bis(1H,1H,2H,2H-heptadecafluorodecyl) 2-aminopentanedioate (4a). 1H,1H,2H, 2H-heptadecafluoro-1-decanol (13.0 g, 28 mmol), L-glutamic acid (1.8 g, 11.9 mmol) and

p-TsOH (2.8 g, 14.7 mmol) were dissolved in toluene (100 ml) and refluxed with Dean-Stark trap for 6 h at 130 ºC. The reaction mixture was concentrated under reduced pressure. The residue was added H_2O and filtered. The crude compound **4a** was white solid and used in next step without purification; ^{1}H NMR (400 MHz, DMSO-d_6): δ = 4.54-4.30 (m, 2H), 4.26-4.07 (m, 2H), 3.67 (t, 1H, *J*= 6.62 Hz), 2.30-2.25 (m, 4H), 2.16-1.88 (m, 4H); MALDI-TOF MS (m/z) calcd. for $C_{25}H_{16}F_{34}NO_4$ $[M+H]^+$: 1040.34; found 1038.42.

FITC-bis(1H,1H,2H,2H-heptadecafluorodecyl) 2-aminopentanedioate (4). The molecule **4a** (1.7g, 1.6 mmol) in DMF (10 ml) was added triethylamine (50 µl, 0.5 µmol). After stirring for 1 min, the reaction mixture was concentrated under reduced pressure. The residue was added FITC-I (0.2 g, 0.7 mmol) in DMF (15 ml). The reaction mixture was stirred for 24 h at 35 ºC and then concentrated under reduced pressure to give **4** as yellow oil; MALDI-TOF MS (m/z) calcd. for $C_{46}H_{27}F_{34}N_2O_9S$ $[M+H]^+$: 1429.72; found 1428.23.

Bis(3,6,9,12,15,18-hexaoxatriacontyl) 2-aminopentanedioate (5a). 3,6,9,12,15,18-hexaoxatriacontan-1-ol (2.5 g, 5.6 mmol), L-glutamic acid (0.4 g, 2.4 mmol) and *p*-TsOH (0.6 g, 3.0 mmol) were dissolved in toluene (30 ml) and refluxed with Dean-Stark trap for 4 h at 130 ºC then stirred overnight. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$-MeOH (9:1) then $CHCl_3$-MeOH (8:2) to give **5a** (1.0 g, 42.0 %) as white solid; R_f = 0.28 (silica, EtOAc/MeOH = 8/2); ^{1}H NMR (400 MHz, CD_3OD): δ = 4.47-4.12 (m, 4H), 3.78-3.53 (m, 45H), 3.46 (t, 4H, *J*= 6.63 Hz), 2.61 (t, 2H, *J*= 7.16 Hz), 2.25-2.12 (m, 2H), 1.61-1.51 (m, 4H), 1.40-1.20 (m, 36H), 0.90 (t, 6H, *J*= 6.72 Hz); MALDI-TOF MS (m/z) calcd. for $C_{53}H_{106}NO_{16}$ $[M+H]^+$: 1013.40; found 1012.01.

FITC-bis(3,6,9,12,15,18-hexaoxatriacontyl) 2-aminopentanedioate (5). The molecule **5a** (0.6 g, 0.6 mmol) in toluene (3 ml) was added triethylamine. After stirring for 1 min, the reaction

mixture was concentrated under reduced pressure. The residue was added FITC-I (0.3 g, 0.7 mmol) in DMF (5 ml). The reaction mixture was stirred for 4 h at room temperature and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$-MeOH (9:1) to give **5** (0.2 g, 27.9 %) as yellow solid; R_f= 0.47 (silica, $CHCl_3$/MeOH = 9/1); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.00 (d, 1H, *J*= 1.64 Hz), 7.75 (dd, 2H, *J*= 1.88, 8.08 Hz), 7.34 (d, 2H, *J*= 8.08 Hz), 6.70-6.68 (m, 4H), 6.57 (dd, 2H, *J*= 2.38, 8.76 Hz), 4.36-4.18 (m, 4H), 3.84-3.50 (m, 45H), 3.43 (t, 4H, *J*= 6.62 Hz), 2.67-2.52 (m, 2H), 2.39-2.18 (m, 2H), 1.61-1.47 (m, 4H), 1.40-1.18 (m, 36H), 0.89 (t, 6H, *J*= 6.88 Hz); MALDI-TOF MS (m/z) calcd. for $C_{74}H_{117}N_2O_{21}S$ $[M+H]^+$: 1402.78; found 1400.39.

11,14,17,20-tetraoxadocosan-1-ol (6a). The solution of 2-(2-(2-ethoxyethoxy)ethoxy)ethanol (10 g, 56.1 mmol) dissolved in THF (30 ml) was stirred for 3 min at -20 ºC. The solution was added NaH (1.9 g, 79.2 mmol) and stirred for 10 min at -20 ºC. The reaction mixture was added 10-bromo-1-decanol (5.0 g, 21.1 mmol) and stirred overnight under N_2 atmosphere at room temperature. The reaction mixture was added MeOH and concentrated under reduced pressure. The residue was extracted with $CHCl_3$. The organic layer was concentrated under reduced pressure, and the residue was purified by column chromatography on silica gel with EtOAc to give **6a** (2.0 g, 28.4 %); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.96-3.50 (m, 16H), 3.45 (t, 2H, *J*= 6.80 Hz), 1.60-1.26 (m, 16H), 1.21 (t, 6H, *J*= 7.02 Hz); MALDI-TOF MS (m/z) calcd. for $C_{18}H_{39}O_5$ $[M+H]^+$: 335.49; found 335.14.

Bis(11,14,17,20-tetraoxadocosanyl) 2-aminopentanedioate (6b). The molecule **6a** (2.0 g, 6.0 mmol), L-glutamic acid (0.4 g, 2.6 mmol) and *p*-TsOH (0.6 g, 3.2 mmol) were dissolved in toluene (50 ml) and refluxed with Dean-Stark trap for 5 h at 130 ºC. The reaction mixture was concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with EtOAc-MeOH (8:2) to give **6b** (1.8 g, 88.0 %); R_f = 0.67 (silica, EtOAc/MeOH =

8/2); ^{1}H NMR (400 MHz, CD_3OD): δ = 4.22-4.10 (m, 2H), 4.10-4.03 (m, 2H), 3.70-3.50 (m, 29H), 3.46 (t, 4H, *J*= 6.58 Hz), 2.53-2.41 (m, 2H), 2.10-1.87 (m, 2H), 1.71-1.26 (m, 32H), 1.18 (t, 6H, *J*= 7.04 Hz); MALDI-TOF MS (m/z) calcd. for $C_{41}H_{82}NO_{12}$ $[M+H]^+$: 781.08; found 779.12.

FITC-bis(11,14,17,20-tetraoxadocosanyl) 2-aminopentanedioate (6). The molecule **6b** (0.5 g, 0.6 mmol) in toluene (3 ml) was added triethylamine. After stirring for 1 min, the reaction mixture was concentrated under reduced pressure. The residue was added FITC-I (0.3 g, 0.8 mmol) in DMF (5 ml). The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$/MeOH (9:1) to give **6** (0.1 g, 18.7 %) as yellow solid; R_f= 0.42 (silica, $CHCl_3$/MeOH = 9/1); ^{1}H NMR (400 MHz, CD_3OD): δ = 8.25 (d, 1H, *J*= 1.76 Hz), 7.85 (dd, 1H, *J*= 2.14, 8.08 Hz), 7.15 (d, 1H, *J*= 8.28 Hz), 6.75-6.62 (m, 4H), 6.53 (dd, 2H, *J*= 2.36, 8.62 Hz), 4.18 (t, 2H, *J*= 6.36 Hz), 4.06 (t, 2H, *J*= 6.62 Hz), 3.69-3.46 (m, 33H), 3.42 (td, 4H, *J*= 1.60, 6.60 Hz), 1.73-1.65 (m, 2H), 1.64-1.58 (m, 2H), 1.57-1.47 (m, 4H), 1.45-1.23 (m, 28H), 1.16 (t, 6H, *J*= 7.08 Hz); MALDI-TOF MS (m/z) calcd. for $C_{62}H_{93}N_2O_{17}S$ $[M+H]^+$: 1170.46; found 1169.34.

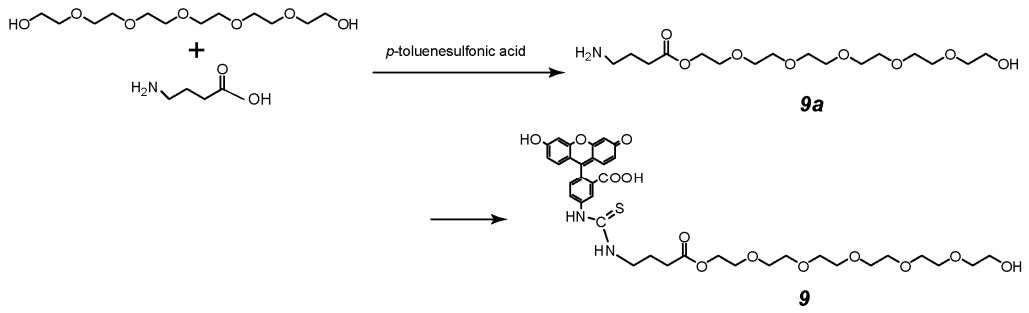
Dioctadecyl 2-aminopentanedioate (7a). 1-Octadecanol (10 g, 37.0 mmol), L-glutamic acid (2.3 g, 15.7 mmol) and *p*-TsOH (3.7 g, 19.5 mmol) were dissolved in toluene (100 ml) and refluxed with Dean-Stark trap for 5 h at 130 ºC. The reaction mixture was concentrated under reduced pressure then the residue was purified by column chromatography on silica gel with $CHCl_3$-EtOAc (1:1) to give **7a** (0.2 g, 1.8 %) as white solid; R_f = 0.42 (silica, $CHCl_3$/EtOAc = 1/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.11 (t, 2H, 6.84 Hz), 4.07 (t, 2H, *J*= 6.88 Hz), 3.51-3.42 (m, 1H), 2.46 (t, 2H, *J*= 7.60 Hz), 2.16-2.01 (m, 1H), 1.92-1.76 (m, 1H), 1.66-1.56 (m, 8H), 1.31-1.22 (m, 56H), 0.88 (t, 6H, *J*= 6.90 Hz); MALDI-TOF MS (m/z) calcd. for $C_{41}H_{82}NO_4$

$[M+H]^+$: 653.09; found 651.90.

FITC-dioctadecyl 2-aminopentanedioate (7). The molecule **7a** (0.1 g, 0.1 mmol) in DMF (5 ml) was added FITC-I (0.05 g, 0.1 mmol) and triethylamine (21.2 µl, 0.1 mmol, d = 0.70 g/ml). The reaction mixture was stirred for 1 h at room temperature and then concentrated under reduced pressure. The residue was purified by PLC with $CHCl_3$-EtOAc (1:1) to give **7** as yellow solid; R_f= 0.25 (silica, $CHCl_3$/EtOAc = 1/1); MALDI-TOF MS (m/z) calcd. for $C_{62}H_{93}N_2O_9S$ $[M+H]^+$: 1042.47; found 1040.66.

Di(Z)-octadec-9-enyl 2-aminopentanedioate (8a). Oleyl alcohol (10.7 g, 39.9 mmol), L-glutamic acid (2.5 g, 17.0 mmol) and *p*-TsOH (4.0 g, 21.1 mmol) were dissolved in toluene (100 ml) and refluxed with Dean-Stark trap for 5 h at 130 ºC. The reaction mixture was concentrated under reduced pressure. The residue was washed sequentially with saturated Na_2CO_3 (2 × 10 ml), dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$-EtOAc (99:5) then $CHCl_3$-EtOAc (3:1) to give **8a** (7.7 g, 70.4 %) as yellow oil; R_f = 0.17 (silica, $CHCl_3$/EtOAc = 9/1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.43-5.30 (m, 4H), 4.11 (t, 2H, *J*= 6.84 Hz), 4.07 (t, 2H, *J*= 6.84 Hz), 3.46 (dd, 1H, *J*= 5.20, 8.36 Hz), 2.46 (t, 2H, *J*= 7.52 Hz), 2.13-2.05 (m, 1H), 2.05-1.92 (m, 8H), 1.92-1.77 (m, 1H), 1.69-1.54 (m, 4H), 1.37-1.22 (m, 44H), 0.88 (t, 6H, *J*= 6.84 Hz); MALDI-TOF MS (m/z) calcd. for $C_{41}H_{78}NO_4$ $[M+H]^+$: 649.05; found 648.40.

FITC-di(Z)-octadec-9-enyl 2-aminopentanedioate (8). The molecule **8a** (0.1 g, 0.1 mmol) in DMF (7 ml) was added FITC-I (0.05 g, 0.1 mmol). The reaction mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was purified by column chromatography on silica gel with $CHCl_3$-MeOH (95:5) to give **8** (10 mg, 6.9 %) as yellow oil; R_f= 0.34 (silica, $CHCl_3$/MeOH = 9/1).

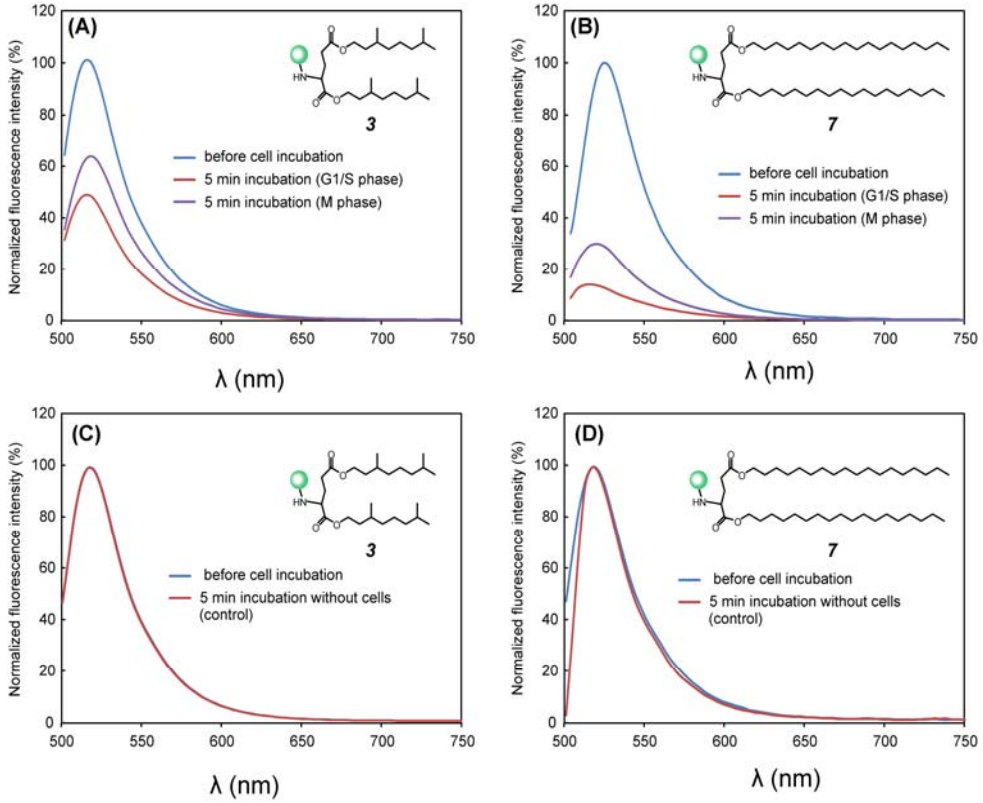


Supporting Scheme 2. Synthesis of lipid-like molecule **9**.

17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-aminobutanoate (9a). Hexa(ethyleneglycol) (6.43 g, 22.8 mmol), 4-aminobutanoic acid (1.0 g, 9.7 mmol) and *p*-TsOH (2.3 g, 12.0 mmol) were dissolved in toluene (50 ml) and refluxed with Dean-Stark trap for 5 h at 130 ºC then for overnight at room temperature . The reaction mixture was concentrated under reduced pressure. The residue was purified by reverse chromatography on Wakogel 50C18 to give **9a**; R_f = 0.67 (silica, $CHCl_3$/MeOH = 9/1); ^{1}H NMR (400 MHz, CD_3OD): δ = 4.29-4.29 (m, 2H), 3.65-3.63 (m, 22H), 2.50 (t, 1H, *J*= 6.84 Hz), 2.44 (t, 1H, *J*= 7.16 Hz), 2.37 (s, 2H), 2.00-1.88 (m, 2H); MALDI-TOF MS (m/z) calcd. for $C_{16}H_{34}NO_8$ $[M+H]^+$: 368.44; found 368.49.

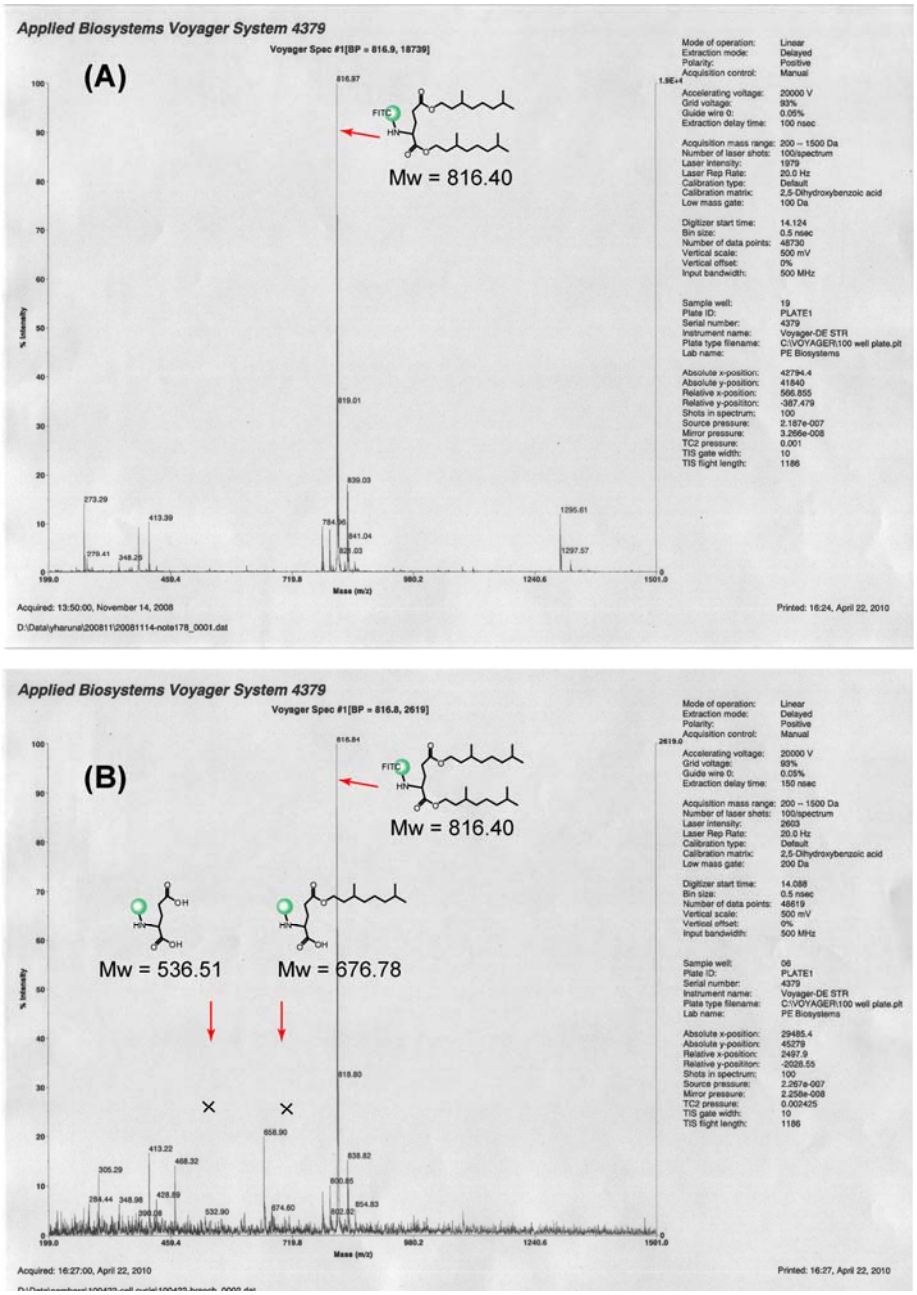
FITC-17-hydroxy-3,6,9,12,15-pentaoxaheptadecyl 4-aminobutanoate (9). The molecule **9a** (0.05 g, 0.1 mmol) in DMF (4 ml) was added FITC-I (0.05 g, 0.1 mmol) and triethylamine (50 µl, 0.5 mmol). The reaction mixture was stirred 2.5 h at room temperature and then concentrated under reduced pressure. The crude was purified by PLC with EtOAc-MeOH (3:1) to give **9**; R_f= 0.66 (silica, EtOAc/MeOH = 2/1); MALDI-TOF MS (m/z) calcd. for $C_{37}H_{45}N_2O_{13}S$ $[M+H]^+$: 757.82; found 756.29.

Monitoring of fluorescence intensity of medium containing lipid-like molecules during cell culture. HeLa cells (1.0×10^5 cells) were seeded onto 35-mm tissue culture dishes and cells were then treated with lipid-like molecules **3** and **7**. After incubation for 5 min at 37 ºC, the supernatant was removed. Measurement of fluorescence intensity was performed by NanoDrop 3300 fluorospectrometer (Thermo) and measured using a 470 nm excitation source. In the control, lipid-like molecules **3** and **7** were incubated in 35-nm tissue culture dishes in the absence of HeLa cells.



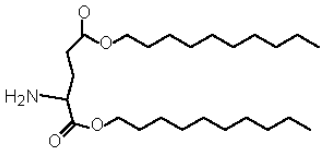
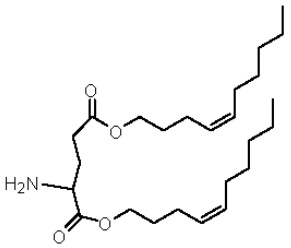
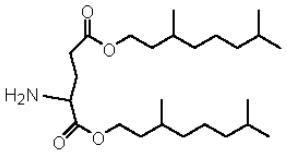
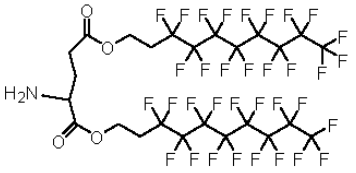
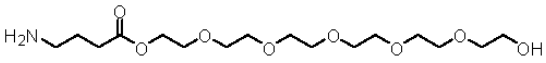
Supporting Figure 1. Fluorescence intensity of culture medium containing lipid-like molecules with or without synchronized HeLa cells. Lipid-like molecules (A) **3** and (B) **7** were incubated with synchronized HeLa cells for 5 min. Lipid-like molecules (C) **3** and (D) **7** were incubated in culture medium for 5 min in the absence of HeLa cells (control).

Degradation test of lipid-like molecules 3 and 7 using rabbit reticulocyte lysate (RRL). RRL was purchased from Promega. Lipid-like molecule **3** (final concentration: 1μM) was treated with RRL (20 μL) for 5 min at 37 ºC. After adding chloroform (30 μL), the organic layer was measured by MALDI-TOF MS spectrometry.



Supporting Figure 2. MALDI-TOF mass spectrometry of lipid-like molecule **3** (A) before and (B) after treatment with RRL.

Supporting Table I. Calculated logarithm of lipophilicity coefficients (LogP). LogP was calculated with ChemBioDraw 11.0 software (HULINKS).

	lipid-like molecules	log P
1a		6.6
2a		6.0
3a		6.3
4a		10.1
9a		-1.9