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Synthesis of phospholipids containing perfluorooctyl group and their interfacial properties

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Abstract

Highly fluorinated single-chained and/or double-chained phospholipids containing the perfluorooctyl group as the terminal segment of hydrophobic chains and a phosphocholine moiety as the hydrophilic headgroup were synthesized in order to investigate the effect of fluorinated segments on the stability of phospholipid monolayers formed at the air–water interface. Judging from the equilibrium spreading pressures (π_es) of their monolayers at the air–water interface, all of the fluorinated phospholipids formed more stable monolayers than the corresponding non-fluorinated counterparts. In addition, the fluorinated double-chained phosphatidylcholine containing C–C triple bond (monoyne group) formed stable and fluid vesicle membranes in water, although the single-chained phospholipids did not form vesicle membranes but micellar solutions under the present conditions.

Keywords: Perfluorooctyl; Glycerophospholipid; Equilibrium spreading pressure; Monolayer stability; Vesicle

1. Introduction

Fluorinated amphiphiles (surfactants, lipids) have been extensively investigated in terms of industrial (detergents, surface modifiers, repellents, etc.), biotechnological (protein solubilizer, protein crystallizers, etc.), and/or medical applications (DDS matrices, MRI agents, etc.) [1–4]. In particular, partially fluorinated amphiphiles are considered to show interesting physicochemical characteristics, since (i) the fluorinated segment in a molecule is rigid (physically stable) as well as chemically stable, whereas the non-fluorinated one is flexible; (ii) intermolecular interaction among fluorinated segments is much weaker than that among non-fluorinated ones; (iii) interaction of fluorinated amphiphiles with cells is weak, e.g., fluorinated amphiphiles exhibit low hemolytic activity due to a low affinity of fluorocarbon chains for non-fluorinated biomaterials and weak adhesion to cells [2–4].

We have already reported the synthesis of highly fluorinated C_{18} fatty acids and their interfacial properties [5]. These fatty acids containing the longer fluorinated segment, perfluorooctyl group, were synthesized in good yields (Fig. 1). Equilibrium spreading pressures (π_es) of their monolayers at the air–water interface were measured to consider the effect of fluorine substituents on their interfacial stability, and as a result, the introduction of fluorinated segments was found to improve the interfacial stability of monolayers.

We extended syntheses from fluorinated fatty acids to fluorinated phospholipids (Fig. 2) since phospholipids are expected to be more biocompatible than fatty acids. Although the synthesis of several phosphorylated perfluoroalkyl amphiphiles has been reported [1,6–9], phosphorylated amphiphiles containing highly fluorinated C_{18} alkyl chains, of which the chain length is considered to be compatible with biomembrane components, have not been synthesized or reported so far.

In this paper, we will describe the synthesis of highly fluorinated single-chained and/or double-chained phospholipids in order to investigate the effect of fluorinated segments on the stability of phospholipid monolayers formed at the air–water interface, and discuss the contribution of fluorinated segments to their interfacial properties. In addition, we will examine molecular aggregation behavior of the proposed fluorinated phospholipids in water.

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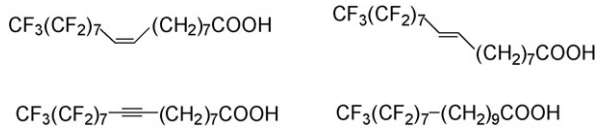


Fig. 1. Fluorinated fatty acids.

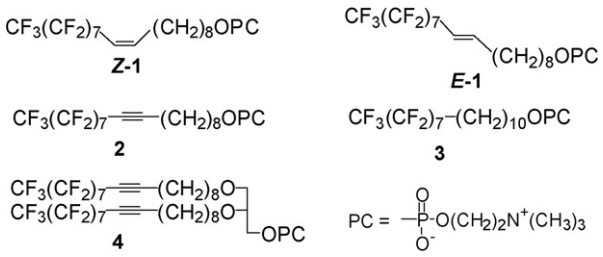


Fig. 2. Synthesis of fluorinated phospholipids.

2. Results and discussion

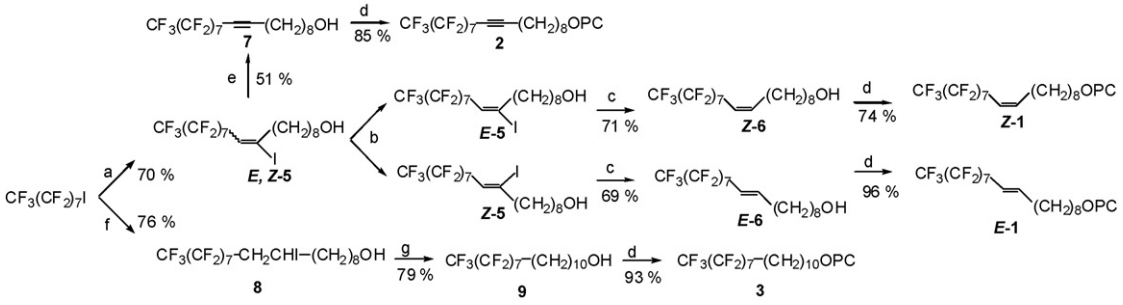
We achieved synthesis of the highly fluorinated single-chained phospholipids, (*Z*)-11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluoro-9-octadecenylphosphocholine (**Z-1**) in which a perfluorooctyl moiety is bonded directly to a C–C double bond, the analogs of **Z-1** (*E*-isomer: (*E*)-11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluoro-9-octadecenyl-phosphocholine (**E-1**); alkyne type: 11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluoro-9-octadecynylphosphocholine (**2**); saturated type: 11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluorooctadecylphosphocholine (**3**)) as shown in Scheme 1 and the double-chained phosphatidylcholine, 1,2-di-*O*-(11′,11′,12′,12′,13′,13′,14′,14′,15′,15′16′,16′,17′,17′,18′,18′,18′-heptadecafluoro-9′-octadecynyl)-*sn*-glycero-3-phosphocholine (**4**) as shown in Scheme 2. In addition, we measured equilibrium spreading pressures (π_es) of their monolayers at the air–water interface in order to demonstrate how the degree of unsaturation in the hydrophobic chain, the geometric isomerization, and the presence of fluorinated segments influence the monolayer stability.

Z-1 and **E-1** were synthesized by the following procedures (Scheme 1) [5,10]. Perfluorooctyl iodide was reacted with 9-decyn-1-ol in the presence of $Na_2S_2O_4$ as a free radical initiator to give the alkenyl iodide **5** in 70% yield as *E*/*Z* mixture (*E*:*Z* = 8:2). **5** was separated into the *E*-isomer **E-5** and the *Z*-isomer **Z-5** by column chromatography. Each configuration was agreement with chemical shifts and coupling constants of ^1H and ^{19}F NMR spectra in our previous report [5]. **E-5** was treated with *n*-BuLi for 4 h at −78 °C to give the alcohol compound **Z-6** in 71% yield. **Z-6** was phosphorylated with 2-bromoethylphosphorodichloridate in the presence of triethylamine, then stirred with water to give the phosphorylated intermediate. The crude intermediate was heated at 60 °C with excess trimethylamine to afford **Z-1** in 74% yield. **E-1** was prepared similarly from **Z-5** in 66% overall yield.

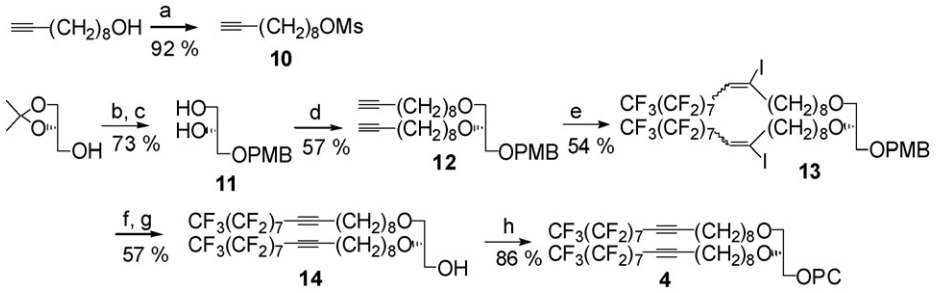
The alkynol **7** was synthesized from the *E*/*Z* mixture of iodide **5** by the treatment with *t*-BuOK in 51% yield [5]. By the standard method to introduce the phosphocholine group [10], **7** was converted into the compound **2** in 85% yield.

In the synthesis of the compound **3**, perfluorooctyl iodide was reacted with 9-decen-1-ol in the presence of $Na_2S_2O_4$ to give the alkyl iodide **8** in 76% yield, which was treated with Zn-$NiCl_2$ to provide the compound **9** in 79% yield [5]. The introduction of the phosphocholine group into **9** gave **3** in 93% yield [10].

We further carried out the synthesis of a new type of double-chained fluorinated phosphatidylcholine **4** containing a C–C triple bond (monoyne group) in a hydrophobic chain, which is distinct from natural phospholipids such as monoene- and/or polyene-type phospholipids. The introduction of a triple bond (monoyne group) into a hydrophobic chain is much easier than that of a double bond (monoene group) because we found previously that the oleic acid and/or elaidic acid analogs (**E-** and **Z-5**, **E-** and **Z-6**, etc.) should be separated by repeated column chromatography [5]. The phospholipid **4** was synthesized by the following procedures (Scheme 2). 9-Decyn-1-ol was reacted with methanesulfonyl chloride in the presence of triethylamine to give the compound **10** in 92% yield. The starting compound D-1,2-*O*-isopropylidene-*sn*-glycerol was benzylated with 4-methoxybenzyl chloride, and the acetonide group was hydrolyzed by a catalyst of *p*-toluenesulfonic acid monohydrate to afford the mono-PMB-protected triol **11** in 73% overall yield [11]. Alkylation of **11** with **10** gave the compound **12** in 57% yield [12] which was



Scheme 1. Synthesis of fluorinated single-chained phospholipids. (a) 9-Decyn-1-ol, $Na_2S_2O_4$, $NaHCO_3$, CH_3CN/H_2O, rt, 4 h; (b) silica gel column (EtOAc:*n*-hexane = 1:3, **E-5**:**Z-5** = 7:3); (c) (i) *n*-BuLi, Et_2O, −78 °C, 4 h, (ii) MeOH; (d) (i) $Br(CH_2)_2OP(O)Cl_2$, Et_3N, benzene, rt, 18 h, (ii) H_2O, rt, 8 h, (iii) Me_3N aq., $CHCl_3/CH_3CN/i$-PrOH, 60 °C, 18 h; (e) *t*-BuOK, Et_2O, −20 °C, 1 h, then 0 °C, 2 h; (f) 9-decen-1-ol, $Na_2S_2O_4$, $NaHCO_3$, CH_3CN/H_2O, rt, 4 h; (g) Zn, $NiCI_6$ (cat.), THF/H_2O, rt, 3 h.



Scheme 2. Synthesis of fluorinated double-chained phosphatidylcholine (**4**). (a) MsCl, Et_3N, CH_2Cl_2, rt, 4 h; (b) PMBCl, Et_3N, CH_2Cl_2, rt, 4 h; (c) $AcOH/H_2O$, 90 °C, 2 h; (d) 10, NaH, DMF, rt, 18 h; (e) $F(CF_2)_8I$, $Na_2S_2O_4$, $NaHCO_3$, CH_3CN/H_2O, rt, 4 h; (f) *t*-BuOK, Et_2O, −20 °C, 2 h; (g) DDQ, THF/H_2O, rt, 4 h; (h) (i) $Br(CH_2)_2OP(O)Cl_2$, Et_3N, benzene, rt, 18 h, (ii) H_2O, rt, 8 h, (iii) Me_3N aq., $CHCl_3/CH_3CN/i$-Pr0H, 60 °C, 18 h.

Table 1
Equilibrium spreading pressures (π_e's) of fluorinated phospholipids and their non-fluorinated counterparts at 25 °C

	π_e (mN/m)		π_e (mN/m)		π_e (mN/m)
$CF_3(CF_2)_7$(CH=CH)$(CH_2)_8OPC$ **Z-1**	51.2	$CF_3(CF_2)_7$(CH=CH)$(CH_2)_8OPC$ **E-1**	51.1	$CF_3(CF_2)_7$–C≡C–$(CH_2)_8O$ $CF_3(CF_2)_7$–C≡C–$(CH_2)_8O$ OPC **4**	54.8
$CF_3(CF_2)_7$–C≡C–$(CH_2)_8OPC$ **2**	51.2	$CF_3(CF_2)_7(CH_2)_{10}OPC$ **3**	51.1		
$CH_3(CH_2)_7$–C≡C–$(CH_2)_8OPC$ **15**	33.5	$CH_3(CH_2)_{17}OPC$ **16**	34.4		

reacted with perfluorooctyl iodide to give the alkenyl iodide **13** in 54% yield [5]. The reaction of **13** with *t*-BuOK, followed by treatment with dichlorodicyanoquione [13] afforded the precursor **14** which provided **4** in 34% overall yield via the introduction of the phosphocholine group [10].

In order to investigate the effect of the fluorine substituent on the monolayer stability, the equilibrium spreading pressures (π_es) of their monolayers (**Z-1**, **E-1**, **2**, **3**, and the corresponding non-fluorinated lipids, i.e., 9-octadecynylphosphocholine (**15**), octadecylphosphocholine (**16**)) at the air–water interface were measured at 25 °C [5], and the results are listed in Table 1. At 25 °C, the double-chained phosphatidylcholine **4** rapidly spread on the water surface and exhibited the highest π_e value among the phospholipids examined, while non-fluorinated counterparts, **15** and **16**, spread to the relatively low π_e of around 34 mN/m. As the equilibrium spreading pressure is considered to be a criterion for monolayer stability [14], it is clear that fluorinated phospholipids form more stable monolayers with higher spreading pressures at the air–water interface as compared to their non-fluorinated counterparts. It is noteworthy that single-chained phospholipids showed approximately identical pressures irrespective of the degree of unsaturation or geometric isomerization in the hydrophobic chain. In addition, it is also clear that each fluorinated phospholipid forms more stable monolayers as compared to the fluorinated fatty acid form in which the structure of the hydrophobic chain is identical to the corresponding fluorinated phospholipid [5]. These results indicate that the presence of the hydrophilic phosphocholine headgroup in addition to fluorinated segments predominantly contributes to the monolayer stability of fluorinated phospholipids, while the degree of unsaturation and geometric isomerization in the hydrophobic chain in fluorinated phospholipids have a minor effect. As expected, the introduction of an additional hydrophobic chain into a single-chained phospholipid molecule also enhances the monolayer stability.

In addition to the interfacial properties, molecular aggregation behavior of the proposed fluorinated phospholipids in

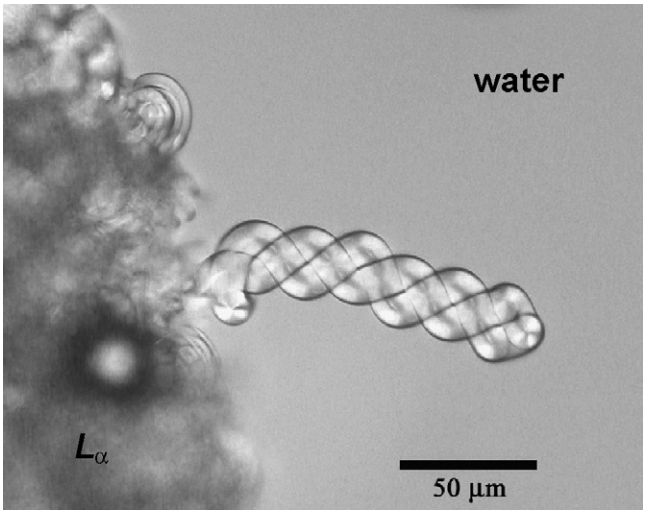


Fig. 3. Myelin figure growth after the fluorinated double-chained phosphatidylcholine (**4**) came into contact with water from the right side at 25 °C.

water was preliminarily examined. It is well known that double-chained surfactants [15–17] and/or natural phospholipids can often form vesicle membranes, which is also the case for fluorinated amphiphiles. A variety of fluorinated surfactants, including even some single-chained fluorinated surfactants, have been so far found to form stable vesicle membranes [1,3,4,7,9,17]. On the basis of polarizing microscope observation, although water penetration into a powder of the fluorinated double-chained phosphatidylcholine **4** proceeded more slowly as compared to natural phospholipids such as egg yolk PC, the phospholipid **4** was also found to form fluid liquid-crystalline membranes, i.e., myelin figures (Fig. 3) and vesicle membranes (Fig. 4) at 25 °C. On the other hand, the single-chained fluorinated phospholipids in this study, e.g., the phospholipid **2**, were found to form at least two distinct optically anisotropic textures in water at 25 °C as can be seen in Fig. 5. In the concentrated lipid region (the left side of Fig. 5), the phospholipid **2** exhibited Maltese-crosses and oily-streaks characteristic of lamellar phase (L phase). In the diluter lipid region (the mid section of Fig. 5), however, it formed another texture, which could be attributed to normal hexagonal phase (H_I phase) by referring to published pictures [18,19]. The separate small-angle X-ray scattering experiments showed that its lamellar phase in the concentrated lipid region (=60 wt.% lipid) is in the liquid-crystalline state (L_α phase) and the transition from L_α phase to H_I phase occurs within the lipid concentration region from 50 to 60 wt.% lipid (data not shown). Unlike some single-chained fluorinated surfactants containing perfluorooctyl group [1,7,9], however, the proposed single-chained phospholipids did not form definite myelin figures nor vesicle membranes, but micellar solutions under the present conditions. This finding suggests the subtle geometry of lipid molecules may control the molecular aggregation behavior in water.

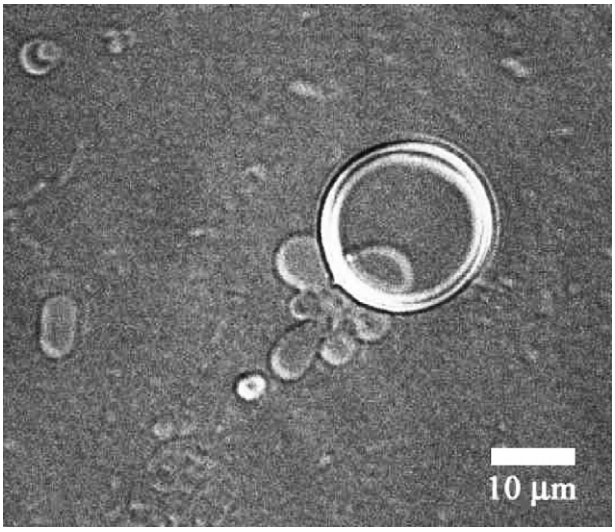


Fig. 4. Vesicle formation of the fluorinated double-chained phosphatidylcholine (**4**) in water at 25 °C observed by DIC microscope.

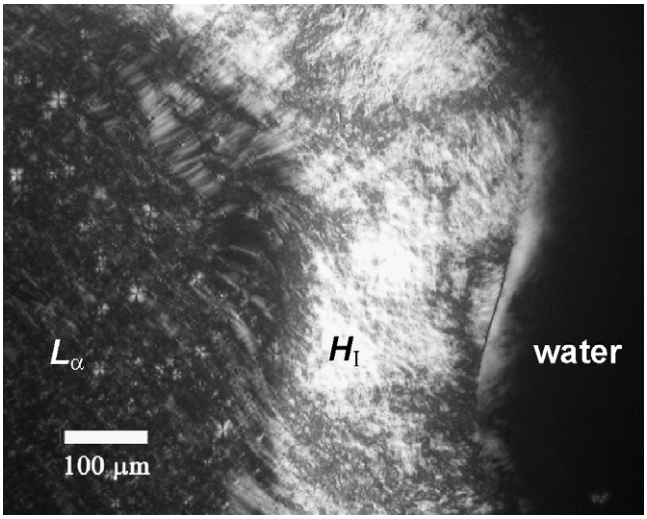


Fig. 5. Polarizing micrograph of liquid-crystalline phases formed after the fluorinated single-chained phospholipid (**2**) came into contact with water from the right side at 25 °C. L_α, liquid-crystalline lamellar phase; H_I, normal hexagonal phase.

In conclusion, we found highly fluorinated single-chained phospholipids (**Z-1**, **E-1**, **2**, **3**) and double-chained phosphatidylcholine (**4**) could be successfully synthesized and their π_es were much higher than those of their non-fluorinated counterparts. The double-chained phospholipid **4** also formed stable and fluid vesicle membranes in water. This observation suggests that the proposed fluorinated phospholipid analogs are a new class of phospholipids as promising materials for industrial, biotechnological, and/or medical applications.

3. Experimental

3.1. Instruments

^{1}H NMR spectra were recorded on a JEOL JNM-LA 500 FT-NMR system (500 MHz) using TMS as an internal standard. ^{19}F NMR spectra were measured with a JEOL JNM-LA 500 FT-NMR system (500 MHz) or a JEOL JNM-LA 300 FT-NMR system (300 MHz) using benzotrifluoride (BTF) as an internal standard. Mass spectra (MS) were measured with a JEOL JMS-700T Tandem MStation. IR spectra were measured with a JASCO FT-IR-680plus.

3.2. Materials

Perfluorooctyl iodide was purchased from Daikin Fine-chemical Laboratory (Osaka) and its purity was over 95% (GC). 2-Bromoethylphosphorodichloridate was prepared by the method of Baumann and co-workers [10]. (*E*)- and (*Z*)-11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluoro-9-octadecen-1-ol (**E-6** and **Z-6**), 11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluoro-9-octadecyn-1-ol (**7**), 11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluoro-9-iodooctadecan-1-ol (**8**), and 11,11,12,12,13,13,14,14,15,15,16,16,17,17,18,18,18-heptadecafluorooctadecan-1-ol (**9**) were synthesized according to our previous report [5], and were assigned by ^{1}H and ^{19}F NMR. The precursor of **15**, 9-octadecyn-1-ol, was synthesized from octyl

bromide and lithium acetylide of 9-decyn-1-ol [20]. All other chemicals and reagents were commercially available.

Water for interfacial chemical measurements was prepurified with a homemade purification system (RO membrane, ion-exchange column, and 0.22 μm filter) and was further purified with a Milli-Q Labo system (Millipore Corp., Bedford, MA) and distillation in an all-glass still [21]. Its resistivity was higher than 18 MΩ cm.

3.3. General procedure for the synthesis of phosphocholine

Alcohol (1.0 equiv.) was treated with 2-bromoethylphosphorodichloridate (1.5 equiv.) in benzene containing triethylamine (1.5 equiv.) at room temperature for 18 h, and then the reaction mixture was evaporated. The residue was extracted with $CHCl_3$ after being treated with water. The solvent was removed under atmospheric pressure to afford the phosphoryl intermediate. Me_3N aq. was added to the intermediate in a mixture of $CHCl_3$/*i*-PrOH/CH_3CN (3/5/5). The solution was stirred at 60 °C for 18 h. After the removal of the solvent, the crude compound was purified by column chromatography (SiO_2 (Merck, 7734): $CHCl_3$/MeOH/H_2O = 3/2/0 to 65/35/4 and Sephadex LH20: MeOH) to give the phosphocholine compound in a high yield [10].

Z-1: colorless waxy solid. FAB-MS *m/z*: 740 $(M+H)^+$. HRMS (FAB) calcd for $C_{23}H_{32}O_4NF_{17}P$ $(M+H)^+$ 740.1797; Found: 740.1806. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 6.12 (1H, dtt, J = 11.88, 7.92, 2.23 Hz), 5.49 (1H, td, J = 15.68, 11.88 Hz), 4.25 (2H, m), 3.85 (2H, q, J = 6.71 Hz), 3.66 (2H, m), 3.26 (9H, s), 2.30 (2H, m), 1.61 (2H, m), 1.40 (2H, m), 1.33–1.30 (8H, m). ^{19}F NMR ($CDCl_3$-CD_3OD) ppm: −18.05 (3F, t, J = 10.70 Hz), −43.86 (2F, m), −58.71 (2F, bs), −59.18 (4F, bs), −59.96 (2F, bs), −60.98 (2F, bs), −63.37 (2F, bs). IR (neat) υ_{max}: 2929, 2857, 1662,1481, 1203, 1147, 1085 and 968 cm^{-1}.

E-1: colorless sticky solid. FAB-MS *m/z*: 740 $(M+H)^+$. HRMS (FAB) calcd for $C_{23}H_{32}O_4NF_{17}P$ $(M+H)^+$ 740.1797; Found: 740.1797. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 6.40 (1H, dtt, J = 15.84, 7.01, 2.13 Hz), 5.59 (1H, dt, J = 15.84, 11.86 Hz), 4.26 (2H, bs), 3.84 (2H, q, J = 6.40 Hz), 3.72 (2H, m), 3.31 (9H, s), 2.19 (2H, m), 1.61 (2H, m), 1.44 (2H, m), 1.35–1.29 (8H, m). ^{19}F NMR ($CDCl_3$-CD_3OD) ppm: −18.03 (3F, t, J = 9.72 Hz), −48.50 (2F, m), −58.73 (2F, bs), −59.25 (4F, bs), −60.01 (2F, bs), −60.82 (2F, bs), −63.40 (2F, bs). IR (neat) υ_{max}: 2935, 2859, 1674, 1488, 1238, 1201, 1149, 1076 and 970 cm^{-1}.

2: colorless waxy solid. FAB-MS *m/z*: 738 $(M+H)^+$. HRMS (FAB) calcd for $C_{23}H_{30}O_4NF_{17}P$ $(M+H)^+$ 738.1641: Found: 738.1639. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 4.27 (2H, bs), 3.84 (2H, q, J = 6.60 Hz), 3.71 (2H, bs), 3.29 (9H, s), 2.35 (2H, quin, J = 6.45 Hz), 1.62–1.53 (4H, m), 1.43–1.25 (8H, m). ^{19}F NMR ($CDCl_3$-CD_3OD) ppm: −18.08 (3F, t, J = 10.04 Hz), −33.43 (2F, m), −58.44 (2F, brs), −59.24 (4F, brs), −59.86 (2F, brs), −60.06 (2F, brs), −63.43 (2F, brs). IR (neat) υ_{max}: 2933, 2859, 2256, 1484,1238, 1205, 1149, 1087 and 968 cm^{-1}.

3: colorless sticky solid. FAB-MS *m/z*: 742 $(M+H)^+$. HRMS (FAB) calcd for $C_{23}H_{34}O_4NF_{17}P$ $(M+H)^+$ 742.1954: Found: 742.1946. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 4.27 (2H, brs), 3.83 (2H, q, J = 6.69 Hz), 3.75 (2H, brs), 3.33 (9H, s), 2.04 (2H, m), 1.60 (4H, m), 1.35–1.29 (12H, m). ^{19}F NMR ($CDCl_3$-CD_3OD) ppm: −18.01 (3F, t, J = 10.04 Hz), −51.72 (2F, m), −59.21 (6F, brs), −60.02 (2F, brs), −60.83 (2F, brs), 63.40 (2F, brs). IR (neat) υ_{max}: 2927, 1482, 1243, 1197, 1149, 1081 and 968 cm^{-1}.

4: colorless waxy solid. [α] + 0.0094 (c 0.1, MeOH). FAB-MS *m/z*: 1366 $(M+H)^+$. HRMS (FAB) calcd for $C_{44}H_{51}O_6NF_{34}P$ $(M+H)^+$ 1366.2911: Found: 1366.2905. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 4.28 (2H, bs), 3.89 (2H, t, J = 5.63 Hz), 3.68 (2H, m), 3.64–3.52 (4H, m), 3.48–3.38 (3H, m), 3.28 (9H, s), 2.35 (4H, quin, J = 6.40 Hz), 1.61–1.52 (8H, m), 1.39 (4H, m), 1.29 (12H, bs). ^{19}F NMR ($CDCl_3$-CD_3OD) ppm: −18.03 (6F, t, J = 10.71 Hz), −33.36 (4F, bs), −58.34 (4F, bs), −59.13 (8F, bs), −59.78 (4F, bs), −59.95 (4F, bs), −63.34 (4F, m). IR (neat) υ_{max}: 2931, 2859, 2258, 1671, 1236, 1205, 1147, 1093, 968 and 894 cm^{-1}.

15: colorless waxy solid. FAB-MS *m/z*: 432 $(M+H)^+$. HRMS (FAB) calcd for $C_{23}H_{47}O_4NP$ $(M+H)^+$ 432.3243: Found: 432.3246. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 4.26 (2H, bs), 3.84 (2H, q, J = 6.70 Hz), 3.66 (2H, m), 3.26 (9H, s), 2.13 (4H, t, J = 6.55 Hz), 1.61 (2H, quin, J = 7.08 Hz), 1.47 (4H, m), 1.36–1.28 (18H, m), 0.88 (3H, t, J = 6.85 Hz). IR (neat) υ_{max}: 2927, 2854, 1463, 1236, 1089 and 968 cm^{-1}.

16: colorless sticky solid. FAB-MS *m/z*: 436 $(M+H)^+$. HRMS (FAB) calcd for $C_{23}H_{51}O_4NP$ $(M+H)^+$ 436.3556: Found: 436.3557. ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 4.26 (2H, bs), 3.84 (2H, q, J = 6.70 Hz), 3.69 (2H, m), 3.28 (9H, bs), 1.61 (2H, quin, J = 7.01 Hz), 1.25 (30H, m), 0.88 (3H, t, J = 7.01 Hz). IR (neat) υ_{max}: 2915, 2848, 1471, 1243, 1149, 1079 and 968 cm^{-1}.

3.3.1. Synthesis of *13*

The compound (**12**) was obtained from D-1,2-*O*-isopropylidene-*sn*-glycerol according to the method described in the literature [12]. To a solution of **12** and perfluorooctyl iodide (1.870 g, 3.42 mmol) in CH_3CN/H_2O (2/1) was added to $NaHCO_3$ (0.249 g, 2.96 mmol) and 85% $Na_2S_2O_4$ (0.578 g, 2.88 mmol), and the mixture was stirred at room temperature for 4 h. The mixture was poured into ice, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl and dried over $MgSO_4$. After the solvent was evaporated under atmospheric pressure, the residue was separated by column chromatography (SiO_2, EtOAc:*n*-hexane = 3:7) to give a *E*/*Z* mixture (*E*/*Z* = 7/3) of **13** (1.210 g, 54%).

E-13: ^{1}H NMR ($CDCl_3$-CD_3OD) δ: 7.26 (2H, dt, J = 8.83, 2.13 Hz), 6.87 (2H, dt, J = 8.83, 2.13 Hz), 6.31 (2H, t, J = 14.41 Hz), 4.47 (2H, s), 3.80 (3H, s), 3.60–3.39 (9H, m), 2.61 (4H, m), 1.56 (10H, m), 1.30 (14H, bs). ^{19}F NMR ($CDCl_3$) ppm: −18.08 (9F, t, J = 9.71 Hz), −42.70 (3F, m), −58.81 (4F, bs), −59.25 (8F, bs), −60.06 (4F, m), −60.60 (4F, bs), −63.46 (4F, m).

Z-13: ^{1}H NMR ($CDCl_3$) δ: 7.26 (2H, dt, J = 8.83, 2.13 Hz), 6.87 (2H, dt, J = 8.83, 2.13 Hz), 6.22 (2H, t, J = 11.35 Hz), 4.47 (2H, s), 3.80 (3H, s), 3.60–3.39 (9H, m), 2.64 (4H, m), 1.56 (10H, m), 1.30 (14H, bs). ^{19}F NMR ($CDCl_3$) ppm: −18.08 (6F, t, J = 9.71 Hz), −45.78 (4F, m), −58.81 (4F, bs), −59.25 (8F, bs), −60.06 (4F, m), −60.60 (4F, bs), −63.46 (4F, m).

*3.3.2. Synthesis of **14***

To a suspension of *t*-BuOK (0.410 g, 3.65 mmol) in dry Et_2O (10 mL) from CaH_2 cooled at −20 °C was added slowly a solution of **13** (1.151 g, 0.73 mmol) in dry Et_2O (5 mL), and the mixture was stirred at −20 °C for 1 h and then at 0 °C for 2 h. The mixture was poured into 10% HCl and ice and then extracted with Et_2O. The Et_2O layer was washed with saturated $NaHCO_3$ and saturated NaCl, and then dried over $MgSO_4$. After the evaporation of the solvent, the residue was separated by column chromatography (SiO_2, EtOAc:*n*-hexane = 1:9) to give mono-PMB-protected diyne intermediate (0.654 g, crude).

Dichlorodicyanoquione (2.0 equiv.) was added to the protected diyne intermediate (0.731 g, crude) in CH_2Cl_2 (6.0 mL) and pH 7.0 phosphate buffer (0.6 mL) at 0 °C. The mixture was stirred at room temperature for 3 h, poured into iced water, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with saturated NaCl and dried over $MgSO_4$. After the evaporation of the solvent, the residue was separated by column chromatography (SiO_2, EtOAc:*n*-hexane = 3:7) to give **14** (0.557 g, 84% on the basis of **13**).

14: ^{1}H NMR ($CDCl_3$) δ: 3.75–3.71 (1H, m), 3.64–3.58 (2H, m), 3.55–3.41 (6H, m), 2.35 (4H, m), 2.15 (1H, m), 1.61–1.52 (8H, m), 1.39–1.31 (16H, m). ^{19}F NMR ($CDCl_3$) ppm: −18.14 (6F, t, J = 9.71 Hz), −33.54 (4F, bs), −58.47 (4F, bs), −59.30 (8F, bs), −59.94 to ∼−60.09 (8F, m), −63.47 (4F, m).

3.4. Equilibrium spreading pressure measurements

Equilibrium spreading pressures of phospholipids were measured by Wilhelmy technique using a Pt plate attached to a KSV electronic balance (pressure sensitivity: 0.01 mN/m; KSV Instruments, Helsinki, Finland) as described previously [5]. A phospholipid sample was sprinkled onto the clean surface of pure water (pH ∼ 6) in a Teflon vessel at 25.0 ± 0.2 °C. The equilibrium spreading pressures, π_es were reproduced within ± 0.2 mN/m.

3.5. Optical microscope observations

Lyotropic mesophase behavior of the fluorinated phospholipids was examined by the use of either an Olympus BHSP polarizing microscope equipped with an Olympus C-4040 digital camera or a Nikon Eclipse TE2000 inverted microscope with DIC optics and a Nikon DS-2Mv CCD camera. A fine powder of phospholipid on a slide glass mounted on a microscope stage was covered with a cover slip. A water drop was introduced from the edge of the cover slip and then the textures of phospholipid/water mixtures were observed at 25 °C.

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