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Synthesis and monolayer properties of double-chained phosphatidylcholines containing perfluoroalkyl groups of different length

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ABSTRACT

A series of double-chained phosphatidylcholines (PCs), 1,2-dioctadec-9′-ynoyl-*sn*-glycero-3-phosphocholine analogs containing perfluoroalkyl moieties (CF_3, C_2F_5, *n*-C_4F_9 or *n*-C_8F_{17}) as the terminal segment in two hydrophobic chains, **1a–d**, were synthesized. Equilibrium spreading pressures of these fluorinated PCs at the air–water interface were measured as an indication of monolayer stability, in order to obtain the minimal fluorine content in PC molecule efficient to exhibit monolayer stabilizing effect. The monolayer stability sigmoidally increased with the fluorine content in PC molecule and subsequently leveled off above a certain fluorine content, i.e., *n*-C_4F_9 moiety, at 25 °C. Under this condition, the replacement of at least five hydrogen atoms at the terminal hydrophobic segment in double-chained PC molecule by fluorine atoms, i.e., CF_3CF_2 moiety, is required to exhibit the monolayer stabilizing effect, whereas further fluorination of double-chained PC ($F(CF_2)_n$; $n > 4$) has a minor effect on the monolayer stability.

1. Introduction

Fluorinated amphiphiles (surfactants and lipids) exhibit unique properties such as weak cohesive force due to low polarizability of fluorine atom, low surface energy, biological inertness, etc. [1–5]. Therefore, the fluorinated amphiphiles have been investigated from the viewpoint of industrial (detergents, surface modifiers, etc.), biotechnological (protein solubilizer, protein crystallizer, etc.), and/or medical applications (DDS matrices, MRI reagent, etc.) [2,3,6–10]. Many studies on syntheses and interfacial chemical characterization of fluorinated phosphatidylcholines (PCs) as fluorinated amphiphiles have been carried out so far [4,11–18]. Vierling and co-workers reported the syntheses of fluorinated PCs containing C_{17} or C_{19} carbon chains [13–15]. The proposed PCs formed stable liquid-condensed monolayers at the air–water interface with higher collapse pressures than dipalmitoylphosphatidylcholine (DPPC) or distearoylphosphatidylcholine (DSPC) [13–15]. The proposed fluorinated PCs, however, formed crystalline or gel phase bilayers at ambient temperatures [16], which is not preferable for biotechnological uses. In general, the molecules containing long perfluoroalkyl moiety tend to form rigid self-assemblies in water. Therefore, the introduction of perfluoroalkyl chain into the target molecules is limited in chain length for ease of handling. In addition, highly fluorinated lipids tend to be incompatible with many kinds of biomaterials (proteins, lipids, etc.) [12,19–22].

In our previous study [23], we synthesized C_{18} double-chained PC containing perfluorooctyl groups with C–C triple bond (monoyne bond), and observed it shows high monolayer stability at the air–water interface. In addition, we observed the fluorinated PC forms stable and fluid vesicle membranes in water [23], suggesting the fluorinated PCs with monoyne bonds are expected to be suitable lipid materials for biotechnological uses. We have already reported the synthesis of a series of fluorinated C_{18} fatty acids with monoyne bond, i.e., fluorinated stearolic acids containing $F(CF_2)_n$ ($n = 1$, 2, 3, 4, 8), and estimated their monolayer stability by measuring their equilibrium spreading pressures, π_es, at the air–water interface and at 25 °C. Under this condition, the replacement of at least five hydrogen atoms at the terminal hydrophobic segment in stearolic acid molecule by fluorine atoms (CF_3CF_2 group) was required to alter the bulk property of stearolic acid and exhibit the monolayer stabilizing effect, whereas further fluorination of stearolic acid ($F(CF_2)_n$; $n > 4$) had a minor effect on the monolayer stability [24,25].

In this paper, based on the information regarding the relationship between fluorine content in fluorinated stearolic acid and their monolayer stability, we tried to synthesize a series of double-chained PCs with fluorinated stearolic acids, i.e., 1,2-dioctadec-9′-

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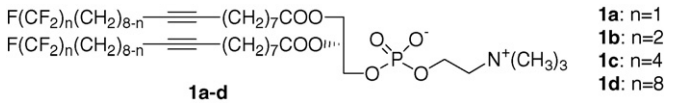


Fig. 1. Novel partially fluorinated double-chained phosphatidylcholines (**1a**–**d**).

ynoyl-*sn*-glycero-3-phosphocholine analogs (Fig. 1) containing perfluoroalkyl moieties (CF_3, C_2F_5, *n*-C_4F_9 or *n*-C_8F_{17}) as the terminal segment in two hydrophobic chains. Further, we tried to estimate their monolayer stability, in order to obtain the minimal fluorine content in PC molecule sufficient to exhibit monolayer stabilizing effect from the viewpoint of biocompatibility.

2. Results and discussion

We achieved syntheses of a series of double-chained PCs **1a**–**d** containing perfluoroalkyl moieties (CF_3, C_2F_5, *n*-C_4F_9 or *n*-C_8F_{17}) and the corresponding non-fluorinated counterpart **1e** as shown in Scheme 1.

The fluorinated stearolic acids **2a**–**d**, the corresponding non-fluorinated counterpart, i.e., stearolic acid (**2e**), and (*R*)-3-*O*-(4′-methoxybenzyloxy)-1,2-propanediol (**3**) were provided by a known procedure [23–26]. The esterification of **2a**–**e** with **3** proceeded at room temperature in the presence of *N*, *N*′-dicyclohexyl carbodiimide (DCC) to afford **4a**–**e** in high yields. **4a**–**e** were treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to give **5a**–**e** in high yields [27]. **5a**–**e** were phosphorylated with 2-bromoethylphosphorodichloriate in the presence of triethylamine to furnish the phosphorylated intermediates. The crude intermediates were heated at 60 °C with excess trimethylamine to produce **1a**–**e** in good yields [28].

We measured equilibrium spreading pressures, π_es of the synthesized PCs at the air–water interface as an indication of monolayer stability [29,30]. The bulk solid of **1a**–**e** rapidly spread at the air–water interface, and as a result, they formed the stable liquid-expanded monolayers. The relationship between the value of π_e and the fluorinated carbon number in a single hydrophobic chain, *N*, is shown in Fig. 2.

The monolayer stability sigmoidally increased with the fluorine content in PC molecule and subsequently leveled off above a certain fluorine content, i.e., *n*-C_4F_9 moiety, at 25 °C. Under this condition, the replacement of at least five hydrogen atoms at the terminal hydrophobic segment in double-chained PC molecule by fluorine atoms, CF_3CF_2 moiety, is required to exhibit the monolayer stabilizing effect, whereas further fluorination of double-chained PC ($F(CF_2)_n$; $n > 4$) has a minor effect on the monolayer stability, suggesting the surface structures of the air side of monolayers formed with PCs containing $F(CF_2)_n$ ($n = 4$–8) should be almost the same. The analogous relations between fluorinated chain length and interfacial property have been reported in cases of wettability

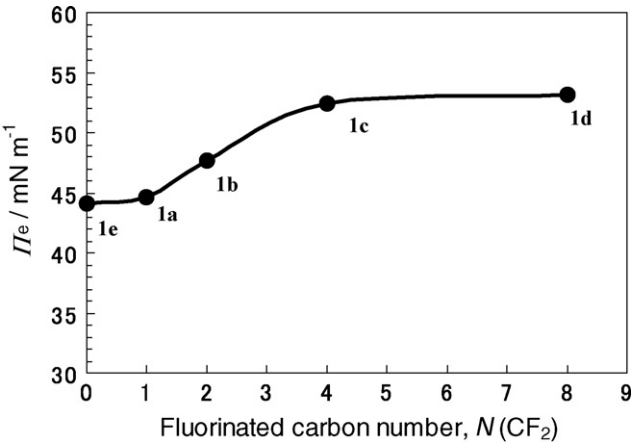
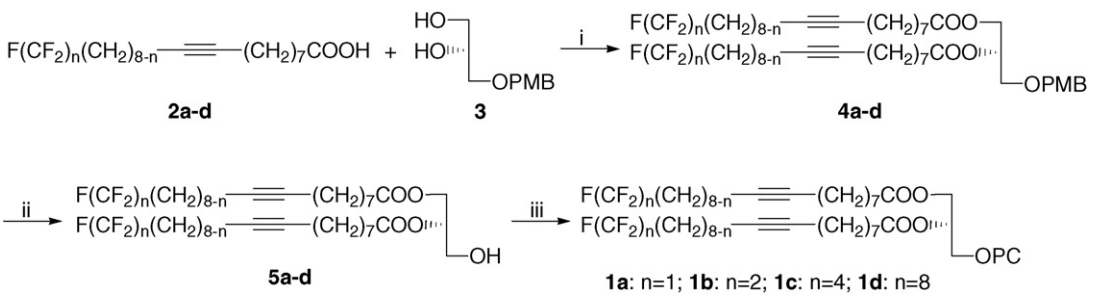


Fig. 2. Effect of the fluorine content in the hydrophobic chain on the equilibrium spreading pressures (π_es) of the partially fluorinated phosphatidylcholine analogs **1a**–**e** at the air–water interface and 25 °C. Subphase is pure water (unbuffered water, pH ~6).

of fluorinated monolayers [31] and polymers with fluorinated side chains [34]. In these cases, wettability decreased with increasing fluorination of the chains or surface fluorine concentration to a minimum wettability for the highly fluorinated surface [31]. The monolayer stability should be also controlled by the same molecular factors determining the wettability, which is much sensitive to the chemical composition and physical structure of the outermost layer of materials [35].

It is noteworthy that the monolayer stability of non-fluorinated PC counterpart **1e** and CF_3-termenated PC **1a** is unexpectedly almost the same in contrast with a series of fluorinated stearolic acids, where the introduction of terminal CF_3-group significantly raises the equilibrium surface pressure of stearolic acid at 25 °C [25]. One factor affecting the relationship between the value of π_e and the fluorinated carbon number should be a difference in the bulk state, i.e., hydrated stearolic acid and its CF_3-terminated analog are in the solid state at the air–water interface while the more fluorinated stearolic acid analogs and all of the PCs in this study are considered to be in the liquid one. Another factor may be attributed to the fluorinated chain length-dependent additional interaction, i.e., the mutual repulsive interaction between the strong dipoles associated with CF_3–CH_2 bond [31,32] as shown in Fig. 3. At the most closely packed chain state, the orientational order of CF_3–CH_2 dipoles may become higher and the repulsive interaction among the orientated dipoles might contribute to the reduction of the monolayer stability in the fluorinated chain length-dependent manner.

In conclusion, we could successfully synthesized double-chained PCs, 1,2-dioctadec-9′-ynoyl-*sn*-glycero-3-phosphocholine analogs



Scheme 1. Synthesis of partially fluorinated double-chained phosphatidylcholines (**1a**–**d**). (i) DCC, DMAP, CH_2Cl_2, rt, 3 h; (ii) DDQ, pH 7.0 H_3PO_4 buffer, CH_2Cl_2, rt, 3 h; (iii) (a) $Br(CH_2)_2OP(O)Cl_2$, Et_3N, benzene, rt, 18 h, (b) H_2O, rt, 8 h, (c) Me_3N aq., $CHCl_3/CH_3CN/i$-PrOH, 60 °C, 18 h.

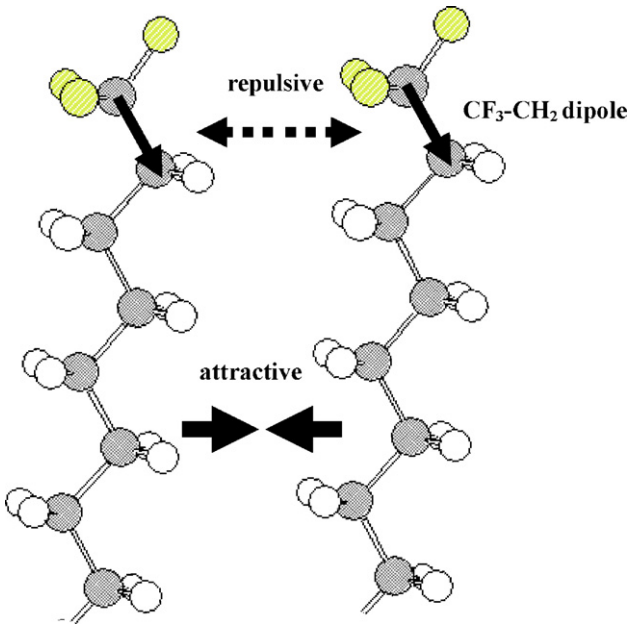


Fig. 3. Illustrative diagram of the terminal CF_3 group effect on the interactions among the hydrophobic chains (only terminal C_8 segment was shown) in CF_3-terminated PC monolayer.

containing perfluoroalkyl moieties (CF_3, C_2F_5, n-C_4F_9 or n-C_8F_{17}) as the terminal segment in two hydrophobic chains, **1a**–**d**. We also found the monolayer stability sigmoidally increased with the fluorine content in PC molecule and subsequently leveled off above a certain fluorine content, (i.e., n-C_4F_9 moiety) at 25 °C. The fluorination at only the terminal methyl groups in the PC unexpectedly exhibited no effect on the monolayer stability. At present, it is not obvious whether this finding is derived from the bulk property of lipids or monolayer packing state. In the future, we will reexamine the fluorination effect on monolayer stability employing the fluorinated fatty acids of which bulk state is in the liquid one.

3. Experimental

3.1. Instruments

^{1}H- and ^{19}F NMR spectra were measured on a JEOL JNM-LA 500 FT NMR system (500 MHz) using TMS and benzotrifluoride as internal standards, respectively. FT-IR spectra were measured on a JASCO FT-/IR-680 plus. Mass spectra (ESI-MS) were measured on a JEOL JMS-700T Tandem MStation. Column chromatography purifications were carried out using silica gel 60 (Merck 7734). HPLC analyses were carried out on an HP Model 1100 system (Hewlett-Packard/Agilent Technologies) equipped with an ERC-7515A RI detector (ERC Inc., Tokyo) and an Inertsil ODS-3 column (4.6 mm i.d. × 150 mm, GL Sciences Inc.), which was maintained at 37 °C in the system itself, with acetonitrile/water/formic acid (=90:10:0.05) as the mobile phase at a flow rate of 0.8 ml/min. The eluate was monitored with the UV detector at 215 nm and the RI detector.

3.2. Materials

3.2.1. Synthesis of 1,2-dioctadec-9′-ynoyl-sn-glycero-3-phosphocholine (1e)

3.2.1.1. 1,2-Dioctadec-9′-ynoyl-3-O-4″-methoxybenzyl-sn-glycerol (4e). To a solution of stearolic acid (**2e**) (2.08 g, 5.62 mmol), (*R*)-3-*O*-4′-methoxybenzyloxy-1,2-propanediol (**3**) (477 mg, 2.25 mmol) and *N*,*N*-dimethyl-4-aminopyridine (687 mg, 5.62 mmol) in CH_2Cl_2 (28 ml) in ice bath was added a solution of *N*,*N*′-dicyclohexyl carbodiimide (1.16 g, 5.62 mmol) in CH_2Cl_2 (14 ml) over a period of 5 min. The mixture was stirred in ice bath for 10 min, and then stirred at room temperature for 3 h. After filtration, the reaction mixture was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with 2 M HCl, satd. $NaHCO_3$ and satd. NaCl, and dried over $MgSO_4$. After the solvent was evaporated, the residue was separated by column chromatography (SiO_2, EtOAc/*n*-hexane; 10%) to give 1,2-dioctadec-9′-ynoyl-3-*O*-4″-methoxybenzyl-*sn*-glycerol (**4e**) (2.06 g, quantitative).

4e: ^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, J = 7.01 Hz), 1.21–1.41 (32H, m), 1.47 (8H, quint, J = 6.99 Hz), 1.55–1.66 (4H, m), 2.13 (8H, t, J = 6.99 Hz), 2.27 (2H, t, J = 7.61 Hz), 2.31 (2H, t, J = 7.61 Hz), 3.52–3.59 (2H, m), 3.81 (3H, s), 4.17 (1H, dd, J = 11.88, 6.39 Hz), 4.33 (1H, dd, J = 11.88, 3.64 Hz), 4.47 (2H, ABq, J = 11.88, 9.73 Hz), 5.18–5.26 (1H, m), 6.87 (2H, dt, J = 9.13, 2.45 Hz), 7.23 (2H, dt, J = 9.13, 2.45 Hz).

3.2.1.2. 1,2-Dioctadec-9′-ynoyl-sn-glycerol (5e). 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (0.91 g, 4.03 mmol) was added to a solution of **4e** (1.98 g, 2.69 mmol) in CH_2Cl_2 (27 ml) and pH 7.0 phosphate buffer (2.7 ml) at 0 °C. The mixture was stirred at room temperature for 3 h, quenched with iced satd. $NaHCO_3$, and extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with satd. NaCl and dried over $MgSO_4$. After the evaporation of the solvent, the residue was separated by column chromatography (SiO_2, EtOAc/*n*-hexane; 20–30%) to give 1,2-dioctadec-9′-ynoyl-*sn*-glycerol (**5e**) (1.35 g, 81%).

5e: ^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, J = 7.31 Hz), 1.21–1.42 (32H, m), 1.47 (8H, quint, J = 7.31 Hz), 1.58–1.68 (4H, m), 2.13 (8H, t, J = 6.09 Hz), 2.33 (2H, t, J = 7.31 Hz), 2.35 (2H, t, J = 7.31 Hz), 3.68–3.78 (2H, m), 4.24 (1H, dd, J = 12.18, 6.09 Hz), 4.32 (1H, dd, J = 12.18, 4.87 Hz), 5.08 (1H, quint, J = 4.87 Hz).

3.2.1.3. 1,2-Dioctadec-9′-ynoyl-sn-glycero-3-phosphocholine (1e). To a solution of 2-bromoethylphosphorodichloridate (1.32 g, 5.45 mmol) in benzene (11 ml) containing triethylamine (0.76 ml, 5.45 mmol) at ice bath was added a solution of **5e** (2.24 g, 3.63 mmol) in benzene (54 ml) under nitrogen. The mixture was stirred at room temperature for 18 h, and then the reaction mixture was evaporated. The residue was extracted with $CHCl_3$ after being stirred with water (36 ml) at room temperature for 4 h. After the reaction mixture was extracted with $CHCl_3$, the solvent was removed under atmospheric pressure to afford the phosphoryl intermediate. Trimethylamine aq. was added to the intermediate in a mixture of 2-propanol (18 ml), acetonitrile (18 ml) and $CHCl_3$ (11 ml). The solution was stirred at 60 °C for 18 h. After removal of the solvent, the crude compound was purified by column chromatography (SiO_2, $CHCl_3$:MeOH:H_2O = 3:2:0 to 65:35:8 and Sephadex LH20, MeOH) to give 1,2-dioctadec-9′-ynoyl-*sn*-glycero-3-phosphocholine (**1e**) (1.42 g, 50%).

1e: colorless waxy solid. $[\alpha]_D$ +5.625° (c 0.01, MeOH). FAB-MS m/z: 782 $(M+H)^+$. HRMS (FAB) calcd for $C_{44}H_{81}NO_8P$ $(M+H)^+$ 782.5700: Found 782.5703. ^{1}H NMR ($CDCl_3$) δ: 0.88 (6H, t, J = 7.31 Hz), 1.22–1.40 (32H, m), 1.47 (8H, quint, J = 7.31 Hz), 1.53–1.63 (4H, m), 2.13 (8H, t, J = 7.31 Hz), 2.13 (8H, t, J = 7.31 Hz), 2.28 (2H, t, J = 7.31 Hz), 2.30 (2H, t, J = 7.31 Hz), 3.38 (9H, s), 3.79–3.89 (2H, m), 3.93–4.05 (2H, m), 4.13 (1H, dd, J = 12.18, 7.31 Hz), 4.37 (2H, bs), 4.40 (1H, dd, J = 12.18, 2.44 Hz), 5.17–5.27 (1H, m).

3.2.2. Synthesis of 1,2-di(18′,18′,18′-trifluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1a)

1a was prepared from 9-octadecynoic acid (**2e**) and **3** in the same manner as **1e**.

3.2.2.1. 1,2-Di(18′,18′,18′-trifluorooctadec-9′-ynoyl)-3-O-4″-methoxybenzyl-sn-glycerol (4a). **4a** (quantitative): ^{1}H NMR ($CDCl_3$) δ: 1.25–1.67 (40H, m), 1.99–2.10 (12H, m), 27 (2H, t, J = 7.79 Hz), 2.32 (2H, t, J = 7.79 Hz), 3.56 (2H, d, J = 5.04 Hz), 3.81 (3H, s), 4.17 (1H, dd, J = 11.91, 6.41 Hz), 4.33 (1H, dd, J = 11.91, 3.66 Hz), 4.47 (2H, ABq, J = 11.91, 6.41 Hz), 5.22 (1H, quint, J = 5.04 Hz), 6.88 (2H, d, J = 8.24 Hz), 7.23 (2H, d, J = 8.24 Hz). ^{19}F NMR ($CDCl_3$) ppm: −3.65 (6F, t, J = 10.46 Hz).

3.2.2.2. 1,2-Di(18′,18′,18′-trifluorooctadec-9′-ynoyl)-sn-glycerol (5a). **5a** (86%): ^{1}H NMR ($CDCl_3$) δ: 1.25–1.43 (24H, m), 1.43–1.52 (8H, m), 1.52–1.68 (8H, m), 1.90–2.10 (4H, m), 2.10–2.18 (8H, m), 2.33 (2H, t, J = 7.31 Hz), 2.35 (2H, t, J = 7.31 Hz), 3.68–3.78 (2H, m), 4.23 (1H, dd, J = 12.18, 6.09 Hz), 4.32 (1H, dd, J = 12.18, 4.87 Hz), 5.08 (1H, quint, J = 4.87 Hz). ^{19}F NMR ($CDCl_3$) ppm: −3.65 (6F, t, J = 10.45 Hz).

3.2.2.3. 1,2-Di(18′,18′,18′-trifluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1a). **1a** (56%): colorless waxy solid. $[\alpha]_D$ +4.752° (c 0.01, MeOH). ^{1}H NMR ($CDCl_3$) δ: 1.23–1.42 (24H, m), 1.42–1.52 (8H, m), 1.51–1.64 (8H, m), 1.99–2.13 (4H, m), 2.08–2.19 (8H, m), 2.28 (2H, t, J = 7.33 Hz), 2.30 (2H, t, J = 7.33 Hz), 3.37 (9H, s), 3.75–3.86 (2H, m), 3.90–4.04 (2H, m), 4.13 (1H, dd, J = 11.91, 7.33 Hz), 4.34 (2H, bs), 4.40 (1H, dd, J = 11.91, 2.75 Hz), 5.14–5.28 (1H, m). ^{19}F NMR ($CDCl_3$) ppm: −3.65 (6F, t, J = 10.45 Hz).

3.2.3. Synthesis of 1,2-di(17′,17′,18′,18′,18′-pentafluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1b)

1b was prepared from 17′,17′,18′,18′,18′-pentafluorooctadec-9′-ynoic acid (**2b**) and **3** in the same manner as **1a**.

3.2.3.1. 1,2-Di(17′,17′,18′,18′,18′-pentafluorooctadec-9v-ynoyl)-3-O-4″-methoxybenzyl-sn-glycerol (4b). **4b** (quantitative): ^{1}H NMR ($CDCl_3$) δ: 1.21–1.53 (28H, m), 1.53–1.66 (8H, m), 1.92–2.07 (4H, m), 2.27 (2H, t, J = 7.31 Hz), 2.31 (2H, t, J = 7.31 Hz), 3.49 (2H, dd, J = 4.87, 1.22 Hz), 3.80 (3H, s), 4.17 (1H, dd, J = 12.18, 6.09 Hz), 4.32 (1H, dd, J = 12.18, 3.65 Hz), 4.46 (2H, q, J = 10.96 Hz), 5.21 (1H, quint, J = 4.87 Hz), 6.87 (2H, d, J = 8.53 Hz), 7.23 (2H, d, J = 8.53 Hz). ^{19}F NMR ($CDCl_3$) ppm: −22.44 (3F, s), −55.19 (2F, t, J = 18.34 Hz).

3.2.3.2. 1,2-Di(17′,17′,18′,18′,18′-pentafluorooctadec-9′-ynoyl)-sn-glycerol (5b). **5b** (98%): ^{1}H NMR ($CDCl_3$) δ: 1.27–1.53 (26H, m), 1.55–1.67 (10H, m), 1.94–2.08 (4H, m), 2.10–2.18 (8H, m), 2.32 (2H, t, J = 7.31 Hz), 2.35 (2H, t, J = 7.31 Hz), 3.68–3.78 (2H, m), 4.23 (1H, dd, J = 12.18, 4.87 Hz), 4.32 (1H, dd, J = 12.18, 4.87 Hz), 5.08 (1H, quint, J = 4.87 Hz). ^{19}F NMR ($CDCl_3$) ppm: −22.44 (6F, s), −55.19 (4F, t, J = 18.30 Hz).

3.2.3.3. 1,2-Di(17′,17′,18′,18′,18′-pentafluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1b). **1b** (53%): colorless waxy solid. $[\alpha]_D$ +4.845° (c 0.01, MeOH). FAB-MS m/z: 962 (M+H)$^+$. HRMS (FAB) calcd for $C_{44}H_{71}F_{10}NO_8P$ (M+H)$^+$ 962.4758: Found 962.4749. ^{1}H NMR ($CDCl_3$) δ: 1.25–1.53 (28H, m), 1.54–1.64 (8H, m), 2.10–2.19 (8H, m), 2.22–2.33 (4H, m), 3.39 (9H, s), 3.76–3.87 (2H, m), 3.92–4.03 (2H, m), 4.13 (1H, dd, J = 12.18, 7.31 Hz), 4.34 (2H, bs), 4.41 (1H, dd, J = 12.18, 2.44 Hz), 5.17–5.26 (1H, m). ^{19}F NMR ($CDCl_3$) ppm: −22.58 (6F, s), −55.33 (4F, t, J = 18.24 Hz).

3.2.4. Synthesis of 1,2-di(15′,15′,16′,16′,17′,17′,18′,18′,18′-nonafluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1c)

1c was prepared from 15′,15′,16′,16′,17′,17′,18′,18′,18′-nonafluorooctadec-9′-ynoic acid (**2c**) and **3** in the same manner as **1a**.

3.2.4.1. 1,2-Di(15′,15′,16′,16′,17′,17′,18′,18′,18′-nonafluorooctadec-9′-ynoyl)-3-O-4″-methoxy-benzyl-sn-glycerol (4c). **4c** (90%): ^{1}H NMR ($CDCl_3$) δ: 1.24–1.41 (12H, m), 1.42–1.51 (4H, m), 1.53–1.65 (8H, m), 1.68–1.77 (4H, m), 2.01–2.16 (8H, m), 2.18–2.24 (4H, m), 2.27 (2H, t, J = 7.61 Hz), 2.31 (2H, t, J = 7.61 Hz), 3.51–3.59 (2H, m), 3.80 (3H, s), 4.17 (1H, dd, J = 11.88, 6.39 Hz), 4.32 (1H, dd, J = 11.88, 3.14 Hz), 4.46 (2H, ABq, J = 11.58, 10.03 Hz), 5.18–5.26 (1H, m), 6.87 (2H, dt, J = 8.83, 2.25 Hz), 7.23 (2H, dt, J = 8.83, 2.25 Hz). ^{19}F NMR ($CDCl_3$) ppm: −18.32 (6F, t, J = 10.80 Hz), −51.89 (4F, quint, J = 15.97 Hz), −61.68 to −61.88 (4F, m), −63.23 to −63.42 (4F, m).

3.2.4.2. 1,2-Di(15′,15′,16′,16′,17′,17′,18′,18′,18′-nonafluorooctadec-9′-ynoyl)-sn-glycerol (5c). **5c** (81%): ^{1}H NMR ($CDCl_3$) δ: 1.26–41 (12H, m), 1.47 (4H, quint, J = 7.31 Hz), 1.53–1.67 (8H, m), 1.68–1.77 (4H, m), 2.00–2.17 (8H, m), 2.18–2.24 (4H, m), 2.32 (2H, t, J = 7.31 Hz), 2.34 (2H, t, J = 7.31 Hz), 3.68–3.78 (2H, m), 4.23 (1H, dd, J = 12.18, 6.09 Hz), 4.31 (1H, dd, J = 12.18, 4.87 Hz), 5.08 (1H, quint, J = 4.87 Hz). ^{19}F NMR ($CDCl_3$) ppm: −18.01 (6F, t, J = 10.45 Hz), −51.54 (4F, quint, J = 15.68 Hz), −61.32 to −61.54 (4F, m), −62.90 to −63.10 (4F, m).

3.2.4.3. 1,2-Di(15′,15′,16′,16′,17′,17′,18′,18′,18′-nonafluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1c). **1c** (55%): colorless waxy solid. $[\alpha]_D$ +3.901° (c 0.01, MeOH). FAB-MS m/z: 1106 (M+H)$^+$. HRMS (FAB) calcd for $C_{44}H_{63}F_{18}NO_8P$ (M+H)$^+$ 1106.4004: Found 1106.4011. ^{1}H NMR ($CDCl_3$) δ: 1.26–1.42 (12H, m), 1.47 (4H, quint, J = 7.31 Hz), 1.54–1.65 (8H, m), 1.69–1.77 (4H, m), 2.03–2.18 (8H, m), 2.18–2.25 (4H, m), 2.31 (4H, q, J = 8.53 Hz), 3.21 (9H, s), 3.56–3.61 (2H, m), 4.00 (2H, t, J = 6.09 Hz), 4.14 (1H, dd, J = 12.18, 6.09 Hz), 4.24 (2H, bs), 4.40 (1H, dd, J = 12.18, 4.87 Hz), 5.18–5.25 (1H, m). ^{19}F NMR ($CDCl_3$) ppm: −18.01 (6F, t, J = 10.46 Hz), −51.55 (4F, quint, J = 15.69 Hz), −61.31 to −61.92 (4F, m), −62.90 to −63.10 (4F, m).

3.2.5. Synthesis of 1,2-di(11′,11′,12′,12′,13′,13′,14′,14′,15′,15′,16′,16′,17′,17′,18′,18′,18′-heptadecafluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1d)

1d was prepared from 11′,11′,12′,12′,13′,13′,14′,14′,15′,15′,16′,16′,17′,17′,18′,18′,18′-heptadecafluorooctadec-9′-ynoic acid (**2d**) and **3** in the same manner as **1a**.

3.2.5.1. 1,2-Di(11′,11′,12′,12′,13′,13′,14′,14′,15′,15′,16′,16′,17′,17′,18′,18′,18′-heptadeca-fluorooctadec-9′-ynoyl)-3-O-4″-methoxybenzyl-sn-glycerol (4d). **4d** (91%): ^{1}H NMR ($CDCl_3$) δ: 1.27–1.44 (12H, m), 1.53–1.66 (8H, m), 2.27 (2H, t, J = 7.61 Hz), 2.32 (2H, t, J = 7.61 Hz), 2.30–2.38 (4H, m), 3.51–3.59 (2H, m), 3.80 (3H, s), 4.17 (1H, dd, J = 11.87, 6.70 Hz), 4.33 (1H, dd, J = 11.87, 3.65 Hz), 4.46 (2H, ABq, J = 11.87, 10.35 Hz), 5.19–5.26 (1H, m), 6.87 (2H, dt, J = 8.83, 2.74 Hz), 7.23 (2H, dt, J = 8.83, 2.74 Hz). ^{19}F NMR ($CDCl_3$) ppm: −18.32 (6F, t, J = 10.46 Hz), −33.74 (4F, s), −58.67 (4F, s), −59.26 to −59.65 (8F, m), −60.08 (4F, bs), −60.28 (4F, bs), −63.54 to −63.82 (4F, m).

3.2.5.2. 1,2-Di(11′,11′,12′,12′,13′,13′,14′,14′,15′,15′,16′,16′,17′,17′,18′,18′,18′-heptadeca-fluorooctadec-9′-ynoyl)-sn-glycerol (5d). **5d** (97%): ^{1}H NMR ($CDCl_3$) δ: 1.28–1.45 (12H, m), 1.53–1.68 (8H, m), 2.29–2.39 (8H, m), 3.68–3.78 (2H, m), 4.23 (1H, dd, J = 11.88, 5.49 Hz), 4.33 (1H, dd, J = 11.88, 4.54 Hz), 5.09 (1H, quint, J = 5.49 Hz). ^{19}F NMR ($CDCl_3$) ppm: −18.06 (6F, t, J = 10.57 Hz), −33.51 (4F, s), −58.41 (4F, s), −59.04 to −59.38 (8F, m), −59.86 (4F, bs), −60.03 (4F, bs), −63.30 to −63.52 (4F, m).

3.2.5.3. 1,2-Di(11′,11′,12′,12′,13′,13′,14′,14′,15′,15′,16′,16′,17′,17′,18′,18′,18′-heptadeca-fluorooctadec-9′-ynoyl)-sn-glycero-3-phosphocholine (1d). **1d** (43%): colorless waxy solid. $[\alpha]_D$ +2.429° (c 0.01, MeOH). FAB-MS m/z: 1394 (M+H)$^+$. HRMS (FAB) calcd for $C_{44}H_{47}F_{34}NO_8P$ (M+H)$^+$ 1394.2496: Found 1394.2502. ^{1}H NMR

($CDCl_3$) δ: 1.26–1.36 (8H, m), 1.35–1.44 (4H, m), 1.58 (8H, quint, J = 7.31 Hz), 2.28 (2H, t, J = 7.31 Hz), 2.30 (2H, t, J = 7.31 Hz), 2.35 (4H, quint, J = 6.09 Hz), 3.38 (9H, s), 3.81 (2H, bs), 3.87–4.01 (2H, m), 4.12 (1H, dd, J = 12.18, 7.31 Hz), 4.31 (2H, bs), 4.41 (1H, dd, J = 12.18, 2.44 Hz), 5.15–5.25 (1H, m). ^{19}F NMR ($CDCl_3$) ppm: −18.40 (6F, t, J = 10.80 Hz), −33.73 (4F, s), −58.66 (4F, s), −59.29 to −59.64 (8F, m), −60.13 (4F, s), −60.29 (4F, s), −63.58 to −63.82 (4F, m).

3.3. Equilibrium spreading pressure measurements

The purity of fluorinated PCs was checked by ^{1}H NMR, ^{19}F NMR and HPLC analysis in 99% purity. The purification of water for interfacial chemical measurements was described in our previous publications [23–25,33]. The PC sample was sprinkled onto the clean surface of unbuffered water (pH ∼ 6) in a thermostated Teflon vessel as one or more visible powders remained on the surface. The surface pressure was monitored by Wilhelmy technique using a sandblasted platinum plate attached to a KSV electronic balance (pressure sensitivity: 0.01 mN/m; KSV Instruments, Helsinki, Finland) at 25.0 ± 0.2 °C. This set-up was housed in a clean box to reduce any contaminations. The equilibrium spreading pressures, π_es were reproduced within ±0.5 mN/m.

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