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Synthesis of Two New Phospholipidic Fluorescent Probes for Membrane Studies.

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Abstract: Two new fluorenyl-phospholipidic probes have been prepared in good yield by a Pd^0/CuI-catalyzed cross-coupling reaction.

Introduction

An important property of phospholipidic biomembranes is their fluidity. It is considered that many other properties, such as bilayer permeability and optimal activity of membrane-bound proteins, are directly influenced by this fundamental physical characteristic.

Fluidity can be evaluated by many different methods, and in particular by measuring the anisotropy of fluorescence decay of a lipophilic probe embedded in the double-bilayer, which is related to the extent or the rate of orientational excursions of the lipidic chains surrounding the probe.[1-3] It would be of course important to use probes built in such a way that their fluorescent moiety is maintained at a precise depth in the membrane, and this has led recently to the introduction of novel probes, in which a fatty acid group, serving as an anchoring polar head, is linked by an alkyl chain (or two) to a fluorenyl [4,5] or a pyrenyl group.[6] By varying the length of the alkyl linkers, it should in principle be possible to explore the fluidity of the double-layer at various depths. The use of C-C bonds between the fluorophore and the alkyl chains should ensure a better lipophilicity than with the ester groups used previously with an anthroyloxy probe.[7]

Another interesting question in the study of biomembranes is to determine the depth from the membrane surface of a specific site of a membrane protein. Among different methods employed for this purpose, fluorescence energy transfer and fluorescence quenching[8-11] have been used to determine the positions of fluorophores, for example the tryptophanes of membrane proteins. This method, however, requires prior estimates of the average positions of the fluorescence quenchers (for example, bromine atoms in bilayers formed from bromolipid probes) in order to obtain the accurate position of the donor fluorophore.[12,13] Moreover, an extensive dynamic disorder of the phospholipid matrix or the probe is unavoidable, leading obviously to a loss of depth selectivity. The concept of transmembrane immobilization of the fluorescent probe in bilayers could provide a solution to such problems.[14,15]

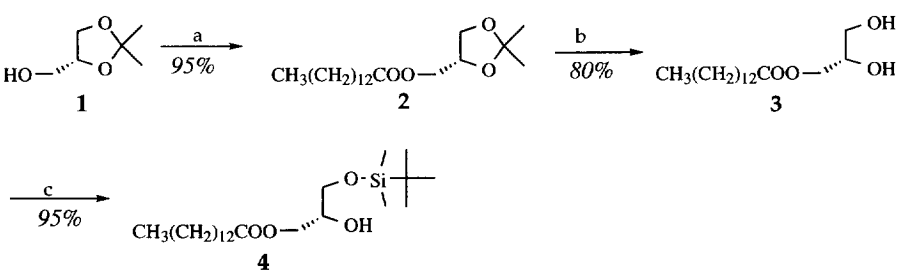
We have previously developed membrane probes using a different principle, the photoactivation of a benzophenone chromophore, introduced by Breslow *et al.*,[16] and showed that it is possible to improve dramatically the order of the bilayer + probe system in two ways : the construction of a phospholipid double-headed system, with the photoactivatable group located in the middle of a long-transmembrane chain, and the addition of large amounts of cholesterol, the well known reinforcer of eucaryotic membranes. The introduction of the phospholipidic polar heads ensures an optimal compatibility of the probe with the membrane it is supposed to report on without disturbing it, as shown in particular by the binary phase diagram and by solid-state ^{2}H-NMR.[15] The use of cholesterol at high, but physiologically reasonable, concentrations (up to 33 % molar) is essential to obtain a good site selectivity.

We describe now the synthesis of two new fluorescent probes based on these principles: both combine a phosphatidyl choline head (or two) with acyl chains : at C-1 of glycerol a normal fatty acid (myristoyl) chain, and at C-2, either an alkyl chain terminated by the fluorene group, or the sequence: alkyl chain-fluorene-alkyl chain-second phosphatidylcholine, supposed to maintain the fluorene in the middle of the double layers with minimal pertubation. This synthesis makes use of a palladium-copper catalyzed cross-coupling of the fluorenyl group with an acetylenic chain, and of a protection of the glycerol as an acetonide, which can be removed with boron trichloride without acyl migration.

Results and discussion

The synthetic scheme adopted in the synthesis of 1-acyl-3-t-butyldimethylsilyloxy-*sn*-glycerol is shown below. To obtain optically pure mixed-chain phosphatidylcholines, a hemi-synthesis pathway is widely used, i.e. a phosphatidylcholine is deacylated with phospholipase A_2 and the resulting lysolecithine is reacylated with the desired carboxylic acid.[17] We present here another approach : the total synthesis of the target fluorescent phospholipid **10** from 1,2-isopropylidene-*sn*-glycerol **1**, easily obtained from mannitol.[18] This method requires the stepwise regioselective acylation of the vicinal hydroxyls of the glycerol moiety to form an 1,2-diacyl-*sn*-glycerol; this is followed by the introduction of the phosphocholine moiety. Migration of the substituents and racemization must be avoided.

Our synthesis starts with the elaboration of 1-acyl-3-t-butyldimethylsilyloxy-*sn*-glycerol **4**. Esterification of the primary hydroxyl group of 1,2-isopropylidene-*sn*-glycerol **1** was realized by the conventional method using dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in



Reagents: (a) Myristic acid, DCC, DMAP, $CHCl_3$, 20°C, 16 hrs; (b) BCl_3, CH_2Cl_2, -78°C, 2 min.; (c) TBDMSiCl, imidazole THF, 20°C, 6 hrs.

chloroform.[19] 1-Myristoyl-2,3-isopropylidene-*sn*-glycerol **2** was obtained in high yield and in a large scale. This method permits to prepare the probe bearing fatty acids of different lengths, and thus to vary the

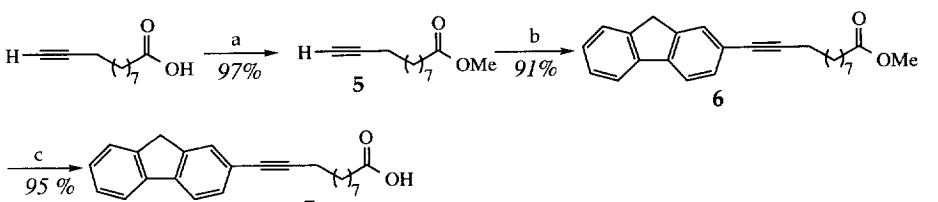
depth of the fluorophore incorporated within the bilayer. For the removal of the isopropylidene group, the classical acetonide cleavage reagents such as aqueous hydrochloric or acetic acid could not be used : acyl migration has been reported in these conditions for the synthesis of enantiomeric monoacyl-glycerols.[20] The intermediate **2** could be hydrolyzed to 3-myristoyl-*sn*-glycerol **3** by the procedure of Baer et Fischer,[21] which uses dry hydrogen chloride in diethyl ether at a low temperature, or by the method used by Mikkilinesii *et al.*[22] which employs a cation exchange resin (ethanolysis). Kodali *et al.*[19] described a modification of the first procedure: the diol **3** was obtained by application of the protected monoacyl derivative **2** to silicic acid and treatment with hydrogen chloride at -75°C for about 5-10 min. It appeared, however, that another approach using a Lewis acid such as boron trichloride - through the formation of an intermediate complex with the ether oxygens - might effect the desired cleavage of the acetonide. Boron trichloride (BCl_3) has indeed been used to remove acetonide groups in the field of sugar chemistry.[23]

The acetonide **2** was dissolved in methylene chloride and treated with 1.1 eq. of boron trichloride for 2 min. at -78 °C. After an aqueous work-up, this gave a white solid which was crystallized in methylene chloride/pentane, and whose ^1H- and ^{13}C-NMR spectra are identical with those of **3** obtained from the method using glycidol and titanium isopropoxide.[24] The mixed-melting point showed no depression, and the NMR spectra confirmed the absence of 2-acyl-*sn*-glycerol in the purified 3-myristoyl-*sn*-glycerol. Therefore, isopropylidene glycerol could be cleaved selectively without acyl-migration.

In this manner, we are able to prepare the required 3-acyl-*sn*-glycerol in two steps in 80 % overall yield, which is much higher than that obtained in the glycidol pathway (one step; 30-40 % yield).[24] Moreover, the reactions were cleaner and there were no problems in the isolation procedures, in contrast to those observed in the hydrolysis and washing out of titanium salts .

The next step is the selective protection of the primary alcohol in the glycol **3**, which ensures the introduction of two different acyl-groups at the 1 and 2 positions of glycerol. A number of protecting groups have been used for this purpose, for example the trityl group, but the yield of tritylation is generally low and the removal requires acidic conditions (hydrochloric acid or acetic acid).[25,26] Thus, the primary hydroxyl group of **3** was protected selectively as a t-butyldimethylsilyl (TBDMS) ether **4** by TBDMSCl / imidazole in THF (95 %). The TBDMS group was stable during the next coupling reaction and could be removed by different methods.

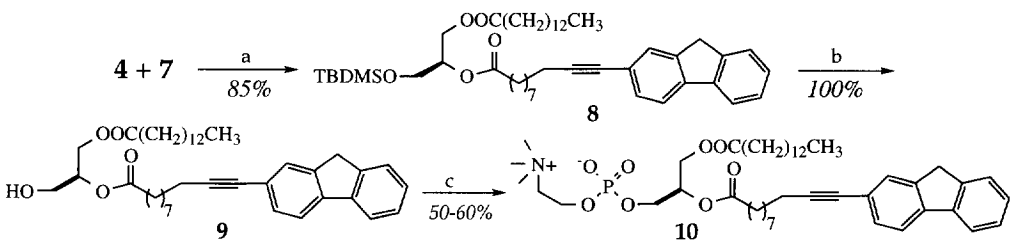
The conversion of 2-bromofluorene into the desired fluorenyl carboxylic acid derivative **7** with which the alcohol group at C-2 of the glycerol derivative **3** is acylated implied as the key step the cross-coupling of a terminal alkyne and an aryl halide catalyzed by palladium and copper, apparently used for the first time in the fluorene series.



Reagents : (a) $SOCl_2$, MeOH, -15 °C→ rt; (b) 2-bromofluorene, $PdCl_2(PPh_3)_2$, CuI, Et_2NH, reflux; (c) KOH, EtOH 95 %, reflux.

Coupling of the yne-ester **5** with 2-bromofluorene proceeded in 6 hrs at reflux in the presence of 20 mol % of $PdCl_2(PPh_3)_2$ and 10 mol % of CuI in diethylamine to give **6** in 91 % isolated yield. The conditions were sufficiently mild to avoid oxidation of the fluorene to the fluorenone, a reaction that has caused us often some problems. The ester **6** was prepared in a gram-scale and crystallized from hot pentane. The fluorenyl carboxylic acid derivative **7** was obtained by alkaline hydrolysis (95 % ethanol, KOH, reflux).

Acylation of **7** with **4** in the presence of DCC and DMAP in chloroform yielded the silylated diacyl glycerol **8** in 85 % yield. The removal of the TBDMS group was a delicate step : the commonly used tetrabutylammonium fluoride method should be avoided because it is known that during this type of deprotection, transacylation occurs very rapidly.[27] Moreover, we observed in our trial experiments the oxidation of the 9-methylene group of the fluorene. Another method, using N-bromosuccinimide / DMSO / THF / water was developed by Burgos *et al.*.[24] When we applied this method to the silyl ether **8**, deprotection was totally achieved but the partial oxidation of the fluorene group to fluorenone was observed. More recently, Morgans *et al.*[28] described a new method to regenerate the alcohol : treatment of the silyl ether in methanol with 5N HCl at 5°C. We tried this method with slight modifications (see Experimental Part). Neither transacylation, nor oxidation were observed during the reaction, and the alcohol **9** was obtained quantitatively.



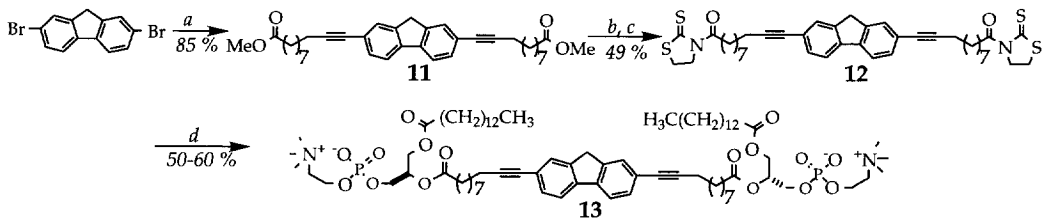
Reagents: (a) DCC, DMAP, $CHCl_3$, 20°C, 18 hrs; (b) HCl 5M, MeOH/CH_2Cl_2, 0°C, 3 hrs ; (c) i. $POCl_3$, Et_3N, $CHCl_3$, -20°C; ii. Choline tosylate, pyridine, 20°C; iii H_2O.

This alcohol was used without purification in the next reaction. Its phosphorylation with phosphorus oxychloride in chloroform at -20 °C, followed by condensation with choline tosylate[29] in the presence of pyridine, provided the desired fluorenylphospholipid **10** in 50-60 % yield.

To synthesize the transmembrane fluorescent probe **13**, we have employed a hemi-synthetic pathway, which has been previously developed in our group for the synthesis of a transmembrane probe possessing a benzophenone as the photosensitive group, using a thiazolidine-thione as the acyl-activating group and CsF as a catalyst.[30] The choice of this method was justified by the low yield reported by Just *et al.*[31] in the synthesis of a dipolaramphiphilic molecule from a lysolecithin and a carboxylic acid by the usual DCC / DMAP method. Here, we report the use of a palladium-catalyzed reaction to build up the core of the probe, like for the half-probe. We could obtain the diester **11** in gram quantities (yield: 85 %). After alkaline hydrolysis, the diacid obtained (yield : 90 %), was converted into the corresponding

bis(thiazolidine-2-thione) **12** by reaction with 2-chloro-1-methylpyridinium iodide and 2-mercaptothiazoline in 54 % yield.

The activated synthon **12** was coupled with the lysomyristoylphosphatidylcholine-cadmium chloride complex (LMPC-$CdCl_2$) in the presence of CsF in dry DMF at room temperature during 6 days. The transmembrane probe **13** was obtained in 50-60 % yield. This step is very sensitive to the presence of oxygen in the reaction medium : oxidation of the fluorene to fluorenone occurred unless great care was taken (deoxygenation of the solvent; an inert and dry atmosphere must be kept during the 6 days).



Reagents: (a) ester 5, $PdCl_2(PPh_3)_2$, CuI, Et_2NH, 8 hr; (b) KOH, 95 % EtOH , 1 hr;
(c) 2-chloro-1-methylpyridinium, 2-mercaptothiazoline, Et_3N, CH_2Cl_2, 3 hrs; (d) LMPC-$CdCl_2$, CsF, DMF, 6 d.

Thus, we have synthesized the first transmembrane fluorenyl-type probe. The procedure described here provides an easy and economically reasonable access to a new series of fluorescent probes. In particular, the Pd°-CuI catalyzed cross-coupling described here should provide a useful method for the synthesis of other aryl fluorophore groups. Thanks to the rigid and straight core of the fluorene-conjugated with two triple bonds, it was expected that the orientation of the probe in bilayers would be improved, and that the perturbation of the membrane would be smaller than with the benzophenone probe.[15] Preliminary experiments of the steady-state and time-dependent fluorescence depolarizations by Dr. G. Duportail have shown that the two synthetic probes **10** and **13** have indeed the desired fluorescence properties : the former is well adapted for the study of the fluidity of biomembranes and the latter for the analysis of the topography of proteins in bilayers. The biophysical properties of these fluorescent probes incorporated in membranes will be described in detail later.

Experimental

Melting points were measured on a Reichert hot stage microscope and are uncorrected. IR spectra were recorded in KBr on a Perkin-Elmer 881 infrared spectrophotometer. $[\alpha]_D$ were measured on a Perkin-Elmer 241 spectrophotometer. UV spectra were measured on a Kontron-Uvikon 810 UV-VIS spectrophotometer. NMR spectra were recorded on Bruker SY (200 MHz), Bruker AM (400 MHz) and AMX (500 MHz) spectrometers using $CDCl_3$ (δ = 7.26 ppm), MeOD-d_4 (δ = 3.30 ppm) and CD_2Cl_2 (δ = 5.32 ppm) as internal standards for ^1H NMR and $CDCl_3$ (δ = 77.0 ppm) and MeOD-d_4 (δ = 43 ppm) as internal standards for ^{13}C NMR. H_f design protons of the fluorene part; H_g protons of the glycerol part; H_m protons of the myristoyl chain and H_u protons of the undecynoyl chain. The chemical shifts are reported in ppm downfield from TMS. The nature of the different carbons (C, CH, CH_2 or CH_3) was determined by ^{13}C to ^1H polarization transfer (DEPT). Mass spectra (MS) were measured on a VG Analytical ZAB-HF apparatus in the FAB mode and on a Trio 2000 Fisons by direct introduction

(EI^+) ot (CI^+, with NH_3). TLC were run on pre-coated silica gel plates 60 F 254 (Merck, 0.25 mm). In order to reveal the compounds, TLC plates were exposed to UV-light, dipped in a solution of vanillin (1 g) in EtOH/H_2SO_4 (95/5, 1 l) and heated on a hot plate to reveal the compounds. Medium pressure chromatography (P = 0.5 - 1.1 bar) was carried out using silica gel (40 - 63 µm, Merck) columns. All solvents were freshly distilled before use. Air- or moisture- sensitive reactions were conducted in flame-dried glassware and under an inert atmosphere.

1-Myristoyl-2,3-isopropylidene-*sn*-glycerol [2].

To a solution of isopropylidene glycerol **1** (2.48 ml; 20 mmol) and of myristic acid (1.1 eq.; 5.02 g; 22 mmol) in 200 ml of dry $CHCl_3$ was added a solution of DCC (1.1 eq.; 4.53 g; 22 mmol) and DMAP (0.7 eq.; 1.71 g; 14 mmol) in 50 ml of dry $CHCl_3$. The solution was stirred at room temperature for 16 hrs and filtered, and the solvent was removed. The residual oil was extracted with ether, washed with aqueous saturated K_2CO_3, then with water until the washings were neutral, and with brine. The organic layer was dried, evaporated and chromatographed using hexane/ether (60:40) to give 6.1 g (90 %) of **2** as a colorless oil.

2 **^{1}H NMR** 200 MHz ($CDCl_3$) δ: 0.86 (t; $3H_m$; H-14); 1.24 (m; $16H_m$; H-4 to 13); 1.38 (s; $3H_g$; H-1'); 1.42 (s; $3H_g$; H-2'); 1.61 (q; $2H_m$; H-3; J=7.3 Hz); 2.32 (t; $2H_m$; H-2; J=7.3 Hz); 3.72 (dd; $2H_g$; H-3; J_1=8.3 Hz; J_2=6.1 Hz); 4.09 (m; $2H_g$; H-1); 4.30 (m; $1H_g$; H-2). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 173.6; 109.8; 73.6; 66.3; 64.5; 34.1; 31.9; 29.4; 29.3; 29.2; 29.1; 26.6; 25.4; 24.9; 22.6; 14.1. **Mass (relative intensity): m/z=** 341 ($M-H^+$, 1); 327 (100); 284 (14); 129 (24); 116 (38); 101 (73).

1-Myristoyl-*sn*-glycerol [3].

A solution of **2** (4.8 g; 14.2 mmol) in 20 ml of dry CH_2Cl_2 was cooled to -78°C under argon. Boron trichloride in CH_2Cl_2 (1.02 eq.; 14.5 ml; 14.5 mmol) was added and after 2 minutes the reaction was quenched with 10 ml of THF. The mixture was extracted with ether, washed with aqueous 10 % $NaHCO_3$, with water until neutral, and with brine. The organic layer was dried and evaporated. The crude product was crystallized in CH_2Cl_2/pentane at -20°C to yield 3.04 g (76 %) of **3**.

3 **^{1}H NMR** 200 MHz ($CDCl_3$) δ: 0.88 (t; $3H_m$; H-14); 1.25 (m; $20H_m$; H-4 to 13); 1.63 (m; $2H_m$; H-3); 2.08 (t; $1H_g$; OH-3; J=6.1 Hz); 2.35 (t; $2H_m$; H-2; J=7.3 Hz); 2.58 (d; $1H_g$; OH-2; J=5.1 Hz); 3.65 (m; $2H_g$; H-3); 3.94 (m; $1H_g$; H-2); 4.20 (m; $2H_g$; H-1; $J_{1a\text{-}1b}$=11.6 Hz; $J_{1a\text{-}2}$=5.7 Hz; $J_{1b\text{-}2}$=4.9 Hz). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 174.4; 70.3; 65.1; 63.4; 34.2; 31.9; 29.5; 29.3; 29.2.; 29.1; 24.9; 22.7; 14.1. **Mass** (relative intensity): **m/z=** 303 ($M+H^+$, 19); 285 (24); 229 (20); 211 (54); 134 (55); 98 (94); 57 (100). **mp** 63-64°C. $[\alpha]_D$=- 4 (c 2.8, pyridine).

1-Myristoyl-3-(t-butyldimethylsilyloxy)-*sn*-glycerol [4].

A solution of **3** (900 mg; 2.98 mmol), imidazole (2.3 eq.; 467 mg; 6.85 mmol), and *t*-butyldimethylchlorosilane (4.3 eq.; 1.93g; 12.8 mmol) in dry THF (25 ml) was stirred overnight at room temperature. The reaction mixture was filtered and solvents were evaporated. The crude product was flash-chromatographed over silica gel (elution: 400 ml hexane/ether 90/10 then 400 ml 50/50) and gave 1.18 g (95 %) of pure **4**.

4 **^{1}H NMR** 200 MHz ($CDCl_3$) δ: 0.08 (s; 6H; CH_3-Si); 0.86 (t; $3H_m$; H-14; J=6.7 Hz); 0.90 (s; 9H; $(CH_3)_3$-C); 1.26 (m; $22H_m$; H-4 to 13); 1.60 (m; $2H_m$; H-3); 2.31 (t; $2H_m$; H-2; J=7.4 Hz); 2.55 (d; $1H_g$; OH-2; J=4.9 Hz); 3.63 (dd; $2H_g$; H-3; $J_{3a\text{-}3b}$=10.1Hz; $J_{3a\text{-}2}$=5.1 Hz; $J_{3b\text{-}2}$=4.6 Hz); 3.81 (m; $1H_g$; H-2); 4.09 (dd; $2H_g$; H-1; $J_{1a\text{-}1b}$=5.25 Hz; $J_{1a\text{-}2}$=1.8 Hz; $J_{1b\text{-}2}$=1.6 Hz). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 174.0; 70.0; 65.0; 63.8; 34.2; 32.0; 29.7; 29.5; 29.4; 29.3; 29.2; 25.9; 25.0; 22.7; 18.3; 14.1; -5.4. **Mass** (relative intensity): **m/z=** 416 (M^+; 1); 359 (52); 285 (13); 211 (18); 131 (100); 115 (17); 745 (44); 57 (42). $[\alpha]_D$=+ 2 (c 5.7, $CHCl_3$).

Methyl 10-undecynoate [5].

A solution of 10-undecynoic acid (5 g; 27.4 mmol.) in 100 ml of dry methanol was cooled to -15°C under argon. Thionyl chloride (1.5 eq.; 3 ml; 41.2 mmol) was added dropwise upon cooling in a ice bath. Attend the addition was over, the ice bath was removed. After 1 hour, the reaction was quenched with water and extracted with ether. The ether layer was washed with water until the washings were neutral, then with brine, dried and evaporated. Flash chromatography using 30 % CH_2Cl_2 in hexane as eluent gave 5.18 g (95 %) of the methyl ester **5** as a colorless oil.

5 **^{1}H NMR** 400 MHz ($CDCl_3$) δ: 1.30 (m; $6H_u$; H-5;6;7); 1.38 (m; $2H_u$; H-4); 1.50 (q; $2H_u$; H-8; J=6.8 Hz); 1.60 (q; $2H_u$; H-3; J=7.2 Hz); 1.92 (t; $1H_u$; H-11; J=2.6 Hz); 2.16 (dt; $2H_u$; H-9; J_1=2.6 Hz; J_2=6.8 Hz); 2.29 (t; $2H_u$; H-2; J=7.2 Hz); 3.63 (s; 3H;Me). **^{13}C NMR** 100 MHz ($CDCl_3$) δ: 173.9; 84.4; 67.0; 51.2; 33.8; 28.9; 28.8; 28.7; 28.5; 28.3; 24.7; 18.2. **Mass** (relative intensity): **m/z=** 195 ($M\text{-}H^+$,19); 181 (7); 167 (24); 163 (46); 121 (52); 87 (30); 81 (80); 74 (100).

Methyl 11-(2-fluorenyl)-10-undecynoate [6].

A mixture of 2-bromofluorene (2 g; 2.16 mmol), ester **5** (3 eq.; 4.8 g; 24.48 mmol), $Pd(PPh_3)_2Cl_2$ (0.2 eq.; 1.14g; 1.63 mmol) and CuI (0.1 eq.; 155 mg; 0.82 mmol) in diethylamine (300 ml) was stired under reflux for 6 hrs. The mixture was filtered and solvent evaporated. The dark oil was diluted in pentane and the solid was removed by filtration. After evaporation the residual oil was purified by chromatography on silica gel (3 % ether in hexane) to give 2.63 g (91 %) of **6** as a white solid.

6 **^{1}H NMR** 200 MHz ($CDCl_3$) δ: 1.26-1.52 (m; $10H_u$; H-4 to 8); 1.63 (m; $2H_u$; H-3); 2.32 (t; $2H_u$; H-2, J=7.6 Hz); 2.44 (t; $2H_u$; H-9, J=6.8 Hz); 3.67 (s; 3H; Me); 3.87 (s; $2H_f$; H-9) 7.26-7.40 (m; $3H_f$; H-3.6.7); 7.52 (d; $1H_f$; H-8; J=7.6 Hz); 7.58 (s; $1H_f$; H-1); 7.71 (d; $1H_f$; H-4; J=7.9 Hz); 7.77 (d; $1H_f$; H-5; J=6.8 Hz). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 174.3; 143.5; 143.1; 141.3; 141.2; 131.6; 130.4; 128.2; 126.9; 125.1; 122.3; 120.1; 119.7; 90.3; 81.3; 51.5; 37.6; 36.7; 34.1; 29.2; 29.1; 28.9; 25.0; 19.6. **Mass** (relative intensity): **m/z=** 360 (M, 54); 218 (48); 205 (100); 203 (90). **mp** 57-58°C. **UV** (MeOH) λ_{max}: 314 (ε 22000), 302 (ε 21000); 288 (ε 31000) and 284 nm (ε 30000).

11-(2-Fluorenyl)-10-undecynoic acid [7].

A solution of **6** (1 g; 2.78 mmol) in 100 ml of 95 % ethanol, and KOH (5 eq.; 780 mg; 13.9 mmol) was refluxed during 45 minutes. The mixture was acidified with aqueous 1 N HCl (50 ml). The precipitation was finished by addition of water (50 ml), the solid was filtered and the acid was dissolved in CH_2Cl_2. The solution was dried over $MgSO_4$ and evaporated to give 961 mg (95 %) of **7** as a white solid, crystallized from CH_2Cl_2/Hexane.

7 **^{1}H NMR** 200 MHz ($CDCl_3$) δ: 1.35-1.46 (m; $10H_u$; H-4 to 8); 1.62 (m; $2H_u$; H-3); 2.36 (t; $2H_u$; H-2; J=7.5 Hz); 2.43 (t; $2H_u$; H-9; J=6.8 Hz); 3.88 (s; $2H_f$; H-9); 7.26-7.41 (m; $3H_f$; H-3.6.7); 7.54 (m; $2H_f$; H-1.8); 7.68 (d; $1H_f$; H-4); 7.75 (d; $1H_f$; H-5). **^{13}C NMR** 50 MHz (CD_2Cl_2) δ: 179,9; 143.2; 142.92; 140.9; 130.1; 127.8; 126.7; 126.6; 124.7; 122.0; 119.7; 119.3; 89.9; 80.9; 36.3; 33.9; 19.1. **Mass** (relative intensity): **m/z=** 346 (M, 29); 327 (8); 302 (4); 217 (22), 205 (78); 123 (59); 55 (100). **mp** 135-137°C. **UV** (MeOH) λ_{max}: 314 (ε 16000), 302 (ε 16000); 288 (ε 23000) and 284 nm (ε 22000).

1-Myristoyl-2-[11-(2-fluorenyl)-10-undecynoyl]-3-(t-butyldimethylsilyloxy)-*sn*-glycerol [8].

The monoester silyl ether **4** (0.95 eq.; 731 mg; 1.75 mmol) and the acid **7** (640 mg; 1.84 mmol) were disolved in freshly distilled $CHCl_3$ (50 ml) and stirred at room temperature. Then DCC (1.5 eq.; 542 mg; 2.76 mmol) and DMAP (0.7 eq.; 157 mg; 1.29 mmol) were added to the mixture. The reaction was stirred during 18 hrs at room temperature. The

dicyclohexylurea formed was removed by filtration and the solvent was evaporated. The crude oil obtained was chromatographed using hexane/ether (90/10) to give 1.12 g (85 %) of **8** as a white waxy solid.

8 **^{1}H NMR** 200 MHz ($CDCl_3$) δ: 0.05 (s; 6H; CH_3-Si); 0.88 (t; $3H_m$; H-14; J=6.8 Hz); 0.89 (s; 2H; $(CH_3)_3$-C); 1.26-1.90 (m; $34H_{m-u}$; CH_2 aliph); 2.28 (t; $2H_u$; H-2; J=7.6 Hz); 2.30 (t; $2H_m$; H-2; J=7.55 Hz); 2.43 (t; $2H_u$; H-9); 3.50 (d; $2H_g$; H-3; J=5.3 Hz); 3.88 (s; $2H_f$; H-9); 4.15 (dd; $1H_g$; H-1a; J_{1a-1b}=11.8 Hz; J_{1a-2}= 6.2 Hz); 4.37 (dd; $1H_g$; H-1b; J_{1b-1a}=11.8 Hz; J_{1b-2}= 3.7 Hz); 5.07 (m; $1H_g$; H-2); 7.26-7.77 (m; $7H_f$; H-Ar). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 173.5; 173.1; 143.5; 143.1; 141.3; 141.1; 130.4; 128.2; 126.9; 125.1; 122.3; 120.1; 119.6; 90.3; 81.3; 71.8; 62.5; 61.5; 36.7; 35.0; 34.4; 34.2; 32.0; 29.7; 29.5; 29.4; 29.2; 29.1; 29.0; 28.9; 25.8; 25.5; 25.0; 24.7; 22.7; 19.6; 18.2; 14.2; -5.4. **Mass** (relative intensity)**: m/z=** 762 ($M+NH_4^+$, 60); 687 (8); 401 (1); 346 (6).

1-Myristoyl-2-[11-(2-fluorenyl)-10-undecynoyl]-*sn*-glycerol [9].

The ether **8** (225 mg; 0.3 mmol) is dissolved in 30 ml of a mixture of MeOH/CH_2Cl_2 (14/1) and cooled to 0°C. Aqueous HCl 5M (0.9 ml) was added dropwise over 5 minutes. The reaction was stirred at 0°C for 4 hrs. When deprotection was complete (followed by ccm), the mixture was rapidly extracted with ether, washed with water until washings were neutral and then with brine. The organic layer was dried and evaporated (temperature bath<25°C). The crude diacylglycerol **9** was used after 2 hrs of drying under vacuum for the phosphorylation reaction, without purification .

1-Myristoyl-2-[11-(2-fluorenyl))-10-undecynoyl]-*sn*-glycero-3-phosphocholine [10].

The diacylglycerol **9** (191 mg; 302 µmol) in 1 ml of ethanol-free $CHCl_3$ was added dropwise to a solution of phosphorus oxychloride (1.25 eq.; 36 µl; 378 µmol) and triethylamine (1.25 eq.; 53 µl; 378 µmol) in 2 ml of dry $CHCl_3$ cooled at -20°C. The reaction was left at room temperature during 30 min, and choline tosylate (1.5 eq.; 125 mg; 454µmol) and dry pyridine (250 µl) were added. The reaction was continued during 16 hrs and quenched with 200 µl of water. After 30 min, solvents were evaporated (temperature bath<30°C). The residual oil was dissolved in 10 ml of CH_2Cl_2/toluene (1:1) and filtered. The solvents were evaporated and the crude product was chromatographed. Elution was as followed: 100 ml of 5 % MeOH in CH_2Cl_2, 70 ml of 20 % MeOH in CH_2Cl_2, and 200 ml of 65/25/4 (v/v) CH_2Cl_2/MeOH/H_2O. The pure probe **10** was obtained as a white waxy solid : 155 mg (65 %).

10 **^{1}H NMR** 500 MHz ($CDCl_3$/MeOH. 1/1) δ: 0.85 (t; $3H_m$; H-14; J=6.85 Hz); 1.24-1.48 (m; $36H_{m-u}$; H-aliph); 1.61 (m; $4H_{m-u}$; H-3); 2.30 (t; $2H_u$; H-2; J=7.7 Hz); 2.34 (t; $2H_m$; H-2; J=7.55 Hz); 2.42 (t; $2H_u$; H-9; J=7.05 Hz); 3.22 (s, 9H; $(CH_3)_3$-N); 3.64 (m; 2H; CH_2-N); 3.83 (s; $2H_f$; H-9); 4.03 (t; $2H_g$; H-3; J_{3-2}=5.6 Hz); 4.14 (dd; $1H_g$; H-1a; J_{1a-1b}=11.8 Hz; J_{1a-2}=3.2 Hz); 4.29 (m; 2H, CH_2-O); 4.40 (dd; $1H_g$; H-1b; J_{1b-1a}=11.8 Hz; J_{1b-2}=3 Hz); 5.24 (m; $1H_g$; H-2); 7.28 (dt; $1H_f$; H-6; J_{6-8}=1.1 Hz; J_{6-7}= 7.45 Hz); 7.35 (t; $1H_f$; H-7; J=7.45 Hz); 7.39 (d; $1H_f$; H-3; J_{3-4}=7.8 Hz); 7.53 (d; $1H_f$; H-8; J=7.4 Hz); 7.55 (s; $1H_f$; H-1); 7.69 (d; $1H_f$; H-4; J=7.8 Hz); 7.76 (d; $1H_f$; H-5; J=7.5 Hz). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 173.4, 173.0, 143.3, 143.0, 141.2, 141.0, 130.3, 128.9, 128.0, 126.8, 124.9, 122.1, 119.9, 119.5, 90.1, 81.2, 69.9, 65.9, 64.3, 62.4, 60.2, 54.3, 36.6, 34.2, 34.0, 31.9, 29.6, 29.3, 29.2, 29.0, 28.9, 28.8, 24.8, 22.7, 19.5, 14.1 **Mass** (FAB^+): 796.5 ($M+H^+$); 613.4; 568; 385.1. **UV** ($CHCl_3$): λ_{max} 277 (ε 22000), 285 (ε 25700), 292 (ε 29500), 307 (ε 20900), 315 (ε 23300) nm.

Dimethyl 11,11'-(2,7-fluorenyl)-bis-10-undecynoate [11].

11 **^{1}H NMR** 400 MHz ($CDCl_3$) δ:1.34-1.46 (m; $16H_u$; H-aliph.); 1.65 (m; $8H_u$; H-3,7); 2.30 (t; $4H_u$; H-2; J=7.4 Hz); 2.42 (t; 4 H_u; H-9; J=7.0 Hz); 3.67 (s; $6H_u$; H-1'); 3.83 (s; $2H_f$; H-9); 7.40 (d; $2H_f$; H-3,6; J=7.9 Hz); 7.55 (s; $2H_f$; H-1,8); 7.64 (d; $2H_f$; H-4,5; J=7.9 Hz). **^{13}C NMR** 50 MHz ($CDCl_3$) δ: 174.2; 143; 140.5; 130.4; 122.3; 119.7; 90.5; 81.1;

51.4; 36.4; 34.0; 29.1; 29.0; 28.9; 28.8; 28.7; 28.2; 24.9; 19.4. **Mass** (relative intensity): 574 ($M+NH_4^+$, 100); 555 ($M+H^+$,29); 373 (15). **mp**: 53-55°C.

11,11'-(2,7-fluorenyl)-bis-10-undecynoic acid [11, CO_2H instead of CO_2Me].

^1H NMR 400 MHz (DMSO-d_6) δ: 1.28-1.56 (m; 24H_u; H-aliph.); 2.18 (t; 4H_u; H-2; J=7.3 Hz); 2.43 (t; 4H_u; H-9; J=6.9 Hz); 3.89 (s; 2H_f; H-9); 7.37 (d; 2H_f; H-3,6; J=7.9 Hz); 7.57 (s; 2H_f; H-1,8); 7.83 (d; 2H_f; H-4,5; J=7.9 Hz). **Mass** (relative intensity): 544 ($M+NH_4^+$, 46); 526 (M, 49); 136 (21); 129 (100); 100 (32). **mp**: 174-177°C.

3,3'-[11,11'-(2,7-fluorenyl)-bis-10-undecynoyl]-bis-thiazolidine-2-thione [12].

To a suspension of 2-chloro-1-methylpyridinium iodide (3 eq.; 729 mg; 2.85 mmol) in dichloromethane (25 ml) were added the diacid (500 mg; 0.95 mmol) and 2-thiazoline-2-thiol (4 eq.; 453 mg; 3.8 mmol) at room temperature under argon. Triethylamine (10 eq.; 1.3 ml; 9.5 mmol) was added dropwise with stirring to the reaction mixture. The mixture was stirred for 3 hrs under reflux. Toluene was added to precipitated salts and the solution was filtered and evaporated. The crude oil was purified by chromatography on silica gel (solvent: hexane/CH_2Cl_2 50/50 then 25/75) to give 374 mg (54 %) of **12** as a yellow solid.

12 **^1H NMR** 400 MHz ($CDCl_3$) δ: 1.33-1.50 (m; 16H_u; H-aliph.); 1.56-1.68 (m; 8H_u; H-3.8); 2.25 (d; 4H_u; J=7.5 Hz); 2.40 (d; 4H_u; H-9; J=7 Hz); 3.20 (t; 4H; CH_2-N; J=7.5 Hz); 3.82 (s; 2H_f; H-9); 4.56 (t; 4H; CH_2-S; J=7.5 Hz); 7.38 (d; 2H_f; H-3.6; J=8 Hz); 7.54 (s; 2H_f; H-1.8); 7.64 (d; 2H_f; H-4.5; J=8 Hz). **^{13}C NMR** 100 MHz ($CDCl_3$) δ: 201.1; 174.4; 143.1; 140.2; 130.1; 127.8; 122.1; 119.5; 90.4; 80.9; 57.8; 38.13; 36.2. **IR** (KBr) cm^{-1}: 2920, 2851, 2215, 1715, 1467, 1698, 1467, 1369, 1152, 822. **Mass** (relative intensity): **m/z**=728(M^+, 1); 655 (62); 627 (43); 536 (25); 490 (19); 446 (18); 243 (38); 119 (100).

1,1'-Dimyristoyl-2,2'-[11,11'-(2,7-fluorenyl)-bis-(10-undecynoyl)]-*sn*-glycero-3-phosphocholine [13].

All products were dried over P_2O_5 in vacuo before use. A mixture of 12 (74 mg; 0.1 mmol), the LMPC . $CdCl_2$ complex (5 eq.; 754 mg; 0.5 mmol) and CsF (30 eq.; 463 mg; 3 mmol) in dry deoxygenated DMF (15 ml) was stirred at room temperature for 6 days under argon. After evaporation of the solvent, the residual solid was diluted in $CHCl_3$/MeOH/H_2O and then passed slowly on a column of mixed ion-exchange resins (IRC-50/A-21 1/1 (v/v)). The eluate was evaporated to dryness and chromatographed on silica gel (elution with $CHCl_3$/MeOH/NH_3 30 % 10/6/1 then $CHCl_3$/MeOH/H_2O 10/6/1). The probe **13** was obtained as a white waxy solid, 89 mg (60 %).

13 **^1H NMR** 500 MHz ($CDCl_3$/MeOH 1/1) δ: 0.84 (t; 6H_m; H-14; J=6.85 Hz); 1.20-1.47 (m; 72H_{m-u}; H-aliph.); 1.59 (m; 8H_{m-u}; H-3); 2.28 (t; 4H_m; H-2; J=7.7 Hz); 2.33 (t; 4H_u; H-2; J=7.55 Hz); 2.40 (t; 4H_u; H-9; J=7.1 Hz); 3.18 (s; 18H; $(CH_3)_3$-N); 3.59 (m; 4H; CH_2-N); 3.82 (s; 2H_f; H-9); 3.97 (t; 4H_g; H-3; J=5.6 Hz); 4.14 (dd; 1H_g; H-1a; J_{1a-1b}=11.8 Hz; J_{1a-2}=3.2 Hz); 4.22 (m; 2H; CH_2-O); 4.40 (dd; 1H_g; H-1b; J_{1b-1a}=11.8 Hz; J_{1b-2}=3 Hz); 5.22 (m; 1H_g; H-2); 7.35 (d; 2H_f; H-3,6; J_{3-6}=8.4 Hz); 7.66 (d; 2H_f; H-4,5; J_{4-3}=8 Hz); 7.72 (s; 2H_f; H-1,8). **Mass (FAB$^+$)**: 1447 ($M+Na^+$, 35); 1426 ($M+2H^+$, 85); 1367 (10); 1243 (16); 1082 (36); 959 (11); 877 (37); 855 (100); 833 (49). **UV** ($CHCl_3$): 267 (ε 5300), 295 (ε 13700), 308 (ε 18300), 316 (ε 18200), 323 (ε 18200), 331 (ε 30300).

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