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SYNTHESIS OF FLUORESCENT PROBES FOR LOCALIZED MEMBRANE FLUIDITY MEASUREMENTS

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Abstract : *The synthesis of two long-chain homologues of the fluorescent membrane probes TMA-DPH and DPHpPC is described. The long-chain phosphatidylcholine could be synthesized from palmitoyl-lysophosphatidylcholine and 21-(diphenylhexatrienyl) henicosanoic acid only when the promoting agent was 1,1'-thionyldiimidazole.*

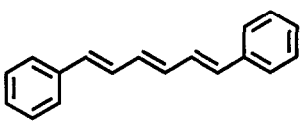
1,6-Diphenylhexa-1,3,5-triene (DPH) **1** and molecules containing the DPH moiety are among the most popular probes used to study membrane fluidity by fluorescence anisotropy.[1] However, in intact cells, a precise location of DPH is precluded since labelling is followed by a progressive incorporation of the probe into intracellular membranes.[2] This drawback can be overcome, at least partially, by using amphiphilic probes such as TMA-DPH **2**[3] or the phospholipidic derivative DPHpPC **5**.[4] Besides, both leaflets of cell plasma membranes have a different lipid composition and different physical properties,[5] so that fluorescent probes allowing fluidity measurements of the inner leaflet only, would be useful. We have recently synthesized to this end the phosphatidylethanolamine analogue of the amphiphilic probe DPHpPC **5** and tested its partitioning properties.[6] In this paper, we report the synthesis of the long-chain (Lc) homologues **4** and **6** of the fluorescent probes TMA-DPH **2** and DPHpPC **5**. These homologues bear the DPH fluorophore respectively at the end of a C_{22} and of a C_{21} spacer, a stucture that should allow the location of the DPH moiety in the inner leaflet of cell plasma membranes whereas the ammonium or the phosphorylcholine polar head would remain at the surface of the outer leaflet.[7]

RESULTS AND DISCUSSION

In our initial approach to the long-chain probes,[8] we planned to couple a functionnalized *p*-methyl DPH synthon **7** (X=leaving group) and the Grignard reagent derived from 20-bromo-1-(tetrahydropyranyloxy)icosane **8**, using dilithium tetrachlorocuprate.[9] We expected to

obtain in this way protected 21-DPH-henicosan-1-ol **9** that we would then have transformed on the one hand into 21-DPH-henicosyltrimethylammonium iodide **3** and on the other into 21-DPH-henicosanoic acid **10**, an intermediate in the synthesis of the phosphatidylcholine (PC) **6**. But the synthesis of these long-chain DPH derivatives was more difficult than expected. In spite of several attempts under different conditions,[10] we were unable to obtain reasonable yields of long-chain Grignard reagents from the corresponding bromides. Furthermore, the preparation and purification of DPH derivatives **7** (X = Br, OMs, OTs) from the corresponding benzyl alcohol was also rather difficult. It appeared to us that polar DPH derivatives were poorly soluble in most solvents, and consequently it was difficult to work with, particularly when low temperatures were required.

Taking this into consideration, we decided to introduce the rigid DPH feature only in the last steps.[8] Moreover, since it was difficult to prepare an organomagnesium reagent from the long-chain bromide **8**, we choose to use the latter to alkylate a benzylic carbanion. The carbanion derived from *p*-toluic acid seemed well suited since the carboxy group, in addition



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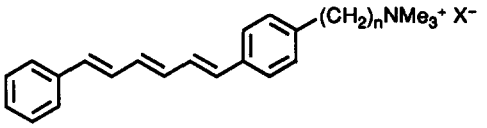
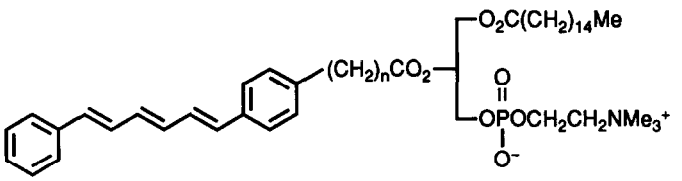
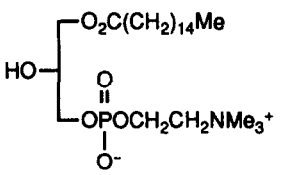


2 n = 0 X = TsO
3 n = 21 X = I
4 n = 22 X = I



5 n = 2
6 n = 20



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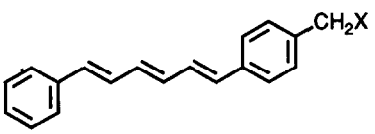

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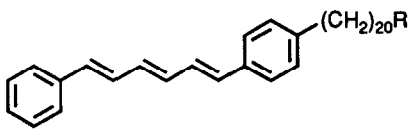

9 R = CH_2OH
10 R = CO_2H

to helping stabilize the benzylic negative charge, would also allow later to complete the DPH moiety. We succeeded in preparing alcohol **9** along these lines, but it was also found to be difficult to handle because of its low solubility and of the sensitivity of the DPH moiety to certain reaction conditions. In our hands, it could neither be transformed into a halide nor oxidized into acid **10**. We then modified the preceding route in order to introduce even the dimethylamino or the carboxy group before the DPH moiety. These modified reaction sequences led to the probes **4** and **6** as described below.

Synthesis of LcTMA-DPH 4

The 20-bromo-1-(tetrahydropyranyloxy)icosane **8** needed in our syntheses was prepared from icosane-1,20-diol **11** which was obtained in two ways: i) from a mixture of natural acetates containing 33% icosane-1,20-diol diacetate; ii) by reduction of icosane-1,20-dioic acid[11] with borane-dimethyl sulfide complex.[12]

The diol **11**, suspended in petroleum ether (bp 110-130 °C), was refluxed with 48% aqueous hydrobromic acid for 24 h and gave a mixture of bromohydrin **12**, unreacted diol **11**, and 1,20-dibromoicosane§ (Scheme 1). The bromohydrin **12** was converted into the tetrahydropyran-2-yl ether (THP ether) **8** with dihydropyran and pyridinium *p*-toluenesulfonate (PPTS).[15]

p-Toluic acid treated at 0 °C with two molar equivalents of lithium diisopropylamide gave a dianion[16] which was reacted with the bromide **8** to give the acid **13**. The yield was only 56% after purification of **13** by recrystallisation, but the unreacted bromide **8** could easily be recovered from the mother liquors and was recycled. After esterification of the acid **13** with diazomethane, the resulting methyl ester **14** was transformed in a one-pot sequence into the cyano ester **15** by treatment with triphenylphosphine dibromide in dichloromethane followed by sodium cyanide in dimethyl sulfoxide.[17] Reduction of the cyano ester **15** with lithium aluminium hydride in the presence of aluminium chloride[18] gave the amino alcohol **16** which is a bolaform amphiphile[19] and is poorly soluble in most solvents. It could however be extracted from the reaction medium with a 2:1 chloroform-methanol mixture, and after drying by azeotropic distillation of toluene, was used in the next step without further purification. It was transformed into the more soluble tertiary amine **17** by the Eschweiler-Clarke procedure[20] (formic acid, aqueous formaldehyde, reflux) which also produced some formate of compound **17**. Therefore an alkaline treatment (potassium hydroxide, methanol) was required before work-up, to hydrolyze this amino ester into the amino alcohol **17**. Oxidation of the latter with Collins reagent[21] gave the amino aldehyde **18** which was allowed to undergo a Wittig-Horner-Emmons reaction with the phosphonate **19**,[22] prepared in three steps from (2*E*,4*E*)-5-phenylpenta-2,4-dienal[23] (Scheme 2). The resulting amine **20** was treated with a large excess of iodomethane to give the 22-DPH-docosyltrimethylammonium iodide **4** (LcTMA-DPH).

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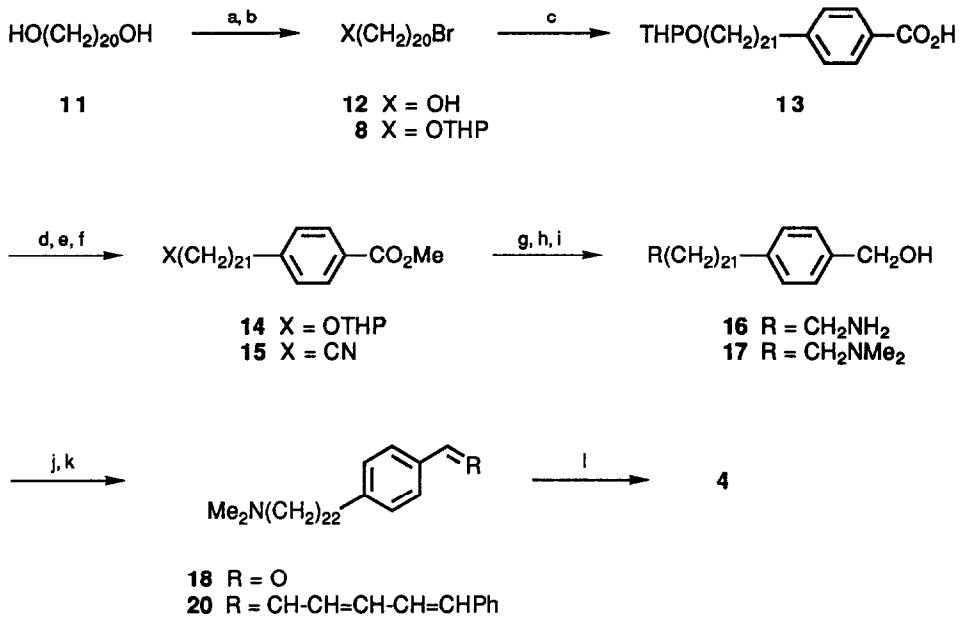
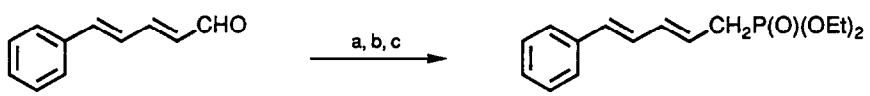
§ In our case, this procedure was found more convenient than the continuous liquid-liquid extraction method which is usually used to prepare halohydrins from smaller α,ω-diols.[13] The dibromide could be retransformed into diol **11** by treatment with water in N-methyl-2-pyrrolidone (15:85 v/v) at 130 °C (71% yield).[14]



Scheme 1. a) 48% aqueous HBr, petroleum ether (bp 110-130 °C), reflux, 24 h (49% yield); b) dihydropyran, PPTS, CH_2Cl_2, 20 h (100%); c) *p*-$LiCH_2C_6H_4CO_2Li$, THF, 0 °C, 30 min (56%); d) CH_2N_2, THF, 0 °C, 15 min (89%); e) PPh_3Br_2, THF, r. t., THF, 30 min ; f) NaCN, DMSO, reflux, 20 h (71% from **14**); g) $LiAlH_4$, $AlCl_3$, THF, 20 h; h) aqueous HCHO, HCO_2H, reflux, 20 h; i) KOH, MeOH, reflux, 30 min (63% from **15**); j) CrO_3-2 pyridine, CH_2Cl_2, r. t., 15 min; k) Ph-CH=CH-CH=CH-$CH_2P(O)(OEt)_2$, MeONa, THF, 0 °C then r. t., 2.5 h (48%); l) MeI, toluene, acetone, 70 °C, 3 h (86%).



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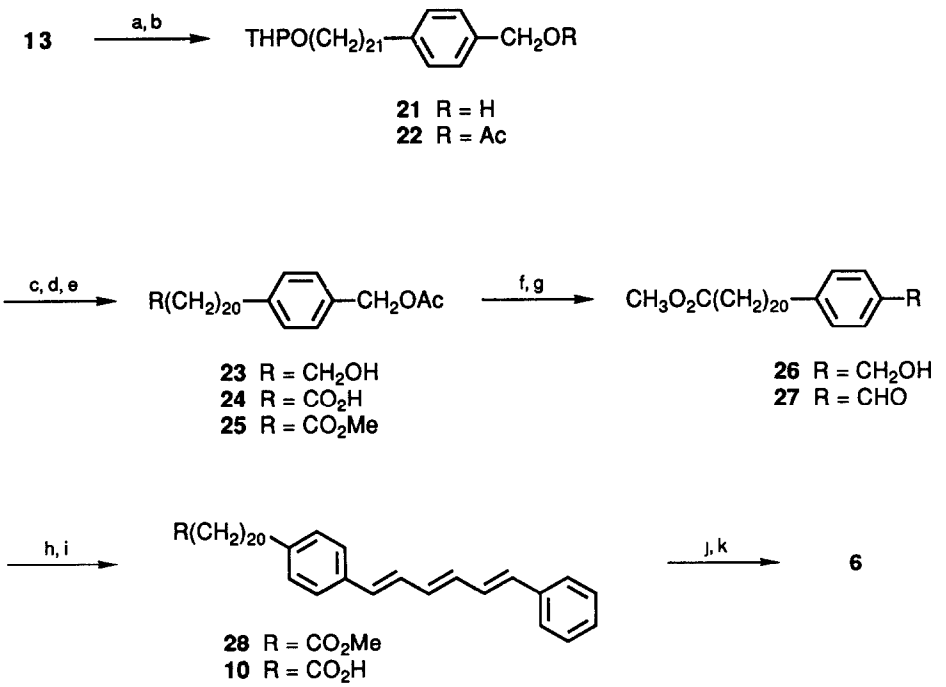
Scheme 2. a) $(i\text{-Bu})_2AlH$, THF, 0 °C then r. t., 2 h (97%); b) PCl_3, THF, 0 °C then r. t., 10 h; c) $P(OEt)_3$, 130 °C, 7 h.

Synthesis of LcDPHpPC 6

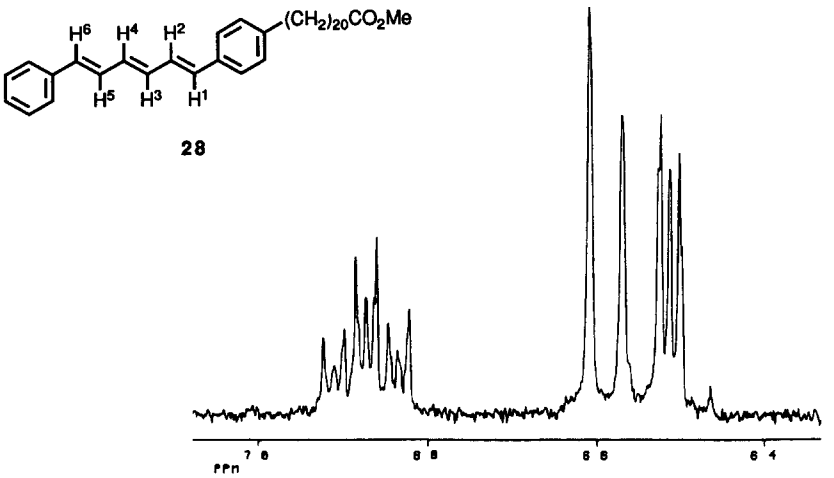
The acid **13** was reduced into the alcohol **21** with borane-dimethyl sulfide complex[12] (Scheme 3). Acetylation of the benzylic hydroxy of **21** followed by removal of the THP protecting group[15] led to the acetoxy alcohol **23** which was oxidized with Jones reagent[24] into the acetoxy acid **24**. This compound was converted into the methyl ester **25** prior to the selective hydrolysis of the acetate with potassium carbonate in anhydrous methanol. Oxidation of the resulting alcohol **26** with Collins reagent[21] gave the aldehyde **27** which, by treatment with phosphonate **19**[22] and sodium methoxide in THF, afforded the DPH methyl ester **28**. Saponification of the latter furnished the acid **10**.



Scheme 3. a) BH_3-SMe_2, THF, 0 °C then r. t., 20 h (67% yield); b) Ac_2O, DMAP, pyridine, CH_2Cl_2, r. t., 20 h (85%); c) PPTS, EtOH, reflux, 1 h (95%); d) Jones reagent, acetone, r. t. (87%); e) CH_2N_2, THF, 0 °C (78%); f) K_2CO_3, MeOH, THF, r. t., 45 min (69%); g) CrO_3-2 pyridine, CH_2Cl_2, r. t., 15 min (76%); h) Ph-CH=CH-CH=CH-$CH_2P(O)(OEt)_2$, MeONa, THF, 0 °C, 30 min (66%); i) KOH, MeOH, toluene, reflux, 20 h (82%); j) TDI, THF, r. t., 1 h; k) lysoPC **29**, $CHCl_3$, 50 °C, 5 days (8%).

That the DPH moiety thus formed was all-*trans* could be shown by second order analysis of the olefinic pattern in the ^{1}H-NMR spectrum of the ester **28** (Scheme 4). Use of the Bruker routine PANIC gave the following alkenic coupling constants in chloroform-d : $J_{1,2}$=15.6 Hz; $J_{3,4}$=14.7 Hz; $J_{5,6}$=15.4 Hz.[25]

After obtention of the acid **10**, we focussed our efforts on its esterification with the lysophosphatidylcholine (lysoPC) **29**. The lysoPC acylation reaction is well documented[26] and works usually fairly well when common fatty acids (*i.e.*, C_{18} or so) are involved. But in our case, it was complicated by the size of the acid **10** (C_{39}) which resulted in poor solubility and low reactivity, and by the presence of the acid-sensitive DPH moiety. Acylation methods involving a mixed anhydride (from **10** and pivaloyl chloride[4]), the symmetric anhydride (either preformed or prepared *in situ* with 1,3-dicyclohexylcarbodiimide),[27] an azaarene,[28] or the imidazolide prepared with 1,1'-carbonyldiimidazole (CDI)[29] were explored, but none led to the probe **6**. We found however that when acid **10** was reacted with CDI and excess methanol, methyl 21-DPH-henicosanoate was formed in 50% yield. Commercially available CDI is sometimes partially hydrolyzed.[30] Since preparation of fresh CDI in the laboratory requires the use of phosgene, we decided to make the imidazolide of acid **10** with the more



Scheme 4. Olefinic pattern of the ^{1}H-NMR spectrum of the ester **28** (400 MHz; $CDCl_3$).

reactive 1,1'-thionyldiimidazole (TDI)[31] which can be conveniently prepared from thionyl chloride and imidazole in THF just before use. Reaction of the so obtained imidazolide in THF with the lysoPC **29** in chloroform for 5 days at 50 °C gave LcDPHpPC **6** in 8% yield.

EXPERIMENTAL

Melting points were determined on a Buchi SMP-20 apparatus and are uncorrected. IR spectra were obtained from carbon tetrachloride solutions or potassium bromide pellets on a Perkin-Elmer 457 or 1310 spectrophotometer and are reported in cm^{-1}. ^{1}H-NMR spectra were recorded, unless otherwise stated, in $CDCl_3$ solutions on a Hitachi-Perkin-Elmer R-24A (60 MHz), on a Bruker WP-200 Sy (200 MHz) or a Bruker AM-400 (400 MHz) instrument; chemical shifts are reported in ppm downfield from tetramethylsilane and coupling constants J are in Hz. FAB mass spectra were recorded on a ZAB HF spectrometer (VG Analytical). Electron-impact mass spectra (EIMS) were recorded on a LKB 9000 S spectrometer. Elemental analyses were performed at the Institut de Chimie, Strasbourg. Column chromatography was carried out under pressure on Merck 9385 silica gel (Kieselgel 60, 40-63 μm particle size). Thin layer chromatography (TLC) was carried out on Merck 5715 plates (Kieselgel 60 F_{254}). The detection of phospholipids on TLC was performed with a molybdenum spray.[32] All reactions were run under a positive pressure of dry argon.

***20-Bromoicosan-1-ol* 12.** A suspension of icosane-1,20-diol **11** (mp 104 °C; Lit.[33] mp 103 °C) (5.02 g; 16 mmoles) in petroleum ether (bp 110-130 °C; 60 ml) and 48% aqueous HBr (70 ml) was refluxed for 24 h. After cooling to room temperature, the mixture was filtered on a Büchner funnel. The organic layer of the filtrate contained mainly 1,20-dibromoicosane

which was retransformed into diol **11** according to ref. 14. The solid on the Büchner funnel was dissolved in CH_2Cl_2 (500 ml), and filtered again to retain the poorly soluble unreacted diol **11**. The CH_2Cl_2 solution was washed with aqueous $NaHCO_3$ until neutral, dried with $MgSO_4$, evaporated *in vacuo*, and chromatographed on a silica gel column (toluene-AcOEt 9:1) to give bromohydrin **12** (2.98 g; 49%). TLC R_f 0.25 (hexane-AcOEt 8:2); mp 67-68 °C ; IR (KBr) 3280, 2910, 2840, 1470, 1460 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.56 (m; 2 H); 1.86 (m; 2 H); 3.40 (t; *J* 6.8; 2 H); 3.63 (t; *J* 6.5; 2 H) ; Anal. Calcd for $C_{20}H_{41}BrO$: C, 63.64; H, 10.95. Found : C, 63.8; H, 10.8.

***20-Bromo-1-THP-oxyicosane* 8.** 3,4-Dihydro-2*H*-pyran (2.0 ml; 22 mmoles) and PPTS (200 mg; 0.80 mmole) were added to a solution of bromoalcohol **12** (2.98 g; 7.9 mmoles) in CH_2Cl_2 (200 ml). The mixture was allowed to react for 20 h at room temperature. It was then evaporated *in vacuo* and chromatographed on a silica gel column (hexane-AcOEt 95:5) to yield compound **8** (3.64 g; 100%). TLC R_f 0.86 (hexane-AcOEt 8:2) ; mp 39-41 °C (recristallized in hexane) (Lit.[34] 46-48 °C) ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.45-1.95 (m; 10 H); 3.40 (t; *J* 6.9; 2 H); 3.32-3.95 (m; 4 H); 4.58 (m; 1 H).

***4-(21-THP-oxyhenicosyl)benzoic acid* 13.** A 0.5 *M* solution of lithium diisopropylamide (8 ml) in THF was added to a solution of *p*-toluic acid (272 mg; 2.0 mmoles) in dry THF (5 ml) at 0 °C. After 30 min, a solution of bromide **8** (922 mg; 2.0 mmoles) in THF (2 ml) was added to the red solution and the mixture was allowed to react for 30 min. It was then quenched with water, acidified with 10% aqueous HCl, and extracted three times with ether. The organic layers were washed twice with brine, dried over $MgSO_4$, evaporated *in vacuo*, and recristallized (hexane) to give the acid **13** (578 mg; 56%) which was sufficiently pure for the following step. The mother liquors were evaporated and chromatographed on a silica gel column (eluent: CH_2Cl_2) to recover unreacted bromide **8** (313 mg; 0.68 mmole). An analytical sample of acid **13** was obtained by a rather difficult chromatography of crude **13** on a silica gel column (THF-CH_2Cl_2 1:9) followed by recrystallization in hexane. TLC R_f 0.5 (hexane-AcOEt 1:1) ; mp 100-101 °C ; IR (KBr) 3100-2500, 1680, 1610, 1470, 1420, 1320, 1280, 1030 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 36 H); 1.45-1.93 (m; 8 H); 2.68 (t; *J* 7.6; 2 H); 3.30-4.00 (m; 4 H); 4.59 (m; 1 H); 7.27 and 8.01 (2 d; *J* 8.2; 4 H) ; Anal. Calcd for $C_{33}H_{56}O_4$: C, 76.69; H, 10.92. Found : C, 76.8; H, 11.1.

***Methyl 4-(21-THP-oxyhenicosyl)benzoate* 14.** A solution of ethereal diazomethane was slowly added to a solution of acid **13** (930 mg; 1.8 mmole) in THF (100 ml) at 0 °C, until the yellow color persisted. After 15 min, the solution was evaporated *in vacuo* and chromatographed on a silica gel column (CH_2Cl_2) to give ester **14** (858 mg; 89%). TLC R_f 0.53 (hexane-AcOEt 9:1) ; mp 57-58 °C (from hexane) ; IR (KBr) 2910, 2840, 1710, 1290, 1110, 1030 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 36 H); 1.45-1.93 (m; 8 H); 2.65 (t; *J* 7.7; 2 H); 3.30-4.00 (m; 4 H); 3.90 (s; 3 H); 4.58 (m; 1 H); 7.23 and 7.94 (2 d; *J* 8.4; 4 H) ; Anal. Calcd for $C_{34}H_{58}O_4$: C, 76.93; H, 11.01. Found : C, 76.8; H, 10.9.

***Methyl 4-(21-cyanohenicosyl)benzoate* 15.** A solution of triphenylphosphine dibromide was prepared by reacting triphenylphosphine (263 mg; 1.0 mmole) in dry CH_2Cl_2 (5 ml) with bromine (51 µl; 1.0 mmole). A white solid precipitated while the red color of bromine disappeared. A solution of ester **14** (483 mg; 0.91 mmole) in CH_2Cl_2 (3 ml) was added to this mixture and was allowed to react for 30 min. Sodium cyanide (171 mg; 3.5 mmoles) and dry DMSO (5 ml) were then added and the mixture was refluxed for 20 h. It was then diluted with water and extracted with CH_2Cl_2. The combined organic layers were washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$ and evaporated *in vacuo*. The crude product was chromatographed on a silica gel column (CH_2Cl_2) to yield cyano ester **15** (293 mg; 71%). TLC R_f 0.38 (hexane-AcOEt 9:1) ; mp 62-64 °C (from hexane) ; IR (CCl_4) 2920, 2850, 1725, 1280 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.30-1.75 (m; 6 H); 2.33 (t; *J* 7.0; 2 H); 2.65 (t; *J* 7.6; 2 H); 3.90 (s; 3 H); 7.23 and 7.94 (2 d; *J* 8.5; 4 H). EIMS m/e 455 (M^+, 8%), 423 (100%),163 (5%), 150 (5%), 131 (26%) ; Anal. Calcd for $C_{30}H_{49}NO_2$: C, 79.06; H, 10.84; N, 3.07. Found : C, 79.1; H, 11.0; N, 3.1.

***4-(22-Dimethylaminodocosyl)benzyl alcohol* 17.** Aluminium chloride (160 mg; 1,2 mmole) was added to a solution of lithium aluminium hydride (46 mg; 1.2 mmole) in dry THF (4 ml). After 10 min, a solution of cyano ester **15** (253 mg; 0.56 mmole) in THF (3 ml) was added and the mixture was allowed to react for 20 h at room temperature. It was then hydrolyzed and extracted five times with $CHCl_3$-MeOH 2:1 (slow phase separation). The combined organic layers were evaporated *in vacuo* to yield amino alcohol **16** as a white poorly soluble solid. TLC R_f 0.54 ($CHCl_3$-MeOH-H_2O 70:26:4). This crude compound was refluxed for 20 h in a 1:1 mixture of formic acid and 37% aqueous formaldehyde (1 ml). After cooling to room temperature, potassium hydroxide (1.0 g) in MeOH (100 ml) was added and the mixture was refluxed for 30 min. It was then diluted with $CHCl_3$-MeOH, 2:1 and washed with MeOH-aqueous KOH 1:1 (pH>11). The combined organic layers were evaporated *in vacuo* and chromatographed on a silica gel column ($CHCl_3$-MeOH 8:2) to give amino alcohol **17** (162 mg; 63%). TLC R_f 0.32 ($CHCl_3$-MeOH 9:1) ; IR (KBr) 2910, 2840, 1465, 1380, 1010 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 36 H); 1.37-1.67 (m; 4 H); 2.20 (s; 6 H); 2.23 (m; 2 H); 2.59 (t; *J* 7.7; 2 H); 4.65 (s; 2 H); 7.10-7.32 (m; 4 H).

***4-(22-Dimethylaminodocosyl)benzaldehyde* 18.** A solution of amino alcohol **17** (272 mg; 0.59 mmole) was added to a solution of pyridine (1 ml; 12 mmoles) and chromium trioxide (600 mg ; 6 mmoles) in dry CH_2Cl_2 (6 ml). After 15 min at room temperature, the suspension was diluted with CH_2Cl_2, washed with aqueous KOH and with water, dried with $MgSO_4$, and evaporated to yield amino aldehyde **18** (283 mg) which was used in the next step without purification. TLC R_f 0.32 ($CHCl_3$-MeOH 8:2) ; ^{1}H-NMR (60 MHz) 1.23 (broad s; 36 H); 2.17 (s; 6 H); 2.66 (m; 2 H); 7.35 and 7.83 (2 d; *J* 8; 4 H); 9.98 (s; 1 H).

***Diethyl [(2E,4E)-5-phenylpenta-2,4-dienyl]phosphonate* 19.** A 1 *M* solution of DIBAH in THF (4.8 ml) was added to a solution of 5-phenylpenta-2,4-dienal (500 mg; 3.16 mmoles) in THF (20

ml) at 0 °C. The reaction was stirred at room temperature for 2 h, quenched slowly with 10% aqueous HCl, and extracted three times with ether. The organic layers were washed with aqueous $NaHCO_3$ and with brine, dried over $MgSO_4$ and evaporated to give 5-phenylpenta-2,4-dienol (490 mg; 97%) ; ^{1}H-NMR (200 MHz) 4.26 (d; *J* 5.8; 2 H); 5.96 (dt; *J* 5.8, 15.1; 1 H); 6.43 (ddt; *J* 10.3, 15.1, 1.4; 1 H); 6.55 (d; *J* 15.6; 1 H); 6.79 (dd; *J* 10.3, 15.6; 1 H); 7.15-7.45 (m; 5 H). A solution of this alcohol (400 mg; 2.50 mmoles) in dry ether (10 ml) was reacted with PCl_3 (80 µl; 0.91 mmole) at 0 °C for 15 min and at room temperature for 10 h. It was then hydrolyzed with water and extracted with ether. The combined organic layers were washed with water, aqueous $NaHCO_3$, and with brine, dried over $MgSO_4$, and evaporated *in vacuo* to give 5-phenylpenta-2,4-dienyl chloride (356 mg) which was heated to 130 °C with triethylphosphite (365 mg; 2.2 mmoles) for 7 h. The unreacted triethylphosphite and the other volatiles were distilled from the reaction medium (180 °C, 0.5 mm Hg) to leave the crude phosphonate **19** (308 mg) as a syrup. ^{1}H-NMR (200 MHz) 1.34 (t; *J* 7; 6 H); 2.71 (dd; *J* 7.5, 22.5; 2 H); 4.12 (quint.; *J* 7.0; 4 H); 5.77 (dq; *J* 7.5, 15.0; 1 H); 6.35 (m; 1 H); 6.51 (dd; *J* 2.3, 15.5; 1 H); 6.78 (dd; *J* 10.5, 15.5; 1 H); 7.31 (m; 5 H).

***N,N-Dimethyl-22-DPH-docosylamine* 20.** A solution of aldehyde **18** (300 mg; 0.66 mmole) and of crude phosphonate **19** (420 mg) in THF (10 ml) was added dropwise to a suspension of sodium methoxide (54 mg; 1.0 mmole) in THF (2 ml) at 0 °C. Immediately a brownish precipitate was formed. After 10 min, the mixture was allowed to warm to room temperature and was stirred for 2 h. It was then quenched with water and filtered on a Hirsch funnel. The solid was washed with cold MeOH, chromatographed on a silica gel column ($CHCl_3$-MeOH 8:2) and recrystallized (CH_2Cl_2-hexane) to give amine **20** (185 mg; 48%). TLC R_f 0.59 ($CHCl_3$-MeOH 8:3) ; mp 175 °C ; IR (KBr) 2910, 2840, 1470, 1380, 995 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 36 H); 1.32-1.70 (m; 4 H); 2.21 (s; 6 H); 2.10-2.26 (m; 2 H); 2.58 (t; *J* 7.5; 2 H); 6.40-6.95 (m; 6 H); 7.05-7.45 (m; 9 H).

***22-DPH-docosyltrimethylammonium iodide (LcTMA-DPH)* 4.** A suspension of amine **20** (100 mg; 0.17 mmole) in 1:1 toluene-acetone (30 ml) was heated to 70 °C until homogeneous. The solution was then allowed to cool to 30 °C and methyl iodide (4 ml) was added. Immediately a precipitate formed. The mixture was then heated to 70 °C for 3 h, cooled to room temperature, and filtered on a Hirsch funnel. The solid was washed with toluene and dried *in vacuo* to give the ammonium iodide **4** (106 mg; 86%). TLC R_f 0.55 ($CHCl_3$-MeOH-H_2O 70:26:4) ; mp 217-220 °C ; ^{1}H-NMR (200 MHz; DMSO-d_6 dilute solution) 1.24 (broad s; 36 H); 3.01 (s; 9 H); 6.53-7.05 (m; 6 H); 7.05-7.50 (m; 9 H) ; UV ($CHCl_3$-MeOH 1:4) 360 nm (ε_M 40900 OD M^{-1} cm^{-1}); FAB-MS (*m*-nitrobenzyl alcohol) 598.7 (M-I, 100%), 538.6 (2), 352.5 (5) ; Anal. Calcd for $C_{43}H_{68}NI$: C, 71.14; H, 9.44; N, 1.93. Found : C, 70.9; H, 9.2; N, 1.8.

***4-(21-THP-oxyhenicosyl)benzyl alcohol* 21.** A solution of 2 *M* BH_3-Me_2S in THF (5 ml) was added to a solution of acid **13** (2.01g; 3.9 mmoles) in dry THF (40 ml) at 0 °C. The mixture was then stirred for 20 h at room temperature, quenched with water and extracted three times

with ether. The combined organic phases were washed rapidly with 10% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, dried over $MgSO_4$ and evaporated. The crude product was chomatographed on a silica gel column (CH_2Cl_2-EtOH 95:5) and recrystallized (absolute EtOH) to yield the alcohol **21** (1.31 g; 67%). TLC R_f 0.39 (CH_2Cl_2-EtOH 95:5) ; mp 61-62 °C ; IR (CCl_4) 3610, 2910, 2840, 1450, 1025 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 36 H); 1.43-1.93 (m; 8 H); 2.60 (t; *J* 7.6; 2 H); 3.30-3.95 (m; 4 H); 4.57 (m; 1 H); 4.66 (s; 2 H); 7.12-7.33 (m; 4 H) ; Anal. Calcd for $C_{33}H_{58}O_3$: C, 78.82; H, 11.63. Found: C, 79.0; H, 11.9.

***4-(21-THP-oxyhenicosyl)benzyl acetate* 22.** Pyridine (1 ml; 12 mmoles), acetic anhydride (1 ml; 10 mmoles), and 4-(dimethylamino)pyridine (DMAP; 27 mg; 0.22 mmole) were added to a solution of alcohol **21** (1.09 g; 2.17 mmoles) in CH_2Cl_2 (100 ml). The mixture was allowed to react for 20 h at room temperature. It was then diluted with water and extracted three times with CH_2Cl_2. The combined organic phases were washed with 10% aqueous HCl, saturated aqueous $NaHCO_3$, and brine, dried with $MgSO_4$ and evaporated. The crude product was chomatographed on a silica gel column (hexane-AcOEt 9:1) and recrystallized (absolute EtOH) to yield the acetate **22** (1.01 g; 85%). TLC R_f 0.29 (hexane-AcOEt 9:1) ; mp 43-46 °C ; IR (CCl_4) 2910, 2840, 1735, 1460, 1225, 1035 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 36 H); 1.38-1.73 (m; 8 H); 2.10 (s; 3 H); 2.59 (t; *J* 7.5; 2 H); 3.30-3.95 (m; 4 H); 4.59 (m; 1 H); 5.08 (s; 2 H); 7.10-7.35 (m; 4 H) ; EIMS m/e 544 (M^+; 2%); 484 (20); 400 (91); 384 (16); 85 (100) ; Anal. Calcd for $C_{35}H_{60}O_4$: C, 77.15; H, 11.10. Found : C, 77.3; H, 11.4.

***4-(21-Hydroxyhenicosyl)benzyl acetate* 23.** A solution of acetate **22** (933 mg; 1.71 mmole) and of PPTS (43 mg; 0.17 mmole) in absolute EtOH (125 ml) was refluxed for 1 h. It was then evaporated, chromatographed on a silica gel column (CH_2Cl_2-EtOH 95:5) and recrystallized (hexane) to give the hydroxy acetate **23** (752mg; 95%). TLC R_f 0.43 (CH_2Cl_2-EtOH 95:5) ; mp 81-82.5 °C ; IR (KBr) 3270, 2920, 2840, 1735, 1460, 1245 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 34 H); 1.43-1.70 (m; 4 H); 2.09 (s; 3 H); 2.59 (t; *J* 7.6; 2 H); 3.64 (t; *J* 6.5; 2 H); 5.07 (s; 2 H); 7.10-7.30 (m; 4 H) ; Anal. Calcd for $C_{30}H_{52}O_3$: C, 78.20; H, 11.38. Found : C, 78.0; H, 11.5.

***21-[4-(Acetoxymethyl)phenyl]henicosanoic acid* 24.** Jones reagent[23] was added dropwise to a solution of the hydroxy acetate **23** (677 mg; 1.47 mmole) in acetone (200 ml) until the characteristic orange color of the reagent persisted. The chromium salts were then filtered off and the acetonic solution was evaporated. The residue was dissolved in CH_2Cl_2, washed with aqueous 0.01 *N* HCl, and evaporated. The crude product was recrystallized in hexane to yield pure acetoxy acid **24** (611 mg; 87%). TLC R_f 0.38 (CH_2Cl_2-EtOH 95:5) ; mp 83-84 °C ; IR (KBr) 3300-2500, 2920, 2850, 1740, 1705, 1250 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.50-1.73 (m; 4 H); 2.09 (s; 3 H); 2.35 (t; *J* 7.5; 2 H); 2.60 (t; *J* 7.5; 2 H); 5.07 (s; 2 H); 7.10-7.33 (m; 4 H) ; Anal. Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found : C, 76.0; H, 10.7.

***Methyl 21-[4-(acetoxymethyl)phenyl]henicosanoate* 25.** A solution of ethereal diazomethane was added dropwise to a solution of acetoxy acid **24** (541 mg; 1.14 mmole) in THF (50 ml) at 0 °C until the yellow color persisted. The solvent was then evaporated and the residue was

chromatographed on a silica gel column (CH_2Cl_2) and recrystallized (hexane) to give acetoxy ester **25** (435 mg; 78%). TLC R_f 0.41 (CH_2Cl_2) ; mp 77-78 °C ; IR (CCl_4) 2930, 2860, 1740, 1230 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.45-1.73 (m; 4 H); 2.09 (s; 3 H); 2.30 (t; *J* 7.5; 2 H); 2.60 (t; *J* 7.7; 2 H); 3.66 (s; 3 H); 5.07 (s; 2 H); 7.10-7.30 (m; 4 H); Anal. Calcd for $C_{31}H_{52}O_4$: C, 76.18; H, 10.72. Found : C, 76.3; H, 10.7.

***Methyl 21-[4-(hydroxymethyl)phenyl]henicosanoate* 26**. Potassium carbonate (276 mg; 2 mmoles) was added to a solution of acetoxy ester **25** (385 mg; 0.79 mmole) in a mixture of dry THF (20 ml) and absolute MeOH (10 ml) at room temperature. After 45 min the mixture was neutralized with 10% aqueous HCl, extracted three times with CH_2Cl_2, washed with brine, dried with $MgSO_4$ and evaporated. The crude product was chromatographed on a silica gel column (CH_2Cl_2) and recrystallized (hexane) to give hydroxy ester **26** (248 mg; 69%). TLC R_f 0.28 (hexane-AcOEt 8:2) ; mp 76-77 °C ; IR (KBr) 3340, 2920, 2840, 1735, 1460, 1170 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.50-1.73 (m; 4 H); 2.30 (t; *J* 7.5; 2 H); 2.60 (t; *J* 7.7; 2 H); 3.66 (s; 3 H); 4.66 (s; 2 H); 7.10-7.30 (m; 4 H) ; Anal. Calcd for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found : C, 78.1; H, 11.1.

***Methyl 21-(4-formylphenyl)henicosanoate* 27**. A solution of hydroxy ester **26** (211 mg; 0.47 mmole) in CH_2Cl_2 (5 ml) was added to a solution of pyridine (0.5 ml; 6 mmoles) and of chromium trioxide (300 mg; 3 mmoles) in dry CH_2Cl_2 (3 ml). The reaction was stirred for 15 min at room temperature. The resulting suspension was poured on a short silica gel column (CH_2Cl_2) and the filtrate was evaporated and recrystallized (hexane) to give the formyl ester **27** (160 mg; 76%). TLC R_f 0.64 (hexane-AcOEt 8:2) ; mp 69-70 °C ; IR (CCl_4) 2930, 2860, 2740, 1735, 1700, 1605, 1170 ; ^{1}H-NMR (200 MHz) 1.25 (broad s; 32 H); 1.50-1.73 (m; 4 H); 2.30 (t; *J* 7.5; 2 H); 2.68 (t; *J* 7.7; 2 H); 3.66 (s; 3 H); 7.33 and 7.79 (2 d; *J* 8.0; 4 H); 9.97 (s; 1 H) ; Anal. Calcd for $C_{29}H_{48}O_3$: C, 78.32; H, 10.88. Found : C, 78.6; H, 11.0.

***Methyl 21-DPH-henicosanoate* 28**. A solution of aldehyde **27** (160 mg; 0.36 mmole) and crude phosphonate **19** (250 mg) in dry THF (5 ml) was added to a suspension of sodium methoxide (35 mg; 0.65 mmole) in dry THF (7 ml) at 0 °C. Immediatly a yellow precipitate formed. After 30 min, the mixture was quenched with water, acidified with 10% aqueous HCl, and extracted three times with CH_2Cl_2. The combined organic layers were washed with saturated aqueous $NaHCO_3$ and with brine, dried with $MgSO_4$ and evaporated. The crude product was chromatographed on a silica gel column (toluene) to remove the polar impurities and recrystallized (hexane) to yield ester **28** (135 mg; 66%). TLC R_f 0.64 (toluene) ; mp 110-111 °C ; IR (KBr) 2920, 2860, 1740, 1175, 995 ; ^{1}H-NMR (400 MHz) 1.25 (broad s; 32 H; alkyl chain); 1.55-1.70 (m; 4 H; -$CH_2CH_2CO_2Me$ and -CH_2CH_2DPH); 2.30 (t; *J* 7.5; 2 H; -CH_2CO_2Me); 2.59 (t; *J* 7.7; 2 H; -CH_2DPH); 3.67 (s; 3 H; -OCH_3); 6.51 (m; 2 H; olefinic H); 6.59 (d; *J* 15.6; 2 H; olefinic H); 6.82-6.94 (m; 2 H; olefinic H); 7.15-7.50 (m; 9 H; aromatic H) ; ^{13}C-NMR (100 MHz) 25.0; 29.1; 29.2; 29.3; 29.4; 29.5; 29.6; 29.7; 31.4; 34.1; 35.7; 51.4; 116.6; 126.3; 127.4; 128.2; 128.6; 128.7; 129.2; 132.3; 132.8; 133.0; 133.8; 134.8; 137.5; 142.6; 174.3 ; EIMS m/e

570 (M^+, 47%); 296 (16); 268 (9); 245 (8); 243 (15); 231 (16); 111 (28); 83(78); 55 (100) ; Anal. Calcd for $C_{40}H_{58}O_2$: C, 84.15; H, 10.24. Found : C, 84.2; H, 10.3.

***21-DPH-henicosanoic acid* 10**. Potassium hydroxide (300 mg) was added to a solution of ester **28** (130 mg; 0.23 mmole) in toluene-MeOH 1:1 (200 ml) and the mixture was refluxed for 20 h. It was then cooled to room temperature, acidified to pH 2 with 10% aqueous HCl, and extracted three times with $CHCl_3$-MeOH 2:1. The organic layers were washed with acidic MeOH-H_2O 1:1 (acidified to pH 2 with concentrated aqueous HCl) and dried with $MgSO_4$. Recrystallization (THF-EtOH) afforded pure acid **10** (105 mg; 82%). TLC R_f 0.44 (toluene-ethyl acetate) ; mp 141-142 °C ; IR (KBr) 3010, 2920, 2850, 1700, 1470, 995 ; ^{1}H-NMR (400 MHz; THF-d_8) 1.36 (broad s; 34 H); 2.27 (t; *J* 7.4; 2 H); 2.65 (t; *J* 7.7; 2 H); 6.60 (m; 2 H); 6.65 and 6.66 (2 d; *J* 15.6; 2 H); 6.95-7.07 (m; 2 H); 7.18 and 7.41 (2 d; *J* 8.1; 4 H); 7.20-7.50 (m; 5 H); . Anal. Calcd for $C_{39}H_{56}O_2$: C, 84.12; H, 10.14. Found : C, 83.9; H, 10.1.

***1-Palmitoyl-2-(21-DPH-henicosanoyl)-sn-glycero-3-phosphocholine (LcDPHpPC)* 6**. *Preparation of a solution of 1,1'-thionyldiimidazole (TDI):* Two double necked flasks were connected by a glass tube equipped with a fritted filter. Imidazole (1.09 g; 16 mmoles) and dry THF (16 ml) were introduced into the first flask and were cooled to 0 °C. Addition of freshly distilled thionyl chloride (0.29 ml; 4.0 mmoles) resulted in the formation of TDI and of a precipitate of imidazolium hydrochloride. Transfer of the mixture through the glass frit into the other flask gave a clear 0.25 *M* solution of TDI.

Acylation of lysoPC **29** *:* Acid **10** (406 mg; 0.73 mmole) and lysoPC (400 mg; 0.81 mmole; 8:2 mixture of palmitoyl lysoPC **29** and stearoyl lysoPC) were separately dried, first by azeotropic distillation of dry toluene (3x20 ml) under reduced pressure (30 °C, 1 mm Hg) and then overnight under reduced pressure (50 °C, 1mm Hg). The acid **10** was then dissolved in dry THF (30 ml) and treated for 1 h with the TDI solution (4.0 ml; 1 mmole) prepared above, to give the imidazolide. That the latter had actually been obtained was shown by the quantitative formation of the methyl ester **28** when an aliquot of the solution was reacted with dry MeOH. Chloroform (30 ml; freed of ethanol and water by five extractions with water, drying overnight with $CaCl_2$ and distillation over P_2O_5) was then added to the lysoPC, followed by the imidazolide solution (33 ml). The mixture was allowed to react for 5 days at 50 °C. It was then brought to pH 2 with 10% aqueous HCl and extracted three times with chloroform-MeOH 2:1. The organic layers were washed with acidic MeOH-H_2O 1:1 (acidified to pH 2 with concentrated aqueous HCl), evaporated *in vacuo*, dried by azeotropic distillation of toluene, and chromatographed on a silica gel column with the following eluents : $CHCl_3$-MeOH 95:5 (500 ml); $CHCl_3$-MeOH 2:1 (500 ml; elution of the unreacted acid **10**); $CHCl_3$-MeOH-H_2O 70:26:4 [1000 ml; elution of the fluorescent phospholipid **6** (60 mg; 8%)]. TLC R_f 0.45 ($CHCl_3$-MeOH-H_2O 70:26:4) ; ^{1}H-NMR (400 MHz; $CDCl_3$-CD_3OD 2:1) 0.84 (t; *J* 6.8; 3 H; -Me); 1.22 (broad s; 56 to 60 H; alkyl chains); 1.57 (m; 6 H; -$CH_2CH_2CO_2$- and -CH_2CH_2DPH); 2.28 and 2.30 (2 t; *J* 7.7; 4 H; -CH_2CO_2-); 2.55 (t; J 7.6; 2 H; -CH_2DPH); 3.18 (s; 9 H; -NMe_3); 3.57 (m; 2 H; -$CH_2N^{\oplus}$); 3.96 (m; 2H; $^{\ominus}$CHCH_2OPO_3-); 4.13 and 4.39 (2 dd; *J* 12.0, 7.0, 3.1; 2 H;

-CH_2O_2C-); 4.28 (m; 2 H; -$OCH_2CH_2N\equiv$); 5.21 (m; 1 H; $\equiv$CHO-); 6.48 (m; 2 H; olefinic H); 6.54 (d; *J* 15.4; 2 H; olefinic H); 6.78-6.90 (m; 2 H; olefinic H); 7.08-7.39 (m; 9 H; aromatic H) ; UV ($CHCl_3$-MeOH 2:1) 360 nm (ε_M 41800 OD M^{-1} cm^{-1}) ; FAB-MS (*m*-nitrobenzyl alcohol) 1056.8 ($M+Na^+$, 1%) and 1034.8 ($M+H^+$, 4) for the palmitoyl PC; [1084.9 ($M+Na^+$, 0.3) and 1062.8 ($M+H^+$, 1) for the stearoyl PC]; 184.1 (100).

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