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Properties of unusual phospholipids. III: Synthesis, monolayer investigations and DSC studies of hydroxy octadeca(e)noic acids and diacylglycerophosphocholines derived therefrom

Lars Negelmann [a], Sandra Pisch [b], Uwe Bornscheuer [b], Rolf D. Schmid [b,*]

[a] *Bereich Enzymtechnologie/Naturstoffchemie der GBF, Gesellschaft für Biotechnologische Forschung mbH, Mascheroder Weg 1, D-38124 Braunschweig, Germany*
[b] *Institut für Technische Biochemie, Universität Stuttgart, Allmandring 31, D-70569 Stuttgart, Germany*

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Abstract

Diacylglycerophosphocholines containing (*R*)-3-, (*R*)-12-, (*R*)-17-hydroxy octadeca(e)noic acids and the corresponding racemates were synthesized and purified to homogeneity. The influence of the position of the hydroxy group on the monolayer packing properties of these fatty acids and their phosphatidylcholines was studied by Langmuir techniques and 1,2-di-[(*R*)-12-hydroxy-octadec-*cis*-9-enyl]-*sn*-glycero-3-phosphocholine displayed the largest lift-off area (330 Å^2/molecule). This result was in line with the thermotropic phase behavior of these phospholipids, as measured by differential scanning calorimetry (DSC): the gel- to liquid-crystalline phase transition temperature (T_m) passed through a minimum of -15.1°C for 1,2-di-[(*R*)-12-hydroxy-octadec-*cis*-9-enyl]-*sn*-glycero-3-phosphocholine.

Keywords: Unusual phospholipids; Hydroxy fatty acids; Langmuir film; Differential scanning calorimetry

Abbreviations: R3HOA = (*R*)-3-Hydroxy octadecanoic acid; 3HOA = (*R,S*)-3-Hydroxy octadecanoic acid; R12HOA = (*R*)-12-Hydroxy octadecanoic acid; 12HOA = (*R,S*)-12-Hydroxy octadecanoic acid; R17HOA = (*R*)-17-Hydroxy octadecanoic acid; 17HOA = (*R,S*)-17-Hydroxy octadecanoic acid; TBDMS-octadeca(e)noic acid = *t*-Butyldimethylsilyl octadeca(e)noic acid; DSPC = 1,2-Di-[octadecanoyl]-*sn*-glycero-3-phosphocholine; D3HSPC = 1,2-Di-[3-hydroxy-octadecanoyl]-*sn*-glycero-3- phosphocholine; DR3HSPC = 1,2-Di-[(*R*)-3-hydroxy-octadecanoyl]-*sn*-glycero-3-phosphocholine; DR12HEPC = 1,2-Di-[(*R*)-12-hydroxy-octadec-*trans*-9-enyl]-*sn*-glycero-3-phosphocholine; DR12HOPC = 1,2-Di-[(*R*)-12-hydroxyoctadec-*cis*-9-enoyl]-*sn*-glycero-3-phosphocholine; DR12HSPC = 1,2-Di-[(*R*)-12-hydroxyoctadecanoyl]-*sn*-glycero-3-phosphocholine; DHSPC = 1,2-Di-[12-hydroxyoctadecanoyl]-*sn*-glycero-3- phosphocholine; DR17HSPC = 1,2-Di-[(*R*)-17-hydroxyoctadecanoyl]-*sn*-glycero-3-phosphocholine; D17HSPC = 1,2-Di-[17-hydroxyoctadecanoyl]-*sn*-glycero-3- phosphocholine; DEPC = 1,2-Di-[octadec-*trans*-9-enylic acid]-*sn*-glycero-3-phosphocholine; DOPC = 1,2-Di-[octadec-*cis*-9-enylic acid]-*sn*-glycero-3- phosphocholine.

* Corresponding author. Fax: +49 711 6853196; e-mail: Rolf.D.SchmidÅ.uni-stuttgart.de

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1. Introduction

Natural fatty acids occur as components of triacylglycerols, wax and membrane lipids, of which phospholipids are the major constituents. Their acyl chains are composed of fatty acids with different degrees of saturation and a chain length between 14 and 24 carbon atoms. In contrast to this limited number of fatty acids in phospholipids, many plants and animals have developed biosynthetic pathways for more than 1000 varieties of fatty acids, e.g. branched, *trans*-unsaturated, acetylenic, and hydroxylated fatty acids (Pohl and Wagner, 1972). Non-typical saturated and unsaturated hydroxy octadecanoic acids have mainly been found as components in oils and waxes of terrestrial plants, yeast and bacteria (Matic, 1956; Bharucha and Gunstone, 1957; Riley, 1960; Downing et al., 1960; Gorin et al., 1961; Carter et al., 1961; Thiele, 1964; Tulloch and Spencer, 1964; Fiegert, 1970; Herz and Sharma, 1976; Dictionary of Organic Compounds, 1982; Stothers, 1982; Brüschweiler et al., 1989). Ricinoleic acid is synthesized in the seeds of *Ricinus communis* (James et al., 1965). As it is readily available at low price, ricinoleic acid is used for the synthesis of nylon 11 and sebacinic acid in industry (Weissermehl and Arpe, 1988). Optically active 3-hydroxy fatty acids with more than ten carbon atoms have been found in plant toxins (Herz and Sharma, 1976), microorganisms (Parker and Rathnum, 1975; Rietschel, 1984) and animals (Boylan and Scheuer, 1967). Hydroxy octadeca(e)noic acids occur in glycolipid 'biosurfactants', indicating their role in substrate solubilization. The structure, dynamics and function of biological membranes composed of phospholipids containing 'standard' fatty acids have been studied in great detail (Buddecke, 1985), but little information is available on the biological properties of phospholipids containing unusual fatty acids. Consequently, we have begun to synthesize such phospholipids with the intent to study their physicochemical properties and to explore their application potential, e.g. for stabilizing membrane proteins. In two earlier reports we have described the synthesis and properties of phosphatidylcholines composed of several acetylenic (Rürup et al., 1994a) and branched (Rürup et al., 1994b) acyl chains. Here we present the synthesis and properties of diacylglycerophosphocholines containing hydroxy octadeca(e)noic acids.

2. Materials and methods

2.1. Chemicals

sn-Glycero-3-phosphocholine (GPC) was obtained from Lukas Meyer (Hamburg, Germany). 1,2-Di-(oleoyl)-*sn*-glycero-3-phosphocholine (DOPC), 1,2-di-(elaidyl)-*sn*-glycero-3-phosphocholine (DEPC), elaidic acid, ricinelaidic acid methyl ester, *n*-octyl-β-D-glucopyranoside (OG) and 2-hydroxy octadecanoic acid were obtained from Sigma (Deisenhofen, Germany). All other chemicals were purchased from Fluka (Buchs, Switzerland) and Aldrich (Steinheim, Germany).

2.2. Analytical methods

2.2.1. NMR

^{1}H and ^{13}C NMR spectra were recorded at 27°C on Bruker (Karlsruhe, Germany) AM 300 and WM 400. Chemical shifts are given in ppm relative to internal tetramethylsilane.

2.2.2. Mass spectrometry

Phospholipids and hydroxy fatty acid methyl esters were characterized by EI–MS, the phosphatidylcholines by Liquid SIMS (Kratos MS 50 and MS 9, Shimadzu, Duisburg, Germany).

2.2.3. Preparative HPLC

The system consisted of a Latek Preparation 500 system, a spectrophotometric detector SPD-2A (Latek Labortechnik, Heidelberg, Germany) set at 207 nm, and evaporation scattering detector, Sedex 45 (S.E.D.E.R.E., Vitry/Seine, France). The column (250×4 mm i.d., VDS Optilab, Berlin, Germany), packed with ODS-Nucleosil-C18, 7 μm, was eluted in gradient mode using the solvent system methanol/water (80:20) to 100% methanol in 20 min with an elution rate of 50 ml/min.

2.2.4. Analytical HPLC

The high resolution liquid chromatography system consisted of a Knauer HPLC system, pump 64 and programmer 50 (Knauer, Hamburg, Germany), a programmer absorbance detector Techlab SA 6503 (Svern Analytical) set at 207 nm and a light scattering detector, Sedex 45 (S.E.D. E.R.E., Vitry/Seine, France). Analysis was carried out on a 250 × 4 mm i.d. column (VDS Optilab, Berlin, Germany) packed with Nucleosil-C18, 7 μm. The column was eluted in gradient mode using the solvent system methanol/water (80:20) to 100% methanol in 20 min with an elution rate of 1 ml/min.

2.2.5. TLC

The qualitative analysis of phospholipids was done with petrolether/diethylether/acetic acid 80:20:2 and chloroform/methanol/water 65:25:4 as mobile phase. The phospholipids were detected at 365 nm after spraying with 0.1% 8-anilinonaphthalin-1-sulfonic acid (ANS).

2.2.6. Refractometry

To identify fluid substances, the refractive index (n_D^{20}) was measured on a Zeiss-refractometer (Oberkochen, Germany) and with reference to the D-line of sodium light. The temperature was kept at 20°C with a thermostat.

2.2.7. Polarimetry

The optical rotation (α) was measured with a Polarimeter 241 (Perkin Elmer, Überlingen, Germany) with reference to the D-line of sodium light. The temperature was kept at 20°C with a thermostat. The concentration (c) is given as g/100 ml solution.

2.2.8. Langmuir film behavior

Surface pressure isotherms were measured using a Langmuir-Blodgett film balance from Lauda (Königshofen, Germany) equipped with a continuous pressure measuring system. Monolayers were spread from chloroform solutions of fatty acids and phospholipids onto a water subphase. The experiments were carried out from 4° to 40°C and the temperature was kept constant with a Lauda compact thermostat R6 equipped with the Lauda film balance. The monolayer was compressed during 30 min after waiting 5 min for the solvent to evaporate.

2.2.9. Differential Scanning Calorimetry

T_m and ΔH values of PC–water dispersions were measured with a calibrated MC-2 microcalorimeter (Microcal, USA). The temperature was regulated by a Haake-thermostat F3/Q.

2.2.10. Preparation of small unilamellar vesicles

Pure 1,2-diacyl-*sn*-glycero-3-phosphocholine (10 mg) was solubilized in 25 ml chloroform, and the solvent was slowly evaporated at 30°C. A thin lipid-film could be obtained on the glass flask. The sample was dried at 10^{-2} torr and dispersed in 10 ml 20 mM Tris buffer (pH 8) above the main transition temperature T_m, to form small multilamellar vesicles. Afterwards, the vesicles were homogenized by ultra wave with a Branson Sonifier.

2.3. Synthesis of isomeric hydroxy octadecanoic acids

2.3.1. Synthesis of (R)-3-hydroxy octadecanoic acid

2,2-Dimethyl-1,3-dioxane-1,4-dione (240 mmol) was acylated with palmitoylchloride (264 mmol) in 300 ml dichloromethane and 38 ml pyridine as catalyst at 0°C during 90 min in a N_2-atmosphere (Nakahata et al., 1982). The organic layer was washed three times with 80 ml 10% HCl-solution, once with 80 ml water, dried with sodium sulfate and then evaporated to dryness. The intermediate was decarboxylated with 600 ml methanol to give 3-oxostearoyl methyl ester in 16% yield.

The crude product was purified by column chromatography using *n*-hexane/diethylether (80:20) and then hydrolyzed to the acid with 1 N NaOH. The β-keto acid (14.08 mmol) was reduced with a solution of *Saccharomyces cervisiae* (84 g *Saccharomyces cerevisiae*, 42 g glucose, 315 mg KH_2PO_4 in 250 ml water) at pH 5.7–5.8 and 29°C during 12 h to yield (*R*)-3-hydroxy octadecanoic acid (Utaka et al., 1990). For purification by column chromatography using *n*-hexane/diethylether (80:20) the acid was esterified with 10%

borontrifluoride solution during 20 min at 80°C (general procedure for esterification) in 18% yield (Morrison and Smith, 1964; Pörschmann et al., 1982). The enantiomeric purity of the ester was determined by ^{1}H NMR with Eu(hfc)$_3$ as shift agent (Nakahata et al., 1982). The methyl ester (3 mmol) was then hydrolyzed by addition of 7 ml methanol and 6 ml 1 N potassium hydroxide solution. The mixture was stirred at 80°C for 5 h and then diluted with 25 ml water and 50 ml ethyl acetate. The solution was neutralized with 1 N HCl and the water layers were extracted with 50 ml ethyl acetate/diethyl ether (1:1). The combined organic layers were washed with 50 ml water, dried over magnesium sulfate and evaporated to dryness to give (*R*)-3-hydroxy octadecanoic acid (general hydrolysis procedure) (Fröhling, 1990); m.p. = 87–89°C, $[\alpha]_D^{20} = -11.8°$ ($c = 0.21$ in chloroform), analytical HPLC: R_t (min^{-1}) = 17.137, purity = 99.99%.

2.3.1.1. (R)-3-Hydroxy octadecanoic ester methyl ester. ee-value = 92%, yield = 18%, m.p. = 55–56°C, $[\alpha]_D^{20} = -14.4°$ ($c = 0.66$ in chloroform).

Analytical HPLC: R_t (min^{-1}) = 21.197 (99.6%), GC–MS: m/z (%) = 314 (0.1) [M$^+$], 296 (2) [M$^+$ –H$_2$O], 264 (7), 222 (7), 199 (4), 194 (4), 180 (4), 166 (4) 152 (4), 103 (100), 96 (42), 82 (53), 74 (35), 69 (49), 55 (71), 43 (90).

IR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3540 [ν(OH)], 3010, 2920 and 2860 [$\nu(CH_2, CH_3)$], 1730 [$\nu(C{=}O)_{Ester}$], 1600, 1460, 1440, 1400, 1360, 1330, 1295, 1220, 1175, 990.

^{1}H NMR (300 MHz, DMSO-d$_6$): $\delta = 0.88$ (t, J = 6.9 Hz, 3H, C18–CH_3), 1.24 (m, 26H, $(CH_2)_{13}$), 1.40 (m, 2H, C4–CH_2), 2.29 (dd, ^{2}J = 14.7 Hz, ^{3}J = 8.2 Hz, 1H, C2–CH_b), 2.47 (dd, ^{2}J = 14.7 Hz, ^{3}J = 4.9 Hz, 1H, C2–CH_a), 2.51 (d, J = 3, 1H, OH), 3.58 (s, 3H, CO_2CH_3), 3.81 (m, 1H, C3–CH).

^{13}C NMR (75.5 MHz, DMSO-d$_6$): $\delta = 13.87$ (C18), 22.05 (C17), 25.00 (C5), 28.67–29.00 (C6–C15), 31.26 (C16), 36.87 (C4), 42.36 (C2), 51.02 (OCH_3), 67.02 (C3), 171.74 (C1).

2.3.2. Synthesis of (R)-12-hydroxy octadec-cis-9-enylic acid

Crude acid (50.3 g; purchased from Fluka) was purified by low pressure chromatography with hexane/diethylether 55:45 and silica gel as adsorbent to afford 25.96 g ricinoleic acid (N_2-pressure = 0.3–0.5 bar). For spectroscopic characterization, 2.9 g acid were methylated with 100 ml 10% methanolic borontrifluoride solution according to the general procedure. The resulting methyl ester was purified through preparative HPLC to afford 1.8 g of pure product in 60% yield.

$n_D^{20} = 1.4719$, $[\alpha]_D^{20} = +3.8°$ ($c = 1.10$ in chloroform), analytical HPLC: R_t (min^{-1}) = 10.093, purity = 98.8%.

2.3.2.1. (R)-12-hydroxy octadec-cis-9-enylic acid methyl ester. $n_D^{20} = 1.4626$, $[\alpha]_D^{20} = +3.4°$ ($c = 8.52$ in chloroform) analytical HPLC: R_t (min^{-1}) = 14.905 (99.7%). GC–MS: m/z (%) = 310 (1) [M$^+$ –2H], 294 (8) [M$^+$–H$_2$O], 279 (2), 263 (4), 198 (9), 166 (41), 124 (40), 113 (40), 96 (42), 81 (40), 74 (41), 67 (43), 55 (100), 43 (56).

^{1}H NMR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3620 [ν(OH)], 3005 [ν(=CH)], 2940 and 2860 [$\nu(CH_2, CH_3)$], 1730 [$\nu(C{=}O)_{Ester}$], 1460, 1440, 1360, 1215, 1175, 1015, 855, 720 [δ(=CH)].

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.85$ (t, J = 6.9 Hz, 3H, C18–CH_3), 1.26–1.27 (m, 16H, $(CH_2)_8$), 1.43 (m, 2H, C13–CH_2), 1.62 (qui, J = 7.2 Hz, 2H, C3–CH_2), 2.01 (qua, J = 6.6 Hz, 2H, C8–CH_2), 2.18 (t, J = 6.7 Hz, 2H, C11–CH_2), 2.27 (t, J = 7.5 Hz, 2H, C2–CH_2), 3.58 (qui, J = 5.9 Hz, 1H, C12–CH), 3.63 (s, 3H, CO_2CH_3), 5.39 (m, 1H, C9–CH), 5.51 (m, 1H, C10–CH).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.03$ (C18), 22.58 (C17), 24.88 (C3), 25.68 (C14), 27.34 (C8), 29.05–29.54 (C4–C7, C15), 31.81 (C16), 34.04 (C2), 35.34 (C11), 36.84 (C13), 51.38 (OCH_3), 71.48 (C12), 125.23 (C9), 133.23 (C10), 174.25 (C1).

2.3.3. Synthesis of (R)-12-hydroxy octadec-trans-9-enylic acid (ricinelaidic acid)

To (*R*)-12-hydroxy-octadec-*trans*-9-enylic methyl ester (3 mmol), 7 ml methanol and 6 ml 1 N KOH-solution were added. The solution was refluxed at 80°C for 5 h, diluted with 25 ml water, 50 ml ethyl acetate and then neutralized with 1 N

HCl. The water layers were extracted with 50 ml ethyl acetate/diethylether (1:1); the combined organic layers were washed with 50 ml water, dried over magnesium sulfate and evaporated to dryness. The crude product was recrystallized twice from acetone.

m.p. = 45–46°C, $[\alpha]_D^{20} = +3.7°$ ($c = 0.41$ in chloroform); analytical HPLC: R_t (min^{-1}) = 10.582 (99.99%).

2.3.3.1. (R)-12-hydroxy octadec-trans-9-enylic acid methyl ester. m.p. = 27–28°C, $[\alpha]_D^{20} = -0.2°$ ($c = 2.44$ in chloroform); analytical HPLC: R_t (min^{-1}) = 15.51 (99.99%).

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.86$ (t, J = 6.9 Hz, 3H, C18–CH_3), 1.24–1.28 (m, 16H, $(CH_2)_8$), 1.36 (m, 2H, C13–CH_2), 1.60 (qui, J = 7.2 Hz, 2H, C3–CH_2), 1.96 (qua, J = 5.6 Hz, 2H, C8–CH_2), 2.02 (dd, ^{2}J = 14.3 Hz, ^{3}J = 15.0 Hz, 1H, C11–CH_b), 2.22 (ddd, ^{2}J = 14.3 Hz, ^{3}J = 13.9 Hz, ^{3}J = 13.0 Hz, 1H, C11–CH_a), 2.28 (t, J = 7.5 Hz, 2H, C2–CH_2), 3.60 (qui, J = 5.7 Hz, 1H, C12–CH), 3.65 (s, 3H, CO_2CH_3), 5.31–5.45 (m, 2H, C9–u. C10–CH).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.14$ (C18), 22.68 (C17), 25.01 (C3), 25.35 (C14), 29.17–29.64 (C4–C7, C15), 31.93 (C16), 32.70 (C8), 34.16 (C2), 36.88 (C13), 40.77 (C11), 51.45 (OCH_3), 72.57 (C12), 126.77 (C9), 132.60 (C10), 174.25 (C1).

2.3.4. Synthesis of (R)-12-hydroxy octadecanoic acid

In a hydrogen atmosphere, ricinoleic acid (30 mmol) was hydrogenated with rhodium (500 mg) on activated aluminum oxide as catalyst (Berkowitz et al., 1979) in 20 ml ethyl acetate (dried over molecular sieve) to (*R*)-12-hydroxy octadecanoic acid. The reaction was initiated at −30° and then stirred at room temperature for another 4.5 h (Rylander, 1985). The solid product was solubilized with methanol, the solution was filtered through a silica column and then evaporated to dryness. The crude product was recrystallized twice from methanol/petrolether to afford 8.97 g (*R*)-12-hydroxy octadecanoic acid (97% yield). For further purification by HPLC, the acid (2.1 g) was methylated with 80 ml borontrifluoride solution according to the general procedure to yield 1.3 g (61%) of pure, solid product. The methyl ester was then hydrolyzed (Fröhling, 1990) and the crude acid was recrystallized twice from acetone.

m.p. = 80°C, $[\alpha]_D^{20} = -0.5°$ ($c = 0.54$ in chloroform); analytical HPLC: R_t (min^{-1}) = 11.710; purity = 99.6%.

2.3.4.1. (R)-12-Hydroxy octadecanoic acid methyl ester. Yield = 61%; m.p. = 57°C; $[\alpha]_D^{20} = -0.4°$ ($c = 4.84$ in chloroform); analytical HPLC: R_t (min^{-1}) = 17.102 (99.9%). GC–MS: m/z (%) = 296 (1) [$M^+ - H_2O$], 264 (9), 242 (3), 229 (6), 222 (5), 197 (41), 97 (41), 87 (52), 83 (35), 74 (45), 69 (55), 55 (100), 43 (64), 41 (68).

IR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3620 [ν(OH)], 2920 and 2860 [$\nu(CH_2, CH_3)$], 1730 [$\nu(C{=}O)_{Ester}$], 1460, 1440, 1365, 1240, 1175, 1115, 1010.

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.84$ (t, J = 6.9 Hz, 3H, C18–CH_3), 1.26 (m, 22H, $(CH_2)_{11}$), 1.40 (m, 4H, C11–u. C13–CH_2), 1.59 (qui, J = 7.2 Hz, 2H, C3–CH_2), 2.28 (t, J = 7.5 Hz, 2H, C2–CH_2), 3.57 (qui, J = 6.0 Hz, 1H, C12–CH), 3.64 (s, 3H, CO_2CH_3).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.05$ (C18), 22.60 (C17), 24.93 (C3), 25.62 (C10, C14), 29.37–29.68 (C4–C9, C15), 31.83 (C16), 34.09 (C2), 37.49 (C11, C13), 51.40 (OCH_3), 72.00 (C12), 174.31 (C1).

2.3.5. Synthesis of (R)-17-hydroxy octadecanoic acid

Sophorose lipid-1′,4″-lactone-6′,6′-diacetate (146 mmol) from *Torulopsis bombicola* (grown on glucose/oleic acid; Asmer et al., 1988) was hydrolyzed and methanolized with 1200 ml methanol and 870 ml of 0.5 N methanolic KOH-solution at room temperature in 10 h to give (*S*)-17-hydroxy octadecanoic acid methyl ester. The reaction mixture was refluxed for 7 h at pH 5, which was adjusted with concentrated HCl. The solution was extracted four times with 600 ml *n*-hexane, the combined organic layers were washed with $NaHCO_3$-solution and then dried over sodium sulfate. After evaporation to dryness, the crude product was recrystallized twice from petrolether in 70% yield. (*S*)-17-Hydroxy octade-

canoic acid methyl ester (47 mmol) was transformed to (*S*)-17-toluene-4-sulfonyl octadecanoic acid methyl ester by reaction with tosylchloride (118 mmol) and pyridine (238 mmol) in 148 ml absolute dichloromethane. Then, 200 ml ice cold water was added, the water layer was extracted twice with 150 ml dichloromethane, the combined organic layers were dried over magnesium sulfate and the solvent was evaporated. The product was separated from the methyl ester by silica gel column chromatography, using dichloromethane in 56% yield. The Walden Umkehr (Kropf and Maher-Detweiler, 1977; Sykes, 1982) was performed by refluxing the tosylate (26.5 mmol) with 140 ml acetic acid anhydride and potassium acetate (137 mmol) at 120°C for 6 h to yield the (*R*)-17-acetoxy octadecanoic acid methyl ester. The solvent was evaporated, the residue dissolved in 450 ml dichloromethane and extracted twice with 350 ml NaCl solution, twice with 350 ml 5%-$NaHCO_3$ solution and finally with 200 ml NaCl solution. The organic layer was dried over sodium sulfate and then evaporated. The dark brown product was purified with silica gel column chromatography using *n*-hexane/diethylether (80:20) in 19% yield. To the (*R*)-17-acetoxy octadecanoic acid methyl ester (9.1 mmol), 70 ml ethanol and 50 ml 1 N KOH solution were added and the solution stirred for 24 h at room temperature. The solvent was evaporated and the residue dissolved in 100 ml water. The water layer was washed twice with 100 ml diethylether, its pH set to 2 with 2 N HCl and then extracted three times with 100 ml diethylether. The combined organic layers were dried over sodium sulfate and then evaporated. The residue was recrystallized twice from acetone to yield 2.54 g of (*R*)-17-hydroxy octadecanoic acid (93% yield) (Tachibana and Kambara, 1965). To obtain analytical data, the acid (8.3 mmol) was methylated with 100 ml methanolic borontrifluoride solution. After purification by preparative HPLC, 2.05 g (78%) of (*R*)-12-hydroxy octadecanoic acid methyl ester were obtained. According to the general procedure, the methyl ester was hydrolyzed to the acid, which was then recrystallized twice from acetone; m.p. = 79–81°C, $[\alpha]_D^{20} = -5.4°$ ($c = 0.71$ in chloroform); analytical HPLC: R_t (min^{-1}) = 11.89; purity = 99.99%.

2.3.5.1. (R)-17-Hydroxy octadecanoic acid methyl ester. m.p. = 53–54°C, $[\alpha]_D^{20} = -3.5°$ ($c = 1.49$ in chloroform); analytical HPLC: R_t (min^{-1}) = 18.482 (99.99%). GC–MS: m/z (%) = 312 (1) [$M^+ - 2H$], 296 (2) [$M^+ - H_2O$], 264 (24), 222 (26), 111 (22), 97 (43), 87 (66), 83 (47), 74 (81), 69 (64), 55 (100), 43 (86), 41 (51).

IR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3620 [ν(OH)], 2930 and 2860 [$\nu(CH_2, CH_3)$], 1730 [$\nu(C{=}O)_{Ester}$], 1460, 1440, 1370, 1205, 720.

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 1.14$ (d, J = 6.2 Hz, 3H, C18–CH_3), 1.22 (m, 24H, $(CH_2)_{12}$), 1.36 (m, 2H, C16–CH_2), 1.56 (qui, J = 7.2 Hz, 2H, C3–CH_2), 2.27 (t, J = 7.5 Hz, 2H, C2–CH_2), 3.63 (s, 3H, CO_2CH_3), 3.75 (m, 1H, C17–CH).

2.3.6. Synthesis of (R,S)-3-, 12- and 17-hydroxy octadecanoic acids

The hydroxy octadecanoic methyl esters (32 mmol) were dissolved in 100 ml concentrated acetic acid and oxidized with a solution of sodium dichromate-dihydrate (22 mmol) in a mixture of 8 ml water, 3.5 ml conc. sulfuric acid and 60 ml conc. acetic acid. The temperature was kept below 40°C by cooling with an ice bath. The reaction mixture was stirred for 2 h at room temperature and then the reaction was stopped with 200 ml ice cold water. The water phase was extracted twice with 100 ml *n*-hexane and the combined organic layers were washed twice with Na_2CO_3 solution, once with 50 ml water and then dried over sodium sulfate (Nichols and Schipper, 1958). After evaporation of the solvent, the keto-group of the methyl ester (0.019 mol) was reduced with sodium borohydride (0.028 mol) in 40 ml toluene/methanol 3:1 at 4°C. After stirring at room temperature for 4 h, the pH was adjusted to 2 with 0.1 N HCl. Then, the organic layer was separated and the water layer was extracted twice with ethyl acetate (Feichter et al., 1990). The crude methyl esters were purified with HPLC using a gradient of *n*-hexane/diethylether (80:20 to 20:80). The hydroxy methyl esters were hydrolyzed to the hydroxy acids according to the general procedure and then recrystallized twice from acetone.

(*R,S*)-3-Hydroxy octadecanoic acid: m.p. = 88–90°C, analytical HPLC: R_t (min^{-1}) = 17.593, purity = 99.99%.

(*R*,*S*)-3-Hydroxy octadecanoic acid methyl ester: yield = 58%.

(*R*,*S*)-12-Hydroxy octadecanoic acid: m.p. = 77–79°C, analytical HPLC: R_t (min^{-1}) = 17.442, purity = 99.6%.

(*R*,*S*)-12-Hydroxy octadecanoic acid methyl ester: yield = 50%.

(*R*,*S*)-17-Hydroxy octadecanoic acid: m.p. = 77–79°C, analytical HPLC: R_t (min^{-1}) = 12.442, purity = 99.3%.

(*R*,*S*)-17-Hydroxy octadecanoic acid methyl ester: yield = 37%.

2.4. General procedure for the synthesis of 1,2-diacyl-sn-glycero-3-phosphatidylcholines of isomeric hydroxy octadecanoic(enylic) acids

2.4.1. TBDMS-octadeca(e)noic acids

In order to protect the hydroxyl group, the hydroxy octadeca(e)noic acid methyl esters (4 mmol) were solubilized in 10 ml pyridine, 4 mmol (0.66 g) *t*-butyldimethylchlorosilane (TBDMSCl) and a catalytic amount of *N*,*N*-dimethyl-4-aminopyridine was added (Fröhling, 1990). The reaction was stirred for 3 days at room temperature. The solution was evaporated to dryness, dissolved in 50 ml diethylether and 50 ml water. The organic layer was separated and the water layer was extracted three times with 50 ml diethylether. The combined organic layers were washed with 100 ml 0.1 N sulfuric acid, then twice with 100 ml water, dried over magnesium sulfate and evaporated to dryness. The crude products were purified through silica gel column chromatography using *n*-hexane/diethylether (80:20). The TBDMS-octadeca(e)noic methyl esters (3 mmol) were then hydrolyzed (Fröhling, 1990) by addition of 7 ml methanol and 6 ml 1 N potassium hydroxide solution. The mixture was stirred at 80°C for 5 h and diluted with 25 ml water and 50 ml ethyl acetate. The solution was neutralized with 1 N HCl, the water layers were extracted with 50 ml ethyl acetate/diethylether (1:1), and the combined organic layers were washed with 50 ml water, dried over magnesium sulfate and evaporated to dryness to give the TBDMS-octadecanoic (enylic) acids.

2.4.2. 1,2-Di-[TBDMS-octadecanoyl(enyl)]-sn-glycero-3-phosphatidylcholines

The TBDMS-octadeca(e)noic acids (2.8 mmol) were transformed into their imidazolides by reaction with *N*,*N*-carbonyldiimidazole (CDI) (3 mmol) in 10 ml chloroform during 30 min at room temperature. *sn*-Glycero-3-phosphocholine (GPC) (1.4 mmol) and 0.6 ml 1,8-diazabicyclo-[5.4.0]undec-7-en (DBU) were added and the reaction mixture stirred for 24 h at room temperature (Rürup et al., 1994; Paltauf, pers. comm.). The solution was evaporated to dryness and dissolved in 10 ml acetone. The suspension was kept at −20°C for 24 h, filtered and the solid product was used without further purification for the next reaction.

2.4.3. 1,2-Di-octadecanoyl(enyl)-sn-glycero-3-phosphatidylcholine

1,2 - Di - (TBDMS - octadecanoyl(enyl)) - *sn* - glycero-3-phosphatidylcholine (1.4 mmol) was dissolved in 2 ml THF, 6 ml acetic acid and 2 ml water were added and the solution was stirred for 28 h at room temperature. The solvent was evaporated, the crude product dried under high vacuum and purified through preparative HPLC (Corey and Venkateswarlu, 1972).

2.4.4. 1,2-Di-[3-hydroxy-octadecanoyl]-sn-glycero-3-phosphocholine

Yield: 72 mg (2% relative to hydroxy octadecanoic acid methyl ester). $[\alpha]_D^{20} = +4.8°$ ($c = 1.25$ in chloroform). IR ($CHCl_3$): υ (cm^{-1}) = 3540 and 3500–3100 (OH), 2910 and 2860 (CH_2, CH_3), 2500, 1725 (CO–ester), 1460, 1370, 1240, 1070, 965, 910, 870, 815, 720.

^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.86$ (t, 6.9 Hz, 6H, C18–2CH_3), 1.24 (m, 52 H, 2$(CH_3)_{13}$), 1.53 (m, 4H, C4–2CH_2), 2.35–2.50 (m, 4H, C2–2CH_2), 3.30 (s, 9H, N$(CH_3)_3$), 3.75 (m, 2H, CH_2N), 3.82–3.84 (m, 2H, C3–2CH), 3.85–3.99 (m, 2H, glycerol–C3–$H_{a,b}$), 4.03–4.10 (dd, ^2J = 12 Hz, $^3J = 7.6$ Hz, 1H, glycerol–C1-H_b), 4.11–

4.31 (m, 2H, PO–CH_2), 4.35–4.39 (dd, $^2J = 12$ Hz, $^3J = 2.8$ Hz, glycerol–C1–H_a), 5.09–5.19 (m, 1H, glycerol-C2-H).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.11$ (C18), 22.71 (C17), 24.95 (C5), 29.20–29.74 (C6–C15), 31.95 (C16), 34.20 (C4), 36.00 (C2), 54.53 ($N(CH_3)_3$), 59.28 (PO–CH_2), 63.06 (glycerol–C1), 63.41 (glycerol–C3), 66.50 (CH_2N), 66.50 (C3), 70.59 and 70.69 (d, glycerol C2), 173.21 (C1–chain 1), 173.56 (C1–chain 2).

^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 0.386$ and 0.784.

LSIMS positive (3-NBA): m/z (%) = 822.5 (22) $[M + H]^+$, 224.1 (39), 184.1 (100).

2.4.5. 1,2-Di-[(R)-3-hydroxy-octadecanoyl]-sn-glycero-3-phosphocholine

Yield: 5.1 mg (1% relative to (*R*)-3-hydroxy-octadecanoic acid methyl ester).

$[\alpha]_D^{20} = -12.0°$ ($c = 0.30$ in chloroform).

LSIMS positive (3-NBA): m/z (%) = 822.6 (25) $[M + H]^+$, 224.1 (5), 184.1 (100).

NMR-data: see 1,2-Di-[3-hydroxy-octadecanoyl)-*sn*-glycero-3-phosphocholine.

2.4.6. 1,2-Di-[(R)-12-hydroxy-octadec-trans-9-enyl]-sn-glycero-3-phosphocholine

Yield: 120 mg (5% relative to (*R*)-12-hydroxy octadec-*trans*-9-enylic acid methyl ester).

$[\alpha]_D^{20} = +5.2°$ ($c = 0.77$ in chloroform).

IR ($CHCl_3$): υ (cm^{-1}) = 3620–3100OH), 3010 (CH), 2935 and 2860 (CH_2, CH_3), 2500, 1735 (CO–ester), 1465, 1370, 1375, 1250, 1075, 965, 910, 870, 830.

^1H NMR (300 MHz, $CDCl_3$): $\delta = 0.85$ (t, 6.6 Hz, 6 H, C18–$2CH_3$), 1.25 (m, 32 H, $2(CH_3)_8$), 1.39 (m, 4H, C13–$2CH_2$), 1.55 (m, 4H, C3–$2CH_2$), 1.97 (q, J = 6.6 Hz, 4H, C8–$2CH_2$), 2.05 (dd, $^2J = 14.3$ Hz, $^3J = 15.0$ Hz, 1H, C11–$2CH_b$), 2.20 (ddd, $^2J = 14.3$ Hz, $^3J = 13.9$ Hz, $^3J = 13.0$ Hz, 1H, C11–$2CH_a$), 2.24 (t, J = 7.5 Hz, 2H, C2–CH_2–chain 1), 2.26 (t, J = 7.2 Hz, 2H, C2–CH_2–chain 2), 3.31 (s, 9H, $N(CH_3)_3$), 3.54 (q, J = 5.7 Hz, 2H, C12–2CH), 3.73 (m, 2H, CH_2N), 3.86–3.90 (m, 2H, glycerol–C3–$H_{a,b}$), 4.06–4.12 (dd, $^2J = 12$ Hz, $^3J = 7.2$ Hz, 1H, glycerol–C1–H_b), 4.25 (m, 2H, PO–CH_2), 4.33–4.37 (dd, $^2J =$ 12 Hz, $^3J = 2.8$ Hz, glycerol–C1–H_a), 5.15 (m, 1H, glycerol–C2–H), 5.33–5.42 (m, 2H, 9–2CH), 5.44–5.53 (m, 2H, 10–2CH).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.06$ (C18), 22.59 (C17), 24.79 (C3–chain 1), 24.84 (C3–chain 2), 25.63 (C14), 28.92 (C7), 29.03–29.35 (C4–C6, C15), 31.81 (C16), 32.58 (C8), 34.05 (C2–chain 1), 34.23 (C2–chain 2), 36.72 (C13), 40.67 (C11), 54.31 ($N(CH_3)_3$), 59.24 (PO–CH_2), 62.95 (glycerol–C1), 63.34 (glycerol–C3), 66.30 (CH_2N), 70.44 and 70.54 (d, glycerol C2), 173.21 (C1–chain 1), 70.87 (C12), 126.13 (C9), 134.12 (C10–chain 1), 134.16 (C10–chain 2) 173.56 (C1–chain 2), 173.50 (C1–chain 2).

^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 0.20188$.

LSIMS positive (3-NBA): m/z (%) = 818.6 (32) $[M + H]^+$, 224.1 (39), 184.1 (100).

Elementary analysis for $C_{44}H_{84}NO_{10}P$: *calc*. C, 64.60%; H, 10.35%; N, 1.71%; P, 3.79%; *found* C, 64.59%; H, 10.08%; N, 1.45%; P, 3.78%.

2.4.7. 1,2-Di-[(R)-12-hydroxy-octadec-cis-9-enoyl]-sn-glycero-3-phosphocholine

Yield: 274 mg (9% relative to (*R*)-12-hydroxy octade-*cis*-9-enylic acid methyl ester).

$[\alpha]_D^{20} = +7.1°$ ($c = 3.95$ in chloroform); analytical HPLC: R_t (min^{-1}) = 19.698 (99.99%).

IR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3620–3100 [ν(OH)], 3005 [ν(=CH)], 2940 and 2860 [$\nu(CH_2, CH_3)$], 2500, 1730 [$\nu(C{=}O)_{Ester}$], 1465, 1370, 1250, 1075, 965, 910, 870, 830, 660 [δ(=CH)].

^1H NMR (400 MHz, $CDCl_3$): $\delta = 0.86$ (t, J = 6.9 Hz, 6H, C18–$2CH_3$), 1.23–1.27 (m, 32H, $2(CH_2)_8$), 1.43 (m, 4H, C13–$2CH_2$), 1.57 (m, 4H, C3–$2CH_2$), 2.02 (qua, J = 6.6 Hz, 4H, C8–$2CH_2$), 2.18 (t, J = 6.7, 4H, C11–$2CH_2$), 2.26 (t, J = 7.3 Hz, 2H, C2–CH_2–chain 1), 2.28 (t, J = 7.1 Hz, 2H, C2–CH_2–chain 2), 3.31 (s, 9H, $N(CH_3)_3$), 3.58 (qui, J = 5.9 Hz, 2H, C12–2CH), 3.75 (m, 2H, CH_2N), 3.87–3.98 (m, 2H, glycerol–C3–$H_{a,b}$), 4.08–4.13 (dd, $^2J = 12.0$ Hz, $^3J = 7.0$ Hz, 1H, glycerol–C1–H_b), 4.27 (m, 2H, $POCH_2$), 4.36–4.39 (dd, $^2J = 12.0$ Hz, $^3J = 2.9$ Hz, 1H, glycerol–C1–H_a), 5.15–5.21 (m, 1H, glycerol–C2–H), 5.35–5.42 (m, 2H, C9–2CH), 5.47–5.54 (m, 2H, C10–2CH).

^{13}C NMR (100.6 MHz, $CDCl_3$): $\delta = 14.10$ (C18), 22.64 (C17), 24.83 (C3–chain 1), 24.89 (C3–chain 2), 25.76 (C14), 27.38 (C8), 29.07–29.70 (C4–C7, C15), 31.87 (C16), 34.10 (C2–chain 1), 34.29 (C2–chain 2), 35.41 (C11), 36.89 (C13), 54.49 ($N(CH_3)_3$), 59.31 ($POCH_2$), 62.97 (glycerol–C1), 63.44 (glycerol–C3), 66.48 (CH_2N), 70.55 and 70.62 (d, glycerol C2), 71.42 (C12), 125.52 (C9–chain 1), 125.56 (C9–chain 2), 132.93 (C10–chain 1), 132.96 (C10–chain 2), 173.17 (C1–chain 1), 173.53 (C1–chain 2).

^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 0.28227$.

LSIMS positive (3-NBA): m/z (%) = 818.5 (22) $[M + H]^+$, 224.1 (2), 184.1 (100).

HRMS for $C_{44}H_{84}NO_{10}P$: *calc*. 818.5911, *found* 822.5938.

Elementary analysis for $C_{44}H_{84}NO_{10}P$: *calc*. C, 64.60%; H, 10.35%; N, 1.71%; P, 3.79%; *found* C, 64.61%; H, 10.11%; N, 1.55%; P, 3.66%.

2.4.8. 1,2-Di-[(R)-12-hydroxy octadecanoyl]-sn-glycero-3-phosphocholine

Yield: 107 mg (5% relative to (*R*)-12-hydroxy octadecanoic acid methyl ester). $[\alpha]_D^{20} = +5.3°$ ($c = 0.31$ in chloroform); analytical HPLC: R_t (min^{-1}) = 22.772 (99.99%).

IR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3620 and 3500–3100 [ν(OH)], 2930 and 2860 [$\nu(CH_2, CH_3)$], 2500, 1735 [$\nu(C{=}O)_{Ester}$], 1465, 1370, 1260, 1210, 1090, 1065, 970, 725.

^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.86$ (t, J = 6.9 Hz, 6H, C18–$2CH_3$), 1.25 (m, 44H, $2(CH_2)_{11}$), 1.40 (m, 8H, C11–u. C13– $4CH_2$), 1.56 (m, 4H, C3–$2CH_2$), 2.26 (t, J = 7.3 Hz, 2H, C2–CH_2–chain 1), 2.28 (t, J = 7.2 Hz, 2H, C2–CH_2–chain 2), 3.34 (s, 9H, $N(CH_3)_3$), 3.55 (m, 2H, C12–2CH), 3.77 (m, 2H, CH_2N), 3.87–3.94 (m, 2H, glycerol–C3–$H_{a,b}$), 4.09–4.13 (dd, ^{2}J = 12.0 Hz, ^{3}J = 7.2 Hz, 1H, glycerol–C1–H_b), 4.28 (m, 2H, $POCH_2$), 4.36–4.39 (dd, ^{2}J = 12.0 Hz, ^{3}J = 2.8 Hz, 1H, glycerol–C1–H_a), 5.17 (m, 1H, glycerol–C2–H).

^{13}C NMR (75.5 MHz, $CDCl_3$): $\delta = 14.11$ (C18), 22.63 (C17), 24.84 (C3–chain 1), 24.91 (C3–chain 2), 25.68 (C10, C14), 29.06–29.73 (C4–C9, C15), 31.87 (C16), 34.11 (C2–chain 1), 34.29 (C2–chain 2), 37.50 (C11, C13), 54.46 ($N(CH_3)_3$), 59.27 ($POCH_2$), 62.94 (glycerol–C1), 63.42 (glycerol–C3), 66.42 (CH_2N), 70.46 and 70.55 (d, glycerol–C2), 71.85 (C12), 173.22 (C1–chain 1), 173.50 (C1–chain 2). ^{31}P NMR (162 MHz, $CDCl_3$): $\delta =$ 0.41360.

LSIMS positive (3–NBA): m/z (%) = 822.5 (18) $[M + H]^+$, 224.1 (3), 184.1 (100).

HRMS for $C_{44}H_{88}NO_{10}P$: calc. 822.6224, *found* 822.6236.

Elementary analysis for $C_{44}H_{88}NO_{10}P$: *calc*. C, 64.28%; H, 10.79%; N, 1.70%; P, 3.77%; *found* C, 64.28%; H, 10.61%; N, 1.69%; P, 3.83%.

2.4.9. 1,2-Di-[12-hydroxy octadecanoyl]-sn-glycero-3-phosphocholine

Yield: 75 mg (1% relative to 12-hydroxy octadecanoic acid methyl ester). $[\alpha]_D^{20} = +5.8°$ ($c = 0.96$ in chloroform); analytical HPLC: R_t (min^{-1}) = 22.120 (99.79%).

^{31}P NMR (162 MHz, $CDCl_3$): $\delta = 0.41734$.

LSIMS positive (3-NBA): m/z (%) = 822.6 (19) $[M + H]^+$, 224.1 (3), 184.1 (100).

HRMS for $C_{44}H_{88}NO_{10}P$: *calc*. 822.6224, *found* 822.6632.

NMR-data: see 1,2-Di-[(*R*)-12-hydroxy-octadecanoyl)-*sn*-glycero-3-phosphocholine.

2.4.10. 1,2-Di-[(R)-17-hydroxy octadecanoyl]-sn-glycero-3-phosphatidylcholine, DR17HSPC

Yield: 380 mg (14% relative to (*R*)-17-hydroxy octadecanoic acid methyl ester). $[\alpha]_D^{20} = +3.2°$ ($c = 2.68$ in chloroform); analytical HPLC: R_t (min^{-1}) = 22.880 (99.89%).

IR ($CHCl_3$): $\bar{\nu}$ (cm^{-1}) = 3620 and 3500–3100 [ν(OH)], 2930 and 2860 [$\nu(CH_2, CH_3)$], 2500, 1725 [$\nu(C{=}O)_{Ester}$], 1465, 1370, 1260, 1230, 1085, 1060, 970, 860, 825.

^{1}H NMR (300 MHz, CD_3OD): $\delta = 1.18$ (d, J = 6.2 Hz, 6H, C18–$2CH_3$), 1.34 (m, 52H, $2(CH_2)_{13}$), 1.64 (m, 4H, C3–$2CH_2$), 2.36 (t, J = 7.5 Hz, 2H, C2–CH_2–chain 1), 2.38 (t, J = 7.4 Hz, 2H, C2–CH_2–chain 2), 3.27 (s, 9H, $N(CH_3)_3$), 3.68 (m, 2H, CH_2N), 3.74 (m, J = 6.0 Hz, 2H, C17–2CH), 4.02–4.06 (m, 2H, glycerol–C3–$H_{a,b}$), 4.18–4.25 (dd, ^{2}J = 12.0 Hz, ^{3}J = 6.9 Hz, 1H, glycerol–C1–H_b), 4.31 (m, 2H, $POCH_2$), 4.45–4.51 (dd, ^{2}J = 12.0 Hz, ^{3}J = 3.1 Hz, 1H, glycerol–C1–H_a), 5.27 (m, 1H, glycerol–C2–H).

^{13}C NMR (75.5 MHz, CD_3OD): $\delta = 21.89$ (C18), 24.40 (C3), 25.31 (C15), 28.60–29.19 (C4–C14), 33.29 (C2–chain 1), 33.47 (C2–chain 2), 38.62 (C16), 53.02 ($N(CH_3)_3$), 58.84 ($POCH_2$), 62.04 (glycerol–C1), 63.24 (glycerol–C3), 65.83 (CH_2N), 66.93 (C17), 70.11 and 70.22 (d, glycerol C2), 172.96 (C1–chain 1), 173.26 (C1–chain 2).

^{31}P NMR (162 MHz, CD_3OD): $\delta = 0.71947$.

LSIMS positive (3-NBA): m/z (%) = 822.5 (26) $[M + H]^+$, 224.1 (2), 184.0 (100).

HRMS for $C_{44}H_{88}NO_{10}P$: *calc*. 822.6224, *found* 822.6228.

Elementary analysis for $C_{44}H_{88}NO_{10}P$: *calc*. C, 64.28%; H, 10.79%; N, 1.70%; P, 3.77%; *found* C, 64.24%; H, 10.49%; N, 1.71%; P, 3.65%.

2.4.11. 1,2-Di-[17-hydroxy octadecanoyl]-sn-glycero-3-phosphocholine, D17HSPC

Yield: 173 mg (5% relative to 17-hydroxy octadecanoic acid methyl ester). $[\alpha]_D^{20} = +4.0°$ (c = 1.42 in chloroform); analytical HPLC: R_t (min^{-1}) = 22.952 (99.79%).

^{31}P NMR (162 MHz, CD_3OD): $\delta = 0.71949$.

LSIMS positive (3-NBA): m/z (%) = 822.6 (26) $[M + H]^+$, 224.1 (3), 184.1 (100).

HRMS for $C_{44}H_{88}NO_{10}P$: *calc*. 822.6224, *found* 822.6511.

NMR-data: see 1,2-Di-[(*R*)-17-hydroxy-octadecanoyl)-*sn*-glycero-3-phosphocholine.

Elementary analysis for $C_{44}H_{88}NO_{10}P$: *calc*. C, 64.28%; H, 10.79%; N, 1.70%; P, 3.77%; *found* C, 64.26%; H, 10.85%; N, 1.63%; P, 3.66%.

3. Results

3.1. Synthesis of hydroxy octadeca(e)noic acid

The chemical synthesis of the hydroxy octadeca(e)noic acids followed procedures described in literature. (*R*)-3-Hydroxy octadecanoic acid was synthesized according to Nakahata et al. (1982) and Utaka et al. (1990) starting from 2,2-dimethyl-1,3-dioxane-1,4-dione. The enantiomeric purity of the methyl ester as determined by ^{1}H NMR with $Eu(hfc)_3$ was 92% ee.

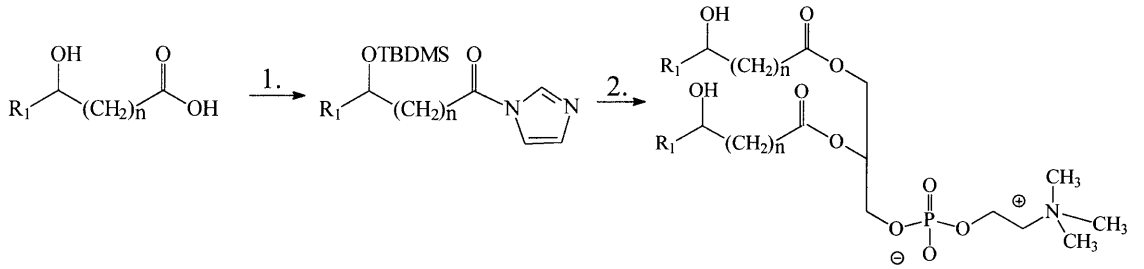
Berkowitz et al. (1979) described the synthesis of (*R*)-12-hydroxy octadecanoic acid from ricinoleic acid using rhodium on activated aluminum oxide (5%) as catalyst. Side reactions — for example hydrogenolysis of the homoallylic function — could be avoided by initiating the reaction at −30°C.

(*R*)-17-Hydroxy octadecanoic acid was synthesized from (*S*)-17-hydroxy octadecanoic acid methyl ester by Walden Umkehr. The (*S*)-methyl ester was prepared from sophorose lipid-1′,4″-lactone-6′,6″-diacetate, a fermentation product of the yeast *Torulopsis bombicola*. According to Asmer et al. (1988), the sophorose lipid was hydrolyzed under alkaline conditions and then methylated.

(*R*,*S*)-3-, 12-, 17-hydroxy octadecanoic acids were prepared according to Nichols and Schipper (1958). (*R*)-12 and *R*-(17)-hydroxy octadecanoic acid methyl esters were oxidized with sodium dichromate to the corresponding oxooctadecanoic acid methyl esters. 3-Oxo octadecanoic acid methyl ester was obtained during the synthesis of the (*R*)-3-hydroxy octadecanoic acid. Following the procedure of Feichter et al. (1990), (*R*,*S*)-hydroxy octadecanoic acid methyl esters were synthesized from the oxo octadecanoic acid methyl esters with sodium borohydride in toluene/methanol (3:1) with yields between 37–58%.

3.2. Synthesis of diacylglycerophosphocholines containing hydroxylated fatty acids

Fig. 1 shows a general scheme. Phosphocholines were synthesized through acylation of free *sn*-glycero-3-phosphocholine (GPC) with activated fatty acids and 1,8-diazabicyclo[5.4.0]undec-7-en(1,5-5) (DBU) as catalyst in chloroform according to the method of Paltauf (pers. comm.), based on a procedure by Warner and Benson (1977) by which fatty acids are activated with *N*,*N*-carbonyldiimidazole (CDI) under mild conditions to prevent racemisation. Before activation with CDI, the hydroxyl group had to be protected. To this end, the methyl esters were suspended in pyridine and the hydroxyl group was etherified with *t*-butyl dimethylchlorosilane and *N*′,*N*′-dimethylaminopyridine (DMAP) as catalyst in yields from 66–93%, according to Fröhling



1. i) BF_3/MeOH ii) TBDMSCl iii) KOH iv) CDI/$CHCl_3$ **2.** i) GPC/DBU ii) THF/HOAc/H_2O

Fig. 1. Synthesis sequence of diacylglycerophosphocholines.

(1990). The *t*-butyldimethyl (TBDMS) protecting group was stable under alkaline conditions necessary for the hydrolysis of the methyl ester prior to their activation with CDI. The TBDMS-ether group of the TBDMS-*sn*-glycero-3-phosphocholines was cleaved according to Corey and Venkateswarlu (1972) with tetrahydrofurane/acetic acid/water (1:3:1) to the corresponding diacylglycerophosphocholines, resulting in low yields between 1% and 14%. The diacylglycerophosphocholines were purified by preparative HPLC.

3.3. Monolayer investigation

Lift-off areas, coexistence points π_E, coexistence areas A_E, collapse areas and collapse values of the hydroxy fatty acids are summarized in Table 1. All fatty acids produced liquid-expanded films upon compression, except for racemic and chiral 3-hydroxy octadecanoic, which, similar to stearic acid, formed condensed films only. Fig. 2 shows the surface-area curves of saturated hydroxy octadecanoic acids on a water subphase at 20°C. Compared to the chiral hydroxy octadecanoic acids, the racemic isomers were more expanded and exhibited higher film pressures at the transition to the coexistence area. On moving the hydroxy group from position 3 to 12 of the alkyl chain, the lift-off area profoundly increased (Menger et al., 1989), while going from position 12 to 17 (Steffen, 1989), only a slight increase could be observed. Usually, *trans* unsaturated fatty acids form less expanded films than their corresponding *cis* confomers. Nevertheless, the introduction of a secondary hydrophilic group resulted in an inversion of this behavior. During investigations on the influence of temperature on the monolayer state, a dependence on the position of the hydroxy group was observed. Thus, (*R*,*S*)- and (*R*)-3-hydroxy octadecanoic acid showed liquid-expanded to condensed phase transitions above 25°C.

Fig. 3 presents π/A-isotherms of DR12HSPC at different temperatures. At 20°C, only DSPC showed a condensed phase, whereas D3HSPC additionally exhibited an expanded phase. Between 5–40°C, D12HSPC and DR12HSPC produced gaseous, liquid expanded, liquid condensed and solid condensed phase transitions, whereas D17HSPC and DR17HSPC formed no totally condensed films. In opposition to their fatty acids, DR12HSPC and DR17HSPC were of the more expanded type than their racemic isomers. The surface pressure-area curves on a water subphase of the chiral phospholipids in comparison to DSPC are illustrated in Fig. 4. Lift-off areas, coexistence points π_E, coexistence areas A_E, collapse areas and collapse values are presented in Table 2.

Fig. 5 depicts π/A-isotherms of DR12HEPC, DR12HOPC, DEPC and DOPC. In contrast to the fatty acids, DR12HOPC formed more expanded phases than the *trans* unsaturated phospholipid DR12HEPC, which corresponds with the behavior of DOPC compared to DEPC. Except for

Table 1
Characteristic pressure and area values of isomeric hydroxy octadeca(e)noic acids (HSS) and standards at 20°C

Hydroxy octadeca(e)noic acids and standards	Lift off	Transition point K_E		Collapse point	
	A (Å^2/molecule)	A_E (Å^2/molecule)	π_E (mN/m)	A (Å^2/molecule)	π (mN/m)
2HOA	26	—	—	19	47
R3HOA	35	—	—	19	46
3HOA	34	—	—	19	46
R12HOA	154	91	7	18	44
12HOA	159	92	8	18	41
R17HOA	158	80	10	17	33
17HOA	159	81	11	17	32
Octadecanoic acid	25	—	—	20	50
Ricinelaidinic acid	180	—	—	20	19
Ricinoleic acid	174	—	—	22	17
Elaidinic acid	55	—	—	31	26
Oleic acid	56	—	—	32	32

D3HSPC, which produced an expanded film above 20°C, no temperature dependence between 5°C and 40°C on the monolayer state could be detected (Fig. 4).

3.4. Differential scanning calorimetry investigations of diacylphosphocholines containing hydroxy octadecanoic acids

T_m, $\Delta T_{1/2}$ and ΔH values for the phospholipids are shown in Table 3.

PC-derivatives, except the racemic 3-hydroxy octadecanoic-derivatives, showed higher T_m and ΔH values compared to standard phospholipids DSPC, DOPC and DEPC. PC-derivatives with unsaturated acyl chains had notably lower T_m and ΔH values than PC-derivatives with saturated acyl chains. Except for DSPC, no pretransition was observed.

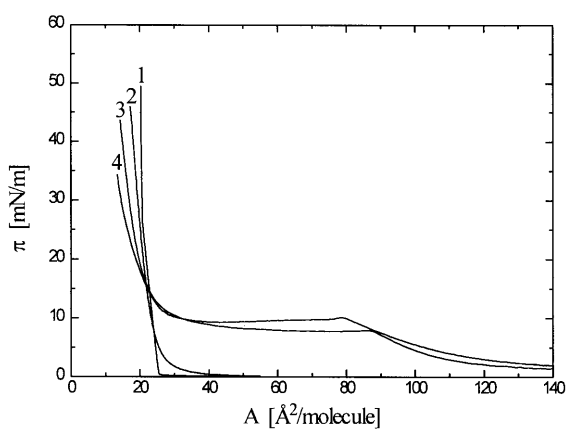


Fig. 2. π/A-isotherms of phosphocholines of octadecanoic acid (1), R3HOA (2), R12HOA (3), R17HOA (4) at 20°C.

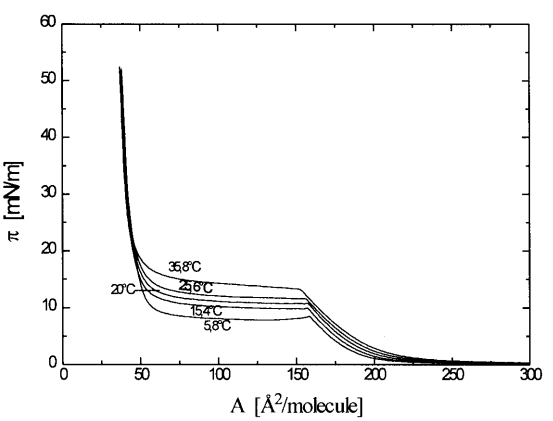


Fig. 3. π/A-isotherms of DR12HSPC at different temperatures.

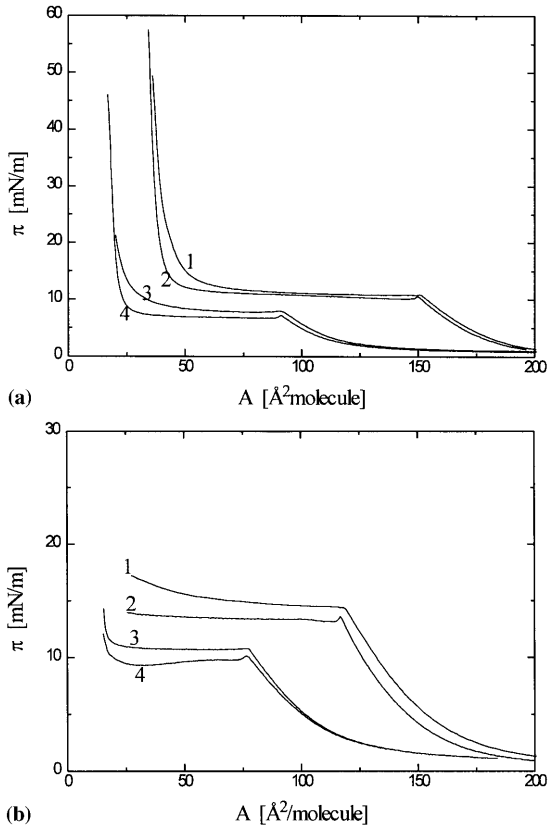


Fig. 4. (a) Surface pressure–area curves on a water subphase of DR12HSPC (1), D12HSPC (2), 12HOA (3) and R12HOA (4) at 20°C. (b) Surface pressure–area curves on a water subphase of DR17HSPC (1), D17HSPCm (2), 17HOA (3) and R17HOA (4) at 20°C.

4. Discussion

4.1. Synthesis and analysis of the hydroxy octadecanoic(enylic) acids derivatives

The synthesis of the hydroxy octadecanoic acids was carried out according to standard methods. The asymmetric reduction of 3-oxooctadecanoic acid methyl ester with *Saccharomyces cervisiae* gave very low yields. For the (*R*)-12- and (*R*)-17-hydroxy octadecanoic acids, reduction and inversion-reactions could be modified.

Acylation of *sn*-glycero-3-phosphocholine (GPC) with activated fatty acids, e.g. imidazolides and anhydrides (Gupta and Radhakrishnan, 1977), is a standard procedure in diacylglycerophosphocholine synthesis. All our attempts, however, to synthesize phosphocholines using activated hydroxy fatty acid imidazolides, glycerophosphatidylcholine and DBU or methylsulfinylmethid (Warner and Benson, 1977) as catalyst were unsuccessful. We assume that instead of the desired intermolecular reaction of the imidazolide with GPC an intra- or intermolecular reaction with the hydroxy group of the fatty acid took place. Thus it was necessary to protect the fatty acid hydroxy group prior to activation. Following the procedure of Nicholas et al. (1983), van Boom and Burgers (1978) and Hassner et al. (1975), we tried to react 4-oxopentanoic acid (levulinic acid) and dicyclohexylcarbodiimid with ricinoleic acid and pyridine to the levulinic ester (data not shown). Because of the hydrolytic instability of the acid anhydride, we also investigated several silyl protecting agents, e.g. *N*,*O*-bis-[trimethylsilyl]-acetamide, hexamethyldisilazane and trimethylchlorosilane (data not shown), but the silyl group at the carboxyl function could neither be cleaved selectively with methanol nor thionylchloride. It proved possible, however, to protect the hydroxy group of the fatty acid in a selective reaction with *t*-butyldimethylchlorosilane. Even in this reaction sequence, the cleavage of the silyl protecting group under aqueous-acidic conditions led to a partial hydrolysis of the glycerol ester bond, resulting in low overall yields of hydroxy fatty acid phosphatidylcholines.

4.2. Monolayer investigations

4.2.1. Influence of position of the hydroxy group and unsaturation

Lift-off-values of isomeric hydroxy octadecanoic acids and stearic acid are given in Table 4 (Menger et al., 1989). The monolayer behavior of fatty acids and phospholipids is influenced by their acyl chain structure. In order to investigate this influence, a Langmuir-type film balance was used to study the effect of an additional polar group in the fatty acid of diacylglycerophosphocholines on monolayer packing properties. Stearic acid, oleic acid, elaidinic acid and the corresponding phosphocholines were used as reference stan-

Table 2
Characteristic pressure and area values of PC-derivatives of isomeric hydroxy octadeca(e)noic acids and PC-standards at 20°C

Phospholipids and standards	Lift off	Transition point K_E		Collapse point	
	A (Å^2/molecule)	A_E (Å^2/molecule)	π_E (mN/m)	A (Å^2/molecule)	π (mN/m)
D3HSPC	90	87	2	41	55
DR12HSPC	240	155	11	39	51
D12HSPC	237	154	10	38	53
DR17HSPC	241	9118	15	—	—
D17HSPC	240	9117	12	—	—
DSPC	58	—	–	47	56
DR12HEPC	320	107	23	40	46
DR12HOPC	330	116	25	49	42
DEPC	110	—	—	51	49
DOPC	110	—	—	58	47
Lyso-DOPC	97	—	—	34	36

dards. Stearic acid manifested the usual behavior of a long-chain saturated fatty acid. Introduction of a hydroxy group led to a decreased acyl chain interaction in the monolayer, resulting in larger film expansion (Kellner and Cadenhead, 1978; Tachibana et al., 1979; Nagarajan and Shah, 1981; Rakshit et al., 1981; Bois and Baret, 1988; Menger et al., 1989) as indicated by an increased area under the PA curve (Fig. 2). The liquid expanded region was sensitive to the location of the hydroxy group. The lift-off area represents the beginning of observable intermolecular forces between adjacent molecules in the film. Lift-off values increased by moving the hydroxy group away from the carboxyl function towards the end of the molecule (Table 3). A similar behavior for a series of hydroxy hexadecanoic acids was reported by Kellner and Cadenhead (1978). Molecules which contact the water subphase with both polar groups (hydroxy- and carboxy group) and the hydrophobic chain require a minimum area of 117 Å^2/molecule (Menger et al., 1988a,b). In the case of 3-hydroxy octadecanoic acid, we conclude from the observed lift-off area of 34 Å^2/molecule that the hydrophobic chain beyond the hydroxy group stands out of the subphase. In contrast, the lift-off areas for 12- and 17-hydroxyoctanoic acid are

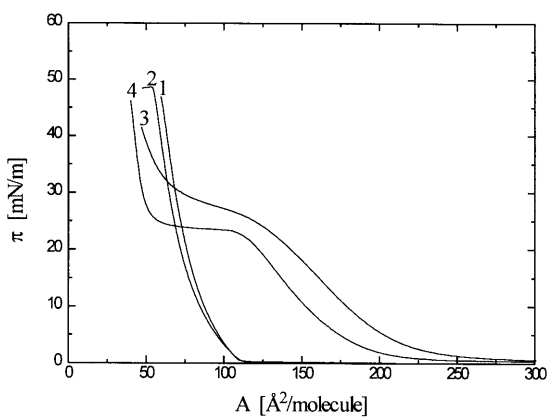


Fig. 5. π/A-isotherms of DOPC (1), DEPC (2), DR12HOPC (3) and DR12HEPC (4).

Table 3
Gel- to liquid-crystalline phase transition temperature (T_m) of PC-derivatives of isomeric hydroxy octadeca(e)noic acids and PC-standards

PC	T_m (°C)	ΔH (kJ mol^{-1})	$\Delta T_{1/2}$ (°C)
D3HSPC	39.6	44.2	1.04
DR12HSPC	67.1	51.4	1.87
D12HSPC	69.1	53.5	1.53
DR17HSPC	77.3	55.5	4.13
D17HSPC	80.7	59.2	0.83
DR12HOPC	−15.1	31.8	2.51
DR12HEPC	33.1	32.1	4.65
DSPC	54.3	49.7	0.51
DOPC	−20.7	31.3	2.94
DEPC	11.8	31.9	0.53

Table 4
Characteristic pressure and area values of isomeric hydroxy octadeca(e)noic acids and standards at 20°C

Fatty acid	Stearic acid	2HOA	3HOA	8HOA[a]	10HOA[a]	12HOA[a]	12HOA	16HOA[a]	17HOA
Lift off A (Å^2/molecule)	25	26	34	108	127	145	159	141	159
A_E (Å^2/molecule)	—	—	—	56	74	90	92	87	81
π_E (mN/m)	—	—	—	12	9	7	8	7	11

[a] Values at 23°C according to Menger et al. (1988).

higher than 117 Å^2/molecule, indicating that the whole molecule contacts the subphase. During further compression, the polar hydroxy group remains in contact with the subphase while the hydrophobic segment beyond the hydroxy group stands out into the gaseous phase. This leads to the formation of arched molecules (Menger et al., 1988), whose compressibility decreases on reducing the number of methylene groups between the two polar groups. Surprisingly, 17-hydroxy octadecanoic acid is more compressible than 12-hydroxy octadecanoic acid (Asmer et al., 1992). Possibly, an arch consisting of 15 methylene groups is extraordinarily stable.

In the liquid condensed condition, all hydroxy octadecanoic acids showed extremely small molecule areas of 17–19 Å^2/molecule and stable films, reflecting strong chain interactions due to strong hydrogen bonding. This assumption is corroborated by the higher melting points (75–91°C) of hydroxy octadecanoic acids as compared to stearic acid (69.5°C).

It has been suggested before that the condensed films of (*R*)- 12- and 17-octadecanoic acids are thermodynamically more stable than the films of the racemic compounds due to a sterically more favorable hydrogen bonding (Tachibana et al., 1979).

Monolayers of the unsaturated hydroxy octadecanoic acids were more expanded compared to saturated hydroxy acids. The film of the ricinelaidinic acid (*trans* conformation) was more expanded than the *cis*-conformer, because the C1 and C12 hydroxy groups of the *cis*-compound are 'out of plane' and cannot form an arched but rather a vertical conformation (Nagarajan and Shah, 1981).

Monolayer films of phospholipids showed properties similar to those of the corresponding fatty acids. Thus DSPC, a saturated long-chain diacylglycerophosphocholine, formed condensed monolayer films at room temperature in the same way as stearic acid. Above 20°C, diacylglycerophosphocholines with hydroxy substituted acyl chains exhibited a strong tendency to form expanded phases (Figs. 3 and 4), similar to their hydroxy octadecanoic acid building blocks. If compared to DSPC, the diacylphosphatidylcholines with hydroxy substituted acyl chains had bigger lift-off areas, an extensive phase coexistence area of the fluid expanded and fluid condensed phase and a highly condensed solid phase with smaller molecular areas and film pressures. In both series of isomers, lift-off values increased by moving the hydroxy group from the carboxyl function to the end of the molecule (Table 2). As Menger et al. (1989) determined a similar type of phase characteristics for the 1,2-diacylphosphatidylcholines of isomeric oxo octadecanoic acids which was attributed to the formation of arched molecules, our results seem to confirm that PC-derivatives of hydroxy- or oxooctadecanoic acids form arched conformations. However, the 'arch', which is formed in the fluid expanded condition of the choline head group and the hydroxy group is more stable due to stronger chain–water-interactions. Furthermore the PC-derivatives of ricinoleic acid and ricinelaidinic acid possessed a more or less distinct phase coexistence area at 20°C, indicating that even the PC-derivatives of ricinoleic acid can develop arched molecules on the subphase in contrast to their fatty acid building blocks. This might be due to the greater distance of the two polar groups, allowing for a wider variety of conformations.

The PC-derivatives of the (*R*)-fatty acids exhibited larger expanded phases than the derivatives of the racemic fatty acids, suggesting reduced hydrogen bonding due to sterical hindrance. In contrast to DSPC, we found small molecule areas at the collapse point for the PCs constituted of hydroxy octadecanoic acids (38–41 Å^2/molecule) which are probably due to intermolecular hydrogen bonding. This conclusion is confirmed by the fact that the PCs of the (*R*)- 12- and 17-hydroxy octadecanoic acids were more expanded and had a higher film pressure at the transition point at the same molecule area than the corresponding racemic PC-derivatives. It is obvious that the formation of chain-water-interactions and hydrogen bonding is less favorable for PCs with two optically active acyl chains and hydroxy groups in the same orientation than for PCs with irregularly oriented hydroxy groups.

4.3. Differential scanning calorimetry investigations of diacylphosphocholines containing hydroxy octadecanoic acids

The thermally induced transition of phospholipid bilayers from the gel to the liquid-crystalline phase was monitored by differential scanning calorimetry (DSC). We concentrated principally on the 'main transition', indicating the 'melting' of the phospholipid-acyl chains and the effect of the hydroxy group position on their molecular packing. The DSC heating thermogram of DSPC shows a sharp transition at $T_m = 55.6$°C. The introduction of the hydroxy group (except D3HSPC) leads to the stabilization of the gel-crystalline phase, probably by inter- and intramolecular dipole–dipole-interactions. Therefore, the phosphocholines with hydroxylated fatty acids show increased T_m and ΔH values (Table 4) (Menger et al., 1988). The T_m values of saturated diacylglycerophosphocholines increased stepwise as the position of the hydroxy group moved from the glycerol backbone towards the terminal part of the acyl chain. The dependence of T_m from the position of the hydroxy group is illustrated in Fig. 6. All phospholipids exhibited just one direct transition from a lamellar-gel to a fluid lamellar phase without the formation of a ripple phase (P_β). The

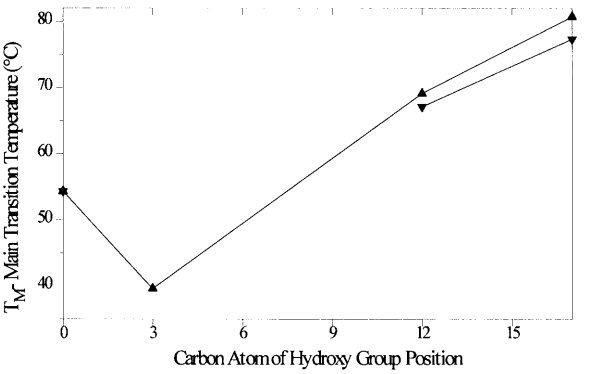


Fig. 6. Dependence of main transition temperature T_m on hydroxy group position of phospholipids for chiral phospholipids (▲) and racemic phospholipids (▼).

appearance of membrane ripples in diacylglycerophosphocholine bilayers is explained by lamellar-to-periodic gel transition. In a structural model it is inferred that hydrocarbon chains have to slide along each other freely in order to form a ripple phase. Hydroxylated acyl chains, stabilized through hydrogen bonds, are less able to slide along and thus phospholipids containing hydroxy substituted acyl chains may not be able to form undulating bilayers.

We showed in our monolayer investigations that hydrogen-bonding in racemic PC-derivatives, due to their irregularly placed hydroxy groups, is stronger than in diacylglycerophosphocholines containing chiral hydroxy fatty acids. This notion was confirmed by our DSC measurements. In comparison with D12HSPC and D17HSPC, the chiral derivatives showed lower T_m (ca. 2–3°C) and lower ΔH values (2–4 kJ/mol).

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