Pergamon

Tetrahedron 57 (2001) 10219–10227

TETRAHEDRON

Synthesis of phosphatidylcholines containing ricinoleic acid

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Received 3 July 2001; revised 25 September 2001; accepted 18 October 2001

Abstract—1,2-Diricinoleoyl- and 1-ricinoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine were synthesised with good yields. The synthesis started with the preparation of ricinoleic acid from castor oil. The choice of a suitable agent to protect the –OH group of ricinoleic acid was a key factor to afford the final products. Several protecting groups were assayed but only β-methoxyethoxymethyl chloride (MEMCl) and 2,2,2-trichloroethyl chloroformate (TRECCl) gave reasonable yields and good optical purities of the final products. The overall yields for 1,2-diricinoleoyl-*sn*-glycero-3-phosphocholine and 1-ricinoleoyl-2-oleoyl-*sn*-glycero-3-phosphocholine were 32.1% (with respect to ricinoleic acid methyl ester using TREC as protecting group) and 10.3% (with respect to 1-trityl-glycero-3-phosphocholine), respectively.

1. Introduction

Ester derivatives of glycero-phosphocholines are key intermediates of oil metabolism in plants. Ricinoleic acid is an unsaturated hydroxylated acid which constitutes about 85–90% of the fatty acids fraction (castor oil) stored in mature *Ricinus communis* seeds.[1] Although castor oil is one of the most relevant plant commodities for non-food application,[2] ricinoleic acid in vivo metabolism is not yet fully understood and metabolites are difficult to isolate. Diricinoleoyl-glycero-3-phosphocholine is an obligatory intermediate in oil formation in developing seeds, being the substrate of important regulatory enzymes such as specific endoplasmatic acyl- and phospho-transferases and phospholipase A.[3,4] 1-Ricinoleoyl-2-oleoyl-glycero-3-phosphocholine, the actual substrate of the enzyme oleate-β-12-hydroxylase,[5] is the key enzyme of ricinoleic acid synthesis in *R. communis*. The enzyme specifically promotes the hydroxylation of the oleoyl moiety in position 2 of the diacyl-glycero-3-phosphocholine, forming the corresponding ricinoleoyl group. The aim of this contribution is to define a synthesis of those enzymatic substrates which may be useful to biochemists to improve knowledge on plant metabolic pathways of uncommon fatty acids.

A key step of the synthesis is the suitable protection of the ricinoleic acid hydroxyl group. Fröling[6] was able to synthesise well defined oligoricinoleoyl oligoglycerols. The secondary –OH of ricinoleic acid was successfully protected with *t*-butyl dimethylchlorosilane. It was then possible to carry on the specific esterification of (oligo)-glycerols with protected (oligo)ricinoleic acids.

Negelmann et al.[7] proposed an elegant synthesis of hydroxy fatty acid derivatives of glycero-phosphocholines. The author used the same protecting group of the ricinoleic –OH as Fröling did. The protection successfully withstood the alkaline conditions used to complete the synthesis of the glycero-phosphocholines. However, the final yield of the diricinoleoyl-glycero-3-phosphocholine was low (9%).

Following previous findings,[8] the present work has improved the method for preparing the diricinoleoyl-glycero-3-phosphocholine (final yield 32.1%) by changing the protecting groups and the reaction conditions. For instance, the hydrolysis of the ricinoleic methyl ester, protected with a group sensitive to strong alkaline conditions, was accomplished by enzymatic catalysis in mild conditions (neutral pH, 35°C). Moreover, in this work the synthesis of a mixed type of glycero-phosphocholine (1-ricinoleoyl-2-oleoyl-glycero-3-phosphocholine) it is also presented, which, to our knowledge, has not been reported yet.

2. Results and discussion

The synthesis of 1,2-di-[(*R*)-12-hydroxy-octadec-9-enoyl])-*sn*-glycero-3-phosphocholine (diricinoleoyl-glycero-3-phosphocholine) is summarised in Scheme 1. The synthesis of glycero-phosphocholines containing ricinoleoyl group(s) has not been described in the literature until recently.[7,8] The difficulty of synthesising these compounds is related to the

Keywords: ricinoleic acid; glycero-phosphocholine; OH-protection.
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Abbreviations: CDI, *N*,*N*-carbonyl diimidazole; DCC, *N*,*N*′-dicyclohexyl carbodiimide; DEE, diethyl ether; DIPEA, *N*,*N*-diisopropyl-*N*-ethylamine; EMK, ethylmethylketone; GPC, *sn*-glycero-3-phosphoryl choline; MEMCl, β-methoxyethoxymethyl chloride; PPTS, pyridinium-*p*-toluensulfonate; PPyr, pyrrolidinopyridine; pyr, pyridine; TRECCl, 2,2,2-trichloroethyl-chloroformate; tritylCl, triphenylmethyl chloride.

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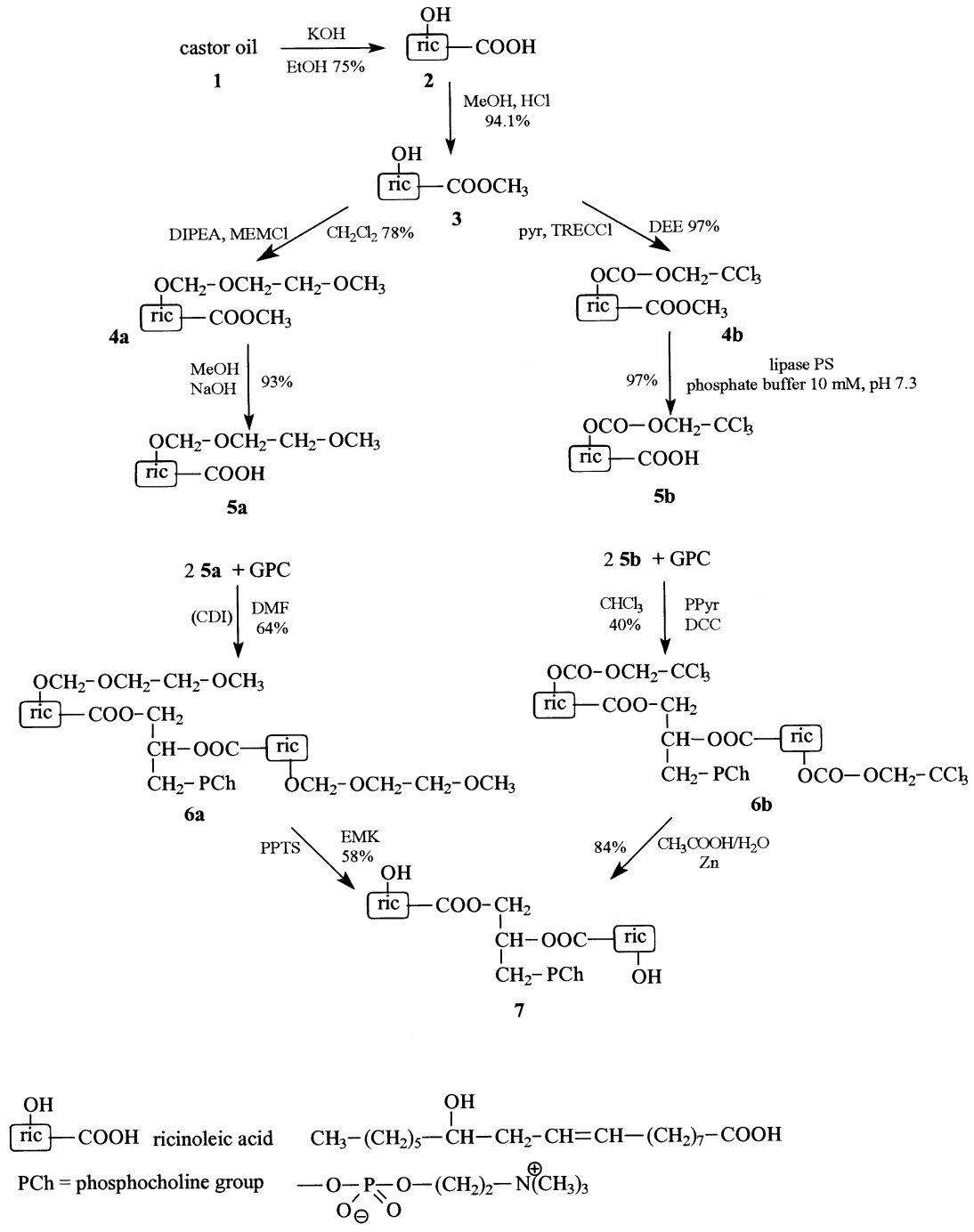
PII: S0040-4020(01)01057-2

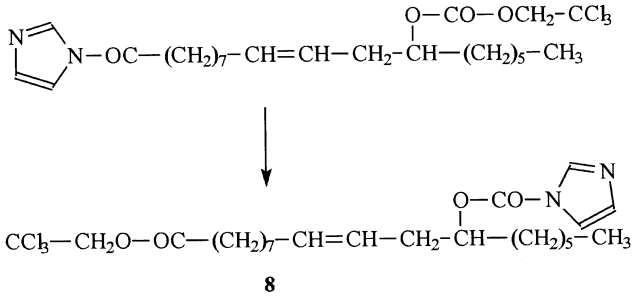


Scheme 1. Synthesis of 1,2-di-[(*R*)-12-hydroxy-octadec-9-enoyl]-*sn*-glycero-3-phosphocholine.

presence of the 12-OH group of the ricinoleic acid. As a matter of fact, direct reaction between L-glycero-3-phosphorylcholine (GPC) and ricinoleic acid following the procedure described in the literature for saturated fatty acids,[9] does not yield the desired product. Instead of diricinoleoyl-glycero-3-phosphocholine only condensation products between the 12-OH group and carboxylic acid groups were observed. Therefore it was necessary to protect the 12-OH group before the acylation of GPC.

Ricinoleic acid (**2**) was obtained by alkaline hydrolysis of castor oil (**1**). By carrying out the hydrolysis according to standard procedures[10] the purity of **2** does not usually exceed 90%. Further purification (>96%) was achieved by extraction with a suitable solvent (for instance diethyl ether or THF), added to the dry low-purity product just enough to form a paste. The $[\alpha]_D$ agreed well with the literature data.[7] Complete removal of the residual impurities was easily accomplished in the next synthetic step, the methyl ester formation.

Several –OH protecting groups were assayed without success. For example, chloroacetylchloride Cl–CH_2–CO–Cl was a promising group since activation of the free acid with imidazole and protection afforded high yields (>80%). However, GPC acylation gave poor results because the



Scheme 2. Transesterification and transamidation within the *O*-TREC-protected ricinoleic acid.

protecting group was partially removed under the reaction conditions.

Chloromethyl-methylether $Cl–CH_2–OCH_3$ is another protecting group which was supposed to be easily removable. The protection was carried out as described in the literature with formaldehyde dimethyl acetal in the presence of P_2O_5.[10] Unfortunately, the reaction yield was low (<30%).

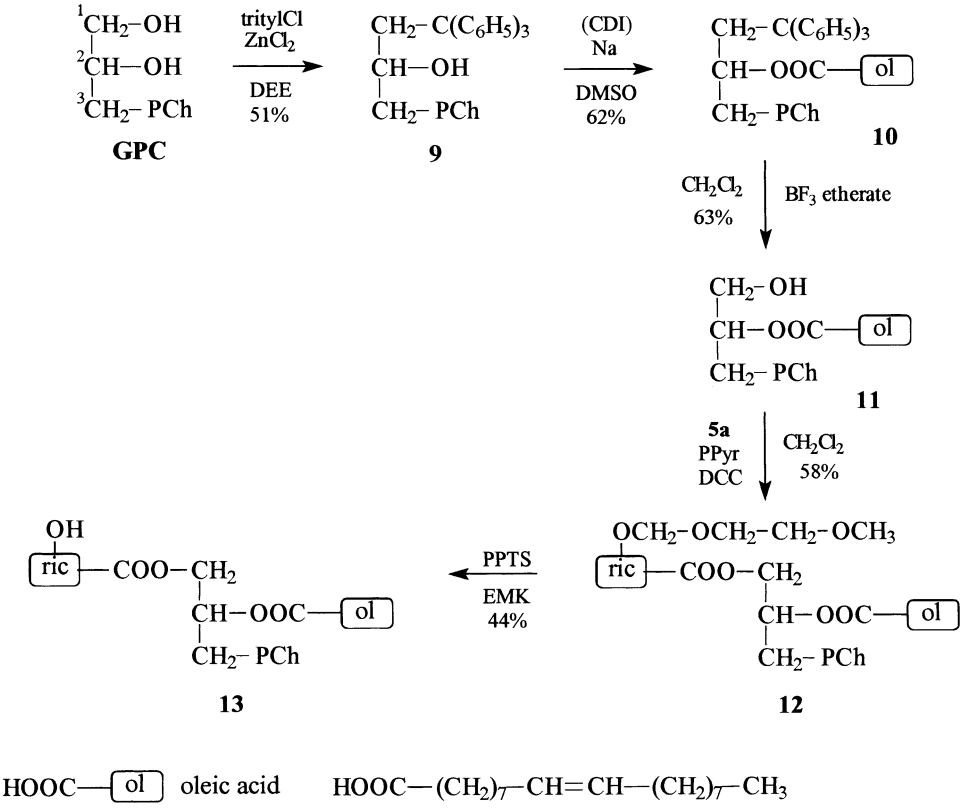
A good yield (97%) of the protected ester was obtained with 2,2,2-trichloroethyl chloroformate (TREC). Unfortunately, in the following acylation step, performed in DMF in the presence of an anhydrous carbonate, the acylation yield was poor (<2%) even if the protection was not removed. The reason of such low yield was due to the concurrent transesterification and transamidation reaction within the protected ricinoleic acid derivative, leading to the formation of unreactive compound **8**, as assessed by GC–MS and NMR analysis (Scheme 2). However, TREC can be still conveniently used if different acylation conditions are chosen.

The best results were obtained with β-methoxyethoxymethyl chloride (MEMCl) and TREC group (if used with specific acylation conditions), affording a reaction yield of 78 and 97%, respectively. The use of each protecting agent, MEM or TREC, has both positive and negative aspects. The *O*-MEM derivative is stable under strong alkaline conditions. Consequently, the methyl ester **4a** can be readily hydrolysed with NaOH. On the other hand, MEM removal at the end of the synthesis is difficult due to its intrinsic stability and can be only accomplished over a long period of time (several hours) causing a concurrent partial degradation of the compound of interest (**7**). A chromatographic step is then needed to purify the remaining product. Conversely, the TREC protecting group is not stable in strong alkaline conditions. The hydrolysis of ester **4b** cannot be done in the presence of NaOH and has to be carried on with specific non-chemical methods such as enzymatic catalysis. Several hydrolytic enzymes were assayed but only the lipase from *Pseudomonas cepacea* readily hydrolyses **4b** with high yields (98–99%). At the end of the synthesis, unlike MEM, TREC can be easily removed without degradation of the final product **7**. It should be noted that the final yield (with respect to ricinoleoyl methyl ester **3**) of the 'TREC synthetic route' (Scheme 1) is slightly higher (32.1%) than the 'MEM route' (27.2%).

The GPC acylation with the protected acid was first assayed using the conditions described in the literature.[11] Anhydride fatty acid derivatives are able to acylate GPC with good yields in the presence of 4-dimethylaminopyridine or 4-pyrrolidinopyridine. The drawbacks of this reaction are that a large molar excess of the anhydride with respect to the OH group is usually necessary and that half of the anhydride group is lost as acid. A more convenient method is that described by Warner and Benson.[12] The fatty acid is activated by preparing the corresponding imidazolyl derivative. Acylation is then carried out in the presence of $NaCH_2SOCH_3$ and DMSO with a slight molar excess of the activated acid. However, also in this case there are some disadvantages, such as the difficulty of DMSO removal (which may be accomplished at high temperatures where product degradation may occur) and the presence of $NaCH_2SOCH_3$ which induces a complete destruction of the TREC protection. Because of these difficulties, the GPC acylation reaction was further improved as follows: the reaction was still carried out with the imidazolyl-derivative of the *O*-protected ricinoleic acid, but DMF (easier to remove) was used as solvent instead of DMSO. Furthermore, the reaction was optimised by adding anhydrous carbonate such as Na_2CO_3, K_2CO_3 or Cs_2CO_3 (molar ratio carbonate/GPC 5:1). These bases are mild, did not hydrolyse the *O*-TREC group and gave the best yields (64%) also with the *O*-MEM derivative.

The final removal of –OH protecting group is the last critical step of the synthesis. MEM removal was the most difficult to achieve. For example, the hydrolysis of MEM with $ZnBr_2$ or $TiCl_4$ as catalysts[13] afforded only degradation by-products, all containing some halogen atoms. Better results were obtained by using the complex of cathecol with BBr_3,[14] but the overall yield was poor. The best way to remove the MEM protecting group is the method described by Monti et al.[15] As suggested by the authors, the use of pyridinium *p*-toluenesulfonate dissolved in ethylmethylketone (EMK) was effective. Although the long reaction time (40 h) at high temperature induced some degradation of **7**, nevertheless, after purification, the final product **7** was recovered with a reasonable yield (58%) and good optical purity. Unlike MEM, TREC protecting group is more easily removable. The best method consisted on the use of Zn in CH_3COOH[16] (reaction yield 84%). Compound **7** was obtained with high purity and could be used without further purification.

The synthesis of 1-(12-hydroxy-octadec-9-enoyl)-2-(octadec-9-enoyl)-*sn*-glycero-3-phosphocholine (1-ricinoleoyl-2-oleoyl-GP) is summarised in Scheme 3. The synthesis follows the procedures described in the literature for fatty acids without hydroxyl groups.[17] A key point was the selection of a protecting group of the 1-OH of GPC. Since protection with MEM (as suggested by the elegant synthesis of phosphatidylcholines containing very long chain polyunsaturated fatty acids)[18] was not satisfactory, triphenylmethyl chloride (tritylCl) was used as protecting agent of the glycerol 1-OH group[19] (reaction yield 51%). TritylCl is large enough to react with only the 1-OH group of glycerol without limiting the accessibility of the 2-OH to the incoming activated oleic acid (yield 62%). Moreover, the group was efficiently removed since good yield (63%) of the deprotection reaction was observed.



Scheme 3. Synthesis of 1-(12-hydroxy-octadec-9-enoyl)-2-(octadec-9-enoyl)-*sn*-glycero-3-phosphocholine.

It should be noted that under the conditions (boron trifluoride etherate in methylene chloride) described in the literature,[17] the deprotection reaction should have been concurrent to the acylation reaction in the presence of ricinoleic acid anhydride. Only deprotection was observed and consequently the acylation of **11** was carried on with DCC and pyrrolidinopyridine, as described.

Since migration between position 1 and 2 may have occurred in the deprotected sample, position 2 being thermodynamically favoured with respect to 1, the isomer distribution was controlled by selective hydrolysis by phospholipase A_2 of the ester in position 2. Good yield (>95%) of the correct isomer confirmed the positional selectivity afforded by the synthesis.

In conclusion, the synthesis of phosphocholines containing ricinoleic acid was accomplished with reasonably good yields, improving significantly (yield 32.1%) the literature results (9%)[7] in the case of diricinoleoyl-glycero-3-phosphocholine. The choice of the protecting groups and the modulation of the reaction media were the two main factors of the yield improvements. These may be significant enough to prepare the radiolabelled counterparts of these compounds for specific biological studies of castor oil metabolism.

3. Experimental

3.1. General methods

Chemicals. L-α-Glycerophosphorylcholine (GPC) was a generous gift from CHEMI s.p.a. (Patrica, FR, Italy). Castor oil and phospholipase A_2 obtained from bee venom (1220 U/mg) were from Sigma (Milano, Italy). *Pseudomonas cepacea* lipase PS (1000 U/mg) was from Amano (Nagoya, Japan). Zinc powder was from Merck (Italy).

Molecular mass analysis. Mass analysis was performed with the Electron Spray (ESI) technique by using a Finnigan MAT LCQ spectrometer, ESI (electron spray) ionisation source: sheath gas flow rate 50, spray voltage 5 kV, capillary temperature 210°C, capillary voltage 26 and −35 V (positive and negative ions, respectively) tube lens offset 15 and −45 V (positive and negative ions, respectively). HRMS measurements were performed with a HR Finnigan MAT FAB/MS instrument.

NMR. ^{1}H, ^{13}C and ^{31}P NMR spectra were recorded with Varian VXR 300 instrument. The references for ^{13}C and ^{1}H $CDCl_3$ were $CDCl_3$ at 77.0 and 7.26 ppm, respectively. The detailed assignment of all the hydrogen atoms was completed by recording the COSY spectra. ^{13}C NMR spectra were well resolved, allowing the identification of the signal of all the 44 C atoms.

Polarimetry. The optical rotation (α) was measured with a Perkin–Elmer Polarimeter 241 at the sodium D-line. Concentrations (*c*) are given as g/100 mL solution.

Gas chromatography. Gas chromatographic analysis was performed with a HRGC 5300 gas chromatograph Carlo Erba (Milano, Italy) equipped with a Supelco SPB-5 column. Temperature gradient: from 80 to 300°C, 10°C/min.

3.2. Synthesis of 1,2-di[(*R*)-12-hydroxy-octadec-9-enoyl])-*sn*-glycero-3-phosphocholine (Scheme 1)

3.2.1. (*R*)-12-Hydroxy-octadec-*cis*-9-enylic acid (ricinoleic acid) (2). Two hundred grams of castor oil (**1**) were dissolved in 400 mL of ethanol containing KOH (40 g), refluxed for 15 min and dried under vacuum. The solid residue was finely dispersed and washed twice in diethyl ether and then dissolved in 1 L of iced water acidified with HCl. The oil was extracted with hexane, treated with Na_2SO_4 overnight at 4°C and then dried under vacuum. 150 g (75% yield by weight) of ricinoleic acid (**2**) as colourless oil were obtained, 97% pure (from GC analysis after esterification with diazomethane, the major contaminants were: linoleic acid 0.3%, oleic acid 1.3%, stearic acid 0.4%). $[\alpha]_D^{20}=+3.9°$ (c=1.0, $CHCl_3$), data consistent with literature ($[\alpha]_D^{20}=+3.8°$).[7]

3.2.2. (*R*)-12-Hydroxy-octadec-*cis*-9-enylic acid methyl ester (ricinoleic acid methyl ester) (3). Into a solution of **2** (100 g, 335 mmol) in methanol (300 mL) at 0°C, gaseous HCl (30 g) was bubbled under stirring at room temperature. After 30 min water (500 mL) and then hexane (200 mL) were added. The extract phase was washed twice with water, once with $NaHCO_3$ (10% w/v) and then treated with Na_2SO_4. After drying the residue under vacuum, 106 g of **3** were obtained (purity by GC: 96%). Further purification was achieved by silica gel chromatography. A column (80×400 mm) was eluted with hexane/acetone 90:10. After evaporation of the solvent from the fractions containing the methyl ester, 98.5 g (315 mmol, yield 94.1%) of pure **3** as a colourless oil were obtained (purity: 99.9% by GC). [Found: C, 73.30; H, 11.80. $C_{19}H_{36}O_3$ requires C, 73.01; H, 11.72]. $[\alpha]_D^{23}=+3.4$ (c=1.5, $CHCl_3$), result consistent with literature ($[\alpha]_D^{20}=+3.4°$).[7] ν_{max} (liquid film, neat) 3360 [$(OH)_{ass}$], 3010 [(═CH)], 2924 and 2852 [(CH_2, CH_3)], 1743 [(C═O)], 1192 and 1168 [$(C–O)_{ester}$], 724 [$\rho(CH_2)_{3\ chain}$]. δ_H (300 MHz, $CDCl_3$): 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, C2–CH_2). 2.27 (t, J=7.5 Hz, 2H, C″–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, C10CH).

3.2.3. (*R*)-12-*O*-MEM-octadec-*cis*-9-enylic acid methyl ester (ricinoleic acid methyl ester-*O*-MEM) (4a). To a solution of **3** (38 g, 121.6 mmol) and *N*,*N*-diisopropyl-*N*-ethylamine (DIPEA, 25 mL, 137 mmol) in CH_2Cl_2 (200 mL), MEMCl (20 g, 160 mmol) was slowly added at 0°C in 2 h. The solution was stirred at room temperature overnight. The reaction mixture was diluted with HCl (20% in water), washed with water and with $NaHCO_3$ (10% w/v). After the addition of Na_2SO_4, the product **4a** (38 g, 94.9 mmol, yield 78.01%) was dried under reduced pressure as a colourless oil. [Found: C, 68.81; H, 10.95. $C_{23}H_{44}O_5$ requires C, 68.95; H, 11.08]. $[\alpha]_D^{23}=+21.3$ (c=2.5, CH_3COCH_3). ν_{max} (neat liquid film) 3010 [(═CH)], 2920 and 2860 [(CH_2, CH_3)], 2818 [$(OCH3)_{MEM}$], 1742 [(C═O)], 1460, 1430, 1192 and 1168 [$(C–O)_{ester}$], 1110 [$(C–O–C)_{MEM}$], 1040 [$(O–C–C)_{MEM}$], 850 [$(C–O–C)_{MEM}$], 723 [$\rho(CH_2)_{3\ chain}$]. δ_H (300 MHz, $CDCl_3$): 0.78 (3H, t, J=6.5 Hz, C18–CH_3), 1.02–1.34 (16H, m, C(4–7)– CH_2, C(14–17)– CH_2), 1.34–1.45 (2H, m, C13–CH_2), 1.45–1.65 (2H, m, C3–CH_2), 1.82–2.05 (2H, m, C8–CH_2), 2.17 (2H, m, C11–CH_2), 2.20 (2H, t, J=8.5 Hz, C2–CH_2), 3.29 (3H, s, OCH_{3MEM}), 3.56 (3H, s, OCH_3), 3.40–3.68 (4H, m, OCH_2CH_2O), 4.57–4.73 (2H, m, OCH_2O), 5.20–5.45 (2H, m, C9–CH═, C10–CH═).

3.2.4. (*R*)-12-*O*-TREC-octadec-*cis*-9-enylic acid methyl ester (ricinoleic acid methyl ester-*O*-TREC) (4b). To the reaction mixture, containing **3** (23 g, 80.06 mmol), diethyl ether (DEE, 150 mL), pyridine (10 mL), 2,2,2-trichloroethyl chloroformate (TREC-Cl, 18 g, 84,9 mmol) was slowly added in 2 h at 5°C. After few hours at room temperature, hexane (100 mL) was added and the pyridine HCl was filtered off. The product was dried under reduced pressure and then under high vacuum at 50°C. Pure product **4b** (37.8 g, 77.7 mmol, yield 97.1%) was obtained as a colourless viscous oil. [Found: C, 54.21; H, 7.77; Cl, 21.44. $C_{22}H_{37}Cl_3O_5$ requires C, 54.30; H, 7.67; Cl, 21.58]. $[\alpha]_D^{23}=+19.4°$ (c=4.4, $CHCl_3$). ν_{max} (neat, film) 3010 [(═CH)], 2925 and 2860 [(CH_2, CH_3)], 1742 [$(C═O]_{ester}$, 1755 [$(C═O)]_{TREC}$, 1460, 1430, 1370, 1250 [(O–CO–O)], 1070, 1020, 820 [$(CCl)_3$], 785 [$\pi(O–CO_2]$, 730 [$(CCl)_3$]. δ_H (300 MHz, $CDCl_3$): 0.81 (3H, t, J=6.5 Hz, C18–CH_3), 1.05–1.40 (16H, m, C(4–7)–CH_2, C(14–17)–CH_2), 1.4–1.68 (4H, m, C3–CH_2, C13–CH_2), 1.85–2.07 (2H, m, C8–CH_2), 2.23 (2H, t, J=7 Hz, C2–CH_2), 2.31 (2H, dd, J=13, 7 Hz, C11–CH_2), 3.59 (3H, s, OCH_3), 4.60–4.80 (3H, m, $COCH_2$+CH–C12), 5.20–5.50 (2H, m, C9–CH═, C10–CH═).

3.2.5. (*R*)-12-*O*-MEM-octadec-*cis*-9-enylic acid (ricinoleic acid *O*-MEM) (5a). The product **4a** (38 g, 94.9 mmol) was dissolved in methanol (100 mL) and NaOH–water (20 g, 500 mmol, in 100 mL). After few hours the hydrolysis was complete. The reaction mixture was diluted with water (500 mL) and acidified with HCl (20%). The product was extracted with hexane. The extract was washed with water, treated with Na_2SO_4 and dried under reduced pressure. Pure ricinoleic acid *O*-MEM-protected **5a** (34.1 g, 88.27 mmol, yield 92.99%) was obtained (purity 99% according to TLC and NMR analysis) as a colourless oil. [Found: C, 68.21; H, 11.12. $C_{22}H_{42}O_5$ requires C, 68.34; H, 10.96]. ν_{max} (neat liquid film) 3400–2500 br[$(OH)_{ass}$)], 3009 [(═CH)], 2921 and 2861 [(CH_2, CH_3)], 2818 [$(OCH3)_{MEM}$], 1710 [$(C═O)_{acid}$], 1410, 1130 and 1110 [$(C–O–C)_{MEM}$], 1040 [$(O–C–C)_{MEM}$], 850 [$(C–O–C)_{MEM}$], 723 [$\rho(CH_2)_{3\ chain}$]. δ_H (300 MHz, $CDCl_3$): 0.88 (3H, t, J=7 Hz, C18–CH_3), 1.05–1.38 (16H, m, C(4–7)–CH_2, C(14–17)–CH_2), 1.38–1.52 (2H, m, C13–CH_2), 1.52–1.72 (2H, m, C3–CH_2), 1.86–2.10 (2H, m, C8–CH_2), 2.10–2.28 (2H, m, C11–CH_2), 2.30 (2H, t, J=7.5 Hz, C2–CH_2), 3.35 (3H, s, OCH_3), 3.45–3.80 (4H, m, OCH_2CH_2O), 3.60 (1H, m, C12–CH), 4.60–4.90 (2H, m, OCH_2O), 5.25–5.50 (2H, m, C9–CH═, C10–CH═).

3.2.6. (*R*)-12-O-TREC-octadec-*cis*-9-enylic acid (ricinoleic acid *O*-TREC) (5b). The hydrolysis of the ester **4b** was accomplished by using enzymes as catalysts. The best results were obtained with lipase PS from *Pseudomonas cepacea*, a commercial product from Amano (Japan). Compound **4b** (25 g, 51.4 mmol) was suspended in phosphate buffer 10 mM pH 7.3 (300 mL) under vigorous

agitation and lipase PS (4 g) was added. The reaction was carried on for 24 h at 35°C. The pH was kept constant by means of the continuous addition of 0.5 M NaOH in a automatic titration device (pH stat). The reaction mixture was then acidified with HCl 20% and the product was extracted with hexane, washed with water, treated with Na_2SO_4 and dried under reduced pressure. This procedure yielded 23.6 g of **5b** as a viscous oil (49.9 mmol, yield 97.2), containing 1–2% of the starting substrate **4b**. The product may be used without further purification in the next synthetic step, otherwise may be purified to homogeneity by silica gel chromatography (solvent for elution: hexane/acetone 85/15). [Found: C, 53.51; H, 7.37; Cl, 21.99. $C_{21}H_{35}Cl_3O_5$ requires C, 53.37; H, 7.47; Cl, 22.22]. ν_{max} (neat, film) 3400–2500 [br$(OH)_{ass}$], 3010 [(=CH)], 2920 and 2861 [(CH_2, CH_3)], 1755 [$(C{=}O)_{TREC}$], 1710 [$(C{=}O)_{acid}$], 1370, 1250 [(O–CO–O) and $(C–O)_{acid}$], 820 [$(CCl)_3$], 785 [$\pi(O–CO_2$], 730 [$(CCl)_3$]. δ_H (300 MHz, $CDCl_3$): 0.83 (3H, t, J=7 Hz, C18–CH_3), 1.00–1.38 (16H, m, C(4–7)–CH_2, C(14–17)–CH_2), 1.38–1.69 (4H, m, C3–CH_2, C13–CH_2), 1.88–2.03 (2H, m, C8–CH_2), 2.12 (2H, t, J=7 Hz, C2–CH_2), 2.15–2.52 (2H, m, C11–CH_2), 4.51 (2H, s, $COCH_2$), 4.86 (1H, m, CH–C12), 5.30–5.55 (2H, m, C9–CH=, C10–CH=).

3.3. 1,2-Di-(*O*-MEM-octadec-9-enoyl)-*sn*-glycero-3-phosphocholine (6a)

The acylation of *sn*-glycero-3-phosphorylcholine (GPC) was assayed by using two methods: the first method consisted on forming in situ an anhydride intermediate with *N*,*N*′-dicyclohexylcarbodiimide (DCC), whereas the second one consisted on the activation of the ester with *N*,*N*-carbonyldiimidazole (CDI).

3.3.1. Synthesis of 6a with DCC. Under nitrogen atmosphere, methanol (50 mL), **5a** (15.5 g, 40.12 mmol) and GPC (2,57 g, 10.0 mmol) were mixed until complete dissolution was obtained. Methanol was removed under reduced pressure and the residue heated several hours at 65°C. Chloroform (30 mL) was added and then a solution of 4-pyrrolidinopyridine (PPyr, 6 g, 40.4 mmol in 20 mL chloroform) and DCC (8.3 g, 40.2 mmol) was added slowly (30 min) at 45°C. The reaction was allowed to proceed overnight under stirring. The mixture was diluted with diethyl ether, the salt filtered and the solvent evaporated under reduced pressure. The residue was dissolved in chloroform/methanol 1:1 and the solution was purified with a column of Amberlist 15 to eliminate 4-pyrrolidinopyridine. The product was dried and dissolved in chloroform and purified by silica gel chromatography: the product was eluted first with $CHCl_3/CH_3OH/H_2O$ 65:25:2 and then by gradually increasing the water content from 2 to 4. After drying the fractions containing the product, 4.6 g (4.63 mmol, yield 23.0%) of pure **6a** as white waxy paste were obtained. [Found: C, 62.70; H, 10.29; N, 1.37; P, 3.06. $C_{52}H_{100}NO_{14}P$ requires C, 62.80; H, 10.14; N, 1.41; P, 3.12]. $[\alpha]_D^{22}$=+20.2° (c=1.75, $CHCl_3$). ν_{max} (neat) 3009 [(=CH)], 2926 and 2855 [(CH_2, CH_3)], 1742 and 1730 [$(C{=}O)_{overlap}$], 1466, 1365, 1240 [(P=O)], 1199 and 1171 [$(C–O)_{ester}$], 1135 [$(C–O–C)_{MEM}$], 1091 and 1045 [$(P–O–C)_{choline}$ and $(O–C–C)_{MEM}$], 970. δ_H (300 MHz, $CDCl_3$): 0.82 (6H, t, J=7 Hz, C18–CH_3, C36–CH_3), 1.03–1.35 (32H, m, C(4–7)–CH_2, C(14–17)– CH_2, C(22–25)–CH_2, C(32–35)–CH_2), 1.35–1.46 (4H, m, C13–CH_2, C31–CH_2), 1.46–1.65 (4H, m, C3–CH_2, C21–CH_2), 1.73–2.08 (4H, m, C8–CH_2, C26–CH_2), 2.08–2.36 (8H, m, C2–CH_2, C20–CH_2,C11–CH_2, C29–CH_2), 3.30 (6H, s, –OCH_3), 3.31 (9H, s, $N(CH_3)_3$), 3.40–3.70 (8H, m, OCH_2CH_2O), 3.55 (2H, m, C12–CH, C30–CH), 3.85 (2H, m, CH_2–N), 3.92 (2H, m, glycerol-C3), 4.05 (1H, dd, J=12, 7 Hz, glycerol-C1–H_b), 4.24 (2H, m, PO–OCH_2), 4.35 (1H, dd, J=12, 2.5 Hz glycerol-C1–H_a), 4.60–4.80 (4H, m, O–CH_2–O), 5.15 (1H, m, glycerol-C2–H), 5.25–5.50 (4H, m, C9–CH=, C10–CH=, C27–CH=, C28–CH=). m/z 1016 $[MNa]^+$ (100%), 2009 $[2M+Na]^+$, 957 $[MNa-N(CH_3)_3]^+$; m/z 978 ($[M-CH_3]^-$ 100%), 907 $[(M-CH_3)- C_4H_9N]^-$, 385 [Ricinol. *O*-MEM–H ac.]$^-$.

3.3.2. Synthesis of 6a with CDI. The reaction was performed after activation of the acid with *N*,*N*-carbonyldiimidazole (CDI), as follows. **5a** (8 g, 20.70 mmol) was dissolved in anhydrous THF (20 mL) under a gentle stream of nitrogen. CDI (6.6 g, 40.7 mmol) was then added slowly, allowing the temperature to rise. After the completion of the reaction (end of CO_2 production), THF was evaporated under reduced pressure. To the residue, the 'activated' ricinoleic acid O-protected (9 g, 20.58 mmol, yield 99%), GPC (2,57 g, 10 mmol), DMF (20 mL) and K_2CO_3 (4 g) were added. The mixture of reactants and solvent was stirred for 24 h at room temperature. Diethyl ether was added to dilute the suspension, then the salts were filtered off and the products were dried under reduced pressure and high vacuum. The residue was dissolved in chloroform and purified as described earlier (Section 3.3.1). After drying the fractions containing the product, 6.6 g (6.54 mmol, yield 64.2%) of pure **6a** were obtained. $[\alpha]_D^{22}$=+20.2 (c=1.75, $CHCl_3$).

3.3.3. 1,2-Di-(12-*O*-TREC-octadec-9-enoyl)-*sn*-glycero-3-phosphocholine (6b). Acylation of GPC with *O*-TREC ricinoleic acid was assayed only with the method of anhydride formed in situ, as described in Section 3.3.1 in the case of the *O*-MEM derivative (the method with DMF and K_2CO_3 induced the unwanted rearrangement shown in Scheme 2). The reaction was carried on by mixing GPC (1.8 g, 7.0 mmol), adduct **5b** (3.3 g, 6.78 mmol), 4-pyrrolidinopyridine (PPyr, 4.15 g, 28 mmol) and DCC (5.8 g, 28.1 mmol) in chloroform. After purification by silica gel chromatography, 3.2 g (2.74 mmol, yield 40.5%) of **6b** as white waxy paste were obtained. [Found: C, 51.60; H, 7.31; N, 1.37; P, 2.60; Cl, 17.85. $C_{50}H_{86}NO_{14}Cl_6P$ requires C, 51.48; H, 7.44; N, 1.20; P, 2.66; Cl, 18.00]. $[\alpha]_D^{23}$= +20.9° (c=1.2, $CHCl_3$). ν_{max} (neat) 3010 [(=CH)], 2925 and 2860 [(CH_2, CH_3)], 1750 [$(C{=}O)_{TREC}$ and $(C{=}O)_{ester}]_{overlap}$, 1465, 1390, 1240 [(P=O)], 1280 [(O–CO–O)$_{TREC}$], 1175 [$(C–O)_{ester}$], 1090 and 1060 [(P–O–C)], 970 [$\rho(CH_3)_{choline}$], 725 [$(CCl)_3$]. δ_H (300 MHz, $CDCl_3$): 0.84 (6H, t, J=6.5 Hz, C18–CH_3, C36–CH_3), 1.05–1.45 (32H, m, C(4–7)–CH_2, C(14–17)–CH_2, C(22–25)–CH_2, C(32–35)–CH_2), 1.45–1.73 (8H, m, C13–CH_2, C31–CH_2, C3–CH_2, C21–CH_2), 1.88–2.12 (4H, m, C8–CH_2, C26–CH_2), 2.17–2.28 (4H, m, C20–CH_2, C2–CH_2), 2.28–2.48 (4H, m, C11–CH_2, C29–CH_2), 3.35 ppm (9H, s, $N(CH_3)_3$), 3.79 ppm (2H, m, glycerol-C3), 3.90 (2H, m, CH_2–N), 4.09 ppm (1H, dd, J=12, 7 Hz, glycerol-C1–H_b), 4.28 ppm (2H, m, PO–OCH_2), 4.37 ppm (1H, dd,

J=12, 2.5 Hz, glycerol-C1–H_a), 4.75 ppm (2H, m, C12–CH, C30–CH), 4.73 ppm (4H, s, CH_2CCl_3), 5.16 ppm (1H, m, glycerol-C2–H), 5.24–5.55 (4H, m, C27–CH═, C9–CH═, C28–CH═, C10–CH═). *m/z* 1188 $[MNa]^+$, 1190 (100%), 1192, 1194, 1196, 2353 $[2M+Na]^+$, 1129 $[MNa-N(CH_3)_3]^+$; *m/z* 1150 $[M-CH_3]^-$, 1152 (100%), 1154, 1156, 1158, 471 [ricinol. ac. *O*-TREC–H]$^-$.

3.3.4. 1,2-Di-[(*R*)-12-hydroxy-octadec-*cis*-9-enoyl]-*sn*-glycero-3-phosphocholine (7). Deprotection of the derivative **6a** containing MEM protecting group. The reactants, **6a** (2 g, 2.01 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 3 g, 11.9 mmol) were dissolved in ethylmethylketone (EMK) (45 mL) and the solution was refluxed for 42 h. After dilution with diethyl ether, the precipitated material was filtered. The products were recovered after evaporation of the solvent under reduced pressure, dissolved in chloroform and separated with silica gel chromatography. The column was first eluted with $CHCl_3$, then with a stepwise increase of CH_3OH (up to 30% v/v) and finally with addition of water (up to 4%). The fractions containing the product were dried and the residue dissolved in diethyl ether and washed with $NaHCO_3$ (10% w/v). After the addition of Na_2SO_4 and evaporation of the solvent, 0.96 g (1.17 mmol, yield 58.4%) of pure **7** as a waxy solid were obtained.

The overall yields were 27.2% (from ricinoleic acid methyl ester via DMF–carbonate route) and 9.7% (via DCC and pyrrolidinopyridine route), respectively. $[\alpha]_D^{23}=+7.3°$ (c=1.5, $CHCl_3$).

3.3.5. 1,2-Di-[(*R*)-12-hydroxy-octadec-*cis*-9-enoyl]-*sn*-glycero-3-phosphocholine (7). Deprotection of the derivative **6b** containing TREC protecting group. The compound **6b** (1.5 g, 1.28 mmol) was dissolved in acetic acid (15 mL) and water (1.5 mL). Powdered Zn (1.5 g washed with HCl 5% (in order to remove inert oxide), water, methanol, ether and then dried under vacuum) was added in small aliquots for 10 min, keeping the temperature as low as 10–15°C. The mixture was stirred for 2 h at room temperature, poured in water (50 mL) and adjusted to neutrality by adding $NaHCO_3$ (10% w/v). The product was extracted with diethyl ether and then recovered after addition of Na_2SO_4 and evaporation of the solvent. The white solid was then further purified as described in Section 3.3.4. After the addition of Na_2SO_4, and evaporation of the solvent from the fraction of the chromatography column, 0.88 g (1.076 mmol, yield 84.09%) of pure **7**, a waxy white solid, was obtained. On the assumption that the unreacted protected acid (two fold molar excess of the stoichiometric quantity) used in the acylation reaction is fully recovered, the overall was yield 32.1% with respect to ricinoleic acid methyl ester. [Found: C, 64.68; H, 10.21; N, 1.60; P, 3.69. $C_{44}H_{84}NO_{10}P$ requires C, 64.58; H, 10.35; N, 1.71; P, 3.79]. $[\alpha]_D^{23}=+7.3°$ (c=1.5 in $CHCl_3$). ν_{max} (neat) 3600–3100 [$(OH)_{ass}$], 3010 [(═CH)], 2921 and 2852 [(CH_2, CH_3)], 1742 and 1730 [$(C═O)_{ester}$], 1234 [(P═O)], 1162 [$(C–O)_{ester}$], 1088 and 1057 [(P–O–C)], 964 [$\rho(CH_3)_{choline}$]. δ_H (300 MHz, $CDCl_3$): 0.88 (6H, t, J=7 Hz, C18–CH_3, C36–CH_3), 1.19–1.40 (32H, m, C(4–7)– CH_2, C(14–17)–CH_2, C(22–25)–CH_2, C(32–35)–CH_2), 1.40–1.51 (4H, m, C13–CH_2, C31–CH_2), 1.51–1.65 (4H, m, C3–CH_2, C21–CH_2), 1.98–2.10 (4H, m, C8–CH_2, C26–CH_2), 2.19 (4H, t, J=7 Hz, C11–CH_2, C29–CH_2,), 2.27 (2H, t, J=7.5 Hz, C20–CH_2), 2.29 (2H, t, J=7 Hz, C2–CH_2), 3.33 (9H, s, $N(CH_3)_3$), 3.49–3.67 (2H, m, C12–CH, C30–CH), 3.67–3.84 (2H, m, CH_2–N), 3.84–4.03 (2H, m, glycerol-C3–$H_{a,b}$), 4.11 (1H, dd, J=12.2, 7.3 Hz, glycerol-C1–H_b), 4.19–4.34 (2H, m, PO–CH_2), 4.37 (1H, dd, J=12.2, 3 Hz, glycerol-C1–H_a), 5.18 (1H, m, glycerol-C2–H), 5.30–5.60 (4H, m, C27–CH═, C9–CH═, C28–CH═, C10–CH═). δ_{13C} (75.42 MHz, $CDCl_3$):13.96 (C18, C36) (2CH_3), 22.5(2), 24.70, 24.76, 25.61(2), 27.25(2), 28.90, 28.96(4), 29.02(2), 29.25(2), 29.46(2) (19CH_2), 31.73(2), 33.96, 34.13, 35.25(2), 36.72(2) (8CH_2), 54.19 ppm (3C), 59.21 (CH–OH), 62.87 (–CH_2–N), 63.29 (glycerol), 66.17 (glycerol), 70.42 (glycerol), 71.26 (PO–CH_2–), 125.40, 125.43, 132.69, 132.72 CH(9)═CH(10), CH(27)═CH(28),173.06, 173.42 CO(1), CO(13). δ_{31P} (81.0 MHz, $CDCl_3$)=0.95. *m/z* 840 $[MNa]^+$(100%), 1657 $[2M+Na]^+$, 781 $[MNa-N(CH_3)_3]^+$; *m/z*802 $[M-CH_3]^-$ (100%), 731 $[(M-CH_3)-C_4H_9N]^-$, 297 [ricinoleic ac.-H]$^-$. HRMS (3-NBA) MH^+, found 818.5930; $C_{44}H_{85}NO_{10}P$ requires 818.5911.

3.4. Synthesis of 1-(12-hydroxy-octadec-9-enoyl)-2-(octadec-9-enoyl)-*sn*-glycero-3-phosphocholine (Scheme 3)

3.4.1. 1-Trityl-glycero-phosphocholine (1-trityl-GPC) (9). *sn*-Glycero-3-phosphorylcholine (GPC, 26 g, 101 mmol) and $ZnCl_2$ (14 g, 102 mmol) were dissolved in 220 mL of anhydrous DMF and stirred for 3 h at room temperature. Triphenylmethyl chloride (tritylCl, 28.5 g, 102 mmol) was then added at 5°C. The reaction was stirred overnight and diluted with 1 L of diethyl ether. The organic phase was dissolved in $CHCl_3$/isobuthanol 2:1 and washed with NH_4OH (4%). After adding Na_2SO_4, the mixture was filtered and concentrated down to 400 mL. After addition of diethyl ether (2 L), 1-trityl-GPC (**9**) precipitated from solution (25.1 g, 51.8 mmol, yield 51.2%) as a white powder. [Found: C, 66.72; H, 7.45; N, 2.77; P, 6.35. $C_{27}H_{35}NO_5P$ requires C, 66.91; H, 7.28; N, 2.89; P, 6.40]. $[\alpha]_D^{23}=-10.5°$ (c=2.5, CH_3OH). δ_H (300 MHz, $CDCl_3$): 3.16 (9H, s, $N(CH_3)_3^+$), 3.16 (2H, m, glycerol-C3), 3.48–3.65 (2H, m, –CH_2–N), 3.83–4.00 (2H, m, glycerol-C1), 4.00–4.10 (1H, m, glycerol-C2), 4.14–4.30 (2H, m, PO–OCH_2), 7–7.50 (15H, $(C_6H_5)_3$).

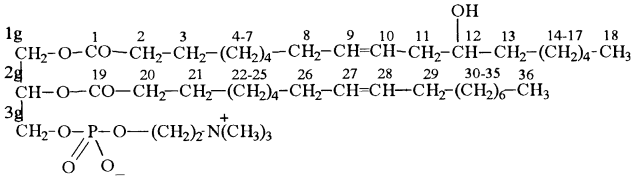
3.4.2. 1-Trityl-2-(octadec-*cis*-9-enoyl)-*sn*-glycero-3-phosphocholine (10). The reaction was carried on after activation of oleic acid with *N*,*N*-carbonyldiimidazole (CDI). Oleic acid (5.64 g, 19.9 mmol) was dissolved in THF and then CDI (3.95 g, 24.3 mmol) was slowly added. After stirring few hours, the solvent was evaporated and the residue suspended in DMSO (25 mL) containing compound **9** (5.1 g, 10.53 mmol). Na (0.69 g, 30 mmol, in 55 mL DMSO) was added and the mixture was stirred for 30 min at 5°C. Acetic acid (63 mL, 1000 mmol) was added to the solution which was further diluted with $CHCl_3$/CH_3OH 2:1 (1.0 L), washed with acetic acid/water (250 mL, 1:1) and concentrated NH_4OH (2.5 mL). The formation of two well separated phases was favoured by methanol (200 mL). The phase containing chloroform was distilled after dilution with toluene and methanol. The residue was dried under vacuum for several hours, dissolved in CH_3Cl and purified by silica gel chromatography. The product was eluted first

with $CHCl_3/CH_3OH/NH_3$ 70:30:2 and then with $CHCl_3/CH_3OH/NH_3$ 50:50:2. The fractions containing the product were dried under reduced pressure and the adduct **10** (4.9 g, 6.54 mmol, yield 62.1%) was recovered as a colourless viscous residue. [Found: C, 72.01; H, 9.26; N, 1.75; P, 4.35. $C_{45}H_{67}NO_6P$ requires C, 72.15; H, 9.02; N, 1.87; P, 4.14]. δ_H (300 MHz, $CDCl_3$): 0.85 (3H, t, J=6 Hz, C36–CH_3), 1.05–1.45 (20H, m, C(22–25)–CH_2, C(30–35)–CH_2), 1.45–1.73 (2H, m, C21–CH_2), 1.82–2.10 (4H, m, C26–CH_2, C29–CH_2), 2.30 (2H, t, J=8 Hz, C20–CH_2), 3.20 (9H, s, $N(CH_3)_3^+$), 3.20 (2H, m, glycerol-C1), 3.5–3.75 (2H, m, –CH_2–N), 3.96 (2H, t, J=6 Hz, glycerol-C3), 4.05–4.25 (2H, m, PO–OCH_2), 5.25 (1H, m, glycerol-C2), 5.28–5.45 (2H, m, C27–CH═, C28–CH═), 7–7.50 (15H, $(C_6H_5)_3$). m/z 786 $[MNa]^+$(100%), 1549 $[2M+Na]^+$, 727 $[MNa-N(CH_3)_3]^+$=243 $[C(C_6H_5)_3]^+$. m/z 748 $[M-CH_3]^-$ (100%), 677 $[(M-CH_3)-C_4H_9N]^-$.

3.4.3. 2-(Octadec-*cis*-9-enoyl)-*sn*-glycero-3-phosphocholine (11). According to literature, deprotection and acylation with *O*-MEM–ricinoleic acid anhydride could be obtained in a one pot reaction.[17] 2.7 g (3.60 mmol) of **10** and 5.5 g (7.3 mmol) of *O*-MEM–ricinoleic acid anhydride (prepared from 5.6 g (14.5 mmol) of *O*-MEM–ricinoleic acid **5a** and 1.5 g (7.3 mmol) of DCC in 50 mL of CCl_4) in CH_2CL_2 (90 mL) were treated at 0°C with BF_3 etherate (3.8 mL). After 1 h the solution was neutralised with 10 g of $NaHCO_3$ suspended in 20 mL of water. Methanol (40 mL) was added and the organic phase separated and evaporated to dryness. The residue was dissolved in $CHCl_3$ and purified by silica gel chromatography. The product was eluted first with $CHCl_3/CH_3OH/H_2O$ 60:40:2 and then the eluent composition was changed to 50:50:4. The recovered product (as a white waxy viscous paste) were 1.2 g (2.29 mmol, yield 63.6%) of 2-oleoyl-GPC (**11**) suggesting that in these conditions deprotection was not followed by the acylation reaction. [Found: C, 59.61; H, 10.39; N, 2.59; P, 5.75. $C_{26}H_{53}NO_7P$ requires C, 59.73; H, 10.23; N, 2.68; P, 5.93]. ν_{max} (neat) 3005 [(═CH)], 2925 and 2854 [(CH_2, CH_3)], 1731 [$(C═O)_{ester}$], 1467, 1232 [(P═O)], 1086 and 1059 [(P–O–C)], 970 [$\rho(CH_3)_{choline}$]. δ_H (300 MHz, $CDCl_3$): 0.85 (3H, t, J=6 Hz, C36–CH_3), 1.05–1.45 (20H, m, C(22–25)– CH_2, C(30–35)– CH_2), 1.45–1.70 (2H, m, C21–CH_2), 1.82–2.15 (4H, m, C26–CH_2, C29–CH_2), 2.27 (2H, t, J=7.5 Hz, C20–CH_2), 3.32 (9H, s, $N(CH_3)_3^+$), 3.60–3.86 (2H, m, –CH_2–N), 3.86–3.90 (2H, m, glycerol-C1), 4.00–4.15 (2H, m, glycerol-C3), 4.15–4.38 (2H, m, PO–OCH_2), 4.38–4.60 (1H, m, glycerol-C2), 5.20–5.45 (2H, m, C27–CH═, C28–CH═). m/z 544 $[MNa]^+$, 1065 $[2M+Na]^+$, 485 $[MNa-N(CH_3)_3]^+$; m/z 506 $[M-CH_3]$, 435 $[(M-CH_3)-C_4H_9N]^-$.

3.4.4. 1-(12-*O*-MEM-octadec-9-enoyl)-2-(octadec-*cis*-9-enoyl)-*sn*-glycero-3-phosphocholine (12). The reaction mixture containing **11** (1 g, 1.91 mmol), MEM-protected ricinoleic acid **5a** (2.5 g, 6.5 mmol), 4-pyrrolidinopyridine (1 g, 6.7 mmol) and DCC (1.35 g, 6.55 mmol, added slowly in 30 min) in CH_2Cl_2 (20 mL) was stirred for 6 h at room temperature. After dilution with hexane, the suspension was filtered and the solvent evaporated. The residue was dissolved in $CHCl_3/CH_3OH/H_2O$ 60:30:4 and purified on a column of Amberlist 15. The fractions containing the product were dried and passed through a silica gel column eluted with the same solvent. After evaporation of the solvent, 1-(*O*-MEM)ricinoleoyl-2-oleoyl-GPC (**12**) was recovered (1 g, 1.12 mmol, yield 58.6%) as a white waxy paste. [Found: C, 64.51; H, 10.62; N, 1.72; P, 3.65. $C_{48}H_{93}NO_{11}P$ requires C, 64.67; H, 10.52; N, 1.57; P, 3.48]. ν_{max} (neat) 3006 [(═CH)], 2926 and 2855 [(CH_2, CH_3)], 1741 and 1732 [$(C═O)_{ester}$ and $(C═O)_{ester}]_{overlap}$, 1466, 1241 [(P═O)], 1172 [$(C–O)_{ester}$], 1090 and 1044 [$(P–O–C)_{choline}$ and $(O–C–C)_{MEM}$], 970 [$\rho(CH_3)_{choline}$]. δ_H (300 MHz, $CDCl_3$): 0.84 (6H, t, J=6 Hz, C18–CH_3, C36–CH_3), 1.00–1.40 (36H, m, C(4–7)– CH_2, C(14–17)– CH_2, C(22–25)– CH_2, C(30–35)– CH_2), 1.40–1.60 (6H, m, C3–CH_2, C13–CH_2, C21–CH_2), 1.87–2.12 (6H, m, C8–CH_2, C26–CH_2, C29–CH_2), 2.12–2.38 (6H, t, C2–CH_2, C11–CH_2, C20–CH_2), 3.20–3.45 (12H, s, $N(CH_3)_3^+$+OCH_3), 3.45–3.75 (4H, m, OCH_2CH_2O), 3.58 (1H, m, C12–CH), 3.75–3.85 (2H, m, glycerol-C3), 3.85–4.00 (2H, m, –CH_2–N), 4.07 (1H, dd, J=12, 7 Hz, glycerol-C1–H_b), 4.20–4.35 (2H, m, PO–OCH_2), 4.35 (1H, dd, J=12, 3 Hz, glycerol-C1–H_a), 4.65–4.83 (2H, m, O–CH_2–O), 5.15 (1H, m, glycerol-C2), 5.25–5.50 (4H, m, C27–CH═, C28–CH═, C9–CH═, C10–CH═). m/z 912 $[MNa]^+$, 1801 $[2M+Na]^+$, 853 $[MNa-N(CH_3)_3]^+$; m/z 874 $[M-CH_3]^-$, 803 $[(M-CH_3)-C_4H_9N]^-$, 385 [ricinol. *O*-MEM ac.-H]$^-$.

3.4.5. 1-(12-hydroxy-octadec-9-enoyl)-2-(octadec-*cis*-9-enoyl)-*sn*-glycero-3-phosphocholine (13). The reaction solution containing **12** (1 g, 1.12 mmol) and pyridinium *p*-toluenesulfonate (PPTS, 1 g, 3.98 mmol) dissolved in ethylmethylketone (EMK 20 mL) was refluxed for 42 h. After dilution with diethyl ether, the precipitated material was filtered. The products were recovered after evaporation of the solvent under reduced pressure, dissolved in chloroform and separated with silica gel chromatography. The column was first eluted with $CHCl_3/CH_3OH/H_2O$ 65:25:2 and then the water content was doubled. After evaporation of the solvent, pure 1-ricinoleyl-2-oleoyl-GP (**13**), a waxy white solid, was obtained (0.4 g, 0.498 mmol, yield 44.4%). The overall yield was 10.3% from trityl-GPC **9**. [Found: C, 64.68; H, 10.76; N, 1.68; P, 3.79. $C_{44}H_{85}NO_9P$ requires C, 65.79; H, 10.67; N, 1.74; P, 3.86]. $[\alpha]_D^{23}$=+5.1 (c=0.63, $CHCl_3$). ν_{max} (neat) 3600–3100 $[(OH)_{ass}]$, 3006 [(═CH)], 2926 and 2854 [(CH_2, CH_3)], 1740 and 1731 [$(C═O)_{ester}$ and $(C═O)_{ester}]_{overlap}$, 1466, 1242 [(P═O)], 1174 [$(C–O)_{ester}$], 1090 and 1066 [$(P–O–C)_{choline}$ and $(O–C–C)_{MEM}$], 969 [$\rho(CH_3)_{choline}$].



δ_H (300 MHz, $CDCl_3$): 0.87 (6H, t, J=6.5 Hz, C18–CH_3, C36–CH_3), 1.12–1.39 (36H, m, C(4–7)– CH_2, C(14–17)–CH_2, C(22–25)– CH_2, C(30–35)– CH_2), 1.39–1.49 (2H, m, C13–CH_2), 1.49–1.68 (4H, t, C3–CH_2, C21–CH_2), 1.87–2.10 (6H, m, C8–CH_2, C26–CH_2, C29–CH_2), 2.18 (2H, t, J=6.5 Hz, C11–CH_2), 2.26 (2H, t, J=7 Hz, C20–CH_2), 2.28 (2H, t, J=7 Hz, C2–CH_2), 3.32 (9H, s, $N(CH_3)_3$),

3.52–3.66 (1H, m, C12–CH), 3.66–3.81 (2H, m, CH_2–N), 3.81–4.02 (2H, m, glycerol-C3–$H_{a,b}$), 4.11 (1H, dd, J=12, 7 Hz, glycerol-C1–H_b), 4.18–4.33 (2H, m, PO–CH_2), 4.33 (1H, dd, J=12, 3 Hz, glycerol-C1–H_a); 5.18 (1H, m, glycerol-C2–H), 5.24–5.36 (2H, m, C27–CH=, C28–CH=), 5.36–5.62 (2H, m, C9–CH=, C10–CH=). δ_{13C} (75.42 MHz, $CDCl_3$) 14.06, 14.07 (C18, C36), 22.59, 22.64, 24,85 (2), 25.72, 27.17, 27.19, 27.31 ($8CH_2$), 28.97, 29.01, 29.07, 29.13, 29.16, 29.22, 29.28, 29.29, 29.35, 29.50, 29.52, 29.73(2) ($13CH_2$), 31.83, 31.87, 34.09, 34.23, 35.36, 36.83 ($6CH_2$) 54.38 ppm (3), 59.34 (CH–OH), 62.96 (CH_2–N), 63.37 (glycerol), 66.35 (glycerol), 70.52 (glycerol), 71.35 (PO–CH_2–), 125.50, 132.89 CH(9)=CH(10), 129.98, 129.66 CH(27)=CH(28), 173.14, 173.50 CO(1), CO(13). δ_{31P} (81.0 MHz, $CDCl_3$)= 0.74. *m/z* 824 $[MNa]^+$(100%), 1625 $[2M+Na]^+$, 802 $[MH]^+$, 765 $[MNa-59]^+$ (59=$N(CH_3)_3$); *m/z* 800 $[M-H]^-$, 786 $[M-CH_3]^-$, 715 $[R-HPO_4]^-$ (100%). HRMS (3-NBA) MH^+, found 803.6060; $C_{44}H_{86}NO_9P$ requires 803.6040.

3.4.6. Positional purity of 13. The positional specificity of the synthesis of **13** was controlled by specific enzymatic hydrolysis with phospholipase A_2, which selectively hydrolyses the acyl group of position 2 of GPC. Compound **13** (32 mg), phospholipase A_2 (2 mg, dissolved in 0.25 mL of tris buffer 100 mM, pH 7.5, $CaCl_2$ 20 mM) were added to 5 mL of diethylether/methanol 10:1. The progression of the reaction was followed by TLC (eluent: $CHCl_3$/MeOH/CH_3–COOH/H_2O 170:30:20:7). After 4 h at 30°C, the reaction was analysed by GC-MS. It was found that the content of the correct isomer (ricinoleic group in position 1) was better than 95%.

Acknowledgements

This work has been carried out with the financial support of the Ministero dell'Università e della Ricerca Scientifica e Tecnologica, Progetto Biogene.

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