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SYNTHESIS OF NEW ETHER GLYCEROPHOSPHOLIPIDS STRUCTURALLY RELATED TO MODULATOR†

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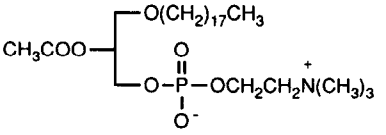
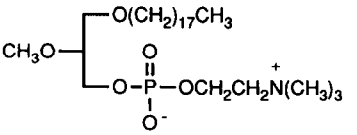
Abstract: *A series of new glycerophospholipids, bearing a short-chain carboxylic acid in position sn-1 and phosphocholine or phosphoserine in postion sn-3 of glycerol, have been prepared in good overall yields. Compound* ***11****, 1-O-(6-carboxyhexyl)-sn-glycero-3-phosphoserine, a strict analog of the structure proposed for modulator, has been synthesized in a stereoselective way from (R)-1,2-isopropylideneglycerol* ***1****.*

Alkyl glycerophospholipids, characterized by the presence of an ether bond in position *sn*-1 of the glycerol backbone, are present in mammalian cells in considerable amount[1] and their physiological role, as well as their involvement in some pathological conditions, has recently attracted great interest.[2] Platelet activating factor (PAF, **I**), an extremely potent biological mediator, is the most studied representative of this class.[3] Some synthetic analogues of the naturally occurring ether lipids are endowed with cytotoxic properties[4] and are commonly named alkyl-lysophospholipids (ALP) or, more simply, ether lipids (EL). They originate from lysophosphatidylcholine and usually present a non-metabolizable group in position *sn*-2, as in the structure of the compound ET-18-OCH_3 (**II**), the first component of this family to reach clinical studies.[5]



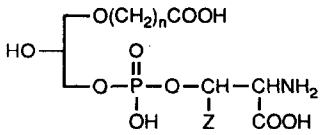
PAF (I)



ET-18-OCH_3 (II)

Recently, a structure of ether aminophosphoglyceride has been proposed for the *modulator*, an endogenous cytosolic low-molecular weight substance, which inhibits the activation of the glucocorticoid-receptor complex.[6,7] The structure proposed is showed in formula **III** and is characterized by the presence of a short-chain carboxylic acid in postion *sn*-1 and by the aminoacidic residue replacing the choline moiety in position *sn*-3. The nature and position of the group Z has not been totally clarified, being the CH_2NH_2 group the most likely candidate.

† A preliminary report of this work has been presented at the 81º Annual Meeting of the American Association for Cancer Research, May 23-26, 1990, Washington, DC (Abstr. 2454).

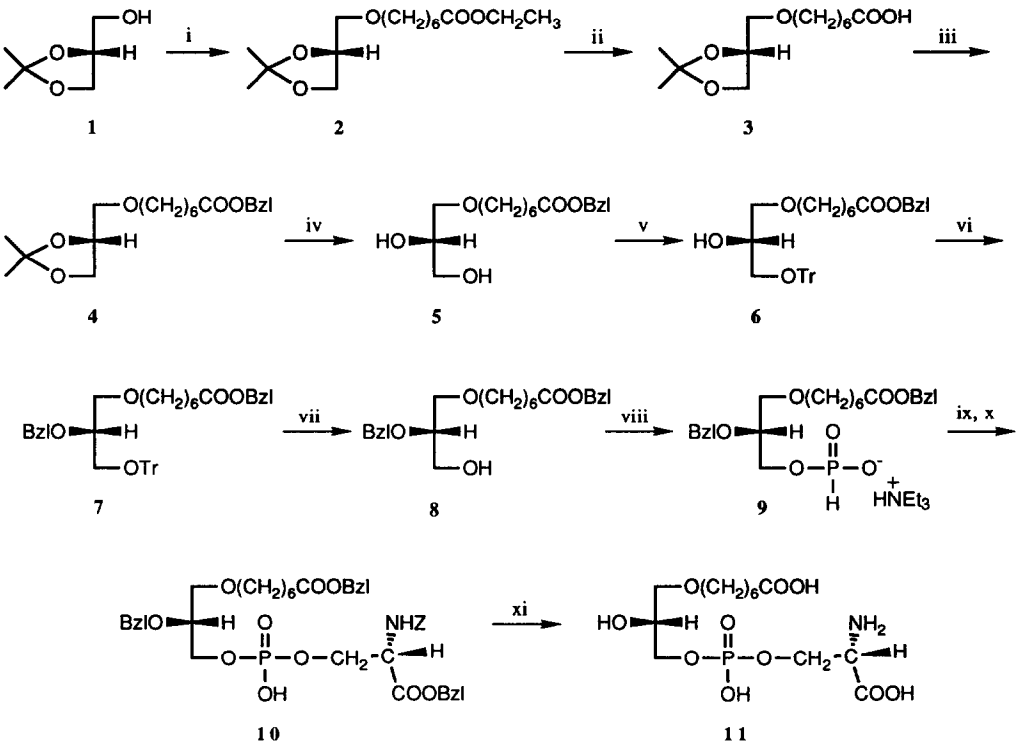


Modulator (III) n = 4-7

As a contribution to the elucidation of the exact structure of *modulator*, we describe here the synthesis of compound **11**, which corresponds to the basic framework, i.e. without the group Z, proposed in formula **III**. Since the stereochemistry of *modulator* has not been determined, it is unknown which stereoisomer of compound **III** can be responsible of the biological activity. Thus, in addition to the enantiomerically pure compound **11**, whose configuration *R,S* corresponds to that of natural phosphoserines, we have synthesized a mixture of **11** and its epimer on position 2 of glycerol. We also describe here the synthesis of some analogs, compounds **18**, **22**, **24**, and **26**, which can be considered as novel ALP having the unusual feature of a short-chain carboxylic acid in position 1 of the glycerol backbone. In three of them a methoxy group is present in C_2 of the glycerol backbone, since it is known that for ALP a hydroxyl group in that position reduce their cytotoxic activity.[8]

The synthesis of compound **11** is depicted in Scheme I. The synthetic strategy is based on the use of (*R*)-1,2-isopropylideneglycerol (**1**) as the chiral precursor. Alkylation of **1** with ethyl 7-bromoheptanoate and NaH in THF afforded the corresponding ether **2** in 35 % yield. Subsequent alkaline hydrolysis of the ester group using NaOH in dioxane-water gave the corresponding carboxylic acid **3**, which was converted to the benzyl ester **4** by alkylation of the carboxylate group with benzyl chloride in DMF. After cleavage of the acetonide group using HCl in THF-water (82% yield), the primary hydroxyl group of the resulting diol **5** was selectively blocked as the trityl ether and the remaining hydroxyl group in **6** was protected with benzyl bromide. Removal of the trityl group was carried out by treatment with HCl in dioxane-water, to give the alcohol **8** in 34 % overall yield from **6**. The optical purity of this alcohol **8** was determined by conversion into its MTPA ester (see below).

Attempts to convert alcohol **8** into a phosphoserine derivative, by phosphorylation with methyl dichlorophosphite and coupling with the appropriate protected serine, resulted in low yields and impure products. Therefore, the phosphoserine **10** was synthesized from **8** in 33 % overall yield, by using the procedure via H-phosphonate intermediate recently developed by Stawinski.[9] Thus, the H-phosphonate **9** was prepared by reaction of alcohol **8** with phosphorus trichloride, imidazole and triethylamine, in acetonitrile-toluene solution. The intermediate **9** was rendered anhydrous by repeated evaporation of a pyridine solution and then condensed with *N*-benzyloxycarbonyl-(L)-serine benzyl ester, in the presence of pivaloyl chloride as the coupling agent. The final oxidation of the phosphonate group to phosphate **10** was performed by addition of a iodine solution in water. Simultaneous removal of the four benzyl protecting groups on carboxyl, amino and hydroxyl functions was carried out by hydrogenation over palladium hydroxide, giving the desired lysophosphoserine **11** in 80 % yield.

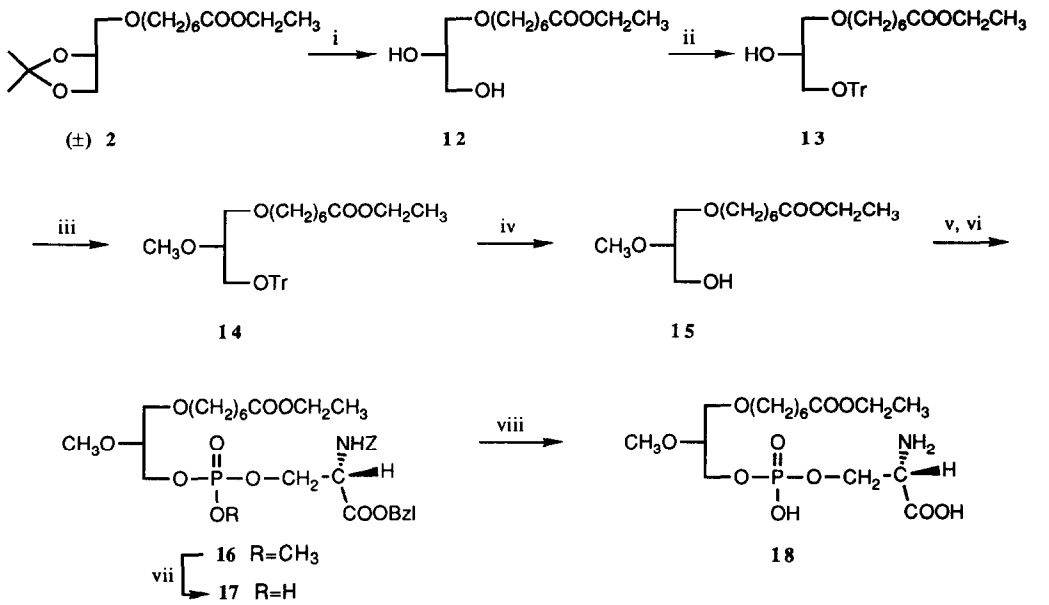


Scheme I. i) $Br(CH_2)_6COOEt$, NaH, DMF; ii) NaOH, dioxane; iii) BzlCl, $NaHCO_3$, DMF; iv) 2 M HCl, THF; v) TrCl, pyridine; vi) BzlBr, NaH, THF; vii) 1 M HCl, dioxane; viii) PCl_3, imidazole, Et_3N, CH_3CN/toluene; ix) Z-Ser-Bzl, pyridine, PVCl; x) I_2, pyridine, H_2O; xi) H_2, $Pd(OH)_2$-C, MeOH/H_2O.

An epimeric mixture of *R,S*- and *S,S*-**11** was obtained in similar yields, following the same synthetic pathway and starting from racemic isopropylidene glycerol (*rac*-**1**). No spectroscopic differences (^1H- and ^{13}C-NMR) were seen for the epimeric mixture or the pure enantiomer **11**. Thus, to ascertain that no racemisation had occurred during the synthesis of alcohol *R*-**8**, this compound and the racemic *R,S*-**8** were converted into their (+)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) esters by treatment with (+)-MTPA chloride.[10] The diastereomeric ratio of the resulting mixtures was analyzed by 300 MHz ^1H-NMR. Small but significant differences were observed for the following three signals: the triplet corresponding to the OCH_2 group in the *sn*-1 chain (*2R*-epimer δ 3.34, J=6.4 Hz; *2S*-epimer δ 3.35, J=6.7 Hz), the quartet for the methoxy group in MTPA (*2R*-epimer δ 3.50; *2S*-epimer δ 3.51), and the doublet of doublets for one of the hydrogens of the *sn*-3 CH_2 group of glycerol (*2R*-epimer δ 4.33, J = 11.6 and 6.4 Hz; *2S*-epimer δ 4.32, J = 11.6 and 5.5 Hz). The absence of the signals corresponding to the *2S*-epimer in the spectrum of the MTPA ester of **8** indicates that the optical purity of this alcohol is higher than 95 %.

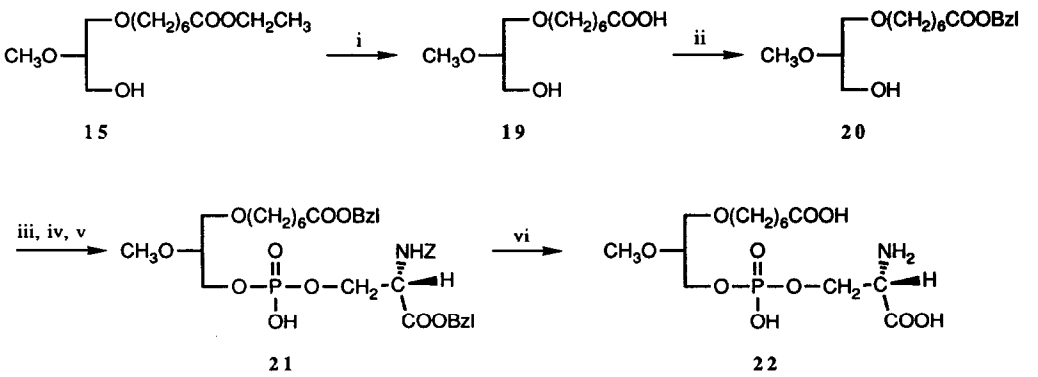
Compounds **18** and **22**, 2-*O*-methylated analogs of the phosphoserine **11**, containing an esterified or free carboxyl group respectively, were prepared following a procedure (Schemes II and III) similar to the above described for the synthesis of **11**.

Thus, racemic 1,2-isopropylideneglycerol was alkylated with ethyl 7-bromoheptanoate to give *rac*-**2**, which was deprotected in HCl solution to diol **12**. The reaction sequence comprising selective monotritylation, methylation of the 2-hydroxyl group, and detritylation led to the required methylated intermediate **15**. In contrast with the results obtained in the synthesis of **11**, in this case the phosphoserine moiety could be introduced by the phosphite-triester method,[11] through condensation of alcohol **15** with methyl dichlorophosphite and *N*-benzyloxycarbonyl-(L)-serine benzyl ester, followed by oxydation with hydrogen peroxide solution. Removal of the phosphate methyl ester in **16** was carried out by nucleophilic substitution with sodium iodide in butanone, and the resulting phosphoric acid **17** was hydrogenated with palladium on charcoal, to deprotect the serine carboxylate and amino groups. The final phosphoserine **18** was obtained in 11 % overall yield from the starting diol **12**.



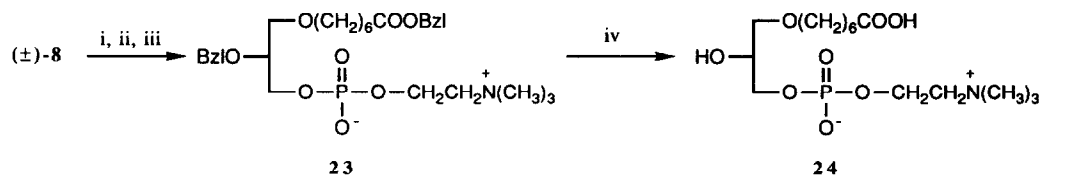
Scheme II. i) 2M HCl, THF; ii) TrCl, pyridine; iii) MeI, KH, benzene; iv) 1M HCl, dioxane; v) Z-Ser-Bzl, Cl_2POMe, $(iPr)_2EtN$, THF; vi) H_2O_2, CH_2Cl_2, vii) NaI, MeCOEt; viii) H_2, Pd-C, AcOH.

The corresponding carboxylic acid **22** was synthesized in 9 % overall yield from the same methylated intermediate **15** (Scheme III). After hydrolysis in alkaline solution to the acid **19**, protection of the carboxylate group was done by alkylation with benzyl chloride to the ester **20**, which was then converted into an H-phosphonate derivative by PCl_3 treatment and hydrolysis. Condensation with the above mentioned protected serine and oxidation on the phosphorus atom with iodine gave the phosphoserine **21**, whose three benzyl groups were removed by hydrogenation as described for **11**.

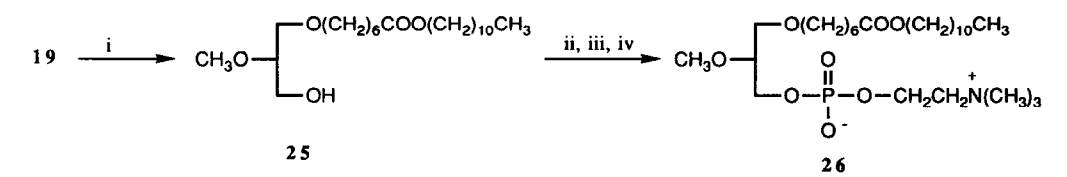


Scheme III. i) NaOH, dioxane; ii) BzlCl, $NaHCO_3$, DMF; iii) PCl_3, imidazole, Et_3N, CH_3CN/toluene; iv) Z-Ser-Bzl, pyridine, H_2O; v) I_2, pyridine, H_2O; vi) H_2, $Pd(OH)_2$-C, MeOH/H_2O.

To study the possible influence of the serine moiety on the biological activity of these ALP, two compounds containing the phosphocholine group, **24** and **26**, were synthesized (Schemes IV and V). Compound **24** was obtained from racemic dibenzylated alcohol **8**, which was phosphorylated with 2-bromoethyl phosphorodichloridate and triethylamine in diethyl ether solution. The intermediate bromoethyl phosphate obtained after hydrolysis was converted into the phosphocholine **23** by treatment with anhydrous trimethylamine in chloroform. The final debenzylation step was carried out by hydrogenation over palladium hydroxide, to afford racemic **24** in 39 % overall yield.



Scheme IV. i) $Cl_2P(O)OCH_2CH_2Br$, Et_3N, Et_2O; ii) KCl, H_2O; iii) Me_3N, $CHCl_3$, 65°C; iv) H_2, $Pd(OH)_2$-C, MeOH/H_2O.



Scheme V. i)$Br(CH_2)_{10}CH_3$, Li_2CO_3, DMF, 90°C; ii) $Cl_2P(O)OCH_2CH_2Br$, Et_3N, Et_2O; iii) KCl, H_2O; iv) Me_3N, $CHCl_3$, 65°C; v) H_2, $Pd(OH)_2$-C, MeOH/H_2O.

Finally, compound **26**, having the same short-chain carboxylic moiety esterified with a highly lipophilic alcohol, was synthesized from the methylated acid **19** (Scheme V), through esterification with undecyl bromide and lithium carbonate to give **25** and subsequent phosphorylation using the same procedure described for the synthesis of **24**.

Compounds **11, 18, 22, 24** and **26** are under pharmacological evaluation; preliminary results will be published elsewhere.[12]

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EXPERIMENTAL

Silica gel TLC plates (Merck silica gel 60 F_{254}) were used to monitor reactions, developing with 5 % phosphomolibdic acid in ethanol. Silica gel plates (Merck 20x20 cm, 0.5 mm thickness, without fluorescent indicator) were used for preparative TLC. MN silica gel 60 (70-230 mesh ASTM) was used for flash chromatography. Solvents were made anhydrous as follows: chloroform and dichloromethane were dried under calcium oxide, acetonitrile was distilled from calcium hydride, tetrahydrofuran and diethyl ether were refluxed over sodium/benzophenone just prior to use. The other solvents were absolute grade and were stored over molecular sieves. Reagents were purchased from Aldrich, except for (*R*)-1,2-isopropylideneglycerol and ethyl 7-bromoheptanoate which were obtained from Shell Chemicals and Imhausen-Chemie GMBH, respectively. 2-Bromoethyl phosphorodichloridate was prepared by literature procedures[13]. ^{1}H- and ^{13}C-NMR were taken on a Varian Gemini 300 instrument. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. Elemental analyses were performed by "Centro de Investigación y Desarrollo", C.S.I.C., Barcelona (Spain).

1-O-(6-Ethoxycarbonylhexyl)-2,3-isopropylidene-sn-glycerol (**2**)

A solution of (*R*)-1,2-isopropylideneglycerol (15.7 g, 11.9 mmol) in DMF (30 ml) was added dropwise to a suspension of NaH (14.13 mmol, 4.24 g of 80% dispersion in mineral oil, washed with petroleum ether under N_2) in dry DMF (350 ml). The mixture was stirred at room temperature for 2.5 h and a solution of ethyl 7-bromoheptanoate (33.8 g, 14.2 mmol) was added dropwise. The resulting mixture was kept at room temperature for 48 h, diluted with water (100 ml), extracted with ether (3x200 ml), and dried. Removal of solvents afforded an oil, which was purified by distillation under vacuum (130°C/0.5 torr) to give **2** (11.7 g, 35 % yield); $[\alpha]_D^{25}$= -3.83° (c= 4.5, MeOH); ^{1}H-NMR ($CDCl_3$) δ 1.20 (t, J=7.5 Hz, 3H, $OCH_2\underline{CH_3}$); 1.30 (br s, 7H, 2 CH_2 and CH_3); 1.37 (s, 3H, CH_3); 1.55 (m, 4H, $2CH_2$); 2.25 (t, J=7.5 Hz, 2H, CH_2CO); 3.40 (m, 4H, CH_2OCH_2); 3.67 (dd, half ABC pattern, J_{AB}=7.0 Hz, J_{BC}=6.2 Hz, 1H, $CH_A\underline{H_B}O$); 4.00 (m, 3H, half ABC and $OC\underline{H_2}CH_3$); 4.18 (m, 1H, CHO).

1-O-[6-(Benzyloxycarbonyl)hexyl]-2,3-isopropylidene-sn-glycerol (**4**)

A solution of 1.6 M NaOH (51.4 ml) was added to a solution of **2** (11.7 g, 40.9 mmol) in 275 ml of dioxane. After 18 h of stirring at room temperature, the mixture was neutralized with 0.1 M HCl. The solution was extracted with ethyl acetate, the combined organic extracts were dried, and solvents were evaporated under reduced pressure to give an oil (10.4 g), which was identified as 1-*O*-(6-carboxyhexyl)-2,3-isopropylidene-*sn*-glycerol (**3**) and was used without further purification in next step. ^{1}H-NMR ($CDCl_3$) δ 1.30 (br s, 7H, 2 CH_2 and CH_3); 1.35 (s, 3H, CH_3); 1.53 (m, 4H, $2CH_2$); 2.25 (t, J=7.5 Hz, 2H, CH_2CO); 3.40 (m, 4H, CH_2OCH_2); 3.67 (dd, half ABC pattern, J_{AB}=7.5 Hz, J_{BC}=6.8 Hz, 1H, $CH_A\underline{H_B}O$); 4.00 (dd, half ABC pattern, J_{AB}=7.5 Hz, J_{AC}=8.3 Hz, 1H, $C\underline{H_A}H_BO$); 4.18 (m, 1H, CHO).

To a solution of the above oil in dry DMF (50 ml) was added $NaHCO_3$ (4.7 g, 5.6 mmol) and dropwise a solution of benzyl chloride (16.1 ml, 140 mmol) in DMF (10 ml). The mixture was stirred at 40°C for 18h and water (100 ml) was then added. The mixture was extracted with ethyl acetate (3x150 ml), dried, and evaporated to give a clear oil. Purification by column chromatography (silica gel, petroleum ether-ether, 1:1) afforded 5.3 g of the benzyl ester **4** (84 % yield); $[\alpha]_D^{25}$=-3.38° (c= 0.59, MeOH); ^{1}H-NMR ($CDCl_3$) δ 1.30 (br s, 7H, 2 CH_2 and CH_3); 1.38 (s, 3H, CH_3); 1.53 (m, 2H, CH_2); 1.61 (m, 2H, CH_2); 2.32 (t, J=7.5 Hz, 2H, CH_2CO); 3.42

(complex m, 4H, CH_2OCH_2); 3.69 (dd, half ABC pattern, J_{AB}=8.2 Hz, J_{BC}=6.4 Hz, 1H, $CH_A\underline{H}_BO$); 4.02 (dd, half ABC pattern, J_{AB}=8.2 Hz, J_{AC}=6.4 Hz, 1H, $C\underline{H}_AH_BO$); 4.25 (m, 1H, CHO); 5.09 (s, 2H, $COOCH_2Ph$); 7.32 (br s, 5H, Ph); ^{13}C-NMR ($CDCl_3$) δ 25.61 and 26.97 ($2CH_3$), 25.03, 25.91, 29.07 and 29.54 ($4CH_2$), 34.37 ($\underline{C}H_2CO$), 66.34 ($COO\underline{C}H_2Ph$), 67.17 (CH_2O cycle), 71.94 (OCH_2), 72.17 (CH_2O), 75.09 (CHO), 109.77 ($\underline{C}(CH_3)_2$), 128.71, 129.08 and 136.73 (Ph), 174.14 (CO).

1-O-[6-(Benzyloxycarbonyl)hexyl]-sn-glycerol (**5**)

A solution of the acetal **4** (7.0 g, 20.0 mmol) in THF (825 ml) was mixed with 260 ml of 2 M HCl. After stirring for 4 h at room temperature, the solution was neutralized with $NaHCO_3$, the layers were separated, and the aqueous layer was extracted with ethyl acetate (3x200 ml). The combined organic extracts were dried and evaporated under reduced pressure to give a crude product, which was purified by column chromatography (silica gel, petroleum ether-EtOAc, 3:7), affording 5.1 g of the diol **5** (82 % yield); $[\alpha]_D^{25}$=+0.43° (c= 0.69, MeOH); ^{1}H-NMR ($CDCl_3$) δ 1.27 (m, 4H, 2 CH_2); 1.52 (m, 2H, CH_2); 1.59 (m, 2H, CH_2); 2.31 (t, J=7.5 Hz, 2H, CH_2CO); 3.40 (m, 4H, CH_2OCH_2); 3.55 (dd, half ABC pattern, J_{AB}=11.2 Hz, J_{BC}=5.6 Hz, 1H, $CH_A\underline{H}_BO$); 3.65 (dd, half ABC pattern, J_{AB}=11.2 Hz, J_{AC}=3.7 Hz, 1H, $C\underline{H}_AH_BO$); 3.81 (m, 1H, $C\underline{H}OH$); 5.02 (s, 2H, $COOCH_2Ph$); 7.30 (br s, 5H, Ph); ^{13}C-NMR ($CDCl_3$) δ 25.02, 25.89, 29.06 and 29.52 ($4CH_2$), 34.43 ($\underline{C}H_2CO$), 64.45 (CH_2OH), 66.50 ($COO\underline{C}H_2Ph$), 71.05 (CHOH), 71.93 (OCH_2), 72.66 (CH_2O), 128.77, 129.14 and 136.65 (Ph), 174.44 (CO).

1-O-[6-(Benzyloxycarbonyl)hexyl]-3-O-trityl-sn-glycerol (**6**)

Trityl chloride (3.95 g, 14.18 mmol) was added portionwise to a solution of diol **5** (2.25 g, 7.26 mmol) in dry pyridine (40 ml). The mixture was stirred under N_2 at room temperature for 17 h. Then, it was poured into water-ice and extracted with ether (3x100 ml). The organic layer was washed with 0.1 M HCl until acidic pH, then washed with 5 % $NaHCO_3$ solution and water. After driyng and evaporation, the residue was treated with CH_2Cl_2 and the crystallized triphenylmethanol was removed by filtration. Evaporation of the filtrate afforded a crude product, which was purified by column chromatography (silica gel, petroleum ether-ether, 1:1), to give 2.95 g of the protected alcohol **6** (80 % yield); $[\alpha]_D^{25}$=+1.49° (c= 0.67, MeOH); ^{1}H-NMR ($CDCl_3$) δ 1.30 (br s, 4H, 2 CH_2); 1.52 (m, 2H, CH_2); 1.63 (m, 2H, CH_2); 2.31 (t, J=7.5 Hz, 2H, CH_2CO); 3.15 (dd of d, ABC pattern J_{AB}=10.5 Hz, J_{AC}=J_{BC}= 5.2 Hz, 2H, CH_2OTr), 3.44 (complex m, 4H, CH_2OCH_2); 3.92 (m, 1H, $C\underline{H}OH$); 5.10 (s, 2H, $COOCH_2Ph$); 7.3 (complex m, 20H, 4Ph); ^{13}C-NMR ($CDCl_3$) δ 25.13, 26.02, 29.19 and 29.72 ($4CH_2$), 34.52 ($\underline{C}H_2CO$), 65.09 (CH_2OTr), 66.50 ($COO\underline{C}H_2Ph$), 70.28 (CHOH), 71.83 (OCH_2), 72.57 (CH_2O), 87.11 (CPh_3), 127.70-129.33, 136.79 and 144.61 (4Ph), 174.34 (CO).

1-O-[6-(Benzyloxycarbonyl)hexyl]-2-O-benzyl-sn-glycerol (**8**)

A solution of **6** (2.89 g, 5.6 mmol) in 20 ml of DMF was added dropwise to a suspension of NaH (13.3 mmol, 0.4 g of 80% dispersion in mineral oil, washed with petroleum ether under N_2) in dry DMF. After stirring for 45 min at room temperature, a solution of benzyl bromide (2.27 g, 13.3 mmol) in 10 ml of DMF and a few crystals of tetrabutylammoniun iodide were added. The resulting mixture was stirred at room temperature for 24 h, diluted with water (25 ml), extracted with ether (3x50 ml), and dried. The solvents were removed under reduced pressure to give a crude oil, which was purified by column chromatography (silica gel, petroleum ether-ether, 8:2) affording 2.10 g of 1-*O*-[6-(benzyloxycarbonyl)hexyl]-2-*O*-benzyl-3-*O*-trityl-*sn*-glycerol (**7**) (62 % yield); $[\alpha]_D^{25}$=+4.3° (c= 1.13, MeOH); ^{1}H-NMR ($CDCl_3$) δ 1.26 (br s, 4H, 2 CH_2); 1.50 (m, 2H, CH_2); 1.60 (m, 2H, CH_2); 2.31 (t, J=7.5 Hz, 2H, CH_2CO); 3.19 (d, J=5.0 Hz, 2H, CH_2OTr), 3.35 (t, J=6.6 Hz, 2H, OCH_2); 3.53 (m, 2H, CH_2O); 3.70 (m, 1H, CHOBzl); 4.63 (dd, AB pattern, J_{AB}=12.7 Hz, 2H, $CHOC\underline{H}_2Ph$), 5.08 (s, 2H, $COOCH_2Ph$); 7.3 (complex m, 25H, 5Ph).

A mixture of the above protected alcohol **7** (1.0 g, 1.63 mmol), 15 ml of dioxane, and 4.5 ml of 1 M HCl was stirred at 80°C for 4 h. After cooling, it was neutralized with saturated aqueous $NaHCO_3$ solution and extracted with ethyl acetate (3x50 ml). The organic layer was dried, evaporated and the residue was purified by column chromatography (silica gel, petroleum ether-ether, 7:3), giving 360 mg of alcohol **8** (55 % yield); $[\alpha]_D^{25}$=-0.40° (c= 1.75, MeOH); ^{1}H-NMR ($CDCl_3$) δ 1.30 (br s, 4H, 2 CH_2); 1.52 (m, 2H, CH_2); 1.63 (m, 2H, CH_2); 2.32 (t, J=7.5 Hz, 2H, CH_2CO); 3.40 (t, J=6.6 Hz, 2H, OCH_2); 3.55 (complex m, 4H, $2CH_2O$); 3.70 (m, 1H, CHOBzl); 4.65 (dd, AB pattern, J_{AB}=12.7 Hz, 2H, $CHOC\underline{H}_2Ph$), 5.10 (s, 2H, $COOCH_2Ph$); 7.30 (br s, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$) δ 25.07, 25.97, 29.12 and 29.64 ($4CH_2$), 34.47 ($\underline{C}H_2CO$), 63.36 (CH_2OH), 66.47 ($COO\underline{C}H_2Ph$), 71.44 (OCH_2), 72.04 ($CHOC\underline{H}_2Ph$), 72.48 (CH_2O), 78.35 ($\underline{C}HOCH_2Ph$), 128.40-129.15 and 138.93 (2Ph), 174.36 (CO). Anal. Calcd. $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 71.85; H, 8.13.

General procedure for preparation of Mosher Esters of **8** *and racemic* **8**

Thionyl chloride (4 ml) was added to 100 mg (0.43 mmol) of commercial R-(+)-MTPA. The mixture was refluxed for 4h and the excess of thionyl chloride was removed under reduce pressure to afford the Mosher acid chloride, which was used without further purification. To a solution of 0.06 mmol of the appropriate alcohol (**8** or racemic **8**) in dry $CHCl_3$ (0.6 ml) were added 0.124 mmol of (R)-(+)-MTPA chloride and 2 drops of pyridine. After stirring for 4 h at room temperature, the mixture was acidified with 0.1 M HCl, extracted with $CHCl_3$ (3x5ml) and dried. Solvents were evaporated under reduced pressure to give a crude product, which was purified by preparative TLC (silica gel, petroleum ether:ether, 2:8), giving the corresponding Mosher ester of **8** or *rac*-**8** in 90% yield. ^{1}H-NMR of (+)-**8** ($CDCl_3$) δ 1.28 (br s, 4H, 2 CH_2); 1.50 (m, 2H, CH_2); 1.60 (m, 2H, CH_2); 2.32 (t, J=7.7 Hz, 2H, CH_2CO); 3.34 (t, J=6.4 Hz, 2H, OCH_2); 3.44 (dd of d, ABC pattern J_{AB}=9.9 Hz, J_{AC}=J_{BC}= 5.8 Hz, 2H, CH_2O); 3.50 (q, J=1.2 Hz, 3H, CH_3O); 3.78 (m, 1H, CHOBzl); 4.33 (dd, half ABC pattern, J_{AB}=11.6 Hz, J_{BC}=6.4 Hz, 1H, CH_AH_BO); 4.55 (s, 2H, $CHOCH_2Ph$); 4.56 (dd, half ABC pattern, J_{AB}=11.6 Hz, J_{AC}=3.6 Hz, 1H, CH_AH_BO); 5.08 (s, 2H, $COOCH_2Ph$); 7.30 (br s, 15H, 3Ph)

1-O-(6-carboxyhexyl)-sn-glycero-3-phosphoserine, (**11**)

To a stirred and ice-cooled solution of imidazole (267 mg, 3.9 mmol, evaporated from dry acetonitrile and dried over P_2O_5) in 7 ml of acetonitrile was added dropwise PCl_3 (0.104 ml, 1.2 mmol), followed by Et_3N (0.578 ml, 4.1 mmol). After 15 min of stirring, a solution of alcohol **8** (110 mg, 0.27 mmol, evaporated from dry toluene and dried over P_2O_5) in 4 ml of toluene was added dropwise, during a period of 25 min. The mixture was stirred under N_2 at room temperature for 4 h. The reaction was quenched by addition of water (2 ml) and the resulting solution was stirred for an additional period of 30 min. The mixture was first evaporated under reduced pressure and then co-evaporated with pyridine:Et_3N 4:1. After partitioning of the residue between $CHCl_3$ and water, the aqueous phase was extracted with $CHCl_3$ (4x20 ml) and dried. The solvents were removed to give a semi-solid oil (160 mg), which contained mostly the triethylammonium salt of 1-*O*-[6-(benzyloxycarbonyl)hexyl]-2-*O*-benzyl-*sn*-glycero-3-*H*-phosphonic acid (**9**). This product was used without further purification in the next step. ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, J=7.5 Hz, 9H, $HN^+(CH_2CH_3)_3$); 1.30 (br s, 4H, 2 CH_2); 1.49 (m, 2H, CH_2); 1.58 (m, 2H, CH_2); 2.30 (t, J=7.5 Hz, 2H, CH_2CO); 2.95 (q, J=7.5 Hz, 6H, $HN^+(CH_2CH_3)_3$); 3.34 (t, J=6.6 Hz, 2H, OCH_2); 3.55 (m, 2H, CH_2O); 3.72 (m, 1H, CHOBzl); 3.94 (m, 2H, CH_2OP); 4.62 (br s,2H,$CHOCH_2Ph$); 5.05 (s,2H,$COOCH_2Ph$); 6.85 (d,J_{P-H}= 622.5 Hz, PH); 7.30 (br s, 10H, 2Ph).

A mixture of the above glycerophosphonate **9** (160 mg, evaporated from dry pyridine), *N*-benzyloxycarbonyl-(L)-serine benzyl ester (145 mg, 0.44 mmol, evaporated from dry pyridine), 10 ml of pyridine, and pivaloyl chloride (0.067 ml, 0.58 mmol) was stirred at room temperature for 3.5 h. Iodine (128 mg, 0.50 mmol) and water (0.148 ml) were added to the reaction mixture, which was stirred for an additional period of 30 min. Then 50 ml of $CHCl_3$ and 10 ml of 5% aqueous sodium bisulfite solution were added. The layers were separated and the aqueous one was extracted with $CHCl_3$ (3x25 ml). After driyng, solvents were evaporated under reduced pressure to give a crude product, which was purified by column chromatography (silica gel, $CHCl_3$-MeOH, 95:5) to give 71 mg of **10** (33 % overall yield from **8**); ^{1}H-NMR ($CDCl_3$) δ 1.30 (br s, 4H, $2CH_2$); 1.50 (m, 2H, CH_2); 1.62 (m, 2H, CH_2); 2.23 (t, J=7.5 Hz, 2H, CH_2CO); 3.26 (m, 2H, OCH_2); 3.36 (m, 2H, CH_2O); 3.61 (m, 1H, CHOBzl); 4.09 (m, 2H, CH_2OP); 4.19 (m, 1H, serine); 4.35 (m, 1H, serine); 4.47 (m, 1H, serine); 4.53 (dd, AB pattern, J_{AB}=8.3 Hz, $CHOCH_2Ph$); 5.05 (complex m, 6H, $3COOCH_2Ph$); 7.25 (br s, 20H, 4Ph); ^{13}C-NMR ($CDCl_3$) δ 25.08, 25.93, 29.14 and 29.60 ($4CH_2$), 34.47 (CH_2CO), 54.80 (d, J_{P-C}=7.3 Hz, CH serine), 66.48 ($COOCH_2Ph$), 67.50 ($COOCH_2Ph$ serine), 67.63 (d, J_{P-C}= 6.3 Hz, $POCH_2$), 68.08 ($COOCH_2Ph$ serine), 70.04 (CH_2OP), 71.96 ($2CH_2O$), 72.61 ($CHOCH_2Ph$), 76.70 (m, $CHOCH_2Ph$), 128.32-129.21, 135.69, 136.74, 136.79 and 138.72 (4Ph), 156.75 (NHCOO), 169.65 (COOBzl serine), 174.39 (COOBzl).

A mixture of **10** (63 mg, 0.080 mmol), 20 % $Pd(OH)_2$ on charcoal (135 mg), methanol (4 ml), and water (0.4 ml) was hydrogenated at room temperature and atmospheric pressure, until not more H_2 was consumed (about 18 h). The catalyst was filtered off and the solvents were removed under reduced pressure, affording 24 mg of phosphoserine **11** (80 % yield); $[\alpha]_D^{25}$=+2.75° (c= 1.09, $CHCl_3$-MeOH, 2:1); ^{1}H-NMR (CD_3OD) δ 1.26 (br s, 4H, $2CH_2$); 1.50 (m, 4H, $2CH_2$); 2.20 (t, J=7.5 Hz, 2H, CH_2CO); 3.40 (m, 4H, CH_2OCH_2); 3.81 (m, 3H, CH_2OP and 1H serine); 4.19 (complex m, 2H, serine); ^{13}C-NMR (CD_3OD) δ 26.30, 27.09, 30.25 and 30.75 ($4CH_2$), 35.13 (CH_2CO), 55.43 (d, J_{P-C}=7.0 Hz, CH serine), 64.79 (d, J_{P-C}= 4.6 Hz, $POCH_2$), 68.73 (d, J_{P-C}=5.5 Hz, CH_2OP), 71.36 (t, J_{P-C}= 7.3 Hz, CHOH), 72.88 (OCH_2), 73.18 (CH_2O), 170.45 (CO serine), 178.52 (COOH).

*1-O-(6-carboxyhexyl)glycero-3-phosphoserine, (rac-***11**)

Racemic protected alcohol *rac-***8** was obtained from racemic isopropylideneglycerol, using the same procedure described for the synthesis of **8**. A diastereomeric mixture of **11** was obtained in 34 % overall yield, starting from *rac-***8** and *N*-benzyloxycarbonyl-(L)-serine benzyl ester, using the same procedure which has been described above for the preparation of **11**. It hasn't been observed any spectroscopic difference between this mixture and the chiral compound **11**.

1-O-[6-Ethoxycarbonyl)hexyl]-3-O-tritylglycerol (**13**)

Racemic 1-*O*-[6-(ethoxycarbonyl)hexyl]glycerol (**12**) was obtained in 89 % yield, starting from 1-*O*-[6-(ethoxycarbonyl)hexyl]-2,3-isopropylideneglycerol (*rac-***2**) and using the same procedure which has been described above for the preparation of **5**. The compound was used without purification in the next step.

This diol **12** (7.26 g, 14.82 mmol) was converted into the trityl derivative **13** as described above for the preparation of **6**. The yield of chromatographically purified **13** was 12.1 g (84 %); ^{1}H-NMR ($CDCl_3$) δ 1.22 (t, J=7.3 Hz, 3H, $OCH_2C\underline{H}_3$), 1.30 (br s, 4H, 2 CH_2); 1.52 (m, 2H, CH_2); 1.60 (m, 2H, CH_2); 2.25 (t, J=7.5 Hz, 2H, CH_2CO); 3.15 (m, 2H, CH_2OTr), 3.45 (complex m, 4H, CH_2OCH_2); 3.94 (m, 1H, C<u>H</u>OH); 4.10 (q, J=7.3 Hz, 2H, $OC\underline{H}_2CH_3$); 7.30 (complex m, 15H, 3Ph).

1-O-[6-(Ethoxycarbonyl)hexyl]-2-O-methylglycerol (**15**)

A solution of the alcohol **13** (6.86 g, 14.0 mmol) in 20 ml of dry benzene was added dropwise to a suspension of KH (21 mmol, 4.20 g of 20 % dispersion in oil, washed with dry petroleum ether under argon) in 150 ml of dry benzene. The mixture was stirred at room temperature for 30 min, then methyl iodide (13.92 g, 98 mmol) was added dropwise, and the resulting mixture was stirring at room temperature for an additional period of 3.5 h. After addition of water, the reaction mixture was extracted with ether (3x100 ml), the combined organic extracts were dried, and solvents were evaporated under reduced pressure to give an oil (6.77 g), which contained mostly 1-*O*-[6-(ethoxycarbonyl)hexyl)-2-*O*-methyl-3-*O*-tritylglycerol(**14**). This compound was used without further purification in the next step.

Deprotection of the above trityl derivative **14** (4.46 g, 8.85 mmol) to give the alcohol **15** was carried out as described above for the preparation of **8**, by the use of 1 M HCl (4 ml) and dioxane (95 ml). The yield of **15** was 1.6 g (69 %); ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, J=7.2 Hz, 3H, $OCH_2C\underline{H}_3$),1.40 (m, 4H, $2CH_2$); 1.70 (m, 4H, $2CH_2$); 2.29 (t, J=7.5 Hz, 2H, CH_2CO); 3.44 (t, J=6.6 Hz, 2H, OCH_2); 3.46 (s, 3H, CH_3O); 3.52 (complex m, 3H, $C\underline{H}_2O$ and $C\underline{H}OCH_3$); 3.62 (dd, half ABC pattern, J_{AB}=11.5 Hz, J_{BC}=5.2 Hz, 1H, $CH_A\underline{H}_BOH$); 3.73 (dd, half ABC pattern, J_{AB}=11.5 Hz, J_{AC}=4.2 Hz, 1H $C\underline{H}_AH_BOH$); 4.11 (q, J=7.2 Hz, $OC\underline{H}_2CH_3$); ^{13}C-NMR ($CDCl_3$) δ 14.30 ($OCH_2\underline{C}H_3$), 24.96, 25.84, 28.99 and 29.48 ($4CH_2$), 34.36 ($\underline{C}H_2CO$), 57.96 (CH_3O), 60.39 ($O\underline{C}H_2CH_3$), 62.55 (CH_2OH), 70.66 (OCH_2), 71.86 (CH_2O), 80.43 ($\underline{C}HOCH_3$), 174.37 (CO). Anal. Calcd. $C_{13}H_{26}O_5$: C, 59.52; H, 9.99. Found: C, 59.55; H, 10.13.

1-O-[6-(Ethoxycarbonyl)hexyl]-2-O-methylglycero-3-phosphoserine (**18**)

A solution of *N*-benzyloxycarbonyl-(L)-serine benzyl ester (526 mg, 1.59 mmol) in a small volume of THF was added dropwise to a solution of methyl dichlorophosphite (0.180 ml, 1.91 mmol) and *N,N*-diisopropylethylamine (615 mg, 4.76 mmol) in 5 ml of dry, oxygen free THF (5 ml), maintained at -78°C by external cooling. After stirring for 10 min at -78°C, a solution of alcohol **15** (500 mg, 1.91 mmol) in a minimum volume of THF was added slowly. The resulting suspension was stirred for 3 h at -78°C, the cooling bath was removed, and the suspension was stirred for an additional 1 h. The solvent was evaporated under reduced pressure, the residue suspended in ethyl acetate, and the solids were separated by filtration through a celite pad. The filtrate was evaporated under reduced pressure to give the phosphite triester derivative of **15**, which was used without further purification in the oxidation step.

After dissolving this residue in CH_2Cl_2 (10 ml), 0.24 ml of 30 % H_2O_2 were added. The mixture was stirred at room temperature for 2.5 h, saturated aqueous NaCl solution (5 ml) was added and the layers were separated. The aqueous layer was extracted with CH_2Cl_2 (3x25 ml), the combined organic phases were dried and evaporated under reduced pressure. Purification was effected by column chromatography (silica gel, petroleum ether-EtOAc, 1:1) affording 403 mg of **16** (38 % overall yield from **15**); ^{1}H-NMR ($CDCl_3$) δ 1.25 (t, J=7.2 Hz, 3H, $OCH_2C\underline{H}_3$); 1.30 (m, 4H, $2CH_2$); 1.55 (m, 4H, $2CH_2$); 2.28 (t, J=7.5 Hz, 2H, CH_2CO); 3.41 (complex m, 8H, CH_2OCH_2 and $CHOCH_3$); 3.66 (m, 3H, CH_3OP); 4.05 (m, 2H, CH_2OP); 4.12 (q, J=7.2 Hz, $OC\underline{H}_2CH_3$); 4.38 (m, 1H, CH serine); 4.48 (m, 1H, CH serine); 4.63 (m, 1H, CH serine); 5.13 (s, 2H, $COOCH_2Ph$); 5.22 (s, 2H, $COOCH_2Ph$); 7.35 (br s, 10H, 2 Ph).

A mixture of **16** (540 mg, 0.83 mmol) and sodium iodide (146 mg, 0.97 mmol) in 24 ml of butanone was

refluxed for 18 h. After cooling at room temperature, the solvent was removed under reduced pressure and the residue was diluted with $CHCl_3$ (100 ml), washed with saturated aqueous NaCl solution, and dried. The organic phase was evaporated under reduced pressure to give a crude oil, which was purified by column chromatography (silica gel, $CHCl_3$-MeOH, 9:1) to give 380 mg of the phosphoric acid derivative **17** (72 % yield); ^{1}H-NMR ($CDCl_3$) δ 1.21 (t, J=7.2 Hz, 3H, $OCH_2\underline{CH_3}$),1.28 (br s, 4H, $2CH_2$); 1.48 (m, 2H, CH_2); 1.52 (m, 2H, CH_2); 2.22 (t, J=7.6 Hz, 2H, CH_2CO); 3.31 (s, 3H, CH_3O); 3.36 (m, 5H, $\underline{CH_2}OCH_2$ and $\underline{CH}OCH_3$); 3.40 (m, 2H, CH_2OP); 4.05 (q, J=7.2 Hz, $O\underline{CH_2}CH_3$); 4.25 (m, 1H, CH serine); 4.40 (m, 1H, CH serine); 4.56 (m, 1H, CH serine); 5.06 (s, 2H, $COOCH_2Ph$); 5.15 (dd, AB pattern J_{AB}=12.4 Hz, 2H, $COOCH_2Ph$); 7.25 (br s, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$) δ 13.85 ($OCH_2\underline{CH_3}$), 24.50, 25.33, 28.55 and 28.97 ($4CH_2$), 33.93 ($\underline{CH_2}CO$), 54.23 (d, J_{P-C}=7.6 Hz, CH serine), 57.74 (CH_3O), 59.99 ($O\underline{CH_2}CH_3$), 66.63 (d, J_{P-C}=5.2 Hz, $POCH_2$), 66.88 ($COO\underline{CH_2}Ph$), 67.47 ($COO\underline{CH_2}Ph$), 68.87 (CH_2OP), 71.42 ($2CH_2O$), 78.47 (d, J_{P-C}=6.9 Hz, $\underline{CH}OCH_3$), 128.05-128.63, 135.10 and 136.22 (2Ph), 156.21 (NHCOO), 169.12 (COOBzl), 174.06 (COOEt).

A suspension of **17** (495 mg, 0.76 mmol), 10 % palladium on charcoal (79 mg) in 7 ml of acetic acid and 7 ml of MeOH was shaken under hydrogen, at room temperature and atmospheric pressure, until not more H_2 was consumed (about 18 h). The catalyst was filtered off and the solvents were removed under reduced pressure to yield 232 mg of the phosphoserine **18** (71 %); ^{1}H-NMR (CD_3OD) δ 1.00 (t, J=7.2 Hz, 3H, $OCH_2\underline{CH_3}$),1.10 (m, 4H, $2CH_2$); 1.34 (m, 4H, $2CH_2$); 2.05 (t, J=7.6 Hz, 2H, CH_2CO); 3.17 (s, 3H, CH_3O), 3.25 (complex m, 5H, $\underline{CH_2}OCH_2$ and $\underline{CH}OCH_3$); 3.65 (m, 2H, CH_2OP); 3.85 (q, J=7.2 Hz, 2H, $O\underline{CH_2}CH_3$); 4.05 (m, 3H, serine); ^{13}C-NMR (CD_3OD) δ 14.85 ($OCH_2\underline{CH_3}$), 26.24, 27.13, 30.24 and 30.79 ($4CH_2O$), 35.31 ($\underline{CH_2}CO$), 55.21 (d, J_{P-C}=5.9 Hz, CH serine), 58.64 (CH_3O), 61.73 ($O\underline{CH_2}CH_3$), 64.83 ($POCH_2$), 66.49 (d, J_{P-C}=5.1 Hz, CH_2OP), 71.39 (OCH_2), 72.92 (CH_2O), 81.05 (d, J_{P-C}=7.2 Hz, $\underline{CH}OCH_3$), 170.45 (CO serine), 176.20 (COOEt). Anal. Calcd. $C_{16}H_{32}NO_{10}P.H_2O$: C, 42.95; H, 7.66; N,3.13. Found: C, 42.92; H, 7.65; N, 3.33.

1-O-[6-(Benzyloxycarbonyl)hexyl]-2-O-methylglycerol (**20**)

The procedure above described for the preparation of **4** was applied to the synthesis of the benzyl ester **20**. In the first step, 1-*O*-[6-(ethoxycarbonyl)hexyl]-2-*O*-methylglycerol (**15**) was hydrolyzed with NaOH in dioxane-water to the corresponding carboxylic acid **19**, in 71 % yield. Carboxylate alkylation of **19** with benzyl chloride gave **20** in 42 % yield; ^{1}H-NMR ($CDCl_3$) δ 1.25 (br s, 4H, 2 CH_2); 1.49 (m, 2H, CH_2); 1.58 (m, 2H, CH_2); 2.29 (t, J=7.5 Hz, 2H, CH_2CO); 3.35 (s, 3H, CH_3O); 3.40 (complex m, 5H, $\underline{CH_2}OCH_2$ and $\underline{CH}OCH_3$); 3.55 (dd, half ABC pattern, J_{AB}=11.5 Hz , J_{BC}=5.2 Hz, 1H, $CH_A\underline{H_B}OH$); 3.65 (dd, half ABC pattern, J_{AB}=11.5 Hz , J_{AC}=4.2 Hz, 1H, $\underline{CH_A}H_BOH$); 5.10 (s, 2H, $COOCH_2Ph$); 7.25 (br s, 5H, Ph). Anal. Calcd. $C_{18}H_{28}O_5$: C, 66.64; H, 8.70. Found: C, 66.72; H, 8.77.

1-O-(6-Carboxyhexyl)-2-O-methylglycero-3-phosphoserine (**22**)

The intermediate protected phosphoserine **21** was obtained in 34 % overall yield from alcohol **20**, through the same procedure which has been described above for the preparation of **10**. ^{1}H-NMR ($CDCl_3$) δ 1.28 (br s, 4H, $2CH_2$); 1.48 (m, 2H, CH_2); 1.52 (m, 2H, CH_2); 2.25 (t, J=7.5 Hz, 2H, CH_2CO); 3.31 (s, 3H, CH_3O); 3.40 (complex m, 5H, $\underline{CH_2}OCH_2$, $\underline{CH}OCH_3$); 4.00 (m, 2H, CH_2OP); 4.25 (m, 1H, serine); 4.40 (m, 1H, serine); 4.56 (m, 1H, serine); 5.05 (s, 4H, $2COOCH_2Ph$); 5.15 (dd, AB pattern, J_{AB}=12.4 Hz, $COOCH_2Ph$); 7.25 (br s, 15H, 3Ph).^{13}C-NMR ($CDCl_3$) δ 25.07, 25.90, 29.14 and 29.57 ($4CH_2$), 34.47 ($\underline{CH_2}CO$), 54.88 (d, J_{P-C}=7.6 Hz, CH serine), 58.34 (CH_3O), 66.47 ($COO\underline{CH_2}Ph$), 67.25 (d, J_{P-C}=4.7 Hz, $POCH_2$), 67.47 ($COO\underline{CH_2}Ph$), 68.05 ($COO\underline{CH_2}Ph$), 69.51 (CH_2OP), 72.00 ($2CH_2O$), 79.08 (d, J_{P-C}=6.7 Hz, $\underline{CH}OCH_3$), 128.65-129.21, 135.69, 136.81 and 136.79 (3Ph), 156.80 (NHCOO), 169.69 (COOBzl serine), 174.45 (COOBzl).

Debenzylation of **21** (252 mg, 0.35 mmol) to **22** was carried out by hydrogenation, as described above. The yield of **22** was 118 mg (84 %); ^{1}H-NMR (CD_3OD) δ 1.12 (br s, 4H, $2CH_2$); 1.35 (m, 4H, $2CH_2$); 2.05 (t, J=7.6 Hz, 2H, CH_2CO); 3.22 (s, CH_3O); 3.28 (complex m, 5H, $\underline{CH_2}OCH_2$ and $\underline{CH}OCH_3$); 3.68 (m, 2H, CH_2OP); 4.05 (m, 3H, serine); ^{13}C-NMR (CD_3OD) δ 26.24, 27.11, 30.24 and 30.74 ($4CH_2O$), 35.16 ($\underline{CH_2}CO$), 55.18 (d, J_{P-C}=6.7 Hz, CH serine), 58.58 (CH_3O), 64.68 (d, J_{P-C}=3.7 Hz, $POCH_2$), 66.41 (d, J_{P-C}=4.4 Hz, CH_2OP), 71.32 (OCH_2), 72.91 (CH_2O), 81.08 (d, J_{P-C}=7.8 Hz, $\underline{CH}OCH_3$), 170.26 (CO serine), 178.45 (COOH). Anal. Calcd. $C_{14}H_{27}NO_{10}PNa.H_2O$: C, 38.10; H, 6.62; N,3.17. Found: C, 38.30; H, 6.46; N,3.47.

1-O-[6-(Benzyloxycarbonyl)hexyl]-2-O-benzylglycero-3-phosphocholine (**23**)

A solution of 242 mg (0.61 mmol) of *rac*-**8** in 5 ml of dry ether was added at 0°C to a solution of 2-bromoethyl phosphorodichloridate (271 mg, 1.12 mmol) and Et_3N (0.31 ml, 2.24 mmol) in 7 ml of dry ether. The mixture was stirred at room temperature for 24 h and a solution of 1.1 ml of 0.1 M potassium chloride was

added. After stirring for 1.25 h, the layers were separated, the aqueous layer was extracted with ether (3x15 ml), and the combined extracts were dried. The solvent was evaporated under reduced pressure to give the corresponding 2-bromoethylphosphate derivative (321 mg), which was used without further purification in the next step.

Dry trimethylamine (4 ml) was added to a solution of the above compound (321 mg) in 12 ml of dry $CHCl_3$, placed in a thick-walled glass flask. The flask was sealed and the mixture was stirred at 65°C for 28 h. After cooling, the solvent was removed under reduced pressure and the residue was purified by column chromatography (silica gel, $CHCl_3$-MeOH, 4:6) to give the phosphocholine **23** (190 mg, 62 % yield); ^{1}H-NMR ($CDCl_3$) δ 1.15 (br s, 4H, $2CH_2$); 1.38 (m, 2H, CH_2); 1.49 (m, 2H, CH_2); 2.20 (t, J=7.6 Hz, 2H, CH_2CO); 3.00 (s, 9H, $N^+(CH_3)_3$); 3.25 (t, J=5.6 Hz, 2H, OCH_2); 3.48 (m, 4H, CH_2O and CH_2N^+); 3.60 (m, 1H, CHOBzl); 3.78 (m, 2H, CH_2OP); 4.02 (m, 2H, $POCH_2$); 4.52 (dd, AB pattern, J_{AB}=11.25 Hz, 2H, $CHOC\underline{H}_2Ph$); 4.95 (s, 2H, $COOCH_2Ph$); 7.19 (m, 10H, 2Ph); ^{13}C-NMR ($CDCl_3$) δ 25.29, 26.25, 29.31 and 29.91 ($4CH_2$), 34.61 ($\underline{C}H_2CO$), 54.34 ($N^+(CH_3)_3$), 59.59 (d, J_{P-C}=4.8 Hz, $POCH_2$), 65.75 (d, J_{P-C}=6.1 Hz, CH_2N^+), 66.57 ($\underline{C}H_2OP$ and $COO\underline{C}H_2Ph$), 71.14 (d, J_{P-C}=7.8 Hz, CHOBzl), 71.46 ($CHO\underline{C}H_2Ph$), 71.92 (OCH_2), 72.51 (CH_2O), 127.85-128.77, 136.53 and 139.27 (2Ph), 173.88 (CO).

1-O-(6-Carboxylhexyl)glycero-3-phosphocholine (**24**)

Compound **24** was obtained from **23** in 63 % yield, by the same procedure above described for the preparation of **11**. ^{1}H-NMR (CD_3OD) δ 1.12 (m, 4H, $2CH_2$); 1.35 (m, 4H, $2CH_2$); 2.05 (t, J=7.3 Hz, 2H, CH_2CO); 3.01 (s, 9H, $N^+(CH_3)_3$); 3.22 (m, 4H, CH_2OCH_2); 3.45 (m, 2H, CH_2N^+); 3.70 (m, 3H, $C\underline{H}OH$ and CH_2OP); 4.10 (m, 2H, $POCH_2$).^{13}C-NMR (CD_3OD) δ 26.27, 27.13, 30.25 and 30.78 (4 CH_2), 35.17 ($\underline{C}H_2CO$), 55.06 ($N^+(CH_3)_3$), 60.99 (d, J_{P-C}=4.8 Hz, $POCH_2$), 67.75 (m, CH_2N^+), 69.04 (d, J_{P-C}=5.7 Hz, CH_2OP), 71.25 (d, J_{P-C}=7.3 Hz, CHOH), 72.88 (CH_2O), 73.21 (CH_2O), 178.30. Anal. Calcd. $C_{15}H_{32}NO_8PNa.2H_2O$: C, 42.75; H, 8.61; N,3.32. Found: C, 42.55; H, 8.36; N,3.25.

1-O-[6-(Undecyloxycarbonyl)hexyl]-2-O-methylglycerol (**25**)

To a solution of **19** (200 mg, 0.86 mmol) in dry DMF (10 ml) was added 1-bromoundecane (389 mg, 1.69 mmol) and Li_2CO_3 (125 mg, 1.69 mmol). The mixture was stirred at 90°C for 24 h, 10 ml of water were added, and the solution was extracted with ethyl acetate (3x25 ml). Evaporation of the dried extracts afforded a crude oil, which was purified by column chromatography (silica gel, petroleum ether-ether, 7:3) to yield 233 mg of **25** (69 %); ^{1}H-NMR ($CDCl_3$) δ 0.82 (t, J=5.6 Hz, 3H, CH_3); 1.25 (m, 20H, $10CH_2$); 1.52 (m, 6H, $3CH_2$); 2.25 (t, J=7.5 Hz, 2H, CH_2CO); 3.40 (s, 3H, OCH_3); 3.45 (m, 5H, $C\underline{H}_2OC\underline{H}_2$ and $C\underline{H}OCH_3$); 3.65 (complex m, 2H, $C\underline{H}_2OH$); 4.02 (t, J=6.8 Hz, 2H, $COOCH_2$); ^{13}C-NMR ($CDCl_3$) δ 14.27 (CH_3), 22.87-34.52 ($14CH_2$), 58.10 (CH_3O), 62.91 ($O\underline{C}H_2(CH_2)_9CH_3$), 64.80 ($CH_2OH$), 70.93 ($CH_2O$), 72.07 ($CH_2O$), 80.29 ($\underline{C}HOCH_3$), 174.66. Anal. Calcd. $C_{22}H_{43}O_5$: C, 68.18; H, 11.18. Found: C, 68.19; H, 11.21.

1-O-[6-(Undecyloxycarbonyl)hexyl]-2-O-methylglycero-3-phosphocholine (**26**)

Phosphocholine **26** was prepared in 36 % overall yield, by the same procedure as described above for the synthesis of **23**, starting from the alcohol **25** (63 mg, 0.16 mmol); ^{1}H-NMR ($CDCl_3$) δ 0.82 (t, J=5.6 Hz, CH_3); 1.25 (m, 20H, $10CH_2$); 1.50 (m, 6H, $3CH_2$); 2.25 (t, J=7.5 Hz, 2H, CH_2CO); 3.30 (br s, 9H, $N^+(CH_3)_3$); 3.35 (s, 3H, CH_3O); 3.30-3.45 (complex m, 5H, $C\underline{H}_2OC\underline{H}_2$ and $C\underline{H}OCH_3$); 3.75 (m, 2H, CH_2N^+); 3.80 (m, 2H, CH_2OP); 3.95 (t, J=6.7 Hz, 2H, $COOCH_2$); 4.20 (m, 2H, $POCH_2$); ^{13}C-NMR ($CDCl_3$) δ 14.29 (CH_3), 22.86-34.48 (14 CH_2), 54.63 ($N^+(CH_3)_3$), 58.08 (OCH_3), 59.56 (d, J_{P-C}=4.9 Hz, $POCH_2$), 64.80 ($O\underline{C}H_2(CH_2)_9CH_3$), 65.11 (d, J_{P-C}=5.6 Hz, CH_2OP), 66.6 (d, J_{P-C}=7.1 Hz, CH_2N^+), 70.69 (CH_2O), 71.91 (CH_2O), 80.09 (d, J_{P-C}=7.1 Hz, $\underline{C}HOCH_3$),174.66. Anal. Calcd. $C_{27}H_{56}NO_8P.2H_2O$: C, 53.74; H, 10.31; N, 2.33. Found: C, 53.59; H, 9.98; N, 2.31.

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