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Synthesis of enantiomerically pure 1-*O*-phosphocholine-2-*O*-acyl-octadecane and 1-*O*-phosphocholine-2-*N*-acyl-octadecane

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Abstract

This is the first report on the chemical synthesis of enantiomerically pure R- or S-1-*O*-phosphocholine-2-*O*-acyl-octadecanes and R- or S-1-*O*-phosphocholine-2-*N*-acyl-octadecanes. From a stuctural point of view these phospholipids are intermediates between phosphatidylcholine and sphingomyelin. The synthesis of these model compounds is based on R- or S-1.2-*O*-isopropyliden-glyceraldeyde for chain elongation in a Wittig reaction with pentadecane-triphenylphosphine bromide. The resulting 1.2-*O*-isopropylidene-octadec-3-en is converted to R- or S-1.2-octadecanediol by catalytic hydrogenation of the double bond and by acidic removal of the isopropylidene protecting group. Tritylation of R- or S-1.2-octadecanediol results in the general intermediates R- or S-1-*O*-trityl-2-hydroxy-octadecane. These are the key intermediates for the synthesis of the phosphatidylcholine- or sphingomyelin-like end products. R- or S-1-*O*-phosphocholine-2-*O*-acyl-octadecane is obtained from the tritylated intermediates via benzylation in position 2, acidic detritylation and conversion of the R- or S-1-hydroxy-2-benzyl-octadecanes to the respective phosphocholines via the phosphoethanolamines. Catalytic hydrogenolysis of the benzyl group results in R- or S-1-O-phosphocholine-2-hydroxy-octadecane, which is converted to the phosphatidylcholine-like end products by acylation. R- or S-1-*O*-phosphocholine-2-*N*-acyl-octadecane is obtained from the tritylated intermediate by conversion of the R- or S-2-hydroxy group into the *N*-phthalimido group, which is achieved by inversion of the configuration using the Mitsunobu reaction with phthalimid. After acidic detritylation, the product is converted to the respective S- or R-1-*O*-phosphocholine derivative in a similar sequence of reactions, The phthalimido group is converted to the 2-amino group, and acylation results in the sphingomyelin-like end products.

Key words: Synthetic phospholipids; Phospholipid analogues; Synthesis; Chiral pool; Isopropylidene-glyceraldehyde; Optical purity; Mitsunobu reaction

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1. Introduction

Phospholipase C and sphingomyelinase are closely related enzymes. For an understanding of their mechanism of hydrolysis, we have prepared enantiomerically pure model compounds that contain stepwise alterations of the chemical structure. Starting from phosphatidylcholine as substrate for phospholipase C via 1-*O*-phosphocholine-2-*O*-acyl-octadecanes and 1-*O*-phosphocholine-2-*N*-acyl-octadecanes, we finally reach sphingomyelin as substrate for sphingomyelinase. With these new model compounds we have also studied the hydrolytic mechanism and the inhibition of phospholipase A_2.

Model compounds based on racemic long-chain 1.2-alkanediols have already been studied as analogues of the platelet activating factor (PAF) [1,2]. Correspondingly, amido model compounds based on enantiomerically pure short-chain 2-aminoalcohols (R configuration) and racemic long-chain 2-aminoalcohols have also been investigated for phospholipase A_2 inhibition [3]. For detailed studies of the structural requirements in phospholipids which result in substrate or inhibitor properties for these enzymes we need optically pure long-chain model compounds in both configurations, since phospholipase C [4], sphingomyelinase [5] and phospholipase A_2 [6] are stereoselective.

2. Synthesis

2.1. R- or S-1-O-trityl-2-hydroxy-octadecanes

The R- and S-2.3-*O*-isopropylidene-glyceraldehydes (**R-6/S-6**) are the central educts for all compounds. The aldehyde in the R configuration (Fig. 1) was made from D-mannitol (**1**) by selective protection of the 1.2- and 5.6-diolgroups as acetonide with acetone and $ZnCl_2$ [7], followed by cleavage of the obtained 1.2:5.6-di-*O*-isopropylidene-D-mannitol (**2**) with sodium metaperiodate [8]. The aldehyde with S configuration (**S-6**) was prepared from L-ascorbic acid (**3**), which is converted into the L-gulono-1.4-lactone (**4**) by catalytic hydrogenation [9]. The selective protection of the 5.6-diol group with isopropenylmethylether under *p*-toluenesulfonic acid catalysis [10] leads to the 5.6-*O*-isopropylidene-L-gulono-1.4-lactone (**5**). The yield of this step was increased by optimizing the working up procedure (85% yield in comparison with 70% yield [10]). The cleavage of **5** with sodium metaperiodate under aqueous, pH-controlled conditions [10] gave the enantiomerically pure S-**6** (Fig. 2).

Other methods for synthesizing the enantiomerically pure aldehydes **R-6** and **S-6**, for instance the oxidation of R- or S-1.2-*O*-isopropylideneglycerol with pyridinium chlorochromate [11,12] or by the procedure of Swern with dimethylsulfoxide and oxalylchloride [13,14], have been tested. According to our experimental experience, only the oxidative cleavage of the protected D-mannitol or the L-gulono-1.4-lactone with sodium metaperiodate in pH-controlled aqueous solution resulted in enantiomerically pure aldehydes. We think that the reason for this was the chosen aqueous conditions in the cleavage reaction. In aqueous solution the very active aldehydes **R-6** and **S-6** are immediately protected by hydroxylation [15], the carbonyl activity is now on a low level and no racemisation (via a keto-enol-tautomeric process)

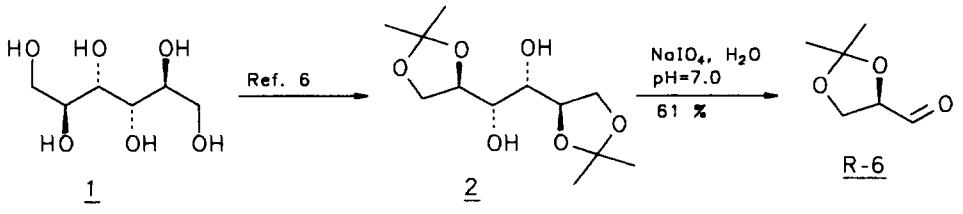

Fig. 1. Synthesis of R-2.3-*O*-isopropylidene-glyceraldehyde (**R-6**) from D-mannitol.

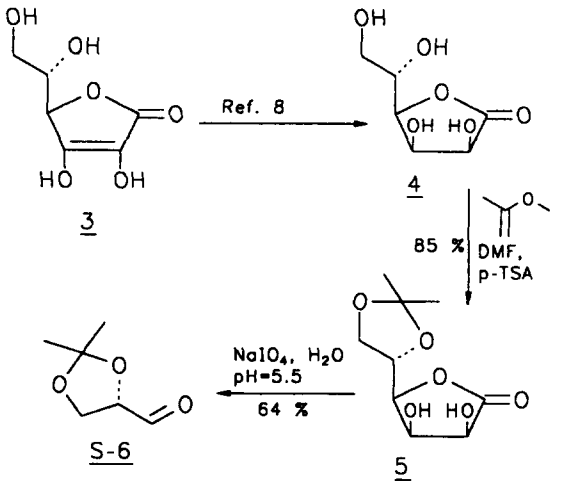

Fig. 2. Synthesis of S-2.3-*O*-isopropylidene-glyceraldehyde (S-**6**) from L-ascorbic acid.

can occur. Furthermore, the distilled and water-free aldehydes are protected by polymerisation and form paraformaldehyde-like products. The decrease of the optical rotation of the pure aldehyde with time, which was first described by Bear and Fischer [15], is the consequence of a higher level of polymerisation and not of racemisation. The use of the polymeric aldehydes or the use of freshly distilled aldehydes in the following reaction (Wittig reaction) leads to products with the same ee-values.

The condensation of the enantiomerically pure C_3 aldehydes (R-**6**, S-**6**) with the C_{15} chain using pentadecane triphenylphosphine bromide (**7**) was achieved in 76% yield with *n*-butyllithium at −78°C in THF as solvent [16]. The Wittig salt was prepared from triphenylphosphine and pentadecane bromide without solvent at 130°C in 90% yield [17]. The optical rotation of the oily Wittig products R- or S-1.2-*O*-isopropylidene-octadec-3-en (R-**8**, S-**8**) was −4.6° for the R-enantiomer and +4.6° for the S-isomer. The *cis/trans* ratio of the double bond was not detected, because in the next reaction step the double bond in R- and S-**8** was catalytically hydrated with 10% Pd/C in THF/water (100:1, v:v). Deacetonation of the hydrated intermediate with hydrochloric acid led to the R- or S-1.2-octadecanediols (R-**9**, S-**9**). Selective protection of the 1-hydroxyl group in both enantiomers was possible by tritylation with trityl-chloride and triethylamine in boiling toluene [18]. The R- or S-1-trityl-2-hydroxy-octadecanes (R-**10**, S-**10**) as key intermediates were obtained in yields of ~90%.

2.2. *R- or S-1-O-phosphocholine-2-O-acyl-octadecanes*

Educts for the preparation of the phosphatidylcholine-like model compounds are the trityl intermediates R- or S-**10**. Protection of the 2-hydroxy group as benzylether was achieved by benzylation with benzylchloride/potassium *tert*-butylate in

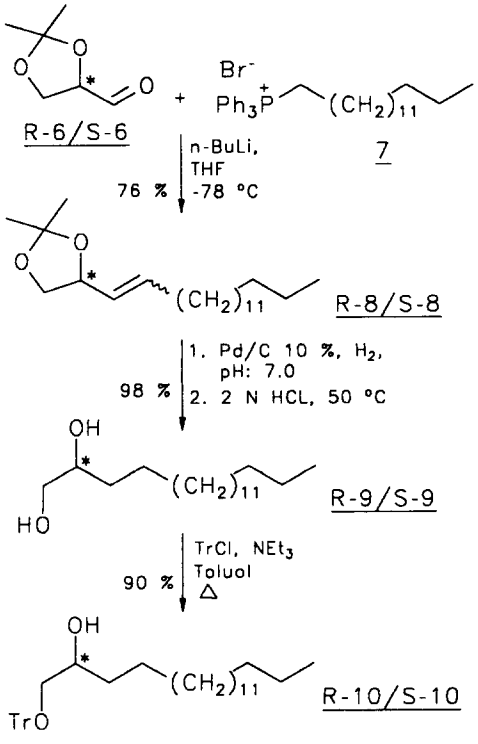

Fig. 3. Synthesis of R- and S-1-*O*-trityl-2-hydroxy-octadecane (R-**10**, S-**10**).

THF [19]. Methanolysis of the trityl group with sulfuric acid in dioxane/methanol [20] resulted in R- or S-1-hydroxy-2-*O*-benzyl-octadecanes (**R-11**, **S-11**). Phosphorylation of the free primary hydroxyl groups in R- or **S-11** with phosphoroxychloride, phospholane ring formation with *N*-methyl-aminoethanol and specific hydrolysis of the P-N bond generates the respective phospho-*N*-methylethanolamines, which were converted to the respective phosphocholine by methylation (for details see Experimental section). This phosphorylation procedure has been developed in our laboratory as described earlier [21]. However, the reaction was now performed as a one-pot reaction [22]. The catalytic cleavage of the benzyl ethers of the resulting phosphocholine compounds R- and **S-12** with Pd/C in methanol/THF led to R- and S-1-*O*-phosphocholine-2-hydroxy-octadecanes (**R-13**, **S-13**). The acylation of R and **S-13** with various fatty acids resulted in the phosphatidylcholine-like ester model compounds in both enantiomeric forms. As acylating agents, fatty acid chlorides were used in the presence of dimethylamino pyridine (DMAP) as catalyst and base [23,24]. Acylation was also performed with fatty acids using the dicyclohexylcarbodiimide (DCC)/DMAP method [24,25]. The acylations must be supported by ultrasonication, otherwise the reaction stops at 40% of conversion.

2.3. R- or S-1-O-phosphocholine-2-N-acyl-octadecanes

As in the case of the phosphatidylcholine-like compounds, the educts for the preparation of the sphingomyelin-like compounds were the trityl intermediates R- and **S-10**. The free hydroxyl groups of these compounds must be converted in a stereospecific way into the amino groups. Favoured above other methods with respect to stereoselectivity of the *N*-protected intermediate and yield is the Mitsunobu reaction, which converts the free hydroxyl groups of the trityl compounds in the R or S configuration into phthalimido groups under complete inversion of the configuration [26]. Thus, R-1-*O*-trityl-2-hydroxy-octadecane resulted in enantiomerically pure S-*O*-1-trityl-2-*N*-phthalimido-octadecane and

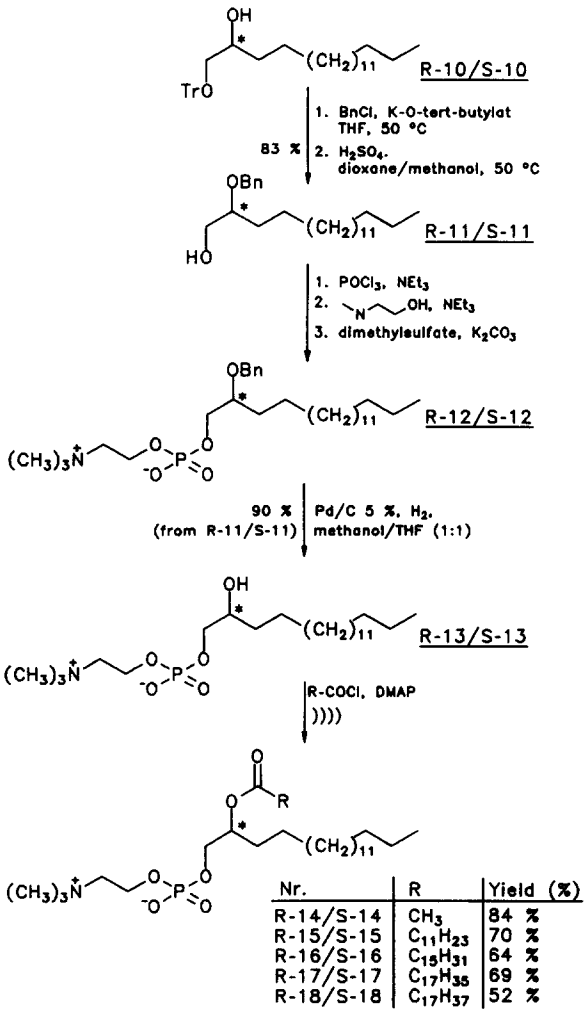

Fig. 4. Synthesis of enantiomerically pure 1-*O*-phosphocholine-2-*O*-acyl-octadecanes.

S-1-*O*-trityl-2-hydroxy-octadecane, respectively, in R-1-*O*-trityl-2-*N*-phthalimido-octadecane. Acidic hydrolysis of the tritylether [20] of the phthalimido compounds resulted in the R- or S-1-hydroxy-2-*N*-phthalimido-octadecanes (**R-19**, **S-19**), respectively. Phosphorylation of R- or **S-19** to the R- or S-1-*O*-phosphocholine-2-*N*-phthalimido-octadecanes (**R-20**, **S-20**) was as described for the phosphatidylcholine-like compounds R- and **S-12**. Removal of the phthalimido

protecting groups according to Osby was achieved through reduction with sodium borohydride in 2-propanol/water, followed by mild cleavage of the resulting 2-methylhydroxy benzoic acid amide by formation of phthalan [27]. In 2-propanol/water the *N*-phthalimids R- and S-**20** possibly form overstructures, and therefore the reductions were inhibited. However, with the addition of toluene the reduction resulted in high yields. In our case the high stability of 1-*O*-phosphocholine-2-amino-octadecanes at acidic pH values allowed the hydrolysing of the 2-methylhydroxy-benzoic acid amide intermediates with 6 N hydrochloric acid at 80°C. The cleavage reaction was now supported by the drastic acid conditions instead of the cyclisation process of the 1-methylhydroxy benzoic acid. In addition, the use of hydrochloric acid resulted in a very simple cleaning procedure. The products R- and S-**21** were precipitated as their hydrochlorides from acetone. The acylation of R- and S-**21** with various fatty acids led to the sphingomyelin-like model compounds. The acylating procedure was the same as for the phosphatidylcholine-like model compounds, also supported by ultrasonication.

2.4. Optical purity

Most important for meaningful biological experiments is the optical purity of the model compounds. The R- and S-enantiomers and the racemic mixtures of the key intermediate 1-*O*-trityl-2-hydroxy-octadecane and the compounds R- and S-1-hydroxy-2-*O*-benzyl-octadecane and R- and S-1-hydroxy-2-*N*-phthalimido-octadecane were converted to the respective 'Mosher esters' [28] with the 'Mosher acid', R-(+)-1-methoxy-1-phenyl-trifluoromethyl-acetic acid (DCC/DMAP) method [24,25]). As demonstrated for the RR- or the RS-diastereomers of the 'Mosher esters' by ^{19}F-NMR, the de-values were better than 99%. For visualization, the ^{19}F-NMR spectras of the Mosher esters of R-, S- and *rac*-1-hydroxy-2-*N*-phthalimido-octadecane are shown in Fig. 6. It is experimental proof that the high optical purity of the chiral pool compounds D-mannitol and L-ascorbic acid was not decreased by the periodate cleavage reaction of vicinal hydroxyl groups, by

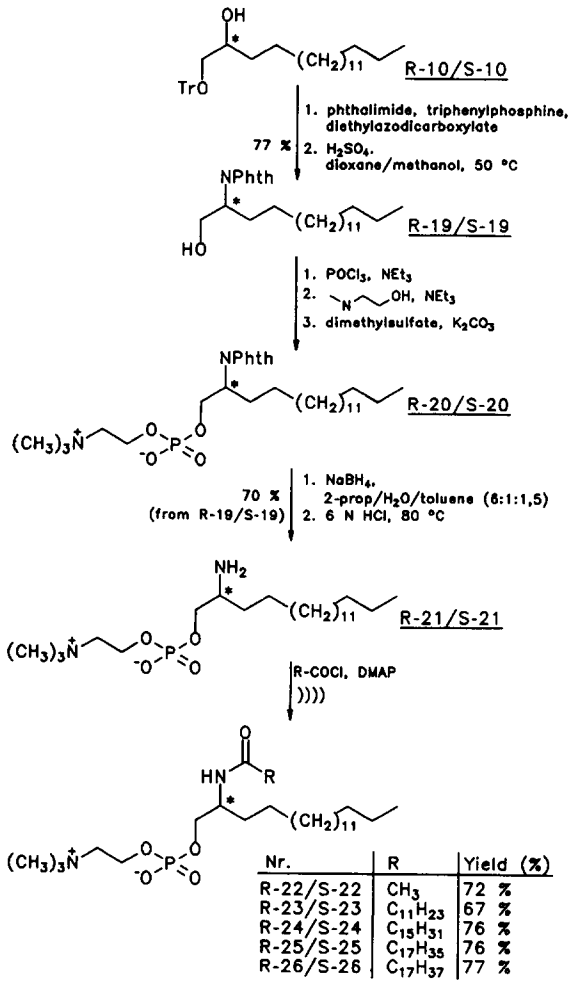

Fig. 5. Synthesis of enantiomerically pure 1-*O*-phosphocholine-2-*N*-acyl-octadecanes.

the Wittig reaction or by the additional chemical conversions, including the Mitsunobu reaction. According to these studies, the Mitsunobu reaction led to complete inversion of the configuration by the exchange of the hydroxyl group versus the amino group.

3. Materials and methods

^{1}H-NMR spectra were recorded with a Bruker MSL300 spectrometer (300 MHz, TMS = 0 ppm) and ^{19}F-NMR-spectra with a Bruker WR360 (338.87 MHz, $CFCl_3$ = 0 ppm, internal standard: C_6F_6 = 162.28 ppm). Infrared spectra were

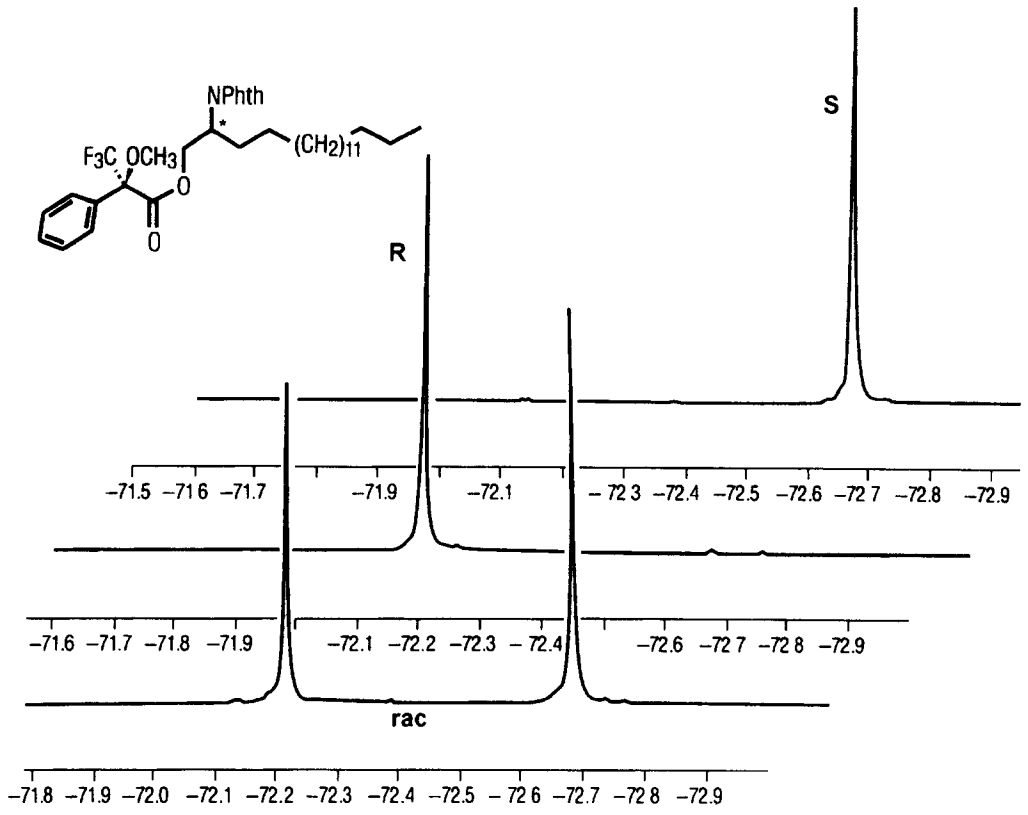

Fig. 6. ^{19}F-NMR spectras of the 'Mosher esters' of R-, S- and *rac*-1-hydroxy-2-*N*- phthalimido-octadecane.

measured using a Perkin-Elmer 1420 spectrophotometer. A Büchi 520 apparatus was used to measure melting points. Elemental analysis of the compounds was performed by Mikroanalytisches Laboratorium Beller (Göttingen). Silica gel (Kieselgel 60, 35–70 mesh) and TLC plates (art. 5721) were obtained from Merck (Darmstadt). Solvent mixtures for chromatography are given in volume ratios, and ammonia stands for a 25% aqueous solution. Chemicals and solvents were of p.a. grade from Aldrich, Baker, Fluka and Merck and used without further purification. Removal of solvents, if not otherwise specified, was done in a rotatory evaporator under reduced pressure.

1.2:5.6-Di-*O*-isopropylidene-D-mannitol (**2**) was synthesized from D-mannitol (**1**) according to Baer [7]. L-Gulono-1.4-lactone (**4**) was prepared from L-ascorbic acid (**3**) by the method of Andrews et al. [9].

*3.1. R-2.3-O-Isopropylidene-glyceraldehyde (R-**6**)*

The protected D-mannitol **2**, 140 g (0.534 mol), was dispersed in 700 ml water at room temperature. The dispersion was stirred vigorously, and 148 g (0.694 mol) $NaIO_4$ were added in 0.5 g portions. Care was taken that the pH of the reaction mixture never dropped below 6.0 by continuos addition of 2N NaOH. Stirring was continued for 1.5 h. NaCl, 300 g, was added and the precipitate was filtered off (membrane filter) and washed with 200 ml of saturated NaCl solution. The water phase

was extracted five times with 300 ml dichloromethane and four times with 300 ml ethylacetate. The combined organic phases were dried with Na_2SO_4. The solvent of the organic phase was removed. The product was purified by distillation; $bp_{9\ mbar}$ 30–40°C. Yield: 84.2 g (0.674 mol, 61%). $[\alpha]_{20} = +71.6°$ (c = 6.02, benzene). ^{1}H-NMR ($CDCl_3$): δ = 1.43 (s, 3 H, $-CH_3$); 1.50 (s, 3 H, $-CH_3$); 4.07–4.21 (m, 2 H, $-CH_2-$); 4.38–4.43 (m, 1 H, —CH—); 9.75 (s, 1 H, aldehyde proton). IR (film): [cm^{-1}] 3000, 2950, 2900, 1770, 1480, 1220, 1070, 850.

3.2. 5.6-O-Isopropylidene-L-gulono-1.4-lactone (5)

L-Gulono-1.4-lactone (**4**), 150 g (0.824 mol) and 1.1 g (6.70 mmol) *p*-toluene sulfonic acid were dissolved in 1.3 l DMF and cooled to 10°C. Isopropenyl-methyl-ether, 103 ml (78.9 g, 1.10 mol), was added dropwise and the reaction mixture kept at 20°C for 24 h. After the addition of 150 g sodium carbonate decahydrate the dispersion was stirred vigorously for a further 3 h. Filtration and removal of DMF from the filtrate by high-vacuum distillation (0.05 mbar at 50–60°C) resulted in a residue that was dissolved in 600 ml dichloromethane. After addition of 2 l toluene, a precipitate was formed after 48 h at 4°C. The product was isolated by filtration and washed with 1 l hexane/ethanol (9:1). Yield: 156 g (0.713 mol, 85%). R_f (chloroform/methanol 9:1): 0.27; R_f (educt) (chloroform/methanol 9:1): 0.0. $[\alpha]_D$: +37.4° (c = 1.0, methanol); lit.: +38.3° (c = 0.7, methanol) [10]. F_p: 163°C; lit.: 167–168°C [10]

3.3. S-2.3-O-Isopropylidene-glyceraldehyde (S-6)

The lactone **5**, 105 g (0.482 mol), was dispersed in 400 ml water. The dispersion was stirred vigorously, and 206 g (0.965 mol) $NaIO_4$ was added in 0.5 g portions while carefully maintaining the pH at 5.5 through continuos addition of 2 N NaOH. After stirring at 20°C for 1.5 h, 300 g NaCl was added and the pH adjusted to 7.0. The precipitate was filtered with a membrane filter and washed with 200 ml of saturated NaCl-solution. The filtrate was extracted five times with 350 ml dichloromethane and four times with 350 ml ethylacetate. The combined organic extracts were dried with Na_2SO_4. Solvent was removed and the product was purified by distillation; $bp_{9\ mbar}$ 30–40°C. Yield: 40.6 g (0.311 mol, 64%). $[\alpha]_{20} = -68.2°$ (c = 6.10, benzene). ^{1}H-NMR ($CDCl_3$): δ = 1.43 (s, 3 H, $-CH_3$); 1.50 (s, 3 H, $-CH_3$); 4.07–4.21 (m, 2 H, $-CH_2-$); 4.38–4.43 (m, 1 H, —CH—); 9.75 (s, 1 H, aldehyde proton). IR (film): [cm^{-1}] 3000, 2950, 2900, 1770, 1480, 1220, 1070, 850.

3.4. Pentadecane triphenylphosphine bromide (7)

Pentadecane bromide, 202 g (0.692 mol), and triphenylphosphine, 182 g (0.692 mol), were heated for 12 h at 130°C under continuos stirring in a 2-l flask. After cooling to 90°C, 400 ml THF was added slowly through the reflux condenser under intensive stirring. The reaction mixture was poured into a 5-l beaker, and 2 l diethylether was added under continuous stirring. The precipitation was complete after 12 h at 4°C. The product was filtered off (membrane filter) and washed with 500 ml diethylether. Yield: 394 g (0.631 mol, 91%).

3.5. R- or S-1.2-O-Isopropylidene-octadec-3-ene (R-8, S-8)

The Wittig salt **7**, 168 g (304 mmol), was dissolved in 1.2 l THF under stirring and cooled to 0°C. *n*-Butyllithium (2.5 M in hexane), 146 ml (364 mmol), was added carefully with a syringe. After stirring for ~ 10 min at 0°C, the solution was cooled to −78°C. R-**6** or S-**6**, 47.4 g (364 mmol), in 150 ml THF were added dropwise over 30 min. Cooling was continued for 20 min. The cooling bath was removed, and the reaction mixture reached 20°C by stirring for 12 h. The organic phase was extracted with 1 l water. The aqueous phase was re-extracted with 150 ml diisopropylether. The combined organic phases were freed from solvents and the residue dissolved in 1 l pentane. The solution was stirred vigorously and cooled to 0°C. The precipitate was filtered off and washed with 150 ml pentane. The combined organic phases were extracted with 350 ml saturated NaCl solution, and the solvent of the organic phase was removed. The residue was filtered over 500 g silica gel with hex-

ane. The apolar byproducts were removed with hexane/diisopropylether (20:1). A ratio of 4:1 eluted the product. Yield: 75.4 g (232 mmol, 76%). R_f: 0.25 (hexane/diisopropylether 4:1). $[\alpha]_D$: −4.60° (R-**8**); +4.60° (S-**8**) (without solvent). ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.7 Hz, 3 H, —CH_3); 1.27 (s, 22 H, alkyl-(6–17)-CH_2—); 1.40 (s, 3 H, acetonide-CH_3); 1.43 (s, 3 H, acetonide-CH_3); 2.02–2.18 (m, 2 H, alkyl-5-CH_2—); 3.53 (dd, $^2J_{1',1}$ = 8.1 Hz; $^3J_{1',2}$ = 8.1 Hz; 1 H, 1′-H); 4.06 (dd, $^2J_{1,1'}$ = 8.1 Hz; $^3J_{1,2}$ = 6.1 Hz; 1 H, 1-H); 4.80–4.90 (m, 1 H, 2-CH—); 5.34–5.45 (m, 1 H, 4-CH=); 5.60–5.69 (m, 1H, 3-CH=). IR (film): [cm^{-1}] 3020, 2960, 2890, 1480, 1080, 880. $C_{21}H_{40}O_2$ (324.55) calc. C 77.71% H 12.42%; found C 77.98% H 12.56%.

3.6. *R- or S-1.2-Octadecanediol (R-9, S-9)*

The educts R-**8** or S-**8**, 35.5 g (110 mmol), were dissolved in 900 ml THF, and 20 g 10% Pd/C was added. Hydrogenation was performed at 20°C at atmospheric pressure until completion of the hydrogen uptake. The catalyst was removed by filtration. After addition of 130 ml 2N HCl, the reaction mixture was heated to 60°C for 30 min. The organic phase was extracted with 1 l K_2CO_3 solution (40 g/l). The aqueous phase was re-extracted with 300 ml THF. The solvents of the combined organic phases were removed and the residue was dried by azeotropic distillation with toluene. Yield: 31.2 g (106 mmol, 98%). R_f: 0.19 (chloroform/methanol 20:1). R_f: (1.2-*O*-isoproylidene-octadecanediol) 0.50 (hexane/diisopropylether 4:1). R_f (educt): 0.56 (hexane/diisopropylether 4:1). ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.7 Hz, 3 H, —CH_3); 1.24 (s, 28 H, alkyl-(4–17)-CH_2—); 1.40–1.51 (m, 2 H, alkyl-3-CH_2—); 1.65 (m, 1 H, —OH); 1.98 (m, 1 H, —OH); 3.46 (dd, $^2J_{1',1}$ = 11.1 Hz; $^3J_{1',2}$ = 8.1 Hz; 1 H, 1′-H); 3.68 (dd, $^2J_{1,1'}$ = 11.1 Hz; $^3J_{1,2}$ = 3.8 Hz; 1 H, 1-H); 4.76–4.84 (m, 1 H, 2-CH—). $C_{18}H_{38}O_2$ (286.50) calc. C 73.21% H 9.98% N 4.10; found C 73.10% H10.25% N 4.25.

3.7. *R- or S-1-O-Trityl-2-hydroxy-octadecane (R-10, S-10)*

The compounds R-**9** or S-**9**, 41.3 g (144 mmol), and 30.2 ml (21.9 g, 216 mmol) triethylamine were heated under reflux in 370 ml toluene. Tritylchloride, 42.2 g (165 mmol), in 180 ml toluene was added dropwise within 10 min. Heating under reflux was continued for a further 1 h. The solvents were removed and the residue was dispersed in 800 ml of a 1:1 mixture of saturated NaCl solution and Na_2CO_3 solution (40 g/l). After extraction with 800 ml diisopropylether containing 2 ml triethylamine, the organic phase was filtered through 100 g silica gel. The product was completely eluted with an additional 500 ml diisopropylether containing 0.5 ml triethylamine. The solvents were removed and the product was recrystallized from 800 ml pentane. Colourness needles were obtained after 48 h at −20°C. Yield: 68.6 g (130 mmol, 90%). R_f: 0.70 (diethylether/pentane 1:1). R_f (educt): 0.09 (diethylether/pentane 1:1) ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.7 Hz, 3 H, —CH_3); 1.23 and 1.26 (2 s, 24 H, alkyl-(6–17)-CH_2—); 1.37 (s (b), 2 H, 5-CH_2—); 1.43 (s (b), 2 H, 4-CH_2—); 1.85 (m, 2 H, 3-CH_2—); 2.30 (s (b); 1 H, —OH); 3.02 (dd, $^2J_{1',1}$ = 9.3 Hz; $^3J_{1',2}$ = 7.8 Hz; 1 H, 1′-H); 3.17 (dd, $^2J_{1,1'}$ = 9.3 Hz; $^3J_{1,2}$ = 3.3 Hz; 1 H, 1-H); 3.73–3.84 (m, 1 H, 2-CH—); 7.21–7.48 (m, 15 H. arom. H).

3.8. *R- or S-1-Hydroxy-2-O-benzyl-octadecane (R-11, S-11)*

The compounds R-**10** or S-**10,** 35.3 g (66.8 mmol), and 8.90 g (80.1 mmol) potassium-*tert*-butylate were dissolved in 300 ml THF. The solution was warmed to 50°C, and 9.22 ml (10.1 g, 80.1 mmol) benzylchloride in 100 ml THF was added dropwise. The mixture was stirred for ~2 h. After the addition of 4.0 g (36.0 mmol) potassium-*tert*-butylate, 4.1 ml (4.5 g, 36.0 mmol) benzylchloride in 40 ml THF was added dropwise. The mixture was stirred continuosly for ~2 h at 50°C. 300 ml NaCl solution (100 g/l) was added, and the phases were separated. The organic phase was freed from solvents and the residue was dissolved in 1.1 l dioxane/methanol (1:1). Sulfuric acid, 10 ml, was added slowly and the mixture was stirred for 1.5 h at 50°C. 800 ml potassium carbonate solution (40 g/l) and 100 ml saturated NaCl solution were added. The aqueous phase was extracted with 1 l and then with 300 ml diisopropylether. The sol-

vents of the combined organic phases were removed. The crude product was filtered over 500 g silica gel. First the tritylmethyl ether was eluted with hexane and hexane/diisopropylether (20:1) and then the product with diethylether/pentan (1:1). Yield: 21.0 g (55.7 mmol, 83%). R_f: 0.35 (diethylether/pentane 1:1). R_f: (1-*O*-trityl-2-*O*-benzyl-octadecane) 0.80 (diethylether/pentane 1:1). R_f (educt): 0.70 (diethylether/pentane 1:1). ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.6 Hz, 3 H, $-CH_3$); 1.24 (s, 30 H, $-CH_2-$);1.88–1.94 (s (b), 1 H,—OH); 3.47–3.59 (m, 2 H, 1-CH_2—); 3.65–3.75 (m, 1 H, 2-CH—); 4.54 (d, ^2J = 11.5 Hz; 1 H, benzyl-CH*H*); 4.65 (d, ^2J = 11.5 Hz; 1 H, benzyl-C*H*H); 7.28–7.39 (m, 5 H, arom. benzyl-H). IR (film): [cm^{-1}] 3450, 3020, 3060, 3080, 2915, 2840, 1490, 1460, 1450, 1065, 1090, 730, 690. $C_{25}H_{44}O_2$ (376.62) calc. C 79.73% H 11.78% O 8.49; found C 79.81% H 11.82% O 8.27.

3.9. R- or S-1-O-Phosphocholine-2-O-benzyl-octadecane (R-12, S-12)

A solution of 16.6 g (44.1 mmol) R- or S-**11** and 10.8 ml (7.80 g, 77.2 mmol) triethylamine in 60 ml THF was added dropwise to 4.63 ml (7.78 g, 50.7 mmol) phosphoroxychloride. The temperature of the vigorously stirred reaction mixture was controlled, and should not exeed 10°C. Stirring was continued for 10 min, then a solution of 4.7 ml (4.41 g, 58.7 mmol) *N*-methyl-ethanolamine and 10.8 ml (7.80 g, 77.2 mmol) triethylamine in 32 ml THF was added dropwise. The temperature of the reaction mixture should not exceed 40°C. The precipitate was filtered off and the filtrate was added to 10 ml 6 N HCl. After 10 min the pH value was adjusted to 7.0 with ammonia. The organic solvent was removed and the aqueous residue dissolved in 120 ml chloroform and 150 ml methanol. After extraction with 100 ml water, the solvents of the organic phase were removed. The residue was dissolved in 120 ml dichloromethane/2-propanol (1:3), and dimethylsulfate, 10.2 ml (13.3 g, 106 mmol), was added. The mixture was warmed to 40°C, and a solution of 15.4 g (110 mmol) potassium carbonate in 50 ml water was added in 1 min while the mixture was intensively stirred. Stirring was continued for 30 min at 40°C and the reaction mixture was cooled to 20°C. After phase separation, the solvents of the organic phase were removed and the residue was dissolved in 300 ml methanol and 250 ml chloroform. The organic phase was extracted with 250 ml water, the organic solvents were removed and the residue was dried by azeotropic distillation with toluene. The residue was dissolved in 25 ml dichloromethane and precipitated with 250 ml acetone. After 12 h the precipitated crude product was used in the next step without further purification. For analytical purposes, a small amount of the crude product was purified by column chromatography with chloroform/methanol/ammonia (6%) (60:40:6). R_f: 0.17 (chloroform/methanol/ammonia (6%) 60:40:6). ^{1}H-NMR ($CDCl_3$/CD_3OD 1:1): δ = 0.88 (t, ^3J = 6.5 Hz, 3 H, $-CH_3$); 1.28 (s (b), 28 H, $-CH_2-$); 1.57 (s (b), 2 H, 3-CH_2—); 3.22 (s, 9 H, $N(CH_3)_3$); 3.60 (m, 2 H, XX′ part of the XX′MM′ system (PO-CH_2—C*H*$_2$—N)); 3.60–3.70 (m, 1 H, H-1′); 3.83–3.93 (m, 1 H, H-1); 3.94–4.03 (m, 1 H, 2-CH—); 4.15–4.24 (m, 2 H, MM′ part of the MM′XX′ system (PO-C*H*$_2$—CH_2—N)); 4.60 (d, ^2J = 11.3 Hz, 1 H, benzyl-CH*H*—); 4.69 (d, ^2J = 11.3 Hz, 1 H, benzyl-C*H*H—); 7.25–7.24 (m, 5 H, arom. benzyl-H). IR (KBr): [cm^{-1}] 3060, 3020, 2910, 2840, 1470, 1240, 1080, 960.

3.10. R- or S-1-O-Phosphocholine-2-hydroxy-octadecane (R-13, S-13)

The crude R- or S-product **12** (~44.1 mmol) was dissolved in 200 ml methanol/THF (1:1). 5 g palladium on activated charocoal (5%) in 20 ml water and 20 ml 1N HCl were added. Hydrogenolysis was performed under intensive stirring at room temperature and atmospheric pressure until hydrogen uptake was completed. The catalyst was filtered off (membrane filter) and the pH value of the mixture was adjusted to 7.0 with ammonia. The solvents were removed. The residue was dissolved in 250 ml methanol and 200 ml chloroform and extracted twice with 200 ml NaCl solution (150 g/l). The solvents of the organic phase were removed and the residue was dried by azeotropic distillation with toluene. The residue was dispersed under reflux in 70 ml chloroform and precipitated with 700 ml acetone. After 48 h at 4°C the precipitate was collected by filtration.

Yield: 18.0 g (39.0 mmol, 90%). R_f: 0.12 (chloroform/methanol/ammonia (6%) 60:40:6). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.88 (t, ^3J = 6.4 Hz, 3 H, $-CH_3$); 1.28 (s (b), 28 H, $-CH_2-$); 1.46 (s (b), 2 H, 3-CH_2-); 3.22 (s, 9 H, $N(CH_3)_3$); 3.60 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.5 Hz, $J_{X'M}$ = 6.7 Hz, $J_{X'M'}$ = 2.1 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2-CH_2-N)); 3.64–3.80 (m, 2 H, 1-CH_2-); 3.84–3.93 (m, 1 H, 2-CH—); 4.21–4.30 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2-CH_2-N)). $C_{23}H_{50}O_5NP$ (453.63) calc. C 61.17% H 11.16% N 3.10 P 6.86; found C 61.15% H 11.27% N 3.15 P 6.85%.

3.11. R- or S-1-O-Phosphocholine-2-O-acyl-octadecanes (R-14, S-14, R-15, S-15, R-16, S-16, R-17, S-17, R-18, S-18)

R- or S-13, 2.00 g (4.44 mmol), 1.14 g (9.32 mmol) DMAP and X_1 ml (X_2 g, 8.88 mmol) of the fatty acid chlorides or of acetic anhydride were dissolved in alcohol-free chloroform. The acylations were performed in stoppered flasks at 30°C and supported by ultrasonication. After 16 h, 1 ml methanol was added and the solvents were removed. The residues were dissolved in X_3 ml chloroform and the crude products were precipitated at X_4°C by the addition of 250 ml acetone. The precipitates were collected by filtration and then dissolved in 70 ml chloroform and 80 ml methanol, respectively. The organic phases were extracted with 60 ml water, and the upper aqueous phases were re-extracted with 90 ml chloroform/methanol (2:1). In the case of the acetyl esters R- or S-14, the chloroform/methanol mixture was extracted with conc. NaCl solution. The solvents of the organic phases were removed and the residues were dried by azeotropic distillation with toluene. The products were finally purified by column chromatography with 200 g silica gel. First, apolar byproducts were eluted with chloroform/methanol/ammonia (60:40:2); then the polarity of the elution system was increased. The products R- and S-14 were finally eluted with chloroform/methanol/ammonia (50:50:9). The products were dried by azeotropic distillation with toluene. Yield: X_5 g.

3.11.1. 1-O-Phosphocholine-2-O-acetyl-octadecane (14)

R_f: 0.13 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 130–150°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 3 H, $-CH_3$); 1.27 (s, 28 H, $-CH_2-$); 1.54–1.67 (m, 2 H, 3-CH_2-); 1.98 (s, 3 H, acetyl-CH_3); 3.22 (s, 9 H, $N(CH_3)_3$); 3.60 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.9 Hz, $J_{X'M'}$ = 2.3 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2-CH_2-N)); 3.83–4.00 (m, 2 H, 1-CH_2-); 4.18–4.29 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2-CH_2-N)); 4.97–5.07 (m, 1 H, 2-CH—). IR (KBr): [cm^{-1}] 2940, 2870, 1745, 1480, 1250, 1100, 980. $C_{25}H_{52}O_6NP$ (493.66) calc. C 60.82% H 10.61% N 2.83%, found C 59.86% H 10.06% N 2.71%.

3.11.2. 1-O-Phosphocholine-2-O-laurinoyl-octadecane (15)

R_f: 0.32 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 130–150°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.27 (s, 44 H, acyl-(4–11) and alkyl-(4–17)-CH_2-); 1.56–1.69 (m, 4 H,

Table 1

Prod.	Chloride/anhydride	X_1 (ml)	X_2 (g)	X_3 (ml)	X_4 (°C)	X_5 (g, mmol, %)
14	Acetic anhydride	0.85	0.91	4	−25 (12 h)	1.83, 3.71, 84
15	Laurinoyl chloride	2.11	1.94	8	−20 (12 h)	1.98, 3.12, 70
16	Palmitoyl chloride	2.70	2.44	30	4 (2 h)	1.94, 2.81, 64
17	Oleoyl chloride	12.4[a]	2.67	10	−20 (12 h)	2.18, 3.00, 69
18	Stearoyl chloride	3.00	2.69	45	rt (24 h)	1.66, 2.31, 52

[a]0.35 M (THF).

acyl-3-CH_2— and alkyl-3-CH_2—); 2.32 and 2.33 (2 t, 2 H, signals of the diastereotopic acyl-2-CH_2 protons); 3.21 (s, 9 H, $N(CH_3)_3$); 3.59 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.8 Hz, $J_{X'M'}$ = 2.3 Hz, 2H, XX′ part of the XX′MM′ system (PO-CH_2-CH_2-N)); 3.82–3.98 (m, 2 H, alkyl-1-CH_2—); 4.18–4.29 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)); 4.98–5.08 (m, 1 H, alkyl-2-CH—). IR (KBr): [cm^{-1}] 2940, 2870, 1745, 1480, 1255, 1100, 980. $C_{35}H_{72}O_6NP$ (633.94) calc. C 66.31% H 11.44% N 2.20%, found C 65.32% H 11.58% N 2.16%.

*3.11.3. 1-O-Phosphocholine-2-O-palmitoyl-octadecane (**16**)*

R_f: 0.24 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 135–150°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.26 (s, 52 H, acyl-(4–15) and alkyl-(4–17)-CH_2—); 1.57–1.69 (m, 4 H, acyl-3-CH_2— and alkyl-3-CH_2—); 2.32 and 2.33 (2 t, 2 H, signals of the diastereotopic acyl-2-CH_2 protons); 3.21 (s, 9 H, $N(CH_3)_3$); 3.60 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.8 Hz, $J_{X'M'}$ = 2.2 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2-CH_2-N)); 3.83–3.99 (m, 2 H, alkyl-1-CH_2—); 4.17–4.30 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)); 4.98–5.09 (m, 1 H, alkyl-2-CH—). IR (KBr): [cm^{-1}] 2930, 2850, 1735, 1470, 1250, 1090, 970. $C_{39}H_{80}O_6NP$ (690.04) calc. C 67.88% H 11.68% N 2.02%, found C 67.56% H 11.78% N 2.02%.

*3.11.4. 1-O-Phospholcholine-2-O-oleoyl-octadecane (**17**)*

R_f: 0.28 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 120–135°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.27 and 1.34 (2 s, 50 H, acyl-(3–7, 12–17) and alkyl-(4–17)-CH_2—); 1.54–1.71 (m, 4 H, acyl-3-CH_2— and alkyl-3-CH_2—); 1.97–2.10 (m, 4 H, acyl-8 and 11-CH_2—); 2.33 and 2.34 (2 t, 2 H, signals of the diastereotopic acyl-2-CH_2 group); 3.22 (s, 9 H, $N(CH_3)_3$); d = 3.59 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.6 Hz, $J_{X'M'}$ = 2.2 Hz, 2H, XX′ part of the XX′MM′ system (PO-CH_2—CH_2—CH_2—N)); 3.83–4.00 (m, 2 H, alkyl-1-CH_2—); 4.18–4.32 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)); 4.98–5.10 (m, 1 H, alkyl-2-CH—); 5.30–5.40 (m, 2 H, acyl-9 and 10-CH=). IR (KBr): [cm^{-1}] 3020, 2940, 2875, 1740, 1470, 1250, 1100, 980. $C_{41}H_{82}O_6NP$ (716.08) calc. C 68.77% H 11.54% N 1.95%, found C 68.74% H 12.00% N 2.07%.

*3.11.5. 1-O-Phosphocholine-2-O-stearoyl-octadecane (**18**)*

R_f: 0.30 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 130–150°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.27 (s, 56 H, acyl-(4–17) and alkyl-(4–17)-CH_2—); 1.57–1.68 (m, 4 H, acyl-3-CH_2— and alkyl-3-CH_2—); 2.32 and 2.33 (2 t, 2 H, signals of the diastereotopic acyl-2-CH_2 protons); 3.22 (s, 9 H, $N(CH_3)_3$); 3.59 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.8 Hz, $J_{X'M'}$ = 2.2 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2—CH_2—N)); 3.83–3.99 (m, 2 H, alkyl-1-CH_2—); 4.18–4.30 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)); 4.98–5.12 (m, 1 H, alkyl-2-CH—). IR (KBr): [cm^{-1}] 2920, 2850, 1745, 1470, 1255, 1100, 970. $C_{41}H_{84}O_6NP$ (718.10) calc. C 68.57% H 11.79% N 1.95%, found C 67.99% H 11.59% N 2.02%.

*3.12. R- or S-1-Hydroxy-2-N-phthalimido-octadecane (R-**19**, S-**19**)*

R- or S-**10**, 45.0 g (85.1 mmol), 15.1 g (102 mmol) phthalimid and 32.4 g (123 mmol) triphenylphosphine were dissolved in 500 ml THF. The solution was cooled to 0°C, and 19.3 ml (21.6 g, 124 mmol) diethylazodicarboxylate in 100 ml THF was added dropwise. The reaction mixture was stirred for 2 h at room temperature, then 1 l K_2CO_3 solution (40 g/l) and 100 ml NaCl solution (saturated) was added. The aqueous solution was extracted with 1 l diisopropylether. The organic phase was extracted with 200 ml of NaCl solution, then the solvents of the organic phase were combined and removed. The residue was dissolved in 40 ml diisopropylether and freed from triphenylphosphineoxide by column chromatography on 550 g silica gel with hexane/diisopropylether

(2:1) + 1% triethylamine. After elution of triphenylphosphineoxide the 1-*O*-trityl-2-*N*-phthalimido-octadecane was eluted with diisopropylether. The organic solvent was removed and the residue was dissolved in 1 l dioxane/methanol (1:1). Sulfuric acid, 10 ml, was added slowly and the mixture was stirred for 1.5 h at 60°C. Potassium carbonate solution, 1 l (40 g/l), was added. The aqueous phase was extracted with 1 l diisopropylether. The solvent of the organic phase was removed. The crude product was filtered over 500 g silica gel. First the tritylmethyl ether was eluted with hexane/diisopropylether (4:1). Then the product was eluted with diethylether/pentan (1:1). Yield: 27.3 g (66.7 mmol, 77%). R_f: 0.29 (ethylacetate/hexane 1:2). R_f: (1-*O*-trityl-2-*N*-phthalimido-octadecane) 0.67 (ethylacetate/hexane 1:2). R_f (educt): 0.69 (ethylacetate/hexane 1:2). ^{1}H-NMR ($CDCl_3$): δ = 0.88 (t, ^3J = 6.6 Hz, 3 H, —CH_3); 1.22 and 1.28 (2 s, 28 H, —CH_2—); 1.40–1.51 (m, 2 H, alkyl-3-CH_2—); 1.52–1.68 (m, 1 H, 3-CH*H*—); 1.68–2.02 (m, 1 H, 3-C*H*H—); 2.66 (dd, $^3J_{OH,1}$ = 3.5 Hz; $^3J_{OH,1'}$ = 9.9 Hz; 1 H, —OH); 3.87 (ddd, $^2J_{1,1'}$ = 11.8 Hz; $^3J_{1,2}$ = 3.3 Hz, $^3J_{1,OH}$ = 3.5 Hz; 1 H, 1-H); 4.04 (ddd, $^2J_{1',1}$ = 11.8 Hz; $^3J_{1',2}$ = 7.4 Hz, $^3J_{1',OH}$ = 8.9 Hz; 1 H, 1'-H); 4.30–4.41 (m, 1 H, 2-CH—); 7.70–7.77 (m, 2 H arom. phthalimid-H_b); 7.81–7.88 (m, 2H. arom. phthalimid-H_a). IR (KBr): [cm^{-1}] 3500, 2910, 2840, 1695, 1470, 1395, 1050, 715. $C_{26}H_{44}O_3N$ (415.62) calc. C 73.21% H 9.98% N 4.10; found C 73.10% H 10.25% N 4.25%.

*3.13. R- or S-1-O-Phosphocholine-2-N-phthalimido-octadecane (R-**20**, S-**20**)*

A solution of 27.3 g (65.2 mmol) R- or S-**19** and 16.0 ml (11.6 g, 114 mmol) triethylamine in 90 ml THF was added dropwise under stirring to 9.90 ml (11.5 g, 75.0 mmol) phosphoroxychloride. The temperature of the reaction mixture should not exceed 10°C. Stirring was continued vigorously for 10 min. Then a solution of 7.00 ml (6.51 g, 86.7 mmol) *N*-methyl-ethanolamine and 16.0 ml (11.6 g, 114 mmol) triethylamine in 50 ml THF was added dropwise. The temperature of the reaction mixture was kept below 40°C. The precipitate was filtered off and the filtrate was added to 10 ml 6 N HCl. After 10 min the pH value was adjusted to 7.0 with ammonia. The organic solvent was removed. The aqueous residue was dissolved in 250 ml chloroform and 300 ml methanol and extracted with 200 ml water. The solvents of the organic phase were carefully removed. The residue was dissolved in 180 ml dichloromethane/2-propanol (1:3), and 15.0 ml (19.7 g, 157 mmol) dimethylsulfate was added. The mixture was warmed to 40°C, and 22.5 g (163 mmol) potassium carbonate in 75 ml water was added in 1 min by intensively stirring the reaction mixture. After an additional 30 min at 40°C the mixture was cooled to room temperature. The phases were separated, the solvents of the organic phase were removed and the residue was dissolved in 300 ml methanol and 250 ml chloroform. The organic phase was extracted with 250 ml water, the organic solvents were removed and the residue was dried by azeotropic distillation with toluene. The crude product was used in the next step without further purification. For analytical purposes, small amounts of the pure product were obtained by chromatographic purification on silica gel using chloroform/methanol/ammonia (60:40:10) for elution. R_f: 0.17 (chloroform/methanol/ammonia (6%) 60:40:6). ^{1}H-NMR ($CDCl_3$/CD_3OD 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 3 H, —CH_3); 1.22 and 1.26 (2 s, 28 H, —CH_2—); 1.63–1.78 (m, 1 H, 3-H'); 1.94–2.13 (m, 1 H, 3-H); 3.20 (s, 9 H, $N(CH_3)_3$); 3.60 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.7 Hz, $J_{X'M'}$ = 2.1 Hz, 2 H, XX' part of the XX'MM' system (PO-CH_2-CH_2-N)); 4.21–4.30 (m, 2 H, MM' part of the MM'XX' system (PO-CH_2—CH_2—N)); 4.35–4.57 (m, 3 H, 1-CH_2— and 2-CH—); 7.53–7.69 (m, 4 H, arom. phthalimid-H).

*3.14. R- or S-1-O-Phosphocholine-2-amino-octadecane (R-**21**, S-**21**)*

The crude products R- or S-**20** (~80.2 mmol) were dissolved in 250 ml 2-propanol/water/toluene (6:1:1.5). The solution was cooled to 0°C, and 6.0 g (160 mmol) $NaBH_4$ was added. The mixture was stirred overnight at room temperature. An additional 1.5 g $NaBH_4$ was added and the mixture was stirred vigorously for 1 h. The solvents of the

mixture were carefully removed in a rotatory evaporator under reduced pressure. The residue was dispersed with 300 ml water in a 4-l beaker. Under intensive stirring and cooling to 30°C with an ice bath, 300 ml conc. HCl were added *slowly*. The dispersion was stirred for 10 h at 80°C. The mixture was cooled to room temperature and the pH value was adjusted to 9.0, first with solid NaOH, then with 6N NaOH. The mixture was extracted with 2.4 l chloroform/methanol (1:1). In addition, the aqueous phase was extracted twice with 500 ml chloroform. The material partly precipitated in the chloroform phases. The solvents of the combined organic phases were removed. The residue was dispersed in 100 ml conc. HCl, and 450 ml acetone was added. After cooling to 0°C for 1 h the precipitate was filtered off, 4.5 l acetone was added and the product was kept at −20°C for 24 h. The precipitate was filtered off and dissolved in 300 ml methanol and 280 ml chloroform. The solution was extracted with 300 ml ammonia (12%). The upper phase was additionally extracted with 200 ml chloroform/methanol (8:1). The organic solvents were removed and the residue was dried by azeotropic distillation with toluene. Yield: 25.2 g (56.0 mmol, 70%). R_f: 0.05 (chloroform/methanol/ammonia (6%) 60:40:6). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.88 (t, ^3J = 6.7 Hz, 3 H, $-CH_3$); 1.27 (s (b), 30 H, $-CH_2-$); 2.92–3.03 (m, 1 H, 1′-H); 3.20 (s, 9 H, $N(CH_3)_3$); 3.57 (m, 3 H, H-1 and XX′ part of the XX′MM′ system (PO-CH_2-CH_2-N)); 3.84–3.95 (m, 1 H, 2-CH—); 4.20–4.31 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)). IR (KBr): [cm^{-1}] 2950, 2860, 1640, 1470, 1250, 1100, 1060. $C_{23}H_{51}O_4N_2P$ (450.64) calc. C 61.30% H 11.55% N 6.21%, found C 60.55% H 11.17% N 5.76%.

3.15. R- or S-1-O-Phosphocholine-2-N-acyl-octadecanes (R-22, S-22, R-23, S-23, R-24, S-24, R-25, S-25, R-26, S-26)

R- or S-**21**, 2.00 g (4.44 mmol), 1.14 g (9.32 mmol) DMAP and X_1 ml (X_2 g, 8.88 mmol) of the fatty acid chloride or of acetic anhydride were dissolved in alcohol-free chloroform. The acylations were performed in stoppered flasks at 30°C and supported by ultrasonication for 10 h. 1 ml methanol was added and the solvents were removed. The residues were dissolved in X_3 ml chloroform and the crude products were precipitated at X_4°C by the addition of 250 ml acetone. The crude products were filtered off and were dissolved in 70 ml chloroform and 80 ml methanol. The organic phases were extracted with 60 ml water, and the upper aqueous phases were re-extracted with 90 ml chloroform/methanol (2:1). For complete extraction of R- or S-**22** the aqueous phases were extracted three more times with 50 ml chloroform/methanol (8:1). The solvents of the combined organic phases were removed and the residues were dried by azeotropic distillation with toluene. The products were finally purified by column chromatography on 200 g silica gel. First, the apolar byproducts were eluted with chloroform/methanol/ammonia (60:40:2), then polarity was increased and the products were eluted with chloroform/methanol/ammonia (60:40:7). R- and S-**22** were eluted with chloroform/methanol/ammonia (50:50:9). The products were dried by azeotropic distillation with toluene. Yield: X_5 g.

Table 2

Prod.	Chloride/anhydride	X_1 (ml)	X_2 (g)	X_3 (ml)	X_4 (°C)	X_5 (g, mmol, %)
22	Acetic anhydride	0.85	0.91	4	−20 (12 h)	1.56, 3.20, 72
23	Laurinoyl chloride	2.11	1.94	8	4 (12 h)	1.87, 3.00, 67
24	Palmitoyl chloride	2.70	2.44	30	rt (2 h)	2.33, 3.38, 76
25	Oleoyl chloride	12.4[a]	2.67	10	4 (12 h)	2.40, 3.36, 76
26	Stearoyl chloride	3.00	2.69	45	rt (3 h)	2.46, 3.43, 77

[a]0.35 M (THF).

*3.15.1. 1-O-Phosphocholine-2-N-acetyl-octadecane (**22**)*

R_f: 0.12 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 200–210°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 3 H, $-CH_3$); 1.29 (s, 28 H, $-CH_2-$); 1.35–1.60 (s (b), 2 H, 3-CH_2—); 1.98 (s, 3 H, acteamide-CH_3); 3.22 (s, 9 H, $N(CH_3)3$); 3.59 (quint, J_{XM} = 4.6 Hz, $J_{XM'}$ = 4.6 Hz, $J_{X'M}$ = 6.7 Hz, $J_{X'M'}$ = 2.5 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2-CH_2-N)); 3.77–3.91 (m, 2 H, 1-CH_2—); 3.92–4.03 (m, 1 H, 2-CH—); 4.11–4.30 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2-CH_2-N)). IR (KBr): [cm^{-1}] 3250, 3060, 2910, 2840, 1650, 1470, 1240, 1080, 970. $C_{25}H_{53}O_5N_2P$ (492.68) calc. C 60.95% H 10.84% N 5.69%, found C 60.08% H 11.07% N 5.65%.

*3.15.2. 1-O-Phosphocholine-2-N-laurinoyl-octadecane (**23**)*

R_f: 0.31 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 183–199°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.29 (s, 46 H, acyl-(3–11) and alkyl-(4–17)-CH_2—); 1.35–1.60 (s (b), 2 H, 3-CH_2—); 1.53–1.68 (m, 2 H, alkyl-3-CH_2—); 2.13–2.28 (m, 2 H, acyl-2-CH_2—); 3.22 (s, 9 H, $N(CH_3)_3$); 3.59 (quint, J_{XM} = 4.4 Hz, $J_{XM'}$ = 4.4 Hz, $J_{X'M}$ = 6.8 Hz, $J_{X'M'}$ = 2.4 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2—CH_2—N)); 3.75–3.90 (m, 2 H, alkyl-1-CH_2—); 3.93–4.05 (m, 1 H, alkyl-2-CH—); 4.20–4.31 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)). IR (KBr): [cm^{-1}] 3300, 3060, 2940, 2860, 1650, 1470, 1250, 1100, 970. $C_{35}H_{73}O_5N_2P$ (632.95) calc. C 66.41% H 11.62% N 4.42%, found C 65.23% H 11.70% N 4.36%.

*3.15.3. 1-O-Phosphocholine-2-N-palmitoyl-octadecane (**24**)*

R_f: 0.25 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 201°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.88 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.27 (s, 50 H, acyl-(3–15) and alkyl-(4–17)-CH_2—); 1.54–1.70 (m, 2 H, alkyl-3-CH_2—); 2.13–2.28 (m, 2 H, acyl-2-CH_2—); 3.22 (s, 9 H, $N(CH_3)_3$); 3.61 (quint, J_{XM} = 4.5 Hz, $J_{XM'}$ = 4.5 Hz, $J_{X'M}$ = 6.8 Hz, $J_{X'M'}$ = 2.4 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2—CH_2—N)); 3.78–3.90 (m, 2 H, alkyl-1-CH_2—); 3.93–4.05 (m, 1 H, alkyl-2-CH—); 4.18–4.31 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)). IR (KBr): [cm^{-1}] 3260, 3040, 2910, 2850, 1640, 1470, 1250, 1090, 970. $C_{39}H_{81}O_5N_2P$ (689.06) calc. C 67.98% H 11.84% N 4.06%, found C 67.69% H 11.78% N 4.03%.

*3.15.4. 1-O-Phosphocholine-2-N-oleoyl-octadecane (**25**)*

R_f: 0.26 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 168°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.89 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.26 and 1.34 (2 s, 50 H, acyl-(3–7, 12–17) and alkyl-(4–17)-CH_2—); 1.53–1.70 (m, 2 H, alkyl-3-CH_2—); 2.12–2.28 (m, 2 H, acyl-2-CH_2—); 3.22 (s, 9 H, $N(CH_3)3$); 3.59 (quint, J_{XM} = 4.4 Hz, $J_{XM'}$ = 4.4 Hz, $J_{X'M}$ = 6.7 Hz, $J_{X'M'}$ = 2.4 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2—CH_2—N)); 3.76–3.89 (m, 2 H, alkyl-1-CH_2—); 3.93–4.05 (m, 1 H, alkyl-2-CH—); 4.17–4.29 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)). IR (KBr): [cm^{-1}] 3020, 2940, 2875, 1650, 1480, 1250, 1100, 980. $C_{41}H_{83}O_5N_2P$ (715.10) calc. C 68.86% H 11.69% N 3.91%, found C 68.32% H 11.90% N 3.72%.

*3.15.5. 1-O-Phosphocholine-2-N-stearoyl-octadecane (**24**)*

R_f: 0.19 (chloroform/methanol/ammonia (6%) 60:40:6). F_p: 193–202°C (decomp.). ^{1}H-NMR ($CDCl_3/CD_3OD$ 1:1): δ = 0.88 (t, ^3J = 6.7 Hz, 6 H, acyl and alkyl-CH_3); 1.27 (s, 58 H, acyl-(3–17) and alkyl-(4–17)-CH_2—); 1.56–1.69 (m, 2 H, alkyl-3-CH_2—); 2.12–2.28 (m, 2 H, acyl-2-CH_2—); 3.22 (s, 9 H, $N(CH_3)_3$); 3.58 (quint, J_{XM} = 4.5 Hz, $J_{XM'}$ = 4.5 Hz, $J_{X'M}$ = 6.7 Hz, $J_{X'M'}$ = 2.3 Hz, 2 H, XX′ part of the XX′MM′ system (PO-CH_2—CH_2—N)); 3.76–3.89 (m, 2 H, alkyl-1-CH_2—); 3.92–4.08 (m, 1 H, alkyl-2-CH—); 4.18–4.29 (m, 2 H, MM′ part of the MM′XX′ system (PO-CH_2—CH_2—N)). IR (KBr): [cm^{-1}] 2930, 2860, 1650, 1480, 1250, 1100, 980. $C_{41}H_{85}O_5N_2P$ (717.11) calc. C 68.67% H

Table 3

No.	Alcohol	X_1 (mg)	X_2 (mg)	X_3 (mmol)	X_4 (%)	R_f
1	**11**	94	147	0.247	99	0.62
2	**19**	105	117	0.185	74	0.41
3	**10**	132	134	0.195	78	0.43

^{19}F-NMR ($CDCl_3$) (d): 'Mosher ester' of

rac-**11**	R-**11**	S-**11**	*rac*-19	R-**19**	S-**19**	*rac*-**10**	R-**10**	S-**10**
−71.83	−71.82	—	−72.11	−72.11	—	−72.06	−72.06	—
−72.26	—	−72.26	−72.58	—	−72.58	−72.12	—	−72.12

11.94% N 3.90%, found C 67.92% H 11.97% N 4.06%.

*3.16. 'Mosher ester' ((R)-(+)-a-methoxy-a-triflouromethyl-phenylacetic acid ester) of the alcohols R-**10**, S-**10**, R-**11**, S-**11**, R-**19**, S-**19** and their racemic mixtures, respectively*

X_1 mg (0.250 mmol) of the respective alcohol and 79.6 mg (0.386 mmol) DCC, 82.0 mg (0.350 mmol) (R)-(+)-a-methoxy-a-triflouromethyl-phenylacetic acid and 2 mg DMAP were stirred for ~3 h in 1.5 ml chloroform (alcohol-free). The solvent was removed and the products were purified chromatographically on a 25-g silica gel column. The 'Mosher esters' of the alcohols **11** and **19** were eluted with ethylacetate/hexane (1:4), and the 'Mosher esters' of the alcohol **10** were eluted with diisopropylether/hexane (1:7). Yield: X_2 mg (X_3 mmol, X_4%).

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