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CHEMICAL SYNTHESIS OF SOME LECITHIN ANALOGUES POTENTIAL INHIBITORS OF PHOSPHOLIPASE A

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The chemical synthesis of a number of lecithin analogues is described. These compounds were prepared to study inhibitory properties in the enzymatic hydrolysis of normal lecithins by porcine pancreatic phospholipase A. The structures of the synthesized products resemble very much those of the normal lecithins, but differ in most cases in that part of the molecule which is supposed to be important either for the binding with phospholipase A or for the catalytic reaction. The synthesized compounds include stereo- and structural isomers of the normal lecithins (= substrates for phospholipase A) and lecithins modified in the alkyl chain, the ester bond, the glycerol backbone, and the phosphate moiety.

I. Introduction

3-*sn*-phosphatidylcholines are usually good substrates for phospholipase A. Sometimes, especially in the case of compounds with long-chain fatty acid esters, addition of a detergent (i.e. bile salts) or organic solvents is required for enzymatic hydrolysis. Recently De Haas et al.[1]) reported the use of short-chain lecithins as substrates for porcine pancreatic phospholipase A. In particular, the lecithins with a chain length varying from 6 to 8 carbon atoms were found to be very useful to study enzyme parameters as maximal velocity and binding constants. These authors were able to evaluate a reaction mechanism for the hydrolysis of lecithins. The main advantage of these short-chain lecithins is that addition of a detergent is not required.

Preliminary studies on the inhibition of enzyme action with substrate analogues showed that compounds with a net charge different from that of the substrates or with acyl chain lengths very different from those of the substrates had a pronounced effect on the enzyme parameters. It was, however, in most cases impossible to separate inhibitory properties due to

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a structural modification from inhibitory or activating properties due to alteration of the physico-chemical properties of the lipid-water interface. In order to study, therefore, purely the inhibition caused by the structural modification, it was found preferable to use compounds which resemble, from a physico-chemical point of view, as much as possible the normal substrates. This means that all compounds, except one, have a phosphorylcholine moiety and a chain length varying from 6 to 8 carbon atoms.

In this paper we report the chemical synthesis of a number of lecithin analogues, which differ from the normal substrate in that part of the molecule which is supposed to be important for the enzymatic action. The minimal substrate requirements which could be established from hydrolysis experiments of a large variety of phospholipids are a phosphate moiety (including one negative ionization) bound to a *vic*-glycol with a carboxylic ester bond adjacent to it[2]). Of the optically active compounds, only one of the isomers is a substrate. It is mainly in this part of the lecithin molecule that modifications were made. (See encircled part of fig. 1.)

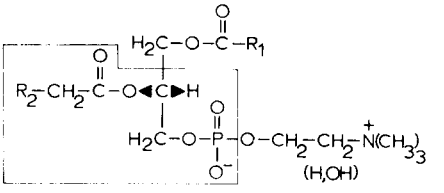


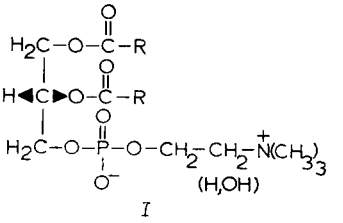
Fig. 1. Structure of a 3-*sn*-phosphatidylcholine.

A. Stereoisomers of the normal substrate

The 1-*sn*-phosphatidylcholines (I, fig. 2) are not hydrolyzed by phospholipase A[2]). This fact offers the possibility of obtaining these compounds by preparing a racemic mixture of the lecithin and by removal of the 3-*sn*-phosphatidylcholine by means of a hydrolysis with phospholipase A. Two procedures were employed to prepare the racemic lecithins: (1) a chemical synthesis involving the acylation of *rac*-1-deoxy-1-iodoglycerol followed by a reaction with the silver salt of dibenzylphosphate, catalytic hydrogenolysis and conversion of the phosphatidic acid into a lecithin, and (2) acylation of *rac*-glycero-1-phosphate, followed by the introduction of the choline moiety. In this way the dihexanoyl-, diheptanoyl-, and dioctanoyl derivatives of 1-*sn*-phosphatidylcholine were prepared.

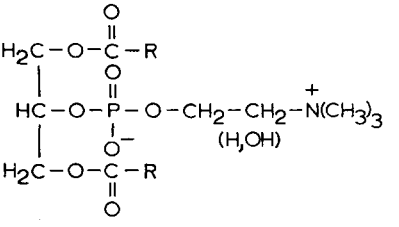
B. Structural isomer of the normal substrate

rac-1,3-diacylglycerol-2-phosphorylcholines (II, fig. 3) were already found



I

Fig. 2. Structure of 1-*sn*-phosphatidylcholine (I)



II

Fig. 3. Structure of *rac*-1,3-diacylglycero-2-phosphorylcholine (II).

to be substrates for phospholipase A [3]). However, they are hydrolyzed much more slowly than the 1,2-diacylglycerol-3-phosphorylcholines. So it was interesting to see which enzyme parameter was affected by the use of the structural isomer. The chemical synthesis was performed by preparing a 1,3-diglyceride followed by the introduction of the phosphorylcholine moiety.

C. Modification of the acyl-chain at the 2-position of phosphatidylcholines

Introduction of a branch in the acylchain at the methylene group adjacent to the carbonyl function was supposed to have influence on the kinetic parameters, e.g. by steric hindrance. In this series a 2-monomethylacyl-, a 2,2-dimethylacyl- and a benzoyl derivative were prepared (see fig. 4). For reasons of comparison also a 3,3-dimethylacyl ester was prepared. The introduction of branched-chain fatty acids into 1-acyl lysolecithins appeared to be more difficult than with straight-chain fatty acids. The benzoyl and 2,2 dimethylacyl group could not be introduced at all and the corresponding lecithins were, therefore, prepared starting from glycerol iodohydrin.

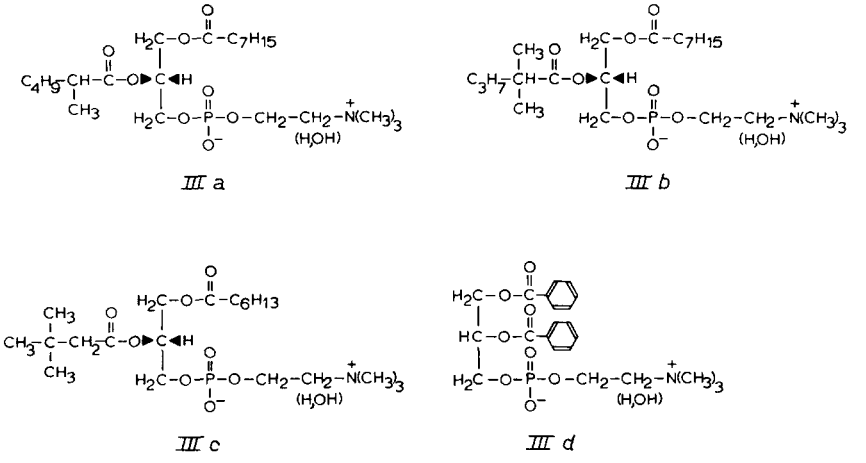


Fig. 4. Structures of lecithins medified in the acylchain at the 2-position. IIIa: 1-octanoyl-2-(2 methyl)hexanoyl-*sn*-glycero-3-phosphorylcholine. IIIb: 1-octanoyl-2-(2,2-dimethyl)-pentanoyl-*sn*-glycero-3-phosphorylcholine. IIIc: 1-heptanoyl-2-(3,3-dimethyl)butyryl-*sn*-glycero-3-phosphorylcholine. IIId: *rac*-1,2-dibenzoylglycero-3-phosphorylcholine.

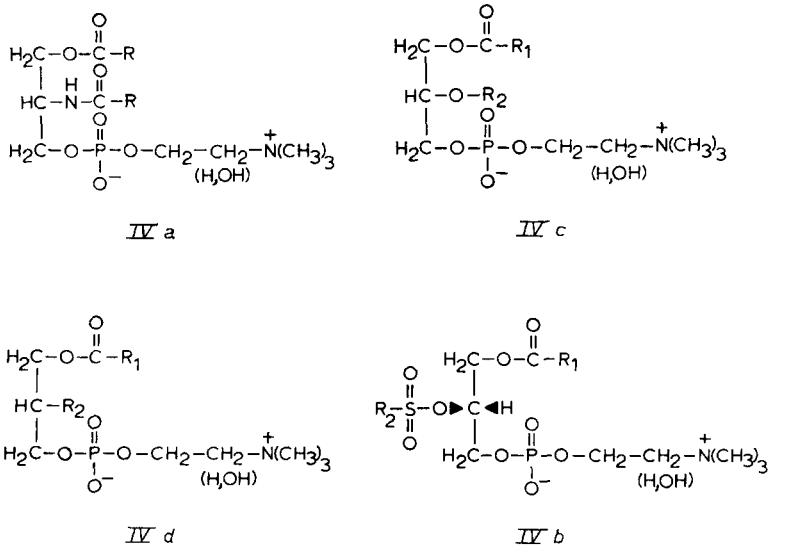


Fig. 5. Structures of lecithins modified in the ester bond at the 2-position. IVa: *rac*-1-acyl-2-acylamido-2-deoxyglycero-3-phosphorylcholine. IVb: 1-acyl-2-alkanesulfonyl-*sn*-glycero-3-phosphorylcholine. IVc: *rac*-1-acyl-2-alkylglycero-3-phosphorylcholine. IVd: *rac*-1-acyl-2-alkyl-2-deoxyglycero-3-phosphorylcholine.

D. Modification of the ester linkage at the 2-position of phosphatidylcholines

The ester linkage at the 2-position is apparently very important for enzymatic hydrolysis[2]). So modification of this ester will certainly have a dramatic effect on enzymatic action. The formation of an amide bond instead of an ester bond (IVa, fig. 5) leaves the carbonyl function intact, but judged from the chemical difference between an ester and an amide, a strong effect on the kinetic parameters was anticipated. In another compound of this series, the carbonylester was replaced by a sulfonyl ester (IVb). Two compound lacking the ester linkage completely are an ether derivative and an alkyl derivative with a C–O–C and C–C linkage respectively between the alkyl chain and the glycerol backbone (IVc and IVd). All four compounds were obtained by a complete chemical synthesis.

E. Compounds without an acyl-chain at the 2-position of phosphatidylcholines

One of the reaction products of enzymatic hydrolysis is a 1-acyl lysolecithin. Because of the fact that the lyso derivative of the short-chain substrates used in the kinetic investigations are completely water soluble and probably not

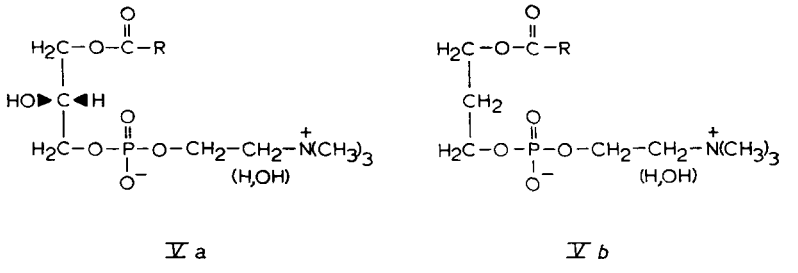
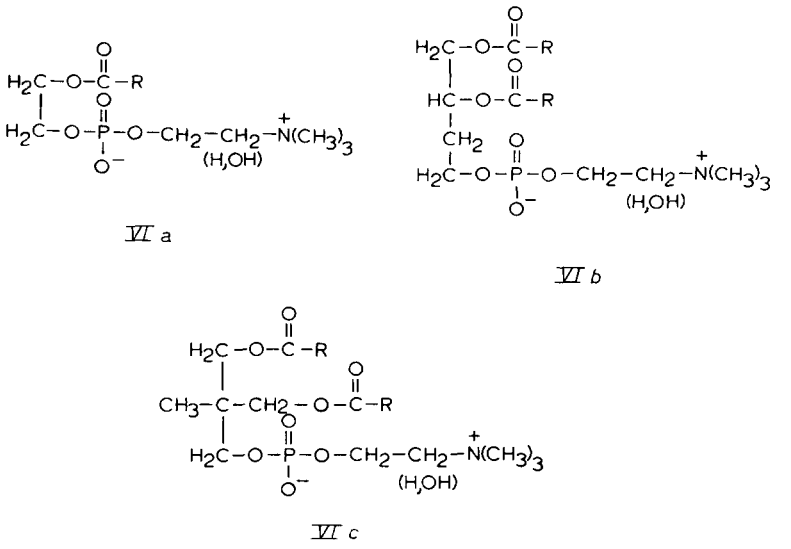


Fig. 6. Structures of lecithins lacking an acyl chain at the 2-position. Va: 1-acyl-*sn*-glycero-3-phosphorylcholine. Vb: 1-acyl-2-deoxyglycero-3-phosphorylcholine.

built in in the substrate micelles (which are supposed to be the only species to be hydrolyzed by phospholipase A), long-chain lysolecithins were prepared as well as a compound lacking the hydroxyl function at the 2-position (2-deoxy-lysolecithin, Vb, fig. 6).

F. Modification of the glycerol backbone

Although not having a modification in the encircled part (fig. 1) a glycol-lecithin (VIa, fig. 7) was supposed to have some influence on enzymatic hydrolysis of a good substrate. Two other compounds in this series with a backbone of 1,2,4-butane triol (VIb) and 1,1,1-trihydroxylmethylethane (VIc) both have an increased distance between the phosphate moiety and the



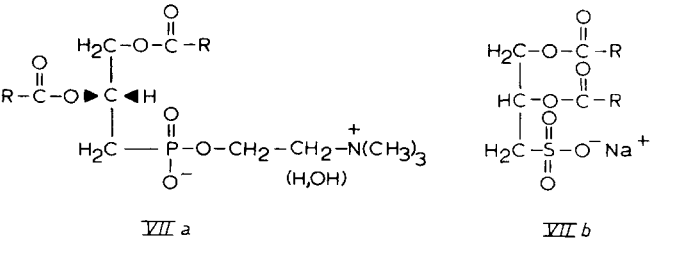
VI a VI b VI c

Fig. 7. Structures of lecithins modified in the glycerol backbone. VIa: 1-acylglycol-2-phosphorylcholine. VIb: *rac*-1,2-diacylbutanetriol-4-phosphorylcholine. VIc: 2,2-(diacylhydroxymethyl)propanol-1-phosphorylcholine.

carbonyl function. The effect of this modification might give some information about the importance of the geometry of the substrate molecule. The latter compounds were synthesized starting from the corresponding polyalcohols.

G. Modification of the phosphate group

Another possibility of changing the distance between the negative ionization and the carbonyl function (at the 2-position) was thought to be found when the phosphate was converted into a phosphonate group with a direct C–P bond between the "glycerol" backbone and phosphorus. For this purpose two phosphono analogues of 1,2-diacylglycerol-3-phosphorylcholine (VIIa,



VII a VII b

Fig. 8. Structures of phospholipids with a modified phosphate moiety. VIIa: 1,2-diacyl-3-deoxy-*sn*-glycero-3-phosphonylcholine. VIIb: *rac*-1,2-diacyl-3-deoxyglycero-3-sulfonic acid (as sodium salt).

fig. 8) were prepared. A compound which does not contain a choline moiety and does not properly fit in this context, is a sulfonic acid derivative of a 1,2-diglyceride (VIIb). This sulfonic acid is particularly interesting because it was anticipated that this product was not a substrate for phospholipase A, but it turned out to be a relatively good substrate. Apparently the phosphate moiety itself is not a prerequisite for hydrolysis by phospholipase A. The negative charge, however, seems to be important because phosphotriesters of phospholipids are not degraded at all[2]).

In a subsequent paper the results of the kinetic studies with these substrate analogues will be reported.

II. Experimental part

A. Methods

Element analyses were carried out under supervision of Mr. W. J. Buis at the Micro-Analytical Department of the Institute for Organic Chemistry TNO, Utrecht, The Netherlands. Melting points were determined on a Leitz Mikroskopheiztisch 350 and are uncorrected. Optical rotations were measured in a Lichtelektrisches Präzisions Polarimeter, with a limit of accuracy 0.005° (Carl Zeiss, Jena, Germany).

The purity of intermediates and end products was checked by thin-layer chromatography on microscope slides coated with silica gel G (Merck, Germany). Column chromatography was performed on Mallinckrodt silicic acid sieved to 60–140 mesh and activated at 120° for several hours before use. Alumina (neutral, activity grade I) was a product of M. Woelm (Germany) and was washed repeatedly with chloroform-methanol (1:1, v/v) before use. All lecithins were finally purified by column chromatography on silicic acid with mixtures of chloroform and methanol to elute the products followed by a rapid elution over alumina with chloroform-methanol (1:1, v/v) as solvent.

Identification methods used were: iodine for all lipids, the molybdate reagent for phosphate containing compounds, the ninhydrin reagent for free amino groups, the periodate-Schiff reagent for vicinal hydroxyl groups and charring with 30% (v/v) H_2SO_4 (only on thin-layer plates).

B. Materials

Straight-chain fatty acids, 2,2-dimethylvaleric acid, 1-octanol, 1-bromoctane, triethylphosphite, *rac*-glycero-1-phosphate (di-sodium salt), benzyloxycarbonylchloride and dicyclohexylcarbodiimide (DCC) were products of Fluka AG (Switzerland). 2-methylhexanoic acid (K+F Laboratories,

U.S.A.), L-serine and diethyloxalate (Baker Chemical Co., U.S.A.), 2,4,6-trisopropylbenzene sulfonylchloride (Aldrich, U.S.A.) and trichloroacetonitrile (Merck, Germany). Dibenzylphosphite, 1,2,4-butanetriol, 1,1,1-trihydroxymethylethane and phospholipase A_2 from *Crotalus adamanteus* were purchased from Light and Co., England.

3,3-dimethylbutyric acid was prepared as described by Hommelen[4]). Fatty acid anhydrides were synthesized from the corresponding fatty acids with DCC[5]). The preparation of 3-deoxy-3-iodo-*sn*-glycerol and *rac*-1-deoxy-1-iodo-glycerol have been described by Baer and Fischer[6]) and Turner et al.[7]), respectively. *rac*-2-benzyl-glycerol was prepared via *rac*-1,3-benzylideneglycerol as described by Porck and Craig[8]). Dibenzylphosphoric acid and its silver salt were prepared according to the procedure of Atherton et al.[9]). 2-bromoethylphosphoryldichloride was obtained as described by Hirt and Berchtold[10]), and choline tosylate as described by Rosenthal[11]). Phospholipase A_2 from porcine pancreas was isolated as described earlier[13]). Phospholipase C from *Bacillus cereus* was a gift from Drs. R.F.A. Zwaal and B. Roelofsen.

III. Syntheses

A. 1-sn-*phosphatidylcholines – stereoisomers*

1. Synthesis starting from rac-*1-deoxy-1-iodoglycerol*

20 g of *rac*-1-deoxy-1-iodoglycerol were acylated at 0° with 31 g of heptanoylchloride in the presence of 17 ml of pyridine and chloroform as solvent. After the completion of the reaction (approximately 15 hr) the chloroform solution was washed successively with 0.5 N H_2SO_4, 5% bicarbonate solution and water. After drying over sodium sulfate, the solvent was removed by evaporation. The residue was thoroughly dried in vacuo over P_2O_5. Yield 30 g (70%).

27 g of the *rac*-1-deoxy-1-iodo-2,3-diheptanoylglycerol were reacted with 25 g of the silver salt of dibenzylphosphoric acid in boiling benzene. After 4 hr the silver iodide was removed by centrifugation and the supernatant washed with 0.5 N H_2SO_4, 5% $NaHCO_3$ solution and water. The benzene solution was dried over sodium sulfate and the solvent removed in vacuo. Yield 22 g (80%).

The obtained phosphotriester (8.5 g) was subjected to catalytic hydrogenolysis with 10% Pd/C as catalyst to remove the benzyl groups. This gave 4.6 g (81%) of pure *rac*-1,2-diheptanoylglycero-3-phosphoric acid, after chromatography on silicic acid.

4,6 g of this phosphatidic acid was reacted with choline tosylate (21 g) in the presence of trichloroacetonitrile (50 ml) as condensing agent in

pyridine, as described by Rosenthal[11]). After 48 hr at 55° most of the phosphatidic acid was converted into lecithin. After evaporation of the solvent and excess of trichloroacetonitrile the dark brown reaction mixture was dissolved in methanol and treated with active carbon overnight. This resulted in a slightly yellowish solution which was percolated over a mixture of Amberlites IRC-50 and IR-45. Chromatography on silicic acid yielded a pure and colorless lecithin. Yield 4.5 g (80%).

The so-obtained *rac*-1,2-diheptanoylglycero-3-phosphorylcholine was treated with 100 mg of phospholipase A_2 (*Crotalus adamanteus*) in a borate buffer (0.1 M) containing 5 mM $CaCl_2$. After 24 hr the mixture was evaporated in vacuo. The residue was dissolved in absolute ethanol and heated to boiling temperature and centrifuged to remove denatured protein. This procedure was repeated twice. The mixture was then separated by column chromatography on silicic acid with mixtures of chloroform and methanol. This procedure yielded 2.1 g of pure 1-*sn*-phosphatidylcholine (93%) and 1.6 g of lysolecithin (92%). The lecithin was indistinguishable from the corresponding 3-*sn*-phosphatidylcholine by chromatographic means. $[\alpha]_D^{20} = -9.9°$ (c,8 in chloroform-methanol 4:1, v/v). The corresponding 3-*sn*-phosphatidylcholine (diheptanoyl) had $[\alpha]_D^{20} = +9.8°$ (c,10 in chloroform-methanol 4:1, v/v).

2. Synthesis starting from rac-*glycero-1-phosphoric acid*

Acylation of *rac*-glycero-1-phosphoric acid was carried out as described by Lapidot et al.[14]), with a mixture of the anhydride of the desired fatty acid and the tetraethylammonium salt of the same fatty acid. The obtained phosphatidic acids were purified by column chromatography on silicic acid. Introduction of the choline moiety was done as described above (Ia). The pure racemic lecithins were then treated with snake venom phospholipase A_2, leaving the 1-*sn*-phosphatidylcholines intact, while the stereoisomer was broken down to a 1-acyl-*sn*-glycero-3-phosphorylcholine (lysolecithin) and fatty acid. With this method the 2,3-dihexanoyl-, 2,3-diheptanoyl- and 2,3-dioctanoyl-*sn*-glycero-1-phosphorylcholines were prepared. The yields of the end products varied from 20 to 50% based on racemic glycerophosphate.

Optical rotations:

2,3-dihexanoyl-*sn*-glycero-1-phosphorylcholine: $[\alpha]_D^{20} = -10.5°$ (c,9 in chloroform-methanol 4:1, v/v).
2,3-diheptanoyl-*sn*-glycero-1-phosphorylcholine: $[\alpha]_D^{20} = -9.8°$ (c,8 in cholroform-methanol 4:1, v/v).
2,3-dioctanoyl-*sn*-glycero-1-phosphorylcholine: $[\alpha]_D^{20} = -9.35$ (c,9 in chloroform-methanol 1:1, v/v).

The second method will sometimes give rise to complications. It was found that especially with very short-chain fatty acids (hexanoic acid and lower homologues) appreciable amounts of 1,3-diacylglycero-2-phosphorylcholine can be formed due to isomerization of *rac*-glycero-1-phosphate during the reaction. Because of the complete hydrolysis of these side products by phospholipase A, the remaining lecithin is pure 1-*sn*-phosphatidylcholine. The yields in these cases are, however, lower.

B. rac-*1,3-diheptanoylglycero-2-phosphorylcholine – structural isomer*

To a solution of 4.5 g of *rac*-2-0-benzylglycerol in anhydrous chloroform at 0° were added 5 ml of pyridine and 7.4 g of heptanoylchloride. When the reaction was complete (3 hr) the mixture was poured into ice cold 0.5 N H_2SO_4, the organic phase washed with a 5% $NaHCO_3$ solution and water. The solution was dried over sodium sulfate and the solvent removed by evaporation. *rac*-1,3-diheptanoyl-2-0-benzylglycerol was obtained as a slightly yellowish oil (yield 76%) and was used without further purification.

The benzyl group was released by catalytic hydrogenolysis with 10% palladium on coal as a catalyst in absolute ethanol-acetic acid (1:1, v/v). The catalyst was removed by filtration and the filtrate brought to dryness in vacuo. The 1,3-diglyceride was obtained as a colorless oil in a yield of 95%.

5.6 g of the 1,3-diglyceride was reacted with 5.7 g of 2-bromoethylphosphoryldichloride in the presence of 6.4 g of triethylamine in chloroform as described by Hirt and Berchtold[10]). The obtained 2-bromoethylester of phosphatidic acid was further converted into a lecithin with trimethylamine, as described by Eibl et al.[15]). Chromatography on silicic acid yielded *rac*-1,3-diheptanoyl-glycero-2-phosphorylcholine (II) as a waxy solid in a yield of 21%.

Found: C = 53.7; H = 9.5; N = 3.0; P = 6.1.
Calc. for $C_{22}H_{46}NO_9P$ (M = 499.6): C = 53.0; H = 9.3; N = 2.8; P = 6.2.

C. Lecithins with a modification in the acylchain at the 2-position

*1. 1-octanoyl-2-(2-methyl)hexanoyl-*sn*-glycero-3-phosphorylcholine (IIIa)*

3.2 g of 1-octanoyl-*sn*-glycero-3-phosphorylcholine as $CdCl_2$-adduct (prepared from 1,2-dioctanoyllecithin with phospholipase A_2 from *Crotalus adamanteus*) was acylated in anhydrous chloroform with 4.8 g of 2-methylhexanoylchloride (bp = 163°/760 mm) and 3.7 g of pyridine. The mixture was vigorously stirred for 1 hr at 0° and for 3 hr at room temperature. After evaporation of the solvent in vacuo, the residue was taken up in 90% aqueous methanol and percolated over a mixed Amberlite column (IR-45

and IRC-50). The final purification was done on silicic acid giving IIIa in a yield of 20%, $[\alpha]_D^{20} = +7.9°$ (C,10 in chloroform).

The introduction of this branched-chain fatty acid into a 1-acyllysolecithin apparently gives a much lower yield than the comparable reactions with straight-chain fatty acids (usually 50–70%).

2. 1-octanoyl-2-(2,2-dimethyl)pentanoyl-sn-*glycero-3-phosphorylcholine (IIIb)*

1-octanoyl-3-deoxy-3-iodo-*sn*-glycerol ($[\alpha]_D^{20} = +2.4°$, C,12.5 in chloroform) was prepared from 3-deoxy-3-iodo-*sn*-glycerol as described by Bird et al.[16].

Found: C=41.1; H=6.7; I=37.5.
Calculated for $C_{11}H_{21}IO_3$ (M=328.2): C=40.3; H=6.5; I=38.6.

Acylation with 2,2-dimethylpentanoylchloride provided the desired diacyl derivative. The introduction of this branched-chain fatty acid was found to be quite cumbersome. More drastic conditions than usual for acylations were required (48 hr at 40°). Condensation of this iodo compound with the silver salt of dibenzylphosphoric acid[9]) yielded the dibenzylester of phosphatidic acid. Colorless oil. $[\alpha]_D^{20} = +5.9°$ (C,10 in chloroform).

Found: C=65.2; H=8.1; P=5.3.
Calculated for $C_{32}H_{47}O_8P$ (M=590.7): C=65.1; H=8.0; P=5.3.

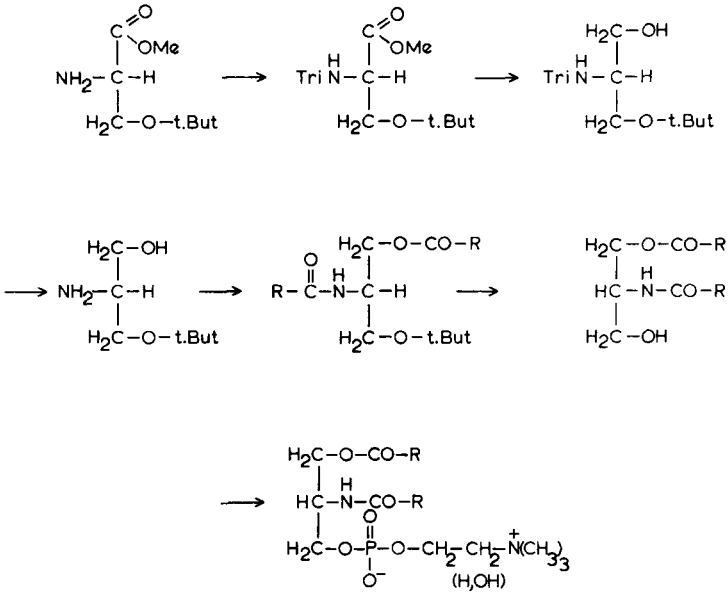
Catalytic hydrogenolysis gave the corresponding phosphatidic acid which was converted into the lecithin IIIb with choline tosylate in the presence of trichloroacetonitrile, as described by Rosenthal[11]). $[\alpha]_D^{20} = +10.4°$ (C,9 in chloroform).

Found: C=54.3; H=9.5; N=2.8; P=6.0.
Calculated for $C_{23}H_{48}NO_9P$ (M=513.0): C=53.8; H=9.4; N=2.7; P=6.0.

3. 1-heptanoyl-2-(3,3-dimethyl)butyryl-sn-*glycero-3-phosphorylcholine (IIIc)*

Acylation of 1-heptanoyllysolecithin with 3,3-dimethylbutyrylchloride (bp 132°/760 mm) as described for IIIa gave the lecithin IIIc in a yield of 70%. $[\alpha]_D^{20} = +8.8°$ (C,10 in chloroform).

Found: C=52.6; H=9.4; N=2.8; P=6.2.
Calculated for $C_{21}H_{44}NO_9P$ (M=487): C=52.0; H=9.1; N=2.9; P=6.4.



Scheme 1. Chemical synthesis of *rac*-1-acyl-2-acylamido-2-deoxyglycero-3-phosphoryl-choline.

4. rac-*1,2-dibenzoylglycero-3-phosphorylcholine (IIId)*

rac-1-deoxy-1-iodoglycerol was acylated with benzoylchloride as described by Verkade[17]). Condensation with the silver salt of dibenzylphosphoric acid and catalytic hydrogenolysis has previously been described for similar compounds[18]). The dibenzoylphosphatidic acid was converted into the lecithin, as described by Rosenthal[11]). *rac*-1,2-dibenzoyl-glycero-3-phosphorylcholine (IIId) was obtained as a colorless waxy material. This lecithin was found to be very soluble in organic solvents and water.

Found: C = 55.2; H = 6.8; N = 3.2; P = 6.1.
Calc. for $C_{22}H_{30}NO_9P$ (M = 483.4): C = 54.8; H = 6.3; N = 2.9; P = 6.4.

D. Lecithins modified in the ester moiety at the 2-position

1. rac-*1-acyl-2-acylamido-2-deoxyglycero-3-phosphorylcholines (IVa)*

The synthesis of these acylamido lecithins was attempted for the optical active derivatives. During one of the reaction steps, however, racemization caused by acylmigration* took place. The acylmigration in a 1-acyl-2-acyl-

* A nucleophilic attack, however, of the glycerol-C_3 hydroxyl group on the carbonyl carbon atom at C_1 might also explain the observed racemisation.

amido-2-deoxy-*sn*-glycerol was not anticipated because of the stability of an acylamido group. Although migration and the racemization occurred during the acid-catalyzed removal of the t.butylgroup, the product which was isolated still contained an acylamido group. The end products (IVa) are, therefore, racemic. Because the synthesis was started with L-serine, the first steps in the synthesis are described for the optically active materials (see scheme 1).

0-t.butyl-L-serine methyl ester. L-serine was esterified with methanol in the presence of thionylchloride, as described by Guttmann and Boissonnas[19]). The amino function was then blocked with a benzyloxycarbonyl group according to the procedure of Baer et al.[20]). Alkylation of N-benzyloxycarbonyl-L-serine methylester with isobutene and subsequent release of the N-protection was performed, as described by Poduška and Titov[21]). *0-t.butyl-N-trityl-L-serine methylester.* 10 g of 0-t.butyl-L-serine methylester (as HCl salt) were dissolved in chloroform. At 0° 9.55 g of triethylamine and 13.5 g of tritylchloride dissolved in chloroform were added. The mixture was kept at room temperature for 16 hr. Judged from TLC the reaction had virtually gone to completion. The solvent was removed in vacuo after washing with water and drying over sodium sulfate. Crystallization from pentane at −15° gave a white crystalline product in a yield of 87%. mp 66–68°. $[\alpha]_D^{20} = +30.6°$ (C,11.5 in chloroform).

Found: C=77.3; H=7.5; N=3.3.
Calc. for $C_{27}H_{31}NO_3$ (M=415.5): C=78.0; H=7.5; N=3.4.

2-deoxy-2-tritylamino-3-0-t.butyl-sn-*glycerol.* 10 g of 0-t.butyl-N-trityl-L-serine methylester were dissolved in 100 ml of ether (freshly distilled from $LiAlH_4$) and added to a stirred suspension of 2.3 g of $LiAlH_4$ in 200 ml of dry ether at 0°. The mixture was stirred at 0° for 1 hr after addition of the ester and then refluxed for 3 hr. Excess of $LiAlH_4$ was decomposed with 10 ml of methanol and 10 ml of water. The precipitate was removed by filtration and the filtrate dried over sodium sulfate. The solvent was removed by evaporation and the residue dried in vacuo over P_2O_5. Yield 95%. Only a trace of starting material was still present, as could be seen on TLC. For the element analysis and rotation a small part was purified on silicic acid with toluene-ether mixtures as eluents, giving the alcohol as a colorless sirup. $[\alpha]_D^{22} = +14.3°$ (C,10 in chloroform).

Found: C=79.7; H=8.3; N=3.5.
Calc. for $C_{26}H_{31}NO_2$ (M=387.4): C=80.1; H=8.1; N=3.6.

2-amino-2-deoxy-3-0-t.butyl-sn-*glycerol.* The above-mentioned trityl derivative was freed from its trityl protecting group according to the procedure

described by Billimoria and Lewis[22]). The compound, dissolved in 90% aqueous acetic acid, was heated on a boiling waterbath for 10 min, and kept at room temperature overnight. The residue, after removal of the solvent, was dissolved in a 5% bicarbonate solution, extracted twice with chloroform to remove tritylalcohol and a trace of starting material. No or very little 2-amino-2-deoxy-3-0-t.butyl-*sn*-glycerol was extracted in this way. The aqueous solution was then concentrated to a small volume and saturated with sodium chloride. The compound could be isolated by repeated extraction with chloroform, and was found to be pure on TLC. The solvent was removed in vacuo, after drying over sodium sulfate, and the residue, a colorless oil, was extensively dried in vacuo over P_2O_5. Yield 86%. $[\alpha]_D^{22} = +3.1°$ (C,9 in chloroform).

Found: C = 56.5; H = 11.4; N = 9.4.
Calc. for $C_7H_{17}NO_2$ (M = 147.2): C = 57.1; H = 11.6; N = 9.5.

1-octanoyl-2-deoxy-2-octanamido-3-0-t.butyl-sn-*glycerol.* 2.7 g of 2-amino-2-deoxy-3-0-t.butyl-*sn*-glycerol were dissolved in dry chloroform and acylated with octanoylchloride (6.3 g) in the usual manner. Chromatography on silicic acid with toluene and ether as solvents gave the title compound in a yield of 78% (colorless oil). $[\alpha]_D^{22} = +5.3$ (C,16 in chloroform).

Found: C = 68.6; H = 11.4; N = 3.5.
Calc. for $C_{23}H_{45}NO_4$ (M = 399.6): C = 69.2; H = 11.3; N = 3.5.

rac-*1-octanoyl-2-deoxy-2-octanamidoglycerol.* The t.butylgroup of the foregoing product was released by a treatment with anhydrous hydrogenchloride in chloroform at 0°. After 10 hr excess of hydrogenchloride was removed with N_2 and the solution evaporated in vacuo. The residue was kept in vacuo over KOH. Purification was done on silicic acid (toluene-ether mixtures as eluents), yielding the product as a colorless oil in a yield of 63%. Neither before nor after the chromatographic purification could a ninhydrin positive product be detected, indicating that the amino function was blocked. The optical rotation ($[\alpha]_D^{22} = 0°$, C,9 in chloroform) indicates that racemization, probably due to acylmigration, had occurred.

Found: C = 66.3; H = 10.9; N = 4.0.
Calc. for $C_{19}H_{37}NO_4$ (M = 343.5): C = 66.5; H = 10.9; N = 4.1.

rac-*1-octanoyl-2-deoxy-2-octanamidoglycero-3-phosphorylcholine (IVa).* Introduction of the phosphorylcholine moiety into the "diglyceride" was carried out as described by Eibl et al.[15]) with 2-bromoethylphosphoryl-dichloride followed by a reaction with trimethylamine. Presumably due to an enhanced reactivity of the amido function, side reactions were found to take

place to a large extent during the phosphorylation reaction, giving rise to rather low yields (20–25%). The phosphorylation mixture was therefore kept at 0° for 48 hr instead of at 40° overnight. Purification on silicic acid provided the lecithin analogue IVa as a colorless waxy material.

Found: C = 54.6; H = 9.9; N = 5.2; P = 6.0.
Calc. for $C_{24}H_{51}N_2O_8P$ (M = 526.6): C = 54.8; H = 9.8; N = 5.3; P = 5.9.

Following the same procedure also *rac*-1-heptanoyl-2-deoxy-2-heptanamido-glycero-3-phosphorylcholine (IVa) was prepared.*

Found: C = 53.7; H = 9.6; N = 5.6; P = 6.4.
Calc. for $C_{22}H_{47}N_2O_8P$ (M = 498.6): C = 53.0; H = 9.5; N = 5.6; P = 6.2.

2. rac-1-octanoyl-2-octane sulfonyl-glycero-3-phosphorylcholine (IVb)

Octane sulfonylchloride was prepared by a modification of the method of Latimer and Bost[12]). 35 g 1-bromoctane was suspended in a solution of 45 g of sodium sulfite in 270 ml of water. The mixture was stirred and refluxed for 16 hr. The resulting clear solution was evaporated and the solid residue was stirred with dry methanol to remove excess sodium sulfite. Sodium-bromide was removed by addition of an aqueous solution of one equivalent of silver acetate. The sodium salt of the sulfonic acid was converted into the sulfonylchloride with an excess of phosphoruspentachloride using thionylchloride as solvent. After 3 hr the solvent was removed in vacuo and excess PCl_5 was decomposed by addition of ice-water. The sulfonyl-chloride was extracted with ether and after drying and evaporation of the solvent, the residue was distilled in vacuo. Colorless oil. Yield 50% calc. on 1-bromoctane. Boilingpoint 108°/2mm. $N_D^{24} = 1.4590$.

Found: C = 45.4; H = 8.1; Cl = 16.9; S = 14.7.
Calc. for $C_8H_{17}ClO_2S$ (M = 212.7): C = 45.2; H = 8.1; Cl = 16.7; S = 15.1.

1,3-dibenzyl-2-octane sulfonylglycerol. 0.5 g 1,3-dibenzylglycerol[35]) dissolved in anhydrous pyridine was stirred with 0.4 g of octane sulfonyl-chloride for 20 hr at room temperature. The solvent was evaporated in vacuo and the residue, dissolved in chloroform was washed subsequently with 0.5 sulfuric acid, 5% sodium bicarbonate and water. The solution was dried over sodium sulfate and evaporated in vacuo. Purification on silicic acid

* Hydrolysis of this amido lecithin with phospholipase C from *Bacillus cereus* gave a complete breakdown into the corresponding "diglyceride" and phosphorylcholine. The negative reaction with ninhydrin was an indication that the amino group in the formed "diglyceride" was acylated.

(ether-hexane mixture as eluents) yielded the product as a colorless oil. Yield 0.4 g (61%).

Found: C=67.0; H=7.9; S=7.3.
Calc. for $C_{25}H_{36}O_5S$ (M=448.5): C=66.9; H=8.1; S=7.2.

2-octanesulfonylglycerol was prepared from the foregoing compound by catalytic hydrogenolysis. Large amounts of palladium/carbon were required to remove both benzyl groups, probably due to sulphur-poisoning of the catalyst. The desired product was obtained in pure form after chromatography on silicic acid (chloroform as eluent). Colorless oil. Yield 60%. The negative Schiff-reaction showed the absence of vicinal OH groups.

Found: C=49.0; H=8.8; S=11.7.
Calc. for $C_{18}H_{30}O_5S$ (M=358.4): C=49.2; H=9.0; S=11.9.

rac-*1-octanoyl-2-octane sulfonoyl-glycero-3-phosphorylcholine.* 0.11 g of 2-octane sulfonoylglycerol was acylated with 0.06 g of octanoylchloride in dry chloroform in the presence of 0.05 g of anhydrous pyridine. After 3 hr at room temperature, the solution was washed with 0.5 N sulfuric acid, 5% sodium bicarbonate and water, dried over sodium sulfate and evaporated in vacuo. The crude "diglyceride" after extensive drying in high vacuo over P_2O_5 was directly converted into the corresponding lecithin by the procedure described by Eibl et al.[15]). Chromatography on silicic acid yielded the pure lecithin as a colorless waxy material. Yield: 40%.

Found: C=48.7; H=8.7; N=2.9; P=5.8; S=5.5.
Calc. for $C_{24}H_{52}NO_{10}PS$ (M=417.6): C=49.9; H=9.0; N=2.4; P=5.4; S=5.5.

3. rac-*1-heptanoyl-2-octylglycero-3-phosphorylcholine (IVc)*

rac-*2-octylglycerol.* In a 3-necked bottle with a stirrer and reflux condenser 2.6 g of potassium in dry benzene was slowly heated with stirring until the metal had been finely granulated. 11.9 g of *rac*-1,3-benzylideneglycerol were added slowly and the mixture was stirred and refluxed. After $2\frac{1}{2}$ hr a solution of 17 g of *p*-toluene sulfonyloctanol in benzene were added. The reaction mixture was stirred and refluxed for 24 hr. The excess of potassium was decomposed with wet ether, the solution washed with water and dried over sodium sulfate. The residue, after evaporation of the solvent, was mixed with 700 ml of 0.5 N HCl and refluxed for 3 hr. The cooled solution was extracted with ether, the extract washed with a 5% $NaHCO_3$ solution and water, and dried over sodium sulfate. The residue was distilled in high vacuum. The fraction boiling from 120 to 150°C/0.001 mm was collected

and further purified on silicic acid with chloroform as eluent. *rac*-2-octylglycerol was obtained as a colorless oil in a yield of 48%.

Found: C=63.8; H=11.8.
Calc. for $C_{11}H_{24}O_3$ (M=204.3): C=64.6; H=11.8.

rac-*1-heptanoyl-2-octylglycerol.* 6.4 g of *rac*-2-octylglycerol were acylated in the usual manner with 4.2 g heptanoylchloride. The resulting mixture of starting material, the mono and diacylderivative was separated on silicic acid giving *rac*-1-heptanoyl-2-octylglycerol as a colorless sirup in a yield of 42%.

Found: C=68.4; H=11.5.
Calc. for $C_{18}H_{36}O_4$ (M=316.5): C=68.3; H=11.5.

rac-*1-heptanoyl-2-octylglycerol-3-phosphorylcholine (IVc).* Introduction of the phosphorylcholine moiety into the acyl-alkylglycerol derivative was carried out as described by Eibl et al.[15]). Chromatography on silicic acid and alumina gave the pure lecithin analogue IVc as a colorless waxy material in a yield of 55%.

Found: C=54.9; H=10.2; N=6.6; P=2.7.
Calc. for $C_{23}H_{50}NO_8P$ (M=499.6): C=55.3; H=10.1; N=6.2; P=2.8.

The synthesis of a similar lecithin with a longchain fatty acid and ether function has been reported by Slotboom et al.[23]).

4. rac-*1-octanoyl-2-deoxy-2-hexylglycero-3-phosphorylcholine (IVd)*

rac-*2-deoxy-2-hexylglycerol.* Hexylmalonic ester was prepared according to the procedure of Floyd and Miller[24]) from the ethyl ester of octanoic acid and diethyloxalate. 46 g of hexylmalonic ester were added to a stirred suspension of 12 g of $LiAlH_4$ in anhydrous ether and the mixture was refluxed for 3 hr. The excess of $LiAlH_4$ was decomposed with 20 ml of methanol and 20 ml of water. The precipitate was removed by filtration and the residue dried over sodium sulfate. After removal of the solvent, the residue was distilled in vacuo. bp 95–100°/0.001 mm. Yield of the colorless liquid being 85%.

rac-*1-octanoyl-2-deoxy-2-hexylglycerol* was prepared from the foregoing product by acylation with octanoylchloride in the usual manner. Chromatography on silicic acid (with hexane-ether mixtures as eluents) provided this compound as a colorless sirup in a yield of 67%.

Found: C=70.5; H=11.8.
Calc. for $C_{17}H_{34}O_3$ (M=286.4): C=71.3; H=12.0.

rac-*1-octanoyl-2-deoxy-2-hexylglycero-3-phosphorylcholine (IVd).* *rac*-1-octanoyl-2-deoxy-2-hexylglycerol was converted into a lecithin analogue with 2-bromoethylphosphoryldichloride, followed by a treatment with trimethylamine, as described by Eibl et al.[15]). The lecithin analogue IVd was obtained after chromatography on silicic acid and alumina as a colorless waxy material in a yield of 44%.

Found: C = 56.6; H = 10.4; N = 3.4; P = 6.3.
Calc. for $C_{22}H_{48}NO_7P$ (M = 469.6): C = 56.4; H = 10.3; N = 3.0; P = 6.6.

The synthesis of a similar lecithin with a longchain fatty acid ester and a long alkyl chain has been described previously by Slotboon et al.[23]).

E. Lecithins lacking an acyl-chain at the 2-position

1. 1-acyl-sn-*glycero-3-phosphorylcholines (Va)*

These compounds (lysolecithins) were obtained from the corresponding 3-*sn*-phosphatidylcholines by hydrolysis of the ester bond at the 2-position by phospholipase A_2 (from *Crotalus adamanteus* or porcine pancreas), as described earlier[25]).

2. 1-palmitoyl-2-deoxyglycero-3-phosphorylcholine (Vb)

This lysolecithin analogue was prepared from the monopalmitoyl ester of 1,3-propanediol by a reaction with 2-bromoethylphosphoryldichloride followed by a substitution reaction with trimethylamine as outlined by Eibl et al.[15]).

F. Modifications in the glycerol-backbone

1. 1-palmitoyl-glycol-2-phosphorylcholine (glycollecithin) (VIa)

Starting from monopalmitoylglycol the glycollecithin VIa was obtained according to the procedure as described by Eibl et al.[15]).

2. rac-*1,2-dihexanoyl-butanetriol-4-phosphorylcholine (VIb)*

rac-*1,2-isopropylidenebutanetriol* was synthesized by condensing 1,2,4-butanetriol with acetone in the presence of *p*-toluenesulfonic acid as catalyst according to the method described by Quinn et al.[26]). bp 95°/14 mm.

Found: C = 57.3; H = 9.6.
Calc. for $C_7H_{14}O_3$ (M = 146.2): C = 57.5; H = 9.6.

rac-*1,2-dihexanoylbutanetriol-4-phosphorylcholine (VIb).* 1,2-isopropylidene butanetriol was phosphorylated with tetrabenzylpyrophosphate as described by Bonsen et al.[27]) for the glycerol analogue (yield about 70%). The isopropylidene group was then released with boric acid in trimethyl-

borate according to the procedure of Hartman[28] (yield 69%). Acylation of obtained 1,2-butanetriol-4-(dibenzyl)phosphate with hexanoylchloride was carried out as described above (see §III. B). The dibenzylphosphatidic acid was freed from its blocking group by catalytic hydrogenolysis with palladium as a catalyst. Conversion of the phosphatidic acid into the lecithin was done with choline tosylate in the presence of trichloroacetonitrile as described by Rosenthal[11]), giving after chromatographic purification the lecithin analogue VIb as a colorless waxy product in a yield of about 40% calculated on *rac*-1,2-butanetriol-4-(dibenzyl)phosphate.

Found: C = 52.5; H = 9.4; N = 3.0; P = 6.3.
Calc. for $C_{21}H_{44}NO_9P$ (M = 485.5): C = 51.9; H = 9.1; N = 2.9; P = 6.4.

3. Lecithin derived from 1,1,1-trihydroxymethylethane (VIc)

2,2-(dioctanoylhydroxymethyl)propan-1-ol. 8 g of 1,1,1-trihydroxymethylethane were suspended in chloroform with 6 ml of dry pyridine. 23.4 g octanoylchloride were added slowly and the solution was stirred at room temperature for 16 hr. After the usual work-up procedure and chromatography on silicic acid, the pure "diglyceride" was obtained as a colorless oil in a yield of 78%.

Found: C = 68.1; H = 11.0.
Calc. for $C_{21}H_{40}O_5$ (M = 372.5): C = 67.7; H = 10.8.

2,2-dioctanoylhydroxymethyl-propanol-1-phosphorylcholine (VIc). Introduction of the phosphorylcholine moiety into the dioctanoyl ester of trihydroxyethane was carried out as described for similar compounds by Eibl et al.[15]). Chromatography on silicic acid and alumina yielded the pure lecithin analogue VIc as a waxy solid (45%).

Found: C = 52.5; H = 9.4; N = 3.0; P = 6.3.
Calc. for $C_{26}H_{54}NO_9P$ (M = 555.7): C = 51.9; H = 9.1; N = 2.9; P = 6.4.

G. Modification of the phosphate moiety of lecithins

*1. 1,2-diacyl-3-deoxy-*sn*-glycero-3-phosphonyllecithin (VIIa)*

1,2-isopropylidene-*sn*-glycerol was prepared from 1,2,5,6-diisopropylidene-D-mannitol[29]), as described by LeCoq and Ballou[30]).

*1,2-isopropylidene-3-deoxy-3-iodo-*sn*-glycerol* was synthesized from the foregoing by tosylation and substitution of the tosyl group by iodine, as described by Baer and Fischer[6]).

*3-deoxy-*sn*-glycero-3-phosphonic acid (as barium salt)* was obtained from a condensation reaction of 1,2-isopropylidene-3-iodo-*sn*-glycerol with triethylphosphite, followed by hydrolysis of the isopropylidene group and the ethyl groups with acid, as described by Baer and Basu[31]).

1,2-dioctanoyl-3-deoxy-sn-*glycero-3-phosphonic acid.* The barium salt of 3-deoxy-*sn*-glycero-3-phosphoric acid was converted into the pyridinium salt. Acylation with octanoic anhydride in the presence of the tetraethylammonium salt of octanoic acid was performed as described by Lapidot et al.[14]). The phosphatidic acid analogue was obtained in an almost pure form after silicic acid chromatography in a yield of 76%. Recently Baer and Basu[32]) described the synthesis of long-chain derivatives of this phosphatidic acid analogue using the same procedure.

1,2-dioctanoyl-3-deoxy-sn-*glycero-phosphonylcholine (VIIa, $R=C_7H_{15}$).* The above-mentioned phosphatidic acid analogue was allowed to react with choline tosylate with trichloroacetonitrile as condensing agent in pyridine as solvent, according to the procedure of Rosenthal[11]). This resulted in a pure phosphonolecithin after purification on silicic acid and alumina in a yield of 72%. $[\alpha]_D^{22} = +16.9°$ (C,6 in chloroform).

Found: C = 57.0; H = 9.9; N = 3.1; P = 5.8.
Calc. for $C_{24}H_{50}NO_8P$ (M = 511.6): C = 56.5; H = 9.9; N = 2.8; P = 6.0.

1,2-dihexanoyl-3-deoxy-sn-*glycero-phosphonylcholine (VIIa, $R=C_5H_{11}$).* With the same procedure as described for the dioctanoyl derivative, the dihexanoyl phosphonolecithin VIIa ($R=C_5H_{11}$) was prepared. $[\alpha]_D^{24} = +19.8°$ (C,8 in chloroform).

Found: C = 52.3; H = 9.5; N = 3.3; P = 6.6.
Calc. for $C_{20}H_{42}NO_8P$ (M = 455.5): C = 52.7; H = 9.3; N = 3.1; P = 6.8.

rac-*1,2-dioleoyl-3-deoxy-glycero-3-phosphonylcholine (VIIa, $R=C_{17}H_{33}$).* This lecithin analogue was prepared from *rac*-1,2-isopropylidene-3-deoxy-3-iodoglycerol following the same procedure as described above.*

Found: C = 66.4; H = 10.7; N = 1.8; P = 3.7.
Calc. for $C_{44}H_{86}NO_8P$ (M = 788.1); C = 67.0; H = 11.0; N = 1.8; P = 3.9.

This lecithin has recently been used by Kinsky et al.[34]) for the preparation of immunologically responsive liposomes.

2. rac-*1,2-diheptanoyl-3-deoxy-glycero-3-sulfonic acid (as sodium salt) (VIIb)*

rac-*3-deoxyglycero-3-sulfonic acid (as sodium salt).* 3.5 g of *rac*-3-deoxy-3-

* An attempt to use the procedure of Aneja et al.[33]) for the synthesis of this lecithin analogue, viz. condensation of the phosphatidic acid analogue with dimethylethanol with 2, 4, 6-trisopropylbenzene sulfonylchloride, followed by methylation with methyliodide gave a more complex reaction mixture and a much lower yeild (about 30%).

iodoglycerol and 4.4 g of sodium sulfite were dissolved in 25 ml of water and refluxed under N_2 for 16 hr. The mixture was, after cooling, evaporated in vacuo and the residue dissolved in 90% aqueous methanol. Inorganic salts were removed by filtration and the filtrate concentrated to a small volume. Addition of an excess of absolute ethanol gave a white precipitate, which was recrystallized from water-ethanol, giving the sodium salt of *rac*-3-deoxyglycero-3-sulfonic acid, complexed with sodium iodide, as a white powder. Yield 90%. mp 207–209°. A good analysis could not be obtained because recrystallization resulted in the loss of a part of the sodium iodide.

rac-*1,2-diheptanoyl-3-deoxyglycero-3-sulfonic acid (as sodium salt VIIb).* 3 g of the above mentioned compound was treated with a slight excess of silver acetate in boiling water. The precipitate (AgI) was removed by filtration and the filtrate percolated through a column of Dowex-50 (H^+ form). The eluate was evaporated in vacuo and the residue converted into the pyridinium salt (2.6 g). Acylation with heptanoic anhydride, as described by Lapidot et al.[14]) gave the title compound VIIb after chromatography on silicic acid and elution over Dowex-50 (Na^+ form) in a yield of 63%.

Found: C = 50.0; H = 7.7; S = 8.0.
Calc. for $C_{17}H_{31}NaO_7S$ (M = 402.5): C = 50.8; H = 7.8; S = 7.9.

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