INVESTIGATIONS IN THE FIELD OF COMPLEX LIPIDS

SYNTHESIS OF 3-[(1',2'-DISTEAROYLGLYCERYLOXY)(AMINOETHOXY)-PHOSPHINYL]-1-GLYCYLGLYCEROL

M. A. Grum-Grzhimailo, L. V. Volkova,
and R. P. Evstigneeva

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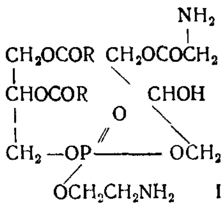
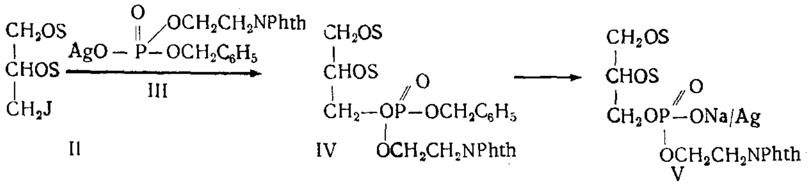
In bacterial membranes, mainly in Gram-positive species, in addition to phosphatidylglycerols there are considerable amounts of their amino-acid derivatives [1, 2]. The structural and functional roles of these compounds in cell membranes have not been definitely established. The question of the possibility of the presence in cell membranes, especially those of the bacterial cell, of lipo-amino-acid complexes containing a phosphoric triester bond is under discussion [3-6].

One of the approaches to the solution of this problem is the directed synthesis of model triester structures and the study of their stability. Continuing investigations on the synthesis of phosphatidylglycerol derivatives [7-8], we have synthesized the model compound (I) with a phosphoric triester link.


I

To protect the amino group in the aminoethyl and the amino-acid residues we used phthaloyl (Phth) and benzyloxycarbonyl (Z) groups, respectively. The choice of different protective groupings was due to the fact that their separate elimination may give additional information on the stabilities of the final and intermediate products of the synthesis. The condensation of the two component parts – a N-phthaloylcephalin and an amino-acid derivative of a substituted glycerol – was based on the silver-salt method. The cephalin structure (V) was obtained by a known method through the reaction of silver benzyl phthalimidoethyl phosphate [9] and 1,2-distearoyl-3-iodo-3-deoxyglycerol (II).



The amino-acid component (VIII) was synthesized from 1-(N-benzyloxycarbonylglycyl)-2-benzylglycerol (VI) through the stage of the formation of 2-benzyl-1-(N-benzyloxycarbonylglycyl)-3-tosylglycerol (VII) by the action of tosyl chloride, with the subsequent replacement of the tosyl group by iodine under the action of sodium iodide.

The treatment of (VI) with a twofold excess of tosyl chloride at 50°C for 3 h led to the formation of 15-20% of the byproduct (IX) in addition to 80-85% of the main substances (VII).

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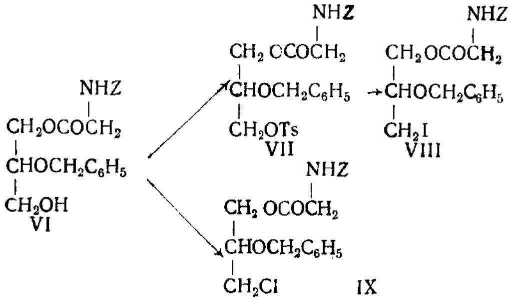
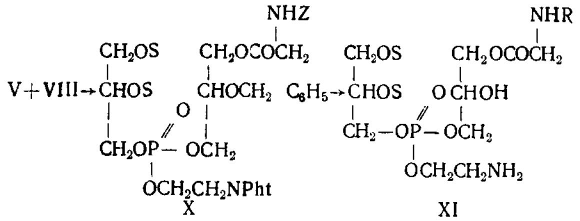


The condensation of (V) and (VIII) in benzene (80-90°C) gave a substance having the triester structure (X) with a yield of 56.5%. (Its IR spectrum is given in Fig. 1a.)



When the phthaloyl protection in compound (X) was removed with a 15-fold excess of hydrazine hydrate (20-22%), substance (XI, R=Z) was obtained. The presence of a free NH_2 group in it was shown by the characteristic color of the chromatographic spot (under the action of a solution of ninhydrin) and by the IR spectrum, which had the absorption bands of an NH_2 group — a doublet at 1530-1625 cm^{-1} and 3300-3430 cm^{-1} (Fig. 1b). An increase in the time of hydrazinolysis led to the cleavage of the phosphoric triester bond. The substance (XI, R=Z) was hydrogenated in ethyl acetate and in ethanol. In ethyl acetate the protective groups were eliminated after 5-6 h, and in ethanol after 15-18 h. In the hydrogenolysis process, an increase in the time led to a greater decomposition of the triester structure (TLC results). Compound (XI, R=H) was separated preparatively in a yield of 35.6% on the (XI, R=Z). This compound is unstable and partially decomposes even during the process of removing the protection and also in the process of purification. The IR spectrum of (X, R=H, Fig. 1b) lacks the absorption bands characteristic for a benzyl group.

To confirm the structure, acid and alkaline hydrolyses of (XI, R=H) were performed. Acid hydrolysis with 4 N sulfuric acid at 100°C for 2 h led to the complete decomposition of the triester compound (XI, R=H) with the liberation of a diglyceride, ethanolamine, glycine, and inorganic phosphorus.

After alkaline hydrolysis with sodium methoxide at 37°C for 20 min, glycine, methyl stearate, and lysocephalin were identified in the reaction mixture. The presence of the decomposition products in the hydrolytic media was established by comparing them with samples of these compounds by TLC and paper chromatography.

EXPERIMENTAL

Thin-layer chromatography was performed in a nonfixed layer of silica in the following systems: 1) petroleum ether—diethyl ether (1:1); 2) benzene—ether (1:1); 3) chloroform—methanol—acetone (3:1:1); 4) chloroform—methanol—acetone (9:1:1); and 5) ether.

The IR spectra were taken on a Perkin-Elmer (model 257) instrument in paraffin oil. The presence of phosphorus in the compounds was shown by treating the chromatograms with a solution of molybdenum blue [10]. The analyses of all the compounds corresponded to the calculated figures.

3-[(Benzyloxy)(phthalimidoethoxy)phosphinyl]-1,2-distearoylglycerol (IV). A mixture of 3.3 g of compound (II) and 2.4 g of the silver salt (III) was boiled in 100 ml of benzene for 6 h. A substance $C_{56}H_{90}NO_{10}P$ (IV) was isolated on a column of silica (100 g) with elution by hexane—ether (1:1). Yield 3.72 g (85.9%). Mp 38-38.5°C; $R_{f\,IV}$ 0.87 (2), 0.15 (1).

Silver Salt of 3-(Phthalimidoethoxyphosphinyl)-1,2-distearoylglycerol (V). A mixture of 1.3 g of substance (IV) in 25 ml of acetone and 0.4 g of sodium iodide was boiled for 3 h. Then it was allowed to stand for 12 h, and the sodium salt was filtered off, washed with water and acetone, and dried. Yield 1.2 g (99.9%). Mp 55-57°C; R_f 0.56 (3).

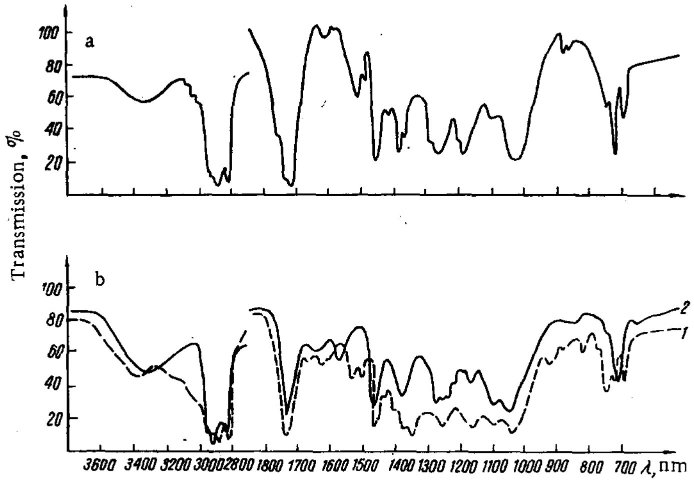


Fig. 1. IR spectra of 2-benzyl-1-(N-benzyloxycarbonylglycyl)-3-[(1',2'-distearoylglyceryloxy)(phthalimidoethoxy)phosphinyl]glycerol (X) (a), of 2-benzyl-1-(N-benzyloxycarbonylglycyl)-3-[(1',2'-distearoylglyceryl)aminoethoxy)phosphinyl]glycerol, and of 3-[(1',2'-distearoylglyceryloxy)(aminoethoxy)phosphinyl]-1-glycylglycerol (paraffin oil) (b): 1) (XI, R = Z); 2) (XI, R = H).

A solution of 0.16 g of silver nitrate in a mixture of 5 ml of acetone and 10 ml of water at 40°C was added to a solution of 0.5 g of the Na salt in 50 ml of acetone heated to 40°C. After 3 h, the precipitate that had deposited was filtered off at 0°C and was washed with water and acetone and dried. Yield 0.53 g (96.8%). Mp 87-88.5°C.

2-Benzyl-1-(N-benzyloxycarbonylglycyl)-3-tosylglycerol (VII). At 0°C, 2.64 g of tosyl chloride was added to 2.6 g of substance (II) in 15 ml of pyridine. After the mixture had been kept at 50°C for 3 h, it was poured into water and extracted with chloroform (3×30 ml), and the extract was washed with 10% sulfuric acid (4×20 ml) and with sodium bicarbonate solution (2×20 ml), dried, and evaporated. The residue was separated on a column of silica. A substance $C_{27}H_{29}NO_8S$ (VIII) was eluted with a mixture of petroleum ether and diethyl ether (1 : 1) and a substance $C_{20}H_{22}NO_5Cl$ (IX) with mixtures of petroleum ether and diethyl ether (9 : 1 and 8.5 : 1.5). An oily substance was isolated. The yield of (VII) was 2.94 g (80.0%); R_f 0.56 (1); 0.83 (2).

The yield of oily substance (IX) was 0.41 g (15%); R_f 0.73 (1).

2-Benzyl-1-(N-benzyloxycarbonylglycyl)-3-iodo-3-deoxyglycerol (VIII). A mixture of 3.12 g of compound (VII) in 50 ml of acetone and 1.84 g of sodium iodide was heated at 70-75°C for 24 h and it was then diluted with ether (100 ml), and the precipitate was filtered off. The filtrate was treated with 10% sodium hyposulfite solution (2×20 ml), washed with water and dried, and the solvent was driven off. This gave substance (VIII) with the composition $C_{20}H_{22}NO_5I$ in the form of a viscous oil. Yield 2.57 g (88.5%), R_f 0.72 (nonfixed layer of KSK silica gel, system 5).

2-Benzyl-1-(N-benzyloxycarbonylglycyl)-3-[(1',2'-distearoylglyceryloxy)(phthalimidoethoxy)phosphinyl]glycerol (X). To a mixture of 0.75 g of the silver salt (V) in 20 ml of benzene was added 0.37 g of compound (VIII) in 10 ml of benzene, and the mixture was heated at the boil for 10 h. Then the precipitate was filtered off and the solvent was distilled off. The residue was separated on a column of silica (20 g), being eluted with benzene–ether (4 : 1 and 7 : 3). It was repurified on silica (preparatively) in the benzene–ether (1 : 1) system. The composition of (X) was $C_{69}H_{105}N_2O_{15}P$. Yield 0.53 g (56.5%), mp 56-56.5°C, R_f 0.65 (2).

2-Benzyl-1-benzyloxycarbonylglycyl-3-[(1',2'-distearoylglyceryloxy)(aminoethoxy)phosphinyl]glycerol (XI, R = Z). To 0.1 g of substance (X) in 5 ml of ethanol was added 0.35 g of hydrazine hydrate, and after 3 h the pH of the mixture was brought to 6.5-7.0 with hydrochloric acid and it was kept at 0°C for 1 h, treated with 20 ml of chloroform and filtered, and the solvent was driven off (at 30°C). The substance was eluted with chloroform–methanol (9 : 1 and 8.5 : 1.5). The composition of (XI, R = Z) was $C_{61}H_{103}N_2O_{13}P$. Yield 0.045 g (50.5%), mp 45-46°C (softening point 40.5-41°C); R_f 0.40 (4), R_f 0.31 (2).

3-[(1',2'-Distearoylglyceryloxy)(aminoethoxy)phosphinyl]-1-glycylglycerol (XI, R = H). Compound (XI, R = Z) (0.040 g) was hydrogenated in freshly distilled ethyl acetate in the presence of 0.05 g of palladium catalyst. After 6 h, the reaction mixture was filtered and the solvent was driven off at −10°C (0.2 mm). Preparative chromatography in the chloroform–methanol–acetone (18 : 3.5 : 3.5) system gave substance (XI). Yield 0.0113 g (35.6%). The substance is labile and decomposes on standing. IR spectrum: 3400-3320 s, 1740 s, 1670-1570 w, 1050-1030 s, 760 m, 720 m.

SUMMARY

1. The synthesis of 3-[(1',2'-distearoylglyceryloxy)(aminoethoxy)phosphinyl]-1-glycylglycerol has been effected.

2. The considerably greater lability of the phosphoric ester bond with the aminoacyl derivative of glycerol in compound (I) as compared with the bonds in a cephalin structure has been shown.

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