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# SYNTHESIS OF PHOSPHOLIPIDS. III. SYNTHESIS OF 1,2-DIPALMITOYL-*rac*-GLYCERYL-3-PHOSPHORYL-3′β-CHOLESTEROL AND 1,2-DIPALMITOYL-*rac*-GLYCERYL-3-PHOSPHORYL-20′-(3β-HYDROXY NORPREGN-5-ENE)

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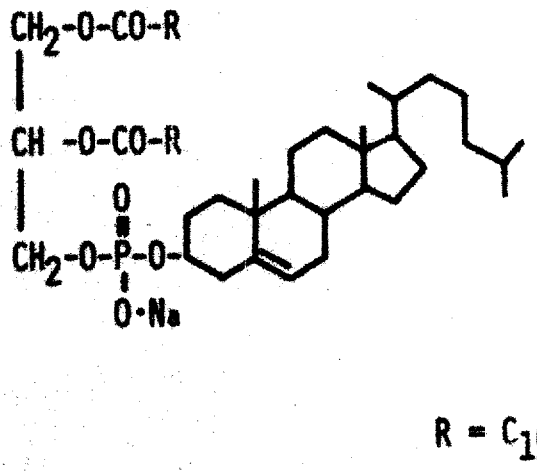
The chemical synthesis of two new glycerophosphatide analogues containing steroid groups, i.e., 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-3′β-cholesterol and 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-20′-(3β-hydroxy norpregn-5-ene) is described.

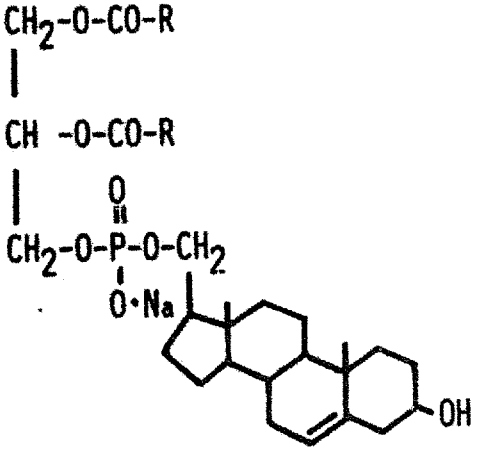
## I. Introduction

Structural analogues of naturally occurring glycerophosphatides, obtained by chemical synthesis, are of interest in physicochemical and biochemical investigations [1]. We have previously synthesized such analogues of phosphatidyl ethanolamine, i.e., 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-propanolamine, -*iso*propanola-




R = $C_{15}H_{31}$

( I )



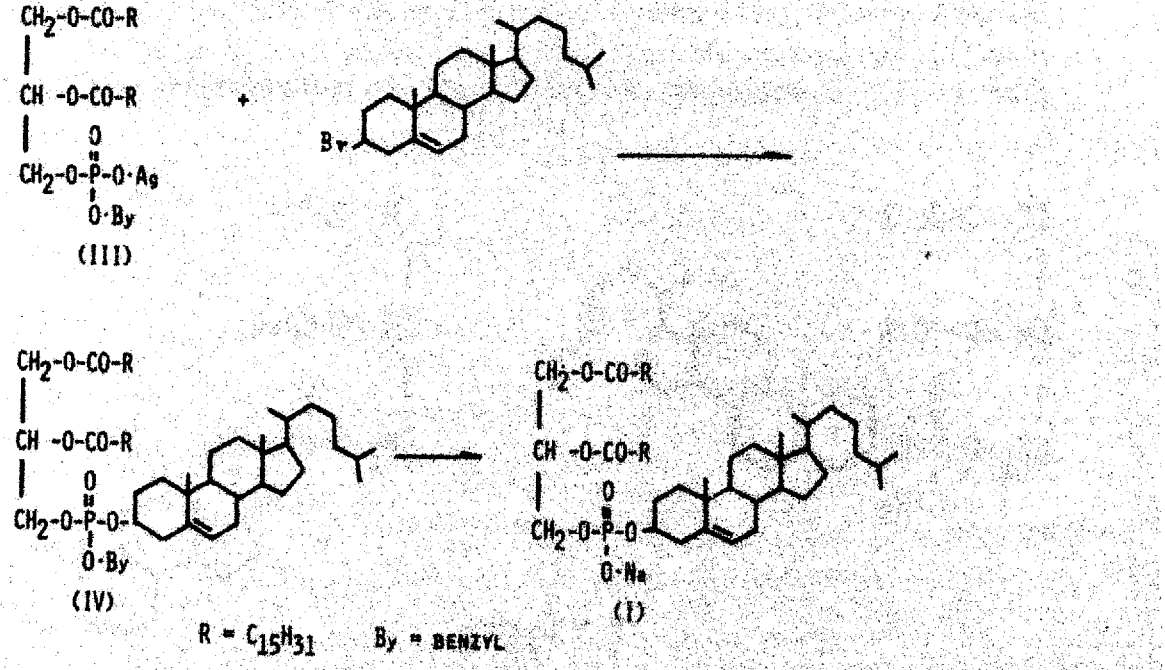

( II )

mine, -butanolamine and -pentanolamine, and compared their physical properties with those of phosphatidylethanolamine [2–6]. We report here the synthesis of two new glycerophosphatides, 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-3′β-cholesterol (I) and 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-20′-(3β-hydroxy norpregn-5-ene) (II) which do not occur naturally. The fact that these compounds contain steroid groups makes them of interest not only for physicochemical studies, but also for immunochemical investigations on phospholipids and steroids. Immunochemical reactivities of some phospholipids and steroids *in vitro* have been previously reported by one of the authors [7,8].

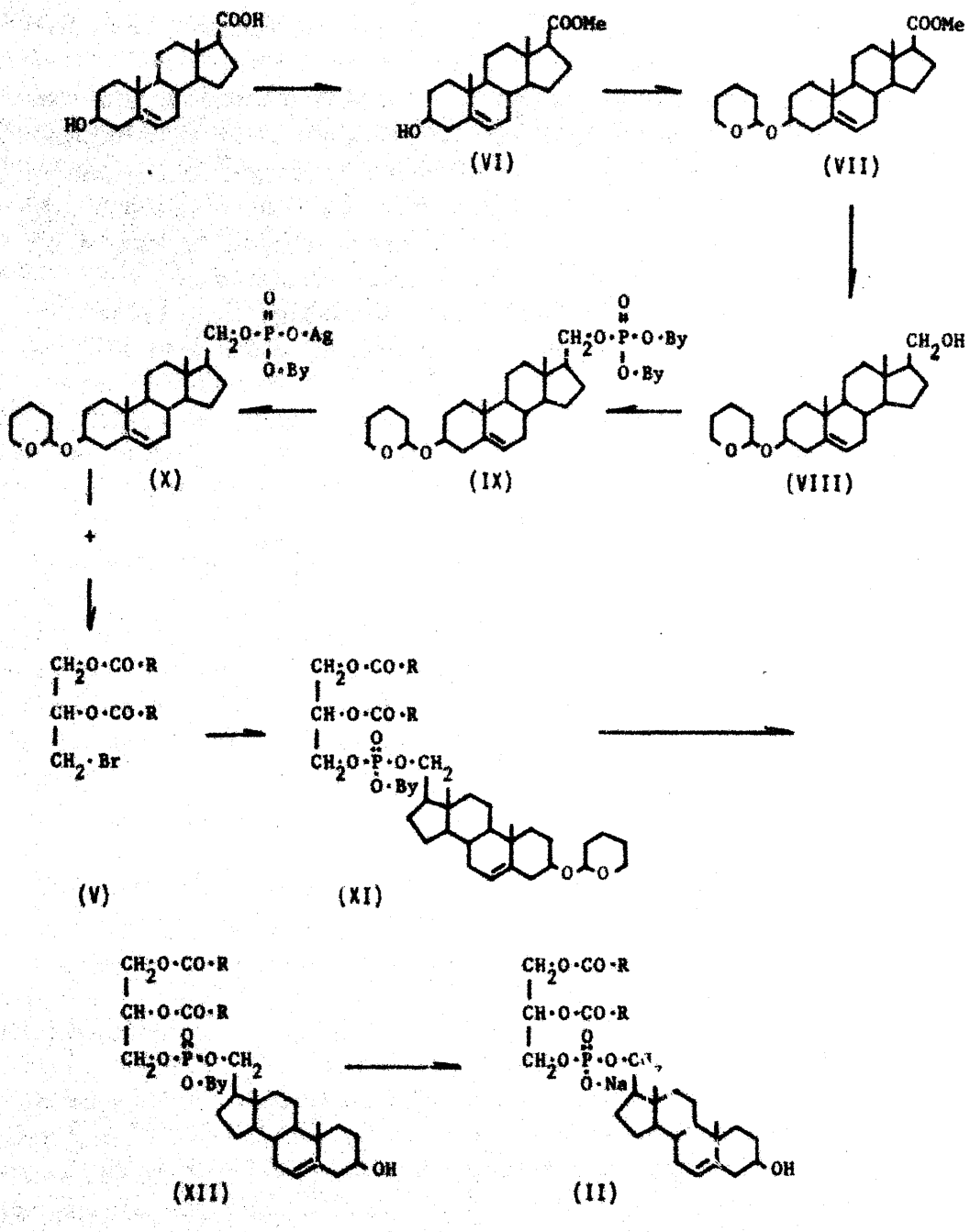
## II. Results and discussion

1,2-Dipalmitoyl-*rac*-glyceryl-3-phosphoryl-3′β-cholesterol (I) was synthesized by a condensation reaction of 1,2-dipalmitoyl-*rac*-glyceryl-3-silver benzylphosphate (III) and 3β-cholesteryl bromide. The reaction was carried out in acetonitrile in the dark, and after debenzylation of the condensation product, the final compound (I) was obtained as sodium salt in good yield. The reaction sequence is outlined in scheme 1.

Attempts to obtain 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-20′-(3β-hydroxy norpregn-5-ene) (II) by a similar condensation reaction were unsuccessful. Therefore, compound (II) was obtained by condensation of dipalmitoyl-*rac*-1-glycerol




SCHEME 1




R = $C_{15}H_{31}$, By = benzyl

Scheme 2

bromohydrin (V) with 3β-(2′-tetrahydropyranyloxy)-norpregn-5-ene-20-silver benzylphosphate (X), followed by removal of the protective groups as outlined in scheme 2.

For the synthesis of compound (X) commercially available 3β-hydroxyetiochol-5-enic 17β-acid was used. It was esterified with anhydrous methanol–hydrochloric

acid, and the 3β-hydroxy group was protected with 2,3-dihydropyran. The methyl ester (VI) was suspended in anhydrous benzene and a small molar excess of 2,3-dihydropyran was added as well as a catalytic amount of 70% perchloric acid. The mixture was stirred until a clear solution was obtained. Only 20 min at room temperature or 5 min at 40°C were required for complete reaction, and 3β-(2′-tetrahydropyranyloxy)-etiochol-5-enic 17β-acid methyl ester (VII) was obtained in quantitative yield. Perchloric acid was used as catalyst rather than hydrochloric acid or *p*-toluenesulfonic acid because the latter required longer reaction times.

Reduction of the methyl ester (VII) with lithium aluminum hydride in anhydrous diethyl ether produced 3β-(2′-tetrahydropyranyloxy)norpregn-5-en-20-ol

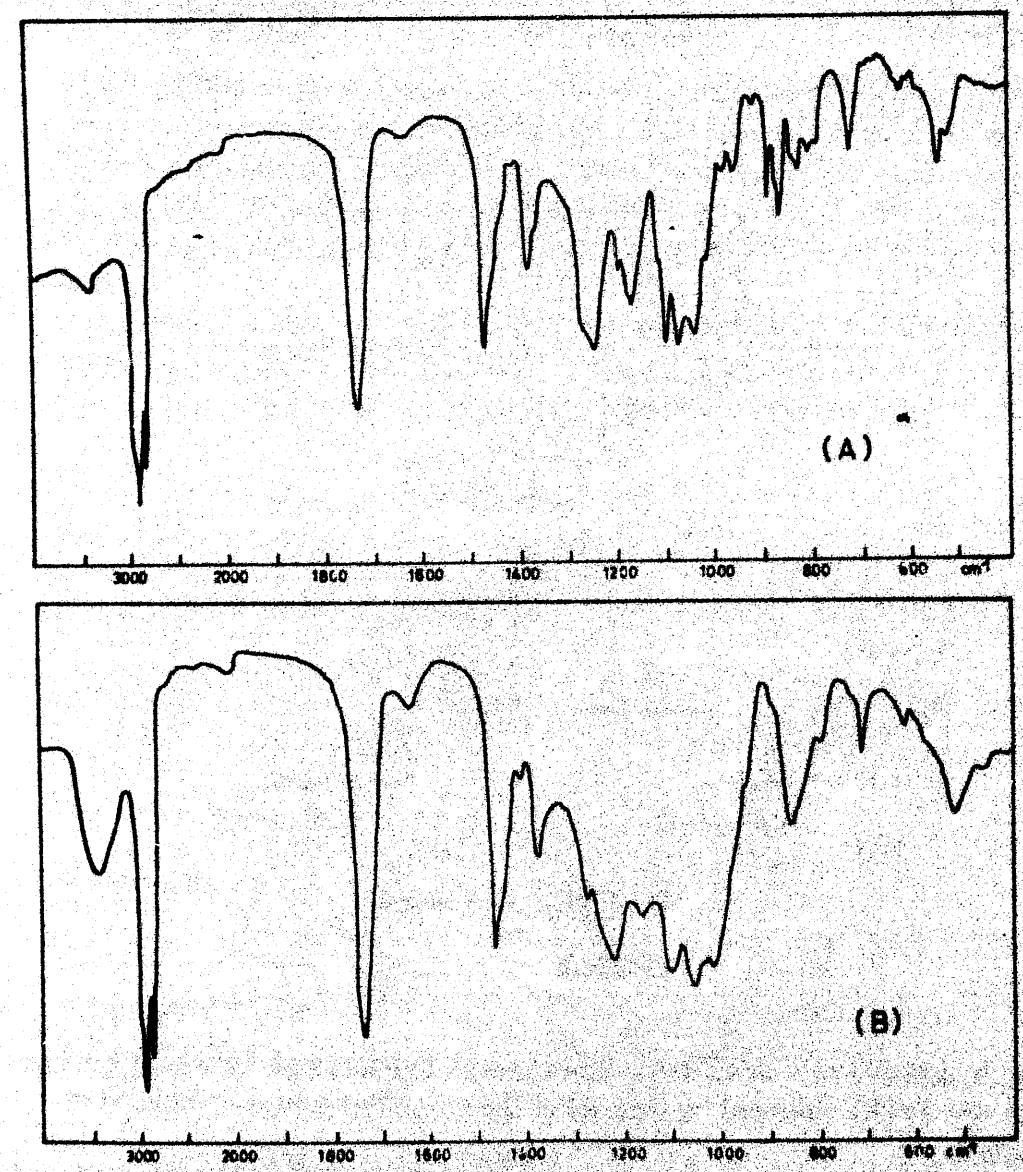



Fig. 1. Infrared spectra of 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-3′β-cholesterol (A) and 1,2-dipalmitoyl-*rac*-glyceryl-3-phosphoryl-20′-(3β-hydroxy norpregn-5-ene) (B).

(VIII) which was phosphorylated with dibenzylphosphochloridate, followed by mono-debenzylation with sodium iodide in anhydrous acetone. The resulting sodium salt was converted to the silver salt, and thus 3β-(2′-tetrahydropyranyloxy) norpregn-5-ene-20-silver benzylphosphate (X) was obtained. The silver salt (X) was condensed with 1,2-dipalmitoyl-*rac*-glycerol-3-bromohydrin (V) in boiling anhydrous benzene in the dark. After removal of the protective groups by acid hydrolysis, followed by reaction with sodium iodide, the final product (II) was obtained as the sodium salt.

IR spectra of both compounds (I) and (II) are shown in fig. 1.

Attempts to obtain compound (II) by condensation of 1,2-dipalmitoyl-*rac*-glycer-yl-3-silver benzylphosphate (III) and 3β-hydroxy- or 3β-(2′-tetrahydropyranyloxy) norpregn-5-ene-20-bromide under various conditions failed. The latter compound was synthesized from the corresponding 20-ol (VIII) *via* the methanesulfonate according to Baumann et al. [9] or by bromination of the methanesulfonate with anhydrous sodium bromide in *N,N*-dimethyl formamide at 70°C for 16 h.

The fact that, in contrast to the reaction with the 3β-bromide, condensation of the silver benzylphosphate (III) with the 20-bromide did not occur, can be explained by the presence of the double bond at C-5 of the steroid which may facilitate the condensation reaction. This assumption is supported by the fact that condensation of the silver benzylphosphate (III) with 3β-cholestanyl bromide also failed under the same conditions. *rac*-Glycerol-1-bromohydrin used as the starting material in the preparation of compound (II) was easily prepared from commercially available epibromohydrin by acid hydrolysis; this one-step reaction should be convenient for the synthesis of optically inactive phospholipids.

The physicochemical, biochemical and immunochemical properties of compounds (I) and (II) will be discussed elsewhere.

## III. Experimental

Melting points were determined on a Kofler heating stage (Leitz, Model 350) and were corrected. Elemental analyses were carried out by the Faculty of Pharmacy, Chiba University, by the Faculty of Science, Tokyo Metropolitan University and by the Laboratory of Microanalysis, Eisai Co. Infrared spectra were recorded with a Nihon Bunko Model IR-G spectrophotometer on KBr disks, and ultraviolet spectra were recorded in ethanol with a Hitachi Model 124 spectrophotometer. NMR were recorded with a Nihon Denshi Model MH 100 spectrometer in $CDCl_3$. Optical rotations were measured in chloroform with a Nihon Bunko Model Dip-SL automatic polarimeter. Analytical thin-layer chromatography was carried out on layers of silica gel H (Merck), 0.5 mm thick, in tanks lined with filter paper and fractions were made visible by spraying with Vaskovsky reagent [10] for phosphorus determination or with 50% $H_2SO_4$ and heating. In preparative TLC layers, 2 mm thick, were made by the method of Schmid et al. [11], using silica gel H or silica gel 60 $PF_{254}$ (Merck).

The fractions were eluted with chloroform–methanol, 2 : 1. The solvents were distilled and dried over Molecular Sieve Type 3A (Nishio Industrial Co.). Epibromohydrin, 3β-cholesteryl bromide and 2,3-dihydropyran were purchased from Tokyo Kasei Co. and 3β-hydroxyetiochol-5-enic 17β-acid (m.p. 268–275°C, $[\alpha]_D^{20°}$ = −12 to −18°) was from Merck. Dibenzylphosphite was synthesized according to the method of Atherton et al. [12], and was chlorinated with *N*-chlorosuccinimide by the method of Kenner et al. [13]. 1,2-Dipalmitoyl-*rac*-glyceryl-3-bromohydrin and 1,2-dipalmitoyl-*rac*-glyceryl-3-silver benzylphosphate were synthesized by the method of Bird et al. [14] except for the preparation of *rac*-glycerol-1-bromohydrin.

*A. Preparation of rac-glycerol-1-bromohydrin*

Epibromohydrin, 137 g, was heated with 54 ml of distilled water and 5 ml of 2 N $H_2SO_4$ at 100°C; a violent reaction occurred at once and continued for about 10 min. The homogeneous solution was heated for about 3 h at 100°C to complete the reaction, cooled and diluted with 3 vol of distilled water and passed through a column of Amberlite IR-45 (OH type). The column was washed with 1 liter of water and the eluent was concentrated under reduced pressure. The slightly yellow viscous liquid was purified by distillation in vacuo. Pure *rac*-glycerol-1-bromohydrin was obtained as a colorless liquid; b.p. 105–106°C/5 mm Hg, $n_D^{25°}$ = 1.5156, yield 82 g (52.9%).

*B. Preparation of 1,2-dipalmitoyl-rac-glyceryl-3-phosphoryl-3'β-cholesterol (I)*

*1,2-Dipalmitoyl-rac-glyceryl-3-benzylphosphoryl-3'β-cholesterol (IV)* Compound (III), 1.6 g, and 850 mg of 3β-cholesteryl bromide (purified by column chromatography on silica gel (Mallinckrodt, 100 mesh), eluted with benzene, and recrystallized from ethanol) were dissolved in 30 ml of anhydrous acetonitrile and refluxed for 5 h in the dark. The solution was cooled, diluted with 3 vol of ether and filtered. The precipitate was washed with several portions of ether and the filtrate was collected and concentrated under reduced pressure. The remaining oily material was purified by preparative TLC with benzene–diethyl ether, 50 : 50, as a solvent and 1.46 g (70.0%) of waxy but pure condensation product (IV) was obtained. (m.p. 38–39°C, TLC (benzene–diethyl ether, 50 : 50) $R_f$ = 0.49, UV $\lambda_{max}$ = 257.5 nm (log ε = 2.42), $[\alpha]_D^{22°}$ = −12.84° (c = 1.46).)

Analysis. Calcd. for $C_{69}H_{119}O_8P$ (1107.677): C 74.82, H 10.83, P 2.97.
Found: C 74.73, H 10.69, P. 2.61.

*1,2-Dipalmitoyl-rac-glyceryl-3-phosphoryl-3'β-cholesterol (I).* Compound (IV), 2.1 g, was refluxed with 430 mg (1.5 molar equivalent) of sodium iodide in 30 ml of anhydrous acetone for about 6 h. The solution was cooled and held overnight at −15°C. The precipitate was filtered, washed with cold acetone and dried in

vacuo. The sodium salt was obtained as a slightly yellow powder (1.88 g; 97.5%). The colored material was purified by washing with boiling acetone and 1.39 g (72.1%) of white, pure sodium salt (I) was obtained. (m.p. 198°C (decomp.), TLC (chloroform–methanol–28% aqueous ammonia, 70 : 30 : 7) $R_f = 0.80$, $[\alpha]_D^{22^\circ} = -23.16^\circ$ (c = 0.95), NMR: δ 5.25 ppm (C = C*H*).)

Analysis Calcd. for $C_{62}H_{112}O_8PNa$ (1039.534): C 71.64, H 10.86, P. 2.98.
Found: C 71.72, H 10.87, P. 3.06.

*C. Preparation of 1,2-dipalmitoyl-rac-glyceryl-3-phosphoryl-20'-(3β-hydroxynorpregn-5-ene) (II)*

*3β-Hydroxyetiochol-5-enic 17β-acid methyl ester (VI).* 3β-Hydroxyetiochol-5-enic 17β-acid, 4.09 g, was refluxed with 90 ml of anhydrous 3% HCl–MeOH for about 5 hr. After cooling, the solution was allowed to stand overnight at −15°C. The crystalline material was filtered, washed with cold methanol and recrystallized from methanol. The methyl ester (VI) (3.83 g; 89.8%) was obtained as needles. (m.p. 179–180°C, TLC (benzene–diethyl ether, 50 : 50) $R_f = 0.43$, $[\alpha]_D^{22^\circ} = -14.34^\circ$ (c = 1.06).)

Analysis Calcd. for $C_{21}H_{32}O_3$ (332.486): C 75.86, H 9.70.
Found: C 75.65, H 9.73.

*3β-(2'-Tetrahydropyranyloxy)etiochol-5-enic 17β-acid methyl ester (VII).* Compound (VI), 3.5 g, and 5 ml of freshly distilled 2,3-dihydropyran were suspended in 200 ml of anhydrous benzene. To this suspension, 5 μl of 70% perchloric acid was added and stirring was continued at 40°C until the starting material was dissolved (about 5 min). After stirring for 0.5 hr at room temperature, 1 g of anhydrous potassium carbonate was added and stirring was continued for 0.5 hr. The filtered and slightly yellow colored benzene solution was concentrated and a colored crystalline material was obtained. The crude material was recrystallized once from a small volume of methanol, and 3.96 g (90.2%) of pure 3β-(2'-tetrahydropyranyloxy)etiochol-5-enic 17β-acid methyl ester (VII) was obtained. (m.p. 152–153°C, TLC (benzene–diethyl ether, 50 : 50) $R_f = 0.60$, $[\alpha]_D^{22^\circ} = -15.03^\circ$ (c = 1.53).)

Analysis Calcd. for $C_{26}H_{40}O_4$ (416.610): C 74.96, H 9.68.
Found: C 75.05, H 9.79.

*3β-(2'-Tetrahydropyranyloxy)norpregn-5-en-20-ol (VIII).* Compound (VII), 1.6 g was dissolved in 70 ml of anhydrous ether and added dropwise to 40 ml of a diethyl ether solution of $LiAlH_4$ (1.0 g) under stirring. After stirring for about 2 hr at room temperature, water was added and the ether layer was separated, washed with water and dried over $Na_2SO_4$. The solvent was evaporated and 1.4 g (93.9%) of pure alco-

hol (VIII) was obtained. (m.p. 151–152°C (recrystallized from methanol), TLC (benzene–diethyl ether, 50 : 50) $R_f = 0.37$, $[\alpha]_D^{22^\circ} = -50.49^\circ$ (c = 1.03).)

Analysis. Calcd. for $C_{25}H_{40}O_3$ (388.600): C 77.27, H 10.38.
Found: C 77.20, H 10.58.

*3β-(2'-Tetrahydropyranyloxy)norpregn-5-ene-20-dibenzyl-phosphate (IX).* To an ice-cold solution of 4.86 g of compound (VIII) in 50 ml anhydrous benzene and 8 ml pyridine were added dropwise 140 ml of an anhydrous benzene solution containing 15.6 g (ca. 4 times molar excess) of dibenzylphosphochloridate, within 2 hr under vigorous stirring. After the solution had been stirred for 3 h under the same conditions, it was allowed to stand overnight at room temperature. The resulting precipitate was filtered off, 2 ml of water was added to the filtrate which was further stirred about 3 hr. The solvent was evaporated under reduced pressure and a yellow oily material was obtained; it was purified by column chromatography on silica gel (100 g of Iatrobeads 6RS-80100 (spherical silica gel), 100 mesh), eluted with benzene–diethyl ether, 3 : 2. The first 400 ml of eluent were concentrated and the remaining colorless oily material was dissolved in 30 ml of acetone. *n*-Hexane (200 ml) was added to the acetone solution and held overnight at –18°C. The resulting crystalline material was filtered and washed well with *n*-hexane, and 5.89 g (72.6%) of pure, white dibenzylphosphate (IX) was obtained. (m.p. 104–105°C, TLC (benzene–diethyl ether, 50 : 50) $R_f = 0.48$, UV $\lambda_{max} = 257.5$ nm ($\log \epsilon = 2.65$), $[\alpha]_D^{22^\circ} = -27.18^\circ$ (c = 1.03).)

Analysis. Calcd. for $C_{39}H_{53}O_6P$ (648.819): C 72.20, H 8.23, P 4.77.
Found: C 72.18, H 8.33, P 4.58.

*1,2-Dipalmitoyl-rac-glyceryl-3-benzylphosphoryl-20'-[3β-(2'-tetrahydropyranyloxy) norpregn-5-ene] (XI).* Compound (IX), 1.56 g, was refluxed with 570 mg of sodium iodide in 30 ml of anhydrous acetone for 7 hr; after standing overnight at –15°C, the sodium salt was filtered and washed with cold acetone and boiling acetone. The sodium salt was obtained as a powder (1.23 g; 87.9%). Silver nitrate (500 mg) was dissolved in 10 ml of distilled water and was added to 25 ml of a solution of the sodium salt in a distilled water as described above. After stirring for about 2 hr, the solution was placed overnight in a refrigerator; the silver salt was filtered off, washed with cold water and dried in vacuo over $P_2O_5$. The silver salt (X) (decomposed at 155–156°C) was obtained (1.2 g; 85.1%). Silver salt (X) (1.0 g) and 1.0 g of 1,2-dipalmitoyl-*rac*-glycerol-1-bromohydrin (V) were dissolved in 50 ml of anhydrous benzene and refluxed for 12 h in the dark. After cooling, the silver bromide was filtered off and the filtrate was concentrated under reduced pressure; 1.56 g of oily material was purified by preparative TLC using benzene–diethyl ether, 50 : 50, as a solvent. The main fraction ($R_f$ about 0.7) was collected and 1.34 g (80.7%) of colorless oily material (XI) (solidified in the refrigerator) was obtained. (m.p. 24–25°C, TLC

(benzene–diethyl ether, 50 : 50) $R_f$ = 0.63, UV $\lambda_{max}$ = 257.5 nm (log $\epsilon$ = 2.35), $[\alpha]_D^{22°}$ = –18.35° (*c* = 1.09).)

Analysis. Calcd. for $C_{67}H_{113}O_{10}P \times H_2O$ (1127.613): C 71.37, H 10.28, P 2.75.
Found: C 71.19, H 10.29, P 2.91.

*1,2-Dipalmitoyl-rac-glyceryl-3-benzylphosphoryl-20'-(3β-hydroxynorpregn-5-ene) (XII).* Compound (XI), 1.33 g, was refluxed with 1 ml of 1 N HCl in 60 ml of methanol until the oily material was dissolved (about 20 min); it was cooled and held overnight at –15°C. The white powdery precipitate was filtered and washed with cold methanol; 1.01 g (81.6%) of pure 3β-hydroxy compound (XII) was obtained. (m.p. 27–28°C, TLC (benzene–diethyl ether, 50 : 50) $R_f$ = 0.43, UV $\lambda_{max}$ = 257.5 nm (log $\epsilon$ = 2.33), $[\alpha]_D^{22°}$ = –20.36° (c = 1.11).)

Analysis. Calcd. for $C_{62}H_{105}O_9P$ (1025.480): C 72.62, H 10.32, P 3.02.
Found: C 72.45, H 10.16, P 2.98.

*1,2-Dipalmitoyl-rac-glyceryl-3-phosphoryl-20'-(3β-hydroxynorpregn-5-ene) (II).* Compound (XII), 2.18 g, was refluxed with 500 mg of sodium iodide in 40 ml of anhydrous acetone for about 6 hr. The solution was cooled and held overnight at –15°C, yielding 1.9 g (91.8%) of a powdery precipitate which was filtered and washed well with cold acetone. It was then purified by washing it several times with boiling acetone. A white, powdery final product (II) (1.70 g; 82.1%) was obtained as sodium salt. (m.p. 151–152°C, TLC (chloroform–methanol–28% aqueous ammonia, 70 : 30 : 7) $R_f$ = 0.74, $[\alpha]_D^{22°}$ = –18.80° (c = 1.06), NMR: δ 5.25 ppm (C = C*H*).)

Analysis. Calcd. for $C_{55}H_{98}O_9PNa \times H_2O$ (975.376): C 67.73, H 10.33, P 3.18.
Found: C 67.90, H 10.23, P 3.20.

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