Chemistry of Natural Compounds and Bioorganic Chemistry

Synthesis of modified ether phospholipids

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A number of modified ether phospholipids with additional substituents in the 2 position of the C(1)-alkyl chain, 1-*O*-[2′-(*R,S*)-hydroxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine, 1-*O*-[2′-(*R,S*)-acetoxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine, 1-*O*-[2′-(*R,S*)-hydroxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine, and 1-*O*-[2′-(*R,S*)-acetoxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine, have been synthesized.

Key words: ether phospholipids; analogs of platelet activating factor; modified phospholipids.

Ether lipids possess a broad spectrum of biological activities. A representative of this class of substances, 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor, PAF), is known to be a potent wide-range biological regulator. PAF has been shown to be a mediator of a number of biological processes. It stimulates platelet degranulation and aggregation;[1] causes the contraction of smooth muscles, bronchoconstriction, and coronary vasoconstriction;[1] increases vascular permeability;[1,2] and stimulates immune response.[3–5] PAF also plays an important role in a number of physiological and pathological processes, such as allergy, hypotension, anaphylaxis, thrombosis, ischemia, acute infections in transplantation, nephritis, gastric ulcer, *etc.*[6]

A large number of PAF structural analogs were synthesized to study the mechanism of PAF interaction with target cells. Modifications of the structure of the PAF molecule made it possible to separate various types of biological activities. For example, substances with more potent platelet aggregating and hypotensive effects than that of PAF; specific PAF antagonists;[7] and immunomodulators without platelet aggregating activity, which are hardly metabolizable as compared to PAF,[8–10] have been obtained. Many PAF structural analogs have been found to possess antitumor activity.[11] Some ether phospholipids are shown to inhibit HIV-1 replication in infected T-cells.[12] Thus, ether phospholipids are of considerable interest as potential therapeutic preparations because of the variety of their biological effects.

At present the main directions of the modifications of phospholipid structure, which cause the appearance or the disappearance of various biological activities, are already known. For example, the substitution of the ether bond in the *sn*-1 position by a thioether, amide, or

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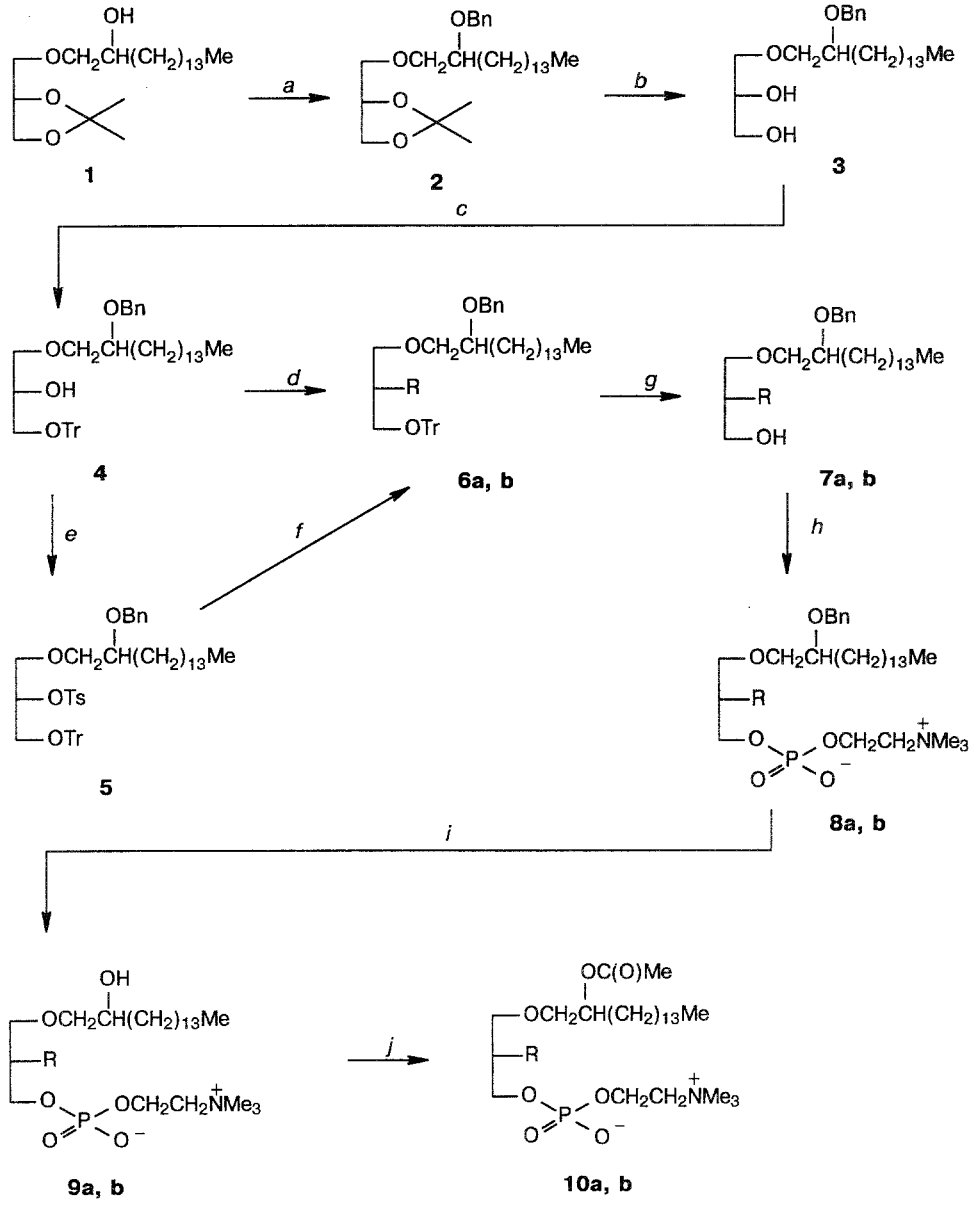
carbamoyl function or the replacement of the acetyl group in the *sn*-2 position with a small hardly metabolizable moiety (*e.g.*, a short chain alkoxy group or a halogen atom) cause a lack of platelet aggregating activity.[7] Sometimes such modifications lead to the appearance of PAF antagonism[7] and an antitumor effect.[11] The absence of platelet aggregating and hypotensive activities is indispensable in the synthesis of substances with possible antitumor and immunomodulating effects.

In confirming the studies on the structure—activity relations of ether phospholipids, we synthesized the compounds with additional substituents in the 2 position of the long alkyl chain. The additional moiety influences the steric surroundings in closest proximity to the glycerol backbone, which we expected to cause a decrease or disappearance of PAF-like activities. Such structures are of interest, because 1-*O*-[2′-(*R*)-methoxyhexadecyl]-*sn*-glycerol, which was isolated from natural

Scheme 1



R = OMe (**a**), Cl (**b**)

Reagents and conditions: *a*. BnCl, KOH, C_6H_6, 80 °C; *b*. TsOH, MeOH; *c*. TrCl, Py, 7 °C; *d*. MeI, KOH, C_6H_6, 80 °C; *e*. TsCl, Py, 0 °C; *f*. LiCl, DMF, 80 °C; *g*. Me_3SiCl, silica gel; *h*. $POCl_3$, Et_3N, $CHCl_3$; $HOCH_2CH_2N^+Me_3OTs^-$, Py; H_2O; *i*. H_2, Pd/C, MeOH; *j*. Ac_2O, $HClO_4$, $CHCl_3$.

sources, characterized, and then obtained by chemical synthesis, is known to inhibit tumor growth and to modulate the immune response in mice during oral administration.

In our previous communication[15] we described the synthesis of modified phosphatidylcholines with 2-methoxy substituents in the long alkyl chain. Now we report the synthesis of modified ether phospholipids (**9a,b** and **10a,b**) with hydroxy and acetoxy groups in the 2 position of the long alkyl chain. These substances are of interest as possible PAF antagonists, immunomodulators, and antitumor agents.

Initially, 1-*O*-[2′-(*R,S*)-hydroxyhexadecyl]-2,3-di-*O*-isopropylidene-*rac*-glycerol (**1**)[16] was refluxed with KOH powder in C_6H_6 with azeotropic distillation of water followed by alkylation with BnCl (Scheme 1) to give derivative **2**. The isopropylidene protective group of **2** was removed by methanolysis in the presence of a catalytic amount of TsOH. 1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-*rac*-glycerol (**3**) was tritylated to protect the primary hydroxy group, and the trityl derivative **4** was alkylated with MeI to yield 1-*O*-[2′-(*R,S*)-benzyloxyhexadecyl]-2-*O*-methyl-3-*O*-trityl-*rac*-glycerol (**6a**).

In the synthesis of 2-chloro-2-deoxy derivatives compound **4** was treated with TsCl in the presence of Py in $CHCl_3$ to obtain the tosyl derivative **5**. The tosyl group of **5** was then substituted by a chlorine atom by refluxing with LiCl in DMF *via* the Finkelstein reaction to give **6b**. Using DMF as solvent made it possible to avoid the partial detachment of the trityl group, which took place when the reaction was performed in MeC(O)Et. Then compounds **6a** and **6b** were treated with Me_3SiCl in the presence of silica gel to remove the trityl groups under mild conditions.[17] The resulting 1-*O*-[2′-(*R,S*)-benzyloxyhexadecyl]-2-*O*-methyl-*rac*-glycerol (**7a**) and 1-*O*-[2′-(*R,S*)-benzyloxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycerol (**7b**) were converted to phosphatidylcholines (**8a** and **8b**) by subsequent treatment with $POCl_3$ in the presence of Et_3N and with $HOCH_2CH_2N^+Me_3OTs^-$ in the presence of Py in $CHCl_3$ according to Brockerhoff *et al.*[18]

The catalytic hydrogenolysis of **8a** and **8b** in the presence of Pd/C in MeOH to remove the benzyl protective groups afforded 1-*O*-[2′-(*R,S*)-hydroxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (**9a**) and 1-*O*-[2′-(*R,S*)-hydroxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine (**9b**).

The 2′-hydroxyl functions of **9a** and **9b** were acetylated with Ac_2O in the presence of $HClO_4$ (see Ref. 19) to give 1-*O*-[2′-(*R,S*)-acetoxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (**10a**) and 1-*O*-[2′-(*R,S*)-acetoxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine (**10b**).

The identity and structure of the compounds obtained were confirmed by TLC, IR, ^1H and ^{31}P NMR spectroscopy, and mass spectrometry data.

Experimental

IR spectra (films) were recorded on a Shimadzu IR-435 spectrometer. ^1H NMR spectra were registered on Bruker MSL-200 (200 MHz for **3**, **5**, **7a,b**, and **8a**) and WM-500 (500 MHz for **9a,b** and **10a,b**) spectrometers in $CDCl_3$ for neutral lipids and in a mixture of $CDCl_3$—CD_3OD—D_2O (1 : 1 : 0.15) for phosphocholines with $SiMe_4$ as internal standard. Broad-band ^{31}P NMR spectra with proton decoupling were obtained on a Bruker MSL-200 instrument (81 MHz with H_3PO_4 as external standard) in a mixture of $CDCl_3$—CD_3OD—D_2O (1 : 1 : 0.15). Mass spectra were taken on an MSBKh (Sumy, Ukraine) plasma desorption mass spectrometer with ionization by the products of californium-252 fission and at an accelerating voltage of ±5 or ±20 eV. TLC was performed on Silufol UV-254 (Kavalier, Czechoslovakia) plates using the following developing systems: Et_2O (*A*); hexane—Et_2O, 4 : 1 (*B*); hexane—Et_2O, 1 : 1 (*C*); CHCl—MeOH—H_2O, 65 : 25 : 4 (*D*). The substances were detected by burning or treating with molybdenum blue (in the case of phospholipids). Column chromatographic purification was carried out on L 40/100 μm (Chemapol, Czechoslovakia) silica gel.

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2,3-di-*O*-isopropylidene-*rac*-glycerol (2). To a solution of 4.16 g (11.2 mmol) of 1-*O*-[2′-(*R,S*)-hydroxyhexadecyl]-2,3-di-*O*-isopropylidene-*rac*-glycerol (**1**) in 50 mL of C_6H_6 5.0 g (99.8 mmol) of KOH powder was added with vigorous stirring. The mixture was refluxed for 6 h with stirring and azeotropic distillation of water. Then 4.18 g (33.0 mmol, 3.80 mL) of BnCl was added, and the mixture was refluxed for 4 h with stirring. After the reaction mixture was cooled, the solvent and the excess of BnCl were removed *in vacuo*. The residue was dissolved in 100 mL of Et_2O and washed with water (3×100 mL) up to pH 7. The organic phase was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (30×300 mm; hexane—Et_2O, 95 : 5, as the eluent) and dried *in vacuo* (130 Pa, 60 °C, 8 h) to yield 4.40 g (85 %) of **2**, R_f 0.62 (system *B*). IR, ν/cm^{-1}: 3100—3050 (w, C—H arom.); 2900, 2840 (s, C—H); 1460 (s, C—H); 1280, 1270, 1250, 1210 (m, C—H, $C(CH_3)_2$); 1100—1150 (s, C—O—C); 840, 715, 695 (m, π,* C—H arom.); 720 (m, C—H).

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-*rac*-glycerol (3). Isopropylidene derivative **2** (4.4 g) was dissolved in 50 mL of MeOH in the presence of a catalytic amount of TsOH, and the solvent was removed *in vacuo*. The operation was repeated 5 times, up to the absence of the initial compound. The resulting residue was chromatographed on a silica gel column (30×300 mm; Et_2O as the eluent) and dried *in vacuo* to give 2.73 g (68 %) of **3**, R_f 0.45 (system *A*). IR, ν/cm^{-1}: 3400 (m, O—H); 3100—3050 (w, C—H arom.); 2900, 2840 (s, C—H); 1470, 1450 (m, C—H); 1120 (s, C—O—C); 1080 (s, C—O, C—OH); 715 (m, C—H); 700, 690 (m, π, C—H arom.). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.22 (br.s, 24 H, $(CH_2)_{12}$); 1.50 (m, 2 H, $CH(OBn)C\underline{H}_2$); 3.43—3.70 (m, 7 H, $C\underline{H}_2OH$, $C\underline{H}_2OC\underline{H}_2C\underline{H}OBn$); 3.82 (m, 1 H, $C\underline{H}OH$); 4.57 (dd, 2 H, $C\underline{H}_2Ph$, J = 11.5 and 13.0 Hz); 7.2—7.35 (m, 5 H, Ph). MS, *m/z* (I_{rel} (%)): 424.0 $[M+H]^+$ (100).

* π is extraplanar vibration.

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-3-*O*-trityl-*rac*-glycerol (4). A solution of 5.43 g (12.8 mmol) of **3** in 15 mL of anhydrous $CHCl_3$ and 5 mL of anhydrous Py was cooled to 7 °C, and 3.57 g (12.8 mmol) of TrCl was added with stirring. The mixture was stirred for 4 h at 7 °C, diluted with 35 mL of $CHCl_3$, and washed with 1 % HCl (50 mL) and then with water (2×50 mL) up to pH 7. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated. The residue was dried *in vacuo* (130 Pa, 50 °C, 6 h) to give 9.10 g of crude **4**, R_f 0.27 (system *B*). Compound **4** was used for the preparation of **5** and **6a** without purification.

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-*O*-tosyl-3-*O*-trityl-*rac*-glycerol (5). To a solution of 2.21 g (3.31 mmol) of **4** in 15 mL of dry Py cooled to 0 °C 0.75 g (3.97 mmol) of TsCl was added with stirring. The mixture was stirred for 20 h at 20 °C and then concentrated *in vacuo*. The residue was dissolved in 5 mL of C_6H_6. The solution was filtered to remove TsOH and Py · HCl and was evaporated *in vacuo*. The residue was dissolved in Et_2O and passed through Al_2O_3. After concentrating *in vacuo* the residue was chromatographed on a silica gel column (25×300 mm; hexane—Et_2O, 85 : 15, as the eluent) and dried *in vacuo* (130 Pa, 40 °C, 6 h). Yield 2.20 g (81 %), R_f 0.52 (system *C*). Found (%): C, 76.02; H, 8.03; S, 3.95. $C_{52}H_{66}O_6S$. Calculated (%): C, 76.25; H, 8.12; S, 3.91. IR, ν/cm^{-1}: 3100—3050 (w, C—H arom.); 2900, 2840 (s, C—H); 1480, 1430 (m, C—H); 1350, 1160 (s, S=O); 1110 (s, C—O—C); 900 (s, S—O); 790, 740 (m, π, C—H arom.); 720 (m, C—H); 670 (s, π, C—H arom.). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.24 (br.s, 24 H, $(CH_2)_{12}$); 1.32 (m, 2 H, $CH(OBn)C\underline{H}_2$); 2.37 (s, 3 H, $C\underline{H}_3$ (OTs)); 3.18—3.42 (m, 5 H, $C\underline{H}_2OC\underline{H}_2C\underline{H}OBn$); 3.59, 3.65 (dd, 1 H, $C\underline{H}_2OTr$, J = 5 and 11 Hz); 4.44 (dd, 13.0, 2 H, $C\underline{H}_2Ph$, J = 11.5 Hz); 4.66 (m, 1 H, $C\underline{H}OTs$); 7.14—7.39 (m, 22 H, 4Ph; *m*-H (OTs)); 7.77 (dt, 2 H, *o*-H (OTs), J = 8 and 2 Hz).

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-*O*-methyl-3-*O*-trityl-*rac*-glycerol (6a). To a solution of 1.90 g (2.85 mmol) of **4** in 25 mL of C_6H_6 0.8 g (14.3 mmol) of KOH powder was added. The mixture was refluxed for 3 h with stirring and azeotropic distillation of water and then cooled to 30 °C. MeI (2.02 g, 14.3 mmol) was added, and the mixture was stirred for 6 h at 50 °C. The solvent and the excess MeI were removed *in vacuo*. The residue was dissolved in 100 mL of Et_2O and washed with water (3×100 mL) up to pH 7. The organic layer was separated, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (25×200 mm; hexane—Et_2O, 9 : 1, as the eluent) and dried *in vacuo* (130 Pa, 50 °C, 6 h). Yield 1.63 g (84 %), R_f 0.54 (system *B*). Found (%): C, 81.28; H, 9.17. $C_{46}H_{62}O_4$. Calculated (%): C, 81.37; H, 9.20. IR, ν/cm^{-1}: 3100—3050 (w, C—H arom.); 2900, 2840 (s, C—H); 1490 (m, C=C arom.); 1460, 1450 (m, C—H); 1110 (s, C—O—C); 1090 (s, C—O, C—OH); 760, 740, 630 (m, π, C—H arom.); 725 (m, C—H); 695 (s, π, C—H arom.).

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-chloro-2-deoxy-3-*O*-trityl-*rac*-glycerol (6b). To a solution of 2.06 g (2.52 mmol) of tosyl derivative **5** in 25 mL of DMF 0.56 g (13.1 mmol) of LiCl was added, and the mixture was stirred for 7 days at 80 °C. The solvent was removed *in vacuo*. The residue was dissolved in 25 mL of Et_2O; the solution was washed with water (2×25 mL) and concentrated *in vacuo*. The residue was dried *in vacuo* (130 Pa, 40 °C, 6 h) to give 1.73 g of crude **6b**, R_f 0.74 (system *C*). Compound **6b** was then detritylated without purification.

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-*O*-methyl-*rac*-glycerol (7a). To a solution of 1.43 g (2.10 mmol) of **6a** in 20 mL of anhydrous $CHCl_3$ 5.0 g of activated (110 °C, 8 h) silica gel (L 40/100 μm) was added with stirring. Then a solution of 1.14 g (10.5 mmol, 1.32 mL) of Me_3SiCl in 20 mL of anhydrous $CHCl_3$ was gradually poured in, and the reaction mixture was stirred for 1 h at 20 °C. Py—H_2O mixture (1 : 2, 60 mL) was added; the reaction mixture was stirred for 1 h, filtered and washed with water up to pH 7. The solvents were removed *in vacuo*. The resulting residue was crystallized from hexane, chromatographed on a silica gel column (25×200 mm; toluene—Et_2O, 7 : 3, as the eluent), and dried *in vacuo* (130 Pa, 50 °C, 6 h). Yield 0.842 g (91 %), R_f 0.16 (system *C*). IR, ν/cm^{-1}: 3400 (m, O—H); 3100—3050 (w, C—H arom.); 2900, 2840 (s, C—H); 1460, 1450 (m, C—H); 1120 (s, C—O—C); 1080 (s, C—O, C—OH); 715 (m, C—H); 710, 690 (m, π, C—H arom.). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.23 (br.s, 24 H, $(CH_2)_{12}$); 1.30 (m, 2 H, $CH(OBn)C\underline{H}_2$); 3.31—3.65 (m, 8 H, $C\underline{H}_2OH$, $C\underline{H}OMe$, $C\underline{H}_2OC\underline{H}_2C\underline{H}OBn$); 3.35 (s, 3 H, OMe); 4.40 (dd, 2 H, $C\underline{H}_2Ph$, J = 11 and 19 Hz); 7.0—7.25 (m, 5 H, Ph). MS, *m/z* (I_{rel} (%)): 437.7 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycerol (7b) was obtained by detritylation of **6b** under the conditions of the previous experiment. Yield 0.843 g (76 % from **5**), R_f 0.36 (system *C*). Found (%): C, 70.77; H, 10.18. $C_{26}H_{45}O_3Cl$. Calculated (%): C, 70.80; H, 10.28. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.26 (br.s, 24 H, $(CH_2)_{12}$); 1.50 (m, 2 H, $CH(OBn)C\underline{H}_2$); 3.45—3.92 (m, 7 H, $C\underline{H}_2OH$, $C\underline{H}_2OC\underline{H}_2C\underline{H}OBn$); 4.09 (m, 1 H, $C\underline{H}Cl$); 4.58 (dd, 2 H, $C\underline{H}_2Ph$, J = 12 and 17 Hz); 7.21—7.36 (m, 5 H, Ph). MS, *m/z* (I_{rel} (%)): 441.7 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (8a). To a solution of 0.37 g (2.41 mmol, 0.22 mL) of $POCl_3$ in 5 mL of anhydrous $CHCl_3$ 0.842 g (1.93 mmol) of **7a** and 0.244 g (2.41 mmol, 0.334 mL) of dry Et_3N in 10 mL of anhydrous $CHCl_3$ were added dropwise with stirring. After 30 min 1.13 mL of anhydrous Py was added, and the reaction mixture was stirred for 30 min. Then 1.06 g (3.86 mmol) of $HOCH_2CH_2N^+Me_3OTs^-$ was added, and the reaction mixture was stirred for an additional 4 h. Then it was diluted with 25 mL of $CHCl_3$ and washed with 3 % aqueous solution of Na_2CO_3 (20 mL), 5 % HCl (20 mL), and water (3×20 mL) up to pH 7. The water layer was extracted twice with $CHCl_3$. The combined organic extract was evaporated *in vacuo*. The residue was chromatographed on a silica gel column (20×250 mm; $CHCl_3$—MeOH—H_2O, 65 : 25 : 4, as the eluent) and dried *in vacuo* (130 Pa, 20 °C, 12 h) to yield 0.804 g (69 %) of **8a**, R_f 0.20 (system *D*). ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.21 (br. s, 24 H, $(CH_2)_{12}$); 1.47 (m, 2 H, $CH(OBn)C\underline{H}_2$); 3.19 (s, 9 H, $N^+(CH_3)_3$); 3.34—3.66 (m, 8 H, CH_2N, $C\underline{H}OMe$, $C\underline{H}_2OC\underline{H}_2C\underline{H}OBn$); 3.47 (s, 3 H, OCH_3); 3.93 (m, 2 H, CH_2OP); 4.24 (m, 2 H, $POCH_2$); 4.61 (dd, 2 H, $C\underline{H}_2Ph$, J = 11.5 and 24 Hz); 7.25—7.45 (m, 5 H, Ph). MS, *m/z* (I_{rel} (%)): 604.4 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Benzyloxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine (8b) was obtained from 0.829 g (1.88 mmol) of **7b**, 0.36 g (2.35 mmol) of $POCl_3$, and 1.03 g (3.76 mmol) of $HOCH_2CH_2N^+Me_3OTs^-$ in the presence of 0.238 g (2.35 mmol, 0.326 mL) of anhydrous Et_3N and 1.19 g (15.03 mmol, 1.21 mL) of anhydrous Py in 20 mL of anhydrous $CHCl_3$ under the conditions of the previous experiment. The product was chromatographed on a silica gel column (25×200 mm; $CHCl_3$—MeOH—H_2O, 65 : 25 : 4, as the eluent) and dried *in vacuo* (130 Pa, 20 °C, 12 h) to give 0.923 g (81 %) of **8b**, R_f 0.20 (system *D*). MS, *m/z* (I_{rel} (%)): 607.4 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Hydroxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (9a). To 0.10 g of 10 % Pd/C (previously activated in an H_2 atmosphere at 100 °C and cooled to 20 °C) a solution of 0.80 g (1.33 mmol) of **8a** in 15 mL of anhydrous MeOH was added, and the mixture was stirred in the H_2 atmosphere for 8 h at 20 °C. The catalyst was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was chromatographed on a silica gel column (25×250 mm; $CHCl_3$—MeOH—H_2O, 65 : 25 : 4, as the eluent) and dried *in vacuo* (130 Pa, 20 °C, 12 h) to give 0.513 g (75 %) of **9a**, R_f 0.11 (system *D*). Found (%): C, 56.55; H, 10.59; N, 2.71. $C_{25}H_{54}O_7PN \cdot H_2O$. Calculated (%): C, 56.67; H, 10.66; N, 2.65. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.26 (br.s, 24 H, $(C\underline{H}_2)_{12}$); 1.42 (m, 2 H, CH(OH)C$\underline{H}_2$); 3.21 (s, 9 H, $N^+(CH_3)_3$); 3.29—3.66 (m, 7 H, CH_2N, C$\underline{H}_2$OC$\underline{H}_2$, C$\underline{H}$OMe); 3.45 (s, 3 H, OCH_3); 3.72 (m, 1 H, C$\underline{H}$OH); 3.94 (m, 2 H, CH_2OP); 4.25 (m, 2 H, $POCH_2$). ^{31}P NMR, δ: 1.13. MS, *m/z* (I_{rel} (%)): 512.8 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Hydroxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine (9b) was obtained by catalytic hydrogenolysis of 0.854 g of **8b** under the conditions of the previous experiment. Yield 0.431 g (59 %), R_f 0.12 (system *D*). Found (%): C, 53.99; H, 10.06; N, 2.70. $C_{24}H_{51}O_6PNCl \cdot H_2O$. Calculated (%): C, 53.90; H, 10.19; N, 2.62. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.26 (br.s, 24 H, $(C\underline{H}_2)_{12}$); 1.42 (m, 2 H, CH(OH)C$\underline{H}_2$); 3.22 (s, 9 H, $N^+(CH_3)_3$); 3.34—3.53 (m, 2 H, $CH_2OC\underline{H}_2$); 3.61 (m, 2 H, CH_2N); 3.67—3.80 (m, 3 H, C$\underline{H}$OH, C$\underline{H}_2$OCH_2); 4.09 (m, 2 H, CH_2OP); 4.21 (m, 1 H, CHCl); 4.27 (m, 2 H, $POCH_2$). ^{31}P NMR, δ: 0.65. MS, *m/z* (I_{rel} (%)): 517.1 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Acetoxyhexadecyl]-2-*O*-methyl-*rac*-glycero-3-phosphocholine (10a). To a solution of 0.273 g (0.534 mmol) of **9a** in 5 mL of $CHCl_3$ 1.96 g (16.21 mmol, 1.81 mL) of Ac_2O was added. After stirring for 30 s the reaction mixture was cooled to 0 °C, 0.91 mL of 8 *N* $HClO_4$ was added dropwise, and the mixture was stirred for 10 s. Then 18 mL of ice-cold water, 19.5 mL of $CHCl_3$ and 20 mL of MeOH were added. The organic layer was separated and extracted with MeOH—H_2O, 10 : 9 (2×38 mL). The water layer was extracted twice with $CHCl_3$. The combined organic extract was evaporated *in vacuo*. The residue was chromatographed on a silica gel column (10×200 mm; $CHCl_3$—MeOH—H_2O, 65 : 25 : 4, as the eluent) and dried *in vacuo* (130 Pa, 20 °C, 12 h) to yield 0.177 g (60 %) of **10a**, R_f 0.21 (system *D*). Found (%): C, 55.86; H, 10.13; N, 2.46. $C_{27}H_{56}O_8PN \cdot 1.5H_2O$. Calculated (%): C, 55.82; H, 10.25; N, 2.41. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.26 (br.s, 24 H, $(C\underline{H}_2)_{12}$); 1.47 (m, 2 H, CH(OAc)C$\underline{H}_2$); 2.07 (s, 3 H, $OC(O)CH_3$); 3.21 (s, 9 H, $N^+(CH_3)_3$); 3.41—3.67 (m, 7 H, CH_2N, C$\underline{H}_2$OC$\underline{H}_2$, C$\underline{H}$OMe); 3.45 (s, 3 H, OCH_3); 3.88 (m, 2 H, CH_2OP); 4.24 (m, 2 H, $POCH_2$); 5.0 (m, 1 H, C$\underline{H}$OAc). ^{31}P NMR, δ: 1.17. MS, *m/z* (I_{rel} (%)): 554.7 $[M+H]^+$ (100).

1-*O*-[2′-(*R,S*)-Acetoxyhexadecyl]-2-chloro-2-deoxy-*rac*-glycero-3-phosphocholine (10b) was obtained by treating **9b** (0.196 g, 0.379 mmol) with Ac_2O (1.39 g, 13.64 mmol) in the presence of $HClO_4$ (1 mL of 8 *N* solution, 0.777 mmol) under the conditions of the previous experiment. Yield 0.164 g (77 %), R_f 0.15 (system *D*). Found (%): C, 54.34; H, 9.62; N, 2.39. $C_{26}H_{53}O_7PNCl \cdot H_2O$. Calculated (%): C, 54.23; H, 9.63; N, 2.43. ^1H NMR, δ: 0.86 (t, 3 H, $(CH_2)_{12}C\underline{H}_3$, J = 7 Hz); 1.25 (br.s, 24 H, $(C\underline{H}_2)_{12}$); 1.55 (m, 2 H, CH(OAc)C$\underline{H}_2$); 2.08 (s, 3 H, $OC(O)CH_3$); 3.22 (s, 9 H, $N^+(CH_3)_3$); 3.51—3.83 (m, 4 H, C$\underline{H}_2$OC$\underline{H}_2$); 3.61 (m, 2 H, CH_2N); 4.04 (m, 2 H, CH_2OP); 4.18 (m, 1 H, CHCl); 4.27 (m, 2 H, $POCH_2$); 5.0 (m, 1 H, C$\underline{H}$OAc). ^{31}P NMR, δ: 0.64. MS, *m/z* (I_{rel} (%)): 559.2 $[M+H]^+$ (100).

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