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New Type of Cationic Glycerophospholipids

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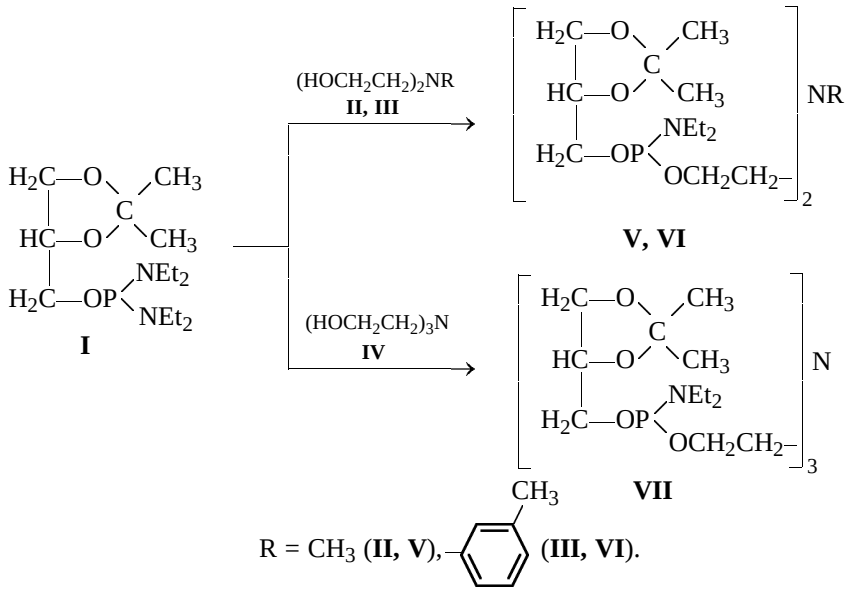
Abstract — A synthetic approach to an original type of model glycerophospholipids on the basis of glycerophosphoramidites and oligoethanolamines is developed.

Natural nitrogen-containing glycerophospholipids are components of biological membranes, and, therefore, synthesis of such compounds has been actively investigated [1, 2]. At the same time, for solving actual problems of modern lipidology, for example, for designing phospholipid-based liposoms, of great interest are structural analogs of nitrogen-containing glycerophospholipids [3, 4]. Among such analogs, a prominent place belongs to phospholipids containing polynitrogenous compounds, for example, polyamines [5] or peptides [6, 7]. Polynitrogenous glycerophospholipids, unlike natural compounds, contain more than one nitrogen atom per one phosphoglycerol residue, which explains their wide practical use [8, 9].

The aim of this work was design of an original type of glycerophospholipid analogs with an inverted, compared with the above-mentioned ones, phosphorus : nitrogen ratio. Such compounds may prove valuable starting materials for creating unusual biological and technical membranes. Research into the synthesis of modified glycophospholipids enriched with phosphoglycerol radicals we begun from studying phosphorylation of diethanolamine derivatives, such as methyldiethanolamine (**II**), *m*-tolyldiethanolamine (**III**), and triethanolamine (**IV**), with 1,2-*O*-isopropylideneglycerolphosphorodiamidite **I**.



Reactions of glycerophosphoramidite **I** with diethanolamines **II, III** were carried out at a 3 : 1 molar ratio in toluene, and with triethanolamine (**IV**), at a 4.5 : 1 molar ratio in dioxane at 65°C for 6 h with distillation of the diethylamine formed at a slightly reduced pressure. Using smaller amounts of glycerophosphoramidite **I** increases the reaction time and decreases the yield of the target products **V**–**VII**.

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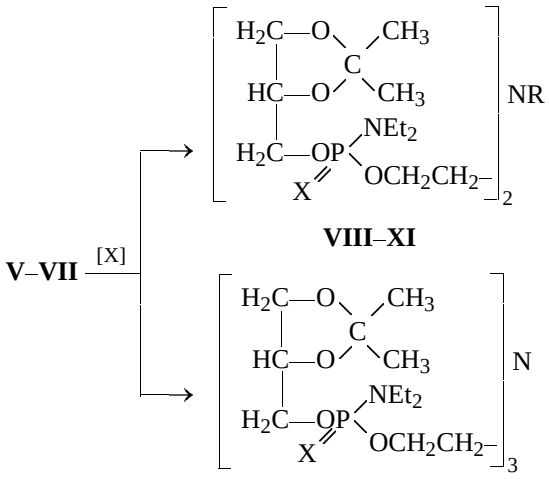
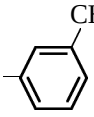
No difference in the reactivities of di- and triethanolamines was found. Reaction progress was controlled by TLC and ^{31}P NMR. The ^{31}P NMR spectra of glycerophosphoramidites **V**–**VII** contained a single broadened signal at 147–148 ppm, characteristic of alkyl phosphoramidites [3]. According to TLC and ^{1}H NMR data, compounds **V**–**VII** were sufficiently pure. Therefore, they were brought into subsequent transformations without additional purification.

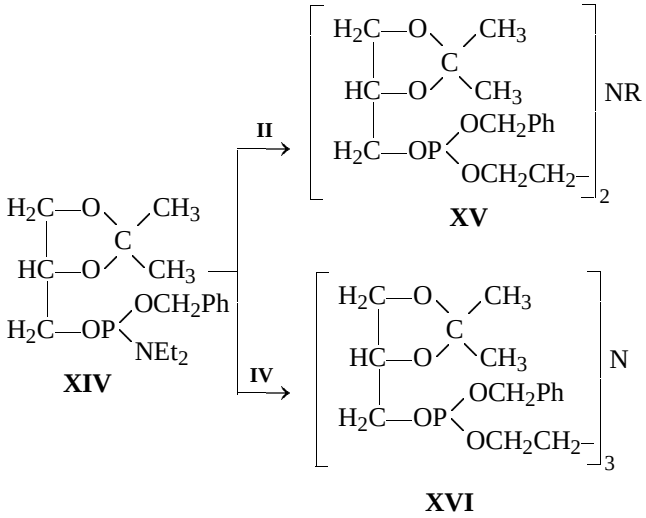
Glycerophosphoramidites **V**–**VII** were further converted into previously unknown glycerophosphoramidates **VIII, X, XII**, by oxidation with urea hydrogen peroxide adduct and to glycerophosphoramidothioates **IX, XI, XIII** by reaction with elemental sulfur.



X = O (**VIII, X, XII**), S (**IX, XI, XIII**); R = CH_3 (**VIII, IX**),  (**X, XI**).

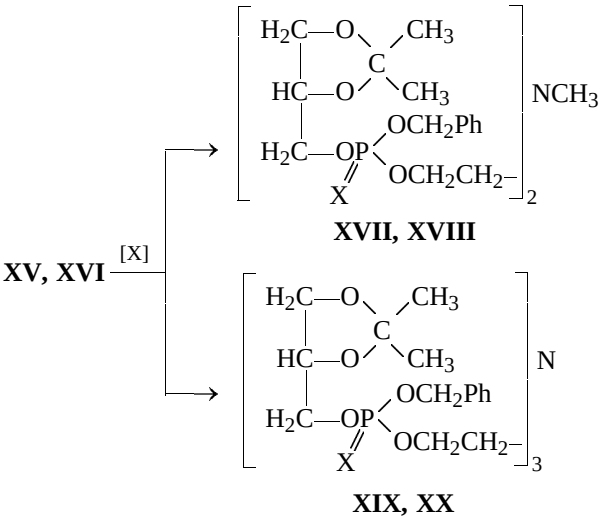
The oxidation and sulfurization were carried out at 20°C for 12 h. Glycerophosphoramidates **VIII, X, XII** and glycerophosphoramidothioates **IX, XI, XIII** were isolated by column chromatography on silica gel. Their yields after two stages (per alkanolamines **II**–**IV**) were 61–82%. The purity and structure of glycerophosphates **VIII**–**XIII** were established by means of TLC and ^{31}P and ^{1}H NMR spectroscopy. The ^{31}P NMR spectra of diastereomeric mixtures of glycerophosphates **VII, X, XII** and glycerophosphothioates **IX, XI, XII** contained broadened singlets at δ_P 10–11 and 76–77 ppm, respectively. Separate signals of diastereomers could not be detected because of the insufficient resolution of the spectrometer. The survey ^{1}H NMR spectra of phosphoramidates **VIII**–**XIII** showed signals of all proton groups with expected integral intensities. Thus, at δ 1.04 ppm and 3.03 ppm there were a triplet of methyl protons and a multiplet of methylene protons of the $PNCH_2CH_3$ group. At δ 1.34 ppm and 1.40 ppm, two singlets of methyl groups from the isopropylidene protective group were observed. The triplet at δ 2.91 ppm and the multiplet at δ 3.92 ppm were assigned to methylene protons of the NCH_2CH_2– and $POCH_2CH_2$ groups. The multiplets at δ 3.60, 3.81, and 4.04 ppm relate to the OCH_2CH– and –$CHCH_2OP$ methylene protons, respectively, and the CH_2CHCH_2 methine protons give a multiplet at δ 4.23 ppm. Along with the above-mentioned signals, the spectra of *N*-methyl derivatives **VIII, IX** display singlets of the NCH_3 methyl protons at δ 2.30–2.38 ppm, and the spectra of *N*-tolyl derivatives **X, XI**, signals of the tolyl methyl protons at δ 2.27–2.30 ppm. Furthermore, the $RNCH_2$ methylene signals of *N*-tolyl derivatives **X, XI** (δ 3.64–3.67 ppm) are shifted downfield compared with those of *N*-methyl derivatives **VIII, IX**.

In the next stage we made use of benzyl glycerophosphoramidite **XIV** as the phosphorylating agent.



The synthesis of trialkyl phosphites **XV, XVI** was performed in toluene or dioxane, respectively, at 90°C for 10 h and a 1.5-fold excess of the phosphorylating agent, like in the synthesis of glycerophosphoramidites **V**–**VII**. Unlike the reactions of glycerophosphoramidite **I** with ethanolamines **II**–**IV**, the reactions of benzyl glycerophosphoramidite **XIV** with amino alcohols **II, IV** occurred at higher temperatures and required more time to complete, which is connected with the lower reactivity of the amido group in glycerophosphoromonoamidites compared to glycerophosphorodiamidites [3]. The ^{31}P NMR spectra of **XV, XVI** contained a single broadened signal at δ_P 138–139 ppm, characteristic of trialkyl phosphites.

Crude benzyl glycerophosphites **XV, XVI** were converted into the corresponding phosphates **XVII, XIX** and phosphorothioates **XVIII, XX** under conditions analogous to those used for preparing glycerophosphoramidates **VIII**–**XIII**.



XVII, XVIII

XIX, XX

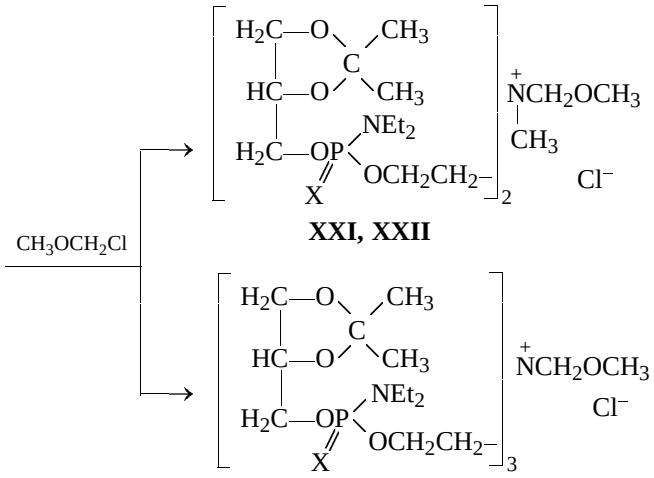
X = O (**XVII, XIX**), S (**XVIII, XX**).

Glycerophosphates **XVII, XIX** and glycerophosphorothioates **XVIII, XX** were isolated by column chromatography in 70–80% yields. The ^{31}P NMR spectra of phosphates **XVII, XIX** contained a broadened singlet at δ_P –1 ppm, and of phosphorothioates **XVIII, XX**, at δ_P 65–66 ppm. The ^1H NMR spectra of benzyl glycerophosphates **XVII**–**XX** were largely similar to those of glycerophosphoramidites **VII, IX, XII, XIII**. But, as would be expected, the NCH_2CH_3 methyl and methylene proton signals have disappeared, and a doublet of methylene protons at δ 5.1 ppm and a multiplet of aromatic protons at δ 7.4 pmm, belonging to the benzyl radical, appeared (see Experimental).

Note that among the newly synthesized polyglycerophosphamides of trivalent phosphorus, benzyl glycerophosphites **XV, XVI** are more stable to handling than glycerophosphoramidites **V**–**VII**. But, according to TLC and ^3P NMR data, both compounds **XV, XVI** and **V**–**VII** can be handled in closed vessels in toluene solutions under argon at 5°C for some days without noticeable decomposition. As would be expected, glycerophosphates **VII**–**XIII, XVII**–**XX** are more stable than the corresponding P(III) compounds and can be handled in toluene solutions under argon at 5°C for some weeks. At the same time, when handled in air at room temperature without solvent, glycerophosphates **VIII, X, XII, XVII, XIX** undergo disproportionation within several days, and glycerophosphorothioates **IX, XI, XIII, XVIII, XX**, even within 12 h under the same conditions.

In the final stage of this work, to obtain a series of model of nitrogen-containing cationic glycerophospholipids and their thio derivatives, we alkylated glycerophosphoramidates **VIII, IX**, derived from methyldiethanolamine, and glycerophosphoramidates **XII, XIII**, derived from triethanolamine, by the nitrogen atom with chloromethyl methyl ether.

VIII, IX, XII, XIII



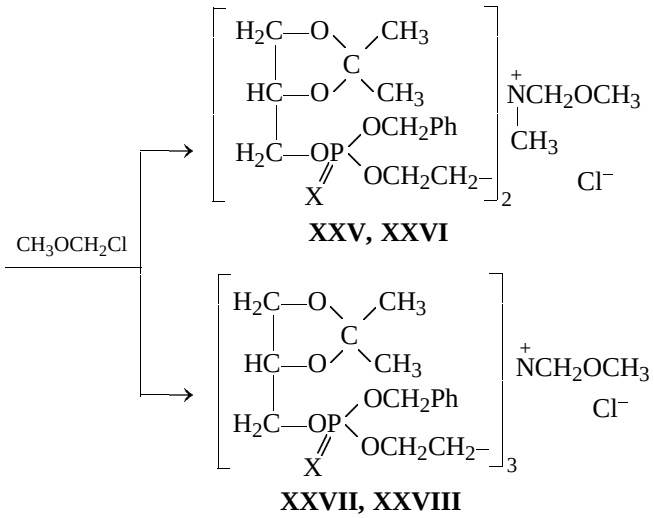
XXI, XXII

XXIII, XXIV

X = O (**XXI, XXIII**), S (**XXII, XXIV**).

As models of cationic glycerophospholipids and their thio analogs we synthesized compounds **XVII**–**XX**.

XVII–**XX**

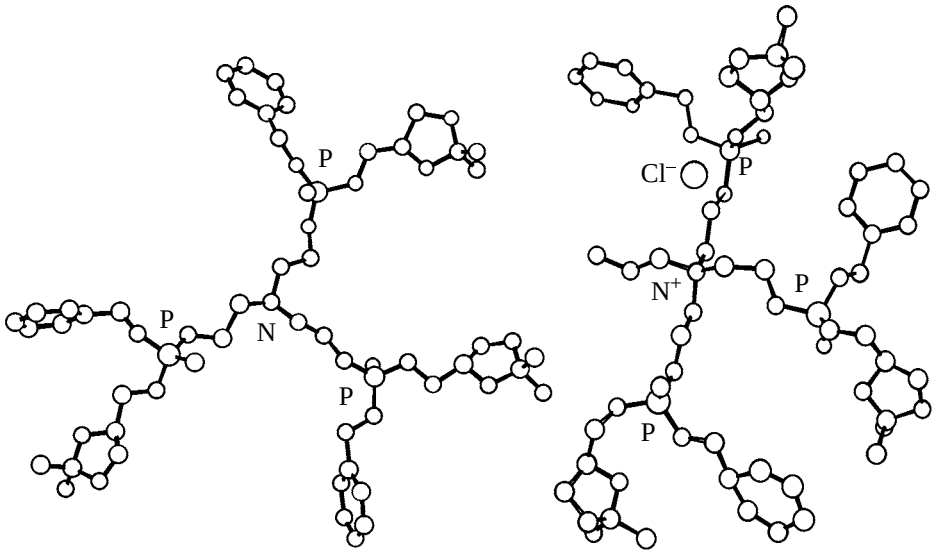


XXV, XXVI

XXVII, XXVIII

X = O (**XXV, XXVII**), S (**XXVI, XXVIII**).

The quarternization of phosphates **VIII, IX, XII, XIII, XVII**–**XX** was carried out at room temperature



Spatial arrangement of atoms in the molecules of phosphotrialkylamine **XIX** and its ammonium form **XXVII**.

for 1 h. Reaction progress was controlled by TLC. Ammonium compounds **XXI**–**XXVIII** were isolated by dissolution in ether followed by precipitation with hexane. The yields of the salts of benzyl glycerophosphates **XXV**–**XXVIII** reached 92%, whereas the salts of glycerophosphoramidates were isolated in lower yields (83%), probably, because salts **XXV**–**XXVIII** are poorer soluble in hexane. The ^{31}P NMR spectra of ammonium salts of phosphates **XXI, XXIII** and **XXV, XXVII** and phosphorothioates **XXII, XXIV** and **XXVI, XXVIII** contained broadened singlets at δ_P 10–11, –1, 76–77, and 66–67 ppm, respectively. In the ^{1}H NMR spectra of these salts, methyl and methylene signals of the CH_3OCH_2– group are observed at δ 3.65–4.05 ppm. Furthermore, the methylene proton signals of the diand triethanolamine residues were shifted downfield by 0.3–0.5 ppm compared with the parent phosphates.

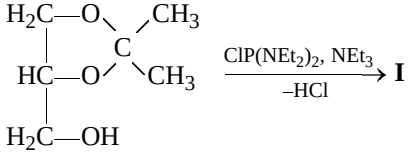
Evidently, for steric reasons we have not met with success on attempted quarternization with chloromethyl methyl ether of glycerophosphoramidates **X, XI** derived from *m*-tolyldiethanolamine. Alkylation by nitrogen did not take place at room temperature and occurred by phosphorus at 80°C. The latter fact was confirmed by changes in the ^{31}P NMR spectra of the reaction mixtures. Attempted alkylation by nitrogen of glycerophosphoramidates **VIII, XII** with alkyl halides, such as CH_3I and CH_3Br, failed. Here, too, alkylation by phosphorus was observed. At the same time, on alkylation of benzyl glycerophosphates **XVII, XIX** with methyl iodide and bromide we observed cleavage of the benzyl radical, which was confirmed by ^{1}H NMR spectroscopy.

Note in conclusion that on the basis of diethanolamine and triethanolamine derivatives we prepared model nitrogenous glycerophospholipids containing two or three phosphoglycerol residues per one nitrogen atom. These compounds are easily alkylated by the nitrogen atom, forming phosphoammonium derivatives that model cationic glycerophospholipids.

Using Dreiding models we found optimal spatial arrangements of atoms in the molecules of the synthesized glycerophosphates and their ammonium forms. It can be proposed that these molecules are round-shaped, which is most clearly pronounced in glycerophosphoramidates. The steric structures of phosphotrialkylamine **XIX** and its ammonium form **XXVII** are presented in the figure to illustrate this statement.

As seen from the figure, the nitrogen atom locates in the center of the creative construction and is surrounded by phosphoglycerol residues. Therefore, such nitrogen-containing glycerophospholipids can be considered as a prototype of inverted micelles in oil [10]. Up to now such compounds have been unknown. Their advantage is that they allow facile drawing a preset hydrophilic–lipophilic balance by means of chemical methods. For example, with cationic amphiphiles, this makes possible creation of chemical containers for transport of negatively charged biologically active compounds, such as nucleic acids or their fragments incorporated in genosoms.

The starting glycerophosphorodiamidite **I** was synthesized by the following scheme.



Previously 1,2-*O*-isopropylideneglycerophosphorodiamidite **I** was prepared by reaction between 1,2-*O*-isopropylideneglycerol and hexaethylphosphorous triamide [11]. Therewith, irrespective of the reagent ratio, diglycerophosphoramidite formed along with the target glycerophosphorodiamidite **I**. These compounds were separated by high-vacuum distillation. The yield of compound **I** under optimal conditions was no higher than 65%. The synthesis of glycerophosphorodiami-dite **I** by phosphorylation of 1,2-*O*-isopropylideneglycerol with tetraethylphosphorodiamidous chloride, proposed in the present work, is more convenient, and the yield of glycerophosphorodiamidite **I** reaches 82%.

EXPERIMENTAL

The ^{1}H NMR specra of compounds **V**–**XIII, XV**–**XXVIII** in $CDCl_3$ were recorded on a Bruker WM-250 (250 MHz) spectrometer against internal TMS. Signal assignment was made on the basis of double-resonance data. The ^{31}P–{^{1}H} NMR spectra of compounds **V**–**VIII, XV**–**XVIII** in $CHCl_3$ were recorded on a Bruker WP-80 SY (32.4 MHz) spectrometer against external 85% phosphoric acid. Column chromatography was performed carried out on a column 15 mm in diameter, filled on Silica gel L (100–160 μm). Thin-layer chromatography was performed on Silufol UV-254 plates, eluents 3:1 benzene–dioxane (A), 3:1 hexane–dioxane (B), 3:1 chloroform–methanol (C), 1:1 benzene–dioxane (D), benzene (E), and 1:1 hexane–ethyl acetate (F); development in iodine vapor or by calcination at 250–300°C.

All the syntheses with P(III) derivatives were carried out under dry argon. All solvents were dried by conventional procedures. 1,2-*O*-Isopropylideneglycero-3-(tetraethylphosphorodiamidite) (**I**) was prepared according to [11], and 1,2-*O*-isopropylodeneglycero-3-(benzyl diethylphosphoramidite) (**XIV**), according to [12]. Physicochemical constants of these compounds were in agreement with published data.

Bis[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinoyloxy]ethyl]methylamine (VIII). To a solution of 0.92 g of glycerophosphorodiamidite **I** in 5 ml of toluene, 0.12 g of (diethanol)methylamine (**II**) was added with stirring. The reaction mixture was kept for 6 h at 65°C and a residual pressure of 500 mm. Reaction progress was controlled by ^{31}P NMR spectroscopy and TLC. Excess glycerophosphorodiamidite **I** was removed in a vacuum, and the resulting glycerophosphoramidite **V** was kept at 65°C in a vacuum (1×10^{-4} mm) for 1 h. R_f 0.32 (A), 0.30 (B), 0.40 (D). ^{31}P NMR spectrum, δ_P, ppm: 147.82 br.s. Then to a solution of glycerophosphoramidite **V** in 5 ml of toluene, 0.4 g an of oxidant (urea hydrogen peroxide adduct) was added at 5°C and with vigorous stirring. The reaction mixture was kept for 12 h at room temperature, filtered, and the solvent was removed in a vacuum. Glycerophosphoramidate **VIII** was purified on a column of silica gel (25 g), filled with benzene. Compound **VIII** was eluted with 150 ml of a 5:1 benzene–dioxane mixture. The solvent was removed, and the residue kept in a vacuum (10^{-4} mm) for 2 h at 40°C. The yield of glycerophosphoramidate **VIII** per (diethanol)methylamine (after two stages) was 0.51 g (83%), n_D^{20}, R_f 0.10 (A), 0.78 (B), 0.22 (D). ^{31}P NMR spectrum, δ_P, ppm: 10.76 br.s. ^{1}H NMR spectrum, δ, ppm: 1.08 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.15 Hz), 1.33 s (6H), 1.39 s [6H, $C(CH_3)_2$], 2.35 (3H, NCH_3), 2.76 t (4H, NCH_2CH_2O, $^2J_{HH}$ 6.05 Hz), 3.05 m (8H, NCH_2CH_3, $^3J_{PH}$ 11.55 Hz), 3,80 m (2H), 3.90 m (2H, CH_2CHCH_2OP), 4.01 m (4H, NCH_2CH_2O), 4.04 m (4H, CH_2CHCH_2OP), 4.28 m (2H, CH_2CHCH_2OP). Found, %: C 48.59, H 8.70, P 10.08. $C_{25}H_{53}N_3O_{10}P_3$. Calculated, %: C 48.61, H 8.65, P 10.03.

Bis[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinothioyloxy]ethyl]methylamine (IX). To the solution of glycerophosphoramidite **V**, prepared from 0.92 g of glycerophosphorodiamidite **I** and 0.12 g of (diethanol)methylamine (**II**) in 5 ml of toluene, 0.1 g of sulfur was added, and the resulting mixture was kept for 12 h at room temperature. Excess sulfur was filtered off, and the solvent was removed in a vacuum. Glycerophosphoramidothioate **IX** was purified on a column of silica gel (25 g), filled with hexane. Compound **IX** was eluted with 150 ml of 5:1 hexane–ethyl acetate. The solvent was removed, and the residue was kept in a vacuum (1×10^{-4} mm) for 2 h at 40°C. The yield of glycerophosphoramidothioate **IX** per amine **II** (after two stages) was 0.52 g (80%), n_D^{20} 1.4884, R_f 0.70 (A), 0.95 (B), 0.07 (E). ^{31}P NMR spectrum, δ_P, ppm: 76.86, br.s. ^{1}H NMR spectrum, δ, ppm: 1.09 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.17 Hz), 1.35 s (6H), 1.40 s [6H, $C(CH_3)_2$], 2.32 s (3H, NCH_3), 2.73 t (4H, NCH_2CH_2O, $^3J_{HH}$ 6.08 Hz), 3.20 m (8H, NCH_2CH_3, $^3J_{PH}$ 11.55 Hz), 3.82 m (2H), 3.91 m (2H, CH_2CHCH_2OP), 4.01 m (4H, NCH_2CH_2O), 4.04 m (4H, CH_2CHCH_2OP), 4.27 m (2H, CH_2CHCH_2OP), 4.27 m (2H, $CH_2CH\cdot CH_2OP$). Found, %: C 46.21, H 8.22, P 9.53. $C_{25}H_{53}\cdot N_3O_8P_2S_2$. Calculated, %: C 46.17, H 8.30, P 9.59.

Bis[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinoyloxy]ethyl](*m*-tolyl)amine (X). First glycerophosphoramidite **VI** was prepared analogously to glycerophosphoroamidite **V** from 0.94 g of glycerophosphorodiamidite **I** and 0.2 g of (diethanol)(*m*-tolyl)amine (**III**). R_f 0.74 (A), 0.33 (B).

^{31}P NMR spectrum, δ_P, ppm: 147.61 br.s. Then, analogously to the synthesis of compound **VIII**, glycerophosphoramidite **VI** was oxidized with 0.4 g of urea hydrogen peroxide adduct to obtain phosphoramidate **X.** The yield of compound **X** per amine **III** (after two stages) was 0.42 g (61%), n_D^{20} 1.4871, R_f 0.18 (A), 0.89 (B), 0.38 (D). ^{31}P NMR spectrum, δ_P, ppm: 10.84 br.s. ^{1}H NMR spectrum, δ, ppm: 10.84 br.s. ^{1}H NMR spectrum, δ, ppm: 1.06 t (12H, $NCH_2 \cdot CH_3$, $^3J_{HH}$ 7.15 Hz), 1.33 s (6H), 1.39 s [6H, $C(CH_3)_2$], 2.27 s (3H, $NC_6H_4CH_3$), 3.03 m (8H, NCH_2CH_3, $^3J_{PH}$ 12.65 Hz), 3.64 t (4H, NCH_2CH_2O, $^3J_{HH}$ 6.04 Hz), 3.81 m (2H), 3.89 m (2H, $CH_2CH \cdot CH_2OP$), 4.01 m (4H, NCH_2CH_2O), 4.02 m (4H, CH_2CHCH_2OP), 4.26 m (2H, CH_2CHCH_2OP), 6.53 m (3H, C_6H_5), 7.08 t (1H, CH_3CCHCH, $^3J_{HH}$ 7.20 Hz). Found, %: C 53.60, H 8.35, P 8.87. $C_{31}H_{57} \cdot N_3O_{10}P_2$. Calculated, %: C 53.67, H 8.28, P 8.93.

Bis[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinothioyloxy]ethyl](*m*-tolyl)amine (XI) was prepared analogously to **IX** from glycerophosphoramidite **VI** (prepared from 0.94 g of glycerophosphorodiamidite **I** and 0.2 g of amine **III**) and 0.1 g of sulfur. The yield of glycerophosphoramidothioate **XI** per amine **III** (after two stages) was 0.51 g (70%), n_D^{20} 1.5183, R_f 0.90 (A), 0.13 (E), 0.65 (F). ^{31}P NMR spectrum, δ_P, ppm: 77.16 br.s. ^{1}H NMR spectrum, δ, ppm: 1.07 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.15 Hz), 1.35 s (6H), 1.40 s [6H, $C(CH_3)_2$], 2.29 s (3H, $NC_6 \cdot H_4CH_3$), 3.18 m (8H, NCH_2CH_3, $^3J_{PH}$ 12.65 Hz), 3.64 t (4H, NCH_2CH_2O, $^3J_{HH}$ 6.04 Hz), 3.79 m (2H), 3.86 m (2H, CH_2CHCH_2OP), 4.03 m (4H, $NCH_2 \cdot CH_2OP$), 4.28 m (2H, CH_2CHCH_2OP), 6.56 m (3H, C_6H_5), 7.09 t (1H, CH_3CCHCH, $^3J_{HH}$ 7.69 Hz). Found, %: C 51.32, H 7.95, P 8.57. $C_{31}H_{57}N_3O_8P_2S_2$. Calculated, %: C 51.29, H 7.91, P 8.53.

Tris[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinoyloxy]ethyl]amine (XII). First glycerophosphoramidite **VII** was prepared analoously to glycerophosphoramidite **V** from 1.40 g of glycerophosphorodiamidite **I** and 0.15 g of triethanolamine (**IV**) in 5 ml of dioxane. R_f 0.30 (A), 0.27 (B), 0.38 (D). ^{31}P NMR spectrum: δ_P 147.99 br.s. Then, similarly to the synthesis of compound **VIII**, from glycerophosphoramidite **VII** and 0.5 g of urea hydrogen peroxide adduct, glycerophosphoramidate **XII** was prepared. The yield of compound **XII** per amine **IV** (after two stages) was 0.74 g (82%), n_D^{20} 1.4622, R_f 0.18 (A), 0.93 (B), 0.25 (D). ^{31}P NMR spectrum, δ_P, ppm: 10.65 br.s. ^{1}H NMR spectrum, δ, ppm: 1.08 t (18H, NCH_2CH_3, $^3J_{HH}$ 6.95 Hz), 1.34 s (9H), 1.40 s [9H, $C(CH_3)_2$], 2.89 t (6H, NCH_2CH_2O, $^3J_{HH}$ 6.01 Hz), 3.05 m (12H, NCH_2CH_3 , $^3J_{PH}$ 11.55 Hz), 3.80 m (3H), 3.88 m (3H, CH_2CHCH_2OP), 3.93 m (6H, NCH_2CH_2OP), 4.06 m (6H, CH_2CHCH_2OP), 4.27 m (3H, CH_2CHCH_2OP). Found, %: C 48.12, H 8.48, P 10.28. $C_{36}H_{75}N_4O_{15}P_3$. Calculated, %: C 48.21, H 8.43, P 10.36.

Tris[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinothioyloxy]ethyl]amine (XIII) was obtained analogously to compound **IX** from glycerophosphoramidite **VII** (prepared from 1.40 g of glycerophosphoramidite **I** and 0.15 g of amine **IV**) and 0.1 g of sulfur. The yield of glycerophosphoramidothioate **XIII** per amine **IV** (after two stages) was 0.70 g (74%), n_D^{20} 1.4939, R_f 0.61 (A), 0.93 (B), 0.11 (E). ^{31}P NMR spectrum, δ_P, ppm: 76.81 br.s. ^{1}H NMR spectrum, δ_P, ppm: 76.81. ^{1}H NMR spectrum, δ, ppm: 1.09 t (18H, NCH_2CH_3, $^3J_{HH}$ 7.15 Hz), 1.34 s (9H), 1.40 s [9H, $C(CH_3)_2$], 2.88 t (6H, NCH_2CH_2O, $^3J_{HH}$ 6.05 Hz), 3.20 m (12H, NCH_2CH_3, $^3J_{PH}$ 12.20 Hz), 3.80 m (3H), 3.87 m (3H, CH_2CHCH_2OP), 3.96 m (6H, NCH_2CH_2O), 4.06 m (6H, $CH_2CHCH_2 \cdot OP$), 4.30 m (3H, CH_2CHCH_2OP). Found, %: C 45.69, H 8.01, P 9.78. $C_{36}H_{75}N_4O_{12}P_3S_3$. Calculated, %: C45.75, H 8.00, P 9.83.

Bis[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinoyloxy]ethyl]methylamine (XVII). First benzyl glycerophophoramidite **XV** was prepared analogously to glycerophosphoramidite **V** from 1.03 g of benzyl glycerophophoramidite **XIV** in 5 ml of toluene and 0.12 g of amine **II** within 10 h at 90°C. R_f 0.30 (A), 0.69 (B). ^{31}P NMR spectrum, δ_P, ppm: 138.60 br.s. Then, analogously to the synthesis of compound **VIII**, benzyl glycerophosphoramidate **XVII** was obtained from compound **XIV** and 0.4 g of urea hydrogen peroxida adduct. The yield of compound **XVII** per amine **II** (after two stages) was 0.58 g (84%), n_D^{20} 1.4996, R_f 0.09 (A), 0.86 (B), 0.44 (D). ^{31}P NMR spectrum, δ_P, ppm: –1.20 br.s. ^{1}H NMR spectrum, δ, ppm: 1.33 s (6H), 1.39 s [6H, $C(CH_3)_2$], 2.29 s (3H, NCH_3), 2.68 t (4H, NCH_2CH_2O, $^2J_{HH}$ 6.01 Hz), 3.75 (2H), 3.94 m (2H, CH_2CHCH_2OP), 4.02 m (4H, NCH_2CH_2O), 4.05 m (4H, CH_2CHCH_2OP), 4.24 m (2H, $CH_2CH \cdot CH_2OP$), 5.08 d (4H, $OCH_2C_6H_5$, $^3J_{PH}$ 8.53 Hz), 7.37 m (10H, $OCH_2C_6H_5$). Found, %: C 54.10, H 6.92, P 8.95. $C_{31}H_{47}NO_{12}P_2$. Calculated, %: C 54.15, H 6.89, P 9.01.

Bis[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinothioyloxy]ethyl]-methylamine (XVIII) was prepared analogously to compound **IX** from benzyl glycerophosphoramidite **XV** (prepared from 1.03 g of benzyl glycerophosphoramidite **XIV** and 0.12 g of amine **II**) and 0.1 g of

sulfur. The yield of compound **XVIII** per amine **II** (after two stages) was 0.57 g (79%), n_D^{20} 1.5284, R_f 0.75 (A), 0.98 (B), 0.06 (E). ^{31}P NMR spectrum, δ_P, ppm: 69.01 br.s. ^{1}H NMR spectrum, δ, ppm: 1.34 (6H), 1.40 s [6H, $C(CH_3)_2$], 2.35 s (3H, NCH_2), 2.75 t (4H, NCH_2CH_2O, $^3J_{HH}$ 6.05 Hz), 3.76 m (2H), 3.96 m (2H, CH_2CHCH_2OP), 4.00 m (4H, $NCH_2 \cdot CH_2O$), 4.12 m (4H, CH_2CHCH_2OP), 4.25 m (2H, CH_2CHCH_2OP), 5.11 d (4H, $OCH_2C_6H_5$, $^3J_{PH}$ 10.59 Hz), 7.37 m (10H, $OCH_2C_6H_5$). Found, %: C 51.70, H 6.63, P 8.58. $C_{31}H_{47}NO_{10}P_2S_2$. Calculated, %: C 51.73, H 6.58, P 8.61.

Tris[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinoyloxy]ethyl]amine (XIX). First benzyl glycerophosphoramidite **XVI** was prepared similarly to glycerophosphoroamidite **V** from 1.50 g of benzyl glycerolphosphoramidite **XIV** in 5 ml of dioxane and 0.15 g of triethanolamine (**IV**) within 10 h at 90°C. R_f 0.32 (A), 0.27 (B), 0.40 (C). ^{31}P NMR spectrum δ_P, ppm: 138.65 br.s. Then, analogously to the synthesis of compound **VIII**, benzyl glycerophosphoramidate **XIX** was prepared from compound **XVI** and 0.5 g of urea hydrogen peroxide adduct. The yield of compound **XIX** per amine **IV** (after two stages) was 0.78 g (78%), n_D^{20} 1.5015, R_f 0.20 (A), 0.90 (C), 0.50 (D). ^{31}P NMR spectrum, δ_P, ppm: –0.45 br.s. ^{1}H NMR spectrum, δ, ppm: 1.33 s (9H), 1.39 s [9H, $C(CH_3)_2$], 2.81 t (6H, NCH_2CH_2O, $^3J_{HH}$ 6.11 Hz), 3.73 m (3H), 3.97 m (3H, $CH_2CH \cdot CH_2OP$), 4.01 m (6H, NCH_2CH_2O), 4.04 m (6H, CH_2CHCH_2OP), 4.23 m (3H, CH_2CHCH_2OP), 5.07 d (6H, $OCH_2C_6H_5$, $^3J_{PH}$ 8.69 Hz), 7.36 m (15H, $OCH_2C_6H_5$). Found, %: C 53.82, H 6.68, P 9.16. $C_{45}H_{66}NO_{18}P_3$. Calculated, %: C 53.94, H 6.64, P 9.27.

Tris[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinothioyloxy]ethyl]amine (XX) was obtained analogously to compound **IX** from benzyl glycerophosphoramidite **XVI** (prepared from 1.50 g of benzyl glycerophosphoramidite **XIV** and 0.15 g of amine **IV**) and 0.1 g of sulfur. The yield of compound **XX** per amine **IV** (after two stages) was 0.74 g (70%), n_D^{20} 1.5182, R_f 0.73(A), 0.96 (B), 0.15 (E). ^{31}P NMR spectrum, δ, ppm: 65.07 br.s. ^{1}H NMR spectrum, δ, ppm: 1.33 s (9H), 1.39 s [9H, $C(CH_3)_2$], 2.84 t (6H, NCH_2CH_2O, $^3J_{HH}$ 6.08 Hz), 3.74 m (3H), 3.93 m (3H, CH_2CHCH_2OP), 3.98 m (6H, $NCH_2 \cdot CH_2O$), 4.03 m (6H, CH_2CHCH_2OP), 4.23 m (3H, $CH_2CHCHd2OP$), 5.07 d (6H, $OCH_2C_6H_5$, $^3J_{PH}$ 8.68 Hz), 7.36 m (15H, $OCH_2C_6H_5$). Found, %: C 51.38, H 6.35, P 8.80. $C_{45}H_{66}NO_{15}P_3S_3$. Calculated, %: C 51.47, H 6.33, P 8.85.

Chloromethoxymethylates of glycerophosphates XXI–XXVIII. To a solution of 1 mmol of compound **VIII, IX, XII, XIII, XVII–XX** in 3 ml of benzene, a solution of 10 mmol of chloromethyl methyl ether in 2 ml of benzene was added dropwise with vigorous stirring, and the reaction mixture was stirred for 1 h at room temperature. The solvent and excess chloromethyl methyl ether were removed in a vacuum, and the resulting ammonium salts were dissolved in 2 ml of diethyl ether and precipitated with 10 ml of hexane. Products **XXI–XXVIII** precipitated as oils and were kept in a vacuum (1×10^{-4} mm) for 2 h at 40°C.

Bis[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinoyloxy]ethyl]methylamine chloromethoxymethylate (XXI). Yield 0.61 g (87%), n_D^{20} 1.4723, R_f 0.09 (C), 0.03 (D). ^{31}P NMR spectrum, δ_P, ppm: 11.13 br.s. ^{1}H NMR spectrum, δ, ppm: 1.10 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.15 Hz), 1.34 s (6H), 1.40 s [6H, $C(CH_3)_2$], 2.77 br.s (3H, NCH_3), 3.06 m (8H, NCH_2CH_3, $^3J_{PH}$ 11.55 Hz), 3.25 br.s (4H, $NCH_2 \cdot CH_2O$), 3.80 m (2H), 3.90 m (2H, CH_2CHCH_2OP), 4.06 m (4H, CH_2CHCH_2OP), 4.07 s (3H, CH_3OCH_2), 4.31 m (4H, NCH_2CH_2O), 4.31 m (2H, $CH_2CHCH_2 \cdot OP$), 4.33 br.s (2H, CH_3OCH_2). Found, %: C 46.59, H 8.45, P 8.90. $C_{27}H_{58}ClN_3O_{11}P_2$. Calculated, %: C 46.45, H 8.37, P 8.87.

Bis[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinothioyloxy]ethyl]methylamine chloromethoxymethylate (XXII). Yield 0.62 g (85%), n_D^{20} 1.4933, R_f 0.58 (C), 0.05 (D). ^{31}P NMR spectrum, δ_P, ppm: 76.96 br.s. ^{1}H NMR spectrum, δ, ppm: 1.10 t (12H, NCH_2CH_3, $^3J_{HH}$ 7.17 Hz), 1.35 s (6H), 1.41 s [6H, $C(CH_3)_2$], 2.57 br.s (3H, NCH_3), 3.01 br.s (4H, NCH_2CH_2O, $^3J_{HH}$ 6.08 Hz), 3.21 m (8H, $NCH_2 \cdot CH_3$, $^3J_{PH}$ 11.55 Hz), 3.82 m (2H), 3.89 s (3H, $CH_3 \cdot OCH_2$), 3.96 m (2H, CH_2CHCH_2OP), 4.05 m (4H, NCHd2CH_2O), 4.08 m (4H, CH_2CHCH_2OP), 4.15 br.s (2H, CH_3OCH_2), 4.31 m (2H, $CH_2CHCH_2 \cdot OP$). Found, %: C 44.46, H 8.08, P 8.53. $C_{27}H_{58} \cdot ClN_3O_9P_2S_2$.

Tris[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinoyloxy]ethyl]amine chloromethoxymethylate (XXIII). Yield 0.83 g (85%), n_D^{20} 1.4730, R_f 0.08 (C), 0.03 (D). ^{31}P NMR spectrum, δ_P, ppm: 11.18 br.s. ^{1}H NMR spectrum, δ, ppm: 1.09 t (18H, NCH_2CH_3, $^3J_{HH}$ 7.15 Hz), 1.33 s (9H), 1.39 s [9H, $C(CH_3)_2$], 3.06 m (12H, NCH_2CH_3, $^3J_{PH}$ 11.55 Hz), 3.55 br.t (6H, NCH_2CH_2O, $^3J_{HH}$ 4.95 Hz), 3.67 s (3H, CH_3OCH_2), 3.76 m (3H), 3.84 m (3H, $CH_2 \cdot CHCH_2OP$), 3.93 m (6H, NCH_2CH_2O), 4.04 m (6H, CH_2CHCH_2OP, 4.27 m (3H, CH_2CHCH_2OP),

4.40 br.s (2H, CH_3OCH_2). Found, %: C 46.71, H 8.28, P 9.50. $C_{38}H_{80}N_4O_{16}P_3$. Calculated, %: C 46.69, H 8.25, P 9.51.

Tris[1-[1,2-*O*-isopropylideneglycero-3-(diethylamino)phosphinothioyloxy]ethyl]amine chloromethoxymethylate (XXIV). Yield 0.85 g (83%), n_D^{20} 1.5004, R_f 0.71 (C), 0.06 (D). ^{31}P NMR spectrum, δ_P, ppm: 76.95 br.s. ^{1}H NMR spectrum, δ, ppm: 1.11 t (18H, NCH_2CH_3, $^3J_{HH}$ 7.06 Hz), 1.36 s (9H), 1.45 s [9H, $C(CH_3)_2$], 2.99 br.t (6H, NCH_2CH_2O, $^3J_{HH}$ 5.98 Hz), 3.22 m (12H, NCH_2CH_3, $^3J_{PH}$ 14.04 Hz), 3.80 m (3H), 3.86 m (3H, CH_2CHCH_2OP), 3.89 s (3H, CH_3OCH_2), 4.03 m (6H, CH_2CHCH_2OP), 4.07 m (6H, NCH_2CH_2O), 4.32 m (3H, CH_2CHCH_2OP). Found, %: C 44.38, H 7.94, P 9.02. $C_{38}H_{80}ClN_4O_{13}P_3S_3$. Calculated, %: C 44.50, H 7.86, P 9.06.

Bis[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinoyloxy]ethyl]methylamine chloromethoxymethylate (XXV). Yield 0.7 g (91%), n_D^{20} 1.5122, R_f 0.59 (C),).11 (G). ^{31}P NMR spectrum, δ_P, ppm: –0.98 br.s. ^{1}H NMR spectrum, δ, ppm: 1.34 s (6H), 1.41 s [6H, $C(CH_3)_2$], 2.73 br.s (3H, NCH_3), 3.23 br.t (4H, NCH_2CH_2O), 3.70 m (2H), 3.99 m (2H, CH_2CHCH_2OP), 4.02 m (4H, $NCH_2 \cdot CH_2O$), 4.07 m (4H, CH_2CHCH_2OP), 4.12 s (3H, CH_3OCH_2), 4.24 m (2H, CH_2CHCH_2OP), 4.45 br.s (2H, CH_3OCH_2), 5.13 d (4H, $OCH_2C_6H_5$, $^3J_{PH}$ 9.83 Hz), 7.41 m (10H, $OCH_2C_6H_5$). Found, %: C 51.64, H 6.90, P 8.00. $C_{33}H_{52}ClNO_{13}P_2$. Calculated, %: C 51.60, H 6.82, P 8.06.

Bis[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinothioyloxy]ethyl]methylamine chloromethoxymethylate (XXVI). Yield 0.72 g (90%), n_D^{20} 1.5358, R_f 0.88 (C), 0.20 (D). ^{31}P NMR spectrum, δ_P, ppm: 69.55 br.s. ^{1}H NMR spectrum, δ, ppm: 1.34 s (6H), 1.12 s [6H, $C(CH_3)_2$], 2.75 br.s (3H, NCH_3), 3.33 br.t (4H, NCH_2CH_2O, $^3J_{HH}$ 6.05 Hz), 3.68 m (2H), 3.93 m (2H, $CH_2CH \cdot CH_2OP$), 4.02 m (4H, NCH_2CH_2O), 4.03 s (3H, CH_3OCH_2), 4.04 m (4H, CH_2CHCH_2OP), 4.26 m (2H, CH_2CHCH_2OP), 4.47 br.s (2H, CH_3OCH_2), 5.15 d (4H, $OCH_2C_6H_5$, $^3J_{PH}$ 10.59 Hz), 7.37 m (1H, $OCH_2C_6H_5$). Found, %: C 49.48, H 6.60, P 7.80. $C_{33}H_{52}ClNO_{11}P_2S_2$. Calculated, %: C 49.53, H 6.55, P 7.74.

Tris[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinoyloxy]ethyl]amine chloromethoxymethylate (XXVII). Yield 0.99 g (92%), n_D^{20} 1.5058, R_f 0.30 (C), 0.05 (D). ^{31}P NMR spectrum, δ_P, ppm: –0.9 br.s. ^{1}H NMR spectrum, δ, ppm: 1.30 s (9H), 1.38 [9H, $C(CH_3)_2$], 3.00 br.t (6H, NCH_2CH_2O, $^3J_{HH}$ 5.51 Hz), 3.70 m (3H), 3.94 m (3H, CH_2CHCH_2OP), 3.98 m (6H, CH_2CHCH_2OP), 4.02 s (3H, CH_3OCH_2), 4.19 m (6H, NCH_2CH_2O), 4.23 m (3H, CH_2CHCH_2OP), 4.24 br.s (2H, CH_3OCH_2), 5.07 d (6H, $OCH_2C_6H_5$, $^3J_{PH}$ 8.72 Hz), 7.37 m (15H, $OCH_2C_6H_5$). Found, %: C 52.03, H 6.68, P 8.55. $C_{47}H_{71}ClNO_{19}P_3$. Calculated, %: C 52.15, H 6.61, P 8.58.

Tris[1-[1,2-*O*-isopropylideneglycero-3-[(benzyloxy)(diethylamino)phosphinothioyloxy]ethyl]amine chloromethoxymethylate (XXVIII). Yield 1.05 g (89%), n_D^{20} 1.5264, R_f 0.83 (B), 0.41 (D). ^{31}P NMR spectrum, δ_P, ppm: 66.57 br.s. ^{1}H NMR spectrum, δ, ppm: 1.34 s (9H), 1.41 s [9H, $C(CH_3)_2$], 2.98 br.t (6H, NCH_2CH_2O), 3.75 m (3H), 3.96 m (3H, $CH_2CH \cdot CH_2OP$), 3.99 m (6H, NCH_2CH_2O), 4.02 m (6H, CH_2CHCH_2OP), 4.06 s (3H, CH_3OCH_2), 4.14 br.s (2H, CH_3OCH_2), 4.24 m (3H, CH_2CHCH_2OP), 5.11 d (6H, $OCH_2C_6H_5$, $^3J_{PH}$ 8.68 Hz), 7.37 m (15H, $OCH_2C_6H_5$). Found, %: C 49.90, H 6.37, P 8.23. $C_{47}H_{71}ClNO_{16}P_3S_3$. Calculated, %: C 49.93, H 6.33, P 8.22.

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