Synthesis of amphiphilic glycophospholipids based on β-cyclodextrins*

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An approach to the synthesis of amphiphilic glycophospholipids based on β-cyclodextrin (β-CD) was proposed. Distinctive features of the proposed method are the use of highly efficient trivalent phosphorus derivatives in the first steps of the synthesis and the regioselective monophosphorylation of nonprotected β-CD. The efficiency of using ^{13}C NMR spectroscopy for the determination of the position and number of phospholipid residues introduced into the CD composition was shown.

Key words: β-cyclodextrin, phospholipidocyclodextrins, regioselected monophosphorylation, NMR spectroscopy.

It is known that β-cyclodextrin (β-CD) and its numerous derivatives found wide use in pharmacology mainly as drug "containers" due to their unique ability to "encapsulate" various hydrophobic compounds (formation of host—guest inclusion compounds).[1,2] This encapsulation protects the included drug from biodegradation, increases its solubility, and, which is especially important, favors the efficient and selective delivery of the drug to the required site within the predetermined period. Attention has recently been given to studies of covalent "linking" (conjugation) of drugs to CD, which in several cases makes it possible to develop drugs of more prolonged and purposeful effect.[3—5]

In addition to the extending role of drug mediators, CD are used for the extraction of bioregulators (first of all, cholesterol) and study of their metabolism in live biological systems.[2] It was found[6] that hydroxypropylated β-CD can extract virion-bound** cholesterol and affect cholesterol bound to HIV-infected cells. This extraction of cholesterol strongly decreases the formation of viruses, and the respective virions released from the cells that have been liberated from cholesterol were minimally virulent. In addition, CD can *in vivo* affect the transfer of cell signals through the biological membrane[6,7] changing the composition of the so-called lipid rafts.[8]

The progress in the considered directions could be the combination of the said specific properties of CD with the transport properties of organized structures, such as vesicles and micelles, or the introduction of CD derivatives into pre-organized lipid matrices, for example, liposomes or their analogs (see, *e.g.*, Ref. 9). First representatives of a new class of amphiphilic CD-based derivatives, namely, β-CD-bound cholesterol (**A**) and phospholipidocyclodextrins (**B**), have recently been synthesized aimed at design of fragments close in structure and nature of the lipid cholesterol matrix localized in the cell membranes.[10—13]

These compounds contain hydrophobic lipid fragments conjugated with the narrow part of the CD rim, which imparts the amphiphilic properties and remains a wide part of the CD rim free for "guest" inclusion. Cholesterol was chosen as a hydrophobic residue because of its presence in biological membranes, due to which studies of its role and influence on the behavior of bilayers are of special interest.[14] The second representative of amphiphilic CD (**B**) contains the phospholipid residue attached to the CD framework through the spacer. It is considered that the control of the length of this spacer can optimize the incorporation of the molecule into the lipid matrix to form the so-called "membrane anchor" and thus induce lesser structural changes in the matrix.[10—12] It is of interest that the methylated analogs of the phospholipid systems (R = Me) are more soluble in water than the nonprotected ones (R = H), which is important for the formation of self-organized objects (vesicles and micelles). The low values of critical micelle concentrations suggest strong

* Dedicated to Academician A. I. Konovalov on his 75th birthday.
** Virion is the completely formed virus particle consisting of a nucleic acid and a protein shell. Virion stores and transfers the genetic material from one cell to another.

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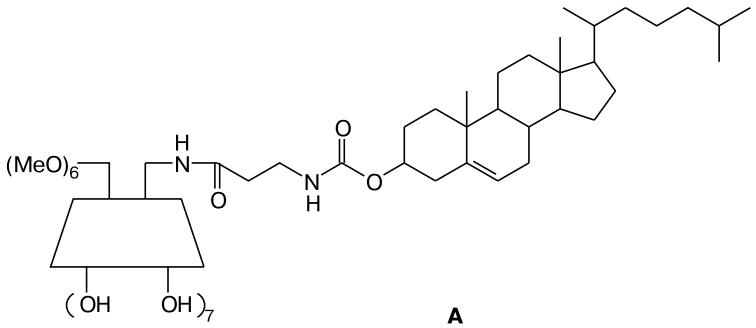
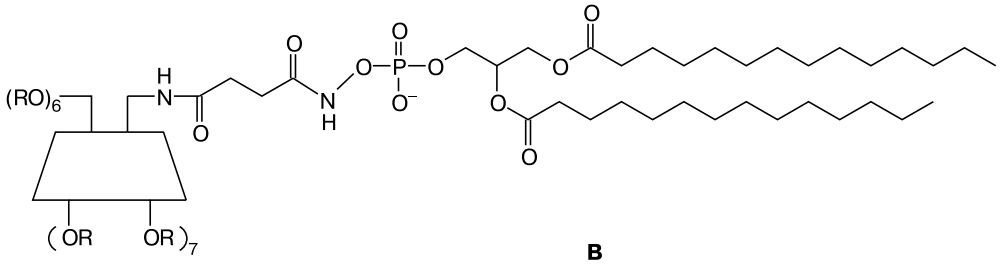


A



B

R = H, Me

proneness of these amphiphilic molecules to self-organization in water. It was shown by static and dynamic light scattering that in aqueous solutions these amphiphilic CD behave as long fluctuating "twigs" ("rods") corresponding to infinitely long micelles with bulky CD heads rocking at the sides. The NMR spectra recorded with the known guest molecules showed that even in the highly organized form these amphiphilic CD completely retain their ability to include "guests" into the CD cavity.[10–12]

In addition, it is necessary to provide for any efficient drug carrier to be able to penetrate through biological barriers. Among the latter, the most important is the blood-brain barrier (BBB) that protects the central nervous system from foreign substances circulating in blood and controls the composition and properties of the spinal fluid. The effective use of, *e.g.*, a neuroactive drug assumes that the drug can penetrate this selective barrier. The studies for the model system showed that some phospholipids containing the β-CD residue can penetrate through this barrier and, which is especially important, do not decompose it. It is considered that the conjugates mentioned are the first example of drug mediators capable of penetrating the BBB without violating its integrity.[10–12] Thus, due to a combination of properties of CD as host molecules with the transport efficiency of self-organized amphiphilic molecules, the conjugates can become valuable objects of special interest for the point delivery of drugs and studying their metabolism.

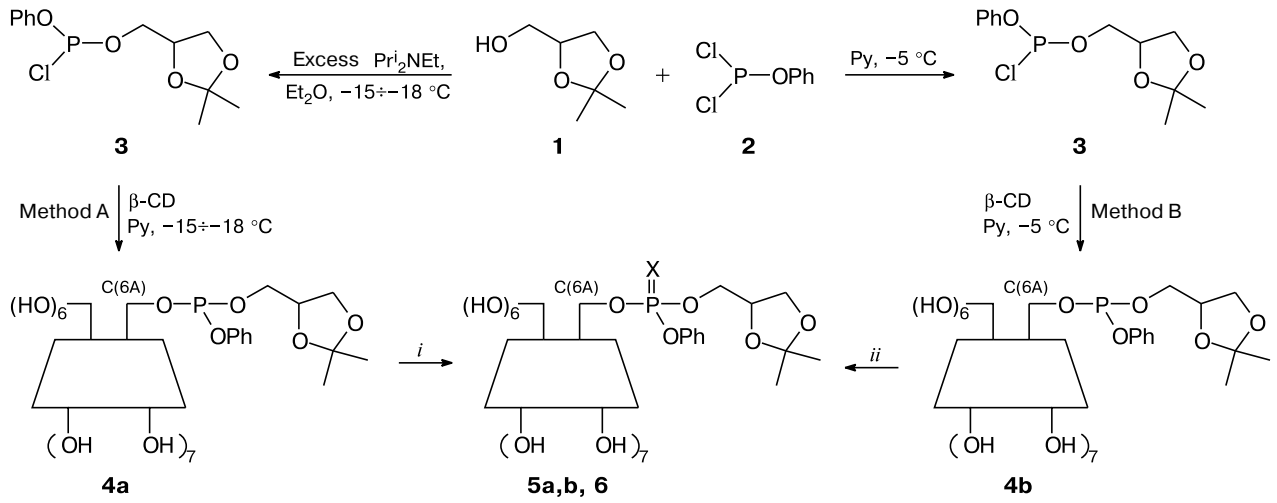
Taking into account our data on a possibility of regioselective acylation[15] and monophosphorylation of the primary hydroxy groups in the presence of the secondary groups,[16] we intended to develop other approaches to the synthesis of amphiphilic CD belonging to the class of phospholipidocyclodextrins. Amphiphilicity of these compounds should be determined by the presence of hydrophilic hydroxy groups of CD molecules and the presence of hydrophobic residues of aliphatic fatty acids, and the ability to inclusion is determined by the presence of the CD cavity. What we propose is the use of CD with free hydroxy groups. Since the stated problem is experimentally difficult, we decided first to select optimal conditions for the synthesis of compounds containing CD linked through the phosphate group with the 1,2-*O*-isopropylideneglycerol residue (**1**) modeling natural 1,2-diglycerides. Phenyl phosphodichloridite (**2**) was used as the starting phosphorylating agent (Scheme 1).

The reaction was carried out in diethyl ether at −15 to −18 °C in the presence of a tenfold molar excess of sterically hindered base, ethyldiisopropylamine (hydrogen chloride acceptor), to suppress the possible phosphorylation of the secondary hydroxy groups of CD in the subsequent stage of phosphorylation[16,17] (method *A*) or in pyridine at −5 °C (method *B*). The reaction mixture containing chloride **3** was added to a solution of CD in pyridine (molar ratio 1 : 1) at −15 to −18 °C, and triphosphite **4a** that formed was oxidized by the hydrogen peroxide—urea adduct ("hydroperit") to the corresponding phosphate **5a** (Scheme 1)*.

Alternatively, chloride **3** was added to a solution of CD in pyridine at −5 °C, and the resulting triphosphite **4b**

* For discussion convenience, products **4** and **5** synthesized by methods *A* and *B* (see further) were designated as **4a** and **4b**, **5a** and **5b**, respectively.

Scheme 1



X = O (**5a,b**), S (**6**)

i. $H_2O_2 \cdot C(O)(NH_2)_2$; *ii*. $H_2O_2 \cdot C(O)(NH_2)_2$; S

was oxidized to the corresponding phosphate **5b** or treated with sulfur to form thiophosphate **6**.

At all steps of the syntheses, the compositions of the reaction mixtures and the course of the reaction were monitored by ^{31}P NMR spectroscopy. Several important features were observed for phosphorylation in the mentioned solvents (among the solvents that are not proton donors, β-cyclodextrin is rather well soluble only in pyridine and DMF). For example, when the reactions are carried out at ambient temperature, the decomposition products of P^{III} derivatives are rapidly accumulated (mainly due to disproportionation). Under these phosphorylation conditions, chloride **3** turned out to be especially unstable, and triphosphites **4a** and **4b**, whose content in the reaction mixture was ~50%, were more stable. Small distinctions of the ^{31}P NMR chemical shifts of compounds **4a** and **4b** with the same structure should be specially mentioned: broadened singlets at δ 136.6 (**4a**) and 138.0 (**4b**).* The low total yields of phosphates **5a** and **5b** and thiophosphate **6** (12—15% over three steps) are mainly related to losses upon crystallization from water (compound **5a**) and column chromatography (compounds **5b** and **6**). According to the data from ^{13}P NMR spectroscopy, they appear as singlets at δ 4.6 (**5a**, **5b**) and 53.2 (**6**). According to the ^{1}H and ^{13}C NMR data, ethyldiisopropylamine is included in product **5a**. The replacement of diethyl ether by toluene as a solvent resulted in inclusion of toluene in product **5a** under the same conditions of synthesis and isolation. Thus, method *B* should be preferred, because products **5b** and **6** contained no inclusion compounds according to the data from ^{1}H NMR spectroscopy. An important feature of the one-pot syntheses of compounds **5** and **6** according to methods *A* and *B* is that all the three steps before the isolation of the final P^V products should be carried out at the indicated reduced temperatures for at most 20—30 min to avoid the accumulation of considerable amounts of poorly separable by-products.

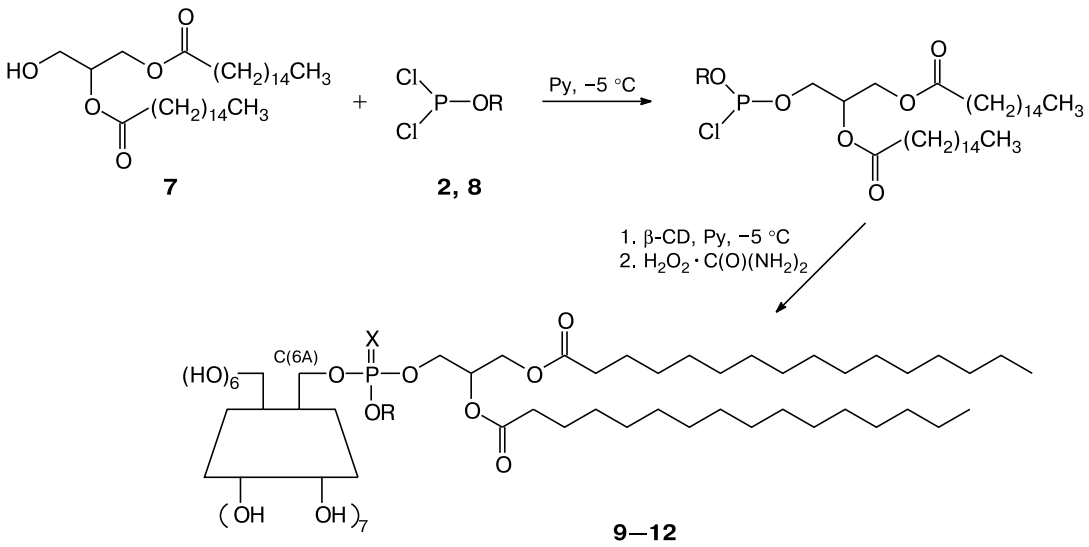
The structures of isolated compounds **5a**, **5b**, and **6** were confirmed by the data from ^{1}H, ^{13}C, and ^{31}P NMR spectroscopy. The ^{1}H NMR spectrum of compound **5a** contains additional signals for ethyldiisopropylamine included into the CD cavity: the broadened signal for protons of the CH_3 groups at δ 1.27 and the multiplet of protons of the CH_2 and CH groups at δ 3.18—3.22. The signals for ethyldiisopropylamine included in compound **5a** also appear in the ^{13}C NMR spectrum as four singlets: signals for the $\underline{C}H_3CH_2$ and $(\underline{C}H_3)_2CH$ groups at δ 14 and 23 and signals for the $\underline{C}H_2$ and CH groups at δ 44 and 46, respectively. Note that the signals for ethyldiisopropylamine do not disappear after additional recrystallizations from water and drying. Among other features, we can mention good resolution of the signals for protons of the CD framework, which is usually observed for the inclusion of an appropriate guest[18] into the CD cavity, most likely, because the CD framework becomes more rigid.

Based on the analysis of the ^{13}C NMR spectra, we can conclude that the phosphorylation of β-CD by phosphodichloridite **3** involved, as expected,[15,16] the primary hydroxy groups. We observed the spin-spin coupling constant $^2J_{P,C(6A)}$** and the downfield shift of the signals

* Different chemical shifts for compounds of the same structure are probably caused by the inclusion of Pr$^i_2$NEt into the cavity of CD **4a** (see further).

** The atom of the glucose CD fragment containing the substituent is marked.

Scheme 2



R = Ph (**2**, **9**, **11**), CH_2CH_2CN (**8**, **10**, **12**); X = LEP (**9**, **10**), O (**11**, **12**)

for the C(6A) atoms in products **5a** and **5b** (δ_C 63.4) compared to the corresponding signal in free β-CD (δ_C 61.4) and the upfield characteristic shift of the C(5) signal at δ_C 73.7 (in free β-CD) to δ_C 71.1 for C(5A).[19—22] It is important that no shifts of signals for the secondary C(2) and C(3) carbon atoms of the CD framework were observed in the ^{13}C NMR spectra of products **5a** and **5b**, *i.e.*, these positions were not affected by phosphorylation. The individual character of isolated compounds **5a**, **5b**, and **6** was confirmed by the TLC data. Compounds **5a** and **5b** had the same chromatographic mobilities and slightly different temperatures of melting onset (with decomposition): 164 °C (**5a**) and 167 °C (**5b**), which is evidently related to the fact that product **5a** is an inclusion compound (**5a** = **5b** ⊃ Pr^i_2NEt).

Thus, we showed for the first time that highly reactive phosphochloridites can be used for the regioselective monophosphorylation of the primary hydroxy groups of β-cyclodextrin in the presence of unprotected secondary hydroxy groups.

Taking into account the data from model experiments, we synthesized amphiphilic CD derivatives containing the covalently linked phospholipid residue using method *B*. 1,2-Di-*O*-palmitoylglycerol **7** reacted with freshly distilled phenyl dichloridite **2** or β-cyanoethyl phosphodichloridite **8**. Treatment of β-CD in pyridine at −5 °C with the chlorides formed and oxidation of β-CD triphosphites **9** and **10** with hydroperit gave the corresponding phosphates **11** and **12**.

The course of the reaction and compositions of the reaction mixtures were monitored by ^{31}P NMR spectroscopy. Triphosphites **9** and **10** appeared in the ^{31}P NMR spectra as broadened singlets at δ 134 and 140, and phosphates **11** and **12** gave broadened singlets at δ 4.2 and 4.5, respectively.

The structures of isolated compounds **11** and **12** were confirmed by the data from ^1H, ^{13}C, and ^{31}P NMR spectroscopy and MALDI-TOF mass spectrometry.

Note that the ^1H NMR spectrum of compound **11** exhibits well resolved signals for protons of the CD framework, unlike the ^1H NMR spectrum of compound **12**, which is probably a consequence of the inclusion of the phenyl radical into the CD cavity.

Similarly to compounds **5a** and **5b**, we analyzed the ^{13}C NMR spectra and made a conclusion that the phosphorylation of β-CD involved the primary hydroxy groups. We observed the spin-spin coupling constant $^2J_{P,C(6A)}$ and the downfield shift of signals from the C(6A) atoms in products **11** and **12** (δ_C 63.1 for C(6A)) compared to the corresponding signals for C(6) in free β-CD (δ_C 61.4). The characteristic upfield shift of the signal for C(5) at δ_C 73.7 (in free β-CD) to δ_C 71.3 for C(5A) was also observed.[19—22] In addition, in the ^{13}C NMR spectra the signals for the carbonyl groups of compounds **11** and **12** appear as four signals and the cyano group in compound **12** is observed as two signals suggesting the presence of diastereomers.

An additional confirmation of the structures of compounds **11** and **12** was obtained by the integration of the signals for the carbon atoms of these compounds in the ^{13}C NMR spectra recorded with a large delay (RD = 5) between pulses (Fig. 1).

Thus, we proposed the new routes for the synthesis of the CD—phospholipid derivatives. The proposed approach differs from the known method[10—12] in the use (at the first step of the design of complicated systems) of the corresponding highly reactive trivalent phosphorus derivatives instead of the reactant traditional for the chemistry of natural compounds and in the phosphorylation of β-CD

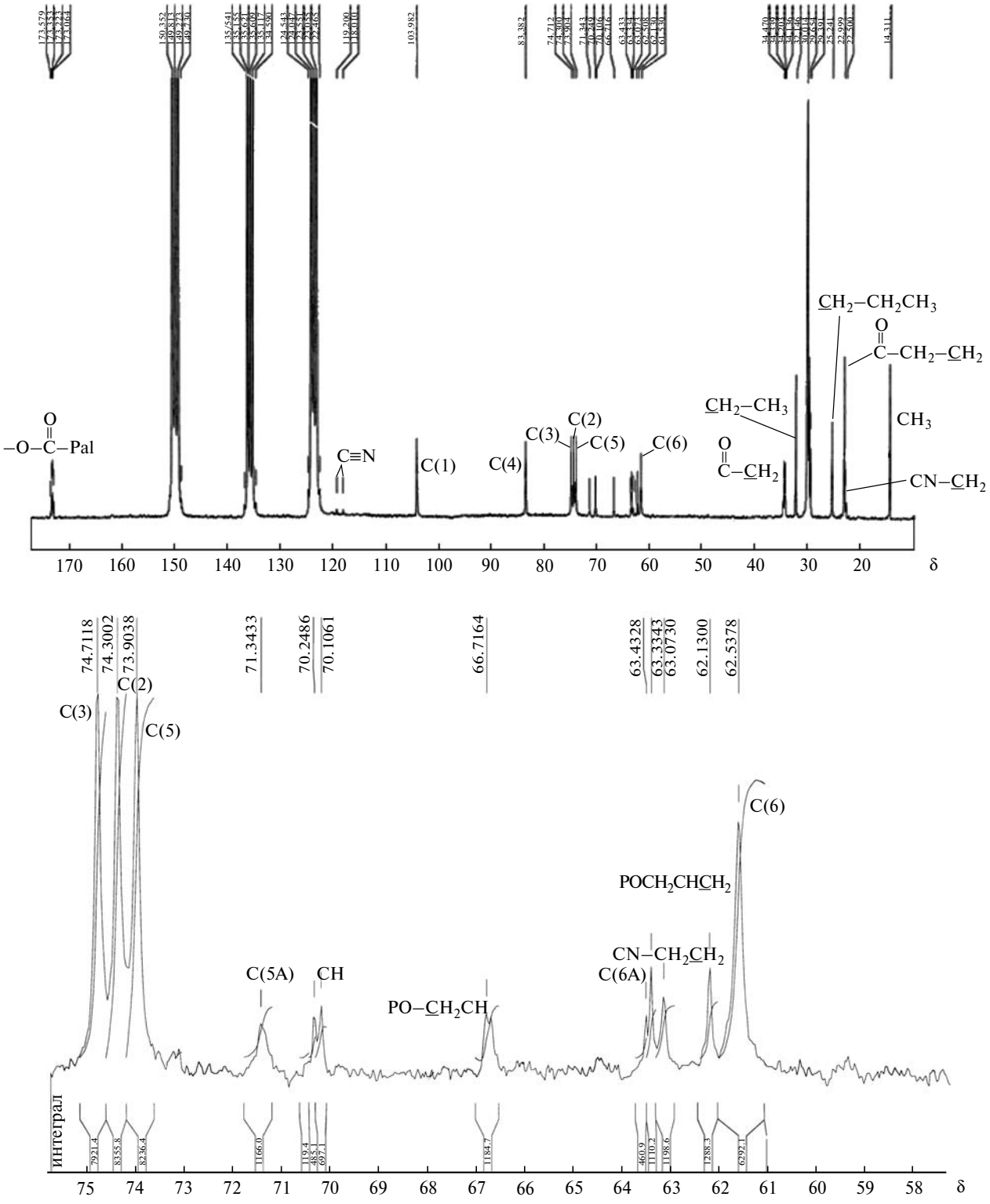


Fig. 1. ^{13}C NMR spectrum (Py-d_5) of compound **12**.

containing no protecting groups by the proposed reactants. It can be expected that the new route of synthesis and the synthesized compounds will find practical use in studies of functioning cellular membranes and metabolism of a series of biologically important compounds, as well as in works devoted to problems of targeted drug delivery.

Experimental

All experiments with trivalent phosphorus compounds were carried out in anhydrous solvents purified according to standard procedures and under an inert atmosphere. ^{1}H NMR spectra were recorded on Bruker WM-250 and Bruker AC-200 instruments (250 and 200 MHz, respectively). ^{13}C NMR spectra were measured on a Bruker AC-200 instrument (50.32 MHz). The ^{1}H NMR chemical shifts are presented relative to the residual signal of Py (δ 8.75 for the downfield signal (s)), and the ^{13}C NMR chemical shifts are given relative to the signal for Py-d_5 (δ 149.8 for the downfield signal (t)). ^{31}P{H} NMR spectra were recorded on Bruker WP-80SY and Bruker AC-200 instruments (compounds **11** and **12**) (32.4 and 82.2 MHz) relative to 85% H_3PO_4 as the external standard. Preparative column chromatography was carried out on Kieselgel 40/60 (Merck), and TLC analysis was performed on glass plates with silica gel 60 F 254 (Merck), detection of spots in iodine vapor or under the UV light after spraying with anisaldehyde. The systems for TLC and column chromatography were acetonitrile—methanol—water, 3 : 3 : 1 (*A*) and acetonitrile—chloroform—methanol, 2 : 2 : 1 (*B*).

Mass spectra were recorded on a Bruker Ultra Flex instrument with the time-of-flight (TOF) detector by matrix-activated laser desorption and ionization (MALDI) (λ = 337 nm) using 2,5-dihydroxybenzoic acid as the matrix.

Commercial β-CD (Novodex, Russia) was subjected to additional thorough dehydration.[15] Phenyl phosphodichloridite was obtained by an earlier described procedure.[23] 1,2-*O*-Isopropylideneglycerol was from Sigma and hydroperit was from Tatkhimfarmpreparaty (Russia).

***rac*-6-*O*-[(2,3-Isopropylidenedioxypropoxy)phenoxy)-phosphoryl]-β-cyclodextrin (5). Method *A*.** 1,2-*O*-Isopropylideneglycerol (**1**) (0.123 g, 1 mmol) and ethyldiisopropylamine (1.423 g, 11.0 mmol) in diethyl ether or toluene (3 mL) were added to a solution of phenyl phosphodichloridite (**2**) (0.195 g, 1 mmol) in diethyl ether or toluene (3 mL) at −15 to −18 °C for 5 min. The reaction mixture was immediately (without isolation of intermediate phosphodichloridite **3**) added with stirring to a solution of β-CD (1.00 g, 0.88 mmol) in pyridine (20 mL) at −15 to −18 °C for 10 min; the ^{31}P NMR spectrum of the reaction mixture, δ_P: 136.6 br.s (compound **4a**). Crushed hydroperit (0.103 g, 1.1 mmol) was added to the reaction mixture, which was stirred for 24 h at 20 °C. The solvent was distilled off *in vacuo*. Compound **5a** was purified by recrystallization from water (2′50 mL). The crystalline product was kept *in vacuo* (1 Torr) for 5 h at 70 °C. The yield was 0.148 g (12% over three steps), m.p. (decomp.) 164 °C, R_f 0.74 (*A*). ^{1}H NMR* (Py-d_5), δ: 1.31 (s, 3 H, CH_3); 1.41 (s, 3 H, CH_3); 4.14 (dd, 7 H, C(2)H, $^3J_{H(1),H(2)}$ = 2.9 Hz, $^3J_{H(2),H(3)}$ = 9.5 Hz); 4.30 (dd, 7 H, C(4)H, $^3J_{H(3),H(4)}$ = 9.1 Hz, $^3J_{H(4),H(5)}$ = 9.9 Hz); 4.46—4.57 (m, 21 H, C(5)H, C(6)H_2, $^3J_{H(4),H(5)}$ = 9.9 Hz); 4.82 (dd, 7 H, C(3)H); 4.88—5.46 (m, 5 H, CH_2—CH—CH_2); 5.68 (d, 7 H, C(1)H); 7.20—7.49 (m, 5 H, H-arom.). ^{13}C NMR* (Py-d_5), δ: 26.9 (1 C, CH_3); 29.7 (1 C, CH_3); 61.4 (6 C, C(6)); 62.1 (1 C, $POCH_2CH\underline{C}H_2$); 63.4 (C(6A), $^2J_{C(6),(A)P}$ = 5.0 Hz); 66.7 (1 C, $PO\underline{C}H_2CH$, $^2J_{C,P}$ = 3.6 Hz); 70.1 (1 C, $POCH_2\underline{C}H$, $^3J_{C,P}$ = 6.8 Hz); 71.1 (1 C, C(5A); 73.7 (6 C, C(5)); 74.1 (7 C, C(3)); 74.5 (7 C, C(2)); 83.2 (7 C, C(4)); 103.8 (7 C, C(1)); 109.9 (1 C, $\underline{C}(CH_3)_2$); 120.4 (2 C, $C_{o\text{-arom}}$); 125.7 (1 C, $C_{p\text{-arom}}$); 130.0 (2 C, $C_{m\text{-arom}}$); 161.7 (1 C, $C_{ipso\text{-arom}}$). ^{31}P NMR (Py), δ: 4.6 br.s.

Method *B*. 1,2-*O*-Isopropylideneglycerol (**1**) (0.132 g, 1.00 mmol) in pyridine (3 mL) was added with stirring at −5 °C over 5 min to a solution of phenyl phosphodichloridite **2** (0.195 g, 1.00 mmol) in pyridine (3 mL). The reaction mixture was immediately (without isolation of intermediate phosphodichloridite **3**) was added with stirring to a solution of β-CD (1.00 g, 0.88 mmol) in pyridine (20 mL) at −5 °C over 10 min; the ^{31}P NMR spectrum of the reaction mixture, δ_P: 138.0 br.s (compound **4b**). Crushed hydroperit (0.103 g, 1.10 mmol) was added to the reaction mixture, which was stirred for 24 h at 20 °C. The solvent was distilled off *in vacuo*. Compound **5b** was purified by column chromatography eluting with system *A*. The solvents were removed *in vacuo*, and the residue was kept *in vacuo* (1 Torr) for 5 h at 70 °C. The yield was 0.185 g (15% for three stages), m.p. (decomp.) 167 °C, R_f 0.74 (*A*). Found (%): C, 46.18; H, 6.06. $C_{54}H_{85}O_{40}P$. Calculated (%): C, 46.16; H, 6.10. The ^{1}H, ^{13}C NMR (Py-d_5, δ) and ^{31}P NMR spectra are identical to those of sample **5a**. MALDI-TOF, *m/z*: 1405.3 $[M]^+$.

***rac*-6-*O*-[(2,3-Isopropylidenedioxypropoxy)(phenoxy)thiophosphoryl]-β-cyclodextrin (6).** 1,2-*O*-Isopropylideneglycerol (**1**) (0.132 g, 1.00 mmol) in pyridine (3 mL) was added with stirring to a solution of phenyl phosphodichloridite (**2**) (0.195 g, 1.00 mmol) in pyridine (3 mL) at −5 °C for 5 min. The reaction mixture was immediately added with stirring to a solution of β-CD (1.00 g, 0.88 mmol) in pyridine (20 mL) at −5 °C over 10 min; the ^{31}P NMR spectrum of the reaction mixture; δ_P: 138.0 br.s (compound **4b**). Finely divided sulfur (0.035 g, 1.10 mmol) was added to the reaction mixture, which was stirred for 24 h at 20 °C. The solvent was distilled off *in vacuo*. Compound **6** was purified by column chromatography eluting with system *A*. The solvents were removed *in vacuo*, and the residue was kept *in vacuo* (1 Torr) for 5 h at 70 °C. The yield was 0.163 g (13%), R_f 0.71 (*A*), m.p. (decomp.) 176 °C. Found (%): C, 45.68; H, 6.00. $C_{54}H_{85}O_{39}PS$. Calculated (%): C, 45.63; H, 6.03. ^{1}H NMR (Py-d_5), δ: 1.30 (s, 3 H, CH_3); 1.42 (s, 3 H, CH_3); 4.12 (dd, 7 H, C(2)H, $^3J_{H(1),H(2)}$ = 2.8 Hz, $^3J_{H(2),H(3)}$ = 9.4 Hz); 4.32 (dd, 7 H, C(4)H, $^3J_{H(3),H(4)}$ = 9.0 Hz, $^3J_{H(4),H(5)}$ = 9.9 Hz); 4.46—4.55 (m, 21 H, C(5)H, C(6)H_2, $^3J_{H(4),H(5)}$ = 9.9 Hz); 4.84 (dd, 7 H, C(3)H); 4.91—5.44 (m, 5 H, CH_2—CH—CH_2); 5.67 (d, 7 H, C(1)H); 7.21—7.48 (m, 5 H, H-arom). ^{31}P NMR (Py), δ: 53 br.s.

***rac*-6-*O*-[(1,2-Dipalmitoyloxypropyloxy)(phenoxy)phosphoryl]-β-cyclodextrin (11).** 1,2-Di-*O*-palmitoylglycerol[23] (**7**) (0.50 g, 0.88 mmol) in pyridine (3 mL) was added with stirring to a solution of phenyl phosphodichloridite **2** (0.195 g, 1.00 mL) in pyridine (3 mL) at −5 °C for 5 min. The reaction mixture was immediately added with stirring to a solution of β-CD (1.00 g, 0.88 mmol) in pyridine (20 mL) at −5 °C for 10 min; the ^{31}P NMR spectrum of the reaction mixture, δ_P: 134.0 br.s (compound **9**). Crushed hydroperit (0.103 g, 1.10 mmol) was added to the reaction mixture, which was stirred for 24 h at 20 °C. The solvent was distilled off *in vacuo*. Compound **11** was purified by column chromatography eluting with system *B*. The solvents were removed *in vacuo*, and the residue was kept *in vacuo* (1 Torr) for 5 h at 70 °C. The yield was 0.194 g (12%), m.p. (decomp.) 186 °C, R_f 0.57 (*B*). Found (%): C, 54.20; H, 7.76. $C_{83}H_{141}O_{42}P$. Calculated (%): C, 54.12; H, 7.72. ^{1}H NMR (Py-d_5), δ: 0.87 (m, 3 H, CH_3); 1.03 (m, 3 H, CH_3); 1.29 (m, 48 H, $(CH_2)_{12}$); 1.68 (m, 4 H, $C(O)CH_2C\underline{H}_2$); 2.42

* For the signals of ethyldiisopropylamine, see discussion of the obtained results.

(m, 4 H, C(O)CH_2); 4.13 (dd, 7 H, C(2)H, $^3J_{H(1),H(2)}$ = 2.9 Hz, $^3J_{H(2),H(3)}$ = 9.9 Hz); 4.28 (dd, 7 H, C(4)H, $^3J_{H(3),H(4)}$ = 8.0 Hz, $^3J_{H(4),H(5)}$ = 9.1 Hz); 4.48 (dd, 7 H, C(6)H, $^3J_{H(5),H(6')}$ = 9.9 Hz, $^2J_{H(6),H(6')}$ = 14.3 Hz); 4.53 (dd, 7 H, C(6)H′, $^3J_{H(5),H(6')}$ = 9.9 Hz, $^2J_{H(6),H(6')}$ = 14.3 Hz); 4.67 (m, 7 H, C(5)H, $^3J_{H(4),H(5)}$ = 9.1 Hz, $^3J_{H(5),H(6')}$ = 9.9 Hz, $^3J_{H(5),H(6)}$ = 9.9 Hz); 4.80 (dd, 7 H, C(3)H); 4.50—5.10 (m, 5 H); 5.64 (d, 7 H, C(1)H); 7.15 (m, 2 H, $H_{m\text{-arom}}$); 7.40 (m, 1 H, $H_{p\text{-arom}}$); 7.52 (d, 2 H, $H_{o\text{-arom}}$). ^{13}C NMR (Py-d_5), δ: 14.3 (2 C, CH_3); 22.8 (2 C, C(O)$CH_2\underline{C}H_2$); 25.2 (2 C, $\underline{C}H_2CH_2CH_3$); 29.1—29.6 (20 C, CH_2); 31.9 (2 C, $\underline{C}H_2CH_3$); 34.0, 34.2 (2 C, C(O)$\underline{C}H_2CH_2$); 61.4 (6 C, C(6)); 62.0 (1 C, $POCH_2CH\underline{C}H_2$); 63.3 (C(6A), $^2J_{C(6A),P}$ = 4.9 Hz); 66.7 (1 C, $PO\underline{C}H_2CH$, $^2J_{C,P}$ = 4.0 Hz); 70.2 (1 C, $POCH_2\underline{C}H$, $^3J_{C,P}$ = 7.1 Hz); 71.4 (1 C, C(5A)); 73.9 (6 C, C(5)); 74.3 (7 C, C(3)); 74.7 (7 C, C(2)); 83.2 (7 C, C(4)); 103.7 (7 C, C(1)); 119.9 (2 C, $C_{o\text{-arom}}$); 125.39 (1 C, $C_{p\text{-arom}}$); 129.78 (2 C, $C_{m\text{-arom}}$); 163.52 (1 C, $C_{ipso\text{-arom}}$). ^{31}P NMR (Py-d_5), δ: 4.2 br.s. MALDI-TOF, *m/z*: 1841.8 $[M]^+$.

***rac*-6-*O*-[(2,3-Dipalmitoyloxypropyloxy)(2-cyanoethoxy)-phosphoryl]-β-cyclodextrin (12).** Dipalmitoylglycerol **7** (0.50 g, 0.88 mmol) in pyridine (3 mL) was added with stirring to a solution of β-cyanoethyl phosphodichloridite[24] (**8**) in pyridine (3 mL) at −5 °C for 5 min. The reaction mixture was immediately added with stirring to a solution of β-CD (1.00 g, 0.88 mmol) in pyridine (20 mL) at −5 °C over 10 min; the ^{31}P NMR spectrum of the reaction mixture, δ_P: 140.0 br.s (compound **10**). Crushed hydroperit (0.103 g, 1.1 mmol) was added to the reaction mixture, which was stirred for 24 h at 20 °C. The solvent was distilled *in vacuo*. Compound **12** was purified by column chromatography eluting with system *B*. The solvents were removed *in vacuo*, and the residue was kept *in vacuo* (1 Torr) for 5 h at 70 °C. The yield was 0.176 g (11%), m.p. (decomp.) 196 °C, R_f 0.55 (*B*). Found (%): C, 52.78; H, 7.80. $C_{80}H_{140}NO_{42}P$. Calculated (%): C, 52.83; H, 7.76. ^{1}H NMR (Py-d_5), δ: 0.88 (m, 6 H, CH_3); 1.28—1.30 (m, 48 H, $(CH_2)_{12}$); 1.71 (m, 4 H, C(O)$CH_2\underline{C}H_2$); 2.39 (m, 4 H, C(O)CH_2); 3.16 (m, 2 H, CH_2CN); 4.11 (dd, 7 H, C(2)H, $^3J_{H(1),H(2)}$ = 3.4 Hz, $^3J_{H(2),H(3)}$ = 9.5 Hz); 4.22 (t, 7 H, C(4)H, $^3J_{H(3),H(4)}$ = 9.5 Hz, $^3J_{H(4),H(5)}$ = 9.5 Hz); 4.45—4.56 (m, 23 H, C(5)H, C(6)H_2, $C\underline{H}_2CH_2CN$, $^3J_{H(4),H(5)}$ = 9.5 Hz); 4.25—4.90 (m, 5 H, CH_2—CH—CH_2); 4.75 (t, 7 H, C(3)H); 5.61 (d, 7 H, C(1)H). ^{13}C NMR (Py-d_5), δ: 14.3 (2 C, CH_3); 22.6 (1 C, $\underline{C}H_2CN$); 23.0 (2 C, C(O)$CH_2\underline{C}H_2$); 25.2 (2 C, $\underline{C}H_2CH_2CH_3$); 29.1—30.0 (20 C, CH_2); 32.2 (2 C, $\underline{C}H_2CH_3$); 34.1, 34.2, 34.3, 34.5 (2 C, C(O)$\underline{C}H_2CH_2$); 61.5 (6 C, C(6)); 62.1 (1 C, $POCH_2CH\underline{C}H_2$); 63.1 (1 C, $PO\underline{C}H_2CH_2CN$); 63.4 (C(6A), $^2J_{C(6)(A)P}$ = 5.0 Hz); 66.7 (1 C, $PO\underline{C}H_2CH$, $^2J_{C,P}$ = 4.1 Hz); 70.2 (1 C, $POCH_2\underline{C}H$, $^3J_{C,P}$ = 7.2 Hz); 71.3 (1 C, C(5A)); 73.9 (6 C, C(5)); 74.3 (7 C, C(3)); 74.7 (7 C, C(2)); 83.4 (7 C, C(4)); 104.0 (7 C, C(1)); 118.0, 119.2 (1 C, CN); 173.1, 173.2, 173.4, 173.6 (2 C, C(O)). ^{31}P NMR (Py-d_5), δ: 4.5 br.s. MALDI-TOF: *m/z*, 1818.7 $[M]^+$.

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