Antitumor liposomes bearing a prodrug of combretastatin A-4 and a tetrasaccharide ligand of selectins

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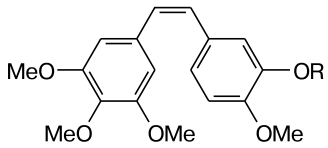
Therapeutic liposomes with an average diameter of 100 nm based on natural phospholipids (phosphatidylcholine and phosphatidylinositol) containing palmitoyl or oleoyl derivatives of the antimitotic agent combretastatin A-4 were constructed. The cytotoxicity of liposomes with the oleoyl derivative in the human breast cancer cell culture turned out to be only three times lower than that of combretastatin A-4, thus indicating the probability of facile intracellular hydrolysis of the prodrug. To achieve selective drug delivery to the tumor tissue *in vivo*, the diglyceride conjugate of the tetrasaccharide ligand of selectins, *viz.*, Sialyl—Lewis X (SiaLe^X, 2 mol.%) was incorporated into the liposomes. The SiaLe^X-equipped liposomes loaded with the lipophilic prodrug showed a reliable inhibition of tumor growth on the model of spontaneous breast cancer in mice.

Key words: combretastatin A-4, lipophilic prodrugs, liposomes, Sialyl—Lewis X, model of spontaneous breast cancer.

Cell protein tubulin is the main component of microtubes and the most important target of antitumor therapy.[1] Depending on the molecular structure, antitubulin agents can interact with various regions of this protein: with the colchicine and taxane sites, as well as with the *vinca* alkaloid site.[2] Combretastatin A-4 (CA-4)[3] is one of the most promising agents of the colchicine site: it efficiently prevents the formation of a mitotic spindle in tumor cells, inhibiting tubulin polymerization. In the present time, water-soluble combretastatin A-4 phosphate (CA4P) undergoes the third phase of clinical trials in a combination with the known chemiotherapeutic drugs.[4] Unfortunately, CA-4 induces undesirable side effects in healthy tissues,[5] which is characteristic, however, of the predominant majority of antitumor drugs.[6]



R = H (CA-4), PO_3Na_2 (CA4P)

One of the methods for surmounting systemic toxicity of antitumor agents is the use of nanosized carrier of drugs, in particular, liposomal nanoparticles.[7] The inclusion of a drug into liposomes with a diameter of 50—150 nm makes it possible to decrease its overall toxic effect due to a decrease in the concentration in the blood stream and the effect of passive accumulation in tumors related to the

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increased permeability of neoplasm capillaries.[8] The further increase in the selectivity of the effect of liposomal drugs can be achieved by the inclusion in their composition of molecular "addresses" (antibodies, RGD-peptide, oligosaccharides, *etc*.) with high affinity to tumor cells.[7]

A technological method for obtaining liposome formulations is the delivery of a water-soluble drug inside a liposome against the concentration gradient of some salts. The method is applicable only to a restricted number of drugs having the nature of amphiphilic acids and bases.[9] An alternative method for the construction of preparations based on nanosized liposomes can be the incorporation of drugs into the lipid bilayer as lipophilic prodrugs.[10] This approach makes it possible to improve the pharmacological properties of drugs due to a decrease in losses when the liposomal membrane is damaged in the circulation and upon the interaction with the target cell and to facilitate intracellular unloading of the liposome.

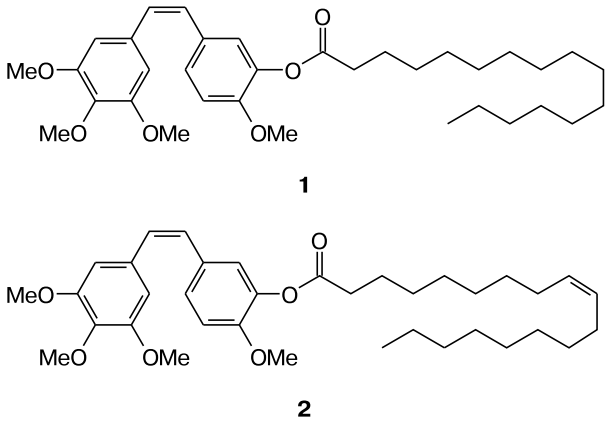
The purpose of this work is the construction of liposomal forms of acyl prodrugs CA-4 equipped with the tetrasaccharide ligand of selectins Sialyl—Lewis X (SiaLe^X-ligand) and the approbation of their antitumor effect on the model of spontaneous breast cancer.

Two examples of using CA-4 derivatives in the liposomal forms are known[11,12] to the present time. It has recently[11] been proposed to include water-soluble CA4P into Stealth® liposomes carrying the full-size monoclonal antibody (mAb) to E-selectin on the distal residue of polyethylene glycol (PEG). Liposomes Stealth® contain PEG residues (degree of polymerization 42—48) grafted on the surface to protect from premature removal from the blood stream by immunocompetent cells of the reticule-endothelial system.[7,9] Liposomes containing the water-insoluble initial form of CA-4 in the lipid bilayer and targeted by the cyclic RGD-peptide to αvβ3-integrins have earlier been obtained.[12] However, the capacity of stable load of these liposomes CA-4 is lower than 3 mol.% and, hence, this preparation is not promising for the systemic administration in animals.

For the more efficient inclusion of CA-4 into the lipid bilayer of a liposome, we propose to use biodegradable combretastatin A-4 derivatives: the palmitoyl (**1**) and oleoyl (**2**) derivatives.

The ester bond easily undergoes hydrolysis by intracellular esterases, which are widely presented in all tissues. At the same time, in the liposome formulation the lipophilic prodrugs (diglyceride ester conjugates of melphalan and methotrexate) demonstrate considerable resistance to premature hydrolysis by esterases of human blood plasma.[13]

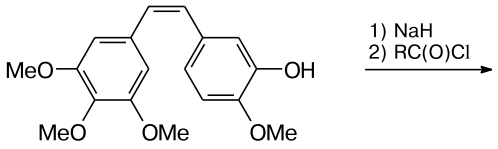
Biodegradable lipophilic derivatives **1** and **2** were synthesized by the acylation of CA-4 sodium salt obtained by the known procedures[14] with palmitoyl and oleoyl chlorides, respectively (Scheme 1). Target products **1** and **2** were isolated in 80 and 67% yields, respectively.



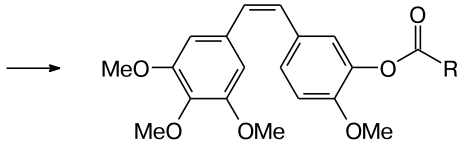
1

2

Scheme 1



CA-4



1, **2**

R = $C_{15}H_{31}$ (**1**), $C_{17}H_{33}$ (**2**)

The vector for drug-loaded liposomes neoglycolipid **3** (Fig. 1), *viz*., conjugate of 3-aminopropyl glycoside SiaLe^X and *rac*-1,2-dioleoyl-3-carboxymethylene[poly(8—15)-oxyethylene]oxyacetylamidopropionylglycerol, was synthesized as described previously.[15] The flexible polar inset of PEG (degree of polymerization 8—15) between the carbohydrate residue and diglyceride membrane anchor makes it possible to exposure the ligand at a sufficient distance from the liposome surface, which provides contact with the cell receptors.

The use of tetrasaccharide SiaLe^X as a molecular address is due to the fact that it is a common epitope for selectins, which are cell adhesion proteins expressing on activated cells of endothelium (E- and P-selectins), leucocytes (L-selectin), and thrombocytes (P-selectin).[16] Selectins are involved in many (patho)physiological processes, including processes of development of the inflammatory response and metastasis dissemination.[17] It was shown that the use of the selectin SiaLe^X-ligand for the selective

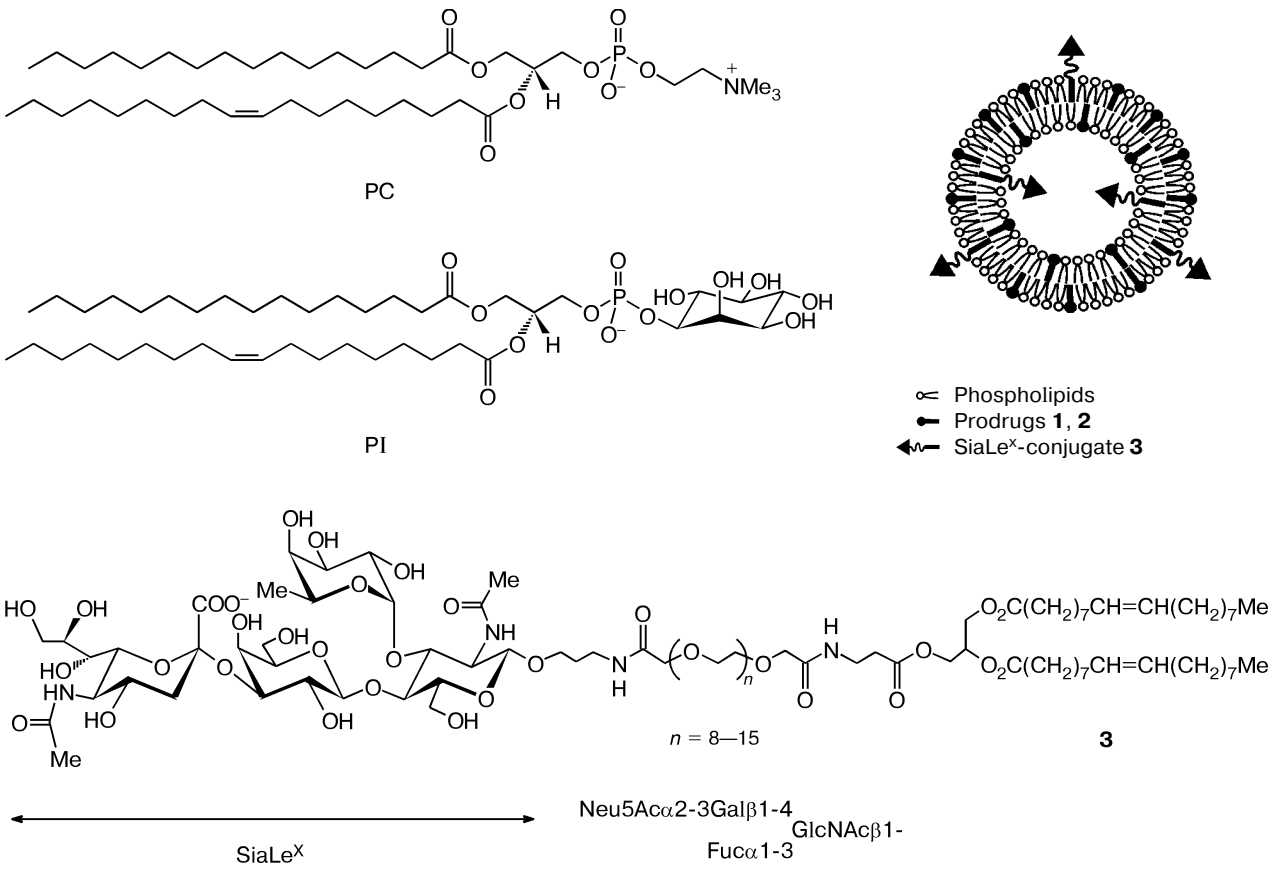


Fig. 1. Structures of phospholipids (phosphatidylcholine (PC) and phosphatidylinositol (PI)) and lipophilic SiaLe^X-conjugate **3** and the scheme of the targeted drug-loaded liposome.

delivery of liposomes containing a lipophilic prodrug of sarcolysin[18] or doxorubicin[19] substantially increased the therapeutic effect.

Liposomes were obtained from mixtures of natural phospholipids, namely, egg phosphatidylcholine (PC) and phosphatidylinositol (PI) from baker´s yeast, with prodrugs **1** and **2** and with SiaLe^X-conjugate **3** by the standard method of extrusion through 100-nm filters.[10,13,20] This affords monolamellar liposomes carrying the modified drug and carbohydrate ligand. The scheme of the liposome structure is shown in Fig. 1. According to the dynamic laser light scattering data, the average diameter of the obtained liposomes ranged from 89±30 to 98±33 nm.

The liposomal bilayer is basically (more than 80 mol.%) formed by phospholipids containing a half of the saturated acyl residues (palmitoyl and stearoyl), as well as the unsaturated oleoyl residues and a smaller amount of linoleoyl residues. Liposomes Stealth® are formed from phopholipids containing the saturated acyl residues (usually hydrogenated soya phosphatidylcholine) and cholesterol (up to 30%), because a rigid membrane is necessary to decrease drug leakage in the blood stream.[7,9,11] At the same time, the low efficiency of intracellular unloading caused by the rigidity of the lipid bilayer (its phase transition temperature exceeds 60 °C) and impeded confluence with cell membranes is one of substantial drawbacks of Stealth® liposomes. The lipophilic prodrugs themselves are the components of the lipid bilayer and do not leave the liposome after its damage, as it has been shown for the lipid conjugates of the water-soluble drugs.[10,13] The liquid lipid bilayer can include a larger amount of a prodrug. Phosphatidylinositol protects liposomes from the adsorption of plasma proteins and premature removal from the blood stream by the reticule-endothelial system. Indeed, the polar fragments of phosphatidylinositol molecules composing ~10% of the bilayer create a highly hydrated sterically stabilizing shell on the membrane surface. Similarly to the PEG residues, the shell stabilizes Stealth® liposomes in the blood stream.[21,22] However, a series of undesirable side effects for the use of liposomes Stealth® is caused by the presence of PEG.[23]

The content of prodrugs in liposomes was determined spectrophotometrically after the fractionation of dispersions by gel chromatography, as described previously[10,13] for other preparations. The capacity of inclusion of derivative CA-4 **2** in liposomes was 15 mol.%, and the prodrug was included into the lipid bilayer almost quantitatively.

In the case of compound **1**, the same efficiency of bilayer loading under the extrusion conditions at 22—40 °C was obtained for a liposomal capacity of ~7 mol.%. This result indicates a greater correspondence of the oleoyl derivative CA-4 to the lipid bilayer packing under these conditions.

It is known[24] that the pentasaccharide conjugate (SiaLe^X)1-3Galβ linked to the distearoylphosphatidylethanolamine by PEG residue (degree of polymerization 42—48) through the modified *N*-acetyl group of glucosamine is well incorporated into the lipid bilayer of liposomes Stealth®. Tetrasaccharide SiaLe^X-conjugate **3** (see Fig. 1) contains a considerably shorter residue of PEG, which enhances the affinity of the molecule to the lipid bilayer. It can be assumed with high reliability that neoglycolipid **3** composing 2 mol.% of the initial mixture of the amphiphilic components is completely incorporated into the liposome membrane.

The dose of CA4P used for a single injection to animals with tumor is very high (81 mg kg^{-1}).[11] In this connection, the capacity of the drug carrier gains a decisive significance, and it becomes unreasonable to use liposomes with prodrug **1** for the systemic injections to animals. Therefore, we carried out biological experiments with the liposomal preparations of compound **2**. The antiproliferative properties of CA-4 and prodrug **2** in liposomes were compared in the human breast cancer culture. After incubation for 48 h, the both preparations induced changes in the cell morphology. The data on survival of the cells are presented in Fig. 2. The calculated values of IC_{50} were 0.0075±0.002 and 0.023±0.004 μmol L^{-1} for CA-4 and the preparation of liposomes with prodrug **2**, respectively. When working *in vitro* with the cell lines, the usual multiple decrease in the toxicity of the drugs in the composition of nanosized carriers is explained by a change in the endocytosis mechanism and by the additional stage of intracellular unloading. The stage of prodrug hydrolysis also retards the manifestation of cytotoxicity. In our case, the decrease in the cytotoxicity by three times only may indicate fast rates of releasing of prodrug **2** in cells and subsequent hydrolysis with the formation of CA-4.

In order to estimate the antitumor effect of prodrug **2** in the targeted liposomal form *in vivo*, we chose the spontaneous model of breast cancer in mice BLRB/BYRB, which is characterized by the natural process of tumor formation.[25] Spontaneous models are most adequate to the human breast cancer, since they have important morphological and immunological features compared to the transplanted ones.[26] The preparations of SiaLe^X-liposomes with prodrug **2** were injected to recipient mice (n = 4) intravenously three times at an interval of one week after the tumors reaches a diameter of ~10 mm (*i.e.*, volume ~0.5 cm^3). Tumors of these sizes already have a high degree of vascularization (our data). Control mice (n = 6) obtained no injections. The growth dynamics of the individual tumors is presented in Fig. 3. An analysis of the curves shows that the tumor growth stopped for three recipients treated on the 4th, 5th, and 6th weeks, and the tumor regress was observed in one case. Only one animal had no antitumor effect. The drug was injected to this

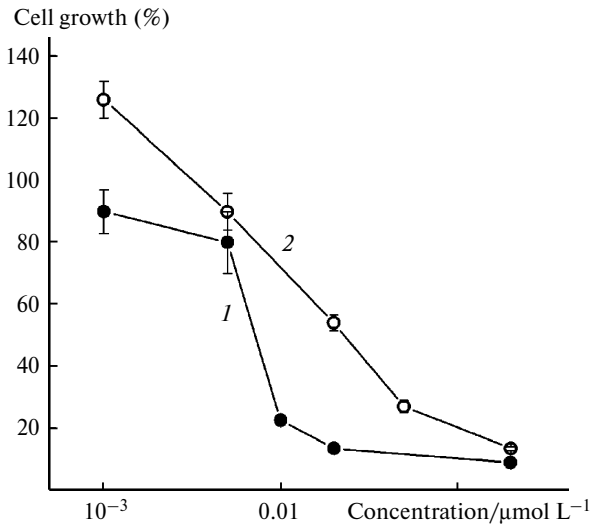


Fig. 2. Estimation of the cytotoxicity of CA-4 (*1*) and prodrug **2** (*2*) in the liposomal form in the human breast carcinoma cell culture HBL-100. After incubation for 48 h, the amount of living cells was determined by the inclusion of trypan blue dye. The percent of living cells was calculated as a ratio of the amount of living cells in experiment to the amount of living cells in the control multiplied by 100. The average values ±SE are given for each experiment performed in duplicate.

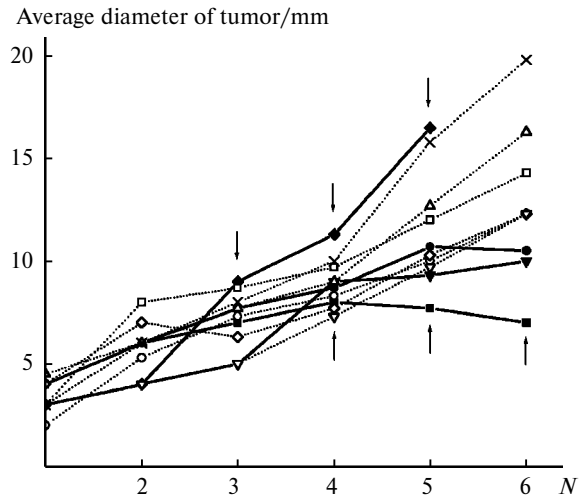


Fig. 3. Individual curves of the dynamic of tumor growth in the female mice BLRB/BYRB (n = 4) with spontaneous breast cancer upon the intravenous injection of 0.2 mL of dispersions of SiaLe^X liposomes loaded with prodrug **2** in an equivalent dose of 22 mg kg^{-1} by CA-4 three times at an interval of one week (solid lines). The days of drug injection are marked by vertical arrows. The control curves for intact animals (n = 6) are shown by dotted lines; *N* indicates weeks after the detection of palpable tumors.

animal a week earlier than for other animals because of the intense growth of its tumor. Evidently, in this case, injections should be started earlier and carried out more frequently than once a week.

Thus, SiaLe^X-liposomes with prodrug **2** in the dose used by the CA-4 residue (22 mg kg^{-1}) showed the efficient antitumor effect in the regime of monotherapy. A single injection of CA4P to mice with transplanted breast cancer cells MCa-4 in a dose accepted in the clinic practice (81 mg kg^{-1}) did not inhibit the tumor growth.[11] Noticeable inhibition was achieved only if the preparation was injected after irradiation (by ~30% on the first 10 days), and the curve of tumor growth almost coincided with the curves obtained after irradiation without using any preparation or after four injections of CA4P at an interval of a day. The strong inhibition of breast cancer growth (by ~80% during 15—20 days) was achieved due to the introduction of CA4P (15 mg kg^{-1}) in immunoliposomes Stealth® carrying mAb to E-selectin and only after the irradiation of the tumor, which considerably increases selectin expression.[11] The effect of immunoliposomes on tumor growth without irradiation almost did not differ from the effect of CA4P after irradiation, and only the dose of the preparation introduced in liposomes was 5.4 times smaller.[11] Note that in the cited work[11] the tests were carried out on the model of rapidly growing breast cancer (during 10 days after the beginning of the treatment the tumors in the control group increased in volume from 1 to 4 cm^3). It is known[27] that rapidly growing aggressive tumors are more sensitive to chemotherapy. The spontaneous model of slowly growing breast cancer, which better reflects the corresponding human disease, was used in our work.

Thus, we obtained the new liposome construction bearing the antimitotic agent combretastatin A-4 as the oleoyl derivative and the carbohydrate ligand of selectins SiaLe^X. The results of primary testing *in vivo* showed a good antitumor effect exemplified by therapy of breast cancer. The use of CA-4 as an antivascular antitumor remedy for systemic injection can be improved due to the inclusion of its lipophilic prodrug into liposomes equipped with the molecular address to the angiogenic endothelium of tumors.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX 400 spectrometer (400 and 101 MHz, respectively) in $CDCl_3$. Chemical shifts are presented in the δ scale relative to Me_4Si. Elemental analysis was carried out on a Perkin—Elmer Series II CHN/O Analysis 2400 instrument. Commercially available reagents (Aldrich, Alfa Aesar) were used as received. Prior to use solvents were purified by standard methods. Petroleum ether with b.p. 40—70 °C was used. Combretastatin A-4 was synthesized by the known procedure.[14]

5-[(*Z*)-2-(3,4,5-Trimethoxyphenyl)ethenyl]-2-methoxyphenyl palmitate (1). Oxalyl chloride (49 μL, 0.57 mmol) was added to a solution of palmitic acid (50 mg, 0.19 mmol) in anhydrous THF (2 mL) under an inert atmosphere. The reaction mixture was stirred for 3 h at 25 °C. The mixture was dissolved in 1 mL of THF under an inert atmosphere and added dropwise to sodium phenolate of CA-4, which was obtained by the addition of NaH (4.2 mg, 0.18 mmol) as a 60% suspension in mineral oil to a solution of CA-4 (50 mg, 0.16 mmol) in anhydrous THF (0.5 mL). The reaction mixture was stirred for 1 h at 25 °C and then concentrated by evaporation. The residue was dissolved in ethyl acetate (20 mL) and extracted three times with a 5% solution of NaOH (10 mL). The organic layer was dried over anhydrous Na_2SO_4. The product was isolated by column chromatography on silica gel (ethyl acetate—petroleum ether (1 : 4) mixture as eluent). Product **1** was obtained as a white solid in a yield of 71 mg (80%). Found (%): C, 73.29; H, 9.09. $C_{34}H_{50}O_6$. Calculated (%): C, 73.61; H, 9.08. ^{1}H NMR, δ: 0.88 (t, 3 H, Me, J = 8.0 Hz); 1.25 (m, 24 H, CH_2); 1.71 (m, 2 H, C(O)CH$_2$C$\underline{H}_2$); 2.52 (t, 2 H, C(O)C$\underline{H}_2$CH$_2$, J = 8.0 Hz); 3.70 (s, 6 H, OMe); 3.79, 3.83 (both s, 3 H each, OMe); 6.43, 6.47 (both d, 1 H each, HC=C, J = 12.0 Hz); 6.50 (s, 2 H, H(2′), H(6′)); 6.84 (d, 1 H, H(5″), J = 8.0 Hz); 7.00 (d, 1 H, H(2″), J = 2.0 Hz); 7.11 (dd, 1 H, H(6″), J = 2.0 Hz, J = 8.0 Hz). ^{13}C NMR, δ: 14.1, 22.7, 25.0, 29.0, 29.2, 29.3, 29.5, 29.6, 29.5, 29.7, 31.9, 33.9, 55.8, 55.9, 60.9, 105.7, 111.9, 123.1, 127.6, 128.6, 129.4, 130.0, 132.4, 137.0, 139.5, 150.2, 152.9, 171.7.

5-[(*Z*)-2-(3,4,5-Trimethoxyphenyl)ethenyl]-2-methoxyphenyl oleate (2). Oxalyl chloride (80 μL, 0.87 mmol) was added to a solution of oleic acid (0.21 g, 0.76 mmol) in anhydrous THF (2 mL) under an inert atmosphere. The reaction mixture was stirred for 3 h at 25 °C. The mixture was evaporated and dried. The residue was dissolved in THF (2 mL) under an inert atmosphere, and added dropwise to sodium phenolate of CA-4, which was obtained by the addition of NaH (15 mg, 0.64 mmol) as a 60% suspension in mineral oil to a solution of CA-4 (0.18 g, 0.58 mmol) in anhydrous THF (1 mL). The reaction mixture was stirred for 1 h at 25 °C and concentrated by evaporation. The residue was dissolved in ethyl acetate (30 mL) and extracted three times with a 5% solution of NaOH (10 mL). The organic layer was dried over anhydrous Na_2SO_4. The product was isolated by column chromatography on silica gel (ethyl acetate—petroleum ether (1 : 4) as eluent). Compound **2** (230 mg, 67%) was obtained as a colorless oil. Found (%): C, 74.29; H, 9.04. $C_{36}H_{52}O_6$. Calculated (%): C, 74.45; H, 9.02. ^{1}H NMR, δ: 0.88 (m, 3 H, Me); 1.31 (m, 20 H, CH_2); 1.72 (m, 2 H, C(O)CH$_2$C$\underline{H}_2$); 2.03 (m, 4 H, C$\underline{H}_2$CH=CH); 2.52 (t, 2 H, C(O)C$\underline{H}_2$CH$_2$, J = 8.0 Hz); 3.70 (s, 6 H, OMe); 3.79, 3.83 (both s, 3 H each, OMe); 5.35 (m, 2 H, CH=CH); 6.43, 6.47 (both d, 1 H each, ArHC=C, J = 12.0 Hz); 6.50 (s, 2 H, H(2′), H(6′)); 6.84 (d, 1 H, H(5″), J = 8.0 Hz); 6.99 (d, 1 H, H(2″), J = 2.0 Hz); 7.11 (dd, 1 H, H(6″), J = 2.0 Hz, J = 8.0 Hz). ^{13}C NMR, δ: 14.1, 22.7, 25.0, 27.2, 29.0, 29.2, 29.3, 29.5, 29.7, 31.9, 33.9, 55.8, 55.9, 60.9, 105.9, 112.0, 123.2, 127.6, 128.6, 129.5, 129.7, 130.0, 132.4, 137.2, 139.5, 150.3, 153.0, 171.7.

Preparation of dispersions of drug-loaded liposomes. Phosphatidylcholine from egg yolk and PI from *S. cerevisiae* (Reakhim, Russia) were used. SiaLe^X-conjugate **3** was synthesized from biscarboxymethyl ether of PEG (average weight 600 Da), *rac*-1,2-dioleyl-3-(3-aminopropionyl)glycerol, and 3-aminopropyl glycoside SiaLe^X as described recently.[15] Buffers were prepared with a 1 m*M* solution of EDTA: PBS, pH 7.06 is a physiological solution on a phosphate buffer (KH_2PO_4, 0.2 g L^{-1};

$NaH_2PO_4 \cdot 2H_2O$, 0.15 g L^{-1}; Na_2HPO_4, 1.0 g L^{-1}; KCl, 0.2 g L^{-1}; NaCl, 8.0 g L^{-1}); HBS, pH 7.2 is a physiological solution on buffer HEPES (HEPES-Na, 25 mmol L^{-1}; NaCl, 140 mmol L^{-1}); HEPES is *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid (Flow Laboratories).

Mixtures PC—PI—prodrug **1** (8 : 1 : 0.7 (mol.)), PC—PI—prodrug **2** (7.5 : 1 : 1.5 (mol.)), or PC—PI—prodrug **2**—$SiaLe^X$-conjugate **3** (7.5 : 1 : 1.5 : 0.2 (mol.)) were co-evaporated in round-bottom tubes from solutions in chloroform on a rotary evaporator at the temperature not higher than 40 °C. Each mixture contained 81 mg of PC, 11.4 mg of PI, and 5 mg (9.2 μmol) of compound **1** or 54 mg of PC, 8 mg of PI, 8 mg (14 μmol) of compound **2**, and 4 mg (~1.87 μmol) of $SiaLe^X$-conjugate **3**. Lipid films were dried for 30 min at 5 Pa and then hydrated for 2 h at room temperature in 2 mL of buffer PBS when obtaining liposomes for the experiment in the cell culture or with animals or in buffer HBS to determine the inclusion in liposomes. The suspension was shaken, subjected to a fivefold procedure of freezing—thawing (liquid nitrogen—+40 °C), and 20 times extruded through polycarbonate membrane filters (Nucleopore, USA) with the pore size 100 nm using a Mini-extruder setup (Avanti Polar Lipids, USA). The liposome sizes were determined by dynamic laser light scattering on a Brookhaven Particle Analyzer 90+ technique (USA). The concentrations of the prodrugs in dispersions were determined after the liposomes were destructed by 20-fold dilution in EtOH: the UV spectra were recorded and the absorbance at the absorption maximum (λ_{max} = 287 nm, $\varepsilon \approx 13440$) was measured on an SF-256-UVI two-beam spectrophotometer (Lomofotonika, St. Petersburg). Prodrug losses on the filters were monitored by determining their amounts in solutions obtained by macerating the filters in EtOH followed by UV spectra recording; the losses were at most 3%. The composition of the liposomes was determined after gel chromatography on a column with Sepharose CL-4B: the fractions were analyzed for phospholipid phosphorus by the colorimetric method,[28] and prodrugs were determined spectrophotometrically after the fivefold dilution of EtOH as described for other drugs.[10,13] Dispersions of liposomes were stored at +4 °C for at most 24 h.

Determination of cytotoxicity *in vitro*. Human breast carcinoma cells of the line HBL-100 were cultured at 37 °C under CO_2 (4%) in medium RPMI-1640 (ICN Biomedicals Inc., USA) with the addition of $NaHCO_3$ (0.2%), L-glutamine (2 mmol L^{-1}), gentamicin G (50 μg mL^{-1}), Streptomycin (100 μg mL^{-1}), and 10% calf embryonic serum inactivated by heating (Gibco BRL, Great Britain), pH 7, and passaged twice a week. The cells were incubated for 48 h in a cultural medium in 24-well plates with the liposome samples containing compound **2** in the concentrations 0.005—1 μmol L^{-1} or with the initial CA-4 added as solutions in DMSO, whose final concentration in the medium with cells did not exceed 0.001—0.2 μmol L^{-1}. Control cells were incubated with an aliquot of PBS with 1% DMSO. The number of living cells was determined by the standard test with trypan blue; the percent of living cells was calculated as the ratio of the amount living cells in experiment to that in control multiplied by 100. Each experiment was carried out in duplicate. The cytotoxicity (IC_{50}) was calculated by the Origin 6.0 program (MicroCal Software Inc., USA).

Tests of antitumor activity *in vivo*. Ten female mice BLRB/BYRB with the chromosomal translocation Rb(8.17)1Iem and a high frequency (>90%) of appearance of spontaneous MMTV-dependent breast cancer (MMTV is mouse mammary tumor virus) were used.[25] The average age of the females at the moment of primary establishment of breast cancer was 10 months. The mice were kept under conventional conditions. The moment of achieving a size of 2—4 mm in diameter by the tumor was accepted to be the beginning of the clinic stage of breast cancer, after which the tumor size was measured weekly with a sliding calipers. The average diameter of the tumor was calculated by the formula $(a + b + h)/3$, where a is the maximum length of the tumor, b is the maximum width of the tumor, and h is the average height of the tumor. The state of health of the animals was evaluated daily. The data on the tumor growth in intact females were used as control. The control group consisted of 6 mice, of which 2 mice were the current control, and others were historical control. Animals with similar values of such parameters as the age at the moment of visual appearance of the tumor, localization, and the initial dynamics of the tumor growth were taken to the experimental (4 females) and control groups. After the tumor reached 7—8 mm in diameter, 0.2 mL of dispersions of liposomes with the composition PC—PI—prodrug **2**—$SiaLe^X$-conjugate **3** (7.5 : 1 : 1.5 : 0.2 (mol.)) in a dose equivalent by CA-4 (22 mg kg^{-1}) was injected into the tail vein of each mouse of the experimental group. The antitumor effect was estimated by the dynamics of tumor growth.

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