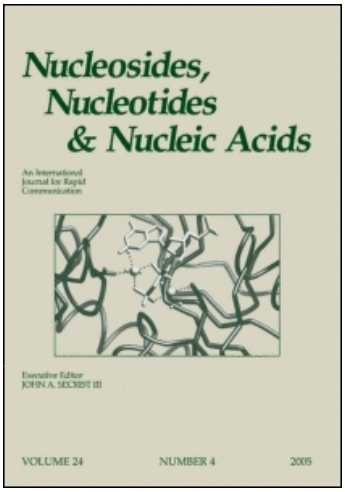

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DESIGN OF THE NEW MOLECULAR TRANSPORT SYSTEMS FOR THE NUCLEOSIDES - PHARMACOPHORES CARRYING.

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ABSTRACT. The structure design of a specific and nonspecific transport systems for nucleosides was proposed. A number of model compounds were synthesized.

The nucleoside analogues are extensively used in therapy of viral and cancer diseases. The most known such medicines are azidothymidine (AZT)[#] **(1)**, 3'-deoxy-2',3'-didehydrothymidine (d4T) **(2)**, and also some 2',3'-dideoxy-nucleosides (ddC, ddI) etc[1]. These compounds show both high activity and toxity. Its toxity caused by high efficient concentration of these compounds in the blood.

The nucleoside analogues are hydrophilic and don't have ability to the passive transport through cellular membrane. A lot of nucleoside derivatives with various hydrophobic residues as lipids, hydrophobic amino acids and peptides[2-11] were described within last five years. Almost all described prodrugs are able to the nonspecific membrane transport.

Also molecular systems with fragments (ligands), capable to specific interaction with membrane receptors present real significance. The examples of similar transport systems

AZT - 2 ,3'-dideoxy-3'-azidothymidine, d4T - 3'-deoxy-2',3'-didehydrothymidine, ddC -2',3'-dideoxcytidine, ddI -2',3'- dideoxyinosine, DMAP - 4-N,N-dimetylaminopyridyne, DCC - dicyclohexylcarbodiimide.

are described for derivatives fat-soluble vitamins with nucleosides[12]. The capacity to specific transport and the antiviral effect was reported for nucleoside analogues of the vitamin B_{12} coenzyme few years ago[13].

As a rule the prodrug design based on medicines with known action requires the presence at least of three fragments in the molecule. First of all is analogue of the nucleoside, second fragment is the spacer, and, finally, the unit providing specific or nonspecific membrane transport. Usually phosphodiester bridge was used as the spacer, however such derivatives showed high activity *in vitro* but did not exhibit adequate activity *in vivo* that can be explained by their fast cleavage by nonspecific blood enzymes.

We developed an approach to structure design of a specific and nonspecific transport systems for nucleoside analogues based on the more stable ester bonded spacer.

The synthesis of such derivatives **(3-5, 7-9)** was carried out according to **SCHEME 1**. Available and pharmacologically well known thymidine analogues - 3'-deoxy-3'-azidothymidine **(1)** and 3'-deoxy'-2',3'-didehydrothymidine **(2)** were chosen as nucleoside components; as spacer was selected succinic acid. There were synthesized two groups of compounds:

-for nonspecific transport with residues of geraniol **(3)** and **(4)**, dihexadecylglicerol **(8)** and dipalmitoylglicerol **(7).**

-for specific transport with residues of retinol **(5)** and tryptamine. **(9)**.

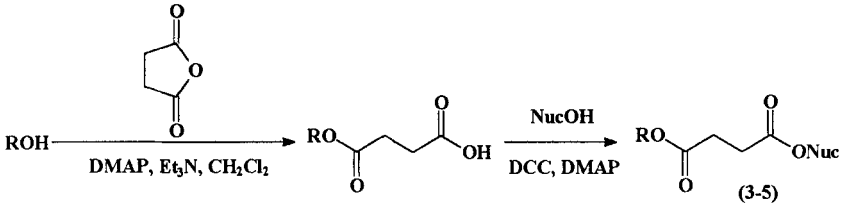
Because our researches on the creation of combinatorial nucleoside derivatives library for study of active and passive pharmacophores transport are in initial stage, we plan to test the biological properties of new syntesized compounds soon. Preliminary investigations showed that **(3)** and **(4)** do not cause sizable decomposition of the model bilayer membranes from egg phosphatydilcholine (according to ^{31}P-ЯМР data) in concentrations up to 20% (by weight)[14]. This is lets us to hope on the possibility of successful application of these and similar compounds.

EXPERIMENTAL

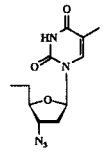
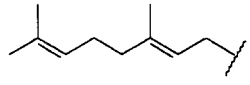
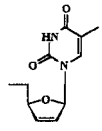
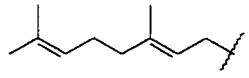
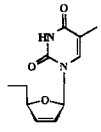
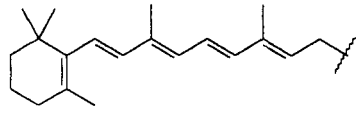
General

The following solvents were distilled prior to use: CH_2Cl_2 (from P_2O_5), ethylacetat (from $CaCl_2$), acetonitrile (from CaH_2), pyridine (from CaH_2), DMF. Chemicals were bought from Lancaster (United Kingdom) and ChiMed (Russia).

A.



Where

	Nuc	R
(3)		
(4)		
(5)		

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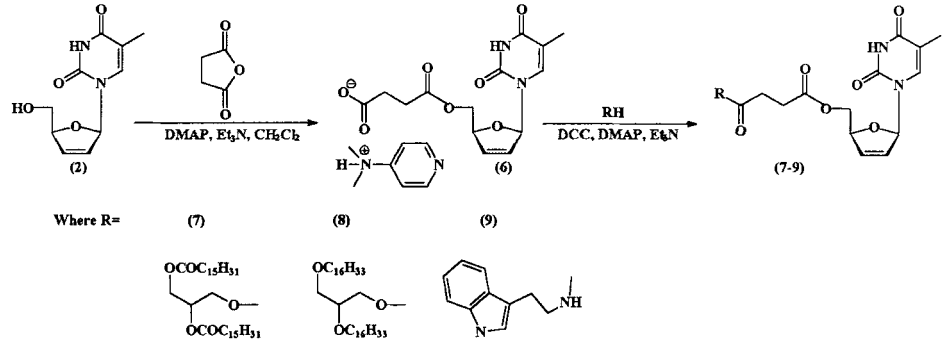

SCHEME 1

NMR spectra run in $CDCl_3$, DMSO-D_6 or mixture of $CDCl_3$ - CD_3OD 1:1 v/v [with tetramethylsilane (δ 0 ppm) as internal standard] at Bruker MSL-200 (operating at 199,5 MHz for ^1H). For signal designations the IUPAC retinoid numbering system is used[15]. There were used next abbreviations in spectra description: Ger - geraniol residue; Succ - succinic ester residue; Ret - retinol residue; Glycer - glycerol residue; Thy - thymidine analogue residue; Trp - tryptamine residue; Alk - residues of alkyl lipid chains; term - terminal methyls.

Evaporation of solvents is performed *in vacuo* (at 20 mm Hg). Purification is performed by flash column SiO_2 (Kieselgel 60/200, Merck, Germany) using ether/petroleum ether or chloroform/methanol as eluent. Reactions were followed by TLC.

Monosuccinic ester of geraniol. Geraniol 0.77 g (5 mmol) was dissolved in 20 ml of dichloromethane, then DMAP 0.31 r (3 mmol), triethylamine 0.51 r (5 mmol), succinic anhydride 0.75 r (8 mmol) were added and mixture was stirred during 12 h at room temperature. Reaction mixture washed by 5% HCl. After drying and evaporation of organic phase residue was purified by column chromatography. Yield 0.54 g (42.5%). ^1H-NMR (in $CDCl_3$), δ 5.34 ppm (1H; m; 2-CH-Ger); 5.08 (1H; m; 6-CH-Ger); 4.62 (2H; d; *J* 7.03; 1-CH_2-Ger); 2.67 (4H; m; -$(CH_2)_2$-Succ); 2.10 - 2.06 (4H; m; 4+5-$(CH_2)_2$-Ger); 1.70-1.68 (6H; m: term-$(CH_3)_2$-Ger); 1.60 (3H; s; 3-CH_3-Ger).

Mixed succinat of geraniol and AZT (3). To the solution of AZT **(1)** 0.06 g (0.22 mmol), DMAP 0.04 g (0.33 mmol) and geraniol succinat 0.06 g (0.24 mmol) in 3 ml of dry ethylacetate was added DCC 0.05 g (0.26 mmol) and mixture was stirred during 48 h at room temperature. Then the reaction mixture was filtered off, filtrate was evaporated and residue was purified by column chromatography. Yield 0.082 g (73%). ^1H-NMR (in $CDCl_3$), δ 9.72 ppm (1H; s; 3-NH-Thy); 7.31 (1H; s; 6-CH-Thy); 6.17 (1H; t; *J* 6.50; 1'-CH-Thy); 5.33 (1H; m; 2-CH-Ger); 5.08 (1H; m; 6-CH-Ger); 4.61 (2H; d; *J* 7.03; 1-CH_2-Ger); 4.57-4.45 (1H; m; 4'-CH-Thy); 4,38-4.20 (2H; m; 5'-CH_2-Thy); 4.04 (1H; m; 3'-CH-Thy); 2.69 (4H; m; -$(CH_2)_2$-Succ); 2.43 (2H; m; 2'-CH_2-Thy); 2.10-2.06 (4H; m; 4+5-$(CH_2)_2$-Ger); 1.94 (3H; s; 5-CH_3-Thy); 1.69 (6H; s; term-$(CH_3)_2$-Ger); 1.60 (3H; s; 3-CH_3-Ger).

Mixed succinat of geraniol and d4T (4). was obtained by the same manner as **(3)** from 0.09 g (0.402 mmol) of **(2)**, 0.074 g (0.603 mmol) of DMAP, 0.112 g (0.442 mmol) of

geraniol succinat and 0.099 g (0.482 mmol) of DCC in 4.5 ml of ethylacetate. Yield 0.114 g (61.6 %). ^{1}H-NMR (in $CDCl_3$), δ 9.80 ppm (1H; s; 3-NH-Thy); 7.20 (1H; d; *J* 1.32; 6-CH-Thy); 6.94 (1H; m; 1'-CH-Thy); 6.20 (1H; dt; J_1 6.00; J_2 1.50; 3'-CH-Thy); 5.84 (1H; m; 2'-CH-Thy); 5.25 (1H; m; 2-CH-Ger); 4.96 (2H; 6-CH-Ger + 4'-CH-Thy); 4.53 (2H; d; *J* 7.03; 1-CH_2-Ger); 4.50-4.10 (2H; m; 5'-CH_2-Thy); 2.57 (4H; m; -$(CH_2)_2$-Succ); 2.01-1.98 (4H; m; 4+5-$(CH_2)_2$-Ger); 1.84 (3H; s; 5-CH_3-Thy); 1.62 (3H; s; term-CH_3-Ger); 1.59 (3H; s; term-CH_3-Ger);1.60 (3H; s; 3-CH_3-Ger).

Monosuccinic ester of retinol. Retinol was prepared from 3.28 g (0.01 mol) of retinolacetat by action of 0.1 g (0.025 mol) NaOH in methanol without purification. Retinol monosuccinat was obtained from retinol, DMAP 0.61 g (0.005 mol), succinic anhydride 1.5 g (0.015 mol), triethylamine 1.4 ml (0.01 mol) in 30 ml dichloromethane by the same manner as geraniol succinat. Yield 3.4 g (88%). ^{1}H-NMR (in $CDCl_3$), δ 6.61 (1H; dd: J_1 11.5; J_2 16.0; 11-H-Ret); 6.24 (1H; d; *J* 16.0; 7-CH-Ret); 6.11 (1H; d; *J* 11.5; 10-CH-Ret); 6.08 (1H; d; *J* 16.0; 12-CH-Ret); 6.06 (1H; d; *J* 16.0; 8-CH-Ret); 5.57 (1H; t; *J* 7.03; 14-CH-Ret); 4.73 (2H; d; *J* 7.03; 15-CH_2-Ret); 2.62 (4H; s; -$(CH_2)_2$-Succ); 1.99 (2H; m; 4-CH_2-Ret); 1.93 (3H; s; 13-CH_3-Ret); 1.85 (3H; s; 9-CH_3-Ret); 1.68 (3H; s; 5-CH_3-Ret); 1.58 (2H; m; 3-CH_2-Ret); 1.45 (2H; m; 2-CH_2-Ret); 0.99 (6H; s; 1-$(CH_3)_2$-Ret).

Mixed succinat of retinol and d4T (5) was obtained by the same manner as **(3)** from 0.075 g (0.33 mmol) of **(2)**, 0.060 g (0.500 mmol) of DMAP, 0.140 g (0.36 mmol) of retinol monosuccinat and 0.082 g (0.4 mmol) of DCC in 3 ml of dry ethylacetate. Yield 0.090 g (45.8 %) ^{1}H-NMR (in $CDCl_3$), δ 8.27 (1H; s; 3-NH-Thy); 7.24 (1H; s; 6-CH-Thy); 6.98 (1H; m; 1'-CH-Thy); 6.62 (1H; dd: J_1 11.5; J_2 16.0; 11-CH-Ret); 6.27 (1H; d; *J* 6.15; 3'-CH-Thy); 6.23 (1H; d; *J* 16.0; 12-CH-Ret); 6.18 (1H; d; *J* 16.0; 7-CH-Ret); 6.06 (1H; d; *J* 11.5; 10-CH-Ret); 6.05 (1H; d; *J* 16.0; 8-CH-Ret); 5.87 (1H; d; *J* 6.15; 2'-CH-Thy); 5.57 (1H; t; *J* 7.03; 14-CH-Ret); 4.96 (1H; m; 4'-CH-Thy); 4.73 (2H; d; *J* 7.03; 15-CH_2-Ret); 4.30 (1H; dd; J_1 4.0 J_2 12.5; 5'-CH-Thy); 4.20 (1H; dd; J_1 4.0 J_2 12.5; 5'-CH-Thy); 2.63 (4H; s; $(CH_2)_2$-Succ); 1.97 (2H; m; 4-CH_2-Ret); 1.95 (3H; s; 13-CH_3-Ret); 1.89 (3H; s; 5-CH_3-Thy);1.87 (3H; s; 9-CH_3-Ret); 1.62 (3H; s; 5-CH_3-Ret); 1.58 (2H; m; 3-CH_2-Ret); 1.45 (2H; m; 2-CH_2-Ret); 0.99 (6H; s; 1-$(CH_3)_2$-Ret).

1/2 4-N,N-Dimethylaminopyridinium salt of d4T monosuccinic ester of (6). To a stirring solution of **(2)** 0.300 g (1.3 mmol) in 10 ml dichloromethane were added 0.08 g (0.7 mmol) DMAP, 0.14 g (1.3 mmol) triethylamine and 0.200 g (2.0 mmol) succinic anhydride. The mixture was stirred over night at room temperature and then cooled down to 0°C. The precipitate was filtered, washed twice with dichloromethane and dried in vacuo. Yield 0.19 g (44%). ^{1}H-NMR (in DMSO-D_6), δ 8.09 ppm (1H; d; *J* 5.71; 2-CH+6-CH of DMAP); 7.27 (1H; s; 6-CH-Thy); 6.80 (1H; m; 1'-CH-Thy); 6.69 (1H; d; *J* 5.71; 3-CH+5-CH of DMAP); 6.38 (1H; d; *J* 6.15; 3'-CH-Thy); 5.99 (1H; d; *J* 6.15; 2'-CH-Thy); 4.96 (1H; m; 4'-CH-Thy); 4.34-4.12 (2H; m; 5'-CH_2-Thy); 2.96 (3H; s; -N-$(CH_3)_2$ of DMAP); 2.48 (4H; m; -$(CH_2)_2$-Succ); 1.78 (3H; s; 5-CH_3-Thy).

Mixed succinat of dipalmitoylglicerol and d4T (7). To a stirred solution of **(6)** 0.132 g (0.1 mmol), dipalmitoylglicerol 0,050 g (0.09 mmol), 0.017 g (0.14 mmol) of DMAP in 4 ml dry acetonitrile was added 0,023 g (0.11 mmol) of DCC. The mixture was stirred overnight at room temperature. The precipitate was filtered off. The filtrate was concentrated in vacuo, and the residue was purified on a silica gel chromatography column ($CHCl_3$ - MeOH, 95:5 v/v). Yield 0.26 g (30%). ^{1}H-NMR (in $CDCl_3$), δ 8.21 ppm (1H; s; 3-NH-Thy); 7.26 (1H; d; *J* 1.32; 6-CH-Thy); 6.98 (1H; m; 1'-CH-Thy); 6.27 (1H; dt; J_1 6.00; J_2 1.50; 3'-CH-Thy); 5.87 (1H; m; 2'-CH-Thy); 5.10-4.94 (2H; m; 2-CH-Glycer + 4'-CH-Thy); 4.46-3.90 (6H; m; α-CH_2-Alk + 5'-CH_2-Thy); 3.75-3.40 (4H; m; 1-CH_2 + 3-CH_2-Glycer); 2.82-2.55 (4H; m; -$(CH_2)_2$-Succ); 1.91 (3H; s; 5-CH_3-Thy); 1.61 (4H; m; β-CH_2-Alk); 1.22 (48H; m; Alk); 0.85 (6H; t; *J* 6.59; term-CH_3-Alk).

Mixed succinat of dihexadecylglicerol and d4T (8) was obtained by the same manner as **(7)** from 0.050 g (0.15 mmol) of **(6)**, 0.025 g (0.21 mmol) of DMAP and 0.075 g (0.14 mmol) of dihexadecylglicerol, 0.034 g (0.17 mmol) of DCC in 3 ml of dry acetonitrile. Yield 0.030 g (23%). ^{1}H-NMR (in $CDCl_3$), δ 8.06 ppm (1H; s; 3-NH-Thy); 7.24 (1H; s; 6-CH-Thy); 6.97 (1H; m; 1'-CH-Thy); 6.27 (1H; dt; J_1 6.00; J_2 1.50; 3'-CH-Thy); 5.88 (1H; m; 2'-CH-Thy); 5.02 (1H; m; 4'-CH-Thy); 4.47-4.05 (4H; m; 3-CH_2-Glycer + 5'-CH_2-Thy); 3.62-3.37 (7H; m; 1-CH_2 + 2-CH-Glycer + α-CH_2-Alk); 2.63 (4H; m; -$(CH_2)_2$-Succ); 1.90 (3H; d; *J* 1.32; 5-CH_3-Thy); 1.54 (4H; m; β-CH_2-Alk); 1.23 (52H; m; Alk); 0.86 (6H; t; *J* 7.03; term CH_3-Alk).

Mixed succinat of tryptamine and d4T (9) was obtained by the same manner as **(7)** from 0.046 g (0.14 mmol) of **(6)** and 0.021 g (0.13 mmol) of tryptamine, 0.032 g (0.16 mmol) of DCC in the mixture of 4 ml of dry acetonitrile and 1 ml of dry pyridine. Yield 0.020 g (30%). ^{1}H-NMR (in $CDCl_3$ - CD_3OD), δ 7.60-7.00 ppm (6H; m; arom-CH-Trp + 6-CH-Thy); 6.94 (1H; m; 1'-CH-Thy); 6.30 (1H; dt; J_1 6.00; J_2 1.50; 3'-CH-Thy); 5.88 (1H; m; 2'-CH-Thy); 5.00 (1H; m; 4'-CH-Thy); 4.30-4.00 (2H; m; 5'-CH_2-Thy); 3.52 (2H; t; J 6.59; 2-CH_2-Trp); 2.95 (2H; t; J 6.59; 1-CH_2-Trp); 2.70-2.35 (4H; m; -$(CH_2)_2$-Succ); 1.89 (3H; d; J 1.32; 5-CH_3-Thy).

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