

NEW N^4 -HYDROXYCYTIDINE DERIVATIVES: SYNTHESIS AND ANTIVIRAL ACTIVITY

Maksim A. IVANOV^a, Elena V. ANTONOVA^a, Aleksey V. MAKSIMOV^a,
Lyudmila K. PIGUSOVA^a, Evgenii F. BELANOV^b and
Lyudmila A. ALEKSANDROVA^{a1,*}

^a Engelhardt Institute of Molecular Biology, Russian Academy of Sciences,
32 Vavilov Str., Moscow 119991, Russian Federation; e-mail: ¹ ala2004@zmail.ru

^b State Research Center of Virology and Biotechnology "Vektor",
Koltsovo, Novosibirsk Region 633159, Russian Federation; e-mail: belanov@vector.nsc.ru

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday in recognition of his invaluable contributions to the area of medicinal chemistry.

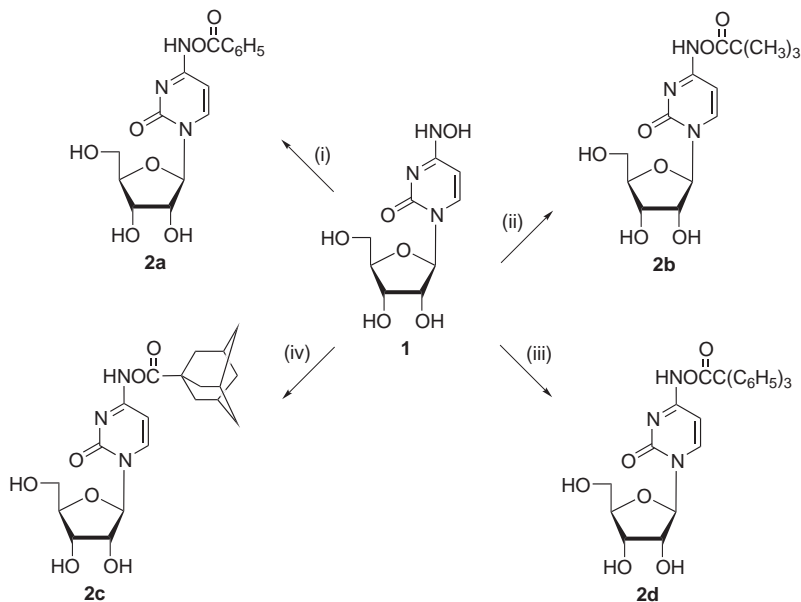
Two series of N^4 -hydroxycytidine derivatives were synthesized and evaluated as potential antipox virus agents. Acylation of N^4 -hydroxycytidine (**1**) with an excess of acyl chloride or imidazolidine yielded the corresponding N^4 -(acyloxy) derivatives. 5'-Phosphonates of **1** were prepared by the reaction of cytidine 5'-phosphonates with aqueous hydroxylamine hydrochloride (pH 6.0). Nucleoside **1** and its N^4 -(acyloxy) derivatives inhibited replication of pox viruses in cell cultures, N^4 -(pivaloyloxy)- and N^4 -(benzoyloxy)cytidines being the most potent. The synthesized 5'-phosphonates were more cytotoxic than the parent nucleoside.

Keywords: Nucleosides; Nucleoside analogs; N^4 -Hydroxycytidine; Nucleoside phosphonates; Hydroxylamine; Pyrimidines; Antiviral agents; Pox viruses.

Nucleoside analogues show a high antiviral potential. Nucleoside-derived drugs are approved for therapy of AIDS and infections induced by herpes virus, varicella zoster virus, and hepatitis B virus. Of their shortcomings, one can note their cytotoxicity and insufficient bioavailability, which could be improved by proper modifications of their structures. N^4 -Hydroxycytidine (**1**) was shown to have a broad spectrum of antiviral activity. In particular, **1** inhibited in cell culture assays some clinically important infections, induced by viruses of severe acute respiratory syndrome, influenza A and B ¹, measles¹, hepatitis C ² and some others. In this paper we describe the synthesis of new derivatives of **1** and evaluation of their antiviral prop-

erties in Vero and LLC-MK2 cell cultures infected with viruses of the pox family.

We prepared a series of N^4 -(acyloxy) derivatives of **1** based on the data on the positive effect of hydrophobic substituents in the molecules of antivirals³. The synthesis of N^4 -(acyloxy) derivatives (**2a–2d**) was performed according to Scheme 1. Acylation of **1** in pyridine with a small excess of the acyl chloride led to benzoyl (**2a**) or pivaloyl (**2b**) derivatives of **1**. In the case of N^4 -(adamantane-1-carbonyloxy)cytidine (**2c**) and N^4 -(triphenylacetoxy)cytidine (**2d**), imidazolides of adamantane-1-carboxylic or triphenylacetic acid were used as acylating agents. The products were isolated on a silica gel column in a gradient of concentrations of MeOH in CHCl_3 in 54–79% yields. We did not observe the formation of the corresponding N -acyl derivatives, which correlates with Mertes's and Smrt's data⁴ on the exclusive formation of O -derivatives when modified the N^4 -(hydroxy-amino) group of N^4 -hydroxy-6-azacytidine.



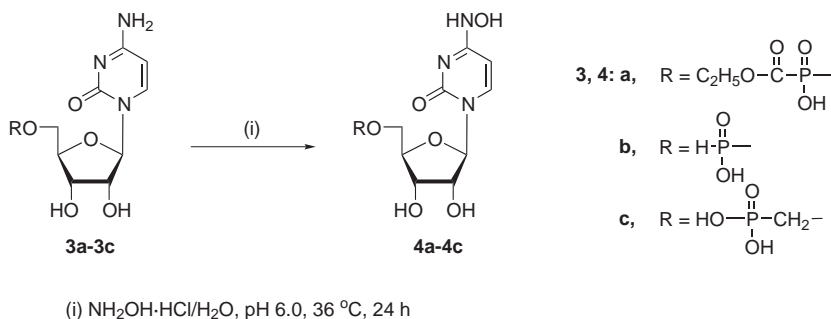
(i) benzoyl chloride, Py, 4 °C, 20 h; (ii) pivaloyl chloride, Py, 4 °C, 20 h; (iii) 1-(adamantane-1-carbonyl)imidazolide, DMF, 20 °C, 20 h; (iv) 1-(triphenylacetyl)imidazolide, DMF, 20 °C, 20 h

SCHEME 1

Another approach of designing new antivirals involves the introduction of 5'-phosphonate fragments into molecules of nucleoside analogues. For example, 5'-phosphonate of 3'-deoxy-3'-azidothymidine is used in med-

ical practice as an anti-HIV drug (Necivir®)⁵. Phosphonate derivatives of acyclic nucleosides have a broad spectrum of an antiviral activity and three of them are already marketed – Hepsera™ (adenovir, PMEA), Viread™ (tenofovir, PMPA) and Vistide™ (cidofovir, HPMPC)⁶.

5'-O-Phosphonate derivatives of cytidine **3a–3c** were prepared according to the reported procedures⁷. The reaction of **3a–3c** with aqueous hydroxylamine hydrochloride (pH 6) under the conditions described in ref.⁸ (Scheme 2) afforded derivatives **4a–4c**, which were purified by reversed-phase silica gel chromatography and isolated in 56–83% yields.



SCHEME 2

The structures of the synthesized compounds were confirmed by UV, ¹H, ¹³C and ³¹P NMR, and mass spectra. It is noteworthy that substitution of the 4-amino group in the cytosine base by a hydroxylamine group resulted in a significant upfield shift of H-6, H-5 and H-1' resonances in the ¹H NMR spectra compared with the parent cytidine phosphonates.

Stability of compounds **2a**, **2b** was evaluated in PBS and normal human blood serum at 37 °C according to the reported procedures⁹. Compounds **2a**, **2b** were stable in PBS to give only traces of **1** after 10 days. Half-life periods of compounds **2a**, **2b** in human blood serum were shown to be 40 ± 5 and 45 ± 5 min, respectively, to give only **1**.

Antiviral activity of the synthesized compounds was studied in Vero and LLC-MK2 kidney cell cultures infected with various pox viruses: vaccinia (VV), monkeypox (MPV) and cowpox (CPV) viruses. Antiviral efficiency was determined using a Neutral Red uptake assay according to ref.¹⁰ The data were graphed as shown in Fig. 1 for **2a** and summarized in the Table I. Compound **1** effectively suppressed replication of pox viruses (VV, MPV, CPV) and was moderately toxic. Antiviral activities of **1** were higher than those of cidofovir, used as reference preparation in the antipox virus study¹⁰, although the latter demonstrated a considerably lower toxicity. As a

result, selectivity indexes (SI) of **1** were comparable with those of cidofovir (3.6–15.3 versus 1.2–9.5 for different viruses and cell cultures). Antiviral properties of compounds **2a** and **2b** were higher than those of parent **1** due to lower cytotoxicity and higher activity in both cell cultures. In LLC-MK-2 cells SI values of compounds **2a** and **2b** achieved 50–100, whereas SI values of **1** were 5–15 for different viruses. The similar results were obtained in Vero cells (SI 10–67 for compounds **2a** and **2b** versus 3.6–5.6 for parent **1**). The introduction of more bulky substituents into the N^4 -position of the heterocyclic base (compounds **2c** and **2d**) dramatically increased cytotoxicity and did not effect antiviral activity.

As a rule, nucleoside phosphonates exhibit a lower activity in cell assays if compared with parent nucleosides, but due to a more potent reduction of cytotoxicity, the SI of these compounds are higher^{5,11}. Cytotoxicities of N^4 -hydroxycytidine 5'-phosphonate derivatives **4** in Vero cell culture were higher then that of the parent nucleoside (CC_{50} 5.4–38 μ M) and SI values of compounds **4a** and **4b** were low (3 and 4.4 correspondingly) Compound **4c** was inactive in these experiments. A similar result was obtained for 4'-thio-5-ethyl-2'-deoxyuridine, whose phosphonates were neither more active as anti herpes simplex virus agents nor less toxic⁹.

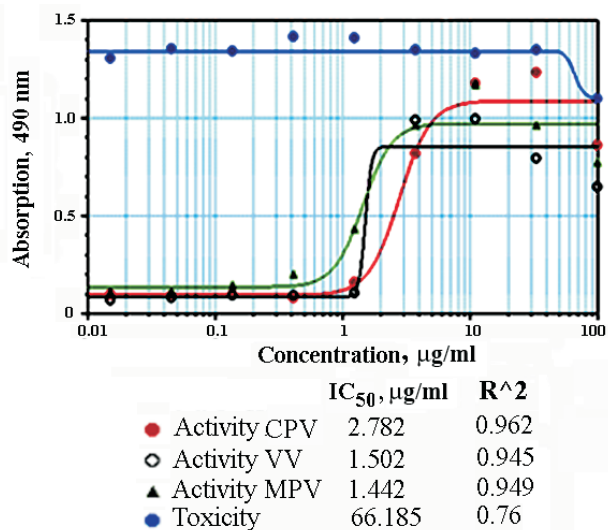


FIG. 1

Representative data from a single experiment for **2a** against various pox viruses on LLC-MK2 cells. Data are shown for a single replicate set of assays. IC_{50} values (μ g/ml) for each strain are shown below the graph, as the R^2 fit of the points to the curve

TABLE I
Antiviral activity and cytotoxicity of *N*⁴-alkyloxycytidines **2a–2d** and 5'-phosphonate derivatives of *N*⁴-hydroxycytidine **4a–4c** in Vero and LLC-MK2 cell cultures

Compd	Cell culture	CC ₅₀ , μM	VV		MPV		CPV	
			IC ₅₀ , μM	IS	IC ₅₀ , μM	IS	IC ₅₀ , μM	IS
1	Vero	50	8.9	5.6	10.1	5.0	14.0	3.6
	MK-2	77	15.1	5.1	5.0	15.3	13.9	5.76
2a	Vero	165	4.1	40.2	7.9	20.7	19.7	8.4
	MK-2	275	2.7	102	2.3	118	4.8	57.5
2b	Vero	175	2.6	67	8.4	21	18.0	9.7
	MK-2	480	4.7	103	2.6	186	6.7	77
2c	Vero	31	6.7	4.7	4.6	6.7	5.0	6.2
	MK-2	102	7.3	13.1	11.4	8.9	31.6	3.2
2d	Vero	23	1.3	17.7	2.9	8.2	6.4	3.6
	MK-2	47	1.4	34	4.0	11.7	22.5	2.1
4a	Vero	6.6	1.5	4.4	n.d.	n.d.	n.d.	n.d.
4b	Vero	5.4	1.8	3.0	n.d.	n.d.	n.d.	n.d.
4c	Vero	38	38	1	n.d.	n.d.	n.d.	n.d.
Cidofovir	Vero	360	44.1	8.1	37.6	9.5	47.7	7.5
	MK-2	360	83.5	4.3	112.2	3.0	298.7	1.2

VV, vaccinia virus; MPV, monkeypox virus; CPV, cowpox virus.

CC₅₀ is the minimal concentration of the compound, at which 50% cell death occurs.

IC₅₀ is the concentration of compounds at which proliferation of virus is inhibited by 50%.

IS = CC₅₀/IC₅₀; n.d., not determined.

To summarize, modification of *N*⁴-hydroxycytidine **1** by acylation of the *N*⁴-position may have some hopeful prospects, whereas its 5'-phosphonylation seems to be a dead lock.

EXPERIMENTAL

UV spectra (λ, nm; ε) were measured on a UV-2401 spectrophotometer (Shimadzu, Japan).

¹H NMR spectra were recorded on an AMX III-400 (Bruker, U.S.A.) with a working frequency of 400 MHz for ¹H NMR (Me₄Si and sodium 3-(trimethylsilyl)propane-1-sulfonate (DSS) were internal standards for CDCl₃ or DMSO-*d*₆ and D₂O solutions, respectively), 101 MHz for ¹³C NMR and 162 MHz for ³¹P NMR. ³¹P NMR spectra were recorded with ¹H-decoupling and 85% H₃PO₄ as an external standard. Chemical shifts (δ-scale) are given in ppm; cou-

pling constants (J) in Hz. Mass spectra were registered on a Compact MALDI 4 spectrometer (Kratos Analytical, U.S.A.). Melting points were determined on a Kofler block and are uncorrected.

Starting Materials and Solvents

Adamantane-1-carboxylic acid, Et_3N , benzoyl chloride and pivaloyl chloride were purchased from Fluka; pyridine, dioxane, DMF, triphenylacetic acid, 1,1'-carbonyldiimidazole were from Aldrich; Silica gel 60 (40–63 μm) and LiChroprep RP-18 (25–40 μm) were from Merck, hydroxylamine hydrochloride was from Reakhim (Russia). Cytidine 5'-(ethoxycarbonyl)-phosphonate (**3a**), cytidine 5'-phosphonate (**3b**) and 5'-*O*-(phosphonomethyl)cytidine (**3c**) were prepared according to published procedures⁷. Cidofovir was a kind gift of Dr Rosenberg (Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic).

N^4 -(Benzoyloxy)cytidine (**2a**) and N^4 -(Pivaloyloxy)cytidine (**2b**)

Benzoyl chloride or pivaloyl chloride (0.34 mmol) was added to a precooled ($-20\text{ }^\circ\text{C}$) solution of **1** (80 mg, 0.31 mmol) in dry pyridine (2 ml). The mixture was kept at $4\text{ }^\circ\text{C}$ for 20 h, MeOH (1 ml) was added and the solvents were evaporated. The residue was dissolved in chloroform (0.5 ml), applied onto a silica gel column ($2 \times 22\text{ cm}$), and eluted with a gradient of methanol in chloroform (0→20%, 750 ml). The target fractions were evaporated, the residue was crystallized from MeOH to give **2a** (76 mg, 72%) and **2b** (89 mg, 79%).

N^4 -(Benzoyloxy)cytidine (**2a**): For $\text{C}_{16}\text{H}_{17}\text{N}_3\text{O}_7$ MS: m/z 362.3 (M^+). M.p. 208–209 $^\circ\text{C}$. UV (MeOH): λ_{max} 276 (7200), 231 (7300). ^1H NMR (CD_3OD): 7.54 m, 6 H (C_6H_5 , H-6); 5.91 d, 1 H, $J_{1',2'} = 4.7$ (H-1'); 5.82 d, 1 H, $J_{5,6} = 8.1$ (H-5); 4.18 m, 2 H (H-2', H-3'); 3.99 t, 1 H, $J_{4',3'} \sim J_{4',5'} = 2.8$ (H-4'); 3.82 dd, 2 H, $J_{\text{gem}} = 12.7$, $J_{5'a,4'} = 3.9$ (H-5'a); 3.76 dd, 2 H $J_{\text{gem}} = 12.7$, $J_{5'b,4'} = 3.7$ (H-5'b). ^{13}C NMR (CD_3OD): 61.09 (C-5'); 69.99 (C-2'); 72.93 (C-3'); 84.78 (C-4'); 87.31 (C-1'); 96.32 (C-5); 128.34 (CH-*m*-Bz); 129.86 (CH-*i*-Bz); 130.01 (CH-*o*-Bz); 133.03 (CH-*p*-Bz); 133.30 (C-6); 134.35 (C-2); 149.07 (C-4); 179.07 (C=O).

N^4 -(Pivaloyloxy)cytidine (**2b**): For $\text{C}_{14}\text{H}_{21}\text{N}_3\text{O}_7$ MS: m/z 342.3 (M^+). M.p. 119–120 $^\circ\text{C}$. UV (MeOH): λ_{max} 277 (7100), 227 (7150). ^1H NMR (CD_3OD): 7.52 d, 1 H, $J_{5,6} = 8.1$ (H-6); 6.07 d, 1 H (H-5); 5.88 d, 1 H, $J_{1',2'} = 4.9$ (H-1'); 5.87 d, 1 H, $J_{1',2'} = 4.4$ (H-2'a); 4.18 m, 2 H (H-2'b, H-3'); 4.02 m, 1 H (H-4'); 3.8 dd, 2 H, $J_{\text{gem}} = 12.7$, $J_{5'a,4'} = 3.86$ (H-5'a); 3.76 dd, 2 H, $J_{5'b,4'} = 3.7$ (H-5'b); 1.22 m, 9 H (3 CH_3). ^{13}C NMR (CD_3OD): 26.83 ((CH_3)₃); 39.75 (C(CH_3)₃); 61.09 (C-5'); 70.01 (C-2'); 72.99 (C-3'); 87.38 (C-4'); 91.27 (C-1'); 96.51 (C-5); 133.92 (C-6); 136.35 (C-2); 149.05 (C-4); 187.12 (C=O).

N^4 -(Adamantane-1-carbonyl)oxy]cytidine (**2c**) and N^4 -(Triphenylacetoxy)cytidine (**2d**)

1,1'-Carbonyldiimidazole (81 mg, 0.5 mmol) was added to a solution of adamantane-1-carboxylic or triphenylacetic acid (0.33 mmol) in DMF (5 ml) and the mixture was kept at $20\text{ }^\circ\text{C}$ for 1 h, then the mixture was added to a precooled ($-20\text{ }^\circ\text{C}$) solution of **1** (78 mg, 0.3 mmol) in DMF (3 ml). After 20 h at $4\text{ }^\circ\text{C}$, the solvent was evaporated and the residue was partitioned between chloroform (20 ml) and water (5 ml). The organic layer was washed with water (5 ml), dried over anhydrous Na_2SO_4 , and evaporated. The residue was dissolved in chloroform (0.5 ml), applied onto a silica gel column ($2.5 \times 9\text{ cm}$), and eluted with a gradient of methanol in chloroform (0→7%, 300 ml). The target fractions were evaporated in vacuum to give 68 mg of **2c** (54%) or 94 mg of **2d** (59%) as a white foam.

N^4 -[(Adamantane-1-carbonyl)oxy]cytidine (**2c**): For $C_{20}H_{27}N_3O_7$ (421.2) MS: m/z 420 (M^+). UV (MeOH): λ_{\max} 276 (7200), 230 (7250). 1H NMR: ($CDCl_3$): 9.75 s, 1 H (3-NH); 7.05 d, 1 H, $J_{6,5} = 8.1$ (H-6); 5.82 d, 1 H, $J_{1',2'} = 5.6$ (H-1'); 5.8 d, 1 H (H-5); 4.27 t, 1 H, $J_{2',1'} \sim {}^3J_{2',3'} = 5.6$ (H-2'); 4.17 t, 1 H, $J_{3',2'} \sim {}^3J_{3',4'} = 5.6$ (H-3'); 4.04 m, 1 H (H-4'); 3.7 dd, 2 H, $J_{\text{gem}} = 12.7$, ${}^3J_{5'a,4'} = 3.1$ (H-5'a); 3.61 dd, 2 H, $J_{5'b,4} = 4.4$ (H-5'b); 1.94 m, 15 H (Ada).

N^4 -(Triphenylacetoxycytidine (**2d**): For $C_{29}H_{27}N_3O_7$ (529.6) MS: m/z 528 (M^+). UV (MeOH): λ_{\max} 270 (7400), 228 (7300). 1H NMR: ($CDCl_3$): 9.45 s, 1 H (3-NH); 7.46 d, 1 H, $J_{6,5} = 8.06$ (H-6); 7.2 m, 15 H (3 C_6H_5); 6.81 d, 1 H (H-5); 5.86 d, 1 H, $J_{1',2'} = 5.0$ (H-1'); 4.27 t, 1 H, $J_{2',1'} \sim J_{2',3'} = 5.2$ (H-2'); 4.16 t, 1 H, $J_{3',2'} \sim J_{3',4'} = 5.1$ (H-3'); 4.12 m, 1 H (H-4'); 3.82 dd, 2 H, $J_{\text{gem}} = 12.7$, $J_{5'a,4'} = 3.8$ (H-5'a); 3.76 dd, 2 H, $J_{5'b,4'} = 4.2$ (H-5'b).

N^4 -Hydroxycytidine 5'-(Ethoxycarbonyl)phosphonate (**4a**), N^4 -Hydroxycytidine 5'-Phosphonate (**4b**) and N^4 -Hydroxy-5'-*O*-(phosphonomethyl)cytidine (**4c**)

A solution of hydroxylamine hydrochloride (174 mg, 2.5 mmol) in water (3 ml, pH 6.0) was added to the solution of corresponding cytidine derivative **3a–3c** (0.5 mmol) in water (3 ml). The mixture was kept at 36 °C for 24 h, then concentrated to 3 ml. The residue was applied onto a LiChroprep RP-18 column (2 × 17 cm) and eluted in a linear gradient of MeOH (0–5%, 600 ml) in aqueous 0.01 M NH_4HCO_3 . The target fractions were evaporated, co-evaporated with water (2 × 5 ml), the residue was dissolved in water (5 ml) and freeze-dried to yield 148 mg (72%) of **4a**, 121 mg (71%) of **4b** or 153 mg (83%) of **4c** as ammonium salts.

N^4 -Hydroxycytidine 5'-(ethoxycarbonyl)phosphonate (**4a**): For $C_{12}H_{18}N_3O_{10}P$ (395.3) MS: m/z 394 (M^+). UV: (H_2O , pH 2) λ_{\max} 282 (10400), 221 (8990); (H_2O , pH 7) λ_{\max} 271 (8100), 235 (8400). 1H NMR (D_2O): 6.88 d, 1 H, $J_{6,5} = 8.4$ (H-6); 5.64 d, 1 H, $J_{1',2'} = 6.5$ (H-1'); 5.51 d, 1 H (H-5); 4.04–4.00 m, 2 H (H-2', H-3'); 3.97–3.95 m, 2 H (CH_2CH_3); 3.93 m, 1 H (H-4'); 3.91–3.87 m, 2 H (H-5'); 1.18 t, 3 H, $J_{CH_2,CH_3} = 7.1$ (CH_2CH_3). ^{31}P NMR (D_2O): -4.51 s.

N^4 -Hydroxycytidine 5'-phosphonate (**4b**): For $C_9H_{14}N_3O_8P$ (323.2) MS: m/z 322 (M^+). UV: (H_2O , pH 2) λ_{\max} 282 (10300), 222 (8950); (H_2O , pH 7) λ_{\max} 269 (8150), 235 (8600). 1H NMR (D_2O): 6.85 d, 1 H, $J_{5,6} = 8.1$ (H-6); 6.49 d, 1 H, $J_{H,P} = 641$ (H-P); 5.52 d, 1 H (H-5); 5.64 d, 1 H, $J_{1',2'} = 6.5$ (H-1'); 4.05–3.99 m, 2 H (H-2', H-3'); 3.92 m, 1 H (H-4'); 3.83–3.73 m, 2 H (H-5'). ^{31}P NMR (D_2O): 7.12 s. ^{13}C NMR (D_2O): 60.48 d, $J_{C,P} = 2.28$ (C-5'); 67.46 (C-2'); 69.85 (C-3'); 80.28 d, $J_{C,P} = 7.63$ (C-4'); 85.69 (C-1'); 96.27 (C-5); 128.62 (C-6); 144.11 (C-2); 148.64 (C-4).

N^4 -Hydroxy-5'-*O*-(phosphonomethyl)cytidine (**4c**): For $C_{10}H_{16}N_3O_9P$ (353.2) MS: m/z 352 (M^+). UV: (H_2O , pH 2) λ_{\max} 281 (10500), 221 (9050); (H_2O , pH 7) λ_{\max} 271 (8200), 235 (8500). 1H NMR (D_2O): 7.40 d, 1 H, $J_{6,5} = 5.3$ (H-6); 5.72 m, 2 H (H-5, H-1'); 4.17 t, 1 H, $J_{2',1'} = 5.3$, $J_{2',3'} = 5.3$ (H-2'); 4.10 t, 1 H, $J_{3',2'} = 4.4$, $J_{3',4'} = 5.0$ (H-3'); 4.00 d, 1 H, $J_{4',3'} = 3.1$ (H-4'); 3.75 dd, 1 H, $J_{\text{gem}} = 11.2$, $J_{5'a,4'} = 2.5$ (H-5'a); 3.65 dd, 1 H, $J_{5'b,4'} = 3.4$ (H-5'b); 3.55 d and 3.57 d, 2 H, $J_{Ha,P} = J_{Hb,P} = 9.0$ (PCH_2O). ^{31}P NMR (D_2O): 15.94 s. ^{13}C NMR (D_2O): 68.72 d, $J_{C,P} = 156$ (C-P); 70.46 (C-2'); 72.37 (C-3'); 73.82 d, $J_{C,P} = 9.8$ (C-5'); 83.41 (C-4'); 86.89 (C-1'); 98.33 (C-5); 130.28 (C-6); 143.46 (C-2); 149.45 (C-4).

The *in vitro* cytotoxicity and antiviral effects of synthesized compounds in Vero, and LLC-MK2 kidney cell cultures infected with viruses of pox virus family (vaccinia, monkeypox and cowpox viruses) were determined using a neutral red uptake assay as described previously¹⁰. The data were plotted and analyzed by using the software program (Molecular Devices, Menlo Park, U.S.A.).

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REFERENCES

1. Barnard D. L., Hubbard V. D., Burton J., Smee D. F., Morrey J. D., Otto M. J., Sidwell R. W.: *Antivir. Chem. Chemother.* **2004**, *15*, 15.
2. a) Stuyver L. J., Whitaker T., McBrayer T., Hernandez-Santiago B. I., Lostia S., Tharnish P. M., Ramesh M., Chu C., Jordan R., Shi J., Rachakonda M., Watanabe K. A., Otto M. J., Schinazi R. F.: *Antimicrob. Agents Chemother.* **2003**, *47*, 244; b) Hollecker L., Shi J., Du J., Wang P., Hassan A., Rachakonda S., Patterson S. E., Khan N., Hobbs A., Clark J. L., Chun B.-K., Iyodogan P., Lostia S., Mcbrayer T. R., Tharnish P. M., Otto M. J., Stuyver L. J., Schinazi R. F., Pankeiwicz K. W. in: *Frontiers in Nucleosides and Nucleic Acids* (R. F. Schinazi and S. E. Patterson, Eds), p. 319. IHL Press, Tucker, U.S.A. 2004.
3. a) Parang K., Wiebe L. I., Knaus E. E.: *Curr. Med. Chem.* **2000**, *7*, 995; b) Wan W. B., Beadle J. R., Hartline C., Kern E. R., Ciesla S. L., Valiaeva N., Hostetler K. Y.: *Antimicrob. Agents Chemother.* **2005**, *49*, 656.
4. Mertes M. P., Smrt J.: *Collect. Czech. Chem. Commun.* **1968**, *33*, 3304.
5. a) Krayevsky A. A., Watanabe K. A.: Modified Nucleosides as Anti-AIDS Drugs: Current Status and Perspectives, *Bioinform Press* **1993**; b) Krayevsky A. A.: *Mol. Biol. (Moscow)* **1999**, *33*, 295; c) Machado J., Salomon H., Oliveira M., Tsoukas C., Krayevsky A. A., Wainberg M. A.: *Nucleosides Nucleotides* **1999**, *18*, 901; d) Yurin O. G., Krayevsky A. A., Afonina L. Yu., Balaganin V. A., Burova N. V., Voronin E. E., Kolesnik A. N., Molodov I. B., Moshkovich G. F., Pokrovskii V. V.: *Epidemiol. Infect. Dis.* **2001**, *43*; e) Krayevsky A. A., Tarusova N. B., Zhu Q.-Y., Vidal P., Chou T.-C., Baron P., Polsky B., Jiang X.-Y., Matulic-Adamic J., Rosenberg I., Watanabe K. A.: *Nucleosides Nucleotides* **1992**, *11*, 177.
6. a) Holy A.: *Curr. Pharm. Design* **2003**, *9*, 2567; b) Holý A.: *Collect. Czech. Chem. Commun.* **1993**, *58*, 649; c) De Clercq E.: *Clin. Microbiol. Rev.* **2003**, *16*, 569; d) Naesens L., De Clercq E.: *Nucleosides Nucleotides* **1997**, *16*, 983; e) Bray M., Roy C. J.: *Antimicrob. Chemother.* **2004**, *54*, 1.
7. a) Jasko M., Shipitsin A., Shirokova E., Krayevsky A., Polsky B., Baron P., MacLow C., Ostrander M., O'Hara B.: *Nucleosides Nucleotides* **1993**, *12*, 879; b) Shirokova E. A., Jasko M. V., Shipitsin A. V., Krayevsky A. A., Ostrander M., O'Hara B., Baron P., MacLow C., Polsky B.: *Mol. Biol. (Moscow)* **1994**, *28*, 224; c) Yasko M. V., Novikov, N. A., Tarusova N. B.: *Bioorg. Khim.* **1994**, *20*, 50; d) Holý A., Rosenberg I.: *Collect. Czech. Chem. Commun.* **1982**, *47*, 3447.
8. Budowsky E. I., Sverdlov E. D., Shibaeva R. S., Monastyrskaya G. S., Kochetkov N. K.: *Biochim. Biophys. Acta* **1971**, *246*, 300; b) Janion C.: *Mutat. Res.* **1978**, *56*, 225.
9. Aleksandrova L. A., Andronova V. L., Karpenko I. L., Skoblov Yu. S., Adani A., Galegov G. A.: *Bioorg. Khim.* **2002**, *28*, 455.
10. Baker R. O., Bray M., Huggins J. W.: *Antiviral Res.* **2003**, *57*, 13.
11. Kukhanova M. K., Shirokova E. A.: *Frontiers in Nucleosides and Nucleic Acids* (R. F. Schinazi and Patterson S. E., Eds), p. 339. IHL Press, Tucker, U.S.A. 2004.