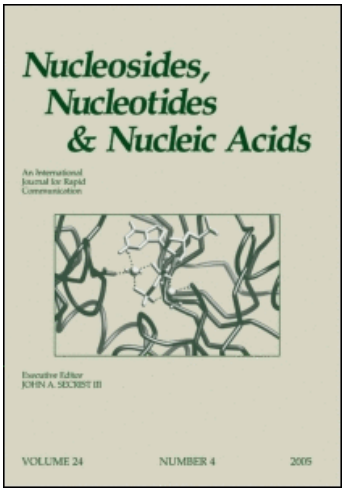

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P-(Alkyl)-Nucleoside 5'-Hydrogenphosphonates as Depot Forms of Antiviral Nucleotide Analogues

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NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 19(10–12), 1795–1804 (2000)

P-(ALKYL)-NUCLEOSIDE 5'-HYDROGENPHOSPHONATES AS DEPOT FORMS OF ANTIVIRAL NUCLEOTIDE ANALOGUES

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ABSTRACT. *P*-(Alkyl)esters of AZT 5'-hydrogenphosphonate were synthesized and their stabilities in the phosphate buffer and human serum were evaluated. The esters bearing residues of primary and secondary alcohols were degraded to give AZT, whereas those containing tertiary alkyl groups yielded AZT 5'-hydrogenphosphonate. The corresponding derivatives of d2A and d4T showed the same properties.

Abbreviations: Ada, adamantyl-1; AZT, 3'-azido-3'-deoxythymidine; d2A, 2',3'-dideoxyadenosine; d4T, 2',3'-didehydro-2',3'-dideoxythymidine

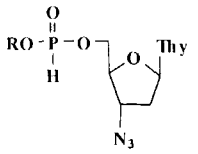
INTRODUCTION

Numerous attempts have been made to synthesize precursors of nucleoside 5'-phosphates to circumvent the dependence of nucleoside analogues, such as AZT [1, 2], d2A and 2',3'-didehydro-2',3'-dideoxyadenosine [3], d4T [4-6], and 3'-fluoro-3'-deoxyuridine [7], upon nucleoside kinases. The approach used was based on the preparation of neutral lipophilic phosphorotriesters that might penetrate into cells by passive diffusion and revert by chemical and enzymatic hydrolysis to the ionic nucleoside 5'-phosphate.

It was recently reported that 3'-azido-3'-deoxythymidine 5'-hydrogenphosphonate (**I**) may be an attractive anti-AIDS drug displaying a higher selectivity index than that of

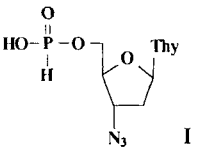
AZT [8-10]. The residue of phosphorous acid introduced prevents AZT from glucuronidation in blood [11]. Unlike AZT, hydrogenphosphonate **I** is responsible for a single codon mutation [8], which is a positive indication in regard of the potential of compound **I**.

AZT 5'-*H*-phosphonate (**I**) is less hydrophilic than the corresponding phosphate. It can penetrate into cells generating AZT 5'-monophosphate, the accumulation of the latter being slower in the cell than in the case of AZT [12]. However, the pathway of transformation of hydrogenphosphonate **I** into AZT 5'-monophosphate remains unclear. It may be either due to direct oxidation to the corresponding phosphate or hydrolysis to AZT followed by enzymatic phosphorylation.

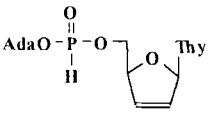


II a-i

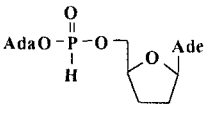
a R = Me; **b** R = Et; **c** R = Me_3CCH_2; **d** R = $PhCH_2$; **e** R = iso-Pr; **f** R = cyclohexyl; **g** R = cholesteryl; **h** R = *tert*-Bu; **i** R = Ada



I



II j



II k

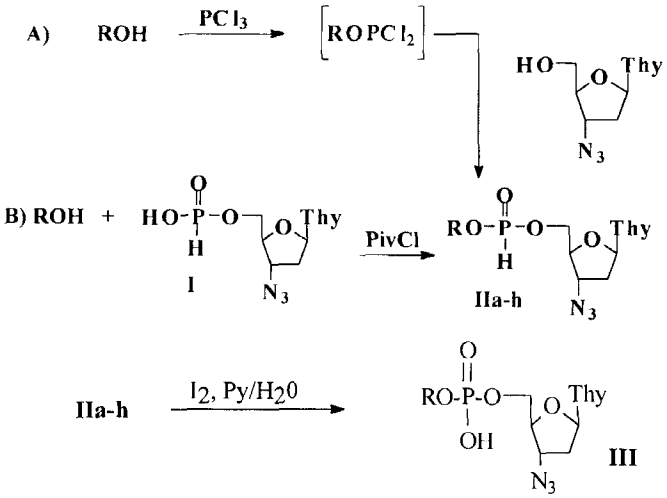
Hydrogenphosphonate **I** was shown to be stable to alkaline phosphatases and 5'-nucleotidases [12, 13]. The elucidation of the mode of action of **I** may be important for the development of other nucleotide-based antivirals, for example, for 5'-*H*-phosphonate of d2A, whose anti-HIV activity is higher than that of the parent nucleoside [8, 9].

Lipophilic esters of compound **I** as its metabolic precursors could be a useful tool for studying biochemical properties of nucleoside hydrogenphosphonates. *P*-alkyl derivatives of hydrogenphosphonate **I** bearing primary alcohol residues were earlier reported [14]. Some of them revealed higher activity in HIV-infected cells than parent **I**, probably, due to the better membrane permeability. However, chemical properties of these compounds and their stability in biological media have not been investigated. We synthesized a series of alkyl esters of AZT, d2A, and d4T hydrogenphosphonates (**IIa-k**)

bearing different types of alkyl substituents, studied their chemical hydrolysis, and determined their stability in human blood serum. In addition, we compared the ability to chemical oxidation of the esters obtained with that of parent hydrogenphosphonate **I**.

RESULTS AND DISCUSSION

We synthesized compounds of type **II** using two approaches. First, we simplified the procedure described in [14] for **IIa,b** carrying out a one-pot reaction of phosphorus trichloride with an alcohol in the presence of a base followed by the addition of a nucleoside as is shown in Scheme (A) for AZT derivatives. The second approach involved a coupling of the corresponding nucleoside 5'-*H*-phosphonate with an alcohol in the presence of pivaloyl chloride [Scheme (B)] [15, 16]. The yields of the products in both methods used were comparable and rather high (Table 1). Compounds **II** were purified on silica gel, and shown to be homogeneous using HPLC analysis.



Scheme

The structures of compounds **II** were confirmed by UV, mass, ^{1}H, and ^{31}P NMR spectra (Tables 1 and 2). In the ^{1}H NMR spectra of all the esters synthesized, clear doublets with coupling constants about 700 Hz were observed, which are typical for derivatives of phosphorous acid. The ^{31}P NMR spectra patterns contained two groups of signals (triplet doublets for **IIh-k** or two multiplets for **IIa-g**) corresponding to a 1 : 1 mixture of diastereomers. When proton decoupled, these signals were converted to two singlets (see Fig. 1).

TABLE 1. Yields and physicochemical properties of the esters **II** synthesized

Comp	R	Method	Yield, %	UV (MeOH), λ_{max}, nm	M/e, $[M]^+$
II a	Me	A B	67 48	265	345.3
II b	Et	B	74	265	359.3
II c	Me_3CCH_2	A	47	266	401.35
II d	$PhCH_2$	B	62	266	421.3
II e	iPr	B	84	265	373.3
II f	cyclohexyl	B A	62 52	265	413.4
II g	cholesteryl	B	70	265	699.9
II h	Me_3C	A	80	266	387.3
II i	adamantyl-1	B	67	266	465.9
II j	adamantyl-1	A	79	265	422.4
II k	adamantyl-1	B	68	257	431.3

TABLE 2. ^1H and ^{31}P NMR spectra data of the diesters synthesized

Com	^1H NMR of **II**, CD_3CN, δ, ppm (*J*, Hz) *[1]				^{31}P, CD_3CN, δ, ppm (proton decoupled)	
	H-6	5-Me	R	H-P (J_{H-P})	Comp **II**	Comp **III**
c	7.28 s, 7.31s	1.86 s	0.92 s (9H), 3.73 d, 3.70 d (*J* 6 Hz, 2H)	6.87 d (706), 6.85 d (705)	8.99 s, 8.40 s	1.73 s
d	7.48 s, 7.46 s	1.88 s	5.10 m (2H), 7.30 m (5H)	6.83 d (700)	8.75 s, 8.73 s	0.54 s
e	7.39 s, 7.38 s	1.87 s	1.35 d (*J* 6.3 Hz, 6H), 4.75 m (1H)	6.86 d (700)	10.9 s, 10.1 s	1.10 s
f	7.37 s	1.90 brs (5H)*[2]	1.28 m , 1.55 m, 1.73 m (8H)	6.87 d (697)	6.33 s, 6.18 s	0.58 s
g	7.76 s, 7.72 s	1.94 s	0.94-1.62 m (15H), 2.30-2.34 m (29H), 5.56 m (1H)	6.87 d (702)	6.57 s, 6.96 s	-0.39 s
h	7.39 s	1.85 s 1.86 s	1.5 s (9H)	6.90 d (697)	3.62 s, 3.96 s	-3.46 s
i*[3]	7.38 s, 7.37 s	1.96 s	2.16 brs (3H), 2.06 m (6H), 1.64 m (6H)	6.93 d (696)	3.36 s, 3.08 s	-3.58 s
j*[3]	7.32 s, 7.27s	1.93 s	2.16 brs (3H), 2.03 m (6H), 1.65 m (6H)	6.93 d (696), 6.91 d (696)	2.92 s, 2.04 s	-3.52 s

*[1] Spectra of compounds **IIa,b** were in coincidence with [14]. The signals of the carbohydrate moiety for all the compounds synthesized were typical.

*[2] Overlapped with the signals of two (2"a + 6"a) cyclohexyl protons

*[3] in $CDCl_3$

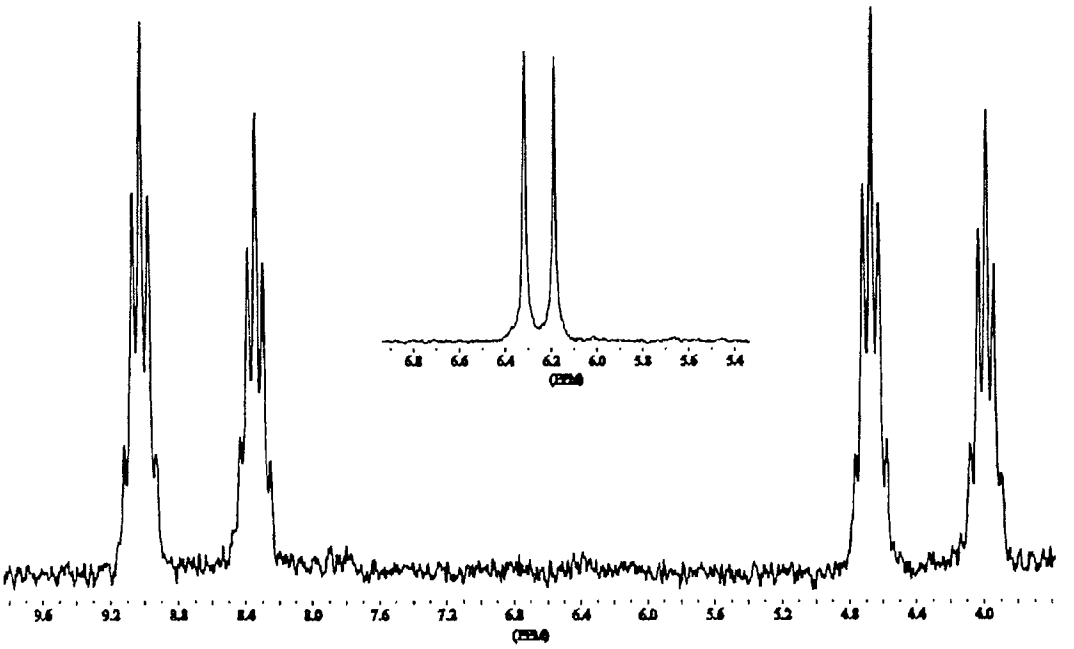


FIG.1 ^{31}P NMR patterns of ester **IIf**

Oxidation of phosphonates **I** and **II** was performed by I_2 in pyridine at room temperature (Scheme) [16]. In contrast to compound **I**, diesters **II** were oxidized very rapidly, the reaction being completed in 5-20 min *versus* > 24 h for **I**. The phosphates obtained were purified by chromatography on LiChroprep RP-8 and analyzed by NMR spectroscopy (Table 2).

To determine the degradation pathway of diesters **II**, we studied their behavior in the phosphate buffer (pH 7.5) and in human blood serum. The products were analyzed by TLC and HPLC (Table 3), the product composition correlating in both liquids. The data obtained demonstrated that diesters with primary and secondary aliphatic alkyl groups were cleaved to give solely AZT. Hydrolysis of **IIg** could not be studied because of its insolubility in water solutions. Another way of cleavage was observed for compounds **IIh-k** bearing tertiary alkyl substituents. The only nucleoside-derived products were the corresponding 5'-hydrogenphosphonates. Hence, stability of the compounds under study depended both on the nature of the alkyl group and properties of the leaving group,

TABLE 3. Stability of compounds **II**

Comp	Retention time, min	Half-life in buffer	Initial concentration in serum, M	Half-life in serum	Product
IIa	-	1 h	2×10^{-3}	2 min	AZT
IIb	-	1 h	„	15 min	AZT
IIc	38.52	18 h	„	2 h	AZT
IId	42.73	1.5 h	„	30 min	AZT + **I** (7:3)
IIe	36.48	7 h	„	1 h	AZT
IIf	41.78	8 h	„	3 h	AZT
IIh	40.32	0.5 h	„	5 min	**I**
IIi	47.41	1.5 h	5×10^{-4}	30 min	**I**
IIj	44.83	2 h	„	50 min	**I**
IIk	46.23	2 h	„	60 min	**I**
I	12.41	240 h	2×10^{-3}	50 h	AZT
AZT MP*	10.05	not determined	5×10^{-4}	50 min	AZT

which agreed well with the formation of two products, AZT and hydrogenphosphonate **I**, as was in the case of compound **IId**.

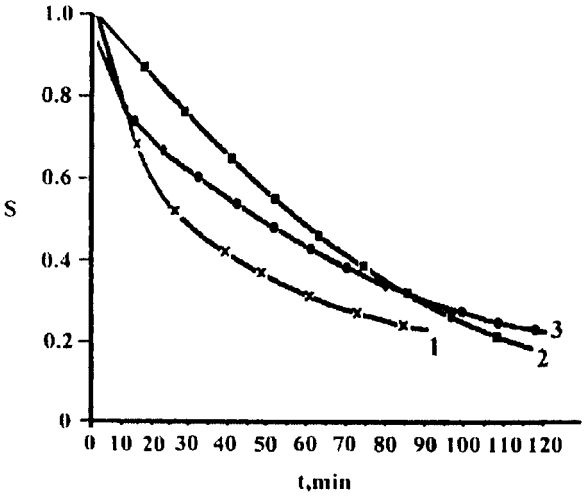
The cleavage in serum proceeded faster than in the buffer, although resulted in the same products. The hydrolysis rate also depended on the substituent introduced and was very high in the case of **IIa,b,h**. The profile of cleavage of compounds **IIi-k** in human serum is shown in Fig. 2.

The results obtained imply that nucleoside *H*-phosphonate esters may serve either as depot forms of antiviral nucleoside *H*-phosphonates or corresponding nucleosides, which can be governed by the type of the alkyl introduced.

The experiments in cell cultures are in progress.

EXPERIMENTAL

Pivaloyl chloride and phosphorus trichloride were from Fluka; pyridine was from Aldrich. *H*-Phosphonate of d2A was obtained as in [17]. The alcohols used for esterification were dried and distilled before use. Human serum was a kind gift of Dr. V.Chernikov (Institute of Gene Biology, RAMS). UV spectra were recorded on a Shimadzu UV-1201 spectrophotometer. ^{1}H and ^{31}P NMR spectra were registered on a



(1) **Ii**; (2) **IIj**; (3) **IIk** ; S, the amount of **II** at t min (%).

FIG. 2. Kinetic curves of cleavage of compounds **II** in blood serum.

Bruker WP-200 SY spectrometer (200 MHz) in CD_3CN if not stated otherwise. Mass spectra were registered on a COMPACT MALDI-4 (Kratos Analytical, United States) spectrometer. Column chromatography was performed on Kieselgel 60 (40-60 μ); TLC was carried out on Kieselgel 60 F254 plates (Merck) in chloroform-ethanol 95 : 5 (system A) or dioxane-25% aq. ammonia 4 : 1 (system B). For the quantitative analysis of hydrolysis, spots of starting compounds and products were cut out from TLC plates, eluted, evaporated, and the amount was determined by UV spectroscopy. HPLC was performed on a Gilson chromatograph using a Durasil S8 (ARO Company, Russia) column (13 μ, 4 x 150 mm) in a gradient of MeOH (0% for 5 min; 0→80% for 40 min; 80% for 5 min) in 5 mM potassium phosphate buffer (pH 6.0); flow rate 0.6 ml/min; detection at 265 nm.

***P*-(*tert*-Butyl)-3'-azido-3'-deoxythymidine 5'-hydrogenphosphonate (IIh).**

Method A. A solution of PCl_3 (135 mg, 88 μl, 1 mmol) in dichloromethane (2 ml) was cooled to 1-2°C, and *tert*-BuOH (75 mg, 96 μl, 1 mmol) and pyridine (80 μl, 1 mmol)

were added under stirring. A solution of AZT (130 mg, 0.5 mmol) in pyridine (80 μl) and acetonitrile (3 ml) was added at 4-5°C. The reaction mixture was stirred for 3 h at ambient temperature and quenched with a cooled solution of saturated $NaHCO_3$ (3 ml) and chloroform (5 ml). The organic layer was washed with water, dried with Na_2SO_4, and evaporated. The residue was purified on a Kieselgel column (2 x 25 cm) eluting with system A to give compound **IIh** (150 mg, 80%). ^{1}H NMR (CD_3CN), δ, ppm: 7.39s (1H, H6,), 6.15t (1H, *J* 6.0 Hz, H1'), 6.90d (1H, *J* 697 Hz, H-P), 4.35m (1H, H3'), 4.19m (2H, H5'), 4.01m (1H, H4'), 2.38t (2H, *J* 6.0 Hz, H2'), 1.85s, 1.86s (3H, CH_3), 1.50s (9H, *tert*-Bu). ^{31}P NMR (CD_3CN), δ, ppm: 3.95dt, 3.63dt (*J* 697; 7.6). For proton decoupled ^{31}P NMR, see Table 2.

***P*-(Adamantyl)-2',3'-dideoxyadenosine 5'-hydrogenphosphonate (IIk).**
Method B. A water solution of 2',3'-dideoxyadenosine 5'-hydrogenphosphonate Na-salt (70 mg, 0.25 mmol) was passed through a Dowex-50 (Py^+) column (1 x 5 cm) eluting with water. The eluate was evaporated to dryness and coevaporated with pyridine (3 x 2 ml). The residue was dissolved in MeCN (3 ml) and pyridine (1 ml), and adamantanol-1 (60 mg, 0.4 mmol) was added under stirring. The mixture was cooled to -10°C, and pivaloyl chloride (90 mg, 91 μl, 0.75 mmol) was added. Cooling was removed, and the mixture was stirred for 30 min. The mixture was diluted with chloroform (5 ml) and washed with a saturated solution of $NaHCO_3$ (3 ml) and water (3 x 3 ml). The organic layer was dried with Na_2SO_4, evaporated, and coevaporated with toluene. The crude product was purified on a Kieselgel column (15 x 2.5 cm) eluting with system A to give compound **IIk** (73 mg). For the yield and physicochemical data, see Table 1. ^{1}H NMR [$CDCl_3$, δ, ppm (*J*, Hz)]: 8.28 s, (H, 1H-2), 8.11 s, 8.07 s (1H, H-8), 6.88 d, 6.86 d (1H, 698, P-H), 6.29 m (1H, H-1'), 6.09 brs (1H, NH), 4.33 m (1H, H-4'), 4.24 m (2H, H-5'), 2.53 m (2H, H-2'), 2.20 m (2H, H-3'), 2.14-2.18 m (3H, adamantyl), 2.03 m (6H, adamantyl), 1.56 m (6H, adamantyl). ^{31}P NMR: CD_3CN, δ, ppm (*J*, Hz): 2.74 s, 2.53 s.

***P*-(Alkyl)nucleoside 5'-phosphates (III c-f, h-k).** Diesters **II** (0.1 mmol) were dissolved in 0.1 M solution of I_2 in pyridine-water 98 : 2 (2 ml) at 20°C. After 5-45 min (TLC control, system B), the solvents were evaporated, the residue was dissolved in water and loaded on a LiChroprep RP-8 column eluting with a gradient of MeOH in

water (0→20%). The target fractions were pooled and evaporated to give 80-86% of compounds **III**. ^{31}P NMR spectra data are given in Table 2.

Chemical hydrolysis of compounds II. Compounds **II** (15-20 mg) were dissolved in acetonitrile (0.25 ml) and added to the 0.1 M potassium phosphate buffer (pH 7.5, 0.25 ml) at room temperature. Aliquots were taken out and analyzed by TLC. The data are given in Table 3.

Hydrolysis of compounds II in human serum. Human serum (100%, 99 μl) was added to 100 mM solution of **II** in formamide (0.5 μl), and the mixture was incubated at 37°C. Aliquots (10 μl) were taken after certain intervals, acetone (40 μl) was added, the mixture was kept for 20 min at –20°C, and centrifuged. The supernatant was evaporated, the residue was dissolved in water (50 μl), and analyzed by HPLC or TLC (Table 3).

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REFERENCES

1. Lefebvre, I.; Perigaud, C.; Pompon, A., Aubertin, A.M.; Girardet, J.L.; Kirn A.; Gosselin G.; Imbach J.L. J. Med. Chem., **1995, *38***, 3941-3950.
2. Egron, D.; Lefebvre, I.; Perigaud, C.; Beltran T.; Pompon, A.; Aubertin, A.M.; Gosselin, G.; Imbach, J.L. J. Bioorg. Med. Chem. Letters, **1998, *8***, 1045-1050.
3. Balzarini, J.; Kruining, J.; Wedgewood, O.; Pannecooque, C.; Aquaro, S.; Perno, C.F.; Naezens, L.; Witvrouw, M.; Heijtink, R.; De Clercq, E.; McGuigan, C. FEBS Lett. **1997, *410*** (2-3), 324-328.
4. Balzarini, J.; Karlsson, A.; Aquaro, S.; Perno, C.F.; Cahard, D.; Naesens, L.; De Clercq, E.; McGuigan, C. Proc. Natl. Acad. Sci. USA **1996, *93***, 7295-7299.
5. Meier, C.; Lorey, M.; De Clercq, E.; Balzarini, J. J. Bioorg. Med. Chem. Letters, **1997**, *7*, 99-104.

6. Naesens, L.; Cahard, D.; Salgano, A.; Bidois, L., De Clercq, E.; McGuigan, C. Adv. Exp. Med. Biol. **1998**, ***431***, 753-757.
7. Farquhar, D.; Khan, S.; Srivastva, D.N.; Saunders, P.P. J. Med. Chem. **1994**, ***37***, 3902-3909.
8. Machada, J.; Salomon, H.; Oliveira, M.; Tsoukas, C.; Krayevsky, A.A.; Wainberg, M.A. Nucleosides & Nucleotides **1999**, ***18***, 901-906.
9. Tarussova, N.B.; Kukhanova, M.K.; Krayevsky, A.A.; Karamov, E.K.; Lukashov, V.V.; Kornilayeva, G.B.; Rodina, M.A.; Galegov, G.A. Nucleosides & Nucleotides **1991**, ***10***, 351-354.
10. Krayevsky, A.A.; Tarussova, N.B.; Lhu, Q.U.; Vidal, P.; Chon, T.C.; Baron, P.; Polsky, B.; Jiang, X.I.; Matulic-Adamic, J.; Rosenberg, I.; Watanabe, K.A. Nucleosides & Nucleotides **1992**, ***11***, 177-196.
11. Jarchoan, R.; Mitsuya, H.; Myers, C.E.; Broder, S. New Eng. J. Med. **1989**, ***321***, 726-738.
12. Boal, J.H.; Iyer, R.P.; Egan, W.; Nucleosides & Nucleotides **1993**, ***12***(10), 1075-1084.
13. Kukhanova, M.K.; Tarussova, N.B.; Yasko, M.V.; Arzumanov, A.A.; Gudima, S.O.; Krayevsky, A.A.; Chidgeavadze, Z.G.; Beabealashvilli, R. Sh. Mol. Biologia (Russian), **1992**, ***26***, 1148-1159.
14. McGuigan, C.; Bellevergue, P.; Jones, B.C.N.M.; Mahmood, N.; Hay, A.I.; Petric, J.; Karpas, A.; Antiviral Chemistry and Chemotherapy, **1994**, ***5***(4), 271-277.
15. Froehler, B.C.; Ng, P.G.; Matteucci, M.D. Nucleic Acid Res. **1986**, ***14***, 5399-5407.
16. Kers,A.; Kers, I.; Kraszewski, A.; Sobkowski, M.; Szabo, T.; Thelin, M.; Zain, R.; Stawinski, J. Nucleosides & Nucleotides **1996**, ***15***, 361-378.
17. Matulic-Adamic, J.; Rosenberg, I.; Arzumanov, A.A.; Dyatkina, N.B.; Shirokova, E.A.; Krayevsky, A.A.; Watanabe, K.A. Nucleosides & Nucleotides **1993**, ***12***, 1085-1092.