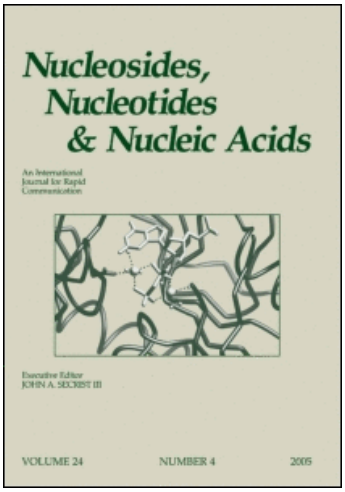

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5'-PHOSPHONATES OF RIBONUCLEOSIDES AND 2'-DEOXYRIBONUCLEOSIDES: SYNTHESIS AND ANTIVIRAL ACTIVITY

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Abbreviations used: HIV - human immunodeficiency virus; HSV - human herpes symplex virus; CMV - human cytomegalovirus; VV -vaccinia virus, FluA influenca A virus.

ABSTRACT: 5'-Phosphonates of natural 2'-deoxynucleosides and ribonucleosides were synthesized by condensation of 3'-O-acylated 2'-deoxynucleosides or 2',3'-substituted (2',3'-O-isopropylidene, 2',3'-O-methoxymethylene or 2',3'-O-ethoxymethylene) ribonucleosides. As condensing agents, either N,N'-dicyclohexylcarbodiimide or 2,4,6-triisopropylbenzenesulphonyl chloride were used. Nucleoside 5'-ethoxycarbonylphosphonates were converted into corresponding nucleoside 5'-aminocarbonylphosphonates by action of ammonia in methanol or aqueous ammonia. 5'-Hydrogenphosphonothioates of thymidine and 3'-deoxythymidine were obtained by reaction of phosphinic acid in the presence of pivaloyl chloride with 3'-O-acetylthymidine or 3'-deoxythymidine, respectively, followed by addition of powedered sulfur. 5'-O-methylenephosphonates of thymidine and 2'-deoxyadenosine were prepared by intramolecular reaction of corresponding 3'-O-iodomethylphosphonates under basic conditions. All compounds were tested for inhibition of several viruses, including HSV-2 and CMV, but showed no activity. A few compounds insignificantly inhibited HIV-1 reproduction. Thymidine 5'-hydrogenphosphonate neutralized anti-HIV action of 3'-azido-3'-deoxythymidine (AZT) and it indirectly showed that even some nucleoside 5'-phosphonates could be partly hydrolyzed in cell culture to corresponding nucleosides.

5'-Phosphonates of modified 2'-deoxynucleosides in which one group in a phosphate residue is substituted for hydrogen, alkyl or other groups, have shown to be potent biologically

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active agents. Some of them, such as 5'-phosphonates of 3'-azido-2',3'-dideoxynucleosides[1-4], 2',3'-dideoxynucleosides and others[4-6], prove to be effective inhibitors of HIV reproduction in cell cultures. All these compounds are modified at the carbohydrate moiety and their properties are determined by this modification alone. At the same time as research of DNA polymerases was proceeding, substrate analogs modified in triphosphate residues in cell-free systems with different DNA polymerases have demonstrated that a phosphate modification can be responsible for the inhibitory properties of nucleotide analogues[7,8]. This has also been shown for thymidine 5'-(α-methylphosphonyl)-β,γ-diphosphate and its 3'-substituted analogs[9,10]. The above mentioned modified thymidine phosphonate diphosphate shows the difference in specificity toward different DNA polymerases, being a strong inhibitor of retroviral reverse transcriptases. This was the impetus to attempt more detailed investigations into the activity of various 5'-phosphonates of natural ribo- and 2'-deoxyribonucleosides.

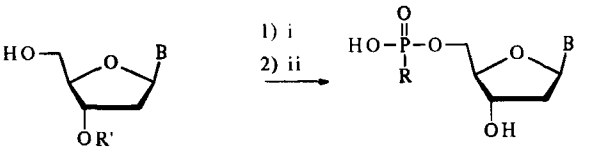
Published research[11] on ribonucleoside 5'-carboxymethylphosphonates showed a lack of inhibition of HSV-1 and HSV-2 reproduction in cell culture. Negative results were also obtained for adenosine and guanosine 5'-ethoxycarbonylphosphonates and 5'-aminocarbonylphosphonates, as well as 2'-deoxyadenosine and 2'-deoxyguanosine 5'-carboxyphosphonates[12] against HSV-1, HSV-2 and VV in cell culture. Adenosine 5'-methylphosphonate was, though, found to possess fairly high inhibitory activity against different RNA viruses *in vitro*, including VV, FluA, Semliki Forest virus, Coxsackie virus, and Columbia SK virus[13]. This compound also exhibited therapeutic activity inhibiting production of VV, FluA and especially mouse Semliki Forest virus[13], with concurrent low toxicities.

Another previously described series of 5'-phosphonates of nonmodified nucleosides are 5'-O-phosphonylmethylnucleosides. 5'-O-Phosphonylmethyl 2'-deoxynucleosides with uracil and thymine bases didn't inhibit HIV-1, HSV-1, HSV-2, VV and vesicular stomatitis virus production[14]. The same results were obtained for 5'-O-phosphonylmethylribonucleosides[15].

The present paper describes the synthesis of several types of nonmodified nucleoside 5'-phosphonates and investigation of their inhibition against HIV-1, HSV-2 and CMV in cell culture.

Chemistry

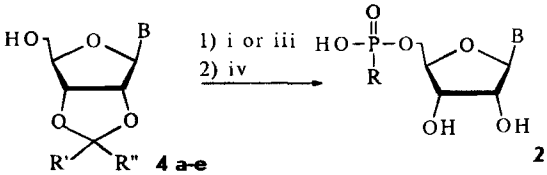
The syntheses of 2'-deoxyribonucleoside 5'-phosphonates (**1**) and ribonucleoside 5'-phosphonates (**2**) are carried out by condensation of corresponding nucleosides (**3** and **4**) and phosphonic acids in the presence of N,N'-dicyclohexylcarbodiimide (DCC) or 2,4,6-triisopropylbenzenesulphonyl chloride (TPSCl)[1,11](Schemes 1 and 2). 2'-Deoxynucleoside 5'-aminocarbonylphosphonates (**1d** and **1m**) are synthesized by methods previously described[16]. Ribonucleoside 5'-chloromethyl phosphonates (**2i** and **2g**) are obtained by reaction of **4a** and **4b** with chloromethylphosphonic dichloride according to method[17] (Scheme 3). Ribonucleoside 5'-aminocarbonyl phosphonates (**2k**, **2l**, **2m**, and **2n**) were prepared by the reaction of corresponding ribonucleoside 5'-ethoxycarbonyl phosphonates (**2i**, **2d**, **2f** and **2h**) in



3a-d **1a-o**

3a	R' =Ac	B =	Thy	1a	R=	Me	B = Thy
3b	Ac		Ade	**1b**		CH_2OH	Thy
3c	Ac		Cyt(N^4-Ac)	1c		COOH	Thy
3d	Bz		Gua	**1d**		$CONH_2$	Thy
				1e		H	Thy
				1f		Me	Ade
				1g		CH_2OH	Ade
				1h		COOH	Ade
				1i		COOEt	Ade
				1j		Me	Cyt
				1k		COOH	Cyt
				1l		COOEt	Cyt
				1m		$CONH_2$	Cyt
				1n		Me	Gua
				1o		COOH	Gua

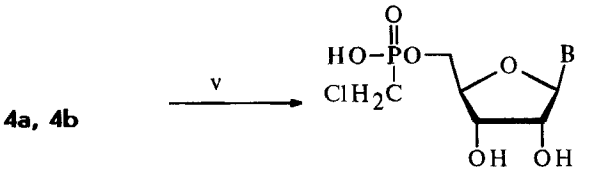
Scheme 1



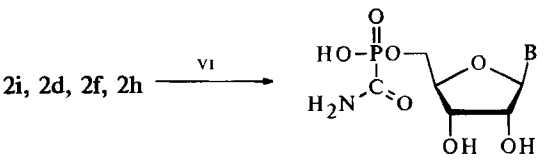
4 a-e **2 a-h**

4a	R'=	Me	R"=	Me	B=	Ura	2a	R=	Me	B=	Ura
4b		H		OMe		Ade	**2b**		COOEt		Ura
4c		Me		Me		Cyt(N^4-Bz)	2c		COOMe		Ura
4d		H		OEt		Cyt	**2d**		COOEt		Ade
4e		H		OMe		Gua	2e		Me		Cyt
							2f		COOEt		Cyt
							2g		Me		Gua
							2h		COOEt		Gua

Scheme 2



Scheme 3



Scheme 4

aqueous ammonia (Scheme 4). The reaction was completed in several hours and predominantly yielded the desired amide. Using methanolic or ethanolic ammonia solution we could isolate after 6-7 days only 20-30% of amide. Homogeneity of the prepared products was achieved by subsequent ion-exchange and reverse phase chromatography. The structure of the prepared compounds is proved by UV , NMR and mass-spectra (Tables 1,2).

5'-O-Methylenephosphonates of thymidine and 2'-deoxyadenosine (**5a,b**) are obtained by intramolecular replacement reaction of corresponding nucleoside 3'-O-iodomethylphosphonates (**6a,b**) in the presence of *t*-BuOK. This approach provides for high yields of the products. All physical chemical data of the synthesized phosphonates support the assigned structures[13] The prepared **5b** is the first example of 5'-O-methylenephosphonates of 2-deoxynucleosides with purine base.

Thymidine and 3'-deoxythymidine 5'-hydrogenphosphonothioates (**7a** and **7b**) are synthesized according to previously published methods[17] (Scheme 5). They are the only reaction products, isolated with an 80% yield. Deprotection of 3'-acetyl group occurred in the reaction mixture when 2 M triethylammonium bicarbonate buffer was added.The products are solids, stable in neutral and alkaline media. Their structure is proved by elemental analysis and spectral data. The spectrum ^{31}P NMR with ^{1}H-decoupling showed 1:1 ratio singlets of hydrogenphosphonothioate groups at 54.69 and 54.80 ppm for **7a** and 54.54 and 54.67 ppm for **7b**. The ^{1}H NMR spectrum showed a 1:1 ratio of two doublets at 7.97 ppm (J_{P-H}=591 Hz) and 7.99 ppm (J_{P-H}=592 Hz) for **7a** and at 7.92 ppm (J_{P-H}=592 Hz) and 7.93 ppm (J_{P-H}=593 Hz) for **7b**. The coupling patterns of signals of P and H atoms (Table 4) indicate that the reaction products are a mixture of diastereomers with chiral center at phosphorous that correlates with previous data[17].

Table 1. Yields and characteristics of the NH_4^+-salts of the prepared compounds

Compound	Reagent	Yield %	UV-spectra, $\lambda_{max}(\varepsilon)$ at pH 12 and 2, nm	Mol. mass	Mass spectra m/e
1a	$MeP(O)(OH)_2$	64	266 (8200) 266.5 (9050)	320	321
1b	$AcOCH_2P(O)(OH)_2$	66	266 (8100) 266.5 (8900)	336	337
1c	$EtOOCP(O)(OH)_2$	68	266 (7000) 266.5 (8800)	378	379
1d	$EtOOCP(O)(OH)_2$	57	266 (7000) 266.5 (9000)	349	350
1f	$MeP(O)(OH)_2$	52	259 (13900) 259.5 (13100)	329	330
1g	$AcOCH_2P(O)(OH)_2$	54	260 (14800) 259 (14300)	345	346
1h	$EtOOCP(O)(OH)_2$	41	261 (14800) 258 (13100)	359	360, 377
1i	$EtOOCP(O)(OH)_2$	64	261 (15000) 259 (13300)	387	388
1j	$MeP(O)(OH)_2$	44	271 (8800) 279 (11000)	305	306
1k	$EtOOCP(O)(OH)_2$	37	270 (9000) 278 (11950)	335	336, 353
1l	$EtOOCP(O)(OH)_2$	48	271 (10000) 279 (11800)	363	364
1m	$EtOOCP(O)(OH)_2$	31	270 (10900) 280 (12100)	334	335
1n	$MeP(O)(OH)_2$	39	259 (11400) 254 (11900)	345	346
2a	$EtOOCP(O)(OH)_2$	33	256 (11000) 255 (11850)	375	376, 393
2b	$MeP(O)(OH)_2$	72	262 (8100) 261.5 (9050)	322	323, 340
2c	$EtOOCP(O)(OH)_2$	57	263 (7000) 261.5 (9100)	380	381, 398
2d	$EtOOCP(O)(OH)_2$	65	260 (15000) 257 (13400)	403	404, 421
2e	$MeP(O)(OH)_2$	35	271.5(7200) 280 (9700)	321	322
2f	$EtOOCP(O)(OH)_2$	30	269 (8500) 278 (11000)	379	380, 397
2g	$MeP(O)(OH)_2$	44	259 (10700) 255 (11500)	361	362
2h	$EtOOCP(O)(OH)_2$	41	256 (11000) 254 (12000)	419	420
2i	$ClCH_2P(O)Cl_2$	58	262 (6800) 262.5 (8600)	356.5	357,359, 374,376
2j	$ClCH_2P(O)Cl_2$	71	260 (12200) 259.5(11300)	379.5	380,382
2k	-	78	264 (6700) 265.5 (8200)	351	352
2l	-	75	259(13200) 257 (14000)	374	375
2m	-	40	268(8700) 279(10900)	350	-
2n	-	67	260 (10900) 254 (12000)	380	381
5a	-	35	267 (8000) 267 (8800)	336	337
5b	-	28	259 (14100) 258 (13600)	345	346
6a	$ICH_2P(O)(OH)_2$	60	266 (7900) 267 (8600)	446	447
6b	$ICH_2P(O)(OH)_2$	55	59 (14300) 257 (13700)	455	456
7a	$NH_4H_2PO_2$	69	267 (6900) 267 (8600)	322	323
7b	$NH_4H_2PO_2$	81	267 (7000) 268 (9800)	306	307

Table 2. ^{1}H-NMR spectra of the synthezised phosphonates [D_2O, δ, ppm (J Hz)]

Com-pound	Protons					
	1'	3'	4'	5'	2'	Phosphonate moiety protons
1a	6.30t(7)	4.50-4.65m	4.02-4.23m		2.30-2.49 m	1.35d(16)(CH_3)
1b	6.29t(7)	4.51-4.61m	4.06-4.22m		2.31-2.49m	3.77d (7)(CH_2)
1c	6.31t(7)	4.51-4.67m	4.06-4.24m		2.29-2.49m	-
1d*	6.18t(7)	4.22-4.36m	3.80-4.02m		1.96-2.2m	7.3br.s(NH_2CO)
1f	6.37t(7.5)	4.66-4.76m	3.98-4.14m	2.52-2.94m	2.61-2.73m	1.34d(16)(CH_3)
1g	6.47t(7)	4.75-4.83m	4.31-4,37m	4.11-4.23m	2.61-2.73m 2.79-2.82m	3.77d(7)(CH_2)
1h	6.38t(7)	4.67-4.37m	4.05-4.22m	2.55-2.89m	2.55-2.89m	
1i	6.41t(7)	4.70-4.77m	4.26-4,32m	4.15-4,20m	2.57-22.77m 2.68-2.90m	4.04-4.15m (CH_2);1.16dt (7.5;1)(CH_3)
1j	6.26t(6.5)	4.45-4.63m	4.14-4.26m	3.99-4.14m	2.22-2.58m	1.33d(16)(CH_3)
1k	6.26t(7)	4.48-4.66m	4.05-4.33m		2.20-2.59m	
1l	6.25t(7)	4.49-4.67m	4.15-4.35m		2.26-2.62m	4,25m(CH_2);1.33 dt(7.5;1)(CH_3)
1m*	6.15t(7)	4.22-4.30m	3.86-3.94m		1.82-2.01m 2.05-2.16m	7.2br.s.(NH_2);7.3 br.s.(NH_2CO)-
1n	6.27t(7)	4.69-4.29m	4.22-4.29m	4.01-4.08m	2.53-2.64m 2.75-2.88m	1.3d(16.5)(CH_3)
1o	6.34dd	4.76-4.85m	4.28-4.34m	4.13-4.19m	2.52-2.63m 2.78-2.90m	-
2a	5.81d(2)	4.80-4.90m	3.90-4.05m		4.7m	1.39d(16)(CH_3)

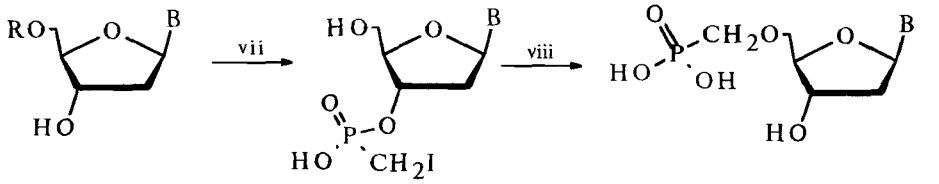
Table 2 Continued.

2b	6.01d(4)	4.25-4.40m				4.25-4.4m (CH_2);1.31dt(7; 1) (CH_3)
2c	6.01d(4.5)	4.36-4.40m	4.25-4.35m		4.36-4.40m	-3.83d(1)(CH_3)
2d	6.00d(5)	4.10-4.50m				4.1-4.5m(CH_2) 1.25dt(7.5;1) (CH_3)
2e	5.95d(3)	5.2m	4.10-4.35m		5.0m	1.35d(16)(CH_3)
2f	6.0d(3.5)	4.2-4.3m				4.2-4.3m(CH_2) 1.3dt(7;1.5)(CH_3)
2g	5.98d(5)	4.8m	4.38d(4)	4.1-4.2m	4.50-4.60m	1.34d(15)(CH_3)
2h	5.88d(5)	4.6-4.8m	4.1-4.4m	4.6-4.8m		4.1-4.2m(CH_2) 1.2dt(7.5;1)(CH_3)
2i	6.05d(5)	474m	4.3m	4.2m	4.45m	3.6d(10)($ClCH_2$)
2j	6.09d(5)	4.65d	4.36-4.42m	4.19-4.25m	4.55m	3.45d(10) ($ClCH_2$)
2k*	5.80d(5)	4.00-4.10m	3.85-3.95m		4.00-4.10m	7.1br.s.(NH_2CO)
2l*	5.98d(5)	4.65dd (6;4)	4.05-4.40m		4.3dd(6;4)	7.2br.s. (NH_2);7.36br.s (NH_2CO)
2m*	5.92d(3)	4.2-4.3m	4.05-4.15m		4.2-4.3m	7.2br.s.(NH_2CO)
2n	5.96d(5)	4.6-4.8m	4.15-4.4m		4.6-4.8m	7.2br.s.(NH_2CO)
5a*	6.26t(7)	4.52m	4.13m	3.84m	2.38m	3.72d(8.5)(O-CH_2-P)
5b	6.32t(6.5)	4.65m	4.25m	3.82m	2.44-2.92m	3.68d(8.6) (O-CH_2 -P)
6a	6.3d(7)	4.9m	4.26m	3.9m	2.54m	3.12d(9) (I-CH_2)
6b	6.23t(7)	5.02m	4.33m	3.87m	2.68m	3.13d(9)I-CH_2)
7a	6.42t(7)	4.65m	4.2-4.3m		2.45-2.5m	7.97d(591), 7.97d(592)(P-H)
7b	6.13-6.18m	2.1-2.5m	4.39-4.44d	4.15-4.21m	2.1-2.5m	7.92d(592), 7.93d(593)(P-H)

* Spectra were recorded in DMSO-d_6

Table 3. ^{31}P-NMR spectra of phosphonylmethylnucleosides **5a,b** and hydrogenphosphonothioathes **7a,b**

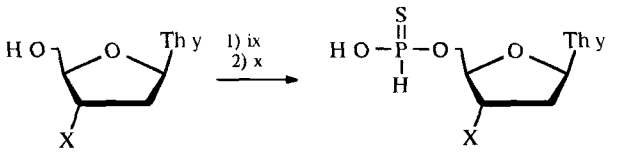
	δ, ppm	$^{1}J_{P-H}$, Hz	$^{2}J_{P-H}$, Hz	$^{3}J_{P-H}$, Hz
5a	15.8t		8.5	-
5b	15.7t		8.6	-
7a	54,68dt	591	-	8.8
	54.80dt	592		8.6
7b	54.54dt	592	-	9.1
	54.67dt	593		9.3



B= Thy R= CPh_3 **6a** B= Thy **5a** B=Thy

Ade *t*-$BuMe_2Si$ **6b** Ade **5b** Ade

Scheme 5



X= OAc, H **7a** X'= OH

7b H

(i) DCC/ Py, phosphonic acid (ii) NH_3/EtOH for **1i, l** and KOH/H_2O for others; (iii) TPSCl /Py , phosphonic acid for **2a, e**; (iv) 60% HCOOH; (v) $ClCH_2POCl_2$/$PO(OEt)_3$; (vi) aq NH_3; (vii) $ICH_2PO(OH)_2$, TPSCl/Py; (viii) *t*-BuOK/DMSO; (ix) $NH_4H_2PO_2$, PivCl/Py; (x) S_8.

Scheme 6

Table 4. Effect of some nucleoside 5'-phosphonates on AZT activity in H9 cells

	Percent Inhibition of HIV-1 with AZT, μM						ED_{50}+
	10	2	0.4	0.08	0.016	0.0032	
AZT	98	97	86	23	0	0	0.098
AZT+**2a***+100μM	99	98	89	68	16	0	0.047
AZT+**1e****+100 rM	96	30	3	0	0	0	2.33
AZT+**1d****+100 rM	98	96	96	6	23	3	0.191

+ ED_{50} -concentration at which the inhibition of HIV productionis equal to 50%.

*Compound **2a** inhibits HIV-1 production by 77% at 100 rM and 0% at 50rM;

****1e** and **1d** showed no inhibition of HIV-1 production.

Thymidine 5'-hydrogenphosphonate (**1e**) was synthesized according to methods previously described[18].

Antiviral activity

All synthesized compounds were tested as inhibitors against HIV-1 in H9 and peripheral blood lymphocytes (PBL) cells, HSV-2 in vero cells and CMV in HeLa cells, respectively.

With few exceptions none of the compounds inhibited HIV replication at concentrations to 100 rM. For the 2'-deoxynucleotides, 50% inhibition of viral replication by **1n** was 2-10 rM, in the ribonucleotides line the ED_{50} for **2i** was 1-10 rM, **2k** (1-10 rM), **2n** (1-10 rM) and **2m** (1-5 rM) in H9 cells. The ED_{50} of the AZT controls were approximately 0.1 rM in H9 cells and 0.001 rM in PBLs.

No activity up to concentrations of 200 rM has been obtained for both other above mentioned viruses with the exception of **1a** (50% inhibition of CMV reproduction is obtained at 48 rM). No marked toxicities were observed for any compound in all cell cultures up to concentrations of 200 rM (for H9 and PBL up to 1 mM).

Table 4 shows the effects of three nucleoside 5'-phosphonates on the activity of AZT on inhibition of HIV replication in H9 cells. As one can see from Table 4, compound **1e** strongly inhibits AZT activity. At the same time **2a** possesses the additive effect with AZT, and **1d** has an intermediate action.

Discussion

To date, the molecular mechanism of action of nucleoside 5'-phosphonates has not been investigated. Modified nucleosides such as 3'-azido-2',3'-dideoxynucleosides and others form corresponding 5'-triphosphates, incorporate into the 3'-termini of growing DNA chains and terminate their elongation thus inhibiting biosynthesis of proviral DNA. Not all modified nucleosides, however, can undergo triphosphorylation. This process is affected by specificity of corresponding phosphorylating enzymes[19-21]. Additionally, phosphorylating enzyme levels vary greatly in different types of cells. If this mechanism is possible for 5'-phosphonates of modified nucleosides, their phosphorylation to nucleoside 5'-(α–phosphonyl)-β,γ–diphosphates should be expected. The base, at least in one case, can incorporate into the growing DNA chain and inhibit its synthesis[10]. An alternative reaction route, however, can be suggested by hydrolysis of 5'-phosphonates of modified nucleosides to corresponding nucleosides catalyzed by cell phosphatases or phosphoesterases followed by routine anabolic triphosphorylation of nucleosides. However, higher activity of 5'-phosphonates or modified nucleosides (in some cases 50-100 fold higher) in comparison with corresponding nucleosides[4-6] contradicts this hypothesis. In addition, a number of reports find high stability of nucleoside 5'-phosphonates to different phosphatases[22,23]. Other mechanisms of nucleoside 5'-phosphonate action are also possible.

The lack of marked antiviral activity of the compound types **1** and **2** can be explained by their partial hydrolysis to corresponding nucleosides, the latter being transformed to natural nucleoside 5'-triphosphates completely compensates for the activity of probably formed nucleoside 5'-(α-phosphonyl)-β,γ-diphosphates. At the same time, hydrolysis has not fully occurred (at least for type **1** compounds) because an excess of any natural 2'-deoxynucleoside over the three other 2'-deoxynucleosides in a cell is bound to interrupt DNA replication on account of disturbances in the pool of four natural dNTPs. This would increase the toxicity of **1** in cell culture.

Table 4 shows indirect evidence to support this viewpoint. In the case of 2'-deoxynucleoside phosphonates **1e** and **1d** thymidine has to be obtained as a result of enzymatic hydrolysis as it competes with AZT. Hydrolysis of **1e** is probably deeper and therefore its influence on AZT action more powerful. The hydrolysis of **2a** produces uridine which does not compete with AZT. Moreover **2a** shows an additive effect of inhibition of HIV reproduction summarizing strong AZT and weak 2a action.

These data demonstrate the small chance to find highly active inhibitors of DNA virus and retrovirus reproduction among 2'-deoxynucleoside 5'-phosphonates as well as inhibition of RNA virus among the ribonucleoside 5'-phosphonates. Nevertheless, exceptions may be found, especially after proper development of research of hydrolyzing enzymes of human blood.

Materials and Methods

2',3'-Protected ribonucleosides and 3'-protected 2'-deoxyribonucleosides were synthesized according to methods previously reported[23,24]. Anhydrous pyridine, DMF and triethylphosphate were used. Methylphosphonic and alkoxycarbonylphosphonic acids were prepared from the corresponding dichlorides. TPSCl, DCC, pivaloyl chloride, *t*.-butyldimethylsilyl chloride were from Fluka, LiChroprep RP-18 (25-40μ) and Kieselgel 60 F_{251} were from Merck, DEAE-cellulose DE-32 from Whatman. System for TLC: isopropanol:NH_4OH: water 7:1:2 (v/v). UV-Spectra were registered on a Specord-M10 spectrophotometer, ^{1}H-NMR-spectra - on a Varian XL 100 15 and a Bruker 250, inner standard *t*-BuOH. ^{31}P-NMR Spectra were recorded on an MS-200 spectrophotometer, inner standard trimethylphosphate. FAB-Mass spectra were made on a Kratos MS 50TC mass-spectrometer. Samples were mixed with glycerol in the probe tip. Xenon was used for the fast atom gun at 8 keV.

General procedure of synthesis of 5'-phosphonates of 2'-deoxynucleosides (1a-d,f-o)

The pyridinium salt of the corresponding phosphonic acid (1.2 mmol) was dissolved in pyridine (3 ml) and the obtained solution was added into a solution of **3** (1 mmol) in pyridine (2 ml) and then DDC (2.5 mmol) was added with stirring. The reaction mixture was stirred for 10 hours at room temperature and than diluted with water (5 ml). Stirring was continued for 30 min and the residue was separated. The solution was evaporated and the remaining material was dissolved in 1M KOH (5 ml). In the cases of **1i** and **1l** ethanol saturated with ammonia (5 ml) was used. Solutions were kept at room temperature (3 hours for **1i**, 18 hours for **1l** or 5 days for **1d** and **1m**), and evaporated, residues were dissolved in water (100 ml), neutralized to pH 7.5 and purified by chromatography on a DEAE-cellulose (HCO_3^-) column (20 x 2.5 cm) with elution by a linear gradient (0-->0.15M) of ammonium bicarbonate buffer (pH 7.5), total volume 1l. The substances were eluted by 0.08-0.1 M buffer. Solutions were evaporated, reevaporated with water (5 x 5 ml), ethanol (3 x 5 ml) and were purified on LiChroprep RP-18 column (15 x 2.5 cm) with elution by water. Fractions with the desired substances were freeze-dried. Yields and characteristics are shown in Tables 1 and 2.

General procedure of synthesis of 5'-alkoxycarbonylphosphonyl ribonucleosides (2b,c,d, 2f and 2h)

A solution of the pyridinium salt of methoxy- or ethoxycarbonylphosphonic acid (1.2 mmol) in pyridine (3 ml) was added to nucleoside **4** (1 mmol) in pyridine (2 ml) to which DCC (2.5 mmol) was then added. The reaction mixture was stirred for 10 hours at room temperature and diluted with water (5 ml). Stirring was continued for 30 min and precipitate was removed. The solution was evaporated and remaining material in 60% HCOOH (10 ml)

was stirred at room temperature for 30 min. The solution was evaporated and the substance was purified by the same methods used for **1**. Yields and spectral data are given in Tables 1 and 2.

5'-Methylphosphonylguanosine (2g) was prepared from **4e** and methylphosphonic acid using the same procedure. Yield and spectral data are given in Tables 1 and 2.

General procedure of preparation of 5'-aminocarbonylphosphonyl ribonucleosides (2k-n)

The solution of ribonucleoside 5'-ethoxycarbonylphosphonate (1 mmol) in 25% aqueous ammonia (5 ml) was left at room temperature for 1 hour. The reaction mixture was evaporated and residue was applied onto a column (15 x 2 cm) with LiChroprep RP-18. Elution by water followed by freeze drying gave the desired aminocarbonylphosphonates. Yields and spectral data are shown in Tables 1 and 2.

Synthesis of 5'-methylphosphonates of uridine (2a) and cytidine (2e)

A solution of methylphosphonic acid (1 mmol) in pyridine (5 ml) was stirred with tri-n-butylamine (1.1 mmol) for 15 min, and evaporated. The residue was dissolved in pyridine (3 ml) and added to the 2',3'-O-isopropylideneuridine (**4a**) or 2',3'-O-isopropylidene-N^4-acetylcytidine (**3c**) (7 mmol) and TPSCl (1 mmol) in pyridine (3 ml). The reaction mixture was stirred for 12 hours and evaporated. The remaining material was kept in 60% HCOOH for 30 min at room temperature. Solvent was evaporated, water (10 ml) was added and precipitate was removed. Filtrate in the case of **2c** was diluted with 25% aqueous ammonia (5 ml), and after keeping for 10 hours evaporated. The crude product was purified on a column (16 x 2 cm) with DEAE-cellulose as described above. The phosphonate was eluted with 0.05-0.06 M buffer, evaporated, coevaporated with water as above and purified on a LiChroprep RP-18 column (15 x 2 cm). Yields and characteristics are shown in Tables 1 and 2.

Synthesis of 5'-chloromethylphosphonates of uridine and adenosine (2i and 2j)

To the suspension of **4a** or **4b** (0.3 mmol) in triethylphosphate (1ml), chloromethylphosphic dichloride (0.5 mmol) was added. The reaction mixture was left at 4°C for 20 hours and diluted with ether (10 ml). The precipitate was filtered, dissolved in water (5 ml) and kept at room temperature for 0.5 hour (pH 1.0). Water was evaporated, residue was reevaporated with water and purified on DEAE-cellulose and LiChroprep RP-18 columns as was described above. Yields and spectral data are shown in Tables 1 and 2.

Synthesis of 3'-O-iodomethylphosphonates of thymidine and 2'-deoxyadenosine (6a and 6b)

To the solution of 5'-O-triphenylmethylthymidine or 5'-O-*t*-butyldimethylsilyl-2'-deoxyadenosine (0.8 mmol) in dry pyridine (40 ml) the solution of iodomethylphosphonic acid

(1 mmol) in pyridine (5 ml) and TPSCl (1 mmol) were added. The reaction mixture was stirred for 3 hours at room temperature, diluted with water (20 ml) and evaporated to dryness. The residue was subsequently coevaporated with water (20 ml x 3), ethanol (20 ml x 3) and dichloroethane (20 ml x 3) and diluted with dichloroethane (30 ml). Then trifluoroacetic acid (1 ml) was added and the mixture was kept at room temperature for 3 hours and evaporated. The crude material was dissolved in water (200 ml), put onto a column (10 x 3 cm) with DEAE cellulose (HCO_3^-). The substance was eluted by a linear gradient (0--> 0.15M) of ammonium bicarbonate buffer, total volume 1l. Fractions with the desired product were evaporated, coevaporated with 10% ethanol (10 ml x 5) and freeze dried. Yields and spectral data are shown in Tables 1 and 2.

Synthesis of 5'-O-methylenephosphonates of thymidine and 2'-deoxyadenosine (5a and 5b)

To the solution of **6a** or **6b** (0.45 mmol) in dry DMSO (300 ml) *t*-BuOK (5.4 mmol) was added and the mixture was stirred for 24 hours at 37°C. Then cold water was added (100 ml), the resulting solution was neutralized with 0.1 M HCl and purified by column chromatography as it was described for **6a,b**. Yields and spectral data see in Tables 1 and 2.

Synthesis of hydrogenphosphonothioates of thymidine and 3'-deoxythymidine (7a and 7b)

Ammonium phosphinate (1 mmol) in water (5 ml) with Et_3N (1.2 mmol) was evaporated, twice coevaporated with pyridine, combined with 3'-O-acetylthymidine or 3'-deoxythymidine (1.5 mmol), again reevaporated with pyridine and dissolved in pyridine (5 ml). The reaction solution was cooled to 0°C and pivaloyl chloride (1.5 mmol) was added. After 10 min stirring, cooling was removed and powdered sulphur (1.5 mmol) was added. The reaction mixture was stirred at room temperature for 2 hours, diluted with 2 M triethylammonium bicarbonate buffer (pH 7.5), kept for 0.5 hour and evaporated. The residue was purified on DEAE-Cellulose and LiChroprep RP-18 as shown above. Yields and characteristics are shown in Tables 1-3.

Testing for anti-HIV effect in H9 cells

Compounds to be tested, including AZT and other appropriate controls, were diluted 5-10 fold in culture media, 3-6 dilutions total, and aliquoted into duplicate wells of 96-well microtiter plates. HIV-1 stock supernatant was incubated with H9 cells, washed and resuspended in culture media, aliquoted into wells and incubated 37 °C/7 days. At day 7, supernatants were harvested and tested for HIV-1 p24 antigen using a commercial viral capture assay (Dupont). Toxicity was assessed via the trypan-blue exclusion method for cell viability. Drug efficacy was determined by percent inhibition of HIV-1 reproduction for each drug concentration and the ED_{50} of each drug by calculations using data reduction softwear[27].

Testing for inhibition of CMV (strain AD 169) was made in human foreskin fibroblasts. Testing HSV type 2 inhibition involved a derivative of the Patton strain lacking the viral thymidine kinase gene and was carried out using Vero cells. Cells were plated on day 1 at $4x4.10^4$cells per well. On day 2, nucleoside analogues were added to give final concentrations (after addition of virus) ranging from 1 to 200 rM. Virus was then added to a multiplicity of infection of 0.01. Growth of virus was measured on day 3 (HSV) or 7 (CMV) using assays to be described (manuscript in preparation).

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