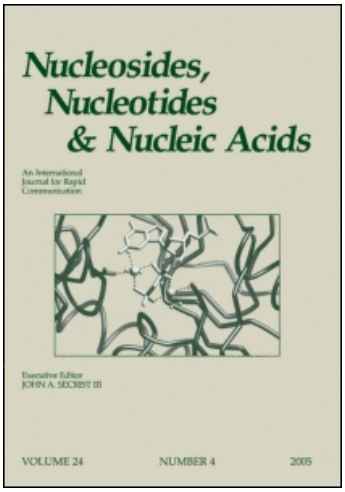

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 09 August 2003

To cite this Article Shirokova, Elena A. , Jasko, Maxim V. , Khandazhinskaya, Anastasiya L. , Yanvarev, Dmitry V. , Skoblov, Yury S. , Pronayeva, Tatyana R. , Fedyuk, Nina V. , Pokrovsky, Andrey G. and Kukhanova, Marina K.(2003) 'New Lipophilic Derivatives of AZT and d4T 5'-Phosphonates', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 981 — 985
To link to this Article: DOI: 10.1081/NCN-120022718
URL: http://dx.doi.org/10.1081/NCN-120022718

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 981–985, 2003

New Lipophilic Derivatives of AZT and d4T 5′-Phosphonates

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ABSTRACT

5′-Aminocarbonylphosphonyl and aminocarbonylmethylphosphonyl diesters of AZT and d4T were synthesized as potential anti-HIV agents.

Key Words: Anti-HIV agents; AZT and d4T derivatives; Nucleoside 5′-phosphonates.

Going on the studies of antiviral agents in the series of nucleotide derivatives, we paid attention on AZT conjugated with phosphonoformic (PFA) and phosphonoacetic (PAA) acids. Earlier diesters and triesters of type (**I**) were prepared.[1–3] Their antiviral effect was much higher than that of PFA and comparable with that of AZT. A poor increase in the efficacy could be due to high instability in water solutions of the triesters and poor penetration through cell membranes of the diesters.[3]

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DOI: 10.1081/NCN-120022718
1525-7770 (Print); 1532-2335 (Online)

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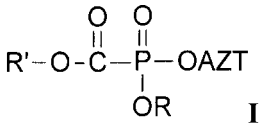
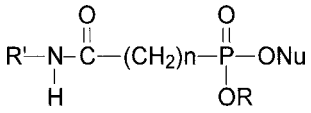


I

R = t-butyl-SATE, Et, long-chain alkyls
R' = H, Me, Et, AZT

We synthesized a set of uncharged 5′-aminocarbonyl- and 5′-aminocarbonylmethylphosphonate derivatives of AZT and d4T (**II–V**) and evaluated their activity in cell cultures infected with HIV-1.



AZT-derived				d4T-derived			
Comp	n	R′	R	Comp	n	R′	R
IIa	0	H	Et	**IVa**	0	H	Et
IIb	0	Me	Et	**IVb**	0	Me	Et
IIc	0	$PhCH_2CH_2$	Et	**IVc**	0	$PhCH_2CH_2$	Et
IIIa	1	H	Et	**Va**	1	H	Et
IIIb	1	H	cyclohexyl	–			
IIIc	1	Me	Et	–			

The target amides **II–V** were prepared by treatment of the corresponding 5′-ethoxycarbonylphosphonyl nucleosides with primary amines followed by esterification with various alcohols. Esterification was carried out using two procedures: in the presence of TPSCl and under Mitsunobu conditions. In the case of secondary alcohols, the first method was preferential, whereas for primary alcohols the second procedure resulted in higher yields.

The resulting compounds were mixtures of diastereoisomers (due to a chiral phosphorus atom). Isomers A and B of amide **IIa** were separated by reverse-phase chromatography, their retention times differing in about 2 min.

Chemical stability of the synthesized amides was estimated in 0.05 M phosphate buffer at 37°C. Figure 1 shows the stability of amide **IIa** under various pH. Obviously, it was strongly pH-dependent. In the range of pH 5.5–7.8 its half-life time decreased from 6 h to less than 30 min. At physiological pH 7.2–7.4 its half-life time was about 1 h.

The composition of the reaction mixture in the process of chemical hydrolysis of amide **IIa** was studied at pH 7.2 at 37°C. We observed the formation of two products, a major one being AZT and a minor one - 5′-aminocarbonylphosphonyl-AZT. The formation rate of the former was considerably higher than that of the latter (Fig. 2). The corresponding PAA-AZT analogue was degraded under similar conditions to give only the nucleoside.

As a whole, PAA derivatives in both series were noticeably more stable than the corresponding PFA ones (for example, 7 h for **IVa** vs. $\gg$ 24 h for **V**). d4T derivatives

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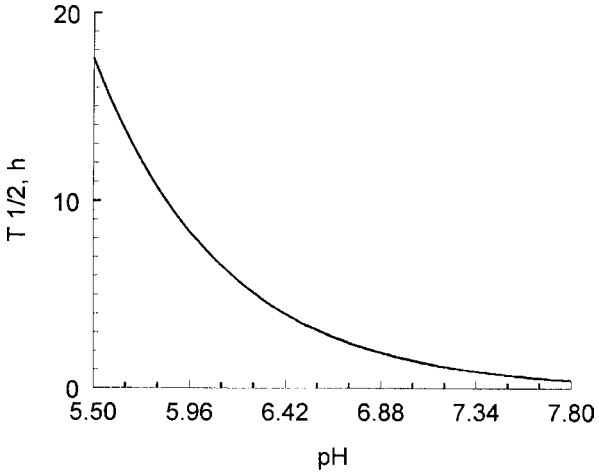


Figure 1. pH-Dependence of half-life time of amide **IIa**.

had longer half-lives than the corresponding AZT derivatives (7 h for **IVa** against 2.5 h for **IIa**). The substitution at the amide group increased the chemical stability (2.5 h for **IIa,** 6 h for **IIb** and >10 h for **IIc**).

Antiviral effect of the compounds under study was evaluated in MT-4 cells infected with HIV-1 strain GKV-4046 (Table 1). The tested compounds were added into the culture after the virus adsorption. The activity was measured by the amount of viral p24 antigen using immunoassay. Cytotoxicity was measured by the colorimetric method.

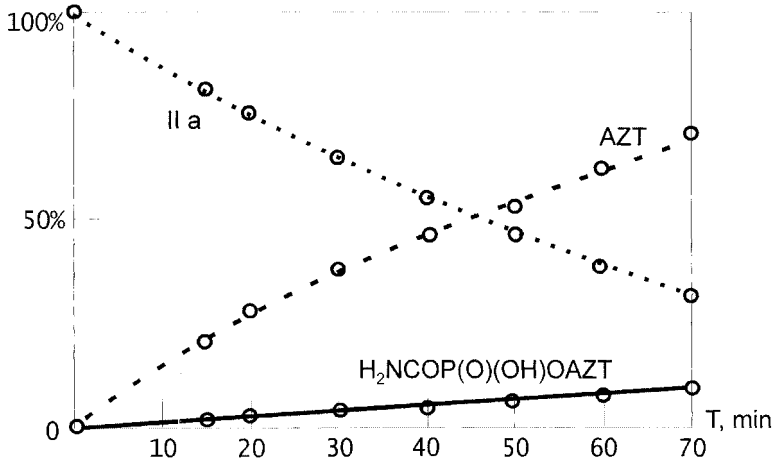
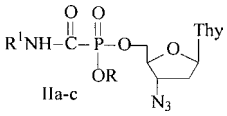
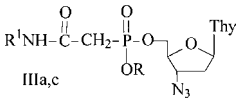
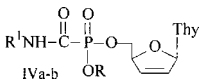
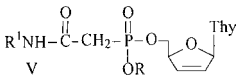


Figure 2. Chemical hydrolysis of amide **IIa** in phosphate buffer at pH 7.2.

Table 1. Anti-HIV properties of the synthesized compounds.

		CD_{50}* (μM)	ID_{50}** (μM)	ID_{90}** (μM)	IS***
 IIa-c	**IIa** R = Et, R^1 = H	298.3	0.0009	0.004	346800
	IIb R = Et, R^1 = Me	112.9	0.00024	0.007	470400
	IIc R = Et, R^1 = $PhCH_2CH_2$	177.7	< 0.0002	0.0002	> 888500
 IIIa,c	**IIIa** R = Et, R^1 = H	456.3	0.29	1.68	1573
	IIIc R = cyclohexyl, R^1 = H	170.1	0.0085	–	20012
 IVa-b	**IVa** R = Et, R^1 = H	573.5	0.9	2.1	637
	IVb R = Et, R^1 = Me	85.7	0.04	0.27	2187
 V	**V** R = Et, R^1 = H	267.9	5.4	18.8	50
AZT		187.5	0.018	0.35	11030
d4T		314	0.24	23.8	1308

*$\mathbf{CD_{50}}$ – Compound concentration required for 50% inhibition of cell proliferation.
**$\mathbf{ID_{50}}$ and $\mathbf{ID_{90}}$ – Compound concentration required for 50% or 90% inhibition of HIV reproduction, respectively.
***SI – selectivity index, $\mathbf{CD_{50}/ID_{50}}$.

As is seen from the table, this modification seems to be promising in the case of PFA-AZT series: as a rule a significant increase in the activity resulted in an increase of the selectivity index. For d4T analogues, this gain was not so obvious.

ACKNOWLEDGMENTS

The work was supported by ISTC, project 1244, and Russian Foundation for Basic Research, project 02-04-49009.

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