

# Antiviral Activity of Novel NRTI

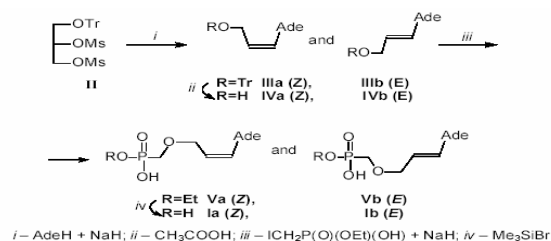
Edward Karamov<sup>\*1</sup>, Marina Kukhanova<sup>2</sup>, Galina Kornilaeva<sup>1</sup>, Tatiana Pavlova<sup>1</sup>, and Maxim Jasko<sup>2</sup>  
Ivanovsky Institute of Virology, Moscow, Russia<sup>1</sup> Engelhardt Institute of Molecular Biology, Moscow, Russia<sup>2</sup>

Edward Karamov (Karamov [2004@yandex.ru](mailto:2004@yandex.ru))

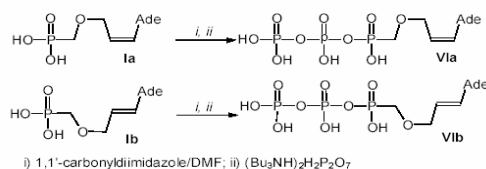
## Background:

We previously reported that phosphonate derivative of thymidin - Nikavir - a potent nucleotide reverse transcriptase inhibitor (NRTI), was active against a wide spectrum of HIV-1 isolates, including a variety of laboratory and drug-resistant HIV-1 strains *in vitro*. Nikavir possessed both high anti-HIV activity and low cytotoxicity (6-8-fold less toxic as compared to other NRTI *in vitro*). The compound has successfully passed all clinical trials in Russia and has been approved for the treatment of HIV infection. We evaluated some groups of novel NRTI, among them the acyclic nucleoside phosphonates. The acyclic nucleoside phosphonates are wellknown group of compounds, which demonstrate a broad spectrum of activity against retroviruses. Acyclic phosphonate derivatives of adenine (Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]adenine were selected in a search for novel NRTI bearing a double bond conjugated with the adenine base.

## A GENERAL SCHEME FOR SYNTHESIS OF (Z)- AND (E)-9-[3-(PHOSPHONOMETHOXY)PROP-1-EN-1-YL]ADENINE



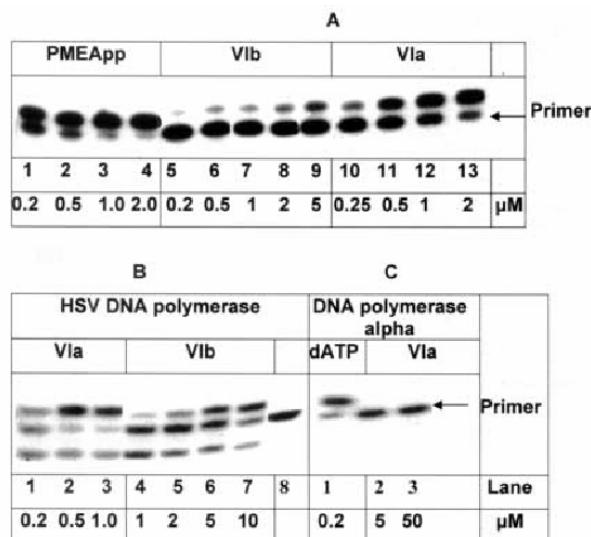
## A GENERAL SCHEME FOR SYNTHESIS OF DIPHOSPHATES (Z)- AND (E)-9-[3-(PHOSPHONOMETHOXY)PROP-1-EN-1-YL]ADENINE



## Methods:

(Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]adenine and their diphosphates were subjected to an *in vitro* evaluation of antiviral potency and toxicity. Cytotoxicity of compounds was defined using T-lymphoblastoid cell lines. Antiviral activity was profiled using CEMss cells and HIV-1 strains. The level of virus reproduction in infected cells was assessed as p24 production by p24 ELISA. Pharmacokinetics was evaluated on dogs and rabbits.

## PAAG OF DOSE-DEPENDENT INCORPORATION OF THE COMPOUNDS INTO THE 3'-END OF THE [5'-32P] PRIMER-TEMPLATE COMPLEX CATALYZED BY RT



(A): PMEApp (lanes 1-4), Vib (lanes 5-9) and Via (lanes 10-13); (B) by HSV DNA polymerase: Via(lanes 1-3) and Vib (lanes 4-7); (C) by human DNA polymerase alpha: Via(lanes 2-3). lane 8 shows the [5'-32p]-primer position; lane 1 presents the datpincorporation by DNA polymerase alpha.

## Results:

Both isomers (Z)- and (E)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]adenine inhibited HIV replication without apparent cellular toxicity up to 3300 μM, albeit with different efficiency. Z-isomer was about tenfold more effective inhibitor of HIV replication than E-isomer and displayed twice higher anti-HIV activity than that found for PMEA 9-[2-(phosphonomethoxy)ethyl]adenine (adefovir). All compounds possessed lower cytotoxicity as compared with adefovir and AZT. The selectivity index of Z-isomer exceeds that for PMEA by 2.5-3-fold. The T1/2 of both isomers were greater than T1/2 of AZT (3-10-fold).

## THE ANTIVIRAL EFFECT OF COMPOUNDS IA AND IB IN CELL CULTURES

Compound	Anti-HIV-1 activity in CEMss cell culture <sup>a</sup>			Anti-HSV-activity in Vero cell culture <sup>b</sup>					
				HSV-1/L <sub>2</sub>			HSV-1/ACV <sup>R</sup>		
	CD <sub>50</sub> , μM	ID <sub>50</sub> , μM	SI	CD <sub>50</sub> , μM	ID <sub>50</sub> , μM	SI	ID <sub>50</sub> , μM	SI	
Z-isomer (Ia)	>3300	81	>40	>3100	95	>34	106	>30	
E-isomer (Ib)	>3300	660	>5	>3100	387	>8	437	>7	
PMEA	2860	182	16	>344	80	>4.5	77	>4.5	

<sup>a</sup>Dose-response curves were plotted using six drug concentrations varied from 0.3 to 3500 μM, each in triplicate. <sup>b</sup>The data on the anti-HSV activity were published by us earlier (Ivanov et al., 2005). HSV-1/ACV<sup>R</sup>, acyclovir-resistant HSV strain.

## Conclusions:

Our results demonstrate that antiviral activity of (Z)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]adenine is comparable or even higher than that was established for PMEA, while E-isomer was about tenfold less active than Z-isomer. Both compounds were less toxic in cell cultures as compared with adefovir. These data warrant that (Z)-9-[3-(phosphonomethoxy)prop-1-en-1-yl]adenine be further developed as potential therapeutic for HIV infection and potential microbicide.

