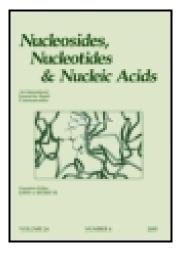
This article was downloaded by: [Tulane University] On: 11 October 2014, At: 15:25 Publisher: Taylor & Francis Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Nucleosides and Nucleotides

Publication details, including instructions for authors and subscription information: http://www.tandfonline.com/loi/lncn19

Novel Open-Chain Nucleotides Imitating 2',3'-Dideoxy-2',3'-Didehydronucleotides: Synthesis and Substrate Properties Toward DNA Polymerases

E. A. Shirokova^a, N. B. Tarussova^a, A. V. Shipitsin^a, D. G. Semizarov^a, M. Hieber^{a b} & A. A. Krayevsky^a ^a Engelhardt Institute of Molecular Biology Russian Academy of Sciences, 32 Vavilov st., Moscow, 117984, Russia

^b Universitat des Saarlandes im Stadwald , 66123, Saarbrucken, Germany

Published online: 16 Feb 2007.

To cite this article: E. A. Shirokova , N. B. Tarussova , A. V. Shipitsin , D. G. Semizarov , M. Hieber & A. A. Krayevsky (1995) Novel Open-Chain Nucleotides Imitating 2',3'-Dideoxy-2',3'-Didehydronucleotides: Synthesis and Substrate Properties Toward DNA Polymerases, Nucleosides and Nucleotides, 14:3-5, 749-751

To link to this article: <u>http://dx.doi.org/10.1080/15257779508012464</u>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms &

Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

NOVEL OPEN-CHAIN NUCLEOTIDES IMITATING 2',3'-DIDEOXY-2',3'-DIDEHYDRONUCLEOTIDES: SYNTHESIS AND SUBSTRATE PROPERTIES TOWARD DNA POLYMERASES

E.A. Shirokova^{*}, N.B. Tarussova, A.V. Shipitsin, D.G. Semizarov, M. Hieber[#], A.A. Krayevsky

Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 32 Vavilov st., Moscow 117984, Russia;

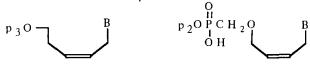
Abstract. A new series of acyclic nucleotide diphosphates was synthesized and evaluated as potential inhibitors of HIV reverse transcriptases.

To acquire new data on structure-function relationship of DNA polymerases novel groups of modified nucleotides have been synthesized and evaluated in the last few years. Specifically, compounds of type I demonstrated good substrate properties towards different DNA polymerases [1]. With the aim of developing selective inhibitors of viral DNA polymerases, HIV reverse transcriptase in particular, we undertook the synthesis of phosphonate diphosphates (IIa-d) isosteric to the parent I. These compounds also reveal conformational rigidity of the pseudosugar residue due to the presence of a *cis* double bond, but contain a nonhydrolyzable bond between the triphosphate residue and the acyclic fragment.

Originally we planned to synthesize $(HO)_2P(O)CH_2OCH_2C \equiv CCH_2OH$ but all our attempts to alkylate $RCH_2C \equiv CCH_2OBz$ (R = OH, Br, OMs) with $R'OCH_2P(O)(OEt)_2$ (IIIa,b, a R' = Ts, b R' = H) as described in [2,3] failed. Therefore, we followed an alternative route and prepared $HOCH_2C \equiv CCH_2B$ (IVa-d) for subsequent condensation with IIIa. To prepare IVa, we used the hydride procedure [4], while for IVbd, the maximal yield was achieved when the nucleic base (for IVd chloroaminopurine) was stirred with 4-mesyloxybut-2-ynol (V) and K_2CO_3 in DMF. Alkylation with V simplified the procedure [4,5]. Since the prepared IVa remained intact in the reaction with IIIa, we

[#]present address:Universitat des Saarlandes im Stadwald, 66123 Saarbrucken, Germany

mesylation, but only traces of the attempted to activate it by target MsOCH₂C=CCH₂Ade(VI) were observed. Moreover, alkyne VI synthesized from 1,4dimesyloxybut-2-yne and adenine manifested unusual properties in the routine nucleophylic reactions. When VI, KBr or KI, and dibenzo-18-crown-6 were refluxed in acetone, TLC revealed only the starting compounds in the reaction mixture. An increase of temperature up to $80-90^{\circ}$ C (in the presence of a small amount of DMF) resulted in its degradation. The reaction of mesylate VI with phosphonate IIIb was also unsuccessful. Therefore, we hydrogenated alkynols IV over the Lindlar catalyst. Z-Configuration of the resulting VIIa-d was confirmed by comparison of their H¹ NMR spectra with those for the same compounds reported in [5], as well as with the spectra for the corresponding E isomers prepared by reduction of IV with LiAlH₄.

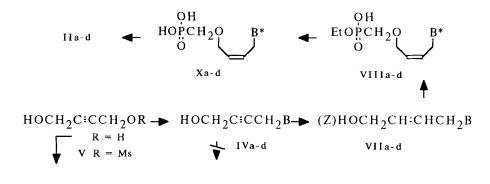


I B = Thy, Ade, Cyt IIa-d if not stated otherwise a B=Ade, b B=Thy, c B=Cyt, d B=Gua; $p_2=P_2H_3O_6$; $p_3=P_3H_4O_9$

Butenols VIIb,d were directly converted to phosphonates VIIIb,d by the reaction with ethyl p-toluenesulfonyloxymethylphosphonate (IX). Compounds VIIa,c were first Nbenzoylated, and then condensed with IX providing VIIIa,c. Deblocking of VIIIa-d with Me₃SiBr afforded phosphonate acids (Xa-d). They were then routinely pyrophosphorylated, and the adenine and cytosine derivatives were treated with aqueous ammonia. The structure of the target compounds was confirmed by UV, ¹H NMR and ³¹P NMR data. The ¹H NMR spectra of phosphonates IIa-d, VIIIa-d and Xa-d revealed a clear doublet of the CH₂-P group at 3.6-3.8 ppm (J = 8.5-9.5 Hz) characteristic of compounds containing an oxymethylphosphonate group [2]. For all these compounds the vinylic proton patterns were similar. In the ³¹P NMR spectra of Xa-d obtained with ¹H decoupling, a phosphorus singlet was observed at 16.0-17.0 ppm. The ³¹P NMR spectra of IIa-d were as expected.

The substrate properties of IIa-d were evaluated in cell free systems containing various DNA polymerases including viral reverse transcriptases. They were recognized only by HIV-1 and AMV reverse transcriptases. The [IIc/dCTP] concentration ratio at which DNA synthesis is inhibited by 50% was 1.3-fold higher than that for corresponding I [1], implying a slightly lower affinity of II to the DNA-synthesizing complex. Moreover, appreciable termination was observed for IIa-d and I at the same [analog/dNTP] concentration ratios. The tested II were not utilized by human DNA polymerases α and ε , β from rat liver, *E. coli* DNA polymerase I, and HSV-1 and CMV DNA polymerases. Phosphonates Xb-d and alkenols VIIa-d displayed no activity in HIV-1 infected MT-4

cells; Xa was moderately effective (ED₅₀ 9 μ M). This is presumably due to a low efficiency of intracellular phosphorylation and may imply the existence of a different mechanism of action for adenine derivatives.



$$MsOCH_{2}C:CCH_{2}OMs \rightarrow MsOCH_{2}C:CCH_{2}Ade \rightarrow (EtO)_{2}PCH_{2}OCH_{2}C:CCH_{2}Ade$$

$$VI$$

$$a B^{*}=Ade^{N-Bz}, b B^{*}=Thy, c B^{*}=Cyt^{N-Bz}, d B^{*}=Gua$$

Acknowledgment. This work was supported by Russian Fund of Fundamental Research, grant N 93-04-20 524 and Program "National Priorities in Medicine and Public Health, AIDS," grant 38.

References

- Krayevsky, A.; Victorova, L.; Mozzherin, D.; Kukhanova, M. Nucleosides & Nucleotides 1993, 12, 83-93.
- Rosenberg, I.; Holy, A.; Masojidkova, M. Coll. Czechoslovak. Chem. Commun. 1988, 53, 2753-77.
- (3) Hóly, A.; Rosenberg, I. Coll. Czechoslovak. Chem. Commun. 1982, 47, 3447-63.
- Borchering, D.R.; Narayanan, S.; Hasobe, M.; McKee, J.G.; Keller, B.T.; Borchardt, R.T. *J. Med. Chem.* 1988, 31, 1729-1738.
- (5) Phadtare, S.; Kessel, D.; Corbett, T.H.; Renis, H.E.; Court, B.A.; Zemlicka, J. J. Med. Chem. 1991, 34, 421-429.