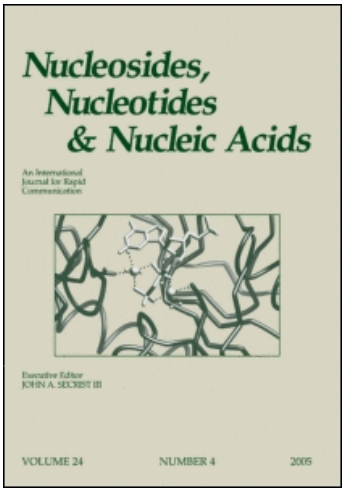

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Novel Open-Chain Nucleotides Imitating 2',3'-Dideoxy-2',3'-Didehydronucleotides: Synthesis and Substrate Properties Toward DNA Polymerases

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

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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 IVb-d, 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

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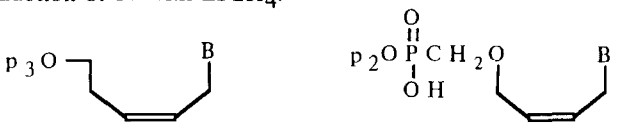
attempted to activate it by mesylation, but only traces of the target $MsOCH_2C{=}CCH_2Ade$(VI) were observed. Moreover, alkyne VI synthesized from 1,4-dimesyloxybut-2-yne and adenine manifested unusual properties in the routine nucleophylic reactions. When VI, KBr or KI, and dibenzo-18-crown-6 were refluxed in acetone, TLC revealed only the starting compounds in the reaction mixture. An increase of temperature up to 80-90^0 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 H^1 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 $LiAlH_4$.



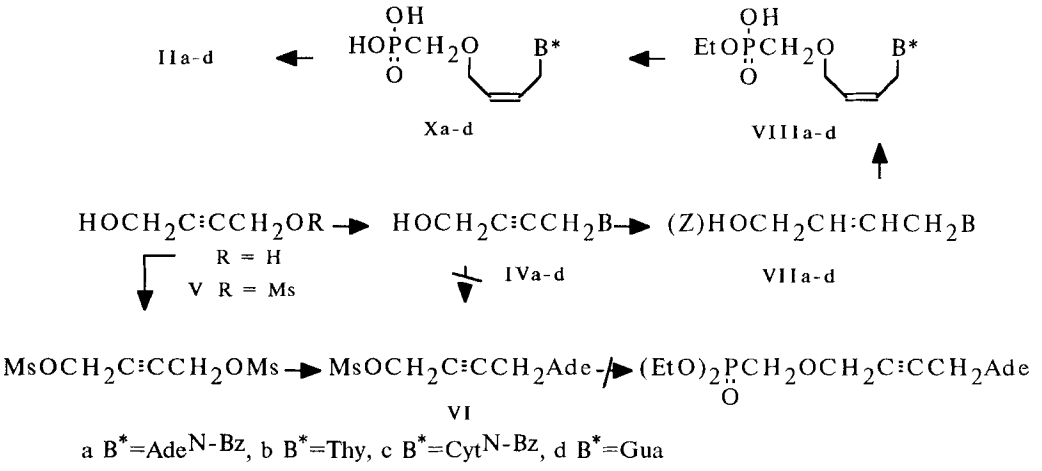
I B = Thy, Ade, Cyt **IIa-d**

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 N-benzoylated, and then condensed with IX providing VIIIa,c. Deblocking of VIIIa-d with Me_3SiBr 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 ^{31}P NMR data. The ^1H NMR spectra of phosphonates IIa-d, VIIIa-d and Xa-d revealed a clear doublet of the CH_2-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 ^{31}P NMR spectra of Xa-d obtained with ^1H decoupling, a phosphorus singlet was observed at 16.0-17.0 ppm. The ^{31}P 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 (ED_{50} 9 μM). 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.



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