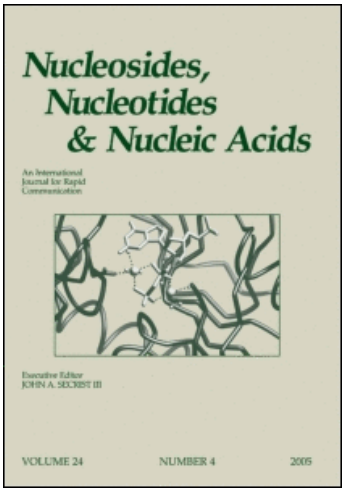

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Holger Winter[a]; Yosuke Maeda[b]; Hiroaki Mitsuya[b]; Jiri Zemlicka[a]
[a] Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan [b] The Experimental Retrovirology Section, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

To cite this Article Winter, Holger , Maeda, Yosuke , Mitsuya, Hiroaki and Zemlicka, Jiri(1997) 'Phosphodiester Amidates of Unsaturated Nucleoside Analogues as Anti-HIV Agents', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 1341 — 1345
To link to this Article: DOI: 10.1080/07328319708006184
URL: http://dx.doi.org/10.1080/07328319708006184

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 1341-1345 (1997)

PHOSPHODIESTER AMIDATES OF UNSATURATED NUCLEOSIDE ANALOGUES AS ANTI-HIV AGENTS

Holger Winter[a], Yosuke Maeda[b], Hiroaki Mitsuya[b] and Jiri Zemlicka*,[a]

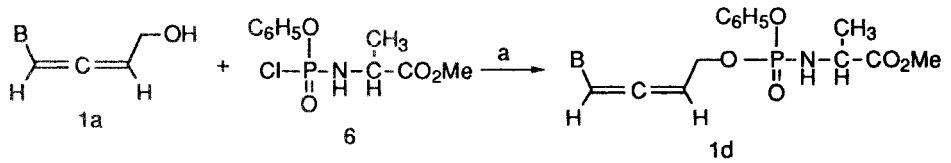
[a]Barbara Ann Karmanos Cancer Institute, Wayne State University School of Medicine, Detroit, Michigan 48201-1379

[b]The Experimental Retrovirology Section, Medicine Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

ABSTRACT. Lipophilic phosphodiester L-alaninates of acyclic unsaturated nucleoside analogues **1d**, **1e**, **2d**, **2e**, **3d**, **3e**, **4d** and **5d** were prepared and their antiretroviral activity was examined in ATH8 cell culture infected with HIV-1. A possible mechanism of action of these analogues is discussed.

In the past several years a new class of lipophilic prodrugs of antiretroviral nucleoside analogues such as AZT (zidovudine, Retrovir) and d4T (stavudine, Zerit) comprising a phosphodiester L-alaninate moiety was developed[1,2]. This concept was also applied to activation of inactive analogues, such as L-2',3'-dideoxy-3'-oxaadenosine[3,4] (isoddA). Our laboratory and others extensively investigated acyclic unsaturated nucleoside analogues and their biological activity[5]. Allenic derivatives, particularly adenallene[6] (**1a**) and cytallene[7] (**1b**), are among those which exhibit a potent anti-HIV activity and analogue **1b** is also effective[8] against HBV. It was therefore of interest to examine the effect of a phosphodiester L-alaninate moiety on the biological activity of these and related acyclic unsaturated analogues. Two groups of derivatives were investigated: (i) phosphoalaninates **1d** and **2d** derived from biologically active adenallene (**1a**) and R-enantiomer **2a** and (ii) compounds **1e**, **2e**, **3d**, **3e**, **4d** and **5d** related to inactive allenes **1c**, **2c** and unsaturated analogues **3a**, **3c**, **4a**, **5a** which are also devoid of antiretroviral effect.

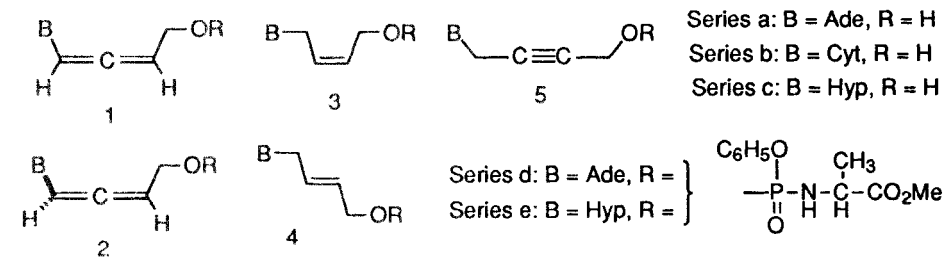
Synthesis of all these compounds followed the route described previously for aforementioned nucleoside analogues[1] as shown in Scheme 1. For example, **1a** was



a. N-Methylimidazole, tetrahydrofuran.

Scheme 1

phosphorylated with phosphorochloridate **6** and N-methylimidazole in tetrahydrofuran to give phosphamidate **1d** (71 %). The obtained phosphodiester L-alaninates were tested for anti-HIV activity in ATH8 cell culture[9]. As expected, the most active analogues were adenallene phosphamidates **1d** and **2d** (EC_{50} 0.88 and 0.21 μM, respectively) which were more effective[10] than parent analogues **1a** and **2a**. Some activity was also noted with hypoxallene phosphamidates **1e** and **2e** derived from inactive analogues **1c** and **2c**.



Phosphodiester amidate **3d** derived from Z-alkene **3a** was the most active compound (EC_{50} <1 μM) in the group of analogues containing only a single double or triple bond in the side-chain. The corresponding hypoxanthine derivative **3e** was inactive. Apparently, activating mechanism[11] important for anti-HIV activity of 2',3'-dideoxy-inosine (ddI, didanosine, Videx) and requiring a prior phosphorylation which to some extent may function in case of phosphodiester alaninates **1e** and **2e** is ineffective with **3e**. The E-alkene derivative **4d** was virtually inactive; only some toxicity was apparent above 1 μM. The antiretroviral activity of acetylenic phosphamidate **5d** was not well separated from cytotoxicity in the range of 0.1 - 100 μM. In general, an increased anti-HIV activity of these analogues is accompanied by elevated cytotoxicity levels.

Hydrolysis of phosphodiester alaninates **1d**, **1e**, **3d**, **3e** and **4d** gave the following results. Quite surprisingly, phosphamidates **1d** and **1e** are relatively stable in strong acid ($t_{1/2}$ 24.4 and 23.4 h at pH 1.2, FIGURE 1). Base-catalyzed hydrolysis is much faster ($t_{1/2}$ 151 and 308 min at pH 9.8, FIGURE 2) giving phosphoalaninate monoesters **7a** and **7b** stable at pH 7.0 and above. Analogue **3d** was hydrolyzed in triethylamine - water[2] to afford **8a**. This procedure avoids using buffers and, therefore, it

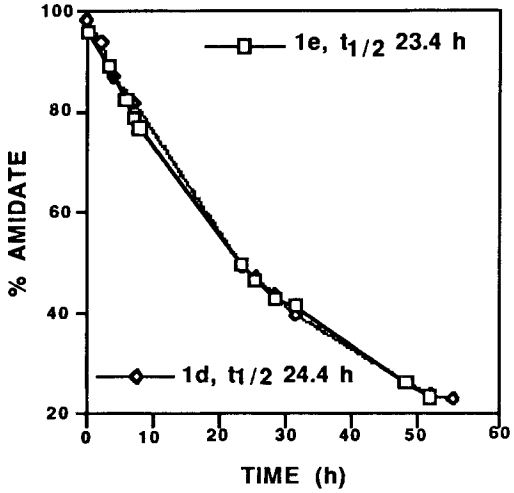


FIGURE 1. pH 1.2

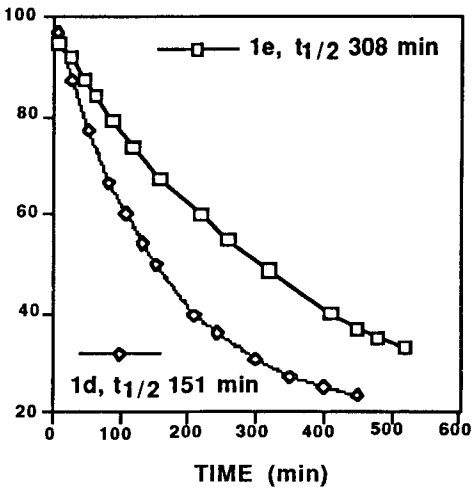
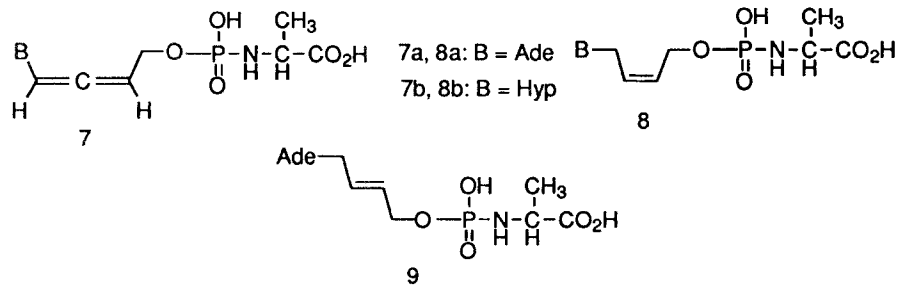


FIGURE 2. pH 9.8

is more convenient from a preparative standpoint. Hydrolysis of phosphodiester alaninates Hydrolysis of phosphodiester L-alaninates **1d** and **1e** at room temperature.



1d, **1e**, **3d**, **3e** and **4d** at pH 7.4 catalyzed by pig liver esterase led also to products **7a**, **7b**, **8a**, **8b** and **9**. The mechanism postulated[10] for transformation of **1d** to **7a** includes cleavage of ester function followed by a nucleophilic displacement of the phenoxy group by carboxylate anion and hydrolysis of the resultant cyclic anhydride to give phosphoalaninate **7a**. Such a reaction course is also anticipated in case of other analogues. Intracellular hydrolysis of phosphodiester L-alaninates of d4T and isoddA afforded similar phosphoalaninate monoesters[2,4].

It can then be assumed that cellular metabolism of phosphodiester alaninates **1d**, **2d** and **3d** which exhibit a potent antiretroviral activity follows the similar lines to give the respective monoester amidates **7a** (and the corresponding R-enantiomer) as well as **8a**. It seems likely that the latter derivatives are transformed to the respective monophosphates which are ultimately converted to triphosphates necessary for inhibition of viral reverse transcriptase by a nucleotide kinase-assisted mechanism. At the present time it is not clear which enzyme is responsible for this activation. Phosphodiesterases were postulated as possible converting enzymes in case of similar phosphomonoester alaninates[4] derived from isoddA. However, compound **7a** is resistant to snake venom phosphodiesterase from *Crotallus durissus*. Further studies are clearly needed to elucidate all steps of metabolism of lipophilic prodrugs based on phosphodiester L-alaninate structure.

Acknowledgment. This study was supported by a research grant CA32779 from the National Cancer Institute, Bethesda, Maryland and the United Way of Southeastern Michigan.

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