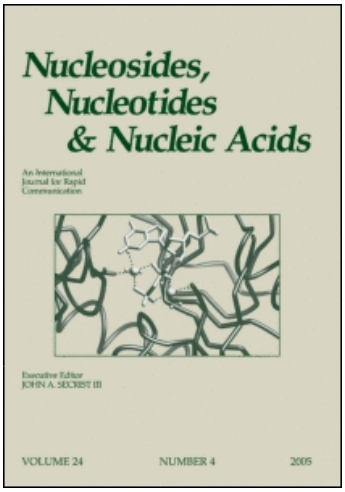

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Phosphorodiamides as Prodrugs for Antiviral Nucleosides

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ABSTRACT

New phosphorodiamides of modified nucleoside monophosphates were synthesized and their antiviral properties were evaluated.

INTRODUCTION

Nucleoside monophosphate esters and their analogues displaying activity towards a wide range of viruses as well as the corresponding phosphoroamide derivatives have been thoroughly studied.[1,2] The mechanism of action of these nucleosides involves a cascade of intracellular triphosphorylation followed by inhibition of viral polymerases by the resulting triphosphates. The use of monophosphate derivatives allows bypass of the first stage of triphosphorylation cascade, which is often a limiting stage.

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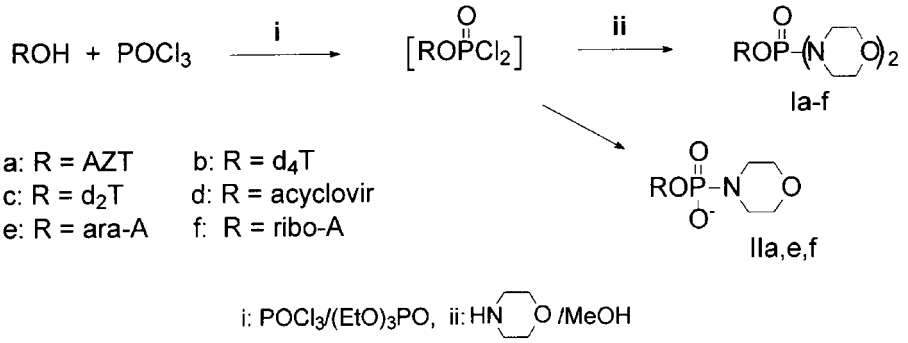

Scheme 1.

Nucleoside phosphorodiamidates are studied to a lesser extent. However, for some members of this class, a decrease in toxicity was observed[3,4]. The goal of this work was the synthesis of new nucleoside 5′-phosphorodiamidates and evaluation of their antiviral properties.

RESULTS AND DISCUSSION

Dimorpholidates **(Ia–f)** were prepared by the reaction of the corresponding nucleoside with phosphorus oxychloride followed by treatment of the resulting phosphodichloridate with an excess of morpholine in methanol (Sch. 1).

In the case of AZT and d4T the yield was about 60%.

Bis(methoxyamides) **(IIIa,b)** and bis(diphenylmethylamides) **(IVa,b) exemplify** another type of AZT and d4T monophosphate derivatives. They were synthesized **analogously** to dimorpholidates **(Ia,b)** in yields of 45 and 35% and 30 and 25%, respectively. For the synthesis of diamides **(III)** we used methoxyamine hydrochloride and an excess of triethylamine to remove the releasing hydrogen chloride. In the case of diamides **(IV)** dioxane was taken as a solvent, since both diphenylmethylamine and methanol reacted with the intermedia phosphodichloridate to give a considerable amount of phosphoromethoxyamidate.

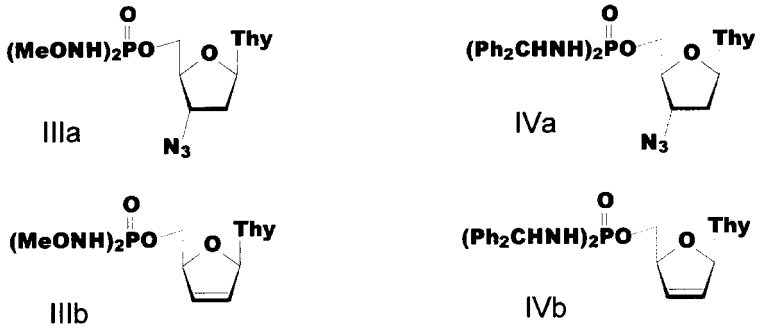

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Table 1.

Compound	CD_{50} (μM)[a]	ID_{50} μM)[b]	SI[c]	Compound	CD_{50} (μM)[a]	ID_{50} (μM)[b]	SI[c]
AZT	187.5	0.018	10,400	d4T	314	0.01	31,400
Ia	>206	7.2	>30	Ib	>226	0.0022	>1,00,000
IIIa	740	0.012	61,700	IIIb	828	0.028	29,600
IVa	6.2	1.7	3.6	IVb	47.3	0.55	77

[a]CD_{50} – 50% cytotoxic dose.
[b]ID_{50} – 50% inhibiting dose.
[c]SI – selectivity index (CD_{50}/ID_{50} ratio).

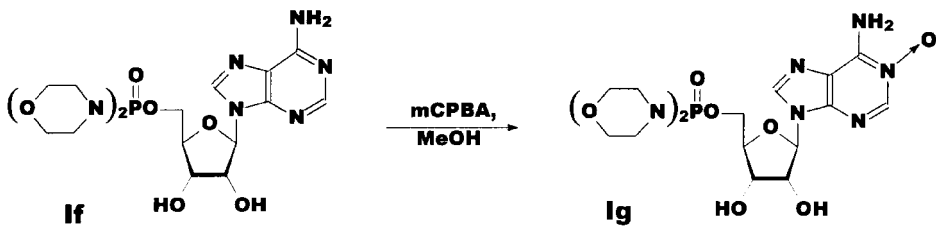
The results of biochemical tests of these compounds are shown in Table 1. The amides were evaluated in MT-4 cells infected with HIV-1 strain GKV-4046. As is seen from the table, diamides **(I)** and **(III)** of the AZT and d4T series dramatically differed in their antiviral activity. For example, dimorpholidate **Ib** was substantially more active than the parent nucleoside, whereas in the case of compounds **(III)**, this modification was more advantageous for the AZT series. It is interesting that the activity of monomorpholidate AZT **(IIa)** prepared according to Sch. 1 with an additional stage of treating the intermediate phosphorodichloridate with water, displayed the activity similar to that of **Ib** (ID_{50} 0.0023 μM and SI ≈ 1,00,000). High toxicity of AZT bis(diphenylmethylamido)phosphate is also noteworthy.

Phosphorodimorpholidates of d4T **(Ic)** and acyclovir **(Id)** were synthesized according to Sch. 1 in yields of 70 and 30%, respectively. Both of them were inactive against HIV-1 and HSV-1, respectively.

Using this scheme, we also prepared phosphorodimorpholidate of arabinoadenosine **(Ie)**, which is active against many viruses but is highly toxic.[5] Unlike thymidine derivatives, we obtained a mixture of phosphoromono- and -dimorpholidates **(IIe, Ie)** (15 and 55%, respectively). The tests with cell cultures infected with cytomegalovirus and poxviruses are in progress.

Adenosine N^1-oxide is known to be rather active against the poxvirus group.[5] We recently showed that it was highly active against smallpox virus with ID_{50} 0.57 μM, the CD_{50} being 19 μM. With the goal to decrease the toxicity, we synthesized its phosphorodimorpholidate **(Ig)**.

However, direct interaction of the nucleoside with $POCl_3$ and morpholine under aforementioned conditions yielded less than 10% of the target **(Ig)**. As an alternative,



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we synthesized compound **(If)** and oxidized it with *m*-chloroperbenzoic acid in methanol in the presence of tributylamine (Sch. 2). The overall yield of phosphorodimorpholidate **(Ig)** was 35%.

It is noteworthy that while preparing riboadenosine derivative **(If)**, we isolated about 10% of monomorpholidate **(IIf)** in addition to the target diamide.

Thus, the introduction of a phosphorodiamidate residue in the molecule of nucleosides displaying antiviral activity in some cases strongly affects their antiviral properties. Therefore, further investigations in this field seem to be promising.

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