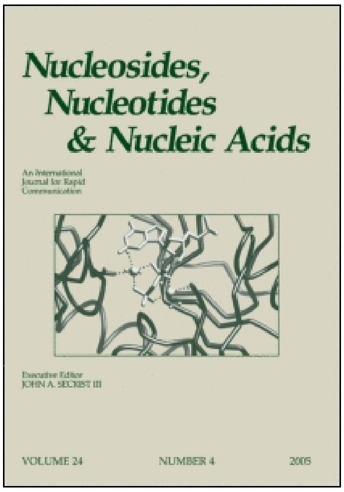

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SYNTHESIS AND STUDY OF A NEW SERIES OF PHOSPHORAMIDATE DERIVATIVES AS MONONUCLEOTIDE PRODRUGS

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SYNTHESIS AND STUDY OF A NEW SERIES OF PHOSPHORAMIDATE DERIVATIVES AS MONONUCLEOTIDE PRODRUGS

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ABSTRACT

The synthesis and the study of new mononucleoside phosphoramidate diesters bearing *S*-acyl-2-thioethyl (SATE) groups and an alkylamino residue are reported. The studied compounds appear to be able to deliver the corresponding 5′-mononucleotide inside the cells, and could be considered as prototypes for a new kind of mononucleotide prodrugs (pronucleotides).

In order to overcome the dependence of nucleoside analogues upon nucleoside kinase activation, various research groups have focused their attention on the study of mononucleotide prodrugs, namely pronucleotides (1). Our group was particularly interested in the development of monophosphorylated derivatives incorporating *S*-acyl-2-thioethyl (SATE) groups as biolabile phosphate protections (2). Thus, illustrated on the basis of the (L)-methoxyalaninyl derivative **1** (Fig.), we have previously demonstrated that phosphoramidate diesters containing a *S*-pivaloyl-2-thioethyl group (*t*BuSATE) and a methyl-esterified amino acid allow the intracellular delivery of the corresponding 5′-mononucleotide (3). The proposed mechanism for the decomposition of this new series of pronucleotides may

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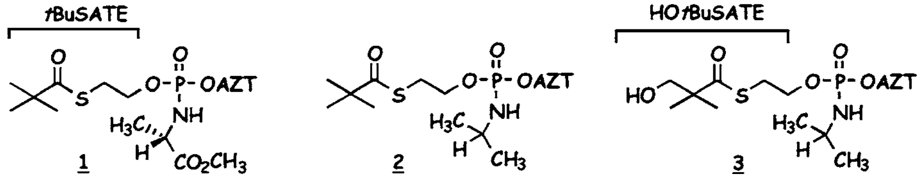


Figure.

involve successively an esterase and then a phosphoramidase hydrolytic steps. As part of the therapeutic potential of such phosphoramidate diesters, we studied the effect of structural modifications on the amidate moiety. Here, we would like to report the synthesis, *in vitro* anti-HIV-1 activity and stability studies of two new SATE phosphoramidate diesters of AZT 2 and 3 (Fig.), bearing an isopropylamino residue as a first model of alkylamino groups.

The synthesis of phosphoramidates 2 and 3 was carried out according to a general procedure, involving the reaction of isopropylamine with the appropriate *H*-phosphonate diesters in Atterton-Todd conditions (4). In the case of the derivative 3, the trityl group was selected as transient protection for the hydroxyl function.

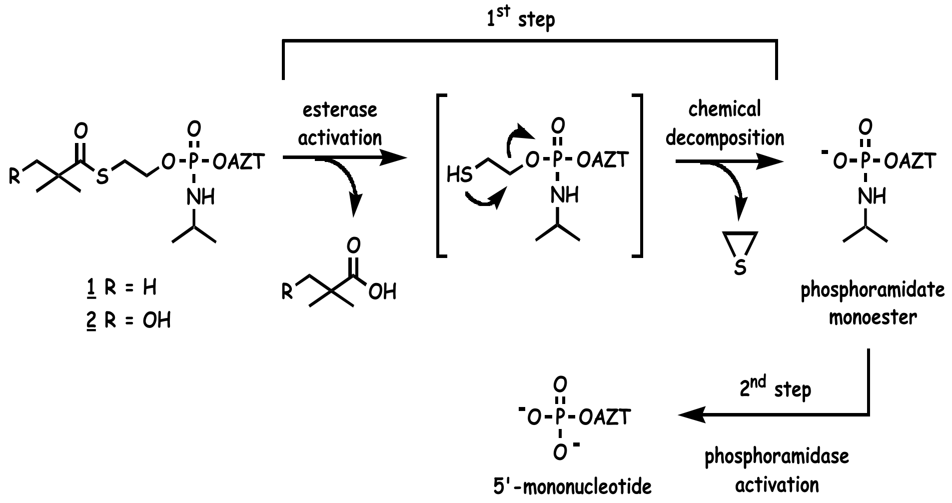
The phosphoramidate diesters 2 and 3 were evaluated for their inhibitory effects on HIV-1 replication (Table 1) in CEM-SS and thymidine kinase-deficient CEM cells (CEM/TK$^-$) in comparison to the parent nucleoside and to the (L)-methoxyalaninyl phosphoramidate 1 as reference pronucleotide.

In contrast to AZT, the phosphoramidate diester 2 exhibited a significant anti-HIV effect in CEM/TK$^-$ cells with an EC_{50} value at submicromolar concentration which was lower that the one observed for the reference pronucleotide. This result seems to demonstrate that 2 is able to give rise to the intracellular delivery of AZTMP and can be considered as a lead compound in the design of a new series of pronucleotides. Compared to its analogue 2, the moderate anti-HIV-1 activity observed for the phosphoramidate 3 in the studied cell lines could be related to a slower rate of decomposition as previously observed in other series of SATE pronucleotides (5).

Table 1.

	CEM-SS		CEM/TK$^-$	
	EC_{50} (μM)[a]	CC_{50} (μM)[b]	EC_{50} (μM)	CC_{50} (μM)
1[c]	0.05	92	4.3	>100
2	0.02	98	0.75	>100
3	0.65	>100	12	>100
AZT	0.006	>100	>100	>100

[a] EC_{50}: 50% Effective Concentration or Concentration Required to Inhibit the Replication of HIV by 50%; [b] CC_{50}: 50% Cytotoxic Concentration or Concentration Required to Reduce the Viability of Uninfected Cells by 50%; [c] Previously Published Data (3).



Scheme.

In this respect, the stability of **2** and **3** was evaluated in total CEM-SS cell extract (TCE) and in human serum (HS). In TCE, the observed decomposition pathway of **2** and **3** was similar and gave rise to the formation of the phosphoramidate monoester after a first esterase activation (Scheme). This metabolite accumulated to a significant level and then was slowly converted to AZTMP, in a second step through a putative phosphoramidase-mediated hydrolysis (6).

Comparison of the half-lives of SATE phosphoramidates **2** and **3** in TCE and HS (Table 2) evidently proved that **3** exhibits a higher resistance against esterase hydrolysis compared to **2**.

Two SATE phosphoramidate derivatives of AZT **2** and **3** have been synthesized using hydrogenphosphonate chemistry. The anti-HIV activity observed for the compound **2** in CEM/TK$^-$ cells showed that the presence of an amino acid moiety is not essential for the efficient delivery of the 5′-mononucleotide, and can be replaced by an isopropylamino residue. Finally, as demonstrated by the study of the hydroxylated derivative **3**, a decrease of the esterase hydrolysis rate of phosphoramidate diesters could be obtained by a simple chemical modification on the SATE group. From the lead SATE phosphoramidate diester **2**, various structural

Table 2.

	Half-Lives of the Studied Compounds[a]	
Biological media	**2**	**3**
total CEM-SS cell extract	1.2 h	7.5 h
human serum	10 h	25 h

[a] Determined According to a Previously Published ⟨⟨on-Line ISRP Cleaning⟩⟩ HPLC Method (7).

modifications could be envisaged in order to avoid a presystemic metabolism of this new series of pronucleotides and to promote the delivery of the corresponding 5′-mononucleotide in infected cells or tissues: (i) on the SATE moiety for the control of the enzymatic stability, and (ii) on the amidate moiety in order to reach a suitable equilibrium between aqueous solubility and lipophilicity. In this context, the development of this new series of pronucleotides as possible anti-HIV candidates is warranted, and the study of SATE phosphoramidates bearing various alkylamino residues is currently in progress in our group.

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