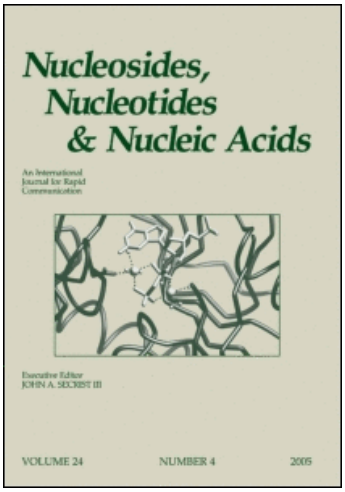

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The Pronucleotide Approach. I. Synthesis, Anti-HIV Activity and Preliminary Stability Studies of Mononucleoside *S,S*'-Bis(O-acyl-2-oxyethyl) Phosphorodithiolates

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THE PRONUCLEOTIDE APPROACH. I. SYNTHESIS, ANTI-HIV ACTIVITY AND PRELIMINARY STABILITY STUDIES OF MONONUCLEOSIDE *S,S'*-BIS(*O*-ACYL-2-OXYETHYL) PHOSPHORODITHIOLATES

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ABSTRACT: The synthesis, *in vitro* anti-HIV activity, and preliminary stability studies of several mononucleoside phosphorodithiolate derivatives incorporating a new kind of bio-labile phosphate-protection, namely *O*-pivaloyl-2-oxyethyl, are reported. Our preliminary results strongly support the hypothesis that such pronucleotides exert their biological effects *via* intracellular delivery of the corresponding 5'-mononucleotide.

We have recently demonstrated that mononucleoside phosphotriesters incorporating *S*-acyl-2-thioethyl *carboxyesterase-labile transient phosphate protections* (SATE pronucleotides, FIG. 1), allow the intracellular delivery of the corresponding 5'-mononucleotides.[1] Among the SATE groups, the *S*-pivaloyl-2-thioethyl group (tBuSATE) emerged as the most promising bio-labile protection for *in vivo* experiments on the basis of its pharmacokinetic properties.

We decided to extend our investigations in the design of new kinds of bio-labile phosphate protections by the synthesis and study of an isomeric form of SATE pronucleotides, namely mononucleoside *S,S'*-bis(*O*-acyl-2-oxyethyl) phosphorodithiolates (isoSATE pronucleotides, FIG. 1). Here, we report the synthesis, antiviral evaluation and preliminary pharmacokinetic data of phosphorodithiolate derivatives of 2',3'-dideoxyadenosine (ddA) and 2',3'-didehydro-2',3'-dideoxythymidine (d4T), **1** and **2** respectively, which incorporate the *O*-pivaloyl-2-oxyethyl [tBu(iso)SATE, FIG. 1] bio-

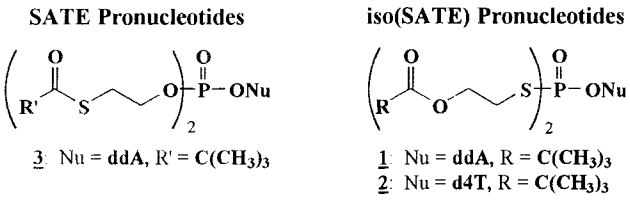


Figure 1: *Structure of the studied pronucleotides*

labile phosphate protecting group. The studies were performed in comparison to the tBu(SATE) pronucleotide of ddA **3** (FIG. 1).

SYNTHESIS

The mononucleoside phosphorodithiolates **1** and **2** were obtained following an one-pot procedure adapted from a published oligonucleotide-phosphorodithioates synthesis,[2] involving (pyrrolidino)phosphoramidites and 1*H*-tetrazole activation (Scheme 1).

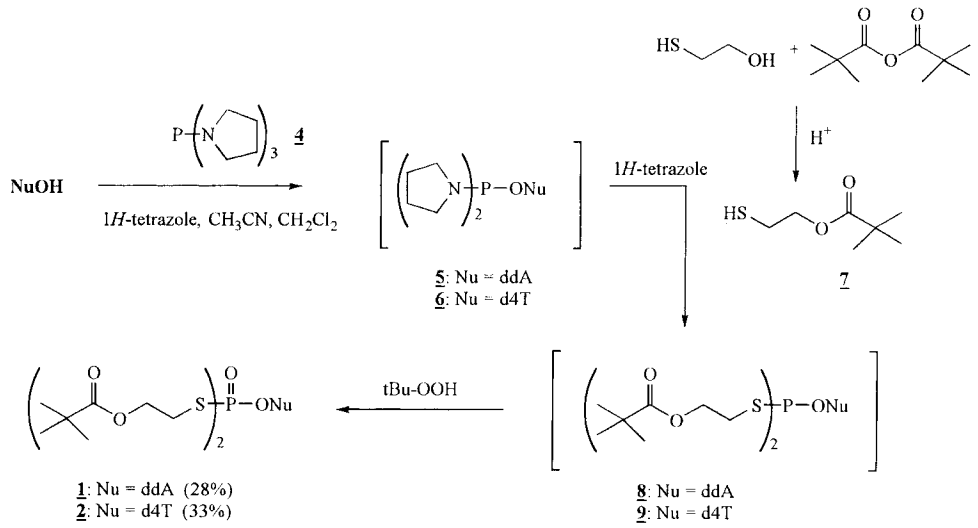
ANTI-HIV-1 ACTIVITY

The isoSATE pronucleotides **1** and **2** were evaluated for their inhibitory effects on the replication of HIV-1 in CEM-SS and in thymidine-kinase deficient cell lines (CEM/TK$^-$) (TABLE 1). For comparison, the parent nucleosides ddA and d4T (and in the case of ddA, the bis(tBuSATE) pronucleotide **3**) were evaluated in the same experiments.

In the two cell culture systems, the anti-HIV-1 activities of the tBu(iso)SATE pronucleotide **1** were similar to those of their corresponding tBu(SATE) pronucleotide **3**, both types of isomeric pronucleotides being more potent inhibitors than ddA. Furthermore, the d4T derivative **2** showed high inhibitory effects in thymidine-deficient CEM cells while, as expected, d4T was weakly active in this cell line.

PRELIMINARY STABILITY STUDIES

The decomposition pathways and kinetic data of the isoSATE pronucleotide **1** were performed in culture medium and in total cell extracts. The proposed decomposition pathway (Scheme 2) may involve: (a) esterase-mediated activation leading to **(A)**; (b) nucleophilic attack of the resulting free hydroxyl function on the phosphorus atom, giving the five-covalent intermediate **(B)**; (c) conversion of **(B)** into the 2-mercaptoethyl

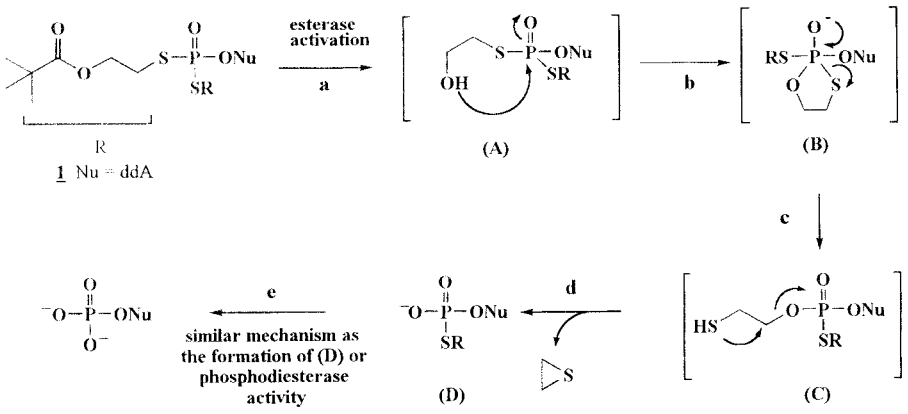


Scheme 1: *Synthesis of the tBu(iso)SATE pronucleotides 1 and 2*

Table 1: *Anti-HIV-1 activity of the pronucleotides 1-3 compared to their parent nucleosides ddA and d4T*

	CEM-SS		CEM/TK$^-$	
	EC_{50} (M)[a]	CC_{50} (M)[b]	EC_{50} (M)[a]	CC_{50} (M)[b]
1	$1.2\ 10^{-10}$	$> 10^{-5}$	$7.7\ 10^{-9}$	10^{-5}
3	$1\ 10^{-10}$	$7.9\ 10^{-6}$	$1.3\ 10^{-9}$	$1.4\ 10^{-6}$
ddA	$4.9\ 10^{-7}$	$> 10^{-4}$	$4.3\ 10^{-7}$	$> 10^{-4}$
2	$4.4\ 10^{-9}$	$> 10^{-5}$	$5.9\ 10^{-9}$	$8\ 10^{-6}$
d4T	$4\ 10^{-8}$	$> 10^{-4}$	$1.5\ 10^{-5}$	$9.2\ 10^{-5}$

[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%



Scheme 2: *Proposed decomposition pathway of an isoSATE pronucleotide*

phosphotriester **(C)**; (d) spontaneous elimination of episulfide, affording the corresponding phosphorothiolate diester **(D)**; (e) hydrolysis of **(D)** into the corresponding 5'-monophosphate by a similar mechanism (a-b-c-d) or by action of phosphodiesterases.

CONCLUSION

The present results demonstrate that isoSATE pronucleotides allow the efficient intracellular delivery of their parent nucleoside 5'-monophosphates. Further studies of isoSATE pronucleotides, particularly on the definite mechanisms involved in their decomposition, are in progress in our laboratory.

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