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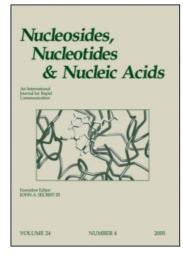
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### Nucleosides, Nucleotides and Nucleic Acids

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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 biolabile 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. 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), <u>1</u> and <u>2</u> respectively, which incorporate the *O*-pivaloyl-2-oxyethyl [tBu(iso)SATE, FIG. 1] bio-

SATE Pronucleotides iso(SATE) Pronucleotides 
$$\begin{pmatrix} O & O & P \\ P-ONu & R & S \end{pmatrix} \begin{pmatrix} O & P \\ P-ONu & R$$

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  $\underline{1}$  and  $\underline{2}$  were obtained following an one-pot procedure adapted from a published oligonucleotide-phosphorodithioates synthesis, involving (pyrrolidino)phosphoramidites and 1H-tetrazole activation (Scheme 1).

#### **ANTI-HIV-1 ACTIVITY**

The isoSATE pronucleotides <u>1</u> and <u>2</u> 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 <u>3</u>) were evaluated in the same experiments.

In the two cell culture systems, the anti-HIV-1 activities of the tBu(iso)SATE pronucleotide <u>1</u> were similar to those of their corresponding tBu(SATE) pronucleotide <u>3</u>, both types of isomeric pronucleotides being more potent inhibitors than ddA. Furthermore, the d4T derivative <u>2</u> 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 <u>1</u> 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

NuOH

$$\begin{array}{c}
P = N \\
\hline
N \\
\hline
1H - tetrazole, CH_3CN, CH_2Cl_2
\end{array}$$

$$\begin{array}{c}
S : Nu = ddA \\
\hline
\underline{6} : Nu = d4T
\end{array}$$

$$\begin{array}{c}
D \\
\hline
1 : Nu = ddA \\
\hline
2 : Nu = ddA \\
\hline
2 : Nu = ddA
\end{array}$$

$$\begin{array}{c}
D \\
\hline
1 : Nu = ddA \\
\hline
2 : Nu = ddA
\end{array}$$

$$\begin{array}{c}
D \\
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2 : Nu = ddA
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$$\begin{array}{c}
D \\
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2 : Nu = ddA
\end{array}$$

$$\begin{array}{c}
D \\
\hline
2 : Nu = ddA
\end{array}$$

**Scheme 1**: Synthesis of the tBu(iso)SATE pronucleotides <u>1</u> and <u>2</u>

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

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

<sup>&</sup>lt;sup>a</sup> EC<sub>50</sub>: 50% effective concentration or concentration required to inhibit the replication of HIV by 50%; <sup>b</sup> CC<sub>50</sub>: 50% cytotoxic concentration or concentration required to reduce the viability of uninfected cells by 50%

1324 SCHLIENGER ET AL.

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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