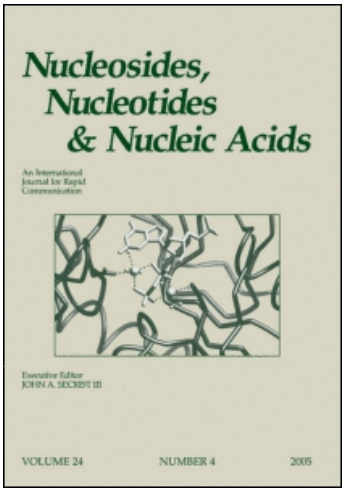


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Effect of the Thioalkyl Chain Variation in the Efficiency of Sate Pronucleotides

D. Egron[a]; C. Perigaud[a]; A. -M. Aubertin[b]; J. -L. Imbach[a]; G. Gosselin[a]
[a] Laboratoire de Chimie Bioorganique, UMR CNRS-USTL 5625, Université, Montpellier, France [b] Université Louis Pasteur, Institut de Virologie, Strasbourg, France

To cite this Article Egron, D. , Perigaud, C. , Aubertin, A. -M. , Imbach, J. -L. and Gosselin, G.(1998) 'Effect of the Thioalkyl Chain Variation in the Efficiency of Sate Pronucleotides', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 1725 — 1729
To link to this Article: DOI: 10.1080/07328319808004707
URL: http://dx.doi.org/10.1080/07328319808004707

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 1725-1729 (1998)

EFFECT OF THE THIOALKYL CHAIN VARIATION IN THE EFFICIENCY OF SATE PRONUCLEOTIDES

D. Egron[1], C. Périgaud[1]*, A.-M. Aubertin[2], J.-L. Imbach[1] and G. Gosselin[1]

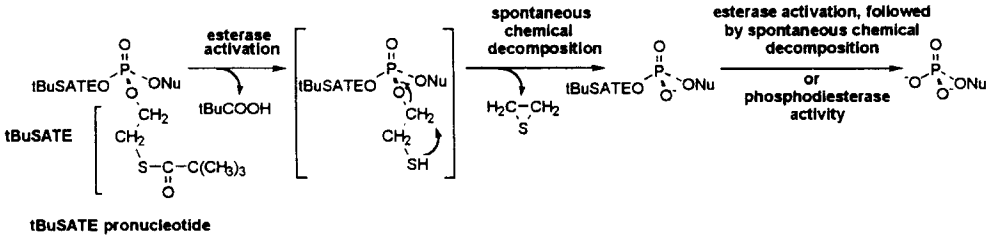
[1]Laboratoire de Chimie Bioorganique, UMR CNRS-USTL 5625, Université Montpellier II, Case Courrier 008, Place E. Bataillon, 34095 Montpellier Cedex 5, France; [2]Université Louis Pasteur, Institut de Virologie, INSERM U 74, 67000 Strasbourg, France

ABSTRACT: The synthesis and the anti-HIV activity of two mononucleoside phosphotriester derivatives of 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxyadenosine (ddA) incorporating a new kind of phosphate protecting group, namely *S*-pivaloyl-4-thiobutyl (tBuSATB), are reported.

INTRODUCTION

The use of nucleotide prodrugs (pronucleotides) constitutes an attractive strategy in order to improve the therapeutic potential of nucleoside analogues.[1,2] In this respect, recent examples of pronucleotides that display *in vitro* activity against human immunodeficiency virus (HIV) include mononucleoside phosphotriesters bearing esterase-labile phosphate protecting groups. For instance, the *S*-pivaloyl-2-thioethyl (tBuSATE, SCHEME 1) group has been proposed as an efficient transient protection for antiviral 5'-mononucleotide or phosphonate analogues.[3,4]

Herein, we report the synthesis and the anti-HIV-1 activity of mononucleoside phosphotriester derivatives of 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxyadenosine (ddA) incorporating a new kind of esterase-labile phosphate-protecting group, namely *S*-pivaloyl-4-thiobutyl (tBuSATB, Figure 1). This study was performed in order to evaluate the effect of the thioalkyl chain length on the efficiency of the corresponding tBu(SATE) pronucleotides of AZT and ddA (FIGURE 1).



Scheme 1. *Simplified mechanism proposed for the decomposition of tBuSATE pronucleotides*

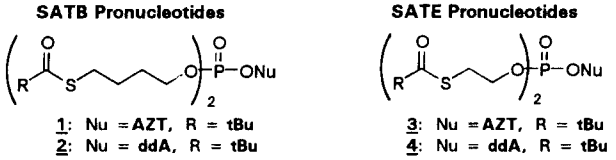


Figure 1. *Structures of the studied mononucleoside phosphotriesters derivatives*

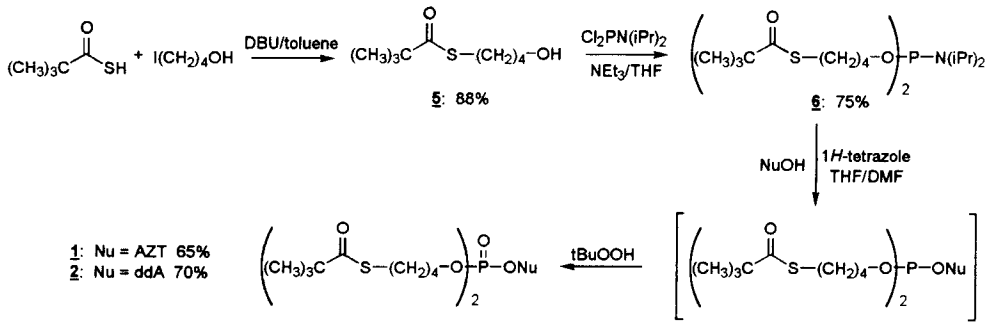
SYNTHESIS

The phosphotriesters **1** and **2** were obtained (SCHEME 2) following an adapted procedure from the synthesis of SATE pronucleotide.[3] The thioester **5** precursor was prepared by reaction of thiopivalic acid with 4-iodobutanol; the latter being not commercially available, was prepared according to a published procedure.[5]

The P(III) reagent **6** was obtained by treatment of *N,N*-diisopropylphosphorodichloridite[6] with 2 equivalents of **5** in the presence of triethylamine, followed by flash chromatography. Coupling of AZT or ddA with the phosphoramidite reagent **6** in the presence of 1*H*-tetrazole, followed by *in situ* oxidation with *tert*-butylhydroperoxide gave, after purification by silica gel column chromatography, the corresponding mononucleoside phosphotriester derivatives, respectively **1** and **2**.

ANTI-HIV ACTIVITY

Compounds **1**-**4** were evaluated for their inhibitory effects on the replication of HIV-1 in two cell lines, and in comparison to their parent nucleosides (TABLE).



Scheme 2. *Synthesis of the bis(tBuSATB)phosphotriester derivatives 1 and 2*

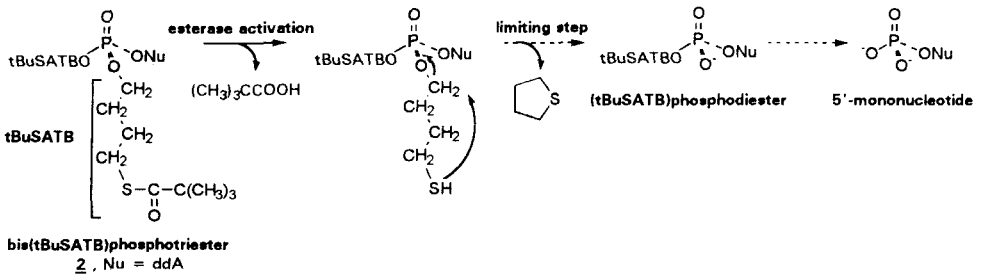
Table. *Antiviral activity of the pronucleotides 1-4 compared to their parent nucleosides, AZT and ddA, in two cell lines infected with HIV-1*[a]

	CEM-SS		CEM/TK$^-$	
	EC_{50}[b]	CC_{50}[c]	EC_{50}[b]	CC_{50}[c]
AZT	0.0012	> 100	> 100	> 100
1	1.0	> 10	> 10	> 10
3	0.0004	> 10	0.11	> 10
ddA	1.5	> 100	1.0	> 100
2	> 10	> 10	> 10	> 10
4	0.0002	6.6	0.0011	1.4

[a] All data represent average values for at least three separate experiments. The variation of these results under standard operating procedures is below ±10%.

[b] EC_{50}: 50% effective concentration (μM) or concentration required to inhibit the replication of HIV by 50%.

[c] CC_{50}: 50% cytotoxic concentration (μM) or concentration required to reduce the viability of uninfected cells by 50%.



Scheme 3. *Proposed explanation for the inefficient decomposition of the bis(tBuSATB)phosphotriester derivatives in cell culture experiments.*

Striking differences were found in the antiviral activities of the test compounds. As previously reported, the tBuSATE pronucleotide of AZT **3** proved to be markedly active in inhibiting the HIV-1 replication in thymidine kinase-deficient (TK^-) CEM cells, demonstrating its ability to circumvent the first anabolic phosphorylation step of AZT catalyzed by cytosolic thymidine kinase.[3] In contrast, the bis(tBuSATB)phosphotriester derivative **1** proved to be inactive against HIV-1 replication in CEM/TK^- cells at concentrations up to 10 μM. Moreover, the pronucleotide **1** exhibited a low antiretroviral activity in wild-type [thymidine kinase positive (TK^+)] human T_4-lymphoblastoid CEM-SS cells. Similar results were obtained from the evaluation of the phosphotriester derivatives of ddA, **2** and **4**. We previously reported that the potency of ddA can be significantly enhanced *in vitro* by the use of its corresponding SATE pronucleotides.[7] In accordance with our previous data, the tBuSATE pronucleotide of ddA **4** showed a very potent anti-HIV-1 activity in the two cell lines studied, and proved to be superior to ddA with regard to the antiviral efficiency. At the opposite, the bis(tBuSATB)phosphotriester derivative **2** was inactive against HIV-1 replication at concentrations up to 10 μM.

CONCLUSION

The present results demonstrate that the mononucleoside phosphotriester derivatives **1** and **2** which incorporate the tBuSATB phosphate-protecting group were not able to deliver the corresponding 5'-mononucleotide inside the cells. Moreover, the low anti-HIV-1 activity in CEM-SS cells of these compounds, in comparison to their parent

nucleoside, supports the hypothesis that such phosphotriester derivatives are inefficient to liberate the corresponding nucleoside analogue in the culture medium or intracellularly.

In relation to the decomposition mechanism of SATE pronucleotides (Scheme 1), data obtained from the *in vitro* evaluation of bis(tBuSATB)phosphotriesters may reflect their relative chemical stability in biological media. Indeed, preliminary stability studies seem to indicate that the phosphotriester derivative of ddA **2** is a substrate for esterases (data not shown). Consequently, the limiting step in the decomposition pathway of these compounds may be related to an inefficient intramolecular nucleophilic displacement into the corresponding (tBuSATB)phosphodiesters and tetrahydrothiophene (Scheme 3). Complete and detailed studies will be necessary to investigate this hypothesis.

ACKNOWLEDGEMENTS

These investigations were supported by grants from the CNRS, the Agence Nationale de Recherches sur le SIDA (ANRS, France), and the Association pour la Recherche sur le Cancer (ARC, France). One of us (D.E.) is particularly grateful to ARC for a fellowship. We thank Dr. I. Lefebvre and Dr. A. Pompon for preliminary stability studies.

REFERENCES

1. Périgaud, C., Girardet, J.-L., Gosselin, G., Imbach, J.-L. In *Advances in Antiviral Drug Design*; De Clercq, E., Ed.; JAI Press: London, 1996; Vol. 2, pp 147-172.
2. Krise, J.P., Stella, V.J., *Adv. Drug Delivery Rev.*, **1996**, *19*, 287-310.
3. Lefebvre, I., Périgaud, C., Pompon, A., Aubertin, A.-M., Girardet, J.-L., Kirn, A., Gosselin, G., Imbach, J.-L., *J. Med. Chem.*, **1995**, *38*, 3941-3949.
4. Benzaria, S., Pélicano, H., Johnson, R., Maury, G., Imbach, J.-L., Aubertin, A.-M., Obert, G., Gosselin, G., *J. Med. Chem.*, **1996**, *39*, 4958-4965.
5. Mandal, A.K., Soni, N.R., Ratnam, K.R., *Synthesis*, **1985**, 274-275.
6. Tanaka, T., Tamatsukuri, S., Ikehara, M., *Tetrahedron Lett.*, **1986**, *27*, 199-202.
7. Périgaud, C., Aubertin, A.-M., Benzaria, S., Pelicano, H., Girardet, J.-L., Maury, G., Gosselin, G., Kirn, A., Imbach, J.-L., *Biochem. Pharmacol.*, **1994**, *48*, 11-14.