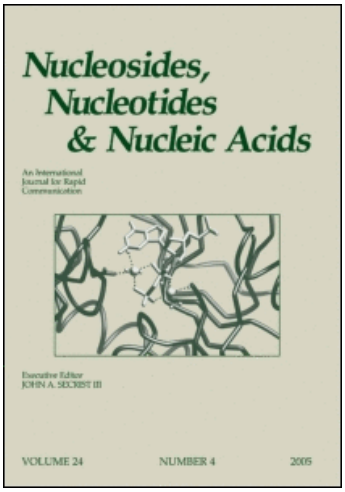

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The Pronucleotide Approach. III. Synthesis, Anti-HBV Activity and Stability Studies of the Bis(*S*-pivaloyl-2-thioethyl) Phosphotriester Derivative of Acyclovir

Gilles Valette[a]; Jean-Luc Girardet[a]; Alain Pompon[a]; Christian Périgaud[a]; Gilles Gosselin[a]; Brent Korba[b]; Olivier Hantz[c]; Jean-Louis Imbach[a]
[a] Laboratoire de Chimie Bioorganique, UMR CNRS-USTL 5625, Université Montpellier II, Montpellier, FRANCE [b] Virology Branch, DMID, NIH, Bethesda, MD, USA [c] Unité de Recherche sur les Hépatites, le SIDA et les Rétrovirus Humains, Lyon, FRANCE

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THE PRONUCLEOTIDE APPROACH. III. SYNTHESIS, ANTI-HBV ACTIVITY AND STABILITY STUDIES OF THE BIS(*S*-PIVALOYL-2-THIOETHYL) PHOSPHOTRIESTER DERIVATIVE OF ACYCLOVIR

Gilles Valette[1], Jean-Luc Girardet[1], Alain Pompon[1] *, Christian Périgaud[1], Gilles Gosselin[1], Brent Korba[2], Olivier Hantz[3] and Jean-Louis Imbach[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]*Virology Branch, DMID, NIH, Solar Bldg, 6003 Executive Blvd, Bethesda, MD 20892, USA.*
[3]*Unité de Recherche sur les Hépatites, le SIDA et les Rétrovirus Humains, INSERM U 271, 151, cours Albert Thomas, 69424 Lyon cedex 3, FRANCE.*

Abstract: The nucleoside analog Acyclovir (ACV) is used in the treatment of *herpes simplex* (HSV) and *varicella-zoster* (VZV) diseases. The possibility to extend the application field of ACV by using the bis[SATE] pronucleotide approach in order to deliver ACVMP inside the cell was investigated. And actually, the title compound has potent anti-hepatitis B activity in cell culture experiments. Here, we also report its synthesis and stability in various media.

Several nucleoside analogues (Nu) are antiviral agents. In most cases, Nu must be phosphorylated by cellular kinases in three steps (Nu → NuMP → NuDP → NuTP). NuTP is the true drug, able to interfere with viral polymerases. Commonly, the first step (Nu → NuMP) is limiting and pronucleotides are designed to overcome this step. In this regard, we had introduced new phosphate bio-labile protecting groups, namely *S*-acyl-2-thioethyl (SATE) which were designed to be removed from the parent phosphotriesters by carboxylesterase-mediated hydrolysis inside the cells. We had already valided this approach by two ways [1-5]: (i) bis[SATE] derivatives of various NuMPs have shown anti-HIV activity, even when the cells were unable to undergo the first phosphorylation step (e.g. bis[SATE]AZTMP in TK^- cell types); (ii) decomposition pathways and kinetic parameters of these compounds have been determined in various biological media mimicing the extracellular content (tissue culture medium, human serum and gastric juice) or the intracellular content (cell extracts of lymphocytes). In cell extracts, all the investigated pronucleotides were quickly metabolized, giving rise to the corresponding

NuMPs. Furthermore, the pronucleotides were significantly more stable in extracellular media than in cell extracts, and it was possible to modulate the stability and the lipophilicity of the bis[SATE] pronucleotides as well as the release of NuMPs by modifying the nature of the acyl moiety (methyl, *iso*propyl, *tertio*butyl, ...). In all cases, the bis[(*t*-butyl)SATE]NuMPs were the most stable pronucleotides.

The nucleoside analog Acyclovir (ACV) is used to treat *herpes simplex* (HSV) and *varicella-zoster* (VZV) diseases. The first phosphorylating step of ACV is mediated by herpes virus-induced thymidine kinases [6]. This particular mechanism explains that ACV, which is not substrate for cellular nucleoside kinases, is inactive against other viruses. Therefore, we investigated the possibility to extend the application field of ACV by using the bis[SATE] pronucleotide approach in order to deliver ACVMP inside the cell. And actually, we found that the title compound bis[(*t*-butyl)SATE]ACVMP (**1**, Scheme 1)[7] has potent anti-hepatitis B activity in cell culture experiments.

SYNTHESIS. The title compound **1** was prepared by direct condensation of bis(*S*-pivaloyl-2-thioethyl)*N*,*N*-diisopropylphosphoramidite[4] with unprotected ACV in THF and in the presence of tetrazole, followed by oxidation with 3-chloroperoxybenzoic acid in methylene chloride. The yield was 83 %.

ANTI-HBV ACTIVITY. The anti-HBV activity of **1** was evaluated in human HBV transfected liver HepG2 cells on day 9. Results were compared with those obtained with the parent ACV, and 2'3'-dideoxyguanosine (ddG) as reference compound (Table 1). As expected, ACV proved to be virtually inactive against HBV-replication at concentrations up to 100 μM. This data illustrates the failure of ACV to undergo conversion to the active triphosphate form in HepG2 cells [6].

On the other hand, the pronucleotide **1** emerged in this cell culture experiment as a potent inhibitor of HBV, with 50% effective concentration (EC_{50}) which was in the same range as the EC_{50} value observed for the reference compound ddG. Moreover, the pronucleotide exhibited low toxicity in mock-infected HepG2 cells and proved to be 20 to 50-fold superior to ddG with regard to its selectivity index.

STABILITY STUDIES. The decomposition of **1** (initial concentration 5.0×10^{-5} M) was studied at 37°C in: (i) RPMI 1640 containing 10 % fetal calf serum (culture medium); (ii)

Table 1. Anti-HBV activity.[a] CC_{50}: 50 % cytotoxic concentration in μM [b] EC_{50}: 50 % effective concentration in μM. [c] SI: Selectivity index (ratio CC_{50}/EC_{50}). [d] NA: Not applicable.

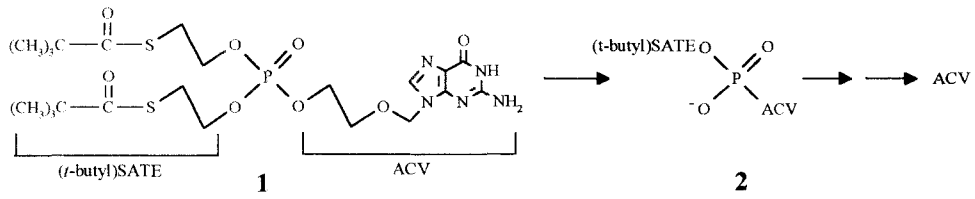
		HBV replicative intermediate		HBV virions	
	CC_{50}[a]	EC_{50}[b]	SI[c]	EC_{50}[b]	SI[c]
ACV	630	> 100	NA[d]	110	6
bis[(*t*-butyl)SATE]ACVMP	1600	1.1	1450	0.2	8000
ddG	220	3.4	65	1.3	170

full cell extract from CEM-SS cells; and (iii) various sera (man, monkey, rat, mouse, duck and woodchuck). During incubation, aliquots were removed and analysed using an "on-line-cleaning" method which allows the direct HPLC analysis of drugs and metabolites in biological samples without any pre-treatment [4,5]. The decomposition pattern and kinetic parameters of **1** and of its first metabolite, the corresponding phosphodiester mono[(*t*-butyl)SATE]ACVMP **2**, are reported on Scheme 1 and Table 2, respectively.

DISCUSSION. The present results illustrate the antiviral potential of the SATE pronucleotide approach. Applied to the well-established anti-herpetic drug ACV, whose the antiviral activity is strictly dependent on thymidine kinases specifically induced by the herpetic viruses, this approach gives rise in cell culture to a potent inhibitor of another virus, namely *Hepatitis B virus*.

The results strongly support the hypothesis that the anti-HBV activity of the bis[SATE]phosphotriester derivative **1** of ACV is related, via the intracellular delivery of the 5'-mononucleotide, to an accumulation of the phosphorylated forms of ACV inside the cell.

The *ex-vivo* decomposition studies of this pronucleotide in sera of various animals point out some insights on future *in-vivo* experimentations of SATE pronucleotides: (i) the stability of bis[(*t*-butyl)SATE]ACVMP is strongly dependent on the origin of the serum (man >> monkey ≅ rat ≅ duck >> woodchuck ≅ mouse). (ii) the first phosphodiester metabolite mono[(*t*-butyl)SATE]ACVMP **2**, once formed, is much more



Scheme 1. Structure and decomposition pathway of bis[(*t*-butyl)SATE]ACVMP

Table 2. Half-live of the investigated pronucleotide (**1**) and its first metabolite (**2**) in tissue culture medium, extract of CEM-SS cells, and sera of man and various animals.

	Culture Medium	Cell Extract	Sera					
			Human	Monkey	Rat	Duck	Wood-chuck	Mouse
$T_{1/2}$ of **1**	6.4 day	5.5 hr	14 hr	1.2 hr	1.1 hr	42 min	5 min	2 min
$T_{1/2}$ of **2**	144 day	12 day	6 day	1.6 day	8.7 hr	2.4 day	12.2 hr	5.8 hr

stable than the parent pronucleotide; furthermore, its stability is also strongly dependent on the serum but follows a different order (man > monkey ≅ duck > woodchuck ≅ rat ≅ mouse). Further experiments on the title compound in cell cultures as well as in animal models are currently in progress in our laboratories.

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