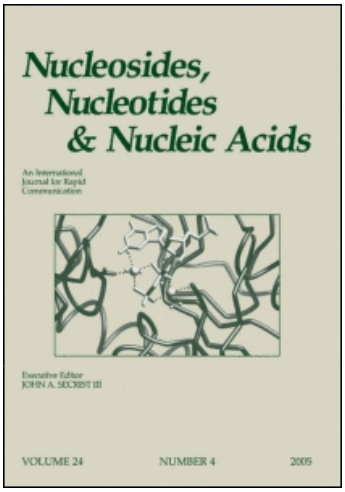

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DESIGN OF NEW MONONUCLEOTIDE PRODRUGS: ARYL (SATE) PHOSPHOTRIESTER DERIVATIVES

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ABSTRACT

Synthesis, biological activities and decomposition kinetics of novel phosphotriester derivatives of 3′-azido-2′,3′-dideoxythymidine (AZT) bearing a *S*-*t*Butyl-2-thioethyl (*t*BuSATE) group and L-tyrosinyl residues are reported. All the derivatives appeared to be potent inhibitors of HIV-1 replication in various cell culture experiments. The proposed decomposition process of these mixed phosphotriesters may involve successively an esterase and then a phosphodiesterase activation.

The antiviral activity of nucleoside analogues is closely related to their ability to be phosphorylated intracellularly. Many strategies have been envisaged to mask or reduce negative charges of nucleoside 5′-monophosphates, thereby forming more lipophilic derivatives (pronucleotides) which would be expected to revert back to the corresponding 5′-mononucleotides inside cells (1). Research progressed in this way with the design of mononucleoside phosphotriester derivatives incorporating two *S*-acyl-2-thioethyl (SATE) groups as biolabile phosphate protections (2). The decomposition pathway of such pronucleotides involves two successive esterase

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activation steps (3). In *vivo* pharmacokinetic studies of bis(*t*BuSATE) phosphotriesters seem to demonstrate that the second enzymate-mediated step could be considered limiting (4,5). Therefore, we decided to explore mixed phosphotriester derivatives bearing only one SATE chain and another moiety susceptible to be hydrolyzed selectively by an enzymatic system other than esterases. We previously showed that the mononucleoside phenyl (*t*BuSATE) phosphotriester derivative of AZT **1** (Fig.) leads *in vitro* to the selective intracellular release of the corresponding 5′-mononucleotide (6). Herein, we would like to report the synthesis and the evaluation of new phosphotriesters **2**–**4** bearing L-tyrosinyl residue as aryl substituent (Fig.).

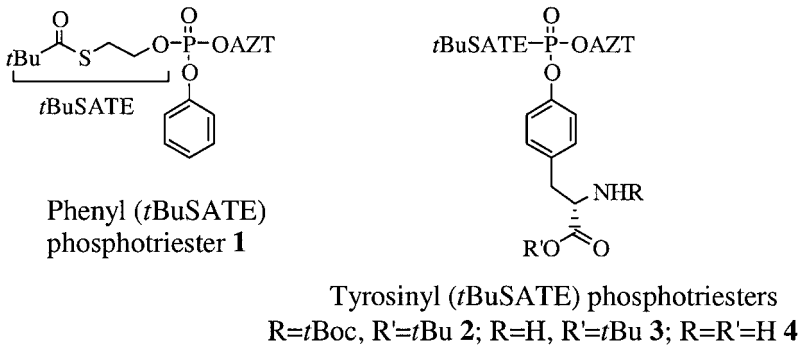

Figure 1. Structure of the studied arylphosphotriester derivatives of AZT.

Synthesis. The targeted compounds were obtained via a phosphoramidite approach (Scheme 1).

The phosphitylating agent **5** bearing the SATE moiety and the desired tyrosinyl residue was obtained from commercially available bis(diisopropylamino) chlorophosphine with subsequent coupling of the SATE chain (7) and then the aryl precursor **6** in presence of 1*H*-tetrazole. The reaction of the tyrosinyl (*t*BuSATE) phosphoramidite intermediate **5** with AZT, followed by *in situ* oxidation using *tert*-butyl hydroperoxyde, afforded the fully protected phosphotriester derivatives **2**. Treatment of compound **2** in various acidic conditions gave rise to partially protected **3** and to the fully deprotected tyrosinyl (*t*BuSATE) phosphotriester derivative of AZT **4**.

Antiviral evaluation. All the derivatives were evaluated for their *in vitro* anti-HIV activity and were potent inhibitors of HIV-1 replication in cell culture experiments (Table 1).

In contrast to AZT, the tyrosinyl (*t*BuSATE) phosphotriesters **2**–**4** exhibited significant anti-HIV effects in thymidine kinase-deficient cells (CEM/TK$^-$) with EC_{50} values in the same range as the one observed for the pronucleotide **1**. These results suggest that the mixed aryl phosphotriesters **2**–**4** were able to deliver intracellularly of the 5′-mononucleotide of AZT (AZTMP) and can be considered as new pronucleotides.

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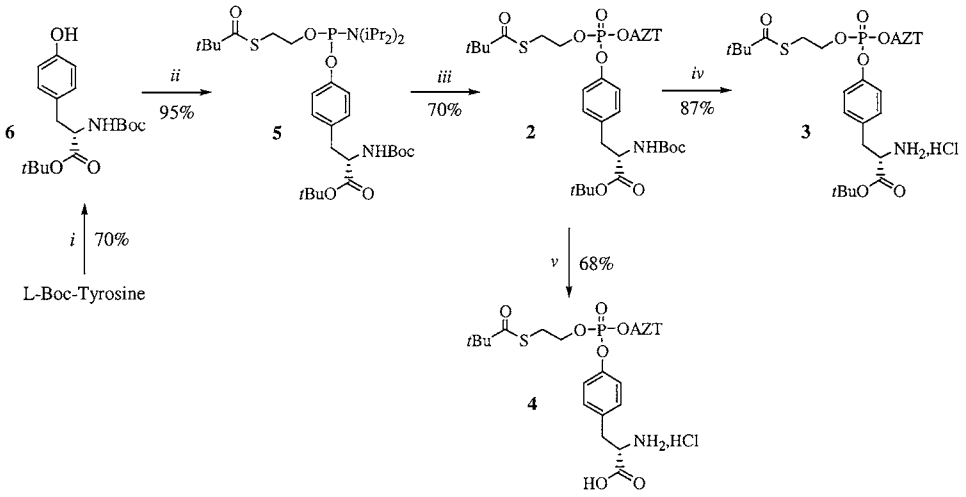


Scheme 1. Synthesis of tyrosinyl (*t*BuSATE) phosphotriester derivatives of AZT Reagents: (*i*) $(CH_3)_2NCH[OCH_2C(CH_3)_3]_2$, *t*BuOH, toluene; (*ii*) *t*BuSATEOP$[N(iPr)_2]_2$, 1*H*-tetrazole, $HN(iPr)_2$, CH_3CN; (*iii*) AZT, 1*H*-tetrazole, CH_3CN, then *t*BuOOH, toluene; (*iv*) 30 % HCl, Et_2O solution; (*v*) 10 % TFA, CH_2Cl_2, then HCl, dioxane solution.

Stability studies. Using a previously published procedure (3), the decomposition pathways and kinetic data of compounds **2**–**4** were studied in total CEM-SS extracts in order to mimic the conditions inside the cells. The metabolites formed during the incubation of the phosphotriester were identified by HPLC-MS and by coinjection with authentic samples. For this purpose, the intermediates **8**, **10** and **11** (Schemes 2 and 3) were synthesized using a H-phosphonate approach (8).

These results indicate that the decomposition mechanism for the fully protected phosphotriester **2** (Scheme 2) occurred following two concomitant

Table 1. Anti-HIV-1 Activity in Various Cell Lines of Mixed Aryl Phosphotriester Derivatives **2**–**4** in Comparison to the Parent Nucleoside AZT and to the Phenyl (*t*BuSATE) Phosphotriester Derivative **1** as Reference Pronucleotide[a]

	CEM-SS		MT-4		CEM/TK$^-$	
Compound	EC_{50}[b]	CC_{50}[c]	EC_{50}[b]	CC_{50}[c]	EC_{50}[b]	CC_{50}[c]
AZT	0.003	>1	0.015	>1	>100	>100
1	0.002	>10	0.070	>10	3.5	>10
2	0.034	>1	0.250	>1	0.90	>1
3	0.004	>10	0.042	>10	2.7	>10
4	0.006	>100	0.075	>10	29	>100

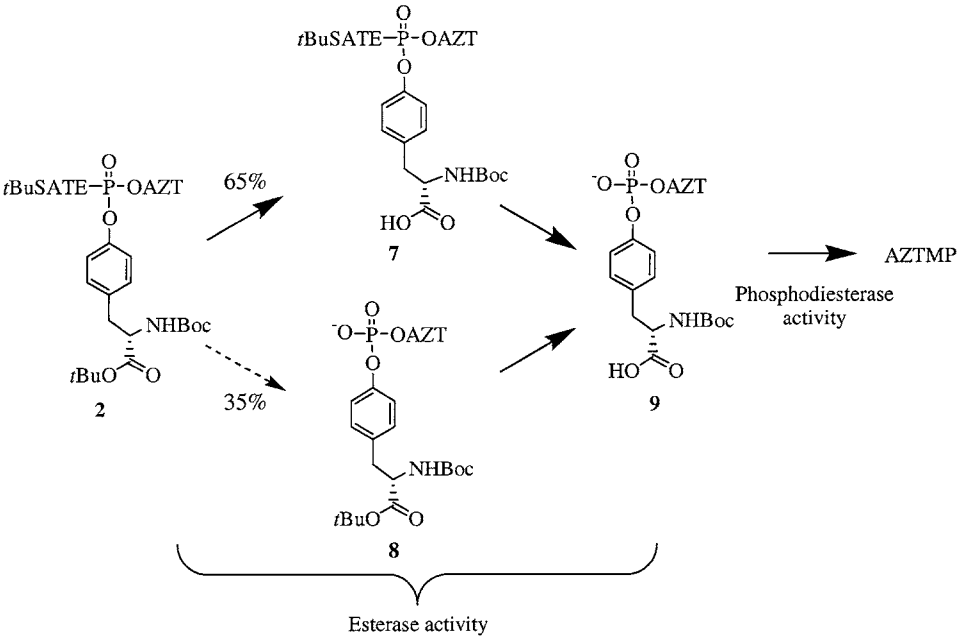
[a]All data represent average values for at least three separate experiments. The variation of these results under standard operating procedures is below ± 10 %. [b]EC_{50}: effective concentration (μM) or concentration required to inhibit the replication of HIV-1 by 50%. [c]CC_{50}: cytotoxic concentration (μM) or concentration required to reduce the viability of uninfected cells by 50%.

esterase-mediated steps: (i) a main decomposition pathway (65%) resulting to the loss of the *t*Butyl group on the tyrosinyl residue, and leading to the phosphotriester **7**; (ii) hydrolysis of the *t*BuSATE chain giving rise to the formation of the corresponding phosphodiester **8**. The metabolites **7** and **8** were then converted to the aryl phosphodiester **9**, which was further hydrolyzed into AZTMP through phosphodiesterase activity.

The two esterase-mediated steps were also observed for phosphotriester **3** in total CEM-SS cell extracts (Scheme 3). Nevertheless, compared to its analog **2**, the major decomposition pathway (93%) of **3** corresponds to the loss of the *t*BuSATE chain from **4** leading to phosphodiester **10**. This is probably due to the presence of the ionisable (at physiological pH) amino function in the vicinity of the ester functionality. Finally, the metabolite **10** is directly converted into AZTMP (phosphodiesterase activity).

The decomposition pathway of the fully deprotected phosphotriester **4** resulted successively in the removal of the SATE moiety (metabolite **11**, Scheme 3) and then of the tyrosinyl residue.

The half-lives of the mononucleoside phosphotriesters and the resulting metabolites are summarized in Table 2. Comparison of the enzymatic stability of the arylphosphodiester **10** and **11** (Scheme 3) demonstrated that the presence of a *t*Butyl ester functionality on the tyrosinyl residue of the phosphodiester derivatives seems to modify the rate of the phosphodiesterase mediated step (Table 2). In order to



Scheme 2. Proposed decomposition mechanism of the fully protected derivative **2** in total CEM-SS cell extracts.

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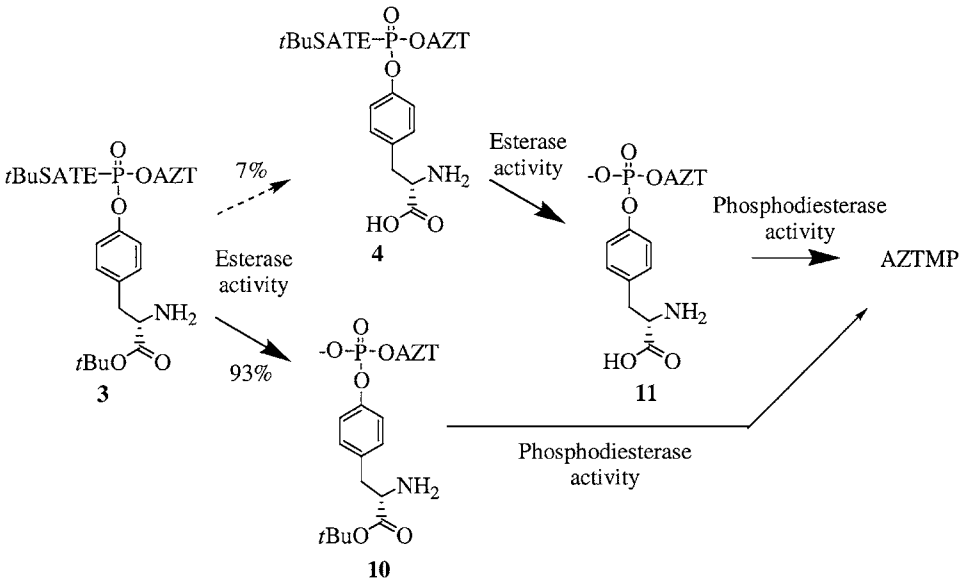

Scheme 3. Proposed decomposition mechanism of derivatives **3** and **4** in total CEM-SS cell extracts.

confirm that the conversion of the aryl phosphodiester metabolites to AZTMP was not due to a chemical process, the stability of metabolite **11** was determined in phosphate buffer (pH 7) and in denatured cell extract. No degradation of this entity was observed after a week of incubation at 37°C.

The comparison of kinetic data, especially the estimated half-life of AZTMP formation, Log P values (which have been correlated with the passive diffusion of compounds through cellular membranes) and EC_{50} in CEM-SS/TK^- cells for the studied compounds, indicates that the observed antiviral activity is closely related to both parameters (Table 3).

It is known that a time-dependent relationship is required between the release of the phosphorylated forms from the pronucleotide and expression of the cellular target in order to observe an antiviral effect (2,9,10). Thus, derivative **4** presents

Table 2. Calculated Half-Lives of the Phosphotriester Derivatives **2**–**4** and Their Metabolites in CEM-SS Cell Extracts, and Corresponding Pseudo First-Order Rate Constants

	Compound	Metabolites		
	2	**7**	**8**	**9**
$t_{1/2}$	<10 min	28 min	40 min	4.3 h
k (min^{-1})	0.061	0.025	0.017	0.0027
	3	**10**	**4**	**11**
$t_{1/2}$	20 min	3.9 h	1.2 h	25 h
k (min^{-1})	0.035	0.0030	0.0093	0.00045

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Table 3. Estimated Half-Lives of AZTMP Formation, Apparent Water Octanol Partition Coefficient (Log P) and EC_{50} in CEM/TK^- Cells

	$t_{1/2}$ of AZTMP Formation	Log P	EC_{50} (μM) in CEM/TK^-
2	5.3 h	4.29	0.9
3	5.1 h	2.90	2.7
4	25 h	0.24	29

the weaker activity whereas fully protected **2** appeared the more potent, due to favorable properties (fast release of AZTMP and high lipophilicity).

CONCLUSION

The present study demonstrates that aryl (*t*BuSATE) phosphotriester derivatives of AZT **2**–**4** are able to deliver the parent 5′-mononucleotide efficiently and they can be considered as novel pronucleotides. The proposed mechanism of decomposition of these mixed phosphotriesters involves successively an esterase and a phosphodiesterase step. The large number of chemical modifications which could be envisaged on the aryl moiety enables the search for antiviral mononucleotide prodrugs with a balance between aqueous solubility, lipophilicity, and enzymatic stability in order to envisage further *in vivo* pharmacological studies. Work on this area is currently in progress in our group.

ACKNOWLEDGMENTS

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