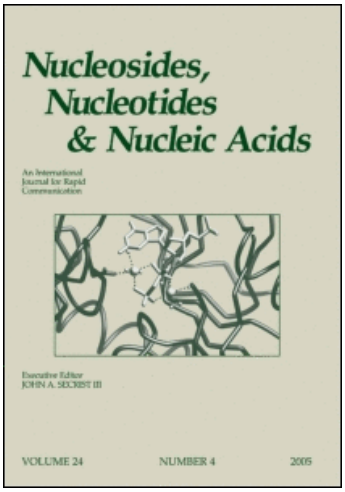

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The Pronucleotide Approach. II: Synthesis and Preliminary Stability Studies of Mononucleoside Glycosyl Phosphotriester Derivatives

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THE PRONUCLEOTIDE APPROACH. II: SYNTHESIS AND PRELIMINARY STABILITY STUDIES OF MONONUCLEOSIDE GLYCOSYL PHOSPHOTRIESTER DERIVATIVES

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ABSTRACT: The synthesis and preliminary stability studies of mononucleoside phosphotriester derivatives of 3'-azido-2',3'-dideoxythymidine (AZT) incorporating a new kind of phosphate protecting group, namely *S*-glycopyranosidyl-2-thioethyl (SGTE), are reported.

INTRODUCTION

We have previously demonstrated that mononucleoside bis(SATE) phosphotriesters,[1] and their isomeric *S,S'*-bis(*O*-acyl-2-oxyethyl) phosphorodithiolate forms [isoSATE pronucleotides, see Part I] allow the intracellular delivery of their parent nucleoside 5'-monophosphates in cell culture experiments. As a part of our anti-HIV drug program, we decided to extend our investigations in the design of new kinds of transient phosphate protections by studying phosphotriester derivatives incorporating *S*-glycopyranosidyl-2-thioethyl groups (SGTE, FIGURE 1).

This class of pronucleotides might be hydrolyzed by glycosidases. Some glycosidases are known to be essentially intracellular,[2] what should induce a more selective delivery of the 5'-mononucleotide. Furthermore, the carbohydrate groups increase the water solubility of the corresponding pronucleotide and might be used as a site-directing moiety toward glycosyl-binding proteins (lectins[3]) on the cell surfaces of macrophages,[4,5] or as substrates for monosaccharide facilitated diffusion transport systems at the blood brain barrier.[6]

Here, we report the synthesis and preliminary stability studies of bis(SGTE) phosphotriester derivatives of 3'-azido-2',3'-dideoxythymidine (AZT) containing

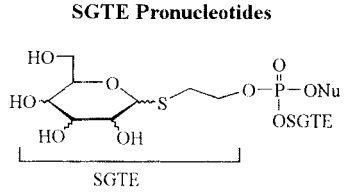


Figure 1

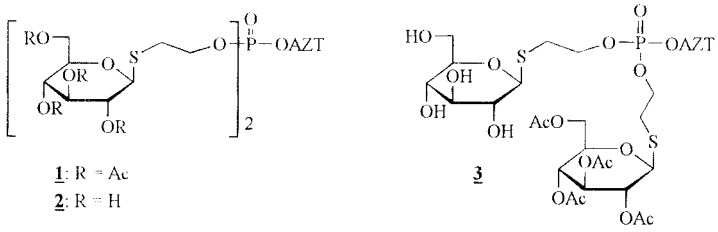


Figure 2: *Structure of the studied glycosyl phosphotriester derivatives*

glucopyranosidyl moieties (FIGURE 2). The acetyl groups of phosphotriesters **1** and **3**, which are likely to be removed by esterases, induce different degrees of lipophilicity and may influence cell penetration as well as enzymatic recognition.

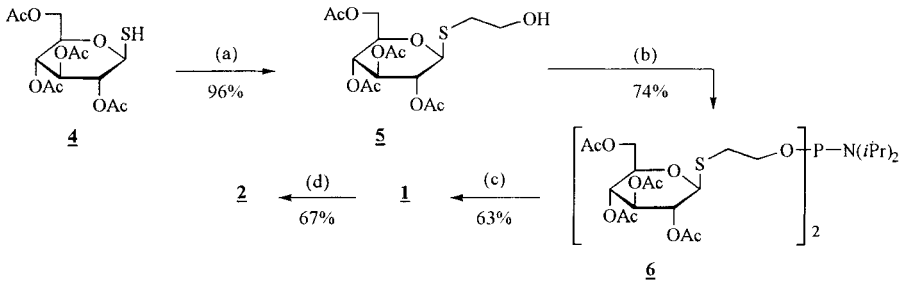
SYNTHESIS

We have previously published the synthesis of SATE pronucleotides using an approach involving trivalent phosphorus intermediates.[1] In this work, the pronucleotides **1** and **2** were prepared according a similar procedure, which involved the synthesis of an appropriate phosphoramidite agent (Scheme 1).

The mixed phosphotriester **3** containing one fully acetylated and one fully deprotected glucopyranoside moiety was synthesized as shown in Scheme 2.

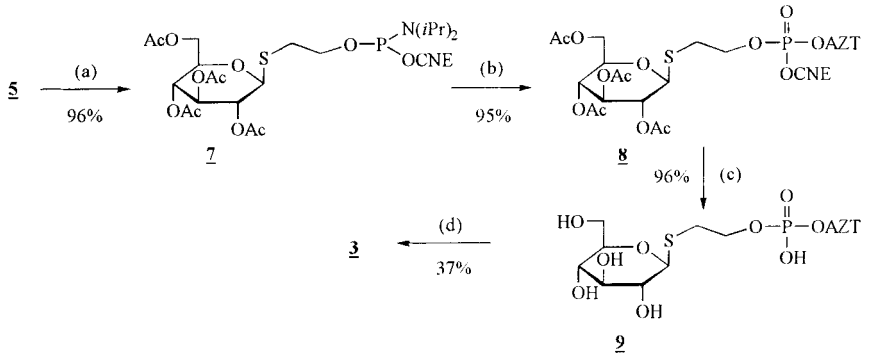
PRELIMINARY STABILITY STUDIES

Using an improved "on-line ISRP cleaning" HPLC method,[1] the half-lives of the pronucleotides **1**-**3** (TABLE 1) were determined in water, RPMI and culture medium (RPMI containing 10% heat-inactivated fetal calf serum).



(a) ICH_2CH_2OH, DBU, toluene; (b) $PCl_2N(i\text{-}Pr)_2$, Et_3N, THF; (c) 1. AZT, 1*H*-tetrazole, THF, 2. *t*BuOOH; (d) Dowex 1X2 (OH^- form), ethanol 95%.

Scheme 1: *Synthesis of bis(glucopyranosidyl)phosphotriester derivatives* **1** *and* **2**

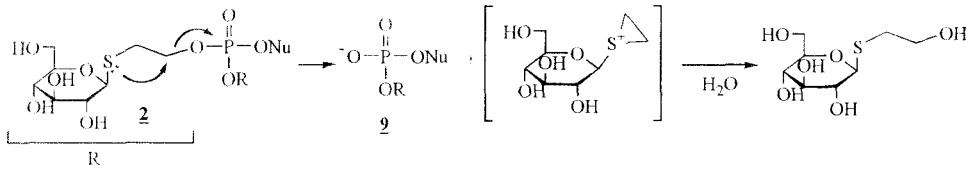


(a) $NCCH_2CH_2OP[N(i\text{-}Pr_2)]_2$, 1*H*-tetrazole, $HN(i\text{-}Pr)_2$, CH_3CN; (b) 1. AZT, 1*H*-tetrazole, CH_3CN, 2. *t*BuOOH; (c) NaOMe, MeOH; (d) 1. Dowex 50WX2 [$(n\text{-}C_4H_9)N^+$ form], 2. 2-iodoethyl 2,3,4,6-tetraacetyl-1-β-D-thioglucopyranoside (**10**), CH_3CN, 80°C.

Scheme 2: *Synthesis of the mixed phosphotriester* **3**

Table 1: *Half-lives of the studied bis(glycosyl) phosphotriester derivatives*

		compound		
		1	**2**	**3**
$t_{1/2}$	water	100 h	4.5 h	8.8 h
	RPMI	35 h	4.5 h	8.3 h
	culture medium	30 h	4.5 h	8.3 h



Scheme 3: *Hypothetical chemical decomposition mechanism for* **2**

The three compounds were hydrolyzed essentially into their corresponding phosphodiesters, except the peracetylated phosphotriester **1**, which yielded a major part of deacetylation products in RPMI and culture medium.

The half-lives of **2** and **3** varied very little in the three considered media and thus, their decomposition seems to be essentially due to chemical mechanisms. Beside possible nucleophilic attacks on the phosphorus atom by nucleophiles present in the media, an additional hydrolytic mechanism involving participation of the sulfur atom (Scheme 3) may be involved.[7]

CONCLUSION

We have synthesized new phosphotriester derivatives of AZT which incorporate two thioglucopyranosidyl groups as transient phosphate protections. Further stability studies and the evaluation of the antiviral activities of compounds **1**-**3** are currently in progress.

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