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SYNTHESIS AND PROPERTIES OF NOVEL TRIPHOSPHATE ANALOGUES: RIBONUCLEOSIDE AND DEOXYRIBONUCLEOSIDE (α -*P*-BORANO, α -*P*-THIO)TRIPHOSPHATES

Jinlai Lin^a, Kenneth W. Porter^a, Barbara Ramsay Shaw^a

^a Paul M. Gross Chemical Laboratory, Department of Chemistry, Duke University, Durham, North Carolina, U.S.A.

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**SYNTHESIS AND PROPERTIES OF NOVEL
TRIPHOSPHATE ANALOGUES:
RIBONUCLEOSIDE AND
DEOXYRIBONUCLEOSIDE (α -*P*-BORANO,
 α -*P*-THIO)TRIPHOSPHATES**

Jinlai Lin, Kenneth W. Porter, and Barbara Ramsay Shaw*

Paul M. Gross Chemical Laboratory, Department of Chemistry,
Duke University, Durham, North Carolina 27708-0346

ABSTRACT

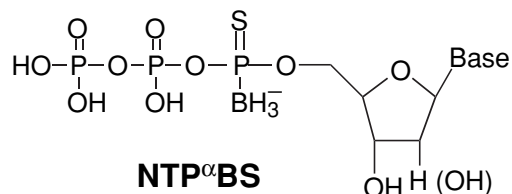
The first ribo- and deoxyribo-nucleoside (α -*P*-borano, α -*P*-thio)triphosphates have been synthesized. The chemical and biochemical properties of adenosine (α -*P*-borano, α -*P*-thio)triphosphate and thymidine (α -*P*-borano, α -*P*-thio)triphosphate have been investigated.

INTRODUCTION

Modified nucleoside triphosphates have received much attention in searches for potential diagnostic and therapeutic agents and as probes in a multitude of biological processes (1-2). Of these modified nucleoside triphosphates, the nucleoside 5'-*O*-(α -*P*-thio)triphosphates (1) and nucleoside 5'-*O*-(α -*P*-borano)triphosphates (2) are among the most promising. By structurally combining the phosphorothioate (3) [S-P=O]⁻ and boranophosphate (4) [O=P-BH₃]⁻, we recently reported the first example of a boranothiophosphate moiety [S=P-BH₃]⁻, dithymidine boranophosphorothioate (5), which is a more nuclease resistant and highly lipophilic phosphodiester analogue of DNA. We expected that the corresponding nucleoside

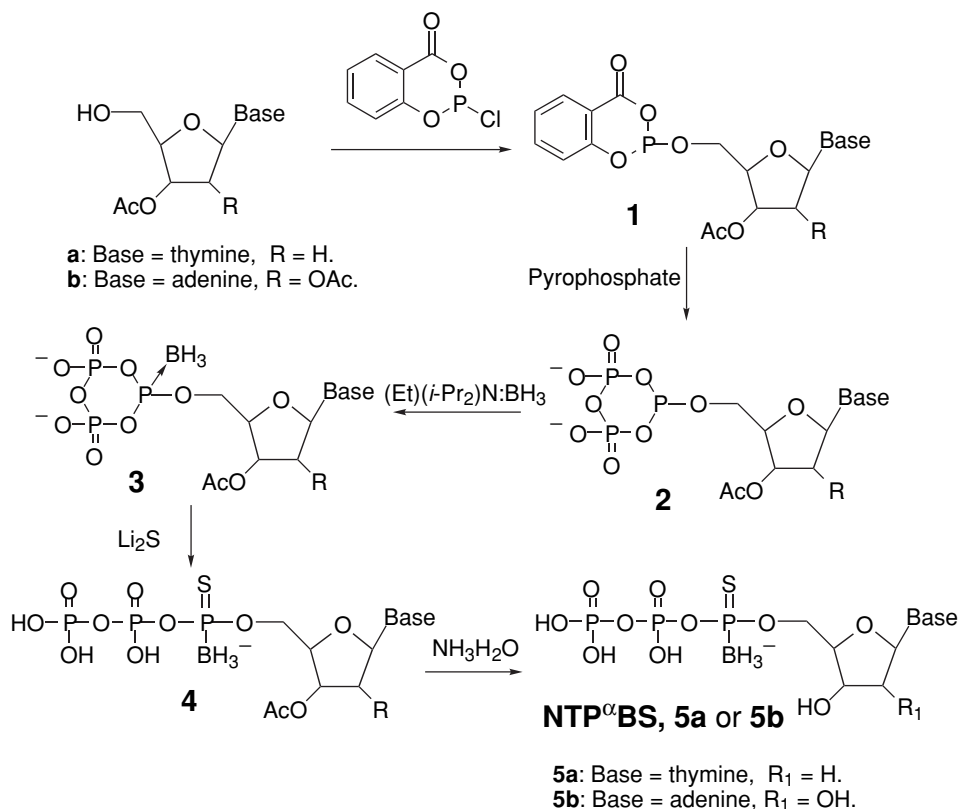
*Corresponding author. E-mail: brs@chem.duke.edu

triphosphate (NTP) analogue, nucleoside α -*P* boranotriphosphate (NTP $^{\alpha}$ BS), could have many useful properties.



Here, we report the first example of deoxyribo- and ribo-nucleoside (α -*P*-borano, α -*P*-thio)triphosphate compounds, specifically the thymidine 5'-*O*-(α -*P*-borano, α -*P*-thio)triphosphate **5a**, and adenosine 5'-*O*-(α -*P*-borano, α -*P*-thio)triphosphate **5b**, their synthesis and properties.

The general procedure for the synthesis of nucleoside 5'-*O*-(α -*P*-borano, α -*P*-thio)triphosphates [nucleoside α -*P*-boranotriphosphorothioates] is shown in Scheme 1. 3'-*O*-Acetyl thymidine or a 2', 3'-*O*-diacetyl adenosine was



Scheme 1.



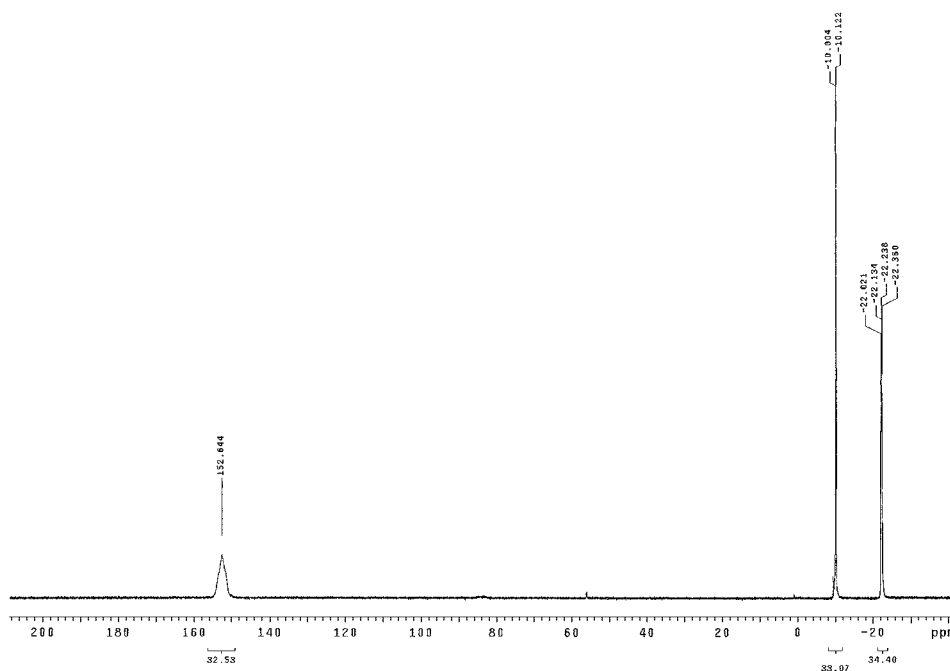


Figure 1. ^{31}P NMR (D_2O) spectrum of $\text{TTP}^\alpha\text{BS}$, **5a**.

phosphitylated to yield two diastereomers of **1**, which were treated with tributylammonium pyrophosphate to form a cyclic intermediate **2**. The borane group was introduced by the reaction of compound **2** with excess borane-diisopropylethylamine complex to afford **3**. The critical step in the synthesis is ring-opening of the cyclic boronated triphosphate **3** by the treatment with lithium sulfide at 55°C to yield **4**, which was converted to compound **5a** or **5b** with $\text{NH}_3\text{H}_2\text{O}/\text{CH}_3\text{OH}$. The overall yield of thymidine 5'-*O*-(α -*P*-borano, α -*P*-thiotriphosphate) **5a** ($\text{TTP}^\alpha\text{BS}$) was about 26%. The chemical structures of **5a** and **5b** were established via spectroscopic methods. ^{31}P NMR (D_2O , 161.9 MHz) δ (ppm) for **5a** [Fig. 1]: 153.1 ppm(br) for α -P, -22.4 ppm and -22.7 ppm for β -P, and -10.0 and -10.1 ppm for γ -P. Successful separation of the two diastereomers (*Rp* and *Sp*) of **5a** or **5b** was achieved by reverse-phase HPLC. The first eluted isomer $\text{TTP}^\alpha\text{BS}$ I (**5a-I**) and the second eluted isomer $\text{TTP}^\alpha\text{BS}$ II (**5a-II**) were characterized by ^{31}P NMR, ^1H NMR and MS. The method used above should be applicable for the synthesis of any ribo- or deoxyribonucleoside (α -*P*-borano, α -*P*-thio)triphosphates.

RESULTS AND DISCUSSION

Our preliminary studies indicated that thymidine 5'-*O*-(α -*P*-borano, α -*P*-thio)triphosphate was a possible substrate for Taq DNA polymerase. To assess the extent to which the thymidine boranothiotriphosphate could serve as a substrate for

DNA polymerase, primer extension experiments were performed with Taq DNA polymerase. Primer was annealed to template and extended in the presence of dATP, dGTP, dCTP and either normal TTP, boranethiotriphosphate $\text{TTP}^\alpha\text{BS}$ (isomer I or isomer II), or no additional thymidine nucleotide. Isomer II supported primer extension no better than the control that contained no TTP nucleotide, indicating practically no incorporation of the second isomer. However, the boranethiotriphosphate $\text{TTP}^\alpha\text{BS}$ isomer I supported primer extension to an extent approximately double that of isomer II, which was about ten percent (10%) that of normal TTP. Therefore, while boranethiotriphosphate $\text{TTP}^\alpha\text{BS}$ isomer II is not a substrate, boranethiotriphosphate $\text{TTP}^\alpha\text{BS}$ isomer I appears to be a substrate for Taq DNA polymerase, albeit a poor one. If nucleoside (α -P-borano, α -P-thio)triphosphates ($\text{NTP}^\alpha\text{BS}$) are strong inhibitors for DNA or RNA polymerases, they might be applied to block virus (such as HIV) replication. Studies of the biochemical properties of ribo- or deoxyribonucleoside (α -P-borano, α -P-thio)triphosphates towards various enzymes and their application in biochemistry and molecular biology as substrates or inhibitors for DNA and RNA polymerases for sequencing, mutagenesis, and the labeling of hybridization probes are currently underway.

To summarize, we have synthesized a new type of doubly modified nucleoside triphosphate analogue, in which the two nonbridging oxygen atoms of an a phosphodiester group have been replaced with a sulfur atom and borane group. The synthesis of the first $[\text{S}=\text{P}-\text{BH}_3]^-$ triphosphate analogue opens the possibility of preparing an entirely new and intriguing class of boron/sulfur modified nucleotides and nucleic acids. Their similarity to natural nucleoside triphosphates and their potential utility as substrates or inhibitors for DNA or RNA polymerases, and as molecular probes for the study of stereochemical aspects of enzymatic and nonenzymatic reactions, make the nucleoside boranethiotriphosphate a promising candidate for further mechanistic and diagnostic applications.

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