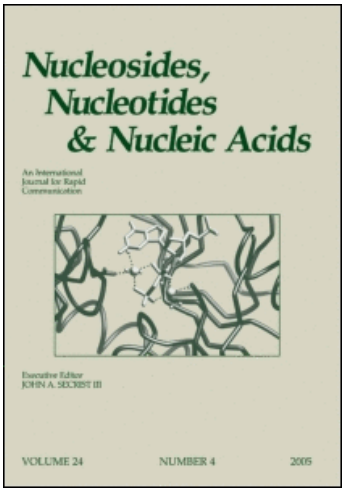

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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

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Online publication date: 31 March 2001

To cite this Article Lin, Jinlai , Porter, Kenneth W. and Shaw, Barbara Ramsay(2001) 'SYNTHESIS AND PROPERTIES OF NOVEL TRIPHOSPHATE ANALOGUES: RIBONUCLEOSIDE AND DEOXYRIBONUCLEOSIDE (α-*P*-BORANO, α-*P*-THIO)TRIPHOSPHATES', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 1019 — 1023
To link to this Article: DOI: 10.1081/NCN-100002482
URL: http://dx.doi.org/10.1081/NCN-100002482

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 1019–1023 (2001)

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

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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) $[O{=}P{-}BH_3]^-$, we recently reported the first example of a boranothiophosphate moiety $[S{=}P{-}BH_3]^-$, dithymidine boranophosphorothioate (5), which is a more nuclease resistant and highly lipophilic phosphodiester analogue of DNA. We expected that the corresponding nucleoside

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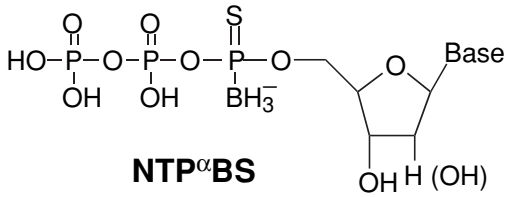
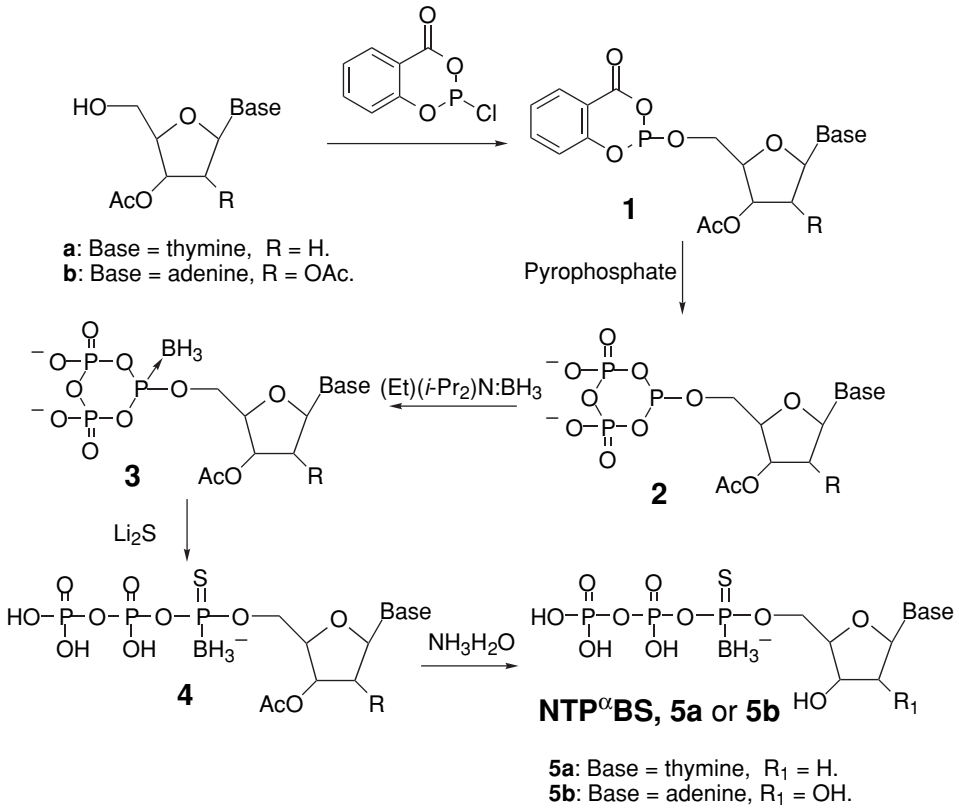
triphosphate (NTP) analogue, nucleoside α-*P* boranothiotriphosphate (NTP$^{\alpha}$BS), could have many useful properties.



NTP$^{\alpha}$BS

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.

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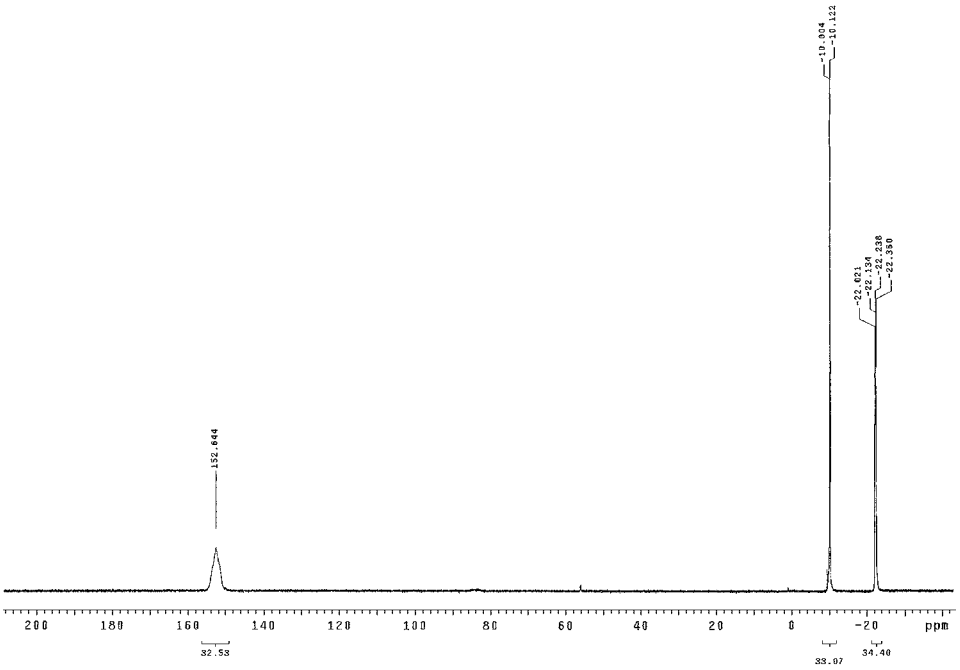

Figure 1. ^{31}P NMR (D_2O) spectrum of **TTPαBS**, **5a**.

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 NH_3H_2O/CH_3OH. The overall yield of thymidine 5′-*O*-(α-*P*-borano, α-*P*-thiotriphosphate) **5a** (TTP$^{\alpha}$BS) was about 26%. The chemical structures of **5a** amd **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 TTP$^{\alpha}$BS I (**5a–I**) and the second eluted isomer TTP$^{\alpha}$BS II (**5a–II**) were characterized by ^{31}P NMR, ^{1}H 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, boranothiotriphosphate TTP$^{\alpha}$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 boranothiotriphosphate TTP$^{\alpha}$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 boranothiotriphosphate TTP$^{\alpha}$BS isomer II is not a substrate, boranothiotriphosphate TTP$^{\alpha}$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 (NTPαBS) 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 $[S{=}P{-}BH_3]^-$ 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 boranothiotriphosphate a promising candidate for further mechanistic and diagnostic applications.

ACKNOWLEDGMENT

This work was supported by grants 1R01-GM57693-01 from NIH and DE-FG05-97ER62376 from DOE to B.R.S.

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