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# Synthesis of Acyclothymidine Triphosphate and α-*P*-Boranotriphosphate and Their Substrate Properties with Retroviral Reverse Transcriptase

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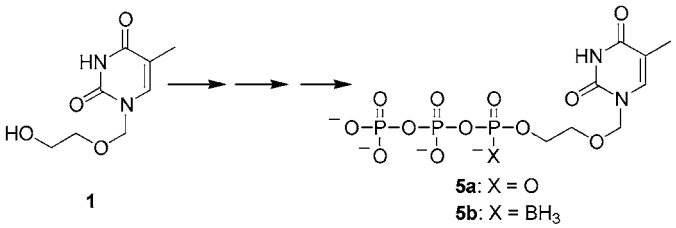
## ABSTRACT




**The first example of an acyclonucleoside α-*P*-boranotriphosphate has been synthesized via a phosphoramidite approach in a one-pot reaction with good yield. The presence of the α-*P*-$BH_3$ in 5b results in a 9-fold increase in efficiency of incorporation by MMLV retroviral reverse transcriptase relative to non-boronated 5a in pre-steady-state conditions. The preliminary results indicate that acyclonucleoside α-*P*-boranotriphosphates may have promising applications as a probe of enzyme mechanisms and in the design of new antiviral drugs.**

At the forefront of antiviral therapeutics has been the design of new classes of nucleosides and nucleotides.[1] Among these, one type of nucleoside modification is an acyclic nucleoside analogue,[2,3] in which the pentafuranosyl sugar ring in the natural nucleoside has been replaced with an acyclic moiety. Such analogues have shown potent antiviral activity. For example, 9-(2-hydroxyethoxymethyl)guanine (acyclovir, ACV)[2] is one of the most effective drugs against the varicella-zoster virus (VZV),[3] Epstein−Barr virus (EBV),[3d] and herpes simplex viruses (HSV-1 and HSV-2).[3b] Most acyclic nucleoside analogues become active after a series of intracellular conversions to the corresponding triphosphates, which are then incorporated into viral DNA and subsequently cause chain termination.[2b,c] Numerous methods,[4a] including pre-steady-state kinetic analyses[4b] of incorporation by HIV-1 reverse transcriptase (RT) and mitochondrial DNA polymerase γ, indicate that acyclonucleoside triphosphates (acycloNTP, Figure 1) may serve as effective antiviral drugs.

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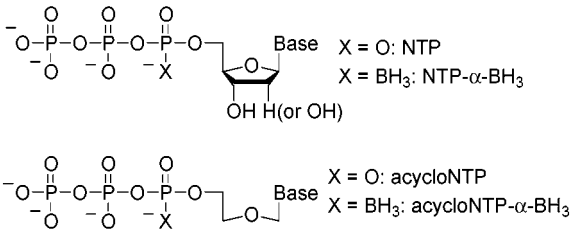



**Figure 1.** Structures of nucleoside triphosphate (NTP) and 5′-(α-P-borano)triphosphate (NTP-α-$BH_3$); acyclonucleoside triphosphate (acycloNTP) and α-*P*-boranotriphosphate (acycloNTP-α-$BH_3$).

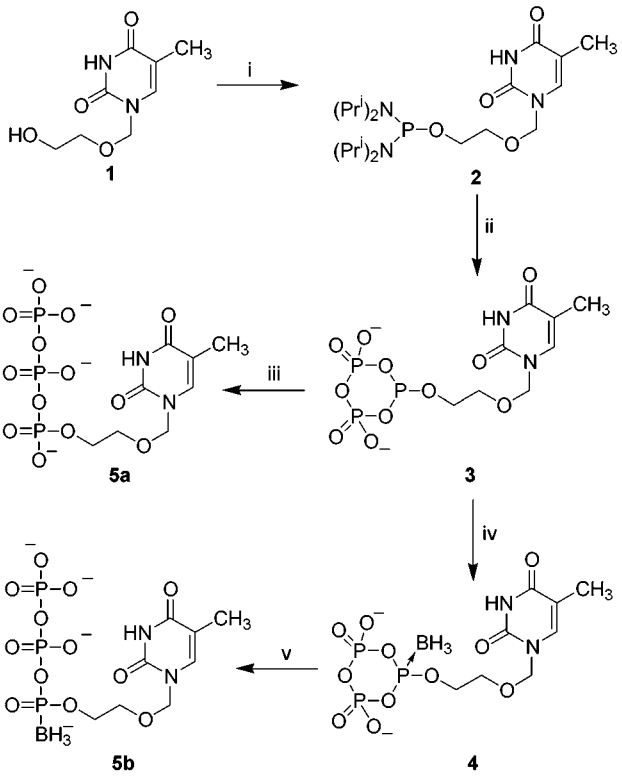
They exhibit low mitochondrial toxicity as well as an absence of HIV-1 RT mutations leading to drug resistance.[4b]

Nucleoside 5′-(α-*P*-borano)triphosphate[5] (NTP-α-$BH_3$, Figure 1) is a new type of nucleotide modification, in which a borane group ($BH_3$) substitutes for one of the nonbridging α-phosphate oxygens in nucleoside 5′-triphosphate (NTP). The presence of the $BH_3$ group at the α-phosphate of triphosphates of clinically relevant dideoxy compounds, such as 3′-azido-3′-deoxythymidine (AZT),[6a] 2′,3′-didehydrodideoxythymidine (D4T),[6a] and 2′,3′-dideoxyadenosine (ddA),[6b,c] improves both phosphorylation by nucleotide diphosphate kinase and incorporation by wild-type[6a] and mutant HIV-1 RTs.[6b,c] Moreover, after an α-*P*-borane group is incorporated into DNA, repair of the blocked DNA chains by pyrophosphorolysis is reduced significantly with mutant RT enzymes from drug-resistant viruses.[6a]

Because of the powerful antiviral activity of acyclonucleosides and the advantages granted by the presence of an α-*P*-borane group in triphosphates, we set out to synthesize an acyclonucleoside α-*P*-boranotriphosphate (acycloNTP-α-$BH_3$, Figure 1) and determine whether it could be a substrate for a viral RT. Specifically, the incorporation of acyclothymidine α-*P*-boranotriphosphate (acycloTTP-α-$BH_3$, **5b**) into viral DNA by moloney murine leukemia virus (MMLV) RT was investigated by using pre-steady-state kinetics.

Although the initial synthesis of NTP-α-$BH_3$ used a phosphoramidite approach,[5a] certain limitations, such as isolation of one intermediate compound and two ion-exchange column chromatography steps, resulted in a low overall yield. However, we thought that with some alterations the phosphoramidite approach would be a viable and efficient way to synthesize α-*P*-boranotriphosphates. Here we demonstrate that the sugar-substituted and α-phosphate-modified triphosphate, e.g., acycloTTP-α-$BH_3$ **5b**, can be synthesized

**Scheme 1**[a]




[a] Reagents and conditions: (i) $[(^iPr)_2N]_2PCl$, DIPEA, DMAP, $CH_3CN$, 15 min; (ii) $(HBu_3N^+)_2P_2O_7^{2-}$, 1*H*-tetrazole, 15 min; (iii) $I_2$/pyridine/$H_2O$, total yield 48% from **1**; (iv) 2 M $BH_3$:$SMe_2$ in THF, 30 min; (v) $H_2O$/$Et_3N$, 5 h, total yield 53% from **1**.

in a one-pot reaction via a phosphoramidite approach (Scheme 1).

Formation of a triphosphate usually requires the use of a phosphitylating reagent. Salicyl phosphochloridite, which has been used extensively in the synthesis of NTP[7] and NTP-α-$BH_3$,[8a−c] is difficult to handle because of its high reactivity and hygroscopicity. As an alternate phosphitylating reagent, we chose a reasonably reactive phosphorus compound, bis-(diisopropylamino)chlorophosphine ($[(^iPr)_2N]_2PCl$). Acyclothymidine **1**[9] was first phosphorylated by $[(^iPr)_2N]_2PCl$ dissolved in dry chloroform to form phosphoramidite **2** in the presence of 4 equiv of diisopropylethylamine (DIPEA) and 0.2 equiv of 1,4-(dimethylamino)pyridine (DMAP). This step is completed in 15 min, and intermediate **2** was identified by the appearance of one signal at $\delta$ 127.72, observed in the $^{31}P$ NMR spectra of the reaction mixture. A large excess of the base, DIPEA, is required for the quick completion of the reaction. Rather than carrying out the boronation step

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after the phosphitylation, as in the previously reported phosphoramidite approach,[5a] compound **2** was treated directly with the solution of pyrophosphate in DMF to form a cyclic intermediate $P^1$-acyclothymidinyl-$P^2$,$P^3$-dioxo-cyclotriphosphite **3**. Formation of intermediate **3** was monitored by $^{31}$P NMR, in which the singlet at $\delta$ 127.72 for phosphoramidite **2** was transformed to a triplet at $\delta$ 105.73 ($P^1$, $J$ = 43.55 Hz) for cyclic intermediate **3** along with the appearance of a doublet at $\delta$ −20.73 ($P^2$,$P^3$, $J$ = 43.39 Hz). Without the addition of 1*H*-tetrazole, the formation of cyclotriphosphite **3** could be sluggish.[10] However, the displacement reaction by pyrophosphate was finished in 15 min when 4 equiv of 1*H*-tetrazole was added.

Cyclotriphosphite **3** was oxidized with iodine/pyridine/water to yield the normal acyclothymidine triphosphate (acycloTTP) **5a**. Alternatively, an in situ boronation of cyclotriphosphite **3** resulted in $P^1$-acyclothymidinyl-$P^1$-borano-$P^2$,$P^3$-dioxo-cyclotriphosphate **4**. The presence of the $P\rightarrow B$ bond in cycloboranophosphate **4** was confirmed by $^{31}$P NMR spectra, which showed a broad peak centered at $\delta$ 90.30 for $P^1$, characteristic of a boranophosphate group.[5a,8] A slight upfield shift of the doublet at $\delta$ 24.47 ($J$ = 45.82 Hz) for $P^2$ and $P^3$ peaks in cyclic compound **4** was also observed.[7,8a−c] Of several borane complexes tried for boronation, a 2 M solution of borane-dimethyl sulfide in THF gave the best results. Cycloboranophosphate **4** was finally treated with water/triethylamine to give the ring-opened product acycloTTP-α-$BH_3$ **5b**. The addition of triethylamine greatly reduced the time for the hydrolysis step.[8] The final products, triphosphates **5a**[11] and **5b**, were purified by ion exchange and HPLC with overall yields of 48% and 53%, respectively.

Single nucleotide incorporation of acycloTTP **5a** or its analogue, acycloTTP-α-$BH_3$ **5b**,[12] into a 5′-HEX-modified 19-mer DNA primer was performed with MMLV RT with use of a 27-mer DNA template. The initial and elongated primers were separated by denaturing polyacrylamide gel electrophoresis and analyzed by fluorescent imaging. Hyperbolic fitting[4b] of the data to the equation $k_{obs} = k_{pol}$[acycloNTP]/($K_d$ + [acycloNTP]) was used to determine values of kinetic constants $k_{pol}$ (rate constant of polymerization) and $K_d$ (equilibrium constant of dissociation). The α-$BH_3$ substitution in acycloTTP increased the efficiency for incorporation ($k_{pol}/K_d$) of acycloTTP-α-$BH_3$ by 9-fold in pre-steady-state conditions with viral reverse transcriptase (Figure 2). This difference in pre-steady-state incorporation of acycloTTP-α-$BH_3$ compared with acycloTTP by MMLV RT involves an approximate 2.5-fold decrease in dissociation constant $K_d$ (from 90 to 36 $\mu$M) and a 3.5-fold increase in rate constant $k_{pol}$ (from 1.3 × $10^{-3}$ $s^{-1}$ to 4.6 × $10^{-3}$ $s^{-1}$).

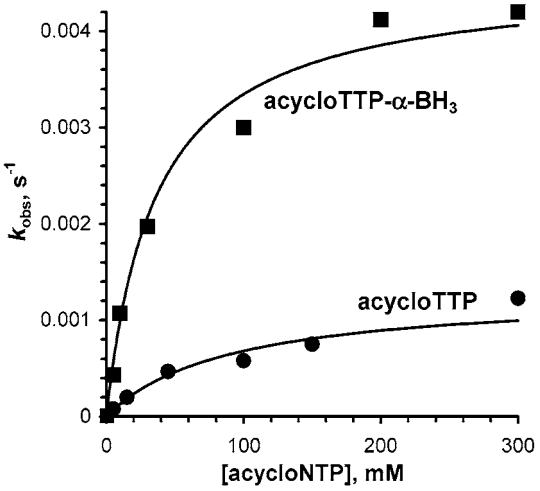


**Figure 2.** Concentration dependence of kinetic rate constants for pre-steady-state incorporation of acycloTTP (●) and acycloTTP-α-$BH_3$ (■) by MMLV RT.

In conclusion, we have successfully synthesized acyclothymidine triphosphate **5a** and acyclothymidine α-*P*-boranotriphosphate **5b** using a phosphoramidite approach in good yield after isolation. The α-*P*-borane substitution in acyclothymidine triphosphate results in a 9-fold increase in the incorporation efficiency compared with the non-boronated triphosphate in pre-steady-state conditions with viral reverse transcriptase. These preliminary results, and the increase in the lipophilicity imparted by the *P*-borane group,[5e] indicate that acyclonucleoside α-*P*-boranotriphosphates may have promising applications as a probe of enzyme mechanisms and in the design of new antiviral drugs.

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**Supporting Information Available:** Spectral data for compounds **5a** and **5b**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) For preliminary investigations, acycloTTP-α-$BH_3$ **5b** was used as a mixture of α-*P*-diastereomers.