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Inhibition of multi-drug resistant HIV-1 reverse transcriptase by nucleoside β-triphosphates

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ABSTRACT

Despite the success of potent reverse transcriptase (RT) inhibitors against human immunodeficiency virus type 1 (HIV-1) in combination regimens, the development of drug resistant RTs constitutes a major hurdle for the long-term efficacy of current antiretroviral therapy. Nucleoside β-triphosphate analogs of adenosine and nucleoside reverse transcriptase inhibitors (NRTIs) (3′-azido-2′,3′-dideoxythymidine (AZT), 3′-fluoro-2′,3′-dideoxythymidine (FLT), and 2′,3′-didehydro-2′,3′-dideoxythymidine (d4T)) were synthesized and their inhibitory activities were evaluated against wild-type and multidrug resistant HIV-1 RTs. Adenosine β-triphosphate (**1**) and AZT β-triphosphate (**2**) completely inhibited the DNA polymerase activity of wild type, the NRTI multi resistant, and nonnucleoside RT inhibitors (NNRTI) resistant HIV-1 RT at 10 nM, 10 and 100 μM, respectively.

During HIV-1 replication, the viral RNA genome is reverse transcribed into an integrated competent double stranded DNA by the virally encoded multifunctional enzyme reverse transcriptase (RT).[1] HIV-1 RT remains a prime target for continued development of antagonists to inhibit virus replication and stem the devastating consequences of AIDS. HIV-1 RT is a heterodimeric enzyme composed of 66 and 51 kD subunits (p66 and p51) possessing RNA- and DNA-dependent DNA polymerase and RNase H activities.[2] DNA polymerase activity is essential for the synthesis of a RNA:DNA heteroduplex from the single stranded viral RNA genome. RNase H hydrolyzes the RNA strand of the RNA:DNA heteroduplex generated during reverse transcription and creates the primer for plus strand DNA synthesis. Thus, both DNA polymerase and RNase H activities of HIV-1 RT have been considered as potential targets for antiretroviral therapy.[3]

Two classes of drugs belonging either to the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) or to the nonnucleoside reverse transcriptase inhibitors (NNRTIs) have been used in the clinic as part of the antiretroviral therapy against HIV/AIDS.[4] NRTIs compete with the natural deoxynucleoside triphosphates (dNTPs) during DNA synthesis and act as chain terminators.[5] In contrast, NNRTIs are noncompetitive inhibitors that bind at an allosteric nonsubstrate binding site, which is distinct from the substrate binding site of HIV-1 RT.[6] While the unique pharmacology of these inhibitors has rendered their use in highly active antiretroviral therapy (HAART) therapy, HIV-1 has the ability to develop drug resistance mutations for both NRTI and NNRTIs.[7] Thus, design of novel lead compounds that can inhibit wild-type and drug resistant HIV-1 RTs is a subject of major interest in antiviral research.

Modified nucleoside triphosphates that mimic naturally occurring deoxyribo and ribonucleoside triphosphates have been used as probes in several biochemical pathways involving DNA and RNA synthesis, and as potential diagnostic and therapeutic agents.[8,9] The structural similarity of modified nucleotides to natural deoxyribo and ribonucleoside triphosphates makes them useful reagents as substrates or inhibitors for DNA or RNA polymerases.[10,11] A number of approaches have focused on modifications and/or substitutions on the base,[12,13] carbohydrate[14–19] and linear triphosphate moieties[20–25] to design modified nucleotides for diverse applications in nucleic acid and antiviral research.

We have previously reported the synthesis of nucleoside 5′-*O*-α,β-methylene-β-triphosphates and 5′-*O*-β,γ-methylenetriphosphates and their potency towards the enzymatic function of wild-type HIV-1 RT.[26,27] In continuation of our efforts to design a diverse array of modified nucleoside triphosphates as RT

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inhibitors, we herein report the synthesis of nucleoside β-triphosphate analogs (**1**–**4**) of adenosine and NRTIs, such as 3′-azido-3′-deoxythymidine (zidovudine, AZT), 3′-fluoro-3′-deoxythymidine (alovudine, FLT), and 2′,3′-didehydro-2′,3′-dideoxythymidine (stavudine, d4T) (Fig. 1) and their inhibitory activity against the DNA polymerase of wild-type and multidrug resistant RTs. To the best of our knowledge, this is the first report of the evaluation of nucleoside β-triphosphate analogs as RT inhibitors.

The synthesis of a β-triphosphitylating reagent from phosphorus trichloride has been previously reported by us in multi-step reactions.[28] The reaction mixture containing β-triphosphitylating reagent was immediately used in coupling reactions with polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol for the synthesis of a number of nucleoside β-triphosphates.[28] Our research on the solid-phase synthesis of organophosphorus and organosulfur compounds revealed that the polymer-bound *p*-acetoxybenzyl alcohol containing amide linker (**5**) was more stable than polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol even in basic conditions and was used to generate sulfonamides and other organophosphorus compounds in high yields and without the need for extensive purifications of final products.[29,30] Thus, polymer-bound linker **5** instead of polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol was selected for the reaction with β-triphosphitylating reagent **6** to generate a new polymer-bound β-triphosphitylating reagent **7** that was used for preparation of nucleoside β-triphosphates including two novel compounds **3** and **4** (Scheme 1).

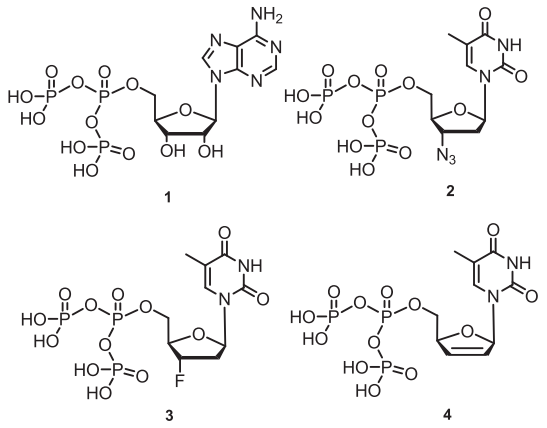
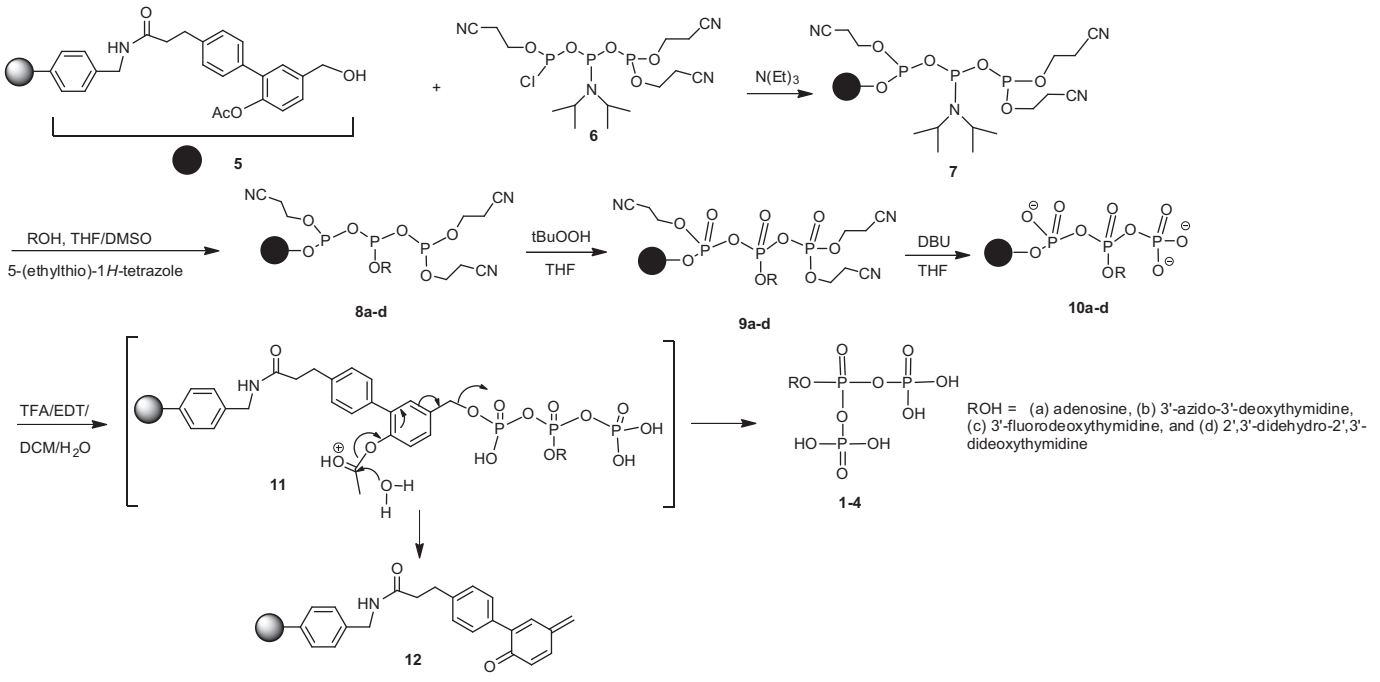


Figure 1. Chemical structures of nucleoside 5′-*O*-β-triphosphates (**1**–**4**).

Scheme 1 shows the synthesis of nucleoside 5′-*O*-β-triphosphates (**1**–**4**). The aminomethyl polystyrene resin-bound *p*-acetoxybenzyl alcohol (**5**, 3.85 g, 0.65 mmol/g) was subjected to reaction with the β-triphosphitylating reagent (**6**, ~10 mmol) in the presence of triethylamine (10 mmol) to produce the corresponding polymer-bound β-triphosphitylating reagent **7**. Unprotected nucleosides (e.g., adenosine (**a**), AZT (**b**), FLT (**c**), and d4T (**d**) were reacted with polymer-bound reagent **7** in the presence of 5-(ethylthio)-1*H*-tetrazole to yield **8a**–**d**. Oxidation with *t*-butyl hydroperoxide followed by removal of the cyanoethoxy group with DBU, afforded the corresponding polymer-bound nucleoside 5′-*O*-β-triphosphotriesters (**10a**–**d**). The cleavage of polymer-bound compounds was carried out under acidic conditions (TFA). The linker-trapped resin (**12**) was separated from the final products by filtration. The crude products had a purity of 87–93% (Table 1) and were purified by using small C_{18} Sep–Pak cartridges and appropriate solvents to afford nucleoside 5′-*O*-β-triphosphates (**1**–**4**) in 76–90% overall yield (calculated from polymer-bound reagent **7** in the four-step reaction sequence) (Table 1). Only one type of monosubstituted compound was produced with high selectivity as a result of this sequence possibly because of the rigidity of polymer-bound β-triphosphitylating reagent **7**. In case of adenosine, the most reactive hydroxyl group of unprotected nucleoside reacted selectively with hindered polymer-bound reagent **7** when an excess of nucleoside was used. The chemical structures of the final products (**1**–**4**) were determined by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, and ^{31}P NMR), high-resolution time-of-flight electrospray mass spectrometry, and quantitative phosphorus analysis.

Enzyme p66/p51 HIV-1 RT was purified according to the protocol described by Le Grice et al.[31] DNA-synthesis was measured on



Scheme 1. Synthesis of polymer-bound β-triphosphitylating reagent **7** and nucleoside 5′-*O*-β-triphosphates **1**–**4** using polymer-bound linker **5**.