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Synthesis of β-triphosphotriester pronucleotides

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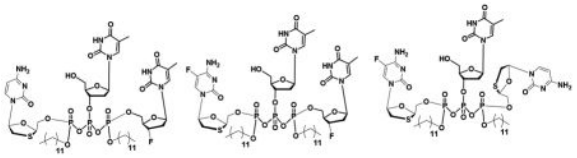
Abstract

Dinucleoside phosphorochloridite were synthesized from phosphorus trichloride and three nucleoside analogues, 3′-fluoro-2′,3′-dideoxythymidine (FLT), 2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC), and 2′,3′-dideoxy-3′-thiacytidine (3TC), in a multistep synthesis. Polymer-bound *N*-Boc *p*-acetoxybenzyl 5′-*O*-2′-deoxythymidine was reacted with dinucleoside phosphorochloridite in the presence of 2,6-lutidine, followed by the reaction with dodecyl alcohol and 5-(ethylthio)-1*H*-tetrazole, oxidation with *tert*-butyl hydroperoxide, and acidic cleavage, respectively, to afford the β-triphosphotriester derivatives containing three different nucleosides.

Graphical abstract



Keywords

Fatty chains; Nucleosides; Nucleotides; Pronucleotides; Triphosphotriesters

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Supplementary Material

Supplementary data associated with this article including experimental procedures and characterization of compounds can be found in the online version.

During HIV-1 replication, the viral RNA genome is reverse transcribed into a double stranded DNA by the virally encoded multifunctional enzyme reverse transcriptase (RT).[1] HIV-1 RT remains a major target for continued development of antagonists to inhibit virus replication and stem the devastating consequences of AIDS.

Two classes of drugs belonging either to the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs) or to the non-nucleoside reverse transcriptase inhibitors (NNRTIs) have been used in the clinic as part of the antiretroviral therapy against HIV/AIDS.[2] NRTIs compete with the natural deoxynucleoside triphosphates (dNTPs) during DNA synthesis and act as chain terminators.[3] In contrast, NNRTIs are non-competitive inhibitors that bind at an allosteric nonsubstrate binding site, which is distinct from the substrate binding site of HIV-1 RT.[4] 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.[5] Thus, design of novel lead compounds that can inhibit wild-type and drug resistant HIV-1 RTs is a subject of major interest in anti-HIV research.

The structural similarity of modified nucleotides to natural ribo- and deoxyribonucleoside triphosphates makes them useful reagents as substrates or inhibitors for DNA or RNA polymerases.[6,7] A number of approaches have focused on modifications and/or substitutions on the base,[8,9] carbohydrate[10–15] and linear triphosphate moieties[16–21] to design modified nucleotides for diverse applications in nucleic acid and antiviral research.

Negatively-charged nucleotides have limited cell-permeability. Masking the phosphate residues with a lipophilic chain could generate pronucleotides with improved cellular permeability. Prodrugs are chemically modified analogous of the active metabolite that can improve pharmacokinetics and pharmacodynamics (PK/PD) properties of the active drug. However, intracellular chemical transformation needs to be occurred in the presence of different enzymes to convert prodrugs to their corresponding pharmacologically potent compounds in *in vivo* systems. Prodrug approach offers several advantageous, such as enhancing water solubility, improved chemical stability, decreased toxicity, and insufficient brain penetration.[22] Herein we hypothesized that lipophilic pronucleotides can act as prodrugs of nucleotide analogs.

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.[23,24] In continuation of our efforts to design a diverse array of modified nucleoside triphosphates as RT inhibitors, we report here the synthesis of *β*-triphosphotriester pronucleotides (**1a–c**) of NRTIs, including 3′-fluoro-3′-deoxythymidine (Alovudine, FLT), 2′,3′-dideoxy-3′-thiacytidine (Lamivudine, 3TC), and 2′,3′-dideoxy-5-fluoro-3′-thiacytidine (Emtricitabine, FTC) (Fig. 1). To the best of our knowledge, this is the first report of the synthesis of *β*-triphosphotriester pronucleotides containing two RT inhibitors.

Scheme 1 illustrates the synthesis of nucleoside *β*-triphosphitylating reagents containing NRTIs **(6a–c)**. Phosphorus trichloride (PCl_3, 2 mmol) was reacted with the nucleosides e.g.

FLT, 3TC, or FTC (2 mmol) in the presence of 2,6-lutidine (2 mmol) to yield intermediate 5′-*O*-nucleoside phosphorus dichloride (**2a**–c). In situ reaction of **2a–c** with *N*,*N*-diisopropylamine (2 mmol) in the presence of 2,6-lutidine (2 mmol) afforded intermediate 5′-*O*-nucleoside *N*,*N*-diisopropylphosphoramidochloridite (**3a–c**). Addition of water (2 mmol) and 2,6-lutidine (2 mmol) gave 5′-*O*-nucleoside *N*,*N*-diisopropyl hydroxyphosphoramide (**4a**–c) that were reacted with phosphorus trichloride (2 mmol) in situ in the presence of 2,6-lutidine (2 mmol) to afford intermediate compounds **5a–c**. The intermediates were used immediately for the next reaction under extremely dry conditions and nitrogen.

The reaction of equimolar amounts of **4a–c** and **5a–c** produced 5′-*O*-5′-*O*-dinucleoside phosphorochloridite (ROH = FLT, R′-OH = 3TC (**6a**); ROH = FLT, R′-OH = FTC (**6b**); and ROH = FTC, R′-OH = 3TC (**6c**). The chemical structures of **6a–c** were confirmed by high-resolution time-of-flight electrospray mass spectrometry of hydroxyl form of the compounds as shown in the Supporting Information.

To accomplish the synthesis of dendritic *β*-triphosphotriester pronucleotides, a diffrentially protected 3′-*O*-TBDMS-2′-deoxythymidine (**8**) was synthesized according to the previously reported procedures[25–27] (Scheme 2). In this regard, the 5′- and 3′-hydroxyl groups of 2′-deoxythymidine were protected by *tert*-butyldimethylsilyl (TBDMS) by the reaction of the unprotected nucleoside with *tert*-butyldimethylsilyl chloride in the presence of imidazole in DMF.[25,26] The selective removal of 5′-*O*-TBDMS group in the presence of AcOH/H_2O/THF[27] afforded 3′-*O*TBDMS-2′-deoxythymidine (**8**).

Our research on the solid-phase synthesis of organophosphorus and organosulfur compounds revealed that the polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol (**9**) is a versatile solid-phase linker system for the phosphorylation of organic compounds[27]. In this regard the polymer-bound *N*-Boc *p*-acetoxybenzyl alcohol (**9**) was prepared according to our previously reported procedure[28] and was used as a loading system in this study. Then the polymer-bound *N*-Boc *p*-acetoxybenzyl trichloracetimidate (**10**) was prepared from the reaction of (**9**) with trichloroacetonitrile in the presence of DBU according to previously reported procedure.[28]

Scheme 3 illustrates the synthesis of dendritic nucleoside *β*-triphosphate analogs (**1a**–**c**). The 2′-deoxy TBDMS protected 2′-deoxythymidine (**8**, 3 mmol) was attached to **10** (1.5 mmol) through 5′-hydroxyl group in the presence of BF_3.OEt_2 as acidic catalyst[29] to afford **11**. The deprotection of 3′-*O*-TBDMS group in **11** with tetrabutylammonium fluoride (TBAF) in THF afforded (**12**, ~1.50 mmol), which was divided to three portions (~0.50 mmol each). Each portion of **12** underwent *β*-triphosphitylation of 3′-hydroxyl group with *β*-triphosphitylating reagents (**6a–c**, ~2 mmol, 4 equiv. of 2′-deoxy functions) under extremely dry conditions and nitrogen to afford (**13a–c**). In this regard, the prepared reaction mixture containing **6a–c** in THF (~2 mmol) was added to a swelled solution of polymer-bound *N*-Boc *p*-acetoxybenzyl 5′-*O*-2′-deoxythymidine **12** (~0.50 mmol) and 2,6-lutidine (2 mmol) in anhydrous THF. The mixture was shaken for 28 h with increasing of temperature from −20 °C to room temperature. The resin was collected by filtration, washed with THF and MeOH, respectively, and was dried overnight under vacuum to give **13a–c**.

1-Dodecanol was used to mask the negatively-charged cell-impermeable phosphate residues and to improve the lipophilicity of the pronucleotides. Thus, 1-dodecanol (4.0 mmol) and 5-(ethylthio)-1*H*-tetrazole (4.0 mmol) were added to **13a–c** in anhydrous THF. The mixtures were shaken for 24 h at room temperature, then the resins were collected by filtration, washed with DCM and MeOH, respectively, and dried under vacuum to give **14a–c**. *tert*-Butyl hydroperoxide in decane was used for the oxidation of **14a–c** to **15a–c**. Finally, the cleavage of polymer-bound compounds was carried out under acidic conditions (DCM/TFA/H_2O/1,2-ethanedithiol). The linker-trapped resin **10** was separated from the final products by filtration. After filtration, the solvents were removed using lyophilization and the crude products were purified (>98%) using HPLC system to afford pure **1a–c** products in 51–53% overall yield calculated from **10**. The chemical structures of the final products (**1a**–**c**) were determined by nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR, and ^{31}P NMR), SELDI-TOF mass spectrometer, and quantitative phosphorus analysis.

In conclusion, a polymer-bound *N*-Boc *p*-acetoxybenzyl 5′-*O*-2′-deoxythymidine was reacted with three dinucleoside phosphorochloridites containing FLT, FTC, and 3TC in the presence of 2,6-lutidine in a solid phase reaction. Subsequent conjugation with dodecyl alcohol to mask the negatively charged phosphate in the presence of 5-(ethylthio)-1*H*-tetrazole, oxidation with *tert*-butyl hydroperoxide, and acidic cleavage, respectively, afforded the *β*-triphosphotriester nucleotide derivatives **1a–c** containing three different nucleosides and a dodecyl chain. The compounds will be further evaluated for anti-HIV activities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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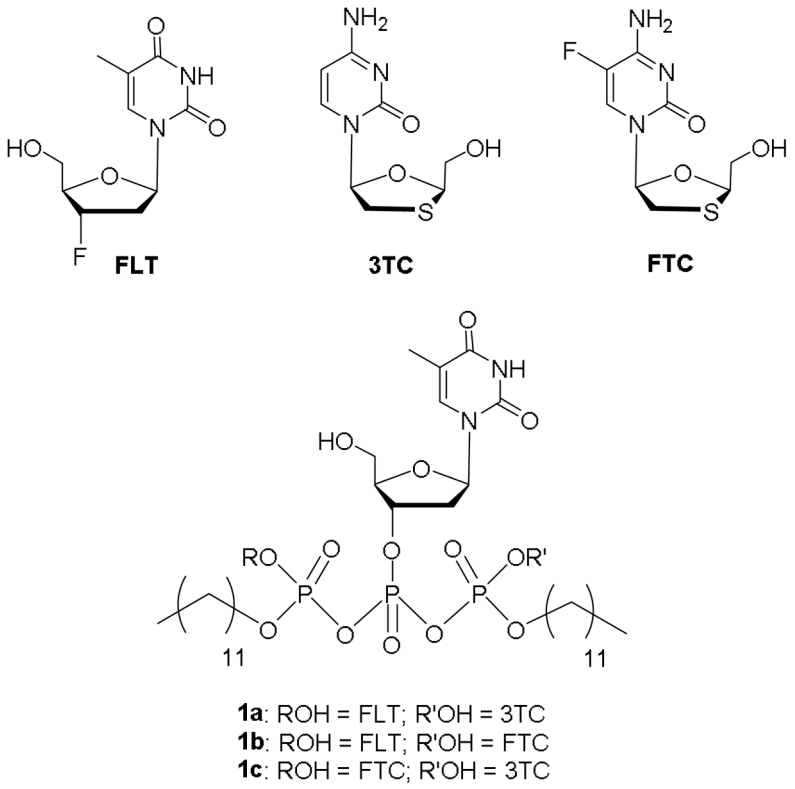
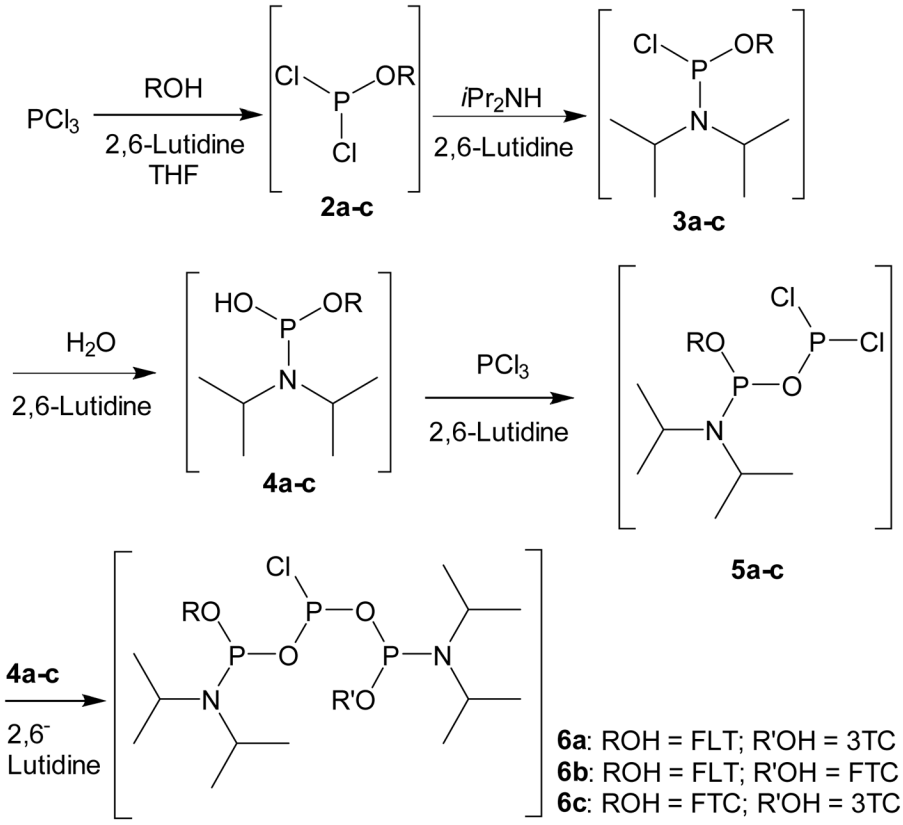
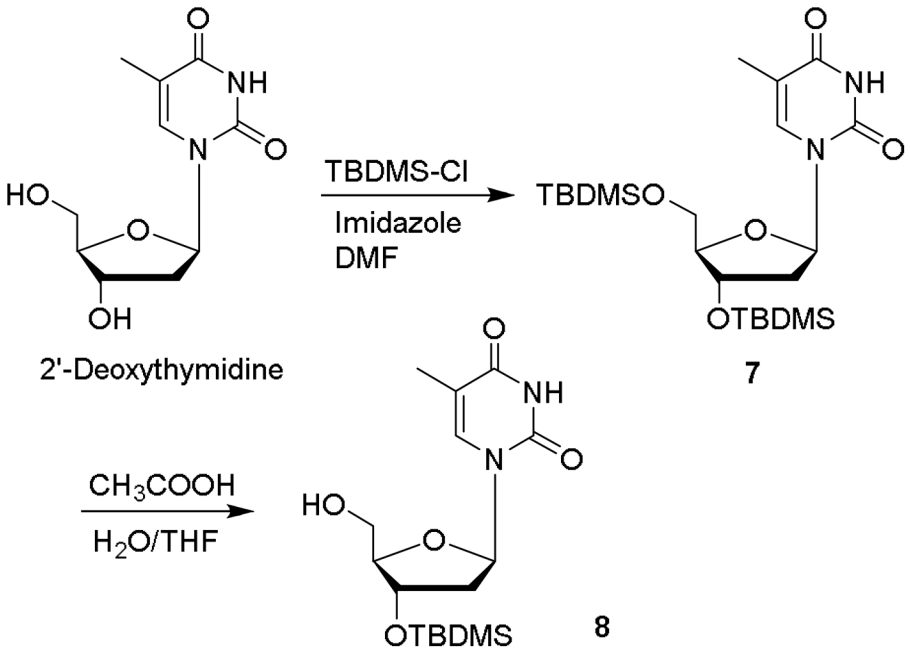


Fig. 1.
Chemical structures of β-triphosphotriester pronucleotides (**1a–c**).

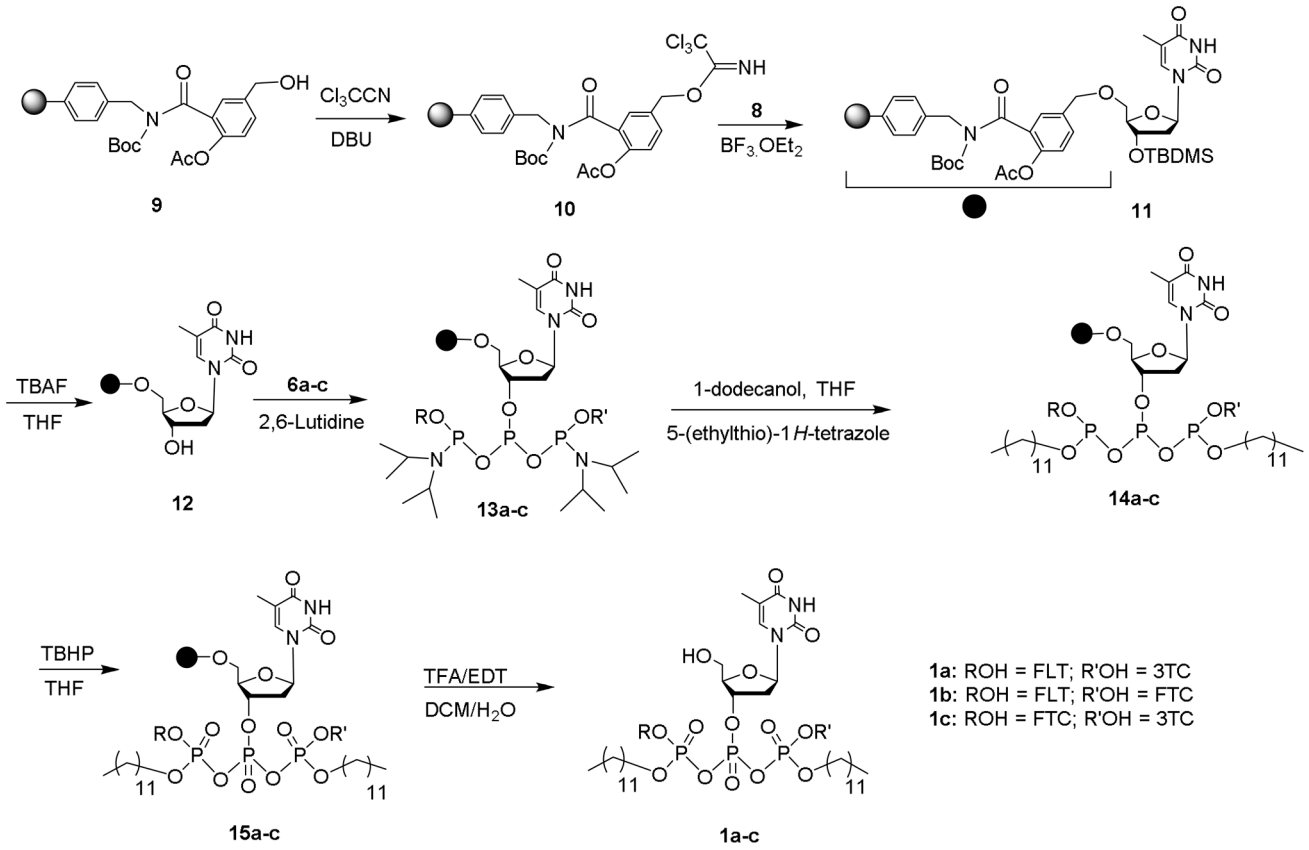


Scheme 1.

Preparation of nucleoside *β*-triphosphitylating reagents containing NRTIs **6a–c**.



Scheme 2.
Preparation of 3′-*O*-TBDMS-2′-deoxythymidine **8**.



Scheme 3.

Preparation of pronucleotide derivatives of 3′-fluoro-3′-deoxythymidine, 2′,3′-dideoxy-5-fluoro-3′-thiacytidine, and 2′,3′-dideoxy-3′-thiacytidine **1a–c**.