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Yousef Ahmadibeni
University of Rhode Island

Rakesh Tiwari
University of Rhode Island

See next page for additional authors

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Synthesis and anti-HIV activities of bis-(*cyclo*Saligenyl) pronucleotides derivatives of 3′-fluoro-3′-deoxythymidine and 3′-azido-3′-deoxythymidine

Yousef Ahmadibeni[a,b,*], **Rakesh Tiwari**[a], **Chelsie Swepson**[c], **Jui Pandhare**[c], **Chandravanu Dash**[c], **Gustavo F. Doncel**[d], and **Keykavous Parang**[a,*]

[a]Department of Biomedical and Pharmaceutical Sciences, College of Pharmacy, The University of Rhode Island, Kingston, RI 02881, USA

[b]Department of Chemistry, Columbus State University, Columbus, GA 31907, USA

[c]Centre for AIDS Health Disparities Research, Meharry Medical College, Nashville, TN 37208, USA

[d]CONRAD, Department of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, VA 23507, USA

Abstract

Anti-HIV nucleoside monophosphates have limited cellular uptake due to the presence of negatively-charged phosphate group. Bis-(*cyclo*Saligenyl) derivatives containing two anti-HIV nucleosides, 3′-fluoro-3′-deoxythymidine (FLT) and 3′-azido-3′-deoxythymidine (AZT) were synthesized to increase intracellular delivery of nucleoside monophosphates. 2,5-Bis(hydroxymethylene)benzene-1,4-diol was selected as a monocyclic bidentate scaffold and synthesized by three different methods from bis(hydroxymethylene)cyclohexan-1,4-diene-1,4-diol, or diethyl 2,5-dihydroxyterephthalate. The reaction of the tetraol with diisopropylphosphoramidous dichloride in the presence of 2,6-lutidine, followed by conjugation reactions with nucleosides (i.e., FLT and AZT) and oxidation afforded symmetrical and unsymmetrical bis-(*cyclo*Saligenyl) diphosphate triester products, AZT–AZT, FLT–FLT, and FLT–AZT conjugates, in 63–74% overall yields and modest anti-HIV activities (IC_{50} = 2.8–69.6 μM).

Keywords

Nucleosides; Bis-(*cyclo*Saligenyl); AZT; FLT; Anti-HIV

Nucleoside analogs are commonly used as antiviral or anticancer agents.[1] Cellular nucleoside kinases mediate three phosphorylation steps, which convert the nucleosides into

*Corresponding authors. kparang@gmail.com (K. Parang).

Supplementary data

Supplementary data (experimental procedures and characterization of final compounds with ^{1}H NMR, ^{13}C NMR, ^{31}P NMR, and high-resolution ESI-mass spectrometry) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.12.038.

their active nucleoside triphosphate analogs (Fig. 1).[2] The first phosphorylation to nucleoside monophosphate is often the rate-limiting step.[3] Negatively charged nucleoside monophosphates cannot be used as therapeutic agents because they are unable to cross cell membranes efficiently, and they are readily dephosphorylated on cell surfaces and in extracellular fluids by non-specific enzymes. Thus, several masked nucleoside phosphate mimics have been synthesized as prodrugs with the aim of delivering the corresponding 5′-monophosphate derivative intracellularly and bypassing the initial phosphorylation step.[4] *cyclo*Saligenyl (*cyclo*Sal) pronucleotide system has been introduced as a prodrug approach for cellular delivery of several nucleoside monophosphates. The intracellular cleavage of an optimal *cyclo*Sal pronucleotide to the monophosphate analog is based on intracellular pH-driven chemical hydrolysis.[5]

Two or more masking groups are required to generate neutral phosphate triester prodrug of nucleosides.[6,7] The *cyclo*Sal pronucleotide system releases one molecule of drug per masking unit (mask to drug ratio, 1:1) as compared to some other pronucleotide delivery systems with higher ratios of masking unit, for example, two to four for a single unit of drug (2:1–4:1). Ducho et al.[8] recently reported synthesis of symmetrical 3,3′-bis-(*cyclo*-Sal) derivatives of 2′,3′-dideoxy-2′,3′-didehydrothymidine (d4T) using a bicyclic tetraol to deliver two similar nucleosides that led to a significant improvement of anti-HIV activity when compared to the parent nucleoside d4T. Gisch et al.[9] synthesized 5,5′-bis-*cyclo*Sal-d4T to deliver intracellularly two molecules of d4T-MP that showed improved anti-HIV activity when compared to 3,3′-bis-*cyclo*Sal-d4T derivatives in thymidine kinase-deficient cells.

The reported synthesis of the bis-(*cyclo*Sal) derivatives is challenging due to a number of synthetic steps for the synthesis of bicyclic tetraol, side reactions between the reactants and reagents, tedious purification steps, low yield, and instability of the intermediates. Therefore, alternative synthetic methodologies are still required to improve the synthesis of bis-(*cyclo*Sal) derivatives and their substituted pronucleotides. Furthermore, to the best of our knowledge the synthesis of unsymmetrical bis-(*cyclo*Sal) derivatives having two different nucleosides has not been previously reported. Herein, we report the synthesis of novel symmetrical and unsymmetrical derivatives of bis-(*cyclo*Sal) derivatives of AZT and FLT (Fig. 2) from a new scaffold bis(hydroxymethylene)benzene-1,4-diol (**4**). It was expected that the individual components of multifunctional compounds (nucleoside monophosphates) would exhibit biological activity, even enhanced efficacy, when released intracellularly by degradative metabolism of the conjugate. Development of resistant virus is a serious problem for any antiviral drug. This combination of nucleosides using unsymmetrical bis-(*cyclo*Sal) may enhance the activity of conjugates against resistant strains. The development of resistance to nucleosides would occur at a slower rate than to either compound alone.

Bis(hydroxymethylene)benzene-1,4-diol (**4**) was introduced as a novel monocyclic tetraol skeleton for the synthesis of bis-*cyclo*Sal derivatives of dideoxynucleosides. The scaffold was synthesized by using three different methods and was reacted with phosphorous trichloride to afford the *cyclo*Sal chlorophosphite. The intermediate was reacted with nucleosides under the controlled conditions of stoichiometry and temperature to afford the

trivalent phosphite derivatives of nucleosides. Subsequent oxidation using *tert*-butyl hydroperoxide (TBHP) and purification afforded bis-(*cyclo*Sal) derivatives of AZT and FLT in moderate yields.

Scheme 1 illustrates the synthesis of 2,5-bis(hydroxymethylene) benzene-1,4-diol **4**. 2,5-Bis(methoxycarbonyl)cyclohexa-1,4-diene-1,4-diol **1** was oxidized by refluxing in the presence of excess of activated manganese dioxide in toluene to afford the 2,5-bis(methoxycarbonyl)benzene-1,4-diol **2**. Reduction of compound **2** using lithium borohydride in methanol and anhydrous diethyl ether or lithium aluminium hydride in THF afforded the corresponding tetraol **4** after purification in 44% and 52% overall yields, respectively. An alternative method was used to synthesize **4** (60% overall yield) by reducing the corresponding diethyl-2,5-dihydroxyterephthalate **3** with BH_3·THF under anhydrous conditions. In all cases the crude product **4** was purified by column chromatography and characterized by NMR and high-resolution time-of-flight electrospray mass spectrometry (ESI-TOF), and was used as a scaffold for the synthesis of bis-(*cyclo*Sal) derivatives.

The reaction of tetraol **4** (0.5 mmol) with phosphorous trichloride (1 mmol) and 2,6-lutidine (2 mmol) in anhydrous tetrahydrofuran afforded the bis-(*cyclo*Sal chlorophosphite) **5** under controlled temperature (−78 °C to −15 °C) (Scheme 2). The addition of 2,6-lutidine (1 mmol) followed by the dry nucleoside (AZT or FLT) (1 mmol), to the same reaction mixture under the controlled conditions of the temperature (−55 °C to −10 °C) afforded the corresponding symmetrical phosphite triester derivatives **6a** and **6b**. The oxidation of **6a** and **6b** by TBHP and purification by preparative HPLC afforded the symmetrical bis-(*cyclo*Sal) phosphate triester derivatives FLT–FLT (**7a**, 74%) and AZT–AZT (**7b**, 71%) as a diastereomeric mixture. The separation of diastereomers was not possible for **7a** and **7b**.

In a separate reaction, tetraol **4** was reacted with phosphorus trichloride in the presence of 2,6-lutidine at −78 °C in anhydrous tetrahydrofuran to afford intermediate **5** as described above (Scheme 2). Then the reaction temperature was kept at −55 °C using dry ice and acetone. To the reaction mixture was added 2,6-lutidine (1 mmol) followed by dry nucleoside AZT (0.5 mmol) (Scheme 3). The reaction was continued for 85 min with steady increase of the temperature to −50 °C. The second nucleoside FLT (0.5 mmol) was added and the temperature was raised to −10 °C over a period of 95 min. Oxidation with TBHB and HPLC purification afforded the unsymmetrical bis-(*cyclo*Sal) phosphate triester of AZT–FLT **10** (63%) as a diastereomeric mixture (Scheme 3).

Compound **7b** showed only modest antiviral activity (IC_{50} = 69.6 μM), significantly lower than that of the parent nucleoside, FLT (IC_{50} = 0.8 μM), when tested using a single-round infection assay[10] using HIV-1 ×4 tropic strains and transformed HeLa cells expressing HIV receptors (CD4) and coreceptors (CXCR4). Compounds **7a** and **10** exhibited IC_{50} values of 12.3 and 2.8 μM, respectively, while control scaffold **4** had an IC_{50} value of 86.5 μM. These data suggest that the presence of both AZT and FLT in the same conjugate generates higher anti-HIV activity as was shown in **10** when compared with **7a** and **7b**. There are several factors which determine in vitro anti-HIV activity of each analog that may include stability, rate of cellular uptake, nature of nucleoside, and extracellular and intracellular rate of

hydrolysis. Structure–function analysis revealed that the anti-HIV activity of bis-(*cyclo*Saligenyl) diphosphate triester products, AZT–AZT, FLT–FLT, and FLT–AZT was clearly dependent on the nature of the nucleoside in the conjugate. These results suggest that the increased inhibition by **10** may be due to the higher cellular uptake, enhanced intracellular release of FLT and AZT, and/or stability of the conjugate when compared with **7a** and **7b**. The extracellular hydrolysis of phosphate triester derivatives of FLT and AZT has been previously reported.[11] The partial or complete premature extracellular hydrolysis of the bis-(*cyclo*Sal) group in all analogs may produce negatively-charged phosphate derivatives with limited cellular uptake and decreased anti-HIV activity. The rate of extracellular hydrolysis remains to be determined to compare the stability of the conjugates. Cytotoxicity assays revealed that compounds were not cytotoxic at the highest concentration tested (CC_{50} >100 μM).

To the best of our knowledge, this is first report on the synthesis of symmetrical and unsymmetrical bis-(*cyclo*Sal) derivatives of FLT and AZT from a monocyclic scaffold, bis(hydroxymethylene)benzene-1,4-diol. This one-pot strategy from a tetraol to the target compounds minimizes side reactions and eliminates the need for the purification of intermediates. The synthesis of these compounds provides insights for design of more optimal scaffolds of bis-(*cyclo*Sal) nucleoside derivatives with higher stability, cellular uptake, and anti-HIV activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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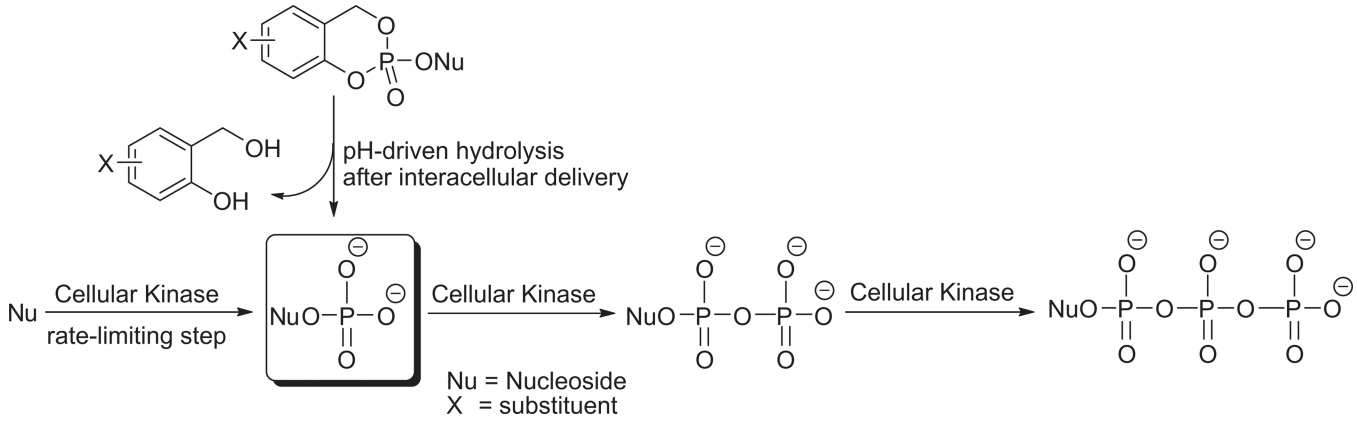


Figure 1.

Nucleoside phosphorylation and *cyclo*Sal delivery system of nucleoside monophosphate.[5]

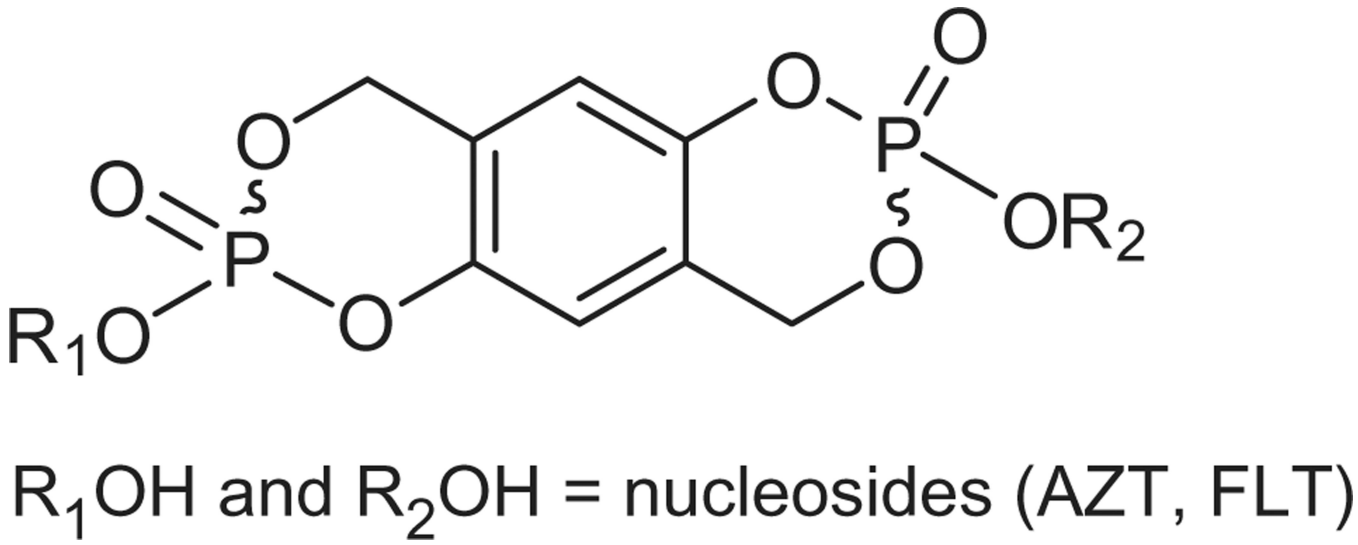
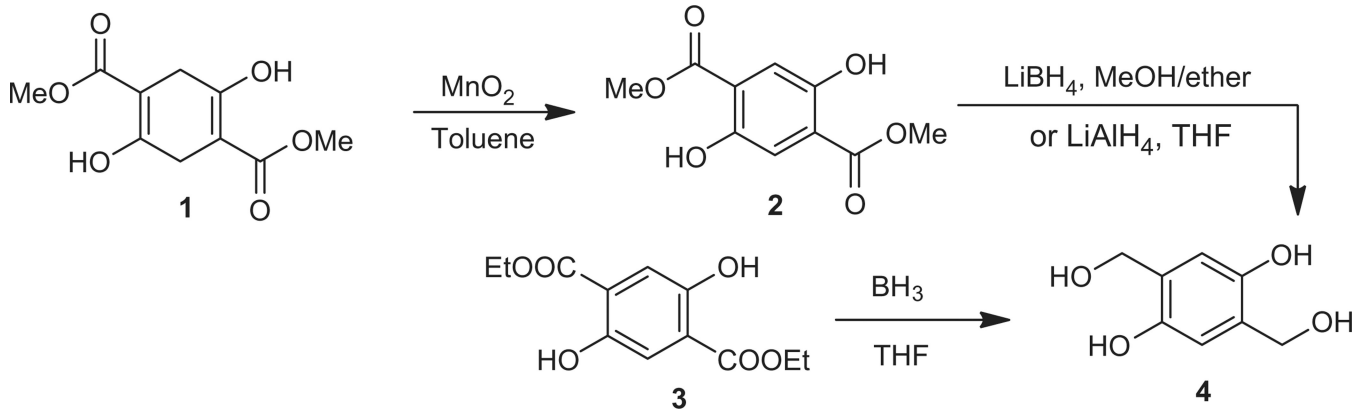
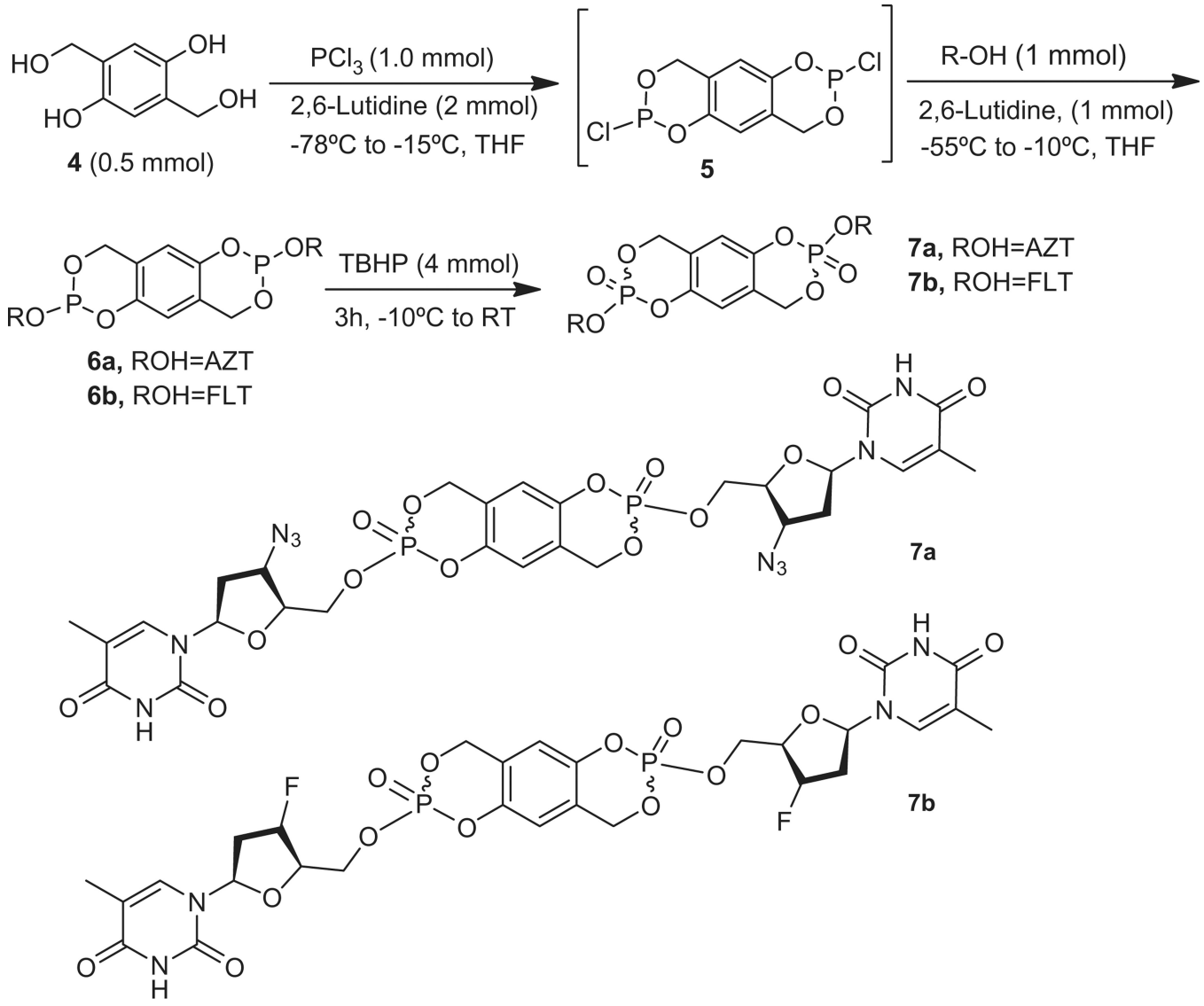


Figure 2.
Symmetrical and unsymmetrical derivatives of bis-(*cyclo*Sal) derivatives of AZT and FLT.

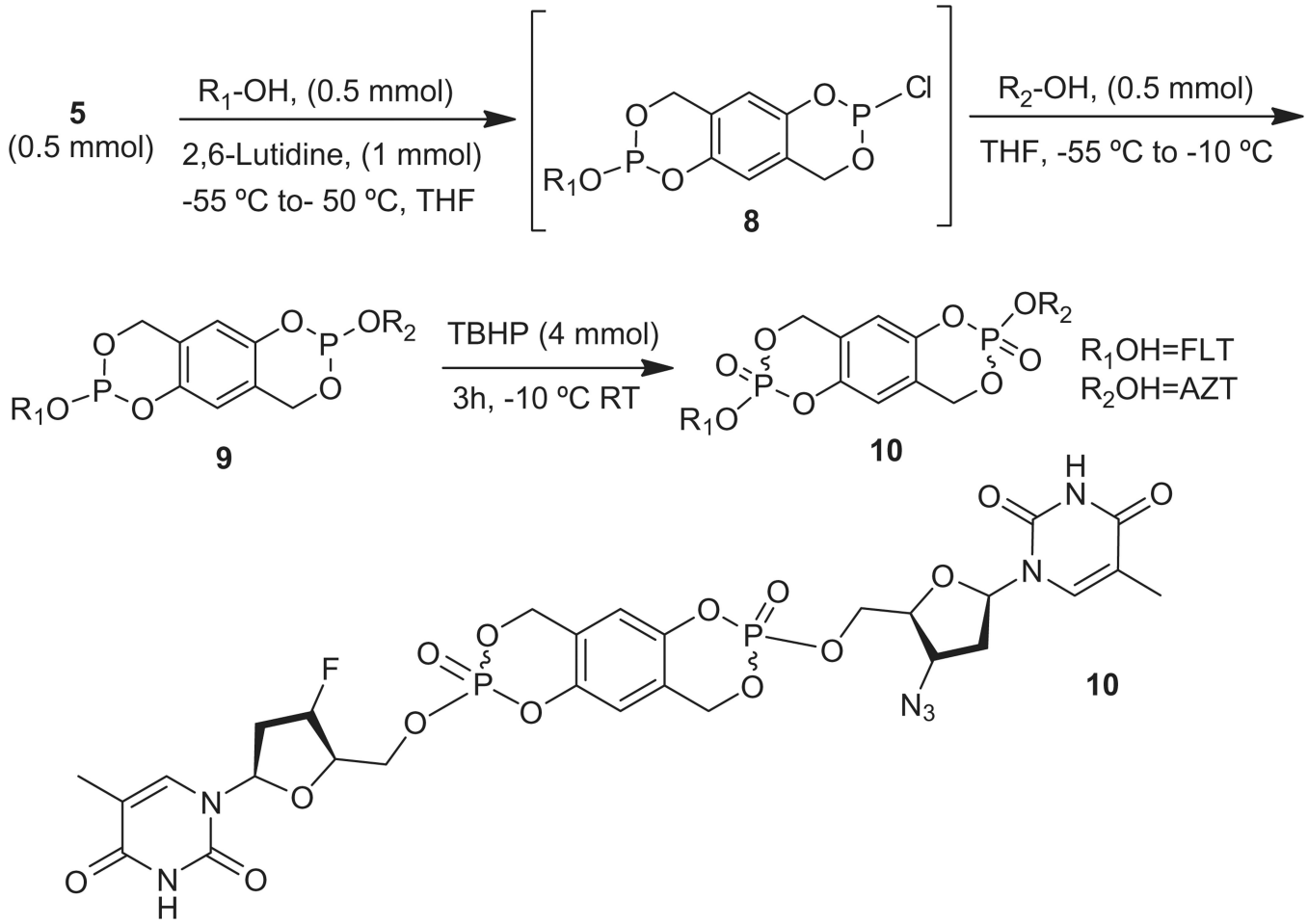


Scheme 1.
Synthesis of 2,5-bis(hydroxymethylene)benzene-1,4-diol **4**.



Scheme 2.

Synthesis of symmetrical bis-(*cyclo*Sal) derivatives of AZT–AZT **7a** and FLT–FLT **7b**.



Scheme 3.

Synthesis of unsymmetrical derivative of bis-(*cyclo*Sal) analog of FLT and AZT (**10**).