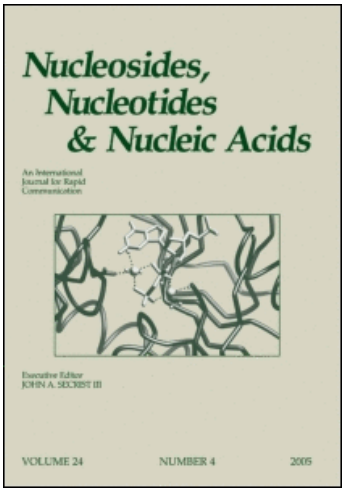

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Thiated Analogues of 2′,3′-Dideoxy-3′-fluorothymidine and Their Phosphorylated and Phosphonylated Derivatives: Synthesis, Interaction with HIV Reverse Transcriptase, and In Vitro Anti-HIV Activity

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INTRODUCTION

The search for new, modified 2′,3′-dideoxynucleosides, potential reverse transcriptase (RT) inhibitors, is still of special interest; bone marrow[1] toxicity and rapidly developing resistance of currently used nucleosides[2] point to the need for new RT inhibitors. 2′,3′-Dideoxy-3′-fluorothymidine (FLT), a pyrimidine 3′-deoxy-3′-substituted thymidine analogue, is one of the most potent in vitro inhibitors of HIV and its reverse transcriptase, but exhibits hematologic toxicity in vivo.[3–5] It was previously shown that some 5-substituted 2′,3′-dideoxyuridine derivatives exhibited more selective anti-HIV activity.[6] It appeared to us that an introduction to FLT of the substituents decreasing the pK_a value for dissociation of N(3)-H, and enhancing hydrophobic properties of pyrimidine moiety may affect its inhibitory properties, with improved selectivity. It was therefore of interest to synthesize and to test the activity and cytotoxicity of hydrophobic analogues of FLT, and investigate its

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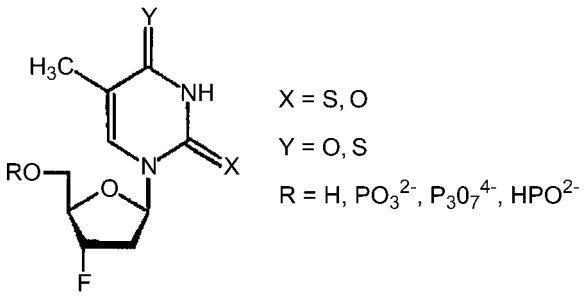
prodrugs with better solubility in water and/or better penetration through the animal cell membrane and blood-brain barrier. To obtain the compounds with decreased toxicity we decided to synthesize a range of FLT, FLTMP, FLTTP and FLT 5′-phosphonate (FLTHP) analogues thiated at 2- and/or 4-position of the pyrimidine ring.

SYNTHESES

Thiated analogues of FLT were prepared with the use of multistep procedures involving as final steps fluorination of the 3′-position of carbohydrate moiety of threo-thymidine with the use of DAST followed by thiation of 2- and/or 4 position of the thymine ring with the use of hydrogen sulfide or Lawesson reagent. Formerly 2′,3′-dideoxy-3′-fluoro-2-tiothymidine (S^2FLT) was synthesized by Lewis-acid catalyzed nucleoside condensation to give the mixture of α- and β-anomers, difficult to separate.[7] At present new synthesis based on the transformation of the known compound *erythro*-thymidine retains the β-anomeric configuration of the starting compound. S^2FLT was then subjected to further modifications leading to its 5′-monophosphate, 5′-phosphonate or 5′-triphosphate.



The nucleoside 5′-monophosphate was prepared by regioselective enzymatic phosphorylation of a nucleoside employing wheat shoot phosphotransferase system. 5′-Triphosphates were synthesized from the corresponding nucleosides with the use of a modified Ludwig procedure employing direct phosphorylation of nucleosides with $POCl_3$, tri-n-butylamine and bis-tri-n-butylammonium pyrophosphate mixture. 5′-H-phosphonate of S^2FLT was prepared by the reaction of the nucleoside with $[(CF_3)_2CHO]_3P$ in anhydrous pyridine.

ANTI-HIV ACTIVITY AND REVERSE TRANSCRIPTASE INHIBITION

Preliminary tests of antiviral activity in vitro were carried out, using the syncytia-inducing laboratory HIV-1 (cat#3) strain in CEM-T_4, MT-2 and PBMC cells. S^2FLT showed inhibition of viral growth with ED_{50} 0.5 μM vs. 0.1 μM for AZT.

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The same concentration of tested compounds showed similar inhibitory effect in primary cultures of PBMC from infected patients. Drug cytotoxicity was observed for AZT between 50 μM−100 μM. For S^2FLT there was no observed cytotoxicity at the highest tested concentration 200 μM. Similar results were obtained for S^2FLTMP and S^2FLTHP. The overall results underline the potent antiviral activity and high selectivity index for above mentioned compounds.

Reverse transcriptase activity was determined using nonisotopic colorimetric method with the use of recombinant reverse transcriptase HIV-1 (Roche Molecular Biochemicals). It was found that 5′-triphosphate of 2′,3′-dideoxy-3′-fluoro-2-thiothymidine (S^2FLTTP) employing the template/primer hybrid poly (A) • oligo $(dT)_{15}$, Dig-dUTP, biotin-UTP and TTP as substrates potently inhibited HIV-1 reverse transcriptase with $IC_{50} = 0.175\,\mu M$, while 2′,3′-dideoxy-3′-fluorothymidine 5′-triphosphate (FLTTP), when investigated with the same template and substrates exhibited $IC_{50} = 0.035\,\mu M$. These results suggest that the antiviral activity of S^2FLTTP is associated with its inhibitory properties towards HIV-1 RT.

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