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Synthesis and Potent Anti-HIV Activity of L-2′,3′-Didehydro-2′,3′-dideoxy-2′-fluoro-4′-thiocytidine

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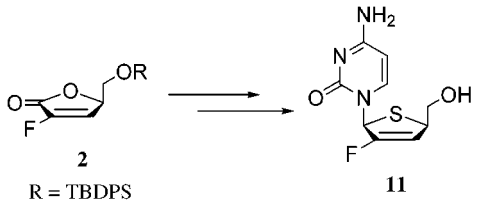
ABSTRACT



L-2′-Fluoro-4′-thio-2′,3′-unsaturated cytidine 11 was synthesized from (*R*)-2-fluorobutenolide 2, which was prepared from 2,3-*O*-isopropylidene-L-glyceraldehyde 1. The synthesized compound 11 shows potent antiviral activity against HIV-1.

Synthetic nucleosides, which have no hydroxyl groups at 2′- and 3′-positons, can be categorized as (a) 2′,3′-dideoxynucleosides such as ddC[1] and ddI,[1] (b) 2′,3′-dideoxy-3′-oxa- or -3′-thia-nucleosides (dioxolane or oxathiolane nucleosides) such as DAPD[2] and 3TC,[3] and (c) 2′,3′-didehydro-2′,3′-dideoxynucleosides such as d4T.[4] Generally, nucleosides in these categories show unique antiviral activities and toxicity profiles. Often both nucleosides with natural and unnatural configurations (D and L) are active against viruses, but cytotoxicity resides, in some cases, mainly in one of the isomers. For example, 3TC[3a] (L-2′,3′-dideoxy-3′-thiacytidine) and FTC[5] (L-2′,3′-dideoxy-3′-thia-5-fluorocytidine) show antiviral activity against human immunodeficiency virus type 1 (HIV-1) with EC_{50} values of 0.07 and 0.009 μM in CEM cells, respectively, and both exhibit no cytotoxicity up to 100 μM in CEM cells. In contrast, the D-isomers, (+)-BCH-189 and D-FTC, are less active against HIV-1 (EC_{50} 0.2 and 0.84 μM in CEM cells, respectively) than the L-isomers. (+)-BCH-189, however, does show cytotoxicity (IC_{50} 2.7 μM in CEM cells). Therefore, it is of interest to investigate the dideoxynucleosides in search of potent antiviral agents with favorable toxicity profiles.

Although various modifications on the carbohydrate moiety and heterocyclic bases of natural nucleosides have been made, 2′,3′-unsaturated 4′-thionucleosides have not been well investigated as a result of the synthetic difficulties. The first synthesis of 4′-thio congeners of natural 2′-deoxynucleosides, 2′-deoxy-4′-thionucleosides, was reported in 1991 by Walker et al.[6] and Secrist et al.;[7] these exhibited

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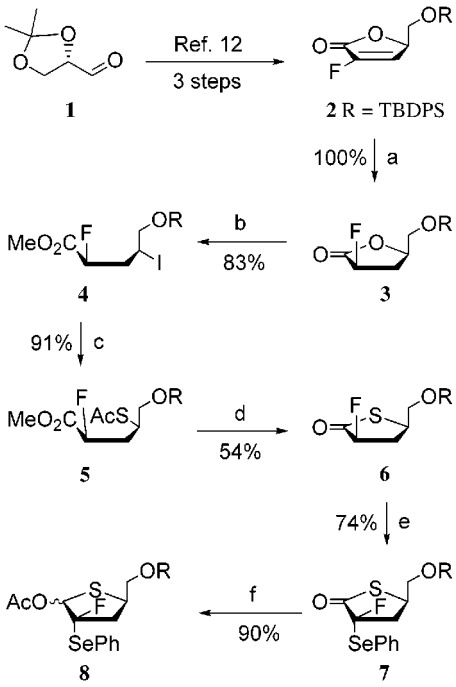
antiviral activities along with cytotoxicity. Several 4′-thionucleosides such as 2′,3′-dideoxy,[8] 2′,3′-dideoxy-3′-*C*-hydroxymethyl,[9] and 2′,3′-didehydro-2′,3′-dideoxy-4′-thionucleosides[10] have been synthesized. Among these, L-2′,3′-didehydro-2′,3′-dideoxy-4′-thionucleosides drew our attention because of their anti-HIV activity, as well as the possibility to improve their chemical stabilities by isosteric replacement of 2′-hydrogen with 2′-fluorine, which has been demonstrated by our previous work.[11] It is well-established that 2′,3′-dideoxy and 2′,3′-didehydro-2′,3′-dideoxy purine nucleosides are quite unstable under acidic conditions, resulting in the cleavage of a glycosidic bond. When a hydrogen atom is replaced by a fluorine atom at the 2′ position, the stability of the glycosidic bond in acidic media is greatly increased. Herein we describe the preliminary enantiomeric synthesis and anti-HIV activity of L-2′,3′-didehydro-2′,3′-dihydroxy-2′-fluoro-4′-thiocytidine (**11**).

The target compound **11** was synthesized from (*R*)-2-fluorobutenolide **2**, which was prepared from 2,3-*O*-isopropylidene-L-glyceradehyde **1** in three steps by a known method[12] (Scheme 1). (*R*)-2-Fluorobutenolide **2** was hydrogenated by treatment with 5% Pd−C under H_2 to allow complete conversion to *β*-2-fluorolactone **3** in a quantitative yield. ^{1}H NMR of compound **3** showed only a single isomer. The lactone **3** was converted to an iodoester **4** in three consecutive steps. Hydrolysis using NaOH in aqueous EtOH followed by methylation of corresponding carboxylic acid gave a hydroxy methylester, which was treated with iodine, triphenylphospine, and imidazole in toluene at 60 °C for 4 h to give the iodoester **4** in 83% overall yield. During the iodination, however, high temperature and longer reaction time resulted in partial epimerization at C4. Currently, we are optimizing the conditions, which prevent the epimerization. A thiolacetate group was introduced by nucleophilic displacement of the iodide group with potassium thiolacetate in DMF to give the corresponding thiolacetate **5** in 91% yield. DIBAL-H induced cyclization of the thiolacetate **5** followed by the Moffatt-type oxidation of the resulting thiolactol gave a thiolactone **6** in 54% yield. The thiolactone **6** was deprotonated by LiHMDS and trapped as a TMS enol ether, which was phenylselenylated using PhSeBr to introduce the 2-phenylselenyl group exclusively at α position of lactone **7** in 74% yield. 2-*β*-Fluoro-2-α-phenylselenyl thiolactone **7** showed no contamination by its *β*-isomer, 2-α-fluoro-2-*β*-phenylselenyl thiolactone, because the sterically demanding phenylselenyl group selectively occupied the α position instead of the sterically crowded *β* position during phenylselenylation of the TMS enol ether. The thiolactone **7** was reduced by DIBAL-H to give the corresponding lactol, which was acetylated to afford the acetate **8** in 90% yield.

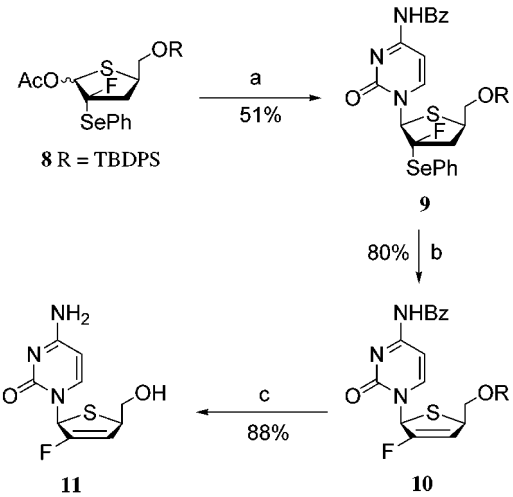
Condensation of the acetate **8** with N^4-benzoylcytosine in Vorbrüggen conditions gave the corresponding cytidine analogue **9** in 51% yield, which underwent mCPBA oxidation followed by elimination to give the N^4,5′-protected 2′,3′-unsaturated 2′-fluoro-4′-thiocytidine **10** in 80% yield. Under the carefully controlled oxidation conditions, no significant sulfoxide formation was obtained. After deprotection of TBDPS and benzoyl groups, the target compound **11**[13] was obtained in 88% yield (Scheme 2).

Scheme 1[a]



[a] Reagents and conditions: (a) H_2, Pd(0), EtOAc; (b) (i) NaOH, aq. EtOH, (ii) dimethyl sulfate, DMSO, (iii) I_2, Ph_3P, imidazole, toluene, 60 °C; (c) KSAc, DMF; (d) (i) DIBAL-H, toluene, −78 °C, (ii) Ac_2O, DMSO; (e) LiHMDS, TMSCl, PhSeBr, THF, −78 °C; (f) (i) DIBAL-H, toluene, −78 °C, (ii) Ac_2O, TEA, CH_2Cl_2.

Scheme 2[a]



[a] Reagents and conditions: (a) silylated N^4-benzoylcytosine, TMSOTf, CH_3CN; (b) mCPBA, CH_2Cl_2, −78 °C; pyridine, rt; (c) (i) TBAF, THF, (ii) NH_3, MeOH.

The anti-HIV activity of the cytidine analogue **11** was evaluated in vitro in human peripheral blood mononuclear

(PBM) cells, in which the compound **11** showed potent anti-HIV activity (EC_{50} 0.12 μM) without cytotoxicity up to 100 μM in PBM, CEM, and Vero cells.

In summary, we have developed the enantiomeric synthesis of 2′,3′-unsaturated L-2′-fluoro-4′-thiocytidine **11** from L-glyceraldehyde derivative **1**, which exhibits potent anti-HIV activity in vitro. This interesting biological result prompted us to synthesize other purine and pyrimidine nucleoside derivatives and optimize the procedures.

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(13) Compound **11**: mp 89−91 °C (dec); $[\alpha]^{26}_{D}$ 205.3° (c 0.14, MeOH); UV(H_2O) λ_{max} 279.5 nm (ϵ 19,900, pH 2), 272.0 nm (ϵ 15,900, pH 7), 272.5 nm (ϵ 16,200, pH 11); ^{1}H NMR (DMSO-d_6) δ 7.83 (d, J = 7.4 Hz, 1H), 7.33, 7.37 (2 br s, 2H), 6.85 (s, 1H), 5.94 (s, 1H), 5.84 (d, J = 7.5 Hz, 1H), 5.28 (t, J = 5.4 Hz, 1H, D_2O exchangeable), 4.02 (m, 1H), 3.59−3.62 (m, 2H); ^{13}C NMR (MeOH-d_4) δ 167.5, 158.4, 156.6 (d, $^2J_{C-F}$ = 276 Hz), 143.0, 112.6 (d, $^3J_{C-F}$ = 17 Hz), 97.4, 65.7, 62.7 (d, $^3J_{C-F}$ = 24 Hz), 48.9. Anal. Calcd for $C_9H_{10}FN_3O_2S\cdot 0.32CH_2Cl_2$: C, 41.39; H, 3.97; N, 15.54; S, 11.86. Found: C, 41.15; H, 4.10; N, 15.55; S, 11.82.