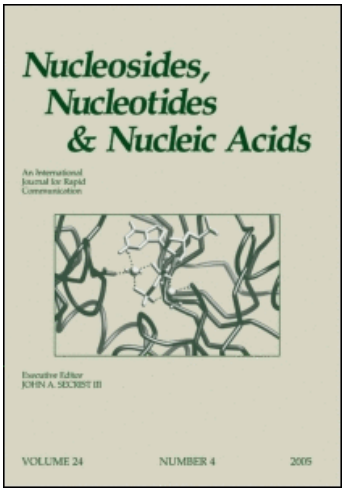

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Structure-Activity Relationships Among a New Class of Antiviral Heterosubstituted 2',3'-Dideoxynucleoside Analogues

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STRUCTURE-ACTIVITY RELATIONSHIPS AMONG A NEW CLASS OF ANTIVIRAL HETEROSUBSTITUTED 2',3'-DIDEOXYNUCLEOSIDE ANALOGUES.

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Abstract. 3'-Oxa-4'-thiocytidine nucleoside analogues **14-17** were prepared from oxathiolanes **10** and **11**, and evaluated for activity against HIV-1 and HBV *in vitro*. The nucleoside analogues were found to possess potent activities against HIV-1 in a panel of cell lines. Compound **16** is moderately active against HBV in 2.2.15 cells.

INTRODUCTION

The replacement of the ribofuranose ring oxygen moiety of nucleosides by a sulfur atom was first demonstrated by Reist *et al.* in the synthesis of 4'-thioadenosine and its L-enantiomer about thirty years ago.[1] The rationale behind this modification is that the 4'-thio analogues were anticipated to possess improved metabolic stability to the phosphorylases which cleave the glycosidic bond in normal nucleosides. Indeed, 4'-thioinosine was subsequently found to be resistant to cleavage by purine nucleoside phosphorylase.[2] Recently, 4'-thiothymidine was reported to be comparable in activity against human cytomegalovirus to ganciclovir in MRC5 cells but was also somewhat toxic.[3] (E)-5-(2-bromovinyl)-4'-thio-2'-deoxyuridine exhibited significant activity against herpes simplex virus type 1 and varicella zoster virus in the same cell line without any apparent cytotoxicity.[4] Moreover, the cytotoxicities of 2'-deoxy-4'-thiocytidine, 2'-deoxy-4'-

thiouridine and 4'-thiothymidine have been reported by Secrist *et al.* in three neoplastic cell lines.[3]

In the 2',3'-dideoxy series of antiviral nucleoside analogues, replacement of the 3'-carbon by sulfur or oxygen moieties resulted in potent activities of some analogues against the human immunodeficiency virus[5] (HIV) and hepatitis B virus (HBV).[6] As a consequence, the issue of stereochemistry emerged favoring enantiomers with β-L-configuration of cytosine[7] and 5-fluorocytosine[8] in the 1,3-oxathiolane series. Thus, the antiviral effects resulting from the transposition of the O,S heteroatoms in the racemic 2,5-disubstituted series were investigated recently by Belleau *et al.* with the five natural bases as well as 5-fluorocytosine[9] (FIG.1). It was also found that the (-)-adenine analogue is comparable in activity to 2',3'-dideoxyinosine against HIV-1 in MT-4 cells and possesses the β-D configuration as evidenced by enzymatic resolution[9] and asymmetric synthesis.[10] Herein, we describe the anti-HIV-1 and anti-HBV activities of both enantiomeric forms of 2'-deoxy-3'-oxa-4'-thiocytidine and their 5-fluoro analogues.

CHEMICAL SYNTHESIS

The target compounds can be considered as 4'-thio analogues of dioxolane nucleosides (FIG. 2) which are accessible from D-mannose for the 2R analogues,[11] L-gulose for the corresponding 2S isomers[12] and D-mannitol[13] or L-ascorbic acid [13,14] for both 2R and 2S isomers.

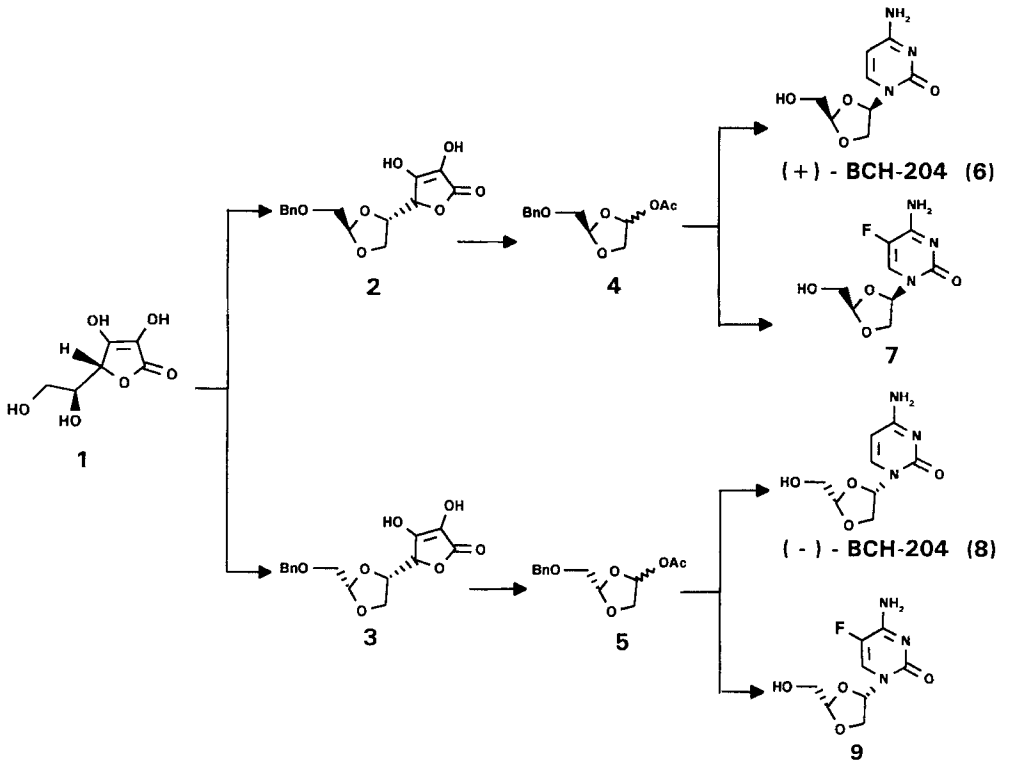
The latter route is particularly attractive as it relies on an efficient fractional recrystallization step of key acetal intermediates **2** and **3** and assessment of their diastereomeric purity by an HPLC method on a reverse-phase Whatman Partisil ODS-3 column.[15] Successive degradation of **2** or **3** with basic H_2O_2, ruthenium catalyzed Wolfe oxidation followed by lead tetracetate oxidation furnished **4** or **5** suitable for coupling with persilylated N-acetylcytosine or its 5-fluoro derivative under Vorbrüggen conditions. Chromatographic separation followed by deprotection gave enantiomerically pure *cis* nucleosides **6-9** (SCHEME 1, trans isomers not shown).

The oxathiolane nucleoside analogues of interest in this study were prepared from (1'R,2'S,5'R)-menthyl-(5R)-acetoxy-1,3-oxathiolan-(2R)-carboxylate (**10**)[16] and its (2S) isomer **14** by a reductive-oxidative process to produce the oxathiolane sugars **12** and **13**, respectively.[10] Chromatographic separation and deprotection as in the case of dioxolanes furnished the *cis* nucleosides **14-17** (SCHEME 2, *trans* isomers not shown).

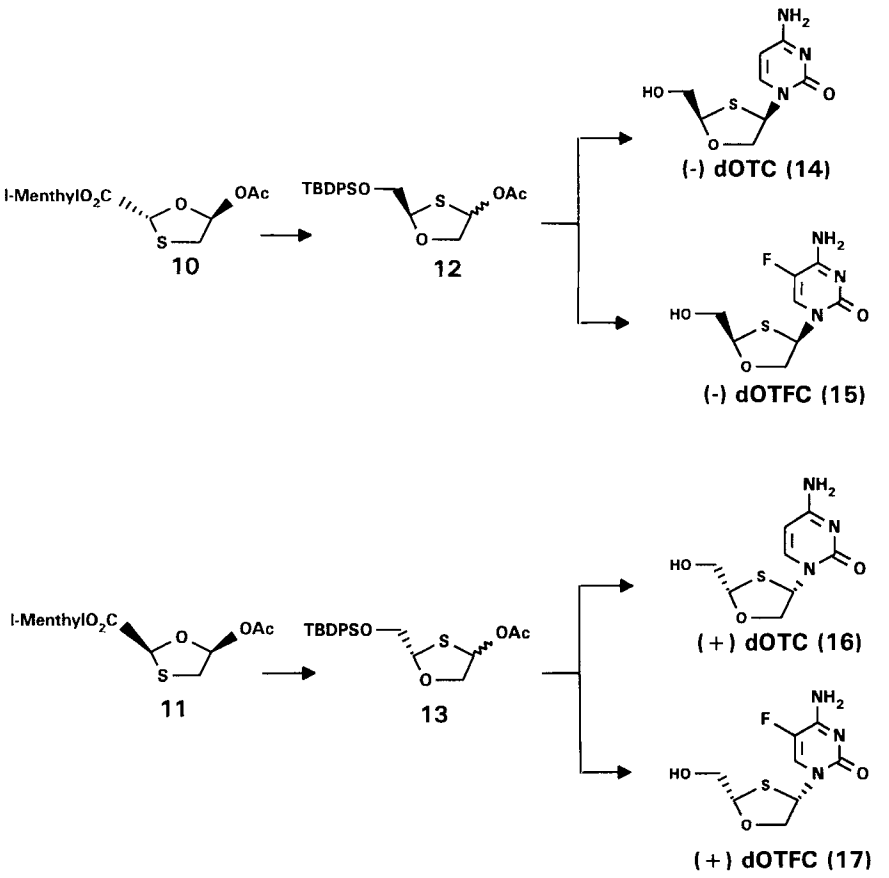
FIG. 1

Base = cytosine, 5-fluorocytosine

FIG. 2



SCHEME 1



SCHEME 2

We have extensively relied on chiral HPLC methods to confirm the optical purity of the synthesized nucleosides prior to biological evaluation.[15] The nomenclature of these analogues follows that of lamivudine[7]: (-)-2'-deoxy-3'-oxa-4'-thiocytidine((-) dOTC (**14**); (-)-2'-deoxy-3'-oxa-4'-thio-5-fluorocytidine (-) dOTFC (**15**); (+)-2'-deoxy-3'-oxa-4'-thiocytidine (+) dOTC (**16**) and (+)-2'-deoxy-3'-oxa-4'-thio-5-fluorocytidine (+) dOTFC (**17**).

ANTIVIRAL RESULTS

The anti-HIV-1 activity of the 2,4-disubstituted and 1,3-oxathiolanes **14-17** was evaluated in MT-4 (human T helper) cells infected with HIV-1 strain IIIB and compared with the inhibition ability of AZT [TABLE 1]. In these assays, all of the nucleosides

TABLE 1. Anti-HIV-1 Activity (IC_{50}) and Cytotoxicity (CC_{50}) of 14-17 in MT-4 Cells.

Compound	IC_{50} (μM)	CC_{50} (μM)	Selectivity Index
(-) dOTC (**14**)	2.8	>500	>178
(-) dOTFC (**15**)	3.2	>500	>156
(+) dOTC (**16**)	0.9	>500	>555
(+) dOTFC (**17**)	3.0	>500	>166
AZT	0.005	110	22,000

exhibited antiviral activity with no cytotoxicity up to 500 μM. In contrast, dioxolane **6** displayed inhibitory activity (IC_{50} 0.2 μM) but exhibited cytotoxicity (CC_{50} 47 μM), whereas **8** and **9** were considerably cytotoxic in MT-4 cells ($CC_{50} \leq 0.5$ μM).

The ability of the nucleosides **14-17** to inhibit HIV-1 in the acutely infected cell lines is demonstrated in cord blood mononuclear cells (CBMCs) and U937 (human monocyte) cell lines [TABLE 2]. All of the nucleosides exhibited appreciable antiviral activity in the cell lines studied, most notably in CBMCs and U937 cells. However, **16** displayed lower selectivity than the other nucleosides in these cell lines.

The anti-HBV activity of 1,3-dioxolanes **6** and **8** and 1,3-oxathiolanes **14-17** was determined in the transfected human hepatoma cell line 2.2.15 [TABLE 3].

(-)-BCH-204 (**8**) emerged as the most potent nucleoside being 3500-fold more potent than its 4'-thio analogue (+) dOTC (**16**) and 28-fold more potent than its D-enantiomer **6**. Unfortunately, **8** was also cytotoxic. Chu and co-workers[12] reported similar potency of **8** and **6** against HBV and were able to determine the anti-HIV-1 activity of **8** in human peripheral blood mononuclear (PBM) cells.

DISCUSSION

The results presented here demonstrated several unexpected findings in the 4'-thio series of dideoxynucleoside analogues. First, despite the antiviral activity of (+) dOTC (**16**) against HIV and HBV, this nucleoside is less selective than its dioxolane analogue, (-)-BCH-204 (**8**), against HBV *in vitro* and at least 300-fold less cytotoxic. Second, the 5-fluoro

TABLE 2. Anti-HIV-1 Activity and Cytotoxicity of Nucleosides 14-17 in Acutely Infected Cell Lines.

Compound	IC50 (μM)		CC_{50} (μM)		Selectivity Index	
	CBMCs	U937	CBMCs	U937	CBMCs	U937
(-) dOTC (**14**)	0.4	0.4	>500	>500	>1250	>1250
(-) dOTFC (**15**)	0.2	0.4	>500	>500	>2500	>1250
(+) dOTC (**16**)	0.3	0.3	105	22	365	73
(+) dOTFC (**17**)	0.3	0.6	>500	>500	>1667	>833
AZT	0.03	0.04	45	110	1500	2750

TABLE 3. Anti-HBV Activity and Cytotoxicity of Nucleoside Analogues in 2.2.15 Cells.

Compound	IC50 (μM)	Cytotoxicity (μM)
(+) -BCH-204 (**6**)	0.14	>47
(-) -BCH-204 (**8**)	0.005	0.5
(-) dOTC (**14**)	>45	>45
(-) dOTFC (**15**)	>45	>45
(+) dOTC (**16**)	17.5	>50
(+) dOTFC (**17**)	>45	>45
Lamivudine	0.02	>45

enantiomers (-) dOTFC (**15**) and (+) dOTFC (**17**) appear to possess very similar profile being almost equipotent in the cell lines investigated. This finding is rather unusual in view of the current knowledge available on anti-HIV nucleoside analogues where activity resides solely in the β-D form as in AZT,[17,18] ddI[19] and d4T,[19] or in the case of BCH-189, where selectivity was obtained in the clinical candidate 3TC™ with β-L configuration.[20] Despite the scarcity of antiviral activity of L-nucleosides, β-L-ddC and β-L-5FddC were recently reported to be inhibitory to HIV and HBV replication *in vitro*.[21-24] Third, the 5-fluorocytidine analogue (+) dOTFC (**17**) is a more selective anti-HIV-1 agent than the corresponding cytidine analogue (+) dOTC (**16**).

In summary, we have demonstrated that the 4'-thio moiety present in the enantiomers of dOTC confers significantly lower cytotoxicity relative to the corresponding dioxolane enantiomers of BCH-204. As analogues of 2',3'-dideoxynucleoside analogues, the 4'-thiocytidines exhibit greater selectivity towards HIV than HBV. The equipotency of (-) dOTFC (**15**) and (+) dOTFC (**17**) towards HIV-1 and the lack of cytotoxicity suggest that the absolute configuration of a dideoxynucleoside analogue may play a significant role in the selectivity towards viral and cellular polymerases.

EXPERIMENTAL

Cell Cultures. MT-4 cells and Jurkat Clone E6-1 were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH. U937 cells were obtained from the American Type Culture Collection. All cells were cultured at 37°C in RPMI-1640 (Gibco, Burlington, Ontario, Canada), supplemented with 10% fetal calf serum (ICN Chemical, Montreal, Canada), 2mN glutamine (Sigma, St.Louis, USA), 100 U/ml of penicillin (ICN), and 100 μg/ml of streptomycin (ICN).

Cord blood mononuclear cells (CBMC). CBMCs obtained from HIV-seronegative donors, were isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Cultures were PHA stimulated, and after 3 days, pools of cells from three CBMC donors were established and used as feeder cultures.

HIV-1 strain. The HIV-1 IIIB was derived from chronically infected H9 cells (kindly supplied by Robert Gallo).

Screening for anti-HIV-1 efficacy. Cells were infected with HIV-1 positive supernatant for 2 hr. The HIV-1 inoculum ranged from 200 to 20 50% tissue culture

infective dose per cell ($TCID_{50}$); for CBMC it ranged from 2000 to 1000 $TCID_{50}$. After infection, cells were washed and dispersed in 96-well plates in the presence of various concentrations of drugs. Control wells treated with AZT were also evaluated for comparison of the agent's potential anti-HIV effect. These experiments were performed in duplicate. The culture medium was changed at day 4, such that half of the medium was replaced with fresh medium containing the original drug concentration in each well. Cell free supernatant fluid was harvested at day 7 and assayed for p24 antigen production by ELISA (Abbott Laboratories, North Chicago, Ill.) In the case of CBMC, HIV-1 replication was assayed by measuring the reverse transcriptase activity in cell culture fluid. We established the 50% inhibitory dose (IC_{50}) of each drug on the basis of p24 antigen levels or reverse transcriptase activity in culture fluids vs. concentration of drug.

The effect of nucleosides on cellular growth. For toxicity studies, uninfected cells were cultured in 96-well plates in the presence of compound. Cultures were set up in duplicate, over a selected range of concentration. Untreated control wells were also maintained in parallel. These experiments were performed prior to anti-HIV testing in order to determine the maximum tolerated dose. A medium change was performed on day 4 and on day 7, and each culture was counted to assess cell proliferation. The percentage of cell growth vs. concentration was plotted for each drug in different cells. Cells cultured in the absence of drug represent 100% growth. CC_{50} is determined as the concentration of drug which inhibits 50% of cellular growth.

Inhibition of human hepatitis B virus. The method used for this test is described in detail by Korba and Milman.[25]

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