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SYNTHESIS, ANTI-HUMAN IMMUNODEFICIENCY VIRUS, AND ANTI-HEPATITIS B VIRUS ACTIVITY OF PYRIMIDINE OXATHIOLANE NUCLEOSIDES

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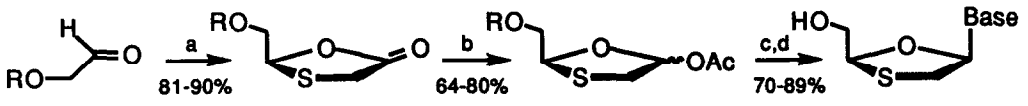
The discovery that certain 2',3'-dideoxynucleosides, such as 3'-azido-3'-deoxythymidine (AZT), 2',3'-dideoxyinosine (DDI), and 2',3'-dideoxycytidine (DDC), are phosphorylated *in vivo* and that the resulting 5'-triphosphates inhibit the reverse transcription of human immunodeficiency virus (HIV)-derived RNA to DNA became the basis of the clinical use of these nucleosides for treatment of HIV-1 infections.[1] However, while these therapies have proven to be reasonably successful, the manifestation of undesirable side effects (*e. g.*, bone marrow suppression, pancreatitis, peripheral neuropathy, *etc.*) and the development of resistant strains of the virus has prompted the search for agents which are more potent, less toxic and not cross-resistant with existing AIDS therapies. Of the candidates which have emerged over the past few years, the 3'-thianucleoside, **BCH-189, 1** (**R = H**, see **Table 1**), was clearly one of the most promising. For example, when the (-)-enantiomer of 2',3'-dideoxy-3'-thiacytidine [(-)-BCH-189 or 3TC], was compared to AZT and DDC in several cell lines acutely infected with HIV-1, it was found to have a therapeutic index of > 1,000 with no cellular toxicity at therapeutic levels.[2] Moreover, when it was tested against AZT-, DDI- or TIBO-resistant HIV, (-)-BCH-189 was not found to be cross-resistant.[3a] The clinical trials, which are now in progress, will demonstrate whether or not these encouraging preliminary findings can be translated into a clinically-efficacious drug.[3a,b]

Because of the interesting properties exhibited by **1** and its enantiomers, we decided to prepare a number of uracil- and cytosine-substituted oxathiolane analogues for evaluation of their anti-HIV activity and cellular toxicity. To accomplish this, we employed the route which we have previously developed for preparing BCH-189 and probe its generality for adding other bases.[4-7] It is noteworthy that when using this approach, which is briefly summarized in **Scheme 1**, we could achieve almost complete β-selectivity (> 95%) in the glycosylation of the oxathiolane ring *in every case reported here* when stannic chloride was used as the Lewis acid catalyst.[8] Since little or no stereoselectivity is generally observed in standard glycosylation reactions with 2'-deoxyribose systems,[9,10] the results observed here were surprising and therefore warranted further study.

Scheme 1



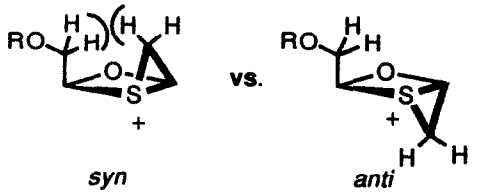
a. Thioglycolic acid, toluene, Δ; b. DIBAL/toluene, -78°C, then Ac_2O, 25°C; c. Silylated base, $SnCl_4$, CH_2Cl_2; d. Deprotection.

R = TBDPS or Butyryl

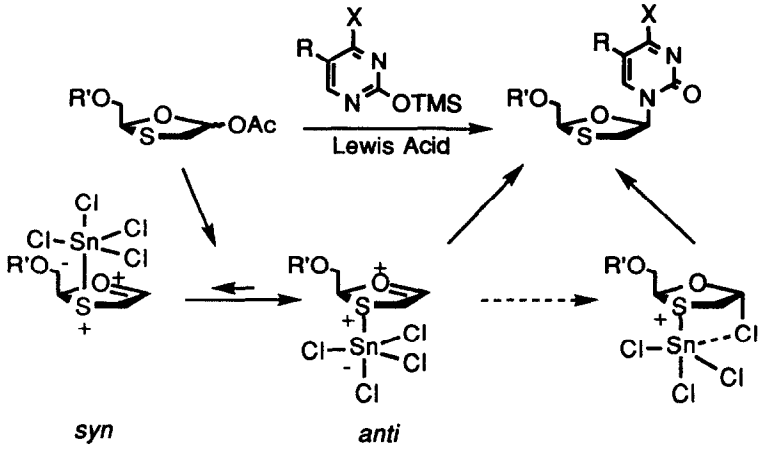
Initially, we rationalized the stereoselectivity based on the preferred formation of the intermediate *anti*-episulfonium ion shown in **Scheme 2**. However, this hypothesis was rejected because of its incompatibility with the high Lewis acid dependency exhibited in these reactions. The tin-sulfur

complexation mechanism shown in **Scheme 3** seems more plausible. Specifically, stannic chloride complexation *anti*- to the protected hydroxymethyl group avoids the severe non-bonding interactions that are present in the *syn*-complex. As a consequence of this selective interaction, the steric bias exerted by the only facial differentiating agent in the system (*i. e.*, the protected hydroxymethyl group) is reversed and amplified. Thus, the formation of this *anti*-complex effectively blocks attack of the silylated base on the α-face, resulting in the formation of the desired β-isomer.

Scheme 2

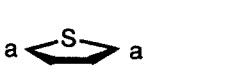


Scheme 3

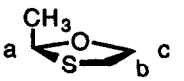


Spectroscopic evidence was obtained which supports the complexation hypothesis. Specifically, **Scheme 4** lists the changes in the ^{13}C chemical shifts ($CDCl_3$) of tetrahydrothiophene and 2-methyloxathiolane due to the presence of the Lewis acids, trimethylsilyl triflate and stannic chloride. In both cases the hard Lewis acid, trimethylsilyl triflate, had no effect on the observed chemical shifts. However, the softer acid, stannic chloride, exerted a profound effect on the chemical shifts of the carbons, suggesting the formation of a complex and providing strong support for the role of these complexes in controlling the facial selectivity of the glycosylation reaction.

Scheme 4



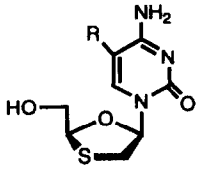
$\Delta\delta_a$ (TMSOTf) < 0.1 ppm
$\Delta\delta_a$ ($SnCl_4$) = 8 ppm



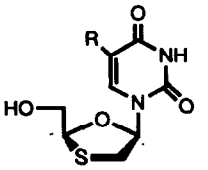
$\Delta\delta_a$ (TMSOTf) < 0.1 ppm
$\Delta\delta_a$ ($SnCl_4$) = 5 ppm
$\Delta\delta_b$ ($SnCl_4$) = 3.5 ppm
$\Delta\delta_c$ ($SnCl_4$) = 0.5 ppm

The results of the anti-HIV activity and cytotoxicity evaluations of a series of uracil and cytosine oxathiolanes prepared using the synthetic protocol described above are listed in **Table 1**. Although none of the uracil derivatives showed any significant activity (*i. e.*, EC_{50} values of > 10 μM) against HIV-1_{LAI} in human peripheral blood mononuclear (PBM) cells, several of the cytosine derivatives exhibited reasonable activity in the same system.[11] In order to further probe the activity profile of these compounds, all of the oxathiolane derivatives were also evaluated in acutely infected MT-4 cells. In this system only the parent cytosine, **1a (BCH-189)**, and the 5-fluorocytosine, **1c (FTC)** displayed any activity. The apparent activity differences exhibited by **1e** and **1f** in PBM compared to MT-4 cells are most likely the result of poor anabolism of these compounds in MT-4 cells. However, further studies will be required to verify this hypothesis. No toxicity in either PBM or Vero cells was found up to concentrtions of 100 μM for any of the compounds tested.

Table 1. Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentrations of Oxathiolane Pyrimidine Nucleosides in Acutely HIV-1-Infected PBM and MT-4 Cells, HBV-Infected Hep G2 (2.2.15) Cells, and Uninfected PBM and Vero Cells.



1



2

No.	R	Anti-HIV-1 EC_{50}, μM		Anti-HBV % Inhib. at [c] μM	Cytotoxicity IC_{50}, μM	
		PBM	MT-4	HepG2 (2.2.15)	PBM	Vero
1a	H	0.06	7.0	87 at 1	> 100	> 100
1b	Me	> 100	> 200	0 at 25	> 100	> 100
1c	F	0.01	2.1	90 at 1	> 100	> 100
1d	Cl	38.7	> 200	58 at 25	> 100	> 100
1e	Br	2.4	> 200	84 at 25	> 100	> 100
1f	I	0.72	> 200	55 at 25	> 100	> 100
2a	H	> 100	> 200	0 at 25	> 100	> 100
2b	Me	64.4	> 200	0 at 25	> 100	> 100
2c	F	> 100	> 200	75 at 25	> 100	> 100
2d	Cl	60.8	> 200	59 at 25	> 100	> 100
2e	Br	> 100	> 200	11 at 25	> 100	> 100
2f	I	> 100	> 200	0 at 25	> 100	> 100

* The EC_{50} values for the more active (-)-enantiomers of **1a** and **1c** are 9 and 10 nM, respectively.

Since hepatitis-B virus (HBV) is known to encode for an enzyme that possesses reverse transcriptase activity, we decided to examine whether these compounds might also act as HBV inhibitors. This was particularly interesting to us because of our previous report which disclosed that **1a** and **1c** were both potent inhibitors of the HBV in the transfected hepatoma cell line 2.2.15.[12,13] As noted in **Table 1**, although several of these derivatives (*i. e.*, **1d**, **1e**, **1f**, **2c** and **2d**) displayed modest anti-HBV activity, none were nearly as potent as either **1a** and **1c**.

In summary, we have demonstrated the generality of our synthetic approach for preparing oxathiolane nucleosides and have provided strong evidence in support of the intermediacy of tin-sulfur complexes which are presumably responsible for the stereoselectivity consistently observed in the glycosylation of the "thiacarbohydrate". We have evaluated the anti-HIV, anti-HBV and cytotoxicities of several 5-substituted uracil and cytosine oxathiolanes and found that the parent cytosine, **BCH-189**, and the corresponding 5-fluoro derivative, **FTC**, were the most promising.

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