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THE SYNTHESIS AND ANTI-HIV ACTIVITY OF PYRIMIDINE DIOXOLANYL NUCLEOSIDES

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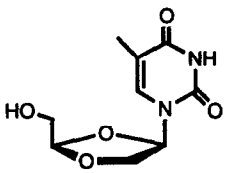
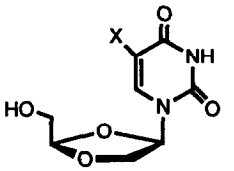
Abstract. A series of 5-substituted uracil and cytosine Dioxolanyl nucleosides were synthesized as potential anti-HIV agents. Compounds 3a and 3c were found to be extremely potent in acutely infected human lymphocytes.

Dioxolane-T, 1, is a nucleoside analogue that exhibits modest anti-HIV activity *in vitro* without acute cellular toxicity.[1] In an effort to more fully examine the potential of this class of compounds, we carried out a structure-function study to evaluate the effects of pyrimidine nucleoside base alterations (*e. g.*, 2 and 3) on anti-HIV activity. However, because these nucleosides contain an "unnatural" Dioxolanyl group instead of the usual 2'-deoxyribose units, they can not be easily accessed from naturally-occurring starting materials. Thus, to carry out this study, an efficient synthetic method for assembling various dioxolane nucleoside analogues with the requisite β-stereochemistry had to be devised.



Dioxolane-T, 1



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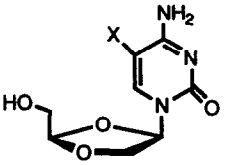

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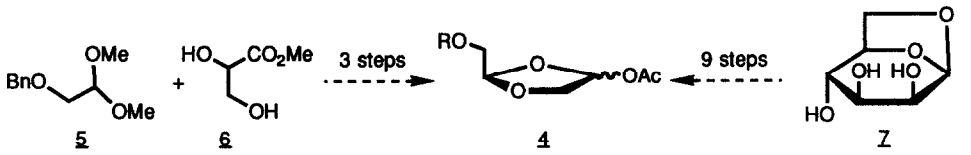
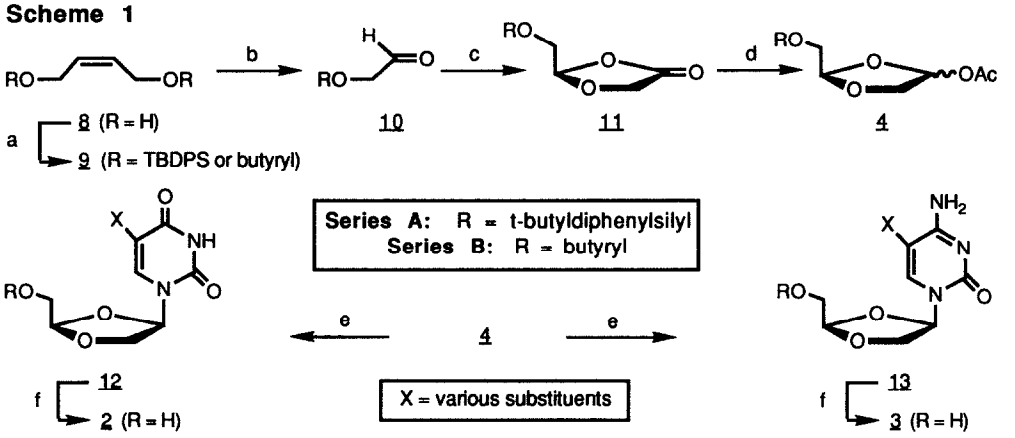
Prior to our synthesis,[2] two other syntheses of 1 had been reported.[1,3] Both employed a protected form of 4 as a key synthetic intermediate and both utilized nitrogen glycosylation reactions which were non-stereoselective. The first approach, a racemic synthesis by Norbeck *et al.*,[1] made use of the simple starting materials, methyl glycerate, 6, and benzyloxyacetaldehyde dimethylacetal, 5. These were subjected to sequential cyclization, saponification and oxidative decarboxylation, producing the required acetate, 4 (R = Bn). The second approach, a chiral synthesis by Chu *et al.*,[3] involved the nine step conversion of 1,6-anhydro-D-mannose, 7, (available from mannose in a two

step, one pot procedure[4]) to one of the enantiomers of acetate 4 (R = TBDPS). Both approaches then employed a coupling of 4 with silylated thymine under Vorbruggen conditions (trimethylsilyl triflate (TMSOTf)) to give an approximately equal mixture of the α– and β-anomers.[5] The β–isomer was separated and deprotected to give dioxolane-T.



Neither of these approaches appeared attractive to us for preparing the derivatives needed for our structure-function study. In particular, since individual enantiomers usually exhibit different activity / toxicity profiles, it seemed imprudent to employ a synthetic protocol which only provides access to only one of the possible enantiomers of each substrate.[6] Moreover, the lack of selectivity in the nitrogen glycosylation reactions resulted in diastereomeric mixtures which were difficult to separate, thereby providing a significant impediment for conveniently preparing a large series of compounds for biological evaluation. Therefore, we developed an efficient approach which produced these materials as racemates, but which, if desired, could provide convenient access to individual enantiomers *via* a kinetic resolution protocol. The approach selected is shown in **Scheme 1**.[2]

Scheme 1



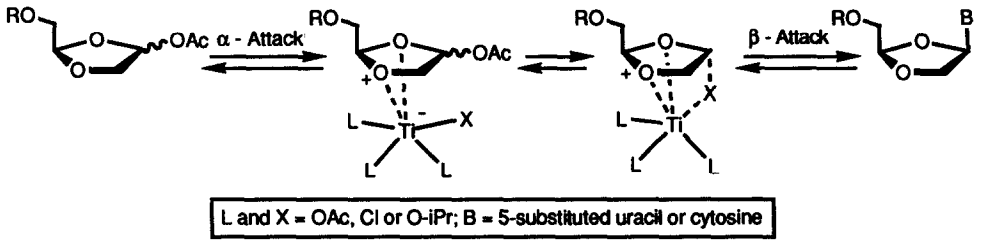
Series A: a. TBDPSCl, NEt_3, DMAP, CH_2Cl_2; b. O_3, CH_2Cl_2, -78°C / Me_2S; c. $HOCH_2CO_2H$, $(ClCH_2)_2$, p-TsOH (28% from 8); d. $LiAlH(OtBu)_3$ / Ac_2O (55%); e. Silylated pyrimidine, $TiCl_3(OiPr)$; f. n-Bu_4NF, THF, 25°C.
Series B: a. C_3H_7COCl, Pyr., DMAP, 0°C (70%); b. O_3, CH_2Cl_2, -78°C / Me_2S (89%); c. $TMSOCH_2CO_2TMS$, $(ClCH_2)_2$, TMSOTf (92%); d. $LiAlH(OtBu)_3$ / Ac_2O (69%); e. Silylated pyrimidine, $TiCl_3(OiPr)$; f. $NaOCH_3$ / CH_3OH.

Protection of *cis*-2-buten-1,4-diol, 8, as its *bis*-*t*-butyldiphenylsilyl (TBDPS) ether was accomplished using standard silylation conditions. This material was then subjected to sequential ozonolysis, reduction, and cyclization with glycolic acid to give the corresponding oxalactone 11 (28%

yield for the four steps). Previously, Norbeck *et al.* had observed that the lactol derived from reduction of 11 (R = Bn) underwent a facile fragmentation to acetaldehyde and benzyloxy-acetaldhyde.[1] To circumvent this problem, lactone 11 was first reduced by diisobutylaluminum hydride (DIBAL) and then acetylated *in situ* with acetic anhydride. Although the reaction produced the desired acetate 4 in 22% yield, the major products (ethyleneglycol, mono-TBDPS ether and ethyleneglycol, mono-TBDPS ether, mono-acetate) were apparently derived from a fragmentation / reduction process (39% yield). Reasoning that the undesired by-products resulted from DIBAL functioning as both a Lewis acid (which promoted C-O cleavage) and a reducing agent (for the resulting oxonium ion), we decided to examine the use of reducing agents which were incapable of Lewis acid behavior (*e. g.*, a tetravalent aluminum hydride). Thus, when lactone 11 was exposed to lithium tri-*t*-butoxyaluminum hydride in tetrahydrofuran at 0°C, followed by *in situ* acetylation, acetate 4 was formed in 50-60% yield and only trace amounts (< 5%) of the by-products were observed.

Although the reactions of 4 with silylated thymine and most Lewis acids (*e. g.*, TMSOTf or $SnCl_4$) proceeded with little, if any, diastereofacial selectivity, one group of catalysts, the oxaphilic titanium Lewis acids, $TiCl_4$, $TiCl_3(O\text{-}iPr)$ and $TiCl_2(O\text{-}iPr)_2$, gave excellent selectivities favoring the β-isomer. The facial selectivity of these nitrogen glycosylation reactions can be rationalized on the basis of the preferential heteroatom / Lewis acid interactions depicted in **Scheme 2**.[2] *Anti*-ligation of the titanium to the ring oxygens is highly favored over the alternative *syn*-mode which suffers from severe steric interactions between the titanium ligands and the protected hydroxymethyl group. Activation of the anomeric center would result in an oxonium ion whose α-face is extremely hindered. Alternatively, another complex may form in which the associated titanium delivers a ligand to the α-face of the anomeric carbon. In either case, however, these complexes should possess a sufficient facial bias to permit preferential β-attack to form the β-*N*-glycoside.

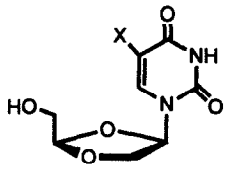
Scheme 2



Using this approach, we were able to prepare a number of uracil- and cytosine-substituted dioxolane analogues for evaluation of their anti-HIV activity and cellular toxicity. Although little effort was made to optimize the reaction conditions for variations in the X- and R-substituents, the observed β-selectivity remained high for virtually all the cases studied. In **Series A** all of the cytosine derivatives gave complete β-selectivity using $TiCl_3(O\text{-}iPr)$.[7] However, three of the uracil derivatives

(X = I, CF_3 and Cl) gave variable amounts of α-product. Although we have not definitely established the origins of the α-isomers in these cases, preliminary evidence indicates that these materials are secondary products which result from epimerization of the kinetically-formed β-isomer.[8]

Table 1. Median Effective (EC_{50}) and Inhibitory (IC_{50}) Concentrations of Dioxolane Pyrimidine Nucleosides in HIV-1-Infected PBM, Uninfected PBM, Vero, CEM and MT-4 Cells.



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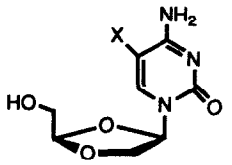

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Cmpd.	X	Anti-HIV-1 EC_{50}, μM[a] HIV-infected PBM	Cytotoxicity IC_{50}, μM[a] PBM	Vero	CEM	MT-4
1	Me	0.09	>100	>100	-	>200
2a	H	57.5	>100	>100	-	>200
2c	Et	>100	>100	>100	-	30%@200
2d	CF_3	93.0	>100	>100	-	>200
2e	F	28.5	>100	>100	>100	-
2f	Cl	1.7	>100	>100	-	>200
2g	Br	2.8	>100	>100	-	>200
2h	I	>100	>100	>100	-	>200
3a	H	0.005	>100	0.17	0.08	66%@200
3b	Me	39.5	>100	>100	-	>200
3c	F	0.002	>100	0.66	0.24	65%@200
3d	Br	15.7	>100	>100	-	75%@200
3e	I	0.69	>100	≥100	29.9	75%@200
(-)-3c	F	0.002	>100	0.3	0.19	63%@200
(+)-3c	F	0.06	>100	4.6	4.3	68%@200

[a] Mean value of at least two determinations.

The results of the anti-HIV activity and cytotoxicity evaluations of a series of uracil and cytosine dioxolanes are listed in **Table 1**.[9,10,11] Over half of the compounds tested showed EC_{50} values of <20μM against HIV-1 (strain LAV) in human peripheral blood mononuclear (PBM) cells. In the uracil series these included the 5-methyl (dioxolane-T, 1), 5-chloro (2f) and 5-bromo (2g) derivatives. These compounds exhibited moderate activity and no toxicity at concentrations up to 100 μM in either slow growing PBM cells or rapidly dividing Vero and MT-4 cells.[10] In the cytosine series several potent anti-HIV agents were discovered. These include the parent cytosine (3a),[12] as well as the 5-fluoro (3c) and 5-iodo (3e) derivatives. While these materials were non-toxic to PBM cells at concentrations up to 100 μM, two of these compounds, 3a and 3c, proved to be quite toxic to Vero, CEM and MT-4 cells.

In order to probe whether the toxicity of 3a and 3c was primarily associated with only one enantiomer, as has been demonstrated to be the case for the enantiomers of the analogous

oxathiolanes, BCH-189 (2',3'-dideoxy-3'-thiacytidine) and FTC (2',3'-dideoxy-5-fluoro-3'-thiacytidine),[13,14] we resolved the more potent of these compounds, 3c. Initial attempts at resolution using classical techniques (*e. g.*, *via* chiral acids) failed uniformly. Although some reasonable degrees of enantiomeric enrichments were obtained using chiral HPLC,[15] we were never able to resolve the enantiomers to a sufficient extent to unequivocally assess the question of differential toxicity. However, we developed a procedure which permitted a highly enantioselective enzyme-catalyzed resolution of 3c based on pig liver esterase-mediated hydrolysis of its butyrate ester derivative.[16,17] Upon subsequent evaluation, both enantiomers of 3c were found to exhibit potent activity against HIV in PBM cells at nanomolar concentrations. However, both showed toxicity in Vero, MT-4, and CEM cells, but not in PBM cells (see last two entries in **Table 1**). Interestingly, the (-)-enantiomer was at least one order of magnitude more toxic than its (+)-counterpart in the rapidly dividing CEM and Vero cells. This is unusual, since previous work has indicated that in the corresponding oxathiolane series, the (-)-enantiomer was less toxic than the (+)-enantiomer.[14,18]

With the exception of compound 1 (EC_{50} = 39 μM), none of the compounds listed in **Table 1** demonstrated any antiviral activity in MT-4 cells. Some of the compounds, in particular 3a, 3c, 3d and 3e, were toxic to uninfected MT-4 cells at 200 μM. The lack of activity against HIV-1 and low toxicity in uninfected MT-4 cells could be the result of these compounds not being phosphorylated and/or transported in these cells. In contrast, in the related oxathiolane series the 5-fluoro derivative (FTC) was found to be extremely potent and non-toxic in both acutely infected PBM and MT-4 cells. It is interesting that the replacement of oxygen with sulfur at the 3'-position has such a profound effect on the toxicities of these compounds.[13,14]

The finding that both enantiomers of 3c are highly potent and selective against HIV-1 in primary human lymphocytes, but are toxic in Vero and CEM cells makes it worthwhile to further evaluate these compounds in other rapidly dividing cells, such as human bone marrow cells, as well as for acute and chronic toxicity in small animals.

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2. Choi, W.-B.; Wilson, L. J.; Yeola, S.; Liotta, D. C.; Schinazi, R. F. *J. Amer. Chem. Soc.*, **1991**, *113*, 9377.
3. Chu, C. K.; Ahn, S. K.; Kim, H. O.; Beach, J. W.; Alves, A. J.; Jeong, L. S.; Islam, Q.; Van Roey, P.; Schinazi, R. F. *Tetrahedron Lett.*, **1991**, *32*, 3791.
4. Zottola, M. A.; Alonso, R.; Vite, G. D.; Fraser-Reid, B. *J. Org. Chem.*, **1989**, *54*, 6123.
5. Although in reference 3 it is claimed that the coupling of 4 with silylated thymine in the presence of stannic chloride produces the desired β-isomer with only a trace of the undesired α-isomer, no

details of this reaction (yield, isomer ratio, *etc.*) are provided. In our hands this reaction produces a 1:1 mixture of isomers.[2]

6. For an example of this approach using Dioxolanyl nucleosides, see: Kim, H. O.; Ahn, S. K.; Alves, A. J.; Beach, J. W.; Jeong, L. S.; Choi, B. G.; Van Roey, P.; Schinazi, R. F.; Chu, C. K. *J. Med. Chem.*, **1992**, *35*, 1987. In this study (-)-dioxolane-T, the only enantiomer synthesized, was less active (0.39 μM) than the racemate (0.09 μM) against HIV-1 in human lymphocytes.
7. The β-stereochemistry was established by the observation of NOE enhancements between $H_{4'}$ and $H_{1'}$ (~2 - 4%). In addition, the anomeric proton of the β-isomers consistently exhibited chemical shifts which were approximately 0.2 ppm higher field than their α-counterparts.
8. **Series B** appears to be more tolerant of variations in the Lewis acid employed. For example, in the coupling reaction leading to 13c, the use of either one equivalent of $TiCl_3$(O-iPr) with 0.15 equivalents of $TiCl_4$ or one equivalent each of $TiCl_3$(O-iPr) and $TiCl_4$ resulted in virtually identical yields of coupling product (44% vs. 48%, respectively) with complete β-selectivity.
9. The antiviral assays were conducted in mitogen stimulated human peripheral blood mononuclear (PBM) cells infected with HIV-1 (strain LAV). For further details, see reference 10.
10. Schinazi, R. F.; Sommadossi, J.-P.; Saalmann, V.; Cannon, D. L.; Xie, M.-Y.; Hart, G. C.; Smith, G. A.; Hahn, E. F. *Antimicrob. Agents Chemother.* **1990**, *34*, 1061.
11. All final compounds (1, 2a-h, 3a-e) were fully characterized by ^1H NMR, ^{13}C NMR, IR, Mass Spectrometry and Combustion Analysis.
12. For the first report of the anti-HIV activity of the parent cytosine, 3a, see: Belleau, B. *European Patent Application* 0 337 713.
13. Schinazi, R. F.; Chu, C. K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L.-S.; Beach, J. W.; Choi, W.-B.; Yeola, S.; Liotta, D. C. *Antimicrob. Agents Chemother.* **1992**, *36*, 672.
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15. A Chiralpak AS - 10 μm, 25 cm x 4.6 mm column (J. T. Baker) was used for the separation of the enantiomers of 3c. The mobile phase was a mixture of 30:70 HPLC grade ethanol:hexane (Fisher Scientific). The flow rate was 0.9 ml/min. The eluent was monitored by UV detection at 271 nm. The retention times for (-)-, and (+)-enantiomers were 9.7 and 13.2 min, respectively.
16. The use of a butyrate ester also facilitates the separation of the optically-enriched, unreacted substrate from the reaction medium by extraction with $CHCl_3$. Cleavage of the ester with sodium methoxide/methanol gave the (-)-enantiomer of 3c (98%ee). Lyophilization of the aqueous layer and subsequent purification by crystallization gave the (+)-enantiomer of 3c (95%ee).
17. For another example of the use of enzyme-mediated resolution of related oxathiolane nucleosides, see: Hoong, L. K.; Strange, L. E.; Liotta, D. C.; Koszalka, G. W.; Burns, C. L.; Schinazi, R. F. *J. Org. Chem.*, **1992**, *57*, 5563.
18. While the absolute configurations of (+)-3c and (-)-3c are not known, we assume that (-)-3c has the same absolute configuration as its oxathiolane analogue (*i. e.*, (-)-FTC) since the 5'-butyrates of both of these materials are approximately three orders of magnitude less reactive than their (+)-counterparts to pig liver esterase.