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(±)-DIOXOLANE-T

((±)-1-[(2β,4β)-2-(hydroxymethyl)-4-dioxolanyl]thymine)

A NEW 2',3'-DIDEOXYNUCLEOSIDE PROTOTYPE WITH *IN VITRO* ACTIVITY AGAINST HIV

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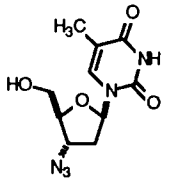
Abstract: A novel analogue of 3'-deoxythymidine, in which the 3'-carbon is replaced by oxygen, was synthesized in 5 steps from benzyloxyacetaldehyde dimethyl acetal and (±)-methyl glycerate. In ATH8 cells, this analogue showed significant inhibition of the infectivity and cytopathic effect of HIV at a concentration of 20 uM, while the growth of the uninfected control cells was not affected by concentrations as high as 200 uM. X-ray crystallographic analysis confirmed the assignment of stereochemistry and established a $^{3}T_{4}$ type conformation of the dioxolane ring.

Although antiretroviral therapy with AZT (**1**) has reduced morbidity and mortality in AIDS patients,[1] the emergence of AZT resistant HIV variants,[2] the hematologic toxicity of AZT,[3] and the transience of AZT's clinical benefit[4] provide a powerful impetus for the discovery of superior therapeutic agents. In this regard, the finding[5] that 2',3'-dideoxynucleosides inhibit the replication of HIV *in vitro* has elicited a search for other nucleoside based inhibitors of the viral reverse transcriptase. Several of these compounds, such as ddI (**2**), ddC (**3**), d4T (**4**), and AZDU (**5**), have entered or will soon enter clinical trials.[6a] In particular, ddI has been shown to suppress HIV replication in patients with AIDS and ARC, and thus far, this agent appears to be the least toxic of the nucleoside analogues tested as antiretroviral drugs.[6b]

Because most of these compounds are derivatives of naturally occurring nucleosides, the possibilities for structural variation have been largely limited to substituent modification. Here we report our initial findings on a new, totally synthetic 2',3'-dideoxynucleoside prototype, dioxolane-T (**6**), in which the 3'-methylene carbon of the normal nucleoside is replaced by oxygen.



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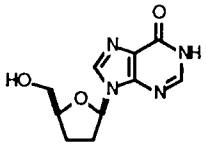

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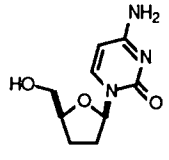

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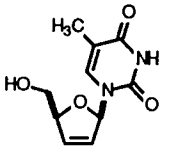

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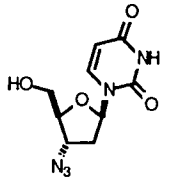

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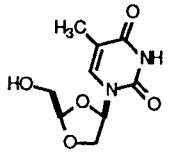

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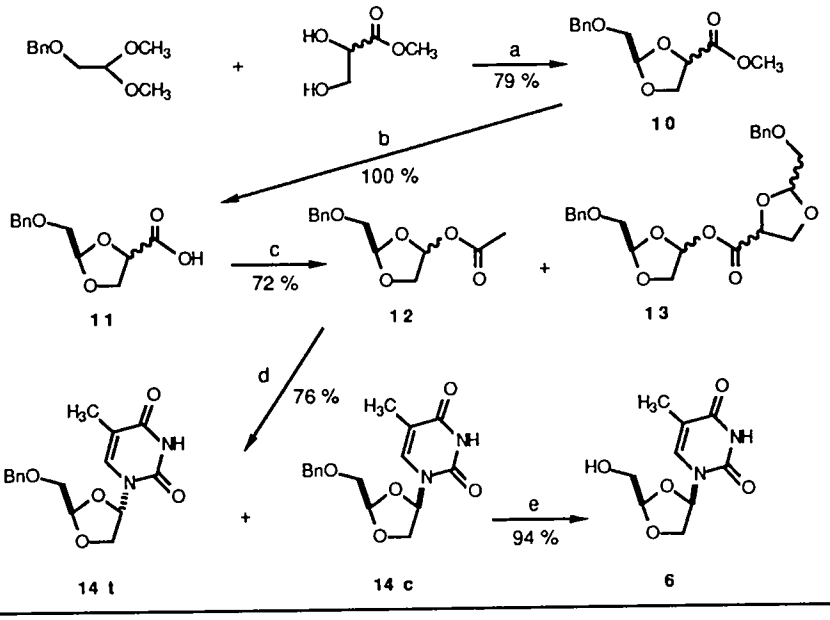
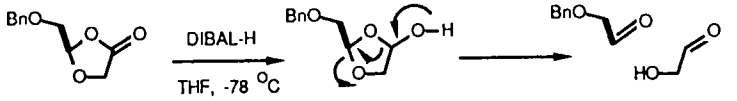
SCHEME. SYNTHESIS OF (±)-DIOXOLANE-T[a]



[a] (a) 0.31 M acetal, 0.37 M diol, 0.01 M p-TsOH, CH_3CN: heat 1 h at reflux, distill off 20% of total volume over 45 m, then heat 1 h at reflux; (b) 0.17 M ester, 0.19 M LiOH, 4:1/THF:H_2O: heat 1.5 h at reflux; (c) 0.21 M acid, 0.27 M $Pb(OAc)_4$, 0.23 M pyridine, CH_3CN: 6 h at 25 °C; (d) 0.19 M acetate, 0.37 M TMS_2-thymine, 0.06 M TMSOTf: 66 h at 25 °C; (e) 0.009 M benzyl ether, 1.3 eq $Pd(OH)_2$ (20% on C), 1 atm H_2, EtOH: 30 m at 25 °C.

As detailed in the Scheme above, an indirect approach to the activation of the anomeric carbon was taken in order to circumvent the potential unravelling of the lactol **8**. We were in fact unable to isolate this lactol by reduction of the lactone **7**.



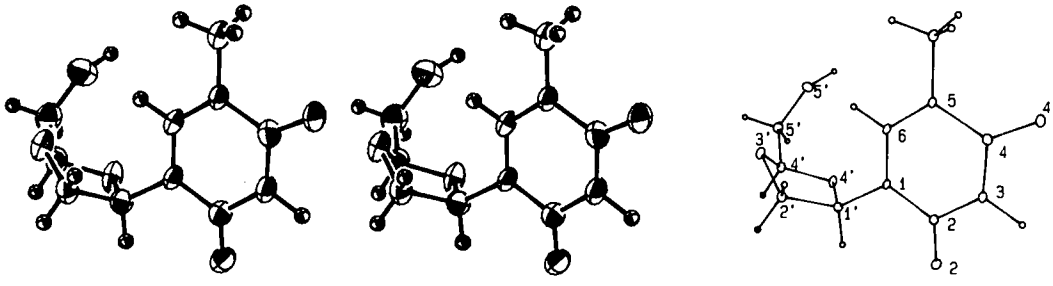
7 **8** **9**

In the successful route, condensation of benzyloxyacetaldehyde dimethyl acetal with (±)-methyl glycerate provided a 79% yield of the dioxolanes **10** as a racemic, 1:1 mixture of diastereomers. Surprisingly, preparation of methyl glycerate[7] by Fischer esterification of the commercially available calcium glycerate salt had not been described in the literature; a procedure for this transformation is detailed in the endnotes.[8] Saponification of the dioxolane methyl esters with LiOH in aqueous THF followed by acidification afforded the carboxylic acids **11** as an oil. In the key step, treatment of **11** with 1.1 equivalents of pyridine and 1.30 equivalents of $Pb(OAc)_4$ in dry acetonitrile at room temperature for 5 h effected a smooth, oxidative decarboxylation, producing a 72 % chromatographed yield of the acetates **12** as a 1.7:1 mixture of cis and trans isomers. Mass spectral and ^1H NMR analysis indicated that the more polar components of the reaction consisted of the diastereomeric esters **13** resulting from self-coupling. Despite the obtention of these by-products, these conditions[9a]

appear to be milder than the original procedure of Kitagawa[9b] which called for 3.6 equivalents of $Pb(OAc)_4$ in refluxing benzene.

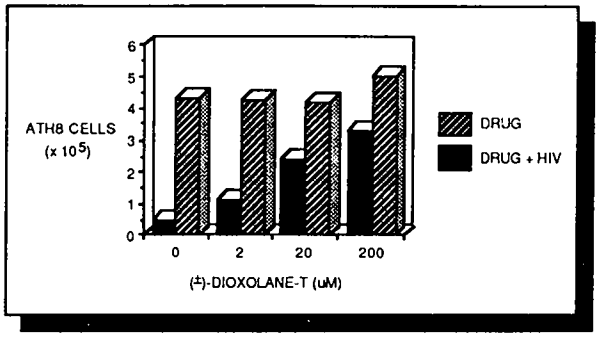
Condensation of the acetates **12** with trimethylsilylthymine according to the general protocol of Vorbruggen[10] proceeded uneventfully to yield the fully elaborated dioxolanes **14** as a 1:1 mixture of diastereomers. Following chromatographic separation on silica gel (7:3 to 10:0/EtOAc:hexane gradient), the relative stereochemistry of the two products was easily assigned by difference NOE spectra. Hydrogenolysis of the desired, cis isomer over $Pd(OH)_2$ on carbon in EtOH followed by crystallization from acetone afforded (±)-dioxolane-T as tiny white needles (mp 149-150 °C).

Crystals suitable for X-ray analysis were obtained from 1:3/methanol:acetone by vapor diffusion against acetone; the structure was solved by a direct methods program. As depicted below, the dioxolane ring adopts the $^3T_4$ conformation ($P=32^o$, $\theta_m = 42.4^o$) commonly observed in ribonucleosides.[11] While the 04'-C1'-N1-C2 torsional angle of -112° positions the thymine base in the normal[12] anti conformation and the O3'-C4'-C5'-O5' torsional angle of 66° places the hydroxymethyl substituent in a typical[12] gauche-gauche conformation, the conformation of the dioxolane ring is quite distinct from the distorted $_3E$ conformations ($166^o \leq P \leq 215^o$) observed in AZT,[12] AZDU,[12] ddA,[13] ddC,[13] and 3'-deoxy-3'-fluorothymidine,[14] all of which exhibit potent *in vitro* activity against HIV. Although the $_3E$ conformation may not be an absolute requirement, its absence is generally predictive of less active compounds.[15]



The anti-HIV activity of (±)-dioxolane-T appears to be consistent with this trend. At 20 uM, (±)-dioxolane-T provides a 50% protective effect against the infectivity and cytopathic effect of HIV-1. In contrast, AZT exerts a 50% protective effect at approximately 0.5 uM and a 100% protective effect at 5 uM.[16] Curiously, a 10 fold increase in the concentration of (±)-dioxolane-T to 200 uM raises the level of protection to only 74%. Even if one assumes that only the "natural" enantiomer of (±)-dioxolane-T has anti-HIV activity, AZT still appears to be the more potent compound, at least on a molar basis. AZT's anti-retroviral activity, however, is accompanied by substantial *in vitro* toxicity: 11% at 5 uM and 50% at 50 uM.[16] Bone marrow suppression has been the dose limiting toxicity of AZT in many patients.[3] The relative lack of toxicity exhibited by (±)-dioxolane-T *in vitro* would warrant further exploration of this and the related 1,3-oxathiolane series.[17]

ANTI-HIV ACTIVITY OF (±)-DIOXOLANE-T



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(8) To a stirred suspension of 75.0 g (0.262 mol) of (±)-calcium glycerate dihydrate in 1.50 L of MeOH was added 30.0 mL (0.540 mol) of 96% H_2SO_4 over 2 m. After 24 h at 25 °C, a trace of methyl orange was added, the reaction mixture cooled to 0 °C, and then rapidly neutralized with 50% sodium hydroxide to an apparent pH of 6-6.5. The reaction mixture was filtered through celite, the celite bed washed with 1 L of MeOH, and then the combined filtrates were concentrated. Kugelrohr distillation (130-160 °C /15 torr) of the residue afforded 58 g (92 %) of (±)-methyl glycerate[7] as an oil.

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(17) Following the completion of this work, we learned that these compounds have been independently synthesized and evaluated by Belleau, B.; Dixit, D.; Nguyen-Ba, N.; Kraus, J.L. *Abstracts of Papers*, Fifth International Conference on AIDS, Montreal; International Development Research Centre: Ottawa, Ontario, 1989; T.C.O.1.

(18) 300 MHz 1H NMR. (a) **(±)-14c**: ($CDCl_3$, TMS= δ 0.00) δ 1.64 (d, 3H, J=1.3 Hz, CH_3), 3.83 (d, 2H, J=2.3 Hz, $CH_2$5'), 4.13 (dd, 1H, J=10.0, J'=5.6 Hz, H2'), 4.19 (dd, 1H, J=10.0, J'=2.0 Hz, H2'), 4.63, 4.65 (2d, 2H, J=12.9 Hz, $ArCH_2O$), 5.10 (dd, 1H, J=J'=2.3 Hz, H4'), 6.38 (dd, 1H, J=5.6, J'=2.0 Hz, H1'), 7.30-7.37 (m, 5H, C_6H_5), 7.65 (q, 1H, J=1.3 Hz, H6), 8.71 (bs, 1H, NH); R_f = 0.39 (SiO_2, EtOAc) (b) **(±)-14t**: ($CDCl_3$, TMS= δ 0.00) δ 1.93 (d, 3H, J=1.3 Hz, CH_3), 3.59 (d, 2H, J=3.6 Hz, $CH_2$5'), 4.03 (dd, 1H, J=9.6, J'=2.6 Hz, H2'), 4.43 (d, 1H, J=9.6, J'=5.6 Hz, H2'), 4.61 (s, 2H, $ArCH_2O$), 5.59 (dd, 1H, J=J'=3.6 Hz, H4'), 6.31 (dd, 1H, J=5.6, J'=2.6 Hz, H1'), 7.18 (q, 1H, J=1.3 Hz, H6), 7.29-7.39 (m, 5H, C_6H_5), 9.13 (bs, 1H, NH); R_f = 0.45 (SiO_2, EtOAc) (c) **(±)-6**: (D_2O, HOD= δ 4.81) δ 1.89 (d, 1H, J=1 Hz, CH_3), 3.85, 3.91 (2 dd, 2H, J=13, J'=2 Hz, $CH_2$5'), 4.25 (dd, 1H, J= 10, J'=6 Hz, H2'), 4.39 (dd, 1H, J=10, J'=1.5 Hz, H2'), 5.12 (dd, 1H, J=J'=2 Hz, H4'), 6.40 (dd, 1H, J=6, J'=1.5 Hz, H1'), 7.78 (q, 1H, J=1 Hz, H6).

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