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4'-Substituted Nucleosides as Inhibitors of HIV: An Unusual Oxetane Derivative.[1]

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ABSTRACT: *The fused oxetane derivative of thymidine* **3a** *inhibits HIV replication in A301 (Alex) cells with remarkably low bone marrow toxicity.*

In the previous communication[2] we described the preparation and potent HIV-inhibitory activity of the 4'-modified nucleoside, 4'-cyanothymidine. As an extension of this work on 4'-substituted nucleosides as potential therapies for AIDS, we saw an opportunity to prepare 4'-substituted analogs of known HIV-inhibitory compounds, culminating in the synthesis of oxetane **3a** and related compounds.

Reaction of diol **1a**[2] with one equivalent of 4,4'-dimethoxytrityl chloride (dmTrCl) (CH_2Cl_2/TEA) gave largely a single dmTr ether. Although intuitively one might assign this as the 5'-ether **1b**, later work (see below) showed this in fact to be the 4'-hydroxymethyl protected compound **2b**![3] The reason for this preference remains unclear. Believing this to be the 5'-trityl ether, we sought to prepare the 4'-azidomethyl analog of the potent 4'-azidothymidine.[4] Mesylation (2 eq MsCl/TEA/CH_2Cl_2) to give **2c** and reaction with LiN_3 (16 eq) (DMA, 110°, 18 h) gave 20% of an azide believed to be **1d**, but shown to be the epimer **2d** after deprotection (TBAF to give **2e** then 80% AcOH/THF, 6:1) by NOE experiments on the final product **2f**. In order to prepare the desired isomer **1f**, it was hoped to take advantage of the greater reactivity of the 4'-hydroxymethyl group seen above. However, reaction of **1a** with 1 eq MsCl (TEA/CH_2Cl_2) gave a 2:1 mixture of mono- and di-mesylates (and remaining diol) with the mono-mesylates **1g/2g** being separated as a 1:1 mixture. Reaction of this mixture with LiN_3 (8 eq, HMPA, 100°, 5h)[5] gave a mixture now containing the two azides **1f** (13%) and **2f**, and a third major product identified as the oxetane **3a** (15%). Separation was achieved by flash chromatography, or more readily by prior conversion of **2f** to the isopropylidene derivative **2h** [$(p\text{-}O_2NC_6H_4O)_2P(O)OH{\cdot}H_2O/Me_2C(OMe)_2$/acetone].[6] The loss of the silyl protecting group under these conditions is noteworthy. The stereochemistry of **1f** and **2f** was proved conclusively by NOE experiments and by the failure of **1f** to form an isopropylidene derivative. The biological activity of **1f** and **2f** (below) prompted the synthesis of further analogs.

Reaction of the mixture of mesylates **1g/2g** with NaI (10 eq, 2,5-hexanedione, 130°C, 2h) gave the mixture of iodomethyl compounds **1i/2i**. Deprotection (CsF/DMF) and flash chromatography gave the pure 4'-iodomethyl analog **1j** and its 4'-epimer **2j**. Alternatively, catalytic hydrogenation of the iodides **1i/2i** (10% Pd/C, NaOH, MeOH, 25°C) to give **1k/2k** and deprotection (CsF/DMF) followed by chromatography gave 4'-methylthymidine **1l** and its epimer **2l** (32% and 39% respectively from **1g/2g**). NOE experiments again permitted assignment of stereochemistry to these compounds, and therefore their precursors.

In the expectation that the above compounds could be incorporated in the newly formed DNA without chain termination, we also investigated 3′-desoxy analogs. Protection of **1a** as the isopropylidene derivative **1m** (conditions as above) and desilylation (F^-) gave **1n**. Routine mesylation gave **1o** which upon heating with NaOH (10 eq 4N, EtOH/THF) gave about a 75% yield of the dehydro compound **4a**, converted (80% AcOH/THF) to the diol **4b**. Mesylation (1 eq MsCl, TEA, CH_2Cl_2) gave a mixture of monomesylates **4c/4d** which was separated from dimesylate and unreacted diol. Heating with LiN_3 (HMPA, 15 h, 90°) gave the azidomethyl compounds **4e/4f** (1:1 ratio) in 35% yield, after separation by flash chromatography and recrystallization. Conversion of the mesylate **1o** to the 3′β-alcohol **5a** (3-5 eq 2N NaOH/MeOH, 50°)[7] and reaction with DAST[8] did not give the desired 3′α-fluoro analog. Rather, use of DAST in CH_2Cl_2/imidazole gave **4a** (80%) only. (This compound was also obtained from **5a** using 5 eq 1N NaOH/EtOH/THF 8:1 reflux).[9] Mesylation of 3′β-alcohol **5a** and reaction with LiN_3 (8 eq, DMF, 90°) gave 15% of the 3′-azidocompound **1p** (and 30% of **4a**). Deprotection of **1p** (80% AcOH/THF) then gave diol **1q**.

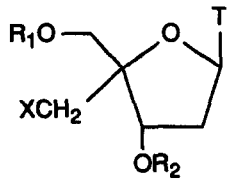
In an attempt to improve the potency of the oxetane **3a**, it was converted to the 5′-hydrogen phosphonate derivative **3b** (24%) by reaction with PCl_3 and Et_3N (excess imidazole, MeCN, 0°)[10] and purification by flash chromatography, adsorption on and elution from Amberlite XAD-2 (a non-polymeric adsorbant) followed by reverse-phase HPLC. The sodium salt **3c** was formed using $NaClO_4$ (0.5 M in acetone). Although preparation of a hydrogen phosphonate of AZT increases the antiviral activity[11] by avoiding the often rate-limiting first phosphorylation, this strategy did not improve the activity of **3c** (see below).

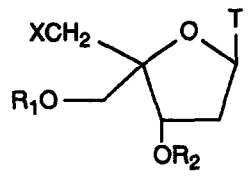
In view of the need for larger quantities of oxetane **3a** for *in vivo* studies, a directed synthesis was developed. Protection of the diol **1a** ($Me_2C(OMe)_2/H^+$) to give **1m** followed by N^3-benzoylation (PhCOCl/DMAP/pyr) and conversion to the triol **1r** (H^+ then nBu_4NF) proceeded routinely. Cyclization of triol **1r** under modified Mitsunobu conditions (Ph_3P/DEAD) to give **3d** and deprotection (NH_4OH/MeOH) gave oxetane **3a** in 5% overall yield.

The *in vitro* anti-HIV activity of the 4′-substituted nucleosides was evaluated using HIV-1 LAV strain-infected A301 (Alex) cells[12] with EC_{50}'s (μM) of **1f** 0.45-2.1; **1l** 3.5; **1p** 16; **1q** 12.5; **3a** 0.14-0.31; **3c** 8.5; **4a** 16; AZT 0.001-0.009. No activity was seen for **1n**, **2f**, **2l**, **4b**, **4e**, **4f** or **5a** (>200). Compound **3a** showed partial toxicity to Alex cells only at 1000 μM whereas **1f** showed full toxicity at 1000 μM but none at 300 μM.

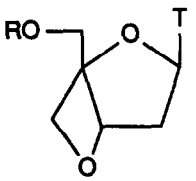
The most potent compound from this series, oxetane **3a**, was assayed for its effect on myelopoiesis *in vitro* using nonadherent human mononuclear cells and human bone marrow mononuclear cells.[13] The IC_{50} values (μM) relative to AZT were found to be 46.41, 44.81 (AZT 1.99, 1.96) in 2 experiments for the effect on proliferation and differentiation of human myeloid precursors in bone marrow (CFU-GM) and 49.59±9.91 (n=8) (AZT 8.09±8.44) using peripheral blood mononuclear cells, confirming the relative lack of toxicity for this compound.

In conclusion, we have prepared a series of 4′-substituted nucleosides as potent inhibitors of HIV. The discovery of oxetane **3a** showing remarkably low bone marrow toxicity is noteworthy.





1a	R_1 = H, X = OH, R_2 = TBDMS	
1b	R_1 = dmTr, R_2 = TBDMS, X = OH	2b
1c	R_1 = dmTr, R_2 = TBDMS, X = OMs	2c
1d	R_1 = dmTr, R_2 = TBDMS, X = N_3	2d
1e	R_1 = dmTr, R_2 = H, X = N_3	2e
1f	R_1 = R_2 = H, X = N_3	2f
1g	R_1 = H, X = OMs, R_2 = TBDMS	2g
	R_1 - R_2 = $C(Me)_2$, X = N_3	2h
1i	R_1 = H, X = I, R_2 = TBDMS	2i
1j	R_1 = R_2 = H, X = I	2j
1k	R_1 = X = H, R_2 = TBDMS	2k
1l	R_1 = R_2 = X = H	2l
1m	R_1 - X = $C(Me)_2O$, R_2 = TBDMS	
1n	R_1 - X = $C(Me)_2O$, R_2 = H	
1o	R_1 - X = $C(Me)_2O$, R_2 = Ms	
1p	R_1 - X = $C(Me)_2O$, OR_2 = N_3	
1q	R_1 = R_2 = H, X = OH	
1r	R_1 = R_2 = H, X = OH, T = N^3Bz thymine	

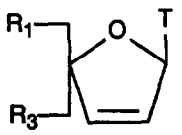


3a R = H

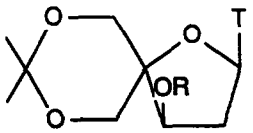
3b R = 

3c R = 

3d R = H, T = N^3Bz thymine



4a R_1R_3 = $OC(Me_2)O$
4b R_1 = R_3 = OH
4c R_1 = OH, R_3 = OMs
4d R_1 = OMs, R_3 = OH
4e R_1 = OH, R_3 = N_3
4f R_1 = N_3, R_3 = OH



5a R = H
5b R = Ms

Acknowledgement

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References and Notes

1. Contribution No. 841 from the Institute of Organic Chemistry.

2. O-Yang, C.; Wu, H.Y.; Fraser-Smith, E.B.; Walker, K.A.M. Preceding paper in this issue.

3. All final compounds and key intermediates gave satisfactory spectral data. Elemental composition was established by HRMS and/or combustion analysis.

4. (a) Maag, H.; Prisbe, E.J.; Verheyden, J.P.H. Eur. Pat. Appl. EP 371,366 (1990). (b) Maag, H.; Chu, N.; Crawford-Ruth, D.; Eugui, E.; McRoberts, M.J.; Mirkovich, A.; Pettibone, M.; Prisbe, E.J.; Rydzewski, R.M. and Verheyden, J.P.H. *Antiviral Res.*, **1991**, *Suppl 1*, 43.

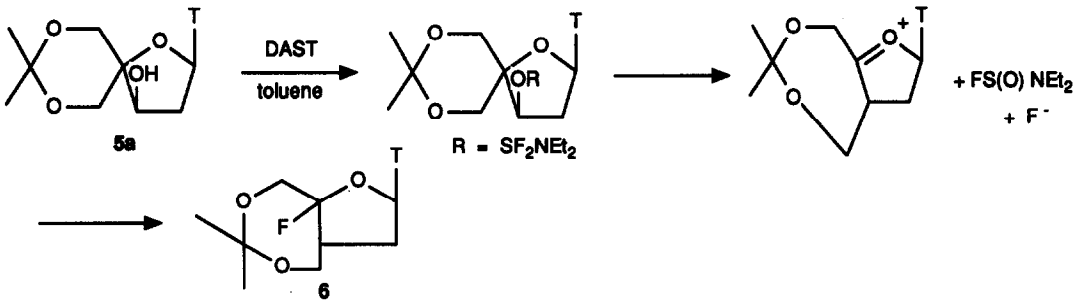
5. Leboeuf, M.; Cave, A. and Goutarel, R. *Compt. Rend.*, **1967**, *264*, 1090.

6. Hampton, A. *J. Am. Chem. Soc.*, **1961**, *83*, 3640.

7. Fox, J.J.; Miller, N.C. *J. Org. Chem.*, **1963**, *28*, 936.

8. Middleton, W.J. *J. Org. Chem.*, **1975**, *40*, 574.

9. It is noteworthy that treatment of 5a with DAST in toluene containing a small amount of CH_2Cl_2 (for solubility) gave a rearranged compound **6** along with the olefin **4a** (ratio 2-3:1). The structure of **6** was assigned on the basis of ^1H, ^{13}C NMR and mass spectroscopy. The proposed mechanistic pathway leading to **6** is shown below:



10. Lindh, I.; Stawinski, J. *J. Org. Chem.*, **1989**, *54*, 1338.

11. Tarussova, N.B.; Kukhanova, M.K.; Krayevsky, A.A.; Karamov, E.K.; Lukashov, V.V.; Kornilayeva, G.B.; Rodina, M.A.; Galegov, G.A. *Nucleosides and Nucleotides*, **1991**, *10*, 351.

12. Sidwell, R.W. and Huffman, J.H. *Appl. Microbiol.*, **1971**, *22*, 797.

13. Sommadossi, J.P.; Carlisle, R. *Antimicrob. Agents Chemother.*, **1987**, *31*, 452.

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