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Synthesis of a Highly Active New Anti-HIV Agent 2′,3′-Didehydro-3′-deoxy-4′-ethynylthymidine

Kazuhiro Haraguchi,[a] Shingo Takeda,[a] Hiromichi Tanaka,[a,*] Takao Nitanda,[b] Masanori Baba,[b] G. E. Dutschman[c] and Yung-Chi Cheng[c]

[a]*School of Pharmaceutical Sciences, Showa University, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan*
[b]*Center for Chronic Viral Diseases, Division of Human Retroviruses, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima 890-8520, Japan*
[c]*Department of Pharmacology, School of Medicine, Yale University, 333 Cedar street, New Haven, CT 06520, USA*

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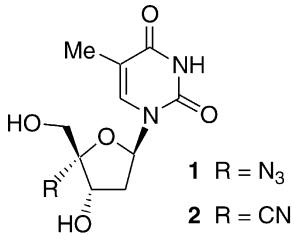
Abstract—Compounds having methyl, vinyl, and ethynyl groups at the 4′-position of stavudine (d4T: 2′,3′-didehydro-3′-deoxythymidine) were synthesized. The compounds were assayed for their ability to inhibit the replication of HIV in cell culture. The 4′-ethynyl analogue (**15**) was found to be more potent and less toxic than the parent compound stavudine.

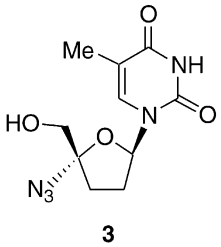
The finding that thymidine derivatives bearing 4′-azido (**1**)[1] and 4′-cyano (**2**)[2] substituents show significant inhibitory activity against HIV proliferation has stimulated the synthesis of 4′-substituted nucleoside analogues. In fairly recent studies along this line, 4′-ethynylnucleosides have also been shown to be promising anti-HIV agents.[3] In these studies, effort has been focused mostly on the synthesis of 2′-deoxynucleosides. This is due to the reported structure–activity relationship (SAR) of the 4′-azidothymidine derivatives,[1] in which the presence of the 3′-hydroxyl group has been observed to be critical for anti-HIV activity: both **3** and **4** are completely devoid of the activity.

In our previous study on the synthesis of 4′-substituted nucleosides from 4′,5′-epoxy precursors,[4] it was demonstrated that ring opening of **5** with $AlMe_3$ results in the dominant formation of **6** (64%), the desired product **7** being isolated only in 5% yield. This unsatisfactory stereochemical outcome was ascribed to conformational preference of the oxonium intermediate, depicted as **A**, which can avoid the steric repulsion between the 5′-*O*-aluminate and the 3′-*O*-TBDMS group.

The present study was undertaken initially to evaluate the above steric influence. Thus, the 4′,5′-epoxide **8** having the opposite 3′-configuration to **5** was prepared from the corresponding 4′,5′-unsaturated derivative[5] by oxidizing with dimethyldioxirane[6] (CH_2Cl_2/−30 °C/0.5 h). When **8** was reacted with Me_3Al under the conditions shown in Scheme 1 (a possible intermediate is depicted as **B**), exclusive formation of **9** (72%) having the expected 4′-configuration was observed. This was also the case for trivinylaluminum, although



1 R = N_3
2 R = CN



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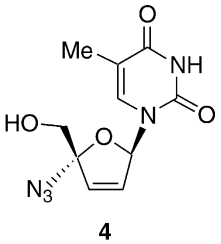

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*Corresponding author. Tel.: +81-3-3784-8186; fax: +81-3-3784-8252; e-mail: hirotnk@pharm.showa-u.ac.jp

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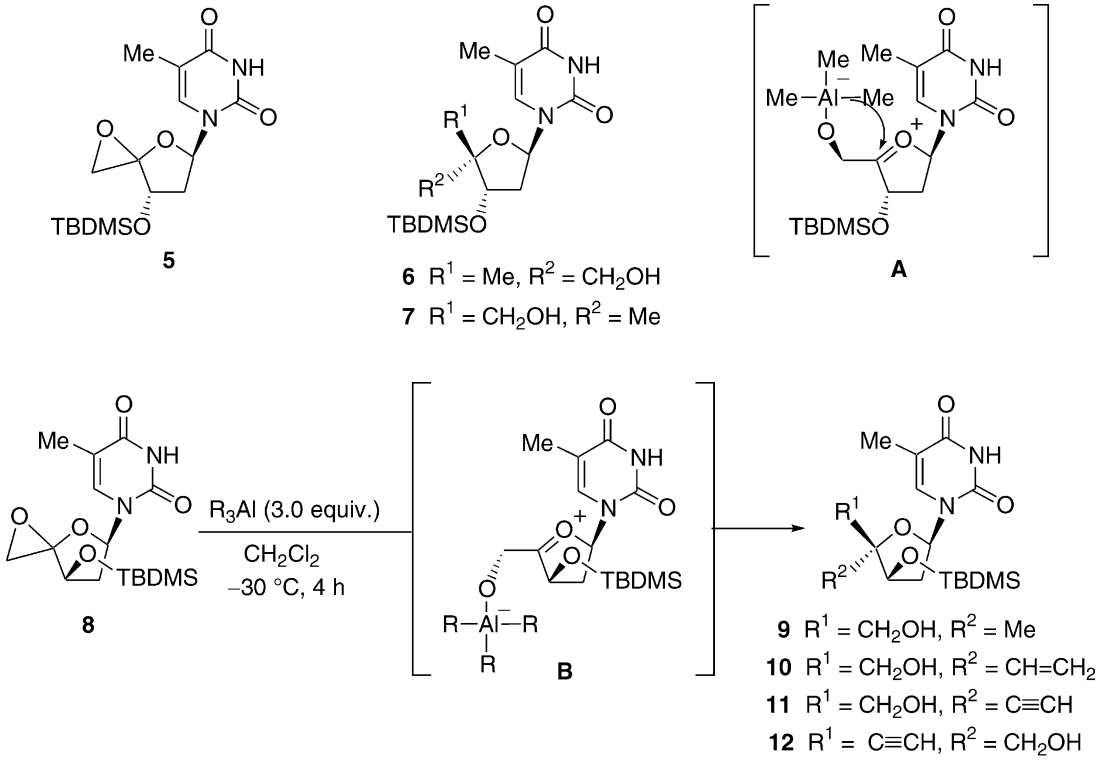
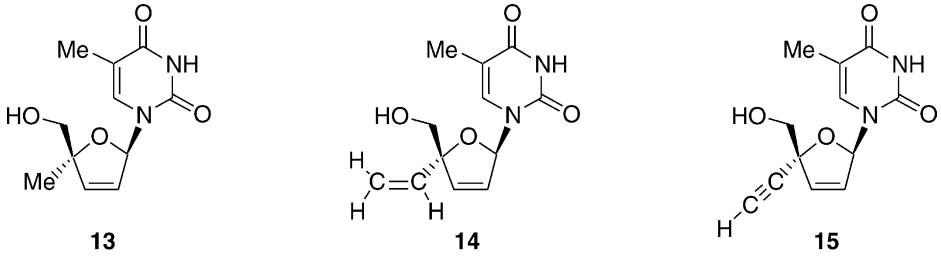


Scheme 1. Synthesis of **9**–**12** by ring opening of **8** with R_3Al.



the yield of **10** (27%) was rather low.[7] In contrast to these two reactions, both the 4′-α- (**11**: 57%) and 4′-β- (**12**: 31%) substituted products were isolated upon reacting **8** with triethynylaluminum.[8,9] At the present time, we have no clear explanation for the formation of **12**.

Transformation of **9**–**11** to the respective 4′-substituted analogue of stavudine was carried out, simply by sequential conventional reactions: acetylation of the 5′-hydroxyl group, desilylation (TBAF/THF), 3′-*O*-mesylation, elimination of MsOH (DBN/CH_3CN), and deacetylation (NH_3/MeOH). Compounds **13**–**15**[10] were obtained in good overall yields.

The anti-HIV-1 III_B activities of **13**–**15** were evaluated,[11] and the results are summarized in Table 1. Although weak anti-HIV activity of **13** has been reported,[12] this compound showed no activity in our assay system. The potent activity of **15** is particularly interesting, and this constitutes counterevidence to the accepted SAR that the 3′-hydroxyl group is necessary for 4′-substituted nucleosides to be active against HIV. It is also noteworthy that **15** is less toxic to CEM cell growth and less inhibitory to mitochondrial DNA synthesis than stavudine (data not shown).

Table 1. Anti-HIV-1 III_B activity of **13**–**15** in MT-2 cells

Compd	IC_{50} (μM)[a]	CC_{50} (μM)[b]
13	> 100	> 100
14	> 100	> 100
15	0.20	> 100
Stavudine	2.8	100

[a]Inhibitory concentration required to achieve 50% protectiom of MT-2 cells against the cytopathic effect of HIV-1 III_B.
[b]Cytotoxic concentration required to reduce the viability of mock-infected MT-2 cells by 50%.

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5. This compound, 1-[3-*O*-(*tert*-butyldimethylsilyl)-2,5-dideoxy-β-L-*glycero*-pent-4-enofuranosyl]thymine, was prepared in four steps from 1-(2,5-dideoxy-5-iodo-β-D-*threo*-pentofuranosyl)thymine: Joshi, B. V.; Reese, C. B. *Tetrahedron Lett*. **1992**, *33*, 2371.
6. For the preparation of an acetone solution of dimethyldioxirane, see: Adam, W.; Bialas, J.; Hadjiarapoglou, L. *Chem. Ber*. **1991**, *124*, 2377.
7. Formation of thymine was a major event in this particular reaction.
8. Triethynylaluminum was prepared by reacting ethynylmagnesium chloride (0.5 M THF solution) with $AlCl_3$ in CH_2Cl_2. After evaporation of the solvents, the residue was dried and suspended into CH_2Cl_2 to give a dark brown suspension of this reagent.
9. The depicted stereochemistry of **9**–**12** was confirmed by ^1H NMR NOE experiment.
10. Physical data for **15** are as follows: solid (mp 207–209 °C); UV (MeOH) λ_{max} 264 nm (ε 10800), λ_{min} 235 nm (ε 4800); ^1H NMR ($CDCl_3$) δ 1.83 (3H, s, Me), 2.63 (1H, s, C≡CH), 3.47 (1H, br, OH), 3.88 (1H, d, J_{gem} = 12.5 Hz, H-5′a), 3.96 (1H, d, J_{gem} = 12.5 Hz, H-5′b), 5.91 (1H, dd, $J_{1',2'}$ = 1.1 Hz and $J_{2',3'}$ = 5.9 Hz, H-2′), 6.30 (1H, dd, $J_{1',3'}$ = 2.0 Hz and $J_{2',3'}$ = 5.9 Hz, H-3′), 7.16–7.17 (1H, m, H-1′), 7.44 (1H, d, $J_{6,Me}$ = 1.1 Hz, H-6), 9.06 (1H, br, NH); FAB-MS *m*/*z* 249 ($M^+ + H$). Anal. calcd for $C_{12}H_{12}N_2O_4 \cdot 1/6H_2O$: C, 57.37; H, 4.95; N, 11.15. Found: C, 57.36; H, 4.69; N, 10.98.
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