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# Synthesis and Biological Evaluation of L- and D-Configuration 1,3-Dioxolane 5-Azacytosine and 6-Azathymine Nucleosides

Mei-Zhen Luo, Mao-Chin Liu, Diane E. Mozdziesz, Tai-Shun Lin,[†]
Ginger E. Dutschman, Elizabeth A. Gullen, Yung-Chi Cheng and Alan C. Sartorelli*

*Department of Pharmacology and Developmental Therapeutics Program, Cancer Center, Yale University School of Medicine, New Haven, CT 06520-8066, USA*

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**Abstract**—Novel L- and D-configuration dioxolane 5-azacytosine and 6-azathymine nucleosides have been synthesized and evaluated for biological activity. (−)-(2*S*,4*S*)-1-[2-(Hydroxymethyl)-1,3-dioxolan-4-yl]-5-azacytosine (**6**) showed significant activity against HBV, whereas the D-configuration analogue (**14**) has been found to exhibit potent anti-HIV activity. 

Nucleoside analogues play an important role in cancer and viral chemotherapy. Recently, several interesting nucleosides such as (−)-L-β-1,3-oxathiolanyl cytosine (3TC, Lamivudine) and (−)-L-β-1,3-dioxolanyl cytosine [(−)-OddC], in which the 3′-methylene carbon of the normal nucleoside was replaced by a heteroatom, have been reported.[1–6] 3TC is being clinically used as an anti-AIDS and anti-HBV drug. (−)-OddC showed significant activity against both solid and lymphoid tumors both in vitro and in vivo,[6] and also exhibited potent anti-HIV and anti-HBV activity.[5] Various azanucleoside analogues, in which the carbon atoms in the natural base have been replaced by a bioisosteric nitrogen, have shown significant anticancer and/or antiviral activity. For example, 5-azacytidine (AZC),[7,8] 5-aza-2′-deoxycytidine (dAZC),[9] and 6-azauridine[10] have exhibited significant antitumor activity. Based upon these findings, it was of interest to evaluate the effects of combining the structural feature of the oxygen-containing sugar of (−)-OddC with various bases of active azanucleosides. In this paper, we report the synthesis and biological evaluation of the L- and D-configurations of 1,3-dioxolane nucleoside derivatives of 5-azacytosine and 6-azathymine.

The synthesis of the dioxolane azanucleosides is shown in Scheme 1. Condensation of (2*S*)-4-acetoxy-2-[(benzyloxy)-methyl]dioxolane (**1a**)[11,12] with silylated 5-azacytosine and 6-azathymine in the presence of TMSOTf in 1,2-dichloroethane produced a mixture of anomers (50–60%), which were separated by silica gel chromatography to give the nucleoside isomers **2–5**. Removal of the benzyl group by transfer hydrogenolysis afforded the desired L-configuration 5-azacytidine and 6-azathymidine derivatives **6–9** (∼80%), respectively.[13] Similarly, coupling of (2*R*)-4-acetoxy-2-[(benzyloxy)methyl]dioxolane (**1b**)[11,12] with silylated 5-azacytosine and 6-azathymine, followed by separation and deprotection of the intermediates **10–13** afforded the corresponding D-configuration 5-azacytidine and 6-azathymidine derivatives **14–17**.[14]

The assignment of the anomeric configuration of these nucleosides was made on the basis of the characteristics of the proton NMR spectra. The 4′-H protons of the α-anomers appeared at a lower field than those of the β-anomers. Conversely, the 5′-H protons of the α-anomers appeared at a higher field than those of the β-anomers (Table 1). These shifts were attributed to the fact that protons at the *syn*-position relative to the base are more deshielded than those in an *anti*-position to the base. The 4′-H protons of the α-anomers and the bases are on the same side of the sugar ring and those of the β-anomers are on the opposite side. In contrast, the 5′-H protons of the α-anomers and the bases are on the opposite side of the sugar ring, whereas those of the β-anomers are on the same side. The findings are consistent with reports by others with similar pyrimidine nucleosides.[15,16]

The synthesized compounds **6–9** and **14–17** were evaluated in vitro for their cytotoxicities against the L1210

*Corresponding author. Tel.: +1-203-785-4533; fax: +1-203-737-2045; e-mail: alan.sartorelli@yale.edu
[†]Deceased.

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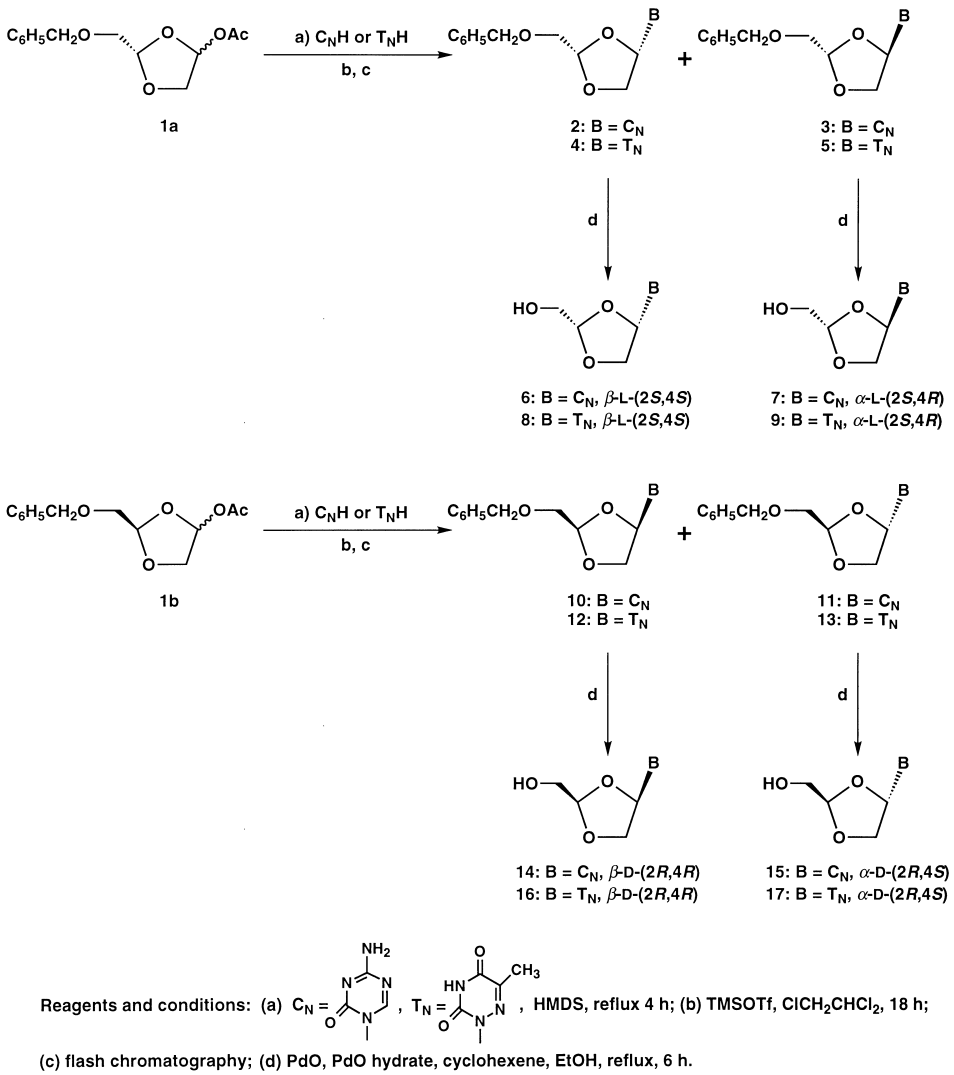


**Scheme 1.**

**Table 1.** Proton NMR chemical shifts δ (ppm)

| Compound | 4′-H[a] | Δδ | 5′-H[a] | Δδ |
|---|---|---|---|---|
| **6** (β) | 4.97 *(anti)* | 0.52 | 3.67 *(syn)* | 0.22 |
| **7** (α) | 5.49 *(syn)* | | 3.45 *(anti)* | |
| **8** (β) | 5.00 *(anti)* | 0.26 | 3.49 *(syn)* | 0.06 |
| **9** (α) | 5.26 *(syn)* | | 3.43 *(anti)* | |

[a]Stereochemistry relative to the base.

and P388 leukemias, CCRF-CEM lymphoblastic leukemia, and $B_{16}F_{10}$ melanoma cell lines and the results are shown in Table 2. The L-configuration 5-azacytosine analogue, (−)-(2*S*,4*S*)-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]-5-azacytosine (**6**) produced $EC_{50}$ values of 70, 90, 20, and 70 μM and its α-isomer (**7**) produced $EC_{50}$ values of 70, 65, 30, and 70 μM against L1210, P388, CCRF-CEM, and $B_{16}F_{10}$ cells, respectively. The corresponding D-configuration compound (+)-(2*R*,4*R*)-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]-5-azacytosine (**14**) and its α-isomer (**15**) had similar activity. The 6-azathymine analogues **8**–**9** and **16**–**17** had no activity up to 100 μM against these neoplastic cell lines.

**Table 2.** Evaluation of dioxolanyl nucleoside analogues of 5-azacytosine and 6-azathymine against L1210, P388, CCRF-CEM, and $B_{16}F_{10}$ neoplastic cell lines in vitro

| | $EC_{50}$ (μM)[a] | | | |
|---|---|---|---|---|
| Compound | L1210 | P388 | CCRF-CEM | $B_{16}F_{10}$ |
| **6** (β) | 70 | 90 | 20 | 70 |
| **7** (α) | 70 | 65 | 30 | 70 |
| **8** (β) | >100 | >100 | >100 | >100 |
| **9** (α) | >100 | >100 | >100 | >100 |
| **14** (β) | 90 | 70 | 80 | 60 |
| **15** (α) | 80 | 70 | 70 | 80 |
| **16** (β) | >100 | >100 | >100 | >100 |
| **17** (α) | >100 | >100 | >100 | >100 |

[a]$EC_{50}$ values represent the drug concentration (μM) required to inhibit 50% of cancer cell replication. The compounds were tested up to a concentration of 100 μM.

Antiviral assays were performed against herpes simplex Type 1 virus (KOS strain, HSV-1), herpes simplex Type 2 virus (333 strain, HSV-2), hepatitis B virus (HBV), and human immunodeficiency virus (HIV-IIIB) in vitro

**Table 3.** Evaluation of dioxolanyl nucleoside analogues of 5-azacytosine and 6-azathymine against herpes simplex Type 1 virus (HSV-1), herpes simplex Type 2 virus (HSV-2), hepatitis B virus (HBV), and human immunodeficiency virus (HIV-IIIB) in vitro

| | $EC_{50}$ (μM)[a] | | | | $IC_{50}$ (μM)[b] | | |
|---|---|---|---|---|---|---|---|
| Compound | HSV-1[c] | HSV-2[c] | HBV[d] | HIV-IIIB[e] | Vero[f] | 2.2.15[g] | MT-2[h] |
| **6** (β) | 32 | >50 | 0.6 | 5 | 12 | 17 | 17 |
| **7** (α) | >50 | >50 | 6.5 | >100 | 31 | 17 | 18 |
| **8** (β) | >50 | >50 | >20 | >100 | >50 | >50 | >100 |
| **9** (α) | >50 | >50 | >20 | >100 | >50 | >50 | >100 |
| **14** (β) | >50 | >50 | 6.3 | 1 | 30 | 27 | 32 |
| **15** (α) | >50 | >50 | >20 | >100 | 28 | 25 | 27 |
| **16** (β) | >50 | >50 | >20 | >100 | >50 | >50 | >100 |
| **17** (α) | >50 | >50 | >20 | >100 | >50 | >50 | >100 |
| **ACV**[i] | 23 | 35 | ND[j] | ND | >50 | ND | ND |
| **ddC**[k] | ND | ND | 2.3 | ND | ND | >37 | ND |
| **d4T** | ND | ND | >20 | 0.6 | ND | ND | >100 |

[a]$EC_{50}$ values represent the drug concentration (μM) required to inhibit 50% of viral replication.
[b]$IC_{50}$ values represent the drug concentration (μM) required to inhibit 50% of host cell replication.
[c]The compounds were tested up to a concentration of 50 μM.
[d]The compounds were tested up to a concentration of 20 μM.
[e]The HIV assays were performed using a viral multiplicity of 0.1 $TCID_{50}$/cell and the compounds were tested up to a concentration of 100 μM.
[f]$IC_{50}$ values of Vero cells used in HSV-1 and HSV-2 studies.
[g]$IC_{50}$ values of human hepatoma cells (2.2.15) used in HBV studies.
[h]$IC_{50}$ values of MT-2 cells used in HIV studies.
[i]ACV: acyclovir.
[j]ND: not determined.
[k]ddC: dideoxycytidine.

as previously described[17,18] and the results are shown in Table 3. The L-configuration 5-azacytosine analogue (**6**) showed significant activity against HBV ($EC_{50}$ = 0.6 μM) and HIV ($EC_{50}$ = 5 μM), whereas the D-configuration analogue (**14**) demonstrated potent anti-HIV activity on a par with the clinical antiviral agent 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (d4T, Stavudine, Zerit). Compound **14** produced an $EC_{50}$ value of 1 μM against HIV while the known antiviral agent d4T used as a positive control had an $EC_{50}$ value of 0.6 μM. Compound **14** was also more active against HBV than d4T ($EC_{50}$ = 6.3 and >20 μM, respectively). In addition, compound **6** demonstrated modest activity against HSV-1 with an $EC_{50}$ value of 32 μM. Except for compound **7**, the α-isomer of **6**, which showed activity against HBV ($EC_{50}$ = 6.5 μM), the remaining compounds showed little or no activity against these various virus strains up to their maximum tested concentrations.

In summary, a novel class of dioxolane azapyrimidine nucleoside analogues has been synthesized. The L-configuration azacytosine derivative, (−)-(2*S*,4*S*)-1-[2-(hydroxymethyl)-1,3-dioxolan-4-yl]-5-azacytosine (**6**) showed significant activity against HBV, whereas the D-configuration analogue (**14**) was found to exhibit potent anti-HIV activity.

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13. Compound **6**: mp 184–186 °C; $[\alpha]_D^{22}$ −18.6° (*c* 0.1, MeOH); $^1$H NMR (DMSO-$d_6$) δ 3.67 (m, 2H, 5′-H), 4.11 (dd, 1H, *J* = 4.5, 9.6 Hz, 2′-$H_A$), 4.28 (dd, 1H, *J* = 2.0, 9.6 Hz, 2′-$H_B$), 4.97 (t, 1H, *J* = 1.8 Hz, 4′-H), 5.26 (t, 1H, 5′-OH, $D_2O$ exchangeable), 6.12 (dd, 1H, 1′-H, *J* = 2.0, 9.6 Hz), 7.53 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.57 (s, 1H, 6H). Compound **7**: mp 168–170 °C; $[\alpha]_D^{22}$ +41.3° (*c* 0.1, MeOH); $^1$H NMR (DMSO-$d_6$) δ 3.45 (m, 2H, 5′-H), 4.08 (dd, 1H, *J* = 3.0, 9.0 Hz, 2′-$H_A$), 4.29 (dd, 1H, *J* = 5.7, 9.0 Hz, 2′-$H_B$), 5.01 (t, 1H, 5′-OH, $D_2O$ exchangeable), 5.49 (t, 1H, *J* = 3.6 Hz, 4′-H), 6.00 (dd, 1H, 1′-H, *J* = 3.0, 5.7 Hz), 7.53 (s, 2H, $NH_2$, $D_2O$ exchangeable), 8.25 (s, 1H, 6H). Compound **8**: mp 208–210 °C; $[\alpha]_D^{22}$ +64.7° (*c* 0.1,

MeOH); $^1$H NMR (DMSO-$d_6$) δ 2.09 (s, 3H, $CH_3$), 3.49 (m, 2H, 5′-H), 4.06 (dd, 1H, $J$ = 6.3, 9.0 Hz, 2′-$H_A$), 4.21 (dd, 1H, $J$ = 2.7, 9.0 Hz, 2′-$H_B$), 4.90 (t, 1H, 5′-OH, $D_2O$ exchangeable), 5.00 (t, 1H, $J$ = 4.2 Hz, 4′-H), 6.36 (dd, 1H, 1′-H, $J$ = 2.7, 9.0 Hz), 12.05 (brs, 1H, NH, $D_2O$ exchangeable). Compound **9**: mp 178–180 °C; $[\alpha]_D^{22}$ −48.5° (*c* 0.1, MeOH); $^1$H NMR (DMSO-$d_6$) δ 2.10 (s, 3H, $CH_3$), 3.43 (m, 2H, 5′-H), 4.13 (dd, 1H, $J$ = 4.2, 8.7 Hz, 2′-$H_A$), 4.21 (dd, 1H, $J$ = 6.6, 8.7 Hz, 2′-$H_B$), 4.94 (t, 1H, 5′-OH, $D_2O$ exchangeable), 5.26 (t, 1H, $J$ = 3.6 Hz, 4′-H), 6.39 (dd, 1H, 1′-H, $J$ = 4.2, 6.6 Hz), 12.20 (brs, 1H, NH, $D_2O$ exchangeable).

14. Compound **14**: mp 186–187 °C; $[\alpha]_D^{22}$ +53.7° (*c* 0.1, MeOH). Compound **15**: mp 167–169 °C; $[\alpha]_D^{22}$ −39.1° (*c* 0.1, MeOH). Compound **16**: mp 209–211 °C; $[\alpha]_D^{22}$ −20.7° (*c* 0.1, MeOH). Compound **17**: mp 178–180 °C; $[\alpha]_D^{22}$ +16.7° (*c* 0.1, MeOH). $^1$H NMR spectra of compounds **14**–**17** are similar to their corresponding L-configuration counterparts.

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