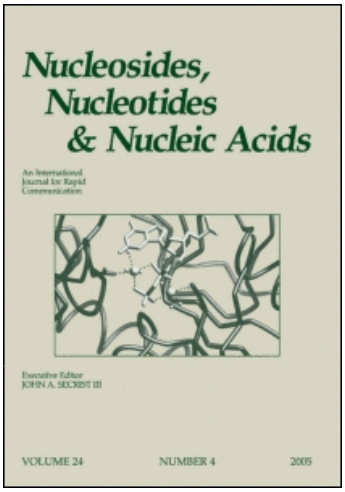


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# Synthesis, Lipophilicity, and Anti-HIV Activity of a New Brominated Analog of Zidovudine

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NUCLEOSIDES & NUCLEOTIDES, 18(3), 337-351 (1999)

# SYNTHESIS, LIPOPHILICITY AND ANTI-HIV ACTIVITY OF A NEW BROMINATED ANALOG OF ZIDOVUDINE

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**ABSTRACT:** A novel cyclic bromine zidovudine analog, **(-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (2)**, and its diastereoisomer **(+)-*trans*-(5R,6R)-(3)** were synthesized and characterized by spectroscopic methods, obtaining **3** in very low yields. The major product **2** presents a selectivity index ($CCID_{50}/IC_{50}$) similar to zidovudine but 55.5 times with higher lipophilicity, which should increase the ability of **2** to cross the blood-brain barrier by a non facilitated diffusion mechanism.

The brain is an important site of human immunodeficiency virus type 1 (HIV-1) replication, which partially accounts for the serious neurologic dysfunction commonly seen in patients with acquired immunodeficiency syndrome (AIDS) and AIDS-related complex.[1,2] **Although 3'-azido-3'-deoxythymidine (Zidovudine, AZT, 1),[3,4] the first nucleoside analog licensed for treating patients with AIDS, has shown to improve neurologic problems in HIV-1 infected patients, its brain concentration is very low and does not provide sufficient therapeutic concentrations to inhibit completely viral replication in the brain.[2]**

It has been demonstrated that anti HIV drugs with high lipophilicity and molecular weight lower than 400 improve brain permeability, through their enhanced ability to cross the blood-brain barrier (BBB) by a non facilitated diffusion mechanism.[5]

Agents that can cross BBB more readily may achieve a therapeutic cephalic concentration and decrease viral replication in the central nervous system (CNS). Since increase the lipophilicity of a drug, we report the synthesis, anti-HIV activity and lipophilicity of a new cyclic bromine derivative of AZT, **(-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (2)**. Characterization of the co-product **(+)-*trans*-(5R,6R)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (3)** is also reported.

## RESULTS AND DISCUSSION

### Chemistry

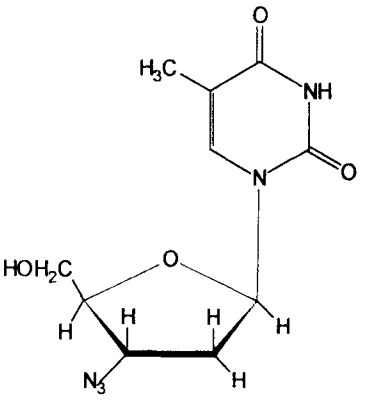
The reaction of 3'-azido-3'-deoxythymidine (AZT, **1**) with N-bromosuccinimide (NBS) in anhydrous N,N-dimethylformamide (DMF), at 25°C and a molar ratio AZT:NBS of 1:1.5, afforded a mixture of **(-)-*trans*-(5S,6S)-2** and **(+)-*trans*-(5R,6R)-3,** the diastereoisomers of 5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido -3'-deoxythymidine (FIG. 1).

The levorotatory diastereoisomer-**2** was isolated in 30% yield whereas diastereoisomer-**3** which was formed in very low yields and could not be separated by chromatography methods, could be attributed dextrorrotatory properties, according to literature data,[8,9] since its experimental determination was not possible. When other reaction conditions were employed, a significant decrease was observed in the overall yields of **2** and no more quantities of **3** were detected.

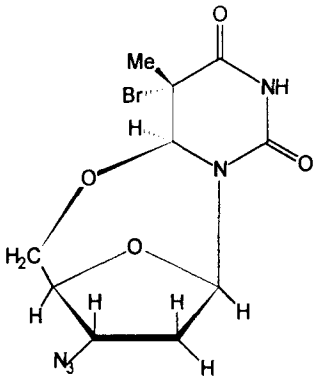
Diastereoisomers **2** and **3** have different configurations in the pyrimidinic ring and different conformations in the sugar moiety. Their configuration has been determined by comparing their respective chemical shifts with those of structurally related compounds,[5,8,9] for which the absolute configuration is known.

All spectroscopic analyses ($^1$H-NMR, $^{13}$C-NMR, IR, MS and UV) were carried out on **2** and the mixture of **2** and **3**. In this way, $^1$H-NMR and $^{13}$C-NMR spectra of pure **2** and the mixture of **2** and **3** allowed us to confirm their structures unequivocally.

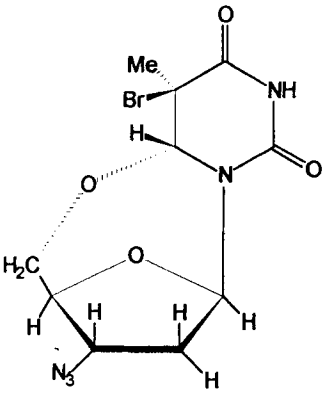
In $^1$H-NMR spectra the signals corresponding to the methyl groups appear as a singlet at δ 1.80 for **2** and δ 1.85 for **3**, which indicates that C-5 is a quaternary carbon. The most distinct differences in $^1$H-NMR chemical shift positions for these spectra were observed for H-1', H-2'$_a$, H-2'$_b$, H-5'$_a$ and H-5'$_b$ protons in the sugar moiety and for H-6 proton of the base, which have been observed for other nucleoside diastereoisomers with a saturation of the 5,6 ethylenic bond.[8]



AZT, 1



(-)-*trans*-(5S,6S)-2



(+)-*trans*-(5R,6R)-3

**FIG. 1.** Molecular structure of the 3′-azido-3′-deoxythymidine (AZT, **1**), (-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (**2**) and (+)-*trans*-(5R,6R)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (**3**).

The H-1' signals of **2** and **3** appear as two double doublets at δ 6.09 in **2** and at δ 6.43 in **3** while the H-2'$_b$ and H-2'$_a$ protons are observed as two multiplets respectively, one at δ 2.23-2.42 (H-2'$_b$-**2**, H-2'$_a$-**3** and H-2'$_b$-**3**) and the other at δ 2.52-2.64 (H-2'$_a$ of **2**). The chemical shifts of H-5'$_a$ and H-5'$_b$ appear as a double doublet at δ 3.82 for H-5'$_b$-**2**, a multiplet at δ 3.88-3.94 for the two protons in **3** and a doublet at δ 4.08 for H-5'$_a$-**2**. The H-6

chemical shifts (δ 7.6-AZT; δ 5.30-**2** and δ 5.00-**3**) were assigned with COSY heteronuclear C-H spectra. The assignment of all exchangeable protons was confirmed by the addition of $D_2O$ which showed that only the imide proton (NH) disappears and that no hydroxyl groups are present in the molecule. In addition, it was observed that there is no tautomerism between the $C_2$=O and $C_4$=O of the pyrimidinic base.

The $^{13}$C-NMR spectra revealed the heavy atom effect in C-5 signals related to the lead compound (AZT, **1**), since the chemical shift at δ 107.59 in **1** appears at δ 56.76 in **2** and at δ 52.42 in **3**. In addition, the change of hybridization from $sp^2$ to $sp^3$ in C-6 was confirmed by the upfield chemical shifts in **2** and **3** (δ 134.10-**1**; δ 89.54-**2** and δ 85.18-**3**) and for characteristic chemical shifts in the carbon methyl group (δ 10.20-**1**; δ 21.90-**2** and δ 21.94-**3**).

In the IR absorption spectra, the typical peak at 2116.6 $cm^{-1}$ showed that the azido group of **1** remains intact in these compounds and no absorption band corresponding to a hydroxyl group was observed. However, the peaks around 1273 $cm^{-1}$ representing the C-O-C vibrations were much more complex and pronounced in the spectra of these compounds than in **1**, as expected from the additional ether group in their structures.

The mass spectra showed characteristic isotopic clusters in their molecular ions (m/z = 345/347) and in all fragments containing a bromine atom.

The UV spectra showed that **1** has two important absorptions at λ 265.1 and λ 206.1 nm as a result of the conjugated chromophors $C_4$=O and $C_5$=$C_6$. However, **2** and **3** show only one absorption near λ 194.0 nm due to the saturation of the $C_5$=$C_6$ double bond.

Thermal analyses together with lipophilicity and antiviral activity were measured only for compound **2**, which decomposes without melt at 174-175°C (FIG. 2).

The DTA curve shows only an exothermic peak between 170-173°C, that could be attributed to a decomposition process, which was confirmed by simultaneous mass loss in the TG curve.[10] The absence of the endothermic peak in the DTA curve suggests that **2** decomposes without melt.

**Mechanism**

The 5-bromo-6,5'-epoxy-5,6-dihydro derivatives **2** and **3** most likely arise through an ionic mechanism with an initial formation of 5,6-bromonium ion intermediates which are


**FIG. 2.** Differential thermal analysis (DTA) and thermogravimetry analysis (TG) of (-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (**2**).



susceptible to regioespecific intramollecular nucleophilic attack by the hydroxyl group of the sugar moiety,[11] at the sterically less hindered C-6 position (FIG. 3), in a similar way to halohydrine/**haloalkoxy** formations.[12-15]

When radical inhibitors such as benzoquinone[16] and hydroxyquinone[17] were employed, improved yields were not observed and undesired co-products turned the reaction work up very troublesome.

**Stability assays**

**The stability assays in organic solvents (methanol and dimethylformamide, twenty days, 37°C) and in isotonic plasma buffer phosphate solution (pH 7.4) during twenty days at 37° and four days at 70°, demonstrated that 2 remained unchanged during all assays period.**

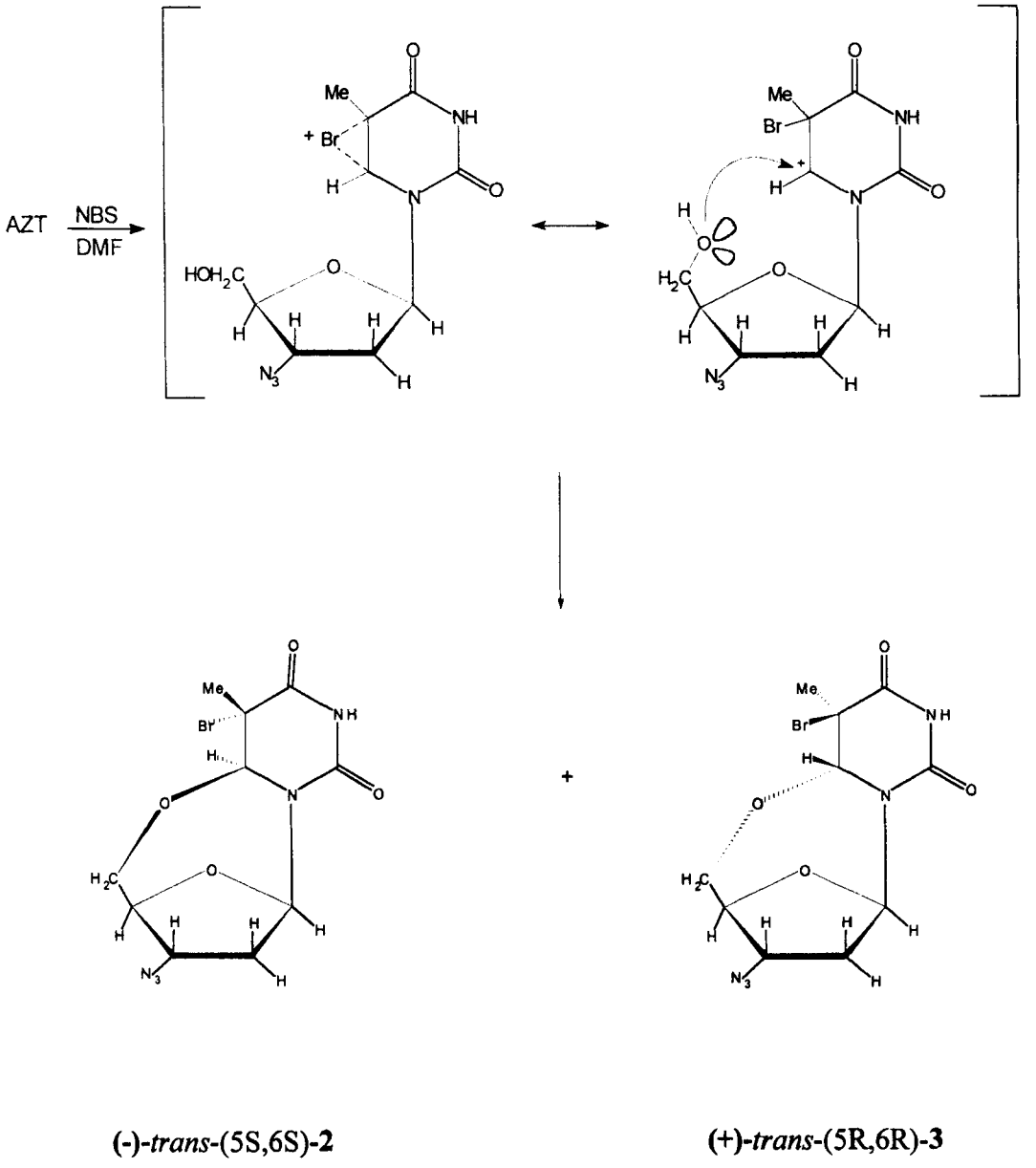



FIG. 3. Proposed mechanism for the conversion of zidovudine (AZT, **1**) to diastereoisomers (-)-*trans*-(5S,6S)-**2** and (+)-*trans*-(5R,6R)-**3**

**Lipophilicity measurements**

The lipophilic feature often seems to be the most important physicochemical parameter in the design and development of new bioactive compounds. The log P defined as the logarithm of the ratio of concentrations of a substance between the n-octanol and aqueous phases of a system under equilibrium conditions (**P = Corg/Caq**),[18] has been established as the reference system. n-Octanol is the organic solvent used as a model due to its long alkyl chain and polar hydroxyl group, which resembles the biological constituent of the membranes.[18]

The partition coefficient of **2** was determined in n-octanol/water using the shake-flask method.[19] The concentrations were measured by means of second derivative UV spectrophotometric analysis,[20] at $\lambda$ 245.0 nm.

The linearity between the second derivative absorbance and the concentration of each drug in aqueous saturated phase was determined with the Beer's graph at the selected wavelength, in the studied range of concentrations. The partition coefficients found for AZT and **2** were log P = 0.02 (lit. 0.02)[21] and log P = 1.11 respectively, which shows that **2** is 55.5 times more lipophilic than AZT.

As an expression of the lipophilic character, the partition coefficient (P) serves as an indicator of the ability of a compound to cross the BBB,[5] and be distributed in the brain.

It has been demonstrated that log P = 0.9-2.5 is the optimal range for drugs designed to cross the BBB by virtue of their lipid solubility.[22] In this way, compound **2** whose lipophilicity falls within this range (log P = 1.11), may display enhanced transcellular diffusion and increased CNS availability.[2]

**Anti-HIV-1 activities and cytotoxicities**

The ability of **2** to inhibit p24 antigen synthesis and to reverse transcriptase activity of HIV-1 IIIB in MT4 cell line and Cord Blood Mononuclear Cells (CBMC) was compared with the inhibitory effects of AZT. The novel nucleoside exhibited antiviral activity similar to that of AZT. Nevertheless, this antiviral activity proved to be most notable in MT4 cell line. Furthermore, the analysis of cytotoxicity was performed by evaluating the $CCID_{50}$ for MT-4 and CBMC, and the results obtained showed no significant differences between AZT and **2** (Table 1).

When **2** was tested against the AZT intermediate resistant strains panel obtained from NIH,[23,24] determinations of $IC_{50}$ were not significantly different from AZT (Table 2).

**TABLE 1.** Determination of 50% Inhibitory Concentration ($IC_{50}$) in HIV-1 IIIB and evaluation of 50% Cell Culture Inhibitory Dose ($CCID_{50}$). Acutely Infected Cells in the presence of 3′-azido-3′-deoxythymidine (**1**) and (-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (**2**).

| | $IC_{50}$ (μM) | | $CCID_{50}$ (μM) | |
|---|---|---|---|---|
| | **1** | **2** | **1** | **2** |
| **MT-4 Cell line** | 0.007 | 0.008 | 80 | 90 |
| **Cord Blood Mononuclear Cells** | 0.002 | 0.001 | 60 | 80 |

**TABLE 2.** Determination of 50% Inhibitory Concentration ($IC_{50}$) in Cord Blood Mononuclear Cells with different 3′-azido-3′-deoxythymidine (**1**) resistant strains panel of HIV-1.

| | $IC_{50}$ (μM) | | | |
|---|---|---|---|---|
| | **AZT intermediate resistant strains panel from NIH[a]** | | | |
| **Compounds** | **1073** | **1074** | **1075** | **1082** |
| **1** | 0.02 | 0.01 | 0.65 | 1.3 |
| **2** | 0.02 | 0.01 | 0.75 | 2.5 |

**1**, 3′-azido-3′-deoxythymidine (AZT).
**2**, (-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine.
**a, Refs. 29-31**



As observed from Tables 1 and 2, the increased lipophilicity of **2** did not reduce their *in vitro* antiviral activity and the $CCID_{50}$ values were similar to those of AZT.

## Conclusions

**It is important to point out that the chemical stability of 2 in isotonic plasma phosphate buffer solutions and in organic solvents, showed that 2 remains unchanged during all stability assays. In addition, taking into account that the activity of AZT is mediated through AZT-triphosphate, and the fact that 2 could not be phosphorylated due to the formation of the 5′,6-anhydro bond, this analog could be intracellularly**

**converted back to some species with a free 5'-OH, prior to its antiviral action as previously reported.[15]**

Since **2** presents a selectivity index ($CCID_{50}/IC_{50}$) similar to AZT but an important additional lipophilicity, this analog can offer some advantages such as wider distribution of the compound throughout the body and higher brain levels by a non facilitated diffusion mechanism.

Furthermore, it is possible to rationalize that this novel compound would protect the 5'-OH of AZT from glucuronidation and increase metabolic stability, enhancing bioavailability and reducing dose-related toxicity. For these reasons, **(-)-*trans*-(5S,6S)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (2)** could result in an important therapeutics against HIV-1.

## EXPERIMENTAL SECTION



All chemicals and reagents were of analytical grade. DMF (Sintorgan) was dried with 4 Å molecular sieves. The nucleoside 3'-azido-3'-deoxythymidine (AZT, **1**) was a generous gift of Filaxis (Paraná, Entre Rios, Argentina), and was used without purification. Quantitative ultraviolet measurements (P values) were carried out with Shimadzu Model UV-160A spectrophotometers using 1 cm quartz cuvettes. The $^{1}$H-NMR and $^{13}$C-NMR spectra were recorded on a 200.13 MHz Bruker spectrometer (with TMS as an internal standard), using DMSO-$d_6$ (99%, SIGMA) as solvent. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of $D_2O$. $^{13}$C-NMR spectra were obtained by using the DEPT technique. IR spectra were obtained from potassium bromide discs on a Nicolet 5 SXC FT-IR. The mass spectra were recorded on a Finnigan Model 3300 F-100 Quadrupole Mass spectrometer and in a high resolution (HRMS) ZAB-SEQ4F (UMYMFOR Laboratories, Buenos Aires, Argentina). Thermoanalytical (DTA-TG) measurements were made with a Netzsch Simultaneous Thermal Analyzer 429 (CIMM, Córdoba, Argentina) in nitrogen and oxygen atmospheres using 5mg samples and at a heating rate of 5°C/min. Chromatotron Model 7924T was used for preparative radial chromatography (PRC). The coating rotor was prepared with Silicagel 60 $PF_{254}$ with calcium sulfate (Merck). Analytical (TLC) and preparative (PTLC) thin layer chromatography were performed on the corresponding precoated silicagel 60 $F_{254}$ plates (Merck). Specific rotation was measured in Jasco Dip digital polarimeter.

**The HPLC measurements were determined on a Konik chromatograph, using an UV detector at λ 265 nm. Chromatography was performed by means of a Shimadzu L.C. column shim-pack CLC-ODS(N)PN 228-17873-91(15 cm) QTY:2, packed with a $C_{18}$ (octadecyl silane) chemically bonded non-polar stationary phase. Methanol-water mixtures of 30-80 methanol concentration were employed as the mobile phase at a flow-rate of 1 mL/min. The samples were injected into the column by a 20 μL loop. The experiments were performed by triplicate at room temperature. A Haake D8 thermostat bath with ± 0.1°C precision was used for temperature control. An Orion Model 5A 520 pHmeter was used for measuring the pH of the buffer solution.**

**(-)-*trans*-(5S,6S)-5-Bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (2) and (+)-*trans*-(5R,6R)-5-bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (3).**

N-bromosuccinimide (NBS, 0.6 g, 3.37 mmoles) in dry N,N-dimethylformamide (DMF, 1 ml) was added to a solution of **1** (0.6 g, 2.24 mmoles) in DMF (1 ml) with magnetic stirring in a bath at 25°C under light protected conditions. The progress of the reactions was monitored by thin layer chromatography (TLC), using ethyl acetate - petroleum ether 60-80 (5.5:4.5 and 3:7) as solvent systems. After stirring the reaction mixture for 15 minutes, 25 ml of cold water was added, a white precipitate was obtained and the solvent was removed *in vacuo*. Compound **2** and the mixture of diastereoisomers **2** and **3** were separated first on PRC using petroleum ether 60-80 and ethyl acetate (85:15) as development solvent and then on PTLC using chloroform-acetone (95:5).

**Diastereoisomer 2:** (0.1507 g, 19.4% yield); $[\alpha]^{24}_{D}$ = -31.8°(*c* 1.00, DMF). $^1$H-NMR, DMSO-$d_6$, δ 1.80 (s, 3H, $CH_3$); 2.22-2.34 (m, 1H, H-2'$_b$), 2.52-2.64 (m, 1H, H-2'$_a$), 3.81 (dd, $J_{5'b,4'}$ = 2.24Hz, $J_{5'a,5'b}$ = 12.60 Hz, 1H, H-5'$_b$), 4.07 (d, $J_{5'a,5'b}$ = 12.60Hz, 1H, H-5'$_a$), 4.29-4.33(m, 1H, H-3'), 4.44-4.52 (m, 1H, H-4'), 5.30 (s, 1H, H-6), 6.09 (dd, $J_{1',2'a}$ = 1.80Hz, $J_{1',2'b}$ = 6.84Hz, 1H, H-1'), 11.0 (s, 1H, NH); $^{13}$C-NMR, DMSO-$d_6$, δ 21.90 ($\underline{C}H_3$ of C-5), 40.76 (C-2'), 56.76 (C-5), 62.15 (C-3'), 73.36 (C-5'), 86.08 (C-4'), 86.48 (C-1'), 89.54 (C-6), 150.14 (C-2), 167.54 (C-4); M.S.H.R. m/z $M^+$+1 = 346.0144 (expected: $M^+$+1 = 346.0108), MS (%); m/z 347 (0.09, $M^+$), 345 (0.11, $M^+$), 266 (0.94), 236 (26.86), 207 (16.99), 205 (17.76), 153 (74.17), 110 (100), 69 (89.11), 54 (93.62). $\lambda_{max.}$(water)/nm 194.0; $\nu_{max.}$ (KBr)/cm$^{-1}$ 2116.6 ($N_3$); 1734.6 (C=O); 1093.9 (C-O-C). DTA and TG analyses showed that this compound decomposed without melt at 174-175°.

**Diastereoisomers 2 and 3:** The MSHR, MS, IR and $\lambda_{max}$ (water) spectra and the DTA-TG analyses for the mixture of **2** and **3** were the same as that observed for diastereoisomer **2**. $^1$H-NMR, DMSO-$d_6$, δ 1.80 (s, 3H, $CH_3$, **2**), 1.85 (s, 3H, $CH_3$, **3**), 2.23-2.42 (m, 3H, H-2'$_b$, **2**, H-2'$_a$-**3** and H-2'$_b$-**3**), 2.52-2.64 (m, 1H, H-2'$_a$, **2**), 3.82 (dd, $J_{5'b,4'}$ = 2.24Hz, $J_{5'a,5'b}$ = 12.60Hz, 1H, H-5'$_b$, **2**), 3.88-3.94 (m, 2H, H-5'$_a$ and H-5'$_b$, **3**), 4.08 (d, $J_{5'a,5'b}$ = 12.60Hz, 1H, H-5'$_a$, **2**), 4.30-4.34(m, 1H, H-3', **2**), 4.41-4.53 (m, 2H, H-4', **3** and **2**), 4.78-4.88 (m, 1H, H-3', **3**), 5.00 (s, 1H, H-6, **3**), 5.30 (s, 1H, H-6, **2**), 6.09 (dd, $J_{1',2'a}$ = 1.80Hz, $J_{1',2'b}$ = 6.84Hz, 1H, H-1', **2**), 6.43 (dd, $J_{1',2'a}$ = 1.36Hz, $J_{1',2'b}$ = 7.23Hz, 1H, H-1', **3**), 11.01 (s, 1H, NH, **2**); 11.14 (s, 1H, NH, **3**); $^{13}$C-NMR, DMSO-$d_6$, δ 21.90 ($\underline{C}H_3$ of C-5, **2**), 21.94 ($\underline{C}H_3$ of C-5, **3**), 35.44 (C-2', **3**), 40.76 (C-2', **2**), 52.42 (C-5, **3**), 56.76 (C-5, **2**), 62.15 (C-3', **2**), 66.82 (C-3', **3**), 71.31 (C-5', **3**), 73.36 (C-5', **2**), 83.39 (C-4', **3**), 85.18 (C-6, **3**), 85.71 (C-1', **3**), 86.08 (C-4', **2**), 86.48 (C-1', **2**), 89.54 (C-6, **2**), 150.14 (C-2, **2**), 152.02 (C-2, **3**), 167.48 (C-4, **2** and **3**).



**Lipophilicity measurements of (-)-*trans*-(5S,6S)-5-Bromo-6,5'-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (2).**

The lipophilic character was measured by their octanol-water partition coefficient, as described elsewhere,[25-27] by means of the shake-flask method, using water obtained from a Milli-Q water system as the polar phase and n-octanol as the lipidic phase. Both phases were mutually saturated prior to use. Normal and second derivative absorption spectra of standard solution of **2** in saturated octanol phase were recorded between 200 and 350 nm against octanolic phase as blank. For the Beer's graph of **2**, a stock solution was prepared in octanolic phase (4.04 x $10^{-3}$ M). Aliquots of 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml were taken in duplicate of stock solution and diluted with octanolic phase to obtain final concentrations in the range (4.04 - 16.18) x $10^{-4}$ M. The second derivative values ($\delta^2$Abs/$\delta\lambda^2$) at 245.0 nm were measured and the plot of $\delta^2$Abs/$\delta\lambda^2$ vs Conc. (mol/L) was linear at the concentration range examined.

For the partitioning process, 14 mg of **2** was dissolved in 25 ml of octanolic phase (1.62 x $10^{-3}$ M) and 75 ml of aqueous phase was added; the bottles were then shaken gently for *ca* 30 min. at 25°C and then centrifuged for 10 min. The partition coefficient of **2** was calculated from eq 1,

$$P = \left(\frac{D_o^f}{D_o^i - D_o^f}\right)\left(\frac{V_w}{V_o}\right) \qquad (1)$$

where $D_o^i$ and $D_o^f$ are the second derivative absorbance at 245.0 nm in the n-octanol phase before and after partitioning respectively; $V_w$ is the volume of the aqueous phase and $V_o$ is the volume of the octanolic phase. The values were expressed as log P.

**Stability assays**

**Stability assays of 2 were carried out in buffer phosphate solutions of pH 7.4 ($Na_2HPO_4$- $NaH_2PO_4$, $\mu$ = 300 mOsm), for 20 days at 37°C and 4 days at 70°C as well as in methanol and dimethylformamide 20 days at 37°C. Evaluation of the corresponding samples were conducted in triplicate using a high performance liquid chromatography (HPLC) technique.**

**Cells**

Cord blood mononuclear cells (CBMC), obtained from HIV-1 seronegative donors, were isolated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient centrifugation. Cultures were stimulated with phytohemmaglutinin, for 3 days and pools of cells from three CBMC donors were then established and used as feeder cultures.[28] MT-4 cells were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH.[24,25] The cells were cultured at 37°C in RPMI-1640 (Gibco, Burlington, Ontario, Canada) supplemented with 10% fetal calf serum (ICN Chemicals, Montreal, Quebec, Canada), 2 mM glutamine, 100 U/ml penicillin (ICN), and 100 mg/ml streptomycin (ICN).



**HIV-1 strains**

HIV-1 IIIB was derived from chronically infected H9 cells (kindly supplied by Robert Gallo). An AZT Intermediate Isolate Panel (Catalog Number 1073, 1074, 1075, 1082) was obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH and was a gift from Dr. Douglas Richman, University of California, San Diego.[29-31]

**Screening and evaluation of single agents for anti HIV-1 activity.**

Cells were infected with different strains of HIV-1 positive supernatant for 2 h. The HIV-1 inoculum used to infect MT-4 cells ranged from 20 to 200, 50% tissue culture infective dose ($TCID_{50}$) per 1 x $10^6$ cells; for CBMC, the range was 1000 to 2000 $TCID_{50}$ per $10^6$

cells. After infection, cells were washed and dispersed in 96-well plates in various concentrations of drugs. AZT was used as a control, with each experiment performed in duplicate. Culture medium was changed at day 4, so that half of the fluid was replaced with fresh medium containing the original drug concentration. Cell-free supernatant fluids were harvested at day 7, and assayed for production of p24 antigen by ELISA (Abbott Laboratories, North Chicago, Illinois, USA). In the case of CBMC, HIV-1 replication was assayed by measuring levels of reverse transcriptase (RT) activity in culture cell fluid as described.[32] The 50% effective dose ($IC_{50}$) of each drug was calculated on the basis of p24 antigen levels or RT activity as appropriate.

**Effect of (-)-*trans*-(5S,6S)-5-bromo-6,5-epoxy-5,6-dihydro-3'-azido-3'-deoxythymidine on the cellular growth.**

For toxicity studies, uninfected cells were cultured in duplicate in 96-well plates at varying concentrations of the compounds. Changes of the culture medium were performed on days 4 and 7, and viable cell numbers were determined by trypan blue assay in order to assess the concentration of drug that inhibited cellular growth by 50% ($CCID_{50}$).



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