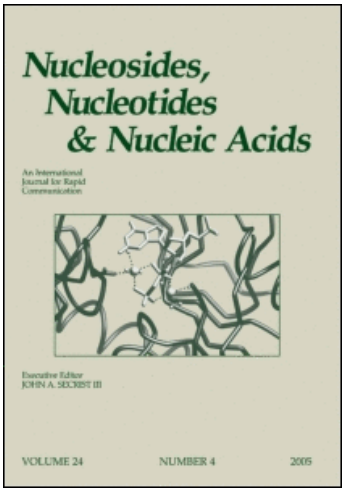

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Synthesis, *In Vitro* Biological Stability, and Anti-HIV Activity of 5-Halo (or Methoxy)-6-Alkoxy (Azido or Hydroxy)-5,6-Dihydro-2',3'-Didehydro-3'-Deoxythymidine Diastereomers as Potential Prodrugs of 2',3'-Didehydro-3'-deoxythymidine (D4T)

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SYNTHESIS, *IN VITRO* BIOLOGICAL STABILITY, AND ANTI-HIV ACTIVITY OF 5-HALO (OR METHOXY)-6-ALKOXY (AZIDO OR HYDROXY)-5,6-DIHYDRO-2',3'-DIDEHYDRO-3'-DEOXYTHYMIDINE DIASTEREOMERS AS POTENTIAL PRODRUGS OF 2',3'-DIDEHYDRO-3'-DEOXYTHYMIDINE (D4T)

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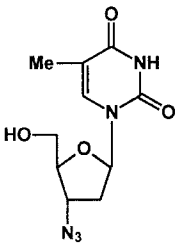
ABSTRACT: A new class of 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-2',3'-didehydro-3'-deoxythymidines (**4-17**) were investigated as potential anti-AIDS drugs. These 5,6-dihydro derivatives, which are also potential prodrugs of 2',3'-didehydro-3'-deoxythymidine (D4T) were designed to have properties which would enhance their duration of action, lipophilicity and cephalic delivery to the central nervous system. The 5,6-dihydro derivatives of D4T (**4-15**), which differ in configuration at the C-5 and C-6 positions, were synthesized by the regiospecific addition of XR (X = Br, Cl, I; R = OMe, OEt, N_3, OH) to the 5,6-olefinic bond of D4T. These 5,6-disubstituted-5,6-dihydro analogs of D4T are more lipophilic (P = 0.70 - 4.0 range) than D4T (P = 0.12) and are stable to *E. coli* thymidine phosphorylase. Regeneration of the 5,6-olefinic bond to give D4T, upon incubation of the 5-bromo- and 5-iodo-6-methoxy-5,6-dihydro derivatives (**6, 7, 10, 11**) with glutathione or a mouse liver soluble enzyme fraction, was extensive (50-95%). The most potent anti-HIV-1 agents, 5-iodo-6-methoxy (**10, 11**), 5-bromo-6-azido (**14, 15**) and 5-methoxy-6-hydroxy (**16, 17**) derivatives of D4T, exhibited anti-HIV activities comparable to D4T.

INTRODUCTION

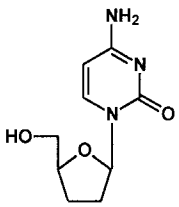
Acquired immunodeficiency syndrome (AIDS), which is attributed to infection by human immunodeficiency virus (HIV-1), is a degenerative disease of the immune and central nervous systems. Since the HIV virus invades the central nervous system (CNS) early in the course of the systemic infection,[1] failure to stop its progression leads to AIDS

Dedicated to Professor Yoshihisa Mizuno, on the occasion of his 75th birthday.

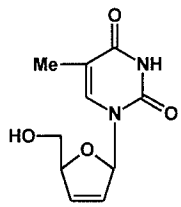
dementia and other neurological manifestations of HIV infection. The HIV virus may replicate more rapidly in the CNS than in other tissues and the CNS may act as a sanctuary for the virus.[2-4] Thus, the ability of antiretroviral agents to effectively penetrate the CNS and provide a therapeutic brain drug concentration constitutes an important requirement for the treatment of HIV infection. 3'-Azido-3'-deoxythymidine (**1**, AZT) is a potent inhibitor of HIV replication which resulted in its introduction as the first drug to treat AIDS.[5-7] AZT, which has a large partition coefficient (P = 1.26) relative to thymidine (P = 0.064)[8] enters cerebrospinal fluid (CSF)[9,10] primarily by passive diffusion, and it delays the onset of HIV-1 induced neurological dysfunction in some patients.[11] However, AZT causes dose-related bone marrow toxicity manifested as severe anemia and leukopenia.[7,12] Other pyrimidine nucleoside analogs currently used in the treatment of AIDS include 2',3'-dideoxycytidine (**2**, DDC)[13-16] and 2',3'-didehydro-3'-deoxythymidine (**3**, D4T).[17-20] D4T was found to be as effective as AZT in protecting ATH8 cells against HIV-1 induced cytopathogenicity *in vitro*, to exhibit a higher chemotherapeutic index,[19] and to be five-fold more potent than DDC[17] against HIV-1



1, AZT



2, DDC



3, D4T

infected MT4 cells. Moreover, D4T has been found to be less toxic than AZT to bone marrow stem cells,[21] and less inhibitory to mitochondrial replication.[22] Pharmacokinetic studies indicated that D4T is rapidly cleared from plasma with a terminal half-life of 17 minutes[23] that necessitates more frequent dosing to maintain an adequate therapeutic level in plasma. The low level to which D4T penetrates the CNS[23] is likely due, at least in part, to its low lipophilicity. The 1-octanol-water partition coefficient, which is a measure of lipophilicity that is generally predictive of blood-brain-barrier (BBB) penetration,[24,25] was 0.23 for D4T[26] compared to 1.26 for AZT. The low lipophilicity of D4T could diminish its ability to provide an effective therapeutic brain concentration,[26] and hence its ability to

suppress viral replication in brain. Furthermore, we recently observed that D4T undergoes partial glycosidic bond cleavage (10%) upon incubation with thymidine phosphorylase, which would also reduce its efficacy.[27]

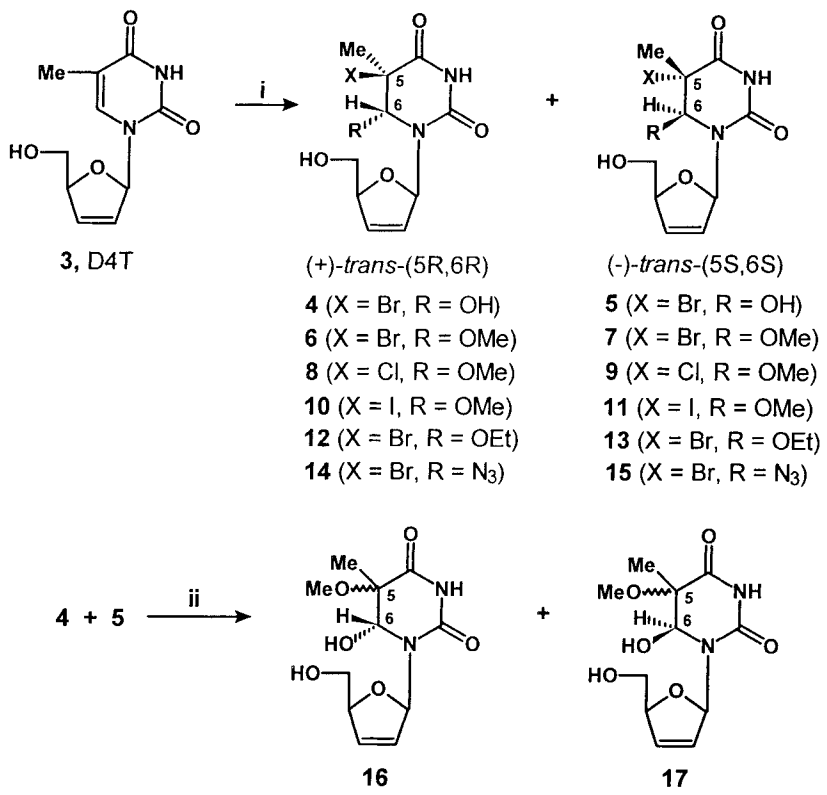
Derivatives of active drugs are often designed as prodrugs to improve their pharmacokinetic and biodistribution properties, increase drug stability, and reduce toxicity. A number of different types of prodrugs to D4T possessing 5'-acetate, 5'-methoxyacetate or 5'-cyclohexylcarbonate,[28] 5'-alkyl monophosphate,[29] and methyl and phenylphosphonate and phosphothionate[30] substituents have been reported. The rationale for making these prodrugs was based on the assumption that the prodrug will be converted *in vivo* to either D4T or a 5'-monophosphate by chemical or enzymatic hydrolysis. Interest in masked phosphate prodrugs originated due to the observation that the metabolic phosphorylation of D4T in human cells is very different from that of AZT. Conversion of D4T to D4T monophosphate is the rate-limiting step, whereas subsequent phosphorylation to D4T diphosphate and D4T triphosphate occurs readily. Thus, there is no accumulation of D4T monophosphate which could saturate the enzyme thymidylate kinase and result in inhibition of thymidine phosphorylation that would lead to decreased levels of thymidine triphosphate (TTP) required for normal host cell DNA synthesis. Decreased levels of TTP have been postulated to be one of the factors associated with the toxicity of AZT.[21] The preparation of 5'-[(1,4-dihydro-1-methyl-3-pyridinyl)carbonyl] ester[26] and 4-thiopyrimidine[31] analogs of D4T have been reported as brain targeted prodrugs. However, attempts to design lipophilic or brain targeted prodrugs of D4T have not yet provided compounds with a therapeutic superior efficacy to D4T.

We recently reported 5-halo-6-alkoxy-5,6-dihydro derivatives of the anti-HIV agents 3'-fluoro-3'-deoxythymidine (FLT)[32] and AZT[33] as potential prodrugs. These 5,6-dihydro derivatives were found to be more lipophilic than the parent compound, and the 5-iodo and 5-bromo analogs exhibited activities comparable to FLT and AZT, respectively. The amount of radioactivity in mouse brain after intravenous injection of [2-^{14}C]-labelled (5R,6R)- or (5S,6S)-5-bromo-6-methoxy-5,6-dihydro analogs of AZT, or (5R,6R)-5-bromo-6-ethoxy-5,6-dihydro-3'-azido-3'-deoxythymidine, were 2-4 fold higher than that for [2-^{14}C]-AZT ($P < 0.05$).[27] 5-Fluoro-5-halo-6-methoxy-5,6-dihydro-2'-deoxyuridine diastereomers have been investigated as prodrugs[34] of the anticancer agent 5-fluoro-2'-

deoxyuridine (FUDR). These latter prodrugs exhibited potent antileukemic activity and possessed improved pharmacokinetic parameters relative to FUDR.[34] All three classes of these 5,6-dihydro compounds (FLT, AZT, FUDR) are resistant to pyrimidine phosphorylase catalyzed glycosidic bond cleavage. These 5,6-dihydro compounds act as slow releasers of the parent nucleoside, due to regeneration of the 5,6-olefinic bond, upon incubation with glutathione. This beneficial combination of desirable physico-chemical properties and favorable pharmacokinetic characteristics for 5,6-dihydro analogs of FLT and AZT[27,32,33] prompted us to extend the structure-activity studies to include 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro analogs of D4T as potential anti-HIV drugs. We now report the synthesis, anti-HIV activity and some biochemical properties of 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-2',3'-didehydro-3'-deoxythymidines (**4-17**) as potential anti-HIV agents and/or prodrugs of D4T that may possess improved pharmacokinetic and/or biodistribution properties.

CHEMISTRY

The target (+)-*trans*-(5R,6R)-**4** and (-)-*trans*-(5S,6S)-**5** diastereomers of 5-bromo-6-hydroxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine were synthesized by reaction of D4T with N-bromosuccinimide in water at 0 °C in 43 and 33% yields, respectively (see Scheme 1). A similar reaction of D4T with molecular bromine in methanol gave a mixture of (+)-*trans*-(5R,6R)-**6** and (-)-*trans*-(5S,6S)-**7** diastereomers of 5-bromo-6-methoxy-5,6-dihydro-D4T. The (+)-*trans*-(5R,6R)-**8** and (-)-*trans*-(5S,6S)-**9** diastereomers of 5-chloro-6-methoxy-5,6-dihydro-D4T, and the (+)-*trans*-(5R,6R)-**10** and (-)-*trans*-(5S,6S)-**11** diastereomers of 5-iodo-6-methoxy-5,6-dihydro-D4T were synthesized by reaction of D4T with N-chlorosuccinimide or N-iodosuccinimide in methanol, respectively. Diastereomers **6-11** were separated by preparative HPLC. Reaction of D4T with bromine in ethanol afforded a mixture of (+)-*trans*-(5R,6R)-**12** and (-)-*trans*-(5S,6S)-**13** diastereomers of 5-bromo-6-ethoxy-5,6-dihydro-D4T in 77% yield. A mixture of (+)-*trans*-(5R,6R)-**14** and (-)-*trans*-(5S,6S)-**15** diastereomers of 5-bromo-6-azido-5,6-dihydro-D4T was obtained by the reaction of D4T with sodium azide in 1,2-dimethoxyethane in 58% yield. Diastereomers **12** and **13**, and **14** and **15**, could not be separated by flash silica gel column chromatography or preparative thin layer



Reagents: i, N-bromosuccinimide, H_2O, 0 °C (**4, 5**); Br_2, MeOH, 25 °C (**6, 7**); N-chlorosuccinimide, MeOH, HOAc, 25 °C (**8, 9**); N-iodosuccinimide, HOAc, MeOH, 25 °C (**10, 11**); Br_2, EtOH, 25 °C (**12, 13**); N-bromosuccinimide, NaN_3, 1,2-dimethoxyethane, -5 °C to 25 °C (**14, 15**); ii, NaOH, MeOH, 25 °C (**16, 17**).

Scheme 1

chromatography (PTLC). Reaction of a mixture of (+/-)-*trans*-5-bromo-6-hydroxy-5,6-dihydro-D4T diastereomers (**4, 5**) with sodium hydroxide in methanol yielded a mixture of two 5-methoxy-6-hydroxy-5,6-dihydro-D4T diastereomers (**16, 17**) in 60% yield. The configuration at the C-5 position for **16** and **17** has not been established since it is not known whether the reaction proceeds by a S_N1 or S_N2 mechanism. The formations of these 5-halo-6-alkoxy (azido or hydroxy)-5,6-dihydro derivatives **4-15** of D4T most likely occurs via the initial formation of a 5,6-halonium ion intermediate which is susceptible to regiospecific nucleophilic attack by the alcohol, water or azide anion, at the

sterically less hindered C-6 position. The configuration of compounds **4-15** at the C-5 and C-6 positions was assigned by comparing the optical rotation, X-ray analysis for (5R,6R)-**8**,[35] and ^{1}H NMR spectral data with that of similar compounds, for which the absolute configuration is known, such as 5-bromo-6-methoxy (or hydroxy)-5,6-dihydrothymidine[36,37] diastereomers. The most distinct differences in chemical shift positions in the ^{1}H NMR spectra of these diastereomers occur for the H-1', H-2' and H-3' protons in the sugar moiety and the H-6 proton of the base.

RESULTS AND DISCUSSION

The objective of this study involved the design of 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-D4Ts (**4-17**) as brain-targeted drugs, and/or prodrugs of D4T, for evaluation as anti-HIV agents. The partition coefficient (P), which is an indicator of the ability of a compound to cross the BBB,[38] for 5-halo (or methoxy)-6-alkoxy (or hydroxy)-5,6-dihydro analogs (**4-13, 16, 17**) was determined in a 1-octanol/water system at 37 °C. The P values for the 5-halo (or methoxy)-6-alkoxy (or hydroxy)-5,6-dihydro-D4T compounds were 6 to 33-fold higher (P = 0.70 - 4.0) than that of D4T (P = 0.12) as illustrated in Table 1. In the absence of an active nucleoside transport system, the enhanced lipophilicity of the 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro analogs should increase their ability, relative to D4T, to cross the BBB by a nonfacilitated diffusion mechanism. This postulate is based on the observation that increasing the lipophilicity of compounds with a molecular weight of less than 400 has been reported to improve brain permeability.[39] Furthermore, the parabolic relationship between log P values and brain extractability for a group of ^{11}C-labelled compounds suggests an optimal log P range of 0.9-2.5 for a radiopharmaceutical designed to cross the BBB by virtue of lipid solubility.[40]

The nature of the halogen atom at the C-5 position (5S,6S-series, Br-**7** > I-**11** > Cl-**9**; 5R,6R series, Br-**6** > I-**10** = Cl-**8**), and the configuration at the C-5 and C-6 positions [(5R,6R) > (5S,6S); **4** > **5**, **6** > **7**, **8** > **9**, **10** > **11**] were determinants of lipophilicity. Compounds having a C-6 methoxy substituent were more lipophilic than the corresponding analogs possessing a C-6 hydroxyl group (**6** > **4**, **7** > **5**). Increasing the length of the C-6 alkoxy substituent also increased lipophilicity (OEt > OMe; **12**, **13** > **6**

TABLE 1. Partition Coefficients (P) and Biochemical Properties of 5-Halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-2',3'-didehydro-3'-deoxythymidines.

no.	P[a]	% Phosphorolysis[b]	Regeneration of 5,6-olefinic bond (% D4T)	
			Glutathione[c]	Mouse liver soluble enzyme fraction[d]
4	2.50	0	ND[e]	ND
5	1.00	0	0	ND
6	3.39	0	85	75
7	1.45	0	50	52
8	2.60	0	0[f]	0
9	1.00	0	0[f]	0
10	2.60	0	> 95	85
11	1.25	0	> 95	95
12,13	4.00	0	ND	ND
14,15	ND	0	ND	ND
16,17	0.70	0	0[f]	ND
D4T	0.12	10	-	-
Thymidine		72		

[a]P = concentration in 1-octanol/concentration in water at 37 °C, n = 5. [b]The % of phosphorolysis upon incubation of the test compound with *E. coli* thymidine phosphorylase at 37 °C for 10 min, n = 2. [c]The % of D4T formed upon incubation of the test compound with glutathione using a test compound:glutathione molar ratio of 1:2 for 30 min at 37 °C, n = 2. [d]The % of D4T formed upon incubation of the test compound with the soluble enzyme fraction of mouse liver for 30 min at 37 °, n = 1. [e]ND = not determined. [f]No D4T was detected for an incubation of 24 hours.

and **7**). It has been reported that D4T could not be detected in mouse brain by HPLC (detection limit of 0.1 μg/g wet tissue) following injection of a 10 mg/kg dose, although scintillation counting showed a level of 0.44 μg/g wet tissue after injection of a 25 mg/kg dose of [methyl-^{3}H]-D4T.[26] The enhanced lipophilicity of 5,6-dihydro derivatives of D4T, relative to D4T, may conceivably provide a higher concentration in the brain.

The efficacy of nucleoside drugs can be increased by enhancing their stability towards phosphorolysis.[41] One potential approach to prevent the undesirable phosphorolytic cleavage of pyrimidine nucleosides is reduction of the 5,6-double bond which substantially increases the stability of the glycosidic bond.[32,33] Thymidine phosphorylase is a specific enzyme which cleaves the base — sugar N — C bond present in pyrimidine deoxyribonucleosides.[42] To be active *in vivo*, the nucleoside drug must not undergo phosphorolysis prior to activation to the triphosphate nucleotide. An *in vitro* phosophorolysis study which involved incubation of 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-D4Ts (**4-17**) with *Escherichia coli* thymidine phosphorylase for 10 minutes at 37 °C indicated that these 5,6-dihydro analogs were completely stable since no phosphorolysis occurred. In contrast, D4T and the physiological nucleoside thymidine undergo 10% and 72% phosphorolysis, respectively under these experimental conditions (Table 1).

The utility of 5-halo (or methoxy)-6-methoxy (or azido)-5,6-dihydro derivatives of D4T as potential prodrugs of D4T would be dependent upon their pharmacokinetic properties, tissue biodistribution, and their rate of conversion to D4T. It was anticipated that bioconversion of these 5,6-dihydro diastereomers to D4T would be essential for trapping the active drug in the brain, since this would prevent, or delay, egress of the active drug from the brain. The *in vitro* incubation of the 5,6-dihydro compounds (**5-13**, **16**, **17**) with the model thiol glutathione (GSH) was therefore investigated (substrate:GSH ratio = 1:2 for a 30 minute incubation at 37 °C) to determine the ability of GSH to regenerate the 5,6-olefinic bond present in D4T. In mammalian tissues, the GSH concentration is in the 0.5-1 mM range, whereas the cysteine concentration is in the 0.03-0.1 mM range.[43,44] It is conceivable that *in vivo* dehalogenation and elimination to generate D4T could occur by a chemical reaction with GSH or cysteine, and/or an enzymatic reaction, with a thiol-containing enzyme. Regeneration of the 5,6-olefinic bond to afford D4T, upon incubation with GSH, was dependent upon the nature of the C-5 halo substituent in the 5-halo-6-methoxy-5,6-dihydro series **6-11** where the relative 5,6-olefinic bond regeneration order was I > Br >> Cl (see Table 1). The nature of the C-6 substituent was also a determinant of 5,6-double bond regeneration since compounds possessing a C-6 hydroxyl substituent (**5, 16, 17**) were completely inert to GSH.

Similarly, the 5-chloro-6-methoxy-5,6-dihydro derivatives (**8, 9**) did not undergo conversion to D4T, even for incubations with GSH for 24 hours. No product other than D4T could be detected by HPLC analysis following incubation of **6, 7, 10** or **11** with GSH at 37 °C. The reaction of the 5,6-dihydro analogs of D4T with GSH (R-SH) to afford D4T could occur via two possible mechanisms (E2 Hal or S_N2 displacement) as described previously for 5,6-dihydro analogs of AZT.[33]

There are alternate mechanisms, other than GSH, which could regenerate D4T from the 5,6-dihydro analogs of D4T. The *in vitro* incubation of the 5-halo-6-methoxy-5,6-dihydro compounds **6-11** with the soluble enzyme fraction from mouse liver was therefore investigated to determine the potential utility of 5,6-dihydro-D4T compounds as prodrugs. The incubation of **6-11** with the mouse liver soluble enzyme fraction for 30 minutes at 37 °C showed a similar profile to that observed in the GSH study for regeneration of the 5,6-olefinic bond where the extent of conversion to D4T was 85-95% for the 5-iodo-6-methoxy compounds **10** and **11**, 52-75% for the 5-bromo-6-methoxy compounds **6** and **7**, and 0% for the 5-chloro-6-methoxy compounds **8** and **9** (see Table 1). The results from these *in vitro* regeneration studies upon incubation with GSH or a mouse liver soluble enzyme fraction indicate that prodrugs **6, 7, 10** and **11** are converted extensively to D4T. Although these prodrugs may diffuse out of cells at the same rate at which they diffuse into cells ($K_1 = K_{-1}$, Figure 1), intracellular regeneration of the 5,6-olefinic bond would produce the significantly more hydrophilic D4T that would diffuse out of cells at a lower rate ($K_3 << K_{-1}$). Subsequent intracellular conversion of D4T to its highly hydrophilic monophosphate (5'-P-D4T) by cellular kinases should result in complete intracellular trapping of the active D4T nucleotide.

The 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro analogs of D4T (**4-17**) were evaluated by the U.S. National Institutes of Health, Antiviral Research Branch in an *in vitro* anti-HIV screen using HIV-1 infected T4 lymphocytes (CEM cell line), and the results are shown in Table 2. Compounds **4-17**, except for diastereomers **4** and **5**, were tested as a mixture of the two diastereomers. In the C-6 OH and C-6 OMe series of compounds (**4-11, 16, 17**), the C-5 substituent (I, Br, Cl, OMe) was a determinant of anti-HIV-1 activity where the potency order was I > Br > Cl (C-6 OMe series) and OMe > Br (C-6 OH series). In the 5-bromo series of compounds, the C-6 substituent was also a

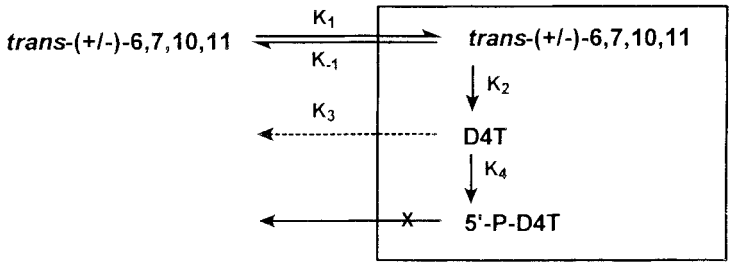
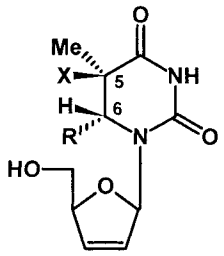


FIGURE 1. Diffusion of 5,6-dihydro analogs of D4T into cells, regeneration of the 5,6-olefinic bond, and metabolic trapping as the 5'-monophosphate (5'-P-D4T).

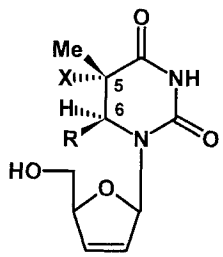
determinant of activity where a C-6 azido substituent exhibited a greater potency than the corresponding weakly active C-6 OMe, and inactive C-6 OH and OEt analogs. The most potent anti-HIV agent, a mixture of (+/-)-*trans*-5-iodo-6-methoxy-5,6-dihydro-D4T diastereomers (**10, 11**) were marginally more active than the parent drug D4T. The increased inhibition of HIV replication by the mixture of 5-iodo-6-methoxy-5,6-dihydro, relative to the corresponding 5-bromo and 5-chloro analogs, may be due to their more efficient conversion to D4T under the assay conditions employed. The modest anti-HIV activity exhibited by the 5-chloro-6-methoxy-5,6-dihydro diastereomers (**8, 9**) is likely due, at least in part, to their inability to undergo conversion to D4T *in vitro*. The mixture of (+/-)-*trans*-5-bromo-6-azido-5,6-dihydro-D4T diastereomers (**14,15**) exhibited a similar anti-HIV activity to that of D4T, although they were more cytotoxic to uninfected CEM cells. The observation that the mixture of the two 5-methoxy-6-hydroxy-5,6-dihydro-D4T diastereomers (**16, 17**), which do not undergo *in vitro* conversion to D4T in the presence of either GSH or a mouse liver soluble enzyme fraction, exhibited equipotent anti-HIV activity as compared to D4T suggests these 5-methoxy-6-hydroxy analogs do not require 5,6-olefinic bond regeneration to exhibit anti-HIV activity.

In conclusion, two (5R,6R)- and (5S,6S)-5-iodo-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine diastereomers **10** and **11** have been identified as a new class of potential prodrugs of D4T that i) possess a much higher lipophilicity (P = 2.60 and 1.25) than D4T (P = 0.12); ii) unlike D4T are completely stable to *E. coli* thymidine

TABLE 2. *In Vitro* Anti-HIV Activity of 5-Halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-2',3'-didehydro-3'-deoxythymidines in HIV-1 Infected CEM Cells.[a]



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



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

no.	X	R	Configuration	IC_{50} (M)[b]	EC_{50} (M)[c]
4	Br	OH	5R,6R	$> 2.0 \times 10^{-4}$	Inactive
5	Br	OH	5S,6S	$> 2.0 \times 10^{-4}$	Inactive
6, 7	Br	OMe	5R,6R; 5S,6S	$> 1.28 \times 10^{-4}$	5.46×10^{-5}
8, 9	Cl	OMe	5R,6R; 5S,6S	$> 1.03 \times 10^{-3}$	3.75×10^{-4}
10, 11	I	OMe	5R,6R; 5S,6S	6.60×10^{-5}	3.76×10^{-7}
12, 13	Br	OEt	5R,6R; 5S,6S	$> 1.4 \times 10^{-5}$	Inactive
14, 15	Br	N_3	5R,6R; 5S,6S	4.47×10^{-5}	9.18×10^{-7}
16, 17	OMe	OH	5?,6R; 5?,6S	$> 1.7 \times 10^{-4}$	7.82×10^{-6}
D4T[d]				$2.3 - 3.5 \times 10^{-4}$	$0.8 - 25 \times 10^{-6}$

[a]Testing was performed by the U. S. National Institutes of Health, Antiviral Research Branch, AIDS antiviral screening program. [b]The IC_{50} value is the test drug concentration which results in a 50% survival of uninfected untreated control CEM cells (eg. cytotoxicity of the test compound). [c]The EC_{50} value is the test drug concentration which produces a 50% survival of HIV-1 infected cells relative to uninfected untreated controls (eg. *in vitro* anti-HIV-1 activity). [d]The EC_{50} value, defined as the dose required to effect a 50% reduction in the cytopathic effect of HIV-1 in CEM cells was taken from reference #31 which was determined under identical conditions in the same AIDS Antiviral Screening Program.

phosphorylase; iii) undergo extensive conversion to D4T upon incubation with GSH or a mouse liver soluble enzyme fraction; and iv) exhibit *in vitro* anti-HIV-1 activity that is marginally superior to that of D4T. The desirable physico-chemical, biochemical and biological properties of these D4T prodrugs indicates the 5,6-dihydro pyrimidine nucleoside prodrug concept warrants consideration in antiviral drug design as a method to enhance cephalic drug delivery.

EXPERIMENTAL SECTION

Melting points were determined with a Buchi capilliary apparatus and are uncorrected. Nuclear magnetic resonance spectra (^{1}H NMR, ^{13}C NMR) were determined using a Bruker AM-300 spectrometer. The assignment of all exchangeable protons (OH, NH) was confirmed by the addition of D_2O. ^{13}C NMR spectra were acquired using the J modulated spin echo technique where methyl and methine carbon resonances appear as positive peaks, and methylene and quaternary carbon resonances appear as negative peaks. Specific rotations were measured using an Optical Activity Ltd. digital polarimeter. Thin layer chromatography (TLC) was performed using Whatman MK6F silica gel microslides (250 μm thickness). Preparative thin layer chromatography (PTLC) was carried out using Whatman PLK5F plates (1 mm in thickness). Silica gel column chromatography was carried out using Merck 7734 (60-200 mesh) silica gel. HPLC analyses were performed using a Water's system consisting of a Model 860 automated gradient controller, Models 510 and M-45 solvent pumps, Model U6K injector, and Model 481 LC ultraviolet detector with detection at 230 nm (5,6-dihydro compounds). Separations were performed with a Whatman Partisil M9 10/25 ODS reverse phase column (preparative column) or a Water's C-18 Radial-Pak reverse phase column (analytial column). 2',3'-Didehydro-3'-deoxythymidine (**3**, D4T) was prepared using a literature procedure.[45] **Warning:** Halogenated solvents such as dichloromethane must not be used in certain reactions, such as those described for the preparation of the 6-azido products **14** and **15**, since its reaction with sodium azide may produce potentially explosive polyazidomethane.

(+)-*trans*-(5R,6R)-5-Bromo-6-hydroxy-5,6-dihydro-2',3'-didehydro-3'-deoxy-thymidine (4) and (-)-*trans*-(5S,6S)-5-Bromo-6-hydroxy-5,6-dihydro-2',3'-dide-hydro-3'-deoxythymidine (5).

N-bromosuccinimide (80 mg, 0.44 mmol) was added in aliquots to a solution of D4T (0.1 g, 0.44 mmol) in water (5 mL) at 0 °C with stirring. The initial yellow color produced upon addition of each aliquot of N-bromosuccinimide disappeared rapidly. After all the N-bronosuccinimide had been added, the reaction mixture was stirred for 20 min at 0 °C. Removal of the solvent *in vacuo*, dissolution of the residue in ethyl acetate (5 mL), adsorption onto silica gel (1 g), removal of the solvent *in vacuo* and application of this material to the top of a silica gel column followed by elution with chloroform-methanol (96:4, v/v) afforded **4** and **5**, respectively.

Diastereomer 4: $[\alpha]^{25}_{D}$ = +31.9° (c 0.26, MeOH); R_f 0.42 ($CHCl_3$-MeOH, 9:1, v/v); mp 94-95 °C; yield (60 mg, 43%); ^{1}H NMR (CD_3OD) δ 1.82 (s, 3H, C*H*$_3$), 3.66-3.80 (m, 2H, H-5'), 4.78-4.82 (m, 1H, H-4'), 5.15 (s, 1H, H-6), 5.90 (dd, $J_{1',2'}$=1.2, $J_{2',3'}$=4.8 Hz, 1H, H-2'), 6.30 (d, $J_{2',3'}$=4.8 Hz, 1H, H-3'), 6.82 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 23.38 (*C*H$_3$), 55.29 (C-5), 62.56 (C-5'), 81.76 (C-6), 87.38 (C-4'), 91.77 (C-1'), 127.14 (C-2'), 135.35 (C-3'), 153.59 (C-2 *C*=O), 169.55 (C-4 *C*=O). Anal. calcd. for $C_{10}H_{13}BrN_2O_5$: C, 37.40; H, 4.07; N, 8.72. Found: C, 37.81; H, 4.15; N, 8.62.

Diastereomer 5: $[\alpha]^{25}_{D}$ = -32.7° (c 0.11, MeOH); R_f 0.35 ($CHCl_3$-MeOH, 9:1, v/v); oil; yield (47 mg, 33%); ^{1}H NMR (CD_3OD) δ 1.82 (s, 3H,C*H*$_3$), 3.74 (m, 2H, H-5'), 4.70-4.80 (m, 1H, H-4'), 5.28 (s, 1H, H-6), 5.95 (m, 1H, H-2'), 6.24 (m, 1H, H-3'), 6.78 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 23.30 (*C*H$_3$), 54.68 (C-5), 65.07 (C-5'), 80.12 (C-6), 87.71 (C-4'), 90.79 (C-1'), 127.80 (C-2'), 133.96 (C-3'), 152.87 (C-2 *C*=O), 169.92 (C-4 *C*=O). Anal. calcd. for $C_{10}H_{13}BrN_2O_5$: C, 37.40; H, 4.07; N, 8.72. Found: C, 37.40; H, 4.23; N, 8.93.

(+)-*trans*-(5R,6R)-5-Bromo-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (6) and (-)-*trans*-(5S,6S)-5-Bromo-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (7).

A freshly prepared solution of methyl hypobromite was added dropwise to a solution of D4T (50 mg, 0.223 mmol) in methanol (5 mL) with stirring until the light yellow color of the reaction mixture persisted. The reaction was allowed to proceed for 20 min at 25 °C prior to neutralization (pH 6-7) with methanolic sodium hydroxide. Removal of the solvent *in vacuo*, dissolution of the residue in methanol (5 mL), adsorption onto silica gel

(1 g), removal of the solvent *in vacuo*, and application of this material to the top of a silica gel column followed by elution with chloroform-methanol (98:2, v/v) as eluent afforded a mixture of **6** and **7** (60 mg, 80%) as a viscous oil, which was separated by preparative HPLC (PHPLC) using a Whatman Partisil M9 ODS reverse phase column using H_2O:MeOH (70:30, v/v) as the eluent at a flow rate of 2 mL/min (retention times of 11.8 and 21.1 min) with UV detection at 230 nm.

Diastereomer 6: $[\alpha]^{25}_D$ = +66.0° (c 0.6, MeOH); R_f 0.57 ($CHCl_3$-MeOH, 9:1, v/v); mp 83-85 °C dec.; yield (30 mg, 40%); ^{1}H NMR (CD_3OD) δ 1.87 (s, 3H, C*H*$_3$), 3.48 (s, 3H, OC*H*$_3$), 3.76 (m, 2H, H-5'), 4.78-4.82 (m, 1H, H-4'), 5.10 (s, 1H, H-6), 6.05 (m, 1H, H-2'), 6.25 (d, $J_{2',3'}$=4.8 Hz, 1H, H-3'), 6.74 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 23.30 (*C*H$_3$), 54.86 (C-5), 57.62 (O*C*H$_3$), 64.33 (C-5'), 87.40 (C-4'), 88.08 (C-6), 91.59 (C-1'), 128.46 (C-2'), 133.76 (C-3'), 153.11 (C-2 *C*=O), 169.83 (C-4 *C*=O). Anal. calcd. for $C_{11}H_{15}BrN_2O_5.1/4H_2O$: C, 38.89; H, 4.59; N, 8.24. Found: C, 38.98; H, 4.44; N, 8.05.

Diastereomer 7: $[\alpha]^{25}_D$ = -80.3° (c 0.36, MeOH); R_f 0.57 ($CHCl_3$-MeOH, 9:1, v/v); oil; yield (20 mg, 27%); ^{1}H NMR (CD_3OD) δ 1.92 (s, 3H, C*H*$_3$), 3.48 (s, 3H, OC*H*$_3$), 3.80-3.86 (m, 2H, H-5'), 4.78-4.84 (m, 1H, H-4'), 5.38 (s, 1H, H-6), 5.96 (m, 1H, H-2'), 6.30 (d, $J_{2',3'}$=4.8 Hz, 1H, H-3'), 6.70 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 23.08 (*C*H$_3$), 53.97 (C-5), 58.73 (O*C*H$_3$), 64.57 (C-5'), 87.99 (C-4'), 89.13 (C-6), 92.04 (C-1'), 128.61 (C-2'), 134.03 (C-3'), 152.72 (C-2 *C*=O), 169.77 (C-4 *C*=O). Anal. calcd. for $C_{11}H_{15}BrN_2O_5.1/4H_2O$: C, 38.89; H, 4.59; N, 8.24. Found: C, 38.49; H, 4.99; N, 8.58.

(+)-*trans*-(5R,6R)-5-Chloro-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (8) and (-)-*trans*-(5S,6S)-5-Chloro-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (9).

N-chlorosuccinimide (190 mg, 1.42 mmol) was added with stirring in aliquots to a solution of D4T (0.1 g, 0.44 mmol) in methanol (10 mL) and glacial acetic acid (0.4 mL) at 25 °C. After all the N-chlorosuccinimide had been added, the reaction mixture was stirred for 18 h at 25 °C, prior to neutralization with methanolic sodium hydroxide. Removal of the solvent *in vacuo*, dissolution of the residue obtained in ethyl acetate (5 mL), adsorption onto silica gel (1 g), removal of the solvent *in vacuo* and application

of this material to the top of a silica gel column followed by elution with chloroform-methanol (95:5, v/v) as the eluent afforded a mixture of **8** and **9**. The diastereomers **8** and **9** were separated by PHPLC (retention times of 11.6 and 20.9 min) using the conditions employed for the separation of **6** and **7**.

Diastereomer 8: $[\alpha]^{25}_{D}$ = +75.4° (c 0.41, MeOH); R_f 0.52 ($CHCl_3$-MeOH, 9:1, v/v); mp 138-139 °C; yield (28 mg, 36%); ^{1}H NMR (CD_3OD) δ 1.70 (s, 3H, C*H*$_3$), 3.50 (s, 3H, OC*H*$_3$), 3.78 (m, 2H, H-5'), 4.78-4.86 (m, 1H, H-4'), 5.05 (s, 1H, H-6), 5.95 (dd, $J_{1',2'}$=1.2, $J_{2',3'}$=4.8 Hz, 1H, H-2'), 6.25 (d, $J_{2',3'}$=4.8 Hz, 1H, H-3'), 6.78 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 22.07 (*C*H$_3$), 57.50 (O*C*H$_3$), 62.45 (C-5), 64.28 (C-5'), 87.07 (C-4'), 88.06 (C-6), 91.59 (C-1'), 128.50 (C-2'), 133.76 (C-3'), 153.09 (C-2 *C*=O), 169.20 (C-4 *C*=O). Anal. calcd. for $C_{11}H_{15}ClN_2O_5$: C, 45.44; H, 5.20; N, 9.63. Found: C, 45.44; H, 5.29; N, 9.46.

Diastereomer 9: $[\alpha]^{25}_{D}$ = - 30.3° (c 0.25, MeOH); R_f 0.52 ($CHCl_3$-MeOH, 9:1, v/v); oil; yield (9 mg, 12%); ^{1}H NMR (CD_3OD) δ 1.70 (s, 3H, C*H*$_3$), 3.44 (s, 3H, OC*H*$_3$), 3.78-3.82 (m, 2H, H-5'), 4.73-4.80 (m, 1H, H-4'), 5.28 (s, 1H, H-6), 5.94 (dd, $J_{1',2'}$=1.2, $J_{2',3'}$=4.8 Hz, 1H, H-2'), 6.28 (m, 1H, H-3'), 6.70 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 21.89 (*C*H$_3$), 58.70 (O*C*H$_3$), 61.88 (C-5), 64.51 (C-5'), 87.96 (C-4'), 88.71 (C-6), 91.89 (C-1'), 128.58 (C-2'), 134.23 (C-3'), 152.85 (C-2 *C*=O), 169.23 (C-4 *C*=O). Anal. calcd. for $C_{11}H_{15}ClN_2O_5$: C, 45.44; H, 5.20; N, 9.63. Found: C, 45.31; H, 5.60; N, 9.65.

(+)-*trans*-(5R,6R)-5-Iodo-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (10) and (-)-*trans*-(5S,6S)-5-Iodo-6-methoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (11).

N-iodosuccinimide (45 mg, 0.2 mmol) was added slowly with stirring to a solution of D4T (45 mg, 0.2 mmol) in methanol (10 mL) and glacial acetic acid (0.1 mL) during a period of 5 min. The reaction was allowed to proceed at 25 °C for 4 h with stirring and the solvent was removed *in vacuo*. The residue obtained was dissolved in chloroform and the chloroform layer was washed with cold water, dried over Na_2SO_4 and the solvent was removed *in vacuo*. The product was purified by preparative thin layer chromatography (PTLC) using chloroform-methanol (95:5, v/v) as the developing solvent to yield a

mixture of diastereomers **10** and **11** which were separated by PHPLC using a Whatman Partisil M9 ODS reverse phase column employing H_2O:MeOH (4:1, v/v) as eluent at a flow rate of 2.5 mL/min with UV detection at 230 nm.

Diastereomer 10: $[\alpha]^{25}_D$ = +82.5° (c 0.45, MeOH); R_f 0.54 ($CHCl_3$-MeOH, 9:1, v/v); oil; yield (14 mg, 18%); ^{1}H NMR (CD_3OD) δ 2.06 (s, 3H, C*H*$_3$), 3.45 (s, 3H, OC*H*$_3$), 3.75 (m, 2H, H-5'), 4.76-4.82 (m, 1H, H-4'), 5.11 (s, 1H, H-6), 6.17 (dd, $J_{1',2'}$=1.2, $J_{2',3'}$=4.8 Hz, 1H, H-2'), 6.26 (m, 1H, H-3'), 6.72 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 26.20 (*C*H$_3$), 35.43 (C-5), 57.44 (O*C*H$_3$), 64.45 (C-5'), 88.14 (C-4'), 89.16 (C-6), 91.68 (C-1'), 128.40 (C-2'), 133.85 (C-3'). Anal. calcd. for $C_{11}H_{15}IN_2O_5$.1/4H_2O: C, 34.16; H, 4.03; N, 7.24. Found: C, 34.19; H, 3.81; N. 6.93.

Diastereomer 11: $[\alpha]^{25}_D$ = -41.8° (c 0.30, MeOH); R_f 0.54 ($CHCl_3$-MeOH, 9:1, v/v); oil; yield (8 mg, 10.5%); ^{1}H NMR (CD_3OD) δ 2.08 (s, 3H, C*H*$_3$), 3.45 (s, 3H, OC*H*$_3$), 3.82-3.86 (m, 2H, H-5'), 4.76-4.82 (m, 1H, H-4'), 5.36 (s, 1H, H-6), 5.96 (d, $J_{1',2'}$=1.2, $J_{2',3'}$=4.8 Hz, 1H, H-2'), 6.26 (m, 1H, H-3'), 6.66 (d, $J_{1',2'}$=1.2 Hz, 1H, H-1'); ^{13}C NMR (CD_3OD) δ 25.99 (*C*H$_3$), 34.54 (C-5), 58.61 (O*C*H$_3$), 64.58 (C-5'), 88.02 (C-4'), 90.89 (C-6), 92.25 (C-1'), 128.70 (C-2'), 133.76 (C-3'). Anal. calcd. for $C_{11}H_{15}IN_2O_5$: C, 34.57; H, 3.95; N, 7.33. Found: C, 34.63; H, 3.84; N, 7.58.

(+)-*trans*-(5R,6R)-5-Bromo-6-ethoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (12) and (-)-*trans*-(5S,6S)-5-Bromo-6-ethoxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (13).

A freshly prepared solution of ethyl hypobromite was added dropwise to a solution of D4T (200 mg, 0.89 mmol) in ethanol (20 mL) at 25 °C with stirring until the light yellow color of the reaction mixture persisted. The reaction was allowed to proceed for one hour at 25 °C prior to neutralization (pH 6-7) with ethanolic sodium hydroxide. Removal of the solvent *in vacuo* and purification of the residue on a silica gel column using chloroform-methanol (98:2, v/v) as eluent yielded a mixture of **12** and **13** (240 mg, 77%) as a viscous oil. The diastereomers **12** and **13** could not be separated by column chromatography or preparative thin layer chromatography (PTLC). ^{1}H NMR (CD_3OD δ 1.12 and 1.14 (ratio 2:1) (2 t, J=7.0 Hz, 3H total, OCH_2CH_3), 1.90 and 1.92 (ratio 2:1) (2 s, 3H total, C*H*$_3$), 3.54-3.95 (m, 4H, H-5', O*CH*$_2$CH$_3$), 4.76-4.82 (m, 1H, H-4'), 5.13

and 5.40 (ratio 2:1) (2 s, 1H total, H-6), 5.92 and 6.06 (ratio 1:2) (m, 1H, H-2'), 6.22-6.28 (m, 1H, H-3'), 6.65 and 6.73 (ratio 1:2) (two d, $J_{1',2'}$=1.2 Hz, 1H total, H-1'); ^{13}C NMR (CD_3OD) δ 15.42 (OCH_2CH_3), 23.18 and 23.40 (CH_3), 54.96 and 54.18 (C-5), 64.39 and 64.49 (C-5'), 66.22 and 67.38 (OCH_2CH_3), 86.38, 87.80, 87.97 and 88.02 (C-6, C-4'), 92.07 and 91.65 (C-1'), 128.52 (C-2'), 133.69 and 134.0 (C-3'), 152.88 and 153.17 (C-2 *C*=O), 163.91 (C-4 *C*=O). Anal. calcd. for $C_{12}H_{17}BrN_2O_5.1/2\ H_2O$: C, 40.23; H, 5.06; N, 7.82. Found: C, 40.44; H, 4.89; N, 7.93.

(+)-*trans*-(5R,6R)-5-Bromo-6-azido-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (14) and (-)-*trans*-(5S,6S)-5-Bromo-6-azido-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine (15).

N-bromosuccinimide (0.2 g, 1.1 mmol) was added in aliquots to a precooled (-5 °C) suspension prepared by a mixing solution of D4T (0.224 g, 1.0 mmol) in 1,2-dimethoxyethane (20 mL) and a solution of sodium azide (0.282 g, 4.0 mmol) in water (0.7 mL) with stirring. The initial yellow color produced upon addition of each aliquot of N-bromosuccinimide disappeared rapidly. When all of the N-bromosuccinimide had reacted, the reaction mixture was stirred for 30 min at 25 °C, then poured onto ice water (50 mL) and extracted with ethyl acetate (3 x 50 mL). Washing the ethyl acetate extract with cold water (2 x 5 mL), drying (Na_2SO_4), and removal of the solvent *in vacuo* gave a residue which was separated by silica gel column chromatography using chloroform-methanol (97:3, v/v) as the eluent to yield a mixture of diastereomers **14** and **15** (0.2 g, 58%) as a syrup. The diastereomers **14** and **15** could not be separated by PTLC or flash silica gel column chromatography. ^{1}H NMR ($CDCl_3$) δ 1.90 and 1.94 (ratio 2:1) (2 s, 3H total, CH_3), 2.88 and 2.94 (ratio 2:1) (2 br s, 1H total, 5'-OH), 3.80-4.04 (m, 2H, H-5'), 4.80-4.90 (m, 1H, H-4'), 5.60 and 5.96 (ratio 2:1) (2 s, 1H total, H-6), 5.88-5.94 (m, 1H, H-2'), 6.20-6.30 (m, 1H, H-3'), 6.84 and 6.90 (ratio 1:2) (two d, $J_{1',2'}$=1.2 Hz, 1H total, H-1'), 9.10 and 9.13 (ratio 1:2) (2 s, 1H total, N*H*); ^{13}C NMR ($CDCl_3$) δ 22.78 and 22.93 (CH_3), 52.16 and 53.0 (C-5), 62.97 and 63.38 (C-5'), 71.99 and 72.84 (C-6), 86.22 and 86.37 (C-4'), 89.16 and 89.42 (C-1'), 127.20 and 127.41 (C-2'), 133.21 and 133.39 (C-3'), 150.14 and 150.32 (C-2 *C*=O), 166.47 and 166.53 (C-4 *C*=O). Anal. calcd. for $C_{10}H_{12}BrN_5O_4$: C, 34.69; H, 3.49; N, 20.23. Found: C, 35.01; H. 3.51; N, 19.88.

5-Methoxy-6-hydroxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidine Diastereomers (16,17).

To a solution of (+/-)-*trans*-5-bromo-6-hydroxy-5,6-dihydro-2',3'-didehydro-3'-deoxythymidines (**4, 5**, 55 mg, 0.17 mmol) in methanol (10 mL) was added a solution of methanolic sodium hydroxide until a pH value of 9 was obtained from the reaction mixture. The reaction was allowed to proceed for 30 min at 25 °C with stirring. Removal of the solvent *in vacuo* gave a residue which was purified by PTLC using chloroform-methanol (85:15, v/v) as the developing solvent to yield a mixture of **16** and **17** (28 mg, 60%), which could not be separated by PTLC or flash silica gel column chromatography: mp 185-190 °C dec.; ^{1}H NMR (CD_3OD) δ 1.32 and 1.38 (ratio 4:1) (2 s, 3H total, CH_3), 3.42 and 3.48 (ratio 1:4) (2s, 3H total, OCH_3), 3.74-3.80 (m, 2H, H-5'), 4.70 and 4.90 (ratio 4:1) (2 s, 1H total, H-6), 4.72-4.76 and 4.80-4.82 (ratio 1:4) (2 m, 1H total, H-4'), 5.92-5.96 (m, 1H, H-2'), 6.24-6.32 (m, 1H, H-3'), 6.64-6.70 and 6.78-6.82 (ratio 4:1) (2 m, 1H total, H-1'); ^{13}C NMR (CD_3OD) δ 23.23 and 23.74 (*C*H_3), 57.68 and 59.15 (O*C*H_3), 64.53 and 64.58 (C-5'), 73.83 and 74.31 (C-5), 87.63 and 87.69 (C-4'), 88.15 (C-6), 90.72 and 91.95 (C-1'), 128.40 and 128.49 (C-2'), 134.03 and 134.34 (C-3'), 153.54 (C-2 *C*=O), 175.79 (C-4 *C*=O). Anal. calcd. for $C_{11}H_{16}N_2O_6.1/2H_2O$: C, 46.97; H, 6.09; N, 9.96. Found: C, 47.06; H, 5.84; N, 9.75.

Partition Coefficients (P).

The test compounds (**4-13, 16, 17**) were partitioned between equal volumes of presaturated 1-octanol and water by mixing in a mechanical shaker for 4.5 h at 37 °C. The two phases were separated, and the concentration of the test compound in the 1-octanol layer was determined by UV quantitation at 230 nm. Partition coefficients (P) were calculated as the ratio of the concentration in the 1-octanol phase to the concentration in the water phase ($P = C_{1\text{-octanol/water}}$).

***In Vitro* Phosphorolysis.**

The extent of *in vitro* phosphorolysis of 5-halo (or methoxy)-6-alkoxy (azido or hydroxy)-5,6-dihydro-D4'T diastereomers (**4-17**) was carried out by incubating the test compound with *E. coli* thymidine phosphorylase (Sigma Chemical Co.) at 37 °C for 10 min using the previously reported method.[33]

In Vitro Regeneration of the 5,6-Olefinic Bond.

The extent of D4T regeneration from 5-halo (or methoxy)-6-alkoxy (or hydroxy)-5,6-dihydro-D4Ts was determined by incubating the test compound with glutathione (GSH, reduced), or a mouse liver soluble enzyme fraction, using the methods previously reported.[33]

In Vitro Anti-HIV-1 Assay.

The ability of the test compound to protect HIV-1 infected T4 lymphocytes (CEM cells) from cell death was determined using the reported procedure.[46] Small amounts of HIV-1 were added to the cells, and a complete cycle of virus reproduction was allowed to take place to obtain the required cell killing. Agents that interact with virions, cells, or virus gene-products to interfere with viral activities will protect cells from cytolysis. All tests were compared with a positive (AZT-treated) control performed at the same time under identical conditions.

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