Synthesis, *in Vitro* Biological Stability, and Anti-HIV Activity of 5-Halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidine Diastereomers as Potential Prodrugs to 3′-Azido-3′-deoxythymidine (AZT)

Rakesh Kumar, Lili Wang, Leonard I. Wiebe, and Edward E. Knaus*

Faculty of Pharmacy and Pharmaceutical Sciences, University of Alberta, Edmonton, Alberta, Canada T6G 2N8

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A new class of 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines was investigated as potential anti-AIDS drugs. These 5,6-dihydro derivatives, which are also potential prodrugs to 3′-azido-3′-deoxythymidine (AZT), were designed in an effort to enhance the duration of action, lipophilicity, and cephalic delivery to the central nervous system. The 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines, 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 = alkoxy, azido) to the 5,6-olefinic bond of AZT. The 5-halo-6-methoxy-5,6-dihydro derivatives of AZT are more lipophilic (P = 3.3–18.8 range) than the parent compound AZT (P = 1.29). These 5-halo-6-methoxy-5,6-dihydro compounds, like AZT, did not undergo glycosidic bond cleavage upon incubation with *Escherichia coli* thymidine phosphorylase. Regeneration of the 5,6-olefinic bond to give AZT, upon incubation of the 5-halo-6-methoxy-5,6-dihydro compounds with glutathione, mouse blood, or mouse liver homogenate, was dependent upon the nature of the 5-halo substituent (I > Br). No 5,6-olefinic bond regeneration was observed for the 5-chloro analogs. The ability of these 5-halo-6-alkoxy (or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines to protect CEM cells against HIV-induced cytopathogenicity was evaluated. Structure–activity studies showed that the C-5 substituent (I, Br, Cl) was a determinant of anti-HIV-1 activity where the potency order was I ≥ Br > Cl. In the 5-bromo series of compounds, the C-6 substituent was also a determinant of activity where 6-OMe and 6-OEt substituents exhibited a greater potency than the corresponding 6-*i*-PrO, 6-(1-octyloxy), 6-(1-hexadecyloxy), and 6-azido analogs. All of the 5-chloro-6-substituted-5,6-dihydro compounds were inactive, except for the approximately equipotent 6-OMe and 6-azido diastereomeric mixtures which were 2–3 log units less active than the reference drug AZT. The configuration at the C-5 and C-6 positions also influenced potency where the activity of the 5*R*,6*R*-diastereomer was generally greater than that of the corresponding 5*S*,6*S*-diastereomer. The most potent anti-HIV-1 agents, which included the (5*R*,6*R*)-5-bromo-6-methoxy, (5*R*,6*R*)-5-iodo-6-methoxy, (5*S*,6*S*)-5-iodo-6-methoxy, and (5*R*,6*R*)-5-bromo-6-ethoxy analogs of AZT, were equipotent to the reference drug AZT. These 5-iodo(bromo)-6-methoxy-5,6-dihydro derivatives of AZT are potential prodrugs to AZT that provide a rapid release of AZT *in vivo*.

Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS), invades the central nervous system (CNS), ultimately culminating in severe neurological disorders and increased susceptibility to persistent infections due to suppression of the immune system.[1-6] The life cycle of the HIV virus is dependent upon HIV-encoded reverse transcriptase (RT) which has been a major target for the design of anti-HIV agents.[7,8] In this regard, 3′-azido-3′-deoxythymidine (**1**, AZT)[9] has been developed as a clinically useful drug for the treatment of AIDS.[10-12] AZT acts as an inhibitor of viral reverse transcriptase after its conversion to AZT-5′-triphosphate by host cell kinases.[4] Although AZT appears to be temporarily effective in decreasing mortality and morbidity in some patients with AIDS, the therapeutic efficacy of AZT is limited by serious side effects. Significant dose-related toxicities associated with the administration of AZT include bone marrow suppression, anemia, and leukopenia, all of which detract from its effective clinical utilization in various treatment strategies.[12,13] Pharmacokinetic studies indicate that the plasma half-life of AZT is approximately 1 h,[14] and therefore frequent administration of AZT is necessary to maintain therapeutic drug levels. Furthermore, the ability of AZT to cross the blood brain barrier (BBB) is less than optimal. Therefore, AZT does not effectively suppress viral replication in the brain.[15] In addition, resistance to AZT may occur upon prolonged treatment.[16]

In order to overcome these limitations of AZT, which include rapid blood clearance and a less than optimal ability to cross the BBB, and to increase its efficacy, a variety of AZT prodrugs have been investigated. These studies involved esterification of the 5′-hydroxyl group of AZT,[17-20] attachment of lipophilic functional groups to the base moiety,[21,22] and utilization of masked phosphate nucleotides.[23,24] Unfortunately, these modifications have not provided effective AZT prodrugs.

5,6-Dihydropyrimidine nucleosides have attracted attention as potential anticancer and antiviral agents.[25] Physiological dihydro nucleosides play an important role in nucleic acid metabolism and appear frequently in the sequence of t-RNA.[26] Previous studies have shown that 5,6-dihydro analogs of thymidine (**2a**)[25,27,28] can act as competitive substrates, to thymidine, for thymidine kinase. 5-Fluoro-5-halo-6-methoxy-5,6-dihydro-2′-deoxy-

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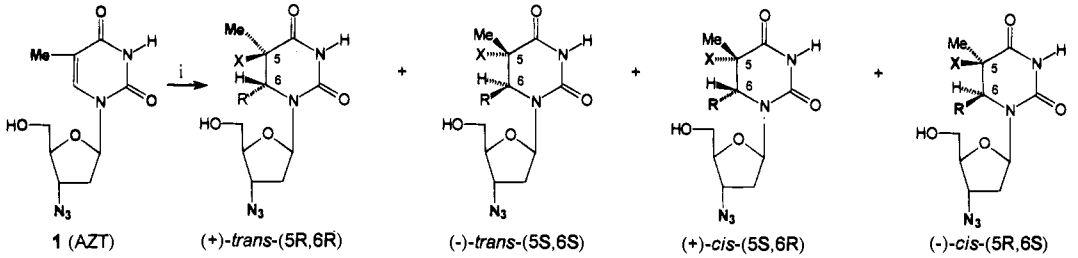
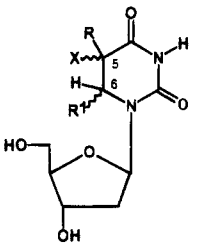
Scheme 1[a]



(5R,6R)-**3** (X = Br, R = OMe) (5S,6S)-**4** (X = Br, R = OMe)
(5R,6R)-**5** (X = Cl, R = OMe) (5S,6S)-**6** (X = Cl, R = OMe) (5S,6R)-**7** (X = Cl, R = OMe)
(5R,6R)-**8** (X = I, R = OMe) (5S,6S)-**9** (X = I, R = OMe)
(5R,6R)-**10** (X = Br, R = OEt) (5S,6S)-**11** (X = Br, R = OEt)
(5R,6R)-**12** (X = Cl, R = OEt) (5S,6S)-**13** (X = Cl, R = OEt) (5S,6R)-**14** (X = Cl, R = OEt)
(5R,6R)-**15** (X = Br, R = $OCH(Me)_2$
(5R,6R)-**16** (X = Cl, R = $OCH(Me)_2$
(5R,6R)-**17** (X = Br, R = $O(CH_2)_7Me$
(5R,6R)-**18** (X = Cl, R = $O(CH_2)_7Me$
(5R,6R)-**19** (X = Br, R = $O(CH_2)_{15}Me$
(5R,6R)-**20** (X = Cl, R = $O(CH_2)_{15}Me$
(5R,6R)-**21** (X = Br, R = N_3) (5S,6S)-**22** (X = Br, R = N_3) (5R,6S)-**23** (X = Br, R = N_3)
(5R,6R)-**24** (X = Cl, R = N_3) (5S,6S)-**25** (X = Cl, R = N_3) (5S,6R)-**26** (X = Cl, R = N_3) (5R,6S)-**27** (X = Cl, R = N_3)

[a] Reagents: (i) Br_2, MeOH, 25 °C (products **3**, **4**); *N*-chlorosuccinimide, MeOH, HOAc, 25 °C (method A; **5**, **7**); Cl_2, MeOH, 25 °C (method B; **5**–**7**); *N*-iodosuccinimide, MeOH, HOAc, 25 °C (products **8**, **9**); Br_2, EtOH, 25 °C (products **10**, 11); Cl_2, EtOH, 25 °C (products **12**–**14**); Br_2, 2-propanol (**15**), 1-octanol (**17**), 1-hexadecanol (**19**), 25 °C; Cl_2, 2-propanol (**16**), 1-octanol (**18**), 1-hexadecanol (**20**), 25 °C; *N*-bromosuccinimide, NaN_3, 1,2-dimethoxyethane, 0 °C (products **21**–**23**); *N*-chlorosuccinimide, NaN_3, 1,2-dimethoxyethane, 25 °C (products **24**–**27**).

uridine diastereomers (**2b,c**) have been investigated as prodrugs[29] to 5-fluoro-2′-deoxyuridine (FUDR). These 5-halo-6-methoxy diastereomers could potentially act as slow releasers of FUDR either by spontaneous regeneration of the 5,6-double bond or by reaction with a tissue nucleophile such as glutathione. A correlation between the ability of diastereomers **2b** to undergo regeneration of the 5,6-double bond *in vitro* to form FUDR and FU and an *in vivo* antileukemic effect was observed. Furthermore, the plasma half-life of FUDR in cancer patients treated with the (+)-diastereomer of **2b** was 10-fold longer than that attained using FUDR.[29] In previous studies, some 5-halo-6-alkoxy-5,6-dihydro derivatives (**2d**–**f**) of 5-ethyl-2′-deoxyuridine (EDU)[30,31] were designed as potential prodrugs to EDU. The 5-bromo-6-methoxy-5,6-dihydro derivatives (**2d**) exhibited potent antiherpes activities against HSV-1 and HSV-2.[30] The 5-halo-6-alkoxy-5,6-dihydro diastereomers (**2d**–**f**) were 5–31-fold more lipophilic than the parent compound EDU, which may enhance their ability to enter cells more readily by diffusion. [4-^{14}C]-(5*R*,6*R*)-5-Bromo-5-ethyl-6-ethoxy-5,6-dihydro-2′-deoxyuridine (**2f**, BEEDU)[31] provided a significantly higher brain concentration of EDU than did its parent compound EDU in mice after iv dosing. These 5,6-dihydro compounds could serve as a slow releaser (prodrug) of the parent nucleoside *in vivo* which may arise either by spontaneous regeneration of the 5,6-double bond or by consecutive dehalogenation elimination reactions upon reaction with a tissue nucleophile such as glutathione. 5-Chloro-5-ethyl-6-methoxy-5,6-dihydro-2′-deoxyuridine (**2e**, CEMDU)[30] provided a longer blood residence time compared to EDU. It appears that (+)-*trans*-5-bromo-5-fluoro-6-methoxy-5,6-dihydro-2′-deoxyuridine (**2b**) and the 5-ethyl-5-halo-6-alkoxy-5,6-dihydro-2′-deoxyuridine diastereomers (**2d**–**f**) act as a reservoir for the *in vivo* release of FUDR or EDU, respectively. These beneficial properties of the 5,6-dihydropyrimidines **2a**–**f** prompted us to investigate 5-halo-6-alkoxy(or azido)-5,6-dihydro analogs of AZT as potential anti-HIV drugs.



2

2a, R = Me, X = Br, R^1 = OMe
2b, R = F, X = Br, R^1 = OMe
2c, R = F, X =Cl, R^1 = OMe
2d, R = Et, X = Br, R^1 = OMe
2e, R = Et, X = Cl, R^1 = OMe
2f, R = Et, X = Br, R^1 = OEt

We now report the synthesis, anti-HIV activity and some biochemical properties of the 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines as potential anti-HIV agents and/or prodrugs to AZT that may possess improved pharmacokinetic and/or biodistribution properties.

Chemistry

Reaction of 3′-azido-3′-deoxythymidine (**1**, AZT) with molecular bromine in methanol at 25 °C afforded the (+)-*trans*-(5*R*,6*R*)-**3** and (−)-*trans*-(5*S*,6*S*)-**4** diastereomers of 5-bromo-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine in 21 and 52% yields, respectively (see Scheme 1). A similar reaction (method A) of AZT with *N*-chlorosuccinimide in methanol in the presence of glacial acetic acid gave the (+)-*trans*-(5*R*,6*R*)-**5** (40%) and (+)-*cis*-(5*S*,6*R*)-**7** (18%) diastereomers of 5-chloro-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine. The reaction of AZT with molecular chlorine in methanol (method B) yielded the (+)-*trans*-(5*R*,6*R*)-**5** (53%), (−)-*trans*-(5*S*,6*S*)-**6** (6.6%), and (+)-*cis*-(5*S*,6*R*)-**7** (32%) diastereomers of 5-chloro-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine. The (+)-*trans*-(5*R*,6*R*)-**8** (28%) and (−)-*trans*-(5*S*,6*S*)-**9** (4.4%) diastereomers of 5-iodo-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine were

synthesized by reaction of AZT with *N*-iodosuccinimide in methanol. Reaction of AZT with bromine in ethanol afforded the (+)-*trans*-(5*R*,6*R*)-**10** and (−)-*trans*-(5*S*,6*S*)-**11** diastereomers of 5-bromo-6-ethoxy-5,6-dihydro-3′-azido-3′-deoxythymidine. In contrast, reaction of AZT with chlorine in ethanol afforded the (+)-*trans*-(5*R*,6*R*)-**12** (61.5%), (−)-*trans*-(5*S*,6*S*)-**13** (3.7%), and (+)-*cis*-(5*S*,6*R*)-**14** (26.7%) diastereomers of 5-chloro-6-ethoxy-5,6-dihydro-3′-azido-3′-deoxythymidine. Similar reactions of AZT with bromine (or chlorine) in 2-propanol, 1-octanol, and 1-hexadecanol yielded the corresponding (+)-*trans*-(5*R*,6*R*)-**15**−**20** diastereomers, respectively. Reaction of AZT with *N*-bromosuccinimide and sodium azide in 1,2-dimethoxyethane at 0 °C afforded the (+)-*trans*-(5*R*,6*R*)-**21**, (−)-*trans*-(5*S*,6*S*)-**22**, and (−)-*cis*-(5*R*,6*S*)-**23** diastereomers of 5-bromo-6-azido-5,6-dihydro-3′-azido-3′-deoxythymidine. In contrast, reaction of AZT with *N*-chlorosuccinimide and sodium azide in 1,2-dimethoxyethane at 25 °C gave a mixture of the (+)-*trans*-(5*R*,6*R*)-**24**, (−)-*trans*-(5*S*,6*S*)-**25**, (+)-*cis*-(5*S*,6*R*)-**26**, and (−)-*cis*-(5*R*,6*S*)-**27** diastereomers of 5-chloro-6-azido-5,6-dihydro-3′-azido-3′-deoxythymidine in 87% yield. These 5-halo-6-alkoxy(or azido)-5,6-dihydro derivatives **3**−**27** of AZT most likely arise via the initial formation of a 5,6-halonium ion intermediate which is susceptible to regiospecific nucleophilic attack by the alcohol or the azide anion, at the sterically less hindered C-6 position. The configuration of the substituents at the C-5 and C-6 positions of compounds **3**−**27** was assigned by comparing their optical rotation and ^{1}H NMR chemical shifts with those of structurally related compounds such as the 5-bromo-6-methoxy(hydroxy)-5,6-dihydrothymidine diastereomers[32,33] for which the absolute configuration is known. The most distinct differences in ^{1}H NMR chemical shift positions for spectra of these diastereomers occur for the H-1′, H-2′, and H-2″ protons in the sugar moiety and the H-6 proton of the base. The 5,6-dihydro compounds (**3**−**27**) are stable products that were separated by silica gel column chromatography and/or preparative thin layer chromatography (PTLC) with the exception of diastereomers **21**−**22** and **24**−**27** which were isolated as mixtures.

Results and Discussion

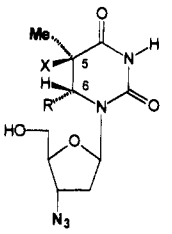
The objective of this study involved the design of 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines (**3**−**27**) as brain-targeted drugs, and/or prodrugs to AZT, for *in vitro* evaluation as anti-HIV agents. The partition coefficients, which serve as an indicator of the ability of a compound to cross the BBB,[34] for the 5-halo-6-methoxy-5,6-dihydro analogs of AZT (**3**−**5**, **7**, **8**−**9**) were all larger (P = 3.3−18.8) than that of AZT (P = 1.29) as summarized in Table 1. In the absence of an active nucleoside transport system,[35] the enhanced lipophilicity of these 5-halo-6-alkoxy-5,6-dihydro analogs, relative to AZT, should increase their ability 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.[36] A parabolic relationship between log P values and brain extractability for a group of ^{11}C-labeled compounds suggests an optimal log P range of 0.9−2.5 for radiopharmaceuticals designed to cross the BBB by virtue of their lipid solubility.[37] Thenature of the halogen atom at the C-5 position (5*S*,6*S*-series, I-**9** ≥ Br-**4**; 5*R*,6*R*-series, Br-**3** > I-**8** > Cl-**5**) and the configuration at the C-5 and C-6 positions (5*S*,6*S*)-I-**9** > (5*R*,6*R*)-I-**8**; (5*S*,6*S*)-Br-**4** > (5*R*,6*R*)-Br-**3**; (5*R*,6*R*)-Cl-**5** > (5*S*,6*R*)-Cl-**7**] were determinants of lipophilicity. Although AZT penetrates into the cerebral spinal fluid (CSF) to provide AZT CSF:plasma ratios ranging from less than 0.1 to 2.05 in patients with AIDS or AIDS-related complex (ARC),[38] a study using a carotid artery injection technique in rats showed that less than 1% of the injected AZT penetrated into the brain.[15] The enhanced lipophilicity of the 5,6-dihydro derivatives of AZT, relative to AZT, may conceivably provide a higher concentation in the brain.

The utility of 5-halo-6-alkoxy(or azido)-5,6-dihydro derivatives of AZT (**3**−**27**) as potential prodrugs to AZT would be dependent upon their rate of bioconversion to AZT, pharmacokinetic properties, and tissue biodistribution. Thymidine phosphorylase is a specific enzyme which cleaves the base−sugar N−C bond present in pyrimidine deoxynucleosides.[39] To be active *in vivo*, the nucleoside drug must not undergo phosphorolysis prior to bioactivation to the triphosphate nucleotide. An *in vitro* phosphorolysis study which involved incubation of the 5-halo-6-alkoxy-5,6-dihydro compounds (**3**−**7**, **8**−**11**) with *Escherichia coli* thymidine phosphorylase for 10 min at 37 °C indicated that these 5,6-dihydro analogs of AZT, like AZT, were completely stable since no phosphorolysis occurred. In contrast, the physiological nucleoside thymidine undergoes 72% phosphorolysis using these experimental conditions (see Table 1).

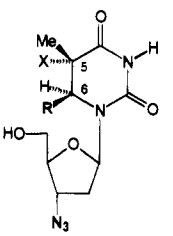
The *in vitro* incubation of the 5,6-dihydro compounds (**3**−**11**) with the model thiol glutathione (GSH) was investigated (substrate:GSH ratio = 1:2 for a 30 min incubation at 37 °C) to determine the ability of GSH to regenerate the 5,6-olefinic bond present in AZT. In mammalian tissues, the GSH concentration is in the 0.5−1 mM range, whereas the cysteine concentration is in the 0.03−0.1 mM range.[40,41] It is possible that dehalogenation and elimination to generate AZT *in vivo* 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 AZT, 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 (**3**−**9**) where the relative 5,6-olefinic bond regeneration order was I (100%) > Br (77−80%) > Cl (0%) (see Table 1). The reaction of the 5,6-dihydro analogs of AZT with GSH (R-SH) could occur by two mechanisms (see Figure 1). Nucleophilic attack by R-SH on the C-5 halo substituent (X) would give the carbanion or enolate anion (**ii**) (E2 Hal mechanism, pathway A) Alternatively, a S_N2 displacement of X by R-SH to give **iii** (S_N2 mechanism, pathway B) and a subsequent reaction with GSH would also yield the carbanion (**ii**). Elimination of the alkoxide, or azide, anion from the carbanion intermediate (**ii**) would regenerate the 5,6-olefinic bond to afford AZT. Similar mechanisms have been proposed for the dehalogenation of 5-bromo-6-methoxy-5,6-dihydrothymine by cysteine.[42] Nucleophilic attack on halogen at C-5 and sulfur attack at sulfur bonded to C-5 in 5,6-dihydrouracils have been reported previously.[41,43,44]

There are alternate mechanisms, other than GSH, which could regenerate AZT from the 5,6-dihydro ana logs of AZT. The *in vitro* incubation of the 5-halo-6-

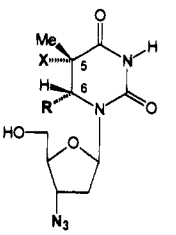
Table 1. Partition Coefficients (*P*), *in Vitro* Incubation Studies with Thymidine Phosphorylase, Glutathione, Mouse Blood, and Mouse Liver Homogenate, and *in Vitro* Anti-HIV Activity and Selectivity[a] of 5-Halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines in HIV-1-Infected CEM Cells



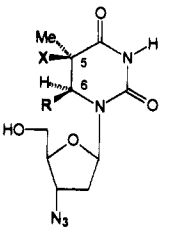
3,5,8,10,12,15-21,24
(+)-*trans*-(5R,6R)



4,6,9,11,13,22,25
(-)-*trans*-(5S,6S)



7,14,26
(+)-*cis*-(5S,6R)



27
(-)-*cis*-(5R,6S)

						regeneration of 5,6-olefinic bond (% AZT)					
no.	X	R	configuration	*P*[b]	% phosphorolysis[c]	glutathione[d]	mouse blood[e]	mouse liver homogenate[f]	IC_{50} (M)[g]	EC_{50} (M)[h]	TI[i]
3	Br	OMe	5*R*,6*R*	13.2	0	80	59	26	1.72×10^{-5}	3.27×10^{-9}	5260
4	Br	OMe	5*S*,6*S*	16.7	0	77	88	44	4.25×10^{-5}	2.80×10^{-7}	152
5	Cl	OMe	5*R*,6*R*	7.6	0	0[k]	0	0	ND[j]	ND	ND
6	Cl	OMe	5*S*,6*S*	ND	0	0[k]	0	0	ND	ND	ND
7	Cl	OMe	5*S*,6*R*	3.3	0	0[k]	0	0	ND	ND	ND
5, 7[l]	Cl	OMe	5*R*,6*R*; 5*S*,6*R*	ND	ND	ND	ND	ND	$>8.9 \times 10^{-4}$	5.79×10^{-6}	155
8	I	OMe	5*R*,6*R*	10.6	0	100	100	79	1.87×10^{-5}	3.17×10^{-9}	5899
9	I	OMe	5*S*,6*S*	18.8	0	100	100	96	6.42×10^{-6}	5.15×10^{-9}	1246
10	Br	OEt	5*R*,6*R*	ND	0	100[m]	ND	ND	1.85×10^{-5}	6.75×10^{-9}	2741
11	Br	OEt	5*S*,6*S*	ND	0	85[m]	ND	ND	2.22×10^{-5}	2.37×10^{-8}	937
12	Cl	OEt	5*R*,6*R*	ND	ND	ND	ND	ND	$>2.0 \times 10^{-4}$	inactive	ND
13	Cl	OEt	5*S*,6*S*	ND	ND	ND	ND	ND	$>2.0 \times 10^{-4}$	inactive	ND
14	Cl	OEt	5*S*,6*R*	ND	ND	ND	ND	ND	$>2.0 \times 10^{-4}$	inactive	ND
15	Br	$OCH(Me)_2$	5*R*,6*R*	ND	ND	ND	ND	ND	$>2.0 \times 10^{-4}$	5.72×10^{-6}	35
16	Cl	$OCH(Me)_2$	5*R*,6*R*	ND	ND	ND	ND	ND	$>2.0 \times 10^{-4}$	inactive	ND
17	Br	$O(CH_2)_7Me$	5*R*,6*R*	ND	ND	ND	ND	ND	1.24×10^{-5}	8.65×10^{-7}	14
18	Cl	$O(CH_2)_7Me$	5*R*,6*R*	ND	ND	ND	ND	ND	3.75×10^{-5}	inactive	ND
19	Br	$O(CH_2)_{15}Me$	5*R*,6*R*	ND	ND	ND	ND	ND	1.10×10^{-5}	inactive	ND
20	Cl	$O(CH_2)_{15}Me$	5*R*,6*R*	ND	ND	ND	ND	ND	3.10×10^{-5}	inactive	ND
21, 22[l]	Br	N_3	5*R*,6*R*; 5*S*,6*S*	ND	ND	ND	ND	ND	1.76×10^{-4}	2.0×10^{-4}	<1
24–27[l]	Cl	N_3		ND	ND	ND	ND	ND	3.5×10^{-4}	1.49×10^{-6}	235
AZT				1.29	0	–	–	–	5×10^{-4}	3×10^{-9}	>10000
thymidine				0.06[n]	72	–	–	–	–	–	–

[a] Testing was performed by the National Cancer Institute's Developmental Therapeutic Program, AIDS antiviral screening program. All of the data listed were compared to the corresponding test results for AZT which served as the treated control, performed at the same time. [b] *P* = concentration in 1-octanol/concentration in water; *n* = 2. [c] The percent of phosphorolysis upon incubation of the test compound with *E. coli* thymidine phosphorylase for 10 min at 37 °C. [d] The percent of 3′-azido-3′-deoxythymidine (AZT) formed upon incubation of the test compound with glutathione using a test compound:glutathione mole ratio of 1:2 for 30 min at 37 °C. [e] The percent of 3′-azido-3′-deoxythymidine formed upon incubation of the test compound with mouse blood for 10 min at 37 °C. [f] The percent of 3′-azido-3′-deoxythymidine formed upon incubation of the test compound with mouse liver homogenate for 30 min at 37 °C. [g] The IC_{50} value is the test drug concentration which results in a 50% survival of uninfected untreated control cells (e.g., cytotoxic activity of the test drug). [h] The EC_{50} value is the test drug concentration which produces a 50% survival of HIV-1-infected cells relative to uninfected untreated controls (e.g., *in vitro* anti-HIV activity). [i] TI = therapeutic index (IC_{50}/EC_{50}). [j] ND = not determined. [k] No AZT was detected for an incubation time for 24 h. [l] Tested as a mixture of diastereomers. [m] Substrate:glutathione mole ratio was 1:5. [n] The *P* value was taken from ref 35.

methoxy-5,6-dihydro compounds **3**–**9** with mouse blood and mouse liver homogenate was therefore investigated to determine the potential utility of 5,6-dihydro-AZT compounds as prodrugs. Thus, incubation of **3**–**9** with mouse blood for 10 min at 37 °C resulted in 100% conversion to AZT (5-iodo-6-methoxy analogs **8** and **9**), 59% 5*R*,6*R* and 88% 5*S*,6*S* conversion for the 5-bromo-6-methoxy analogs **3** and **4**, and 0% conversion to AZT for the (5*R*,6*R*)-**5**, (5*S*,6*S*)-**6**, and (5*S*,6*R*)-**7** 5-chloro-6-methoxy-5,6-dihydro compounds (see Table 1). A similar profile for regeneration of the 5,6-olefinic bond was observed upon incubation with mouse liver homogenate for 30 min at 37 °C where the extent of conversion to AZT was 79–96% for the 5-iodo-6-methoxy compounds **8** and **9**, 26–44% for the 5-bromo-6-methoxy compounds **3** and **4**, and 0% for the 5-chloro-6-methoxy diastereomers **5**–**7**.

A number of 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines (**3**–**5**, **7**–**22**, **24**–**27**) were evaluated by the U.S. National Cancer Institute Antiviral Evaluations Branch in an *in vitro* anti-HIV screen using HIV-1-infected T4 lymphocytes (CEM cell line), and the results are summarized in Table 1. Agents that interact with virions, cells, or virus gene products to interfere with viral activities will protect cells from cytolysis and the killing of T4 lymphocytes by the HIV-1 virus.[45] The C-5 substituent (I, Br, Cl) was a determinant of anti-HIV-1 activity where the potency order was I ≥ Br > Cl [(5*R*,6*R*)-5-I-6-OMe-**8** ≃ (5*R*,6*R*)-5-Br-6-OMe-**3** and (5*S*,6*S*)-5-I-6-OMe-**9** > (5*S*,6*S*)-5-Br-6-OMe-**4** > (5*R*,6*R*)-5-Cl-6-OMe-**5** and (5*S*,6*S*)-5-Cl-6-OMe-**7**; (5*R*,6*R*)-5-Br-6-OEt-**10** > (5*S*,6*S*)-5-Br-6-OEt-**11** ≫ (5*R*,6*R*)-5-Cl-6-OEt-**12** and (5*S*,6*S*)-5-Cl-6-OEt-**13**; (5*R*,6*R*)-5-Br-6-*i*-PrO-**15** > (5*R*,6*R*)-5-Cl-6-*i*-PrO-**16**;

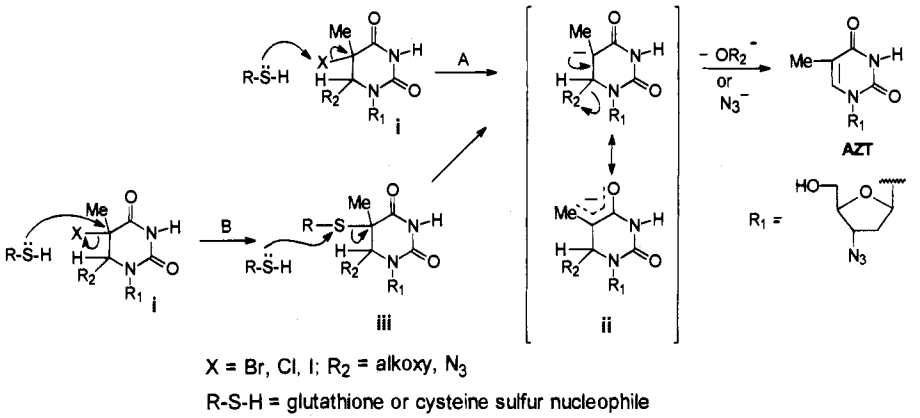


Figure 1. Proposed mechanisms for the conversion of 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines to AZT: pathway A (E2 Hal mechanism), elimination under the influence of a nucleophile, and pathway B (S_N2 mechanism), nucleophilic substitution of X by R-SH followed by elimination of R-S under the influence of a second nucleophile.

(5*R*,6*R*)-5-Br-6-(1-octyloxy)-**17** > (5*R*,6*R*)-5-Cl-6-(1-octyloxy)-**18**]. In the 5-bromo series of compounds, the C-6 substituent was also a determinant of activity where 6-OMe and 6-OEt substituents exhibited a greater potency than the corresponding 6-*i*-PrO, 6-(1-octyloxy), 6-(1-hexadecyloxy), and 6-azido analogs [(5*R*,6*R*)-5-Br-6-OMe-**3**, (5*S*,6*S*)-5-Br-6-OMe-**4**, (5*R*,6*S*)-5-Br-6-OEt-**10**, and (5*S*,6*S*)-5-Br-6-OEt-**11** > (5*R*,6*R*)-5-Br-6-*i*-PrO-**15**, (5*R*,6*R*)-5-Br-6-(1-octyloxy)-**17**, (5*R*,6*R*)-5-Br-6-(1-hexadecyloxy)-**19**, and the 5-Br-6-azido diastereomeric mixture of **21** and **22**]. All of the 5-chloro-6-substituted compounds (**12**–**14**, **16**, **18**, **20**) were inactive, except for the approximately equipotent 6-OMe (**5**, **7**) and 6-azido (**24**–**27**) diastereomeric mixtures which were 2–3 log units less active than the reference drug AZT. In addition, the configuration at the C-5 and C-6 positions also influenced potency where the activity of the 5*R*,6*R*-diastereomer was generally greater than that of the corresponding 5*S*,6*S*-diastereomer [(5*R*,6*R*)-5-Br-6-OMe-**3** > (5*S*,6*S*)-5-Br-6-OMe-**4**; (5*R*,6*R*)-5-I-6-OMe-**8** ≥ (5*S*,6*S*)-5-I-6-OMe-**9**; (5*R*,6*R*)-5-Br-6-OEt-**10** > (5*S*,6*S*)-5-Br-6-OMe-**11**].

The most potent anti-HIV-1 agents, (5*R*,6*R*)-5-Br-6-OMe-**3**, (5*R*,6*R*)-5-I-6-OMe-**8**, (5*S*,6*S*)-5-I-6-OMe-**9**, and (5*R*,6*R*)-5-Br-6-OEt-**10**, were equipotent to the reference drug AZT. The anti-HIV-1 activity exhibited by **3** and **8**–**10**, in view of their similar potency, may be related to their conversion (regeneration of the 5,6-olefinic bond) in the culture media and/or intracellular bioconversion to AZT. This postulate is based on the *in vitro* incubation results described and the following observations. Incubation of the 5-bromo-5-fluoro-6-methoxy-5,6-dihydro, but not the 5-chloro-5-fluoro-6-methoxy-5,6-dihydro, analogs of 5-fluoro-2′-deoxyuridine (FUDR) revert, in part, to FUDR when incubated with reduced glutathione at pH 7 and 35 °C.[29] A 2 h incubation of (5*R*,6*R*)-5-bromo-5-ethyl-6-ethoxy-5,6-dihydro-2′-deoxyuridine (BEEDU) with rat whole blood or brain homogenate gave 5-ethyl-2′-deoxyuridine (EDU) in 53 and 16% yields, respectively.[31] The lower anti-HIV-1 activity of the 5-chloro-6-alkoxy-5,6-dihydro compounds investigated, relative to the corresponding 5-iodo and 5-bromo analogs, may also be due to the fact that 5-chloro compounds do not undergo, or undergo a low rate of, 5,6-olefinic bond regeneration to give AZT under the *in vitro* assay conditions. It was not determined whether the 5-chloro-6-alkoxy (other than methoxy)-5,6-dihydro analogs regenerate AZT. The observation that these latter derivatives either are inactive or exhibit weak anti-HIV activity suggests that they are not converted to AZT under the *in vitro* assay conditions and therefore are not acting as prodrugs. This postulate is consistent with the results of an earlier study which showed that the 5*R*,6*R*- and 5*S*,6*S*-diastereomers of 5-bromo-5-ethyl-6-methoxy-5,6-dihydro-2′-deoxyuridine gave a higher concentration of EDU at 15 min in rat blood (iv dose), relative to the corresponding 5-chloro-5-ethyl-6-methoxy-5,6-dihydro-2′-deoxyuridine diastereomers.[30] It is anticipated that these 5-iodo(bromo)-6-methoxy(ethoxy)-5,6-dihydro derivatives of AZT will serve as prodrugs providing a rapid release of AZT *in vivo*, due to enzymatic and/or chemical conversion to AZT. These 5,6-dihydro derivatives of AZT, which are much more lipophilic than AZT, may be more useful brain-targeted prodrugs compared to AZT to treat HIV infection of the central nervous system. Precedence for this claim comes from the observation that the accumulation of radioactivity in mouse brain after iv administration of [4-^{14}C]BEEDU was significantly higher than that after administration of [4-^{14}C]EDU.[31]

Summary

A new class of 5-halo-6-alkoxy(or azido)-5,6-dihydro-3′-azido-3′-deoxythymidines (**3**–**27**) have been designed. Their enhanced lipophilicity (P = 3.3–18.8 range), relative to the parent compound AZT (P = 1.29), may enhance their ability to cross the BBB to provide a higher concentration of active drug in the brain. Compounds possessing a 5-bromo or 5-iodo substituent may serve as prodrugs to AZT due to regeneration of the 5,6-olefinic bond. It may be posssible to control the rate of AZT release, *in vivo*, which would influence the blood half-life and toxicity, by selecting the appropriate combination of C-5 and C-6 substituents. The 5-halo-6-alkoxy(or azido)-5,6-dihydro compounds described, some of which are equipotent to AZT, could serve as useful lead compounds for the development of a drug (5-chloro analogs), or a prodrug to AZT (5-iodo or 5-bromo analogs), that possesses superior pharmacokinetic and biodistriubtion (enhanced brain and decreased bone uptake) properties.

Experimental Section

Melting points were determined with a Buchi capillary apparatus and are uncorrected. Nuclear magnetic resonance

spectra (^{1}H NMR, ^{13}C NMR) were determined on a Bruker AM-300 spectrometer. Chemical shifts are given in ppm downfield from tetramethylsilane as an internal standard (^{1}H NMR). 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 on an Optical Activity Ltd. digital polarimeter. Quantitative UV measurements (*P* values) were recorded using a Shimadzu UV 160 spectrophotometer. Quantitative HPLC analyses were performed using a Waters HPLC system comprised of model 501 pumps, model U6K injector, and model 486 variable wavelength detector using a C_{18} Radial Pak cartridge (8 mm i.d. × 10 cm length, 10 μm particle size). 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 thickness). Silica gel column chromatography was carried out using Merck 7734 (60–200 mesh) silica gel. 1-Octanol (99%, distilled prior to use) and glutathione (reduced, 98%) were purchased from the Aldrich Chemical Co. Thymidine and *E. coli* thymidine phosphorylase (1200 units/mL in 0.5 M potassium phosphate buffer containing 2 mM uracil, 0.02% sodium azide, and bovine serum albumin) were purchased from the Sigma Chemical Co. A Tris buffer solution (10 mM Tris·HCl, pH 7.3) was prepared by dissolving the Tris base (Trizma, 0.141 g) and $Na_2EDTA{\cdot}2H_2O$ (74.4 mg) in distilled water (90 mL) prior to adjustment of the pH to 7.3 using 10 N HCl and dilution to a 100 mL volume using distilled water. Microanalysis were within ±0.4% of theoretical values for all elements listed, unless otherwise indicated. 3′-Azido-3′-deoxythymidine (1, AZT) was prepared using the literature procedure.[9] **Warning:** Halogenated solvents such as dichloromethane must not be used in certain reactions, such as those described for the preparation of products **21**–**27**, since reaction with sodium azide may produce potentially explosive poly(azidomethane).

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (3) and (−)-*trans*-(5*S*,6*S*)-5-Bromo-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (4). A freshly prepared solution of methyl hypobromite (bromine in methanol) was added dropwise to a solution of AZT (0.2 g, 0.75 mmol) in methanol (10 mL) at 25 °C with stirring until the light yellow color of the reaction mixture persisted. The reaction was allowed to proceed at 25 °C for 20 min prior to neutralization to pH 6 using a solution of 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 (95:5, v/v) afforded a mixture of diastereomers **3** and **4** (0.225 g, 79%) as a viscous oil. The two diastereomers **3** and **4**, were separated on preparative silica gel chromatography using chloroform-methanol (95:5, v/v) as development solvent.

Diastereomer 3: $[\alpha]^{25}_D$ = +71.7° (*c* 0.30, MeOH); R_f 0.61 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (60 mg, 21%); ^{1}H NMR ($CDCl_3$) δ 1.96 (s, 3H, C*H*$_3$), 2.32 (ddd, $J_{2',2''}$ = 14.0, $J_{1',2''}$ = 6.6, $J_{2'',3'}$ = 3.3 Hz, 1H, H-2″), 2.68 (quintet, $J_{2',2''}$ = 14.0, $J_{2',3'}$ = 6.6, $J_{1',2'}$ = 6.6 Hz, 1H, H-2′), 3.46 (s, 3H, OC*H*$_3$), 3.76–3.84 (m, 1H, H-5′), 3.90–3.97 (m, 2H, H-4′, H-5″), 4.34 (ddd, $J_{3',4'}$ = 3.0, $J_{2',3'}$ = 6.6, $J_{2'',3'}$ = 3.3 Hz, 1H, H-3′), 4.95 (s, 1H, H-6), 5.90 (t, $J_{1',2'}$ = $J_{1',2''}$ = 6.6 Hz, 1H, H-1′), 8.64 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 22.82 (*C*H$_3$), 37.04 (C-2′), 53.21 (C-5), 57.41 (O*C*H$_3$), 60.06 (C-3′), 62.12 (C-5′), 84.02 (C-4′), 86.66 (C-1′), 89.16 (C-6), 150.58 (C-2 *C*=O), 167.10 (C-4 *C*=O). Anal. ($C_{11}H_{16}BrN_5O_5{\cdot}^1/_2H_2O$) C, H, N.

Diastereomer 4: $[\alpha]^{25}_D$ = −43.3° (*c* 0.21, MeOH); R_f 0.63 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (148 mg, 52%); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H, C*H*$_3$), 2.26 (ddd, $J_{2',2''}$ = 14.4, $J_{1',2''}$ = 7.2, $J_{2'',3'}$ = 3.0 Hz, 1H, H-2″), 2.95 (quintet, $J_{2',2''}$ = 14.4, $J_{2',3'}$ = 7.2, $J_{1',2'}$ = 7.2 Hz, 1H, H-2′), 3.60 (s, 3H, OC*H*$_3$), 3.70–3.80 (m, 1H, H-5′), 3.90–4.0 (m, 1H, H-5″), 4.01–4.03 (m, 1H, H-4′), 4.52 (ddd, $J_{3',4'}$ = 3.0, $J_{2',3'}$ = 7.2, $J_{2'',3'}$ = 3.0 Hz, 1H, H-3′), 4.59 (s, 1H, H-6), 5.27 (t, $J_{1',2'}$ = $J_{1',2''}$ = 7.2 Hz, 1H, H-1′), 8.53 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 22.66 (*C*H$_3$), 35.01 (C-2′), 53.34 (C-5), 57.15 (O*C*H$_3$), 61.48 (C-3′), 62.86 (C-5′), 85.05 (C-4′), 92.56 (C-1′), 95.27 (C-6), 150.51 (C-2 *C*=O), 166.83 (C-4 *C*=O). Anal. ($C_{11}H_{16}BrN_5O_5$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Chloro-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (5), (−)-*trans*-(5*S*,6*S*)-5-Chloro-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (6), and (+)-*cis*-(5*S*,6*R*)-5-Chloro-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (7). Method A (5, 7). *N*-Chlorosuccinimide (0.2 g, 1.5 mmol) was added to a solution of AZT (0.2 g, 0.75 mmol) in methanol (10 mL) and glacial acetic acid (0.6 mL) with stirring, and the reaction was allowed to proceed at 25 °C for 15 h. At this time, additional *N*-chlorosuccinimide (0.2 g, 1.5 mmol) and glacial acetic acid (0.6 mL), were added and the reaction was allowed to proceed at 25 °C for 24 h with stirring prior to neutralization to pH 6.5 using a solution of methanolic sodium hydroxide. Removal of the solvent in vacuo gave a residue which was dissolved in chloroform (5 mL), the chloroform solution was washed with cold water (2 × 5 mL) and dried (Na_2SO_4), and the solvent was removed in vacuo. The residue obtained was purified by elution from a silica gel column using chloroform–methanol (95:5, v/v) as eluent to yield a mixture of diastereomers **5** and **7**. The two diastereomers, **5** and **7**, were separated by preparative silica gel chromatography using chloroform–methanol (95:5, v/v) as development solvent.

Diastereomer 5: $[\alpha]^{25}_D$ = +74.7° (*c* 0.38, MeOH); R_f 0.57 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (0.1 g, 40%); ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H, C*H*$_3$), 2.30 (ddd, $J_{2',2''}$ = 13.8, $J_{1',2''}$ = 6.6, $J_{2'',3'}$ = 3.0 Hz, 1H, H-2″), 2.63 (ddd, $J_{2',2''}$ = 13.8, $J_{2',3'}$ = 6.0, $J_{1',2'}$ = 6.6 Hz, 1H, H-2′), 3.46 (s, 3H, OC*H*$_3$), 3.88–3.98 (m, 1H, H-5′), 3.90–4.0 (m, 2H, H-4′, H-5″), 4.30–4.38 (m, 1H, H-3′), 4.90 (s, 1H, H-6), 5.92 (t, $J_{1',2'}$ = 6.6, $J_{1',2''}$ = 6.0 Hz, 1H, H-1′), 8.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 21.60 (*C*H$_3$), 36.95 (C-2′), 57.36 (O*C*H$_3$), 60.04 (C-3′), 60.88 (C-5), 62.05 (C-5′), 83.95 (C-4′), 86.39 (C-1′), 88.62 (C-6), 150.66 (C-2 *C*=O), 166.71 (C-4 *C*=O). Anal. ($C_{11}H_{16}ClN_5O_5$) C, H, N.

Diastereomer 7: $[\alpha]^{25}_D$ = +39.3° (*c* 0.59, MeOH); R_f 0.54 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (45 mg, 18%); ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3H, C*H*$_3$), 2.33 (ddd, $J_{2',2''}$ = 14.4, $J_{1',2''}$ = 6.9, $J_{2'',3'}$ = 3.0 Hz, 1H, H-2″), 2.76 (quintet, $J_{2',2''}$ = 14.4, $J_{2',3'}$ = 6.9, $J_{1',2'}$ = 6.9 Hz, 1H, H-2′), 3.56 (s, 3H, OC*H*$_3$), 3.76–3.85 (m, 1H, H-5′), 3.92–4.03 (m, 1H, H-4′, H-5″), 4.38–4.44 (m, 1H, H-3′), 4.76 (s, 1H, H-6), 5.78 (t, $J_{1',2'}$ = 6.9, $J_{1',2''}$ = 6.9 Hz, 1H, H-1′), 8.28 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 26.05 (*C*H$_3$), 37.0 (C-2′), 58.23 (O*C*H$_3$), 60.35 (C-3′), 62.34 (C-5′), 66.88 (C-5), 84.25 (C-4′), 88.18 (C-1′), 91.46 (C-6), 150.57 (C-2 *C*=O), 167.02 (C-4 *C*=O). Anal. ($C_{11}H_{16}ClN_5O_5$) C, H, N.

Method B (5–7). Chlorine gas was bubbled slowly into a suspension of AZT (0.2 g, 0.75 mmol) in methanol (10 mL) at 25 °C with stirring until the pale yellow color of the solution persisted. The pH of the solution was adjusted to 6.5 using a solution of methanolic sodium hydroxide, and the mixture was filtered. Removal of the solvent from the filtrate in vacuo and separation of the residue obtained by elution from a silica gel column using chloroform–methanol (95:5, v/v) as eluent afforded **5** (53%), **6** (6.6%), and **7** (32%), respectively. Products **5** and **7** were identical (^{1}H NMR) with the same products prepared by method A.

Diastereomer 6: $[\alpha]^{25}_D$ = −25° (*c* 0.32, MeOH); R_f 0.60 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (16.5 g, 6.6%); ^{1}H NMR ($CDCl_3$) δ 1.80 (s, 3H, C*H*$_3$), 2.22 (ddd, $J_{2',2''}$ = 13.2, $J_{1',2''}$ = 6.6, $J_{2'',3'}$ = 3.0 Hz, 1H, H-2″), 2.90 (quintet, $J_{2',2''}$ = 13.2, $J_{2',3'}$ = $J_{1',2'}$ = 6.6 Hz, 1H, H-2′), 3.54 (s, 3H, OC*H*$_3$), 3.72–4.0 (m, 3H, H-4′, H-5′), 4.28–4.30 (m, 1H, H-3′), 4.46 (s, 1H, H-6), 5.26 (t, $J_{1',2'}$ = $J_{1',2''}$ = 6.6 Hz, 1H, H-1′), 9.0 (s, 1H, NH). Anal. ($C_{11}H_{16}ClN_5O_5{\cdot}^1/_2H_2O$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Iodo-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (8) and (−)-*trans*-(5*S*,6*S*)-5-Iodo-6-methoxy-5,6-dihydro-3′-azido-3′-deoxythymidine (9). *N*-Iodosuccinimide (0.17 g, 0.75 mmol) was added slowly with stirring to a solution of AZT (0.2 g, 0.75 mmol) in methanol (10 mL) and glacial acetic acid (0.2 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

product was purified by elution from a silica gel column using chloroform–methanol (95:5, v/v) as eluent to yield a mixture of diastereomers **8** and **9**. The two diastereomers, **8** and **9**, were separated by preparative silica gel chromatography using chloroform–methanol (90:10, v/v) as development solvent.

Diastereomer 8: $[\alpha]^{25}_D = +87.3°$ (*c* 0.55, MeOH); R_f 0.57 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (90 mg, 28%); ^1H NMR ($CDCl_3$) δ 2.18 (s, 3H, C*H*$_3$), 2.40 (ddd, $J_{2',2''} = 13.6$, $J_{1',2''} = 6.8$, $J_{2'',3'} = 3.3$ Hz, 1H, H-2''), 2.76 (quintet, $J_{2',2''} = 13.6$, $J_{2',3'} = J_{1',2'} = 6.8$ Hz, 1H, H-2'), 3.46 (s, 3H, OC*H*$_3$), 3.80–3.88 (m, 1H, H-5'), 3.92–4.02 (m, 2H, H-4', H-5''), 4.40 (ddd, $J_{2',3'} = 6.8$, $J_{2'',3'} = 3.3$, $J_{3',4'} = 3.0$ Hz, 1H, H-3'), 5.02 (s, 1H, H-6), 5.88 (t, $J_{1',2'} = J_{1',2''} = 6.8$ Hz, 1H, H-1'), 8.58 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 25.63 (*C*H$_3$), 34.46 (C-5), 37.07 (C-2'), 57.35 (O*C*H$_3$), 60.09 (C-3'), 62.07 (C-5'), 84.04 (C-4'), 86.74 (C-1'), 90.84 (C-6), 150.84 (C-2 *C*=O), 169.31 (C-4 *C*=O). Anal. ($C_{11}H_{16}IN_5O_5$) C, H, N.

Diastereomer 9: $[\alpha]^{25}_D = -46.2°$ (*c* 0.37, MeOH); R_f 0.63 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (14 mg, 4.4%); ^1H NMR ($CDCl_3$) δ 2.18 (s, 3H, C*H*$_3$), 2.34 (ddd, $J_{2',2''} = 14.4$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 3.02 (quintet, $J_{2',2''} = 14.4$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.61 (s, 3H, OC*H*$_3$), 3.70–4.04 (m, 3H, H-4', H-5'), 4.54 (ddd, $J_{2',3'} = 7.2$, $J_{2'',3'} = 3.0$, $J_{3',4'} = 3.0$ Hz, 1H, H-3'), 4.65 (s, 1H, H-6), 5.28 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 7.90 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 25.56 (*C*H$_3$), 34.67 (C-5), 35.15 (C-2'), 57.17 (O*C*H$_3$), 61.59 (C-3'), 63.01 (C-5'), 85.14 (C-4'), 92.78 (C-1'), 97.18 (C-6), 150.38 (C-2 *C*=O). Anal. ($C_{11}H_{16}IN_5O_5$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-ethoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (10) and (−)-*trans*-(5*S*,6*S*)-5-Bromo-6-ethoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (11). A freshly prepared solution of ethyl hypobromite was added dropwise to a solution of AZT (0.9 g, 3.3 mmol) in ethanol (50 mL) at 0 °C with stirring until the light yellow color of the reaction mixture persisted. The reaction was allowed to proceed for 5 h at 0 °C prior to neutralization to pH 6.5 with ethanolic 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 (97:3, v/v) yielded 11 (0.25 g, 19%) as a viscous oil: $[\alpha]^{25}_D = -37.4°$ (*c* 0.35, MeOH); R_f 0.75 ($CHCl_3$–MeOH, 9:1, v/v); ^1H NMR ($CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.97 (s, 3H, C*H*$_3$), 2.22 (ddd, $J_{2',2''} = 14.0$, $J_{1',2''} = 7.0$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.93 (quintet, $J_{2',2''} = 14.0$, $J_{2',3'} = J_{1',2'} = 7.0$ Hz, 1H, H-2'), 3.56 (m, $J_{gem} = 15.0$, $J_{vic} = 7.5$ Hz, 1H, OCH'*H*''CH$_3$), 3.68–4.04 (m, 4H, H-4', H-5', OC*H*'H''CH$_3$), 4.48 (ddd, $J_{2',3'} = 7.0$, $J_{2'',3'} = J_{3',4'} = 3.0$ Hz, 1H, H-3'), 4.52 (s, 1H, H-6), 5.22 (t, $J_{1',2'} = 7.0$, $J_{1',2''} = 7.0$ Hz, 1H, H-1'), 8.04 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.78 (OCH$_2$*C*H$_3$), 22.75 (*C*H$_3$), 34.94 (C-2'), 53.51 (C-5), 61.57 (C-3'), 62.99 (C-5'), 65.56 (O*C*H$_2$CH$_3$), 85.14 (C-4'), 92.84 (C-1'), 94.16 (C-6), 150.50 (C-2 *C*=O), 166.82 (C-4 *C*=O). Anal. ($C_{12}H_{18}BrN_5O_5$) C, H, N.

Further elution with the same solvent yielded **10** (0.1 g, 75%) as a foam: mp 123–5 °C; $[\alpha]^{25}_D = +76.6°$ (*c* 0.37, MeOH); R_f 0.68 ($CHCl_3$–MeOH, 9:1, v/v); ^1H NMR ($CDCl_3$) δ 1.12 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.92 (s, 3H, C*H*$_3$), 2.30 (ddd, $J_{2',2''} = 14.0$, $J_{1',2''} = 7.0$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.64 (quintet, $J_{2',2''} = 14.0$, $J_{2',3'} = J_{1',2'} = 7.0$ Hz, 1H, H-2'), 3.54 (m, $J_{gem} = 15.0$, $J_{vic} = 7.5$ Hz, 1H, OCH'*H*''CH$_3$), 3.72–3.95 (m, 4H, H-4', H-5', OC*H*'H''CH$_3$), 4.28–4.35 (m, 1H, H-3'), 4.98 (s, 1H, H-6), 5.82 (t, $J_{1',2'} = J_{1',2''} = 7.0$ Hz, 1H, H-1'), 8.12 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.91 (OCH$_2$*C*H$_3$), 22.80 (*C*H$_3$), 36.96 (C-2'), 53.30 (C-5), 60.04 (C-3'), 61.98 (C-5'), 65.70 (O*C*H$_2$CH$_3$), 83.97 (C-4'), 86.55 (C-1'), 87.69 (C-6), 150.89 (C-2 *C*=O), 167.54 (C-4 *C*=O). Anal. ($C_{12}H_{18}BrN_5O_5$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Chloro-6-ethoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (12), (−)-*trans*-(5*S*,6*S*)-5-Chloro-6-ethoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (13), and (+)-*cis*-(5*S*,6*R*)-5-Chloro-6-methoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (14). Chlorine gas was bubbled slowly into a suspension of AZT (1.0 g, 3.74 mmol) in 98% ethanol (50 mL) at 0 °C with stirring until the light yellow-green color of the resulting solution persisted. The pH of the solution was adjusted to 6.5 using a solution of sodium hydroxide in ethanol, and the mixture was filtered. Removal of the solvent from the filtrate in vacuo and separation of the residue obtained by elution from a silica gel column using chloroform–methanol (97:3, v/v) as eluent afforded **12**–**14**, respectively.

Diastereomer 12: $[\alpha]^{25}_D = +63.0°$ (*c* 1.9, MeOH); R_f 0.67 ($CHCl_3$–MeOH, 9:1, v/v); mp 118–20 °C; yield (0.8 g, 61.5%); ^1H NMR ($CDCl_3$) δ 1.16 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.82 (s, 3H, C-5 C*H*$_3$), 2.32 (ddd, $J_{2',2''} = 13.6$, $J_{1',2''} = 6.8$, $J_{2'',3'} = 3.3$ Hz, 1H, H-2''), 2.66 (ddd, $J_{2',2''} = 13.6$, $J_{2',3'} = 6.6$, $J_{1',2'} = 6.8$ Hz, 1H, H-2'), 3.56 (m, $J_{gem} = 15.0$, $J_{vic} = 7.5$ Hz, 1H, OCH'*H*''CH$_3$), 3.75–3.98 (m, 4H, H-4', H-5', OC*H*'H''CH$_3$), 4.30–4.38 (m, 1H, H-3'), 4.92 (s, 1H, H-6), 5.84 (t, $J_{1',2'} = J_{1',2''} = 6.8$ Hz, 1H, H-1'), 8.30 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.93 (OCH$_2$*C*H$_3$), 21.76 (C-5 *C*H$_3$), 37.04 (C-2'), 60.10 (C-3'), 60.94 (C-5), 62.20 (C-5'), 65.55 (O*C*H$_2$CH$_3$), 84.01 (C-4'), 87.04 (C-1'), 87.92 (C-6), 150.62 (C-2 *C*=O), 166.62 (C-4 *C*=O). Anal. ($C_{12}H_{18}ClN_5O_5$) C, H, N.

Diastereomer 13: $[\alpha]^{25}_D = -15.3°$ (*c* 2.8, MeOH); R_f 0.72 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (0.05 g, 3.7%); ^1H NMR ($CDCl_3$) δ 1.10 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.68 (s, 3H, C-5 C*H*$_3$), 2.10 (ddd, $J_{2',2''} = 13.2$, $J_{1',2''} = 6.6$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.78 (quintet, $J_{2',2''} = 13.2$, $J_{2',3'} = J_{1',2'} = 6.6$ Hz, 1H, H-2'), 3.50 (m, $J_{gem} = 15.0$, $J_{vic} = 7.5$ Hz, 1H, OCH'*H*''CH$_3$), 3.60–3.92 (m, 4H, H-4', H-5', OC*H*'H''CH$_3$), 4.36 (ddd, $J_{2',3'} = 6.6$, $J_{2'',3'} = J_{3',4'} = 3.0$ Hz, 1H, H-3'), 4.48 (s, 1H, H-6), 5.18 (t, $J_{1',2'} = J_{1',2''} = 6.6$ Hz, 1H, H-1'), 9.04 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.72 (OCH$_2$*C*H$_3$), 21.58 (C-5 *C*H$_3$), 34.94 (C-2'), 61.09 (C-5), 61.56 (C-3'), 62.96 (C-5'), 65.50 (O*C*H$_2$CH$_3$), 85.11 (C-4'), 92.78 (C-1'), 93.86 (C-6), 150.49 (C-2 *C*=O), 166.25 (C-4 *C*=O). Anal. ($C_{12}H_{18}ClN_5O_5$) C, H, N.

Diastereomer 14: $[\alpha]^{25}_D = +42.1°$ (*c* 0.9, MeOH); R_f 0.61 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (0.35 g, 26.7%); ^1H NMR ($CDCl_3$) δ 1.18 (t, J = 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.78 (s, 3H, C-5 C*H*$_3$), 2.30 (ddd, $J_{2',2''} = 14.0$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.3$ Hz, 1H, H-2''), 2.70 (ddd, $J_{2',2''} = 14.0$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.65 (m, $J_{gem} = 15.0$, $J_{vic} = 7.5$ Hz, 1H, OCH'*H*''CH$_3$), 3.72–3.98 (m, 4H, H-4', H-5', OC*H*'H''CH$_3$), 4.32–4.40 (m, 1H, H-3'), 4.82 (s, 1H, H-6), 5.64 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 8.80 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.84 (OCH$_2$*C*H$_3$), 25.88 (C-5 *C*H$_3$), 36.89 (C-2'), 60.34 (C-3'), 62.16 (C-5'), 66.67 (O*C*H$_2$CH$_3$), 66.98 (C-5), 84.15 (C-4'), 87.88 (C-1'), 89.79 (C-6), 151.10 (C-2 C=O), 167.79 (C-4 C=O). Anal. ($C_{12}H_{18}ClN_5O_5$) C, H, N.

Synthesis of the Nucleosides 15–20. Chlorine gas (in the case of compounds **16**, **18**, and **20**) or a solution of bromine in 1,2-dimethoxyethane (1 mL) (in the case of compounds **15**, **17**, and **19**) was added to a suspension of AZT (1 equiv) in the selected alcohol (4 equiv) in 1,2-dimethoxyethane (1 mL) at 25 °C. The reaction was vigorously stirred until TLC indicated that no starting material remained. The solvent was removed in vacuo, and the product was purified by silica gel column chromatography using chloroform–methanol (95:5, v/v) as eluent to afford compounds **15**–**20**.

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-isopropoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (15). $[\alpha]^{25}_D = +72.6°$ (*c* 0.34, MeOH); R_f 0.69 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (50%); ^1H NMR ($CDCl_3$) δ 1.12 and 1.16 (2 d, J = 6.0 Hz, 6H total, OCH(C*H*$_3$)$_2$), 1.94 (s, 3H, C*H*$_3$), 2.36 (ddd, $J_{2',2''} = 14.4$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.73 (ddd, $J_{2',2''} = 14.4$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.80–4.06 (m, 4H, H-4', H-5', OC*H*(CH$_3$)$_2$), 4.32–4.40 (m, 1H, H-3'), 5.08 (s, 1H, H-6), 5.74 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 8.53 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 21.31 (*C*H$_3$), 23.13 and 23.25 (OCH(*C*H$_3$)$_2$), 37.36 (C-2'), 53.69 (C-5), 60.07 (C-3'), 62.13 (C-5'), 70.89 (O*C*H(CH$_3$)$_2$), 84.09 (C-4'), 86.04 (C-1'), 87.30 (C-6), 151.01 (C-2 *C*=O), 167.49 (C-4 *C*=O). Anal. ($C_{13}H_{20}BrN_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Chloro-6-isopropoxy-5,6-dihydro-3'-azido-3'-deoxythymidine (16). $[\alpha]^{25}_D = +70.3°$ (*c* 1.2, MeOH); R_f 0.69 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (75%); ^1H NMR ($CDCl_3$) δ 1.11 and 1.15 (2 d, J = 6.0 Hz, 6H total, OCH(C*H*$_3$)$_2$), 1.72 (s, 3H, C*H*$_3$), 2.30 (ddd, $J_{2',2''} = 13.8$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.62 (ddd, $J_{2',2''} = 13.8$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.66–4.07 (m, 4H, H-4', H-5', OC*H*(CH$_3$)$_2$), 4.28–4.34 (m, 1H, H-3'), 5.02 (s, 1H, H-6), 5.80 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 8.74 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 21.16 (*C*H$_3$), 21.97 and 23.08 (OCH(*C*H$_3$)$_2$), 37.21 (C-2'), 60.01 (C-3'), 61.13 (C-5), 61.96 (C-5'), 70.58 (O*C*H(CH$_3$)$_2$), 84.00 (C-4'),

85.29 (C-1'), 86.80 (C-6), 150.97 (C-2 *C*=O), 167.07 (C-4 *C*=O). Anal. ($C_{13}H_{20}ClN_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-(1-octyloxy)-5,6-dihydro-3'-azido-3'-deoxythymidine (17). $[\alpha]^{25}_D = +41.6°$ (*c* 0.55, MeOH); R_f 0.81 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (65%); ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.0$ Hz, 3H, $OCH_2(CH_2)_5CH_2CH_3$), 1.20–1.35 (m, 10H, $OCH_2(CH_2)_5CH_2CH_3$), 1.44–1.60 (m, 2H, $OCH_2(CH_2)_5CH_2CH_3$), 1.98 (s, 3H, CH_3), 2.32 (ddd, $J_{2',2''} = 14.0$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.72 (ddd, $J_{2',2''} = 14.0$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.42–4.04 (m, 5H, H-4', H-5', $OCH_2(CH_2)_5CH_2CH_3$), 4.32–4.40 (m, 1H, H-3'), 4.96 (s, 1H, H-6), 5.80 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 8.20 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 13.97 ($OCH_2(CH_2)_5CH_2CH_3$), 22.82 (CH_3), 22.53, 25.89, 29.43, 29.17, 29.07, 31.68 ($OCH_2(CH_2)_6CH_3$), 37.04 (C-2'), 53.30 (C-5), 60.12 (C-3'), 62.17 (C-5'), 70.11 ($OCH_2(CH_2)_5CH_2CH_3$), 84.08 (C-4'), 87.32 (C-1'), 88.53 (C-6), 150.70 (C-2 *C*=O), 167.22 (C-4 *C*=O). Anal. ($C_{18}H_{30}BrN_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Chloro-6-(1-octyloxy)-5,6-dihydro-3'-azido-3'-deoxythymidine (18). $[\alpha]^{25}_D = +37.7°$ (*c* 0.48, MeOH); R_f 0.84 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (60%); ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.0$ Hz, 3H, $O(CH_2)_7CH_3$), 1.22–1.40 (m, 10H, $OCH_2(CH_2)_5CH_2CH_3$), 1.50–1.66 (m, 2H, $O(CH_2)_6CH_2CH_3$), 1.85 (s, 3H, CH_3), 2.35 (ddd, $J_{2',2''} = 13.8$, $J_{1',2''} = 6.8$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.72 (m, 1H, H-2'), 3.42–4.02 (m, 5H, H-4', H-5', $OCH_2(CH_2)_6CH_3$), 4.33–4.40 (m, 1H, H-3'), 4.90 (s, 1H, H-6), 5.84 (t, $J_{1',2'} = J_{1',2''} = 6.8$ Hz, 1H, H-1'), 8.38 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.0 ($O(CH_2)_7CH_3$), 21.76 (CH_3), 22.55, 25.91, 29.37, 29.18, 29.09, 31.70 ($OCH_2(CH_2)_6CH_3$), 37.01 (C-2'), 60.11 (C-3'), 60.94 (C-5), 62.22 (C-5'), 70.02 ($OCH_2(CH_2)_6CH_3$), 84.03 (C-4'), 87.33 (C-1'), 88.29 (C-6), 150.59 (C-2 C=O), 166.59 (C-4 C=O). Anal. ($C_{18}H_{30}ClN_5O_5$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-(1-hexadecyloxy)-5,6-dihydro-3'-azido-3'-deoxythymidine (19). $[\alpha]^{25}_D = +27.6°$ (*c* 0.85, MeOH); R_f 0.84 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (43%); ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.0$ Hz, 3H, $O(CH_2)_{15}CH_3$), 1.22–1.40 (m, 26H, $OCH_2(CH_2)_{13}CH_2CH_3$), 1.48–1.60 (m, 2H, $O(CH_2)_{14}CH_2CH_3$), 2.00 (s, 3H, CH_3), 2.36 (ddd, $J_{2',2''} = 14.2$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.68 (ddd, $J_{2',2''} = 14.2$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.43–4.02 (m, 5H, H-4', H-5', $OCH_2(CH_2)_{14}CH_3$), 4.34–4.42 (m, 1H, H-3'), 4.98 (s, 1H, H-6), 5.82 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 8.22 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.06 ($O(CH_2)_{15}CH_3$), 22.96 (CH_3), 22.63, 25.94, 29.63, 29.47, 29.30, 31.88 ($OCH_2(CH_2)_{14}CH_3$), 37.09 (C-2'), 53.30 (C-5), 60.16 (C-3'), 62.27 (C-5'), 70.11 ($OCH_2(CH_2)_{14}CH_3$), 84.11 (C-4'), 87.49 (C-1'), 88.71 (C-6), 150.50 (C-2 *C*=O), 167.02 (C-4 *C*=O). Anal. ($C_{26}H_{46}BrN_5O_5 \cdot {}^1/_2H_2O$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Chloro-6-(1-hexadecyloxy)-5,6-dihydro-3'-azido-3'-deoxythymidine (20). $[\alpha]^{25}_D = +28.6°$ (*c* 0.32, MeOH); R_f 0.81 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (40%); ^1H NMR ($CDCl_3$) δ 0.90 (t, $J = 7.0$ Hz, 3H, $O(CH_2)_{15}CH_3$), 1.20–1.38 (m, 26H, $OCH_2(CH_2)_{13}CH_2CH_3$), 1.47–1.60 (m, 2H, $O(CH_2)_{14}CH_2CH_3$), 1.82 (s, 3H, CH_3), 2.32 (ddd, $J_{2',2''} = 14.0$, $J_{1',2''} = 7.2$, $J_{2'',3'} = 3.0$ Hz, 1H, H-2''), 2.68 (ddd, $J_{2',2''} = 14.0$, $J_{2',3'} = J_{1',2'} = 7.2$ Hz, 1H, H-2'), 3.40–4.00 (m, 5H, H-4', H-5', $OCH_2(CH_2)_{14}CH_3$), 4.30–4.38 (m, 1H, H-3'), 4.88 (s, 1H, H-6), 5.80 (t, $J_{1',2'} = J_{1',2''} = 7.2$ Hz, 1H, H-1'), 8.10 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 14.06 ($O(CH_2)_{15}CH_3$), 21.81 (CH_3), 22.64, 25.95, 29.63, 29.49, 29.40, 29.30, 31.88 ($OCH_2(CH_2)_{13}CH_2CH_3$), 37.05 (C-2'), 60.14 (C-3'), 60.97 (C-5), 62.28 (C-5'), 70.04 ($OCH_2(CH_2)_{14}CH_3$), 84.06 (C-4'), 87.42 (C-1'), 88.38 (C-6), 150.50 (C-2 *C*=O), 166.50 (C-4 *C*=O). Anal. ($C_{26}H_{46}ClN_5O_5$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Bromo-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (21), (−)-*trans*-(5*S*,6*S*)-5-Bromo-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (22), and (−)-*cis*-(5*R*,6*S*)-5-Bromo-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (23). *N*-Bromosuccinimide (0.036 g, 2 mmol) was added in aliquots to a precooled (−5 °C) suspension prepared by mixing a solution of AZT (0.052 g, 2 mmol) in 1,2-dimethoxyethane (10 mL) and a solution of sodium azide (0.052 g, 8 mmol) in water (0.125 mL) with stirring. The initial yellow color produced upon addition of each aliquot of *N*-bromosuccinimide quickly disappeared. When all of the *N*-bromosuccinimide had reacted, the reaction mixture was stirred for 30 min at 0 °C, poured onto ice–water (25 mL), and extracted with ethyl acetate (3 × 50 mL). Washing the ethyl acetate extract with cold water (2 × 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 as eluent to yield a mixture of diastereomers **21** and **22** and **23** respectively.

Diastereomers 21 and 22: R_f 0.63 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (0.03 g, 39%); ^1H NMR ($CDCl_3$) δ 1.98 and 2.0 (ratio 2:1) (2 s, 3H total, CH_3), 2.26–2.74 (m, 2H total, H-2'), 3.72–4.02 (m, 3H total, H-4', and H-5'), 4.28–4.42 (m, 1H total, H-3'), 5.42 and 5.64 (ratio 1:2) (2 s, 1H total, H-6), 5.76 and 6.20 (ratio 1:2) (2 t, $J_{1',2'} = 7.2$ Hz, 1H total, H-1'), 8.60 and 8.68 (ratio 2:1) (2 s, 1H total, NH); ^{13}C NMR ($CDCl_3$) δ 22.76 and 23.08 (CH_3), 35.99 and 36.71 (C-2'), 52.31 and 52.79 (C-5), 60.04 and 60.52 (C-3'), 61.72 and 62.35 (C-5'), 73.88 and 76.64 (C-6), 83.78 and 84.22 (C-4'), 87.81 (C-1'), 149.88 and 150.02 (C-2 *C*=O), 166.11 (C-4 *C*=O).

Diastereomer 23: $[\alpha]^{25}_D = -47.5°$ (*c* 0.16, MeOH); R_f 0.61 ($CHCl_3$–MeOH, 9:1, v/v); oil; yield (20 mg, 26%); ^1H NMR ($CDCl_3$) δ 1.98 (s, 3H, CH_3), 2.30–2.54 (m, 2H, H-2'), 3.86–4.06 (m, 3H total, H-4', H-5'), 4.32–4.40 (m, 1H, H-3'), 5.74 (s, 1H, H-6), 6.04 (t, $J_{1',2'} = 7.2$ Hz, 1H, H-1'), 8.25 (s, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 27.63 (CH_3), 36.02 (C-2'), 60.98 (C-3'), 61.75 (C-5), 62.65 (C-5'), 74.75 (C-6), 83.56 (C-4'), 85.05 (C-1'), 149.66 (C-2 *C*=O), 166.26 (C-4 *C*=O). Anal. ($C_{10}H_{13}BrN_8O_4$) C, H, N.

(+)-*trans*-(5*R*,6*R*)-5-Chloro-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (24), (−)-*trans*-(5*S*,6*S*)-5-Chloro-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (25), (+)-*cis*-(5*S*,6*R*)-5-Chloro-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (26), and (−)-*cis*-(5*R*,6*S*)-5-Bromo-6-azido-5,6-dihydro-3'-azido-3'-deoxythymidine (27). *N*-Chlorosuccinimide (0.125 g, 0.8 mmol) was added slowly to a precooled (−5 °C) suspension prepared by mixing a solution of AZT (0.1 g, 4 mmol) in 1,2-dimethoxyethane (10 mL) and a solution of sodium azide (0.1 g, 1.6 mmol) in water (0.25 mL). After the reaction mixture was stirred at 25 °C for 24 h, additional aliquots of *N*-chlorosuccinimide (0.07 g, 0.5 mmol) and sodium azide (0.025 g, 0.8 mmol) in water (0.15 mL) were added. The reaction was allowed to proceed for 24 h at 25 °C, and the mixture was poured onto ice–water (25 mL) and extracted with ethyl acetate (4 × 50 mL). Washing the ethyl acetate extract with cold water (2 × 10 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 (99:1, v/v) as eluent to yield a mixture of diastereomers **24–27** (0.12 g, 87%) as a syrup: ^1H NMR ($CDCl_3$) (mixture of diastereomers **24–27**) δ 1.75, 1.77, 1.78, and 1.80 (4 s, 3H total, CH_3), 2.23–2.65 (m, 2H, H-2'), 3.70–4.02 (m, 3H, H-4', H-5'), 4.25–4.38 (m, 1H, H-3'), 5.41, 5.58, 5.60, and 5.70 (4 s, 1H total, H-6), 5.78, 6.02, 6.08, and 6.20 (4 t, 1H total, H-1'), 8.88, 8.92, 8.94, and 8.96 (4 s, 1H total, NH); ^{13}C NMR ($CDCl_3$) δ 21.57, 21.82, 25.74, and 26.19 (CH_3), 35.80, 35.96, 36.59, and 37.13 (C-2'), 60.23, 60.61, and 60.89 (C-3'), 60.70 and 62.35 (C-5'), 61.74, 61.96, 66.87, and 68.66 (C-5), 73.40, 73.64, 74.96, and 75.94 (C-6), 83.61, 83.82, 84.01, 84.12, 84.33, 84.73, and 87.39 (C-4', C-1'), 150.02 and 150.59 (C-2 *C*=O), 165.63, 165.69, 166.83, and 166.92 (C-4 *C*=O). Anal. ($C_{10}H_{13}ClN_8O_4 \cdot {}^1/_2H_2O$) C, H, N.

Partition Coefficients (*P*). The nucleoside test compound (**3–5**, **7–9**) was partitioned between equal volumes of presaturated 1-octanol and water by mixing in a mechanical shaker for 4.5 h at 25 °C. The two phases were separated, and the concentration of the test compound in the 1-octanol layer was measured (UV quantitation at 230 nm). Partition coefficients (*P*) were calculated as the ratio of the concentration in the 1-octanol to the concentration in the water phase ($P = C_{1\text{-octanol/water}}$).

***In Vitro* Phosphorolysis.** The diluted enzyme (130 μL, prepared by diluting a 5 μL aliquot of a 1200 units/mL solution with Tris buffer (3.9 mL)) was transferred to a 1.5 mL microcentrifuge tube, and potassium dihydrogen phosphate buffer (20 μL of a 100 mM, pH 7.1 solution) was added with Vortex mixing. The closed tube was then warmed to 37 °C (Haake E3 water bath), and the phosphorolysis reaction was initiated by adding the substrate solution (250 mmol, 50 μL

of a stock solution in Tris buffer) to the prewarmed mixture. The contents of the tube were mixed well prior to a 10 min incubation at 37 °C. The reaction was terminated by removing the tube from the water bath and adding ice-cold methanol (200 μL) with thorough mixing. This tube was then placed in an ice bath for 10 min prior to centrifugation at 12 800 rpm in an Eppendorf microcentrifuge for 3 min at 4 °C. An aliquot of the clear supernatant solution was subjected to quantitative HPLC analysis with UV detection at 230 nm using water:methanol concentrations ranging from 7:3 to 9.5:0.5, v/v, as eluent at a flow rate of 2 mL/min for the different substrates analyzed. The disappearance of the substrate at its predetermined retention time, or the appearance of the expected phosphorolysis product which included thymine or the 5-halo-6-methoxy-5,6-dihydrouracil, after incubation of the test compound with the enzyme was taken as evidence of phosphorolysis. Each experiment was peformed at least twice. Thymidine was used as a control substrate for comparison purposes.

***In Vitro* Regeneration of the 5,6-Olefinic Bond.** Regeneration of AZT from the 5-halo-6-alkoxy-5,6-dihydro-3′-azido-3′-deoxythymidine diastereomers (**3**–11) was determined by incubating the test compound with either glutathione (reduced), mouse blood, or a mouse liver homogenate.

1. **Glutathione (GSH):** The test compound was incubated with GSH using a test compound:GSH mole ratio of 1:2 (compounds **3**–**9**) or 1:5 (compounds **10** and 11) in phosphate buffer (pH 7.4) at 37 °C for times up to 24 h (see Table 1). The incubation sample was subjected to HPLC analysis with UV detection at both 230 nm (5,6-dihydro compound) and 265 nm (AZT), using water:methanol (7:3, v/v) as eluent at a flow rate of 2 mL/min to quantitate the amount of AZT produced by 5,6-olfefinic bond regeneration.

2. **Mouse blood:** Blood (100 μL, drawn into a heparinized syringe after cardiac puncture) was transferred to a 1.5 mL microcentrifuge tube, mixed with a solution of the test compound (100 μL of a stock solution, 1 mg/mL in water), and allowed to incubate at 37 °C for 10 min. To terminate the reaction, ice-cold methanol (200 μL) was added with vortex mixing. The resultant mixture was maintained at ice bath temperature for 10 min prior to centrifugation at 12 800 rpm for 3 min. The supernatant solution was dried under a stream of nitrogen gas at 35 °C, the residue obtained was redissolved in methanol (0.1 mL), and a 40 μL aliquot was subjectd to quantitative HPLC analysis to determine the amount of AZT produced due to regeneration of the 5,6-olefinic bond as described in the glutathione incubation procedure described above.

3. **Mouse liver homogenate:** The livers from three Balb/c mice were washed with ice-cold 0.1 M Tris buffer (pH 8.2) and homogenized in two volumes (w/v) of the 0.1 M Tris buffer (pH 8.2), and the homogenate was centrifuged at 49000*g* for 2 h at 4 °C. The supernatant (soluble enzyme fraction) was used to determine the regeneration of AZT from the test 5,6-dihydro compounds. Individual test compounds (100 μL of a 1 mg/mL stock solution) were incubated with the liver soluble enzyme fraction (50 μL) for 30 min at 37 °C. This incubation mixture was mixed with an equal volume of methanol prior to centrifugation at 12 800 rpm for 3 min. The supernatant obtained (200 μL) was dried under a stream of nitrogen gas and then redissolved in methanol (100 μL). An aliquot (25 μL) was subjected to quantitative HPLC analysis using methanol:water (3:7, v/v) as eluent at a flow rate of 2 mL/min with UV detection at 230 nm (5,6-dihydro compound) and 265 nm (AZT).

***In Vitro* Anti-HIV Drug Assay.**[45] The ability of the test compound to protect HIV-1-infected T4 lymphocytes (CEM cells) from cell death was determined using the reported procedure.[45] Small amounts of HIV were added to 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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