Synthesis and Anti-human Immunodeficiency Virus Type 1 (HIV-1) Activity of 3-Substituted Derivatives of 3′-Azido-3′-deoxythymidine (AZT), and Inhibition of HIV-1 Reverse Transcriptase by Their 5′-Triphosphates[1)]

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Received September 27, 1991

Various 3-substituted 3′-azido-3′-deoxythymidine analogs (2a—i) were prepared by the reaction of 3′-azido-3′-deoxythymidine (1), AZT with *N,N*-dimethylformamide dialkylacetal or alkyl bromide in the presence of base and their activities against human-immunodeficiency virus type-1 (HIV-1) were evaluated. The corresponding 5′-triphosphate analogs (9) were also synthesized in order to examine inhibition of HIV-1 reverse transcriptase activity. Beyond expectation, some N^3-derivatives of AZT were found to reserve the anti-HIV-1 activity to some extent. Among the compounds (2a—i) obtained, 3-allyl-AZT (2e) was the most active against HIV-1 replication in MT-4 cells *in vitro* with an EC_{50} value of 0.9 μM. 3-Allyl-AZT 5′-triphosphate (9e), however, exhibited no inhibition of HIV-1 reverse transcriptase activity.

Keywords AZT; 3′-azido-3′-deoxythymidine; 3-substituted AZT; AZT triphosphate; anti-human immunodeficiency virus activity; inhibition; reverse transcriptase

There is an urgent need for compounds that are effective in the treatment of acquired immunodeficiency syndrome (AIDS) resulting from infection with human immunodeficiency virus (HIV). Although several nucleosides have been shown to have an *in vitro* anti-HIV activity commensurate with development to clinical trials,[2)] 3′-azido-3′-deoxythymidine (AZT, Zidovudine, **1**) is currently the only drug approved for the treatment of AIDS.[3)] AZT is well known to exert its activity as 5′-triphosphate by inhibiting the HIV reverse transcriptase (RT).[4,5)] Furthermore, if incorporated into a growing viral deoxyribonucleic acid (DNA) chain, AZT would halt further DNA synthesis since it lacks the 3′-hydroxy group. Since AZT was found to have excellent anti-HIV activity, a number of 3′-substituted 3′-deoxythymidines possessing various substituents instead of the 3′-azido group of AZT have been synthesized and their anti-HIV activity has been evaluated.[6,7)] These results suggest that the 3′-azido group of AZT is of critical importance for inhibiting the activity of HIV RT. Therefore, chemical modification of AZT with retaining the 3′-azido group has been widely investigated.[8-10)] For example, displacement of the 5-methyl group with other functional groups[6,8)] and introduction of fluorine and hydroxy groups into the 2′-position[9)] have been documented. On the other hand, synthesis and anti-HIV activity of N^3-substituted AZT have been scarcely reported.[10)]

It is generally accepted that the N^3-hydrogen on thymidine 5′-triphosphate plays an important role in the DNA replication for the recognition of base pairing with template DNA. Since RT is a kind of DNA polymerase, it is comprehended that introduction of substituents into the N^3-position of AZT causes the abolishment of its base-pairing capacity and results in an extreme decrease of its anti-retroviral activity. Huff and Topal reported that N^3-methylthymidine triphosphate is not incorporated by *E. coli* DNA polymerase I in the DNA synthesis but is sparingly incorporated by avian myeloblastosis virus RT.[11)] These results might indicate that the substrate-specificity of the RT would not be so strict.

In this paper, our attention is focused on the effect of the N^3-substituent of AZT on anti-HIV type 1 activity. We have embarked on the synthesis and biological evaluation of AZT derivatives bearing several N^3-substituents and their 5′-triphosphates in order to procure more selective and potent antiviral agents against HIV-1, and some mechanistic insights into the HIV-1 RT-dependent DNA synthesis.

Chemistry Various 3-substituted analogs of AZT were synthesized by the methodology previously described[12)] with some minor modifications. Treatment of **1** with *N,N*-dimethylformamide (DMF) dimethylacetal in DMF at 60 °C for 3 h resulted in the formation of 3′-azido-3′-deoxy-3-methylthymidine (3-methyl-AZT) (**2a**) in 59% yield. In a similar manner, 3-butyl-AZT (**2g**) was obtained in 49% yield by treatment with DMF–dibutylacetal. 3-Ethyl-AZT (**2b**), however, was not isolated successfully using DMF–diethylacetal. Thus, 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (**3**) was employed as a starting material for the preparation of 3-ethyl derivative (**2b**): analogous treatment of **3** with DMF–diethylacetal led to the smooth formation of 3′-azido-3′-deoxy-3-ethyl-5′-*O*-tritylthymidine (**4b**) (82% yield), which was deprotected with 80% acetic acid to give the desired 3-ethyl-AZT (**2b**), quantitatively. On the other hand, other 3-substituted AZT derivatives, namely 3-propyl-AZT (**2c**), 3-isopropyl-AZT (**2d**), 3-(2,3-epoxypropyl)-AZT (**2e**), 3-allyl-AZT (**2f**), and 3-benzyl-AZT (**2h**) were prepared by the reaction of **1** with corresponding halides such as propyl bromide, isopropyl bromide, epibromohydrin, allyl bromide, and benzyl bromide in the presence of anhydrous potassium carbonate in 56—87% yields. Synthesis of 3-(3-methyl-2-butenyl)-AZT (**2i**) was accomplished by the reaction of 5′-*O*-trityl-AZT (**3**) with 1-bromo-3-methyl-2-butene in the presence of sodium hydride and subsequent deprotection with 80% acetic acid though the direct alkylation of (**1**) was unsuccessful. 2′,3′-Didehydro-3′-deoxy-3-methylthymidine (**6**) was also synthesized by the reaction of 2′,3′-didehydro-3′-deoxythymidine (D4T) (**5**) with DMF–dimethylacetal.

In order to examine the inhibition potentials against the

RT activity, several 5′-triphosphate analogs (**9**) of the 3-substituted nucleosides (**2**) were prepared as depicted in Chart 3. Phosphorylation of the nucleosides by phosphorus oxychloride in trimethyl phosphate gave the corresponding 5′-monophosphates (**8**),[13] which were allowed to react with *N*,*N*′-carbonyldiimidazole and were subsequently treated with tributylammonium pyrophosphate to give the desired 5′-triphosphates (**9**).[14] 5′-Triphosphate derivative (**11**) of 3-methyl-D4T (**6**) was obtained by analogous reaction *via* the corresponding 5′-monophosphate (**10**). The structures of these nucleoside 5′-monophosphates and 5′-triphosphates were supported by both proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy and high performance liquid chromatographic (HPLC) analysis (see Table I).

Biological Activity The compounds[15] synthesized in this study were tested against HIV-1 (HTLV-III$_B$) strain in MT-4 cells and the results are shown in Table II. The procedure to measure anti-HIV-1 activity in MT-4 cells has

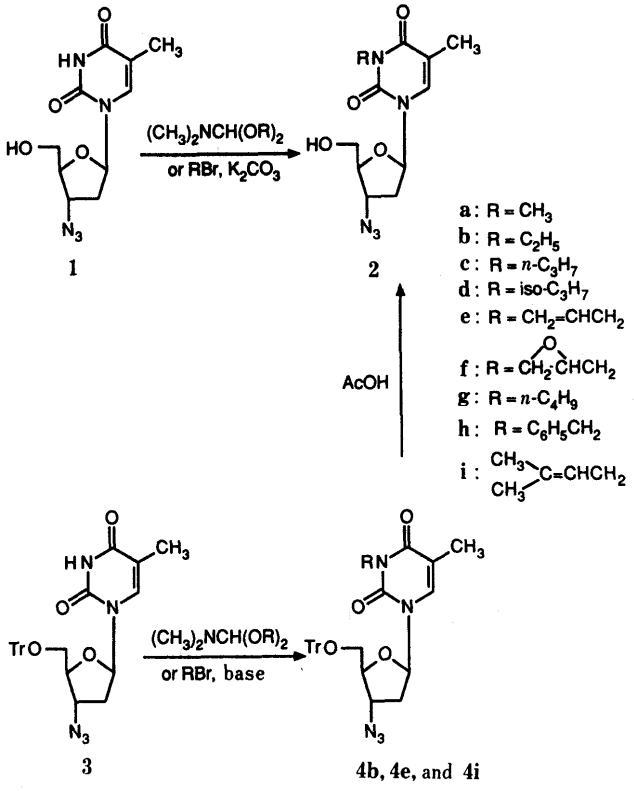


Chart 1

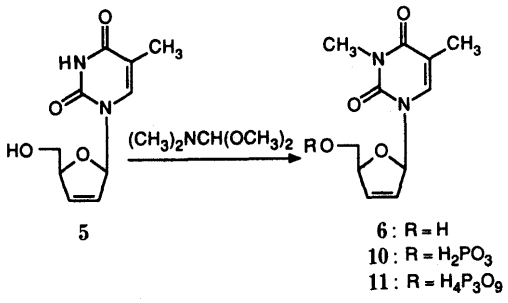


Chart 2

TABLE I. Retention Times of HPLC of 3-Substituted Nucleosides and Nucleotides

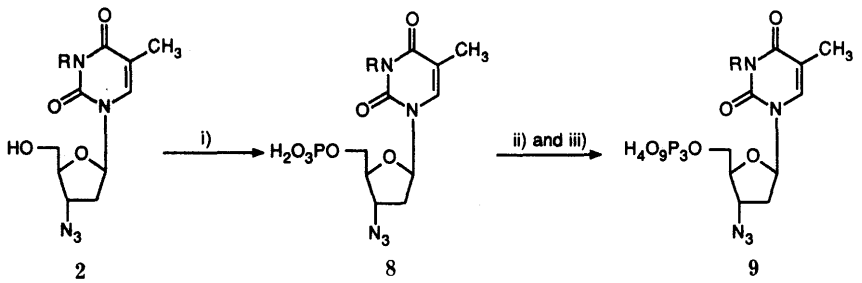
Compound	File 1[a]	File 2[b]	Compound	File 1[a]	File 2[b]
3-Me-AZT (**2a**)	8.63	36.72	3-Allyl-AZTTP (**9e**)		26.28
3-Me-AZTMP (**8a**)		24.57	3-Bu-AZT (**2g**)	55.83	ND
3-Me-AZTTP (**9a**)		19.77	3-Bu-AZTMP (**8g**)		37.22
3-Et-AZT (**2b**)	14.23	41.13	3-Bu-AZTTP (**9g**)		34.35
3-Et-AZTMP (**8b**)		29.31	3-Benzyl-AZT (**2h**)	ND	51.05
3-Et-AZTTP (**9a**)		23.76	3-Benzyl-AZTMP (**8h**)		36.25
3-Pr-AZT (**2c**)	25.28	43.95	3-Benzyl-AZTTP (**9h**)		34.22
3-Pr-AZTMP (**8c**)		34.57	3-Me-D4T (**6**)	4.45	26.57
3-Pr-AZTTP (**9c**)		29.76	3-Me-D4TMP (**10**)		19.99
3-iso-Pr-AZT (**2d**)	26.39	ND	3-Me-D4TTP (**11**)		15.95
3-iso-Pr-AZTMP (**8d**)		33.95	AZT (**1**)	5.29	28.98
3-iso-Pr-AZTTP (**9d**)		27.37	AZTMP		19.03
3-Allyl-AZT (**2e**)	16.04	35.32	AZTTP		14.76
3-Allyl-AZTMP (**8e**)		31.08			

a) Chromatograph performed on Wakosil 5C18 (Wako) reverse-phase column using methanol–water (1 : 1) at 1 ml/min. *b*) Chromatograph performed on Wakosil 5C18 (Wako) reverse-phase column using a gradient of 0 to 50% buffer B (methanol–water, 1 : 1) over 30 min in buffer A (50 mM ammonium phosphate, pH 7.0) at 1 ml/min.

TABLE II. Inhibitory Effects of 3-Substituted Nucleosides on HIV-1 Cytopathogenicity in MT-4 Cells

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]
2a	6.6	>500	>76
2b	16	282	17.6
2c	>345	>345	<1
2d	6.7	278	41.5
2e	0.9	214	192.6
2f	>359	359	<1
2g	6.2	330	53.2
2h	45	210	4.7
2i	10	151	15.1
6	>359	359	<1
1	0.0041	19	4630
5	0.068	7.8	1147

MT-4 cells were infected with HIV-1 (HTLV-III$_B$) at a MOI of 0.02 and incubated in the presence of the test compounds. After a 4 d-incubation, the number of viable cells was determined by the MTT method. *a*) EC_{50}: 50% antiviral effective concentration. *b*) CC_{50}: 50% cytotoxic concentration. *c*) Selectivity index: ratio of CC_{50}/EC_{50}.



i) $POCl_3$ in $(CH_3O)_3PO$ ii) *N*, *N*′-carbonyldiimidazole iii) $H_3P_2O_7^-$ n-Bu_3NH^+

Chart 3

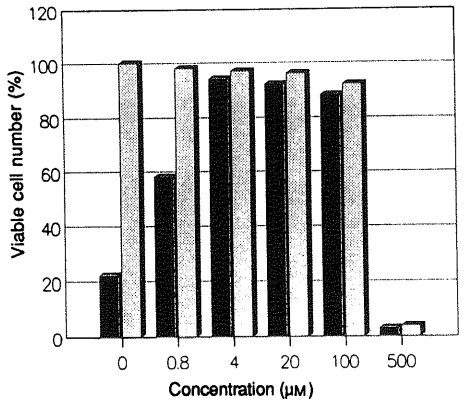


Fig. 1. Inhibitory Effect of 3-Allyl-AZT (**2e**) on HIV-1 Replication in MT-4 Cells

MT-4 cells were infected with HIV-1 (HTLV-III$_B$) and incubated with various concentrations of the test compound for 4 d. The viability of virus-infected cell (■) and mock-infected cells (▨) were determined by the MTT method. The number of viable cells is expressed as a percentage of the number of mock-infected control cells.

TABLE III. Inhibition of HIV-1 RT Activity by 3-Substituted Nucleoside 5′-Triphosphates

Compound	IC_{50}[a] (μM)	Compound	IC_{50}[a] (μM)
9a	2	**9g**	15
9b	> 50	**9h**	> 50
9c	> 50	**11**	12
9d	30	AZTTP	0.3
9e	> 50		

a) IC_{50}: 50% inhibition concentration.

been described previously.[16] Among the 3-substituted AZT derivatives (**2a**—**i**), 3-allyl-AZT (**2e**) is the most active against HIV-1 replication in MT-4 cells *in vitro* with a EC_{50} value of 0.9 μM (see Fig. 1). The 3-methyl, 3-isopropyl, and 3-butyl derivatives of AZT, compounds **2a**, **2d**, and **2g** also showed significant antiviral activity with EC_{50} values of 6.6, 6.7, and 6.2 μM, respectively. The 3-(3-methyl-2-butenyl), 3-ethyl, and 3-benzyl derivatives of AZT, **2i**, **2b**, and **2h**, demonstrated moderate antiviral activity, giving EC_{50} values of 10, 16, and 45 μM, respectively. Both 3-propyl-AZT (**2c**) and 3-(2,3-epoxypropyl)-AZT (**2f**) were found to be inactive (EC_{50} > 50 μM). Unlike 3-methyl-AZT (**2a**), 3-methyl analog (**6**) of D4T had no activity against HIV-1 cytopathogenicity.

The inhibitory effects of the 3-substituted AZT 5′-triphosphates (AZTTP) (**9** and **11**) on HIV-1 RT were also examined and the results are shown in Table III. Among the 5′-triphosphates, 3-methyl-AZTTP (**9a**) was the most potent in inhibition of HIV-1 RT (IC_{50} 2 μM) under the standard assay conditions as described in Experimental. This 5′-triphosphate (**9a**), however, was 7 times less active than AZTTP (IC_{50} 0.3 μM). On the other hand, **9a** was virtually inactive against human DNA polymerase α with an IC_{50} value of > 50 μM (data not shown). Two of the other 3-substituted AZTTP analogs, *i.e.* 3-iso-Pr-AZTTP (**9d**) and 3-Bu-AZTTP (**9g**), moderately inhibited HIV-1 RT with IC_{50} values of 30 and 15 μM, respectively. The other 3-substituted AZTTP derivatives such as 3-Et-AZTTP (**9b**), 3-Pr-AZTTP (**9c**), 3-allyl-AZTTP (**9e**), and 3-benzyl-AZTTP (**9h**) had no inhibitory effect on HIV-1 RT. On the other hand, 3-methyl-D4TTP (**11**) was active with an IC_{50} value of 12 μM (see Table III) although 3-methyl-D4T (**6**) had no inhibitory activity against HIV-1 cytopathogenicity. This observation might indicate that **6** is not a substrate of thymidine kinase.

Discussion

We found that some N^3-substituted AZTs reserve the anti-HIV-1 activity to some extent. Although 3-allyl-AZT (**2e**) possesses a strong antivital activity against HIV-1, 3-allyl-AZTTP (**9e**) has no inhibitory activity against HIV-1 RT. This finding suggests that 3-allyl-AZT (**2e**) may exert its anti-HIV-1 activity through an unknown mechanism different from that of AZT.[4,5] The release of AZT *via* the biological degradation of **2e** under the assay conditions, however, can not be thoroughly denied.

In the effect of the N^3-alkyl substituent on the RT inhibitory potentials of 3-alkylated AZTTP, 3-Et-AZTTP (**9b**) and 3-Pr-AZTTP (**9c**) showed less inhibitory activities than 3-Me-AZTTP (**9a**) and 3-Bu-AZTTP (**9g**) was more active than **9b** and **9c**. The HIV-1 RT inhibitory activities of these compounds are thus in the order of 3-Me-AZTTP (**9a**) > 3-Bu-AZTTP (**9g**) > 3-Pr-AZTTP (**9c**) = 3-Et-AZTTP (**9b**). Analogous observation in the relationship between alkyl chain length and activity has been reported in the inhibitory effects of 5-alkylated 1-β-D-arabinofuranosyluracil 5′-triphosphates (ara-UTPs) on the activity of DNA polymerase α.[17]

Although the modification at the N^3-position of thymine base has been considered to be taboo because of the necessity of this position for base pairing with adenosine through a hydrogen bond, the results obtained in the present study show that the chemical modification at the N^3-position of pyrimidine nucleosides is applicable to the preparation of new nucleoside analogs possessing an anti-HIV-1 activity.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded at 270 MHz on a JEOL JNX-270 spectrometer in $CDCl_3$, $(CD_3)_2SO$, or D_2O. Chemical shifts are quoted in parts per million (s = singlet, d = doublet, t = triplet, m = multiplet, q = quartet, br = broad, dd = double doublet, dt = double triplet). Mass spectra (MS) were measured at 70 eV with a JEOL JMS-D300 spectrometer. Column chromatography was carried out on silica gel (Wako gel C-300). HPLC was performed on a Shimadzu SPD-6A apparatus. The column employed was a Wakosil 5C18 (Wako).

3′-Azido-3′-deoxy-3-methylthymidine (2a) A mixture of compound **1** (0.084 g, 0.3 mmol) and DMF dimethylacetal (0.4 ml, 3 mmol) in dry DMF (1 ml) was heated at 50 °C for 3.5 h. The reaction mixture was evaporated under reduced pressure and the residue was chromatographed on a silica gel column with chloroform–methanol (50 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2a** (0.047 g, 59%): ^{1}H-NMR ($CDCl_3$) δ: 1.93 (s, 3H, 5-CH_3), 2.36—2.59 (m, 2H, 2′-H), 3.33 (s, 3H, N-CH_3), 3.79—4.02 (m, 3H, 4′-H and 5′-H), 4.38—4.44 (m, 1H, 3′-H), 6.09 (t, 1H, J = 6.6 Hz, 1′-H), and 7.46 (s, 1H, 6-H). High resolution mass spectrum (HRMS) m/z: 281.1127 (M^+, Calcd for $C_{11}H_{15}N_5O_4$: 281.1124).

3′-Azido-3′-deoxy-3-ethylthymidine (2b) A mixture of 3′-azido-3′-deoxy-5′-*O*-tritylthymidine (**3**) (0.119 g, 0.2 mmol) and DMF diethylacetal (0.294 g, 2 mmol) in dry DMF (1 ml) was heated at 100 °C for 24 h. The reaction mixture was evaporated under reduced pressure and the residue was chromatographed on a silica gel column with toluene–ethyl acetate (20 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give 3′-azido-3′-deoxy-3-ethyl-5′-*O*-tritylthymidine (**4b**) (0.089 g, 82%). A mixture of **4b** (0.073 g, 0.136 mmol) and 80% acetic acid (10 ml) was heated at 90 °C for 45 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml). The resulting undissolved

material was removed by filtration and the filtrate was evaporated under reduced pressure to give **2b** (0.037 g, 98%). ^{1}H-NMR ($CDCl_3$) δ: 1.21 (t, 3H, J=7.1 Hz, $CH_2\underline{CH_3}$), 1.93 (s, 3H, 5-CH_3), 2.54—2.64 (m, 2H, 2'-H), 3.75—4.04 (m, 5H, 4'-H, 5'-H, and N-$\underline{CH_2}CH_3$), 4.39—4.45 (m, 1H, 3'-H), 6.04 (t, 1H, J=6.6 Hz, 1'-H), and 7.30 (s, 1H, 6-H). HRMS m/z: 295.1275 (M^+, Calcd for $C_{12}H_{17}N_5O_4$: 295.1280).

3'-Azido-3'-deoxy-3-propylthymidine (2c) A mixture of **1** (0.133 g, 0.5 mmol), propyl bromide (0.246 g, 6 mmol), and potassium carbonate (0.083 g, 0.6 mmol) in DMF (5 ml) was heated at 70 °C for 3 h. The resulting undissolved potassium carbonate was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column with chloroform–methanol (50 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2c** (0.113 g, 73%). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (t, 3H, J=7.3 Hz, $CH_2\underline{CH_3}$), 1.57—1.68 (m, 2H, $CH_2\underline{CH_2}CH_3$), 1.93 (s, 3H, 5-CH_3), 2.35—2.44 (m, 1H, 2'-H), 2.53—2.64 (m, 1H, 2'-H), 3.79—3.98 (m, 5H, 4'-H, 5'-H, and N-$\underline{CH_2}CH_2$), 4.39—4.46 (m, 1H, 3'-H), 6.02—6.06 (t, 1H, J=6.3 Hz, 1'-H), and 7.33 (s, 1H, 6-H). HRMS m/z: 309.1453 (M^+, Calcd for $C_{13}H_{19}N_5O_4$: 309.1437).

3'-Azido-3'-deoxy-3-isopropylthymidine (2d) A mixture of **1** (0.03 g, 0.112 mmol), isopropyl bromide (0.055 g, 0.448 mmol), and anhydrous potassium carbonate (0.0186 g, 0.134 mmol) in dry DMF (1 ml) was heated at 60 °C for 20 h. The resulting undissolved potassium carbonate was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column with chloroform–methanol (80 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2d** (0.03 g, 87%). ^{1}H-NMR ($CDCl_3$) δ: 1.46 (d, 6H, J=6.8 Hz, $CH(\underline{CH_3})_2$), 1.91 (s, 3H, 5-CH_3), 2.36—2.60 (m, 2H, 2'-H), 2.68—4.01 (m, 3H, 4'-H and 5'-H), 4.37—4.43 (m, 1H, 3'-H), 5.13—5.24 (m, 1H, $\underline{CH}(CH_3)_2$), 6.06 (t, 1H, J=6.4 Hz, 1'-H), and 7.31 (s, 1H, 6-H). HRMS m/z: 309.1455 (M^+, Calcd for $C_{13}H_{19}N_5O_4$: 309.1437).

3-Allyl-3'-azido-3'-deoxythymidine (2e) A mixture of **3** (0.51 g, 1 mmol), allyl bromide (0.302 g, 2.5 mmol), and anhydrous potassium carbonate (0.207 g, 1.5 mmol) in dry DMF (10 ml) was heated at 60 °C for 4 h. The resulting undissolved potassium carbonate was removed by filtration and the filtrate was dissolved in water (50 ml). The mixture was extracted with chloroform (50 ml × 3) and the combined organic extracts were washed with water (50 ml × 2). The extract was dried over $MgSO_4$ and the solvent was removed *in vacuo* to give 3-allyl-3'-azido-3'-deoxy-5'-*O*-tritylthymidine (**4e**) (0.426 g, 78%). A mixture of **4e** (0.352 g, 0.641 mmol) and 80% acetic acid (30 ml) was heated at 90 °C for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in water (20 ml). The resulting undissolved material was removed by filtration and the filtrate was extracted with pentane. The water layer was evaporated under reduced pressure to give **2e** (0.111 g, 56%). ^{1}H-NMR ($CDCl_3$) δ: 1.94 (s, 3H, 5-CH_3), 2.36—2.55 (m, 2H, 2'-H), 3.80—3.84 (d, 1H, J=10.7 Hz, 5'-H), 3.85—3.96 (m, 1H, 4'-H), 3.98—4.02 (d, 1H, J=10.7 Hz, 5'-H), 4.38—4.44 (m, 1H, 3'-H), 4.55 (d, 2H, J=5.9 Hz, Hd), 5.18 (dd, 1H, J=1.4, 10 Hz, H_a), 5.22 (dd, 1H, J=1.4, 17 Hz, H_b), 5.85 (m, 1H, H_c) $\left(\begin{matrix}H_a\\H_b\end{matrix}\!\!>\!C\!=\!C\!<\!\begin{matrix}H_c\\(H_d)_2N\end{matrix}\right)$, 6.07 (t, 1H, J=6.6 Hz, 1'-H), and 7.38 (s, 1H, 6-H). HRMS m/z: 307.1290 (M^+, Calcd for $C_{13}H_{17}N_5O_4$: 307.1280).

Alternative Preparation of 2e A suspension of **1** (0.068 g, 0.25 mmol), ally bromide (0.061 g, 0.5 mmol), and anhydrous potassium carbonate (0.035 g, 0.25 mmol) in dry DMF (2 ml) was stirred at room temperature for 6 h. The undissolved material was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column with toluene–ethyl acetate (5 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2e** (0.067 g, 87%), which was identical with the compound prepared above.

3'-Azido-3'-deoxy-3-(2,3-epoxypropyl)thymidine (2f) A mixture of **1** (0.08 g, 0.30 mmol) and anhydrous potassium carbonate (0.05 g, 0.36 mmol) in dry DMF (4 ml) was heated at 60 °C for 1 h. The resulting undissolved potassium carbonate was removed by filtration and the filtrate was evaporated under reduced pressure. The residue was chromatographed on a silica gel column with benzene–ethyl acetate (1 : 1) containing 3% of triethylamine. The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2f** (0.094 g, 37%). ^{1}H-NMR ($CDCl_3$) δ: 1.94 (s, 3H, 5-CH_3), 2.47 (m, 2H, 2'-H), 2.75 (m, 2H, $\underline{CH_2}$–CH–), 3.25 (m, 1H, CH_2–$\underline{CH}$–), 3.82 (m, 1H, 5'-H), 4.01 (m, 3H, N-CH, 4'-H, and 5'-H), 4.24 (ddd, 1H, J=2.0, 3.4, 6.3 Hz, N-CH), 4.41 (dd, 1H, J=4.9, 7.3 Hz, 3'-H), 6.11 (dt, 1H, J=2.9, 3.4 Hz, 1'-H), and 7.46 (s, 1H, 6-H). HRMS m/z: 323.1215 (M^+, Calcd for $C_{13}H_{17}N_5O_5$: 323.1229).

3'-Azido-3-butyl-3'-deoxythymidine (2g) A mixture of **1** (0.191 g, 0.5 mmol) and DMF dibutylacetal (3 ml) in dry DMF (1 ml) was heated at 65 °C for 50 h. The reaction mixture was evaporated under reduced pressure and the residue was chromatographed on a silica gel column with chloroform–methanol (50 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2g** (0.08 g, 49%). ^{1}H-NMR ($CDCl_3$) δ: 0.97 (t, 3H, J=7.3 Hz, CH_2-$\underline{CH_3}$), 1.33—1.46 (m, 2H, $\underline{CH_2}$–CH_3), 1.57—1.68 (m, 2H, N–CH_2–$\underline{CH_2}$–CH_3), 1.95 (s, 3H, 5-CH_3), 2.41—2.58 (m, 2H, 2'-H), 3.84—4.05 (m, 5H, 5'-H, 4'-H, and N-$\underline{CH_2}$–CH_2), 4.39—4.47 (m, 2H, 3'-H), 6.18 (t, 1H, J=6.4 Hz, 1'-H), and 7.59 (s, 1H, 6-H). HRMS m/z: 323.1590 (M^+, Calcd for $C_{14}H_{21}N_5O_4$: 323.1593).

3'-Azido-3-benzyl-3'-deoxythymidine (2h) A suspension of AZT (0.134 g, 0.5 mmol), benzyl bromide (0.257 g, 1.5 mmol), and anhydrous potassium carbonate (0.207 g, 1.5 mmol) in dry DMF (4 ml) was heated at 50 °C for 20 h. The resulting undissolved potassium carbonate was removed by filtration. The solvent was removed under reduced pressure and the residue was chromatographed on a silica gel column with toluene–ethyl acetate (1 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **2h** (0.128 g, 71%). Recrystallization from ethanol gave analytically pure sample: mp 147 °C. ^{1}H-NMR ($CDCl_3$), δ: 1.94 (s, 3H, 5-CH_3), 2.33—2.58 (m, 2H, 2'-H), 3.78—4.01 (m, 3H, 4'-H and 5'-H), 4.36—4.42 (m, 1H, 3'-H), 3.78—4.01 (m, 3H, 4'-H and 5'-H), 4.36—4.42 (m, 1H, 3'-H), 5.11 (s, 2H, $\underline{CH_2}C_6H_5$), 6.07 (t, 1H, 1'-H), and 7.25—7.50 (m, 6H, $CH_2\underline{C_6H_5}$, 6-H). HRMS m/z: 357.1451 (M^+, Calcd for $C_{17}H_{19}N_5O_4$: 357.1437). *Anal.* Calcd for $C_{17}H_{19}N_5O_4$: C, 57.13; H, 5.36; N, 19.60. Found: C, 57.09; H, 5.23; N, 19.46.

3'-Azido-3'-deoxy-3-(3-methyl-2-butenyl)thymidine (2i) A mixture of **3** (0.248 g, 0.5 mmol), 1-bromo-3-methyl-2-butene (0.09 g, 0.6 mmol), and sodium hydride (0.024 g, 1 mmol) in dry DMF (4 ml) was heated at 60 °C for 4 h. The solvent was removed *in vacuo* and the residue was chromatographed on a silica gel column to give 3'-azido-3'-deoxy-3-(3-methyl-2-butenyl)-5'-*O*-tritylthymidine (**4i**) (0.281 g, 64%). A mixture of **4i** (0.120 g, 0.207 mmol) and 80% acetic acid (9 ml) was heated at 90 °C for 1 h. The solvent was removed under reduced pressure and the residue was dissolved in water (20 ml). The resulting undissolved material was removed by filtration and the filtrate was extracted with pentane. The water layer was evaporated under reduced pressure to give **2i** (0.025 g, 36%). ^{1}H-NMR ($CDCl_3$) δ: 1.70 (s, 3H, C–CH_3), 1.81 (s, 3H, C–CH_3), 1.94 (s, 3H, 5-CH_3), 2.34—2.63 (m, 2H, 2'-H), 3.79—4.02 (m, 3H, 4'-H and 5'-H), 4.38—4.44 (m, 1H, 3'-H), 4.53 (d, 2H, J=6.8 Hz, CH_2N), 5.18—5.24 (m, 1H, CH), 6.02—6.07 (t, 1H, J=6.6 Hz, 1'-H), 7.28 (m, 1H, 6-H). HRMS m/z: 335.1575 (M^+, Calcd for $C_{15}H_{21}N_5O_4$: 335.1593).

2',3'-Didehydro-3'-deoxy-3-methylthymidine (6) A mixture of 3'-deoxy-2',3'-dehydrothymidine (**5**) (0.03 g, 0.134 mmol) and DMF dimethylacetal (180 μl, 1.34 mmol) in dry DMF (1 ml) was heated at 60 °C for 12 h. The reaction mixture was evaporated under reduced pressuure and the residue was chromatographed on a silica gel column with chloroform–methanol (100 : 1). The appropriate fractions containing the product were collected and the solvent was removed under reduced pressure to give **6** (0.022 g, 69%). ^{1}H-NMR ($CDCl_3$) δ: 1.89 (s, 3H, 5-CH_3), 3.35 (s, 3H, N–CH_3), 3.86 (m, 2H, 5'-H), 4.93 (s, 1H, 4'-H), 5.87 (br d, 1H, J=6.4 Hz, 3'-H), 6.34 (dt, 1H, J=6.4 Hz, 2'-H), 7.04 (dd, 1H, J=1.5, 2.0 Hz, 1'-H), and 7.44 (s, 1H, 6-H). HRMS m/z: 238.0969 (M^+, Calcd for $C_{11}H_{14}N_2O_4$: 238.0953).

Preparation of Nucleoside 5'-Monophosphates (8 and 10)[13] General Procedure: A mixture of nucleoside (0.1 mmol) in trimethyl phosphate (1 ml) was allowed to stand in an ice-cooled bath for 5 min. Phosphorous oxychloride (0.120 ml, 0.4 mmol) was added dropwise to the solution and the mixture was stored in the refrigerator overnight. The mixture was poured into ice-cold water (30 ml) and extracted with ether (30 ml × 3). The water-layer was neutralized with ammonium hydroxide. The solution was diluted with water to 300 ml and applied to a diethylaminoethylcellulose Sephadex A-25 column (1.6 × 20 cm) preequilibrated with water. Elution was with a linear gradient of 0 (600 ml) to 0.2 M (600 ml) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were collected and concentrated *in vacuo*. Water was added and the solvent was evaporated from the resulting residue to remove the residual triethylammonium bicarbonate. The desired products were isolated as the triethylammonium salt. Their structure were confirmed by enzymatic degradation using alkaline phosphatase.

Preparation of Nucleoside 5'-Triphosphates (9 and 11)[14] General

Procedure: A mixture of a 5'-monophosphate and *N,N'*-carbonyldiimidazole (10 eq) was dissolved in dry DMF. The reaction mixture was stirred for 2 h and methanol (0.01 ml) was added to the mixture. After stirring for 30 min, a solution of tributylammonium pyrophosphate (0.4 M, 1—2 ml) was added and the mixture was stirred overnight. The solution was diluted with water to 300 ml and applied to a DEAE-Sephadex A-25 column (1.6 × 20 cm) preequilibrated with water. Elution was with a linear gradient of 0 (600 ml) to 0.5 M (600 ml) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were collected and concentrated *in vacuo*. Water was added and the solvent was evaporated to remove the residual triethylammonium bicarbonate. The desired products (**9** and **11**) were isolated as the triethylammonium salts. Their structures were confirmed by enzymatic degradation using alkaline phosphatase.

Enzymatic Degradation 5'-Dephosphorylation of 5'-monophosphates and 5'-triphosphates was carried out *via* alkaline phosphatase exposure using 0.4 OD_{258} of the substrate and 0.06 unit of the enzyme in Tris–acetate (0.2 M, pH 8.8), $MgCl_2$ (0.001 M), and a total volume of 100 μl. Incubation was at 37 °C for 2 h. The digested products were confirmed by comparison with the corresponding nucleosides, respectively, using HPLC.

Antiviral Assay Procedures The anti-HIV-1 assay was based on the inhibition of the virus-induced cytopathic effect in MT-4 cells as previously described.[16] Briefly, MT-4 cells were suspended in culture medium at 1.0×10^5 cells/ml and infected with 2000 $CCID_{50}$ (50% cell culture infective dose) of HIV. Immediately after virus infection, 100 ml of the cell suspension was brought into each well of a flat-bottomed microtiter tray containing various concentrations of the test compounds. After a 4-d incubation at 37 °C, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cytotoxicity of the compounds was assessed in parallel with their antiviral activity. It was based on the viability of mock-infected host cells as determined by the MTT method.

Assay for HIV-1 RT RT activity was measured with $(rA)_n \cdot (dT)_{12-18}$ as the template·primer under the optimized reaction conditions specified for HIV-1 RT. The reaction mixture contained the following components: 50 mM Tris–HCl, pH 8.0; 3 μg/ml $(rA)_n \cdot (dT)_{12-18}$ (base ratio, 2 : 1); 10 μM [^{3}H]dTTP (400 cpm/pmol); 5 mM dithiothreitol; 100 mM KCl; 15% (v/v) glycerol; and 6 mM $MgCl_2$. All incubations (50 μl) were carried out at 37 °C for 30 min, and the reaction was stopped by adding 20 μl of 0.2 M ethylenediaminetetraacetic acid (EDTA) and immersing the mixture in ice. Then, 50 μl of the mixture was transferred to a DE81 filter paper disc and processed for radioactivity counting as previously described.[18]

References and Notes

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