Preparation and Anti-HIV Activity of N-3-Substituted Thymidine Nucleoside Analogs

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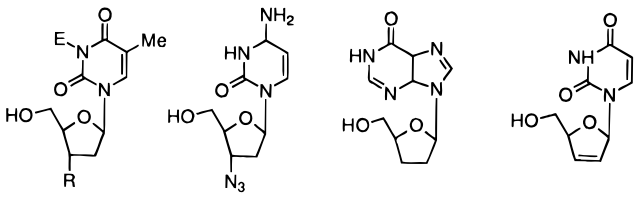
A series of 22 derivatives of AZT substituted at the N-3 position of the thymine base were prepared and evaluated for anti-HIV activity in cell culture (Lai strain of HIV-1 in CEM-c113 cells). The AZT analogs bearing a N-3 amino group (**7**), a hydroxyalkyl chain (**12f**), and a phosphonomethyl (**12k**) substituent displayed activities in the 0.045−0.082 μM range. The analogs **12d**, **12e**, **12q**, **15**, and **19** were active at <0.5 μM concentration. Compound **18** in which two molecules of AZT are connected at N-3 via a two-carbon link and "dimer" **11** also displayed significant activity. To obtain information concerning the mechanism of RT inhibition by these AZT analogs, compounds **7**, **12d**, **12e**, and **12q** were incubated with recombinant HIV-1 RT in the presence of poly(A)-oligo[$dT_{(12-18)}$] and poly(C)-oligo[$dG_{(12-18)}$] template−primers. In contrast to AZT−TP (control), none of these nucleosides displayed any significant inhibition of RT in the recombinant enzyme assay, indicating that phosphorylation is a necessary prerequisite for activity.

The discovery that the 2′,3′-dideoxynucleoside analogs 3′-azido-3′-deoxythymidine (AZT, **1**),[1] ddI (**2**),[2] and ddC (**3**)[3] are potent inhibitors of HIV reverse transcriptase (RT) marked a decisive advance in the search for anti-AIDS drugs. However, the long-term administration of these compounds to AIDS patients is limited by a number of severe side effects[4] and by the appearance of drug resistant HIV strains.[5] In the search for new, longer acting, more selective, and less toxic anti-HIV agents, a wide range of 2′,3′-dideoxynucleoside analogs have been prepared.[6] Interested by structure−activity relation studies in this area, we initiated a program several years back to examine the influence of substitution at the N-3 position of the thymine base of AZT, and other 2′,3′-dideoxynucleoside analogs, on HIV replication.[7−9] Assuming that such molecules inhibit RT in the same manner as AZT itself, one might, *a priori*, anticipate that N-3 substitution would alter base−base interactions at several levels and, in particular, in their capacity to act as chain terminators.[10] In initial experiments it was found that, in contrast to the N-3 amino derivatives of ddT (**4**), 3′-FddT (**5**), and D4T (**6**), which display little or no activity, N-3 amino AZT **7** (RP67042)[7,11] inhibited the HIV-induced cytopathic effect in CEM-c113 cells 7 days after infection with an EC_{50} of 45 nM and a selectivity index ($SI = CC_{50}/EC_{50}$) of up to 10 000 as measured by the MIT method. Furthermore, although **7** displayed reduced antiviral activity *in vitro* (anti-HIV assay) and *in vivo* (anti-FR-MuLV assay) relative to ACT (EC_{50} = 0.003 μM), it was determined to be potentially less toxic *in vivo* and to have a longer plasmatic half-life in mice.



1 AZT E = H, R = N_3 **2 ddC** **3 ddI** **6 D4T**
4 ddT E = R = H
5 FddT E = H, R = F
7 (RP67042) E = NH_2, R = N_3

Building upon these observations, we describe in the present paper the synthesis and evaluation for anti-HIV activity in cell culture (Lai strain of HIV-1 in CEM-c113 cells) of a series of 22 new AZT analogs (compounds **11**, **12a**−**q**, **15**, **17**, **18**, and **19**) bearing structurally and electronically diverse substituents at the N-3 position of the thymine ring.

Chemistry

The AZT employed in this work was prepared by the reaction of the 5′-*O*-(thexyldimethylsilyl)-protected anhydrothymidine derivative **8** with NaN_3 in hot DMF, followed by O-deprotection of intermediate **9** (R = thexyldimethylsilyl) (Scheme 1).[12] Although an excess of azide ion was generally used to obtain **9**, on several occasions formation of minor amounts of the dimer **10** was also observed. The structure of this minor component was deduced from its NMR and mass spectra. The formation of **10** can be seen to arise from competitive reaction of **8** with the N-3 anion of AZT, generated as an intermediate during N_3^--promoted anhydronucleoside ring opening. This supposition was confirmed in a separate experiment in which the N-3 anion of **9** (R = thexyldimethylsilyl) was generated and reacted with **8** in the absence of added sodium azide. Although exten-

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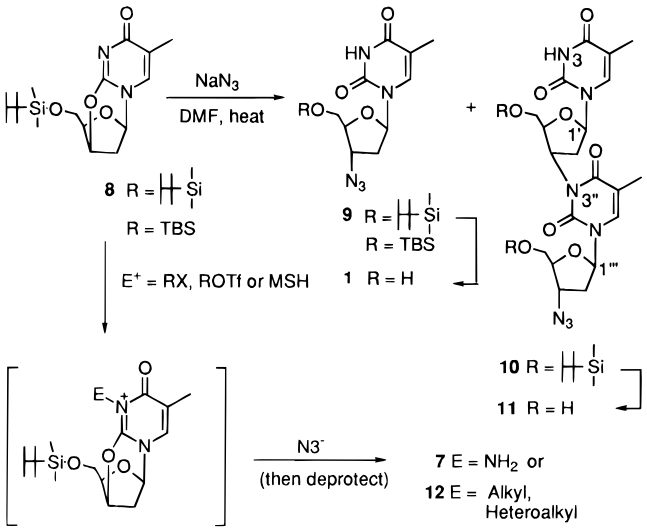
Scheme 1



sive decomposition of the anhydronucleoside occurred under these conditions, significant quantities of dimer **11** were isolated after O-deprotection.

As was previously shown, N-3-substituted derivatives of AZT can be prepared by reaction of anhydronucleoside **8** with an electrophile prior to addition of azide ion and ring opening (Scheme 1).[13] This approach works well for the formation of compounds **7** and **12a**. However, for electrophiles larger than a methyl group, there is competing N- and O-alkylation of **8**, the latter reaction leading to products wherein azide is introduced at C-1′ of the sugar component. For this reason, compounds **12a**−**q**, **15**, **17**, **18**, **19**, and dimer **11** were prepared by N-3 alkylation of AZT as a discrete step (see Tables 1 and 2 and Schemes 1 and 2).

For almost all cases, the preparation of the AZT derivatives **12** was conveniently achieved by deprotonation of AZT with NaH in DMF at 20 °C for 1 h (method A of Table 1) followed by addition of the appropriate electrophile. Use of excess NaH/alkylating agent led in some cases to the formation of small amounts of N-3,O-5′-dialkylated AZT byproducts. For the preparation of **12l**, glycidyl triflate proved far superior as an alkylating agent than the corresponding tosylate. In certain instances the use of method A failed, notably for the reactions with (diethylphosphono)-methyl triflate and bromoacetaldehyde diethyl acetal wherein AZT was recovered largely unreacted. This problem was resolved using potassium carbonate in DMF to deprotonate either AZT (method B) or its 5′-*O*-(*tert*-butyldimethylsilyl)-protected derivative **9** (method C). Analogs **12p** and **12q** were both prepared using 1-(2-bromoethyl)-4-methoxy-5-methylpyrimidin-2-one as the electrophile. In these cases the method C route proved advantageous, as it was more convenient to separate chromatographically the common silylated intermediate **13p** from the slight excess of alkylating agent prior to deprotection. The deprotection of **13p** to give **12p** was achieved using either tetrabutylammonium fluoride in THF or aqueous hydrochloric acid. The latter conditions served to simultaneously deprotect the O-5′ silyl ether and liberate the thymine ring carbonyl leading to **12q**. The use of 1-(2-bromoethyl)-5-methylpyrimidine-2,4-dione instead of the 4-methoxy analog as an electrophile for direct preparation of **12q** led, not surprisingly, to formation of substantial amounts of a product containing two molecules of alkylating agent.

With the exception of epoxide **12l**, which on contact with atmospheric moisture was observed to undergo slow nucleophilic ring opening to form diol **14** (Scheme 2), the entire series of AZT derivatives **12** proved stable under ambient conditions. For compound **12l**, it is likely that epoxide opening is assisted by participation of the thymine ring carbonyl group at C-2 (or C-4) as illustrated in the scheme. A similar mechanism involving an O-alkylated pyrimidinium ion intermediate is proposed to account for the formation of N-3 hydroxyethyl derivative **12d** in the reaction of O-5′ TBS AZT with excess 1,2-dibromoethane and potassium carbonate in hot methanol. These conditions were employed in an attempt to improve the yield of bromoethyl AZT derivative **12g** beyond that obtained by method A. In this instance there was a simultaneous exchange of the terminal bromo function by a hydroxyl group and deprotection of the O-5′ silyl ether affording **12d** directly in 95% yield.

The N-3 bromoethyl-substituted analog **12g** and its O-5′ TBS derivative **16** were also further elaborated to compounds **15**, **17**, and **18** (Table 2). Use of amberlite IR 400 (N_3^-) resin provided azide **15** (91%), and reaction of **16** with potassium phthalimide followed by HF afforded **17** in high yield. Reaction of **16** with **9** (R = TBS) gave the bridged dimer **18**.

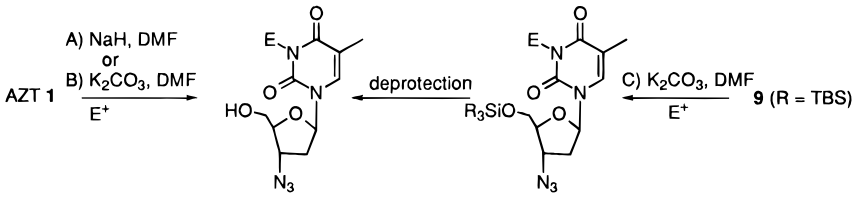
Finally compound **19**, containing an isoxazoline system attached to AZT at N-3 (Scheme 2), was prepared in two steps (55% overall yield) involving a 1,3-dipolar cycloaddition reaction between O-TBS **12c** and the nitrile oxide generated from the O-TBS derivative of 2-nitroethanol, and subsequent double deprotection.

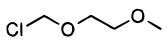
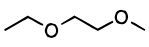
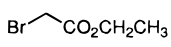
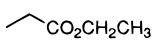
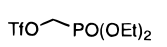
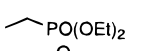
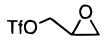
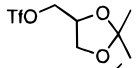
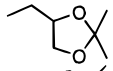
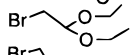
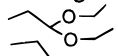
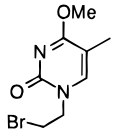
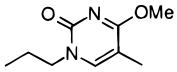
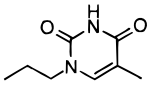
Biological Results and Discussion

Following the same protocols used to evaluate the anti HIV activity of the lead compound **7** (RP67042),[7,11] the N-3-substituted AZT analogs **12a**−**q** synthesized in this study were tested and compared to AZT for cytotoxicity and the ability to inhibit HIV replication in CEM-c113 cells (Lai strain[14]). The HIV-1-induced cytopathic effect (CPE) was monitored by the MTT viability assay to determine EC_{50MTT} and CC_{50} as previously described.[15] HIV replication was also followed by measurement of the reverse transcriptase activity in the culture supernatant (EC_{50RT}). The results obtained are shown in Table 3.

Compound **12a**, substituted at N-3 by a nonpolar methyl group, exhibited an EC_{50} of 940 nM. Increasing the length of the alkyl chain to *n*-pentyl (**12b**) offered no improvement in anti-HIV activity. Indeed, in both cases, the presence of an alkyl chain led to a 20-fold decrease in activity compared to RP67042, **7**. The introduction of an allyl double bond (**12c**) or a terminal bromine atom (**12g**) into the chain also leads to a significant drop in activity. Contrasting with this observation is the good level of activity observed when an azide function is present in the ethyl chain as in compound **15**. The absence of activity for compound **12i** indicated that a benzyl group is not tolerated at the N-3 position. This may be a consequence of both steric and electronic effects, as compounds **12o** and **17**, which are also substituted by bulky and relatively nonpolar aromatic substituents, are inactive.

Table 1



TRIAL	E^+	METHOD	PRODUCT	E	YIELD	FORMULA
1	$2,4\text{-}(O_2N)_2C_6H_3ONH_2$	A	7	$-NH_2$	85%	$C_{10}H_{14}N_6O_4$
2	CH_3I	A	12a	$-CH_3$	85%	$C_{11}H_{15}N_5O_4$
3	$C_5H_{11}Br$	C	12b	$-C_5H_{11}$	96%	$C_{15}H_{23}N_5O_4$
4	$BrCH_2CH{=}CH_2$	A	12c	$-CH_2CH{=}CH_2$	86%	$C_{13}H_{17}N_5O_4$
5	$BrCH_2CH_2OH$	A	12d	$-CH_2CH_2OH$	88%	$C_{12}H_{17}N_5O_5$
6	$Br\text{-}(CH_2)_3\text{-}OH$	A	12e	$-(CH_2)_3\text{-}OH$	68%	$C_{13}H_{19}N_5O_5$
7	$Br\text{-}(CH_2)_6\text{-}OH$	A	12f	$-(CH_2)_6\text{-}OH$	85%	$C_{16}H_{25}N_5O_5$
8	$BrCH_2CH_2Br$	A	12g	$-CH_2CH_2Br$	56%	$C_{12}H_{16}BrN_5O_4$
9		A	12h		74%	$C_{14}H_{21}N_5O_6$
10		A	12i		86%	$C_{17}H_{19}N_5O_4$
11		A	12j		66%	$C_{14}H_{19}N_5O_6$
12		C	12k		54%	$C_{15}H_{24}N_5O_7P$
13		A	12l		81%	$C_{13}H_{17}N_5O_5$
14		A	12m		73%	$C_{16}H_{23}N_5O_6$
15		B	12n		63%	$C_{16}H_{25}N_5O_6$
16		A	12o		77%	$C_{15}H_{17}N_5O_5$
17		C	12p		96%	$C_{18}H_{23}N_7O_6$
18		C	12q		74%	$C_{17}H_{21}N_7O_6$

Compounds **12a**, **12c**, and **12i** have recently been examined for their anti-HIV activity in MT4 cells by Kitade *et al.*[8] In their study, the N-3 allyl-substituted analog **12c** was found to display significant activity, but in keeping with our findings **12i** was also determined to be inactive.

Interestingly, the presence of a terminal hydroxy group on the alkyl chain as in compounds **12d**−**f** restored activity to a level comparable to that of **7**. In fact, for this set of compounds, activity was observed to increase with increasing chain length. The influence of a terminal hydroxyl substituent on anti-HIV activity was most striking for compound **19** (EC_{50} = 0.5 μM), which like **12o** (EC_{50} = 143 μM) contains a heterocyclic component in the side chain. However, simply incorporating an oxygen atom into the N-3 substituent does not appear to be responsible for this effect, as activity dropped to the micromolar range for the (methoxyethoxy)methyl ether **12h** as well as for ketals **12m** and **12n**, epoxide **12l**, and the methyl ester **12j**. A noted exception is the diethyl phosphonate analog **12k** which displays an EC_{50} of 0.071 μM.

Looking further to determine the extent to which large substituents are tolerated at the N-3 position of AZT, it was found that analog **12q**, and especially compound **18** where two AZT components are linked via a two-carbon tether, are potent inhibitors, with EC_{50}'s of 0.48 and 0.7 μM, respectively. Impressive also was the finding that the deprotected dimer **11**, where a 3′-deoxythymidine residue is attached directly to the N-3 position of AZT, displays significant capacity to inhibit HIV-1 replication. However, for **12q** activity was totally lost on conversion of the C-4 amide carbonyl to the corresponding *O*-methyl imidate, as in **12p**.

From the results obtained, it is clear that a varied range of substituents can be introduced at the N-3 position of the pyrimidine ring in AZT. Indeed, 11 of the 22 N-3 functionalized AZT analogs studied inhibited HIV replication *in vitro* with 50% effective concentrations in the micromolar range or lower. Of these,

Table 2

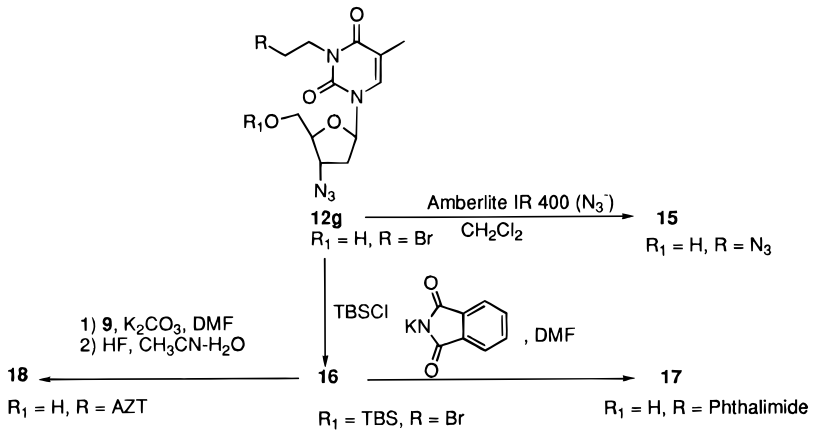
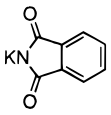
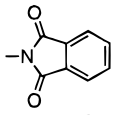
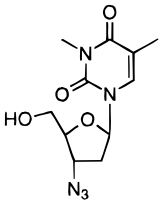
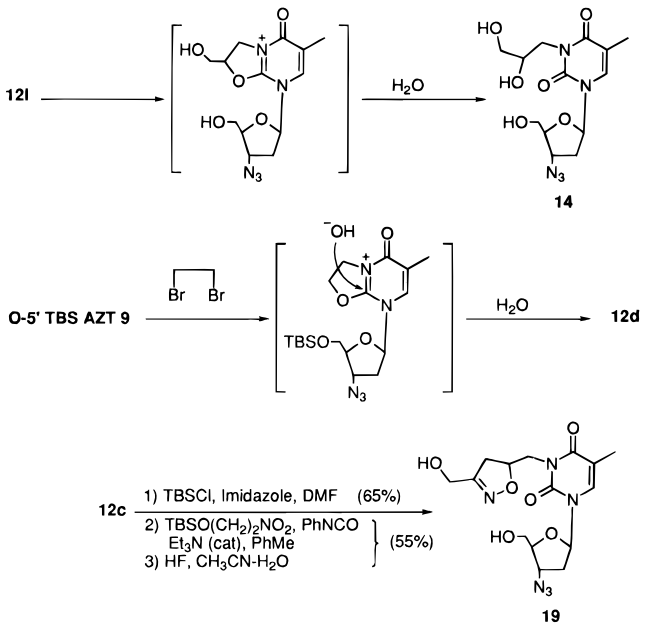


TABLE II

TRIAL	SUBSTRATE	Nu⁻	PRODUCT	R	YIELD	FORMULA
1	**12g**	Resin (N_3)	**15**	N_3	91%	$C_{12}H_{16}N_8O_4$
2	**16**		**17**		82%	$C_{20}H_{20}N_6O_6$
3	**16**	**1** (R = TBS)	**18**		68%	$C_{22}H_{28}N_{10}O_8$

Scheme 2



compounds **12d**, **12e**, **12q**, **15**, and **19** were active at less than 0.5 μM concentration, and compounds **12f** and **12k** were essentially equipotent to the lead compound **7** (RP67042) in CEM-c113 cells (i.e. EC_{50}'s = 50 nM).

Having established that biological activity can be maintained upon substitution of the N-3 position of AZT, a number of fundamental questions arise. Firstly, do these compounds interact with RT at the nucleotide binding site, and if so, do they inhibit RT in a similar manner to AZT? For AZT it has been clearly shown that the drug must initially be phosphorylated by cellular kinases and that it is AZT triphosphate which inhibits RT through competitive binding to the DNA synthesis site and by chain termination upon incorporation into the growing DNA chain. Secondly, if these N-3-substituted analogues do bind to the DNA synthesis site, does the added functionality on the base component spill into empty space in the cleft normally occupied by the template primer (or growing DNA chain), or does it interact in some way with the neighboring "allosteric" binding site?[16] Thirdly, can the presence of the N-3 substitution alter the manner in which these AZT analogues interact with RT to the point where, like the HEPT and TSAO series of compounds,[17,18] they associate preferentially with RT at the allosteric binding site?[19]

In view of both the bulky nature of the functionality present at N-3 in certain of our ACT analogs, and the substrate specificity generally displayed by cellular kinases, it was not immediately obvious that our compounds inhibit RT through an AZT-type mechanism. Concerning this problem, Kitade et al.[8] found that the triphosphate derivatives of N-3 allyl, and several other N-3 alkyl derivatives of AZT, are indeed moderate inhibitors of RT in *in vitro* assays. However, as the molecules studied by these authors correspond to only weakly active members of our series, this point required further evaluation. Thus, compounds **7**, **12d**, **12e**, and **12q** were incubated with recombinant HIV-1 RT[20] in the presence of poly(A)-oligo[$dT_{(12-18)}$] and poly(C)-oligo-[$dG_{(12-18)}$] template−primers, and the activities were monitored at varying concentrations (up to 100 μM). In

Table 3. In Vitro Anti-HIV Activity of N-3-Substituted AZT Analogs[a]

prod	EC_{50MTT} (μM)[b]	CC_{50} (μM)[c]	EC_{50RT} (μM)[d]	SI[e]
7	0.045	450	0.5	10000
12a	0.940	355	NT	380
12b	0.890	>60	NT	>67
12c	32.0	350	65	11
12d	0.300	161	0.09	537
12e	0.150	154	0.12	1027
12f	0.082	64	1.2	780
12g	2.670	53	NT[f]	20
12h	5.600	281	140	50
12i	NA[f]	28	NT	–
12j	1.470	283	14	193
12k	0.071	240	4.7	3380
12l	6.80	>62	NT	9
12m	1.30	>131	13	100
12n	5.00	130	1.5	26
12o	143.0	>288	143	2
12p	NA	29	>29	–
12q	0.480	>29	NT	60
11	2.2	66	0.1	30
15	0.260	60	1.5	230
17	110.0	227	114.0	2
18	0.700	36	1.4	51
19	0.500	26	1.3	52
AZT	0.003	>5	0.003	1667

[a] All tests were carried out in triplicate, and the results represent mean values for at least two experiments in three wells. [b] EC_{50MTT} (50% antiviral effective dose): concentration (μM) that reduces by 50% the HIV induced cytopathic effect. [c] CC_{50} (50% cytotoxic dose): concentration (μM) required to reduce by 50% the viability of noninfected treated cells. [d] EC_{50RT}: concentration (μM) that reduces by 50% the HIV produced in the supernatant (see ref 15). [e] SI (selectivity index): ratio of CC_{50} to EC_{50}. [f] NA: not active. NT: not tested.

contrast to AZT triphosphate, which was used as a control, none of these nucleosides displayed any significant inhibition of RT in the recombinant enzyme assays. This result contrasts markedly with the activities observed in cell culture and in an indirect fashion strongly supports the hypothesis that N-3-substituted analogs of AZT require transformation to the corresponding triphosphate forms before becoming effective inhibitors of RT. It is very probable therefore that, like AZT, these compounds inhibit RT through competitive binding in the catalytic site for DNA synthesis.

Recent molecular modeling studies carried out in our laboratory in which thymidine triphosphate is fitted into the binding site that it interacts correctly with the three catalytic aspartates and two Mg^{2+} ions show that, in fact, the N-3 position of thymidine points into the enzyme cleft. Thus, at least in the absence of the primer–template, the enzyme experiences little difficulty in accommodating functionality present at this position. Further modeling experiments provide a picture of the spatial relationship between this N-3 functionality and non-nucleoside inhibitors, such as TIBO and nevirapine, which are bound in the adjacent allosteric pocket.[16] Together with recent X-ray crystallographic results, this information serves as a valuable guide in work underway to construct "mixed" site inhibitors.[21]

Experimental Section

Melting points were determined using a Reichert Thermovar apparatus and are uncorrected. Mass spectra were obtained on an MS-50 AEI (EI, 70 eV) or an MS-9 AEI (CI, isobutane) spectrometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ (except where noted) on a Brüker spectrometer (200 or 250 MHz), using tetramethylsilane as an internal standard. Chemical shift data are reported in parts per million (δ in ppm) where s, d, dd, t, q, and m designate singlet, doublet, doublet of doublets, triplet, quartet, and multiplet, respectively. ^{13}C NMR spectra where recorded in $CDCl_3$ on the same instruments. Flash column chromatography was performed using Merck aluminoxid 90 (activity II-III) or silica gel 60 (Art. 9385). In all cases the solvent system used for the chromatographic separations was chosen such that on TLC an R_f of 0.25–0.30 was observed for the compound to be isolated. All microanalytical results for C, H, and N are within ±0.4% of the theoretical value.

Preparation of Dimer 11. To a solution of 5′-*O*-thexyldimethylsilyl AZT **9** (40 mg, 0.098 mmol) in DMF (1 mL), stirred at room temperature (23 °C) under argon, was added sodium hydride (2.90 mg, 0.117 mmol) followed, after 30–60 min, by 5′-*O*-(thexyldimethylsilyl)-protected anhydrothymidine **9** (53.7 mg, 0.147 mmol). The resulting mixture was heated at 120 °C for 20 h. In instances where substantial amounts of unreacted **9** remained (TLC), a further quantity of NaH (0.6 equiv) was added, and heating was continued for an additional 12 h. The solvent was then evaporated *in vacuo*, and a preliminary separation of the O-5′-protected dimer **10** from other components in the product mixture was achieved by preparative layer chromatography (silica gel; EtOAc/heptane). Compound **10** was then obtained pure (8 mg; 11%) after flash column chromatography (silica gel; 45% ethyl acetate/55% heptane). Subsequent treatment of this product with excess TBAF and normal extractive workup provided dimer **11**: ^{1}H NMR (^{1}H–^{1}H correlation; DMSO-d_6) δ 11.25 (s, H-3), 7.80 (s, 2H, H-6, H-6″), 6.57 (t, J = 7.5 Hz, H-1′), 6.15 (t, J = 5 Hz, H-1‴), 5.58 (m, H-3′), 5.25 (t, J = 5 Hz, OH‴), 4.98 (t, OH′), 4.39 (q, J = 6 Hz, H-3‴), 4.12 (m, H-4′), 3.84 (m, H-4‴), 3.63 and 3.54 (2m, 4H, H-5′, H-5‴), 2.50–2.18 (m, 4H, H-2′, H-2‴), 1.84 (s, CH_3), 1.79 (s, CH_3); ^{13}C NMR (DMSO-d_6) δ 163.70 and 162.81 (C-2, 2″), 150.46, 150.25 (C-4, 4″), 136.71, 134.86 (C-6, 6″), 109.62, 108.78 (C-5, 5″), 84.76, 84.42, 84.20, 81.68 (C-1′, 1‴, C-4′, 4‴) 61.97, 60.5 (C-5′, 5‴), 59.65 (C-3‴), 50.71 (C-3′), 36.55, 34.81 (C-2′, 2‴), 12.90, 12.14 (CH_3).

Method A: 3-Amino-3′-azido-3′-deoxythymidine (7). To a solution of AZT (60 mg, 0.225 mmol) in DMF (3 mL), stirred at room temperature (23 °C) under argon, was added sodium hydride (6.47 mg, 0.270 mmol) followed, after 30–60 min, by *O*-(2,4-dinitrophenyl)hydroxylamine (52.5 mg, 0.26 mmol).[22] After an additional 18 h the solvent was removed *in vacuo*, and the residue was flash column chromatographed (silica gel; 25% ethyl acetate/heptane) to afford compound **7** as a colorless solid (54 mg; 85%): mp 75 °C ($EtOH/Et_2O$); all spectral data coincide with the results reported in ref 16.

3-Methyl-3′-azido-3′-deoxythymidine (12a). AZT (100 mg, 0.374 mmol), NaH (11 mg, 0.45 mmol), and CH_3I (0.25 mL, 0.40 mmol). Purification by silica gel flash chromatography (80% EtOAc/heptane). **12a**: colorless oil (90 mg; 85%); IR ($CHCl_3$, cm^{-1}) 3450, 2115, 1686, 1672, 1630; ^{1}H NMR ($CDCl_3$) δ 7.37 (s, H-6), 6.05 (t, J = 6 Hz, H-1′), 4.37–4.43 (m, 1H, H-3′), 3.77–4.02 (m, 3H, CH_2-5′, H-4′), 3.34 (s, NCH_3), 2.74 (br m, OH), 2.57 (ddd, J = 14, 7.5, 6 Hz, 1H, H-2′), 2.40 (ddd, 1H, J = 14, 6, 5 Hz, H-2′), 1.94 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.49 (C-2), 150.64 (C-4), 134.27 (C-6), 109.53 (C-5), 86.12 (C-1′), 84.36 (C-4′), 61.39 (C-5′), 59.68 (C-3′), 37.38 (C-2′), 27.51 (NCH_3), 12.87 (CH_3); CI-MS m/z 282 $(M + 1)^+$. Anal. ($C_{11}H_{15}N_5O_4$) C, H, N.

3-Allyl-3′-azido-3′-deoxythymidine (12c). AZT (299 mg, 1.12 mmol), NaH (27 mg, 1.13 mmol), and allyl bromide (0.14 mL, 1.65 mmol). Purification by silica gel flash chromatography (50% EtOAc/heptane). **12c**: colorless oil (295 mg; 86%); IR (neat, cm^{-1}) 3453, 2106, 1705, 1665, 1635; ^{1}H NMR ($CDCl_3$) δ 7.38 (s, H-6), 6.07 (t, J = 6 Hz, H-1′), 5.95–5.79 (m, CH═), 5.29–5.16 (m, ═CH_2), 4.55 (d, J = 5 Hz, NCH_2), 4.45–4.38 (m, H-3′), 4.04–3.79 (m, 3H, CH_2-5′, H-4′), 2.61–2.50 (m, 2H, H-2′, OH), 2.45–2.35 (m, 1H, H-2′), 1.94 (s, CH_3), ^{13}C NMR ($CDCl_3$) δ 163.43 (CO), 150.81 (CO), 134.87 (C-6), 131.66 (CH═), 118.26 (═CH_2), 110.62 (C-5), 87.53 (C-1′), 84.60 (C-4′), 62.12 (C-5′), 60.04 (C-3′), 43.68 (NCH_2), 37.48 (C-2′), 13.34 (CH_3); EI-MS m/z 307 M^+. Anal. ($C_{13}H_{17}N_5O_4$) C, H, N.

3-(2-Hydroxyethyl)-3′-azido-3′-deoxythymidine (12d). AZT (260 mg, 0.973 mmol), NaH (28 mg, 1.16 mmol), and

2-bromoethanol (0.128 mL, 1.8 mmol). Purification by silica gel flash chromatography (EtOAc/heptane/MeOH, 7/3/1)). **12d**: colorless oil (290 mg; 88%); IR (neat, cm^{-1}) 3443, 2105, 1705, 1670, 1636; ^1H NMR ($CDCl_3$) δ 7.50 (s, H-6), 6.11 (t, J = 6 Hz, H-1′), 4.38 (m, H-3′), 4.20 (t, J = 5 Hz, NCH_2), 4.02−3.75 (m, 5H, CH_2-5′, H-4′, CH_2OH), 3.08 (br s, OH), 2.87 (br s, OH), 2.52−2.38 (m, 2H, CH-2′), 1.93 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.26 (CO), 151.40 (CO), 134.95 (C-6), 110.31 (C-5), 86.56 (C-1′), 84.62 (C-4′), 61.69 and 61.06 (C-5′, CH_2OH), 59.85 (C-3′), 43.73 (NCH_2), 37.63 (CH_2-2′), 13.16 (CH_3); CI-MS m/z 312 $(M + 1)^+$. Anal. ($C_{12}H_{17}N_5O_5$) C, H, N.

3-(3-Hydroxypropyl)-3′-azido-3′-deoxythymidine (12e). AZT (1.60 g, 5.99 mmol), NaH (173 mg, 7.21 mmol), and 3-bromopropanol (0.82 mL, 9.03 mmol). Purification by silica gel flash chromatography (2% MeOH/EtOAc). **12e**: colorless oil (1.22 g; 63%); IR (neat, cm^{-1}) 3439, 2107, 1702, 1660, 1635; ^1H NMR ($CDCl_3$) δ 7.69 (s, H-6), 6.18 (t, J = 6 Hz, H-1′), 4.39 (app q, J = 6 Hz, H-3′), 4.08 (t, J = 6 Hz, CH_2N), 3.98−3.71 (m, 5H, CH_2-5′, H-4′, 2 × OH), 3.56−3.49 (m, 2H, CH_2OH), 2.43 (app t, 2H, J = 6 Hz, CH_2-2′), 1.92 (s, CH_3), 1.85 (p, J = 6 Hz, CH_2); ^{13}C NMR ($CDCl_3$) δ 163.85 (CO), 151.12 (CO), 134.91 (C-6), 110.14 (C-5), 86.36 (C-1′), 84.66 (C-4′), 61.64 (C-5′), 59.89 (C-3′), 58.81 (CH_2), 37.93 and 37.78 (C-2′, NCH_2), 30.42 (CH_2), 13.19 (CH_3); CI-MS m/z 326 $(M + 1)^+$. Anal. ($C_{13}H_{19}N_5O_5$) C, H, N.

3-(6-Hydroxyhexyl)-3′-azido-3′-deoxythymidine (12f). AZT (546 mg, 2.04 mmol), NaH (59 mg, 2.5 mmol), and 6-bromohexanol (0.4 mL, 3.05 mmol). Purification by silica gel flash chromatography (EtOAc). **12f**: colorless oil (640 mg; 85%); IR (neat, cm^{-1}) 3429, 2107, 1702, 1669, 1636; ^1H NMR ($CDCl_3$) δ 7.48 (s, H-6), 6.11 (t, J = 13 Hz, H-1′), 4.37 (app q, J = 6 Hz, H-3′), 4.00−3.74 (m, 5H, CH_2-5′, H-4′, NCH_2), 3.61 (t, 2H, J = 6 Hz, CH_2OH), 3.46 (br s, OH), 2.52−2.38 (m, 2H, CH_2-2′), 2.23 (br s, OH), 1.91 (s, CH_3), 1.70−1.30 (m, 8H, CH_2); ^{13}C NMR ($CDCl_3$) δ 163.49 (CO), 150.85 (CO), 134.63 (C-6), 110.27 (C-5), 86.85 (C-1′), 84.64 (C-4′), 62.62 (CH_2OH), 61.82 (C-5′), 59.99 (C-3′), 41.24 (NCH_2), 37.59 (CH_2-2′), 32.50 (CH_2), 27.41 (CH_2), 26.50 (CH_2), 25.20 (CH_2), 13.23 (CH_3); CI-MS m/z 368 $(M + 1)^+$. Anal. ($C_{16}H_{25}N_5O_5$) C, H, N.

3-(2-Bromoethyl)-3′-azido-3′-deoxythymidine (12g). AZT (1.07 g, 4.00 mmol), NaH (110 mg, 4.58 mmol), and 1,2-dibromoethane (2.0 mL, 23.2 mmol). Purification by silica gel flash chromatography (EtOAc). **12g**: colorless solid (841 mg; 56%); mp 96−98 °C ($CHCl_3$/hexane); IR ($CHCl_3$, cm^{-1}) 3432, 2108, 1700, 1663, 1635; ^1H NMR ($CDCl_3$) δ 7.59 (s, H-6), 6.13 (t, J = 6 Hz, H-1′), 4.40 (app q, J = 6 Hz, H-3′), 4.33 (t, J = 7 Hz, NCH_2), 3.95−4.00 (m, 2H, H-4′; H-5′), 3.84 (dd, 1H, J = 9, 3 Hz, H-5′), 3.53 (t, J = 7 Hz, CH_2Br), 3.22 (br s, OH), 2.40−2.50 (m, 2H, CH_2-2′), 1.92 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163 (CO), 150.53 (CO), 135.10 (C-6), 110.13 (C-5), 86.69 (C-1′), 84.60 (C-4′), 61.80 (C-5′), 59.91 (C-3′), 42.09 (NCH_2), 37.60 (C-2′), 27.26 (CH_2Br), 13.13 (CH_3); CI-MS m/z 376 and 374 $(M + 1)^+$. Anal. ($C_{12}H_{16}BrN_5O_4$) C, H, N.

3-[(Methoxyethoxy)methyl]-3′-azido-3′-deoxythymidine (12h). AZT (500 mg, 1.87 mmol), NaH (54 mg, 2.25 mmol), and methoxyethoxymethyl chloride (0.32 mL, 2.80 mmol). Purification by silica gel flash chromatography (1% MeOH/EtOAc). **12h**: colorless oil (490 mg; 74%); IR (neat, cm^{-1}) 3467, 2119, 1702, 1676; ^1H NMR ($CDCl_3$) δ 7.42 (s, H-6), 6.08 (t, J = 7 Hz, H-1′), 5.47 (s, OCH_2N), 4.23−4.36 (m, H-3′), 4.05−3.51 (m, 7H, CH_2-5′, H-4′, OCH_2CH_2O), 3.55 (s, CH_3O), 2.59−2.33 (m, 3H, CH_2−2′, OH), 1.93 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.01 (CO), 150.43 (CO), 134.87 (C-6), 109.46 (C-5), 85.28 (C-1′), 84.18 (C-4′), 71.13 and 69.07 (OCH_2CH_2O), 70.59 (OCH_2N), 61.14 (C-5′), 59.64 (C-3′), 58.27 (CH_3O), 37.16 (CH_2-2′), 12.54 (CH_3); CI-MS m/z 356 $(M + 1)^+$. Anal. ($C_{14}H_{21}N_5O_4$) C, H, N.

3-Benzyl-3′-azido-3′-deoxythymidine (12i). AZT (224 mg, 0.838 mmol), NaH (25 mg, 1.04 mmol), and benzyl bromide (0.10 mL, 0.925 mmol). Purification by silica gel flash chromatography (EtOAc/heptane, 1/3). **12i**: colorless needles (257 mg; 86%); mp 143−144 °C (methanol); IR ($CHCl_3$, cm^{-1}) 3376, 2113, 1701, 1670, 1654, 1628; ^1H NMR ($CDCl_3$) δ 7.26−7.47 (m, 6H, ArCH, H-6), 6.09 (t, J = 6.5 Hz, H-1′), 5.10 (s, $PhCH_2$), 4.32−4.40 (m, H-3′), 3.70−4.00 (m, 3H, CH_2-5′, H-4′), 2.92−3.00 (m, OH), 2.30−2.55 (m, 2H, CH_2-2′), 1.91 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.47 (CO), 150.78 (CO), 136.52 (ArC), 134.76 (C-6), 128.74 (2 × ArCH), 128.32 (2 × ArCH), 127.57 (ArCH), 110.04 (C-5), 86.42 (C-1′), 84.49 (C-4′), 61.66 (C-5′), 59.86 (C-3′), 44.41 (NCH_2), 37.58 (C-2′), 13.19 (CH_3); CI-MS m/z 358 $(M + 1)^+$. Anal. ($C_{12}H_{16}N_5O_4$) C, H, N.

3-[(Ethylacetoxy)methyl]-3′-azido-3′-deoxythymidine (12j). AZT (205 mg, 0.765 mmol) NaH (25 mg, 1.04 mmol), and ethyl bromoacetate (0.10 mL, 0.902 mmol). Purification by silica gel flash chromatography (50% EtOAc/heptane). **12j**: colorless oil (177 mg; 66%); IR (neat, cm^{-1}) 3483, 2106, 1752, 1708, 1654, 1636; ^1H NMR ($CDCl_3$) δ 7.58 (s, H-6), 6.12 (t, J = 6 Hz, H-1′), 4.68 (s, NCH_2), 4.36 (app q, J = 6 Hz, H-3′), 4.22 (q, J = 7 Hz, OCH_2), 3.74−3.98 (m, 3H, CH_2-5′, H-4′), 3.17 (br t, OH), 2.40−2.47 (m, 2H, CH_2-2′), 1.92 (s, CH_3), 1.29 (t J = 7 Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 168.09 (CO), 162.91 (CO), 150.56 (CO), 135.12 (C-6), 110.20 (C-5), 86.76 (C-1′), 84.66 (C-4′), 61.75 (C-5′ + OCH_2), 59.78 (C-3′), 42.11 (NCH_2), 37.71 (CH_2-2′), 14.12 (CH_3), 13.13 (CH_3); CI-MS m/z 354 $(M + 1)^+$. Anal. ($C_{14}H_{10}N_5O_6$) C, H, N.

3-Glycidyl-3′-azido-3′-deoxythymidine (12l) and Diol 14. AZT (307 mg, 1.15 mmol), NaH (27 mg, 1.12 mmol), and glycidyl triflate[23] (347 mg, 1.68 mmol). Purification by silica gel flash chromatography (50% EtOAc/heptane). **12l**: colorless oil (303 mg; 81%); IR (neat, cm^{-1}) 3432, 2108, 1705, 1675, 1635; ^1H NMR ($CDCl_3$) δ 7.55 (s, H-6), 6.14 (t, J = 6 Hz, H-1′), 4.39 (app q, J = 6 Hz, H-3′), 4.23 (ddd, ^1H, J = 14, 5, 2 Hz, NCH_2), 4.07−3.75 (m, 4 H, CH_2-5′, H-4′, CH_2N), 3.26−3.20 (m, CH), 3.15 (br s, OH), 2.77 (t, 1H, J = 4 Hz, *cis*-H of epoxide CH_2), 2.68 (dd, ^1H, J = 5, 2 Hz, *trans*-H of epoxide CH_2), 2.49−2.41 (m, 2H, CH_2-2′), 1.92 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.35 (CO), 150.87 (CO), 134.95 (C-6), 110.19 (C-5), 86.69 (C-1′), 84.60 (C-4′), 61.83 (C-5′), 59.96 (C-3′), 49.98 (CH), 46.45, 42.82 (CH_2, NCH_2), 37.60 (C-2′), 13.18 (CH_3); CI-MS m/z 324 $(M + 1)^+$. Anal. ($C_{13}H_{17}N_5O_5$) C, H, N.

On standing under ambient conditions, epoxide **12l** slowly added water to form diol **14**: ^1H NMR ($CDCl_3$) δ 7.54 (s, H-6), 6.12 (m, H-1′), 4.40 (app q, J = 6 Hz, H-3′), 4.21−4.18 (m, NCH_2), 4.05−3.82 (m, 4H, CH_2-5′, H-4′ CHOH), 3.55 (d, J = 5 Hz, CH_2OH), 2.68 (br s, 3H, 3 × OH), 2.54−2.44 (m, 2H, CH_2-2′), 1.95 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.71 (CO), 151.85 (CO), 135.26 (C-6), 110.59 (C-5), 87.01 (C-1′), 84.72 (C-4′), 70.67 (CHOH), 63.76 (CH_2OH), 61.86 (C-5′), 59.83 (C-3′), 43.90 (NCH_2), 37.79 (CH_2-2′), 13.29 (CH_3); CI-MS m/z (CI) 342 $(M + 1)^+$.

3-[(2,2-Dimethyl-1,3-dioxolan-4-yl)methyl]-3′-azido-3′-deoxythymidine (12m). AZT (299 mg, 1.12 mmol), NaH (38 mg, 1.58 mmol), and (2,2-dimethyl-1,3-dioxolan-4-yl)methyl triflate (444 mg, 1.67 mmol). Purification by silica gel flash chromatography (75% EtOAc/heptane). **12m**: colorless oil (310 mg; 73%); IR ($CHCl_3$, cm^{-1}) 3458, 2106, 1705, 1665, 1635; ^1H NMR (neat) δ 7.33 (s, H-6), 6.03 (t, J = 6 Hz, H-1′), 4.45−4.29 (m, 3H), 4.08−3.75 (m, 6H), 2.64−2.34 (m, 3H, CH_2-2′, OH), 1.93 (s, CH_3), 1.46 (s, CH_3), 1.32 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.39 (CO), 150.72 (CO), 134.73 (C-6), 109.75 and 109.34 (C-5 and OCO), 86.25 (C-1′), 84.46 (C-4′), 73.00 (OCH), 67.54 (OCH_2), 61.52 (C-5′), 59.82 (C-3′), 43.64 (NCH_2), 37.50 (CH_2-2′), 26.50, 25.33 (CH_3), 13.05 (CH_3); CI-MS m/z 382 $(M + 1)^+$. Anal. ($C_{16}H_{23}N_5O_6$) C, H, N.

3-(3-Furanylmethyl)-3′-azido-3′-deoxythymidine (12o). AZT (433 mg, 1.62 mmol), NaH (50 mg, 2.08 mmol), and 3-(bromomethyl)furan (378 mg, 2.35 mmol). Purification by silica gel flash chromatography (50% EtOAc/heptane). **12o**: colorless oil (433 mg, 77%); IR (neat, cm^{-1}) 3458, 2105, 1698, 1635; ^1H NMR ($CDCl_3$) δ 7.52 (s, H-6), 7.47 (d, J = 0.9 Hz, furan CH-O), 7.31 (t, J = 1.4 Hz, furan CH-O), 6.48 (d, J = 1.4 Hz, furan H-4), 6.11 (t, J = 6 Hz, H-1′), 4.93 (s, NCH_2), 4.39 (dt, J = 7, 5 Hz, H-3′), 3.94−4.01 (m, 2H, H-5′, H-4′), 3.80 (dd, 1H, J = 12.5, 3 Hz, H-5′), 2.32−2.57 (m, 2H, CH_2-2′), 1.90 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.16 (C-2), 150.69 (C-4), 142.66 and 142.30 (furan ring C-2, C-5), 134.81 (C-6), 120.26 (furan ring C-3), 111.60 (furan ring C-4), 110.40 (C-5), 87.00 (C-1′), 84.59 (C-4′), 61.92 (C-5′), 59.99 (C-3′), 37.55 (C-2′), 35.44 (NCH_2), 13.22 (CH_3); CI-MS m/z 348 $(M + 1)^+$. Anal. ($C_{15}H_{17}N_5O_5$) C, H, N.

Method B: 3-(2,2-Diethoxyethyl)-3′-azido-3′-deoxythymidine (12n). To a stirred solution of AZT (200 mg, 0.748

mmol) in DMF (2 mL) at 40 °C under argon was added potassium carbonate (207 mg, 1.50 mmol), potassium iodide (37 mg, 0.22 mmol), 18-crown-6 (39 mg, 0.15 mmol), and bromoacetaldehyde diethyl acetal (225 mL, 1.50 mmol), and after 18 h, further potassium carbonate (207 mg, 1.50 mmol) and bromoacetaldehyde diethyl acetal (0.225 mL, 1.50 mmol) were added. After 48 h the reaction mixture was evaporated, triturated with chloroform, and filtered. The filtrate was evaporated and the residue silica gel flash column chromatographed (50% EtOAc/heptane) to afford **12n** as a colorless oil (180 mg; 63%): IR (neat, cm^{-1}) 3450, 2105, 1707, 1661; ^1H NMR ($CDCl_3$) δ 7.55 (s, H-6), 6.07 (t, $J = 6$ Hz, H-1′), 4.83 (t, 1H, $J = 6$ Hz, C*H*$(OEt)_2$), 4.35 (app q, $J = 5$ Hz, H-3′), 4.05 (d, $J = 5$ Hz, NCH_2), 3.93−3.38 (m, 10H, CH_2-5′, H-4′, OH 2 × OCH_2), 2.42−2.34 (m, 2H, CH_2-2′), 1.86 (s, CH_3), 1.09 (t, $J = 7$ Hz, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ 163.41 (CO), 150.85 (CO), 134.84 (C-6), 109.94 (C-5), 98.59 (*CH*$(OEt)_2$), 86.78 (C-1′), 84.61 (C-4′), 61.94 (2 × OCH_2), 61.77 (C-5′), 60.01 (C-3′), 42.53 (NCH_2), 37.62 (C-2′), 15.19 (2 × CH_3), 13.20 (CH_3); CI-MS *m/z* 384 weak $(M + 1)^+$, 338 $(M - OEt)^+$. Anal. ($C_{16}H_{25}N_5O_6$) C, H, N.

Method C: 3-Pentyl-3′-azido-3′-deoxythymidine (12b). 5′-*O*-TBS AZT **9** (187 mg, 0.490 mmol), K_2CO_3 (168 mg, 1.22 mmol), and pentyl bromide (0.150 mL, 1.21 mmol). Intermediate **13b** was directly treated with HF in CH_3CN, and the residue obtained after workup was purified by silica gel flash column chromatography (50% EtOAc/heptane). **12b**: colorless oil (159 mg; 96%); IR ($CHCl_3$, cm^{-1}) 3449, 2105, 1702, 1670, 1636; ^1H NMR (neat) δ 7.55 (s, H-6), 6.13 (t, $J = 6$ Hz, H-1′), 4.41 (app, q, $J = 6$ Hz, H-3′), 3.78−4.03 (m, 5H, H-4′, CH_2-5′, NCH_2), 3.49 (t, OH), 2.39−2.54 (m, 2H, CH_2-2′), 1.91 (s, CH_3), 1.58 (p, 2H, $J = 7$ Hz, CH_2), 1.24−1.37 (m, 4H, 2 × CH_2), 0.87 (t, 3H, $J = 7$ Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.49 (CO), 150.77 (CO), 134.61 (C-6), 110.16 (C-5), 86.79 (C-1′), 84.60 (C-4′), 61.84 (C-5′), 59.98 (C-3′), 41.45 (NCH_2), 37.59 (C-2′), 29.04, 27.20, 22.32 (3 × CH_2), 13.92 (CH_3), 13.24 (CH_3); CI-MS *m/z* 338 $(M + 1)^+$. Anal. ($C_{15}H_{23}N_5O_4$) C, H, N.

3-[(Diethylphosphono)methyl]-3′-azido-3′-deoxythymidine (12k). 5′-*O*-TBS AZT **9** (343 mg, 0.898 mmol), K_2CO_3 (255 mg, 1.84 mmol), and (diethylphosphono)methyl triflate (411 mg, 1.34 mmol). Intermediate **13k** was directly treated with tetrabutylammonium fluoride (1.0 M in THF; 0.90 mL, 0.90 mmol) and the residue obtained after workup was purified by silica gel flash column chromatography (EtOAc). **12k**: colorless oil (203 mg; 54%). IR (neat, cm^{-1}) 3361, 2104, 1708, 1654; ^1H NMR ($CDCl_3$) δ 7.67 (s, H-6), 6.14 (t, $J = 6$ Hz, H-1′), 4.12−4.43 (m, 8H, H-3′, NCH_2, OH, 2 × OCH_2), 3.99 (br d, 1H, $J = 12$ Hz, H-5′), 3.88−3.91 (m, H-4′), 3.71 (br d, 1H, $J = 12$ Hz, H-5′), 2.26−2.42 (m, 2H, CH_2-2′), 1.88 (s, CH_3), 1.36 (t, $J = 7$ Hz, CH_3), 1.35 (t, $J = 7$ Hz, CH_3); ^{13}C NMR ($CDCl_3$) δ 162.63 (CO), 150.28 (CO), 134.63 (C-6), 109.89 (C-5), 86.33 (C-1′), 84.96 (C-4′), 63.99 (d, $J(^{13}C-^{31}P) = 6$ Hz, $P(O)OCH_2$), 62.78 (d, $J(^{13}C-^{31}P) = 6$ Hz, $P(O)OCH_2$), 61.24 (C-5′), 59.70 (C-3′), 38.18 (C-2′), 36.43 (d, $J(^{13}C-^{31}P) = 156$ Hz, $NCH_2P(O)$), 16.35 (d, $J(^{13}C-^{31}P) = 6$ Hz, $P(O)(OCH_2CH_3)_2$), 13.03 (CH_3); CI-MS *m/z* 418 $(M + 1)^+$. Anal. ($C_{15}H_{24}N_5O_7P$) C, H, N.

3-[2-(4-Methoxy-5-methyl-2-oxo-2-pyrimidin-1-yl)ethyl]-3′-azido-3′-deoxythymidine (12p). 5′-*O*-TBS AZT **9** (145 mg, 0.380 mmol), K_2CO_3 (143 mg, 1.03 mmol), and 1-(2-bromoethyl)-4-methoxy-5-methylpyrimidin-2-one (123 mg, 0.498 mmol). Intermediate **13p** was directly treated with tetrabutylammonium fluoride (1.0 M in THF; 0.40 mL, 0.40 mmol), and the residue obtained after workup was purified by silica gel flash column chromatography (5% MeOH/EtOAc). **12p**: amorphous white solid (158 mg; 96%); mp 126−128 °C ($CHCl_3$/hexane); IR (Nujol, cm^{-1}) 3244, 2090, 1698, 1665, 1636; ^1H NMR ($CDCl_3$) δ 7.73 (s, H-6″), 7.21 (s, H-6), 6.10 (t, $J = 6$ Hz, H-1′), 4.46 (q, $J = 5$ Hz, H-3′), 3.84−4.30 (m, 8H, $N(CH_2)_2N$, CH_2-5′, H-4′, OH), 3.94 (s, OCH_3), 2.35−2.47 (m, 2H, CH_2-2′), 1.92 (s, CH_3″), 1.87 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 170.85 (C-2″), 163.41 (C-2), 157.17 (C-4″), 150.83 (C-4), 144.09 (C-6″), 135.13 (C-6), 109.63 and 105.04 (2 × thymidine ring C-5), 86.51 (C-1′), 85.11 (C-4′), 61.56 (C-5′), 60.12 (C-3′), 54.43 (OCH_3), 47.53 and 39.94 ($N(CH_2)_2N$), 37.65 (C-2′), 13.10 and 11.94 (2 × CH_3); CI-MS *m/z* 434 $(M + 1)^+$. Anal. ($C_{18}H_{23}N_7O_6$) C, H, N.

3-[2-(5-Methyl-2,4-oxo-2-pyrimidin-1-yl)ethyl]-3′-azido-3′-deoxythymidine (12q). 5′-*O*-TBS AZT **9** (315 mg, 0.825 mmol), K_2CO_3 (318 mg, 2.30 mmol), and 1-(2-bromoethyl)-4-methoxy-5-methylpyrimidin-2-one (208 mg, 0.842 mmol). Intermediate **13q** was separated from the excess bromide by silica gel flash column chromatography (EtOAc) and treated with 0.2 N HCl (20 mL) in methanol (10 mL) for 4 d and then neutralized with Amberlite IR 68 ion-exchange resin. After filtration and evaporation, the residue obtained was purified by flushing through a plug of silica gel (10% MeOH/EtOAc). **12q**: amorphous white solid (255 mg; 74%); mp 191−192 °C (MeOH); IR (Nujol, cm^{-1}) 3443, 3168, 2106, 1700, 1669, 1635; ^1H NMR (DMSO-d_6) δ 11.13 (br s, NH), 7.77 (s, thymine ring H-6), 7.36 (s, thymine ring H-6), 6.04 (t, $J = 6$ Hz, H-1′), 5.32 (br s, OH), 4.39 (q, $J = 6$ Hz, H-3′), 3.62-4.08 (m, 7H, $N(CH_2)_2N$, CH_2-5′, H-4′), 2.29 (t, 2H, $J = 6.5$ Hz, CH_2-2′), 1.79 (s, CH_3), 1.69 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 164.17 and 162.59 (2 × thymine ring C-2), 151.06 and 150.27 (2 × thymine ring C-4), 141.15 and 134.60 (2 × thymine ring C-6), 108.26 (2 × thymine ring C-5), 84.56 (C-1′), 84.18 (C-4′), 60.49 (C-5′), 59.69 (C-3′), 45.45 and 39.24 ($N(CH_2)_2N$), 36.54 (C-2′), 12.68 and 11.65 (2 × CH_3); CI-MS *m/z* 420 $(M + 1)^+$. Anal. ($C_{17}H_{21}N_7O_6$) C, H, N.

3-(2-Azidoethyl)-3′-azido-3′-deoxythymidine (15). To a solution of **12g** (286 mg, 0.764 mmol) in CH_2Cl_2 (5 mL) at room temperature (23 °C) was added Amberlite 1R 400 (N_3^-) resin (0.80 g, ca. 2 mmol of N_3^-). After 7 d the reaction mixture was filtered, washing the resin well with chloroform, and the filtrate was evaporated. The residue was flash column chromatographed (silica gel; 50% ethyl acetate/heptane) to afford **15** as a colorless oil (233 mg; 91%): IR (neat, cm^{-1}) 3462, 2108, 1704, 1668, 1635; ^1H NMR ($CDCl_3$) δ 7.50 (s, H-6), 6.11 (t, $J = 6.5$ Hz, H-1′), 4.39 (app q, $J = 6$ Hz, H-3′), 4.19 (t, $J = 6$ Hz, NCH_2), 4.04−3.76 (m, 3H, H-4′, CH_2-5′), 3.53 (t, $J = 6$ Hz, CH_2N_3), 2.92 (br s, OH), 2.52−2.40 (m, 2H, CH_2-2′), 1.93 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.29 (CO), 150.82 (CO), 135.08 (C-6), 110.31 (C-5), 87.06 (C-1′), 84.63 (C-4′), 61.95 (C-5′), 59.97 (C-3′), 48.42 (CH_2N_3), 39.94 (CH_2), 37.58 (C-2′), 13.22 (CH_3); CI-MS *m/z* 337 $(M + 1)^+$. Anal. ($C_{12}H_{16}N_8O_4$) C, H, N.

3-(2-Bromoethyl)-3′-azido-3′-deoxy-5′-*O*-(*tert*-butyldimethylsilyl)thymidine (16). To a stirred solution of **12g** (762 mg, 2.03 mmol) in DMF (10 mL) were added *tert*-butyldimethylsilyl chloride (500 mg, 3.32 mmol) and imidazole (280 mg, 4.11 mmol). After 18 h the reaction mixture was quenched with methanol (ca. 2 mL) and the solvent removed *in vacuo* after 30 min. The residue was coevaporated with xylene and silica gel flash column chromatographed (50% EtOAc/heptane) to afford **16** as a white solid (712 mg, 72%): mp 92−93 °C; IR ($CHCl_3$, cm^{-1}) 2106, 1706, 1668, 1652; ^1H NMR ($CDCl_3$) δ 7.44 (s, H-6), 6.22 (t, $J = 7$ Hz, H-1′), 4.33 (t, $J = 7$ Hz, CH_2), 4.26−4.20 (m, H-3′) 3.98−3.97 (m, 2H, H-5′, H-4′), 3.79 (dd, 1H, $J = 11$, 2 Hz, H-5′), 3.53 (t, 2H, $J = 7$ Hz, CH_2Br), 2.45 (ddd, 1H, $J = 14$, 6, 5 Hz, H-2′), 2.22 (dt 1H, $J = 14$, 7 Hz, H-2′), 1.92 (s, CH_3), 0.92 (s, 9H, CH_3), 0.12 (s, 6H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$) δ 163.02 (CO), 150.62 (CO), 133.52 (C-6), 110.26 (C-5), 85.37 (C-1′), 84.63 (C-4′), 62.94 (C-5′), 60.48 (C-3′), 42.20 (CH_2), 38.17 (C-2′), 27.35 (CH_2Br), 26.01 (CH_3), 18.47 (*C*Si), 13.32 (CH_3), −5.26 (CH_3Si); CI-MS *m/z* 490 and 488 $(M + 1)^+$. Anal. ($C_{18}H_{30}N_5O_4SiBr$) C, H, N.

3-(2-Phthalimidoethyl)-3′-azido-3′-deoxythymidine (17). To a stirred solution of phthalimide potassium salt (113 mg, 0.607 mmol) and 18-crown-6 (38.5 mg, 0.146 mmol) in DMF (3 mL) at 75 °C was added **16** (275 mg, 0.563 mmol). After 18 h the solvent was removed *in vacuo*, and the residue was coevaporated with xylene and then silica gel flash column chromatographed (50% ethyl acetate/heptane) to afford a colorless oil (275 mg), which was directly treated with 1:9 40% HF in CH_3CN (2 mL). After 1.5 h the reaction mixture was evaporated to dryness, and the residue was flash column chromatographed (silica gel; 50% ethyl acetate/heptane) to afford **17** as a colorless oil (204 mg; 82%): IR (neat, cm^{-1}) 3472, 2105, 1717, 1669, 1637; ^1H NMR ($CDCl_3$) δ 7.83−7.68 (m, 4H, Ar-H), 7.30 (d, $J = 1$ Hz, H-6), 5.86 (dd, $J = 7$, 5 Hz, H-1′), 4.37−4.25 (m, 3H, H-3′, CH_2N), 4.05−3.67 (m, 5H, CH_2-5′, CH_2N, H-4′), 2.81 (br m, OH), 2.47 (ddd, 1H, $J = 13$, 7, 5 Hz, H-2′), 2.28 (dt, 1H, $J = 13$, 6 Hz, H-2′), 1.81 (d, $J = 1$ Hz, CH_3);

^{13}C NMR ($CDCl_3$) δ 168.33 (2 × CO), 163.46 (CO), 150.83 (CO), 135.02 (C-5), 133.91 (2 × ArCH), 131.89 (2 × ArC), 123.06 (2 × ArCH), 109.64 (C-5), 87.02 (C-1′), 84.63 (C-4′), 61.45 (C-5′), 59.43 (C-3′), 39.74 (CH_2), 37.36 (CH_2), 35.88 (CH_2), 12.99 (CH_3); CI-MS *m/z* 441 $(M + 1)^+$. Anal. ($C_{20}H_{20}N_6O_6$) C, H, N.

3-[(3‴-Azido-3‴-deoxythymidin-3″-yl)ethyl]-3′-azido-3′-deoxythymidine (18). To a vigorously stirred solution of 5′-*O*-TBS **9** (102 mg, 0.266 mmol) in dry DMF (2 mL) was added activated 4 Å sieve powder, powdered potassium carbonate (94 mg, 0.68 mmol), and bromide **16** (165 mg, 0.338 mmol). After 24 h the reaction mixture was filtered, washing well with chloroform, the filtrate was evaporated, and the residue was flash column chromatographed (silica gel; 25% ethyl acetate/heptane) to remove the excess **16** and afford the silylated product as a viscous oil (163 mg). The oil was treated for 1 h with 40% HF (0.30 mL) in CH_3CN (3 mL), evaporated, and flash column chromatographed (silica gel; EtOAc) to afford the deprotected **18** as a white solid (102 mg; 68%): IR ($CHCl_3$, cm^{-1}) 3450, 2106, 1701, 1670, 1636; ^{1}H NMR ($CDCl_3$) δ 7.31 (s, 2 × H-6), 5.88 (t, J = 6 Hz, 2 × H-1′), 4.35 (q, J = 6 Hz, 2 × H-3′), 4.23 (s, 4H, NCH_2CH_2N), 4.84−4.94 (m, 4H, 2 × H-4′, 2 × H-5′), 3.71 (dd, 2H, J = 12.5, 3 Hz, 2 × H-5′), 3.57 (br s, 2 × OH), 2.55 (ddd, 2H, J = 13, 7.5, 5.5 Hz, 2 × H-2′), 2.34 (dt, 2H, J = 13, 6.5 Hz, 2 × H-2′), 1.82 (s, 2 × CH_3); ^{13}C NMR ($CDCl_3$) (all peaks × 2) δ 163.75 (CO), 151.07 (CO) 135.51 (C-6), 109.75 (C-5), 81.11 (C-1′), 84.87 (C-4′), 61.75 (C-5′), 59.77 (C-3′), 39.35 (NCH_2CH_2N), 37.10 (C-2′), 13.14 (CH_3); CI-MS *m/z* 561 $(M + 1)^+$. Anal. ($C_{22}H_{28}N_{10}O_8$) C, H, N.

3-[[3″-(Hydroxymethyl)isoxazol-5″-yl]methyl]-3′-azido-3′-deoxythymidine (19). To a stirred of **12c** (295 mg, 0.96 mmol) in DMF (10 mL) was added *tert*-butyldimethylsilyl chloride (217 mg, 1.44 mmol) and imidazole (105 mg, 1.54 mmol). After 16 h the reaction mixture was quenched with methanol (ca. 5 mL) and the solvent removed *in vacuo* after 30 min. The residue was coevaporated with xylene and silica gel flash column chromatographed (50% EtOAc/heptane) to afford an oil (263 mg; 65%): ^{1}H NMR ($CDCl_3$) δ 7.45 (s, H-6), 6.25 (t, J = 6.5 Hz, H-1′), 5.78−5.98 (m, C*H*═CH_2), 5.16−5.30 (m, CH═C*H*$_2$), 4.55 (d, J = 5.5 Hz, NCH_2), 4.20−4.27 (m, H-3′), 3.77−3.98 (m, 3H, CH_2-5′, H-4′), 2.40−2.52 (m, 1H, H-2′), 2.16−2.30 (m, 1H, H-2′), 1.94 (s, CH_3), 0.93 (s, 9H, $(CH_3)_3CSi$), 0.13 (s, 6H, $(CH_3)_2Si$).

To a solution of this intermediate in toluene (50 °C) was added triethylamine (25 μL, 0.18 mmol), 2-nitroethyl *tert*-butyldimethylsilyl ether (62 mg, 0.30 mmol), and phenyl isocyanate (0.37 mL, 3.40 mmol). After 3 d the reaction mixture was filtered, evaporated, and treated with 1:9 40% HF in CH_3CN (0.06 mL). After 1 h the reaction mixture was evaporated to dryness, and the residue was flash column chromatographed (silica gel; ethyl acetate/methanol 2%) to afford **19** as a colorless oil (59 mg; 54%): IR (neat, cm^{-1}) 3409, 2107, 1669, 1636; ^{1}H NMR ($CDCl_3$) δ 7.50 (s, H-6), 6.10 (td, 1H, J = 6 Hz, NCH_2), 4.02−4.42 (m, 6H, CH_2-5′, H-4′, H-3′, CH_2OH), 3.49 (s, 2 × OH), 3.12 (dd, 1H, J = 17, 10 Hz, CH_2 isoxazoline), 2.90 (dd, 1H, J = 17, 5.5 Hz, CH_2 isoxazoline), 2.32−2.58 (m, 2H, CH_2-2′), 1.89 (s, CH_3); ^{13}C NMR ($CDCl_3$) δ 163.30 (CO), 159.08 (C═NO), 151.29 (CO), 135.22 (C-6), 110.33 (C-5), 86.83 (C-1′), 84.67 (C-4′), 76.80 (CHO isoxazoline), 61.82 (C-5′), 59.96 (C-3′), 57.90 (CH_2OH), (43.64 (NCH_2), 38.88−37.56 (CH_2′, CH_2 isoxazoline), 13.22 (CH_3); CI-MS *m/z* 381 $(M + 1)^+$. Anal. ($C_{15}H_{20}N_6O_6$) C, H, N.

Reverse Transcriptase Assay. As previously described,[20] yeast cells which have been transformed with the vector pAB 24/RT-4 were used to purify the recombinant HIV-1 RT employed in the bioassays. The reaction mixture contained in a final volume of 0.05 mL, 50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 4 mM dithiothreitol, 0.48 A_{260}/mL of poly(A)-oligo(dT) (5:1), 0.5 μCi [^{3}H]dTTP (46 Ci/mmol), 20 μM dTTP, 80 mM KCl, 1 μg of bovine serum albumin, and 20-50 nM RT. Incubation was carried out at 37 °C for 10 min.

The reactions were stopped by the addition of 1 mL of cold 10% trichloroacetic acid plus 0.1 M sodium pyrophosphate. The precipitates were filtered through nitrocellulose membranes, washed with 2% trichloroacetic acid, dried, and counted in a PPO/POPOP/toluene scintillation mixture. For the inhibition studies, all derivatives were dissolved in DMSO. The same final concentration of DMSO was used in the control experiments.

Antiviral Assay on CEM-c113 Cells and RT Dosage. The antiviral assay on CEM c113 cells were performed as follows.[15] Briefly, 1 × 10^4 cells/wells were incubated in microtitration plates for 1 h with 10-fold diluted concentrations of the compound then infected by HIV-1 LAI strain. Cultures were incubated for 7 days at 37 °C in a CO_2 incubator (5%). At day 7 the supernatant was removed for reverse transcriptase (RT) assay to determine inhibition of viral production (EC_{50RT}), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added in cell suspension to determine cell viability (CC_{50} and EC_{50MTT}) by the MTT assay. The toxicity of the products (CC_{50}) was defined in relation to the viability of uninfected untreated control cells. The inhibition of CPE (EC_{50MTT}) of the products was defined in relation to the infected untreated cells.

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