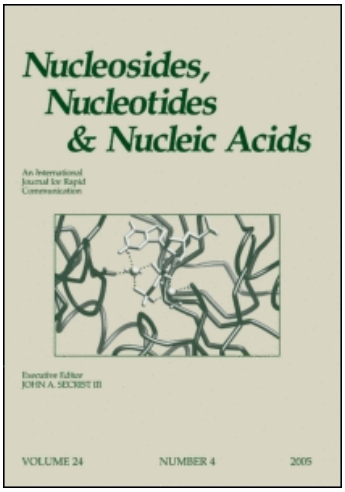

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M. Taourirte[a]; L. Ait Mohamed[b]; A. Rochdi[b]; J. -J. Vasseur[c]; S. Fernández[d]; M. Ferrero[d]; V. Gotor[d]; C. Pannecouque[e]; E. De Clercq[e]; H. B. Lazrek[b]
[a] Faculty of Sciences and Techniques Gueliz, Marrakesh, Morocco [b] Laboratory of Bioorganic Chemistry, Faculty of Sciences Semlalia, Marrakesh, Morocco [c] Laboratoire Chimie Organique Biomoléculaire de Synthèse, CNRS-Université, Montpellier II, France [d] Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, Oviedo, Spain [e] Rega Institute for Medical Research, Leuven, Belgium

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Chemoenzymatic Syntheses of Homo- and Heterodimers of AZT and d4T, and Evaluation of Their Anti-HIV Activity

M. Taourirte,[1] L. Ait Mohamed,[2] A. Rochdi,[2] J.-J. Vasseur,[3] S. Fernández,[4] M. Ferrero,[4] V. Gotor,[4] C. Pannecouque,[5] E. De Clercq,[5] and H. B. Lazrek[2,*]

[1]Faculty of Sciences and Techniques Gueliz, Marrakesh, Morocco
[2]Laboratory of Bioorganic Chemistry, Faculty of Sciences Semlalia, Marrakesh, Morocco
[3]Laboratoire Chimie Organique Biomoléculaire de Synthèse, CNRS-Université, Montpellier II, France
[4]Departamento de Química Orgánica e Inorgánica, Facultad de Química, Universidad de Oviedo, Oviedo, Spain
[5]Rega Institute for Medical Research, Leuven, Belgium

ABSTRACT

Homo- and heterodimers of AZT and d4T, possessing carbonate and carbamate linkers, have been synthesized with the aim to enhance the antiviral activity of their components. Homo- and heterodimer carbamates showed weak anti-HIV activity. On the other hand, dinucleoside carbonates showed marked antiviral activity.

Key Words: Chemoenzymatic syntheses; Homo- and heterodimers; AZT; d4T.

*Correspondence: H. B. Lazrek, Laboratory of Bioorganic Chemistry, Faculty of Sciences Semlalia, Marrakesh, Morocco; E-mail: hblazrek@caramail.com.

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INTRODUCTION

In the treatment of acquired immunodeficiency syndrome (AIDS),[1] the targeting of enzymes necessary for viral replication represents one of the most successful rational approaches of modern antiviral chemotherapy. Thus, many nucleosides have been recognized as potent and selective inhibitors of the replication of human immunodeficiency virus (HIV). The development of reverse transcriptase inhibitors has been, and remains, a key strategy in the fight against AIDS. To date, six 2′,3′-dideoxynucleoside analogues, AZT (zidovudine), d4T (stavudine), ddI (didanosine), ddC (zalcitabine), 3TC (lamivudine) and ABC (abacavir) have been approved for the treatment of HIV infection.[2,3] Although AZT was the first compound approved by FDA, it is still one of the most potent agents active against HIV. Stavudine showed a superior clinical benefit in patients when compared to patients receiving zidovudine. On the other hand, serious side effects are associated with the administration of AZT and d4T, particularly bone marrow suppression, anemia, leukopenia, and neuropathy. These are severe enough to often require cessation of treatment.[4,5] Different approaches to achieve sustained benefits from antiviral therapy with AZT, numerous combination, pro-drugs, and double-drug concept have been reported in the literature.[5–10] For instance, Matsumoto et al.[9,10] have described the design and synthesis of anti-HIV double-drug series consisting of HIV protease inhibitors (dipeptides KNI-272 and KNI-1039) conjugated with a nucleoside reverse transcriptase inhibitor (AZT). Moreover, Tamamura et al.[11] have reported the synthesis and evaluation of bifunctional anti-HIV compounds, which combined a peptide with a specific binding to a chemokine receptor (CXCR4) to AZT (T140 analogues). In most cases, the mechanism of action is based on the hydrolysis of the enzymatically labile 5′-*O*-bonds between the drug (RT inhibitor, PR inhibitor, ...) and the spacer group. The search for new combinations of compounds with improved selectivity, lipophilicity, and efficiency, which could overcome problems of resistance as well as toxicity is a field of great interest.[6]

Based on this strategy, we initiated a program to prepare and evaluate the anti-HIV activity of new series of homo- and heterodimers of AZT and d4T, which contain carbonate and carbamate linkages.[12] Several arguments support this work: 1) as long as the linkage between the nucleosides (AZT and d4T) is not extracellulary hydrolyzed, the delivery and the bioavailability might be enhanced, depending on the lipophilic character of these new models;[7,9,10] 2) some synergetic effects on the inhibition of HIV replication could be expected;[9,10] 3) depending on the nature of the chemical bond between the two nucleosides, intracellular hydrolysis could regenerate the two nucleosides in the cytoplasm. Owing to these facts, the mentioned dimers could be considered as pro-drugs.

RESULTS AND DISCUSSION

To investigate the properties of carbonate dimers **7–9** as well as carbamate dimers **12–14**, their synthesis was undertaken. The synthetic strategy is illustrated below in Schemes 1, 2, and 3.

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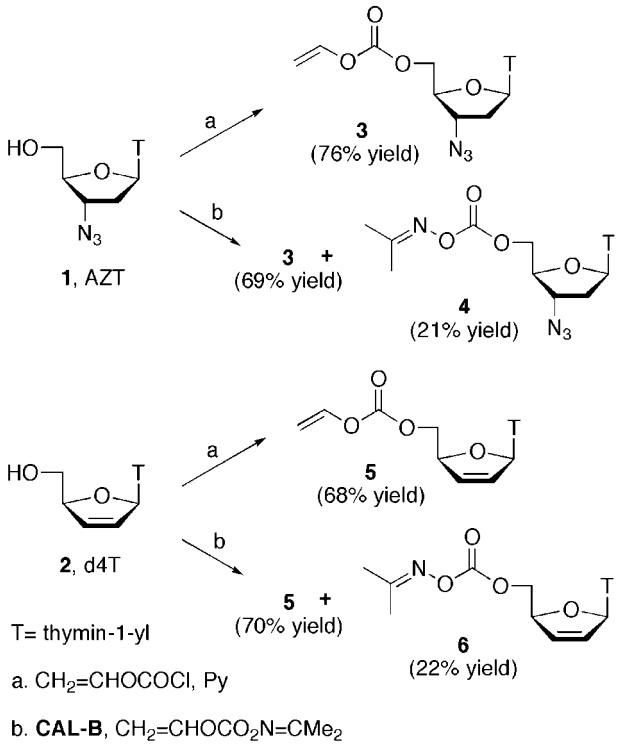
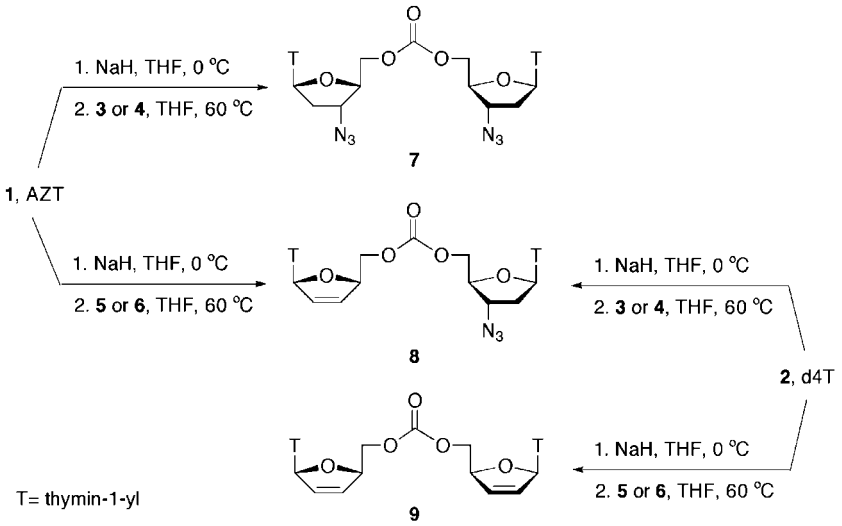


Scheme 1. AZT and d4T converted into 5′-O-alkoxycarbonylated derivatives.

AZT (**1**) and d4T (**2**) were converted into 5′-O-alkoxycarbonylated derivatives **3** and **5** by treatment with an alkoxycarbonyl transferring agent (pathways a, Scheme 1). Thus, the reaction of AZT with vinyl chloroformate in pyridine gave place to 5′-monoprotected derivative **3** with a yield of 76% after flash column chromatography. Similarly, treatment of **2** in the same conditions afforded derivative **5** in 68% isolated yield. In these chemical processes side products were obtained, which correspond to dialkoxycarbonylated derivatives from the reaction of both 5′-OH and the NH of the base with vinyl chloroformate. To avoid these side reactions, an enzymatic methodology was applied taking into account the potential of *Candida antarctica* lipase B (CAL-B) as well as other enzymes in nucleoside chemistry[13,14] (pathways b, Scheme 1). Thus, 5′-*O*-vinyloxycarbonylated nucleosides **3** and **5** were prepared from parent nucleosides **1** and **2** when CAL-B was used as catalyst in THF at 60°C with acetone *O*-[(vinyloxy)carbonyl]oxime acting as alkoxycarbonylating reagent. In addition, 5′-*O*-acetonoximecarbonylated nucleosides **4** and **6** were obtained as minor compounds. Vinyl carbonates as well as oxime carbonates can be used as intermediates in the next step when they react with AZT or d4T. Using an enzymatic procedure it was possible to obtain activated carbonates in higher yields (90% and 92% isolated combined yields, respectively) without side-products, which highly simplifies the



Scheme 2. Preparation of homo- and heterodimer carbonates with vinyl and oxime esters derived from AZT and d4T.

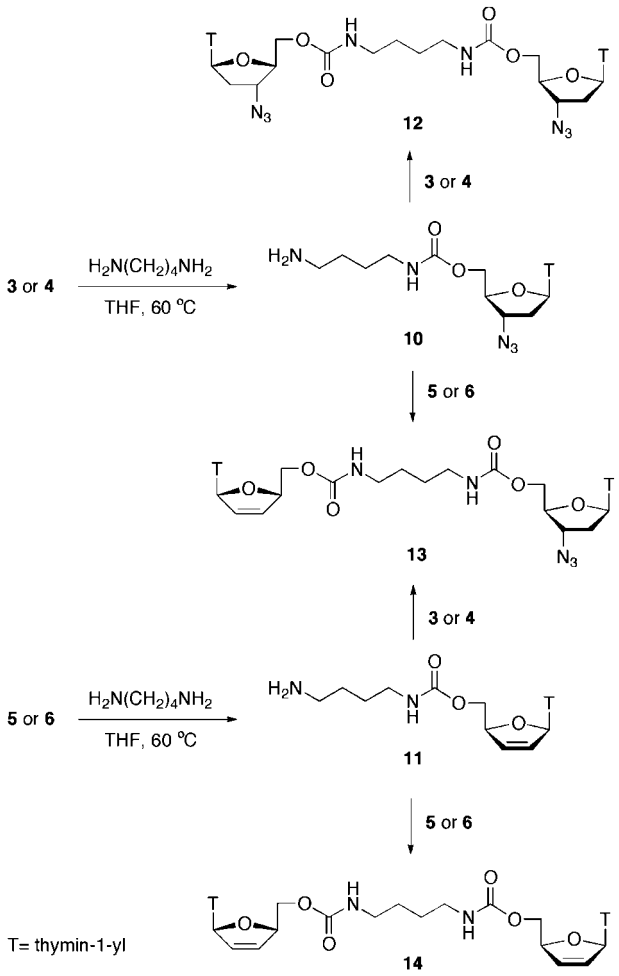
isolation work-up. Also, it is noteworthy that enzymes are cheap, reusable, and ecological catalysts.

Synthesis of Dinucleoside Carbonates

To prepare homo- and heterodimer carbonates, vinyl and oxime esters derived from AZT and d4T were used (Scheme 2). Thus, AZT reacted with NaH in THF at 0°C, giving an alkoxide, which subsequently reacted with activated nucleosides **3** or **4**. After work-up and purification on silica gel chromatography column, the homodimer **7** was obtained in 85% isolated yield. The heterodimer **8** can be prepared by reaction of the alkoxide either from AZT or d4T. The process was carried out by reaction of the AZT alkoxide with activated derivatives **5** and **6** or by reaction of d4T alkoxide with activate derivatives **3** and **4**. In any case **8** was obtained in 74% isolated yield. Finally, the homodimer **9** was prepared following the same procedure with an isolated yield of 84%.

Synthesis of Dinucleoside Carbamates

5′-*O*-Carbamate-AZT **10** and 5′-*O*-carbamate-d4T **11** were obtained by condensation of 5′-*O*-vinylcarbonate-AZT **3** (or 5′-*O*-oximecarbonate-AZT **4**) or 5′-*O*-vinylcarbonate-d4T **5** (or 5′-*O*-oximecarbonate-d4T **6**) with 1,4-diaminobutane in THF at 60°C, respectively (Scheme 3).[15–18] Then, those amino carbamates **10** and **11** were condensed with activated carbonates derived from AZT (**3** or **4**) and d4T (**5** or **6**). When compound **10** was reacted with **3**, **4**, or with a mixture of both, the homodimer dicarbamate **12** was formed in 83% isolated yield. The heterodimer dicarbamate **13** was



Scheme 3. 5′-*O*-Carbamate-AZT and 5′-*O*-carbamate-d4T obtained by condensation of **3**, **4**, **5**, or **6** at 60°C.

prepared reacting **10** with **5** (or **6**) or reacting **11** with **3** (or **4**) in 80% yield. Finally, the homodimer **14** was prepared in 81% yield from the amino carbamate **11**.

Lipophilicity and Chemical Stabitity Studies

According to Zimmermann et al.,[19] AZT crosses cell membranes by non-facilitated diffusion and its uptake is not sensitive to nucleoside transport inhibitors. This indicates that the lipophilicity of AZT analogues, which is reflected by their partition coefficient (log P), might have a significant role in their diffusion.[20] Thus, by increasing the lipophilicity of a drug, we could expect an increase in the intracellular uptake of the parent drug. Therefore, the calculated log P (Clog P) values (partition in

Table 1. In vitro anti-HIV activity and cytotoxicity of compounds **1–5**, **7–9**, and **12–14** against HIV-1 (III_B) and HIV-2 (ROD) from acutely infected MT-4 cells.

Compound	Strain	EC_{50}[a] (μM)	CC_{50}[b] (μM)	SI[c]	Log P
1	III_B	0.0022	168.5	76591	0.10
	ROD	0.0007	168.5	240714	
2	III_B	0.27	299	1107	−0.53
	ROD	0.08	299	3738	
3	III_B	0.009	370.9	41211	−0.23
	ROD	0.009	370.9	41211	
4	III_B	0.029	182	6275	0.48
	ROD	0.073	155	2127	
5	III_B	0.085	217.6	2560	−0.43
	ROD	0.057	217.6	3818	
7	III_B	0.0028	119.2	42571	0.24
	ROD	0.0017	128.1	75353	
8	III_B	0.0063	174	27619	−1.21
	ROD	0.0038	174	45789	
9	III_B	0.24	>52.7	>219.6	−0.21
	ROD	0.33	>52.7	>158.3	
12	III_B	2.84	153.7	54	−0.72
	ROD	2.58	200	78	
13	III_B	96.3	217.3	2	−2.27
	ROD	19.0	186	10	
14	III_B	26.0	178.5	7	−1.89
	ROD	22.5	178.5	8	

[a]50% Effective concentration or concentration required to protect cells against the cytopathogenicity of HIV by 50%.
[b]50% Cytotoxicity concentration required to reduce virus-induced cytopathogenicity by 50%.
[c]Selectivity index: CC_{50}/EC_{50}.

n-octanol and water) were determined for all compounds, using Interactive Analyses Log P (ChemSilico). The results in Table 1 showed that Clog P values are in the range of +0.48 to −2.27. Moreover, it is evident that an increase of the lipophilicity of a prodrug does not necessarily mean that its anti-HIV efficacy will be improved.

The stability of the carbonate linkages of dimers (d4T-CO-d4T is taken as an example) in pH = 7 and in pH = 11.7 was undertaken. The results obtained indicate the following observations: 1) under neutral conditions (0.05 to 0.1 mg of the dimer were dissolved in 50 μl of 0.05 M aqueous triethylammonium acetate at pH 7), HPLC analysis of the resulting solution (5 μl) after 24 h showed only the desired dimer (Rt 17.52 min); 2) under strong basic conditions (0.05 to 0.1 mg of the dimer were dissolved in 40 μl of 5 mM aqueous sodium hydroxide, pH 11.7), the total amount of d4T-CO-d4T dimer hydrolysis product was 40% after 1 h. After 4 h the dimer was totally hydrolyzed. The identity of the latter compound was ascertained by a coinjection with a pure sample of d4T (Rt 5.84 min). The release was calculated from the ratio of the corresponding peaks monomer integration area/(dimer area + monomer

area). These data showed that the dimer is relatively stable under neutral pH and instability at high pH.

Antiviral Activities

The central idea to synthesize homo- and heterodimers was to determine to what extent the individual AZT or d4T retain their capacity to inhibit HIV infection. Three series, monomers **3–5**, carbonates **7–9**, and carbamates **12–14**, were evaluated for their inhibitory effects on HIV replication, monitored by the efficiency of compounds to reduce the cytopathogenicity of HIV after infection of MT-4 cell cultures.[21,22] From the results reported in Table 1, it can be deduced that the carbonate **3** showed comparable activity and less toxicity than AZT. Therefore, for the carbonate **5** the EC_{50} is 3 times and the SI value is 2 times higher than d4T. Moreover, when the vinyloxycarbonyl group was replaced by the oxime (carbonate **4**) the EC_{50} increased from 0.009 to 0.029 μM. However, The series **7–9** showed similar activities to AZT and d4T. Indeed, the homodimer of AZT **7** was the most active prodrug of these series. The carbonate prodrugs could suffer from fast chemical and/or enzymatic hydrolytic cleavage. These results suggest that sensitivity to chemical and/or enzymatic hydrolysis of the homo- and heterodimers depends on the nature of their 5′-*O*-functions.[6] In addition, the result obtained with the heterodimer **8** showed that the anti-HIV activity could be attributed to the action of the more potent ddN (AZT) from the two coupled components.[23,24] On the other hand, the carbamate series **12–14** displayed low anti-HIV activities.The carbonate **7–9** and carbamate **12–14** prodrugs appeared to be slightly more toxic than AZT and less toxic than the d4T. Further investigations are needed to understand these results.

All abovementioned prodrugs **3–14** were evaluated for their inhibitory effect against *Mycobacterium tuberculosis* H_{37} Rv (ATCC 27294) in BACTEC 12B medium. No anti-tuberculosis activity was noted at concentrations up to 6.25 μg/ml.

In conclusion, the homo- and heterodimers prepared were bifunctional anti-HIV agents. They exhibited significant anti-HIV activity and selectivity indexes, presumably resulting in the efficient release of AZT in vitro. No synergetic effects on the inhibition of HIV replication was detected for the carbonates and carbamates dimers.

EXPERIMENTAL SECTION

General. *Candida antarctica* lipase B (CAL-B, 7300 PLU/g) was a gift from Novo Nordisk Co. ^{1}H, ^{13}C NMR, and DEPT spectra were recorded with a Bruker AC-250 spectrometer. Chemical shifts are expressed as δ units (ppm) downfield from TMS. Fast Atom Bombardment (FAB^+ or FAB^-) mass spectral analyses were obtained by Dr. Astier (Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France) on JEOL DX-100 using a cesium ion source and glycerol/thioglycerol (1:1) or *m*-nitrobenzoyl alcohol (NBA) as matrix. IR spectra were recorded on a Perkin–Elmer FTIR 1605 spectrophotometer. Thin-layer chromatography was performed using silica gel plates 0.2 mm thick ($60F_{254}$ Merck). Preparative flash chromatography column was carried out on silica gel (230–240 mesh, G60 Merck). Analysis of the degradation of

the dimers in aqueous medium was performed by reverse phase HPLC on a Waters–Millipore instrument equipped with a Model 600 E solvent delivery system, a Model U6K injector, and a ND Model 486 absorbance detector. A reverse-phase C_{18} (5 μM) Nucleosil column (150 × 4.6 mm, Macherey–Nagel) was used with linear gradients of acetonitrile (10–90%) in 0.05 M aqueous triethylammonium acetate (pH = 7) in 40 min.

Synthesis of 5′-*O*-[(Vinyloxy)carbonyl]-3′-azido-3′-deoxythymidine (3) and 5′-*O*-[(Vinyloxy)carbonyl]-2′,3′-dideoxy-2′,3′-didehydrothymidine (5).

Method A: *Enzymatic alkoxycarbonylation of AZT and d4T with acetone O-[(vinyloxy)carbonyl]oxime*. To a solution of AZT (**1**) or d4T (**2**) (0.38 mmol) in 8 ml of dry THF was added acetone *O*-[(vinyloxy)carbonyl]oxime (1.14 mmol) and CAL-B (1:1 w/w with respect to starting nucleoside). The suspension was stirred (250 rpm) at 30°C, and the progress of the reaction was followed by TLC (90% EtOAc/hexane) until no further reaction was apparent (2–3 days). After removal of the enzyme by filtration and evaporation of the solvent, the residual mixture was purified by flash chromatography column (gradient eluent 0–80% EtOAc/hexane) to give 5′-vinyloxycarbonylated derivatives **3** (69%) and **5** (70%) as major products and 5′-*O*-acetonoximecarbonylated derivatives **4** (21%) and **6** (22%), respectively.

Method B: Vinyl chloroformate (0.94 mmol) was added dropwise into an ice-cold solution of AZT (220 mg, 0.85 mmol) or d4T (190.4 mg, 0.85 mmol) in 10 ml of dry pyridine. Pyridine was removed under reduced pressure. Then the residue was subject to flash chromatography column (eluent 60% EtOAc/hexane) to give compounds **3** (76%) and **5** (68%), respectively.

5′-*O*-[(Vinyloxy)carbonyl]-3′-azido-3′-deoxythymidine (3). ^{1}H NMR ($CDCl_3$): δ 1.92 (s, 3H, CH_3), 2.4–2.5 (m, 2H, H-2′a,b), 4.1–4.2 (m, 1H, H-4′), 4.42–4.65 (m, 3H, H-3′ + H-5′a,b), 4.68 (dd, 1H, $J_{H7''a,H\text{-}6''}$ = 6.2, $J_{H7''a,H\text{-}7''b}$ = 2.1 Hz, H-7″a), 5.96 (dd, 1H, $J_{H7''b,H\text{-}6''}$ = 13.9, $J_{H7''a,H\text{-}7''b}$ = 2.1 Hz, H-7″b), 6.25 (t, 1H, J = 6.1 Hz, H-1′), 7.19 (dd, 1H, $J_{H\text{-}6'',H7''a}$ = 6.2, $J_{H6'',H\text{-}7''b}$ = 13.84 Hz, H-6″), 7.57 (s, 1H, H-6), 11.5 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 12.08 (CH_3), 37.07 (C-2′), 59.64 (C-3′), 66.55 (C-5′), 80.98 (C-4′), 84.72 (C-1′), 98.18 (C-7″), 111.01 (C-5), 135.12 (C-6), 142.02 (C-6″), 150.27 (C-2), 151.84 (C-4), 163.90 (OCO); MS (ES, *m/z*): 360 $(M + Na)^+$, 338 $(M + H)^+$.

5′-*O*-[(Acetonoxime)carbonyl]-3′-azido-3′-deoxythymidine (4). ^{1}H NMR ($CDCl_3$): δ 1.89 (s, 3H, CH_3), 2.00 (s, 3H, H-8″), 2.02 (s, 3H, H-9″), 2.3–2.5 (m, 2H, H-2′a,b), 4.1–4.15 (m, 1H, H-4′), 4.25–4.35 (m, 3H, H-3′), 4.49 (m, 2H, H-5′a,b), 6.20 (t, 1H, J = 6.1 Hz, H-1′), 7.32 (s, 1H, H-6), 9.41 (s, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 12.44 (CH_3), 16.74 (C-8″), 21.58 (C-9″), 37.46 (C-2′), 60.38 (C-3′), 66.89 (C-5′), 81.53 (C-4′), 85.06 (C-1′), 111.22 (C-5), 135.15 (C-6), 150.15 (C-2), 153.34 (C-4), 163.68 (C = N), 164.21 (OCO); MS (ES, *m/z*): 389 $(M + Na)^+$.

5′-*O*-[(Vinyloxy)carbonyl]-2′,3′-dideoxy-2′,3′-didehydrothymidine (5). ^{1}H NMR ($CDCl_3$): δ 1.87 (s, 3H, CH_3), 1.98 (s, 3H, H-8″), 2.03 (s, 3H, H-9″),

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4.37–4.61 (m, 2H, 2H-5′a,b), 5.06 (m, 1H, H-4′), 5.90 (d, 1H, H-2′), 6.31 (d, 1H, H-3′), 7.01 (t, 1H, H-1′), 7.28 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$): δ 12.14 (CH_3), 16.79 (C-8″), 21.63 (C-9″), 67.85 (C-5′), 84.05 (C-4′), 89.40 (C-1′), 111.04 (C-5), 127.72 (C-2′), 132.53 (C-3′), 135.64 (C-6), 150.72 (C-2), 153.73 (C-4), 163.72 (C = N), 164.26 (OCN); MS (ES, *m/z*): 333 $(M + K)^+$, 317 $(M + Na)^+$.

5′-*O*-[(Acetonoxime)carbonyl]-2′,3′-dideoxy-2′,3′-didehydrothymidine (6). ^{1}H NMR ($CDCl_3$): δ 1.88 (s, 3H, CH_3), 1.98 (s, 3H, H-8″), 2.03 (s, 3H, H-9″), 4.4–4.6 (m, 2H, H-5′a,b), 5.06 (m, 1H, H-4′), 5.90 (m, 3H, H-3′), 6.3 (t, 1H, J = 6.1 Hz, H-1′), 7.02 (m, 2H, H-2′), 7.29 (s, 1H, H-6); ^{13}C NMR ($CDCl_3$): δ 12.36 (CH_3), 16.03 (C-8″), 21.83 (C-9″), 67.85 (C-5′), 84.05 (C-4′), 89.40 (C-1′), 111.04 (C-5), 127.72 (C-3′), 132.53.46 (C-2′), 135.64 (C-6), 150.72 (C-2), 153.73 (C = N), 163.72 (C-4), 164.26 (OCN); MS (ES, *m/z*): 364 $(M + Na)^+$, 362 $(M + K)^+$.

Synthesis of Dinucleoside Carbonates 7–9. A solution of **1** or **2** (0.38 mmol) in dry THF (1.5 ml) was added to a suspension of NaH (0.41 mmol) in dry THF (1.5 ml) at 0°C under nitrogen atmosphere. Then, a solution of vinylcarbonate **3** (or oximecarbonate **4**) or vinylcarbonate **5** (or oximecarbonate **6**) (0.41 mmol) in dry THF (1.5 ml) was added slowly. The mixture was stirred at 60°C overnight and then diluted with CH_2Cl_2 and washed with NH_4Cl. After the solvent was removed, the crude was purified by flash chromatography column (80% EtOAc/hexane).

Carbonate of bis(3′-Azido-3′-deoxythymidin-5′-yl) (7). Yield: 85%; ^{1}H NMR (DMSO-d_6): δ 1.8 (s, 6H, 2CH_3), 2.4 (m, 2H, 2H-2′a,b), 4.1 (m, 2H, 2H-4′), 4.5 (m, 6H, 2H-3′ + 2H-5′a,b), 6.3 (t, 1H, 2H-1′), 7.35 (s, 2H, H-6); ^{13}C NMR (DMSO-d_6): δ 12.78 (CH_3), 38.15 (C-2′), 56.02 (C-3′), 62.04 (C-5′), 83.29 (C-4′), 86.54 (C-1′), 112.15 (C-5), 137.85 (C-6), 152.27 (C-2), 157.08 (C-4), 166.49 (OCO); MS (FAB$^-$, *m/z*): 559 $(M-H)^-$.

Carbonate of 3′-Azido-3′-deoxythymidin-5′-yl and 2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl (8). Yield: 82%; ^{1}H NMR (DMSO-d_6): δ 1.83 (s, 3H, CH_3), 1.88 (s, 3H, CH_3), 2.47 (m, 2H, H-2′a,b, AZT), 3.71–3.73 (m, 1H, H-4′, d4T), 4.08–4.09 (m, 1H, H-4′, AZT), 4.45 (m, 5H, H-3′, AZT + 2H-5′a,b, AZT + d4T), 6.11 (d, 1H, J = 6.2 Hz, H-2′, d4T), 6.24 (t, 1H, J = 6.0 Hz, H-1′, AZT), 6.54 (d, 1H, J = 5.9 Hz, H-3′, d4T), 6.95 (d, 1H, J = 1.2 Hz, H-1′, d4T), 7.38 (s, H, H-6, AZT), 7.56 (s, H, H-6, d4T); ^{13}C NMR (DMSO-d_6): δ 12.02 (CH_3), 12.16 (CH_3), 35.41 (C-2′, AZT), 59.96 (C-3′, AZT), 67.37 (C-5′), 80.31 (C-4′), 83.49 (C-1′, AZT),) 83.85 (C-1′, d4T), 109.72 (C-5, AZT), 109.94 (C-5, d4T), 127.14 (C-2′, d4T), 133.12 (C-3′, d4T), 135.87 (C-6, d4T), 136.06 (C-6, AZT), 150.68 (C-2, d4T), 151.00 (C-2, AZT), 154.15 (C-4, AZT), 164.08 (C-4, d4T); MS (FAB$^+$, *m/z*): 518 $(M + H)^+$; MS (FAB$^-$, *m/z*): 516 $(M-H)^-$.

Carbonate of bis(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl) (9). Yield: 74%; ^{1}H NMR (DMSO-d_6): δ 1.80 (s, 6H, 2CH_3), 3.48 (m, 2H, H-4′), 4.44 (m, 4H, 2H-5′a,b), 6.11–6.15 (dd, 2H, J = 1.5, J = 5.9 Hz, 2H-2′), 6.48-6.53 (dd, 2H, J = 1.7, J = 5.9 Hz, 2H-3′), 6.95 (m, 2H, 2H-1′), 7.33 (s, 2H, 2H-6); ^{13}C NMR (DMSO-d_6): δ 12.18 (CH_3), 62.25 (C-5′), 87.30 (C-4′), 88.87 (C-1′), 108.97 (C-5), 125.96 (C-2′),

134.94 (C-3′), 136.77 (C-6), 150.79 (C-2), 163.86 (C-4), 168.49 (OCO); MS (ES, *m/z*): 513 ($M + K^+$), 497 ($M + Na^+$).

Synthesis of Amino Carbamates 10 and 11. To a solution of vinylcarbonate **3** (or oximecarbonate **4**) or vinylcarbonate **5** (or oximecarbonate **6**) (0.21 mmol) in 3 ml of dry THF was added a solution of 1,4-diaminobutane (0.63 mmol) in 2 ml of dry THF. The solution was stirred under nitrogen atmosphere for 24 h at 60°C. Then, solvent was evaporated under reduced pressure and the residue was subjected to flash chromatography column (gradient eluent 0–50% MeOH/CH_2Cl_2) to give amino carbamates **10** (81%) and **11** (80%).

5′-[[*N*-(4-Aminobutyl)carbamoyl]oxy]-3′-azido-3′-deoxythymidine (10). Yield: 81%; ^{1}H NMR (MeOH-d_4): δ 1.25–1.6 (m, 4H, AZT-O_2C-NH-CH_2-*CH_2*-*CH_2*-CH_2-NH_2), 1.89 (s, 3H, *CH_3*-Thy), 2.39–2.51 (m, 2H, H-2′), 2.70 (br s, 1H, NH_2), 3.25 (m, 2H, AZT-O_2C-NH-CH_2-CH_2-CH_2-*CH_2*-NH_2), 3.35 (m, 2H, AZT-O_2C-NH-*CH_2*-CH_2-CH_2-CH_2-NH_2), 3.95-4.09 (m, 1H, H-4′), 4.15–4.41 (m, 3H, H-3′ + H-5′a,b), 4.81 (br s, 1H, AZT-O_2C-N*H*-CH_2-CH_2-CH_2-CH_2-NH_2), 6.15 (t, 1H, H-1′), 7.39 (s, 1H, H-6); MS (FAB^+, *m/z*): 382 $(M + H)^+$.

5′-[[*N*-(4-Aminobutyl)carbamoyl]oxy]-2′,3′-dideoxy-2′,3′-didehydrothymidine (11). Yield: 80%; ^{1}H NMR (MeOH-d_4): δ 1.29-1.34 (m, 4H, d4T-O_2C-NH-CH_2-*CH_2*-*CH_2*-CH_2-NH_2), 1.72 (s, 3H, *CH_3*), 2.9 (br s, 2H, NH_2), 3.58–3.6 (m, 2H, d4T-O_2C-NH-CH_2-CH_2-CH_2-*CH_2*-NH_2), 3.85–3.96 (m, 2H, d4T-O_2C-NH-*CH_2*-CH_2-CH_2-CH_2-NH_2), 3.91–3.96 (m, 1H, H-5′b), 4.25–4.41 (m, 2H, H-4′ + H-5′a), 4.84 (br s, 1H, d4T-O_2C-N*H*-CH_2-CH_2-CH_2-CH_2-NH_2), 5.91–5.93 (d, *J* = 5.9 Hz, 1H, H-2′), 6.31–6.35 (dd, *J* = 1.6, *J* = 5.9 Hz, 1H, H-3′), 6.91 (m, 1H, H-1′), 7.21 (s, 1H, H-6); MS (FAB^+, *m/z*): 339 $(M + H)^+$.

Synthesis of Dinucleoside Carbamates 12–14. To a solution of 5′-O-carbamate **10** or **11** (0.26 mmol) in 5 ml of dry THF was added activated carbonate **3** or **4** and **5** or **6** (0.29 mmol). The solution was stirred under nitrogen atmosphere for 48 h at 60°C. Then, solvent was evaporated under reduced pressure and the residue was subjected to flash chromatography column (gradient eluent 0–0.5% MeOH/CH_2Cl_2) to give dinucleoside carbamates **12** (81%), **13** (80%) and **14** (81%).

5′-[[*N*-[4-(3′-Azido-3′-deoxythymidin-5′-yl)butyl]carbamoyl]oxy]-3′-azido-3′-deoxythymidine (12). Yield: 83%; ^{1}H NMR (MeOH-d_4): δ 1.27–1.55 (m, 4H, AZT-OC-NH-CH_2-*CH_2*-*CH_2*-CH_2-NH-CO-AZT), 1.90 (s, 6H, 2*CH_3*), 2.41–2.47 (m, 4H, 2H-2′a,b), 3.15 (m, 4H, AZT-OC-NH-*CH_2*-CH_2-CH_2-*CH_2*-NH-CO-AZT), 3.32 (m, 2H, 2H-4′), 4.06-4.40 (m, 6H, 2H-3′ + 2H-5′a,b), 4.86 (br s, 2H, AZT-OC-N*H*-CH_2-CH_2-*CH_2*-CH_2-N*H*-CO-AZT), 6.15 (t, 1H, *J* = 6.7 Hz, H-1′), 7.48 (d, *J* = 1 Hz, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 12.15 (*CH_3*), 26.53 (NCH_2*CH_2*CH_2CH_2N), 35.46 (C-2′), 39.41 ($NCH_2CH_2CH_2$*CH_2*N), 60,53(C-3′), 63.66(C-5′), 83.33 (C-4′), 84.19 (C-1′), 109.87 (C-5), 135.45 (C-6), 150.32 (C-2), 155.22 (OCN), 163.76 (C-4); MS (FAB^+, *m/z*): 675 $(M + H)^+$.

5′-[[*N*-[4-(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)butyl]carbamoyl]oxy]-3′-azido-3′-deoxythymidine (13). Yield: 80%; ^{1}H NMR (MeOH-d_4): δ 1.27–1.49 (m,

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4H, AZT-OC-NH-CH_2-CH_2-CH_2-CH_2-NH-CO-d4T), 1.85 (s, 3H, CH_3), 1.86 (s, 3H, CH_3), 2.38–2.43 (m, 2H, H-2′a,b, AZT), 3.10 (m, 4H, AZT-OC-NH-CH_2-CH_2-CH_2-CH_2-NH-CO-d4T), 3.29 (m, 2H, 2H-4′), 4.05–4.35 (m, 5H, H-3′ + 2H-5′a,b), 4.97 (br s, 2H, AZT-OC-N*H*-CH_2-CH_2-CH_2-CH_2-N*H*-CO-d4T), 5.92 (d, 1H, J = 6 Hz, H-3′), 6.12 (t, 1H, J = 6,7 Hz, H-1′, AZT), 6.40 (dt, 1H, J = 6, J = 1.6 Hz, H-2′, d4T), 6.89 (t, J = 1.7 Hz, H-1′, d4T), 7.34 (s, 1H, H-6), 7.45 (s, 1H, H-6); MS (FAB$^+$, m/z): 632 $(M + H)^+$; ^{13}C NMR (DMSO-d_6): δ 12.03 (CH_3), 12.17 (CH_3), 26.70 ($NCH_2CH_2CH_2CH_2N$), 35.53 (C-2′, AZT), 39.98 ($NCH_2CH_2CH_2CH_2N$), 60,79 (C-3′, AZT), 63.77 (C-5′), 64.67 (C-5′), 81,21 (C-4′, d4T), 83.56 (C-4′), 84.22 (C-1′), 89.10 (C-1′), 109.89 (C-5), 126.39 (C-2′, d4T), 133.88 (C-3′, d4T), 135.83 (C-6), 135.92 (C-6), 150.41 (C-2), 150.97 (C-2), 155.77 (OCN), 155.91 (OCN), 163.65 (C-4), 163.80 (C-4); MS (FAB$^+$, m/z): 632 $(M + H)^+$.

5′-[[N-[4-(2′,3′-Dideoxy-2′,3′-didehydrothymidin-5′-yl)butyl]carbamoyl]oxy-2′,3′-dideoxy-2′,3′-didehydrothymidine (14). Yield: 81%; ^{1}H NMR (MeOH-d_4): δ 1.39–1.5 (m, 4H, d4T-OC-NH-CH_2-CH_2-CH_2-CH_2-NH-CO-d4T), 1.89 (s, 6H, 2CH_3), 3.10–3.19 (m, 4H, d4T -OC-NH-CH_2-CH_2-CH_2-CH_2-NH-CO-d4T), 3.30–3.34 (m, 2H, 2H-4′), 4.29–4.38 (m, 4H, 2H-5′a,b), 5.05 (br s, 2H, d4T-OC-N*H*-CH_2-CH_2-CH_2-CH_2-N*H*-CO-d4T), 5.95 (d, 2H, J = 6 Hz, 2H-2′), 6.43 (dt, 2H, J = 6, J = 1.6 Hz, 2H-3′), 6.93 (t, 2H, J = 1.7 Hz, 2H-1′), 7.39 (s, 1H, H-6); ^{13}C NMR (DMSO-d_6): δ 12.25 (CH_3), 26.92 ($NCH_2CH_2CH_2CH_2N$), 39.76 ($NCH_2CH_2CH_2CH_2N$), 64.70 (C-5′), 65.01 (C-5′), 84.41 (C-4′), 89.29 (C-1′), 110.09 (C-5), 126.58 (C-2′), 134.08 (C-3′), 136.03 (C-6), 151.00 (C-2), 156.09 (OCN), 164.04 (C-4); MS (FAB$^-$, m/z): 587 $(M-H)^-$.

Viruses and Cells. HIV-1(III_B)[25] and HIV-2(ROD)[25] were obtained from the culture supernatant of HIV-1- or HIV-2-infected MT-4 cells.[26]

Antiviral Activity and Cytotoxicity Assays. The inhibitory effects of AZT, d4T and their dimeric derivatives on HIV-1 and HIV-2 replication were monitored by measuring the viability of MT-4 cells 5 days after infection. Cytotoxicity of the compounds was determined in parallel by measuring the viability of mock-infected cells on day 5. The number of viable cells was quantified semi-automatically by a tetrazolium-based colorimetric method using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium (MTT).

The **anti-tuberculosis assay** was performed following the procedure described.[27]

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