New 3′-Azido-3′-deoxythymidin-5′-yl *O*-(ω-Hydroxyalkyl) Carbonate Prodrugs: Synthesis and Anti-HIV Evaluation

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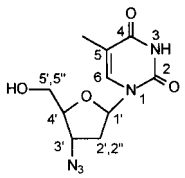
Prodrugs of zidovudine (AZT) have been synthesized in an effort to enhance its uptake by HIV-1 infected cells and its anti-HIV activity. The 5′-OH function of AZT was functionalized with various enzymatically labile alkyl groups using specific procedures. The prodrug moieties included 5′-*O*-carbonate, 5′-*O*-carbamate, and 5′-*O*-ester. Analogues of the 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate series were particularly interesting since they were rearranged through an intramolecular cyclic process during their enzymatic hydrolysis. Evidence of this prodrug rearrangement was confirmed by comparison of the serum half-lives of 5′-*O*-carbonate prodrugs with their corresponding 5′-*O*-ester- and 5′-*O*-carbamate-AZT prodrugs. Interestingly, the anti-HIV-1 activities (EC_{50}) of 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxybutyl) carbonate **10** in acutely infected MT-4 cells and in peripheral blood mononuclear cells (PBMCs) were 0.5 nM and 0.78 nM, respectively. Compound **10** was 30 to 50 times more potent than its parent drug AZT. Our results suggest that the specific intramolecular rearrangement associated with the 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs could explain the remarkable anti-HIV-1 activity of this series of AZT prodrugs. Prodrug **10** may therefore have better clinical potential than AZT for the treatment of AIDS.

Introduction

Intensive efforts are underway worldwide to develop chemotherapeutic agents effective against the human immunodeficiency virus (HIV),[1,2,3] the etiological agent of acquired immunodeficiency syndrome (AIDS).[4] Among the current diversity of compounds active against HIV, the 2′,3′-dideoxynucleosides (ddNs) remain by far the most potent.[5,6] In all cases, the expression of activity requires conversion into its corresponding 5′-*O*-triphosphate analogues.[7,8,9] These may inhibit the replication of the virus by competitive inhibition of the viral reverse transcriptase (RT) and/or by incorporation and subsequent chain termination of the growing viral DNA strand.[10] AZT (zidovudine, Figure 1) was the first ddN that was approved by the Food and Drug Administration (FDA)[11] for the treatment of patients suffering from AIDS.[12,13] Currently, six drugs belonging to the ddNs family are approved by the FDA and are commercially available: zidovudine (AZT, Retrovir),[11] stavudine (d4T, Zerit),[14] zalcitabine (ddC, Hivid),[15] didanosine (ddI, Videx),[16] lamivudine (3TC, Epivir),[17] and abacavir (1592U89, Ziagen).[18] Other families of compounds are also available and approved by the FDA, including the nucleoside analogues such as adefovir dipivoxil (bis-(POM)PMEA, Preveon)[19] and also the nonnucleoside reverse transcriptase inhibitors (NNRTIs), which interact at a specific site in HIV-1. This family includes



AZT
(Zidovudine, Retrovir)

Figure 1. Structure of AZT, the first FDA-approved drug for HIV treatment.

nevirapine (BI-RG-587, Viramune),[20] delavirdine (PNU-90152T, Rescriptor),[21] and efavirenz (DMP-266, Sustiva).[22,23] Protease inhibitors (PIs) are another family of compounds that inhibit the HIV protease. This family includes indinavir (MK-639, Crixivan), ritonavir (ABT-538, Norvir), saquinavir (Ro-31-8959, Invirase and Fortovase), nelfinavir (AG-1343, Viracept), and amprenavir (VX-478, Agenerase).[6,24,25] Although being the oldest compound approved by the FDA, AZT is still one of the most potent agents active against HIV. For many years now, AZT in combination with other nucleoside derivatives (such as lamivudine) and/or some NNRTIs (such as Viramune) and/or different PIs (such as indinavir and nelfinavir) has resulted in a decrease in the mortality rate and frequency of opportunistic infections in AIDS patients.[26] However, a significant dose-related toxicity associated with the administration of AZT, resulting in anemia and leucopenia, remains a limiting factor for its use.[27,28] Moreover, the necessity of administrating high doses of AZT to achieve adequate concentrations in the cerebrospinal fluid (CSF) results in bone marrow toxicity.[27,28] In attempts to overcome the

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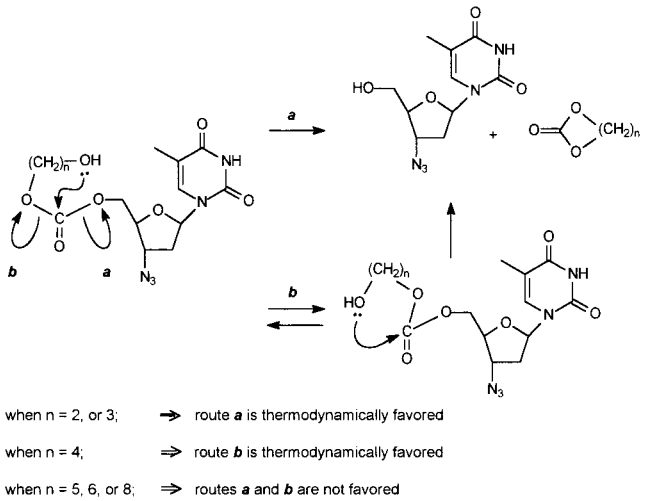


Figure 2. Cyclic intramolecular rearrangement process on 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate which recovers the initial prodrug.

problem of rapid elimination of AZT from the brain and to increase its therapeutic efficacy, numerous AZT prodrugs have been reported in the literature.[29−33] In most of the cases, the mechanism of action of these AZT prodrugs is based on hydrolysis of the enzymatically labile 5′-*O*-bonds between the drug (AZT) and its spacer group. The expected advantages of the AZT prodrugs can be multiple: synergistic drug interactions,[34−36] enhancement of AZT intracellular uptake,[37] increase of AZT brain delivery,[38,39] and bypass of the first AZT phosphorylation step into the cells.[40−43]

In the present paper, we report the specific behavior of new 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs, which are rearranged during the enzymatic hydrolysis of their 5′-*O*-carbonate bond, through an intramolecular cyclic process (Figure 2).

The evidence for this intramolecular cyclic rearrangement, specifically associated with 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs, is confirmed by comparison of their sensitivity to enzymatic hydrolysis and biological properties with various 5′-*O*-ester-, 5′-*O*-carbamate, and other 5′-*O*-carbonate-AZT prodrugs.

With this aim, we have designed and synthesized several series of AZT prodrugs with the general formula shown in Figure 3. We then evaluated their anti-HIV-1 activities in cell cultures.

To study the influence of the terminal functional group X and of the linker moiety Y on the sensitivity of various prodrugs to enzymatic hydrolysis, we arbitrarily fixed the parameter *n* to 3 (Figure 3). We have then studied the influence of the alkyl chain length ($n = 2$, 3, 4, 5, 6, and 8) on the stability against enzymatic hydrolysis and anti-HIV activity of 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs.

Chemistry

In this paper, we describe the synthesis of three series of AZT prodrugs (Figure 3 and Scheme 1).

Ester Series (Y = −C(O)−). Compound **1** was obtained directly by condensation of AZT with glutaric anhydride in the presence of DMAP in DMF in 86% yield.[44] Esterification of acid **1** was achieved through

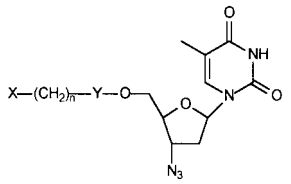


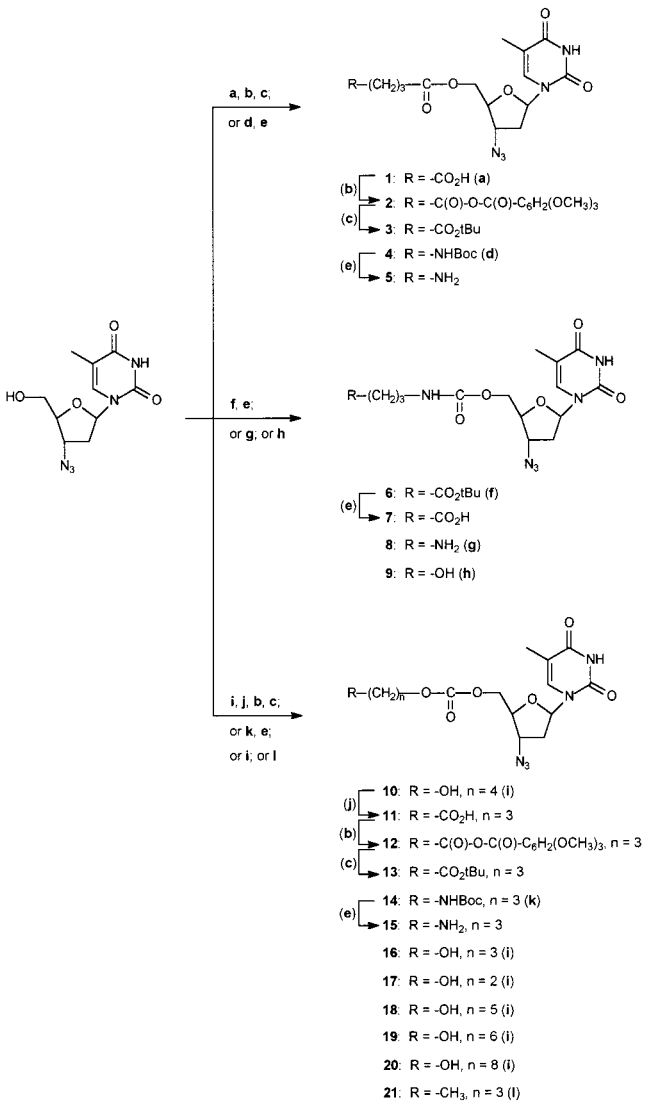
Figure 3. Structure of the three series of 5′-*O*-AZT prodrugs.

the formation of a mixed anhydride using 3,4,5-trimethoxybenzoyl chloride in the presence of Et_3N in EtOAc in 86% yield. The resulting anhydride **2** was converted into its ω-*tert*-butyl ester **3** by addition of *t*BuOH in the presence of DMAP in CH_2Cl_2 in 77% yield.[45] The preparation of compound **4** was performed by esterification of AZT with 4-(*tert*-butoxycarbonyl)-amino-1-butanoic acid in the presence of DCC and DMAP in DMF in 90% yield.[37] *N*-Boc deprotection of compound **4** by TFA in CH_2Cl_2 led to prodrug **5** in quantitative yield. As expected, chemical synthesis of the 4-hydroxy-1-(3′-azido-3′-deoxythymidin-5′-yl) butanoate was impossible because of its in situ lactonisation to γ-butyrolactone.

Carbamate Series (Y = −NH−C(O)−). 5′-*O*-Carbamate-AZT prodrugs were obtained by condensation of AZT with *N*,*N*-carbonyldimidazole (CDI) in the presence of various primary amines.[46] Carbamates **6**, **8**, and **9** were obtained by the condensation of AZT with CDI in DMF or CH_3CN in the presence of 4-amino-1-*tert*-butyl butanoate, 1,3-diaminopropane, and 3-amino-1-propanol in 65%, 78%, and 60% yields, respectively. Hydrolysis of the *tert*-butyl ester group in compound **6** by TFA in CH_2Cl_2 led to prodrug **7** in 94% yield.

Carbonate Series (Y = −O−C(O)−). 5′-*O*-Carbonate-AZT prodrugs were obtained by condensation of AZT with CDI in the presence of various ω-alcohols.[46] In this manner, numerous 5′-*O*-carbonate-AZT prodrugs were obtained in yields ranging from 49% to 85%. Condensation of AZT with CDI in DMF in the presence of 1,4-butanediol gave compound **10** in 85% yield. Its oxidation using TEMPO·HCl and *m*-CPBA in CH_2Cl_2 led to compound **11** in 69% yield.[47] Esterification of acid **11** was achieved via mixed anhydride **12** which was formed by reaction of compound **11** with 3,4,5-trimethoxybenzoyl chloride in the presence of Et_3N in EtOAc in 88% yield. The resulting anhydride **12** was converted into the ω-*tert*-butyl ester **13** by treatment with *t*BuOH in the presence of DMAP in CH_2Cl_2 in 75% yield.[45] Condensation of AZT with CDI in CH_3CN in the presence of 3-(*tert*-butoxycarbonyl)amino-1-propanol led to the *N*-protected intermediate **14** in 68% yield.[46] Its *N*-Boc deprotection by TFA in CH_2Cl_2 gave prodrug **15** in quantitative yield. The condensation of AZT with CDI in DMF in the presence of 1,3-propanediol led to compound **16** in 66% yield.[46] Carbonates **17**, **18**, **19**, and **20** were obtained by condensation of AZT with CDI in

Scheme 1[a]



[a] Reagents: (a) glutaric anhydride, DMAP, DMF; (b) 3,4,5-trimethoxybenzoyl chloride, Et_3N, AcOEt; (c) *tert*-butyl alcohol, DMAP, CH_2Cl_2; (d) BocNH-$(CH_2)_3$-CO_2H, DCC, DMAP, DMF; (e) TFA, CH_2Cl_2; (f) CDI, DMF then H_2N-$(CH_2)_3$-CO_2tBu, DMF; (g) CDI, DMF then H_2N-$(CH_2)_3$-NH_2, DMF; (h) CDI, CH_3CN then H_2N-$(CH_2)_3$-OH, CH_3CN; (i) CDI, DMF then HO-$(CH_2)_n$-OH, DMF; (j) *m*-CPBA, Tempo·HCl, CH_2Cl_2; (k) CDI, CH_3CN then BocNH-$(CH_2)_3$-OH, CH_3CN; (l) CDI, CH_3CN then H_3C-$(CH_2)_3$-OH, CH_3CN.

DMF in the presence of 1,2-ethanediol, 1,5-pentanediol, 1,6-hexanediol, and 1,8-octanediol in 56%, 70%, 49%, and 63% yields, respectively.[46] Finally, the butyl carbonate **21** was obtained by condensation of AZT with CDI in CH_3CN in the presence of 1-butanol in 64% yield.[46]

Results and Discussion

To be clinically useful, a prodrug should fulfill pharmacokinetic and pharmacodynamic profiles which are mainly dependent on the kinetics of their conversion to the parent drug and on its membrane permeation properties.[48] These two properties are related to the sensitivity of the linkage between the prodrug moiety and the parent drug to enzymatic hydrolysis and, on the other hand, to their lipophilicity.

Taking into account that during the course of enzymatic hydrolysis of the AZT prodrugs, the influence of the alkyl chain length of the spacer bearing a terminal hydroxyl group was essential for the intramolecular cyclic rearrangement of the prodrug, we have synthesized various 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs (**17**, **16**, **10**, **18**, **19**, and **20**). To validate the proposed intramolecular cyclic rearrangement of these prodrugs, we have also performed the synthesis and studied the biological properties of various 5′-*O*-ester- (**1**, **3**, **5**), 5′-*O*-carbamate- (**6**−**9**), and other 5′-*O*-carbonate-AZT prodrugs (**11**, **13**, **15**, **21**). In our hypothesis, the rate of the intramolecular cyclic rearrangement might be under the control of the methylene chain length of the spacer. As shown in Figure 2, two different cleavage mechanisms could be considered during the enzymatic hydrolysis of a prodrug by human serum hydrolases (carboxyesterases).

(i) In compounds **17** ($n = 2$) and **16** ($n = 3$): hydrolytic cleavage according to route *a* was likely to occur since the release of AZT would involve the formation of a five- or six-membered ring, respectively, which was thermodynamically favored. In contrast, hydrolytic cleavage according to route *b*, leading to the cyclic rearrangement of the prodrugs **17** and **16** by an intramolecular nucleophilic attack of the terminal hydroxyl function, was not favored.

(ii) In compound **10** ($n = 4$): hydrolytic cleavage according to route *a*, leading to the release of AZT, was thermodynamically unfavorable since this process would involve formation of a seven-membered ring. In contrast, hydrolytic cleavage according to route *b*, leading to the cyclic rearrangement of the prodrug **10** by an intramolecular nucleophilic attack of the terminal hydroxyl function, was favored. Under these conditions, the half-life ($t_{1/2}$) of enzymatic hydrolysis of this prodrug **10** should be enhanced.

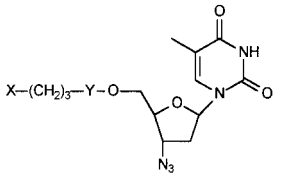
(iii) In compounds **18** ($n = 5$), **19** ($n = 6$), and **20** ($n = 8$): hydrolytic cleavage by route *a*, leading to the release of AZT via the formation of, respectively, an eight-, nine-, or eleven-membered ring was thermodynamically unfavorable. Hydrolytic cleavage by route *b*, leading to the cyclic rearrangement of prodrugs **18**, **19**, or **20**, respectively, by an intramolecular nucleophilic attack of the terminal hydroxyl function, was also not favored because of the growth of their alkyl chain length (which will become too long).

(iv) Compound **21** cannot be cleaved by the intramolecular rearrangement mechanism. This compound did not bear a terminal hydroxyl group, which could help the release of AZT or the cyclic rearrangement of the prodrug by an intramolecular nucleophilic attack. This prodrug **21** was used as a reference compound for prodrug **10** in order to validate the concept.

Serum Hydrolase Stability Studies and Lipophilicity (Tables 1 and 2). Taking into account this hypothesis on chemical reactivity of the carbonate-AZT prodrugs, we determined the enzymatic hydrolysis half-lives ($t_{1/2}$) in human serum of every AZT prodrug synthesized, by an HPLC method. The data reported in Tables 1 and 2 suggest the following conclusions:

(i) Generally the 5′-*O*-carbonate-AZT prodrugs (**11**, **13**, **15**, and **16**) were most sensitive to enzymatic hydrolysis ($t_{1/2}$ values ranging from 8 to 780 min), while the 5′-*O*-ester-AZT prodrugs (**1**, **3**, **5**) were less sensitive to the serum hydrolase action, with $t_{1/2}$ values ranging from

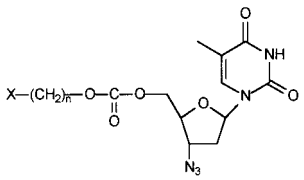
Table 1. Biological Properties of Numerous 5′-*O*-Ester-, 5′-*O*-Carbamate-, and 5′-*O*-Carbonate-AZT Prodrugs



compd	structure X	structure Y	$t_{1/2}$[a] (min)	log *P*[b]	EC_{50}[c] (nM)	CC_{50}[d] (μM)	SI[e]
1	HO_2C-	-C(O)-	1800	−0.52	7	>50	>7143
3	*t*BuO_2C-	-C(O)-	33	1.17	5	>50	>10000
5	H_2N-	-C(O)-	26	−0.90	20	>50	>2500
/	HO-	-C(O)-		−0.95			
7	HO_2C-	-NH-C(O)-	>2880	−0.81	100	>50	>500
6	*t*BuO_2C-	-NH-C(O)-	>2880	0.88	10	>50	>5000
8	H_2N-	-NH-C(O)-	>2880	−1.19	10	>50	>5000
9	HO-	-NH-C(O)-	>2880	−1.24	100	>50	>500
11	HO_2C-	-O-C(O)-	780	−0.55	5	>50	>10000
13	*t*BuO_2C-	-O-C(O)-	16	1.14	5	>50	>10000
15	H_2N-	-O-C(O)-	8	−0.93	20	>50	>2500
16	HO-	-O-C(O)-	10	−0.98	10	>50	>5000
AZT		H		−0.88	25	>100	>4000

[a] $t_{1/2}$ (half-life) is the time required for 50% hydrolysis of prodrugs to AZT at 37 °C upon incubation in human serum (Normal Human Serum). [b] log *P* determinations were performed using ACD (Advanced Chemistry Development, Inc.)/log *P* 1.0 base calculations. [c] EC_{50}: concentration in nM required to inhibit the cytopathicity of HIV-1 by 50% in MT-4 cells. [d] CC_{50}: concentration in μM required to cause 50% death of uninfected MT-4 cells. [e] SI: selectivity index = CC_{50}/EC_{50}.

Table 2. Biological Properties of Various 5′-*O*-Carbonate-AZT Prodrugs, a Study of Their Alkyl Chain Length



compd	structure X	*n*	$t_{1/2}$[a] (min)	log *P*[b]	EC_{50}[c] (nM)	CC_{50}[d] (μM)	SI[e]
11	HO_2C-	3	780	−0.55	5	>50	>10000
13	*t*BuO_2C-	3	16	1.14	5	>50	>10000
15	H_2N-	3	8	−0.93	20	>50	>2500
16	HO-	3	10	−0.98	10	>50	>5000
17	HO-	2	<1	−1.20	10	>50	>5000
10	HO-	4	30	−0.68	0.5	>50	>100000
21	H-	4	9	1.02	8	>50	>6250
18	HO-	5	16	−0.45	10	>50	>5000
19	HO-	6	7	0.08	10	>50	>5000
20	HO-	8	2	1.14	2.5	>50	>20000
AZT				−0.88	25	>100	>4000

[a] $t_{1/2}$ (half-life) is the time required for 50% hydrolysis of prodrugs to AZT at 37 °C upon incubation in human serum (Normal Human Serum). [b] log *P* determinations were performed using ACD (Advanced Chemistry Development, Inc.)/log *P* 1.0 base calculations. [c] EC_{50}: concentration in nM required to inhibit the cytopathicity of HIV-1 by 50% in MT-4 cells. [d] CC_{50}: concentration in μM required to cause 50% death of uninfected MT-4 cells. [e] SI: selectivity index = CC_{50}/EC_{50}.

26 to 1800 min (Table 1). In contrast, the 5′-*O*-carbamate-AZT prodrugs (**7**, **6**, **8**, **9**) appeared to be very stable since they did not undergo enzymatic hydrolytic cleavage for up to 48 h. These results suggested that sensitivity to enzymatic hydrolysis of the three series of AZT prodrugs depends on the nature of their 5′-*O*-functions. Carbamate prodrugs were more stable than

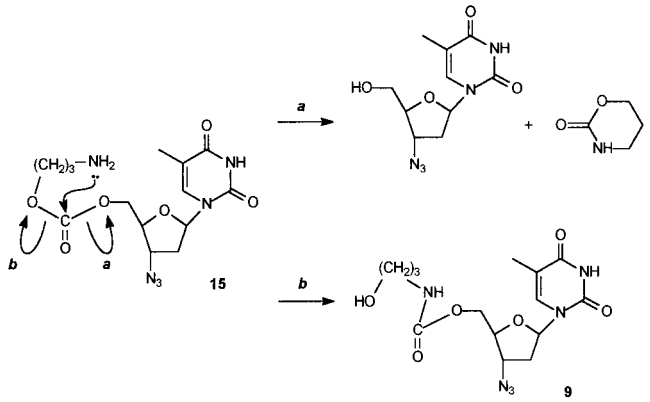


Figure 4. Nucleophilic intramolecular attack of the ω-amino group of compound **15** on its 5′-*O*-carbonate function leading to the generation of the 3′-azido-3′-deoxythymidin-5′-yl *N*-(3-hydroxypropyl) carbonate **9**.

their ester counterparts, which were themselves more stable than their carbonate analogues. This stability of the carbamate function, in human serum, was in accordance with literature data.[49] The influence of the 5′-*O*-terminal groups on the sensitivity to enzymatic hydrolysis in a prodrug (ester or carbonate) series was in the following stability order: carboxylic acid ≫ *tert*-butyl ester > amine ≈ alcohol (Table 1). The terminal carboxylic acid group increased the prodrug enzymatic stability compared to compounds with terminal amino or hydroxyl groups. This result was not surprising since it was known that the presence of a carboxylate group in the structure of a compound inhibits the serum hydrolase activity.[50] Moreover, esterification of the terminal carboxylic acid by a *tert*-butyl group decreased the half-life ($t_{1/2}$) of the resulting *tert*-butyl ester, because of the impossible liberation of carboxylate ions. The presence of an amino or hydroxyl group at the ω-position of the 5′-*O*-spacer enabled the intramolecular nucleophilic attack of this terminal group on the 5′-*O*-ester (e.g., lactonization of 4-hydroxy-1-(3′-azido-3′-deoxythymidin-5′-yl) butanoate to γ-butyrolactone) or 5′-*O*-carbonate functions.

(ii) When the 3′-azido-3′-deoxythymidin-5′-yl *O*-(3-aminopropyl) carbonate **15** was submitted to serum hydrolase, we clearly identified AZT (48%) and the 3′-azido-3′-deoxythymidin-5′-yl *N*-(3-hydroxypropyl) carbamate **9** (52%) on HPLC profile as hydrolysis products. Compound **9** resulted from the nucleophilic intramolecular attack of the ω-amino group on the 5′-*O*-carbonate function within compound **15** (Figure 4). This result confirmed the two possible hydrolytic cleavages (routes *a* or *b*) in prodrug **15**, leading to the release of AZT or to the generation of the carbamate function (prodrug **9**), respectively. This hypothesis was already suggested in the case of 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs (Figure 2).

(iii) We could also observe (Table 2) that compound **10** was the most stable within the 5′-*O*-carbonate series (**17**, **16**, **10**, **18**, **19**, **20**) in human serum ($t_{1/2}$ = 30 min). In comparison, the reference compound **21** possessed a shorter half-life ($t_{1/2}$ = 9 min). These results support our hypothesis of an intramolecular cyclic rearrangement (associated with the intramolecular nucleophilic attack of the ω-hydroxyl group on 5′-*O*-carbonate function) specific to compound **10**, which led to the enhancement

of its stability (increased $t_{1/2}$) toward serum hydrolase. In summary, this cyclic rearrangement process of the prodrug according to route *b* was favored in the case of a four-carbon chain length.

(iv) In contrast, 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs (**17**, **16**, **18**, **19**, and **20**) whose intramolecular cyclic rearrangement process according to route *b* was unfavorable had shorter $t_{1/2}$ values (<1, 10, 16, 7, and 2 min, respectively) than compound **10** (30 min).

In conclusion, these serum hydrolase stability studies were in accordance with our thermodynamic predictions concerning the specific enzymatic hydrolysis process of 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs.

As far as lipophilicity is concerned, it is known that AZT crosses cell membranes by nonfacilitated diffusion and that its uptake is insensitive to inhibitors of nucleoside transport.[48] Moreover, it has been reported that some AZT prodrugs containing a 5′-hydroxyl group esterified with various amino acid ligands exhibited anti-HIV activities in peripheral blood lymphocytes (PBL) greater than that of AZT.[37] 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.[51] Therefore the log *P* values between *n*-octanol and water were determined for all compounds, using ACD/log *P* software from ChemCAD. The results in Tables 1 and 2 show log *P* values are in the range of −1.24 to +1.17 for different series of prodrugs (the log *P* value for AZT itself was −0.88). Moreover, from serum hydrolase stability studies, it is evident that the lipophilicity of the AZT prodrugs does not correlate with their sensitivity to enzymatic hydrolysis.

Antiviral Activity Measurements. The three series of AZT prodrugs (compounds **1**, **3**, **5**−**11**, **13**, **15**−**21**) were evaluated for their inhibitory effects on HIV replication, monitored by the efficiency of the different compounds to inhibit the cytopathicity of HIV after acute infection of MT-4 cell cultures. The 50% effective concentration (EC_{50}) represented the concentration required to inhibit by 50% the cytopathicity of HIV for MT-4 cells. Under these assay conditions, AZT inhibited HIV replication at EC_{50} = 25 nM. Under the same conditions, the anti-HIV activities of most of the AZT prodrugs tested were found to be similar or more pronounced than that of their parent drug (AZT). All of the AZT prodrugs tested elicited anti-HIV activities with EC_{50} values ranging from 0.5 to 100 nM (Tables 1 and 2). The results obtained suggested the following comments:

(i) The prodrugs appeared to be sufficiently lipophilic to cross the cellular membrane by passive diffusion like AZT (without excluding a facilitated transport for these prodrug series) as demonstrated by the antiviral activities. In fact, from data reported in Tables 1 and 2, it can be observed that no correlation between partition coefficient (log *P*) and anti-HIV activity (EC_{50}) can be drawn.

(ii) The overall results were quite intriguing since, depending on both parameters, i.e., (a) the alkyl chain length of the spacer and (b) the terminal functional group X (−COOH, $-CO_2t$Bu, $-NH_2$, −OH, $-CH_3$), notable variations in antiviral activity were observed. It is evident (Tables 1 and 2) that no prodrugs in the ester or carbamate series were more active than their corresponding analogues of the carbonate series (except for compound **8** and its analogue **15**, because compound **15** is partly converted into carbamate **9**).

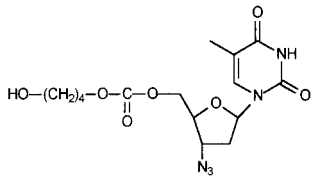
(iii) Under assay conditions, among the compounds bearing a terminal hydroxyl function, the most potent 5′-*O*-carbonate-AZT prodrug was compound **10** (n = 4) with an EC_{50} = 0.5 nM. It was more active than its structural analogues **17**, **16**, **18**, **19**, or **20** (with n = 2, 3, 5, 6, and 8, respectively). These antiviral activities support the suggested intramolecular cyclic rearrangement specific to this new class of 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs. This result appeared to be in complete agreement with the above-mentioned thermodynamic predictions (Figure 2) and with the serum hydrolase studies (Tables 1 and 2). The increased antiviral activity of compound **10** might be related to its favorable serum half-life ($t_{1/2}$ = 30 min) associated with the intramolecular rearrangement process which enhanced both its hydrolytic stability and its intracellular uptake under in vitro conditions. Moreover, we cannot rule out that the prodrugs that are more hydrophobic than its parent drug will have a limited efflux once inside the target cells. Thus, their increased half-life in the cell pool will lead to a prolonged release of AZT from the continuing cleavage of the prodrug.

(iv) In order to confirm the increased antiviral potency of compound **10** vs AZT, anti-HIV evaluation was also performed in PBMCs, infected with different virus strains (HIV-1 III_B and BaL). PBMCs are an interesting research tool, being the primary human targets of HIV. Compound **10** was approximately 10 times more active than AZT (Table 3). These data confirm the activity of compound **10** in MT-4 cells infected by HIV-1, which was found to be 50 times higher compared to AZT (Table 2).

These results show that a subtle balance between the rate of intracellular uptake and the sensitivity of enzymatic hydrolysis of a prodrug under in vitro conditions was required for optimum antiviral activity. Moreover, the antiviral results for 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs were in complete agreement with the above-mentioned thermodynamic predictions for the intramolecular cyclic rearrangement process and the serum hydrolase studies. Another very interesting point is the finding that the antiviral activities of carbamates are similar to AZT. These compounds seem to be quite stable in the cells, so that the formation of significant levels of free intracellular nucleoside is unlikely. It is possible that these carbamates act through another mechanism of action (perhaps, direct inhibition of HIV-reverse transcriptase). This finding warrants further investigation.

Cytotoxicity. The cytotoxicity of AZT and its prodrugs against MT-4 cells was determined using the MTT method and in PBMCs by the trypan blue exclusion method for viability determination. The 50% cytotoxic concentration (CC_{50}) represented the concentration required to cause 50% uninfected cell death. The data reported in Tables 1, 2, and 3 show that most of the AZT prodrugs tested have selectivity indexes (SI) similar or better than that of AZT. We also noted that the

Table 3. Anti-HIV Properties of Compound **10** in MT-4 Cells and in PBMCs Acutely Infected with Different Strains of HIV-1 (BRU, III_B, and BaL)



	MT-4			PBMCs					
	HIV-1 BRU			HIV-1 III_B			HIV-1 BaL		
compd	EC_{50}[a] (nM)	CC_{50}[b] (μM)	SI[c]	EC_{50}[a] (nM)	CC_{50}[b] (μM)	SI[c]	EC_{50}[a] (nM)	CC_{50}[b] (μM)	SI[c]
10	0.5	>50	>100000	0.78	29	37200	0.78	24	30800
AZT	25	>100	>4000	7.5	8	1067	11	11	1000

[a] EC_{50}: concentration in nM required to inhibit the cytopathicity of HIV-1 by 50% on MT-4 cells or to produce inhibition of 50% of HIV-1 replication on PBMCs. [b] CC_{50}: concentration in μM required to cause 50% death of uninfected MT-4 cells or PBMCs. [c] SI: selectivity index = CC_{50}/EC_{50}.

cytotoxic dose of the compound depends on the cell line used. For instance, the most active compound **10** appeared to be slightly more toxic than AZT in MT-4 cells, while in PBMCs it was approximately 2 to 4 times less cytotoxic than the parent drug. In any cases, the SI of compound **10** was approximately 30 times higher than the SI of AZT. These results suggest that the prodrug **10** may have better clinical potential than its parent drug AZT for the treatment of AIDS.

Conclusion

In the present paper, we report the synthesis, biological properties, and the anti-HIV evaluation of a new series of 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs. Among this AZT prodrug series, compound **10** (3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxybutyl) carbonate) was found to be 50 and 30 times more potent than AZT against the replication of HIV in acutely infected MT-4 cells and PBMCs, respectively. Serum hydrolase studies have demonstrated that 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs can be recovered through an intramolecular cyclic rearrangement process (Figure 2). In fact, the initial prodrug is rearranged through an intramolecular cyclic mechanism resulting from the nucleophilic attack of the 5′-*O*-carbonate bond by the terminal hydroxyl function of the spacer. The optimum alkyl chain, for this cyclic rearrangement process, includes 4 methylene groups inserted between the 5′-*O*-carbonate bond of the AZT prodrug and the terminal hydroxyl function (compound **10**). This finding is in complete agreement with our thermodynamic predictions. Prodrug **10** has an enhanced half-life in human serum ($t_{1/2}$ = 30 min) compared with the other 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate prodrugs **16**-**20** ($1 < t_{1/2} < 16$ min, Table 2). This cyclic rearrangement process could explain the observed improvement of anti-HIV activity in prodrug **10**. The increased anti-HIV activity of compound **10** can be also attributed to its resistance to enzymatic hydrolysis (increased serum half-life), its increased cellular uptake (membrane permeation), and its continued cleavage to AZT inside the target cells (prolonged release). We have observed that compound **10** is less cytotoxic than AZT in PBMCs. Our results suggest that this prodrug could enhance AZT bioavailability and result in better clinical potential than its parent drug (AZT) for the treatment of AIDS. However, the mechanism of action of this 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxybutyl) carbonate **10** might be clarified by intramolecular quantification of the amount of AZT-MP, AZT-DP, and AZT-TP, for example.

Consequently, the cyclic intramolecular rearrangement of the carbonate series, specifically associated with the 5′-*O*-(4-hydroxybutyl) carbonate moiety, constitutes a new concept which could be extended to other known drugs and warrants their further investigation as potential clinical candidates.

Experimental Section

Nuclear magnetic resonance spectra were recorded with a Bruker AC-250 spectrometer (^{1}H NMR and ^{13}C NMR); chemical shifts are expressed as δ units (part per million) downfield from TMS (tetramethylsilane). Fast atom bombardment (FAB^+ or FAB^-) mass spectral analyses were obtained by Dr. Astier (Laboratoire de Mesures Physiques-RMN, USTL, Montpellier, France) on a JEOL DX-100 using a cesium ion source and glycerol/thioglycerol (1:1) or *m*-nitrobenzyl alcohol (NOBA) as matrix. Mass calibration was performed using cesium iodide. IR spectra were recorded on a Perkin-Elmer FTIR 1605 spectrophotometer. Microanalyses were carried out by Service Central d'Analyses du CNRS (Venaison, France) and were within 0.4% of the theoretical values. Thin-layer chromatography (TLC) and preparative layer chromatography (PLC) were performed using silica gel plates 0.2, 1, or 2 mm thick ($60F_{254}$ Merck). Preparative flash column chromatography was carried out on silica gel (230−240 mesh, G60 Merck). Analytical HPLC was performed on a Waters 600E instrument with a M991 detector using the following conditions: 4.6 × 150 mm column (Waters Spherisorb S5 ODS2, 5 μM); mobile phases: A = 0.1% TFA in H_2O, B = CH_3OH; flow rate 1 mL/min. All reagents were of commercial quality (Aldrich Company) from freshly opened containers.

Cell Lines, Virus, and Cell Cultures. (i) The CEM cell line and the T-leukemia virus type one (HTLV-I) $CD4^+$ T cell line, MT-4, were cultured in RPMI/10% FCS, and the medium replaced twice a week. The laboratory-adapted strain HIV^{LAV} clade B stock was prepared from the supernatant of infected CEM cell line, and aliquots were kept frozen at −80 °C until use.[1]

(ii) Inhibition of HIV-1 replication was also determined with PBMCs from seronegative donors. Briefly, 10×10^6 stimulated PBMCs were infected, respectively, with HIV-1 III_B and HIV-1 BaL and were incubated for 2 h at 37 °C under 4.5% CO_2. These aliquots were kept frozen at −80 °C until used.

Anti-HIV Activity Assays. (i) Anti-HIV activity was monitored by the efficiency of drug compounds to inhibit the

cytopathicity of HIV in MT-4 cells as already described.[52,53] Briefly, 3×10^5 MT-4 cells were first preincubated with 100 μL of various concentrations of drug compounds dissolved in DMSO or in H_2O and then diluted in phosphate buffer saline solution for 1 h at 37 °C. Then 100 μL of an appropriate virus dilution was added to the mixture, and cells incubated for 1 h at 37 °C. After three washes, cells were resuspended in culture medium in the presence or absence of drug compounds. Cultures were then continued for 7 days at 37 °C under 5% CO_2 atmosphere, and medium was replaced on day 3 post-infection with culture medium supplemented or not with drug compounds. Each culture condition was carried out in duplicate. Viral cytopathicity was followed each day with an inverted optical microscope. Typically, the virus dilution used in this assay (multiplicity of infection of 0.1 TCID/cell) led to cytopathicity on day 5 post-infection. The inhibitory concentration of drug compounds was expressed as the concentration that caused 50% inhibition of viral cytopathicity (EC_{50}) without direct toxicity for the cells. The cytotoxic concentration (CC_{50}) of drug compounds was monitored based on the growth of noninfected cells by trypan blue exclusion method and corresponded to the concentration required to cause 50% cell death. It should be emphasized that when compounds required the addition of DMSO to be solubilized in water, the concentration in volume of DMSO used was always less than 10% with respect to water (the final concentration of DMSO in MT-4 cells incubation medium being less than 2%). As far as DMSO could affect the antiviral activity of the tested drugs,[54] antiviral assays in which solutions containing equal concentrations of DMSO in water were performed and used as standard assays for each tested drug. EC_{50} and CC_{50} reported values were then calculated from these standard assays. Moreover, final DMSO concentrations (1/1000) were very far from the percentages which induced an enhancement of in vitro HIV-1 infection of T-cells.[54]

(ii) PBMCs from healthy donors were isolated by density centrifugation (Lymphoprep; Nycomed Pharma, AS Diagnostics, Oslo, Norway) and stimulated with phytohemagglutin (PHA) (Sigma Chemical Co., Bornem, Belgium) for 3 days. The activated cells (PHA-stimulated blasts) were washed with PBS, and viral infections were done as described by the AIDS clinical trial group protocol.[55] Briefly, PBMCs (2×10^5/200 μL) were plated in the presence of serial dilutions of the test compound and were infected with HIV-1 (III_B) or HIV-1 (BaL) at 1000$CCID_{50}$ per milliliter. At day 4 post-infection, 125 μL of the supernatant of the infected cultures was removed and replaced with 150 μL of fresh medium containing the test compound at the appropriate concentration. At 7 days after plating the cells, the p24 antigen was detected in the culture supernatant by an enzyme-linked immunosorbent assay (ELISA) (NEN, Paris, France).

Hydrolysis of the Prodrugs 1, 3, 5−11, 13, and 15−21 in Human Serum.[37] To 990 μL of normal human serum (NHS) was added 10 μL of a solution of the prodrug (10 mg/mL in DMSO), and the mixture was incubated at 37 °C in a water bath. At various time intervals, the samples (100 μL) were withdrawn and added immediately to ice-cold methanol (400 μL). The resulting samples were centrifuged (7 min, 3000 rpm). The supernatants were filtered through nylon filters (0.45 μm) and then analyzed by HPLC using the following methods. (i) Method I: 20% of solvent B in A to 100% of B in 20 min. (ii) Method II: 40% of solvent B in A to 100% of B in 20 min (A = 0.1% TFA in H_2O, B = CH_3OH). The absorption maximum for all the studied prodrugs is at 267 nm; therefore, this wavelength was used for the HPLC detection. Peak retention times (t_r) were 10.0 min for AZT (method I), 15.2 min for **1** (method I), 17.8 min for **3** (method II), 11.5 min for **5** (method I), 13.4 min for **7** (method I), 16.2 min for **6** (method II), 11.7 min for **8** (method I), 12.9 min for **9** (method I), 15.5 min for **11** (method I), 18.1 min for **13** (method II), 11.9 min for **15** (method I), 14.2 min for **16** (method I), 12.5 min for **17** (method I), 15.7 min for **10** (method I), 20.4 min for **21** (method I), 17.1 min for **18** (method I), 17.9 min for **19** (method I), and 20.1 min for **20** (method I). The $t_{1/2}$ values calculated from peak areas for all the compounds studied, **1**, **3**, **5**−**11**, **13**, and **15**−**21**, in normal human serum are summarized in Tables 1 and 2.

Chemical Syntheses. Mono-(3′-azido-3′-deoxythymidin-5′-yl) Ester 1,5-Pentanedioic Acid 1. To a solution of AZT (0.100 g, 0.374 mmol, 1 equiv) in anhydrous DMF (3 mL), under N_2, containing DMAP (0.043 g, 0.374 mmol, 1 equiv) was added glutaric anhydride (0.047 g, 0.412 mmol, 1.1 equiv). The reaction mixture was stirred at room temperature for 14 h. Then, the solvent was evaporated under reduced pressure. The residual oil was dissolved in CH_2Cl_2 (5 mL) and successively washed with 1 N HCl (2 × 4 mL) and water (2 × 4 mL). The combined aqueous solutions were extracted twice with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The residue was purified by flash chromatography on silica gel, using CH_3OH/CH_2Cl_2 5:95 as eluent, to give the title compound **1** as a white foam (0.122 g, 86%): R_f = 0.28 (CH_3OH/CH_2Cl_2 10:90); MS (FAB^+) 382 $(M + H)^+$; HPLC t_r = 15.2 min (method I). Anal. ($C_{15}H_{19}N_5O_7$) C, H, N.

General Procedure A for the Preparation of Mixed Anhydrides. To a solution of ω-carboxylic acid (1 equiv) in anhydrous EtOAc (5 mL/mmol), under N_2, containing Et_3N (3 equiv) was added dropwise at 0 °C 3,4,5-trimethoxybenzoyl chloride (1.1 equiv) in anhydrous EtOAc (5 mL/mmol). The reaction mixture was then stirred for 4 h at room temperature, diluted with EtOAc, and washed twice with 0.5 N HCl. After extraction, the organic layer was washed twice with water and dried over Na_2SO_4. EtOAc was removed under reduced pressure, and the product slowly crystallized under vacuum. The resulting mixed anhydride was used in the next step without further purification.

Mixed Anhydride of 3,4,5-Trimethoxybenzoic Acid and Mono-(3′-azido-3′-deoxythymidin-5′-yl) Ester 1,5-Pentanedioic Acid 2. According to the general procedure A, the reaction of compound **1** (0.048 g, 0.126 mmol, 1 equiv) afforded the title compound **2** (0.062 g, 86% yield): MS (FAB^+) 576 $(M + H)^+$.

General Procedure B for the Preparation of ω-*tert*-Butyl Esters. To a solution of *tert*-butyl alcohol (5 equiv) in anhydrous CH_2Cl_2 (0.2 mL/mmol), under N_2, containing DMAP (1.2 equiv) was added dropwise mixed anhydride (1 equiv) dissolved in anhydrous CH_2Cl_2 (5 mL/mmol). The reaction mixture was stirred for 14 h at room temperature. The solvent was evaporated under reduced pressure. The residual oil was dissolved in CH_2Cl_2 (5 mL), successively washed with 5% aqueous citric acid (2 × 3 mL), 5% aqueous $NaHCO_3$ (2 × 3 mL), and water (2 × 3 mL). The combined aqueous solutions were extracted twice with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The isolated ω-*tert*-butyl ester was pure as demonstrated by its physicochemical characteristics.

1-(3′-Azido-3′-deoxythymidin-5′-yl),5-(*tert*-butyl) Pentanedioate 3. According to the general procedure B, the reaction of compound **2** (0.062 g, 0.108 mmol, 1 equiv) afforded the title compound **3** as a white foam (0.035 g, 77% yield): R_f = 0.52 (CH_3OH/CH_2Cl_2 10:90); MS (FAB^-) 436 $(M - H)^-$; HPLC t_r = 17.8 min (method II). Anal. ($C_{19}H_{27}N_5O_7$) C, H, N.

4-(*tert*-Butoxycarbonyl)amino-1-(3′-azido-3′-deoxythymidin-5′-yl) Butanoate 4. To a solution of 4-(*tert*-butoxycarbonyl)amino-1-butanoic acid (0.285 g, 1.403 mmol, 2.5 equiv) in anhydrous DMF (3 mL), under N_2, cooled in an ice bath to 0 °C was added DCC (0.139 g, 0.674 mmol, 1.2 equiv). The reaction mixture was stirred at 0 °C for 2 h. Then, a solution of AZT (0.150 g, 0.561 mmol, 1 equiv) in anhydrous DMF (2 mL), containing DMAP (0.069 g, 0.561 mmol, 1 equiv), was added dropwise under N_2. The reaction mixture was stirred at room temperature for 24 h. The solvent was evaporated under reduced pressure. The residual oil was dissolved in EtOAc (8 mL) and filtered. The filtrate was successively washed with 5% aqueous citric acid (2 × 7 mL), 5% aqueous $NaHCO_3$ (2 × 7 mL), and water (2 × 7 mL). The combined aqueous solutions were extracted twice with EtOAc. The combined organic layers were dried over Na_2SO_4, and the

solvent was removed under vacuum. The residue was purified by flash chromatography on silica gel, using CH_3OH/CH_2Cl_2 5:95 as eluent, to give the title compound **4** as a light yellow oil (0.228 g, 90% yield): $R_f = 0.29$ (CH_3OH/CH_2Cl_2 5:95); MS (FAB^+) 453 $(M + H)^+$.

General Procedure C for the Deprotection of *N*-Boc Amino Group and *ω*-*tert*-Butyl Esters. TFA (25 equiv) was added by syringe to a solution of *N*-Boc amino or *ω*-*tert*-butyl ester compound (1 equiv) in anhydrous CH_2Cl_2 (2 mL), under N_2. The reaction mixture was stirred at room temperature for 4 h. The solvent was evaporated under reduced pressure. The *ω*-amino compound was pure as demonstrated by its physicochemical characteristics. In the case of *ω*-carboxylic acid, the residue was dissolved in CH_2Cl_2 (5 mL), washed with 1N HCl (2 × 4 mL) and, water (2 × 4 mL). The combined aqueous solutions were extracted twice with CH_2Cl_2, the combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The *ω*-carboxylic acid was pure as demonstrated by its physicochemical characteristics.

4-Amino-1-(3′-azido-3′-deoxythymidin-5′-yl) Butanoate 5. According to the general procedure C, the reaction of compound **4** (0.200 g, 0.442 mmol, 1 equiv) afforded the title compound **5** as a white foam (0.154 g, quantitative yield): $R_f = 0.14$ (CH_3OH/CH_2Cl_2 10:90); MS (FAB^+) 353 $(M + H)^+$; HPLC $t_r = 11.5$ min (method I). Anal. ($C_{14}H_{20}N_6O_5$) C, H, N.

General Procedure D for the Formation of 5′-*O*-Carbamate- or 5′-*O*-Carbonate-Bonds on AZT Prodrugs. To a solution of AZT (1 equiv) in anhydrous DMF (compounds **6**, **8**, **10**, **16**−**20**) or in anhydrous CH_3CN (compounds **9**, **14**, **21**), *N*,*N*-carbonyldiimidazole (CDI, 1.1 equiv) was added under N_2. The reaction mixture was stirred at room temperature for 2 h. Afterward, the *ω*-amino or *ω*-hydroxyl compound (1.5 equiv) was added, the reaction mixture was stirred overnight at room temperature (in the case of carbamate bond formation) or at 60 °C (in the case of carbonate bond formation). The solvent was removed under reduced pressure, and the residual oil was dissolved in CH_2Cl_2. The organic phase was successively washed with 5% aqueous citric acid and/or 5% aqueous $NaHCO_3$ and/or water and then dried over Na_2SO_4. Evaporation of the solvent under vacuum gave a crude product, which was then purified by flash chromatography on silica gel or preparative layer chromatography (PLC, 1 or 2 mm thick), using hexane/EtOAc 20:80 as eluent.

3′-Azido-3′-deoxythymidin-5′-yl *N*-[3-(*tert*-Butoxycarbonyl)propyl] Carbamate 6. According to the general procedure D, the reaction of AZT (0.060 g, 0.225 mmol, 1 equiv) and 4-amino-1-*tert*-butyl butanoate (0.055 g, 0.338 mmol) in DMF afforded the title compound **6** as a light yellow oil (0.066 g, 65% yield): $R_f = 0.47$ (CH_3OH/CH_2Cl_2 10:90); MS (FAB^-) 451 $(M - H)^-$; HPLC $t_r = 16.2$ min (method II). Anal. ($C_{19}H_{28}N_6O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *N*-(3-Carboxypropyl) Carbamate 7. According to the general procedure C, the reaction of compound **6** (0.040 g, 0.088 mmol, 1 equiv) afforded the title compound **7** as a white foam (0.033 g, 94% yield): $R_f = 0.22$ (CH_3OH/CH_2Cl_2 10:90); MS (FAB^+) 397 $(M + H)^+$; HPLC $t_r = 13.4$ min (method I). Anal. ($C_{15}H_{20}N_6O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *N*-(3-Aminopropyl) Carbamate 8. According to the general procedure D, the reaction of AZT (0.150 g, 0.561 mmol, 1 equiv) and 1,3-diaminopropane (70 μL, 0.842 mmol) in DMF afforded the title compound **8** as a yellow gum (0.161 g, 78% yield): $R_f = 0.43$ (CH_3OH/CH_2Cl_2/AcOH 45:45:10); MS (FAB^+) 368 $(M + H)^+$; HPLC $t_r = 11.7$ min (method I). Anal. ($C_{14}H_{21}N_7O_5$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *N*-(3-Hydroxypropyl) Carbamate 9. According to the general procedure D, the reaction of AZT (0.060 g, 0.225 mmol, 1 equiv) and 1,3-diaminopropane (25 μL, 0.338 mmol) in CH_3CN afforded the title compound **9** as a light yellow oil (0.050 g, 60% yield): $R_f = 0.37$ (CH_3OH/CH_2Cl_2 10:90); MS (FAB^+) 369 $(M + H)^+$; HPLC $t_r = 12.9$ min (method I). Anal. ($C_{14}H_{20}N_6O_6$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(4-Hydroxybutyl) Carbonate 10. According to the general procedure D, the reaction of AZT (0.150 g, 0.561 mmol, 1 equiv) and 1,4-butanediol (75 μL, 0.842 mmol) in DMF afforded the title compound **10** as a white foam (0.182 g, 85% yield): $R_f = 0.27$ (EtOAc/hexane 85:15); MS (FAB^+) 384 $(M + H)^+$; HPLC $t_r = 15.7$ min (method I). Anal. ($C_{15}H_{21}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(3-Carboxypropyl) Carbonate 11. To a solution of compound **10** (0.100 g, 0.261 mmol, 1 equiv) in anhydrous CH_2Cl_2 (2 mL), under N_2, containing Tempo·HCl (40 μL from a 0.2 M solution in CH_2Cl_2, 0.008 mmol, 0.03 equiv) was added dropwise at 0 °C a solution of *m*-CPBA (0.081 g, 0.470 mmol, 1.8 equiv) in anhydrous CH_2Cl_2 (2 mL). The reaction mixture was stirred at room temperature for 2 h. The resulting solution was washed with 5% aqueous citric acid (2 × 5 mL) and water (2 × 5 mL). The combined aqueous solutions were extracted twice with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4, and the solvent was removed under vacuum. The residue was purified by PLC, using CH_3OH/CH_2Cl_2 6:94 as eluent, to give the title compound **11** as a white foam (0.072 g, 69%): $R_f = 0.22$ (CH_3OH/CH_2Cl_2 8:92); MS (FAB^+) 398 $(M + H)^+$; HPLC $t_r = 15.5$ min (method I). Anal. ($C_{15}H_{19}N_5O_8$) C, H, N.

Mixed Anhydride of 3,4,5-Trimethoxybenzoic Acid and 3′-Azido-3′-deoxythymidin-5′-yl *O*-(3-Carboxypropyl) Carbonate 12. According to the general procedure A, the reaction of compound **11** (0.112 g, 0.282 mmol, 1 equiv) afforded the title compound **12** (0.147 g, 88% yield): MS (FAB^+) 592 $(M + H)^+$.

3′-Azido-3′-deoxythymidin-5′-yl *O*-[3-(*tert*-Butoxycarbonyl)propyl] Carbonate 13. According to the general procedure B, the reaction of compound **12** (0.115 g, 0.195 mmol, 1 equiv) afforded the title compound **13** as a light yellow oil (0.067 g, 75% yield): $R_f = 0.33$ (CH_3OH/CH_2Cl_2 5:95); MS (FAB^-) 452 $(M - H)^-$; HPLC $t_r = 18.1$ min (method II). Anal. ($C_{19}H_{27}N_5O_8$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-[3-(*tert*-Butoxycarbonyl)aminopropyl] carbonate 14. According to the general procedure D, the reaction of AZT (0.150 g, 0.561 mmol, 1 equiv) and 3-(*tert*-butoxycarbonyl)amino-1-propanol (0.147 g, 0.842 mmol) in CH_3CN afforded the title compound **14** as a light yellow oil (0.176 g, 68% yield): $R_f = 0.29$ (EtOAc/hexane 80:20); MS (FAB^+) 469 $(M + H)^+$.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(3-Aminopropyl) Carbonate 15. According to the general procedure C, the reaction of compound **14** (0.165 g, 0.352 mmol, 1 equiv) afforded the title compound **15** as a white foam (0.129 g, quantitative yield): $R_f = 0.18$ (CH_3OH/CH_2Cl_2 10:90); MS (FAB^+) 369 $(M + H)^+$; HPLC $t_r = 11.9$ min (method I). Anal. ($C_{14}H_{20}N_6O_6$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(3-Hydroxypropyl) Carbonate 16. According to the general procedure D, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and 1,3-propanediol (50 μL, 0.674 mmol) in DMF afforded the title compound **16** as a light yellow oil (0.109 g, 66% yield): $R_f = 0.20$ (EtOAc/hexane 85:15); MS (FAB^+) 370 $(M + H)^+$; HPLC $t_r = 14.2$ min (method I). Anal. ($C_{14}H_{19}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(2-Hydroxyethyl) Carbonate 17. According to the general procedure D, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and 1,2-ethanediol (40 μL, 0.674 mmol) in DMF afforded the title compound **17** as a light yellow oil (0.089 g, 56% yield): $R_f = 0.18$ (EtOAc/hexane 85:15); MS (FAB^+) 356 $(M + H)^+$; HPLC $t_r = 12.5$ min (method I). Anal. ($C_{13}H_{17}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(5-Hydroxypentyl) Carbonate 18. According to the general procedure D, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and 1,5-pentanediol (70 μL, 0.674 mmol) in DMF afforded the title compound **18** as a white foam (0.124 g, 70% yield): $R_f = 0.30$ (EtOAc/hexane 85:15); MS (FAB^+) 398 $(M + H)^+$; HPLC $t_r = 17.1$ min (method I). Anal. ($C_{16}H_{23}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(6-Hydroxyhexyl) Carbonate 19. According to the general procedure D, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and 1,6-hexanediol (0.080 g, 0.674 mmol) in DMF afforded the title compound **19** as a white foam (0.089 g, 49% yield): $R_f = 0.35$

(EtOAc/hexane 85:15); MS (FAB$^+$) 412 (M + H)$^+$; HPLC t_r = 17.9 min (method I). Anal. ($C_{17}H_{25}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(8-Hydroxyoctyl) Carbonate 20. According to the general procedure D, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and 1,8-octanediol (0.066 g, 0.674 mmol) in DMF afforded the title compound **20** as a white foam (0.125 g, 63% yield): R_f = 0.48 (EtOAc/hexane 85:15); MS (FAB$^+$) 440 (M + H)$^+$; HPLC t_r = 20.1 min (method I). Anal. ($C_{19}H_{29}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-Butyl Carbonate 21. According to the general procedure D, the reaction of AZT (0.100 g, 0.374 mmol, 1 equiv) and 1-butanol (52 μL, 0.561 mmol) in CH_3CN afforded the title compound **21** as a light yellow oil (0.084 g, 64% yield): R_f = 0.65 (EtOAc/hexane 80:20); MS (FAB$^+$) 368 (M + H)$^+$; HPLC t_r = 20.4 min (method I). Anal. ($C_{15}H_{21}N_5O_6$) C, H, N.

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Supporting Information Available: NMR and IR spectroscopic data of the desired compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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