New 3′-Azido-3′-deoxythymidin-5′-yl *O*-(4-Hydroxyalkyl or -Alkenyl or -Alkylepoxide) Carbonate Prodrugs: Synthesis and Anti-HIV Evaluation

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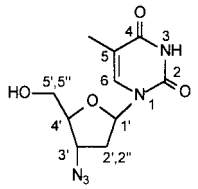
New 5′-*O*-carbonate prodrugs of zidovudine (AZT) have been synthesized in order to enhance its uptake by HIV-1 infected cells, to improve its anti-HIV potency, and to optimize the intramolecular cyclic rearrangement process related to the 5′-*O*-(4-hydroxybutyl) carbonate moiety. Evidence of this prodrug rearrangement was confirmed by comparison of the serum half-lives of the 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxyalkyl or -alkenyl or -alkylepoxide) carbonate prodrugs with our thermodynamic predictions. Interestingly, these 5′-*O*-carbonate prodrug series show increased anti-HIV potencies in conjunction with, or without, reduced cytotoxicity as compared to AZT that lead to a gain in selectivity indexes. The cytotocity of AZT could be reduced with these 5′-*O*-carbonate prodrug series by delaying the 5′-*O*-glucuronidation of AZT, which is one of the major limitations of AZT.

Introduction

Intensive efforts are underway worldwide to develop chemotherapeutic agents effective against the human immunodeficiency virus (HIV),[1−3] the etiological agent of acquired immunodeficiency syndrome (AIDS).[4] The search for an effective chemotherapeutic treatment against HIV infection has led to the development of agents that target specific and critical events in the HIV replicative cycle. Among the current diversity of compounds active against HIV, the 2′,3′-dideoxynucleosides (ddNs) remain by far the most potent.[5,6] The most extensively studied of these agents is 3′-azido-3′-deoxythymidine (AZT, zidovudine, Retrovir; Figure 1).[7] This ddN was the first that was approved by the Food and Drug Administration (FDA)[8] for the treatment of AIDS.[9,10] AZT, after conversion into its 5′-*O*-triphosphate analogue (AZT-TP) by cellular enzymes (kinases),[11−13] inhibits HIV-reverse transcriptase (HIV−RT) by competitive inhibition of the viral reverse transcriptase (RT) and/or by incorporation and subsequent chain termination of the growing viral DNA strand.[14] The major limitations of AZT are due to clinical toxicities that include bone marrow suppression,[15,16] hepatic abnormalities, and myopathy,[17] dependence on host cell kinase-mediated activation,[18−20] limited brain uptake,[21] a short half-life in plasma (that dictates frequent administration to maintain therapeutic drug levels),[22] high susceptibility to catabolism,[23−25] and the rapid development of resistance by HIV-1.[26−28] In attempts to overcome these problems, numerous chemical strategies have been developed by medicinal scientists for designing prodrugs of AZT. Most of these have been prepared by derivatization of AZT at its 5′-*O*-position



AZT
(Zidovudine, Retrovir)

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

to provide two prominent classes of compounds: 5′-*O*-carboxylic ester and 5′-*O*-phosphoroester derivatives. The mechanism of action of these two classes of ester conjugates is based on hydrolysis and/or enzymatic cleavage of their 5′-*O*-bonds between the drug (AZT) and its spacer group, to AZT or to its 5′-*O*-monophosphate (AZT-MP to bypass the first AZT phosphorylation step) into the cells. The expected advantages of these 5′-*O*-substitued AZT prodrugs can be multiple: improvement in anti-HIV activity,[7] synergistic drug interactions,[29−32] enhancement of AZT intracellular uptake,[33] increase of AZT brain delivery,[34,35] bypassing of the first AZT phosphorylation step into the cells,[36−39] and decreased toxicity.[7]

In the present paper, we report the specific behavior of new classes of AZT prodrugs, 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxyalkyl or -alkenyl or -alkylepoxide) carbonates, which are rearranged during the enzymatic hydrolysis of their 5′-*O*-carbonate bond, through an intramolecular cyclic process (Figures 2 and 3).

With this aim, we have previously synthesized several 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs with the general formula shown in Figure 2.[40]

As summarized in Figure 2, we have previously demonstrated (in complete agreement with thermodynamic predictions) that two different cleavage mecha-

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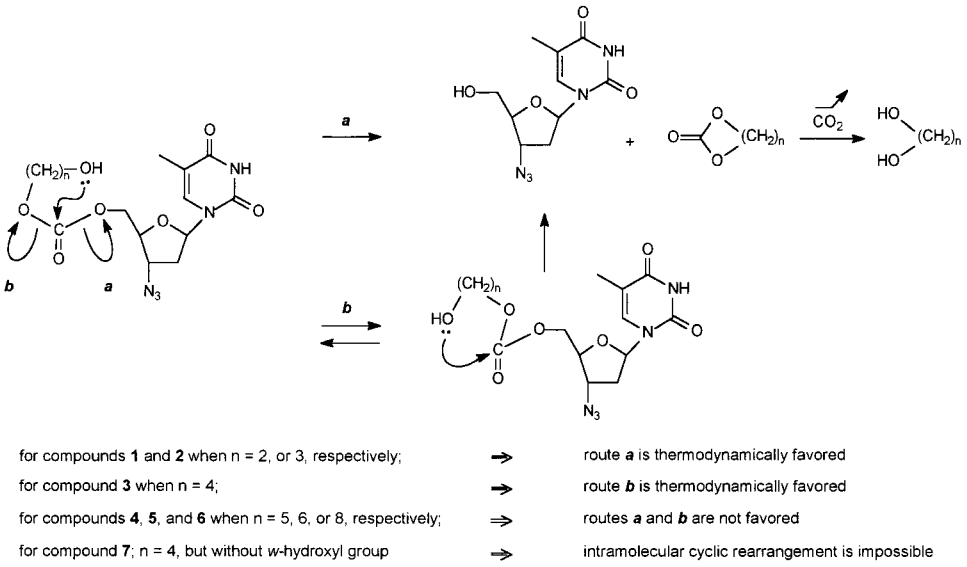


Figure 2. Cyclic intramolecular rearrangement process on 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate that recovers the initial prodrug.

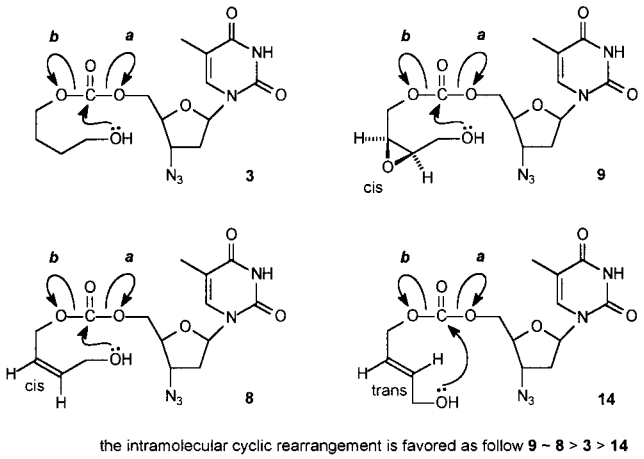


Figure 3. Cyclic intramolecular rearrangement process on compounds **3**, **8**, **9**, and **14** that recovers the initial prodrug (thermodynamic predictions).

nisms (a or b) could be considered during the enzymatic hydrolysis of these carbonate prodrugs by human serum hydrolases (esterases, especially carboxyesterases).[40]

In compounds **1** ($n = 2$) and **2** ($n = 3$), hydrolytic cleavage according to route a is advantaged because the release of AZT involves the formation of a five- or six-membered ring, respectively, which is thermodynamically favored. In contrast, hydrolytic cleavage according to route b, leading to the cyclic rearrangement of the prodrugs **1** and **2** by an intramolecular nucleophilic attack of the terminal hydroxyl function, is not favored.

In compound **3** ($n = 4$), hydrolytic cleavage according to route a, leading to the release of AZT, is thermodynamically disadvantaged, since this process involves the formation of a seven-membered ring. In contrast, hydrolytic cleavage according to route b, leading to the cyclic rearrangement of the prodrug **3** by an intramolecular nucleophilic attack of the terminal hydroxyl function, is favored. Under these conditions, compared with the whole synthesized prodrugs, the human serum half-life ($t_{1/2}$) of prodrug **3** is enhanced.

In compounds **4** ($n = 5$), **5** ($n = 6$), and **6** ($n = 8$), hydrolytic cleavage by route a, leading to the release of AZT via the formation of an eight-, nine-, or eleven-membered ring, respectively, is thermodynamically disadvantaged. Hydrolytic cleavage by route b, leading to the cyclic rearrangement of prodrugs **4**, **5**, or **6**, respectively, by an intramolecular nucleophilic attack of the terminal hydroxyl function, is also not favored because of the growth of their alkyl chain length (which will become too long) compared with compound **3**.

Compound **7** cannot be cleaved or recovered by the intramolecular rearrangement mechanism. This compound does not bear a terminal hydroxyl group, which can help the release of AZT or the cyclic rearrangement of the prodrug by an intramolecular nucleophilic attack. So, its cleavage is only due to the enzymatic lability of its 5′-*O*-carbonate bond. This prodrug **7** is used as a reference for the studied compounds in order to validate the concept of an intramolecular cyclic rearrangement.

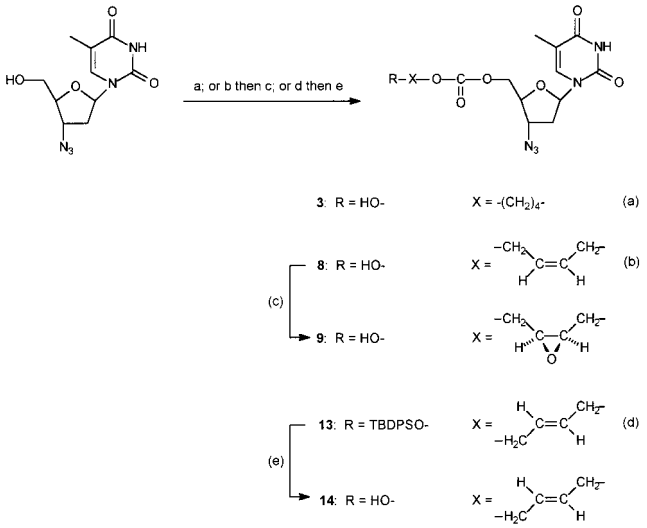
To optimize our concept related to this intramolecular cyclic rearrangement process of 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxyalkyl) carbonate prodrug **3**, we have synthesized other analogues (compounds **8**, **9**, and **14**) like prodrug **3**. We have studied their chemical and human serum half-lives, their lipophilicity and, their anti-HIV-1 activities in different cell cultures.

Chemistry

We describe herein the synthesis of new 5′-*O*-carbonate-AZT prodrugs.

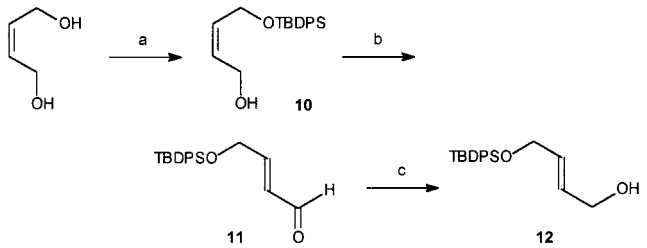
5′-*O*-Carbonate-AZT prodrugs were obtained by condensation of AZT with *N*,*N*-carbonyldiimidazole (CDI) in the presence of various ω-diols. In this manner, numerous 5′-*O*-carbonate-AZT prodrugs **1**−**7** were obtained in yields ranging from 49% to 85% (Figure 2).[40] For example, condensation of AZT with CDI in DMF in the presence of 1,4-butanediol gave the 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxybutyl) carbonate **3** in 85% yield (Scheme 1).[40,41] Condensation of AZT with CDI in DMF in the presence of *cis*-but-2-en-1,4-diol led to the 3′-azido-3′-deoxythymidin-5′-yl *O*-(*cis*-4-hydroxy-

Scheme 1[a]



[a] Reagents: (a) CDI, 1,4-butanediol, DMF; (b) CDI, *cis*-but-2-en-1,4-diol, DMF; (c) *m*-CPBA, CH_2Cl_2; (d) CDI, *trans*-4-(*tert*-butyldiphenylsiloxy)but-2-en-1-ol **12**, DMF; (e) TBAF, THF.

Scheme 2[a]



[a] Reagents: (a) *n*-butyllithium, *tert*-butyldiphenylsilyl chloride, THF; (b) PCC, Celite, CH_2Cl_2; (c) DIBAL-H, Et_2O.

but-2-enyl) carbonate **8** in 67% yield (Scheme 1).[40,41] Its oxidation using *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2 (*syn*-epoxidation) led to the 3′-azido-3′-deoxythymidin-5′-yl *O*-(*cis*-4-hydroxy-2,3-epoxybutyl) carbonate **9** in 70% yield (Scheme 1).[42,43] To synthesize the 3′-azido-3′-deoxythymidin-5′-yl *O*-(*trans*-4-hydroxybut-2-enyl) carbonate **14**, we needed first the synthesis of *trans*-4-(*tert*-butyldiphenylsiloxy)but-2-en-1-ol **12** (Scheme 2).[44] So, *cis*-but-2-en-1,4-diol was monosilylated by *tert*-butyldiphenylsilyl chloride (TBDPS−Cl) in THF in the presence of *n*-butyllithium (*n*-BuLi) to give compound **10** in 81% yield (Scheme 2).[44] This monoalcohol was oxidized by pyridinium chlorochromate (PCC) in CH_2Cl_2 to give compound **11** (*E*-enal) in 83% yield (Scheme 2).[44] Its reduction by diisobutylaluminum hydride (DIBAL-H) in Et_2O gave the *trans*-4-(*tert*-butyldiphenylsiloxy)but-2-en-1-ol **12** in 79% yield (Scheme 2).[44] Then, condensation of AZT with CDI in DMF in the presence of compound **12** gave 3′-azido-3′-deoxythymidin-5′-yl *O*-[*trans*-4-(*tert*-butyldiphenylsiloxy)but-2-enyl] carbonate **13** in 65% yield (Scheme 1).[40,41] Its deprotection by *tetra*butylammonium fluoride (TBAF) in THF gave the 3′-azido-3′-deoxythymidin-5′-yl *O*-(*trans*-4-hydroxybut-2-enyl) carbonate **14** in 85% yield (Scheme 1).[44]

Results and Discussion

Like we have seen previously from numerous 3′-azido-3′-deoxythymidin-5′-yl *O*-(*ω*-hydroxyalkyl) carbonate prodrugs (**1**−**7**), the influence of the alkyl chain length of the spacer (bearing a terminal hydroxyl group) was essential for the intramolecular cyclic rearrangement of a prodrug. The optimum alkyl chain, for this cyclic rearrangement process, includes four methylene groups inserted between the 5′-*O*-carbonate bond of the AZT prodrug and the terminal hydroxyl function (compounds **3** in Figure 2).[40] To improve the human serum half-life of this prodrug series (analogues of compound **3**), we have synthesized a few new prodrugs, **8**, **9**, and **14**. For all of these prodrugs (**3**, **8**, **9**, and **14**), the hydrolytic cleavage according to route a, leading to the release of AZT, is thermodynamically disadvantaged, since this process involves the formation of a seven-membered ring (Figure 3). In contrast, hydrolytic cleavage according to route b, leading to the cyclic rearrangement of the initial prodrug by an intramolecular nucleophilic attack of the terminal hydroxyl function, is more or less favored, depending on the nature of the spacer for the considered prodrug (**3**, **8**, **9**, and **14**). By comparison of prodrug **3**, used as a reference compound, with compounds **8**, **9**, and **14**, we can postulate the following hypotheses (Figure 3):

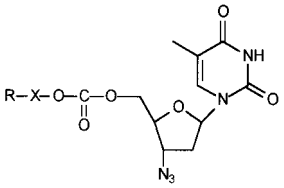
In compound **8**, the introduction of a cis double bond could decrease the thermodynamic mobility of the spacer chain and rigidify it. This cis stereochemistry could also position the hydroxyl function nearer from the 5′-*O*-carbonate bond than in the case of compound **3**. So, for compound **8**, the cyclic rearrangement process should be favored and, consequently its human serum half-life should be enhanced, compared with that of compound **3**.

In compound **9**, the presence of a cis epoxide might have a similar influence to that of the cis double bond of compound **8**. Thermodynamic predictions for the cyclic rearrangement process are difficult to estimate between compounds **8** and **9**. But, in any case, the human serum half-life of compound **9** should be enhanced, compared with that of compound **3**.

In compound **14**, the introduction of a trans double bond could also decrease the thermodynamic mobility of the spacer chain and rigidify it. This trans stereochemistry could position the hydroxyl function further from the 5′-*O*-carbonate bond than in the case of compounds **3**, **8**, and **9**. So, for compound **14**, the cyclic rearrangement process should be disadvantaged, and consequently, its human serum half-life should be reduced, compared with that of compound **3**.

Lipophilicity, and Chemical and Human Serum Stability Studies. As far as lipophilicity is concerned, it is known that AZT crosses cell membranes by non-facilitated diffusion and that its uptake is insensitive to inhibitors of nucleoside transport.[45] 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.[46] So, by increasing the lipophilicity of a prodrug, we could expect an increased intracellular uptake of the parent drug. Therefore, the log *P* values (partition in *n*-octanol and water) were determined for all compounds, using ACD/log *P* software from ChemCAD. The results in Tables 1 and 2 show that log *P* values are in the range of −1.20 to +1.14 for the different series of prodrugs (the log *P* value for AZT itself was −0.88). Moreover, as we will

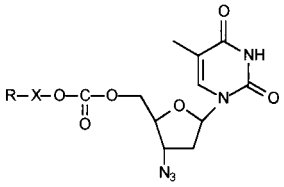
Table 1. Biological Properties of 5′-*O*-carbonate-AZT Prodrugs **1**−**7**



compd	structure R	structure X	$t_{1/2}$[a] (min)	log *P*[b]
1	HO−	$-(CH_2)_{2}-$	<1	−1.20
2	HO−	$-(CH_2)_{3}-$	10	−0.98
3	HO−	$-(CH_2)_{4}-$	30	−0.68
4	HO−	$-(CH_2)_{5}-$	16	−0.45
5	HO−	$-(CH_2)_{6}-$	7	0.08
6	HO−	$-(CH_2)_{8}-$	2	1.14
7	H−	$-(CH_2)_{4}-$	9	1.02
AZT				−0.88

[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, NHS). [b] log *P* determinations were performed using ACD (Advanced Chemistry Development, Inc.)/log *P* 1.0 base calculations.

Table 2. Biological Properties of 5′-*O*-carbonate-AZT Prodrugs **3**, **7**, **8**, **9**, and **14**



compd	structure R	structure X	$t_{1/2}$[a] (min) PBS	$t_{1/2}$[a] (min) NHS	log *P*[c]
3	HO-	$-(CH_2)_4-$	> 1440 (6%)[b]	30	-0.68
8	HO-		> 1440 (8%)[b]	45	-0.59
9	HO-		> 1440 (8%)[b]	80	-0.47
14	HO-		> 1440 (7%)[b]	26	-0.59
7	H-	$-(CH_2)_4-$	> 1440 (0%)[b]	9	1.02
AZT					-0.88

[a] $t_{1/2}$ (half-life) is the time required for 50% hydrolysis of prodrugs to AZT at 37 °C upon incubation in phosphate buffered saline (PBS, 0.01 M, pH 7.4) or in human serum (NHS). [b] (%) of released AZT. [c] log *P* determinations were performed using ACD (Advanced Chemistry Development, Inc.)/log *P* 1.0 base calculations.

see latter, it is evident that an increase of the lipophilicity of a prodrug does not necessary mean that its anti-HIV efficacy will be improved. Indeed, this result is already described in the literature for the 5′-*O*-retinoyl ester of AZT, for example.[33] Starting from the hypothesis that the enzymatic hydrolysis of the 5′-*O*-bond of the prodrugs is likely to be crucial, we have performed chemical (in phosphate-buffered saline, PBS) and human serum stability studies. We determined the enzymatic hydrolysis half-lives ($t_{1/2}$) in PBS and in human serum of the 5′-*O*-carbonate-AZT prodrugs by an HPLC method. The various carbonates of AZT were found to be stable for up to 24 h at 37 °C in PBS. In normal human serum (NHS), we have previously demonstrated that compound **3** was the most stable prodrug in the 3′-azido-3′-deoxythymidin-5′-yl *O*-(ω-hydroxyalkyl) carbonate series (Table 1).[40] This result is in complete agreement with thermodynamic predictions concerning the intramolecular cyclic rearrangement. From the data reported in Table 2, we can suggest the following comments for human serum stability studies.

We observe that compound **9** is the most stable prodrug within these 5′-*O*-carbonate series in human serum ($t_{1/2}$ = 80 min). As we have postulated it, reference compound **7** possesses the shortest serum half-life ($t_{1/2}$ = 9 min). The sensitivity to enzymatic hydrolysis for these prodrug series is in the following stability order: **9** > **8** > **3** > **14**.

These serum hydrolase stability studies are in accordance with our thermodynamic predictions associated with the intramolecular cyclic rearrangement of compound **3**, **8**, **9**, and, **14**. So, as we have seen in our previous studies, the intramolecular cyclic rearrangement of the 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxyalkyl or -alkenyl or -alkylepoxide) carbonate series is confirmed.

Moreover, from the results obtained by the serum hydrolase stability studies, it is evident that the lipophilicity of the AZT prodrugs does not correlate with their sensitivity to enzymatic hydrolysis.

Table 3. Anti-HIV Evaluation of 5′-*O*-carbonate-AZT Prodrugs **3**, **8**, **9**, and **14** on MT-4 Cells (HIV-1 III_B) and PBMCs (HIV-1 BaL)

compd	MT-4 HIV-1 IIIB EC_{50}[a] (nM)	MT-4 HIV-1 IIIB CC_{50}[b] (μM)	MT-4 HIV-1 IIIB SI[c]	PBMCs HIV-1 BaL EC_{50}[a] (nM)	PBMCs HIV-1 BaL CC_{50}[b] (μM)	PBMCs HIV-1 BaL SI[c]
3	6	262	43 667	31	>20	>645
8	18	179	9 944	52	>20	>385
9	13	159	12 231	103	>20	>194
14	8	162	20 250	66	>20	>303
AZT	12	42	3 500	56	15	268

[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}.

Antiviral Activity Results. The different AZT prodrugs were first evaluated for their inhibitory effect on HIV-1 (III_B) replication and their cytotoxicity in MT-4 cells (Table 3). The 50% effective concentration (EC_{50}) represents the concentration required to inhibit the viral cytopathic effect by 50% in these cells. AZT inhibits the HIV-1 (III_B) replication at an EC_{50} value of 12 nM. Evaluated under the same conditions, the carbonate derivatives of AZT gave comparable results, compound **3** being twice as active as compared to AZT. All of the carbonate derivatives of AZT display EC_{50} values ranging from 6 to 18 nM. When considering the cytotoxicity (CC_{50}, 50% cytotoxic concentration, or the concentration that causes 50% toxicity in MT-4 cells) of the carbonates derivatives of AZT, it appears that all of the evaluated compounds show a reduced cytotoxicity as compared to AZT (4−6 times). On the other hand, the different AZT prodrugs were then evaluated for their inhibitory effect on HIV-1 (BaL) replication and their cytotoxicity in peripheral blood mononuclear cells (PBMCs) (Table 3).

AZT inhibits the HIV-1 (BaL) replication at an EC_{50} value of 56 nM. Evaluated under the same conditions, the carbonate derivatives of AZT gave comparable results, compound **3** being the most active compound of this series. All of the carbonate derivatives of AZT display EC_{50} values ranging from 31 to 103 nM. When considering CC_{50} of the carbonate derivatives of AZT, it appears that all of the evaluated compounds show a reduced cytotoxicity as compared to AZT. To conclude, a decreased EC_{50} irrespective of whether it is, or it is not, combined with an increase in CC_{50} leads to a gain in selectivity indexes of the compounds. Perhaps these carbonate derivatives of AZT could prevent or delay the 5′-*O*-glucuronidation of AZT, which is one of the major limitations of AZT.[23,24] However, it is true that at the present time, we cannot exclude that the released diols may have some effect on reducing the toxicity of AZT.

Conclusion

In the present paper, we report the synthesis, biological properties, and the anti-HIV evaluation of new series of 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxyalkyl or -alkenyl or -alkylepoxide) carbonate prodrugs. From serum hydrolase studies, we have demonstrated that the half-lives of these 5′-*O*-carbonate prodrugs are in complete agreement with our thermodynamic predictions. We have observed that these 5′-*O*-carbonate prodrugs of AZT are less cytotoxic than AZT in MT-4 cells and PBMCs, resulting in a gain in their selectivity indexes.

These whole results confirm that a subtle balance between the rate of intracellular uptake (depending on prodrug lipophilicity) and the sensitivity to enzymatic hydrolysis of a prodrug under in vitro conditions was required for optimum antiviral activity. Moreover, we demonstrated that an increase of the serum half-life of a prodrug does not necessary mean that its anti-HIV efficacy will be improved.

The mechanism of action of these 3′-azido-3′-deoxythymidin-5′-yl *O*-(4-hydroxyalkyl or -alkenyl or -alkylepoxide) carbonate prodrugs might now be clarified by intramolecular quantification of the amounts of 5′-*O*-glucuronide of AZT (GAZT) and of AZT-MP, AZT-DP, and AZT-TP, for example.

The intramolecular cyclic rearrangement of these carbonate series, specifically associated with the 5′-*O*-(4-hydroxybutyl) carbonate moiety, constitutes a new concept that could probably be extended to other known drugs possessing a primary hydroxyl function in its structure.

Experimental Section

Nuclear magnetic resonance spectra (^1H and ^{13}C NMR) were recorded with a Bruker AC-250 spectrometer; chemical shifts are expressed as δ units (part per million) downfield from TMS (tetramethylsilane). Fast atom bombardment (FAB^+ or FAB^-) mass spectral analysis 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 Co.) from freshly opened containers.

Cells and Viruses. (i) MT-4 cells[47] were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 0.1% sodium bicarbonate, and gentamicin (20 μg/mL). The origin of the HIV-1 (III_B) stock has been described elsewhere.[48]

(ii) PBMCs were isolated from healthy donors buffy coats by density centrifugation (Lymphoprep; Nycomed Pharma, AS Diagnostics, Oslo, Norway) and stimulated for 3 days in medium containing 2 μg/mL of phytohemagglutin (PHA) (Sigma Chemical Co., Bornem, Belgium) and human interleukin-2 (5 U/mL; Boehringer Mannheim, Mannheim, Germany). The activated PBMCs (PHA-stimulated blasts) were washed with PBS and resuspended in RPMI 1640, supplemented with 2 mM L-glutamine, gentamicin (50 μg/mL), 15% heat-inactivated fetal calf serum, and recombinant human interleukin-2 (10 U/mL). The HIV-1 M-tropic strain BaL was obtained through the Medical Research Council AIDS reagent project (Herts, UK).

Anti-HIV Activity Assays. (i) The inhibitory effects of AZT and its carbonate derivatives on HIV-1 replication were monitored by the inhibition of the virus-induced cytopathicity in MT-4 cells 5 days after infection, as described elsewhere.[49] The cytotoxicity of the compounds was determined by measuring the viability of mock-infected cells on day 5.

(ii) The activated PBMCs (PHA-stimulated blasts) were washed with PBS and viral infections were done as described by the AIDS clinical trial group protocol.[50] 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 (BaL) at 1000 $CCID_{50}$ per milliliter. At 4 days postinfection, 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 3, 8, 9, and 14 in PBS and in Human Serum.[33,40] To 990 μL of PBS or normal human serum (NHS) was added 10 μL of a solution of the desired 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 eluent: 20% of solvent B in A to 100% of B in 20 min. 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, 15.7 min for **3**, 16.5 min for **8**, 14.5 min for **9**, and 16.0 min for **14**. The $t_{1/2}$ values calculated from peak areas for all the compounds studied (**3**, **8**, **9**, and **14**) in PBS and in NHS are summarized in Table 2.

Chemical Syntheses. Syntheses of compounds **1**−**7** have already been described elsewhere.[40]

General Procedure A for the Formation of 5′-*O*-Carbonate Bonds on AZT Prodrugs. To a solution of AZT (1 equiv) in anhydrous DMF was added *N*,*N*-carbonyldiimidazole (CDI, 1.1 equiv) under N_2. The reaction mixture was stirred at room temperature for 2 h. Afterward ω-diol compound (1.5 equiv) was added, and the reaction mixture was stirred overnight at 60 °C to form the carbonate bond. The solvent was removed under reduced pressure and the residual oil was dissolved in CH_2Cl_2. The organic phase was succes-

sively washed with 5% aqueous citric acid and/or 5% aqueous $NaHCO_3$ and/or water and then dried over $MgSO_4$ and filtered. Evaporation of the solvent under vacuum gave a crude product, which was then purified by flash chromatography on silica gel or by preparative layer chromatography (PLC, 1 or 2 mm thick), using hexane/EtOAc 20:80 as eluent.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(4-Hydroxybutyl) Carbonate 3. According to the general procedure A, the reaction of AZT (0.150 g, 0.561 mmol, 1 equiv) and 1,4-butanediol (75 μL, 0.842 mmol) in DMF (5 mL) afforded the title compound 3 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. Anal. ($C_{15}H_{21}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(*cis*-4-Hydroxybut-2-enyl) Carbonate 8. According to the general procedure A, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and *cis*-but-2-en-1,4-diol (56 μL, 0.674 mmol) in DMF (4 mL) afforded the title compound **8** as a pale yellow oil (0.115 g, 67% yield): R_f = 0.37 (EtOAc/hexane 90:10); MS (FAB$^+$) 382 $(M + H)^+$; HPLC t_R = 16.5 min. Anal. ($C_{15}H_{19}N_5O_7$) C, H, N.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(*cis*-4-Hydroxy-2,3-epoxybutyl) Carbonate 9. To a solution of compound 8 (0.100 g, 0.262 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1.5 mL), under N_2, was added dropwise at 0 °C a solution of *m*-CPBA (0.081 g, 0.472 mmol, 1.8 equiv) in anhydrous CH_2Cl_2 (1 mL). The reaction mixture was stirred at 0 °C for 30 min. After the ice bath removal, the reaction mixture was stirred at room temperature for 14 h. Afterward, a 10% Na_2SO_4 aqueous solution (2 mL) was added, and the reaction mixture was stirred at 0 °C for 20 min. The resulting solution was washed with water (2 × 5 mL) and brine (2 × 5 mL). The combined aqueous solutions were extracted twice with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and filtered, and the solvent was removed under vacuum. The residue was purified by PLC, using EtOAc as eluent, to give the title compound **9** as a pale yellow oil (0.073 g, 70% yield): R_f = 0.39 (EtOAc 100); MS (FAB$^+$) 398 $(M + H)^+$; HPLC t_R = 14.5 min. Anal. ($C_{15}H_{19}N_5O_8$) C, H, N.

***cis*-4-(*tert*-Butyldiphenylsiloxy)but-2-en-1-ol 10.** To a solution of *cis*-but-2-en-1,4-diol (0.500 mL, 6.06 mmol, 1.05 equiv) in anhydrous THF (10 mL), under N_2, was added dropwise at −78 °C a solution of *n*-BuLi (3.68 mL, 1.6 M in hexane, 5.89 mmol, 1.02 equiv) in hexane. Afterward, TBDPS−Cl (1.48 mL, 5.77 mmol, 1 equiv) was added, the reaction was allowed to warm to 25 °C over 30 min and heated at reflux for 3.5 h. The reaction mixture was concentrated under vacuum. The residue was purified by flash chromatography on silica gel, using EtOAc/cyclohexane 10:90 as eluent, to give the title compound **10** as a pale green oil (1.53 g, 81% yield): R_f = 0.27 (EtOAc/cyclohexane 10:90); MS (FAB$^-$) 325 $(M - H)^-$.

***trans*-4-(*tert*-Butyldiphenylsiloxy)but-2-en-1-al 11.** To a solution of compound **10** (0.300 g, 0.919 mmol, 1 equiv) in anhydrous CH_2Cl_2 (1 mL), under N_2, was added a mixture of PCC (0.296 g, 1.378 mmol, 1.5 equiv) and Celite (1.5 g) in CH_2Cl_2 (5 mL). The reaction mixture was stirred at 25 °C for 2 h. Afterward, Et_2O (6 mL) was added, and the solids were removed by filtration through a pad of Celite−Florisil (1:1). The resulting solution was concentrated under vacuum. The residue was purified by flash chromatography on silica gel, using Et_2O as eluent, to give the title compound **11** as a pale yellow solid (0.246 g, 83% yield): R_f = 0.52 (Et_2O); MS (FAB$^-$) 323 $(M - H)^-$.

***trans*-4-(*tert*-Butyldiphenylsiloxy)but-2-en-1-ol 12.** To a solution of compound **11** (0.180 g, 0.555 mmol, 1 equiv) in anhydrous Et_2O (4 mL), under N_2, was added dropwise at −78 °C a solution of DIBAL-H (0.61 mL, 1.0 M in hexane, 0.611 mmol, 1.1 equiv) in hexane. The reaction mixture was stirred at −78 °C for 25 min and then at 25 °C for 1 h. Afterward, MeOH (0.8 mL, 5.77 mmol, 1 equiv) was added, and the mixture was stirred for 1.5 h. Saturated aqueous sodium tartrate (2 mL) was added, and the reaction mixture was stirred until two clear layers were formed. The organic phase was separated, and the aqueous layer was extracted with Et_2O (3 × 2 mL). The combined organic extracts were dried over $MgSO_4$, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel, using EtOAc/cyclohexane 15:85 as eluent, to give the title compound **12** as a yellow oil (0.143 g, 79% yield): R_f = 0.16 (EtOAc/cyclohexane 10:90); MS (FAB$^-$) 325 $(M - H)^-$.

3′-Azido-3′-deoxythymidin-5′-yl *O*-[*trans*-4-(*tert*-Butyldiphenylsiloxy)but-2-enyl] Carbonate 13. According to the general procedure A, the reaction of AZT (0.120 g, 0.449 mmol, 1 equiv) and compound **12** (0.220 g, 0.674 mmol) in DMF (4 mL) afforded the title compound **13** as a pale yellow oil (0.181 g, 65% yield): R_f = 0.28 (EtOAc/hexane 40:60); MS (FAB$^-$) 618 $(M - H)^-$.

3′-Azido-3′-deoxythymidin-5′-yl *O*-(*trans*-4-Hydroxybut-2-enyl) Carbonate 14. To a solution of compound **13** (0.150 g, 0.242 mmol, 1 equiv) in anhydrous THF (1 mL), under N_2, was added by syringe at 0 °C a solution of TBAF (0.270 mL, 1.0 M in THF, 0.267 mmol, 1.1 equiv) in THF. The reaction mixture was stirred at room temperature for 2 h and then concentrated under vacuum. The residue was purified by PLC, using CH_2Cl_2/MeOH 95:5 as eluent, to give the title compound **14** as a pale yellow oil (0.078 g, 85% yield): R_f = 0.23 (CH_2Cl_2/MeOH 95:5); MS (FAB$^+$) 382 $(M + H)^+$; HPLC t_R = 16.0 min. Anal. ($C_{15}H_{19}N_5O_7$) 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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