Novel 3′-C/N-Substituted 2′,3′-β-D-Dideoxynucleosides as Potential Chemotherapeutic Agents. 1. Thymidine Derivatives: Synthesis, Structure, and Broad Spectrum Antiviral Properties

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A synthetic scheme for the 3′-oxime derivatives **3E**, **5E**, **5Z**, **7E** and **7Z** of 1-(2,3-dideoxy-β-D-*glycero*-pentofuranosyl)thymine and for 1-(2,3-dideoxy-3-nitro-β-D-*erythro*-pentofuranosyl)-thymine (**10**) has been developed starting from appropriately 5′-protected 3′-ketothymidine. X-ray analysis showed that 3′-*N*-hydroxyimino **3E** and 3′-*N*-methoxyimino **5Z** derivatives have close molecular conformations: *anti* about the N1−C1′ bond, and *gauche*$^+$ about the C4′−C5′ exocyclic bond. Their sugar conformations are C1′-*exo*−O4′-*endo* and C1′-*exo*−C2′-*endo*, respectively. The antiviral assays in cell cultures demonstrated that 3′-*N*-hydroxyimino **3E** and 3′-*N*-acetoxyimino **7E** + **7Z** derivatives are endowed with significant activity against human immunodeficiency virus (HIV) with EC_{50} values ranging between 0.02 and 0.40 μg/mL for both HIV-1 and HIV-2. The other compounds **5E** + **5Z** and **10** were at least 2 orders of magnitude less active. The 3′-*N*-hydroxyimino derivative **3E** also shows promising activity against hepatitis B virus (HBV) (EC_{50} = 0.25 μg/mL) and against herpes simplex virus type 1 (HSV-1) and HSV-2.

Introduction

Modified nucleosides are still the main therapeutic agents in the treatment of patients with acquired immune deficiency syndrome (AIDS).[1,2] The need to search for new modified nucleoside analogues has become especially clear since the appearance of HIV strains resistant to 1-(2,3-dideoxy-3-azido-β-D-*erythro*-pentofuranosyl)thymine (AZT), 2′,3′-dideoxycytidine (ddC), 2′,3′-dideoxyinosine (ddI), and 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)thymine (d_4T), four drugs currently approved for treatment of HIV-infected individuals.[1,2]

The significance of hydroxyl group replacement at C3′ in 2′-deoxynucleosides by different substituents containing nitrogen in order to obtain anti-HIV compounds has been illustrated previously. Thus, the first potent nucleoside analogue used as a commercial anti-HIV drug, AZT, contains an azido group at C3′.[3] 1-(2,3-Dideoxy-3-amino-β-D-*erythro*-pentofuranosyl)thymine (AMT) is also active against HIV, but it did not find a practical application due to strong cytotoxicity[4] resulting from concomitant inhibition of human DNA polymerase α[5] and β[6] by the corresponding 5′-triphosphate. The 3′-N-alkylated derivatives of AMT with methyl or ethyl groups are devoid of anti-HIV activity, but their 5′-triphosphates show more selective inhibitory properties than does the parent nucleotide to reverse transcriptases (RT) of HIV and avian myeloblastosis virus (AMV) and do not inhibit DNA biosynthesis catalyzed by human DNA polymerases.[6,7] Another example of an anti-HIV nucleoside analogue bearing nitrogen at C3′ is 1-(2,3-dideoxy-3-nitro-β-D-*erythro*-pentofuranosyl)-thymine (**10**).[8,9] The main conformational parameters of this molecule, obtained from the X-ray analysis, are very similar to one of the crystallographically independent forms of AZT found in its crystalline state.[10] However, this compound shows moderate inhibition of HIV replication in MT-4 cell culture,[8] though its 5′-triphosphate is a highly effective and selective inhibitor of DNA biosynthesis catalyzed by RT of HIV and AMV.[10] Therefore it was interesting to resynthesize **10** in order to estimate its antiviral potency against a broad number of viruses. These considerations also prompted us to synthesize the *N*-hydroxyimino derivative **3E**, as well as the *N*-methoxyimino **5E** + **5Z** and *N*-acetoxyimino **7E** + **7Z** derivatives (Figure 1) in order to evaluate their biological properties.

The additional rationale for the syntheses of the above mentioned oximes came independently from the concept that the flattened sugar conformation like realized in the title oximes seems preferable for the design of biologically active nucleoside analogues,[11,12] since they are a number of examples of nucleosides with a flattened sugar moiety possessing high biological activity.[11,14]

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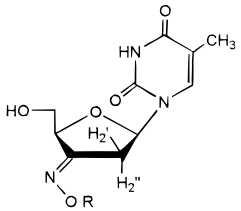
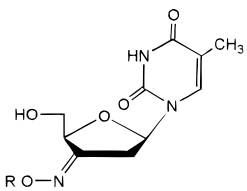


3E : R = H

5E : R = CH_3

7E : R = C(O)-CH_3



5Z : R = CH_3

7Z : R = C(O)-CH_3

Figure 1. Structure of (*E*)-1-[2,3-dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*- pentofuranosyl]thymine (**3E**) and of the (*E*,*Z*)-*O*-methyl ethers (**5E**, **5Z**) and acetyl esters (**7E**, **7Z**).

To the best of our knowledge the oxime **3E** and the acetyl esters **7E** + **7Z** have not been previously synthesized or studied as antiviral agents, although the 5′-*O*-(*tert*-butyldimethylsilyl) derivative of **3E** was recently prepared for synthetical purposes by Tronchet and coworkers.[15] Also, the *O*-methyl ether **5** was recently described by the same group,[16] but no biological evaluation has been reported for this compound.

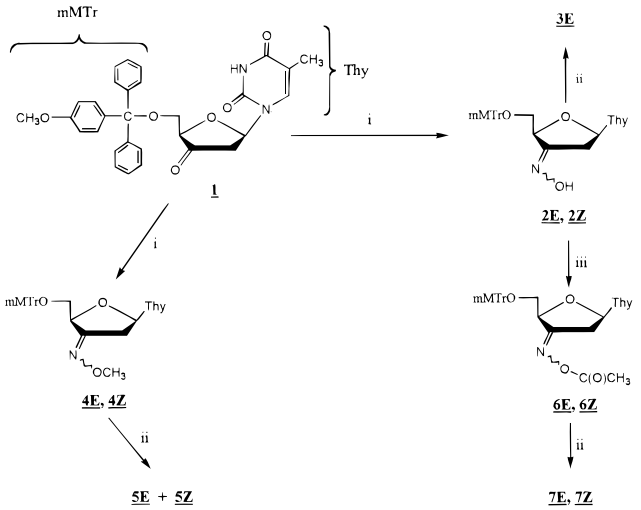
In addition, the 5′-protected oximes **2E** + **2Z**, reported in the present work, appear to be convenient precursors for the synthesis of the 3′-nitro derivative **10**[8,9] which is an interesting subject for antiviral studies. This nucleoside analogue could be easily transformed to the correspondent nitronic salt **11**[8] with more flattened sugar conformation.

The present paper is devoted to the synthesis of the above mentioned new nucleoside analogues as well as conformational studies performed using X-ray analysis for compounds **3E** (*E* isomer) and **5Z** (*Z* isomer) in comparison with **10**[10] and natural thymidine. The antiviral properties of these analogues in various cell cultures infected by a variety of viruses are also reported.

Results

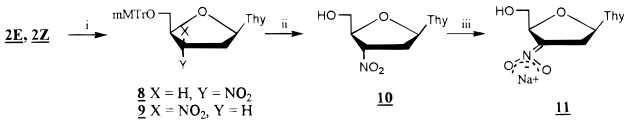
Chemistry. As an appropriate synthon for the synthesis of the desired 3′-oximes derivatives **3E**, **5E** + **5Z**, and **7E** + **7Z**, 5′-(monomethoxytrityl)-3′-ketothymidine (**1**)[17]was chosen (Scheme 1). Compound **1** was treated with a saturated solution of hydroxylamine hydrochloride in pyridine or with *O*-methylhydroxylamine hydrochloride to give, in quantitative yields, the mixture of isomeric protected oximes **2E** + **2Z** and **4E** + **4Z** in a ratio of 3:2 and 3:1, respectively. These mixtures of 5′-protected oximes were separated by preparative TLC to afford the analytical samples of individual *E* and *Z* isomers. Deprotection of the isomeric mixture of **2E** + **2Z** by 80% aqueous acetic acid gave exclusively the *E* isomer **3E.** A similar deprotection of compounds **4E** + **4Z** afforded the mixture **5E** + **5Z** in a ratio of 3:1; the compounds were also separated by preparative TLC in order to isolate analytical samples of the individual isomers. The acylation of the oxime hydroxyls of the **2E** + **2Z** mixture by acetyl chloride in pyridine followed by deprotection of the monomethoxytrityl group yielded an **7E** + **7Z** isomeric mixture in a ratio of 3:2. This pair of **7E** + **7Z** isomers was separable neither by chromatography on silica gel nor by reverse phase chromatography.

Scheme 1[a]



[a] Reaction conditions: (i) hydroxylamine hydrochloride or *O*-methylhydroxylamine hydrochloride/pyridine; (ii) 80% aqueous acetic acid; (iii) $CH_3C(O)Cl$/pyridine.

Scheme 2[a]



[a] Reaction conditions: (i) CF_3CO_3H/CH_3CN; (ii) 80% aqueous acetic acid; (iii) Na_2CO_3/H_2O.

The mixture of the protected oximes **2E** + **2Z** was successfully used to prepare, in only two steps and with an improved overall yield, the previously reported 3′-nitro derivative **10**[8,9] (Scheme 2). Thus, oxidation of **2E** + **2Z** using a solution of pertrifluoroacetic acid in acetonitrile in the presence of anhydrous Na_2HPO_4 and $CO(NH_2)_2$ gave a mixture of the 5′-protected *erythro*- and *threo*- 3′-nitro derivatives **8** and **9** in a ratio of 7:1. Deprotection of this mixture with 80% aqueous acetic acid afforded exclusively the *erythro* diastereomer **10** with the natural configuration of the nitro group. The nucleoside analogue **10** was identical (^{1}H NMR, mass spectrum) to the compound previously reported.[8,9] Finally, treatment of an aqueous solution of **10** with Na_2CO_3 afforded the quantitative formation of the corresponding sodium salt of the nitronic acid **11**.[8]

Physicochemical Properties. The structure of all the reported compounds was ascertained by ^{1}H NMR, UV, mass spectrometry, and elemental C, H, N analysis for final compounds. Also, ^{13}C NMR was recorded for **3E**, **5E**, **5Z**, and **7E**, and X-ray analysis was carried out for the compounds **3E** and **5Z**.

The sugar portion of ^{1}H NMR spectra of all title oximes consists of two separated ABX systems of protons (5′,5″,4′ and 2′,2″,1′). However, these two systems are more complicated due to the long distance *W* coupling constant between H2′ and H4′ ranging from 1 to 2 Hz. The assignment of 2′ and 2″ protons was done in accordance with the C. Altona rule[18] and the presence of long-distance coupling was taken into the consideration during this assignment.

The assignment of the *E* and *Z* isomers was performed on the basis of X-ray analysis for compound **3E** (*E* isomer) and **5Z** (*Z* isomer). The NMR spectra of the oximes **3E** and **5Z** have some characteristic features.

Table 1. Chemical Shifts in ^{1}H NMR Spectra of Nucleoside Analogues[a]

	chemical shift, ppm							
compd	H-6 q	H-1′	H-4′ m	H-5′ dd	H-5″ dd	H-2″	H-2′	Me d
2E[b]	7.62	7.20 pt	4.70	3.61	3.48	3.55 dd	2.79 ddd	1.36
2Z[b]	7.76	6.39 pt	4.94	3.94	3.29	3.23 dd	3.06 ddd	1.29
3E	7.57	6.31 pt	4.64	3.89	3.82	3.30 dd	2.90 ddd	1.82
4E[c]	7.58	6.38 pt	4.65	3.59	3.40	3.44 dd	2.72 ddd	1.37
4Z[c]	7.74	6.39 dd	4.82	3.84	3.23	3.19 dd	3.03 ddd	1.30
5E[c]	7.55	6.28 dd	4.68	3.90	3.82	3.28 ddd	2.92 ddd	1.82
5Z[c]	7.71	6.27 dd	4.65	4.08	3.80	3.08 dd	2.90 ddd	1.84
6E[d]	7.60	6.38 dd	4.82	3.74	3.48	3.62 dd	3.13 ddd	1.37
6Z[d]	7.73	6.48 dd	4.92	*e*	3.47	3.39 dd	3.18 ddd	1.34
7E[d]	7.58	6.30 pt	4.78	3.98	3.91	3.50 dd	3.13 ddd	1.83
7Z[d]	7.73	6.34 dd	5.00	4.13	3.92	3.25 dd	3.10 ddd	1.83
8[f]	7.51	6.48 dd	4.56	3.67	3.46	3.11 ddd	2.54 m	1.54
9[f]	7.63	6.22 dd	4.20	3.54	*e*	2.65 ddd	2.92 m	1.39
10[f]	7.61	6.34 dd	4.58	3.81	3.83	3.10 ddd	2.60 m	1.83
11	7.72	6.27 pt	4.50	4.04	3.84	3.23 ddd	2.84 ddd	1.80

[a] Spectra were recorded in $CDCl_3$ for compounds **2E**, **2Z**, **4E**, **4Z**, **6E**, **6Z**, **8**, and **9** and in D_2O for compounds **3E**, **5E**, **5Z**, **7E**, **7Z**, **10**, and **11**; the chemical shifts of the signals of the protecting group are not cited. [b] N-O**H**: s, 9.01 and 8.69 for compounds **2E** and **2Z**, respectively. [c] N-O**Me**: s, 3.97, 3.89, 3.86, and 3.82 for compounds **4E**, **4Z**, **5E** and **5Z**, respectively. [d] N-OCO**Me**: s, 2.23, 1.94, 2.16, and 1.83 for compounds **6E**, **6Z**, **7E**, and **7Z**, respectively. [e] Signal is overlapped. [f] H3′, m, 5.22, 5.16 and 5.36 for compounds **8**, **9**, and **10**, respectively.

Table 2. Coupling Constants in ^{1}H NMR Spectra of Nucleoside Analogues

	J, Hz							
compd	1′,2′	1′,2″	2′,2″	2′,4′	4′,5′	4′,5″	5′,5″	γ^{+}, %
2E	7.7	6.8	−18.7	1.9	3.0	1.8	−10.5	88
2Z	9.2	6.5	−16.0	0.7	1.7	1.9	−10.2	100
3E	6.3	7.4	−19.1	1.6	2.7	4.1	−12.8	67
4E	7.6	6.8	−18.5	2.0	3.1	2.0	−10.4	85
4Z	8.9	6.3	−16.3	1.3	1.8	1.5	−10.2	>100
5E[a]	6.2	7.3	−19.2	1.9	2.9	4.3	−13.0	63
5Z	8.2	6.5	−17.4	1.8	3.3	2.3	−12.6	80
6E	7.8	6.5	−18.6	1.8	3.0	2.1	−10.7	85
6Z	9.2	6.1	−17.1	1.1	b	1.8	−10.4	
7E	6.2	7.4	−19.6	1.3	2.7	3.9	−16.0	69
7Z	8.2	6.3	−17.7	1.3	3.2	2.2	−12.9	81
8[c]	8.1	6.1	−14.5		3.0	2.8	−10.8	77
9[c]	7.5	6.2	−15.0		5.3	5.3	−10.5	28
10[c]	7.7	6.5	−15.0		3.5	4.0	−12.6	60
11[d]	7.6	6.8	−18.4	3.0	3.7	2.6	−12.4	72

[a] $J_{2'',4'} = 1.0$ Hz. [b] Signal is overlapped. [c] $J_{2',3'} = 8.2$, 7.9, and 8.1 Hz; $J_{2'',3'} = 2.6$, 2.6, and 3.5 Hz; $J_{3',4'} = 3.4$, 6.2, and 4.1 Hz for compounds **8**, **9** and **10**, respectively [d] $J_{2'',4'} = 1.5$ Hz.

These features are conserved and were repeated in all the other oximes (**2E** + **2Z**, **4E** + **4Z**, **5E**, **6E** + **6Z**, and **7E** + **7Z**) reported in this paper. On the basis of these features, the assignment of *E*/*Z* configuration of those other oximes has been carried out. Thus, for each *E*/*Z* isomeric pair the chemical shift of the 2″ proton was always more downfield for the *E*-isomer, the chemical shift of H4′ was usually more upfield for the *E* isomer and the chemical shift of H6 was always more upfield for the *E* isomer (Table 1); $J_{1',2'}$ was bigger for the *Z* isomer, $J_{1',2''}$ was bigger for the *E* isomer, and $J_{2',2'}$ was bigger for the *E* isomer. Also, the population of the γ^+ rotamer around the exocyclic C4′−C5′ bond was always bigger for the *Z* isomer (Table 2).

Regarding the nitro derivatives **8**−**10** (Scheme 2), the assignment of a *threo* configuration for the minor diastereomer **9** in the mixture of **8** + **9** was derived from the "sum rule"[8,19] based on the analysis of the exocyclic $J_{4',5'}$ and $J_{4',5''}$ coupling constants using the equation $P^{\gamma^+} = (13.3 - \sum(J_{4',5'} + J_{4',5''}))/9.7$.[20] For the *threo* isomers the population of the γ^+ rotamer is usually less than 30%.[8] In our case for the major **8** and minor **9** isomers these calculations gave 77% and 29%, and this suggests the *erythro* and *threo* configurations, respectively.

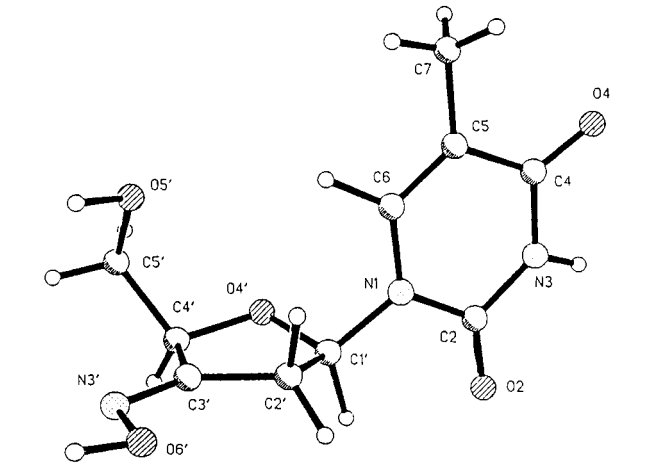
The three-dimensional structures of compounds **3E** and **5Z** were determined by X-ray analysis. Figure 2 shows the obtained conformations and the accepted atom numbering for **3E**, **5Z**, (and **10**),[10] respectively. The conformational parameters of the above mentioned molecules are presented in Table 3, as well as those of natural thymidine[21] and of two crystallographically independent forms of AZT.[22]

It appears that the compound **10** and one crystallographical independent form of AZT (molecule 1) have close conformational parameters. In both cases an *anti* conformation about the N-glycosidic bond and C2′-*endo*−C3′-*exo* conformation of the furanose ring are found in these molecules. Atoms C2′ and C3′ are deviated from the atom planes C1′, C4′, O4′ for 0.307 and 0.181 Å in molecule **10** and for 0.352 and 0.170 Å in the AZT molecule 1. The difference observed in the conformation about the exocyclic C4′-C5′ bond can be due to intermolecular interactions within the crystals.

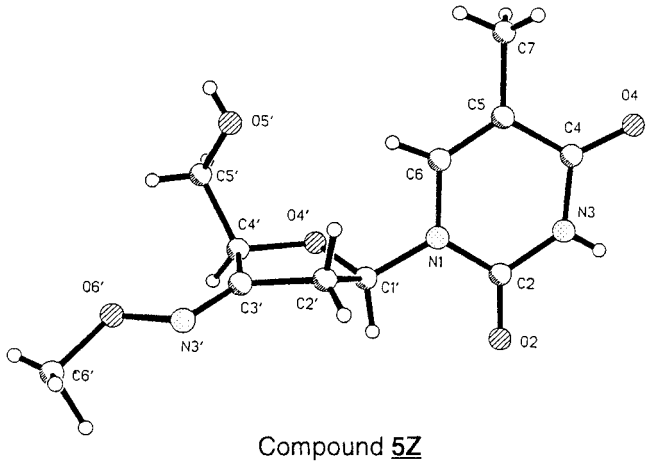
Another group of conformationally similar molecules includes the oximes **3E** and **5Z** (Table 3). The glycosidic torsion angles and conformations about the exocyclic C4′−C5′ bonds in these compounds are coincidental with each other, and the furanose ring puckering belongs to the C1′-*exo* population. In the oxime **3E** the furanose cycle C1′-*exo*−O4′-*endo* ($_1$T^o) conformation is realized. Atoms C1′ and O4′ are deviated to the opposite sides of the plane of atoms C2′, C3′ and C4′ for 0.292 and 0.087 Å, respectively. Atoms of the 3′-oximino group (N3′O6′H) are in the plane of atoms C2′, C3′, and C4′. The value of the glycosidic torsion angle for **3E** is −118.1°, and this value is rather close to the angle of AZT molecule 1 (−125.4°). Always in the case of **3E**, and with the aim to compare the solid state and solution structure, it was not possible to perform complete pseudorotational analysis in order to determine conformation of the sugar ring using ^{1}H NMR owing the absence of endocyclic $^3J_{2',3'}$, $^3J_{2'',3'}$, $^3J_{3',4'}$ coupling constants. However, it has been shown[23] that the using of the equation

$$\%N = (7.9 - {}^3J_{1',2'}) \times 100/6.9$$

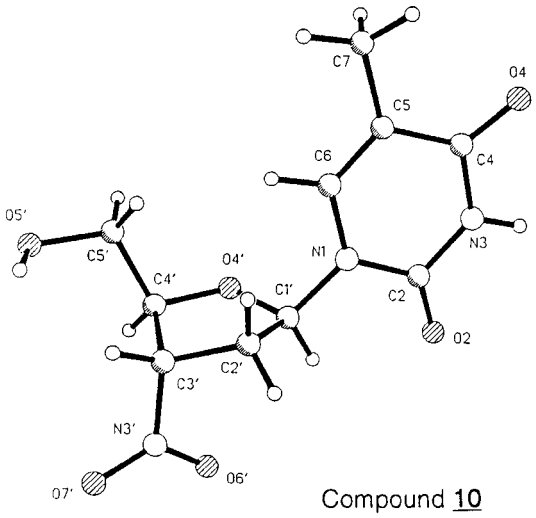
allows us to determine the percentage of the north population (%N) in the two-state north−south equilib-



Compound 3E



Compound 5Z



Compound 10

Figure 2. Three-dimensional structure of the crystal conformation of **3E**, **5Z** (and **10**[10]).

rium on the basis of $^3J_{1',2'}$ coupling constant. This calculation for **3E** with experimental value of $^3J_{1',2'}$ = 6.3 Hz (D_2O) gave 23% for north and 77% for south conformer, respectively. Predominance of south population in solution was in accordance with X-ray data. The similar results were obtained for the *E*-oximes **5E** and **7E** (data are not cited). The conformation around the exocyclic C4′−C5′ bond (γ) can be described through relative distribution (x) of three staggered rotamers population γ^+, γ^-, and γ^i and the following equations[8,20,23] allow to determine the percentage of rotamers:

$$x(\gamma^+) = (13.3 - \sum {}^3J_{4',5'} + {}^3J_{4',5''})/9.7$$

$$^3J_{4',5'} = x(\gamma^+)2.4 + x(\gamma^-)10.6 + x(\gamma^i)2.6$$

$$x(\gamma^+) + x(\gamma^-) + x(\gamma^i) = 1$$

The use of experimental $^3J_{4',5'}$ and $^3J_{4',5''}$ coupling constants (2.7 and 4.1 Hz, respectively) in these equations gave the relative percentages of the staggered rotamers across C4′−C5′ as follows: γ^+ = 67%, γ^- = 3%, and γ^i = 30%. The distributions obtained for the *E*-oximes **5E** and **7E** were very similar (data are not cited). However, the percentage of the γ^+ rotamers for the *Z*-oximes **5Z** and **7Z** were essentially bigger (**5Z** γ^+ = 81%, γ^- = 11%, and γ^i = 8%; **7Z** γ^+ = 83%, γ^- = 10%, and γ^i = 7%). The preference of the γ^+ rotamers is in agreement with X-ray data for the oximes **3E** and **5Z** where the γ^+ conformation was found. Moreover, in order to determine the conformation around the glycosidic C1′−N1′ bond the approach proposed by Davies[24] based on the analysis of experimental $^3J_{H1',C2}$ and $^3J_{H1',C6}$ coupling constants was applied. Using of the experimental coupling constants obtained for **3E** ($^3J_{H1',C2}$ = 2.6 Hz and $^3J_{H1',C6}$ = 4.1 Hz) in the following equations

$$\sum J \approx (A2 + A6)\cos^2 \lambda + (C2 + C6)$$

$$P_a = 0.5 - \{(\Delta J - \Delta C)/2\sum B \cos \lambda\} + \{\Delta A \cos \lambda/2\sum B\}$$

where P_a is the population of *anti* conformer, $\sum J$ = $^3J_{H1',C2}$ + $^3J_{H1',C6}$, ΔJ = $^3J_{H1',C6}$ − $^3J_{H1',C2}$ and the coefficients A, B, and C are the experimental Karplus parameters determined for a number of uridine derivatives (*A2* = 5.0, *A6* = 6.2, *C2* = 0.1, *C6* = 0.1, *B2* = −2.1, *B6* = −2.4 Hz), allowed to determine the population of *anti* conformer P_a = 0.62, suggesting predominant *anti* conformation of **3E** which is typical for pyrimidine nucleosides [the determined value of P_a for the natural thymidine ($^3J_{H1',C2}$ = 2.2 Hz, $^3J_{H1',C6}$ = 3.8 Hz) is 0.65 (error in these calculations is ±0.02)].

For the methylated oxime **5Z**, a C1′-*exo*−C2′-*endo* ($^2T_1$) conformation is observed with C1′ and C2′ atom deviations of 0.183 and 0.302 Å, respectively, to opposite sides of the C3′, C4′, and O4′ atoms plane. In spite of the presence of a double bond between the C3′ and N3′ atoms, the N3′ atom is displaced by 0.312 Å from the plane of atoms C3′, C4′ and O4′, and the 3′-methoxyimino group forms, with this plane, an angle of 26°. The deviation of the atoms of the methoxyimino group from the furanose cycle plane is probably due to the involvement of the N3′ atom in intramolecular hydrogen bonds with N3 atom of the adjacent molecule.

The conformation of the sugar ring of the oximes **3E** and **5Z** is exceedingly different from the conformations found in natural thymidine and in AZT. Some flattening of the furanose cycle is observed for all compounds studied, comparable to thymidine. This flattening is especially apparent for the oxime **3E** where the degree of pucker is 25.7°. The same parameter for thymidine is 37.8°.

Table 3. Conformational Parameters of Some Nucleoside Analogues Obtained from X-ray Studies[a]

	3E	**5Z**	**10**	AZT molecule 1	AZT, molecule 2	Thd
conformation about N1′−C1′ bond	*anti*	*anti*	*anti*	*anti*	*anti*	*anti*
χ (O4′C1′N1C2), deg	−118.1	−118.9	−121.9	−125.4	−172.0	−139.4
P (Phase angle of pseudorotation), deg	115.6	147.7	174.9	173.3	212.2	187.5
Ψ_m (degree of pucker), deg	25.7	31.2	30.5	32.4	36.3	37.8
furanose ring conformation	C1′-*exo*/O4′-*endo*	C1′-*exo*/C2′-*endo*	C2′-*endo*/C3′-*exo*	C2′-*endo*/C3′-*exo*	C2′-*endo*/C3′-*exo*	C2′-*endo*/C3′-*exo*
conformation about C4′−C5′ bond	*gauche*$^+$	*gauche*$^+$	*gauche*$^-$	*gauche*$^+$	*trans*	*trans*
γ (O5′C5′C4′C3), deg	43.1	48.8	−71.9	50.8	173.5	172.8

[a] Conformational parameters for **10**,[10] thymidine[21] (Thd), and AZT[22] (molecules 1 and 2) were calculated from coordinates taken from above mentioned references.

Table 4. Anti-HIV-1 and -HIV-2 Activity and Cellular Toxicity of Nucleoside Analogues in Human MT-4 and CEM Cells[a]

	EC_{50}, μg/mL[b]					CC_{50}, mg/mL[c]			selectivity index HIV-1 and HIV-2	
	HIV-1		HIV-2							
compd	MT-4	CEM	MT-4	CEM	CEM/TK$^-$	MT-4	CEM	CEM/TK$^-$ [d]	MT-4	CEM
3E	0.025	0.40	0.03	0.35	20	1.6	9.9	>100	35−88	25−28
5E + 5Z	3.3	26	4.6	27	>100	182	>200	>200	34−64	8
7E + 7Z	0.05	1.0	0.05	0.80	>20	7.1	104	>200	120−182	104−130
10	1.0	≥100	2.2	100	>100	93	168	>200	48−79	≥1.7
11	0.86	13	1.2	10	>100	91	247	>200	77−102	19−25
AZT	0.0005	0.0007	0.0007	0.0009	>25	1.5	>100	>200	2100−3000	>100000

[a] Experiments were performed with HIV-1 (III_B) and HIV-2 (ROD) in MT-4 or CEM cells. The values reported are the average from two independent experiments. Selectivity index was calculated as follows: SI = average CC_{50}/average EC_{50}. [b] 50% effective concentration or compound concentration required to protect MT-4 cells against cytopathogenicity of HIV or to protect CEM cells against HIV-induced giant cell formation by 50%. [c] 50% cytotoxic concentration or compound concentration required to reduce MT-4 cell viability by 50%. [d] CEM/TK$^-$: thymidine kinase deficient CEM cells.

Antiviral Activity and Cellular Toxicity. Compounds **3E, 5E + 5Z, 7E + 7Z, 10,** and **11** were evaluated for their inhibitory activity against HIV-1 and HIV-2 in cell cultures (Table 4). Compound **3E** and the 3′-acetyl derivative **7E + 7Z** proved markedly inhibitory to HIV-1 and HIV-2 replication in MT-4 and CEM cell cultures. The antiviral potency of these test compounds against HIV-1 and HIV-2 proved 10−20 fold higher when evaluated in MT-4 cell cultures than in CEM cell cultures. Also, the cytostatic activity of the test compounds were 5−15-fold higher against MT-4 than against CEM cells (Table 4). The methyl derivative **5E + 5Z** proved less inhibitory to HIV-1 and HIV-2 by approximately 2 orders of magnitude. The 3′-nitro (**10**) and 3′-nitronate (**11**) derivatives showed an EC_{50} in the range of 1 μg/mL in MT-4 cell cultures and 10−100 μg/mL in CEM cell culture.

The compounds were also evaluated against a panel of DNA and RNA viruses, including herpes simplex virus type 1 (HSV-1), HSV-2, the thymidine kinase deficient strain of HSV-1 (B2006), vaccinia virus (VV) and vesicular stomatitis virus (VSV) in human E_6SM cells, Sindbis virus, Semliki forest virus, parainfluenza virus, Coxsackie virus and reovirus-1 (Reo-1) in Vero cells, Coxsackie virus and VSV in Hela cells, and varicella-zoster virus (wild-type strains OKA and YS, and TK$^-$ strains 07/1 and YS:R) and cytomegalovirus (strains AD169 and Davis) in human embryonic HEL cells. None of the compounds were markedly inhibitory at 200 μg/mL against parainfluenza virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Semliki forest virus, vesicular stomatitis virus, and vaccinia virus, except for compound **7E + 7Z** that had a MIC_{50} of 70 μg/mL against VV, and compound **3E** that had a MIC_{50} of 135 μg/mL against VSV. Compounds **3E** and **7E + 7Z** showed marginal activity against VZV (OKA strain) (MIC_{50}: 11−20 μg/mL).

In contrast, compounds **3E** and **7E + 7Z** proved markedly inhibitory to the replication of several HSV-1 strains (EC_{50}: 0.4−1.3 μg/mL for **3E** and 0.9−5 μg/mL for **7E + 7Z**) (Table 5). Also, these compounds proved inhibitory to HSV-2 strains, albeit at a lower potency than for HSV-1. Compounds **3E** and **7E + 7Z** proved inactive against the thymidine kinase deficient (TK$^-$) HSV-1 strain B2006 (Table 5).

Striking differences were found with regard to the cytostatic activities of the test compounds depending the cell type against which they were evalutated. Whereas the cytostatic effects of the test compounds were similar for L1210, Molt4 (clone 8 and MT-4) cells (Tables 4 and 6), their inhibitory potential to CEM cell proliferation was markedly lower, and no inhibitory activity was found against murine mammary carcinoma FM3A cells at 200 μg/mL (Table 6). Since compounds **3E** and **7E + 7Z** showed a marked inhibitory effect on the replication of TK$^+$ HSV-1 strains (KOS, F, Mc Intyre), but not the TK$^-$ HSV-1 strain B2006, they were also evaluated for their inhibitory effect on FM3A cells transfected by the HSV-1 TK gene (Table 6). However, neither **3E** nor **7E + 7Z** or the other test compounds (**5E + 5Z, 10, 11**) proved active against FM3A/TK$^-$/HSV-1 TK$^+$ cells.

Compounds **3E, 5E + 5Z**, and **10** were also evaluated for their *in vitro* anti-HBV activities in the HBV-transfected 2.2.15 cell line. The derivative **3E** demonstrated a significant anti-HBV activity with a 50% effective concentration (EC_{50}) of 0.25 μg/mL (1 μM) for inhibiting intracellular viral replicative intermediate DNA as compared to control. In contrast, the compounds **5E + 5Z** and **10** exhibited no *in vitro* anti-HBV activity up to a concentration of 10 μM. None of these compounds exhibited *in vitro* cytotoxicity in Hep-G2 cells up to a concentration of 50 μg/mL (200 μM), thus demonstrating the important anti-HBV selectivity (>200) of the **3E** derivative.

Table 5. Cytotoxicity and Antiviral Activity of Nucleoside Analogues in E_6SM and in HEL Cells

	minimum cytotoxic concentration[a]	50% minimum inhibitory concentration (μg/mL)[b]											
		HSV-1 (KOS)		HSV-1 (F)	HSV-1 (Mc Intyre)		HSV-2 (G)		HSV-2 (196)	HSV-2 (Lyons)		HSV-1 TK$^-$ (B2006)	
compd	(μg/mL)	E_6SM	HEL	E_6SM	E_6SM	HEL	E_6SM	HEL	E_6SM	E_6SM	HEL	E_6SM	HEL
3E	>400	0.4	1.4	0.5	1.3	0.85	0.7	0.5	11	1.4	0.31	≥200	>50
5E + 5Z	>200	>200	50	>200	>200	>50	>200	>50	>200	>200	35	>200	>50
7E + 7Z	400	0.9	3.8	5	2	2.7	1	2.8	70	4	2	>200	>5
10	>200	>200	5	>200	>200	20	>200	20	>200	>200	25	>200	>50
11	>200	35	ND[c]	9	35	ND	>200	ND	>200	>200	ND	>200	ND
BVDU	≥300	0.007	0.005	0.02	90	0.003	90	30	>400	>400	5	10	50
ribavirin	>400	60	ND	80	90	ND	50	ND	150	100	ND	150	ND
DHPG	>100	0.001	ND	0.002	0.003	ND	0.002	ND	0.006	0.002	ND	5	ND
ACG	≥400	0.01	ND	0.02	0.006	ND	0.009	ND	0.02	0.004	ND	100	ND

[a] Minimum cytotoxic concentration that causes a microscopically detectable alteration of normal cell morphology after 2 days of incubation. [b] Concentration required to reduce virus-induced cytopathogenicity by 50%. [c] ND, not determined.

Table 6. Inhibitory Effects of Nucleoside Analogues on the Proliferation of Murine Leukemia (L1210), Murine Mammary Carcinoma Cells (FM3A), and Human T-Lymphocyte (Molt4/C8) Cells

	EC_{50}, μg/mL[a]				
compd	L1210	FM3A	FM3A/TK$^-$	FM3A/TK$^-$ HSV-1 TK$^+$	Molt4/C8
3E	1.1 ± 0.3	>200	>200	>200	1.2 ± 0.4
5E + 5Z	112 ± 60	>200	>200	>200	>200
7E + 7Z	19 ± 6	>200	>200	>200	14 ± 3
10	58 ± 2	>200	>200	>200	70 ± 19
11	25 ± 5	>200	>200	>200	17 ± 5

[a] 50% cytotoxic concentration or compound concentration required to reduce cell viability by 50%.

Discussion

Chemistry. The synthetic precursor of the 3′-*N*-hydroxyimino derivative **3E**, namely the 5′-protected 3′-ketothymidine **1** (Scheme 1) is an unstable compound which easily undergoes β-elimination in the presence of protic solvents. However, the nitrogen of the oxime group of **3E** is a much weaker electron acceptor than that of the oxygen of the keto group of **1** which provides strongly increased oxime stability. Thus, only traces of thymine were observed after deprotection of the oximes **2E** + **2Z** under acidic conditions. An aqueous solution of the oxime **3E** is stable at room temperature for several months, and no traces of thymine formation have been observed. The acetate **7E** + **7Z** appears to be hydrolytically unstable, releasing parent **3E,** and its half-life in water solution did not exceed 24 h. This process is accompanied by strong decomposition with release of thymine (data are not cited).

The 5′-monomethoxytritylated oxime **2E** + **2Z** exists in organic solvents as a pair of *E*/*Z* diastereomers. However, after deprotection with 80% aqueous acetic acid the only product identified in aqueous solution was the *E* isomer **3E** (^{1}H NMR data). Nevertheless, in organic solvents such as DMSO-d_6 and CD_3OD, **3E** immediately generates the corresponding *Z* isomer, and a mixture of *E* and *Z* isomers can be observed by ^{1}H NMR (data are not cited).

The 3′-nitro derivative **10** has been previously synthesized by a few independent synthetic routes. These methods include (i) direct oxidation of 5′-toluoyl-3′-amino-3′-deoxythymidine by CF_3COOOH in acetonitrile in the presence of Na_2HPO_4;[8] (ii) reduction of 5′-(monomethoxytrityl)-3′-nitro-3′-deoxy-2′,3′-didehydrothymidine by $NaBH_4$ in ethanol;[8] (iii) reduction of methyl 2,3-dideoxy-3-nitro-5-*O*-toluoyl-2,3-didehydro-α/β-D-pentofuranoside by $NaBH_4$ in ethanol to give exclusively methyl 2,3-dideoxy-3-nitro-5-*O*-toluoyl-α/β-D-*erythro*-pentofuranoside[25] which was consequently condensed with silylated thymine to afford an α and β anomeric mixture of only the *erythro* nucleosides.[8] Deprotection of all these products under acidic or basic conditions gave 3′-nitro-3′-deoxythymidine with the natural *erythro* configuration.[8] The same conclusion that only *erythro*-3′-nitro-3′-deoxythymidine **10** is stable was confirmed by the recent synthesis of this compound by the reaction of mixture of *erythro*- and *threo*-1-(2-deoxy-5-O-trityl-3-deoxy-3-iodopentofuranosyl)-2-methoxy-5-methyl-4(1*H*)-pyrimidinone with $LiNO_2$ following by deprotection in acidic conditions to give exclusively **10**.[9] On the other hand, it has been previously reported that the oxidation of sugar 3-oximes[24] and nucleosides,[8] bearing appropriately protected hydroxyl functions at C2′, provides the formation of mixtures of *erythro* and *threo* compounds.

It was surprising that the oxidation of the 5′-protected oximes **2E** + **2Z** by CF_3COOOH in acetonitrile in the presence of Na_2HPO_4 gave a mixture of *erythro* and *threo* nucleosides **8** and **9** in a ratio of 7:1. The exact reasons for the formation of the 5′-tritylated *threo*-3′-nitro-3′-deoxythymidine **9** during this oxidation are not clear. Probably it is due to the steric hindrances from the β-face of the oximes **2E** + **2Z**, where are disposed the bulky nucleic base and MMTr group, and which might bring about the energetically unfavored *threo* configuration. However, deprotection of the diastereomeric mixture of **8** + **9** afforded only the expected *erythro*-3′-nitro-3′-deoxythymidine (**10**), and this suggests a total instability of *threo*-3′-nitro-3′-deoxythymidine in protic solvents.

Structure−Activity Relationship. The 3′-*N*-hydroxyimino derivative **3E** represents a novel inhibitor of HIV and HSV replication in cell culture. It does not discriminate between HIV-1 and HIV-2 (Table 4) and is also active against both HSV-1 and HSV-2 (Table 5). The compound proved virtually inactive against VZV and CMV in HEL cells. It is noteworthy that compounds **3E** and **7E** + **7Z** are about as active against HSV-1 and -2 in HEL cells as in E_6SM cells, which thus means that their inactivity against VZV in HEL cells must be ascribed to the virus and not to the cells. The inactivity of **3E** and its closely related **7E** + **7Z** derivative against VZV is somewhat surprising in the light of the close similarities of substrate properties between HSV-1 thymidine kinase (TK) and VZV thymidine kinase. Indeed, the observation that **3E** and **7E** + **7Z** are inactive against a thymidine kinase deficient HSV-1

strain suggests that the compound **3E** is dependent on the phosphorylation by HSV-1 (and HSV-2) TK to become active against the virus. Therefore, one may conclude that the TKs of HSV-1 and VZV are endowed with different affinities for these test compounds. However, the inactivity of **3E** and **7E** + **7Z** against VZV (and CMV) can also be explained by differences in cellular metabolism of the test compounds. Indeed, the different anti-HIV properties depending the cell line used in the anti-HIV assays (i.e. MT-4 or CEM), as well as the difference in cytostatic activity of the compounds against cell lines of different origin strongly suggest that cellular metabolism may be an important determinant in the eventual cytostatic and antiviral activity of these test compounds. Likewise, the inactivity of **3E** and **7E** + **7Z** against HSV-1 TK gene transfected FM3A cells may be explained by such cellular factors, including inactivity of the phosphorylated products against the putative cellular target enzymes (i.e. dTMP synthase, DNA polymerases) for cytostatic activity.

The additional clue that a flattened sugar configuration could be preferable for sufficient recognition of modified nucleosides and/or nucleoside 5′-triphosphates by cellular and viral enzymes came from a comparison of the activity of 3′-nitro (**10**) and the corresponding nitronate (**11**) derivatives. Thus, **10** is inactive against HIV-1 and HIV-2 in CEM cell cultures ($EC_{50} \geq 100$ μg/mL for HIV-1 and HIV-2), but the nitronate **11** shows moderate activity (EC_{50} 13 and 10 μg/mL for HIV-1 and HIV-2, respectively) in the same cell line (Table 4). Both the nucleoside analogues **10** and **11** show similar cytotoxicity against CEM cells (168 and 247 μg/mL for **10** and **11,** respectively) (Table 4), which suggests that both of them are phosphorylated at least to the 5′-monophosphate by cellular thymidine kinase. Then the 5′-monophosphate of **11** is probably consequently phosphorylated to the corresponding 5′-triphosphate to inhibit the retroviral reverse transcriptase. As was previously reported, the 5′-triphosphate of 3′-nitro-3′-deoxythymidine **10** is a potent terminator of DNA chain elongation, catalyzed by HIV-1 and HIV-2 RT,[10] an observation which suggests that a loss of activity of **10** by 1 order of magnitude (compared to **11**) is likely due to the poor phosphorylation of 3′-nitro-3′-deoxythymidine 5′-monophosphate to the corresponding 5′-di- or -triphosphate. A similar observation has been made for the activity of **10** and **11** against herpes simplex virus replication. Compound **10** is inactive against both HSV-1 and HSV-2 in E_6SM cells (EC_{50} >200 μg/mL), whereas **11** shows moderate activity against several HSV-1 strains (Table 5). Both **10** and **11** are devoid of toxicity against the E_6SM cell line. Compound **11** may be still able to pass through all steps of activation by cellular kinases, whereas compound **10** cannot. These data demonstrate that recognition of two very similar nucleoside analogues **10** and **11** by cellular and/or viral enzymes of nucleic acid metabolism is preferable for **11**, having a more flattened sugar conformation, isosteric to that of the very active oxime **3E**, and thus supports the design of novel molecules wherein such a conformation is realized.

Conclusion

We have shown that the hitherto unknown 3′-*N*-hydroxyimino **3E** analogue of thymidine is a novel inhibitor of both HIV-1, HIV-2, HSV-1, and HSV-2 replication in cell culture. In addition it is notworthy that this compound is also active against HBV in HBV-transfected 2.2.15 cell line. It would now seem important to know how this compound exerts its biological activities. In this regard, the mechanism of action of **3E** is currently under investigation. Other work in progress in our laboratories is directed toward the application of the synthetic approach described above to the preparation of various other 2′- and/or 3′-oximino nucleoside analogues.

Experimental Section

A. Synthesis. Materials and Methods. The ^{1}H NMR and ^{13}C spectra were recorded on a Bruker AC 250 spectrometer at 25 °C in $CDCl_3$ or D_2O using TMS or MeCN as an internal standard. The accepted abbreviations are as follows: s, singlet; dd, doublet of doublets; ddd, doublet of doublets of doublets; m, multiplet; q, quartet; pt, pseudotriplet. FAB mass spectra were recorded in the positive or negative ion mode on a JEOL DX 300 mass spectrometer operating with a JMA-DA 5000 mass data system using 3-nitrobenzyl alcohol (NBA) as matrix. The UV spectra were recorded on a Uvicon-931 spectrometer in water. TLC was performed on aluminium silica gel F_{254} sheets (Merck, Art. 5554), visualization of products being accomplished by UV absorbance followed by charring with 10% ethanolic sulfuric acid with heating. Column chromatography was carried out on silica gel 60 (Merck, Art. 15111), using methylene chloride and methanol as eluents. Melting points were determined with a Reichert melting point apparatus (Austria) and are uncorrected. Separation of analytical samples of isomeric mixtures was performed on precoated silica gel 60 F_{254} TLC plates (layer thickness 0.5 mm, Merck, Art. 1.05744). Reverse phase chromatography was performed on LiChroprep RP-18 (40−63 μm, Merck, Art. 13900). Compound **1** was prepared as previously reported.[17] The X-ray analyses were carried out on a CAD-4 diffractometer. The structures were solved by a direct method and refined by the full-matrix least squares with anisotropic approximation for nonhydrogen atoms. The hydrogen atom coordinates were determined from the difference of Fourier syntheses and refined using the isotropic temperature factors. Final values of *R* factors were 4.2% and 3.0% for compounds **3E** and **5Z**, respectively. Crystals of all compounds were obtained from water.

1-[5-*O*-(4-Monomethoxytrityl)-2,3-dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]thymine (2E + 2Z). To a saturated solution of hydroxylamine hydrochloride in pyridine (5 mL) was added compound **1**[17] (1.45 g, 2.83 mmol). After 15 min, the reaction mixture was evaporated and partitioned between water (50 mL) and dichloromethane (50 mL). The organic layer was dried with anhydrous Na_2SO_4, evaporated, and re-evaporated with toluene. Column chromatography on silica gel (stepwise gradient of MeOH in CH_2Cl_2, 0 → 2.5%) gave 1.39 g of a mixture of **2E** + **2Z** as a white foam (93%): MS *m/e* (FAB MS < 0, NBA) 527 $(M - H)^-$.

1-[2,3-Dideoxy-3-(*N*-hydroxyimino)-*β*-D-*glycero*-pentofuranosyl]thymine (3E). A solution of **2E** + **2Z** (193 mg, 0.37 mmol) in 80% aqueous acetic acid (5 mL) was stirred overnight, evaporated to dryness, and re-evaporated with toluene. The residue was partitioned between water (5 mL); and methylene chloride (5 mL), the water phase was washed with methylene chloride (5 mL), filtered through a wet paper filter, and evaporated to dryness. The residue was purified by reverse phase chromatography (gradient of MeOH in water, 0 → 5%) to give after freeze drying 64 mg of **3E** as a white foam (68%). Crystallization from water gave 43 mg of crystalline **3E**: mp 117−119 °C; UV λ_{max} 267 nm (ϵ 9600); ^{13}C NMR (20% aqueous CD_3OD) δ 168.2 (C4), 161.6 (C3′), 153.4 (C2), 139.2 (C6), 113.8 (C5), 85.1 (C1′), 81.0 (C4′), 62.4 (C5′), 34.9 (C2′), 12.6 (Me-C5); MS *m/e* (FAB MS < 0, NBA) 254 $(M - H)^-$; (FAB MS > 0, NBA) 256 $(M + H)^+$. Anal. ($C_{10}H_{13}N_3O_5 \cdot H_2O$) C, H, N.

1-[5-*O*-(4-Monomethoxytrityl)-2,3-dideoxy-3-(*N*-methoxyimino)-*β*-D-*glycero*-pentofuranosyl]thymine (4E + 4Z).

The reaction of a saturated solution of *O*-methylhydroxylamine hydrochloride in pyridine (2 mL) with **1** (451 mg, 0.88 mmol), followed by workup and purification on silica gel (stepwise gradient of MeOH in CH_2Cl_2, 0 → 2%) as described for **2E** + **2Z,** gave 431 mg of **4E** + **4Z** as a white foam (91%): MS *m/e* (FAB MS < 0, NBA) 540 $(M - H)^-$.

1-[2,3-Dideoxy-3-(*N*-methoxyimino)-β-D-*glycero*-pentofuranosyl]thymine (5E + 5Z). A solution of **4E** + **4Z** (222 mg, 0.41 mmol) in 80% aqueous acetic acid (5 mL) was stirred overnight and worked up as described for **3E**. Purification by chromatography on silica gel (stepwise gradient of MeOH in CH_2Cl_2, 0 → 7%) gave 79 mg of a mixture of **5E** + **5Z** as a white foam (72%). Crystallization from water gave 45 mg of crystalline **5Z**: mp 121−123 °C. UV λ_{max} 267 nm (ϵ 9600); ^{13}C NMR (20% aqueous CD_3OD) (**5E**) 166.9 (C4), 159.5 (C3′), 152.0 (C2), 112.1 (C5), 84.6 (C1′), 79.8 (C4′), 62.3 (C5′), 61.1 (OMe), 33.1 (C2′), 11.9 (Me-C5); (**5Z**) 166.8 (C4), 159.8 (C3′), 152.1 (C2), 137.7 (C6), 112.7 (C5), 83.4 (C1′), 79.6 (C4′), 62.4 (C5′), 60.8 (OMe), 35.0 (C2′), 12.0 (Me-C5); MS *m/e* (FAB MS < 0, NBA) 268 $(M - H)^-$; (FAB MS > 0, NBA) 270 $(M + H)^+$. Anal. ($C_{11}H_{15}N_3O_5$) C, H, N.

1-[5-*O*-(4-Monomethoxytrityl)-2,3-dideoxy-3-(*N*-acetoxyimino)-β-D-*glycero*-pentofuranosyl]thymine (6E + 6Z). To a solution of a mixture of **2E** + **2Z** (460 mg, 0.87 mmol) in dry pyridine (5 mL) with stirring at 0 °C was added acetyl chloride (71 μL, 1 mmol). The reaction mixture was allowed to warm up to room temperature, and after 6 h, a saturated solution of $NaHCO_3$ in water (2 mL) was added. The solution was evaporated to dryness and worked up and purified on silica gel (stepwise gradient of MeOH in CH_2Cl_2, 0 → 2%) as described for **2E** + **2Z** to give 327 mg of a mixture of **6E** + **6Z** as a white foam (66%): MS *m/e* (FAB MS < 0, NBA) 568 $(M - H)^-$.

1-[2,3-Dideoxy-3-(*N*-acetoxyimino)-β-D-*glycero*-pentofuranosyl]thymine (7E + 7Z). A solution of a mixture of **6E** + **6Z** (340 mg, 0.60 mmol) in 80% aqueous acetic acid (5 mL) was stirred overnight and worked up as described for **3E**. The residue was purified on silica gel (stepwise gradient of acetone in CH_2Cl_2, 0 → 50%) to give a mixture of 110 mg of **7E** + **7Z** as a white hygroscopic foam (62%). UV λ_{max} 267 nm (ϵ 9600); ^{13}C NMR (20% aqueous CD_3OD) (**7E**) 172.0 [*C*(O)-CH_3], 168.9 (C3′), 166.9 (C4), 152.0 (C2), 138.3 (C6), 112.2 (C5), 84.9 (C1′), 80.0 (C4′), 62.1 (C5′), 34.5 (C2′), 19.0 [C(O)*C*H_3], 11.9 (Me-C5); MS *m/e* (FAB MS < 0, NBA) 296 $(M - H)^-$; (FAB MS > 0, NBA) 298 $(M + H)^+$. Anal. ($C_{12}H_{15}N_3O_6 \cdot H_2O$) C, H, N.

1-[5-*O*-(4-Monomethoxytrityl)-2,3-dideoxy-3-nitro-β-D-*erythro*- and -*threo*- pentofuranosyl]thymine (8 + 9). To a stirred solution of a mixture of **2E** + **2Z** (393 mg, 0.75 mmol) containing Na_2HPO_4 (5 g) and urea (10 mg) in acetonitrile (10 mL) at 0 °C was added dropwise a 4 M solution of pertrifluoroacetic acid in acetonitrile (60 mmol, 1 mL) during 15 min. The mixture was allowed to warm up to room temperature, and after 30 min the mixture was diluted with a saturated aqueous solution of $NaHCO_3$ (30 mL) and water (30 mL) and extracted with methylene chloride (2 × 50 mL). The combined organic layers were dried with Na_2SO_4, evaporated to dryness, and purified on silica gel (stepwise gradient of MeOH in CH_2Cl_2, 0 → 2%) to give 255 mg of a mixture of the *erythro* and *threo* isomers **8** + **9** in ratio 7:1 as a slightly yellowish foam (62%): MS *m/e* (FAB MS < 0, NBA) 542 $(M - H)^-$.

1-[2,3-Dideoxy-3-nitro-β-D-*erythro*-pentofuranosyl]thymine (10). A solution of the diastereomeric mixture of **8** + **9** (210 mg, 0.39 mmol) in aqueous 80% acetic acid (5 mL) was stirred overnight and worked up as described for **3E**. Purification by reverse phase chromatography (stepwise gradient of MeOH in water, 0 → 7%) gave, after freeze drying, 85 mg of **10** as a white foam (80%): MS *m/e* (FAB MS < 0, NBA) 270 $(M - H)^-$; (FAB MS > 0, NBA) 272 $(M + H)^+$.

1-[2,3-Dideoxy-3-nitronate-β-D-*glycero*-pentofuranosyl]thymine Sodium Salt (11). A solution of Na_2CO_3 (4.1 mg, 0.039 mmol) in water (0.5 mL) was added to **10** (12.5 mg, 0.045 mmol). After 1 h of stirring, and following freeze drying, 15.7 mg of a white foam was obtained which contained 90% of nitronate **11** and excess of Na_2CO_3. Total conversion of **10** to nitronate **11**, which was not additionally purified, was confirmed by ^1H NMR. MS *m/e* (FAB MS < 0, NBA) 270 $(M - Na^+)^-$; (FAB MS > 0, NBA) 294 $(M + H)^+$.

B. Experiments with cell cultures. Materials and Methods. Antiviral Activity Assays. The antiviral assays, other than the anti-HIV-1 assays, were based on an inhibition of virus-induced cytopathicity in either E_6SM, HeLa, Vero, or HEL cell cultures, following previously established procedures.[26−28] Briefly, confluent cell cultures in microtiter trays were inoculated with 100 $CCID_{50}$ of virus, 1 $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200, 100, ... μg/mL) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures.

Cytostatic Activity Assays. The cytostatic assays were performed as previously described.[29] Briefly, 100 μL aliquots of the cell suspensions (5×10^5 murine leukemia L1210 or murine mammary carcinoma FM3A or 7.5×10^5 human T-lymphocyte Molt-4 or CEM cells/mL) were added to the wells of a microtiter plate containing 100 μL of varying concentrations of the test compounds. After a 2-day (L1210, FM3A) or 3-day (Molt-4 and CEM) incubation period at 37 °C in a humidified CO_2-controlled incubator, the number of viable cells was determined using a Coulter Counter. Cytostatic activity is expressed as the compound concentration that reduced the number of viable cells by 50% (CC_{50}). The cytotoxicity measurements were based on microscopically visible alteration of normal cell morphology (E_6SM, HeLa, Vero) or inhibition of normal cell growth (HEL), as previously described.[28]

Inhibition of HIV-Induced Giant Cell Formation. CEM cell cultures were suspended at 250000−300000 cells/mL of culture medium and infected with HIV-1 (III_B) or HIV-2 (ROD) at 100 $CCID_{50}$/mL. Then, 100 μL of the infected cell suspension were transferred to 200 μL microtiter plate wells containing 100 μL of serial dilutions of the test compound solutions. After 4 days of incubation at 37 °C, cell cultures were examined for syncytium formation as previously described.[30]

Anti-Hepatitis B Virus Assays. The 2.2.15 HBV transfected human hepatoma cells derived from the Hep-G2 cell line were cultured as described by Korba and Guerin[31] with minor modifications. Cells cultured in Dubelcco's modified eagle medium supplemented with 4% fetal bovine serum and 0.5 mM glutamine were treated with drugs for 9 days, and culture medium was changed every 3 days. Hep-G2 cells and untreated 2.2.15 cells were used as negative and positive controls. At harvest, the medium was removed and cells were lysed. Total intracellular DNA was recovered and subjected to southern blot analysis using a ^{32}P-labeled HBV specific probe (pTHBV plamid which contains the full length HBV genome) kindly provided by Dr. Raymond F. Schinazi (Emory University, Atlanta, GA). Inhibition of the viral replicative intermediate DNA in drug-treated cells versus control was determined. Evaluation of compound cytotoxicity was performed in Hep-G2 cells by measuring the uptake of neutral red dye in a 96-well cell culture plate. Cells were cultured and treated under the same conditions as those used for evaluating the antiviral activity.

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Supporting Information Available: Further details of the X-ray data of compounds **3E** and **5Z** including atomic coordinates for all atoms, bond lengths and angles, and thermal parameters (9 pages). Ordering information is given on any current masthead page.

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