3′-C-Branched 2′-Deoxy-5-methyluridines: Synthesis, Enzyme Inhibition, and Antiviral Properties

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A synthesis scheme for 3′-*C*-methyl-2′-deoxynucleosides and 3′-*C*-methylidene-2′,3′-dideoxy-5-methyluridine has been proposed with 2-deoxyribose as the starting material. Methyl 5-*O*-benzoyl-2-deoxyribofuranose was oxidized and the mixture of the 3′-keto derivatives was separated into the α- and β-anomers. The β-keto derivative was converted by reaction with MeMgBr, and after reaction with thymine and subsequent deprotection 1-(3′-*C*-methyl-2′-α-deoxy-α-D-*threo*-pentofuranosyl)thymine and its β-anomer were obtained. The same reactions with the α-keto sugar gave 1-(3′-*C*-methyl-2′-deoxy-α-D-*erythro*-pentofuranosyl)thymine and its β-anomer. 1-(5-*O*-Benzoyl-3′-*C*-methyl-2′-deoxy-α-D-*threo*-pentofuranosyl)thymine was converted to a mixture of 3′-*C*-methylidene-2′,3′-dideoxy-5-methyluridine and 3′-*C*-methyl-2′,3′-dideoxy-2′,3′-didehydro-5-methyluridine, which were separated. The stereoselectivity of the Grignard reagent's attachment to 2-deoxyfuranose 3-ulosides has been ruled by the substitute configuration at Cl. Also, the effect of the hydroxyl or OBz group configuration at C3 on the condensation stereoselectivity of 3-*C*-methyl-2-deoxyfuranosides with silylated thymine has been studied. The structure of the obtained compounds was proved by ^{1}H NMR UV, ^{13}C NMR, and CD spectroscopy, as well as elemental (C, H, N) analysis. The C2′-endo-C1′-exo conformation, the anti conformation of thymine in relation to the glycosidic bond, and the gauche$^+$ conformation in relation to the C4′–C5′ bond are characteristic for the 3′-*C*-methyl-2′-deoxythymidine structure in the crystals. 3′-*C*-Methyl-2′-deoxythymidine 5′-triphosphate was synthesized and proved to be a competitive inhibitor, with respect to dTTP, of a number of DNA polymerases, including the reverse transcriptases of human immunodeficiency virus type 1 (HIV-1) and avian myeloblastosis virus (AMV). None of the DNA polymerases examined were able to incorporate this compound into the growing DNA chain. In contrast, 3′-*C*-methylidene-2′,3′-dideoxy-5-methyluridine 5′-triphosphate was found to be incorporated at the 3′-end of the DNA chain by HIV-1 reverse transcriptase, albeit with very low efficiency. 3′-*C*-Methyl-2′-deoxy-5-methyluridine did not suppress HIV-1 replication in MT-4 cells at 500 μM while its 5′-phosphite derivative exhibited modest anti-HIV-1 activity.

Introduction

Hydroxyl group substitution at C-3′ in 2′-deoxynucleosides by other functional groups with preservation of the hydrogen atom at the C-3′ position has been predominantly used as an approach to synthesize compounds with potential antiretroviral activity.[1] Substitution of alkyl substituents for the hydrogen atom in the C-3′ position with preservation of the 3′-hydroxyl appeared to be very interesting, since these compounds could be potentially involved in polynucleotide chain elongation during DNA synthesis. This alkyl group would decrease the 3′-hydroxyl nucleophilicity in phosphoester bond formation, which in turn would lead to the appearance of new substrate properties. The inhibitory effect of 3′-*C*-methylribonucleosides on vaccinia virus replication is due to inhibition of viral RNA synthesis by the corresponding 5′-triphosphates.[2] 3′-*C*-Methyluridine 5′-triphosphate has also been shown to be a DNA chain terminating substrate for *E. coli* RNA polymerase.[3]

A general scheme of the synthesis of 3′-branched 2′-deoxythymidine is presented in this paper. The Grignard reaction with 3′-keto-2′-deoxynucleosides[4] and 2′-*O*-tosylribonucleosides[5,6] has been used earlier to obtain exclusively 3′-*C*-methyl-2′-deoxynucleosides with the D-threo configuration. The Grignard reaction with protected 2-*O*-tosylribofuranoses resulted in a mixture of 3′-*C*-methyl-D-erythro and 3′-*C*-methyl-D-threo sugar derivatives,[7] which were not transformed to the corresponding

† Moscow Medical Sechenov Academy.
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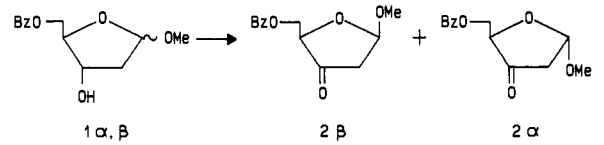
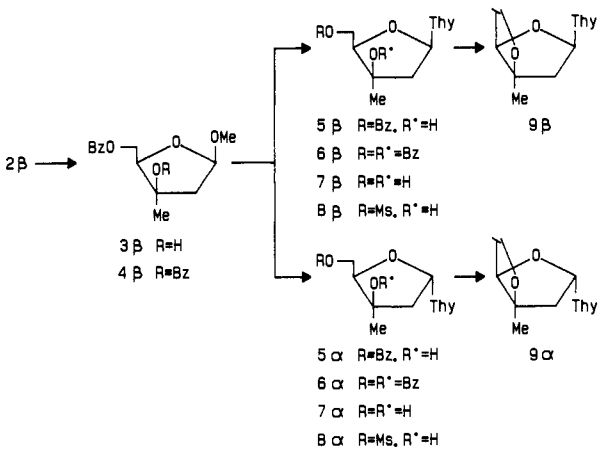
Scheme I



Scheme II



nucleosides. Also, an 18-step 3′-*C*-methyl-2′-deoxyuridine synthesis from D-glucose[8] has been recently described.

Results

Synthesis. The starting compound 2-deoxy-D-ribose was transformed in two steps to yield the anomer mixture of 1-methyl 5-*O*-benzoyl-D-ribofuranosides (**1α,β**). The individual anomers were then separated on silica gel and oxidized to the corresponding ketones **2α** and **2β** (Scheme I). The reaction of **2β** with MeMgI resulted in the formation of D-threo isomer **3β** only (Scheme II). The complete stereoselectivity of the Grignard reagent's attachment can be explained by the stereocontrolling effect of the cis-located OMe and CH_2OBz groups at C1 and C4, which leads to the **2β**·MeMgI attachment from the sterically available site. The partially protected sugar **3β** was further transformed in low yield into the anomer mixture of the thymidine nucleosides **5**. Without intermediate protection of the 3-hydroxyl group,[9] predominantly the **5β** anomer was formed, irrespective of the catalyst $CF_3SO_3SiMe_3$ or $SnCl_4$ (Table I).

Deblocking of the reaction mixture **5α + 5β** and subsequent chromatography on silica gel permitted the isolation of microquantities of the target nucleoside **7β** and nucleoside **7α**. Better results were obtained if **3β** was preliminarily converted into the dibenzoate **4β** by heating with benzoyl chloride in *N*-methylimidazole, since the conventional methods of benzoylating carbohydrate tertiary hydroxyl groups[10,11] resulted in their almost complete destruction. Dibenzoate **4β** was then transformed into the nucleosides **6β** and **6α** under the same reaction conditions as used for **3β**. After hydrolysis of **6β** and **6α**, the mixture of **7α** and **7β** was isolated in pure state.

When $SnCl_4$ was used as a catalyst, the main glycosylation product was the α-nucleoside **6**. $CF_3SO_3SiMe_3$ appeared to be a nonselective catalyst, a mixture of the D-threo nucleosides **6α** and **6β** anomers in approximately equal proportion being formed (Table I). Deblocking followed by chromatography on silica gel permitted the isolation of sufficient quantities of the target nucleoside **7β** and its α-anomer.

The configuration of the 3′-hydroxyl group in the D-threo nucleoside **7β**[4] described earlier was chemically proved (Scheme II). Nucleosides **7β** and **7α** were selectively mesylated at the 5′-position into **8β** and **8α**. Their treatment with an aqueous alcohol solution of NaOH led to closing the 3′,5′-O-anhydrocycle to form the compounds **9α** and **9β**, as indicated by the cis location of the 3′-OH and 5′-CH_2OH groups.

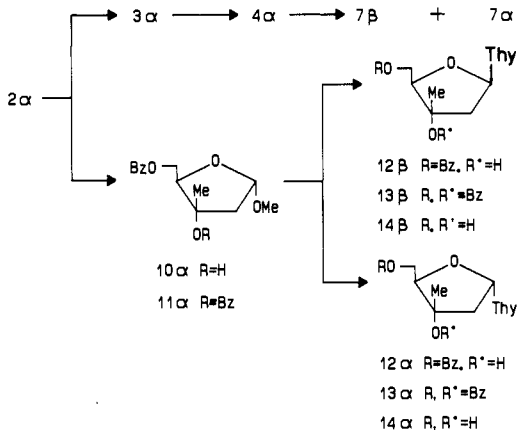
Reaction of the ketone **2α** with MeMgI yielded a mixture of the D-erythro and D-threo isomers **10α** and **3α**, which could be separated chromatographically on silica gel (Scheme III). The formation of a mixture of diastereomers with the prevalence of the D-erythro isomer is related to the trans location of the OMe and CH_2OBz groups. Unprotected sugars **10α** and **3α** were further converted into the dibenzoates **11α** and **4α**, respectively, by heating with benzoyl chloride in *N*-methylimidazole.

The D-threo sugars **3α** and **4α** obtained were converted into nucleosides **5α,β** and **6α,β** similarly to compounds **3β** and **4β**. The stereoselectivity of the glycosylation reaction was preserved: while $SnCl_4$ was used as catalyst, the predominant formation of β-anomer **5** from entirely protected sugar **3α** and the predominant formation of α-anomer **6** from dibenzoate **4α** was observed (Table I).

Table I. Ratio between α- and β-Nucleoside Anomers (**5**, **6** and **12**, **13**) under Glycosylation (^1H NMR Spectroscopy Data)

	ratio, % of the sum; catalyst:			
	$CF_3SO_3SiMe_3$		$SnCl_4$	
initial compound	β-anomer	α-anomer	β-anomer	α-anomer
incompletely protected sugars				
D-threo				
3β	83	17	84	16
3α	82	18	83	17
D-erythro				
10α	20	80	19	79
completely protected sugars				
D-threo				
4β	60	40	15	85
4α	59	41	15	85
D-erythro				
11α	42	58	85	15

Scheme III



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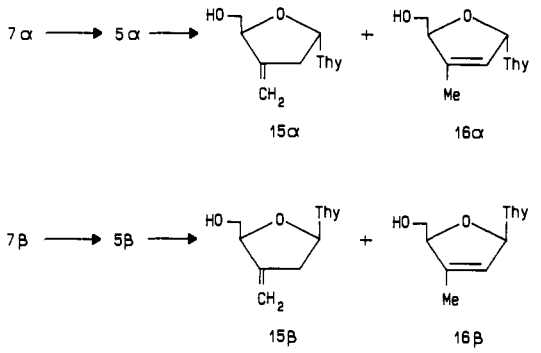
Scheme IV



Incompletely protected D-erythro sugar **10α** was allowed to react with 2,4-bis(trimethylsilyl)thymine [$(Me_3Si)_2$Thy] in the presence of $SnCl_4$ or $CF_3SO_3SiMe_3$, which resulted in the formation of α-anomer **12** (Table I). The yield of nucleosides from incompletely protected sugars under the conditions used did not exceed 10%.

To synthesize sufficient quantities of 3'-*C*-methyl-2'-deoxythymidine **14β**, the sugar **10α** was benzoylated into **11α** in *N*-methylimidazole with subsequent condensation with $(Me_3Si)_2$Thy, $SnCl_4$ being used as catalyst. Under these experimental conditions, predominantly the nucleoside **13β** anomer was formed (Table I). Deblocking of the reaction mixture and subsequent crystallization from methanol permitted the target nucleoside **14β** to be obtained with acceptable yield.

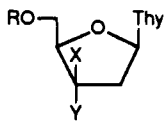
3'-*C*-Methyl-2'-deoxynucleosides appeared to be suitable starting compounds to synthesize 3'-branched unsaturated 2',3'-dideoxynucleosides: 3'-C-methylidene-2',3'-dideoxy-5-methyluridine (**15β**)[12,13] and 3'-*C*-methyl-2',3'-dideoxy-2',3'-didehydro-5-methyluridine (**16β**).[12,14,15] Parenthetically, 3'-*C*-methylidene-2'-3'-dideoxycytidine has been found inhibitory to human leukemic, adenomacarcinomatous, and carcinomatous cells.[13]

To synthesize 3'-branched unsaturated 2',3'-dideoxynucleosides, the nucleosides **7β** and **7α** were converted to the 5'-monobenzoates **5β** and **5α** (Scheme IV). Reaction with thionyl chloride yielded two products, identified as the 5'-*O*-benzoates of 3'-*C*-methylidene-2',3'-dideoxy-5-methyluridine and 3'-*C*-methyl-2',3'-dideoxy-2',3'-didehydro-5-methyluridine. Upon deblocking, the β-anomers of nucleosides **15** and **16** were obtained.

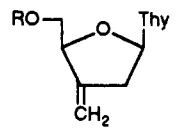
The nucleosides **16** appeared to be hydrolytically unstable and were completely destroyed in aqueous solution during 24 h with the thymine elimination. 2',3'-Dideoxy-2',3'-didehydro-5-methyluridine is also known to be hydrolytically unstable under acidic conditions.[16] The hydrolytical stability of its 3'-C-methylated analog is significantly decreased, probably due to the positive inductive effect of the 3'-*C*-methyl group and the simultaneous presence of the double bond.

The nucleosides that were chosen for further investigation of their interaction with DNA polymerases and reverse transcriptases were transformed into 5'-triphosphates (**17**, **18**, and **21**) by treatment with $POCl_3$ in the presence of triethyl phosphate and then with tetrabutylammonium pyrophosphate in the presence of tributylamine[17] (for **7β**, **14β**), or by the triazole method[18] (for **15β**).

The nucleoside 5'-phosphites are less toxic and more selective inhibitors of virus replication in some cases.[18,19] Therefore, nucleosides **7β** and **14β** were transformed into 5'-phosphites by treatment with PCl_3 in triethyl phosphate, since attempts to obtain these compounds by the conventional methods with the application of H_3PO_3 in the presence of *N*,*N*'-dicyclohexylcarbodiimide[19,20] led to a multicomponent mixture of products scarcely amenable to separation.



17: X = Me, Y = OH, R = $H_4P_3O_9$
18: X = OH, Y = Me, R = $H_4P_3O_9$
19: X = Me, Y = OH, R = H_2PO_2
20: X = OH, Y = Me, R = H_2PO_2



21: R = $H_4P_3O_9$

Physicochemical Properties. The structure of the obtained compounds was proved by ^{1}H NMR, ^{13}C NMR, UV, and CD spectroscopy as well as elemental C,H,N analysis and X-ray analysis of the nucleoside **14β**. The presence of the two separated ABX systems (5'$_a$,5'$_b$,4' and 2'$_a$,2'$_b$,1') is characteristic of the ^{1}H NMR spectra of the 3'-*C*-methyl-2'-deoxynucleosides. Chemical shift values are given in Tables II and III, and spin–spin coupling constants (SSCC) are represented in Table IV.

According to the chemical shift values, the anomers could be assigned to either the α- or β-series. For the anomer sugars **1–3**, the proton signals at C1 and the OMe groups of the α-anomers were more downfield than those of the β-anomers. This dependence was inverse for the nucleosides, and the proton signal at C1' as well as H-6 in the β-anomers was more downfield in all cases. With each anomer pair, the $J_{1',2'a}/J_{1',2'b}$ ratio was larger for the β-anomer than for the α-anomer.

The ^{1}H NMR spectroscopy data enabled us to prove the furanose form of the methylated nucleosides obtained. From the spectra in DMSO-d_6 it appeared that the proton signal of the tertiary hydroxyl in **7β** was a singlet (δ 5.28) and that of the primary hydroxyl was a triplet (δ 5.0, $J <$ 0.5 Hz). A similar picture was observed for **14β**.

The ^{1}H NMR spectra of the unsaturated nucleosides **15β** and **16β** were more complicated. The presence of the methylene group proton pseudo quartet signals in the region of 5.3–5.4 ppm and the extremely complex system of two separated protons at C2' (a total of more than 40 lines) is characteristic of the compound **15β**. The specific

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Table II. Chemical Shifts in ^{1}H NMR Spectra of 2′-Deoxyfuranosides and Their 3′-C-Methylated Derivatives

	chemical shift, ppm ($CDCl_3$/TMC)							
compd	H-1′	H-5′a	H-5′b	H-4′	OMe s	H-2′a	H-2′b	3-C-Me s
1β[a]	5.09 dd	4.56 dd	4.48 dd	4.18 m	3.26	2.27 qd	2.11 qd	
1α[b]	5.10 dd	4.37 m	4.37 m	4.37 m	3.39	2.19 qd	2.05 qd	
2β	5.36 dd	4.65 dd	4.44 br d	4.42 dd	3.38	2.81 dd	2.51 dd	
2α	5.37 d	4.75 dd	4.45 dd	4.26 br t	3.46	2.67 dd	2.47 d	
3β	5.04 m	4.64 dd	4.46 dd	4.12 dd	3.39	2.09 m	2.09 m	1.43
3α	5.16 dd	4.67 dd	4.44 dd	4.07 br t	3.38	2.37 dd	2.00 dd	1.44
4β	5.09 dd	4.82 dd	4.60 dd	4.32 dd	3.35	2.89 dd	2.46 dd	1.34
10α	5.12 dd	4.43 dd	4.22 dd	4.38 br t	3.40	2.10 m	2.10 m	1.38

[a] H-3′, 4.38 m. [b] H-3′, 4.24 m.

Table III. Chemical Shifts in ^{1}H NMR Spectra of 3′-Methyl-2′-deoxynucleosides

	chemical shift, ppm										
compd	NH s	H-6 q	H-1′ dd	H-5′a dd	H-5′ b dd	H-4′ dd	H-2′a dd	H-2′b dd	Me (C-5) d	Me-3′-C s	solvent
6β	8.89	7.28	6.27	4.94	4.88	4.22	3.06	2.73	1.91	1.87	$CDCl_3$
6α	9.04	7.10	6.05	4.71 dd	4.71 dd	4.90 dd	3.53	2.50	1.91	1.87	$CDCl_3$
7β		7.87	6.09	3.94 m	3.94 m	3.94 m	2.62	2.19	1.89	1.42	D_2O[b]
7α		7.44	6.07	3.78 dd	3.62 dd	4.13 dd	2.42	2.14	1.78	1.33	D_2O[b]
9β	9.16	8.05	6.66	4.69 dd	4.59 d	4.08 dd	2.53	2.42	1.94	1.68	$CDCl_3$
9α	9.64	7.15	6.70	4.38 dd	4.75 m	4.75 m	2.57	1.67	1.95	1.69	$CDCl_3$
13β	9.43	a	6.27	4.92 dd	4.70 m	4.70 m	3.40	2.20	1.98	1.66	$CDCl_3$
14β		7.76	6.27	3.81 dd	3.70 dd	4.01 dd	2.63	2.18	1.89	1.41	D_2O[b]

[a] The signal is exceeded by solvent signal. [b] DSS was used as internal standard.

Table IV. SSCC of 2′-Deoxy-3′-C-methylfuranosides and Their Nucleosides

	J, Hz						
compd	1′,2′a	1′,2′b	2′a,2′b	4′,5′a	4′,5′b	5′a,5′b	$J_{1',2'b}/J_{1',2'a}$
1β[a]	1.5	4.6	−13.4	4.5	4.3	−10.1	3.1
1α[b]	4.4	0.5	−13.7	-	-	-	0.1
2β	5.6	1.2	−18.1	2.7	1.0	−11.3	0.2
2α	5.1	-	−18.3	2.9	4.0	−12.1	0.0
3β	-[c]	-	-	4.0	7.0	−11.9	-
3α	3.5	6.1	−14.3	4.8	6.2	−12.0	1.7
4β	2.4	6.0	−14.5	4.5	7.3	−12.0	2.5
10α	2.0	3.5	-	4.2	3.9	−11.0	1.8
6β	3.4	7.7	−15.9	3.8	6.7	−12.2	2.3
6α	6.8	7.7	−14.8	3.5	7.0	−12.0	1.1
7β	2.8	8.1	−15.0	-	-	-	2.9
7α	6.1	7.6	−14.5	3.5	7.5	−12.4	1.2
9β	3.0	8.0	−15.9	4.0	1.7	−8.4	2.7
9α	8.4	5.3	−13.8	4.6	-	−10.4	0.6
13β	5.2	9.0	−14.5	-	3.5	−14.5	1.7
13α	5.8	3.9	...	...	...	...	0.7
14β	5.7	9.3	−13.6	3.6	6.2	−12.3	1.6
14α	5.8	3.3	...	...	...	...	0.6

[a] $J_{2'a,3'}$ = 7.0 Hz, $J_{2'b,3'}$ = 6.2 Hz. [b] $J_{2'a,3'}$ = 6.1 Hz, $J_{2'b,3'}$ = 1.6 Hz. [c] -: SSCC absent. [d] ...: SSCC not obtained, because in the mixture another anomer prevailed.

signals of two protons at C2′ in the region of 1.5–3.5 ppm were absent in the nucleoside 16β spectra, and there appeared a multiplet of proton 2′ in the region of the double bond at 5.7. The spectral characteristics of these compounds are given in the Experimental Section.

The spatial structure of the 3′-C-methyl-2′-deoxy-5-methyluridine molecules was definitively determined by X-ray analysis.[21] The investigated crystals were grown from a saturated solution of 14β in methanol and did not contain the solvent molecules.

The C2′-endo-Cl′-exo conformation of the furanose cycle with displacement of the C2′ atom from the plane C3′C4′O4′ in the direction of the N1 and C5′ atoms on a

(21) Bochkarev, A. V.; Gurskaya, G. V.; Zdanov, A. S.; Kazmina, E. M.; Fedorov, I. I. The molecular and crystal structure of 1-(3′-C-methyl-2′-deoxy-β-D-ribofuranosyl)thymine. *Bioorg. Khim.* 1991, *17*, 1094–1100.

Scheme V. Primer–Template Complex

```
          10        20        30        40
3'-GGGTCAGTGCTGCAACATTTTGCTGCCGGTCACGGTTCGA
5'-CCCAGTCACGACGT
-------------------------------------------
direction of primer elongation

          50        60        70        80        90
ACCCGACGTCCAGCTGAGATCTCCTAGGGCCCGCTCGAGCTTAAGCATTAGTA
```

distance of 0.521 Å was found for **14β** molecules in crystal. Displacement of the C1′ atom to the opposite side of the sugar plane on a distance of 0.063 Å was characteristic of **14β**. The molecular conformation in relation to the exocyclic C4′–C5′ bond is gauche. Natural thymidine has the C4′–C5′-trans conformation and the C2′-endo-C3′-exo conformation, with the C3′ and C2′ atoms moving off the carbohydrate cycle plane at a distance of 0.42 and 0.18 Å, respectively.[22] **14β** is in anti conformation around the N-glycoside bond, as is 2′-deoxythymidine.

On the whole the conformation of **14β** is very similar to that of 3′-*C*-methylcytidine,[23] which, in its 5′-triphosphate form, is a substrate and efficient terminator of the RNA polymerase reaction. From a comparison of the two structures the following general patterns of spatial organization of the 3′-C-methylated pyrimidine nucleosides, not depending on the sugar and nucleic base nature, can be established: first, preferential C2′-endo conformation of the furanose ring resulting in the equatorial position of the pyrimidine base; second, anti conformation around the glycoside bond, the gauche$^+$ conformation in relation to the exocyclic C4′–C5′ bond; third, elongation of the C3′–O3′ bonds accompanying the conversion from the secondary (natural nucleosides) to the tertiary 3′-hydroxyl group (3′-*C*-methyl derivatives).

Biochemical Properties. Compounds **17**, **18**, and **21** were evaluated for their capacity to serve as substrates for the following DNA polymerases: DNA polymerase I (Klenow fragment) from *E. coli* DNA polymerase α and ε from human placenta, DNA polymerase β from rat liver, terminal deoxynucleotidyl transferase from calf thymus, as well as the reverse transcriptases from the avian myeloblastosis virus (AMV), and human immunodeficiency virus type 1 (HIV-1). A model consisting of the DNA template of the M 13 mp10 phage and the deoxytetradecanucleotide oligomer complementary to this template (Scheme V) was used. Compounds **17** and **18** were not incorporated into the growing DNA chain, although they inhibited DNA chain synthesis. Both compounds acted as competitive inhibitors with respect to dTTP. They were inhibitory to DNA polymerase I and AMV reverse transcriptase at concentrations exceeding the dTTP concentration by 400- and 750-fold (Table V).

Compound **21** acted as a DNA chain terminator with both AMV and HIV-1 reverse transcriptases as well as DNA polymerase β. However, compound **21** was not incorporated into the growing DNA chain by DNA polymerases α, ε, or I (data not shown). From Table V it is evident that **21** has a 10-fold higher affinity for AMV reverse transcriptase than for DNA polymerase I. Figure 1 illustrates the DNA chain terminator properties of **21** for AMV reverse transcriptase. All DNA fragments terminate at the dTMP (T39, T40, T45, T51, T56, T58, etc.).

Table V. Inhibition of DNA Polymerase I and AMV Reverse Transcriptase by **17**, **18**, and **21**

	ratio of inhibitor to dTTP (mol) conferring 50% inhibn	
compd	DNA polymerase I	AMV reverse transcriptase
17	400	750
18	500	400
21	400	40

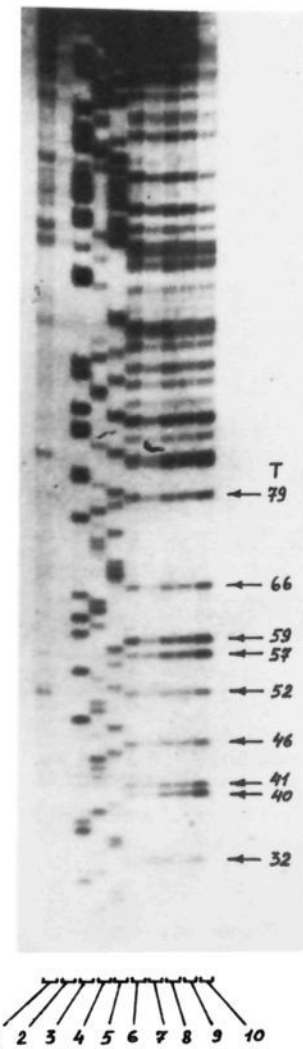

Figure 1. Autoradiography/PAGE analysis of primer elongation catalyzed by AMV reverse transcriptase in the presence of **21**. Track 1: DNA synthesis in the complete system without termination nucleotides. Track 2: identical to 1, but with subsequent chase. Tracks 3–6: control DNA synthesis in the presence of ddATP (3), ddGTP (4), ddCTP (5), or ddTTP (6). Tracks 7–10: identical to 2, but in the presence of **21** at a concentration of 200 μM (7), 400 μM (8), 800 μM (9), or 1600 μM (10).

Compound **21** was also evaluated for its inhibitory effect on terminal deoxynucleotidyl transferase. In this case, it was incorporated in the primer tetradecanucleotide chain as the 15th nucleotide, elongating it by one nucleotide residue (Figure 2). The pentadecanucleotide thus formed was not further elongated upon addition of dTTP. In contrast, the initial tetradecanucleotide present in the mixture not containing compound **21** was readily converted into a polymer upon addition of dTTP (Figure 2).

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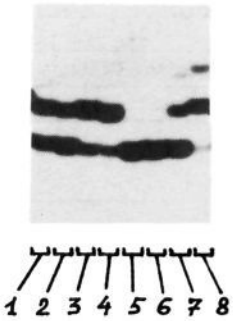


Figure 2. Autoradiography/PAGE analysis of primer elongation catalyzed by terminal deoxynucleotidyl transferase. Tracks 1 and 2: DNA synthesis in the presence of **21** at either 10 μM (1) or 100 μM (2). Tracks 3 and 4: identical to 1 and 2, but with subsequent incubation with 10 μM dTTP. Tracks 5–8: controls: [^{32}P]tetradecadeoxynucleotide only (5), [^{32}P]tetradecadeoxynucleotide and enzyme (6), [^{32}P]tetradecadeoxynucleotide and enzyme and either 3 μM dTTP (7), or 10 μM dTTP (8).

Table VI. Inhibition of HIV-1-Induced Cytopathicity in MT-4 Cells by 3′-*C*-Methyl-2′-deoxy-5-methyluridine (**14β**), Its Threo Isomer **7β**, and Their 5′-Phosphite Derivatives **19** and **20**

compd	EC_{50}[a] (μM)	CC_{50}[b] (μM)
7β	>500	>500
14β	>500	>500
19	72	>500
20	243	>500

[a] EC_{50}, 50% effective concentration, or compound concentration required to inhibit HIV-1-induced cytopathicity in MT-4 cells by 50%. [b] CC_{50}, 50% cytotoxic concentration, or compound concentration required to reduce MT-4 cell viability by 50%.

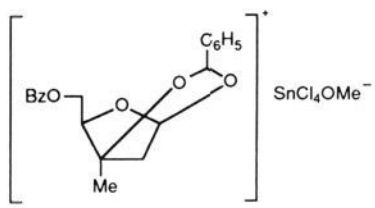
Anti-HIV-1 Activity. The ability of **14β**, **7β**, **19**, and **20** to suppress HIV-1 replication in MT-4 cell cultures was evaluated as previously described.[24] As evident from Table VI, compounds **7β** and **14β** were inactive against HIV-1. Also, **20** was virtually inactive. The 5′-phosphite D-erythro derivative **19** had some inhibitory effect on HIV-1-induced cytopathicity.

Discussion

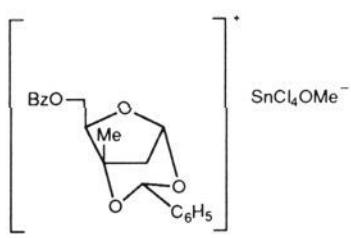
Synthesis. The synthetic scheme presented here for the 3′-*C*-alkyl-2′-deoxynucleosides makes it possible to obtain compounds with both the D-erythro and D-threo configuration. The method of protection of tertiary hydroxyl groups of sugars with low reaction capacity was based on the heating of partially protected sugars with acyl chloride in *N*-methylimidazole. With this technique significantly higher yields of 3-*C*-methyl carbohydrate dibenzoates can be obtained than with previously reported procedures.[10,11]

The data obtained when condensing 3-C-methyl sugars **3α,β**, **4α,β**, **10α**, and **11α** with $(Me_3Si)_2$Thy enabled us to discern a consistent pattern in the stereoselectivity of the glycosylation reactions. The stereoselectivity of this reaction for incompletely protected sugars **3α**, **3β**, and **10α** did not depend on the nature of the catalyst and the initial configuration at the glycoside center, but was determined by the 3-hydroxyl group configuration; the nucleosides with the cis-disposed nucleic bases and 3′-OH groups appeared to be the predominant form. In the case of the completely protected sugars **4α**, **4β**, and **11α**, the stereoorientation was determined by the catalyst used. $Me_3SiSO_3CF_3$ appeared to be nonselective and its application led to the formation of a mixture of the nucleoside α- and β-anomers in approximately equal proportions. Through the use of $SnCl_4$ the nucleoside α- or β-anomers can be obtained selectively: compounds with the trans disposition of the benzoyloxy group and the nucleic base are predominantly formed. The ratio of the nucleoside **13α** and **13β** anomers synthesized from D-erythro sugar **11α** was inversed as compared to the ratio of nucleosides **6** synthesized from the D-threo sugars **4α,β** (Table I). The high stereoselectivity of the glycosylation reactions can be explained by the stereocontrolling effect of the benzoyloxy group in the 3′-position and the formation of the intermediate protected sugar–catalyst complexes (**4α,β**·$SnCl_4$, **9α,β**·$SnCl_4$) with a different spatial structure.

The preparation of the 5′-phosphite derivatives of 3′-*C*-methyl-2′-deoxynucleosides **14β** and **8β** by treatment with PCl_3 in triethyl phosphate followed by hydrolysis is a new technique described in this paper. This technique is superior to the use of H_3PO_3 in the presence of *N*,*N*′-dicyclohexylcarbodiimide,[19] since the present technique avoids the formation of a mixture of 5′-phosphites, 3′-phosphites, 3′,5′-diphosphites of 3′-*C*-methyl-2′-deoxynucleosides as well as a number of unidentified products.



4α,β·$SnCl_4$



11α·$SnCl_4$

Activity–Conformation Relationship. 3′-*C*-Methyl-2′-deoxy-5-methyluridine 5′-triphosphate (**17**) inhibits DNA polymerases in a competitive manner with respect to dTTP, but it is not incorporated into the DNA chain and, consequently, does not form a productive complex. This property seems to be interesting. Compound **17** contains all the functional groups of dTTP and nevertheless is not utilized by the DNA polymerases as a substrate. We presume that the presence of the 3′-*C*-methyl group must have altered the conformation flexibility of **17**. Indeed, X-ray analysis data indicate that the structure of **14β** may be too rigid to allow transition of the **14β** carbohydrate residue to a flat structure. Such flattened conformation has been considered and may be needed for 2′-deoxyribonucleotides to act as substrates of DNA polymerases.[25,26] Further support for this hypothesis stems from the observation that compounds containing a

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Table VII. Analytical Data of Compounds

compd	theoretical, % C	H	N	P	formula	found, % C	H	N	P
2α	62.39	5.64			$C_{13}H_{14}O_5$	62.58	5.71		
2β	62.39	5.64			$C_{13}H_{14}O_5$	62.63	5.40		
3β	63.15	6.81			$C_{14}H_{18}O_5$	62.99	6.92		
4α	68.10	5.99			$C_{21}H_{22}O_6$	68.20	6.20		
4β	68.10	5.99			$C_{21}H_{22}O_6$	68.26	5.77		
5α	59.99	5.59	7.77		$C_{18}H_{20}N_2O_6$	60.06	5.83	7.86	
5β	59.99	5.59	7.77		$C_{18}H_{20}N_2O_6$	60.17	5.78	7.91	
7α	51.56	6.29	10.94		$C_{11}H_{16}N_2O_5$	51.39	6.06	10.81	
7β	51.56	6.29	10.94		$C_{11}H_{16}N_2O_5$	51.61	6.31	11.18	
9α	55.46	5.92	11.86		$C_{11}H_{14}N_2O_4$	55.20	5.99	11.58	
9β	55.46	5.92	11.86		$C_{11}H_{14}N_2O_4$	55.31	6.12	11.90	
10α	63.15	6.81			$C_{14}H_{18}O_5$	62.96	6.70		
11α	68.10	5.99			$C_{21}H_{22}O_6$	67.95	6.12		
14β	51.55	6.29	10.94		$C_{11}H_{16}N_2O_5$	51.77	6.02	11.10	
15β	55.46	5.92	11.76		$C_{11}H_{14}N_2O_4$	55.49	6.11	11.66	
16β[a]	55.46	5.92	11.76		$C_{11}H_{14}N_2O_4$	55.58	6.01	11.60	
19	39.17	5.98	12.46	9.18	$C_{11}H_{20}N_3O_7P \cdot NH_3$	39.01	6.16	12.30	9.04
21	37.19	6.24	11.83	8.72	$C_{11}H_{20}N_3O_7P \cdot NH_3 \cdot H_2O$	36.94	6.44	12.06	8.64

[a] Analysis should be made for freshly isolated substance because of its unstability.

flattened carbohydrate residue, such as 2',3'-dideoxy-2',3'-didehydronucleoside 5'-triphosphates,[27,28] and 2',3'-riboanhydro- and 2',3'-lyxoanhydronucleoside 5'-triphosphates,[28,29] can act as DNA chain terminators. Also, 3'-C-methylidene-2',3'-dideoxy-5-methyluridine 5'-triphosphate (21) appeared to act as a terminator substrate for some DNA polymerases, as shown in Figure 1. Reverse transcriptases (i.e. AMV or HIV reverse transcriptase) seem to be less restrictive in recognizing terminator substrates than other DNA polymerases (i.e. DNA polymerase α).[28-31]

Experimental Section

The NMR spectra were obtained on a Bruker WM-250 (250 MHz) spectrometer for ^1H and 62.8 MHz for ^{13}C at 20 °C ($CDCl_3$, CD_3OD or D_2O, internal standard TMS, t-BuOH or DSS, respectively). The ^{31}P NMR spectra were obtained with 101.3 MHz in D_2O, internal standard $(MeO)_3PO$. The accepted abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; br s, broad singlet; m, multiplet; pt, pseudotriplet; pq, pseudoquartet. The UV spectra were obtained on a Specord UV M-40 spectrometer, and the CD spectra were obtained on a Jobin Ivon dichrograph in water or methanol. The X-ray analysis was carried out on a CAD-4 difractometer. HPLC was performed on a Gilson chromatograph at 268 nm, column 4.6 × 250 mm, carrier Silasorb C-18 (5 μm), flow rate 1 mL/min. The nucleosides were isolated in the following buffers: A, 0.04 M Et_3NHHCO_3, pH 7.4; B, 50% MeCN in water, the gradient 5 → 30%, 20 min; B, 5 → 10% was also used for the nucleoside 5'-triphosphates. TLC, including the preparative TLC, was performed on Merck plates in the following systems: C, chloroform–EtOH, 97:3; D, chloroform–EtOH, 9:1. The column chromatography was performed on silica gel L (40/100) (CSFR). Analytical data are shown in Table VII.

Synthesis. Methyl 5-O-Benzoyl-2-deoxy-α-D-ribofuranoside (1α) and Its β Anomer (1β). To a solution of acetyl chloride (3 mL) in dry MeOH (250 mL) was added 2-deoxy-D-ribose (20 g, 149 mmol), and after 15 min dry pyridine (10 mL) was added. The solution was evaporated to dryness and reevaporated with pyridine (3 × 20 mL). The residue was dissolved in pyridine (50 mL), and benzoyl chloride (17.3 mL, 148.9 mmol) in dichlorethane (30 mL) was added over 1 h at −5 °C. The reaction mixture was allowed to be warmed up to 0 °C, and then it was poured into water with ice (500 mL) and extracted with chloroform (5 × 100 mL). The chloroform extract was washed with 1% HCl (5 × 100 mL), saturated $NaHCO_3$ (2 × 100 mL), and water (2 × 100 mL), dried with Na_2SO_4, and evaporated. The substance was separated on a silica gel column and eluted with EtOH in chloroform (0 → 5%). The yield as yellow syrups was 6.02 g (16%) of 1α (C: R_f 0.24) and 8.07 g (21.5%) of 1β (C: R_f 0.18). The sugar 1β was crystallized upon standing at 0 °C, mp 63–64 °C. ^{13}C NMR ($CDCl_3$) δ 1α 105.3 (C1), 84.5 (C4), 72.8 (C3), 64.5 (C5), 54.7 (OMe), 40.9 (C2); 1β 105.0 (C1), 83.6 (C4), 72.0 (C3), 65.3 (C5), 54.8 (OMe), 41.4 (C2).

Methyl 5-O-Benzoyl-2-deoxy-α-D-*glycero*-pentofuranosid-3-ulose (2α) and Its β-Anomer (2β). Dry pyridine (10 mL), acetic anhydride (5 mL), and freshly dried CrO_3 (6 g, 60.0 mmol) were dissolved in dry CH_2Cl_2 (140 mL) and stirred for 30 min. A solution of 1α or 1β (5 g, 19.8 mmol) in CH_2Cl_2 (10 mL) was added for 5 min to the first solution. After 5 min, ethyl acetate (300 mL) was added, and the solution was filtered through silica gel, evaporated to the volume of 50 mL, washed with saturated $NaHCO_3$ (50 mL), and then with water (50 mL). The organic extract was dried with Na_2SO_4, evaporated to dryness, dissolved in ethyl acetate (20 mL), kept for 1 h at 0 °C, and filtered through silica gel. The silica gel was washed with ethyl acetate (50 mL), and the combined solutions were evaporated in vacuo. Ulosides 2α or 2β (4.84 g, 97.6%) in the form of yellow syrup were obtained. The ulosides could be kept for one week only.

Methyl 5-O-Benzoyl-3-C-methyl-2-deoxy-β-D-*threo*-pentofuranoside (3β). To a solution of uloside 2β (1.65 g, 6.6 mmol) in dry ether (220 mL) at 0 °C was added for 5 min the solution of MeMgI obtained from magnesium (1.7 g, 70.8 mmol) and MeI (4.0 mL, 62.6 mmol) in ether (50 mL) with stirring. The reaction mixture was stirred for 10 min, a cooled mixture of NH_4Cl (150 g), water (360 mL), and ether (200 mL) was added, and stirring was continued for 10 min. The ether layer was separated, dried with Na_2SO_4, and evaporated to dryness. The yield was 1.45 g (83%) of a yellow syrup: ^{13}C ($CDCl_3$) δ 104.7 (C1), 85.5 (C4), 77.1 (C3), 65.0 (C5), 54.9 (OMe), 47.0 (C2), 23.0 (Me-3C).

1-(3'-C-Methyl-2'-deoxy-α-D-*threo*-pentofuranosyl)thymine (7α) and Its β-Anomer (7β). To a solution of 3β or 3α (100 mg, 0.38 mmol) in dry dichloroethane (4 mL) were added 2,4-bis(trimethylsilyl)thymine (0.5 mL, 1.82 mmol) and $CF_3SO_3SiMe_3$ (0.5 mL, 1.96 mmol), and the mixture was boiled for 30 min, then cooled to room temperature, stirred for 30 min with saturated $NaHCO_3$ (10 mL), and filtered through silica gel. Silica

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gel was washed with water, $NaHCO_3$ (10 mL), and dichloroethane (20 ml). Organic extracts were dried with Na_2SO_4 and evaporated. The residue was dissolved in 0.5 M solution of MeONa in MeOH (12 mL) and stirred for 1 h at 20 °C. Then MeOH (5 mL) and Dowex-50 (H^+) were added to adjust the pH to 5. The mixture was filtered, the residue on the filter was washed with MeOH (5 mL) and water (5 mL), and the filtrates were evaporated in vacuo. The substances were separated by preparative TLC in the D system (two developments), and after elution with water, freeze-dried **7β** (12.5 mg, 12.9%; D-R_f 0.35) and **7α** (2.6 mg, 2.7%; D-R_f 0.23) were obtained: ^{13}C NMR (D_2O) δ **7α** 167.0 (C2), 152.1 (C4), 138.3 (C6), 111.8 (C5), 88.3 (C1′), 86.8 (C4′), 79.0 (C3′), 60.8 (C5′), 46.8 (C2′), 22.5 (Me-C3′), 12.1 (Me-C5); **7β** 167.1 (C2), 152.2 (C4), 139.7 (C6), 111.1 (C5), 88.3 (Cl′), 85.2 (C4′), 79.2 (C3′), 60.8 (C5′), 46.8 (C2′), 23.9 (Me-C3′), 12.1 (Me-C5); CD spectra (MeOH) **7α** λ_{max} 273 nm ($\Delta\epsilon$ = −1.1), **7β** λ_{max} 272 nm ($\Delta\epsilon$ = +1.3).

Methyl 3,5-Di-*O*-benzoyl-3-*C*-methyl-2-deoxy-α-D-*threo*-pentofuranoside (4α), Its β-Anomer (4β), and Methyl 3,5-Di-*O*-benzoyl-3-*C*-methyl-2-deoxy-α-D-*erythro*-pentofuranoside (11α). **3α**, **3β**, or **10α** (0.48 g, 1.80 mmol) were dissolved in *N*-methylimidazole (4.3 mL), benzoyl chloride (0.26 mL) was added, and the mixture was heated at 150 °C for 10 min with stirring. The reaction mixture was cooled to 20 °C, again benzoyl chloride (0.26 mL) was added, and the mixture was heated at 150 °C for 10 min. Then cooling, benzoyl chloride addition, and heating was repeated for the third time. A total of 0.78 mL (6.71 mmol) benzoyl chloride were added. On cooling, the reaction mixture was poured into water with ice (10 g) and extracted with chloroform (3 × 5 mL). The chloroform extract was washed with 1% HCl (2 × 5 mL), saturated $NaHCO_3$ (2 × 5 mL), and water (2 × 5 mL), dried with Na_2SO_4, and evaporated. The substances were isolated on a silica gel column with the chloroform in hexane (50 → 100%) as the eluent. **4α** (0.42 g, 63%) or **4β** (0.36 g, 54%) or **11α** (0.42 g, 63%) was obtained in the form of a yellow syrup.

1-(3′-*C*-Methyl-2′-deoxy-α-D-*threo*-pentofuranosyl)thymine (7α) and Its β-Anomer (7β). Method A. Dibenzoate **4β** or **4α** (120 mg, 0.32 mmol) were dissolved in dichloroethane (3 mL), 2,4-bis(trimethylsilyl)thymine (0.25 mL, 0.91 mmol) was added, then $SnCl_4$ (0.25 mL, 2.14 mmol) in dichloroethane (0.3 mL) was added, and the mixture was boiled for 15 min. After cooling, saturated $NaHCO_3$ (10 mL) was added, and then the procedure was continued as described for the synthesis of nucleosides **7α,β** from sugars **3β** or **3α**. After separation of the anomers **7** by preparative TLC in system D, 8 mg (9.6%) of **7β** and 43 mg (52%) of **7α** were obtained.

Method B. 2,4-Bis(trimethylsilyl)thymine (1 mL, 3.64 mmol) was added to **4β** or **4α** (530 mg, 1.43 mmol) in dichloroethane (10 mL), then a solution of $CF_3SO_3SiMe_3$ (0.50 mL, 1.96 mmol) in dichloroethane (3 mL) was added, and the mixture was boiled for 1 h. The mixture was then cooled and, after addition of saturated $NaHCO_3$ (15 mL), stirred for 30 min and filtered through silica gel. The residue was washed with saturated $NaHCO_3$ (5 mL) and then with dichloroethane (15 mL). The organic layer was washed with water (2 × 5 mL), dried with Na_2SO_4, and evaporated. The resulting syrup was treated with 0.5 M MeONa in MeOH (6 mL), allowed to stand for 1 h at 20 °C, diluted with MeOH (30 mL), neutralized with Dowex-50 (H^+) to adjust the pH to 5, and filtered. Then it was washed with water (15 mL) and methanol (15 mL), concentrated to make up the volume to 10 mL, and extracted with chloroform (5 × 1 mL). The aqueous layer was evaporated, and 320 mg (87%) of a mixture of **7α,β** were obtained. After separation with preparative TLC (as described in the synthesis of **7α,β**) and evaporation, 126 mg (34.2%) of **7β** and 174 mg (47.3%) of **7α** were obtained.

1-(3′-*C*-Methyl-2′-deoxy-3′,5′-*O*-anhydro-α-D-*threo*-pentofuranosyl)thymine (9α) and Its β-Anomer (9β). Mesyl chloride (0.045 mL, 0.58 mmol) was fluxed into solution of nucleoside **7β** or **7α** (74 mg, 0.29 mmol) in dry pyridine (0.5 mL). The solution was stirred for 15 min at 20 °C, saturated $NaHCO_3$ (1 mL) was added, and the mixture was evaporated to dryness. The residue was extracted with chloroform (10 × 3 mL), and the solution was filtered, evaporated, and reevaporated with toluene (3 × 3 mL). A solution of 0.1 M NaOH (0.3 mL), water (0.7 mL), and ethanol (3 mL) was added to the residue, and the mixture was boiled for 15 min. After cooling, the reaction mixture was neutralized with Dowex-50 (H^+) to adjust the pH to 6 and filtered. The residue on the filter was washed with ethanol (5 mL) and water (5 mL). The filtrate was evaporated, benzene (3 mL) and dry EtOH (0.5 mL) were added to the residue, and the mixture was evaporated. The substances were isolated by preparative TLC in the C system. The product was eluted from silica gel with chloroform–ethanol (1:1), the solutions were evaporated and the residues were extracted with chloroform (5 mL) and evaporated: yield of **9α** 53 mg (76.4%) (D-R_f 0.74) and **9β** (D-R_f 0.81).

Methyl 5-*O*-Benzoyl-3-*C*-methyl-2-deoxy-β-D-*threo*-Pentofuranoside (3α) and Methyl 5-*O*-Benzoyl-3-*C*-methyl-2-deoxy-α-D-*erythro*-pentofuranoside (10α). **2α** (1.65 g, 6.6 mmol) was dissolved in ether (220 mL), and then the procedure was followed as described for the synthesis of compound **3β**. The mixture of the **3α** and **10α** diasteromers was separated on the silica gel column in the C system. **3α** (0.55 g, 31.2%) (C-R_f 0.27) and **10α** (0.90 g, 51.2%) (C-R_f 0.36) were obtained: ^{13}C NMR ($CDCl_3$) δ **10α** 103.8 (Cl), 81.7 (C4), 77.0 (C3), 63.1 (C5), 55.3 (OMe), 49.2 (C2), 21.7 (Me-C_3).

1-(3′-*C*-Methyl-2′-deoxy-α-D-*erythro*-pentofuranosyl)thymine (14α) and Its β-Anomer. Method A. **10α** (100 mg, 0.38 mmol) was dissolved in dry dichloroethane (4 mL), and then the procedure was followed as described for the synthesis of **7α,β**. As a result, 20 mg of a mixture (20.7%) containing 80% of **14α** and 20% of **14β** was obtained (^1H NMR spectroscopy data). They were not separated into the individual anomers.

Method B. To the solution of dibenzoate **11α** (120 mg, 0.32 mmol) in dichloroethane (3 mL) were added 2,4-bis(trimethylsilyl)thymine (0.25 mL, 0.91 mmol) and $SnCl_4$ (0.25 mL, 2.14 mmol) in dichloroethane (0.3 mL) and then followed up as described for the synthesis of the nucleosides **7α,β** from sugars **3β** or **3α**. After deblocking, 60 mg (73.2%) of crude mixture was obtained. Crystallization of this mixture from MeOH (0.2 mL) yielded 30 mg (36.6%) of **14β**: mp 182–183 °C (D-R_f 0.20); ^{13}C NMR (D_2O) δ 167.0 (C2), 152.0 (C4), 138.2 (C6), 112.0 (C5), 89.1 (C1′), 85.6 (C4′), 79.1 (C3′), 62.0 (C5′), 44.4 (C2′), 21.8 (Me-C3′), 12.3 (Me-C5′).

Method C. To a solution of **11α** (120 mg, 0.32 mmol) in dichloroethane (3 mL) were added 2,4-bis(trimethylsilyl)thymine (0.25 mL, 0.91 mmol) and $CF_3SO_3SiMe_3$ (0.25 mL, 1.3 mmol) in dichloroethane (1 mL), and the mixture was boiled for 1 h. Saturated $NaHCO_3$ (15 mL) was added, the mixture was stirred for 30 min and filtered through silica gel, and the adsorbent was washed with saturated $NaHCO_3$ (5 mL) and then with dichloroethane (15 mL). Organic layer was washed with water (2 × 5 mL), dried with Na_2SO_4, and evaporated. The resulting syrup was stirred for 1 h with 0.5 M MeONa in MeOH (6 mL) at 20 °C, MeOH (30 mL) was added, and the solution was neutralized with Dowex-50 (H^+) to adjust the pH to 5 and filtered. The residue on the filter was washed with a mixture of water (15 mL) and MeOH (15 mL), and the filtrate was concentrated to a volume of 10 mL and extracted with chloroform (5 × 1 mL). The solution was evaporated to dryness, and a mixture of **14α** and **14β** (71 mg, 87%) was obtained. According to ^1H NMR, the ratio of **14α** to **14β** was 58:42.

1-(5′-*O*-Benzoyl-3′-*C*-methyl-2′-deoxy-α-D-*threo*-pentofuranosyl)thymine (5α) and Its β-Anomer (5β). To a solution of **7α** or **7β** (386 mg, 1.5 mmol) in dry pyridine (3 mL) was added benzoyl chloride (0.27 mL, 2.3 mmol). The reaction mixture was stirred for 10 min, saturated $NaHCO_3$ (10 mL) was added, and the solution was evaporated to dryness. The residue was extracted with chloroform (5 × 5 mL), the solution was evaporated, and the residue was reevaporated five times with toluene–EtOH (5:1, 5 mL). **5α** or **5β** (512 mg, 95%) was obtained as a colorless syrup.

3′-*C*-Methylidene-2′,3′-dideoxy-5-methyluridine (15β) and 3′-*C*-Methyl-2′,3′-dideoxy-2′,3′-didehydro-5-methyluridine (16β). A solution of **5β** (200 mg, 0.56 mmol) in $SOCl_2$ (2 mL) was boiled for 2 min, cooled to −20 °C, and diluted with cold saturated $NaHCO_3$ (15 mL). The mixture was evaporated, the residue was extracted with chloroform (5 × 5 mL), and the solution was evaporated. Saturated NH_3 in MeOH (5 mL) was added to residue, and the solution was allowed to stand for 24 h at 20 °C and evaporated. The mixtures of nucleosides **15β**, **16β** were separated by preparative HPLC and eluted with 10% MeOH in water. The fractions containing the nucleosides were immediately evaporated at room temperature. **15β** (15 mg, 11%) and **16β** (45 mg, 34%) were obtained in the form of colorless syrups: ^1H NMR spectra (CD_3OD) δ **15β** 8.01 q (1H, H6), 6.34 pt (1H, $J_{1'2'}$ = 6.8 Hz, H1′), 5.43 pq (1 H, J = 2.2 Hz, 4.4 Hz, CH_2-C3′), 5.31 pq (1H,

CH_2-C3'), 4.73 m (1H, H4'), 4.09 dd (1H, $J_{5'a,4'}$ = 2.9 Hz, $J_{5'a,5'b}$ = 12.1 Hz, H5'a), 3.96 dd (1H, $J_{5'b,4'}$ = 4.0 Hz, H5'b), 3.28 m (1H, $J_{2'a,2'b}$ = 11.4 Hz, H2'a), 2.94 m (1H, H2'b), 2.09 d (3H, Me-C5); 16β 8.02 q (1H, H6), 7.05 m (1H, H1'), 5.72 m (1H, H-2'), 4.81 m (1H, H4'), 4.09 m (2H, H5'a, H5'b), 2.12 q (Me-C3', 3H), 2.04 d (Me-C5, 3H).

1-(3'-C-Methyl-2'-deoxy-β-D-*erythro*-pentofuranosyl)thymine 5'-Triphosphate (Ammonium Salt) (17). To the solution of 14β (25.7 mg, 0.1 mmol) in triethyl phosphate (0.4 mL) was added $POCl_3$ (18 μL, 0.19 mmol) at 0 °C, and the solution was allowed to stand for 48 h at 0 °C. Then a mixture of 0.5 M solution of tetra(tributylammonium) pyrophosphate in DMF (0.55 mL) and tributylamine (0.14 mL) at 0 °C was added. The reaction mixture was stirred for 30 min at 20 °C and then neutralized with 1 M $Et_3NH_2CO_3$ to adjust the pH to 7.5, evaporated at 20 °C, and reevaporated with 5% aqueous EtOH. The solid in 300 mL of water was applied onto a DEAE-column (DE-32, Whatman, HCO_3^- form, 10 × 0.5 cm). The substances were eluted with a linear gradient of NH_4HCO_3, pH 7.5 (0 → 0.5 M; 2 L). 17 was eluted with 0.3 M buffer. The fractions containing 17 were evaporated at 20 °C and reevaporated with 5% EtOH (5 × 10 mL). After freeze-drying, 17 (29 mg, 58.5%) was obtained: ^{1}H NMR (D_2O, t-BuOH) δ 7.81 d (1H, H6), 6.25 dd (1H, $J_{1',2'a}$ = 5.5. Hz, $J_{1',2'b}$ = 9.5 Hz, 1H'), 4.07–4.21 m (3H, H4' + 2H5'a,b), 2.21–2.60 m (2H, H2'a,b), 1.93 d (3H, Me-C5), 1.43 s (3H, Me-C3'); ^{31}P NMR (D_2O) δ −9.16 d (J_{P^α,P^β} = 19.1 Hz, P^α), −11.65 d (J_{P^γ,P^β} = 18.9 Hz, P^γ), −22.41 t (J_{P^β,P^α} = J_{P^β,P^γ} = 19.06 Hz, P^β).

1-(3'-C-Methyl-2'-deoxy-β-D-*threo*-pentofuranosyl)thymine 5'-Triphosphate (Ammonium Salt) (18). The compound was obtained from 7β using the same conditions as for the synthesis of 17β. 18: yield 13 mg (26.1%); ^{1}H NMR (D_2O, t-BuOH) δ 7.88 q (1H, H6), 6.11 dd (1H, $J_{1',2'a}$ = 3.0 Hz, $J_{1',2'b}$ = 8.0 Hz, 1H'), 4.05–4.26 m (3H, H4' + 2H5'a,b), 2.25–2.57 m (2H, H2'a,b), 1.95 d (3H, Me-C5), 1.45 s (3H, Me-C3'); ^{31}P NMR (D_2O) δ −9.20 d (J_{P^α,P^β} = 18.8 Hz, P^α), −11.67 d (J_{P^γ,P^β} 18.9 Hz, P^γ), −22.16 t (J_{P^β,P^α} = J_{P^β,P^γ} = 19.21 Hz, P^β).

1-(3'-C-Methyl-2'-deoxy-β-D-*erythro*-pentofuranosyl)thymine 5'-Phosphite (Ammonium Salt) (19). A solution of 14β (25.7 mg, 0.1 mmol) in triethyl phosphate (375 μL) was cooled to 0 °C, PCl_3 (20 μL, 0.23 mmol) was added, and the mixture was stirred for 24 h at 0 °C, neutralized with 1 M $Et_3NH_2CO_3$ to adjust the pH to 7.5, and followed up as described for the synthesis of 17. The compound was isolated using 0.25 M NH_4HCO_3 and eluted with 0.07 M buffer. After freeze-drying, 19 was obtained: yield 28 mg (82.7%); ^{1}H NMR (D_2O, t-BuOH) δ 7.80 q (1H, H6), 6.24 dd (1H, $J_{1',2'a}$ = 5.5 Hz, $J_{1',2'b}$ = 9.0 Hz, 1H'), 4.08–4.21 m (3H, H4' + 2H5'a,b), 2.61 m (2H, H2'a), 2.12 m (1H, 2'b), 1.88 d (3H, Me-C5); ^{31}P NMR (D_2O) δ −6.63d t ($^1J_{P,H}$ = 632 Hz, $^3J_{P,H5'}$ = 6.3 Hz).

1-(3'-C-Methyl-2'-deoxy-β-D-*threo*-pentofuranosyl)thymine 5'-Phosphite (Ammonium Salt) (20). The compound was obtained from 7β using the same conditions as for the synthesis of 19. 20: yield 28 mg (82.7%); ^{1}H NMR (D_2O, t-BuOH) δ 7.92 q (1H, H6), 6.15 dd (1H, $J_{1',2'a}$ = 3.0 Hz, $J_{1',2'b}$ = 8.0 Hz, 1H'), 4.22 m (1H, H4'), 4.06–4.19 m (2H, 2H5'a,b), 2.60 m (1H, H2'a), 2.16 m (1H, 2'b), 1.92 d (3H, Me-C5), 1.43s (3H, Me-C3'); ^{31}P NMR (D_2O) δ −6.60 dt ($^1J_{P,H}$ = 636 Hz, $^3J_{P,H5'}$ = 6.3 Hz).

3'-C-Methylidene-2',3'-dideoxy-5-methyluridine 5'-Triphosphate (Ammonium Salt) (21). 1,2,4-Triazole (28 mg, 0.4 mmol) was reevaporated with acetonitryl (3 × 5 mL) and dissolved in acetonitryl (0.6 mL), then triethylamine (55 μL, 0.4 mmol) and $POCl_3$ (12 μL, 0.13 mmol) were added at 0 °C, the mixture was stirred for 40 min at 20 °C, solid was separated by centrifugation, and the solution was added to 15β (8 mg, 0.03 mmol). The reaction mixture was stirred for 40 min at 20 °C and then 0.5 M tetra(tributylammonium) pyrophosphate in DMF (0.5 mL) was added, and the mixture was stirred for 30 min at 20 °C and further followed up as described for the synthesis of 17. 21: yield 2.1 mg (15%); ^{1}H NMR (D_2O, t-BuOH) δ 8.05 q (1H, H6), 6.35 pt (1H, $J_{1',2'}$ = 7.0 Hz, 1H'), 5.45 pq (1H, CH_2-C3'), 5.33 pq (1H, CH_2C3'), 4.79 m (1H, H4'), 4.12–4.28 m (2H, 2H5'a,b), 3.31 m (1H, H2'a), 2.97 m (1H, 2'b), 2.03 d (3H, Me-C5); ^{31}P NMR −9.21 d (J_{P^α,P^β} = 19.0 Hz, P^α), −11.65d (J_{P^γ,P^β} = 18.8 Hz, P^γ), −22.35 t (J_{P^β,P^α} = J_{P^β,P^γ} = 19.20 Hz, P^β).

Experiments with Cell-Free Enzymes. The DNA of M13 pm10 phage was isolated as described in ref 32. The following enzymes were used: T4 polynucleotide kinase (Amersham), calf thymus terminal deoxynucleotidyl transferase (Amersham), *E. coli* DNA polymerase I, Klenow fragment (Amersham), avian myeloblastosis virus (AMV) reverse transcriptase (Omutninsk, USSR), and the DNA polymerases α and ε from human placenta and DNA polymerase β from rat liver (kindly provided by D. Mozzherin and A. Atrazhev, Institute of Molecular Biology, Moscow). The HIV-1 reverse transcriptase was a gift of Dr. W. Egan, Center for Biologics Evaluation and Review, Bethesda. Radioactive dNTP was from Radioisotops USSR ([γ-^{32}P]ATP, 2000 Ci/mmol, [α-^{32}P]dATP, 4000 Ci/mmol). Tetradecadeoxynucleotide primer (Scheme V) was labeled at the 5'-position with T4 polynucleotide kinase.[32] Template-dependent elongation of the [5'-^{32}P]primer was achieved as described in ref 33, and the reaction products were analyzed by electrophoresis as described in ref 33.

Inhibition of DNA Synthesis in the Presence of the Substrate Analogs. The assay mixture (total volume 6 μL) contained the enzymes in the appropriate buffers, 0.02 μM primer–template complex, 20 μM of the substrates (dATP and dGTP, or dCTP and dGTP) each, 1 μM dTTP, and the test compounds at different concentrations. The reaction was performed for 15 min at 20 °C with 0.5 unit of DNA polymerase I or for 15 min at 40 °C with 3–4 units of reverse transcriptase and then stopped by the addition of EDTA, pH 8.0, up to 50 μM. The aliquots were brought on DE-81 filters (Whatman) and counted after washing with 0.2 M NaCl and 0.5 μM EDTA, pH 8.0, and fixation with ethanol.

For the chase experiments, just before termination 70 μM of each of the four dNTP were added to the reaction mixture, the incubation was continued for 10 min at 40 °C, and then the reaction was stopped and the assay mixture treated as described above. The termination of the DNA synthesis in the presence of ddNTP or test compounds was assayed under standard conditions[32] after electrophoresis in 8% denaturing PAGE.

Anti-Human Immunodeficiency Virus Assay. The procedure for the anti-retroviral assays has been described[24] and is based on the examination of HIV-1-induced cytopathicity in human lymphocyte MT-4 cells by trypan blue exclusion.

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