

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

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Received February 12, 1992

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  $\alpha$ - and  $\beta$ -anomers. The  $\beta$ -keto derivative was converted by reaction with MeMgBr, and after reaction with thymine and subsequent deprotection 1-(3'-C-methyl-2'- $\alpha$ -deoxy- $\alpha$ -D-threo-pentofuranosyl)thymine and its  $\beta$ -anomer were obtained. The same reactions with the  $\alpha$ -keto sugar gave 1-(3'-C-methyl-2'-deoxy- $\alpha$ -D-erythro-pentofuranosyl)thymine and its  $\beta$ -anomer. 1-(5-O-Benzoyl-3'-C-methyl-2'-deoxy- $\alpha$ -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 C1. 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 <sup>1</sup>H NMR UV, <sup>13</sup>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<sup>+</sup> 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  $\mu$ M 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.<sup>1</sup> 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.<sup>2</sup> 3'-C-Methyluridine 5'-triphosphate has also been

shown to be a DNA chain terminating substrate for *E. coli* RNA polymerase.<sup>3</sup>

A general scheme of the synthesis of 3'-branched 2'-deoxythymidine is presented in this paper. The Grignard reaction with 3'-keto-2'-deoxynucleosides<sup>4</sup> and 2'-O-tosylribonucleosides<sup>5,6</sup> 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,<sup>7</sup> which were not transformed to the corresponding

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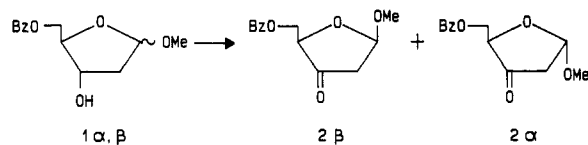
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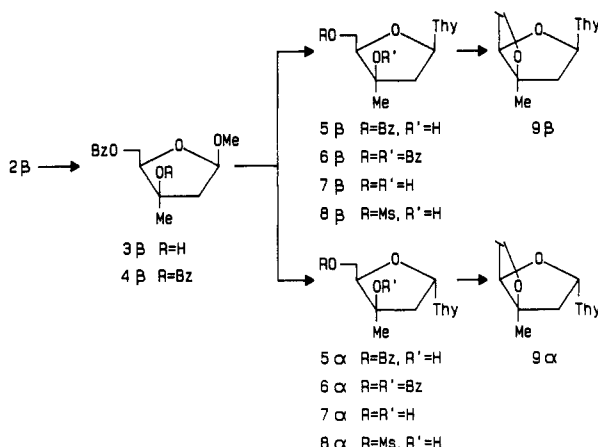
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## Scheme I



## Scheme II



nucleosides. Also, an 18-step 3'-C-methyl-2'-deoxyuridine synthesis from D-glucose<sup>9</sup> has been recently described.

## Results

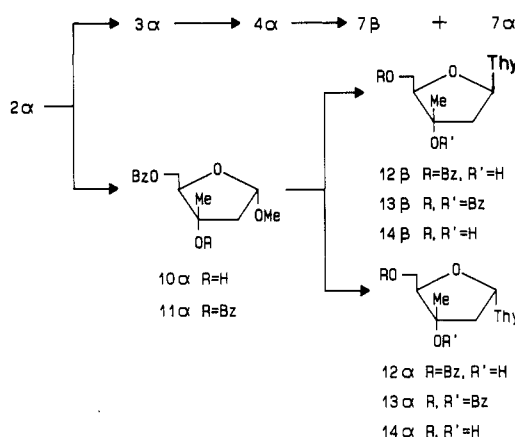
**Synthesis.** The starting compound 2-deoxy-D-ribose was transformed in two steps to yield the anomer mixture of 1-methyl 5-O-benzoyl-D-ribofuranosides (1α,β). The individual anomers were then separated on silica gel and oxidized to the corresponding ketones 2α and 2β (Scheme I). The reaction of 2β 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<sub>2</sub>OBz 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,<sup>9</sup> predominantly the 5β anomer was formed, irrespective of the catalyst CF<sub>3</sub>SO<sub>3</sub>SiMe<sub>3</sub> or SnCl<sub>4</sub> (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<sup>10,11</sup> resulted in their almost complete destruction. Dibenzoate 4β was then transformed into the nucleosides 6β and 6α under the same reaction

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

initial compound	ratio, % of the sum; catalyst:			
	CF <sub>3</sub> SO <sub>3</sub> SiMe <sub>3</sub>		SnCl <sub>4</sub>	
	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



conditions as used for 3β. After hydrolysis of 6β and 6α, the mixture of 7α and 7β was isolated in pure state.

When SnCl<sub>4</sub> was used as a catalyst, the main glycosylation product was the α-nucleoside 6. CF<sub>3</sub>SO<sub>3</sub>SiMe<sub>3</sub> 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<sup>4</sup> 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<sub>2</sub>OH 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<sub>2</sub>OBz 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<sub>4</sub> 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).

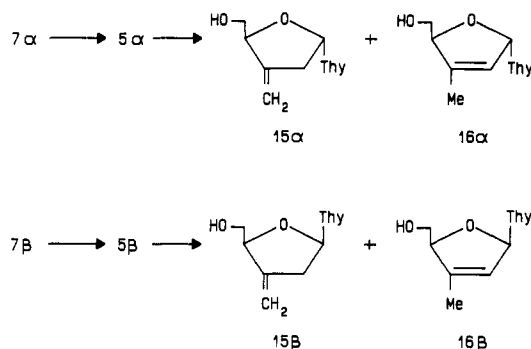
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## Scheme IV



Incompletely protected D-erythro sugar  $10\alpha$  was allowed to react with 2,4-bis(trimethylsilyl)thymine [(Me<sub>3</sub>Si)<sub>2</sub>Thy] in the presence of SnCl<sub>4</sub> or CF<sub>3</sub>SO<sub>3</sub>SiMe<sub>3</sub>, which resulted in the formation of  $\alpha$ -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  $14\beta$ , the sugar  $10\alpha$  was benzoyleated into  $11\alpha$  in *N*-methylimidazole with subsequent condensation with (Me<sub>3</sub>Si)<sub>2</sub>Thy, SnCl<sub>4</sub> being used as catalyst. Under these experimental conditions, predominantly the nucleoside  $13\beta$  anomer was formed (Table I). Deblocking of the reaction mixture and subsequent crystallization from methanol permitted the target nucleoside  $14\beta$  to be obtained with acceptable yield.

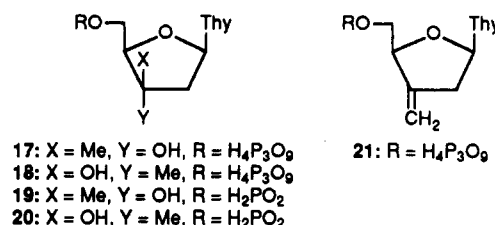
3'-C-Methyl-2'-deoxynucleosides appeared to be suitable starting compounds to synthesize 3'-branched unsaturated 2',3'-dideoxynucleosides: 3'-C-methylidene-2',3'-dideoxy-5-methyluridine ( $15\beta$ )<sup>12,13</sup> and 3'-C-methyl-2',3'-dideoxy-2',3'-didehydro-5-methyluridine ( $16\beta$ ).<sup>12,14,15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

To synthesize 3'-branched unsaturated 2',3'-dideoxynucleosides, the nucleosides  $7\beta$  and  $7\alpha$  were converted to the 5'-monobenzoates  $5\beta$  and  $5\alpha$  (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  $\beta$ -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.<sup>16</sup> 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<sub>3</sub> in the presence of triethyl phosphate and then with tetrabutylammonium pyrophosphate in the presence of tributylamine<sup>17</sup> (for  $7\beta$ ,  $14\beta$ ), or by the triazole method<sup>18</sup> (for  $15\beta$ ).

The nucleoside 5'-phosphites are less toxic and more selective inhibitors of virus replication in some cases.<sup>18,19</sup> Therefore, nucleosides  $7\beta$  and  $14\beta$  were transformed into 5'-phosphites by treatment with PCl<sub>3</sub> in triethyl phosphate, since attempts to obtain these compounds by the conventional methods with the application of H<sub>3</sub>PO<sub>3</sub> in the presence of *N,N'*-dicyclohexylcarbodiimide<sup>19,20</sup> led to a multicomponent mixture of products scarcely amenable to separation.



**Physicochemical Properties.** The structure of the obtained compounds was proved by <sup>1</sup>H NMR, <sup>13</sup>C NMR, UV, and CD spectroscopy as well as elemental C,H,N analysis and X-ray analysis of the nucleoside  $14\beta$ . The presence of the two separated ABX systems (5',5'b,4' and 2'a,2'b,1') is characteristic of the <sup>1</sup>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  $\alpha$ - or  $\beta$ -series. For the anomer sugars 1-3, the proton signals at C1 and the OMe groups of the  $\alpha$ -anomers were more downfield than those of the  $\beta$ -anomers. This dependence was inverse for the nucleosides, and the proton signal at C1' as well as H-6 in the  $\beta$ -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  $\beta$ -anomer than for the  $\alpha$ -anomer.

The <sup>1</sup>H NMR spectroscopy data enabled us to prove the furanose form of the methylated nucleosides obtained. From the spectra in DMSO-*d*<sub>6</sub> it appeared that the proton signal of the tertiary hydroxyl in  $7\beta$  was a singlet ( $\delta$  5.28) and that of the primary hydroxyl was a triplet ( $\delta$  5.0,  $J$  < 0.5 Hz). A similar picture was observed for  $14\beta$ .

The <sup>1</sup>H NMR spectra of the unsaturated nucleosides  $15\beta$  and  $16\beta$  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  $15\beta$ . The specific

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**Table II.** Chemical Shifts in  $^1\text{H}$  NMR Spectra of 2'-Deoxyfuranosides and Their 3'-C-Methylated Derivatives

compd	chemical shift, ppm ( $\text{CDCl}_3/\text{TMC}$ )							
	H-1'	H-5'a	H-5'b	H-4'	OMe s	H-2'a	H-2'b	3-C-Me s
1 $\beta^a$	5.09 dd	4.56 dd	4.48 dd	4.18 m	3.26	2.27 qd	2.11 qd	
1 $\alpha^b$	5.10 dd	4.37 m	4.37 m	4.37 m	3.39	2.19 qd	2.05 qd	
2 $\beta$	5.36 dd	4.65 dd	4.44 br d	4.42 dd	3.38	2.81 dd	2.51 dd	
2 $\alpha$	5.37 d	4.75 dd	4.45 dd	4.26 br t	3.46	2.67 dd	2.47 d	
3 $\beta$	5.04 m	4.64 dd	4.46 dd	4.12 dd	3.39	2.09 m	2.09 m	1.43
3 $\alpha$	5.16 dd	4.67 dd	4.44 dd	4.07 br t	3.38	2.37 dd	2.00 dd	1.44
4 $\beta$	5.09 dd	4.82 dd	4.60 dd	4.32 dd	3.35	2.89 dd	2.46 dd	1.34
10 $\alpha$	5.12 dd	4.43 dd	4.22 dd	4.38 br t	3.40	2.10 m	2.10 m	1.38

<sup>a</sup> H-3', 4.38 m. <sup>b</sup> H-3', 4.24 m.**Table III.** Chemical Shifts in  $^1\text{H}$  NMR Spectra of 3'-Methyl-2'-deoxynucleosides

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

<sup>a</sup> The signal is exceeded by solvent signal. <sup>b</sup> DSS was used as internal standard.**Table IV.** SSCC of 2'-Deoxy-3'-C-methylfuranosides and Their Nucleosides

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

<sup>a</sup>  $J_{2'a,3'} = 7.0$  Hz,  $J_{2'b,3'} = 6.2$  Hz. <sup>b</sup>  $J_{2'a,3'} = 6.1$  Hz,  $J_{2'b,3'} = 1.6$  Hz. <sup>c</sup> -: SSCC absent. <sup>d</sup> ...: 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 16 $\beta$  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.

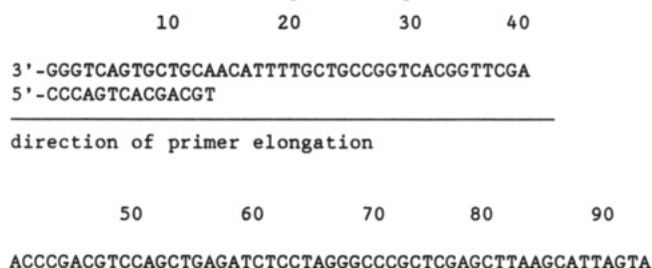
The spatial structure of the 3'-C-methyl-2'-deoxy-5-methyluridine molecules was definitively determined by X-ray analysis.<sup>21</sup> The investigated crystals were grown

from a saturated solution of 14 $\beta$  in methanol and did not contain the solvent molecules.

The C2'-endo-C1'-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- $\beta$ -D-ribofuranosyl)thymine. *Bioorg. Khim.* 1991, 17, 1094–1100.

## Scheme V. Primer-Template Complex



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.<sup>22</sup> 14β is in anti conformation around the N-glycoside bond, as is 2'-deoxythymidine.

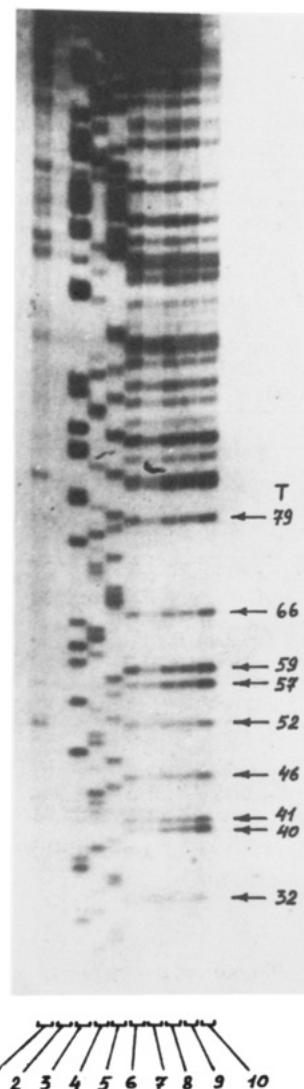
On the whole the conformation of 14β is very similar to that of 3'-C-methylcytidine,<sup>23</sup> which, in its 5'-triphosphate form, is a substrate and efficient terminator of the RNA polymerase reaction. From a comparison of the two structures the following general patterns of spatial organization 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<sup>+</sup> 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

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

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



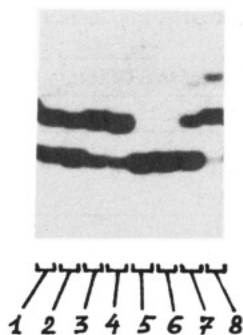
**Figure 1.** Autoradiography/PAGE analysis of primer elongation catalyzed by AMV reverse transcriptase in the presence of 21. Track 1: DNA synthesis in the complete system without termination nucleotides. Track 2: identical to 1, but with subsequent chase. Tracks 3-6: control DNA synthesis in the presence 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).

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.).

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  $\mu\text{M}$  (1) or 100  $\mu\text{M}$  (2). Tracks 3 and 4: identical to 1 and 2, but with subsequent incubation with 10  $\mu\text{M}$  dTTP. Tracks 5–8: controls: [ $^{32}\text{P}$ ]tetradecadeoxynucleotide only (5), [ $^{32}\text{P}$ ]tetradecadeoxynucleotide and enzyme (6), [ $^{32}\text{P}$ ]tetradecadeoxynucleotide and enzyme and either 3  $\mu\text{M}$  dTTP (7), or 10  $\mu\text{M}$  dTTP (8).

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

compd	EC <sub>50</sub> <sup>a</sup> ( $\mu\text{M}$ )	CC <sub>50</sub> <sup>b</sup> ( $\mu\text{M}$ )
7 $\beta$	>500	>500
14 $\beta$	>500	>500
19	72	>500
20	243	>500

<sup>a</sup> EC<sub>50</sub>, 50% effective concentration, or compound concentration required to inhibit HIV-1-induced cytopathicity in MT-4 cells by 50%. <sup>b</sup> CC<sub>50</sub>, 50% cytotoxic concentration, or compound concentration required to reduce MT-4 cell viability by 50%.

**Anti-HIV-1 Activity.** The ability of 14 $\beta$ , 7 $\beta$ , 19, and 20 to suppress HIV-1 replication in MT-4 cell cultures was evaluated as previously described.<sup>24</sup> As evident from Table VI, compounds 7 $\beta$  and 14 $\beta$  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.

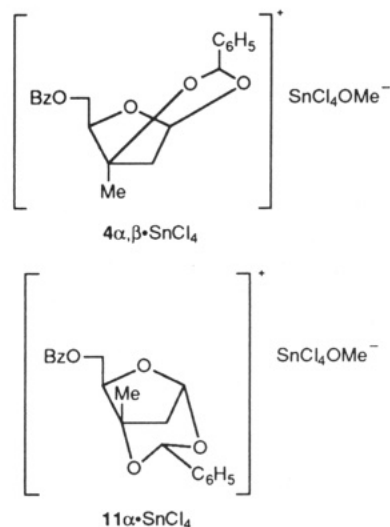
## Discussion

**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.<sup>10,11</sup>

The data obtained when condensing 3-C-methyl sugars 3 $\alpha,\beta$ , 4 $\alpha,\beta$ , 10 $\alpha$ , and 11 $\alpha$  with (Me<sub>3</sub>Si)<sub>2</sub>Thy enabled us to discern a consistent pattern in the stereoselectivity of the glycosylation reactions. The stereoselectivity of this reaction for incompletely protected sugars 3 $\alpha,\beta$ , and 10 $\alpha$  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 4 $\alpha,\beta$ , and 11 $\alpha$ , the stereoorientation was determined by the catalyst used. Me<sub>3</sub>-

SiSO<sub>3</sub>CF<sub>3</sub> appeared to be nonselective and its application led to the formation of a mixture of the nucleoside  $\alpha$ - and  $\beta$ -anomers in approximately equal proportions. Through the use of SnCl<sub>4</sub> the nucleoside  $\alpha$ - or  $\beta$ -anomers can be obtained selectively: compounds with the trans disposition of the benzyloxy group and the nucleic base are predominantly formed. The ratio of the nucleoside 13 $\alpha$  and 13 $\beta$  anomers synthesized from D-erythro sugar 11 $\alpha$  was inverted as compared to the ratio of nucleosides 6 synthesized from the D-threo sugars 4 $\alpha,\beta$  (Table I). The high stereoselectivity of the glycosylation reactions can be explained by the stereocontrolling effect of the benzyloxy group in the 3'-position and the formation of the intermediate protected sugar-catalyst complexes (4 $\alpha,\beta$ -SnCl<sub>4</sub>, 9 $\alpha,\beta$ -SnCl<sub>4</sub>) with a different spatial structure.

The preparation of the 5'-phosphite derivatives of 3'-C-methyl-2'-deoxynucleosides 14 $\beta$  and 8 $\beta$  by treatment with PCl<sub>3</sub> in triethyl phosphate followed by hydrolysis is a new technique described in this paper. This technique is superior to the use of H<sub>3</sub>PO<sub>3</sub> in the presence of *N,N'*-dicyclohexylcarbodiimide,<sup>19</sup> 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.



**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 14 $\beta$  may be too rigid to allow transition of the 14 $\beta$  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.<sup>25,26</sup> 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, %				formula	found, %			
	C	H	N	P		C	H	N	P
2 $\alpha$	62.39	5.64			C <sub>13</sub> H <sub>14</sub> O <sub>5</sub>	62.58	5.71		
2 $\beta$	62.39	5.64			C <sub>13</sub> H <sub>14</sub> O <sub>5</sub>	62.63	5.40		
3 $\beta$	63.15	6.81			C <sub>14</sub> H <sub>18</sub> O <sub>5</sub>	62.99	6.92		
4 $\alpha$	68.10	5.99			C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>	68.20	6.20		
4 $\beta$	68.10	5.99			C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>	68.26	5.77		
5 $\alpha$	59.99	5.59	7.77		C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub>	60.06	5.83	7.86	
5 $\beta$	59.99	5.59	7.77		C <sub>18</sub> H <sub>20</sub> N <sub>2</sub> O <sub>6</sub>	60.17	5.78	7.91	
7 $\alpha$	51.56	6.29	10.94		C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	51.39	6.06	10.81	
7 $\beta$	51.56	6.29	10.94		C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	51.61	6.31	11.18	
9 $\alpha$	55.46	5.92	11.86		C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	55.20	5.99	11.58	
9 $\beta$	55.46	5.92	11.86		C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	55.31	6.12	11.90	
10 $\alpha$	63.15	6.81			C <sub>14</sub> H <sub>18</sub> O <sub>5</sub>	62.96	6.70		
11 $\alpha$	68.10	5.99			C <sub>21</sub> H <sub>22</sub> O <sub>6</sub>	67.95	6.12		
14 $\beta$	51.55	6.29	10.94		C <sub>11</sub> H <sub>16</sub> N <sub>2</sub> O <sub>5</sub>	51.77	6.02	11.10	
15 $\beta$	55.46	5.92	11.76		C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	55.49	6.11	11.66	
16 $\beta^a$	55.46	5.92	11.76		C <sub>11</sub> H <sub>14</sub> N <sub>2</sub> O <sub>4</sub>	55.58	6.01	11.60	
19	39.17	5.98	12.46	9.18	C <sub>11</sub> H <sub>20</sub> N <sub>3</sub> O <sub>7</sub> P-NH <sub>3</sub>	39.01	6.16	12.30	9.04
21	37.19	6.24	11.83	8.72	C <sub>11</sub> H <sub>20</sub> N <sub>3</sub> O <sub>7</sub> P-NH <sub>3</sub> -H <sub>2</sub> O	36.94	6.44	12.06	8.64

<sup>a</sup> Analysis should be made for freshly isolated substance because of its instability.

flattened carbohydrate residue, such as 2',3'-dideoxy-2',3'-didehydronucleoside 5'-triphosphates,<sup>27,28</sup> and 2',3'-riboanhydro- and 2',3'-lyxoanhydro-nucleoside 5'-triphosphates,<sup>28,29</sup> 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  $\alpha$ ).<sup>28-31</sup>

## Experimental Section

The NMR spectra were obtained on a Bruker WM-250 (250 MHz) spectrometer for <sup>1</sup>H and 62.8 MHz for <sup>13</sup>C at 20 °C (CDCl<sub>3</sub>, CD<sub>3</sub>OD or D<sub>2</sub>O, internal standard TMS, *t*-BuOH or DSS, respectively). The <sup>31</sup>P NMR spectra were obtained with 101.3 MHz in D<sub>2</sub>O, internal standard (MeO)<sub>3</sub>PO. 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 diffractometer. HPLC was performed on a Gilson chromatograph at 268 nm, column 4.6 × 250 mm, carrier Silasorb C-18 (5  $\mu$ m), flow rate 1 mL/min. The nucleosides were isolated in the following buffers: A, 0.04 M Et<sub>3</sub>NHCO<sub>3</sub>, pH 7.4; B, 50% MeCN in water, the gradient 5 → 30%, 20 min; C, 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.

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**Synthesis. Methyl 5-O-Benzoyl-2-deoxy- $\alpha$ -D-ribofuranoside (1 $\alpha$ ) and Its  $\beta$  Anomer (1 $\beta$ ).** 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 dichloroethane (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<sub>3</sub> (2 × 100 mL), and water (2 × 100 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, 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 1 $\alpha$  (C: *R*<sub>f</sub> 0.24) and 8.07 g (21.5%) of 1 $\beta$  (C: *R*<sub>f</sub> 0.18). The sugar 1 $\beta$  was crystallized upon standing at 0 °C, mp 63-64 °C. <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  1 $\alpha$  105.3 (C1), 84.5 (C4), 72.8 (C3), 64.5 (C5), 54.7 (OMe), 40.9 (C2); 1 $\beta$  105.0 (C1), 83.6 (C4), 72.0 (C3), 65.3 (C5), 54.8 (OMe), 41.4 (C2).

**Methyl 5-O-Benzoyl-2-deoxy- $\alpha$ -D-glycero-pentofuranoside-3-ulose (2 $\alpha$ ) and Its  $\beta$ -Anomer (2 $\beta$ ).** Dry pyridine (10 mL), acetic anhydride (5 mL), and freshly dried CrO<sub>3</sub> (6 g, 60.0 mmol) were dissolved in dry CH<sub>2</sub>Cl<sub>2</sub> (140 mL) and stirred for 30 min. A solution of 1 $\alpha$  or 1 $\beta$  (5 g, 19.8 mmol) in CH<sub>2</sub>Cl<sub>2</sub> (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<sub>3</sub> (50 mL), and then with water (50 mL). The organic extract was dried with Na<sub>2</sub>SO<sub>4</sub>, 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 2 $\alpha$  or 2 $\beta$  (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- $\beta$ -D-threo-pentofuranoside (3 $\beta$ ).** To a solution of uloside 2 $\beta$  (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<sub>4</sub>Cl (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<sub>2</sub>SO<sub>4</sub>, and evaporated to dryness. The yield was 1.45 g (83%) of a yellow syrup: <sup>13</sup>C (CDCl<sub>3</sub>)  $\delta$  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- $\alpha$ -D-threo-pentofuranosyl)thymine (7 $\alpha$ ) and Its  $\beta$ -Anomer (7 $\beta$ ).** To a solution of 3 $\beta$  or 3 $\alpha$  (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<sub>3</sub>SO<sub>3</sub>-SiMe<sub>3</sub> (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<sub>3</sub> (10 mL), and filtered through silica gel. Silica

gel was washed with water, NaHCO<sub>3</sub> (10 mL), and dichloroethane (20 mL). Organic extracts were dried with Na<sub>2</sub>SO<sub>4</sub> 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<sup>+</sup>) 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 7 $\beta$  (12.5 mg, 12.9%; D-R<sub>f</sub> 0.35) and 7 $\alpha$  (2.6 mg, 2.7%; D-R<sub>f</sub> 0.23) were obtained: <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  7 $\alpha$  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); 7 $\beta$  167.1 (C2), 152.2 (C4), 139.7 (C6), 111.1 (C5), 88.3 (C1'), 85.2 (C4'), 79.2 (C3'), 60.8 (C5'), 46.8 (C2'), 23.9 (Me-C3'), 12.1 (Me-C5); CD spectra (MeOH) 7 $\alpha$   $\lambda_{\max}$  273 nm ( $\Delta\epsilon$  = -1.1), 7 $\beta$   $\lambda_{\max}$  272 nm ( $\Delta\epsilon$  = +1.3).

**Methyl 3,5-Di-O-benzoyl-3-C-methyl-2-deoxy- $\alpha$ -D-threo-pentofuranoside (4 $\alpha$ ), Its  $\beta$ -Anomer (4 $\beta$ ), and Methyl 3,5-Di-O-benzoyl-3-C-methyl-2-deoxy- $\alpha$ -D-erythro-pentofuranoside (11 $\alpha$ ).** 3 $\alpha$ , 3 $\beta$ , or 10 $\alpha$  (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  $\times$  5 mL). The chloroform extract was washed with 1% HCl (2  $\times$  5 mL), saturated NaHCO<sub>3</sub> (2  $\times$  5 mL), and water (2  $\times$  5 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, and evaporated. The substances were isolated on a silica gel column with the chloroform in hexane (50  $\rightarrow$  100%) as the eluent. 4 $\alpha$  (0.42 g, 63%) or 4 $\beta$  (0.36 g, 54%) or 11 $\alpha$  (0.42 g, 63%) was obtained in the form of a yellow syrup.

**1-(3'-C-methyl-2'-deoxy- $\alpha$ -D-threo-pentofuranosyl)thymine (7 $\alpha$ ) and Its  $\beta$ -Anomer (7 $\beta$ ).** **Method A.** Dibenzate 4 $\beta$  or 4 $\alpha$  (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<sub>4</sub> (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<sub>3</sub> (10 mL) was added, and then the procedure was continued as described for the synthesis of nucleosides 7 $\alpha,\beta$  from sugars 3 $\beta$  or 3 $\alpha$ . After separation of the anomers 7 by preparative TLC in system D, 8 mg (9.6%) of 7 $\beta$  and 43 mg (52%) of 7 $\alpha$  were obtained.

**Method B.** 2,4-Bis(trimethylsilyl)thymine (1 mL, 3.64 mmol) was added to 4 $\beta$  or 4 $\alpha$  (530 mg, 1.43 mmol) in dichloroethane (10 mL), then a solution of CF<sub>3</sub>SO<sub>3</sub>SiMe<sub>3</sub> (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<sub>3</sub> (15 mL), stirred for 30 min and filtered through silica gel. The residue was washed with saturated NaHCO<sub>3</sub> (5 mL) and then with dichloroethane (15 mL). The organic layer was washed with water (2  $\times$  5 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, 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<sup>+</sup>) 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  $\times$  1 mL). The aqueous layer was evaporated, and 320 mg (87%) of a mixture of 7 $\alpha,\beta$  were obtained. After separation with preparative TLC (as described in the synthesis of 7 $\alpha,\beta$ ) and evaporation, 126 mg (34.2%) of 7 $\beta$  and 174 mg (47.3%) of 7 $\alpha$  were obtained.

**1-(3'-C-Methyl-2'-deoxy-3',5'-O-anhydro- $\alpha$ -D-threo-pentofuranosyl)thymine (9 $\alpha$ ) and Its  $\beta$ -Anomer (9 $\beta$ ).** Mesyl chloride (0.045 mL, 0.58 mmol) was fluxed into solution of nucleoside 7 $\beta$  or 7 $\alpha$  (74 mg, 0.29 mmol) in dry pyridine (0.5 mL). The solution was stirred for 15 min at 20 °C, saturated NaHCO<sub>3</sub> (1 mL) was added, and the mixture was evaporated to dryness. The residue was extracted with chloroform (10  $\times$  3 mL), and the solution was filtered, evaporated, and reevaporated with toluene (3  $\times$  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<sup>+</sup>) 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 9 $\alpha$  53 mg (76.4%) (D-R<sub>f</sub> 0.74) and 9 $\beta$  (D-R<sub>f</sub> 0.81).

**Methyl 5-O-Benzoyl-3-C-methyl-2-deoxy- $\beta$ -D-threo-Pentofuranoside (3 $\alpha$ ) and Methyl 5-O-Benzoyl-3-C-methyl-2-deoxy- $\alpha$ -D-erythro-pentofuranoside (10 $\alpha$ ).** 2 $\alpha$  (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 3 $\beta$ . The mixture of the 3 $\alpha$  and 10 $\alpha$  diastereomers was separated on the silica gel column in the C system. 3 $\alpha$  (0.55 g, 31.2%) (C-R<sub>f</sub> 0.27) and 10 $\alpha$  (0.90 g, 51.2%) (C-R<sub>f</sub> 0.36) were obtained: <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  10 $\alpha$  103.8 (C1), 81.7 (C4), 77.0 (C3), 63.1 (C5), 55.3 (OMe), 49.2 (C2), 21.7 (Me-C<sub>3</sub>).

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

**Method B.** To the solution of dibenzate 11 $\alpha$  (120 mg, 0.32 mmol) in dichloroethane (3 mL) were added 2,4-bis(trimethylsilyl)thymine (0.25 mL, 0.91 mmol) and SnCl<sub>4</sub> (0.25 mL, 2.14 mmol) in dichloroethane (0.3 mL) and then followed up as described for the synthesis of the nucleosides 7 $\alpha,\beta$  from sugars 3 $\beta$  or 3 $\alpha$ . 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 14 $\beta$ : mp 182–183 °C (D-R<sub>f</sub> 0.20); <sup>13</sup>C NMR (D<sub>2</sub>O)  $\delta$  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 11 $\alpha$  (120 mg, 0.32 mmol) in dichloroethane (3 mL) were added 2,4-bis(trimethylsilyl)thymine (0.25 mL, 0.91 mmol) and CF<sub>3</sub>SO<sub>3</sub>SiMe<sub>3</sub> (0.25 mL, 1.3 mmol) in dichloroethane (1 mL), and the mixture was boiled for 1 h. Saturated NaHCO<sub>3</sub> (15 mL) was added, the mixture was stirred for 30 min and filtered through silica gel, and the adsorbent was washed with saturated NaHCO<sub>3</sub> (5 mL) and then with dichloroethane (15 mL). Organic layer was washed with water (2  $\times$  5 mL), dried with Na<sub>2</sub>SO<sub>4</sub>, 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<sup>+</sup>) 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  $\times$  1 mL). The solution was evaporated to dryness, and a mixture of 14 $\alpha$  and 14 $\beta$  (71 mg, 87%) was obtained. According to <sup>1</sup>H NMR, the ratio of 14 $\alpha$  to 14 $\beta$  was 58:42.

**1-(5'-O-Benzoyl-3'-C-methyl-2'-deoxy- $\alpha$ -D-threo-pentofuranosyl)thymine (5 $\alpha$ ) and Its  $\beta$ -Anomer (5 $\beta$ ).** To a solution of 7 $\alpha$  or 7 $\beta$  (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<sub>3</sub> (10 mL) was added, and the solution was evaporated to dryness. The residue was extracted with chloroform (5  $\times$  5 mL), the solution was evaporated, and the residue was reevaporated five times with toluene-EtOH (5:1, 5 mL). 5 $\alpha$  or 5 $\beta$  (512 mg, 95%) was obtained as a colorless syrup.

**3'-C-Methylidene-2',3'-dideoxy-5-methyluridine (15 $\beta$ ) and 3'-C-Methyl-2',3'-dideoxy-2',3'-didehydro-5-methyluridine (16 $\beta$ ).** A solution of 5 $\beta$  (200 mg, 0.56 mmol) in SOCl<sub>2</sub> (2 mL) was boiled for 2 min, cooled to -20 °C, and diluted with cold saturated NaHCO<sub>3</sub> (15 mL). The mixture was evaporated, the residue was extracted with chloroform (5  $\times$  5 mL), and the solution was evaporated. Saturated NH<sub>3</sub> 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 15 $\beta$ , 16 $\beta$  were separated by preparative HPLC and eluted with 10% MeOH in water. The fractions containing the nucleosides were immediately evaporated at room temperature. 15 $\beta$  (15 mg, 11%) and 16 $\beta$  (45 mg, 34%) were obtained in the form of colorless syrups: <sup>1</sup>H NMR spectra (CD<sub>3</sub>OD)  $\delta$  15 $\beta$  8.01 q (1H, H6), 6.34 pt (1H, J<sub>1,2</sub> = 6.8 Hz, H1'), 5.43 pq (1H, J = 2.2 Hz, 4.4 Hz, CH<sub>2</sub>-C3'), 5.31 pq (1H,

CH<sub>2</sub>-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); 16 $\beta$  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- $\beta$ -D-erythro-pentofuranosyl)thymine 5'-Triphosphate (Ammonium Salt) (17).** To the solution of 14 $\beta$  (25.7 mg, 0.1 mmol) in triethyl phosphate (0.4 mL) was added POCl<sub>3</sub> (18  $\mu$ L, 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<sub>3</sub>NH<sub>2</sub>CO<sub>3</sub> 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<sub>3</sub><sup>-</sup> form, 10  $\times$  0.5 cm). The substances were eluted with a linear gradient of NH<sub>4</sub>HCO<sub>3</sub>, pH 7.5 (0  $\rightarrow$  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  $\times$  10 mL). After freeze-drying, 17 (29 mg, 58.5%) was obtained: <sup>1</sup>H NMR (D<sub>2</sub>O, *t*-BuOH)  $\delta$  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'); <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  -9.16 d ( $J_{P\alpha,P\beta} = 19.1$  Hz, P $\alpha$ ), -11.65 d ( $J_{P\gamma,P\beta} = 18.9$  Hz, P $\gamma$ ), -22.41 t ( $J_{P\beta,P\alpha} = J_{P\beta,P\gamma} = 19.06$  Hz, P $\beta$ ).

**1-(3'-C-Methyl-2'-deoxy- $\beta$ -D-threo-pentofuranosyl)thymine 5'-Triphosphate (Ammonium Salt) (18).** The compound was obtained from 7 $\beta$  using the same conditions as for the synthesis of 17 $\beta$ . 18: yield 13 mg (26.1%); <sup>1</sup>H NMR (D<sub>2</sub>O, *t*-BuOH)  $\delta$  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'); <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  -9.20 d ( $J_{P\alpha,P\beta} = 18.8$  Hz, P $\alpha$ ), -11.67 d ( $J_{P\gamma,P\beta} = 18.9$  Hz, P $\gamma$ ), -22.16 t ( $J_{P\beta,P\alpha} = J_{P\beta,P\gamma} = 19.21$  Hz, P $\beta$ ).

**1-(3'-C-Methyl-2'-deoxy- $\beta$ -D-erythro-pentofuranosyl)thymine 5'-Phosphite (Ammonium Salt) (19).** A solution of 14 $\beta$  (25.7 mg, 0.1 mmol) in triethyl phosphate (375  $\mu$ L) was cooled to 0 °C, POCl<sub>3</sub> (20  $\mu$ L, 0.23 mmol) was added, and the mixture was stirred for 24 h at 0 °C, neutralized with 1 M Et<sub>3</sub>NH<sub>2</sub>CO<sub>3</sub> 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<sub>4</sub>HCO<sub>3</sub> and eluted with 0.07 M buffer. After freeze-drying, 19 was obtained: yield 28 mg (82.7%); <sup>1</sup>H NMR (D<sub>2</sub>O, *t*-BuOH)  $\delta$  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); <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  -6.63 d ( $J_{P,H} = 632$  Hz,  $J_{P,H5'} = 6.3$  Hz).

**1-(3'-C-Methyl-2'-deoxy- $\beta$ -D-threo-pentofuranosyl)thymine 5'-Phosphite (Ammonium Salt) (20).** The compound was obtained from 7 $\beta$  using the same conditions as for the synthesis of 19. 20: yield 28 mg (82.7%); <sup>1</sup>H NMR (D<sub>2</sub>O, *t*-BuOH)  $\delta$  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'); <sup>31</sup>P NMR (D<sub>2</sub>O)  $\delta$  -6.60 dt ( $J_{P,H} = 636$  Hz,  $J_{P,H5'} = 6.3$  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 acetonitrile (3  $\times$  5 mL) and dissolved in acetonitrile (0.6 mL), then triethylamine (55  $\mu$ L, 0.4 mmol) and POCl<sub>3</sub> (12  $\mu$ L, 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 15 $\beta$  (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%); <sup>1</sup>H NMR (D<sub>2</sub>O, *t*-BuOH)  $\delta$  8.05 q (1H, H6), 6.35 pt (1H,  $J_{1',2'} = 7.0$  Hz, 1H'), 5.45 pq (1H, CH<sub>2</sub>-C3'), 5.33 pq (1H, CH<sub>2</sub>-C3'), 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); <sup>31</sup>P NMR -9.21 d ( $J_{P\alpha,P\beta} = 19.0$  Hz, P $\alpha$ ), -11.65d ( $J_{P\gamma,P\beta} = 18.8$  Hz, P $\gamma$ ), -22.35 t ( $J_{P\beta,P\alpha} = J_{P\beta,P\gamma} = 19.20$  Hz, P $\beta$ ).

**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  $\alpha$  and  $\epsilon$  from human placenta and DNA polymerase  $\beta$  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 ( $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ , 2000 Ci/mmol,  $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ , 4000 Ci/mmol). Tetradecadeoxynucleotide primer (Scheme V) was labeled at the 5'-position with T4 polynucleotide kinase.<sup>32</sup> Template-dependent elongation of the  $[\gamma\text{-}^{32}\text{P}]\text{primer}$  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  $\mu$ L) contained the enzymes in the appropriate buffers, 0.02  $\mu$ M primer-template complex, 20  $\mu$ M of the substrates (dATP and dGTP, or dCTP and dGTP) each, 1  $\mu$ M 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  $\mu$ M. The aliquots were brought on DE-81 filters (Whatman) and counted after washing with 0.2 M NaCl and 0.5  $\mu$ M EDTA, pH 8.0, and fixation with ethanol.

For the chase experiments, just before termination 70  $\mu$ M 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<sup>32</sup> after electrophoresis in 8% denaturing PAGE.

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

**Acknowledgment.** We express our profound gratitude to Dr. S. N. Mikhailov, Engelhardt Institute of Molecular Biology, Moscow, for his assistance, to S. G. Rosenberg, Institute of the Pharmacology, Moscow, for obtaining some of the NMR spectra, to Ann Absillis for excellent technical assistance with the antiviral experiments, and to Christiane Callebaut for precious editorial assistance.

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