SYNTHESIS, STRUCTURE AND CERTAIN BIOCHEMICAL PROPERTIES OF 3[1]-BRANCHED THYMIDINES AND THEIR 5[1]-PHOSPHATE DERIVATIVES

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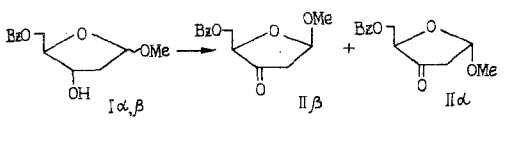
For the synthesis of compounds with potential antiretroviral activity, the conventional approach is preferentially applied. This method is based on the substitution of a hydroxyl group in the 3'-position in 2'-deoxynucleosides by other functional groups with the retention of the hydrogen atom at the 3'-position [14]. It was also of interest to introduce an alkyl substituent into the 3'-position in place of the hydrogen atom while retaining of the 3'-hydroxyl group, which could potentially participate in the extension of the polynucleotide chain during a synthesis of DNA. The nucleophilicity of these deoxynucleosides in the formation of a phospho-ester bond is thereby substantially decreased, which may impart new properties to the substrate. In this way antiviral activity has been previously established in 3'-C-methylribonucleosides with respect to the smallpox vaccine virus due to their probable 5-triphosphorylation in the cell and inhibition of the synthesis of the viral RNA [30]. It was shown that 3'-C-methyluridine 5'-triphosphate is a terminating substrate for the RNA-polymerase of E. coli [1]. In the present work a general scheme of a synthesis of 3'-branched 2'-deoxythymidines is presented. Previously the Grignard reaction was used with 3'-keto-2'-deoxynucleosides [33] and with 2'-O-tosylribonucleosides [5, 6] for their preparation, which made it possible to obtain 3'-C-methyl-2'-deoxynucleosides with an exclusively D-threo-configuration. The Grignard reaction with protected 2-O-tosylribofuranoses probably leads to a mixture of 3-C-methyl-D-erythro- and D-threo-sugars [23], which are not transformed into the corresponding nucleosides. An 18-step synthesis of 3'-C-methyl-2'-deoxyuridine from D-glucose was recently described [7].

The Synthesis. The starting compound – 2-deoxy-D-ribose was converted in two steps into an anomeric mixture of 1-methyl-5-O-benzoyl-D-ribofuranosides (Iα, β), which was separated by chromatography on silica gel into the individual anomers, which were then oxidized into the corresponding ketones IIα and IIβ (scheme 1). The reaction of IIβ with MeMgI led to the formation of only the D-threo-isomer IIIβ (scheme 2). The complete stereoselectivity of the addition of the Grignard reagent can be explained by the stereo-controlling action of cis-positioned OMe and HC_2OBz groups at C1 and C4, which leads to an attack on IIβ by MeMgI in

Scheme 1



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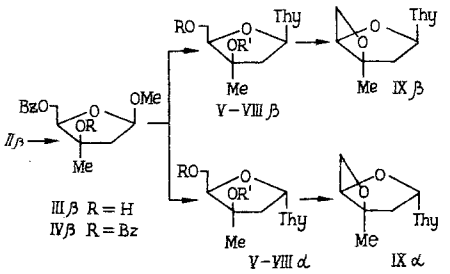
Scheme 2

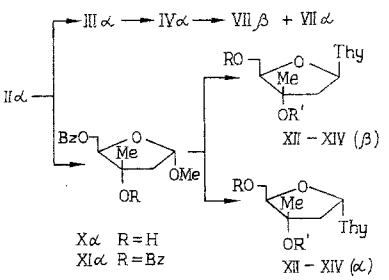


R=Bz(Vα, β), Ms(VIIIα, β); R′=H(Vα, β, VIIIα, β). R= =R′=H(VIIα, β). Bz(VIα, β)

TABLE 1. Ratio between the α- and β-Anomers of Nucleosides (V, VI and XII, XIII) during Glycosylation (the ^{1}H NMR spectroscopy data)

Starting compound	Ratio, % of total catalyst: $CF_3SO_3SiMe_3$ β-anomer	$CF_3SO_3SiMe_3$ α-anomer	$SnCl_4$ β-anomer	$SnCl_4$ α-anomer
Partially protected sugars				
D-threo-				
IIIβ	83	17	84	16
IIIα	82	18	83	17
D-erythro				
Xα	20	80	19	79
Completely protected sugars				
D-threo				
IVβ	60	40	15	85
IVα	59	41	15	85
D-erythro				
XIα	42	58	85	15

the sterically accessible direction. The partially protected sugar IIIβ was then transformed in a low yield into an anomeric mixture of 5'-monobenzoates of thymine nucleosides V without the intermediate protection of the 3-hydroxyl group in the presence of catalytic amounts of $CF_3SO_3SiMe_3$ or $SnCl_4$ [32]. Thus, independently of the catalyst, a β-anomer of nucleoside V was preferentially formed (Table 1). The deblocking of the reaction mixture and the subsequent chromatography on silica gel made it possible to isolate microamounts of nucleoside VIIβ and nucleoside VIIα. Better results were obtained, if the sugar IIIβ was preliminarily converted into dibenzoate IVβ by heating with benzoyl chloride in N-methylimidazole, since the conventional methods of benzoylation of tertiary hydroxyl groups of sugars [19, 29] led to its almost complete degradation. Dibenzoate IVβ was then transformed into nucleosides VIβ and



R=Bz(XIIα, β); R′=H(XIIα, β); R=R′=H(XIVα, β). Bz(XIIIα, β)

VIα under the same conditions as IIIβ. During catalysis with $SnCl_4$ the preferential glycosylation product is the α-nucleoside VI, while $CF_3SO_3SiMe_3$ acted as a nonselective catalyst, and as a result, a mixture of α- and β-anomers of threo-nucleosides was formed in an approximately equal ratio (see Table 1). Their deblocking and subsequent chromatography on silica gel made it possible to isolate the desired β-nucleoside VIIβ and its α-anomer on a preparative scale.

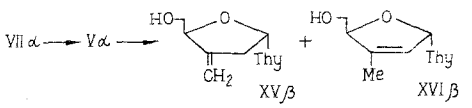
The configuration of the hydroxyl group in the 3'-position in the previously described D-threo-nucleoside VIIβ [33] was confirmed chemically (see scheme 2). Nucleosides VIIβ or VIIα were selectively mesylated at the 5'-position into VIIIβ or VIIIα. Their treatment with an aqueous alcoholic solution of NaOH led to closure of the 3,5'-O-anhydro ring with the formation of compounds IXα or IXβ, which was determined by the cis-disposition of the 3'-OH and 5'-CH_2OH groups.

The reaction of ketone IIα with MeMgI led to the formation of a mixture of D-erythro and D-threo-isomers of Xα and IIIα, which were separated by chromatography on silica gel (scheme 3). In this case the formation of a mixture of diastereomers with D-erythro-isomer predominating is due to the trans-disposition of the OMe and CH_2OBz groups. The incompletely protected sugars Xα and IIIα were later converted into dibenzoates XIα and IVα, respectively by heating with benzoyl chloride in N-methylimidazole.

The D-threo-sugars IIIα and IVα obtained, were transformed similarly to sugars IIIβ and IVβ, into nucleosides Vα, β or VIα, β. The stereoselectivity of the glycosylation reactions was thereby retained: during catalysis with $SnCl_4$ the β-anomer of V was preferentially formed from the incompletely protected sugar IIIα, and the α-anomer of VI from dibenzoate IVα (see Table 1).

The incompletely protected D-ertythro-sugar Xα was reacted with 2,4-bistrimethylsilylthymine [$(Me_3Si)_2Thy$] in the presence of $SnCl_4$ or $CF_3SO_3SiMe_3$, which resulted in the preferential formation of α-anomer of XII (see Table 1). In the condensations the yield of the incompletely protected sugars did not exceed 10%.

For the synthesis of 3'-C-methyl-2'-deoxythymidine XIVβ on a preparative scale, sugar Xα was benzoylated in N-methylimidazole into XIα, which was then (see scheme 3) condensed with $(Me_3Si)_2Thy$ using $SnCl_4$ as a catalyst. The β-anomer of nucleoside XIII was thus pre-



ferentially formed (see Table 1). The deblocking of the reaction mixture and subsequent crystallization from methanol made it possible to obtain the desired nucleoside XIVβ in a satisfactory yield.

3'-C-Methyl-2'-deoxynucleosides were found to be satisfactory starting compounds for the synthesis of 3'-branched unsaturated 2',3'-dideoxynucleosides: 3'-C-methylidene-2',3'-dideoxythymidine (XVβ) [27, 31] and 3'-C-methyl-2',3'-dideoxy-2',3'-didehydrothymidine (XIVβ) [12, 27, 28]. The good prospects for studying the biochemical properties of these compounds are confirmed by the fact that 3'-C-methylidene-2',3'-dideoxycytidine has antineoplastic activity with respect to human leukemic, adenocarcinoma and carcinoma cells [28].

For their synthesis, nucleosides VIIβ or VIIα were converted into 5'-monobenzoates Vβ or Vα (scheme 4). Their reaction with thionyl chloride led to two products identified as 3'-C-methylidene-2',3'-dideoxythymidine and 3'-C-methyl-2',3'-dideoxy-2',3'-didehydrothymidine 5'-O-benzoates. Deblocking of these compounds led to α-, or β-anomers of nucleosides XV or XVI.

Nucleosides XVI were found to be hydrolytically unstable and decomposed completely in aqueous solutions in the course of 24 h with the elimination of thymine. As known, 2',3'-dideoxy-2',3'-didehydrothymidine is also hydrolytically unstable in an acid medium [20]. The hydrolytic stability of its 3'-C-methylated analog is strongly decreased, which is explained by a positive inductive effect of the 3'-C-methyl group and by the simultaneous presence of a double bond.

TABLE 2. Values of Chemical Shifts in ^1H NMR Spectra of 2'-Deoxyfuranosides and Their 3-C-Methyl Derivatives

Compound	Chemical shift, ppm							
	H—1′	H—5′a	H—5′b	H—4′	OMe s	H—2′a	H—2′b	3—C—Me s
Iβ*	5,09 dd	4,56 dd	4,48 dd	4,18 m	3,26	2,27 g	2,11 g	—
Iα**	5,10 dd	4,37 m	4,37 m	4,37 m	3,39	2,19 g	2,05 g	—
IIβ	5,36 dd	4,65 dd	4,44 br.d	4,42 dd	3,38	2,81 dd	2,51 dd	—
IIα	5,37 d	4,75 dd	4,45 dd	4,26 br.g	3,46	2,67 dd	2,47 d	—
IIIβ	5,04 m	4,64 dd	4,46 dd	4,12 dd	3,39	2,09 m	2,09 m	1,43
IIIα	5,16 dd	4,67 dd	4,44 dd	4,07 br.t	3,38	2,37 dd	2,00 dd	1,44
IVβ	5,09 dd	4,82 dd	4,60 dd	4,32 dd	3,35	2,89 dd	2,46 dd	1,34
Xα	5,12 dd	4,43 dd	4,22 dd	4,38 br.t	3,40	2,10 m	2,10 m	1,38

Note. $CDCl_3$/TMS; asterisk – H-3' – 4.38 m; two asterisks – H-3' – 4.24 m.

TABLE 3. Values of Chemical Shifts in ^1H NMR Spectra of 3-C-Methyl-2'-deoxynucleosides

Compound	Chemical shift, ppm										
	NH m	H—6 g	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	Solvent
VIβ	8,89	7,28	6,27	4,94 dd	4,88 dd	4,22 dd	3,06	2,73	1,91	1,87	$CDCl_3$
VIα	9,04	7,10	6,05	4,71 dd	4,71 dd	4,90 dd	3,53	2,50	1,91	1,87	$CDCl_3$
VIIβ	—	7,87	6,09	3,94 m	3,94 m	3,94 m	2,19	2,62	1,89	1,42	D_2O
VIIα	—	7,44	6,07	3,78 dd	3,62 dd	4,13 dd	2,42	2,14	1,78	1,33	D_2O
IXβ	9,16	8,05	6,66	4,69 dd	4,59 d	4,08 dd	2,53	2,42	1,94	1,68	$CDCl_3$
IXα	9,64	7,15	6,70	4,38 dd	4,75 m	4,75 m	2,57	1,67	1,95	1,69	$CDCl_3$
XIIIβ	9,43	*	6,27	4,92 dd	4,70 m	4,70 m	3,40	2,20	1,98	1,66	$CDCl_3$
XIVβ	—	7,76	6,27	3,81 dd	3,70 dd	4,01 dd	2,63	2,18	1,89	1,41	D_2O

Note. Asterisk – the signal is overlapped by the solvent signal.

Prospective nucleosides for the investigation of the substrate specificity with respect to DNA-polymerases and reverse transcriptases were transformed into 5'-triphosphates (XVII-XVIII and XXI) by the action of $POCl_3$ in triethyl phosphate and then tetra-(tributylammonium) pyrophosphate in the presence of tributylamine [26] (for VIIβ, XIVβ, XIVβ), or by the triazole method [3] for XVβ.

Several 5'-phosphites of nucleosides are known to be less toxic and more selective inhibitors of the replication of viruses [4, 8]. Nucleosides VIIβ and XIVβ were therefore transformed into 5'-phosphites by treatment with PCl_3 in triethyl phosphate, since an attempt to prepare them by the conventional method using H_3PO_3 in the presence of N,N'-dicyclohexylcarbodiimide [8] led to a difficultly separable mixture of a number of products.

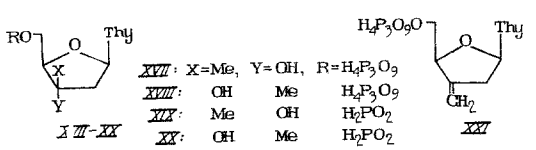


TABLE 4. Spin-spin Coupling Constants (SSCC) of 2'-Deoxy-3'-C-methyl-furanosides and Their Nucleosides

Compound	Chemical shift, ppm						
	1′, 2′a	1′, 2′b	2′a, 2′b	4′, 5′a	4′, 5′b	5′a, 5′b	$\frac{J_{1',2'b}}{J_{1',2'a}}$
Iβ*	1,5	4,6	—13,4	4,5	4,3	—10,1	3,1
Iα**	4,4	0,5	—13,7	—	—	—	0,1
IIβ	5,6	1,2	—18,1	2,7	1,0	—11,3	0,2
IIα	5,1	—	—18,3	2,9	4,0	—12,1	0,0
IIIβ	—	—	—	4,0	7,0	—11,9	—
IIIα	3,5	6,1	—14,3	4,8	6,2	—12,0	1,7
IVβ	2,4	6,0	—14,5	4,5	7,3	—12,0	2,5
Xα	2,0	3,5	—	4,2	3,9	—11,0	1,8
VIβ	3,4	7,7	—15,9	3,8	6,7	—12,2	2,3
VIα	6,8	7,7	—14,8	3,5	7,0	12,0	1,1
VIIβ	2,8	8,1	—15,0	—	—	—	2,9
VIIα	6,1	7,6	—14,5	3,5	7,5	—12,4	1,2
IXβ	3,0	8,0	—15,9	4,0	1,7	8,4	2,7
IXα	8,4	F 3	—13,8	4,6	—	—10,4	0,6
XIIIβ	5,2	9,0	—14,5	—	3,5	—14,5	1,7
XIIIα	5,8	3,9	...	...	...	...	0,7
XIVβ	5,7	9,3	—13,6	3,6	6,2	—12,3	1,6
XIVα	5,8	3,3	...	...	...	...	0,6

Note. The SSCC is absent; $J_{2'a,3'}$ 7.0 Hr, $J_{2'b,3'}$ 6.2 Hr; the constants were not obtained, since a spectrum of a mixture of anomers was obtained with the other anomer predominating; asterisk — $J_{2a',3'}$ 7.0 Hz, $J_{2'b,3'}$ 6,2 Hz; two asterisks — $J_{2'a,3'}$ 6.1 Hz, $J_{2'b,3'}$ 1.6 Hz.

PHYSICOCHEMICAL PROPERTIES

The structure of the compounds obtained was confirmed by the ^1H, ^{13}C, NMR, UV and CD spectroscopy data, elemental analysis and also by x-ray diffraction analysis for nucleoside XIVβ.

The UV spectra of the nucleoside analogs obtained did not differ substantially from the UV spectrum of 2'-deoxythymidine (no data are given).

In contrast to the natural nucleosides, the ^1H NMR spectra of 3'-C-methyl-2'-deoxynucleosides are characterized by the presence of two resolved ABX systems ($5'_a$, $5'_b$, 4' and $2'_a$, $2'_b$, 1'). The values of the chemical shifts for the main compounds obtained are listed in Tables 2-3, and the spin-spin coupling constants (SSCC) are given in Table 4.

The values of the chemical shifts were used to assign the pairs of anomers to an α- or β-series. For the anomeric sugars I, II, III, the proton signals at the C1 atom and the OMe group in the α-anomers appeared in weaker fields than in the β-anomers. For the nucleoside analogs this relationship was reversed, and the proton signal at C1' and of H-6 in the β-anomers in all cases was located in a weaker field.

The SSCC $J_{1',2'a}$ and $J_{1'2'b}$ values also enabled carrying out this assignment. For each pair of anomers, the ratio $J_{1'2'a}/J_{1'2'b}$ was higher for the β-anomer than for the α-anomer.

The ^1H NMR spectroscopy data made it possible to verify the furanose form of the methylated nucleosides obtained. In the spectra obtained in DMSO-D_6, in compound VIIβ, the signal of the tertiary hydroxyl proton was a singlet (δ = 5.28), and that of the primary hydroxyl — a triplet (δ = 5.0, $J < 0.5$ Hz). A similar pattern was observed in XIVβ.

The ^1H NMR spectrum of the unsaturated nucleosides XV and XVI were considerably more complex. Compounds XVα, β are characterized by the presence of signals of the methylidene group protons in the 5.3-5.4 ppm region (each signal is a pseudoquartet) and an immensely complex system of two resolved protons at C2' (total more than 40 lines). In the spectrum of nucleosides XVIα, β the characteristic signals of two protons at C2' in the 1.5-3.5 ppm region were absent, and a multiplet of proton 2' at the double bond appeared in the 4.8 ppm region. The spectral characteristics of these compounds are given in the experimental part.

Finally, the ^1H NMR data enabled carrying out a conformational analysis for the 3'-C-methyl analog XIVβ in solution, based on the fact that the introduction of the methyl group does not substantially affect the accuracy of the calculation of the S-N equilibrium [13]. Since the values of SSCC $J_{3',4'}$ are required for the calculations, they were carried out starting from the sum of $J^{1',2'}a$ and $J_{3',4'}$ constants (where $J_{1',2'_a}$ – trans-SSCC), which, for the natural 2'-deoxythymidine were equal to 6.1 and 5.5 Hz, respectively. The following formulas were used for the calculation:

$$^{S}X=(J_{1',2''_a}/J_{1',2'_a}+J_{3',4'}), \quad (1)$$

$$N_X+{}^{S}X=1. \quad (2)$$

For the calculation of the molar fractions of the rotamers with respect to the C-4'–CH_2OH bond the following relationships were used:

$$P_+=13-(J_{4',5'_a}+J_{4'5'_b})/10, \quad (3)$$

$$P_a=(J_{4',5'_b}-1{,}5)/10, \quad (4)$$

$$P_-=(J_{4',5'_b}-1{,}5)/10, \quad (5)$$

$$P_++P_a+P_-=1, \quad (6)$$

where ^{S}X and ^{N}X are the molar fractions of the S and N conformers, respectively, $J_{1',2''_a}$ is the SSCC of compound XIVβ; $J_{1',2'_a}$ is the SSCC of thymidine; $P_{+\pm}$, P_a, P_- are the molar fractions of gauche-gauche, gauche-trans and trans-gauche rotamers, and the weakest field 5' proton was assumed to be the $5'_a$ proton.

The values for compound XIVβ determined according to these formulas are equal to: ^{S}X = 0.80; ^{N}X = 0.20; P_+ = 0.32, P_a = 0.47; P_- = 0.21. The corresponding values for the natural 2'-deoxythymidine are: ^{S}X = 0.53; ^{N}X = 0.47; P_+ = 0.62, P_a = 0.13; P_- = 0.25.

The introduction of the relatively bulky methyl group into the 3'-position probably leads to interference with the conformational transitions of the sugar residue.

The steric structure of the molecules of 3'-C-methyl-2'-deoxythymidine was finally established by an x-ray diffraction analysis [2]. The crystals studied were grown from a saturated solution of XIVβ in methanol and were free from the solvent molecules.

The molecules of XIVβ in a crystal are characterized by a C2'-endo-C1'-exo-conformation of the furanose ring with deviations of the C2' atom from the C3'C4'O4' plane in the direction of N1 and C5' by 0.521 Å and of the C1' atom in the opposite direction by 0.063 Å. The conformation of the molecules relative to the exocyclic C4'-C5' bond is gauche$^+$. In the natural 2'-desoxythymidine a trans-conformation is observed relative to the C4'-C5' bond and a C2'-endo-C3'-exo-conformation of 2-deoxyribose with deviation of the C3' and C2' atoms from the eing plane of the sugar by 0.42 Å and 0.18 Å, respectively [34]. As in the natural 2-deoxythymidine, XIVβ is present in an anti-conformation relative to the N-glycoside bond.

The conformation of the molecules of XIVβ is on the whole virtually the same as the conformation of the molecules of 3'-C-methylcytidine [16], which is a substrate and effective terminator of the RNA polymerases. Comparison of these two structures makes it possible to establish general patterns of the steric organization of the molecules of 3'-C-methylated pyrimidine nucleosides, which are independent of the nature of the sugar and the nucleic base. These patterns should include: firstly, the preferential endo-disposition of the C2' atoms of the furanose rings, taking out into the case an equatorial position, as a result of which the possible steric hindrances between the atoms of the nucleic bases and the atoms of the relatively bulky 3'-C-methyl group decrease; secondly, an anti-conformation around the glycoside bond and gauche$^+$ conformation relative to the exocyclic C4'–C5' bond; thirdly, elongation of the C3'–O3' bond, accompanying the transition from a secondary (in natural nucleosides) to the tertiary 3'-hydroxyl group in 3'-C-methyl derivatives, which leads to a change in their reactivity and considerably influences the activity of these compounds in biological transormations.

TABLE 5. Inhibition of Synthesis of DNA by XVII, XVIII, and XXI During the Catalysis of the Process by DNA Polymerase 1 (A) and Reverse Transcriptase FMV (B)

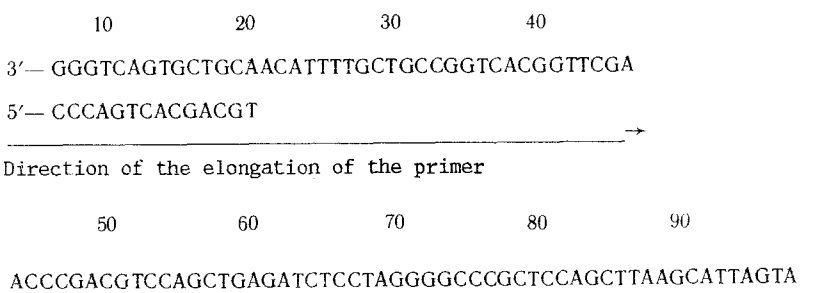
Compound	Ratio of compound/dTTP, corresponding to 50% inhibition	
	A	B
XVII	400	750
XVIII	500	400
XXI	400	40

The data obtained during an x-ray structural analysis and the data of calculations carried out on the basis of ^{1}H NMR spectra are in good agreement.

Enzymology. An investigation was made of the properties of compounds XVII, XVIII, and XXI in noncellular systems with DNA polymerases: I, the Klenov fragment from E. coli, α and ε from human placenta, β from rat liver, terminal deoxynucleotidyl-transferase from calf thymus, and also with reverse transcriptases from fowl myeloblastosis virus (FMV) and the human immunodeficiency virus (HIV-1). A system was used from the DNA matrix of phage 13, mp 10, and of deoxytetradecanucleotide complementary to this matrix (scheme 5), which were preliminarily bound to one another.

For compounds XVII and XVIII is was shown that none of the enzymes incorporated then in the DNA chain, although it has been observed, that, firstly, the synthesis of the DNA chain is generally inhibited; and secondly in the scheme of the synthesis in PAAG pauses were observed on the nucleotide residues preceding the 2'-deoxythymidilic acid (no data are given). The two compounds thus exhibit the properties of inhibitors, concurrent with respect to dTTP, but they do not form a productive complex. Their affinity to DNA-polymeases in low (Table 5).

At the same time compound XXI displayed terminatory properties with two reverse transcriptases (in Fig. 1, as an example, the scheme of the synthesis is shown catalyzed by the reverse transcriptase FMV and DNA polymerase β), but was not incorporated in the DNA chain by DNA polymerases α, ε, and I (nodata aregiven). Table 5shows in fact that compound XXI has



low affinity for DNA polymerase I, but has a 10 times higher affinity for the reverse transcriptase from FMV.

The analysis of the photograph of the gel in Fig. 1 shows that the strips of all the DNA fragments end with a thymidylic acid residue (T39, T40, T45, T51, T56, T58, etc. - see Fig. 1). These data unequivocally illustrate the terminatory properties of XXI.

In the case of the terminal deoxynucleotidyltransferase, XXI is also included in the chain of the priming tetradecanucleotide, lengthening it by one unit (Fig. 2, tracks 1, 2). The resulting pentadecanucleotide is not even later eluted, while the initial tetradecanucleotide present in the initial mixture completely disappears when dTTP is subsequently introduced, being converted into a polymeril compound (tracks 3, 4). Tracks 5-8 are controls for these tracks.

Experiments with HIV-1 Infected Cells. The ability of XIVβ, VIIβ, XIX and XX to inhibit the production of HIV-1 was studied on a culture of MT-4 cells as described in [9]. It is

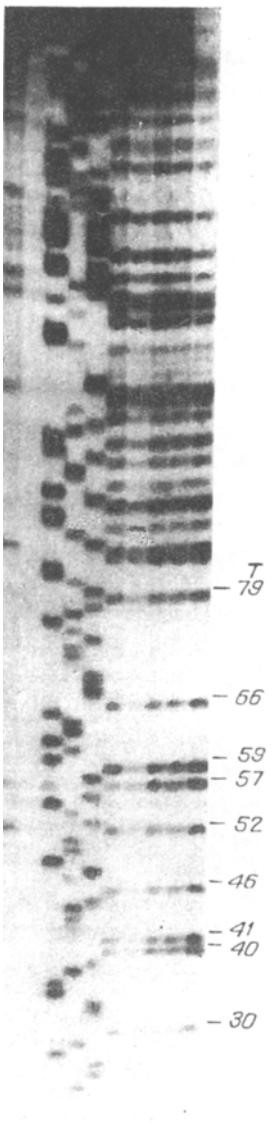
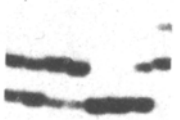


Fig. 1. Electrophorogram of a elongation of DNA in the presence of XXI catalyzed by RT FMV. Tracks: 1) synthesis in a complete system without terminatory nucleosides; 2) the same with subsequent overtaking; 3, 4, 5, 6) synthesis in the presence of controlling terminatory substrates 2',3'-dideoxy-nucleotide-5'-triphosphates (ddNTP) (3) with ddATP, 4) with ddGTP, 5) with ddCTP, 6) with ddTTP). 7, 8, 9, 10) The same as track 2, but into the first incubation, besides the natural substrates, XXI was introduced in a concentration of 200 μM (track 7), 400 μM (track 8), 800 μM (track 9) and 1600 μM (track 10).



1 2 3 4 5 6 7 8

Fig. 2. Electrophorogram of elongation of tetradecadeoxyoligonucleotide in the presence of terminal deoxynucleotidyltransferase. Tracks: 1, 2) synthesis in the presence of XXI, concentration 10 μM (track 1) and 100 μM (track 2). 3, 4) The same as tracks 1, 2, with subsequent elongation in the presence of 10 μM of dTTP. 5, 6, 7, 8) Controls: 5) [^{32}P]tetradecadeoxyoligonucleotide; 6) the same with addition of enzyme and dTTP, 3 μM (track 7) and 10 μM (track 8).

TABLE 6. Action of 3'-C-Methyl-2'-deoxythymidine XIVβ, its Threo-isomer and the 5'-Phosphites XIX and XX Thereof on the Production of HIV-1 in the Culture of MT-4 Cells

Compound	Concentration inhibiting the production of virus, μM	Concentration inhibiting the growth of the cells by 50%, μM
XIVβ	>500	>500
XIX	72	>500
VIIβ	>500	>500
XX	243	>500

seen from Table 6 that all the compounds are virtually inactive, although the 5'-phosphite of D-erythro derivative XIx nevertheless inappreciably inhibits the production of the virus, but these properties are of no practical interest.

DISCUSSION

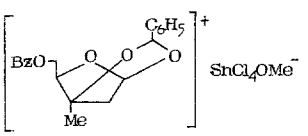
Synthesis. The scheme of synthesis of 3-C-alkyl-2-deoxysaccharides and their nucleosides proposed in the present work is a comprehensive one and enables the preparation of compounds having both the D-erythro- and the D-threo configuration.

A method has been developed for the protection of the slightly reactive tertiary hydroxyl groups of sugars, which is based on heating partially protected sugars with an acyl chloride in N-methylimidazole. This method makes it possible to obtain considerably higher yields of dibenzoates of 3-C-sugars, than by the presently existing methods [19, 29].

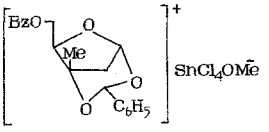
To confirm the fact that the reaction of ulosides IIα and IIβ similarly proceeds with other Grignard compounds, we studied the stereoselectivity of the addition of EtMgBr to them. The stereodirectivity of the addition was retained (no data are given), and this example illustrates the universality of the proposed method.

The data obtained from the condensation of 3-C-methylsugars IIIα, β; IVα, β, Xα, XIα with 2,4-bistrimethylsilylthymine showed a regularity in the stereoselectivity of the glycosylation reaction. The directivity of these reactions for incompletely protected sugars IIIβ, and Xα and Xα did not depend on the nature of the catalyst and the initial configuration of the glycoside center, but was determined exclusively by the configuration of the 3 -hydroxyl group: Nucleosides with the cis-disposition of the nucleic base and the 3'-OH group were found to be the predominating form. In the case of the completely protected sugars IVα, IVβ and XIα, the stereodirectivity was determined by the catalyst employed. Trimethylsilyl triflate was found to be nonselective and its use resulted in the formation of a mixture of α- and β-anomers of the nucleosides in approximately equal reations. The use of $SnCl_4$ made possible the selective formation of the α- and β-anomers of nucleosides; compounds with a

trans-disposition of the benzoyloxy group in the 3'-position and nucleic base were preferentially obtained. Thus, the ratio between the α- and β-anomers of nulceosides XIII obtained during their synthesis of the D-etythro-sugar XIα changed to a reversed ratio compared with the corresponding ratio during the synthesis of nucleosides VI from D-threo-sugars IVα, β (see Table 1). The high selectivity of the glycosylation reaction thus observed can be explained by the stereocontrolling influence of the benzoyloxy group at the 3'-position and the formation of intermediate protected sugar-catalyst complexes (IVα, β $SnCl_4$, XIα $SnCl_4$) with a different steric structure:



IVα,β · $SnCl_4$



IVα · $SnCl_4$

In the present work, we have also developed a method of synthesis of 5'-phosphites of 3'-C-methyl-2'-deoxynucleosides XIVβ and VIIIβ by treatment with PCl_3 in triethyl phosphate, and subsequent hydrolysis. This method has advantages for these compounds, compared with the synthesis using H_3PO_3 in the presence of N,N-dicycloexyl carbodiimide [8], since the formation of a difficultly separable mixture consisting of 5'-phosphites, 3'-phosphites, 3'-5'-diphosphites, 3'-C-methyl-2'-deoxynucleosides and several other unidentified products can be avoided.

Enzymology. The study of the compounds obtained in noncellular systems with DNA-polymerases showed that 3'-C-methyl-2'-deoxythymidine 5'-triphosphate (XVII) exhibits the properties of an inhibitor competing with dTTP, but is not incorporated in the DNA chain, and hence, does not form a productive complex. This property of XVII appears to us very interesting. This compound contains all the functional groups of dTTP, and nevertheless is not utilized by DNA-polymerases as a substrate. We assume that the influence of the 3'-C-methyl group on the properties of XVII is realized through the substantial changes in its conformational mobility. In fact the NMR spectroscopy data and the x-ray diffraction analysis of XIVβ showed that its structure is fairly rigid and the transition of the conformation of the sugar residue of XIVβ into a planar structure is hindered. This situation supports the hypothesis on the compressed conformation of the 2'-deoxyribose residue in the substrates in the productive complex with DNA-polymerases [17, 18]. The probability of this hypothesis is also indicated by the activity of the terminatory substrates with a compressed structure of the sugar residue, such as 2',3'-dideoxy-2',3'-didehydronucleoside 5'-triphosphates [15], 2',3'-riboanhydro and 2',3'-lyxoanhydronucloside 5'-triphosphates [24, 25]. At the same time, 3'-C-methylidene-2',3'-dideoxythymidine 5'-triphosphate (XXI) exhibited the properties of a terminatory substrate for certain DNA-polymerases, although these properties are weakly expressed. The ability of this compound to be recognized by reverse transciptases of retroviruses and not to be recognized by DNA-polymerases α and ε conforms with the rule established by us for the lowest specificity of reverse transcriptases in comparison with other DNA-polymerases [6, 11, 24].

EXPERIMENTAL

The NMR spectra were run on a Bruker WM-250 spectrometer (250 MHz for ^1H and 62.8 MHz for ^{13}C), 20°C, $CDCl_3$, CD_3OD or D_2O, using TMS, tert-BuOH or DCC, respectively, as internal standards. Accepted designations: s) singlet; d) doublet; t) triplet; q) quartet; pt) pseudotriplet; pq) pseudoquartet; dd) doublet of doubles; br. s) broadened singlet; m) multiplet. The UV spectra were taken on a Specord UV-M-40 spectrophotometer, the CD spectra on a Jobin Ivon dichrograph in water or methanol. The x-ray diffraction analysis was carried out on a CAD-4 diffractometer. The HPLC was carried out on a Gilson chromatograph at 268 nm, using 4.6 × 250 mm columns, a Silasorb C-18 carrier (5 μm), at a flow rate of 1 ml/min. The nucleosides were isolated in buffers: A) 0.04 M $Et_3NH_2CO_3$, pH 7.4, B) 50% MeCN in water, gradient 5 → 30%, 20 min. For the nucleoside 5'- triphosphates B, 5 > 10% was also used. The TLC, including the preparative variant, was carried out on Merck plates in systems: C) chloroform—EtOH, 97.3, D) chloroform—EtOH, 9:1. The column chromatography was carried out on L (40/100) silica gel (CSFR).

Synthesis. 1-Methyl-5-O-benzoyl-2-deoxy-α-D-ribofuranoside (Iα) and Its β-anomer. A 3 ml portion of acetyl chloride was dissolved in 250 ml of abs. MeOH, and after 5 min 20 g (149.1 mmoles) of 2-deoxy-D-ribose was added with stirring to the solution, and then after 15 min 10 ml of absolute pyridine was added. The solution was evapored to dryness, and thrice reevaporated with 20 ml of pyridine. The residue was dissolved in 50 ml of absolute pyridine, and a solution of 17.3 ml (148.9 mmoles) of benzoyl chloride in 30 ml of dichloroethane was added at −5°C in the course of 1 h. The reaction mixture was allowed to warm up to 0°C, was poured into 500 ml of ice water and extracted with chloroform (5 × 100 ml). The combined chloroform extracts were washed with 1% HCl in water (5 × 100 ml), a saturated $NaHCO_3$ solution (2 × 100 ml), water (2 × 100 ml), dried over Na_2SO_4 and evaporated. The compounds were separated on a column with silica gel, eluting with EtOH in chloroform (0 → 5%). Yield after the evaporation, 6.02 g (16%) of Iα ($C-R_f$ 0.24) and 8.07 g (21.5%) of Iβ ($C-R_f$ 0.18) − yellowish syrups. Sugar Iβ crystallized after prolonged standing in cold. (Mp 63-64°C), ^{13}C NMR spectra in $CDCl_3$. Iα, δ: 105.3 (C1), 84.5 (C4), 72.8 (C3), 64.5 (C5), 54.7 (OMe), 40.9 (C2), Iβ δ: 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-glyceropentofuranos-3-uloside (IIα) and Its β-anomer (IIβ). A 10 ml portion of absolute pyridine, 5 ml of acetanhydride, and 6 g (60.0 mmoles) of freshly dried CrO_3 were dissolved in 140 ml of absolute CH_2Cl_2 and the mixture was stirred for 30 min. A 5 g portion (19.8 mmoles) of Iα or Iβ in 10 ml of absolute CH_2Cl_2 was added to the solution obtained in the course of 5 min. After 5 min 300 ml of ethyl acetate was added, and the solution was filtered through a silica gel, evaporated to 50 ml, washed with 50 ml of a saturated $NaHCO_3$ solution, and then with 50 ml of water. The organic layer was dried over Na_2SO_4, then evaporated, and the residue was dissolved in 20 ml of ethyl acetate. The solution was allowed to stand for 1 h at 0°C and was filtered again through silica gel. The silica gel was washed with 50 ml of ethyl acetate, and the combined solutions were evaporated in vacuo. Yield, 4.84 g (97.6%) of uloside IIα or IIβ − yellowish syrups. The ulosides are stored for not more than one week.

Methyl-5-O-benzoyl-3-C-methyl-2-deoxy-β-D-threo-pentofuranoside (IIIβ). A 1.65 g portion (6.6 mmoles) of uloside IIβ was dissolved in 220 ml of absolute ether, the solution was cooled to 0°C and solution of MeMgI [obtained from 1.7 g (70.8 mmoles) of magnesium, 4.0 ml (62.6 mmoles) of MeI in 50 ml of ether] cooled to 0°C was added with stirring in the course of 5 min. The reaction mixture was stirred for 10 min, a cooled mixture of NH_4Cl, 360 ml of water, and 200 ml of ether were added, and the mixture was stirred for 10 min. The ether layer was separated, washed with water (2 × 500 ml), dried over Na_2SO_4, and evaporated in vacuo. Yield, 1.54 g (83%) of yellowish syrup. ^{13}C NMR spectrum of IIIβ in $CDCl_3$, 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'-desoxy-α-D-threo-pentofuranosyl)thymine (VIIα) and Its β-anomer (VIIβ). A 100 mg portion (0.38 mmole) of IIIβ or IIIα was dissolved in 4 ml of absolute dichloroethane, 0.5 ml (1.82 mmole) of 2,4-bistrimethylsilylthymine and 0.50 ml (1.96 mmole) of $CF_3SO_3SiMe_3$ were added, and the mixture was boiled for 30 min. It was then cooled, and 10 ml of a saturated $NaHCO_3$ solution was added. The mixture was stirred for 30 min, and filtered through silica gel. The residue on the filter was washed with 10 ml of a saturated $NaHCO_3$ solution and 20 ml of dichloroethane. The organic layer was separated, dried over Na_2SO_4 and evaporated. The residue was dissolved in 12 ml of a 0.5 M solution of MeONa in MeOH, the solution was stirred for 1 h at 20°C, and 5 ml of MeoH and then Dowex-50 (H^+) were added to pH 5.0. The mixture was filtered, the residue on the filter was washed with 5 ml of MeOH and 5 ml of water, and the filtrates were evaporated in vacuo. The residue was separated by preparative TLC in system D (two elutions) and after evaporation and lyophilization from water 12.5 mg (12.9%) of VIIβ (D−R_f 0.35) and 2.6 mg (2.7%) of VIIα (D − R_f 0.23) were obtained. ^{13}C NMR spectra in D_2O. VIIα, δ: 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). VIIβ, δ: 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 in methanol: VIIIα-λ_{max}, 273 nm ($\Delta\varepsilon$ = −1.1); VIIβ-λ_{max}, 272 nm ($\Delta\varepsilon$ = +1.3).

Methyl-3,5-di-O-benzoyl-3-C-methyl-2-deoxy-α-D-threo-pentofuranoside (IVα), its β-anomer (VIβ) and methyl-3,5-di-O-benzoyl-3-C-methyl-2-deoxy-α-D-erythro-pentofuranoside (XIα). A 0.48 g portion (1.80 mmole) of IIIα or IIIβ or Xα was dissolved in 4.3 ml of N-methylimidazole, 0.26 ml of benzoyl chloride was added and the mixture was heated at 150°C for 10 min with stirring. The reaction mixture was cooled to 20°C, a further 0.26 ml of benzoyl chloride was added, and the mixture was heated for 10 min at 150°C. The operation of cooling, addi-

tion of benzoyl chloride and heating was then repeated for the third time. Altogether, 0.78 ml (6.71 mmoles) of benzoyl chloride was added. After cooling, the reaction mixture was poured onto 10 g of ice, and the mixture was extracted with chloroform (3 × 5 ml). The combined chloroform extracts were washed with 1% HCl in water (2 × 5 ml), a saturated $NaHCO_3$ solution (2 × 5 ml), and water (2 × 5 ml), dried over Na_2SO_4 and evaporated. The compounds were separatred by column chromatography in a chloroform gradient in hexane (50 → 100%). Yield, 0.42 g (63%) of IVα or 0.36 g (54%) of IVβ and 0.42 g (63.0%) of XIα – yellowish syrups.

1-(3'-C-Methyl-2'-deoxy-α-D-threo-pentofuranosyl)thymine (VIIα) and Its β-anomer (VIIβ) Method A. A 120 mg portion (0.32 mmole) of dibenzoate IVβ or IVα was dissolved in 3 ml of dichloroethane, 0.25 ml (0.91 mmole) of 2,4-bistrimethylsilylthymine and then 0.25 ml (2.14 mmoles) of $SnCl_4$ in 0.3 ml of dichloroethane were added, and the mixture was boiled for 15 min. After cooling, 15 ml of a saturated $NaHCO_3$ solution was added, and the procedure was continued as described for the synthesis of nucleosides VIIα, β from sugars IIIβ or IIIα. After the separation of the anomers of VII by preparative TLC, 8 mg (9.6%) of VIIβ and 43 mg (52%) of VIIα were obtained.

Method B. A 1 ml portion (3.64 mmoles) of 2,4-bistrimethylsilylthymine and then a solution of 0.50 ml (1.96 mmole) of $CF_3SO_3SiMe_3$ in 3 ml of dichloroethane were added to a solution of 530 mg (1.43 mmole) of IVβ or IVα in 10 ml of dichloroethane and the mixture was boiled for 1 h. After cooling, 15 ml of a saturated $NaHCO_3$ solution was added, the mixture was stirred for 30 min and filtered through silica gel. The residue was washed with 5 ml of a saturated $NaHCO_3$ solution and then with 15 ml of dichlorethane. The organic layer was wahed with water (2 × 5 ml), dried over anhydrous sodium sulfate, and evaporated. The syrup obtained was treated with 6 ml of a 0.05 M MeONa solution in MeOH. The mixture was allowed to stand for 1 h at 20°C, and after adding 30 ml of methanol, was neutralized with Dowex-50 (H^+) to pH 5.0 and filtered. The filtrate was washed with 15 ml of water and 15 ml of methanol, concentrated to a volume of 10 ml, and extracted with chloroform (5 × 1 ml). The aqueous layer was evaporated to yield 320 mg (87%) of a mixture of VIIα, β. After separation by preparative TLC, as described in the synthesis of nucleosides VIIα, β, and evaporation, 126 mg (34.2%) of VIIβ and 174 mg (47.3%) of VIIα was obtained.

1-(3'-C-methyl-2'-deoxy-3',5'-O-anhydro-α-D-threo-pentofuranosyl)thymine (IXα) and Its β-anomer (IXβ). A 0.045 ml portion (0.58 mmole) of mesyl chloride was added to a solution of 74 mg (0.29 mmole) of nucleoside VIIβ or VIIα in 0.5 ml of absolute pyridine. The mixture was stirred for 15 min at 20°C, and after adding 1 ml of a saturated solution of $NaHCO_3$. was evaporated to dryness. The residue was extracted with chloroform (10 × 3 ml), the solution was filtered off, evaporated and reevaporated with toluene (3 × 3 ml). To the residue, a solution of 0.3 ml of a 0.1 M NaOH, and 0.7 ml of water in 3 ml of ethanol was added and the mixture was boiled for 15 min. After cooling, the reaction mixture was neutralized with Dowex-50 (H^+) to pH 6.0, filtered, and the residue on the filter was washed with 5 ml of ethanol and 5 ml of water. The filtrate was evaporated, to the residue 3 ml of benzene and 0.5 ml of absolute EtOH were added, and the solution was evaporated. The product was isolated by preparative TLC in system C. It was eluted from silica gel by a mixture of chloroform and ethanol (1:1), the solution was filtered and evaporated to dryness. The residue was extracted with chloroform (5 × 1 ml), and evaporated. Yield, 53 mg (76.4%) of IXα (D–R_f 0.74) or IXβ (D–R_f 0.80).

Methyl-5-O-benzoyl-3-C-methyl-2-deoxy-β-D-threo-pentofuranoside (IIIα) and Methyl-5-O-benzoyl-3-C-methyl-2-deoxy-α-D-erythropentofuranoside (Xα). A 1.65 g portion (6.6 mmoles) of IIα was dissolved in 220 ml of ether and the procedure was then continued as described in the synthesis of compound IIIβ. The mixture of diastereomers IIIα and Xα was separated by column chromatography in system C. Yield 0.55 g (31.2%) of IIIα (C–R_f 0.27) and 0.90 g (51.2%) of Xα (C–R_f 0.36). ^{13}C NMR spectrum of Xα in $CDCl_3$, δ: 103.8 (C1), 81.7 (C4), 77.0 (C3), 63.1 (C5), 55.3 (OMe), 49.2 (C2), 31.7 (Me–C3).

1-(3'-C-Methyl-2'-deoxy-β-D-erythro-pentofuranosyl)thymine (XIVα) and Its β-anomer Method A. A 100 mg portion (0.38 mmole) of Xα was dissolved in 4 ml of absolute dichlorethane and the procedure was then continued as described in the synthesis of compounds VIIα, β. Yield 20 mg of a mixture (20.7%) containing 80% of XIVα and 20% of XIVβ (the ^1H NMR spectroscopy data), which was not separated into the individual anomers.

Method B. A 120 mg protion (0.32 mmole) of dibenzoate XIα was dissolved in 3 ml of dichloroethane, 0.25 ml (0.91 mmole) of 2.4-bis-trimethylsilylthymine, and then 0.25 ml

(2.14 mmoles) of $SnCl_4$ in 0.3 ml of dichloroethane were added, and the procedure was continued as described in the synthesis of nucleosides VIIα, β from sugars IIIβ or IIIα. After deblocking, 60 mg (73.2%) of unpurified mixture was obtained, crystallization of which from 0.2 ml MeOH gave 30 mg (36.6%) of XIVβ (D–R_f 0.20) (mp 182-183°C), which were then further used for the x-ray diffraction analysis, and after the purification by HPLC from a 2'-deoxythymidine impurity, for the preparation of 5'-triphosphates and 5' hydrophosphonates. ^{13}C NMR spectrum of XIVβ in 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. A 120 mg portion (0.32 mmole) of XIα was dissolved in 3 ml of dichloroethane, 0.25 ml (0.91 mmole) of 2,4-bistrimethylsilylthymine and 0.25 ml (1.3 mmole) of $CF_3SO_3SiMe_3$ in 1 ml of dichloroethane were added, and the mixture was boiled for 1 h. To the solution, 15 ml of saturated $NaHCO_3$ solution was added, the mixture was stirred for 30 min, then was filtered through silica gel, and the residue was washed with 5 ml of a saturated $NaHCO_3$ solution, and with 15 ml of dichloroethane. The organic layer was washed with water (2 × 5 ml), dried over Na_2SO_4, and evaporated. The syrup obtained was treated with stirring for 1 h at 20°C with 6 ml of a 0.5 M solution of MeONa in MeOH, 30 ml of MeOH was added, and the mixture was neutralized with Dowex-50 (H^+) to pH 5.0 and filtered. The residue on the filter was washed with 15 ml of water and 15 ml of MeOH, concentrated to a volume of 10 ml and extracted with chloroform (5 × 1 ml). The aqueous layer was evaporated in vacuo to yield 71 mg (87%) of a mixture of XIVα and β containing according to the ^1H NMR spectrosocpy data 58% of XIVα and 42% of XIVβ, which was not separated into individual anomers.

1-(5'-O-Benzoyl-3'-C-methyl-2'-deoxy-α-D-threo-pentofuranosyl)thymine (Vα) and Its β-anomer (Vβ). A 386 mg portion (1.5 mmole) of nucleoside VIIα or VIIβ was dissolved in 3 ml of absolute pyridine, and 0.27 ml (2.3 mmoles) of benzoyl chloride was added. After 10 min, 10 ml of a saturate $NaHCO_3$ solution was added with stirring and the mixture was evaporated to dryness. The residue was extracted with chloroform (5 × 5 ml), the solution was filtered and evaporated again. The residue obtained was reevaporated 5 times with a mixture of 5 ml of toluene and 1 ml of absolute EtOH to yield 512 mg (95%) of Vα or Vβ as colorless syrups.

3'-C-Methylidene-2',3'-dideoxythymidine (XVβ), 3'-C-methyl-2',3'-dideoxy-2',3'-didehydrothymidine (XVI) and Their α-anomers (XVα), (XVIα). A solution of 200 mg (0.56 mmole) of Vα or Vβ in 2 ml of $SOCl_2$ was boiled for 2 min, was then cooled to –20°C and 15 ml of a cold saturated $NaHCO_3$ solution was added. The mixture was evaporated, the residue was extracted with chloroform (5 × 5 ml) and the solution was evaporated. To the residue 5 ml of a saturated solution of NH_3 in MeOH was added, the mixture was allowed to stand for 24 h at 20°C and evaporated. The mixture of nucleosides XVα, XVIα or XVβ, XVIβ obtained was separated by preparative HPLC, eluting with 10% MeOH in water. The fractions containing nucleosides were evaporated quickly at room temperature. Yield, 15 mg (11%) of XVα or XVβ and 45 mg (34%) of XVIα or XVIβ – colorless syrups. ^1H NMR spectra in CD_3OD, XVβ, δ: 8.01 q (1H, H6), 6.34 pt (1H, $J_{1',2'}$ 68 Hz, H1'), 5.43 pq (1H, 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, $J_{5'4'}$ 2.9 Hz, $J_{5'a,5'b}$ – 11.1 Hz, H5'$_a$), 3.96 dd (1H, $J_{5'b,4'}$ 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). XVIβ, δ: 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 Salts) (XVII). A 25.7 mg portion (0.1 mmole) of erythro-nucleoside XIVβ, preliminarily purified by HPLC was dissolved in 0.4 ml of triethyl phosphate, 18 μliter (0.19 mmole) of $POCl_3$ was added at 0°C, and the mixture was allowed to stand for two days at 0°C. A cold (0°C) solution of 0.14 ml of tributylamine in 0.55 ml of a solution of 0.5 M tetra(tributylammonium) pyrophosphate in DMF was added. The reaction mixture was held for 30 min at 20°C, neutralized with 1 M $Et_3NH_2CO_3$ to pH 7.5, evaporated at 20°C, and reevaporated thrice with 5% aqueous EtOH. The residue in 300 ml of water was deposited on a column with DEAE-cellulose (DE-32, Whatman). The compounds were eluted with NH_4HCO_3 (pH 7.5) (0 → 0.5 M), total volume 2 liter): the 5'-triphisphate XVII was eluted with 0.3 M buffer. The fraction containing XVII was evaporated at 20°C, and was reevaporated 5 times with 5% EtOH in water. After lyophilization 29 mg (58.5%) of XVII was obtained.

1-(3'-C-methyl-2'-deoxy-β-D-threo-pentofuranosyl)thymine 5'-triphosphate (Ammonium Salt) (XVIII). A 25.7 mg portion (0.1 mmole) of VIIβ was dissolved in 0.4 ml of triethyl phosphate and the procedure was continued as described for the synthesis of XVII. After lyophilization 13 mg (26.1%) of XVIII was obtained.

1-(3'-C-Methyl-2'-desoxy-β-D-erythro-pentofuranosyl)thymine 5'-phosphite (Ammonium Salt) (XIX). A 25.7 mg portion (0.1 mmole) of XIVβ was dissolved in 375 μliter of triethyl phosphate. The mixture was cooled to 0°C, and after adding 20 μliter (0.23 mmole) of pCl_3, was held at 0°C for 24 h, neutralized with 1 M $Et_3NH_2CO_3$ to pH 7.5, and then the procedure was continued as described for the synthesis of XVII. The isolation was carried out using a 0.25 M solution of NH_4HCO_3, whereby the desired fraction was obtained by concentrating a 0.07 M buffer solution of the product. After lyophilization 28 mg (82.7%) of XIX was obtained.

1-(3'-C-methyl-2'-deoxy-β-D-threo-pentofuranosyl)thymine 5'-phosphite (Ammonium Salt) (XX). A 25.7 mg portion (0.1 mmole) of VIIβ was dissolved in 600 μliter of triethyl phosphate and the procedure was then continued as described for the synthesis of XIX. After lyophilization 20 mg (62.5%) of XX was obtained.

3'-C-Methylidene-2',3'-dideoxythymidine 5'-triphosphate (XXI). A 28 mg portion (0.4 mmole) of 1,2,4-triazole was thrice reevaporated with 5 ml of acetonitrile, the residue was dissolved in 0.6 ml of acentonitrile and 55 μliter (0.4 mmole) of triethylamine was added. To the solution obtained 12 μliter (0.13 mmole) of $POCl_3$ was added at 0°C, the mixture was allowed to stand for 40 min at 20°C, the precipitate was separated by centrifugation and the solution was added to 8 mg (0.03 mmole) of nucleoside XVβ. The reaction mixture was stirred for 40 min at 20°C, and 0.5 ml of a 0.5 M tetra(tributylammonium) pyrophosphate solution in DMF was added, the mixture was stirred for 30 min at 20°C and then the procedure was continued as described for the synthesis of XVII. Yield, 2.1 mg (15%) of XXI, which was purified by HPLC.

Experiments in Noncellular Systems. The DNA of phage M13 mp 10 (+chain) was isolated from the culture liquid of E. coli R12 XXI as described in [5]. The following enzymic T4 were used: polynucleotidylkinase (Amersham), the terminal deoxynucleotidyltransferase from a calf thymus (Amersham), DNA polymerase I, a Klenov fragment from E. coli (Amersham), a reverse transcriptase from FMV (the Omutninsk Chemical Works), DNA polymerases α and ε from human placentas and DNA polymerase β from a rat liver (kindly supplied by D. Yu. Mozzherin and A. A. Atrazhev of the Institute of Molecular Biology, Moscow), the reverse transcriptase HIV was kindly supplied by Dr. W. Igo (the National Cancer Institute, Bethesda, USA). The radioactive nucleoside trihosphates ("Radioisotope" Tashkent): [γ-^{32}P] FTZ 2000 Ci/mmole, [α-^{32}P] dATP 400 Ci/mole. The 14-unit oligonucleotide primer (see scheme 5) was labelled at the 5'-position with ^{32}P, as recommended in [5], and after reprecipitation with ethanol, was used in the polymerase reactions. The elongation of the [5'-^{32}P] oligonulceotides in the presence of natural dNTP or their analogs was studied under the conditions described in [10].

Matrix-dependent Elongation of [5'-^{32}P] Primer, Catalyzed by DNA-polymerases. The incubation mixtures (5-6 μliter) contained: (i) for DNA polymerase I, 10 μM Of tris-HCl, pH 7.9, 5 μM of $MgCl_2$, 1 μM of dithiothreitol; (ii) for reverse transcriptases 10 μM of tris-HCl, pH 8.2, 5 μM of $MgCl_2$, 40 μM of KCl, 1 μM of dithiothreitol; (iii) for DNA polymerases α and ε 10 μM of tris-HCl, pH 7.4, 6 μM of $MgCl_2$, 0.4 μM of dithiothreitol; (iv) for DNA polymerse β 10 μM of tris-HCl, pH 8.5, 6 μM of $MgCl_2$, 1 μM of dithiothreitol; the [5'-^{32}P] primer–matrix complex (0.02-0.05 μM), dNTP and their analogs. The reactions were carried out for 20 min at 37°C (10 min at 20°C for DNA-polymerase I), were arrested by adding 2 μliter of formamide dye [5], and the products were analyzed by electrophoresis in 20% denaturated PAAG. For the terminal dexynucleosidyltransferase: a mixture containing in a volume of 5 μliter 100 μM of sodium cacodylate, pH 7.2, 10 μM of $MgCl_2$, 1 μM of $CaCl_2$, 1 μM of thiothreitol, 50 μM of dTTP or its analogs (100 μM), 0.6 μM of [5'-^{32}P]-oligonucleotide and 3 activity units of the enzyme was incubated for 40 min at 37°C. The reaction was arrested by adding 2 μliter of formamide dye (deionized formamide containing 0.1% xylenecyanol and bromphenol blue) and 20 μM of EDTA, pH 8.0. The reaction products were analyzed by electrophoresis in a 20% denaturated PAAG.

Inhibition of DNA Synthesis in the Presence of Substrate Analogs. Mixtures of 6 μliter in volume contained enzymes in the corresponding buffers, 0.02 μM of primer-matrix complex, 20 μM portions of the substrates (dATP and dGTP or dCTP and dGTP), 10 μM of [α-^{32}P]-dCTP (or dATP), 1 μM of dTTP, and the analogs tested in various concentrations. The reactions were carried out for 15 min at 20°C with 0.5 activity units of DNA-polymerase I, or for 15 min at 40°C with 3-4 activity units of reverse transcriptase and were arrested by adding EDTA at pH 8.0 up to 50 μM. The aliquots were checked on Whatman DE-81 filters after washing with 0.2 M NaCl solution with 0.5 μM of EDTA, pH 8.0, and fixation with ethanol.

Conditions with Additional Synthesis. After carrying out the reaction before its arrest, 70 μM portions of four dNTP were introduced into the mixture, the incubation was carried out for 10 min at 40°C, and the reaction was arrested and treated further as described above. The termination of the DNA synthesis in the presence of ddNTP or the analogs studied was verified under standard conditions [10] after electrophoresis in an 8% denaturated PAAG.

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